About the Cover:

Model depicting Vps4 remodeling of ESCRT-III filaments during the process of HIV-1 budding. The model is based on the recent cryo-EM structure of an active Vps4 hexamer in complex with an ESCRT-III substrate. "Structural basis of protein translocation by the Vps4-Vta1 AAA ATPase" Nicole Monroe, Han Han, Peter S. Shen, Wesley I. Sundquist, Christopher P. Hill eLife 2017;6:e24487 DOI: 10.7554/eLife.24487
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DAY 1

8:00 – 8:15  **Maureen Goodenow** (NIH Office of AIDS Research)
Opening Remarks

Session I: Reports from Specialized Centers (P50); Session Chair: Irwin Chaiken

8:15 – 9:15  The Pittsburgh Center for HIV Protein Interactions (PCHPI)

**Zandrea Ambrose** (University of Pittsburgh)
*Strangers on a Train: Imaging of HIV-1 and Host Proteins on Microtubules*

**Peijun Zhang** (University of Pittsburgh)
*Cryo-EM Structure of MxB Assembly Reveals a Novel Oligomerization Interface Critical for HIV Restriction*

**Peter Cherepanov** (The Francis Crick Institute)
*Cryo-EM structures of the Maedi-Visna Virus Intasome*

9:15 – 10:15  The Center for HIV Accessory and Regulatory Complexes (HARC)

**Nevan Krogan** (University of California San Francisco)
*The HARC Center: Overview and Progress*

**John Gross** (University of California San Francisco)
*Structure and Mechanism of Fab-Based Inhibitors of the HIV-1 Vif E3 Ubiquitin Ligase*

**James Hurley** (University of California Berkeley)
*HIV-1 Nef as a Cargo-Selective Allosteric Switch in Hijacking Clathrin Coated Vesicles*

10:15 – 10:45  BREAK

Session II: Reports from Specialized Centers (P50) continued; Session Chair: Celia Schiffer

10:45 – 11:45  The Center for HIV RNA Studies (CRNA)

**Alice Telesnitsky** (University of Michigan)
*Introduction to the Center for HIV-1 RNA Studies*

**Amanda Hargrove** (Duke University)
*The Distinguishing Properties of Bioactive RNA-Targeted Small Molecules*

**Janet Smith** (University of Michigan)
*APOBEC3H Catalytic Activity and RNA Binding*
Paul Bieniasz (Institution)
_Evasion of Intrinsic Host Defenses Drives HIV-1 Nucleotide Composition_

11:45 – 1:00 LUNCH

1:00 – 3:00 POSTER SESSION

Session III: Reports from Specialized Centers (P50) continued; Session Chair: Nevan Krogan

3:00 – 4:00 The Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)

_Wesley Sundquist_ (University of Utah)
*Introduction to the Center*

_Adam Frost_ (University of California San Francisco)
*Structures and Functions of ESCRT-III Filaments*

_Chris Hill_ (University of Utah)
*Structure and Mechanism of the Vps4 ATPase*

_Nels Elde_ (University of Utah)
*Evolution of Antiviral Retrogenes*

4:00 – 5:00 The HIV Interaction and Viral Evolution Center (HIVE)

_Bruce Torbett_ (The Scripps Research Institute)
*The HIV Interaction and Viral Evolution (HIVE) Center: Overview and Progress*

_Mamuka Kvaratskhelia_ (Ohio State University)
*Critical Structural Determinants for ALLINI-Induced Aggregation of HIV-1 Integrase*

_Ronald Levy_ (Temple University)
*Exploring Fitness and Energy Landscapes of HIV Proteins*

_Stefan Sarafianos_ (University of Missouri)
*The HIV Interaction and Viral Evolution (HIVE) Center: Future Directions*

5:00 ADJOURN FOR DAY
DAY TWO

Session IV: Envelope Function and Targeting, Session Chair: Wes Sundquist

8:00 – 8:30  Michael Root (NIH Invited Speaker; Thomas Jefferson University)
Receptor Utilization by HIV-1 Env During Viral Entry

8:30 – 9:15  P01: Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

  Irwin Chaiken (Drexel University)
Targeting HIV-1 Env for Inhibition and Inactivation

  Walther Mothes (Yale University)
Associating HIV-1 Env Trimer Structures with Functional Env Conformational States by smFRET Analysis

  Amos B. Smith III (University of Pennsylvania)
Design, Synthesis and Biological Evaluation of Small-Molecule Inhibitors of HIV-1 Entry: A Potential Long-Term Strategy for Curbing the AIDS Epidemic

Session V: Gag Assembly and Targeting, Session Chair: Alice Telesnitsky

9:15 – 9:45  David Millar (Selected Poster Talk; The Scripps Research Institute)
Single-Molecule Studies of HIV-1 Gag Assembly

9:45 – 10:15 Eric Hu (NIH Invited Speaker; Gilead Sciences)
Disrupting HIV Capsid Assembly by Small Molecule Modulators

10:15 – 10:45 BREAK

Session VI: APOBEC, Session Chair: Bruce Torbett

10:45 – 11:15 Yuri Lyubchenko (Selected Poster Talk; University of Nebraska)
Structure and Dynamics of Complexes of APOBEC3G With RNA at Nanoscale

11:15 – 11:45 Reuben Harris (Selected Poster Talk; University of Minnesota)
Structural Basis for Targeted DNA Cytosine Deamination and Mutagenesis by APOBEC Enzymes

11:15 – 12:45 LUNCH
(SAB Lunch, Room B; Please bring your lunch.)

12:45 – 2:45 POSTER SESSION
(12:45 – 2:45 NIGMS Centers Scientific Review Board; Room B)
Session VII: Protease; Session Chair: Stefan Sarafianos

2:45 – 3:30  P01: The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease

Celia Schiffer (UMass Medical School)
The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease, a Case Study

T. Whitfield (UMass Medical School)
Mechanistic Characterization of Inhibitor Resistance in HIV-1 Protease via Machine Learning and Atomistic Simulation

Celia Schiffer (UMass Medical School)
Interdependence of Inhibitor Recognition in HIV-1 Protease

Session VIII: Imaging Infection; Session Chair: Angela Gronenborn

3:30 – 4:00  Collin Kieffer (NIAID Invited Speaker; Caltech)
Multiscale Imaging of HIV Spread in Animal Models

4:00 – 4:30  João Mamede (Selected Poster Talk; Northwestern University)
Live Cell Imaging of HIV-1 Reverse Transcription

4:30 – 5:00  Gregory Melikyan (Selected Poster Talk; Emory University)
Loss of Capsid Protein and the Nuclear Membrane is a Pre-Requisite for Translocation of HIV-1 Pre-Integration Complexes into the Nucleus

5:00  ADJOURN MEETING

Mark Your Calendars!

Structural Biology Related to HIV/AIDS – 2018

Thursday June 28 – Friday June 29, 2018

Natcher Conference Center, Bethesda, Maryland
About the Poster Sessions

Thursday 1:00 – 3:00 T-numbered posters
Friday 1:00 – 3:00 F-numbered posters

Posters may be put up the morning of your session and left all day. Please remember to remove your poster from the board at the end of the assigned day. There are too many for posters to be left up for the whole meeting.
Thursday Posters

**Vpu**

T1. The Vpu-Interacting Protein ATP6V0C Regulates Expression of Tetherin and HIV-1 Release
Abdul A. Waheed, Ariana Gitzen, Maya Swiderski, and Eric O. Freed
Virus-Cell Interaction Section, HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD 21702

T2. Expression and Purification of Vpu and CD4 Complex
Yi-Liang Liu¹, Ignacio Asial¹², and Robert M. Stroud¹
¹Department of Biochemistry and Biophysics, University of California, San Francisco, California, USA; and ²School of Biological Sciences, Nanyang Technological University, Singapore, Singapore

T3. Molecular Basis of How Phosphoserine Acidic Cluster Motifs in HIV-1 Vpu and the Host Proteins Furin and Serinc3 Interact with Clathrin Adaptors
Rajendra Singh¹, Christopher Lim², Xiaofei Jia⁴ Charlotte Stoneham¹, Yong Xiong², and John Guatelli¹³
¹Department of Medicine, University of California San Diego (UCSD), La Jolla, California, USA; ²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, USA; ³The VA San Diego Healthcare System, San Diego, California, USA; and ⁴Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth, Dartmouth, Massachusetts, USA

**Nef**

T4. High Resolution Cryo-EM Structure of a Nef-Inhibited AP-1 Clathrin Adaptor Complex
Kyle L. Morris, Cosmo Buffalo, Xuefeng Ren, and James H. Hurley
Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA 94720, USA

T5. Structural Studies of Tec-Family Kinase Interactions with HIV-1 Nef
Kindra N. Whittlatch¹², John Jeff Alvarado², Rebecca Eells¹³, Haibin Shi², Mathias Losche¹³, and Thomas E. Smithgall¹²
¹Molecular Biophysics and Structural Biology Graduate Program, University of Pittsburgh and Carnegie Mellon University; ²Dept. Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine; and ³Department of Physics, Carnegie Mellon University

T6. Role of the Dileucine Motif in Nef-Induced Trimerization of the Arf1:AP-1 Clathrin Adaptor Complex
Cosmo Z. Buffalo, Kyle L. Morris, Xuefeng Ren, and James H. Hurley
Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA 94720, USA
T7. Analysis of HIV-1 Nef Dimerization and Binding Partner Interactions by Hydrogen Exchange Mass Spectrometry
Jamie A. Moroco¹, John Jeff Alvarado², Thomas E. Smithgall², and John R. Engen¹
¹Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115; and ²Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219

T8. Biochemical and Functional Characterization of a Cargo Selective Allosteric Switch in HIV-1 Nef
Xuefeng Ren¹, Qing-Tao Shen¹, Kyle Morris¹, Cosmo Buffalo¹, Claudia Firrito², Ajit Chande², Massimo Pizzato², and James H. Hurley¹
¹Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences, University of California-Berkeley; and ²Centre for Integrative Biology, University of Trento, Italy

T9. Physical Properties of the HIV-1 Capsid from All-Atom Molecular Dynamics Simulations
Juan R. Perilla¹,² and Klaus Schulten¹
¹Department of Physics & Beckman Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA; and ²Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716, USA.

T10. Structure Determination of HIV-1 Capsid Assemblies at Atomic Resolution Using Magic Angle Spinning NMR
Manman Lu¹,³, Charles D. Schwieters⁴, Guangjin Hou¹, Huilan Zhang¹,³, Gongpu Zhao²,³, Juan R. Perilla⁵, Jinwoo Ahn²,³, In-Ja L. Byeon²,³, Christopher Aiken³,⁸, Peijun Zhang²,³,⁷,⁸, Angela M. Gronenborn²,³, and Tatyana Polenova¹,³
¹University of Delaware, Department of Chemistry and Biochemistry, Newark, DE; ²Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA; ³Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh, Pittsburgh, PA; ⁴Center for Information Technology, National Institutes of Health, Building 12A, Bethesda, MD; ⁵Center for Biophysics and Computational Biology and Beckman Institute for Advanced Science and Technology and Departments of Physics, Chemistry, and Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL; ⁶Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN; ⁷Division of Structural Biology, University of Oxford, The Henry Wellcome Building for Genomic Medicine, Headington, Oxford OX3 7BN, U.K.; and ⁸Electron Bio-Imaging Centre, Diamond Light Sources, Harwell Science and Innovation Campus, Didcot OX11 0DE, U.K.

T11. Characterization of a Host Cell Activity that Stimulates HIV-1 Capsid Assembly in Vitro
Upul D. Halambage and Christopher Aiken
Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, Tennessee
T12. Building Soluble and Homogeneous HIV-1 Capsid Platforms Recognizable by Host Factors
Brady Summers, Ivy Huang, Sarah Smaga, Wei Wang, Katherine Digianantonio and Yong Xiong
Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, CT

T13. Fight AIDS @ Home: High-throughput Virtual Screening of the HIV-1 Mature Capsid Protein
Pierrick Craveur, Stefano Forli, and Arthur Olson
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA

T14. Expanding the Horizons for Structural Analysis of HIV-1 Capsid Assemblies by NMR Spectroscopy at MAS Frequencies Above 100 kHz
Manman Lu1,3, Caitlin M. Quinn1,3, Mingzhang Wang1,3, Guangjin Hou1, Xingyu Lu1,3, Jodi Kraus1, Loren Andreas4, Jan Stanek4, Daniela Lalić4, Guido Pintacuda4, Anne Lesage4, Jochem Struppe5, Werner Maas5, Angela M. Gronenborn2,3, and Tatyana Polenova1,3
1University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716; 2Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260; 3Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh, Pittsburgh, PA 15260; 4Centre de RMN à Très Hauts Champs, Institut des Sciences Analytiques, UMR 5280 CNRS / Ecole Normale Supérieure de Lyon, 5 rue de la Doua, 69100 Villeurbanne (Lyon), France; and 5Bruker Biospin Corporation, 15 Fortune Drive, Billerica, MA, United States

T15. Structural and Dynamic Studies of HIV-1 Capsid by 19F Solution and Magic Angle Spinning NMR
Mingzhang Wang1,3, Manman Lu1,3, In-Ja Byeon2,3, Guangjin Hou1, Chang-Hyeock Byeon2,3, Jochem Struppe5, Tatyana Polenova1,3, and Angela M. Gronenborn2,3
1Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716; 2Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260; 3Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 Fifth Ave., Pittsburgh, PA 15261; and 4Bruker Biospin Corporation, 15 Fortune Drive, Billerica, MA

Maturation

T16. Dynamic Regulation of HIV-1 Capsid Morphology and Viral Maturation
Caitlin M. Quinn1,3, Xingyu Lu1,3, Manman Lu1,3, Mingzhang Wang1,3, Ryan Russell1,3, Guangjin Hou1, Eric Freed4, Chris Aiken5, Angela M. Gronenborn2,3, and Tatyana Polenova1,3
1University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716; 2Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260; 3Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh, Pittsburgh, PA 15260; 4HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Building 535, Room 110, Frederick, MD 21702; and 5Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN 37232
T17. MicroED Structure of a CTD-SP1 Construct of HIV-1 Gag Reveals Binding Interactions with the Maturation Inhibitor Bevirimat
Michael Purdy1, Dan Shi5, Jakub Chrustowicz1, Johan Hattne5, Tamir Gonen5 and Mark Yeager1,2,3,4
University of Virginia School of Medicine, Department of Molecular Physiology and Biological Physics1, Department of Medicine, Division of Cardiovascular Medicine2, Center for Membrane and Cell Physiology3, and Cardiovascular Research Center4, Charlottesville, VA 22908; and 5Howard Hughes Medical Institute, Janelia Research Campus, Ashburn, VA 20147

T18. Structural Studies of HIV-1 Maturation by Magic Angle Spinning NMR
Ryan W. Russell1,3, Caitlin M. Quinn1,3, Mingzhang Wang1,3, Christopher L. Suiter1,3, Guangjin Hou1,3, Manman Lu1,3, Jinwoo Ahn2,3, Sherimay D. Ablan4, Emiko Urano4, Eric O. Freed4, Angela M. Gronenborn2,3, and Tatyana Polenova1,3
1University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716; 2Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260; 3Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh, Pittsburgh, PA 15260; and 4HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Building 535, Room 110, Frederick, MD 21702

T19. Characterization of the Molecular Mechanism for Maturation Inhibitors Against the HIV-1 Capsid-SP1 Domain
Carly A. Sciandra1, Pengfei Ding1, Eric O. Freed2, and Michael F. Summers1
1Department of Chemistry & Biochemistry, Howard Hughes Medical Institute, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21052; and 2Virus-Cell Interaction Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702

Protease and Its Inhibition

T20. Investigating the Effects of Drug Resistance Mutations on Conformational Sampling Patterns in HIV-1 Protease Subtype C in Response to Nelfinavir
Larry Nguyen, Trang Tran, and Gail Fanucci
Department of Chemistry, University of Florida, Gainesville, FL 32610

T21. The Structural and Dynamic Basis for the Interdependence of Drug Resistance in HIV-1 Protease
Shahid N. Khana, John D. Personsa, Janet L. Paulsenb, Michel Guerreroa, Celia A. Schifferb, Nese Kurt Yilmazb, and Rieko Ishimaa
aDepartment of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA15260; and bDepartment of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA01605

T22. Substrate Envelope Guided Design of HIV-1 Protease Inhibitors Incorporating Stereochemically Defined Novel P2’ Ligands
Akbar Ali,1 Linah N. Rusere,1 Gordon L. Lockbaum,1 Sook-Kyung Lee,2 Ronald Swanstrom,2 and Celia A. Schiffer1
1Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, United States; and 2Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States
T23. Development of a Genetic Algorithm for DOCK to Aid in De Novo Design
Courtney D. Singleton¹ and Robert C. Rizzo²,³,⁴
¹Department of Pharmacology, ²Department of Applied Mathematics & Statistics, ³Institute of Chemical Biology & Drug Discovery, and ⁴Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

T24. Mechanistic Characterization of Inhibitor Resistance in HIV-1 Protease via Machine Learning and Atomistic Simulation
Troy W. Whitfield, Debra A. Ragland, Nese Kurt-Yilmaz, Konstantin Zeldovich and Celia A. Schiffer
Department of Medicine, Biochemistry and Molecular Pharmacology and the Program in Bioinformatics, UMass Medical School, Worcester, MA

T25. Interdependence of Inhibitor Recognition at the Active Site of HIV-1 Protease
Janet L. Paulsen, Florian Leidner, Debra A. Ragland, Nese Kurt Yilmaz, and Celia A. Schiffer
Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605, United States

T26. A Computational Approach to Identify and Incorporate Bridging Water Molecules in Drug-lead Discovery
Jiaye Guo¹ and Robert C. Rizzo²,³,⁴
¹Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794; ²Department of Applied Mathematics & Statistics, Stony Brook University, Stony Brook, New York 11794; ³Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York 11794; ⁴Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York 11794.

T27. Analyzing the Hydration Structure of HIV-1 Protease using Molecular Dynamics Simulations
Florian Leidner, Janet Paulsen, and Celia Schiffer
Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA, USA; florian.leidner@umassmed.edu

T28. The Homodimeric HIV-1 Protease Uses Bi-Specific S2/S2' Subsites to Achieve Optimal Processing of Two Evolutionarily Conserved Cleavage Site Motifs
Marc Potempa¹, Sook-Kyung Lee¹, Nese Kurt Yilmaz², Ellen Nalivaik², Amy Rogers¹, Celia Schiffer², and Ronald Swanstrom¹
¹Department of Biochemistry and Biophysics, and the UNC Center for AIDS Research, University of North Carolina, Chapel Hill, NC; and ²Department of Biochemistry and Molecular Pharmacology, University of Massachusetts, Worcester, MA

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Envelop Incorporation

T29. Investigation of the Role of Rab11-FIP1C in HIV-1 Glycoprotein Incorporation
Melissa V. Fernandez and Eric O. Freed
HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702-1201, USA
T30. Mutations at the HIV-1 Matrix Trimer Interface Prevent Envelope Glycoprotein Incorporation
Mariia Novikova, Philip R. Tedbury, and Eric O. Freed
National Cancer Institute, Frederick, MD

Gag-Membrane Interaction

T31. Structural Basis for the Unique Myristoylation Signal of the Feline Immunodeficiency Virus Matrix Protein
1Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Bxaltimore, MD 21250, USA; and 2Virus-Cell Interaction Section, HIV Drug Resistance Program, National Cancer Institute at Frederick, Frederick, MD 21702-1201, USA

T32. A Novel Class of HIV-1 Budding Inhibitor Blocks Ubiquitin Recognition by the ESCRT-I Protein Tsg101
Madeleine Strickland, Lorna S. Ehrlich, Susan Watanabe, Steven M. Bonn, Christina M. Camara, Marie-Paule Strub, David Fushman, Carol Carter, and Nico Tjandra
1Laboratory of Molecular Biophysics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, 20892; 2Department of Molecular Genetics & Microbiology, School of Medicine, Stony Brook University, Stony Brook, NY 11794-5222; and 3Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742.

T33. Structural Basis of ESCRT-III Membrane Assembly
Nathaniel Talledge, Henry C. Nguyen, John McCullough, Dawn M. Wenzel, Wesley I Sundquist, and Adam Frost
1Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94158, USA; and 2Department of Biochemistry, University of Utah, Salt Lake City, UT 84112, USA; *: Equal contributions; †: Corresponding authors

T34. Towards In Vitro Reconstitution of HIV-1 Budding and Release
Thomas G. Flower, Lars-Anders Carlson and James H. Hurley
Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences, University of California-Berkeley, Berkeley, CA 94720

Gag Trafficking and Assembly

T35. Probing HIV-1 Gag Polyproteins with SuFEx Compounds
Theresa Tiefenbrunn Sample, Hua Wang, Lynda Tuberty, Joseph D. Bauman, Joseph Marcotrigiano, Eddy Arnold, Stefano Forli, Suhua Li, Qinheng Zheng, Gencheng Li, Grant Bare, Arthur J. Olson, John H. Elder, Stefan G. Sarafianos, K. Barry Sharpless, and Bruce E. Torbett
1Dept. of Immunology and Microbiology and 2Department of Chemistry, The Scripps Research Institute, La Jolla, CA; 3Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ; 4Laboratory of Infectious Diseases, NIAID, Bethesda, MD; 5Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA; and 6Department of Molecular Microbiology and Immunology, Columbia, MO.
T36. Progress Toward Selection of FANA Xeno-Nucleic Acid Aptamers that Bind with High Affinity to HIV-1 Gag and NC Proteins
Irani Alves Ferreira-Bravo, Kevin Rose, and Jeffrey J. DeStefano
1Cell Biology and Molecular Genetics, Bioscience Research Building, University of Maryland, College Park, MD 20742, USA; 2Maryland Pathogen Research Institute, College Park, MD 20742, USA; and 3HIVE: HIV Interaction and Viral Evolution Center

T37. Cryo-Electron Tomography of HIV-1 Mutants Reveals a Structural Element Critical for Assembly of the Immature Virion
Lucas J. Adams, Mariia Novikova, Dennis C. Winkler, Juan Fontana, Eric O. Freed, and Alasdair C. Steven
1Laboratory of Structural Biology Research, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD; 2Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD; and 3Astbury Centre for Structural Molecular Biology, University of Leeds, UK

T38. Immature HIV-1 Assembly Dynamics from Coarse-Grained Molecular Simulations
Alexander J. Pak, John M. A. Grime, and Gregory A. Voth
1Department of Chemistry, Institute for Biophysical Dynamics, and James Franck Institute, The University of Chicago, Chicago, IL, 60637; *E-mail: ajpak@uchicago.edu

Rajan Lamichhane, Raymond Pauszek, Ilean Chai, Yisong Deng, John Hammond, Bruce Torbett, James R. Williamson and David P. Millar
Department of Integrative Structural and Computational Biology and Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla CA 92037

T40. Toward Understanding Gag Assembly in vivo
Yisong Deng, Ilean Chai, John A. Hammond, Bruce E. Torbett, and James R. Williamson
1Equal Contribution; 1University of California, San Diego, Department of Biomedical Sciences, La Jolla, CA 92093; 2The Scripps Research Institute, Department of Integrative Structural and Computational Biology, La Jolla, CA 92037; and 3The Scripps Research Institute, Department of Immunology and Microbiology, La Jolla, CA 92037

T41. Identification of HIV-1 Interacting Cellular Factors during Gag Trafficking and Assembly
Ilean Chai, Petra Minder, Yisong Deng, John A. Hammond, John R. Yates, III, James R. Williamson, and Bruce E. Torbett
1Equal Contribution; 1Department of Biomedical Sciences, University of California San Diego, La Jolla, CA; 2Department of Immunology and Microbiology, 3Department of Integrative Structural and Computational Biology, and 4Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA
RNA Structure and Targeting

T42. Influence of the 5’ Transcription Start Site Heterogeneity on HIV RNA Structure and Fate
Joshua D Brown1, Siarhei Kharytonchyk2, Lindsay Glang1, Hannah Carter1, Aishwarya Iyer1, Steven Choi1, Michael Lopresti1, Matthew Orellana3, Ubiomo Oboh1, Tatiana Rodriguez1, Jana Hiji1, Yash Desai1, Alice Telesnitsky2 and Michael F. Summers1
1Howard Hughes Medical Institute and Department of Chemistry and Biochemistry, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; and 2Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor MI 48109-0620

T43. Development of Site-Specifically Labeled Nucleotides to Address Problems in NMR Spectroscopy
Andrew P. Longhini, Regan M. Leblanc, and Theodore K. Dayie
University of Maryland, College Park

T44. Structural Study of Noncoding RNAs via NMR Spectroscopy
Hyeyeon Nam, Andrew Longhini, Owen Becette, and T. Kwaku Dayie
University of Maryland College Park

T45. Ensemble Computational Approaches to Human Endogenous Retroviral RNA
Susan J. Schroeder, Xiaobo Gu, and Nathan Sloat
Department of Chemistry & Biochemistry, Department of Microbiology & Plant Biology
University of Oklahoma, Norman, OK 73019 USA

T46. Small Molecule Targeting of Viral and Virus-associated RNAs
Joanna Sztuba-Solinska1, Fardokht Abulwerdi1, Regan LeBlanc1, Shilpa R. Shenoy2,3, Joe S. Matario2, Jennifer Miller1, Jason Rausch1, Tom Kenderdine4, Denise Whitby5, Daniel Fabris4, Barry R. O’Keefe2 and Stuart F.J. Le Grice1.
1Basic Research Laboratory, 2Molecular Targets Laboratory, National Cancer Institute, Frederick MD, USA; 3Leidos Biomedical Research Inc., Frederick, USA; 4SUNY, Albany NY, USA; and 5AIDS and Cancer Virus Program, National Cancer Institute, Frederick MD, USA.

T47. Imine-Based Dynamic Combinatorial Chemistry for Discovery of Multivalent RNA-Binding Ligands
Aline Umuhire Juru1, Adina Jan1, and Amanda E. Hargrove1,2
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T48. Impact of the HLA B*57 Allele on Intra-Host HIV-1 Capsid-Coding RNA Secondary Structure Diversity
Brittany D. Rife1,2, Carla N. Mavian1,2, Susanna L. Lamers4, David J. Nolan1,2, Frederick M. Hecht6, Annika C. Karlsson5, and Marco Salemi1,2
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T49. HIV 5'UTR Forms Multiple Structures in CD4+ T Cells
Phil Tomezsko1,2, Vincent Corbin1, Paromita Gupta1, Sitara Persad1, Margalit Glasgow1, and Silvi Rouskin1
1Whitehead Institute, 2Harvard University

RNA Packaging

T50. Characterization of HIV-1 Matrix Interactions with tRNA\textsuperscript{Lys3}.
Christy R. Gaines, Amalia Rivera-Owen, Emre Tkacik, Ae Lin Yang, Phoebe Somani, Alecia Achimovich, Tawa Alabi, and Michael Summers
Howard Hughes Medical Institute at the University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

T51. HIV-1 Genomic RNA Determinants and Stoichiometry of Specific Gag Binding Reveal Insights into Selective Genome Packaging
Erik D. Olson, Samantha H. Hinckley, Joshua-Paolo Reyes, Shuhui Liu, Vicki H. Wysocki, and Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH

T52. HIV-1 gRNA Packaging is Initiated in a Unique Host RNA Granule that Assembling Gag Enters via a Two-Step Mechanism
Motoko Tanaka, Brook C. Barajas, Bridget A. Robinson, Daryl J. Phuong, Kasana Chutiraka, Jonathan C. Reed, and Jaisri R. Lingappa
Dept. of Global Health, University of Washington, Seattle, WA

T53. Selective HIV-1 Gag-RNA Interaction Requires a Series of Specific Interactions Distributed Across the 5’-UTR
Mauricio Comas-Garcia, Siddhartha Datta, Laura Baker, and Alan Rein
Retroviral Assembly Section, HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD

RRE and RNA Export

T54. Action Mechanism of DDX3X: A DEAD-box Helicase Implicated in HIV-1 mRNA Transport for the Nucleus to the Cytoplasm.
Anthony F.T. Moore, Aliana Lopez de Victoria, and Eda Koculi
Department of Chemistry, University of Central Florida

T55. Exploring the Structural and Functional Landscape of the HIV-1 Rev Protein.
Jason F. Fernandes, Bhargavi Jayaraman, Shumin Yang, Amber M. Smith, Yifan Cheng and Alan Frankel
Center, University of California, San Francisco, San Francisco, CA 94158.

T56. Structure of HIV-1 Rev Protein Recognizing the Host Co-Factor Tat-SF1
Steven Horner, Justin Leach, Mary Pulvino, Jermaine L. Jenkins, and Clara L. Kielkopf
Center for RNA Biology, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642
T57. Uncovering Conformational Excited States in HIV-1 Rev Response Element RNA as New Small Molecule Targets
Chia-chieh Chu¹ and Hashim M Al-Hashimi¹.²
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T58. Structural Characterization of the HIV-1 Rev Response Element
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RNA Splicing

T59. CLIP-seq Reveals a Key Role for hnRNP H1 in Regulation of HIV-1 Alternative mRNA Splicing
Sebla B. Kutluay¹, Ann Emery², Srinivas Penumutchu³, Dana Townsend¹, Michaela Madison¹, Amanda Stukenbroeker¹, Chelsea Powell⁴, David Jannain⁴, Blanton S. Tolbert³, Ronald Swanstrom⁴, and Paul D. Bieniasz⁵.⁶
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T60. Control of HIV-1 Splicing by Cis-Acting Sequence Elements
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Latency and Transcriptional Control

T61. Crystal Structure of the HIV1 Tat:AFF4:P-TEFb:TAR Complex at 4Å Resolution
Ursula Schulze-Gahmen¹ and James H. Hurley¹.²
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T62. Structural and Biochemical Analysis of the Brd4:P-TEFb Complex
Hersh K. Bhargava, Ursula Schulze-Gahmen, Goran Stjepanovic, and James H. Hurley
Department of Molecular and Cell Biology and California Institute of Quantitative Biosciences,
University of California, Berkeley

T63. Molecular mechanism of host factor HLTF recruitment to the CRL4-DCAF1 E3
ubiquitin ligase by HIV-1 Vpr protein
Xiaohong Zhou¹, Maria DeLucia¹, Caili Hao², Kasia Hrecka², Christina Monnie¹, Jacek
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T64. PJA2 Ubiquitinates the HIV-1 Tat Protein with Low Site Specificity to Activate Viral
Transcription
Tyler B. Faust¹, Yang Li², Gwendolyn M. Jang³, Jeffrey R. Johnson³, Shumin Yang⁴,⁵, Amit
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Francisco, San Francisco, California, USA and ⁵School of Medicine, Tsinghua University,
Beijing, China.

T65. HIV-1 Infection of Primary CD4+ T-Cells Regulates the Expression of Specific HERV-
K (HML-2) Elements
Sandra N. Terrya,1, George R. Youngc,1, Lara Manganaroa,1, Alvaro Cuesta-Domingueza,1,
Gintaras Deikusd,1, Juan Ayllon¹, Laura Campisi¹, Ana Fernandez-Sesmaa,1, Robert Sebra¹,2,
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London NW1 1AT, UK; and ⁴Department of Genetics and Genomic Sciences, Icahn School of
Medicine at Mount Sinai, New York, New York, USA; ⁵These authors contributed equally

T66. Towards the Anti-Viral Response of FACT Complex (SUPT16H and SSRP1)
Huachao Huang¹, Netty Santoso¹, Sydney Simpson¹, Hongyu Miao¹, Stephen Elledge², and Jian
Zhu¹
¹University of Rochester Medical Center, Rochester, NY; and ²Brigham and Women’s Hospital,
Boston, MA

T67. CBF-1 Promotes the Establishment and Maintenance of HIV Latency by Recruiting
Polycomb Repressive Complexes, PRC1 and PRC2, at HIV LTR
Sonia Zicari¹,2, Kalamo Farley¹, Lin Sun¹, Liam Spurr¹, Ashok Chauhan¹, Gary Simon¹ and Mudit
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of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA;
and ³Department of Microbiology, Immunology and Tropical Medicine, George Washington
University, Washington, DC 20037; Corresponding Author
T68. Curaxin CBL0100 Blocks HIV-1 Replication and Reactivation Through Targeting of FACT
Maxime Junior Jean, Tsuyoshi Hayashi, Huachao Huang, Justin Brennan, James Kobie, Netty Santoso, and Jian Zhu.
Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, 14642, USA.

T69. Smac Mimetics as a Novel Class of HIV-1 Latency Reversing Agents
Lars Pache¹, Miriam S. Dutra¹, Adam M. Spivak², John M. Marlett³, Jeffrey P. Murry³§, Young Hwang⁴, Mitchell Vamos¹, Peter Teriete¹, Renate König¹,⁵, Nicholas D. P. Cosford¹, Frederic D. Bushman⁴, John A. T. Young³#, Vicente Planelles² and Sumit K. Chanda¹
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Friday Posters

**Integrase**

**F1. Targeting HIV-1 Integrase Multimerization for Inhibitor Design: Modeling Higher-Order Protein Aggregation Mediated by ALLINIs**
Nanjie Deng, Pratibha Koneru, Nicholas Cockroft, James Fuchs, Mamuka Kvaratskhelia, and Ronald Levy

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**F2. Structural Basis for the ALLINI-induced Aggregation of HIV Integrase**
Kushol Gupta, Grant Eilers, Audrey Allen, Young Hwang, Vesa Turkki, Frederic D. Bushman, and Gregory D. Van Duyne

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**F3. Modeling HIV-1 Integrase Multimerization and Allosteric Inhibitor Binding**
Krati Sharma, George W. Merkel, Margret B. Einarson, Yan Zhou, Anna Marie Skalka, and Mark D. Andrake

**F4. Specific Mutations that alter HIV-1 Capsid Stability Modulate the Activity and Composition of HIV-1 Preintegration Complexes**
Muthukumar Balasubramaniam, Jing Zhou, Jui Pandhare, Christopher Aiken, and Chandravanu Dash

1Center for AIDS Health Disparities Research, 2Department of Biochemistry and Cancer Biology, and 3School of Graduate Studies and Research, Meharry Medical College, Nashville, TN; and 4Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN

**F5. Allosteric HIV-1 Integrase Inhibitors Lead to Premature Degradation of the Viral RNA Genome and Integrase in Target Cells**

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F6. Title
Zhaoyong Xi¹, Ryan L. Slack¹, Karen A. Kirby³, Michael A. Parniak², Zhengqiang Wang⁴, Stefan G. Sarafianos³, and Rieko Ishima¹
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F7. 2-Hydroxyisoquinoline-1,3-diones and 3-Hydroxypyrimidine-2,4-diones as Novel HIV-1 RNase H Inhibitors
Karen A. Kirby¹, ², Andrew D. Huber¹, ³, Mary C. Casey¹, ², Atsuko Hachiya⁴, Jing Tang⁵, Sanjeev K. V. Vernekar⁵, Bulan Wu⁵, Nataliya A. Myshakina⁶,⁷, Martin T. Christen⁸, Yue-Lei Chen⁵, Daniel Wilson⁶, Qiongying Yang¹, ², Michael A. Parniak⁶, Rieko Ishima⁸, Zhengqiang Wang⁶, and Stefan G. Sarafianos¹, ², ⁹
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F8. HIV Capsid Pattern Sensing by the Human Antiviral Protein MxB
Sarah S Smaga, Brady J Summers, Katherine M Digianantonio, and Yong Xiong
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

Tatsuya Maehigashi, Lydia R. Studdard, Sarah A. Mereby and Baek Kim
Center for Drug Discovery, Emory School of Medicine, Pediatrics, Atlanta, GA

F10. SAMHD1 Mediates Mx2 Anti-Viral Activity by Targeting HIV-1 Capsid
Wei Wei¹, Haoran Guo¹, Qimeng Gao², Weiming Yang², and Xiao-Fang Yu³
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Nuclear Entry

F11. Inhibition of HIV-1 Nuclear Entry by a Novel Antiviral, JT-4-173
Mary C. Casey\textsuperscript{1,2}, Obiaara B.Ukah\textsuperscript{1,2}, Andrew D. Huber\textsuperscript{1,3}, Jing Tang\textsuperscript{4}, Karen A. Kirby\textsuperscript{1,2}, Atsuko Hachiya\textsuperscript{5}, Seongmi Kim\textsuperscript{6}, Vincent Yapo\textsuperscript{1,2}, Qiongying Yang\textsuperscript{1,2}, Kamalendra Singh\textsuperscript{1,2}, Philip R. Tedbury\textsuperscript{1,2}, Michael A. Parniak\textsuperscript{7}, Zhengqiang Wang\textsuperscript{4}, and Stefan G. Sarafianos\textsuperscript{1,2,6}
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F12. HIV-1 Utilizes CypA to Protect Against Cytoplasmic CPSF6
Joshua Schwartz, Hyun Jae Yu, Wendy Yuen, Vineet N. KewalRamani, and KyeongEun Lee
Basic Research Laboratory, National Cancer Institute, Frederick, MD

F13. Soluble Host Factors Modulate the HIV-1 Nuclear Entry Pathway
Guangai Xue\textsuperscript{1}, Hyun Jae Yu\textsuperscript{1}, Shih Lin Goh\textsuperscript{2}, Anna T. Gres\textsuperscript{3}, KyeongEun Lee\textsuperscript{1}, Stefan G. Sarafianos\textsuperscript{5}, Jeremy Luban\textsuperscript{2}, and Vineet N. KewalRamani\textsuperscript{1}
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F14. CPSF6 Regulates the Intranuclear Trafficking of HIV-1
Hyun Jae Yu, KyeongEun Lee, and Vineet N. KewalRamani
National Cancer Institute, Basic Research Laboratory, Frederick, MD

F15. HIV-1 Co-Localizes with CPSF6 and TNPO3 on Microtubules in the Perinuclear Region
Zhou Zhong, Christopher Kline, Douglas K. Fischer, Simon C. Watkins, and Zandrea Ambrose
Pittsburgh Center for HIV Host Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA

F16. Loss of Capsid Protein at the Nuclear Membrane is a Pre-Requisite for Translocation of HIV-1 Pre-Integration Complexes into the Nucleus
Ashwanth C. Francis\textsuperscript{1}, and Gregory B. Melikyan\textsuperscript{1,2}
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Reverse Transcription

F17. Live Cell Imaging of HIV-1 Reverse Transcription
João I Mamede and Thomas J Hope
Northwestern University, Department of Cell and Molecular Biology, Chicago, IL
F18. Structural Investigation of the HIV-1 Reverse Transcriptase Initiation Complex by HDX, SAXS, and Cryo-EM
Chelsy Chesterman1, Steven Tuske1, Jie Zheng2, Youngmin Jeon3, Cheng Zhang3, William
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Molecular Therapeutics, The Scripps Research Institute Florida; 3Laboratory of Genetics and
Helmsley Center for Genomic Medicine, Salk Institute for Biological Studies; and 4 Department
of Chemistry and Biochemistry, Ohio State University

F19. Potent Activity of 4'-ethynyl-2-fluoro-2'-deoxyadenosine Against Diverse Primary
Isolates of HIV-1
Philip R. Tedbury1,2, Obiaara B. Ukah1,2, Seongmi Kim1,3, Leonard C. Rogers1, Kamalendra
Singh1,4, Eiichi N. Kodama4, Hiroaki Mitsuya5,6, Michael A. Parniak7 and Stefan G. Sarafianos1,2,8
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Health, Bethesda, MD; 4Department of Microbiology and Molecular Genetics, University of
Pittsburgh School of Medicine, Pittsburgh, PA; 8Department of Biochemistry, University of
Missouri, Columbia, MO

F20. Energetics of Nucleotide Translocation Through HIV-1 CA Hexamer
Chaoyi Xu and Juan R. Perilla
Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware

Vif and APOBECs

F21. Structure and Dynamics of Complexes of APOBEC3G with RNA at Nanoscale
Yangang Pan, Zhiqiang Sun, Atanu Maiti, Tapan Kanai, Hiroshi Matsuo, Ming Li, Reuben S.
Harris, Luda S. Shlyakhtenko, and Yuri L. Lyubchenko
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986025 Nebraska Medical Center, Omaha, NE 68198-6025

F22. Structure Determination of APOBEC3G-Vif-CRL5 by Cross-linking Mass
Spectrometry and Integrative Modeling
Robyn M Kaake1, Ignacia Echeverria2, Seung Joong Kim2, Gwendolyn Jang3, Hai Ta4, John
Gross4, Andrej Sali2,4,5, and Nevan Krogan1,3,5
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San Francisco, CA; 4University of California – San Francisco, Dept. of Pharmaceutical
Chemistry, San Francisco, CA; and 5California Institute of Quantitative Biosciences, University
of California San, Francisco, San Francisco, United States
F23. Integrative Structure Modeling of HIV Vif in Complex with Host Proteins
Ignacia Echeverria¹, Seung Joong Kim¹, Robyn Kaake², Hai Ta³, John Gross³, Nevan Krogan², and Andrej Sali¹,³,⁴
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F24. Molecular Characterization of a Unique Restriction Factor, APOBEC3H
Jennifer Bohn¹,², W. Clay Brown², Paul Bieniasz³, Theodora Hatzioannou³, and Janet Smith¹,²
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F25. Structural Basis for Targeted DNA Cytosine Deamination and Mutagenesis by APOBEC Enzymes
K. Shi¹,²,³, M.A. Carpenter¹,²,³,⁴,⁵, S. Banerjee⁶, N.M. Shaban¹,²,³,⁴, K. Kurahashi¹,²,³, D.J. Salamango¹,²,³,⁴, J.L. McCann¹,²,³,⁴, G.J. Starrett¹,²,³,⁴, J.V. Duffy¹,²,³, Ö. Demir⁷, R.E. Amaro⁷, D.A. Harki²,⁸, H. Aihara¹,²,³, and R.S. Harris¹,²,³,⁴,⁵
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F26. Model and Dynamics of a Full A3G Monomer
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F27. Fab-Based Inhibitors Separate Ubiquitin Dependent and Independent Functions of HIV Vif Neutralization of APOBEC3
Jennifer M. Binning1, Amber M. Smith2,3, Judd F. Hultquist4,5,6, Charles S Craik1, Nathalie Caretta1, Melody G. Campbell2,3, Lily Burton1, Florencia La Greca1, Michael J. McGregor4,5,6, Hai M. Ta1, Koen Bartholomeeusen7,8,9, B. Matija Peterlin7,8,9, Nevan J. Krogan1,5,6, Natalia Sevillano1, Yifan Cheng2,3, and John D. Gross1
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TRIMs and Uncoating

F28. Distinct Capsid Uncoating and Host Cell Infectivity Phenotypes in HIV-1NL4-3 and HIV-1LAI are Caused by Minor Sequence Differences in CA
Douglas K. Fischer1,2,4, Simon C. Watkins3,4, Masahiro Yamashita4,5, and Zandrea Ambrose1,2,4
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F29. Reverse Transcription-Driven Uncoating of Mature HIV Capsids
Ioulia Rouzina1 and Robijn Bruinsma2
1Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH; 2Department of Physics and Astronomy, University of California, Los Angeles, CA. 1Ohio State University, Department of Biochemistry, Columbus, OH; and 2University of California, Department of Physics and Astronomy, Los Angeles, CA

F30. Novel and Distinct Functions of Diaphanous-Related Formins Regulate HIV-1 Uncoating and Transport
Michael Keegan Delaney, Viacheslav Malikov, Guangyuan Zhao, and Mojgan H. Naghavi
Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA.

