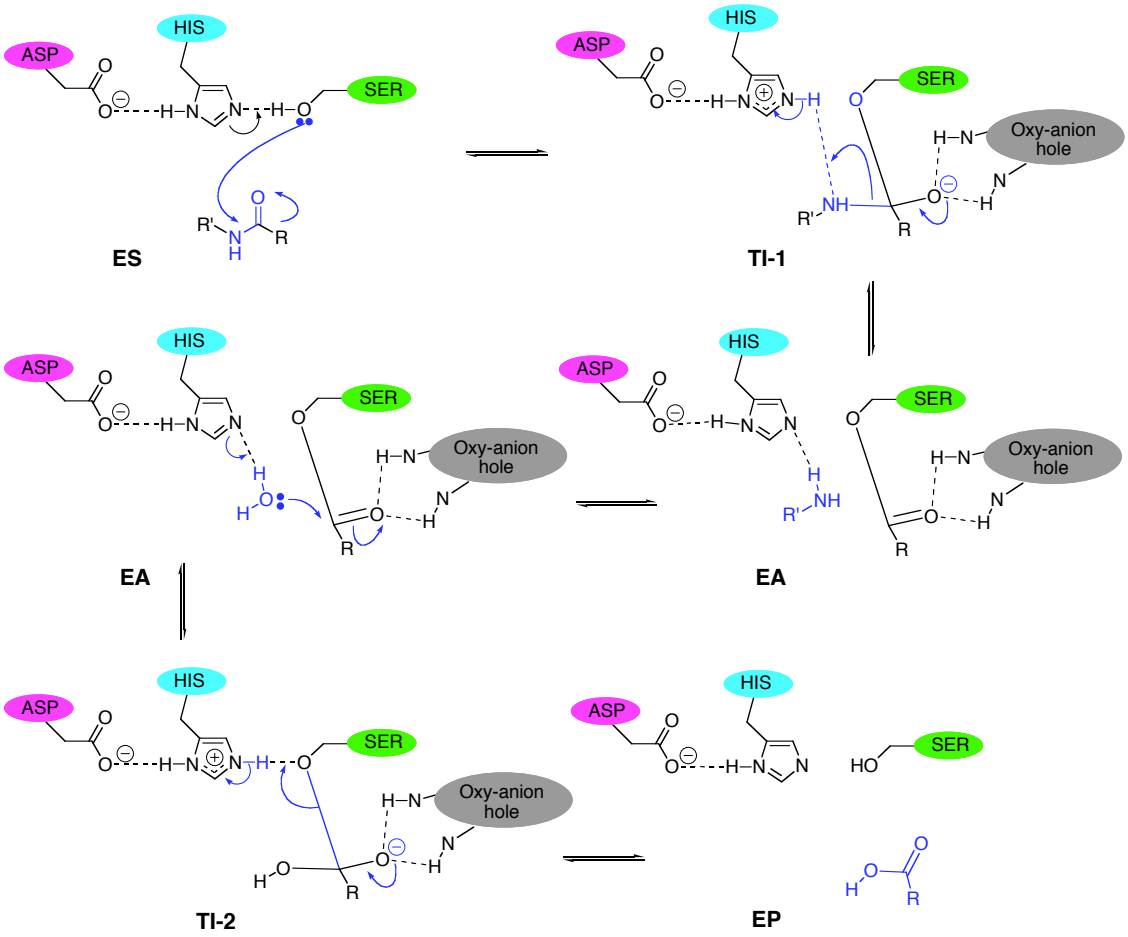
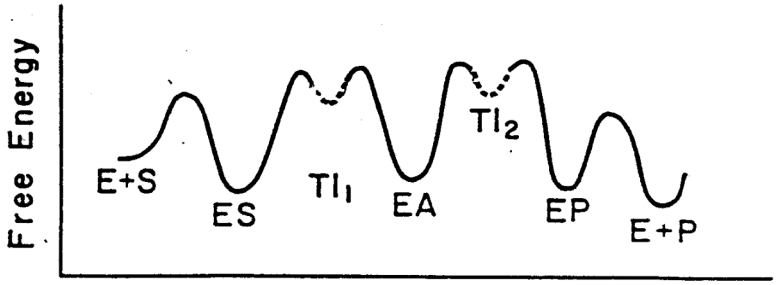
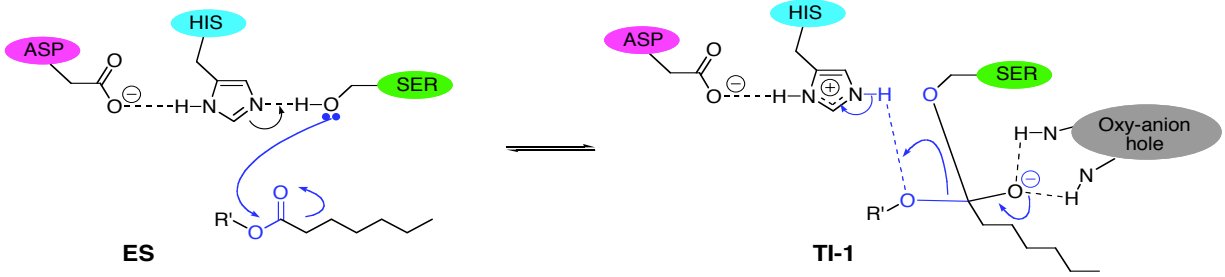
Serine Hydrolases



