BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

NAME: Xiong, Yong

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

POSITION TITLE: Professor of Molecular Biophysics and Biochemistry

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
Tsinghua University, China		05/1994	Physics
The Ohio State University	Ph.D.	05/2000	Biophysics

A. Personal Statement

My lab employs a multidisciplinary approach that integrates structural biology, biochemistry, computational biology, and functional studies to investigate biological processes at the molecular level, with a particular focus on host immune defenses against pathogens. We have made substantial contributions to the field of retrovirology by elucidating the structural and mechanistic details of various host-viral interaction systems. These include how HIV-1 Vif hijacks the cellular ubiquitin ligase machinery to degrade APOBEC3 proteins, the mechanism of viral tethering by the restriction factor BST2/tetherin, and how HIV-1 Vpu counteracts this tethering. We have also uncovered how HIV-1 Nef evades host adaptive immunity, how HIV-1 capsids are recognized by TRIM5α, and how host factors such as SAMHD1 and MxB inhibit HIV-1 replication by blocking reverse transcription and nuclear import, respectively. Beyond HIV, our research has provided insights into protein-DNA complexes in the Fanconi anemia pathway involved in DNA damage repair, the inhibition of host translation by the coronavirus Nsp1 protein, and the development of monospecific and bispecific antibodies that effectively neutralize multiple SARS-CoV-2 variants of concern. To facilitate these studies, we have advanced methodologies in X-ray crystallography, cryo-electron microscopy (cryo-EM), and cryo-electron tomography (cryo-ET). Specifically, we have pioneered a cutting-edge *in situ* cryo-EM pipeline that enables high-throughput, high-resolution studies of viral processes directly within heterogeneous cellular and animal environments. These advancements, combined with our deep expertise in host-viral interactions and structural biology, uniquely position me to lead my research and drive new discoveries in the field.

I have dedicated over 20 years to training students and researchers at Yale, serving in roles such as Director of Graduate Studies for the Department of Molecular Biophysics and Biochemistry, co-director of the Biochemistry, Quantitative Biology, Biophysics, and Structural Biology track, and currently as Director of the Yale Biophysics Training Program, Advisory Committee member for the Yale Virology Training Program, and advisory board member for the Structural Biology and Molecular Biophysics Training Program at the University of Pennsylvania. I have mentored 19 undergraduate researchers, 27 predoctoral students, and 26 postdoctoral associates, many of whom have achieved successful careers in academia and industry. In addition, I have served on the thesis committees of 88 graduate students at Yale. My extensive experience in research and education equips me to effectively guide my trainees.

Ongoing projects that I would like to highlight include:

5T32GM008283
National Institutes of Health
Xiong (PI)
7/1/2020 - 6/30/2028
Predoctoral Program in Biophysics

5R37AI116313
National Institutes of Health
Xiong (PI)
6/15/2021 - 5/31/2026
Multifaceted interactions between lentiviral Vif and host molecules for viral infectivity enhancement

1R01AI162260
National Institutes of Health
Xiong (PI)
10/1/21 – 8/31/26
Using DNA-origami nuclear pore mimics to reveal nuclear entry mechanisms of HIV-1 capsid

R01AI192025
National Institutes of Health
Xiong (PI)
04/24/2025 - 03/31/2030
Molecular investigations of HIV-1 maturation pathways and inhibitor actions in situ

U54AI170791
National Institutes of Health
Gronenborn (PI), Role: Project leader
7/01/2022 - 4/30/2027
Mechanisms of retroviral capsid sensing by host proteins

R01AI160922
National Institutes of Health
Mothes (PI), Role: Co-Investigator
7/1/21 – 6/30/26
Antibody-mediated immune response against the SARS-CoV-2 spike protein

R01AI178846
National Institutes of Health
Perilla (PI), Role: Co-Investigator
8/15/23 – 7/31/28
Determining the molecular mechanisms of HIV-1 maturation

B. Positions, Scientific Appointments, and Honors

Professional Experience

2019-	**Professor,** Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut.
2011-2019	**Associate Professor,** Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut.
2006-2011	**Assistant Professor,** Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut.
2001-2006	**Postdoctoral Associate** with Dr. Thomas Steitz, Department of Molecular Biophysics & Biochemistry, Yale University, Howard Hughes Medical Institute, New Haven, Connecticut.

2000-2001 **Postdoctoral Associate** with Dr. Muttaiya Sundaralingam, Chemistry Department, The Ohio State University, Columbus, Ohio.

Awards and Fellowships

2021- MERIT Award, National Institutes of Health
2010-2012 Innovation Award, Alex's Lemonade Stand Foundation
2010-2012 Basil O'Connor Starter Scholar Research Award, March of Dimes Foundation
2008-2010 Child Health Research Award, Charles H. Hood Foundation
2006-2008 Smith Family New Investigator Award, Richard and Susan Smith Family Foundation
2004 Sidhu Award for the best contribution to crystallography from an investigator within five years of Ph.D. The Pittsburgh Diffraction Society

C. Contribution to Science

We have made significant contributions to the scientific community by furnishing in-depth structural and mechanistic results for a range of new protein-protein/ligand interaction systems important in host-viral interplays. Moreover, the experimental methods devised during our research will provide valuable new tools for the study of host-viral interactions and other challenging biological problems. Our work has resulted in 140 peer-reviewed publications.

