More Than Just A Pretty Picture

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Structural biology has become one of the most visible and fast-expanding areas of biological research. The ability to define the precise atomic structures of proteins and their complexes with other molecules (DNA, other proteins, ligands, inhibitors) has enormous implications for understanding biology and for applications in drug design and biotechnology. The pictures are nice but the secrets are in the detail.

Our laboratory at the University of Auckland was born almost two years ago, in rebuilt space in the School of Biological Sciences. It now numbers some 24 researchers, with a distinctly international flavour; China, England, France, Germany, Ireland, Sweden, USA and even New Zealand are represented. Our research is built around the use of X-ray crystallography to determine biomolecular structure, but also involves elements of mutagenesis, protein expression and purification, crystallization, computing, and interactive graphics. Here, from a range of projects that also include transport proteins, insect communication proteins, heat-stable enzymes, rab proteins and several important enzymes, we highlight four projects:

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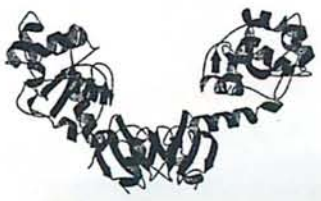

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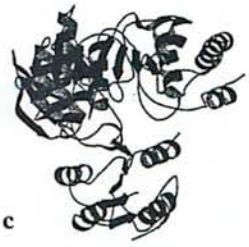

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Those toxic bacteria

Pathogenic bacteria secrete a variety of toxins that enable them to invade or disable a human host. In a project led by Ted Baker, we are focusing on two types of toxins from the human pathogen *Streptococcus pyogenes*. The first is a group of highly potent proteins known as superantigens, that cause massive overstimulation of the human immune system by binding to T-cell receptors and MHC class II molecules. These are causative agents of diseases such as toxic shock syndrome. In collaboration with John Fraser (University of Auckland), Vic Arcus and Jill Sigrell have determined the structures of several new superantigens, and found evidence of remarkable diversity in how they bind to their human receptors. We are now actively trying to crystallize complexes, in order to see exactly how these interactions occur.

The second type of toxin is a streptococcal cysteine protease that is directly involved in initiating the notorious "flesh-eating" disease, necrotising fasciitis. In collaboration with Jim Musser (Baylor College of Medicine), Todd Kagawa and Jakki Cooney (Massey) are making exciting progress towards a high resolution structure for this potential drug design target.

Shuffling protein disulfide bonds

Many proteins contain disulfide bonds in addition to the numerous hydrogen bonds and hydrophobic interactions which maintain their folded three-dimensional structures. Disulfide bonds join only particular pairs of cysteine residues in the polypeptide chain, a small fraction of the possible cysteine pairs in the unfolded polypeptide. Cellular enzymes collectively termed 'foldases' catalyse not only protein folding and the formation of disulfide bonds, but also the rearrangement of incorrect disulfides that form during protein folding.

Protein disulfide bond isomerases rearrange disulfide bonds and a project led by Peter Metcalf concerns the extensively characterised disulfide bond isomerase DsbC from the bacterium *Escherichia coli*. The atomic structure of this molecule suggests intriguing mechanisms for how DsbC functions to ensure folding protein molecules end up with only correct disulfide bonds. The question of how disulfide bond isomerases promote protein folding is more than just academic: Overexpression in *E.coli* is the most common method for recombinant protein production, and folding problems together with incorrect disulfide bond formation often prevent success. We expect that knowledge gained from the atomic details of this first disulphide bond isomerase structure will contribute both to an improved understanding of protein folding and to the development of better methods of producing recombinant proteins in *E.coli*.

Eat your greens - what to do with folic acid

Folates play an essential role in all cells, where they are carriers of one-carbon units involved in DNA and amino acid biosynthesis. Antifolates, in contrast, are modified folates that can kill bacterial and cancer cells by interrupting cell growth mechanisms. This project, led by Clyde Smith, in collaboration with Andy Bognar (University of Toronto), targets a key enzyme involved in the activation of folates and antifolates. This requires the addition of a polyglutamate tail, catalysed by the enzyme folylpolyglutamate synthetase (FPGS).

We have recently determined the crystal structure of FPGS from of its two domains (see Figure 1) bears a striking and totally unexpected resemblance to dihydrofolate reductase. We are now focusing on site-specific mutants, and on binding studies with different folates. One mutant shows a major structural rearrangement in the active site. As we learn more about the structure of this enzyme and how it works, we expect that new or improved antifolate drugs can be designed, with higher efficacy, and more efficient modification by FPGS.

Beyond genome sequences...

The large number of genome sequencing projects now coming to fruition is already beginning to transform the biological sciences. In its wake comes the question of how to use this rapidly-accumulating information. It is already clear that our ignorance is profound; in fully-sequenced genomes at least 50% of gene products are of unknown function and 80-90% are of unknown structure. At the same time, this opens exciting new opportunities.

We have joined with structural biology groups at UCLA, Berkeley and Los Alamos National Laboratory (USA) in one of the first large-scale structural genomics exercises. The aims are ambitious: to systematically clone, express and solve the structures for all the "unknown" gene products of a model organism, the archaebacterium *Pyrobaculum aerophilum*. This is aimed at finding representative structures for as many protein families as possible, discovering presently-unknown folds, and discovering evolutionary and functional relationships that are not apparent at the level of protein sequence. The suggestion that one could determine structures for all proteins in a bacterium would have been considered absurdly optimistic only a few years ago. That this task is now realistic reflects the extraordinary advances in crystallographic methods and technology, and the parallel growth of structural biology in Auckland makes us well placed to participate.

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