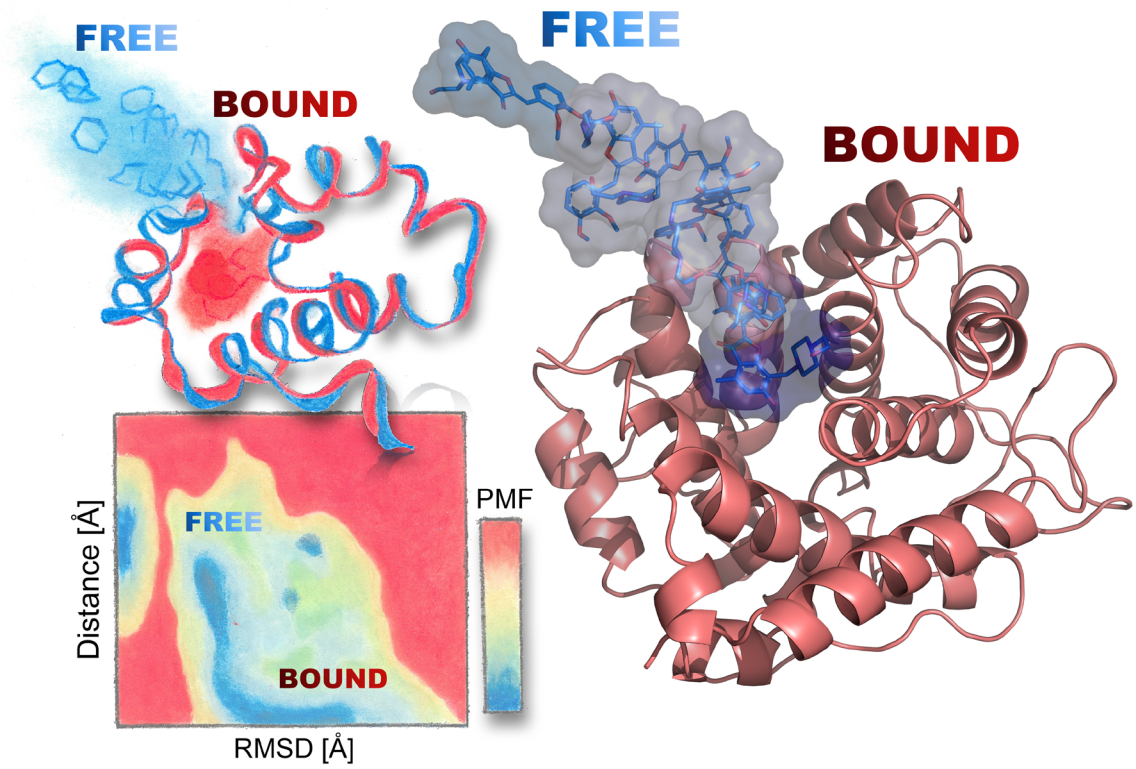
# The Morikis Memorial Symposium



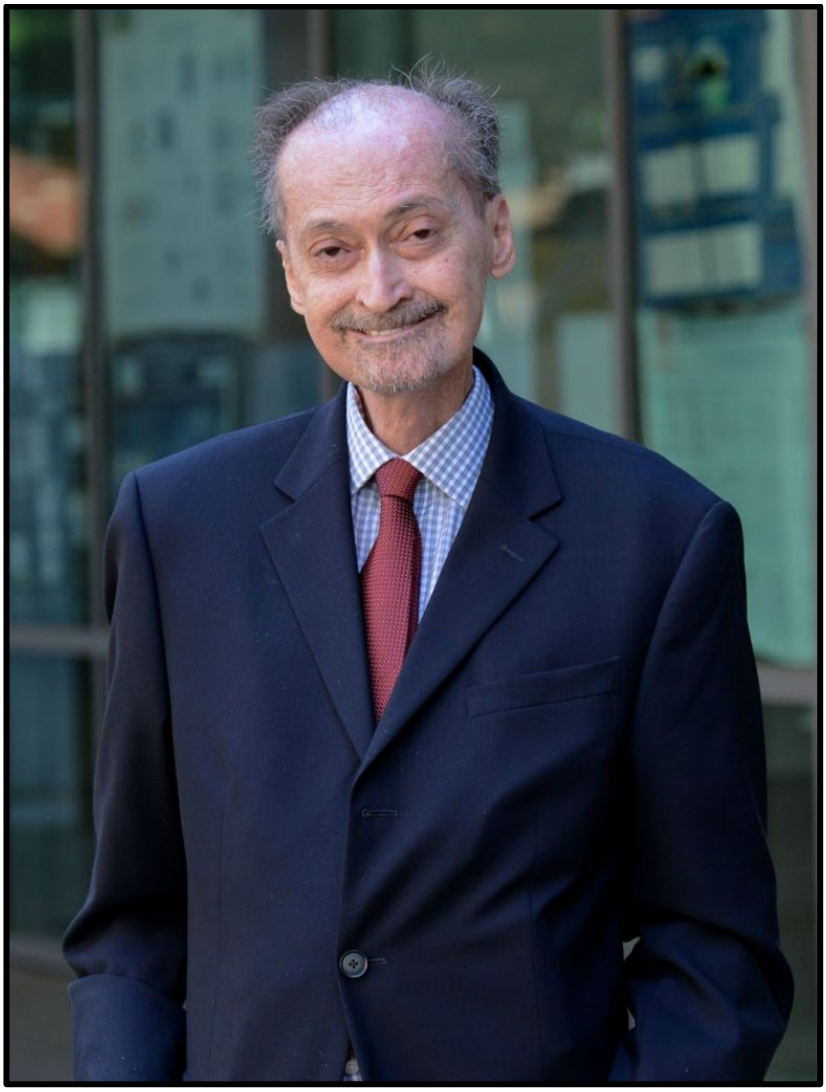

***February 10, 2020 – University of California Riverside***

***A tribute to the work and accomplishments of***
***Professor Dimitrios Morikis***

**Cover Picture: Professor Morikis studies on protein ligand binding**

Adapted from: Gorham D. G., Nuñez V., Lin J., Rooijakkers S. M. H., Vullev I. V. and Morikis D. Discovery of Small Molecules for Fluorescent Detection of Complement Activation Product C3d. *J. Med. Chem.* **2015**, *58*, 9535−9545; and from: Amaro R. E., Madura J. D., Wong C. F. Tribute to J. Andrew McCammon. *J. Phys. Chem. B*. **2016**, *120*, 8055-8056

# Prof. Dimitrios Morikis



Professor Morikis earned a Ph.D. in Physics from Northeastern University and, following his postdoctoral work at Scripps Research Institute and UC San Diego, he began his professorial career at UC Riverside's Department of Chemical and Environmental Engineering. In 2006, he became a founding faculty member of the Department of Bioengineering and later became part of the faculty of the graduate program in Biomedical Sciences of the School of Medicine and of the Institute for Integrative Genome Biology.

He published more than 130 peer-reviewed research publications, facilitated more than $4 million in extramural research funds, and was named a Fellow of the American Association for the Advancement of Science and a Fellow of the American Institute for Medical and Biological Engineering.

