Molecular Biophysics Training Grant (MBTG) Annual Retreat

May 5, 2017

Program and Abstracts

UC San Diego

mbtg.ucsd.edu

UCSD Molecular Biophysics Training Grant Annual Retreat

Friday, May 5, 2017

Institute of the Americas

1:00PM	**Poster set-up**
1:15PM – 1:45PM *Deutz Room*	**Elena Koslover** "Emergent Physics of Intracellular Soft Matter, from DNA to Cytoplasm"
1:45PM – 2:05PM *Deutz Room*	**Lightning Talks Part I** Bryan Arias Lewis Churchfield
2:10 – 2:40PM *Deutz Room*	**Dmitry Lyumkis** "Structural Insights into Retroviral Integration using Cryo-EM"
2:45 – 3:15PM *Deutz Room*	**Lightning Talks Part II** Chris Lee Kristen Ramsey Jeffrey Wagner
3:20-4:30PM *Arango Foyer*	**Poster Presentations** DeeAnn Asamoto Samantha Borowski Colin Deniston Benjamin Dick Benjamin Jagger Samuel Kantonen Evan Kobori Sarah Kochanek Noah Kopcho Ryan Lumpkin Adam Maloney Jeff Mindrebo Riley Peacock Kevin Sweeney Sarah Ur
4:30-5:00PM *Deutz Room*	**Poster Awards**
5:00-6:00PM *Friend Plaza*	**BBQ Dinner**

POSTER PRESENTORS INDEX

MBTG Annual Retreat

1. DeeAnn Asamoto

“Investigation of OmpA folding in small unilamellar vesicles and nanodiscs”

2. Samantha Borowski

”Exploring Antimicrobial Peptide Interactions with Outer Membrane Protein A Using Spectroscopic Methods”

3. Colin Deniston

“Structure Elucidation of the Parkinson’s Disease Protein LRRK2 using Cryo-Electron Microscopy”

4. Benjamin Dick

“Structural Approach to Elucidating Metalloenzyme Inhibitor Selectivity”

5. Benjamin Jagger

“Multiscale estimation of kinetic rates of trypsin with benzamidine using a hybrid molecular dynamics, brownian dynamics, and milestoning approach”

POSTER PRESENTORS INDEX

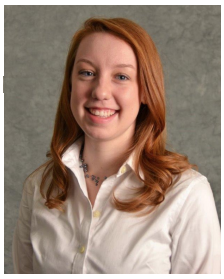
MBTG Annual Retreat

6. Samuel Kantonen

“Towards Improved Potential Functions for MD Simulations”

7. Evan Kobori

“Understanding Conformational States of PKA”



8. Sarah Kochanek

“Whole-Virion Simulation of Influenza Reveals Novel Druggable Pockets on Surface Glycoproteins”

9. Noah Kopcho

“A rapid in vitro method for generating target-specific nanobodies”

10. Ryan Lumpkin

“Dynamics and Assembly of ASB-containing E3 Ubiquitin Ligases”

POSTER PRESENTORS INDEX

MBTG Annual Retreat

11. Adam Maloney

“A Minimal In Vitro Translation for Structural Studies of FMRP”

12. Jeff Mindrebo

“Trapping Acyl Carrier Protein Interactions With a Type III PKS system”

13. Riley Peacock

“Probing the Dynamics Behind the Substrate Specificity of Thrombin”

14. Kevin Sweeney

“Lanthanide Cofactors for Triphosphorylation Ribozymes”

15. Sarah Ur

“The Msh4-Msh5 complex’s role in meiotic crossover formation”

LIGHTNING TALK PRESENTORS

MBTG Annual Retreat

1. Bryan Arias

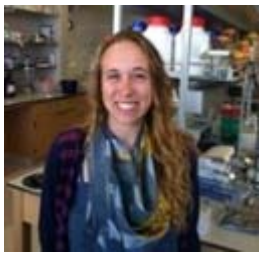
"RNA Modulates the Interaction Between NS1 and PABP1"

2. Lewis Churchfield

"Examining the allosteric crosstalk within a designed disulfide-linked Zn-binding metalloprotein"

3. Chris Lee

"Investigating transport properties with multiscale computable mesh models from heterogeneous structural datasets"



4. Kristen Ramsey

"DNA and IκBα both induce long-range conformational changes in NFκB"

5. Jeffrey Wagner

"Toward Comparative Biophysics of APOBEC3 Enzymes"

Poster 1

Investigation of OmpA folding in small unilamellar vesicles and nanodiscs

DeeAnn K. Asamoto, Guipeun Kang, and Judy E. Kim
University of California, San Diego

The insolubility of membrane proteins in water and their tendency to denature outside of the membrane represent an impediment to performing biophysical studies on membrane proteins, including the folding and insertion mechanisms into lipid membranes. The goal of our research is to investigate the folding dynamics of OmpA in nanodiscs. We present a combination of SDS-PAGE differential mobility and digestion studies with steady-state fluorescence and UV resonance Raman spectroscopy to confirm the folding of OmpA into preformed nanodiscs. Fluorescence and digestion studies with Arg-C protease indicate that the refolding yields and kinetics for nanodiscs are similar to those for small unilamellar vesicles (SUVs). UV resonance Raman spectroscopy revealed an enhanced hydrogen- out- of- plane mode of OmpA folded in nanodiscs, and this structural signature is also observed under refolding conditions in the presence of detergent micelles and SUVs. These data indicate that nanodiscs are an excellent alternative to SUVs for folding experiments, and offer experimental benefits of optical clarity, better control of protein- to- nanodisc ratios, and greater stability compared to traditional SUVs.

