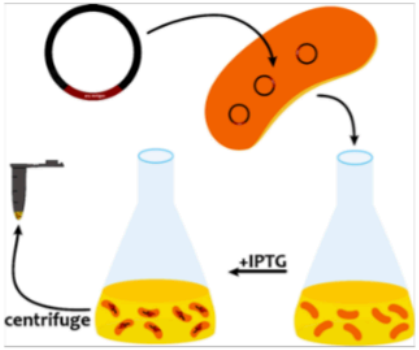
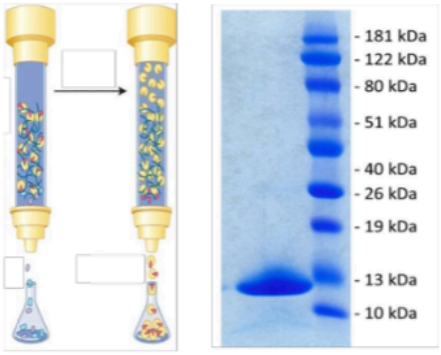
Our path to evaluate FKBP12 ligands



in silico cloning; overexpress FKBP12

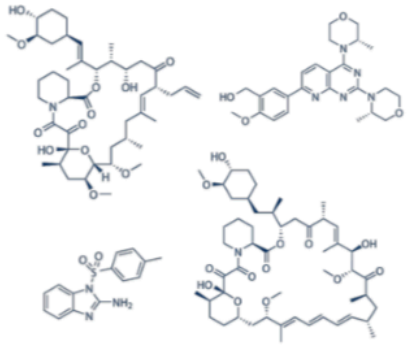
lab days 1 & 2



purify and analyze FKBP12

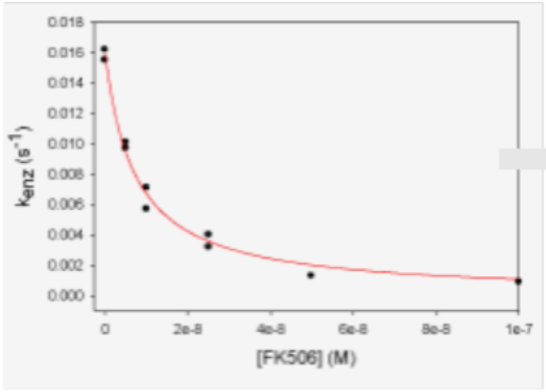
lab days 3 and 4

+



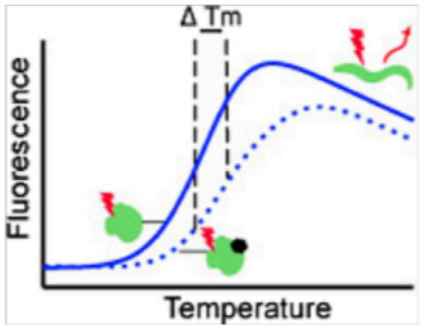
re-analyze screen data

lab day 2



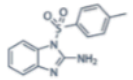
test FKBP12 and ligands in enzyme activity assay

lab day 5



test ligands in FKBP12 binding assay

lab day 6



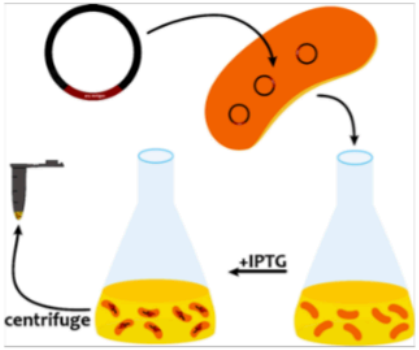
IC_{50} = X μM

ΔT = Y °C

complete data analysis prioritize best ligands

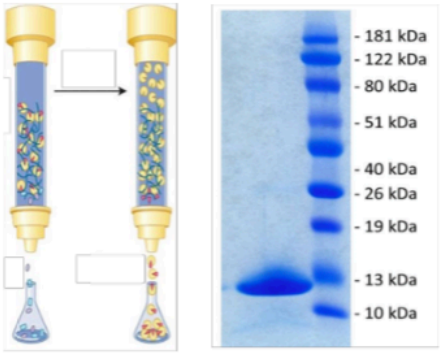
lab day 7

Our path to evaluate FKBP12 ligands



in silico cloning; overexpress FKBP12

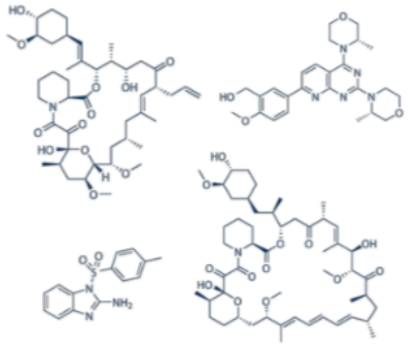
lab days 1 & 2



purify and analyze FKBP12

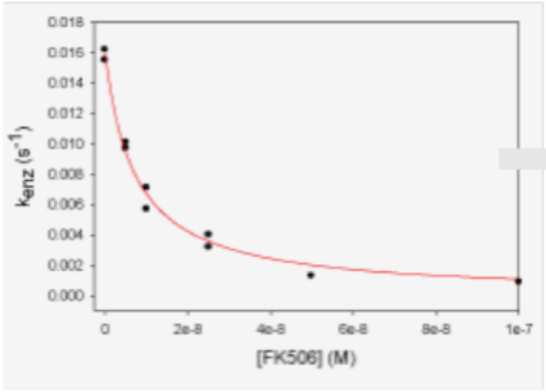
lab days 3 and 4

+



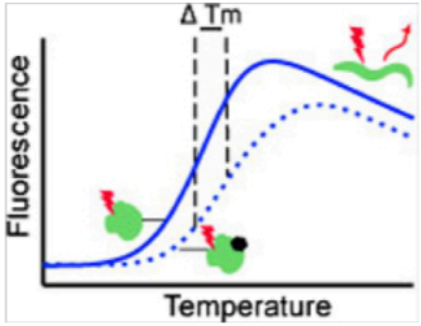
re-analyze screen data

lab day 2



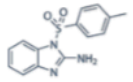
test FKBP12 and ligands in enzyme activity assay

