BIOGRAPHICAL SKETCH

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NAME: Linxiang Yin

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POSITION TITLE: Postdoctoral Research Fellow

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
China Agricultural University, Beijing, China	B.S.	07/2012	Biochemistry and molecular biology
Peking University, Beijing, China		07/2013	Biochemistry
Iowa State University, Ames, IA	Ph.D.	05/2018	Biochemistry
The Johns Hopkins University, Baltimore, MD		03/2017	Biophysics
Boston Children's Hospital, Harvard Medical School, Boston, MA	Postdoctoral		

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

I am currently a postdoc researcher in the Department of Microbiology of Harvard Medical School (HMS) and the Department of Urology at Boston Children's Hospital (BCH). I received my Ph.D. at the Iowa State University. My graduate studies focused on singlemolecule FRET (smFRET) studies of the functional dynamics of several membrane proteins within liposomes or nanodiscs: SNARE proteins and regulators, multi-drug efflux pumps, and mixed lineage kinase domain-like (MLKL) protein. From 2014 to 2017, I published three publications on single-molecule FRET studies as the first or co-first author in Biochemical Journal (2016), Structure (2017), and Nature Communications (2017). I also have one first author manuscript now in preparation. During my Ph.D., I was in a graduate program mainly focuses on biochemistry and protein biophysics, where I gained a solid training in studying protein structures and dynamics, protein-lipid interactions, and protein engineering. My postdoctoral studies focus on the structural and molecular mechanisms of how bacterial toxins target and disrupt host cellular functions and developing toxin-derived scientific tools and novel therapeutics. Particularly, the cell membrane is a major protective barrier and the ability to penetrate the cell membrane is key to develop effective therapeutics targeting intracellular proteins and signaling pathways. Bacterial toxins such as Clostridium difficile toxin B (TcdB) and BoNTs are naturally evolved to penetrate the cell membrane efficiently through its membrane translocation process, and then deliver the light chain domain as its cargo into the cytosol. Recently, a cover article published in Science Translational Medicine from our lab reported that tamed BoNTs efficiently delivery antibodies into neurons and cure diseases within hours. How TcdB and BoNTs achieve this complicated membrane translocation process is still a major mystery.

The intermediate structures of TcdB membrane translocation are also a long-standing open question and remain largely elusive. I plan to use high-resolution single particle cryo-electron microscopy (EM) and single-molecule fluorescence resonance energy transfer (smFRET), in combination with liposome and nanodisc lipid bilayer systems to decipher the structures and functional dynamics of TcdB intermediates during translocation. In this project, I will work closely with Dr.Jonathan Abraham and Dr. Maofu Liao, who pioneered high resolution cryo-EM. The Min Dong lab focus on bacterial toxins studies and have already well established many toxin-

related functional research techniques. The mentorship of Dr.Jonathan Abraham and Dr. Maofu Liao on cryo-EM, my previous smFRET and lipid system experience, as well as the great platform of Min Dong's group provide me a great opportunity to do cryo-EM research and strengthen my toxin functional research experience to significantly enhance my abilities to accomplish my proposed research successfully. Also, I plan to have my independent lab after my postdoctoral training. I will further my research on the translocation of various bacterial toxins using smFRET and cryo-EM to develop toxin-based efficacious drug delivery platforms.

Publications (*, 1st author)
A first author manuscript is in preparation.

Botulinum Toxins A and E Inflict Dynamic Destabilization on t-SNARE to Impair SNARE Assembly and Membrane Fusion.
Khounlo R*, Kim J*, **Yin L***(surnames alphabetical order) Shin YK. **Structure**. 2017 Oct 4. pii: S09692126(17)30295-2. doi: 10.1016/j.str.2017.09.004.

Structures and transport dynamics of a Campylobacter jejuni multidrug efflux pump.
Su CC*, **Yin L***, Kumar N*, Dai L, Radhakrishnan A, Bolla JR, Lei HT, Chou TH, Delmar JA, Rajashankar KR, Zhang Q, Shin YK, Yu EW. **Nat Commun**. 2017 Aug 1;8(1):171. doi: 10.1038/s41467-017-00217-z.

Complexin splits the membrane-proximal region of a single SNAREpin.
Yin L*, Kim J, Shin YK. **Biochem J**. 2016 Jul 15;473(14):2219-24. doi: 10.1042/BCJ20160339. Epub 2016 May 24.

