BIOGRAPHICAL SKETCH

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NAME: Jason T Kaelber

eRA COMMONS USER NAME (credential, e.g., agency login): kaelber

POSITION TITLE: Assistant Research Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University	B.A.	05/2011	Chemistry; Biology
Baylor College of Medicine	Ph.D.	09/2017	Molecular Virology & Microbiology

A. Personal Statement

I am extremely enthusiastic to be collaborating with Dr. Eddy Arnold on his studies of SARS-CoV-2 polyproteins. I lead the Rutgers New Jersey Cryo-Electron Microscopy and Tomography Core Facility and serve as a field expert in cryoelectron microscopy technologies for our state. I am a structural biologist and virologist with experience in every aspect of the cryoelectron microscopy workflow. My broad background in virology and structural biology spans from classical virology and cell biology techniques to purification to cryoelectron microscopy, structure determination, and qualitative and quantitative interpretation of electron density maps. At the Rutgers New Jersey CryoEM/ET Core Facility, my recent structure of AAV strain hu.37 is among the highest-resolution cryoEM structures ever published from a 200 kV instrument (paper *a*), demonstrating that my team and I are operating at an internationally-competitive level.nIn addition to work I initiated or drove, I have a demonstrated track record of catalyzing investigator-initiated projects in microbiology, cryoelectron microscopy, and image processing.

At Rutgers I wear two hats: director of the core facility, and as a researcher/educator. These roles interact in a virtuous cycle of workflow optimization, methods development, and training. Through my independent projects (e.g. paper *a*) I continually push the limits of technology at our site, leading to improved capabilities for the community. My active research helps me better relate to and communicate with collaborators with a biochemistry (e.g. paper *b*) or cell biology emphasis. I also serve on the ORED Advisory Committee for Core Facilities.

Building on my prior experience as a teaching assistant of software workshops in cryoEM and as co-organizer of the Center for HIV RNA cryoEM workshop, I co-taught the Rutgers course “Cryo-Electron Tomography” in 2018 and lectured on single-particle reconstruction in 2019 at Rutgers and Cornell Universities. I have also trained numerous scientists hands-on in vitrification, operation of cryoelectron microscopes, and image processing.

My participation in this application pairs my skills in all facets of cryoelectron microscopy (see papers *a-d*) with the skills of principal investigator Dr. Eddy Arnold, whose structural biology discoveries during the AIDS

pandemic were pivotal to the development of small-molecule drugs against HIV. In addition to working towards the highest possible resolutions in single-particle reconstruction, I've also invented novel methods to deal with specimen heterogeneity (paper *c*), which may be beneficial in resolving polyproteins complexes. I am confident that, working together, we will resolve the structures of SARS-CoV-2 polyproteins to gain insight into drug design, structure-function relationships, and the fundamental biology of polyprotein activity and processing in SARS-CoV-2.

a. Kaelber JT, Yost SA, Webber KA, Firlar E, Liu Y, Mercer AC. Structure of the AAVhu.37 capsid by cryoelectron microscopy. *Acta Cryst* 2020 February 5; F76(2).
b. Wang C, Molodtsov V, Firlar E, Kaelber JT, Blaha G, Su M, Ebright RH. Structural basis of transcription-translation coupling. *Science* 2020 Aug 06. PMID: 32820061.
c. Yin Z, Kaelber JT, Ebright R. Structural basis of Q-dependent antitermination. *Proc Natl Acad Sci U S A*. 2019 Sep 10;116(37):18384-18390. PMID: 31455742. PMCID: PMC6744881
d. Kaelber JT, Jiang W, Weaver SC, Auguste AJ, Chiu W. Arrangement of the Polymerase Complexes inside a Nine-Segmented dsRNA Virus. *Structure*, 2020 Feb 7. PMID: 32049031. PMC Journal - In Process

B. Positions and Honors

2009	Stagiaire, Unité de Populations Virales & Pathogenèse, Institut Pasteur
2009	Intern, Microbiology Division, New England Primate Research Center, Harvard Medical School
2007-2011	Laboratory Asst, Dept of Microbiol & Immunol, Baker Institute for Animal Health, Cornell Univ
2011-2017	Predoctoral fellow, National Center for Macromolecular Imaging, Baylor College of Medicine
2017-	Assistant Research Professor, Institute for Quantitative Biomedicine, Rutgers Univ
2017-	Director, Rutgers New Jersey Cryo-Electron Microscopy and Tomography Core Facility
2019-	Member, Cancer Institute of New Jersey

