Glycosylation analysis in glycoproteins using enzymatic digestion and mass spectrometry (Prof. Heather Desaire) The main objective of the Desaire group in glycoprotein characterization is to obtain molecular-scale information about carbohydrates attached to proteins. These studies are critically needed because glycosylation of proteins can affect protein folding, receptor binding, and degradation or excretion of a protein. The tools available for studying these structure-function relation-ships are currently limited, especially for proteins with multiple glycosylation sites. The traditional analytical tools for carbohydrate characterization typically do not provide glycosylation information in a site-specific manner, despite its importance: changes in glycosylation at one site on the protein may dramatically impact protein folding, whereas changes at another site may exclusively affect other factors, e.g., protein binding. Thus, Prof. Desaire's group is developing methods to profile carbohydrates in a glycosylation site-specific fashion, by analyzing glycopeptides. REU students will use enzymatic digestion and mass spectrometry as primary tools to obtain information about glycosylation on proteins. The participants will address important research questions such as what is the best enzyme or series of enzymes to use for generating good MS data of glycopeptides? REU students will acquire a variety of research skills, e.g., preparation/analysis of biochemical assays, enzymatic digestion techniques, sample purification using reverse-phase HPLC and affinity chromatography, and instrumental techniques including ESI-MS and MALDI-MS. Since arriving at KU in 2002, Prof. Desaire has published seven papers with eleven undergraduate co-authors including two NSF REU students.⁴⁵⁻⁵¹

- Bandu, M. L.; Watkins, K. R.; Bretthauer, M. L.; Moore, C. A.; Desaire, H. "Prediction of MS/MS data. 1. A focus on pharmaceuticals containing carboxylic acids." *Anal. Chem.* 2004, 76, 1746-1753.
- Dalpathado, D. S.; Jiang, H.; <u>Kater, M. A.</u>; Desaire, H. "Reductive amination of carbohydrates using NaBH(OAc)₃." *Analytical and Bioanalytical Chemistry* 2005, *381*, 1130-1137.
- 3 Yi, L.; <u>Dratter, J.</u>; Wang, C.; Tunge, J. A.; Desaire, H. "Identification of sulfation sites of metabolites and prediction of the compounds' biological effects." *Analytical and Bioanalytical Chemistry* 2006, 386, 666-674.
- Dalpathado, D. S.; <u>Chang, Q.</u>; **Burkett, C. M.**; Bandu, M. L.; Desaire, H. "Application of the statistical test of equivalent pathways (STEP) method to the triple quadrupole mass spectrometer." *Rapid Commun. Mass Spectrom.* 2007, *21*, 3365-3372.
- 5. Rebecchi, K. R.; <u>Wenke, J. L.</u>; Go, E. P.; Desaire, H. "Label-free quantitation: A new glycoproteomics approach." *J. Am. Soc. Mass. Spectrom.* **2009**, *20*, 1048-1059.
- 6. Bandu, M. L.; <u>Grubbs, T.; Kater, M.;</u> Desaire, H. "Collision induced dissociation of alpha hydroxy acids: Evidence of an ion-neutral complex intermediate." *Int. J. Mass spectrom.* **2006**, *251*, 40-46.
- Woodin, C. L.; Hua, D.; <u>Maxon, M.</u>; Rebecchi, K. R.; Go, E. P.; Desaire, H. "GlycoPep Grader: A Web-Based Utility for Assigning the Composition of N-Linked Glycopeptides." *Anal. Chem.* 2012, 84, 4821-4829.