

Biotin - Streptavidin Interactions: Measuring Conformational Change and Differentiating between Specific and Non-Specific Binding

Introduction

Dual Polarisation Interferometry (DPI) is an important enabling technology for the rapid and sensitive monitoring of interactions between proteins and small molecules and the differentiation between specific and non-specific binding ⁽¹⁾.

DPI provides density and dimensional measurements, showing mass capture events and revealing conformational changes in proteins that are indicative of a response to specific binding. The sub-atomic resolution of DPI allows the detection of small molecules binding to large, immobilised proteins. This means that DPI can be used to determine whether small molecules change the conformation or structure of a protein upon binding, opening new insights into **structurally informed drug discovery**, giving a level of information beyond that provided by kinetic parameters alone.

Most molecules of pharmaceutical interest as drug candidates have a molecular weight below 500 Daltons, whilst the 'target' proteins they interact with are often of the order of tens or hundreds of thousands of Daltons. The ability to reliably detect the specific binding of such small molecules to such large proteins is one of the more demanding applications for any tagless analytical system.

This application note demonstrates the unique benefits of DPI for studying protein-small molecule interactions. We were interested in using the well-characterised biotin-streptavidin system as a model to gain direct insights into protein function associated with specific binding through conformational change, and to provide a distinct differentiation signature between specific and non-specific interactions. These studies also demonstrate the provision of a DPI surface for the capture of biotinylated molecules.

Experimental

The DPI experiments were performed on a Farfield **AnaLight**[®] instrument. The surface used in all studies was an amine functionalised silicon oxynitride **AnaChip**[™] with no further pre-treatment. The temperature of the samples was controlled throughout to 20°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.

Immobilisation of Streptavidin: The **AnaChip**[™] was firstly covalently modified *ex situ* to provide a biotin surface. The **AnaChip**[™] was incubated with sulpho-NHS-LC-biotin (2mg/ml) in PBS (10mM phosphate, 150mM NaCl, pH7.4) for 5 minutes, followed by water and isopropanol rinses. The **AnaChip**[™] was then mounted in the **AnaLight**[®] instrument and, after standard calibration, streptavidin (15µg/ml in PBS) was flowed over the **AnaChip**[™] surface at 20µl/min followed by PBS washes until the streptavidin surface had stabilised.

Streptavidin-Biotin Interactions: After stabilising the immobilised streptavidin, both the working channel and second, unmodified reference channel were simultaneously subjected to flow of D-biotin (1µM in PBS) at 20µl/min and the subsequent binding events were measured.

Results and Discussion

Immobilisation of Streptavidin: **Figure 1** shows the streptavidin immobilisation data. The layer thickness after immobilisation of streptavidin increases by 6.6nm. This correlates well with the X-ray crystal structure for streptavidin, which gives a long axis dimension of 6.8nm at pH7.5 ⁽²⁾, and indicates that the protein has bound in a viable orientation as a monolayer on the surface. The simultaneous measurement of both thickness and density provided by DPI allows calculation of the bound mass, and hence the surface coverage, of the protein layer. The combination of approximately 90% surface coverage and the dimensions of the layer confirm that the streptavidin bound as a monolayer.

Streptavidin-Biotin Interactions: **Figure 2** shows the expanded detail of the free D-biotin injections and binding of D-biotin to the immobilised streptavidin layer, with the data resolved into the core DPI parameters of thickness and density. The first aliquot of D-biotin clearly binds instantaneously to the streptavidin, as would be expected given its high binding affinity. Immediately upon binding D-biotin, the streptavidin layer decreases sharply in thickness (by approx. 0.5Å) with a concurrent sharp increase in density. This is followed by an unspecified secondary process, which leads to a slower increase in thickness but remains density neutral. This secondary process is reversed through

PBS washing, and is interpreted as non-specific attachment of further D-biotin to the streptavidin layer once all the streptavidin binding sites are saturated.

