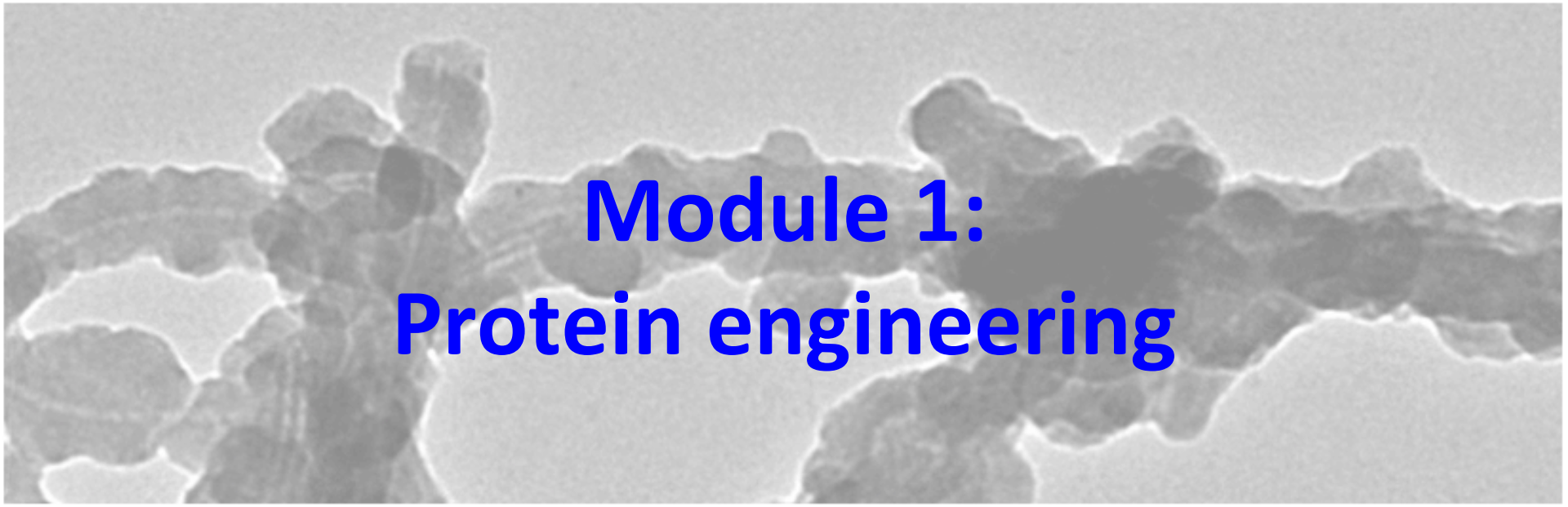
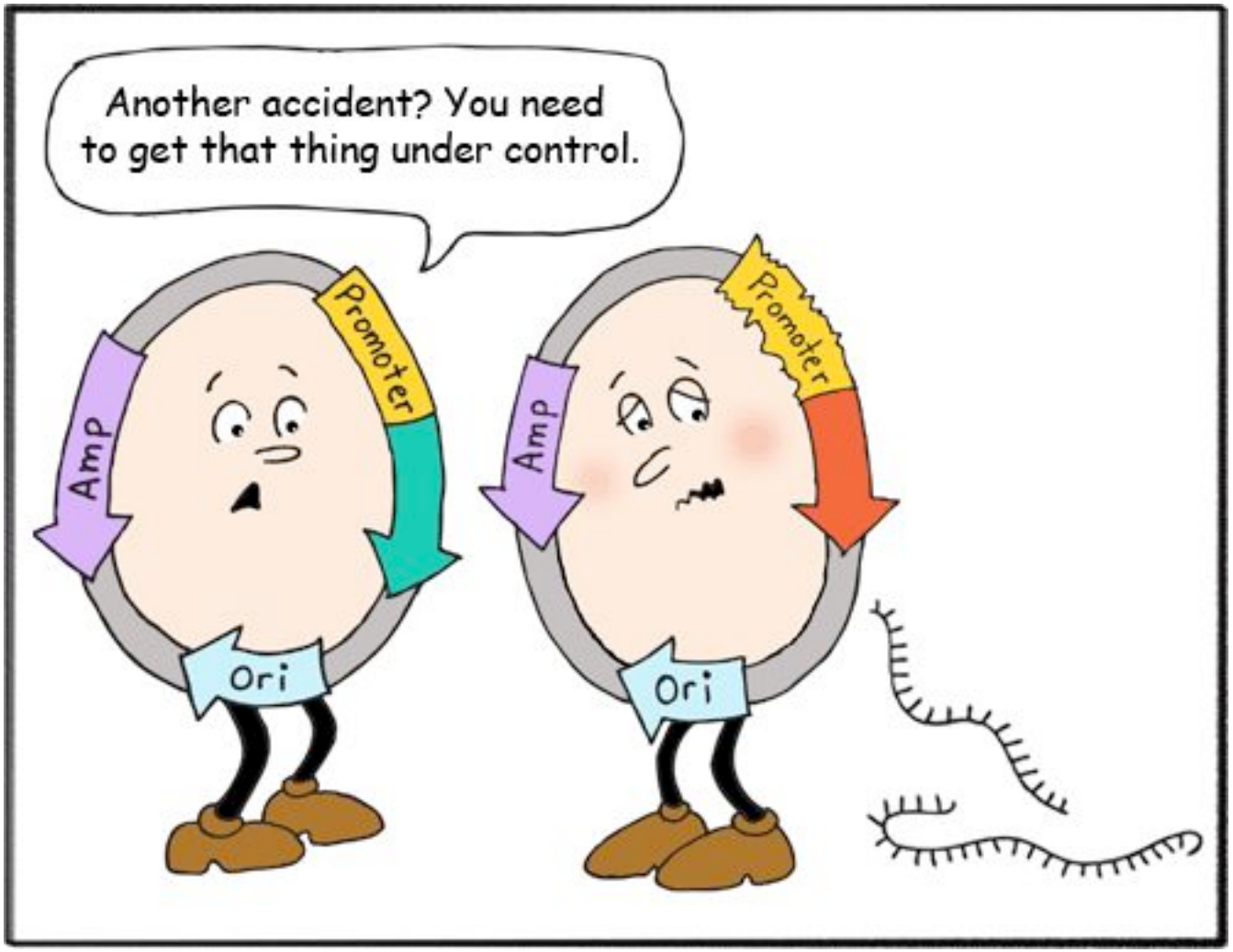


