Key personnel:

Yong Xiong (PI)

Yingxia Hu (primary user)

Shuai Yuan (secondary user)

BIOGRAPHICAL SKETCH

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NAME: Xiong, Yong

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

POSITION TITLE: Associate 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	B.S.	05/1994	Physics
Ohio State University	Ph.D.	12/2000	Biophysics

A. Personal Statement

My lab uses a multidisciplinary approach including structural, biochemical, computational, and functional studies to investigate the mechanisms of host-pathogen interactions at the molecular level. My research program encompasses a wide range of host-virus interactions in the HIV life cycle, centering on HIV suppression by host innate immune restriction factors and viral immune evasion strategies. We have obtained important results for numerous newly discovered host-viral interaction systems. Specifically for this application, 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 single particle cryo-EM studies, and identify new host factors that only recognize patterns in the assembled HIV capsid.

a. 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 (in press).
b. Huang P, Summers BJ, Xu C, Perilla JR, Malikov V, Naghavi MH & Xiong Y. (2019). FEZ1 is recruited to a conserved cofactor site on capsid to promote HIV-1 trafficking. Cell Reports (in press)
c. Smaga SS, Xu C, Summers BJ, Digianantonio KM, Perilla JR, Xiong Y (2019). MxB restricts HIV-1 by targeting the tri-hexamer interface of the viral capsid. Structure (in press)
d. Fribourgh J, Nguyen H, Matreyek KA, Alvarez FJD, Summers BJ, Dewdney TG, Aiken C, Zhang P, Engelman A & Xiong Y (2014). Structural Insight into HIV-1 Restriction by Myxovirus Resistance Protein 2 (MxB). Cell Host Microbe 16, 627-638. PMCID: PMC4252739
e. Yang H, Ji X, Zhao G, Ning J, Zhao Q, Aiken C, Gronenborn AM, Zhang P & Xiong Y (2012). Structural Insight into HIV-1 Capsid Recognition by Rhesus TRIM5α. Proc. Natl. Acad. Sci. USA. 109,18372-18377. PMCID: PMC3494900

B. Positions and Honors

Positions and Employment

2000-2001 Postdoctoral Associate with Dr. Muttaiya Sundaralingam, Chemistry Department, Ohio State University, Columbus, Ohio.
2001-2006 Postdoctoral Associate with Dr. Thomas Steitz, Department of Molecular Biophysics & Biochemistry, Yale University, Howard Hughes Medical Institute, New Haven, Connecticut.
2006-2011 Assistant Professor, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut.
2011- Associate Professor, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut.

Awards and Fellowships

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
1999-2000 Dissertation Fellowship, The Ohio State University
1994-1995 Bennett Fellowship, The Ohio State University
1994-1995 University Fellowship, The Ohio State University

C. Contributions to Science

Besides research on HIV capsid-host protein interactions, we have made significant other contributions to the scientific community by furnishing in-depth structural and mechanistic results for a range of new host-viral interaction systems. 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 67 peer-reviewed publications.

1. 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.
    f. Jia X, Weber E, Tokarev A, Lewinski M, Rizk M, Suarez M, Guatelli J & Xiong Y (2014). HIV-1 VpuMediated BST2 Antagonism via Hijacking of the Clathrin Adaptor Protein Complex 1. eLife 3: e02362. PMID: 24843023
    g. 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. PMID: 22705789
    h. 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. PMID: 20940320

2. 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. 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. doi:10.1073/pnas.1805593115

b. 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. DOI: 10.1038/s41467-017-02783-8.

c. Tang C, Ji X, Wu L & Xiong Y (2015). Impaired dNTPase Activity of SAMHD1 by Phosphomimetic Mutation of T592. J. Biol. Chem. 290, 26352-26359. PMID: 26294762

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

e. Ji X, Wu Y, Yan J, Mehrens J, Yang H, DeLucia M, Hao C, Gronenborn AM, Skowronski J, Ahn J & Xiong Y (2013). Mechanism of Allosteric Activation of SAMHD1 by dGTP. Nat. Struct. Mol. Biol. 20, 1304-1309. PMCID: 3833828

3. 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. Ziegler SJ, Liu C, Landau M, Buzovetsky O, Desimmie BA, Zhao Q, Sasaki T, Burdick RC, Pathak VK, Anderson1 KS, Xiong Y (2017). Insights into DNA substrate selection by APOBEC3G from structural, biochemical and functional studies. PLoS ONE 13(3): e0195048.

