Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 9:

Serine Proteases and Crystallography

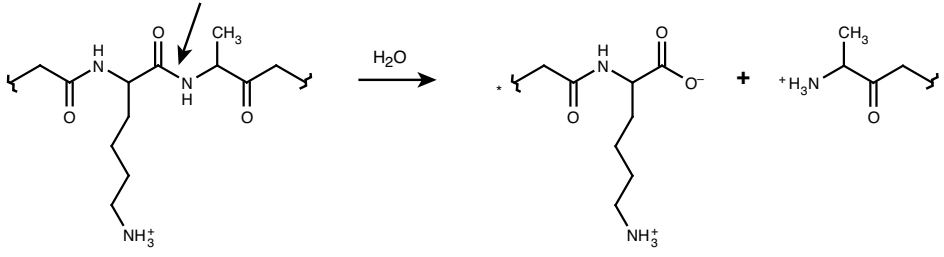
Tuesday, 6 February 2023

©David P. Goldenberg
University of Utah
goldenberg@biology.utah.edu

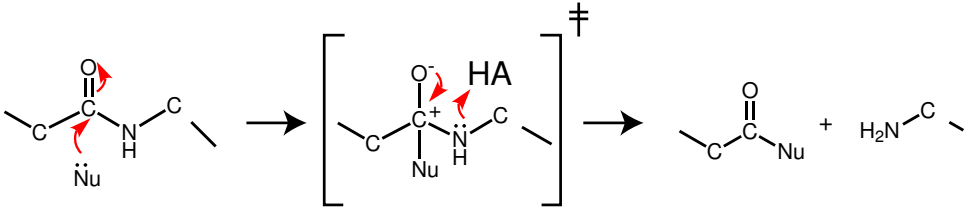
Announcements

- Computer Labs this week.
  - Molecular modeling with PyMOL
  - Start at 1:00 PM
  - Room 150 Biology Building
- Quiz on Thursday, 9 February
  - Will cover lectures through last week and Experiments 1 and 2.
  - 25 min, in second half of class session.
- Review session with TAs
  - Wednesday, 8 February, at 5:00 PM
  - 210 ASB

