BIOGRAPHICAL SKETCH

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NAME: Michael K. Rosen

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POSITION TITLE: Professor and Chair, Department of Biophysics; Investigator, HHMI

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 Michigan, Ann Arbor	B.S./B.S.E.	05/1987	Chemistry/Chem.Engin.
Cambridge University, England	C.P.G.S.	07/1988	Chemistry
Harvard University, Cambridge	Ph.D.	07/1993	Chemistry
Mount Sinai Hospital & U. of Toronto, Canada	Postdoc	11/1995	Mol. Biol./NMR

A. Personal Statement

Research in my group focuses on understanding the physical mechanisms and biological consequences of macromolecular phase separation. Several years ago we discovered that interactions between multivalent actin regulatory proteins and their multivalent ligands cause oligomerization of the proteins and concomitant phase separation. In three dimensions this produces liquid droplets suspended in aqueous media. When one of the components is attached to a membrane this produces two-dimensional puncta. The signaling pathways emanating from the cell surface receptors Nephrin and T Cell Receptor are our workhorses for these studies, in both two- and three-dimensional platforms. We have reconstituted both pathways in vitro on supported lipid bilayers, and used them to discover important functional consequences of phase separation, including increased specific activity of the components toward Arp2/3 complex-mediated actin assembly. We have also studied them in cells using quantitative imaging to characterize interactions between two-dimensional phase separated clusters and the cortical actomyosin cytoskeleton.

B. Positions and Honors

Positions Held:

1982-1987 Honors Chemistry & Chemical Engineering Student, University of Michigan: Graduated With Highest Honors in Chemistry, Summa Cum Laude in Chemical Engineering
1987-1988 Winston Churchill Scholar, University of Cambridge
1988-1993 Graduate Student in Organic Chemistry, Harvard University: NSF Graduate Fellow
1993-1995 Damon Runyon-Walter Winchell Post-Doc. Fellow, Lunenfeld Res. Inst. and U. of Toronto
1996-2000 Assist. Member, Cellular Biochem. and Biophys. Prog., Memorial Sloan-Kettering Cancer Center
1996-2000 Assistant Professor, Dept. of Biochem. & Struct. Biol., Weill Medical College of Cornell Univ.
2000-2001 Assistant Investigator, HHMI (position relinquished on move to UTSW, December, 2001)
2000-2001 Assoc. Mem., Cellular Biochem. & Biophys. Prog., Memorial Sloan-Kettering Cancer Center
2000-2001 Assoc. Professor, Department of Biochemistry and Structural Biology, Weill Medical College
2001-2005 Associate Professor, Department of Biochemistry, UT Southwestern Medical Center
2006-2009 Carolyn R. Bacon Professor of Medical Science and Education
2005- Professor, Department of Biochemistry, UT Southwestern Medical Center
2005- Investigator, HHMI
2009- Mar Nell and F. Andrew Bell Distinguished Chair in Biochemistry
2012- Inaugural Chair, Department of Biophysics, UT Southwestern Medical Center

Honors:

1987 Outstanding Graduating Senior, Department of Chemical Engineering, University of Michigan.

1987 Outstanding Graduating Senior, Department of Chemistry, University of Michigan.
1987 National Science Foundation Graduate Research Fellowship.
1987 Winston Churchill Foundation Scholarship.
1989 Harvard University Danforth Center Distinguished Teaching Award.
1991 ACS, Div. of Organic Chemistry, Graduate Fellowship sponsored by Merck, Sharp & Dohme.
1993 Damon Runyon-Walter Winchell Post-Doctoral Fellowship.
1997 Beckman Young Investigator Award.
1997 Presidential Early Career Award for Scientists and Engineers (PECASE).
1998 Kimmel Scholar Award, Sidney Kimmel Foundation for Cancer Research.
2000 Howard Hughes Medical Institute.
2001 Boyer Award, Memorial Sloan-Kettering Cancer Center.
2005 Howard Hughes Medical Institute.
2006 Inaugural Edith and Peter O'Donnell Award, Texas Acad. of Medicine, Engineering & Science.
2013 John T. Edsall Lecture, Dept. of Molecular & Cellular Biology, Harvard University.
2013 Kensal E. van Holde Lectureship, Marine Biological Laboratory, Woods Hole.
2015 Richard D. Berlin Lecture, Dept. of Cell Biology, U. Connecticut Health Center.
2016 Martin Kamen Lecture, Dept. of Chem. & Biochem., UC San Diego.

