

5. Jim McDonnell

Molecular structures and interactions in immune responses and apoptosis

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Our lab is primarily interested in understanding the structural basis of macromolecular recognition, with a focus on immunological receptors and signalling proteins, and the application of this information in developing inhibitors of these interactions. We used the methodologies of structural biology and biophysics for defining structures and interaction characteristics, synthetic chemistry and combinatorial screening methods for inhibitor development, and molecular biology and biochemical assays to better understand functional biochemistry of the systems. Our work in the year 2003/2004 has focused on defining the structures and recognition events for several interaction networks.

In the IgE network, a set of proteins that control allergic and inflammatory reactions, we have characterized the structures of the Ce3 domain from IgE and the CD23 molecule (the low-affinity IgE receptor). The Ce3 domain undergoes a disordered to ordered transition upon binding to FcεRI (the high-affinity IgE receptor), which results in a complicated kinetic and thermodynamic control of ligand binding. We are investigating the nature of how IgE binds to its receptors using surface plasmon resonance, studying binding over a range of temperatures. This has given information on the thermodynamic parameters of association and dissociation and the energetic barriers that control these rates. We use site-directed mutagenesis to couple this information to structural features of the proteins.

The CD23 molecule exists in multiple active forms - membrane integrated and soluble, with both forms existing in equilibrium between monomeric and oligomeric structures - with different functional activities ascribed to the different structures. The binding of different forms of CD23 to different ligands results in a complex, sometimes seemingly contradictory, role for CD23 in immune regulation. The solution structure of CD23, solved using heteronuclear NMR methods, has given a number of insights into the functional biology of this molecule. Oligomerization appears to be an important factor in controlling ligand specificity, affinity and the functional effects of receptor binding. The trimerization of CD23 allows for high-level cross-linking of IgE which results in a more efficient activation of IgE-positive B-cells (figure 1).

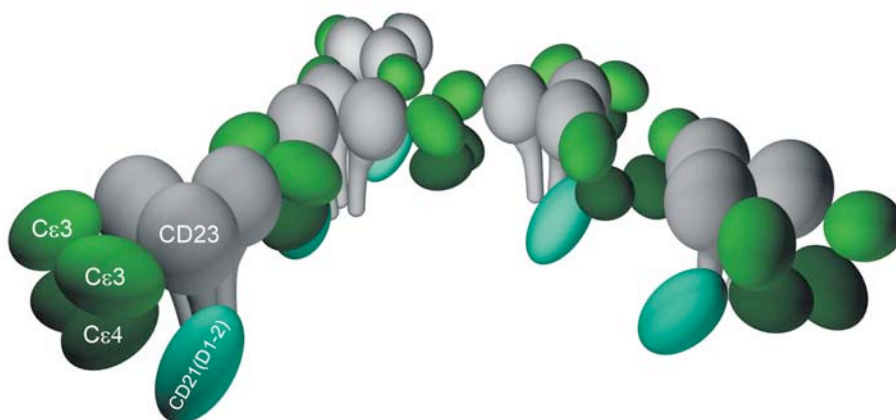


Fig. 1: Cartoon illustrating the ability of trimeric CD23 molecule (grey) to co-crosslink IgE (green) with CD21 (cyan).

In collaboration with Professors Endicott, Johnson and Noble we have been studying the recruitment site on cyclins; binding to this site plays an important event in determining substrate specificity for cyclin-dependent kinases. Using information from X-ray crystallographic analyses we have used structure-based methods to design and synthesize high-affinity recruitment site inhibitors (figure 2). We are also investigating how the recruitment site communicates with the catalytic site.

