

## High quality circular dichroism spectroscopy of proteins in highly scattering solutions or suspensions

### Initial Information

June 2006

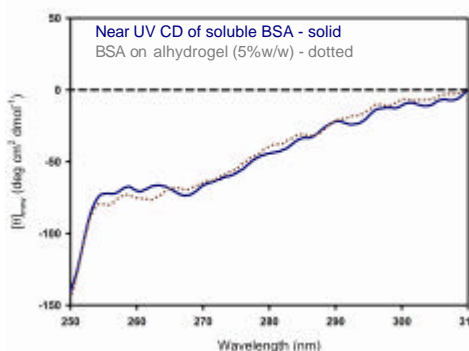
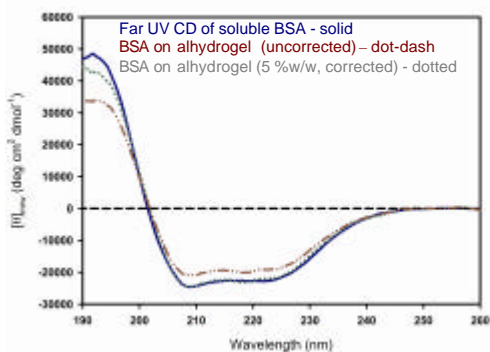
### Introduction

A technique for measuring good quality circular dichroism spectra of proteins in the presence of scattering particles was recently developed in a joint collaboration between scientists at the Universities of Glasgow and Strathclyde<sup>1</sup>. For the first time this provides a reliable way to study the secondary and tertiary structure of proteins

- i) bound to or within microparticles or microcrystals
- ii) as suspensions of lyophilised powders
- iii) in aqueous in the presence of scattering particles or as aggregates.

Measurements can be made in aqueous or in suitable inert solvents.

This enabling spectroscopic technique has been further developed at XstalBio by Dr Ashok Ganesan, the first author on the original publication. For example its applicability to the study of antigens bound to alhydrogel in aqueous (see spectra below) and to suspensions of immobilised protein particles in organic solvents was reported at the AAPS National Biotechnology Meeting in Boston, June 2006<sup>2</sup>.



### The Technology

The circular dichroism measurements are carried out in a specially-designed motor-driven rotating cylindrical cell-holder that can be used to prevent particles from sedimenting during the spectral acquisition. With appropriate modifications to the optical path this cell can be used in conventional spectropolarimeters such as the Jasco J-810. By application of appropriate know-how and sample preparation it is then possible to obtain good quality spectra of proteins from a wide range of particles suspended in various UV transparent solvents including aqueous buffer, ethanol, propanol and acetonitrile. Where necessary correction algorithms (Q correction) may be applied to take into account the absorption flattening commonly observed in such systems. The

resultant far UV CD spectra are often of sufficient quality to allow secondary structural analysis to be carried out with freely available software packages. For example CDSSTR from DICHROWEB was used to analyse the above far UV spectra of BSA in solution and bound to alhydrogel as shown in the table below.

#### Secondary structure analysis of BSA and BSA-alhydrogel suspension in PBS

Protein	Protein loading	Q <sub>2</sub> correction	Helix	Sheet	Turns	Unordered
X-ray (HSA)	-	-	70	0	15	15
BSA (sol)	-	-	73	5	7	15
BSA 1	5	No	62	8	11	19
BSA 1	5	Yes	70	8	9	14

The analysis shows there are only small changes to secondary structure on binding of BSA to alhydrogel. Interestingly, this is contrary to a recent published study where infrared was used to deduce the protein became more disordered<sup>3</sup>. From the near UV spectra it is clear that subtle changes in BSA tertiary structure do take place on binding. This is consistent with published fluorescence measurements showing a blue shift upon adsorption to alhydrogel<sup>3</sup>.

#### What we are offering:

XstalBio is offering to provide companies interested in the delivery and stabilisation of proteins or vaccines an immediate competitive advantage by rapidly transferring this important new technique into their analytical laboratories. Thus, if an initial evaluation study is successful XstalBio will undertake a technology transfer programme and tailor the methodology and cell design for the research requirements of the interested party.

#### References

1. Circular dichroism studies of subtilisin Carlsberg immobilised on micron sized silica particles, A. Ganesan, N. C. Price, S. M. Kelly, I. Petry, B. D. Moore, P. J. Halling *Biochim. Biophys. Acta* **2006**, 1764, 6, 1119-1125.
2. Circular dichroism for assessing the solid-state structure of proteins or antigens A. Ganesan, P. J. Halling, S. M. Kelly, N. C. Price, C. Lyle, and B. D. Moore, *AAPS National Biotechnology Conference 2006*, Boston, Abstract T2003.
3. Effects of adsorption to aluminum salt adjuvants on the structure and stability of model protein antigens, L. S. Jones, L. J. Peek, J. Power, A. Markham, B. Yazzie, C. R. Middaugh *J. Biol. Chem.* **2005**, 280, 14, 13406–13414

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