## 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: Lingling Chen

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

POSITION TITLE: Professor of Molecular and Cellular Biochemistry

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 |
|---|---|---|---|
| Xiamen University, China | B.S. | 07/1988 | Physical Chemistry |
| Stanford University, CA | Ph.D. | 05/1996 | Biophysical Chemistry |
| Yale University, CT | Postdoc. | 12/2000 | Protein Crystallography |

### A. Personal Statement

My current research program has focused on understanding the molecular mechanism of human mitochondrial chaperonin system mHsp60-mHsp10. mHsp60-mHsp10 is essential for mitochondrial function, and mutations in mHsp60 are associated with neurological diseases. mHsp60 has been proposed as potent biomarkers and drug targets; however, the lack of detailed mechanistic understandings of mHsp60 at the molecular and structural level hinders its therapeutic developments.

Lack of direct mechanistic information on mHsp60-mHsp10 is due to the difficulties in mHsp60 preparation and dynamic of mHsp60-mHsp10 system. We have developed a protocol to consistently obtain active mHsp60, and we propose to study structures of the mHsp60-mHsp10 system using cryoEM. CryoEM is most suitable for structural studies of the dynamic systems such as mHsp60-mHsp10, because it can capture, sort out and determine the different conformations.

As a structural biologist, I have a lasting and strong track record on mechanistic understandings of the chaperonin system. Starting from my postdoctoral training at Yale University, my work on the *E. coli* chaperonin GroEL-substrate interaction using combinatory biology and particularly protein X-ray crystallography provides structural basis for how GroEL recognizes substrate proteins. After I started my independent lab at IU, I have continued structure-function studies of the chaperonin system with a focus on mechanistic investigations on the human mitochondrial mHsp60. As mentioned, purified mHsp60 is unstable; to overcome this difficulty, in our early research we focused on creating stable, single-ring, and functional chaperonin systems as model system for detailed molecular investigations on mHsp60-mHsp10. Notably, we created a novel reagent, *groES*$^{7}$, which concatenates seven copies of *E. coli* cochaperonin GroES genes to express the cochpaperonin in a continuous polypeptide. Using *groES*$^{7}$, we identified several GroES$^{7}$ variants to activate the single-ring GroEL$^{SR}$ that is otherwise inactive with GroES. Our work together demonstrates that the chapaperonin system can function in a single-ring mechanism, a conclusion directly relevant to the single-ring mHsp60-mHsp10. We also find several molecular properties paralleled between the active GroEL$^{SR}$-GroES$^{7}$ and mHsp60-mHsp10 systems, highlighting the relevance of the model systems. Recently, we developed a purification protocol to consistently obtain <u>*active, single-ring*</u> mHsp60, allowing us for <u>*direct*</u> investigations on the mechanism of mHsp60-mHsp10 at the molecular and structural level. With our extensive experience in the chaperonin system combined with our structural/biochemical expertise, I believe that we are in a unique position to elucidate the molecular mechanism of mHsp60-mHsp10. Our mechanistic understandings will provide insights on mHsp60-associated human diseases, and on designing mHsp60-targeted therapeutics.

As mentioned, cryo-EM is most suitable for structural studies on dynamic systems such as mHsp60-mHsp10. To better lead the project and to become proficient in cryoEM, I am applying for the training program

at the NIH-funded National Center for CryoEM Access and Training (NCCAT) in New York City. I expect to learn a thorough experimental skillset associated with cryoEM structural determination, including cryo-specimen preparation, basics of microscope operation, data collection, image processing, structural refinement, and map deposition. So far, I have much experience with image processing using relion, and have obtained the EM maps at 6.4A. As a structural biologist, I have learned solution x-ray scattering in my Ph.D. study at Stanford, protein x-ray crystallography in my postdoctoral training at Yale, and NMR in my tenure at IU. I am confident that I will become a competent cryo-EM researcher to lead and work on the cryoEM studies. Like my expertise in x-ray crystallography, my expertise in cryoEM will greatly benefit our local EM and structural biology communities.