1. HIV capsid sensing by host protein factors. We strive to understand the mechanisms by which host antiviral protein factors achieve the species-specific but shape-independent recognition of retrovirus capsids. We have determined the crystal structures of MxB and the capsid-binding domain of TRIM5α, which allowed us to build capsid-binding models to guide the ongoing research on HIV-1 capsid assembly and its interaction with many host-binding partners. We have further developed technically innovative disulfide and isopeptide crosslinking methods. The methods enable us to overcome a major hurdle in the research community to create an array of soluble, homogeneous capsid assemblies that capture a diverse range of capsid patterns. These novel capsid assemblies have opened up important new avenues for us to interrogate previously illusive host factor-capsid interfaces, produce homogenous complexes with host factors for X-ray crystallography and/or single particle cryo-EM studies, and identify new host factors that only recognize patterns in the assembled HIV capsid.

a. Arizaga F Jr, Freniere C, Rey JS, Cook M, Wu C, Perilla JR & **Xiong Y.** (2025). Exploring the Structural Divergence of HIV and SRLV Lentiviral Capsids. J. Am. Chem. Soc. DOI: 10.1021/jacs.5c09436.
b. Shen Q, Feng Q, Wu C, Xiong Q, Tian T, Yuan S, Shi J, Bedwell GJ, Yang R, Aiken C, Engelman AN, Lusk CP, Lin C, **Xiong Y**. (2023) Modeling HIV-1 nuclear entry with nucleoporin-gated DNA-origami channels. ***Nat Struct Mol*** **Biol** 30, 425-435. PMCID: PMC10121901
c. Shen Q, Kumari S, Xu C, Jang, S, Burdick RC, Xiong Q, Wu C, Devarkar SC, Tian T, Tripler TN, Hu Y, Yuan S, Temple J, Shi J, Aiken C, Engelman AN, Perilla JR, Pathak VK, Lin C, **Xiong Y**. (2023). The capsid lattice engages a bipartite NUP153 motif to mediate nuclear entry of HIV-1 cores. ***Proc. Natl. Acad. Sci. USA.*** 120 (13) e2202815120. PMCID: PMC10068764
d. Summers BJ, Digianantonio KM, Smaga SS, Huang P, Zhou K, Gerber EE, Wang W& **Xiong Y.** (2019). Modular HIV-1 Capsid Assemblies for Investigating Diverse Host-Capsid Recognition Mechanisms. ***Cell Host & Microbe*** 26, 203-216. PMCID: PMC6777739

2. Mutation of viral DNA by APOBEC3 proteins and their antagonization by HIV Vif. Members of the APOBEC3 protein family potently inhibit HIV and many other retroviruses. To evade this host defense, the lentivirus-encoded protein Vif hijacks cellular E3 ubiquitin ligases to target APOBEC3 proteins for proteasome-mediated degradation. We aim to establish the mechanisms by which APOBEC3 proteins mutate viral DNA and how Vif sequesters these antiviral proteins. Toward this end, we have solved the crystal structures of molecular complexes explaining how HIV-1 Vif hijacks the human E3 ligase components EloB/EloC and how the ligase components Cul2 and Cul5 are selected. We further dissected Vif interactions with various components of the host E3 ligase. These results provide structural and mechanistic information on the complicated interactions in these host-viral interplays, which is also critical for the design of Vif inhibitors.

a. Hu Y, Delviks-Frankenberry KA, Wu C, Arizaga F, Pathak VK & **Xiong Y**. (2024) Structural Insights into PPP2R5A Degradation by HIV-1 Vif. ***Nat Struct Mol Biol***. https://doi.org/10.1038/s41594-024-01314-6
b. Hu Y, Gudnadóttir RB, Knecht KM, Arizaga F, Jónsson SR, **Xiong Y**. (2023). Structural basis for

recruitment of host CypA and E3 ubiquitin ligase by maedi–visna virus Vif. ***Science Advances*** Vol 9, Issue 2. DOI: 10.1126/sciadv.add3422. PMCID: PMC9839330

c. Hu Y, Desimmie BA, Nguyen HC, Ziegler SZ, Cheng TC, Chen J, Wang J, Wang H, Zhang K, Pathak VK & **Xiong Y**. (2019). Structural basis of antagonization of human APOBEC3F by HIV-1 Vif. ***Nat. Struct. Mol. Biol.*** 26, 1176–1183. PMCID: PMC6899190

d. Fribourgh J, Nguyen H, Wolfe L, DeWitt DC, Zhang W, Yu XF, Rhoades E & **Xiong Y** (2014). CBFβ plays a critical role facilitating the assembly of the Vif-Cul5 E3 ubiquitin ligase. ***J. Virol.*** 88, 3309-19. PMCID: 2898223

3. Host interactions with coronavirus proteins. To mitigate the impact of COVID-19 and prepare for potential future coronavirus outbreaks, we engaged in extensive research for the development, cryo-EM structure studies and functional analyses of distinct potent monospecific and bispecific antibodies that neutralize SARS-CoV-2 and its variants of concern. In addition, we made substantial progress in investigating the mechanisms of the non-structural protein 1 (Nsp1) of coronaviruses, which is an essential pathogenicity factor that potently restricts host gene expression.

a. Ren P, Hu Y, Peng L, Yang L, Suzuki K, Fang Z, Bai M, Zhou L, Feng Y, Zou Y, **Xiong Y***, Chen S*. (2023). Function and Cryo-EM structures of broadly potent bispecific antibodies against multiple SARS-CoV-2 Omicron sublineages. ***Sig Transduct Target Ther*** 8, 281. PMCID: PMC9387138
* Corresponding authors.

b. Devarkar SC, Vetick M, Balaji S, Lomakin IB, Yang L, Jin D, Gilbert W, Chen S, **Xiong Y**. (2023). Structural basis for translation inhibition by MERS-CoV Nsp1 reveals a conserved mechanism for betacoronaviruses. ***Cell Reports*** 42, 113156.

