

BIOGRAPHICAL SKETCH

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NAME: Huan Bao

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

POSITION TITLE: Assistant 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 graduate and postdoctoral studies. During that time, I have developed expertise and skillsets that are directly related to my program, which is centered on biophysics and synthetic biology of membrane proteins. First, I used an elegant design of lipid nanoparticles known as nanodiscs to interrogate membrane protein complexes using an array of biochemical techniques (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, we attempt to expand the function and geometry of lipid nanoparticles for mechanistic dissections of membrane biology, with the focus on exocytosis mediated by the SNARE proteins. On this front, the first goal of my research is to establish a mechanistic framework of the SNARE cycle at the level of defined protein-protein/protein-lipid interactions and associated conformational changes. In conjunction with unbiased genomic and proteomic screens, the second goal is to define how protein-protein interactions are steered toward aberrant vesicle fusion involved in pathophysiological states. Together, these studies will not only advance mechanistic understandings of regulated exocytosis, but will also shed light on new methods to control abnormal secretion in diseases. My lab is currently supported by the NIH Director's New Innovator Award to develop next-generation nanodiscs for the structural and functional studies of membrane proteins (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.

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

2019 – Present	Assistant Professor, Department of Molecular Medicine, The Scripps Research Institute
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

Scientific Appointments

2019 - Present	Editorial Board, <i>Frontiers in Neuroscience</i> , <i>Endocrinology</i> , <i>Journal of Clinical and Vaccine Immunology</i> .
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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 enables 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, which allows us to reconstitute 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. I believe that these circularized MSPs will significantly promote the use of nanodiscs for mechanistic dissections of membrane proteins, thereby conferring access to the biochemical space unattainable in previous studies.

Ren Q, Zhang S, and **Bao H** (2022) isenND: a robust fluorescent sensor for membrane binding and remodeling reactions. **Submitted**.

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.

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

2. Mechanistic understanding of membrane fusion.

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 μ sec 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*. In press.

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.

3. 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 MalFGK₂ 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 MalFGK₂ 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 MalFGK₂; 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 MalFGK₂. *PLoS One*. 7(4): e34836. PMID: 22529943; PMCID: PMC3328499.

*equal contribution

Complete list of published work: <https://scholar.google.fi/citations?user=5ZIJY8IAAAJ&hl=en>