BIOGRAPHICAL SKETCH

NAME: **Pingwei Li**

eRA COMMONS USER NAME: **PINGWEI06**

POSITION TITLE: **Professor of Biochemistry and Biophysics**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Northwest University, Xi'an, China		06/1989	Chemical Engineering
Peking University, Beijing, China	M.Sc.	07/1992	Physical Chemistry
Peking University, Beijing, China	Ph.D.	07/1996	Structural Biology
Fred Hutchinson Cancer Res. Center, Seattle, WA	Post-doc	09/2001	Structural Biology
California Institute of Technology, Pasadena, CA	Post-doc	07/2005	Structural Biology

A. Personal Statement

The research in my lab focuses on elucidating the structural basis of microbial nucleic acids sensing in innate immunity over the last eight years. Biochemical, biophysical, cellular, and structural approaches are employed to investigate the molecular basis of viral RNA sensing by the RIG-I-like receptors (RLRs) and microbial DNA sensing through the cGAS-STING pathway. We have determined the crystal structures of LGP2 CTD bound to dsRNA, MDA5 CTD in isolation, and RIG-I CTD bound to 5′ triphosphate and blunt-ended dsRNA. These studies provided important insights into the mechanism of viral RNA recognition by the RLRs. Recently, we are investigating the molecular mechanisms of cytosolic dsDNA sensing through the cGAS-STING pathway. We have determined the crystal structures of cGAS catalytic domain in isolation and in complex with dsDNA and elucidated the mechanism of cGAS activation by dsDNA. In addition, we have determined the structures of STING in isolation and in complex with either canonical or noncanonical cyclic dinucleotides (CDNs) including c-di-GMP, c-di-AMP, and cGAMP, providing critical insights into the mechanism of STING activation by the CDNs. We have determined the structure of the protein kinase TBK1 downstream of STING, revealing the mechanism of TBK1 activation and regulation. Moreover, we have recently determined the structure of phosphorylated STING C-terminal peptide bound to IRF-3 and the structure of a phosphomimetic dimer of IRF-3. I was trained as a crystallographer and have over twenty years continuous experience in structural studies of proteins and protein/ligand complexes involved in immunity, protein ubiquitination, and other important biological processes. We have determined over 30 protein structures independently since I started at Texas A&M University in 2005. I also have strong expertise in biochemical and biophysical characterization of protein and protein/ligand interactions by various techniques. Recently, we have established our tissue culture facility and started conducting cell-based studies, including luciferase reporter assays, ELISA, immunoprecipitation, immunoblot, and confocal microscopy. In sum, I have a demonstrated record of successful and productive research in the field of structural biology. My expertise and experience prepared me to carry out the proposed studies.

B. Positions and Honors

Positions and Employment

1996-1998 Research Associate, Institute of Biophysics, Chinese Academy of Science
1998-2001 Post-doctoral Research Associate, Fred Hutchinson Cancer Research Center
2001-2003 Staff Scientist, Department of Molecular Biology, Princeton University
2003-2005 Postdoctoral Scholar, Division of Biology, California Institute of Technology

2005-2011 Assistant Professor of Biochemistry and Biophysics, Texas A&M University
2011-2017 Associate Professor of Biochemistry and Biophysics, Texas A&M University
2017-Present Professor of Biochemistry and Biophysics, Texas A&M University

Other Experience and Professional Memberships

2008-2009 Member of the American Chemical Society (ACS)
2010 Member of the American Crystallographic Association (ACA)
2016 Member of the American Association of Immunologist (AAI)
2011 Ad Hoc Grant Reviewer, NIH Study Section MSFC
2013 Reviewer for NIAID P01 Grant Proposal
2013 Reviewer for NIAID P19 Grant Proposal
2012 Reviewer for the French National Research Agency (ANR)
2011 Reviewer for Hong Kong Research Grants Council

