

Monitoring the assembly of antibody-binding membrane protein arrays using polarised neutron reflection

Anton P. Le Brun · Stephen A. Holt · Deepan S. Shah · Charles F. Majkrzak · Jeremy H. Lakey

Received: 29 September 2007 / Revised: 1 February 2008 / Accepted: 11 February 2008 / Published online: 4 March 2008
© EBSA 2008

Abstract Protein arrays are used in a wide range of applications. The array described here binds IgG antibodies, produced in rabbit, to gold surfaces via a scaffold protein. The scaffold protein is a fusion of the monomeric *E. coli* porin outer membrane protein A (OmpA) and the Z domain of *Staphylococcus aureus* protein A. The OmpA binds to gold surfaces via a cysteine residue in a periplasmic turn and the Z domain binds immunoglobulins via their constant region. Polarised Neutron Reflection is used to probe the structure perpendicular to the gold surface at each stage of the assembly of the arrays. Polarised neutrons are used as this provides a means of achieving extra contrast in samples having a magnetic metal layer under the gold surface. This contrast is attained without resorting to hydrogen/deuterium

exchange in the biological layer. Polarised Neutron Reflection allows for the modelling of many and complex layers with good fits. The total thickness of the biological layer immobilised on the gold surface is found to be 187 Å and the layer can thus far be separated into its lipid, protein and solvent parts.

Abbreviations

BME	2-Mercaptoethanol
DTT	Dithiothreitol
IgG	Immunoglobulin G
IMAC	Immobilized metal affinity chromatography
IPTG	Isopropyl- β -D-thiogalactopyranoside
OG	<i>n</i> -Octylglucoside
OmpA	Outer membrane protein A
OmpAZ	Circularly permuted OmpA with two SpA Z domains fused at the N terminus
PNR	Polarised Neutron Reflection
Q	Momentum transfer
SDS	Sodium dodecyl sulphate
SLD	Scattering length density
SpA	<i>Staphylococcus aureus</i> protein A
TCEP	Tris(2-carboxy-ethyl)phosphine hydrochloride
thioPEG	Polyethylene glycol with a thiolalkane functional group

Advanced neutron scattering and complementary techniques to study biological systems. Contributions from the meetings, “Neutrons in Biology”, STFC Rutherford Appleton Laboratory, Didcot, UK, 11–13 July and “Proteins At Work 2007”, Perugia, Italy, 28–30 May 2007.

A. P. Le Brun · J. H. Lakey (✉)
Institute for Cell and Molecular Biosciences,
The Medical School, University of Newcastle upon Tyne,
Framlington Place, Newcastle upon Tyne NE2 4HH, UK
e-mail: j.h.lakey@ncl.ac.uk

A. P. Le Brun
e-mail: anton.le-brun@ncl.ac.uk

S. A. Holt
ISIS, STFC Rutherford Appleton Laboratory,
Didcot, Oxfordshire OX11 0QX, UK

D. S. Shah
Orla Protein Technologies Ltd, Nanotechnology Centre,
Herschel Building, Newcastle upon Tyne NE1 7RU, UK

C. F. Majkrzak
NIST Centre for Neutron Research,
Gaithersburg, MD 20899, USA

Introduction

Protein arrays

Protein arrays are becoming important tools for various applications including: use as scaffolds for tissue engineering (Liu et al. 2007), proteomics (Unwin et al. 2006) and point of use diagnostics (Kingsmore 2006). Unlike nucleic

acid arrays, proteins used in arrays should maintain their 3D structure in order to be fully functional. Methods which are able to study the structure of immobilised protein arrays in contact with aqueous solutions are therefore required.

Molecules can be immobilised to a variety of different surfaces using various different chemistries. For gold surfaces molecules can be immobilised using thiol chemistry (Dubois and Nuzzo 1992). The sulphur atom in thiol alkanes will form strong gold-thiolate bonds with the gold. The monolayer that is formed is strong, stable and orientated (Bain et al. 1989). Thiol bearing cysteine residues can be introduced into strategic locations in proteins using conventional molecular biology techniques. A single cysteine in the protein will bond the protein to the gold surface in a specific orientation.

