OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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NAME: Wang, Lie

POSITION TITLE: Instructor

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*)*	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
East China Univ. of Sci. and Tech.	BS	09/2007	06/2011	Biotechnology
Shanghai Jiao Tong Univ.	MS	09/2011	06/2014	Pharmacy
Baylor College of Medicine	PhD	08/2016	08/2021	Biochemistry and molecular biology

A. Personal Statement

My long-term career goal is to conduct basic research as a principle investigator in an academic laboratory. I am interested in understanding mechanisms of membrane proteins involved in lipid metabolism. I have a strong foundation in enzymology, protein biochemistry and pharmacology, and I have worked extensively on projects that integrates multiple disciplines including molecular biology, biochemistry, biophysics, and chemistry.

My research interests mainly focus on the structure and mechanism of membrane enzymes and transporters. With cryo-electron microscopy, I solved novel structures of multiple membrane enzymes that are involved in lipid metabolism. With the structures and biochemical assays, I identified their substrate binding sites and revealed their reaction mechanism. I am also working on the structures of critical membrane transporters that are responsible for solute transport in plants and humans. With both structure and functional studies, we got a better understanding of their transport mechanism. For my long-term career, I will continue the study of the native structure and complex of lipid metabolism-related membrane proteins, which could be potential drug targets for lipid-accumulation related diseases. In addition, drug discovery and study of structure-activity relationships based on structural biology will be another potential research direction.

1. **Lie Wang**, Anthony Hoang, Eva Gil-Iturbe, Arthur Laganowsky, Matthias Quick, Ming Zhou. Mechanism of anion exchange, small-molecule inhibition, and lipid regulation of pendrin. **Nat Commun**, 2024, 15, 346.

2. **Lie Wang**., Ming Zhou. Structure of a eukaryotic cholinephosphotransferase-1 reveals mechanisms of substrate recognition and catalysis. **Nat Commun** 2023, 14, 2753.

3. **Lie Wang**., Kehan Chen. & Ming Zhou. Structure and function of an Arabidopsis thaliana sulfate transporter. **Nat Commun**, 2021, 12, 4455.

4. **Lie Wang**., Hongwu Qian., Yin Nian., Yimo Han., Zhenning Ren., Hanzhi Zhang., Liya Hu., B. V. Venkataram Prasad., Arthur Laganowsky., Nieng Yan. & Ming Zhou. Structure and mechanism of human diacylglycerol O-acyltransferase 1. **Nature**, 2020, 581: 329–332.

B. Positions and Honors

Positions and Employment

2014 - 2016 Research Assistant, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, China

2021 - 2022 Postdoctoral associate, Baylor College of Medicine, Houston, Texas.

2022 - Instructor, Baylor College of Medicine, Houston, Texas.

Other Experience and Professional Memberships

2017 - Member, Biophysical Society

C. Contribution to Science

1. **Graduate Career:** My graduate research contributions focused on two parts:

1.1 Mechanism in catalytic reaction and substrate recognition of human diacylglycerol-O-acyltransferase 1 (hDGAT1). DGAT1 is an enzyme that catalyzes the reaction between acyl-CoA and diacylglycerol and the production of triacylglycerol. DGAT1 is critical in dietary fat absorption and considered as a critical target for diabetes and fat-metabolism related diseases. Results from my research provided new details into the catalytic reaction and substrate recognition of hDGAT1 and established a framework for further development of hDGAT1 inhibitors. I developed a protocol for the purification of hDGAT1 in large amount for structure determination, and I developed a novel assay for hDGAT1 enzymatic activity analysis. I solved the structure of hDGAT1 in complex with one of its substrates, oleoyl-CoA, and validated the substrate binding site by mutational studies. These results were reported in a recent manuscript.

a. **Wang L.**, Qian H., Nian Y., Han Y., Ren Z., Zhang H., Hu L., Prasad BVV., Laganowsky A., Yan N., Zhou M. Structure and mechanism of human diacylglycerol O-acyltransferase 1. ***Nature***. 2020; 581:329-332.

