

BIOGRAPHICAL SKETCH

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NAME: DAmico, Kevin

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

POSITION TITLE: Postdoctoral Associate

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)	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Rowan University	BS	09/2012	05/2016	Biological Sciences
Princeton University	MA	09/2018	01/2020	Molecular Biology
Princeton University	PHD	09/2018	01/2024	Molecular Biology
Rutgers University	Postdoctoral Associate	02/2024	Present	Virology/Cryo-electron Microscopy

A. Personal Statement

My prior research experience and training, specifically in cryo-electron microscopy (cryo-EM) allow me to make high-quality contributions to the field of structural biology. During my tenure as a research specialist with Dr. Ronen Marmorstein at the University of Pennsylvania, my work on the structural characterization of epigenetic DNA-binding proteins introduced me to the fields of both X-ray crystallography and cryo-EM. My time as a graduate student with Dr. Fred Hughson at Princeton University allowed me to accelerate these learning goals. From a crystallography perspective, I was able to contribute to the structural characterization of several proteins involved in intracellular trafficking at high-resolution. The majority of my time, however, was spent developing a skillset in cryo-EM. While my group initially lacked this expertise, I was able to receive training on this method and related techniques through work with core facilities and collaborators, as well as by attending training seminars and conferences. These efforts allowed me to publish my group's first protein structure generated by cryo-EM: a large- and flexible-heteropentameric complex involved in vesicle capture and membrane tethering. Through this process, I was able to not only gain personal proficiency but establish workflows for other members of the lab to follow. This process included generating protocols, training new users on methodologies, and becoming a lab advocate for advancing technologies regarding both hardware and software. Additionally, as my particular research target was poorly expressed, I gained proficiency in troubleshooting and optimizing protein expression of both individual proteins and heterocomplexes.

Now, as a member of the Dr. Eddy Arnold's lab, I apply my expertise to the field of HIV-1 polyprotein structures. Dr. Arnold is a recognized leader in this field, having published the only available structure of an HIV-polymerase polyprotein as well as having a robust pipeline for expressing and purifying larger polyprotein constructs. As of writing, I have been a member of the Arnold lab for 13 months. In this time, I have led the lab's efforts to establish cryo-EM as a standard method for all members of our group to utilize. This has included training new users on methods like grid preparation and vitrification, screening and data collection at the microscope, and data processing with software including CryoSPARC. My and my coworker's efforts have transformed the lab from a group that collects perhaps one EM dataset per year to one that collects one high-resolution dataset every two weeks. This preliminary success has been awarded in the form of approval for data collection time at facilities such as LBMS at Brookhaven National Laboratory and NCEF at the National Cancer Institute. I am confident that the quality of the data we have collected, as well as the rapid nature in which we have achieved these workflows, is indicative of the success that we will have with the structure

determination of these polyproteins moving forward. Beyond the scope of this project, my individual career goals include pushing forward the field of cryo-EM, especially in regard to flexible proteins that would otherwise be characterized as difficult targets. I also aim to continue educating and training others on the theory and techniques involved in cryo-EM, in order to help others achieve their research and career objectives.

1. Travis S, **DAmico K**, Yu I, McMahon C, Hamid S, Ramierz-Arellano G, Jeffrey P, Hughson F. Structural basis for the binding of SNAREs to the multisubunit tethering complex Dsl1. *The Journal of Biological Chemistry*. 2020 Jul 24; 295(30): 10125-10135 PMID: PMC7383367
2. **DAmico K**, Stanton A, Shirkey J, Travis S, Jeffrey P, Hughson F. Structure of a membrane tethering complex incorporating multiple SNAREs. 2024 Jan 9, 31(2): 246-254 PMID: PMC10923073

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2024 – Present	Postdoctoral Associate, Rutgers University
2018 – 2024	Graduate Student Researcher, Princeton University
2016 – 2018	Laboratory Manager / Research Specialist, University of Pennsylvania
2015 – 2016	Undergraduate Researcher, Rowan University

Honors

2023	Award for best poster at the Molecular Membrane Biology Gordon Research Conference
2018	Award for best mini-proposal in MOL504: Cellular Biochemistry at Princeton University
2016	B.S. awarded with honors, Rowan University

C. Contributions to Science

1. **Early Career:** As an undergraduate researcher at Rowan University, my work focused on the mechanism of viral entry in HSV-1. My tasks included generating specialized mammalian cultures via transfection to express or lack cell-surface receptors of interest. This project resulted in acknowledgements in a collaborator's publication. As a research specialist at The University of Pennsylvania, my projects included the biochemical characterization and structural determination of DNA binding proteins, especially those involved in epigenetics. This involved expressing and purifying constructs for both my own work and that of our collaborators.

