BIOGRAPHICAL SKETCH

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NAME: Luke H. Chao

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

POSITION TITLE: Assistant 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
Brandeis University	B.S./M.S.	05/2003	Biochemistry
University of California, Berkeley	Ph.D.	06/2010	Molecular and Cell Biology
Harvard Medical School	Postdoc	08/2016	Biophysics

A. Personal Statement

Our group is fascinated by how proteins collectively shape the form and function of cellular membranes – a complex molecular symphony mis-regulated in aging, cancer and neurodegeneration. Our central focus is to understand the mitochondrion's remarkable pleiomorphic morphology. As an independent investigator, our research program reconstitutes key membrane rearrangement events [**1-3**] and visualizes dynamic membrane and protein complexes [**4**], to describe fundamental mechanisms for cell ultrastructure rearrangement [**5**].

My record in structural biology and molecular biophysics demonstrates creative use of multiple systems to develop deep insight into fundamental principles of membrane rearrangement by dynamic macromolecular assemblies. My group recently *in vitro* reconstituted mitochondrial inner-membrane fusion, revealing a mechanism for gating the final pore-opening step [**1,2**]. Our team has characterized conformational states of signaling complexes by single particle electron cryo-microscopy (cryo-EM) [**4**] and revealed mechanisms of membrane rearrangement by electron cryo-tomography (cryo-ET) [**5**].

Our inclusive international team of scientists actively develops community outreach programs (see Contributions to Science 5.)

Selected publications (5 out of 18):

1. Ge Y, Shi X, Boopathy S, McDonald J, Smith AW, **Chao LH**. Two forms of Opa1 cooperate to complete fusion of the mitochondrial inner-membrane. *eLife* 2020; 9:e50973 10.7554/eLife.50973 PMID: 31922487, PMC7299343.
2. Ge Y, Boopathy S, Smith AW, **Chao LH**. A model membrane platform for reconstituting mitochondrial membrane dynamics. *J. Vis. Exp.* 2020 (163), e61620, doi:10.3791/61620 PMID: 32955498. PMCID in process.
3. Ge Y, Boopathy S, Nguyen TH, Lugo CM, **Chao LH.** Absence of cardiolipin from the mitochondrial inner membrane outer leaflet restricts Opa1-mediated fusion. *Front. Mol. Biosci.* 2021 (8) 1309, doi: 10.3389/fmolb.2021.769135, PMID: 35004847, PMCID PMC8728091.
4. Sloutsky R*, Dziedzic N*, Dunn MJ, Bates RM, Torres-Ocampo AP, Boopathy S, Page B, Weeks JG, **Chao LH**^, Stratton MM^. Heterogeneity in human hippocampal CaMKII transcripts reveals an allosteric role for the hub domain in activity regulation. *Science Signaling 2020* Jul 21;13(641): eaaz0240. 10.1126/scisignal.aaz0240 PMID: 32694170 PMCID PMC7654443 *denotes equal contribution, ^co-corresponding authors.

a. 5. Navarro PP[#], Vettiger A[#], Ananda VY, Llopis PM, Allolio C, Bernhardt TG*, **Chao LH***. Cell wall synthesis and remodeling dynamics determine bacterial division site architecture and cell shape. *Nat Microbiol.* 2022 Sep 12;. doi: 10.1038/s41564-022-01210-z. PMID: 36097171. [#]equal contribution, *co-corresponding

B. Positions, Scientific Appointments and Honors

Positions and Employment

2016-present	Assistant Professor	Dept. of Molecular Biology, Massachusetts General Hospital Dept. of Genetics, Harvard Medical School
2011-2016	Postdoctoral Fellow	Dept. of Biol. Chem. & Mol. Pharm., Harvard Medical School

Honors

2020	Eukaryogenesis Project Award	Moore Foundation & Simons Foundation
2018	Child Health Research Award	Charles H. Hood Foundation
2015-2016	Fellow	Charles A. King Trust
2012-2015	Frederic M. Richards Fellow	Jane Coffin Childs Memorial Fund for Medical Research
2011	Fellow	Society for General Physiology
2011	Milton L. Shifman Scholarship	Marine Biology Laboratory
2005, 2009	Departmental Travel Grants	University of California, Berkeley
2003	William P. Jencks Prize	Brandeis University

