Lipid Nanodiscs assembling Protocol

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A – Materials and Reagents

1- Main reagents

- MSP-protein (for special properties and information related to protein expression and purification, please refer to us directly), stored at -80°C
- Lipids, stored at -20°C in powder form or in Chloroform
- Protease inhibitor cocktail (EDTA free), 100x stock solution, stored at -20°C
- DTT (dithiothreitol), stored in powder at 4C, or in liquid stock at -20°C
- Detergent; usually Triton TX-100 (MW=647g/mol), stored at RT
- Bio-Beads SM-2(from BioRad, Cat. #152-3920), stored at RT

2- Buffer preparation

"MSP1D1 Buffer" contains: 25mM Tris HCl, 100mM NaCl, 0.5mM EDTA, prepared at pH=7.5

3- Lipid preparation: 50mM/200mM (lipid/detergent)

Prepare a 200mM Triton X-100 solution in MSP1D1 Buffer

Weight correct amount of lipid to get a 20 to 50 mM conc. in the previous detergent solution

[Note: To obtain completely dissolved lipids in detergent solution, it can take some time; sonication can help]

[Note: If lipid is originally in Chloroform, a drying step under N2 is required]

B – Nanodiscs assembling reaction

[Before assembling Nanodiscs including target protein, one has to adjust the ratio of MSP protein to lipid ratio]

Assemble the nanodiscs in the following order:

- 1: Lipids solution in detergent
- 2: Add protease inhibitor + DTT
- 3: Add MSP1D1
- 4: Adjust to expected final volume with MSP1D1 Buffer

Let the reaction standing for 2 hours

[Note: If target protein is directly included in Nanodiscs assembling, it can be added either before or after MSP1D1 protein]

C – Removing detergent with Biobeads

(to allow self-assembling of Nanodiscs)

[Note: 1g of biobeads is required to remove 0.07g of TX-100]

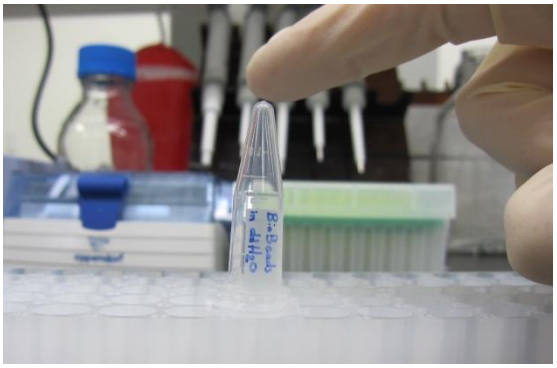
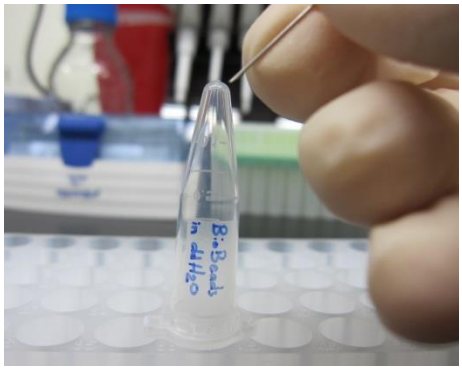
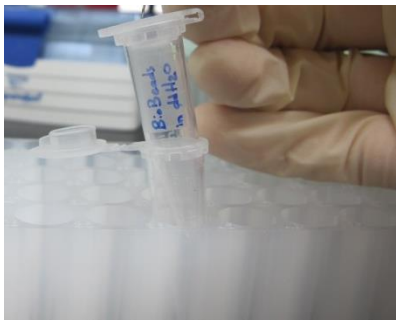
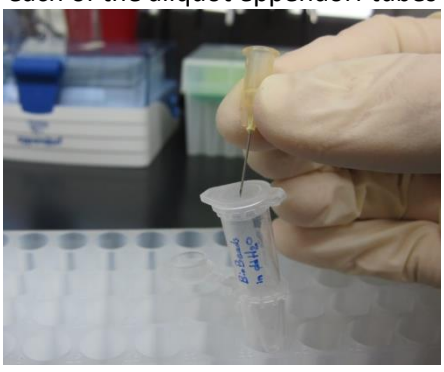
1- Weight the amount of biobeads require to remove detergent in all samples

Wash Biobeads with water, then MeOH, then rinse with a lot of water

2- Add a known amount of water to separate biobeads in aliquots (same number of aliquots as the number of samples)

[Note: For pipetting biobeads in water, it is usually prefered to use a 1000uL pipette, and cut the extremity of the pipette to allow Biobeads to be sucked into the tip]

3- Dry Biobeads: follow the special trick to make sure biobeads are completely dry before adding it to the Nanodiscs samples

(1) Invert the eppendorf tube and tap gently to discard all the biobeads at the top of the eppendorf tube. 	(2) Using the syringe, pierce three holes at the bottom of each of the aliquots eppendorf tubes 
(3) Re-invert the eppendorf tube and put in on a second empty (and not pierced) eppendorf tube 	(4) Using the syringe, pierce three holes at the top of each of the aliquot eppendorf tubes 
(5) Centrifuge the "two eppendorf system" aliquots to dry the biobeads, at around 4000rpm. Water will go through the holes and be discard in the second eppendorf tube	(6) Biobeads are then dry (ddH20 in the second Eppendorf can be discard)