   b. Nguyen H, Yang H, Fribourgh J, Wolfe LS & Xiong Y (2015). Insights into Cullin-RING E3 ubiquitin ligase recruitment: Structure of the VHL–EloBC–Cul2 complex. Structure 23, 1-9. PMID: 25661653

   c. 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

   d. Wolfe LS, Stanley BJ, Liu C, Eliason WK, & Xiong Y (2010). Dissection of HIV Vif interaction with the human E3 ubiquitin ligase. J. Virol. 84, 7135-7139. PMCID: 2898223

   e. Stanley BJ, Ehrlich ES, Short L, Yu Y, Xiao Z, Yu XF & Xiong Y (2008). Structural Insight into the HIV Vif SOCS Box and Its Role in Human E3 Ubiquitin Ligase Assembly. J. Virol. 82, 8656-63. PMCID: 2519636

4. Fanconi Anemia (FA) pathway of DNA damage repair. FA is a genetic disease characterized by developmental abnormalities, bone marrow failure, chromosome fragility, and a much-elevated incidence of cancer. Studies of the FA pathway of DNA damage response have led to new paradigms of how defects in DNA crosslink (ICL) repair lead to cancer pathogenesis. We have made significant contribution to the elucidation of the FA pathway by examining the protein complexes that function at critical steps in this pathway.

   a. Zhao Q, Xue X, Longerich S, Sung P & Xiong Y (2014). Structural Insights into 5' Flap DNA Unwinding and Incision by the Human FAN1 Dimer. Nat. Commun. 5:5726 doi:10.1038/ncomms6726. PMID: 25500724

   b. Zhao Q, Saro D, Sachpatzidis A, Singh RR, Schlingman D, Zheng X, Mack A, Tsai MS, Mochrie S, Regan L, Meetei AR, Sung P & Xiong Y (2014). MHF complex senses branched DNA via binding a pair of crossover DNA duplexes. Nat. Commun. 5:2987-98. PMCID: 3967914

   c. Singh TS, Saro D, Ali AM, Zheng X, Du C, Killen MW, Wahengbam K, Pierce AJ, Sachpatzidis A, Xiong Y, Sung P & Meetei AR (2010). MHF1-MHF2, a Histone-Fold-Containing Protein Complex, Participates in the Fanconi Anemia Pathway via FANCM. Mol. Cell 37, 879-86. PMID: 20347429

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/collections/bibliography/40672264/

D. Research Support

Ongoing

R01AI116313 Xiong (PI) 07/01/15 – 06/30/20
National Institute of Health
Recognition of Viral DNA by APOBEC3 Proteins and their Antagonization by HIV Vif
The major goal of this project is to determine the APOBEC3-DNA and the APOBEC3-Vif interactions.
Role: PI

R21AI136737 Xiong (PI) 05/01/18 – 04/30/20
National Institute of Health
Comparative structure and function analyses of human and mouse SAMHD1 proteins
The major goal of this project is to determine the mechanistic differences of human and mouse SAMHD1.
Role: PI

R01AI129706 Guatelli (PI) 04/01/17 – 03/31/22
National Institute of Health
Enhancement of Infectivity by HIV-1 Nef via Antagonism of SERINC Proteins
The major goal of this project is to define the biochemical and structural basis of HIV inhibition by SERINC proteins and their antagonization by HIV Nef.
Role: Co-Investigator

P50GM082251 Gronenborn (PI) 08/01/17 – 7/31/22
National Institute of Health
Mechanisms of retroviral capsid sensing by host protein binding partners
The major goal is to establish the viral capsid pattern-sensing mechanisms of TRIM5 and MxB proteins.
Role: Project Leader

P01 CA016038 Dimaio (PI) 09/01/15 – 08/31/20
National Institute of Health
Molecular Basis of Cancer Virus Replication, Transformation, and Innate Defense
The major goal is to obtain structural information of ZEBRA-DNA interactions.
Role: Co-Investigator

Completed

R01AI102778 Xiong (PI) 01/01/13 – 06/30/19
National Institute of Health
Viral Hijacking of Host Membrane Trafficking Pathways
The major goal is to investigate the immune evasion by HIV Nef and Vpu through trafficking pathways
Role: PI