C. Contributions to Science

1. Organelle membrane dynamics and ultrastructure: In my independent group, we study membrane-protein assemblies involved in membrane dynamics and ultrastructure. A central focus is mitochondrial morphology. Mitochondrial membranes have an intricate, highly specialized structure dependent on dynamic protein assemblies. The regulation of these proteins integrates organelle and cell state and is frequently mis-wired in disease. Recently, we uncovered a regulatory mechanism where membrane potential-dependent proteolytic processing by mitochondrial inner-membrane proteases gates efficient and fast pore opening [**a**]. This advance equips us to investigate membrane dynamics in a variety of contexts. We explore protein assembly using supported lipid bilayer and proteoliposomes that allow control of lipid composition, bilayer asymmetry and environmental conditions [**b, c**]. We analyze *in situ* membrane structures and protein arrangements using electron cryo-tomography (cryo-ET) [**d, e**].

b. Ge Y, Shi X, Boopathy S, McDonald J, Smith AW, **Chao LH**. Two forms of Opa1 cooperate to complete fusion of the mitochondrial inner-membrane. *eLife* 2020; 9:e50973 10.7554/eLife.50973 PMID 31922487, PMC7299343.

c. Ge Y, Boopathy S, Smith AW, **Chao LH**. A model membrane platform for reconstituting mitochondrial membrane dynamics. *J. Vis. Exp.* 2020 (163), e61620, doi:10.3791/61620 PMID: 32955498 PMCID in process.

d. Ge Y, Boopathy S, Nguyen TH, Lugo CM, **Chao LH.** Absence of cardiolipin from the mitochondrial inner membrane outer leaflet restricts Opa1-mediated fusion. *Front. Mol. Biosci.* 2021 (8) 1309, doi: 10.3389/fmolb.2021.769135, PMID: 35004847, PMCID PMC8728091.

e. Allouche J, Rachmin I, Adhikari K, Pardo LM, Lee JH, McConnell AM, Kato S, Fan S, Kawakami A, Suita Y, Wakamatsu K, Igras V, Zhang J, Navarro PP, Lugo CM, Noonan HR, Christie KA, Itin K, Mujahid N, Lo JA, Won CH, Evans CL, Weng QY, Wang H, Osseiran A, Lovas A, Németh I, Cozzio A, Navarini AA, Hsiao J, Nguyen N, Kemeny J, Iliopoulos O, Berking C, Ruzicka T, Gonzalez-José R, Bortolini M-C, Canizales-Quinteros S, Acuna-Alonso V, Gallo C, Poletti G, Bedoya G, Rothhammer F, Ito S, Schiaffino MV, **Chao LH**, Kleinstiver BP, Tishkoff S, Zon LI, Nijsten T, Ruiz-Linares A, Fisher DE, Roider E. NNT-mediated redox changes enable UV- and MITF-independent skin pigmentation. *Cell* 2021; Jun 29;S0092-8674(21)00757-1, PMID: 34233163 PMCID: PMC8349839.

f. Navarro PP[#], Vettiger A[#], Ananda VY, Llopis PM, Allolio C, Bernhardt TG*, **Chao LH***. Cell wall synthesis and remodeling dynamics determine bacterial division site architecture and cell shape. *Nat Microbiol.* 2022 Sep 12;. doi: 10.1038/s41564-022-01210-z. PMID: 36097171. [#]equal contribution, *co-corresponding

2. Viral membrane fusion: Flaviviruses (a family which includes, Dengue, West Nile and Zika) are a major international public health concern. The membrane fusogens of enveloped viruses (including those on influenza and coronaviruses), undergo a conserved series of conformational rearrangements to mediate membrane fusion. The structure and lifetime of these transition intermediates remain unknown, yet their understanding is essential

for new therapeutics. As a Postdoctoral Fellow, I built single particle assays to determine kinetic bottlenecks in West Nile and Dengue virus envelope membrane fusion [**a**]. I determined several cryo-EM reconstructions of viral membrane fusion proteins. These approaches relate the structure of conformational intermediates, with steps of membrane fusion such as tethering, hemifusion (lipid-mixing) and pore opening. I also performed computational simulations to understand the behavior of an array of membrane proteins at the virus/cell membrane contact site, and measured the stoichiometry of inhibition by small molecules [**b**].

a. **Chao LH**, Klein DE, Schmidt AG, Peña JM, and Harrison SC. Sequential conformational change in flavivirus membrane fusion. *eLife* 2014; 10.7554. PMID: 25479384, PMC4293572.
b. **Chao LH**, Jang J, Johnson A, Nguyen A, Gray NS, Yang PL, Harrison SC. How small-molecule inhibitors of dengue-virus infection interfere with viral membrane fusion. *eLife* 2018; 7; PMID: 29999491, PMC6056230.

