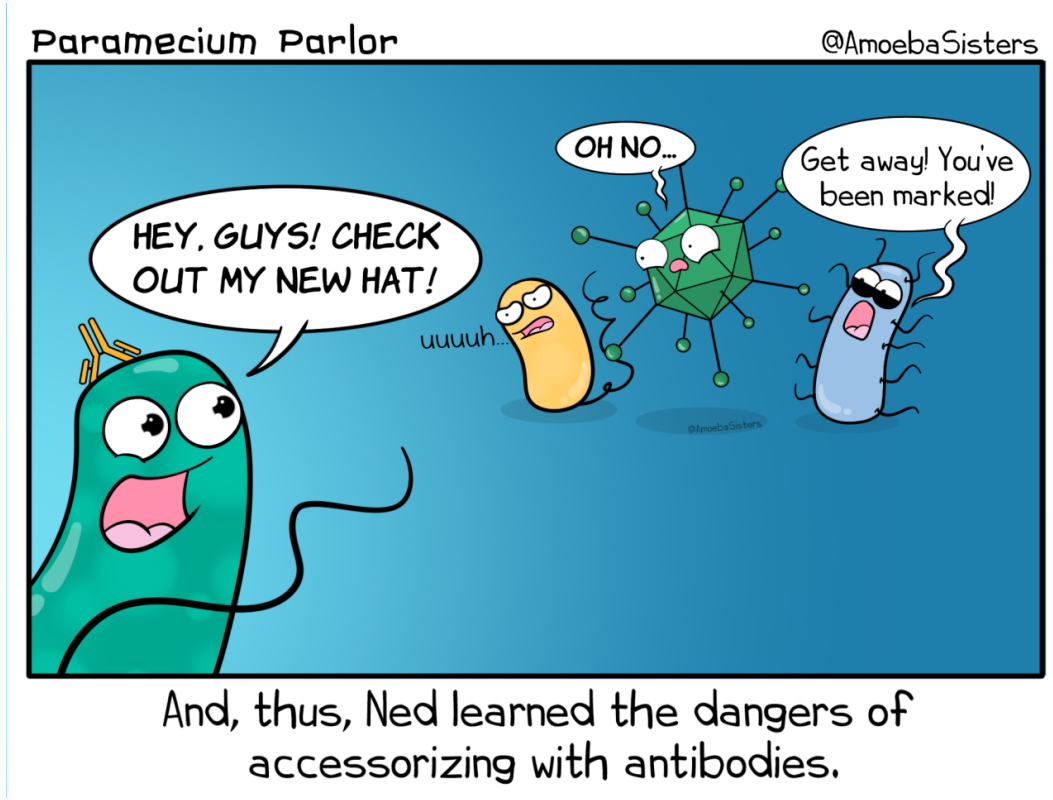
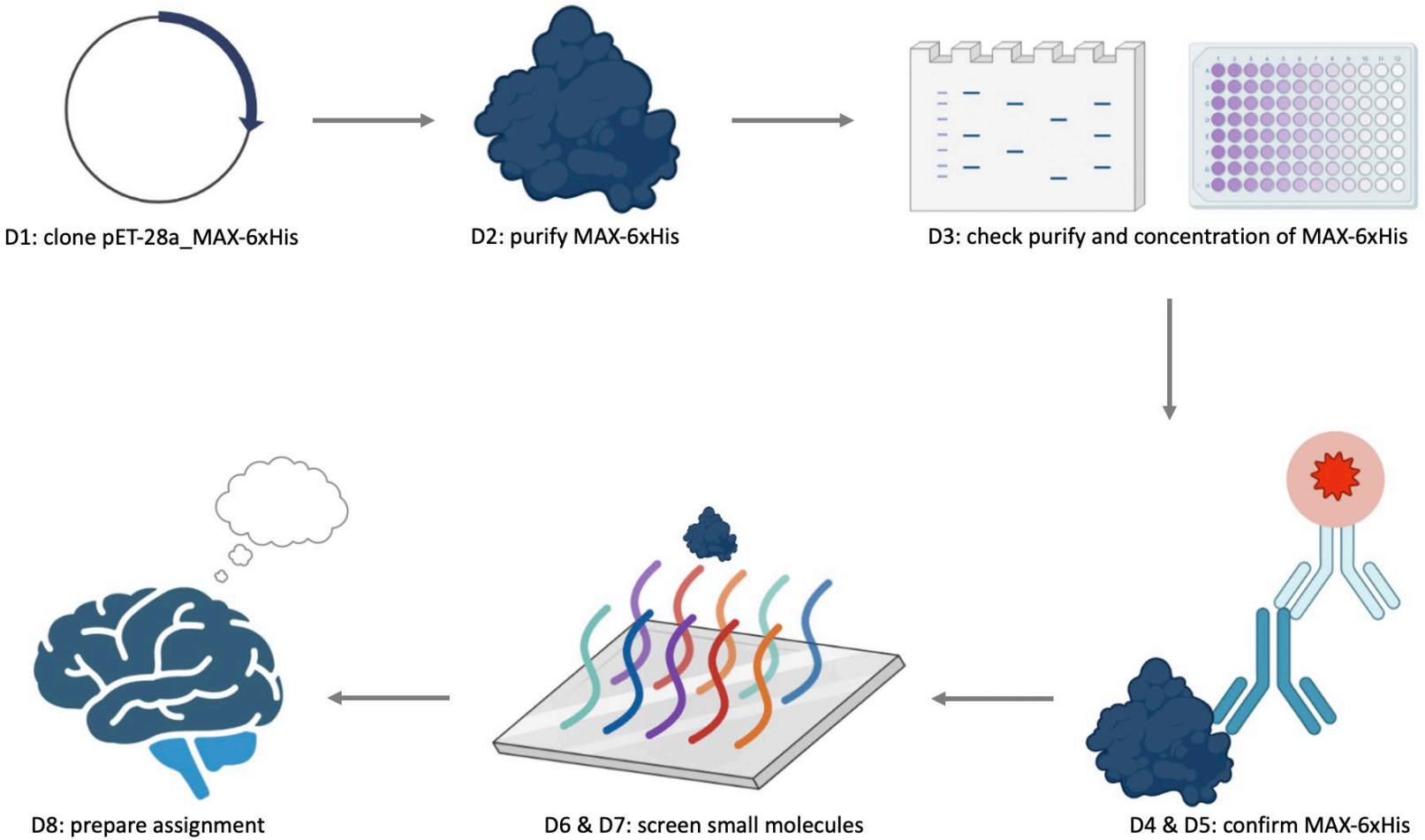
M1D5:
Image Western blot of purified protein