lab day 5



test ligands in FKBP12 binding assay

lab day 6



IC_{50} = X μM

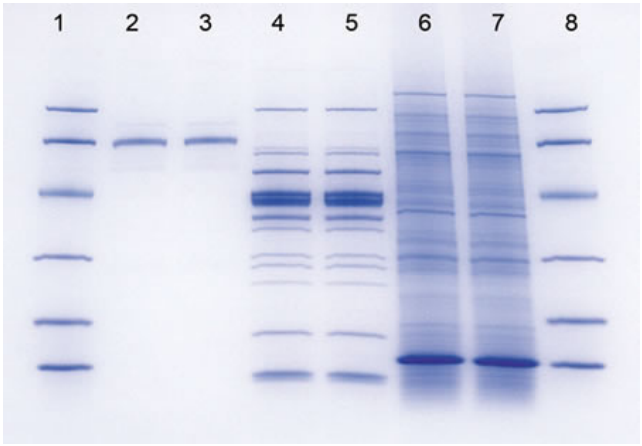
ΔT = Y °C

complete data analysis prioritize best ligands

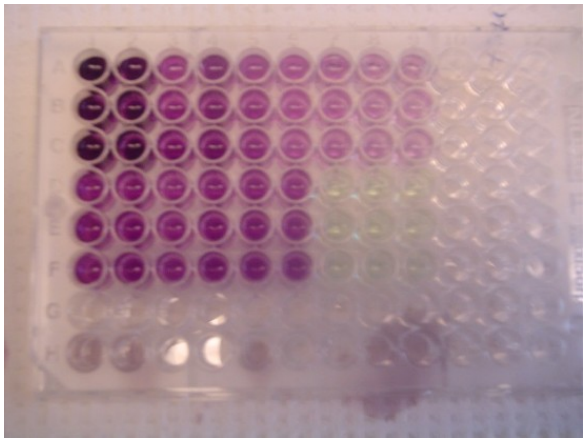
lab day 7

M1D4: Evaluate purity & concentration of FKBP12

Part 1 and Part 2: Desalt and concentrate purified FKBP12

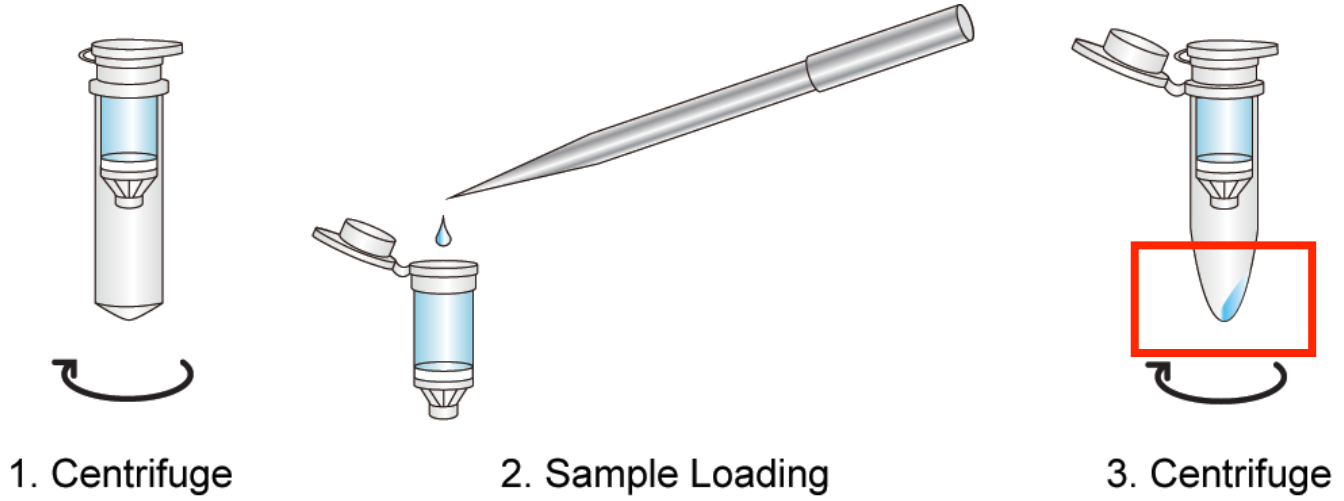


Part 3: Visualize purified protein with Coomassie



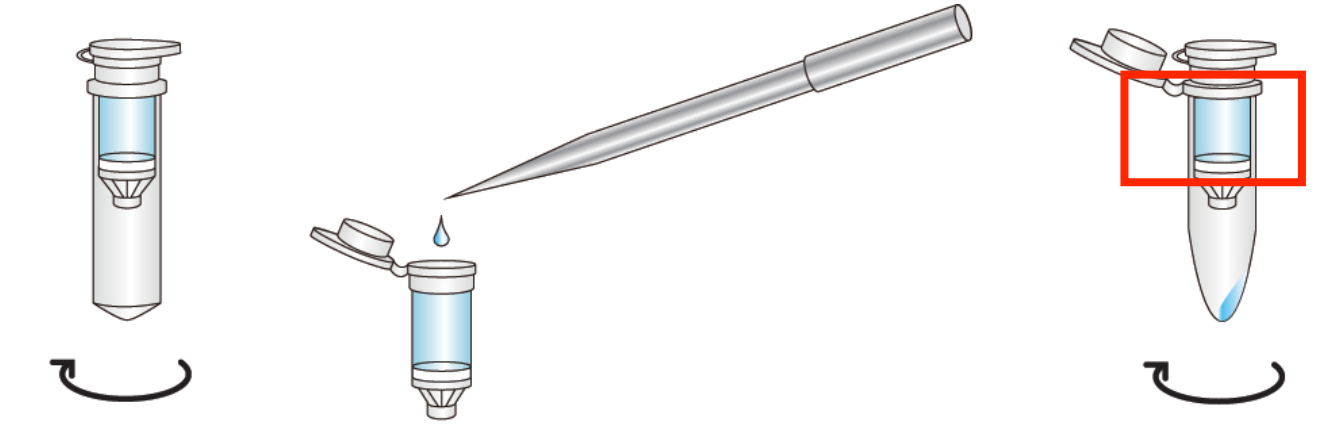
Part 4: Measure protein concentration (BCA)

Must remove imidazole, because it may interfere with secondary assays



1. Buffer exchange with PBS

Must remove imidazole, because it may interfere with secondary assays



5 Minute Purification

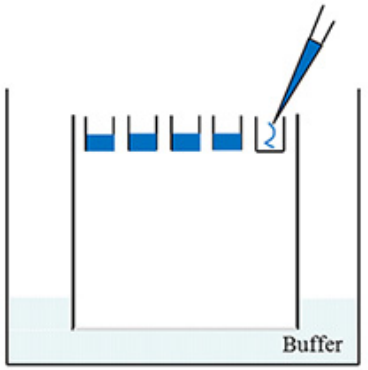
1. Buffer exchange with PBS
2. Concentrate protein

$$\text{Concentration factor} = \frac{\text{Starting volume}}{\text{Final volume}}$$