F31. Charge-Charge Interaction is Important for Fez1-Capsid Interaction During HIV Infection
Ivy Huang, Brady Summers, and Yong Xiong
Yale University, Department of Molecular Biophysics and Biochemistry, New Haven, CT
F32. Hexagonal Lattice Assembly of TRIMCyp
Jonathan Wagner, Devin Christensen, Akash Bhattacharya, Marcin D. Roganowicz, Yueping Wan, Dmitri Ivanov, Barbie K. Ganser-Pornillos, Wesley I. Sundquist, and Owen Pornillos

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F33. Defects in Assembly Explain the Reduced Antiviral Activity of the G249D Polymorphism in Human TRIM5α
Sevnur Komurlu, Margret Bradley, Nikolai Smolin, Sabrina Imam, Raymond F. Pauszek III, Seth L. Robia, David Millar, and Edward M. Campbell

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F34. Structural Basis of Retroviral Capsid Recognition by the Restriction Factor, TRIM5α
Katarzyna Skorupka, Yueping Wan, Marcin Roganowicz, Owen Pornillos, and Barbie K. Ganser-Pornillos

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F35. Structure-Based Optimization of Small-Molecule CD4-Mimics: Inhibitors of HIV-1 Cell Entry and Viral Neutralization Agents
Sharon M. Kirk, Melissa C. Grenier, Bruno Melillo, Andrés Finzi, Navid Madani, Joseph Sodroski, and Amos B. Smith, III

Department of Chemistry, University of Pennsylvania

F36. Expanding Chemical Space for Optimizing Macrocyclic HIV-1 Inactivators
Ann Haftl, Adel A. Rashad, Kriti Acharya, Rachna Aneja, Andrew P. Holmes, and Irwin Chaiken

Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania

F37. Pharmacophore Modeling of Human CC-Chemokine Receptor 4 Allosteric Antagonists
Gulriz Aytekin-Kurban, Xiang S. Wang, Mugizi R. Rwebangira, and Chunmei Liu

Howard University

F38. Computational And Experimental Screening for Small Molecules Obstructing HIVgp41 N-Heptad Repeat Trimer

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Targeting Envelope
Lauren Prentis¹, Steven Telehany², William J. Allen³, Brian C. Fochtman¹, Trent E. Balius⁴, and Robert C. Rizzo¹,²,³,⁵,⁶
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F40. Liposome Nanoparticle Encapsulation and Surface Modification with Macrocyclic Peptide Triazole HIV-1 Inactivators
Rachna Aneja¹, Aakansha Nangaria¹, Adel A Rashad¹, Antonella Grigoletto², Gianfranco Pasut², Steven Wrenn³, Jeffrey M. Jacobson⁴, and Irwin Chaiken¹
¹Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, 245N 15th St, Philadelphia, PA 19102; ²Department of Pharmaceutical and Pharmacological Sciences, University of Padua, via Marzolo 5, Padua 35131, Italy; ³Department of Chemical and Biological Engineering, Drexel University, Philadelphia, PA 19104; and ⁴Departments of Medicine and Neuroscience and Center of Translational AIDS Research, Lewis Katz School of Medicine, Temple University, Philadelphia, PA 19140.

F41. Restricted HIV-1 Env Glycan Engagement by Lectin-Reengineered DAVEI Protein Chimera is Sufficient for Lytic Inactivation of the Virus
Bibek Parajuli¹, Kriti Acharya¹, Harry C. Bach¹,², Shiyu Zhang¹, Bijay Parajuli¹, Cameron F. Abrams¹,³, and Irwin Chaiken¹
¹Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102, United States; ²Department of Biomedical Engineering, Drexel University, Philadelphia, Pennsylvania 19102, United States; and ³Department of Chemical and Biological Engineering, Drexel University, Philadelphia, Pennsylvania, 19104, United States

F42. Generation of HIV-1 Escape Mutants to Peptide Triazole Entry-Inhibiting Virus Inactivators
Andrew P. Holmes¹,², Shiyu Zhang¹,³, Alexej Dick¹, Adel A. Rashad¹, Lauren D. Bailey¹, Michael J. Root⁴, and Irwin M. Chaiken¹
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Env Structure and Function

F43. Associating HIV-1Env Trimer Structures with Functional Env Conformational States by smFRET Analysis
Maolin Lu1, Xiaochu Ma1, Luis R. Castillo-Menendez2,3, Utz Ermel1, Daniel S. Terry4, Jay Gorman5, Nick Reichard1, Kevin Wang1, James B. Munro6, Peter D. Kwong5, Scott C. Blanchard4, Joseph Sodroski2,3 and Walther Mothes1
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F44. The HIV-1 Env Trimer Opens Through an Asymmetric Intermediate in Which Only a Single CD4 Engages the Trimer
Xiaochu Ma1, Maolin Lu1, Priyamvada Acharya2,3, Jason Gorman2, Daniel S. Terry4, Xinyu Hong1, Zhou Zhou4, Hong Zhao4, Roger B. Altman4, James Arthos5, Clint S. Potter3, Scott C. Blanchard4, Bridget Carragher3, Peter D. Kwong2, James B. Munro6, and Walther Mothes1
1Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06536, USA; 2Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; 3Simons Electron Microscopy Center, The National Resource for Automated Molecular Microscopy, New York Structural Biology Center, NY 10027, USA; 4Department of Physiology and Biophysics, Weill Cornell Medical College of Cornell University, New York, NY 10021, USA; 5Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA; and 6Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111, USA.

F45. Single Molecule Fluorescence Spectroscopy of HIV Envelope in Solution
Department of Biochemistry and Molecular Biology and Institute of Human Virology, University of Maryland School of Medicine, Baltimore, Maryland, USA

F46. Selection of Env Mutations that Globally Rescue HIV-1 Replication Despite Impaired Cell-Free Infectivity: Implications for Overcoming Antiretroviral Therapy
Rachel Van Duyne1, Lillian Kuo, Ken Fujii, and Eric O. Freed
Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD

F47. HIV-1 Entry Depends on Virus-Induced Cell Surface Exposure of Phosphatidylinerine
Elena Zaitseva1, Eugene Zaitsev1, Kamran Melikov1, Anush Arakelyan2, Mariana Marin3, Rafael Villasmiǐ4, Leonid B. Margolis2, Gregory B. Melikyan3, and Leonid V. Chernomordik1
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F48. The Conformational Stability of SOSIP
Arne Schöhn and Ernesto Freire
The Johns Hopkins University; ef@jhu.edu

F49. Quaternary Configuration of the Functional Cd4-Binding Site in the HIV-1 Env Trimer
Qingbo Liu¹, Priyamvada Acharya²,³, Michael A Dolan⁴, Peng Zhang¹, Christina Guzzo¹, Jacky Lu¹, Alice Kwon¹, Deepali Gururani¹, Huiyi Miao¹, Tatsiana Bylund², Gwo-Yu Chuang², Aliaksandr Druz², Tongqing Zhou², William J Rice³, Christoph Wigge³, Bridget Carragher³, Clinton S Potter³, Peter D. Kwong² and Paolo Lusso¹
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Host Factors and Discovery

F50. Post-Translational Regulation on PQBP1/cGAS Innate Immune Sensing Complex During HIV-1 Infection
Sunnie M. Yoh, Felipe Galvez, and Sumit K. Chanda
Sanford Burnham Prebys Medical Discovery Institute

F51. Global Proteomics of HIV-1 Infection Uncovers Mechanisms of Host Cellular Pathway Rewiring
J. Johnson, R. Hüttenhain, L. Lundrigan, J. Hultquist, D. Crosby, E. Verschueren, B. Newton, T. Johnson, and N. Krogan

F52. High-Throughput Genome Engineering for the Interrogation of Host-Pathogen Interactions in Primary Cell Types
Judd F. Hultquist¹,², Joseph Hiatt³,⁴, Kathrin Schumann³, Michael J. McGregor¹,², Alexander Marson³, and Nevan J. Krogan¹,²
¹Department of Cellular and Molecular Pharmacology, California Institute for Quantitative Biosciences, QBI, University of California, San Francisco, CA 94158, USA; ²J. David Gladstone Institutes, San Francisco, CA 94158, USA; ³Department of Microbiology and Immunology, Innovative Genomics Initiative, University of California, San Francisco, CA 94143, USA; and ⁴Medical Scientist Training Program, Biomedical Sciences Graduate Program, University of California, San Francisco, CA, 94143, USA.

Communicating HIV Science

F53. Animating the Science of HIV
Janet H. Iwasa
University of Utah, Department of Biochemistry
F54. CellPAINT-HIV: Interactive Illustration of HIV Structure for Education and Hypothesis Generation
Adam Gardner, Ludovic Autin, Arthur J. Olson and David S. Goodsell
Department of Integrative Structural and Computational Biology, The Scripps Research Institute
Strangers on a Train: Imaging of HIV-1 and Host Proteins on Microtubules

Zandrea Ambrose

Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh School of Medicine, Pittsburgh, PA

Multiple host factors have been shown to interact with HIV-1 complexes early after entry into the cell, many of which have been shown to bind to capsid. To understand the complex, coordinated nature of host protein interactions with virus complexes, we performed rapid multi-color, live-cell microscopy to visualize trafficking of HIV-1 nucleic acid complexes towards the nucleus of cells expressing fluorescently labeled host proteins and microtubules. We observed microtubule-associated movement of WT HIV-1 integrase with CPSF6 and TNPO3 in the perinuclear region of cells. The capsid mutant N74D HIV-1 also utilized microtubules, but association with CPSF6 was not observed. Truncation of the C-terminus of CPSF6 renders it unable to bind to TNPO3 and shifts expression to the cell periphery. Infection with WT HIV-1 particles induced formation of higher order complexes of truncated CPSF6, which did not form when capsid binding to CPSF6 was disrupted by virus mutations (CA N74D or A77V) or with a small molecule inhibitor (PF-74). In addition, premature binding of truncated CPSF6 to HIV-1 capsid induced more rapid capsid permeabilization and affected microtubule movement of virus particles. Our results suggest that premature access of CPSF6 by WT HIV-1 capsid leads to alteration of cytoplasmic trafficking towards the nucleus, whereas the virus normally encounters CPSF6 and TNPO3 on microtubules in the perinuclear region and this complex may facilitate capsid dissociation and nuclear import of HIV-1 DNA.
CryoEM Structure of MxB Assembly Reveals a Novel Oligomerization Interface Critical for HIV Restriction

Peijun Zhang1,2,3, Frances J. D. Alvarez2,3, Shaoda He4, Juan R. Perilla5, Sooin Jang3,6,7, Klaus Schulten5, Alan N. Engelman3,6,7, and Sjors H.W. Scheres4

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Human dynamin-like, interferon-induced myxovirus resistance 2 (Mx2/MxB) is a potent inhibitor of HIV-1 infection and a potential agent for the treatment of HIV/AIDS. MxB directly interacts with the HIV-1 capsid and blocks nuclear import of pre-integration complexes and subsequent chromosomal integration of the viral cDNA. Anti-HIV-1 activity and capsid-binding require the N-terminal domain of MxB and protein oligomerization, yet each of these has eluded structural determination due to difficulties in protein preparation. Here, we report that full-length wild-type MxB purifies as discrete oligomers and further self-assembles into helical arrays in physiological salt. Intriguingly, GTP, but not GDP, binding to MxB results in array disassembly, while subsequent GTP hydrolysis allows its re-formation. Using cryoEM, we determined the MxB assembly structure at 4.6 Å resolution, revealing novel oligomerization and higher-order assembly interfaces that were absent or are distinct from the crystal structure of MxB that lacked the N-terminal domain and harbored interface mutations. The structure suggests that salt bridges mediate MxB higher-order assembly, which is disrupted by GTP-induced conformational changes. More importantly, mutational analysis combined with viral infectivity assays revealed that MxB oligomers, not the dimer or higher-order assemblies, are in fact the active species against HIV-1 infection, and demonstrated a critical role for one of these interfaces in HIV-1 restriction. Moreover, this first high-resolution assembly structure of the mammalian dynamin-like large GTPases allows us to propose a new GTP-dependent assembly/disassembly model.
Cryo-EM Structures of the Maedi-Visna Virus Intasome

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Retroviral integration is mediated by the intasome, a nucleoprotein complex comprising a multimer of integrase (IN) assembled on viral DNA ends. The lack of structural information on the lentiviral intasome impeded the development of HIV IN inhibitors and the understanding of the mechanisms of viral resistance to these small molecules. Unfavourable biochemical properties of HIV-1 IN necessitate the use of hyperactive and solubilizing mutations, which, by their nature, dramatically change properties of the protein. Taking an alternative approach, we sought to identify lentiviral INs that are amenable for structural studies as wild type proteins. We found that the IN from the Maedi-visna virus (MVV), an ovine lentivirus, is highly proficient at concerted integration in the presence of the common lentiviral host factor LEDGF/p75. We assembled and isolated the functional MVV intasome and determined its structure at 4.9 Å resolution using single-particle cryo-electron microscopy. Strikingly, the nucleoprotein complex comprises a homo-hexadecamer of IN with a tetramer-of-tetramers architecture. The conserved intasomal core, previously observed in simpler retroviral systems, is formed between two MVV IN tetramers, with a pair of C-terminal domains from flanking tetramers completing the synaptic interface. The MVV intasome incorporates salient features observed in partial HIV-1 IN structures accumulated over the past 22 years. The hexadecameric architecture of the intasome is necessitated by the alpha-helical configuration of the linker connecting the core and C-terminal domains in MVV IN and the propensity of the protein to form tetramers in solution. Crucially, both of these properties are shared by HIV-1 IN, arguing that the overall structure is conserved among lentiviral intasomes. More generally, retroviral intasomes contain tetramers of IN species found in solution. We are currently investigating the function of the supramolecular lentiviral intasome assembly and the role LEDGF/p75 plays in its context.
Inhibition of HIV-1 Maturation via Small Molecule Targeting of the Amino-Terminal Domain in the Viral Capsid Protein

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The HIV-1 capsid protein is an attractive therapeutic target owing to its multifunctionality in virus replication and the high fitness cost of amino acid substitutions in capsid to HIV-1 infectivity. To date, small molecule inhibitors have been identified that inhibit HIV-1 capsid assembly and/or impair its function in target cells. Here we describe the mechanism of action of the previously reported capsid-targeting HIV-1 inhibitor, BI compound 1 (C1). We show that C1 acts during HIV-1 maturation to prevent assembly of a mature viral capsid. However, unlike the maturation inhibitor Bevirimat, C1 did not significantly affect the kinetics or fidelity of Gag processing. HIV-1 particles produced in the presence of C1 contained unstable capsids that lacked associated electron density and exhibited impairments in early postentry stages of infection, most notably reverse transcription. C1 inhibited assembly of recombinant HIV-1 CA in vitro and induced aberrant crosslinks in mutant HIV-1 particles capable of spontaneous intersubunit disulfide bonds at the interhexamer interface in the capsid lattice. Resistance to C1 was conferred by a single amino acid substitution within the compound-binding site in the N-terminal domain of the CA protein. Our results demonstrate that the binding site for C1 represents a new pharmacological vulnerability in the capsid assembly stage of the HIV-1 life cycle.
The HARC Center: Overview and Progress

Yifan Cheng$^{1,2,3}$, Charles Craik$^{2,4}$, Jennifer Doudna$^{2,3,5}$, Alan Frankel$^{1,2}$, John Gross$^{2,4}$, James Hurley$^{2,5}$, Matt Jacobson$^{2,4}$, Nevan Krogan$^{2,6}$, Matija Peterlin$^7$, and Robert Stroud$^{1,2}$

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The HARC Center mission is to elucidate the molecular basis of systems that are essential for, or contribute to, the pathogenesis of HIV/AIDS. Our focus is on the HIV proteins involved in regulatory and accessory functions and their complexes with host proteins and nucleic acids that are critical to perpetuate the viral life cycle. To accomplish this, we have brought together a diverse team of investigators and collaborators that span the technological and biological expertise needed to achieve a “Systems to Structure” pipeline for the elucidation of HIV-host biology. Through these efforts, the Center has made significant progress in characterizing structural interactions between HIV and host cell protein complexes, particularly those involving Tat, Rev, Vif and Nef as well as systems-wide discovery of novel complexes. The next frontier is to test the function of HIV-host macromolecular complexes in primary human cells. This requires the ability to perturb specific genetic sequences in human cells and assess the consequences. CRISPR-Cas9 genome editing provides unprecedented opportunities to accomplish these functional genetic studies in human primary cells. In close collaboration with the Marson lab at UCSF, we recently overcame longstanding hurdles in performing genome editing in primary human T cells by achieving highly efficient knock-out and knock-in genome engineering in human CD4+ T cells using Cas9:guide RNA ribonucleoproteins (Cas9 RNPs), developing a robust platform for arrayed editing and functional testing of isogenic primary human T cells and applying it to genes involved in HIV pathogenesis. The CRISPR/Cas9 platform is currently being employed for the functional interrogation of novel host factors and novel host factor interactions, with implications for both active and latent HIV infection and other disease states. Overall, this work and other ongoing work in the Center is increasing mechanistic understanding of the virus life cycle and identifying new targets and protein interfaces for intervention as well as expanding the technologies that can be brought to bear on such problems. The Center is achieving a deeper understanding of how the virus subverts the host machinery to control transcription, RNA trafficking, protein degradation, and other essential cellular processes. Updates on two of these ongoing works will be discussed in the companion talks.
Structure and Mechanism of Fab-based Inhibitors of the HIV-1 Vif E3 Ubiquitin Ligase

Jennifer Binning, Amber Smith, Judd Hultquist, Natalia Sevillano, Nathalie Caretta, Lily Burton, Melody Campbell, Mike Trnka, Florencia La Greca, Koen Bartholomewes, B. Matija Peterlin, Nevan Krogan, Charles Craik, Yifan Cheng, and John Gross

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The lentiviral protein Viral Infectivity Factor (Vif) counteracts the antiviral effects of host APOBEC3 (A3) innate immune proteins and contributes to persistent HIV infection. Vif engages A3 as part of a multi-protein complex consisting of Vif, Core-binding Subunit Factor β and the Cullin-RING ubiquitin ligase (CRL5), resulting in the ubiquitination and subsequent targeting of A3 for proteasomal degradation. HIV Vif counteracts multiple APOBEC3 (A3) restriction factors but the structural basis for this phenomenon is unclear. To validate Vif as a therapeutic target, to further probe Vif functions, and to generate novel tools for Vif structural and biochemical studies, we generated a panel of antibody antigen-binding fragments (Fabs) against the A3 substrate receptor of the Vif E3, which is comprised of Vif, CBFβ and EloBC (VCBC). In biochemical and cellular assays two Fabs, 3C9 and 1D1, inhibited Vif-mediated ubiquitination and degradation of A3. 3C9 inhibits A3F but not A3G ubiquitination by the Vif-E3 in vitro and in cells, and rescues packaging of A3F, but not A3G, into virus-like particles (VLPs). In contrast, 1D1 was able to restore cellular levels of all tested A3 proteins; however, the restored A3 proteins were unable to be packaged into VLPs, suggesting that 3C9 and 1D1 inhibit Vif through distinct mechanisms. Electron microscopy structure determination of the VCBC-3C9-1D1 "double Fab" complex combined with biochemical studies have allowed us to determine the mechanism of action for each Fab. 3C9 impairs ubiquitination through a non-competitive mechanism, shielding A3 from ubiquitin transfer but does not preclude assembly of the Vif-holoenzyme. In contrast, the unique functional properties of 1D1 are linked to its ability to inhibit VCBC interactions with Cul5/Rbx2. Collectively, our data illuminate a ubiquitination-independent mechanism by which Vif prevents A3 packaging, and highlight the potential of diverse Fab panels as functional probes to guide HIV drug discovery.
HIV-1 Nef as a Cargo-Selective Allosteric Switch in Hijacking Clathrin Coated Vesicles

Kyle Morris¹, Xuefeng Ren¹, Cosmo Buffalo¹, Claudia Firrito², Ajit Chande², Massimo Pizzato², Qing-Tao Shen¹ and James H. Hurley¹

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HIV Nef downregulates cell surface proteins such as MHC-I and SERINC5 into clathrin coated vesicles (CCVs) by hijacking the AP-1 and AP-2 clathrin adaptors. HIV-1 Nef cooperates with the GTPase Arf1 to induce oligomerization and activation of AP-1, assembling as either closed or open trimers in vitro. Whereas the open trimer is proposed to form an inner hexagon ring to facilitate clathrin cage formation, the role of the closed trimer is less defined. The 3.5 Å cryo-EM structure of the closed trimer reveals a unique set of contacts at the AP-1:Nef interface not found in the open state. Using biochemical and virological experiments, mutational analysis of the newly identified closed trimer interface of AP-1:Nef shows that formation of this interface is essential for downregulation of MHC-I by Nef in cells, although it is less crucial for HIV infectivity. We propose that the closed trimer serves to exclude non-preferred cargoes (such as tetherin for NL4-3 Nef) from CCVs while the open trimer promotes the inclusion of preferred cargoes such as MHC-I into CCVs. We further propose a model wherein Nef dynamics serve as a regulatory switch to sense the nature of the cargo and thereby control the conformation of the AP-1:Arf1:Nef trimer.
Introduction to the Center for HIV-1 RNA Studies (CRNA)

Alice Telesnitsky

University of Michigan Medical School

This introduction to the CRNA will provide a brief overview of the Center and its work elucidating processes and structures in the HIV-1 replication cycle that involve RNA.
The Distinguishing Properties of Bioactive RNA-Targeted Small Molecules

Brittany S. Morgan,¹ Jordan E. Forte,¹ Rebecca N. Culver,¹ and Amanda E. Hargrove¹,²

¹Department of Chemistry, and ²Department of Biochemistry, Duke University, Durham, NC

While small molecules offer a unique opportunity to target structural and regulatory elements in HIV RNA, selectivity has been a recurrent challenge in small molecule:RNA recognition. In particular, RNAs tend to be more dynamic and offer less chemical functionality than proteins, and biologically active ligands must compete with the highly abundant and highly structured RNA of the ribosome. Nonetheless, the last few years have seen an increase in successful reports of RNA-targeted chemical probes. In order to gain fundamental insights into small molecule:RNA recognition, we are pursuing the parallel discovery of RNA-biased small molecule chemical space and RNA topological space privileged for differentiation. Here we will present the identification of physicochemical, structural, and spatial properties of biologically active RNA ligands that are distinct from those of protein-targeted ligands. We are currently expanding these studies with the ultimate goal of applying these insights to the rapid development of ligands with high affinity and specificity for a wide range of RNA elements.
The APOBEC3 (A3) family of cytidine deaminases cause lethal hypermutation of retroviruses via deamination of newly reverse-transcribed viral DNA. Their ability to bind RNA is essential for virion infiltration and antiviral activity, yet the mechanisms of viral RNA recognition are unknown. Among the A3 proteins that restrict HIV-1, APOBEC3H (A3H) uniquely employs a single Zn-binding domain for deamination and RNA binding. Seven A3H haplotypes (hap) have been identified in humans, but only three (hap II, hap V, hap VII) restrict HIV-1. In contrast, A3H is highly polymorphic in pig-tailed macaques (pgt), where 13 allelic variants, each with two spliced isoforms, were found in 15 animals. Four purified, recombinant, naturally occurring A3Hs (three pgtA3H variants and huA3H) had potent cytidine deaminase activity, even when bound to RNA. Viral RNA binding preferences of pgtA3H and huA3H (hap II) are similar. Like huA3H [1], pgtA3H bound preferentially to G-rich viral RNA sequences, as demonstrated by CLIP-Seq analysis of pgtA3H from infected cells, whereas A-rich sites were the least frequently bound. JAB is supported by the Cellular Biotechnology Training Program (NIH T32 GM008353).

Evasion of Intrinsic Host Defenses Drives HIV-1 Nucleotide Composition

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The ~9.5 kilobase HIV-1 genome contains RNA sequences and structures that control many aspects of viral replication, including transcription, splicing, nuclear export, translation, packaging and reverse transcription. Nonetheless, chemical probing and other approaches suggest that the HIV-1 genome may contain many more RNA secondary structures of unknown importance and function. To determine whether there are additional, undiscovered cis-acting RNA elements in the HIV-1 genome that are important for viral replication, we undertook a global silent mutagenesis experiment. Sixteen mutant proviruses containing clusters of ~50 to ~200 synonymous mutations covering nearly the entire HIV-1 protein coding sequence were designed and synthesized. Analyses of these mutant viruses resulted in their division into three phenotypic groups. Group 1 mutants exhibited near wild-type replication, Group 2 mutants exhibited replication defects accompanied by perturbed RNA splicing, and Group 3 mutants had replication defects in the absence of obvious splicing perturbation. The three phenotypes were caused by mutations that exhibited a clear regional bias in their distribution along the viral genome. Analysis of group 3 mutants revealed the existence of a novel form antiviral defense that appears to act by discriminating non-self RNA based on compositional features. The nucleotide composition of HIV-1 appears to have adapted to evade this host defense.
Introduction to the Center (CHEETAH)

Wesley Sundquist

University of Utah
Structures and Functions of ESCRT-III Filaments

Adam Frost

University of California San Francisco and CHEETAH Center

The cellular ESCRT pathway mediates a series of important membrane remodeling and fission events, including HIV-1 budding. During these processes, participating subunits from the ESCRT-III protein family appear to open and polymerize into filaments that remodel membranes and facilitate fission. I will describe high resolution cryo-EM reconstructions of helical copolymers comprising two different human ESCRT-III subunits, CHMP1B and IST1. The one-start helical structures are composed of a double-stranded helical filament that contains an outer strand of “closed” IST1 subunits and an inner strand of “open” CHMP1B subunits that interlock in an elaborate domain-swapped configuration. The interior surface of the CHMP1B strand is highly basic and it binds and remodels lipid bilayers into constricted tubules within the lumen of the IST1-CHMP1B assembly. Our studies reveal how these ESCRT-III filaments bind and stabilize positive membrane curvature, as is required to tubulate endosomal membranes that project into the cytoplasm. I will also discuss how other related ESCRT-III filaments could bind and stabilize negative membrane curvature, as is required for HIV budding.
Structure and Mechanism of the Vps4 ATPase

Chris Hill

University of Utah

Many important cellular membrane fission reactions are driven by ESCRT pathways, which culminate in disassembly of ESCRT-III polymers by the AAA ATPase Vps4. We report a cryo-EM structure of the active Vps4 hexamer with its cofactor Vta1, ADP·BeF₆, and an ESCRT-III substrate peptide. Four Vps4 subunits form a helix whose interfaces are consistent with ATP-binding, is stabilized by Vta1, and binds the substrate peptide. The fifth subunit approximately continues this helix but appears to be dissociating. The final Vps4 subunit completes a notched-washer configuration as if transitioning between the ends of the helix. We propose that ATP binding propagates growth at one end of the helix while hydrolysis promotes disassembly at the other end, so that Vps4 “walks” along ESCRT-III until it encounters the ordered N-terminal domain to destabilize the ESCRT-III lattice. This model may be generally applicable to other protein-translocating AAA ATPases.
Evolution of Antiviral Retrogenes

Diane Downhour, Lara Rheinemann, Alesia McKeown, Wesley Sundquist, and Nels C. Elde

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Viruses constantly compromise the sanctity of cells. These associations often become conflicts over replication and survival, which over time leads to dramatic adaptations transiently favoring viruses or hosts. While conspicuous bouts of adaptation occur for many dedicated immune functions, less clear is the impact of host-virus conflicts on core cellular processes, such as membrane trafficking functions exploited by pathogenic viruses. Many enveloped viruses require interactions with components of the endosomal sorting complex required for transport (ESCRT) pathway to escape from infected cells. Our evolutionary analysis of ESCRT genes across diverse primates identified numerous duplicated gene copies. These genes arose as processed pseudogenes, a byproduct of retrotransposition, and based on previous studies are candidates for antiviral restriction of viruses, including HIV. We observed striking inhibition of HIV replication with the expression of a CHMP3 retrogene from squirrel monkeys. Importantly, retroCHMP3 exhibited reduced cytotoxicity compared with synthetic CHMP3 genes. Combining evolutionary reconstruction with confocal microscopy allowed us to narrow the genetic basis of reduced cytotoxicity to a handful of amino acid substitutions in retroCHMP3 compared to the parental gene. These findings suggest a mechanism where retrogenes derived from conserved host genes adapt to encode activities against pathogens. Our genetic survey also revealed additional retrogenes encoding unrelated core cell functions manipulated by viruses. These observations support the emerging idea that a substantial share of species-specific interactions with viruses leads to species-specific immune functions.

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The HIV Interaction and Viral Evolution (HIVE) Center

Eddy Arnold¹, Jeffrey DeStefano², John Elder³, Alan Engelman⁴, M.G. Finn⁵, Patrick Griffin⁶, Stephen Hughes⁷, Roger Jones¹, Mamuka Kvaratskhelia⁸, Ron Levy⁹, Dmitry Lyumkis¹⁰, Joe Marcotrigiano¹¹, Karin Musier-Forsyth¹², Arthur Olson¹³, Alan Rein⁷, Stefan Sarafianos¹⁴, K. Barry Sharpless¹⁵, Dave Stout¹³, and Bruce Torbett³

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The HIV Interaction and Viral Evolution (HIVE) Center is focused on understanding, at the atomic, biophysical and evolutionary level, the system interdependency of interacting HIV macromolecules and their assemblies which shape the HIV life cycle. HIVE Center Members explore the structural and biophysical interactions of HIV Gag and Gag-Pol polyproteins, capsid, reverse transcriptase, and integrase and their evolutionary relationships. Our research extends to cellular factors that inform the structural and macromolecular dynamics of events in reverse transcription, assembly, and integration. Studies on HIV drug resistance link genetic and structural perspectives, based on mutational correlations that are due to constraints on protein structural stability and function, which shapes fitness. The Center’s biological and computational research strength provides insights into the interrelationships of viral and host mechanisms, enabling the discovery of new drug targets and therapeutic strategies that may ultimately lead to a functional cure.

The presentation will highlight the nature of the collaborations and research within the HIVE Center, with selected examples of our findings over the past 5 years that emphasize the connections between experimental and computational approaches to understanding HIV structures and the evolution of anti-viral drug resistance.
Critical Structural Determinants for ALLINI-Induced Aggregation of HIV-1 Integrase

Pratibha C Koneru1, Nanjie Deng2, Min Li3, Stephen H. Hughes4, Robert Craigie3, Alan N. Engelman4, Ronald Levy5, and Mamuka Kvaratskhelia1

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Allosteric HIV-1 integrase (IN) inhibitors (ALLINIs) are an emerging class of anti-retroviral therapies that interfere with correct virus particle maturation through inducing the hyper-multimerization of IN and preventing its binding to the viral RNA genome in virions. Previous studies have revealed that quinoline-based ALLINIs promote bridging interactions between the catalytic core domain (CCD) of one IN dimer and the C-terminal-domain (CTD) of another dimer. Here, we have identified that the N-terminal domain (NTD) is also critically important for inhibitor induced aggregation of IN. A truncation mutant that lacked the NTD as well as various substitutions in the NTD conferred marked resistance to ALLINIs. Furthermore, our studies have revealed the importance of the CCD-CTD helical linker for hyper-multimerization of IN. Alterations of the linker structure through insertions of Pro or small flexible segments as well as single amino acid deletions markedly altered inhibitor activities. We have also compared hyper-multimerization of IN in the presence of two distinct classes of ALLINIs: quinoline- and pyridine-based compounds. Consistent with previous crystallographic and modeling studies, quinoline-based compounds induced IN multimerization at the 2:2 stoichiometry of inhibitor-to-IN. However, unexpectedly pyridine-based inhibitors exhibited 2:4 (inhibitor:protein) stoichiometry. These findings suggest that quinoline-based compounds bridge between IN dimers, whereas pyridine-based inhibitors promote interactions between IN tetramers. The SAR studies using various substitutions in IN further highlighted important differences between these two classes of inhibitors. These experimental results together with molecular modeling calculations suggest that quinoline and pyridine compounds differently orient the CTD relative to the CCD-CCD dimer at the IN-IN intersubunit interface, which in turn determines the differential assembly patterns of the inhibitor induced hyper-multimers. These new SAR findings are expected to facilitate the development of second generation ALLINIs.
Exploring Fitness and Energy Landscapes of HIV Proteins

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My talk will review work in our lab concerning the construction and analysis of fitness and energy landscapes of HIV Proteins. Both sequence based and structure based approaches will be described. Multiple sequence alignments of drug-experienced HIV-1 protease sequences contain networks of many pair correlations which can be used to build a maximum entropy (Potts) model of these mutation patterns. The statistical energies of the model are correlated with the fitness of individual proteins as estimated by measurements of protein stability and viral infectivity. We show that the penalty of acquiring primary resistance mutations depends on epistatic interactions with the sequence background. Primary mutations which lead to drug resistance can become highly advantageous (or entrenched) by the complex mutation patterns which arise in response to drug therapy despite being destabilizing in the wildtype background.

