BIOGRAPHICAL SKETCH

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NAME: ERUMBI, Rangarajan S.

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

POSITION TITLE: Staff Scientist

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
University of Madras, Chennai, India	B.Sc.	05/1991	Chemistry
University of Pune, Pune, India	M.Sc.	05/1993	Biochemistry
University of Pune, Pune, India	Ph.D.	03/2001	Biochemistry
McGill University, Montreal, Canada	Post-doc	11/2006	Biochemistry/Crystallography
St. Jude Children Research Hospital	Post-doc	06/2007	Biochemistry/Crystallography
The Scripps Research Institute, Florida	Research Associate	10/2010	Biochemistry/Crystallography/ Molecular Biology/Cell Biology

A. Personal Statement

I am a self-motivated and innovative scientist who cherishes working in challenging scientific assignments and possess necessary training and expertise to carry out the proposed research project. As a graduate student, I was trained in protein biochemistry and enzymology to carry out protein characterization and functional analysis of an extracellular nuclease from a fungal strain of *Rhizopus stolonifer*. As a post-doctoral fellow in the laboratory of Dr. Mirek Cygler at McGill University, Montreal, I gained experience in all facets of protein crystallography, including recombinant protein expression, purification, crystallization, X-ray diffraction data collection, and structure determination. As a heavy user, I also became highly experienced with data collection using our in-house Rigaku system equipped with rotating anode X-ray source and synchrotron beam lines (X8c, X12b, X25, and X29). During this period, I used various automated platforms for crystal screen setup (HyrdaIIplus) and crystal image monitoring (Bruker's crystal farm). Subsequently, working in the cell adhesion laboratory at Scripps Florida, my expertise was expanded to accommodate remote Xray diffraction data collection using synchrotron beamlines from APS (SER-CAT 22 ID/BM) and SSRL (11-1) as well as in molecular biology (cloning, transformation and expression in bacterial, mammalian, yeast, and insect cells) and cell biology (transfection, expression analysis, western blot, immunoprecipitation, confocal microscopy, *etc*.). I carried out various both *in vitro* and *in vivo* studies to address important biological questions related to focal adhesions and adherens junctions that I published in many peer-reviewed articles. In addition, I gained working knowledge on biophysical characterization of proteins using SEC-MALS and SAXS (SYBILS beamline) through formal workshops at Wyatt Light Scattering University and Lawrence Berkeley National Laboratory, respectively. Collectively, I have developed the management skills to accommodate various needs of the laboratory and research. Overall, my experience and expertise will significantly aid the success of the current proposal.

B. Positions, Scientific Appointments, and Honors

Positions

2010-present Staff Scientist, Department of Cancer Biology, The Scripps Research Institute, Jupiter, FL
2007-2010 Research Associate, Department of Cancer Biology, The Scripps Research Institute, Jupiter, FL
2006-2007 Post-doctoral Research Associate, Department of Oncology, St. Jude Children's Research Hospital, Memphis, TN
2001-2006 Post-doctoral Fellow, Department of Biochemistry, McGill University, Montreal, Quebec, Canada

Scientific Appointments

2016 Member of the American Heart Association

Honors

2016 Electron Microscopy training (April 11-13) in Dr. Andrew Ward's laboratory at The Scripps Research Institute, La Jolla, CA, USA
2014 MALS Training (December 3-5) at Wyatt Light Scattering University, Santa Barbara, CA.
2014 Frontiers of Biological SAXS (October 7-8) at SYBILS beamline, Lawrence Berkeley National Laboratory, Berkeley, CA
1993 CSIR Fellowship for Doctoral Research (Council for Scientific and Industrial Research, Government of India)
1993 GATE Scholarship (Indian Institute of Technology, India)
1993 University Grants Commission, Government of India Fellowship for Doctoral Research
1991 M.Sc. Merit Scholarship (National Chemical Laboratory, Pune, India)

