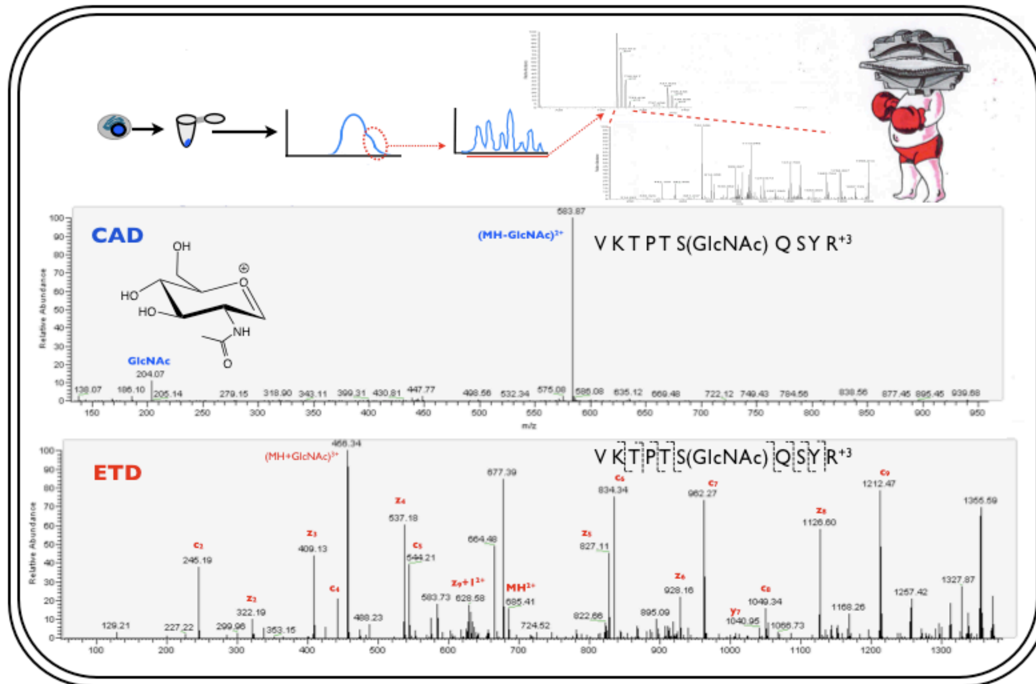
Chemical Biology in the Bay Area (CBBA)

A research day for students, postdocs, and faculty from UC Berkeley, Davis, Santa Cruz, and San Francisco



Saturday May 14, 2022 9:00am-4:35pm
University of California San Francisco
Mission Bay Campus

Talks: Genentech Hall Byers Auditorium
Posters: Genentech Hall Atrium

We would like to thank the following organizations for making this event possible:
Bristol Myers Squibb
Merck
UC Berkeley, UC Davis, UC Santa Cruz, UCSF Integrated Funding Model

Chemical Biology in the Bay Area Research Day
Saturday May 14, 2022
UCSF, Mission Bay Campus
Genentech Hall, Byers Auditorium

Agenda

9:00-9:30am – Poster set up and continental breakfast
9:30-9:45am – Introduction, Jason Gestwicki (UCSF Chemistry & Chemical Biology Program Director)

Session 1
Session Chair – Quinn Edmondson (UCSF)

9:45-10:10 – Doug Wassarman (UCSF)
Tissue-Restricted Inhibition of mTOR Using Chemical Genetics

10:10-10:35 – Vanha Pham (UC Berkeley)
Multi-omics approaches to deciphering formaldehyde and the one-carbon metabolism/signaling interface

10:35-11:00- Robert Tombari (UC Davis)
Novel Scaffolds for Photoswitchable Tools

11:00-11:15 – Break

Session 2
Session Chair – Amanda Bischoff (UC Berkeley)

11:15-11:40 – Justin Faris (UC Santa Cruz)
Bringing Permeability to mRNA-Display: A Scaffold Based Approach

11:40-12:05- Kelly Montgomery (UCSF)
The Chemical Features of Polyanions Modulate Tau Aggregation and Conformational States

12:05-12:30 – Deark Garcia Almedina (UC Berkeley)
Tyrosinase-enabled modular construction of bispecific antibodies

