UC San Diego

MBTG 2019 Annual Retreat

Friday, April 5th, 2019

Program Book

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UC San Diego

Molecular Biophysics Training Grant (MBTG)
2019 Annual Retreat

Friday, April 5th, 2019, 3:00PM
Institute of the Americas

2:30PM — *Arango Foyer*
Poster set-up

3:00PM — *Deutz Room*
Welcome Remarks: Betsy Komives

3:05PM — *Deutz Room*
Faculty Talk: Tatiana Mishanina
RNA polymerase: Stop-and-go traffic on the DNA highway

3:35PM — *Arango Foyer*
Poster Presentations: MBTG Trainees

Bryce Ackermann
Joshua Arriola
Joshua Corpuz
Cyrus De Rozieres
Mounir Fizari
John Gillies
Sonjiala Hotchkiss
Dominic McGrosso
Riley Peacock
Kira Podolsky
Elizabeth Porto
Clara Posner
Hannah Rutledge
Bryce Timm
Hetika Vora
Douglas Zhang

5:00PM — *Deutz Room*
Faculty Talk: Mark Herzik
Bringing Mitochondrial Transport into View Using Novel cryo-EM Methodologies

5:30PM — *Deutz Room*
Lightning Talks: MBTG Alumni

1. Robert Alberstein
2. DeeAnn Asamoto
3. Colin Deniston
4. Benjamin Jagger
5. Evan Kobori
6. Sarah Kochanek
7. Noah Kopcho
8. Christopher Lee
9. Ryan Lumpkin
10. Jeffrey Mindrebo
11. Sarah Ur

6:30PM — *Friend Plaza*
Barbeque Dinner

6:45PM — *Friend Plaza*
Poster Awards: Betsy Komives

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MBTG 2019 Annual Retreat

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Muller Lab

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The Molecular Biophysics Training Grant is supported by NIH Grant T32 GM008326.

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The Molecular Biophysics Training Grant is supported by NIH Grant T32 GM008326.

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Sarah Ur
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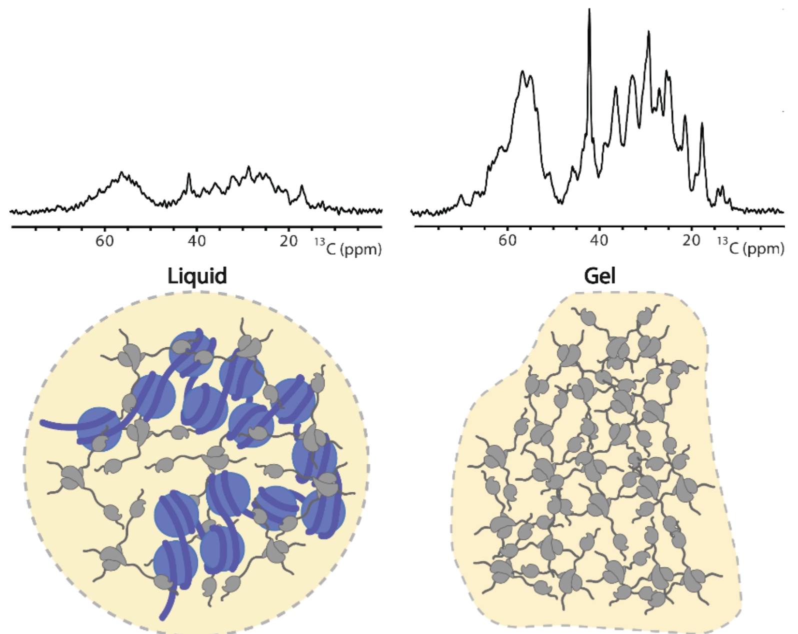
Corbett Lab

The Molecular Biophysics Training Grant is supported by NIH Grant T32 GM008326.

Heterochromatin protein HP1α gelation dynamics revealed by solid-state NMR spectroscopy

Bryce Ackermann
Debelouchina Lab
Poster #1

Heterochromatin protein 1α (HP1α) undergoes liquid-liquid phase separation (LLPS) and forms liquid droplets and gels *in vitro*, properties that also appear to be central to its biological function in heterochromatin compaction and regulation. Here we use solid-state NMR spectroscopy to track the conformational dynamics of phosphorylated HP1α during its transformation from the liquid to the gel state. Using experiments designed to probe distinct dynamic modes, we identify regions with varying mobilities within HP1α molecules and show that specific serine residues uniquely contribute to gel formation. The addition of chromatin disturbs the gelation process while preserving the conformational dynamics within individual bulk HP1α molecules. Our study provides a glimpse into the dynamic architecture of dense HP1α phases and showcases the potential of solid-state NMR to detect an elusive biophysical regime of phase separating biomolecules.



Combinatorial Identification of Short Catalytic Motifs from Hundreds of Ribozymes

Joshua Arriola
Muller Lab
Poster #2

Introduction: The RNA World hypothesis states that RNA acted both as genetic storage and as the only genome-encoded catalyst during an early stage of life. To explore how an RNA world organism could have functioned, catalytic RNAs (ribozymes) have been developed by in vitro selection from large pools (usually $>10^{14}$ different sequences) of randomized RNA (Bartel & Szostak 1993). Because the prebiotic synthesis of RNA likely favored shorter sequences (Huang & Ferris 2003) it is important to determine what the shortest RNA motifs are that can catalyze important reactions relevant for an RNA world organism. Previously, the catalytic core of certain ribozymes was identified by successive truncation from individual, selected sequences (Akoopie & Muller 2016). Here we present a combinatorial method to identify the shortest RNA motifs that result from in vitro selections.