Module 1:
Protein engineering

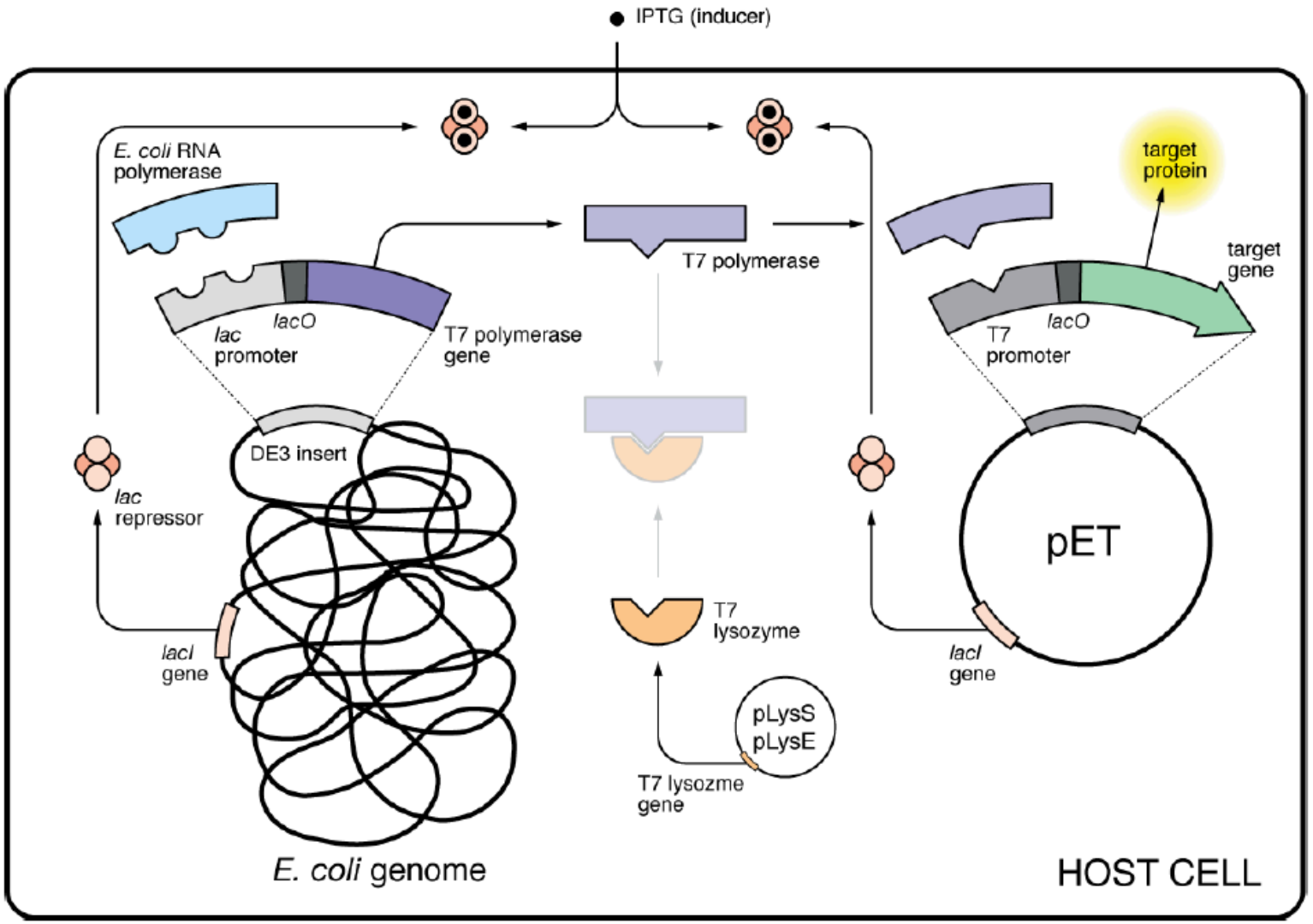
I Protein purification

II Applications

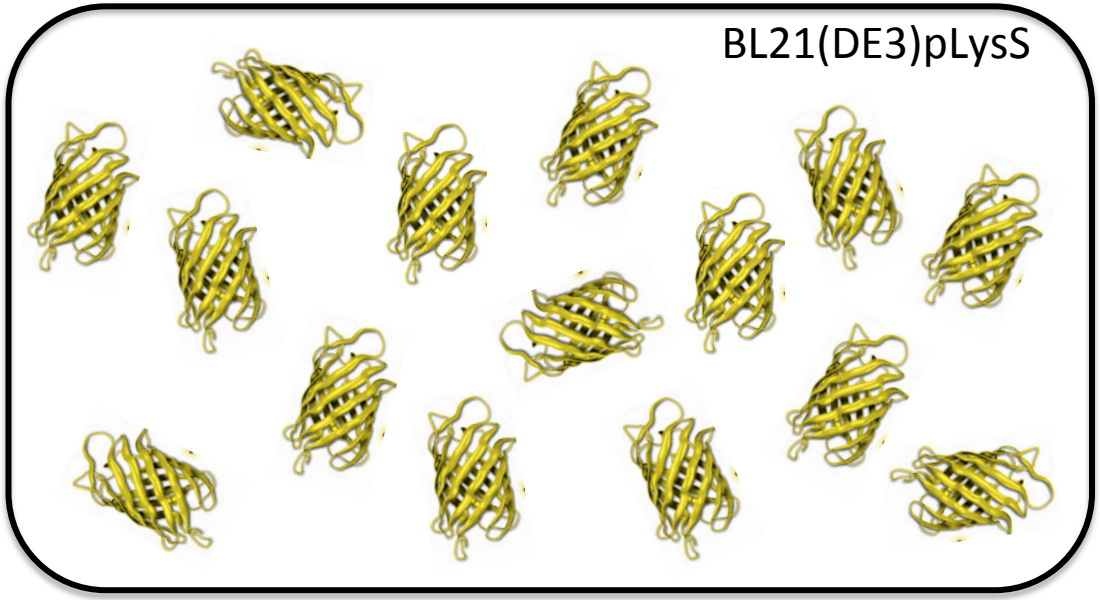
2/25/16


Another accident? You need to get that thing under control.
Amp
Promoter
Ori
Amp
Promoter
Ori

