Measuring α-Synuclein Aggregation in Wild Type and Mutant Forms using Dual Polarisation Interferometry

Introduction

Dual Polarisation Interferometry (DPI) is an important tool for the study of the structural events that underpin biomolecular function and interaction [(1)]. This application note describes the use of DPI to produce pioneering real time, quantitative data on the structural processes behind *in vitro* aggregation of three different forms of α-synuclein.

A key feature of Parkinson's disease is the formation and accumulation of plaques on the surface of neuronal cells in the brain leading to subsequent neurodegeneration. The formation of these plaques is implicated in the parthenogenesis of Parkinson's disease. These plaques are thought to result from an aggregation process involving α-synuclein peptides. The molecular mechanisms behind the aggregation of α-synuclein and the formation of plaques, and how these might be linked to the degeneration of neuronal cells, are areas of interest in the search for drugs to treat Parkinson's disease. However, the lack of sensitivity provided by many analytical techniques has made the study of the mechanism of aggregation extremely challenging.

We were interested in using DPI to gain a real time understanding of the α-synuclein aggregation process by comparing the behaviour of wild type α-synuclein, which is known not to aggregate sufficiently to cause disease, with two mutant forms that are known to form aggregates. DPI has also been demonstrated to be a valuable technique for the study of the peptide aggregation processes behind Alzheimer's disease ***(Farfield Application Note 004)***.

Experimental

The DPI experiments were performed on a Farfield ***Ana*Light**® instrument. The surface used in these studies was a silicon oxynitride ***Ana*Chip**™ functionalised with glutaraldehyde to immobilise the initial α-synuclein layer. The temperature of the samples was controlled throughout to 37°C. Water used in buffer and reagent preparation was deionised and free from organic impurities. All buffers and reagents were analytical grade or higher, and solutions were degassed prior to use.

α-Synuclein Aggregation: The three forms of α-synuclein peptide used for these studies were the non-aggregating wild type **WT** and two aggregating mutants with single amino acid substitutions, **A30P** and **A35T**. The α-synuclein samples (50μM in 10mM phosphate buffer, 150mM NaCl, pH7.4) were each introduced to the ***Ana*Light**® instrument fluidics. The flow was stopped when the peptide solution covered the ***Ana*Chip**™ surface. The accumulation of peptide on the ***Ana*Chip**™ surface measured during incubation (37°C) for a total of 72 hours, after which the ***Ana*Chip**™ was removed from the instrument and SEM images were obtained in order to confirm that the formation of mature α–synuclein aggregates had occurred (see **Figure 4**).

Results and Discussion

α-Synuclein Aggregation: Protein and peptide aggregation is often monitored using techniques that typically require aggregation processes to proceed for several hours under similar conditions before meaningful measurements can be taken. **Figure 1**, however, demonstrates that DPI can be used to study the very earliest stages of aggregation processes and shows the first ***two minutes*** of the α-synuclein aggregation experiments. **Figure 1** displays only the raw signals from a single polarization. Whilst this clearly demonstrates that there are differences in the kinetics of adlayer formation between the three forms of α-synuclein, it is not possible to draw strong conclusions about the molecular processes occurring in the three different α-synuclein samples from this data alone. However, when data from both polarizations is resolved into the core DPI parameters of thickness (**Figure 2**) and density (**Figure 3**), it is clear that dramatically different processes are occurring in the case of the two mutant forms.

In the case of the **WT** sample, **Figures 2 & 3** show that a thin (approx. 7Å), dense layer of α-synuclein is immobilized to the ***Ana*Chip**™ surface *via* the glutaraldehyde functionality. There is little evidence from either the thickness or the density profiles that **WT** proceeds to form aggregate structures, and this was confirmed when this thin, dense layer did not change significantly throughout the remaining 72 hours of the experiment.