C. Contributions to Science

1. As a post-doctoral fellow, my work focused on structure-function studies of bacterial proteins from different genomes (K12 and157) that I carried out under the Montreal-Kingston Structural Genomics Initiative. The basic goal of the project was to determine the crystal structures of E. coli proteins of known or unknown function with the main focus on small-molecule metabolic pathway enzymes, RNA binding proteins, and proteins that may participate in bacterial pathogenesis. I was involved in crystallization of many enzymes that are part of bacterial biosynthetic pathway and include HisB, PseG, PglD, YdiF, and CaiB, for which structural snapshot were provided using either their substrate, intermediate, or product and aided the elucidation of structure based catalytic mechanisms. Additionally, I determined the structures of hypothetical proteins YdiF and YjhS for which the functions were not known, however, based on the structure the functions was deciphered and validated through biochemical analysis. My efforts led to the publication of 13 articles in peer-reviewed journals and resulted in 27 PDB depositions with the RCSB database.

    a. **Rangarajan ES**, Li Y, Ajamian E, Iannuzzi P, Kernaghan SD, Fraser ME, Cygler M & Matte A (2005)
    Crystallographic trapping of the glutamyl-CoA thioester intermediate of family I CoA transferases
    J Biol Chem 280:42919-42928

    b. **Rangarajan ES**, Li Y, Iannuzzi P, Cygler M & Matte A (2005)
    Crystal structure of *Escherichia coli* crotonobetainyl-CoA: carnitine CoA-transferase (CaiB) and its complexes with CoA and carnitinyl-CoA
    Biochemistry 44:5728-5738

    c. **Rangarajan ES**, Proteau A, Wagner J, Hung MN, Matte A & Cygler M (2006)
    Structural snapshots of *Escherichia coli* histidinol phosphate phosphatase along the reaction pathway
    J Biol Chem 281:37930-3794

d. **Rangarajan ES**, Ruane KM, Sulea T, Watson DC, Proteau A, Leclerc S, Cygler M, Matte A & Young NM (2008)
Structure and active site residues of PglD, an *N*-acetyltransferase from the bacillosamine synthetic pathway required for N-glycan synthesis in *Campylobacter jejuni*
Biochemistry 47:1827-1836

2. My expertise gained in studying the structure-function relationship of bacterial proteins provided an exciting opportunity to work on human proteins, namely metavinculin, an isoform of vinculin, which plays an important role in the cytoskeleton organization in the heart and its mutants has been implicated in various cardiomyopathy conditions. I obtained stability as well as the X-ray diffraction quality of metavinculin crystals by developing a streak seeding protocol. This allowed me to solve the metavinculin structures (wild type as well as its cardiomyopathy associated Leu-954 deletion mutant) to about 3.4 Å resolution. Importantly, the vinculin project as a whole provided an excellent opportunity to carry out various *in vivo* studies, which boosted my overall expertise on the functional side as well. In addition, we also performed structural and functional studies involving vinculin in complex with raver1, a ribonucleoprotein that shuttles between the nucleus and cytoplasm.

   a. Lee JH, **Rangarajan ES**, Yogesha SD & Izard T (2009)
   Raver1 interactions with vinculin and RNA suggest a feed-forward pathway in directing mRNA to focal adhesions
   Structure 17:833-842

   b. **Rangarajan ES**, Lee JH, Yogesha SD & Izard T (2010)
   A helix replacement mechanism directs metavinculin functions
   PLoS One 5:e10679

   c. **Rangarajan ES**, Lee JH & Izard T (2011)
   Apo raver1 structure reveals distinct RRM domain orientations
   Protein Sci 20:1464-1470

   d. **Lee JH**, Rangarajan ES, Vonrhein C, Bricogne G & Izard T (2012)
   The metavinculin tail domain directs constitutive interactions with raver1 and vinculin RNA
   J Mol Biol 422:697-704

3. In another cell adhesion project, I studied the structural aspects of α-catenin, which is a scaffold protein similar to vinculin that participates in adherens junctions to interlink the E-cadherin, a single pass transmembrane protein, to the actin cytoskeleton. Human α-catenin, has been shown to exist both as a monomer and as a dimer. I was able to improve the initial X-ray diffraction from 6 Å to 3.7 Å through systematic optimization of a novel phosphate and malonate mediated dehydration protocol. Furthermore, I identified the distinct orientation of the two F-actin binding domains and identified the mechanism of interaction with F-actin. This was a huge accomplishment as a-catenin was long thought to be a protein 'not suitable for crystallization studies'. In addition, I was able to solve the structure of vinculin *N*-terminal domain (Vh1) with the vinculin-binding domain of α-catenin that showed that the latter could dimerize upon binding to vinculin. Collectively, the vinculin and α-catenin projects have yielded 10 PDB depositions and exciting peer-reviewed articles.

