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Enabling Translational Science

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ICBS 2023 Trainee Symposium
Sunday, October 8, 2023

Sarah Canarelli	Identifying Covalent Peptide Inhibitors Using Phage Display
Yi Liao	Facilitating novel target and drug discovery in drug-refractory lung cancer by harnessing integrated photoactivatable fragment probes and chemical proteomics.
Alyssa Winkler	Small-molecule GAS41 YEATS domain inhibitors in non-small cell lung cancer.
Jose Reyes Franceschi	A Chemotranscriptomic Pipeline for RNA-Targeted Ligand Discovery.
Frederik Simonsen Bro	Identification of Inhibitors of Sterol Transport Proteins Through the Synthesis of a Cholic Acid-Inspired Compound Collection
Maylynn Hu	Targeting p38/MK2 protein-protein interaction for therapeutic discovery in human diseases.
Zhengyuan Pang	Volumetric click reaction for organ level in situ small molecule drug visualization.
David Y. Nie	The discovery and characterization of potent and selective chemical degraders of the histone methyltransferase NSD2.
Ingrid Wertz	Why I Love Being a Scientist. **Keynote lecture.**

ICBS 2023 Annual Conference
Sunday, October 8, 2023

Stefan Knapp	Development and validation of PROTACs as selective chemical tools. **Keynote lecture.**

Monday, October 9, 2023

Ingrid Wertz	Co-opting the Ubiquitin System for Therapeutic Benefit. **Keynote lecture.**
Weiping Tang	Enabling Platforms for the Development of Degraders.
Amit Choudhary	Protein editing using small molecules.
Jobette Santos	Discovery of a PTPN2 degrader in vivo tool compound for the study of cancer immunotherapy.
Billy Wai-Lung Ng	Targeted Protein O-GlcNAcylation Using Bifunctional Small Molecules.
David Lombard	High throughput screening reveals a role for BRD4 in potentiating cadmium cytotoxicity via nuclear-mitochondrial crosstalk.
Toren Finkel	Small Molecule Therapies That Can Modulate Aging.
Boris Vauzeilles	New Borinic Probes for Fast Detection and Imaging of Cellular Hydrogen Peroxide.
Antonio Pineda-Lucena	Develop potent and isoform selective PRMT inhibitors.

Essam Eldin A. Osman	Development of Sigma 2 Receptor Ligands for Cancer and Neurodegenerative Disorders.
Yi Tang	Combinatorial Biosynthesis of Protease Inhibitors.
Ralph Kleiner	Illuminating RNA biology with metabolically incorporated ribonucleoside probes.
Tania Lupoli	Activation and Incorporation of Rare Sugars into Bacterial Surfaces.
Marco Di Antonio	RNA G-quadruplex structures are responsible for insoluble aggregates characteristic of the neurodegenerative disease ALS.

Tuesday, October 10, 2023

Shaomeng Wang	Our journey in the discovery of clinical-quality PROTAC degraders for the treatment of human diseases. **Keynote lecture.**
John Cranfill	Title TBD.
Britto Sandanaraj	Directed Chemical Evolution of Self-Assembling Artificial Proteins.
Joomyung (Vicky) Jun	Gene Therapy Without the Genes: Intracellular Delivery of Therapeutic Proteins via Traceless Bioreversible Esterification Strategy.
James W. Checco	Unraveling the signaling mechanisms of endogenous cell-cell signaling peptides.
Minkui Luo	Exploring Cofactor Analogue Inhibitors of Protein Methyltransferases.
Tomek Cierpicki	SET domain dynamics facilitates targeting histone methyltransferases.
Jun Qi	Targeting multi-domain proteins for pediatric cancer therapy.
William C. K. Pomerantz	New Chemical Probes for the Nucleosome Remodeling Factor, NURF, an Emerging Therapeutic Target in Adult and Pediatric cancers.
Cheryl Arrowsmith	Mapping the druggability of WD40 Repeat (WDR) proteins.
Timothy Cernak	Chemical Synthesis Enabled by Information.
Matthieu Schapira	Computational hit finding: a long way to go.
Serah Kimani	Discovery and rational development of novel DCAF1 WD40 domain ligands.
Sam Giannakoulias	Representation Learning with Quantum Chemistry.
Pat Walters	Artificial Intelligence in Drug Discovery – Revolution, Evolution, or Complete Nonsense
James Petersson	Identification of Ligands for PET Imaging of Alpha-Synuclein in Parkinson’s Disease.
Carter Mitchell	Leveraging Bioinformatics and Machine Learning in Biologics Drug Discovery Workflows.

Wednesday, October 11, 2023

Raphaël Rodriguez	Chemical Control of Cell Plasticity. **2023 ICBS Global Lectureship Award Lecture**
Brian Liau	Leveraging CRISPR genome editing for chemical genetic approaches at scale.
Alexis Komor	Understanding and Engineering Precision Genome Editing Tools.
Abhijit Parolia	NSD2 is a requisite subunit of the AR neo-enhanceosome in promoting prostate tumorigenesis.
Amanda Garner	Enabling Technologies for Revealing the Druggability of RNA-Protein Interactions.
Lara Mahal	Rethinking the Paradigm: New roles for miRNA in controlling the proteome and glycome.
Peng Wu	Targeting RNA-binding proteins and RNA modification with small-molecule inhibitors
Enver Cagri Izgu	Investigating Biological Targets Using Functional Small Molecules and Biomimetic Systems
Shuibing Chen	Human Pluripotent Stem Cell, Organoids and Drug Screening
Xiling Shen	Patient-Derived Micro-Organospheres Enable Precision Oncology.
Elena S. Reckzeh	Identifying drugs effective to target head and neck squamous cell cancer using a drug repurposing library on organoids.
Haian Fu	Neo-protein-protein interactions as a new dimension of cancer genome: discovery and intervention.
Pamela Chang	Title TBD.

Mariko Takahashi	DrugMap: A quantitative pan-cancer analysis of cysteine ligandability.
Matthew Maitland	A chemical probe to modulate human GID4 Pro/N-degron interactions.

Poster presentations
Monday, October 9th, 2023

1	Evan Moriarity	Using CDD Vault for Data Management as Part of Interdisciplinary Research Projects
2	Soham Maity	Light Controlled Reversible Michael Addition of Cysteine: A New Tool for Dynamic Site-Specific Labeling of Proteins
3	Mariia Zmyslia	Harnessing Encapsulins for Intracellular Organometallic and Enzymatic Catalysis
4	Sahil Sharma	Epichaperome imaging probes for precision medicine in Alzheimer's disease
5	Jiho Song	An activity-based covalent photoacoustic imaging probe targeting NCEH1 for in vivo tumor imaging
6	Pooja Kumari	Chemical biology approaches to target the “undruggable” SMAD4 protein
7	Eita Sasaki	Creation of Novel Proteinaceous Shell Structures through Molecular Evolution of Bacterial Microcompartment (BMC) Shell Proteins
8	Ying Meng	α-N-methylation of Protein Arginine Deiminase 1 (PAD1) Affects its Stability and Protein-protein Interactions
9	Kenjiro Hanaoka	Design strategy to control the emission of fluorophores via a twisted intramolecular charge transfer (TICT) process
10	Aylin Binici	Identification of small molecule-enhancers of natural killer cell cytotoxicity
11	Casandra Sandoval Hurtado	Designing an Engineered Biosynthetic Pathway to Access Complex Diketopiperazines
12	Hongyue(Nicole) Chen	Unearthing druggable vulnerabilities in cancer with Averon Notebook: Leveraging mutant-directed protein-protein interactions
13	Grace McIntyre	Development of miRNA Targeted Therapies for High Grade Serous Cancer
14	Tehreem Anwar	In silico modeling and analysis of small molecules binding to the PHLPP1 protein by molecular dynamics simulation
15	Toru Komatsu	Synthesis-based on affinity separation (SAS) strategy for preparation of fluorogenic substrate library for single-molecule enzyme activity analysis
16	Wukun Ouyang	A versatile multiplexed TR-FRET assay for detection of SMAD4-SMAD3-DNA complex and high-throughput chemical probe discovery
17	Stephen DeAngelo	Revisiting the Se-ntral role of GPx4 in Ferroptosis: the Selenoprotein TXNRD1 emerges as a primary target of RSL3
18	Kota Koike	Simple purification of small-molecule-labelled peptides via palladium enolate formation from β-ketoamide tags
19	Maylynn Hu	Targeting p38/MK2 protein-protein interaction for therapeutic discovery in human diseases
20	Bradley Clegg	Development and Evaluation of Covalent NSD3 Inhibitors
21	Brandon Datuin	Development of Enantiomerically Pure 1,4-thiazepanes and Their Evaluation as Domain-Selective BET Bromodomain Inhibitors
22	David Fairlie	Class IIa Histone Deacetylases And Their Inhibitors
23	Guang Huang	Structure-guided Design of New ASH1L Inhibitors As Potential Anti-leukemic Agents
24	Haru Kudo	Decitabine inhibits DNA methyltransferases activity and suppresses vasculogenic mimicry formation in tumor cells
25	Sydney Musser	Polycomb repressive complex 1 inhibitors demonstrate potent activity in acute leukemia models
26	Joshua Ray	Characterizing MLL-binding affinity of clinically resistant Menin mutations

Poster presentations
Tuesday, October 10th 2023

27	Emma Seipp	The biological evaluation of NatD bisubstrate inhibitors in lung cancer
28	Miranda Simes	Structure-guided development of potent small-molecule inhibitors of PRC1
29	Yuting Yang	Structural studies of intrinsically disordered MLL-fusion protein AF9 in complex with peptidomimetic inhibitors
30	Franchesca Fonseca-Lanza	The ENL YEATS epigenetic reader domain critically links MLL-ENL to leukemic stem cell frequency in t(11;19) Leukemia
31	Sophia West	Targeting triple-negative breast cancer through the MYC-MKK3 complex
32	Joyeeta Roy	Design and Synthesis of Orally Active Quinolyl Pyrazinamides as Sigma 2 Receptor Ligands for the Treatment of Pancreatic Cancer
33	Ryan Hippman	Synthetic optimization of a small-molecule ATG14L-Beclin 1 protein-protein interaction inhibitor for selective autophagy inhibition.
34	Dong Chen	Development of the menin-MLL1 inhibitors targeting menin patient mutations
35	Thomas Whitmarsh-Everiss	Synthesis of Small Molecule Autophagy Modulators as Potential Therapeutics for Alzheimer's Disease
36	Forrest FitzGerald	Benzoxaborolone: An Oxidatively Stable Arylboronic Acid Pharmacophore
37	Kotaro Ochiai	Ferrocene as a Three-Dimensional Platform for Molecular Building: Development of Ferrocene-Based Nuclear Receptor Ligands
38	Tom Schulz	Unveiling a Novel Gα-Pocket in PDGFRA and KIT: Avapritinib-Based SAR Studies Provide Unique Structural Insights into Kinase Inhibition and Acquired Drug Resistance
39	Jennifer Nguyen	Farming more than Fishes: Bioactive Potential of Pseudomonas spp. Isolated from an Aquaculture Facility
40	Xiling Shen	Patient-Derived Micro-Organospheres Enable Precision Oncology
41	Zhixiang Chen	Discovery of ERD-3111 as a Potent and Orally Efficacious Estrogen Receptor PROTAC Degrader with Strong Antitumor Activity
42	Zhixiang Chen	Discovery of CBPD-409 as a Highly Potent, Selective, and Orally Efficacious CBP/p300 PROTAC Degrader for the Treatment of Advanced Prostate Cancer
43	Atsunori Kaneshige	Discovery of a potent and selective small-molecule degrader of STAT6 as a new class of Immuno-Oncology Therapy
44	Joyce Kariuki	Development of a TR-FRET assay to screen for neo-protein-protein interaction inhibitors
45	Maria Kutera	The Structural Characterization of a Potently Selective NSD2 Degrader in Ternary Complex with a Putative E3 Ligase
46	Ally Su	Ultrahigh-Throughput Screening Assays to Identify 14-3-3 Isoform-Selective Modulators
47	Georg Goebel	Discovery of Aminothiazolones as Small-molecule Inhibitors of the RNA–modifying protein METTL16
48	Kira Holton	Cellular Context For mRNA Substrate Selection By Pseudouridine Synthase 7 (PUS7)
49	Rachel O'Rourke	Biological characterization of small molecule eIF4E inhibitors to assess their therapeutic potential in cancer
50	Dailia Soueid	Cell-Based Assay Development Strategies for the Detection and Validation of Aberrant mRNA-Protein Interactions
51	Gabriela Vega-Hernandez	A Live-Cell Assay to Detect eIF4E-mRNA Interactions
52	Noah Puleo	Unraveling the function of TRAF2 and NCK interacting kinase (TNIK) in high-grade serous ovarian cancer

Identifying Covalent Peptide Inhibitors Using Phage Display

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Activity-based protein profiling (ABPP), which has traditionally relied on small molecule chemical probes, is a powerful proteomic platform to interrogate enzyme activity. As many proteins lack small molecule binding pockets, peptide-based probes, which can better bind a larger/flat area on the protein surface, can be employed to target previously “undruggable” proteins. The recent application of peptide scaffolds in probe design has been made possible by the inclusion of electrophilic warheads to covalently bind nucleophilic residues on proteins. When incorporated into the peptide scaffold, these warheads allow for a covalent mechanism of action. Compared to conventional non-covalent peptides, covalent peptides offer enhanced potency, *in vivo* efficacy, selectivity, and versatility. Using cysteine-targeting electrophilic warheads, we are working on developing covalent peptide probes to study thioredoxin-domain containing proteins and protein-arginine deiminases (PADIs) with incomplete functional annotation and clinical relevance. In our latest efforts, we are using phage display to access large peptide libraries, rather than using a chemically synthesized library. The development and use of covalent peptide-based ABPP probes will facilitate our understanding of protein activity and function in various (patho)physiological systems.

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Facilitating novel target and drug discovery in drug-refractory lung cancer by harnessing integrated photoactivatable fragment probes and chemical proteomics

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Background: Photoreactive fragment-like probes can facilitate the identification of proteins that constitute novel cellular vulnerabilities, and their fragment scaffolds can also provide the chemical basis for targeted drug discovery[1]. However, probe library design and target identification/prioritization by mass spectrometry (MS) remain challenges.

Methods: 20 BioCore[2] or BioCore-like fragments featuring natural product and lead-like structural elements were selected and fully functionalized with orthogonal diazirine and alkyne moieties. These probes were used for protein crosslinking in live cells, target enrichment via "click chemistry", and subsequent target identification through label-free quantitative LC-MS/MS analysis. Target profile comparison was enabled by performing experiments using a panel of 20 probes, as well as a negative control probe. High-confidence targets of each probe were queried against a pharmacogenomic database (DepMap) and prioritized through cross-comparison with other probes. The top-ranked probe-binding target was validated using a competitive affinity assay and RNA interference. Protein tyrosine reactivity was profiled by using the sulfur-triazole exchange chemistry (SuTEx) probe, HHS-482[3]. The tyrosine phosphorylation of proteins was investigated using pY-100 antibody and western blotting.

Results: MS analysis identified >4,000 proteins, of which ~1,500 qualified as target candidates based on pair-wise comparison with a blunted negative control. However, stringent prioritization via cross-comparisons against the entire probe panel identified 31 high confidence target candidates, such as glutathione S-transferase zeta 1 (GSTZ1), with selective affinity for individual probes. Knocking down GSTZ1 significantly sensitized non-small cell lung cancer (NSCLC) cells to oncogenic inhibition by targeted clinic drugs. Proteome-wide tyrosine reactivity profiling suggested GSTZ1 modulated amino acid metabolism and several signaling pathways mediated by KRAS, MAPK and Receptor Tyrosine Kinases, which are known to cause resistance to the clinical KRAS G12C inhibitor sotorasib[4]. Consistently, GSTZ1 knockdown remarkably decreased the tyrosine phosphorylation of FGFR1 in H520 cells and the phosphorylation of AKT and ERK in KRAS G12C-mutant, but sotorasib-refractory H1792 cells. In addition, re-activation of AKT upon treatment with sotorasib was inhibited by GSTZ1 knockdown. Furthermore, the identified BioCore probe inhibited the enzymatic activity of GSTZ1 and may serve as a chemical base for developing pharmacological inhibitors of GSTZ1.

Conclusion: By applying integrated phenotypic fragment screening, chemical proteomics, and systems biology approaches, we have established a fragment-based chemical biology platform for discovering novel targetable proteins and their binding molecules. GSTZ1 was found to cooperate with oncogenic alterations in supporting refractory NSCLC cell survival signaling, which may form the biological basis for developing novel GSTZ1 inhibitors to improve the therapeutic efficacy of oncogene-directed targeted drugs.

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Small-molecule GAS41 YEATS domain inhibitors in non-small cell lung cancer

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Glioma-amplified sequence 41 (GAS41) is an oncogene overexpressed in multiple cancers including brain, colorectal, and non-small cell lung cancers (NSCLC). GAS41 protein contains a YEATS domain, a subtype of epigenetic reader domains that recognize lysine residues on histone proteins that have been post-translationally modified with acetyl groups. Acetylation reader activity is associated with gene expression in cancer. GAS41 preferentially recognizes acetylated histone H3 lysine 27; this mechanism recruits GAS41 to the promoters of actively transcribed genes. The GAS41 YEATS domain acetylation binding activity has been shown to be necessary for the proliferation of NSCLC cellular and tumors in mouse xenograft models. Disrupting GAS41 YEATS domain chromatin recognition represents an attractive target for small molecule inhibitors. Using protein NMR-based fragment screening of an in-house fragment-like small molecule library, we have identified and optimized a series of GAS41 YEATS domain histone acetylation reader inhibitors. Extensive medicinal chemistry efforts improved the activity of inhibitors by 30-fold in biochemical assays. Binding experiments with other YEATS domain family proteins show that these inhibitors are highly selective for GAS41. Leading compounds have been tested in NSCLC cell lines and have been shown to inhibit NSCLC cell proliferation with sub-micromolar affinity, with limited activity in normal lung fibroblasts. These inhibitors have also been shown to disrupt the GAS41-H3 interaction in mammalian cells. This class of compounds has novel therapeutic potential for the treatment of NSCLC.

A Chemotranscriptomic Pipeline for RNA-Targeted Ligand Discovery

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Dysregulation of RNA functions has been implicated in multiple human pathologies, such as bacterial and viral infections as well as playing a role in other diseases including cancers.[1,2] Because of RNAs' involvement in diseases, the transcriptome has become an area of interest in the field of drug discovery.[3,4] Current efforts for targeting RNA with small molecules have yielded therapeutics that treat bacterial infections and cancer; however, these target a small number of RNA molecules meaning that this field is still vastly underexplored.[5] One big reason for the lack of RNA-targeting therapeutics is the lack of technologies for accurately screening RNA-binding small molecules in cells. This is a major issue since RNA is a very dynamic molecule whose structure depends upon its surrounding environment.[6] Therefore, it is important to develop new approaches that allow us to screen for RNA-binding small molecules in living cells. **In this proposal, we explore the synthesis and biological characterization of novel chemical probes that can help elucidate small molecule-RNA interactions in cells.** This probe will consist of a small molecule fragment of interest linked to an RNA-selective covalent warhead that will react with the RNA to enable its covalent modification and subsequent identification via affinity purification and sequencing. To address the efficiency of these probes they will be tested in model systems with known RNA binders such as the $PreQ_1$-Riboswitch and possible off target binding partners. We will also leverage this platform to also test putative RNA binding small molecules inside of cellular environments. Small molecule-RNA interactions identified by this project will help shed new light into new motifs that can bind to RNA sequences and structures in cellular environments, as well as give us a new tool to screen for molecules that can be further developed into RNA targeting small molecule therapeutics.

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Identification of Inhibitors of Sterol Transport Proteins Through the Synthesis of a Cholic Acid-Inspired Compound Collection

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Sterol transport proteins (STPs) all bind and transport sterols, and have high structural similarities, particularly in their sterol-binding domain (SBD).[1] Despite these similarities, STPs possess distinct tissue distributions as well as intracellular localisation and functions. Furthermore, STPs not only mediate intracellular sterol transport, but also organelle contacts and lipid metabolism more generally. Their mis-regulation has been associated with lipid storage disorders, atherosclerosis, and a wide range of cancers.[2] Therefore, the development of selective small molecule STP inhibitors is of great significance for both basic and translational lipid biology. Crucially, very few STP inhibitors have been reported, often with little or no selectivity annotations, and the majority of these target a small fraction of STPs, highlighting a significant gap in the field.

The pseudo-natural product (PNP) strategy has proven efficient in identification of active molecules including inhibitors of STPs.[3-6] Thus, this strategy was applied to design and synthesise a compound collection of small molecules inspired by cholic acid in order to identify new STP inhibitors. The *cis*-decalin scaffold as found in cholic acid was used as the primary sterol scaffold to act as a "bait" for STPs. The primary scaffold was fused with natural product fragments (the secondary scaffolds) resulting in heterocyclic edge- and spiro-fused analogues. Additional analogues were accessed from the resulting pseudo-natural products using a "complexity-to-diversity" approach to give ring-distorted analogues.[7] The analogues have been thoroughly characterised including several crystal structures. The biological activity of the analogues has been tested against a panel of different STPs by fluorescence polarisation (FP) and differential scanning fluorimetry (DSF). An interesting scaffold obtained by an oxidative ring contraction followed by a spontaneous intramolecular condensation was identified as a selective and potent new chemotype Aster-A inhibitor (Figure 1). Structure-activity relationship (SAR) studies and molecular docking has been employed to rationalise compound binding modes.

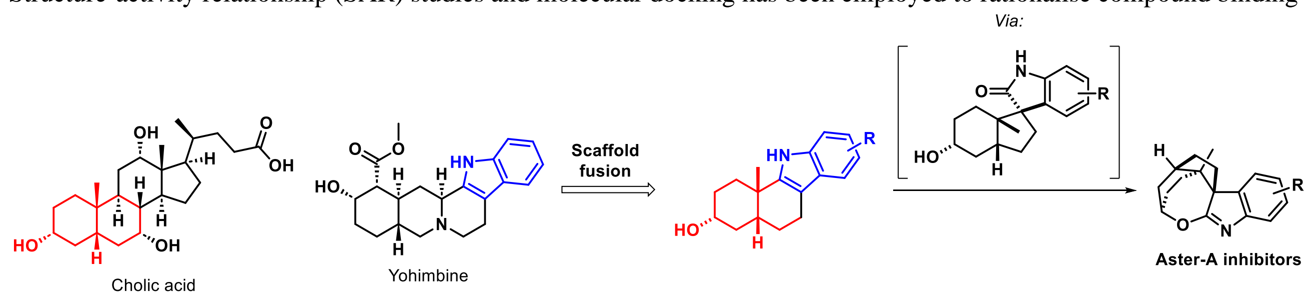

Figure 1: The identification of Aster-A inhibitors through the synthesis of a cholic acid-inspired compound collection.

