OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 01/31/2026)

BIOGRAPHICAL SKETCH

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NAME: Huan Bao

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

POSITION TITLE: Associate Professor

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
Wuhan University, China	B.S.	06/2005	Biological Science
Chinese Academy of Sciences, China	M.S.	06/2008	Biochemistry
University of British Columbia, Canada	Ph.D.	05/2014	Biochemistry
HHMI & University of Wisconsin, Madison, WI	Postdoc	05/2019	Neuroscience

A. Personal Statement

I have obtained extensive training in membrane biology throughout my graduate and postdoctoral studies. During that time, I have developed expertise and skillsets that are directly related to my research program, which is centered on designing lipid nanoparticles to probe and reprogram membrane biology. First, I employed an elegant design of lipid nanoparticles known as nanodiscs to interrogate membrane protein complexes using an array of biochemical techniques (Bao & Duong, *Plos One* 2012; Bao & Duong, *JBC* 2013; Bao & Duong, *JBC* 2014; Bao & Duong, *JBC* 2015). Second, I provided novel insights into the molecular mechanism of vesicle exocytosis (Bao et al., *NSMB* 2016; Bao & Das et al., *Nature* 2018; Das & Bao et al., *Nat Commun* 2020), using biochemical reconstitution, cell biology, protein engineering, and single-molecule biophysics. In my lab, we seek to develop biochemical and genetic tools that would transform basic and translational research of membrane biology. Specifically, the overarching goal of our research is to expand the structure and function of lipid nanoparticles (LNPs) to advance mechanistic understandings of membrane proteins and control human diseases through steering immunity. Currently, my lab is supported by NIH to develop next-generation nanodiscs for research and therapeutic applications of membrane biology involved in synaptic transmission, neurodegenerative and infectious diseases (DP2GM140920 and R21AG078699). During the past few years, we have created a new set of enabling nanodisc tools that are highly useful for probing the molecular mechanism of membrane proteins (Bao et al., 2021 *Commun Biol*; Zhang et al., 2021 *Nat Commun*; Ren et al., 2022 *Commun Biol*; Courtney et al., 2022 *NSMB;* Xiong et al., 2022 *Cell*).

Since the beginning of my independent career at Scripps-Florida in the summer of 2019, I have re-invented the trajectory of my research program both technically and biologically. Fueled by our passion for LNPs and membrane trafficking, we discovered a new regulator (Annexin A6) of mRNA-LNP biology and its connection with exosome biogenesis and structural organizations of cell membranes (Shin et al., 2023 Nano Lett, in revision). In order to assay the utility of our new nanodisc toolkit for structural biology, I have extended our research into pore-forming proteins and went through a whole learning process to establish the workflow for single particle cryoEM microscopy and analysis in my own lab (Xiong & Zhang et al., 2023 in preparation; Ren et al., 2024 submitted). This was achieved by taking trainings myself in multiple workshops from PNCC and NCCAT because I do not have previous training in this powerful technique nor access to cryoEM on our campus. It is particularly challenging due to the hit of COVID-19 in early 2020 and the shockingly abrupt decision from The Scripps Research Institution (TSRI) to sell our campus to the University of Florida in late 2021. I was forced to go along with the transfer because I was told that I would lose all of my grants and also my job if I did not agree to do so. The short notice and timeframe (6 months) of that transfer in April 2022 caused chaos in grant applications, recruitment for students and postdocs, and the stability of the campus (about one-third of research

groups have left since the transfer). Despite these difficulties, I have produced over twelve papers in the past four years, and mentored five postdocs, one high-school student, four undergraduates, and one graduate student who just won a F31 scholarship (F31GM147951-01A1).