Poster 2

Exploring Antimicrobial Peptide Interactions with Outer Membrane Protein A Using Spectroscopic Methods

Samantha Borowski
University of California, San Diego

Understanding the mechanisms by which antimicrobial peptides (AMPs) disrupt the lipid membranes of invading pathogens, as well as their structures within these membranes, is necessary in furthering the design of peptide-based therapeutics. Previous studies have demonstrated the ability of various AMPs to interact with target proteins present in the outer membrane of bacteria. Our studies aim to probe interactions between different AMPs and Outer Membrane Protein A (OmpA), a beta-barrel protein abundant in the outer membrane of gram-negative bacteria and highly conserved amongst the *Enterobacteriaceae* family. Initial experiments focus on fluorescence studies to probe the extent of vesicle disruption by melittin in the presence and absence of OmpA, while future work includes vibrational methods to probe AMP-protein molecular interactions.

Poster 3

Structure Elucidation of the Parkinson's Disease Protein LRRK2 using Cryo-Electron Microscopy

Colin Deniston
University of California, San Diego

Roughly 60,000 new cases of Parkinson's Disease (PD) are reported each year in the US. Recent research efforts have found the Leucine Rich Repeat Kinase 2 (LRRK2) protein to be a main contributor to both sporadic and familial forms of PD. Despite this significance, relatively little is known about LRRK2's functional role and biological interactions in both healthy and PD neurons. In order to better understand LRRK2's role in disease progression I am utilizing cryo-electron microscopy (cryo-EM) to solve the high-resolution structure of the full-length WT LRRK2 protein. I am currently working to optimize the expression and purification of WT LRRK2 from HEK293T cells.

In addition to the native structure of LRRK2 there are multiple other structural targets of interest as long term goals of the project, the dimer of WT LRRK2 being one. Recent studies have suggested that dimerization of LRRK2 may be critical for its biological function and that monomerization may be a means of regulating or "turning off" LRRK2 activity in the cell. LRRK2 function is also altered via serval mutations in its kinase and GTPase domains which strongly correlate with PD progression. Currently, DNA constructs of these point-mutants have been made by collaborator Tsan-wen but expression and purification efforts are not yet underway.

Poster 4

Structural Approach to Elucidating Metalloenzyme Inhibitor Selectivity

Benjamin L. Dick[§], Ashay Patel[§], Cy Credille[§], James A. McCammon[§¶‡°], Seth M. Cohen[§]
[§]Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0358
[¶]Department of Pharmacology, University of California San Diego, La Jolla, California, United States of America
[‡]Howard Hughes Medical Institute, University of California San Diego, La Jolla, California, United States of America
[°]National Biomedical Computation Resource, University of California San Diego, La Jolla, California, United States of America

Metalloenzymes comprise a significant portion of known clinical targets, yet inhibitors of these enzymes are underrepresented in approved therapeutics. In this limited number of approved inhibitors there is an even more limited number of metal binding motifs used. To assist in developing inhibitors of metalloenzymes, a better understanding of the fundamental metal-inhibitor interactions is needed. Factors that may influence inhibitor specificity and potency include active site architecture, identity of the active site metal, and identity of the coordinating atoms of the inhibitor. To better understand the factors that affect inhibitor binding the mono-nuclear metalloenzyme human Carbonic Anhydrase II (hCA II) was used as a model system to explore the role of metal-inhibitor interactions. Mutational studies with hCA II showed that the identity of coordinating atoms of the inhibitor influence how an inhibitor responds to a mutation which decreases steric crowding in the enzyme active site. This allowed for different binding geometries than were previously observed with wild type hCA II and for developing a better understanding of how coordinating atom identity influences the interactions an inhibitor has with the enzyme active site.[1]

To gain a deeper understanding of the metal-inhibitor interactions that affect potency of inhibitors of dinuclear metalloenzymes, the clinically relevant metalloenzyme influenza endonuclease was studied. Structural studies of a select number of inhibitors with different coordination motifs bound to the active site Mn^{2+} ions were performed. Based on the mode of coordination and the range of inhibitory activity exhibited by the selected inhibitors, some of the factors that affect the potency of dinuclear metalloenzyme inhibitors were determined. The results reported in these studies, can provide a better understanding of the interactions and factors that influence metalloenzyme inhibitor selectivity and ultimately guide the development of future metalloenzyme inhibitors.

1. Dick BL, Patel A, McCammon JA, Cohen SM. Effect of donor atom identity on metal-binding pharmacophore coordination. J Biol Inorg Chem. 2017 Apr 7. doi: 10.1007/s00775-017-1454-3. [Epub ahead of print]. PubMed 28389830.

