BIOGRAPHICAL SKETCH

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NAME: CHEN, LINGLING

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

POSITION TITLE: 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)	END DATE MM/YYYY	FIELD OF STUDY
Xiamen University, Xiamen, Fujian	BS	07/1988	Chemistry
Stanford University, Stanford, CA	PHD	05/1996	Chemistry
Yale University, New Haven, CT	Postdoctoral Fellow	12/2000	Protein Crystallography

A. Personal Statement

As a structural biologist, I have a strong and sustained track record in understanding the mechanistic aspects of the chaperonin system. My postdoctoral work at Yale focused on *E. coli* chaperonin GroEL-substrate interactions using combinatorial biology and X-ray crystallography, providing a structural basis for how GroEL recognizes substrate proteins. At IU, my research has centered on mechanistic investigations of the human mitochondrial chaperonin hmHsp60. To address the challenges in purifying hmHsp60, we initially developed stable, single-ring, and functional chaperonin systems as models for hmHsp60-hmHsp10. This led to the creation of a novel gene, *groES*^{7}, which fuses seven copies of the *E. coli* cochaperonin *groES* gene to produce continuous polypeptides. Using *groES*^{7}, we identified several variants to activate the single-ring inactive mutant GroEL^{SR}. More recently, we have developed a purification protocol that consistently yields active, single-ring hmHsp60 without the need for tags, refolding, reconstitution, or gene concatenation. Our publications have elucidated the structural basis for subunit association dynamics of hmHsp60 (Sci Rep, 2021), the gain-of-activity (GOA) of the pathological V72I mutation (Sci Rep, 2022), and the structural and molecular dynamic basis for the GOA of the V72I mutation (Structure, 2024). Combining with the forthcoming cryo-EM studies of ATP-bound hmHsp60 and hmHsp60^{V72I}, our lab is establishing itself as a leading contributor in the field.

1. Syed, A., Zhai, J., Guo, B., Zhao, Y., Wang, J. C., Chen, L. Cryo-EM structure and molecular dynamic simulations explain the enhanced stability and ATP activity of the pathological chaperonin mutant. *Structure*, 2024 May 2;32(5):575-584.e3. Epub 2024 Feb 26. PubMed PMID: 38412855; PubMed Central PMCID: PMC11069440
2. Chen, L., Syed, A. & Balaji, A. Hereditary spastic paraplegia SPG13 mutation increases structural stability and ATPase activity of human mitochondrial chaperonin. *Sci Rep*. 2022 Oct 31;12(1):18321. PubMed PMID: 36316435; PubMed Central PMCID: PMC9622745.
3. Wang JC, Chen L. Structural basis for the structural dynamics of human mitochondrial chaperonin mHsp60. Sci Rep. 2021 Jul 20;11(1):14809. PubMed Central PMCID: PMC8292379.
4. Illingworth M, Ellis H, Chen L. Creating the Functional Single-Ring GroEL-GroES Chaperonin Systems via Modulating GroEL-GroES Interaction. Sci Rep. 2017 Aug 29;7(1):9710. PubMed Central PMCID: PMC5575113.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2019 -	Professor, Dept. Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN
2009 - 2018	Associate Professor, Dept Molecular and Cellular Biochemistry, Indiana University, Bloomington, IN
2001 - 2008	Assistant Professor, Biology Department, Indiana University, Bloomington, IN

1996 - 2000	Postdoctoral Fellow, Dept. Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

Honors

2010 - 2019	Minjian Fellow, Xiamen University, China
2016	Residential Fellow, Institute for Advance Study, Indiana University
2003	Faculty Summer Fellowship, Indiana University
1997 - 2000	Helen Hay Whitney Postdoctoral Fellowship, Helen Hay Whitney Foundation
1990	Guang-Hua Scholarship, Graduate School, Xiamen University, China
1989	Lu Jia-Xi & Cai Qi-Rui Scholarship, Graduate School, Xiamen University, China
1984 - 1988	University Fellowship, Xiamen University
1988	Presidential Award: Chen Jia-Geng Fellowship, Xiamen University
1985	Zhong-Xue Scholarship, Chemistry Department, Xiamen University, China

C. Contribution to Science

1. Mechanism of chaperonins in protein folding

GroEL, the chaperonin 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 I 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 beta-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 the 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 alpha-helix conformation, strengthening the notion that unlike other chaperone GroEL interacts with the secondary structures in the substrates. Recently, my lab has focused on studying the mechanism of human mitochondrial mHsp60. To overcome the mHsp60 instability, we developed novel regents to activate a single ring form $GroEL^{SR}$ that is otherwise inactive and non-functional. Mimicking the unstable mHsp60, these single-ring $GroEL^{SR}$ -based chaperonin systems revealed important biochemical determinants that allow chaperonin to function as a 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 3.4 Å cryo-EM structure of mHsp60 reveals the structural basis for the subunit association dynamics of mHsp60. Our recent study on the pathological V72I mutant uncovers the unexpected V72I allosteric effects on increasing subunit association and ATPase activity, supporting the functional role of subunit association dynamics of mHsp60. I directed all these studies.

