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: Chen, Bing

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

POSITION TITLE: Rosalind Franklin PhD Professor of Pediatrics

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
FUDAN UNIVERSITY, Shanghai	BS	07/1988	Biochemistry
OHIO STATE UNIVERSITY, Columbus, OH	PHD	08/1997	Biochemistry
OHIO STATE UNIVERSITY, Columbus, OH	Postdoctoral Fellow	02/1998	Biochemistry
HARVARD MEDICAL SCHOOL/BOSTON CHILDREN'S HOSPITAL, Boston, MA	Postdoctoral Fellow	02/2004	Structural Biology

A. Personal Statement

I have over 24 years' experience in biochemistry and structural biology of HIV/SIV envelope proteins. My laboratory has produced strictly homogeneous, rigorously characterized forms of HIV-1 Env in various fusion-related conformational states, as well as its complex with receptor CD4 and coreceptor CCR5. These preparations, challenging to produce, are essential for proper analysis of Env structure, function, antigenicity and immunogenicity. Using these preparations, we have made substantial progress in understanding structure, antigenic and immunogenic properties of HIV-1 Env, as well as molecular mechanisms of neutralization by broadly neutralizing antibodies and viral entry. Since the beginning of the COVID-19 pandemic, my group has also made significant contributions to understanding the structures of the intact spike proteins of SARS-CoV-2 and its variants, and we have also designed ACE2-based fusion inhibitors. I currently lead multiple projects focusing on viral entry and inhibition. Overall, I have a proven track record in working on challenging research projects in structural virology, and my expertise, experience and management skills have prepared me to lead the proposed studies in this application.

1. Zhang J, Xiao T, Cai Y, Lavine CL, Peng H, Zhu H, Anand K, Tong P, Gautam A, Mayer ML, Walsh RM, Jr., Rits-Volloch S, Wesemann DR, Yang W, Seaman MS, Lu J, Chen B. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. Science. 2021 Dec 10;374(6573):1353-1360.
2. Zhang J, Cai Y, Xiao T, Lu J, Peng H, Sterling SM, Walsh RM, Rits-Volloch S, Zhu H, Woosley AN, Wei Y, Sliz P, Chen B. Structural impact on SARS-CoV-2 spike protein by D614G substitution. Science. 2021 Apr 30;372(6541):525-530. PubMed PMID: 33727252; PMCID: PMC8139424.
3. Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh Jr RM, Rawson S, Rits-Volloch S, Chen B. Distinct conformational states of SARS-CoV-2 spike protein. Science. 2020 Sep 25;369(6511):1586-1592. PubMed PMID: 32694201; PMCID: PMC7464562.
4. Shaik MM, Peng H, Lu J, Rits-Volloch S, Xu C, Liao M, Chen B. Structural basis of coreceptor recognition by HIV-1 envelope spike. Nature. 2019 Jan;565(7739):318-323. PubMed PMID: 30542158; PMCID: PMC6391877.

B. Positions and Honors

Positions and Employment

1988-1990 Graduate Research Assistant, Masters Program in Biochemistry, Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, China
1990-1991 Graduate Student, Ohio State Biochemistry Program, Ohio State University, Columbus, OH
1991-1993 Graduate Teaching Associate, Department of Biochemistry, Ohio State University, Columbus, OH

1993-1997	Graduate Research Associate, Department of Molecular Genetics, Ohio State University, Columbus, OH
1997-1998	Postdoctoral Fellow, Department of Molecular Genetics, Ohio State University, Columbus, OH
1998-2004	Research Fellow in Biological Chemistry and Molecular Pharmacology, Harvard Medical School/Boston Children's Hospital, Boston, MA
2004-2006	Instructor in Pediatrics, Boston Children's Hospital/Harvard Medical School, Boston, MA
2006-	Tutor in Biochemical Sciences, Harvard University, Cambridge, MA
2006-2012	Assistant Professor of Pediatrics, Department of Pediatrics, Harvard Medical School, Boston, MA
2007-	Associate Scientific Staff, Department of Medicine, Boston Children's Hospital, Boston, MA
2012-2020	Associate Professor of Pediatrics, Harvard Medical School, Department of Pediatrics, Boston, MA
2020-	Professor of Pediatrics, Harvard Medical School, Department of Pediatrics, Boston, MA
2022-	Rosalind Franklin, PhD Professor of Pediatrics, Harvard Medical School, Department of Pediatrics, Boston, MA