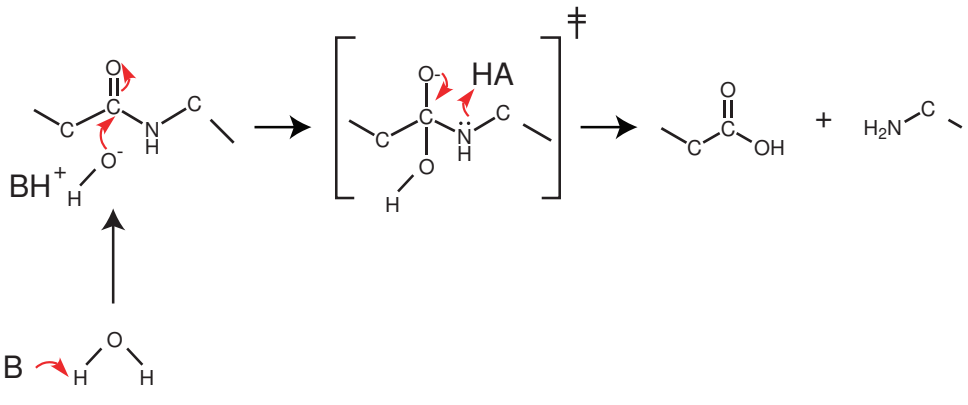
The General Protease Reaction



General Protease Mechanism is Nucleophilic Substitution

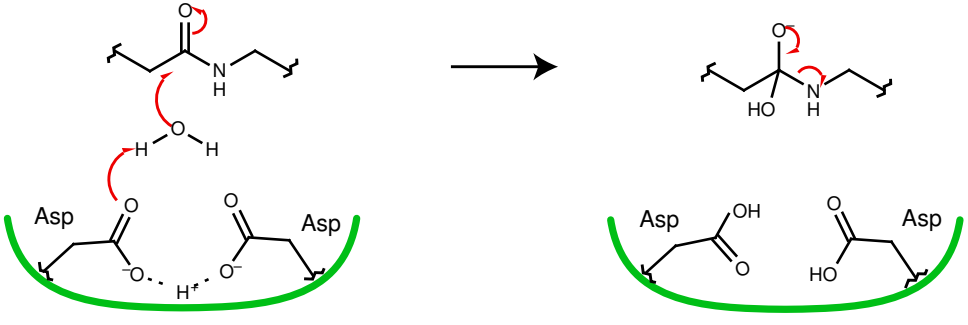


Water Can Act as the Nucleophile, but Must be Activated by a Base



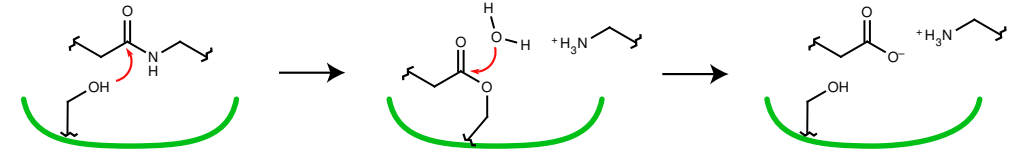
- Why is this reaction so slow in the absence of an enzyme?
- How do enzymes enhance the rate?

Carboxyl Groups Activate H_2O in Aspartyl Proteases



- Examples include pepsin and HIV protease

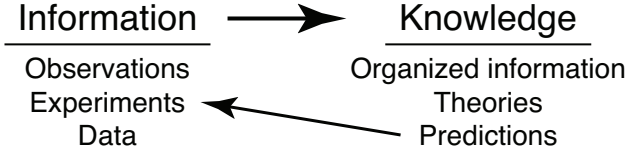
Serine Proteases Employ a Two-Step Mechanism



Enzyme-Substrate Complex | Acyl Intermediate | Enzyme + Products

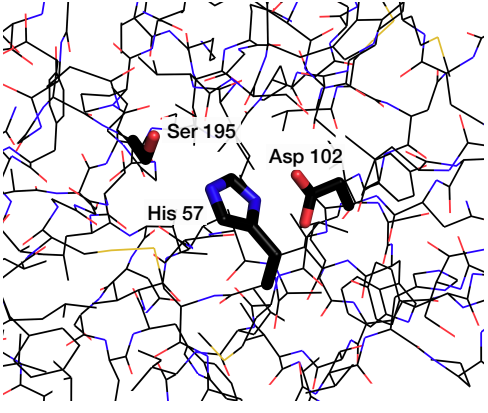
- In step 1, a serine hydroxyl is the nucleophile.
- In step 2, a water molecule is the nucleophile.
- Both steps require activation by a base.
- Both steps require an acid to protonate the leaving group.

How Do We Know What We Know About Serine Proteases?



- Chemical and biochemical data:
  - Enzyme kinetics
  - Studies with inhibitors
  - Chemical analysis, *e.g.,* active site labeling
- Structural analysis, mostly X-ray crystallography

The Catalytic Triad

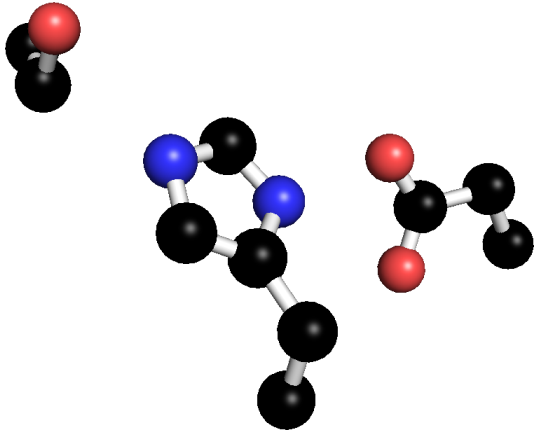


- Prior to first serine protease structure determination (chymotrypsin, 1967):
  - Ser 195 identified by unusual chemical reactivity as a nucleophile.
  - His 57 *also* identified by unusual reactivity as a nucleophile.
- Identified in chymotrypsin structure:
  - Asp 102 close to His 57.

Blow, D. M., Birktoft, J. J. & Hartley, B. S. (1969). Role of a buried acidic group in the mechanism of action of chymotrypsin. *Nature*, 221, 337–340. http://dx.doi.org/10.1038/221337a0

Blow, D. M. (1997). The tortuous story of Asp. . . His. . . Ser: Structural analysis of α-chymotrypsin. *Trends Biochem. Sci.*, 22, 405–408. http://dx.doi.org/10.1016/S0968-0004%2897%2901115-8

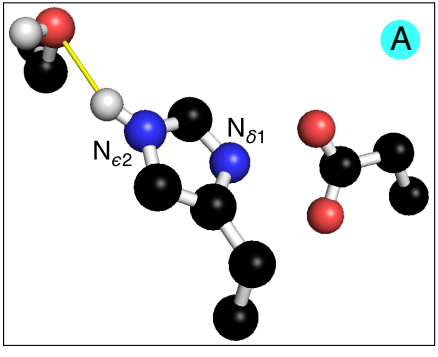
Where do Hydrogen Atoms Belong?



