BIOGRAPHICAL SKETCH

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NAME: **Reisler, Emil**

eRA COMMONS USER NAME (credential, e.g., agency login): REISLER2

POSITION TITLE: Distinguished Professor of Biochemistry

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
Hebrew Univ., Jerusalem, Israel	B.S.	1964	Chemistry
The Weizman Inst. Sci., Rehovot, Israel	M.S.	1966	Physical Chemistry
The Weizman Inst. Sci., Rehovot, Israel	Ph.D.	1971	Physical Chemistry
The Johns Hopkins Univ., Baltimore, MD	Postdoc	1972-74	Biology

A. Personal Statement

I have been always fascinated by structures and functions of protein assemblies. My recent focus has been on understanding actin filaments structures, properties, interactions, remodeling, and biological functions, all of which are vital to cells. We made major contributions to clarifying the dynamics and function of actin filaments. Our work helped to clarify a key cellular process – how ADF/cofilin proteins sever actin filaments. We included in these investigations the analysis of cofilin's partner proteins in actin disassembly, and the filaments severing by other proteins (Inverted Formin 2 and Mical). We continue our investigation of the remodeling of neuronal cytoskeleton in dendritic spines and the function and interactions with actin of its main protein partner, drebrin. All along we benefit from collaborations which bring excellent expertise in electron microscopy, crystallography, mass spectrometry, EPR spectroscopy to questions of actin structure and dynamics. My group has provided research opportunities to undergraduate, Ph.D. and postdoctoral fellows. The new project builds on our prior and recent results and the collaborations that we have developed. Our goal is to achieve detailed understanding of actin filaments disassembly via their oxidation by Mical and severing by cofilin, the up-regulation of cofilin's action by "assisting" proteins, and their contribution to cellular processes. We hope also to gain structural understanding of drebrin's effect on actin and its role in cytoskeleton function in dendritic spines.

An ongoing project that I would like to highlight is:

R01 GM077190 Reisler (PI) 07/01/1978 – 06/30/2022
NIH/NIGMS
Actin Dynamics, Interactions and Function
Remodeling of actin cytoskeleton is one of the key steps in many cell functions, including cell division, metastasis, and tumor cell migration and invasion. Our investigation of actin filaments severing and disassembly via their specific enzymatic oxidation, and through interactions with cofilin and its partner proteins, will deepen the understanding of actin dynamics and remodeling, leading to potential therapeutic applications. Drebrin is a critical component of actin cytoskeleton in neuronal cells and our work on its interactions with actin can reveal potential routes for intervention in the diseased state of these cells.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2006-present Distinguished Professor of Biochemistry, UCLA

2003-2009 Dean of Life Sciences, UCLA
1984-2006 Professor of Biochemistry, UCLA
1989-1991 Vice-Chairman, 1997-2001; Chairman, Dept. of Chemistry & Biochemistry, UCLA
1977-1980 Asst. Professor, 1980-1984 Assoc. Professor of Biochemistry, UCLA
1974-1976 Senior Scientist, Polymer Dept., The Weizmann Inst. Sci., Rehovot, Israel

Honors

2009 Judy Lengyel Award for extraordinary teaching, research or service, MCDB, UCLA
2006 McCoy Award, UCLA, Chem. and Biochem., Excellence in Research
1991-present The Johns Hopkins Society of Scholars, Member
1995 Hanson-Dow Award, UCLA, Excellence in Teaching
1984-1985 Visiting Professor, The Weizman Institute of Science
1984 College Institute Award, UCLA
1971-1974 Hopkins-Weizman Exchange Fellow
1970 John F. Kennedy Award, The Weizman Inst. of Science. 1970.

Other Experience and Professional Memberships

2010 Ad hoc Member, Board of Scientific Counselors, NHLBI
1997 -2003 Member, Editorial Board, Biophys. J.
1992 - 2008 Member, Ad Hoc, NIH Study Sections
Member, Biophysical Society, AAAS, American Soc. Biological Chemists, ACS, Cell Biology.
1991-1994 Member, Editorial Board, Protein Science
1990-1994 Member, Advisory Board of the J. Lewis Center for Neuromuscular Disease, UCLA
1990-1994 Member, Research Committee of the American Heart Association, Los Angeles Chapter, Study Section Chairman, 1993.

