PBC Day 1 Recitation Notes

Agenda:
I. Why purify proteins
II. Why does purification work?
III. β-galactosidase Intro
IV. Module Overview
V. β-galactosidase activity assay

I. Why purify proteins

- structure determination
- enzymatic activity
- to determine binding partners in the cell
- antibody production

II. Why does protein purification work (what properties can we take advantage of?)

- proteins have different charges
- proteins have different hydrophobicity
- proteins have different substrates (and binding affinity to those substrates)
- proteins have different sizes and quaternary structures
- proteins have different solubilities

III. β-galactosidase intro

- functions as a tetramer
- breaks down lactose in the cell (see overhead)

IV. Module Overview and Day 1 techniques (see handout)

V. β-galactosidase activity assay (see handout as well)

- Information that you can get from this assay:
  - total activity of a sample
  - yield (how much at each step of a purification)
  - total activity + total protein --> specific activity --> measure of purity
- Can be quantitative (using spec) or qualitative (by eye)
- When doing assays, need to time accurately!
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Please see:-
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Help Aliaa with the β-galactosidase Purification Scheme!

**Bacterial cell pellet**

- Thaw at 37°C

**A. Disrupt Cells**
1. Freeze thaw
2. Lysozyme
3. Triton X-100 + ddH₂O

**B. Treat to Remove DNA**
1. DNase I

**Centrifuge**

**Crude Lysate**

- Other proteins + β-gal
- from lysis cells

Notes:
- Two strains: CS936 or H15461
- Freeze thaw - outer membrane
- Lysozyme - break down cell wall
- TritonX-100 - bursts inner membrane
- DNase - break down genomic DNA
- Removes insoluble material
Crude Lysate

C. Precipitate Proteins
   1. Ammonium Sulfate (45%)

D. Column purification
   1. PD-10 (desalting)

1. DEAE (ion exchange)
2. PD-10
1. APTG-affinity

Purified β-gal
ONPG \text{ (Substrate for $\beta$-gal)}

$2$-Nitrophenyl-$\beta$-D-galactopyranoside

$\beta$-gal cleaves here

Galactose \quad O-nitrophenol (ONP)
\text{YELLOW absorbs @ 420nm}

$\beta$gal $\times$ ONPG cleavage $\times$ ONP product