22nd R. Bryan Miller Symposium

Poster Session Abstract Book

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1. The Impact of Metals on the Structure and Dynamics of the Intermediate Filament Protein Vimentin

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Vimentin intermediate filaments (IFs) are an integral component of the cell cytoskeleton. Disruptions in the organization and assembly of vimentin IFs have been implicated in over 100 human IF diseases including cataracts, neuromuscular disorders, and cancer. The hierarchical assembly process of vimentin begins with its monomeric form and further self-associates into different intermediate structures until assembling into intact full-length filaments. The binding of metal ions to the tail domain of vimentin can affect filament assembly, organization, and interactions. Despite the known assembly process and metal ion interactions of vimentin IFs, the structure and protein-metal interactions of the intact full-length filaments of vimentin have not been thoroughly characterized. This project aims to elucidate the structure of the intact full-length vimentin IFs by investigating how the tail domain interacts with metals and how these interactions affect the functional higher-order organization of full-length vimentin filaments. This work employs a ‘divide and conquer’ approach where the impact of metals on the conformation and dynamics of peptides and protein fragments of vimentin are investigated to better understand these interactions in the full-length vimentin protein. To determine amino acid-specific interactions of metals with peptides from the tail domain, various spectroscopic methods are used including circular dichroism, solution-state nuclear magnetic resonance (NMR), and UV-visible absorption spectroscopy. Vimentin protein fragments containing the entire tail domain will be characterized using similar methods to provide further insight into the structural and conformational changes of the tail domain upon metal binding. These highly complementary studies will enable a comprehensive characterization of the interactions between the vimentin tail domain and metals, and will aid in the understanding of the structure and dynamics of the tail domain in the full-length vimentin IF assembly and network. The outcomes of this work will provide an experimental framework for investigating the structural properties and metal-protein interactions of other intermediate filaments, and how these interactions potentially play a role in the various human IF diseases.
2. Investigating the Role of the 5-HT$_2$A Receptor in Somatic Cell Growth and Proliferation

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Recent findings in the field of neuroscience demonstrate that serotonergic psychedelics promote neuronal growth through a 5-HT$_2$A receptor-dependent mechanism. We wished to investigate whether serotonergic agents are capable of promoting growth of other cell types expressing the 5-HT$_2$A receptor, as this would provide insight into whether activation of this receptor exclusively promotes growth in neurons or does so in all cell types. To test this, we expressed the 5-HT$_2$A receptor in a cancer cell line via plasmid DNA transfection, then treated the cells with a series of small molecules. After a 72-hour incubation period, we imaged the cells and quantified the average cell diameter and number of nuclei per field of view (FOV) to determine the difference in cell growth and proliferation. Preliminary results suggest that serotonin (5-HT) has a mitogenic effect. Determining if the 5-HT$_2$A receptor promotes growth and proliferation upon activation is important because of its ramifications in oncology. If activation of this receptor does promote cell growth and proliferation, then consideration of this effect will be imperative when treating cancer patients suffering from stress-related psychiatric disorders.

List of small molecules used:
3. Synthesis and Development of Silanol-containing Ligands for Asymmetric Catalysis

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The synthesis and development of novel chiral silanol-containing ligand scaffolds for asymmetric catalysis will be presented. Silanol compounds contain a unique Si−OH bond and have been previously utilized as ancillary ligands to stabilize low-valent, coordinatively unsaturated metal species for metal-catalyzed transformations. Using silanol-containing ligands for asymmetric catalysis offers advantages including enhancement of reactivity on the metal center and better steric modulating ability; however, the application of such ligands has been relatively under-explored in enantioselective catalysis due to challenges in synthesis and limited investigations. We have synthesized several novel chiral metal-chelating bidentate ligands combining both heterocycle and silanol motifs, which have been explored in asymmetric reactions that are important for the synthesis of chiral building blocks useful for materials and medicinal chemistry. Preliminary results show the potential of using these chiral silanol ligands to induce enantioselectivity for a Cu-catalyzed N−H insertion reaction to access enantioenriched chiral amines. The optimization, validation and application to other enantioselective X−H insertions will be discussed.

**Enantioselective Cu-catalyzed N−H insertion**

![Enantioselective Cu-catalyzed N−H insertion](image)
4. Identification and removal of oxidatively modified bases in G-quadruplexes by DNA glycosylase enzymes NEIL1 and NEIL3

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DNA base modifications resulting from reactions with exogenously and endogenously produced reactive oxygen and nitrogen species can contribute to genomic instability. In order to protect genomic integrity, cells employ the base excision repair (BER) pathway to repair DNA damage. The endonuclease VIII-like (NEIL) family of repair enzymes are DNA glycosylases that initiate the BER pathway by cleaving the N-glycosidic bond between the base and the sugar when abnormal bases are detected. NEIL1 and NEIL3 preferentially excise modified bases from double-stranded and single-stranded DNA respectively, and exhibit high removal activity toward the hydantoin lesions, guanidino- and spiriminodi-hydantoin (Gh and Sp), which block DNA polymerases and lead to mutagenesis. More recently, we have demonstrated the removal of the Gh base modification from G-quadruplex (G4) structures by NEIL1 and NEIL3. Notably, because G4 structures are found in oncogene and repair enzyme promoter regions, the unique ability of the NEIL enzymes to remove DNA damage from G4s could reveal a role beyond genome maintenance. To further investigate the ability of the NEIL enzymes to remove DNA damage from G4 structures, we performed kinetic assays under single-turnover (STO) conditions with the G4 DNA sequence from the KRAS promoter region. We evaluated the ability of NEIL1 and NEIL3 to remove various base modifications from the G4, including 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG), 5-hydroxyuracil (5-OHU), and an abasic site (AP). Our results indicate that both NEIL enzymes are capable of excising FapyG, 5-OHU, and AP from the KRAS promoter region G4. This showcases that the NEIL family of enzymes are involved in the recognition and excision of multiple modified bases from G4s and may play an important role in protection of genomic integrity as well as gene regulation.
5. Divergent Stereochemical Outcomes: Insertion of Donor/Donor Carbenes into the C–H Bonds of Stereogenic Centers

