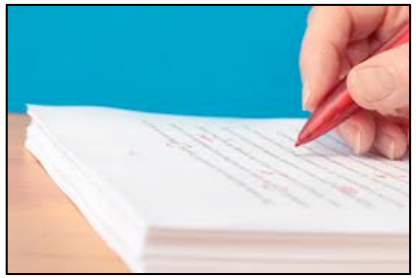
M1D6:
Purify protein

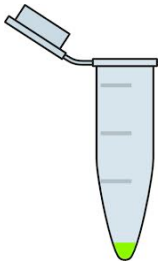
02/26/2016

Assignments on the horizon

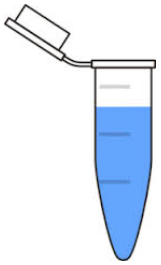


- for M1D7:
  - Background & Motivation
    - topic sentences + bullet points + references
  - schematic of M1 approach
- on M1D7:
  - Jing will grade one lab notebook entry
- for M1D8:
  - revise and add to Methods section
  - *optional:* outline of mini-presentation
- on M1D8:
  - quiz
- wrapping up M1:
  - protein engineering summary draft due 03/12

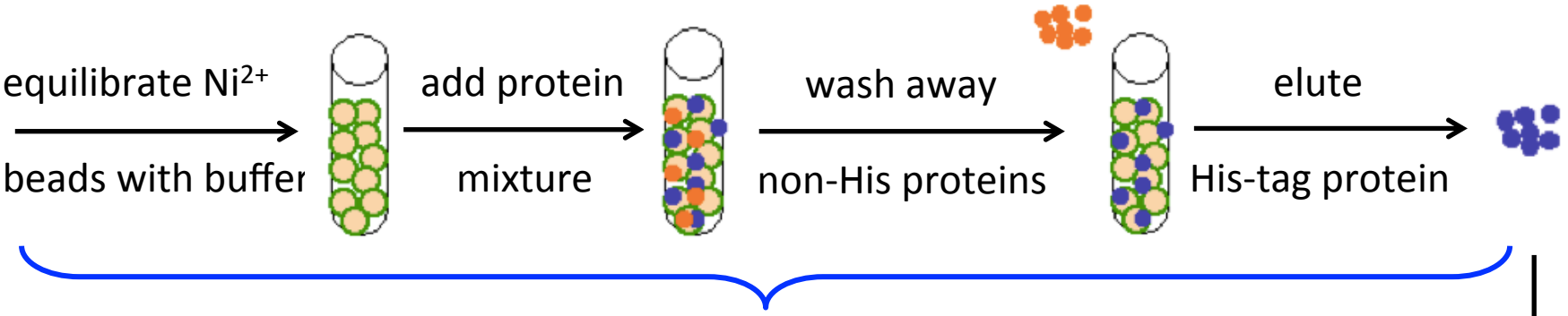
Protein purification: protocol overview



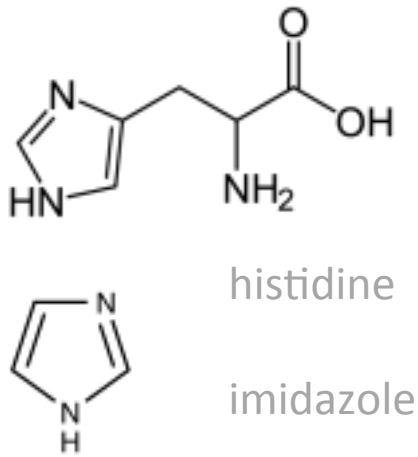
lyse with BugBuster (and extract supernatant)



- lysozyme: **breaks open the bacterial cell walls**
- DNAase (deoxyribonuclease): **chews up DNA**
- protease inhibitor cocktail: **prevents protein degradation**
- bovine serum albumin (BSA): "carrier", **stabilizer**

