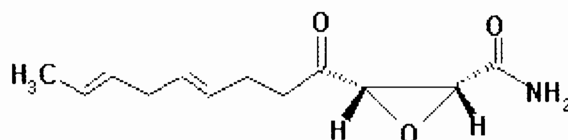
5.08 TD1

β-ketoacyl-ACP synthase I (FabB)

I. Background

- E. coli enzyme involved in elongation of fatty acids; works in concert with two other synthases (II and III)
- Catalyzes Claisen condensation between ACP-acyl fatty acid + ACP-malonyl
- His-His-Cys catalytical triad in active site
- Inhibited irreversibly by natural product **cerulenin**, which forms covalent adduct with active site cysteine (IC_{50} = 3 uM).



II. Crystal structure of cerulenin in complex with FabB

- Reference: J. Biol. Chem., Vol. 276, Issue 9, 6551-6559, March 2, 2001. Inhibition of beta-Ketoacyl-Acyl Carrier Protein Synthases by Thiolactomycin and Cerulenin.
- At end of paper, Protein Data Bank code for structure given: 1FJ8
- To download structure from the Protein Data Bank:
  - Google search for PDB; go to http://www.rcsb.org/pdb/
  - Search for "1FJ8" – open page
  - Click left side: Download/display file
  - Download the structure file, PDB, no compression
  - Save; filename = 1FJ8.pdb
- To view structure:
  - Install DSViewerPro (download software from course website); open
  - Drag 1FJ8.pdb into the DSViewerPro screen
  - Get used to the Rotate, Translate, and Zoom buttons
  - Universal color code: red = oxygen, blue = nitrogen, yellow = sulfur. H atoms not shown.
  - Note that structure may be a dimer/multimer. To convert to a monomer (for easier viewing):
    - Open pdb file with Wordpad
    - Save as different filename
    - Note that there are 4 chains, ABC and D, all with same amino acid sequence
    - Delete chains B,C, and D. save file
  - Open monomer structure
- Find the cerulenin buried inside
  - Double click on one amino acid to highlight it. Double click on it again to highlight the entire protein chain. Go to view/Display Style and click "off". Protein chain disappears, leaving only cerulenin
  - Double click on cerulenin, go to view/Display Style, and select green color.

- Unclick everything. Go back to view/Display Style and select "Line". Protein should reappear

- Use ribbon mode to view alpha helices and beta sheets
  - Unclick everything. Go to view/Display style. Select the "protein" tab. Select "line ribbon." Go to "atom" tab and select "none" to make the protein chain disappear (improves clarity).
  - Make "plumbing diagram" showing helices and sheets and residue numbers involved. Indicate tertiary interactions. Sheets = arrow. Helix = cylinder.
- View the cerulenin binding site
  - Double click cerulenin to highlight it
  - Go to Tools/enter command. Type "SelectBy Radius inside 7.0 aminoacid. This will highlight all amino acids within 7 angstroms of cerulenin.
  - Go to view/show only. This will make all the rest of the protein invisible.
- Measure distances between cerulenin and protein
  - [it may help to show cerulenin by atom color and in 'stick' mode]
  - highlight one atom by clicking on it; the highlight a second atom as well by clicking on it while holding down the "shift" key
  - Go to Tools/monitors/distance. Green bond and distance in angstroms should appear
  - Hydrophobic interactions max distance ~5 Å(ex. C-H--H-C distance)
  - Hydrogen bond max distance ~4 Å (ex. O-H--O distance)

III. BLAST analysis of FabB

- Obtain the amino acid sequence for FabB (from E. coli)
  - Google search "GenBank". Go to: http://www.ncbi.nlm.nih.gov/
  - Search under "protein" for "FabB E. coli"
  - Click on the entry that is synthase I from E. coli; scroll to bottom for sequence.
  - Check a few residues against the sequence from the PBD file just to make sure
- Use BLAST to find proteins homologous to FabB
  - Google search "BLAST". Go to: http://www.ncbi.nlm.nih.gov/BLAST/
  - Click on "protein-protein BLAST"
  - Paste in the amino acid sequence. Numbers don't matter. Hit "BLAST"
  - Click on "format"
  - You get a bunch of hits. Cursor over the red lines tells you identity of each hit (enzyme name and species)
  - Select some for the same enzyme from different organisms; paste into a text file

IV. ClustalW alignment of FabB protein sequences from different organisms

- Google search "ClustalW". Go to: http://www.ebi.ac.uk/clustalw/
- Paste in data in Fasta (or other) format; format very important or data will be rejected. Click on HELP and then "your sequences" for more info.
- Hit "run"