

Measurement of Conformational Change in Transglutaminase on Calcium Ion Binding

Introduction

Dual Polarisation Interferometry (DPI) is an important enabling technology for the rapid and sensitive monitoring of interactions between proteins and metal cations, and the measurement of resulting conformational changes in proteins.

DPI provides density and dimensional measurements, showing mass capture events and revealing structural changes in proteins that are indicative of a response to specific binding. The sub-atomic resolution of DPI allows the detection of metal cations binding to large, immobilised proteins. This means that DPI can be used to determine whether metal cations known to be essential for protein **function** actually change the **structure** of a protein when they bind, giving a level of information beyond that provided by traditional biosensors and other kinetic techniques.

Transglutaminases (**Figure 1**) are implicated in a range of protein modifying activities including roles as diverse as fusing fibrinogen to increase its mechanical strength as part of the blood clotting cascade ⁽¹⁾ to cross-linking cytoskeletal and membrane proteins and programmed cell death ⁽²⁾. Regulatory binding sites are known for Ca^{2+} and GTP, which reciprocally modulate the cross-linking and signaling activities of transglutaminase ⁽³⁾.

Protein structure is intimately related to function, activity and regulation of activity. Transglutaminase is presumed to undergo conformational changes on binding both Ca^{2+} and GTP, bringing about the modulation in activity. Indirect analysis using Shallow Angle Neutron Scattering (SANS) ⁽⁴⁾ and Circular Dichroism Spectroscopy ⁽⁵⁾ has suggested that the gyration radius of transglutaminase increases by approximately 0.8nm on binding of Ca^{2+} ions. There is no clear crystal structure data available to support this.

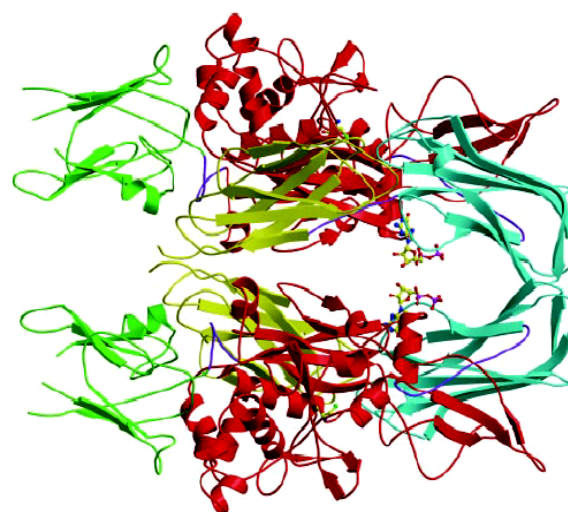


Figure 1: The structure of transglutaminase

This application note demonstrates the use of DPI for the analysis of the conformational changes taking place in guinea pig liver transglutaminase (tTG, 77,000 Da) when it binds calcium ions (Ca^{2+} , 40 Da), and to validate this measurement by calculating the affinity constant K_D for the interaction from the DPI data for comparison with published values for the transglutaminase-calcium interaction.

Experimental

The DPI experiments were performed on a Farfield **AnaLight**[®] instrument. The surface used in all studies was an amine functionalised silicon oxynitride **AnaChip**[™]. The temperature of the samples was controlled to 20°C throughout. 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 Transglutaminase: The **AnaChip**[™] was calibrated by injecting an 80% (w/w) ethanol/water solution into a stream of PBS running buffer (10mM, 150mM NaCl, pH7.4) at a flow rate of 50µl/min (**Farfield Technical Note 001**). The amine-amine linker BS³ (bis[sulfosuccinimidyl]suberate, 4mg/ml in PBS) was added to both channels (experimental and reference) for 8 minutes at 10µl/min. Guinea pig liver transglutaminase solution (1mg/ml in PBS) was then added to the experimental channel only for 10 minutes at 10µl/min. Tris buffer (3M, pH8.0) was then added to both channels for 8.5 minutes at 50µl/min to block unreacted BS³ (**Farfield Technical Note 003**).