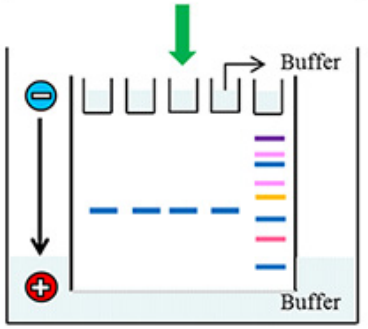
Assess purity of protein with Coomassie

1. Run SDS-PAGE
   - Separate proteins by MW
2. Stain gel with Coomassie
   - Visualize all proteins in sample

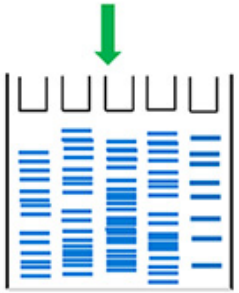
Assess purity of protein with Coomassie



Protein samples and marker loaded in vertical SDS-PAGE system

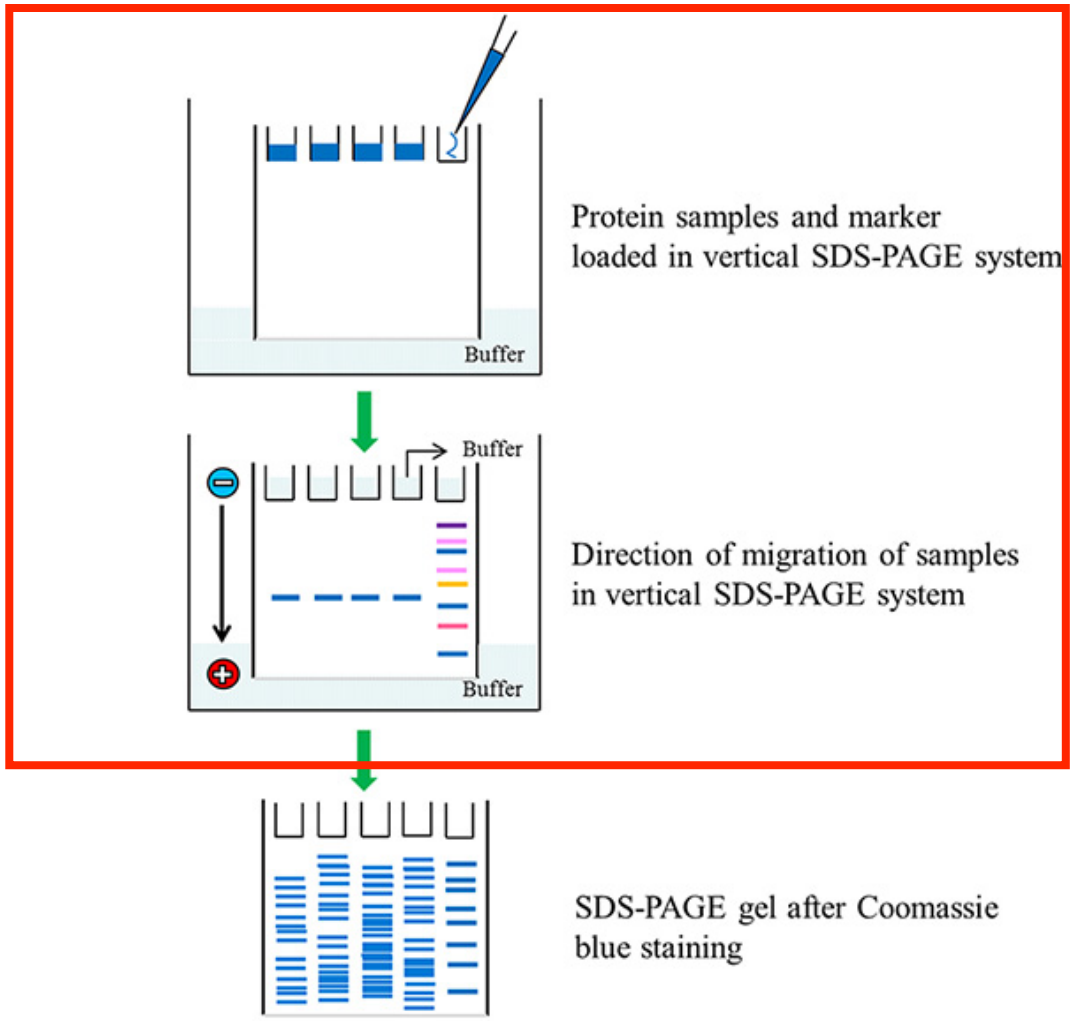


Direction of migration of samples in vertical SDS-PAGE system



SDS-PAGE gel after Coomassie blue staining

Assess purity of protein with Coomassie



Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
2. Boil sample mixed with loading dye
3. Load gel w/ladder and samples
4. Run gel 200V ~40 min

Separate proteins by MW using SDS-PAGE

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Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
    - SDS
    - β-mercaptoethanol
    - Bromophenol blue
    - glycerol

What do these reagents do to the sample?

Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
    - SDS – detergent used to denature proteins and coat protein is uniform negative charge
    - β-mercaptoethanol – breaks disulfide bonds
    - Bromophenol blue – visualize sample
    - Glycerol – weigh down sample and enable loading

Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
2. Boil sample mixed with loading dye

What does this do to the sample?

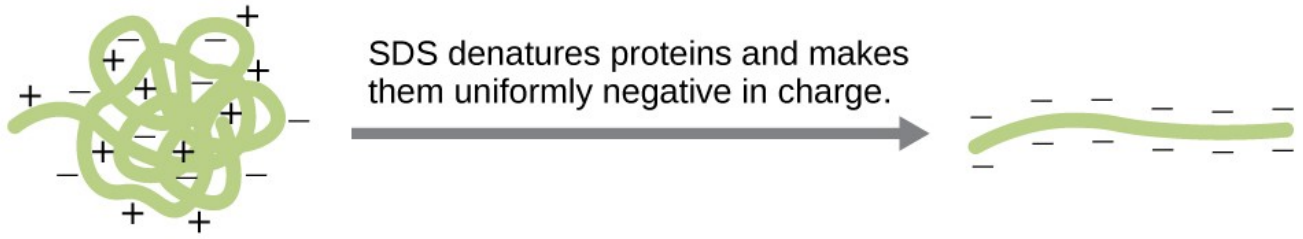
Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
2. Boil sample mixed with loading dye
   - Denatures higher order structures

Protein is now a linear, negatively charged molecule



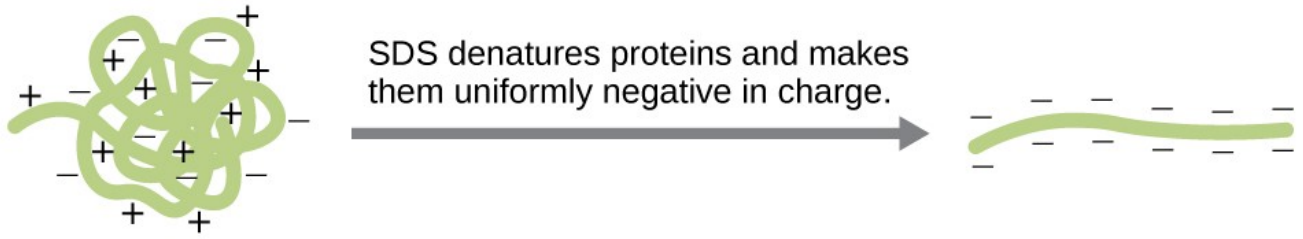
Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
2. Boil sample mixed with loading dye

What would happen if we just loaded the sample into the gel without denaturing or boiling it (like a DNA gel)?

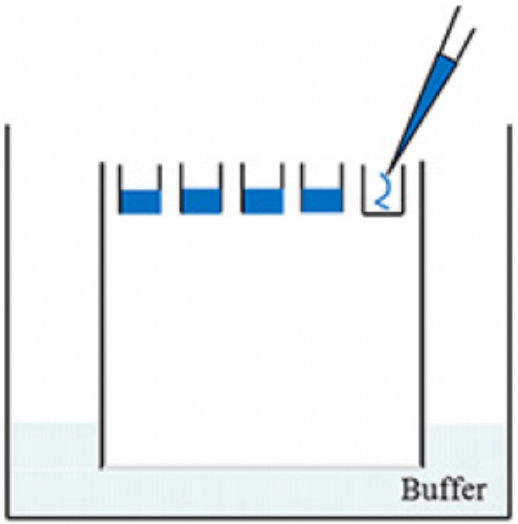


Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
2. Boil sample mixed with loading dye
3. Load gel w/ladder (x2) and sample (x8)
    - Ladder (one stained, one unstained)
    - Lysate (+/-IPTG)
    - Washes (x3)
    - Elutions (x2)
    - Purified protein