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Targeting p38/MK2 protein-protein interaction for therapeutic discovery in human diseases

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P38 kinases are essential components of signal transduction. Four known p38 isoforms (p38α, p38β, p38g, and p38d) serve non-redundant functions by binding various substrates, including transcription, signal transduction, protein folding, and cytoskeleton maintenance[1,2]. The inhibition of p38 activity has emerged as a highly appealing therapeutic strategy in multiple human diseases, including cancer, Alzheimer's disease (AD), and different metabolic conditions. Although many p38 inhibitors have been developed, none of them have been approved as a drug due to a limited selectivity against p38 isoforms and other kinases. The failure of p38 inhibitors in clinical trials highlights the urgent need for new therapeutic approaches to p38 regulation. To address this unmet medical need, we develop a novel approach to control p38 activity by selectively targeting p38 protein-protein interaction (PPI) with its major substrate the MAPK-activated protein kinase 2 (MK2), coded by MAPKAPK2. MK2 is one of the most clinically important p38-regulated proteins which play a key role in AD-associated neuroinflammation and neurotoxicity, as well as tumor growth and DNA damage response in cancer metastasis and proliferation[3]. The discovery of potent p38/MK2 PPI inhibitors may open new avenues for p38-based therapeutic development. Our Time-Resolved Fluorescence Energy Transfer (TR-FRET) and affinity pulldown assays revealed that MK2 has a significantly higher binding affinity to p38a and p38b compared to p38g and p38d isoforms. Using computational structural analysis, we identified multiple contact sites on the p38/MK2 PPI interface that are critical for p38/MK2 interaction. The calculated druggability scores and contribution of p38 and MK2 residues to the MK2/p38 free binding energy allowed us to prioritize two pockets suitable for small molecule p38/MK2 disruptor binding. Based on the analysis, we experimentally confirmed the p38/MK2 interface, and designed short inhibitory peptides to disrupt the p38/MK2 PPI. Through a computational screening, we have uncovered and validated the first small molecule p38/MK2 PPI inhibitors. We have further optimized and miniaturized the TR-FRET assay for the high-throughput screening (HTS) 384- and ultra-HTS 1,536-well plate formats. We have shown that both p38a/MK2 and p38b/MK2 PPIs demonstrated strong TR-FRET signal with >20 signal/background ratio, which was stable for more than 48 hours and tolerated >10 % DMSO. Togetgher, our data indicate the p38/MK2 complex druggability, provides the first chemical tools to regulate this clinically significant complex, and establish a new robust assay to discover small molecule MK3/p38 PPI inhibitors to facilitate therapeutic discovery towards cancer, neurological, and metabolic diseases.

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Volumetric click reaction for organ level *in situ* small molecule drug visualization

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Precise mapping of drug actions across cell types can be highly desirable due to the complex cellular compositions across tissue compartments. However, direct visualization of small molecule drug binding in mammalian tissue has long been challenging due to insufficient imaging resolution. By strategically integrating tissue clearing and click reaction, we developed a strategy termed clearing-assisted tissue click chemistry (CATCH) for highly specific on-target covalent drug binding mapping with cellular resolution[1]. Building upon the initial CATCH work primarily associated with 2D characterizations, we explored the principles to enable click reaction in large tissue samples. We identified maintaining sufficient catalytic loading as the key factor for efficient deep tissue click labeling. Furthermore, we found that cleared tissue is compatible with multiple rounds of click reactions without generating excessive surface background labeling, highlighting the high specificity of click reactions. Utilizing an in-house tissue clearing pipeline HYBRiD that is well suited for complex tissue compositions[2], we systematically optimized click reaction procedures and achieved high-resolution drug mapping in whole mouse organs, paving the way for unbiased drug binding registration across regions/cell types.

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The discovery and characterization of potent and selective chemical degraders of the histone methyltransferase NSD2

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Nuclear receptor-binding SET domain-containing 2 (NSD2) is a methyltransferase responsible for the formation of dimethylated lysine 36 of histone 3 (H3K36me2), an epigenetic mark associated with chromatin regions of active transcription[1]. For that, NSD2 plays a significant role in gene regulation, and NSD2 dysregulation has been reported to be the oncogenic driver in many cancers[1-3]. Recently, we reported the development of an NSD2 selective degrader, UNC8153, with sub-micromolar potency that can reduce the cellular levels of NSD2 protein and the associated H3K36me2 chromatin mark[4]. UNC8153 is based on a novel E3 recruiting warhead that contains a simple primary alkylamine serendipitously discovered to confer neddylation-, ubiquitination-, and proteosome-dependent degradation of NSD2[4]. The degradation of NSD2 by UNC8153 results in the down-regulation of pathological phenotypes in multiple myeloma cells, including mild anti-proliferative effects in MM1.S cells containing an activating point mutation in NSD2 and anti-adhesive effects in KMS11 cells harboring the t(4;14)-translocation which up-regulates NSD2 expression[4]. Despite these promising effects, the mechanism of action of UNC8153 was not known. In recent efforts, we used proximity-dependent biotin identification (BioID) to characterize the degrader-mediated protein-protein interactions of NSD2. This resulted in candidate E3s being studied for target engagement and mechanism of action validation. We further made optimizations to UNC8153 to yield UNC8732 and achieved nanomolar potency. We anticipate that these new chemical degraders will be valuable tools for the epigenetics community to investigate the therapeutic potential of NSD2 and that the novel E3-recruiting warhead could open doors to new strategies in targeted protein degradation.

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Keynote Lecture

Why I Love Being a Scientist

Ingrid Wertz
Lyterian Therapeutics
(no abstract)

Keynote Lecture

Development and validation of PROTACs as selective chemical tools

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Small molecule degraders such as PROTACs (PROtein Targeting Chimeras) have emerged as new promising pharmacological modalities but currently their development is limited by the small number of ligands targeting E3 ligases. We developed a workflow developing PROTACs interacting with new E3 ligases and established assay systems for their validation as highly selective degrader chemical tools.

Keynote Lecture

Co-opting the Ubiquitin System for Therapeutic Benefit

Ingrid Wertz
Lyterian Therapeutics

The ubiquitin system is a primary conduit for the regulated degradation of cellular proteins. Ubiquitination describes the covalent modification of protein substrates with ubiquitin, a small protein that binds the proteasome and directs substrate degradation. Our goal is to co-opt the ubiquitin system, and more broadly protein homeostasis, to irreversibly regulate challenging therapeutic targets for enhanced clinical efficacy. Our efforts are focused on targets for which there is a clear rationale for homeostatic regulation over target inhibition. We will discuss strategies to co-opt protein homeostasis, highlight mechanisms for how selective targeting can be achieved, and review the cellular and physiological consequences of target regulation, with an emphasis on maximizing safety and therapeutic benefit for patients.

Enabling Platforms for the Development of Degraders

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I will present our recent progresses for the development of proteolysis targeting chimeras (PROTACs), molecular glues, and lysosome-targeting chimeras (LYTACs) for the degradation of intracellular and extracellular proteins.[1-3] In the area of PROTACs and molecular glues, I will focus on the development of two platforms: rapid synthesis of PROTACs (Rapid-TAC) and rapid synthesis of molecular glues (Rapid-Glue), and their applications for the degradation of intracellular proteins. In the area of LYTACs, I will focus on the development of tissue-selective degraders with in-vitro and in-vivo activities.

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Protein editing using small molecules.

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CRISPR-based technologies have made editing of modifications of DNA/RNA facile. However, technologies to “write” or “erase” posttranslational modifications (PTMs) on proteins, which dramatically alter protein structure and function, are non-existent. Our long-term goal is to develop CRISPR-equivalent platform technologies for robust PTM editing. Towards that goal, we recently reported PHosphorylation-Inducing Chimeric Small molecules (PHICS) that selectively induce phosphorylation of a protein-of-interest by bringing the kinase and target protein in proximity.

Platform development: The 1st-generation PHICS brought Ser/Thr kinases (AMPK or PKC) or Tyr kinase (ABL) in proximity to a target protein, inducing target phosphorylation even for non-substrates (i.e., neo-substrates) of the kinase (platform 1). However, these PHICS linked the target binder to an *allosteric*, *non-inhibitory* kinase binder, which is scarce. To expand the applications of PHICS, we developed linker chemistries to generate PHICS from kinase inhibitors (abundantly available), allowing rapid development of PHICS for >30 kinases (platform 2).

Novel bioactivities of PHICS: PHICS induced known phosphorylations with diverse bioactivities, including signal transduction, phase separation, and 14-3-3 mediated sequestrations. Furthermore, PHICS-induced neo-phosphorylations (unobserved naturally) adversely affected the target protein’s ability to interact with negatively charged biomolecules (e.g., ATP, GTP, phospholipids). For example, PHICS-mediated phosphorylation disrupted the ability of membrane-translocating KRAS to interact with GTP/phospholipids. “Homo-PHICS” (i.e., a dimer of kinase binder) inhibited the kinase by inducing phosphorylations in the ATP-binding pocket. This novel mechanism of action allowed PHICS to efficaciously kill cancer cells resistant to known drugs (e.g., imatinib/asciminib for CML, ibrutinib for CLL).

In summary, we have developed PTM editors for phosphorylation that exhibit novel bioactivities and linker technologies that will enable the rapid development of editors of other PTMs.

Discovery of a PTPN2 degrader *in vivo* tool compound for the study of cancer immunotherapy

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Protein tyrosine phosphatase non-receptor type 2 (PTPN2) is involved in the intracellular oncogenic signaling JAK-STAT pathway[1]. Furthermore, PTPN2 was identified from an *in vivo* tumor CRISPR screen to synergize with anti PD-1 treatment, which led to an enhanced anti-tumor effect via further immune activation[2]. Modulating PTPN2 activity is of high therapeutic interest as an oncoprotein and potential immunomodulator. However, the phosphatase class of proteins only recently have begun to discharge the mantle of undruggability with inhibitors within the class entering clinical studies[3]. The phosphate binding site is highly polar[4], which has led to substrate-competitive inhibitors that can exhibit compromised physicochemical property space and developability[3]. We hypothesized that a heterobifunctional degrader has an advantage over inhibitors as it possesses a catalytic mode-of-action, since PTPN2 degradation would suppress the target signaling through the JAK-STAT pathway signaling for an extended period thereby separating PK from PD effects. The potential for enhanced PD at lower exposures opens a pathway to a developable drug for this target. Herein, we describe our efforts to advance a thiadiazolidinone (TZD)-derived chemical series to identify a PTPN2 degrader *in vivo* tool compound. Structure- and property-based drug design enabled the identification of productive ternary complexes for degradation, control of physicochemical properties, and *in vivo* anti-tumor activity. Our studies culminated in the discovery of compound **17**, a highly potent, selective, efficacious PTPN2 degrader.

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Targeted Protein O-GlcNAcylation Using Bifunctional Small Molecules

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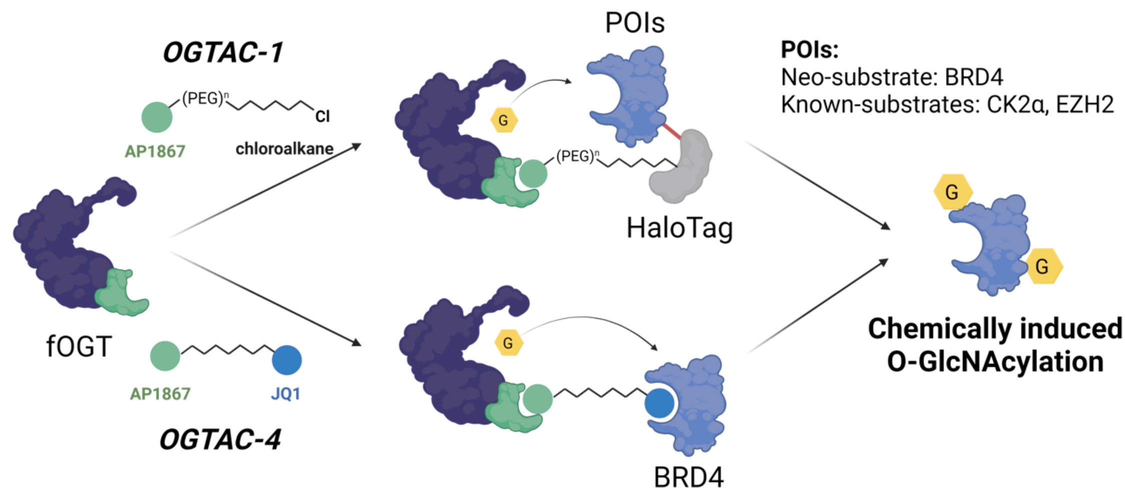
Protein O-linked β-*N*-acetylglucosamine modification (O-GlcNAcylation) plays a crucial role in regulating essential cellular processes. The disruption of O-GlcNAcylation homeostasis has been linked to various human diseases, including cancer, diabetes, and neurodegeneration. However, there are limited chemical tools for protein- and site-specific O-GlcNAc modification, rendering the precise study of O-GlcNAcylation challenging. To address this, we have developed first-in-class heterobifunctional small molecules, named O-GlcNAcylation targeting chimeras (OGTACs), which enable protein-specific O-GlcNAcylation in cells. OGTACs promote O-GlcNAcylation of proteins such as BRD4, CK2α, and EZH2 *in cellulo* by recruiting O-GlcNAc transferase (OGT), with temporal and magnitude control. Proteomics analysis revealed that OGTACs induced site-selective O-GlcNAcylation of BRD4. Overall, OGTACs represent a promising approach for inducing protein-specific O-GlcNAcylation, thus enabling functional dissection and offering new directions for O-GlcNAc-targeting therapeutic development.



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HIGH THROUGHPUT SCREENING REVEALS A ROLE FOR BRD4 IN POTENTIATING CADMIUM CYTOTOXICITY VIA NUCLEAR-MITOCHONDRIAL CROSSTALK

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We previously performed high throughput screening to identify small molecules that enhance cellular resistance to multiple stressors, including cadmium (Cd) (Lombard *et al.*, *Sci Adv* 2020). Cd is a widespread environmental pollutant that affects millions of individuals worldwide. Cd exposure in humans most often occurs through Cd's many industrial applications, smoking, or consumption of contaminated food. Due to its extremely long biological half-life, Cd persists for decades in tissues. Cd exerts numerous deleterious effects, including bone, reproductive, neurodevelopmental, and pulmonary toxicities, and carcinogenesis. The kidneys are the major target of Cd toxicity, particularly the proximal tubular epithelial cells, injury to which hampers tubular reabsorption. Despite the many sequelae associated with Cd exposure in humans, molecular mechanisms of Cd toxicity are poorly understood, and no specific therapies exist to mitigate the effects of Cd exposure.

Based on our initial screens, we find that multiple chemically distinct Bromodomain and Extra-Terminal motif inhibitors (BETi) can suppress cytotoxicity occurring in response to Cd treatment, as well as Cd-associated perturbations in cellular metabolite levels, mitochondrial function, and gene expression. BETi inhibit the interaction of BET family proteins with chromatin, and are in clinical trials for a variety of neoplastic and inflammatory diseases. The best-characterized BET protein is BRD4. We have identified the Succinate Dehydrogenase (SDH) enzyme complex, aka mitochondrial complex II, as one key target of Cd. In genome-wide as well as locus-specific ChIP studies, BRD4 occupancy increases in response to Cd exposure upstream of metabolically important genes, including genes encoding SDH subunits. Crucially, our *in vivo* studies reveal that the BETi JQ1 can mitigate acute Cd-induced kidney injury *in vivo*. Based on these findings, we propose that the interplay between the epigenome and mitochondrial function represents an important target of Cd toxicity, with potential translational significance.

Small Molecule Therapies That Can Modulate Aging

Toren Finkel
University of Pittsburgh Small Molecule

I plan to discuss our group's approach to defining tractable targets in aging biology and how we have begun to develop small molecules that influence these targets. In particular, I will discuss our attempt to modulate the autophagy-lysosomal pathway by targeting the transcription factor TFEB. In addition, I will review our attempts to reverse the age-dependent decline in NAD^+ levels by developing a small molecule that augments the activity of NAMPT, the rate-limiting enzyme in the NAD^+ salvage pathway. Finally, I will review our strategy to developing novel, rational-based senolytic drugs. This approach leverages a whole genome CRISPR screen we have executed in order to identify a set of potential specific senolytic targets. Together, these three programs identify an approach to develop small molecules that might positively influence human healthspan.

New Borinic Probes for Fast Detection and Imaging of Cellular Hydrogen Peroxide

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Reactive oxygen species (ROS) are by-products of aerobic metabolism. Among them, hydrogen peroxide (H_2O_2) plays a crucial role in a wide range of physiological processes.[1] However, when our cells are subjected to oxidative stress, its overproduction is directly or indirectly responsible for numerous damages at the molecular level. This process is associated with aging, as well as cancer and several neuro-degenerative diseases such as Alzheimer's or Parkinson's.[2] The development of selective and sensitive tools allowing H_2O_2 detection in a biological context represents a great challenge for a better understanding of H_2O_2-mediated signaling in physiological and pathological processes.

To date, several "off-on" small fluorescent probes triggered by H_2O_2 have been developed for its detection. Among them, probes based on boronate oxidation are amongst the most effective for the detection of H_2O_2 *in cellula*.[3] But these probes suffer from lack of reactivity, which is not fully satisfactory for biological applications. To address this issue, we envisioned the use of borinic acids which due to electronic effects could be more prone to oxidation compared to their boronic acid counterparts.

Recently, we reported the design, synthesis, and kinetic properties of the first borinic sensor for the fast detection of hydrogen peroxide.[4] Furthermore, a comparative study with its boronic acid counterpart was presented *in vitro* and in a cellular context.

We are currently further optimizing this strategy for biological applications and will present our latest results in this field.[5]

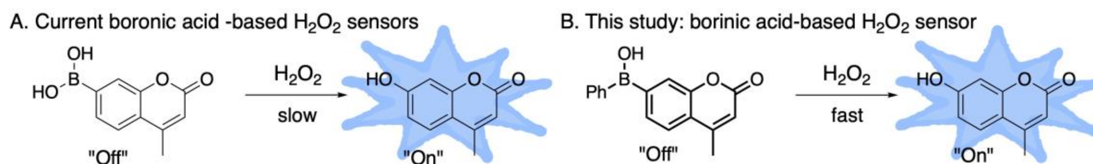

Fig. 1 Schematic representation of our strategy using borinic probes, versus established boronic probes.

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Develop potent and isoform-selective PRMT inhibitors

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Abstract Protein arginine methyltransferases (PRMTs) are an emerging drug target class. However, the lack of selective chemical probes hampers pharmacological interrogation of PRMT biology. One challenge is the conserved active site among all PRMTs. Here, we report a versatile strategy to build a SAH analog library embracing a diverse set of 100 compounds in a 5-step reaction sequence through a one-compound one purification manner. By identifying promising hits for each tested PRMT, we observed a clear chemical preference for each test PRMT, supporting subtle differences in the active site of highly conserved PRMTs. Specifically, we identified a selective and potent inhibitor for PRMT1 and PRMT4 with an $IC_{50} < 5$ nM. Collectively, the preliminary structure-activity relationships generated from this work would provide general guidance to design inhibitors with improved potency and isoform selectivity to each PRMT. In addition, we anticipate that this focused library can be adaptable to glimpse the active site of other methyltransferases to direct and accelerate the discovery of inhibitors.

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Development of Sigma 2 Receptor Ligands for Cancer and Neurodegenerative Disorders

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GPCR-related σ receptors (σRs) were originally considered as a subtype of opioid receptors (μ, κ, and σ). Subsequent studies showed their distinction and classified these receptors into σ_1 and σ_2 subtypes. σRs interfere with ion channels and GPCR receptors activity modulating several physiological pathways. σRs are implicated in various CNS disorders, neurodegenerative diseases, traumatic brain injury, drug abuse, COVID-19, and cancer. Until now, only a few σR ligands have entered clinical trials to treat different conditions, including neurodegenerative diseases, mental disorders, and pain. However, no σR ligands for the treatment of cancer have reached the clinic, probably due to inadequate understanding of the signaling pathways and inconsistent preclinical data. Nevertheless, a growing body of evidence supports the use of small molecule σRs ligands as therapeutic agents to treat select cancers. Elevated levels of σ1R protein and apoptosis-inducing effects of select σ1R antagonists suggest that functional σ1R is required for tumorigenesis and tumor progression. σ2R is overexpressed in a broad range of cancers including breast and pancreatic cancers. σ2R has been used as a biomarker of proliferation and σ2R radioligands can be used for tumor imaging. Importantly, several σ2R ligands have shown *in vivo* efficacy in mouse xenograft models of pancreatic cancer. While the exact mechanism of action of σR ligands in cancer is still elusive, the cytotoxic effects of σ2R ligands are attributed to multiple oncogenic signaling including ROS, p53 and caspase-independent/dependent apoptotic pathways. Additionally, the recently solved cocrystal structures of σRs with several ligands and the discovery of diverse chemical structures that bind to σRs with various affinities and degrees of selectivity will inform the development of more selective σRs ligands for cancer therapy. We will present our efforts on the design and synthesis of a novel series of quinolyl pyrazinamides as selective and potent σ2R ligands with nanomolar potency in pancreatic cancer cell lines. Our optimized compound **JR1-157** is water soluble, metabolically stable, orally active, cytotoxic across a panel of pancreatic cancer cell lines, and increases the levels of autophagy marker LC3B and the ER stress marker GRP78. JR1-157 shows single agent efficacy in a syngeneic mouse model of pancreatic cancer without significant toxicity. Our findings warrant development of **JR1-157** as an effective treatment for pancreatic cancer.

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Acknowledgments

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Combinatorial Biosynthesis of Protease Inhibitors

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Natural product biosynthesis has been focused on biosynthetic pathways that have anchoring core enzymes, such as polyketide synthase, nonribosomal peptide synthetase and terpene cyclase. This approach is conceptually simple and bioinformatically straightforward to execute. However, nature encodes many biosynthetic pathways that do not use core enzymes, which makes these difficult to identify and challenging to predict metabolite structure and enzyme functions. In recent years, our lab has focused on the mining and engineering of biosynthetic pathways that are referred to as "Unknown-unknowns", which represents unknown biosynthetic logic and unknown products.[1] In this presentation, we will discuss the mining of unknown unknowns in fungi to identify a short pathway to a potent protease inhibitor natural product. Previous annotated hypothetic proteins were discovered to catalyze biosynthetically and synthetically interesting reactions. With enzyme catalyzed, we performed large scale combinatorial biosynthesis of the pathway, taking advantage of the broad substrate promiscuity of the enzymes. This approach led to biosynthesis of hundreds of analogs and identification of more potent analogs that inhibit proteases of interest.

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Illuminating RNA biology with metabolically incorporated ribonucleoside probes

Ralph Kleiner
Princeton University

RNA plays a central role in biological processes and characterizing the regulatory mechanisms governing its behavior can reveal fundamental insights into gene expression programs in normal and disease contexts. Towards this end, our lab has developed general approaches based upon RNA metabolic labeling with artificial nucleoside probes in order to study RNA-associated processes transcriptome-wide in live cells and organisms. In the first part, we present RNA-mediated activity-based protein profiling (RNABPP), a chemoproteomic strategy using mechanism-based nucleosides that enables characterization of RNA modifying enzymes and their associated post-transcriptional RNA modifications. We apply RNABPP with diverse C5-modified pyrimidine nucleosides in order to identify RNA methyltransferase, dihydrouridine synthase, and dioxygenase enzymes. Further, we combine quantitative RNA mass spectrometry and modification-specific sequencing technologies in order to characterize the abundance and distribution of individual RNA modifications and understand their function in cellular processes. In the second part, we combine protein engineering of the nucleotide salvage pathway with bioorthogonal and fluorescent nucleoside chemistry to develop an approach for live-cell imaging of global RNA dynamics. We apply our method to study RNA trafficking and metabolism upon oxidative stress. Taken together, our work provides powerful and general strategies for interrogating RNA regulation and reveals the presence of novel mechanisms controlling RNA function in biological systems.