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Intramolecular C–H insertions with donor/donor dirhodium carbenes provide a concise and highly stereoselective method to set two contiguous stereocenters in a single step. Herein, we report the insertion of donor/donor carbenes into stereogenic carbon centers allowing access to trisubstituted benzodihydrofurans in a single step. This study illuminates, for the first time, the stereochemical impact on the carbene center and delineates the structural factors that enable control over both stereogenic centers. Sterically bulky, highly activated C–H insertion centers exhibit high substrate control yielding a single diastereomer and a single enantiomer of product regardless of the catalyst used. Less bulky, less activated C–H insertion centers exhibit catalyst control over the diastereomeric ratio (dr), where a single enantiomer of each diastereomer is observed with high selectivity. A combination of experimental studies and DFT calculations elucidate the origin of these results. First, hydride transfer from the stereogenic insertion site proceeds with high stereoselectivity to the carbene center, thus determining the absolute configuration of the product. Second, the short lived zwitterionic intermediate can diastereoselectively ring-close by a hitherto unreported S_{E2} mechanism that is either controlled by the substrate or the catalyst. These results demonstrate that donor/donor carbenes undergo uniquely stereoselective reactions that originate from a stepwise reaction mechanism, in contrast to the analogous concerted reactions of carbenes with one or more electron-withdrawing groups attached. Current work focuses on applying this stereoselective methodology as the key step in the targeted synthesis of a subclass of xanthone natural products known as dihydroxybenzoxanthenes.
6. Rational design of oligonucleotide guide strands for site-directed RNA editing

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Adenosine Deaminases Acting on RNA (ADARs) convert adenosine to inosine in double stranded RNA. Inosine is recognized by cellular machinery as guanosine, affording an A-to-G transition. Therefore, endogenous human ADARs can be used to correct disease-causing point mutations at the RNA level by directing them to target sites in the transcriptome via complementary guide strands. The use of endogenous editing enzymes can minimize barriers associated with off-target editing and enzyme delivery, and presents a potentially safer alternative to genome editing for therapeutic applications. This strategy requires the use of guide oligonucleotides that can stimulate efficient directed editing, leading to efforts aimed at optimizing guide RNAs. Here we use structural information for ADAR2-RNA complexes to guide the design of nucleoside analogs for the position in the guide strand that contacts a conserved glutamic acid residue in ADARs (E488 in ADAR2), where mutation of this residue to glutamate (E488Q) results in enzyme hyperactivity. We designed and evaluated chemical modifications that mimic this activating mutation, and found that Benner’s Z nucleotide (a cytidine analog) increased the observed rate when compared to the preferred canonical base. We obtained a high-resolution X-ray crystal structure of ADAR2 complexed with a duplex containing the Z nucleotide, which revealed its effect on the contact with E488. Finally, we report that this single nucleotide modification increased directed editing yields in human cells and mouse primary liver fibroblasts. Our results show that modification of the guide RNA can mimic the effect of hyperactive mutants of ADARs, and lays the groundwork for additional rational design of ADAR guide strands.
7. Inhibition of ADAR1 editing by an RNA duplex bearing 8-azanebularene

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Though essential for proper cellular function, A-to-I editing by ADARs has also been implicated in various cancers and other metabolic disorders. Studies have shown that ADAR1 deletion results in increased tumor sensitivity to immunotherapy and in cell death for some cancers characterized by elevated levels of interferon-stimulated genes. However, there are no potent, targeted inhibitors of ADARs to date. Here, we designed an ADAR1 inhibitor from a short RNA duplex derived from HER1, a yeast RNA which we previously found to be preferentially edited by human ADAR1. The duplex inhibitor also bears the adenosine analog, 8-azanebularene (8-azaN), at the edited A position; a feature that we’ve previously shown to help in trapping the base-flipped conformation of ADAR2-dsRNA structures. In vitro deamination experiments in the presence of the duplex inhibitor showed inhibition of 5-HT2c and NEIL1 editing by ADAR1, with an IC50 of 13.2 ± 2.1 nM and 8.9 ± 0.9 nM, respectively. We found that this inhibition is 8-azaN-dependent and that the potency is affected by the length of the oligonucleotide relative to 8-azaN. Additionally, we have shown that this duplex does not inhibit 5-HT2c or NEIL1 editing by ADAR2, and hence appears to be ADAR1-selective.

8. Origins and implications of post-transition state bifurcations in Rh-promoted C-H insertion reactions

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C-H insertion reactions catalyzed by dirhodium catalyst are utilized as a powerful strategy in synthetic chemistry. We discovered that a class of reported Rh-catalyzed β-lactonization reactions suffers from a post-transition state bifurcation (PTSB), forming β-lactones (P1) and ketene+ketone fragmentation products (P2) from the same transition state structure (TSS). In PTSB cases, a single "ambimodal" transition state structure (TSS) leads directly to two products without any intervening minima. Under this circumstance, traditional transition state theory (TST) cannot explain the preference between two products because there is only one barrier for them to share. Herein, we implemented both density functional theory (DFT) calculation and ab initio molecular dynamics (AIMD) simulations to estimate the yield and selectivity of β-lactone formation. Detailed investigation with different TSSs demonstrated that transition state conformational effects dominate activation energies and dynamic behaviors. Weak interactions between the catalyst and substrate were studied by energy decomposition analysis (EDA) and non-covalent interaction (NCI) plots to unmask the specific role of the 2-bromophenyl substituent, revealing that a pair of beneficial interactions facilitates reactions. In terms of dynamics, our study on potential energy surfaces (PES) implies that the slope of vectors towards P1 and P2 controls the preference between P1 and P2, hinting at the long-lasting impact of conformational effects post-transition state. At the beginning of this research, we used Rh2(OAc)4, a simplified catalyst model to describe one of the systems. More recently, we tried to rationalize the high yield of β-lactone given by chiral D2-symmetric catalyst Rh2(s-TCPTTL)4. Surprisingly, a PTSB still exists with this large catalyst, with a strong dynamic preference for fragmentation. But with a "cage" effect support by bulky ligands on Rh2(s-TCPTTL)4, β-lactone can be formed via a subsequent [2+2]-cyclization at room temperature.
9. Molecular Packing within Lipid Nanoconstructs

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This work aims to produce phospholipid nanoconstructs following the designed geometry and molecular packing. 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) features with complex and controlled geometries were made using controlled assembly, a concept developed by our team. This new means uses AFM-based microfluidic delivery system for the printing of minute volume (<fL) of solution containing lipid molecules on solid and modified surfaces. The solvent is left to air dry, during which the spatial confinement controls the assembly of POPC molecules. The resulting features form bilayers and amorphous state lipid features. Atomic Force Microscopy (AFM)-based force spectroscopy experiments were carried out to explore the nanomechanics of POPC nanosized features with complex and controlled geometries and molecular packing. AFM-based force spectroscopy has been largely used to explore the nanomechanics of lipid bilayer stacks, in which the nano-sized probe tip applies force on supported lipid bilayer stacks and the resulting force-distance curve reveals the rupture events of the bilayers. Here we apply the method to both the POPC nanosized bilayer stacks and the amorphous POPC assemblies to shed light on the different inner molecular packing.