- a. Mawhinney M, Liu R, Lu F, Maksimoska J, **DAmico K**, Marmorstein R, Lieberman P, Urbanc B. CTCF-induced circular DNA complexes observed by atomic force microscopy. *Journal of Molecular Biology*. 2018 Mar 16; 430(6): 759-776 PMID: PMC5860984

2. **Graduate Career:** As a PhD candidate at Princeton University, I studied the structure of membrane tethering complexes and their interactions with membrane-bound SNARE proteins. This involved the structural determination, by both X-ray crystallography and cryo-EM, of protein complexes involved in intracellular trafficking. My first-author work, detailing the first structure of a complete tethering complex interacting with SNARE proteins, identified a paradigm of tethering complex organization. This work revealed similarities in architecture between protein complexes that were previously thought to operate through separate mechanisms.

- a. Travis S, **DAmico K**, Yu I, McMahon C, Hamid S, Ramierz-Arellano G, Jeffrey P, Hughson F. Structural basis for the binding of SNAREs to the multisubunit tethering complex Dsl1. *The Journal of Biological Chemistry*. 2020 Jul 24; 295(30): 10125-10135 PMID: PMC7383367
- b. **DAmico K**, Stanton A, Shirkey J, Travis S, Jeffrey P, Hughson F. Structure of a membrane tethering complex incorporating multiple SNAREs. 2024 Jan 9, 31(2): 246-254 PMID: PMC10923073

3. **Postdoctoral Career:** My role as a postdoctoral Associate at Rutgers University is to accelerate the lab's efforts to determine the structure of HIV-1 polyproteins by cryo-EM. While these efforts have not yet resulted in a preprint or publication, we have made significant advancements to our EM workflow that have allowed for the rapid data collection and data processing of these targets.

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 diseases and to apply the insights gained to the development of better treatments. I have authored more than 280 peer-reviewed publications (>200 on the topic of HIV) which have garnered substantial citations (>34,500; h-index=98). A central focus of our studies has been the enzyme reverse transcriptase (RT), which is an essential component of the AIDS virus and the target of numerous widely used anti-AIDS drugs. Using the techniques of X-ray crystallography, our team 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 drug-resistant variants (etravirine/Intelence and rilpivirine/Edurant) and six licensed medicines currently used for treating HIV infection.

In addition to continuing to characterize key functional states of HIV-1 RT by crystallography, we used cryo-EM to determine the structure of the HIV Pol precursor polyprotein in collaboration with Dmitry Lyumkis (Salk). We are extending this work by cryo-EM characterization of Gag-Pol constructs and have seen encouraging improvement of the resolution of the PR region suggesting possible mechanisms of PR activation, one of the central questions in maturation of HIV. We are currently using cryo-EM to investigate the mechanism of selective destruction of HIV-infected cells by TACK NNRTIs, by elucidating the molecular details of how the TACK inhibitors bind to Gag-Pol precursors and accelerate PR activation.

My laboratory has provided a strong training environment for scientists at all levels over a 37-year period, including research faculty, >50 postdoctoral and >25 graduate fellows, >50 undergraduates, and many senior researchers and laboratory scientists. I currently mentor two graduate students, four postdoctoral fellows, and one research faculty member. Scientists I have mentored have established independent research programs at

top universities in both the U.S. and internationally and have also attained leadership positions in industry and government.

Our cross-disciplinary team uses a broad swath of tools and techniques from molecular biology, crystallography, cryo-EM, virology, chemistry, biochemistry, biophysics, and computational chemistry to pursue investigations of biological structure aimed at guiding drug design targeting serious human diseases. I have mentored 8 NIH pre-doctoral trainees and 11 F32 NIH postdoctoral trainees. I have 1) led two NIGMS P01 multi-investigator program projects, and 2) been the recipient of two consecutive MERIT awards, 3) been continuously funded by NIH since 1987, and 4) been the recipient of many other grants from NIH, foundations, institutions, and industry.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

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

Honors and Awards:

2021	Antonin Holy Award from the International Society for Antiviral Research
2020	Rutgers Chancellor's Award for Pioneering Research (Inaugural)
2019	Raymond F. Schinazi Distinguished Lecturer, Emory University
2016	Pennsylvania Drug Discovery Institute Award
2014	Elected Fellow of the American Crystallographic Association
2013	Hyacinth Award "Honoring Outstanding Achievements in the Struggle Against HIV/AIDS," from the Hyacinth AIDS Foundation
2009 - 2019	NIH MERIT Awardee (Second consecutive award)
2006	Elected Fellow of the American Academy of Microbiology
2005 - 2011	Chair, Biological Macromolecules Commission, International Union for Crystallography
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
1999 - 2008	NIH MERIT Awardee
1996	Recognized as one of the most cited scientists in the field of AIDS research. <i>ScienceWatch</i>
1994	Distinguished Lecturer at the European Molecular Biology Laboratory, Heidelberg, Germany
1990 - 1992	Alfred P. Sloan Research Fellowship
1985 - 1987	NIH Postdoctoral Fellow
1982 - 1984	Damon Runyon-Walter Winchell Postdoctoral Fellow
1979 - 1982	National Science Foundation Predoctoral Fellowship, Cornell University
1979 - 1980	Merz Prize in Organic Chemistry, Cornell University; Cornell University Graduate Fellowship

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 and 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 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. PMCID: PMC46920.
- 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. PMCID: PMC145536.
- 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. PMCID: PMC2881421.
- 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. PMCID: PMC6462067.

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. 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-cyanoethenyl]-pyrimidinyl 2,6-dimethylphenyl]amino]-2-amino]-benzonitrile (R278474, rilpivirine). *J. Med. Chem.* **48**:1901-1909. PMID: 15771434.
- 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. PMID: 15572156.
- 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. PMCID: PMC3359132.
- 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. PMCID: PMC3906421.

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 an 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. PMID: 7525966.
- 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. PMID: 15115397.
- 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. PMCID: PMC2234167.
- 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. PMCID: PMC2987654.

4. Bacterial RNA polymerase structure, function, and inhibition; structural basis of transcription; influenza virus structure, function, and drug targeting.

We determined structures of complexes of bacterial multisubunit RNA polymerases (RNAPs) with inhibitors and nucleic acid with implications for understanding the structural basis of transcription in all living cells. Structures and biochemical studies of RNAP complexed with antibiotics elucidated their respective binding sites and inhibition mechanisms. We determined structures of the influenza NS1B C-terminal domain and the influenza A cap-snatching endonuclease. Crystallographic fragment screening with endonuclease yielded small molecule leads, one of which was evolved to derivatives with antiviral activity in cell culture.

- a. 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. PMCID: PMC3593053.
- b. 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. PMCID: PMC5438085.
- c. 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. PMCID: PMC2957899.
- d. 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. PMCID: PMC3928712.

5. Structural insights into viral polyproteins and processing by proteases (HIV, PFV, and SARS-CoV-2):

We have been pursuing structural and functional studies of retroviral [HIV, prototype foamy virus (PFV)] and coronaviral polyproteins and their processing by their cognate viral proteases. We determined the structure of PFV PR-RT, a retroviral polyprotein that is mature but contains both PR and RT domains, providing insights into the conformational maturation of retroviral Pol polyproteins. With Dmitry Lyumkis at Salk, we published the cryo-EM structure of HIV PR-RT within the Pol polyprotein, which has a dimeric arrangement similar to mature RT.

Beginning early in the COVID-19 pandemic we investigated the biochemical and structural basis for determining the order of polyprotein cleavage of the SARS-CoV-2 nsp7-8 and nsp7-11 intermediates, which upon maturation are cofactors of the SARS-CoV-2 RNA-dependent RNA polymerase nsp12. Additionally, we studied the polyprotein intermediates' interaction with the viral protease Mpro, which has functional and drug discovery implications.

Recently with the laboratories of Jun Wang (Rutgers) and Xufang Deng (Oklahoma State University), we contributed crystallographic structures to the structure-based drug discovery and design of the first SARS-CoV-

2 papain-like protease (PL^{pro}) inhibitor with antiviral efficacy in a mouse model. Our laboratory determined the structures of a covalent lead and eight non-covalent leads that were essential in the discovery of a novel binding site, Val70^{Ub}. We have shown that the lead inhibitor **Jun12682** efficiently inhibits not only the protease but also the deubiquitinase activity of PL^{pro}, as the bound Val70Ub site impedes binding of both ubiquitin and ISG-15. Oral administration of a rationally designed PL^{pro} inhibitor, Jun12682, efficiently inhibited SARS-CoV-2 replication and mitigated SARS-CoV-2 induced lung lesions *in vivo*.