Activation and Incorporation of Rare Sugars into Bacterial Surfaces

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Our planet is inhabited by trillions of bacteria that live inside and outside of humans. The "skin", or surface, of bacteria is called the cell envelope, which mediates infection of the host and protects bacteria from host immune defense tactics. While Gram-negative bacteria contain a protective outer membrane layer absent in most Gram-positives, almost all bacteria contain polymers composed of unique monosaccharides that extend from the cell surface. Gram-negative bacteria typically contain lipopolysaccharide (LPS) in the outer leaflet of the outer membrane with attached polysaccharides called O-antigens that help mediate interactions with the environment. O-antigens are composed of repeating oligosaccharides that define particular bacterial serotypes, which distinguishes bacterial strains within a single species. Foundational chemical biology work has contributed to our understanding of eukaryotic cell surface composition. However, we still lack a clear understanding of assembly of bacterial surface glycan polymers that contain prokaryote-specific or "rare" sugars. Here, we describe synthetic and chemoenzymatic methods to construct rare nucleotide sugars to study substrate recognition by bacterial glycosyltransferases that build O-antigens. We identify key regions in sugar substrates that are required for substrate binding and activity, and we use this knowledge to design chemical probes that will be used for the construction of synthetic O-antigens and small molecule inhibitors that will stall O-antigen synthesis. This work will expand our understanding of cellular mechanisms underlying bacterial polysaccharide synthesis, and will teach us about the roles that rare sugars play in bacterial cellular interactions.

RNA G-quadruplex structures are responsible for insoluble aggregates characteristic of the neurodegenerative disease ALS.

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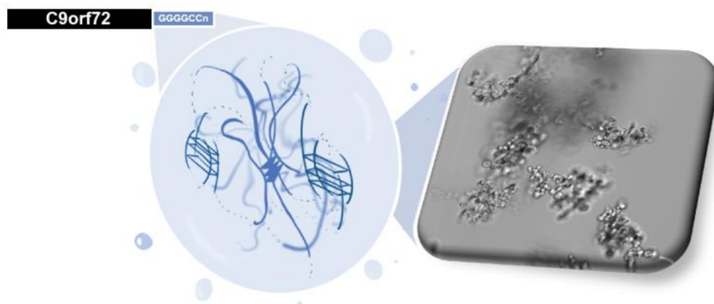
Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are just two of the neurodegenerative diseases characterised by the presence of pathological aggregates[1]. 40% of familiar cases in ALS and FTD have been correlated to expansion of the hexanucleotide repeat $(GGGGCC)_n$, which has been previously shown to assemble into G-quadruplexes (G4s) structures[2]. In this talk I will discuss our recent work, where we investigated the role of nucleic acids and secondary structure formation in the generation of ALS/FTD aggregates, something that has often been relegated as a peripheral effect in the protein-led aggregation[3]. We showed a correlation between the emergence of multimolecular G4s (mG4s) formed by the DNA $(GGGGCC)_n$ repeats and the formation of protein free insoluble aggregates. Aggregation is dependent on K^+ concentration and repeat-length, indicating that G4-formation is essential to observe aggregates. G4-structures were detected in the aggregates by staining with the G4-specific fluorescent dye NMM. G4-unfolding promoted by NMM-mediated guanine photo-oxidation led to prompt disassembly of the insoluble aggregates, further confirming a G4-based aggregation mechanism. To reinforce the physiological relevance of our observations, we characterised the aggregation of RNA $(GGGGCC)_n$, which is thought to contribute to pathological aggregation in ALS/FTD. We observed that RNA repeats can aggregate at significant lower concentrations compared to DNA, suggesting that under physiological conditions RNA repeats can aggregate in the absence of any protein. Using patient-derived ALS cell lines, we validated our model by observing the same G4-based RNA aggregates in the pathological RNA *foci* that are characteristic of this disease, suggesting that nucleic acids targeting could be the key to treat neurodegenerative diseases in the future. Our findings constitute the first evidence supporting the formation of multimolecular G4-structures to drive protein-free aggregation in neurodegenerative diseases, challenging the current dogmas on the mechanisms responsible of neurodegeneration and associate protein led aggregate formation.[3]



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Our journey in the discovery of clinical-quality PROTAC degraders for the treatment of human diseases

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Abstract: In recent years, induced protein degradation using the PROTAC technology has become a very attractive strategy for the discovery and development of novel therapeutics for the treatment of human diseases. While PROTAC degraders have been reported for a large number of protein targets, over 99% of such compounds lack the desirable potency, selectivity, physiochemical properties, pharmacokinetic properties, pharmacodynamic effect, efficacy and toxicity for potential clinical development. In this lecture, I will present our journal in the discovery of clinical-quality PROTAC degraders for the treatment of human diseases and the large number of barriers we had to overcome for clinical development of PROTAC molecules for the treatment of human diseases.

Directed Chemical Evolution of Self-Assembling Artificial Proteins

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The vast diversity of proteins with spectacular structure and function is the result of natural evolution with a time scale of millions of years. With the invention of DNA recombinant technology, biologists exploited directed evolution principles to create a vast number of self-assembling artificial proteins (SAPs) in a very short time. However, most of the SAPs made through genetic methods lack functions due to reasons they are restricted to the standard set of twenty amino acids. In this talk, I will discuss our laboratory efforts to design and synthesize SAPs with rich chemical/structural diversity through a directed chemical evolution method powered by a micelle-assisted protein labeling technology (MAPLabTech). The designer proteins self-assemble to form protein assemblies of defined size and shape and also exhibit exotic functions.

Gene Therapy Without the Genes: Intracellular Delivery of Therapeutic Proteins via Traceless Bioreversible Esterification Strategy

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Following the inception of the first recombinant protein therapeutic—human insulin—into the clinic in 1982, the use of protein-based drugs has flourished, often with greater target specificity and lower toxicity compared to small molecules. Yet, despite several blockbuster therapies, these protein-based drugs have been restricted to extracellular targets due to the intrinsic cell impermeability of proteins, leaving approximately 80% of intracellular protein-related diseases undruggable. Conventional delivery techniques fail to address this fundamental problem in that protein cargo is predominantly delivered into cells via endocytosis, leading to degradation pathways. Thus, the ability to modulate protein surface to interrogate factors important for cell permeability is highly desired in both biological research and protein therapeutics.

Addressing the fundamental limitation, we developed a bioreversible esterification strategy to endow proteins with the ability to enter the cytosol of human cells. Specifically, we developed a tricomponent molecule, α-aryl-α-diazoacetamide, synthesized in three steps, that incorporates a diazo moiety for chemoselective esterification of carboxyl groups, a pyridyl disulfide group for late-stage functionalization with thiolated ligands, and a self-immolative carbonate group for esterase-mediated cleavage. Using cytochrome *c* (Cyt c) and the green fluorescent protein (GFP) as models, we generated protein conjugates modified with diverse domains for cellular delivery that include a small molecule, targeting and cell-penetrating peptides (CPPs), and a large polysaccharide and PEG group, to assist the delivery of proteins into the cytosol of live mammalian cells in the presence of serum.

To further expand the utility of α-aryl-α-diazoacetamides in protein delivery applications *in vivo*, we propose a new therapeutic platform involving nanoparticles for the intracellular delivery of therapeutic proteins. Lipid and polymer-based nanoparticles have revolutionized gene delivery into the cell both *in vivo* and in the clinic. However, these nanoparticles have seldom been applied for protein delivery. This is because proteins are extremely diverse, varying in size, surface character, charge, and hydrophobicity/hydrophilicity, or simply not "anionic" enough. Here, we propose to chemically "esterify" therapeutic proteins (e.g., STING, Cas9, PTEN) that will facilitate encapsulation within clinically promising nanoparticle formulations. This strategy focuses on the fine-tuning of electrostatic interactions between the protein cargo and the nanoparticle carrier. Our strategy represents a significant advance over previous reversible strategies because protein mutagenesis is not required, modifications are done under mild conditions, the probe is synthetically accessible, and most importantly, our strategy is potentially compatible with virtually any protein of interest.

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Unraveling the signaling mechanisms of endogenous cell-cell signaling peptides

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Endogenous peptides (neuropeptides and peptide hormones) act as cell-to-cell signaling molecules to carry out complex tasks in living systems, including regulating metabolism, pain perception, stress response, circadian rhythm, and more. Characterizing the molecular mechanisms of endogenous peptides represents a significant goal to understand how living systems function in both healthy and disease states, and may identify novel therapeutic targets. Understanding cellular communication requires not only knowledge of the transmitter (i.e., the peptide ligand), but also information regarding the cognate receptor that mediates signaling on the partner cell. However, there exist a relatively large number of biologically active peptides whose cell-surface receptor(s) are not known, primarily because of a lack of techniques to reliably identify peptide receptors *de novo*.

To better understand the molecular mechanisms of intercellular communication, our group focuses on developing and utilizing chemical biology approaches to understand endogenous peptides and probe peptide-receptor interactions.[1-3] One major project in the group is focused on developing methods to label neuropeptide receptors on the surface of living cells for the purpose of probing known peptide-receptor interactions and discovering novel receptors for bioactive peptides.[4] In parallel, we are also exploring the impact of an understudied endogenous peptide post-translational modification (l- to d-residue isomerization) on receptor recognition and signaling in nature, and have discovered a neuropeptide signaling system in which peptide isomerization is utilized to alter selectivity between distinct family members.[5] Overall, our research combines approaches from chemical biology, bioanalytical chemistry, and synthetic chemistry to advance our understanding of specific cell-cell signaling pathways, identify new pathways for further exploration, and provide innovative starting points for future therapeutics.

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Exploring Cofactor Analogue Inhibitors of Protein Methyltransferases

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Epigenetic regulations are involved in establishing cell-lineage diversity and errors in these processes have been linked to many diseases including cancer. Among the key biochemical modifications in epigenetics is protein methylation, a process orchestrated by around 100 human protein methyltransferases (PMTs). Given the importance of PMTs, we leveraged multiple approaches to identify PMT inhibitors. Our effort was made to explore cofactor analogues as selective PMT inhibitors. We found that even closely related PMTs can adopt distinct conformational states and thus be selectively recognized by structurally matched cofactor analogues. This presentation will outline the decade-long journey of the Luo lab to develop cofactor analogue inhibitors of PMTs through unconventional modes of interaction.

SET domain dynamics facilitates targeting histone methyltransferases

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Family of histone methyltransferases involved in mono and di-methylation of H3K36 contains four members: NSD1, NSD2, NSD3 and ASH1L. These methyltransferases play important role in epigenetic regulation of gene expression and have been implicated in multiple cancers. Amplifications, translocations or mutations of H3K36 methyltransferases are found in acute leukemia, breast and brain cancers, multiple myeloma, which prompted multiple efforts to discover small molecule inhibitors of these enzymes. The unique feature of the catalytic SET domain in H3K36 methyltransferases is the presence of autoinhibitory loop blocking the access to enzyme active site. Such an autoinhibitory loop has been attributed to difficulty in discovery of small molecule inhibitors. However, inherent dynamics of the autoinhibitory loop may also offer an opportunity to develop potent and selective inhibitors. In this presentation we will discuss dynamics of H3K36 methyltransferases and progress to develop SET domain inhibitors.

Targeting multi-domain proteins for pediatric cancer therapy

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Among all the cancer type, the pediatric cancers posted significant challenge for effective therapy development. Despite intense study and cooperative group trials, patients diagnosed with aggressive, high-risk pediatric tumors such as sarcomas, neuroblastoma, high grade gliomas and embryonal brain tumors have poor overall survival outcomes. In addition to the limited efficacy of conventional chemotherapy and radiation in such patients, these modalities are fraught with toxicities, leaving survivors with long-term deficits including infertility, heart failure, endocrine disruption and a risk of secondary malignancies. Thus, one of the most pressing clinical needs in pediatric oncology remains the identification of agents with potent anti-tumor activity that have limited toxicities. We have discovered that the robust epigenetic landscape plays a vital role in gene expression via its ability to promote or repress transcription. Reprogramming of these processes can lead to genomic instability, which may propagate a cancerous state, and offered a great group of targets for novel therapy development. Thus, our research focuses to combat this reprogramming utilizing novel small molecules to target epigenetic proteins.

Among all the targets that play critical roles in pediatric cancers, we have identified several unique dependencies, such as EP300, CBP, SMARCA2/4, that are all multi-domain proteins in pediatric cancers. While targeting these multi-domain proteins selectively remained challenging, advancements in chemical biology, particularly the development of a novel group of molecules, called *proteolysis-targeted chimaeras* (**PROTACs**) or degrader offered additional approach to go after these multi-domain proteins. We have established understanding and design principle to effectively targeting these important proteins selectively. More importantly, we identify the unique biological system that depend on these targets as unique dependencies, and assessed the therapeutic potential of our chemical biology approach. Overall, our chemical biology approach combined with cancer biology discovery entitled us to establish the function of targeted proteins in different cancers, and provide an opportunities for developing novel and effective therapy for cancer, particularly for pediatric cancers.

New Chemical Probes for the Nucleosome Remodeling Factor, NURF, an Emerging Therapeutic Target in Adult and Pediatric cancers

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Protein members of nucleosome remodeling complexes are emerging therapeutic targets. One protein of interest, the Bromodomain PHD Finger Transcription Factor, BPTF, is an essential member of the human nucleosome remodeling factor NURF. In recent years, BPTF has become increasingly identified as a pro-tumorigenic factor, prompting investigations into the molecular mechanisms associated with BPTF function. We recently have shown BPTF inhibition can enhance the effects of chemotherapeutics in triple negative breast cancer[1] and in unpublished data, we have now uncovered a new role for BPTF in neuroblastoma, an aggressive pediatric cancer, through regulation of both MYC and MYCN expression. Despite a wealth of genetic data supporting the oncogenic role of BPTF, the functional role of the individual chromatin binding domains in driving tumor progression are poorly understood. Chemical probes and genetic tools are envisioned to serve as useful chemical biology resources for probing BPTF function both in normal and pathophysiology. Here I will describe our recent efforts developing, small molecule inhibitors of two different chromatin binding domains of BPTF, as well as early efforts developing protein degraders. Here, the role of the individual chromatin binding domains will be discussed using a combination of inhibitor studies, genetic knockdowns, and new CRISPR-edited cells in neuroblastoma cell lines.

To date, our lab has developed novel screening approaches using protein-based ^{19}F NMR, protein crystallography, and supporting biophysical methods to develop both the first inhibitor of the BPTF bromodomain AU1,[2] and a more potent second generation inhibitor BZ1[3]. Our bromodomains inhibitors have been used in both cell-based assays as well as in vivo. Subsequently, we have now identified the first small molecules engaging the PHD domain, the first such chemical matter for this domain which we will discuss for the first time. These tool compounds have helped demonstrate the importance of the bromodomain for mediating transcription as well as serving as a mechanism for reducing MYC occupancy on chromatin. Most recently they have showed synergistic effects with chemotherapeutic drugs in metastatic breast cancer.[1] Finally, I will discuss our initial efforts at developing the first degraders of BPTF as an alternative mechanism to phenocopy genetic knockdowns.[4] Together, these studies set the stage to study the role of the chromatin binding domains of BPTF and subsequently NURF function in neuroblastoma and highlights a potential new anticancer strategy.

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Mapping the druggability of WD40 Repeat (WDR) proteins

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WD40 Repeat (WDR) proteins comprise one of the largest protein families with ~350 WDR encoding genes in the human genome. Many WDR genes have strong genetic links to human diseases and are involved in a wide variety of cellular processes including epigenetics, ubiquitin signaling, DNA repair, RNA splicing, and immune system signaling (Schapira *et al, Nat Rev Drug Disc.* 2017). Despite the strong links to disease, an unlike other large target classes such as GPCRs or kinases, the WDR family is under-explored with respect to drug discovery. Based on our previous discoveries of chemical probes to modulate the WDR-containing epigenetic regulators WDR5 and EED, we hypothesized that the WDR protein family harbours many more druggable members, and set out to test this hypothesis by systematically evaluating a broad collection of WDR proteins, evaluating their amenability to several assays used in early drug discovery. As part of this strategy we employed a novel approach in which machine learning (ML) was applied to the output of DNA encoded library (DEL) screens to predict commercially available compounds that were likely to bind to the WDR protein of interest. This approach was previously demonstrated by McCloskey et al (*J. Med Chem* 2020) for a single member of each of the highly druggable protein kinase, hydrolase, and nuclear receptor target classes. Using the same approach, our results support the concept that many of the understudied WDR domain proteins are generally ligandable, and that DEL-ML is a feasible strategy to identify new WDR ligands.

Chemical Synthesis Enabled by Information.

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The invention of medicines by a design-make-test cycle of molecular ideation, chemical synthesis, and bioassay is influenced by the availability of robust chemical reactions such as the amide coupling. In an effort to expand access to chemical space, towards safer and more efficacious medicines, we have explored novel strategies for reaction invention, pursuing novel amine-acid coupling reactions that complement the classic amide coupling. Miniaturized high-throughput experimentation has been essential to the discovery and development of such novel amine-acid couplings. Additionally, the coupling of the design-make-test cycle wherein reaction mixtures are directly subjected to biochemical assays promises to further accelerate the design-make-test cycle. This presentation will highlight some of our recent work in using informatics tools and automation in chemical synthesis, towards a goal of the rapid invention of safe and selective medicines.

Computational hit finding: a long way to go

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To discover chemical modulators for all druggable human proteins by year 2035 (the mission of Target2035), more efficient hit-finding technologies need to be developed and validated. Given recent advances in machine learning, artificial intelligence is expected to enable a breakthrough in computational hit finding, as it did with alphaFold for protein structure prediction. To accelerate this process, the Structural Genomics Consortium is initiating a two-pronged approach. First, generate ultra-large, high-quality experimental screening data that can serve as training set for machine learning. Second, organize benchmarking challenges modeled after CASP to engage the AI community and identify the most promising computational methods. The status of these novel initiatives, preliminary results and lessons learned from the CACHE hit finding challenge will be presented.

Discovery and rational development of novel DCAF1 WD40 domain ligands

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DCAF1 (DDB1 and CUL4 Associated Factor 1) is the substrate recognition subunit of Cullin 4 RING ubiquitin E3 ligase (CRL4) complex that is involved in the host ubiquitin proteasome-mediated protein degradation pathway, and is implicated in various cellular processes including DNA replication, transcription, cell cycle progression among many others[1,2]. It has been identified as a driver of tumorigenesis and other disease processes[3,4] as well as a potential target to enable the proteasome-mediated degradation of therapeutic targets. DCAF1, a 1507 residue multi-domain protein utilizes its WD40 repeat (WDR) domain to recruit substrate proteins to the CRL4 E3 ligase complex; a key process we aim to modulate or exploit using small-molecule chemical probes for development of proteolysis-targeting chimeras (PROTACs).

We used two different approaches to discover small-molecule ligands targeting the WD40 domain of DCAF1: DNA-encoded library (DEL) screening combined with ZebiAI's Machine Learning (ML) technology[5] and a proteome-wide drug-target interaction prediction ML technology implemented in Recursion's MatchMaker[6]. Hits emerging from these efforts were tested experimentally using Surface Plasmon Resonance (SPR), which resulted in the identification of two chemical classes of DCAF1 binders. Co-crystallization studies coupled with structure-activity relationship efforts have led to the synthesis and development of more potent compounds from one of the chemical series, with binding affinities in the lower nanomolar range. Further extension of this hit to develop a PROTAC handle is underway.

Here, we report the discovery and development of novel small-molecule ligands targeting the WD40 domain of DCAF1 by combining DEL screening, ML, structural and biophysical tools.

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Representation Learning With Quantum Chemistry

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This work presents a novel approach that integrates deep learning techniques with quantum chemistry calculations to enhance molecular representation. By combining the strengths of both methodologies, we achieve more accurate and comprehensive molecular descriptors. These improved representations have demonstrated utility in predicting a wide range of downstream properties, including physical characteristics and structure-activity relationships in protein binding.

Artificial Intelligence in Drug Discovery – Revolution, Evolution, or Complete Nonsense

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Many have claimed that Artificial Intelligence (AI) will bring about a revolution in drug discovery and development, while others have argued that we are reaching the zenith of a hype cycle that will lead to a period of disillusionment. As is often the case, the truth lies somewhere between these extremes. This presentation will focus on areas where AI is having a real impact on drug discovery and highlight factors that are necessary to increase its utility.

Identification of Ligands for PET Imaging of Alpha-Synuclein in Parkinson's Disease

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Synucleinopathies are a family of neurodegenerative diseases characterized by the formation of fibrillar aggregates composed primarily of the neuronal protein α-synuclein, including Parkinson's Disease (PD), dementia with Lewy bodies, and multiple system atrophy (MSA). Positron emission tomography (PET) probes have proved invaluable for monitoring the progression of protein aggregation in Alzheimer's disease and for the development of first in class antibody therapeutics. Currently, there are no PET probes available to enable similar progress for PD and the other synucleinopathies. We have used a variety of computational methods, including structural modeling, ultra-high throughput virtual screening, and machine learning guided structure activity relationship analysis to identify several candidate PET ligands with 1-10 nM affinity for α-synuclein fibrils.[1,2] These ligands are being tested in tissue autoradiography, mouse disease models, and non-human primates to optimize them for human imaging in PD and MSA patients.[3,4]

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Leveraging Bioinformatics and Machine Learning in Biologics Drug Discovery Workflows

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The field of biologics drug discovery has witnessed remarkable advancements in recent years, driven in large part by the integration of bioinformatics and machine learning approaches. Biologics, including antibodies, enzymes, and gene therapies, represent a growing class of pharmaceuticals with immense potential for targeting complex diseases. This presentation explores the pivotal role of bioinformatics and machine learning in expediting the discovery and development of biologics through prediction of process/sequence risks, identification of critical sequence elements, and critical process parameters.

2023 ICBS Global Lectureship

Chemical Control of Cell Plasticity

Raphaël Rodriguez
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(no abstract)

Leveraging CRISPR genome editing for chemical genetic approaches at scale

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Chemical genetic approaches are powerfully enabling for biological discovery and therapeutics development. The identification of mutant alleles that enhance or suppress the activity of chemical probes may not only validate on-target mechanism but also drive deeper understanding of a small molecule's binding interactions, molecular mechanism of action, and downstream biological effects. Recent breakthroughs in CRISPR genome editing technologies enable the systematic mutation of endogenous proteins at scale and directly in cells, opening new research paradigms for chemical genetic approaches[1]. Exploiting these new technologies, we have developed and employed in situ CRISPR-mutational scanning approaches to systematically profile protein target(s) sequence-function relationships in their native cellular environment. When leveraged with chemical biology, these mutations in the target can be exploited as discovery tools to study small molecule mechanism of action and target biology, allowing us to uncover mechanisms of allosteric regulation, cell signaling, and cancer vulnerabilities.

We showcase these capabilities through two vignettes exploring the mechanisms of chromatin regulator complexes and the small molecules that target them. First, through the discovery of resistance alleles, we revise the mechanistic role of lysine-specific histone demethylase 1a (LSD1) in leukemia, highlighting how LSD1 active site inhibitors are serendipitously transcription factor inhibitors. By diving deeper into the mechanism of unexpected resistance mutations in the intrinsically disordered N-terminus of LSD1, we show how this region plays a key role in leukemia differentiation, revealing new principles of how unstructured domains can control protein-protein interactions. In the second vignette, we implemented base editor protein scanning to identify mechanisms that regulate DNA methyltransferase 3A (DNMT3A) activity. Utilizing base editor scanning, we have annotated the function of several DNMT3A clinical mutations and uncovered new allosteric mechanisms that govern catalytic activity, nominating potential protein sites for the development of small molecule agonists. I will then conclude by discussing potential future directions of CRISPR genome editing approaches for catalyzing chemical biology research.