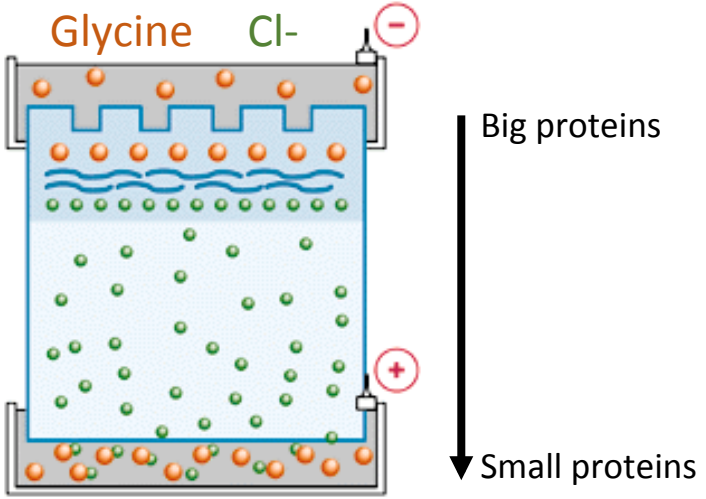
Separate proteins by MW using SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

1. Mix sample with Laemmli sample buffer / loading dye
2. Boil sample mixed with loading dye
3. Load gel w/ladder and samples
4. Run gel 200V ~40 min
    - Tris-HCl-Glycine-SDS (TGS) Running Buffer (pH 8.3)

↑ Polyacrylamide % ↓ Pore size



Separate proteins by MW using SDS-PAGE

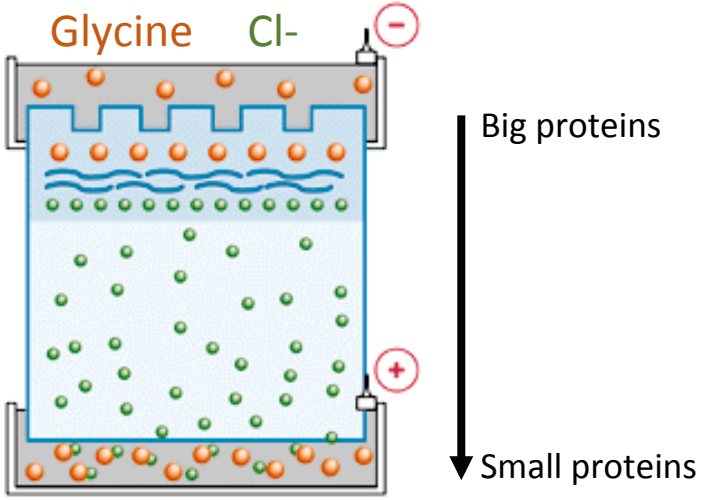
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Steps:

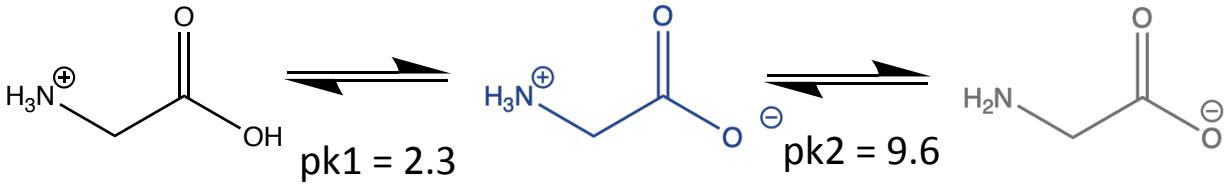
Why don't we just use agarose like a DNA gel?

3. Load gel w/ladder and samples
4. Run gel 200V ~40 min
   - Tris-HCl-Glycine-SDS (TGS) Running Buffer (pH 8.3)

↑ Polyacrylamide % ↓ Pore size



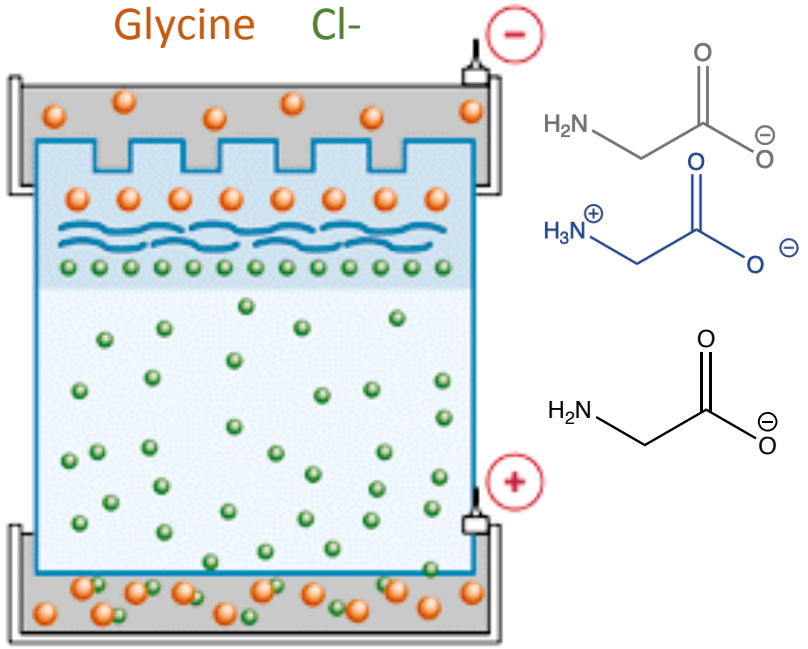
Separate proteins by MW using SDS-PAGE



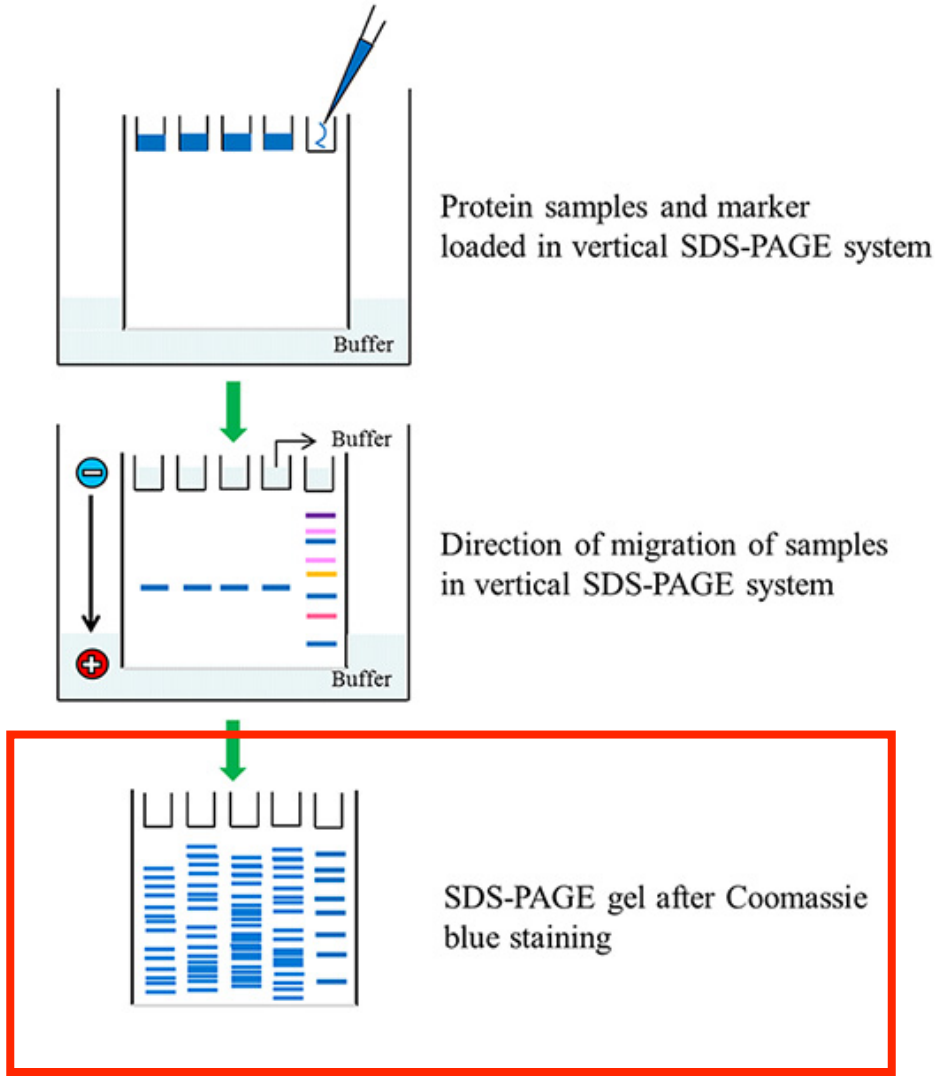
Tris-HCl-Glycine-SDS (TGS) Running Buffer (pH 8.3)

