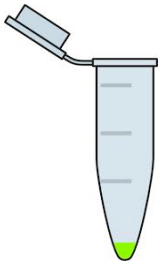
M1D6: Purify protein

2/25/2016

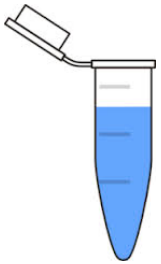
1. Prelab lecture
2. Lyse bacteria and prep SDS-PAGE samples
3. Purify protein
4. Measure purified protein with microBCA assay

Protein purification: protocol overview



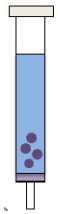
lyse with BugBuster (and extract supernatant)

- lysozyme: damages bacterial cell walls
- DNAase (deoxyribonuclease): cleaves DNA
- protease inhibitor cocktail: blocks protein degrdation
- bovine serum albumin (BSA): "carrier", protein stabilizer



Equilibrate Ni^{2+} beads w/ buffer → add protein mixture → wash away non-His proteins → elute His-tag protein

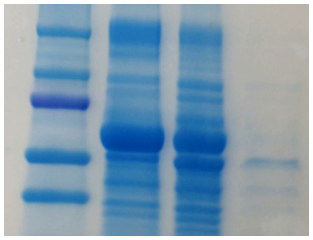
affinity purification



desalt

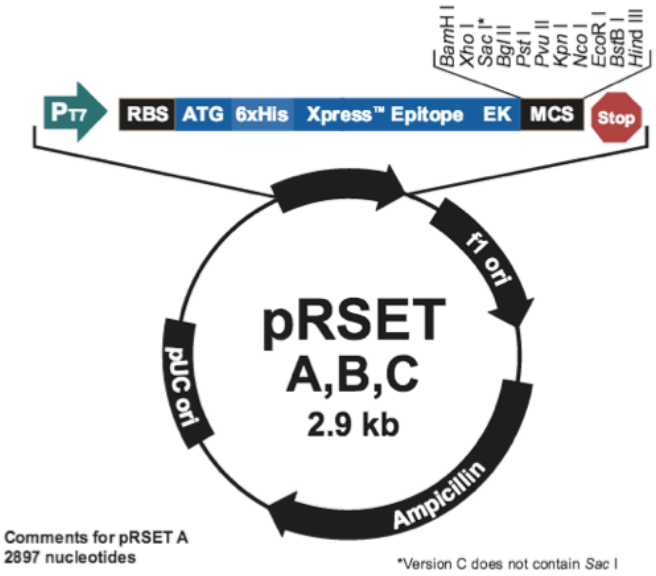
Evaluate [Protein]