The performance of our re-engineered nanodiscs has been widely appreciated in the community of membrane biology. Our constructs have been requested over 300 times from Addgene by different groups all over the world in the past two years. Several studies have already demonstrated the unique advantage of these tools for elucidating the molecular mechanisms of membrane protein-lipid interactions (Dalal et al., 2024 Nat Commun). The recognition of our work is also reflected that I have been invited to review manuscripts in membrane biology and protein biophysics for multiple journals: Nature Communication (3), EMBO J (1), ACS Nano (1), eLife (2), Communications Biology (5), Communications Chemistry (1), Journal of Molecular Biology (3), Frontiers in Neuroscience (3), Frontiers Molecular Biosciences (5), Scientific Rep (2), Channels (2). In addition, I have served as an editorial board member of Communications Biology since 2022 and have handled over 30 manuscripts. Finally, our research is also selected for presentation in the Future of Biophysics Burroughs Wellcome Fund Symposium during the 2023 Biophysical Society annual meeting.

Over the years of my career, I realized the importance of promoting diversity, equity, and inclusion (DEI) for the development of our next-generation scientists. Therefore, I have been actively recruiting undergraduates, graduate students and fellows from diverse backgrounds and underrepresented minorities. Most of my current trainees are women and underrepresented minorities with little experience or knowledge in membrane biology before joining the lab. In addition, I have been seeking teaching opportunities to promote the recognition and practice of DEI in my classroom, even though teaching at Scripps-Florida (later UF-Scripps) is voluntary, and I need to cover 95% of my salary from grants. Further, I have served as a mentor in the local Tri-Institutional Network for Women in Science (3NIW) group to provide resources and support for their career success. Beyond direct mentoring, I have also volunteered in several outreach programs to change the historical lack of inclusion and equity in science. For example, I provided research opportunities to high school students through the Kenan Fellows Summer Internship in Florida and the Starr Hill program in Virginia. I also gave lectures to middle school students from underrepresented districts through the Scientist in Every Florida School (SEFS) program.

I decided to move my lab to the University of Virginia in Oct 2023 because of the membrane biology community here and a much more stable research environment. The state-of-the-art facility, especially the cryoEM and cell imaging core, provides everything I need to investigate the molecular mechanism of membrane proteins. The proposed study is the beginning of a whole new chapter to apply and refine our new nanodisc toolkit for a wide spectrum of membrane biology research.

Courtney KC, Wu L, Mandal T, Swift M, Zhang Z, Alaghemandi M, Wu Z, Bradberry MM, Deo C, Lavis LD, Volkmann N, Hanein D, Cui Q, **Bao H** and Chapman ER. (2022) The complexin C-terminal amphipathic helix stabilizes the fusion pore open state by sculpting membranes. ***Nat Struct Mol Biol***. 29(2): 97-107. PMID: 35132256; PMCID: PMC 8857072. Funding: DP2GM140920.

Zhang S, Ren Q, Novick SJ, Strutzenberg TS, Griffin PR and **Bao H** (2021) One-step construction of circularized nanodiscs using SpyCatcher-SpyTag. ***Nat Commun***. 12(1): 5451. PMID: 34521837; PMCID: PMC8440770. Funding: DP2GM140920.

Bao H, Das D, Courtney N, Jiang Y, Briguglio J, Lou X, Roston D, Cui Q, Chanda B and Chapman ER. (2018) Dynamics and number of trans-SNARE complexes determine nascent fusion pore properties. ***Nature*** 554 (7691): 260-263. PMID: 29420480; PMCID: PMC5808578.

Bao H, Goldschen-Ohm M, Jeggle P, Chanda B, Edwardson JM and Chapman ER. (2016) Exocytotic fusion pores are composed of both lipids and proteins. ***Nat Struct Mol Biol***. 23(1): 67-73. (Cover paper) PMID: 26656855; PMCID: PMC4756907.