Results: To identify the shortest RNA motif from a previous in vitro selection (Moretti & Muller 2014) that generated hundreds of ribozymes (Pressman et al. 2017) we generated truncated versions of the selected pool and fractionated it by size. Each of the ten size fractions was subjected to one round of selection. The seven largest fractions showed catalytic activity while the three shortest fractions did not. The shortest, active fraction was analyzed, containing only a single, active motif. The resulting ribozyme is smaller than the smallest previously isolated ribozyme for the same catalytic activity.

Conclusion: We have generated a combinatorial method that can identify small catalytic motifs from pools. This method can now be used to identify the smallest motifs from libraries that resulted from other in vitro selection experiments.

References:

Akoopie, A., & Muller, U. F. 2016, Phys. Chem. Chem. Phys., 18, 20118
Bartel, D. P., & Szostak, J. W. 1993, Science, 261, 1411
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Pressman, A., Moretti, J. E., Campbell, G. W., Muller, U. F., & Chen, I. A. 2017, Nucleic Acids Res, 45, 8167

Elucidating the Protein-Protein Interface of the Peptidyl Carrier Protein and Adenylation Domain in Pyoluteorin Biosynthesis

Joshua Corpuz

Burkart Lab

Poster #3

Many pharmaceutical compounds are derived from nonribosomal peptide synthetase (NRPS) products. NRPSs are modular enzymes that, like an assembly line, incorporate specific amino acids into a short polypeptide chain. Traditional type I NRPSs are megasynthetases that have multiple enzymatic domains in a single polypeptide chain. Type II NRPSs consist of the stand-alone enzymatic domains that rely on protein-protein interactions for accurate protein communication and efficient product synthesis. Type II NRPSs have diverse tailoring domains and also commonly participate in hybrid biosynthetic pathways. The architecture and diversity of type II NRPSs makes them ideal for combinatorial biosynthesis, however, engineering efforts depends on understanding the important protein-protein interactions that govern product fidelity and formation.

This project involves the structural elucidation of the protein-protein interactions between type II peptidyl carrier proteins (PCP) and their cognate adenylation domain (A domain), which are essential NRPS domains. Structural analysis, including nuclear magnetic resonance and x-ray crystallography, on the type II PCP and A domain of pyoluteorin biosynthesis, PltL and PltF, reveals a largely hydrophobic protein-protein interface (Figure 1). Furthermore, the interface contact with PltF almost solely consists of the dynamic loop 1 motif of PltL. The PltL-PltF interface contrasts the interfaces of recently obtained type I and type II PCP-A domain structures, which reveals the diversity of PCP and partner protein binding modes across NRPS systems. This difference in the PCP-partner protein interface adds a layer of complexity in the combinatorial biosynthesis of NRPS systems.