C. Contributions to Science

1. Elucidated the molecular mechanisms of cytosolic DNA sensing via the cGAS-STING pathway

Microbial nucleic acids induce potent innate immune responses by stimulating the expression of type I interferons. cGAS is a cytosolic dsDNA sensor mediating the innate immunity to microbial DNA. cGAS is activated by dsDNA and catalyzes the synthesis of a cyclic dinucleotide (CDN) cGAMP, which binds to the adaptor STING and mediates the recruitment and activation of TBK1 and IRF-3. In addition, STING is also a direct sensor of bacterial CDNs such as c-di-GMP and c-di-AMP. As a leading player in the structural characterization of the cGAS-STING signaling pathway, we have contributed broadly and significantly to this field. For example, we have determined the crystal structures of cGAS in isolation and in complex with an 18 bp dsDNA. These structures reveal that cGAS is activated by dsDNA induced oligomerization instead of simple 1:1 binding as proposed by others. We have also determined the structures of STING ligand binding domain in isolation and in complex with c-di-GMP and cGAS. These structures show that cGAMP binding induces a major conformational change of the STING dimer. Moreover, we have solved the structures of mouse TBK1 bound to two inhibitors, providing insights into the mechanism of TBK1 activation and inhibition. Recently, we have determined the structure of phosphorylated STING C-terminal tail bound to IRF-3 C-terminal domain (CTD), which reveals the molecular basis of IRF-3 recruitment by phosphorylated STING. Furthermore, we have determined the structure of an IRF-3 phosphomimetic dimer, which demonstrated that an internal *p*LxIS motif of IRF-3 mediates its dimerization and activation. These extensive structural studies have significantly advanced our understanding of DNA sensing through the cGAS-STING pathways. As the PI, I oversaw the entire project and directed staff scientists and graduate students to design and perform the experiments.

a. Baoyu Zhao[#], Chang Shu[*#], Xinsheng Gao, Banumathi Sankaran, Fenglei Du, Catherine L. Shelton, Andrew B. Herr, Jun-Yuan Ji, and **Pingwei Li*** (2016). Structural basis for concerted recruitment and activation of IRF-3 by innate immune adaptor proteins. ***Proc Natl Acad Sci U S A*** 113, E3423-E3430. doi:10.1073/pnas.1603269113. ([#] These authors made equal contribution to the work).
b. Xin Li[#], Chang Shu[#], Guanghui Yi, Catherine T. Chaton, Catherine L. Shelton, Jiasheng Diao, Xiaobing Zuo, C Cheng Kao, Andrew B. Herr, and **Pingwei Li*** (2013). Cyclic GMP-AMP synthase is activated by double-stranded DNA-induced oligomerization. **Immunity** 39, 1019-103. ([#] These authors contributed equally.)
c. Chang Shu, Banumathi Sankaran, Catherine T. Chaton, Andrew B. Herr, Ashutosh Mishra, Junmin Peng and **Pingwei Li*** (2013). Structural insights into the functions of TBK1 in innate antimicrobial immunity. **Structure** 21, 1137-1148.
d. Chang Shu, Guanghui Yi, Tylan Watts, C. Cheng Kao, **Pingwei Li*** (2012). Structure of STING bound to c-di-GMP reveals the mechanism of cyclic dinucleotide recognition by the immune system. **Nature Structural and Molecular Biology** 19, 722-724.

2. Determined the structural basis of viral RNA recognition by the RIG-I like receptors

Host immunity to viruses involves recognition of viral genetic material to promote host defense. In mammals, the RIG-I like receptors (RLRs), RIG-I, MDA5 and LGP2, sense viral RNA in the cytosol and play crucial roles in antiviral immunity. The RLRs recognize 5′ triphosphate (ppp) single-stranded (ssRNA) or double-stranded

RNA (dsRNA), which are signature structures of viral RNA. Stimulation of the RLRs lead to the induction of type I interferons, conferring antiviral activity to the host and activating acquired immune responses. We have conducted extensive RNA binding studies using the C-terminal domain (CTD) of the RLRs, demonstrating that RIG-I recognizes 5′ ppp ssRNA and dsRNA with or without triphosphate, while LGP2 and MDA5 recognize dsRNA with no preference for RNA with triphosphate. We have determined the crystal structure of human LGP2 CTD bound to an 8 bp blunt-ended dsRNA and demonstrated for the first time that the termini of dsRNA is recognized by the RLR. We also determined the 1.45 Å resolution structure of MDA5 CTD and mapped its RNA binding surface by NMR. To elucidate the structural basis of RNA recognition by RIG-I, we determined the structures of RIG-I CTD bound to dsRNA with and without 5′ triphosphate and elucidated the structural basis of dsRNA recognition by RIG-I. Our structural and biochemical studies demonstrated that RIG-I CTD is a versatile RNA binding module, using overlapping sets of residues to recognize various forms of RNA. These studies provided critical insights into the structural basis of viral RNA recognition by the RIG-I like receptors. As the PI of this work, I oversaw the entire project and directed staff scientists and graduate students to design and perform the experiments.