Small heat shock protein IbpB acts as a robust chaperone in living cells by hierarchically activating its multi-type substrate-binding residues.
Fu X*, Shi X*, **Yin L**, Liu J, Joo K, Lee J, Chang Z. **J Biol Chem**. 2013 Apr 26;288(17):11897-906. doi: 10.1074/jbc.M113.450437. Epub 2013 Mar 13.

PDIp is a major intracellular oestrogen-storage protein that modulates tissue levels of oestrogen in the pancreas.
Fu X*, Wang P, Fukui M, Long C, **Yin L**, Choi HJ, Zhu BT. **Biochem J**. 2012 Oct 1;447(1):115-23. doi: 10.1042/BJ20120868.

Emerging enterococcus pore-forming toxins with MHC/HLA-I as receptors
Xiong, X., Tian, S., Yang, P., Lebreton, F., Bao, H., Sheng, K., **Yin L**,... & Dong, M. **Cell** 185 (7), 1157-1171. e22

B. Positions and Honors

Positions and Employment

2010-2011 Undergraduate Research Assistant, China State Key Laboratory of Plant Physiology and Biochemistry, Beijing, China
2011-2013 Research Assistant, China State Key Laboratory of Protein and Plant Gene Research (Peking University), Beijing, China
2013-2017 Research Graduate Assistant, Iowa State University, Ames, IA
2017 Visiting PHD Student, The Johns Hopkins University, Baltimore, MD
2018- Postdoc, Boston Children's Hospital, Harvard Medical School, Boston, MA

Honors

China Agricultural University, Outstanding Student Award
China Agricultural University, Outstanding Volunteer
2016 World Life Science Conference, Travel Award, Beijing, China
2019 Harvard Medical School Department of Microbiology Annual Retreat, Best Poster Award, Cape Cod, Massachusetts

C. Contribution to Science

1. The first project during my Ph.D. is about a major regulator of SNARE-dependent membrane fusion: complexin. I got the data and finished the main work around 12 months after I joined my Ph.D. lab. For the current structural basis of the inhibitory role of complexin during membrane fusion, there are two completely different models: the binding model proposed by Dr. Josep Rizo and Dr. Thomas Südhof's groups and the Zigzag array model proposed by Dr. James Rothman's groups. Both models were proposed mainly based on X-ray structure studies. The binding model shows that Cpx binds to the surface groove between the VAMP2 and syntaxin SNARE motifs of one SNARE core complex. The Zigzag array model proposes that one Cpx crosslinks two adjacent partially zippered SNARE core complex. However, both studies were carried out in the absence of two opposite membranes and just SNARE motifs rather than full-length SNARE proteins were used. We proposed to use nanodisc to mimic the native lipid environment and to solubilize the full-length SNARE membrane proteins. Our results showed that complexin has the capacity to split t- and v-SNAREs at the C-terminal half while maintaining the core structure at the N-terminal half. A similar paper came out right after ours (1). They used soluble VAMP as well as supported bilayer membrane-tethered soluble syntaxin but somehow got the similar splitting effect. Very recently, a ~41 Å resolution cryo-ET study of SNARE-complexin-synaptotagmin complex membrane fusion interfaces morphologies within proteoliposomes membrane environment was published. Their results show that complexin increased the membrane separation between synaptic and target membranes. This is in line with our results. Our work is also appreciated by recent papers. A recent review paper mentioned our work: "beautiful experiments using the surface force apparatus and single-molecule FRET studies using nanodisc-anchored SNAREs showed that complexin indeed hinders C-terminal zippering of trans-SNARE complexes, arresting them in a half-zippered state". In this project, I designed the experiment with my advisor and completed all the experiments.

    a. Yin, L., Kim, J., & Shin, Y. K. (2016). Complexin splits the membrane-proximal region of a single SNAREpin. Biochemical Journal, 473(14), 2219-2224.
    b. Choi, U. B., Zhao, M., Zhang, Y., Lai, Y., & Brunger, A. T. (2016). Complexin induces a conformational change at the membrane-proximal C-terminal end of the SNARE complex. Elife, 5, e16886.
    c. Gipson, P., Fukuda, Y., Danev, R., Lai, Y., Chen, D. H., Baumeister, W., & Brunger, A. T. (2017). Morphologies of synaptic protein membrane fusion interfaces. Proceedings of the National Academy of Sciences, 114(34), 9110-9115.
    d. Rizo, J. (2018). Mechanism of neurotransmitter release coming into focus. Protein Science.