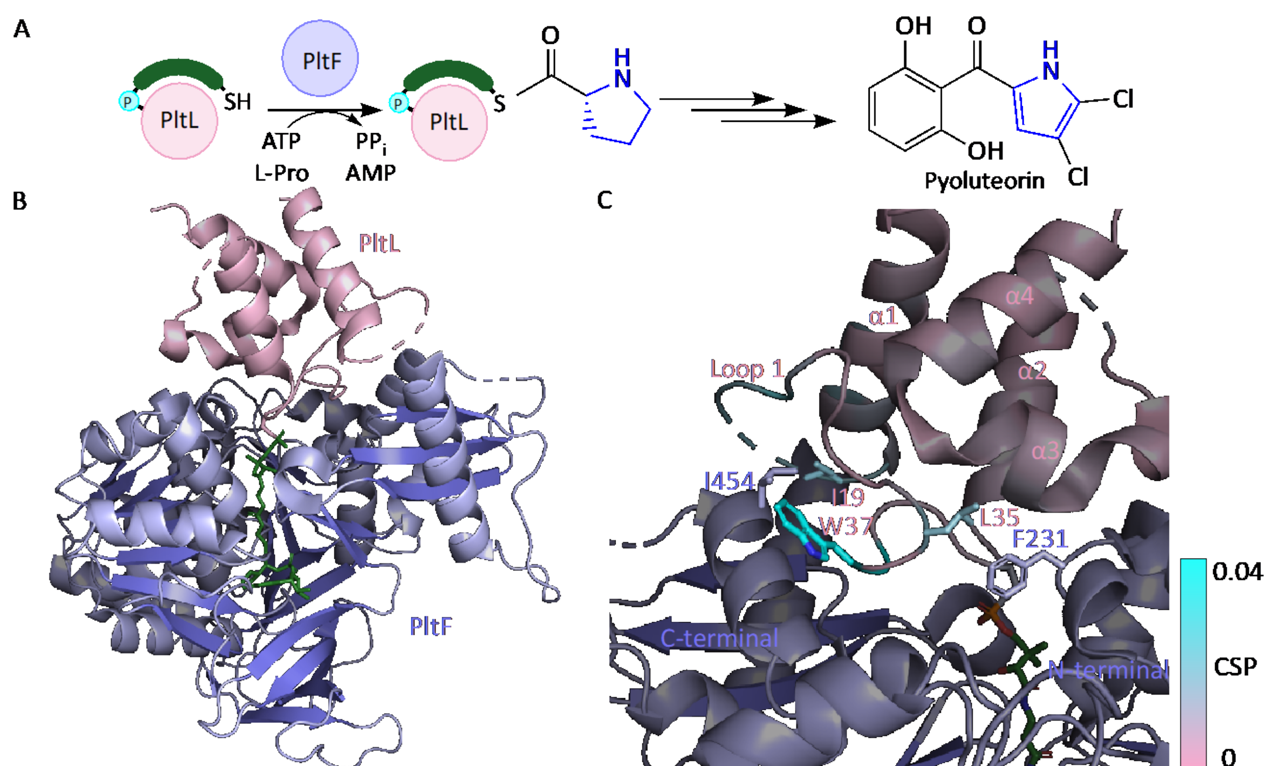


Figure 1. Interaction between the PCP, PltL (blue), and the A domain, PltF (pink). A) PltF adenylates and thiolates L-proline onto the phosphopantetheine arm (green) of PltL. PltL transfers the prolyl moiety to downstream enzymes for functionalization and incorporation into pyoluteorin. B) 2.1 Å crystal structure of the PltL-PltF complex trapped with a mechanism-based inhibitor. C) Close up of the interface with important interactions shown for clarity. Chemical shift pertubations from NMR titrations of PltL with PltF are mapped onto PltL. The structure in panel C is rotated 180° from panel B.

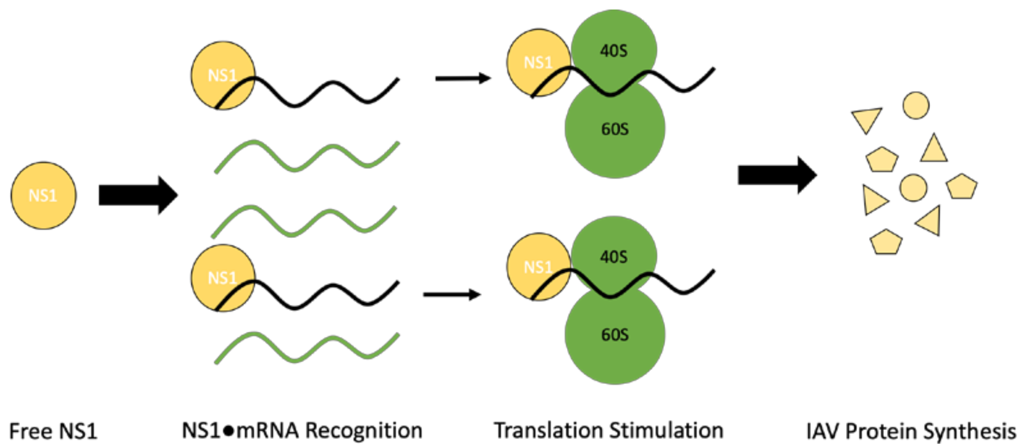
Translation Stimulation Mediated via the Selective RNA Binding of the Influenza NS1 Protein

Cyrus de Rozieres

Joseph Lab

Poster #4

Influenza A virus (IAV) is the cause for the seasonal flu whereby infected human lung epithelial cells serve as the host for viral replication. Infection by IAV cause upper respiratory tract difficulties that can range from mildly debilitating to fatal. The CDC estimates that flu related deaths worldwide range from 250,000 to 500,000 every year. Currently, the best measures against the flu are vaccination-based prophylactics. However, with a ~60% efficiency, alternative modes of treatment targeting essential viral functions are of importance. A key step of the IAV life cycle involves shutting down host cell processes in order to direct resources towards viral proliferation. The viral protein Non-Structural Protein 1 (NS1) is implicated in a variety of functions that serve to shut down host cell processes in order to direct cellular machinery toward viral replication. As part of its function, NS1 is known to enhance translation by some unknown mechanism. One method that may be important towards its ability to stimulate translation may be due to NS1s capacity to bind to RNA, however specific sequences or motifs have yet to be identified. Here I report NS1's affinity towards a particular sequence that is generated during the viral life cycle. Using anisotropy and gel-shift based approaches with purified NS1 and in-vitro transcribed RNAs, I have measured binding coefficients for varying RNA. This binding correlates with the translation upregulation of mRNA containing these sequences, as seen in transfection and in vitro translation assays. This suggests a possible mode by which NS1 may selectively target specific sequences that are unique to viral mRNA, in order to have them translated over non-viral mRNA. This specific interaction makes both NS1 and its target RNA sequence possible targets for therapeutic intervention that may help mitigate future outbreaks of this pathogen.



Maximum force generated by the phage phi29 DNA packaging motor assessed by optical tweezers measurements

Mounir Fizari
D. Smith Lab
Poster #5

Viral DNA packaging motors play a key role viral assembly. In collaboration with Paul Jardine's lab, we use optical tweezers to measure DNA translocation by the phi29 motor and a rapid force jump technique to investigate the maximum force generation. Preliminary measurements suggest that the motor can translocate briefly against applied forces of ~60 pN at low capsid filling. Measurements at high capsid filling suggest that the motor can generate even higher forces. However, frequent slipping occurs, such that there is no net packaging. The preliminary findings suggest the force is limited by the strength of the motor's grip.

