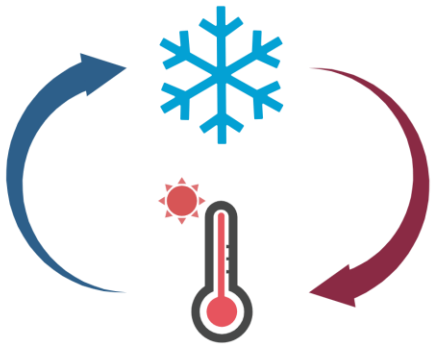
Lab on cell-free synthetic biology

Week 4

Outline

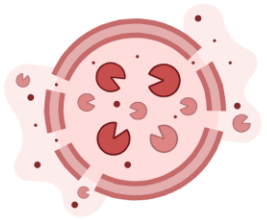
- Protein purification
- SDS-PAGE

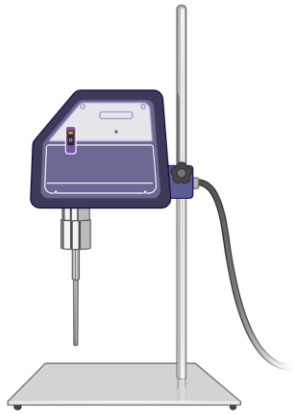
How to lyse the bacteria?



