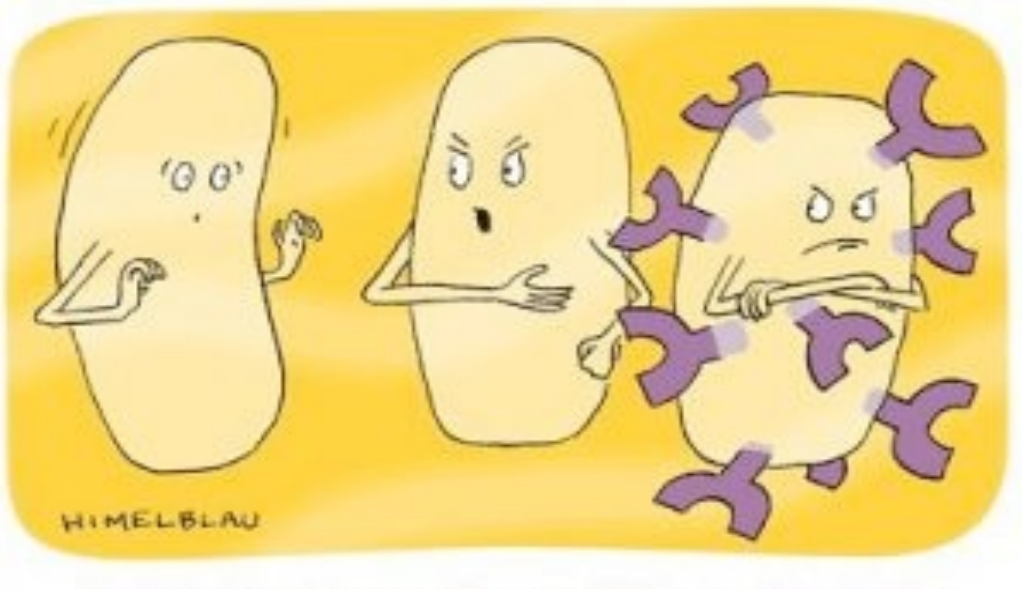
M2D2: Perform protein purification protocol

1. Prelab discussion
2. Protein purification
3. Assess RE digest



"Don't pick it up," I say, and he says, "It's just a *plasmid*, what harm could it do?" Well just look at him now…who knows *what* protein he's expressing!

Homework

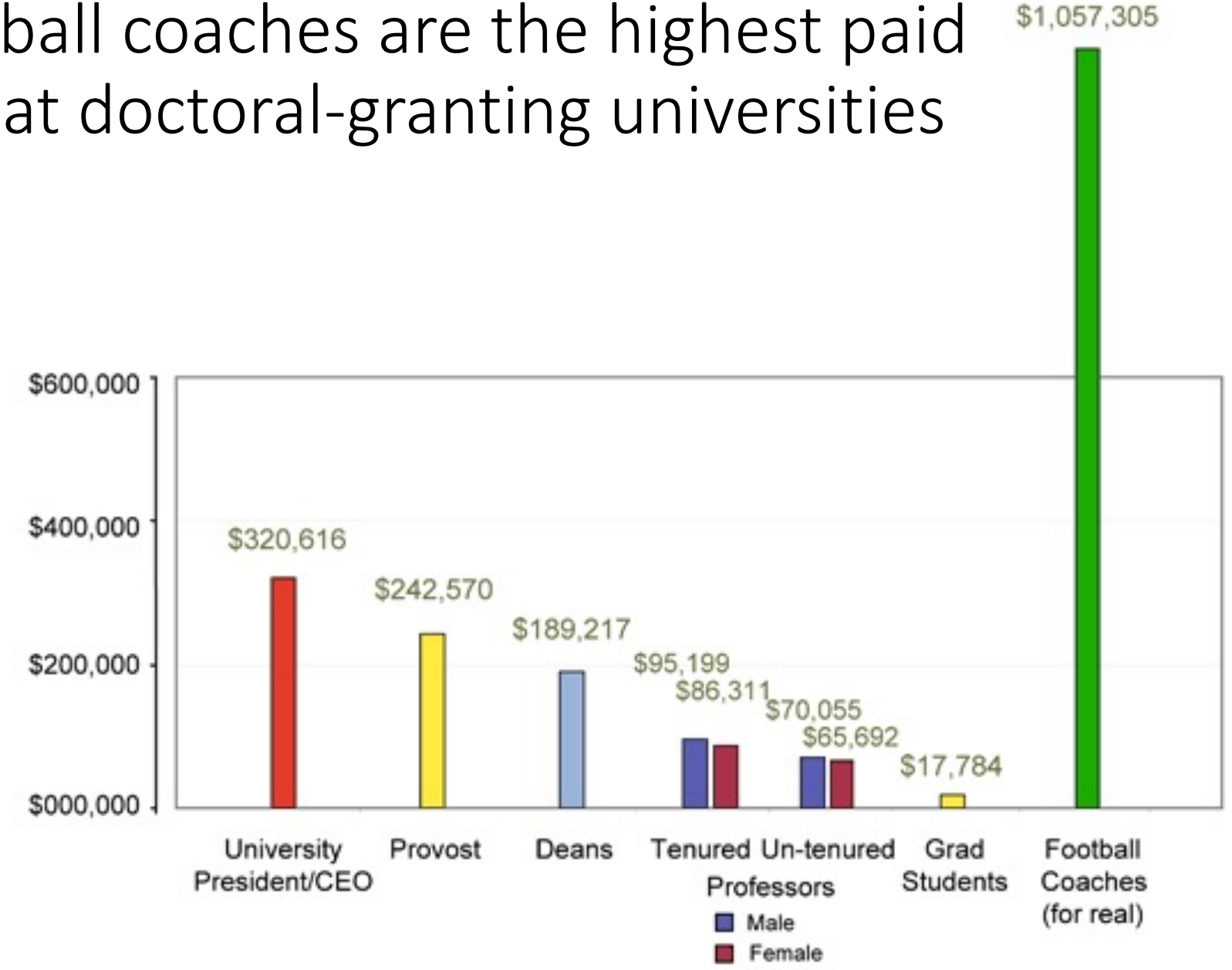
Crafting a slide for the Journal Article Presentation

Craft 1-2 slides using your journal article so you present key data from 1 figure

- Each slide should show a single message
- The title should state the take-home message of the data that are shown.
- Your slide(s) should show the data and highlight the key finding(s).
- The information should be clear and large enough to read.
- Keep text to a minimum. (NO figure captions on slide!)