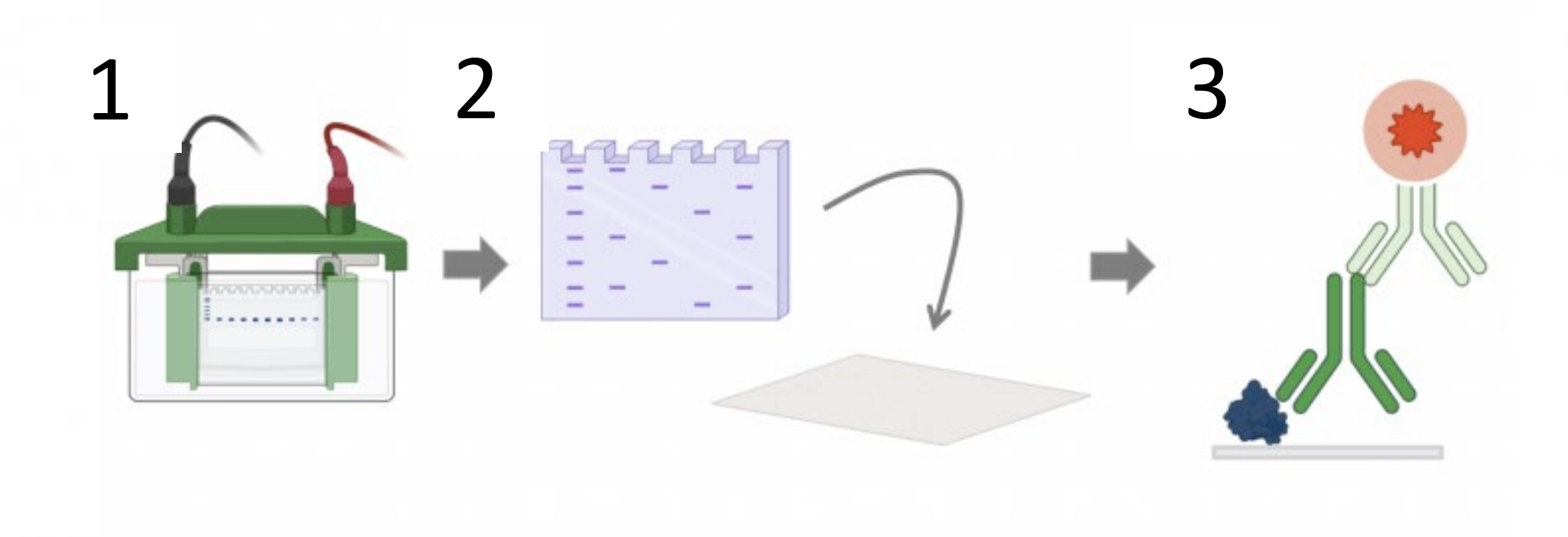
1. Prelab discussion
2. *Complete western blot experiment – moved to Thursday!*
3. Prepare Data summary slide