C. Contributions to Science (over 200 peer reviewed publications)

1. Self-assembly, structure and function of glutamic dehydrogenase and myosin.
During my Ph.D. thesis work I was fascinated by the unknown structure and assembly of bovine liver glutamic dehydrogenase, and how they determined its different activities. I characterized the hexameric structure and function of this enzyme, and it's self-assembly into linear oligomers. In my first independent position (at UCLA) I discovered that 16-18 myosin molecules can form homogenous bipolar minifilaments, similar to those present in some non-muscle cells. We showed that the formation of mini-filaments is an intrinsic property of myosin, and simplified studies of assembled myosin interactions with actin. We were the first to show by proteolytic methods the organization of myosin heads (subfragment 1; S1) into three subdomains (20, 25, and 50 kDa). We then clarified the link between structural elements of S1 and its ATPase activity and actin binding.

a. Eisenberg, H., Josephs, R., and Reisler, E. (1976). Bovine Liver Glutamate Dehydrogenase. Adv. Prot. Chem. 30, 101-181. PMID:7109
b. Reisler, E., Smith, C., and Seegan, G. (1980). Myosin Minifilaments. J. Mol. Biol. 143, 129-145. PMID: 7441758
c. D. Applegate, and Reisler, E. (1983). Protease sensitive regions in Myosin Subfragment-1. Proc. Natl. Acad. Sci. U.S.A., 80, 7109-7112. PMCID: PMC390002
d. Bobkov, A.A., Bobkova, E.A., Lin, S.H., and Reisler, E. (1996). The role of surface loops (residues 204-216 and 627-646) in the motor Function of the Myosin head. Proc. Natl. Acad. Sci. U.S.A. 93, 2285-2289. PMCID: PMC39787

2. Actin filaments structure, dynamics and function.
Our initial interest in actin filaments (F-actin) was in how they activate myosin's ATPase to produce force and motion, where myosin binds on them, and how they interact with the ADP and ATP states of myosin. We were the first to start mapping actin's sites for the weak (in the presence of ATP) and strong (rigor type, in the presence of ADP) myosin binding. With site specific antibodies (Fab) we identified charged residues on actin that drive its weak interactions with myosin. We then adopted yeast actin as a good model for a highly productive mutational analysis of the role of its specific residues in myosin interactions, and motion and force generation. The use of

fluorescent and EPR spin probes on actin mutants enabled us to examine the effects of actin binding proteins on local environments on actin. Our results made us fascinated with the local dynamics of actin filaments and how it governs their diverse functions. We discovered that limiting actin dynamics by cross-linking inhibited strongly its contribution to motion generation by myosin. In a comprehensive study employing mutants of virtually all residues of D-loop in actin (a.a. 40-50) we demonstrated the structural plasticity of this loop via disulfide cross-linking, EPR spectroscopy and other approaches. Our experience with targeted actin cross-linking enabled us to prepare and crystalize cross-linked dimers and to analyze the inter-actin contacts in them. This, in turn, helped us to define the atomic level contacts between subdomains 3 and 4 (on adjacent protomers) and confirmed the plasticity of subdomains 2 and 1 contacts in F-actin.

a. Miller, C.J., Wong, W.W., Bobkova, E., Rubenstein, P.A., and Reisler, E. (1996). Mutational analysis of the role of the N-terminus of actin in actomyosin interactions. Comparison with other mutant actins and implications for the cross-bridge cycle. Biochemistry 35, 16557-16565. PMID:8987990
b. Kim, E., Bobkova, E., Hegyi, G., Muhlrad, A., and E. Reisler, E. (2002). Actin cross-linking and the inhibition of the actomyosin motor. Biochemistry41, 86-93. PMID:11772006
c. Oztug Durer, Z.A., Sept D., Kudryashov, D.S., and E. Reisler, E. (2010). F-actin structure destabilization and DNase-I binding loop fluctuations. J. Mol. Biol. 395, 544-557. PMCID: PMC3070609.
d. Kudryashov, D.S., Sawaya, M.R., Adisetiyo, H., Norcoss, T., Hegyi, G., Reisler, E., and Yeates, T.O. (2005). The crystal structure of a cross- linked actin dimer suggests a detailed molecular interface in F-actin. Proc. Natl. Acad. Sci. USA 102, 13105-13110 (2005). PMCID: PMC1196358

