XMAP215 Expression & Purification

(1) Transfection SF+ Cells
in a sterile 24 deep well plate (QIAGEN #19583)

400 µl SF900II medium
2 µl Midi prep (BACMID)
2 µl Midi prep (XMAP215-His_6-GFP in pOEM vector)

400 µl SF900II medium
12 µl Escort IV (SIGMA #L-3287)

- Combine two reactions above and leave at RT 15-45 min.
- Add reaction to 200 µl SF+ cells (10^6 cells total).
- Incubate o/n at 200 rpm.
- Next day add 1 ml SF900II medium with antibiotics.

(2) Make P1

- 5 days after transfection.
- Filter 2 ml through a 45µ filter.
- Store filtrate at 4°C.

(3) Make P2
in a sterile 24 deep well plate

- Infect 2 ml cells (10^6 cells/ml) with 100 µl P1.
- Make P2 (filter, store at 4°C) 48 h after infection.

(4) Make BIIC stock.

- Infect 50 ml SF+ cells (10^6 cells/ml) with 0.5 ml P2.
- Monitor cells 24 h after transfection on CASY counter.
- Harvest and freeze when cells are 22-23 µm in diameter, uninfected cells will be around 19 µm.
- Harvest at 1500 rpm for 5 min.
- Resuspend cells in 2 ml freezing medium (90% SF900II medium, 10% DMSO).
- Store 400-500 µl aliquots at -80°C or LN.

(5) Expression in SF+ Cells

- Infect 500 ml SF+ cells (10^6 cells/ml) with BIIC stock.
- Harvest cells after 54 hrs of infection.
- Resuspend pellet in ice-cold lysis buffer (50 mM HEPES pH 7.5, 50 mM NaCl, 5% glycerol, 0.1% Triton-X-100, 10 U/ml Benzonase and 1x protease inhibitors mix (1 µg/ml AMPSF, 10 µg/ml antipain-HCl, 6 µg/ml chymostatin, 2

µg/ml aprotinin, 0.7 µg/ml pepstatin A, 0.5 µg/ml leupeptin, and 3.6 µg/ml E64).
- Homogenize using a Dounce homogenizer.
- Clarify crude lysate by centrifugation. Spin in TPC 10 ml BECKMAN tubes (# 355647) in a MLA80 rotor at 50k for 40 min at 4°C.

(6) Cation exchanger

- Load supernatant onto an SP-sepharose column (HiTrap SP-HP, GE Healthcare) equilibrated with cation buffer (6.7 mM HEPES-/KOH pH 7.5, 6.7 mM MES, 6.7 mM Na-acetate, and 200 mM NaCl).
- Wash the column with equilibration buffer.
- Elute the protein with a continuous salt gradient (200 mM-600 mM NaCl) using a BioCAD SPRINT system.
- Pool the peak fractions.

(7) HisTrap

- Equilibrate Ni_2–sepharose column (GE Healthcare HisTrap HP) with imidazole buffer (50 mM $NaPO_4$ pH 8.0, 300 mM NaCl, 15 mM imidazole, 10% glycerol, 1 mM $MgCl_2$, 10 µM Mg-ATP).
- Bring the pooled peak fractions to 15 mM imidazole.
- Load fractions on the Ni_2–sepharose column.
- Washed the column with 30 mM imidazole and 60 mM imidazole buffers.
- Elute XMAP215 with 500 mM imidazole.

(8) Size exclusion
- Pool peak Ni-column fractions.
- Buffer exchange to storage buffer (10 mM Bis-Tris Propane, 10 mM TrisHCl, 330 mM NaCl, 1 mM DTT, 10% glycerol) using a NAP25 desalting column (GE Healthcare).
- Pass XMAP215 through a size exclusion chromatography column (GE Healthcare Superdex 200 16/60) pre-equilibrated with elution buffer: BRB80 (80 mM PIPES-/KOH pH 6.9, 1 mM $MgCl_2$, 1 mM EGTA), 150 mM KCl, and 1 mM DTT.
- Aliquots of were flash frozen with glycerol added to 10% and stored in liquid N2 .
- (Only the single peak fraction from the size exclusion chromatography column was used for TIRFM, mostly using freshly purified protein.)