

Affinity Studies of Glycoprotein-Lectin Interactions

Introduction

Dual Polarisation Interferometry (DPI) is an important enabling tool for biochemistry and cell biology. Farfield's **AnaLight**[®] DPI instrument embodies a truly quantitative analytical technique, rather than a simple 'mass sensor' response, providing absolute mass, dimensional and density measurements of proteins at high resolution⁽¹⁾. Changes in these physical parameters can be directly related to both structural and functional elements of the interactions of biomolecules and can be used to differentiate specific from non-specific binding events.

Post-translational modification of proteins is a complex process, and protein glycosylation in particular has significant effects on molecular recognition and cell signalling, protein folding, conformation, stability and activity. Glyco-molecules play critical roles in myriad physiological and pathological reactions including immunity, blood clotting, cell death and development. Oligosaccharides are major structural components of many cell surface and secreted proteins. The majority of proteins targeted by therapeutic compounds are glycoproteins, including some of the world's best-selling pharmaceutical products for heart disease, cancer, neurodegenerative diseases and diabetes. Therapeutic peptides may not perform optimally unless they are correctly glycosylated, so a reliable method for assessing glycosylation may assist in gauging the suitability of a therapeutic candidate prior to further investigation. While measurement of the identity of proteins is readily addressed using mass spectrometry, there are no consistent methods for characterising protein activity.

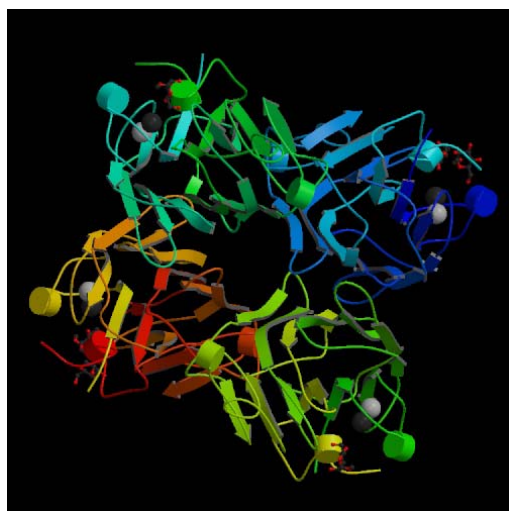


Figure 1 shows concanavalin A (ConA), a naturally occurring lectin which exists as a tetramer (MW 104kDa) at physiological pH and specifically binds α -mannose and α -glucose. Lectins are proteins that specifically bind certain carbohydrates. In the following study, ConA has been challenged with a number of oligosaccharide peptide conjugates revealing the interaction constants using the **AnaLight**[®].

This application note details how **AnaLight**[®] can be used to quantify the efficiency of binding of RNaseB, a bonded mannose glycoprotein⁽²⁾, and various smaller mannose glycopeptides to ConA. In addition, the interactions between ConA and mannose monosaccharide and the known non-binder galactose were also studied. DPI can be used to measure the affinity of ConA for a range of carbohydrate binding.

With the continued focus on the study of biological molecule interactions in the drug discovery process, **AnaLight**[®] provides an unequivocal approach for the analysis of these interactions through the provision of a unique combination of data.

Figure 1: Schematic representation of concanavalin A tetramer at pH 7.4

Experimental

DPI experiments were performed on a Farfield **AnaLight**[®] instrument. The surface used in these studies was a thiol functionalised **AnaChip**[™] activated with sulpho-GMBS. The temperature of the samples was controlled throughout to 24°C. Water used in buffer preparation was deionised and free from organic impurities. All buffers and reagents were analytical grade or higher, and solutions were degassed prior to use.

Calibration: The thiol functionalised **AnaChip**[™] was calibrated using 80% ethanol and pure water (**Farfield Technical Note 001**). Oxidised thiol groups were then reduced using 5mM DTT.