Lis1 promotes the formation of maximally activated dynein complexes

John P. Gillies
Reck-Peterson Lab
Poster #6

Cytoplasmic dynein-1 (dynein) is a microtubule-based motor that facilitates the long-distance transport of many different cargos toward the minus end of microtubules. Human dynein does not move processively on its own. Instead, it must form a complex with dynactin and a coiled-coil-containing activating adaptor, such as BICD2, to achieve motility1,2. Complexes containing two dynein dimers move faster3. Another highly conserved dynein regulator, Lis1, is also required for nearly all of dynein's functions. In vitro Lis1 increases the velocity of activated human dynein complexes4,5, but the mechanistic basis for this remains unclear. Using in vitro reconstitution of pure proteins and single-molecule imaging, we have found that this increase in velocity is due to Lis1 recruiting an additional dynein dimer to the complex. The requirement for Lis1 to increase dynein's velocity can be bypassed by adding excess dynein when activated complexes are being assembled, or by using an activating adaptor that already maximally promotes dynein complex assembly. We hypothesize that Lis1 has a general role in the formation of optimally activated dynein complexes.

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Development of Allosteric Inhibitors of IKK2

Sonjiala Hotchkiss
G. Ghosh Lab
Poster #7

Nuclear factor kappa B (NF-κB) is a family of transcription factors that play a crucial role in the immune response. In the NF-κB activation pathway, the signaling cascade can be initiated by cytokines, cellular stress, viral or bacterial agents, and ionizing radiation. NF-κB acts to enhance or repress genes important in the regulation of inflammation, apoptosis, proliferation, and cell survival. As aberrant activation of the NF-κB pathway underlies many inflammatory diseases and cancers, the development of specific small molecule inhibitors of this pathway would have important therapeutic applications. The key regulator of the NF-κB pathway is IκB kinase (IKK) complex. IKK complex contains two catalytic components, IKK1 and IKK2, and one scaffolding component NF-kappa-B essential modulator (NEMO). In the canonical pathway, the catalytic subunit IKK2 phosphorylates IκBα, an inhibitor that holds NF-κB dimers dormant in the cytoplasm. Phosphorylated IκBα is then polyubiquitinated and undergoes proteasomal degradation thus releasing NF-κB to travel to the nucleus to activate or repress transcription. Experimental data show that the small molecules we developed are effective allosteric inhibitors that target IKK2. Cellular assays show that IκBα degradation is blocked. In vitro assays show that while kinase function of activated IKK is blocked when IKK2 is incubated with known ATP competitive inhibitor SC514, this function is not blocked when IKK2 is incubated with our inhibitor #65.5. In addition, we use hydrogen-deuterium exchange mass spectrometry (HDX-MS) to show where the inhibitors bind IKK2 and how the inhibitors affect the protein dynamics of IKK2. Our HDX-MS data support that our inhibitors work allosterically through examination of differences in IKK2 structural dynamics when IKK2 is partnered with ATP, with known ATP-competitive inhibitor TPCA, and with our inhibitor #65.5.3. Following identification of residues of inhibitor binding using HDX-MS, we use tandem mass spectrometry (MS/MS) to confirm the identify of specific binding residues. Using the collected data, we developed a model of how our small molecule inhibitors disrupt the activation of IKK.

Ligand Activated Molecular Probes

Dominic McGrosso
G. Chang Lab
Poster #8

The field of biosensors has a need for portable, robust systems that are both rapid and inexpensive for on-site measurements of small molecules with minimal sample preparation and technical expertise. Current techniques used for the analysis of small molecules are typically performed in the lab and include High Pressure Liquid Chromatography, Gas or Liquid Chromatography coupled to Mass Spectrometry, Enzyme-Linked Immunosorbent Assay, and various spectrophotometric methods. These analytical methods have major drawbacks including the need for technical expertise and are prohibitively expensive, making them less than ideal for rapid on-site condition investigations. Biosensors are broadly classified based by: (1) the type of bioreceptor recognition element (antibody, nucleic acid, enzyme, whole cell, aptamers, etc.) and (2) the transduction method signaling ligand binding and detection (optical, electrochemical, piezo-electrical, etc.).

Despite great advances in transducer technologies and recent methods using molecular evolution or rational structure-based/computational design, no clear strategy has emerged for the general design of a biosensor scaffold capable of high affinities and specificities for a diverse range of small molecule compounds. Even fewer methods combine the receptor and transducer elements into a ligand-activated fluorescent biosensor scaffold. Consequently, most field biosensors today still cannot fully compete with the accuracy and reproducibility of conventional lab-based analytical methods and, conversely, many of the lab-based sensors cannot withstand typical environmental conditions for field applications (heat, salinity, lack of power source, etc.). Further, the development of these sensors is often slow, costly and cannot keep pace with the growing list of small molecule targets prevalent in biomedical research today. As such, there is increasing need for a reliable, inexpensive, and portable biosensor platform that can be easily made to not only specifically bind a diverse range of small molecules, but also to transduce a signal with tunable output for convenient detection.