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Understanding and Engineering Precision Genome Editing Tools

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Currently, less than 0.5% of identified human single nucleotide variants (SNVs) have a defined clinical interpretation. This represents a major barrier to precision medicine efforts, and new laboratory-based methods to clinically classify genetic variants are needed. Base editors (BEs) enable the programmable installation of point mutations with high efficiency and precision and are thus highly valuable tools for variant classification. Two major classes of BEs exist that convert C•G base pairs to T•A (CBEs) and A•T base pairs to G•C (ABEs). Here, I will describe the development and characterization of aptamer-derived CBEs and ABEs that can be multiplexed with each other orthogonally. These multiplexed orthogonal base editor (MOBE) systems are a valuable addition to the genome editing toolbox, and will aid significantly in efforts to study/model combinations of genetic variants, such as those found in haplotypes, polygenic disorders, and driver-passenger mutation pairs. I will also describe our efforts to characterize the cellular DNA repair factors that process CBE intermediates. This mechanistic information is in turn useful for the development of CBEs with enhanced efficiency and precision.

NSD2 is a requisite subunit of the AR neo-enhanceosome in promoting prostate tumorigenesis

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Abstract:

The androgen receptor (AR) is a ligand-responsive transcription factor that binds FOXA1-pioneered enhancers as a homodimer at palindromic DNA response elements. Prostate cancer (PCa) is highly dependent on the AR enhanceosome complex, and in castration-resistant disease, this dependency is reinforced through alterations in the AR pathway. This involves extensive redistribution of AR on the chromatin, away from its physiologic pro-differentiation sites, to gain *de novo* binding at cancer-specific enhancers (aka neo-enhancers) that activate hyper-proliferative and metastatic gene programs. However, essential subunits of the AR neo-enhanceosome and its exclusive target genes remain uncharacterized. Here, we CRISPR-engineered an endogenous AR reporter system by fusing the mCherry sequence in-frame with the *KLK3* gene and conducted a knock-out screen to identify druggable chromatin/epigenetic cofactors of AR. Ranked alongside BRD4 and TRIM24, we identified NSD2 as a novel AR coactivator. NSD2 is a histone 3 lysine 36 di-methyltransferase that activates gene expression. In PCa cells, inhibition of NSD2's catalytic function attenuated the expression of AR targets without affecting the abundance of AR or FOXA1. ChIP-seq profiles of AR in NSD2-deficient PCa cells showed its dramatic off-loading from over 40,000 genomic sites (>65% of the cistrome). Strikingly, motif analyses of the NSD2-dependent AR sites identified a marked enrichment of a hexameric 5′-AGAACA-3′ AR half-site juxtaposed to the FOXA1 DNA sequence. In contrast, NSD2-independent AR sites, a large fraction of which showed increased AR binding, housed the canonical 15-bp palindromic AR motif with two invertedly-oriented half-sites recognized by each half of the AR homodimer. Meta-analyses of primary AR cistromes from patient samples revealed AR loading at degenerate half-sites to be a defining feature of the tumor-specific neo-enhancer circuitry. Concordantly, NSD2-inactivation in PCa cells attenuated cancer hallmark phenotypes such as colony formation, invasion, hyper-proliferation, and *in vivo* grafting. Further, we found that NSD2 through its HMG domain interacts with the AR transcriptional complex, and that NSD2-deficiency engendered enhanced dependency on its paralog NSD1 in PCa cells. To therapeutically exploit these findings, we developed a first-in-field dual NSD1/2 PROTAC, called LLC0150. This compound showed preferential cytotoxicity in AR/FOXA1+ PCa relative to the AR-negative disease, as well as other cancer and normal cell lines from 22 distinct lineages. In a pan-cancer screen comprising over 120 cell lines, treatment with LLC0150 triggered apoptotic death in AR/FOXA1+ tumors, notably including NSD2-driven acute lymphocytic leukemia and multiple myeloma. Altogether, we identify NSD2 as a novel AR neo-cofactor that assists FOXA1 in loading the AR enhanceosome at degenerate, low-affinity AR motifs, thereby wiring its oncogenic gene programs. Furthermore, we develop a dual NSD1/2 PROTAC that selectively kills AR-driven prostate and NSD2-altered tumors, positioning NSD-targeted agents and novel tools of cancer therapy.

Enabling Technologies for Revealing the Druggability of RNA-Protein Interactions

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RNAs are invariably bound to and often modified by RNA-binding proteins (RBPs), which regulate many aspects of coding and non-coding RNA biology. Disruption of this network of RNA-protein interactions (RPIs) has been implicated in a number of human diseases and targeting RPIs has arisen as a new frontier in RNA-targeted drug discovery. This talk will highlight newly developed technologies by the Garner laboratory for validating and screening RPIs to enable RBP-targeted drug discovery.

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Rethinking the Paradigm: New roles for miRNA in controlling the proteome and glycome

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microRNAs (miRNAs, miRs) are small non-coding RNA that tune protein expression through modulation of mRNA. The canonical view of miRNAs is that they are posttranscriptional repressors, binding to the 3'-untranslated region (UTR) of mRNA and causing mRNA destabilization and/or loss of translation. Using our recently developed high-throughput fluorescence assay (miRFluR)[1], we have comprehensively mapped the miRNA regulatory landscape of multiple proteins, including glycosylation enzymes[2-3], and discovered, contrary to expectations, miRNA predominantly upregulate protein expression of some messages. Our results upend common assumptions surrounding miRNA, arguing that upregulation by these non-coding RNA is common. Here I discuss my laboratory's latest work on miRNA-mediated protein upregulation and underlying mechanisms.

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Targeting RNA-binding proteins and RNA modification with small-molecule inhibitors

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The pervasive interactions between RNAs and RNA-binding proteins (RBPs) regulate all aspects of RNA biology and metabolism. Dysregulation of the interplay between RNAs and RBPs is closely associated with diseased cellular states, leading to a wide range of human disorders. Consequently, the identification of small molecules targeting either RBPs or structured RNAs has emerged as an attractive strategy for the development of biological probes and therapeutic candidates. Whereas small molecules have been reported for different RNA species with varied structured elements, the majority of RBPs remain challenging targets to be addressed by small molecules, partially due to the lack of a classical deep ligand-binding pocket and corresponding discovery approaches. Therefore, we aim to develop RBP-targeting small-molecule modulators by using a multifaceted approach combining screening and rational design methods for chemical biology and medicinal chemistry research. In this talk, I will present our latest results in the identification of such small molecules by targeting RNA-binding proteins involved in the recognition and installation of the m6A RNA modification, which is the most abundant and conversed RNA modification in eukaryotes. The identified first-in-class small-molecule inhibitors targeting m6A-binding and modifying proteins offer an opportunity to study protein–RNA interactions and RNA metabolism at the post-transcriptional level.

Investigating Biological Targets Using Functional Small Molecules and Biomimetic Systems

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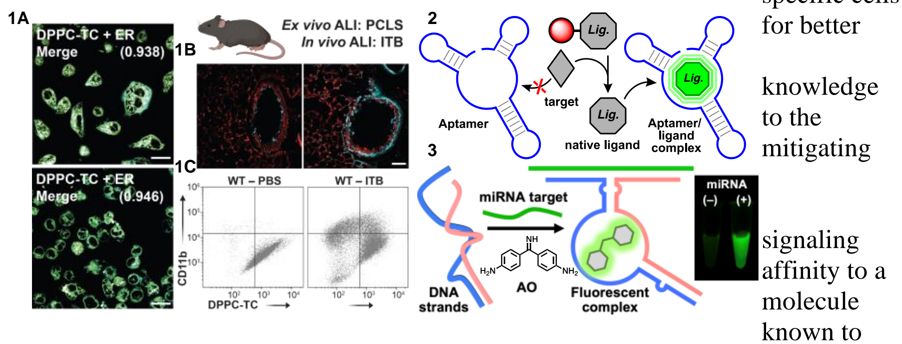
Selectivity, adaptability, and biostability are key challenges that limit the potential of molecular tools designed for biosensing and therapeutics. A major goal in our lab is to address these challenges by developing sophisticated design criteria for functional small molecules and nucleic acid polymers that interact with (patho)physiologically significant targets or interfaces.

Nitrative Stress on Lipid Membranes and Surfactants: Nitrative stress, a critical redox condition characterized by the presence of peroxynitrite ($ONOO^-$), can alter the integrity of biomolecules and its dysregulation is associated with a number of human pathologies (1). We have developed a membrane-localized phospholipid (**DPPC-TC-ONOO**$^-$) that allows, *for the first time*, the high-fidelity detection of nitrative stress in diverse lipid environments and interfaces: biomimetic vesicles, cell compartments, and within the lung lining (Fig. 1) (2). Real-time confocal imaging demonstrated substantial fluorescence enhancement of the ER in HeLa and RAW cells under nitrative stress induce d through endogenous stimulation (Fig. 1A). Further, we successfully demonstrated the biological potential of **DPPC-TC-ONOO**$^-$ through murine models of *ex vivo* and *in vivo* acute lung injury (ALI). We observed iNOS-dependent nitrative stress around bronchioles in precision-cut lung slices (Fig. 1B) and in pulmonary macrophages (Fig. 1C) following ALI. Assessment of nitrative stress pathways in specific cells and tissue environments opens the opportunity for better understanding lung homeostasis and inflammation resolution. In the long term, knowledge gained from these investigations could contribute to the development of therapeutic approaches for mitigating lung injury and pulmonary fibrosis.



Advancing the Application Space of Aptasensors: Many biological targets (e.g., signaling molecules, metabolites) have no fluorogenicity or affinity to a known aptamer. Recently, we developed a small-molecule approach that allows a single RNA aptamer, known to bind a specific organic ligand, to detect structurally diverse and non-fluorogenic inorganic targets (Fig. 2) (3). We successfully adapted this method to *E. coli* competent cells (BL21-DE3), which transformed them into cell-based biosensors. This approach substantially expands the capabilities of aptamers for diagnostics and synthetic biology.

In addition, we studied the detection of miR-21, miR-19b, and miR-92a, which are closely associated with dysfunctional physiology, especially cancer (Fig. 3) (4). MiR-21 downregulates tumour-suppressing proteins (5), and miR-19b and miR-92a are part of the oncogenic miR-17/92 cluster, which promotes Myc-dependent tumour growth (6). All there miRNAs are upregulated in a number of cancer types. However, each has a hybridization characteristic or secondary structure that challenges the design of a typical aptasensor or molecular beacon. We developed split DNA aptamers that enhance fluorescence of 4,4'-(iminomethylene)bis(*N*,*N*-dimethylaniline) (AO) in the presence of the miRNA target. With minimal sample handling, we achieved single-nucleotide specificity in the medium collected from cultured HeLa cells, human serum, and plasma.

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Human Pluripotent Stem Cell, Organoids and Drug Screening

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Organoids are miniaturized, in vitro-grown organs that closely resemble the functional, structural, and biological complexity of in vivo organs. They can be derived from either human pluripotent stem cell (hPSCs) or adult tissues. Notable, hPSC-derived organoids offer enhanced complexity and scalability compared to those derived from adult tissue-derived organoids. In the early years of my scientific career, we conducted high throughput/content chemical screens and identified several small molecules promoting hPSC differentiation toward pancreatic β cells, pancreatic ductal epithelial cells, cardiac pacemaker cells, colon organoids, and more. We established an hPSC platform encompassing over 14 types of hPSC-derived cells and organoids, including endoderm derivatives, such as pancreatic endocrine, colonic, small intestinal, alveolar, airway, liver organoids, mesoderm derivatives, such as cardiomyocytes, endothelial cells, pacemaker cells, macrophages, etc, and ectoderm derivatives, such as cerebral organoids.

We are interested in applying this multi-organoid platform to study common diseases. Compared to traditional in vitro models, human organoids more accurately recapitulate the intricate cellular components and three-dimensional tissue architecture, making them increasingly popular for modeling human diseases. While most previous organoid-based disease modeling has focused on rare diseases caused by single gene/mutation, common diseases affect entire populations, involve multiple organs, and are associated with complex genetics. In our previous studies, we have used this multi-organoid platform to demonstrate the key concepts of applying organoid to study common diseases, including isogenic hPSCs to study the individual genetic variants identified from population-based genome-wide associate studies (GWAS), induced pluripotent stem cell (iPSC)-based GWAS, multiple organoids models to examine organ/tissue-specific host response, and immune-host mini-tissue to mimic the immune cell-mediated host response. Our current focus is on applying the hPSC-derived organoid platform to systematically investigate the impact of genetic and environmental factors on disease progression, with the aim of developing precision medicine for common diseases.

Patient-Derived Micro-Organospheres Enable Precision Oncology

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Patient-derived xenografts (PDX) and organoids (PDO) have been shown to model clinical response to cancer therapy. However, it remains challenging to use these models to guide timely clinical decisions for cancer patients. Here we used droplet emulsion to rapidly generate thousands of Micro-Organospheres (MOS) from low-volume patient tissues, which serve as an ideal patient-derived model for clinical precision oncology. A clinical study of newly diagnosed metastatic colorectal cancer (CRC) patients using a MOS-based precision oncology pipeline reliably predicted patient treatment outcome within 14 days, a timeline suitable for guiding treatment decisions in clinic. Furthermore, MOS capture original stromal cells and allow T cell penetration, providing a clinical assay for testing immuno-oncology (IO) therapies such as PD-1 blockade, bispecific antibodies, and T cell therapies on patient tumors. Lastly, we demonstrate an ultra high-throughput MOS screening platform that provides "virtual clinical trials" to capture patient diversity for determining drug efficacy.

Identifying drugs effective to target head and neck squamous cell cancer using a drug repurposing library on organoids.

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Advanced stage head and neck squamous cell carcinoma (incidence >700.000 yearly worldwide) is generally treated with surgery combined with adjuvant (chemo)radiotherapy.1 Despite these interventions, up to 60% of patients relapse or do not qualify for treatment due to inadequate fitness. Hence, therapeutic alternatives are urgently needed for these patients. Organoids have been shown to recapitulate the patients' tumor and treatment response.2,3 Hence, we conducted a high-throughput drug repurposing screen (> 5,600 compounds) in patient-derived head and neck squamous cell carcinoma (HNSCC) organoids and identified novel potential clinical candidates, which we validated for their efficacy, tumor selectivity, mode-of-action and toxicology profile. We identified two final hits with extraordinary sensitivity differences between normal and tumor organoid lines from 4 and 10 distinct donors, respectively. The final hits are tested in MicroOrganoSpheres™, which are generated directly from tissue and include cancer-associated fibroblasts, tumor organoids and immune cells, to estimate the potential cancer selectivity.4 Ultimately, we aim to conduct clinical studies in HNSCC. Drug repurposing screens have been described in patient-derived organoids before.5,6 However, to our knowledge, this is the largest drug screen ever reported in patient-derived organoids.

From initial drug and compound screens to mode-of-action investigations, toxicology studies and pharmacokinetic predictions, success stands and falls with the right *in vitro* models. The era of stem cell-derived organoid models has the potential to increase clinical relevance for all of these research areas, which could accelerate successful drug development and reduce failure of potential drugs in clinical stages.

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Neo-protein-protein interactions as a new dimension of cancer genome: discovery and intervention

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Oncogenic mutations represent cancer-specific features that offer unprecedented opportunities for mutation-directed therapeutic strategies. Here we describe the discovery of prevalent variant-enabled neo-protein-protein interactions (neoPPI) and mutation-disabled hypo-PPIs. Analysis of the established neoPPI network suggests unique oncogenic re-wiring mechanisms supported by chemical synthetic lethal studies, revealing the BRAFV600E-KEAP1 signaling axis. On the other hand, mutation-disabled hypo-PPIs offer opportunities to develop mutation-specific molecular glues. A case study with mutated SMAD4 will be presented to illustrate our systematic approach to identify small molecule glues, which can restore the PPI and re-activate its lost function. Our studies highlight a functional dimension of the cancer genome to inform variant-directed therapeutic strategies for precision medicine.

Deciphering the chemical crosstalk of host-gut microbiota interactions

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The metagenome of the gut microbiome encodes tremendous potential for the biosynthesis and transformation of small-molecule metabolites through the activity of many enzymes expressed by the intestinal bacteria. The metabolic activity of this gut bioreactor provides numerous important functions for the host, including breaking down indigestible components of our diet, biosynthesizing essential vitamins and nutrients, and regulating the development of our immune system. Accordingly, elucidating the metabolic potential of the multitude of potential enzymatic reactions is critical for understanding how the activities of the gut microbiota contribute to human health and physiology. Therefore, there is a critical need for new technologies that can determine the enzymatic activities of the gut microbiome because these activities cannot be directly determined by existing metagenomic approaches used to profile microbial taxonomy by genetic content. Activity-based probes, which are mechanism-based covalent tags for enzyme active sites, represent a powerful chemical approach for directly identifying and profiling enzymatic activities. Here, we have developed activity-based probes for a class of cysteine proteases known as bile salt hydrolases (BSHs), which are gate keeper enzymes of gut microbial transformation of host-derived, or primary, bile acids into a large and diverse group of secondary bile acids. These important metabolites regulate myriad host biological processes, including metabolism, gut barrier function, and immune homeostasis. In addition, changes in bile acid levels are known to accompany different physiological states. However, the role of BSH activity in these processes remains poorly understood due to the lack of adequate tools to address its function. To address this critical gap in knowledge, we have applied our chemical approach to profile BSH activity in gut microbiomes using healthy and diseased samples.

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DrugMap: A quantitative pan-cancer analysis of cysteine ligandability

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Cysteine-focused chemical proteomic platforms have expanded the repertoire of protein targets for covalent inhibitors. However, it remains unknown how cysteine targeting varies across multiple cancer contexts. To address this question, we compiled an atlas of cysteine ligandability across 417 cancer cell lines. We unexpectedly find that cysteine ligandability fluctuates across cancers, which we attribute to differences in cellular redox state, protein expression and mutation. Focusing on lineage-restricted liganding, we identify a highly actionable cysteine in SOX10, leveraging this information to develop covalent inhibitors that disrupt the transcription factor. We find the SOX10 ligand functions as a covalent molecular glue and disrupts transcriptional activity in a dominant negative manner, leading to inhibition of melanoma proliferation. Our findings reveal pervasive heterogeneity in cysteine ligandability across cancer, pinpoint cell-intrinsic features driving cysteine targeting, and exemplify the use of covalent probes to disrupt oncogenic transcription factor activity.

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A chemical probe to modulate human GID4 Pro/N-degron interactions

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Protein turnover is an incompletely understood aspect of biology, important for various processes including adaptation to environmental stimuli. In *Saccharomyces cerevisiae*, glucose triggers ubiquitination and proteasomal degradation of gluconeogenic enzymes which bear N-terminal Proline degron sequences (Pro/N-degrons)[1]. The Pro/N-degrons are recognized by a β-barrel substrate binding pocket in Gid4, a substrate receptor for the GID/CTLH multi-subunit E3 ligase[2,3]. Though GID4 and this E3 ligase complex are evolutionary conserved, the targets of the Pro/N-degron pathway beyond *S. cerevisiae* remain undefined. Here, we report PFI-7, a potent, selective, and cell-active chemical probe that binds in the human GID4 β-barrel pocket to antagonize Pro/N-degron binding[4]. Use of PFI-7 in proximity-dependent biotinylation proteomics enabled the identification of dozens of endogenous GID4-interacting proteins that bind via the GID4 substrate binding pocket. GID4 interactors, including those with canonical Pro/N-degron sequences, are enriched for nuclear and nucleolar proteins including RNA helicases. GID4 antagonism by PFI-7 altered protein levels of several proteins including RNA helicases as measured by label-free quantitative proteomics. Together, PFI-7 and the proteomic datasets profiled the Pro/N-degron pathway in humans, which revealed insights into GID4 and CTLH complex biology and their potential use for targeted protein degradation. Overall, the work demonstrates the utility of combining chemical probes and proteomics for biological discovery which we will continue to exploit going forward.

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1. Using CDD Vault for Data Management as Part of Interdisciplinary Research Projects

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As the science field advances and evolves, the need for platforms to efficiently organize and manage data becomes increasingly relevant. As a company, Collaborative Drug Discovery (CDD) started out as a way for chemists and biologists to share data easily as part of the classical model of drug discovery projects. In the current state, "drugs" are starting to look less like small molecules and start to resemble entities that are larger and cross into the realm of biologics. Tracking structure activity relationships of biologics is just as important as that of small molecule medicinal chemistry.

Collaborative Drug Discovery (CDD) provides a whole solution for today's biological and chemical data needs, including the ability to register many different types of entities like DNA, RNA, and amino acid sequences. CDD Vault® is differentiated from other platforms by ease-of-use and superior collaborative capabilities. The software includes Activity & Registration, Visualization, Inventory, and ELN capabilities. Researchers can archive, mine, and securely collaborate within CDD Vault. Collaborative hypothesis generation and evaluation allow multiple perspectives for multi-parameter optimization.

2. Light Controlled Reversible Michael Addition of Cysteine: A New Tool for Dynamic Site-Specific Labeling of Proteins

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The use of cysteine-based Michael addition is a popular method for covalently bonding proteins, peptides, and drugs together. However, this reaction is typically irreversible, making it difficult to control. Our team conducted spectroscopic analyses and X-ray crystallography to demonstrate how an engineered cysteine residue in human Cellular Retinol Binding Protein II (hCRBPII) can undergo Michael addition with a coumarin analog, resulting in a non-fluorescent complex. By using UV-illumination, the conjugation can be reversed, yielding a fluorescent species through a retro-Michael process. This process can be repeated between a bound and non-bound form of the cysteine, allowing for ON-OFF control of fluorescence. We were able to confirm the mechanism by recreating the process in light-irradiated single crystals.

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3. Harnessing Encapsulins for Intracellular Organometallic and Enzymatic Catalysis

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Subcellular compartmentalization is a fundamental strategy used by all organisms to enable regulation and fine-tuning of metabolic processes. Cells utilize compartmentalization to create specific microenvironments, segregate incompatible biochemical reactions and pathways, or protect themselves from toxic by-products [1]. Encapsulins (Enc), self-assembling protein-based bacterial nanocompartments, are a highly attractive platform for enzyme compartmentalization. Encapsulins are temperature and pH-stable [2] and promote highly selective packaging of co-expressed proteins through a conserved amino acid sequence (encapsulin localization sequence, ELS) at their C-terminus [3–5]. Non-native cargo proteins can be directed inside Enc via simple tagging with ELS, enabling self-assembly of the new functional compartment [3–5].

In this study, we describe the first targeted incorporation of an organometallic catalyst to the interior of Enc from *Mycobacterium smegmatis* (Enc_{SM}) enabled by a HaloTag-ELS construct (Enc_{SM}{HaloTag}). The approach is versatile, as the HaloTag-ELS strategy enables the introduction of many different metal/ligand systems new to biology, provided they operate in water. We explore, as a proof of concept reaction, the ruthenium-catalyzed deallylation of a profluorophore inside the Enc nanoreactor.

Upon installing a transition metal catalyst inside the capsule, we show that catalysis inside this capsule is possible and that encapsulation affects reaction yields. The deallylation of a profluorophore takes place with lower yields with the HaloTag-PEG_3-Ru construct outside of the capsule compared to within encapsulin. We go on to demonstrate that the same reaction is also efficiently catalyzed inside a living cell line that takes up the engineered Enc and produces localized fluorescent vesicles [6].

