Scientific Background

Cellular signalling is a complicated process regulating and coordinating the myriad of processes in the cell in response to a variety of factors. Dysfunction in the signalling pathways in cells can cause diseases such as …. [ref]. These diseases place a burden of approximately $ X billion per year on the health system in Australia, and this sum is projected to double in the next decade [ref].

ABCDs are a class of protein (~70kDa) involved in regulating … [ref]. These proteins have a variety of binding partners that modulate their function. X is one such binding partner (~30kDa), that prevents binding of ABCDs to their target in response to … [ref]. In some individuals the ABCD:X interactions are linked with a range of chronic diseases [ref].

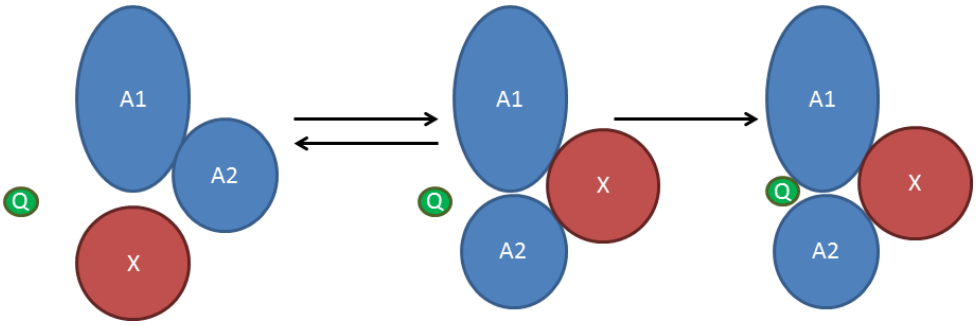

Figure 1: Proposed reaction causing dysfunction upon Protein A binding X. The binding of X is reversible, but if Q binds irreversibly to the new interface between domain A1 and A2, Protein A is unable to return to its original state

Protein A (an ABCD protein) and its ligand X has been linked to … in … individuals [ref]. There are a number of schools of thought on the issue of dysfunction caused by Protein A:X interactions. Our research group has evidence that when Protein A interacts with X, a small molecule, Q, can bind irreversibly [ref] (Fig. 1). We intend to better understand the molecular details of the ProteinA:X binding in order to develop strategies to prevent and treat …

Aim

We believe that binding of X induces a large conformational change in Protein A that exposes a binding area for Q. To test whether ligand X induces a large scale conformational change in Protein A:

- We propose a small-angle neutron scattering experiments with contrast variation to determine the low-resolution structure of Protein A with X

Experimental Details, Preliminary Data and Time Justification

To accurately determine the nature of any structural rearrangements in Protein-A upon binding to X, we propose a small-angle neutron contrast variation experiment. X is a 30kDa protein that will be deuterated to the 75% level, such that it is contrast matched in ~100% D_2O. Unlabelled Protein-A (70kDa, with a match point of ~40% D_2O) will be complexed with ^{D}X, and small-angle neutron contrast variation data will be measured at a range of D_2O concentrations including the two stated match points (Fig 2A). This data should allow us to determine the structure of both Protein-A and X in the complex.

We routinely prepare this complex at concentrations of 10mg/mL, however, preliminary SAXS studies suggest that there is a measurable structure factor above 5mg/mL (see Fig 2B). Our preliminary SAXS data also show that the Protein-A:X complex is ~150Å in diameter (see Fig 2C), thus a q-range of 0.01-0.40Å^{-1} will be appropriate. This will require sample to detector distances of 8m (low-q) and 2m (high-q). Protein is stable at 4ºC, hence we wish to maintain this temperature throughout the experiment. At 5 mg/mL we foresee collecting for 1h (0%), 2h (20%), 4h (40%), 2h (80%) , 1h (100%) at 8m, and half these times at 2m. Thus, for 5 samples and 5 buffers, we will require 30 hours + instrument configuration, empty cell etc. Thus we are asking for 2 days beam time.

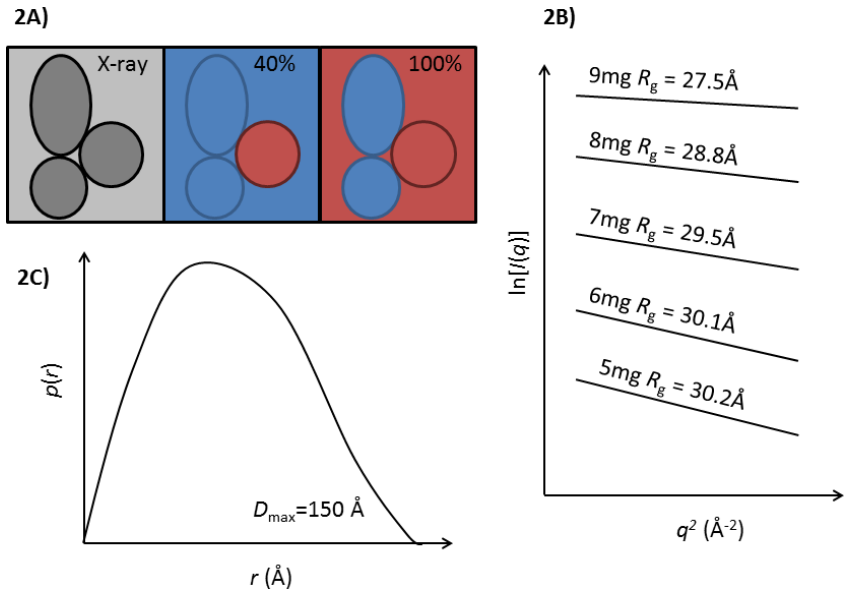

Figure 2A: It can be seen that as both subunits have the same contrast, we are unable to determine the position of X (as X and domain A2 are the same size). The use of contrast variation shows this clearly. 2B: Guinier plots of a concentration series showing inter-particle interference at concentrations above 5mg/mL. 2C: Pair-distance distribution function shows a maximum dimension of the complex is 150Å.

Choice of Instrument

Because SAXS gives the structure of the entire complex, it is difficult to reach solid conclusions regarding changes in shape of each component in the complex. In order to accurately determine the structure of each component in the complex, we require contrast between the two components, which can be achieved using small-angle neutron contrast variation. Thus, Quokka is the most appropriate instrument to carry out this research.

Data Analysis Overview

Data quality will be assessed via linearity of Guinier plots [ref] and estimates of the mass of the scattering particles. We will then use Stuhrmann analysis [ref] to yield basic structural parameters relating to the complex. If data quality is appropriate will use conventional shape restoration programs (such as DAMMIN [ref]), to determine the low resolution structure of the complex and that of Protein A and X. By comparing these low resolution structures to those obtained from SAXS on Protein A and X in isolation, we should be able to determine the nature of any large conformational changes.

References

[1] XXX; [2] XXX; [3] XXX; [4] XXX; [5] XXX; [6] XXX; [7] XXX; [8] XXX; [9] XXX; [10] XXX