I will also describe features of the structure and (free) energy landscape by which allosteric HIV-1 integrase inhibitors promote aberrant protein multimerization by directly mediating inter-subunit interactions between IN dimers. We have built atomic models of the inter-subunit interfaces in IN multimers by incorporating information from hydrogen-deuterium exchange (HDX) to drive protein-protein docking, and developed novel free energy simulation methods to estimate the effects of ALLINI binding on the association of CCD and CTD.

This work has been carried out in collaboration with the members of the HIVE Center (HIV Interaction and Viral Evolution Center) (NIH P50GM103368).

The HIV Interaction and Viral Evolution (HIVE) Center: Future Directions

Eddy Arnold¹, Jeffrey DeStefano², Alan Engelman³, Patrick Griffin⁴, Stephen Hughes⁵, Mamuka Kvaratskhelia⁶, Ron Levy⁷, Dmitry Lyumkis⁸, Michael Malim⁹, Joe Marcotrigiano¹⁰, David Millar¹¹, Karin Musier-Forsyth¹², Arthur Olson¹¹, Stefan Sarafianos¹³, Barry Sharpless¹⁴, Bruce Torbett¹⁵, and James Williamson¹¹

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The HIVE Center members will build on their productive collaborative work of the past four years, to further understand, at the atomic, biophysical and evolutionary level, the system interdependency of interacting HIV macromolecules and their assemblies which shape the HIV life cycle. Work will focus on four major research areas:

- the structural biology of retroviral polyproteins and their components in retroviral assembly and maturation, including study of structural determinants for integrase pleiotropism in viral maturation and assembly, and maturation of HIV and PFV polyproteins;
- interactions of HIV with host factors during reverse transcription and integration, including initiation of reverse transcription, inhibition by APOBEC3 family proteins, and the mechanisms of integration into cellular chromatin and their consequences on the formation and reactivation of latent proviruses;
- evolution of antiviral resistance mutations and their biological and biophysical implications, building on computational analysis of full-length genomes obtained from patient samples using an innovative new sequencing technology;
- development and characterization of small molecule probes to understand the biological function of critical molecules and assemblies in the HIV life cycle, including the innovative SuFEx chemistry approach for discovering highly selective covalent inhibitors and computational approaches for discovering new molecules and characterizing the large mesoscale assemblies that are targets of these molecules.

These research aims will be addressed using a variety of experimental methods (biophysical, crystallographic, NMR, molecular & cell biology, virology, and synthetic chemistry) combined with computational modeling approaches. New principal investigators add complementary relevant expertise: Lyumkis in cryoelectron microscopy, Musier-Forsyth in the use of SHAPE and SAXS, Millar in single-molecule fluorescence methods, and Williamson in RNA biophysics.

The presentation will highlight how research groups will participate in the future HIVE collaborative projects and how the new administrative structure will oversee the Center’s research activities.
Receptor Utilization by HIV-1 Env During Viral Entry

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The homotrimeric HIV-1 envelope glycoprotein (Env) undergoes receptor-triggered structural changes that mediate viral entry through membrane fusion. Although Env contains three CD4 and three chemokine-receptor binding sites, full occupancy is not required for function. To investigate a role for multivalent receptor interactions with Env, we have altered receptor binding stoichiometry by titrating receptor levels on target cells and eliminating competent receptor-binding sites on Env trimers. Here, we utilize these approaches to characterize the synergy between chemokine-receptor antagonists (CoRAs) and fusion inhibitors (FIs). Synergy between CoRAs and FIs has been attributed to a CoRA-dependent decrease in the rate of viral membrane fusion that extends the lifetime of the intermediate state targeted by FIs. We demonstrate that the magnitude of synergy depends on the stoichiometry of chemokine-receptor binding to trimeric Env. Reducing chemokine receptor levels on target cells or eliminating competent chemokine-receptor binding sites on Env trimers results in a loss of synergistic activity. The data imply that the stoichiometry required for CoRA/FI synergy exceeds that required for HIV-1 entry. The analysis suggests two distinct roles for chemokine-receptor binding, one to trigger formation of the FI-sensitive intermediate state and another to facilitate subsequent conformational transitions. Our results could explain the wide variety of previously reported activities for CoRA/FI combinations and have implications for the combined use of CoRAs and FIs in antiviral therapies.
Program Project Overview: Targeting HIV-1 Env for Inhibition and Inactivation

Irwin Chaiken, Cameron Abrams, Andres Finzi, Ernesto Freire, Wayne Hendrickson, Walther Mothes, Amos B. Smith III and Joseph Sodroski

Our Program develops strategies for inhibiting HIV-1 Env trimer, the only virus-specific protein exposed on the virion surface. HIV-1 Env is metastable and activated by traversing an energetically favorable cascade of host cell CD4 and co-receptor interactions that lead to virus-cell fusion. To antagonize the metastable HIV-1 Env, we identify and develop small-molecule inhibitors as prototypes to hijack the machinery built into the Env protein for potential use in disease intervention, prevention and eradication. The developed inhibitors in turn serve as probes to define fundamental concepts, mechanisms and targetable vulnerabilities of the HIV-1 Env protein complex. A hallmark achievement of the Program has been to identify stages along the “activation trajectory” at which different classes of molecules can block progression to entry. At the same time, small Env inhibitors have been identified that can divert the conformationally dynamic Env trimer towards an “inactivation trajectory” to disrupt Env trimer assembly before cell encounter and inactivate virus. The binding sites for inhibitors and inactivators are being investigated by a multi-disciplinary approach including crystallography, computation, synthetic design, mutagenesis and patterns of virus resistance. Strikingly, inhibitors have been identified that can promote activation and inactivation with different functional outcomes, differentially capturing intrinsic conformational transitions built into the Env trimer but with overlapping binding sites in the highly conserved CD4 binding site that represents a crucial drug target. The structural mechanisms of binding of these inhibitors are being used to advance potent inhibitor designs. Importantly, inhibitor mechanisms and consequent designs are increasingly being informed by the rapidly expanding definition of high-resolution Env trimers revealed by cryogenic electron microscopy, crystallography and the definition of conformational states by single molecule fluorescence resonance energy transfer. The potential to identify and explore an expanded repertoire of binding sites in Env trimers, and to correlate Env inhibitor investigations of the Program Project with the rapid expansion of high-resolution structures and conformational dynamics mechanisms of the HIV-1 Env protein entry machine, will comprise major driving forces in the development of the Program Project going forward.

NIH P01GM56550
Associating HIV-1 Env Trimer Structures with Functional Env Conformational States by smFRET Analysis

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The HIV-1 envelope glycoprotein (Env) trimer is the sole viral protein exposed on the virus surface and as such represents a main target for small molecule inhibitors and a vaccine against HIV-1/AIDS. The functional Env trimer mainly exists in a closed conformation (State 1), which is driven by CD4 binding through an intermediate conformation (State 2) to the open CD4-bound conformation (State 3). These functional Env states can be visualized by single-molecule Fluorescence Resonance Energy Transfer (smFRET). Using smFRET to measure the conformational consequences of antibody binding we found that most broadly neutralizing antibodies are specific for State 1. Thus, immunogens designed to elicit bNAbs should present State 1. A breakthrough in the structural characterization of the HIV-1 Env trimer has been the generation of recombinant cleaved soluble gp140 SOSIP.664 trimers stabilized by a disulfide bond between the gp120 and gp41 subunits (SOS), an I559P change in gp41 (IP), and a truncation at gp41 residue 664. Parallel cryoelectron microscopy studies have been performed with the mature HIV-1JR-FL Env in complex with the PGT151 neutralizing antibody. Both approaches resulted in similar structures. It is currently generally assumed that these structures represent the ground state of HIV-1 Env (State 1). Here we apply smFRET to probe the conformational state of HIV-1 Env in these constructs and antibody complexes. Fluorophores were introduced at the identical positions in the HIV-1 Env proteins used for structural studies and the native Env on the surface of virions, and the resulting smFRET values compared. Surprisingly, smFRET data reveal that both the soluble gp140 SOSIP.664 and PGT151-HIV-1JR-FL Env structures correspond to the State 2 gp120 conformation observed on the virus. Our data suggest that the all-important structure of State 1 of HIV-1 Env, which is the target of the majority of broadly neutralizing antibodies, remains unknown. We believe that smFRET can serve as a useful tool to guide structural studies towards the characterization of State 1 HIV-1 Env. Determining the structure of this additional conformation observed on native virions should allow the design of second generation immunogens that specifically present the State 1 conformation of HIV-1 Env.
Design, Synthesis and Biological Evaluation of Small-Molecule Inhibitors of HIV-1 Entry: A Potential Long-Term Strategy for Curbing the AIDS Pandemic


Department of Chemistry, University of Pennsylvania

With approximately 36.7 million people living with HIV worldwide and with 2.1 million new infections reported each year, the need for novel strategies, both to prevent transmission and to suppress viral reservoirs, remains a critical priority. Over the past several years, our highly interdisciplinary NIH Project Team (GM 56550) has developed a detailed understanding of both the structural/functional mechanism of the HIV-1 glycoprotein envelope (Env) complex in the viral entry process, which has led to a potential long-term strategy for intervention. Based on the initial observations by Debnath and colleagues at the New York Blood Center, we have designed and optimized a series of potent, broadly active small molecule CD4-mimetic entry inhibitors exemplified by (+)-(R,R)-BNM-IV-147. Small molecule CD4-mimetic compounds directly inhibit virus infection by blocking Env binding to the receptor, CD4, and by prematurely activating the metastable Env complex. CD4-mimetic compounds also expose Env epitopes that are recognized by readily elicited antibodies that can neutralize the HIV-1 virus and kill infected cells via antibody-dependent cellular cytotoxicity (ADCC). Thus, small molecule CD4-mimetic compounds hold the promise of enhancing the efficacy of the immune response against both the HIV virus itself and against infected cells. NIH P01GM56550
Single-Molecule Studies of HIV-1 Gag Assembly

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The Gag polyprotein is the key structural protein mediating assembly of virions in HIV-1-infected cells. Previous studies have shown that Gag will spontaneously assemble into virus-like particles in vitro in the presence of a variety of nucleic acids, although the mechanism of this assembly and the role of nucleic acid are not fully understood. We have developed a single-molecule method to monitor Gag assembly in a defined in vitro system. The system utilizes fluorescently-labeled recombinant Gag polyprotein (enzymatically labeled with ATTO 488 or expressed as a fusion with eGFP) and a model nucleic acid immobilized on a microscope slide. Using TIRF microscopy, individual Gag monomers are resolved as they spontaneously assemble around the immobilized nucleic acid. These experiments probe early steps in the Gag assembly pathway, the equilibrium distribution of assembly intermediates and the rate constants for each step of assembly. Studies are performed with various model nucleic acids, to elucidate how the nucleic acid structure dictates the early steps of the Gag assembly pathway.

In cells, Gag assembly takes place on the plasma membrane, in the presence of viral RNA and various cellular proteins. Previous studies have shown that discrete Gag assembly intermediates of varying sizes can be separated by sucrose gradient fractionation. However, the Gag stoichiometry and the identity of the cellular proteins in each intermediate are not well characterized. We have developed a single-molecule pull down assay to analyze the Gag assembly intermediates. Lysates from mammalian cells transfected with Gag-GFP are fractionated on a sucrose gradient, individual fractions are applied to a microscope cover slip coated with anti-Gag or anti-GFP antibodies and the number of Gag-GFP molecules at each location on the slide is determined by single-molecule photobleaching analysis. The results reveal the distribution of Gag monomers within each of the Gag assembly intermediates (10S, 40S, 80S, 150S, 500S etc.), as well as any heterogeneity that may exist (subpopulations). The advantage of this approach is that Gag assembly occurs in the native cellular environment and all natural cofactors are present. Supported by NIH P50 grant GM082545.
Disrupting HIV Capsid Assembly by Small Molecule Modulators

Eric Hu, Ph.D.

Structural Chemistry, Gilead Sciences

Two distinct categories of HIV capsid inhibitors and activators were identified to disrupt in vitro capsid assembly. Inhibitors bind in the capsid protein N-terminus while activators bind in the interface of neighboring full length capsid proteins. In vitro binding and assembly assays, together with X-ray co-crystal structures, helped to elucidate compounds’ antiviral effect. Successful medicinal chemistry optimization led to selection of activators with exquisite antiviral potency, favorable resistance profile and properties suitable for long-acting antiviral administration.
Structure and Dynamics of Complexes of APOBEC3G with RNA at Nanoscale

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APOBEC3G (A3G) belongs to the APOBEC3 family of DNA-editing cytidine deaminases that are responsible for deamination of HIV-1 cDNA during reverse transcription in the absence of viral infectivity factor (Vif). However, there are evidences that deaminase-independent mechanisms are also involved in antiviral activity of A3G. There is a “road block” mechanism by which A3G physically blocks the elongation of reverse transcribed proviral ssDNA. Interaction of A3G with RNA is another rather unexplored area of A3G properties. It was also shown that A3G binds to diverse cellular RNA and binding RNA to the CTD domain of A3G inhibits A3G catalytic activity. Here we focus on a fundamental problem of how A3G protein, a potent innate anti viral agent interacts with RNA. To accomplish our goals, we, for the first time, directly visualized the A3G-RNA complexes with AFM. A remarkable difference in assemblies of A3G on ssDNA and RNA substrates was discovered. Together with similar A3G-ssDNA complexes, when one RNA molecule bound to A3G, we also observed two RNA molecules bound to A3G. Complexes containing two molecules of ssDNA bound to A3G have not been observed. By analyzing the data for each A3G domain independently, we show RNA forms complexes with both NTD domain and CTD domain, while ssDNA is not able to form such complexes. Together, these results indicate the existence of an additional, different from ssDNA region on A3G that is capable of binding RNA. The dynamic behavior of the A3G-RNA complexes suggests that the two binding regions of A3G have different affinities to RNA. Based on these results a model for RNA binding to A3G is discussed. Overall, our study provides a fundamental step toward the mechanistic understanding of how A3G interacts with the RNA target and the role of domains of A3G in RNA binding.
Structural Basis for Targeted DNA Cytosine Deamination and Mutagenesis by APOBEC Enzymes

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APOBEC-catalyzed cytosine-to-uracil deamination of single-stranded (ss)DNA has beneficial functions in immunity and detrimental roles in cancer. APOBEC enzymes have intrinsic dinucleotide specificities that impart hallmark mutation signatures. Despite numerous structures, mechanisms for global ssDNA recognition and local target sequence selection remain unclear. We report crystal structures of human APOBEC3A and a chimera of human APOBEC3B and APOBEC3A bound to ssDNA at 3.1 and 1.7 angstroms resolution, respectively. These structures reveal a U-shaped DNA conformation, with the specificity-conferring -1 thymine flipped out and the target cytosine inserted deep into the zinc-coordinating active site pocket. The -1 thymine base fits between flexible loops in a groove that forms upon binding ssDNA, and it makes direct hydrogen bonds with the protein accounting for the strong 5'-TC preference. These studies explain both conserved and unique properties among APOBEC family members, and provide a basis for the rational design of inhibitors to impede the evolvability of viruses and tumors.
The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease, a Case Study

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In this highly collaborative proposal we hypothesize that key pathways and coupled mechanisms confer drug resistance to therapeutic targets. Together, we seek to define the sequence, structural and dynamic, and temporal evolutionary constraints of the interdependency of drug resistance: 1) to recognize the pathways and mechanisms by which resistance occurs and 2) to devise drug design strategies for developing potent inhibitors that are more robust against resistance. We combine analysis of sequence diversity, structure, dynamics and energetics to elucidate the underlying pathways and additional macromolecular pressures that contribute to drug resistance. In this year we have probed the interdependency of both inhibitors and resistant variants that arose from viral passaging, through a combination of structural, dynamic and mutational methods. In each case we bridge experimental and computational techniques and are developing a database where multiple data sets can be probed to elucidate their interdependencies. An overview of all the accomplishments of all the Projects and the Cores will be presented.
Mechanistic Characterization of Inhibitor Resistance in HIV-1 Protease via Machine Learning and Atomistic Simulation

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Darunavir is an HIV-1 protease inhibitor with high binding affinity that can be effective against strains where resistance to other inhibitors has developed. Despite this effectiveness and the associated delay in the onset of protease resistance to darunavir inhibition, however, resistance has been observed in the presence of multiple simultaneous mutations. In order to gain mechanistic insight on the role that specific mutations play in this resistance, molecular dynamics simulations were carried out on a selection of 15 HIV-1 protease variants, chosen to include susceptible variants and wild-type controls, along with strains that are resistant to darunavir in vivo and/or in vitro. The resulting high-dimensional space of thermodynamic observables among this set of sequence variants can be reduced to interpretable variables using machine learning, with a particular emphasis on supervised methods.
Interdependence of Inhibitor Recognition in HIV-1 Protease

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Molecular recognition is a highly interdependent process. Subsite couplings within the active site of proteases are most often revealed through conditional amino acid preferences in substrate recognition. However, the potential effect of these couplings on inhibition and thus inhibitor design are largely unexplored. We examined the interdependency of subsites in HIV-1 protease using a focused library of protease inhibitors, to aid in future inhibitor design. Our series of DRV analogs enables systematically probing the S1' and S2' subsites. All-atom molecular dynamics simulations starting from co-crystal structures were performed, and systematically analyzed in terms of atomic fluctuations, intermolecular interactions and water structure. These relationships intricately link the HIV-1 protease subsites, and are critical to understanding of molecular recognition and inhibitor binding. More broadly, the interdependency of subsite recognition within an active site requires consideration in the selection of chemical moieties in drug design; this strategy is in contrast to what is traditionally done with independent optimization of chemical moieties of an inhibitor.
Multiscale Imaging of HIV Spread in Animal Models

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Despite extensive efforts to characterize HIV-1 pathogenesis, surprisingly little is known about the biological mechanisms of HIV-1 spread in tissues at the resolution of individual cells and virions. To address this challenge, we have developed a multiscale imaging platform for visualizing HIV-1 spread in lymphoid tissues from humanized mice (hu-mice). We utilized tissue clearing methods combined with immunofluorescence (IF) staining and confocal and light sheet fluorescence microscopy (LSFM) to visualize individual infected cells within large tissue volumes (mm³–cm³) and applied serial blockface electron microscopy (SBEM) and electron tomography (ET) to gain higher resolution, ultrastructural information.

LSFM analysis of HIV-1-infected tissues from hu-mice, harvested at specific times post-infection, revealed the density and spatial localization of infected cells, allowing us to identify infectivity profiles unique to individual tissues. SBEM of adjacent regions of HIV-1-infected tissues provided 3D ultrastructural information that enabled us to model the entire volume of HIV-1 infected cells within tissue. These analyses revealed hundreds of virions accumulated in numerous “pools” around infected cells, which were often localized at regions of plasma membrane immediately adjacent to the nucleus. ET of spleen from hu-mice revealed the release of thousands of free-virions from distinct sites of plasma membrane, allowed the quantification of mature, immature, or budding virions, and identified electron dense assemblies associated with budding virions. Analysis of bone marrow from hu-mice by ET identified infected T-cells and macrophages as early as 10-days post-infection, before several other lymphoid tissues showed evidence of infection. The density of infected cells and free-virus remained low and constant out to 121-days post infection, suggesting a potential tissue source of virus that endures after after infectivity in other tissues peaks. Our ET studies also detected unique budding profiles exhibited by T-cells and macrophages in bone marrow and identified phagocytic invaginations in proximity to virus budding regions on the surfaces of infected macrophages. Cumulatively, our multiscale imaging platform provided temporal, spatial, and structural information that allowed us to generate models of HIV-1-pathogenesis within hu-mice and we are currently expanding our studies to include non-human primate and human patient samples.
Live Cell Imaging of HIV-1 Reverse Transcription

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Following HIV-1 fusion with the cell membrane the conical capsid containing the viral RNA genome is delivered to the cytoplasm. There is general agreement that the process of reverse transcription facilitates the disruption of the conical core through a process known as uncoating. Ultimately, a double stranded provirus is generated through the completion of reverse transcription and the provirus must reach host nuclear DNA where it will integrate to complete the early life cycle of HIV. The site of the completion of reverse transcription is a contentious issue. Some models suggest that reverse transcription is initiated and completed in the cytoplasm while others suggest that reverse transcription is (or can be) completed in the nuclear pore or nucleus. To gain insights into these processes by live cell imaging during infection we have developed a method to label viral dsDNA.

The binding of tetR to dsDNA tetO sequences has been used extensively in inducible expression systems. We devised a dsDNA detection system where we have inserted an array of tet operons (tetO) into an HIV-1 viral vector that expresses a GFP reporter gene (HIVtetO-GFP). We also designed a panel of cell lines stably expressing tetR-tdTomato fusion proteins. The binding of tetR-tdTomato to the dsDNA tetO repeats recruits the tdTomato fluorescent protein to tag the DNA provirus. This DNA tethering allows the monitoring of formation and trafficking to the viral DNA genome by fluorescence microscopy of fixed or living cells. Here we used VSV-G pseudotyped particles carrying the tetO arrays that are also labeled with IN-GFP. This configuration makes it possible to follow nascent reverse transcribed viral DNA, from the moment when the double-stranded DNA encoding the tetO arrays becomes accessible to the expressed tetR-tdTomato, until integration. The specificity of the tetR-tdTomato interaction can be evaluated through the addition of tetracycline that disrupts tetR-tetO binding.

Initial validation studies revealed that cells stably transduced with HIVtetO-GFP contained small puncta of nuclear tdTomato that was sensitive to the presence of tetracycline. Live cell microscopy detects the recruitment of the tetR-tdTomato to the IN-GFP labeled viral complexes in the cytoplasm approximately 2 hours post viral challenge. The recruitment of the tetR-tdTomato in the cytoplasm reveals that the viral dsDNA becomes exposed to cytoplasmic factors relatively early, consistent with the early uncoating model. This newly developed live-cell imaging reverse transcription system will allow answering many key questions of the HIV-1 early-steps of infection, such as the exact location of reverse transcription and the interaction of viral DNA with the innate sensing machinery.
Loss of Capsid Protein at the Nuclear Membrane is a Pre-Requisite for Translocation of HIV-1 Pre-Integration Complexes into the Nucleus

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HIV-1 cores released into the cytoplasm as a result of virus-cell fusion undergo reverse transcription and shed a major portion of the capsid protein (CA) as they traffic to the nucleus. The extent, the sites, and the dynamics of CA loss (referred to as uncoating) are poorly understood. We have recently visualized single HIV-1 uncoating in living cells using a novel fluorescent CA marker, cyclophilin-A DsRed (CypA-DsRed). CypA-DsRed binds tightly to the viral core without considerably affecting infectivity and is lost concomitantly with CA. Imaging single cores co-labeled with integrase-GFP (IN-GFP) and CypA-DsRed revealed distinct uncoating phenotypes in the cytoplasm: an abrupt loss of CypA-DsRed from cores at early times post-infection vs. gradual release of the CA marker over several hours, which also occurred near the nuclear envelope (NE). To assess the relevance of these uncoating phenotypes to productive infection, we tracked single IN-GFP labeled cores that entered the nucleus and thus had reasonable probability of integrating in the host genome. We examined temporal and spatial characteristics of CA release from IN-GFP labeled cores entering the nucleus by 3-color live cell imaging of TZM-bl cells expressing a nuclear membrane marker, EBFP2-lamin. Nearly all IN-GFP complexes entering the nucleus (n=91) contained detectable amounts of CypA-DsRed upon arrival at the NE. These complexes docked at the NE for 36 min on average, during which time they lost CypA-DsRed. Importantly, the loss of this CA marker always preceded the nuclear entry of IN-GFP complexes. By contrast, double-labeled particles docked at the NE never entered the nucleus under our experimental conditions, suggesting that loss of CA at the nuclear pore is a necessary step for nuclear import. This notion is further supported by the observed block in nuclear entry for the hyper-stable E45A CA mutant that retained CypA-DsRed and accumulated in the perinuclear area. We also did not detect nuclear complexes for the unstable CA mutant, K203A, that abruptly uncoated shortly after virus fusion. The lack of K203A nuclear import was most likely due to the loss of IN-GFP signal in the cytoplasm within approximately 30 min after CypA-DsRed shedding. Of note, a fraction of nuclear complexes contained very small amounts of CypA-DsRed and stained for p24/CA, indicating that CA shedding at the nuclear pore does not always reach completion. Collectively, our results suggest that early uncoating in the cytoplasm may result in disintegration of the HIV-1 core, whereas partial or full loss of CA at the NE is a prerequisite for nuclear entry of pre-integration complexes. This work was supported by the NIH R01 GM054787 and AI129862 grants to GBM.
T1. The Vpu-Interacting Protein ATP6V0C Regulates Expression of Tetherin and HIV-1 Release

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The HIV-1 accessory protein Vpu enhances virus release by counteracting the host restriction factor tetherin, and this antagonism involves Vpu-mediated down-regulation of cell-surface tetherin. To further understand the role of host proteins in Vpu function, we carried out yeast two-hybrid (Y2H) screening and identified five Vpu-interacting proteins, including the small glutamine-rich tetratricopeptide repeat-containing protein (SGTA) and the V0 subunit C of vacuolar ATPase (ATP6V0C). Previously, we reported that overexpression of SGTA inhibited HIV-1 release and stabilized a 23-kDa, non-glycosylated tetherin species (Waheed et al., Sci. Rep. 6 2016). In the current study, we examined the role of ATP6V0C in Vpu-mediated tetherin degradation and HIV-1 release.

We observed that knockdown of ATP6V0C in HeLa cells impairs Vpu-mediated tetherin degradation and HIV-1 release. This inhibition of HIV-1 release imposed by ATP6V0C knockdown can be rescued by knockdown of tetherin. Interestingly, knockdown of other V-ATPase subunits has differential effects; knock-down of ATP6V0C has no effect on HIV-1 release or tetherin degradation, whereas knockdown of ATP6V0A1 inhibited tetherin degradation and HIV-1 release. Overexpression of ATP6V0C, primarily in the absence of Vpu, resulted in stabilization of 26-kDa, high-mannose-enriched tetherin. The stabilization of 26-kDa tetherin is specific to ATP6V0C as overexpression of ATP6V0C has no effect on the expression of 26-kDa tetherin. Further, the ATP6V0C-mediated stabilization is specific to tetherin, as overexpression of ATP6V0C did not stabilize CD4 or interfere with its Vpu-mediated down-regulation. Immunofluorescence localization studies showed that the ATP6V0C-stabilized 26-kDa tetherin was sequestered in LAMP1-enriched intracellular lysosomal compartments. Treating cells with kifunensine, an inhibitor of -mannosidase I, resulted in a 26-kDa tetherin species as observed with the overexpression of ATP6V0C, and this high-mannose-enriched tetherin species inhibited HIV-1 release to an extent similar to that of complex-type glycosylated tetherin. Flow cytometric analysis shows kifunensine treatment does not significantly alter the cell surface expression of tetherin. This result suggests that complex-type N-linked glycosylation is dispensable for the ability of tetherin to restrict virus release. These studies indicate that the Vpu-interacting protein ATP6V0C plays a role in regulation of tetherin expression and HIV-1 assembly and release.
T2. Expression and Purification of Vpu and CD4 Complex

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Viral Protein U (Vpu) plays a vital role in associating with host proteins to initiate downstream interactions. Vpu is the only transmembrane protein among the four accessory proteins and is responsible for downregulating several host factors including CD4, the receptor for entry, and restriction factor BST-2 (tetherin), so facilitating entry and release of viral particles, respectively. It does so through the E3 ligase system (Cul1-βTrCP-Skp1-Rbx1). Importantly, Vpu also acts functionally as a viroporin as a homopentamer that controls the membrane potential and regulates membrane permeability. More recently, we have broken through this roadblock by devising a system to screen thousands of random mutants for high expression of stable and functional Vpu. Random Vpu mutants were made by error-prone PCR via Mega Primer PCR of Whole Plasmid (MEGAWHOP) and rapidly screened for membrane proteins with enhanced yield.

Vpu gets phosphorylated at two serines at positions 52 and 56. Phophorylated Vpu interacts with the single transmembrane spanning C-terminal region of CD4 and subsequently recruits βTrCP-Skp1 to ubiquitinate CD4 for proteosomal degradation. Therefore, understanding the direct interaction between Vpu and CD4 will facilitate understanding its mechanism of action on CD4. Currently, we have co-expressed Vpu with a TM and cytoplasmic domain of CD4 in a monodisperse manner and are using this complex to analyze the basis for selectivity.
T3. Molecular Basis of How Phosphoserine Acidic Cluster Motifs in HIV-1 Vpu and the Host Proteins Furin and Serinc3 Interact with Clathrin Adaptors

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Specific protein-protein interactions are the molecular basis of protein sorting in the endosomal system, a process that controls the trafficking of both host and viral membrane proteins and is largely dependent on clathrin and clathrin adaptors. We aim to understand the molecular and structural basis of how phosphoserine acidic cluster (PSAC) motifs in cellular and viral membrane proteins are recognized by the clathrin adaptor protein complexes 1 and 2 (AP-1 and AP-2). AP-1 and AP-2 mediate the trafficking of many transmembrane proteins (cargos) between cellular compartments. The AP-complexes recognize specific sorting signals such as leucine-based, tyrosine-based, and PSAC motifs in the CDs of transmembrane cargo proteins. Whereas the molecular and structural basis of the interactions of AP complexes with leucine-based and tyrosine-based motifs is known, exactly how PSAC motifs interact with AP complexes is not known. To determine this, we pursued the examples of the PSAC motifs found in the HIV-1 protein Vpu and in the host proteins Furin and Serinc3. HIV-1 Vpu is an accessory protein that provides evasion of the host immune response. Furin is a trans-Golgi network membrane associated endoprotease that cleaves certain cellular and viral precursor proteins in both biosynthetic and endocytic pathways. Serinc3 is a multipass transmembrane protein originally known for stimulating the incorporation of serine into membrane lipids. Recently, it has been identified as a restriction factor for viral infectivity that is counteracted by lentiviral Nef proteins. We used in vitro methods and recombinant proteins to establish that the PSACs in each of these proteins mediate a direct interaction with the medium (µ) subunit of certain AP complexes. This interaction depends on serine-phosphorylation. We determined the binding affinities of these interactions using biolayer interferometry (the Octet system). The µ subunits are basic in nature and contain several basic patches on their surfaces that might bind the PSAC motifs. Our preliminary studies suggest a divergence in the mode of binding of viral and host proteins to the µ subunits. Two viral proteins, HIV-1 Nef and Vpu, exploit the same basic regions on µ for binding, but the cellular proteins Furin and Serinc3 do not appear to bind these regions. We aim to map in detail the interactions between these PSACs and the µ subunits of AP-complexes by employing cellular, biochemical, and structural approaches.
The lentiviral protein Nef promotes infectivity and subverts immune surveillance of infected cells by downregulating cell surface proteins such as SERINC5 and MHC-I. The Nef proteins of HIV-1 group O, HIV-2, and SIV also target the restriction factor tetherin. These downregulatory activities are mediated by Nef hijacking the clathrin adaptor proteins AP-1 and AP-2 so as to redirect cargoes to the lysosome. Nef hijacks AP-1 in part by enhancing its Arf1-dependent trimerization. Nef and Arf1 in combination promote structurally and functionally distinct closed (trapped) and open (active) trimeric conformations. A new 3.5 Å cryo-EM structure of the AP-1:Arf-1:NL4-3 Nef:tetherin closed trimer reveals novel contacts that stabilize this hijacked complex. These data are interpreted in terms of a mechanism for trapping non-preferred cargoes such as tetherin with respect to HIV-1 M and N-group Nefs.
T5. Structural Studies of Tec-Family Kinase Interactions with HIV-1 Nef

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Nef is an HIV-1 accessory factor that is essential for virus infectivity, host immune evasion and AIDS progression. Nef lacks intrinsic catalytic activity and functions instead via interactions with multiple classes of host cell proteins involved in signal transduction and endocytic trafficking. For example, Nef interacts with the Src-family tyrosine kinases Hck and Lyn through their SH3 domains, resulting in constitutive kinase activity that enhances viral replication and MHC-I downregulation. More recent work has shown that Nef also binds to select members of the Tec-family of tyrosine kinases, including Btk, Bmx, and Itk, all of which are expressed in HIV-1 target cells. Of particular interest is Itk, which is expressed in CD4+ T cells and is activated by Nef. Selective Itk inhibitors block Nef-dependent enhancement of HIV-1 infectivity and replication, suggesting an important role in the viral life cycle. While the interaction between Itk and Nef has been demonstrated at the plasma membrane in cell-based fluorescence complementation assays, the structural basis of this interaction has not been reported. Like Src-family kinases, Itk has a core region consisting of sequential SH3, SH2 and kinase domains. In addition, Itk has an N-terminal pleckstrin homology (PH) domain important for membrane targeting as well as a Tec homology (TH) region involved in kinase regulation. To explore the structure of the Nef:Itk complex, we have created a panel of bacterial expression constructs for the Itk regulatory region. These include the entire PH-TH-SH3-SH2 region, the SH3-SH2 region, and the isolated SH3 domain, all of which have yielded mg amounts of soluble protein. Surface plasmon resonance (SPR) experiments show that the Itk SH3 domain alone does not form high-affinity complexes with Nef, in contrast to the SH3 domain from the Src-family kinase Hck. However, Nef binding was increased with the tandem Itk SH3-SH2 dual domain, suggesting that contacts outside of SH3 are important for interaction. The recombinant Nef core protein also forms stable complexes with Itk SH3-SH2 in solution, a first step towards an X-ray crystal structure of this complex. In complementary studies, we are also exploring the structure of Nef:Itk complexes at model lipid bilayers. To this end, we have produced recombinant, N-terminally myristoylated (Myr) Nef in bacteria, a post-translational modification essential for Nef membrane localization in cells. Preliminary SPR studies show that Myr-Nef binds membrane bilayers with low µM affinity in a Myr-dependent manner. Neutron reflectometry (NR) of Myr-Nef on a sparsely tethered bilayer lipid membrane reveals a protein conformation similar to published results Myr-Nef on a lipid monolayer. Our next aim is to build a complex of Myr-Nef with Itk on the lipid bilayer for NR studies, with the ultimate goal of modeling the overall domain organization of the active, membrane-bound Nef:Itk complex.
HIV Nef downregulates cell surface host proteins, including SERINC5, CD4, and MHC-I, by redirecting them into clathrin coated vesicles (CCVs) for lysosomal degradation. Nef achieves this by hijacking the AP-1 and AP-2 clathrin adaptors. HIV-1 Nef cooperates with the host GTPase Arf1 to induce oligomerization and activation of AP-1, assembling as trimers in vitro. AP complexes’ normal function is to sort two main classes of cargo proteins into CCVs. These cargo classes are defined by the presence of a tyrosine-based YXXφ motif or an acidic-dileucine motif DE[XXX][LI]. Nef-induced downregulation of MHC-I involves the hijacking of both the the tyrosine and dileucine cargo binding sites of AP-1. Arf1 promotes dimerization of AP-1, while in the presence of Arf1 and Nef together, AP-1 trimers predominate. The combination of dimer and trimer interfaces lead to formation of a hexagonal lattice. In order to determine the contribution of the Nef dileucine motif to trimerization, a cryo-EM analysis of the AP-1:Arf1:NefLLAA complex was carried out. From this analysis, we conclude that the dileucine motif of Nef is important for promoting the trimerization of the Arf1:AP-1 clathrin adaptor complex and subsequent clathrin cage assembly.
The HIV-1 accessory protein Nef supports high-titer viral replication, immune evasion of HIV-infected cells, and is essential for AIDS progression. Efficient replication of M-tropic HIV-1 in primary human macrophages requires expression of the myeloid Src-family kinase, Hck. Nef provides a crucial link between HIV-1 and this host cell kinase, interacting with Hck through its SH3 domain and driving constitutive kinase activation. In addition to Hck, Nef also interacts with the cellular endocytic adaptor proteins, AP-1 and AP-2, which are responsible for downregulation of MHC-I and CD4, respectively. Mutagenesis and fluorescence complementation studies, based on previous crystal structures of Nef dimers, have suggested that Nef dimerization is required for Hck activation, MHC-I and CD4 downregulation as well as enhancement of virus replication. The requirement for dimerization in multiple Nef functions identifies the dimerization interface as an attractive target for small molecule inhibitors. While the X-ray crystal structure of the complete Nef:Hck complex remains elusive, the structures of the Nef core domain in complex with either the Hck SH3 or tandem SH3-SH2 domains illustrate the mechanisms of Nef:SH3 domain binding and Nef dimerization. In order to understand the dynamic changes in the Nef protein associated with Hck complex formation we used hydrogen deuterium exchange mass spectrometry (HDX MS) to analyze the smaller Nef:Hck complexes in solution. The X-ray crystal structures of the HIV-1 Nef core protein in complex with an isolated SH3 domain (PDB: 1EFN) and the tandem SH3-SH2 domains of Hck (PDB: 4U5W) both form dimers of complexes, with the Nef core forming the dimer interface. The Nef dimer interface is formed by the αB-helix in both complex structures, though the orientations of the αB-helices are distinct between the two. To better understand these different structures and the dynamic changes that accompany their formation in solution, the Nef core protein was analyzed by HDX MS either alone or in complex with the Hck SH3 and SH3-SH2 domains. HDX MS revealed that the SH3-binding region of Nef was protected from exchange when bound to the SH3 or SH3-SH2 domains at early time points, indicative of SH3 engagement as expected. At later time points, however, the Nef dimer interface was protected in the complexes when compared to the Nef core alone. While the Nef dimer interface is helical and incorporates minimal deuterium in the isolated protein alone, the protection of this region in both complexes suggests that partner protein binding stabilizes the Nef dimer through this interface. These data also suggest that the Nef core is unlikely to dimerize in solution when not bound to Hck. Substantial literature precedent supports a role for dimerization in Nef functions related to HIV-1 replication and immune evasion. Our data suggest that interaction with Hck or other cellular binding partners with SH3 domains are required to induce the Nef dimer formation that is necessary for function.
T8. Biochemical and Functional Characterization of a Cargo Selective Allosteric Switch in HIV-1 Nef

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HIV Nef downregulates cell surface proteins by hijacking the AP-1 and -2 clathrin adaptors. Previously, we showed that HIV-1 Nef cooperates with the GTPase Arf1 to induce the oligomerization and activation of AP-1, assembling together as closed trimers or open trimers in vitro. The open trimer is proposed to assemble into a hexagonal inner layer of the clathrin coat. In this study, we investigate the functions of closed trimer on the basis of a novel AP-1:Nef interface revealed by its high resolution cryo-EM structure. Mutation of this interface shows that it is important for clathrin cage assembly in vitro and MHC-I downregulation in HIV infected cells.
T9. Physical Properties of the HIV-1 Capsid from All-Atom Molecular Dynamics Simulations

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Human immunodeficiency virus type 1 (HIV-1) infection is highly dependent on its capsid. The capsid is a large container, made of 1,300 proteins with altogether 4 million atoms. Although the capsid proteins are all identical, they nevertheless arrange themselves into a largely asymmetric structure made of hexamers and pentamers. The large number of degrees of freedom and lack of symmetry pose a challenge to studying the chemical details of the HIV capsid. Simulations of over 64 million atoms for over 1 micro-second allow us to conduct a comprehensive study of the chemical–physical properties of an empty HIV-1 capsid including its electrostatics, vibrational and acoustic properties, and the effects of solvent (ions and water) on the capsid. The simulations reveal critical details about the capsid with implications to biological function.

