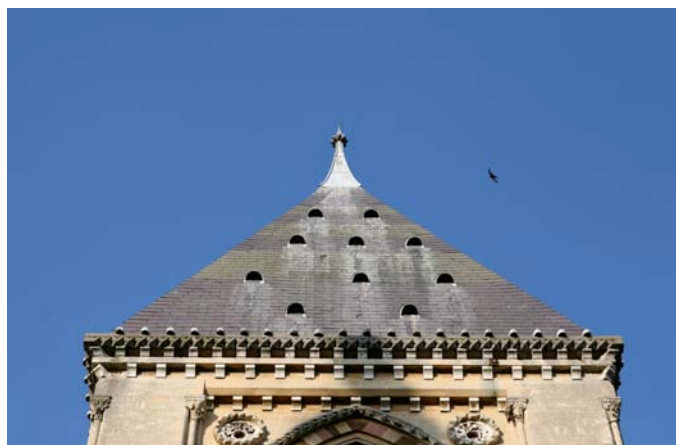


*Oxford Science Lecture Series:
Professor Robert Huber

12 June, 2006

Sponsored by the Oxford Biochemical Society

The Oxford Biochemical Society, with support from the UK Biochemical Society, sponsored the lecture entitled *Molecular Machines for Protein Degradation* given by Professor Robert Huber (1) on 12 June 2006 at the Oxford University Museum. The museum provided a quintessential Oxonian venue for this talk by the Nobel laureate. As expected, a significant portion of Oxford's aspiring and established scientists turned out for the occasion.



Spire of the University Museum

<http://www.oum.ox.ac.uk/>

Huber focused the lecture on his more recent work at the Max-Planck-Institut, involving the application of the novel methods of protein crystallography that he has developed and refined throughout his career. He began with a brief introduction, describing the incredible diversity that is characteristic of proteins and proteases (the enzymes responsible for the intracellular maintenance and elimination of proteins). A natural result of the staggering structural range of proteins and proteases, as Huber subsequently pointed out, is an abundance of mechanisms for the regulation of related substrate/enzyme functional relationships. These mechanisms vary in complexity from simple interactions dependent on

substrate inhibition or specificity, through interactions involving multiple molecules, such as co-localization and co-factor binding, to the most complex regulatory interactions involving multiple molecules and allosteric effects. It is these complex allosteric interactions — or molecular machines — that Huber focused on for most of the duration of his talk. Simple cartoon drawings provided comprehensible background material for even those scientists unfamiliar with structural biology. The body of the talk provided several examples of the mechanical protein/protease systems that he has investigated recently: the simplest case of serine protease activation (2) and the very large multisubunit proteases (3). In some cases the Free-Mounting System™ for optimization of protein crystals was essential (4).

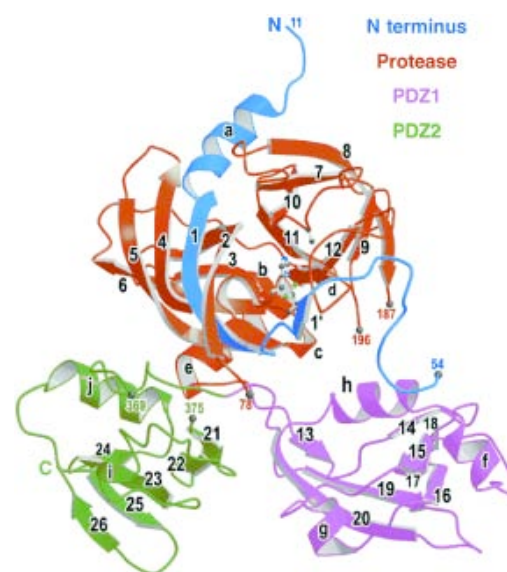
Huber detailed classical serine protease activation as a well-understood example of co-factor binding with allosteric interactions, in order to elucidate less-obvious systems of protease regulation. In serine protease, a specific peptide bond must be cleaved by the presence of a co-factor before a protease can bind and have an effect. The bond cleavage triggers a mechanical allosteric interaction that allows physical access of a specific protease to its binding site within the three-dimensional serine protease structure. This multi-phase regulation of protease binding with serine protease is essential for the survival of the cellular system; a more accessible binding site could be dangerous, in that resultant protease binding and proteolysis could deplete the serine concentration within the cell. In fact, several viruses have been identified in this system (and others) that mimic the mechanical regulatory effects of a specific protease. Again, Huber generously incorporated images and animations into his slides, to clearly depict the intricacies of protein–protease binding and to illustrate why these molecules can be classified as fundamentally mechanical.