2. My second main project during my Ph.D. is a structure and dynamic study of a multi-drug efflux pump with a team of collaborators. My work on this project emphasized a single-molecule study of the multidrug efflux transporter CmeB real-time functional dynamics, the first single-molecule investigation of bacterial transporters. The data suggest that the CmeB protomers may have three different states: the "resting" state, the "binding" state, and the "extrusion" state. CmeB protomers prefer the "resting" conformation without the energy source. The process of transitioning from the "resting" state to the "binding" state may require energy. Each protomer within the CmeB trimer can independently transitions through three different states, which lead to the extrusion of substrate.

    a. Su, C. C., Yin, L., Kumar, N., Dai, L., Radhakrishnan, A., Bolla, J. R., ... & Zhang, Q. (2017). Structures and transport dynamics of a Campylobacter jejuni multidrug efflux pump. Nature Communications, 8(1), 171.
    b. Lin, J., Michel, L. O., & Zhang, Q. (2002). CmeABC functions as a multidrug efflux system in Campylobacter jejuni. Antimicrobial agents and chemotherapy, 46(7), 2124-2131.
    c. Shen, Z., Su, C. C., Edward, W. Y., & Zhang, Q. (2013). Multidrug Efflux Transporters in Campylobacter. Microbial Efflux Pumps: Current Research, 223.

3. Botulinum toxins (BoNTs) A and E target and cleave the same target protein SNAP-25 but have different effects on blocking SNARE-mediated membrane fusion. Our structural studies revealed that BoNT A and E eliminate SNARE-mediated membrane fusion via different mechanisms. After BoNT E cleavage, SNAP-25 is not able to support SNARE pairing and vesicle docking. In contrast, after BoNT A cleavage, SNAP-25 still facilitates unimpaired SNARE pairing and vesicle docking with diminished SNARE zippering. Our

results provide insights into the detailed structure/dynamics mechanism by which BoNT A and E inhibit membrane fusion.

   a. Khounlo, R., Kim, J., Yin, L., & Shin, Y. K. (2017). Botulinum Toxins A and E Inflict Dynamic Destabilization on t-SNARE to Impair SNARE Assembly and Membrane Fusion. Structure, 25(11), 1679-1686.
   b. Schiavo, G., Santucci, A., Dasgupta, B. R., Mehta, P. P., Jontes, J., Benfenati, F., ... & Montecucco, C. (1993). Botulinum neurotoxins serotypes A and E cleave SNAP‐25 at distinct COOH‐terminal peptide bonds. FEBS letters, 335(1), 99-103.
   c. Rossetto, O., Pirazzini, M., & Montecucco, C. (2014). Botulinum neurotoxins: genetic, structural and mechanistic insights. Nature Reviews Microbiology, 12(8), 535.

4. It has been proposed that a lipid-binding loop (LBL) of Botulinum toxins interacts with lipid membranes and contribute to neuron binding. However, the direct interaction of the LBL and lipid has not been detected due to the intrinsically transit and weak interaction. Here we established a system that can directly measure the interaction by combining and optimizing the biolayer interferometry assay with the nanodisc approach. With this system, we show that Hc/DC, Hc/C, and Hc/G, but not Hc/B and Hc/D directly interact with ganglioside-free lipid bilayer. Our study also show that the interaction is mediated by the aromatic residues at the tip of the LBL. Based on this knowledge, we designed a Hc/B mutant (I1248W/V1249W) that shows increased binding ability to the lipid bilayers mimicking human neuron membrane as well as neuron cell. This mutant also shows extended duration of paralysis as well as lower systemic diffusion in mice Digit Abduction Score assays. This work established the mechanism of LBL-lipid interactions and designed a BoNT/B mutant with enhanced therapeutic effects.
   a. Yin L, Masuyer G, Zhang S, Zhang J, Miyashita SI, Burgin D, Lovelock L, Coker SF, Fu TM, Stenmark P, Dong M. PLoS Biol. 2020 Mar 17;18(3):e3000618. doi: 10.1371/journal.pbio.3000618. eCollection 2020 Mar. Characterization of a membrane binding loop leads to engineering botulinum neurotoxin B with improved therapeutic efficacy.
   b.
5. In the previous study, we designed a Hc/B mutant (I1248W/V1249W) that shows enhanced therapeutic effects. We further improved the design and get a mutant with about four times the lipid binding capabilities that of the Hc/B mutant (I1248W/V1249W). We are applying a patent and the patent application.

Complete List of Published Work in MyBibliography:
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