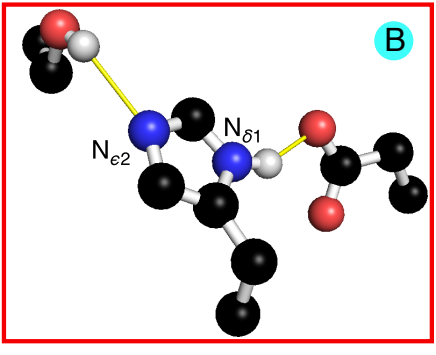
- Hydrogen atoms are usually not observable in X-ray crystal structures.
- Positions of hydrogen atoms must be inferred.
- What functional groups will be protonated or not at neutral pH?
  - On Ser 195?
  - On His 57?
  - On Asp 102?

Clicker Question #1

Where do hydrogen atoms belong?



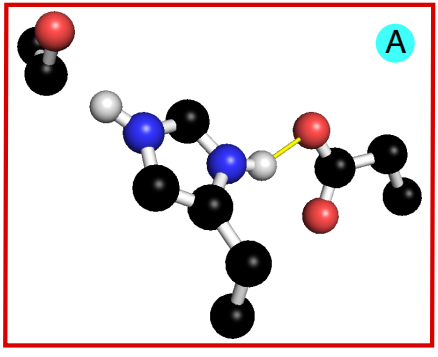
OR



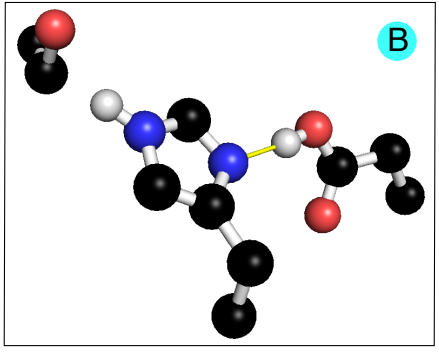
All answers count for now.

Clicker Question #2

When Ser 195 is deprotonated, where do hydrogen atoms go?

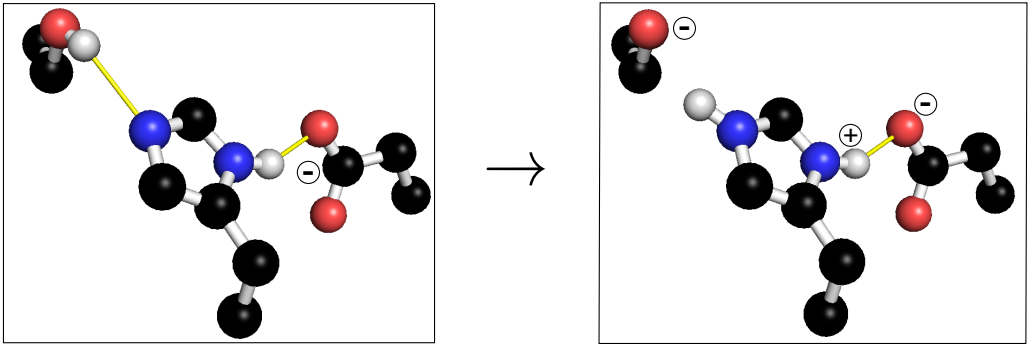


OR



All answers count for now.

The “Charge-Relay System”



Charge of protonated His is stabilized by negative charge of Asp 102.

Blow, D. M., Birktoft, J. J. & Hartley, B. S. (1969). Role of a buried acidic group in the mechanism of action of chymotrypsin. *Nature*, 221, 337–340. http://dx.doi.org/10.1038/221337a0

Warning!

Direction Change

X-ray Crystallography

Clicker Question #3: How Big is an Enzyme?

