Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2023

Lecture 18

SARS-CoV-2 Proteases and Inhibitors and Introduction to Electrophoresis

16 March 2023

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The Coronavirus Lifecycle

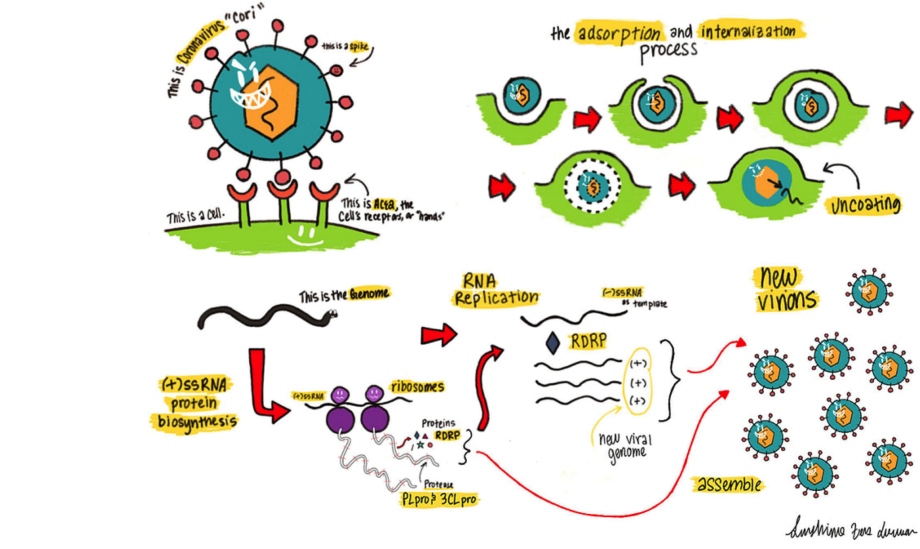
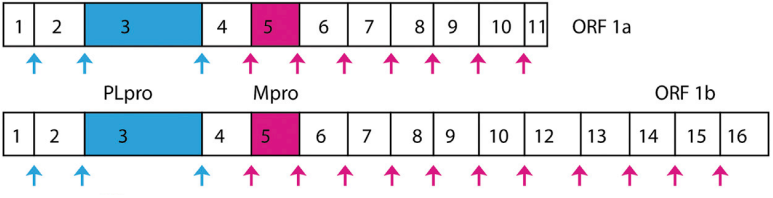


Illustration from: Morse, J. S., Lalonde, T., Xu, S. & Liu, W. R. (2020). Learning from the past: Possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. *ChemBioChem*, 21. https://doi.org/10.1002/cbic.202000047

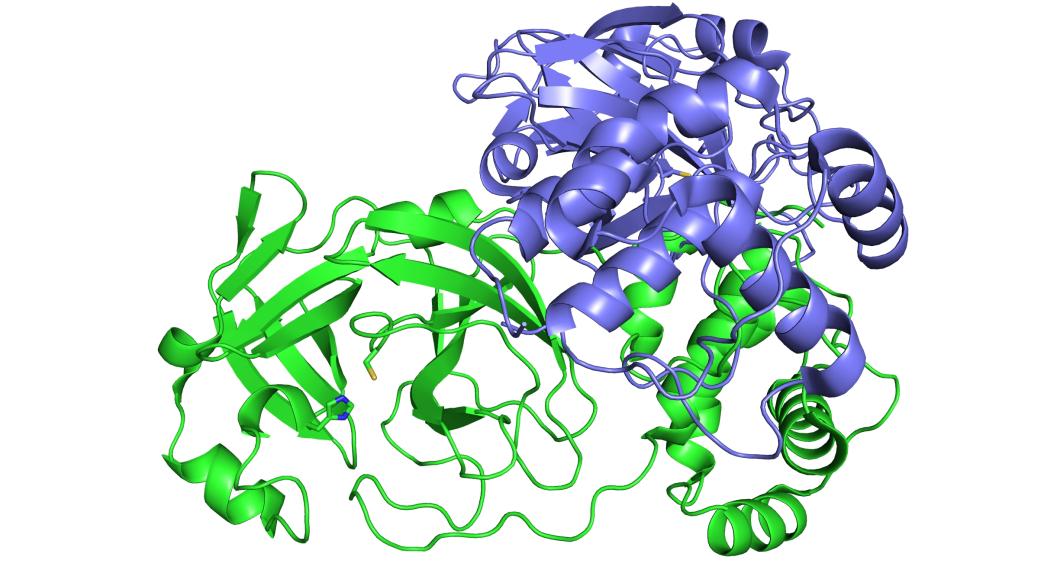
Processing of SARS-CoV-2 Non-structural Proteins (nsps)