- a. Harrison JJEK, Tuske S, Das K, Ruiz FX, Bauman JD, Boyer PL, DeStefano JJ, Hughes SH, and **Arnold E**. Crystal Structure of a Retroviral Polyprotein: Prototype Foamy Virus Protease-Reverse Transcriptase (PR-RT). *Viruses*. 2021. PMCID: PMC8402755.
- b. Harrison JJEK*, Passos DO*, Bruhn JF, Bauman JD, Tuberty L, DeStefano JJ, Ruiz FX, Lyumkis D[#], and **Arnold E**[#]. Cryo-EM structure of the HIV-1 Pol polyprotein provides insights into virion maturation. *Sci Adv*. 2022. (#Corresponding authors). PMCID: PMC9258950.
- c. Yadav R*, Courouble VV*, Dey SK, Harrison JJEK, Timm J, Hopkins JB, Slack RL, Sarafianos SG, Ruiz FX[#], Griffin PR[#], **Arnold E**[#]. Biochemical and structural insights into SARS-CoV-2 polyprotein processing by Mpro. *Sci Adv*. 2022. (#Corresponding authors). PMCID: PMC9733933.
- d. Tan B*, Zhang X*, Ansari A*, Jadhav P*, Tan H, Li K, Chopra A, Ford A, Chi X, Ruiz FX[#], **Arnold E**[#], Deng X[#], Wang J[#]. Design of SARS-CoV-2 papain-like protease inhibitor with antiviral efficacy in a mouse model. *Science* 2024. (#Corresponding authors). PMID: 38547259.

(285 peer-reviewed publications; 34,500 citations; h-index=98)

Link: <https://scholar.google.com/citations?user=3ALJe7MAAAAJ>

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ruiz, Francesc Xavier

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

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
Universitat Autònoma Barcelona, Spain	B.S.	09/2001	Biochemistry
Universitat Autònoma Barcelona, Spain	M.Sc.	04/2003	Biochemistry and Molecular Biology
Universitat Autònoma de Barcelona, Spain	Ph.D.	09/2010	Enzymology
<i>Institut de Génétique et de Biologie Moléculaire et Cellulaire</i> (IGBMC), France	Postdoc	12/2014	Structural biology & drug discovery
Center for Advanced Biotechnology and Medicine (CABM), Rutgers University, NJ, United States of America (USA)	Postdoc	02/2018	Structural virology & drug discovery

A. Personal Statement

Enzymes are proteins acting as biological catalysts that are essential for all living organisms as well as viruses. My research is focused on studying the structure and function of human and viral enzymes—human aldol-keto reductases in my early career stage, HIV-1 reverse transcriptase (RT) since my second postdoctoral stage—with an emphasis on catalysis and inhibition for drug discovery. My deep interest in HIV (given the AIDS epidemic I witnessed growing up) and RNA viruses with pandemic potential (due to the recent COVID-19 pandemic) have led me to broaden my research to viral polyproteins, and viral RNA polymerases and proteases.

I have extensive experience in end-to-end mechanistic studies and X-ray crystallography (gene to structure) of target protein-inhibitor complexes, gained during my academic career in Spain, France, and the United States. For the past decade I have been focusing on one of the most sought viral targets, HIV RT, and finding novel insights into the molecular mechanisms of catalysis and inhibition, as well as drug resistance. In collaboration with biochemists, computational and medicinal chemists, I have contributed to the development of improved diarylpyrimidine-based non-nucleoside inhibitors and characterized in atomic detail novel mechanisms of inhibition of RT, including nucleotide-competing RT inhibitor INDOPY-1 and inhibitors binding in the primer grip bridging the NNRTI and NRTI sites. More recently, I have supervised the research on structure determination of HIV RT (+) strand initiation complexes that may explain high sensitivity to NNRTI inhibition, spearheaded by Shawn Rumrill (whose PhD I co-supervised with Prof. Eddy Arnold).

Regarding viral polyproteins and proteases, my research has significantly contributed to an improved understanding of the structural basis of viral polyprotein processing and maturation of retroviruses and coronaviruses. These include retroviral prototype foamy virus PR-RT and HIV Pol polyproteins' structures, as well as coronaviral polyprotein integrative structures. The Pol structure suggests that RT dimerization in the polyprotein may help positioning the protease (PR) subunits in a dimeric arrangement that could support PR activity and virion maturation. Our preliminary cryo-EM data with Gag-Pol constructs further supports this notion. In a recent collaboration with medicinal chemist Dr. Jun Wang (Rutgers), our group has provided key

crystallographic structures that facilitated the structure-based design and discovery of the first papain-like protease inhibitor with antiviral efficacy in a mouse model (Tan et al., Science, 2024, in which I was a senior author).

