Proposal Title: X-ray Crystallographic Study of Novel Protein Folds and Virulence Factors of *Helicobacter pylori*

Wenching Wang(007771), Jaishin Liu (0007768), WenChi Cheng(0014087), YuNing Chang(0009880)
Dept. of Life Science, National Tsing-Hua University, Taiwan

Helicobacter pylori is a Gram-negative microaerophilic bacterium which colonizes the gastric mucosa of the human stomach. Infection with *H. pylori* is associated with gastritis, gastric ulcer, and duodenal ulcer. The current triple-therapy treatment of *H. pylori* infection includes antibiotics and a combination of a proton pump inhibitor. However, a higher frequency of therapy failure is emerging due to antimicrobial resistance. The HP0157 protein involved in the shikimate pathway that is essential in the bacterium but absent in mammals is a potential new target for developing non-toxic anti-microbial agents. In this beamline study, we collect the data to ~2.3-Å resolution. The data would be processed by the Amore and Refimac5 software to solve the overall structure.

Aspartic protease (AP) is a prototypic dizinc exopeptidase that can remove the N-terminal hydrophobic amino acids of a polypeptide chain. Since the number of hydrophobic amino acids contributes to the intensity of bitterness in a polypeptide chain, AP from GRAS microorganisms is potentially useful for debittering protein hydrolysates with N-terminal hydrophobic amino acids, which are commonly used as clinical nutrition supplements.

The crystals diffract to ~1.1 Å with the mosaicity of 0.23 and the overall R_{merge} below 10%. We also collected the anomalous signal of sulfur in the wavelength of 1.7 Å. Combination of the high resolution and the anomalous datasets, the protein structure presumes to be determined soon.

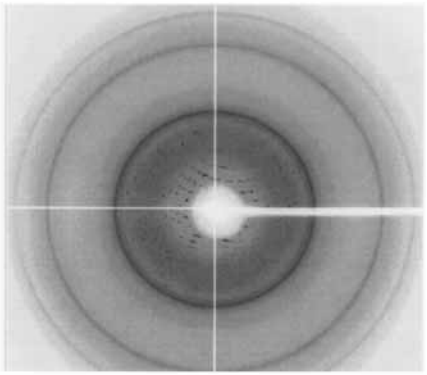


Figure1 Diffraction of HP0157 crystal to 2.3 Å resolution.

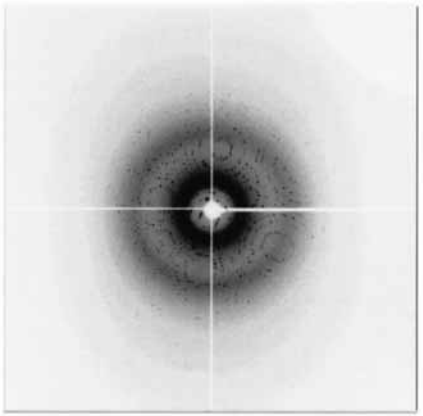


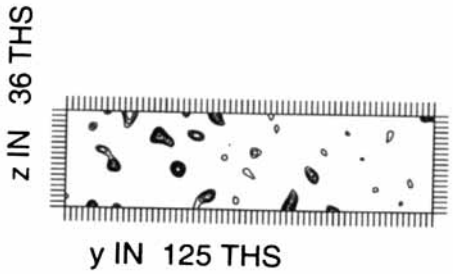
Figure 2 Diffraction of AP crystal to 1.1-Å resolution

Anaerobic Structure of Ferredoxin II from *Desulfovibrio gigas*

Hong-Hsiang Guan (9947)[1,2], Yun-Ling Wang (9946) [1,2] ,and Chun-Jung Chen[1](6903)
[1] Structural Biology Group, National Synchrotron Radiation Research Center, Hsinchu, Taiwan
[2] Institute of Bioinformatics and Structural Biology, National Tsing-Hua University, Hsinchu, Taiwan

Ferredoxin II (Fd II) is a small electron transfer protein, isolated from the strict anaerobic sulfate-reducing bacterium, *Desulfovibrio gigas*. It contains 58 amino acids and a [3Fe-4S] cluster. . Anaerobic Fd II crystals have been grown using hanging-drop vapor diffusion method under anaerobic condition. Fd II crystals diffracted to a resolution of 1.4Å and belong to space group $P2_12_12$, with unit-cell parameters a= 32.685 Å, b= 82.281 Å, c= 22.953 Å. The structure of anaerobic Fd II has been determined and refined. Its [3Fe-4S] cluster is bound with Cys8, Cys14 and Cys50, and Cys11 extends away from cluster. Cys18 and Cys42 form a disulfide bond to maintain foldings. In addition to a [3Fe-4S] cluster, five isolated heavy electron densities around the anaerobic ferredoxin II are located, and the density heights are similar to those of cluster irons. These extra irons are bound with Glu, Asn and Asp, respectively, which reveals the unique iron-storage function and electron transfer pathway of ferredoxin II. Cardiotoxin (CTX) is a major component of cobra toxin. Cobra cardiotoxins (CTXs) are basic proteins, composed of 60-62 amino acids, in which b-sheets form three finger-loop structures.

When cobra bite animals, CTX can induce tissue inflammation. However, the CTX major target on cell membrane remains unclear. The previous studies have proven that the heparan sulfate is the most potential target for CTX on cell membranes. We have determined the complex structure of CTX A3 and hexasaccharide which shows the loop 2 of CTX A3 is the major heparin binding location. Positively-charged amino acid residues near the tip of loop 2 play an important role on binding heparin through ionic interactions with *O*-sulfates. It is suggested that the loop 2 is a heparin-binding loop. CTXs are aggregated on cell membrane through loop 2 binding to heparan sulfates to enhance its toxin.



Crystal structure of CTX A3 and hexasaccharide heparin complex from *Naja atra*.