C. Contributions to Science

Research in my lab combines structural biology (NMR spectroscopy and x-ray crystallography) with biochemistry and light microscopy to elucidate the structural and energetic mechanisms by which signaling pathways control the actin cytoskeleton. Dynamic rearrangements of actin play a central role in diverse cellular processes, including motility, division, membrane trafficking and bacterial/viral infection. Actin dynamics are controlled *in vivo* on nm to μm spatial scales and second to minute temporal scales. A deep understanding of actin regulation requires quantitative studies of the structural and dynamic properties of nm-scale proteins/complexes, as well as the mechanisms by which these proteins are organized into μm-scale cellular assemblies with macroscopic properties and behaviors. Our work has provided such a multi-scale understanding of actin biology through a series of important discoveries and conceptual advances. These can be grouped into the five areas described below.

1. Mechanisms of actin regulatory signaling by WASP proteins

The earliest work from my lab focused on understanding how proteins in the Wiskott-Aldrich Syndrome protein family integrate diverse upstream signals to activate the Arp2/3 complex, producing branched actin filament networks. Our NMR studies revealed the structural and energetic bases of autoinhibition in the archetypal WASP protein, and how the Rho family GTPase, Cdc42, relieves autoinhibition, driving activation of the Arp2/3 complex. This work provided the first structural explanation of GTPase control of actin assembly. We also revealed that and how WASP integrates signals from Cdc42 and kinase/phosphatases. We showed that this mechanism enables long-term storage of information by WASP following decay of GTPase signals. This biochemical circuitry allows WASP to respond to the levels and timing of GTPase and kinase signals, and provides mechanisms to specifically achieve transient or persistent actin remodeling, as well as long-lasting potentiation of actin-based responses to kinases. Later we found that dimerization greatly enhances the affinity of WASP proteins for the Arp2/3 complex, an observation that unified many previously unexplained biochemical and biological observations under a new hierarchical model for signaling through these pathways. In addition to informing deeply on actin regulatory pathways, these findings established basic principles in biophysics, structural biology, signal transduction and medicine. By learning how Cdc42 relieves autoinhibition in WASP, we were the first to discover the molecular mechanism by which a Ras-family GTPase activates a downstream effector. This mechanism also provided the basis for understanding the human disease X-linked neutropenia and its relationship to the Wiskott-Aldrich Syndrome. Our discovery that part of WASP could adopt 3 different structures in 3 different contexts represented one of the first and strongest demonstrations of how an intrinsically unstructured protein domain could adopt functionally distinct structures in different contexts. Our studies of WASP phosphorylation were paradigmatic demonstrations of how an apparently simple signaling switch can display complex behaviors, including noise filtering and molecular memory.

1. Abdul-Manan, N., Aghazadeh, B., Liu, G. A., Majumdar, A., Ouerfelli, O., Siminovitch, K. A., Rosen, M. K.: Structure of Cdc42 in Complex with the GTPase Binding Domain of the Wiskott-Aldrich Syndrome Protein, *Nature*, **1999**, *399*, 379-383.

2. Kim, A. S., Kakalis, L. T., Abdul-Manan, N., Liu, G. A., Rosen, M. K.: Autoinhibition and activation mechanisms of the Wiskott-Aldrich syndrome protein, *Nature,* **2000**, *404*, 151-158.
3. Torres, E. and Rosen, M. K.: Contingent Phosphorylation/Dephosphorylation Provides Mechanisms of Signal Integration and Molecular Memory in WASP, *Molecular Cell*, **2003**, *11*, 1215-1227.
4. Padrick, S. B., Cheng, H.-C. C., Ismail, A., Panchal, S. C., Doolittle, L. K., Kim, S., Skehan, B.M., Umetani, J., Brautigam, C. A., Leong, J. M., Rosen, M. K., Hierarchical Regulation of WASP/WAVE Proteins, *Mol. Cell*, **2008**, *32*, 426-438. PMC2680354.