1.2 Mechanism of iron recognition and transport by mammalian ferroportin. Ferroportin is a critical transporter that responsible for intracellular iron Results from this research project provided novel details about the structure and iron binding sites of ferroportin, and revealed that ferroportin functions as an iron-proton antiporter. I mainly contributed to the structure determination of ferroportin-Fab complex with cryo-EM.

b. Pan Y., Ren Z., Gao S., Shen J., **Wang L.**, Xu Z., Yu Y., Bachina P., Zhang H., Fan X., Laganowsky A., Yan N., Zhou M. Structural basis of ion transport and inhibition in ferroportin. ***Nat Commun***. 2020;11(1):5686.

2. **Postdoctoral research:** My postdoctoral research contributions focused on two parts:

2.1 Structure and mechanism of lipids synthesis enzymes. This project is aimed to reveal the mechanism in catalytic reaction and substrate recognition of phosphatidylcholine synthesis enzyme. Results from my research provided details into the catalytic reaction and substrate recognition of choline phosphotransferase 1 (CHPT1). I overexpressed and purified CHPT1 for structure determination in cryo-EM, and I developed a novel assay for CHPT1 enzymatic activity analysis. I solved the structure of CHPT1 in complex with one of its substrates, CDP-choline and Mg^{2+}, and validated their binding sites by mutational studies. These results were reported in a manuscript.

c. **Lie Wang**., Ming Zhou. Structure of a eukaryotic cholinephosphotransferase-1 reveals mechanisms of substrate recognition and catalysis. **Nat Commun**, 2023, 14, 2753.

2.2 Structure and transport of SLC26 transporters. This project is aimed to reveal the mechanism in substrate recognition and transport of SLC26 transporters, including a plant sulfate transporter and Pendrin. The plant sulfate transporter is responsible for the storage and transport of sulfate ions in plant cells. Pendrin, which is SLC26A4, is responsible for the transport of iodide (I^-) or bicarbonate (HCO_3^-) ions in exchange of chloride ion (Cl^-). We determined the structures of both SLC26 transporters with their substrates in high-resolution. With both structural and functional results, we provided new details into the substrate recognition and transport of SLC26 family transporters and established a framework for further development of small molecule inhibitors.

d. **Lie Wang**, Anthony Hoang, Eva Gil-Iturbe, Arthur Laganowsky, Matthias Quick, Ming Zhou. Mechanism of anion exchange, small-molecule inhibition, and lipid regulation of pendrin. **Nat Commun**, 2024, 15, 346.

e. **Lie Wang**., Kehan Chen. & Ming Zhou. Structure and function of an Arabidopsis thaliana sulfate transporter. **Nat Commun**, 2021, 12, 4455.

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NAME: Zhou, Ming

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

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)*	Completion Date MM/YYYY	FIELD OF STUDY
Fudan University, Shanghai, China	BS	07/1990	Biochemistry
Jiaotong University, Shanghai, China	MS program	01/1996	Chemistry
State University of New York at Buffalo, Buffalo, NY	PhD	07/1999	Biophysics
Rockefeller University, New York, NY	Postdoc Fellow	07/2004	Structural Biology

A. Personal Statement

Research in my lab focuses on membrane-embedded enzymes and ion transporters with the goals of 1) visualizing these proteins in different functional states and in the presence of lipid and protein partners, and 2) elucidating the mechanisms of catalysis or transport in terms of the physical and chemical basis of their functions. Although a project often starts with structure determination of the target protein, our focus is on understanding the mechanisms by rigorously testing structure-based hypotheses, which requires developing precise and quantitative functional assays and integrating multiple approaches that are tailored to each protein. For structure determination, I transitioned from X-ray crystallography to cryo-electron microscopy since 2014 and have become proficient with the new approach.

I am committed to training and mentoring the next generation molecular biophysicists within a safe, supportive, and inclusive environment. I have trained ten PhD students and sixteen postdocs. I have been actively involved in promoting women and underrepresented groups in STEM: seven of the past and current trainees are women and two are from underrepresented groups. To improve my mentoring, I have taken evidence-based National Research Mentor Network training workshops. I uphold the highest standards for ethical, responsible, and rigorous scientific research.