This poster reports the feasibility of controlling the geometry of the produced POPC features verified by AFM topographic imaging. The molecular packing of POPC features with complex and controlled geometries is explored using AFM-based force spectroscopy and Confocal Laser Scanning Microscopy. The stability of POPC features with different molecular packing will also be addressed. Some of the lipid assemblies were validated using molecular simulation (MD). In conclusion, precise controlled assembly and 3D characterization have been achieved with POPC molecules. This poster introduces the promising combination of Force Spectroscopy, AFM, Confocal, and MD for the study of lipid nanoconstructs, allowing for the topographical and 3D characterization of phospholipids assemblies, providing a valuable tool in controlling and designing complex lipid systems.

(A) An AFM topographic image of a POPC assembly on a plasma-cleaned glass slide. (B) A cursor profile as indicated in (A). (C) A 3D display of (A). (D) The histogram analysis on (A). (E) A diagram showing the layer thickness.
10. Feasibility Studies for IR Cryo-photochemistry Approaches on $H_{\text{ox}}(H_2)$ Species in Hydrogenases

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Nature processes $H_2$ with remarkable efficiency via metalloenzymes known as hydrogenases—H$_2$ases. To fully understand these energy converting pathways, information on how reaction coordinates and surrounding ligands guide catalysis for small molecules are essential. Experimental constraints in current methods, prevent us, from identifying and stabilizing short-lived catalytic intermediates and obtaining structures, such as the bound-H$_2$ species which may be the first step in the $H_2$-splitting reaction and key to understanding the mode of initial $H_2$ interaction in both [FeFe]-hydrogenase and the regulatory [NiFe]-hydrogenases. One approach, called cryoenzymology, provides new ways to investigate enzyme-action by working suboptimally, specifically taking measurements at temperatures below 0°C. Thus, by reducing reaction kinetics sufficiently, it is possible to accumulate intermediates that cannot be observed under normal conditions by slowing down their kinetics of formation, by changes in the rate limiting step or by shifts in equilibria. IR spectroscopy is a useful probe to monitor intermediates in situ and obtain high fractions of a single turnover, where complement spectroscopies can be used. To maintain the fluidity at subzero temperatures a “cryoprotectant” must be added to prevent the crystallization of water, usually miscible organic solvents. In particular, we discuss the importance of choosing the right solvent: this requires an extensive feasibility study to understand both temperature-effect and cryosolvent-effect on the protein, enzyme activity, and ensuring reliable IR signatures of CO and CN$^-$ ligands. In this study, cryosolvent candidates included aqueous mixtures of glycols, alcohols, and dimethylsulfoxide, as well as a ternary mixture. Our ‘caged-hydrogen’ is supplied to the system by a combination of cryobiochemistry and photochemistry using near infrared (NIR) radiation to stimulate release of substrate from NH$_3$NH$_3$ (ammonia borane). To use NIR, we employ nanoparticle photocatalysts that operate using plasmon surface resonance combined with 2-photon upconversion to catalyze $H_2$ evolution. We illustrate the usefulness of cryoenzymology by running IR experiments and activity assays on two classes of hydrogenase, both [FeFe] and [NiFe] hydrogenase.
11. Synthesis of 2-Bromo-8-oxo-2’-deoxyinosine oligonucleotides to aid in the investigation of the MutY substrate recognition mechanism

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MutY is the base-excision-repair (BER) enzyme responsible for repairing the DNA mismatch of 8-oxoguanosine (8-OG) with adenine by cleaving the mispaired adenine base. Though previous study has shown that the 2-position amino is important for MutY’s substrate recognition of the OG:A mispair, the precise mechanism of recognition remains unclear. Our work will help uncover the target recognition mechanism of MutY. To achieve this goal, we are synthesizing numerous 8-OG analogs, including my current synthesis target, 2-Bromo-8-oxo-2’-deoxyinosine. I employed a combination of diazotization, deamination, and radical reaction to achieve the bromination at the 2-position on the purine ring. Experimental results showed that not only does our selected radical reaction successfully brominate the desired 2-position, but it also creates a di-brominated product that is essential for proceeding to the oxidation reaction at the 8-position. New compounds are confirmed via ESI-MS, HNMR, CNMR, HSQC, and COSY. This analog is part of a rationally designed suite of 2-modified 8-OG analogs that will be critical to revealing MutY’s mechanism for substrate recognition and will generate new ideas for MutY-targeted inhibitors and cancer therapies.

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Adenosine (A) to inosine (I) editing is an important post-transcriptional modification carried out by Adenosine Deaminase Acting on RNA (ADAR) on specific double-stranded RNA substrates. The ADAR1 protein plays a critical role as a modulator of innate immunity by preventing aberrant activation of dsRNA dependent innate immune pathways. Loss of function mutations in ADAR1 results in an autoimmune disorder, Aicardi Goutières Syndrome (AGS). Seven of these mutants lie directly in the catalytic domain of the protein. However, how the mutations affect substrate binding, catalysis, and protein protein interactions is poorly understood. These studies are complicated by the concentration-dependent aggregation of ADAR1. Improved purification methods for the ADAR1 protein have enabled the characterization of these mutations in vitro. Here we perform systematic studies of AGS mutants in the context of truncated and full-length protein, to reveal the fundamental mechanism by which these mutations impact ADAR1 structure and function.