As Associate Research Professor in the Arnold group, I am directly supervising 2 graduate students and 4 postdoctoral associates and have provided significant intellectual contributions for securing multiple awarded NIH grants. In this application, I will be taking a leading role as co-Investigator, in light of my primary scientific expertise in HIV structural biology and my scientific role in directing the project alongside the PI Eddy Arnold.

Ongoing and recently completed projects that I would like to highlight include:

Ongoing projects

NIH U54AI170855

Torbett (PI). Role: Arnold, Co-I

06/01/2022-05/30/2027

Behavior of HIV in Viral Environments (B-HIVE)

NIH U19AI171292

Baric (PI). Role: Arnold, Co-I

05/01/2022-04/30/2025

Rapidly emerging antiviral drug development initiative AViDD Center (READDI-AC)

NIH U19A171110

Krogan (PI), Role: Arnold, Co-I

05/01/2022-04/30/2025

QCRG Pandemic Response Program

Recently completed projects

New Jersey Health Foundation Grant # PC 131-22

Francesc Xavier Ruiz (PI). Role: Arnold Co-I

02/01/2022-01/31/2023;

Title: Structure-based Design of HIV-1 Reverse Transcription Inhibitors Acting Through Novel Mechanisms of Action

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2019-Current	Faculty (Assistant Professor: 2019-23; Associate Professor: 2023-current). CABM, Rutgers University, Piscataway, NJ, US. PI: Prof. Eddy Arnold
2015-2019	Postdoctoral Associate (2015-18) & Senior Associate (2018-19). CABM, Rutgers University, Piscataway, NJ, US. PI: Prof. Eddy Arnold
2013-2014	Postdoctoral fellow, IGBMC, France. PI: Dr. Alberto Podjarny
2011-2012	Industrial funding from "The Institutes for Pharmaceutical Discovery LLC", CT, USA; & "Mutabilis", France. Location: IGBMC, France. PIs: Dr. Alberto Podjarny & Dr. Marc Ruff
2011	EMBO short term Fellowship, IGBMC, France. PI: Dr. Alberto Podjarny
2007-2010	Teaching Associate, Universitat Autònoma de Barcelona (UAB), Spain
2003-2010	Predocotrinal Research Fellow (PhD Advisers: Prof. Xavier Parés / Prof. Jaume Farrés)

Honors and Awards:

2024	Peer-reviewer of proposals to the MacCHESS Synchrotron beamlines
2023	Guest editor of a Special Issue in the journal "Viruses"
2023	Associate Editor for Structural Biology in the journal "Frontiers in Molecular Biosciences"
2023	Wellcome Trust Career Development Award reviewer # 227831/Z/23/Z. Topic: coronaviral polyproteins
2021	Guest editor of a Special Issue in the journal "Molecules"

2018	Best poster presentation award in the HIV DART meeting, Miami, FL, US
2014	Selected attendee to the 47 th Course of Erice International School of Crystallography: "Structural Basis of Pharmacology: Deeper Understanding of Drug Discovery through Crystallography", Erice, Italy
2014	Talk in the 23rd International Union of Crystallography meeting (2014), Montreal, Canada
2013-2014	Postdoctoral Fellowship "Fondation pour la Recherche Médicale" (Paris, France), Code FRM: SPF20121226275
2011	EMBO short term Fellowship ref. EMBO ASTF 500-2010
2003-2007	Predocotrinal Fellowship, Science and Technology Ministry, Spain

C. Contributions to Science

1. Structural insights into HIV-1 reverse transcriptase (RT) inhibition and catalysis:

In my current stay at the Arnold lab, I am performing structural studies related to the inhibition and catalysis of RT. Taking advantage of an aptamer that is a template-primer mimic (PMID: 26296781), I have optimized a crystal form that diffracts consistently between 2.5-3 Å. This crystal form allows dNTP/NRTI binding and incorporation, but not translocation, in a structural enzymology fashion, allowing characterization of nucleoside inhibitors with different mechanisms (including L-nucleosides), as well as the first described nucleotide-competing RT inhibitor (NcRTI), INDOPY-1. This knowledge will facilitate design of nucleotide-competing inhibitors of RT and other polymerases.