- PLpro: Papain-like protease.
- Mpro: Main protease. (Also called C3Lpr: C3-like protease.)
- Both are cysteine proteases.

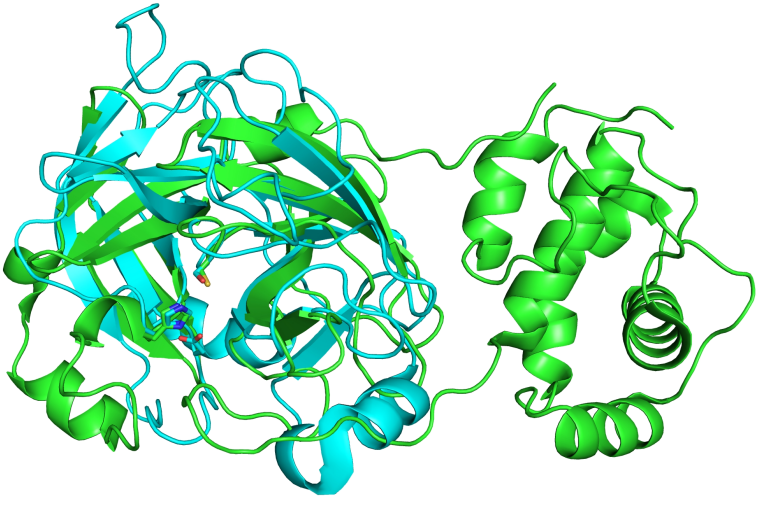
Illustration adapted from:
Lv, Z., Cano, K. E., Jia, L., Drag, M., Huang, T. T. & Olsen, S. K. (2022). Targeting SARS-CoV=2 Proteases for COVID-19 antiviral development. *Front. Chem.*, 9, 819165.
https://doi.org/10.3389/fchem.2021.819165

Crystal Structure of SARS-CoV-2 Main Protease



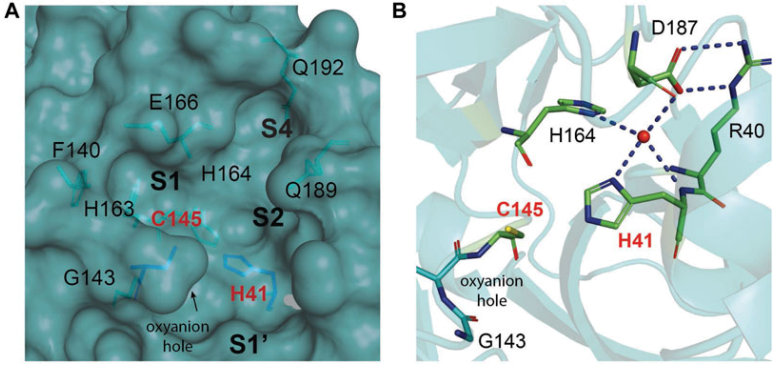
Protein Data Bank entry 6LU7, deposited 26 January 2020. X. Liu, B. Zhang, Z. Jin, H. Yang and Z. Rao.

SARS-CoV-2 Main Protease and Bovine Trypsin



Protein Data Bank entries 1CE5 and 6LU7.