C. Contributions to Science

Reconstructing ancient events in viral evolution

The rapid evolution of viruses and lack of universally conserved genes obscure insights into their ultimate provenance from sequence comparison of extant viruses. While the most common approach to paleovirology is searching for endogenized viruses in host genomes, these events are rare and only practicable for events within the last few hundred million years. To query the history of a non-endogenized virus, I used the signatures of selective pressure it left in host genomes. Integrating reverse genetics, bioinformatics, and cell biology, I reconstructed ancient host-cell binding events to reconstruct the history of canine parvovirus. I showed its ancestor infected ancient canids until they evolved a defense, but the virus was maintained in other Carnivora until a 20th-century spillover (paper *e*). Because this technique cannot reconstruct the most ancient events, I moved to **structure-based inference of deep evolutionary events**. After establishing the Fako virus system and considering the architecture of the last universal ancestor of *Spinareovirinae* (paper *f*), I solved the atomic structure of Fako virus and am using this to shed light on the evolutionary mechanisms of architectural variation in this family (conference presentation *g*). To enhance my skills in this area I also collaborate with George Fox (co-discoverer of Archaea) to solve structures of archaean ribosomal insertions and use these to better understand ribosomal evolution (conference proceedings *h*). My long-term goal is to determine how many origins of viruses there were, where they came from, and to elucidate principles involved in transitions in virion architecture.

e. Kaelber JT, Demogines A, Harbison CE, Allison AB, Goodman LB, Sawyer SL, Parrish CR. Evolutionary reconstructions of the transferrin receptor of Caniforms supports canine parvovirus being a re-emerged and not a novel pathogen in dogs. *PLos Pathogens* 2012 May;**8**(5):e1002666. PMID: 22570610 PMCID: PMC3342950

f. Auguste JA,* Kaelber JT,* Fokam E,* Guzman H, Carrington C, Erasmus JH, Kamgang B, Popov VL, Jakana J, Liu X, Wood TG, Widen SG, Vasilakis N, Tesh RB, Chiu W, Weaver SC. A newly-isolated reovirus has the simplest genomic and structural organization of any reovirus. *J Virol*. 2015 Jan;89(1):676-687 PMID: 25355879 PMCID: PMC4301156 (* denotes co-first authors)
g. Kaelber JT, Jiang W, Weaver SC, Auguste AJ, Chiu W. CryoEM structures of the capsid, non-icosahedral replicases, and dsRNA in the simplest reovirus. Presented at: Gordon Research Conference Physical Virology; 2017 Jan 30; Lucca, Italy.
h. Tirumalai MR, Kaelber JT, Park D, Chiu W, Fox GE. Complexity in Ribosomal Evolution — A Case Study of an Evolutionarily Divergent Recent Insertion in the 5S RNA. In: *XVIIIth International Conference on the Origin of Life*. 2017 Jul 16-21; San Diego, California. Houston: Lunar and Planetary Institute; 2017. #4225.

Facilitating investigator-initiated cryoEM projects through technical excellence

For projects driven by other investigators (such as this one), I have a **track record of enabling discoveries by providing cutting-edge technical expertise and collaboration** (papers *i-l*). The 3Å structure of quorum-sensing protease Rgg3 in complex with its cognate peptide (66 kDa dimer) is among the lowest molecular weight complexes to be resolved by high-resolution single-particle reconstruction (paper *i*). I repurposed single-particle techniques to resolve the structure of the helical NrdEF filament, whose pitch is too extreme for traditional helical reconstruction (paper *j*). I collaborate with academic labs (paper *k*) and with companies for cryoEM to support translational projects in therapeutics or vaccines. Also, ongoing work on the expressome is solving long-standing, fundamental questions about how translation occurs in bacteria (paper *l*).

