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BIOGRAPHICAL SKETCH

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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 DateMM/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**

**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 X-ray crystallography provides structural basis for how GroEL recognizes substrate proteins. My chaperonin research at IU has been focused on mechanistic investigations on the human mitochondrial mHsp60. As in the proposal, purified mHsp60 is unstable and dissociates to nonfunctional monomers. To overcome this difficulty, in our early research we focused on creating stable, single-ring, and functional chaperonin systems as model systems for detailed molecular investigations on mHsp60-mHsp10. Notably, we created a novel reagent, *groES7*, which concatenates seven copies of *E. coli* cochaperonin GroES genes to express the cochpaperonin in a continuous polypeptide. Using *groES7*, we identified several GroES7 variants to activate the single-ring inactive GroELSR. 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 GroELSR-GroES7 and mHsp60-mHsp10 systems, highlighting the relevance of the model systems. Lately, we have developed a purification protocol to consistently obtain active, single-ring mHsp60, and have used it to obtain a 3.4** Å **cryo-EM structure of mHsp60 (manuscript submitted). Finally, I recently completed** the intensive immersion TP1 training at National Center for Cryo-EM Access and Training (NCCAT). **With our extensive experience in chaperonin, I believe that we are in a unique position to elucidate the molecular mechanism of mHsp60-mHsp10.**

**My research program has been greatly impacted by a series of challenging including both work and tragic life events. Near the end of my last R01 funding cycle, I lost my 6-year old son tragically. He was born with multiple genetic issues; however, his unexpected passing was caused by cultural indifference in the community. (I have established a memorial fund in his honor to promote cultural awareness. The fund supported activities for K-12 teachers and students as well as IU students for nine years.) Years after my son’s passing, I gave birth to a healthy boy and became a single mother. While raising my son, I also took care of my mother who passed away this year at 97. In 2018, I was diagnosed with breast cancer, pausing my research temporally. In addition, my research has been impacted by my teaching and service obligations. In 2008, my appointment was transferred to the new Biochemistry Department; as one of the four founding members, I have taken on the new department’s heaviest teaching load, including designing new intensive writing and lab courses for the new undergraduate degree majors. As the department’s only female faculty, I have severed in various committees at the departmental, college and university levels. While coping with the decade-long life misfortunes and work challenges, I managed to maintain the research activity as much as possible. Now with my cancer treatments finished and my son in high school, I have been able to devote my energy to research. The recently published paper on cryo-EM structure of mHsp60 speaks for my strong comeback to research. Support from this grant will accelerate our research effort, and I do expect a high productivity of quality research from my lab.**

**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

2017-19 Oversea Review Panelist, National Science Foundation of China

2017- Reviewing Editorial Board Member, *Cell Stress & Chaperones*

2018- 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 chaperonins 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. I also showed that substrate adopts -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. 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 a peptide in -helix conformation, 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 mHsp60 that had not been amenable for biochemical and structural studies until recently. We have developed novel regents to activate a single ring form GroELSR that is otherwise inactive and nonfunctional. Combining with functional GroELSR mutants, we identified biochemical determinants that allow chaperonin to function as single ring. Most recently, we developed a purification protocol to obtain active, single-ring mHsp60, enabling us to launch detailed biochemical and structural investigations directly on mHsp60-mHsp10. Our recent reported 3.4 A cryo-EM structure of mHsp60 reveals structural basis for the subunit association dynamics of mHsp60. I directed all 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](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3162399/)

c. Melissa Illingworth, Holly Ellis and **Lingling Chen** (2017). Creating the Functional Single-Ring GroEL-GroES Chaperonin Systems via Modulating GroEL-GroES Interaction. Sci. Rep., 7:9710, PMID: 5575113

d. Joseph Wang and **Lingling Chen** (2021). Structural Basis for the Structural Dynamics of Human Mitochondrial Chaperonin mHsp60. Sci. Rep., Jul 20;11(1):14809, PMC8292379

**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 o**nce 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, and 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](http://www.ncbi.nlm.nih.gov/pmc/articles/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](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2034266/)

d. Mair Churchill and **Lingling Chen** (2011). “Structural Basis of Acyl-homoserine Lactone-Dependent Signaling”, *Chem. Rev*. 111: 68-85. [PMC3494288](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3494288/)

**3. Coupling activation of T3SS activation with secretion**

Many Gram-negative pathogens, including *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](http://www.ncbi.nlm.nih.gov/pmc/articles/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](http://www.ncbi.nlm.nih.gov/pmc/articles/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](http://www.ncbi.nlm.nih.gov/pmc/articles/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 the 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

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<https://www.ncbi.nlm.nih.gov/myncbi/lingling.chen.2/bibliography/public/>