13. Molecular Level Structural Characterization of Self-Assembled Monolayers of Functionalized Bidentate Aromatic Thiols

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Self-Assembled monolayers (SAMs) are versatile and used in a wide range of fields, their importance has increased as the size in which we operate has decreased. Some examples include tissue engineering, controlled assembly, 3D printing, biosensors, lubrication, protection, and purification. The objective of this research is to provide molecular-level structural information on dithiol SAMs by investigating 5-(octyloxy)-1,3-phenylenedimethanethiol (OPDT) SAMs on Au(111) surfaces, using combined high-resolution scanning tunneling microscopy (STM), atomic force microscopy (AFM), and nanolithography. Desorption of these OPDT SAMs leads to the formation of ordered domains known as the striped phases, whose unit mesh is revealed as commensurate with the underlying Au(111) lattice. OPDT molecules are lying-down, with the benzene ring and the zigzag plane of the alkyl chain parallel to the Au(111) surface. Using these ordered structures as internal standards in situ, the structure of the high-coverage OPDT SAMs is revealed: a mixture of standing-up and lying-down molecules randomly distributed on Au(111); as such, these SAMs exhibit little long-range order or ordered domains.
14. Investigation of a conserved histidine residue and its importance in the DNA damage repair activity of adenine glycosylase *MutY*

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Exposure to reactive oxygen species (ROS) causes oxidation of guanine (G) to 8-oxo-7,8-dihydroguanine (OG), a common form of DNA damage. This damage is problematic because OG preferentially pairs with adenine during replication, resulting in potential G:C to T:A transversion mutations. The adenine glycosylase *MutY* (MUTYH in humans) plays an important role in maintaining genome integrity by removing adenine bases mispaired across from OG. Inherited biallelic mutations in the MUTYH gene are linked to a cancer predisposition syndrome known as MUTYH associated polyposis (MAP), underscoring the essential role of MUTYH in protecting the genome. A cellular assay is employed in the present work which uses mutation frequency as a proxy for evaluating *MutY* function. Bacterial cell cultures which expressed either wild type *MutY*, no *MutY*, or a variant of *MutY* in which a key residue is changed were grown, then plated the following day on agar plates with rifampicin antibiotic, and incubated at 37°C overnight. Cultures with compromised mutation suppression are likely to acquire resistance to rifampicin, as identified by an increase in the number of colonies on rifampicin plates. This assay was employed to compare wild type *MutY* function with H292A *MutY*, and with bacteria in which *MutY* is not expressed. The bacteria with no *MutY* demonstrated more colonies on the rifampicin plates than the wild type, indicating a poor capacity for mutation suppression. This was to be expected, considering that bacteria with no *MutY* would be expected to lack the DNA repair ability of *MutY* entirely, while wild type colonies would be expected to completely retain *MutY* repair activity. With these positive and negative control experiments performed and their results in hand, H292A *MutY* expressing colonies were tested. The results aligned with those of the wild type colonies, giving a mutation frequency of approximately zero. These results suggest mutation suppression is not significantly compromised upon removal of the H292 sidechain. More experiments with H292A and other mutations at the 292 position are currently underway and will elucidate the degree of importance of this residue.
15. Effects of Axial Solvent Coordination to Dirhodium Complexes on Reactivity and Selectivity in C-H Insertion Reactions: A Computational Study

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Density functional theory calculations were used to systematically explore the effects of axial ligation by solvent molecules on the reactivity and selectivity of dirhodium tetracarboxylates with diazo compounds in the context of C–H insertion into propane. Insertions on three types of diazo compounds—acceptor/acceptor, donor/acceptor, and donor/donor—promoted by dirhodium tetraformate were tested with and without axial solvent ligation for no surrounding solvent, dichloromethane, isopropanol, and acetonitrile. Magnitudes, origins, and consequences of structural and electronic changes arising from axial ligation were characterized. The results suggest that axial ligation affects barriers for N₂ extrusion and C–H insertion, the former to a larger extent.
16. Investigation of the role of turn residues on β-hairpin catalytic peptides of aldol reactions

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Peptides are intriguing catalysts because they occupy a middle ground between enzymes and small-molecule organocatalysts. Although too short to form tertiary and quaternary structures, peptide catalysts can incorporate secondary structures that have potential benefits for catalytic activity. Our group specifically focused on peptides that can form β-hairpins. A β-hairpin is a structural motif of peptides and proteins that contain two antiparallel β-sheets with turn residues at the bend (Figure 1). Peptides that contain β-hairpin structures have been reported to catalyze a variety of organic reactions. We have been interested in the aldol addition reaction between 4-nitrobenzaldehyde and hydroxyacetone catalyzed by a four-residue peptide that has the potential to form a β-hairpin. More specifically, we aimed to test and compare how the initial rates of reaction and stereoselectivity varied based on different catalyst sequences with the same catalytic group (primary amine in the N-terminus) but different i+1 and i+2 turn residues. We used Nuclear Magnetic Resonance Spectroscopy (NMR) to identify peptide secondary structures, determine peptide concentration and record reaction kinetic data under controlled concentrations. We observed significant variation in the initial rates of reactions catalyzed by peptides with different turn residues, indicating the significant role of structure on catalytic activity. Our studies of peptide catalysts may lead to the discovery of design principles that could be used for the development of next generation catalysts.

Figure 1. The β-hairpin structure of the tetrapeptides used in my project, which have valine as the first residue, leucine as the fourth residue and the second (i+1) and third (i+2) residues (turn residues) varied across different peptide catalysts
17. DNA glycosylase NEIL1 demonstrates lesion specificity from RNA editing

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Conditions of oxidative stress produced from exogenous toxins and toxicants and endogenous cellular processes can result in modifications to all four nucleobases. Such oxidative DNA damage can result in mutations or disrupt normal cellular processes including replication and transcription. The DNA glycosylase, NEIL1, is able to excise lesions arising from all four nucleobases from a variety of DNA contexts. Additionally, the pre-mRNA of NEIL1 is subject to modification by the Adenosine Deaminase Acting on RNA (ADAR1) that leads to a recoding event that converts a lysine to arginine in the lesion recognition loop of NEIL1. This leads to the presence of two isoforms of NEIL1 under different cellular conditions. Notably, the two isoforms display different enzymatic properties on Thymine Glycol (Tg), where the unedited (K242) isoform showed a significantly faster rate of excision compared to edited NEIL1 (R242). We have performed detailed examinations of lesion processing by the two NEIL1 isoforms on a large number of substrates, and many interesting trends have emerged. Notably, unedited NEIL1 demonstrates better excision of oxidized pyrimidines than the edited isoform, but the differences in excision are not as striking as those observed previously with Tg. Calculations were performed in the gas phase to examine lesion tautomer stability and proton affinity, and there is a correlation between the isoform specific excision to the N3 proton affinity of the most stable tautomer. These results suggest that enzyme promoted tautomerization affects the cleavage of the glycosidic bond and the enhanced excision observed with the unedited enzyme. Thus, the differences in activity between the two isoforms of NEIL1 imply a unique regulatory mechanism for DNA repair.
18. Evaluating Laccase Ability to Enzymatically Degrade Aflatoxin