1. [Perilla and Schulte, Nature Communications, 2017 DOI: 10.1038/ncomms15959]
T10. Structure Determination of HIV-1 Capsid Assemblies at Atomic Resolution Using Magic Angle Spinning NMR

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In a mature HIV-1 virion, the viral CA protein assembles into a conical capsid, enclosing the viral genome. The capsid, which plays multiple roles in HIV-1 life cycle, is crucial for viral replication¹. CA assemblies have been characterized by various methods²,³,⁴, but there is still no atomic-resolution structure. Here, we present an atomic-resolution structure of tubular assemblies of CA, determined by an integrated magic angle spinning (MAS) NMR and cryo-EM approach. We have collected intramolecular and intermolecular distance restraints in sparsely and differentially labeled CA assemblies using homo- and heteronuclear experiments, from which the 3D structure of CA monomer was determined. The structure of the hexameric building block was determined using both NMR experimental restraints and the cryoEM density map.

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T11. Characterization of a Host Cell Activity that Stimulates HIV-1 Capsid Assembly in Vitro

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During HIV-1 maturation, the Gag polyprotein is processed into its constituents by proteolytic cleavage, resulting in assembly of the conical viral capsid. HIV-1 capsid assembly can be mimicked with purified CA protein in vitro; in such reactions, the rate of CA assembly is increased in the presence of small molecules that bind to a conserved drug-sensitive pocket at the NTD-CTD intersubunit interface. Based on analogies with picornaviruses and on the existence of the small molecule binding pocket in the CA hexamer, we hypothesized that a cellular small molecule “pocket factor” controls the rate of HIV-1 capsid assembly.

We have detected and initiated efforts to identify an HIV-1 CA Assembly-Stimulating Activity ("CASA") present in extracts of cultured human epithelial cells. CA assembly was monitored by turbidity, and conditions were established under which preincubation with cell extracts potently enhanced CA assembly initiated by addition of salt. Dialysis of recombinant HIV-1 CA into soluble cell extracts resulted in markedly accelerated assembly, suggesting that a dialyzable cell factor was responsible. However, the activity did not pass through a 10 kDa-cutoff ultrafiltration device. CASA was lost upon heating of cell extract and was resistant to treatment with ribonuclease. Addition of purified DNA did not stimulate the assembly reaction. CASA was partially enriched by anion-exchange chromatography; however, it was subsequently lost upon dialysis of the pooled active fractions. Addition of purified ATP, hexacarboxybenzene, or IP6 failed to stimulate CA assembly in vitro, suggesting that CASA was not due to small molecule binding to the recently-described nucleotide pore in the CA hexamer. Our current data suggest that CASA is due to a protein-bound small molecule or peptide. Additional efforts to purify and characterize the activity will be presented. Identification of a host small molecule controlling HIV-1 capsid assembly will result in new insights regarding the control of HIV-1 maturation and provide a new angle on therapeutic development.
Building Soluble and Homogeneous HIV-1 Capsid Platforms Recognizable by Host Factors

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The HIV-1 capsid serves as a molecular platform for the binding of numerous host factors, both beneficial and harmful to the virus. Most well-studied capsid binding factors require high-order capsid lattice patterns for complete binding and do not show appreciable affinity for small capsid building blocks. Although recombinant host factors can often readily bind in vitro assembled capsid tubes, which are large, heterogeneous, and insoluble, they cannot bind the soluble CA assemblies currently available (CA monomers, dimers, hexamers, pentamers). This creates challenges for high-resolution structural and biochemical analysis. Therefore, our mechanistic understanding of host-capsid interactions is well behind that of our knowledge of mature capsid structure.

To overcome this limitation, we design and build a repertoire of soluble capsid assemblies that represent all unique capsid-lattice interfaces. Our first approach uses engineered disulfides to build a variety of disulfide-stabilized CA oligomers (between 50-150 kDa) that contain native di-hexamer and/or tri-hexamer interfaces—interfaces potentially necessary for the binding of many host factors. We have used these small assemblies to gain insight into the binding mode of TRIMCyp, which uses its two cyclophilin A domains to simultaneously bind two CA molecules in a flexible manner. We have also discovered a novel, CA-lattice targeting peptide from the putative capsid-binding cofactor Fez1. This Fez1 peptide specifically recognizes intact CA hexamers.

To build more complete capsid platforms that may be required for the binding of host capsid pattern-sensing factors, we create multi-hexamer assemblies incorporating the SpyCatcher/SpyTag system to form isopeptide bonds among hexamers and pentamers. This has enabled us to build isopeptide/disulfide bond-stabilized CA platforms that are 300/450 kDa and above in size. Importantly, these large platforms can be recognized by TRIM5α and MxB constructs that are only sensitive to CA lattice interfaces. This provides insights into the complex binding mode of these two restriction factors. We believe this work greatly expands our ability to study known host-capsid interactions and provides a framework to facilitate the investigation of the binding modes of novel capsid-binding factors.
The capsid protein (CA) plays crucial roles in the HIV replication cycle. After viral and host cell membrane fusion, the capsid core is released into the cytoplasm. This core, which corresponds to the assembly of ~1200 capsid proteins, contains and protects viral RNA and proteins from degradation. To date, no drugs targeting CA are approved for clinical use.

With the goal of identifying novel active molecules that destabilize the capsid core, we set up a high-throughput virtual screening (VS) campaign in collaboration with the World Community Grid (WCG) from IBM in part of the Fight AIDS At Home (FA@H) project.

Four pockets of interest were selected at the surface of the recently solved hexameric assembly. Structural variability surrounding these pockets was analyzed by comparing this X-ray structure from the PDB (4xfx), and the two full capsid core models assembled by Schulten's lab (3j3q and 3j3y). Based on that, 36 different conformations were selected as targets for the VS, including the X-ray structure and structures from the models. Each target was set both as fully rigid and also with a specific combination of residue side chains defined as flexible.

An extended library of ~1.6 million commercially available compounds where used for the screening. Replicate computations were performed for each docking experiment in order to assess the consistency of the results. In total ~621 million docking computations will be performed on the WCG. Currently, ~50% of the computation is completed, with a projected completion estimated at the end of 2017, if the computation does not increase in speed. However, in 1 month we will be able to propose to our HIVE Center collaborators a selection of compounds (focusing on one of the 4 pockets) for experimental binding and infectivity assays.

Dedicated web-pages (see http://fightaidsathome.scripps.edu/Capsid/index.html) were developed to inform the public and the WCG volunteers on advances in the project. The pages contain an overview of the project, details on targets and the selection process, description of the compound library, an hourly updated status of the computations, and a “people” section where volunteers can appear in the page to be fully part of the project.

An automatic pipeline has been developed in order to constantly post-process the docking results received from the WCG. These post computations involve the High Performance Computing (HPC) cluster from The Scripps Research Institute, and are mainly related to the identification of the interactions between drug candidates and the CA protein. The pipeline ends in filling a MySQL database, which will be made public as soon as it will be stable.

In detail, 3.3TB of compressed data are estimated to be received from the WCG, and 1TB to be generated after post-processing.

T14. Expanding the Horizons for Structural Analysis of HIV-1 Capsid Assemblies by NMR Spectroscopy at MAS Frequencies Above 100 kHz

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Magic angle spinning nuclear magnetic resonance (MAS NMR) is uniquely suited to access atomic-resolution structural and dynamics information on HIV-1 capsid assemblies\textsuperscript{1-3}. The recent breakthroughs in NMR probe technologies have resulted in the development of MAS NMR probes with rotation frequencies exceeding 100 kHz\textsuperscript{4,5}. We demonstrate the cutting edge application of ultrafast MAS to fully protonated tubular assemblies of HIV-1 CA capsid protein. We observe dramatic increases in sensitivity and resolution at MAS frequencies of 110-111 kHz in a novel 0.7 mm HDCN probe that enables structural analysis of fully protonated biological systems. Proton-detected 2D and 3D correlation spectroscopy under such conditions requires only 0.1 – 0.5 mg of sample and a fraction of time compared to conventional \textsuperscript{13}C-detected experiments. With the high sensitivity and resolution, resonance assignments of protons and heteronuclei, \textsuperscript{1}H–\textsuperscript{15}N dipolar and \textsuperscript{1}H CSA tensors, and proton-proton distances are readily accessible from proton-detected 3D experiments. The approach demonstrated here is expected to enable atomic-resolution structural biology of large protein assemblies inaccessible to current methodologies including the \textit{de novo} 3D structure determination of HIV-1 capsid assemblies.

This work was supported by the National Institutes of Health (NIH) National Institute of General Medical Sciences Grant P50 GM082251; the National Science Foundation (NSF) Grant CHE0959496 (for the acquisition of the 850 MHz NMR spectrometer at the University of Delaware); and NIH Grants P30GM103519 and P30GM110758 (for the support of core instrumentation infrastructure at the University of Delaware).

T15. Structural and Dynamic Studies of HIV-1 Capsid by $^{19}$F Solution and Magic Angle Spinning NMR

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The HIV-1 capsid protein (CA) plays essential roles in the HIV-1 life cycle. In mature virions, it assembles into a cone-shaped structure comprised of ~1500 copies of CA, to enclose the viral RNA genome during viral maturation. To determine a full atomic-resolution capsid structure and understand the conformational plasticity of the CA protein, we explored $^{19}$F as a novel NMR structural probe of the HIV-1 capsid and capsid’s complexes with host factors, such as Cyclophilin A (CypA). We have incorporated 5-F-Trp into CA, single Trp CA mutants, and CypA proteins. $^{19}$F solution NMR spectra exhibit excellent resolution and permitted to assign chemical shifts, characterize the CA building blocks (monomer, dimer and hexamer) and understand how the $^{19}$F chemical shifts are affected upon assembly. $^{19}$F MAS NMR spectra of tubular assemblies of CA and its complex with CypA also exhibit remarkably high sensitivity and resolution, permitting identification of individual sites and their assignment. The 2D spin-diffusion experiments of CA and CA/CypA assemblies permitted to establish the $^{19}$F-$^{19}$F correlations between fluorine sites as distant as 23 Å, and the multiple conformers are well resolved. $^{19}$F CSA tensors recorded from MAS NMR experiments vary significantly for the individual sites in CA, and are distinct for CA and CA/CypA. Furthermore, we observed a pronounced perturbation of $^{19}$F chemical shifts between the CA wild type assemblies and cross-linked CA hexameric assemblies, indicating significant conformational changes.

This work was supported by the National Institutes of Health (NIH) National Institute of General Medical Sciences Grants P50 GM082251 and F32GM113452; the National Science Foundation (NSF) Grant CHE0959496 (for the acquisition of the 850 MHz NMR spectrometer at the University of Delaware); and NIH Grants P30GM103519 and P30GM110758 (for the support of core instrumentation infrastructure at the University of Delaware).

T16. Dynamic Regulation of HIV-1 Capsid Morphology and Viral Maturation

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The mature HIV-1 virion contains a conical capsid core, which encapsulates the retroviral RNA and accessory proteins. The capsid lattice is comprised of 216 hexameric subunits, with 12 pentameric subunits incorporated to induce curvature and capsid closure. In addition to structural features, dynamics plays an essential role in HIV infectivity and interactions with host factors. To understand the contribution of dynamics to capsid morphology and stability, we have investigated several capsid sequence polymorphs yielding tubular or conical assemblies by magic angle spinning nuclear magnetic resonance (MAS NMR) spectroscopy including WT tubes, hexamer tubes (A14C/E45C/W184A/M185A), A204C cones, and conical assemblies of mixed 10:1 natural abundance hexamers and 13C/15N-labeled pentamers (N21C/A22C/W184A/M185A). From these samples, on the basis of cross polarization transfer dynamics, we observe that pentameric subunits are more rigid than hexameric subunits on the micro- to millisecond timescale, in agreement with MD simulations. This enhanced rigidity may be an essential feature for stability of the closed capsid. In the conical HIV-1 capsid assemblies of A204C mutant, we have observed attenuated CypA loop dynamics relative to tubular assemblies, as well as structural perturbations, many of which are concentrated in helix 1.

We have also investigated the dynamic regulation of HIV capsid maturation with assemblies of several CA-SP1 and CA-SP1-NC sequences. During HIV-1 maturation, the Gag polyprotein undergoes a sequential cleavage cascade to form the mature capsid. The small-molecule maturation inhibitor Bevirimat (BVM) inhibits the final step of Gag maturation, cleavage of the SP1 peptide. An SP1 mutation, T8I, mimics BVM action by causing accumulation of the CA-SP1 intermediate. Mixed tubular/conical assemblies of CA-SP1-NC have markedly attenuated CypA loop dynamics, which may be due to steric hindrance of the loop in the immature-like capsid lattice as observed by others. The presence of the nucleocapsid (NC) domain stabilizes the highly dynamic SP1 peptide, and increases the helical content of the C-terminal domain (CTD) tail (H226 to L231). In contrast, in conical assemblies of the maturation inhibiting CA-SP1-NC T8I mutant, the SP1 peptide (Q237 to T243) possesses higher helical content compared to CA-SP1 T8I, and the N-terminal β-hairpin is less stable in the longer T8I construct. CA-SP1 T8I and CA-SP1-NC T8I have similar CypA loop dynamics, faster than wild type CA-SP1-NC dynamics, but slower than in the mature capsid. This result agrees with the cryo-EM finding that in the CA-SP1 T8I mutant neither a mature nor an immature lattice is present. The NC domain of the T8I mutant is more dynamic than in the WT sequence, indicating further differences in lattice conformation.

This work was supported by the National Institutes of Health (NIH) National Institute of General Medical Sciences Grants P50 GM082251 and F32GM113452; the National Science Foundation (NSF) Grant CHE0959496 (for the acquisition of the 850 MHz NMR spectrometer at the University of Delaware); and NIH Grants P30GM103519 and P30GM110758 (for the support of core instrumentation infrastructure at the University of Delaware).

HIV-1 protease (PR) cleavage of the Gag polyprotein triggers the assembly of mature, infectious particles containing the characteristic conical capsid. Final cleavage of Gag occurs at the junction helix between the capsid protein CA and the SP1 spacer peptide. In 2016, compelling evidence for the predicted 6-helix bundle of the junction helices was provided by three orthogonal experimental approaches: cryoEM tomography and subtomogram averaging of ΔMA-Gag virus-like particles (VLPs) (1), X-ray crystallography of a Gag construct comprised of the C-terminal domain (CTD) of CA and SP1 (2) and ssNMR spectroscopy of ΔMA-Gag VLPs (3). The cryoEM and X-ray structures revealed that the protease cleavage sites are sequestered on the interior of the 6-helix bundle. The cryoEM map also showed that a single molecule of bevirimat binds at the center of each 6-helix bundle. To achieve a resolution of 3.9 Å, 6-fold averaging was applied to the cryoEM map, which prevented characterization of the interactions between bevirimat and the CTD-SP1 junction helices. Here we delineated the binding interactions of bevirimat using electron diffraction of thin, three-dimensional microcrystals (MicroED) of a CTD-SP1 Gag construct. The 2.9-Å MicroED structure revealed that bevirimat stabilizes the 6-helix bundle via both electrostatic interactions with the dimethylsuccinyl moiety and hydrophobic interactions with the pentacyclic triterpenoid rings. These results provide insight into the mechanism of action of bevirimat and related maturation inhibitors that will inform further drug discovery efforts. This study also demonstrates the capabilities of microED for structure-based drug design. [Funding: NIH R01 GM066087 and NIH P50 GM082545 (CHEETAH)]

The maturation of the HIV-1 virion is a critical process in the viral lifecycle leading to the formation of infectious virions. Maturation occurs through the proteolytic cleavage cascade of the Gag polyprotein and subsequent lattice remodeling. Gag is comprised of the matrix (MA), capsid (CA), nucleocapsid (NC), and p6 proteins as well as spacer peptides 1 and 2 (SP1 and SP2). The final maturation step is the cleavage of the 14-residue SP1 domain, leading to the formation of a conical capsid comprised of ~1500 copies of the 231-residue CA capsid protein.\(^1,2\)

To understand the structure of the immature Gag lattice, we have investigated the assemblies of the 300-residue CA-SP1-NC polyprotein. We have employed multidimensional magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopy to obtain atomic-resolution information on the structure. We have nearly complete chemical shift assignments for the carbon and nitrogen resonances.

From the assigned chemical shifts, we have characterized secondary structure elements of CA-SP1-NC in the assembled state with TALOS-N\(^3\) and by computing the secondary chemical shifts\(^4\) for C\(\alpha\) and C\(\beta\) resonances. The results indicate that the CA domain of CA-SP1-NC is folded with the same secondary structure as mature capsids. We compared the TALOS-N\(^3\) prediction for CA-SP1-NC(T8I) and CA-SP1-NC(WT) with those for CA-SP1(T8I) and CA-SP1(WT) maturation intermediates. We find that (1) the \(\beta\)-hairpin is formed at the N-terminal terminus of the capsid protein in all four assemblies; and (2) the CTD-SP1 junction and SP1 peptide has helical content in all four assemblies.

This investigation sets the stage for 3D structure determination of CA-SP1-NC assemblies at atomic resolution by MAS NMR.

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HIV/AIDS is a global pandemic and infects roughly 40 million people worldwide. Current therapies target fusion, reverse transcription, and integration processes, but serve only to minimize the effects of the disease. Only the maturation process is targeted in the late phase of the HIV replication cycle, which involves proteolytic cleavage of the Gag polyprotein. During viral assembly, thousands of Gag molecules form a hexagonal lattice, while the CA-SP1 junction region transitions from a random coil to a helical structure in order to assemble into a 6-helix bundle. This CA-SP1 junction is cleaved last during viral maturation, and previous work has suggested that maturation inhibitors prevent the cleavage of the CA-SP1 junction by targeting and stabilizing the 6-helix bundle structure. The 6-helix bundle is only formed in assembled Gag molecules, hindering the solution study of its interaction with maturation inhibitors. Meanwhile, efforts to crystallize the assembled CA-SP1/inhibitor complex have not been successful. Thus, the molecular mechanism for the inhibitory function of these compounds still remains elusive. This work aims to provide insight to the mechanism of maturation inhibition, by utilizing a hexameric scaffolding protein to construct an isolated CA-SP1 junction hexamer. This protein will facilitate probing into the binding stoichiometry and binding affinity of maturation inhibitors, exemplified by Bevirimat, to the hexameric protein by isothermal titration calorimetry (ITC) and solution nuclear magnetic resonance (NMR). Once the binding ratio is better understood, NMR structural studies can be performed to probe the binding mechanism of maturation inhibitors to the CA-SP1 junction helix.
T20. Investigating the Effects of Drug Resistance Mutations on Conformational Sampling Patterns in HIV-1 Protease Subtype C in Response to Nelfinavir

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Many studies and highly active antiretroviral therapy (HAART) targeting the enzyme HIV-1 protease (PR) have focused on HIV-1 subtype B, which accounts for less than 10% of global infections, but growing evidence shows that natural polymorphisms in non-subtype B HIV-1 PR have resulted in evolved mutations that promote drug resistance against the available protease inhibitors (PI) (1). Previous studies have shown that the M36I polymorphism in non-subtype B HIV-1 PR is associated with higher rates of drug resistance and evolution of other mutations in response to PI by affecting the conformational sampling patterns (2). HIV-1 Subtype C accounts for over 50% of the global HIV-1 infections and its evolved mutations in response to nelfinavir (NFV) therapy develops cross-resistance against other PI (2). Our study focuses on how the naturally occurring polymorphisms affect the conformational sampling pattern of HIV-1 subtype C PR in response to NFV. From site-directed spin labeling (SDSL) double electron-electron resonance (DEER) investigations, the four conformations of HIV-1 PR are closed, semi-open, curled/tucked, and wide-open. We hypothesize that the combinations of mutations (M36I/D30N/M46I/L90M) in subtype C will change the conformational sampling patterns to stabilize the semi-open population to enhance enzymatic activity or increase the open-like populations over the closed population to promote drug resistance in response to NFV (3). Generated recombinant proteins were site-directed spin labeled and assessed by mass spectrometry and continuous wave (CW) electron paramagnetic resonance (EPR) spectroscopy. The conformational sampling patterns of the recombinant HIV-1 subtype C PRs unbounded and against NFV are collected via SDSL DEER. Taken together, the results from this study will shed light on the effects of drug resistance mutations on conformational sampling patterns in HIV-1 Subtype C in response to NFV.

4. Elucidating a Relationship between Conformational Sampling and Drug Resistance in HIV-1 Protease. Biochemistry, 2013, 52 (19), 3278-3288
T21. The Structural and Dynamic Basis for the Interdependence of Drug Resistance in HIV-1 Protease

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We aim to characterize structural and dynamic changes in Human Immunodeficiency Virus-1 (HIV-1) protease (PR) upon drug interaction and resistance mutations. A slight change in the chemical moiety of an inhibitor or a mutation does not necessarily alter the enzyme structure but often still impacts the molecule’s inhibitory activity. In the era of state-of-the-art inhibitor design and high-resolution structural studies, elucidating how these small changes in inhibitor chemical moieties alter protein structure or dynamics, and interplay between drug-resistance mutations may be critical to further developing inhibitors. In the last two decades, NMR sensitivity and computation capacity increased tremendously. Taking advantage of these technological advancements, we detect conformational and dynamic changes in PR upon inhibitor interaction and mutations at high sensitivity using NMR spectroscopy, and reveal the impacts on inhibition. This year, we will present our optimization of water detection by NMR, chemical shift analysis for the PR-inhibitor interaction studies, and interpretation of data with assistance of molecular dynamics simulations. The work is supposed by NIH P01 GM109767.
T22. Substrate Envelope Guided Design of HIV-1 Protease Inhibitors Incorporating Stereochemically Defined Novel P2’ Ligands

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We have developed a substrate envelope guided strategy to rationally design HIV-1 protease inhibitors with improved potency and resistance profiles. This strategy together with insights from detailed structural analysis of protease-inhibitor complexes led to the design of new inhibitors with improved potency against highly resistant strains of HIV. Here, we describe the substrate envelope guided design, synthesis, biological evaluation, and X-ray crystal structure analysis of a series of novel HIV-1 protease inhibitors. The inhibitors were designed by incorporating stereoisomers of the 4-(1-hydroxyethyl)benzene and 4-(1,2-dihydroxyethyl)benzene moieties as novel P2’ ligands to enhance hydrogen bond interactions within the S2’ binding pocket. Structure-activity relationship (SAR) studies showed that compounds with both the (R)- and (S)-stereoisomer of the 4-(1-hydroxyethyl)-benzene as P2’ ligands showed improved potency than DRV against highly drug resistant HIV-1 variants. The SAR data and molecular insights from X-ray crystal structures may allow further optimization of this novel series of HIV-1 protease inhibitors.
T23. Development of a Genetic Algorithm for DOCK to Aid in De Novo Design

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Genetic algorithms (GAs) are evolution-based computational methods that have emerged as a powerful tool for atomic-level de novo design. GA-based methods can be employed to construct novel small molecules tailored to specific biologically-relevant protein targets by chemically altering an initial ensemble through mating and mutations (insertions, deletions, additions, substitutions). We are developing a GA for the program DOCK6 that incorporates 3D ligand geometry and binding interactions, as quantified by different scoring functions, to tailor the evolution of new molecules for improved binding in the context of a defined protein site. As proof-of-principle, the GA has been applied to several systems, including HIV Protease and Reverse Transcriptase, to evaluate its behavior using distinct starting ensembles, binding sites, and input parameters. Our progress to date will be presented.
T24. Mechanistic Characterization of Inhibitor Resistance in HIV-1 Protease via Machine Learning and Atomistic Simulation

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Darunavir is an HIV-1 protease inhibitor with high binding affinity that can be effective against strains where resistance to other inhibitors has developed. Despite this effectiveness and the associated delay in the onset of protease resistance to darunavir inhibition, however, resistance has been observed in the presence of multiple simultaneous mutations. In order to gain mechanistic insight on the role that specific mutations play in this resistance, molecular dynamics simulations were carried out on a selection of 15 HIV-1 protease variants, chosen to include susceptible variants and wild-type controls, along with strains that are resistant to darunavir in vivo and/or in vitro. The resulting high-dimensional space of thermodynamic observables among this set of sequence variants can be reduced to interpretable variables using machine learning, with a particular emphasis on supervised methods.
T25. Interdependence of Inhibitor Recognition at the Active Site of HIV-1 Protease

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Our lab has developed the substrate envelope hypothesis to link structure to mechanisms of drug resistance. This hypothesis asserts that inhibitors designed to best fit within the recognition site volume of the natural substrates will be less susceptible to resistance. Darunavir (DRV), the most potent and least susceptible to drug resistance of all FDA approved inhibitors targeting HIV-1 protease, fits well with the substrate envelope. Using a focused library of DRV analogs, we examined the interdependency between subsites of the protease active site in inhibitor recognition. These analogs, their co-crystal structures bound to protease, and molecular dynamics (MD) simulations starting from these structures enabled systematically probing the S1′ and S2′ subsites. Analysis of MD simulations in terms of atomic fluctuations, intermolecular interactions, and water structure revealed that the chemical moiety at the S1′ subsite highly influences other subsites. Our results intricately link the HIV-1 protease subsites, providing insights into molecular recognition and inhibitor binding. More broadly, the interdependency between subsites urges consideration of chemical moieties in context of each other, in contrast to independent optimization of chemical moieties in drug design.
Bridging water molecules are often found in protein-ligand complexes that mediate interactions between the ligand and surrounding residues. In drug-lead discovery and refinement, it has been an effective strategy to displace such waters with polar functionalities in rationally designed ligands (e.g. cyclic urea inhibitors developed for HIV-1 protease) in order to improve binding affinity. Another approach to potentially improve potency is to coordinate bridging water molecules (to maintain the favorable water-mediated interactions) with novel ligands. In the present study, we report an interaction energy-based computational approach to specifically identify bridging water molecules and then incorporate (displace or coordinate) them in virtual screens. Solvated molecular footprints (per-residue interaction energy decomposition) are employed to drive water displacement. The proposed approach has been tested on five disease-related complexes (four crystallographic structures and one homology model) and both previously reported and novel bridging water molecules have been identified. Preliminary results from small-scale virtual screens to two of the systems (HIV-1 protease and PARP1) to identify novel ligands that displace or coordinate bridging waters will be presented as proof of concept.
HIV-1 protease is essential for viral maturation and one of the main targets of antiretroviral therapy. Under the selective pressure of therapy, drug resistant strains have emerged. Residues in the active site of HIV-1 protease are particularly prone to resistance mutations if their interactions with the inhibitor outweigh interactions with the enzyme substrate. Recently developed protease inhibitors such as Darunavir fit well within the substrate consensus volume and minimize these contacts, requiring additional mutations distal from the active site for resistance to occur. These distal mutations influence the active site through networks of intramolecular interactions, reducing inhibitor potency while maintaining enzymatic activity. A plethora of studies aimed to understand the intramolecular rearrangements that lead to resistance, however few have looked beyond the macromolecular surface and considered the role of hydration waters in mediating intramolecular interactions. In this study we utilize molecular dynamics simulations to elucidate the role of hydration in the structural ensemble of HIV-1 protease. We identified local maxima in the solvent density distribution where water-protein interactions significantly perturb solvent dynamics. Water molecules occupying these sites differ significantly from bulk water. Our results resolve and characterize the full structure of HIV-1 protease hydration. We demonstrate how water mediated interactions expand the network of intra- and intermolecular interactions. Future work will address how rearrangements of the enzymes structure due to drug resistance mutations correlate with changes in hydration structure. We expect our model to complement the current models of drug resistance and expand our understanding of the molecular mechanisms of drug resistance. This will aid in the future development of more robust inhibitors.
The Homodimeric HIV-1 Protease Uses Bi-Specific S2/S2' Subsites to Achieve Optimal Processing of Two Evolutionarily Conserved Cleavage Site Motifs

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All orthoretroviruses including HIV-1 maintain a Proline at the N terminus of the capsid protein to adapt the functional need for a conformational change during virion maturation. Thus, HIV-1 protease (PR) has evolved to cleave substrates containing a Proline at the P1' position (i.e. the amino acid in the C-terminal side of the scissile bond). However, most cleavage sites in Gag and Gag-Pro-Pol carry large hydrophobic amino acids instead of a Proline at the P1' position, implying that at least two different classes of HIV-1 cleavage sites exist. In this study, we examined amino acid sequence determinants governing the rate of cleavage of the substrates within the Gag and Gag-Pro-Pol regions using a two substrate system. Briefly, the wild type MA/CA site was replaced with mutant cleavage sites and the rate of cleavage was compared to that of the wild type MA/CA site used as an internal control in the two substrate system. Three Glycines were added on both sides of the wild type MA/CA site, -GGGSQNY/PIVQGGG-, to minimize any contextual effects on the rate of cleavage. We generated 150+ mutant substrates representing six canonical HIV-1 PR processing sites. Using these globular protein substrates, we were able to measure proteolysis under near-physiological conditions (pH 6.5, ionic strength 0.15M). Based on the site-specific determinants for cleavage rate assessed from the data collected from the 150+ mutant substrates and previously published structural data, we found that the homodimeric PR differentially binds the two classes of cleavage sites (i.e. with or without a P1' Proline). Optimal cleavage rates appear to be determined by the nature of the P2 and P2' amino acids in ways that are reversed between the two classes of cleavage sites. One of the P2/ P2' residue side chains points back toward the scissile bond to form a hydrophobic interaction with PR, while the other points away from the scissile bond to form hydrogen bonds with PR amino acid D30. Pairing amino acids at the P2/ P2' position to create interaction of either hydrophilic/hydrophobic (with a P1' Proline, as in the MA/CA site) or hydrophobic/hydrophilic (with a P1' hydrophobic amino acid, as in the SP1/NC site) is necessary for optimal processing efficiency. Slowly cleaved sites are improved as they are moved closer to these patterns. Thus, the homodimeric HIV-1 PR accommodates an asymmetry substrate in two ways that represent mirror images of each other, and down-regulate the rate of cleavage by deviating from these patterns.
T29. Investigation of the Role of Rab11-FIP1C in HIV-1 Glycoprotein Incorporation

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The lentiviral envelope glycoprotein complex (Env) is a critical determinant of viral infectivity. Although the structure and function of Env have been extensively studied, the mechanism by which the Env proteins are incorporated into virions remains poorly defined. In most physiologically relevant cell types, the Env cytoplasmic tail (CT) is required for Env incorporation into virions but in some cell lines the CT is not required. For example, the Jurkat T-cell line requires the full-length CT to be present for HIV-1 to replicate, while MT-4 permit replication of CT-deleted Env. The role of the CT was recently linked to the endosomal trafficking factor, Rab11 family interacting protein 1c (FIP1C). In this study, we investigated the role of FIP1C in Env incorporation onto nascent viral particles. We found that FIP1C is expressed in the Jurkat T-cell line but not in MT-4 cells. Furthermore, Env incorporation in progeny virus produced by Jurkat is higher than in virus produced by MT-4. These data are consistent with a role for FIP1C in promoting Env incorporation. Recently, the tyrosine-based motif, YW795, located in the gp41 CT, was found to promote Env incorporation. Defects in Env incorporation and particle infectivity of the gp41 YW795SL mutant were more pronounced in Jurkat than MT-4. shRNA knockdown of FIP1C in Jurkat reduced wildtype Env incorporation and infectivity but had no effect on the YW795SL mutant. Microscopy of HeLa expressing GFP-FIP1C showed a CT-dependent induction of FIP1c relocalization from the perinuclear region to the periphery. Therefore, the YW795 motif located within the gp41 CT is necessary for FIP1c-dependent trafficking of Env to sites of viral assembly in a cell-type-dependent manner. These findings suggest of role for Env recycling in Env incorporation into virions. The data obtained from this study shed light on HIV-1 Env incorporation, thereby providing key insights into the trafficking pathways utilized by Env during particle assembly.
T30. Mutations at the HIV-1 Matrix Trimer Interface Prevent Envelope Glycoprotein Incorporation

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The HIV-1 envelope (Env) glycoprotein is incorporated into the Gag lattice as a trimer of heterodimers, composed of the surface subunit (gp120) and the transmembrane subunit (gp41). The mechanism of Env incorporation is still not completely understood. Several studies demonstrated the involvement of the matrix (MA) domain of the Gag in this process. It has been shown that mutations in MA and in the long cytoplasmic tail of HIV-1 gp41 can prevent Env incorporation, implying that MA and gp41 might interact. It has been proposed that MA forms hexamers of trimers in intact virions. In this arrangement, the MA layer would contain a central aperture into which the gp41 cytoplasmic tail could fit. In our previous study, we developed a glutaraldehyde cross-linking assay and demonstrated a role for MA trimer formation in HIV-1 Env glycoprotein incorporation. MA trimer formation was observed in both immature and mature viral particles. We introduced mutations at the MA trimer interface and analyzed MA trimer formation. We found that two MA mutants, L74E and L74G, lacked detectable MA trimers, did not incorporate Env and were non-infectious. In the current study, we further investigated the relationship between MA trimerization and Env incorporation. We selected for compensatory mutations that rescue the Env incorporation defect for MA trimer-defective mutants. Interestingly, two compensatory mutations, F43I and F43L, were identified in the hydrophobic core of the trimer interface, close to L74. We showed that both mutations F43I and F43L, in combination with another mutation, V34I, completely rescued Env incorporation and restored infectivity and virus replication. We are currently analyzing the formation of MA trimers in the replication-competent V34I/F43I/L74E and V34I/F43I/L74G revertants. Our data confirm that changes in the MA trimer interface influence HIV-1 Env incorporation into viral particles.
T31. Structural Basis for the Unique Myristoylation Signal of the Feline Immunodeficiency Virus Matrix Protein

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N-terminal myristoylation of the matrix (MA) domain is critical for the targeting and assembly of the feline immunodeficiency virus (FIV) Gag polyprotein to the cellular membrane. This myristoylation event is facilitated by N-myristoyltransferase (NMT) which links the myristoyl moiety to the amino terminus of the substrate MA upon recognition of the myristoylation signal. Recent work reported that, although feline proteins follow the prototypical mammalian NMT recognition sequence (M-G-X-X-S/T; X = variable amino acid), the MA proteins of 25 known FIV isolates use the unique, conserved myristoylation sequence M-G-X-X-X-G. To identify structural and functional causes for this preference to select a non-consensus signal, we engineered mutations to FIV MA such that it included the consensus feline protein myristoylation motif (G4L/Q5K/G6S; NOS). Our findings indicate that, upon adoption of the common myristoylation signal, the myristoylation efficiency of FIV MA is decreased in vitro. Progress toward the characterization of the effect of this mutation on virus release and NMR structure determination of FIV myristoylated MA (myrMA), unmyristoylated MA (unmyrMA), and unmyrMA NOS will be presented.
T32. A Novel Class of HIV-1 Budding Inhibitor Blocks Ubiquitin Recognition by the ESCRT-I Protein Tsg101

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A high-throughput screening recently identified two structurally related small-molecule compounds as inhibitors of HIV-1, the causative agent of AIDS. The compounds inhibit viral budding at an early stage, preventing particle formation and transmission to other cells. Tsg101, the human protein that was the target of the ligand screen, is known to be hijacked by HIV-1 Gag through a Pro-Thr-Ala-Pro motif (PTAP), and would normally recruit the ESCRT machinery to pinch off and release the budding HIV-1 particle. In order to develop improved versions of the drugs tailored to the treatment of HIV-1, and to further understand the mechanism of inhibition, we determined the high-resolution NMR structure of Tsg101 with one of the inhibitors identified in the screen. Unexpectedly, the compound did not bind Tsg101 near the PTAP-binding pocket; instead, the drug formed a covalent interaction with a Cys residue near to the ubiquitin recognition site of Tsg101. We therefore set out to identify the reason why blocking a ubiquitin recognition site might affect HIV-1 budding. Tsg101 is known to recognize ubiquitinated proteins in its cellular cargo sorting role, although it is not clear exactly which types of ubiquitin conjugates are important. Proteins can have one or more ubiquitin molecules attached at Lys residues, and those ubiquitin molecules can be monomeric or form polymeric chains, between the C-terminus of one ubiquitin and a Lys residue of the next. For example, the K48-linked polyubiquitin chains target proteins for proteasomal degradation whereas K63-linked polyubiquitination is important for trafficking and HIV-1 budding. We determined whether Tsg101 has a preference for certain ubiquitin chains by assaying the interaction between K48- and K63-linked diubiquitins and Tsg101 using NMR spectroscopy. We did indeed find that Tsg101 has a preference for certain diubiquitin linkages and we characterized the structural basis for that recognition using a combination of chemical shift perturbations and docking. In summary, this work highlights the importance of the Tsg101-ubiquitin interaction and provides us with new mechanistic insight into the interactions between Gag and the cellular ESCRT complexes during HIV-1 budding.
T33. Structural Basis of ESCRT-III Membrane Assembly

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The Endosomal Sorting Complexes Required for Transport (ESCRT) mediate critical membrane remodeling events throughout the mammalian cell cycle, including HIV budding and cytokinetic abscission--among other roles. ESCRT-III proteins assemble into membrane-binding filaments to catalyze these reactions, but the structures and functions of these assemblies remain poorly understood. Our collaborative team recently determined the first atomic-resolution structure of an ESCRT-III filament – an IST1-CHMP1B hetero-polymer. Our structure demonstrated how one of these ESCRT-III subunits, CHMP1B, transitions from a “closed” to an “open” state to form an interlocked and domain-swapped filament. Moreover, we and others have shown that the IST1-CHMP1B copolymer participates in non-canonical, positive-curvature membrane fission pathways. Very recent work on other ESCRT-III proteins indicated that the mechanisms of opening and assembly we reported are conserved, but also raised questions regarding membrane binding and remodeling activities, as well as the generality of hetero-polymerization. To address these gaps in our understanding, we have focused on determining the structural mechanisms governing ESCRT-III activity during both positive- and negative-curvature membrane deforming activities. Our atomic-resolution cryo-EM studies will resolve the protein-protein and protein-lipid interactions governing these processes.
T34. Towards In Vitro Reconstitution of HIV-1 Budding and Release

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HIV-1 particle production occurs at the plasma membrane, where the viral structural protein Gag packages HIV-1 genomic RNA and assembles the new virus particle. This is followed by a membrane fission event where the immature particle is released from the cell surface. This release is mediated via the host ESCRT machinery which is hijacked by the HIV-1 Gag molecule. We plan to use an in vitro reconstituted Giant Unilamellar Vesicle (GUV) based system in combination with optical tweezer force measurements to observe and quantify the effects that these various macromolecular assemblies have on membrane deformation during the HIV-1 budding process.
T35. Probing HIV-1 Gag Polyproteins with SuFEx Compounds

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SuFEx (Sulfur(VI) Fluoride Exchange) reactions have properties that make them particularly suitable in biological environments. When binding to a protein SuFEx activation is achieved when the compound binds in a precise pre-reactive geometry specific conformation, providing a very selective way to target specific residues, especially free tyrosine hydroxyls, within the vicinity of positively charged amino acid side chains (Lys, Arg). The SuFEx-protein binding is covalent. The Sharpless laboratory has assembled an initial library of more than 580 compounds of 8 different classes functionalized with different SuFEx moieties. Of these, ~125 are alkyne-containing compounds suitable for click attachment of N\textsubscript{3}-CY3 or N\textsubscript{3}-biotin, which is ideal for identification of SuFEx-HIV Gag polyprotein complexes and HIV Virus-Like Particles (VLPs) binding.