## B. Positions and Honors

### Positions and Employment

1996-00 Helen Hay Postdoctoral Fellow, Department of Molecular Biophysics and Biochemistry, Yale University, CT
Advisor: Paul Sigler
2001-07 Assistant Professor, Department of Biology, Indiana University, Bloomington, IN
2008-18 Associate Professor, Dept. of Molecular and Cellular Biochemistry, Indiana Univ., Bloomington, IN
2019- Professor, Dept. of Molecular and Cellular Biochemistry, Indiana Univ., Bloomington, IN

### Other Experience and Professional Memberships

2003- Member, American Crystallographic Association
2003- Member, American Society for Microbiology
2006 Panelist (Ad-hoc Member), NSF Signal Transduction Spring Panel
2005, 08,14-16: NSF Ad-hoc Member
2017- Oversea Review Panelist, National Science Foundation of China
2017-20 Reviewing Editorial Board Member, *Cell Stress & Chaperones*
2018-20 Editorial Board Member, *Scientific Reports*

### Honor

1984-88 University Fellowship, Xiamen University, China 1985 Zhong Xue Scholarship, Department of Chemistry, Xiamen University, China
1988 Presidential Award: Chen Jia-Geng Fellowship, Xiamen University, China
1989 Lu Jia-Xi & Cai Qi-Rui Scholarship, Graduate School, Xiamen University, China
1990 Guang-Hua Scholarship, Graduate School, Xiamen University, China
1996-97 Howard Hughes Medical Institute Associate, Yale University 1997-00 Helen Hay Whitney Postdoctoral Fellowship, Yale University
2003 Faculty Summer Fellowship, Indiana University
2010-19 Minjian Fellow, Xiamen University, China
2016 Indiana University Institute for Advance Study Residential Fellow

## C. Contributions to Science

1. Mechanism of molecular chaperone in protein folding

The double ring chaperonin Hsp60, GroEL in *E. coli*, assists folding of numerous proteins involved in fundamental cellular processes. How GroEL recognizes and interacts with such diverse substrate proteins has been central to the GroEL field. To reveal the structural basis for GroEL-substrate interaction, I used a phage display peptide library to identify a peptide with strong affinity for GroEL, and solved the crystal structure of GroEL/peptide complex. My work provides the first structural evidence indicating that conformational flexibility in the substrate-binding site combined with the nature of non-sequence specific hydrophobic interaction contributes to the substrate promiscuity of GroEL. In addition, I also showed that substrate adopts β-sheet conformation while bound to GroEL, supporting that GroEL recognizes and binds the secondary structure of the substrate. I was the primary investigator in this study. Following Dr. Sigler's untimely passing, I carried on the research on mechanistic study of GroEL as an independent investigator at IU. My lab used nuclear magnetic resonance (NMR) to show that GroEL can bind to α-helix, strengthening the notion that unlike other chaperone GroEL interact with the secondary structures in the substrates. Recently, my lab has focused on studying the mechanism of single ring variants including human mitochondrial mtHsp60 that is not amenable for biochemical and structural studies, and we have developed novel regents to activate a single ring form $GroEL^{SR}$ that is otherwise inactive and nonfunctional. Combining with functional $GroEL^{SR}$ mutants, we identified biochemical determinants that allow chaperonin to function as single ring. I directed these studies.

a. **Lingling Chen** and Paul Sigler (1999), "Crystal Structure of a GroEL/Peptide Complex: Plasticity as a Basis for Substrate Diversity", *Cell*, 99, 757-768. PMID: 10619429
b. Melissa Illingworth, Andrew Ramsey, Zhida Zheng, and **Lingling Chen** (2011), "Stimulating the substrate folding activity of a single-ring GroEL variant by modulating the cochaperonin GroES", *J. Biol. Chem*. 286: 30401-30408. PMC3162399
c. Melissa Illingworth, Jared Salisbury, Wenqian Li, Donghai Lin and **Lingling Chen** (2015), "Effective ATPase activity and moderate chaperonin-cochaperonin interaction are important for the functional single-ring chaperonin system", *Biochem Biophys Res Commun* **466**, 15-20. PMID: 26271593
d. Melissa Illingworth, Holly Ellis and **Lingling Chen**. Creating the Functional Single-Ring GroEL-GroES Chaperonin Systems via Modulating GroEL-GroES Interaction. Sci. Rep., 7:9710, 2017 PMID: 5575113