Stacking gel (pH 6.8)

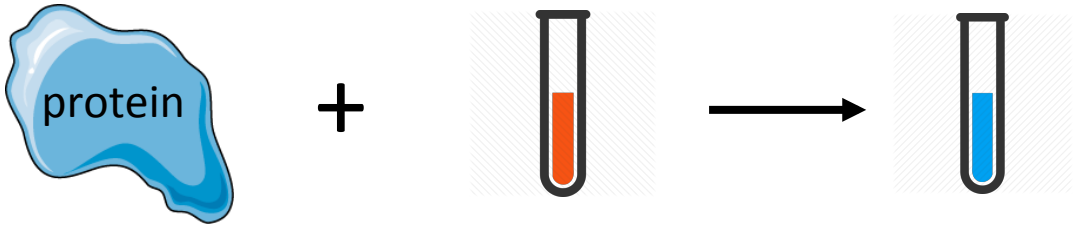
Running gel (pH 8.8)



Assess purity of protein with Coomassie



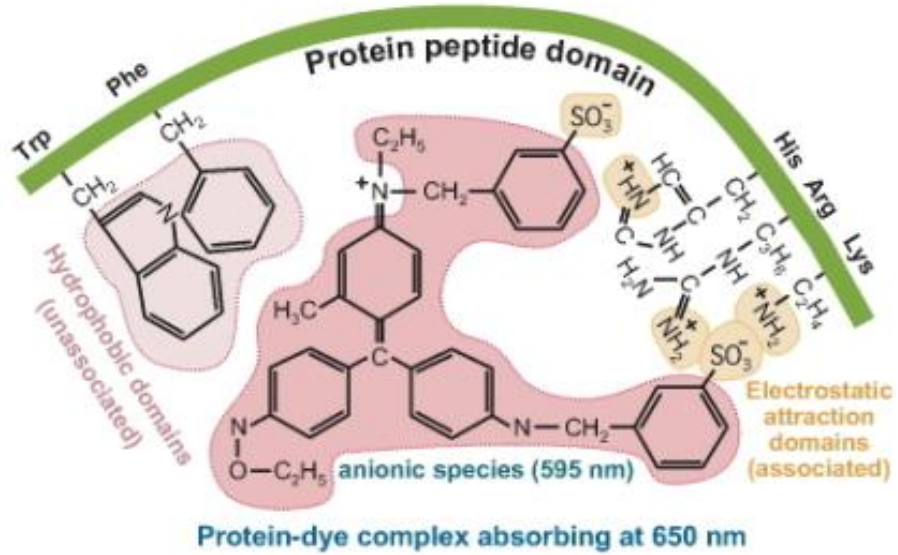
Visualize proteins using Coomassie colorimetric assay



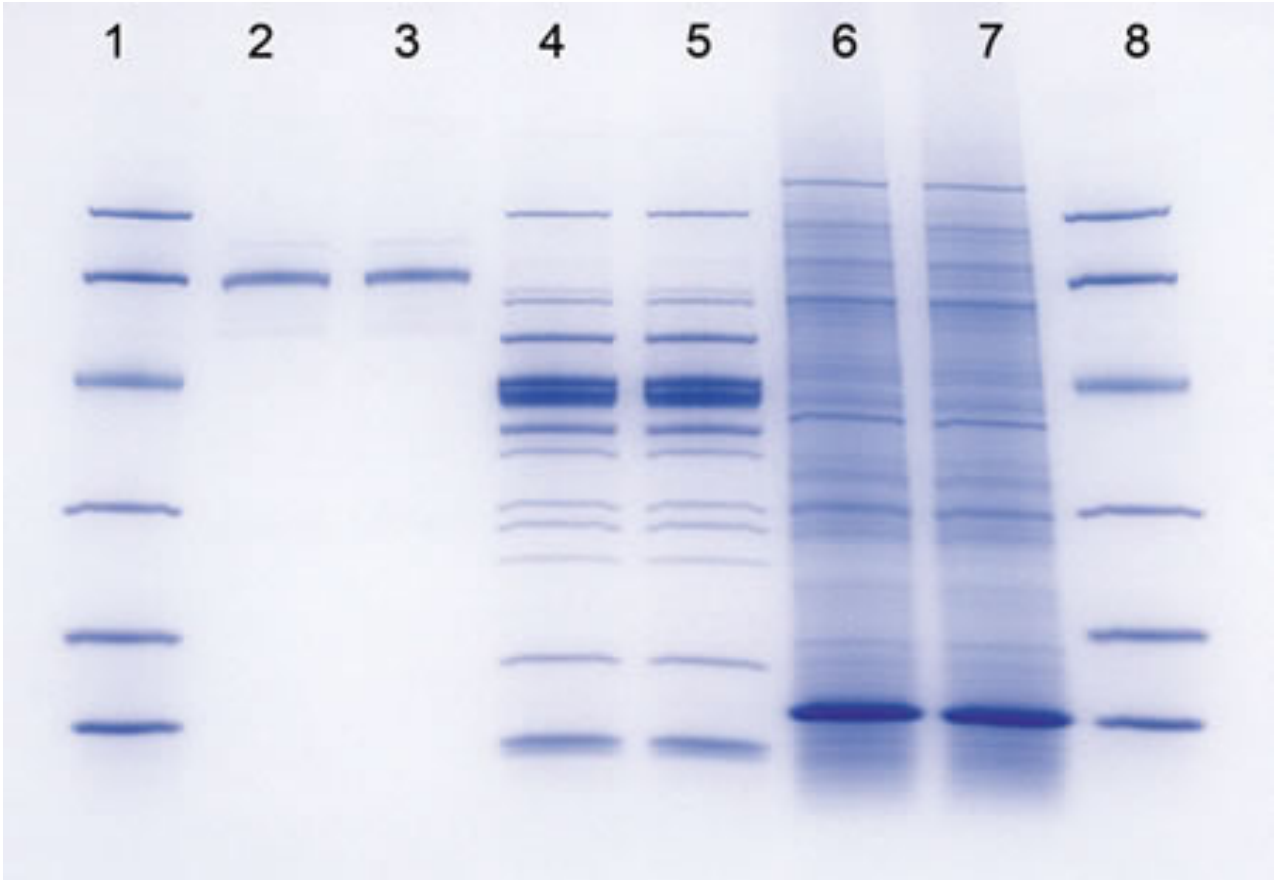
Basic and aromatic side chains

Coomassie G-250 (anionic)

Protein – Dye Complex (cationic)