The availability of the genomes of a multitude of organisms, ranging in complexity from bacteria to humans, has added an exciting new element to the derivation of functional information from structural knowledge (2). Professor Huber described one example of his efforts in achieving just this. By comparing yeast and archaeobacteria genomes, a crystallographic structure of the yeast proteasome was finally derived. Furthermore, the method revealed the configuration of the yeast protein's active binding sites, and thus provided insight into the mechanism of protein/protease regulation.

This work sparked similar studies worldwide, in both academia and industry, using techniques that combined structural and genetic information to characterize the active binding sites of proteases that have been implicated in human disease. By describing binding site structure, it has been possible to pinpoint both natural and synthetic molecules capable of inhibiting these proteases, and thus alleviating associated diseases. The drug Velcade™ is one such example, in which synthetic chemists took structural effects into account to develop a treatment for myeloma. Huber also mentioned lactacystin and epoxomicin as natural inhibitors of the proteasome that have potential as therapeutic agents in the treatment of cancer. Another natural regulator of the proteasome, TMC95A, had been identified, but had provided little benefit because of the difficulty involved in its synthesis. However, Huber described how a structural understanding of the molecule facilitated development of a substitute, with only the mechanistically crucial aspects of the protease targeted in its synthesis. Finally, he mentioned that crystallography also has application in monitoring the coupled regulation of a protein–protease functional assembly, a capability that is essential to both basic science and drug development.

Huber next described his group's recent work in characterizing the protein DegP, a unique molecule capable of acting as both a chaperone (a protease-like molecule that repairs, rather than destroys, damaged proteins) and a destructive protease (3). Crystallographic studies of DegP have suggested that temperature is an important factor in its functional regulation: its destructive function seems to dominate at high temperatures, while its reparative chaperone-type function prevails at low temperatures. This work is novel in that it has elucidated a protease-like molecule with a more elegant system of self-regulation and more extensive capabilities than the proteases previously described.

Finally, Huber gave a brief description of one of the improvements to the crystallographic method that he has pioneered. The Free-Mounting System™, developed with Reiner Kiefersauer, has facilitated more straightforward isolation of single crystals, allowing more accurate crystallographic studies. Conventional methods involved mounting crystals within a closed capillary to protect them from the ambient environment. However, the capillary tube provided a barrier, attenuating incident X-rays, thereby adding an undesirable aspect of anisotropy to crystallography experiments. It also prevented any manipulation of the crystal's environment and thus limited the range of studies that could be performed. The Free-Mounting System™ has eliminated these barriers by isolating protein crystals, via suction, at the tip of a micro-pipette, protected by an air stream. The air stream can be manipulated by the user to supply the crystal with humidity or other volatile chemicals of interest. This system, when used in conjunction with an X-ray crystallography setup, has enabled structural measurements of a single crystal with great precision. Furthermore, the Free-Mounting System™ has allowed quantification of crystal responses to variable environmental factors, most notably humidity, for the first time.



Structure of the DegP protomer in ribbon representation (3).



Professor Huber at the Max Planck Institut
http://www.biochem.mpg.de/xray/load_members.html

Huber concluded his lecture with the statement, “I hope I have shown to you diverse solutions in nature of the great problem of intracellular proteolysis.” With simple and elegant explanations of a number of relevant systems, both in nature and ongoing in his laboratories, he certainly accomplished that modest aim. The most poignant factor to emerge from his lecture, however, was the incredible innovation he has repeatedly shown in applying a well-understood analytical tool to countless molecular systems. Huber has manipulated X-ray crystallography to elucidate a staggering number of biological problems, ranging from his most lauded characterization of a photosynthetic site, to the distinct topic of drug development. Scientists at the University of Oxford are privileged to have been exposed to Huber’s open mind and insightful problem-solving strategies — attributes that all scientists should strive to achieve.

References:

1. Huber Nobel biography, accessed 11/06/06. URL: <http://nobelprize.org/chemistry/laureates/1988/huber-autobio.html>
2. Friedrich *et al.* (2003) Staphylocoagulase is a prototype for the mechanism of co-factor induced zymogen activation. *Nature* **425**, 535-539.
3. Groll *et al.* (2005) Molecular machines for protein degradation. *ChemBioChem* **6**, 222–256.
4. Kiefersauer *et al.* (2000) A novel free-mounting system for protein crystal: transformation and improvement of diffraction by accurately controlled humidity changes. *J. Appl. Cryst.* **33**, 1223-1230.