# 1 Description

**About The Morikis Memorial Symposium**

Dimitrios Morikis, UC Riverside Professor of Bioengineering, passed away on May 27, 2019. Professor Morikis is well known for his work in immunophysics and immunoengineering, where he used physics and engineering approaches to understand molecular mechanisms of immunology, develop disease models, and design new drugs and molecular sensors for autoimmune and inflammatory diseases.

***"Throughout the years, there was a natural evolution from biophysics to bioengineering via structural biology and computational chemistry, which is consistent with the evolution of my research interests and training,"*** Professor Morikis said during an interview about how his research had shifted.

His research focus on immune system function came after a personal struggle with illness. "In 1994, I got sick with a life-threatening disease of the bone marrow. Thanks to modern medicine and after a strenuous process, I recovered and managed to get back to research," he explained during the interview. *"It was in 1995 when I decided to dedicate the rest of my research life in studying the molecular basis of immune system function and trying to develop means to fight immune-mediated diseases."*

Professor Morikis' most recent research was highly cross-disciplinary and focused on utilizing a blend of molecular-level and systems-level science and collaborations with researchers working on cell and tissue levels and in vivo studies. A prime focus of his work was the development of affordable potential pharmaceuticals for rare diseases. He led the immunophysics field as the Editor-in-Chief of BMC Biophysics.

The ***Professor Dimitrios Morikis Memorial Symposium*** is a tribute to the work and accomplishments of Professor Dimitrios Morikis. Professor Morikis was a founding faculty member of the Department of Bioengineering at the University of California, Riverside and his scientific energy was vital in shaping the program into what it is today. His research, however, extended well beyond our campus and has had an international impact. His passing on May 27, 2019 has left us with a tremendous void however, we are grateful for being among those whose lives he has touched.

Professor Morikis was a caring teacher and devoted mentor. To keep his legacy alive, at this memorial symposium, we gather colleagues from all across the world who have been inspired by Dimitrios.

We hope this will inspire young scientists, students and postdocs in biophysics and bioengineering.

# 2 Program

## Morning session

- 8:00 to 9:00 – Check-in, Registration, Poster Setup, and Continental Breakfast

### Welcome Remarks

- 9:00 to 9:10 – **Victor G. J. Rodgers**
  *Professor, Department of Bioengineering, UCR*

- 9:10 to 9:20 – **Christopher Lynch**
  *Dean, Marlan and Rosemary Bourns College of Engineering, UCR*

### Keynote Speaker

- 9:30 to 10:20 – **J. Andrew McCammon**
  *Diffusional Interactions of Biomolecules*

### Coffee Break

- 10:20 to 10:30

### Computational and multi-scale modeling in biology and medicine
### Chair: Giulia Palermo

- 10:30 to 10:50 – **Chris Kieslich**
  *Computational Tools for Optimizing Electrostatic Properties of Proteins*

- 10:50 to 11:10 – **Douglas Tobias**
  *Molecular Dynamics Simulation Studies of the Gating and Pharmacology of the Human HV1 Proton Channel*

- 11:10 to 11:30 – **Roya Zandi**
  *Self-assembly of Mature Conical HIV Particles: The Role of Genome and Membrane*

- 11:30 to 11:50 – **Reed Harrison**
  *Immunoengineering Peptides to Monitor Complement Activation*

### Lunch and Posters

- 12:00 to 1:00 – **Lunch and Posters**

## Afternoon session

### Molecular Biophysics: from NMR to Biophotonics
### Chair: Victor G. J. Rodgers
### Co-Chair: Valentine I. Vullev

- 1:00 to 1:20 – **Srigokul Upadhyayula**
  *High Spatial & Temporal Resolution Biological Imaging Across Scales*
- 1:20 to 1:40 – **Katarzyna Rybicka-Jasińska**
  *Porphyrins as Photoredox Catalysts in Efficient C-C Bond Formation*
- 1:40 to 2:00 – **Paul Champion**
  *Proton Tunneling in Proteins*
- 2:00 to 2:20 – **George P. Lisi**
  *NMR Reveals Dynamic Signatures of Latent Allostery and Redox Sensitivity in Macrophage Migration Inhibitory Factor*
- 2:20 to 2:40 – Intermission
- 2:40 to 3:00 – **Peter Wright**
  *Role of Intrinsically Disordered Proteins in Cellular Signaling and Regulation*
- 3:00 to 3:20 – **Rafael Brüschweiler**
  *Observing Functional Protein Motions on Unchartered Timescales by Nanoparticle-Assisted NMR Spin Relaxation*
- 3:20 to 3:40 – **Patricia Jennings**
  *TBA*

### Coffee Break

- 3:40 to 3:50

### Immunology, immunophysics, and immunoengineering
### Chair: Mark Alber

- 3:50 to 4:10 – **Elias Lolis**
  *TBA*
- 4:10 to 4:30 – **Nehemiah T. Zewde**
  *TBA*
- 4:30 to 4:50 – **Vasilios Morikis**
  *Tensile Force Transmitted Through LFA-1 Bonds Mechanoregulate Neutrophil Inflammatory Response*

**Reflections**
**Chair: Victor G. J. Rodgers**

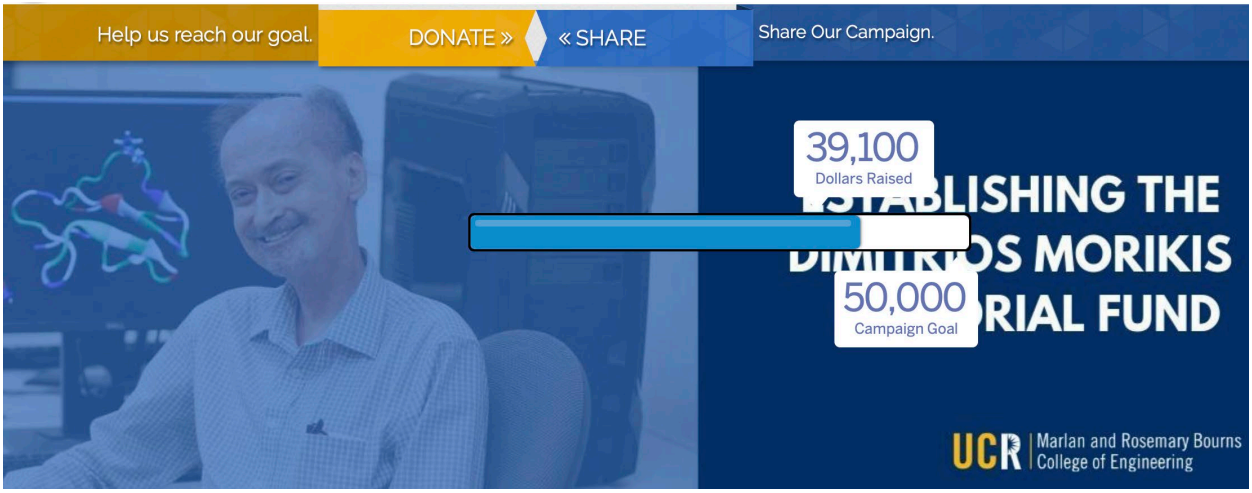
- 4:50 to 5:20 – **Individual remarks**
- 5:20 to 5:40 – **Jed Schwendiman**
- 5:40 to 6:00 – **Gloria Gonzalez-Rivera**

**Reception**

- 6:00 to 7:00

# 3 The Morikis Memorial Fund

***Please join us in honoring Professor Dimitrios Morikis by helping to establish the Dimitrios Morikis Bioengineering Scholarship in his memory***




To honor Professor Dimitrios Morikis' support to young scientists, the Marlan and Rosemary Bourns College of Engineering has started a crow funding campaign. This new endowment will provide annual scholarship support to undergraduate and graduate students in bioengineering.