To investigate enzyme catalysis in Enc_{SM} nanocompartment, we explore encapsulation of nitroreductase NfsB from *E. coli* (NTR). The combination of encapsulated NTR with nitroaromatic prodrugs is a promising approach in enzyme prodrug therapy by minimizing toxicity to healthy cells and increasing the concentration of drugs against cancer cells.

Since NTR is an obligate dimer, we show that a tandem-dimer configuration (tdNTR), where NTR monomers are connected by a floppy 22-residue linker, enhances enzyme stability [8] and ensures correct packaging of the enzyme inside the nanocompartment, retaining its activity. In vitro prodrug activation is confirmed by LC/MS analysis after incubation with free and encapsulated tdNTR (Enc_{SM}{td-NTR}).

In line with this, extracellular prodrug activation proves that Enc_{SM}{td-NTR} and 1-(4-Nitrobenzoyl)piperidine prodrug are a promising combination and prodrug activation leads to a decrease in cell viability. On the other hand, in cellulo prodrug activation is not sufficient for a significant decrease in cell viability.

A performed co-localization study shows that the taken-up Enc particles are found together with Lysotracker dye. Therefore, we hypothesize that the encapsulated enzyme is trapped in lysosomes, where it cannot activate the prodrug, as the required co-factor NADH is not present in the lysosomal environment. Surface modification/optimization of Enc_{SM} shell is necessary to influence the cellular fate of nanoparticles and direct the encapsulated enzyme to the cytosol, where it can unravel its action.

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4. Epichaperome imaging probes for precision medicine in Alzheimer's disease

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Alzheimer's disease (AD) is a multifaceted disorder influenced by a convergence of genetic, epigenetic, and environmental factors, resulting in personalized alterations to brain circuitry and cognitive decline. The identification of therapeutic targets for halting AD progression remains challenging due to the intricate involvement of multiple pathological pathways. Our interdisciplinary team has recently unveiled a crucial link: stressors and vulnerabilities associated with AD impact protein-protein interaction (PPI) networks via specialized structures known as epichaperomes. This discovery offers a central mechanism that unifies the spectrum of AD pathology.

Epichaperomes, long-lived formations uniquely found in afflicted cells, consist of chaperones and other components. Their emergence disrupts essential protein interactions within cells, exerting detrimental effects on neural and cerebral function. Elevated epichaperome levels correlate with more pronounced disruptions in molecular interactions within affected cells, influencing critical AD-associated biological processes including synaptic plasticity, intercellular communication, protein translation, cell cycle re-entry, axon guidance, metabolism, and inflammation. Promisingly, preclinical investigations reveal that targeted intervention with small molecules capable of dismantling epichaperomes restores protein network connectivity and cognitive function to baseline levels. This work establishes epichaperomes as a promising avenue for rectifying functional imbalances linked to AD. Notably, these structures manifest specifically in cells exposed to chronic stressors associated with AD onset and progression, rendering them amenable to imaging and targeting using purpose-designed small molecule probes. However, ensuring selectivity for pathogenic chaperone assemblies (epichaperomes) over physiological chaperone assemblies poses challenges due to the abundance and resemblance of chaperones compared to epichaperomes.

We will present our endeavors in the creation, synthesis, and characterization of small molecule epichaperome probes. Through a spectrum of biochemical and functional assays encompassing in vitro and in vivo experiments, including investigations in both murine and human models, we demonstrate the kinetic selectivity of small molecule HSP90 binders. This selectivity effectively distinguishes the minute fraction of HSP90 residing in epichaperomes from the prevalent HSP90 pools distributed throughout the cell and body. Additionally, we will present evidence derived from murine models, elucidating how an epichaperome imaging probe can unveil region- and age-dependent epichaperome formation within disease-relevant brain regions. Moreover, we outline results from a preliminary clinical feasibility study showcasing the feasibility of imaging and quantifying epichaperomes in human patients through PET scans.

In conclusion, epichaperome imaging tools offer significant diagnostic potential for AD. When amalgamated with anatomical and other neuroimaging techniques, as well as plasma biomarkers, these tools hold promise for diagnosing and quantifying molecular changes underpinning functional deterioration in the AD-afflicted brain, even preceding tau and amyloid pathologies. Additionally, they can serve as indispensable companions in the development of epichaperome-targeting therapeutics, aiding in patient selection, real-time monitoring of target engagement by epichaperome disruptors, and quantitative assessments to optimize dosing and treatment regimens.

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5. An activity-based covalent photoacoustic imaging probe targeting NCEH1 for in vivo tumor imaging

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Photoacoustic (PA) imaging is an emerging modality in biomedical imaging with superior imaging depth and specificity. However, PA imaging still has some obstacles, such as the background noise from endogenous chromophores. To overcome these limitations, we developed a covalent activity-based photoacoustic imaging probe, **NOx-JS013**, targeting the cancer-associated serine hydrolase NCEH1. NCEH1, a highly expressed and activated serine hydrolase in aggressive cancers, has the potential to be employed for the diagnosis of cancers. We show that **NOx-JS013** labels active NCEH1 in live cells with high selectivity relative to other serine hydrolases. **NOx-JS013** also successfully visualizes tumor regions in mouse models of PC3, which is aggressive prostate cancer, while negligibly detected in tumors of non-aggressive LNCaP mouse models. These findings indicate that **NOx-JS013** presents an exciting opportunity to develop a new class of precision PA imaging reagents that report on a marker of metastatic progression in several cancers.
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6. Chemical biology approaches to target the "undruggable" SMAD4 protein

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Transforming growth factor-beta (TGFβ) signaling plays fundamental roles in numerous physiological processes, including embryonic development, tissue differentiation, and homeostasis. Dysregulation of the TGFβ pathway has been closely associated with multiple diseases such as cancer, fibrosis, inflammation, autoimmune diseases, Juvenile Polyposis Syndrome, and Myhre syndrome. Therefore, the TGFβ pathway presents a promising therapeutic target. Currently, therapeutic approaches focus on inhibiting upstream TGFβ and its receptors through neutralizing antibodies, receptor kinase inhibitors, and antisense oligonucleotides for the treatment of cancer and fibrosis. However, no therapeutic agents have received FDA approval. Herein, we present our versatile chemical biology discovery platforms aimed at targeting the SMAD4 protein to expand the existing repertoire of anti-TGFβ therapeutic options. SMAD4, a transcriptional master regulator downstream of TGFβ signaling, forms a heterotrimeric complex with receptor-regulated SMADs, such as SMAD3, and translocate to the nucleus to regulate gene expression for tissue-specific biology. First, we have identified a panel of small molecules functioning as molecular glues that can induce interaction between the mutant SMAD4-SMAD3 complex. These molecular glues not only stabilize the SMAD4 R361H/C mutant interaction with SMAD3 but also restore tumor suppressive TGFβ signaling in cancer cells harboring SMAD4 mutations. These results demonstrate the potential development of mutant SMAD4-SMAD3 PPI molecular glues for novel personalized anti-cancer agents in early-stage pancreatic and colon cancers. Second, we have identified a panel of natural products as inhibitors for wildtype (WT) SMAD4 that can disrupt the SMAD4-SMAD3 PPI. We found that gambogic acid and gambogenic acid can potently inhibit the WT SMAD4-SMAD3 PPI, blocking TGFβ pathway activation and mitigating TGFβ-mediated cancer metastasis. These results suggest novel therapeutic strategies using SMAD4 inhibitors to suppress the TGFβ pathway in advanced metastatic cancer. Last but not least, both SMAD4 molecular glues and inhibitors serve as promising small molecule SMAD4 binders, enabling us to further develop SMAD4-targeted proteolysis targeting chimeric (PROTAC) chemical probes to explore therapeutic opportunities through SMAD4 protein degradation. In summary, our proof-of-concept studies demonstrate the feasibility of developing small molecules to targe the "undruggable" SMAD4 protein which may enable us to target pluripotent TGFβ pathway with the expanded arsenal of SMAD4-targeted chemical probes.

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7. Creation of Novel Proteinaceous Shell Structures through Molecular Evolution of Bacterial Microcompartment (BMC) Shell Proteins

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Proteinaceous shell structures are ubiquitous in nature. They are used for various biological phenomena such as transportation, storage, and production of the specific biomolecules. For example, viruses deliver nucleic acids, ferritins store iron, and bacterial microcompartments (BMCs) encapsulate enzymes which are responsible for various metabolic pathways. BMCs are organelle-like structures widely distributed in majority of bacterial phyla[1] and are thoroughly made of proteins. BMC shell structures are known to be self-assembled from thousands of shell proteins, which are subdivided into hexameric, pseudohexameric, and pentameric proteins[2], however, it is relatively recent that the first intact shell structure of a small (~40 nm) BMC was determined at an atomic resolution[3]. In general, sizes of BMCs are extremely large (ranging from 100 to 400 nm), and the entire shell structures are polyhedral but not necessarily symmetric. Thus, despite availability of the diverse sets of genes encoding BMC shell proteins from many bacterial genomes, it is still challenging to reconstruct the uniform and defined shell structures *in vitro*, and this limits the potential applications using BMC shell proteins in synthetic biology and medicine such as creation of artificial organelles and development of vaccines and drug delivery systems etc. To overcome this problem, we aimed to develop easily reconstructed uniform artificial shell structures consisting of a single BMC shell protein through the process of molecular evolution.

Among many BMC shell proteins, we selected two permuted hexameric proteins, EutS from ethanolamine utilization microcompartment and PduU from propanediol utilization microcompartment found in *Salmonella enterica*. Based on the previous studies[4,5], we predicted that permuted hexameric BMC shell proteins might have potential to form shell structures by themselves. To prove this hypothesis, we applied molecular evolution technique, which was previously developed for evolution of lumazine synthase shell structures[6,7], to the permuted hexameric proteins, EutS and PduU, to alter their self-assembled states into symmetric shell structures.

First, we replaced three basic amino acids on the surfaces of EutS and PduU with negatively charged glutamic acids to make them interact with a toxic protein (HIV-protease) possessing a positively charged polyarginine tag by electrostatic interactions. The resulting variants, named EutS-neg and PduU-neg, were further introduced random mutations throughout the sequences using error-prone-PCR. Second, the resulting shell protein libraries were co-expressed with the toxic HIV-protease in *E. coli*. If the shell proteins form the closed shell structures along with interacting with the toxic protease, they may sequester the protease in the shells and provide growth advantage to the host cells. We applied different selection pressures by controlling the expression levels of HIV-protease in *E. coli* and extracted the plasmids encoding the evolved shell proteins from the survived cells. After three rounds of the evolution, we analyzed the evolved shell protein sequences and selected two PduU variants, PduU-1 and PduU-2, for further characterization. We confirmed that (i) PduU-1 and PduU-2 exhibited the growth advantage over PduU-neg when co-expressed with HIV-protease, (ii) PduU-1 and PduU-2 were stable when heated to 50-80°C, and (iii) PduU-1 and PduU-2 purified by size-exclusion chromatography showed spherical shell structures ranging from 25 to over 120 nm in diameter, but most particularly ~30, ~50, and ~70 nm in diameter, when subjected to transmission electron microscopy (TEM) analysis.

In summary, we succeeded in creating artificial shell structures composed of a single BMC-derived protein through three rounds of molecular evolution. Taking advantage of the flexible and scalable nature of the PduU-derived shells, it may be useful to create custom-made shells that match the size and shape of the cargo molecules for various biological applications in future.

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8. α-N-methylation of Protein Arginine Deiminase 1 (PAD1) Affects its Stability and Protein-protein Interactions

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α-N-terminal (Nα) methylation is catalyzed by N-terminal methyltransferases (NTMT1, NTMT2, METTL13), featuring the transfer of 1-3 methyl group(s) from S-adenosyl-L-methionine (SAM) to the α-N-terminus of substrate proteins. Nα methylation modulates protein cellular distribution, protein stability, protein-protein and protein-DNA interactions in diverse biological systems.[1–4] NTMT1/2 recognizes an N-terminal sequence X-P-K/R (X represents any amino acid other than D/E).[5] Compared to the number of substrates, the function of protein Nα methylation remains largely enigmatic. Protein arginine deiminase 1 (PAD1) catalyzes citrullination, converting the guanidine group on the side chain of arginine to an ureido group.[6] PAD1 possesses an extended N-terminal tail starting with APKR following the cleavage of the initial methionine, conforming to the consensus recognition motif of NTMTs (XPK/R). Here we reported the identification and characterization of Nα methylation of PAD1. We demonstrated that the α-N-terminus of PAD1 is methylated by NTMT1 *in vitro* and human HEK293 cells. Around 30% of transfected PAD1 undergoes Nα methylation in HEK293 cells. In addition, WT-PAD1 displayed extended protein stability compared to a methylation-defective mutant of PAD1. Besides, Nα methylation of PAD1 resulted in decreased protein-protein interactions based on the pull-down assay. Our results also showed that Nα methylation affects neither the enzymatic activity nor the cellular distribution of PAD1, though the N-terminal tail was reported to be essential to its activity. Our study provides new knowledge about the posttranslational regulation of PAD1 and expands the biological functions of protein α-N-methylation.

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9. Design strategy to control the emission of fluorophores via a twisted intramolecular charge transfer (TICT) process

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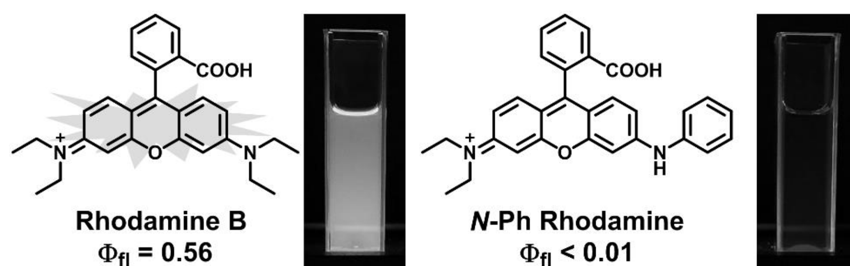
Fluorogenic probes for bioimaging have become essential tools for life science and medicine, and the key to their development is a precise understanding of the mechanisms available for fluorescence off/on control, such as photoinduced electron transfer (PeT) and Förster resonance energy transfer (FRET). Rhodamine dyes are one of the most widely used fluorescent dyes for bioimaging because they have high fluorescence quantum yield and high photostability. In contrast, *N*-Ph rhodamine dyes, which are rhodamine derivatives conjugated with the phenyl moiety at N atoms of 3- or/and 6-position of the xanthene moiety, are known to be non-fluorescent. The application of *N*-Ph rhodamine dyes has been limited to the usage as fluorescence dark quenchers, and the mechanism of the nonradiative decay of *N*-Ph rhodamine dyes has not been fully elucidated. However, some fluorescence probes based on *N*-Ph rhodamine dyes such as RNA aptamer probe and ROS probe were recently reported. So, we tried to analyze the quenching mechanism of *N*-Ph rhodamine dyes and further develop novel fluorescence probes rationally based on this mechanism. First, the energy minimized structure of *N*-Ph rhodamine dyes in the excited state was calculated by TD-DFT method. As a result, it was strongly suggested that the formation of the twisted intramolecular charge transfer (TICT) state is related to the fluorescence quenching of *N*-Ph rhodamine dyes. Then, we synthesized various derivatives of *N*-Ph rhodamine dyes, and the formation of the TICT state of *N*-Ph rhodamine dyes was also supported by their photophysical properties. Based on these results, we established a new molecular design strategy to rationally develop fluorescence probes, which exhibit a fluorescence off/on change in response to target biomolecules, by controlling the twisted intramolecular charge transfer (TICT) process. To illustrate and validate this TICT-based design strategy, we developed fluorescence probes for HaloTag and SNAP-tag. The fluorescence intensity of the probes increased 17 or 25 times by the reaction with HaloTag or SNAP-tag, respectively. Further, we succeeded to visualize the expression of HaloTag and SNAP-tag on cell surface by means of fluorescence confocal microscopy without washing out unreacted excess probes. We further showed that the TICT-controlled fluorescence off/on mechanism is generalizable by synthesizing a Si-rhodamine-based fluorogenic probe for HaloTag, thus providing a palette of chemical dyes that spans the visible and near-infrared range.



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10. Identification of small molecule-enhancers of natural killer cell cytotoxicity

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Natural Killer (NK) cells have the unique ability to sense and eradicate malignant cells without prior sensitization, making them crucial for the mammalian first-line defence against tumor development. Cancer cells and the surrounding supportive tissue within the tumor microenvironment (TME) promote tumor growth by co-opting immune inhibitory pathways and via secretion of immunosuppressive factors such as kynurenine, prostaglandin E2 (PGE-2), and transforming growth factor β (TGFβ). This allows cancer cells to become less immunogenic, which supports the escape of immune cell-mediated cytotoxicity. Many strategies are currently being developed to harness the anticancer potential of NKs and overcome resistance of cancer cells. However, previous clinical studies using NK cells to treat diverse malignancies had mixed outcomes due to lacking efficacy. For this reason, novel strategies to enhance and preserve NK cell tumoricidal activity within the TME are demanded. To harness the power of NKs, we developed a phenotypic co-culture-based approach that mimics the TME using primary lymphocytes. This medium-throughput assay enabled the identification of small molecules that revert the immunosuppressive effect of TME and thereby restore NK cell-mediated cytotoxicity.

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11. Designing an Engineered Biosynthetic Pathway to Access Complex Diketopiperazines

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For decades, researchers have investigated the fungal-derived natural product family of diketopiperazine indole alkaloids with bicyclo[2.2.2]diazaoctane cores because of their diverse biological activities and the intriguing chemistry needed to generate these metabolites.[1-3] However, they can be challenging to access from fungal strains due to their low yields, and synthetically because of their structural complexity and multiple stereocenters.[1,4] A new study by our group shows that introducing a substrate to two flexible biosynthetic enzymes derived from unrelated biosynthetic pathways can produce the desired diketopiperazine precursor.[5] By extending this one-pot biocatalytic cascade in *Escherichia coli*, endogenous and commercially available precursors can be employed as starting materials in an engineered biosynthetic pathway. This pathway is more efficient, sustainable, and enables the stereoselective production of target diketopiperazines compared to previously reported synthetic methods.[4] Our results indicate that key intermediates in the biocatalytic cascade are readily identified and isolated after heterologous co-expression of all enzymes in *E. coli*. The ultimate goal of this project is to apply this platform to access a diverse library of complex diketopiperazines for bioactivity analysis against human microbial pathogens and parasites.

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12. Unearthing druggable vulnerabilities in cancer with Averon Notebook: Leveraging mutant-directed protein-protein interactions

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Cancer, a formidable disease driven by genomic changes, continues to be a major global threat which causes over 10 million deaths annually. The impact of genomic alterations, such as mutations, extends to crucial cellular processes, disrupting protein-protein interaction (PPI) networks, and driving the onset and advancement of this devastating ailment. The quantitative High Throughput differential Screening (qHT-dS) platform we have developed has unveiled numerous mutant-enabled or neomorph protein-protein interactions (neoPPIs). These findings encompass both oncogenic and tumor suppressor mutations, presenting exciting prospects for precision medicine with novel mutant-directed therapeutic strategies (Mo, X., et al., Cell. 2022, 185, 1974-1985). Nevertheless, comprehensively understanding the precise pathogenic functions acquired through mutant-directed neoPPIs and devising clinical approaches to regulate them poses a significant challenge. To address this critical challenge, we developed a novel computation platform termed Averon for discovering Actionable Vulnerabilities Enabled by Rewired Oncogenic Networks. We implemented the Averon as a Python Jupyter Notebook that includes new algorithms and statistical methods to i) assess and analyze the levels of neoPPIs in cancer patients, ii) evaluate their impact on clinical outcomes, iii) determine specific signature gene sets and oncogenic pathways which can be regulated by individual neomorph PPIs, and iv) identify clinically significant neoPPI-regulated genes with available clinical compounds and approved drugs. The Averon Notebook represents the first computational platform that enables the exploration of functional consequences resulting from mutant-induced dysregulation of PPI networks at a precise single residue resolution. Moreover, it sheds light on previously undiscovered clinically actionable dependencies facilitated by neomorph PPIs in cancer patients.

Acknowledgments
This work was supported in part by NCI's Informatics Technology for Cancer Research (ITCR) Program (R21CA274620, A.A.I.), Mary Kay Foundation Grant for Cancer Research (A.A.I), NCI Emory Lung Cancer SPORE (P50CA217691, H.F.), NCI P01CA257906 (H.F.), Career Enhancement Program (A.A.I., P50CA217691), Winship Cancer Institute (NIH 5P30CA138292).

13. Development of miRNA Targeted Therapies for High Grade Serous Cancer

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High grade serous cancer (HGSC) remains the deadliest gynecological cancer and subtype of ovarian cancer. Despite high genomic instability being a key phenotype of HGSC, there are very few genetic drivers contributing to its progression prompting the exploration of new genetic drivers and therapeutic targets. Our group has discovered that microRNA-181a (miR-181a) is highly expressed in HGSC and promotes oncogenesis through the activation of the WNT and TGF-β signaling pathways thus mediating epithelial to mesenchymal transition and transcription of pro-survival signals. Additionally, miR-181a inhibits innate immune function by suppressing STING, making it an attractive therapeutic target. Therefore, in this study we aim to identify a series of small molecule inhibitors specifically targeting miR-181a. To achieve this, we functionally screened >54,000 drug-like compounds using a previously characterized high-throughput miR-181a biosensor drug screen that can simultaneously assess changes in miR-181a function and cell viability. From this collection, 32 compounds reduced miR-181a activity by >20% while also decreasing cellular viability. Interestingly, of the 32 compounds 5 of the compounds clustered as structurally similar RNA binding molecules. At least 4 of these have been shown to target miR-181a post-transcriptionally and at least 1 reduces TGF-β mediated epithelial to mesenchymal transition. Future cell-based profiling of these compounds will confirm and identify our lead hits to be optimized using structure activity relationship analysis. Identification of a miR-181a small molecule inhibitor will be the first of its kind to be used in HGSC and will establish a novel and foundational therapeutic avenue to be exploited in gynecological cancers.

Acknowledgements
I want to express my sincere gratitude to Dr. Analisa DiFeo and the members of the DiFeo lab for providing their mentorship and guidance. I would also like to thank the pathology department and team at the University of Michigan’s drug discovery core for their support of this work.

14. *In silico* modeling and analysis of small molecules binding to the PHLPP1 protein by molecular dynamics simulation

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The PHLPP (Pleckstrin homology domain leucine-rich repeat protein phosphatases) is a newly discovered group of genes which includes PHLPP1 and PHLPP2 and plays an integral part in several cellular processes like apoptosis, cell signaling cell survival, and cell proliferation etc. Both the activation and deactivation of these genes can have vital role in several ailments like heart diseases, circadian rhythm and most importantly the cancer, hence encouraging the growth of novel therapeutic elements. To give new directions into the development of PHLPP1- targeting drugs, the interaction mechanism between PHLPP1 and five important ligands 4IP, B39, 635, ATP and GTA were investigated through docking and Molecular Dynamics Simulation. It is also noteworthy to be mentioned here that there is no previous crystal structure of PHLPP1 available. The *in silico* results can provide potential base for advancements in development of new therapeutic elements targeting different diseases, mainly cancer. In this study, we employed homology modeling technique to develop a high-quality structure model of PHLPP1. The PHLPP1 model was then used in docking interaction analysis and Molecular Dynamics Simulation, to study binding pockets and interactions of PHLPP1 ligands and finding actively contributing residues in binding pocket. In final step, Free Energy Estimation was performed to observe ligand binding's quantitative characteristics.

Keywords
PHLPP1, cancer, molecular docking, homology modeling, molecular dynamics, phosphorylation.