a. Cheng Lu, C. T. Ranjith-Kumar, Lujiang Hao, C. Cheng Kao, **Pingwei Li*** (2011) Crystal structure of RIG-I C-terminal domain bound to blunt-ended double-strand RNA without 5′ triphosphate. **Nucleic Acids Research** 39, 1565-1575.
b. Cheng Lu, Hengyu Xu, C. T. Ranjith-Kumar, Monica T. Brooks, Tim Y. Hou, Fuqu Hu, Andrew B. Herr, Roland K. Strong, C. Cheng Kao, and **Pingwei Li*** (2010). The structural basis of 5′ triphosphate double-stranded RNA recognition by RIG-I C-terminal domain. **Structure** 18, 1032-1043.
c. Xiaojun Li, C. T. Ranjith-Kumar, Monica T. Brooks, Andrew B. Herr, C. Cheng Kao, and **Pingwei Li*** (2009). The RIG-I like receptor LGP2 recognizes the termini of double-stranded RNA. **Journal of Biological Chemistry** 284, 13881-91.

3. Elucidated the molecular mechanism of ligand recognition by the NK cell, γδ T cell receptor NKG2D

NKG2D is an activating immunoreceptor expressed on most NK cells, CD8 αβ T cells, and γδ T cells. The ligands for NKG2D include MICA, MICB and ULBP family in human, H60 and the RAE-1 family in mice, which are distant homologs of the MHC class I molecules. The expression of NKG2D ligands is induced during cellular stress, either as a result of viral infection or genomic stress such as in cancer. The engagement of NKG2D with its ligands results in the activation of NK cells or γδ T cells that mediate the elimination of the infected cells or the cancer cells. To elucidate the mechanism of ligand recognition by NKG2D, I determined the structures of human MICA and mouse RAE-1 in isolation and in complex with NKG2D. These structures revealed that NKG2D binds to the top of the α1α2 platform domain of MICA or RAE-1 in an interaction similar to that in the T cell receptor–MHC class I complex. However, unlike T cell receptors, which recognize the MHC class I protein through the variable domains of the TCR, the homodimer of NKG2D recognizes distinct surfaces of its ligands using different sets of residues and through distinct interactions. These structures reveal the mechanism of how the structurally conserved NKG2D receptor recognizes a wide range of ligands. As the primary scientist for this project, I determined all these structures.

a**. Pingwei Li**, Gerry McDermott, and Roland Strong (2002). Crystal structures of RAE-1β and its complex with activating immunoreceptor NKG2D. **Immunity** 16, 77-86.
b. **Pingwei Li**, Daniel Morris, Benjamin Willcox, Alexander Steinle, Thomas Spies, and Roland Strong (2001). Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. **Nature Immunology** 2, 443-451.
c**. Pingwei Li**, Sirrku T Willie, Stefan Bauer, Daniel Morris, Thomas Spies, and Roland K Strong (1999). Crystal structure of the MHC class I homolog MIC-A, a gd T cell ligand. **Immunity** 10, 577-584.

4. Structural analysis of polyglutamine (polyQ) using a monoclonal antibody

Huntington and related neurological diseases results from expansion of a polyglutamine (polyQ) tract in several proteins. I cloned and expressed the variable domains (V_H and V_L) of a monoclonal antibody (MW1) specific for polyQ and determined the structure of the F_V fragment in isolation and in complex with a polyQ containing peptide $GQ_{10}G$. This structure reveals that polyQ adopts an extended coli-like structure and bound to MW1 F_V diagonally across the V_H and V_L domains. polyQ binding studies showed that multimeric F_v bind more tightly to

longer rather than shorter polyQ tracts and provided evidence to the linear lattice model of polyQ structure. This study provided insights into the structure of polyQ and the mechanisms of polyQ aggregation in neurodegenerative diseases and suggests a strategy to link polyQ binding molecules to create a high avidity therapeutics to prevent the aggregation of proteins with expanded polyQ tracts. As the primary scientist for this project, I cloned of the V_H and V_L genes of MW1, purified the proteins, and determined the structures.