The methods used so far to study proteins on surfaces include spectroscopic techniques such as Surface Plasmon Resonance (SPR), Dual-Polarisation Interferometry (DPI) (Terry et al. 2006), Ellipsometry, FTIR (Terrettaz et al. 2002) and Circular Dichroism (CD) spectroscopy (Keegan et al. 2005). These latter two techniques can provide secondary structural information on the proteins, and using these techniques structural changes caused by the addition of other array components (such as lipids) or denaturants (such as urea) can be followed. Observing these changes in protein conformation/structure indicates how robustly the protein's 3D-structure is retained in the array. The most common technique associated with biosensors and arrays is SPR, which uses refractive index changes to measure how much material is immobilised onto a surface. Microscopy in the form of Atomic Force Microscopy (AFM) is the preferred way of directly imaging single molecules on a surface (Cisneros et al. 2006).

The methodologies described provide either little structural information (SPR) or high resolution over small sample areas (AFM). While AFM can provide information in all three axes (x , y and z), the z -axis (perpendicular to the surface) is probed as a measure of topology or sometimes flexibility. The advantage of also using neutron reflection to study surface immobilised proteins is that a large sample area is "viewed" and structural information is obtained continuously along the z -axis revealing the physical and chemical composition of complex and hidden layers at high resolution.

Polarised Neutron Reflection (PNR)

When studying biological systems using neutron scattering the most commonly used method of achieving contrast between different components is to selectively replace hydrogen in the sample with deuterium. This relies upon the large difference in scattering length density between the

two isotopes and is easily achieved for the aqueous phase of the sample (Thomas 2004). However, producing deuterated biological products is not a trivial task and PNR provides a simpler route to increased contrast.

When depositing gold onto silicon an intermediate metal layer is required as the gold does not 'stick' well to the silicon substrate. If the metal under the gold has magnetic properties (e.g., iron or nickel) then the intermediate layer can provide magnetic contrast in the following way. Neutrons can be polarised to select for their spin property (spin up or spin down) and are reflected differently by magnetic layers (Majkrzak 1991). If the sample is placed in a permanent saturating magnetic field where the direction of the field is in the plane of the interface, the magnetic moment generated in the buried layer will result in the reflectivity profile of the whole sample being spin dependent (Parkin et al. 1990). This gives two independent reflection profiles of the same sample, which both report on the entire layer structure.

Thus the advantage of using magnetic contrast to studying immobilised biological layers is that contrast is achieved without having to change anything in the biological sample (Majkrzak and Berk 1999).

A model protein array

Outer Membrane Protein A (OmpA) is a monomeric outer membrane protein from *E. coli*, which has a beta-barrel structure (Pautsch and Schulz 1998). The protein has been circularly permuted (Koebink and Kramer 1995) (in this case this is where the N- and C-termini have been swapped from the periplasmic side to the exterior of the outer membrane) so that protein tags can be fused to the OmpA scaffold whilst still allowing for surface immobilisation. OmpA is immobilised to gold surfaces through a cysteine residue that has been inserted into the periplasmic turn four (Shah et al. 2007). The circular permutation, beta-barrel structure and gold immobilisation make the OmpA an ideal scaffold protein for use in membrane arrays since protein domains can be genetically fused to its N- or C-terminus. Here we use the Z domain of *Staphylococcus aureus* protein A (SpA). SpA is a pathogenicity factor bound to the cell wall of the bacterium. It has five domains: E, D, A, B, and C (from the N-terminus) (Tashiro and Montelione 1995). The Z domain is effectively a B domain with the mutations A1V and G29A (Nilsson et al. 1987). The constant regions of immunoglobulins (antibodies) bind to the Z domain (Deisenhofer 1981) leaving the variable (antigen binding) domain free to function. Two Z domains have been fused to the N-terminal of the OmpA scaffold to create OmpAZ, so that antibody arrays can be built up upon an oriented protein scaffold.

Aims

The aims of this study are to show that neutron reflection can provide structural information on immunoglobulins bound to a pre-deposited array of OmpAZ molecules immobilised upon gold surfaces. In order to accomplish this demanding objective of defining many layers we will use samples in which a magnetic layer lies under the gold surface so that magnetic contrast can be used.