Freeze-thaw cycle

Lysozyme + detergent



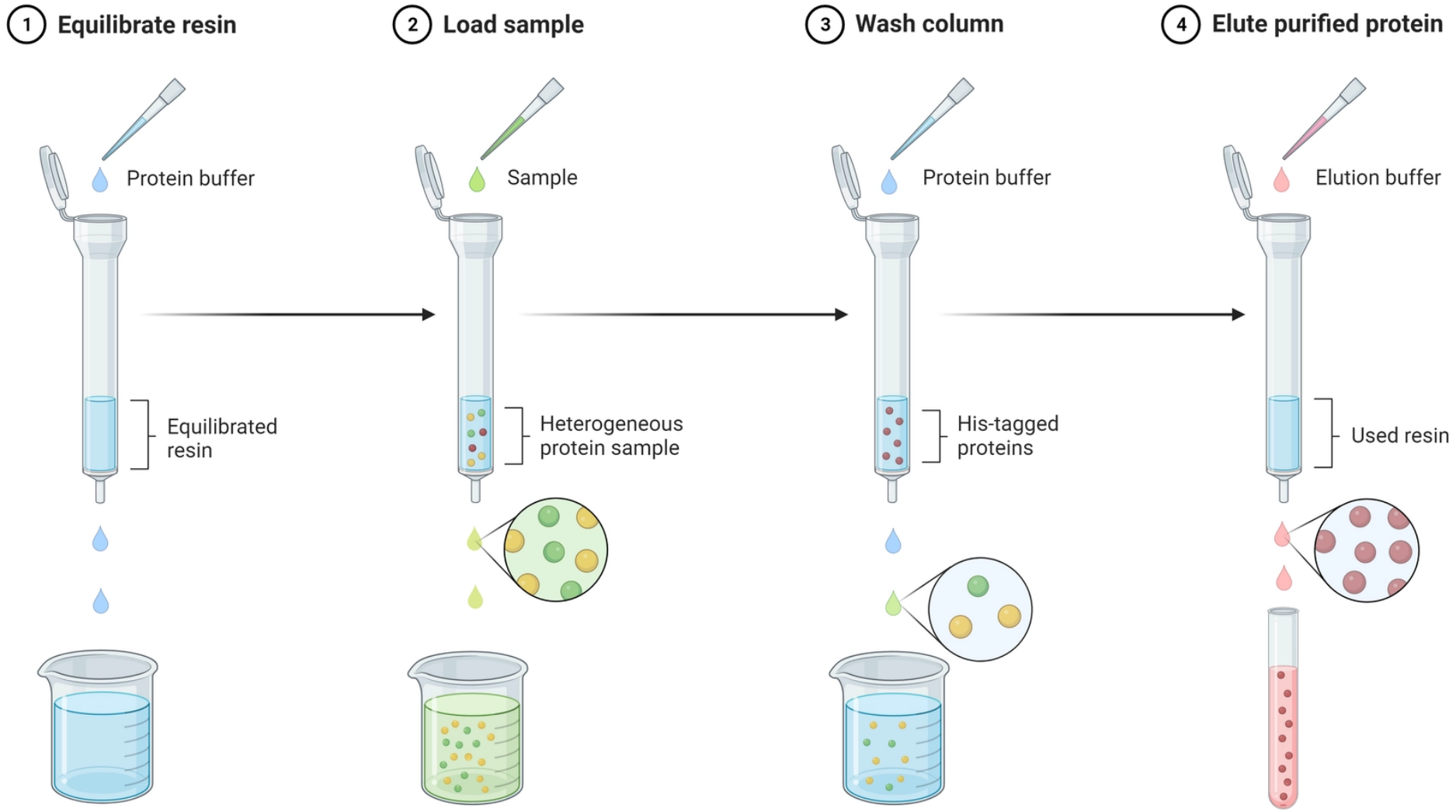


Sonication

How do I choose the method?

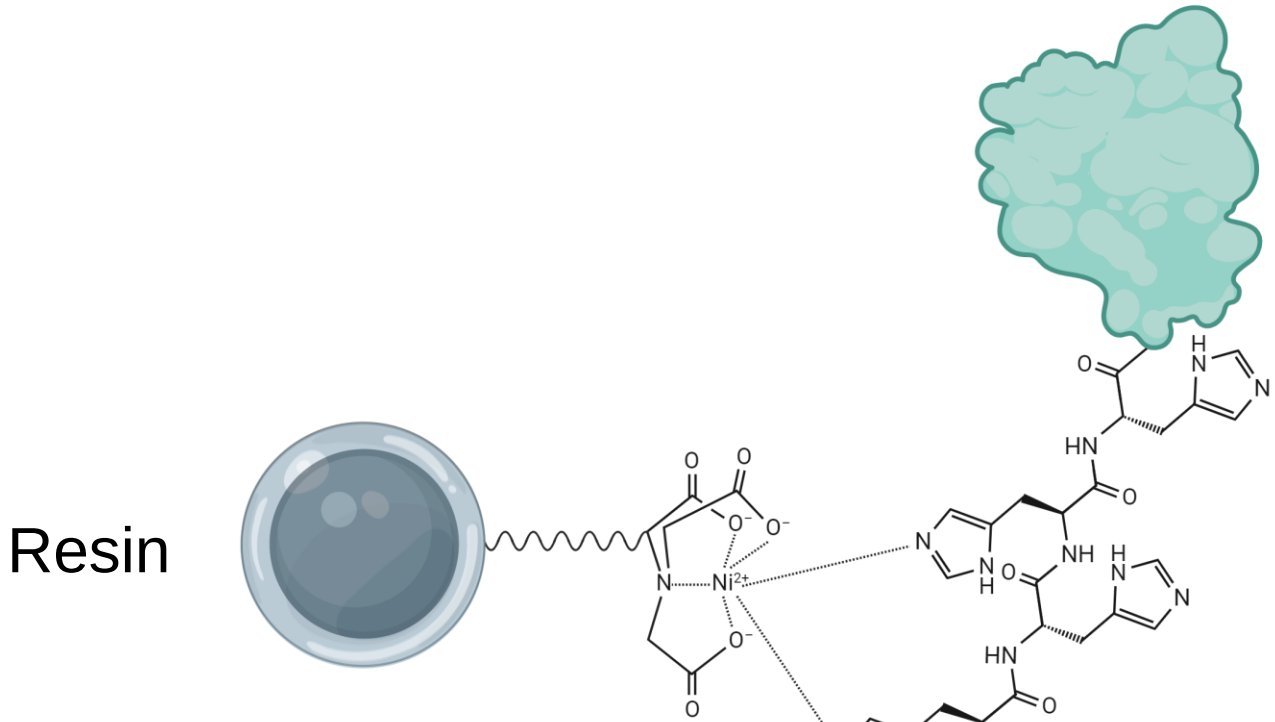
- Equipment (cost)
- Downstream assay
- Sensitivity of the target protein

