Protein homeostasis is essential to maintain normal cellular function, as its deregulation leads to various pathological disorders including cancer. One of the cellular strategies to control protein homeostasis involves modification of proteins after their biosynthesis. There are many types of protein modifications, one of them is the covalent attachment of small protein - SUMO to cellular proteins in a process called SUMOylation. Sumoylation of cellular proteins influences their activity, localization, stability, and interaction with other proteins. An important feature of sumoylation is its reversibility, mediated by SUMO-specific proteases (SENPs). SENPs are also required to activate SUMO proteins by removing a short fragment from the C-terminus of pro-SUMO, thereby tightly regulating the sumoylation levels of cellular proteins necessary for normal cell physiology. Biological studies on SENPs function revealed that these proteases play an important role in the development of human diseases including cancer. Elevated level of SENP proteases is observed in many types of cancer, including colon, breast, lung, ovary, bladder, pancreatic, and liver. Thus, SENP proteases appear as an attractive therapeutic target in drug development. However, the precise role of individual SENPs in normal and pathological states still remains to be elucidated. Selective and potent chemical tools could shed light on SENP possible mechanisms in tumorigenesis, and examine new strategies for cancer therapy. The major challenge in the discovery of SENP selective substrates and activity-based probes lies in their similar substrate specificities. Since SENPs are highly specific toward SUMOs, the most commonly used SENP assay reagents are based on full length or truncated SUMO proteins. Due to the lack of selectivity, utilization of these types of tools prevents from the accurate determination of which of SENP proteases is overexpressed or suppressed in pathological disorders. Taking this into account, the purpose of this project is to design selective SUMO-based chemical tools for SENPs. Recently, our group developed a new chemical approach to obtain selective substrates and probes toward proteases (DUBs) that recognize a small protein called ubiquitin. Ubiquitin-based substrate and probe selectivity toward individual DUBs was achieved by modifying only the C-terminal motif of ubiquitin. Given this, we hypothesize that selective chemical tools for individual SENPs can be obtained by modifying the SUMO C-terminal motif. Importantly, selective substrates and activity-based probes towards individual SENPs have never been reported before, which represents the novelty of the project.

The implementation of the above research goal will involve modification of SUMO C-terminal motif. To select amino acids that can be incorporated into C-terminus, SENPs substrate preferences will be determined. SUMO-based substrates with fluorophore and biotinylated SUMO-based probes will be designed towards each SENP and synthesized. The activity and selectivity of the SUMO-based tools will be evaluated using purified recombinant SENPs and cell lysates.

Our new SUMO-based chemical tools will significantly expand the existing “toolbox” to study SENPs. Selective substrates and activity-based probes towards individual SENPs will be valuable biochemical tools to establish their precise biological function in normal and pathological states. Activity-based probes are the most commonly used chemical tools in proteolytic enzyme investigation, as they enable to monitor protease activity as a function of various biological and chemical perturbations.