i. Capodagli GC, Tylor KM, Kaelber JT, Petrou VI, Federle M, Neiditch MB. Structural basis of Rgg protein binding to their regulatory pheromones and target DNA promoters. *bioRxiv* [Preprint] 2020 Apr 30. Available from: https://doi.org/10.1101/2020.04.30.069369
j. Thomas WC, Brooks FP 3rd, Burnim AA, Bacik JP, Stubbe J, Kaelber JT, Chen JZ, Ando N. Convergent allostery in ribonucleotide reductase. *Nat Commun*. 2019 Jun 14;10(1):2653. doi: 10.1038/s41467-019-10568-4. PMID: 31201319. PMCID: PMC6572854.
k. Erasmus JH, Auguste AJ, Kaelber JT, Luo H, Rossi SL, Fenton K, Leal G, Kim DY, Chiu W, Wang T, Frolov I, Nasar F, Weaver SC. A chikungunya fever vaccine utilizing an insect-specific virus platform. *Nat Med*. 2017 Feb;23(2):192-199. PMID: 27991917 PMCID: PMC5296253
l. Wang C, Molodtsov V, Firlar E, Kaelber JT, Blaha G, Su M, Ebright RH. Structural basis of transcription-translation coupling. *Science* 2020 Aug 20. PMID: 32820061

Elucidating the basis of cellular phenotypes through cryoelectron tomography

Cryoelectron tomography can provide an untargeted ultrastructural census of whole cells. This makes it ideal to pursue known phenotypes with unknown mechanisms. For example, it was known that ovarian cancer causes a change in platelets but the nature of that change was unknown. I showed that based only on tomograms of platelets I can predict whether the person whence they were derived has an ovarian malignancy (paper *m*). I contributed data analysis to my colleagues who demonstrated that the malignancy-related changes to platelets include alterations to the mitochondria and to the marginal band, a platelet-specific cytoskeletal feature. To understand why lateral attachment of the *Trypanosoma* flagellum is required for directional motion, I imaged mutants by cryoET, wrote new code to analyze intermicrotubule relationships and cytoskeletal bending, proposed a model for force transduction in this organism, and collaborated closely with several colleagues to find that beating of the laterally-attached *Trypanosoma* flagellum contorts the cell body in a way necessary for directional motion (paper *n*). Currently, using our recently-acquired cryogenic focused ion beam, my lab is applying cryoelectron tomography of bacterial cells to understand their metabolic regulation as well as collaborating with the Ebright lab to understand heterogeneous transcription structures.

m. Wang R, Stone RL, Kaelber JT, Rochat RH, Nick AM, Vijayan KV, Afshar-Kharghan V, Schmid MF, Dong JF, Sood AK, Chiu W. Electron cryotomography reveals ultrastructure alterations in platelets from patients with ovarian cancer. *PNAS* 2015 Nov 17;112(46):14266-71 PMID: 26578771 PMCID: PMC4655568
n. Sun SY, Kaelber JT, Chen M, Dong X, Nematbakhsh Y, Shi J, Dougherty M, Lim CT, Schmid MF, Chiu W, He CY. Flagellum couples cell shape to motility in *Trypanosoma brucei*. *PNAS* 2018 Jun 11; PMID: 29891682 PMCID: PMC6042131

List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/jason.kaelber.1/bibliography/public/

D. Additional Information: Research Support

COVID-19 Research Proposals in the Biomedical Sciences (Intramural) 06/01/20-06/01/21
"Structural and Small-Molecule Binding Studies of SARS-CoV-2 Polyproteins and Proteins"
We are producing recombinant SARS-CoV-2 polyproteins, characterizing their structures with cryo-electron microscopy and X-ray crystallography, and screening synthetic small molecule fragments for interaction with the purified proteins.
Role: Co-PI

Sponsored research agreement, RegenXBio 05/2019-04/2021
"Characterization of AAV structures by cryo-electron microscopy"
Using structural biology tools I am revealing the mechanisms underpinning host cell tropism and cell penetration.
Role: PI

Pilot grant, Brain Health Institute 01/2020-01/2021
"Architecture of ribosome heterogeneity in developing neocortex under the control ofRNA binding proteins"
I aim to determine the atomic structure of specialized ribosomes purified from neocortical tissue to elucidate mechanisms of translational control by development-specific cofactors.
Role: co-PI

New faculty start-up funds, Rutgers University, Institute for Quantitative Biomedicine
09/2017-09/2020
"The origin(s) of viruses: how many were there?"
I aim to combine high-throughput determination of virion structure with structural bioinformatics to reconstruct the deep phylogeny of viral hallmark proteins, concentrating first on unifying the dsRNA families and understanding transitions between the ssDNA families.
Role: PI