2. Mechanisms of actin regulatory signaling by WAVE proteins

More recently, our efforts have been directed to the WAVE subgroup of the WASP family, whose members control cell spreading and migration, and cell-cell adhesion. In cells, the WAVE proteins are constitutively incorporated into the 400 kDa heteropentameric WAVE Regulatory Complex (WRC). Like WASP, the WRC integrates diverse upstream signals, including those from the Rac GTPase, acidic phospholipids and kinases, and transmits them to the Arp2/3 complex. My lab was the first to reconstitute the WRC recombinantly, enabling definitive demonstration that the complex is basally inhibited toward the Arp2/3 complex. These findings resolved a long-standing debate in the field regarding the biochemical function of the WRC. We went on to determine the crystal structure of the intact WRC. The structure, and complementary biochemical analyses explained the key regulatory functions of the complex--why it is inactive on its own; how it can be activated by Rac and kinases, and recruited to membranes by acidic phospholipids; and how these inputs act cooperatively to promote localized actin assembly in cells. Very recently, using a combination of biochemical, structural, bioinformatic and genetic analyses, we discovered a novel WRC-binding peptide motif that is found in over 100 diverse membrane proteins. In collaborative work, we showed that WRC interactions with this motif are important in oogenesis and neuronal targeting in flies and in synapse formation and axonal branching in worms, consistent with broad roles in organismic development. These findings directly link a large and diverse array of membrane proteins to the WRC and actin cytoskeleton for the first time. Parallel biochemical and EM studies of the WASP family member WASH, which controls actin assembly in membrane trafficking, led to the surprising discovery that WASH also functions as part of a large pentameric assembly that is distantly related (15-21 % sequence identity) to the WRC and can be activated by ubiquitin modification. Through our studies of WASP, WAVE and WASH, my lab has established the core physical principles that control actin assembly by the WASP family. These principles have had substantial impact in signal transduction, cell biology, immunology and neuroscience, and will frame all future studies of the WASP family.

1. Ismail, A. M., Padrick, S. B., Chen, B., Umetani, J., Rosen, M. K.: The WAVE Regulatory Complex is Inhibited, *Nat. Struct. Mol. Biol.,* **2009**, *16(5)*, 561-563. PMC2716658.
2. Chen, Z., Borek, D., Padrick, S. B., Gomez, T. S., Metlagel, Z., Ismail, A., Umetani, J., Billadeau, D. D., Otwinowski, Z., Rosen, M. K.: Cooperative Control Mechanisms of the Actin Regulatory WAVE Complex, *Nature*, **2010**, *468*, 533-538. PMC3085272.
3. Chen, B. S., Chen, Z., Pak, C. W., Brinkmann, K., Liao, Y., Shi, S., Henry, L., Grishin, N. V., Bogdan, S., Rosen, M. K.: The WAVE Reglatory Complex Links Diverse Receptors to the Actin Cytoskeleton, *Cell,* **2014*,*** *156*, 195-207. PMC4059610.
4. Jia, D.†, Gomez, T. S.†, Metlagel, Z., Umetani, J., Otwinowski, Z., Rosen, M. K.*, Billadeau, D. D.*: WASH and WAVE Actin Regulators are Controlled by Analogous Structurally Related Complexes, *Proc. Natl. Acad. Sci. USA,* **2010**, *107(23)*, 10442-10447. PMC2890800.

3. Structural and energetic mechanisms of regulation of the protooncoprotein Vav1

The protooncoprotein Vav1 is a guanine nucleotide exchange factor (GEF) for Rho GTPases, which plays an important role in actin regulatory signaling. Vav1 is also an archetype of multidomain proteins regulated by autoinhibition. Central questions in such systems are: how do the domains act cooperatively to strongly suppress activity, and how does this suppression remain compatible with efficient activation? Initially we showed that in the minimal autoinhibited fragment of Vav1, the active site of the catalytic DH domain is blocked by an adjacent inhibitory helix, and explained how the helix is released by phosphorylation. Later we showed by NMR that phosphorylation and basal GEF catalysis occur through an excited state where the helix is dissociated from the DH domain, since the population of this state is linearly correlated with phosphorylation kinetics (k_{cat}/K_M) and GEF activity. This study was one of the first to demonstrate the quantitative importance of a dynamic equilibrium to protein function. To address the complex dynamics in Vav1, we also developed procedures to quantitatively determine the kinetic and thermodynamic parameters in 4-state-equilibrium systems. Finally, we showed by NMR that the flanking CH, PH and zinc finger domains in Vav1 shift the

autoinhibitory equilibrium in the helix-DH core ~10-fold toward the inhibited state, explaining how these domains cooperatively enhance autoinhibition. We also determined the crystal structure of Vav1, revealing how CH-PH contacts cause this shift in the core equilibrium. We demonstrated that this layered inhibitory construction enables rapid, stepwise activation: initial phosphorylation events first disrupt CH contacts, enabling rapid disassembly of the core through phosphorylation of the inhibitory helix. This work illustrates a likely general mechanism by which the opposing requirements of strong suppression of activity and efficient kinetics of activation can be achieved in multidomain systems.