My lab is currently supported by the following grants from the NIH.

R01 DK122784
Zhou and Tsai (MPI)
07/01/2019-05/31/2024 (NCE to 5/31/2025)
Structure and Mechanism of Mammalian Stearoyl-CoA Desaturases

RM1 GM145416
Zhou, Laganowsky, Baker, and Marty (MPI)
09/15/2022-08/31/2027
Understanding the Role of Lipids in Structure and Function of Membrane Proteins

R01 GM151548
Zhou and Quick (MPI)
09/01/23-06/30/27
Structure and mechanism of pendrin and the mutations that cause Pendred's Syndrome

Citations:

1. Cao Y, Pan Y, Huang H, Jin X, Levin EJ, Kloss B, **Zhou M**. Gating of the TrkH ion channel by its associated RCK protein TrkA. Nature. 2013 Apr 18;496(7445):317-22. doi: 10.1038/nature12056. PMID: 23598339; PMCID: PMC3726529.
2. Zhou X, Levin EJ, Pan Y, McCoy JG, Sharma R, Kloss B, Bruni R, Quick M, **Zhou M**. Structural basis of the alternating-access mechanism in a bile acid transporter. Nature. 2014 Jan 23;505(7484):569-73. doi: 10.1038/nature12811. Epub 2013 Dec 8. PMID: 24317697; PMCID: PMC4142352.
3. Bai Y, McCoy JG, Levin EJ, Sobrado P, Rajashankar KR, Fox BG, **Zhou M**. X-ray structure of a mammalian stearoyl-CoA desaturase. Nature. 2015 Aug 13;524(7564):252-6. doi: 10.1038/nature14549. Epub 2015 Jun 22. PMID: 26098370; PMCID: PMC4689147.
4. Wang, L., Qian, H., Nian, Y., Han, Y., Ren, Z., Zhang, H., Hu, L., Prasad, BVV, Laganowsky, A., Yan, N., **Zhou M.** (2020) Structure and mechanism of human diacylglycerol O-acyltransferase 1. **Nature**, Vol 581:329-332. PMC7255049

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2015-Present Professor (with tenure), Ludwig Bolin Endowed Chair in Biochemistry, Department of Biochemistry and Molecular Pharmacology, Baylor College of Medicine, Houston, TX
2012-2015 Associate Professor (with tenure), Ruth McLean Bowman Bower Endowed Chair, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX
2010-2012 Associate Professor (with tenure), Department of Physiology & Cellular Biophysics, Columbia University, New York, NY
2004-2010 Assistant Professor (tenure track), Department of Physiology & Cellular Biophysics, Columbia University, New York, NY
2014-2018 Senior Investigator, Kunming Institute of Zoology, Kunming, China

Committees

2015-2019 Member, NIH BBM study section
2014-2017 Member, Proposal Review Panel at SLAC National Accelerator Laboratory
2010-2014 Member, Swiss National Science Foundation, Danish Council for Independent Research
2010-2012 Associate Professor (with tenure), Department of Physiology & Cellular Biophysics, Columbia University, New York, NY
2006-2009 Member, American Heart Association Cardiac Electrophysiology Peer Review Study Group

Honors

2006 Pew Scholar in Biomedical Science
2006 Alfred P. Sloan Fellow
2006 Basil O'Connor Starter Scholar Award

C. Contributions to Science

My work as an independent investigator has centered on understanding basic mechanisms of membrane proteins. I initially worked on ion channels and transitioned to solute transporters and membrane-embedded enzymes. Here I briefly summarize ongoing and completed projects, starting with ones that are most relevant to the current proposal.

