

Assignment #7: Data Mining & Molecular Modeling of Protease *Subtilisin*

It is the goal of Assignment #7 to exemplify the concept of using an enzymatic detergent by way of exploring the structure-function relationship of one protease commonly used in enzymatic detergents. You will visit the Protein Data Bank (<http://www.rcsb.org>), search the PDB for crystal structures of the protease subtilisin, read the primary literature to learn about the role of this protease in its biological context, and learn how to employ molecular modeling to support your learning / understanding / discussion of the chemical mechanisms of the protease's function.

(a) Select Protease Crystal Structure. The PDB contains many X-ray crystal structures of hydrolases (hits on 01/23/12) that are (1) proteases (3282 Hits), (2) lipases (132 Hits), (3) amylases (266 Hits), and (4) cellulases (184 Hits). We will focus on the protease subtilisin from *Bacillus licheniformis* (23 hits). Explore these hits and select one of these structures for detailed discussion. In one paragraph, explain the role of this protease in its biological context with proper citation of primary sources and justify your selection of the specific structure.

(b) Analyze the Protease. Using the online tools provided by the PDB portal, locate the active site of the protein and analyze the immediate neighborhood of its active site. Arrange the display such that the pertinent features of the active site can be viewed well, and create one image of the display. You can use several images if that is necessary for a proper appreciation of the active site. Show the image(s) in Figure 1 and include major structural parameters in the Figure caption (not too many, select thoughtfully). "Major structural parameters" include the most characteristic bond lengths, angles and dihedrals. Such parameters usually describe the geometry of functional groups and they include especially any parameters that undergo change during protein hydrolysis. Write one paragraph to describe the structure of the protein's active site.

(c) Molecular Model the Protein Hydrolysis. Using the molecular modeling program Chem3D, build a small model of the active site (which includes the three key residues Asp, His, and Ser) bound to a simple dipeptide model, create images of ball-and-stick, stick, and space-

filling models, show these images in Figure 2 and include major structural parameters in the Figure's caption. Feel free to alter default settings for structure display (i.e., atom size, color, ...) as you see fit. Write one paragraph to describe the interaction of the dipeptide with the active site residues.

(d) Scheme of Mechanism of Protein Hydrolysis. Using the drawing program ChemDraw, create a scheme that shows a small model of the active site (which includes the three key residues Asp, His, and Ser) at the following several stages: (i) simple dipeptide bound to active site, (ii) after serine acylation and amine still bound to His, (iii) after serine acylation and water bound to His, and (iv) after ester hydrolysis. Write one paragraph to describe the mechanism of the protein hydrolysis.

Submission & Target Dates: The assignment must be completed using PDB software, CambridgeSoft modeling software ("Chem3D", "ChemDraw"), and MS Word with *JOC* formatting. Your submission should contain a title, four paragraphs, two Figures, one Scheme and a properly formatted reference section. Submit one Word file "A07_'your name'.docx" by Tuesday, 03/20/12, midnight. Bring one hardcopy in color to class on Wednesday, 03/21/12, for peer-review.