   a. **Rangarajan ES** & Izard T (2012)
   The cytoskeletal protein α-catenin unfurls upon binding to vinculin
   J Biol Chem 287:18492-18499, PMCID=3365723

   b. **Rangarajan ES** & Izard T (2013)
   Dimer asymmetry defines α-catenin interactions
   Nat Struct Mol Biol 20:188-193, PMCID=3805043

   c. K Chinthalapudi, **ES Rangarajan**, D Brown & T Izard (2016)

Differential lipid binding of vinculin isoforms promotes quasi-equivalent dimerization
Proceeding of the National Academy of Sciences USA 113:9539-9544

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47586236/?sort=date&direction=descending

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED 5 PAGES.**

NAME: **IZARD**, Tina

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

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
University of Basel, Biocentre, Switzerland	B.Sc.	06/1989	Biochemistry
University of Basel, Biocentre, Switzerland	M.Sc.	06/1990	Biophysics/Crystallography
University of Melbourne, Australia	Ph.D.	12/1994	Physics/Crystallography
University of Washington, Seattle, WA, USA	Post-doc	08/1996	Biochemistry/Crystallography

A. Personal Statement

Dr. Tina Izard, Principal Investigator (**PI**), is an expert in cell adhesion structure-function studies with decades of experience. She received outstanding training by Drs. Hans Jansonius (Basel, Switzerland), Peter Colman (Melbourne, Australia), and Wim Hol (Seattle, USA). In 1996, the PI began her academic career as a Lecturer at the University of Leicester. In 2000, she joined the faculty of St. Jude Children's Research Hospital (**SJCRH**) as an Assistant Professor. She was promoted to Associate Professor in 2005. In 2007, she was recruited to The Scripps Research Institute (**TSRI**) as an Associate Professor with Tenure and promoted to full Professor in 2017. At TSRI, her duties also include leadership of the macromolecular crystallography and cryogenic electron microscopy and the management of the TSRI SER-CAT beamline share at the Advanced Photon Source at Argonne National Laboratory. The PI serves on National Institutes of Health (**NIH**) Study Sections and is a reviewer for several journals, including *The Journal of Cell Biology, EMBO Journal, Nature, Nature Structural & Molecular Biology*, and *Proceedings of the National Academy of Sciences of the USA*.

Significant service to the scientific community beyond mentoring and committee duties: The PI never had any teaching responsibilities. All her teaching is voluntary. As a daughter of a primary school teacher at her K-12 school, the PI often spent time in her mother's classroom at the German School in Barcelona (Spain). The PI tutored students in various subjects as middle and high schoolers. In graduate school, she tutored a class for the Physics Department at Melbourne University (Australia). During her Lectureship appointment at Leicester University (England), the PI taught undergraduate Chemistry tutorials, and she also privately tutored several middle and high schoolers. After joining the faculty of SJCRH, the PI obtained an adjunct appointment at The University of Tennessee, where she taught '*Physical Chemistry and Applications*' in the Structural Biology Graduate Course. During her tenure at SJCRH, she was also a lecturer at the Graduate Student Journal Club.

Tthe PI was an active mentor in the SJCRH Pediatric Oncology Education (**POE**) Program that provides research training and education to top-tier undergraduate students to promote careers in biomedical research. The PI trained several POE students. She also participated in the Rhodes College/SJCRH Summer Plus, Undergraduate Research Program, by providing training to outstanding young undergraduates at Rhodes College (Memphis, TN). The PI was often approached to serve as a role model to female students, whom she enjoyed mentoring, including under-represented minorities. For example, a female African American undergraduate student from LeMoyne Owen College (a minority college located in Memphis, TN) as part of the McNair Program and a black female graduate student from Paris (France). During her lectureship at Leicester University, the PI trained and hosted a female graduate student from the laboratory of her collaborator, Professor Sygusch (University of Montreal), to prepare seleno-methionine substituted proteins. In addition to mentoring many undergraduate and graduate students, the PI mentored many post-doctoral fellows. The PI participates in the TSRI outreach program by organizing DNA extraction and drug discovery hands-on workshops in middle schools. She participates in the High School Student summer internship program funded by the William R. Kenan, Jr. Charitable Trust. She mentors students for six weeks in the Summer and provided hands-on research experience. The PI also mentors High School students for academic credit. Besides her appointment as a Graduate Program faculty member at TSRI, the PI is heavily involved with a middle school. She recently served as the Chair of the science fair. She used the TSRI demonstration laboratory to bring middle school students hands-on research experience or travels to Bak Middle School of the Arts to give lectures related to their science curriculum.