3. Disassembly of actin filaments by cofilin.

Changes in the helical twist of actin filaments by ADF/cofilin proteins and their critical role in the disassembly of cellular actin filaments ignited our interest in learning how cofilin acts on F-actin. Using a wide range of biophysical methods and EM analysis, we mapped in a series of studies the cooperative effects of cofilin on subdomain 2 of actin - weakening its D-loop interactions with the adjacent actin protomer, while providing alternative filament stabilizing contacts under conditions of binding saturation. We documented cofilin-induced changes in actin-actin contacts and showed that cofilin enhances intrinsic modes of F-actin instability. Based on fluorescence microscopy observations of filaments severing by cofilin we (and others) proposed that this severing is due to dynamic (Brownian) motions between cofilin occupied and cofilin free segments of F-actin, and connected the changes in filaments flexibility to specific divalent cation displacement by cofilin.

a. Bobkov, A.A., Muhlrad, A., Kokabi, K., Vorobiev, S., Almo, S.C., and Reisler, E. (2002). Structural effects of cofilin on longitudinal contacts in F-actin. J. Mol. Biol. 323, 739-750. PMID: 12419261
b. Orlova, A., Shvetsov, A., Galkin, V.E., Kudryashov, D.S., Rubenstein, P.A., Egelman, E.H., and Reisler, E. (2004). Actin destabilizing factors disrupt filaments by means of a time reversal of polymerizations. Proc. Natl. Acad. Sci. USA 101, 17664-17668. PMCID: PMC539747
c. Pavlov, D., Muhlrad, A., Cooper, J., Wear, M., and E. Reisler, E. (2007). Actin filament severing by cofilin. J. Mol. Biol. 365, 1350-1358. PMCID: PMC2572264
d. Galkin, V.E., Orlova, A., Kudryashov, D.S., Solodukhin, A., Reisler, E., Shroder G.F., and Egelman, E.H. (2011).Remodeling of actin filaments by ADF/cofilin proteins. Proc.Natl. Acad. Sci. USA 108, 20568-20572. PMCID: PMC3251117

4. Proteins assisting in actin filaments disassembly.

Because actin filaments severing by cofilin may be too slow for rapidly moving or dividing cells, there is great interest in proteins that may partner with cofilin to assist in its function. One of the main candidates in this category is the ubiquitous protein coronin (in its various isoforms). In a recent collaboration with Dr. Goode we have shown by TIRF microscopy that coronin accelerates several-fold the rate of filaments severing by cofilin. It may do this via local effects on actin structure, as shown in that work, and/or - as suggested by our recent cryo-EM analysis of coronin-actin filaments - because coronin and cofilin can bind to each other on ADP-F-actin (but not on ADP-BeFx-F-actin). In an ongoing study we discovered that a dramatic and potent acceleration of actin severing by cofilin occurs due to a specific oxidation of M44 and M47 on F-actin by the enzyme Mical. The oxidation of M44 and M47 accelerates cofilin binding, destabilizes D-loop contacts with another actin, and – as we found - greatly amplifies filaments severing by cofilin. This work is continued. In another project/collaboration on a different disassembly protein, Inverted Formin 2 (INF2), we found that its severing of actin filaments involves opening of the INF2 dimer and encirclement of filaments, which we could image by AFM. The severing of filaments occurs most likely by insertion of INF2 segment between actin protomers in the filament.

a. Sharma, S., Grintsevich, E.E., Woo, J., Gure, P.S., Higgs, H.N., Reisler, E., and Gimzewski, J.K. (2014). Nanostructured self-assembly of inverted formin 2 (INF2) and F-actin-INF2 complexes revealed by atomic force microscopy. Langmuir 30,7533-7539. PMCID: PMC4082382
b. Ge, P., Otzug Durer, Z.A., Kudryashov, D.S., Zhou, Z.H., and E. Reisler, E. (2014). Cryo-EM reveals different coronin binding modes for ADP- and ADP-BeFx actin filaments. Nature Structural & Molecular Biology 21, 1075-1081. PMCID: PMC4388421.
c. Grintsevich, E.E., Yesilyurt, H.G., Rich, S.K., Hung, R.J., Terman, J.R., and Reisler, E. (2016). F-actin dismantling through a redox-driven synergy between Mical and cofilin. Nat Cell Biol. 18:876-885. PMCID: PMC4966907.
d. Grintsevich, E.E, Ge, P., Sawaya, M.R, Yesilyurt, H.G., Terman, J.R., Zhou, Z.H., Reisler, E. (2017). Catastrophic disassembly of actin filaments via Mical-mediated oxidation. Nat Commun. 19;8(1):2183, 1-10. PMCID: PMC5736627.