Poster 5

Multiscale estimation of kinetic rates of trypsin with benzamidine using a hybrid molecular dynamics, brownian dynamics, and milestoning approach

Benjamin Jagger[1], *Lane Votapka*[1,2], *Alexandra Heyneman*[1], *Rommie Amaro*[1]
[1] *Chemistry and Biochemistry, University of California, San Diego, La Jolla, California, United States;* [2] *Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, New York, United States*

Kinetic parameters are important for determining the in vivo efficacy of drugs. Traditional simulation approaches, however, are unable to access the timescales needed to observe multiple association or dissociation events, and therefore cannot accurately estimate kinetic rates. We present an alternative simulation approach: Simulation Enabled Estimation of Kinetic Rates (SEEKR) that is capable of performing these types of calculations. SEEKR is a suite of open source scripts and tools that facilitate the calculation of kinetic rates using a multiscale, hybrid, molecular dynamics, Brownian dynamics and milestoning approach. We also present the calculation of kinetic rates for the well-studied protein trypsin with its competitive inhibitor benzamidine to demonstrate the accuracy and efficiency of the SEEKR package.

Poster 6

Towards Improved Potential Functions for MD Simulations

Samuel A. Kantonen[1], Hari S. Muddana[2], Niel M. Henriksen[3], Michael K. Gilson[3]
Department of Chemistry[1], Skaggs School of Pharmacy and Pharmaceutical Sciences[3]
University of California, San Diego
Dart Neuroscience LLC[2]

Molecular dynamics simulations are widely utilized for their applications in biophysical chemistry, including determination of protein structure, drug design, and elucidation of underlying physics of non-covalent interactions at the atomic level. While these simulations are useful, they require accurate input parameters, in particular potential functions (force field) used to compute atomic forces at discrete time steps. Minor inaccuracies in potential functions or the parameters used can translate to large discrepancies in the information obtained from simulations. The development and improvement of force fields has become a highly researched topic, ranging from merely optimizing parameters used for popular force fields to design of entirely new potential functions. Here, we utilize a new potential function, based on Slater type orbitals, to model non-bonded interactions, with most of the input parameters coming from quantum mechanical calculations, as opposed to empirically derived. We then evaluate force fields based not only upon pure liquid properties that have been relied on in the past, but also upon noncovalent binding experimental data; our laboratory has begun to synthesize novel host-guest binding systems and characterize their binding using isothermal titration calorimetry. Using various coupling reactions, we have generated a modular host (based on the cyclical oligosaccharide beta-cyclodextrin), which contains different functional groups that can be swapped between host and guest (in particular, adamantane based guests), in order to evaluate potential functions for a diverse range of interactions. We also measure the binding thermodynamics using high-accuracy ITC experiments. We are currently developing computational infrastructure to implement these new potential functions into MD simulations, which can be used to compare with our novel experimental binding data to evaluate the effectiveness of the Slater potential function and related input parameters.

Poster 7

Understanding Conformational States of PKA

Evan Kobori
University of California, San Diego

cAMP dependent protein kinase (PKA) is a ubiquitous kinase that regulates many biological processes and is associated with a variety of diseases and disorders. Physiologically, PKA exists as a tetrameric holoenzyme consisting of two regulatory (R) subunits and two catalytic (C) subunits. There are four structurally and functionally non-redundant R isoforms (RI α/β, RII α/β) that can bind cAMP resulting in a dramatic conformational change that unleashes the catalytic activity of the kinase. My project has focused on characterizing the RIβ holoenzyme conformational changes upon cAMP binding via electron microscopy and X-ray crystallography. Currently, I have negative stain images of the apo RIβ holoenzyme and am in the process of optimizing conditions to eventually obtain 2D class averages. And in the future I plan to obtain 2D class averages of RIβ holoenzyme with cAMP bound using RIβ cyclic nucleotide binding (CNB) domain mutants to control the amount of cAMP bound and to obtain a more homogenous sample.

Additionally, there has been recent evidence to suggest that in primary cultured neurons, RIβ colocalizes with the microtubule binding protein, Map2. I have performed Co-Immunoprecipitations to show that there appears to be an interaction between RIβ and Map2. In addition I am working to set up peptide arrays to determine whether or not there is indeed a direct interaction between RIβ and Map2.

Poster 8

Whole-Virion Simulation of Influenza Reveals Novel Druggable Pockets on Surface Glycoproteins

Sarah E. Kochanek, Jacob D. Durrant, and Rommie E. Amaro
University of California, San Diego

Influenza virus infection continues to be a major healthcare issue, with 3-5 million cases of severe disease reported and 300,000-500,000 deaths worldwide each year. Variation in the sequences and structures of the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), from strain to strain complicate prophylactic and therapeutic approaches. Integrative modeling techniques combining cryo-electron tomography with x-ray crystallography, homology modeling, and protein-protein docking have led to the construction of a whole-virion model of influenza. Using the petascale computing machine Blue Waters, we have performed molecular dynamics (MD) simulations of this virion model. Principal component analysis reveals that simulation of the complete virion allows exploration of greater glycoprotein conformational space as compared to single glycoprotein simulations. Further, Markov state models constructed from the trajectories of the glycoproteins reveal novel druggable pockets in both HA and NA that can be targeted for the development of novel treatments for influenza virus infection. The identification of potentially druggable pockets previously unseen in simulations of individual glycoproteins demonstrates the utility of modeling in the sub-cellular scale and beyond to inform drug discovery.