Close-up of SARS-CoV-2 Main Protease Active Site



Lv, Z., Cano, K. E., Jia, L., Drag, M., Huang, T. T. & Olsen, S. K. (2022). Targeting SARS-CoV-2 Proteases for COVID-19 antiviral development. *Front. Chem.*, 9, 819165.
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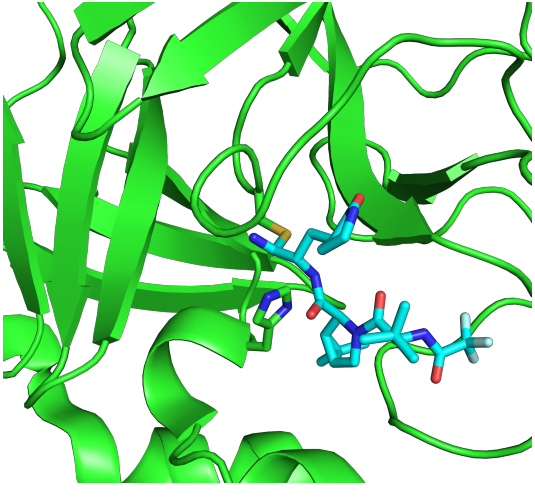
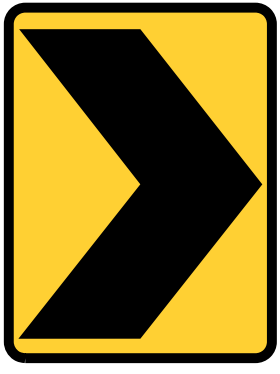
An Inhibitor of SARS-CoV-2 Main Protease: Nirmatrelvir



- Reacts with active-site Cys sulfur atom.
- Inhibition is reversible!
- Active component of Pfizer oral COVID-19 treatment, Paxlovid.

Owen, D. R., Allerton, C. M. N., *et al.* (2021). An oral SARS-CoV-2 M_{pro} inhibitor clinical candidate for the treatment of COVID-19. *Science*, 374, 1586–1593.
https://www.science.org/doi/abs/10.1126/science.abl4784 34 co-authors!

Warning!



Direction Change

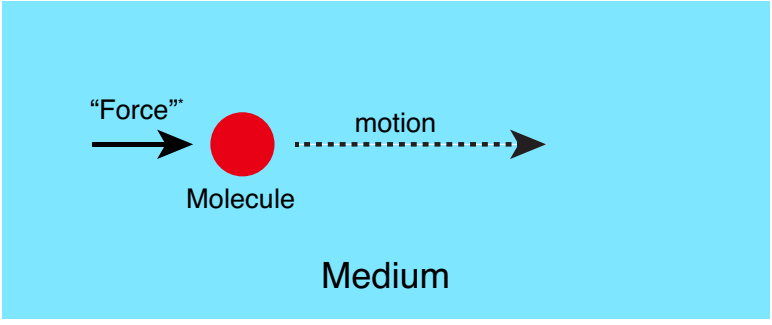
Separation Techniques

Separation Techniques in Biochemistry

- Isolation of pure components
- Analysis of complex mixtures
- Can be the basis of enzyme assays
- Physical characterization:

  Separation methods generally depend on differences in physical properties of molecules, such as size, shape and charge.

Separation Methods: The General Idea



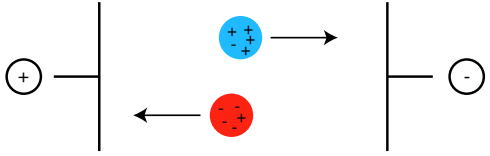
- Something (a “force”*) causes molecules to move through a medium.
- The rate of motion depends on the strength of the force and the interactions of the molecules with the medium.
- Different kinds of molecules move at different rates, allowing them to be separated.

* “Force” is used rather loosely here to describe anything that causes motion of the molecules.

Two Biochemical Separation Methods that We Will Study

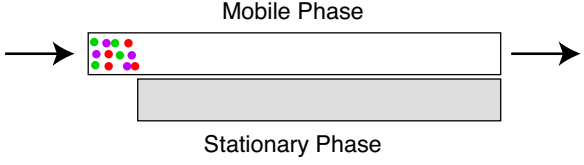
- Electrophoresis

  Charged molecules are subjected to an electric field and move through a medium.