3. Signaling assembly structure and function: The calcium/calmodulin dependent protein kinase II (CaMKII) holoenzyme has a unique dodecameric architecture, with flexibly tethered catalytic domains surrounding a central hub. CaMKII's structure is important for its calcium signaling properties in neurons and cardiac cells. As a graduate student, I determined the first crystal structure of a human CaMKII holoenzyme [**a**]. These studies revealed new mechanisms of allosteric activation and inhibition in a dynamic kinase assembly [**b**]. I performed small-angle X-ray scattering experiments probing isoform-dependent changes in conformational state. In addition, I solved crystal structures of activated CaMKII intermediates and structures of a feed-back inhibited form of the kinase. More recently, I analyzed this dynamic structure using single-particle cryo-EM, to understand how to capture large conformationally dynamic assemblies [**c**]. Our current interests extend to assemblies at membranes [**d**]. I have been invited to review structural studies (X-ray crystallography and cryo-EM) of such assemblies.

a. **Chao LH**, Stratton MM, Lee I-Y, Rosenberg OS, Levitz J, Mandell DJ, Kortemme T, Schulman H, Groves JT, Kuriyan J. A mechanism for tunable autoinhibition in the structure of a human calcium/calmodulin-dependent kinase II holoenzyme. *Cell* 2011 5:732-45. PMID: 21884935, PMC3184253.
b. **Chao LH***, Pellicena P*, Deindl S, Barclay LA, Schulman H, Kuriyan J. Inter-subunit capture of regulatory segments is a component of cooperative CaMKII activation. *Nature Structural and Molecular Biology* 2010 3: 264-72. PMID: 20139983, PMC2855215 *denotes equal contribution.
c. Sloutsky R*, Dziedzic N*, Dunn MJ, Bates RM, Torres-Ocampo AP, Boopathy S, Page B, Weeks JG, **Chao LH**^, Stratton MM^. Heterogeneity in human hippocampal CaMKII transcripts reveals an allosteric role for the hub domain in activity regulation. *Science Signaling 2020* Jul 21;13(641): eaaz0240. 10.1126/scisignal.aaz0240 PMID: 32694170 PMCID PMC7654443. *denotes equal contribution, ^co-corresponding authors.
d. Shi X, Lingerak L, Herting CJ, Ge Y, Kim S, Toth P, Cuizon C, Zheng J, **Chao LH**, Sossey-Alaoui K, Buck M, Singh S, Varadan V, Himanen J, Hambardzumyan D, Nikolov D, Smith AW, Wang B. Cell Surface Multimeric Assemblies Regulate Canonical and Noncanonical EphA2 Receptor Tyrosine Kinase Signaling. *bioRxiv* 2021.04.11.439330.

4. Creative approaches and new tools: To understand unique response properties of macromolecular assemblies, we have an interest in applying new microfluidic and fluorescence-based tools. For example, CaMKII has an intriguing ability to retain a 'molecular memory' of past activation events. This was postulated by Francis Crick as a protein-based means to retain a record of past stimuli. I performed the first FRET-based experiments testing the role of subunit-exchange in stably propagating calcium signals beyond protein turn-over [**a**], and built custom devices to understand CaMKII's calcium frequency response, describing mechanisms for cooperative CaMKII activation and how splice isoforms tune this behavior. Conformation-selective probes are a powerful means to allosterically probe function in large macromolecular assemblies. I performed single-channel recordings with toxin-based protein allosteric modulators to understand potassium channel regulation and contributed to mathematical modeling of reaction schemes [**b**]. Similarly, I performed biochemical characterization of new small molecule viral inhibitors [**c**], and solved crystal structures of patient-derived mutant kinases bound to conformation-specific inhibitors to understand principles of small molecule selectivity [**d**]. My group continues this spirit of creative methods development, currently focused on cryo-EM sample preparation, incorporating monolayers and bilayers on cryo-EM grids, and investigating new cryo-focused ion beam preparation approaches of cellular samples for cryo-ET [**e**].

a. Stratton MM*, Lee I-Y*, Bhattacharyya M, Christensen SM, **Chao LH**, Schulman H, Groves JT, Kuriyan J. Activation-triggered subunit exchange between CaMKII holoenzymes facilitates the spread of kinase activity. *eLife* 2014 3:e01610 arXiv:1312.5376 *denotes equal contribution. PMID: 24473075, PMC3901001.

