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: Bo Hu

eRA COMMONS USER NAME (credential, e.g., agency login): BOHU123

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
Nankai University	B.S.	06/2003	Biological science
Nankai University	Ph.D.	06/2010	Microbiology
University of Texas Medical School at Houston (now McGovern Medical School)	Postdoctoral	04/2016	Cryo electron tomography

A. Personal Statement

With seven years training in microbiology and further seven years experiences in cryo electron tomography (Cryo-ET), I have gained the expertise in cryo electron microscopy, microbiology and biochemistry necessary to successfully carry out the proposed research project. In recent years, we develop a high-throughput Cryo-ET pipeline, our pipeline effectively integrates dose-fractionation in a direct detector device with specific software, allowing massive data collection, drift correction, fiducial model generation, alignment, contrast transfer function (CTF) correction, and reconstruction of several thousands of tomograms at high magnification. This pipeline has been successfully applied to address broad biological questions and produce several peer-reviewed publications. My researches focus on understanding the structure and function of bacterial nanomachines in situ, including phage infection, bacterial flagellar motor and chemoreceptor proteins and bacterial secretion systems. I mastered all required genetic manipulation techniques to make minicells for fitting the thickness limitation of high resolution Cryo-ET. In addition to my research, I have acquired the administrative skills as a research assistant professor and with extensive experiences of collaboration with other scientists, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. The current application builds logically on my prior work.

1. **Hu, B.**, Lara-Tejero M, Kong Q, Galán JE, Liu J. (2017) In situ molecular architecture of the *Salmonella* type III secretion machine. *Cell* 168(6):1065-1074. PMCID: PMC5393631
2. Farley M., **Hu B.**, Margolin W., Liu J. (2016) Minicells, Back in Fashion. *J. Bacteriol.* 198 (8), 1186-1195. PMCID: PMC4859596
3. Morado, D. R., **Hu, B.**, Liu, J. (2016) Using Tomoauto: A Protocol for high-throughput automated cryo-electron tomography. *J. Vis. Exp*. e53608, doi:10.3791/53608. PMCID: PMC4781705
4. Zhao, X., Zhang, K., Boquoi, T., **Hu, B.**, Motaleb, M., Miller, K., James, M., Charon, N. W., Manson, M. D., Norris, S. J. , Li, C. , Liu, J. (2013) : Visualizing the sequential assembly of bacterial flagellum in *Borrelia burgdorferi* by cryo-electron tomography, *Proc. Natl. Acad. Sci. USA* 110: 14390-14395. PMCID: PMC3761569
5. Liu, J., **Hu, B.**, Morado, D. R., Jani, S., Manson, M. D., and Margolin, W. (2012). Molecular architecture of chemoreceptor arrays revealed by cryoelectron tomography of *Escherichia coli* minicells, *Proc. Natl.*

Acad. Sci. USA 109, E1481-1488. PMCID: PMC3384206

B. Positions and Honors

2010-2016 Postdoctoral Fellow, University of Texas Medical School at Houston, Houston, TX
2016-2017 Research Assistant Professor, McGovern Medical School (newly renamed), Houston, TX
09/2017- Assistant Professor, McGovern Medical School, Houston, TX

C. Contributions to Science

1. My early publications directly addressed the biosynthesis and regulation of bacterial surface antigen: O polysaccharide and flagellin, also known respectively as the O and H antigens. My research is primarily concerned with the functions of these glycosyltransferases which have the potential to be applied in the targeted synthesis of specific glycoconjugates. Another project is about the flagellar phase variation in *E. coli* strains. Although the flagellar phase variation in *Salmonella* has been well studied, the mechanism involved in unilateral flagellar phase variation in *E. coli* remains unclear. Using *E. coli* H3 and H17 serotype strains as models, our results demonstrate that the flagellin gene is within a genomic island (GI), and an integrase mediates the excision of the GI from the chromosome, which causes the occurrence of unilateral flagellar phase variation.

a. Brockhausen, I., **Hu, B.**, Liu, B., Lau, K., Szarek, W. A., Wang, L., and Feng, L. (2008) Characterization of two beta-1,3-glucosyltransferases from *Escherichia coli* serotypes O56 and O152, *J Bacteriol.* 190, 4922-4932. PMCID: PMC2446995
b. **Hu, B.**, Perepelov, A. V., Liu, B., Shevelev, S. D., Guo, D., Senchenkova, S. N., Shashkov, A. S., Feng, L., Knirel, Y. A., and Wang, L. (2010) Structural and genetic evidence for the close relationship between *Escherichia coli* O71 and *Salmonella enterica* O28 O-antigens, *FEMS Imm. Med. Microbiol.* 59, 161-169. PMID: 20482625
c. Liu, B., **Hu, B.**, Zhou, Z., Guo, D., Guo, X., Ding, P., Feng, L., and Wang, L. (2012) A novel non-homologous recombination-mediated mechanism for *Escherichia coli* unilateral flagellar phase variation, *Nucleic Acids Res.* 40, 4530-4538. PMCID: PMC3378880

