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Structural Insights at Sub-Ångstrom, Medium and Low Resolution:

Crystallization of Trypsin, Bacterioferritin, Photosynthetic Reaction Center, and Photosynthetic Core Complex

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Akademisk avhandling för filosofie doktorsexamen i Naturvetenskap, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fredagen den 19:e oktober 2012 kl. 10.00 i sal Karl Kylberg (K2320), Institutionen för kemi och molekylärbiologi, Medicinaregatan 7B, Göteborg.

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Abstract
The catalytic action of serine proteases depends on the interplay of a nucleophile, a general base and a general acid. The catalytic triad is composed of serine, histidine and aspartate residues. The serine acts as a nucleophile while the histidine plays a dual role as the general base or acid at different steps of the reaction. However, the role of aspartate is unclear. I recovered an ultrahigh resolution (0.93 Å) X-ray structure of a complex formed between trypsin and a canonical inhibitor. At sub-ångstrom resolution, hydrogen atoms could be visualized, giving a clue to the protonation state of the catalytic residues. By comparing this with the theoretical electron density calculated by density theory functional, the protonation states of the catalytic histidine and aspartate are discussed. Hence, a refined mechanism for serine protease action is proposed in this thesis.
Photosystem harvests energy from sunlight with near 100% quantum yield. To study light-induced structural changes of the photosynthetic reaction center from purple non-sulfur bacterium *Blastochloris viridis* using X-ray crystallography, robust protein crystals with tight crystal packing are prerequisite. In this thesis, lipidic-sponge phase crystallization method was used and yielded well diffracting crystals for structure determination. Crystals showed a type I packing and a 1.86 Å resolution structure was determined with four lipid molecules captured in the structure. Moreover, I demonstrated that an occupied QB binding site can be obtained by co-crystallizing with UQ2 using the sponge phase crystallization method. However, attempting to crystallize the reaction center-light harvesting 1 core complex, a 440 kDa membrane protein complex of total 54 putative subunits, it required different crystallization methods. Here, the resolution has been optimized to beyond 8 Å by using the lipidic bicelle crystallization method.
Conflict between the free but potential toxic Fe(II) and the insolubility of Fe(III) led to the evolution of bacterioferritin in bacteria, which functions as an iron storage and detoxification protein. Bacterioferritin from *Blastochloris viridis* (*Bv* Bfr) was crystallized and the structure was solved to 1.58 Å resolution. With the combination of X-ray structure, redundancy PCR and tandem mass spectrometry, the previously unknown amino acid sequence of Bv Bfr was determined. Conformational states of the ferroxidase center which undergoes reorganization upon different soakings were trapped. One water-like small ligand coordinated to the Fe1 binding site was captured in the Fe(II)-soaked structure. By density functional theory calculations the character of this small legend was rationalized. In addition, the structure and mechanism of iron import of the protein was studied and discussed. Finally, the redox-state of the heme in the crystals with and without Fe(II)-soaking treatment was studied by single crystal UV-VIS microspectrophotometry, before and after the X-ray exposure.