c. Peng L, Hu Y, Mankowski M, Ren P, Chen R, Wei J, Zhao M, Li T, Tripler T, Ye L, Chow R, Fang Z, Wu C, Dong M, Cook M, Wang G, Clark P, Nelson B, Klein D, Sutton R, Diamond M, Wilen C*, **Xiong Y***, Chen S*. (2022). Monospecific and bispecific monoclonal SARS-CoV-2 neutralizing antibodies that maintain potency against B.1.617. ***Nature Communications***, 13(1):1638. doi: 10.1038/s41467-022-29288-3. PMCID: PMC8960874 * Corresponding authors.

d. Yuan S, Peng L, Park JJ, Hu Y, Devarkar SC, Dong MB, Wu S, Chen S, Lomakin I, **Xiong Y**. (2020). Nonstructural protein 1 of SARS-CoV-2 is a potent pathogenicity factor redirecting host protein synthesis machinery toward viral RNA. ***Molecular Cell*** 80, 1–12. PMCID: PMC7833686

4. Viral hijacking of host membrane trafficking pathways. We overcome a major hurdle in studying membrane-bound multicomponent complexes by using a fusion-protein strategy that allows ternary interactions mediated by lipid membranes to be modeled in aqueous solution. This approach has enabled us to reconstitute numerous membrane trafficking complexes and led to the determination of the crystal structures of the HIV-1 Nef/MHC-I/μ1(AP1), HIV-1 Vpu/tetherin/AP1, and HIV-1 Nef/CD4/AP2 (unpublished) complexes. These results provide great insight into the mechanisms by which Nef and Vpu hijack cellular membrane trafficking pathways to evade multiple host defenses. The information obtained will provide the intellectual basis for the development of new antiviral compounds and strategies.

a. Stoneham C, Ramirez WP, Singh R, Suarez M, Debraya A, Lim C, Jia X, **Xiong Y**, Guatelli J. (2020). A conserved acidic cluster motif in SERINC5 confers resistance to antagonism by HIV-1 Nef. ***J Virol.*** pii: JVI.01554-19. PMCID: PMC7081897

b. Jia X, Weber E, Tokarev A, Lewinski M, Rizk M, Suarez M, Guatelli J & **Xiong Y** (2014). HIV-1 Vpu-Mediated BST2 Antagonism via Hijacking of the Clathrin Adaptor Protein Complex 1. ***eLife*** 3: e02362. PMCID: PMC5600163

c. Jia X, Sing R, Homann S, Yang H, Guatelli J & **Xiong Y** (2012). Structural Basis of Evasion of Cellular Adaptive Immunity by HIV-1 Nef. ***Nat. Struct. Mol. Biol.*** 19, 701-706. PMCID: PMC3407041

d. Yang H, Wang J, Jia X, Zang T, McNatt MW, Pan B, Meng W, Wang H, Bieniasz PD & **Xiong Y** (2010). Structural Insight into the Mechanisms of Enveloped Viruses Tethering by Tetherin/BST2. ***Proc. Natl. Acad. Sci. USA.*** 107, 18428-18432. PMCID: PMC2972963

5. Suppression of HIV reverse transcription by SAMHD1. SAMHD1, a deoxyribonucleoside triphosphate triphosphohydrolase (dNTPase), prevents the infection of blood cells by HIV via depleting the cellular dNTP pool available for viral reverse transcription. Mutations in SAMHD1 are associated with chronic lymphocytic leukemia (CLL) and the autoimmune disease Aicardi-Goutieres syndrome (AGS). We have made substantial contributions to the understanding of SAMHD1 functions by elucidating i) the active conformation of SAMHD1 that reveals how it depletes cellular dNTP pool to inhibit viral replication, ii) the complete spectrum of nucleotide complexes of SAMHD1 that delineates how it controls cellular dNTP

levels, and iii) how the activities of SAMHD1 are regulated by its phosphorylation. These results provide deep, mechanistic understandings of the many functions of SAMHD1 while guiding future investigations.

a. Espada CE, Levent S, Cahill MP, Phillips S, Martinez N, Lin M, **Xiong Y**, Wu L. (2023). SAMHD1 impairs type I interferon induction through the MAVS, IKKε, and IRF7 signaling axis during viral infection. J. Biol. Chem. 299,104925
b. Knecht KM, Buzovetsky O, Schneider C, Thomas D, Srikanth V, Tofoleanu F, Reiss K, Ferreirós N, Geisslinger G, Batista VS, Ji X, Cinatl Jr. J, Keppler OT, **Xiong Y** (2018). The structural basis for cancer drug interactions with the catalytic and allosteric sites of SAMHD1. ***Proc. Natl. Acad. Sci. USA.*** 115(43):E10022-E10031. PMCID: PMC6205433
c. Buzovetsky O, Tang C, Knecht K, Antonucci JM, Wu L, **Xiong Y** (2017). The SAM domain of mouse SAMHD1 is critical for its activation and regulation. ***Nat. Commun.*** 9:411. PMCID: PMC5788916
d. Ji X, Tang C, Zhao Q, Wang W & **Xiong Y** (2014) Structural basis of Cellular dNTP regulation by SAMHD1. ***Proc. Natl. Acad. Sci. USA.*** 111, E4305-E4314. PMCID: 4205617

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/yong.xiong.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: Yujie Li

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Tongji University, China	B.S.	07/2019	Chemistry
Tsinghua University, China	Ph.D.	07/2024	Biochemistry

A. Personal Statement

My research centers on using cryo-EM to resolve virus-related macromolecular complexes and reveal how viral machines engage host pathways. During my Ph.D., I applied single-particle cryo-EM to characterize SARS-CoV-2 spike bound to a broadly neutralizing IgG. The structures uncovered multiple binding configurations across spike conformations and a previously unrecognized inter-spike crosslinking mode, providing a structural rationale for breadth and for the reduced potency against immune-evasive variants. As a postdoctoral researcher in the Xiong Lab, I focus on HIV-1 capsid biology—its maturation pathway, interactions with the nuclear pore complex (NPC), and engagement with host factors that either promote or restrict infection. I am integrating both in vitro experiments with high-resolution in situ cryo-EM to define the molecular logic of capsid trafficking and remodeling at near-atomic detail, informing mechanism-driven mutagenesis and therapeutic targeting.

