SFB Guest Lecture Series

December 5, 2024

Evelyn Rampler

Unveiling Glycosphingolipids Using Novel High-Resolution Mass Spectrometric Assays

Katharina Hohenwallner[1], Marlene Pühringer[1], Amirreza Dowlati Beirami[1], Amanda Schütz[1], Ursula Hiden[2], Dominik Egger[3], Leonida Lamp[4], Jürgen Hartler[4], Gavin Reid[5], Evelyn Rampler[1]

[1] University of Vienna, Department of Analytical Chemistry, Vienna, Austria
[2] Department of Obstetrics and Gynecology, Medical University of Graz, Graz, Austria
[3] Biofabrication for Drug Testing, Leibniz University Hannover, Hannover, Germany
[4] University of Graz, Institute of Pharmaceutical Sciences, Graz, Austria
[5] School of Chemistry, University of Melbourne, Parkville, Victoria, Australia

Glycosphingolipids play critical roles in cell-cell communication, cellular growth, host-pathogen interactions, and signal transduction. They extend the diversity of the lipidome beyond what is typically captured by conventional lipidomics assays, which often focus on bulk lipid classes. However, glycosphingolipidomics remains an emerging field due to the limited availability of commercial standards and comprehensive methodologies for measuring and identifying native glycolipids. Traditional analytical approaches only enable class-specific glycolipid detection. Mass spectrometry (MS), by contrast, allows for the simultaneous monitoring of hundreds of glycolipid species with distinct lipid compositions, identifiable by their mass-to-charge ratios and fragmentation patterns. High-resolution MS is particularly advantageous, offering high mass accuracy, reducing hybrid spectra, and minimizing false annotations.To enhance structural resolution in glycosphingolipid analysis, we developed new high-resolution MS (HRMS)-based strategies incorporating: (1) liquid chromatography, (2) field asymmetric ion mobility spectrometry (FAIMS), and (3) alternative fragmentation techniques such as ultraviolet photodissociation (UVPD) and electron-activated dissociation (EAD). These approaches enable precise characterization of glycosphingolipids at the molecular level, detailing the glycan head group, glycolipid class, ceramide, and fatty acid composition. Additionally, we developed a novel automated annotation workflow for global glycosphingolipid profiling, facilitating robust identification of glycosphingolipids in both human and plant samples. This glycosphingolipidomics approach allows us to monitor glycosphingolipid dynamics in various applications including heat stress in plants, human mesenchymal stem cell differentiation, and maternal gestational diabetes.