**TAP PURIFICATION DAY 1**

1. Grow cells to OD$_{600}$ $\approx$ 1.5.
2. Spin cells down using JLA8.1 rotor for 10 minutes at 6000 x g
3. Wash cells with water and centrifuge in the tabletop centrifuge at 4°C for 10 minutes.
4. Resuspend cell pellets in 25 mL of TAP extraction buffer (40mM Hepes-KOH, pH 7.5; 350mM NaCl; 0.1% Tween-20; and fresh 1ug/mL pepstatin A, 2ug/mL Leupeptin, 0.5mM DTT and 1mM PMSF and use a 30 mL syringe with a 21 gauge needle to transfer the whole cell solution into liquid nitrogen.
5. Transfer the frozen whole cell solution to a blender with a few pieces of dry ice and lyse the cells for $\sim$20 seconds.
6. Allow the extract to thaw and clarify by centrifugation. Spin lysate at 3,000-4,000 x g for 30 minutes in a tabletop centrifuge followed by an additional centrifugation at 60,000 rpm in either the 70 Ti or 90 Ti rotor for 1.0 hour.
7. Wash 500uL IgG Sepharose with 5mL TAP Extract Buffer twice.
8. Add 500uL washed IgG Sepharose to the cleared protein lysate and incubate on the rotating wheel overnight at 4°C.

**TAP PURIFICATION DAY 2**

1. Transfer the extract resin suspension to a 30mL Bio-Rad Polyprep column and allow extract to drain by gravity.
2. Wash beads with 20mL TAP extract buffer by gravity flow.
3. Wash beads with 10mL TEV cleavage buffer (10mM Tris, pH 8, 150mM NaCl, 0.1% NP-40, 0.5mM EDTA, and freshly added 1mM PMSF, 2ug/mL Leupeptin, 1ug/mL Pepstatin A, and 1mM DTT).
4. Resuspend beads in 1mL TEV cleavage buffer and transfer to a 1.5mL microfuge tube. Add 10uL of AcTEV protease (Invitrogen) and cleave for 1hr @ 30°C, shaking 900 rpm.
5. Return suspension to the column and elute TEV cleaved products by letting the buffer drip out of the column.
6. Wash the resin with 3 mL Calmodulin binding buffer (10mM Tris, pH 8, 300mM NaCl, 1mM MgOAc, 1mM imidazole, 2mM CaCl$_2$, 0.1% NP-40, and freshly added 1mM PMSF, 2ug/mL Leupeptin, 1ug/mL Pepstatin A, and 0.5mM DTT) in the column and pool the 3mL wash with the TEV cleavage elution.
7. Add 3uL of 1M CaCl$_2$ to the TEV cleavage elution.
8. Wash 0.5mL Calmodulin Sepharose with 5mL Calmodulin binding buffer with 0.3M NaCl x 3.
9. Mix TEV cleavage elution with the washed 0.5mL of Calmodulin Sepharose and incubate overnight on the rotating wheel at 4°C.
1. Wash Calmodulin Sepharose with 3x5mL CBB with 0.3M NaCl followed by 2x5mL CBB with 0.15M NaCl.

2. Obtain a cap and a plug for the 10mL column and elute with Calmodulin elution buffer (CEB) (10mM Tris, pH 8, 0.15M NaCl, 1mM MgOAc, 1mM imidazole, 2mM EGTA, 0.1% NP-40, and freshly added 1mM PMSF, 2ug/mL Leupeptin, 1ug/mL Pepstatin A, and 0.5mM DTT). Elute by adding 1 mL buffer to the CAM Sepharose beads and incubating for 5 minutes on a rotating wheel at room temperature.

3. After 5 minutes, collect the eluate by gravity flow into a 1mL centrifuge tube.

4. Repeat x 4. (5 elutions total, alternatively can perform 10 elutions of 500uL each).