B. Positions, Scientific Appointments, and Honors

Professional Experience

2019- **Postdoctoral Associate** with Dr. Yong Xiong, Department of Molecular Biophysics & Biochemistry, Yale University, Howard Hughes Medical Institute, New Haven, Connecticut.

Awards and Fellowships

2024 Outstanding PhD Graduate
2023 China National Scholarship
2022 Shiyou Scholarship at Tsinghua University
2021 Qingluo Scholarship at Tsinghua University
2019 Shanghai Outstanding Graduate
2016 China National Scholarship

C. Contributions to Science

1) Discovered distict binding modes of antibodies with SARS-CoV-2 WT and Omicron variants by cryo-EM, revealing a novel neutralization mechanism. (*Nat. Commun.* **2024**, *15*, 10578.)
2) Proposed design principles of supramolecular networks based on polymeric molecular dynamics and revealed a new scaling law of molecular diffusion in dynamic networks (*Adv. Mater.* **2024**, *36*, 202307129; *J. Am. Chem. Soc.* **2022**, *144*, 19017.)
3) Developed a cationic lipid-independent strategy to prepare mRNA-lipid nanoparticle (LNP) based on frame-guided assembly, enabling intracellular delivery of target mRNA (*Nano Today* **2023**, *52*, 101991.)

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:	Chunxiang Wu
eRA COMMONS USER NAME (credential, e.g., agency login):	CHUNXIANGWU
POSITION TITLE:	Associate Research Scientist

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
Anhui Agricultural University	BS	06/2011	Biological Sciences
Indiana University	PHD	08/2019	Biochemistry

A. Personal Statement

I was trained in biochemistry and structural biology using X-ray crystallography and cryo-EM. My current study at Yale University involves structural mechanism of the host-viral interactions of HIV, which includes interactions of co-factors Nup358, TrimCyp and small molecule inhibitors targeting HIV capsid.

B. Positions, Scientific Appointments, and Honors

My current position is Associate Research Scientist in Yong Xiong lab at Yale University.

C. Contributions to Science

Chunxiang Wu, Megan E. Meuser, Juan S. Rey, Hamed Meshkin, Rachel Yang, Swapnil Chandrakant Devarkar,Christian Freniere, Jiong Shi, Christopher Aiken, Juan R. Perilla, Yong Xiong. Structural insights into inhibitormechanisms on immature HIV-1 Gag lattice revealed by high-resolution in situ single-particle cryo-EM. bioRxiv 2024.10.09.617473 (in preparation for re-submission)

Fidel Arizaga Jr., Christian Freniere, Juan S. Rey, Matthew Cook, Chunxiang Wu, Juan R. Perilla, and Yong Xiong. Exploring the Structural Divergence of HIV and SRLV Lentiviral Capsids. Journal of the American Chemical Society (2025).

Wang C, Huang H, Valera L, Parcella K, Iwuagwu C, McAuliffe B, Falk PJ, O'Boyle II DR, Rose RE, Padilla RR, Wu C, Xiong Y, Kadow J, Hanumegowda U, Sardo L, Gillis EP, Krystal M, Fridell RA. 0. Preclinical virology profiles of the HIV-1 capsid inhibitors VH4004280 and VH4011499. Antimicrob Agents Chemother 0:e00309-25.

Matthew Cook, Christian Freniere, Chunxiang Wu, Faith Lozano, Yong Xiong. Structural insights into HIV-2 CA lattice formation and FG-pocket binding revealed by single particle cryo-EM. Cell Reports. Volume 44, issue 2, 115245 (2025).

Qingzhou Feng, Martin Saladin, Chunxiang Wu, Eason Cao, Wei Zheng, Amy Zhang,Pushpanjali Bhardwaj, Xia Li,Qi Shen, Larisa E. Kapinos, Toshiya Kozai,Malaiyalam Mariappan, C. Patrick Lusk, Yong Xiong, Roderick Y. H. Lim, Chenxiang Lin. Channel width modulates the permeability of DNAorigami–based nuclear pore mimics. Sci. Adv.10, eadq8773 (2024).

Hu, Y., Delviks-Frankenberry, K.A., Wu, C. et al. Structural insights into PPP2R5A degradation by HIV-1 Vif. Nat Struct Mol Biol 31, 1492–1501 (2024).

Wu, C., Xiong, Y. Enrich and switch: IP6 and maturation of HIV-1 capsid. Nat Struct Mol Biol 30, 239–241 (2023).

Shen, Q., Feng, Q., Wu, C. et al. Modeling HIV-1 nuclear entry with nucleoporin-gated DNA-origami channels. Nat Struct Mol Biol 30, 425–435 (2023).

Duan, D., Lyu, W., Wu K., Wu, C., Xiong, Y., Koleske, A.J. Abl2 mediates microtubule nucleation and repair via tubulin co-condensation. Biophysical Journal 122, 124a (2023). Q. Shen, S. Kumari, C. Xu, S. Jang, J. Shi, R.C. Burdick, L. Levintov, Q. Xiong, C. Wu, S.C. Devarkar, T. Tian, T. N. Tripler, Y. Hu, S. Yuan, J. Temple, Q. Feng, C.P. Lusk, C. Aiken, A.N. Engelman, J.R. Perilla, V.K. Pathak, C. Lin, Y. Xiong, The capsid lattice engages a bipartite NUP153 motif to mediate nuclear entry of HIV-1 cores, Proc. Natl. Acad. Sci. U.S.A. 120 (13) e2202815120

Shen Q, Xiong Q, Zhou K, Feng Q, Liu L, Tian T, Wu C, Xiong Y, Melia TJ, Lusk CP, Lin C. Functionalized DNA-Origami-Protein Nanopores Generate Large Transmembrane Channels with Programmable Size-Selectivity. J Am Chem Soc. 2022 Dec 28. Epub ahead of print.