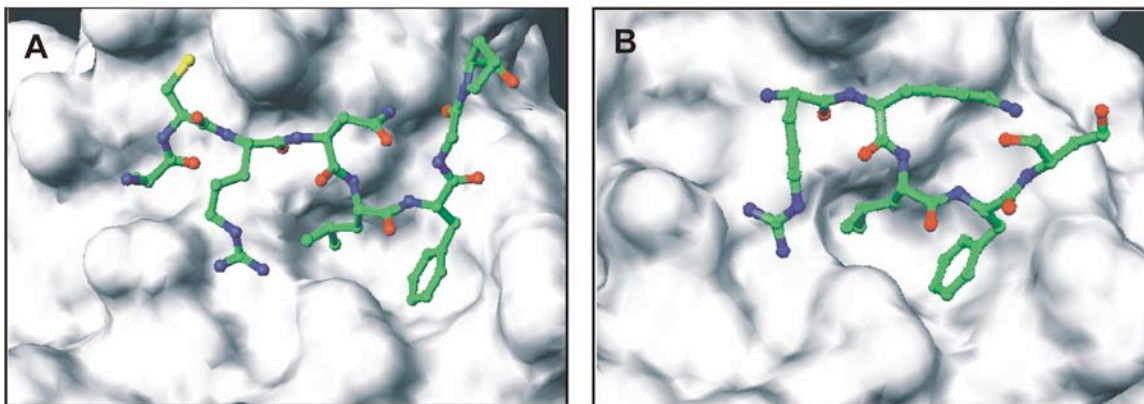


Fig. 2: The crystal structures of bound linear (A) and cyclic (B) recruitment site peptides.

We are also continuing to develop methodologies that allow us to better understand time-resolved changes in protein structure. NMR is a very powerful tool for this because of its potential for atomic resolution structural information and its capacity to study dynamic systems. We are using site-specific isotopic labelling approaches and stopped flow NMR studies to allow new insights into such events as macromolecular recognition, protein folding and allosteric changes (figure 3).

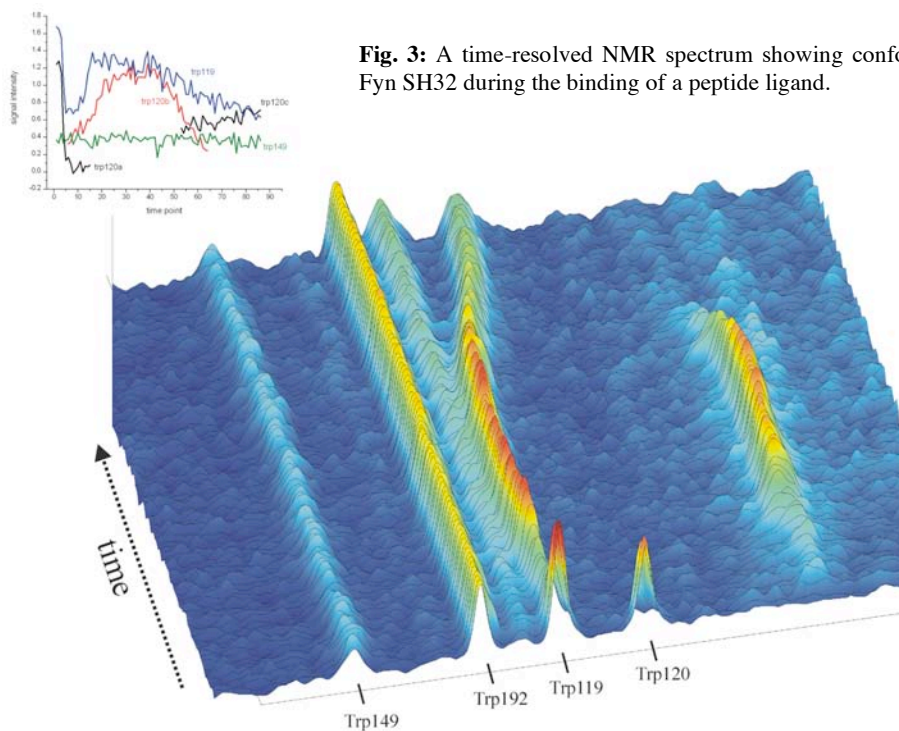


Fig. 3: A time-resolved NMR spectrum showing conformational changes to Fyn SH32 during the binding of a peptide ligand.