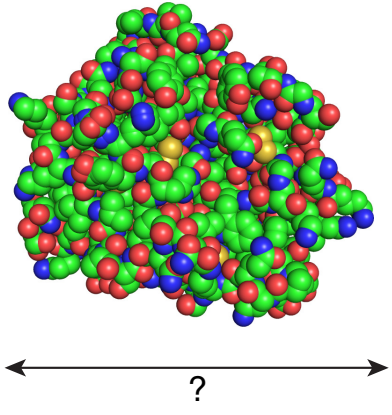
A) 10^{-10} m

B) 10^{-9} m

C) 10^{-8} m

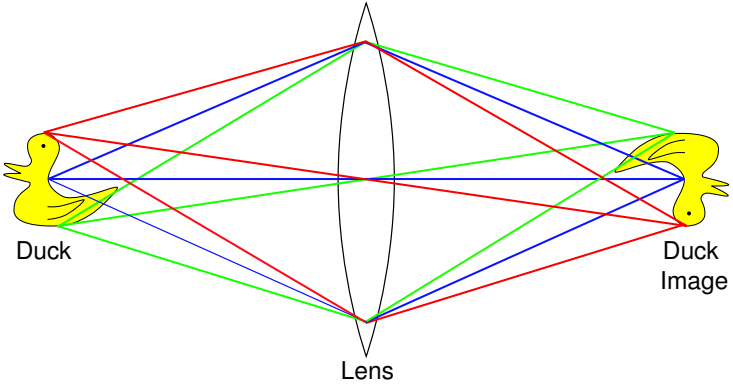
D) 10^{-7} m

E) 10^{-6} m

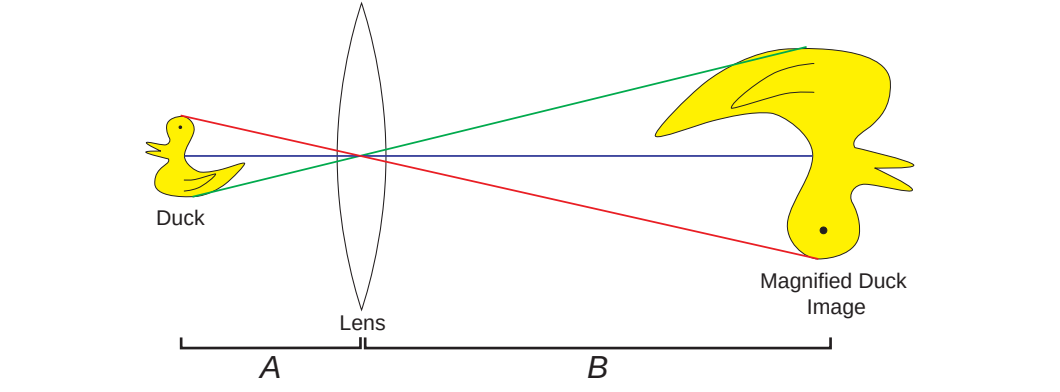


No wrong answers (for now)!

Image Formation with a Lens



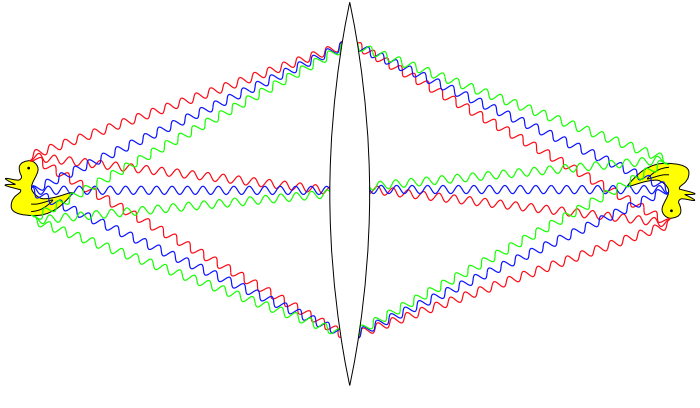
Optical Magnification



As the object is brought closer to the lens:

- Image moves further from the lens and becomes larger.
- Magnification $= B/A$
- Magnification, in principle, is not limited, but resolution is.

Imaging With a Lens - a Wave Interpretation



- Image is formed at points where waves are brought back in phase.
- Points in the object must be separated by at least $\sim 1/2$ wavelength to give rise to separate points in the image.

The Electromagnetic Spectrum

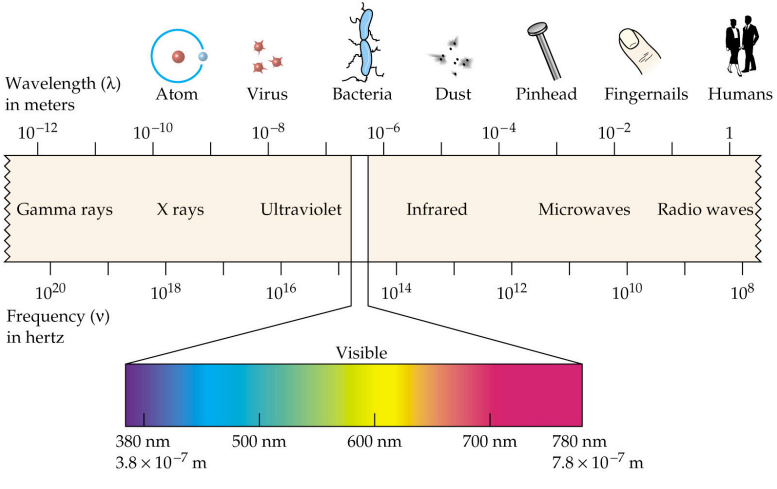
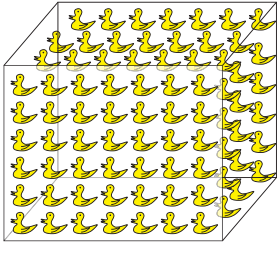


Illustration From: McMurry, J. & Fay, R. (2004). *Chemistry*. Prentice-Hall, 4^{th} edition.