We are developing a method to discover and rapidly generate a new type of biosensor called *Ligand Activated Molecular Probes (LAMPs)*. LAMPs are derived from a library of protein scaffolds based on a protein originating from the eel, *Anguilla japonicus,* that binds a small compound called bilirubin with high specificity and affinity. Upon binding, changes in the structure of the protein act in concert with the electronic structure of bilirubin to produce fluorescence in the visible region. Our method begins by altering key positions at the ligand-binding interface to create a library of mutants that we call LAMPs. This library is displayed on the cell surface of bacteria and challenged with small molecules, using FACS to select for fluorescence generated during binding. Upon enrichment of a fluorescent population, we apply molecular evolution to yield new variants, iteratively re-selecting new LAMPs with desired affinities, specificities, and fluorescent output. Our goal is to produce LAMPs that are selective for small molecules while developing an understanding of the molecular structural basis for ligand binding and fluorescence. We will use several biophysical approaches, including x-ray crystallography, bio-layer interferometry, and spectrofluorometry to characterize binding and quantum yield, respectively.

The Dynamics Behind Thrombin Mutants with Anticoagulative Substrate Activity

Riley Peacock
Komives Lab
Poster #9

The serine protease, thrombin, regulates the balance between the anticoagulation and coagulation in the blood clotting pathway. Binding of thrombomodulin to thrombin switches its catalytic activity from procoagulative substrates (i.e. fibrinogen) to protein C, triggering the switch from the coagulation to the anticoagulation pathway. Recently, Ala mutants replacing Trp215, Glu217, and Phe227 were reported to lose activity towards fibrinogen without significant loss of activity towards protein C, and the double mutant W215A/E217A has been included in clinical studies, because it has an even greater preference for anticoagulative over procoagulative substrates. Our lab has obtained experimental and computational results suggesting that enzyme dynamics are important for connecting the main allosteric site on thrombin, where TM binds, to the active site. To understand how mutation of Trp215, Glu217, and Phe227 may alter thrombin specificity, hydrogen-deuterium exchange experiments (HDXMS) were carried out to compare the dynamics of W215A, W215A/E217A, W215I, F227A, and F227V with those of WT, activity assays were utilized to map out the catalytic effect of each mutant, and Accelerated Molecular Dynamics simulations of W215A were used to shed light on the phenomena behind the HDXMS results. Results indicate that mutations that affect the Trp215-Phe227 pi interaction directly destabilized the 170's, and 220's loops, and indirectly destabilized the N-terminus of the heavy chain of thrombin, resulting in misalignment of the catalytic triad. The W215A/E217A double mutant caused an even greater degree of destabilization within these regions, which extended into additional regions theorized to be important in TM-dependent allostery. Using HDXMS experiments, we show that TM-binding partially compensates for the destabilizing effects of the W215A, E217A, and F227A mutants. Our results provide a mechanism by which TM allows these mutant thrombins to cleave protein C -in the presence of TM- more effectively than procoagulative substrates.

Delineating Kras4b HVR membrane association

Kira Podolsky
Devaraj Lab
Poster #10

RAS (rat sarcoma) gain-of-function missense mutations are oncogenic drivers in almost 30% of all human cancers. Of the three RAS isoforms (HRAS, NRAS, and KRAS), KRAS is the most prevalent driver of cancer. All RAS isoforms are comprised of a GTPase domain and a hypervariable region (HVR) required for RAS localization to the cell membrane. Membrane localization is essential for RAS signaling as it facilitates RAS concentration on a 2D surface in close proximity with downstream effectors. This project aims to construct a thorough mechanism of KRAS4b membrane binding by delineating key thermodynamic and kinetic factors of membrane association and investigating inducible conditions that cause KRAS4b membrane dissociation. These studies provide insight for rational inhibitor design targeting the KRAS4b membrane binding mechanism.

Expanding the Scope of Base Editing for Precise and Therapeutically Relevant Genome Engineering