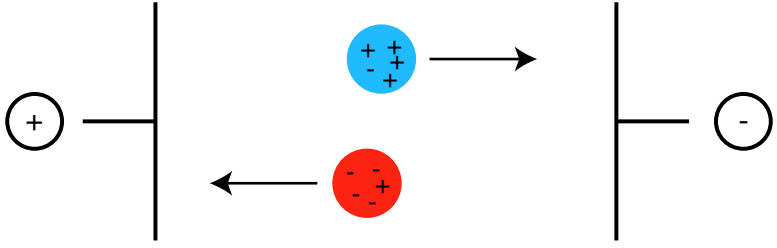


- Chromatography

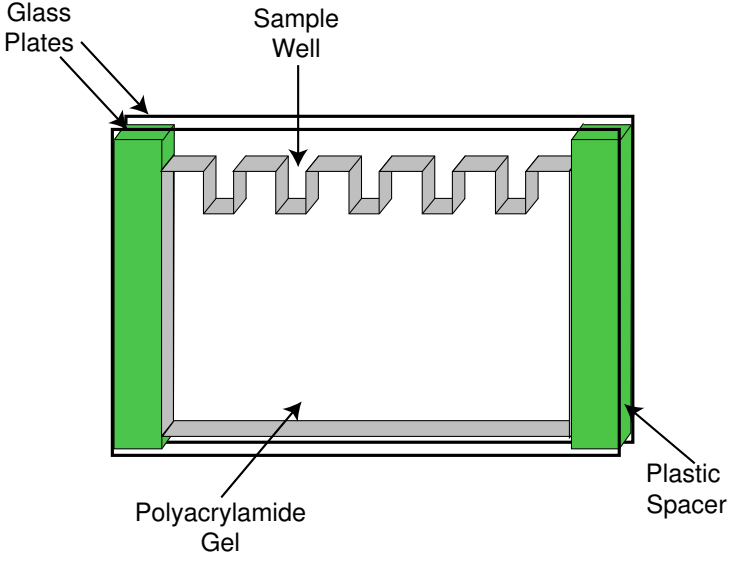
  Molecules are carried by flow of medium in one phase past a second, stationary phase.



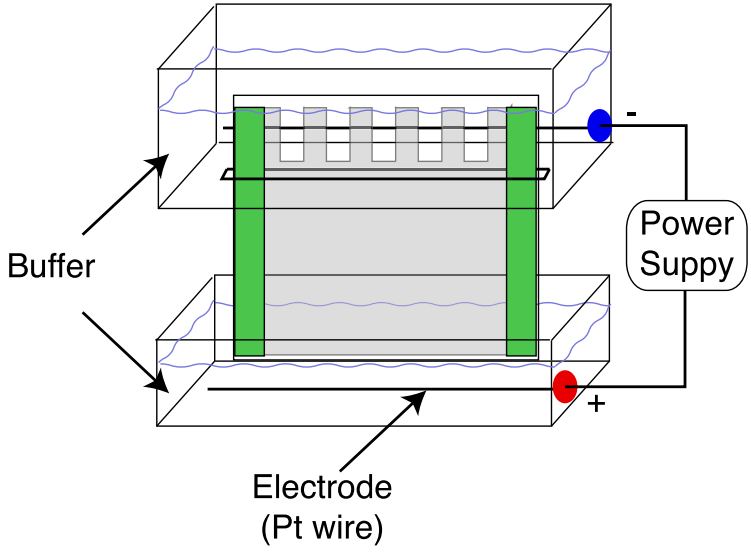
Electrophoresis:
Separation based on movement in an electric field



