Protein based therapeutics, especially those centered on monoclonal antibodies, are a fast growing area of drug development due to their high target-specificity and therefore generally lower toxicity. However, aggregation of proteins is a common issue during product development and manufacturing, as well as during subsequent transportation and storage [1]. Protein aggregation mediated by partial unfolding can be accelerated by inadvertent stresses, such as agitation, exposure to elevated temperatures, chemical degradation, and even exposure to solid-liquid and liquid-water interfaces (e.g. the inside surface of a glass vial or at the air-water interface of trapped bubbles in a tube) [2]. Large aggregate formation is a concern not only due to the potential loss in efficacy and shelf life of a drug but also due to the potential triggering of unwanted and possibly life-threatening immunogenic responses in patients [3].

Association of proteins at bulk interfaces has been empirically implicated in aggregate formation for some proteins but the mechanistic details of the process are not fully known, at least in part, because a majority of characterization techniques measure protein structure and aggregation state only in bulk solution. Therefore the nature of the protein layer at the surface, how its structure changes due to desorption and the correlation with aggregate particle formation in solution are all poorly understood. To target these questions, the interaction of a monoclonal antibody IgG1 with a hydrophilic SiO$_x$ surface has been studied using the unique surface characterization technique of neutron reflectivity (NR). Solution-based methods such as micro flow imaging (MFI) were then used to connect protein surface structure to aggregate particle formation in the aqueous phase.

A schematic of the experimental setup and procedure is shown in Fig. 1. Interpretation of the NR data provides a 1-D profile of the protein density distributed along the surface normal (z-axis) direction from the SiO$_x$ layer. To take advantage of the sensitivity of neutrons to the isotopic state of hydrogen, all measurements were repeated in both H$_2$O and D$_2$O buffer solution and the data were fit simultaneously to a single protein profile structure.

IgG structure at the SiO$_x$ surface was measured at two different pH values, 4.5 and 6.2 and two salt concentrations, 0 mmol/L and 100 mmol/L NaCl resulting in four buffer conditions. Fig. 2 shows the resulting protein layers derived from the NR measurements. At pH 4.5, for both salt conditions, the IgG1 molecules form a narrow single-peak distribution on the SiO$_x$ surface with dimensions consistent with a ‘flat-on’ orientation as depicted in Fig. 2A, insert. At pH 6.2 an alternate layer structure is observed for the low salt condition (Fig. 2B). The broad distribution with a secondary peak can be interpreted as a protrusion of a protein lobe from the surface (Fig. 2B, inset). However the profile could also be due to multiple protein orientations and further measurements are required to distinguish between these cases. During the desorption steps, the structure is unchanged and very little of the protein layer is removed at pH 4.5, indicating a strong surface interaction. Conversely, at pH 6.2, desorption resulted in 20% to 30% of the protein layer removed. This IgG1 molecule was shown to carry a +15e charge at low pH and a greater than 3-fold reduction in surface charge at high pH, [4] suggesting electrostatics play a significant role in promoting surface interactions.