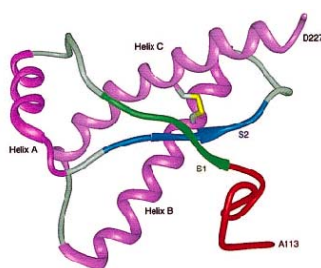


Measurement of Conformational Change in Prion Protein on Copper Binding

Introduction

Metal ions, particularly divalent metal cations, perform a number of essential functions in a wide range of biological processes, including mediation in the interaction between a protein and a ligand, forming bridges between residues and domains within the protein structure and contributing to active site mechanisms as an electron transfer agent or nucleophilic catalyst. Farfield's **AnaLight®** Dual Polarisation Interferometry (DPI) instrument range offers an important enabling technology for the rapid and sensitive monitoring of interactions between proteins and metal ions, due to DPI's class-leading mass sensitivity and ability to measure real-time conformational changes in proteins.

DPI shows mass capture events and provides dimensional and fold density (refractive index) measurements in real time, thereby revealing structural changes in proteins that are indicative of a response to interactions and binding events. The resolution levels provided by DPI allow the detection of metal cations binding to large proteins. This means that DPI can be used quantitatively to measure the conformational change in a protein when metal cations bind, giving a level of information beyond that provided by traditional biosensors and other kinetic techniques.



Prions are a unique form of infectious agent consisting of only protein. Prions are abnormally structured forms of a host protein that are capable of converting normal protein molecules into their abnormal structure.

Figure 1. Structure of Mouse PrP as Revealed by NMR, Showing the Unstructured N-terminal Region (red) in the Absence of Copper

A conformational change of the prion protein (PrP) is responsible for a class of neurodegenerative diseases, including Creutzfeldt-Jacob Disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep.

In the absence of Cu^{2+} ions, PrP has an unstructured N-terminal octapeptide repeat as revealed by NMR (Figure 1, red region). This region has been shown to be highly selective in binding Cu^{2+} (1). Copper binding is implicated in defining the structure of the N-terminus, which then produces functional activity. It is believed that the infectious forms of prion are covalently modified in the region of the Cu^{2+} binding site, resulting in altered binding characteristics. Therefore Cu^{2+} metabolism may be affected by prion disease, resulting in low availability of Cu^{2+} at synapses, and consequent

neurodegenerative symptoms. Unfortunately, NMR cannot be used to study protein with bound copper due to interference with the signal. This makes DPI an invaluable tool for studying protein- Cu^{2+} ion interactions, revealing a level of information not previously available.

This application note demonstrates the unique sensitivity of DPI for the analysis of the conformational changes taking place in recombinant mouse prion protein (PrP, circa 30,000Da) in response to binding divalent copper cations (Cu^{2+} , 64Da).

Results and Discussion

Prion protein (PrP) was immobilised onto an amine functionalised **AnaChip™**. The immobilised protein was then exposed to sequentially increasing concentrations of Cu^{2+} ions in solution, with return to running buffer between each addition.

Figure 2 shows that the maximum mass loading increased as the immobilised PrP was exposed to increasing concentrations of Cu^{2+} . The dimensions of the protein decreased (Figure 3) whilst the fold density (RI) increased (Figure 4). These combined effects are an unambiguous signature of structural change in PrP. This indicates that Cu^{2+} associates with the PrP structure, probably via a binding site or region, resulting in a conformational rearrangement of the PrP represented by a tightening of the protein structure.

It is clear that the mass change did not return to its original value within the timescale of this experiment (180 seconds). Neither did the dimensional or fold density values, indicating that copper formed a strong association with the PrP. This is in stark contrast to various other metal ions studied (Farfield Application Note 058).