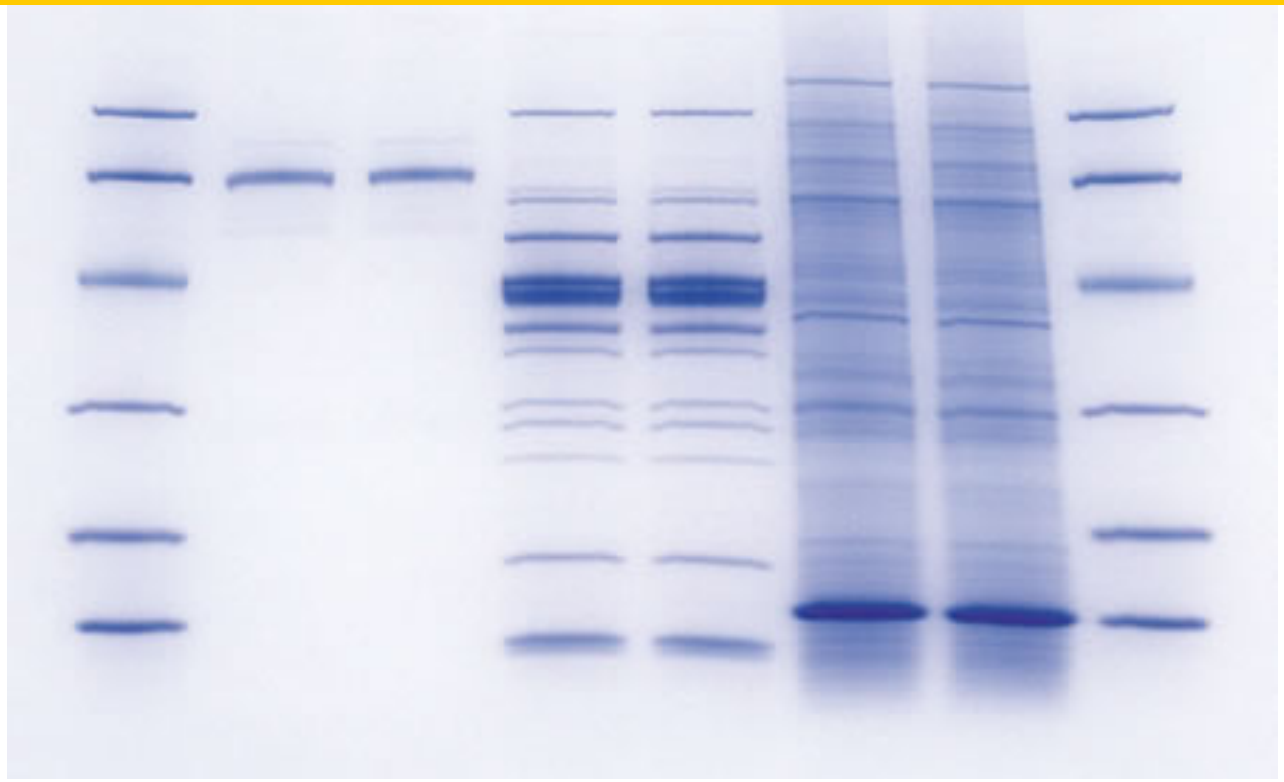


Visualize proteins using Coomassie colorimetric assay



Quantify protein with bicinchoninic acid assay (BCA)

What are you quantifying with a BCA?