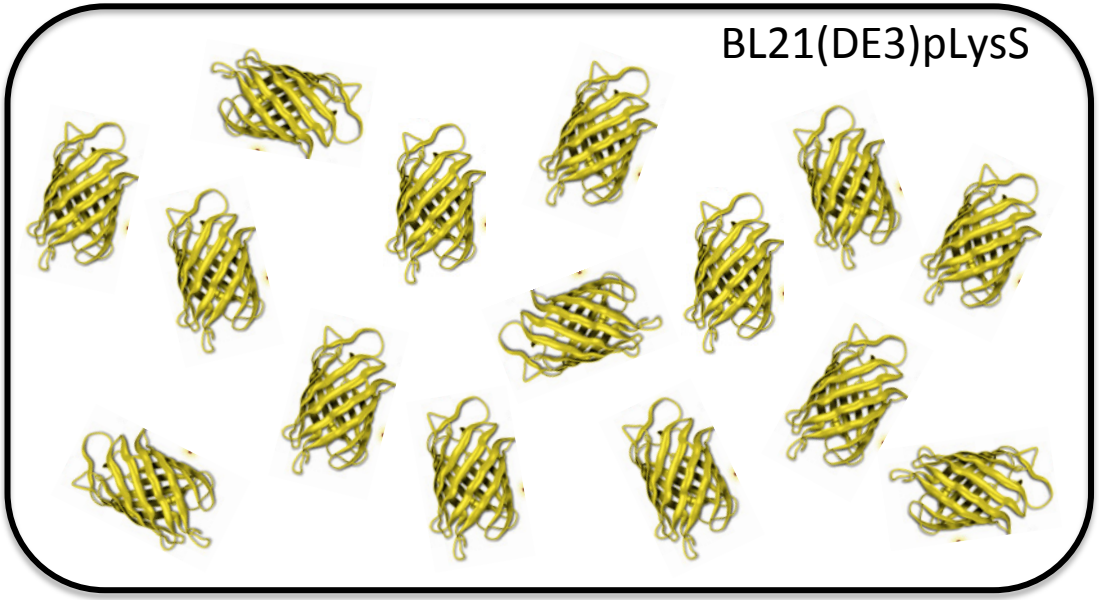
Review of last time…



How do we get *our* proteins?



How do we get *our* proteins?



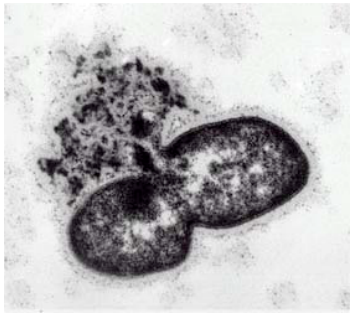
1. Lyse cells
2. Isolate protein of interest

Methods for cell lysis

- Physical disruption of cells

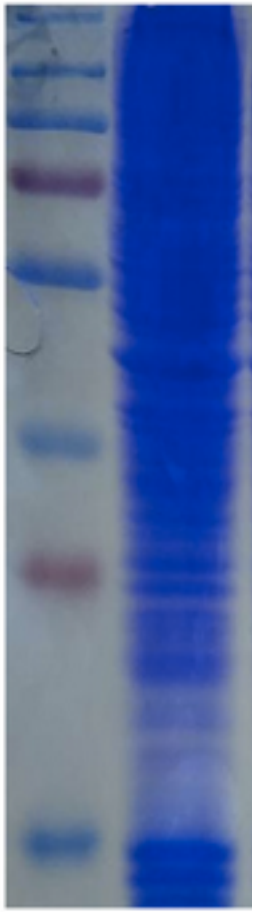



- Chemical disruption of cells



When and how did we do this previously?

Host also produces native proteins



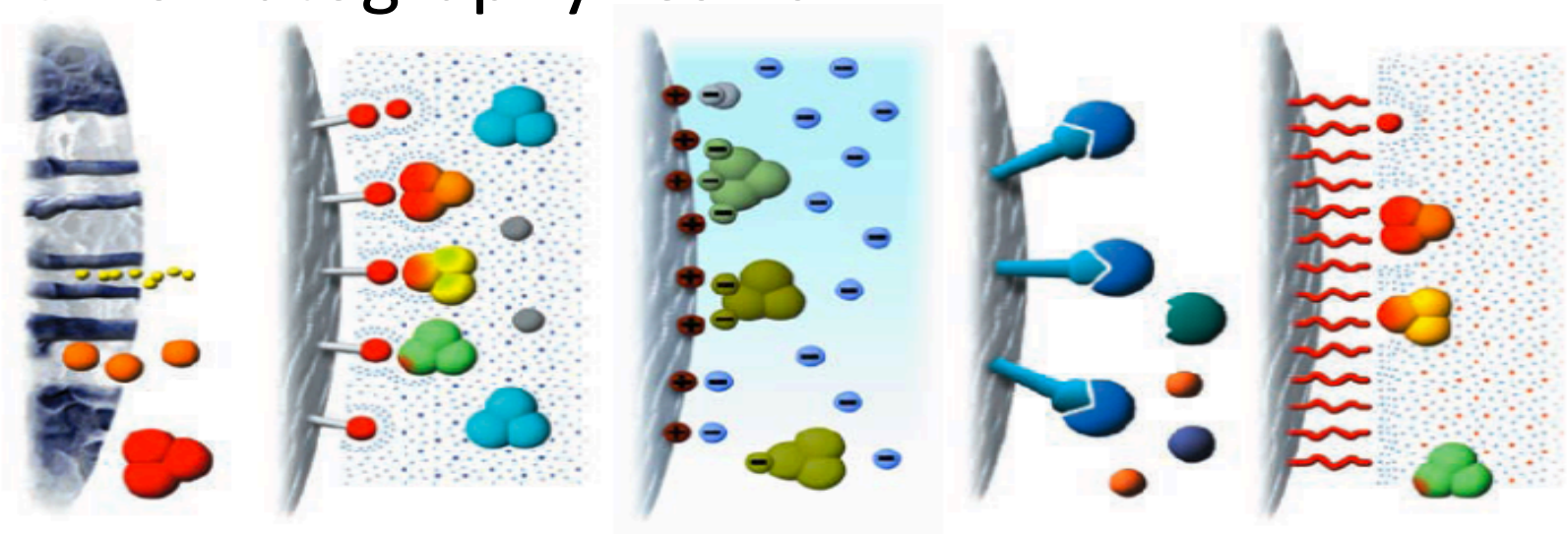
- 2-4 million proteins / fL in native cell

What properties can be used to isolate a specific protein from the cell lysate?

Milo. (2013) *Bioessays* 35:1050-1055.

