The Liu Laboratory protocol — PSII isolation *Arabidopsis*
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1. Chloroplast Isolation:
   A. Harvest and wash Arabidopsis plants grown in flats. Cut and save leaves only.
   B. Fill cold blender cup with leaves, add Chloroplast Isolation Buffer to about the middle of the blender cup, give 1 second burst, push down leaves, give 1 second burst, repeat until all leaves are blending and grind for 7 seconds.
   C. Filter through two layers of cheesecloth and one layer of Mirocloth into cold flask, Save the paste and blend it again for 7 seconds, This can increase 30% thylakoid yield.
   D. Spin 5 min at 3,500 rpm (GSA rotor) pure off supernatant.
   E. Resuspend pellets in small amount of Buffer A (MES 50mM, 25% glycerol, 15mM NaCl. pH6.0). save in -80°C aliquots, pro tease inhibitor.
   F. Read chlorophyll on spec and adjust chloroplast concentration at 0.5 mg/ml with Buffer A. You should get about 90 mg chlorophyll per flat. A/B=2.74 (61308 prep).
2. Detergent treatment
   A. Add protease inhibitor (EDTA free) into the supernatant. Add 1/25 Volume of 10% OTG and 1/10 Volume of 10% DM freshly made in Buffer A and incubate on ice in the dark 30min
   B. Spin 25,000xg for 30 min. Save supernatant. You should see white starch pellet.
3. His-column Isolation of PSII core in the dark (foil wrapped)
   A. At the same time of centrifugation, equilibrate His-column with Buffer A plus 0.04% DM. Load 10mg chlorophyll into the column and invert the column several times until the resin bed is dispersed in the solution. Keep the column in the dark for 20 min and adjust the flow rate at 4-6 drops/min. It takes about one hour to pass the solubilized TK into the column. Save the flow-through. You should see dark column. 0.04% DM and recover
   B. Flash wash the column with 3x column volume Buffer B (25mM HEPES-KOH (pH7.5), 100mM NaCl, 25% glycerol) at maximum flow rate (this procedure is essential to remove most non-specific binding proteins such as free psbQ and 70kD species). Then wash the column with 3x column volume Buffer A (+0.04% DM) at low speed. You should see the column is still moderately dark green.
   C. Elute the column with Buffer C (25mM MES (pH6.), 50mM histidines, 15mM NaCl, 25% glycerol). Connect the column to fraction collector and adjust the collection at 60 drops per tube. Keep 9,10,11,12,13,14 numbered tubes. You should notice tube 10 and 11 are darkest.
4. Concentration by centricon (100,000 kD cutoff)
   A. Spin tube 9,12,13,14 first at 6500 rpm in SS34 rotor. Check every 10 min. Resuspend the pellet formed inside the centricon on the wall. Add tube 10 and 11 PSII core solution. Once the PSII core is more concentrated, add Buffer D (50 mM MES (pH6.0), 0.4M sucrose, 10mM $CaCl_2$ (from Dr.Yocum, personal correspondence)).
   B. Read Spec and check the A/B ratio. You should get A/B=4.5. The total yield is about 2.5%.