BIOGRAPHICAL SKETCH

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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
The University of Washington, Seattle, WA	Post-doc	08/1996	Biochemistry/Crystallography

A. Personal Statement

I am an expert in cell adhesion structure-function studies as documented in my publication record, invitations as a speaker at Gordon Research Conferences, and federal funding since 2004. I received training from Drs. Hans Jansonius (Basel, Switzerland), Peter Colman (Melbourne, Australia), and Wim Hol (Seattle, USA). In 1996, I began my academic career as a Lecturer at the University of Leicester. In 2000, I joined the St. Jude Children's Research Hospital (**SJCRH**) faculty as an Assistant Professor. I was promoted to Associate Professor in 2005. In 2007, I was recruited to The Scripps Research Institute (**TSRI**) as an Associate Professor with Tenure and was promoted to full Professor in 2017. At TSRI, my duties included leadership of the macromolecular crystallography and cryogenic electron microscopy (**cryoEM**) and managing the TSRI SER-CAT beamline share at the Advanced Photon Source at Argonne National Laboratory. I serve on National Institutes of Health (**NIH**) Study Sections. I am a reviewer for several journals, including *CELL, 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: I never had teaching responsibilities. All my teaching is voluntary. As the daughter of a primary school teacher at my K-12 school, I often spent time in my mother's classroom at the German School in Barcelona, Spain. During high school, I tutored middle and high school students in various subjects. During graduate school, I tutored a class for my Physics Department at Melbourne University in Australia. During my Lectureship appointment at Leicester University in England, I taught undergraduate Chemistry tutorials and privately tutored several middle and high school students. After joining the faculty of SJCRH, I obtained an adjunct appointment at The University of Tennessee, where I taught '*Physical Chemistry and Applications*' in their Structural Biology Graduate Course. During my tenure at SJCRH, I was also a Graduate Student Journal Club lecturer.

I was also an active mentor in the SJCRH Pediatric Oncology Education (**POE**) Program, which provides research training and education to top-tier undergraduate students to promote careers in biomedical research. I trained several POE students. I also participated in the Rhodes College/SJCRH Summer Plus Undergraduate Research Program by providing training to outstanding young undergraduates at Rhodes College in Memphis, Tennessee. I was often approached to serve as a role model to female students whom I enjoyed mentoring, including under-represented minorities and undergraduate students from LeMoyne Owen College, a minority college in Memphis, as part of their McNair Program.

Besides my appointment as a Graduate Program faculty member at TSRI, where I mentored many undergraduate and graduate students, I also mentored several post-doctoral fellows. I participated in the TSRI outreach program by organizing hands-on workshops for DNA extraction and drug discovery in middle schools. I regularly accept students from our High School Student summer internship program funded by the William R. Kenan, Jr. Charitable Trust. I mentor students for ten weeks in the Summer and provide hands-on research experience. I also mentor High School students for academic credit and served as the Chair of the middle school science fair. I used our TSRI demonstration laboratory to bring middle school students to our campus to provide hands-on research experience or travel to the Bak Middle School of the Arts to lecture on their science curriculum.

This proposal will allow me to further train students and post-doctoral fellows in cryoEM, which I will bring to an extensive scientific community that does not have cryoEM expertise. Notably, in 2019-2020, I set up

cryoEM at TSRI in Florida. In 2019, NIGMS recognized my leadership by awarding supplemental administrative funds to purchase a plunge freezer, glow discharger, and computer for cryogenic electron microscopy structure determination. I am a member of the Scientific Advisory Group of the University of Florida electron microscopy core and the Florida representative on our South East Regional Collaborative Access Team beamlines.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022 - present UF Scripps
2016 - present The Scripps Research Institute (**TSRI**) Graduate Program Faculty Member
2017 - 2022 Adjunct Professor, Department of Immunology and Microbiology, TSRI
2017 - 2022 Professor, Department of Integrative Structural and Computational Biology, TSRI
2015 - 2017 Adjunct Associate Professor with Tenure, Department of Immunology and Microbiology, TSRI
2007 - 2017 Associate Professor with Tenure, The Scripps Research Institute
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, 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. 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, contact inhibition, and epithelial-to-mesenchymal transitions. Thus, adherens junctions must be regulated dynamically to allow cells to migrate continuously 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 forming and stabilizing 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 the proper assembly and stabilization of these junctions is a fundamental process in cell biology that goes awry in critical 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 and thus solved a long-standing conundrum by showing how α-catenin cannot bind to F-actin and β-catenin simultaneously. We also contributed significantly to another cell-cell junction protein, neurofibromin 2, responsible for neurofibromatosis type II. More recently, we are collaborating with Dr. Daniel Leitha (Madrid, Spain) on the modulation of inositol kinases and phosphatases, which either generate or deplete phosphatidylinositol. Key enzymes involved include PI3K, PTEN, SHIP, and INPP4, which specifically target the 3', 4', and 5' carbons of the inositol ring, respectively. In our collaboration with Dr. Lietha, we focus on SHIP2.