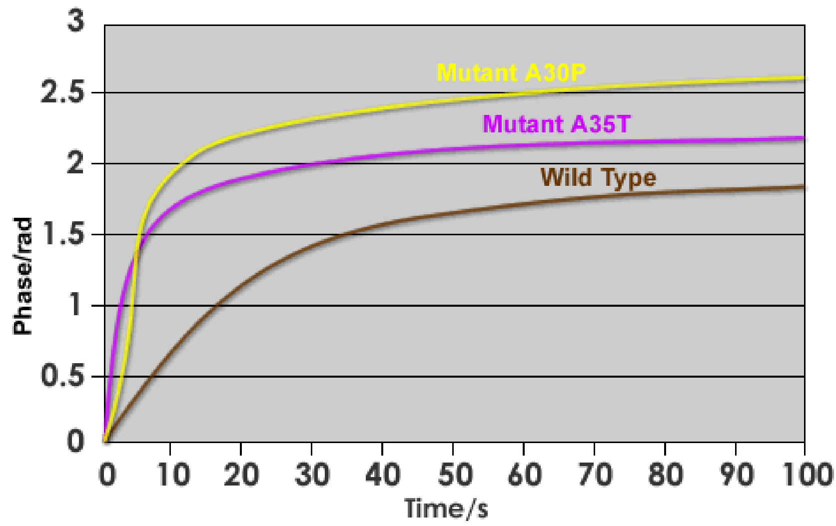

Figure 1: Single polarisation phase response for thin films of wild type and mutant forms of a-synuclein

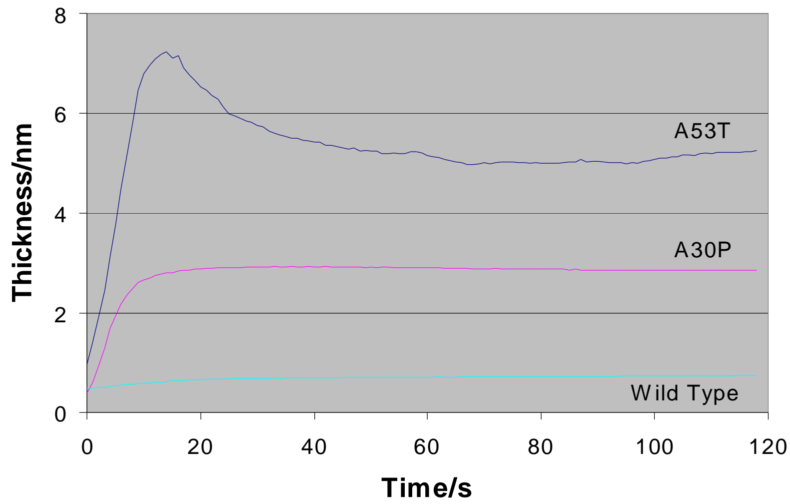

Figure 1: Thickness profile during early stage aggregation of wild type and mutant forms of α-synuclein

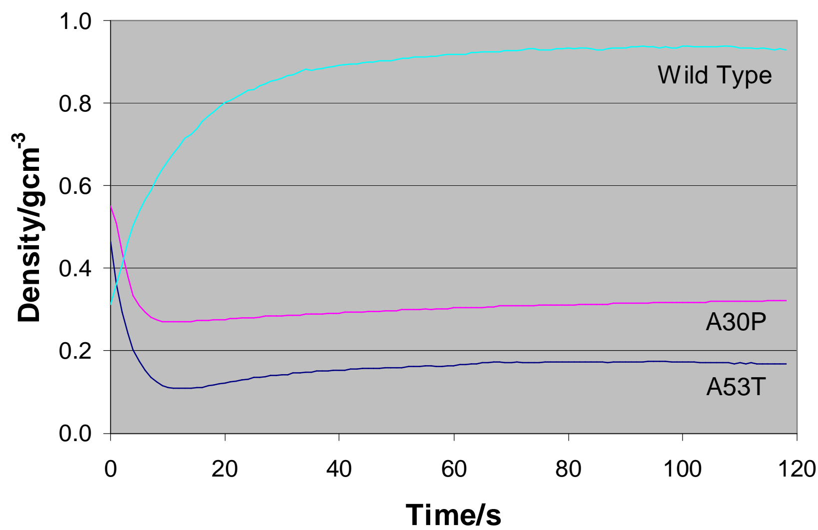

Figure 2: Density profile during early stage aggregation of wild type and mutant forms of α-synuclein