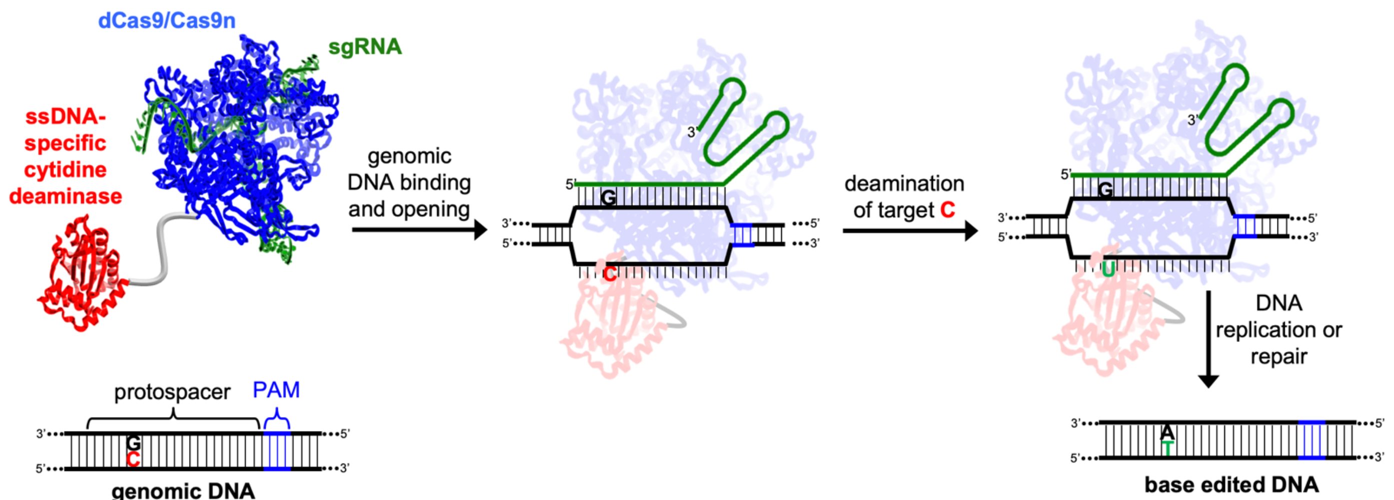
Elizabeth Porto
Komor Lab
Poster #11

Genome editing, the introduction of a user-defined change to the sequence of chromosomal DNA, is quickly becoming an indispensable tool for scientific research. The optimal genome editing tool would irreversibly edit any chromosomal position with high specificity and efficiency, and with zero undesired edits. The use of the clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated protein 9 (Cas9) system has become the standard method for specific genome editing. This system relies on the ability of the Cas9 endonuclease to introduce a double strand break (DSB) at a desired DNA sequence, which is then resolved through homology-directed repair (HDR) or non-homologous end joining (NHEJ). Under most conditions, these two repair processes are in direct competition, with NHEJ oft-winning, leading to high levels of undesired, random insertions and deletions at the site of the DSB. Therefore, DSB-reliant genome editing results in stochastic mixtures of unwanted genome modifications, making it less reliable for therapeutic and commercial use.

Base editing is an alternative approach that enables the direct, irreversible conversion of a single target DNA base in a precise, programmable manner without introducing a toxic DSB or requiring an exogenous donor template. The current mechanistic model employs a catalytically dead Cas9 enzyme (dCas9), where only DNA binding capability is maintained, complexed with a short guide RNA (sgRNA) fused to a single stranded DNA (ssDNA) modifying enzyme. Similar to CRISPR methodology, the sgRNA is used for complementary base pairing to the protospacer, which can be located and bound to through recognition of the protospacer adjacent motif (PAM). After the base editor binds to its editing target, a small stretch of ssDNA is exposed, allowing for nucleotides within this editing window to be modified by the tethered enzyme. *See figure.*

As it stands, base editing technology is limited in its scope by only facilitating C•G to T•A or A•T to G•C base pair conversions through cytidine deaminase and adenine deaminase DNA modifying enzymes. This project seeks to expand the base editor toolkit through engineering a novel ssDNA modifying enzyme from a precursory ssRNA modifier: RlmN. RlmN naturally displays substrate promiscuity, modifying both rRNA and tRNA substrates. This pre-programmed ability will be probed and expanded to include a ssDNA substrate using directed evolution methods. After the mutagenic profile of the methyl-2-adenosine base intermediate induced by RlmN has been determined, DNA editing ability will be examined and optimized. Subsequent characterization of the RlmN-base editor in mammalian cells will reveal possible downstream therapeutic application and ensure directed evolution processes worked given the orthogonality of the study.

This project will advance basic scientific understanding of genome editing enzymes, as well as accelerate novel organism engineering efforts and have a profound impact on the use of human pluripotent stem cells in genetic screening, disease modelling, and cellular therapy development.



A Rationally Enhanced Red Fluorescent Protein Expands the Utility of FRET Biosensors

Clara Posner
J. Zhang Lab
Poster #12

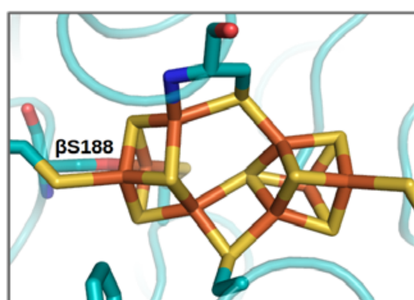
Genetically Encoded Förster Resonance Energy Transfer (FRET)-based biosensors have become powerful tools to illuminate spatiotemporal regulation of cell signaling in living cells, but the utility of the red spectrum for biosensing has been limiting, largely due to a lack of bright and stable red fluorescent proteins. Here, we rationally improve the photophysical characteristics of the coral-derived fluorescent protein TagRFP-T. We show that a new single-residue mutant, super-TagRFP (stagRFP) has nearly twice the molecular brightness of TagRFP-T and negligible photoactivation. StagRFP directly facilitates significant improvements on a number of green-red biosensors and is an efficient FRET donor to an infrared fluorescent protein, forming a red/infrared FRET biosensor. The ability to couple with multiple FRET partners allowed us to examine the signaling activities from Src, Akt, and ERK kinases simultaneously in a single cell following growth factor stimulation. These data suggest that stagRFP will enhance our ability to decipher live cell signaling.