A moderate throughput ELISA-based assay for SuFEx-Gag polyprotein screening was developed utilizing GST-Gag, GST-MA, GST-Capsid (CA), GST-Capsid-P2, or GST-Capsid-P2-NC proteins which can be bound to Glutathione-coated plates and then probed with alkyne-SuFEx compounds. Interactions were identified by clicking N\textsubscript{3}-biotin, followed by the addition of strep-HRP for visualization. SuFEx-Gag polyprotein binding specificity was determined by counter-screening on the various Gag regions. SuFEx-protein binding was further assessed by running the complex on denaturing gels to confirm a potential covalent interaction. From the library of 125 SuFEx alkyne compounds the following binding patterns were determined: Compound (CMPD) 407 bound P2, CMPD-325 bound CA and possibly P2-NC, CMPD-440 and CMPD-441 bound P2-NC, CMPD-399 and CMPD-400 bound P2 and RT. CMPD-440 and CMPD-441 bound delipidated VLPs. Mass spectrometry studies are underway to confirm whether the interaction of SuFEx-CMPDs with Gag polyproteins and VLPs are covalent, as well as the identity of the amino acids responsible for covalent interaction in the protein.
Aptamers are nucleic acid sequences selected for high affinity binding to protein targets. They have several uses including: inhibitors and drugs, biosensors, antibody substitutes, understanding biological processes, and quantification of proteins. Recently in collaboration with Dr. Eddy Arnold’s group at the HIVE, we used a novel primer-template mimicking aptamer to produce a high resolution crystal structure with HIV reverse transcriptase (RT), the first such structure that did not require cross-linking of the nucleic acid to RT. This result suggests that the tight binding of aptamers may help stabilize proteins and make it easier to obtain valuable structural information. In our continuing collaboration with the HIVE, we are currently working on producing aptamers to the HIV Gag and NC proteins with the hope that they may aid in gaining structural insights to these proteins. These proteins, and in particular Gag, have been very difficult targets for aptamer production. In our lab, the most promising approach thus far has been to use 2'-deoxy-2'-fluoroarabinonucleotides (FANA) to select for aptamers to these proteins. FANA is a “xeno-nucleic acid” (XNA) with a modified sugar group that has unique structural properties. Our group recently produced a FANA aptamer to HIV RT that bound with low pM affinity. We will present various aptamers to Gag and NC and discuss future prospects.
T37. Cryo-Electron Tomography of HIV-1 Mutants Reveals a Structural Element Critical for Assembly of the Immature Virion

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Human immunodeficiency virus type 1 (HIV-1) has claimed more than 35 million lives and currently affects approximately 37 million people worldwide. HIV-1 is a retrovirus that primarily infects CD4+ T lymphocytes and other immune cells, crippling the immune system and leading to acquired immune deficiency syndrome (AIDS). Multidrug resistance has necessitated continual development of new antiretrovirals targeting various phases of the HIV-1 life cycle, and viral maturation has emerged as a promising target. HIV-1 is released from infected cells in an immature, non-infectious form whose principal feature is a spherical array of the Gag polyprotein. To trigger maturation, the viral protease (PR) cleaves Gag at specific sites, allowing the capsid protein (CA) to reassemble into a conical core. We have previously shown that certain mutations in Gag can prevent full cleavage of Gag, blocking maturation. Additionally, maturation inhibitors (MI) impede maturation by stabilizing the immature Gag lattice and interfering with proteolysis. In the present study, we are investigating the role of a highly conserved Pro-Pro-Ile-Pro (PPIP) motif (CA residues 122-125) in the loop connecting helices 6 and 7 (H6-H7 loop) of CA in HIV-1 assembly. Data suggest that the mutations P122A and I124A impair virus release, infectivity, and replication in T-cell lines and that the T58S/T107I/P122A mutant reverts to wild type (WT)-like infectivity. We have been using cryo-electron tomography to ascertain how these mutations affect the structure of immature virions whose main constituent in WT HIV-1 is a trilaminar shell of Gag organized in a honeycomb lattice. We find that the P122A and I124A mutations disrupt this array in immature (PR-) virions. Neither mutant produces the lattice seen in WT PR- virions, and the Gag layer is distributed sparsely and discontinuously along the membrane in both mutants. Additionally, P122A and I124A have broader size distributions than WT PR-. The T58S/T107I/P122A revertant, in contrast, exhibits WT-like traits, including an organized, near-continuous Gag lattice and a compact size distribution. These observations suggest that the H6-H7 loop of HIV-1 CA, and particularly the PPIP motif, has an important role in coordinating the immature Gag lattice.
The packaging and release of immature human immunodeficiency virus-1 (HIV-1) particles from host cells is a critical step in the viral replication cycle. Disrupting this highly-regulated process is a potential target for anti-retroviral treatments. It is well-known that Gag (group-specific antigen) polyprotein must multimerize into a protein shell known as the immature lattice, which is also bound to a dimer of the viral ribonucleic acid (RNA) and is enveloped by the cell membrane. However, the molecular details of Gag assembly, especially as influenced by the presence of RNA and the cell membrane, remain unclear. To explore these dynamics, we developed a coarse-grained (CG) computational model that is derived from sub-nanometer resolution structural data and investigated the mechanisms that work in concert between Gag polyproteins, RNA, and the membrane to promote immature lattice growth. Our analysis, which is further supported by single particle tracking photoactivated localization experiments, demonstrates that viral RNA and the membrane are critical constituents that expedite Gag multimerization through scaffolding while short competitor RNA suppresses assembly. We also demonstrate the importance of weak anisotropic interactions at the helical spacer peptide 1 junction to mitigate defects in the otherwise continuous and hexameric Gag lattice. Finally, we reveal that lattice growth kinetics appears to be coupled to membrane deformation dynamics owing to intrinsic curvature that develops in the protein lattice. Overall, these findings elucidate a simple network of interactions that regulate the early stages of HIV-1 assembly and budding.

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The Gag polyprotein is the key structural protein mediating assembly of virions in HIV-1-infected cells. Previous studies have shown that Gag will spontaneously assemble into virus-like particles in vitro in the presence of a variety of nucleic acids, although the mechanism of this assembly and the role of nucleic acid are not fully understood. We have developed a single-molecule method to monitor Gag assembly in a defined in vitro system. The system utilizes fluorescently-labeled recombinant Gag polyprotein (enzymatically labeled with ATTO 488 or expressed as a fusion with eGFP) and a model nucleic acid immobilized on a microscope slide. Using TIRF microscopy, individual Gag monomers are resolved as they spontaneously assemble around the immobilized nucleic acid. These experiments probe early steps in the Gag assembly pathway, the equilibrium distribution of assembly intermediates and the rate constants for each step of assembly. Studies are performed with various model nucleic acids, to elucidate how the nucleic acid structure dictates the early steps of the Gag assembly pathway.

In cells, Gag assembly takes place on the plasma membrane, in the presence of viral RNA and various cellular proteins. Previous studies have shown that discrete Gag assembly intermediates of varying sizes can be separated by sucrose gradient fractionation. However, the Gag stoichiometry and the identity of the cellular proteins in each intermediate are not well characterized. We have developed a single-molecule pull down assay to analyze the Gag assembly intermediates. Lysates from mammalian cells transfected with Gag-GFP are fractionated on a sucrose gradient, individual fractions are applied to a microscope cover slip coated with anti-Gag or anti-GFP antibodies and the number of Gag-GFP molecules at each location on the slide is determined by single-molecule photobleaching analysis. The results reveal the distribution of Gag monomers within each of the Gag assembly intermediates (10S, 40S, 80S, 150S, 500S etc.), as well as any heterogeneity that may exist (subpopulations). The advantage of this approach is that Gag assembly occurs in the native cellular environment and all natural cofactors are present. Supported by NIH P50 grant GM082545.
T40. Toward Understanding Gag Assembly in vivo

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The Gag polyprotein is the major HIV component of assembled virions in infected cells. Previous studies have shown that discrete Gag-containing species can be isolated from Gag-expressing cells. However, the composition and role of these species and their cellular factors in Gag assembly remain unclear.

Using different lysis conditions and density gradient ultracentrifugation analysis, we show that a number of Gag-containing species can be isolated. Species that are found in the highest sucrose concentration fractions appear to be RNase A resistant, while others are not. A “spike” lysis experiment, where monomeric Gag is added during lysis, is then performed to demonstrate that while all of the Gag-containing species are pre-formed before cell lysis, some are highly transient in nature. To elucidate the order of these species in the assembly pathway, a pulse-labeling experiment was performed. Interestingly, our results are inconsistent with the previously proposed linear assembly model. Instead, our data imply the presence of parallel pathways and/or dead-end intermediates, revealing an as yet unappreciated complexity to the assembly process.
T41. Identification of HIV-1 Interacting Cellular Factors during Gag Trafficking and Assembly

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Gag is fully capable of self-assembly in vitro, although it is generally accepted that during infection in cells host factors play a decisive role for immature Gag trafficking and assembly. Lingappa and colleagues have demonstrated that during cellular formation of the immature virion, Gag is found in a progression of discrete assembly intermediates that are distinguishable by their velocity sedimentation values (10S, 80/150S, 500S, 750S). The ever increasing change in the sedimentation values has been argued to be due to host factor involvement coincident with Gag multimerization and maturation. However, less is known about the composition, dynamics, and function of cellular proteins that may contribute to Gag maturation and trafficking from translation through the cell to involvement with the ESCRT pathway. To interrogate the Gag-host protein interactome, we focused on identifying proteins that interact with Gag during cellular trafficking and assembly.

Gag Immunoprecipitation (IPs) methodology was implemented to enrich for Gag-host protein complexes. Chemical crosslinkers were used to capture transient and nearest neighbor protein interactions in situ after transient transfection with an HIV plasmid. Upon mass spectrometry analysis of the Gag-host cell complexes, 702 proteins were identified, including the known Gag binding proteins ALIX, Cyclophilin A, and DDX3X. Of the 702 proteins identified, 196 proteins had peptides exhibiting crosslinking modifications. Based on gene ontology, 29 of the 196 have been associated to viral processes. 60 of our hits have been described as Gag interactors and an additional 78 were listed as potential HIV interactors (NIH HIV interaction database). The remaining 58 proteins were categorized by gene ontology into the following pathways: translational, nucleotide synthesis and hydrolysis, exosomal, cytoskeletal remodeling and protein folding processes. Our findings on the composition of host proteins associated with Gag provide the first hint of molecular pathways potentially co-opted by Gag during trafficking and assembly.

Our IP methodology employed potentially could sample Gag-protein complexes during all stages of cellular trafficking and assembly. To interrogate host proteins associated with Gag during the continuum of the trafficking and assembly process, a linear sucrose gradient fractionation of lysates from HIV transiently transfected cells is underway. This will provide identification of the composition of host proteins interacting with Gag over time during the trafficking and assembly process. Our efforts will continue to provide insights into the HIV-host protein interactome and host protein function during infection.
The function and fate of the HIV-1 RNA genome is decided by its monomer-dimer equilibrium, which is regulated by the highly conserved 5’ leader region. Recently, it was found that all lentiviral genomes are transcribed in infected cells from an integrated proviral DNA that contains a stretch of three sequential guanosines, any of which could potentially serve as the transcription start site. The 5’-capped genomes beginning with one guanosine (1G) favored dimerization and were selected efficiently for packaging. The 5’-capped 2G and 3G genomes favored the monomeric conformation and were enriched on polysomes, apparently preferred for translation and possibly for splicing. Using a nuclear magnetic resonance (NMR) approach and a variety of unique ²H-labeling schemes, we analyzed the start site region of the native 5’ leader in its dimeric and previously elusive monomeric conformation. The additional guanosine(s) enables the disruption of the lower stem of the adjacent polyA stem loop, freeing up residues to base pair with and sequester the palindromic loop of the dimer-promoting DIS hairpin, thereby stabilizing the monomeric form of the RNA. This analysis reveals how a single nucleotide disrupts the dimeric conformation and stabilizes the monomeric conformation with an extended U5:DIS interaction.
Stable isotope labeling is central to NMR studies of nucleic acids, and the development of methods that incorporate isotope labels at specific atomic positions within each nucleotide promises to expand the size range and complexity of RNAs that can be studied by NMR. Combining enzymes from the pentose phosphate and other pathways with chemical synthesis of the ribose and base moieties, we have developed a streamlined chemo-enzymatic method to make nucleotides site specifically. This chemo-enzymatic approach is inexpensive, rapid and produces high yields of up to 90%. To demonstrate the range of applicability, we incorporated these custom made nucleotides into RNAs with sizes ranging from 27 to 59 nucleotides: A-Site (27 nt), the iron responsive elements (29 nt), a frame-shifting element from a human corona virus (59 nt), and fluoride riboswitch from Bacillus anthracis (48 nt), using standard in vitro techniques. Finally, we showcase the dramatic improvement in spectral quality arising from reduced crowding and narrowed linewidths, accurate and quantitative analysis of NMR relaxation dispersion (CPMG) and TROSY-based CEST experiments to measure μs-ms time scale motions, and an improved NOESY walk for resonance assignment.
T44. Structural Study of Noncoding RNAs via NMR Spectroscopy

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Non–protein coding RNAs can undergo conformational transitions in response to ligands to promote or repress downstream gene expression. Although X-ray crystallographic studies of such RNAs provide insight into ligand binding, NMR spectroscopy is the only high resolution technique capable of probing the highly dynamic unbound state. Here we present site-selective labeling strategies and NMR methodologies to study the structure and dynamics of ligand-free RNAs. The combination of our selective labels with customized NMR experiments alleviates problems of rapid relaxation and spectral crowding, thereby allowing us to investigate the structure and dynamics of relatively large RNAs.
T45. Ensemble Computational Approaches to Human Endogenous Retroviral RNA

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Human Endogenous Retroviral RNA (HERV) are key components in the HIV interactome. HERV RNA are part of immune response signaling pathways and interact with tat, gag, rev, and rex proteins. We propose that HERV RNA are RNA shapeshifters that change conformation with different protein binding partners. HERV RNA sequences were studied with Swellix a program that computes all possible non-pseudoknotted folds for an RNA rapidly and efficiently. The Swellix program combines a helix abstraction with a combinatorial approach to the RNA folding problem to thoroughly explore RNA conformational space. The Swellix program builds on the Crumple program and can include experimental constraints on global RNA structures such as the minimum number and lengths of helices from crystallography or cryoelectron microscopy. The conceptual advance in Swellix is to count helices and generate all possible combination of helices rather than counting and combining base pairs. Swellix bundles similar helices and includes improvements in memory use and efficient parallelization. The biological applications of Swellix are demonstrated by computing the reduction in conformational space and entropy for naturally modified nucleotides in tRNA sequences and motif searches in Human Endogenous Retroviral RNA sequences. Thus, Swellix provides a practical alternative to free energy minimization tools when multiple structures, kinetically determined structures, or complex RNA-RNA and RNA-protein interaction are present in an RNA folding problem.
Kaposi’s sarcoma herpesvirus (KSHV) is the etiological agent of Kaposi’s sarcoma (KS), Primary Effusion Lymphoma and the Multicentric Castleman’s Disease. In the HAART era KS remains the second most frequent tumor in HIV-infected patients worldwide, and has become the most common cancer in Sub-Saharan Africa. During lytic infection, KSHV express a highly abundant long noncoding transcript designated polyadenylated nuclear (PAN) RNA, a global regulator of viral and cellular gene expression. At its 3’ terminus, PAN exhibits a unique structure - the expression and nuclear retention element (ENE) - which assumes a triple helix configuration to sequester and shield the poly(A) tail from exonucleases. While this motif imparts distinctive structural properties on PAN, the biological significance of the ENE triple helix remains unclear. We have provided proof-of-principle that structured RNA motifs can be specifically targeted with small molecules in vivo, potentially bypassing problems associated with “macromolecular” therapeutics. Using a small molecule microarray (SMM) strategy and PAN ENE motifs as “bait”, we identified 26 compounds that specifically bind to the ENE triple helix. A luminescence based cytotoxicity test using the specialized cell line iSLK-219 reduced the number of PAN ENE binders for further biological testing. Of these, Compound 15 was found to activate KSHV from latency. A parallel effort in the lab has identified hydroxytropolones as highly potent herpesvirus inhibitors, opening the possibility of a “shock-and-kill” strategy to eliminate KSHV after virus reactivation. Additional viral RNAs that have been targeted using the small molecule microarray, including those carrying m^6A modifications, will be presented.
T47. Imine-Based Dynamic Combinatorial Chemistry for Discovery of Multivalent RNA-Binding Ligands

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Non-protein coding RNA transcripts have been increasingly recognized as potential drug targets owing to their important roles in cellular processes. Since peptide-based and RNA-based therapeutics often exhibit poor in vivo delivery and pharmacokinetics, small molecules offer an excellent alternative tool for targeting RNA. However, due to its unique chemical and structural properties compared to protein targets, RNA has been difficult to target with drug-like small molecules. For example, despite the proven promise of targeting RNA with multivalent ligands, progress in this area has been hampered by current limitations in three-dimensional structure characterization of large RNAs. There is thus a need to develop techniques that do not rely on structure-based design. We are developing an imine-based dynamic combinatorial chemistry (DCC) technique for multivalent ligand discovery for large RNAs. In DCC, a target biomolecule is incubated with a thermodynamically-controlled dynamic library of small molecules, allowing it to select its highest affinity binders. Contrary to structure-based design, in DCC all thermodynamically accessible conformations of the target participate in the ligand discovery process, which offers a more biologically-relevant discovery platform. To date, we have identified favorable conditions for imine formation in aqueous media, conducted comparative studies of amine reactivity towards imine formation, and begun validation studies on a known RNA binding scaffold. For validation studies, an aldehyde scaffold will be incubated with a diverse library of primary amines to discover ligands for the HIV-1 Transactivation Response (TAR) RNA. Upon validating the DCC method, we will set out to identify first multivalent ligands for a number of disease-relevant RNA targets. Ultimately, this work will provide a much-needed platform for multivalent ligand discovery for large RNAs, particularly those that have yet to be structurally characterized.
Impact of the HLA B*57 Allele on Intra-Host HIV-1 Capsid-Coding RNA Secondary Structure Diversity

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The HLA-B*5701 allele, which targets a particular region within p24, has been associated with control of viral replication (Norstrom \textit{et al.}, 2012). For this reason, viral protein evolution in patients with this specific allele has been studied in order to provide insights into modes of protection that are essential for the successful development of vaccine and/or treatment strategies. Until recently, little research has been undertaken to explore the selective pressures at the level of both the protein and the underlying RNA secondary structure, the combination of which likely results in a complex interplay between protein and RNA structural evolution required for productive infection. In particular, since HIV-1-infected subjects usually harbor at any given time a heterogeneous population of viral strains the existence of a single RNA is unlikely. Based on this principle, we hypothesized that selective pressures at the protein level may affect the preservation of the HIV-1 RNA structural landscape within an infected host. Using the existing SHAPE structure (Watts \textit{et al.}, 2009) as well as a variety of phylodynamic and RNA structural prediction methods, we investigated how HIV-1 diversity at the nucleotide and amino acid levels are associated with RNA structural diversity over the course of infection and the importance of the HLA-B*57 allele on RNA phenotypic (structural) evolution. Viral population dynamics were inferred utilizing extensive longitudinal sequence analysis with the Bayesian statistical framework for six HIV-1-infected patients with and six without the B*57 allele. Both RNA-Decoder and Mfold programs were then used to determine intra-host RNA structural diversity over time. Although no clear correlation was observed between changes in viral effective population size over time, a reflection of viral diversity, and temporal patterns in RNA secondary structure, patterns distinguishing patient cohorts were clearly evident. Specifically, subjects carrying the HLA-B*57 allele harbored significantly less stable structured RNA over the entire sampling time course (approximately 7 years post-infection). This allele-specific pattern may play an important role in the reduced viral replication observed within these patients and potentially prove useful as a therapeutic target.
We used DMS-MaPseq (dimethyl sulfate mutational profiling with sequencing), a technique we developed previously, to chemically probe RNA secondary structure at single molecule and single nucleotide resolution of the HIV-1 5' UTR in infected primary T cells. Recently, we developed and validated an algorithm based on Expectation-Maximization (EM) clustering, which uses DMS-MaPseq data to detect the presence of alternative RNA structures forming from the same underlying sequence. This EM-Clustering algorithm can detect as many as five different structures, even when an alternative structure is present in only 3% of the population of RNA molecules. Application of EM-Clustering to in vivo and in vitro DMS-MaPseq data revealed differences of the structures formed by the HIV-1 5' UTR. We identified as many as 4 different stable conformations in infected cells formed by genomic 5' UTR. Surprisingly, we discovered that tRNA(Lys3) only binds the primer-binding site (PBS) of HIV-1 dimeric RNA, while the PBS of HIV-1 genomic monomers or HIV-1 transcripts is partially open and unbound by tRNA. These differences in the structure of the PBS in dimers and monomers suggest that a dimerization-induced opening of the PBS allows for tRNA binding in cells.
T50. Characterization of HIV-1 Matrix Interactions with tRNA^{Lys3}

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The matrix (MA) domain of the HIV-1 Gag polyprotein is responsible for targeting the Gag-genome complex to the plasma membrane for virion assembly. This targeting is mediated through specific interactions between the highly basic patch (HBR) on MA and phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2] on the plasma membrane. The myristoyl group on MA adopts an exposed conformation to anchor Gag to the membrane after initial HBR interactions. Mutations of basic residues in the HBR can lead to retargeting of Gag from the plasma membrane to multivesicular bodies (MVBs) and a decrease in MA's specificity for PI(4,5)P2-containing membranes. In addition to PI(4,5)P2, MA specifically binds to some cellular tRNAs, including the tRNA^{Lys3} which is packaged into virions and serves as the primer for reverse transcription. Isothermal titration calorimetry (ITC) was used to investigate MA and tRNA^{Lys3} interactions, and to determine the role basic patch residues play in MA's ability to bind tRNA^{Lys3}. Mutations that cause retargeting of Gag to MVBs also resulted in decreased affinity of MA for tRNA^{Lys3}, suggesting the residues may play a role in both processes. ITC experiments with myristoyl exposure-deficient mutants indicated a potential role for the myristoyl group in regulating tRNA binding, as increased myristoyl exposure weakened tRNA-MA interactions.
In infected cells, host RNA is present in vast excess to viral RNA, yet retroviruses specifically package full-length, dimeric genomic RNA (gRNA). The Psi element within the 5′-untranslated region (5′UTR) is critical for gRNA packaging through interaction with the nucleocapsid domain of Gag. However, under physiological conditions, Gag binding affinity for Psi versus non-Psi RNAs is not significantly different. To investigate the mechanism of this selectivity in HIV-1, salt-titration binding assays were previously performed (Webb, J.A. et al., RNA 2013), allowing measurement of the strength of the non-electrostatic (i.e. specific) component of binding and the number of charges that mediate the protein-RNA interaction. Compared to non-Psi RNAs tested, HIV-1 Gag bound to Psi RNA with greater specificity and lower charge, whereas binding to non-Psi RNA was less specific and involved more electrostatic interactions. Surprisingly, a Gag mutant lacking MA was less effective at discriminating Psi from non-Psi RNA. We investigated the elements of Psi that contribute to specific HIV-1 Gag interaction and conclude that two G-rich bulges in SL1 and single-stranded G residues that flank SL3 are the most critical (Rye-McCurdy, T. et al., Viruses 2016). Since no single-site mutant reduces Gag binding specificity to the level of non-Psi RNA, we propose that the clustering of several Gag interaction sites in close proximity is an important determinant of specific binding and gRNA packaging. We also investigated the contribution of RNA domains upstream and downstream of Psi to specific recognition by Gag. We find that sequences upstream of Psi reduce specific Gag-RNA binding unless ~50 nt downstream of Psi are also present. However, the presence of Psi plus the downstream sequence does not enhance Gag specificity over levels associated with Psi alone, suggesting that this element acts to block an inhibitory effect of the upstream RNA domains, consistent with a previous proposal (El-Wahab, A. et al., Nature Communications 2014). Finally, we employed native-mass spectrometry to directly probe the stoichiometry of Gag binding to different RNA constructs. We find that monomeric Psi interacts with 3 Gag molecules, while a similarly sized non-Psi RNA does not support protein binding under the same conditions. We propose a model wherein the cluster of 3 high-sensitivity Gag interaction sites in Psi leads to the binding of 3 Gag molecules (6 in a Psi dimer), which nucleates immature particle assembly and facilitates specific packaging of gRNA.
T52. HIV-1 gRNA Packaging is Initiated in a Unique Host RNA Granule that Assembling Gag Enters via a Two-Step Mechanism

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In cells, HIV-1 gRNA would be expected to behave like cellular mRNA – i.e. gRNA should be found either in translating complexes (monosomes and polysomes) or in non-translating RNA granules, which are diverse host ribonucleoprotein complexes involved in mRNA storage, degradation, silencing and transport. Recent structural studies indicate that it is the pool of non-translating HIV-1 gRNA that undergoes packaging. Here we report that in HIV-1-provirus expressing cells, non-translating gRNA is largely in ribonucleoprotein complexes of ~30 - ~80S, and is almost absent from the soluble fraction and small complexes. Importantly, upon expression, assembling Gag enters a class of ~80S gRNA-containing granules and forms an ~80S complex in which Gag first associates with gRNA, termed the packaging initiation complex. WT Gag forms this gRNA-containing packaging initiation complex, as well as a larger gRNA-containing ~500S complex that corresponds to a late packaging/assembly intermediate. These packaging intermediates contain DDX6, a cellular RNA granule marker and RNA helicase, as well as ABCE1, a cellular ATPase, both of which were previously shown to facilitate immature HIV-1 capsid assembly. Thus, our biochemical studies support a model in which Gag targets to a unique host RNA granule to initiate packaging; this granule contains both the gRNA needed for packaging and host proteins that facilitate Gag assembly. WT Gag remains associated with this granule until completion of packaging and assembly at the plasma membrane. Notably, we confirmed this model using two in situ approaches. Using the proximity ligation assay, we showed that assembling Gag co-localizes with ABCE1 and DDX6. Using quantitative immunoelectron microscopy, we demonstrated that DDX6 co-localizes with gRNA at plasma membrane sites of assembly.

Our studies also identified the mechanism by which Gag finds and enters gRNA-containing granules. We found that ABCE1-binding domains within Gag allow Gag to associate with a broad class of ABCE1-containing RNA granules, most of which lack gRNA. Moreover, a Gag construct that contains the ABCE1-binding domains but lacks a gRNA-binding domain successfully completes assembly in the subset of granules that does not contain gRNA, producing virions that also lack gRNA. In contrast, Gag constructs that contain both the ABCE1-binding domains and a gRNA-binding domain (either the native nucleocapsid domain or a heterologous gRNA-binding domain) are directed to the subset of ABCE1-containing ~80S RNA granules that also contain gRNA, where they complete assembly and package gRNA. Based on these findings, we conclude that Gag must co-opt gRNA-containing RNA granules to initiate gRNA packaging, and that Gag has evolved a two-step mechanism for entering the RNA granules that contain gRNA. In step one, Gag binds to ABCE1, which serves as an adaptor that takes Gag to a broad class of ~80S granules containing diverse RNAs; step two requires a gRNA-binding domain in Gag, i.e. the NC domain or a heterologous substitute, which takes Gag to the subclass of RNA granules that contains gRNA.

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Assembly of infectious HIV-1 virions requires selective packaging of the genomic RNA (gRNA). In retroviruses, this process involves a cis-acting RNA element called the packaging signal or “Ψ”. This signal allows the gRNA to be selectively packaged in the presence of a large excess of cellular and spliced viral RNAs. The HIV-1 Ψ is located in the 5′-UTR and part of the Gag ORF in the gRNA.

Although Ψ was identified almost three decades ago, the molecular mechanism that drives the specificity of RNA packaging is not fully understood. In vitro, the structural protein Gag binds to a wide range of nucleic acids with nanomolar affinity. In fact, the difference in binding affinities between Ψ and non-Ψ RNAs (or even ssDNA oligos) is small and seems insufficient to explain packaging selectivity. However, Webb and co-workers showed, by doing salt titrations of Gag/RNA complexes, that binding to Ψ has a specific, non-electrostatic component that is far stronger than that for a control RNA (Webb et al., RNA 2013). Furthermore, recent data from our laboratory demonstrates that when the strength of Gag-Gag or Gag-RNA interactions is reduced by mutations in Gag, or when electrostatic Gag-RNA interactions are impaired by raising the salt concentration, Gag exhibits a very high binding specificity for Ψ. In other words, lowering these interactions greatly decreases the non-specific component of binding.

Originally, stem loop 3 (SL3) was suggested to be the main element required for selective packaging. However, recent evidence implies that SL1 plays a significant role in high-affinity binding (Abd El-Wahab et al., Nat. Comm. 2014). Nevertheless, most deletions and/or mutations in these stem loops are not enough to significantly decrease in vitro binding to Gag, even in supraphysiological salt concentrations. It is possible these two stem loops do not contain all the sites required for specific binding; alternatively, the specific interactions may be obscured by additional, non-specific RNA-binding activity.

To solve these problems, we have used fluorescence correlation spectroscopy and fluorescence quenching to characterize the in vitro interactions between recombinant Gag and Cy5-labeled RNAs. By measuring Cy5 quenching we have determined the binding affinities of Gag to wild-type as well as to several mutant Ψ. Increasing the salt concentration or using Gag mutants with either weaker Gag-Gag or Gag-RNA interactions has revealed the regions within Ψ that are required for specific binding. Furthermore, by monitoring the diffusion coefficient of Cy5-labeled WT Ψ we have carried out head-to-head competition experiments against different mutant RNAs. Our data show that selective Gag/Ψ interactions do not depend on SL1 or SL3 but on at least seven short stretches, distributed across the 5′-UTR, each containing one or more unpaired Guanosines. These results are in agreement with chemical probing data that shows that in the virion these unpaired Guanosines strongly interact with the NC protein (Wilkinson et al., PLoS Biol, 2008). Although these seven clusters of unpaired Guanosines make a stronger contribution to specific binding than either SL1 or SL3, our data suggests that there are other important interactions that are yet to be identified. Furthermore, we have designed an in vivo packaging approach to validate the in vitro binding data and to test the relationship between binding specificity and packaging specificity.
DDX3X is a human DEAD-box RNA helicase implicated in HIV-1 progression. In addition to the RecA-like catalytic core, DDX3X contains N- and C-terminal domains. We investigate the substrate and protein requirements to support the ATPase activity of a DDX3X construct lacking 80 residues from its C-terminal domain. Our data demonstrate that for an RNA molecule to support the ATPase activity of DDX3X it must contain a single-stranded–double-stranded region. We investigated protein and RNA structural reasons for this requirement. First, the RNA substrates consisting only of a double-helix were unable to support DDX3X binding. A single-stranded RNA substrate supported DDX3X binding, while an RNA substrate consisting of a single-stranded–double-stranded region not only supported the binding of DDX3X to RNA, but also promoted DDX3X trimer formation. Thus, the single-stranded–double-stranded RNA region is needed for DDX3X trimer formation, and trimer formation is required for ATPase activity. Interestingly, the dependence of ATP hydrolysis on the protein concentration suggests that the DDX3X trimer hydrolyzes only two molecules of ATP. Lastly, a DNA substrate that contains single-stranded–double-stranded regions does not support the ATPase activity of DDX3X.
Viral proteins must maintain their function in the face of high viral polymerase error rates and competing selective pressure from overlapped reading frames and overlapped RNA structures. The combination of these forces shapes the manner in which overall protein structure and functional domains evolve. Here we investigate the effect of these forces on the HIV-1 Rev protein. We combine analyses of comprehensive mutagenesis of the HIV-1 regulatory protein Rev with structural information to guide the design and testing of Rev mutants that elucidate the protein’s high mutational tolerance. We use truncation, insertion, deletion and mass substitution mutations of these linker regions to reveal previously unknown structural constraints not apparent from systematic point mutational approaches. Together our data provide a detailed examination of the mutational limits of this genetically robust protein, and reveal previously unknown structural and functional constraints. Our approach demonstrates the power in combining analysis of systematic point mutational scanning datasets with structural and functional knowledge in a manner that reveals the true mutational limits of a highly plastic viral protein.
T56. Structure of HIV-1 Rev Protein Recognizing the Host Co-Factor Tat-SF1

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Complex retroviruses such as HIV-1 co-opt the human spliceosome machinery for tightly coordinated production of their spliced mRNAs and genomic RNAs during the early and late stages of the viral life cycle. Tat-SF1 is a host protein that is critical in the production of fully spliced HIV-1 mRNAs in the early stage of the retroviral life cycle. Tat-SF1 is known to associate with the U2 small nuclear ribonucleoprotein subunit (snRNP) of the spliceosome and regulate the splicing of specific human transcripts. At present, the molecular mechanisms responsible for Tat-SF1 activities in human versus HIV-1 RNA splicing remain unknown.

Here, we establish that Tat-SF1 recognizes the HIV-1 Rev protein using a “U2AF Homology Motif” (UHM) domain to bind a “U2AF Ligand Motif” (ULM) of Rev. First, we show that Rev co-immunoprecipitates with Tat-SF1 in transient transfections of a human cell line. To elucidate the molecular interactions, we determined the crystal structure of a Tat-SF1 UHM complex with the HIV-1 Rev ULM at 2.8 Å resolution. Three of four UHMs in the crystallographic asymmetric unit bind a Rev ULM. The binding site of the fourth is partially occluded by crystal packing, and a fifth molecule is disordered. In the typical manner of UHM–ULM family members, basic residues and a tryptophan of the Rev ULM bind acidic residues and a hydrophobic pocket of the Tat-SF1 UHM.

We next determined the affinity of a Rev ULM peptide for Tat-SF1 by isothermal titration calorimetry (ITC). The major interacting UHM residues of Tat-SF1 are required for detectable association with Rev in comparative ITC experiments of structure-guided mutant proteins. We observe that Tat-SF1 binds the HIV-1 Rev ULM with slightly higher affinity than a ULM from the host splicing factor SF3b1, which we have shown associates with Tat-SF1 by co-immunoprecipitation experiments, ITC, and crystal structure determination.

Given the established importance of Tat-SF1 for HIV-1 infectivity, we are in the process of testing whether HIV-1 Rev disrupts host Tat-SF1–SF3b1 splicing factor complexes to produce unspliced viral RNA in the late stages of HIV-1 replication. Altogether, these results suggest a new mechanism of action for HIV-1 to exploit the human splicing machinery.