Evaluate [Protein] and purity

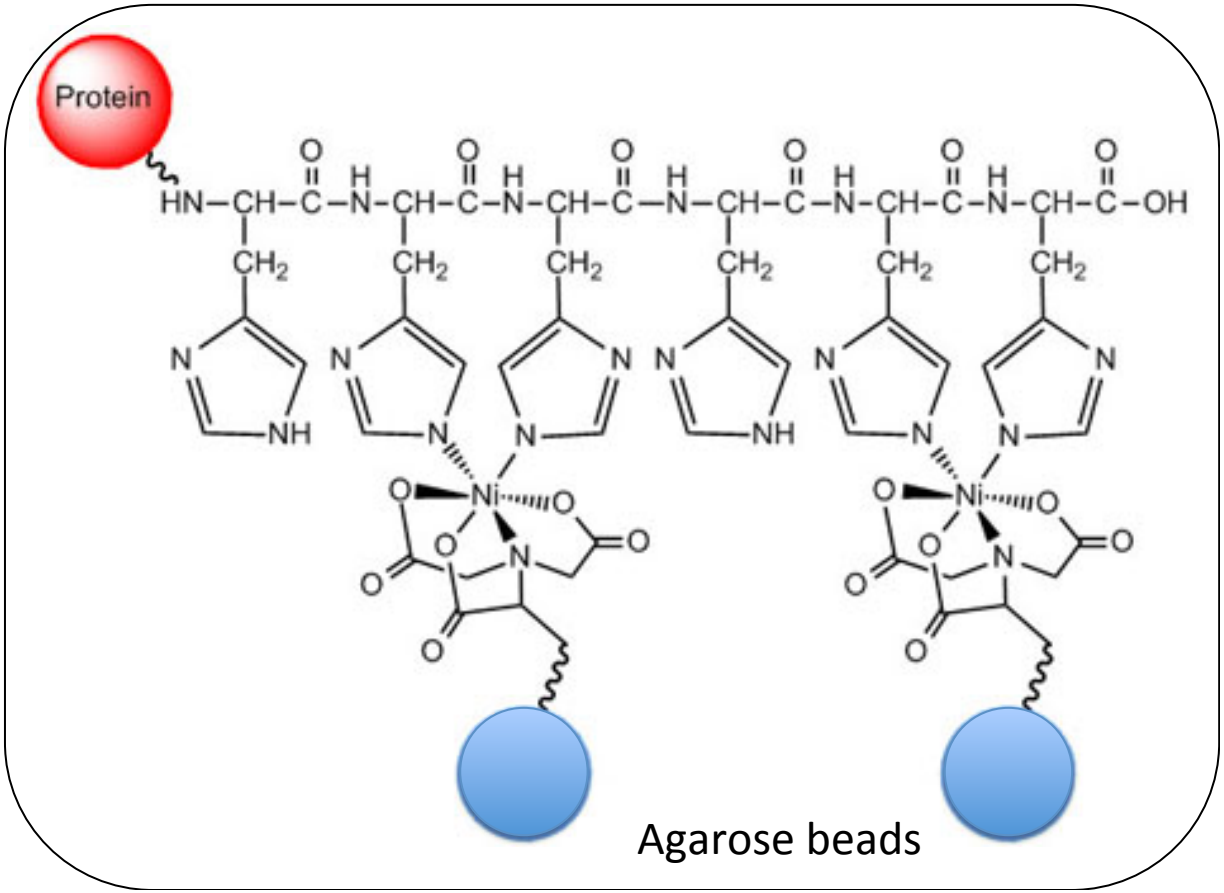


Calcium Titration and analysis M1D7-M1D8

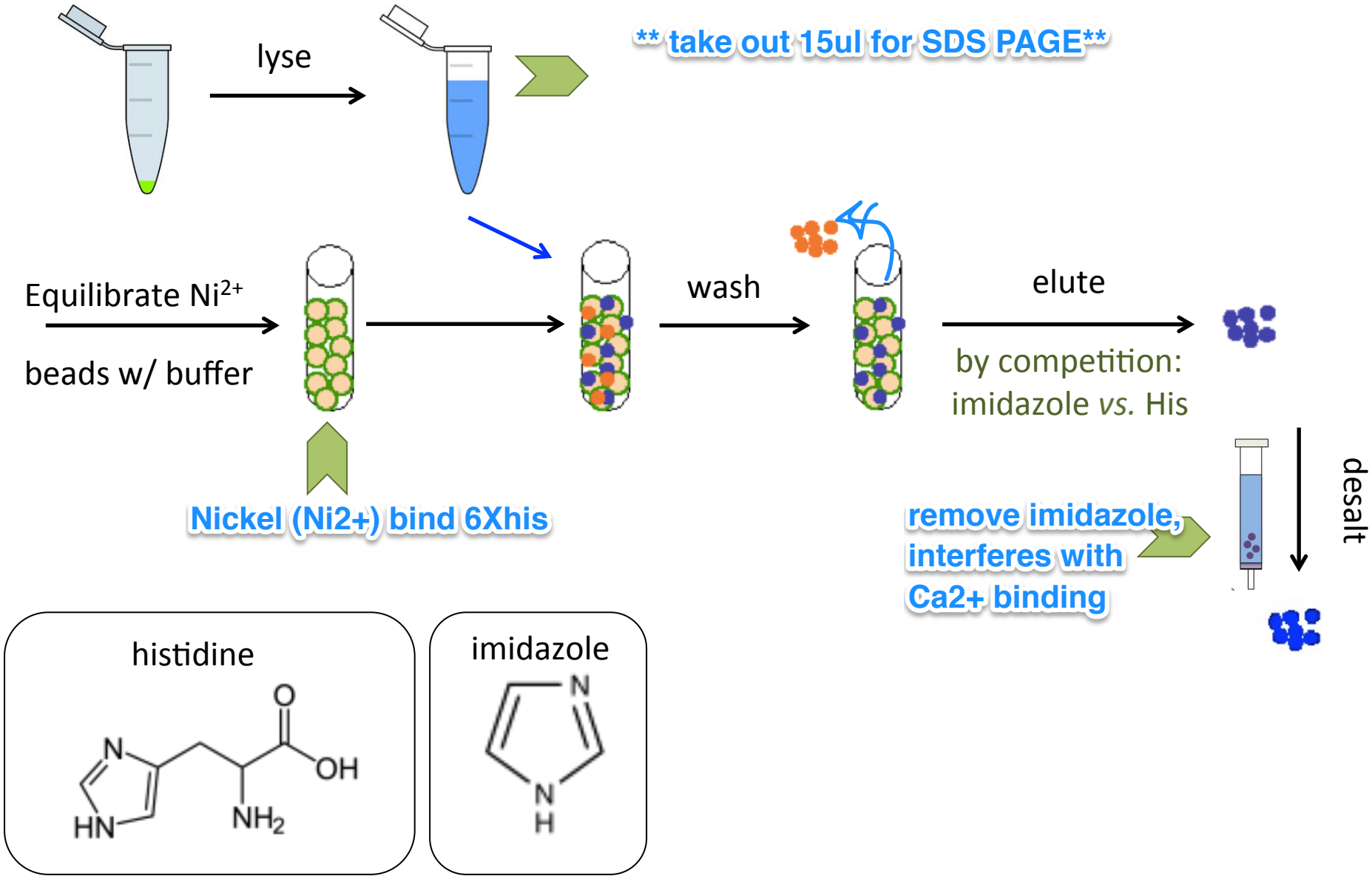
The polyhistidine (6XHis) tag binds nickel



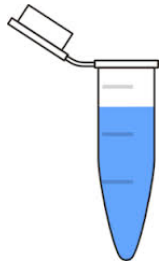