R01AI097064 Xiong (PI) 05/01/11 – 10/31/16
National Institute of Health
Mechanisms of enveloped virus tethering by tetherin and viral countermeasures
The major goals are structural studies of tetherin, and tetherin-Vpu and Vpu-βTrCP interactions.
Role: PI

BIOGRAPHICAL SKETCH

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NAME: Hu, Yingxia

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

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
University of Science and Technology of China	B.S.	08/2005	05/2009	Biotechnology
Oklahoma State University	Ph.D.	08/2009	12/2015	Biochemistry and Molecular Biology
Yale University, New Haven	Postdoc	01/2016	Present	Molecular Biophysics and Biochemistry

A. Personal Statement

My research interest mainly focuses on understanding the interactions between HIV-1 virus and different host restriction factors at the early stage of viral infection. Specifically for this application, my work contributes to how HIV capsid interacts with various host cellular factors on a structural basis. We have successfully assembled soluble capsid oligomers for the characterizations of the interactions between HIV capsid and different host partners, for example, TRIM5α and TRIM-Cyp, which restrict HIV infection after the viral entry. I have also been trained for the toolset of cryo-EM and is currently responsible for the data collection of various projects in the lab.

B. Positions and Honors

Positions and Employment

2009-2015 Graduate Research Assistant, Oklahoma State University
2016-Present Postdoctoral Associate, Yale University

Other Professional Experience

2017-Present Reviewer, *Bio-protocol*

Honors

2005-2006 Outstanding Student Scholarship, University of Science and Technology of China
2009 Outstanding Undergraduate Thesis, University of Science and Technology of China
2015 First Prize for the Graduate Poster Presentation, the 12th Annual Research Symposium of Biological Sciences at Oklahoma State University

C. Contributions to Science

1. **Early career:** My undergraduate research contribution focused on the structural elucidation of the human nascent polypeptide-associated complex (hNAC), which is the first identified cytosolic chaperone that contacts the nascent polypeptide chain emerging from the ribosome to protect it from inappropriate early interactions with cytosolic factors. I purified and optimized the heterodimeric complex and successfully obtained a well-behaved homogenous sample suitable for crystallization. Under the help of a graduate student, we determined the crystal structure of the hNAC domain and identified a new nucleic acid-binding region which indicated its potential role in transcriptional regulation.

    a. Liu Y, **Hu Y**, Li X, Niu L, Teng M. (2010) The crystal structure of the human nascent polypeptide-associated complex domain reveals a nucleic acid-binding region on the NACA subunit. *Biochemistry* 49:2890-2896.

2. **Graduate career:** My graduate research focused on a defense mechanism named pro-phenoloxidase (PPO) activation pathway in insects, which is an important innate immune response to protect insects against pathogens and parasites. My contributions mainly involved the determination of crystal structures of two defense molecules in the pathway. One is Peptidoglycan Recognition Protein (PGRP)-1 from a biochemical model insect, *Manduca sexta.* It recognizes the invading bacteria to trigger the PPO activation cascade. Unique structural features were identified within the peptidoglycan-binding pocket, providing insights into the recognition mechanism of PGRPs. The paper is in preparation now. The second structure is a PPO from the African malaria mosquito, *Anopheles gambiae*. It is a critical enzyme catalyzing melanization in insects upon immune challenge, and in mosquitoes such as *A. gambiae*, melanotic encapsulation is a resistance mechanism against certain parasites that cause malaria and filariasis. In combination with the molecular docking and mutagenesis studies, I revealed a new substrate-binding site and identified the key catalytic residue for the enzymatic activities, elucidating a conserved catalytic mechanism of phenoloxidase (PO) and offered a new model for PPO activation. This work has been published in *BMC Biology*. In additional to structural studies, I also extensively participated in genomic projects by performing computational structure modeling of various immunity molecules identified in *Manduca sexta* genome to investigate their structure-function relationships.