Peng L, Hu Y, Mankowski MC, Ren P, Chen RE, Wei J, Zhao M, Li T, Tripler T, Ye L, Chow RD, Fang Z, Wu C, Dong MB, Cook M, Wang G, Clark P, Nelson B, Klein D, Sutton R, Diamond MS, Wilen CB, Xiong Y, Chen S. Monospecific and bispecific monoclonal SARS-CoV-2 neutralizing antibodies that maintain potency against B.1.617. Nat Commun. 2022 Mar 28;13(1):1638.

Shen Q, Wu C, Freniere C, Tripler TN, Xiong Y. Nuclear Import of HIV-1. Viruses. 2021 Nov 8;13(11):2242. Martin-Sancho, L., Lewinski, M.K., Pache, L., Stoneham, C.A., Yin, X., Becker, M.E., Pratt, D., Churas, C., Rosenthal, S.B., Liu, S., Weston, S., De Jesus, P.D., O'Neill, A.M., Gounder, A.P., Nguyen, C., Pu, Y., Curry, H.M., Oom, A.L., Miorin, L., Rodriguez-Frandsen, A., Zheng, F., Wu, C., Xiong, Y., Urbanowski, M., Shaw, M.L., Chang, M.W., Benner, C., Hope, T.J., Frieman, M.B., García-Sastre, A., Ideker, T., Hultquist, J.F., Guatelli, J., Chanda, S.K. (2021). "Functional landscape of SARS-CoV-2 cellular restriction." Mol Cell 81(12): 2656-2668.e2658.

Chunxiang Wu, Sivinski, J., Ambrose, A.J., Panfilenko, I., Zerio, C.J., Machulis, J.M., Mollasalehi, N., Kaneko, L.K., Stevens, M., Ray, A.M., Park, Y., Wu, C., Hoang, Q.Q., Johnson, S.M., Chapman, E. (2021). "Functional Differences between E. coli and ESKAPE Pathogen GroES/GroEL." mBio 12(1).

Wu, C.X., Liao, J., Park Y., Reed X., Engel V.A., Hoang, N.C., Takagi, Y., Johnson, S.M., Wang, M., Federici, M., Nichols, J., Sanishvili, R., Cookson, M.R., and Hoang Q.Q. (2019) Parkinson's disease-associated mutations in the GTPase domain of LRRK2 impair its nucleotide dependent conformational dynamics. J Biol Chem. 294(15) 5907–5913.

Wu, C.X., Liao, J., Park Y., Reed X., Engel V.A., Hoang, N.C., Takagi, Y., Johnson, S.M., Wang, M., Federici, M., Nichols, J., Sanishvili, R., Cookson, M.R., and Hoang Q.Q. (2020) A revised 1.6 Å structure of the GTPase domain of the Parkinson's disease-associated protein LRRK2 provides insights into mechanisms. (Manuscript in preparation, preprint on bioRxiv).

Huang, X. Wu, C., Park Y., Long X., Hoang, Q.Q., and Liao, J. (2018) The Parkinson's disease–associated mutation N1437H impairs conformational dynamics in the G domain of LRRK2. FASEB J. 33(4):4814-4823.

Wang, W., Nguyen, L.T.T., Burlak, C., Chegini, F., Guo, F., Chataway, T., Ju, S., Fisher, O.S., Miller, D.W., Datta, D., Wu, F., Wu, C.X., Landeru A., Wells, J.A., Cookson, M.R., Boxer, M.B., Thomas, C.J., Gai, W.P., Ringe, D., Petsko, G.A., and Hoang, Q.Q. (2016). "Caspase-1 causes truncation and aggregation of the Parkinson's disease-associated protein alpha-synuclein." Proc Natl Acad Sci, USA, 113(34): 9587-9592.

Mascarenhas, R., Thomas, P.W., Wu, C.X., Nocek, B.P., Hoang, Q.Q., Liu, D., and Fast, W. (2015) Structural and Biochemical Characterization of AidC, a Quorum-Quenching Lactonase with Atypical Selectivity. Biochemistry, 54(28) 4342–4353.

Liao, J., Wu, C.X., Burlak, C., Zhang, S., Sahm, H., Wang, M., Zhang, Z.Y., Vogel, K.W., Federici, M., Riddle, S.M., Nichols, R.J., Liu, D., Cookson, M.R., Stone, T.A., and Hoang, Q.Q. (2014) Proc Natl Acad Sci, USA, 111 (11) 4055-4060.