5. Interactions of neuronal protein Drebrin A with actin.

The need of neuronal cells for both actin filaments remodeling and stabilization focused our attention on the role of drebrin A, a major actin binding protein in neurons, and its interactions with actin. We expressed and purified drebrin and achieved initial mapping of its binding mode and sites on actin. We were able also to image drebrin's clustering on filaments by AFM, and determine its cooperative effect on the helical twist and mechanical properties of filaments. Consistent with the observed changes in filaments structure (longer average length and greater stability) we also found that drebrin stabilizes lateral interactions in the filaments and rescues the assembly of polymerization impaired actin mutants. We showed that drebrin competes with cofilin for binding to actin. We also found that the strongest filaments stabilizing effect of drebrin is at their barbed ends, suggesting its possible interplay with polymerization nucleation factors.

a. Grintsevich, E.E., Galkin, V.E., Orlova, A., Ytterberg, A.J., Mikati, M.M., Kudryashov, D.S., Loo, J.A., Egelman, a. E.H., and Reisler, E. (2010). Mapping of drebrin binding site on F-actin. J. Mol. Biol. 398, 542-554. PMCID: PMC2866048
b. Sharma, S., Grintsevich, E.E., Hsueh, C., Reisler, E., and Gimzewski, J.K. (2012). Molecular cooperativity of drebrin1-300 binding and structural remodeling of F-actin. Biophys. J. 103, 275-283. PMCID: PMC3400778
c. Mikati, M.A., Grintsevich, E.E., and Reisler, E. (2013). Drebrin-induced stabilization of actin filaments. J. Biol. Chem. 288, 19926-19938. PMCID: PMC3707693
d. Grintsevich, E.E., and Reisler, E. (2014). Drebrin inhibits cofilin-induced severing of F-actin. Cytoskeleton 7, 472-483. PMCID: PMC4465285

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BIOGRAPHICAL SKETCH

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NAME: Aguirre, Roman

eRA COMMONS USER NAME (credential, e.g., agency login): RAGUIRRE2

POSITION TITLE: Graduate Student Researcher

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	Completion Date MM/YYYY	FIELD OF STUDY
California Polytechnic State University- Pomona, Pomona CA	B.S	09/2017	05/2021	Chemistry: Biochemistry Option
University of California Los Angeles (UCLA), Los Angeles CA	Ph.D.	09/2021	Present	Biochemistry, Molecular, and Structural Biology

A. Personal Statement

The main objective of the parent R01 grant is to further develop and enhance methods for the determination of high-resolution structures of using cryogenic electron microscopy (cryoEM). The supplement proposed here will further expand the parent grant's goals through the development of a protein imaging scaffold. Through the rigid attachment of a protein of interest (POI) to a much larger biomolecule, a small POI is able to overcome the size limitation that hinders many proteins from being imaged at high resolution. This would allow for the visualization of any protein or macromolecule, further establishing cryoEM as a powerful structural technique. Additionally, the development of a scaffold that can attach any protein of interest opens avenues for other potential applications. One such application is that of vaccine development and delivery of therapeutics through the display of an antigen.

My career as a scientist and involvement in structural biology began at the start of my second year of university at Cal Poly Pomona, where I joined Dr. Kathryn McCulloch's lab as an undergraduate researcher. After getting acquainted with bacterial recombinant systems and protein purification, I started my independent project on the purification and structural characterization of BaiK, a proposed Coenzyme A transferase found in human gut microbiota. This enzyme is part of the bile acid inducible operon (bai) that is actively involved in the dehydroxylation of bile acids, resulting in accumulation that has been linked to gastrointestinal diseases and cancers. During my time in the McCulloch group, I was also able to prepare and present my research through posters on three separate occasions, one being the CSUperb symposium, in which researchers from all 23 California State Universities gather to present and share their findings. At the end of my undergraduate studies, I submitted my thesis titled "Structural and Homology Model Studies of BaiK, a Proposed Coenzyme A Transferase Protein". I was particularly intrigued about the BaiK enzymatic mechanism elucidation using structural techniques, which ultimately influenced me to pursue a PhD in structural biology to learn about other powerful methods such as cryoEM

During the first year of my PhD program, I was in Dr. Todd Yeates' lab, where I worked on developing Design Ankryin Repeat Protein (DARPin) binders for various small proteins of interest for the purpose of imaging them using a self-assembling protein cage as a scaffold. During this time, I became proficient in protein expression and purification, protein design techniques, and library-based directed evolution approaches using yeast cells. Dr. Yeates is currently providing my funding as a Graduate Student Researcher and will continue to do so until the end of the 2023 academic school year.