Poster 9

A rapid in vitro method for generating target-specific nanobodies

Noah Kopcho
University of California, San Diego

Nanobodies are small, single-domain proteins which bind to specific target molecules. Although they are about one tenth the size of conventional antibodies, nanobodies are capable of binding kinetics comparable to typical antibody-antigen interactions. Nanobodies are therefore very well suited for usage as therapeutics and biophysical probes. One severe limitation, however, is that nanobodies are only known to be produced by the immune systems of camelids and cartilaginous fish.

These animals are difficult to maintain and are impractical for large scale cultivation. We plan to circumvent this issue by developing a streamlined process for generating nanobodies using only benchtop equipment.

One unique advantage of our in vitro system is the potential capability to produce nanobodies which only bind to desired conformational states of a particular target. Here we present the isolation of a nanobody which is specific for the ATP binding cassette transporter P-glycoprotein (P-gp) in its ATP bound conformation. The conformational change induced by ligand binding has been verified using hydrogen-deuterium exchange mass spectrometry, and our first generation nanobody shows a distinct preference for P-gp in the presence of the allosteric ligand AMP-PNP. By increasing the binding affinity through gain-of-function recombination, this may serve as a valuable experimental probe and may also aid in crystallization of this structurally uncharacterized P-gp conformation.

Poster 10

Dynamics and Assembly of ASB-containing E3 Ubiquitin Ligases

Ryan Lumpkin
University of California, San Diego

E3 Ubiquitin Ligases (UBLs) facilitate the highly-specific covalent attachment of activated Ubiquitin (Ub) to bound substrate proteins through an isopeptide bond on an exposed lysine residue. Ub signaling regulates cellular protein degradation which is essential for proper cell functioning. The multi-subunit Cullin-RING ligase (CRL) is the largest family of UBLs, and it is responsible for up to 20% of degradation through the proteasome (Lydeard et al. 2013). CRLs share a common structure, composed (in order of arrangement) of a substrate receptor with a suppressor of cytokine signaling(SOCS) domain, one or two adapter proteins, a Cullin (CUL) protein, and a RING-box (RBX) protein that recruits E2 enzymes charged with activated Ub (Petrowski et al. 2005). Among the substrate receptors belonging to this class of UBLs, all 18 proteins in the Ankyrin Repeat and SOCS-box (ASB) family of proteins associate with CUL5 and RBX2 through the Elongin B and C (EB/C) adapter proteins. (Kohroki et al. 2005, Okumura et al. 2016)

ASB9 has been experimentally shown to tightly and specifically bind to Creatine Kinase (CK), but pulldown experiments in recent literature suggest ASB9 may bind other substrates, including histones and several metabolic enzymes. (Balasubramaniam et al. 2015, Andresen et al. 2014) Ankyrin repeat proteins have extended Ankyrin repeat domains, yet they have only previously been shown to bind single substrates in vitro. By studying the ASB9-containing CRL (ASB9-CRL), I aim to further characterize the functional role of Ankyrin repeat proteins and the dynamics of the CRLs that enable ubiquitin transfer across 70 Å distances (Schiffer et al. 2016). I have assembled the full ASB9-CRL at concentrations suitable for biophysical experiments, and I demonstrated the ubiquitination activity of this ligase toward CK through band shift assays and anti-Ub western blots. I have studied the internal dynamics of the ligase through hydrogen deuterium exchange, and I have submitted samples for structural characterization by cryo-electron microscopy.

References

Balasubramaniam D, Schiffer J, Parnell J, Mir SP, Amaro RE, Komives EA. How the ankyrin and SOCS box protein, ASB9, binds to creatine kinase. Biochemistry. 2015; 54(8):1673-80.
Kohroki J, Nishiyama T, Nakamura T, & Masuho Y. ASB proteins interact with Cullin5 and Rbx2 to form E3 ubiquitin ligase complexes. FEBS Letters. 2005; 579(30): 6796–6802. Kwon S, Kim D,
Lydeard JR, Schulman BA, & Harper JW. Building and remodelling Cullin RING E3 ubiquitin ligases. EMBO Rep. 2013; 4(12): 1050–1061.
Okumura F, Joo-okumura A, Nakatsukasa K, Kamura T. The role of cullin 5-containing ubiquitin ligases. Cell Div. 2016; 11:1.
Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. Nat Rev Mol Cell Biol. 2005; 6(1): 9-20.
Schiffer JM, Malmstrom RD, Parnell J, et al. Model of the Ankyrin and SOCS Box Protein, ASB9, E3 Ligase Reveals a Mechanism for Dynamic Ubiquitin Transfer. Structure. 2016; 24(8): 1248-56. Villén, J. & Gygi, S. P. The SCX/IMAC enrichment approach for global phosphorylation analysis by mass spectrometry. Nat Protoc. 2008; 3.