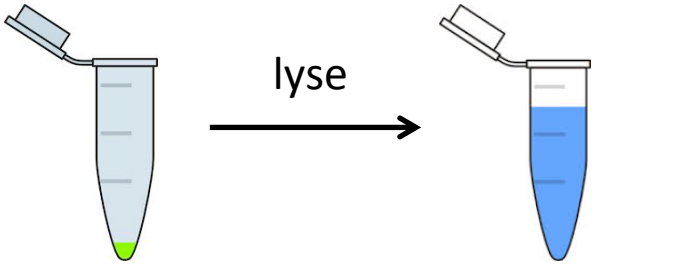


Protein purification: a few notes



histidine

imidazole

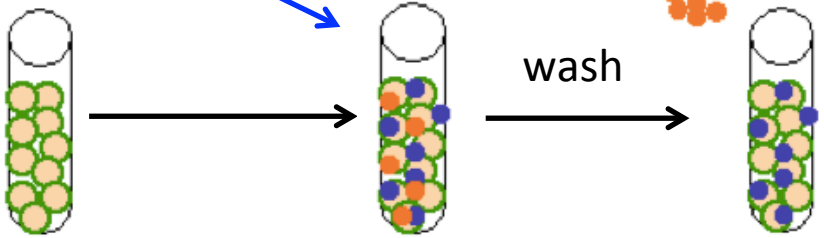


lyse

set aside 15 uL for SDS-PAGE

equilibrate Ni^{2+}

beads with buffer



wash

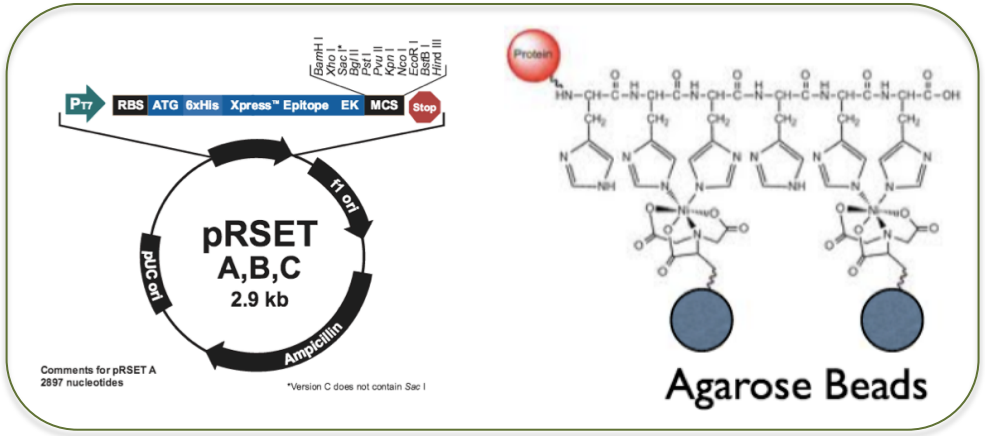
elute

by competition: imidazole *vs.* His

desalt

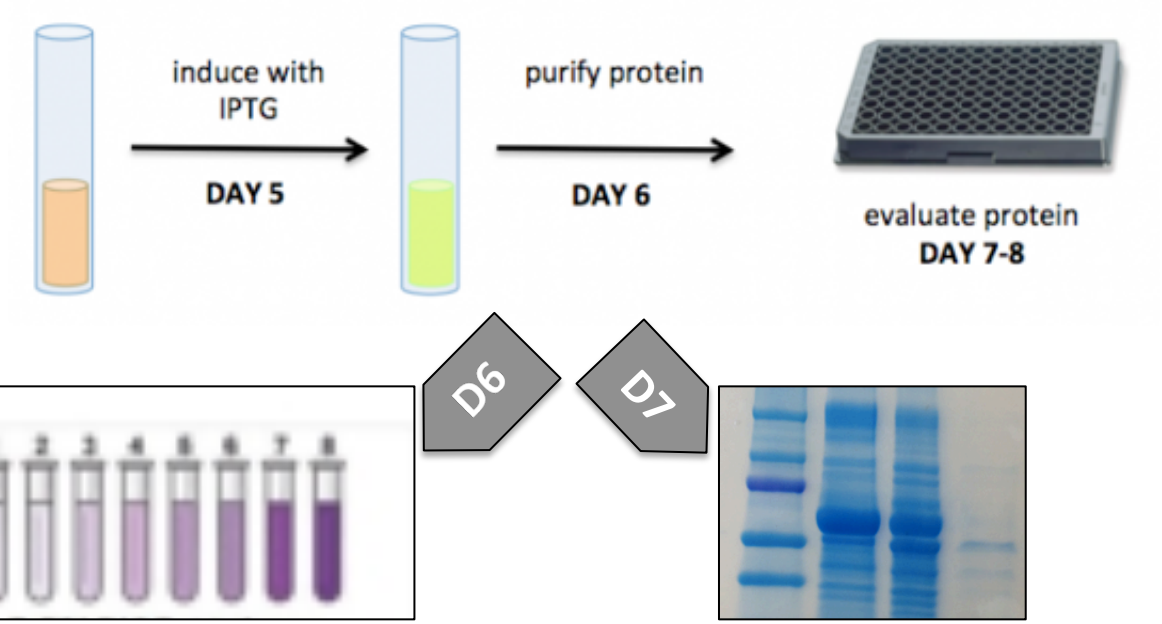
His-tag binds to metals

get rid of imidazole because it interferes with Ca2+ binding



evaluate protein M1D7-M1D8

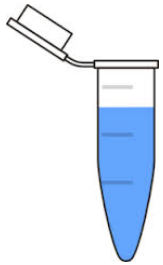
Let's also measure protein concentration



1. microBCA assay
   - [protein]

2. SDS-PAGE
   - [protein]
   - protein purity
   - leaky expression of IPC under T7 promoter

Prepare samples for SDS-PAGE

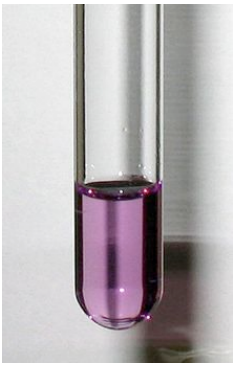


- Set aside whole cell extracts
  - equal number of cells based on OD_{600} (from M1D5)