Protein-Metal Cation Interactions: After stabilising the immobilised transglutaminase with sufficient rinsing with Tris running buffer (50mM, pH7.6) at 50µl/min, the protein was challenged with metal salt solutions. Stock solutions of calcium chloride (CaCl_2 , 30mM) and sodium chloride (NaCl, 60mM) were made up in Tris running buffer. Aliquots of each were introduced to the flow stream of Tris running buffer for 3 minutes at 50µl/min over both channels in a series of nine injections of each solution, alternating between CaCl_2 and NaCl. The injection sequence began with the lowest concentration of CaCl_2 and then the lowest concentration of NaCl and progressed through to the highest

Application Note 005

concentrations. The transglutaminase was washed with Tris running buffer for 4 minutes at 50 μ l/min between metal cation additions. These additions covered a CaCl₂ concentration range of 0.15mM to 30mM and a NaCl concentration range of 0.32mM to 60mM, designed to be equimolar with respect to chloride ion. The density, thickness and mass of the transglutaminase surface were monitored throughout.

Results and Discussion

Immobilisation of Transglutaminase: Transglutaminase (tTG) has a discoid structure that is 15nm in diameter and around 5nm thick. Following immobilisation onto the amine surface using BS³, the thickness of the transglutaminase was measured at 4.6nm by DPI. This is consistent with the expected thickness value if the protein is immobilised with the disc lying parallel to the surface, which is most likely given the immobilisation strategy employed.

Protein-Metal Cation Interactions: A mass signature alone cannot be used as a reliable indicator to quantify protein-metal cation interactions when considering the binding of very low atomic mass metal cations, as the signal becomes diminishingly low with their interactions with much larger proteins. The capabilities of the **AnaLight**[®] instrument series allows for unique levels of sensitivity to metal cation and small molecule detection, by providing direct insight into protein function associated with specific binding through conformational change coupled with density variation.

Whilst looking for the diminutive signals associated with metal cations binding to large proteins, the effects of changes in the buffer or bulk refractive index need to be accounted for if the measurement is to be quantified. Generally speaking, because the **AnaLight**[®] measures in a thin film format, it is adequate to simply subtract the reference channel signals from their counterparts on the experimental channel.

Having compensated for bulk refractive index effects from the high metal cation concentrations present, the resolved thickness data as a function of Ca²⁺ concentration is shown in **Figure 2** and density data as a function of Ca²⁺ concentration is shown in **Figure 3**. Clearly, each Ca²⁺ binding event results in a concentration-dependant decrease in thickness and increase in density of the tTG, an effect not observed in the presence of the Na⁺ ions in the control experiments.

The effects observed for the Ca²⁺ ions indicate a **specific binding** event (density increase associated with mass capture) and a corresponding **conformational change** in transglutaminase (thickness decrease associated with structural tightening). Both of these effects are reversible with buffer washing.