EXAMPLE SLIDE: Football coaches are the highest paid academic employees at doctoral-granting universities

- Data represent expression of Y using method A
- Possibly something about the control(s), if applicable
- Important notes about the data and findings that are not already stated in the title
- Transition to next slide… (can also be done verbally)



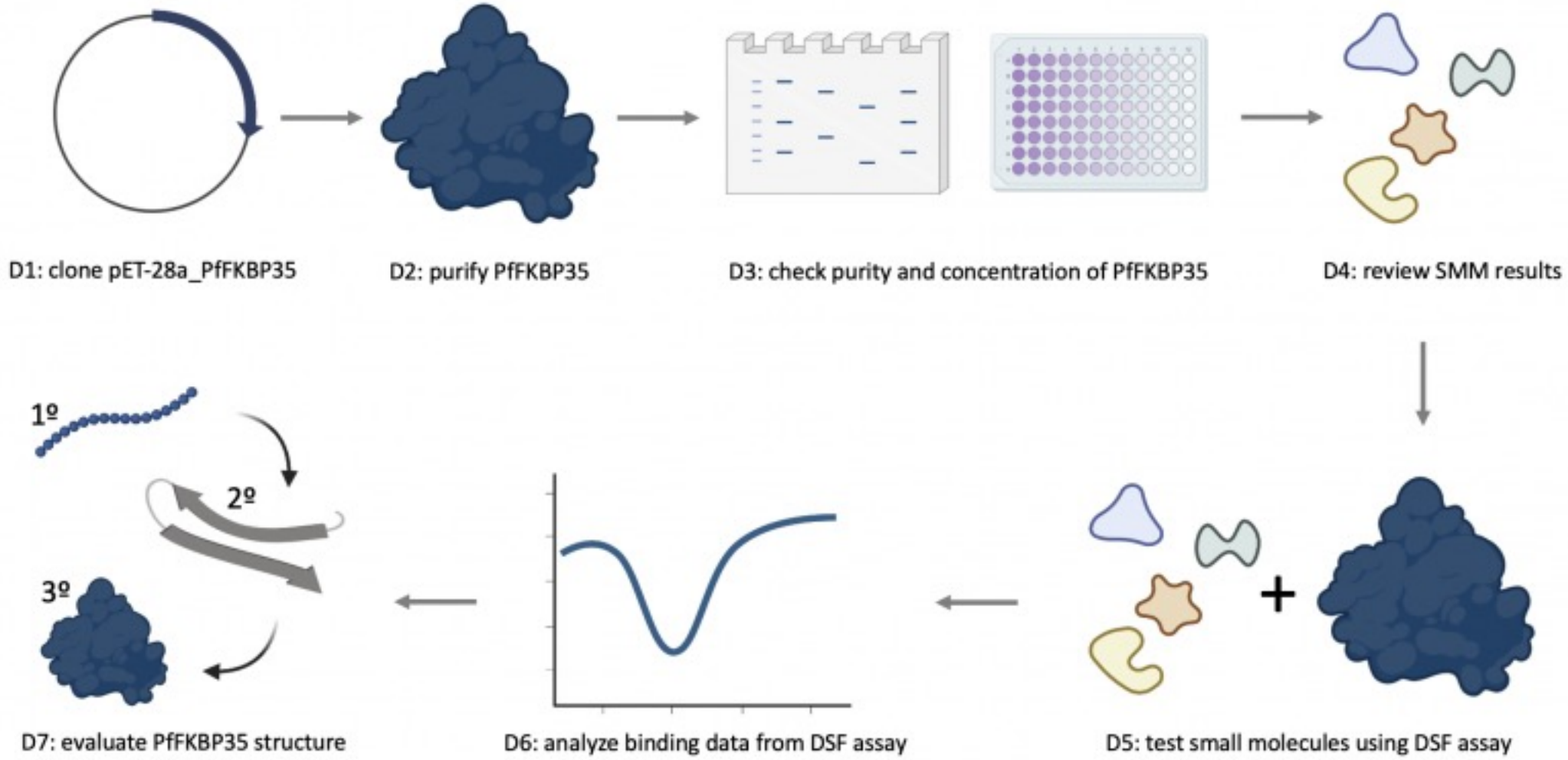
PhD Comics

Labwork

Purification of 6xHis-PfFKBP35

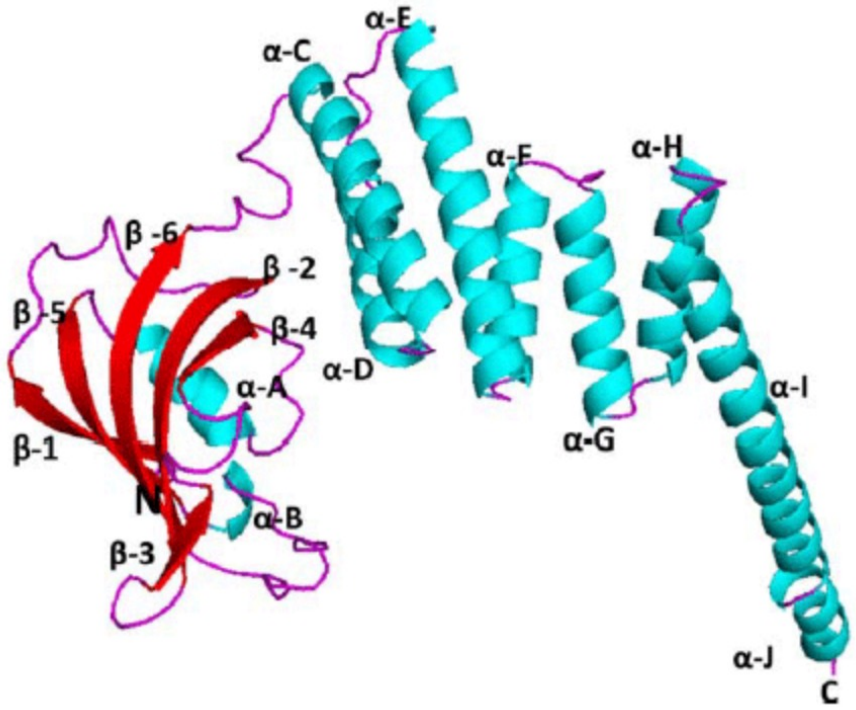
Overview of M2: drug discovery

Research goal: Test small molecules for binding to the *Plasmodium falciparum* FKBP35 protein using a functional assay.