To contribute:
https://crowdfunding.ucr.edu/campaigns/dimitrios-morikis-bioengineering-scholarship

# 4 Abstracts

## Observing Functional Protein Motions on Unchartered Timescales by Nanoparticle-Assisted NMR Spin Relaxation

**Rafael Brüschweiler**

Departments of Chemistry & Biochemistry and Biological Chemistry & Pharmacology,
The Ohio State University, Columbus, Ohio 43210

Multidimensional NMR relaxation experiments have played a pivotal role for the characterization of structural dynamics of many different proteins and their critical role in protein function. However, for NMR methodological reasons the observation of motions on timescales ranging from few nanoseconds to microseconds have remained elusive. A novel approach will be described that opens up this timescale window by the use of silica nanoparticles that transiently interact with the proteins of interest in solution. It is demonstrated how nanoparticle-assisted spin relaxation is able to uncover novel types of protein motions that were previously unobservable and how such motions can be used to validate molecular dynamics (MD) computer simulations.

# Proton Tunneling in Proteins

**Paul M. Champion**
Department of Physics and Center for Interdisciplinary Research on Complex Systems
Northeastern University
Boston, MA 02115
**Contact:** champ@neu.edu

Proton tunneling in proteins over a wide range of temperatures and time-scales has been probed experimentally[1] and analyzed theoretically[2-3]. For the case of thermally driven ground state proton transport (I→A in Fig. 1), associated with the *OH---O* hydrogen bonds of Green Fluorescent Protein (GFP), we observe surprisingly fast (~400ps) vibrationally activated proton tunneling at room temperature (Fig. 2). This suggests how high $pK_a$ amino acid residues, such as serine and threonine, can actively participate in water-based "proton wires" by helping to limit and control the direction of proton flow. A second example involves tunneling-based hydrogen atom abstraction from an aliphatic carbon substrate that is catalyzed by the enzyme soybean lipoxygenase (SLO). In analyzing this type of tunneling reaction, the often neglected anharmonic electronic repulsion between the proton donor and acceptor atoms is found to be a crucial factor. It is shown that a ~1 nN force, arising from a ~80 MV/cm electric field extrinsic to the local $CH\text{---}O^{q-}$ quantum interaction region, is required to establish a donor-acceptor distance distribution that is consistent with the measured SLO tunneling kinetics[4].

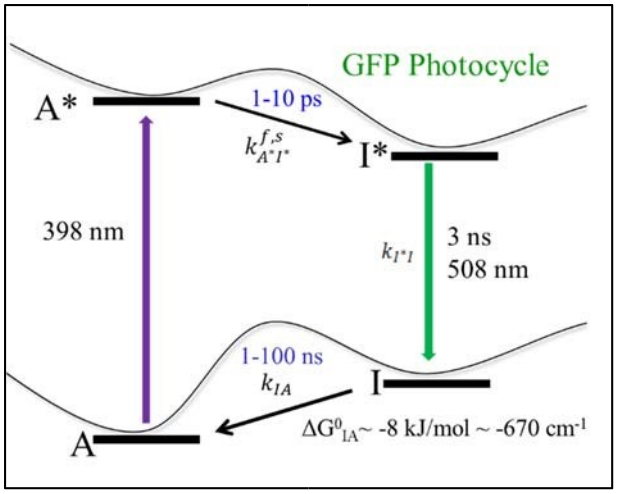



**Figure 1:** Photocycle of GFP showing ground state I→A transition.

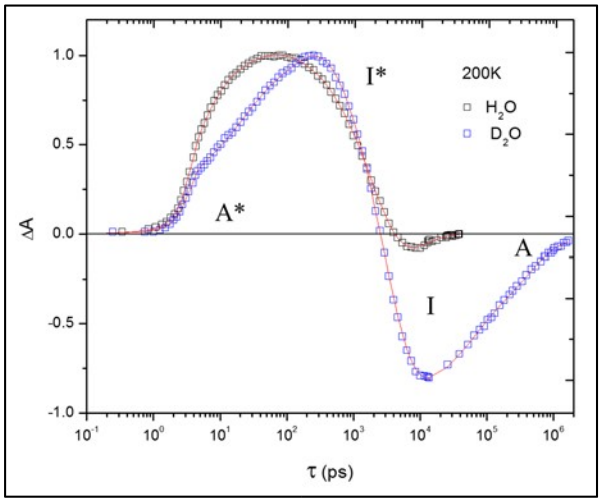



**Figure 2:** Logarithmic time population evolution of GFP and its isotope dependence

**References:**
[1] Salna, B.; Benabbas, A.; Sage, J.T.; van Thor, J.; Champion, P.M., *Nature Chem.* **2016**, *8*, 874-880.
[2] Benabbas, A.; Salna, B.;Sage, J.T.; Champion, P.M., *J. Chem. Phys.* **2015**, *142*, 114101-1-17.
[3] Salna, B.; Benabbas, A.; Champion, P.M., *J. Phys. Chem. A* **2017**, 121, 2199-2207. [4] Salna, B.; Benabbas, A.; Champion, P.M., *J. Phys. Chem. B* **2017**, 121, 6869-6881.

# Immunoengineering Peptides to Monitor Complement Activation

**R.E.S. Harrison, N.T. Zewde, Y.B. Narkhede, R. Hsu, V. Vullev, G. Palermo, D. Morikis**
Department of Bioengineering, University of California, Riverside
**Contact:** gpalermo@engr.ucr.edu

**Introduction:** Accumulation of C3d on membranes of tissues is a hallmark of complement activation. Though most tissues of self will be tagged with some small amount of C3d, an immune response from the complement system will result in more C3b that will bind non-specifically to foreign surfaces and native tissues alike. Complement regulators expressed by native cells then promote degradation of C3b to C3d, leading to accumulation of C3d on tissues of self. As such, C3d is an attractive biomarker to monitor complement activity.

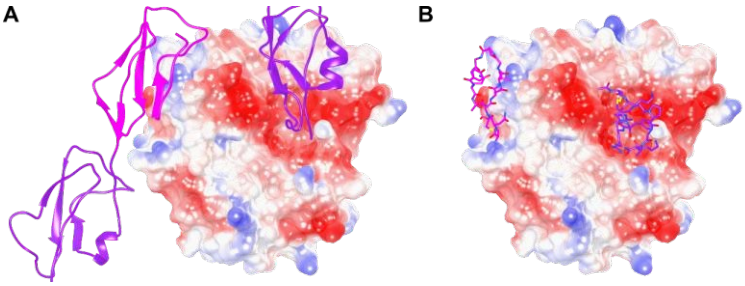



**Figure 1:** (A) Crystallographic complex of factor H in complex with C3d compared to (B) models for peptides in complex with C3d. Coulombic potentials are projected onto the surface of C3d where red indicates a negative potential of -10 kcal/mol/e and blue indicates a positive potential of 10 kcal/mol/e.