Experimental

Protein preparation

The OmpAZ protein was expressed from the plasmid pOR-LA18 in *E. coli* BL21(DE3) cells with induction at $OD_{600} \sim 0.6$ with a final concentration of 1 mM IPTG. The protein formed inclusion bodies, which were isolated by disrupting the cells in Bug Buster (Novagen) with nucleases and lysozyme added. The inclusion bodies were washed three times in a detergent solution (1:10 dilution of Bug Buster) and solubilised in 8 M urea, 20 mM sodium phosphate pH 7.6, 500 mM sodium chloride and 20 mM imidazole. Contaminants were removed by a two-step purification step. Firstly IMAC purification was carried out using a 1 ml HisTrap column (GE Healthcare) with elution in 250 mM imidazole. Secondly, after buffer exchange into 8 M urea and 30 mM ethanolamine pH 9.5 anion exchange chromatography with a 1 ml Q FF HiTrap column (GE Healthcare) with elution in 80 mM sodium chloride was used to complete the purification.

The protein was refolded from the urea denatured state by a slow (approximately 200 μ l of stock urea solubilised protein per hour) one in ten dilution into refold buffer [consisting of 50 mM Tris-HCl pH 8, 1 mM DTT, 0.1 mM EDTA and 1 % (w:v) OG]. When the dilution was complete the protein was left for at least 48 h at 37°C to refold. Before use the protein was passed down a PD10 column (GE Healthcare) and eluted in fresh refold buffer.

Production of gold surfaces

For experiments carried out at the NIST Centre for Neutron Research (NCNR) the gold surfaces were made in-house by magnetron sputtering (Willmott 2004). This is a process where the metal to be deposited (the target) is bombarded with a positively ionised gas (argon) in a high vacuum when a negative potential of approximately -300 V is applied to the target. The bombardment of the target by the gas releases neutral metal atoms that coat the vacuum chamber. The process is maintained by a series of magnets placed behind the target, which keep any elec-

trons that are released in close proximity to the target increasing the chance of the gas being ionised and hence releasing more metal atoms. The vacuum chamber will also contain the silicon substrate to be coated. The sputtered atoms coat the substrate in a four-stage process: nucleation, island growth, coalescence and continuous growth.

The substrates used at CRISP, ISIS are made by ion beam deposition by INESC Microsistemas and Nanotecnologias (Lisbon, Portugal) using a Nordiko 3000 Ion Beam system. The metal to be deposited is evaporated and ionised. The ions are then accelerated through a high electric field and then decelerated to target the substrate.

Preparation of the protein arrays

The gold surfaces are pretreated with a 1% (v/v) solution of β -mercaptoethanol (BME). The thiol group on the β -carbon bonds to the gold surface. This leaves the hydroxyl groups exposed creating a hydrophilic surface. This allows for more specific orientation of the protein when binding to the gold surface as the amount of hydrophobic interactions between the outside of the β -barrel structure of the scaffold protein and the gold surface are reduced. It has also been shown to prevent unfolding of the protein at the gold surface (Terretaz et al. 2002). For a more detailed description see Keegan et al. (2005).

Before the protein is incubated on the surface the thiol group in the protein needs to be reduced to enable specific bonding to the gold surface. This is done using the reducing agent Tris(2-carboxy-ethyl)phosphine hydrochloride (TCEP) to a final concentration of 5 mM. TCEP is the preferred reducing agent because unlike dithiothreitol (DTT) and BME, TCEP will not compete with the protein during assembly by binding to the gold surface. The gold surface is coated with a protein solution at concentrations between 0.2 and 0.5 mg/ml and incubated at room temperature for 3 h on the surface ex situ of the cell. The surface is washed with a 1% (w:v) solution of sodium dodecyl sulphate (SDS). The SDS will remove any protein that is non-specifically bound to the gold surface. The SDS wash is followed with a wash with water.

The final addition to the gold surfaces is of a “thioPEG” [$HS-(CH_2)_{11}-(OCH_2CH_2)_3-OH$] (Prochimia). ThioPEG is diluted to a final concentration of 0.25 mg/ml in a 1 % (w:v) OG solution. Before incubation on the gold surface the thioPEG was incubated at 55°C with a final concentration of 5 mM TCEP. The thioPEG is incubated on the gold surface at room temperature for 3 h with a fresh application of warmed thioPEG every hour. At the end of the incubation in thioPEG the gold surface is once again washed with a 1% (w:v) SDS solution to remove any non-specifically bound thioPEG.