a. **Pingwei Li***, Kathryn E Huey Tubman, Tiyu Gao, Xiaojun Li, Athony P West, Melanie J Bennett, and Pamela Bjorkman (2007). The structure of a polyQ-anti-polyQ complex reveals binding according to a linear lattice model. **Nature Structural and Molecular Biology** 14, 381-387. (*Corresponding author)

5. Determined the molecular mechanism of the UBP family deubiquitination enzymes

Ubiquitination of proteins is involved in the regulation many critical cellular processes. Deubiquitination is an important mechanism in regulating the ubiquitin-dependent pathways. The UBP family deubiqutinating enzymes are involved in regulating a diverse set of cellular processes. For example, HAUSP specifically removes ubiquitin chains from modified p53 and promotes p53-dependent cell growth arrest and apoptosis. USP14 helps to remove the polyubiquitin chains of a protein substrate before its degradation by the proteasome. To determine how the UBP family enzyme recognizes ubiquitin and catalyzes the cleavage of the link between the substrate and ubiquitin, I determined the structures of the catalytic domains of HAUSP and USP14 in isolation and covalently linked to ubiquitin aldehyde. These structures reveal that the catalytic cores of the UBP exhibit a similar fold like an extended hand and recognize ubiquitin through the finger like domain. Binding of ubiquitin aldehyde induces a drastic conformational change in the active site of the enzyme and realigns the catalytic triad for catalysis. These structural studies established the mechanism of ubiquitin recognition and activation of the UBP family deubiquitinating enzymes. As one of the primary scientist for this project, I purified and crystallized USP14 and determined three of the four structures from data collection to structural refinement.

a. Min Hu***, Pingwei Li***, Ling Song, Philip D Jeffery, Tatiana A Chenova, Keith D Wilkinson, Robert Cohen, and Yigong Shi (2005). Structure and Mechanism of the proteasome associated deubquitinating enzyme USP14. **EMBO J**. 24, 3747-3756. (*These authors contributed equally to the work).
b. Min Hu, **Pingwei Li**, Muyang Li, Tingting Yao, Jiawei Wu, Wei Gu, Robert Cohen, and Yigong Shi (2002). Crystal structure of an UBP-family deubiquiting enzyme: in isolation and in complex with ubiquitin aldehyde**.** **Cell** 111, 1041-1054.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47297317/?sort=date&direction=ascending

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

Current Research Support

RP150454, Cancer Prevention and Research Institute of Texas (CPRIT). Unpaid extension to 8/31/2018.
Tumor suppression through the cGAMP/STING pathway.
The goal of this research is to investigate the mechanism of tumor suppression by cGAMP at molecular and cellular level.
Role: PI.

A-1931-20170325, The Welch Foundation, 6/1/2017 to 5/31/2020.
The structural basis of RNA synthesis by Zika virus.
The goal of this research is to elucidate the molecular mechanism of RNA synthesis catalyzed by Zika virus RNA polymerase and identify inhibitors of the enzyme based on the structural studies.
Role: PI.

1 R21 AI140004, National Institute of Allergy and Infectious Diseases (NIAID), 5/23/2018 to 4/30/2020
The secreted effector SseC is a key regulator of retrograde transport during Salmonella enterica serovar Typhimurium infection
The goal of this research is to determine how the Salmonella virulence factor SseC hijacks host retromer.

PI. Robert Watson, Co-PI, Pingwei Li

Completed Research Support

R01 AI087741, National Institute of Health, NIAID, 4/1/2010 to 3/31/2015
The structural basis of viral RNA sensing by the RIG-I like receptors
The goal of this research is to elucidate the molecular mechanism of viral RNA sensing by RIG-I like receptors.
Role: PI

A-1816, The Welch Foundation, 6/1/2013 to 5/31/2016
The structural basis of microbial DNA sensing in innate immunity
The goal of this research is to elucidate the structural basis of ligand recognition by STING and the mechanism of TBK1 activation.
Role: PI.