s1.m8.o1 **Atomic and Ultra-high Resolution Crystallography Reveal Subtleties in Substrate Binding.**

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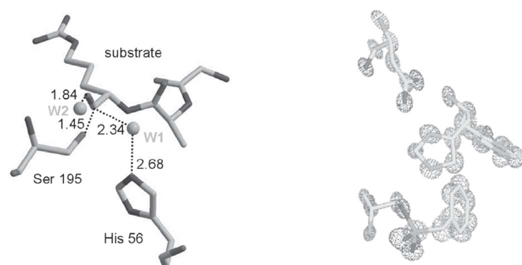
Keywords: Atomic and Ultra-High Resolution; Substrate Recognition; Mechanism

Macromolecular crystallography has become the method of choice for acquiring three-dimensional structural information. X-ray data extending to atomic resolution (< 1.2 Å) offer an extremely powerful tool for the elucidation of protein function. Accurately defined atomic positions and fine details in the electron density allow an in-depth interpretation of electronic features.

The combination of atomic resolution crystallography with *ab initio* quantum chemical calculations and biophysical methods enables to close the gap between the protein structure and the chemical properties. Using the crystal structure as a geometrical template, one can assign a protonation state or electronic state and determine the charge distribution which governs the surface properties, substrate specificity and binding energies. A series of crystal structures of trypsin, containing either a peptide fragment or a covalently bound inhibitor, were determined at atomic and ultra-high resolution and subjected to *ab initio* quantum chemical calculations and multipole refinement. The combined results confirmed the catalytic function of the active site residues and the two water molecules. The crystal structures represented snapshots from the reaction pathway, close to a tetrahedral intermediate [1].

In addition to the electronic features, analysis of the anisotropic atomic displacement parameters at cryo- and at room temperature hint at an induced fit substrate binding step. This is one of the details, particularly in the substrate recognition and binding as well as the product release, which have still not been fully resolved from these previous structures. Therefore, biophysical experiments and further crystallographic studies on structures mimicking the "empty" state of the enzyme as well as a temperature study have been carried out in order to fill the gaps in the reaction pathway from the structural as well as the energetics side. A detailed analysis of the structures in terms of mobility and substrate recognition will be given and the importance of protein flexibility and the benefits of atomic and ultra-high resolution crystallography will be discussed.

[1] Schmidt A *et al.*. *J Biol Chem* **2003**, 278. 43357-43362.



Geometry and anisotropy in the active site of *Fusarium oxysporum* trypsin

s1.m8.o2 **Using Thrombin to Study Protein-Ligand Interactions.**

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Keywords: Thrombin; X-Ray; Fluorine Scan

X-ray structures of closely similar inhibitors may be used to develop an accurate experimental estimate of the energetics of ligand receptor interactions. This requires a well-diffracting crystal system of sufficient interest to be attractive for synthetic chemistry. I will describe in detail the use of the blood coagulation enzyme Thrombin for this purpose, drawing examples both from the literature and from my own work. In particular, the substitution of fluorine for hydrogen has been examined recently in the context of medicinal chemistry, as this can contribute both to improved binding and to improved molecular properties.

References:

[1] Principles of Enzyme Inhibitor Design. Banner, DW. Chapter 7 of the monograph "Molecular Recognition in Protein-Ligand Interactions" (Wiley-VCH), Boehm, H-J and Schneider, G. (Eds) (2003).

[2] A fluorine scan of thrombin inhibitors to map the fluorophilicity/fluorophobicity of an enzyme active site: evidence for C-F...C=O interactions. Olsen JA, Banner DW, Seiler P, Obst Sander U, D'Arcy A, Stihle M, Muller K, Diederich F. *Angew Chem Int Ed Engl.* 2003 Jun 6;42(22):2507-11.