sample	example		wt IPC		selected X#Z	
	- IPTG	+ IPTG	- IPTG	+ IPTG	- IPTG	+ IPTG
OD600	lowest: 0.5	1.0				
sample volume (µL)	15	15*0.5/1.0				
water volume(µL)	0	7.5				
total volume (µL)	15	15	15	15	15	15
add 6x buffer (µL)	3	3	3	3	3	3

- SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel
  - separation by size ? shape ? charge ?

The copper ion-based microBCA (Smith) assay measures protein concentration



① from cupric (Cu^{2+}) to cuprous (Cu^{1+}) ions when binding to peptide (alkaline + temperature, Biuret reaction)
   - proportional to [protein]

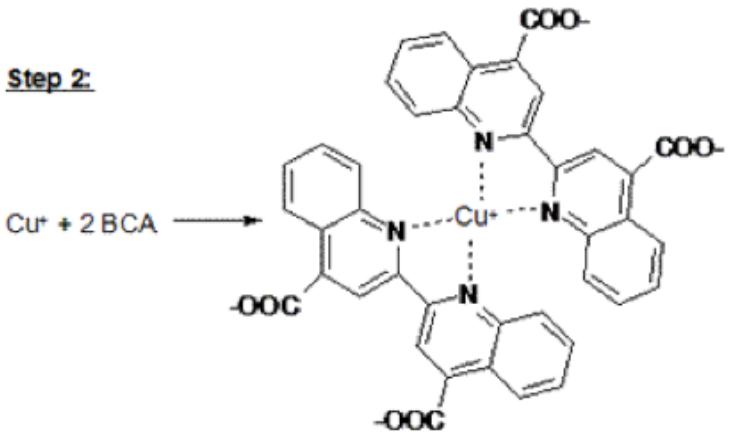
② Cu^{1+} reduces bicinchoninic acid (BCA)
   - BCA turns **purple** = absorbs 562 nm

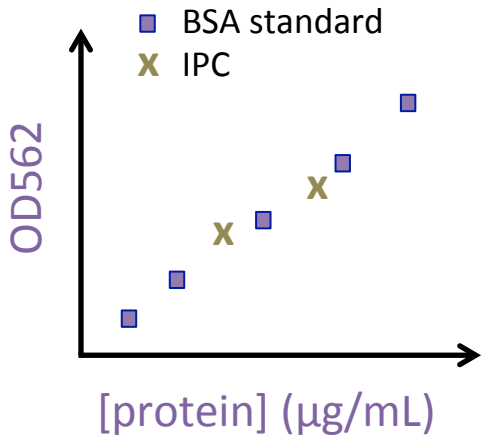
- calibration with bovine serum albumine (BSA)
  - 0.5 – 20 µg/mL