Methods for protein purification

- Solubility
  - Alternate pH, [salt], solvents, temperature
- Chromatography resins



gel filtration (size)

hydrophobic interaction (hydrophobicity)

ion exchange (charge)

affinity (binding)

reverse phase (hydrophobicity)

GE Healthcare. (2007) *Recombinant Protein Purification Handbook*

Which method does our system use?

- For cell lysis:

- For protein purification:

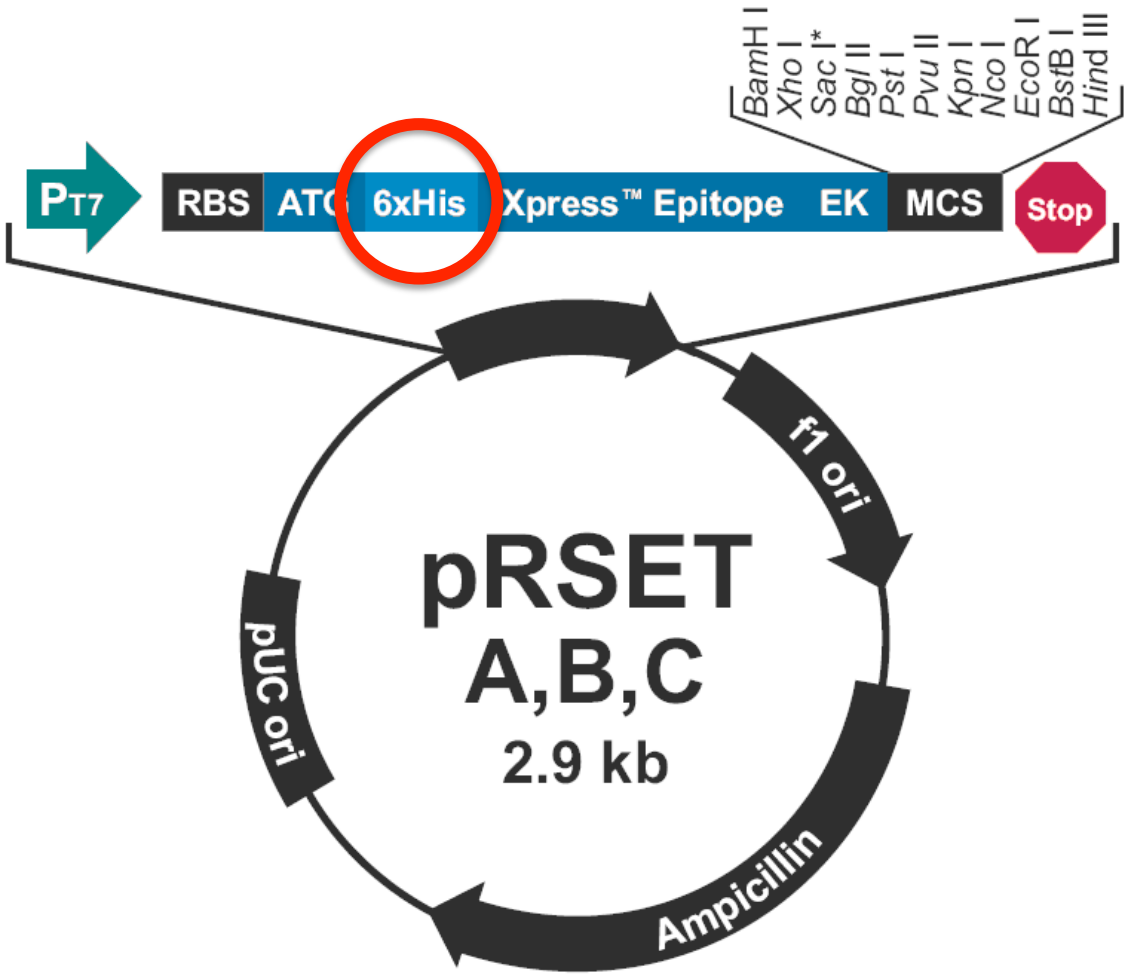
Which method does our system use?

- For cell lysis:
  - BugBuster protein extraction reagent
  - Protease inhibitors
  - Nuclease enzyme

chemical disruption of cells

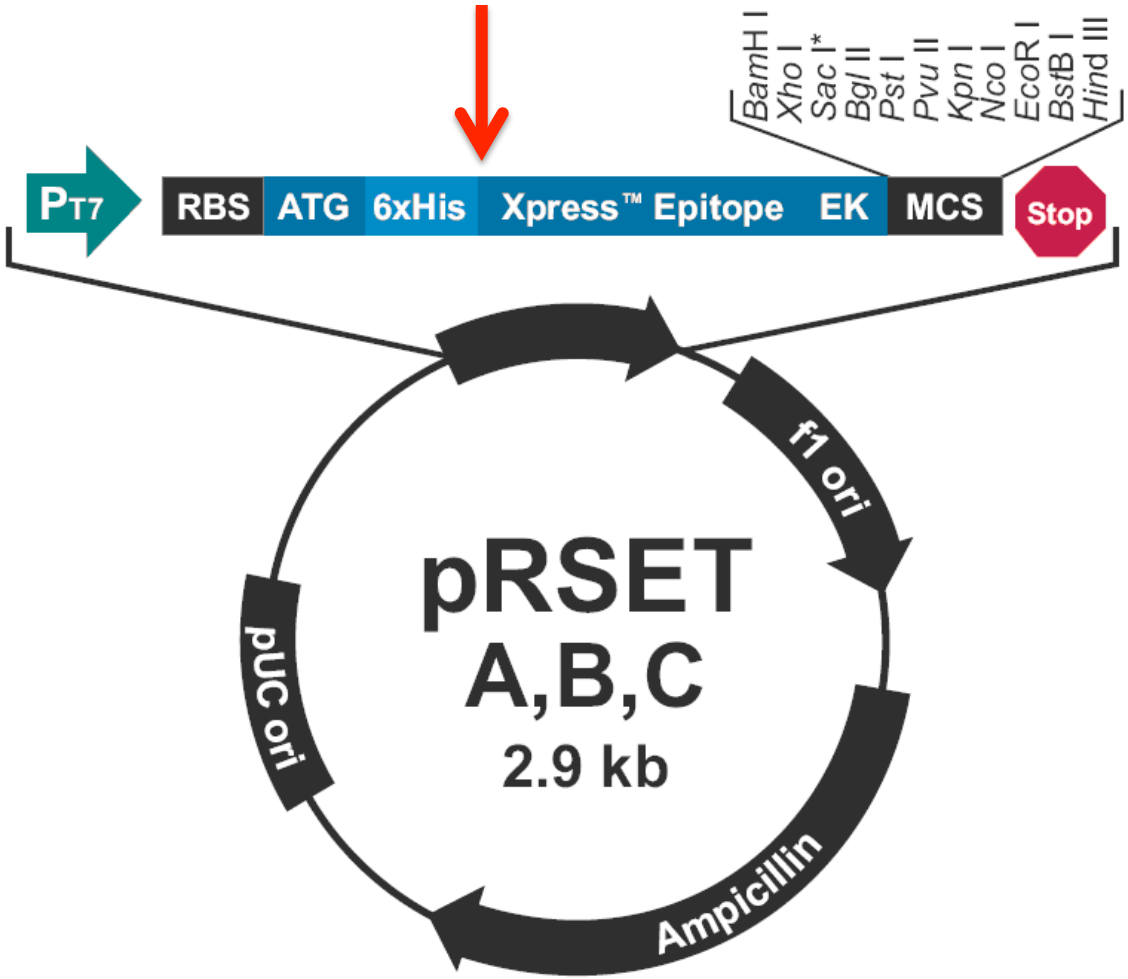
- For protein purification:
  - Affinity tag (6x His residues)