    a. **Hu Y**, Wang Y, Deng J, Jiang H. (2016) The structure of a prophenoloxidase from Anopheles gambiae provides new insights into the mechanism of PPO activation. *BMC Biol* 14:2.
    b. He Y, Cao X, Li K, **Hu Y**, Chen YR, Blissard G, Kanost MR, Jiang H. (2015) A genome-wide analysis of antimicrobial effector genes and their transcription patterns in Manduca sexta. *Insect Biochem Mol Biol* 62:23-37.
    c. Cao X, He Y, **Hu Y**, Wang Y, Chen YR, Bryant B, Clem RJ, Schwartz LM, Blissard G, Jiang H. (2015) The immune signaling pathways of Manduca sexta. *Insect Biochem Mol Biol* 62:64-74.
    d. Rao XJ, Cao X, He Y, **Hu Y**, Zhang X, Chen YR, Blissard G, Kanost MR, Yu XQ, Jiang H. (2015) Structural features, evolutionary relationships, and transcriptional regulation of C-type lectin-domain proteins in Manduca sexta. *Insect Biochem Mol Biol* 62:75-85.
    e. Cao X, He Y, **Hu Y**, Zhang X, Wang Y, Zou Z, Chen Y, Blissard GW, Kanost MR, Jiang H. (2015) Sequence conservation, phylogenetic relationships, and expression profiles of nondigestive serine proteases and serine protease homologs in Manduca sexta. *Insect Biochem Mol Biol* 62:51-63.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Yuan, Shuai

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

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
University of Science and Technology of China	B.S.	05/2012	Biology
The Institute of Biophysics, Chinese academy of sciences	Ph.D.	01/2018	Biochemistry and molecular biology

A. Personal Statement

My research program mainly focuses on the transportation of the HIV capsid using both crystallography and cryo-EM. After the membrane fusion, the HIV capsid containing the viral genome is released into the cell and then transported to the nuclear. Bicaudal D2 (BICD2), which is one of the most important components in the dynein complex, plays a key role in the retro-transportation process. I'm trying to purify the BICD2 protein and test the interaction between the viral capsid and BICD2. We anticipate that our work can provide more detail information about the transportation in the viral infection and help with new antiviral drug design.

B. Positions and Honors

Professional Experience

2018- **Postdoctoral Associate** with Dr. Yong Xiong, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, Connecticut.

Awards and Fellowships

2017-2018 National Scholarship of China
2011-2012 Outstanding graduates of University of Science and Technology of China

C. Contribution to Science

I have made contributions to Science by determining structures of different types of viruses and studying protein-protein interactions between important viruses and corresponding receptors. The optimized methods we used could help with more challenging biological problems.

1. Structure of herpesvirus capsid

By using a "block-based" image reconstruction approach combined with a Ewald sphere correction, we have visualized the HSV capsid by cryo–electron microscopy and have built atomic structures including three types of hexons that contain the major capsid protein VP5 and the small capsid protein VP26, pentons made up of VP5, and triplexes composed of VP23 and VP19C. We also identified several α-herpesvirus-specific structural features, providing insight into how the shell assembles and is stabilized.

a. **Yuan, S**., Wang, J., Zhu, D., Wang, N., Gao, Q., Chen, W., . . . Wang, X. (2018). Cryo-EM structure of a herpesvirus capsid at 3.1 A. Science, 360(6384). doi:10.1126/science.aao7283
b. Wang, J., **Yuan, S**., Zhu, D., Tang, H., Wang, N., Chen, W., . . . Wang, X. (2018). Structure of the herpes simplex virus type 2 C-capsid with capsid-vertex-specific component. Nature Communications, 9(1), 3668. doi:10.1038/s41467-018-06078-4

2. Interaction between hTIM-1 and Ebola GP

We demonstrated a direct interaction between hTIM-1 and EBOV GP in vitro and determined the crystal structures of the Ig V domains of hTIM-1 and hTIM-4. The binding region in hTIM-1 to EBOV GP was mapped by chimeras and mutation assays, which were designed based on structural analysis. The infection assays performed using hTIM-1 and its homologs as well as point mutants verified the location of the GP binding site and the importance of EBOV GP-hTIM-1 interaction in EBOV cellular entry.

a. **Yuan, S**., Cao, L., Ling, H., Dang, M. H., Sun, Y., Zhang, X. Y., . . . Rao, Z. (2015). TIM-1 acts a dual-attachment receptor for Ebolavirus by interacting directly with viral GP and the PS on the viral envelope. Protein & Cell, 6(11), 814-824. doi:10.1007/s13238-015-0220-y

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https://www.ncbi.nlm.nih.gov/myncbi/collections/mybibliography/