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A novel technique for identifying polysialylated proteins in complex mixtures

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Polysialic acid (polySia) is a large homopolymer of alpha-2,8-linked Neu5Ac residues which has profound consequences for the proteins it is attached to. In healthy human adults, polySia is found in the nervous, reproductive, and immune systems where it contributes to cell migration and reduces the immune responses. PolySia is also abnormally expressed in chronic health conditions, including mental health disorders, autoimmune diseases, and cancers. Its expression is strongly correlated to poor prognoses. However, the mechanisms underlying polySia biology are poorly understood, in part because we do not know what proteins are polysialylated. Currently, there remains less than half a dozen identified polysialylated proteins and our analyses indicate that there are many more which have yet to be discovered. Being able to identify polysialylated proteins in complex mixtures, such as cells or serum, is a crucial fist step in understanding the role of polySia in health and disease.

We have developed novel methodology to identify which proteins in a complex mixture are polysialylated. In this strategy, we label alpha-2,8-linked Neu5Ac with 5-Az-NeuAc using a bacterial polysialyltransferase. We can then attach biotin through strain-promoted azide-alkyne cycloaddition and isolate the resulting biotinylated proteins on streptavidin agarose. These isolated proteins undergo protein identification using mass spectrometry. This new methodology substantially improves upon traditional methods like immunoprecipitation because it allows for stringent washing of the captured proteins with detergents to remove non-specifically bound proteins.

We have demonstrated the utility of this method by correctly identifying neural cell adhesion molecule (NCAM) as a polysialylated protein in the non-Hodgkin's lymphoma cell line NK-92.

In addition to improving our understanding of the role polySia plays in health and disease, these identified proteins have the potential to be used for diagnostic and prognostic testing. This proteomics method is versatile and will be useful for identifying polysialylated proteins from various sources such as immune cells, cancers, and other diseases.