PfFKBP35

- Pf = Plasmodium falciparum
- Essential to parasite survival
- Known drug target for:
  - Rapamycin
  - FK506
- Problem: Has a human ortholog in FKBP12
  - FKBP12 -/- is embryonic lethal
- Goal: a drug that targets the parasite protein and <u>not</u> the human protein



Native system in KRX *E. coli*

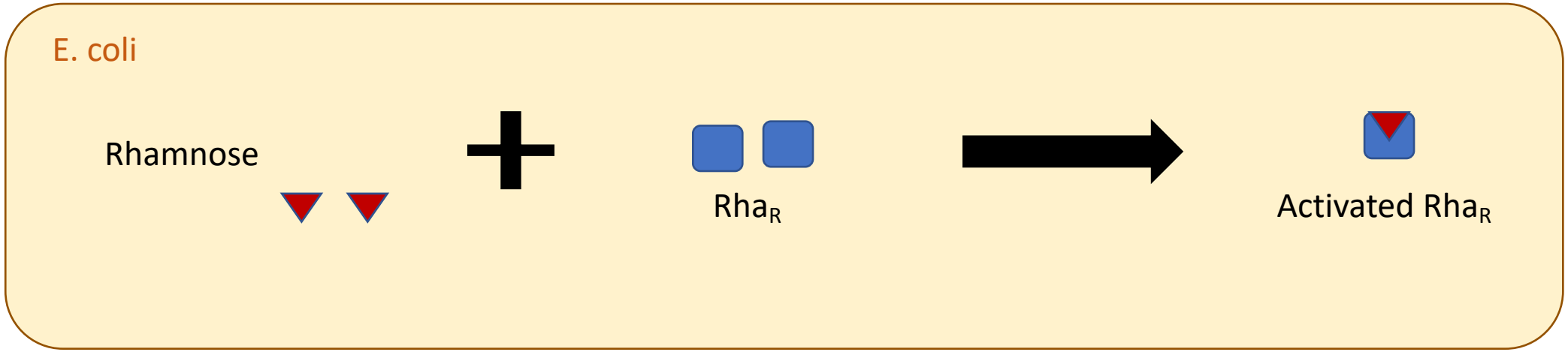
- Rhamnose = deoxy sugar
- *Rha* operon = *rhaR, rhaS, rhaB, rhaA, rhaD*
  - metabolizes rhamnose

- Rhamnose 

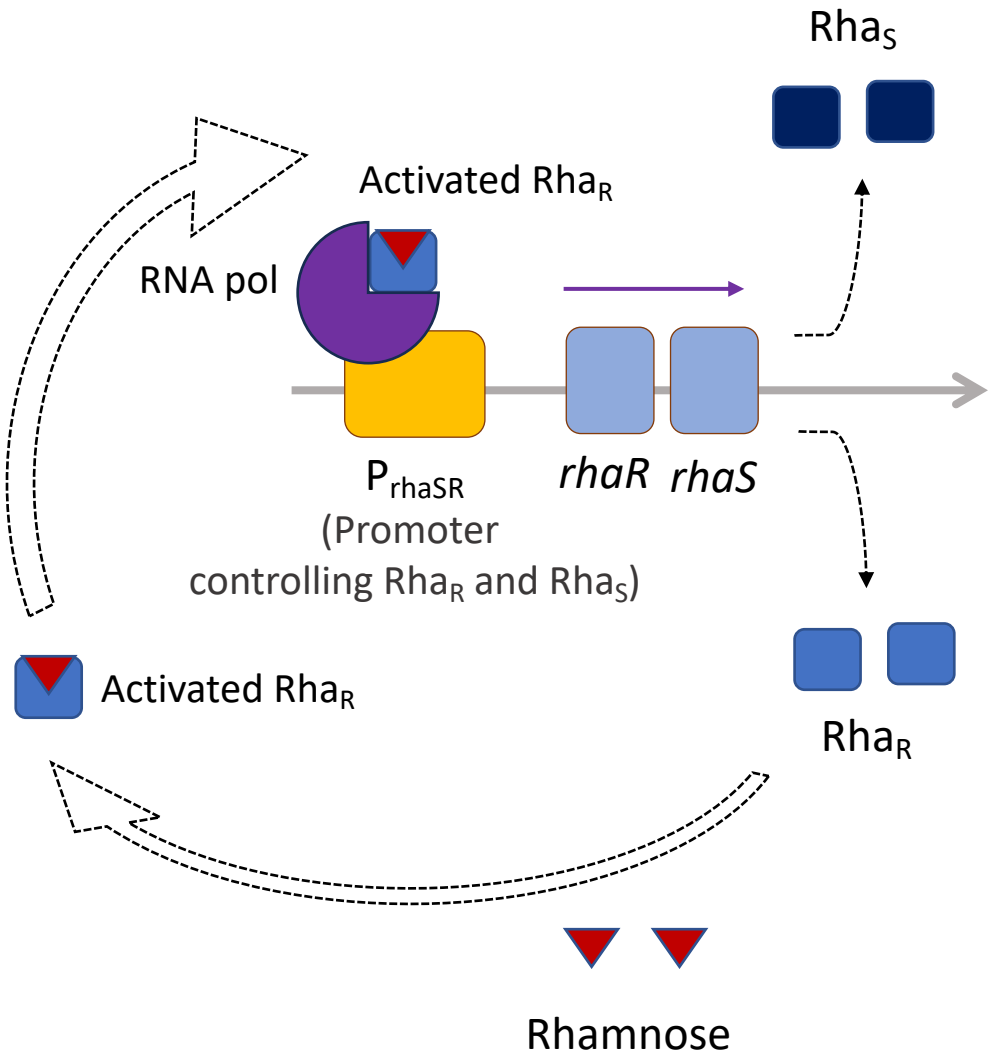
Rha_R + Rha_S 

Rha_{BAD} → rhamnose metabolism

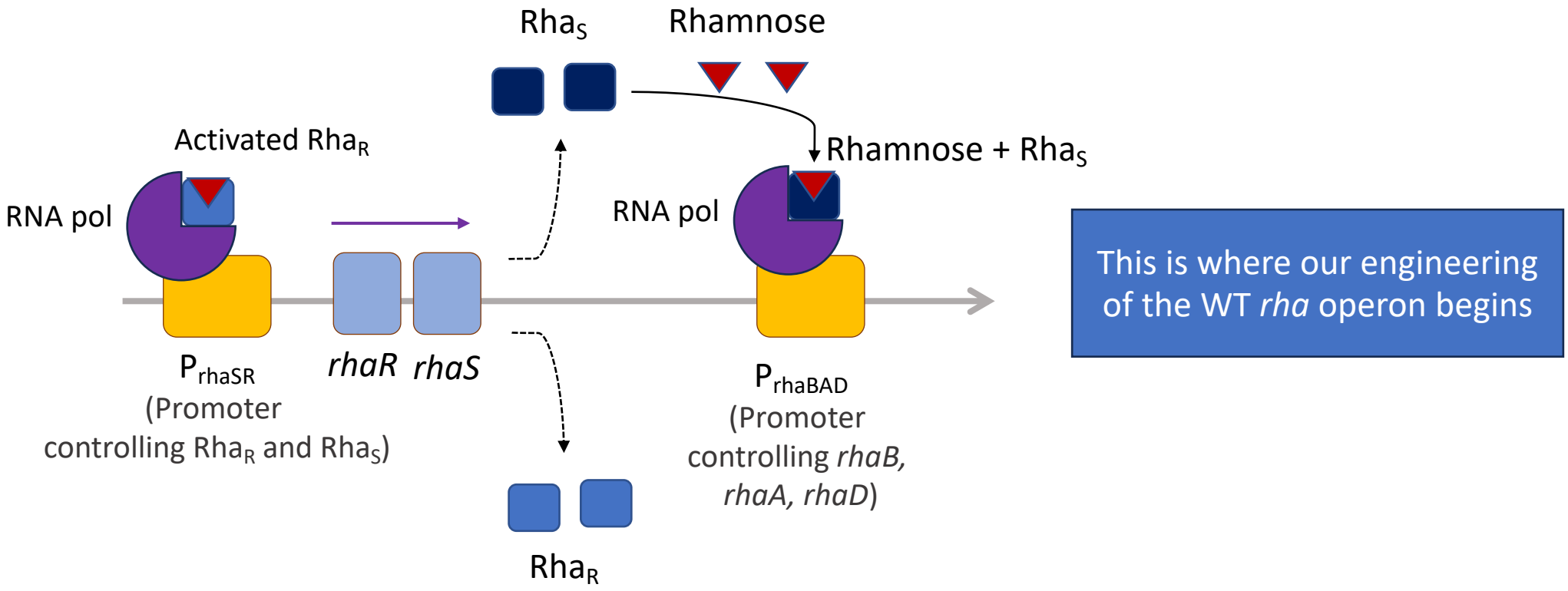
Bacterial induction: Rhamnose activates Rha_R



