**Purifying unfolded OMPs on a benchtop Q column in 8M urea**

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**Purification overview:**

1. Dissolve IB in 8M urea, spin down and filter.
2. Run on Q column, elute with NaCl.
3. Run gel of fractions, combine protein-containing fractions.
4. De-salt/concentrate protein with centrifugal device.
5. Spec to get exact concentration.
6. Aliquot and store protein at -80˚C.

**Buffers used** (make buffers ahead of time and freeze at -20˚C)

Urea buffer: 8M urea, 20 mM Tris, pH 8

Elution buffer: 8M urea, 500 mM NaCl, 20 mM Tris, pH 8

**Note:** When purifying full-length OmpA (OmpA325), buffers should contain 2 mM TCEP to prevent dimerization.

**Prepare OMP sample**

1. Thaw OMP inclusion body (from previous IB prep).
2. Dissolve IB in 6 mL urea buffer and let sit for ~15 min.
3. Aliquot into eppendorf tubes (1 mL each) and spin down in microcentrifuge at max speed for 5 min.
4. Carefully pipet off supernatants, combine, and filter through 0.45 µm syringe filter.
5. Take absorbance scan (dilute ~20 fold with urea buffer) and remove sample for SDS-PAGE (16 µl sample + 4 µl 5X SDS loading buffer).
   1. Calculate A260/A280 to see how dirty protein is (pure protein should have A260/A280~0.6).

**Run through Q column**

1. Use a 6 mL Q column. Run 30 mL of water through to remove ethanol.
2. Equilibrate column in 8M urea by running 40 mL of urea buffer through.
3. Apply OMP sample to column. Collect flow-through.
4. Once OMP has soaked in, run 30 mL urea buffer through to wash. Collect 10 mL fractions.
5. Run 40 mL of 20% elution buffer (100 mM NaCl) to elute OMP. Collect 10 mL fractions.
6. Run 30 mL of 100% elution buffer (500 mM NaCl) to elute nucleic acids. Collect 10 mL fractions.
7. If purifying another sample, return to step 2 and repeat above steps.
8. Run 50 mL of water then 20 mL of 20% ethanol to wash and store column.
9. Wrap top and bottom of column in parafilm to prevent drying out.

**Note:** When purifying Omp85, elute with 30% elution buffer (150 mM NaCl).

**Recover purified OMP (in salt)**

1. Take samples of fractions for SDS-PAGE (16 µl sample + 4 µl 5X SDS LB).
2. Load 4 µl of markers, 5 µl of “before” sample, and 10-15 µl of fractions on 10% or 12% gel.
3. Run at 200V for 30 min. Stain and destain gel.
4. Combine OMP-containing fractions.
5. Take absorbance scan to get approximate concentration and check A260/A280.
6. Also spec high salt elution fractions to see nucleic acid (peak at 260).
7. If necessary, freeze sample at -20˚C overnight.

**De-salt and concentrate OMP**

1. Use a centrifugal filtration device to de-salt the sample (we have 15 mL and 4 mL tubes, with 10 kDa or 3 kDa filters).
   1. Use 10 kDa filters for OmpA325, OmpT, FadL, and Omp85.
   2. Use 3 kDa filters for OmpA171, PagP, OmpX, and OmpW, and OmpLa.
   3. For 15 mL tubes: spin at 5000 rpm (20 min for 10 kDa, 40 min for 3 kDa)
   4. For 4 mL tubes: spin at 7500 rpm (20 min for 10 kDa, 40 min for 3 kDa).
2. Pre-rinse the filter by filling with water and spinning. Pipet out excess liquid after spinning.
   1. Add the water in increments and mark each amount on the side of the tube to help you figure out how much you have later.
3. Adding the maximum volume at a time (15 mL or 4 mL), begin spinning down protein until entire sample has been added to tube. Spin for an additional time to concentrate the sample down to a small volume.
   1. A good practice is to spec the flow-through after the first spin to make sure protein is not leaking through the filter.
   2. **Note:** protein sample must be re-pipetted to mix well because protein will be concentrated toward the bottom after spinning.
4. Add urea buffer to bring the volume to the max amount and spin again. Do this successive times to de-salt the sample.
5. With each dilution, calculate the final salt concentration. Continue with rounds of spinning and diluting until salt concentration is below 1 mM.
6. Spin for additional time to concentrate sample to a few mL.

**Store purified OMP**

1. Recover sample from centrifugal device (re-pipet sample to resuspend protein at bottom, but be careful not to introduce too many air bubbles!)
2. Measure absorbance and calculate exact concentration (make several dilutions and average values from scans).
3. Concentrate further if necessary.
4. Aliquot sample into tubes, label, and store at -80˚C.
5. Make note of the number and volume of tubes, the protein concentration, and the urea concentration the protein is in.