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Measurement of Conformational Change in HSA on Copper Ion Binding

Introduction

Human serum albumin (HSA) is a high molecular weight endogenous plasma protein (MW 67kDa). It is the main component of the blood transport system and reversibly binds a variety of endogenous (vitamins, lipids) and exogenous (drugs, toxins) molecules, distributing them to the target organs. HSA is also known to bind metal cations which play an important role in human growth, development, cell division and synthesis of proteins and DNA [(1)]. It is essential in the transport and metabolism of competitively bound Cu^{2+} in particular [(2)].

Dual Polarisation Interferometry (DPI) is a major enabling tool for biophysics and wider life science research. Farfield's ***Ana*Light**® DPI instrument range embodies a truly quantitative analytical technique, rather than a simple 'mass sensor' response, providing absolute mass, dimensional and fold density measurements of biomolecules and their complexes at high resolution, in real time [(3)]. Changes in these physical parameters can be directly related to both the ***structural*** and ***functional*** elements of the interactions of biomolecules, and can readily detect metal cations binding to large proteins. DPI can therefore be used to determine whether metal cations known to be vital to protein ***function*** actually change the ***structure*** of a protein upon binding, giving a level of information not previously available.

This application note demonstrates how the ***Ana*Light**® can be used to assess the real-time binding and biomolecular interaction of Cu^{2+} with HSA through the measurement of conformational changes within the protein structure, with validation of these measurements by calculation of the affinity constant (K_D) for this interaction.

Results and Discussion

HSA was immobilised onto an amine ***Ana*Chip**™ and then challenged with a range of Cu^{2+} concentrations (1μM – 1000μM). The metal ion-protein complex was allowed to fully dissociate between Cu^{2+} additions.

Immobilised HSA Structure

	Dimension (nm)	Fold Density (gcm^{-3})	Mass ($ngmm^{-2}$)
Human Serum Albumin	**4.12**	**0.6540**	**2.675**

Figure 1: Resolved Structure of Immobilised Human Serum Albumin on an Amine *Ana*Chip™

Changes in HSA on Binding Cu^{2+}

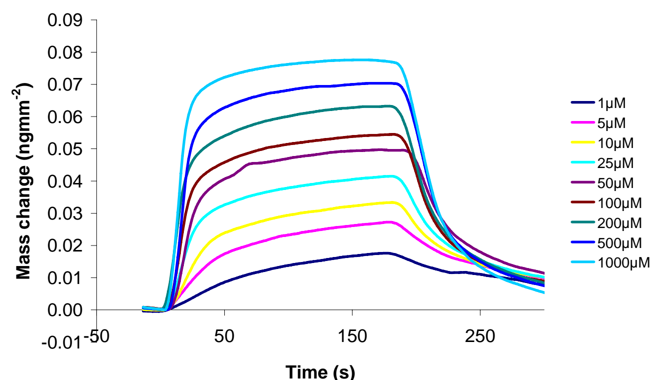

Figure 2: Mass Change in HSA on Cu^{2+} binding as a Function of $[Cu^{2+}]$

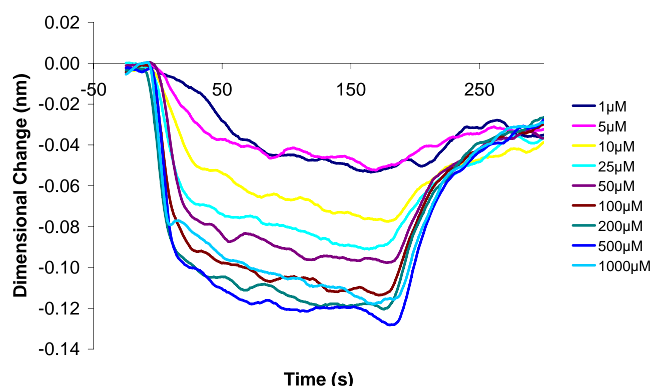

Figure 3: Dimensional Change in HSA as a Function of $[Cu^{2+}]$ Showing Metal Ion-Induced Conformational Changes in the Protein