Affinity purification



https://www.aatbio.com/catalog/affinity-purification

His-tagged protein purification

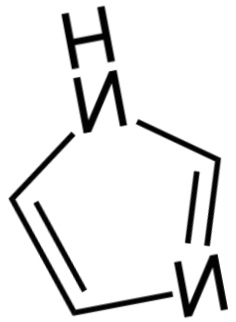


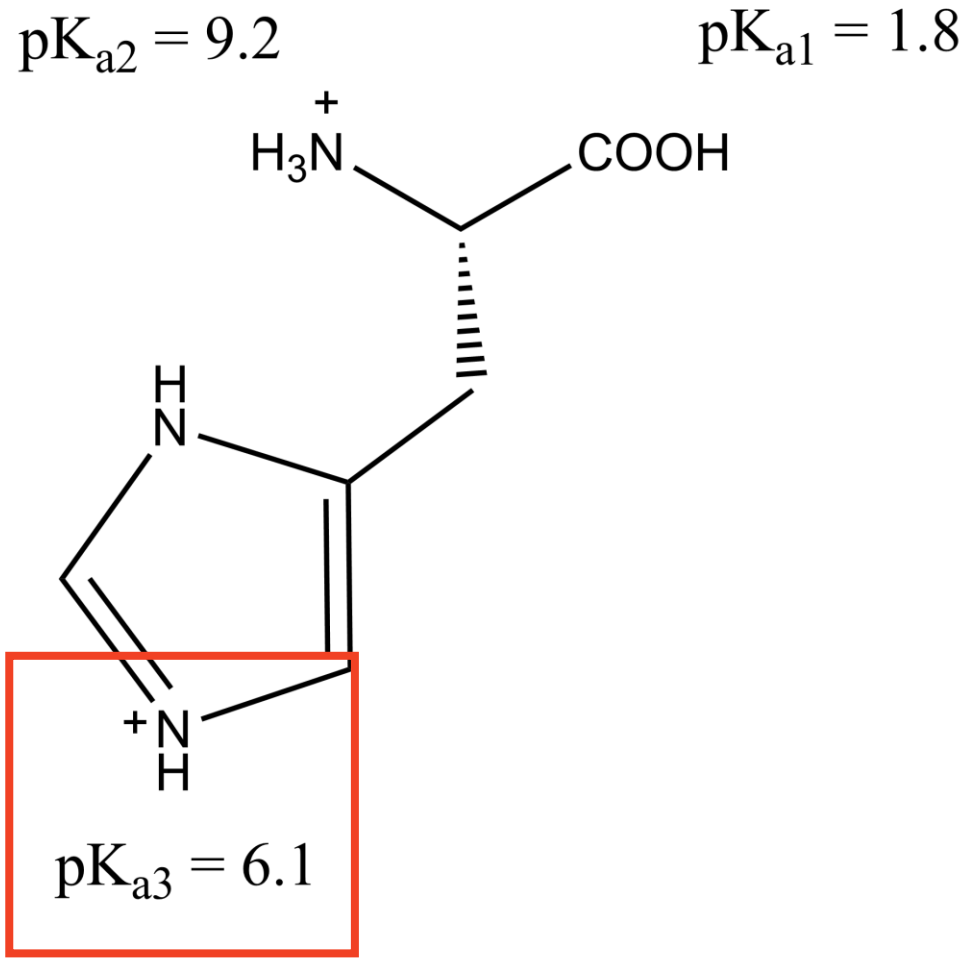
Nitrilotriacetic acid (NTA)