b. Tilley DC, Angueyra JM, Eeum KS, Kim H, **Chao LH**, Peng AW, Sack JT. The tarantula toxin GxTx detains K^+ channel gating charges in their resting conformation. *Journal of General Physiology* 2019. PMID: 30397012 PMC6400525.

c. Chou Y, Cuevas C, Carocci M, Stubbs S, Ma M, Cureton D, **Chao LH,** Evesson F, He K, Yang P, Whelan S, Ross S, Kirchhausen T, and Gaudin R. Identification and characterization of a novel broad spectrum virus entry inhibitor. *J. Virology* 2016; 90(9):4494-510. PMID: 26912630, PMC4836360.

d. Young MA, Shah NP, **Chao LH**, Seeliger M, Milanov ZV, Biggs II WH, Treiber TK, Patel HK, Zarrinkar PP, Lockhart DJ, Sawyers CL, Kuriyan J. Structure of the kinase domain of an imatinib-resistant Abl mutant in complex with the Aurora kinase inhibitor VX-680. *Cancer Research* 2006 66:1007-14. PMID: 16424036.

e. Navarro PP[#], Vettiger A[#], Ananda VY, Llopis PM, Allolio C, Bernhardt TG*, **Chao LH***. Cell wall synthesis and remodeling dynamics determine bacterial division site architecture and cell shape. *Nat Microbiol.* 2022 Sep 12;. doi: 10.1038/s41564-022-01210-z. PMID: 36097171. [#]equal contribution, *co-corresponding

5. Leadership in mentoring students from diverse backgrounds: In addition to mentoring postdoctoral fellows, I mentored two students from under-represented groups through the HHMI Exceptional Research Opportunities Program (EXROP) (both of whom have gone onto graduate studies) and supervised 5 graduate students (all of whom successfully completed their Ph.D. degrees). I also trained four undergraduates at public and private institutions (two of which published their thesis work and went on to successfully earn Ph.D.s) [**a, b**]. I am actively involved in community outreach programs through Mass. General Hospital, focusing on students from under-represented backgrounds. I launched a new program in 2020 for students at local high schools focusing on virus structure/function, membrane biophysics and electron microscopy. This program extends outreach opportunities from an EM consortium at (Harvard Medical School and MIT), where I serve on the executive and steering committees.

a. Chao LH*, Pellicena P*, Deindl S, **Barclay LA**, Schulman H, Kuriyan J. Inter-subunit capture of regulatory segments is a component of cooperative CaMKII activation. *Nature Structural and Molecular Biology* 2010 3: 264-72. PMID: 20139983, PMC2855215 *denotes equal contribution.

b. Chao LH, Klein DE, Schmidt AG, **Peña JM**, and Harrison SC. Sequential Conformational Change in Flavivirus Membrane Fusion. *eLife* 2014; 10.7554. PMID: 25479384, PMC4293572.

A full list of published work can be found at this NCBI My Bibliography link:
https://www.ncbi.nlm.nih.gov/myncbi/luke.chao.1/bibliography/public/

D. Current Support

Moore Foundation & Simons Foundation (PI Chao) 09/01/20 - 08/31/23
Award # 9736 $51,126 DC/year
Project on the Origin of the Eukaryotic Cell
The major goals of this project are ancestral sequence reconstruction of the cristae junction protein Mic60 to understand intermediates in eukaryogenesis.

R35GM142553 09/15/21 - 06/30/26
NIH/NIGMS $250,000 DC/year
Probing structural and biophysical mechanisms of mitochondrial membrane ultrastructure
The goal of this project is to establish a research platform exploring how protein conformational change is influenced by subcellular context, how membrane ultrastructure is regulated by protein factors, and the functional interplay of these elements in physiology and disease. This project explores these concepts through investigating the protein structures that regulate mitochondrial membranes.