Overview of Mod 1 experiments:



Western blots probe for specific proteins



separate proteins using electrophoresis

transfer proteins onto nitrocellulose membrane

probe membrane using antibodies

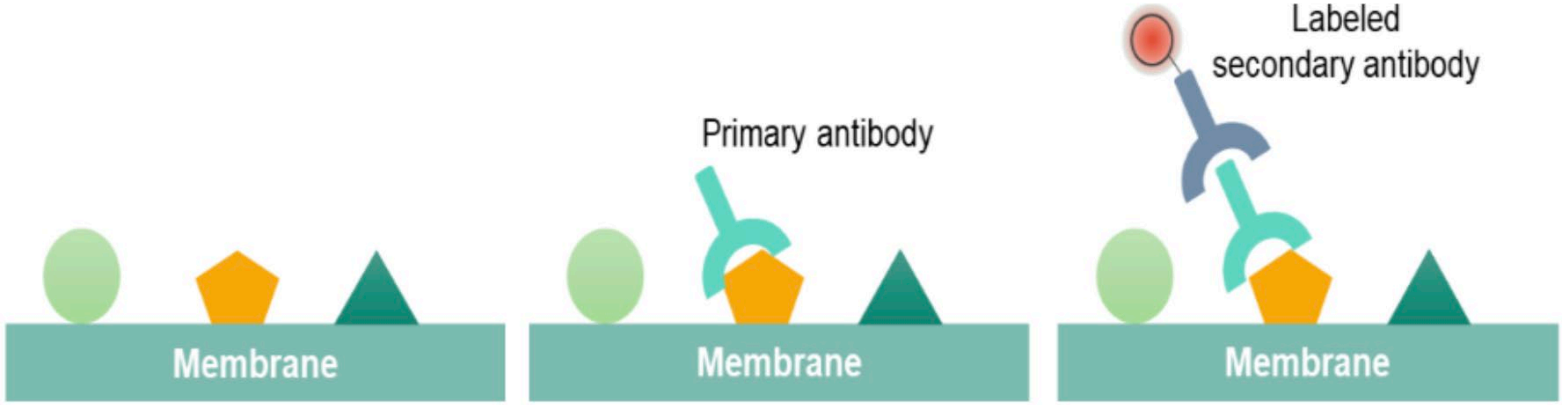
Let’s review the separation and transfer steps…

Step 1: SDS-PAGE used to separate proteins into distinct bands

Step 2: Protein bands from polyacrylamide gel transferred to a nitrocellulose membrane via applying a current

- Why is it necessary to transfer proteins onto a membrane?