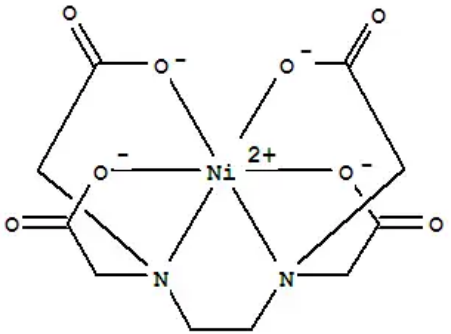
How to elute the His-tagged protein?

- Imidazole (competition)
- Decrease the pH value

  (protonation on histidine)
- EDTA chelation (removal of Ni^{2+})







• 2 H+

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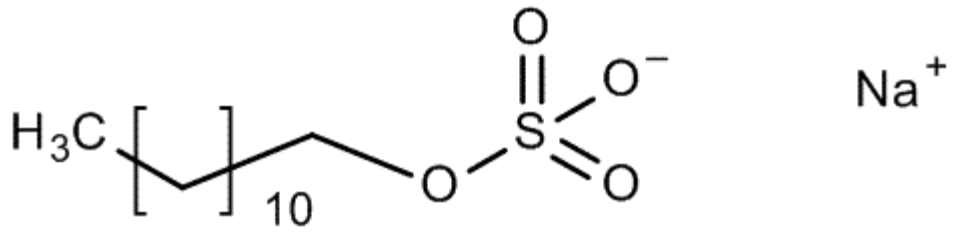
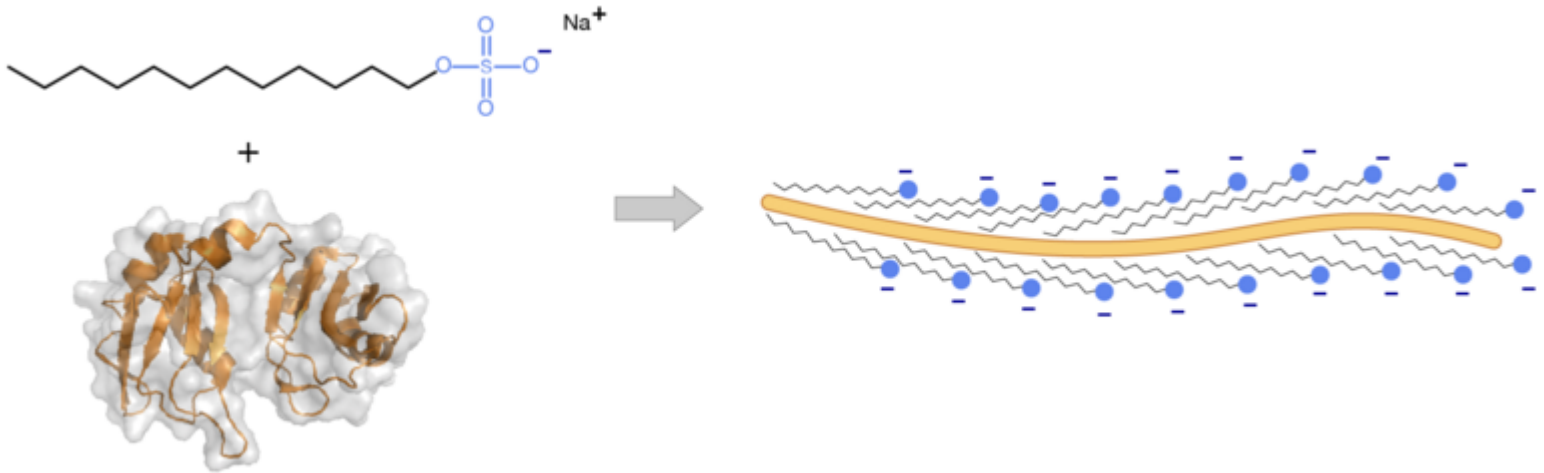
SDS-PAGE (sample prep. & gel prep.)