BIOGRAPHICAL SKETCH

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NAME: Boopathy, Sivakumar

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

POSITION TITLE: Research Fellow

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
Birla Institute of Technology and Science, RJ India	B.E.	08/2009	Electronics and Instrumentation Engineering
Birla Institute of Technology and Science, RJ India	M.Sc.	08/2009	Biological Sciences
University of Massachusetts Medical School, MA USA	Ph.D.	12/2017	Biochemistry and Molecular Pharmacology
Massachusetts General Hospital, MA USA	Research Fellow	present	Molecular Biology

A. Personal Statement

I am aspiring structural biologist. My long-term research goal involves the inter-relationship between protein structure and function and its implication in health and disease. As an undergraduate, I majored in both biology and engineering which puts me in a strong position to adopt multidisciplinary approach to problem solving. During my undergraduate studies, I worked under Dr. Nilashis Nandi, studying protein aggregation in neurodegeneration. During my internship at National Metallurgical Laboratory, I worked in a team and developed a software for calculating uncertainties in thermocouple calibrations. As a project trainee at Honeywell Technology Solutions Lab, I was part of the team that implemented Electronic Device Description Language (EDDL) technology for the Honeywell's distributed control system, Experion PKS. The above two positions helped me develop programming skills. As a doctoral student in the laboratory of Dr. Daryl Bosco at UMMS, I investigated a novel protein mutated in amyotrophic lateral sclerosis. I devised methods for the expression and purification of several challenging proteins. Further, I designed assays to probe the protein function *in vitro* and *in cellulo*. Here I acquired key skills in protein biochemistry, cell biology, fixed and live cell microscopy. I wrote custom scripts for processing single molecule fluorescence and live cell imaging data. In line with my research interests, I am learning cryo-electron microscopy as a research fellow to study membrane remodeling machineries. I learnt the intricacies of membrane protein expression and purification and learnt to optimize samples for cryo-electron microscopy. Throughout my graduate and post-doc career, I had the opportunity to mentor students and technicians. My excellent background in molecular biology, protein biochemistry, cell biology and computer programming put me in a competitive position to pursue challenging biological questions in the future. With my current research experience, I am confident of seeking an independent academic career in the next 2 years.

B. Positions and Honors

Positions and Employment

2018 -	Research Fellow, Massachusetts General Hospital, Boston, MA

Other Experience and Professional Memberships

2008 - 2009	Project Trainee, Honeywell Technology Solutions Lab, Bengaluru, India
2006 - 2007	Representative, BITS Biological Society, Birla Institute of Technology and Science
2006	Intern, National Metallurgical Laboratory, Chennai, India

Honors

2005	First Prize for "Economic Baby Food", Ideas for Rural India, Center for Entrepreneurial Leadership, Birla Institute of Technology and Science
2005	Institute merit-cum-need scholarship, Birla Institute of Technology and Science

C. Contributions to Science

1. Ge Y, Boopathy S, Nguyen TH, Lugo CM, Chao LH. Absence of Cardiolipin From the Outer Leaflet of a Mitochondrial Inner Membrane Mimic Restricts Opa1-Mediated Fusion. Front Mol Biosci. 2021; 8:769135.

2. Schmidt EJ, Funes S, McKeon JE, Morgan BR, Boopathy S, O'Connor LC, Bilsel O, Massi F, Jégou A, Bosco DA. ALS-linked PFN1 variants exhibit loss and gain of functions in the context of formin-induced actin polymerization. Proc Natl Acad Sci U S A. 2021 06 08; 118(23).

3. Ge Y, Boopathy S, Smith A, Chao LH. A Model Membrane Platform for Reconstituting Mitochondrial Membrane Dynamics. J Vis Exp. 2020 09 02; (163).

Compartmentalization of biochemical reactions in membranous organelles is a defining feature of eukaryotic cells. The membranes with its specific lipid composition often facilitate macromolecular complex assembly, protein function, spatial organization of biochemical reactions. This work details the procedure used for fabricating lipid bilayers and reconstituting proteins in them. As an example, the mitochondrial inner membrane fusogen OPA1 was reconstituted in the lipid bilayers mimicking mitochondrial inner membrane for studying different stages of membrane fusion. This procedure was used in the experiments described in (5). The method used here describes general considerations for reconstitution of membrane proteins and can be applied to other membrane systems. My role in this study was to express and purify the OPA1 proteins using the *Pichia pastoris* expression system.

4. Sloutsky R, Dziedzic N, Dunn MJ, Bates RM, Torres-Ocampo AP, Boopathy S, Page B, Weeks JG, Chao LH, Stratton MM. Heterogeneity in human hippocampal CaMKII transcripts reveals allosteric hub-dependent regulation. Sci Signal. 2020 07 21; 13(641).

5. Ge Y, Shi X, Boopathy S, McDonald J, Smith AW, Chao LH. Two forms of Opa1 cooperate to complete fusion of the mitochondrial inner-membrane. Elife. 2020 01 10; 9.