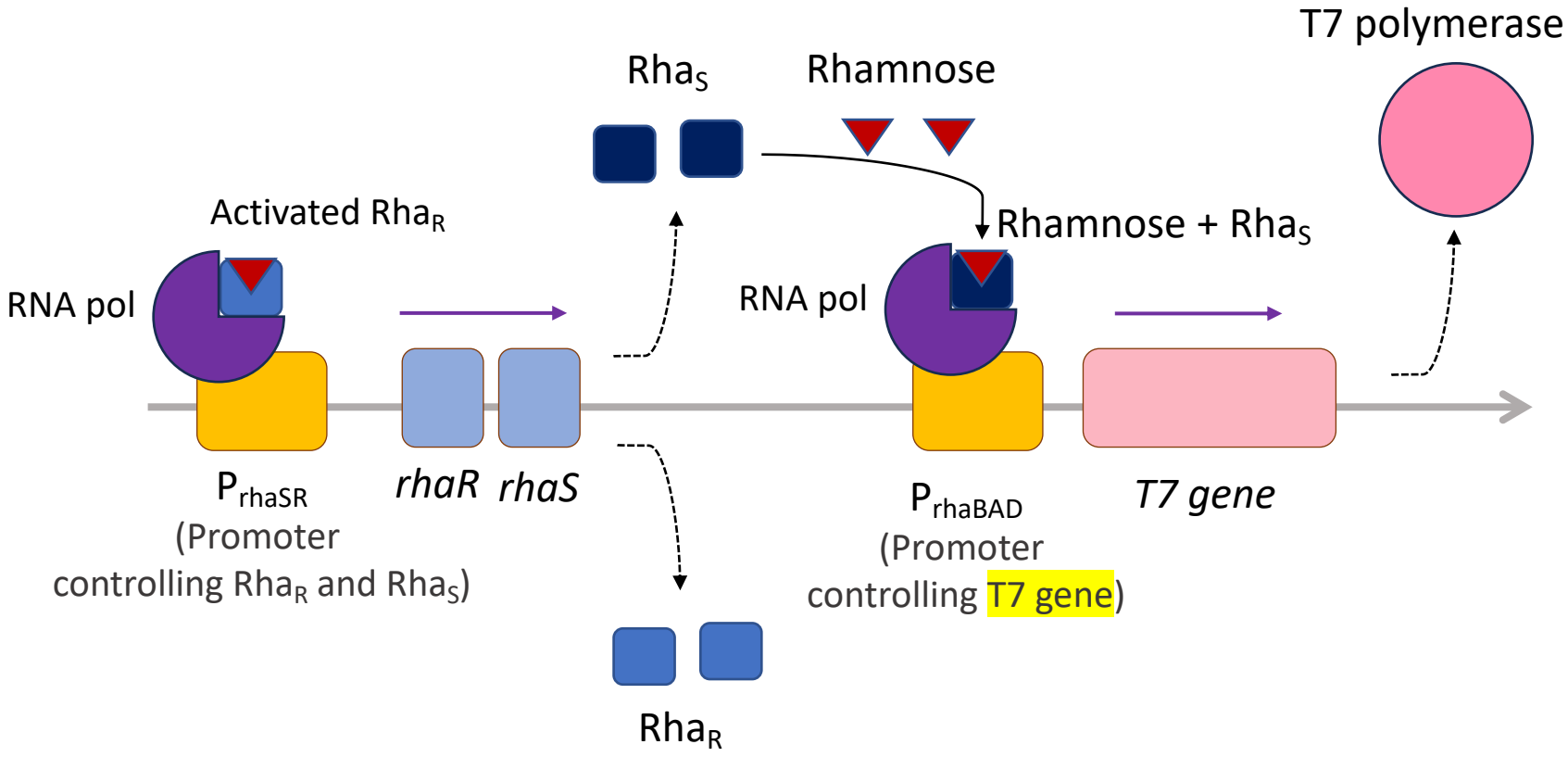
Bacterial induction: Rha_R activates production of Rha_R and Rha_S