Poster 11

A Minimal In Vitro Translation for Structural Studies of FMRP

Adam Maloney
Simpson Joseph Lab, University of California, San Diego

Fragile X syndrome is a common neurological disease that stems from a gene defect in the FMR1 gene. Expanded CGG repeats in FMR1 can cause silencing of the protein FMRP, which is involved in regulation of protein translation in the brain. Previous research from our lab has suggested that FMRP may inhibit translation in neurons by interacting directly with the ribosome or mRNA during translation. A current focus of our research is to better understand this interaction through structural studies of FMRP and the translating ribosome. By investigating the mechanism through which FMRP inhibits translation we hope to gain an understanding of how translation is fundamentally regulated.

Structural studies of translation are challenging using traditional in vitro methods because of the many different protein factors required for to initiate translation. My research is focused on developing a minimal in vitro model of translation that can be used in biophysical studies of FMRP interacting with the ribosome. I am working on utilizing the CrPV IRES, a specialized RNA sequence that facilitates translation initiation, and purified protein and tRNA components to construct a translation system simple enough to be studied using cryo EM. At this point I have managed to purify two proteins, eEF1A1 and eEF2, and have produced and purified an mRNA containing the CrPV IRES. Development of a minimal translation system will eventually be utilized to analyze the mechanism of translation regulation by FMRP using structural and biophysical methods.

Poster 12

Trapping Acyl Carrier Protein Interactions With a Type III PKS system

Jeff Mindrebo
University of California, San Diego

Polyketide synthases (PKSs) are enzymatic assembly lines that produce a wealth of bioactive natural products and antibiotics through the iterative condensation reduction, and cyclization of two carbon units. These systems are divided into three categories; Type I and II systems depend on acyl carrier proteins (ACPs) to carry growing polyketide intermediates, while type III systems use only coenzyme A (CoA) extender units and all reactions are performed in a single active site. Recently, germicidin synthase (Gcs), a type III PKS from *Streptomyces coelicolor*, was shown to intercept ACP derived polyketide starter units from the fatty acid biosynthesis (FAS) pathway. The underlying mechanism for how GCS intercepts ACP intermediates from FAS is currently unknown. To address this, we have developed active site mechanistic crosslinkers that can be loaded onto ACP and reacted with Gcs to trap the Gcs and ACP interaction. Production of crosslinked complex is scalable and future work is targeted towards crystallizing the complex to identify the ACP-Gcs interaction interface. Additionally, with a working crosslinking assay, we can begin to tease out important molecular interactions necessary for ACP mediated cargo delivery to Gcs through site directed mutagensis. This work will lead to a better understanding of the communication between primary and secondary metabolism in Streptomyces as well as new avenues for engineering PKS pathways to produce novel compounds.

Poster 13

Probing the Dynamics Behind the Substrate Specificity of Thrombin

Riley Peacock; Elizabeth Komives
University of California, San Diego

The serine protease, thrombin, regulates the balance between the anticoagulation and coagulation in the blood clotting pathway. Thrombin ordinarily cleaves fibrinogen generating fibrin, which forms the blood clot. Binding of thrombomodulin to thrombin switches its catalytic activity away from fibrinogen and towards activation of protein C, which initiates the anticoagulation pathway. Recently Trp 215 was shown to be a key residue in determining substrate specificity. The mutant W215A was reported to lose significant activity towards fibrinogen without significant loss of activity towards protein C- shifting thrombin's activity toward anticoagulation, even in the absence of thromobomodulin, while the mutant W215I showed a loss of both coagulative and anticoagulative activity.

Our lab has obtained experimental and computational results suggesting that enzyme dynamics are important for connecting the main allosteric site on thrombin, where thrombomodulin binds, to the active site. In order to understand how mutation of Trp215, which is not on the pathway between the allosteric site and the active site may alter thrombin specificity, I carried out hydrogen-deuterium exchange experiments (HDXMS) to compare the enzyme dynamics of the W215A and W215I mutants with those of wild type thrombin. HDXMS results indicate that amide exchange throughout much of the thrombin molecule remains unchanged in both mutants. However, amide exchange in the 170's loop and in the N-terminus of the heavy chain was increased in the both mutant as compared to wild-type, and amide exchange in the beta-sheet leading up to position 215 (res. 209-214) was markedly decreased.

Additionally, chromogenic substrate assay results suggest that only W215I significantly affects the active site, though it is reported that both mutants cleave fibrinogen poorly. We hypothesize that these changes result from the removal of a substrate binding interaction, and the occupation of the active site by the Trp 215 mutant side chains. Thus, it is likely that these mutations directly alter substrate specificity rather than allosteric pathways within thrombin.

Poster 14

Lanthanide Cofactors for Triphosphorylation Ribozymes

Kevin J. Sweeney[1] and Ulrich F. Müller[1],
[1]*Department of Chemistry & Biochemistry, University of California, San Diego*
** e-mail of Correspondence Author: ufmuller@ucsd.edu*

The RNA world hypothesis describes an early stage in the evolution of life in which RNA would have served as genome and the only genome-encoded catalyst. RNA world organisms would have required an energy source for the thermodynamically unfavorable polymerization of RNA. We previously showed that trimetaphosphate (Tmp), a prebiotically plausible energy source, can be used by ribozymes to triphosphorylate RNA 5'-hydroxyl groups, thereby gen-erating chemically activated RNA 5'-phosphates that contain the thermodynamic driving force for RNA polymerization [1]. Analogous 5'-triphosphates could be seen as the precur-sors for ATP, the energy currency in every known form of life.