Honors

2000	Scholar Award, American Foundation for AIDS Research
2008	ICAAC (Interscience Conference on Antimicrobial Agents and Chemotherapy) Young Investigator Award, American Society for Microbiology
2009	Young/Early Career Investigator of the Collaboration for AIDS Vaccine Discovery (CAVD), Bill & Melinda Gates Foundation

C. Contributions to Science

1. **HIV-1 envelope glycoprotein and viral entry.** HIV-1 envelope glycoprotein (Env) fuses viral and cell membranes, allowing entry of the virus into host cells to initiate infection. The Env polypeptide chain is produced as a precursor, gp160, which trimerizes to $(gp160)_3$ and then undergoes cleavage into two noncovalently associated fragments: gp120 and gp41. Three copies each of gp120 and gp41 form the mature envelope spike $(gp120/gp41)_3$. Gp120 binds to host primary receptor CD4 and then to coreceptor (e.g. CCR5 or CXCR4), triggering large conformational changes and a cascade of refolding events in gp41 that lead to membrane fusion. ***1)*** Production of an immunogen to mimic the native HIV-1 Env spikes is an essential goal for vaccine development, but we lacked a reliable description of a native, functional trimer because of its inherent instability and heterogeneity. We have identified three conformationally homogeneous gp160s derived from difficult-to-neutralize HIV-1 primary isolates. Their antigenicity correlates closely with antibody neutralization. Truncation of the gp41 cytoplasmic tail has little impact on membrane fusion, but diminishes binding to broadly neutralizing antibodies (bnAbs), while exposing non-neutralizing epitopes. These results paint a more accurate antigenic picture than hitherto possible of a genuinely untriggered and functional HIV-1 Env spike; they can guide effective vaccine development. ***2)*** To understand the physical coupling (conformation and/or dynamics) between the CT and the ectodomain of HIV-1 Env, which are connected by the transmembrane domain (TMD), we teamed up with Dr. James Chou, who is a world expert on studying membrane proteins by NMR. We first determined an NMR structure of the TMD of Env in bicelles that mimic a lipid bilayer. We find that the TMD forms a well-ordered trimer, and that mutational changes disrupting the TMD trimer alter antibody sensitivity of the ectodomain, suggesting that the TMD contributes to Env stability and antigenicity. We next completed another structure that contains both the membrane proximal external region (MPER) and TMD. The MPER in this trimeric assembly is well ordered and not buried in membrane; the MPER mutations can destabilize the Env ectodomain and shift it towards an open conformation. Thus, the MPER appears to be a control relay that modulates open and closed states of the Env trimer and exposure of other epitopes. Furthermore, we obtained a third structure containing the TMD and part of CT in bicelles. We find that the CT portion also trimerizes through specific inter-chain interactions, wrapping around the TMD to form a support baseplate for the entire Env. These new structures have filled a major gap in our understanding of the HIV-1 Env structure and may explain why the CT can dramatically influence the ectodomain. ***3)*** HIV-1 Env interacts with primary receptor CD4 and coreceptor (e.g. chemokine receptor CCR5 or CXCR4) to promote viral entry by fusing viral and target cell membranes. Encounter of gp120 with the coreceptor was thought to be the most crucial trigger for unleashing the fusogenic potential of gp41. We have determined a cryo-EM structure, at 3.9Å resolution, of a full-length gp120 complexed with a soluble CD4 and an unmodified human CCR5. We find that the V3 loop of gp120 inserts into the chemokine binding pocket formed by seven transmembrane helices of CCR5, while the N-terminus of CCR5 contacts the CD4-induced bridging sheet of gp120. CCR5 induces no obvious allosteric changes in gp120 that can propagate

to gp41, but it brings the Env trimer close to the target membrane. The N-terminus of gp120, gripped by gp41 in the prefusion or CD4-bound Env, flips back in the CCR5-bound conformation and may irreversibly destabilize gp41 to initiate fusion. Our data suggest that the coreceptor probably functions by stabilizing and anchoring the CD4-induced conformation of Env near the cell membrane. These results advance our understanding of HIV-1 entry and may guide development of vaccines and therapeutics.