In age-related macular degeneration, a prevalent condition that leads to blindness, deposits in the eye called drusen are formed in the macula between Bruch's membrane and the retinal pigment epithelium. A mutation in complement regulator factor H confers risk for developing macular degeneration later in life, and formation of drusen in the macula is associated with inflammation mediated by complement. Thus, the complement system may play a pivotal role in progression of age-related macular degeneration.

Thus, we explore the structure-function relationship of factor H and C3d in order to better understand the role of risk polymorphisms and understand how complement proteins interact at the level of individual amino acids. We then leverage this information to design and optimize peptides targeting C3d that may have therapeutic or diagnostic potential.

**Materials and Methods:** Free energy calculations and molecular dynamics simulations are employed to evaluate interactions between C3d and factor H and multiple binding interfaces. Leveraging this information, peptides are designed that preserve key intermolecular interactions. Peptide cyclization is used to reduce conformational entropy of the peptide and promote binding. Peptides are synthesized and C3d binding affinities are determined with microscale thermophoresis.

**Discussion:** Free energy calculations and molecular dynamics simulations suggest two putative binding sites on C3d for complement factor. Interactions between C3d and factor H at Site 1 are dominated by electrostatic interactions, while a mixture of aliphatic, hydrogen bond, and salt bridge interactions comprise interactions at Site 2. These binding modes are potentially synergistic with Site 1 promoting fast association while Site 2 promotes slow dissociation. Moreover, the two modes of binding occur through different domains of factor H, facilitating multivalent interactions with C3d. Peptide designs (**Figure 1**) mimicking key residues of factor H for each binding site were designed and cyclized to reduce conformational entropy in the peptides and promote binding. Molecular dynamics simulations of solvated peptide and peptide in complex with C3d suggest optimizable positions, and microscale thermophoresis was able to determine binding affinities as designs were iteratively optimized.

**Conclusions:** Biophysical methods provide relevant information for peptide design and optimization. Our candidate peptide binds C3d with 61.6 μM binding affinity. This molecule has therapeutic and diagnostic potential for age-related macular degeneration.

## Computational Tools for Optimizing Electrostatic Properties of Proteins

**Chris A. Kieslich**
Department of Chemical Engineering, Auburn University
Contact: kieslich@auburn.edu

Electrostatic interactions contribute significantly to protein structure, dynamics, and consequently function, especially the pH-dependent aspects of function such as association, stability, and catalysis. A computational framework named, Analysis of Electrostatic Similarities of Proteins (AESOP), was developed by the research group of Prof. Dimitrios Morikis to explore the role of electrostatics in protein function and to design proteins with tailored electrostatic properties. AESOP combines computational mutagenesis, electrostatics calculations, and clustering techniques to quantify the role of each charged amino acid in protein-protein interactions. The AESOP framework is based on the idea of perturbative design, and mutations of charged amino acids are used to computationally perturb a protein structure and electrostatic calculations are used to quantify the effects. Electrostatic similarity measures are used in AESOP to compare the global electrostatic character of proteins/mutants, while solvation free energy calculations provide insight into the specific electrostatic interactions driving binding. AESOP was developed with specific interest in protein/peptide design, but has also been applied to areas such as modeling the role of electrostatics in activation and regulation of the complement immune system. In this talk, I will discuss computational/theoretical advances that contributed to the development of the AESOP framework and survey the various applications of AESOP in terms of modeling the role of electrostatics in protein function and designing proteins with tailored electrostatic properties. I will also introduce a novel approach for considering electrostatic interactions in combinatorial protein/peptide design, which builds on the capabilities of the AESOP framework.

# NMR Reveals Dynamic Signatures of Latent Allostery and Redox Sensitivity in Macrophage Migration Inhibitory Factor

**George P. Lisi**
Dept. of Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence, RI

**Introduction:** Protein motions are critical to function, playing a role in optimizing polypeptide scaffolds and propagating biological signals. In proteins with multiple functions or promiscuous interactomes, such as macrophage migration inhibitor factor (MIF), studies of intramolecular dynamic networks can offer mechanistic insight into non-overlapping chemical processes and facilitate novel routes to therapeutic inhibition. MIF is critical to the pathophysiology of inflammation, opposes the immunosuppressive effects of glucocorticoids, and its levels are strongly correlated with the severity of asthma symptoms in patients and murine models. A dynamic mechanism controlling the MIF-induced activation of its pro-inflammatory receptor, CD74, was recently proposed with molecular dynamics simulations. In a new study, we explored the regulation of a second function in MIF, enzymatic tautomerase activity that requires a dynamic pathway connecting to the spatially distant site that regulates CD74 activation. Signal transmission between the allosteric and catalytic sites involves multi-timescale protein dynamics, intramolecular aromatic interactions and a hydrogen bond network formed among residues and ordered water molecules in the MIF solvent channel. The interplay between the N-terminal enzymatic site and solvent channel allosteric site in MIF can now, for the first time, be probed at the molecular level in order to connect the essential functions of MIF within a tightly controlled regulatory pathway (**Fig 1**).

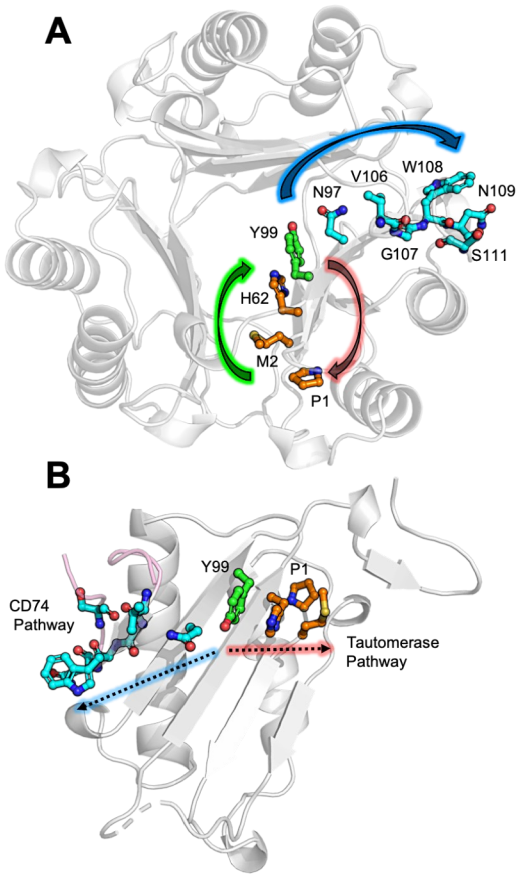



**Fig 1. Allosteric pathway(s) influencing MIF activity. (A)** Communication from the solvent channel (red arrow) to the N-terminal tautomerase site (orange sticks) established in this work. Reciprocity of this effect is shown by NMR studies of Pro1 mutants (green arrow). Tyr99 (green sticks) is also a central node in the CD74 activation network (blue arrow, sticks) shown by MD simulations in Pantouris *et al*, **2018**, *Angew. Chem. Intl. Ed. Engl.* **(B)** Allosteric pathways mapped onto a MIF monomer highlight the majority of the CD74 network residing on the C-terminus of an adjacent subunit (pink cartoon).