1. Aghazadeh, B., Lowry, W.E., Huang, X.-Y., Rosen, M.K.: Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation, *Cell*, **2000**, *102*, 625-633.
2. Li, P., Martins, I. R. S., Amarasinghe, G. K., Rosen, M. K.: Internal Dynamics Control Activation and Activity of the Autoinhibited Vav DH Domain, *Nat. Struct. Mol. Biol.*, **2008**, *15(6)*, 613-618. PMC2512264.
3. Yu, B., Martins, I. R. S., Li, P., Amarasinghe, G. K., Umetani, J., Fernandez-Zapico, M., Billadeau, D. D., Machius, M., Tomchick, D. R., Rosen, M. K.: Structural and Energetic Mechanisms of Cooperative Autoinhibition and Activation of Vav1, *Cell*, **2010**, *140*, 246-256. PMC2825156.
4. Li, P., Martins, I. R. S., Rosen, M. K.: The Feasibility of Parameterizing Four-State Equilibria Using Relaxation Dispersion Measurements, *J. Biomol. NMR*, **2011**, *51*, 57-70. PMC3229927.

4. Mechanisms of actin nucleation by formins, WH2 proteins and the Arp2/3 complex

In addition to studying the signaling pathways that control actin nucleation, we have also made key contributions to understanding the nucleation factors themselves, the formin proteins the so-called WH2 domain-containing proteins and the Arp2/3 complex. In 2005, we reported the first structure of the actin-nucleating FH2 domain of a formin protein in complex with actin monomers. This complex was the only atomic resolution structure of an actin nucleation factor bound to actin from 2005 until 2013. The structure and complementary biochemical analyses provided models to explain many of the key functions of formins. The structure revealed that the FH2 domain binds actin monomers in an orientation closely resembling that in the actin filament, suggesting a templating mechanism for nucleation. FH2 binding leaves the actin pointed end free to bind the bulk filament, but blocks the barbed end, explaining how formins attach to filaments and prevent other molecules from binding the barbed end. The work also suggested how formins can remain attached to the filament barbed end, while the end grows or shrinks through the addition or loss of actin monomers. Our related study explained how formins are activated by Rho-family GTPases. More recently we described the structure of the WH2-based actin nucleation factor VopL, free and in complex with actin. This structure showed remarkable analogy to the FH2-actin complex, in that VopL holds multiple actin monomers in a filament-like organization. The two structures together thus suggest that templating filament-like structures is a general mechanism by which actin nucleation factors function. Finally, our studies of the Arp2/3 complex have shown that, contrary to previous models, the assembly is activated by two WASP proteins, which bind to distinct sites. In collaborative single-molecule studies we also answered a key mechanistic question, showing that the initiation of new filament growth is triggered by dissociation of WASP from an Arp2/3-actin_filament-actin$_2$-WASP$_2$ assembly.

1. Otomo, T., Tomchick, D. R., Otomo, C., Panchal, S. C., Machius, M., Rosen, M. K.: Structural Basis of Actin Filament Nucleation and Processive Capping by a Formin Homology 2 Domain, *Nature,* **2005**, *433*, 488-494.
2. Yu, B., Cheng, H.- C., Brautigam, C. A., Tomchick, D. R., Rosen, M. K.: Mechanism of Actin Filament Nucleation by the Bacterial Effector VopL, *Nat. Struct. Mol. Biol.*, **2011**, *18(9)*, 1068-1074. PMC3168117.
3. Zahm, J. A., Padrick, S. B., Chen, Z., Yunus, A. A., Henry, L., Tomchick, D. R., Chen, Z., Rosen, M. K.: Structure of an Actin Trimer Bound to the Bacterial Effector VopL, *Cell*, **2013***, 155(2)*, 423-34. PMC4048032.
4. Smith, B. A., Padrick, S. B., Doolittle, L. K., Daugherty-Clarke, K., Correa Jr., I. R., Xu, M. -Q., Goode, B. L., Rosen, M. K.*, Gelles, J.*: Three-Color Single Molecule Imaging Shows WASP Detachment from Arp2/3 Complex Triggers Actin Filament Branch Formation, *eLife*, **2013**:2:e01008. PMC3762362.