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T57. Uncovering Conformational Excited States in HIV-1 Rev Response Element RNA as New Small Molecule Targets

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Antiretroviral combination therapies suppressing HIV-1 replication have transformed the fatal illness of AIDS into a long-term controllable disease. However, emergence of drug-resistant HIV-1 strains has raised the risks of transmission of the drug-resistant strains among individuals, and development of drugs targeting new replication steps is needed. Rev response element (RRE) is a highly conserved viral cis-acting RNA element which plays an essential role in promoting the nuclear export of unspliced or incompletely spliced intron-containing HIV-1 RNAs through cooperative Rev binding. Rev protein initially binds to a high affinity purine-rich site in RRE stem-loop IIB, and minor mutations in stem IIB have detrimental effects on viral RNA exportation and replication. In this study, we used NMR spectroscopy to characterize the various conformational states that are sampled by RRE stem-loop IIB (RREIIB). Relaxation dispersion NMR data reveal the existence of low abundance and short-lived conformational excited states that reshuffle mispairs which may play critical roles in Rev recognition. RREIIB mutants stabilizing these excited states were designed to investigate their effects in the context of the Rev-RRE interaction. In the future, we will validate the excited states as therapeutic targets through cell based experiments, and perform computational docking against ensembles of ground and excited state structures to identify new small molecules targeting inactive conformations of RRE to inhibit RRE-Rev interaction.
T58. Structural Characterization of the HIV-1 Rev Response Element

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The HIV-1 Rev response element (RRE) is a highly structured region of the HIV-1 genome associated with nuclear export of intron-retaining viral RNA. It acts as a scaffold on which several copies of the accessory protein Rev assemble, forming a complex that is recognized by host nuclear export machinery.

While recent studies utilizing a wide range of biophysical techniques have provided substantial insight into the structural determinants of this process, high resolution characterization of the RRE has been limited to short synthetic RNA sequences designed to mimic the initial high affinity Rev binding site. We are using nuclear magnetic resonance (NMR) spectroscopy to extend this characterization to a fully native, 232 nucleotide RRE sequence.

The application of NMR spectroscopy to the study of large RNAs such as the RRE is complicated by a number of factors, including limited chemical shift dispersion, undesirable relaxation parameters and a relative lack of long-range dipolar contacts. We have developed a range of approaches designed to mitigate these difficulties, primarily based on the use of RNAs prepared with nucleotide- and atom-specific ²H substitutions, in which structural elements are identified from various combinations of ²H-edited ¹H-¹H NOESY spectra and confirmed by comparison with appropriately designed oligonucleotide fragments. We will illustrate these approaches with reference to our current progress towards 3D structure determination of the RRE.
T59. CLIP-seq Reveals a Key Role for hnRNP H1 in Regulation of HIV-1 Alternative mRNA Splicing

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Alternative splicing of HIV-1 mRNAs regulates transcript abundance, increases the coding potential of the compact viral genome and allows temporal regulation of viral gene expression. This process is in part regulated by the host heterogeneous nuclear ribonucleoproteins (hnRNPs) that bind to poorly defined sequences on HIV-1 RNAs and repress splicing at proximal splice sites. In this study, we employed crosslinking immunoprecipitation coupled with next-generation sequencing (CLIP-seq) to identify the direct binding sites of several hnRNP proteins on viral RNAs in cells. We show that hnRNP A1, hnRNP A2 and hnRNP B1 binding is widespread across viral RNAs and required the presence of ‘AGG’ motifs for binding. In contrast, hnRNP H1 binding occurred at a small number of well-defined purine-rich sequences comprised of four or more consecutive guanosines that are often surrounded by A-rich elements. The sequence specificities of hnRNPs for HIV-1 RNA elements largely mirrored those for elements in cellular introns. In support of these findings, isolated RNA-binding domains of hnRNP H1 exhibited a clear preference for binding to G-rich sequences in vitro. Notably, modulation of hnRNP H1 levels by RNAi and mutations within the most prominent hnRNP H1 binding site on viral RNA decreased use of splice acceptor A1 as revealed by an unbiased next-generation sequencing-based assay. The decrease in A1 usage was accompanied by a defect in Vif expression in infected cells. Overall, these studies revealed an unexpected splice enhancer role for hnRNP H upon binding to a novel cis-regulatory element proximal to splice acceptor A1. Finally, the methods developed herein provide the highly needed experimental framework to study HIV-1 splicing in greater detail.
T60. Control of HIV-1 Splicing by Cis-Acting Sequence Elements

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Background: HIV-1 makes a single full-length transcript used for genomic RNA or as the gag and gag/pro/pol mRNA. 50+ additional HIV-1 mRNAs are made by splicing of this full-length transcript. Spliced transcripts are in one of two size classes, depending on inclusion of the env intron located between splice donor D4 and splice acceptor A7. Shorter (1.8 kb) fully spliced transcripts remove the env intron, are completely spliced, and exit the nucleus by the cellular mRNA export pathway. Longer (4 kb) partially spliced transcripts and full-length transcripts retain the env intron and require the viral protein Rev to access the Crm1 nuclear export pathway. Mechanisms of differential splicing suppression and activation must exist in order to produce these three transcript classes – unspliced, partially spliced, and fully spliced. Cis-acting sequences in the HIV-1 genome act as splicing regulatory elements (SRE), as either activators or repressors of splicing, and are critical to providing the needed balance of HIV-1 transcripts.

Methods: With our Primer ID-tagged deep sequencing assay we can quantify HIV-1 spliced transcripts within the two HIV-1 size classes. We also use a second deep sequencing assay, a Primer ID random reverse primer coupled with a fixed forward primer at the major splice donor. This assay can detect changes in the amounts of full-length, long spliced transcripts (containing the env intron), and short fully spliced transcripts.

Results: We examined the effects of silent mutations on some of the known HIV-1 cis-acting splicing regulatory elements. We found that silent mutations introduced within enhancer elements associated with splice acceptor A1(vif) modulated the frequency of A1 usage but did not dramatically alter the balance of the transcript classes. Silent mutations to splicing suppressor elements associated with acceptors A2(vpr) and A3(tat) affected the usage of these acceptors and also dramatically increased the proportion of spliced transcripts relative to unspliced transcripts, a phenomenon known as over-splicing. Silent mutations of elements that control the use of A7 altered the proportions of the two spliced size classes.

Conclusions: These data suggest that elements that enhance splicing increase the usage of specific acceptors, while elements that suppress splicing control both specific acceptor usage and contribute to an overall suppression of splicing required for the production of unspliced and partially spliced transcripts.
T61. Crystal Structure of the HIV1 Tat:AFF4:P-TEFb:TAR Complex at 4Å Resolution

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HIV-1 Tat recruits the human superelongation complex (SEC) to facilitate proviral transcription. Understanding this transcriptional regulation is critical to develop reagents that can induce viral latency reversal and potentially cure HIV/AIDS. Our previous integrative structure of the Tat:AFF4:P-TEFb:TAR complex provided a first glimpse of TAR interactions with the SEC. We have now determined a crystal structure of the same complex at 4Å resolution. Strong electron density for the TAR loop shows interactions with the Cyclin T TRM region, and the Tat Zn-coordinating loop with much more detail. These results are an important step towards the goal of a high resolution structure of the Tat:AFF4:P-TEFb:TAR complex.
The positive transcription elongation factor b (P-TEFb) is critical to gene transcription regulation in eukaryotes, functioning to selectively release RNA polymerase II (RNAPII) from a promoter-proximally paused state in more than half of metazoan genes. It is essential for transcription from the HIV-1 promoter in humans and thus plays a central role in HIV-1 reactivation from latency. P-TEFb can be recruited from an inactive complex by the bromodomain protein Brd4 to gene promoter regions. During HIV-1 infection, the HIV protein Tat competes with Brd4 in order to recruit P-TEFb to the HIV provirus to enhance its transcription. Despite the large number of genes regulated in this manner, the nature of the interaction between P-TEFb and Brd4 is not well understood. In order to elucidate these interactions and the mechanism of P-TEFb recruitment, we reconstituted a ternary complex containing the P-TEFb components CDK9 and Cyclin T1 bound to Brd4. In addition, we employed hydrogen-deuterium exchange coupled mass spectrometry (HDX-MS) to localize the C-terminal P-TEFb interacting domain (PID) of Brd4 and performed pulldown assays demonstrating that the scaffold protein AFF4 inhibits binding of P-TEFb to Brd4.
T63. Molecular mechanism of host factor HLTF recruitment to the CRL4-DCAF1 E3 ubiquitin ligase by HIV-1 Vpr protein

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The Vpr protein is an accessory virulence factor of HIV-1 that facilitates infection in immune cells. Cellular functions of Vpr are tied to its interaction with DCAF1, a substrate receptor component of the CRL4 E3 ubiquitin ligase. Recent proteomic approaches suggested that Vpr degrades HLTF DNA helicase in a proteasome-dependent manner by redirecting the CRL4-DCAF1 E3 ligase. However, the precise molecular mechanism of Vpr-dependent HLTF depletion is not known. Here, using in vitro reconstitution assays, we show that Vpr mediates polyubiquitination of HLTF, by directly loading it onto the C-terminal WD40 domain of DCAF1 in complex with the CRL4 E3 ubiquitin ligase. Mutational analyses suggest that Vpr interacts with DNA binding residues in the N-terminal HIRAN domain of HLTF in a manner similar to the recruitment of another target, Uracil DNA glycosylase (UNG2), to the CRL4-DCAF1 E3 by Vpr. Strikingly, Vpr also engages a second, adjacent region, which connects the HIRAN and ATPase/helicase domains, with a stronger affinity. Thus, our findings reveal that Vpr utilizes common as well as distinctive interfaces to recruit multiple post-replication DNA repair proteins to the CRL4-DCAF1 E3 ligase for ubiquitin-dependent proteasomal degradation.
T64. PJA2 Ubiquitinates the HIV-1 Tat Protein with Low Site Specificity to Activate Viral Transcription

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Transcription complexes that assemble at the HIV-1 promoter efficiently initiate transcription but generate paused RNA polymerase II downstream from the start site. The virally encoded Tat protein hijacks positive transcription elongation factor b (P-TEFb) to phosphorylate and activate this paused polymerase. In addition, Tat undergoes a series of reversible post-translational modifications that regulate distinct steps of the transcription cycle. To identify additional functionally important Tat cofactors, we performed RNAi knockdowns of sixteen previously identified Tat interactors and found that a novel E3 ligase, PJA2, ubiquitinates Tat in a non-degradative manner and specifically regulates the step of HIV transcription elongation. Interestingly, several different lysine residues in Tat can function as ubiquitin acceptor sites, and variable combinations of these lysines support both full transcriptional activity and viral replication. Further, the polyubiquitin chain conjugated to Tat by PJA2 can itself be assembled through variable ubiquitin lysine linkages. Importantly, proper ubiquitin chain assembly by PJA2 requires that Tat first binds its P-TEFb cofactor. These results highlight that both the Tat substrate and ubiquitin modification have plastic site usage, and this plasticity is likely another way in which the virus exploits the host molecular machinery to expand its limited genetic repertoire.
HIV-1 Infection of Primary CD4+ T-Cells Regulates the Expression of Specific HERV-K (HML-2) Elements

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Human endogenous retroviruses occupy extensive regions of the human genome. Although many if not all of these elements have likely lost their ability to replicate, some of them retain intact open reading frames with the capacity to generate viral proteins. Transcription of retroviral elements is generally tightly controlled by dedicated chromatin silencing systems. Nonetheless it has been reported that some pathologic states, such as viral infections and certain forms of cancer coincide with a heightened transcription of a number of retroelements. HIV-1 infection has been described to induce the transcription of the HML-2 group of HERV-Ks. The conserved nature of these >90 repetitive elements has rendered profiling their specific expression problematic.

We combined enrichment of in vitro HIV infected primary human CD4+ T-cells with single molecule real-time (SMRT) sequencing to generate unbiased, comprehensive HIV-1 specific HERV-K HML-2 expression profiles. First we show that contrary to some previous studies, when compared to uninfected cells, overall HERV-K expression is not upregulated in the HIV-1 infected cells of all donors. This underlines that HIV-1 is insufficient to cause a generalized induction of HERV-K transcription. We find though, that three HERV-Ks (HML-2 6q25.1 HML-2 8q24.3b and HML-2 19q13.42) are consistently up-regulated between 3-5 fold over uninfected controls. The envelope of HML-2 19q13.42, despite being truncated, retained the ability to produce a protein.

Taken together we developed a powerful approach to probe for unbiased profiling of HERV-K expression in primary human cells. We identify three HERV-Ks whose expression in primary human CD4+ T cells is regulated by productive HIV-1 infection. Future studies are needed to dissect the consequences of such HIV:HERV-K interplay.
Towards the Anti-Viral Response of FACT Complex (SUPT16H and SSRP1)

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Our functional genomic RNAi screens have identified the protein components of the FACT (facilitates chromatin transcription) complex, SUPT16H and SSRP1, as top host factors that negatively regulate HIV-1 replication. FACT interacts specifically with histones H2A/H2B to affect assembly and disassembly of nucleosomes as well as transcription elongation. We further investigated the suppressive role of FACT proteins in HIV-1 transcription. First, depletion of SUPT16H or SSRP1 protein enhances Tat-mediated HIV-1 LTR (long terminal repeat) promoter activity. Second, HIV-1 Tat interacts with SUPT16H but not SSRP1 protein. However, both SUPT16H and SSRP1 are recruited to LTR promoter. Third, presence of SUPT16H interferes with the association of Cyclin T1 (CCNT1), a subunit of P-TEFb, with the Tat-LTR axis. Removing inhibitory mechanisms to permit HIV-1 transcription is an initial and key regulatory step to reverse post-integrated latent HIV-1 proviruses for purging of reservoir cells. We therefore evaluated the role of FACT proteins in HIV-1 latency and reactivation. Depletion of SUPT16H or SSRP1 protein affects both HIV-1 transcriptional initiation and elongation, and spontaneously reverses latent HIV-1 in U1/HIV and J-LAT cells. Similar effects were observed with a primary CD4+ T cell model of HIV-1 latency. FACT proteins also interfere with HTLV-1 Tax-LTR mediated transcription and viral latency, indicating that they may act as general transcriptional suppressors for retroviruses. We conclude that FACT proteins, SUPT16H and SSRP1, play a key role in suppressing HIV-1 transcription and promoting viral latency, which may serve as promising gene targets for developing novel HIV-1 latency-reversing agents (LRAs).

Further study showed that SUPT16 is acetylated which sheds light on the antiviral response of host machinery. Future study will further determine how post-translational modifications of FACT complex involve in suppression of viral transcription.
The type of chromatin structure around the LTR promoter of integrated HIV provirus provides critical signals that regulate transcription during both productive and latent HIV infections. The C-promoter binding factor-1 (CBF-1), is a potent and specific inhibitor of the HIV-1 LTR promoter. Here we demonstrate that the knockdown of endogenous CBF-1 in latently infected primary CD4+ T cells, using specific small hairpin RNAs (shRNA), resulted in the reactivation of latent HIV proviruses. By performing Chromatin immunoprecipitation (ChIP) assays, using latently infected primary T cells and Jurkat T-cell lines, we demonstrated that CBF-1 induces the establishment and maintenance of HIV latency by recruiting Polycomb Group (PcG/PRC) corepressor complexes or Polycomb repressive complexes 1 and 2 (PRC1 and PRC2). Knockdown of CBF-1 resulted in the dissociation of PRCs corepressor complexes that enhanced the recruitment of RNA polymerase II (RNAP II) at HIV LTR. Knockdown of certain components of PRC1 and PRC2 leads to the reactivation of latent proviruses. Similarly, treatment of latently infected primary CD4+ T cells with the EZH2 inhibitor, 3-deazaneplanocin A (DZNep), led to their reactivation.

**Importance:** Instead of inhibiting individual enzymes, targeting factors such as CBF-1, which mediate the establishment of multiple repressive epigenetic changes, could be more beneficial in designing strategies to reactivate and subsequently eliminate latent HIV reservoirs.
Despite combination antiretroviral therapy (cART), acquired immunodeficiency syndrome (AIDS), which is predominantly caused by the human immunodeficiency virus type 1 (HIV-1), remains an incurable disease and a public burden. The major barrier to HIV-1 eradication and its cure lies in the virus’ ability to establish a latent infection in HIV/AIDS patients. The effort of HIV-1 cure has been focused on the development of latency reversing agents (LRAs) to complement cART in order to eliminate the latent reservoirs through the “shock and kill” strategy. However, the success of this strategy for a “sterilizing” cure still faces many challenges. Thus, other approaches are being explored as well. Recently, an alternative, namely the “block and lock” strategy, has been proposed to reinforce HIV latency and prevent its sporadic reactivation (“blip”) by using the latency-promoting agents (LPAs). In addition, the use of such compounds could eventually lead to a deep and irreversible latency of HIV-1 and result in a “functional” cure. Our recent studies of the facilitates chromatin transcription (FACT) complex in HIV-1 transcription and latency involve the test of curaxin 100 (CBL0100), a small-molecule that targets FACT, in HIV-1 replication and reactivation. The results showed that CBL0100 has an antiretroviral activity against acute HIV-1 infection in cells including the PBMCs isolated from ART-naïve, HIV-1 viremic patients. Furthermore, we demonstrated that CBL0100 is also able to efficiently block the reactivation of silenced HIV-1 proviruses in cells including the PBMCs isolated from the ART-treated, aviremic patients. The mechanistic studies elucidated that CBL0100 preferentially targets HIV-1 transcriptional elongation and decreases the occupancy of RNA Polymerase II (Pol II) and FACT at the 5’ HIV-LTR region. Overall, our studies identify CBL0100 as a new class of LPA that blocks HIV-1 replication and reactivation, which could potentially benefit the “block and lock” HIV-1 cure strategy and reduce or eliminate HIV-1 latent reservoirs.
Replication of HIV-1 is highly dependent on components of the host cell machinery. Employing a targeted RNAi screen, we have identified host cell factors that interfere with early stages of HIV-1 replication. One of these factors, BIRC2, limits HIV-1 transcription through an NF-κB-dependent mechanism. BIRC2 is a negative regulator of non-canonical NF-κB signaling, suggesting that this pathway activates HIV-1 transcription. Consistently, activation of non-canonical NF-κB signaling through BIRC2 depletion enhances HIV-1 replication. While previous studies have demonstrated that canonical NF-κB signaling is utilized by HIV-1 to increase viral replication, the role of the non-canonical NF-κB pathway has been more elusive. Taken together, our findings suggest that non-canonical NF-κB signaling increases HIV transcription. Interestingly, treatment of latently infected Jurkat (JLat) cells with small-molecule BIRC2 antagonists known as Smac mimetics leads to a reactivation of the virus in this latency model. Moreover, Smac mimetics act synergistically with HDAC inhibitors to reverse latency in JLat cell lines. We further confirmed the latency-reversing activity of these compounds in the more physiological context of resting CD4 T cells isolated from aviremic HIV-infected patients. We have found that Smac mimetic treatment, in combination with the HDAC inhibitor panobinostat, results in the synergistic activation of the latent reservoir in this ex vivo system. Importantly, we did not observe activation of resting CD4 T cells or the release of cytokines upon treatment with these compounds. Activating the non-canonical NF-κB pathway by targeting BIRC2 with Smac mimetics appears as a promising new therapeutic strategy to reactivate latent HIV-1 while limiting toxicity risks associated with systemic activation of canonical NF-κB signaling. We expect that the development of novel compounds in this class, and a careful evaluation of their potential to synergize with other LRAs, will increase the suitability of Smac mimetics as therapeutic agents for shock-and-kill strategies to eliminate the latent HIV reservoir.
F1. Targeting HIV-1 Integrase Multimerization for Inhibitor Design: Modeling Higher-Order Protein Aggregation Mediated by ALLINIs

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The allosteric HIV-1 Integrase Inhibitors (ALLINI) which bind at the dimer interface of the catalytic core domain (CCD) promote higher-order multimerization of Integrase and impairs the encapsidation of the viral ribonucleoprotein complex within the viral capsid during virus maturation. Through our previous modeling guided by HDX-MS experimental data, we have established that the aberrant multimerization is mediated directly by ALLINI binding which promotes intersubunit CCD-CTD interactions between two Integrase dimers.1

In the first part of this poster, we present new evidence from modeling which reveals that different ALLINIs can induce distinct higher-order organization of the IN subunits in the multimer. The modeling results are consistent with and provide a physical explanation for the latest biochemical data which reports the stoichiometry of inhibitor-Integrase association. We also show how our modeling may help explain the critical role of the NTD in promoting the Integrase multimerization.

The second part of the presentation is concerned with rationalizing the activities of ALLINIs by computation. We show that the calculated MM-GBSA energy of ligand binding at the CCD dimer interface provides an efficient and reasonably accurate estimator to quickly assess the activities of different classes of ALLINIs with $r^2 \approx 0.7 – 0.9$. However, there is a notable outlier: for certain compounds such as KF116 which carries an extended R group, the MM-GBSA energy significantly underestimated the potency. We discuss how this inconsistency may be removed when taking into account the presence of the CTD in the multimer intersubunit interface.

F2. Structural Basis for the ALLINI-induced Aggregation of HIV Integrase

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Integration is a required step in HIV replication. The integrase protein has been targeted by FDA-approved drugs that bind to the active site, but resistant viruses emerge, focusing attention on additional targets in integrase. A new class of inhibitors, the allosteric integrase inhibitors (ALLINIs), show a different mechanism of action. The major effects of the ALLINIs are observed during virion maturation, where ALLINI treatment results in IN aggregation and the formation of aberrant particles. We previously crystallized full-length HIV IN bound with an ALLINI and determined the structure of this complex at 4.4 Å resolution. We have extended these studies to include new crystallographic structures with additional representative ALLINIs and resistance mutations. The structures reveal the formation of an open polymer, with dimers of IN interacting in a head-to-tail manner. An interface between the catalytic core domain of one dimer with the C-terminal domain of an adjacent dimer forms around the ALLINI, which is deeply buried by IN surfaces. These surfaces are rich in residues that convey resistance to ALLINIs, as identified by serial viral passage experiments. Escape mutants were found to decrease drug-induced aggregation, and crystallographic studies of escape mutants support a model where ALLINIs disrupt maturation by inducing the formation of the polymer observed in the IN-ALLINI crystal structure. In summary, the new structures show that: 1) Fully functional IN proteins form the same open polymer as the previously studied inactive mutant IN; 2) multiple different ALLINIs can promote formation of the same final IN polymer; and 3) modified INs encoded by escape mutants form the same final open polymer, implying that amino acid substitutions act on the pathway leading to the final structure and not by altering the final structure itself.

To extend our understanding of the IN oligomerization, we performed biophysical analyses informed by these structures using synchrotron size-exclusion chromatography in-line with small angle X-ray scattering (SEC-SAXS). Evolving Factor Analysis-Singular Value Decomposition (EFA-SVD) analysis of this dataset allows for the isolation of monomers, dimers, and tetramers, suggesting a structure for the IN tetramer in the absence of DNA and how this oligomer is stimulated by LEDGF/p75 and the ALLINI class of molecules. Contrast Variation studies of IN assembled on a DNA strand-transfer mimic show that the tetramer assembled on DNA is different from that in the absence of DNA. Characterization of oligomeric intermediates, higher order IN-ALLINI complexes, and resulting escape mutants provides important data for optimizing ALLINI drug design and understanding mechanisms of resistance.
F3. Modeling HIV-1 Integrase Multimerization and Allosteric Inhibitor Binding

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HIV-1 Integrase is the retroviral enzyme that inserts reverse transcribed viral DNA into the host genome and is a validated antiviral target. Three FDA approved drugs that target the active site are in clinical use to treat AIDS patients, but resistant virus variants have been well documented. For Integrase to function it must form multimers and perform a coordinated insertion of two viral DNA ends into the host target DNA, in a reaction called concerted integration. A partial defective reaction occurs when one viral end is inserted (called single end integration), and this reaction likely reflects impaired Integrase multimerization.

To take an alternate approach to the discovery of allosteric inhibitors that target this required Integrase multimerization, we designed a FRET-based assay for dimer formation, and used to screen a 50,000 compound chemical diversity library. Compound hits were cross-validated by testing for those that inhibited integrase catalysis. Most of the 20 compounds that also inhibit catalysis fall into two distinct scaffold classes with intriguing differential effects on Integrase catalytic function. The first class I includes several compounds that are dual inhibitors of single end joining and concerted integration. The second class II scaffold is distinct and has the interesting property of weakly stimulating single end joining, while specifically inhibiting concerted integration. This indicates that class II compounds affect multimerization at a specific interface that is required for concerted activity.

We are now trying to understand the molecular details of the binding mechanism of class II inhibitors. We are modeling the HIV Integrase – DNA complex based on the recently published Cryo-EM structure (PDB code 5U1C, Passos et. al, 2017; PMID: 28059769), with the main challenge to build the missing structural components without changing the biological assembly of the active intasome. Our first goal was to model a full-length tetrameric intasome complex with all loops and linkers, especially as these regions may be important in compound binding. Using protein labeling with class II analogues coupled with proteolysis and mass-spectroscopy, we recently obtained information regarding lysine residues that are proximal to putative class II binding sites. A second goal is focused on using the model and biochemical labeling information in conjunction with various docking methodologies to identify and characterize binding sites at the molecular level. Binding site predictions will be iteratively confirmed with the help of ongoing biochemical and biological experiments, with the ultimate goal to support lead compound optimization.
F4. Specific Mutations that alter HIV-1 Capsid Stability Modulate the Activity and Composition of HIV-1 Preintegration Complexes

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HIV-1 capsid (CA) is a multifunctional viral protein and a clinically unexploited target for novel antiretroviral therapy. CA forms the conical viral capsid that surrounds the viral ribonucleoprotein complex and facilitates early post entry steps in infection. It is well established that the CA is involved in reverse transcription and nuclear entry. Recent studies provide indirect evidence of a potential role of CA in viral DNA integration. Infection studies of HIV-1 CA mutants show altered efficiency of viral DNA integration and a shift in the pattern of viral DNA integration sites in the host genome. Studies using imaging techniques indicate that HIV-1 CA co-stains with the viral replication complex and/or viral integrase (IN) in the cytoplasm and at/near the nuclear membrane of infected cells. In addition, a recent report show that CA can enter the host cell nucleus in association with HIV-1 preintegration complexes. Very recently, the CA-specific inhibitor PF74 has been shown to alter targeting of the viral DNA integration within the host genome. However, biochemical data supporting a direct and functional role of CA in integration are lacking. To probe a functional role of CA in integration, we are studying the activity and composition of HIV-1 preintegration complexes (PICs) isolated from the cytoplasm of acutely infected cells. We have previously shown that PICs formed by the Q63/67A mutant contain elevated levels of CA and are impaired for integration activity, suggesting that HIV-1 integration depends on PIC-associated CA. To further study the specific role of PIC-associated CA on integration, we measured integration activity of PICs of the hyperstable capsid mutant E45A and the revertant double mutant E45A/R132T. The E45A mutant is competent for reverse transcription and is sufficiently infectious to test the effects of CA stability on integration. Activity measurements of PICs of the E45A mutant showed significantly reduced integration activity relative to the wild type PICs. PIC-associated viral DNA levels indicated that the reduced integration activity of the mutant virus is not due to lower levels of reverse transcription. Since cores isolated from the E45A mutant virions retain higher levels of associated CA and uncoat more slowly than wild type cores in vitro, our data suggest that increasing capsid stability reduces the integration activity of PICs. We also measured PIC activity of the double mutant E45A/R132T that partially restores infectivity to E45A. E45A/R132T PICs exhibited significantly elevated levels of integration activity compared to the E45A PICs. Surprisingly, the activity of the double mutant PICs was significantly higher compared to the wild type PICs. Collectively, these data support a functional role of CA in integration and suggest that integration activity of PICs is sensitive to alterations in intrinsic capsid stability. To pinpoint a direct role of PIC-associated CA on the integration activity, we are probing the composition of the wild type and mutant PICs by fractionating through velocity sedimentation on a linear gradient of sucrose. Measurement of integration activity, viral DNA copies and CA levels in the fractions are currently underway and results from these experiments will be presented to test a functional and direct role of CA in viral DNA integration.
**F5. Allosteric HIV-1 Integrase Inhibitors Lead to Premature Degradation of the Viral RNA Genome and Integrase in Target Cells**

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Recent evidence indicates that inhibition of HIV-1 integrase (IN) binding to the viral RNA genome by allosteric integrase inhibitors (ALLINIs) or through Class II IN mutations yields aberrant particles, in which the viral ribonucleoprotein complexes (vRNPs) are eccentrically localized outside the capsid core. These particles are non-infectious and blocked at an early reverse transcription stage in target cells. However, the basis of this reverse transcription defect is unknown. Here we show that the viral RNA genome and IN from ALLINI-treated virions are prematurely degraded in the target cells, whereas reverse transcriptase remains active and stably associated with capsid cores. The aberrantly shaped capsid cores in ALLINI-treated particles can efficiently saturate and be degraded by a restricting TRIM5 protein, indicating that eccentric cores are still composed of capsid proteins arranged in a hexagonal lattice. Notably, fates of viral core components follow a similar pattern in cells infected with eccentric particles generated by mutations within IN that inhibit its binding to the viral RNA genome. We propose that IN-RNA interactions allow for packaging of both the viral RNA genome and IN within the protective capsid cores to ensure subsequent reverse transcription and productive infection in target cells. Conversely, disruption of these interactions by ALLINIs or mutations in IN leads to premature degradation of both the viral RNA genome and IN during early steps of infection.
HIV reverse transcriptase (RT) is a multifunctional enzyme with both DNA polymerase and ribonuclease H (RNH) activities and is essential for HIV replication. Although many inhibitors against the polymerase activity have been developed, there is no clinically approved drug that inhibits the RNH activity of HIV. We have characterized the binding mechanism of the RNH active-site inhibitors by using Nuclear Magnetic Resonance (NMR) spectroscopy and other biophysics methods. Active-site inhibitors exhibited stronger binding, compared to the previously published allosteric inhibitors. Among the active-site inhibitors, a newer inhibitor, with a 3-hydroxypyrimidine-2,4-dione (HPD) scaffold, exhibited more favored binding compared to the previous 2-hydroxyisoquinoline-1,3-dione (HID) inhibitors. Our data clearly indicate recent advancement of the RNH inhibitor design on the molecular-interaction basis. This project is supported by NIH GM105401 and AI100890.
During HIV-1 replication, viral reverse transcriptase (RT) uses both RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase H (RNH) functions to transform the single-stranded RNA viral genome into double-stranded DNA, which is necessary for downstream integration of the viral DNA into the host genome. All FDA-approved against RT only target the polymerase function of the enzyme. The RNH activity of RT is an attractive antiviral target, as it is the only enzymatic function of HIV-1 remaining to be exploited by approved therapies.

We are using a structure-based design approach to develop novel RNH inhibitors (RNHIs) with increased potency against HIV-1. We have synthesized several compounds based on the 2-hydroxyisoquinoline-1,3-dione (HID) and 3-hydroxypyrimidine-2,4-dione (HPD) scaffolds. Several initial HID and HPD analogues demonstrated mid-nanomolar (150-900 nM) potency in RT-associated RNH inhibition assays; however, none were able to inhibit HIV-1 in cell-based assays. Further design of HPD analogues produced compounds with low nanomolar (<50 nM) potency in RT-associated RNH assays and some lead compounds with low micromolar efficacy in cell-based assays. Cell-based mechanistic studies demonstrated that some lead compounds act by inhibiting RNH, some integrase, and some both activities. A crystal structure of an HID compound in complex with HIV-1 RT demonstrates, for the first time, multiple modes of binding at the RNH active site. Additional crystal structures of first- and second-generation HPD analogues in complex with HIV-1 RT provide insights into the molecular details of RNH inhibition. These structures demonstrate the flexibility of inhibitor binding at the RNH active site and provide insights useful for the further design of inhibitors with enhanced antiviral potency.
F8. HIV Capsid Pattern Sensing by the Human Antiviral Protein MxB

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Myxovirus resistance protein B (MxB) is an interferon-inducible restriction factor of HIV that blocks viral infection between reverse transcription and integration. MxB is known to localize with nuclear pores and interact with the capsid core, but its precise mechanism of restriction remains unknown. Previous studies have mapped the restriction activity of MxB to its N-terminus, specifically a triple arginine motif 11RRR13, and demonstrated that oligomerization is an important determinant of restriction. Evidence suggests that MxB does not recognize individual or hexameric CA, but instead recognizes interfaces present only in the assembled capsid lattice. However, the precise details of this interaction have yet to be described.

We demonstrate a direct and specific interaction between an N-terminal MxB peptide and assembled CA tubes using highly purified recombinant proteins. We used novel and established CA mutations (including P207S, G208R, and T210K) to study the CA requirements for interaction with an MxB N-terminal peptide. Our results show that previously characterized CA mutations that escape MxB restriction (G208R and T210K) disrupt MxB binding to CA, but do not change the general lattice architecture. Furthermore, we identified specific mutations in the interface between CA hexamers that disrupt MxB binding to varying degrees. Specifically, E213A dramatically reduces MxB binding. Separately, we have generated a soluble capsid assembly that mimics the interface between three capsid hexamers using engineered disulfides and a trimeric fusion partner. We show that this assembly binds an N-terminal MxB peptide. Together, our results suggest that MxB likely targets the interface between three capsid hexamers.

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SAM domain and HD domain containing protein 1 (SAMHD1) was identified as a myeloid specific host restriction factor against HIV-1. The dNTP triphosphohydrolase (dNTPase) activity of SAMHD1 limits dNTP levels particularly in macrophages and non-dividing cells by degrading dNTPs into deoxynucleosides and triphosphates, which in turn suppresses viral reverse transcription kinetically. SAMHD1 orthologs are found in various species including non-primates. Among them, *Felis catus* encodes highly conserved SAMHD1 gene, and Feline immunodeficiency virus (FIV) is the only non-primate lentivirus that causes AIDS-like syndrome. However, non-primate lentiviruses are considered ancestral lentiviruses compared to primate lentiviruses, and do not encode Vpx like protein found in some SIVs and HIV-2. Therefore, it remains unknown on how and whether the non-primate lentiviruses overcome SAMHD1-mediated restriction in their nondividing myeloid cell types. Here, we examined feline SAMHD1’s biochemical dNTPase activity, and its degradation by FIV. Our study indicates that feline SAMHD1 is indeed a dNTPase, however unlike HIV-2 and some SIVs, FIV do not proteosomally degrade its host SAMHD1 proteins. *Caenorhabditis elegans* (C. elegans) also encodes a SAMHD1 ortholog, ZK177.8, which reportedly induces developmental defects upon the gene knockdown. With recent reports on SAMHD1’s involvement in other diseases, such as Aicardi Goutieres Syndrome (AGS), it is highly desirable to evaluate if *C. elegans* can serve as a model to study SAMHD1-mediated AGS. Here we demonstrate that ZK177.8 is also an allosterically-regulated dNTPase. Its dNTPase activity was also only observed upon incubation with dGTP or GTP as seen in human SAMHD1. ZK177.8 recognized both 2’ OH and triphosphate of the dNTP substrates for the dNTP hydrolysis as it’s unable to degrade NTPs or various nucleotide chain terminators. Finally, we explore structural characterization of these SAMHD1 orthologs through X-ray crystallography. Here, we report our rational and approach on construct design, purification, and current progress on screening process for successful crystal growth.
F10. SAMHD1 Mediates Mx2 Anti-Viral Activity by Targeting HIV-1 Capsid

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Retroviruses, including HIV-1, have evolved to create a delicate viral core (capsid) by recruiting a series of host proteins that are critical for viral uncoating, reverse transcription, nuclear entry, and evasion of the host’s innate immune responses. Consequently, the retroviral core can be sensed by the host as pathogen-associated molecular patterns and subjected to inhibition by host restriction factors such as Fv1, TRIM5α and Mx2/MxB. Here, we identify SAMHD1 as a novel HIV-1 capsid-targeting restriction factor. SAMHD1 mediates this novel anti-viral function at a step post-reverse transcription, distinct from its role as a deoxynucleoside triphosphate triphosphohydrolase (dNTPase), in which it depletes the intracellular dNTP pool to limit HIV-1 cDNA synthesis during reverse transcription. Surprisingly, SAMHD1 engages Mx2/MxB to mediate this anti-viral function. HIV-1 CA mutants resistant to CypA/CPSF6-dependent MX2-mediated anti-viral activity were still sensitive to SAMHD1/MX2-mediated inhibition. Furthermore, interferon treatment enhanced SAMHD1 anti-viral activity by increasing Mx2 expression. Thus, SAMHD1 participates in intrinsic host restriction as well as the innate immune response against HIV-1, and strategies that can augment SAMHD1/Mx2 function may lead to novel therapeutics against HIV-1.
F11. Inhibition of HIV-1 Nuclear Entry by a Novel Antiviral, JT-4-173

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The use of highly active antiretroviral therapy (HAART) has been key to the medical management of HIV-1 infection. There are currently six classes of FDA approved drugs that are categorized by their molecular mechanism and mostly target viral proteins such as the reverse transcriptase, integrase, and protease. Even though HAART has made remarkable strides in managing viral loads throughout the years, there continues to be a need for novel classes of inhibitors directed at alternative targets.