Step 3: probe membrane using antibodies



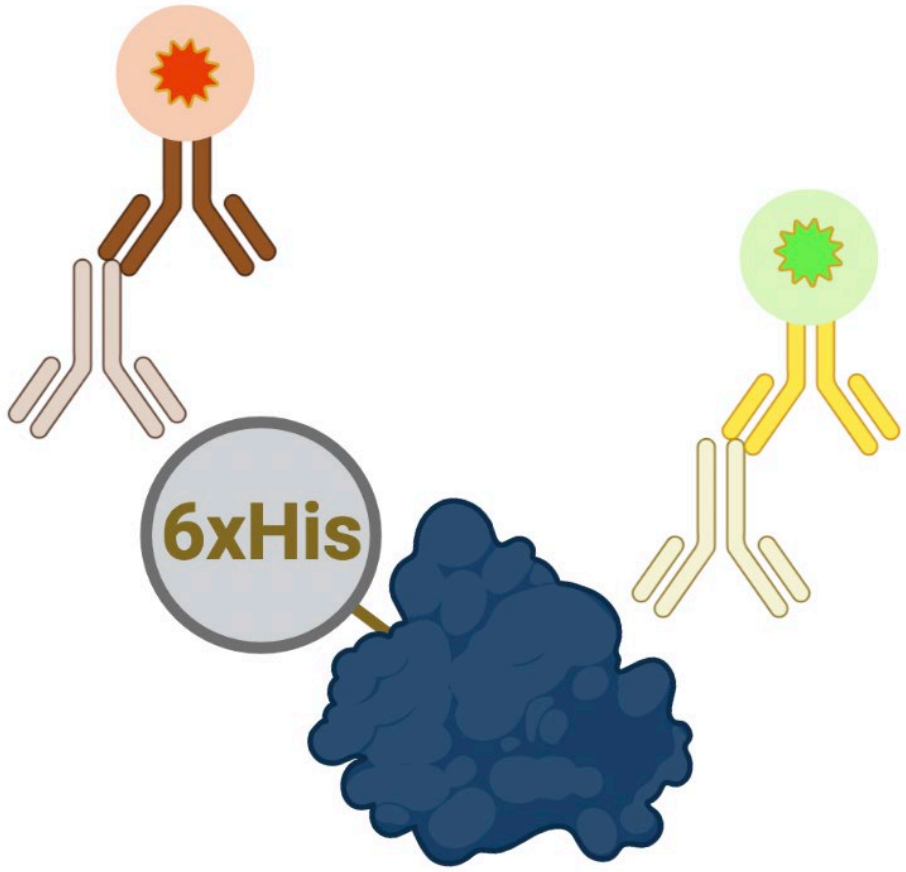
- Primary antibody raised against protein of interest to identify band that corresponds to specific protein on the blot

- Secondary antibody raised against the species of the primary antibody to visualize band that corresponds to specific protein of interest

What antibodies are we using?