BIOGRAPHICAL SKETCH

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NAME: Arnold, Eddy

eRA COMMONS USER NAME (credential, e.g., agency login): EdwardArnold

POSITION TITLE: Resident Faculty Member, CABM, Board of Governors and Distinguished Professor of Chemistry and Chemical Biology, Rutgers University

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University, Ithaca, NY	B.A.	06/1978	Chemistry
Cornell University, Ithaca, NY	Ph.D.	06/1982	Organic Chemistry
Purdue University, West Lafayette, IN	Postdoctoral	06/1987	Virus Crystallography

A. Personal Statement

Starting in 1987 my laboratory has worked to understand the structural and molecular basis of human infectious disease problems and to apply the insights gained to the development of better treatments. A central topic of our studies has been reverse transcriptase (RT), which is an essential component of the AIDS virus and the target of many of the most widely used anti-AIDS drugs. Using the techniques of X-ray crystallography, our team, including Dr. Stephen Hughes of the NCI at Frederick, has solved the three-dimensional structures of HIV-1 RT in complex with antiviral drugs and segments of the HIV genome. These studies have illuminated the working of an intricate and fascinating biological machine in atom-by-atom detail and have yielded numerous novel insights into polymerase structure-function relationships, detailed mechanisms of drug resistance, and structure-based design of RT inhibitors. Synthesis of the information led to the development of two drugs with outstanding potency against known drug-resistant variants (etravirine/Intelence and rilpivirine/Edurant) and five licensed medicines currently used for treating HIV infection. Together with multiple expert collaborators we are continuing to study the molecular basis of HIV RT function, inhibition, and resistance, and are extending these studies to the HIV precursor polyproteins. Success in protein and crystal engineering yielding high-resolution crystals of HIV-1 RT (1.5 Å resolution) has enabled a systematic application of crystallographic fragment screening, revealing several novel allosteric sites for inhibiting RT polymerase and RNase H activity. Other recent projects have included studies of the influenza virus polymerase, in particular the endonuclease domain and structures of bacterial RNA polymerase—the central transcription machinery and a validated target for treatment of tuberculosis. Among the structures analyzed (with Richard Ebright) include RNA polymerase complexes with antibiotics, an open-promoter complex that revealed the structural basis of transcription initiation, and M. *thermophilus* RNA polymerase complexed with nucleic acid and the key tuberculosis drug rifampicin.

In addition to continuing to characterize key functional states of HIV-1 RT by crystallography, my group has teamed up with cryo-EM expert Dmitry Lyumkis to determine the first structures of the HIV precursor polyproteins. This unique opportunity arose because of a breakthrough in my laboratory in obtaining multiple-milligram amounts of the HIV-1 Gag-Pol and Pol polyproteins in soluble and purified forms, permitting successful application of cryo-EM and other biophysical methods that are described in this

proposal. The wide variety of tools we have developed to study HIV-1 RT is applicable to the polyproteins, because the core feature of the Pol dimer structure is RT p66/p51 heterodimer-like. As an example, the newly engineered diabodies that recognize only the p51 subunit in HIV-1 RT are immediately applicable to creating particle displays of HIV Gag-Pol and Pol for both cryo-EM and crystallography. The diabodies were originally engineered as a scaffold for creating larger displays of RT for cryo-EM, and they are now proving advantageous for crystallization, cryo-EM, and other biophysical studies of both RT and the HIV Pol and Gag-Pol polyproteins.

My laboratory has provided a strong training environment for scientists at all levels, including research faculty, postdoctoral and graduate fellows, undergraduates, and laboratory scientists. Scientists I have mentored have established research programs at top universities both in the U.S. and internationally, and have also attained leadership positions in industry and government. The cross-disciplinary research in the group uses a broad swath of tools and techniques from molecular biology, protein chemistry and biochemistry, biophysics, virology, crystallography, cryo-EM, and computational chemistry.