In collaboration with the Zhan/Liu lab (Shandong University, China), I am characterizing structurally a set of evolved substituted diarylpyrimidine derivatives binding RT with high affinity and resilience to resistance mutations raised by rilpivirine and etravirine. In collaboration with Asim Debnath (NY Blood Center), we have co-discovered a novel chemotype that acts as dual gp120 antagonist and RT inhibitor, binding in a pocket that bridges the NNRTI and NRTI sites in RT, showing nM antiviral activity.

Using RT as a proof-of-concept system, I have co-developed a new fragment screening library, the Halo library, containing 46 halogenated fragments, leveraging their higher binding propensity to proteins in comparison to non-halogenated fragments. This small library may thus provide a convenient tool for rapidly assessing the feasibility of a target for X-ray crystallographic fragment screening, mapping hot spots and cryptic sites, as well as finding fragment binders that can be useful for developing drug leads.

Finally, given my expertise in retroviral RTs, I have contributed as a co-author in the landmark study about human LINE-1ORF2p structures, functions and adaptations, recently published in *Nature*. ORF2p contains an RT domain that is central in its transposon activity that makes this element account for one third of the human genome, with some intriguing commonalities and differences with retroviral RTs, with potential implications for pathology and biotechnology.

- Ruiz FX**, Hoang A, Das K, Arnold E. *Structural Basis of HIV-1 Inhibition by Nucleotide-Competing Reverse Transcriptase Inhibitor INDOPY-1*. J Med Chem. 2019. PMID: 31603676 PMCID: PMC7737671.
- Losada N*, **Ruiz FX***, Curreli F, Gruber K, Pilch A, Das K, Debnath AK#, Arnold E#. *HIV-1 gp120 Antagonists Also Inhibit HIV-1 Reverse Transcriptase by Bridging the NNRTI and NRTI sites*. J Med Chem. 2021. PMID: 34735153 PMCID: PMC10655131 (*these authors contributed equally; #Corresponding authors).
- Chopra A, Bauman JD, **Ruiz FX**#, Arnold E#. *Halo Library, a Tool for Rapid Identification of Ligand Binding Sites on Proteins Using Crystallographic Fragment Screening*. J Med Chem. 2023. PMID: 37115705 PMCID: PMC10184123 (#Corresponding authors).
- Baldwin ET*, van Eeuwen T*, Hoyos D*, Zalevsky A*, Tchesnokov EP, Sánchez R, Miller BD, Di Stefano LH, **Ruiz FX**, Hancock M, Işik E, Mendez-Dorantes C, Walpole T, Nichols C, Wan P, Riento K, Halls-Kass R, Augustin M, Lammens A, Jestel A, Upla P, Xibinaku K, Congreve S, Hennink M, Rogala KB, Schneider AM, Fairman JE, Christensen SM, Desrosiers B, Bisacchi GS, Saunders OL, Hafeez N, Miao W, Kapeller R, Zaller DM, Sali A, Weichenrieder O, Burns KH#, Götte M#, Rout MP#, Arnold E#, Greenbaum BD#, Romero DL#, LaCava J#, Taylor MS*#. *Structures, functions and adaptations of the human LINE-1 ORF2 protein*. Nature. 2023. PMID: 38096902 PMCID: PMC10830420 (*these authors contributed equally; #Corresponding authors).

2. Structural insights into viral polyproteins and viral proteases:

I am currently involved in the structural characterization of retroviral (HIV, prototype foamy virus (PFV)) and coronaviral polyproteins. Many retroviral and RNA viruses' proteins are initially translated from unspliced full-length RNA as polyprotein precursors that are subsequently processed by the viral proteases (PR) to yield the

mature forms. While polyprotein maturation is vital for viral replication, it is an understudied and poorly understood part of the viral replication cycle, especially in terms of its structural bases.