Acknowledgments
None.

14. Synthesis-based on affinity separation (SAS) strategy for preparation of fluorogenic substrate library for single-molecule enzyme activity analysis

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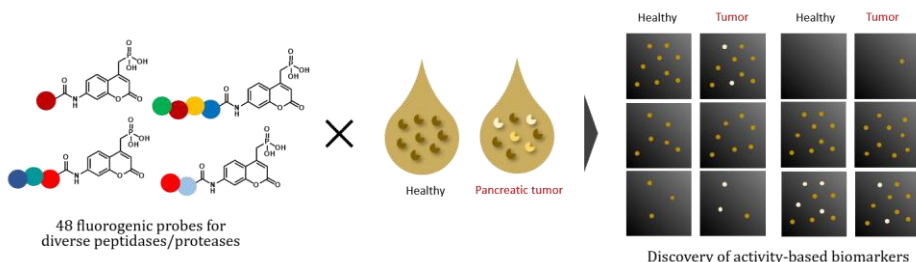
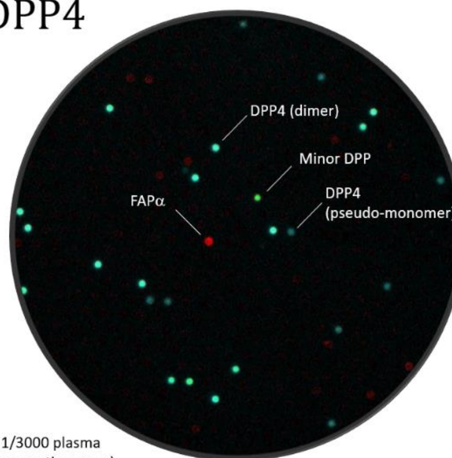
In living systems, there are thousands of enzymes present to maintain the homeostasis, and their alterations are often tightly connected to the onset of diseases. The screening of the enzymatic activities by enzymomics (omics of enzyme) approach is a strategy for the discovery of activity-based biomarkers[1,3], in which we prepare for the library of enzyme substrates and perform the specimen-based screening to identify the activities that showed the differences between the samples of interest. We proposed a concept of single-molecule enzyme activity-based enzyme profiling (SEAP) to detect the lower concentrations of enzymes with the capability of discriminating the enzyme subtypes and showed its suitability to analyse biomarker enzymes in blood samples[4,5]. In this study, we have established the way of preparing the library of substrates useful for the single-molecule enzyme activity analysis to widen the scope of the enzymes targetable by the assay and to perform single-molecule activity-based screening of disease biomarkers.



The single-molecule enzyme activity analysis is performed by preparing a microdevice with 10^5- 10^6 reaction chambers, each with volumes of 10-100 fL, and loading the diluted enzyme solution so that theoretically 0 or 1 target enzyme molecule is included in each chamber. All chambers are loaded with the fluorogenic probes and are sealed by oil. If the target single-molecule enzyme is included in the chamber, the turnover of the enzyme generates the fluorescent product, which quickly accumulate to the detectable concentration in the small chamber. Currently, only limited classes of enzymes, phosphatases and glycosidases, have been analysed in this platform due to the unavailability of the fluorogenic substrates useful for the study. The microdevice and sealing oil are hydrophobic materials, so the fluorogenic probes used in the microdevice-based assays are required to exhibit sufficient hydrophilicity to make them not leaky from the system. We have established the on-demand fluorogenic substrate preparation platform for various enzymes such as amidases, peptidases, and proteases for single-molecule enzymatic assays. We synthesized probe library and performed the activity-based screening to characterize disease-related alterations of single-molecule activities of DPP4, CD13, and pancreatic elastase in blood samples of patients with early-stage (stage I-II) pancreatic tumor[6].



Keywords: chemical biology, enzymes, fluorescent probes, microdevices, liquid biopsy

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15. A versatile multiplexed TR-FRET assay for detection of SMAD4-SMAD3-DNA complex and high-throughput chemical probe discovery

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The remarkable clinical achievements of immune checkpoint inhibitor (ICI) therapy have propelled the rapid advancement of immune-mediated anti-tumor strategies, establishing them as the first-line treatment for various tumor types.[1,2] Despite the paradigm-shifting progress in cancer immunotherapy over the last decade, the majority of patients fail to respond to current monotherapy based on ICIs, and a significant challenge lies in the occurrence of patient relapse following initial response.[3] Consequently, there is an urgent and unmet clinical need to address the requirements of the majority of cancer patients, necessitating renewed endeavors to broaden the scope and efficacy of immune system-targeted strategies.

Transforming growth factor-beta (TGFβ) signaling has emerged as a promising target for cancer immunotherapy.[4,5] While the role of TGFβ signaling in cancer initiation, progression, and metastasis is multifaceted and context-dependent,[6,7] its contribution to the establishment of an immunosuppressive tumor microenvironment (TME) has been extensively documented for both adaptive and innate immune responses.[6,7] For instance, TGFβ hampers anti-tumor immunity by inhibiting the proliferation, maturation, differentiation, and activation of natural killer (NK) cells, macrophages, dendritic cells (DCs), and $CD8^+$ T cells.[6,8,9] Moreover, it promotes the conversion of naïve $CD4^+$ T helper cells into immune suppressive regulatory T (Treg) cells.[10] Building upon these observations, anti-TGFβ signaling therapies have been actively investigated in clinical trials, particularly in combination with immune checkpoint inhibitors (ICIs), across a wide variety of tumor types.

Numerous therapeutic approaches targeting the anti-TGFβ signaling pathway have been developed, focusing on inhibiting upstream TGFβ and its receptors through the use of neutralizing antibodies, receptor kinase inhibitors, and anti-sense oligonucleotides.[4,11] Although promising results have been obtained with these anti-TGFβ therapies in preclinical *in vitro* studies and mouse models, most clinical trials have failed to reproduce these favorable outcomes.[12,13] The formidable challenges encountered in the clinical translation of anti-TGFβ signaling therapy not only necessitate the development of novel mechanism-driven strategies but also underscore the imperative to expand the existing repertoire of anti-TGFβ therapeutic options.

SMAD4 (Mothers against decapentaplegic homolog 4) serves as a critical downstream master regulator in the canonical TGFβ signaling pathway.[7] It functions as a common SMAD (co-SMAD) and acts as an adaptor protein, forming protein-protein interaction (PPI) complex with the receptor-regulated SMADs (R-SMADs), such as SMAD3.[14,15] Following the activation of the TGFβ pathway, the SMAD4-SMAD3 PPI complex translocate into the nucleus, binds to SMAD-binding elements (SBE) containing DNA sequences, and initiates the expression of a wide spectrum of TGFβ target genes. However, SMAD4 has been considered 'undruggable' due to its lack of enzymatic activity and its large protein-protein interaction interface.[16]

To advance the search for SMAD4 inhibitors and broaden the arsenal of anti-TGFβ signaling therapy, we present the development of a multiplexed time-resolved fluorescence resonance energy transfer (TR-FRET) assay capable of simultaneously measuring SMAD4-SMAD3 protein-protein interaction (PPI) and SMAD-SBE protein-DNA interaction (PDI). This assay enables the precise monitoring of the dynamics of SMAD4-SMAD3-SBE PPI and PDI at the resolution of single amino acids within a homogeneous cell lysate-based configuration. Furthermore, the assay has been miniaturized and validated for ultrahigh-throughput screening (uHTS) in 1536-well plate format. This optimized and validated uHTS assay will facilitate future large-scale screening campaigns for SMAD4 inhibitors in the quest for novel anti-TGFβ signaling therapy drugs.

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17. Revisiting the Se-ntral role of GPx4 in Ferroptosis: the Selenoprotein TXNRD1 emerges as a primary target of RSL3

Stephen L DeAngelo, Marwa Elderany, Sofi Dziechciarz, Andrii Balia, Markos Koutmos, Nouri Neamati, Yatrik Shah

Ferroptosis is a regulated Iron dependent form of cell death distinct from apoptosis. Recent literature has shown that Colorectal Cancer (CRC) has heightened sensitivity to ferroptosis due to high accumulation of Iron from the tumor microenvironment. In the presence of high cytoplasmic Iron levels, intracellular hydrogen peroxide (produced as a metabolic byproduct) undergoes a chemical decomposition to form a highly reactive peroxide radical (HO•), one of many cellular Reactive Oxygen Species (ROS). While low levels of ROS are natural and may even aid tumor growth and metastasis, elevated levels of ROS can quickly cause high levels of cellular damage by reacting with proteins, DNA, and lipids, with high levels of lipid peroxides directly leading to ferroptosis. Over the last 10 years the field has focused in on the enzyme Glutathione Peroxidase 4 (GPx4) as a key target in ferroptosis resistance as GPx4 utilizes a catalytic selenocysteine residue to detoxify lipid peroxides. In 2014 the ferroptosis inducing agent (FIN) RSL3 was characterized as an inhibitor of GPx4 and has since been prolifically used throughout the field of cancer biology to study ferroptosis. Driven by the success of RSL3 as a FIN, our laboratory sought to genetically validate GPx4 as a therapeutic target in colorectal cancer. Unexpectedly we observed that genetic depletion of GPx4 in our mouse models of CRC does not lead to increased survival. Furthermore, upon inducible knockdown of GPx4 in our CRC cell lines we see varying levels of response despite high sensitivity to RSL3. Intrigued by these experimental results we synthesized a biotinylated RSL3 derivative to perform IP-MS. Our preliminary data suggest that RSL3 is not an inhibitor of GPx4 but is instead an inhibitor of another selenoprotein, Thioredoxin Reductase 1 (TXNRD1), with many additional selenoprotein off-targets that regulate Endoplasmic Reticulum (ER) stress and Calcium Homeostasis. The gold compound Auranofin (Ridaura) is an FDA approved agent for the treatment of Rheumatoid Arthritis that potently inhibits TXNRD1. Additionally, flavonols are natural product inhibitors of the SERCA calcium pumps that disrupt calcium homeostasis to induce ER stress and ROS formation. Here we show preliminary evidence Auranofin and several flavonols can drastically reduce the proliferation of CRC cell lines. While preliminary we hope that this work may lead to a repurposing study of Auranofin for the treatment of CRC.

18. Simple purification of small-molecule-labelled peptides via palladium enolate formation from *β*-ketoamide tags

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Palladium enolates derived from *β*-ketocarbonyl compounds serve as key intermediates in various catalytic asymmetric reactions.[1] We found that the palladium enolate formed from *β*-ketoamide is stable in air and moisture,[2] and we applied this property to develop a peptide purification system using *β*-ketoamide as a small affinity tag in aqueous media. A solid-supported palladium complex successfully captured *β*-ketoamide-tagged molecules as palladium enolates and released them in high yield upon acid treatment. Optimum conditions for the catch and release of tagged peptides from a mixture of untagged peptides were established. To demonstrate the value of this methodology in identifying the binding site of a ligand to its target protein, we purified and identified a peptide containing the ligand-binding site from the tryptic digest of cathepsin B labelled with a covalent cathepsin B inhibitor containing a *β*-ketoamide tag.[3]

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19. Targeting p38/MK2 protein-protein interaction for therapeutic discovery in human diseases

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P38 kinases are essential components of signal transduction. Four known p38 isoforms (p38α, p38β, p38g, and p38d) serve non-redundant functions by binding various substrates, including transcription, signal transduction, protein folding, and cytoskeleton maintenance[1,2]. The inhibition of p38 activity has emerged as a highly appealing therapeutic strategy in multiple human diseases, including cancer, Alzheimer's disease (AD), and different metabolic conditions. Although many p38 inhibitors have been developed, none of them have been approved as a drug due to a limited selectivity against p38 isoforms and other kinases. The failure of p38 inhibitors in clinical trials highlights the urgent need for new therapeutic approaches to p38 regulation. To address this unmet medical need, we develop a novel approach to control p38 activity by selectively targeting p38 protein-protein interaction (PPI) with its major substrate the MAPK-activated protein kinase 2 (MK2), coded by MAPKAPK2. MK2 is one of the most clinically important p38-regulated proteins which play a key role in AD-associated neuroinflammation and neurotoxicity, as well as tumor growth and DNA damage response in cancer metastasis and proliferation[3]. The discovery of potent p38/MK2 PPI inhibitors may open new avenues for p38-based therapeutic development. Our Time-Resolved Fluorescence Energy Transfer (TR-FRET) and affinity pulldown assays revealed that MK2 has a significantly higher binding affinity to p38a and p38b compared to p38g and p38d isoforms. Using computational structural analysis, we identified multiple contact sites on the p38/MK2 PPI interface that are critical for p38/MK2 interaction. The calculated druggability scores and contribution of p38 and MK2 residues to the MK2/p38 free binding energy allowed us to prioritize two pockets suitable for small molecule p38/MK2 disruptor binding. Based on the analysis, we experimentally confirmed the p38/MK2 interface, and designed short inhibitory peptides to disrupt the p38/MK2 PPI. Through a computational screening, we have uncovered and validated the first small molecule p38/MK2 PPI inhibitors. We have further optimized and miniaturized the TR-FRET assay for the high-throughput screening (HTS) 384- and ultra-HTS 1,536-well plate formats. We have shown that both p38a/MK2 and p38b/MK2 PPIs demonstrated strong TR-FRET signal with >20 signal/background ratio, which was stable for more than 48 hours and tolerated >10 % DMSO. Togetgher, our data indicate the p38/MK2 complex druggability, provides the first chemical tools to regulate this clinically significant complex, and establish a new robust assay to discover small molecule MK3/p38 PPI inhibitors to facilitate therapeutic discovery towards cancer, neurological, and metabolic diseases.

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20. Development and Evaluation of Covalent NSD3 Inhibitors

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The Nuclear Receptor SET domain-containing (NSD) family of proteins are histone lysine methyltransferases (KMTase) that mono and di-methylate histone 3 lysine 36 (H3K36), which is known to activate transcription of euchromatin. There are three enzymes in this family: NSD1, NSD2, and NSD3 with NSD1 and NSD3 sharing 80% sequence similarity in the SET domain. Overexpression of *NSD3* has been found in a variety of different cancers to date such as lung, breast, and pancreatic and is often associated with a poor prognosis. It has been shown that NSD3 drives breast cancer tumor initiation and metastatic progression through H3K36 methylation. The NSD family has been a difficult target for inhibitor development due in part to the presence of an autoinhibitory loop that blocks the entrance of the lysine binding channel. We have previously developed covalent inhibitors of NSD1. Subsequent optimization yielded novel inhibitor BT479, which potently and selectively binds and inhibits NSD3 KMTase activity in biochemical assays. BT479 demonstrates potent anti-proliferative activity in breast cancer models, inhibiting sphere formation in cell lines with high levels of NSD3 expression. Proper characterization of such covalent inhibitors requires analysis of the engagement in biological samples. In order to assess engagement, we have developed several methods utilizing a variety of different probe compounds. Employing a biotinylated probe, we utilized streptavidin bead capture to enrich biotin-tagged proteins for bottom-up proteomics analysis. We are also developing a new approach to establish cellular engagement through employment of a probe compound functionalized with a HaloTag ligand. Together, these approaches can be used to gain valuable information for the development of covalent inhibitors targeting NSD3. These inhibitors will serve as valuable probes to study the potential of targeting the histone methyltransferase activity of the NSD family in cancers. In this presentation, we will describe the development of novel NSD3 inhibitor BT479 as well as several different approaches to establish covalent engagement of potent and selective inhibitors in mammalian cells.

21. Development of Enantiomerically Pure 1,4-thiazepanes and Their Evaluation as Domain-Selective BET Bromodomain Inhibitors

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Heterocyclic compounds represent a significant fraction of pharmaceuticals and materials, with a variety of ring systems present in many natural products and biologically active molecules. One such class of heterocycles are 1,4-thiazepanes—seven-membered rings containing a nitrogen and sulfur atom in their respective positions. Molecules containing 1,4-thiazepane cores exhibit biological activity, with examples of thiazepane-based drugs having been marketed such as thiazesim and diltiazem. However, the lack of representation of non-benzofused-thiazepane syntheses in the literature illustrates the difficulty of medium-sized ring construction. While methods do exist, their limited substrate scope—often paired with long reaction times and low yields—are not amenable to the construction of diverse libraries for medicinal chemistry applications.

Recently, the Pomerantz lab reported a robust method for the construction of racemic 1,4-thiazepanes. A series of thiazepanes were also shown to selectively bind to the second *N*-terminal bromodomain (BD2) of the bromodomain and extra-terminal domain (BET) family of proteins.[1,2] Moreover, one enantiomer demonstrated higher affinity than the other for bromodomain binding.[3] Taken together, these results highlight the potential for further development of the thiazepane scaffold as a BD2-selective BET inhibitor. Here, we show the progress towards the stereoselective synthesis of 1,4-thiazepanes and testing of a small molecule library for improving their affinity and selectivity for BD2 of BET proteins.

The current direction of this project seeks to utilize bifunctional hydrogen bond catalysis to initiate the sulfa-Michael addition used in the synthesis developed by the Pomerantz lab while biasing the reaction to produce enantiomerically pure thiazepanes. Preliminary experiments have shown an improvement in yield over our previously established synthesis while also demonstrating stereoselective enrichment. While the stereoselective method is being developed, several racemic analogues have been synthesized, with guidance from a solved co-crystal structure with BRD2-D2, and evaluated via an AlphaScreen competitive inhibition assay. The effects of acetylated lysine mimics, ring rigidity, and vectors targeting the WPF shelf of the BET bromodomain are being evaluated. Further attempts at functionalizing the thiazepane core itself are also underway.

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22. Class IIa Histone Deacetylases And Their Inhibitors

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Histone deacetylases and their inhibitors[1] regulate the acetylation status of the lysine sidechain in many proteins. However, inhibitors of the zinc-containing class IIa enzymes[2] (HDACs 4, 5, 7, 9), unlike those of class I (HDACs 1, 2, 3, 8), do not lead to hyperacetylation of nuclear proteins called histones[3] and they tend to be much less cytotoxic. We have identified class IIa HDACs capable of regulating cytosolic proteins, metabolic[4] and inflammatory[5] responses, affording new molecular insights into the biology[2] of class IIa proteins. Here we describe different chemical approaches to inhibitors of class IIa HDACs, novel interactions they make with class IIa versus class I HDACs, and their efficacy in models of inflammatory disease.

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23. Structure-guided Design of New ASH1L Inhibitors As Potential Anti-leukemic Agents

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The absent, small, or homeotic-like 1 (ASH1L) protein is a histone lysine methyltransferase responsible for the methylation of histone 3 lysine 36.[1] ASH1L is found to be overexpressed in various cancer types, including acute leukemia, making it an attractive therapeutic target. Herein, we present a new generation of ASH1L inhibitors with improved inhibitory activity and enhanced cellular activity in leukemia cell over our previously developed ASH1L inhibitor, **AS-99**[2]. The structure-based design, synthesis, and structure-activity relationship studies of these compounds will be presented together with their activity in leukemic cells. Furthermore, development of a fluorescence polarization (FP) assay to determine the activity of our new ASH1L inhibitors will be reported. Among these compounds, the most promising lead compound **AS-254** and its close analogs demonstrated significantly improved inhibitory activity and microsomal stability as compared to our previously developed ASH1L inhibitors. Furthermore, our lead compound **AS-254** effectively blocked cell proliferation and induced apoptosis and differentiation in leukemia cells harboring *MLL1* translocation. This work will pave the way for future drug discovery efforts aiming at the development of small-molecule inhibitors of ASH1L as therapeutic agents.

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24. Decitabine inhibits DNA methyltransferases activity and suppresses vasculogenic mimicry formation in tumor cells

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Background: Vasculogenic mimicry (VM) is a phenomenon in which blood vessel-like structure is formed by tumor cells, not vascular endothelial cells like tumor angiogenesis. Tumors intake nutrients and blood cells carrying oxygen through the structures, so VM has been suggested to be associated with tumor aggressiveness and poor patient prognosis. [1, 2] However, its comprehensive mechanism remains unclear. DNA methylation is one of the major epigenetic mechanisms mediated by DNA methyltransferases (DNMTs). DNMTs catalyze the addition of methyl groups onto cytosine residues of DNA and regulate gene expression. Hence, aberrant DNA methylation of oncogenes and tumor suppressor genes is associated with cancer progression. [3, 4] In recent years, some studies have shown that epigenetic aberrance such as hypermethylation of THBS1 is involved in oncogenic angiogenesis. [5] This might imply the relationship between DNA methylation and VM formation, which exhibits the angiogenic phenotype. [1] Therefore, we sought to investigate whether and how DNA methylation contributes to VM formation.

Results: In this study, we show that the treatment with a DNMTs inhibitor, 5-aza-2'-deoxycytidine (decitabine), significantly suppresses VM formation in human fibrosarcoma (HT1080) and breast cancer (MDA-MB-231) cells. Such VM-positive cells form vasculogenic-like networks on Matrigel *in vitro*. Moreover, decitabine treatment on HT1080 cells decreased the protein level of DNMTs, which is caused by trapping them on decitabine-incorporating DNA. [6] It was also checked by bisulfite sequencing (BS-seq) that the DNA methylation level in a genome region accordingly decreased by decitabine treatment. Next, we conducted RNA-seq analysis on decitabine-treated HT1080 cells in order to reveal the regulatory mechanism of VM formation by DNA methylation. As a result of the comparison between DMSO- and decitabine-treated HT1080 cells, 1,962 differentially expressed genes (DEGs) including 1730 upregulated and 232 downregulated genes were identified. Some of the upregulated DEGs have also been reported as methylation-silenced genes. [7] Gene Ontology (GO) analysis demonstrated that GO terms putatively related to VM were significantly enriched such as collagen trimmer and cell junction. Moreover, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis indicated that cytokine signaling pathways could also be activated. Of the upregulated DEGs, we selected ones that were presumably the most contributory genes and examined their effect on VM formation. We established an HT1080 cell line overexpressing vesicle-associated membrane protein 5 (VAMP5). The overexpression of VAMP5 partially suppressed VM formation in HT1080 cells.

Conclusion: These results suggest that DNMTs inhibition by decitabine suppressed VM formation of cancer cells through reprogramming genome-wide DNA methylation and gene expression.

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25. Polycomb repressive complex 1 inhibitors demonstrate potent activity in acute leukemia models

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Polycomb repressive complex 1 (PRC1) is an epigenetic regulatory complex that monoubiquitinates histone H2A lysine 119, resulting in the repression of target genes important for cellular identity, stemness and differentiation[1]. The canonical core of PRC1 is composed of a RING1A/RING1B and BMI1 heterodimer, which elicits its E3 ligase activity[2]. Previous studies have implicated the role of PRC1 in the maintenance and proliferation of leukemia stem cells[3,4]. Furthermore, loss of PRC1 activity via *RING1A*/*RING1B* knockdown impairs leukemic stem cell proliferation and induces leukemic stem cell differentiation[4,5]. Therefore, the development of small molecule inhibitors of PRC1 presents a valuable therapeutic strategy for leukemia treatment. We previously reported the development of first-in-class small molecule inhibitors that bind directly to RING1A/RING1B to block its interaction with the nucleosome and prevent PRC1 E3 ligase activity[6]. Here, we present the development and characterization of a potent, new generation of PRC1 inhibitors with enhanced inhibitory activity. Treatment of acute leukemia cell lines with these inhibitors results in potent anti-proliferative effects at mid-nanomolar concentrations. PRC1 inhibitors display on-target activity as seen by the reduction of the H2A ubiquitination mark. On-target activity is also supported by gene expression analyses revealing the de-repression of *CDKN1A*, a key PRC1 target gene encoding for the p21 tumor suppressor protein important for cell cycle progression and leukemic stem cell differentiation. Global upregulation of p21 levels is also seen by western blot analysis. Additionally, we demonstrate small-molecule inhibition of PRC1 induces differentiation of leukemic cells, as well as apoptosis. ChIP-seq and RNA-seq studies demonstrate the displacement of PRC1 from chromatin and the upregulation of multiple PRC1 target genes upon PRC1 inhibitor treatment in acute myeloid leukemia cells. Furthermore, our PRC1 inhibitors are non-toxic in normal human CD34+ bone marrow cells and have been shown to reduce leukemia burden in *in vivo* mouse models. Our studies suggest that multiple subtypes of acute leukemia are sensitive to PRC1 inhibition, and our small-molecule inhibitors elicit potent effects in both *in vitro* and *in vivo* models of leukemia. These inhibitors offer a new therapeutic approach for leukemia treatment.