To test whether different metal ion cofactors could be used by triphosphorylation ribo-zymes we performed an in vitro selection in the absence of Mg2+ and in the presence of the lanthanide Yb3+. Lanthanides are promising cofactors for triphosphorylation reactions be-cause they activate Tmp for nucleophilic attack, modulated by the lanthanide's coordination status [2]. While the lanthanide's prebiotic relevance is debatable (they are highly enriched in pegmatitites [3] but these minerals may be hard to mobilize) these experiments explore the chemical space accessible to RNA-catalyzed RNA triphosphorylation.

After eight rounds of selection from a pool with 150 randomized nucleotides several active ribozymes were recovered. Interestingly, different sequence clusters displayed very different responses to changes in pH and ion concentrations. This suggests that these lanthanide-using ribozymes employ different catalytic strategies.

References

1. Moretti JE, Muller UF. A ribozyme that triphosphorylates RNA 5'-hydroxyl groups (2014) Nucleic Acids Res. 42 (7), 4767-78.
2. Huskens J, Kennedy AD, van Bekkum H, Peters JA. The Hydrolysis of Trimetaphosphate Catalyzed by Lanthanide(III) Aminopolycarboxylate Complexes: Coordination, Stability, and Reactivity of Intermediate Complexes (1995) J Am Chem Soc. 117, 375-82.
3. Taylor SR, McLennan SM. Distribution of the lanthanides in the earth's crust. Met Ions Biol Syst. 2003; 40:1-38.

Poster 15

The Msh4-Msh5 complex's role in meiotic crossover formation

Sarah Ur[1,2] and Kevin Corbett[2,3]
[1]Biomedical Sciences Graduate Program, University of California, San Diego. [2]LICR, University of California, San Diego. [3]Department of Cellular and Molecular Medicine, University of California, San Diego.

The MutS Homologs (MSH) have been identified in all organisms from *E. coli* to humans, and function in the initial recognition of mismatched base pairs in the conserved mismatch repair pathway. The MSH2-MSH6 and MSH2-MSH3 complexes participate in the repair of mismatches after mitotic DNA replication. In contrast to MSH2-MSH6 and MSH2-MSH3, the MSH4-MSH5 complex does not participate in mitotic mismatch repair, but plays a critical role in meiotic recombination and the segregation of homologous chromosomes during gamete/spore formation. Mutations of Msh4 or Msh5 in *S. cerevisiae* result in defects in the formation of crossovers, the physical links between homologs that enable their proper segregation in meiosis I. The current model for the MSH4-MSH5 complex's function is that it binds and stabilizes an early recombination intermediate, perhaps the D-loop product of initial strand invasion or alternatively a Holliday Junction, and promotes the assembly of crossover-specific machinery. This machinery constitutes a "recombination nodule," a large assembly whose architecture is not known, but which is visible by low-resolution electron microscopy on meiotic chromosomes. In order to further our understanding for the MSH4-MSH5 complex's binding mechanism to recombination intermediates and its roles in meiotic crossover formation, we will first aim to reconstitute the complex, characterize its binding to artificial DNA assemblies mimicking recombination intermediates, and crystallize the complex bound to a favored intermediate. Additional studies will aim to determine the complex's interaction with other proteins such as RecA/Rad51 homologs and downstream repair factors. This work will reveal how MSH4 and MSH5 interact with chromosomes and components of recombination machinery. By researching the MSH4-MSH5 complex and its downstream partners, we will be able to make a leap in understanding the physical basis of crossover formation.

Lightning Talk 1

RNA Modulates the Interaction Between NS1 and PABP1

Authors: Bryan H. Arias (1) and Simpson Joseph (2)
Department of Biological Sciences; (2) Department of Chemistry and Biochemistry. University of California - San Diego, La Jolla, CA 92037

Influenza A Virus is a RNA virus that infects seasonally causing flu and is responsible for nearly fifty thousand deaths annually. Influenza A virus has been proposed to hijack the host translational process using the viral Non-Structural Protein 1 (NS1). NS1 is a small, RNA binding protein that, in addition to preventing host interferon response, is able to bind to several host proteins, including Poly (A) Binding Protein (PABP1). Previous research suggest that NS1 binds to the unique 5'-UTR of viral RNAs, recruits PABP1, which then recruits eIF4G and other factors, resulting in the stimulation of viral mRNA translation. How NS1 is able to distinguish viral and non-viral RNA, and the unique sequences within the viral 5'-UTR, is currently unknown. Moreover, it is not known how RNA modulates the interaction between NS1 and PABP1. Using anisotropy with a GST-tagged NS1, we find that NS1 does not preferentially bind to the single-stranded 5'-UTR nor double stranded viral RNA. However, it binds to G-quadruplex structures and double-stranded G-rich RNA. We also show that NS1 does bind weakly to the truncated version of a viral gene, M1, compared to exogenous RNA, *Renilla luciferase*. Using FRET, we show that the interaction between NS1 and PABP1 is strong (K_D = 18.5 ± 5 nM), but modulated in the presence of RNA. When incubated with Poly (A), the K_D shifts to over 160 nM, but with Poly (C), it shifts to 45 nM. In the presence of tailed M-Del or R-Del, the binding shifts to 45nM and 150 nM, respectively, indicating that RNA weakens the interaction between NS1 and PABP1. Why RNA modulates the interaction is not known and if there are any other factors, such as eIF4G, that stabilize the interaction.