This proposal will allow the PI to further train students and post-doctoral fellows in cryogenic electron microscopy, an incredible asset that she will bring to an extensive scientific community that does not have cryogenic electron microscopy expertise. Notably, the PI recently set up cryogenic electron microscopy at TSRI in Florida. In 2019, NIGMS recognized her leadership by awarding supplementary administrative funds to purchase a plunge freezer, glow discharger, and computer for cryogenic electron microscopy structure determination.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2017 - present Adjunct Professor, Department of Immunology and Microbiology, TSRI
2017 - present Professor, Department of Integrative Structural and Computational Biology, TSRI
2016 - present The Scripps Research Institute Graduate Program Faculty Member
2015 - 2017 Adjunct Associate Professor with Tenure, Department of Immunology and Microbiology, TSRI
2007 - 2017 Associate Professor with Tenure, The Scripps Research Institute (**TSRI**)
2005 - 2007 Associate Faculty Member, Department of Oncology, SJCRH, Memphis, TN
2000 - 2007 Adjunct Assistant Professor, Department of Molecular Sciences, University of Tennessee Health Science Center, Memphis, TN
2000 - 2005 Assistant Faculty Member, St. Jude Children's Research Hospital (**SJCRH**), Memphis, TN
1996 - 1999 Lecturer, Department of Biochemistry, University of Leicester, Leicester, England
1995 - 1996 Research Associate, HHMI, Biological Structure, University of Washington, Seattle, WA
1990 - 1991 Research Assistant, Structural Biology, Biocentre, University of Basel, Switzerland
1987 - 1988 Apprenticeship, Department of Vitamin Research, Hoffmann-La Roche, Basel, Switzerland

Other Experience and Professional Membership

2022 *Ad hoc* Maximizing investigator Research Award C (**MRAC**), NIH, study section Member
2020 - 2021 National Science Foundation Panelist
2019 - present Mentor of The Scripps Research Institute Fellow, Dr. Raktim Roy
2019 - present Board Member, South East Regional Collaborative Access Team, Argonne National Lab
2019 Program Project Grant Reviewer for the National Heart, Lung, and Blood Institute, NIH
2019 Reviewer, Special Emphasis Panel for the National Institutes of Health
2019 The Scripps Research Institute faculty promotion *Ad hoc* committee member
2017 - 2019 The Scripps Research Institute faculty search committee member
2017 *Ad hoc* Reviewer, Intercellular Interactions (**ICI**) Study Section for the NIH
2017 - 2022 Editorial Board Member, The Journal of Biological Chemistry
2015 Reviewer, Special Emphasis Panel, Biological Chemistry & Macromolecular Biophysics (**BCMB**) Study Section for the National Institutes of Health
2014 Reviewer, Special Emphasis Panel, Macromolecular Structure & Function E (**MSFE**) Study Section for the National Institutes of Health
2012 - present Reviewer, Biotechnology and Biological Science Research Council (England) project grant applications
2009 - present Reviewer, The Wellcome Trust (England) project grant applications
2009 - present Reviewer, Macromolecular Crystallography Proposals, Advanced Photon Source
2006 *Ad hoc* Reviewer, Macromolecular Structure & Function B (**MSFB**) Study Section
1997 - present *Ad hoc* Reviewer for Acta Crystallographica D, Acta Crystallographica F, Biochemistry, Cell Communication and Adhesion, Cell Motility and the Cytoskeleton, Communications Biology, eLife, FEBS Letters, FEBS Journal, Journal of Structural Biology, Nature, Nature Communications, Nature Structural & Molecular Biology, Proceedings of the National Academy of Sciences of the USA, Protein Science, Scientific Reports, Structure, The EMBO Journal, The Journal of Cell Biology, The Journal of Biological Chemistry, The Journal of Molecular Biology, Trends in Biochemical Sciences
1997, 1998 The Wellcome Trust Travel Grant
1993 Scholarship from the Society of Crystallography in Australia
1992, 1994 International Union of Crystallography Young Scientist Award
1991 Swiss National Science Foundation
1991 - 1994 Melbourne University Postgraduate Scholarship, Australia
1991, 1993-4 Dr. Max Husmann Foundation, Zürich, Switzerland

1985 - 1990 The Educational Department Bellinzona, Switzerland, scholarship

C. Contributions to Science

C.1. Bacterial enzymes as novel drug targets

I started my academic career with a 3-year Lectureship appointment at Leicester University in England (1997 - 1999). My laboratory, comprised of me only, made significant contributions to understanding several bacterial enzymes and how these could be exploited as novel antibacterial drug targets.