Through a medicinal chemistry and structure-based design approach, we are developing inhibitors targeting virus functions of HIV-1. We identified a novel antiviral, JT-4-173, which displayed strong antiviral potency (EC₅₀ of 0.2 µM) in cell-based assays. To investigate the possible mechanism of action of JT-4-173, we used a combination of in vitro and in vivo studies to determine its mechanism of action. The compound did not exhibit significant inhibition of the DNA polymerization or RNase H activities of HIV reverse transcriptase, or the nuclease strand transfer activities of HIV integrase in vitro (>10 µM). Moreover, in vitro capsid protein (CA) stability assays using cross-linked CA hexamers, and assembly assays using CA monomer, did not establish any direct interaction of the compound with CA, indicating the possibility of another target. In addition, CA mutations that impart PF74 resistance (A105T) or integrase mutations (N155H, Y143C, G140S/Q148H, L74F/V75I/N155H) that affect resistance to INSTIs (Integrase Strand Transfer Inhibitors) did not affect HIV susceptibility to JT-4-173. Using real-time PCR and specific primers and probes, we were able to analyze post-entry events of HIV-1 replication and narrow down the steps of the virus life-cycle that are affected by the compound: We found that while JT-4-173 did not affect the early or late stages of reverse transcription, it had a significant effect on integrated proviral DNA, as judged by Alu PCR assay. Moreover, the compound suppressed formation of 2-LTR circles, suggesting that it blocked nuclear entry of the product of reverse transcription. Hence, these data suggest that JT-4-173 blocks viral replication by targeting a step after the completion of reverse transcription and prior to nuclear entry.
F12. HIV-1 Utilizes CypA to Protect Against Cytoplasmic CPSF6

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We previously identified CPSF6 as an HIV-1 capsid (CA) interacting factor that blocks HIV-1 nuclear entry when mislocalized to the cell cytoplasm. The N74D mutation in HIV-1 CA impairs CPSF6 interaction and protects the virus from infection blocks caused by elevated cytoplasmic levels of CPSF6. Notably, cyclophilin A (CypA) also interacts with HIV-1 CA during infection via the P90 residue. In some cells, this interaction clearly aids the early replication of HIV-1. By contrast, knockdown of CPSF6 in cells will modestly increase wild-type (WT) HIV-1 infection in the same cells. CPSF6 is predominantly localized to the nucleus, however reduced levels of the endogenous protein can be detected in the cytoplasm. CypA is present throughout the cell, but is abundant in the cytoplasm, particularly near the nucleus. Given the potential interaction of both CPSF6 and CypA with CA during early infection steps of HIV-1, we sought to understand whether they were functionally interconnected. Strikingly, we observed that G89V HIV-1 or P90A HIV-1, viruses that are deficient for CypA interactions, significantly increased infection in cells that were depleted of CPSF6. Similarly, in cells depleted for CypA by shRNA, WT HIV-1 infection can be restored to levels observed in control cells if CPSF6 is also depleted. The above findings implied that HIV-1 acquired CA binding to CypA to prevent premature interactions of CPSF6 in the cell cytoplasm. Supporting this conclusion, microscopic examination of infected cells reveal CPSF6 aggregating with HIV-1 CA in the cytoplasm of cells after CypA knockdown. In control cells, CA complexed with CypA but not CPSF6 is observed in the cell cytoplasm. The mechanism by which CypA impairs CPSF6 cytoplasmic targeting of HIV-1 will require further analysis. Notably, the compound of PF-3450074 (PF74), which targets the CPSF6 binding interface of CA, is less potent in the absence of CypA suggesting that CypA binding conformationally affects the proximal CPSF6 binding pocket. We propose that HIV-1 dependence on CypA is in part subject to cytoplasmic CPSF6 levels, and because the virus exploits CPSF6 in the nucleus, rather than lose the CPSF6 binding site, HIV-1 has co-opted CypA as a defense against cytoplasmic CPSF6.
F13. Soluble Host Factors Modulate the HIV-1 Nuclear Entry Pathway

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Here we identify Nup35 and POM121 to aid HIV-1 infection. HIV-1 reliance on Nup35 or POM121 is linked to the viral capsid protein (CA). Nup35 and POM121 are phenylalanine-glycine nucleoporins (FG-Nups). It was previously demonstrated that the N74 pocket of CA interacts with FG-motifs from CPSF6 and Nup153. Notably, HIV-1 interaction with cyclophilin A (CypA) regulates dependence on the FG-Nups. Disruption of the interaction between CA and CypA restores HIV-1 infectivity in Nup35, Nup153, or POM121 knockdown cells. Dependence on CypA is lost in cells depleted of CPSF6. We hypothesize that premature CPSF6 interaction with HIV-1 impairs CA interaction with FG-Nups at the nuclear pore complex (NPC). CypA use by HIV-1, however, prevents CPSF6 access to the N74 pocket in CA until the virus docks at the NPC. We propose that the HIV-1 core, comprised of multimeric CA in association with the viral nucleic acid and enzymatic proteins, directly functions as a nuclear transport receptor and exploits successive FG interactions to achieve transfer through the NPC.
CPSF6 interactions with capsid (CA) promote HIV-1 integration in gene-rich regions of chromatin. The N74D mutation in CA impairs CPSF6 binding and hinders HIV-1 access to gene-rich regions for integration. CPSF6 is present throughout the nucleus partially co-localizing with speckles, nuclear bodies present at 15-30 copies per cell that are enriched in transcription and splicing factors. Infection with wild-type (WT) HIV-1 increases CPSF6 localization to speckles whereas infection with N74D HIV-1 has no effect on CPSF6 distribution in the nucleus. Notably, hotspots for CPSF6-dependent HIV-1 integration associate with speckles, while CPSF6-independent HIV-1 integration hotspots co-localize with nuclear speckles much less. Based on these findings, we asked whether CPSF6 associated with HIV-1 after the virus trafficked to speckles, or whether CPSF6 facilitated HIV-1 trafficking to speckles. Super-resolution microscopy revealed CA-dependent accumulation of CPSF6 at the nuclear interface of nuclear pore complexes after HIV-1 infection. To track viral complexes, we employed Fluorescent in-situ Hybridization (FISH) to detect HIV-1 DNA. Viral DNA was detected in both the cytoplasm and nucleus of infected cells. Signal was lost in the presence of a reverse transcription inhibitor but unaffected by an integrase inhibitor. WT HIV-1 DNA accumulated in speckles with CPSF6 at a high multiplicity of infection (MOI), but N74D HIV-1 DNAs association with speckles was impaired, suggesting a role for CPSF6 in HIV-1 trafficking to speckles. Thus we next examined HIV-1 intranuclear localization after CPSF6 knockdown, challenging cells at different MOIs to understand whether HIV-1 association with speckles was a potentially misleading consequence of overwhelming trafficking pathways or in fact represented a bona fide waypoint for the virus in the nucleus. CPSF6 depletion impaired the trafficking of WT HIV-1 DNA to speckles at MOIs of 2, 8, and 30 to the same extent. WT HIV-1 DNA associated with speckles in CPSF6-knockdown cells at the same diminished level that N74D HIV-1 DNA associated with speckles in control or knockdown cells. Collectively, these data suggest that HIV-1 utilizes CPSF6 to target nuclear speckles, and the association with speckles facilitates access to gene-rich regions of chromatin for virus integration.
F15. HIV-1 Co-Localizes with CPSF6 and TNPO3 on Microtubules in the Perinuclear Region

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Nuclear trafficking of HIV-1 DNA involves multiple host factors and is required for productive infection. Microtubules, which serve as the highway for cellular macromolecule transport, have been shown to mediate trafficking of HIV-1 complexes. Previously, the host proteins CPSF6 and TNPO3 were identified to be involved in HIV-1 DNA nuclear import. While both host factors are predominantly localized in the nucleus, we detected fluorescently tagged CPSF6 and TNPO3 in the perinuclear region of the cytoplasm. We hypothesized that perinuclear CPSF6 binding to HIV-1 capsid is required for proper nuclear trafficking and that premature co-localization of CPSF6 with HIV-1 at the cell periphery would alter HIV-1 trafficking. We performed live-cell frustrated TIRF microscopy of HIV-1 particles containing fluorescently tagged integrase in cells expressing fluorescently labeled CPSF6, TNPO3, and microtubules. We observed microtubule-associated movement of WT HIV-1 integrase with CPSF6 and TNPO3 towards the nucleus. While N74D HIV-1 particles also utilized microtubules, association with CPSF6 was not observed. In cells expressing truncated CPSF6 that does not bind to TNPO3, and thus remains at the cell periphery, WT HIV-1 particles induced formation of higher order complexes of CPSF6. These did not form when capsid binding to CPSF6 was disrupted by virus mutations (CA N74D or A77V) or with a small molecule inhibitor (PF-74). In addition, premature binding of truncated CPSF6 to HIV-1 capsid induced more rapid capsid permeabilization and affected microtubule movement of virus particles. In conclusion, our data suggest that premature access of CPSF6 by WT HIV-1 capsid leads to alteration of cytoplasmic trafficking towards the nucleus, whereas the virus normally encounters CPSF6 and TNPO3 on microtubules in the perinuclear region and this complex may facilitate capsid dissociation and nuclear import of HIV-1 DNA.
F16. Loss of Capsid Protein at the Nuclear Membrane is a Pre-Requisite for Translocation of HIV-1 Pre-Integration Complexes into the Nucleus

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HIV-1 cores released into the cytoplasm as a result of virus-cell fusion undergo reverse transcription and shed a major portion of the capsid protein (CA) as they traffic to the nucleus. The extent, the sites, and the dynamics of CA loss (referred to as uncoating) are poorly understood. We have recently visualized single HIV-1 uncoating in living cells using a novel fluorescent CA marker, cyclophilin-A DsRed (CypA-DsRed). CypA-DsRed binds tightly to the viral core without considerably affecting infectivity and is lost concomitantly with CA. Imaging single cores co-labeled with integrase-GFP (IN-GFP) and CypA-DsRed revealed distinct uncoating phenotypes in the cytoplasm: an abrupt loss of CypA-DsRed from cores at early times post-infection vs. gradual release of the CA marker over several hours, which also occurred near the nuclear envelope (NE). To assess the relevance of these uncoating phenotypes to productive infection, we tracked single IN-GFP labeled cores that entered the nucleus and thus had reasonable probability of integrating in the host genome. We examined temporal and spatial characteristics of CA release from IN-GFP labeled cores entering the nucleus by 3-color live cell imaging of TZM-bl cells expressing a nuclear membrane marker, EBFP2-lamin. Nearly all IN-GFP complexes entering the nucleus (n=91) contained detectable amounts of CypA-DsRed upon arrival at the NE. These complexes docked at the NE for 36 min on average, during which time they lost CypA-DsRed. Importantly, the loss of this CA marker always preceded the nuclear entry of IN-GFP complexes. By contrast, double-labeled particles docked at the NE never entered the nucleus under our experimental conditions, suggesting that loss of CA at the nuclear pore is a necessary step for nuclear import. This notion is further supported by the observed block in nuclear entry for the hyper-stable E45A CA mutant that retained CypA-DsRed and accumulated in the perinuclear area. We also did not detect nuclear complexes for the unstable CA mutant, K203A, that abruptly uncoated shortly after virus fusion. The lack of K203A nuclear import was most likely due to the loss of IN-GFP signal in the cytoplasm within approximately 30 min after CypA-DsRed shedding. Of note, a fraction of nuclear complexes contained very small amounts of CypA-DsRed and stained for p24/CA, indicating that CA shedding at the nuclear pore does not always reach completion. Collectively, our results suggest that early uncoating in the cytoplasm may result in disintegration of the HIV-1 core, whereas partial or full loss of CA at the NE is a prerequisite for nuclear entry of pre-integration complexes. This work was supported by the NIH R01 GM054787 and AI129862 grants to GBM.
F17. Live Cell Imaging of HIV-1 Reverse Transcription

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Following HIV-1 fusion with the cell membrane the conical capsid containing the viral RNA genome is delivered to the cytoplasm. There is general agreement that the process of reverse transcription facilitates the disruption of the conical core through a process known as uncoating. Ultimately, a double stranded provirus is generated through the completion of reverse transcription and the provirus must reach host nuclear DNA where it will integrate to complete the early life cycle of HIV. The site of the completion of reverse transcription is a contentious issue. Some models suggest that reverse transcription is initiated and completed in the cytoplasm while others suggest that reverse transcription is (or can be) completed in the nuclear pore or nucleus. To gain insights into these processes by live cell imaging during infection we have developed a method to label viral dsDNA.

The binding of tetR to dsDNA tetO sequences has been used extensively in inducible expression systems. We devised a dsDNA detection system where we have inserted an array of tet operons (tetO) into an HIV-1 viral vector that expresses a GFP reporter gene (HIVtetO-GFP). We also designed a panel of cell lines stably expressing tetR-tdTomato fusion proteins. The binding of tetR-tdTomato to the dsDNA tetO repeats recruits the tdTomato fluorescent protein to tag the DNA provirus. This DNA tethering allows the monitoring of formation and trafficking to the viral DNA genome by fluorescence microscopy of fixed or living cells. Here we used VSV-G pseudotyped particles carrying the tetO arrays that are also labeled with IN-GFP. This configuration makes it possible to follow nascent reverse transcribed viral DNA, from the moment when the double-stranded DNA encoding the tetO arrays becomes accessible to the expressed tetR-tdTomato, until integration. The specificity of the tetR-tdTomato interaction can be evaluated through the addition of tetracycline that disrupts tetR-tetO binding.

Initial validation studies revealed that cells stably transduced with HIVtetO-GFP contained small puncta of nuclear tdTomato that was sensitive to the presence of tetracycline. Live cell microscopy detects the recruitment of the tetR-tdTomato to the IN-GFP labeled viral complexes in the cytoplasm approximately 2 hours post viral challenge. The recruitment of the tetR-tdTomato in the cytoplasm reveals that the viral dsDNA becomes exposed to cytoplasmic factors relatively early, consistent with the early uncoating model. This newly developed live-cell imaging reverse transcription system will allow answering many key questions of the HIV-1 early-steps of infection, such as the exact location of reverse transcription and the interaction of viral DNA with the innate sensing machinery.
F18. Structural Investigation of the HIV-1 Reverse Transcriptase Initiation Complex by HDX, SAXS, and Cryo-EM

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Over the past 30 years, a concerted effort has been made to understand the full enzymatic cycle of HIV-1 reverse transcriptase (RT) through structural biology, biochemistry, molecular biology, and virology techniques. RT plays an essential role in HIV replication, and is the target of nearly half of the drugs used to treat HIV infection. A critical stage of reverse transcription that has not been visualized is the initial complex, in which a portion of the viral genomic RNA binds to provide the template (primer-binding site) and reverse transcription is primed by tRNA_{Lys}^3 from the host cell. This reverse transcription initiation complex is relatively challenging to produce in sufficient quantity and quality for high-resolution structure determination.

We are using a range of hybrid methods in a multi-laboratory collaboration to determine the most stable variants of this complex to use for high-resolution structural biology techniques such as cryo-electron microscopy (cryo-EM). Among the key factors is choosing truncations of viral RNA and tRNA that combine to form more stable in vitro complexes. A multi-stage workflow has been used to screen multiple truncations by hybrid techniques including size-exclusion chromatography coupled with small angle X-ray scattering and hydrogen-deuterium exchange coupled with mass spectrometry. These techniques allow us to map the interactions between RT and the vRNA/tRNA complexes and select non-aggregated samples that are enriched in the complete complex. This has informed sample preparation for cryo-EM, and allowed us to generate initial cryo-EM structures for the reverse transcription initiation complex. This poster will include our multi-technique workflow and the resulting structures of the reverse transcription initiation complex.
The development of a large number of highly active antiretroviral drugs has enabled the use of combination antiretroviral therapy to treat HIV-1, and has been a major success in the fight against HIV therapy, providing years of healthy life to infected patients. Current treatment regimens completely suppress viral replication; however, the existence of long-lived viral reservoirs necessitates life-long therapy to maintain viral suppression. Consequently, in the absence of a cure, a major interest in HIV-1 therapy is the development of easy-to-follow regimens to enhance patient compliance. Such regimens currently exist as single dose pills containing multiple antiretrovirals for once-daily administration. Current goals include the development of drugs that are highly stable and long acting, such that they may be given at longer intervals, such as once per month. 4'-ethynyl-2-fluoro-2'-deoxyadenosine (EFdA) is an adenosine-mimetic nucleotide reverse transcriptase translocation inhibitor that is highly stable and thus suitable for use in a long acting regimen. In ongoing clinical trials, Merck has shown that EFdA is targeted for use as a single 10-mg dose per >10 days. EFdA is a highly potent inhibitor of HIV-1 reverse transcriptase (RT), acting as a delayed- or immediate-chain terminating agent by diminishing translocation of the enzyme on the nucleic acid substrate. EFdA possesses activity against known NRTI-resistant mutants. Notably, the K65R RT mutation that confers resistance to tenofovir disoproxil fumarate (TDF) increases sensitivity to EFdA. However, these studies have been conducted with HIV-1 from subtype B, which accounts for only 12% of global infections. Hence, we sought to evaluate the activity of EFdA against a range of HIV-1 primary isolates from subtypes A, B, C and D, and circulating recombinant forms 01_AE and 02_AG. The EC_{50} values for TDF and EFdA were determined in TZM-GFP cells for each isolate. Additional studies focused on the susceptibility of purified RTs from various HIV-1 subtypes to EFdA-triphosphate. Our data demonstrate that EFdA is a highly potent inhibitor of HIV-1 from multiple subtypes.
F20. Energetics of Nucleotide Translocation Through HIV-1 CA Hexamer

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The capsid is a proteinaceous container of the virus genome and other viral proteins. During the early phase of HIV-1 replication cycle, the viral DNA is reverse-transcribed with the aid of the capsid. However, little is known about how the DNA nucleotides translocate into the capsid or whether the capsid acts as a gating agent. Recently, it was proposed that the pore in the N-terminal domain of capsid protein could be a nucleotide channel because of its flexibility and electropositive charges. To verify this hypothesis, we performed non-equilibrium simulations of the translocation of dNTP, ATP and ADP; results from the simulations unveil the variations of mechanical forces along the pore coordinate. In addition, equilibrium simulations permitted us to construct potential-of-mean-force (PMF) analyses thus revealing the energetics required for the translocation of different types of nucleotides.
APOBEC3G (A3G) belongs to the APOBEC3 family of DNA-editing cytidine deaminases that are responsible for deamination of HIV-1 cDNA during reverse transcription in the absence of viral infectivity factor (Vif). However, there are evidences that deaminase-independent mechanisms are also involved in antiviral activity of A3G. There is a “road block” mechanism by which A3G physically blocks the elongation of reverse transcribed proviral ssDNA. Interaction of A3G with RNA is another rather unexplored area of A3G properties. It was also shown that A3G binds to diverse cellular RNA and binding RNA to the CTD domain of A3G inhibits A3G catalytic activity. Here we focus on a fundamental problem of how A3G protein, a potent innate anti viral agent interacts with RNA. To accomplish our goals, we, for the first time, directly visualized the A3G-RNA complexes with AFM. A remarkable difference in assemblies of A3G on ssDNA and RNA substrates was discovered. Together with similar A3G-ssDNA complexes, when one RNA molecule bound to A3G, we also observed two RNA molecules bound to A3G. Complexes containing two molecules of ssDNA bound to A3G have not been observed. By analyzing the data for each A3G domain independently, we show RNA forms complexes with both NTD domain and CTD domain, while ssDNA is not able to form such complexes. Together, these results indicate the existence of an additional, different from ssDNA region on A3G that is capable of binding RNA. The dynamic behavior of the A3G-RNA complexes suggests that the two binding regions of A3G have different affinities to RNA. Based on these results a model for RNA binding to A3G is discussed. Overall, our study provides a fundamental step toward the mechanistic understanding of how A3G interacts with the RNA target and the role of domains of A3G in RNA binding.
APOBEC3G (A3G) is a potent restriction factor, which in the absence of HIV-1 Vif, catalyzes the lethal hypermutation of the HIV genome. Vif counters A3G by hijacking cellular components CBFβ and Cullin-5 RING E3 ligase (CRL5), which form a complex that targets A3G for ubiquitylation and proteasomal degradation. Disruption of A3G-Vif-CRL5 interactions could reactivate A3G antiviral activities and lead to new HIV therapies. Characterization of A3G-Vif-CRL5 structures is essential to identifying these “disruptable” interfaces. However, due to the transient nature of enzyme-substrate interactions, poor stability of A3G, and conformational heterogeneity of Cullin ligases, the A3G-Vif-CRL5 complex has resisted structure determination. Structure techniques like crystallography, NMR, and cryo-EM, struggle to accommodate such biochemical and structural challenges. Here I use cross-linking mass spectrometry (XL-MS) as an alternative and complementary approach for structure determination of A3G-Vif-CRL5 complexes. Originally developed to enhance the capture of transient protein interactions from pull-down experiments, XL-MS approaches have evolved into powerful tools for probing macromolecular structures. Identified cross-linked peptides map interaction interfaces that, combined with integrative modeling approaches (e.g. IMP), can determine structures of protein complexes that were previously intractable. Though a lower resolution technique, XL-MS can overcome several limitations by: (1) stabilizing transient interactions; (2) accommodating heterogeneity and flexibility; (3) adapting to various in solution requirements; and 4) providing in cell and in vivo applications. Here we use a XL-MS integrative modeling pipeline to determine the A3G-Vif-CRL5 structure. Our model provides the first physical interaction data describing the full A3G-Vif-CRL5 complex that corroborates previously reported genetic perturbation studies and importantly captures previously undefined structure details. Based on our structure model, we have designed disruption mutations and begun functional validation of key interfaces testing for A3G protein stability, incorporation into virus particles, antiviral activity, as well as full A3G-Vif-CRL5 complex stability. Our results demonstrate the feasibility and applicability of this approach for structure determination of virus-host complexes.
F23. Integrative Structure Modeling of HIV Vif in Complex with Host Proteins

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APOBEC3 (A3) proteins can potently restrict HIV infection by deamination of the HIV-1 proviral DNA. HIV-1 Vif hijacks the host cell’s apparatus to counteract the antiviral roles of certain human APOBEC3 (e.g., A3G, F, and H) proteins by targeting them for ubiquitylation and proteasomal degradation. Vif, which is stabilized by cellular CBF-β, binds simultaneously to EloB/EloC/Rbx2/Cullin5 and APOBEC3G to form a ubiquitin ligase complex, targeting the latter for degradation. This complex (referred to as VIF-CLR5-A3G) has been refractory to traditional structure determination techniques because of its structural heterogeneity and/or conformational flexibility. To overcome this challenge, we applied integrative structure modeling based on data from X-ray crystallography, DSSO-based chemical crosslinking-mass spectrometry (XL-MS), small-angle X-ray scattering (SAXS), and biochemical experiments, allowing us to determine the structure of the VIF-CLR5-A3G complex. We assessed the structural heterogeneity of the complex and characterized the relevant protein-protein interfaces. The structural models were validated using SAXS and published biochemical data. We also designed a series of mutants targeting the relevant protein-protein interfaces to test their impact on the complex stability and to functionally assess how these mutants alter APOBEC3G incorporation into virus particles and HIV infectivity. Finally, this study has shown the feasibility of using DSSO-based XL-MS analysis for integrative modeling of host-pathogen protein complexes, highlighting the power of the integrative structure determination approach. The pipeline is general and can be applied to structurally characterize other HIV-human protein complexes.
F24. Molecular Characterization of a Unique Restriction Factor, APOBEC3H

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Restriction factors play a critical role in protecting the host from viral infections such as HIV-1. Restriction factors in the APOBEC3 family recognize minus strand (\(-\)) DNA (ssDNA) of the HIV-1 genome generated during reverse transcription. This ssDNA is a substrate for zinc-dependent cytidine deamination by APOBEC3 enzymes, resulting in hypermutation of the HIV-1 genome, leading to either degradation of the cDNA or incoherent protein translation. In addition to the cytidine deaminase activity, APOBEC3 proteins bind to host and viral RNAs to facilitate encapsidation into new virions. Their ability to bind RNA is essential for virion infiltration and antiviral activity, yet the mechanisms of viral RNA recognition are unknown.

In two-domain APOBEC3F (A3F) and -3G (A3G) proteins, one zinc-binding domain (Z-domain) binds RNA while the other catalyzes cytidine deamination. APOBEC3H (A3H) is a unique member of the APOBEC3 family in that it potently restricts HIV-1 infectivity using only a single Z-domain that both binds RNA and catalyzes deamination. We have generated protein expression constructs for several natural polymorphic variants of pig-tailed macaque (pgt) A3H, which exhibit varying levels of antiviral activity in cells. Human and pgtA3H variants have been recombinantly expressed and purified for structural studies and to test biochemical activities. The recombinant A3H variants possess a robust cytidine deaminase activity despite the presence of co-purifying RNA.
F25. Structural Basis for Targeted DNA Cytosine Deamination and Mutagenesis by APOBEC Enzymes

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APOBEC-catalyzed cytosine-to-uracil deamination of single-stranded (ss)DNA has beneficial functions in immunity and detrimental roles in cancer. APOBEC enzymes have intrinsic dinucleotide specificities that impart hallmark mutation signatures. Despite numerous structures, mechanisms for global ssDNA recognition and local target sequence selection remain unclear. We report crystal structures of human APOBEC3A and a chimera of human APOBEC3B and APOBEC3A bound to ssDNA at 3.1 and 1.7 angstroms resolution, respectively. These structures reveal a U-shaped DNA conformation, with the specificity-conferring -1 thymine flipped out and the target cytosine inserted deep into the zinc-coordinating active site pocket. The -1 thymine base fits between flexible loops in a groove that forms upon binding ssDNA, and it makes direct hydrogen bonds with the protein accounting for the strong 5'-TC preference. These studies explain both conserved and unique properties among APOBEC family members, and provide a basis for the rational design of inhibitors to impede the evolvability of viruses and tumors.
F26. Model and Dynamics of a Full A3G Monomer

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We built a computer model for the full-size APOBEC3G (A3G) monomer using docking approaches and molecular dynamics simulations, based on the available X-ray and NMR structural data. The model revealed a large-scale dynamics of A3G monomer, where two domains A3G can assume either compact forms or extended dumbbell type forms, with two domains visually separated from each other. To validate A3G model, we perform time-lapse high-speed atomic force microscopy (HS-AFM) experiments enabling us to get images of fully hydrated A3G and directly visualize its dynamics. HS-AFM confirmed that A3G exists in two forms, a globular form (~90% of the time) and a dumbbell form (~10% of the time), and can dynamically switch from one form to the other. The obtained HS-AFM results are in line with the computer modeling, which demonstrate similar distribution between two forms. The revealed dynamic nature of A3G monomer could aid in the target recognition including scanning for cytosine locations along the DNA strand and in participation of A3G in interaction with viral RNA during its packaging into HIV virion.
F27. Fab-Based Inhibitors Separate Ubiquitin Dependent and Independent Functions of HIV Vif Neutralization of APOBEC3

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HIV-1 Viral Infectivity Factor (Vif) counteracts host APOBEC3 (A3) restriction factors by hijacking a Cullin-RING ubiquitin E3 ligase complex (CRL5) and core binding factor beta (CBFβ). To better understand the activities of the Vif E3 assembly and to generate selective reagents for Vif structural studies, we isolated antibody antigen-binding fragments (Fab) against the A3 substrate receptor of the Vif E3. Biochemical and structural studies of the substrate receptor-Fab complex indicate that Fab 3C9 impairs A3F ubiquitination through a non-competitive mechanism, shielding A3 from ubiquitin transfer. In contrast, Fab 1D1 inhibits A3 ubiquitination and packaging into virions by disrupting the CUL5 substrate receptor interactions. Collectively, our data illuminate a ubiquitination-independent mechanism by which Vif prevents A3 packaging, and highlights the potential of diverse Fab panels as functional probes to guide HIV drug discovery.
Distinct Capsid Uncoating and Host Cell Infectivity Phenotypes in HIV-1_{NL4-3} and HIV-1_{LAI} are Caused by Minor Sequence Differences in CA

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After host cell entry, human immunodeficiency virus type 1 (HIV-1) reverse transcribes its RNA genome into double-stranded DNA, which is then translocated into the nucleus for integration. The dissociation of the viral capsid, which shields the viral genome, is key to this HIV-1 life cycle step, through a tightly regulated process called uncoating. A fluorescence-based imaging assay to visualize the kinetics of HIV-1 capsid permeabilization in infected cells revealed that common lab-adapted strains HIV-1_{NL4-3} and HIV-1_{LAI} exhibit different capsid-dependent uncoating kinetics. An examination of the four CA differences between the two strains (amino acids 6, 83, 120, and 208) demonstrated different infectivity phenotypes, including cell cycle and/or Cyclosporine A (CsA) dependence and impaired host factor interactions, in multiple human cell lines and primary human CD4+ T cells. Data suggest that a subset of the four amino acids that differ between HIV-1_{NL4-3} and HIV-1_{LAI} capsid, which are polymorphic and widely prevalent in clinical virus isolates, are integral to these phenotypes. The cell cycle dependent capsid mutant N57A has an infectivity defect that was shown to be 1.5-2-log in HIV-1_{NL4-3} but only 2-5-fold in HIV-1_{LAI}. This defect is rescued by the CsA dependent G94D capsid mutation in HIV-1_{LAI} but not in HIV-1_{NL4-3}. Mutation of CA amino acid 83 in HIV-1_{NL4-3} to the HIV-1_{LAI} residue (L83V) is sufficient to change the infectivity of capsid mutant N57A from the HIV-1_{NL4-3} phenotype to the HIV-1_{LAI} phenotype. However, it is not sufficient to confer rescue of the N57A infectivity defect by the addition of the G94D mutation in HIV-1_{NL4-3}, as is observed in HIV-1_{LAI}. The induction of cell cycle arrest by aphidicolin treatment exacerbates the N57A infectivity defect and prevents its rescue by G94D in HIV-1_{LAI}. Additionally, infectivity of the N57A capsid mutant in virus bearing either the HIV-1_{NL4-3} or HIV-1_{LAI} capsid and in multiple cell types is further impaired by treatment with CsA, particularly in conjunction with cell cycle arrest, suggesting that interaction with host factor cyclophilin A is important for infectivity of the N57A capsid mutant. A better understanding of how different HIV-1 strains interact with host cell processes can provide valuable insight into aspects of the virus life cycle that might be targeted by novel therapeutics.
Until recently it was a commonly accepted notion that reverse transcription (RTion) in retroviruses, including HIV-1, takes place within the cytoplasm of the infected cell after uncoating of the mature capsid. However, accumulating evidence suggests that the RTion process happens largely within the mature capsid core, which protects the viral genome from host factors and maintains high local concentrations of the essential viral proteins within the RTion complex. In this theoretical work, we consider the problem of mature HIV capsid uncoating driven by polymerization of double-stranded (ds) pro-viral DNA during RTion. The millimolar concentrations of nucleocapsid protein (NC) contained within the capsid drives aggregation of both single-stranded (ss) gRNA and dsDNA, provided the capsid is intact. Flexible gRNA is aggregated by NC into a ribonucleoprotein complex occupying only a small fraction of the capsid volume and providing no pressure on the capsid. While the self-volume of the full-length pro-viral DNA (~10^4 bp) is the same as of its diploid gRNA genome, the dsDNA is very rigid, and would destroy the marginally stable mature capsid early in the RTion process, if it were not condensed by NC. Prior studies of dsDNA condensation by multivalent cations suggest that the DNA becomes condensed into a toroidal globule with is the more compact, the stronger is the cation-induced dsDNA self-attraction. For a given capsid stability and the NC-induced DNA self-attraction, we predict there is a critical length of dsDNA length that can be synthesized by RT inside the capsid before it will break open/dissociate at some critical pressure. We compare our predictions with the recent AFM results by Rousso et.al, (2017, JVI), who visualized the accumulation of the “rigid toroidal filament” inside the capsid during the course of RTion, followed by the abrupt capsid rupture at its narrow end. These processes were shown to correlate with the build-up and abrupt decrease in the capsid stiffness. We predict the uncoating process to be “all or nothing”, as the punctured capsid is expected to promptly rapidly loose its NC protein, leading to the dsDNA expansion, and subsequent capsid destruction. We describe the phase diagram that relates the volume of double stranded pro-viral DNA synthesized within the capsid, the strength of its the dsDNA NC-induced self-attraction, and the stability of the capsid at the point of uncoating. This diagram can be used to predict the amount of ds proviral DNA that can be synthesized inside the mature HIV-1 capsid with as a function of its stability, varied modulated by either CA mutations, or various cellular restriction factors.
F30. Novel and Distinct Functions of Diaphanous-Related Formins Regulate HIV-1 Uncoating and Transport

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Diaphanous (Dia)-related formins (DRFs) coordinate cytoskeletal remodeling by controlling actin nucleation and microtubule (MT) stabilization to facilitate processes such as cell polarization and migration, yet the full extent of their activities remains unknown. Here, we uncover two discrete roles and novel functions of DRFs during early human immunodeficiency virus type 1 (HIV-1) infection. Depletion of Dia family members, Dia1 or Dia2 in a variety of human cell types including natural target cells such as human microglial cells (CHME3) or human monocytic cells (Thp-1) differentiated to macrophages potently inhibited early infection with HIV-1 carrying either vesicular stomatitis virus G (VSV-G) or wild type (WT) envelope glycoprotein, suggesting that DRFs promote early HIV-1 infection regardless of the route of viral entry. In addition, independent of their actin regulatory activities, Dia1 and Dia2 facilitated HIV-1-induced MT stabilization and the long-range movement of incoming viral cores toward the nucleus as determined by both fixed and live cell imaging approaches in CHME3 cells infected with fluorescently tagged HIV-1 carrying WT envelope. Moreover, early infection by two other retroviruses, murine leukemia virus (MuLV) and simian immunodeficiency virus (SIV), that did not induce MT stabilization was unaffected by Dia depletion, suggesting that induction of MT stabilization and a requirement for DRFs during early infection was specific to HIV-1. However, DRFs also bound in vitro assembled capsid-nucleocapsid complexes and promoted the disassembly of the HIV-1 capsid shell (a poorly understood process also known as uncoating), assessed using both in situ fluorescence microscopy and fate-of-capsid uncoating assays. Domain analysis and structure modeling of Dia2 mutants revealed that regions of Dia2 that bound viral capsid and mediated uncoating as well as early infection contained coiled-coil (CC) domains, which are present in the limited number of known capsid-binding proteins. Using Dia mutants that specifically induce MT stabilization without regulating actin, we further show that these activities of Dia were genetically separable from effects on MT stabilization. Our findings reveal that HIV-1 exploits CC domains of DRFs to control core stability while independently exploiting their MT stabilizing activity to coordinate the early processes of uncoating with virus transport. This uncovers novel functions for CC domains in the DRFs, and identifies these formins as critical host cofactors exploited by HIV-1 during early infection.
F31. Charge-Charge Interaction is Important for Fez1-Capsid Interaction During HIV Infection

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The trafficking and uncoating events occurring immediately after HIV-1 entry into the host cell are complex and among the most poorly understood aspects of the viral life cycle. Previously, it was discovered that Fez1 is important in capsid nuclear trafficking, as Fez1 knockdowns reduce core accumulation near the nucleus and lessen integration events. In these studies, Fez1 was shown to co-pellet with in vitro assembled capsid tubes. To investigate this Fez1-capsid interaction, we mapped the binding regions of recombinant Fez1 and capsid using size exclusion chromatography and pelleting assays. We tested different Fez1 truncation and mutation constructs and found that binding sites within Fez1 contain acidic, poly-glutamate stretches. Furthermore, our data revealed that Fez1 specifically binds to one complete capsid protein (CA) hexamer, but not with smaller CA oligomers. Interestingly, the CA hexamer contains a positively charged surface and mutations at this region greatly reduced Fez1-CA hexamer interaction. Similar reductive effects were seen in competition assays where Fez1-CA hexamer binding was decreased by the presence of nucleotides that have been shown to bind at a similar location. These findings support a model where Fez1 serves as a unique CA hexamer pattern sensor that specifically recognizes conserved capsid lattice features using charge-charge complementarity.
F32. Hexagonal Lattice Assembly of TRIMCyp

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TRIMCyp from owl monkey is a natural chimera resulting from retrotransposition of cyclophilin A into the TRIM5α gene. The cyclophilin domain is a well-characterized CA binding protein, and so can functionally replace the more common capsid-binding SPRY domain in TRIM5α. TRIM5α with its SPRY domain is known to require the avidity gains provided by higher-order assembly into a hexagonal lattice, in order to productively bind retroviral capsids and restrict viral replication. Higher-order assembly requires the TRIM5α B-box 2 domain, which forms the trimeric vertices of the hexagonal lattice. Previous studies have shown that TRIMCyp is not dependent on B-box self-association because the relatively strong affinity for the CA monomer (about 20 µM) precludes the need for an affinity amplification mechanism. We found, however, that under stringent experimental conditions, TRIMCyp still requires B-box/B-box interactions for maximal restriction activity. Structural studies further confirmed that the TRIMCyp B-box 2 domain can form trimers similar to the TRIM5α B-box trimers. Our studies support the conclusion that both forms of TRIM5 require the avidity gains provided by higher-order assembly in order to efficiently recognize retroviruses and inhibit their replication.
F33. Defects in Assembly Explain the Reduced Antiviral Activity of the G249D Polymorphism in Human TRIM5α

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TRIM5α is an interferon inducible protein, which contributes to the defense mechanism against HIV infection by targeting the HIV capsid protein CA. Although human TRIM5α (huTRIM5α) does not potently inhibit HIV-1 infection, the ability of huTRIM5α to exhibit some control of HIV-1 infection is evidenced by a single nucleotide polymorphism in human TRIM5α, which substituted aspartic acid to glycine at position 249 (G249D) in L2 region, associated with higher susceptibility to HIV-1 infection of Japanese and Indian subjects (Nakayama et al., 2013).

To understand the mechanistic basis for the reduced antiviral activity, we employed biophysical, molecular dynamics simulations and cell biological methods to compare wt and the G249D polymorphism of huTRIM5α. We investigated the differences in conformational dynamics of rhesus and human TRIM5α Coiled Coil(CC) –Linker 2(L2) domain by utilizing circular dichroism and single molecule-Fluorescence Energy Transfer (sm-FRET). These methods revealed that the G249D dimer exhibits secondary structure and conformational dynamics similar to wt huTRIM5α. To better understand how the G249D mutation may influence viral restriction, we performed homology modeling of the huTRIM5α dimer. Modelling revealed that the G249D mutation was present on the hairpin of the antiparallel dimer, in a position which may act to stabilize the adjacent BBox2 domain which mediates the inter-dimeric contacts required for the formation of TRIM5 assemblies. We therefore asked if the G249D mutant forms assemblies in cells with the same efficiency as wt protein by expressing these proteins as YFP fusions and quantifying the number of assemblies in cells. In cells expressing comparable amounts of protein, the G249D mutant formed fewer assemblies than wt protein, in agreement with our homology modeling predictions.

