

DO SURFACTANTS REALLY ASSIST TRYPSIN DIGESTION? AN ASSESSMENT OF CUMULATIVE ENZYME ACTIVITY SAYS OTHERWISE. **Alan Doucette**¹, & Jessica Nickerson², ¹Department of Chemistry, Dalhousie University, Halifax, Nova Scotia; ²Allumiqs Corporation, Halifax, Nova Scotia, Canada.

Multiple reports have demonstrated that certain solution additives, such as surfactants, will enhance enzymatic activity when added at specific concentrations [1]. However, our group has recently shown that enhanced enzyme activity may be short lived, as kinetic assays demonstrate an accelerated decline in sustained trypsin activity [2]. Here, we discuss the impacts of surfactants (sodium dodecyl sulfate, SDS; sodium deoxycholate, SDC), previously employed in surfactant-assisted proteomics workflows to enhance proteome analysis. Although low surfactant concentrations (0.01% SDS or 0.2% SDC), significantly enhances the initial trypsin activity, time course spectroscopic enzyme activity assays revealed accelerated enzyme deactivation, with a 10 to 40-fold reductions in enzyme activity half-life. Quantitative mass spectrometry analysis of a proteome extract, digested with surfactants, consistently revealed a decreased peptide count and lower signal intensity, indicative of lower digestion efficiency compared to a non-surfactant control. Furthermore, detergents did not improve detection of membrane proteins, nor hydrophobic peptides. These results stress the importance of assessing cumulative enzyme activity when optimizing the digestion of a proteome mixture, particularly in the presence of denaturants.

[1] T. Masuda, M. Tomita, Y. Ishihama. *J. Proteome Res.* 7 (2008) 731–740.

[2] J. Nickerson, A. Doucette. *Biology.* 11 (2022) 1444.