2. In addition to the contributions described above, I have focused on visualizing the virus-host interaction by Cryo-ET. With collaboration Dr. William Margolin, we developed methods to genetically modify Gram-negative bacteria to produce minicells, which has proven to be a very powerful subject for structural research by Cryo-ET. I also developed methods to purify minicells from different species. Together with high throughput Cryo-ET and sub-volume averaging, we generated high-resolution reconstructions of cell-virus complexes and were able to capture T7 virions at successive stages of infection. Our structures revealed the first complete pathway of infection initiation by any phage. In addition to virus T7, I also conducted a research project on T4 Bacteriophage, which is a genetically and biochemically well-studied member of the Myoviridae family. What are less well understood, and what my studies have focused on, are the mechanism by which the phage absorbs to the bacterial membrane and the interaction of the phage DNA injection tube with the inner membrane of *E. coli*.

a. **Hu, B.**, Margolin, W., Molineux, I. J., and Liu, J. (2015) Structural remodeling of bacteriophage T4 and host membranes during infection initiation, *Proc. Natl. Acad. Sci. USA* 112 (35), E4919-E4928. PMCID: PMC4568249
b. **Hu, B.**, Margolin, W., Molineux, I. J., and Liu, J. (2013). The bacteriophage t7 virion undergoes extensive structural remodeling during infection. *Science* 339, 576-579. PMCID: PMC3873743

3. Understanding of the mechanisms underlying bacterial pathogenesis in humans is a major focus of microbiological research and the elucidation of the infectious process yields practical applications of new antibiotics and improved vaccines. One example is the bacterial type III secretion system (T3SS). Many infectious bacteria such as *Shigella* and *Salmonella* use type III secretion machines, to transfer virulence proteins into eukaryotic host cells, cause diarrheal disease. In my more recent studies, I conducted a comprehensive study on the structure and function of the bacterial T3SSs from *Shigella* and *Salmonella*. In these projects, I combined advanced imaging and genetic techniques to visualize the frozen-hydrated diarrheal pathogen *Shigella flexneri* and revealed the intact type III secretion machine and its interaction with a host cell for the first time. The structures characterized herein provide new insights into the mechanisms underlying type III secretion and pathogenesis. In a very recent study, we report a high-resolution *in situ* structure of the *Salmonella Typhimurium*

type III secretion machine obtained by high-throughput cryo-electron tomography and sub-tomogram averaging. Through molecular modeling and comparative analysis of machines assembled with protein-tagged components or from different deletion mutants, we determined the molecular architecture of the secretion machine in situ and localized its structural components.

a. **Hu, B.**, Lara-Tejero M, Kong Q, Galán JE, Liu J. (2017) *In situ* molecular architecture of the *Salmonella* type III secretion machine. *Cell* 168(6):1065-1074. PMCID: PMC5393631
b. **Hu, B.**, Dustin R. Morado, William Margolin, John R. Rohde, Olivia Arizmendi, Wendy L. Picking, William D. Picking, and Jun Liu (2015) Visualization of the type III secretion sorting platform of *Shigella flexneri*. *Proc. Natl. Acad. Sci. USA* 112, 1047-1052. PMCID: PMC4313800

4. Structural definition of bacterial type IV secretion systems. Type IV secretion systems (T4SSs) are complex machines used by bacteria to deliver protein and DNA complexes into target host cells. Recently, I and Christie in UT collaborated with Drs. J. Liu, C. Roy, and D. Chetrit at Yale to solve the structure of the *Legionella pneumophila* Dot/Icm T4SS (Chetrit*, Hu* et al., *equal first-authors, in review). The Dot/Icm T4SS is phylogenetically unrelated to the IVA systems, and representative of a second large type IVB subfamily. It is, however, composed of the three signature ATPases of T4SSs, VirB4-like DotO, DotB, and DotL. Using Cryo-ET, we generated a 3D map of the Dot/Icm system at ~3 nm resolution. The new Dot/Icm structure spans both bacterial membranes, and most strikingly is composed of a highly symmetrical IMC subassembly. Structural analyses of mutant machines lacking one of the three ATPases, coupled with the use of GFP as a traceable tag, established that DotO and DotB assemble as stacked hexamers at the entrance to the T4SS channel. Very recently, I and Christie resolved F plasmid-encoded T4SS substructures alone and in association with the F pilus. These data change the existing paradigm for how T4SSs are architecturally configured at the cytoplasmic entrance, and offer a view of how substrates dock and are translocated across the IM.

Complete List of Published Work in MyBibliography: https://www.ncbi.nlm.nih.gov/sites/myncbi/12YI8Oh-l29AW/bibliography/52823422/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

Departmental Start-Up Grant, McGovern Medical School.
Research Start-Up Funds PI: Bo Hu 09/01/17-09/30/20
The purpose of these funds is to set up the PI's laboratory and fund preliminary studies needed for successful grant submissions for extramural research support.

The Texas Medical Center Digestive Diseases Center Pilot Feasibility Project. Supported in part by NIH grant DK056338. PI: Bo Hu; Co-PI Peter Christie 02/15/18-02/14/19
Structure of *H. pylori* Cag Type IV Secretion System.
This project is to solve the structures of WT and mutant variants of the *Helicobacter pylori* Cag type IV secretion system by in situ cryoelectron tomography.