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Aflatoxins are fungal toxins commonly found in spoiled rice, corn, peanuts, and other agricultural goods. It devastates global food systems because 25 percent or more of food crops are destroyed annually due to contamination. The most common metabolite of aflatoxin is aflatoxin B₁ (AFB₁), which is classified as a Group 1 human carcinogen by the Internal Agency for Research on Cancer. If ingested, it can lead to acute liver toxicity or an accumulation of cancerous DNA mutations. Since its discovery in the 1960s, intense research efforts have informed methods of reducing or eliminating AFB₁ in contaminated foods. However, a limit of physical and chemical methods such as UV irradiation and ammonia is the reduction of nutritional value. An example of a more viable method is bioremediation with purified enzymes, where AFB₁ would be converted into a safer byproduct. Enzymes are environmentally friendly and substrate-specific, enabling the preservation of nutritional integrity. In the current study, we used in vitro and in silico methodologies to evaluate the ability of laccases, a common bioremediation enzyme, to degrade AFB₁. We assessed the degradation activity of purified laccases using liquid chromatography to measure AFB₁ concentration throughout the reaction. In addition, we predicted which structural features are most likely to aid the binding of AFB₁ to the enzyme using an extensive computational protein modeling suite, Rosetta. We identified 20 laccases that are capable of AFB₁ degradation out of the 45 laccases tested. Our models suggest that hydrophobic residues on loop structures near the active site can position AFB₁ in an optimal orientation for degradation to occur. These results provide further insight into future design efforts to increase enzymatic AFB₁ degradation.
19. Developing Cu(II)-Responsive Peptide Probe for the Extracellular Space

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Copper imbalance has been linked to diseases such as Alzheimer’s diseases, Wilson’s disease, and promoting angiogenesis in cancer. However, their pathways still remain unknown due to the lack of current methods to trace extracellular Cu(II). To further understand the involvement of copper amongst various diseases, it is necessary to develop a tool to trace extracellular Cu(II) in biological systems. This research describes the strategies towards designing a turn-on imaging probe that is both Cu(II)-responsive and localized in the extracellular space.

We first utilized the one-bead one-compound (OBOC) method to integrate Cu(II)-coordinating amino acids into peptides containing Arg-Gly-Asp (RGD). With the addition of Cu(II)-coordinating amino acids at both the N- and C- terminus of linear RGD peptides, Cu(II) coordinates to the peptide to induce conformational changes, allowing receptor recognition. Screening this peptide library with Cu(II) resulted in favorable binding to pyridines at the N- and C- terminus. UV-Vis was used to confirm the interaction between Cu(II) and the peptide probe.

As a model to test the peptide probe, we targeted surface receptors of brain cancer cells, thus localizing the peptide in the extracellular space. When the copper-coordinating peptides are coupled to an imaging handle, malachite green, we are able to observe an increasing fluorescence signal from the peptide when Cu(II) is present. This Cu(II)-responsive peptide could provide a molecular imaging platform to track extracellular Cu(II) in specific locations and provide valuable insight into copper trafficking and pathways associated with both normal and dysfunctional copper related processes.
DNA damage due to oxidative stress is associated with cancer, aging, neurodegenerative diseases, and metabolic disorders. Reactive oxygen and nitrogen species produced from exogenous sources, such as radiation and cigarette smoke, and endogenous processes, such as metabolism and inflammatory responses, can modify DNA nucleobases compromising the integrity of the genome. DNA glycosylases initiate the base excision repair (BER) pathway by locating and excising the modified nucleobases. While several BER glycosylases work on a single specific substrate, the NEIL family of DNA glycosylases (NEIL1, NEIL2, NEIL3) work on a wide range of oxidative DNA damage and in several DNA contexts, including single-stranded DNA and R-loops in addition to double-stranded DNA. NEIL2 is implicated in transcription-coupled repair and associated with a wide range of disease phenotypes, including increased susceptibility to age-related cataracts and polycystic ovarian syndrome. However, the molecular basis of how NEIL2 dysfunction is associated with disease is unclear, and there is a substantial gap in the literature of the basic biochemical properties, such as substrate scope. We evaluated the ability of NEIL2 to remove oxidative DNA lesions previously shown to be excised by other NEIL glycosylases. A substrate panel of 11 lesions in both single-stranded and double-stranded DNA contexts identified preference of removal from single-stranded DNA by NEIL2. Additionally, NEIL2 was capable of processing guanidinohydantoin, 5-hydroxyuracil, and abasic site lesions, with a demonstrated preference for the abasic site. Our detailed analysis shows that NEIL2 is capable of repairing DNA lesions previously untested in the literature and that NEIL2 has unique preferences from the other NEIL enzymes providing insight into the differing functionality of these glycosylases.
21. Characterization of conserved histidine residue within critical structural motif of the adenine glycosylase MutY