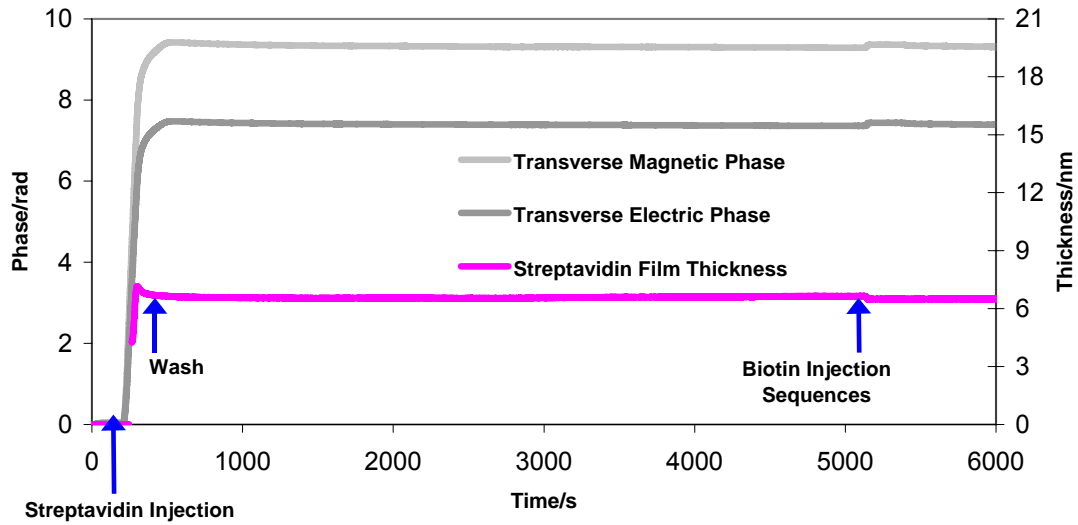


Figure 1: Immobilisation of streptavidin on a biotinylated surface, showing resolved layer thickness (pink) and the measured TE and TM phase changes (grey)

After prolonged PBS washing, addition of the second aliquot of D-biotin leads to another unspecified process with the same thickness increase, density neutral profile and this process is also reversed by PBS washing (Figure 2). This is again interpreted as non-specific attachment of D-biotin to the streptavidin layer, since the binding sites have remained occupied from the first D-biotin addition, and the process is easily reversed on washing.

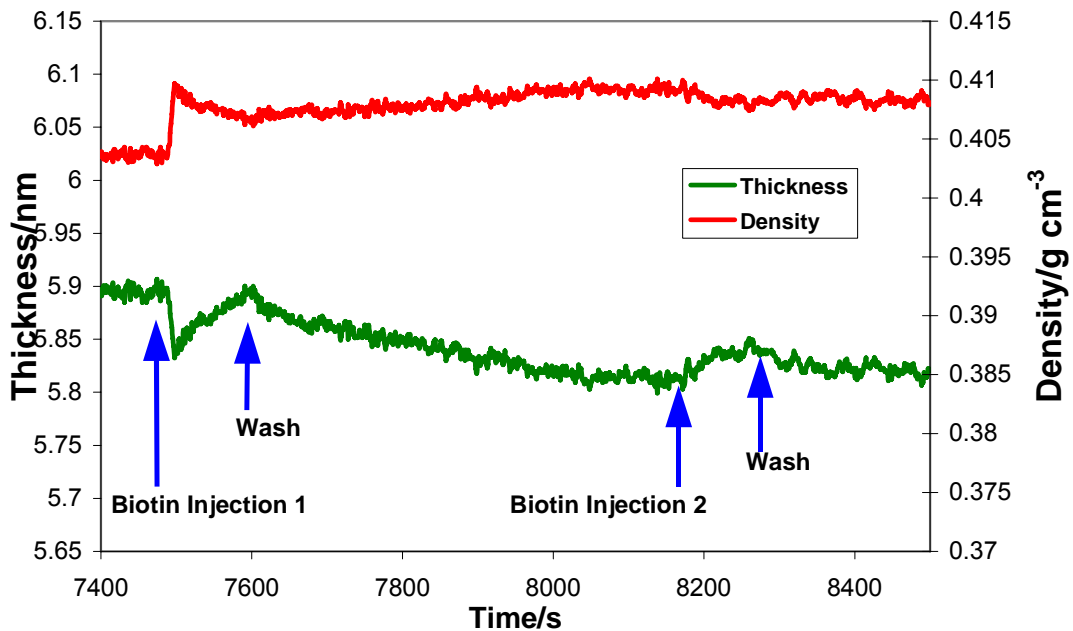


Figure 2: Thickness (green) and density (red) measurements of streptavidin binding free D-biotin (two injections of free D-biotin)

The specific interaction between protein and small molecule will generally elicit a conformational change in the protein, resulting in a change in both thickness and density of the protein layer. In general terms, **specific binding** is typified in DPI measurements by a density increase associated with both a mass capture event and a thickness reduction as a result of conformational change in the protein. Conversely, **non-specific binding** is typically density neutral in DPI measurements, as both the mass and the thickness increase. Furthermore, non-specific binding is always additive so

a thickness reduction, or change in conformation, is uniquely associated with specific binding. This is epitomised in the profile in **Figure 2**, where different thickness and density profiles are obtained for specific and non-specific binding events within the same molecular interaction. A schematic representation of this is shown in **Figure 3**.