1. Structure and Mechanism of Stearoyl-CoA Desaturase-1

Stearoyl-CoA desaturase-1 (SCD1) is a membrane-embedded enzyme that makes the first double bond to a saturated fatty acid. The reaction is catalyzed by a diiron center and is highly stereo-specific (only cis double bond is generated) and regio-specific (only at the 9th position). SCD1 is implicated in lipid and fat metabolism

and is a validated drug target for treating metabolic diseases such as obesity and diabetes, for reducing harmful aggregation of α-synuclein that leads to synucleinopathathies, and for inhibition of many types of cancers. We first determined the structure of mouse SCD1 in complex with oleoyl-CoA in 2015, and although the structure provides understanding of how the stereo- and regio-specificities are achieved, it revealed a challenge that took us several years to overcome. In the initial structure, the diiron center is not occupied by irons and is replaced by two zinc ions. Consequently, the zinc containing SCD1 has no enzymatic activity. In addition, a full enzymatic cycle of SCD1 requires participation of two additional membrane proteins, cytochrome b_5 and cytochrome b_5 reductase, and it is not known how they interact to form an electron transfer chain. We worked out a procedure to produce iron loaded and functional SCD1 and solved structure of SCD1 with a true diiron center. We have also assembled all three proteins into a stable ternary complex and demonstrated that the ternary complex is capable of sustained enzymatic activity and that the rate of electron transfer is significantly faster than simple mixture of individual proteins. The ternary complex is suitable for further structural and functional studies.

a. Bai Y, McCoy JG, Levin EJ, Sobrado P, Rajashankar KR, Fox BG, **Zhou M**. X-ray structure of a mammalian stearoyl-CoA desaturase. Nature. 2015 Aug 13;524(7564):252-6. doi: 10.1038/nature14549. Epub 2015 Jun 22. PMID: 26098370; PMCID: PMC4689147.
b. Shen J, Wu G, Tsai AL, **Zhou M**. Structure and Mechanism of a Unique Diiron Center in Mammalian Stearoyl-CoA Desaturase. J Mol Biol. 2020 Aug 21;432(18):5152-5161. doi: 10.1016/j.jmb.2020.05.017. Epub 2020 May 27. PMID: 32470559; PMCID: PMC7483794.
c. Shen J, Wu G, Tsai AL, **Zhou M**. Transmembrane helices mediate the formation of a stable ternary complex of b_5R, cyt b_5, and SCD1. Commun Biol. 2022 Sep 12;5(1):956. doi: 10.1038/s42003-022-03882-z. PMID: 36097052; PMCID: PMC9468158.
d. Shen J, Wu G, Pierce BS, Tsai AL, **Zhou M**. Free ferrous ions sustain activity of mammalian stearoyl-CoA desaturase-1. J Biol Chem. 2023 Jun 6:104897. doi: 10.1016/j.jbc.2023.104897. Epub ahead of print. PMID: 37290533.

2. Membrane-embedded acyltransferases and phosphotransferases

Synthesis of lipids, such as triglycerides, phospholipids, ceramides and sphingolipids, requires coordinated contributions of multiple membrane-embedded enzymes, many of which have not been well-characterized in terms of substrate recognition, mechanism of catalysis, inhibition by small molecules, and regulation by lipids. It is also not known whether enzymes that are required for the synthesis of a lipid form a stable complex to facilitate the multi-step synthesis. I initiated a research direction aimed at understanding of lipid synthesis at the molecular and atomic levels and we have so far determined the structures of human diacylglycerol acyltransferase-1 (DGAT1, 2020), choline/ethanolamine phosphotransferase (CEPT1, 2023), and phosphatidylserine synthase (PTS, in preparation). For each enzyme, we have also developed functional and biochemical assays to query the activity of the enzyme and the binding of substrates and inhibitors. These efforts have enhanced our competence in working with membrane-embedded enzymes, and established a solid foundation for further explorations.

a. Huang H, Levin EJ, Liu S, Bai Y, Lockless SW, **Zhou M.** Structure of a membrane-embedded prenyltransferase homologous to UBIAD1. PLoS Biol. 2014 Jul;12(7). Doi: 10.1371/journal.pbio.1001911. PMID: 25051182; PMCID: PMC4106721
b. Wang L, Qian H, Nian Y, Han Y, Ren Z, Zhang H, Hu L, Prasad BVV, Laganowsky A, Yan N, **Zhou M.** Structure and mechanism of human diacylglycerol O-acyltransferase 1. Nature. 2020 May;581(7808):329-332. doi: 10.1038/s41586-020-2280-2. PMID: 32433610; PMCID: PMC7255049.
c. Wang L, **Zhou M.** Structure of a eukaryotic cholinephosphotransferase-1 reveals mechanisms of substrate recognition and catalysis. Nat Commun. 2023 May 13;14(1):2753. doi: 10.1038/s41467-023-38003-9. PMID: 37179328; PMCID: PMC10182977.