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Guanine exposure to reactive oxygen species (ROS) causes oxidation to 8-oxo-7,8-dihydroguanine (OG), a frequent form of DNA damage. OG is problematic due to preferential base-pairing to adenine, threatening G:C to T:A transversion mutation formation. Evolutionarily conserved adenine glycosylase MutY (MUTYH in humans) plays an important role in maintaining genome integrity via the selective removal of Adenines mispaired across OG thereby triggering a base excision repair pathway. The David lab’s expertise in holistically characterizing MutY was pivotal in unveiling functional defects of MutY variants led to establishing the first known link between deficit base excision repair and cancer formation referred herein as MUTYH-Associated Polyposis (MAP). The current work in this poster showcases current research centered around revealing how MutY detects OG within DNA. MUTYH variants located in a highly conserved, vital sequence motif within the C-terminal domain (referred to herein as the H_{X}XFSH_{b} motif) have been found in individuals with gliomas and uterine cancer, as well as in MAP patients (H444 N/R/Y respectively). To better understand the role of this residue in MutY-mediated OG:A, In-vitro assays have been employed to measure biochemical metrics including binding affinity, rate of catalysis, and product release. Additionally, a bacteria cellular plasmid-based assay is implemented to observe extent of repair on a OG:A mispair within a longer DNA context. Results suggest an interplay between DNA binding affinity and extent of repair within a cellular context showcasing the interconnected manner MutY functions in repair to oxidatively damaged DNA nucleobases.
The conversion of water to oxygen and hydrogen gases, otherwise known as water splitting, has been an increasingly popular topic for clean fuel research. NiFeO$_x$ and CoO$_x$ catalysts are attractive water splitting catalysts because they not only efficiently facilitate the oxidation of water, but are also relatively cheap, abundant, and durable and may have the potential to ultimately compete with existing energy sources. While these compounds are well studied, the exact mechanisms by which the oxygen evolution reaction progresses are still contested. One way to investigate the mechanism of these catalysts is to quantify triplet O$_2$ ($^1\Sigma_g^+$) isotopologue products ($^{16}$O$_2$, $^{16}$^{18}$O$_2$, $^{18}$O$_2$) when the catalyst is only introduced to H$_2$^{18}$O. We utilize continuous electron paramagnetic resonance (CW EPR) spectroscopy as a method of quantifying these O$_2$ product isotopologues by identifying their transitions at X-band frequencies. Calibration of these signals in the fingerprint region is expected to offer qualitative analysis of O$_2$ isotopologues. We propose that EPR can serve as a relatively fast probe to monitor the oxygen evolution reaction.
23. Molecular probe for high-throughput screening of GLUT5 activity in live cells

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Alterations in facilitative sugar transporters (GLUTs) have been linked to various metabolic disorders, including cancer. Detecting the onset of alterations and measuring the differences in GLUT activity may provide an approach for early detection of disease development and disease diagnosis. Among different GLUTs, fructose-specific transporter GLUT5 has shown a particular link with cancers (breast, lung, ovarian, and other). The differences in GLUT5 expression have been reported between cancer types and sub-types. Here, we present a novel approach to assess the changes in GLUT5 activity in live cells with sugar-like fluorescently labeled turn-on molecular probe. The objective of this research entails the development and validation of turn-on GLUT5-specific probe as tool for quantitative analysis of GLUT5 activity between cell lines. Turn-on fluorescent fructose mimic was synthesized and validated as GLUT5-specific fructose uptake reporter through comparative studies in different cell lines. Metabolism coupled uptake for the probe was validated using temperature studies. GLUT5 specificity was validated through competitive uptake studies. Relative accumulation of turn-on GLUT5 probe in different cell lines was measured with fluorescence microscopy in 96-well plate format and flow cytometry. The turn-on feature of the new probe allowed to alleviate issues with background fluorescence as fluorescence appearance required probe internalization and chemical transformation inside the cell. Differences in acquired fluorescence within the cell paralleled the levels of GLUT5 in the cellular membrane, suggesting the final readout to be independent of the rate of cellular hydrolysis. The turn-on feature of the new probe provided a direct approach to high-throughput screening of multiple cell lines for GLUT5 activity.

Activatable Mannitol Rhodamine (ManRho)
24. Simulation studies of SARS-CoV-2 and MERS-CoV spike proteins binding to sialic acids using alchemical free energy simulations

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Many disease-causing viruses target sialic acids which are known to coat the surface of many cells in the host, especially in the lungs. A number of human beta coronaviridae, known for causing respiratory tract diseases, bind to sialic acids, some preferentially to those with 9-O-Ac-modification. Currently, co-binding of SARS-CoV-2, a beta coronavirus responsible for the COVID-19 pandemic, to human sialic acids is suspected but specific binding pockets are not confirmed. Additionally, O-acetylated sialic acid-protein binding studies are experimentally difficult, due to the ester lability. Chemically similar N-acetylated sialic acids are experimentally more stable and are reasonable mimics. We have used a combination of molecular dynamics and alchemical free energy simulations to study the relative binding free energy differences between 9-O-Ac and 9-NAc sialic acids in a range of potential binding pockets on the SARS-CoV-2 spike protein, giving insight into potential binding domains and how similar the 9-NAc sialic acid mimic may be to 9-O-Ac counterparts in experimental binding studies. From our studies, we have identified multiple weak binding sites, which may combine to increase overall binding. These binding affinities are similar to the values we calculated for another beta coronavirus, MERS-CoV, with its known co-binder 9-OH sialic acid. Our results also suggest that the more stable 9-NAc sialic acids may be a good mimic of their 9-O-Ac counterparts when considering possible binding sites on the SARS-CoV-2 spike protein. The range of sialic acid binding sites may explain the high transmissibility of SARS-CoV-2 spike protein, and the binding similarity of 9-NAc sialic mimics may suggest an experimentally stable mimic to probe sialic acid binding.
25. Sex-Specific Social Effects on Depression-Related Behavioral Phenotypes in Mice

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Social interaction and empathy play critical roles in the emotional well-being of humans. Specific social conditions can exacerbate or mitigate stress-related depression and anxiety. Although it is known that rodents exhibit emotional consolation and empathy-associated behaviors, the effects of group housing on stress-induced phenotypes in both males and females are not well established. In this study, we determined how the presence of stressed or unstressed conspecifics within a cage impact depression-related phenotypes. Male and female C57BL/6J mice housed in same-sex groups were subjected to either 10 days of gentle handling (GH) or daily administration of corticosterone (CORT). The GH and CORT treatment groups were divided into cages of unmixed (GH or CORT) and mixed (GH and CORT) treatments. Depression-related phenotypes were measured using the forced swim test (FST) and sucrose preference test (SPT). We found that mixed housing alters FST behavior in a sex-specific manner. CORT males that were housed in the same cage as gently handled animals exhibited increased immobility, whereas GH females housed with CORT females demonstrated the opposite effect. This study emphasizes the importance of social housing conditions when evaluating stress-induced behavioral phenotypes and suggests that mixed cages of GH and CORT animals yield the greatest difference between treatment groups. The latter finding has important implications for identifying therapeutics capable of rescuing stress-induced behavioral deficits in the FST.