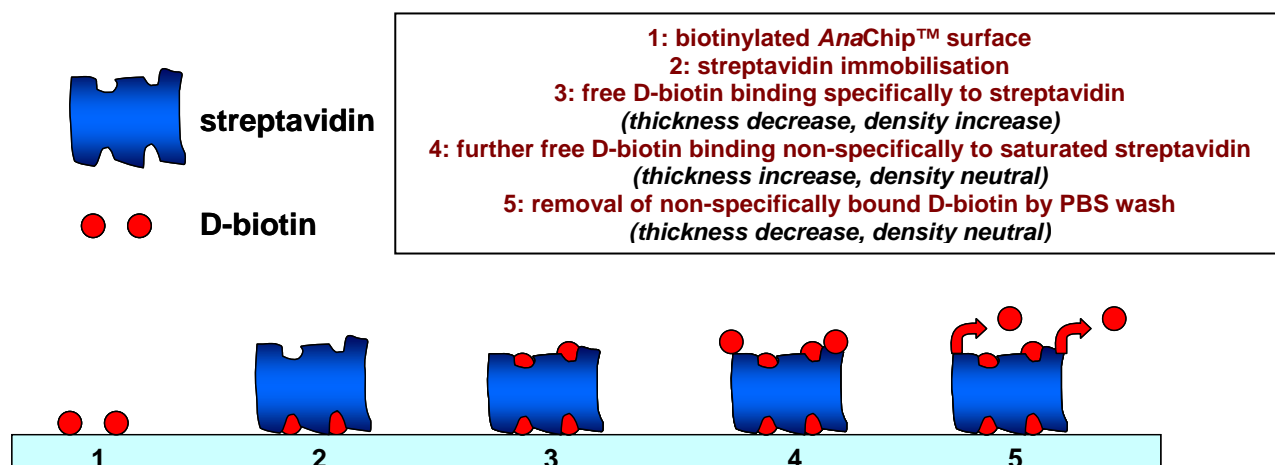


Figure 3: Visual schematic of D-biotin-streptavidin interactions

Conclusions and Benefits

DPI enables the detailed study, in real time, of the intimate link between molecular **structure** and **function** for a diverse range of molecular systems. These experiments show DPI can bring a new level of understanding to the assessment of small molecules as binding candidates for proteins by giving information on both the **functional** binding events and the **structural** response to binding in the protein. In turn, this allows differentiation between **specific** and **non-specific** binding. A mass signature alone cannot be used as a reliable indicator to quantify protein-small molecule interactions when considering the binding of sufficiently low molecular weight drug candidates, as the signal becomes diminishingly low when considering their interactions with much larger proteins.

The core DPI measurements of thickness and density are excellent markers for protein function and give unique levels of sensitivity for the detection of small molecules binding to large proteins (244 Daltons binding to 55,000 Daltons in this example). DPI can also reveal the nature of the binding (specific versus non-specific) and the changes in protein structure that result from the interaction.

The **AnaLight®** instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, density (refractive index) and mass from a bench top technique. The **AnaLight®** is an important enabling tool for drug discovery researchers, giving them the ability to:

- Rapidly and sensitively detect small molecules binding to large proteins
- Differentiate between specific and non-specific binding events
- Connect functional and structural events in a single set of high-content measurements, in real time
- Clearly understand the molecular processes involved in protein-small molecule interactions and screen for similar modes of action amongst small molecule candidate libraries
- Compare the analytical structural data obtained with data from complimentary biophysical techniques such as X-ray crystallography

For further applications information contact: applications@farfield-group.com

Full details and further data from this application can be found in:

M. Swann, L. Peel, S. Carrington & N. Freeman. *Anal. Biochem.* **329** (2004) 190-198

⁽¹⁾ M. Swann, L. Peel, S. Carrington & N. Freeman. *Anal. Biochem.* **329** (2004) 190-198

⁽²⁾ P. Weber, D. Ohlendorf, J. Wendoloski & F. Salemme. *Science* **243** (1989) 85-88