Tashiro, S., Caaveiro, J.M.M., Wu, C.X., Hoang, Q.Q., and Tsumoto, K. (2014) Thermodynamic and Structural Characterization of the Specific Binding of Zn (II) to Human Protein DJ-1. Biochemistry, 53 (14), pp 2218–2220

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Devarkar, Swapnil Chandrakant

eRA COMMONS USER NAME: SDEVARKAR (ORCID ID: 0000-0002-9271-243X)

POSITION TITLE: Associate Research Scientist

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
D.Y. Patil University (Mumbai, India)	B.Tech.	07/2006	08/2010	Biotechnology
Rutgers University (NJ, USA)	Ph.D.	09/2011	10/2017	Biochemistry
Rutgers University (NJ, USA)	Postdoctoral Fellow	11/2017	11/2018	Biochemistry
Yale University (CT, USA)	Postdoctoral Associate	12/2018	02/2023	Structural Biology
Yale University (CT, USA)	Associate Research Scientist	03/2023	Present	Structural Biology

A. Personal Statement

The intersection of human pathogens, our immune system, and therapeutics has always driven my curiosity, and the central theme of my research career has been the structural and mechanistic characterization of RNA-protein complexes integral to the host-viral interface. I work at the interface of biochemistry, enzymology, and structural biology and I am a firm proponent of an interdisciplinary and collaborative approach to scientific research. My graduate research work was carried out in two labs with different scientific backgrounds (Dr. Joseph Marcotrigiano, a structural biologist and Dr. Smita S. Patel, an enzymologist), allowing me to develop a diverse skillset and learn the basic tenets of fostering successful collaborations. My postdoctoral research work at Yale has centered on the use of single-particle (SPA) cryo-EM to solve the structures of challenging RNA-protein complexes and I have leveraged my interdisciplinary expertise to foster collaborations with nine different labs across four departments and coauthored eleven publications via these collaborations. I have also developed and laid down the groundwork for my future research program in the last three years, securing an R21 grant towards Aim 1 of my research program. My research vision is to unravel the mechanistic and structural underpinnings of essential viral mechanisms for hijacking host translation machinery and create novel avenues for structure-guided drug design against emergent viruses.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

03/2023 – Present	Associate Research Scientist, Yale University (USA)
12/2018 – 02/2023	Postdoctoral Research Associate, Yale University (USA)
11/2017 – 11/2018	Postdoctoral Research Fellow, Rutgers University (USA)
09/2011 – 10/2017	Graduate Research Assistant, Rutgers University (USA)
10/2010 – 05/2011	Research Assistant, Advanced Cancer Treatment and Research Centre (ACTREC) (India)

Honors and Awards

2021 – 2023	Spearheaded the grant writing and experimental work for the R21 Grant (AI157890) from NIAID, NIH
2015	'Aaron Shatkin Award' for excellence in Research and Academics, Rutgers University
2011	'Sharadchandra Pawar Gold Medal' for Best Student of the University, D.Y. Patil University
2007 – 2009	Academic Scholarship, D.Y. Patil University

Leadership, Teaching, and Mentoring Experience

2025-Present	Early Career Reviewer (ECR) for the Journal of Biological Chemistry)
2024	Mentored sabbatical for Prof. Jessica A. Brown (Associate Professor, University of Notre Dame) (Cryo-EM sample preparation, data collection, and data processing)
2020-Present	Mentor for graduate thesis research work for Michael Vetick (M.D./Ph.D. candidate) and Shravani Balaji (Ph.D. candidate; NIH F31 awardee [2024]) (Yale University)
2019	Designed the lab workspace for the Xiong lab in the newly constructed Yale Science Building (YSB) and oversaw the relocation of the lab upon completion of the YSB.
2019-2021	Mentor for undergraduate research work of Ethan Littlefield (Yale University)
2014-2017	Mentor for undergraduate research work for Mihai Solotchi M. (Rutgers University)
2015	Mentored summer research projects for 3 high school students; Arjun Gupta (Biotechnology High school, NJ, USA), Jasper Albers (Jacob van Liesveldt, Netherlands) and Neel Patel (Rutgers Prep High School, NJ, USA)
2013-2016	Served as a Judge for New Jersey Regional Science Fair for High School Research
2013-2014	Tutor for the Molecular Biosciences Peer Tutoring Program, Rutgers University
2014	Served as a Judge for Undergraduate Research Symposium, Rutgers University

C. Contributions to Science

1) **Graduate Career:** Cytosolic innate immune receptors like RIG-I, MDA5 and cGAS have rose in prominence in the last decade as the first responders against viral infections. My graduate thesis research work was focused on the innate immune receptor RIG-I, a central player in immune responses against RNA viruses. RIG-I is a DExD/H-box helicase that specifically recognizes viral RNAs and initiates the Type I interferon signaling cascade. I have authored six publications (three as first-author) on RIG-I and these publications have been foundational in understanding the mechanistic and structural basis for the remarkable 'self-versus-non-self' RNA discrimination by RIG-I. My graduate research work provided a comprehensive characterization of the activation pathway for RIG-I and how the RNA-binding, ATPase, and oligomerization activities of RIG-I govern its activation of the Type I interferon signaling cascade. I delineated RNA modifications that can specifically activate or evade RIG-I mediated interferon signaling. My graduate research work established that 2'-O-methylation in cellular Cap-1 RNAs is the critical RNA modification to evade RIG-I recognition and provided a structural basis for how 5'-m7G capped RNAs (Cap-0) can be recognized by RIG-I. These results provided a roadmap for antiviral therapeutic designs targeting viral methyltransferases that render viral transcripts susceptible to immune recognition. These findings were also important for the field of RNA therapeutics wherein earlier clinical trials were marred by autoimmune responses and 2'-O-methylation of the 5'-end of the RNA ligand was used to alleviate the problem.

    a) Ramanathan A.*, **Devarkar S.C.***, Jiang F., Miller M.T., Khan A.G., Marcotrigiano J., Patel S.S. The autoinhibitory CARD2-Hel2i interface of RIG-I governs RNA selection. Nucleic Acids Research, Dec 2015. (PMID: 26612866)
    b) **Devarkar S.C.*,** Wang C.*, Miller M.T., Ramanathan A., Jiang F., Khan A.G., Patel S.S., Marcotrigiano J. Structural basis for m7G recognition and 2'-O-methyl discrimination in capped