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The alkenylation of 2-pyridones is but one example of transition metal-catalyzed C-H activation and C-C coupling reactions. As a nitrogen-containing heterocycle, 2-pyridone is found in a multitude of biologically active natural products and is valued as a useful synthetic intermediate. This is in no small part due to the fact that 2-pyridone rings can be readily converted to piperidine, pyridine, indolizidine and quinolizidine rings. Recent studies demonstrated that Rh complexes can catalyze the oxidative Sonogashira reaction, including key reports from the Walsh and Miura groups. However, these reactions remain plagued by weaknesses such as the use of expensive catalysts and stoichiometric amounts of external oxidants. Recently, Ravikumar et. al reported a series of Co$^{III}$ catalyzed oxidative Sonogashira couplings of 2-pyridones and terminal alkynes with high regioselectivity. This reaction provides one of the first examples of highly regioselective Co-catalyzed C(6) alkenylation of 2-pyridones with terminal alkynes under mild conditions. A mechanistic understanding of the reaction is important as many details remain unclear and resolution of these questions could facilitate the improvement of its substrate scope and the refinement of catalytic conditions.

Calculations were performed at the M06/def2-TZVP(SMD)//B3LYP-D3(BJ)/def2-SVP level of theory. Three main questions were examined: (i) what is the mechanism of the reaction? (ii) what is the origin of the observed high regioselectivity? and (iii) why does the combination of two bulky ligands lead to the most optimal catalyst system?

Regarding question (i), we predict that the overall catalytic cycle consists of three mains steps: (a) C-H activation of 2-pyridone, ortho to the pyridine directing group, (b) alkyne insertion, and (c) protodemetalation. Arene C-H bond activation is predicted to proceed via CMD mechanism and protodemetalation via a retro-CMD mechanism. Regarding question (ii), our calculations reproduce the observed alkyne insertion selectivity, and we highlight the role of dispersion interactions in determining the magnitude of the observed preference. Regarding question (iii), our results indicate that the methyls on Cp* are responsible for lowering the barrier for the protodemetalation step, and indicate that the bulk of pivalate (vs acetate) only slightly affects the overall energetics.
27. Synthesis of Tetralins via a Rhodium Catalyzed [4+2] Cycloaddition Under the Mieldest Conditions

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Tetralin is privileged scaffold found in many natural products of interest because of their biological activity. We have developed a methodology that allows for synthesis of tetralins in moderate to high yields with high diastereo- and regio- selectivity. These compounds are generated from donor-donor dirhodium carbenes at ambient temperatures via a [4+2] cycloaddition of ortho-quinone dimethide (OQD) and an electron poor dienophile. OQD is a reactive diene that is typically generated under harsh conditions. Our methodology employs rhodium to catalyze the generation of this reactive diene at room temperature, and when a dienophile is present the 4+2 cycloaddition is observed. We have produced a large substrate scope that includes tetralins, quinolines and isochromans, which are abundant scaffolds in natural products. This methodology can facilitate the targeted synthesis of natural products with high structural complexity. To highlight the utility of our methodology we have made progress towards racemic synthesis of isolariciresinol which contains a tetralin core. Isolariciresinol is a natural product isolated from the roots of Rubia yunnanensis, and is a target of interest because of its antioxidant and anti-inflammatory activities.

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The opportunities to exploit the unique chemical properties of silicon for derivatives of known drugs often demonstrates enhanced control of stability, solubility, and pharmacokinetic properties. Within the realm of synthetic chemistry, the unique properties of silicon have made the hydrosilylation reaction ubiquitous for the synthesis of silicon-containing molecules and polymers. Here, we contribute to the organosilicon synthetic toolbox by developing a ring-closing dihydrosilylation (RCDHS) strategy. The synthesis of novel silyl-containing macrocycles will allow further investigation of the evolving role of silicon as a design element in bioactive macrocycles. Our initial investigations into the RCDHS methodology using platinum catalysts demonstrate that classic substrates for ring-closing metathesis (RCM), such as diethyl diallylmalonate and diallyl phthalate, proceed with good reactivity using 1,2-bis(dimethylsilyl)benzene and diphenylsilane, respectively. Catalyst, solvent, concentration, temperature, and reaction time have been explored to evaluate optimal conditions. Commercially available [Pt$_2$(dvts)$_3$], Karstedt’s catalyst, was identified to be the optimal catalyst for initial RCDHS substrates. The selectivity towards the formation of the desired silacycles vs linear Si-containing products is examined using $^1$H NMR spectroscopy and MS. Current efforts are focused on applying RCDHS using more complex substrates and derivatized biological building blocks with the goal of synthesizing medicinally relevant silyl-macrocycles, peptides and natural product analogs.
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.
30. Tools of the Trade: The synthesis of modified DNA oligonucleotides as tools for exploring DNA repair.

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The use of modified oligonucleotides as tools of chemical biology has blossomed and has become increasingly prevalent in chemical biology since the automation of solid phase DNA/RNA oligonucleotide synthesis was invented in the early 1980’s. This allowed for DNA/RNA modification at a scale that was previously not possible. Modified nucleosides are extremely valuable in DNA repair research because of their retained similarity to natural DNA substrates, and because nucleosides provide a versatile framework that can be modified on the base, sugar, or backbone. DNA repair systems are essential for maintaining genomic integrity within the cell, and without these repair systems, the cell cannot survive in the oxidative environment of earth.