**Methods:** Protein engineering and expression to generate these data was carried out through standard protocols. The experimental results described in this talk were generated with a combined approach of solution NMR and X-ray crystallography. Biochemical assays measuring tautomerase activity and CD74 activation were used as functional handles to assess the impact of allosteric mutations within MIF. Computational techniques were also employed to assess MIF-CD74 interactions, water network interactions, and water molecule centrality.

**Results and Outlook:** Several investigations of dynamic equilibria in MIF reveal a highly interconnected trimeric assembly with multiple allosteric pathways. Two of these pathways (**Fig 1**), are directly involved in controlling tautomerase function and CD74 activation, while others regulate protein binding at peripheral regions of the MIF trimer. This work revealed Tyr99 of the solvent channel to be a critical node in the primary allosteric pathway of MIF, and mutations at this site disrupt the biochemical activities of MIF to different degrees. This talk will summarize these and other recent studies of MIF allostery and signaling, as well as present evidence of its ability to sample multiple conformations that may be indicative of disease-related states.

# Diffusional Interactions of Biomolecules

**J. Andrew McCammon**
Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, CA

**Introduction:** Diffusion is involved in a vast array of biomolecular processes. Brownian dynamics simulations that treat the diffusing molecules explicitly enable calculation of the rates of many such processes, allowing consideration of intermolecular interactions and other details (1). Such simulations can also be used to study the transfer of intermediates (channeling) among enzymes or proteins involved in metabolism or signal transduction (2). A variety of such applications will be described. The talk will begin with a brief comment on another topic – molecular dynamics simulations that have used new enhanced sampling methods to reveal mechanisms of systems such as CRISPR-Cas9, GPCRs, and other supramolecular assemblages (3).

**Materials and Methods:** All Brownian dynamics simulations were conducted with the software package, BrownDye. The electrostatic forces used in the simulation were calculated with the Poisson-Boltzmann equation, using the software package, APBS. Links to these packages can be found at https://mccammon.ucsd.edu/

**Results and Discussion:** Brownian dynamics simulations have yielded the rate constant for binding of substrates to a number of enzymes. Such simulations have also yielded quantitative information on the throughput of substrates through multienzyme complexes in which electrostatic channeling of intermediates is observed.

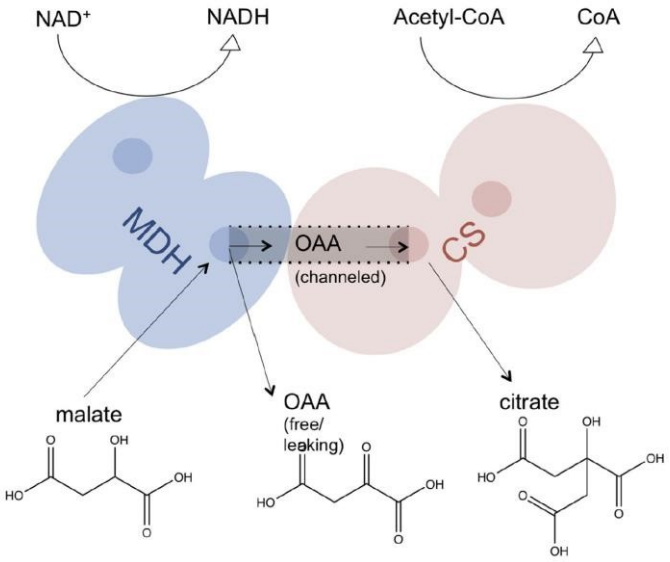



**Figure 1:** Electrostatic channeling of the negatively charged oxaloacetate (OAA) intermediate from malate dehydrogenase to citrate synthase in the Krebs cycle (see ref. 2).

**Conclusions:** Brownian dynamics is an effective tool for simulating biomolecular processes at the molecular and subcellular levels.

**References:**

1. Huber, G., J.A. McCammon: Brownian Dynamics Simulations of Biological Molecules. *Trends in Chemistry*. **2019,** 1, 727-738.
2. Huang, Y.M., G.A. Huber, N. Wang, S.D. Minteer, J.A. McCammon: Brownian dynamic study of an enzyme metabolon in the TCA cycle: Substrate kinetics and channeling. *Protein Science.* **2018,** *27*, 463–471.
3. Palermo, G., C. Ricci, J.A. McCammon: The invisible dance of CRISPR-Cas9. *Physics Today*. **2019,** *72*, 30-36.

# Tensile Force Transmitted Through LFA-1 Bonds Mechanoregulate Neutrophil Inflammatory Response

**Vasilios A. Morikis[1], Myung Hyun Jo[2], Eman Masadeh[1], Taekjip Ha[2], and Scott I. Simon[1*]**

[1]Department of Biomedical Engineering UC Davis, [2]Department of Biophysics and Biophysical Chemistry JHU

**Introduction:** Recruitment of neutrophils in appropriate numbers at vascular sites of inflammation is critical for innate immune surveillance. Lymphocyte function associated antigen-1 (LFA-1) adhesive bonds that support neutrophil deceleration and arrest under fluid shear, also function to mechanotransduce signals that spatially localize calcium flux necessary to guide transendothelial migration. To properly design therapeutics that target neutrophil dysregulation without completely eliminating anti-inflammatory function we must understand how neutrophil adhesion promotes signaling for activation and progression down the neutrophil adhesion cascade, We set out to characterize how bond tension acting on high-affinity (HA) LFA-1 bonds transduce intracellular signaling in a manner proportional to the shear force acting on adherent cells. We hypothesize that there exists a characteristic tensile force conducted across HA bonds between LFA-1 and ICAM-1 catalyzes the sequential assembly of a cytosolic complex that is necessary to achieve efficient $Ca^{2+}$ influx at sites of cell adhesion.

**Materials and Methods:** In order to predict the tensile forces experienced by HA LFA-1/ICAM-1 bonds as they resist the drag of fluid shear stress, we employed a tension gauge tether (TGT) platform. HA inducing LFA-1 antibody is immobilized to a surface through a well-defined noncovalent bond that ruptures at a defined force following receptor engagement. TGT tethers are constructed from two complimentary strands of DNA such that the strength of the bond is precisely engineered by the geometry of where the LFA-1 ligand is placed in relation to the substrate bound avidin/biotin bond. Estimated rupture forces are ~12 pN when unzipped at adjacent points and increases to ~54 pN when dissociated under shear at distant points of attachment. To determine the molecular force thresholds necessary to activate formation of macromolecular LFA-1/Kindlin-3/RACK1/Orai1 complexes a microfluidic, vascular mimetic device was annealed to the surface of the TGT-ICAM-1 tether substrate. Neutrophils were flown over the substrate under physiological shear stress, upon arrest and bond tension, calcium flux and shape change were observed. Spatial association between HA LFA-1/ICAM-1 bonds and cytosolic Kindlin-3 and RACK1 was quantified before and after shear was applied using fluorescence and immunoprecipitation.