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F34. Structural Basis of Retroviral Capsid Recognition by the Restriction Factor, TRIM5α

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TRIM5α is a restriction factor that recognizes incoming retroviral cores by binding to the capsid. Upon capsid binding, TRIM5α accelerates its dissociation, prevents proper reverse transcription of the viral genome, and initiates an interferon response. TRIM5α is proposed to assemble into a hexagonal lattice on the surface of retroviral capsids in an avidity-driven mode of binding. High-resolution structures and detailed molecular interfaces involved in TRIM5α self-assembly have been determined by X-ray crystallography, but the details of how TRIM5α interacts with the capsid are not known. To bridge this gap in knowledge, we have reconstituted TRIM5α/capsid complexes in vitro using purified components. Electron cryo-tomography and sub-tomogram averaging indicate that the complexes consist of capsid-like tubes caged by an outer layer of assembled TRIM5α proteins. We have now confirmed the arrangement of TRIM5α domains predicted from the X-ray crystal structures of individual domains, and have started to define how TRIM5α directly binds retroviral capsids and inhibits capsid function.
F35. Structure-Based Optimization of Small-Molecule CD4-Mimics: Inhibitors of HIV-1 Cell Entry and Viral Neutralization Agents

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Our highly interdisciplinary, synergistic NIH Program Project (GM 56550) explores the structural and functional mechanism of the HIV-1 envelope glycoprotein (Env) complex, which is essential for viral-cell fusion. Based on our growing understanding of the energetic landscape of the metastable Env, we aim to develop potent small-molecule inhibitors of HIV-1 entry that target the well-conserved CD4 binding site. To this end, we have designed and optimized, based on computational, thermodynamic, and crystallographic data, a series of potent, broadly active small-molecule CD4-mimic entry inhibitors, as exemplified by (+)-(R,R)-BNM-IV-147. In addition to entry inhibition, these small-molecule CD4-mimics can be employed in viral neutralization strategies based on their ability to sensitize HIV-1 infected cells to antibody-dependent cellular cytotoxicity (ADCC) and infectious viral particles to neutralization by antibodies. Continued synthetic efforts comprising the indane scaffold and the critical oxalamide linker hold the promise of increasing the therapeutic potential of these small-molecules CD4-mimics.

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HIV-1 entry inhibition remains an urgent need for AIDS drug discovery and development. We previously reported the discovery of cyclic peptide triazoles (cPTs) that retain the HIV-1 irreversible inactivation functions of the parent linear peptides (PTs) and have massively increased proteolytic resistance. Here, we used structural optimization to evaluate the effects of variations in key structural and functional components of the cPT scaffold to produce a next generation of cPTs. We examined the stereochemistry around the cyclization residues to determine the optimal configuration required for activity. We also identified potent cPT analogues by replacing the Trp and Ile residues in the pharmacophore of first generation cPTs with non-natural substituents. To increase the drug-like nature of cPTs, we replaced the ferrocene moiety through examination of different substituents at the triazole ring, with consequent identification of smaller aromatic rings having similar potencies compared to the parent ferrocene-containing cPTs. Based on the observed activity of the biphenyl moiety when added on the triazole ring, we further developed a facile on-resin method to install the bi-aryl system during cPT synthesis. The resultant thiophene containing cPT AAR029N2 (36) showed improved antiviral activity compared to the ferrocene analogue. Findings in this work will guide the ongoing optimization and minimization steps for this important class of HIV-1 killing agents.
Pharmacophore Modeling of Human CC-Chemokine Receptor 4 Allosteric Antagonists

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Human CC-Chemokine Receptor 4 (CCR4) belongs to the G-protein-coupled receptor family and is expressed in Th2 and Th17 cells. CCR4 inhibitors represent a novel therapeutic intervention in diseases where CCR4 has a central role in pathogenesis, such as asthma and various other allergic diseases, the mosquito-borne tropical diseases, cancer and HIV/AIDS. Allosteric regulation is the regulation of a receptor protein by binding at a site distant from the protein’s major active site, thus providing alternative opportunities for modern drug discovery. Procopiou and others examined the structure-activity relationship of indazole arylsulfonamides as allosteric CCR4 antagonists (J. Med. Chem. 56(5), 1946–60 (2013)). In a subsequent paper, Miah and others demonstrated the structure-activity relationship of heteroarylpyrazole arylsulfonamides as another set of allosteric CCR4 antagonists (Org. Biomol. Chem. 12(11), 1779–92 (2014)).

Pharmacophore-modeling searches for common chemical features in the 3D conformations of ligands responsible for the activity. In order to build pharmacophore models, we obtained the chemical structures of two sets of allosteric CCR4 antagonists from the papers above. The first set contained 35 active indazole arylsulfonamides. The second set consisted of the 15 active and two inactive heteroarylpyrazole arylsulfonamides. Pharmacophore modeling was done in two directions. We built pharmacophore models of indazole arylsulfonamides and used the set of heteroarylpyrazole arylsulfonamides in validation and in reverse. All modeling was done with PHASE. For each direction, we created decoys for the validation set in 1:39 ratio using MUDB-Decoymaker developed in the Wang lab.

In the first direction, indazole arylsulfonamides gave us models with three variants: AAHRR\(^1\), AHRRR, and AARRR. Because the set of heteroarylpyrazole arylsulfonamides contained three members with only two aromatic rings, the AAHRR models alone were able to find all among decoys. With PHASE box size 1Å, we were able to compute AAHRR models with very high AUC: 99.6% and 98.6%. However, since small PHASE box size could mean overfitting, we relaxed the box size to 2Å. Yet, the reduction in the accuracy was small, we computed two models with AUC 92.4% and 88.1%. In the other direction, the models of variant AAHRR scored significantly higher than any other variant. At 1Å, PHASE returned only one model with AUC of 97.8%. At 2Å, we had three models with AUC 97.8%, 96.9% and 93.8%.

We built models for two distinct sets of allosteric CCR4 antagonists and demonstrated that they have high accuracy. Our next step is to search the compound libraries for leads and validate the results experimentally.

\(^1\) A: hydrogen bond acceptor, H: hydrophobic group, R: aromatic ring
F38. Computational And Experimental Screening for Small Molecules Obstructing HIVgp41 N-Heptad Repeat Trimer

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HIVgp41 is a validated viral drug target that forms a six-helix bundle to allow membrane fusion, which subsequently facilitates viral entry. One proposed strategy for blocking fusion is to obstruct formation of the inner N-heptad repeat (NHR) trimer. In this work, similarity-based computational screening was used to prioritize compounds for experimental testing, that were related to two hits previously reported by Allen et al (Bioorg. Med. Chem Lett. 2015, 25 2853-59) proposed to act via the NHR obstruction mechanism. The experimental testing led to a promising new hit (compound #11) which showed activity in two different assays (HIV fusion and viral entry) and had low cytotoxicity. Molecular dynamics (MD) simulations showed that while the DOCK-predicted binding geometry of #11 was relatively stable and energetically favorable, there was also the potential to improve affinity via refinement of a benzimidazole group. We employed an in-development version of de novo DOCK to automatically generate ca 50,000 analogs of #11 through sampling of functionality taken from a large fragment library to evaluate and prioritize those with improved electrostatic interactions with residues Thr25 and Thr62. Encouragingly, the de novo refinement protocol led to numerous analogs with improved predicted interaction energies relative to the parent compound. Results will also be presented from MD simulations which were geared to identify compounds that showed further improvement in geometric stability.

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De novo design is a powerful computational approach that can be used to grow small organic molecules from fragment components for compatibility with a protein binding site. Recent efforts in our lab (J Comput Chem 2017, submitted) have focused on developing a robust de novo strategy for the program DOCK6 to assist with two primary types of molecular modeling applications: (1) from-scratch ligand growth starting with fragments oriented in a binding site used to seed layer-by-layer construction of new molecules (de novo generic growth), and (2) scaffold refinement starting from a user-defined molecule binding pose from which select attachment points are then modified (de novo refinement growth). The goal in both cases is to create novel ensembles of molecules that are physically reasonable with strong binding potential. This poster will present an outline of the de novo DOCK procedure including fragment library generation, computational methods of validation, and chemical property assessment of designed ensembles. Example applications targeting HIV glycoprotein 41 (HIV gp41) and Zika virus envelope glycoprotein (ZikaV E) involved in viral entry will also be presented.
F40. Liposome Nanoparticle Encapsulation and Surface Modification with Macrocyclic Peptide Triazole HIV-1 Inactivators

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We developed a cyclic peptide triazole (cPT) class of HIV-1 inactivators that block the binding of viral Env gp120 protein to both host cell CD4 and coreceptor (CCR5/CXCR4), and additionally trigger gp120 shedding. Here, we investigated various liposome formulations with the aim to examine the effects of encapsulation and surface display on pharmacokinetics and potency. cPTs were encapsulated within liposome formulations: DPPC:Cholesterol: mPEG2000-DSPE and DSPC:Cholesterol:mPEG2000-DSPE. In vitro, these liposome formulations released cPTs for up to 5 days after encapsulation under physiological conditions, inhibited cell infection by several HIV-1 subtype B viruses, and exhibited no associated cellular toxicity. Furthermore, in vivo pharmacokinetic analysis in rats showed that the half-life of encapsulated fluorescently labeled cPT was prolonged in circulation and exhibited a reduced clearance rate from serum of at least 15-fold compared to that of free fluorescent cPT. DSPC: DSPE-PEG (2000) Maleimide:Cholesterol (4:01:2) liposomes surface-conjugated with cPT thiols showed 2-fold enhancement in inhibiting HIV-1 infection compared to free cPT. Previously, it was found that surface modification of gold nanoparticles exhibit a multivalency effect on the virus, resulting in virolysis. Similar affect was observed by surface conjugated liposomes upon treatment of HIV-1 pseudovirus alone. The extent of virolysis due to surface modification and consequent multivalency needs further evaluation. The results indicate that liposome nanoparticles could be used for potential delivery and release of cPTs to sites of virus and Env-expressing infected cell accumulation during HIV-1 infection.
F41. Restricted HIV-1 Env Glycan Engagement by Lectin-Reengineered DAVEI Protein Chimera is Sufficient for Lytic Inactivation of the Virus

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We previously reported first generation recombinant constructs of a dual action virus entry inhibitor (DAVEI), composed of cyanovirin-N (CVN) fused to a membrane proximal external region (MPER), that exhibited potent and irreversible inactivation of both pseudotyped and fully infectious HIV-1 viruses. In addition, we used a protein engineering approach to simplify MPER and linker sequences to identify molecular determinants important for virolytic activity. In the CVN-DAVEIs made, the CVN domain binds to gp120 and provides sufficient binding affinity to steer the MPER and MPER surrogates for Env gp41 engagement and consequent virus lysis. However, the promiscuity of CVN to associate with multiple glycosylation sites in gp120 and its multivalency limit current understanding of the molecular arrangement of the DAVEI molecules on trimeric spike needed for virolysis. In the current study, we constructed and investigated the virolytic function of second generation DAVEI molecules using a simpler lectin domain derived from microvirin (MVN). Microvirin (MVN) has a single glycan binding site as revealed by NMR and binds to a small repertoire of gp120 glycans, located in a single patch on the outer domain. MVN is structurally similar to CVN and exhibits no toxicity or mitogenic activity, both of which are liabilities with CVN. We found that, like CVN-DAVEI-L2-3Trp, MVN-DAVEI2-3Trp exploits a similar mechanism of action for inducing virolysis, but by more selective gp120 glycan engagement. By sequence redesign, we were able to significantly increase the potency of MVN-DAVEI2-3Trp protein. Re-engineered MVN-DAVEI2-3Trp(Q81K/M83R) protein binding was competed by 2G12 antibody. 2G12 also suppressed the lytic behavior of MVN-DAVEI2-3Trp(Q81K/M83R), though not CVN-DAVEI2-3Trp. That the lectin domain in DAVEIs can utilize MVN without loss of virolytic function argues that restricted HIV-1 Env glycan engagement via the single glycan binding site in MVN is sufficient for inducing virolysis. Since the reengineered MVN-DAVEI2-3Trp(Q81K/M83R) construct has a defined binding site on gp120 and retains strong antiviral and virolytic potency, it provides an improved tool to elucidate productive molecular arrangements of Env-DAVEI enabling virolysis. This study also opens the way to form DAVEI fusions made up of gp120-binding small molecules linked to Trp3 peptide, and ultimately with simplified surrogates of the Trp3 sequence.
Using infectious virus escape, we investigated resistance patterns of HIV-1 to peptide triazoles that bind to gp120, disrupt the trimeric Env protein and irreversibly inactivate the virus. Generation of HIV-1 escape mutants was accomplished through virus passaging on an immortalized T-cell line with dose escalation of inhibitor. Sequencing of the escape mutant HIV-1 genome revealed common features of escape profiles for both linear and macrocyclic inhibitor variants. Mutations were identified not only within the putative binding site, typified by V255I/T, but also outside this site, typified by S143N. These observed mutations are consistent with the view that peptide triazoles exert inactivating pressure throughout the protein complex, including at the gp120-gp41 interface and the trimer apex. Single and combined mutations were generated in envelope-pseudotyped virus by site-directed mutagenesis in order to investigate the effects of the major escape mutations on individual viruses. Small changes in the side chain at a pivotal V255I/T location within the peptide binding cavity on gp120 had a strong impact on inhibitor efficacy, though this disruption was significantly less for the macrocyclic peptide triazole. Flow cytometric analysis of V255I/T indicated similar shedding sensitivity compared to wild type Env. Flexible molecular docking of peptide triazoles onto HIV-1 Env trimers in the presence and absence of the V255 mutations suggested that the mutations at this site could fill an important binding cavity to block cPT binding. Alternatively, V255 mutations could encourage closure of the cPT binding site by β20-21 loop folding. The results of this work argue that [1] common pathways of HIV-1 occur with different forms of peptide triazoles; [2] escape mutations have a reduced impact on the potency of a protease-resistant macrocyclic peptide triazole compared to linear versions; [3] escape mutations evolve both within the inhibitor binding site and in regions distal to it that are important for Env conformational stability; and [4] structural and functional effects of escape mutation at V255, that has now been observed in multiple passaging experiments, promise to provide insights into how peptide triazoles hijack intrinsic conformational properties of the Env protein complex to cause irreversible inactivation of the protein complex and the virus.
The HIV-1 envelope glycoprotein (Env) trimer is the sole viral protein exposed on the virus surface and as such represents a main target for a vaccine against HIV-1/AIDS. The functional Env trimer mainly exists in a closed conformation (State 1), which is driven by CD4 binding through an intermediate conformation (State 2) to the open CD4-bound conformation (State 3). These functional Env states can be visualized by single-molecule Fluorescence Resonance Energy Transfer (smFRET). A breakthrough in the structural characterization of the HIV-1 Env trimer has been the generation of recombinant cleaved soluble gp140 SOSIP.664 trimers stabilized by a disulfide bond between the gp120 and gp41 subunits (SOS), an I559P change in gp41 (IP), and a truncation at gp41 residue 664. Parallel cryoelectron microscopy studies have been performed with the mature HIV-1JR-FL Env in complex with the PGT151 neutralizing antibody. Both approaches resulted in similar structures. Here we apply smFRET to determine the conformational state of HIV-1 Env in these constructs and antibody complexes. Fluorophores were introduced at the identical positions in the HIV-1 Env proteins used for structural studies and the native Env on the surface of virions, and the resulting smFRET values compared. Surprisingly, smFRET data reveal that both the soluble gp140 SOSIP.664 and PGT151-HIV-1JR-FL Env structures correspond to the State 2 gp120 conformation observed on the virus. The all-important structure of State 1 of HIV-1 Env, which is the target of the majority of broadly neutralizing antibodies, remains unknown. Our data suggests that smFRET can serve as a useful tool to guide structural studies towards the characterization of State 1 HIV-1 Env. Determining the structure of this additional conformation should allow the design of a second generation of immunogens that specifically present the State 1 conformation of HIV-1 Env.
F44. The HIV-1 Env Trimer Opens Through an Asymmetric Intermediate in Which Only a Single CD4 Engages the Trimer

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HIV-1 entry into cells is mediated by the binding of the viral envelope glycoprotein (Env) to receptor CD4 and coreceptor CCR5 or CXCR4. Single molecule fluorescence resonance energy transfer (smFRET) imaging of individual Env molecules previously revealed that the trimer is dynamic, and spontaneously transitions between at least three conformational states. Binding of CD4 and coreceptor surrogate antibody 17b promotes opening of the closed Env (State 1) to stabilize an activated conformation (State 3) by way of at least one structural intermediate (State 2)(1). Here, using smFRET, we identify this intermediate as an asymmetric conformation where only a single CD4 molecule engages the Env trimer and individual protomers adopt distinct conformations. The asymmetric trimer completely opens to State 3 upon engaging either a second CD4 molecule or the coreceptor surrogate antibody 17b, suggesting that either local clustering of CD4 or co-receptor binding promotes opening of the HIV Env trimer. To gain insights into the structure of the asymmetric trimer, we solved an ~8 Å resolution structure of the SOSIP BG505.664 trimer bound to a single CD4 by cryo-electron microscopy. The structure was asymmetric with the CD4-bound protomer best fit by the CD4-bound configuration of gp120, while the two other subunits adopted a somewhat different conformation from both unliganded and CD4-bound Env. These data indicate the HIV Env trimer to be activated through an asymmetric intermediate in which only a single CD4 engages the trimer.

F45. Single Molecule Fluorescence Spectroscopy of HIV Envelope in Solution


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HIV is a subgroup of retrovirus that causes infection and over time AIDS. As a result, increasing interest has emerged in finding preventive measures to block HIV replication prior to host cell attachment/entry. HIV envelope, a heavily glycosylated trimer of the gp120 surface subunit and gp41 transmembrane subunit heterodimers, facilitates viral entry into host cells through a series of conformational changes, and therefore has been the primary target for development of antiretroviral therapies. Due to the heterogeneous nature of the envelope structure, it has become essential to determine the antigenic nature of the HIV virions and how this equates to sensitivity/neutralization by cognate antibodies. Previous studies propose that antibodies are neutralizing only if they bind functional trimers on virion surfaces discounting misfolded structures. However, methodologies used so far have produced conflicting results and can perturb antigenicity profiles and therefore pose problems. Here we employ fluorescence correlation spectroscopy (FCS), a technique that allows real-time analysis of biomolecular interactions in solution to investigate antibody-virion interactions and epitope exposure patterns of HIV envelope on a single virion particle. We show that free HIV particle antigenicity has a preference for functional envelope structures (marked by Anti-gp120 MAbs) over irregular gp120 structures (marked by MAbs against CD4-induced epitopes) by measuring binding efficacy of different anti-HIV envelope monoclonal antibodies (MAbs) with multiple virus types. Moreover, dual color FCS and/or combining FRET with our FCS assay provides new opportunities to interrogate two different MAbs on a single virion or on a single spike of a virion that can provide important information about the structural dispositions and juxtaposition of epitopes. Coincidence FCS and FRET analyses of dual MAb binding signals therefore offers an excellent means to determine if in fact there are different populations of envelope spikes that exhibit distinct signature patterns of epitope exposure. These determinants can be made with all reactants in solution, free from the caveats of capture procedures. FRET signatures that occur between two defined epitope markers provide the average distances between two different MAbs (neutralizing or non-neutralizing) or Fabs bound to the same envelope. FRET measurements using different MAbs and HIV-1 BaL virions suggest an average distance of 7.7nm and 7.1nm between two neutralizing MAb b12 and 2G12 and the two (b12 , 2G12) Fabs bound to a trimer on a single virion particle respectively. Therefore, single molecule spectroscopy facilitates to provide significant information regarding the structural and antigenic dynamics of the HIV envelope.
The p6 domain of HIV-1 Gag contains a YPX\textsubscript{n}L “late” domain that promotes the release of virions through a direct interaction with the ESCRT-associated protein Alix. We previously demonstrated a functional role for the binding between Gag p6 and Alix by introducing mutations within this YPX\textsubscript{n}L motif. The most striking defect observed was a severe delay in virus replication kinetics. To ascertain the nature of the replication defects, we passaged replication-defective Alix-binding site mutants in culture and selected for viral isolates containing compensatory second-site mutations. Sequencing of the viral revertants revealed loss-of-function mutations in Vpu and novel mutations at highly conserved positions in Env. The second-site Env mutations conferred full rescue of the original Gag mutants in Jurkat T-cells. We demonstrate that the Env compensatory mutants alone replicate with wild-type kinetics in Jurkat T-cells; however, they exhibit severe defects in cell-free, single-cycle infectivity. Importantly, the phenotype observed in Jurkat cells was recapitulated in primary T cells, underscoring the physiological relevance of these data. The Env mutations do not affect Env expression or Env incorporation in Jurkat cells; however, they exhibit defects in fusogenicity. Env mutants obtained from vertical scanning mutagenesis reinforced the observed discordance between replication fitness and single-cycle infectivity in Jurkat cells. Despite deficiencies in cell-free infectivity, our data suggest that the Env mutants are capable of efficient cell-cell viral transmission. In addition to rescuing the original p6 Gag mutants, we observe that the Env compensatory mutants can also rescue a replication-defective integrase mutant. Remarkably, we also observe a reduced sensitivity of the Env mutants, relative to WT, to the integrase inhibitor Dolutegravir in spreading infection assays. These results provide insights into the role of Env in mediating HIV-1 cell-cell transfer and suggest that mutations in Env can contribute to HIV drug resistance.
F47. HIV-1 Entry Depends on Virus-Induced Cell Surface Exposure of Phosphatidylserine

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(1) HIV-1 binding to a target cell triggers non-apoptotic exposure of phosphatidylserine (PS) at the cell surface; (2) PS is triggered by specific HIV-1 Env-coreceptor interactions, which initiate calcium signaling and thereby activate the lipid scramblase TMEM16F; (3) suppression of the PS exposure in cells expressing CD4 and co-receptors potently inhibits the fusion stage of HIV-1 infection in cell culture and in an ex vivo tonsillar tissue model; and (4) cell surface PS promotes fusogenic restructuring of HIV-1 Env glycoprotein after the formation of ternary Env-CD4-coreceptor complexes.

HIV-1 predominantly infects activated CD4 T cells. Mechanisms by which complex and poorly defined processes of cell activation facilitate HIV-1 infection and determine which of the cells expressing CD4 and co-receptors will be infected and which will not remain elusive. Phosphatidylserine signaling is a known hallmark of several pathways of activation of immune cells. Thus, the discovery that HIV-1 coopts phosphatidylserine-signaling pathways to enhance cell entry suggests a specific mechanism in which the activation status of a target cell controls the efficiency of viral entry and, thus, the outcome of infection. The signaling pathways that deliver PS to the cell surface and interactions between this lipid and viral Env protein may present new therapeutic targets for suppression of HIV-1 transmission.

In a broader context, the uncovered link between HIV-1 infection and PS externalization identifies a novel bi-directional signaling pathway in which the classic outside-in signaling through GPCR-coreceptor interactions triggers, via intracellular Ca2+ rise, inside-out phosphatidylserine externalization signaling mediated by TMEM16F. We suggest that other viruses also utilize the novel feedback mechanism in which the fusion stage of viral entry depends on the ability of the cell to mount certain signaling pathways.
F48. The Conformational Stability of SOSIP

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The envelope glycoprotein of HIV-1 is a trimer containing three identical gp120 subunits. gp120 is the molecule that binds CD4 and, consequently, the initiator of the infection process and a target for drug development. Since soluble recombinant monomeric gp120 is not a good surrogate of the envelope (e.g. antiviral potency of inhibitors does not correlate with binding affinity), different attempts have been made to develop soluble versions of the trimeric envelope. Of all these trimeric versions, cleaved disulfide stabilized SOSIP gp140 appears to be the most promising and, therefore, a good model system to investigate inter-domain and inter-subunit interactions and how inhibitors with high antiviral potency affect those interactions.

Inter-domain and inter-subunit interactions can be evaluated by measuring and analyzing the heat capacity function associated with the folding/unfolding equilibrium of a protein. We have measured the heat capacity function of unliganded SOSIP (BG505-SOSIP) and SOSIP bound to the inhibitor BMS-626529 (BMS-529), which is the active product of the prodrug BMS-663068 currently in clinical trials. The results indicate that three cooperative domains are involved in the folding/unfolding equilibrium. Neither of those three domains involve gp41, as gp41 is folded at all temperatures studied. Two domains do not participate in inter-subunit interactions; however, these domains are the ones affected by BMS-529 binding. Examination of the crystallographic structure suggests that these domains correspond to the inner and outer domains as these are the ones involved in BMS-529 binding. Finally, one cooperative domain involves all three subunits but is unaffected by BMS-529 binding. This domain most likely involves the so-called trimer association domain which includes the V1/V2 and V3 loops from each subunit. The deconvolution analysis of the heat capacity function provides not only the stability of different domains but the cooperative interactions existing between them. By knowing the effects of effective inhibitors on those interactions it should be possible to develop thermodynamic rules for the development of better and more potent inhibitors. Supported by NIH P01 GM056550.
F49. Quaternary Configuration of the Functional Cd4-Binding Site in the HIV-1 Env Trimer

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The native HIV-1 envelope (Env) spike on the surface of mature virions is a heavily glycosylated trimer of gp120–gp41 heterodimers, which mediates viral attachment and entry. Binding of the gp120 Env trimer to the host CD4 receptor is the first step in the HIV-1 infectious cycle. Although the CD4-binding site of gp120 has been extensively characterized by mutagenesis and co-crystallization with soluble CD4 (sCD4), most of these studies were performed with monomeric gp120 subunits, thus hindering the evaluation of the role of quaternary elements that may be involved in the initial CD4. Moreover, the initial receptor interaction has been difficult to study as gp120 Env trimer, upon CD4 binding, undergoes major structural rearrangements, transitioning to a state that is competent for interaction with the CCR5 or CXCR4 coreceptor.

Here we present the cryo-EM structure of a conformationally constrained HIV-1 Env soluble trimer (DS-SOSIP.664) complexed with sCD4 and a trimer-specific broadly neutralizing antibody, PGT145. DS-SOSIP.664 trimer remains in a pre-fusion, closed conformation after interaction with CD4, permitted to visualize the initial contact with CD4. We found that the initial CD4-contact site in the HIV-1 Env trimer is constituted by a quaternary surface formed by coalescence of the previously defined CD4-binding region in the outer domain of one gp120 protomer with a second CD4-binding site (CD4-BS2) that encompasses discontinuous elements from the inner domain of a neighboring gp120 protomer. Disruption of CD4-BS2 destabilized CD4-trimer interaction and abrogated HIV-1 infectivity by preventing acquisition of coreceptor-binding competence. A corresponding reduction in HIV-1 infectivity occurred upon mutation of CD4 residues that interact with CD4-BS2. We also documented quaternary interactions for selected neutralizing antibodies targeting the CD4 supersite, which suggested an immunogenic nature of CD4-BS2 region in vivo. Thus, the CD4-BS2 region may provide a new molecular target for the development of HIV-1 entry inhibitors or improvement of neutralizing antibodies.
Dendritic cells (DCs) play a critical role in the immune response to viral infection through the facilitation of cell intrinsic antiviral activity and the activation of adaptive immunity. Infection of DCs by HIV-1 triggers cGAS dependent innate immune response. Previously, we have identified PQBP1 protein, through RNAi screen, as a retrovirus specific cofactor of cGAS essential for the innate sensing of retroviral infection. Here, we investigate the regulation on PQBP1/cGAS sensing complex, especially the post-translational modification (PTM) regulation on PQBP1 dependent response to HIV-1 infection. We report that PQBP1 preferentially associate with post-translationally modified cGAS and this interaction specifically enriched by HIV-1 infection but not by introduction of well-established cGAS ligand herring testis DNA (HT-D). We hypothesize that a highly specific affinity of PQBP1 for the modified cGAS provides a specificity for retroviral PAMP sensing as well as enables a dynamic regulation on cGAS sensing in response to cell state.
F51. Global Proteomics of HIV-1 Infection Uncovers Mechanisms of Host Cellular Pathway Rewiring

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The human immunodeficiency virus type-1 (HIV-1) extensively remodels its host environment in order to create conditions optimal for viral replication and immune evasion. The virus has evolved to hijack the ubiquitin proteasome system to promote the degradation of antiviral factors such as APOBEC3G and BST2/tetherin. HIV-1 also modulates myriad signaling pathways affecting the cell cycle, transcriptional regulation, and innate immune processes. Identifying proteins whose post-translational modification state is altered during the course of infection helps to gain a comprehensive understanding of the pathways and processes modulated by the virus.

We applied quantitative proteomics to globally measure changes in host ubiquitylation, phosphorylation, and protein abundance in response to HIV-1 infection. Additionally, mutant viruses were profiled to map the activity of post-translational modification events to specific HIV-1 accessory genes. This approach was able to identify known substrates of HIV-mediated ubiquitination and degradation, including APOBEC3C, tetherin, HLTF, and CD4, as well as known phosphorylation events that are the hallmark of cell cycle arrest induced by HIV-1. We also confirmed recent reports of Vif-mediated degradation of B56-containing protein phosphatase 2A (PP2A-B56) holoenzymes.

Domain analysis identified many proteins ubiquitylated in structured protein domains. Combined with a CRISPR-Cas9 functional screening approach in primary CD4+ T cells, we identified protein domains whose ubiquitylation may alter their activity to the benefit of viral replication, including Ku70 and Ku80 – proteins of the Ku complex that mediate non-homologous end-joining DNA repair – and CPSF6, a pre-mRNA 3’ processing factor.

Global phosphorylation analysis identified a limited set of Vif-dependent phosphorylation events that correlated with PP2A-B56 degradation. One substrate containing 4 regulated phosphosites, GEF-H1, is known to interact with PP2A-B56 proteins, plays a defined role in innate immune signaling, and is known to be inhibited by phosphorylation. We hypothesized that GEF-H1 is a PP2A-B56 substrate, and that HIV-1 degrades PP2A-B56 in order to maintain GEF-H1 in an inactive, phosphorylated state in order to inhibit innate immune activation. However, GEF-H1 knockout in primary CD4+ T cells actually decreased infection in three donors, suggesting that while dephosphorylated GEF-H1 may play a restrictive role in HIV replication, phosphorylated GEF-H1 may play a supportive role in infection.
CRISPR/Cas9 gene editing strategies have revolutionized our ability to engineer the human genome for robust functional interrogation of complex biological processes. We have recently adapted this technology to primary human CD4+ T cells to generate a high throughput platform for analyzing the role of host factors in HIV replication and pathogenesis. CRISPR/Cas9 ribonucleoproteins (crRNPs) are synthesized in vitro and delivered to activated primary human CD4+ T cells by electroporation to achieve high efficiency editing. This platform supports the arrayed generation of hundreds of specific gene manipulations in only a few hours time and is widely adaptable to an array of downstream applications and protocols.

We first used this platform to perform proof-of-principle experiments targeting host factors with defined roles in HIV replication. CXCR4 or CCR5 knock-out primary T cells, for example, are resistant to HIV infection in a tropism-dependent manner. We next bridged this approach to other proteomic and genomic discovery platforms as a secondary screen for host factor functionality, targeting several hundred genes in isogenic donor sets. These efforts have identified several novel dependency and restriction factors that act on or with defined viral proteins or complexes. Additionally, we found that multiplexing of these crRNPs allows for the editing of multiple genes simultaneously, enabling studies of redundancy and epistasis among multiple host and viral factors. Finally, adaptation of this technology to additional primary cell types, such as tissue-derived effector T cells and monocytes, has allowed for the functional interrogation of other diverse biological processes and disease states.

This technology should not only prove useful for discovery-based scientific research, but may also accelerate target validation for pharmaceutical and cell-based therapies. We are currently adapting this technology for the targeted insertion of single nucleotide polymorphisms and for the insertion of larger expression cassettes.
F53. Animating the Science of HIV

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The Science of HIV project seeks to create scientifically accurate and visually stunning animations of the HIV life cycle, highlighting the findings of the P50 Centers for HIV/AIDS-Related Structural Biology. The primary method of outreach to the public will be a multimedia-rich website (http://ScienceofHIV.org). The final animation, which will be approximately 5-10 minutes in length, will tell the compelling story of how a virus is able to hijack its host cell in molecular detail. The visualization will be made available to students, educators and the public in a variety of media contexts, and may act as a powerful means to display the impressive progress that has been made in understanding the molecular mechanisms of HIV infection.
F54. CellPAINT-HIV: Interactive Illustration of HIV Structure for Education and Hypothesis Generation

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Cellular and molecular biology are rapidly becoming integrative disciplines, where data from many spatial and temporal scales are combined to build a complete understanding of the processes of life. We have built an interactive program for integrating diverse biochemical and structural information into an interactive illustration of HIV, blood plasma, and a portion of a cell, depicted with molecular detail. Users build a scene using the molecular components, micrographs, and data from proteomics and interactomics. We are currently testing the program as a tool for HIV education and as a tool for hypothesis generation by HIV structural biologists.
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Some useful notes:

- The conference is located in Natcher (Building 45), southwest of the Gateway Center entrance to campus. The Medical Center Metro stop is next to the Gateway Center.
- Non-NIH employees must undergo inspection and receive temporary ID cards at the Gateway Center (see additional information on following pages).
- Parking garage MLP-11 is for non-NIH employees; NIH employees may park in the garage under Building 45 (requires car safety inspection next to Building 38A) or in other employee parking.
- Dining options on the NIH campus include Eurest Dining Services locations in Buildings 45, 1, 10, and 31, Maryland Business Enterprise Program for the Blind locations in Buildings 38A and 12B, and a concession stand in the Natcher lobby (http://does.ors.od.nih.gov/food/index.htm).
- An ATM is located in the Natcher lobby.
Main Visitor Entrance: NIH Gateway Drive
Gateway Center - Building 66 (for pedestrians entering campus)

Gateway Inspection Station - Building 66A (for vehicles entering campus)

- Monday – Friday: 5am – 10pm; Weekends and After Hours: Closed
  After hours: After 10pm on weekdays, all day weekends and holidays, pedestrians and visitors in vehicles should enter campus via the Commercial Vehicle Inspection Facility (CVIF) - Building 67 (on Rockville Pike between North Drive and Wilson Drive)
- After inspection, vehicles enter campus at Center Drive
- Roadway at Center Drive is for entering campus only; visitors exiting campus may exit from other open locations. To see a list of exits, please see the map.
- All vehicles and their contents will be inspected upon entering the campus.
Multi-Level Parking Garage 11 – MLP-11 (for parking outside of campus)

- Monday – Friday: 6am – 9pm (entrance) 6am – 11pm (exit) Weekends: Closed
- When MLP-11 is closed, visitors can park in lots on the NIH Campus
- Visitors parking in this garage should proceed to the Gateway Center (Bldg. 66) to get a visitor badge
- All visitors traveling in a vehicle are highly encouraged to park in MLP-11 as there is limited visitor parking on the main campus
- No vehicle inspection required to park in MLP-11
- Vehicles left in the MLP-11 parking garage after 11pm on weekdays or during any weekends are subject to ticketing and towing
- Cost: $2 per hour for the first three hours, $12 maximum for the entire day

Directions to NIH Gateway Drive from Rockville Pike/Wisconsin Avenue:

Southbound:
1. Continue on Rockville Pike past South Drive
2. Turn right at NIH Gateway Drive

Northbound – Option 1:
1. Continue on Rockville Pike past South Drive
2. Make a u-turn from the left turn lane at Wilson Drive
3. Continue southbound on Rockville Pike past South Drive
4. Turn right at NIH Gateway Drive

Northbound – Option 2:
1. Continue on Rockville Pike
2. Turn left at Battery Lane
3. Turn right on Old Georgetown Road
4. Turn right on Cedar Lane
5. Turn right on Rockville Pike
6. Continue southbound on Rockville Pike past South Drive
7. Turn right at NIH Gateway Drive

Northbound – Option 3:
1. Continue on Rockville Pike to South Drive
2. Make a u-turn from the left turn lane at South Drive
3. Continue southbound on Rockville Pike
4. Turn right at NIH Gateway Drive
Security Procedures for Entering the NIH Campus:
* All visitors and patients—please be aware: Federal law prohibits the following items on Federal property: firearms, explosives, archery equipment, dangerous weapons, knives with blades over 2 ½ inches, alcoholic beverages and open containers of alcohol.
* The NIH has implemented security measures to help ensure the safety of our patients, employees, guests and facilities. All visitors must enter through the NIH Gateway Center at Metro or the West Gateway Center. You will be asked to submit to a vehicle or personal inspection.
* Visitors over 15 years of age must provide a form of government-issued ID such as a driver's license or passport. Visitors under 16 years of age must be accompanied by an adult.

Vehicle Inspections – All vehicles and their contents will be inspected upon entering the campus. Additionally, all vehicles entering certain parking areas will be inspected, regardless of any prior inspection. Drivers will be required to present their driver’s license and may be asked to open the trunk and hood. If you are physically unable to perform this function, please inform the inspector and they will assist you.
Vehicle inspection may consist of any combination of the following: Detection Dogs Teams (K-9), Electronic Detection Devices and Manual Inspection.
After inspection, you will be issued a vehicle inspection pass. It must be displayed on your vehicle’s dashboard while you are on campus. The inspection pass is not a "parking permit." It only grants your vehicle access to enter the campus. You can only park in designated parking areas.

Personal Inspections – All visitors should be prepared to submit to a personal inspection prior to entering the campus. These inspections may be conducted with a handheld monitoring device, a metal detector and by visible inspection. Additionally, your personal belongings may be inspected and passed through an x-ray machine.

Visitor passes must be prominently displayed at all times while on the NIH campus.
To learn more about visitor and security issues at the NIH, visit:
For questions about campus access, please contact the ORS Information Line at orsinfo@mail.nih.gov or 301-594-6677, TTY - 301-435-1908.