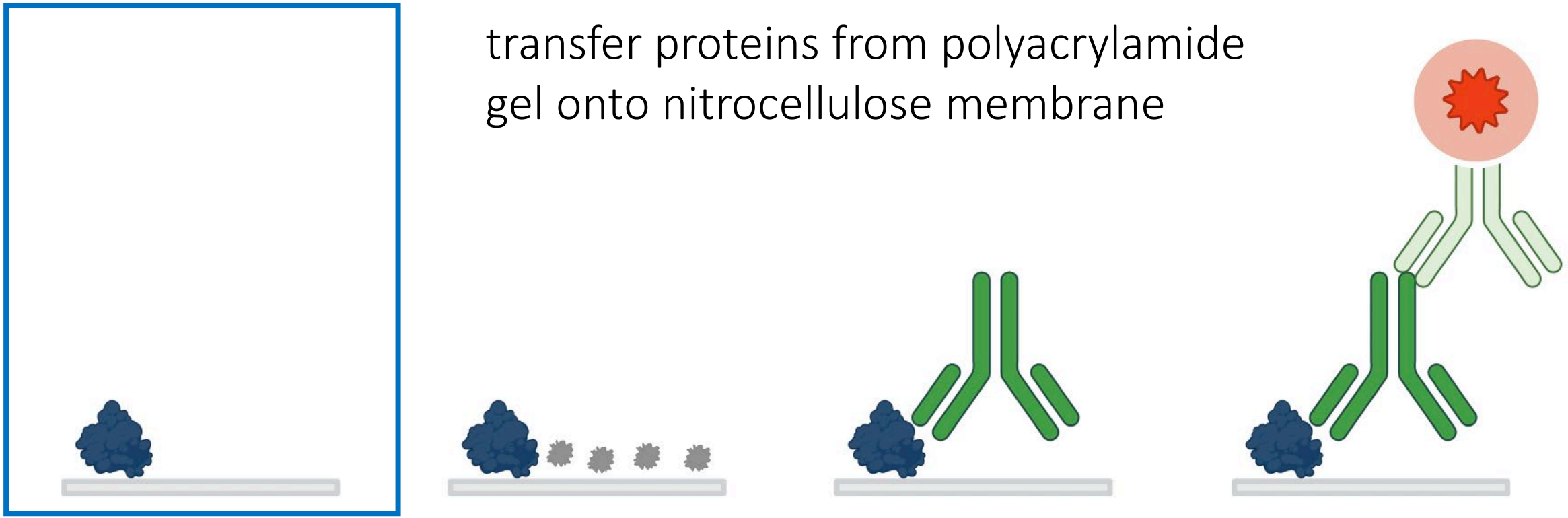
- To probe for MAX…
  - 1° = rabbit α-MAX
  - 2° = donkey α-rabbit IR680

- To probe for 6xHis tag…
  - 1° = mouse α-His
  - 2° = goat α-mouse IR800



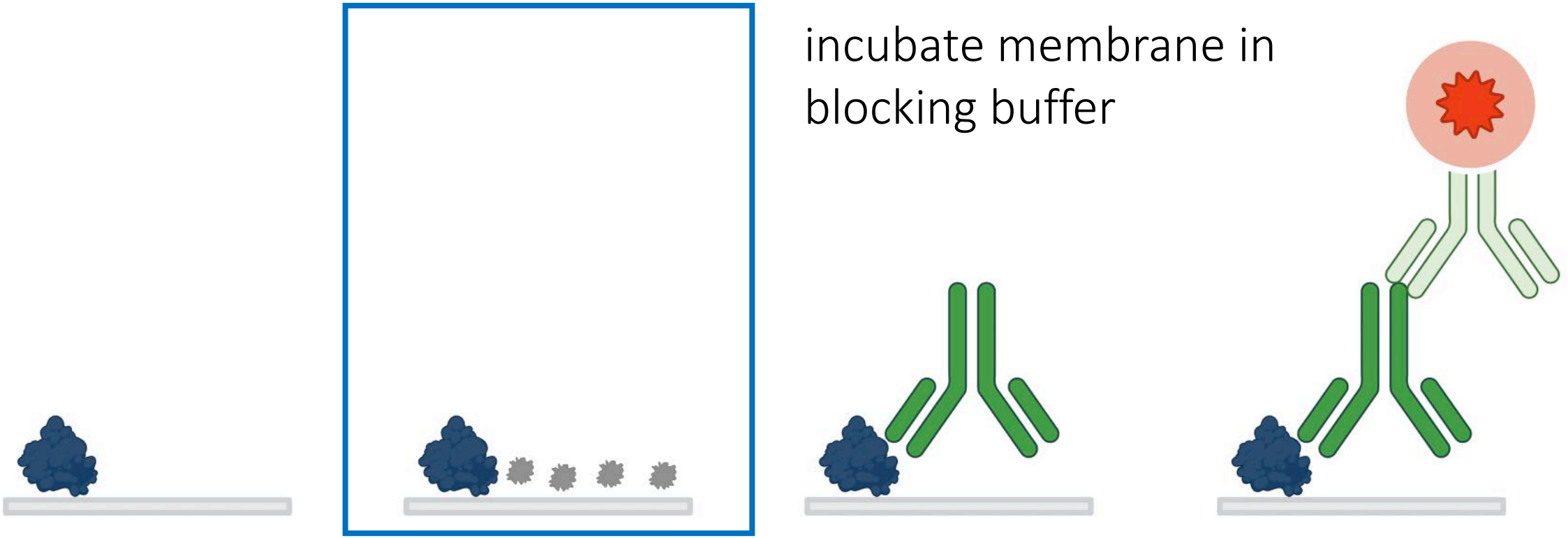
Antibodies used to detect specific protein

