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SSSMuG: Same Sample Sequential Multi-Glycomics

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Summary

The mammalian glycome is structurally complex and diverse, composed of many glycan classes such as N- and O-linked glycans, glycosaminoglycans (GAGs), glycosphingolipids (GSLs) and other distinct glycan features such as polysialic acids (PolySia), sulfation and proteoglycan attachment stubs. Various methods are used to analyze these different components of the glycome, but they require pre-fractionated/partitioned samples to target each glycan class individually. To address this need for a knowledge of the relationship between the different glycan components of a biological system, we have developed a sequential release workflow for analysis of multiple conjugated glycan classes (PolySia, GAGs, GSL glycans, N-glycans O-glycans) from the same tissue lysate, termed SSSMuG – Same Sample Sequential Multi-Glycomics. With this sequential glycan release approach, five glycan classes were characterized (or four glycan classes plus proteomics) using enzymatic or chemical release from a single sample immobilized on a polyvinylidene difluoride membrane. The various released glycan classes were then analyzed by a variety of HPLC and/or MS techniques, such as C18 for DMB-labelled sialic acid quantitation, ZIC-HILIC for 2-AB labelled GAG disaccharide separation, and PGC-LC-MS for reduced GSL, N- and O-glycans. Compared to single glycan class release approaches, SSSMuG was able to identify more glycans and more proteins with higher intensity analytical peaks and provides a better comparative normalization of the different glycan classes of the complex glycome. Applying this to a brain lysate sample, we were able to obtain an in-depth glycomics analysis of the mouse brain. To this end, the SSSMuG technology workflow will be a foundation for a paradigm shift in the field, transforming glyco-analytics and facilitating the push towards multi-glycomics and systems glycobiology.