B. Positions, Scientific Appointments, and Honors

Positions

2023 – Present	Associate Professor, Department of Molecular Physiology and Biological Physics, University of Virginia
2022 – 2023	Assistant Professor, Department of Molecular Medicine, UF Scripps Biomedical Research
2019 – 2022	Assistant Professor, Department of Molecular Medicine, Scripps-Florida

2014 – 2019 Postdoctoral Fellow, Department of Neuroscience, University of Wisconsin-Madison
2008 – 2014 Graduate Student, Department of Biochemistry, University of British Columbia
2005 – 2008 Graduate Student, Department of Biochemistry, Chinese Academy of Sciences
2001 – 2005 Undergraduate Student, School of Biological Sciences, Wuhan University

Scientific Appointments

2019 - Present Editorial Board, *Communications Biology, Frontiers in Neuroscience, Endocrinology, Journal of Clinical and Vaccine Immunology.*

Honors

2020 – 2025 NIH Director's New Innovator Award
2019 – 2022 Human Frontier Science Program Career Development Award (declined as this award requires to move out of the US)
2015 – 2018 Human Frontier Science Program Postdoctoral Fellowship
2013 Chinese Government Award for Outstanding Students Abroad
2010 – 2012 Four Year Fellowship, University of British Columbia

C. Contribution to Science

1. Developing next-generation nanodiscs.
I seek to expand the structure and function of nanodiscs for basic and translational research of membrane biology. First, my lab developed nanodisc-ID that enabled label-free and quantitative characterizations of membrane proteins. By leveraging the power of nanodisc and proximity labeling, nanodisc-ID serves both as scaffolds for encasing biochemical reactions and as sensitive reagents for detecting membrane protein-lipid and protein-protein interactions. Second, we created a simple, one-step approach to ease the construction of circularized nanodiscs (cNDs) using the SpyCatcher-SpyTag technology. This approach increases the yield of cNDs by over 10-fold and is able to rapidly generate cNDs with diameters ranging from 11 to over 100 nm, thereby allowing for the reconstitution of protein-lipid interactions and membrane fusion intermediates that are not possible using small nanodiscs. Building upon the success of the SpyCatcher-SpyTag cNDs, we further used split GFP to circularize nanodiscs into a robust fluorescent probe for reporting membrane binding and remodeling reactions. Based on these achievements, we recently furnished cNDs with fusogenic supercharged GFP and developed novel scaffolds for detergent-free nanodisc reconstitution. Together, I believe that our new nanodisc toolbox will significantly promote the dissection and manipulation of membrane proteins, thereby conferring access to the biochemical space unattainable in previous studies.

Ren Q*, Wang J*, Zhang S*, Idikuda V, Shin JLudlam W, Martemyanov K, Chanda B and **Bao H** (2024) DeFrND: detergent-free reconstitution into native nanodiscs with designer membrane scaffold peptides. *Submitted*. Funding: DP2GM140920.

Ren Q*, Zhang S*, and **Bao H** (2022) isenND: a robust fluorescent sensor for membrane binding and remodeling reactions. ***Commun Biol***. 5(1): 507. PMID: 35618817; PMCID: PMC9135701. Funding: DP2GM140920.

Zhang S*, Ren Q*, Novick SJ, Strutzenberg TS, Griffin PR and **Bao H** (2021) One-step construction of circularized nanodiscs using SpyCatcher-SpyTag. ***Nat Commun***. 12(1): 5451. PMID: 34521837. PMCID: PMC8440770. Funding: DP2GM140920.

Bao H (2021) Developing Nanodisc-ID for label-free characterizations of membrane proteins. ***Commun Biol***. 4(1): 514. PMID: 29420480; PMCID: PMC8087782. Funding: DP2GM140920.

2. **Probing the mechanistic basis of pore-forming proteins, neurotoxins and toxic protein aggregates.**
The interaction of pore-forming proteins and toxins with the plasma membrane is crucial for their ability to disturb cell homeostasis. However, many of these interactions are dependent on the dynamic association of these

proteins with lipids, and often exhibit low affinities that are difficult to capture for biophysical characterizations. Our next-generation nanodiscs are useful tools to dissect these weak protein-lipid interactions and further stabilize them for structural studies. In the past few years, we have successfully utilized our nanodisc toolbox for structural and functional studies of a new type of bacterial pore-forming toxins. Our cNDs are essential to trap this toxin in distinct conformational states for single-particle cryoEM microscopic analysis. In addition, our nanodisc-ID approach robustly identified a new motif in the effector domain of several clostridial neurotoxins that specifically need anionic lipids for their action in the active zone of synapses.