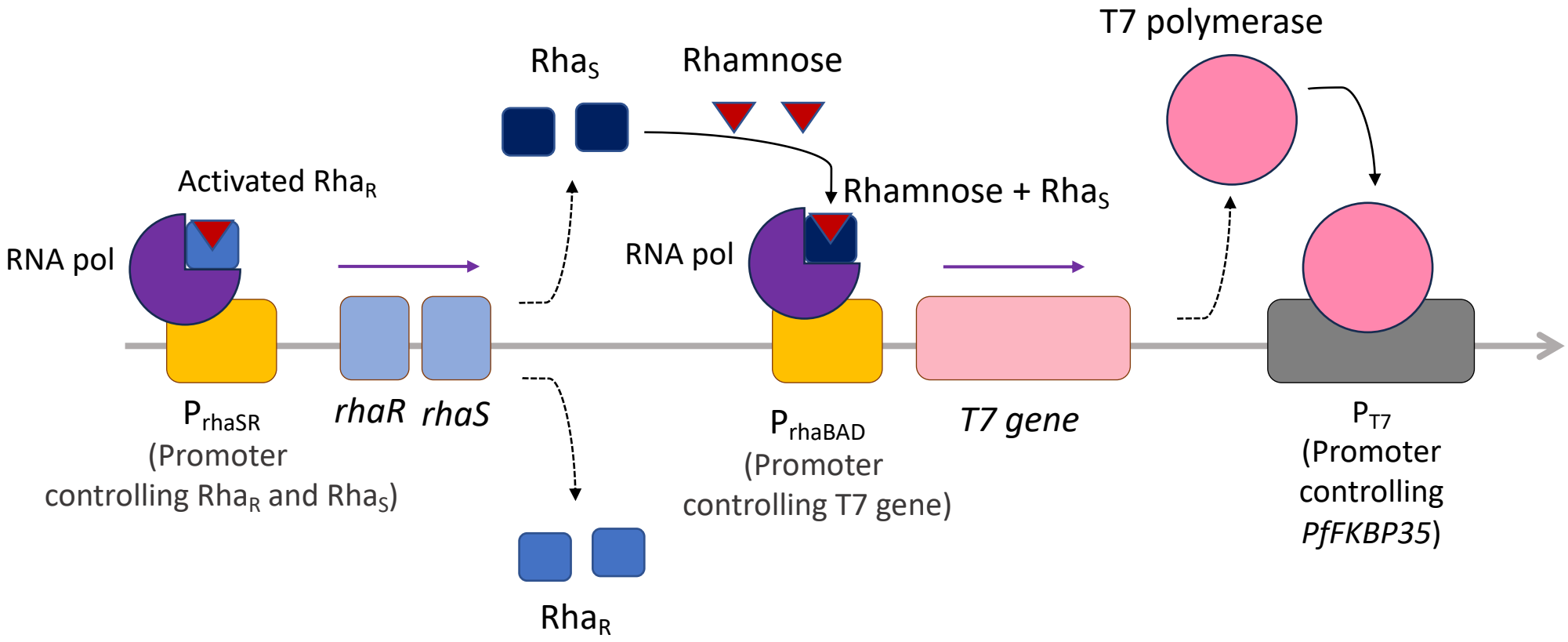
Bacterial induction: Rha_S promotes RNA Pol binding to Rha_{BAD} promoter



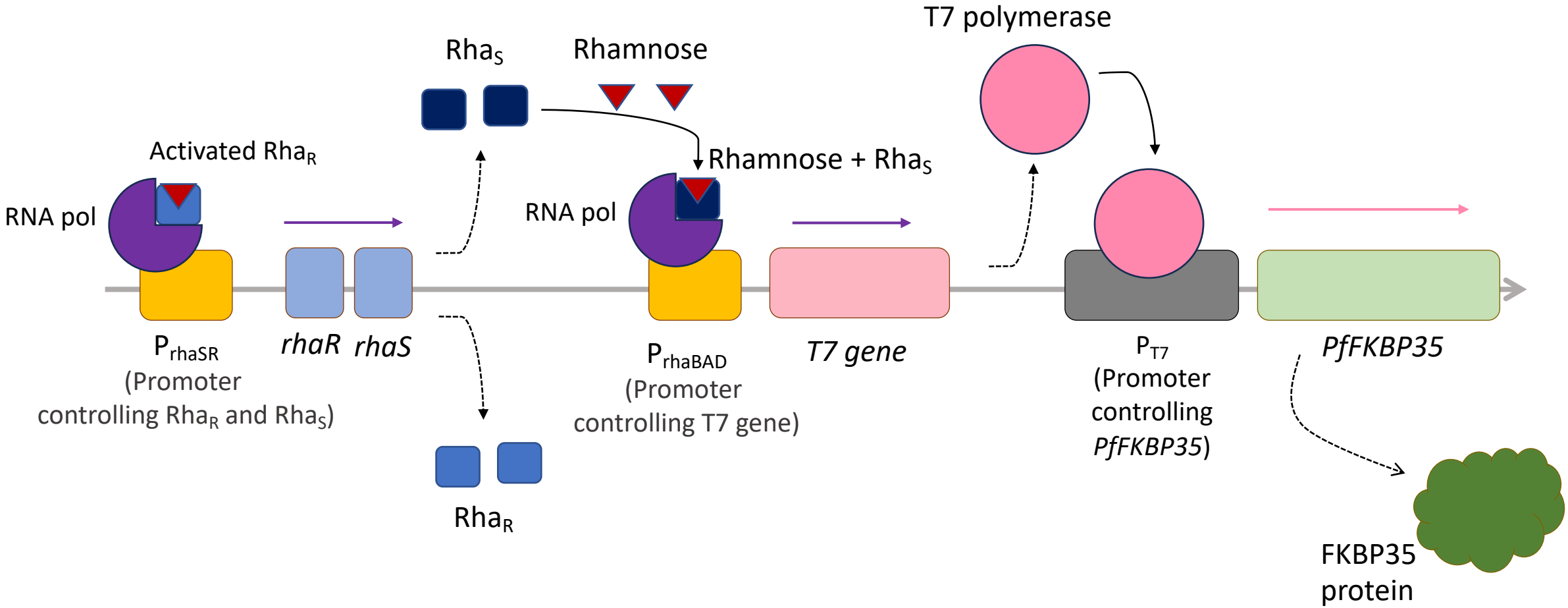
Bacterial induction: Rha_{BAD} promoter controls T7 Pol production