The final step to build up a complete array as seen in Fig. 1 is to add the antibody. Rabbit IgG raised against Human Serum Albumin (HSA) was diluted 1:100 in buffered D₂O and added to the pre-assembled layer in the cell in situ. The antibody was left for 30 min incubation at room temperature before a wash of buffered D₂O (~20 ml) was carried out to remove any unbound/excess antibody.

Carrying out the reflection experiments

The PNR experiments were carried out at the NG-1 instrument at NCNR, USA and the CRISP instrument at ISIS Spallation Source, UK. On both instruments the incident neutron beam was reflected from a polarising supermirror resulting in a polarised neutron beam. The polarising mirror reflects one spin state out of the plane of the neutron beam. Then the neutron beam passes through a spin flipper. If the spin flipper is off (the non-flip state) then the neutrons will pass straight through in their original spin state. If the spin flipper is activated then the neutrons will be flipped to the opposite spin state (the flip state). Neutrons where the spin is parallel to the magnetic fields are designated spin up and those that are antiparallel are designated spin down.

The NCNR is based around a 10 MW reactor with NG-1 viewing a liquid hydrogen moderator that produces cold neutrons. NG-1 employs a focussing pyrolytic graphite monochromator set to produce neutrons at a fixed neutron wavelength of 4.75 Å. The sample is moved through a

series of incident angles (0.23°–7.2°) to achieve a Q range generally up to 0.3 Å⁻¹. At the ISIS spallation pulsed source the time of flight CRISP instrument views a liquid hydrogen moderator with a white beam of neutrons, wavelength range 1.4–6.5 Å in polarised mode. The sample is held at three different fixed angles (0.35°, 0.8° and 1.8°) to achieve the Q range of up to 0.3 Å⁻¹.

The samples were maintained at ambient temperature and pressures. The silicon wafer sample sizes for experiments at NCNR were 50 mm in diameter and for ISIS a sample size of 100 mm in diameter was used.

Data analysis

Analysis of data collected both at NCNR and CRISP was done using the programme GA_refl (Kienzle et al. 2000–2006). The programme defines each layer as a series of slabs each with the property of thickness, scattering length density and interfacial roughness. It uses an optical transfer method (Penfold 1991) for modelling the data and then a genetic algorithm for a least squares refinement of the models. Each pair of spin dependent datasets is refined simultaneously where the only difference between the real space SLD profiles of the two datasets being the SLD of the magnetic layer. The basic model employed consisted of the silicon substrate then layers corresponding to silicon oxide, magnetic reference layer and gold. Extra layers were added to account for the subsequent protein, thioPEG and antibody adsorption.

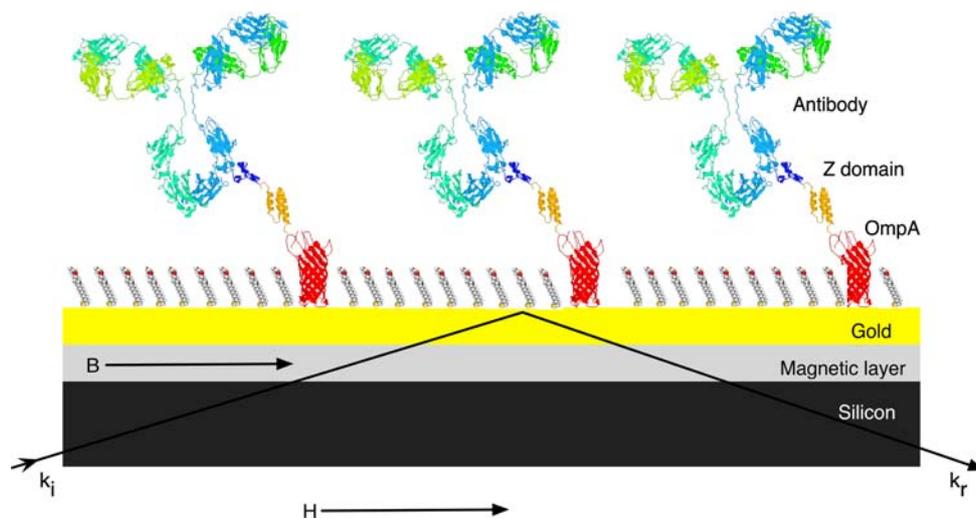


Fig. 1 A schematic (not to scale) of the array to be studied using PNR. Deposited onto the silicon substrate is a layer of an alloy of iron and nickel (μ -metal) that is magnetic and provides contrast between the different neutron spin states. Above the magnetic layer is a layer of gold to which the biological layer is deposited. The protein OmpAZ has a single cysteine residue in periplasmic turn four allowing the protein to bind in a specific orientation to the gold. The space between