pRSET attaches affinity tag to protein



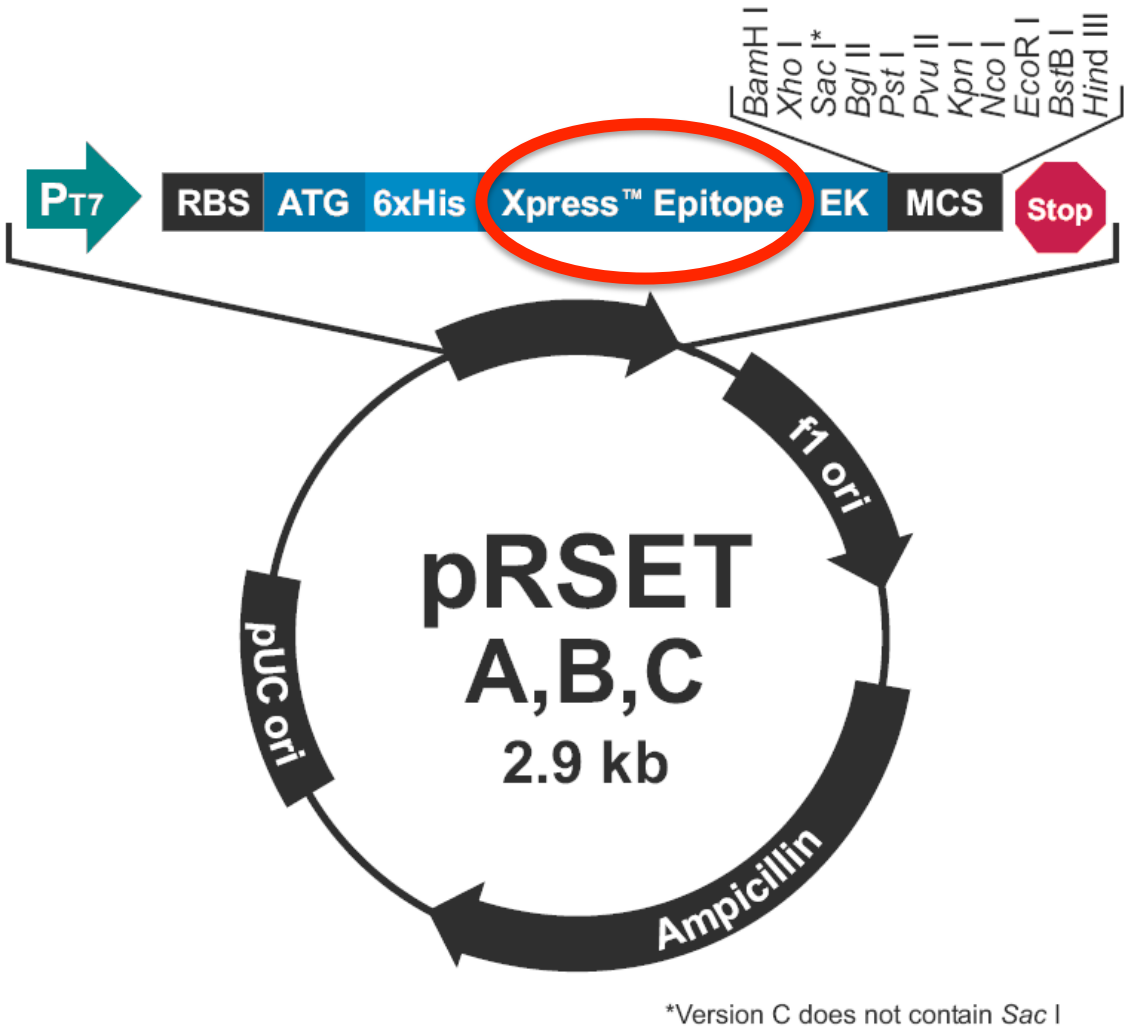
*Version C does not contain *Sac* I

pRSET improves transcript stability



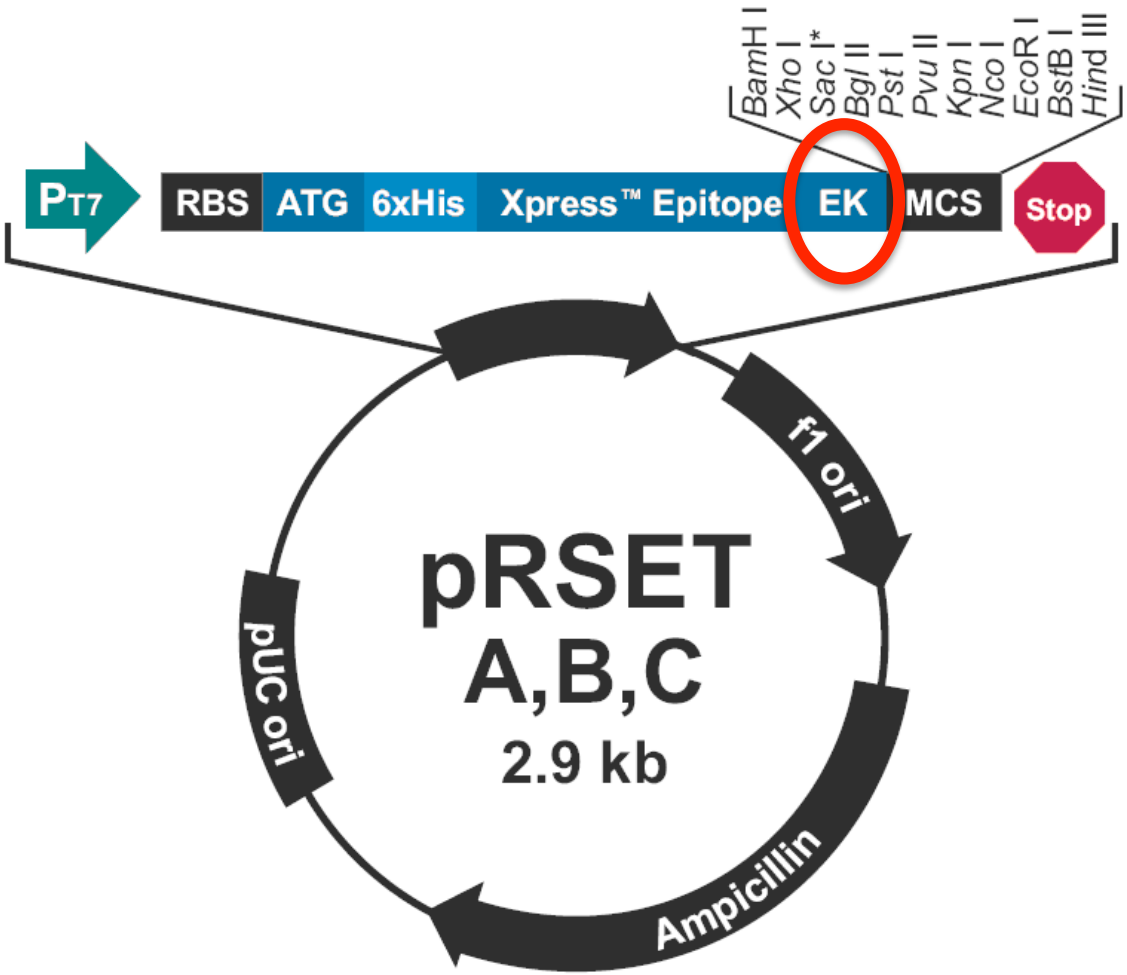
*Version C does not contain *Sac* I

pRSET enables Western blot detection



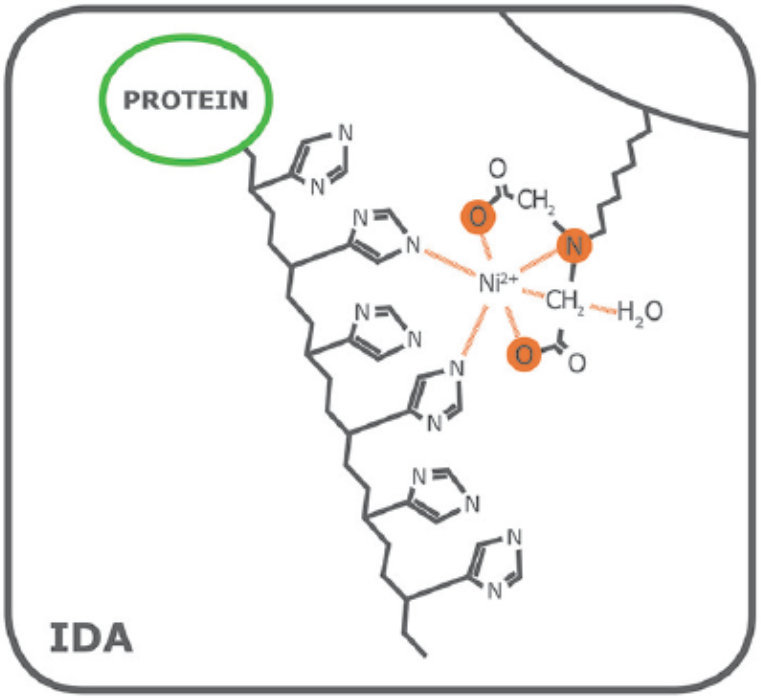
*Version C does not contain *Sac* I

pRSET includes tag removal sequence



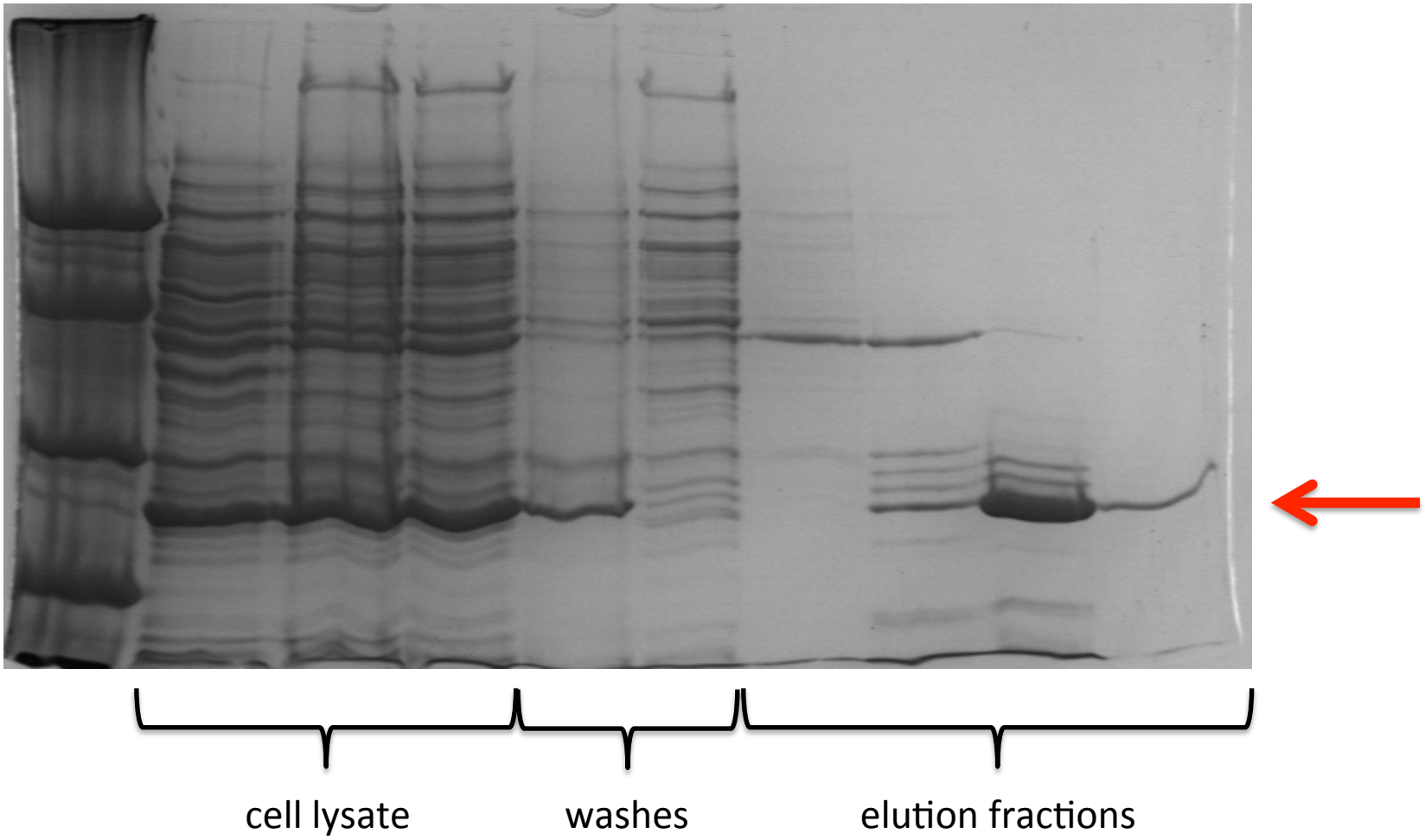
*Version C does not contain *Sac* I