RNAs by the innate immune receptor RIG-I. Proceedings of the National Academy of Sciences of the United States of America, Jan 2016. (PMID: 26733676)

***** Equal Contribution

2) Postdoctoral Career:

RIG-I is an ATPase-powered 3'→5' dsRNA translocase: I spent an additional year in my graduate research lab as a postdoc to complete a biophysical study that established the directionality and rate of RIG-I's ATPase powered translocation. This study provided a 'kinetic proofreading' model for how RIG-I constantly samples self-RNAs in the cytoplasm to detect potential viral RNAs and oligomerizes specifically on viral RNAs to trigger interferon production. The study also provided the basis for how mutations in the RIG-I gene linked to the 'Singleton-Merten' syndrome led to autoimmunity. Through HDX-MS analysis performed by our collaborators at Scripps Institute in Florida, we were also able to directly show the conformational changes in RIG-I mutants that lead to the Singleton-Merten syndrome.

a) **Devarkar S.C.,** Schweibenz B., Wang C., Marcotrigiano J., Patel S.S. RIG-I uses an ATPase-powered translocation-throttling mechanism for kinetic proofreading of RNAs and oligomerization. Molecular Cell, Sep 2018. (PMID: 30270105).
b) Zheng J.*, Wang C.*, Chang M.R., **Devarkar S.C.,** Schweibenz B., Crynen G., Garcia-Ordonez R., Pascal B., Novick S., Patel S.S., Marcotrigiano J., Griffin P. HDX-MS reveals dysregulated checkpoints that compromise discrimination against self RNAs during RIG-I mediated autoimmunity. Nature Communications, Dec 2018 (PMID: 30560918).

Host Translation Shutdown by Betacoronaviruses (β-CoVs): My early tenure as a postdoc at Yale was marked by the onset of the Covid-19 pandemic. As part of Yale's Covid-19 centered research efforts, we collaborated with the Chen lab (Department of Genetics, Yale University) and discovered that the Non-structural protein 1 (Nsp1) of SARS-CoV-2 was the principal pathogenicity factor during SARS-CoV-2 infections. SARS-CoV-2 Nsp1 for shuts down host translation and diverts the host translation machinery towards viral protein production. I was part of a group effort that reported the first high-resolution cryo-EM structure of SARS-CoV-2 Nsp1 in complex with the 40S ribosomal subunit. The CTD of SARS-CoV-2 Nsp1 forms a 'helix-turn-helix' motif that blocks the mRNA entry channel of the 40S ribosome and prevents cellular mRNA loading. Since the large zoonotic reservoir of β-coronaviruses (CoVs) remains a likely source for future outbreaks, I focused my work on understanding whether Nsp1 from other β-CoVs share a similar mechanism for host translation inhibition. MERS-CoV is still actively circulating and is the most distantly related β-CoV to SARS-CoV-2, with modest sequence conservation for Nsp1. I solved a high-resolution cryo-EM structure of MERS-CoV Nsp1 in complex with the human 40S ribosomal subunit and discovered that despite significant sequence divergence from SARS-CoV-2 Nsp1, the CTD of MERS-CoV Nsp1 adopts an identical 'helix-turn-helix' fold and binds in the mRNA entry channel of the 40S ribosomal subunit. Thus, our structural studies with SARS-CoV-2 and MERS-CoV Nsp1 uncovered a highly conserved mechanism across β-CoVs for shutting down host translation. These studies provide a structural guide to the conserved and essential motifs of β-CoV Nsp1 interaction with the 40S ribosomal subunit that can be leveraged in structure-guided drug design of antivirals broadly restricting β-CoVs.

a) Yuan S.*, Peng L.*, Park J.J., Hu Y., **Devarkar S.C.,** Dong M.B., Shen Q., Wu S., Chen S., Lomakin I.B., Xiong Y. Nonstructural Protein 1 of SARS-CoV-2 Is a Potent Pathogenicity Factor Redirecting Host Protein Synthesis Machinery toward Viral RNA. Molecular Cell, Dec 2020 (PMID: 31448897)
b) **Devarkar S.C.***, Vetick M.*, Balaji S., Lomakin I.B., Yang L., Jin D., Gilbert W., Chen S., Xiong Y. Structural basis for translation inhibition by MERS-CoV Nsp1 reveals a conserved mechanism for betacoronaviruses. Cell Reports 2023, 42(10):113156 (PMID: 37733586)

***** Equal Contribution

Human Lysyl tRNA synthetase (h-LysRS), $tRNA^{Lys3}$, and HIV-1 reverse transcription: During my postdoctoral research, I have laid down the groundwork for my future research program. HIV-1 reverse transcription begins at the primer binding site (PBS), an 18-nucleotide region in the HIV-1 genome complementary to the 3'-end of $tRNA^{Lys3}$. Therefore, packaging of host $tRNA^{Lys3}$ into nascent virions for initiating reverse transcription is an indispensable step in the HIV-1 life cycle. h-LysRS, the cellular aminoacylation enzyme