12:30-2:00 – Lunch (pick up box lunches in atrium)

Poster session – Genentech Hall Atrium

Session 3
Session Chair – Guillermo Chacaltana
2:00-2:25 – Albert Liu (UC Davis)
Retracing rubisco evolution reveals plasticity of oligomerization

2:25-2:50 – Jackson Baumgartner (UC Santa Cruz)
Investigating the distribution and role of alkyl quinolone modifying vanadium-dependent haloperoxidases on the microbial battlefield

2:50-3:15 – Neha Prasad (UCSF)
An in vivo CRISPRi screen identifies potential antibacterial targets that synergize with host clearance mechanisms

3:15-3:30 – Break, and poster breakdown

Session 4
Session Chair – Kyle Rouen (UC Davis)

3:30-3:55 Kayli Martinez (UC Berkeley)
Optical quantification of absolute cardiac electrophysiology using VoltageFluor probes

3:55 – Poster winners announced (must be present to win)

4:00-4:35 – Nathanael Gray (Stanford University)
Targeted Protein Degradation as a New Drug Discovery Strate

CBBA Day Speaker Abstracts

Session 1

Doug Wassarman (UCSF)
Tissue-Restricted Inhibition of mTOR Using Chemical Genetics

mTOR is a highly conserved eukaryotic protein kinase that coordinates cell growth and metabolism and plays a critical role in cancer, immunity, and aging. It remains unclear how mTOR signaling in individual tissues contributes to whole-organism processes because mTOR inhibitors, like the natural product rapamycin, are administered systemically and target multiple tissues simultaneously. We have developed a chemical-genetic system, selecTOR, that restricts the activity of a rapamycin analog to specific cell populations through targeted expression of a mutant FKBP12 protein. This analog has reduced affinity for its obligate binding partner FKBP12, which reduces its ability to inhibit mTOR in wild-type cells and tissues. Expression of the mutant FKBP12, which contains an expanded binding pocket, rescues the activity of this rapamycin analog. Using selecTOR, we have shown that selective mTOR inhibition can be achieved in *S. cerevisiae* and human cells, and we have validated the utility of the system in an intact metazoan model organism by identifying the tissues responsible for a rapamycin-induced developmental delay in *Drosophila*.

Vanha Pham (UC Berkeley)
Multi-omics approaches to deciphering formaldehyde and the one-carbon metabolism/signaling interface

One-carbon (1C) metabolism is a universal hub for cellular metabolism and epigenetic regulation. Consisting of the folate and methionine cycles, one-carbon metabolism maintains folate and S-adenosylmethionine (SAM) co-substrates as the primary one-carbon units for metabolic processes. We have identified formaldehyde‚Äîboth an environmental genotoxin and a product of endogenous metabolism‚Äîas another key one-carbon unit. We have employed multi-omics approaches to identify and characterize protein targets of formaldehyde and downstream effects of formaldehyde on cellular methylation status, revealing novel mechanisms of formaldehyde in disrupting metabolic balance and one-carbon metabolism.

Robert Tombari (UC Davis)
Novel Scaffolds for Photoswitchable Tools

Photoswitches capable of accessing two geometric states are highly desirable, especially if their design is modular and incorporates a pharmacophore tethering site. We utilized azide-alkyne click chemistry to access arylazo-1,2,3-triazoles, a previously unexplored class of azoheteroarenes that exhibit high thermal stabilities and near quantitative bidirectional photoconversion. Our strategy enabled us to access a wide variety of photoswitchable compounds from readily available azides. In a similar fashion, we developed a redox isomerization strategy for synthesizing *p*-formylazobenzenes from *p*-nitrobenzyl alcohol, where the resulting azo-aldehydes can be readily converted to photoswitchable compounds with excellent photophysical properties using simple hydrazide click chemistry. The ability to streamline syntheses of photoswitchable compounds with reliably optimal photophysical properties allows us to investigate the mechanism of action of various small molecules in biological systems with the high degree of spatial and temporal control that the field of photopharmacology offers.