Affinity tags are ‘handles’ on your protein



- Immobilized metal affinity chromotography (IMAC)
  - Transition metal chelated to matrix with ligand, iminodiacetic acid (IDA)
- Protein eluted with imidazole
  - High concentrations used to ‘out-compete’ His-Ni^{2+} association

Non-specific protein binding to Ni^{2+}



Native proteins contain 'His-tags'

- Metabolism proteins require metal co-factors
  - Urease
  - Hydrogenase
- Metals must be transported into the cell
  - ZIP family contain His residues in extracellular and intracellular loops

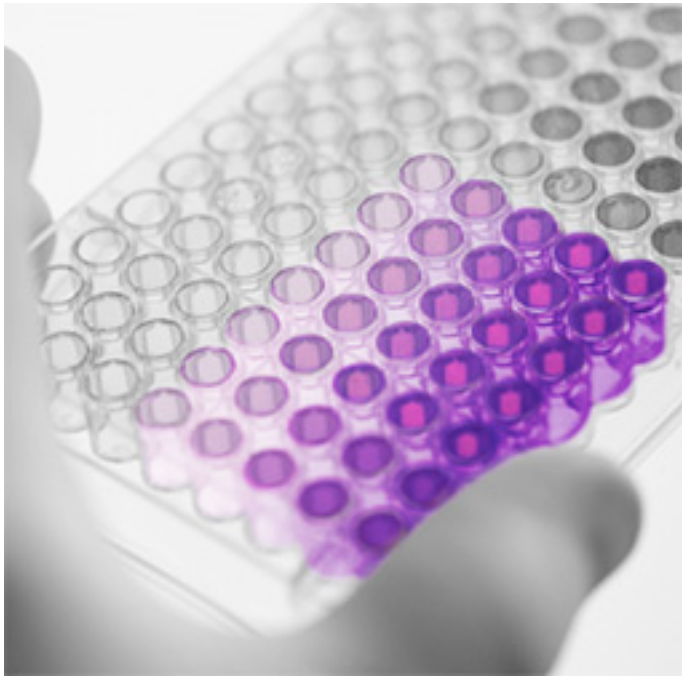
Why are we not concerned with minor non-specific binding?

How do we assess protein yield?

- Directly
  - Sodium dodecyl sulfate (SDS)-PAGE used to separate proteins based on size
- Indirectly
  - MicroBCA assay used to quantify protein concentration

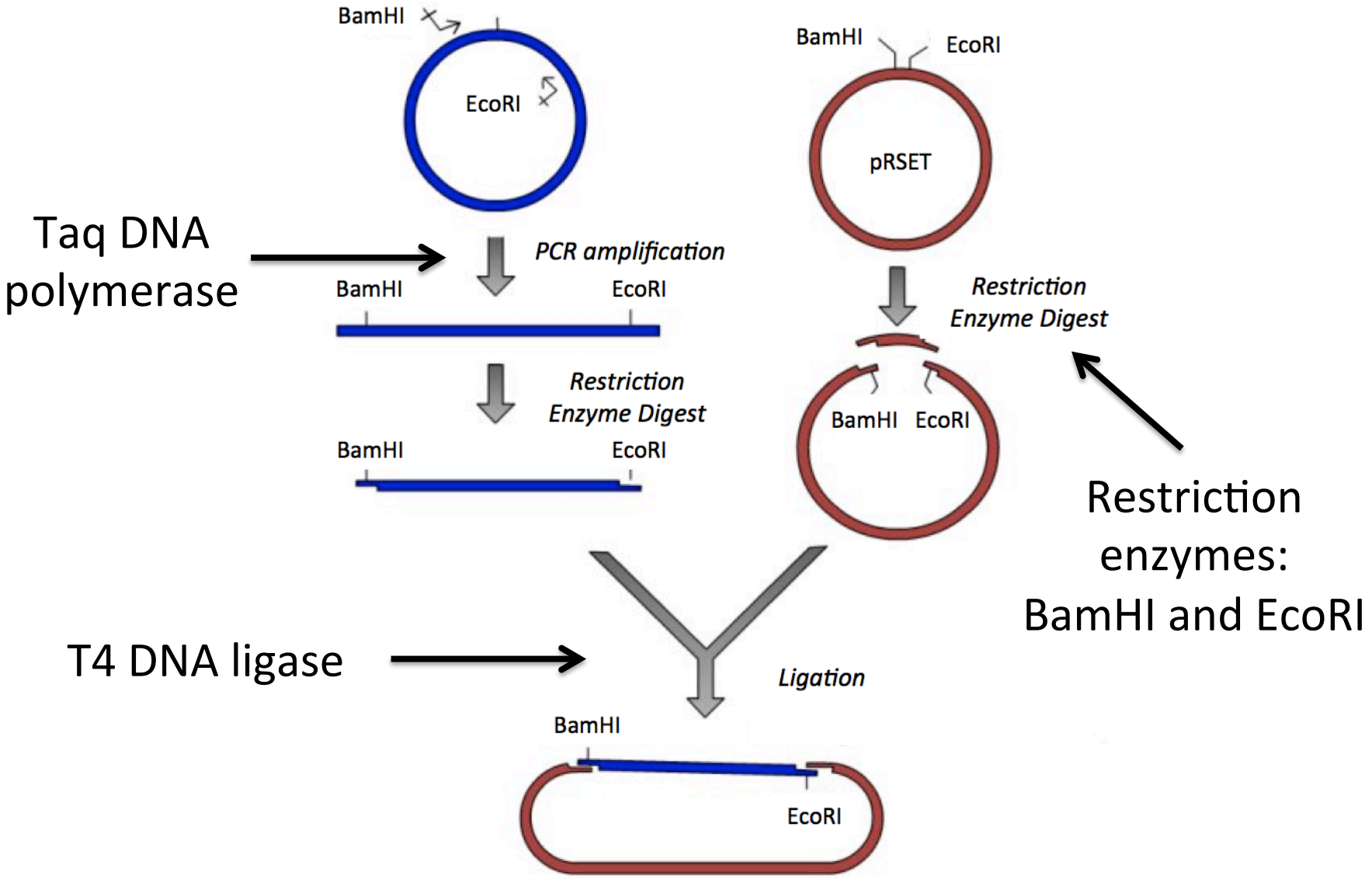
Bicinchoninic acid (BCA) protein detection