(i) We determined the first crystal structure of bacterial phosphopantetheine adenylyltransferase (**PPAT**) alone and bound by several ligands. PPAT catalyzes the penultimate step of coenzyme A (**CoA**) biosynthesis, the primary acyl carrier for all organisms. Our results that we published in 6 papers were the foundation for a pharmaceutical startup company (PanTherix Ltd).

(ii) We determined the crystal structure of chloramphenicol phosphotransferase from *Streptomyces venezuelae*, alone and in complex with ligands. This enzyme inactivates chloramphenicol, which inhibits ribosomal peptidyl transferase activity as we published in 3 papers.

(iii) We determined the crystal structure of the metal-dependent 2-dehydro-3-deoxy-galactarate aldolase from *Escherichia coli* and proposed a novel mechanism that we published in 2 papers.

In collaboration with Drs. Marie-France Carlier (CNRS), Guy Tran Van Nhieu (Pasteur Institute), and Philippe Sansonetti (Pasteur Institute) we have also made significant inroads into our understanding of bacillary dysentery, a major cause of morbidity and mortality. Our work on the *Shigella* invasion IpaA was supported by an NIAID R01 award (2006 - 2010), which scored a 4%. We showed that IpaA harbors two high-affinity binding sites, which bind to and activate vinculin in a novel fashion. This disrupts the contacts of vinculin with talin and α-actinin. We showed that *Shigella* subverts the function of vinculin by molecular mimicry of talin. We found that this interaction is necessary for the efficient entry of *Shigella* into the host cell.

c.1.1. **T Izard*** & A Geerlof (1999)
"The crystal structure of a novel bacterial adenylyltransferase reveals half of sites reactivity"
EMBO J 18:2021-2030

c.1.2. **T Izard*** & J Ellis (2000)
"The crystal structures of chloramphenicol phosphotransferase reveal a novel inactivation mechanism"
EMBO J 19:2690-2700

c.1.3. **T Izard*** & NC Blackwell (2000)
"Crystal structures of the metal-dependent 2-dehydro-3-deoxy-galactarate aldolase suggest a novel reaction mechanism" ***EMBO J*** 19:3849-3856

c.1.4. G Tran Van Nhieu & **T Izard*** (2007)
"Vinculin binding in its closed conformation by a helix addition mechanism" ***EMBO J*** 26:44588-4596

C.2. Cell-matrix interactions in normal and malignant cells

Upon being appointed as a junior faculty member at SJCRH in 2000, I set up a cell adhesion laboratory to study key cell adhesion proteins structurally and functionally. Our studies on the vinculin interactions with talin were supported by an NIGMS R01 award (2004 - 2012), which scored a 5% and a 1% in the renewal. Our structural studies of key linkers of cell-substrate and cell-cell junctions that control the transmission of and responses to force had a major fundamental impact. Our studies showed how lipid binding to vinculin regulates focal adhesion turnover. Our seminal paper in *Nature* showed how a talin-derived vinculin binding site could activate talin. We found that vinculin is autoinhibited. Thus, vinculin cannot bind to the actin cytoskeleton. We discovered a new helix bundle conversion mechanism that we first observed in the talin activation of vinculin. Since full-length talin fails to activate vinculin, the field initially wondered whether our talin-derived vinculin binding sites were physiologically relevant. However, it was then discovered that talin must be partially unfolded by traction forces to bind and activate vinculin. Thus, our work provided the molecular basis of a central event in mechanotransduction. More recently, we established how the phospholipid PIP_2 induces oligomerization of vinculin to promote adhesion turnover and cell migration. These key contributions significantly contributed to the rapid maturing of the field.