Regarding retroviral polyproteins, I have contributed to the determination of the X-ray structure of PFV PR-RT—a retroviral polyprotein that is mature but has PR and RT domains—that provides insights into the conformational maturation of retroviral Pol polyproteins. Regarding HIV, I have contributed to the determination of the cryo-EM structure of HIV PR-RT within the Pol polyprotein—that may be a polyprotein intermediate existing between Gag-Pol and the mature PR—that has a dimeric arrangement similar to mature RT and suggests that PR dimerization and activity within the polyprotein is mediated through RT dimerization. Regarding coronaviral polyproteins, as co-senior author, I have contributed to the understanding of the biochemical and structural basis for determining the order of polyprotein cleavage of the SARS-CoV-2 nsp7-8 and nsp7-11 intermediates, which upon maturation are cofactors of the SARS-CoV-2 RNA-dependent RNA polymerase nsp12. Additionally, we have studied the polyprotein intermediates' interaction with viral protease Mpro, with functional and drug discovery implications. Finally, as part of a collaboration with the laboratories of Jun Wang (Rutgers) and Xufang Deng (Oklahoma State University), as co-senior author, I have contributed to the structure-based drug discovery and design of the first SARS-CoV-2 papain-like protease (PL^{pro}) inhibitor with antiviral efficacy in a mouse model. This molecule represents a promising candidate for further development as an orally bioavailable SARS-CoV-2 antiviral. As such, Rutgers has applied for a PCT patent that covers the PL^{pro} inhibitors reported, of which I am co-inventor (Publication Number WO2024178004), and the manuscript was recently published in *Science*.

- e. Harrison JJEK, Tuske S, Das K, **Ruiz FX**, Bauman JD, Boyer PL, DeStefano JJ, Hughes SH, Arnold E. *Crystal Structure of a Retroviral Polyprotein: Prototype Foamy Virus Protease-Reverse Transcriptase (PR-RT)*. *Viruses*. 2021. PMID: 34452360 PMCID: PMC8402755.
- f. Harrison JJEK*, Passos DO*, Bruhn JF, Bauman JD, Tuberty L, DeStefano JJ, **Ruiz FX**, Lyumkis D#, Arnold E#. Cryo-EM structure of the HIV-1 Pol polyprotein provides insights into virion maturation. *Sci Adv*. 2022. PMID: 35857464 PMCID: PMC9258950 (*these authors contributed equally; #Corresponding authors).
- g. Yadav R*, Courouble VV*, Dey SK, Harrison JJEK, Timm J, Hopkins JB, Slack RL, Sarafianos SG, **Ruiz FX**#, Griffin PR#, Arnold E#. *Biochemical and structural insights into SARS-CoV-2 polyprotein processing by Mpro*. *Sci Adv*. 2022. PMID: 36490335 PMCID: PMC9733933 (*these authors contributed equally; #Corresponding authors).
- h. Tan B*, Zhang X*, Ansari A*, Jadhav P*, Tan H, Li K, Chopra A, Ford A, Chi X, **Ruiz FX**#, Arnold E#, Deng X#, Wang J#. *Design of a SARS-CoV-2 papain-like protease inhibitor with antiviral efficacy in a mouse model*. *Science*. 2024. PMID: 38547259 (*these authors contributed equally; #Corresponding authors).

3. Structural and functional role of human aldo-keto reductases (AKRs) involved in the first step of retinoic acid (RA) biosynthesis:

During my PhD, I found the basis of the high AKR1B10 retinal reductase activity and its relation to retinoic acid biosynthesis, using a combination of enzyme kinetics, mutagenesis, and cellular assays in mammalian cell cultures transfected with AKR1B10. During my postdoc at Dr. Podjarny lab (IGBMC), I solved the first high resolution structure of a human enzyme in complex with a retinoid, which allowed further elucidation of the structural basis for the high all-trans-retinal reductase activity of AKR1B10. In parallel to the study of human AKR1B proteins, I performed a kinetic characterization of human AKR1Cs vs. retinoids and especially relevant was the high retinal reductase activity of recombinant AKR1C3. Through combination of cellular quantification of retinoid metabolites and proliferation assays, I provided evidence that the pro-proliferative action of AKR1C3 in HL-60 leukemia cells involves the RA signaling pathway and that this is in part due to the retinal reductase activity of AKR1C3.

- a. Gallego O., **Ruiz FX**., Ardèvol A., Rovira C., Farrés J., Fita I., Parés X. *Structural basis for the high all-trans-retinaldehyde reductase activity of the tumor marker AKR1B10*. *Proc. Natl. Acad. Sci. USA*, **104**:20764-20769. 2007. PMID: 18087047. PMCID: PMC2410076.
- b. **Ruiz FX**, Porté S, Gallego O, Moro A, Ardèvol A, Del Río A, Rovira C, Farrés J, Parés X. *Retinaldehyde is a substrate for human aldo-keto reductases of the 1C subfamily*. *Biochem. J.* **2011 Dec 15**;440:335-44. 2011. PMID: 21851338
- c. **Ruiz FX**#, Crespo I, Álvarez S, Porté S, Giménez-Dejor J, Cousido-Siah A, Mitschler A, de Lera ÁR, Parés X, Podjarny A, Farrés J#. *Structural basis for the inhibition of AKR1B10 by the C3 brominated TTNPB derivative UVI2008*. *Chem.-Biol. Interact.* **276**:174-181. 2017. PMID: 28161411 (#Corresponding authors).