a. Shaik MM, Peng H, Lu J, Rits-Volloch S, Xu C, Liao M, Chen B. Structural basis of coreceptor recognition by HIV-1 envelope spike. Nature. 2019 Jan;565(7739):318-323. PubMed PMID: 30542158; PMCID: PMC6391877.
b. Fu Q, Shaik MM, Cai Y, Ghantous F, Piai A, Peng H, Rits-Volloch S, Liu Z, Harrison SC, Seaman MS, Chen B, Chou JJ. Structure of the membrane proximal external region of HIV-1 envelope glycoprotein. Proc Natl Acad Sci U S A. 2018 Sep 18;115(38):E8892-E8899. PubMed PMID: 30185554; PMCID: PMC6156635.
c. Dev J, Park D, Fu Q, Chen J, Ha HJ, Ghantous F, Herrmann T, Chang W, Liu Z, Frey G, Seaman MS, Chen B, Chou JJ. Structural basis for membrane anchoring of HIV-1 envelope spike. Science. 2016 Jul 8;353(6295):172-175. PubMed PMID: 27338706; PMCID: PMC5085267.
d. Chen J, Kovacs JM, Peng H, Rits-Volloch S, Lu J, Park D, Zablowsky E, Seaman MS, Chen B. HIV-1 ENVELOPE. Effect of the cytoplasmic domain on antigenic characteristics of HIV-1 envelope glycoprotein. Science. 2015 Jul 10;349(6244):191-5. PubMed PMID: 26113642; PMCID: PMC4701381.

2. **Antibody neutralization mechanisms.** Induction of bnAbs is an important goal for HIV-1 vaccine development. Progress may require in-depth understanding of the molecular mechanisms of neutralization by monoclonal antibodies. We have analyzed the molecular mechanism of neutralization by the bnAbs (2F5, 4E10 and 10E8) that recognize a conserved region on gp41 adjacent to the viral membrane known as the membrane-proximal external region (MPER). We find that their epitopes are only exposed or formed on a fusion-intermediate state during viral entry. We also find that the hydrophobic CDR H3 loops of these antibodies mediate a reversible attachment to the viral membrane and that they are essential for neutralization but not for interaction with gp41. These results reveal a general mechanism of HIV-1 neutralization by MPER-specific antibodies in which they associate with the viral membrane in a required first step and are poised to capture the transient gp41 fusion intermediate. In addition, we also provide biochemical and structural evidence that non-neutralizing antibodies, capable of binding with high affinity to an immunodominant segment adjacent to the neutralizing epitopes in the MPER, only recognize a gp41 conformation when membrane fusion is complete. We suggest that these non-neutralizing antibodies are induced in HIV-1 infected patients by gp41 antigens in a triggered, postfusion form and contribute to production of ineffective humoral responses. These data may guide strategies for the MPER-based immunogen design.

a. Chen J, Frey G, Peng H, Rits-Volloch S, Garrity J, Seaman MS, Chen B. Mechanism of HIV-1 neutralization by antibodies targeting a membrane-proximal region of gp41. J Virol. 2014 Jan;88(2):1249-58. PubMed PMID: 24227838; PMCID: PMC3911647.
b. Frey G, Chen J, Rits-Volloch S, Freeman MM, Zolla-Pazner S, Chen B. Distinct conformational states of HIV-1 gp41 are recognized by neutralizing and non-neutralizing antibodies. Nat Struct Mol Biol. 2010 Dec;17(12):1486-91. PubMed PMID: 21076402; PMCID: PMC2997185.
c. Alam SM, Morelli M, Dennison SM, Liao HX, Zhang R, Xia SM, Rits-Volloch S, Sun L, Harrison SC, Haynes BF, Chen B. Role of HIV membrane in neutralization by two broadly neutralizing antibodies. Proc Natl Acad Sci U S A. 2009 Dec 1;106(48):20234-9. PubMed PMID: 19906992; PMCID: PMC2787149.
d. Frey G, Peng H, Rits-Volloch S, Morelli M, Cheng Y, Chen B. A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. Proc Natl Acad Sci U S A. 2008 Mar 11;105(10):3739-44. PubMed PMID: 18322015; PMCID: PMC2268799.