B. Positions and Honors

Professional Experience:

1978 - 1979	Teaching Associate, Chemistry, Cornell University
1979 - 1982	Graduate Research Assistant, Chemistry, Cornell University (with Jon Clardy)
1982 - 1987	Postdoctoral Research Associate, Biological Sciences, Purdue University (with Michael G. Rossmann)
1987 - present	Resident Faculty Member, Center for Advanced Biotechnology and Medicine
1987 - 1993	Assistant Professor of Chemistry, Rutgers University
1993 - 1996	Associate Professor of Chemistry, Rutgers University
1996 - 1999	Professor of Chemistry, Rutgers University
1999 - present	Professor II (Distinguished Professor) of Chemistry and Chemical Biology, Rutgers University
2010 - present	Board of Governors Professor of Chemistry and Chemical Biology, Rutgers University

Honors and Awards:

1979 - 1980	Merz Prize in Organic Chemistry, Cornell University; Cornell University Graduate Fellowship
1979 - 1982	National Science Foundation Predoctoral Fellowship, Cornell University
1982 - 1984	Damon Runyon-Walter Winchell Postdoctoral Fellow
1985 - 1987	NIH Postdoctoral Fellow
1990 - 1992	Alfred P. Sloan Research Fellowship
1994	Distinguished Lecturer at the European Molecular Biology Laboratory, Heidelberg, Germany
1996	Recognized as one of the most cited scientists in the field of AIDS research. *ScienceWatch*
1999 - 2008	NIH MERIT Awardee
2001	Board of Trustees Award for Excellence in Research at Rutgers, The State University of New Jersey
2001	Elected Fellow of the American Association for the Advancement of Science
2005 - 2011	Chair, Biological Macromolecules Commission, International Union for Crystallography
2006	Elected Fellow of the American Academy of Microbiology
2009 - 2019	NIH MERIT Awardee (Second consecutive award)
2013	Hyacinth Award “Honoring Outstanding Achievements in the Struggle Against HIV/AIDS,” from the Hyacinth AIDS Foundation
2014	Elected Fellow of the American Crystallographic Association
2016	Pennsylvania Drug Discovery Institute Award
2020	Rutgers University Chancellor’s Award for Pioneering Research

C. Contributions to Science

1. **HIV-1 reverse transcriptase structure, function, and drug resistance.** I began studies of HIV-1 RT in 1987 in collaboration with Dr. Stephen Hughes, when no structure of any HIV protein was known. In 1993 we reported the structure of HIV-1 RT in complex with a double-stranded DNA template-primer and an antibody Fab fragment at 3.0 Å resolution. This was the first structure reported for any polymerase

complexed with nucleic acid in a mode relevant for polymerization, which permitted the identification of the roles played by highly conserved motifs that had previously been identified by sequence analysis. The structure of the complex with the template-primer provided insight into the interactions of RT with its nucleic acid substrates, the mechanism of polymerization, and the structural basis of resistance to anti-AIDS drugs targeting RT. The RT/DNA/Fab28 structure appeared shortly after a 3.5 Å resolution structure of HIV-1 RT complexed with the non-nucleoside inhibitor nevirapine from Professor Thomas Steitz and coworkers (Kohlstaedt *et al.*, Science **256**, 1783-1790, 1992). Our work provided the first complete amino acid residue assignment for the p66/p51 heterodimer. We coined the terms "primer grip" and "template grip" to refer to structural elements near the dNTP-binding site that interact with the primer and template strands and act as clamps to position the template-primer for polymerization (and RNase H cleavage). In 2001 we reported the structure of HIV-1 RT in complex with an RNA:DNA template-primer containing the polypurine tract at 3.0 Å resolution. This was the first published example of any protein complexed with an RNA:DNA duplex. The structure of the complex revealed additional contacts with the nucleic acid in the vicinity of the RNase H active site, including a series of contacts between the enzyme and the DNA primer that we named the "RNase H primer grip."

a. Jacobo-Molina, A., J. Ding, R.G. Nanni, A.D. Clark, Jr., X. Lu, C. Tantillo, R.L. Williams, G. Kamer, A.L. Ferris P.Clark, A. Hizi, S.H. Hughes, and **E. Arnold**. 1993. Crystal structure of human immunodeficiency virus type 1 reverse transcriptase complexed with double-stranded DNA at 3.0 Å resolution shows bent DNA. Proc. Natl. Acad. Sci. USA **90**:6320-6324. (1305 citations)

b. Sarafianos, S.G., K. Das, C. Tantillo A.D. Clark, Jr., J. Ding, J. Whitcomb, P.L. Boyer, S.H. Hughes, and **E. Arnold**, 2001. Crystal structure of HIV-1 reverse transcriptase in complex with a polypurine tract RNA:DNA. EMBO J. **20:**1449-1461. (444 citations)

c. Sarafianos, S.G., B. Marchand, K. Das, D.Himmel, M. Parniak, S.H. Hughes, and **E. Arnold**. 2009. Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization, and inhibition. J. Mol. Biol. **385**:693-713. (438 citations)

d. Das, K., S. Martinez, J. DeStefano, and **E. Arnold**. 2019. Structure of HIV-1 RT/dsRNA initiation complex prior to nucleotide incorporation. Proc. Natl. Acad. Sci. USA **116**:7308-7313.