protein molecules is filled with thioPEG. The OmpAZ protein has two SpA Z domains that can bind IgG antibodies in their constant regions. The arrows indicating the direction of travel of the incident and reflected neutron beams are k_i and k_r , respectively. The direction of the permanent external magnetic field is the arrow labelled H and the direction of magnet moment generated in the magnetic layer is labelled B

Results and discussion

Assembly of the OmpAZ array

After the gold surfaces were pretreated with 2-mercaptoethanol, the protein can be bound to the gold surface *ex situ* reaching surface coverages of between 40 and 50% as determined by PNR. The remaining exposed gold surface is filled with the thioPEG molecule (a membrane lipid mimic) making the completed array (Fig. 1). The individual steps of the build up of the array can be followed by neutron reflection. Figure 2 shows the difference in the reflectivity profile between a gold surface with only OmpAZ deposited and a reflectivity profile where thioPEG has been deposited after the protein deposition. The major differences are at the low Q region after the critical edge (the point at which total external reflection occurs) and in the intermediate Q regime where extra fringes are observed. The changes at low Q enable a quick assessment of the thioPEG assembly step. The large changes in the reflectivity signal occur because the environment around the protein has changed from being exclusively D_2O solvent, which has a high SLD, to being predominantly hydrogenous thioPEG, which has a very low SLD.

The height of the OmpAZ molecule is 124 Å (Fig. 3) with a roughness of 29 Å suggesting great flexibility in the two Z domains. The gold layer has its own roughness in the model at 9 Å and so the protein roughness should not be

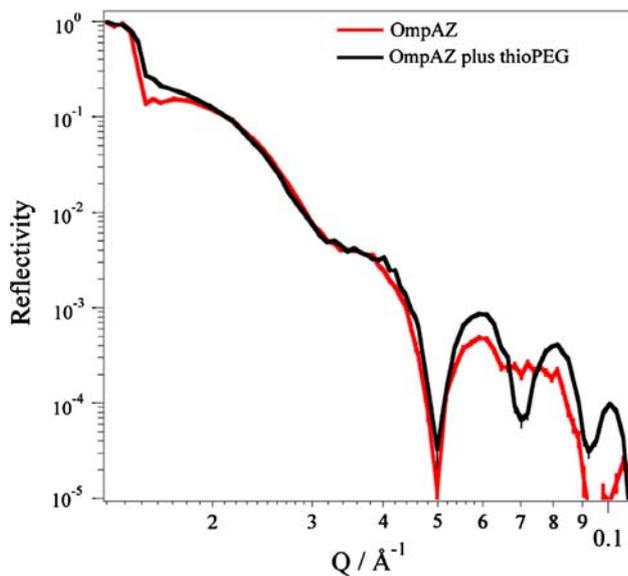


Fig. 2 The build-up of a membrane protein array can be observed using neutron reflection. The reflectivity profile represents the intensity (reflectivity) of neutrons observed at each Q value (spin up state only). The red line is a profile of only protein and BME immobilised onto the gold surface. The black line shows the reflectivity profile in the low Q range after thioPEG deposition. A reduced Q range is displayed highlighting the changes in the low Q region