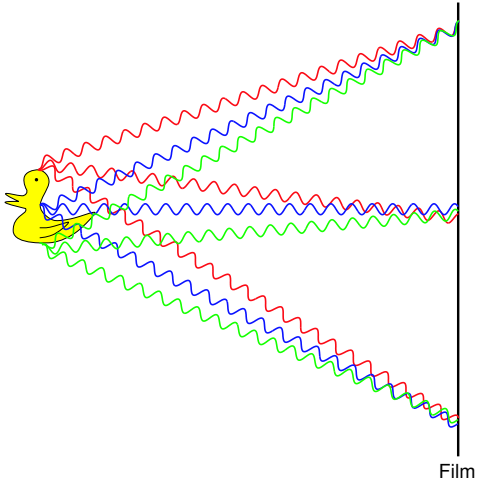
Why Not an X-Ray Microscope?

- Scattering from individual atoms is very weak, especially from elements with low atomic numbers.
- Very difficult to make lenses for X-rays.
- In crystallography:
  - Use crystals to increase the total scattering intensity.

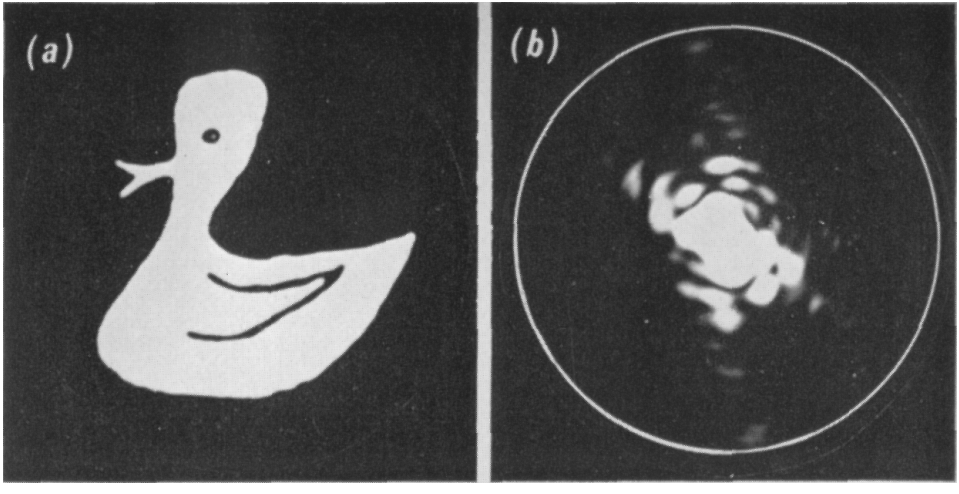


  - Use a mathematical technique, the Fourier transform, to do the job of a lens.

Diffraction from a Duck



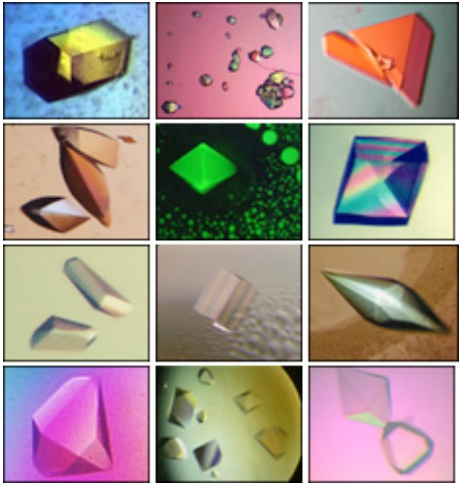
A Real Diffraction Pattern From a Pretend Duck



Taylor, C. & Lipson, H. (1964). *Optical Transforms: Their preparation and application to X-ray diffraction problems*. Cornell Univ. Press, Ithaca, NY.

Steps in Protein Crystallography

1. Grow Crystals



- Entirely empirical and idiosyncratic.
- Protein crystals are about 50% water and are kept suspended in a salt solution; close to physiological conditions.
- Resolution of final structure is highly dependent on how well ordered the crystals are.

Crystal pictures from: http://biophysics.uoguelph.ca/central/facilities.htm