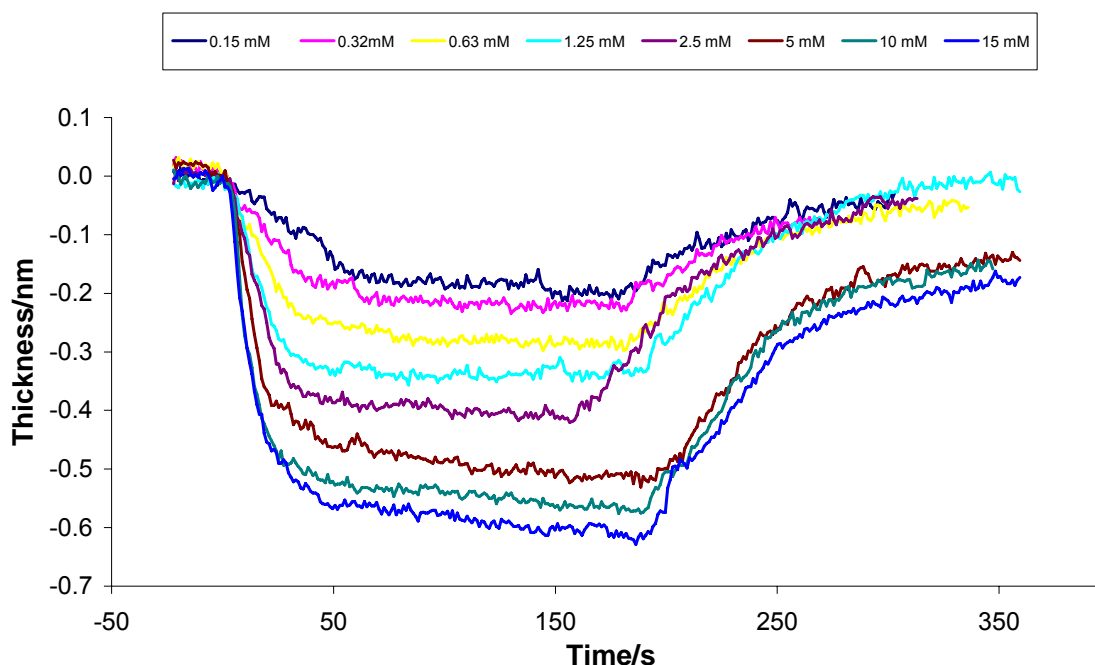


Figure 2: Thickness change in tTG as a function of Ca²⁺ concentration showing Ca²⁺ induced conformational changes in tTG

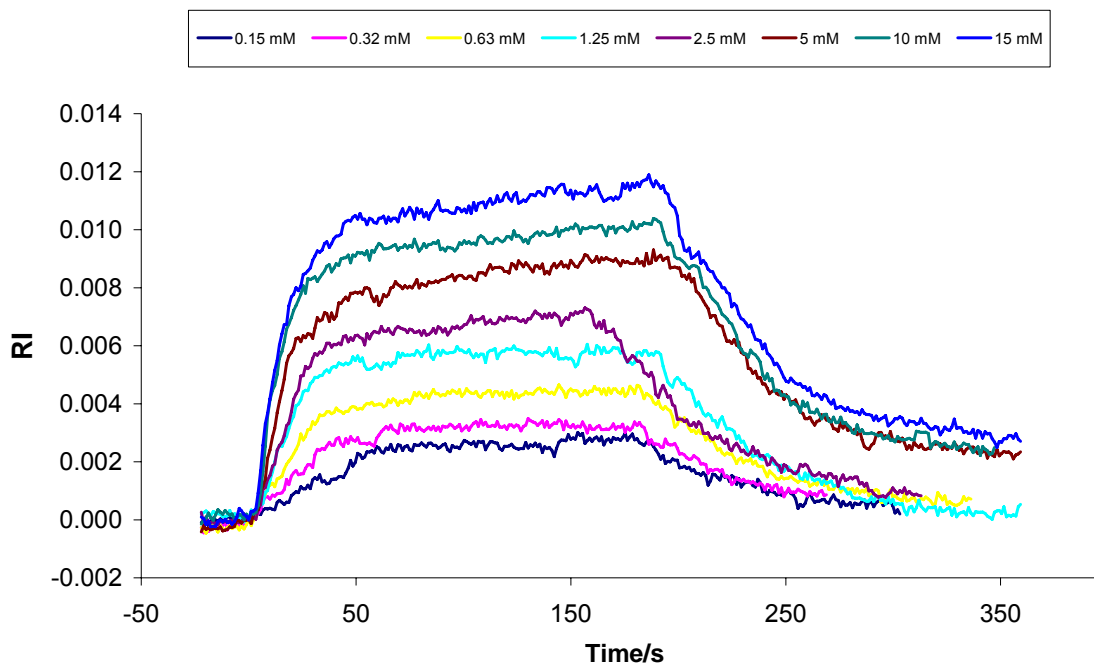


Figure 3: Density (RI) change in tTG as a function of Ca²⁺ concentration

The data in **Figure 2** shows clearly that Ca²⁺ ions induce a conformational change of around 0.6 to 0.7nm. This conformational change is not seen in the presence of Na⁺ ions in the control experiments. A conformational change of this order would be expected in tTG, as the long axis is reported to expand by approximately 3nm on Ca²⁺ binding⁽⁴⁾ which would induce a thickness reduction in the short axis, which is perpendicular to the surface in this immobilisation (above). It is worth noting here that the thickness measurement resolution provided by DPI (**Figure 2**) is better than +/-0.01nm.