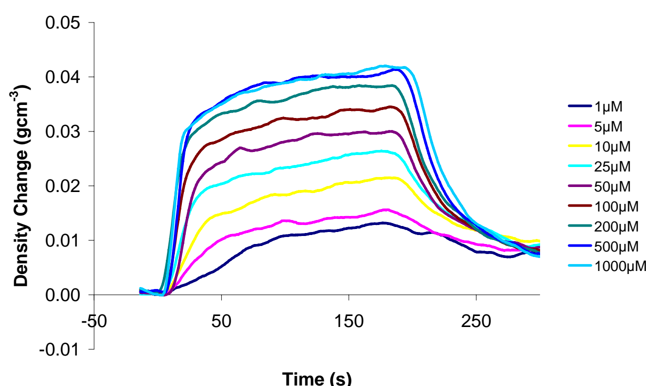

Figure 4: Fold Density (RI) Change in HSA as a Function of $[Cu^{2+}]$ Showing Metal Ion-Induced Conformational Changes in the Protein

A maximum contraction in HAS conformation of approximately 0.13nm occurred with 500µM Cu^{2+}, while an increase in fold density of approximately 6.5% occurred with 1000µM Cu^{2+}.

The effects observed for the concentration range of Cu^{2+} ions indicate a conformational change in HSA (dimensional decrease and fold density increase associated with structural tightening) in response to a Cu^{2+} binding event. Both of these effects are reversible with buffer washing.

Affinity Constant (K_D)

In order to determine whether the structural changes observed in HSA (**Figures 3 and 4**) were directly attributable to Cu^{2+} binding, curve fitting to a plot of relative change in mass of the HSA against Cu^{2+} concentration was analysed (**Figure 5**).

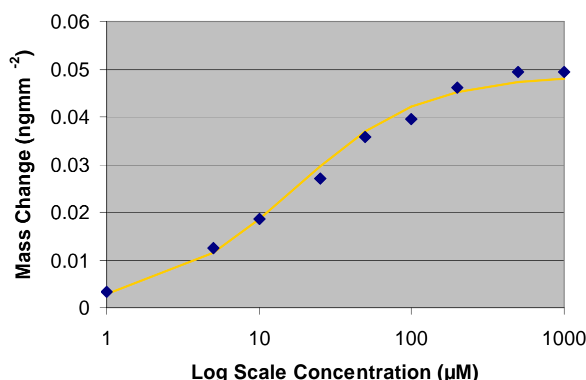

Figure 5: Binding Curve Accounting for Bulk Refractive Index Used to Determine Affinity Constant (K_D) for Cu^{2+} Binding to HSA

The returned K_D value of 16.2µM falls comfortably within the cited literature range of values for the interaction, 40µM to 0.23µM [1], indicating that the observed conformational change in HSA was indeed brought about by Cu^{2+} binding. This was supported by the K_D values additionally calculated from dimensional and fold density data, at 18.8µM and 18.0µM respectively. Thus the interaction affinity can be uniquely qualified by ***Ana*Light®** **from both functional (mass) and structural (dimension and fold density) perspectives.**

Conclusions and Benefits

These experiments show how DPI can be applied to the study of affinity measurements of protein-metal ion interactions through structural changes that occur upon binding. The ***Ana*Light®** instruments and their experimental protocols give the biophysicist a unique combination of high-resolution data in real time on dimensions, fold density (refractive index) and mass to connect the changes in biomolecular structure on metal ion binding with the subsequent changes in biological activity and function, at a resolution not previously available from a laboratory-based technique. ***Ana*Light®** is an important enabling tool for life science researchers, giving them the ability to:

- Rapidly and sensitively detect low atomic weight metal cations binding to large proteins
- Calculate the affinity constants associated with metal ions binding to proteins
- Measure structural changes in proteins, moving the basis for such studies beyond simple measurement of binding affinities and revealing dynamic changes in proteins
- Directly connect structural and functional consequences of metal ions binding to proteins in a single set of high-content measurements, in real time on a bench-top instrument

For further applications information contact:
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(1) Guo et al., ***Analytica Chimica Acta.,*** **443** (2001) 91-99
(2) Masuoka et al., ***J. Bio. Chem.,*** **29**, (1993) 21533-21537
(3) Cross et al., ***Biosens. Bioelectron*** , **19** (2003) 383-390