Bacterial induction: T7 Pol binds to the T7 promoter

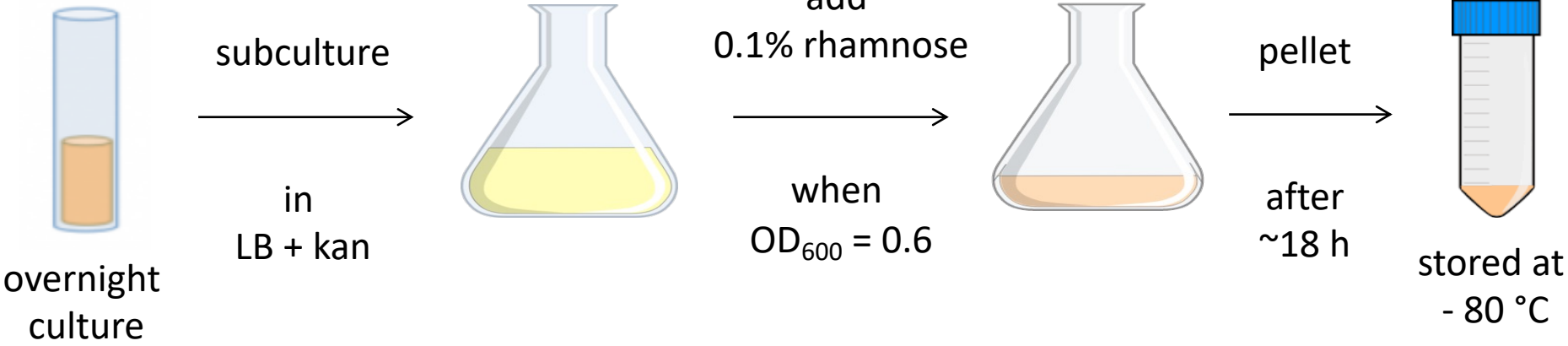


Bacterial induction: T7 promoter controls FKBP35 production

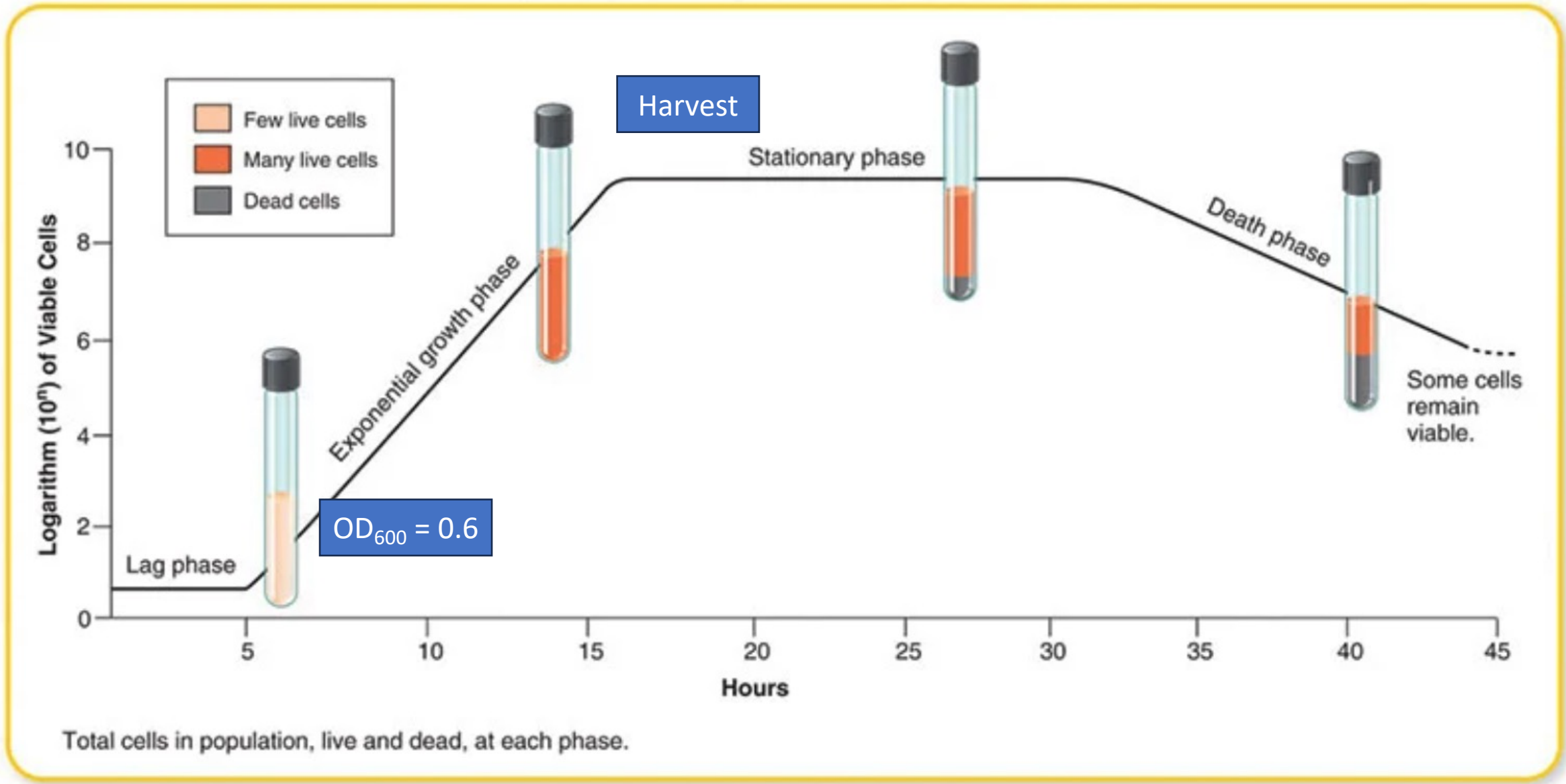


How do we induce protein expression?

E. coli express plasmid and kan resistance genes

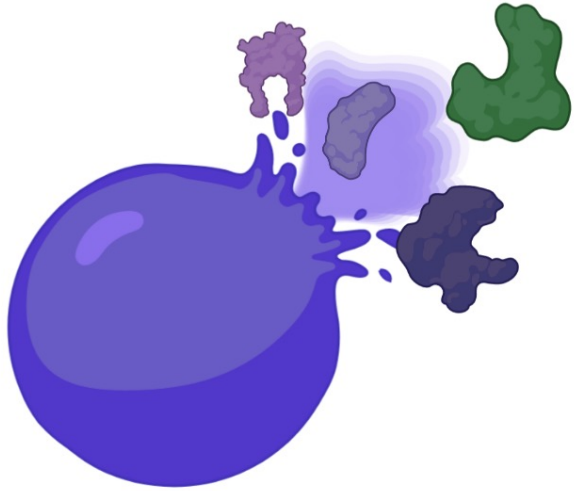


Why do we induce protein expression at OD_{600} = 0.6?



Total cells in population, live and dead, at each phase.

How will you purify PfFKBP35?



First, need to lyse cells to release proteins:

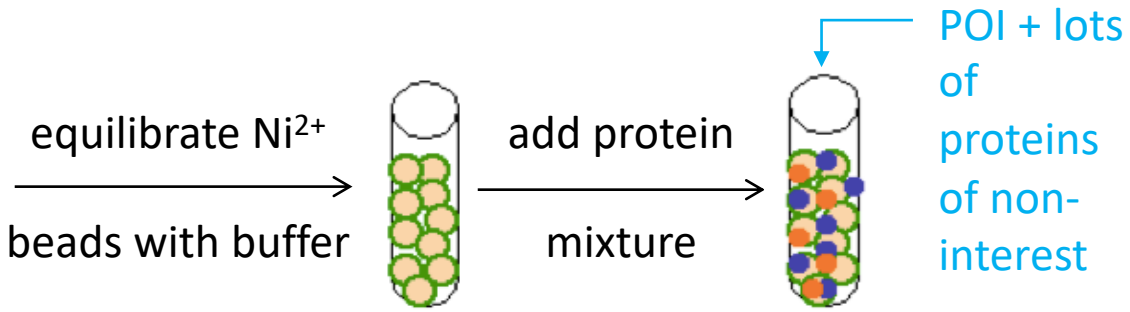
<u>What</u>

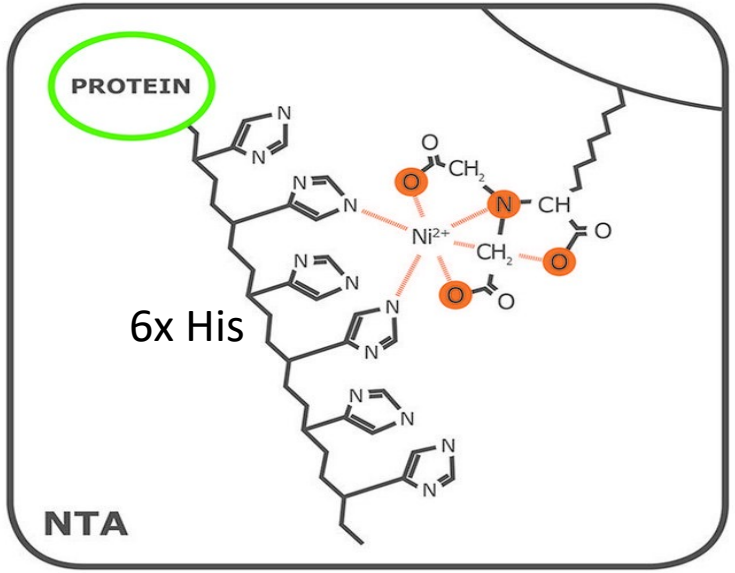
- B-PER bacterial extraction reagent
- Lysozyme
- DNaseI
- Protease Inhibitor Cocktail

<u>Why</u>

- Detergents/buffers rupture membranes
- Break bacterial cell wall
- Digest DNA
- Preserve POI during purification process

6xHis tag binds to Ni^{2+} resin / column to allow purification of protein of interest

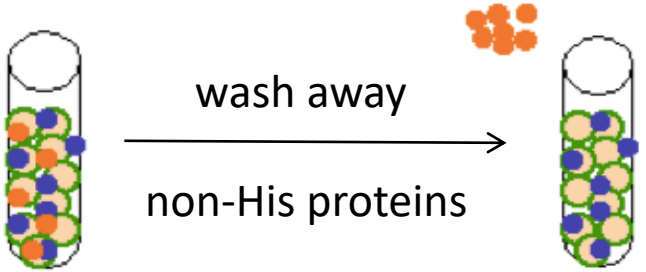


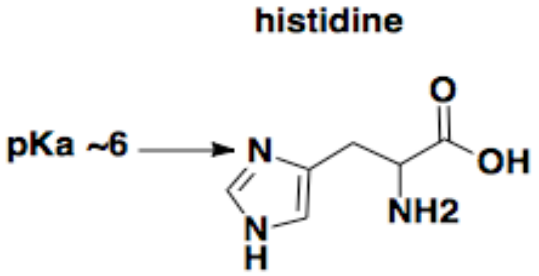


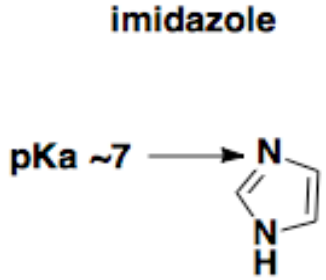
"Affinity purification"

- Ni^{2+} chelated onto agarose resin via nitrilotriacetic acid (NTA) ligand
- His tag chelates to Ni^{2+} causing protein to 'stick' to resin / column

Non-specific binders washed from Ni^{2+} resin / column using a low concentration of imidazole

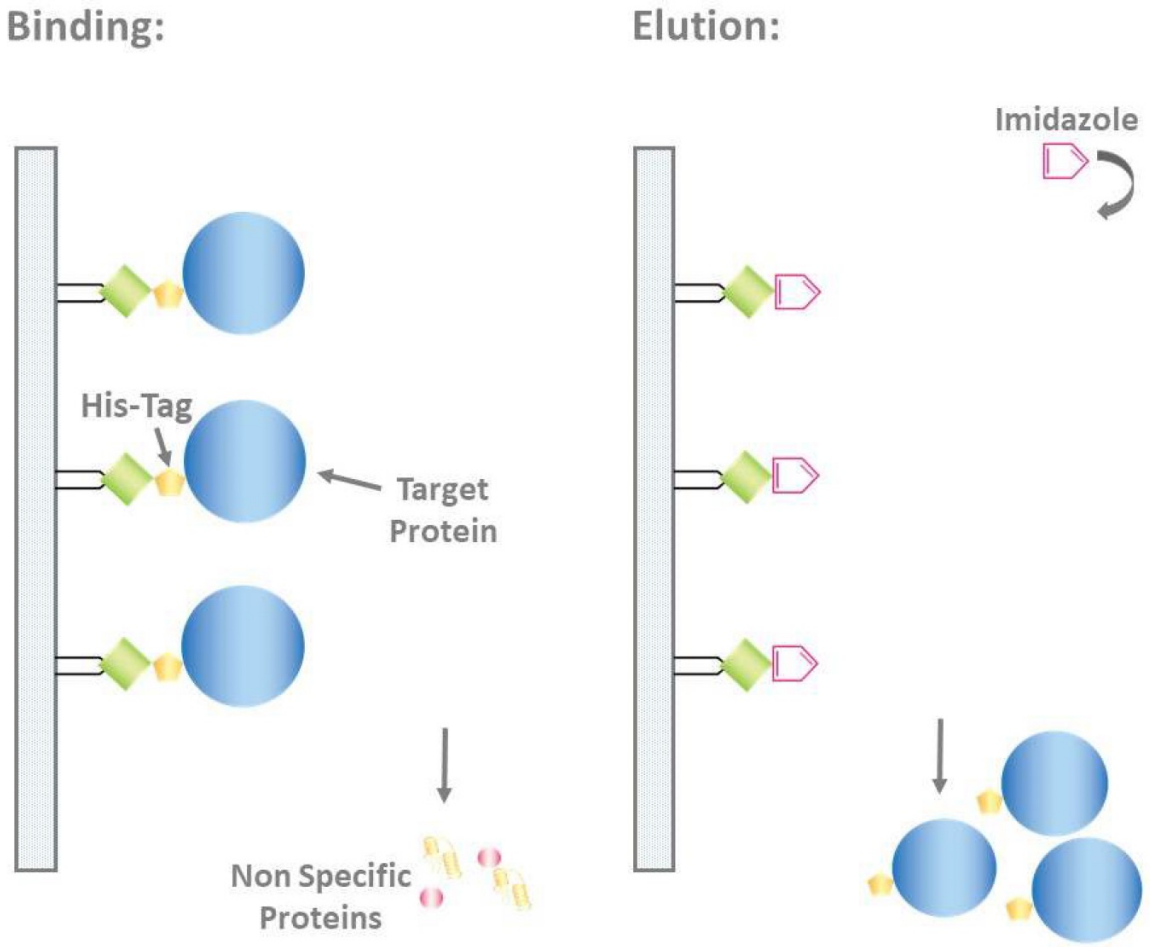






- Low concentration of imidazole included in wash buffer
- Imidazole competes for binding to Ni^{2+} resin
  - Low affinity binders / non-specific binders are outcompeted and released from the resin

