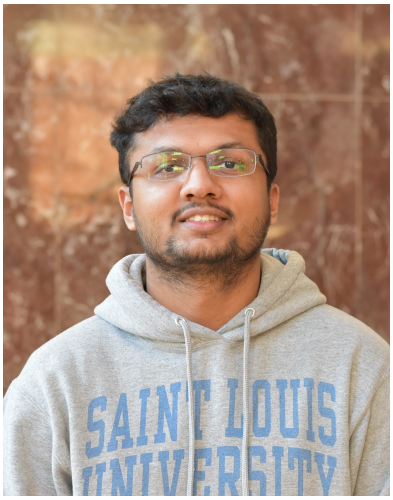
JOSEPH F. FOSTER MEMORIAL
CHEMICAL BIOLOGY AND BIOCHEMISTRY SEMINAR

Monday, December 4, 2023
3:30 PM, BRWN 4102



"Covalent chemistry driven profiling of deubiquitylating enzymes"

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Abstract: Protein ubiquitylation and deubiquitylation is an important post-translational modification (PTM) that regulates key processes in protein degradation and trafficking, mitophagy, cell-cycle control, and DNA damage response. This is all elicited by the joint effort between E1 Ubiquitin (Ub) activating enzymes, E2 Ub conjugating enzymes, and E3 Ub ligases that attach Ub to substrate protein lysine residues. This ligation machinery modularly recognizes its substrates to assemble the many different flavors of Ub and polyUb modifications, in turn assembling a sense of a "Ub code". As with any elicitation of PTMs, ubiquitylation is rendered reversible by proteolytic deubiquitinating enzymes (Dubs) that dynamically recognize diverse sets of Ub modifications. Any deregulation in these processes is often accompanied by disease pathogenesis, a feature resulting to Ub related enzymes having become a focal point in current therapies. This seminar will focus on the development of Dub probes, initially by small fragment molecule libraries and further by genetically encoded unnatural amino acids (UAAs). First, I report the structural basis of fragment inhibition of a UCHL1, a Dub heavily implicated in neuronal degeneration, and the first inhibitor bound structure of UCHL3, a Dub that plays an important role in DNA damage response. The structure of UCHL1 with the lead fragment reveals the role of enzyme plasticity in potency towards different inhibitor scaffolds. Structural comparison between our chloroacetohydrazide scaffold and the recent structurally reported cyanopyrrolidine scaffold show that a lack of one hydrogen bond leads to a substantial loss in inhibition. While these covalent inhibitor fragments represent probes similar to Ub C-terminal electrophiles, I have developed a new class of interactions probes that when coupled with bottom-up proteomics describe multivalent binding modes for Dubs that recognize the diversity of polyUb chains. We discern interaction interfaces through the identification of crosslinked peptides on Dubs from both eukaryotic and prokaryotic origin. Our results depict a more dynamic picture of solution-phase interactions. We capture binding ensembles of Ub-Dub interactions showing both S1 and S1' binding for enzymes, allosteric binding, and support previously observed sites for possible debranching. While further investigations need to be performed to understand the structural and functional relevance of these interactions, we believe that these probes represent a new generation to the widely used Ub-electrophiles such as Ub-glycine vinyl sulfone. Their ability to assess multivalency and tandem application with LC-MS/MS bottom-up proteomics gives a unique dimension to investigate Ub-protein interaction modes.