c.2.1. RA Borgon, C Vonrhein, G Bricogne, PRJ Bois & **T Izard*** (2004)
"Crystal structure of human vinculin" ***Structure*** 12:1189-1197

c.2.2. **T Izard***, G Evans, RA Borgon, CL Rush, G Bricogne & PRJ Bois (2004)
"*Vinculin activation by talin through helical bundle conversion*" ***Nature*** 427:171-175
Commentaries: ***Nature*** 430:513-514 (2004); ***Advanced Photon Source Annual Report*** (2004)
Nature Struc Mol Biol 20:188-193

c.2.3. K Chinthalapudi, ES Rangarajan, DN Patil, EM George, DT Brown & **T Izard*** (2014)
"Lipid binding promotes oligomerization and focal adhesion activity of vinculin"
J Cell Biol 207:643-656; Highlighted "*In This Issue"* of ***J Cell Biol*** 207:572 (2014)

c.2.4. K Chinthalapudi, ES Rangarajan & **T Izard*** (2018)
"The interaction of talin with the cell membrane is essential for integrin activation and focal adhesion formation" ***Proceedings of the National Academy of Sciences USA*** 115:10339-10344

C.3. Specialized cell junctions of the heart and their role in cardiomyopathies

Our work has also had a significant fundamental impact on heart disease by providing clues to the mechanisms of some forms of dilated cardiomyopathy. We defined the structure and regulation of specialized cytoskeletal proteins that regulate the formation and function essential for the coordinated functions of specialized cells in tissues. These include cardiac muscle and how mutations in these proteins lead to defects in development and myopathies. Especially inherited dilated idiopathic cardiomyopathies (**DCM**), the most common form of cardiomyopathy and disease that manifests high morbidity and mortality. Mutations have been described in cardiomyopathies, and notably, these include mutations in *metavinculin*, an alternatively spliced, muscle-specific isoform of vinculin. We solved the structures of human full-length wild type metavinculin (**MV**) and the cardiomyopathy-associated MV deletion mutant. Our structures revealed that the vinculin tail domain (**Vt**) α-helix H1 and its preceding extended coil are replaced in MV by similar residues from the MV specific insert. We showed that the α-helix H1 of Vt is responsible for vinculin to oligomerize in the presence of PIP_2 while MV does not. Our studies unravel the unique properties of MV in interacting with its partners, regulating the actin cytoskeleton, establishing tight cell junctions, and how these regulatory circuits are disrupted in myopathies. Our studies might suggest new avenues for therapeutic intervention for this deadly disease.

c.3.1. JH Lee, ES Rangarajan, C Vonrhein, G Bricogne & **T Izard*** (2012)
"The metavinculin tail domain directs constitutive interactions with raver1 and vinculin RNA"
J Mol Biol 422:697-704

c.3.2. K Chinthalapudi, DN Patil, ES Rangarajan, C Rader & **T Izard*** (2015)
"Lipid-directed vinculin dimerization" ***Biochemistry*** 54:2758-2768

c.3.3. K Chinthalapudi, ES Rangarajan, D Brown & **T Izard*** (2016)
"Differential lipid binding of vinculin isoforms promotes quasi-equivalent dimerization"
Proceedings of the National Academy of Sciences USA 113:9539-9544

c.3.4. ES Rangarajan & **T Izard*** (2021)
"The cryogenic electron microscopy structure of the cell adhesion regulator metavinculin reveals an isoform-specific kinked helix in its cytoskeleton binding domain" ***Int J Mol Sci*** 22:645

C.4. Cell-cell interactions in normal and malignant cells

The formation of cell-cell junctions is critical for developing and maintaining multi-cellular organisms, and a loss of cell-cell junctions is associated with several disease states. The epithelial, endothelial, and neuronal tissues of multi-cellular organisms are held together by specialized cell-cell junctions called adherens junctions. These are required for several biological processes, including wound healing, embryonic morphogenesis, development, differentiation, tissue integrity, homeostasis, and organization. The disassembly of these junctions causes loss of cell polarity and contact inhibition, and epithelial-to-mesenchymal transitions. Thus, adherens junctions need to be regulated dynamically to allow cells to continuously migrate and engage and disengage in adhesive interactions with neighboring cells. Dysregulation of these highly coordinated interactions can lead to the development of cancer and vascular diseases. Changes in cell-cell adhesion reinitiate cell migration during cell turnover or wound healing or allow metastatic cells to scatter to distant organs. At adhesion complexes, the β-catenin-cadherin receptor complex binds to the cytoskeletal protein α-catenin, which is essential for the formation and stabilization of cell-cell junctions. Loss of α-catenin or E-cadherin promotes unrestricted growth of cells and facilitates transformation, tumorigenesis, and metastasis. Thus, understanding the molecular