a. Illingworth M, Hooppaw AJ, Ruan L, Fisher DJ, Chen L. Biochemical and Genetic Analysis of the Chlamydia GroEL Chaperonins. J Bacteriol. 2017 Jun 15;199(12) PubMed Central PMCID: PMC5446618.

b. Illingworth M, Salisbury J, Li W, Lin D, Chen L. Effective ATPase activity and moderate chaperonin-cochaperonin interaction are important for the functional single-ring chaperonin system. Biochem Biophys Res Commun. 2015 Oct 9;466(1):15-20. PubMed PMID: 26271593.

c. Li Y, Gao X, Chen L. GroEL Recognizes an Amphipathic Helix and Binds to the Hydrophobic Side. J Biol Chem. 2009 Feb 13;284(7):4324-31. PubMed Central PMCID: PMC2640968.

d. Illingworth M, Ramsey A, Zheng Z, Chen L. Stimulating the substrate folding activity of a single ring GroEL variant by modulating the cochaperonin GroES. J Biol Chem. 2011 Sep 2;286(35):30401-30408. PubMed Central PMCID: PMC3162399.

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, 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. Chen L. Signal synthesis for a rapid response. Structure. 2010 Sep 8;18(9):1072-3. PubMed PMID: 20826333.
b. Chen G, Jeffrey PD, Fuqua C, Shi Y, Chen L. Structural basis for antiactivation in bacterial quorum sensing. Proc Natl Acad Sci U S A. 2007 Oct 16;104(42):16474-9. PubMed Central PMCID: PMC2034266.
c. Chen G, Wang C, Fuqua C, Zhang LH, Chen L. Crystal structure and mechanism of TraM2, a second quorum-sensing antiactivator of Agrobacterium tumefaciens strain A6. J Bacteriol. 2006 Dec;188(23):8244-51. PubMed Central PMCID: PMC1698194.
d. Chen G, Malenkos JW, Cha MR, Fuqua C, Chen L. Quorum-sensing antiactivator TraM forms a dimer that dissociates to inhibit TraR. Mol Microbiol. 2004 Jun;52(6):1641-51. PubMed PMID: 15186414.

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 these studies.

a. Zheng Z, Ma D, Yahr TL, Chen L. The transiently ordered regions in intrinsically disordered ExsE are correlated with structural elements involved in chaperone binding. Biochem Biophys Res Commun. 2012 Jan 6;417(1):129-34. PubMed Central PMCID: PMC4930836.
b. Zheng Z, Chen G, Joshi S, Brutinel ED, Yahr TL, Chen L. Biochemical characterization of a regulatory cascade controlling transcription of the Pseudomonas aeruginosa type III secretion system. J Biol Chem. 2007 Mar 2;282(9):6136-42. PubMed PMID: 17197437.
c. Lykken GL, Chen G, Brutinel ED, Chen L, Yahr TL. Characterization of ExsC and ExsD self-association and heterocomplex formation. J Bacteriol. 2006 Oct;188(19):6832-40. PubMed Central PMCID: PMC1595525.

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. Pan Y, Wang Y, Fuqua C, Chen L. In vivo analysis of DNA binding and ligand interaction of BlcR, an IclR-type repressor from Agrobacterium tumefaciens. Microbiology (Reading). 2013 Apr;159(Pt 4):814-822. PubMed Central PMCID: PMC4083662.
b. Pan Y, Fiscus V, Meng W, Zheng Z, Zhang LH, Fuqua C, Chen L. The Agrobacterium tumefaciens transcription factor BlcR is regulated via oligomerization. J Biol Chem. 2011 Jun 10;286(23):20431-40. PubMed Central PMCID: PMC3121482.

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. Chen L, Wildegger G, Kiefhaber T, Hodgson KO, Doniach S. Kinetics of lysozyme refolding: structural characterization of a non-specifically collapsed state using time-resolved X-ray scattering. J Mol Biol. 1998 Feb 13;276(1):225-37. PubMed PMID: 9514723.
b. Chen L, Hodgson KO, Doniach S. A lysozyme folding intermediate revealed by solution X-ray scattering. J Mol Biol. 1996 Sep 6;261(5):658-71. PubMed PMID: 8800214.
c. Wilbanks SM, Chen L, Tsuruta H, Hodgson KO, McKay DB. Solution small-angle X-ray scattering study of the molecular chaperone Hsc70 and its subfragments. Biochemistry. 1995 Sep 26;34(38):12095-106. PubMed PMID: 7547949.
d. Chen L, Gavini N, Tsuruta H, Eliezer D, Burgess BK, Doniach S, Hodgson KO. MgATP-induced conformational changes in the iron protein from Azotobacter vinelandii, as studied by small-angle x-ray scattering. J Biol Chem. 1994 Feb 4;269(5):3290-4. PubMed PMID: 8106367.

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