for lysine tRNAs, has been shown to be instrumental in mediating the packaging of lysine tRNAs into nascent HIV-1 virions. Furthermore, tRNAs in humans are extensively modified post-transcriptionally and $tRNA^{Lys3}$ contains nine different modifications and a total of 14 modified nucleotides. Dysregulation of $tRNA^{Lys3}$ modifications has been implicated in metabolic disorders like type-II diabetes and Galloway-Mowat syndrome, whereas mutations in h-LysRS have been linked to neurological disorders like Charcot-Marie-Tooth disease and congenital leukoencephalopathy. Despite the central role played by h-LysRS and $tRNA^{Lys3}$ in protein translation, HIV-1 life cycle, and a wide array of human diseases, the structural and mechanistic basis for $tRNA^{Lys3}$ recognition and aminoacylation by h-LysRS has been lacking. I spearheaded the conceptualization, experimental work, and grant writing for the research program to elucidate the mechanistic and structural basis for h-LysRS-$tRNA^{Lys3}$ packaging by nascent HIV-1 virions, a critical aspect of HIV-1 life cycle that has remained unclear for over 30 years. An R21 grant (AI157890-01A1, Jan 2021-Dec 2023) was awarded for this proposal, and I have recently solved the first structure of h-LysRS bound to a fully-modified cellular $tRNA^{Lys3}$ and a component of the multi-tRNA synthetase complex. I served as the lead corresponding author for this study. Insights from this study have broad implications for understanding the roles of tRNA modifications in governing aminoacylation in humans. This study provides a foundation for understanding the specific packaging of the h-LysRS-$tRNA^{Lys3}$ complex by HIV-1 for priming reverse transcription (Aim 1 of my research program).

a) **Devarkar S.C.†**, Budding C.R., Pathirage C., Kavoor A., Herbert C., Limbach P., Musier-Forsyth K.†, Xiong Y†. Structural basis for aminoacylation of cellular modified $tRNA^{Lys3}$ by human lysyl-tRNA synthetase. *Nucleic Acids Research* 2025 Feb 27;53(5):gkaf114. (*PMID: 40036503)*

[† Corresponding author]

Structural Basis for Translation Inhibition of *Cutibacterium acnes* (*C. acnes*) by Sarecycline: Over the past three years, I have fostered a close collaboration with the Bunick lab (Department of Dermatology, Yale University) and lent my expertise in cryo-EM to address key questions in the Dermatology field. Sarecycline (SAR) is a third-generation tetracycline that was FDA-approved in 2018 for treatment of acne vulgaris. SAR is the only available narrow-spectrum antibiotic against acne and the underlying basis for its narrow-spectrum activity against *C. acnes*, the causative agent for acne, remained unknown. We reported the first high-resolution cryo-EM structure of the *C. acnes* 70S ribosome. We discovered two novel proteins in the *C. acnes* 70S ribosome, bS22 and bL37, that are missing in the ribosomes of widely studied bacteria, E. coli and T. thermophilus. We also observed a novel second binding site for SAR in the nascent peptide exit tunnel (NPET) of the *C. acnes* 70S ribosome, along with the tetracycline canonical binding site in the A-site of the 30S ribosomal subunit. We propose that the narrow-spectrum activity of SAR against C. acnes is due to the dual targeting of the two active centers of the *C. acnes* 70S ribosome – the mRNA decoding center and the peptidyl transferase center. I have recently concluded a follow-up study for the structural characterization of clinically prescribed tetracyclines (Minocycline and Doxycycline) that challenges current knowledge and redefines the mechanism of action for how these antibiotics inhibit bacterial translation (manuscript under preparation). Together, these studies present avenues for design of narrow spectrum tetracyclines, in the mold of Sarecycline, that can target specific pathogenic bacteria.

a) Lomakin I.B.*, **Devarkar S.C.*,** Patel S., Grada A. Bunick C. Acne vulgaris antibiotic sarecycline inhibits protein translation in Cutibacterium acnes 70S ribosome using a two-site mechanism. Nucleic Acids Research, 2023 (PMID: 36864821)
b) **Devarkar S.C.*,** Lomakin I.B.*, Wang J., Grada A., Bunick C.G. Structures redefine the mechanism of action for tetracyclines. (under peer-review, Aug 2025)

***** Equal Contribution

Collaborations: During my time at Yale, I've lent my extensive expertise in characterizing RNA-protein interactions and structural biology towards collaborations with several labs across various departments. Using SEC-MALS and negative staining electron microscopy, I helped the Schlieker lab (MB&B Dept. Yale University) in identifying a key novel regulator of endoplasmic reticulum morphology named Nodal Modulator1 (NOMO1) and determine its native oligomeric state. I also used my expertise in cryo-EM data processing and characterization of RNA-protein complexes to assist the Pyle lab (MCDB Dept., Yale University) in determination of a high-resolution structure of a mobile intron retroelement bound to its target

DNA. I recently led a collaborative project with the Anderson lab (Pharmacology Dept., Yale University) for the development and structural characterization of novel HIV-1 reverse transcriptase inhibitors that can clear latent HIV-1 reservoirs. In total, my collaborative work at Yale has led to eleven publications via nine different collaborations.

a) Chung K.*, Xu L.*, Chai P., Peng J., **Devarkar S.C.,** Pyle A.M. CryoEM Structures of a Mobile Intron Retroelement Poised to Attack Its Structured DNA Target. Science, 2022 (PMID: 36356138)
b) Hollander K.*, **Devarkar S.C.*,** Tang S.*, Tiwari R.*, Ma S., Lee W.G., Denn E., Wang Q., Spasov K.A., Robbins J.A., Frey K.M., Jorgensen W.L., Xiong Y., Shan L., Anderson K.S. Mechanistic basis for a novel dual-function Gag-Pol dimerizer potentiating CARD8 inflammasome activation and clearance of HIV-infected cells. npj Drug Discovery (2025) (PMID: 40904837)
c) Naughton B.S., **Devarkar S.C.**, Todorow V., Malik S., Oxendine S., Junnarkar S., Ren Y., Berro J., Kirstein J., Xiong Y., Schlieker C. Nodal Modulator (NOMO) is a force-bearing transmembrane protein required for muscle differentiation. Journal of Cell Biology (2025) (PMID: 40663102)

* Equal Contribution

D. Complete List of My Published Work:

NIH: https://www.ncbi.nlm.nih.gov/myncbi/swapnil%20chandrakant.devarkar.1/bibliography/public/