electron donor groups from histidine form coordination bonds with tradition metals



His tag affinity purification using Ni-NTA agarose



Prepare samples for SDS-PAGE



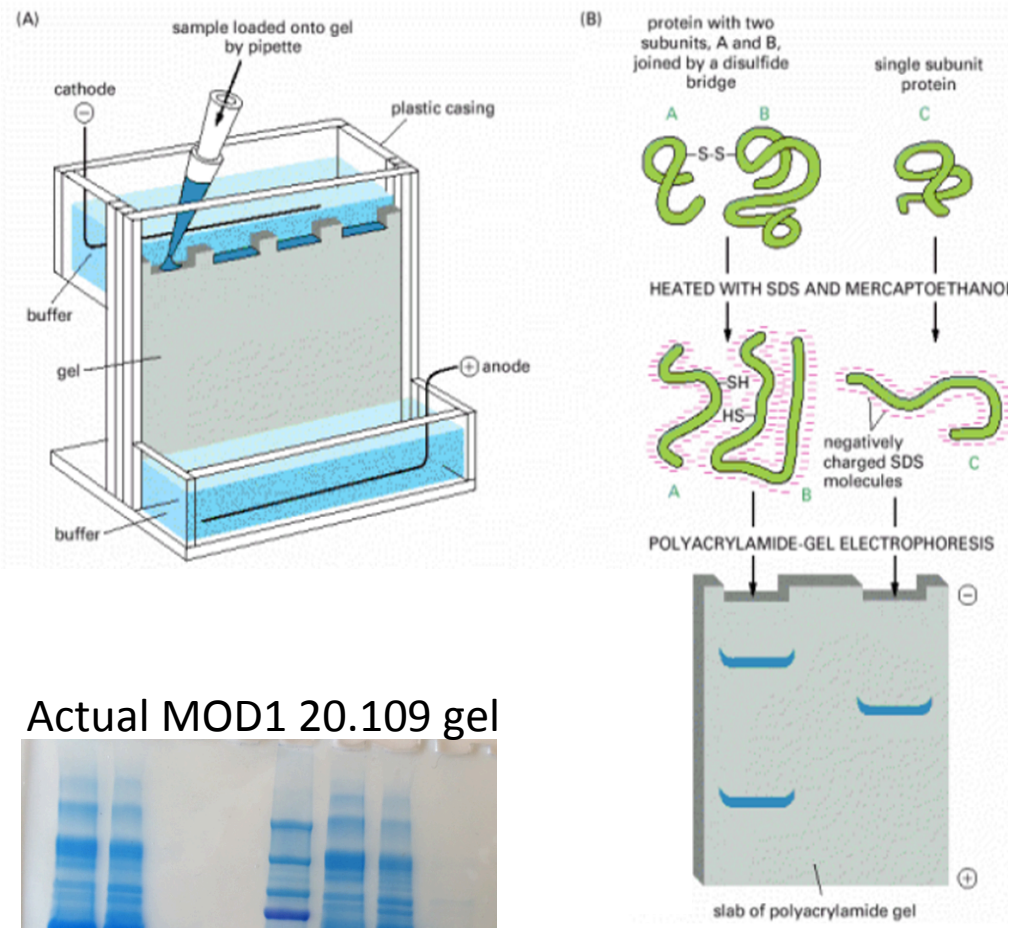
- Set aside whole cell extracts
  - Load relative amount of extract based on OD_{600} (from M1D4)

