The 57th Harden Conference: Proteinase Structure and Function

Oriel College, Oxford, 9–13 September 2003

The 57th Harden Conference was more of a single-subject conference (although it is a pretty big subject). Speakers covered areas such drug targets, the link between cholesterol and Alzheimer’s disease and the origin of BACE (β-site APP-cleaving enzyme) in sea squirts.

The conference took place at Oriel College, Oxford, with dinner in the hall. The portraits had been taken away, not because the Bursar didn’t trust us, but because they were being cleaned (and augmented with a portrait of the Queen). The stained glass remained, glowing dimly in azure, gules, vert and purpure.

After an introduction by Brian Austen (St George’s Hospital Medical School, London), Tim Clausen (Institute for Molecular Pathology, Vienna, Austria) spoke on the crystal structure of DegP (HtrA), a cannon-like cage having an inner cavity with axial pores. The distinction between a protease or a chaperone is usually clear: when proteins lose shape under stress, chaperones repair them. If the situation is hopeless, the protein is degraded by a protease. However, DegP (HtrA) is a temperature-dependent switch, the lateral walls of PDZ domains opening or shutting to make the molecule either a protease or a chaperone, respectively.

Jennifer Rivett (Bristol, UK) gave an account of proteasome function in antigen presentation, unfazed by a technical hitch that delayed her talk for over 15 minutes. She detailed a cell-based assay of proteasome function that used the B-subunit of *Escherichia coli* heat-labile enterotoxin to deliver peptides into the MHC class 1 pathway.

After a coffee break, there was an session on proteolytic enzymes, which account for about 3% of the human genome, and their use in the design of inhibitors. David Fairlie (Queensland, Australia) produced the results of a survey of over 1500 crystal structures for proteinase–ligand complexes and gave a review of inhibitor design. David was followed by four poster presentations, which were followed in turn by a lunch of smoked fish, pasta and salad.

After lunch Chris Southan (Oxford GlycoSciences, UK) spoke on BACE1 and BACE2. BACE (Beta-Site Amyloid Precursor Protein Cleaving Enzyme) is a well-validated drug target with unknown biology, which is good news for drug companies perhaps, but less so for functional genomics. The sequence of this enzyme is very conserved and must have arisen early in vertebrate evolution; probably with the sea squirts.

Raphael Kopan (St Louis, MO, USA) followed with ‘Insights from parallel analysis of Notch 1 and APP proteolysis’. The effects of Notch 1, a discontinuity on the wing of *Drosophila*, were first noted by J.S. Dexter in 1914. Since then, vertebrate homologues have been cloned from many organisms. Raphael raised the possibility of a presenilin dimer at the core of the γ-secretase enzyme.

Sonia Emanuele (Palermo, Italy) gave a presentation on how proteasome inhibitors induce apoptosis in human hepatoma cells by a c-Jun/JNK pathway.

Nikki Copeland (York, UK) and Nick Furnham (Cambridge, UK) were next, filling in for an absent speaker. Nikki spoke on enzymic and kinetic analysis of Lit (an *E. coli* suicide proteinase) and Nick gave a presentation on comparative modelling of BACE1 splice variants. “These are only models,” he said, “so don’t take this as fact, because it isn’t”.

In the evening, and after looking at the posters and eating dinner, the delegates explored Oxford’s pubs. The Bear, with its tie collection, was just around the corner. The Turf, famous from *Inspector Morse* was just down the road.

Chris Southan chaired the Thursday session. It started with Chris Schofield (Oxford, UK) discussing a series of structural studies on acyl enzyme complexes of serine proteases. The formation of stable acyl enzyme complexes is important for the action of various inhibitors of nucleophilic serine enzyme.

Tony Turner (Leeds, UK) then spoke about zinc metalloproteinases as therapeutic targets. There are more than 100 metalloproteinases encoded in the human genome and very little is known about them. NEP (neutral endopeptidase), the prototype of the M13 zinc peptidase family, is a good candidate for protection against Alzheimer’s disease, but, unfortu-
nately, homoeostasis is essential in the body — you can’t just take NEP as if it were a dietary supplement. There is a tantalizing link between cholesterol and Alzheimer’s; the amyloid plaques and their precursors are localized near the cholesterol-rich areas of the plasma membrane.

Bruno Martoglio (Zürich, Switzerland) spoke on intermembrane proteolysis by signal peptide peptidase, concentrating on its role in human cells. Kelvin Cain (Leicester, UK) looked at the role of the apoptosome in caspase activity. Apoptosis proceeds in four stages: decision, execution, engulfment and degradation. Caspases are the executioners. The apoptosome is a large (700 kDa) caspase-activating complex, which, once formed, is unaffected by ionic concentration.

After lunch, Tony Turner took the chair for a session that included a look at the sub-cellular trafficking and phosphorylation of endothelin-converting enzyme-1 and the functional profiling of proteases. The delegates broke for a formal poster session before the meeting dinner in the college hall. The protein and peptide science group of the Royal Society of Chemistry sponsored the poster prizes which were awarded to Cynthia Shuman (Uppsala, Sweden) for ‘Thermodynamic character-ization of HIV-1 protease-inhibitor interactions’ and Michael Engel (Max-Planck-Institut für Biochemie, Martinsried, Germany) for ‘The crystal structure of dipeptidyl peptidase IV (CD26) reveals its functional regulation and enzymatic mechanism’. The runner-up was Nikki Copeland for ‘Enzymatic and kinetic aspects of Lit, an Escherichia coli suicidase protease’.

Friday’s heavy sessions were chaired by Jennifer Rivett in the morning and Robin Leatherbarrow (Imperial College London, UK) in the afternoon. Topics covered included a mutagenesis approach to the understanding of TIMP (tissue inhibitor of metalloproteinases) specificity, the versatility of the plasminogen-activation system, mast-cell tryptases, the substrate specificity of serine proteases, human kallikreins, signal peptidase, protease-inhibitor docking and the molecular structure of human angiotensin-converting enzyme.

After so much heavy science, delegates were taken on a boat trip up the Isis to a barbeque. The conference had been scientifically rich and stimulating. All delegates were grateful to the organizers, Brian Austen, Chris Southan, John Deadman (Thrombosis Research Institute, London, UK) and Robin Leatherbarrow and to all who helped, especially Helen Reed and her colleagues in the Biochemical Society’s Meetings Office.