On the brink of lability: Stabilization of the nitrogenase P-cluster by a redox-switchable ligand

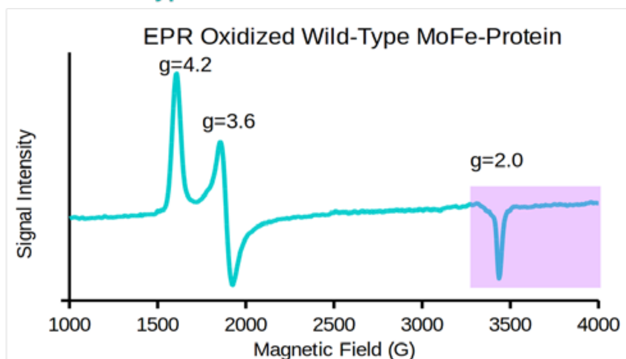
Hannah Rutledge
Tezcan Lab
Poster #13

Nitrogenase is the only known enzyme that can reduce dinitrogen to ammonia. The catalytic MoFe-Protein of nitrogenase contains two unique superclusters: FeMoco, which is the site of catalysis and is extensively studied, and the P-cluster, which is the intermediary electron relay site. Reduction of dinitrogen requires the coordinated transfer of many electrons and protons to FeMoco, a process mediated by the P-cluster. There is growing evidence that the P-cluster behaves as an active gate-keeper in the transfer of electrons to FeMoco. Understanding the P-cluster's dynamic role as an electron conduit in dinitrogen reduction is vital to elucidating the catalytic mechanism of nitrogenase. We hypothesize that mechano-redox coupling opens a conformational gate which makes electron transfer from the P-cluster to FeMoco a favorable process. Such a gate would require the primary coordination sphere of the P-cluster to be fluxional. Gating has been supported by our previous studies that discovered the conservation of a hard, oxygen-based ligand (Ser or Tyr) that ligates the 2-electron oxidized P-cluster. Recently, I constructed Azotobacter vinelandii MoFe-Protein mutants in which the primary coordination sphere ligands of the P-cluster have been altered. Characterization of these mutants with EPR and X-ray crystallography demonstrates that the P-cluster is an intrinsically labile iron-sulfur cluster in which the primary coordination sphere ligands dictate the accessible oxidation states and maintain the cluster on the brink of stability.

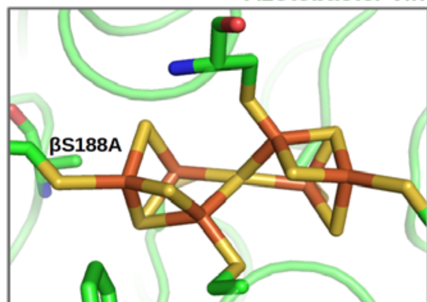
***Azotobacter vinelandii* Wild-Type MoFe-Protein**



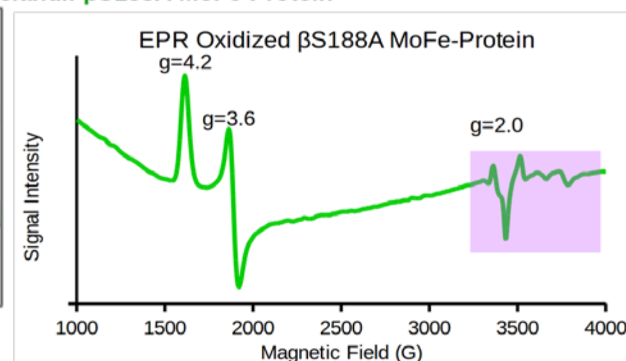
Oxidized P-Cluster:[8Fe-7S] cluster
(PDB ID:2MIN)



***Azotobacter vinelandii* βS188A MoFe-Protein**



Oxidized P-Cluster:[6Fe-7S] cluster
(unpublished)



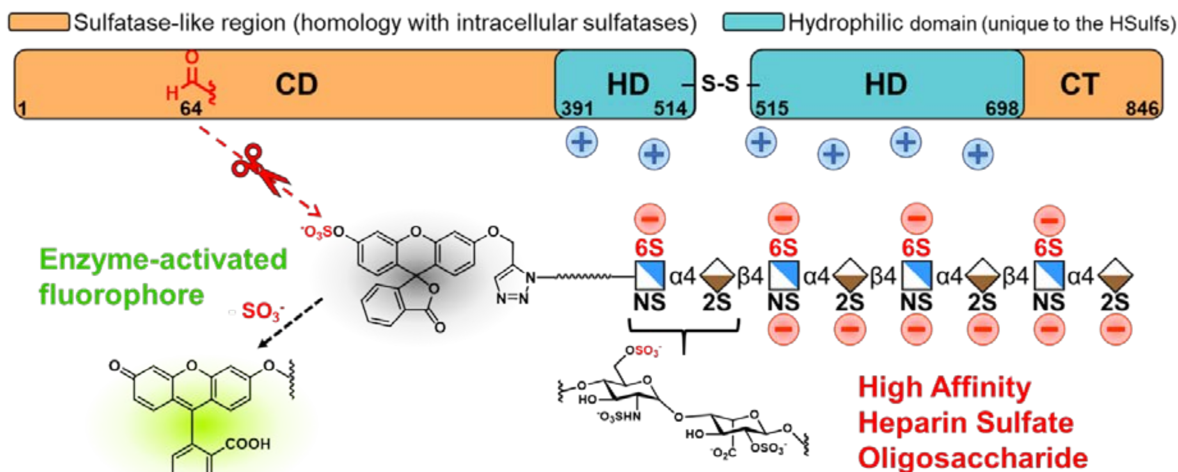
New tools for probing the activities of the human 6-O-endosulfatases: a chemical approach to a challenge in structural biology

Bryce Timm
Godula Lab
Poster #14

The polysaccharides attached to proteoglycans (glycosaminoglycans – GAGs) feature anionic sulfate groups installed during GAG biosynthesis. The unique presentations of anionic sulfate groups are used as recognition motifs for cytokines and growth factors. The "sulfation code" regulates numerous cellular functions such as differentiation, motility, and proliferation by sequestering growth factors and disrupting signaling pathways. In the extracellular environment, the sulfation code is modified by two human ECM 6-O-endosulfatases (hSulf1 and hSulf2). The removal of sulfate groups from the 6-O-position of glycans in the GAG heparin sulfate (HS), mobilizes growth factors and indirectly impacts signaling pathways.

