Sucrose gradient

Sample preparation	Ultracentrifuge has to be reserved in advance in the calendar. Sample - approx. 0.5 mg Chl in thylakoid - there is no standard, but you cannot load too much - 2-3 mg because then the bands won't be distinguishable. Tubes are in the centrifuge room. Use the less transparent, shorter ones.
Sucrose solution (50 mL) 0.5 M sucrose 20 mM HEPES pH 7.5 0.06% α-DM	Making sucrose gradient: **fill** the tubes and freeze them in **-60 °C** or -80 °C for one night (freezer on the left). The tubes should not be completely full - 1 ml less or so. Then take them out and leave them in 4° C for 4 hours or one night in the cold room. Before loading the samples take out the volume you need to load. e.g. you load 500 uL samples, take out 500 uL.
Solution 1 (50 mL) 10 mM HEPES pH 7.5 5 mM EDTA	Add around 300-400 uL and shake the samples a bit. Spin down at 7,000 RMP 10 min, centrifuge in the cold room. Supernatant should be transparent, pellet green. Resuspend the pellet by washing it with the solution and spin down again.
Solution 2 (50 mL) 10 mM HEPES pH 7.5	Wash the samples in Solution 2. Resuspended the pellet and spin down 7,000 RPM 10 min.
Solution 3 (50 mL) 10 mM HEPES pH7.5 1.2% α-DM	Add the same volume of Solution 3 as of Solution 2 in order to make a final α-DM concentration 0.6%. Solubilize the samples for 10 minutes with shaking in between (before loading the samples, they can be also spun down but Chen doesn't do it).

Loading samples	Take out the volume of your loading sample from your sucrose gradient before loading it in the tube. Carefully load, put the pipette tip next to the top. Sucrose concentration is rather low at the top and you don't want to disrupt the gradient.

Balancing tubes	All the tubes have to be balanced (in principle only pairs, but Chen does all) by adding Solution 2. There is a scale in the cold room and special holder for the tubes for weighting - red stand and black tube. Put the tubes very carefully in the holder (2nd drawer in the cold room, there is a special screwdriver for the lids, don't mix the lids).
Ultracentrifuge	Take the rotor (SW41, all black, silver top) and your samples to the centrifuge room. Put the tubes very carefully on the rotor. They have to move a bit (check it multiple times). When you put the rotor in the centrifuge correctly, it will click. Settings: 40 000 RPM, 17 hours, 4 °C Max acceleration No break It doesn't matter if I first press vacuum or start. In the latter case it will stop around 3000 RPM and after the vacuum is established it will go to the max speed. Always **wait until it reaches max speed** to see that everything was done correctly! Write down everything in the notepad.
Collecting bands	The brakes for ultracentrifuge are off, it takes +/- 1 hour to stop. After it is done, press vacuum. **Write down the number** of spins and turn off the setup. Put the tubes in the gray stand (still in the centrifuge room). First drawer in the cold room - syringe with a long needle. Take a picture of the thylakoids separated in the sucrose gradient with the camera from the wet lab. Take all the bands, also the free pigments. When collecting the bands the syringe must go to the lower border of the band. Use liquid nitrogen for freezing the samples and store in -80 °C.
Dilution buffer = sucrose gradient without sucrose	