2. **Design and discovery of two drugs, etravirine/Intelence and rilpivirine/Edurant, that are non-nucleoside RT inhibitor (NNRTI) therapeutics used to treat HIV-1 infections.** We began a structure-based drug design effort with Dr. Paul Janssen in 1990 that led to the design and invention of etravirine/Intelence and rilpivirine/Edurant. Our crystallographic studies of HIV-1 RT complexed with non-nucleoside inhibitors enabled understanding of NNRTI binding principles and fueled the drug discovery effort. We also discovered a hydrophobic drug aggregation phenomenon that explained the nearly 100% oral bioavailability of rilpivirine. More recently we reported a crystallographic fragment screening effort that led to discovery of previously unknown allosteric inhibitory sites of HIV-1 RT.

a. Janssen, P.A.J., P.J. Lewi, **E. Arnold**, F. Daeyaert, M. de Jonge, J. Heeres, L. Koymans, M. Vinkers, J. Guillemont, E. Pasquier, M. Kukla, D. Ludovici, K. Andries, M.-P. de Béthune, R. Pauwels, K. Das, A.D. Clark, Jr., Y.V. Frenkel, S.H. Hughes, B. Medaer, F. De Knaep, H. Bohets, F. De Clerck, A. Lampo, P. Williams, and P. Stoffels. 2005. In search of a novel anti-HIV drug: multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-2cyanoethenyl]- pyrimidinyl 2,6-dimethylphenyl]amino]-2-]amino]-benzonitrile (R278474, rilpivirine). J. Med. Chem. 48:1901-1909. (333 citations)

b. Das, K., P.J. Lewi, S.H. Hughes, and **E. Arnold**. 2005. Crystallography and the design of anti-AIDS drugs: conformational flexibility and positional adaptability are important in the design of non-nucleoside HIV-1 reverse transcriptase inhibitors. Prog. Biophys. Mol. Biol. 88:209-231. (254 citations)

c. Das, K., S.E. Martinez, J.D. Bauman, and **E. Arnold**. 2012. HIV-1 reverse transcriptase complex with DNA and nevirapine reveals nonnucleoside inhibition mechanism. Nat. Struct. Mol. Biol. **9**:253-259. (128 citations)

d. Bauman, J.D., D. Patel, C. Dharia, M.W. Fromer, S. Ahmed, Y. Frenkel, R.S.K. Vijayan, J.T. Eck,W.C.Ho, K. Das, A.J. Shatkin, and **E. Arnold**. 2013. Detecting allosteric sites of HIV-1 reverse transcriptase by X-ray crystallographic fragment screening. J. Med. Chem. **56**:2738-2746. (52 cites)

3. **Structural basis of HIV-1 RT drug resistance, and a general strategy for targeting drug resistance**. Determination of structures of wild-type and drug-resistant variants of HIV-1 RT have elucidated the structural basis of resistance to many of the nucleoside and non-nucleoside drugs that are widely used in the treatment of HIV-1 infections. Based on the work that led to the discovery of etravirine and rilpivirine, which are highly effective in inhibiting drug-resistant variants, we developed a design concept for overcoming resistance called the "strategic flexibility model." Compounds that "wiggle" (structural flexibility) and "jiggle" (compactness) can adapt to mutations in a binding pocket to overcome resistance. We were able to confirm this model using engineered high-resolution crystals of HIV-1 RT and the NNRTI drug

rilpivirine, which is highly resilient to drug resistance. We also have developed a comprehensive model of resistance to the important nucleoside drug AZT/zidovudine, in which an ATP that binds to AZT-resistant HIV-1 RT is used to excise a AZT following its incorporation into the primer strand.