Session 2

Justin Faris (UC Santa Cruz)
Bringing Permeability to mRNA-Display: A Scaffold Based Approach

The effort to modulate challenging "undruggable" intracellular targets has led to a movement toward chemical matter that is larger and more complex than typical small molecule drugs. Discovery in this space has been facilitated by combinatorial techniques such as mRNA display, which, while capable of producing potent lead compounds, rarely generate molecules that are membrane permeable enough to be used towards intracellular targets. Understanding the constraints on passive membrane permeability in mRNA-derived macrocycle libraries would increase the likelihood of identifying lead compounds with permeability values high enough to rationalize an optimization campaign against intracellular targets. Here we investigate the permeabilities of over 250 diverse cyclic 10-mer scaffolds using the thioether cyclization motif commonly found in mRNA display macrocycle libraries. We found the optimal lipophilicity range for achieving permeability in 10-mer cyclic peptides and peptide-peptoid hybrids based on a series of previously identified scaffolds and showed that permeability could be maintained even when multiple stereocenters were inverted. The permeable conformations of these conserved scaffolds were rationalized by NMR spectroscopy and McMD simulations. Furthermore, the inclusion of polar, drug-like side chains among the top performing scaffolds was analyzed. This work provides an example by which pre-existing physicochemical knowledge of a scaffold can benefit the design of mRNA display libraries, pointing toward an approach for biasing libraries toward permeability by design. Moreover, the compounds described herein are further proof that permeability can and does exist far beyond the conventional Rule of 5 chemical space.

Kelly Montgomery (UCSF)
The Chemical Features of Polyanions Modulate Tau Aggregation and Conformational States

The aggregation of tau into insoluble fibrils is a defining feature of neurodegenerative tauopathies. However, tau has a positive overall charge and is highly soluble; so polyanions, such as heparin, are typically required to promote its aggregation in vitro. There are dozens of polyanions in living systems and it is not clear which ones might promote this process. Here, we systematically measure the ability of 30 diverse, anionic biomolecules to initiate tau aggregation, using either wild type (WT) tau or a disease associated P301S mutant. We find that polyanions from many different structural classes can promote fibril formation and that P301S tau is sensitive to a wider range of polyanions (16/30) than WT tau (14/30). We also find that some polyanions preferentially reduce the lag time of the aggregation reactions, while others enhance the elongation rate, suggesting that they act on partially distinct steps. From the resulting structure-activity relationships, the valency of the polyanion seems to be an important chemical feature, such that anions with low valency tend to be weaker aggregation inducers, even at the same overall charge. Finally, the identity of the polyanion contributes to fibril structure, based on electron microscopy and limited proteolysis. These results provide insight into the crucial role of polyanions in regulating both the kinetics of tau aggregation and the conformation of the resulting fibrils.

Diego Garcia Almedina (UC Berkeley)
Tyrosinase-enabled modular construction of bispecific antibodies

Bispecific T-cell Engagers (BiTEs) have emerged as a highly promising cancer immunotherapeutic, overcoming limitations of monoclonal antibodies, which can only bind a single epitope. BiTEs are a class of bispecific antibodies (bsAbs) engineered to recruit T-cells to tumor sites, triggering localized T-cell activation and redirected lysis of cancer cells. These constructs are commonly prepared via genetic fusions for recombinant expression, synthetic methods, or insertion of recognition sequences for enzymatic reactions. Unfortunately, these approaches encounter limitations in the engineering of required tags, linkers, or recognition domains to avoid misfolding, limited positions and orientations of the linkages, use of large excess of coupling partners to attain significant yields in equilibrium reactions, and challenges in isolating the desired product. Therefore, we aim to develop a method for the modular construction of protein-protein immunoconjugates with improved versatility, yield, and efficiency, through an enzymatic oxidative coupling (OC) mediated by tyrosinase. Notably, tyrosinase has been found to rapidly (<30 min) couple full-size protein partners with exposed tyrosine or cysteine residues under mild conditions. We identified nanobodies and scFvs as suitable and accessible protein coupling partners, and using QTOF-LCMS, we confirmed that the tyrosine-tagged and cysteine-bearing building blocks coupled to full conversion with virtually no byproducts. Two bsAbs were produced that bound both HER2 and GFP: one fused at the C-termini, and another fused C-terminus to nanobody framework. Using flow cytometry, we showed that both constructs retain binding for the individual targets, as GFP was captured onto the surface of HER2+ cells in a dose-dependent manner, with equivalent binding constants. Showcasing the versatility of the reaction, we synthesized a nanobody-scFv BiTE, which was able to mediate recruitment and attachment of Jurkat T-cells to HER2+ cancer cells, in addition to mediating PBMC cell cytotoxicity towards HER2+ cancer cells. Through this work, we were able to establish tyrosinase OC of nanobody and scFv building blocks as a suitable method for the facile, rapid, and modular production of bispecific antibodies. Ongoing work is focused on producing higher-order multifunctional constructs, with enhanced combinatorial biological activity compared to bispecific constructs (e.g, increased half-life), through the use of tyrosinases with differential charge sensitivity and an expanded building block library.