Lightning Talk 2

Examining the allosteric crosstalk within a designed disulfide-linked Zn-binding metalloprotein

Lewis Churchfield
University of California, San Diego

Metals in association with protein scaffolds are ubiquitously used in the cell as signaling agents, as structural cofactors, and as catalytic centers. I am interested in enriching our understanding of nature's repertoire of metalloproteins and, and by engineering new functional metalloproteins with non-natural functionalities. My research applies principles of design that draw from protein biophysics and coordination chemistry. Metal ions can serve as templating agents to direct the ordered assembly of oligomeric protein complexes by interacting with strategically-placed chelating motifs on protein surfaces. These oligomeric protein assemblies can be further engineered to assemble by installing combinations of favorable protein-protein contacts and disulfide bond crosslinks, or by altering their metal-coordination sites (**Figure 1a**). The monomeric cytochrome cb562 has been used as a building block in this metal-templated protein design strategy to generate a family of self-assembling metalloprotein scaffolds, the RIDC1 family. RIDC1 self-assembly is sufficiently robust to accommodate up to six disulfide crosslinks in total. Surprisingly, the disulfide crosslinking at a particular site in the scaffold can undergo hydrolysis in a manner that is allosterically coupled to the metal-binding of the protein scaffold (**Figure 1b**). The inter-protein interactions in the protein RIDC1 oligomers can be dissected to better understand this phenomenon by x-ray crystallography and solution-phase biophysical techniques. Additionally, all-atom molecular dynamics simulations provide a means of characterizing this allosteric crosstalk in even greater detail. A thorough understanding of this phenomenon will aid in further efforts to engineer function into the RIDC1 family of proteins, and aid in other efforts to the design metalloproteins from scratch using first principles.

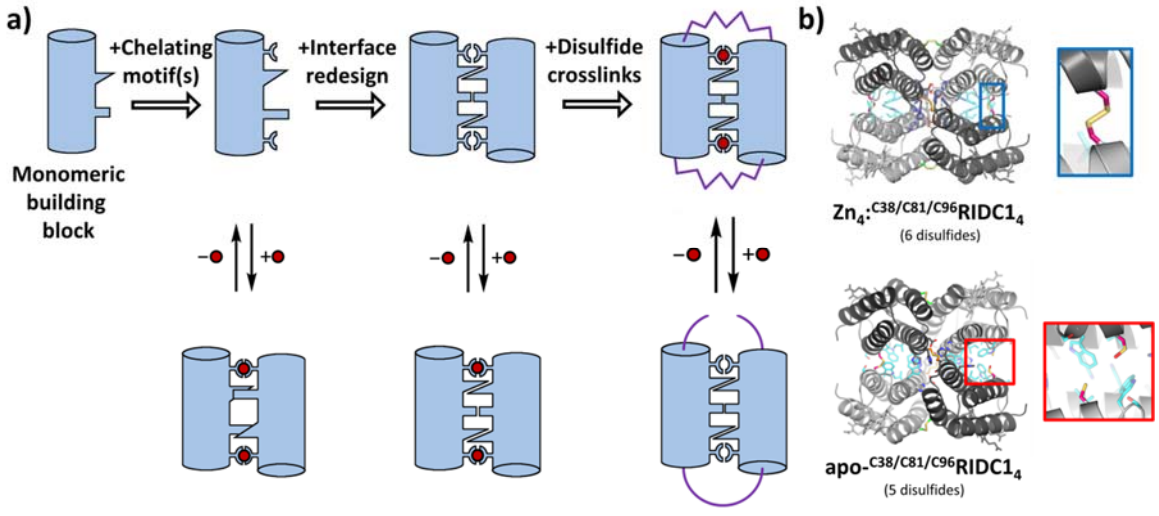
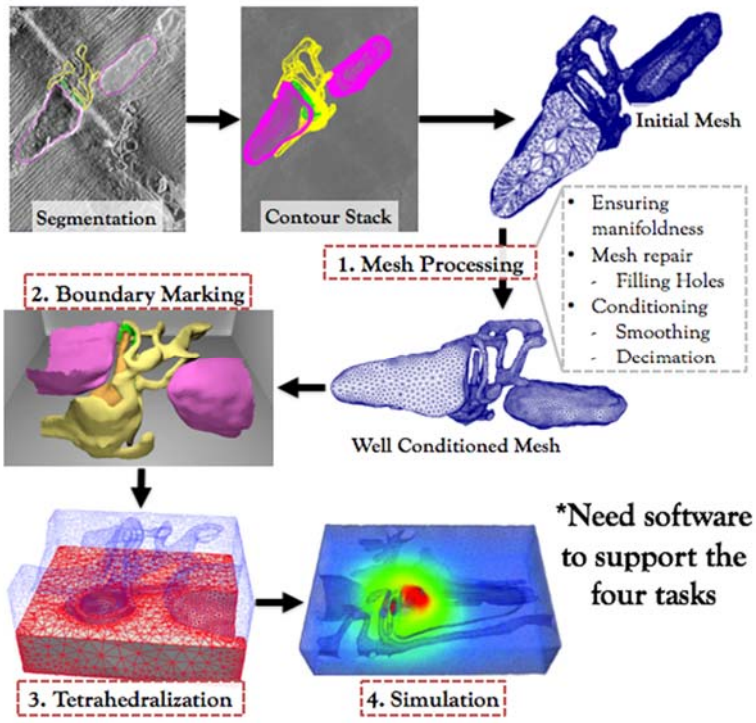


