Announcements

- Discuss mid-term feedback
- FNT heads up: methods, 3Q (got rid of one)
- Day 7: quiz; staggered arrivals (~1 – 1.5hrs)
- Office hours

Pre-lab Lecture

- SDS-PAGE
- Affinity purification recap
- Today in Lab (Mod 2 Day 6)
**SDS-PAGE preparation**

- You will make whole cell extracts with equal cell #s
  - Based on $\text{OD}_{600}$ reading, normalize
    $$V_{\text{max}} = 15\text{mL}$$
    (1) $7.5\text{mL} + 7.5\text{mL} \ H_2\text{O}$
    (2) $15\text{mL}$

- Gel separates proteins based on size, shape, charge

- Sample preparation
  - SDS: coat proteins with negative charge
  - $\beta$-Me: breaks S-S bonds
  - Boiling: denature higher-order structures
  - Sample Buffer has SDS, $\beta$-Me, plus:
    - Glycerol, BPB dye

*Acrylamide - toxic*
SDS-PAGE visualization, analysis

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

- Kaleidoscope
  - In real-time, pre-stained

- Unstained
  - Respond to the stain
  - 150 ng (@20 KDa)
  - 750 ng (@50 KDa)
  - 150 ng (@100 KDa)
Affinity purification

- Basis:
  - His-tag in vector
  - 6x, binds to metals
  - Coat agarose beads with Ni\(^{2+}\)
  - Add protein mixture
  - Wash away non-His proteins
  - Elute His-tagged protein

Courtesy of Life Technologies, Carlsbad, CA. Used with permission.
Today in Lab

- Lyse cell pellets in BPER
  - BSA “carrier,” protease inhibitors
  - Add 4 mL lysis enzymes
- Run a 25 µL aliquot through SDS-PAGE
  - Two ladders also → boil these too
  - Stick with equal volumes if you have < 25
- Purify IPC 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
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