A highly charged region in the protein, termed the hydrophilic domain (HD), is thought to be the main site for GAG recognition. The HD, which spans both subunits is unique to the ECM sulfatases. Interestingly, deletion of the HD impairs endosulfatase catalytic activity, but doesn't affect the enzyme's ability to desulfate arylsulfates, suggesting necessary regulatory elements within the HD that influence the conformation and some activity of the catalytic domain (CD).

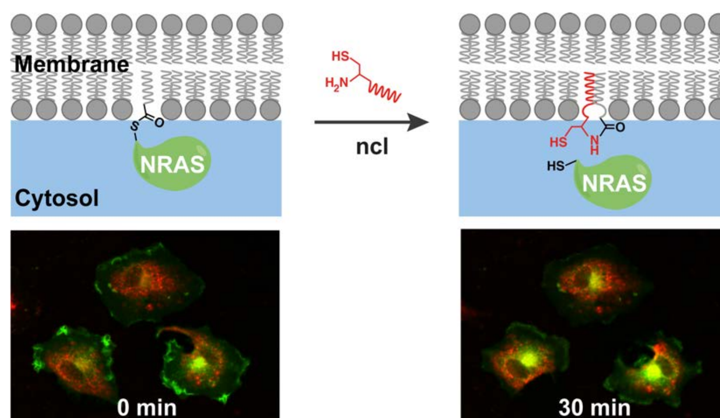
Our group seeks to develop molecules and methods that will aid in the imaging and characterization of the hSulfs. Our first step is the design of a synthetic chemical probe that enables visualization of enzyme activity through enzyme-mediated fluorescence turn-on. Structural features of our probes will be targeted to the enzyme's HD. The implementation of specific sugar chemical moieties will inform enzyme-substrate affinity. We demonstrate work on the fabrication of these tools, and solutions to unique challenges that hinder studying a highly charged and complicated network of GAGs and their associated proteins.



Direct Depalmitoylation of NRAS as a Therapeutic Strategy

Hetika Vora
Devaraj Lab
Poster #15

Protein S-palmitoylation is a reversible post-translational modification that is present on proteins involved in numerous cellular processes including protein trafficking, lipid raft association, and protein complex formation. S-palmitoylation plays a significant role in the signal transduction pathway of the oncogenic NRAS protein, which is mutated in many cancers such as metastatic melanomas and hematopoietic tumors. This post-translational modification allows NRAS to traffic from the Golgi to the plasma membrane, thus enabling its downstream signaling cascade. Mutations in NRAS lead to an overactive protein that turns on downstream proteins involved in the cell growth and survival pathways, which contribute to cancer. About 15–25% of all metastatic melanoma patients harbor activating NRAS mutations; however, there are no currently approved targeted therapies for NRAS-mutated melanomas. Directly targeting the palmitoylation modification of NRAS can be a novel approach used to inhibit NRAS oncogenic activity by preventing association with the plasma membrane using compounds capable of cleaving endogenous S-palmitoyl modifications. Our group has synthesized a class of molecules capable of chemoselective reactions with long chain thioesters, which result in the efficient release of a free thiol. Specifically, live-cell imaging is utilized to study the molecular changes in NRAS localization when disrupted by the depalmitoylating agents. In addition, methods such as western blotting and cell viability assays are used to understand how targeting NRAS protein S-palmitoylation can contribute to changes in downstream signaling pathways in the context of NRAS-mutated melanoma *in vitro* and *in vivo*. These selective and reversible chemical agents for depalmitoylation could potentially serve as new drug candidates able to intercept and shut down cancer signaling through a novel mechanism, specifically for the treatment of NRAS-mutated melanoma.



Mechanism of Depalmitoylation of NRAS
The schematic representation and fluorescence microscopy images show the depalmitoylation of GFP-tagged NRAS mutant protein (green). As a consequence of the native chemical ligation (ncl) reaction with the depalmitoylating agent, NRAS is able to delocalize from the plasma membrane into the Golgi. Golgi apparatus is stained with BODIPY TR Ceramide dye (red).

Versatile Kit of Robust Nanoshapes from RNA and DNA Modules

Douglas Zhang
Hermann Lab
Poster #16

Owing to their ability to encode folding and programmable self-assembly, nucleic acids have emerged as a material for nanotechnology applications. Despite their individual advantages, the two types of nucleic acids, DNA and RNA, have rarely been used in combination to enhance structural diversity or for the partitioning of functional and architectural roles. We have developed a design and screening strategy to integrate combinations of RNA motifs as architectural joints and DNA building blocks as functional modules that allow the programmable self-assembly of a versatile toolkit of polygonal nucleic acid nanoshapes. The design and screening strategy enables systematic development of RNA/DNA hybrid nanoshapes as programmable platforms for applications ranging from molecular recognition and catalyst development to protein interaction studies.