LIGHTNING TALK:

Dr. Roger Ashmus

Development of fluorescence-quenched substrate probes for multiplexed imaging of glycosidase activity within live cells

Roger A. Ashmus[1], Pierre-André Gilormini[1], Sha Zhu[1], Xi Chen[1], Matthew C. Deen[1], David J. Vocadlo[1,2]

[1]Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada
[2]Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada

Glycosidases play an essential role in human health and many have been directly linked to diseases. Developing tools that can modulate their activity is therefore of interest toward further studying these class of enzymes as well as for discovering new therapies (e.g. novel pharmacological chaperones). Few small molecules targeting glycosidases, however, have reached the clinic. This limited process stems, in part, from there being a limited number of tools that enable studying the regulation of these enzymes within their native cellular environment. One such tool that has emerged from efforts aimed at filling this need is the use of substrates that can monitor glycosidase activity directly in tissues.

Fluorescence-quenched substrates (FQS) used as probes are powerful tools for monitoring the activity of hydrolytic enzymes in their native cellular environment. The pocket-shaped active sites of *exo*-glycosidases are, however, sterically demanding making the development of such probes for these enzymes difficult. We previously proposed a strategy to address this problem by use of a bis-acetal motif that conveniently enables linking commercial fluorophores and quenchers to the aglycone leaving group, thereby leaving the carbohydrate core of the substrate intact. Using the design, we developed two novel probes and demonstrated they can for the first-time quantitatively monitor lysosomal α-galactosidase and α-N-acetylgalactosaminidase activities directly in live cells. We then showed these probes can be used to monitor the activity of these enzymes within live Fabry disease patient fibroblasts and measure the efficacy of an FDA-approved pharmacological chaperone. In addition to showcasing the work above, we reveal a new FQS probe capable of quantitatively monitoring another glycosidase activity in live cells including patient fibroblasts having a different lysosomal storage disease. We then highlight the advantage of using these FQS probes in parallel to monitor multiple hydrolytic enzymes by multiplexed quantitative imaging. In addition to facilitating the development of new diagnostic methods, we envision using these FQS will advance current therapeutic approaches as well as aid in the discovery of new lead compounds to treat associated lysosomal storage diseases.