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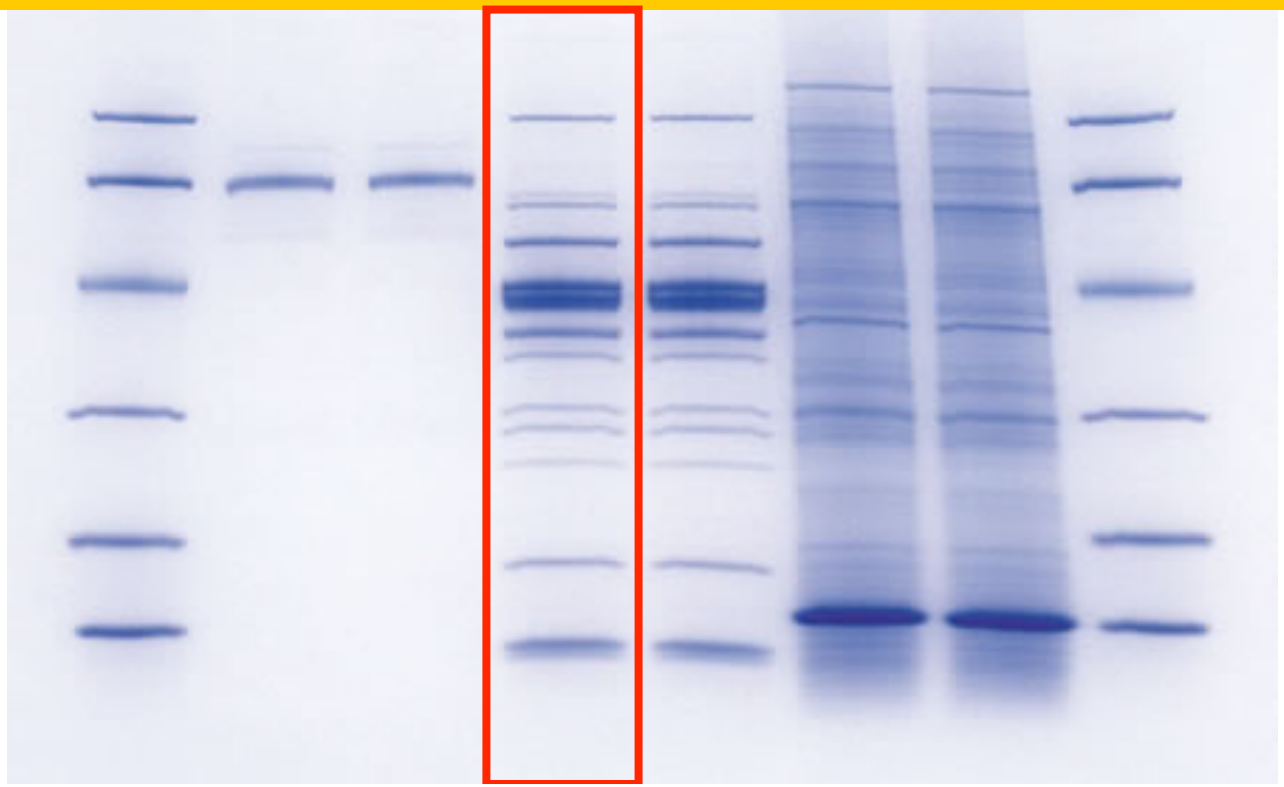
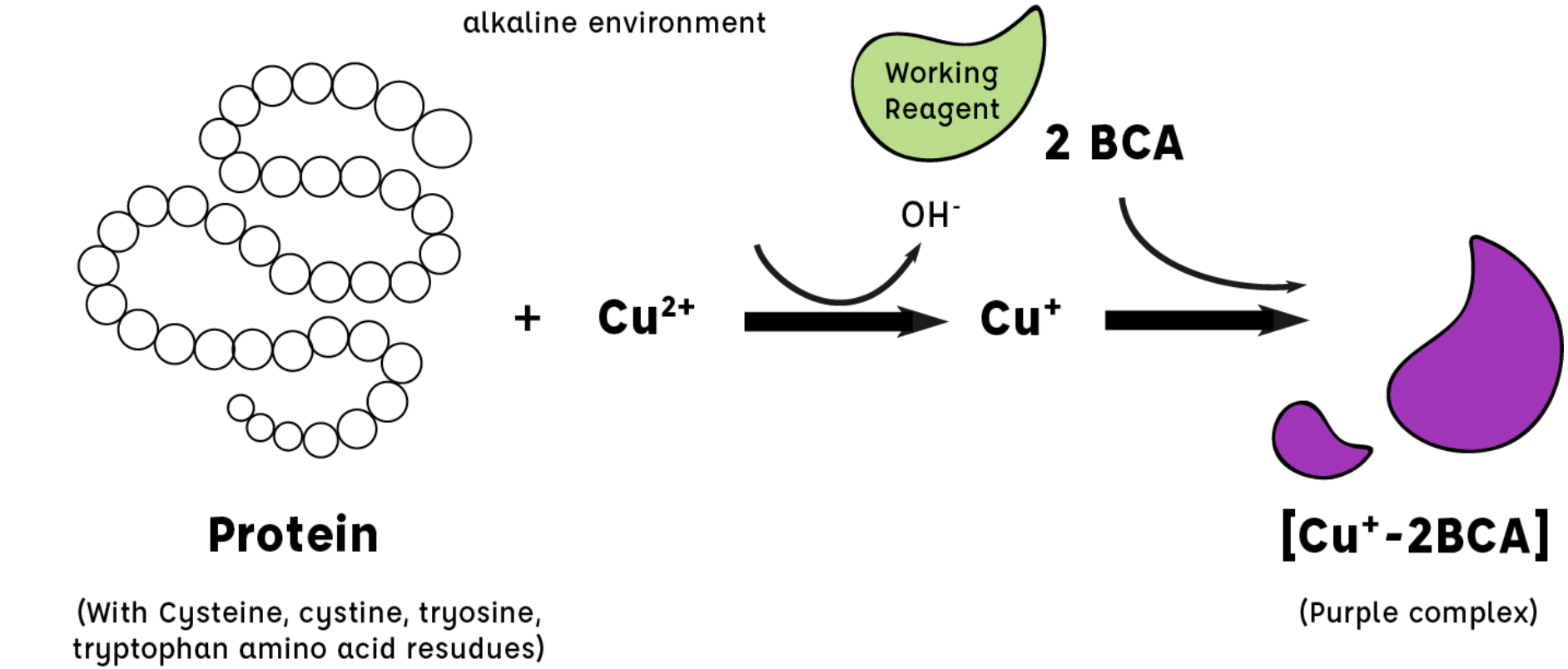
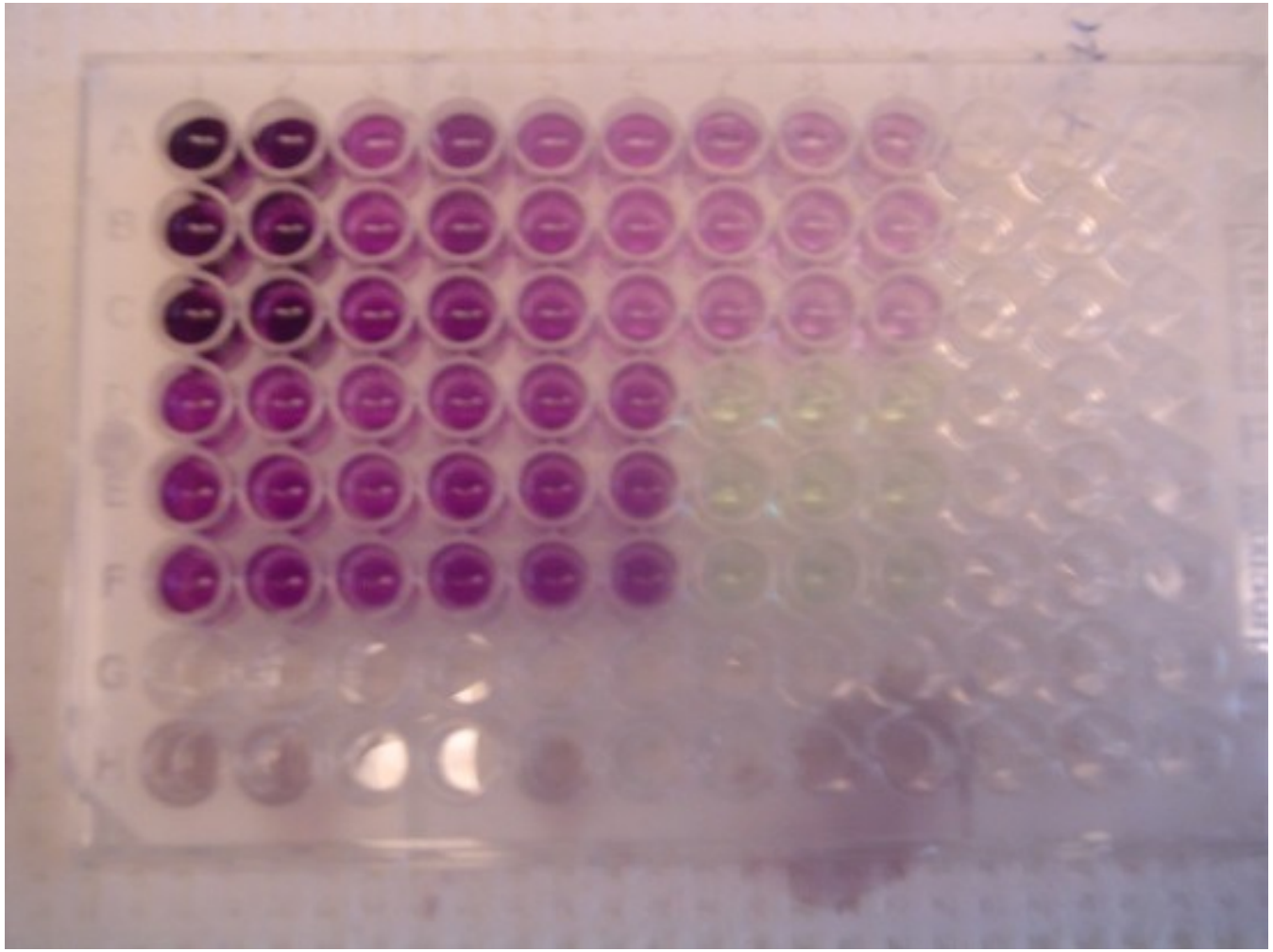


Quantify protein with bicinchoninic acid assay (BCA)

