Session 2: Talk 1

James Ellegate
Purdue University
MCMP, Graduate Student (4th Year)
PI: Carol Post

Customizing a Molecular Dynamics Protocol to Explore an Alternative Binding Orientation of Src Kinase Peptide Substrates

Src is a tyrosine kinase that regulates cellular adhesion, migration, and proliferation, and its aberrant activity in multiple cancers makes it a promising therapeutic target. Due to high conservation of the ATP-binding site among kinases, selectivity of drugs for Src remains an issue. An approach with the possibility of improved selectivity is targeting the substrate-binding site. Given that no structure of substrate-bound Src exists, it is assumed that Src engages its substrates like other tyrosine kinases with known peptide substrate complexes. These complexes orient the C-terminal end of the peptides below the activation loop. However, NMR data suggest that Src may engage its peptide substrates like serine/threonine kinases, which orient the C-terminal end of the peptides above the activation loop.

In this work, NMR data are incorporated into an on-the-fly molecular dynamics simulation approach. This custom workflow exhaustively samples the two binding positions by dynamically iterating between restrained and unrestrained simulations according to the immediate peptide sampling. We anticipate that this approach will help distinguish the structural and energetic features that determine the preferred binding position. This work challenges the current paradigm that Src engages its substrates in an orientation identical to other tyrosine kinases and provides a well-grounded assessment of peptide recognition, with potential to inform the rational design of therapeutics targeting the substrate-binding site.

Session 2: Talk 2

Nick Brauer
Purdue University
Chemistry, Graduate Student (5th Year)
PI: Jean Chmielewski

Advancing a Cure for HIV-1: Design and Development of Small Molecule Dual Agents that Simultaneously Reverse Latency and Prevent New Infection

The advancement of highly active anti-retroviral therapeutics, which can reduce the viral copies of HIV-1 in an infected patient to undetectable levels, has drastically improved patient outcomes in recent years. Suspension of treatment leads to viral rebound, however. This phenomenon is caused by an HIV-1 infected group of resting cells that evades current therapeutics and the host immune system, known as HIV latency.

Several methods to eradicate the latent reservoir have been proposed, including a strategy known as “shock and kill,” which targets enzymes responsible for maintaining the latent state. Although this approach has reached clinical testing, it has not yet successfully reduced the latent reservoir. One possible explanation is that latency reversal can result in the production of mature HIV-1 virions. Herein, we describe a novel dual agent designed to purge the latent reservoir through class I histone deacetylase inhibition while simultaneously preventing new infection through HIV-1 protease inhibition.

Session 2: Talk 3

Nipuni Pannala
Purdue University
Chemistry, Graduate Student (4th Year)
PI: Chittaranjan Das

Substrate biasing in UCHL5 proteoforms by E2-mediated N-terminal ubiquitination

Deubiquitinating enzymes (DUBs) are cysteine and metalloproteases that reverse ubiquitin-mediated post-translational modifications. Although ubiquitination is typically coordinated through the concerted action of E1, E2, and E3 enzymes, UBE2W can mediate E3-independent α-amino methionine ubiquitination. Recent studies using Gly-Gly-Met-specific antibodies identified UCHL1 and UCHL5, members of the ubiquitin C-terminal hydrolase family, as substrates of UBE2W.

Here, we report the crystal structure of N-terminally ubiquitinated UCHL5, revealing ubiquitin bound to a unique site on the backside of the protein opposite the canonical S1 binding site. This represents the first structural characterization of ubiquitin engaging the backside of UCHL5 and uncovers a mechanism by which N-terminal ubiquitination remodels substrate specificity. UBE2W-mediated N-terminal ubiquitination inhibits the ability of UCHL5 to debranch Lys48-linked branch points while activating the enzyme toward mono-ubiquitin substrates. Furthermore, association with Rpn13 relieves this inhibition and restores debranching activity, suggesting that this proteoform functions selectively on the proteasome. Together, these findings reveal a previously undocumented mechanism of substrate biasing in a DUB that is noncanonically ubiquitinated by an E2 enzyme.

Session 2: Talk 4

Cara Trench
Purdue University
Chemistry, Graduate Student (4th Year)
PI: Angeline Lyon

Identification of Isoform-Specific Differences in Phospholipase Cβ Regulation

Cardiovascular disease is the leading cause of death worldwide. Coronary heart disease and arterial disease account for nearly half of all cardiovascular disease-related deaths. Phospholipase C (PLC) enzymes are implicated in these pathologies through their role in cleaving phosphatidylinositol-4,5-bisphosphate, generating signaling molecules that elevate intracellular calcium and activate protein kinase C.

Among the six PLC subfamilies, PLCβ enzymes are of particular interest because they are activated downstream of G protein-coupled receptors through direct interactions with Gαq and Gβγ subunits. Although the four PLCβ isoforms differ in basal activity and responsiveness to activation, the molecular basis of these differences remains poorly understood. We recently determined a cryo-electron microscopy reconstruction of human PLCβ2 that revealed striking differences in the organization of its proximal C-terminal regulatory domain. We are now using cell-based studies to determine how proximal and distal C-terminal domains contribute to basal and G protein-stimulated activities across all four PLCβ isoforms. These studies will reveal isoform-specific regulatory mechanisms that may ultimately guide therapeutic strategies targeting PLCβ signaling in cardiovascular disease.

Session 2: Talk 5

Indranil Arun Mukherjee
Purdue University
Biology, Postdoctoral Researcher
PI: John Tesmer

Structural Insights into GPCR Signal Transduction: From Kinase Regulation to Effector Activation

G protein-coupled receptor (GPCR) signaling is a dynamic process governed by transient interactions with G proteins, kinases, and downstream effectors. This presentation outlines recent cryo-electron microscopy findings that illuminate specific molecular events throughout this signaling continuum.

We capture kinase membrane targeting and basal regulation through structures of the GRK3–Gβγ complex and GRK5 bound to the inhibitor sangivamycin. Receptor engagement is examined through comparisons of GRK2–Rhodopsin and high-resolution GRK5–Rhodopsin complexes. We also present structural studies of atypical GPCR regulation involving ACKR3-CXCL12 and ongoing efforts to characterize transient kinase–receptor intermediates. Finally, structures of the Rho–Trio–Gαq complex and adenylyl cyclase 1 bound to Gαs provide insight into G protein coupling specificity and downstream signal amplification. Together, these structural snapshots contribute to a more precise understanding of GPCR signaling mechanisms.

Session 2: Talk 6

Amala Phadkule
Purdue University
Chemistry, Graduate Student (5th Year)
PI: Mike Reppert

Identifying the Molecular Origins of Lowest-Energy Fluorescent States in Photosystem II Antenna Proteins

Photosystem II is the protein-pigment complex responsible for light-driven water oxidation in oxygenic photosynthesis. Its core antenna subunits, CP47 and CP43, harbor distinct lowest-energy chlorophyll states whose molecular origins have remained debated for decades. Identifying the chlorophylls responsible for these states is essential for understanding energy transfer and photoprotection within Photosystem II.

Using site-directed mutagenesis in the cyanobacterium Synechocystis sp. PCC 6803 together with 77 K fluorescence spectroscopy, we investigated the hydrogen-bonding environment of candidate chlorophylls in both antenna subunits. To isolate Photosystem II fluorescence in whole cells, we engineered a strain with copper-controlled Photosystem I expression and lacking the phycobilisome antenna. Our results confirm chlorophyll B16 as the origin of the CP47 F695 state, resolving a long-standing debate. For CP43, we are employing site-saturated mutagenesis libraries to systematically identify the pigment-protein interactions that define the F685 state.