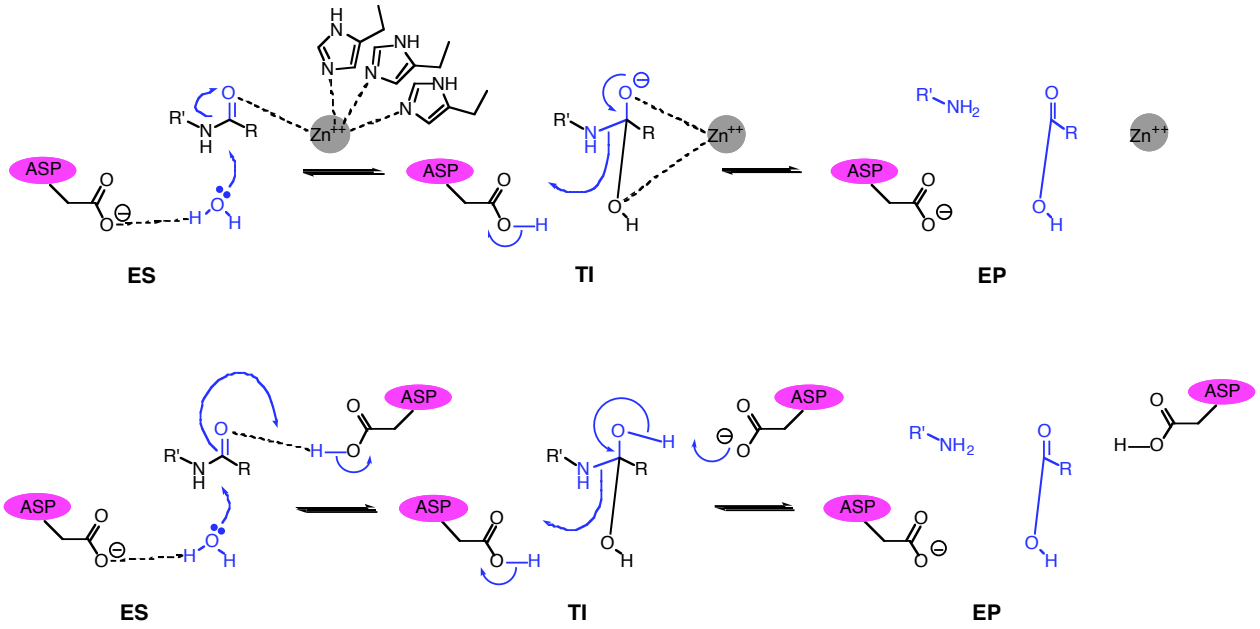


A representation of the expected free energy diagram for serine proteinase catalysis. From evolutionary principles the free energies of all the transition states are expected to be similar, and the energies of all the intermediates are anticipated to be similar

• The amide bond could equally be an ester bond and the same mechanism is used by many lipases:



Metallo/Aspartylproteases



•Here alternative catalytic residues are used but the principles of general acid & base catalysis remain

•Other biocatalysts use alternative bases, acids and nucleophiles (e.g. cysteine proteases such as papain - guess what they use as their nucleophile? **Q:** Is it A). a cyst; B) a cysteine; C) a Cystine chapel?; Answers on a postcard to Michelangelo Buonarroti, Vatican City.)

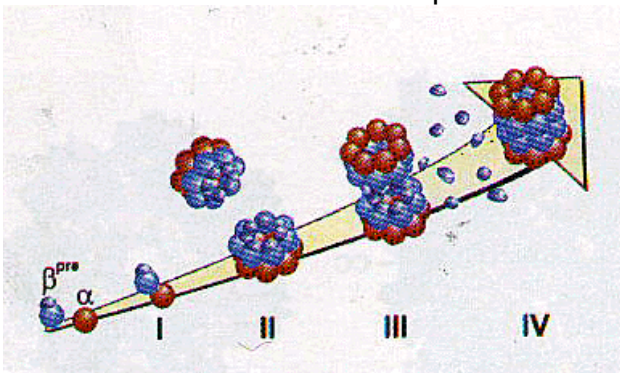
Proteasome

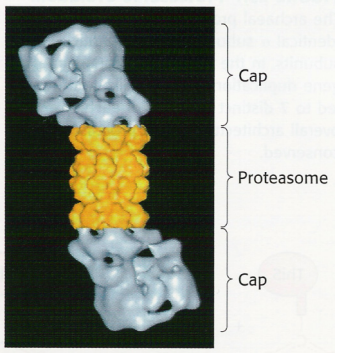
• The proteosome as an amazing cellular machine that degrades unwanted proteins.

