Development of new Bioaffinity phases for Glycoprotein separation and analysis

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The past decade has seen an appreciation of the critical biological significance of glycosylation and the impact that glycans can have on the efficacy, stability and immunogenicity of important glycoproteins. For the biopharma industry the level of product characterization and process monitoring demanded, particularly in the context of glycosylation, is set to increase and will become an even more significant issue with the entry of biosimilar products into the biopharmaceutical arena. In order to meet these demands of the regulatory biopharmaceutical and generic producers alike require rapid , sensitive and high throughput techniques to enable detailed glycoprotein separation and analysis. It is extremely difficult to separate closely related glycoforms using standard chromatographic methods, such as ion exchange chromatography (IEX), gel exclusion chromatography (GEC) and hydrophobic interaction chromatography (HIC).
Here , we report the development of a series of new bio-affinity based phases that are capable of separating closely related glycoproteins/glycoproteins. These phases get their selectivity from a number of recombinant prokaryotic bioligands called lectins. Lectins are proteins that are capable of recognizing and binding reversibly to specific glycan structures. While lectin binding affinities for monosaccharides are generally low they bind to disaccharides and more complex oligosaccharide structures with significantly higher affinities and exquisite specificity.
We have identified, cloned , expressed and purified a series of novel and specific lectin proteins from prokaryotic sources. We have immobilized them onto a number of novel platforms in order to develop a variety of bioaffinity phases. We have interrogated these new bioaffinity phases with various mixtures of closely related glycoproteins and glycoconjugates. We have demonstrated the selective elution of the bound glycoforms from these bioaffinity phases using different combinations of free and modified sugars and that these new phases have the ability to efficiently separate very closely related glycoforms.