UCLA
Protein Expression Lab

Protein Purification and Gel Filtration

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What is Protein Purification?

Target Protein

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Impurities

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- Protein purification is a multi-step process for separating our target protein from a complex mixture of cell membranes, organelles, non-target proteins and other impurities.

Procedure

- This is for a 10g bacterial pellet in which target protein expression has (hopefully) been induced.
- To a 50ml conical vial, add:
  - 50ml native wash buffer
  - 500µl Protease Inhibitor Complex (PIC)
  - 20µl DNAse I
  - 20mg Lysozyme
  - 7.4µl BME
- Resuspend the pellet in the solution.

French Press

- What does the French Press do for us?
  - It forces the cells individually through a tiny opening at high pressure, causing the cells to burst, releasing the contents.
- Steps
  - First clean the French Press with 3 H_2O rinses.
  - Run the sample through the machine twice to ensure full cell lysis.
  - Clean with 2 more H_2O rinses and a final rinse with ethanol.

Centrifuge

Where do I put the laundry?

- Spin down the lysate in the low temp. ultra-centrifuge at 15,000rpm for 30 min.
  - Make sure that everything is balanced, and that you are using the correct plastic centrifuge tubes (small rotor only)
  - Start the centrifuge and run like hell
  - All of the insoluble bodies will be in the pellet, such as lipids, organelles and large or hydrophobic proteins
  - The target protein should be in the supernatant
  - Remove 9µl aliquots of the supernatant and pellet for analysis.

Ni-NTA Beads

- Next we add Ni-NTA Beads to the supernatant, which are made of a resin infused with nickel.
- Our target protein has been engineered to contain a polyhistidine sequence (6xHis). This region binds tightly to the nickel beads, removing the protein from the solution.
- The vial is nutated for an hour at 4 ° C to allow as much of the protein to bind to the beads as possible.

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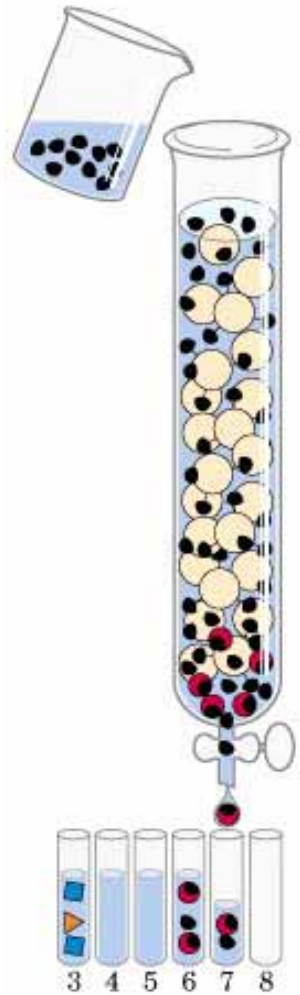
Affinity Chromatography

- Next the contents of the flask are poured into a column, and the flowthrough is run over the beads 4 times. Save the F/T.
  - Add 10ml wash buffer 2x, collect fractions
  - Add 10ml hi-salt wash, collect fraction
  - Add 10ml low-conc. imidizol, collect fraction
  - Elute target protein with 20 1ml washes containing a high concentration of imidizol
- Never allow the column to run dry.
- Hopefully elutions 1-20 contain our purified target protein.



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Phast Gel

- To the first gel add:
  - Broad Standard
  - Supernatant
  - Pellet
  - F/T
  - Wash Buffer 1
  - Wash Buffer 2
  - Hi-salt Rinse
  - Imidizol Rinse
- To the second gel add:
  - Elution 1
  - Broad Standard
  - Elution 2
  - Elution 3
  - Elution 4
  - Elution 5
  - Elution 6
  - Elution 7

Bio-Rad Gel Filtration

- Commercial size exclusion chromatography system
  - The purified protein is loaded into the column, and the target protein, along with any additional impurites is eluted at different times based on protein size
  - A mixture of known proteins is loaded in a separate step for size comparisons
  - Smaller proteins must flow through pores in the gel beads, causing more collisions and longer elution times
  - Larger proteins bypass the pores and simply flow through the spaces between beads, leading to fast elution
  - The absorption of the flow-through at 280nm is plotted and the elution time of the target protein is compared to the standards giving a rough estimate of protein size.
  - The fraction which contains the target protein should hopefully be pure

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The End