

Atomic and Ultra-high Resolution Crystallography Reveal Subtleties in Substrate Binding Andrea Schmidt and Victor S. Lamzin *EMBL Hamburg c/o DESY Notkestrasse 85 D-22603 Hamburg, Germany*

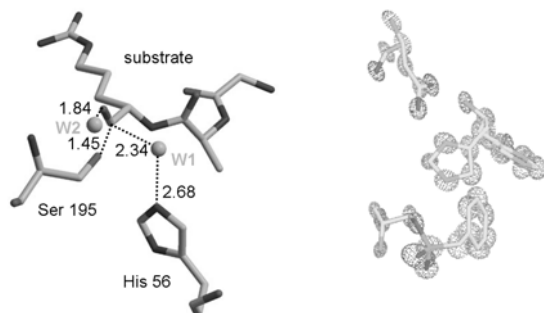
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Macromolecular crystallography has become the method of choice for acquiring three-dimensional structural information. X-ray data extending to atomic resolution ($< 1.2 \text{ \AA}$) offer an extremely powerful tool for the elucidation of protein function. 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