Step 1:

$$\text{Protein} + Cu^{2+} \xrightarrow{OH^-} \text{Protein} + Cu^{+}$$

Step 2:





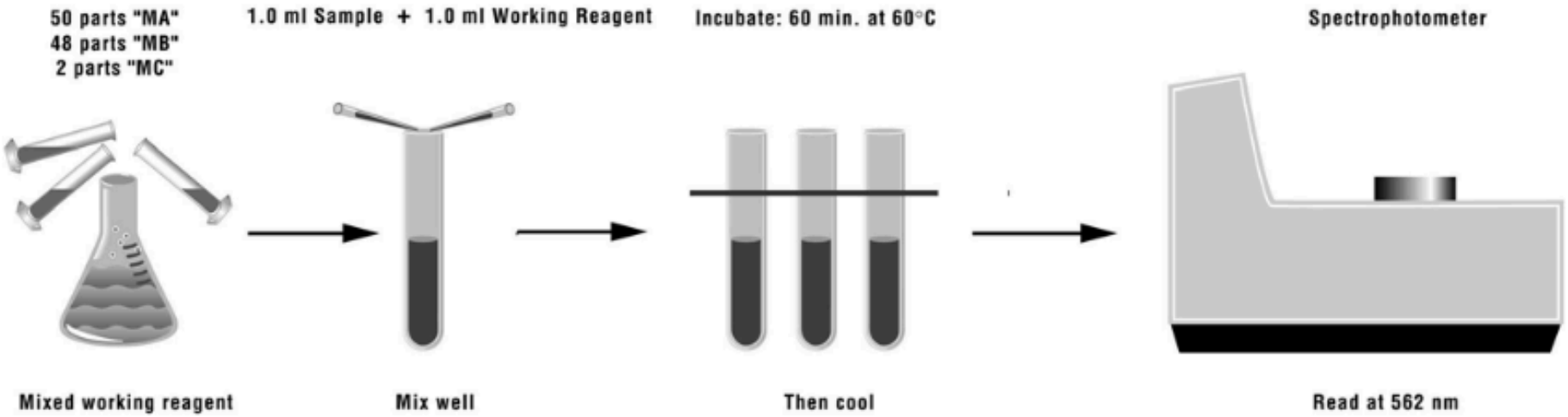
There exists several protein concentration assays

assay	absorption	mechanism	detection limit	advantages	disadvantages
UV absorption	280 nm	tyrosine and tryptophan absorption	0.1-100 ug/ml	small sample volume, rapid, low cost	incompatible with detergents and denaturating agents, high variability
Bicinchoninic acid	562 nm	copper reduction (Cu^{2+} to Cu^{1+}), BCA reaction with Cu^{1+}	20-2000 ug/ml	compatible with detergents and denaturating agents, low variability	low or no compatibility with reducing agents
Bradford or Coomassie brilliant blue	470 nm	complex formation between Coomassie brilliant blue dye and proteins	20-2000 ug/ml	compatible with reducing agents, rapid	incompatible with detergents
Lowry	750 nm	copper reduction by proteins, Folin-Ciocalteu reduction by the copper-protein complex	10-1000 ug/ml	high sensitivity and precision	incompatible with detergents and reducing agents, long procedure

Table 1. Common total protein assays.

Be careful!

- Fresh tips from dilution to dilution
- Mix well (use parafilm to cap tubes)
- (E) to (F) differs from others ☺



Today in lab

- Lyse 4 cell pellets (wt IPC -/+ IPTG and “good” mutant -/+ IPTG)
- Set aside aliquots for SDS-PAGE (M1D7)
  - add Laemmli buffer to each
- Purify protein (1 wt IPC + 1 mutant)
  - 2 steps: affinity purification + desalting
- Immediately aliquot
  - 10 µL for microBCA assay
  - 15 µL for SDS-PAGE
- Stabilize rest of purified protein with BSA
  - ~ 1 mL protein + 10 µL of 10% BSA
  - to be titrated against Ca^{2+} on M1D7
- Assay purified protein concentration with microBCA kit
  - 1 h incubation