Xiong X*, Zhang S*, Wang J, Ren Q, Shin J, **Bao H**† and Dong M† (2023) Structural basis of membrane pore formation by an Enterococci toxin. *In preparation*. Funding: DP2GM140920.

Dallo S*, Xiong X*, Zhang S, Dong M† and **Bao H**† (2023) Lipid electrostatic interactions direct the cleavage of syntaxin by a clostridial-like neurotoxin. *In preparation*. Funding: DP2GM140920.

Xiong X, Tian S, Yang P, Lebreton F, **Bao H**, Sheng K, Yin L, Chen P, Zhang J, Qi W, Ruan J, Wu H, Chen H, Breault DT, Wu H, Earl AM, Gilmore MS, Abraham J, Dong M (2022) Emerging enterococcus pore-forming toxins with MHC/HLA-I as receptors. ***Cell*** 185(7):1157-1171. PMID: 35259335 PMCID: PMC8978092. Funding: DP2GM140920.

Hark TJ, Rao NR, Castillon C, Basta T, Smukowski S, **Bao H**, Upadhyay A, Bomba-Warczak E, Nomura T, O'Toole ET, Morgan GP, Ali L, Saito T, Guillermier C, Saido TC, Steinhauser ML, Stowell MHB, Chapman ER, Contractor A, Savas JN. (2021) Pulse-Chase Proteomics of the App Knockin Mouse Models of Alzheimer's Disease Reveals that Synaptic Dysfunction Originates in Presynaptic Terminals. ***Cell Syst*** 12(2):141-158.e9. Funding: DP2GM140920.

3. **Biogenesis of exosome and mRNA lipid nanoparticles.**
We have extended our interest in membrane trafficking to the unconventional secretion by exosomes and the biogenesis of mRNA-LNPs. Perhaps not surprisingly, these two pathways overlap significantly, and both rely on the fusion of multivesicular bodies with the plasma membrane. Using chemical screens and genetic approaches, we found that Annexin A6 plays an essential role in the fusion of MVB with PM, and can be a potential druggable target to manipulate exosome release and mRNA-LNP efficacies. In addition, we find that mRNA-LNPs do not follow the same route as siRNA-LNPs as proposed previously, calling for future studies to interrogate the underlying molecular mechanism. Finally, we engineered a novel scaffold to enhance the efficacy of mRNA-LNPs based on our understanding of their biogenesis.

Shin J, Zhang S, Ursulaez WC, Ren Q, Chanda B and **Bao H** (2023) Supercharging lipid nanoparticles for membrane engineering and mRNA delivery. *Submitted*. Funding: DP2GM140920.

Shin J, D.C., Zhang S, Seath CP, and **Bao H** (2023) Targeting recycling endosomes to potentiate mRNA lipid nanoparticles. *Nano Lett, in revision.* Funding: DP2GM140920.

4. **Mechanistic understandings of membrane fusion and synaptic transmission.**
I have a long interest in the structure and function of fusion pores and the molecular mechanisms that determine the pore properties. I employed nanodiscs to trap the fusion pores in their initial open state, thus allowing detailed biochemical and biophysical interrogations. My studies have revealed that fusion pores are composed of both proteins and lipids. To gain further insights into the regulatory mechanism of fusion pores, I adapted the planar lipid bilayer method to develop a new system, termed ND-PLB, which is capable of probing pore properties with μsec time resolution, at the single event level. The results showed that both pore size and stability are determined by the number of SNAREs recruited to drive membrane fusion. In addition, this novel method uncovered that the assembly of trans-SNARE complexes between two opposing membranes is highly dynamic and reversible even after fusion pores have opened. Thus, the extremely stable and irreversible four-helix bundle, as shown in the crystal structure of the core SNARE complex, is not fully assembled at the early stage of membrane fusion. The finding that the number of the dynamic trans-SNARE complex dictates fusion pore properties fundamentally revises our understanding of SNARE-mediated vesicle exocytosis. Moreover, ND-PLB has also enabled an array of studies from us and others, elucidating the impact of several regulators such as synaptotagmin, α-synuclein and complexin on fusion pores.