c.1.1. ES Rangarajan & **T Izard*** (2012) PMCID = PMC3365723
"The cytoskeletal protein α-catenin unfurls upon binding to vinculin" ***J Biol Chem*** 287:18492-18499;

c.1.2. ES Rangarajan & **T Izard*** (2013) PMCID = PMC3805043
"*Dimer asymmetry defines α-catenin interactions*" ***Nature Struc Mol Biol*** 20:188-193;
Commentaries: ***Nature Reviews Mol Cell Biol*** 14:66 (2013)

c.1.3. K Chinthalapudi, V Mandati, J Zheng, AJ Sharff, G Bricogne, PR Griffin, J Kissil & **T Izard*** (2018) PMCID = PMC5889391 *"Lipid binding promotes the open conformation and tumor-suppressive activity of neurofibromin 2"* ***Nature Communications*** 9:1338

c.1.4. M Janiszewska, MC Primi & **T Izard** (2020) PMCID = PMC7039572
"Cell adhesion in cancer: Beyond the migration of single cells" ***J Biol Chem*** 295:2495-2505

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 my cell adhesion laboratory to study key cell adhesion proteins structurally and functionally. Our studies on the vinculin interactions with talin were supported by my NIGMS R01 awards (2004 - 2012), which scored 5% and 1% in the renewal. Our structural studies of key linkers of cell-substrate and cell-cell junctions that control the transmission and responses to force had a major fundamental impact. We 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, which 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 traction forces must partially unfold talin to bind and activate vinculin. Thus, our work provided the molecular basis of a major event in mechanotransduction. We also determined how the phospholipid PIP_2 induces the 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. **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)

c.2.2. K Chinthalapudi, ES Rangarajan, DN Patil, EM George, DT Brown & **T Izard*** (2014) PMCID = PMC4259812
"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.2.3. K Chinthalapudi, ES Rangarajan & **T Izard*** (2018) PMCID = PMC6187153
"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.2.4. MC Primi, ES Rangarajan, DN Patil & **T Izard*** (2021) PMCID = PMC8318988 *"Conformational flexibility determines the Nf2/merlin tumor suppressor functions"* ***Matrix Biol Plus*** 12:100074

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 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 defined 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 results might aid the discovery of new avenues for therapeutic intervention for this deadly disease.

c.3.1. JH Lee, ES Rangarajan, C Vonrhein, G Bricogne & **T Izard*** (2012) PMCID = PMC3835166
"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) PMCID = PMC5003255
"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) PMCID = PMC7827843
"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.1. Bacterial enzymes as novel drug targets

I started my academic career with a 3-year temporary Lectureship appointment at Leicester University in England (1997 - 1999). In collaboration with Drs Marie-France Carlier (Centre National de la Recherche Scientifique, Paris, France), Guy Tran Van Nhieu (Pasteur Institute, Paris, France), and Philippe Sansonetti (Pasteur Institute), we contributed to our understanding of bacillary dysentery which is a major cause of morbidity and mortality. Our work on the *Shigella* invasion IpaA was supported by my 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 interaction disrupts the contact of vinculin with talin and α-actinin. We showed that *Shigella* subverts the vinculin function by molecular mimicry of talin. We found that this interaction is necessary for Shigella to efficiently enter the host cell. My laboratory, comprised at the time of me only, made significant contributions to understanding several bacterial enzymes and how these could be exploited as novel antibacterial drug targets, which can be summarized as follows:

(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, which we published in 6 papers, were the foundation for a pharmaceutical startup (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.

c.4.1. **T Izard*** & A Geerlof (1999) PMCID = PMC1171286
"The crystal structure of a novel bacterial adenylyltransferase reveals half of sites reactivity" ***EMBO J*** 18:2021-2030

c.4.2. **T Izard*** & J Ellis (2000) PMCID = PMC212772
"The crystal structures of chloramphenicol phosphotransferase reveal a novel inactivation mechanism" ***EMBO J*** 19:2690-2700

c.4.3. **T Izard*** & NC Blackwell (2000) PMCID = PMC306599
"Crystal structures of the metal-dependent 2-dehydro-3-deoxy-galactarate aldolase suggest a novel reaction mechanism" ***EMBO J*** 19:3849-3856

c.4.4. G Tran Van Nhieu & **T Izard*** (2007) PMCID = PMC2063484
"Vinculin binding in its closed conformation by a helix addition mechanism" ***EMBO J*** 26:44588-4596

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

In 2019, I built the **cryoEM** 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 cryoEM, negative stain data collection, 2D classifications (including sample freezing), and pre-screening representatives for their suitability for cryogenic electron microscopy. NIGMS recognized my leadership by awarding supplemental administrative funds to purchase a plunge freezer, glow discharger, and computer for cryoEM studies. I am a member of the Scientific Advisory Group of the University of Florida EM core.

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) PMCID = PMC8926151
"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) PMCID = PMC6310461 *"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) PMCID = PMC5741099 *"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) PMCID = PMC5546241
"Structural and molecular mechanisms of cytokine-mediated endocrine resistance in human breast cancer cells" ***Molecular CELL*** 65:1122-1135

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