DICKERSON BIOCHEMISTRY SEMINAR SERIES

Midstream Presentation - Spring 2023

Development of a high-throughput SP96 platform for multi-use cysteine chemoproteomic workflows

Flowreen Shikwana

Backus Group

Chemoproteomics has enabled the discovery of chemical probes to study the druggability of several protein classes, notably leading to FDA approved covalent small molecules such as the KRAS G12C inhibitor, sotorasib. While various nucleophilic side chains of amino acids can be targeted using chemical probes, cysteines are of particular interest due to their structural (binding motifs) and functional (active sites, redox potential) roles within proteins. Improving and expanding upon chemoproteomic methodologies to increase coverage of reactive cysteines paves the way for drug discovery efforts towards proteins which have remained untargeted. Here, we present the development of a high-throughput, multiplexed cysteine chemoproteomic workflow (termed SP96) as a multi-use platform for expanding coverage of the cysteinome, screening for covalent molecules, and for thermal proteome profiling (TPP). Expanding the detectable cysteinome will be achieved through cysteine-reactive molecule screens performed on protein fractionated samples within the SP96 format. TPP is used to identify proteome-wide changes in protein thermal stability upon treatment with small molecules; however, high-volume TPP sample preparation necessitates high-throughput platforms such as SP96 to streamline sample preparation and data acquisition. Cysteine-enabled TPP will be utilized for target deconvolution of lead compounds which can be used in the context of covalent and noncovalent molecules.

Characterizing Mitochondrial Double-Stranded RNA (mtdsRNA) Escape and Elicitation of the Innate Immune Response

Connor Short

Koehler Group

PNPase is a 3'-5' exoribonuclease located in the IMS which functions in tandem with SUV3 helicase to regulate mitochondrial RNA by degrading double-stranded RNA. Knock-Down (KD) of either results in accumulation of mtdsRNA as confirmed by immunofluorescent microscopy. PNPase KD displays the additional phenotype of mtdsRNA escape into the cytosol where it forms aggregates. PNPase KD is also accompanied by an increase in type-I INF levels suggesting it may function as a Damage Associated Molecular Pattern (DAMP). Mutational studies seek to elucidate the possible mechanisms of mtdsRNA escape and the role of PNPase in normally preventing this by disambiguating the several functions of this protein. The size of double-stranded RNA would require a transport mechanism or channel for its export, and several putative channel forming proteins have been investigated. Preliminary data suggests Voltage Dependent Anion Channel (VDAC) is the Outer Membrane (OM) channel protein required for the escape as seen in KD and small-molecule inhibition experiments. An in vitro analysis of PNPase mutants in conjunction with SUV3 is explored to investigate the relationship between mtdsRNA degradation and processing with clinical symptoms of mitochondrial dysfunction.

Friday, May 12th at 3:30pm

Mani L. Bhaumik Collaboratory,

Dongwon Yoo Seminar

& Conference Hall (Young Hall 4222)

More information: marla@chem.ucla.edu

UCLA College | Physical Sciences Chemistry & Biochemistry