4. Structural basis of inhibition and selectivity of human AKRs Aldose Reductase and AKR1B10:

In my postdoc at Dr. Podjarny lab (IGBMC), I pursued a structure-based drug design approach of the cancer target AKR1B10. Human AKR Aldose Reductase (AR) is a diabetes target. I performed inhibition assays and X-

ray crystallography. To note that at the start of my PhD there was just 1 PDB deposited for AKR1B10, while >100 for the homologous AR. Right now, there are 20 structures of AKR1B10 deposited, and I co-authored 14 of them (12 of them are protein-ligand complexes). Key for this improvement was the combination of 2 mutations in the enzyme active site and surface lysine methylation. Besides, I also found potent and selective hits for AKR1B10. I screened a set of compounds originated from a marine sponge, finding a benzyluracil acetic acid scaffold that, depending on its decoration, gave highest selectivity and potency for either AR or AKR1B10, both with recombinant protein and in cellular studies. In a subsequent study, through the different decoration of an arylcarbamoyl-phenoxy-acetic acid scaffold with halogen groups, I probed AKR1B10 flexible active site, finding a novel AKR1B10 binding site conformer and several distinctive AKR1B10 features (shape, flexibility, hydration) that can be exploited for drug design.

- a. Cousido-Siah A*, **Ruiz FX***, Crespo I, Porté S, Mitschler A, Parés X, Podjarny A, Farrés J. *Structural analysis of sulindac as an inhibitor of aldose reductase and AKR1B10*. **Chem.-Biol. Interact.** **234**: 290-296. 2014 (*these authors contributed equally). PMID: 25532697.
- b. **Ruiz FX***, Cousido-Siah A*, Porté S, Domínguez M, Crespo I, Rechlin C, Mitschler A, de Lera ÁR, Martín MJ, de la Fuente JÁ, Klebe G, Parés X, Farrés J, Podjarny, A#. *Structural Determinants of the Selectivity of 3-Benzyluracil-1-acetic Acids toward Human Enzymes Aldose Reductase and AKR1B10*. **ChemMedChem** **10** (12, front cover of this issue): 1860-7187. 2015. PMID: 26549844 (*these authors contributed equally; #Corresponding authors).
- c. Cousido-Siah A*, **Ruiz FX***, Fanfrlik J#, Giménez-Dejor J, Mitschler A, Kamlar M, Vesel J, Ajani H, Parés X, Farrés J, Hobza P, Podjarny AD. *IDD388 Polyhalogenated Derivatives as Probes for an Improved Structure-Based Selectivity of AKR1B10 Inhibitors*. **ACS Chem. Biol.** **11** (10), 2693-2705. 2016. PMID: 27359042 (*these authors contributed equally; #Corresponding authors).

5. Structure of the human Papillomavirus protein E6 in complex with cellular ubiquitin ligase E6AP and tumor suppressor p53E6AP/p53 complex:

In collaboration with Dr. Gilles Travé lab (IGBMC, France, while being at Dr. Alberto Podjarny lab), I solved the structure of the ternary complex of human papillomavirus protein E6 with host proteins E6AP and tumor suppressor p53. The structure unveils how the viral protein acts in a similar fashion as a protein degrader [or PROTACs (PROteolysis TARgeting Chimeras)], bringing together E6AP and p53, leading to ubiquitin-mediated degradation of p53, responsible for cervical carcinomas and a growing number of head-and-neck cancers caused by HPV. This has opened avenues for the HPV drug discovery and also for anti-cancer discovery, as the p53 region where the viral E6 binds is distal from any previously described DNA and protein-binding interfaces.

- a. Martinez-Zapien D, **Ruiz FX**, Poirson J, Mitschler A, Ramirez J, Forster A, Cousido-Siah A, Masson M, Vande Pol S, Podjarny A, Travé G, Zanier K. *Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53*. **Nature**. **529(7587)**:541-545. 2016. PMID: 26789255 PMCID: PMC4853763.

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