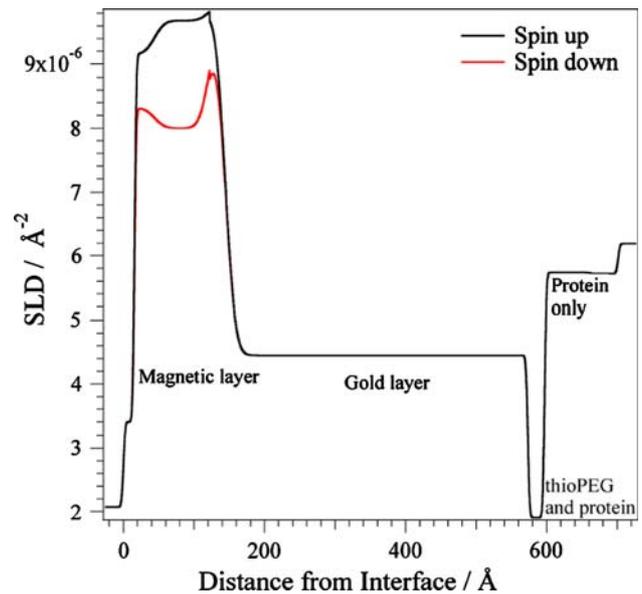


Fig. 3 Scattering length density profile of the OmpAZ array system. The magnetic layer has two scattering length densities depending on the spin state of the neutrons. Between the interfaces of the magnetic layer with the silicon and gold there are magnetic dead zones. The gold layer is modelled to be 430 Å, the mixed protein and thioPEG layer to be 21 Å and the OmpAZ only layer to be 103 Å with a SLD of $5.7 \times 10^{-6} \text{ Å}^{-2}$

affected by the roughness of the gold. The thioPEG layer is 21 Å high and can be split in two distinct parts: the hydrophobic alkyl tail and the more hydrophilic (hydrogen bond accepting only) PEG head group. Splitting the thioPEG into two parts does not add any benefit to the data analysis to the system as similar χ^2 values of approximately 1.8 are obtained for each. Also splitting the PEG adds more layers to the system making it more difficult to model when additional antibody layers are included in later samples. Partitioning the protein into the OmpA and Z domain improves the fits, however non-physical layer thickness is obtained for one or the other. This is most likely because the scattering length densities in the two layers are very similar. A protein layer with no thioPEG added does not fit as well compared to when thioPEG is present (χ^2 of 3.6 and 1.8, respectively).

Contrast using magnetic systems

The magnetic contrast approach enables two datasets to be collected simultaneously from exactly the same biological layers. These independent datasets can be refined simultaneously to good fits (Fig. 4) with real space SLD profiles (Fig. 3) where layers are assigned to different components of the array. Hence more accurate models can be solved. This method of contrast variation enables models of greater complexity to be reliably applied than that published on similar systems (Holt et al. 2005).

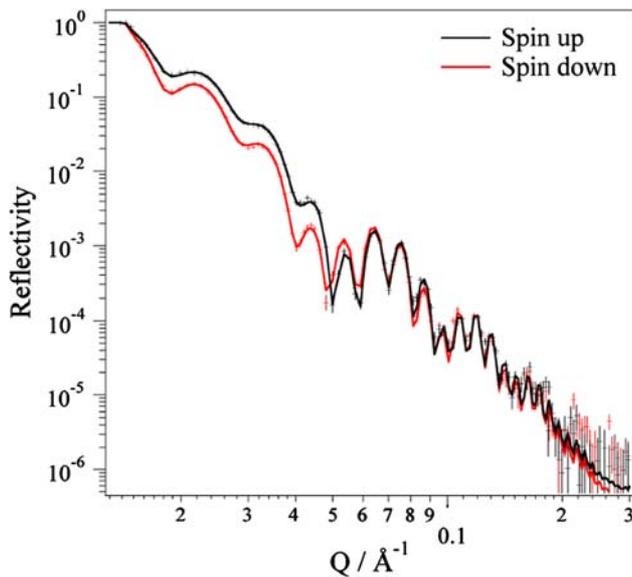


Fig. 4 The reflectivity profile of a silicon substrate with μ -metal and gold layers. Immobilised onto the gold is a layer of OmpAZ and thioPEG. The two different reflectivity profiles are of the same system but the upper profile are of neutrons in the up spin state (black line) and the lower profile of neutrons in the down spin state (red line). The lines are the fitted data fits to a χ^2 of 1.8 and the crosses are the collected data