The protein OPA1 is necessary for mitochondrial inner membrane fusion, maintenance of mitochondrial network and proper respiratory function. OPA1 is commonly mutated in dominant optic atrophy, a devastating pediatric disease leading to blindness. OPA1 exists in a transmembrane anchored l-form and a soluble s-form, but it is unclear why two forms of OPA1 are present and their role in mediating fusion. Using a TIRF-based supported bilayer/liposome assay, a comprehensive study was conducted to investigate the different steps in membrane fusion – tethering, membrane docking, lipid mixing and content release. At each stage of the fusion, the requirements of the two forms of OPA1 and the nucleotide GTP were evaluated. Our study showed how the ratio of l-OPA1 to s-OPA1 regulates fusion – the fusion being maximal at equimolar l- and s-OPA1 and little fusion activity at excess s-OPA1. This study was able to resolve some issues in the field pertaining to the requirement of l-OPA1 vs. s-OPA1 for fusion. For this work, I designed a *Pichia pastoris* expression system for the expression

of OPA1 proteins. I developed a method for purifying the proteins in a functional form. This was essential for all the experiments using the reconstituted model membrane systems. I wrote some sections in the manuscript and critically read the manuscript.

6. Giampetruzzi A, Danielson EW, Gumina V, Jeon M, Boopathy S, Brown RH, Ratti A, Landers JE, Fallini C. Modulation of actin polymerization affects nucleocytoplasmic transport in multiple forms of amyotrophic lateral sclerosis. Nat Commun. 2019 08 23; 10(1):3827.

The process of nucleocytoplasmic transport (NCT) is often disrupted in ALS. This study investigated the role of actin cytoskeletal homoeostasis on NCT. The study revealed that mutant profilin 1 compromise nuclear membrane integrity leading to NCT impairment. This in turn causes mis-localization of ALS-linked RNA-binding proteins, TDP43 and FUS. Importantly, pharmacological disruption of actin polymerization impacted NCT and stimulating actin polymerization using the actin nucleator mDia1 rescued this phenotype. This study was instrumental in describing a link between NCT defects, RNA dysregulation and actin cytoskeletal defects in ALS. My contribution in this study was the design and construction of mDia1 plasmids used in this study, as well as critical reading of the manuscript.

7. Boopathy S, Silvas T, Tischbein M, Jansen S, Shandilya S, Zitzewitz J, Landers J, Goode B, Schiffer C, Bosco D. Structural basis for mutation-induced destabilization of profilin 1 in ALS. Proc Natl Acad Sci U S A. 2015 Jun 30; 112(26):7984-9.

ALS is a debilitating motor neuron disease. The adult-onset disease paralyzes muscles ultimately leading to death. There are many associated genes affecting different cellular pathways such as protein homeostasis, RNA metabolism. This study examined the role of a cytoskeletal protein called profilin 1 in disease pathogenesis. Using various assays, it was shown that profilin 1 mutations severely destabilize the protein leading to rapid turnover in cells. While the overall structure of the protein is preserved in mutants, crystal structures revealed the presence of empty pockets or voids in the interior of the protein explaining a reason for thermodynamic destabilization. Further, the mutations did not overtly affect binding to actin or poly-proline motifs to which profilin normally binds. This study was among the first that described the pathological mechanism of a newly discovered ALS gene at that time, offering insights into the role of actin cytoskeleton in ALS. I was involved in the design of the study and experiments. I expressed and purified WT and mutants of profilin 1, performed biochemical and biophysical experiments with the purified proteins. I collaborated on the X-ray crystallography of the proteins. Along with my advisor, I analyzed, interpreted data, and wrote the manuscript with inputs from co-authors.

Link to Publications
https://pubmed.ncbi.nlm.nih.gov/?term=Sivakumar+Boopathy%5BAuthor%5D&sort=

D. Scholastic Performance

Selected coursework at BITS: Mathematics I (Calculus), Mathematics II (Linear Algebra and Complex Numbers, Mathematics III (Differential Equations), Probability and Statistics, Physics I and II, Chemistry I and II, Microbiology, Biological Chemistry, Biology Project Lab, General Physiology, Cell Biology, Biophysics, Recombinant DNA Technology, Developmental Biology, Ecology, Genetics

Coursework at UMMS: Biochemistry, Molecular Biology and Genetics, Cell Biology, Structural Biology, Molecular Biophysics, Introduction to Neuroscience, Responsible Conduct of Research, Scientific Writing

Self-study: Getting Started in Cryo-EM, Optics