10 ml

sample	example		wt IPC		X#Z	
	- IPTG	+ IPTG	- IPTG	+ IPTG	- IPTG	+ IPTG
OD600	lowest: 0.5	0.75	0.9			
sample volume (µL)	15	15*0.5/0.75	8.3			
water volume(µL)	0	5	6.7			
total volume (µL)	15	15	15	15	15	15
add 6x buffer (µL)	3	3	3	3	3	3

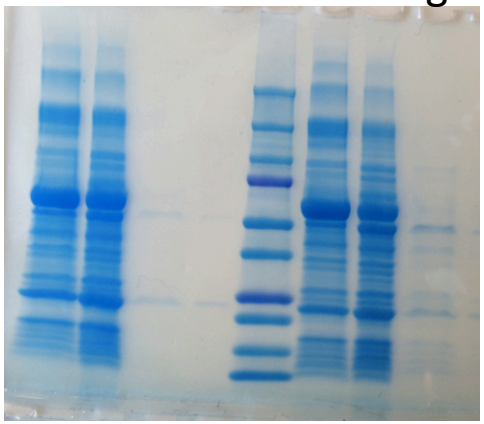
- SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel
  - separation by size ? shape ? charge ?

SDS-PAGE separates proteins by size



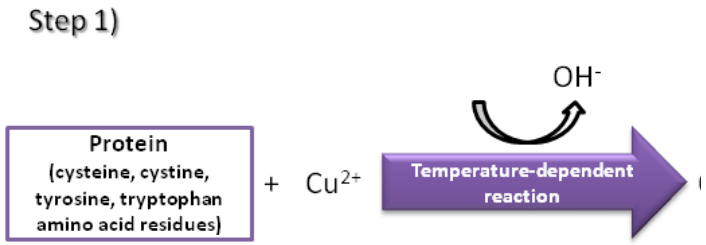
- Laemmli sample buffer:
  - + SDS: surfactant / detergent denatures proteins, coats them with negative charge
  - + β-mercaptoethanol reduces disulfide bonds
  - + bromophenol blue
  - + glycerol
- boiling denatures higher-order structures