3. Structure and Mechanism of PEP Group Translocation

The phosphoenolpyruvate-dependent phosphotransferase system (PTS or PEP group translocation) is a multi-protein complex found in bacteria. PTS transport sugars across cell membrane and phosphorylate the sugar before releasing it into the cytosol. Phosphorylation of the incoming sugar keeps the internal concentration of the sugar at zero to maintain a favorable concentration gradient and the phosphorylated sugar can directly enter the metabolic cycle for energy production. PTS has five components (proteins), HPr,

E1, EIIA, EIIB, and EIIC, that relays a phosphate group originates from the phosphoenolpyruvate, and the membrane-embedded EIIC component is responsible for sugar translocation through cell membrane and phosphorylation of sugar. We determined crystal structures of EIICs from two different bacterial species, and managed to visualize both the inward- and outward-facing conformations of one of the EIICs. We also monitored structural changes with single-molecule fluorescence resonance energy transfer (smFRET) and applied molecular dynamics simulations to validate the structures and to elucidate the dynamics of the EIIC proteins. These results established a mechanism of sugar transport across the cell membrane. We are in the process of solving the structure of an EIIB-EIIC complex to understand the mechanism of sugar phosphorylation.

a. Cao Y, Jin X, Levin EJ, Huang H, Zong Y, Quick M, Weng J, Pan Y, Love J, Punta M, Rost B, Hendrickson WA, Javitch JA, Rajashankar KR, **Zhou M**. Crystal structure of a phosphorylation-coupled saccharide transporter. Nature. 2011 May 5;473(7345):50-4. doi: 10.1038/nature09939. Epub 2011 Apr 6. PMID: 21471968; PMCID: PMC3201810.
b. McCoy JG, Ren Z, Stanevich V, Lee J, Mitra S, Levin EJ, Poget S, Quick M, Im W, **Zhou M**. The Structure of a Sugar Transporter of the Glucose EIIC Superfamily Provides Insight into the Elevator Mechanism of Membrane Transport. Structure. 2016 Jun 7;24(6):956-64. doi: 10.1016/j.str.2016.04.003. Epub 2016 May 5. PMID: 27161976; PMCID: PMC4899283.
c. Lee J, Ren Z, **Zhou M**, Im W. Molecular Simulation and Biochemical Studies Support an Elevator-type Transport Mechanism in EIIC. Biophys J. 2017 Jun 6;112(11):2249-2252. doi: 10.1016/j.bpj.2017.04.040. Epub 2017 May 13. PMID: 28506526; PMCID: PMC5474738.
d. Ren Z, Lee J, Moosa MM, Nian Y, Hu L, Xu Z, McCoy JG, Ferreon ACM, Im W, **Zhou M**. Structure of an EIIC sugar transporter trapped in an inward-facing conformation. Proc Natl Acad Sci U S A. 2018 Jun 5;115(23):5962-5967. doi: 10.1073/pnas.1800647115. Epub 2018 May 21. PMID: 29784777; PMCID: PMC6003338.

4. <u>Structure and Mechanism of an ATP-Gated Ion Channel in Bacteria</u>

TrkH is a membrane protein found in many bacteria. TrkH is composed of four homologous domains in a single polypeptide and each domain resembles a simple K^+ channel. However, the K^+ channel selectivity filter sequence is only partially conserved in TrkH. TrkH is a member of a large superfamily of K^+ transporters (SKT), all of which share a common structural architecture. TrkH assembles with a cytosolic protein TrkA, which is homologous to the cytosolic domain of the high conductance calcium- and voltage-dependent K^+ channels found in higher organisms. It was not known whether TrkH remains selective to K^+, or how TrkA regulates TrkH activity. We expressed and purified TrkH in complex with TrkA and reconstituted the complex into proteoliposomes for patch clamp studies. We recorded single-channel current of TrkH and showed that TrkH is an ion channel that allows permeation of monovalent cations such as Na^+ and K^+ with only a slight preference for K^+. We also showed that ATP binds to TrkA and increases TrkH open probability while ADP inhibits channel opening. Further structural and functional studies show that binding of ATP or ADP induces TrkA into different conformations that in turn open or close TrkH. These results indicate that TrkH is unlikely a K^+ uptake system as previously inferred from cell-based assays, and suggest that TrkH could sense metabolic state of a cell and respond with change of membrane potential. We are now investigating the function of TrkH-TrkA complex in cells.