mechanisms that control proper assembly and stabilization of these junctions is a fundamental process in cell biology that goes awry in important pathological scenarios, especially cancer. We determined the crystal structure of full-length dimeric human α-catenin, and of vinculin-bound α-catenin, and these structures and our biochemical and biological studies defined the roles of the vinculin-α-catenin interaction in the formation and stabilization of adherens junctions. We established that α-catenin unfurls upon binding to vinculin. This solved a long-standing conundrum by showing how α-catenin cannot bind to F-actin and β-catenin simultaneously. We also made significant contributions to another cell-cell junction protein, neurofibromin 2, that is responsible for neurofibromatosis type II.

c.4.1. ES Rangarajan & **T Izard*** (2012)
"The cytoskeletal protein α-catenin unfurls upon binding to vinculin" ***J Biol Chem*** 287:18492-18499

c.4.2. ES Rangarajan & **T Izard*** (2013)
"Dimer asymmetry defines α-catenin interactions" ***Nature Struc Mol Biol*** 20:188-193;
Commentaries**:** ***Nature Reviews Mol Cell Biol*** 14:66 (2013)

c.4.3. K Chinthalapudi, V Mandati, J Zheng, AJ Sharff, G Bricogne, PR Griffin, J Kissil & **T Izard*** (2018)
"Lipid binding promotes the open conformation and tumor-suppressive activity of neurofibromin 2"
Nature Communications 9:1338

c.4.4. M Janiszewska, MC Primi & **T Izard** (2020)
"Cell adhesion in cancer: Beyond the migration of single cells" ***J Biol Chem*** 295:2495-2505

C.5. Collaborations on structure-function studies relevant to diseases

In 2019, I built the cryogenic electron microscopy facility for TSRI in Florida. I provided hands-on leadership in purchasing the Americas' first Japan Electron Optics Laboratory (**JEOL**) 300 kV cryogenic Atomic Resolution Microscope (**cryoARM300**). I also set up the neighboring Max Planck Florida Institute for Neuroscience for automatic data collection of negatively stained protein samples. I ensured the training in cryogenic electron microscopy, negative stain data collection, 2D classifications (including sample freezing), and pre-screening representatives for their suitability for cryogenic electron microscopy. NIGMS recognized our leadership by awarding supplementary administrative funds to purchase a plunge freezer, glow discharger, and computer for cryogenic electron microscopy structure determination.

c.5.1. DN Patil, S Singh, T Laboute, TS Strutzenberg, X Qiu, D Wu, SJ Novick, CV Robinson, PR Griffin, JF Hunt, **T Izard**, AK Singh AK & KA Martemyanov* (2022)
"Cryo-EM structure of human GPR158 receptor coupled to the RGS7-Gβ5 signaling complex"
Science 375:86-91

c.5.2. DN Patil DN, ES Rangarajan, SJ Novick, BD Pascal, DJ Kojetin, PR Griffin, **T Izard*** & KA Martemyanov* (2018)
"Structural organization of a major neuronal G protein regulator, the RGS7-Gβ5-R7BP complex"
eLife 7:e42150

c.5.3. W Cao, H Kayama, ML Chen, A Delmas, A Sun, SY Kim, ES Rangarajan, K McKevitt, AP Beck, CB Jackson, G Crynen, A Oikonomopoulos, PN Lacey, GJ Martinez, **T Izard**, RG Lorenz, A Rodriguez-Palacios, F Cominelli, MT Abreu, DW Hommes, SB Koralov, K Takeda & MS Sundrud (2017)
"The Xenobiotic Transporter Mdr1 Enforces T Cell Homeostasis in the Presence of Intestinal Bile Acids" ***Immunity*** 47:1182-1196.e10

c.5.4. JD Stender, JC Nwachukwu, I Kastrati, Y Kim, T Strid, M Yakir, S Srinivasan, J Nowak, **T Izard**, ES Rangarajan, KE Carlson, JA Katzenellenbogen, XQ Yao, BJ Grant, HS Leong, CY Lin, J Frasor, KW Nettles & CK Glass (2017)
"Structural and molecular mechanisms of cytokine-mediated endocrine resistance in human breast cancer cells" ***Molecular CELL*** 65:1122-1135

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