2. Mechanism of quorum sensing

Quorum sensing (QS) is a well-recognized form of cell-cell communication by which bacteria coordinate their activity in response to population density and diffusivity of their environment**.** In this mechanism, bacteria synthesize specific small molecules that accumulate proportionally to their population density and release them across the bacterial envelope, and once the signal molecule concentration reaches a threshold level, it is perceived via receptor proteins that in turn regulate expression of specific genes. QS-regulated activities include bioluminescence, virulence gene expression, biofilm formation, production of exoenzymes and antibiotics, and in *Agrobacterium tumefaciens* replication and conjugal transfer of tumor-induction (Ti) plasmid. Our biochemical and structural studies have focused on investigating the inhibitory mechanism of the anti-activator TraM on the quorum sensing transcription activator TraR in *A. tumefaciens*. We have solved several crystal structures of TraM and TraR-TraM, utilized a range of biochemical and biophysical techniques to complement structural studies. One of our most significant findings includes revealing a novel mechanism through which TraM antagonizes TraR through allostery. Association of TraM with TraR induces large structural changes in TraR, preventing it from binding to DNA. I directed all these studies.

a. Guozhou Chen, James Malenkos, Mee-Rye Cha, Clay Fuqua and **Lingling Chen** (2004). "Quorum-sensing antiactivator TraM forms a dimer that dissociates to inhibit TraR", *Mol. Micro.* 52:1641-1651. PMID:15186414
b. Guozhou Chen, Chao Wang, Clay Fuqua, Lian-Hui Zhang and **Lingling Chen** (2006). "The crystal structure and mechanism of TraM2, a second quorum sensing antiactivator of *Agrobacterium tumefaciens* strain A6. *J. Bact.* 188:8244-8251. PMC1698194
c. Guozhou Chen, Phillip Jeffery, Clay Fuqua, Yigong Shi and **Lingling Chen** (2007). "Structural basis of TraM anti-activation of quorum sensing transcription factor TraR", *Proc. Natl. Acad. Sci. USA*, 104:16474-16479. PMC2034266
d. Mair Churchill and **Lingling Chen** (2011). "Structural Basis of Acyl-homoserine Lactone-Dependent Signaling", *Chem. Rev*. 111: 68-85. PMC3494288

3. Coupling activation of T3SS activation with secretion

Many Gram-negative pathogens, including *Yersinia pestis*, the pathogen of this grant proposal, and *Pseudomonas aeruginosa,* utilize type III secretion systems (T3SS) to translocate effectors into eukaryotic host cells. Expression of T3SS genes is highly regulated and often coupled to T3SS activity. Transcription of the *P. aeruginosa* T3SS genes is coupled to secretion by a cascade of interacting regulatory proteins (ExsA, ExsD, ExsC, and ExsE). ExsA is an activator of type III gene transcription, ExsD binds ExsA to inhibit transcription, ExsC inhibits ExsD activity, and ExsE inhibits ExsC activity. Transcriptional regulation of T3SS is coupled to T3SS secretion via ExsE, a T3SS secretion substrate. We have shown that although ExsC can form complex with either ExsE or ExsD, it predominately exists as ExsC-ExsE because its binding affinity for ExsE is stronger than for ExsD. We have also shown that the T3SS substrate ExsE is intrinsically disordered and is partially stabilized by interacting with ExsC. Our work supports a model for the efficient activation of T3SS via secretion: secretion of ExsE dissociates ExsE-ExsC complex, allowing ExsC to compete for ExsD from ExsD-ExsA, and the displaced ExsA can bind DNA to activate T3SS genes. The coupling mechanism is immediate, as the intrinsically disordered nature of ExsE allows it to translocate efficiently through the long narrow path of T3SS. I directed all of these studies.