• The unwanted proteins are marked for death by a 76 aa peptide called ubiquitin. This occurs through the formation of an amide bond between Lys side chains in the 'marked' protein and the C-terminus COOH of ubiquitin.

• Ubiquitin (Ubq) contains a Lys at position 48. By adding another Ubq onto this a string of Ubq's can be tagged onto the protein. 4 Ubq's is usually enough to trigger entry into the proteosome.

• The proteosome is made up of 2 sets (α and β) of 14 identical subunits that stack in 4 rings of 7 to make a barrel-like structure called the 20S proteasome.

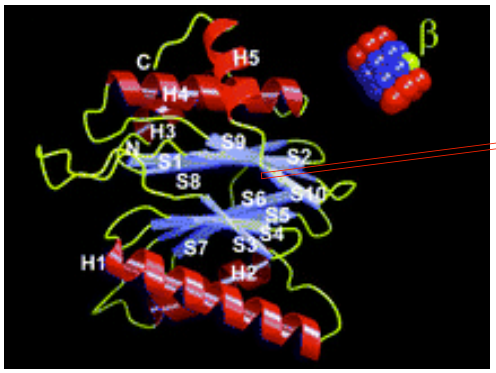


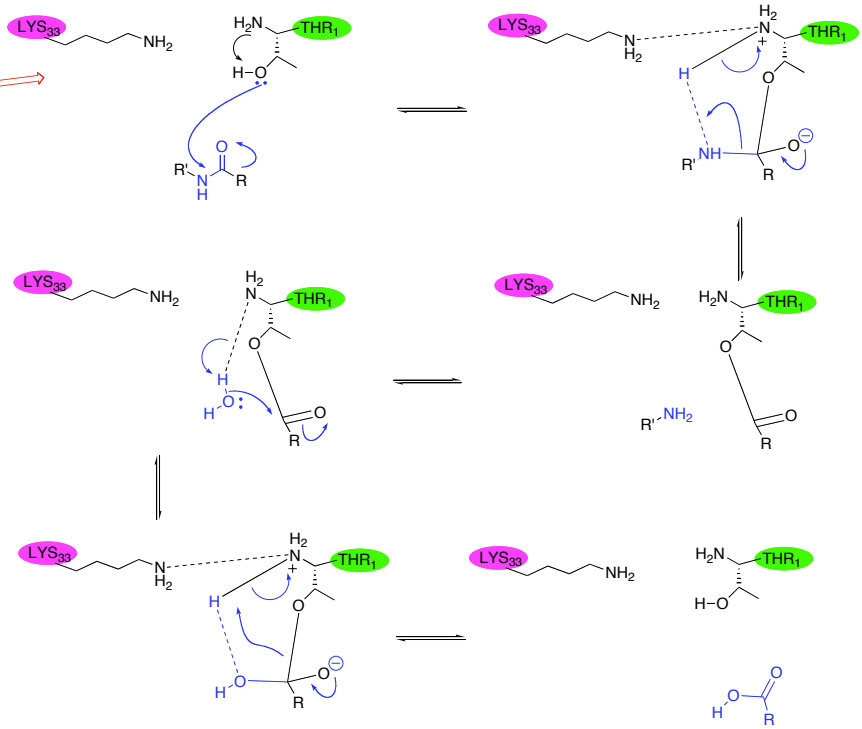


• The proteosome is normally a sealed barrel and access is controlled by two 'caps' on each end of the 20S proteosome. The whole thing together is called the 26S proteosome. The mechanism of the caps is unclear but they recognise the Ubq tags, unfold the 'marked' protein and pipe it in and out of the 20S barrel.

• β subunits with N-terminal Ser or Thr make up the 'active site' of the proteosome.

• A final clipping stage in the 'caps' takes off and recycles the Ubq tags.





Protein Splicing - Inteins and Exteins

- The same principles apply in post-translational protein splicing mechanisms.
- These autocatalytic processes involve nucleophilic catalysis and proximity effects.

- They lead to the 'chopping out' of a peptide segment called an *intein* and result in the joining of *exteins*. The system is in some ways analogous to *exons* and *introns* in pre-mRNA RNA.
- In this example, an Asn amide side chain acts as a nucleophile to cleave the intein by forming a succinimide. Examples with Gln are also known.
- For more see http://bioinformatics.weizmann.ac.il/~pietro/inteins/