In order to determine whether the structural changes observed in tTG (**Figures 2 & 3**) were a direct consequence of Ca²⁺ binding, the results were verified against known parameters from the interaction. The affinity constant K_D for tTG binding calcium has been reported as between 0.2 mM and 3.0 mM⁽⁶⁾. Curve fitting to a plot of the relative change in RI (density) of the tTG layer against Ca²⁺ concentration (**Figure 4**) gives a K_D value of 0.95 ± 0.2mM, which falls well within the range of reported values.

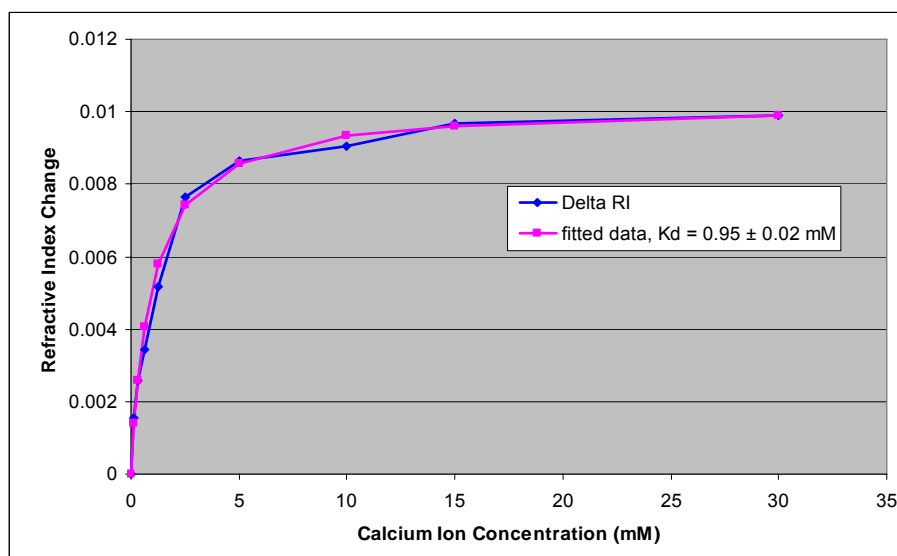


Figure 4: Using the relative change in RI for NaCl and CaCl₂ at the same Cl⁻ concentrations provides a concentration profile from which the affinity constant K_D can be determined

Conclusions and Benefits

DPI enables the detailed study of the intimate link between protein structure and function, activity and regulation of activity. A range of methods is used to elucidate protein structure of which x-ray crystallography provides the most detailed information. Obtaining structural information is often difficult and time consuming, and this structural information cannot always be directly related and compared to functional information obtained from other experimental techniques.

These experiments show how DPI can be used to determine the structural changes occurring when a protein binds metal ions known to play a role in the regulation of its activity. DPI offers a simple, convenient laboratory-based method to unambiguously measure **structural** changes and directly relate these to **functional** aspects of proteins in real time and in a single set of measurements.

The **AnaLight**[®] instruments and their experimental protocols give the biophysicist 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 life science researchers, giving them the ability to:

- Rapidly and sensitively detect low atomic weight metal cations binding to large proteins
- Connect functional and structural events in biological molecules in a single set of high-content measurements, in real time
- Measure structural changes in proteins as a result of metal cation binding, moving the basis for such studies beyond simple measurement of binding affinities and revealing dynamic structural changes in proteins
- Remove the matrix effects which may dominate such measurements on biosensor technologies by using the **AnaLight**[®] reference channel and a little care in experimental design

Farfield gratefully acknowledges that these experiments were carried out in collaboration with Kal Karim, Judith Taylor and David Cullen from the Institute of BioScience & Technology, Cranfield University, Silsoe, Bedfordshire, UK.

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