- Protein concentration measured via detection of Cu^{1+}
  - Reaction involves reduction of Cu^{2+} and oxidation of aromatic residues
  - Purple product formed by chelation of BCA and Cu^{1+}



So what. Now what?

Purified proteins in research



Purified proteins in consumer products

household products

α-amylase, cellulase, protease, lipase included as ‘stain fighters’

cosmetics

botulinum toxin A internalized by specific axons to cause paralysis

supplements

whey isolated from liquid material by-product of cheese production

Purified proteins in industry

phytase

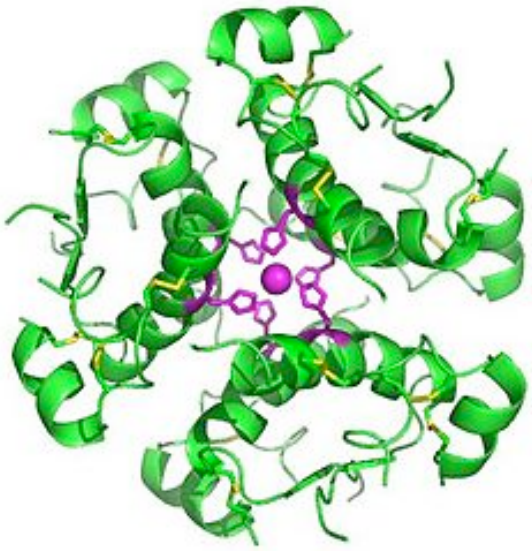
cattle diet
supplement to
increase intake of
phosphorous

laccase

paper production
requires delignification
to breakdown cell walls
in wood and bark

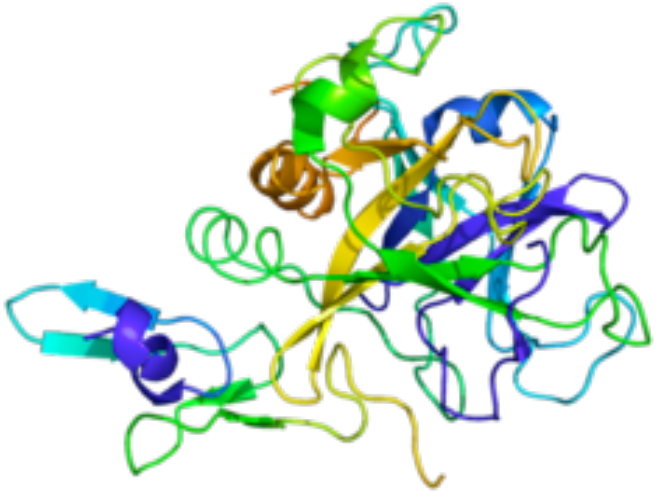
Purified proteins in therapeutics

insulin



diabetes type I results from failure by pancreas to produce insulin

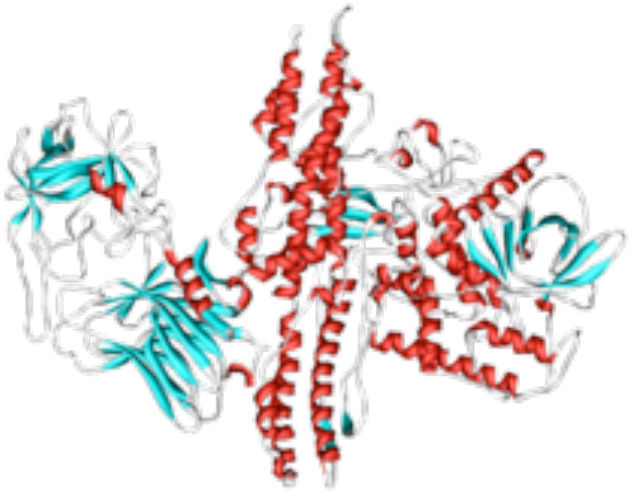
factor X



inability to clot results from genetic mutation, vitamin K deficiency, and some drugs

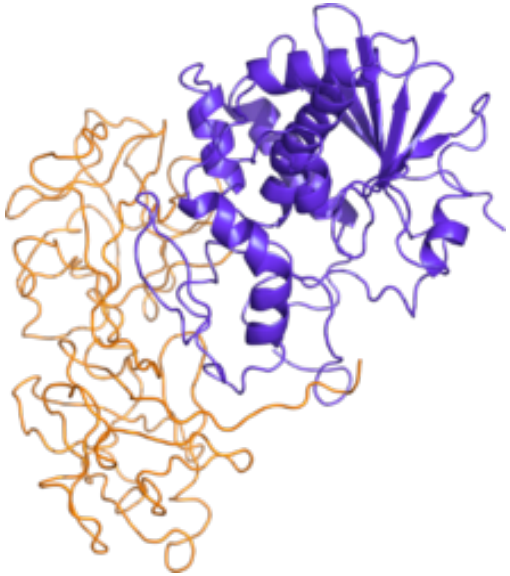
Purified proteins in the ‘wrong hands’

botulinum



neurotoxin produced by bacterium *Clostridium botulinum*

ricin



toxic lectin purified from castor beans

In the laboratory…

- Lyse cells
- Prepare for SDS-PAGE analysis
- Purify protein
- Measure protein concentration