Immobilisation of ConA: After activating the thiol **AnaChip**[™] surface with sulpho-GMBS linker (2mg/ml in H₂O), ConA (1mg/ml) prepared in HEPES buffer (10mM HEPES, 150mM NaCl, 2mM CaCl₂, 2mM MnCl₂, pH7.3) was introduced into the buffer flow and allowed to covalently couple to the **AnaChip**[™]. Excess ConA was washed off with 2% SDS and any unreacted linker or surface groups were blocked with 0.1M ethanolamine (**Farfield Technical Note 004**).

ConA – RNaseB interactions: The glycoprotein RNaseB (MW 16kDa) was introduced at various concentrations in HEPES buffer for a 5 minute association phase with ConA, after which the system was returned to running buffer and allowed to fully dissociate.

In addition, the binding of a series of mannose glycopeptides were compared using galactose as a known negative control using the same methodology.

Results and Discussion

Figure 2 shows the association and dissociation phases of RNaseB, at a range of concentrations, binding to ConA. Clearly the responses are saturating at the highest concentrations allowing curve fitting to determine the K_D . Furthermore, the unique capability of **AnaLight**[®] to resolve the raw data in two polarisations to the dimensions, density and therefore mass of bound material can be used to provide truly quantitative measurements.

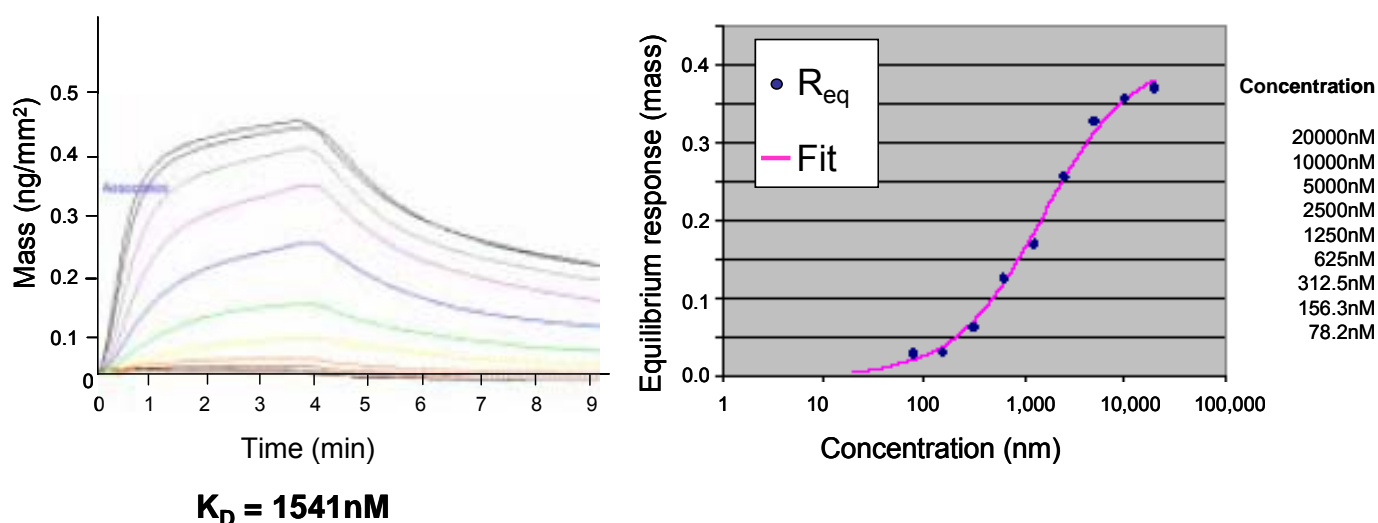


Figure 2: Affinity constant K_D derived from curve fitting for the quantitative mass responses for RNaseB binding to ConA

Molecule	Type	Mol Weight	K_D
Mannose	monosaccharide	180	$5.0 \times 10^{-4}\text{M}$
Galactose	monosaccharide	180	N/A
Man5	glycopeptide	1,590	$2.0 \times 10^{-6}\text{M}$
Man6	glycopeptide	1,750	$1.7 \times 10^{-6}\text{M}$
Man7	glycopeptide	1,910	$1.5 \times 10^{-6}\text{M}$
Man8	glycopeptide	2,010	$<1.5 \times 10^{-6}\text{M}$
RNaseB	glycoprotein	16,000	$1.6 \times 10^{-6}\text{M}$

