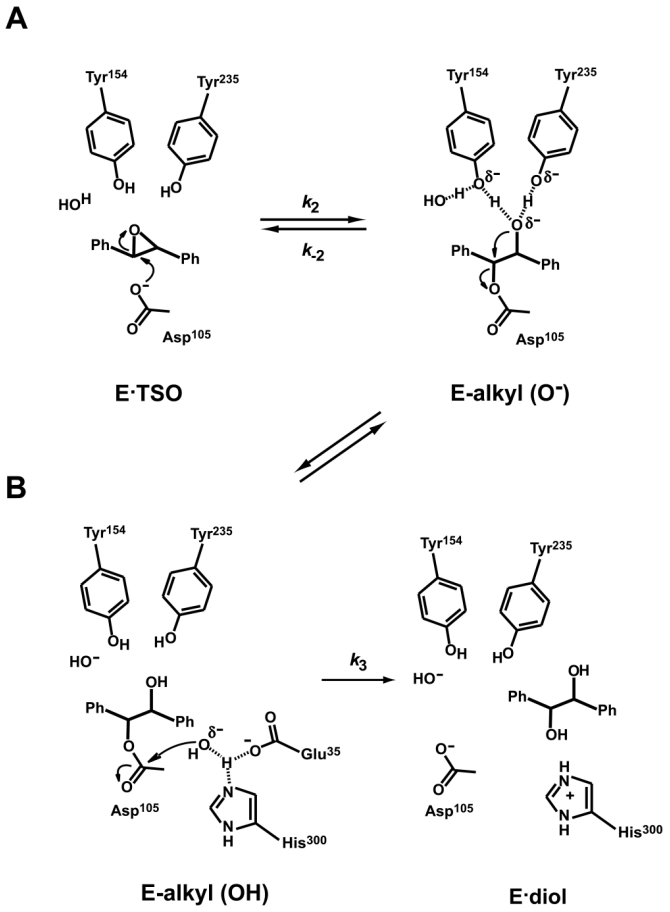
Computer exercise – protein structure and dynamics

Objectives:

(1) Get familiar with general-purpose molecular graphics software.
(2) Get familiar with the anatomy of a protein structure file (a "pdb"-file).
(3) Analyze the results of molecular dynamics simulation of steps in an enzyme-catalyzed reaction.

Background – Model system:
The enzyme epoxide hydrolase StEH1 catalyzes the hydrolysis of *trans*-stilbene oxide (TSO) through the following mechanism:



The only reaction intermediates that can be experimentally detected by kinetic and/or spectroscopic methods are the enzyme-substrate and alkyl-enzyme complexes. In order to better understand additional steps, for example how the last hydrolysis step (may) proceed, we employed molecular dynamics simulations.

The following complexes were modeled:
(1) The enzyme-substrate (Michaelis) complex with *R,R*-TSO.
(2) The alkyl-enzyme complex with the same substrate
(3) A predicted (very short-lived) tetrahedral intermediate that is proposed to result from the nucleophilic attack by a water molecule on the alkyl-enzyme. This intermediate was

modeled in two different protonation states; one with the general-base His^{300} was in its deprotonated imidazole form, and one in the acidic imidazolium form.

Questions that we wanted to address:

(1) Which interactions are made between the epoxide oxygen and the enzyme in the Michaelis complex?
(2) How are these interactions changed after formation of the alkyl-enzyme? Can the interactions explain the (experimentally observed) relative stability of this intermediate?
(3) What may be the function of His^{300} during decomposition of the proposed tetrahedral intermediate (proceeding cleavage of the alkyl-enzyme)?
(4) Should His^{300} be in its protonated (acidic) or deprotonated form for most efficient hydrolysis?

Aiding you in responding/discussing these questions you have a number of different structure files. These are the modeled structures of the different enzyme forms in complex with *R,R*-TSO obtained after a 250 ps MD simulation as follows:

Michaelis complex:

(1) Docking of TSO with GOLD (p. 667 in Leach).

Alkyl-enzyme and tetrahedral intermediates:

(1) Docking with GOLD.
(2) MD with Q (Marelius *et al.* (1998) *J. Mol. Graphics Modell. 16*, 213.) with the OPLS all-atom force field (pp. 210, 228 and 599 in Leach).
(3) Residues within a 20 Å sphere centered at His^{300} was included in the calculations solvated with TIP3P (pp. 216-217 in Leach) water molecules.
(4) Water molecules at the surface were restrained.
(5) Residues outside the 20 Å sphere were restrained to their initial coordinates.
(6) Time step=1 fs
(7) Total simulation=250 ps

Procedure:

(1) If you feel unfamiliar with using the software WeblabViewer Lite start with this tutorial:

http://www.biorg.uu.se/Utbildning/Tutorials/Weblab/index.shtm and follow the steps outlined. If you have received previous training on this program, proceed to (2).

(2) Start up WebLab Viewer Lite (from the Course Program menu).
(3) Go to the following site:
http://beta.uu.se/biorg/Education/Tutorials/Protein+structure+and+dynamics?languageId=1&languageId=3
(4) Download the different structures to the hard drive – give them useful names.
(5) Open one of the files in a text editor (e.g. Notepad) and view how such a file is built up.
(6) Open the files in WebLabViewer and try to respond to/discuss the above questions.

Good Luck,

/micke