**Results and Discussion:** Outside-in signaling requires a sequential set of events beginning with a shift to HA of LFA-1 that binds ICAM-1, a buildup of tension, and assembly of adaptor proteins that terminate in spatial linkage to the CRAC channel Orai-1. Utilizing a molecular platform within our microfluidic devices that regulates the force acting on LFA-1 bonds we determined the characteristic bond tension necessary to transduce signaling (Figure 1). Forces between 33 and 54 pN were sufficient to support LFA-1 binding to Kindlin-3 and promote integrin clustering. However, calcium signaling was only consistent in the 54 pN conditions compared to more sporadic calcium in the 33 pN conditions. We observed that force catalyzed integrin clustering and calcium flux in a Kindlin-3 dependent manner. Kindlin-3 has a dual role as a mechanical anchor that links to adaptors that assemble Orai-1 mediated calcium influx and as a support for LFA1 membrane clustering. Additionally, we show this characteristic force completed a macromolecular complex devoid of RACK1, that still required downstream of signaling of RACK1 to initiate the signaling complex necessary to transduce calcium influx. We identify a molecular circuit catalyzed by a well-defined force that may represent the necessity to assemble Kindlin-3 to LFA-1 and dispel RACK1 for downstream signaling processes.

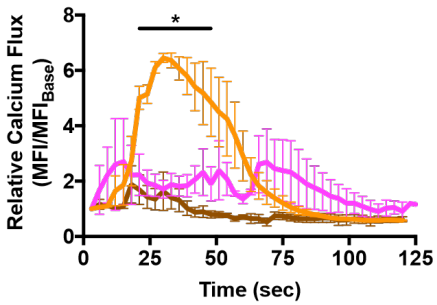



**Figure 1.** Neutrophil were allowed to settle on a substrate of 54, 33, or 12 pN TGT functionalized with HA-inducing LFA-1 antibody and calcium flux (Rhod-2) was observed under continuous force conditions.

**Conclusion:** We report on mechanotransduced regulation of local calcium flux at the region of adhesive contact of neutrophils at the endothelial surface where LFA-1/ICAM-1 bonds form in shear flow. This sensitivity to tensile force and the subsequent spatial calcium flux is mediated by both Kindlin-3 and RACK1. Here we begin to report on the conditions necessary to mediate LFA-1/Kindlin-3/Orai1 signaling circuit that is a key step in informing the neutrophil on where and when within inflamed vessels they should arrest and transmigrate.

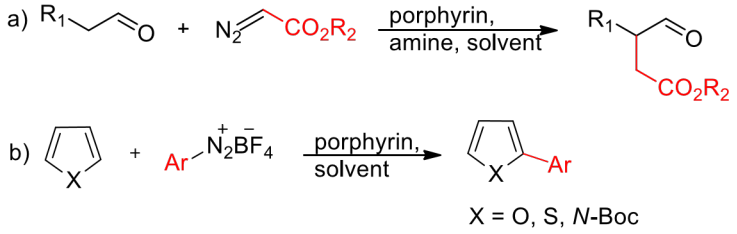
**Acknowledgements:** Funding: 2R01AI047294-18A1 (SIS), AMID T32, Howard Hughes Medical institute (TH).

# Porphyrins as Photoredox Catalysts in Efficient C-C Bond Formation

[1,2] **Katarzyna Rybicka-Jasińska**
[a] Department of Bioengineering, University of California Riverside, CA
[b] Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland

**Introduction:** Porphyrins are natural functional dyes which are utilized by Nature as prosthetic groups of various enzymes not *per se* because of their coordination characteristic, but due to the combination of other properties with absorption of light (which translates to color) being the most important. Efficient C−C bond formation in a green, nontoxic, and inexpensive way has always been a challenge and development of light-induced methodologies is one of the means to achieve such a goal.[1] Photoredox catalysis is based on a photoinduced electron transfer process (PET) between a substrate and a photoredox catalyst, commonly Ru- or Ir-complexes.[2] Though organic dyes have been well-known for their ability to participate in photoinduced electron transfer processes, their use as catalysts in such reactions has been less explored as the replacement of Ir- and Ru-complexes with known organic dyes is not always possible.[3]

**Results and Discussion:** We wondered whether simple porphyrins could be used as photocatalysts. Herein, we demonstrate a successful application of these compounds as efficient photoredox catalysts for C-C bond forming reactions involving the reductive or oxidative quenching. Employing dual catalytic system – photocatalysis merged with enamine-iminium catalysis alkylation of aldehydes and ketones at the α position was accomplished (Scheme 1a).[4] We have also found that porphyrins with tuned physicochemical properties, via tailoring various substituents at the periphery of the macrocycle, are also effective in catalyzing light-induced direct arylation of heteroarenes and cumarins with diazonium salts (Scheme 1b).[5] The reaction afforded arylated products in good yields and tolerates a wide range of functional groups.




**Scheme 1**: a) Alkylation of aldehydes at the α-position b) Arylation of heteroarenes.

**Conclusion:** Porphyrins can now be added to the list of photoredox catalysts that are suitable for photoredox catalysis. As these compounds are easy to synthesize and their optical and electrochemical properties can be tuned by placing a variety of electron-donating or electron-withdrawing substituents at the periphery of the macrocycle, they are perfectly suited for this role. These findings demonstrate unexplored venues in both porphyrin chemistry and photocatalysis.

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# Molecular Dynamics Simulation Studies of the Gating and Pharmacology of the Human HV1 Proton Channel

[1]D. J. Tobias, [1]A. D. Geragotelis, [1]V. T. Lim, and [1]J. A. Freites

[1]Department of Chemistry, University of California, Irvine, CA

**Contact:** dtobias@uci.edu

**Introduction:** The voltage-gated Hv1 proton channel is a ubiquitous membrane protein that plays roles in a variety of cellular processes including proton extrusion, pH regulation, production of reactive oxygen species, proliferation of cancer cells, and increased brain damage during ischemic stroke. A crystal structure of an Hv1 construct in a putative closed state has been reported, and structural models for the channel open state have been proposed, but a complete characterization of the Hv1 conformational dynamics under an applied membrane potential has been elusive, and the lack of atomically detailed structural models of Hv1 has hampered efforts to develop potent small molecule blockers to modulate the function of Hv1.

**Materials and Methods:** We constructed an all-atom model of the human Hv1 (hHv1) channel voltage-sensing domain (VSD) embedded in a lipid bilayer using the mHv1cc crystal structure as a template for the protein chain. The model consists of a monomeric hHv1 VSD (residues 88 to 230) in a POPC bilayer in excess hydration. We generated a ~33-μs MD simulation trajectory under an applied membrane potential according to the schedule in the top panel of Figure 1A. Application of the depolarized potential led to a transition from the closed to the open state of the channel. The open state conformation was used in subsequent docking runs to generate poses for the hHv1 blocker 2-guanidinobenzimidazole (2GBI) within the channel lumen, and alchemical free energy calculations to elucidate the experimentally measured effects of mutations on the 2GBI binding free energy.