Antibody addition

On addition of a rabbit IgG antibody, appreciable binding to the OmpAZ array can be observed using SPR (data not shown). With neutron reflection a change in the reflectivity profile can be clearly seen (Fig. 5). After a wash with 2.5 M citric acid (pH 3.2), which removes any bound antibody, the reflectivity profile reverts to its original shape. The best model fit so far has a χ^2 of 4.02 and includes an extra protein layer for the Z domain with the antibody bound. The sample in this state is now more complex than any biological system yet modelled from NR data. The extra information from magnetic contrast variation is critically important in this analysis and should allow complex membrane models in addition to protein arrays to be studied in the future. Currently the data indicates a total bio-layer thickness near to 187 Å and we are currently refining models to divide this into OmpA, Z domain and antibody. To increase the number of distinct reflectivity profiles that can be used to solve this complexity the contrasts can be varied by deuteration of solvent and the layer components themselves. Solvent mixtures used so far are D_2O , which provides the strongest contrast against the hydrogenated protein and thioPEG molecules and gold matched water (25.1% H_2O and 74.9% D_2O by mass), which highlights the lower boundary of the proteins SLD. Due to the fact that proteins and thioPEG are hydrogen rich, H_2O provides the weakest contrast and the

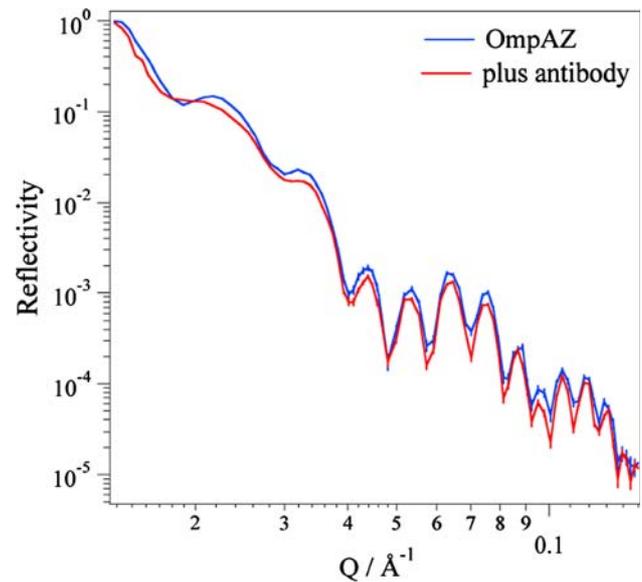


Fig. 5 Reflectivity profiles of an OmpAZ array without antibody (blue line), then with antibody added (red line). Both profiles are of the up spin state. The antibody used is a rabbit IgG raised against human serum albumin

least information for modelling. Future work will use deuterated OmpAZ purified from bacteria grown in deuterated media (from Silantes GmbH, Munich, Germany) and deuterated synthetic thioPEG. Since proteins contain largely carbon and nitrogen their deuteration leads to an approximate change in SLD from $3.5 \times 10^{-6} \text{ \AA}^{-2}$ (for hydrogenated protein in H_2O) to $7.7 \times 10^{-6} \text{ \AA}^{-2}$ (for deuterated protein in D_2O). Lipids on the other hand can show changes from $-0.85 \times 10^{-6} \text{ \AA}^{-2}$ to $7.4 \times 10^{-6} \text{ \AA}^{-2}$ in the CH_2 groups of their alkyl chains. For each set, which as stated above requires significant investment to produce, magnetic contrast variation can again double the amount of useful contrast data obtained. Combinations of these methods may provide the means to model even the most complex biological layers such as bacterial outer membranes. At this stage we have not fully modelled the IgG and an accurate representation of this will need a precise measure of the occupancy. This may be achieved by a complement of PNR and SPR etc.

Conclusions

This work has demonstrated that by using polarised neutrons with an embedded magnetic reference layer it is possible to achieve high-resolution neutron reflection data from adsorbed multilayer biomolecular films without variation of the sample contrast. The total thickness of the OmpAZ layer is 124 Å, which compares well with the

combined known thickness of OmpAZ, 60 Å plus two Z domains at 30 Å each. The IgG as depicted in Fig. 1 would be about 140 Å, if the IgG conformation were such that the two hinges were “flattened” out of the layer would be about 20 Å. Our currently observed layer thickness of around 190 Å indicates that the IgG is probably adsorbed in a conformation intermediate between that depicted and a flattened state.

Acknowledgments The authors would like to thank Frank Heinrich for help with preparation of the gold surfaces. The Biotechnology and Biological Sciences Research Council, the Science and Technology Facilities Council and Orla Protein Technologies Ltd funded this work. The identification of any commercial product or trade name does not imply endorsement or recommendation by the National Institute of Standards and Technology.