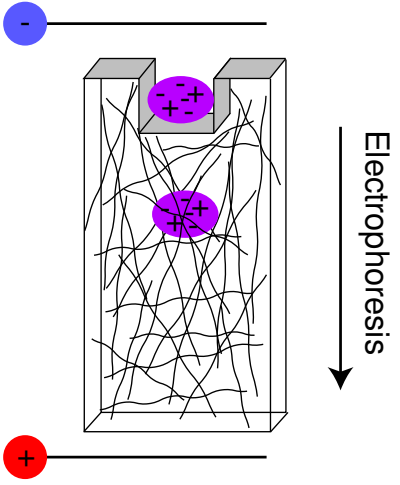
A Gel “Sandwich” for Electrophoresis



Apparatus for Gel Electrophoresis



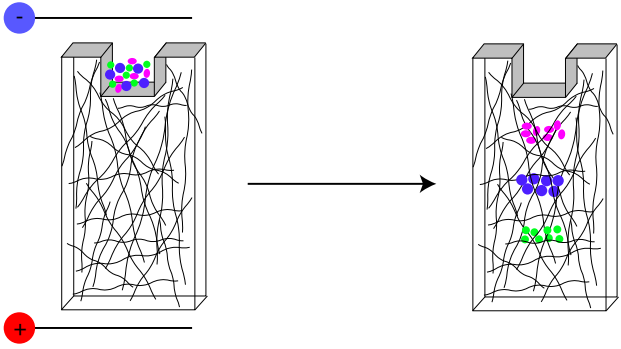
Electrophoresis Through a Gel



Rate of migration through the gel depends on:

- Strength of the electric field.
- Net charge of the protein.
- Size and shape of the protein.
- Density of the gel matrix

Separation of Proteins by Electrophoresis

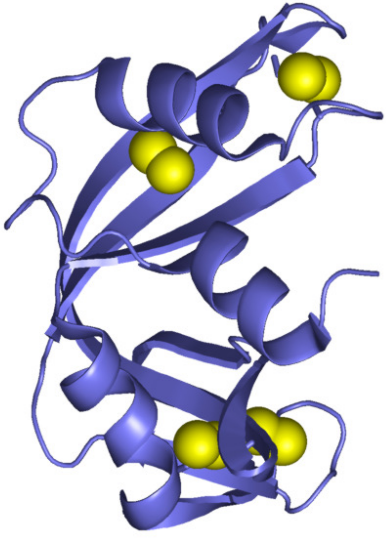


- Proteins with different mobilities migrate as “bands” in the gel.
- Various ways of detecting the proteins in the gel.

Two Major Variants of Gel Electrophoresis for Proteins

1. Non-denaturing ("Native") electrophoresis.
   - Carried out in the absence of denaturants, though sometimes relatively low or high pH values are used.
   - Protein migrates through the gel on the basis of its intrinsic net charge, size and shape, and the sieving effect of the gel.
2. SDS gel electrophoresis
   - Proteins are denatured in the presence of sodium dodecyl sulfate (SDS), a detergent that denatures proteins and complexes.
   - Mobilities reflect molecular weights of polypeptide chains.
   - Very useful for analyzing complex samples and macromolecular complexes composed of multiple polypeptides (*e.g.,* viruses, organelles, membranes).
   - By far the most common form of protein electrophoresis.

Ribonuclease A: A “Classic” Protein Stabilized by Disulfide Bonds

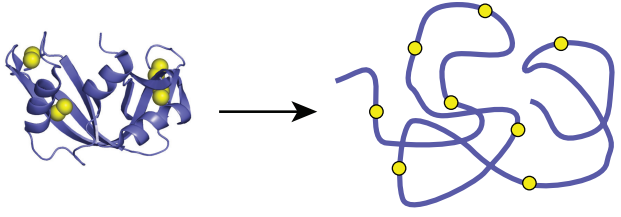


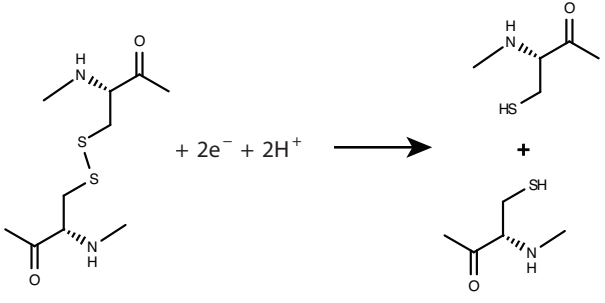
- Hydrolyzes RNA, much as trypsin hydrolyzes proteins.
- Like trypsin, made in pancreas.
- A favorite protein for chemical, enzymatic and structural studies in the 1950s and 1960s. Two Nobel prizes (4 awardees).
- Produced in large quantities (kilogram) by the Armour Meat Packing Company near the end of World War II, and provided at very low price to scientists.
- Close relatives are cytotoxic and are being explored as anti-cancer agents.
- Presence of 4 disulfide bonds allows some neat chemical manipulations of the protein.