3. **HIV-1 Env-based immunogen design and therapeutic development.** HIV-1 Env trimer is the primary target for HIV-1-specific antibodies. Most previous studies on the immunogenicity of HIV-1 envelope oligomers have revealed only marginal improvement over monomers. In collaboration with Dr. Dan Barouch, we have reported that suitably prepared gp140 trimers have nearly all the antigenic properties expected for native viral spikes. These stable, rigorously homogenous trimers have antigenic properties markedly different

from those of monomeric gp120s derived from the same sequences, and they induce potent neutralizing antibody responses for a cross-clade set of viruses with titers substantially higher than those elicited by the corresponding gp120 monomers. These results, which demonstrate that there are relevant immunologic differences between monomers and high-quality envelope trimers, have important implications for HIV-1 vaccine development. Indeed, one of our trimer designs is tested in multiple clinical trials sponsored by Janssen Vaccines & Prevention (NCT02315703-APPROACH, Phase 1/2a, 2014-2017; NCT03060629-Imbokodo, Phase 2b, 2017-2020; NCT03964415-MOSAICO, Phase 3, 2019-2023). Combination antiretroviral therapy (cART) has transformed HIV-1 infection, once a fatal illness, to a manageable chronic condition. The latest cART regimen uses several classes of antiviral therapeutics and a typical therapy requires a combination of three or more drugs from at least two classes. Drug resistance, severe side effects and difficulties in treatment compliance have brought challenges to the implementation of the cART in clinical settings and indicate the need for additional molecular targets. Moreover, the current therapies are not curative since they cannot eliminate latent HIV-1 reservoirs harboring integrated proviruses. Antibody-based therapeutics that can potently inhibit HIV-1 entry and also facilitate killing of Env-expressing cells are promising candidates for reservoir-eliminating strategies. We therefore develop both small-molecule and antibody-derived fusion inhibitors against HIV-1 Env spikes.

a. Xiao T, Frey G, Fu Q, Lavine CL, Scott DA, Seaman MS, Chou JJ, Chen B. Small-molecule fusion inhibitors targeting the membrane proximal external region of HIV-1 envelope spike. Nat Chem Biol., 2020 Mar 9; doi: 10.1038/s41589-020-0496-y.
b. Cai Y, Karaca-Griffin S, Chen J, Tian S, Fredette N, Linton CE, Rits-Volloch S, Lu J, Wagh K, Theiler J, Korber B, Seaman MS, Harrison SC, Carfi A, Chen B. Antigenicity-defined conformations of an extremely neutralization-resistant HIV-1 envelope spike. Proc Natl Acad Sci U S A. 2017 Apr 25;114(17):4477-4482. PubMed PMID: 28396421; PMCID: PMC5410843.
c. Kovacs JM, Noeldeke E, Ha HJ, Peng H, Rits-Volloch S, Harrison SC, Chen B. Stable, uncleaved HIV-1 envelope glycoprotein gp140 forms a tightly folded trimer with a native-like structure. Proc Natl Acad Sci U S A. 2014 Dec 30;111(52):18542-7. PubMed PMID: 25512514; PMCID: PMC4284565.
d. Kovacs JM, Nkolola JP, Peng H, Cheung A, Perry J, Miller CA, Seaman MS, Barouch DH, Chen B. HIV-1 envelope trimer elicits more potent neutralizing antibody responses than monomeric gp120. Proc Natl Acad Sci U S A. 2012 Jul 24;109(30):12111-6. PubMed PMID: 22773820; PMCID: PMC3409750.

4. **Structural studies of SARS-CoV-2 spike protein.** Intervention strategies are urgently needed to control the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) pandemic. The trimeric viral spike (S) protein catalyzes fusion between viral and target cell membranes to initiate infection. We have determined cryo-EM structures of the full-length spike (S) trimers of the Alpha, Beta, Gamma, Kappa and Delta variants, and studied their functional, biochemical and antigenic properties. Mutations in the Alpha protein increase the accessibility of its receptor binding domain and also the binding affinity for receptor ACE2. The enhanced receptor engagement can account for the increased transmissibility. The Beta variant has evolved to reshape antigenic surfaces of the major neutralizing sites on the S protein, rendering complete resistance to some potent neutralizing antibodies. Delta S can fuse membranes more efficiently at low levels of cellular receptor ACE2 and its pseudotyped viruses infect target cells substantially faster than all other variants tested, possibly accounting for its heightened transmissibility. Mutations of each variant rearrange the antigenic surface of the N-terminal domain of the S protein in a unique way, but only cause local changes in the receptor-binding domain, consistent with greater resistance particular to neutralizing antibodies. More recently, we have determined cryo-EM structures of the intact S trimers of the Omicron subvariants (BA.1 and BA.2), and studied their functional, biochemical and antigenic properties. BA.1 S requires a substantially higher level of host receptor ACE2 for efficient membrane fusion than other variants, possibly explaining its unexpected cellular tropism. BA.2 S can fuse membranes more efficiently than Omicron BA.1, mainly due to lack of a BA.1-specific mutation that may retard the receptor engagement, but still less efficiently than other variants. Mutations not only remodel the antigenic structure of the N-terminal domain of the S protein, but also alter the surface of the receptor-binding domain in a way not seen in other variants, consistent with its remarkable resistance to neutralizing antibodies. Both BA.1 and BA.2 viruses replicated substantially faster in animal lungs than the early G614 (B.1) strain in the absence of pre-existing immunity. Thus, Omicron S proteins have acquired an extraordinary ability to evade host immunity by excessive mutations, which also compromise their fusogenic capability, and that both immune evasion and replicative advantage may