transfer proteins from polyacrylamide gel onto nitrocellulose membrane



- Which proteins are transferred to the membrane?

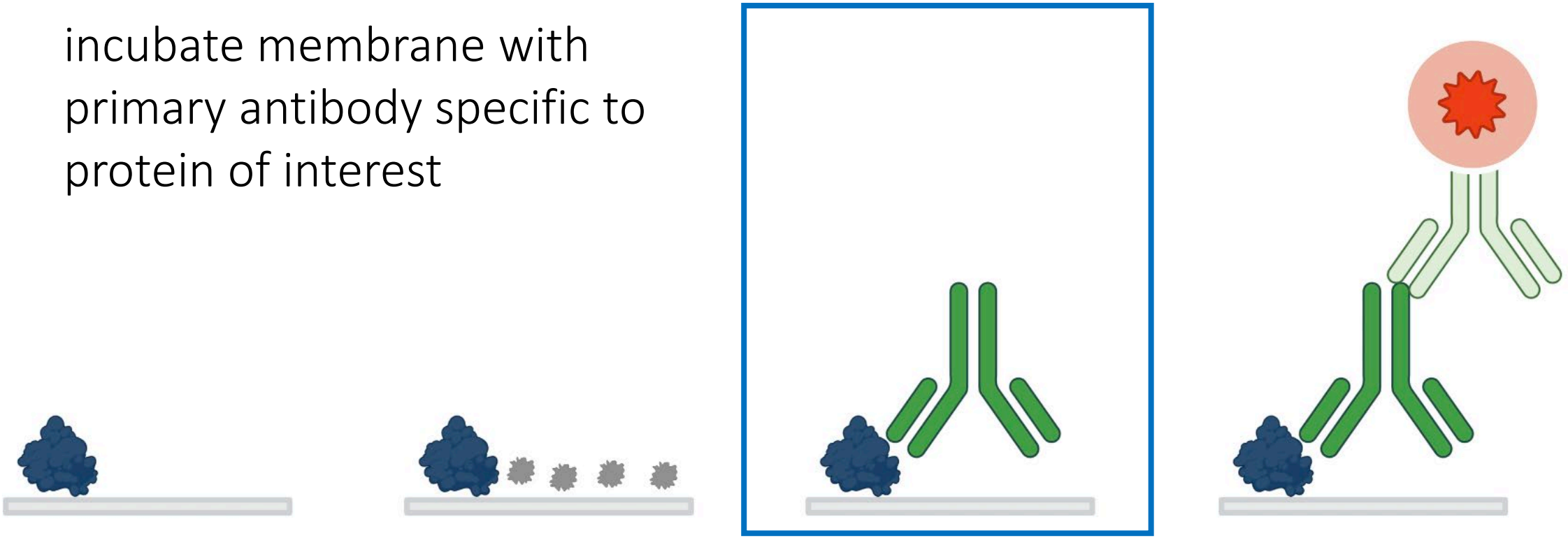
Antibodies used to detect specific protein



- How does blocking buffer prevent non-specific binding of antibodies?