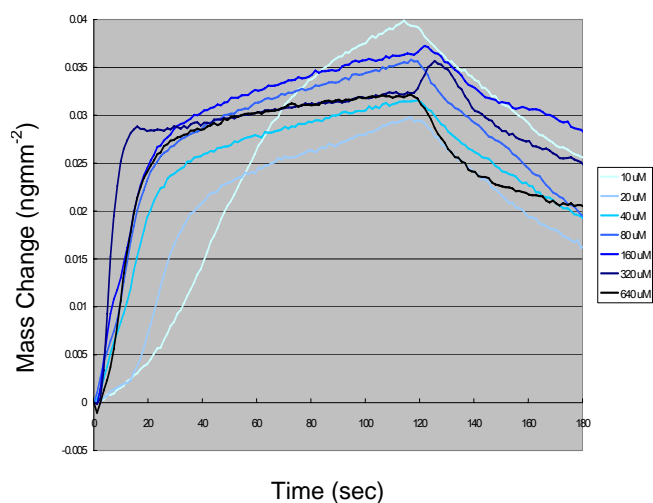


Figure 2: Concentration-Dependent Mass Changes in PrP on Cu^{2+} Binding

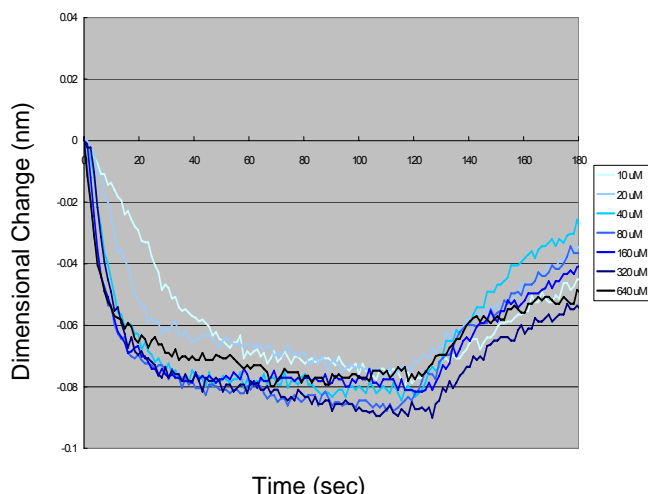


Figure 3: Concentration-Dependent Dimensional Changes in PrP on Cu²⁺ Binding

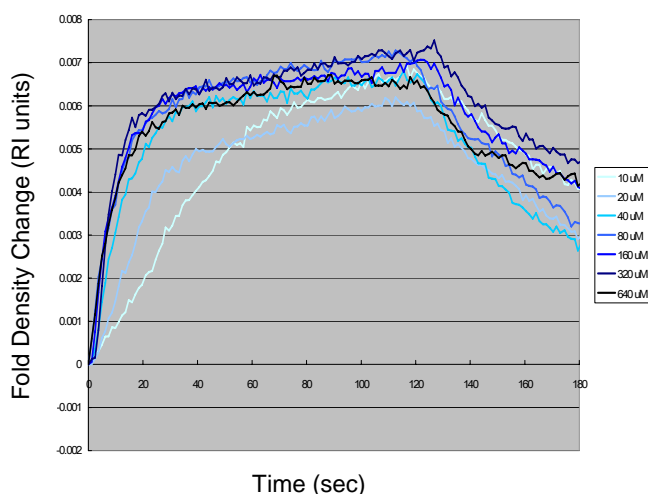


Figure 4: Concentration-Dependent Fold Density (RI) Changes in PrP on Cu²⁺ Binding

The effect of copper at saturating concentration over a longer time period was further investigated in a complementary set of experiments. A different PrP construct was anchored to the **AnaChip™** via a his-tag and exposed to saturating concentrations of copper (1mM Cu²⁺). These experiments showed that copper-induced conformational change was indeed irreversible, and could only be altered by the active removal of Cu²⁺ using chelation with EDTA (data not presented in this note). It was clear that there was a dramatic dimensional effect on the PrP during copper addition. This effect was not reversed by returning the system to running buffer without copper. Only on addition of EDTA, did the PrP dimensions return to their original value.

Conclusions and Benefits

These experiments show how DPI can be applied to the study of conformational change during protein-metal ion interaction. The **AnaLight®** instruments and their experimental protocols give the researcher a unique combination of high-resolution data in real time on mass, dimensions and fold density (RI) in a bench-top instrument. The **AnaLight®** is an important enabling tool for protein biochemists and biophysicists, giving them the ability to:

- Rapidly and sensitively detect low atomic weight metal cations binding to large proteins
- Establish whether observed interactions are structurally and functionally important and 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

Farfield gratefully acknowledges that these experiments were carried out in collaboration with Dr Yuji Kamatari, Dr Tomoharu Matsumoto, Dr Kota Kodama and Dr Kazuo Kuwata from the Division of Prion Research in the Center for Emerging Infectious Diseases (CEID), Gifu University, Gifu, Japan

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

⁽¹⁾ GL Milhauser. Copper binding in the prion protein. *Acc Chem Res.* 2004 Feb;37(2):79-85