a. Guinivere Lykken, Guozhou Chen, Evan Brutinel, **Lingling Chen**, and Timothy Yahr (2006). "Characterization of ExsC and ExsD self-association and heterocomplex formation", *J. Bact.*

188:6832-6840. PMC1595525

b. Zhida Zheng, Guozhou Chen, Evan D. Brutinel, Timothy L. Yahr, and **Lingling Chen** (2007). "Biochemical characterization of a regulatory cascade controlling transcription of the *Pseudomonas aeruginosa* type III secretion system", *J. Biol. Chem.* 282:6136-6142. PMID: 17197437

c. Zhida Zheng, Dejian Ma, Timothy Yahr, and **Lingling Chen** (2012). "The Transiently Ordered Regions in Intrinsically Disordered ExsE Are Correlated with Structural Elements Involved in Chaperone Binding", *Biochem. Biophys. Res. Commun.* 417: 129-134. PMID: 22138394

4. Regulatory mechanism of the IclR transcription family

The IclR transcription factor family controls a wide range of important cellular processes in bacteria, including metabolic pathways, multidrug resistance, aromatic compound degradation, pathogenicity, sporulation, amino acid biosynthesis, and quorum-sensing signal degradation. However, IclR proteins are largely uncharacterized, and molecular understanding of how IclR protein recognizes the promoter DNA and how its DNA-binding activity is regulated is scarce. We have focused on an IclR member, BlcR of *A. tumefaciens*, because it is an experimentally amiable system with a known DNA promoter, a known regulatory ligand, and an in vivo system to confirm the in vitro findings. We have shown that modulating the oligomeric state of BlcR is the mechanism to regulate the DNA-binding function of BlcR. We showed that DNA plays a role in forming the DNA-binding active BlcR tetramer, while the regulatory ligand destabilizes the tetramer leading to dissociation of BlcR from DNA. The BlcR mechanism appears to share among the IclR members, and is drastically different from the only other investigated mechanism adopted by the IclR member TtgV. Our work expands our understanding of the uncharacterized transcription factor family, and our knowledge of how prokaryotes have evolved diverse transcriptional regulators to control transcriptional machinery. I directed all these studies.

a. Yi Pan, Valena Fiscus, Wuyi Meng, Zhida Zheng, Lianhui Zhang, Clay Fuqua, and **Lingling Chen** (2011). "The Agobacterium tumefaciens Transcriptional Factor BlcR Is Regulated via Oligomerization", *J. Biol. Chem.* 286: 20431-20440. PMC3121482

b. Yi Pan, Yi Wang, Clay Fuqua, and **Lingling Chen** (2013). "In vivo Analysis of DNA Binding and Ligand Interaction of BlcR, an IclR-type Repressor from *Agrobacterium tumefaciens*". *Microbiology-SGM*, 159:814-822, 2013. PMC4083662

5. Small angle X-ray scattering (SAXS) studies of biological systems

SAXS is a powerful technique that reveals structural information of biological molecules in their native, aqueous environment, and is particularly useful to study proteins with flexible conformations or refractory to crystallization. The recent increasing utilization of SAXS owes to technical advances in delivering stable and intense X-ray beam, detector technology, and software for data analysis and modeling. However, in the early 90s, the Hodgson/Doniach group was among the few groups exploring the potentials of applying SAXS to study biological systems. My studies on Fe protein of nitrogenase and molecular chaperone Hsp70 demonstrate that binding of nucleotide (ATP) induces large conformational change in protein that is otherwise hard to detect using other structural techniques. In particular, my SAXS results on the ATP-induced compaction in Hsp70 have been validated many years later by crystallographic studies. Moreover, my SAXS work on protein folding is among the pioneers in then the new field of time-resolved x-ray scattering. I was the primary investigator of these studies.