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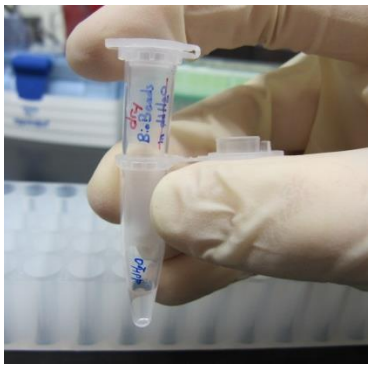
<td> 
Biobeads can then be added to the nanodiscs samples.</td>
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4- Nanodiscs samples containing biobeads are shaken 3 to 4 hours (to overnight) at RT (25°C), to completely remove the detergent.
[At least 3 hours is required to avoid to get Nanodiscs with random shapes, and bad distribution]

5- Remove biobeads from samples (same technique used as for drying biobeads, detailed in part C.3.)
6- Spin down "biobeads free samples" to remove any possible protein precipitation
Transfer to a new eppendorf (and keep the one with the pellet if investigation of precipitated protein is required)

D – Analysis of Nanodiscs

1. First step is to purify the sample by size exclusion chromatography (SEC)
A Superdex 200 prep grade gel filtration column (GE Healthcare) is usually used; with automatic fraction collector.

2. SDS-PAGE gel can be used to make sure that the different fractions of the peak observed in SEC correspond to Nanodiscs (means the gel will include a band for MSP1D1 protein – and one more band of your target protein of this one was included into Nanodiscs assembing)

3. Circular Dichroism (CD) can be used to make sure that the secondary structure of MSP1D1 protein is conserved (alpha helical secondary structure in Nanodiscs); as well as to make sure about the Nanodiscs thermal stability (expected to be between 70 to 80°C)

E – Example of one of our construct

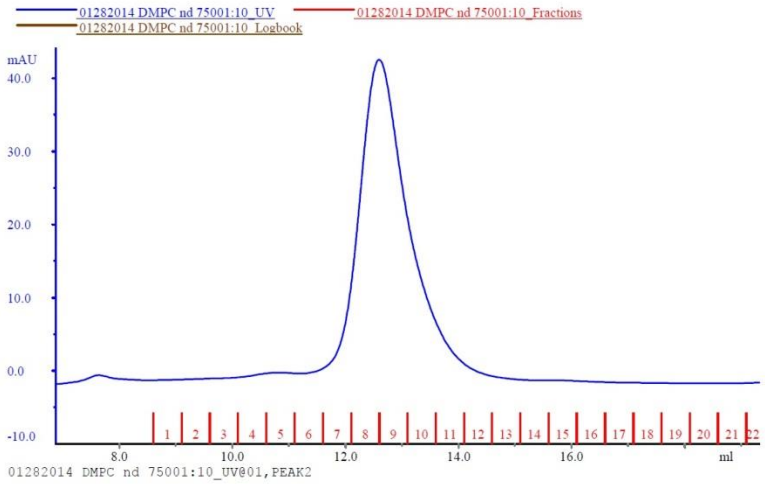
1. Assembling lipid nanodisc with MSP1D1 to DMPC lipid ratio 1 : 75, for a total reaction volume of 150uL
[Note: ratio given is molar ratio]

	MSP1D1 ***(5,09mg/mL, 212uM)***	*Lipid in detergent* ***(50mM/200mM)***	*Protease Inhibitor* ***(100x)***	*DTT* ***(336mM)***	*MSP1D1 Buffer*
Vol (uL)	84,8 **(120uM)**	27 **(9mM)**	1,5 **(1x)**	0.4 **(1mM)**	36,3

Stock concentration
Final concentration

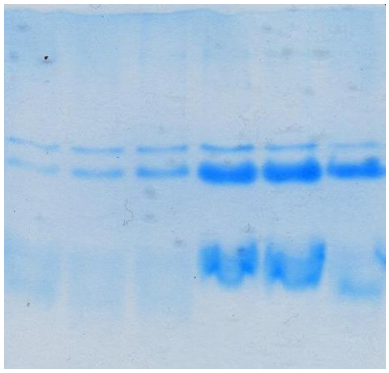
[Note: Each nanodisc contains 2 copy of MSP1D1 protein, so if final concentration of MSP1D1 is 120uM, the final disc concentration would be 60uM]

2. FPLC:
Purification of the nanodiscs by Size Exclusion Column, in order to have an idea of the size (in terms of molecular weight in kDa) of the lipid nanodiscs just assembled:



Regarding our calibration curve, the size of these Nanodiscs is approximately 267 kDa.

3. SDS Page gel: example of Nanodisc including target protein: the upper band corresponding to the target protein, the lower band to the MSP-protein (lower back ground comes from lipids)



3. CD Result

CD Curve: Secondary Structure	Melting Curve: Thermal stability
The alpha-helical structure of MSP1D1 is confirmed by the trend of the following curve	The thermal stability of empty nanodiscs is confirmed to be around 90°C