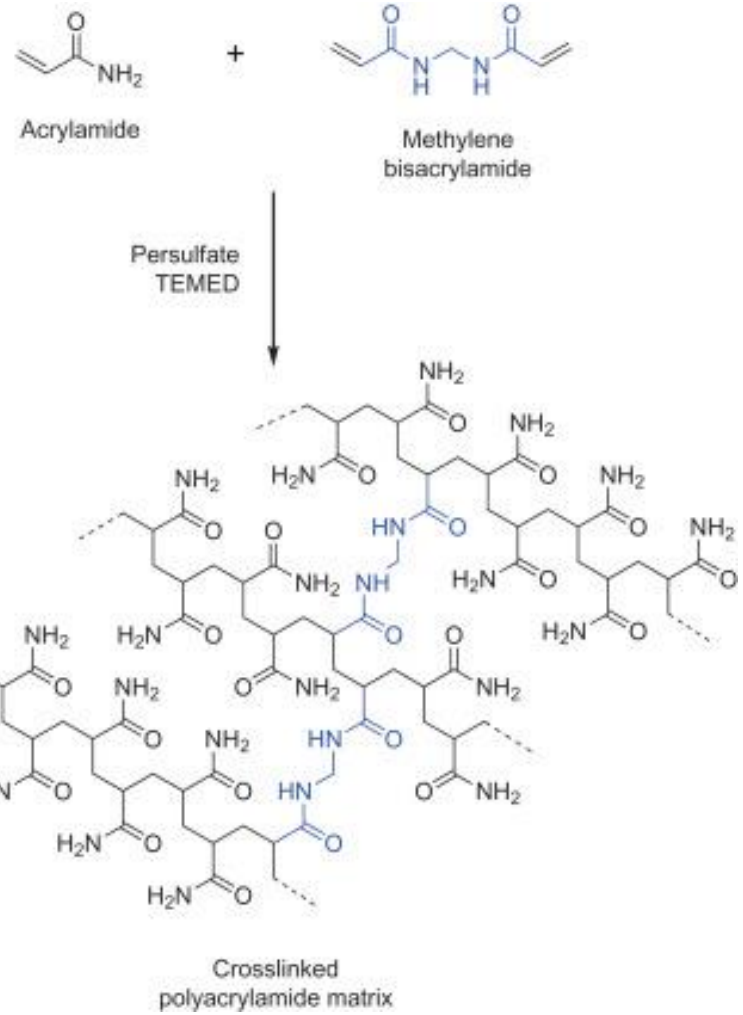
Sodium dodecyl-sulfate polyacrylamide gel electrophoresis



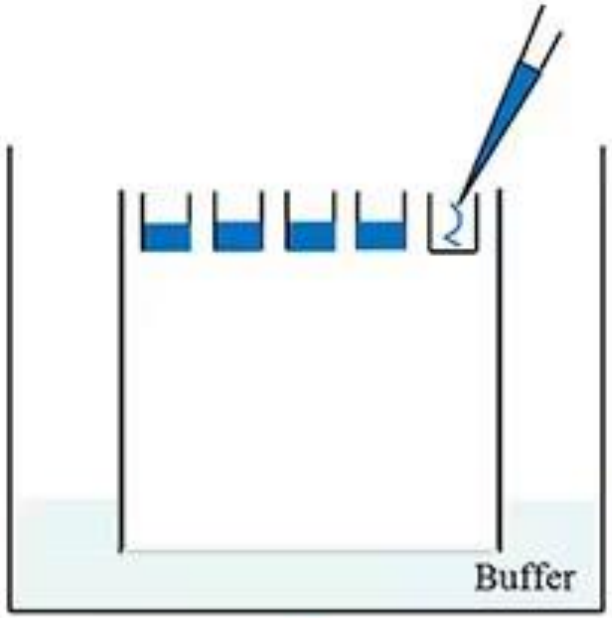
SDS: protein denaturant (strong detergent)