My research in Sheila David’s laboratory focuses on the synthesis of modified DNA oligonucleotides designed to reveal structural and mechanistic details of DNA glycosylases such as MutY and MBD4. 8-Oxo-2’-deoxyguanosine nucleosides modified at the 2-position have been synthesized in this work in order to explore the target recognition mechanism of MutY, a DNA glycosylase that excises adenine bases mispaired with 8-oxoguanine (8-OG). As work from our lab has revealed, the 2-amino of 8-OG is critical for MutY target recognition. My synthetic targets are designed to help reveal the importance of hydrogen bonding and steric effects imparted by the 2-amino of 8-OG on MutY target recognition. To that end, reported synthetic targets vary hydrogen bonding capability and size at the 2-position of 8-OG, and include previously unreported N2-Alkyl-8-oxo-2’-deoxyguanosine analogs, 2-Halo-8-oxo-2’-deoxyinosine analogs, and finally 2-Vinyl- & 2-Ethynyl-8-oxo-2’-deoxyinosine analogs. MutY binding and kinetics data has been collected using our modified oligonucleotides as substrates. We have also synthesized an 8-oxo-2’-deoxyinosine analog oligonucleotide that was used by a collaborator as a substrate built into a DNA tether in single molecule studies using MutY. Additionally, oligonucleotides incorporating 1-azadeoxyribose were synthesized to further delineate the structure and mechanism of DNA glycosylase MBD4. MBD4 excises thymine from G:T mispairs, uracils mispaired with guanine, and modified uracils mispaired with guanine. A high resolution structure of MBD4 bound to our 1-azadeoxyribose oligonucleotide was solved in collaborative work.

Future work involves obtaining MutY binding and kinetics data using our remaining synthetic targets. 2-Halo-8-oxo-2’-deoxyinosine analogs will be explored as convertible nucleosides in addition to their use as MutY substrates. Our 2-Ethynyl- and 2-Vinyl-8-oxo-2’-deoxyinosine analogs will also be tested as DNA tagging tools.
31. One-pot multienzyme (OPME) chemoenzymatic synthesis of brain ganglioside glycans with human ST3GAL II expressed in E. coli

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A human sialyltransferase ST3GAL II (hST3GAL II) was successfully expressed in *Escherichia coli* as an active soluble fusion protein with an N-terminal maltose-binding protein (MBP) and a C-terminal hexa-histidine tag. It was used as an efficient catalyst in a one-pot multienzyme (OPME) sialylation system for high-yield production of the glycans of ganglioside GM1b and highly sialylated brain gangliosides GD1a and GT1b. Further sialylation of GM1b and GD1a glycans using a bacterial α2–8-sialyltransferase in another OPME sialylation reaction led to the formation of the glycans of GD1c and brain ganglioside GT1a, respectively. The lower reverse glycosylation activity of the recombinant hST3GAL II compared to its bacterial sialyltransferase counterpart simplifies the handling of enzymatic synthetic reactions and has an advantage for future use in automated chemoenzymatic synthetic processes.
32. Clickable perfluorocarbon nanoemulsion for $^{19}$F MRI and multi-model cellular detection

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The complexity of the macrophage response to diseases and crucial metabolic events has only recently been appreciated, and it is now understood that macrophages polarize between functions that inhibit or promote disease. Increasingly, distinct macrophage subtypes have been associated with disease progression or resolution. In this work, we describe an *in vivo* imaging probe platform that is readily modifiable to accommodate binding of different molecular targeting moieties and payloads for multi-modal image generation. We demonstrate the utility of perfluorocarbon (PFC) nanoemulsions incorporating dibenzocyclooctyne (DBCO) enabling functionalization, post-emulsification, via click-reaction with azide-containing ligands. Addition of DBCO-lipid to the surfactant in PFC nanoemulsions did not affect nanoemulsion size or nanoemulsion stability. As proof-of-concept, fluorescent dye-azides were conjugated to PFC nanoemulsions, demonstrating the feasibility of functionalization by click reaction. Uptake of the fluorescent PFC by macrophages was demonstrated in situ in an acute inflammation mouse model, where fluorescence imaging and $^1$H/$^{19}$F MRI was used for *in vivo* detection. Overall, these data demonstrate the potential of PFC nanoemulsions incorporating DBCO as a versatile platform for generating functionalized probes. Further, macrophage subtype uptake *in vitro* is undergoing. NP31, a synthetic peptide, was chosen to target CD40 receptor (M1 biomarker). NP31-GGK-azide was clicked on DBCO-PEG-NE and cellular uptake study was performed. CD40 receptor protein expression level for polarized THP-1 derived macrophage were assessed by immunofluorescent staining and flow cytometry (Figure a and b). M1 polarized macrophage showed significantly higher CD40 expression compared to M0 and M2 polarized macrophages. Polarized macrophages uptake efficiency was determined by $^{19}$F-NMR to quantify F atoms per cell (Figure c). M1 polarized macrophages treated with 8mg/mL PFCE of NP31-DBCO-NE showed significantly higher uptake compared to same dose of PEG-NE. Importantly, M1 polarized macrophages showed significantly higher uptake compared to M0 and M2 polarized macrophages. No significant differences for cell uptake were found for M0 and M2 polarized macrophages treated with same dose of NP31-DBCO-NE and PEG-NE. The cellular uptake data is correlated with the CD40 expression, which suggests that NP31-DBCO-NE is promising for future macrophage subtype detection in vivo imaging study.
Figure 1. Preliminary results for in vitro study. a. Representative immunofluorescence images for CD40 staining on polarized THP-1 derived macrophages. The cell nuclei were counterstained with Hoechst 33342. Scale bar = 30 µm. b. Flow cytometry for assessing CD40 surface expression on polarized THP-1 derived macrophages. Unstained cells and AF488 secondary only stained cells were measured as control of auto-fluorescence. c. Quantitative cellular uptake efficiency of polarized THP-1 derived macrophages by $^{19}$F-NMR. The differences were considered significant with p values *<0.05, **<0.01, and ***<0.001 as shown.
33. Substrate and process engineering for biocatalytic synthesis and facile purification of human milk oligosaccharides (HMOs)

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Oligosaccharides are important ingredients in human milk that support the healthy development of breast-fed infants. Many beneficial roles of human milk oligosaccharides (HMOs) have been discovered but the detailed information of specific structure-function relationship is lacking. Accessing structurally defined individual HMOs in sufficient amounts for laboratory research and clinical studies is an actively pursued objective by us and others. We have developed a highly efficient chemoenzymatic strategy for synthesizing structurally complex HMOs. Starting with a lactoside readily obtained by chemical derivatization, HMOs with up to nonasaccharides have been successfully synthesized using sequential one-pot multienzyme (OPME) synthesis with C18-column purification. The efficiency of the strategy has been demonstrated for gram-scale synthesis of a pentasaccharide and preparative-scale synthesis of twenty HMOs.