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 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 with nucleic acid and the 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 (Salk Institute) to determine the first structure 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 our recent manuscript (DOI: 10.1126/sciadv.abn9874). 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.

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 in both 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.

Ongoing projects
NIH R01AI027690
Arnold (PI)
11/01/2019-10/31/2024
Evolving understanding of HIV-1 reverse transcriptase structure, function, and inhibition

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
05/01/2022-04/30/2027
Rapidly emerging antiviral drug development initiative AViDD Center (READDI-AC)

NIH U19A171110
Krogan (PI), Role: Arnold, Co-I
05/01/2022-04/30/2025

Recently completed projects
New Jersey Health Foundation Grant # PC 131-22
Xavi (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

NIH U54AI150472
Arnold (PI)
09/01/2012-08/31/2022
HIV interaction in viral evolution (HIVE)

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments:

2021	Antonin Holy Award from the International Society for Antiviral Research
2020	Rutgers Chancellor's Award for Pioneering Research
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
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:

1979 - 1982	Graduate Research Assistant, Chemistry, Cornell University (with Jon Clardy)
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. *ScienceWatch*
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. 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.
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.
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.
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-[(1*E*)-2-2cyanoethenyl]-pyrimidinyl 2,6-dimethylphenyl]amino]-2-]amino]-benzonitrile (R278474, rilpivirine). J. Med. Chem. 48:1901-1909.
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.
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.
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.

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.
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.
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
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.

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.
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.

Link to Eddy Arnold's Google Scholar citations (~32,300 citations; h-index=96):
https://scholar.google.com/citations?user=3ALJe7MAAAAJ
NCBI publication list (223 publications captured):
https://www.ncbi.nlm.nih.gov/myncbi/edward.arnold.1/bibliography/public/

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 Figueras, Francesc Xavier

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

POSITION TITLE: Associate Research Professor, Center for Advanced Biotechnology and Medicine, 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 de Barcelona, Spain	M.Sc.	04/2003	Biochemistry & Molecular Biology
Universitat Autònoma de Barcelona, Spain	Ph.D.	09/2010	Enzymology
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), France	Postdoc	12/2014	Structural biology & drug discovery
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 aldo-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, viral RNA polymerases and proteases. Complementary to all the previous, I have developed methodological expertise in structure- and fragment-based approaches for inhibitor discovery.

I have extensive experience in end-to-end inhibition mechanistic studies and X-ray crystallography (gene to structure) of target protein-inhibitor complexes, gained during my academic career in Spain, France and USA. In my stage in the US, I have been focusing into one of the most sought viral targets, HIV RT, being capable of finding novel insights into catalysis and inhibition mechanisms, 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.

As Associate Research Professor in the Arnold group, I am directly supervising 4 graduate students and 4 postdoctoral associates (https://cabm.rutgers.edu/research/arnold-lab), and have provided significant intellectual contributions for obtention of multiple awarded NIH grants (~$1M/year funding for the group).

In 2022 I was awarded as PI of a research grant of the “New Jersey Health Foundation”, a seed grant of $35,000 for one year that has provided funds for obtaining the preliminary results detailed in the research proposal.

Complete List of Published Work:

-Manuscripts: https://scholar.google.com/citations?user=SzS4xz8AAAAJ&hl=en&oi=ao

-Manuscripts, peer- and grant-review: https://www.webofscience.com/wos/author/record/335710

-Structures: https://www.ncbi.nlm.nih.gov/structure/?term=Ruiz+FX

https://pdb-dev.wwpdb.org : PDBDEV_00000119 & PDBDEV_00000120

Ongoing projects
No current research support as PI

Recently completed projects
New Jersey Health Foundation Grant # PC 131-22
Xavi (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 Employment

2003-2010	Predoctoral Research Fellow (PhD Advisers: Prof. Xavier Parés / Prof. Jaume Farrés)
2007-2010	Teaching Associate, Universitat Autònoma de Barcelona (UAB), Spain
2011	EMBO short term Fellowship, 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
2013-2014	Postdoctoral fellow, IGBMC, France. PI: Dr. Alberto Podjarny
2015-2019	Postdoctoral Associate (2015-18) & Senior Associate (2018-19). CABM, Rutgers University, Piscataway, NJ, US. PI: Prof. Eddy Arnold
2019-Current	Faculty (Assistant Professor: 2019-23; Associate Professor: 2023-current). CABM, Rutgers University, Piscataway, NJ, US. PI: Prof. Eddy Arnold