Sesssion 3

Albert Liu (UC Davis)
Retracing rubisco evolution reveals plasticity of oligomerization

Oligomerization is a core structural feature that defines the form and function of many proteins. Most proteins form molecular complexes; however, there remains a dearth of diversity-driven structural studies investigating the evolutionary trajectory of these assemblies. Rubisco is one such enzyme that adopts multiple assemblies, though the origins and distribution of its different oligomeric states remain cryptic. Here, we retrace the evolution of ancestral and extant form II rubiscos, revealing a complex and diverse history of oligomerization. We structurally characterize a novel tetrameric rubisco, elucidating how solvent-exposed surfaces can readily adopt new interactions to interconvert or give rise to new oligomeric states. We further use these principles to engineer and demonstrate how changes in oligomerization can be mediated by relatively few mutations. Our findings yield insight into how structural plasticity may give rise to new oligomeric states.

Jackson Baumgartner (UC Santa Cruz)
Investigating the distribution and role of alkyl quinolone modifying vanadium-dependent haloperoxidases on the microbial battlefield

Vanadium-dependent haloperoxidases (VHPOs) are a unique family of enzymes that utilize a histidine-bound vanadate cofactor and hydrogen peroxide to oxidize a halide to hypohalous acid. Previously, Streptomycete homologs were discovered to be involved in the site-selective and substrate-specific halogenation of meroterpenoid natural products (NPs). Since then, site-selective VHPOs have remained under-explored despite their broad species distribution and high biocatalytic potential. Although only a few closely related VHPOs have been characterized, a diverse range of chemical transformations have been observed. Recently, the first bacterial VHPO (VHPO-HZ11) from a non-*Streptomyces* species was characterized to site-specifically brominate alkyl quinolones (AQs). AQs are well known for their role in *Pseudomonas aeruginosa* pathogenesis, however they also play major roles in microbial communities as signaling molecules and bactericides. AQ bromination results in reduced toxicity to the VHPO-containing organism and increased AQ toxicity to other bacteria. VHPO-HZ11 and its homologs represent an intriguing sub-family to both elucidate the mechanism of selective VHPO substrate-modification and to identify the ecological role these enzymes play. We have detected homologs of VHPO-HZ11 primarily in proteobacteria but also in diverse Gram-negative and Gram-positive genera. There were no conserved genomic contexts for these VHPOs and an absence of AQ-producing enzymes in the organisms, implying a role outside of endogenous NP biosynthesis. A representative grouping of 12 VHPO-HZ11 homologs have been selected for heterologous expression and *in vitro* interrogation, which show a conservation of AQ-halogenation activity across diverse bacterial species. Identification of each of these homologs substrate specificities and activities will enable the investigation of the mechanism of VHPO selectivity and lay the groundwork for future biocatalytic application. Of these homologs, the first characterized VHPO from a myxobacteria reveals particularly robust halogenation activity and altered AQ substrate specificity compared to VHPO-HZ11. Future co-culturing studies of VHPO-containing strains will provide insight into the ecological role that VHPOs more broadly play in microbial environments.