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26. Characterizing MLL-binding affinity of clinically resistant Menin mutations

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The Mixed Lineage Leukemia (MLL) family of proteins are histone methyltransferases that tri-methylate histone 3 lysine 4 (H3K4) and regulate important genes for normal hematopoiesis, including *Hox* gene expression. Chromosomal translocations of MLL's C-terminal fragment, which contains its methyltransferase activity, leads to upregulation of *Hox* gene activity, allowing stem cells to avoid differentiation and ultimately cause leukemia. Menin, an oncogenic co-factor of MLL, binds to MLL's N-terminus and is essential in directing MLL to its target genes. In MLL rearranged (MLLr) and NPM1 mutated leukemias, disrupting the Menin-MLL interaction with small molecule inhibitors has shown positive therapeutic outcomes *in vivo* and patient populations. Several menin inhibitors are currently being evaluated in clinical trials, and clinical studies with one of such inhibitors, Revumenib, reported on the discovery of somatic mutations in Menin that disrupt Revumenib's ability to inhibit the Menin-MLL interaction, leading to cancer resistance and progression. Here we characterize the interaction of the most prevalent mutations reported in the Revumenib study with MLL. These mutations include M327I, T349M, G331D, and G331R. Utilizing fluorescence polarization (FP) experiments and isothermal calorimetry (ITC), we have found that these mutations minimally affect the Menin-MLL interaction, giving similar binding as with wild-type Menin. We have also determined crystal structures of Menin mutants in complexes with MLL derived peptides, rationalizing the retention of the high affinity interaction. By understanding if and how these mutations change the Menin-MLL binding interface, we hope to aid the development of a new generation of inhibitors that can overcome resistance caused by somatic mutations in Menin.

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27. The biological evaluation of NatD bisubstrate inhibitors in lung cancer

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Protein N-acetyltransferases (Nats) are a class of enzymes that transfer an acetyl group from acetyl coenzyme A to the alpha-N-terminus of a protein substrate. N-terminal acetyltransferase D (NatD) is a highly selective Nat that acetylates the protein sequence SGRGK on histones H4 and H2A. NatD expression is increased in human lung and colorectal cancer tissues and is associated with poor patient survival. NatD knockdown reduces lung cancer progression by repressing the transcription factor Slug, which prevents epithelial-to-mesenchymal transition. Furthermore, NatD depletion promotes p53-independent apoptosis in colorectal cancer cells. This suggests NatD is a potential epigenetic target for lung and colorectal cancers. A potent and selective NatD inhibitor is needed to unravel the functions of NatD acetyltransferase activity in cancer progression. We previously designed and synthesized a series of potent and selective NatD bisubstrate inhibitors by covalently attaching coenzyme A to peptide substrates via ethyl and propyl linkers.[1] However, these inhibitors were not cell permeable due to their hydrophilic phosphate groups, which limits their application in cellular studies. Our current research aims to improve the cellular permeability of NatD bisubstrate inhibitors using (1) cell-penetrating peptides (CPPs) or (2) lipofectamine delivery. We designed and attached cell-penetrating peptides (CPPs) to bisubstrate inhibitors, which displayed Ki values in the nanomolar range but had limited cell permeability. Alternatively, lipofectamine-aided delivery of NatD bisubstrate inhibitors improved cell permeability and inhibitor treatment decreased proliferation and migration in A549 lung cancer cells. In addition, NatD inhibition led to an increase of cellular phosphorylation of Ser1 on histones H4/H2A and alterations in EMT-related protein markers. Overall, we expect that the biological evaluation of NatD inhibitors can facilitate our understanding of the biological roles of NatD and provide evidence of NatD as a potential therapeutic target.

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28. Structure-guided development of potent small-molecule inhibitors of PRC1

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PRC1 (Polycomb Repressive Complex 1) is a multiunit epigenetic complex responsible for monoubiquitination of lysine 119 on histone H2A. This repressive mark leads to chromatin compaction and transcriptional repression of target genes, which regulate developmental gene expression and maintain stemness.[1] The core of PRC1 that confers its E3 ligase activity is a heterodimeric unit comprised of RING1A or RING1B and one of six PCGF proteins, making six potential PRC1s.[2] Canonical PRC1 possesses PCGF4 (also known as BMI1). Studies implicate RING1B and BMI1 in various cancers and highlight the importance of PRC1 activity in the proliferation and maintenance of leukemic stem cells.[3-4] Therefore, the development of well-characterized, potent small molecule inhibitors directly targeting PRC1 activity may serve as a new approach for therapeutic intervention in leukemia. Our group previously reported the first-in-class PRC1 small molecule inhibitor, RB-3 which blocked proliferation and induced differentiation of primary acute myeloid leukemia samples.[5]

Through an extensive medicinal chemistry campaign facilitated by structure-based design and elaborate biochemical and biophysical characterization, we developed a new generation of PRC1 inhibitors with vastly improved potency in the low-nanomolar range. We solved several high-resolution crystal structures of RING1B-BMI1 in complex with PRC1 inhibitors that informed potent inhibitor design. We demonstrate our inhibitors directly bind to the RING domain of both RING1A and RING1B, classifying them as general PRC1 inhibitors. Biochemical and structural studies identify our PRC1 inhibitors as protein-nucleosome inhibitors, as they block the interaction between RING1A/B and the nucleosome, ultimately preventing the deposition of ubiquitin onto histone H2A. We also solved the first known, high-resolution crystal structures of the RING domains of RING1A-BMI1 and RING1B-PCGF1 in complex with our PRC1 inhibitors. RING1A-BMI1 and RING1B-PCGF1 represent canonical and noncanonical PRC1 E3 ligase cores, respectively. From these structural studies, we observed our PRC1 inhibitors bind similarly to both canonical and noncanonical PRC1 cores. Furthermore, our PRC1 inhibitors block the H2A ubiquitination function of canonical and noncanonical PRC1 cores with similar inhibitory activity. These medicinal chemistry efforts and thorough characterization of small-molecule inhibitors led to the development of YWB-214, a potent PRC1 inhibitor that blocks the proliferation of leukemia cells and reduces leukemia burden in *in vivo* mouse models.

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29. Structural studies of intrinsically disordered MLL-fusion protein AF9 in complex with peptidomimetic inhibitors

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AF9 (MLLT3) and its paralog ENL(MLLT1) are components of the super elongation complex (SEC) and serve as key MLL fusion partners in MLL fusion-transformed acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). The fusion proteins MLL-AF9/ENL recruit the histone methyltransferase DOT1L to the promoters of MLL target genes, inducing H3K79 hypermethylation and aberrant transcriptional activation of these genes, thus promoting leukemogenesis. The interaction between AF9 and DOT1L is mediated by an intrinsically disordered C-terminal ANC1 homology domain (AHD) in AF9, which undergoes folding upon DOT1L binding. Conducting biochemical, biophysical, and functional analyses, we mapped the AHD binding site in DOT1L [1] and demonstrated the crucial role of the interactions between DOT1L and AHD in oncogenic transformation [2]. These findings were confirmed by other groups, which established the consensus binding sequence, LXV(I, L)XIXL(V, I)XXL(V, I) in DOT1L, and identified other binding partners of AF9 AHD. Therefore, blocking these protein-protein interactions represents a viable treatment for MLL-rearranged leukemia. We have developed potent peptidomimetics that disrupt the recruitment of DOT1L by AF9 and ENL, providing a proof-of-concept for targeting these interactions [3]. Understanding the recognition and binding of peptidomimetics to AHD is critical for further optimizing these compounds and facilitating the design and development of potent drugs targeting AHD. In this study, we present a protein engineering strategy by fusing MBP and AF9 AHD using various linkers. This strategy has led to the determination of the first series of X-ray crystal structures of complexes between AF9 AHD and peptidomimetics. The crystal structures provide high-resolution insights into the interactions between AHD and its inhibitors as well as the role of AHD in recruiting various proteins. The findings set the groundwork for future structural investigations of intrinsically disordered proteins and the development of novel therapeutics targeting their interactions.

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30. The ENL YEATS epigenetic reader domain critically links MLL-ENL to leukemic stem cell frequency in t(11;19) Leukemia

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MLL (KMT2a) translocations are found in ~10% of acute leukemia patients, giving rise to the oncogenic MLL-fusions and a subset of aggressive leukemia. In t(11;19) patients, MLL is fused to ENL, the third most common MLL fusion partner. ENL contains a highly conserved N-terminal YEATS domain that binds to acetylated histones and interacts with PAF1 of the PAF1c, an epigenetic regulator protein complex essential for MLL-fusion leukemogenesis. Recently, wild type ENL, and specifically the YEATS domain, was shown to be essential for leukemic cell growth. However, the inclusion and importance of the YEATS domain in MLL-ENL mediated leukemogenesis remains unexplored. We found the YEATS domain is retained in 84.1% of MLL-ENL patients and crucial for MLL-ENL mediated leukemogenesis in mouse models. Mechanistically, deletion of the YEATS domain impaired MLL-ENL fusion protein binding and impacted the epigenetic landscape at and expression of pro-leukemic genes like *Eya1* and *Meis1*. Point mutations that disrupt YEATS domain binding to acetylated histones decreased stem cell frequency and increased MLL-ENL-mediated leukemia latency. Therapeutically, YEATS containing MLL-ENL leukemic cells display increased sensitivity to the YEATS inhibitor SGC-iMLLT compared to control AML cells. Our results demonstrate that the YEATS domain is important for MLL-ENL-mediated leukemogenesis and exposes an "Achilles heel" that may be therapeutically targeted for treating t(11;19) patients. Future directions involve further investigation of other domains present in MLL-ENL that may also play a role in promoting leukemogenesis.

31. Targeting triple-negative breast cancer through the MYC-MKK3 complex

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The triple-negative breast cancer (TNBC) is the most aggressive type of breast cancer and the main cause of breast cancer mortality. The lack of targeted TNBC therapy exposes the urgent need for new therapeutically actionable molecular mechanisms underlying the disease. The transcriptional factor MYC is a major oncogenic driver of TNBC. MYC amplification, found in > 60% of TNBC samples, leads to the aggressive phenotype and poor clinical outcomes in TNBC patients, making MYC a highly appealing therapeutic target. However, MYC-targeted therapy remains unavailable. We discovered that MYC protein-protein interaction (PPI) with Mitogen-Activated Protein Kinase Kinase 3 (MKK3) represents a novel pharmacologically actionable mechanism of MYC activation in TNBC. Using our specially designed bioinformatics workflow we identified MKK3 among the genes whose high expression strongly correlates with worsened clinical outcomes of African American TNBC patients. By integrating patient genomics data with the histopathological image analyses, we showed that MKK3 promotes TNBC aggressiveness in part by activating MYC transcriptional program. Moreover, we discovered that MKK3 directly binds to MYC in TNBC cell lines and enhances both MYC protein stability and transcriptional activity. By combining computational modeling with ultra high-throughput screening, we have discovered the first chemical tools to interrogate MKK3/MYC PPI functions in cancer cells, and demonstrated that the disruption of the MKK3/MYC complex in cells correlates with decreased MYC activity and suppressed viability of TNBC cells. Together, our data suggest a novel mechanism of TNBC tumorigenesis mediated through MKK3-induced MYC activation, defines MKK3/MYC complex as a new druggable target to decrease TNBC-related mortality, and provides the first inhibitors to regulate this oncogenic axis.

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32. Design and Synthesis of Orally Active Quinolyl Pyrazinamides as Sigma 2 Receptor Ligands for the Treatment of Pancreatic Cancer

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Sigma 2 receptor (σ2R) is overexpressed in select cancers and is regarded as a biomarker for tumor proliferation. σ2R ligands are emerging as promising theranostics for cancer and neurodegenerative diseases. Herein, we describe the design and synthesis of a series of novel quinolyl pyrazinamides as selective and potent σ2R ligands that show sub-micromolar potency in pancreatic cancer cell lines. Compounds **14** (JR1-157) and **17** (JR2-298) bind σ2R with K_i of 47 and 10 nM, respectively. Importantly, compound **14** has an oral bioavailability of 60%, $t_{1/2}$ of 6 h, and shows significant *in vivo* efficacy without obvious toxicity in a syngeneic model of pancreatic cancer. The cytotoxicity of the quinolyl pyrazinamides significantly enhanced in the presence of copper and diminished in the presence of the copper-chelator tetrathiomolybdate. The copper complexes of this series of quinolyl pyrazinamides also bind to σ2R. In conclusion, compound **14** is water-soluble, metabolically stable, orally active, cytotoxic in multiple pancreatic cancer cell lines, increases the expression of the autophagy marker LC3B and the ER stress marker GRP78 and warrants further development for the treatment of pancreatic cancer.

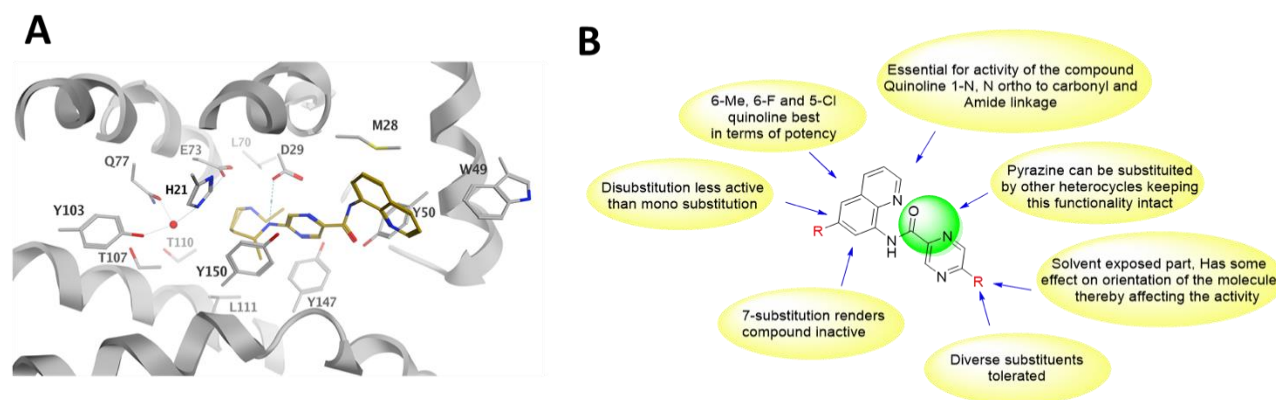

Figure 1. (**A**) Docking pose of compound **1** in the ligand binding site of the human σ2R. (**B**) SAR summary of quinolyl pyrazinamides.

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33. Synthetic optimization of a small-molecule ATG14L-Beclin 1 protein-protein interaction inhibitor for selective autophagy inhibition.

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Autophagy is a major catabolic recycling and degradation process through which cells maintain homeostasis. Cancer cells can utilize the autophagy pathway to promote proliferation and tumorigenesis, thus allowing cell survival within a hypoxic, nutrient-deprived environment. In these cases, autophagy inhibition has been suggested as a potential treatment strategy. The FDA-approved anti-malarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ) have been used in clinical trials to inhibit autophagy by disrupting lysosome function, but their lack of selective for the autophagy pathway leads to undesired off-target effects. Several inhibitors of the lipid kinase VPS34 have recently been developed in an attempt to improve selectivity. However, VSP34 is involved in two multi-protein complexes: the autophagy initiation complex (Complex I) and the vesicle trafficking complex (Complex II), so direct kinase inhibition results in inhibition of both pathways. By contrast, ATG14L is found exclusively in Complex I, where it interacts with Beclin 1 through a coiled-coil domain and is critical for the formation of the autophagosome structure, a required component of the autophagy pathway. We have previously optimized a cellular NanoBRET assay to monitor the interaction of the ATG14L-Beclin 1 protein-protein interaction (PPI) and performed a high-throughput screen to identify small molecules that inhibit this interaction.[1] This led to the discovery of Compound 19, a selective and moderately potent inhibitor of the ATG14L-Beclin 1 PPI. Synthetic strategies were developed to generate the initial hit and diverse analogues, and molecules with improved pharmacokinetic properties and potency are being prioritized. Through iterative synthesis based on previously established structure activity relationships, we will generate analogues which will support in vitro and in vivo efficacy studies. These efficacy studies will help clarify the role of selective autophagy inhibition in cancer and will reveal the utility of autophagy inhibition as a potential therapeutic strategy in cancer.

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34. Development of the menin-MLL1 inhibitors targeting menin patient mutations

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The protein-protein interaction between menin and mixed-lineage leukemia 1 (MLL1) plays an important role in development of acute leukemia with translocations of the *MLL1* or *NUP98* genes as well as with *NPM1* mutations. Indeed, small molecule inhibition of the menin-MLL1 interaction represents a promising therapeutic strategy for the treatment of acute leukemia patients, which has been already validated in clinical studies. However, mutations in the *MEN1* (*Multiple Endocrine Neoplasia 1*) gene, which encodes menin, were detected in leukemia patients undergoing menin inhibitor treatment, leading to drug resistance and relapse in patients. In particular, mutations in M327, G331 and T349 were most frequently found in patients. Interestingly these mutations are located in the inhibitor binding site, leading to dramatically reduced inhibitory activity of structurally distinct classes of menin inhibitors introduced to clinical trials. To overcome drug resistance caused by mutations in menin, we applied structure-based design combined with traditional medicinal chemistry approaches, and discovered a new generation of menin-MLL1 inhibitors, including DCM-262, which showed very potent inhibitory activity for both wild type menin and the menin patient mutants. Importantly, our new menin inhibitors also demonstrate strong activity in leukemia cells harboring *MEN1* patient mutations. These results support that our new generation of menin inhibitors might represent valuable candidates that can be further developed to possibly overcome drug resistance in patients.

35. Synthesis of Small Molecule Autophagy Modulators as Potential Therapeutics for Alzheimer's Disease

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Autophagy is a major catabolic degradation process, crucial for maintaining cellular homeostasis through degradation of damaged proteins in organelles known as autolysosomes.[1] Defects in the autophagic pathway have been implicated in multiple neurodegenerative diseases, including Alzheimer's disease, in which abnormal lysosomal function results in impaired proteolytic function in lysosomes and therefore a significant accumulation of autophagic vesicles and undegraded autophagic cargo in neurons.[2] We hypothesized that compounds capable of restoring and increasing autophagic flux could help reduce neuronal cell death, and potentially find use as therapeutics for the treatment of Alzheimer's disease. Our lab recently performed a high-throughput screen to identify autophagy modulators in a GFP-LC3 reporter cell line, identifying two different series of autophagy inducers, featuring distinct chemical scaffolds and definitive structure-activity relationships. Current research efforts are focused on further optimizing these compound series for in vitro potency and pharmacokinetic properties, in addition to synthesizing biotinylated pulldown probes to facilitate ongoing target identification efforts.

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36. Benzoxaborolone: An Oxidatively Stable Arylboronic Acid Pharmacophore for Medicinal Chemistry

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Boronic acids (R–B$(OH)_2$) are well-known organoboron reagents used for carbon-carbon bond-forming reactions.[1] When a chemist employs these reagents to stitch together smaller chemical fragments into complex, functionally active compounds, the boron moiety is lost as a by-product. However, intact boronic acids have increasingly been leveraged as nucleophile-targeting pharmacophores in small molecule therapeutics due to their mild Lewis acidity and ability to form reversible, covalent bonds in enzyme active sites (e.g., with serine or threonine residues).[2] To date, five boronic acid-containing molecules have been approved by the FDA. Most notably, the proteosome inhibitor Bortezomib is a first-line treatment for multiple myeloma.[3] Unfortunately, boronic acids are highly sensitive to oxidation, resulting in rapid deboronation and short half-lives in biological systems. We recently designed an oxidatively stable phenylboronic acid derivative that contains an *ortho-* carboxylic acid that forms a borolactone, benzoxaborolone (BOL), and engenders a 10,000-fold increase in oxidative stability.[4] To evaluate known substituent effects on BOL stability—which may influence the type of linkage used when appending BOL to molecular scaffolds—we carried out experimental and computational linear free energy analyses with a series of BOL derivatives containing electron-withdrawing or electron-donating groups, uncovering only minor effects. To this end, we demonstrate a variety of synthetic routes for accessing this novel pharmacophore. Finally, to demonstrate BOL as a stable and efficacious boronic acid pharmacophore, we synthesized BOL-containing analogues of the FDA-approved phosphodiesterase-4 (PDE4) inhibitor crisaborole, which contains a boronic acid moiety. While the overall binding affinity of our compound for PDE4 is lower than that of crisaborole, the improved oxidative stability is expected to increase compound half-life, as well as bioavailability due to increased aqueous solubility.

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37. Ferrocene as a Three-Dimensional Platform for Molecular Building: Development of Ferrocene-Based Nuclear Receptor Ligands

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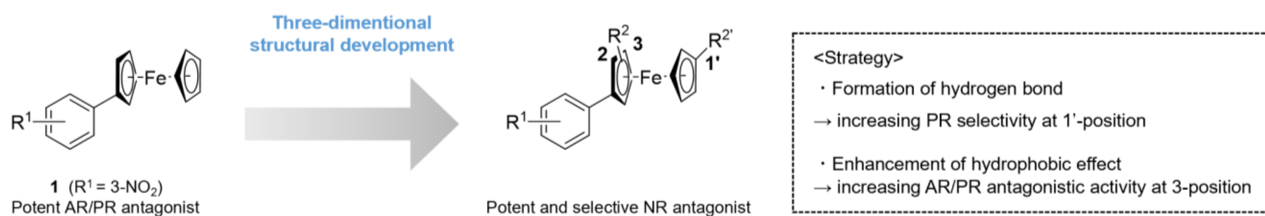
Ferrocene is a stable and non-toxic organometallic species, and has recently attracted attention in the field of medicinal chemistry.[1] Given the three-dimensional characteristics of small molecule-protein interactions, the multisubstituted structure of ferrocene, allowing for the arrangement of various pharmacophores in three dimensions, is a fascinating platform for drug design. In this research, we developed novel nuclear receptor ligands using ferrocene as the three-dimensional platform, with the aim of expanding the chemical space of drug discovery chemistry.

Nuclear receptors (NRs) such as androgen receptor (AR) and progesterone receptor (PR) are important drug targets since their ligands regulate the transcription of various genetic information. Based on the structural similarity between the steroidal CD ring and ferrocene, we previously reported the development of potent AR antagonists such as compound **1** bearing ferrocene as a hydrophobic core.[2] We also found these compounds exhibited cross-activity toward PR, which shares high homology in ligand binding domain (LBD) with AR. The capability of ferrocene for three-dimensional and diverse structural development may be advantageous in tuning the activity profile of compound. We assumed that further structural development based on compound **1** by introducing the second substituent on the ferrocene moiety could lead to develop the potent NR ligands with improved potency and receptor selectivity. Based on these considerations, we designed and synthesized disubstituted ferrocene derivatives and investigated their structure-activity relationships toward AR and PR.