Figure 1 – (a) Design of oligomeric metalloprotein complexes by the installation of metal chelating motifs, redesign of nascent interfaces, and installation of disulfide bond crosslinks. (b) X-ray crystal structures of the disulfide-crosslinked $^{C38/C81/C96}$RIDC1$_4$, highlighting its hydrolysable disulfide bond.

Lightning Talk 3

Investigating transport properties with multiscale computable mesh models from heterogeneous structural datasets

Christopher T. Lee†, John B. Moody‡, Michael J. Holst‡, J. Andrew McCammon†, Rommie E. Amaro†
† *Department of Chemistry and Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0340, United States*
‡ *Department of Mathematics, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0340, United States*



Individual modeling techniques are often applicable in limited spatial-temporal domains leading to gaps in our biological modeling capabilities. To bridge these gaps, new methodological solutions to integrate levels of resolution are necessary. Mesh models can represent spatial data across all size scales, furthermore, they can be used with various levels of simulation resolution. Thus, mesh structures provide an ideal integrative platform for multiscale modeling. In this work, we describe a workflow to convert structural datasets, such as those from electron microscopy or x-ray crystallography, into mesh models suitable for modeling. To ensure that resultant meshes are "compute quality" we employ a redesigned version of GAMer (Geometry-preserving Adaptive MeshER), a mesh generation tool that produces and refines high-quality simplex meshes. GAMer is available as a stand-alone package or it can be accessed as a plug-in to 3D-modeling software Blender. To improve the robustness of mesh generation, we have implemented automated detection and resolution of many common mesh defects. Drastic improvements in structural biology methods have led to an abundance of structural datasets ranging from protein structures to subcellular images. The development of an integrative multiscale meshing platform can yield important new biological insight into the effects of physiology and anatomy on function. These multi-scale models will provide new opportunities for drug discovery and have an impact on human health and longevity.

Lightning Talk 4

DNA and IκBα both induce long-range conformational changes in NFκB

Kristen Ramsey
University of California, San Diego

We recently discovered that IκBα enhances the rate of release of nuclear factor kappa B (NFκB) from DNA target sites in a process we have termed molecular stripping. Coarse-grained molecular dynamics simulations of the stripping pathway revealed two mechanisms for the enhanced release rate: the negatively charged PEST region of IκBα electrostatically repels the DNA, and the binding of IκBα appears to twist the NFκB heterodimer so that the DNA can no longer bind. In this work, we report amide hydrogen/deuterium exchange data that reveal long-range allosteric changes in the NFκB (RelA-p50) heterodimer induced by DNA or IκBα binding. The data suggest that the two Ig-like subdomains of each Rel-homology region, which are connected by a flexible linker in the heterodimer, communicate in such a way that when DNA binds to the N-terminal DNA-binding domains, the nuclear localization signal becomes more highly exchanging. Conversely, when IκBα binds to the dimerization domains, amide exchange throughout the DNA-binding domains is decreased as if the entire domain is becoming globally stabilized. The results help understand how the subtle mechanism of molecular stripping actually occurs

Lightning Talk 5

Toward Comparative Biophysics of APOBEC3 Enzymes

Jeffrey Wagner, Ozlem Demir, Rommie Amaro
Department of Chemistry and Biochemistry. University of California San Diego

The APOBEC3 (A3) family of cytidine deaminase enzymes is suspected to be a driver of mutations in some breast cancers. Each A3 protein consists of either one or two deaminase domains. While these domains all share at least 30% sequence identity, some of them have been biochemically characterized and found to exhibit varied activity profiles and DNA substrate specificities. This background makes the A3 family particularly well-suited for biophysical modeling, as we can predict how uncharacterized A3 proteins will act based on how they differ from characterized A3s. Our computational models of the A3 family are being used to pursue a number of distinct projects with experimental collaborators, including x-ray crystallography, discovery of inhibitory lead compounds, and rationalization of the DNA substrate specificity of the different A3 subtypes. Models also enable us to synergistically combine the results of these projects to more efficiently explore the A3 family's role in disease and potential therapies.