Antibodies used to detect specific protein

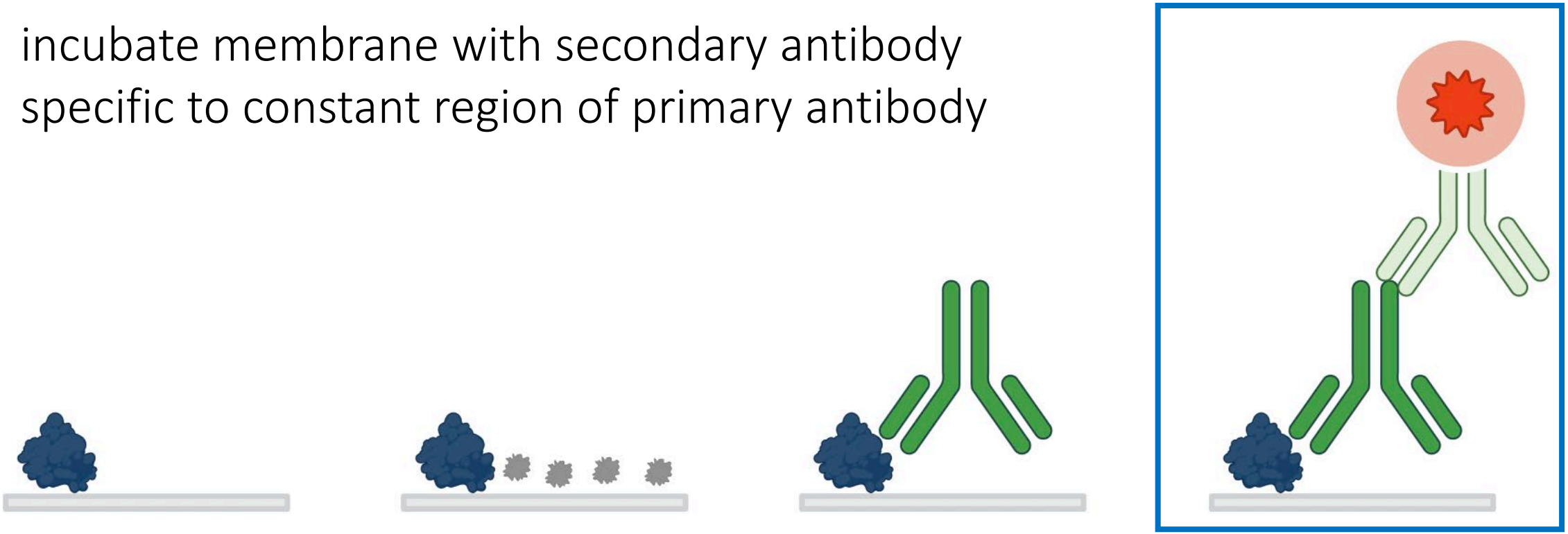
incubate membrane with primary antibody specific to protein of interest



- Why are we using two primary antibodies? For what does each probe?

Antibodies used to detect specific protein

incubate membrane with secondary antibody specific to constant region of primary antibody



- Why use secondary antibodies (rather than labeled primary antibodies)?

For today…

- Complete western blot staining & imaging
- During downtime, draft data summary slide for protein purity and concentration results with your partner
- Complete in-lab methods critique activity.

For M1D6…

- With your laboratory partner, draft methods section
- Submit write-up from meeting with Comm Lab fellow

Notes on methods section…

Include enough information to replicate the experiment

- Cite manufacturer for supplies / equipment (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in **logical order**, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in **full sentences** and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write **concentrations** (when known) rather than volumes

Eliminate 20.109 specific language and obvious details

- Example “labeled Row A, Row B…”
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say “completed by teaching faculty.”

How can you improve this example?

DNA was cut to check insert. Enzymes were used for single and double

digest then run on gel made by adding 1 g of agar to 100 mL of water.

Gel was imaged on a gel box.