Professional Memberships

2003-2023	Member of the Spanish Society for Biochemistry and Molecular Biology (SEBBM)
2014-Current	World Directory of Crystallographers (IUCr ID: 21256)

Honors

2003-2007	Predoctoral Fellowship, Science and Technology Ministery, Spain
2011	EMBO short term Fellowship ref. EMBO ASTF 500-2010
2013-2014	Postdoctoral Fellowship "Fondation pour la Recherche Médicale" (Paris, France), Code FRM: SPF20121226275
2014	Selected attendee to the 47th 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
2018	Best poster presentation award in the HIV DART meeting, Miami, FL, US
2021	Guest editor of a Special Issue in the journal "Molecules" (https://www.mdpi.com/journal/molecules/special_issues/polymerases_human) (closed)
2023	Guest editor of a Special Issue in the journal "Viruses" (https://www.mdpi.com/journal/viruses/special_issues/E2J191I100) (ongoing)
2023	Associate Editor for Structural Biology in the journal "Frontiers in Molecular Biosciences" (https://loop.frontiersin.org/people/47828/overview)
2023	Wellcome Trust Career Development Award reviewer, # 227831/Z/23/Z. Topic: coronaviral polyproteins

C. Contributions to Science

1. 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 *trans*-activation assays in

mammalian cell cultures transfected with AKR1B10. Related to it, during my postdoc at Dr. Podjarny lab, 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.
b. **Ruiz FX**., Gallego O., Ardèvol A., Moro A., Domínguez M., Alvarez S., Alvarez R., de Lera AR., Rovira C., Fita I., Parés X., Farrés J. *Aldo-keto reductases from the AKR1B subfamily: Retinoid specificity and control of cellular retinoic acid levels*. **Chem.-Biol. Interact. 178:171-177.** 2009.
c. **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.
d. **Ruiz FX#**, Crespo I, Álvarez S, Porté S, Giménez-Dejoz 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. (#Corresponding authors)

2. 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, while coordinating collaboration with organic and theoretical chemists, and I also lead manuscript writing. 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 19 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 benzyluracyl 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 job, 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).
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. (*these authors contributed equally; #Corresponding authors).
c. Cousido-Siah A*, **Ruiz FX*#**, Fanfrlík J#, Giménez-Dejoz 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.** (*these authors contributed equally; #Corresponding authors).

3. 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.

4. 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, 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.

a. **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.
b. Kang D, **Ruiz FX**, Feng D, Pilch A, Zhao T, Wei F, Wang Z, Sun Y, Fang Z, De Clercq E, Pannecouque C, Arnold E, Liu X, Zhan P. *Discovery and Characterization of Fluorine-Substituted Diarylpyrimidine Derivatives as Novel HIV-1 NNRTIs with Highly Improved Resistance Profiles and Low Activity for the hERG Ion Channel*. J Med Chem. 2020.

5. Structural insights into HIV-1 and SARS-CoV-2 polyproteins:

In my current stay at the Arnold lab, I have provided mentoring, analysis and data processing in structural studies of HIV-1 and SARS-CoV-2 polyproteins. For HIV-1, the recent cryo-EM structure of Pol provides support to the role of RT in the polyprotein as driving the dimerization of the protease, hence activating it and triggering viral maturation. For SARS-CoV-2, we determined the first structures of polyprotein intermediates using an integrative structure approach, and analyzed its interaction with the viral protease Mpro, providing insights into processing and potential novel targets for drug discovery.

a. Yadav, R., Courouble, V.V., Dey, S.K., Harrison, J.J.E., Timm, J., Hopkins, J.B., Slack, R.L., Sarafianos, S.G., **Ruiz, F.X.** [#]**,** Griffin, P.R. [#], Arnold, E[#]. *Biochemical and structural insights into SARS-CoV-2 polyprotein processing by Mpro*. **Science Advances***.* 2022. **(**[#]Corresponding authors).
b. Harrison, J.J.E.K., Passos, D.O., Bruhn, J.F., Bauman, J.D., Tuberty, L., DeStefano, J.J., **Ruiz, F.X.**, Lyumkis, D., & Arnold, E. *Cryo-EM Structure of the Pol Polyprotein Provides Insights into HIV Maturation*. **Science Advances**. 2022.