a. **Lingling Chen**, Narasaiah Gavini, Hirotsugu Tsuruta, David Eliezer, Barbara K. Burgess, Sebastian Doniach, and Keith O. Hodgson (1994). MgATP-induced conformational changes in the iron protein from *Azotobactor vinelandii*, as studied by small angle x-ray scattering. *J. Bio. Chem*. 269:3290-3294. PMID: 8106367

b. Sigurd M. Wilbanks*, **Lingling Chen***, David B. McKay, Hirotsugu Tsuruta, and Keith O. Hodgson (1995). Solution Small-angle X-ray Scattering Study of A Bovine Heat-Shock Cognate and Its Subfragments. *Biochemistry* 34:12095-12106. (*these two authors contributed equally to this work). PMID: 7547949

c. **Lingling Chen**, Keith O. Hodgson and Sebastian Doniach (1996). A lysozyme folding intermediate revealed by solution x-ray scattering. *J. Mol. Biol.* 261:658-671. PMID: 8800214

d. **Lingling Chen**, Gudrun Wildegger, Thomas Kiefhaber, Keith O. Hodgson and Sebastian Doniach (1998). Kinetics of lysozyme refolding: structural characterization of a non-specifically collapsed state using time-resolved x-ray scattering. *J. Mol. Biol*. 276:225-237. PMID: 9514723

**Complete List of Published Work in MyBibliography:**
http://www.ncbi.nlm.nih.gov/sites/myncbi/1lo2w1j82wM5z/bibliography/48149590/public/?sort=date&direction=ascending

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

**Completed Research Support:**

NIH R01 GM065260 Chen (PI) 05/01/03-04/30/10
Mechanism of GroEL-assisted Protein Folding
The major goal of this project is to understand how GroEL recognizes and interacts with the substrate proteins.
Role: Investigator

NIH R01 GM065260- Administrative Supplement Grant Chen (PI) 01/01/10-04/30/10
Mechanism of GroEL-assisted protein folding
The goal is to construct a stable single ring version of GroEL that is functional active, a model system for the human mitochondrial single ring mtHsp60.
Role: Investigator

NSF MCB-0416447 Chen (PI) 04/01/05-03/31/10
Structural and Functional Analysis of a Quorum-sensing Inhibition Mechanism
The goal of this project is to understand the structural basis for transcriptional inhibition by anti-activator in quorum sensing regulation of plant pathogen *Agrobacterium tumefaciens*.
Role: Investigator

Indiana Clinical and Translational Sciences Institute Chen (PI) 12/01/13-11/30/14
Molecular Mechanism of Human Mitochondrial Chaperonin mtHsp60
The goal is to map the sequence elements in mtHsp60 that are critical for structure, function, and pathology of mtHsp60.
Role: Investigator

IU Collaborative Research Grant, Indiana University Johnson (PI) 04/01/14-09/30/15
Identifying the Structural/Functional Mechanisms of Action of GroEL/ES (HSP60/10) Inhibitors
The goal is to identify small molecules that inhibit the function of the chaperonin GroEL/GroES, and investigate the structural basis of the inhibitor action.
Role: Co-Investigator

IU Institute for Advanced Study Chen (PI) 2016
Preliminary NMR Studies of Type III Secretion Regulator YopK
The goal is to optimize sample conditions of YopK for NMR studies.
Role: Investigator

IU Institute for Advanced Study Chen (PI) 2016
Functional and structural studies of *Chlamydial* secreted proteins
The goal of this study is to characterize the biochemical and structural properties of chlamydial secreted proteins CT311
Role: Investigator

Indiana Clinical and Translational Sciences Institute Chen (PI) 2017-2019
A Chlamydial Nuclear Effector in Host Response to Chlamydial Infection
The goal of this study is to identify the biological processes affected by the chlamydial nuclear effector CT311 and the CT311-interacting proteins from the host cells.
Role: Investigator