References

- Bain CD, Troughton EB, Tao Y-T, Evall J, Whitesides GM, Nuzzo RG (1989) Formation of monolayer films by the spontaneous assembly of organic thiols from solution onto gold. *J Am Chem Soc* 111:321–335
- Cisneros DA, Muller DJ, Daud SM, Lakey JH (2006) An approach to prepare membrane proteins for single-molecule imaging. *Angew Chem Int Ed* 45:3252–3256
- Deisenhofer J (1981) Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8 Å resolution. *Biochemistry* 20:2361–2370
- Dubois LH, Nuzzo RG (1992) Synthesis, structure and properties of model organic surfaces. *Annu Rev Phys Chem* 43:437–463
- Holt SA, Lakey JH, Daud SM, Keegan N (2005) Neutron reflectometry of membrane protein assemblies at the solid/liquid interface. *Aust J Chem* 58:674–677
- Keegan N, Lakey JH, Wright NG (2005) Circular dichroism spectroscopy of folding in a protein monolayer. *Angew Chem Int Ed* 44:4801–4804
- Kienzle PA, Doucet M, McGillivray DJ, O’Donovan KV, Berk NF, Majkrzak CF (2000–2006) GA_refl for simultaneous fitting of reflection data <http://www.ncnr.nist.gov/reflpak>
- Kingsmore SF (2006) Multiplexed protein measurement: technologies and applications of protein and antibody arrays. *Nat Rev Drug Discov* 5:310–320
- Koebink R, Kramer L (1995) Membrane assembly of circularly permuted variants of the *E. coli* outer membrane protein OmpA. *J Mol Biol* 250:617–626
- Liu C, Xia Z, Czernuszka JT (2007) Design and development of three-dimensional Scaffolds for tissue engineering. *Chem Eng Res Des* 85:1051–1064
- Majkrzak CF (1991) Polarized neutron reflection. *Physica B* 173:75–88
- Majkrzak CF, Berk NF (1999) Inverting neutron reflectivity from layered film structures using polarized beams. *Physica B* 268:168–174
- Nilsson B, Moks T, Jansson B, Abrahamsén L, Elmblad A, Holmgren E, Henrichson C, Jones TA, Ulhen M (1987) A synthetic IgG-binding domain based on Staphylococcal protein A. *Protein Eng* 1:107–113
- Parkin SSP, Deline VR, Hilleke RO, Felcher GP (1990) Unidirectionally biased permalloy: a polarized-neutron-reflection experiment. *Phys Rev B* 42:10583–10591
- Pautsch A, Schulz GE (1998) Structure of the outer membrane protein A transmembrane domain. *Nat Struct Biol* 5:1013–1017
- Penfold J (1991) Data interpretation in specular neutron reflection. In: Linder P, Zemb T (eds) *Neutron, X-ray and light scattering*. Elsevier, Amsterdam, pp 223–236
- Shah DS, Thomas MB, Phillips S, Cisneros DA, Le Brun AP, Holt SA, Lakey JH (2007) Self-assembling layers created by membrane proteins on gold. *Biochem Soc Trans* 35:522–526
- Tashiro M, Montelione GT (1995) Structure of bacterial immunoglobulin-binding domains and their complexes with immunoglobulins. *Curr Opin Struct Biol* 5:471–481
- Terrettaz S, Ulrich W-P, Vogel H, Hong Q, Dover LG, Lakey JH (2002) Stable self-assembly of a protein engineering scaffold on gold surfaces. *Protein Sci* 11:1917–1925
- Terry CJ, Popplewell JF, Swann MJ, Freeman NJ, Fernig DG (2006) Characterisation of membrane mimetics on a dual polarisation interferometer. *Biosens Bioelectron* 22:627–632
- Thomas RK (2004) Neutron Reflection From Liquid Interfaces. *Annu Rev Phys Chem* 55:391–426
- Unwin RD, Evans CA, Whetton AD (2006) Relative quantification in proteomics: new approaches for biochemistry. *Trends Biochem Sci* 31:473–484
- Willmott PR (2004) Deposition of complex multielemental thin films. *Prog Surf Sci* 76:163–217