Neha Prasad (UCSF)
An in vivo CRISPRi screen identifies potential antibacterial targets that synergize with host clearance mechanisms

The emergence of multi-drug resistant strains of *Pseudomonas aeruginosa* threatens our ability to use current antibiotics to address high morbidity indications such as ventilator-associated pneumonia and chronic pulmonary inflammation in cystic fibrosis patients. While in vitro antibacterial activity often corresponds to in vivo reduction of bacterial density, host immunity & other factors associated with the infection environment also play important roles in disease resolution. To identify new antibacterial targets that are synergistic with host clearance mechanisms, we conducted an in vivo CRISPRi screen which revealed a set of 170 *P. aeruginosa* genes with greater vulnerability to host clearance in a murine pneumonia model compared to the vulnerability detected in rich media. Notably, of the four in vitro essential genes shown in previous work to be upregulated during human infections (*lptG, lptH, pgsA, cysS*), two are from the lipopolysaccharide transport pathway and known to have attenuated virulence upon genetic inhibition. We further validate the significant depletion of *cysS* and *pgsA* knockdown mutants in mono-infection experiments. Our work provides an avenue for the future development of antibacterial agents that synergize with host mechanisms to eradicate bacterial infections.

Session 4

Kayli Martinez (UC Berkeley)
Optical quantification of absolute cardiac electrophysiology using VoltageFluor probes

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) promise to advance the field of human cardiac biology since they are commercially available, have human protein isoforms, and can be grown in a lab. However, hiPSC-CMs are structurally, transcriptionally, and electrophysiologically immature, making investigation of their fetal-like electrophysiology a particularly important area of research. hiPSC-CM electrophysiology can be quantified via whole-cell patch clamp electrophysiology; however, this technique is difficult and low throughput. To complement electrode methods, we have been developing optical voltage imaging indicators. These voltage-sensitive fluorophores, or VoltageFluor/VF dyes utilize a photoinduced electron transfer (PeT) based mechanism to sense changes in membrane potential (Vmem), are high throughput, and respond with fast kinetics to changes in Vmem. VF dyes offer high temporal and spatial resolution and allow study of single cells, cardiac monolayers, and 3D cultures such as ‚Äúheart-on-a-chip‚Äù microphysiological systems. However, two problems plague fluorescent voltage indicators.

The first is phototoxicity, limiting the length of time imaging can be performed and hindering cell health. To address this challenge, I will discuss our efforts to achieve a generalizable method for reducing phototoxicity of VF dyes by modifying a parent dye with a triplet state quencher. Using this method, we nearly triple the length of time of continuous imaging with VF probes.

A second challenge in using voltage sensitive fluorophores is the inability to measure absolute values of Vmem using intensity-based imaging. While intensity-based imaging allows the study of general physiology and action potential shape and duration, we cannot quantify resting Vmem or absolute changes in voltage during action potentials. I will discuss progress towards the development of a novel method for quantifying absolute values of Vmem using fluorescence lifetime imaging microscopy (FLIM). FLIM allows determination of the fluorescence lifetime (œÑfl) for a given fluorophore, a value independent of dye concentration, loading, or photobleaching. The œÑfl of VF dyes varies with Vmem allowing optical quantification of membrane potential. Together, these two developments will provide a powerful method for optically quantifying electrophysiology in hiPSC-CM monolayers and heart-on-a-chip systems.

Keynote Speaker – Nathanael Gray (Stanford University)
Targeted Protein Degradation as a New Drug Discovery Strategy

Targeted protein degradation (TPD) refers to the use of small molecules to induce ubiquitin-dependent degradation of proteins. TPD is of interest in drug development, as it can address previously inaccessible targets. However, degrader discovery and optimization remains an empirical process due to a lack of understanding of the relative importance of the key molecular events required to induce target degradation. Here I describe our labs efforts develop efficient methods to develop and characterize new degraders focused on protein kinase targets. I will also describe our labs efforts to make mono-valent ‘glue’ degraders for previously difficult-to-drug targets.

Nathanael Gray is a Professor of Chemical and Systems Biology at Stanford, Co-Director of Cancer Drug Discovery, Co-Lead of the Cancer Therapeutics Research Program, member of Chem-H, and Program Leader for Small Molecule Drug Discovery for the Innovative Medicines

Accelerator (IMA). His research uses the tools of synthetic chemistry, protein biochemistry, and cancer biology to discover and validate new strategies for addressing anti-cancer targets, Dr. Gray's research has had broad impact in the areas of kinase inhibitor and degrader design and in circumventing drug resistance. Dr. Gray's generalized strategy for structure-based design of inhibitors that stabilize inactive kinase conformations (type II) has been widely adopted by the research community and has had a significant impact on the development of numerous inhibitors of tyrosine kinases that are currently undergoing clinical development.