Having done research in structural biology labs has reinforced the importance of understanding the underlying molecular mechanisms at the protein level, which has motivated me to pursue resolving high resolution structures using cryoEM in Dr. Zhou's lab. Through my experience working with different methods such as recombinant expression and purification, these techniques will aid me in determining the structures of large complexes. Additionally, through guidance and mentorship from Dr. Zhou, fellow lab members, and EICN staff, I am confident that I will have a support system that will help me thrive. The proposed research supplement will aid me in developing my career as a researcher aspiring to work in an academic setting solving complex structures using cryoEM and cryoET.

B. Positions, Scientific Appointments and Honors

Positions and Employment

2021-present Ph.D. in Biochemistry, Molecular and Structural Biology
2022 Teaching Assistant for Introduction to Biochemistry (Spring 2022) and Physical Biochemistry (Winter and Fall 2022)

Honors

2021 Summa Cum laude, B.S in Chemistry, Biochemistry Option

C. Contributions to Science

1. Structural and enzymatic characterization of BaiK, a proposed coenzyme A transferase in *Clostridum Scindens*: As an undergraduate student researcher in Dr. McCulloch's lab, I was investigating BaiK, a proposed coenzyme A transferase involved in the dehydroxylation of several bile acids found in the human GI tract. As part of my training, I learned how to express and purify protein by taking a recombinant approach using *E. Coli*. This consisted of learning and implementing several techniques such as plasmid preparation and insertion, expression, solubility screening, and purification using immobilizing-metal affinity and size exclusion chromatography. Additionally, I became experienced with homology model and bioinformatic tools such as Modeller and BLAST for the remote portion of this project. During this time, I was able to present research posters on three separate occasions during my time at Cal Poly Pomona. These experiences introduced me to and prepared me for doing research as a graduate level.

2. Development and biophysical characterization of protein cages designed with a protein fragment-based machine learning algorithm and raising DARPin binders for small interest of proteins for cryoEM imaging purposes
As a PhD student in the Yeates lab, my training as a graduate student researcher began by assisting PhD candidate Kyle Meador in his project that consisted of generating *de novo* symmetric protein assemblies for various biomedical applications via a computational approach. It was my task to help with the validation of these designed assemblies through their expression, purification, and characterization. I used traditional *E. Coli* expression and purification techniques to attempt to purify over 40 of these designed constructs. I also used different biophysical methods such as X-ray crystallography, small-angle X-ray scattering, and negative stain electron microscopy to validate designed cages. Afterwards, I began working on my project that focused on generating designed ankyrin repeat protein (DARPin) binders for small therapeutic proteins to image using symmetrical assemblies for cryoEM studies. In this project, I learned how to work with DNA libraries and directed evolution methods using fluorescence-activated cell sorting with yeast cells.

1. Meador K, Castells-Graells R, Aguirre R, Sawaya MR, Arbing MA, Sherman T, Senarathne C, Yeates TO. (2024) A suite of designed protein cages using machine learning and protein fragment-based protocols. **Structure** 32, 1-15**.**

4. Structural characterization of actin binding proteins that sever and shield actin filaments
Through a collaborative effort with Dr. Emil Reilser's group, I am in the process of structurally characterizing various actin binding proteins (ABPs) that regulate filamentous actin and their branched networks using cryoEM. Of particular interest are DrebrinA and Inverted Formin 2, two proteins that regulate F-actin through the shielding and severing of filaments. The dynamic nature of these ABPs make them and the filaments they

are bound to great targets for single particle cryoEM structure determination. I have also using varying concentrations of these proteins to visualize and quantify severing events using negative stain EM.

1. Rajan S, Aguirre R, Zhou HZ, Hauser P, Reisler E. (2024) Drebrin Protects Assembled Actin from INF2-FCC-mediated Severing and Stabilizes Cell Protrusions. **Journal of Molecular Biology** 436 (4).

D. Scholastic Performance

YEAR	COURSE TITLE	GRADE
2021	Chem 269A : Protein Structure	A
2021	Chem 269B: Biocatalysis and Bioenergetics	
2021	Chem 269C: Nucleic Acid Structure	A
2021	Chem 269D: Gene Expression	
2021	Chem 269E: Biomolecular Structure and Regulation	A-
2022	Chem M230B/M230D: Structural Molecular Biology with Laboratory	B/A
2022	Protein Mass Spectrometry	A