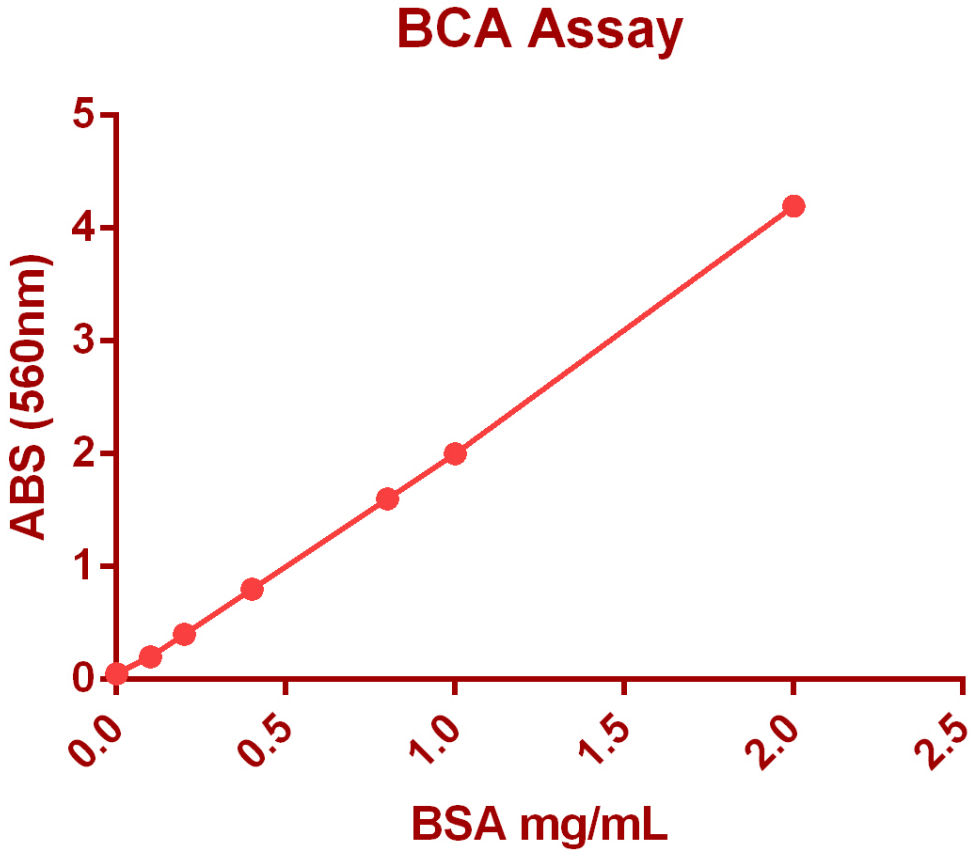


BCA/Copper complex absorbance at 562nm is linearly proportional with protein concentration

Quantify protein with bicinchoninic acid assay (BCA)



Quantify protein with bicinchoninic acid assay (BCA)

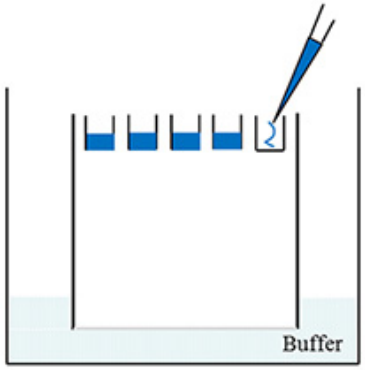


$y = 2{,}0809x - 0{,}0163$

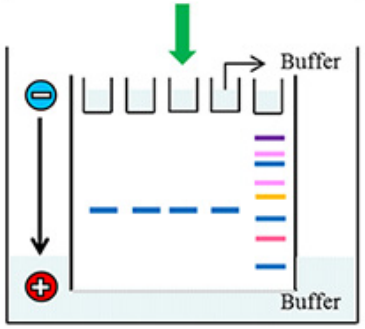
$R^2 = 0{,}9989$

Assess purity and concentration of FKBP12

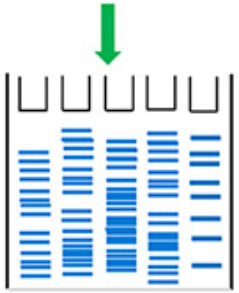
Assess **purity** of FKBP12



Protein samples and marker loaded in vertical SDS-PAGE system

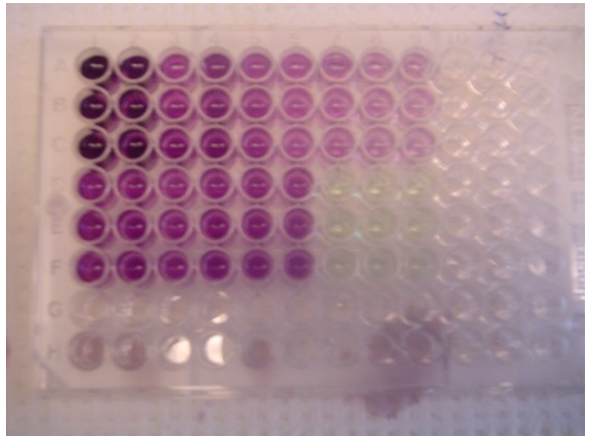


Direction of migration of samples in vertical SDS-PAGE system



SDS-PAGE gel after Coomassie blue staining

Quantify **Concentration** with BCA



M1D5 has a hefty homework assignment due—start on it today!

- Data figure, represents evaluation of purified protein
  - 1 figure = 1 message; Either SDS-PAGE *or* BCA graph
  - Remember title & caption
- Results subsection related to figure
  - Bullet point format—see homework description on wiki
  - The figure and results subsection should both fit on one page (standard 8.5x11, portrait on .pptx)
- Methods subsections (complete with your partners)
  - M1D3 protein purification
  - M1D4 evaluation of protien puritiy and concentration
  - Paragraph form, past tense
- Meet with BE fellow, submit a short summary about your meeting(1-2 paragraphs)

Tips for writing Methods:

- Include enough information to replicate the experiment
  - List manufacturers name and location (City, ST)
  - Be **concise and clear** in your description
- Use clear and concise full sentenes
  - NO tables and lists
  - Passive voice expected
- Use the most flexible units
  - Write concentrations (when known) rather than volumes
- Eliminate 20.109 specific details
  - Example “labeled Row A, Row B…”
  - Do not include details about tubes and water!
  - Assume reader has some biology experience

Methods: sub-sections

- Use sub-sections to group procedures
  - Include descriptive titles
  - Use logical, rather than chronological order (or according to the wiki)
- Separate sub-sections with titles
  - Brief, but specific
- Include an introductory sentence
  - State the purpose or goal of particular method / group of procedures

Methods: language choices

- the protein vs. **FKBP12**
  - Give your products names
- combined or mixed vs. **vortexed**
  - Be precise about the procedure used
- cleaned vs. **purified or isolated**
  - Use the more scientific terminology
- **avoid jargon and define all abbreviations**

Consider sentence structure—
passive voice is often expected in Methods

"FKBP12 was purified using…"

"… purity was examined using polyacrylamide gel electrophoresis (130 V for 30 min) then visualized with a … ."

1. PUT THE SUBJECT OF THE SENTENCE FIRST
2. BE SURE THE SUBJECT AND THE VERB MATCH

Remember the steps you didn't do

- For time reasons, the teaching faculty completed some procedures…
  - You should still include these!
  - Details are provided on the wiki

DO NOT WRITE THAT THESE STEPS WERE COMPLETED BY TEACHING FACULTY

What can you improve in this example?

"Template DNA and primers were mixed with

20 uL of 2.5X Master Mix in a PCR tube. Water

was added to 50 uL. A tube without template

was prepared and labeled control."

Be specific, what was your template? And from what will it be amplified? How much?

What is the sequence of the primers? And and what was the final concentration in the reaction?

"Template DNA and primers were mixed with

Give the final concentration, not the stock concentration.

This information can be assumed by your reader.

20 uL of 2.5X Master Mix in a PCR tube. Water

The volume is not important, just the concentration. Also, include manufacturer information for purchased reagents.

Your reader will know that reaction mixes are prepared in water.

was added to 50 uL. A tube without template

Because the final concentrations are reported, the final volume is not important.

was prepared and labeled control."

Though including a no template control is important, consider a more concise way to include this information by omitting unnecessary details.

Revised example…

"*FKBP100* was amplified from pcDNA3-FK (1 ng/uL) with primers pr1 (5' …AGA… 3') and pr2 (5' …CTC… 3'), each at 2 pmol/uL, using 1X Master Mix (5Prime, City, ST). A control with no template was included."