contribute to the heightened transmissibility for the Omicron subvariants. These findings advance our understanding of SARS-CoV-2 entry and may guide development of vaccines and therapeutics.

a. Zhang J, Xiao T, Cai Y, Lavine CL, Peng H, Zhu H, Anand K, Tong P, Gautam A, Mayer ML, Walsh RM, Jr., Rits-Volloch S, Wesemann DR, Yang W, Seaman MS, Lu J, Chen B. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant. Science. 2021 Dec 10;374(6573):1353-1360.
b. Cai Y, Zhang J, Xiao T, Lavine CL, Rawson S, Peng H, Zhu H, Anand K, Tong P, Gautam A, Lu S, Sterling SM, Walsh RM Jr, Rits-Volloch S, Lu J, Wesemann DR, Yang W, Seaman MS, Chen B. Structural basis for enhanced infectivity and immune evasion of SARS-CoV-2 variants. Science. 2021 Aug 6;373(6555):642-648. PubMed PMID: 33880477; PMCID: PMC8057242.
c. Zhang J, Cai Y, Xiao T, Lu J, Peng H, Sterling SM, Walsh RM, Rits-Volloch S, Zhu H, Woosley AN, Wei Y, Sliz P, Chen B. Structural impact on SARS-CoV-2 spike protein by D614G substitution. Science. 2021 Apr 30;372(6541):525-530. PubMed PMID: 33727252; PMCID: PMC8139424.
d. Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh Jr RM, Rawson S, Rits-Volloch S, Chen B. Distinct conformational states of SARS-CoV-2 spike protein. Science. 2020 Sep 25;369(6511):1586-1592. PubMed PMID: 32694201; PMCID: PMC7464562.

Complete List of Published Work in My Bibliography:
http://www.ncbi.nlm.nih.gov/myncbi/bing.chen.1/bibliography/40934227/public/?sort=date&direction=ascending

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

Ongoing Research Support

01/16/2020–12/31/2024
R01 AI147884, NIH/NIAID
Chen, Bing (PI)
Structure of HIV-1 envelope spike in the context of membrane
In this project, our goal is to visualize novel structural features of the intact Env proteins in the context of membrane and HIV-1 matrix protein, to gain a full understanding of their structure-function and to facilitate Env-based immunogen design for vaccine development.

05/15/2018 – 04/30/2023
R01 AI141002, NIH/NIAID
Chen, Bing (PI)
Structural Basis of Coreceptor Recognition by HIV-1 Envelope Spike
We plan to understand molecular details of how HIV-1 coreceptors recognize Env by obtaining atomic structures of the Env-coreceptor complexes and analyzing functional roles of the coreceptor in viral infection.

05/20/2016-04/30/2026
R01 AI127193 (MPI), NIH/NIAID
Chen, Bing (co-PI)
Structure-function studies of the membrane-interacting domains of HIV-1 Env spike
Chen and Dr. James Chou (Harvard Medical School, Boston, MA) are the co-PIs for this proposal. In this project, we will combine structural biology approaches and functional assays to elucidate the roles of the membrane-interacting domains of HIV-1 Env.

08/01/2020-07/31/2022
COVID-19 Fast grant by Emergent Ventures, a project of the Mercatus Center at George Mason University
Chen, Bing (PI)
Fusion inhibitors targeting the spike (S) protein of SARS-CoV-2
In this project, we plan to design or search for effective inhibitors targeting SARS-CoV-2 spike protein to provide additional potent weapons in the antiviral arsenal.