a. Tantillo, C., J. Ding, A. Jacobo-Molina, R.G. Nanni, P.L. Boyer, S.H. Hughes, R. Pauwels, K. Andries, P.A.J. Janssen, and **E. Arnold**. 1994. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase: implications for mechanisms of drug inhibition and resistance. J. Mol. Biol. **243**:369-387. (604 cites)

b. Das, K., A.D. Clark, Jr., P. Lewi, J. Heeres, M. de Jonge, L. Koymans, M. Vinkers, F. Daeyaert, D.W. Ludovici, M.J. Kukla, B. De Corte, R.W. Kavash, C. Ho, H. Ye, M.A. Lichtenstein, K. Andries, R. Pauwels, M.-P. de Béthune, P.L. Boyer, P. Clark, S.H. Hughes, P.A.J. Janssen, and **E. Arnold**. 2004. Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. J. Med. Chem. **47**: 2550- 2560. (533 citations)

c. Das, K., J.D. Bauman, A.D.Clark, Jr., Y.V. Frenkel, P.J. Lewi, A.J. Shatkin, S.H.Hughes, and **E. Arnold**. 2008. High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: strategic flexibility explains potency against resistance mutations. Proc. Natl. Acad. Sci. **105**:1466-1471 (302 citations)

d. Tu, X., K. Das, Q. Han, J.D. Bauman, A.D. Clark, Jr., X. Hou, Y.V. Frenkel, B.L. Gaffney, R.A. Jones, P.L. Boyer, S.H. Hughes, S.G. Sarafianos, and **E. Arnold**. 2010. Structural basis of HIV-1 resistance to AZT by excision. Nat. Struct. Mol. Biol. **17**:1202-1209. (98 citations)

4. **Bacterial RNA polymerase structure, function, and inhibition; structural basis of transcription**. With Professor Richard Ebright we have solved the structures of complexes of *T. thermophilus* and *E. coli* multisubunit RNA polymerases (RNAPs) with inhibitors and nucleic acid. The structure of the RNAP open-promoter complex was highly informative regarding how the RNAP holoenzyme recognizes, unwinds, and positions the promoter to initiate transcription. This structure has implications for understanding the structural basis of transcription initiation in all living cells. Structures and biochemical studies of RNAP complexed with the antibiotics streptolydigin, myxopyronin, and salinamide A have elucidated their respective binding sites and inhibition mechanisms, and are enabling structure-based design of more potent analogs with the goal of producing new antibiotics for treatment of key human infections caused by bacteria including MRSA and tuberculosis.

a. Tuske, S., S.G. Sarafianos, X. Wang, B. Hudson, E. Sineva, J. Mukhopadhyay, J. J. Birktoft, O. Leroy, S. Ismail, A.D. Clark, Jr., C. Dharia, A. Napoli, O. Laptenko, J. Lee, S. Borokhov, R. Ebright, and **E.Arnold**. 2005. Inhibition of bacterial RNA polymerase by streptolydigin: stabilization of a straight-bridge-helix active-center conformation. Cell. 122:541-552.

b. Mukhopadhyay, J., K. Das, S. Ismail, D. Koppstein, M. Jang, B. Hudson, S. Saratianos, S. Tuske, J. Patel, R. Jansen, H. Irschik, **E. Arnold**, and R.H. Ebright. 2008. The RNA polymerase "switch region" is a target for inhibitors. Cell **135**:295–307.

c. Zhang, Y., Y. Feng, S. Chatterjee, S. Tuske, M.X. Ho, **E. Arnold**, and R.H. Ebright. 2012. Structural basis of transcription initiation. Science **338**:1076-1080.

d. Lin, W., Mandal, S., Degen, D., Liu, Y., Ebright, Y.W., Li, S., Feng, Y., Zhang, Y., Mandal, S., Jiang, Y., Liu, S., Gigliotti, M., Talaue, M., Connell, M., Das, K., E. Arnold, and R.H. Ebright; Structural basis of Mycobacterium tuberculosis transcription and transcription inhibition; Molec. Cell, 66:169-179, 2017.

5. **Influenza virus structure, function, and drug targeting.** We have solved the structures of the influenza NS1B C-terminal domain and the influenza A cap-snatching endonuclease. Both proteins had significant implications for anti-flu drug discovery. From crystallographic fragment screening with endonuclease, we obtained small molecule leads, one of which was elaborated to derivatives with antiviral activity in cell culture.

a. Das, K., L.-C. Ma, R. Xiao, B. Radvansky, J.Aramini, L. Zhao, J. Marklund, R.-L. Kuo, K. Twu, **E. Arnold**, R.M. Krug, and G.T. Montelione. 2008. Structural basis for suppression of a host antiviral response by influenza A virus. Proc. Natl. Acad. Sci. 105:13093-13098.