Courtney KC, Wu L, Mandal T, Swift M, Zhang Z, Alaghemandi M, Wu Z, Bradberry MM, Deo C, Lavis LD, Volkmann N, Hanein D, Cui Q, **Bao H** and Chapman ER. (2022) The complexin C-terminal amphipathic helix stabilizes the fusion pore open state by sculpting membranes. ***Nat Struct Mol Biol***. 29(2): 97-107. PMID: 35132256; PMCID: PMC 8857072. Funding: DP2GM140920.

Das D*, **Bao H***, Courtney K, Wu L and Chapman ER. (2020) Resolving kinetic intermediates during the regulated assembly and disassembly of fusion pores. ***Nat Commun***. 11(1): 231. PMID: 31932584; PMCID: PMC 6957489.

Bao H*, Das D*, Courtney N, Jiang Y, Briguglio J, Lou X, Roston D, Cui Q, Chanda B and Chapman ER. (2018) Dynamics and number of trans-SNARE complexes determine nascent fusion pore properties. ***Nature*** 554 (7691): 260-263. PMID: 29420480; PMCID: PMC5808578.

Bao H, Goldschen-Ohm M, Jeggle P, Chanda B, Edwardson JM and Chapman ER. (2016) Exocytotic fusion pores are composed of both lipids and proteins. ***Nat Struct Mol Biol***. 23(1): 67-73. (Cover paper) PMID: 26656855; PMCID: PMC4756907.

5. Molecular Mechanisms of ABC Transporters.

My career started from addressing how ABC transporters couple ATP hydrolysis to the import and export of a large array of substances across lipid bilayers in all kingdoms of life. Since substrate transport mediated by ABC transporters consumes cellular energy, this type of reaction must be regulated according to the need of cells. I utilized the Escherichia coli maltose transporter $MalFGK_2$ as a prototype to understand the regulatory mechanisms of ABC importers. Biochemical and biophysical approaches were employed to investigate how the transport reaction is modulated by maltose, the maltose-binding protein MalE and the glucose-specific enzyme $EIIA^{Glc}$. I demonstrate that 1) ATP facilitates MalE binding to $MalFGK_2$ to catalyze maltose transport; 2) If the external maltose level exceeds that required, maltose is able to limit the maximal transport rate by promoting the dissociation of MalE from $MalFGK_2$; 3) If the preferred carbon source-glucose is available, $EIIA^{Glc}$ binds to MalK through the interaction of its N-terminal amphipathic helix with acidic phospholipids, thereby inhibiting ATP hydrolysis and maltose transport. These results, combined with previous genetic, biochemical and structural studies, provided a new mechanistic framework for the regulation of the maltose transport system.

Bao H and Duong F. (2014) Nucleotide-free MalK drives the transition of the maltose transporter to the inward-facing conformation. ***J Biol Chem***. 289(14):9844-51. PMID: 24526688; PMCID: PMC3975029.

Bao H and Duong F. (2013) Phosphatidylglycerol directs binding and inhibitory action of $EIIA^{Glc}$ protein on the maltose transporter. ***J Biol Chem***. 288(33): 23666-74. PMID: 23821551; PMCID: PMC3745313.

Bao H and Duong F. (2013) ATP alone triggers the outward-facing conformation of the maltose ABC transporter. ***J Biol Chem***. 288(5): 3439-48. PMID: 23243313; PMCID: PMC3561562.

Bao H and Duong F. (2012) Discovery of an auto-regulation mechanism for the maltose transporter $MalFGK_2$. ***PLoS One***. 7(4): e34836. PMID: 22529943; PMCID: PMC3328499.

*equal contribution; †co-corresponding author

Complete list of published work: https://www.ncbi.nlm.nih.gov/myncbi/huan.bao.1/bibliography/public/