

The Liu Laboratory protocol — PSII_OPQ isolation
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ISOLATION OF MSP SEPARATELY FROM 24 KD AND 17 KD PROTEINS FOLLOWING OEP MEMBRANE

1mg chl/ml in 1 M NaCl-RB (save some OEP membrane for control)

--Resuspend at 1mg chl/ml in 1 M NaCl-RB (2M Stock) on ice in dark for 1h. ✓

--Spin 18,000 rpm 25min, save pellet.

--Resuspend at 1mg chl/ml in 1 M NaCl-RB Up and Down (homogenizer)

--Spin 18,000 rpm 25min, save pellet. [SAVE]

--Resuspend pellet at 1mg chl/ml in 1 M CaCl₂-RB, incubate on ice in dark for 1 h

--Spin 18,000 rpm for 25 min, and save supernatant (MSP in it) at -80C. Pellet? **SAVE**

--Dialysis of supernatant:

Dialysis in 4 liter 5 mM Mes, pH 6.0, overnight. Change fresh buffer in evening.

--Pour dialysis solution into beaker; pour solution into centrifuge tube.

--Spin 18,000 rpm for 25 min, ^{optional}

--Filter supernatant in Nalgene filter,

--Concentrate in Centricon Plus-20 (MW30,000 kD cutoff) and harvest with pipettor, store in centrifuge tube on ice. (final 200ul to 500ul)

RECONSTITUTION MEMBRANE PREPARATION: New OEP membrane

--Resuspend with 1 mg chl/ml in 1 M CaCl₂-resuspension buffer for 1 hr in dark on ice.

--Spin 18,000 rpm for 25 min, keep pellet.

--Resuspend up and down with homogenizer with 1mg chl/ml CaCl₂-RB

--Spin 18,000 rpm for 25 min, keep pellet.

--2 up and down washes in homogenizer with RB to remove CaCl₂ followed by 18,000rpm 25min centrifugation, keep pellet.

--Resuspend pellet in 100mM NaCl in RB and 25 min centrifugation

--PAGE to check whether MSP is completely washed off, also the MSP sample.