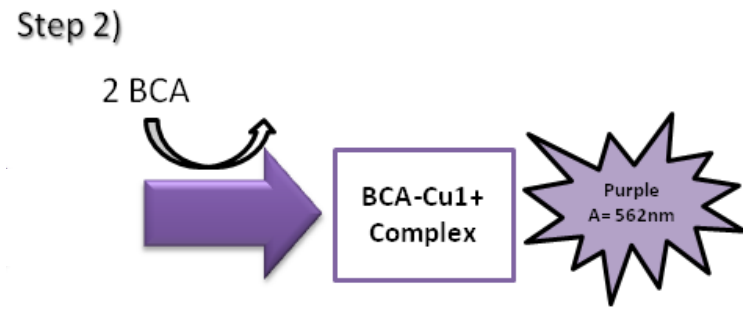
Actual MOD1 20.109 gel

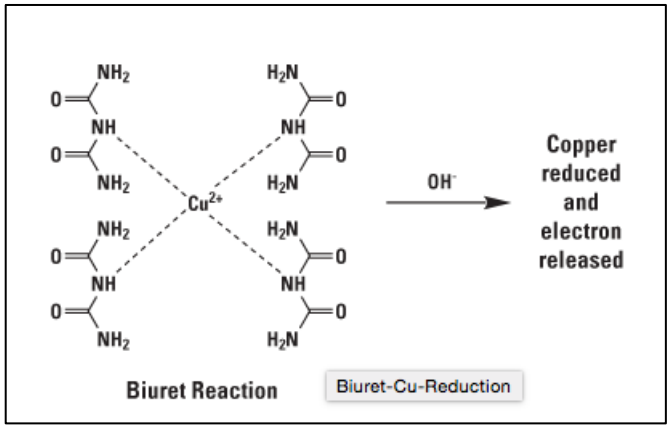


blue color=coomassie

BCA protein assay





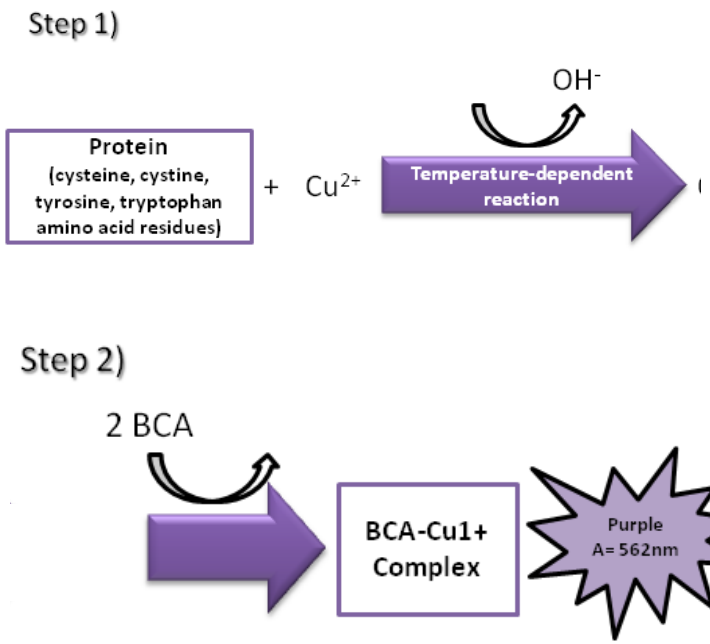


- BCA: bicinchoninic acid is a chromogenic reagent used to detect Cu^{1+}
- amount of Cu^{2+} reduced to Cu^{1+} is a function of protein concentration (also temperature dependent)
- BCA mixed with Cu^{1+} results in purple which absorbs at 562 nm
- Today we will use a microBCA assay with a working range of 0.5ug/ml-20ug/ml

BCA protein assay

State of most of the copper reagent if….

1) No protein in your elution (Cu^{2+} / Cu^{1+})
2) 10ug/ml protein in your elution (Cu^{2+} / Cu^{1+})
3) You didn't heat your Copper/Protein mix (Cu^{2+} / Cu^{1+})
4) You had protein in your elution but didn't add BCA reagent before reading your sample on the spectrophotometer (Cu^{2+} / Cu^{1+})



Today in lab

- Lyse 4 cell pellets (wt IPC -/+ IPTG and "good" mutant -/+ IPTG)
- Set aside aliquots for SDS-PAGE (M1D7)
  - add 6X sample buffer (Laemmli buffer) to each
- Purify protein (1 wt IPC + 1 mutant)
  - 2 steps: affinity purification + desalting
- Immediately aliquot 10 µL for microBCA assay and 15µL 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 assay
  - 1 hour incubation
  - Work quickly and purposefully