**Results and Discussion:** We report structural models of the hHv1 voltage-sensing domain (VSD), in both hyperpolarized and depolarized states, which we obtained from voltage-dependent conformational changes during our 10-μs timescale atomistic MD simulation (Figure 1B,C). We demonstrate that our structural models are consistent with several experimental observations. Specifically, the total gating charge displacement associated with transition from the closed to open state is consistent with experimental estimates. In addition, our molecular docking calculations confirm the proposed mechanism for the inhibitory action of (2GBI) derived from electrophysiological measurements and mutagenesis, and our computed binding free energies correlate well with corresponding experimental values. Finally, the depolarized structural model is also consistent with the formation of a metal bridge between residues located in the core of the VSD.

**Conclusions:** We have generated voltage-dependent structural models of the Hv1 proton channel using multi-μs MD simulations, and we have demonstrated that our models are consistent with a variety of experimental data. Our docking and free energy calculations provide insights into the structural and energetic aspects of the binding of the prototypical blocker 2GBI within the hHv1 channel.

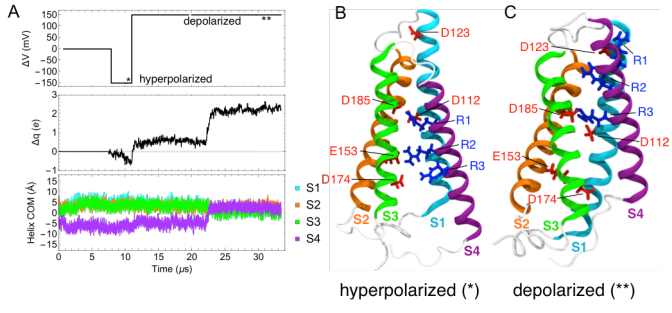


**Figure 1:** (A) Top: Applied membrane potential protocol; Middle: total charge displacement in the membrane electric field; Bottom: trajectories of the centers-of-mass of the S1-S4 helices. Snapshots of (B) the hyperpolarized configuration at 11 μs (*) and (C) the fully depolarized configuration at 30 μs (**).

## High Spatial & Temporal Resolution Biological Imaging Across Scales

**Srigokul Upadhyayula, PhD**
Scientific Director, Advanced Bioimaging Center;
Faculty Scientist, Lawrence Berkeley National Laboratory;
Assistant Professor in Residence of Cell and Developmental Biology,
Department of Molecular Cell Biology, UC Berkeley

Lattice Light Sheet Microscopy is a transformative imaging technology which enables physiological observation of subcellular features over the relevant scales in space and time because of the wide resolution range and large volumetric acquisition capability. This new imaging method allows us to record dynamics at a scale of nanometers and milliseconds, determine their consequences at a scale of microns and hours, and visualize their long-term outcome at a scale of up to several millimeters over several days. I'll briefly present our past work on combined lattice light sheet microscopy with adaptive optics to achieve (*Science,* **2018**), across large multicellular volumes, noninvasive aberration-free imaging of subcellular dynamics *in vivo*. Next, I'll discuss the combination of lattice light-sheet with the physical expansion of samples (Expansion Microscopy) that enables scalable super-resolution volumetric imaging of large tissues (*Science,* **2019**) including the complete fly brain, columns of mouse brain – datasets spanning several hundred terabytes. Finally, I will introduce our next-generation microscope design– dubbed the "Swiss army knife microscope", which combines several different modes of imaging and is designed to alleviate the tradeoffs related to resolution, speed, invasiveness and imaging depth, which precludes any single optical microscopy to function optimally for a diverse set of biological specimens.

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## Role of Intrinsically Disordered Proteins in Cellular Signaling and Regulation

**Peter E. Wright**
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA
wright@scripps.edu

Intrinsically disordered proteins (IDPs) are highly abundant in the human proteome and are strongly associated with numerous devastating diseases, including cancers, age-related neurodegenerative disorders (such as Alzheimer's and Parkinson's diseases), diabetes, cardiovascular diseases, and infectious diseases. IDPs mediate critical regulatory functions in the cell, including transcription, translation, the cell cycle, and numerous signal transduction events. The lack of stable globular structure confers numerous functional advantages on IDPs, allowing them to exert an exquisite level of control over cellular signaling processes. IDPs frequently function as central hubs in dynamic regulatory networks, where their propensity for posttranslational modifications and their ability to interact with multiple target proteins makes them well adapted for precise transduction of cellular signals. The role of IDPs in cellular signaling will be illustrated by reference to pathways regulated by the general transcriptional coactivators CBP (CREB binding protein) and p300.

# Self-assembly of Mature Conical HIV Particles: The Role of Genome and Membrane

**Roya Zandi**
Department of Physics and Astronomy, University of California, Riverside, CA

**Introduction:** In recent years, there have been several studies aimed at understanding the physical principles governing the formation of viral particles for both their material and biological applications. The assembly of retroviruses has been, in particular, receiving additional attention due to their oncogenic activity and the fact that human immuno-deficiency virus (HIV) is connected to the AIDS pandemic. Retroviruses, like all other viruses, contain a proteineous shell (or capsid) that encloses viral genetic material. It is remarkable that under special formation conditions mature HIV capsids will readily self-assemble from their isolated capsid protein and nucleic acid components. The spontaneous formation of conical structures is the distinguishable feature of mature HIV capsids, which gives rise to a number of important physical questions. Does spontaneous curvature determine the shape of the viral capsids? Do kinetics rather than thermodynamics determine the final structure of capsids? Does RNA play an active role in the final assembly products?

**Material and methods:** Since it is not feasible to perform all-atomistic simulations for even the smallest virus particles as it requires more than one million atoms, in most computational studies to date, coarse-grained models are employed to mimic capsid proteins, RNA molecules and lipid bilayers. To study the kinetics of assembly of HIV-1 capsid, we have developed a coarse-grained model for both protein subunits and genome. We also consider the impact of membrane on the formation of HIV conical capsid through a number of computer simulations.

**Results and conclusion:** In this talk, I will discuss the kinetic pathways of HIV assembly and show that the continuum theory of elastic shells combined with the non-equilibrium assembly process is able to predict the formation of structures pertinent to retroviruses (such as HIV). The minimal model of our assembly yields a unified one-dimensional phase diagram in which the appearance of spherical, irregular, conical and cylindrical structures of retroviruses is seen to be governed by the spontaneous curvature of protein subunits. Figure 1 shows the snapshots of formation of a conical capsid. I will also discuss the impact of RNA and membrane on the assembly pathways of HIV capsid formation. As illustrated in Fig. 2, we find that both RNA and membrane contribute to the stability and formation of H IV conical capsids.