a. Cao Y, Jin X, Huang H, Derebe MG, Levin EJ, Kabaleeswaran V, Pan Y, Punta M, Love J, Weng J, Quick M, Ye S, Kloss B, Bruni R, Martinez-Hackert E, Hendrickson WA, Rost B, Javitch JA, Rajashankar KR, Jiang Y, **Zhou M**. Crystal structure of a potassium ion transporter, TrkH. Nature. 2011 Mar 17;471(7338):336-40. doi: 10.1038/nature09731. Epub 2011 Feb 13. PMID: 21317882; PMCID: PMC3077569.
b. Cao Y, Pan Y, Huang H, Jin X, Levin EJ, Kloss B, **Zhou M**. Gating of the TrkH ion channel by its associated RCK protein TrkA. Nature. 2013 Apr 18;496(7445):317-22. doi: 10.1038/nature12056. PMID: 23598339; PMCID: PMC3726529.
c. Zhang H, Pan Y, Hu L, Hudson MA, Hofstetter KS, Xu Z, Rong M, Wang Z, Prasad BVV, Lockless SW, Chiu W, **Zhou M**. TrkA undergoes a tetramer-to-dimer conversion to open TrkH which enables changes in membrane potential. Nat Commun. 2020 Jan 28;11(1):547. doi: 10.1038/s41467-019-14240-9. PMID: 31992706; PMCID: PMC6987127.

5. <u>Mechanism of SLC family of solute transporters</u>

We have interrogated solute transporters from the SLC superfamily, with the goal of visualizing their structures and understanding their mechanisms of solute recognition, transport and inhibition. We have worked extensively on urea transporter (SLC14A1, PMC2871279 and PMC3396522) and iron exporter (SLC40A1, PMC7655804, PMC10404463 and PMC9883014). We have determined structures of a mammalian peptide transporter (SLC15A2, PMC10404463), a mammalian pendrin which is an iodide and bicarbonate transporter (SLC26A4, revision), a plant homolog of sulfate transporter (SLC26A1), and a bacterial homolog of ascorbic acid transporter (SLC23A1, PMC10438392). Our study provides insight into the structural basis of solute permeation and selectivity.

a. Levin EJ, Quick M, **Zhou M**. Crystal structure of a bacterial homologue of the kidney urea transporter. Nature. 2009 Dec 10;462(7274):757-61. Doi: 10.1038/nature08558. PMID: 19865084; PMCID: PMC2871279.
b. Pan Y, Ren Z, Gao S, Shen J, Wang L, Xu Z, Yu Y, Bachina P, Zhang H, Fan X, Laganowsky A, Yan N, **Zhou M.** Structural basis of ion transport and inhibition in ferroportin. Nat Commun. 2020 Nov 10;11(1):5686. doi: 10.1038/s41467-020-19458-6. PMID: 33173040; PMCID: PMC7655804.
c. Shen J, Wilbon AS, **Zhou M**, Pan Y. Mechanism of Ca(2+) transport by ferroportin. Elife. 2023 Jan 17;12. doi: 10.7554/eLife.82947. PMID: 36648329; PMCID: PMC9883014.
d. Weng J, Zhou X, Wiriyasermkul P, Ren Z, Chen K, Gil-Iturbe E, **Zhou M,** Quick M. Insight into the mechanism of H^+-coupled nucleobase transport. Proc Natl Acad Sci U S A. 2023 Aug 15;120(33):e2302799120. doi: 10.1073/pnas.2302799120. PMID: 37549264; PMCID: PMC10438392.

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