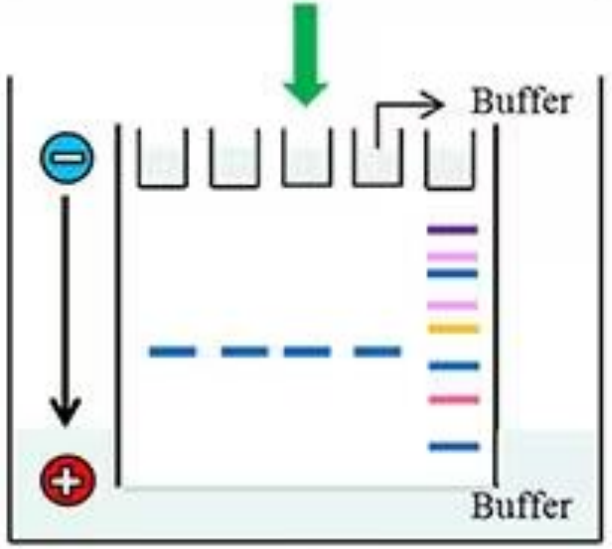
https://commons.wikimedia.org/wiki/File:Protein-SDS_interaction.png



SDS-PAGE (electrophoresis)



Protein samples and marker
loaded in vertical SDS-PAGE system

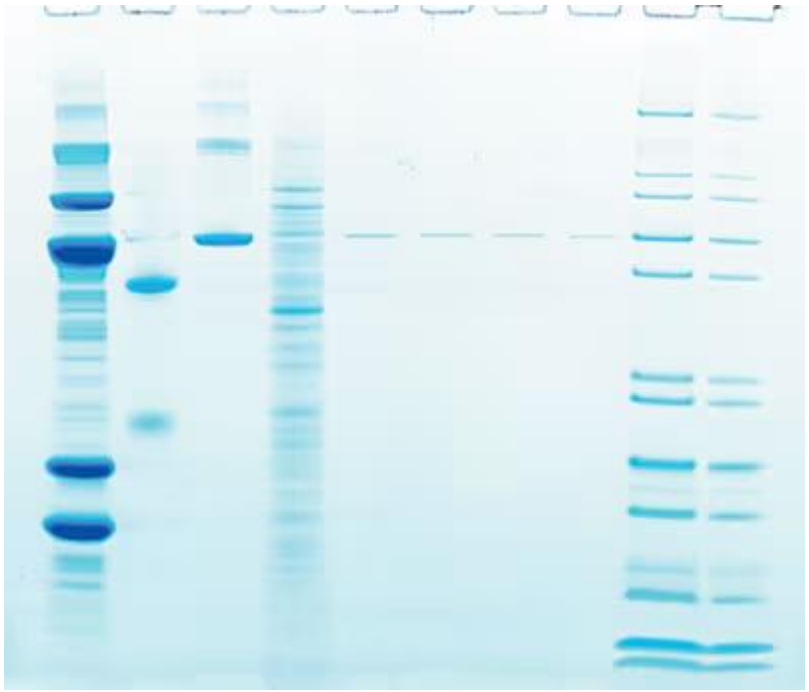


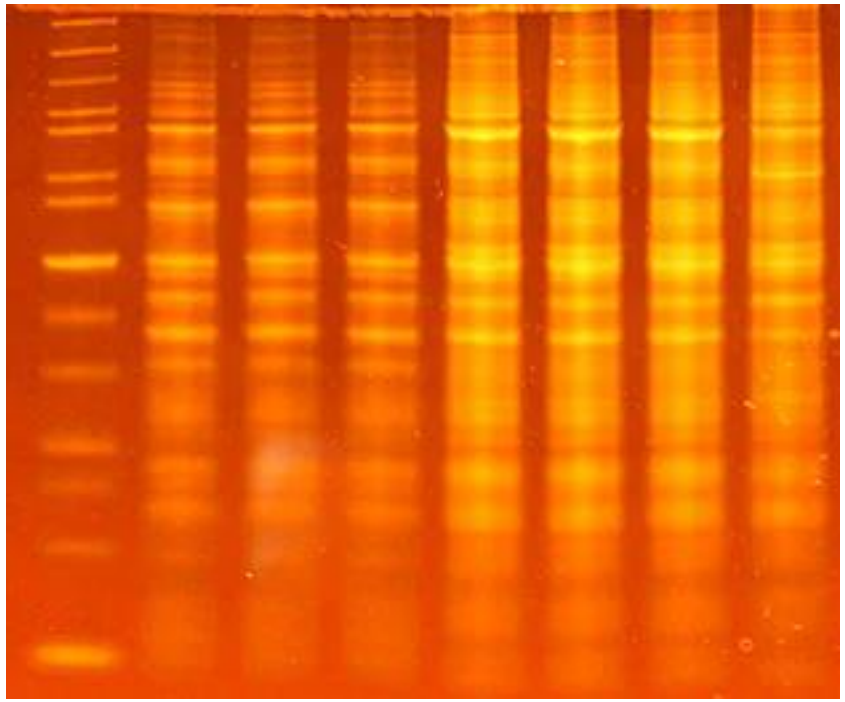
Direction of migration of samples
in vertical SDS-PAGE system

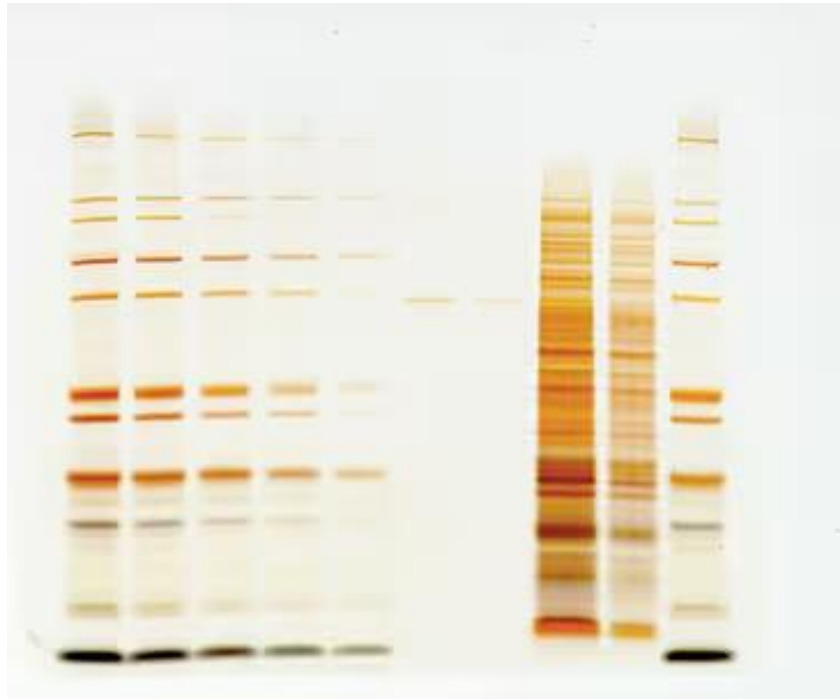
https://www.sigmaaldrich.com/CH/en/technical-documents/protocol/protein-biology/gel-electrophoresis/sds-page

Visualization of proteins in the gel

- Coomassie blue (~ 0.1–0.5 µg)
- Fluorescent dye staining (~ 4–8 ng)
- Silver staining (~ 0.25–0.3 ng)







https://www.thermofisher.com/order/catalog/product/S6650

Next week

- Onepot PURE protein production