Announcements

- Pre-lab Lecture
  - Briefly, data from last time
  - SDS-PAGE
  - Affinity purification recap
  - Today in Lab (Mod 1 Day 6)
Diagnostic digests, etc.

- What might really large bands be?
- Why might really small bands not show up?
- What might the identities of extra bands be?
- What could *unaccounted for* extra bands indicate?

- Besides cell normalization purposes, why might getting –IPTG and +IPTG OD values be useful?
SDS-PAGE preparation

- You will make whole cell extracts with equal cell #s
  - Based on OD$_{600}$ reading, normalize
    \[
    \frac{7.5 \text{ mL}}{1.0} + \frac{7.5 \text{ mL} \text{ H}_2\text{O}}{0.5} = \max \leq 15 \text{ mL}
    \]

- Gel separates proteins based on size, shape, charge

- Sample preparation
  - SDS: coats proteins w/uniform (-) charge
  - β-Me: breaks S-S bonds
  - Boiling: denature → use locks!
  - Sample Buffer has SDS, β-Me, plus glycerol, dye (BPB)
SDS-PAGE visualization, analysis

- Visualization: Coomassie stain (binds certain AA)
- Two ladders: visualization, quantification

Kaleidoscope

Unstained

150 ng (@100 KDa)

750 ng (@50 KDa)

150 ng (@20 KDa)
Affinity purification

- Basis: 6x His tag in vector bound to metals with high [imidazole]
Today in Lab

• Lyse cell pellets in BPER
  – BSA “carrier”, protease inhibitors

• Run a 15 µL aliquot through SDS-PAGE
  – Two ladders also

• Purify protein from the rest (long!)
  – Immediately take 10 µL aliquot and measure concentration
  – The rest is stabilized w/BSA, to be titrated against calcium next time