b. Das, K. J.M. Aramini, L.-C. Ma, R.M. Krug, and **E. Arnold**. 2010. Structures of influenza A proteins and insights into antiviral drug targets. Nat. Struct. Mol. Biol. **17**:530-538.

c. Bauman, J.D., Patel, D., Baker, S.F., Vijayan, R.S.K., Xiang, A., Parhi, A., Martinez-Sobrido, L., LaVoie, E.J., Das, K., and **E. Arnold.** 2013. Crystallographic Fragment Screening and Structure-Based Optimization Yields a New Class of Influenza Inhibitors. ACS Chem. Biol. **8**:2501-2508. PMID: 23978130.

d. Parhi, A.K., Xiang, A., Bauman, J.D., Patel, D., Vijayan, R.S.K., Das, K., **E. Arnold**, and LaVoie, E.J. 2013. Phenyl substituted 3-hydroxypyridin(1H)-2-ones: Inhibitors of influenza A endonuclease.

Bioorg. Med. Chem. **21**:6435-6446. PMID: 24055080.

Link to Eddy Arnold's Google Scholar citations (~29,000 citations; h-index=87; 281 publications captured): https://scholar.google.com/citations?user=3ALJe7MAAAAJ

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01 AI027690 Arnold (PI) 12/01/2019-11/30/2024
Evolving Understanding of HIV Reverse Transcriptase Structure, Function, Inhibition, and Resistance
This major grant to the the Arnold laboratory, now in its 31st year, supports structural studies of wild-type and mutant HIV-1 RT complexed with nucleic acid substrates and inhibitors, and studies of the HIV Pol polyprotein precursor.
Role: PI

U54 AI150472 Torbett (PI, Scripps, HIVE Center) 09/01/2017-08/31/2022
HIV Macromolecular Interactions and Impact on Viral Evolution of Drug Resistance
This grant supports crystallographic fragment screening of HIV-1 RT, CA, and IN, and structural studies by of HIV-1 (Gag-Pol and Pol) and prototype foamy virus (Pol) polyproteins. The efforts on structure determination of the HIV-1 Pol polyprotein by cryo-EM supported by this grant are entirely complementary to the proposed engineering, crystallization, and biophysical studies of HIV-1 Pol proposed in the current application.
Role: Project Leader

R01 GM118012 Sarafianos (PI, Emory) 07/01/2016-06/30/2020
Reverse Transcriptase Multi-Class Drug Resistance and Rilpivirine Susceptibility in Diverse HIV-1 Subtypes
This project studies how resistance to two classes of HIV reverse transcriptase inhibitors develops in different subtypes of HIV.
Role: Co-Investigator

Completed Research Support

NHMRC Grant 1064900 Tachedjian (PI) 01/01/2017-12/31/2019
Novel Class of HIV Reverse Transcriptase Inhibitor for HIV Prevention
This grant supports crystallographic fragment screening by the Arnold group of compounds related to novel inhibitors of HIV-1 RT discovered by principal investigator Gilda Tachedjian and colleagues.
Role: Co-Investigator and Project Leader

R01 AI104660 Ebright (PI) 01/15/2013-12/31/2017
Therapeutics for Drug-Resistant Bacteria: Pseudouridimycins
The major goal of this project is structure-based design, synthesis, and efficacy testing of novel pseudouridimycin analogs effective against drug-resistant bacterial pathogens.
Role: Co-Investigator

P50 GM103368 Olson (PI) 09/01/2012-08/31/2017
HIV Macromolecular Interactions and Impact on Viral Evolution of Drug Resistance
This grant supports studies of HIV-1 and prototypic foamy virus Gag-Pol and Pol polyproteins, crystallographic fragment screening of HIV-1 RT and IN, and chemical synthesis of RT and RNase H inhibitors with improved activity (in collaborations with other Center Project laboratories including Roger Jones and Joseph Marcotrigiano at Rutgers).
Role: Co-Investigator and Project Leader

R21 AI119321 Arnold (PI) 05/01/2015-04/30/2017
Drug Development Targeting Influenza A Cap-Snatching Endonuclease
This R21/R33 supports research at Rutgers University (Arnold and LaVoie laboratories) and University of Rochester (Martinez-Sobrido laboratory) aimed at developing drug candidates targeting the influenza A endonuclease using a structure-guided optimization platform.
Role: PI