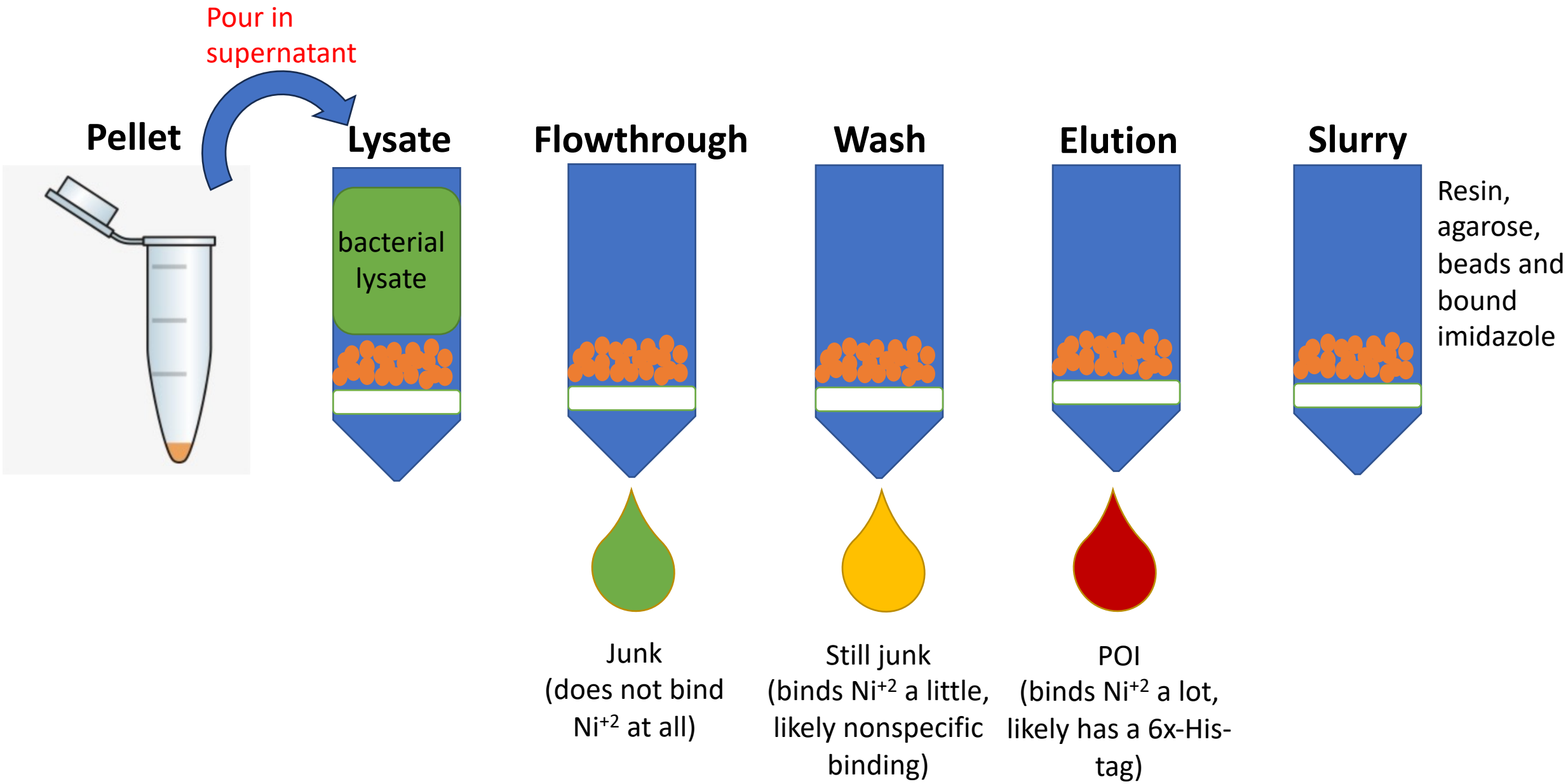
High concentration of imidazole is used to elute the protein from the Ni^{2+} resin / column



- Similar concept to wash
  - Wash uses 50mM imidazole
  - Elution used 250mM imidazole
- Instead of competing away non-specific binding, we can now out-compete the His Tag

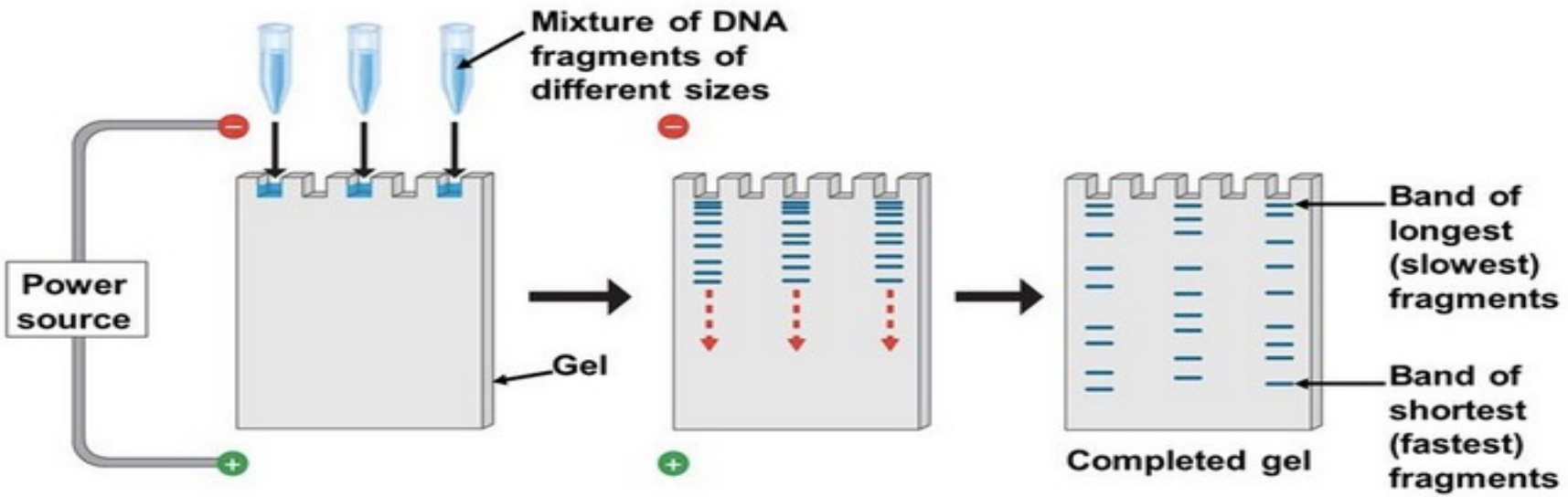
GoldBio

Purification process (and where you will save samples)



DNA electrophoresis review

1% agarose gel



How do you visualize the migration through the gel?

Tracking dye – bromophenol blue

How do you visualize DNA bands in the gel?

SYBR safe

For today…

1. Purify your protein for validation assay
2. During a centrifugation step, electrophorese your RE digest