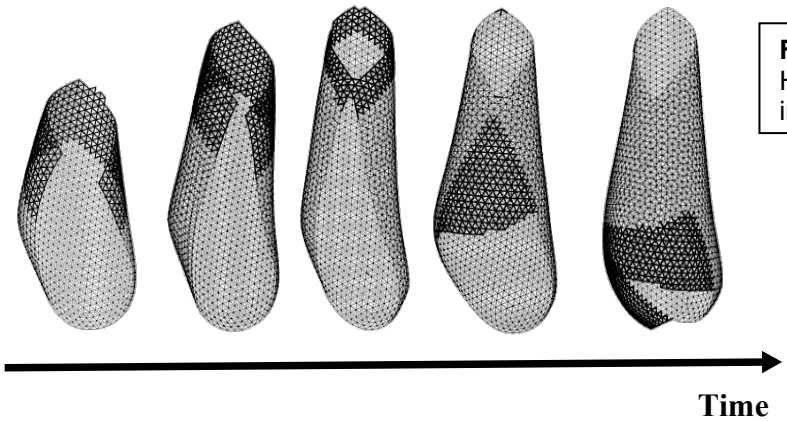


**Figure 1:** Snapshots of formation of HIV conical capsid. Darker color indicates most recent accretion.

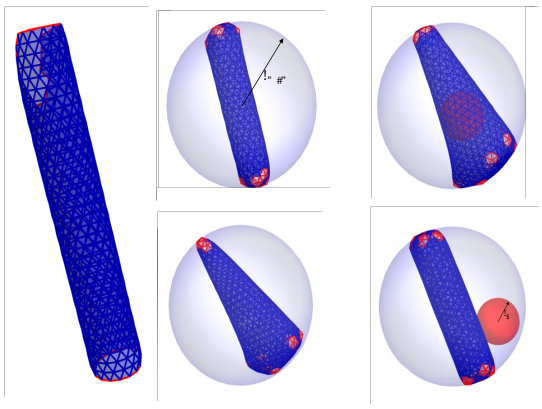

**Figure 2:** Role of membrane and genome in the formation of mature core. (A) Formation of open tube in a free environment. (B) The presence of membrane (the gray sphere) limits the size of the cylinder. Membrane can also facilitate formation of pentamers resulting into the formation of cones. (C) During the assembly process, if the genome (ball) remains attached to a few subunits, a conical capsid forms. Otherwise, a cylindrical shell assembles with genome remaining outside.

# 5 Participant list

## Organizers

**Prof. Mark Alber**
University of California Riverside, United States
malber@ucr.edu

**Rohaine Hsu**
University of California Riverside, United States
rhsu001@ucr.edu

**Prof. Giulia Palermo**
University of California Riverside, United States
giulia.palermo@ucr.edu

**Prof. Victor G. J. Rodgers**
University of California Riverside, United States
vrodgers@engr.ucr.edu

**Prof. Valentine I. Vullev**
University of California Riverside, United States
vullev@ucr.edu

## Assistant Organizers

**Melissa Gomez**
Financial and Administrative Officer
University of California Riverside, United States
melissa.gomez@ucr.edu

**Nadine A. Okuns**
Financial and Administrative Officer
University of California Riverside, United States
nokuns@engr.ucr.edu

## Participants

**Mark Alber,** malber@ucr.edu
**Pablo Arantes,** pablitoarantes@gmail.com
**Aihik Banerjee,** aihik.banerjee@email.ucr.edu
**Yuliana Bosken,** ydavi002@ucr.edu
**Samuel Britton,** sbrit004@ucr.edu
**Rafael Bruschweiler,** bruschweiler.1@osu.edu
**Paul Champion,** champ@neu.edu
**Chia-en Chang,** chiaenc@ucr.edu

**Justin Chartron,** jchartron@engr.ucr.edu
**Weitao Chen,** weitaoc@ucr.edu
**Heyrim Cho,** heyrimc@ucr.edu
**Yat Tin Chow,** yattinc@ucr.edu
**Vasileios Christopoulos,** vchristo@engr.ucr.edu
**John Clark,** jclar019@ucr.edu
**Daniel Collister,** dcoll010@ucr.edu
**Kiera Donoghue,** kdono001@ucr.edu
**Zied Gaieb,** ziedgaieb@gmail.com
**Ron Gorham,** rgorham2@gmail.com
**William Grover,** wgrover@engr.ucr.edu
**Reed Harrison,** r1harrison@eng.ucsd.edu
**Shane Hoang,** shoan012@ucr.edu
**Rohaine Hsu,** rhsu001@ucr.edu
**Chris Kieslich,** kieslich@auburn.edu
**Lauren Lastra,** llast001@ucr.edu
**Mario León López,** mleon059@ucr.edu
**Wayne Leu,** wleu001@ucr.edu
**George Lisi,** george_lisi@brown.edu
**Huinan Liu,** huinan.liu@ucr.edu
**Jaclyn Lock,** jylock10@gmail.com
**Aliana López de Victoria,** alianaldv@gmail.com
**Brian Lupish,** blupi001@ucr.edu
**Vipul Madahar,** vmada001@ucr.edu
**Jacopo Manigrasso,** jacopo.manigrasso@iit.it
**J. Andrew McCammon,** jmccammon@ucsd.edu
**Robert McKee,** rmckee@ucr.edu
**Marco Medrano,** mmedr005@ucr.edu
**Yinglong Miao,** miao@ku.edu
**Christian Michael,** cmich004@ucr.edu
**Brandon Mitchell,** brandonpmitchell@outlook.com
**Rohith Mohan,** rohithmohan@gmail.com
**Maryann Morales,** mmora039@ucr.edu
**Joshua Morgan,** jmorgan@engr.ucr.edu
**Vasilios Morikis,** vamorikis@ucdavis.edu
**Jin Nam,** jnam@engr.ucr.edu
**Łukasz Nierzwicki,** nierzwicki.l@gmail.com
**Noor Nindra,** nnind001@ucr.edu
**Giulia Palermo,** giulia.palermo@ucr.edu
**Francesco Pancaldi,** francesp@ucr.edu
**Hyle Park,** hylepark@engr.ucr.edu
**Alireza Ramezani,** arame002@ucr.edu
**Jennifer Rangel Ambriz,** jrang008@ucr.edu
**Mahima Ravoori,** mravo001@ucr.edu

**Victor Rodgers,** victor.rodgers@gmail.com
**Wilson Rodriguez,** wrodr2013@gmail.com
**Katarzyna Rybicka-Jasińska,** katarzyr@ucr.edu
**Aakash Saha,** asaha011@ucr.edu
**Lisa Salmeron,** lsalm003@ucr.edu
**Betul Senay Aras,** betuls@ucr.edu
**Frances Sladek,** lifescidean@ucr.edu
**Mahesh Sunkula,** maheshs@ucr.edu
**Isaac Tate,** itate001@ucr.edu
**Venkatesh Tavva,** vtavv001@ucr.edu
**Douglas Tobias,** dtobias@uci.edu
**Thamidul Islam Tonmoy,** ttonm001@ucr.edu
**Alysha Toomey,** atoom001@ucr.edu
**Kevin Tsai,** ytsai003@ucr.edu
**Gokul Upadhyayula,** sup@berkeley.edu
**Val Vullev,** vullev@ucr.edu
**Qixuan Wang,** qixuanw@ucr.edu
**Nolan Winicki,** nwini001@ucr.edu
**Peter Wright,** wright@scripps.edu
**Kingsley Wu,** kwu030@ucr.edu
**Hong Xu,** hongxu@engr.ucr.edu
**Jennifer Yang,** jyang033@ucr.edu
**Raymond Yeung,** ryeun003@ucr.edu
**Roya Zandi,** royaz@ucr.edu
**Nehemiah Zewde,** nzewd001@ucr.edu

# 6 Location

## The UCR Alumni & Visitors Center

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