Docking simulations of compound **1** with each receptors suggested that there is a hydrophobic cavity in the ligand-binding pocket around the ferrocene unit of **1** and also the possibility of additional hydrogen-bonding with the both AR-LBD and PR-LBD receptor. Thus, we designed a series of disubstituted ferrocene derivatives with a hydrophobic substituent or a hydrogen bond donor. Since there are three isomers of disubstituted ferrocenes, namely, 1,1'-isomer, 1,2-isomer and 1,3-isomer, we had set to synthesize all isomers. The AR antagonistic activity and PR antagonistic activity of synthesized ferrocene derivatives was evaluated by androgen-dependent SC-3 cell proliferation and progesterone-dependent alkaline phosphatase assay using T47D cells, respectively. Regarding the AR antagonistic activity, introduction of hydrogen bond donors decreased potency in all isomers, probably due to the inability to form hydrogen bond in an appropriate manner, which is inconsistent with the docking simulation. In contrast, interestingly, the PR antagonistic activity of the 1,1'-disubstituted compounds was greater than that of lead compound **1**, suggesting that additional hydrogen bond was probably formed in case of PR. Thus, introduction of a hydrogen bond donor significantly increased the selectivity for PR over AR. Introduction of a hydrophobic methyl group on 1'- or 2-position led to retention or decrease of the both AR and PR antagonistic activity. On the other hand, 3-methyl group enhanced both AR and PR antagonistic activity, consistent with the results of docking simulation. These results indicate that ferrocene is a versatile three-dimensional platform for biologically active compounds with improved potency and selectivity. The detail of compound design, synthesis and structure-activity relationship will be discussed.



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38. Unveiling a Novel Gα-Pocket in PDGFRA and KIT: Avapritinib-Based SAR Studies Provide Unique Structural Insights into Kinase Inhibition and Acquired Drug Resistance

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Avapritinib is the only potent and selective inhibitor approved for the treatment of D842V-mutant gastrointestinal stromal tumors (GIST), the most common primary mutation of the platelet-derived growth factor receptor α (PDGFRA).[1] The approval was based on the NAVIGATOR trial, which revealed overall response rates of more than 90%.[2] Despite this transformational activity, patients eventually progress, mostly due to acquired resistance mutations or following discontinuation due to mostly neuro-cognitive side effects.[3,4] These patients have no therapeutic alternative and face a dismal prognosis. Notable, little is known about this drug's binding mode and its medicinal chemistry development, which is instrumental for the development of the next generation of drugs.[5] Against this background, we have solved the first crystal structures of avapritinib in complex with wild-type and mutant PDGFRA and stem cell factor receptor (KIT), which provide evidence and understanding of type I inhibitor binding and lead to the identification of a unique sub-pocket (Gα-pocket). We utilized this information to design, synthesize and characterize avapritinib derivatives for the determination of key pharmacophoric features to overcome drug resistance and limit potential blood-brain barrier penetration.

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39. Farming more than Fishes: Bioactive Potential of *Pseudomonas* spp. Isolated from an Aquaculture Facility

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The widespread use of antibiotics in healthcare settings has led to the emergence of multi-drug resistant bacteria.[1,2] The term "ESKAPE" encompasses six resistant bacterial pathogens responsible for the majority of nosocomial infection with growing multidrug resistance and virulence, all of which were recently included by the World Health Organization in the list of 12 bacteria against which new antibiotics are urgently needed.[3] Historically, natural products have been harnessed for their antimicrobial potential. Natural products discovery from probiotic microbes presents an avenue to treat and prevent antimicrobial resistant pathogens while also granting the opportunity to identify bioactive compounds that may develop less resistance.[4,5] Amongst several hundred bacteria isolated from a commercial trout farm, six *Pseudomonas* strains were shown to exhibit antimicrobial activities against various ESKAPE pathogens. In this study, we employed co-culture of these six bioactive *Pseudomonas* strains with a human pathogen, *Pseudomonas aeruginosa*, to promote secondary metabolism and elicit the production of bioactive metabolites. Comparison of the bioactivity and metabolome of the monocultures and co- cultures of each strain led to the putative identification of pseudomonine as the bioactive metabolite against methicillin-sensitive *Staphylococcus aureus* (MSSA) from the trout farm isolate, *Pseudomonas fontis.* Additionally, similar comparison across other strains in this study yielded unknown masses that suggests the presence of new chemical structures with antimicrobial activities. This study contributes to the development of a natural product discovery pipeline integrating genomics, metabolomics, and bioactivity applicable to wide-scale investigation of complex microbial consortia for discovery of new classes of antibiotics.

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40. Patient-Derived Micro-Organospheres Enable Precision Oncology

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Patient-derived xenografts (PDX) and organoids (PDO) have been shown to model clinical response to cancer therapy. However, it remains challenging to use these models to guide timely clinical decisions for cancer patients. Here we used droplet emulsion to rapidly generate thousands of Micro-Organospheres (MOS) from low-volume patient tissues, which serve as an ideal patient-derived model for clinical precision oncology. A clinical study of newly diagnosed metastatic colorectal cancer (CRC) patients using a MOS-based precision oncology pipeline reliably predicted patient treatment outcome within 14 days, a timeline suitable for guiding treatment decisions in clinic. Furthermore, MOS capture original stromal cells and allow T cell penetration, providing a clinical assay for testing immuno-oncology (IO) therapies such as PD-1 blockade, bispecific antibodies, and T cell therapies on patient tumors. Lastly, we demonstrate an ultra high-throughput MOS screening platform that provides "virtual clinical trials" to capture patient diversity for determining drug efficacy.

41. Discovery of ERD-3111 as a Potent and Orally Efficacious Estrogen Receptor PROTAC Degrader with Strong Antitumor Activity

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Estrogen receptor α (ERα) is a prime target for the treatment of ER-positive (ER+) breast cancer. Despite the development of several effective therapies targeting ERα signaling, clinical resistance remains a major challenge. Herein, we report the discovery of a new class of potent and orally efficacious ERα degraders using the PROTAC technology with ERD-3111 being the most promising compound. ERD-3111 potently and effectively induced degradation of ERα protein in ER+ MCF-7 and T47D cells. Importantly, it achieved an excellent pharmacokinetic profile in rats, mice and dogs and good oral bioavailability in these species. ERD-3111 shows excellent microsomal stability and exhibits no significant hERG or CYP inhibition. PK/PD studies demonstrated that oral administration of ERD-3111 is highly effective in reducing the levels of wild-type and mutated ERα proteins in xenograft tumor tissues and achieves high plasma and tumor tissue exposures. Consistent with effective depletion of wild-type and ERα mutated proteins in tumor tissues, ERD-3111 demonstrates strong antitumor activity and is capable of achieving persistent tumor regression in the MCF-7 ER wild-type and *ESR1*^{D538G} xenograft tumor models or 100% of tumor growth inhibition in the MCF-7 *ESR1*^{Y537S} mutated xenograft tumor model. Significantly, ERD-3111 treatments did not cause animal weight loss or exhibit other signs of toxicity in mice. Taken together, our data show that ERD-3111 is a potent, orally bioavailable and highly efficacious ERα PROTAC degrader, and represents a promising lead compound for extensive evaluations for the treatment of ERα+ breast cancer.

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42. Discovery of CBPD-409 as a Highly Potent, Selective, and Orally Efficacious CBP/p300 PROTAC Degrader for the Treatment of Advanced Prostate Cancer

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CBP/p300 are critical transcriptional co-activators of the androgen receptor (AR) and are promising therapeutic targets. Herein, we report the discovery of highly potent, selective, and orally bioavailable CBP/p300 degraders using the PROTAC technology with CBPD-409 being the most promising compound. CBPD-409 achieved DC_{50} values of 0.2-0.4 nM and D_{max} >90% against CBP/p300 proteins in the VCaP, LNCaP and 22Rv1 prostate cancer cell lines. Proteomic analysis showed that CBPD-409 is highly selective in reducing CBP/p300 proteins over other 5,000 proteins examined in the VCaP cell line. CBPD-409 was highly potent and effective in inhibition of cell growth with IC_{50} values of 1-2 nM in the VCaP, LNCaP and 22Rv1 cell lines and are much more potent than two CBP/p300 inhibitors (GNE-049 and CCS1477), AR degrader (ARV-110) and AR antagonist (Enzalutamide). Mechanistic studies revealed that CBPD-409 potently and effectively reduced the expression of AR, KLK3 and c-MYC genes in the VCaP, LNCaP and 22Rv1 cell lines and are much more potent and effective than GNE-049 and CCS1477, ARV-110 and Enzalutamide. CBPD-409 displayed a good PK profile in mice and achieved 50% of oral bioavailability. Pharmacodynamic study showed that a single oral dose of CBPD-409 at 1 and 3 mg/kg attained nearly complete depletion of CBP and p300 proteins in the VCaP tumor tissue at 3 and 24 h time-points. Our efficacy experiment demonstrated that CBPD-409 was highly efficacious in inhibition of tumor growth at 0.3 and 1 mg/kg daily dosing and was more efficacious than GNE-049, CCS1477 and Enzalutamide at 20-30 mg/kg. Furthermore, CBPD-409 at 1 mg/kg daily dosing was also more efficacious than ARV-110 at 10 mg/kg daily dosing. Interestingly, CBPD-409 at 1 mg/kg dosed every other day was also very effective in inhibition of tumor growth. Of significance, CBPD-409 was well tolerated in our efficacy experiment and showed no signs of toxicity in mice at its highly efficacious dose-schedules. Taken together, CBPD-409 represents a promising, highly potent and orally efficacious CBP/p300 degrader and warrants extensive investigations as a potential new therapy for the treatment of human prostate cancer and other types of human cancers.

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43. Discovery of a potent and selective small-molecule degrader of STAT6 as a new class of Immuno-Oncology Therapy

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STAT6 is an attractive therapeutic target for not only the treatment of Th2 inflammation driven diseases such as asthma and atopic dermatitis, but also the modulation of tumor microenvironment to treat human cancers. Here, we present the discovery of a potent, selective STAT6 degrader that is effective *in vivo*. Starting from a very weak STAT6 ligand with elusive selectivity, we developed a STAT6 ligand with a low nanomolar affinity and excellent selectivity to STAT6. We devised the STAT6 ligand into STAT6 PROTAC degraders with AK-1690 as the best compound. We obtained the first co-crystal structure of AK-1690 bivalently complexed with STAT6. AK-1690 induces an efficient degradation of STAT6 as well as $pSTAT6^{Y641}$ in cells with DC_{50} of as low as 1 nM while showing minimal effect on the expression levels of other STAT family proteins. AK-1690 also effectively depletes STAT6 in mouse tissues. AK-1690 activated cytotoxic T-cells and slowed tumor growth in a 4T1 xenograft model. AK-1690 will serve as an excellent tool compound in the study where STAT6 is involved and potentially is a nice lead compound for the development of STAT6 targeted therapeutics.

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44. Development of a TR-FRET assay to screen for neo-protein-protein interaction inhibitors

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Small molecule inhibitors to attenuate $BRAF^{V600E}$-mediated oncogenic activity are crucial to therapeutically treat $BRAF^{V600E}$ driven cancers. However, patients often exhibit intrinsic or acquired resistance to these drugs. Therefore, there is a need to develop innovative, and precise strategies that could be used to treat $BRAF^{V600E}$ driven cancers. We have previously discovered that $BRAF^{V600E}$ has an increased interaction with the tumor suppressor KEAP1, forming a mutation-created neo-protein-protein interaction (PPI). This neoPPI leads to NRF2 upregulation even in the absence of oxidative stress, possibly exerting a cytoprotective effect on cancer cells. We hypothesize that inhibiting $BRAF^{V600E}$ and KEAP1 interaction with small molecules would provide an avenue to understand the role of $BRAF^{V600E}$ and KEAP1 PPI in tumorigenesis and therefore offer novel therapeutics for $BRAF^{V600E}$ driven cancers. In order to discover $BRAF^{V600E}$/ KEAP1 PPI inhibitors, we developed a time resolved fluorescence resonance energy transfer (TR-FRET) assay in a 1536-well ultrahigh-throughput screening format (uHTS) to monitor the $BRAF^{V600E}$-KEAP1 PPI. A pilot screen with 12,807 Emory Bioactive library identified several primary hits. The inhibitory effect of one of the positive hits was further confirmed in an orthogonal anti-flag immunoprecipitation assay. This study has not only validated the TR-FRET assay for uHTS, but also provided the proof-of-concept that the $BRAF^{V600E}$ and KEAP1 PPI can be disrupted by small molecules. This study set the stage for the large-scale screening to discover novel $BRAF^{V600E}$/KEAP1 disrupting chemotypes for therapeutics development.

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45. The Structural Characterization of a Potently Selective NSD2 Degrader in Ternary Complex with a Putative E3 Ligase

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In the field of epigenetic therapeutics, targeted protein degradation (TPD) has become a growing modality in eliminating difficult to inhibit proteins [1]. TPD uses a bifunctional molecule to recruit an E3-ubiquitin ligase to the target protein, resulting in a ternary complex formation that facilitates the ubiquitination and subsequent proteasomal degradation of the target [2]. Previous efforts to inhibit NSD2, a histone lysine methyltransferase, have been unsuccessful until the recent development of a series of bifunctional molecules to selectively degrade NSD2 [3]. However, the E3 being recruited remained unkown. Through recent efforts in characterizing the unknown degrader mechanism by proximity-based biotinylation (BioID), potential candidate proteins responsible for the degrader mediated effects were deduced. One particular candidate E3 was of interest and has been further subjected to biophysical and structural studies. This handle has been tested via Size Exclusion Chromatography, Differential Scanning Fluorimetry, as well as Hydrogen Deuterium Exchange Mass Spectrometry to confirm its involvement and elucidate the interaction dynamics of this ternary complex formation. With results confirming the formation of this complex, crystallography is being pursued to further understand the interactions taking place. The structural characterization of this degrader in ternary complex with the candidate E3 holds much value as it will allow us to further understand the compounds structure-activity relationship as well as to facilitate improved optimization of the chemical degrader. This also creates opportunities for further advancements in novel therapeutics that could be developed towards other un-targetable oncogenic proteins.

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46. Ultrahigh-Throughput Screening Assays to Identify 14-3-3 Isoform-Selective Modulators

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14-3-3 is a master cellular regulator with hundreds of binding partners, many of that are involved in disease-relevant pathways such as cancer. The implications of 14-3-3's role in disease progression have previously led to the characterization of 14-3-3 modulators. Yet, there are seven isoforms of 14-3-3 proteins and the multitude of binding partners. Therefore, the discovery of an isoform-specific and protein-protein interaction (PPI)-specific small molecule modulators is greatly needed. In this study, we developed fluorescent polarization (FP) assays for each 14-3-3 isoform in a 1536-well ultrahigh-throughput screening format (uHTS) for small molecule modulators of Bcl-2-antagonist of cell death (BAD) and Yap. The two 14-3-3 binding partners, BAD and Yap, are highly dysregulated in cancer due to their modulation of apoptosis and cellular proliferation, respectively, underlining them as prominent proteins of interest. The primary hits from the screening were selected for validation with anti-GST immunoprecipitation assays and functional evaluation. This study demonstrates the capability of FP assays to identify isoform-specific and PPI-specific modulators, allowing the exploration of the PPI's role in cancer and has significant therapeutic importance in precision medicine.

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47. Discovery of Aminothiazolones as Small-molecule Inhibitors of the RNA–modifying protein METTL16

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Modulation of post-transcriptional modifications by targeting RNA-binding and modifying proteins *via* small molecules has emerged as a new frontier in the chemical biology and medicinal chemistry research. One such RNA-binding protein is the methyltransferase-like protein 16 (METTL16) that deposits N^6-methyladenosine (m^6A) in substrate transcripts. Besides the methyltransferase activity in the nucleus, METTL16 has been associated with gene regulation by promoting the translation of mRNA substrates in the cytosol. Together with the eukaryotic initiation factors 3a and -b (eIF3a/b), METTL16 was shown to play a potent oncogenic role in hepatocellular carcinoma. To date, no potent small-molecule inhibitor interfering with the protein–RNA interaction (PRI) of METTL16 and corresponding RNAs has been reported, highlighting the unmet need to develop such METTL16-targeting small molecules. Herein, we described the identification of a series of first-in-class aminothiazolones METTL16 inhibitors *via* a discovery pipeline started with a fluoresce polarization-based (FP) screening. Structural optimization of the initial hit yielded inhibitors with improved inhibitory potencies. The most potent compound GG416 showed potent single-digit micromolar inhibition activity against METTL16 in a methyltransferase activity assay. The identified aminothiazolone inhibitors not only will be useful probes to elucidate the biological function of METTL16 upon perturbation but also set the foundation for the further development of more potent inhibitors for therapeutic applications.

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48. Cellular Context For mRNA Substrate Selection By Pseudouridine Synthase 7 (PUS7)

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Human pseudouridine synthase (PUS) enzymes isomerize uridine bases to pseudouridine (Ψ) in noncoding and mRNAs. Altered pseudouridylation by PUS7 is implicated in several diseases. The mechanisms of PUS7-depedent substrate modification and how Ψ contributes to disease pathogenesis are unclear. Without understanding the cellular contexts that direct PUS7 target selection, we cannot predict the functional consequences of PUS7-dependent Ψ in mRNA. We hypothesize that distinct RNA structural contexts and protein-mRNA interactions drive selection of Ψ sites by PUS7 in cells. We are employing live-cell chemical probing combined with sequencing technologies to identify these cellular contexts. Preliminary data from interaction network probing in human cells suggest a trend for limited protein-binding at Ψ sites compared to unmodified sites, though it is not statistically significant with the current number of targets. We plan to sequence additional targets and repeat probing in PUS7-knockout human cells to identify molecular features in the absence of Ψ. Structural probing experiments will also be performed in the same human cell lines. We anticipate that the knowledge generated from this research will allow us to predict novel PUS7-dependent Ψ sites and may be suggestive of a novel gene regulatory mechanism based on RNA modifications.

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49. Biological characterization of small molecule eIF4E inhibitors to assess their therapeutic potential in cancer

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Dysregulation of translation is a hallmark of cancer that enables rapid changes in the cell proteome to shape oncogenic phenotypes that promote tumor survival.[1] The predominant signaling pathways leading to dysregulation of translational control in cancer are the PI3K-AKT-mTORC1, RAS-RAF-MAPK, and MYC pathways,[2,3,4] which all converge on eukaryotic translation initiation factor 4E (eIF4E), an RNA-binding protein which binds to the m^7GpppX cap structure at the 5' end of mRNAs to initiate cap-dependent translation.[2,5] eIF4E is the rate-limiting factor of translation initiation, and its overexpression is known to drive oncogenic transformation, progression, and chemoresistance across many cancers, establishing it as an attractive therapeutic target.[2] Traditionally, target transcripts of eIF4E have been characterized as mRNAs with long 5'-UTR motifs,[6,7] but recent studies have demonstrated that select pools of eIF4E-sensitive transcripts are modulated depending on its regulation status.[8-10] Therefore, it is hypothesized that mechanistically distinct eIF4E inhibitors will elicit different responses in tumors based on the dysregulation of select pools of eIF4E-sensitive transcripts. Research in our laboratory focuses on developing mechanistically distinct eIF4E inhibitors, including m^7GpppX prodrug derivatives which bind directly to the cap-binding pocket of eIF4E. Biological characterization of these molecules, as well as efforts towards translational profiling will be discussed.

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50. Cell-Based Assay Development Strategies for the Detection and Validation of Aberrant mRNA-Protein Interactions

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RNA-binding proteins (RBPs) make up a class of over 2,000 proteins that bind to and regulate the diverse functions of various types of RNAs, and accordingly, are involved in controlling many cellular processes. Disruption of RNA-Protein Interactions (RPIs), consequently, has been implicated in human diseases ranging from neurodegenerative and autoimmune diseases to several human cancers. Hence, targeting RBPs and RPIs has surfaced as a new frontier in RNA-targeted drug discovery which takes advantage of the endogenous regulation of messenger RNA (mRNA). This is an alternative strategy to methods currently used in the field to target transcripts which rely on antisense oligonucleotides (ASOs) or microRNAs, resulting in the degradation and silencing of mRNA, respectively. ASO- or miRNA-mediated direct targeting of RNA transcripts poses challenges, as these compounds often possess issues with specificity, delivery, and tolerability. The aim of this work is to characterize the high-affinity interactions of RBPs with mRNA motifs through live-cell detection using an assay previously developed for the detection of pre-miRNAs and their RBP partners, RNA-interaction with Protein- mediated Complementation Assay (RiPCA). Our goal is to be able to expand RiPCA to allow us to study other more complex RPIs in cells composed of RNAs that are larger and more structurally diverse than pre-miRNAs and that bind to proteins which perform a variety of cellular functions. Initial studies to accomplish this included three representative examples motifs found in 3' untranslated regions (UTRs) of mRNA such as (1) expanded repeat RNA, (2) UAGUAG target sequence, and (3) AU-rich elements (AREs). My efforts towards developing these assays will be discussed, as well as future directions aimed at further improvement of this assay technology. Through RiPCA optimization, we hope to generate a platform for detecting and validating various RPIs in live cells to enable screening and drug discovery efforts.

51. A Live-Cell Assay to Detect eIF4E-mRNA Interactions

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Translation initiation of eukaryotic mRNAs (mRNA) begins with the recognition of the 5' m^7GpppN cap by the cap-binding protein known as eukaryotic translation initiation factor 4E (eIF4E).[1] Overexpression of eIF4E, often the case in human cancers, results in increased translation of mRNAs encoding oncoproteins and growth factors.[2-3] Consequently, identifying high-affinity ligands that inhibit eIF4E levels has been of high interest as a potential therapeutic strategy.[4-6] To enable these efforts, we have developed a live-cell assay system for detecting the interaction of eIF4E and m^7G capped mRNA using our laboratory's **R**NA **i**nteraction with **P**rotein-mediated **C**omplementation **A**ssay (RiPCA) technology.[7] Development of this methodology, as well as its application towards small molecule inhibitor testing will be discussed.

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52. Unraveling the function of TRAF2 and NCK interacting kinase (TNIK) in high-grade serous ovarian cancer

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The large majority of high grade serous ovarian cancer (HGSC) patients will eventually become resistant to platinum-based chemotherapy. Therefore, novel therapeutics are required to better treat recurrent, chemotherapy resistant disease. Using an artificial intelligence (AI)-enabled drug discovery platform developed by BenevolentAI, we sought to identify unique therapeutic targets to treat these cancers. Using two patient derived platinum-resistant models along with non-malignant fallopian tube secretory cells (FTSE), we screened an AI-generated commercially available library of compounds predicted to target known or postulated resistance mechanisms that was not previously identified in HGSC. Through this screen, we discovered a drug known to target TNIK, a critical driver of the Wnt/B-catenin pathway, as the top hit to specifically kill HGSC spheres and not non-malignant fallopian tube cells from this screen. Interestingly, several other TNIK inhibitors in the screen did not affect tumor sphere viability, suggesting that an alternative target in conjunction with TNIK may be contributing to the compounds potency or that the TNIK was inhibiting another kinase. Intriguingly, using AI in conjunction with bench-side experimentation, we identified CDK9 as a critical mediator of the downstream effects of this compound. This work demonstrates the combinatorial ability of using AI, patient-derived models, and in-silico modeling to uncover novel targets for cancer treatment and expedite the drug discovery.

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