Figures 2 & 3 show clearly that the adlayer structures formed by the mutants **A35T** and **A30P** are different from the structure obtained with **WT**. Both mutants initially form much thicker adlayers (55Å in the case of **A35T** and 30Å in the

case of **A30P**), both of which are also much less dense than in the case of the **WT**. The adlayer dimensions suggest that the mutant forms may well have aggregated in solution before they are immobilized to the ***Ana*Chip**™ surface. Once the mutant adlayers have formed, they subsequently exhibit the typical characteristics of fibrillar aggregation, with the thickness of the adlayer increasing whilst its density decreases. Fibrillar structures are inefficient in terms of packing, hence the observed density decrease, but lead to an increase in the adlayer thickness and mass as they grow.

The experiment was allowed to continue for 72 hours after which diffuse layers of around 61Å in thickness were observed for the mutant forms. In the case of **WT**, the thin, dense layer initially formed did not evolve further. Scanning Electron Microscopy was used to confirm the presence of aggregate structures on the surface of the ***Ana*Chip**™ at the end of the experiment (**Figure 4**).

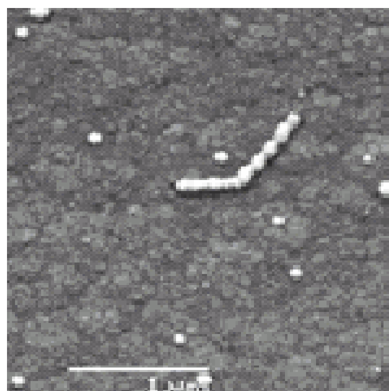

Figure 4: Scanning Electron Micrograph of an α-synuclein protofibril on the surface of the *Ana*Chip™ at the end of the 72-hour experiment, confirming that an aggregation process has occurred

Conclusions and Benefits

This study demonstrates DPI as an enabling technique to study and compare molecular aggregation processes, even at the very earliest stages. Aggregates can be formed simply by immobilizing α-synuclein to the ***Ana*Chip**™ surface using glutaraldehyde and observing the accumulation of aggregates in real time. DPI can be used to rapidly screen peptides to determine their propensity to aggregate in a matter of minutes, rather than the much longer timescales required by other analytical techniques. DPI also displays unsurpassed instrument stability to allow these processes to be measured over a time period of over 72 hours.

The ***Ana*Light**® instrument range and associated experimental protocols give the researcher a unique combination of high-resolution data in real time on thickness, refractive index (density) and surface coverage from a bench top technique, providing an important enabling tool to:

- Clearly understand the molecular processes involved in early stage aggregation
- Differentiate between fibrillar and non-fibrillar aggregation processes
- Obtain real time data on the growth characteristics of WT and mutant forms of α-synuclein aggregation
- Compare the analytical data obtained with complimentary techniques
- Postulate the condition of the α-synuclein variants when they are immobilized to the ***Ana*Chip**™ surface
- Utilize unsurpassed instrument stability allows processes to be measured over several days

Farfield gratefully acknowledges that these experiments were carried out according to a protocol and using samples provided by Professor David Allsop from the Department of Biological Sciences, Lancaster University, Lancaster UK and Dr Omar El Agnaf from the Department of Biochemistry, United Arab Emirates University, UAE.

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(1) M. Swann, N. Freeman, S. Carrington, G. Ronan & P. Barrett. ***Letters in Peptide Science*** 10 (2003) 487-494