Outline of Experiment 5

- Day 1:
  1. Preparation of modified RNase A
- Day 2:
  1. Non-denaturing gel electrophoresis of native and modified RNase A
  2. Trypsin treatment of RNase A forms
- Day 3:
  1. SDS gel electrophoresis of trypsin-treated RNase A samples
  2. Image capture of non-denaturing gel
- Day 3+1 (first day of experiment 6):
  1. Image capture and quantitation of SDS gel

Unfolding RNAse A by Reducing its Disulfides

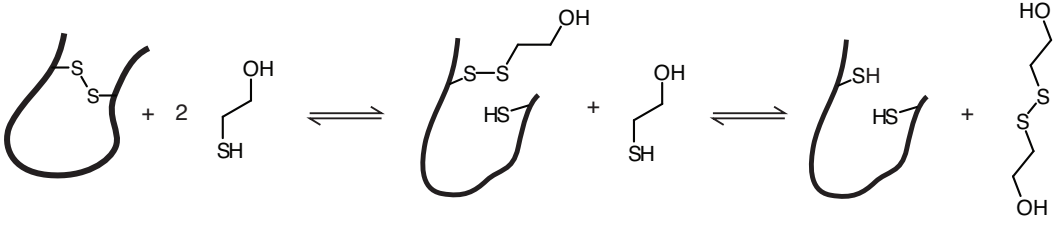




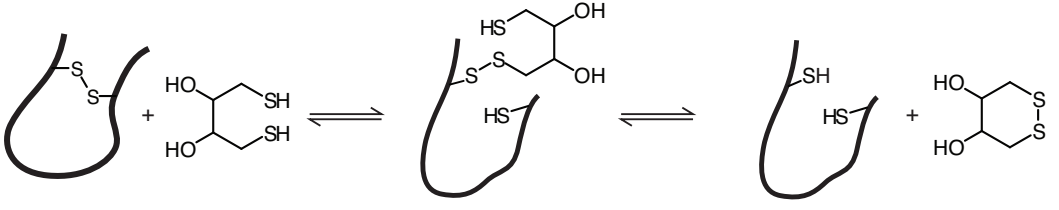
- Without disulfides, the folded protein conformation is unstable.
- Unfolded protein is a broad ensemble of rapidly interconverting conformations.
- Reaction is shown here as a reductive half-reaction.
- There are a variety of ways to promote the reduction reaction.

Reduction of Protein Disulfides by Thiol-Disulfide Exchange

- By 2-mercaptoethanol (β-mercaptoethanol, BME)



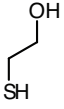
- With dithiothreitol (DTT, Cleland's reagent)



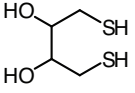
Clicker Question #1

Which reagent (at equal concentrations) will reduce protein disulfides more rapidly:

A) 2-mercaptoethanol



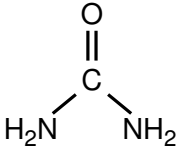
B) Dithiothreitol



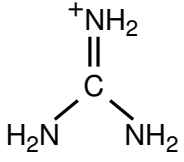
All answers count for now.

Reduction of Disulfides in RNAse A

- Rate is much higher in presence of strong denaturants, such as 8 M urea or 6 M GuHCl (guanidinium chloride).



Urea



Guanidinium

- Urea and GuHCl destabilize folded proteins. Why?
  ~~Probably by weakening the hydrophobic effect~~
  Probably by interacting with the polypeptide backbone (as of 2023).