5. Mechanisms and functions of protein phase separation

Three years ago we reported a discovery that has taken us in new, exciting directions. Drawing from classical polymer science, we reported that interactions between multivalent macromolecules can produce sharp, liquid-liquid demixing phase transitions, generating micron-sized liquid droplets in aqueous solution. This macroscopic transition corresponds to a molecular sol-gel transition between small complexes and large, dynamic supramolecular polymers. These effects are highly general, and are observed for a variety of protein-protein and also protein-RNA systems, both *in vitro* and in cells. The assembly and disassembly of the phase separated structures can be rapidly controlled by covalent modifications that change the valency of the

interacting species, explaining how these transitions could be controlled *in vivo*. The similarity in composition (multivalent macromolecules) and physical properties (dynamic liquids) of the phase separated droplets to so-called cellular bodies—the numerous membrane-less cellular compartments such as PML nuclear bodies and P bodies—suggests that multivalent phase separation may be the mechanism by which these compartments form in the cell. This idea represents the first physical framework to understand the structure, composition and function of these enigmatic cellular compartments. More recently, we showed that analogous processes can generate micron-sized, dynamic clusters of membrane proteins, and thus could account for the observed puncta of numerous membrane proteins observed *in vivo*. In the actin regulatory system composed of the adhesion receptor, nephrin, and its intracellular targets Nck and N-WASP, clustering corresponds to a sharp increase in activity toward the Arp2/3 complex, explaining how clustering can be used to control actin assembly. Although still largely untested in vivo, the concept of multivalent polymerization and phase separation could provide a very general mechanism to control spatial organization, dynamics, biochemical composition and cellular function of numerous micron scale cell structures, ranging from receptor clusters at membranes to RNA-protein granules to chromatin domains.

1. Li, P., Banjade, S., Cheng, H.- C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J. V., King, D. S., Banani, S. F., Russo, P. S., Jiang, Q-. X., Nixon, B. T. Rosen, M. K.: Phase Transitions in the Assembly of 2. Multi-Valent Signaling Proteins, *Nature*, **2012**, *483*, 336-40. PMC3343696.
Banjade, S. and Rosen, M.K.: Phase Transitions of Multivalent Proteins Can Promote Clustering of Membrane Receptors, *eLife*, **2014**, *3*, e04123. PMC4238058.
3. Lin, Y., Protter, D. S. W., Rosen, M. K.*, Parker, R.*: Formation and Maturation of Phase Separated Liquid Droplets by RNA Binding Proteins, *Mol. Cell*, **2015**, *60(2),* 208-19. PMC4609299.
4. Su, X.†, Ditlev, J. A.†, Hui, E., Banjade, S., Okrut, J., King, D. S., Taunton, J., Rosen, M. K.*, Vale, R. D.*: Phase Separation of Signaling Molecules Promotes T Cell Receptor Signal Transduction, *Science*, **2016**, *352*, 595-9. PMC4892427.

Complete List of Published Work in Pubmed: http://www.ncbi.nlm.nih.gov/pubmed/?term=rosen+mk

D. Research Support

Current:

NIH RO1 (Rosen P.I.), #GM56322 04-01-16 to 03-31-20
"Structural Study of GTPase Regulators and Effectors"
Structural, biochemical and biophysical studies of the actin monomer. We seek to understand the conformational dynamics of actin, and how they are modulated by ligands including adenine nucleotides, actin filament nucleation factors, profilin and cofilin, and also by various mutations.
Role: PI.

Welch Foundation Grant (Rosen P.I.), #I1544 06-01-17 to 05-31-20
"2D Phase Separated Protein Polymers: Interactions with Actin"
Analysis of the interactions between actin filaments and phase separated polymers in the T cell receptor and Nephrin signaling pathways.
Role: PI

HHMI 01-01-2017 to 12-31-22
Funds support programs in phase separation of multivalent proteins involved in PML nuclear bodies, RNA granules and the immunological synapse.

Pending:

None