Table 1: The determined K_D values for a range of mannose glycopeptides, the monosaccharide mannose and a negative control, galactose

Con A was challenged with RNaseB, the monosaccharides mannose and galactose and a series of mannose glycopeptides (see **Figure 3**) allowing their respective K_D values to be determined. **Table 1** shows that RNaseB and the glycopeptide series have very similar affinities for ConA, much higher than the mannose monosaccharide. This indicates that the binding element of RNaseB is a branched mannose chain and that a single mannose molecule has far lower affinity for ConA. The similarity of the K_D values also suggests that RNaseB binding is via the mannose side chain rather than the protein moiety. ConA does not bind galactose, confirming the specificity of the model.

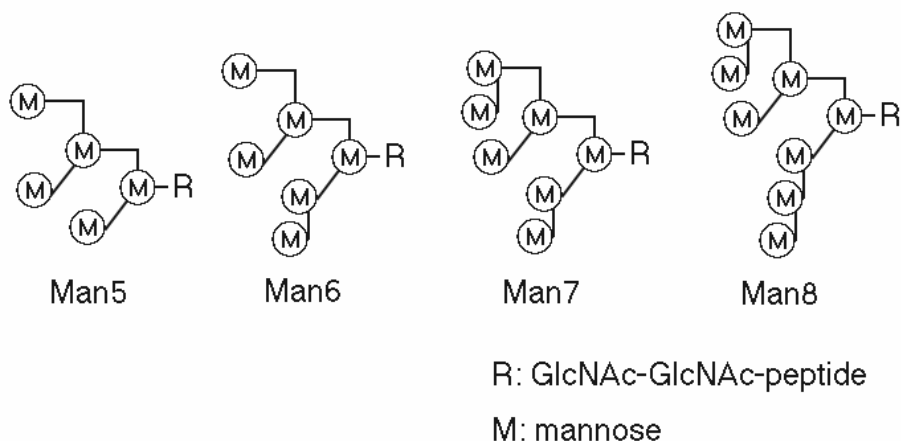


Figure 3: Structure of the mannose glycopeptide series used in these studies

Conclusions and Benefits

These studies demonstrate **AnaLight**[®] as an enabling tool for the study of biomolecular interactions and the determination of affinity constants. A series of monosaccharides and oligosaccharide-peptide complexes binding to a lectin was studied using the **AnaLight**[®] system demonstrating the power of DPI to study glycosylated protein interactions. Resolving mass quantitatively reveals the true kinetic performance without invoking additional assumptions.

These experiments show DPI can be applied to the study of affinity measurement. The **AnaLight**[®] instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on dimension, density and mass in a benchtop instrument. The **AnaLight**[®] is an important enabling tool for protein biochemists and biophysicists, giving them the ability to:

- Generate high-quality affinity parameter data for biomolecular interactions to help in the understanding of the molecular mechanisms underlying glycoprotein-lectin interactions
- Sensitively and directly measure the affinity a wide range of molecular weights (monosaccharides at 180Da to glycoproteins at 16KDa) binding to ConA (104KDa) in one experimental series
- Study carbohydrate binding without any potential interference from sugar-based hydrogel matrices, because these are not required on the **AnaChip**[™], therefore potentially providing a useful tool for determining the glycosylation status of recombinant or target proteins
- Avoid the limitations and ambiguities that are inherent in other established techniques for such studies, and provide quantitative results and analysis rapidly

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⁽¹⁾ G. Cross, A. Reeves, S. Brand, J. Popplewell, L. Peel, M. Swann & N. Freeman. *Biosens. Bioelectron.* **19** (2003) 383-390

⁽²⁾ D. Solis, M. Bruix, L. Gonzales, T. Diaz-Maurino, M.Rico, J. Barbero-Jimenez & T. Feizi. *Glycobiology* **11**(1) (2001) 31-36