Reconstitution of membrane proteins into nanodiscs using his-tagged MSP1D1 protein and POPC phospholipids

Overview

This protocol describes the reconstitution of membrane proteins - already purified and solubilized in detergent micelles- into nanodiscs using membrane scaffold protein (MSP)1D1-His, the phospholipid Palmitoyl-oleoyl-phosphatidylcholine (POPC) and the detergent sodium cholate. After integration into nanodisc particles, membrane proteins are stable in buffered solutions without any detergent. They can be used in a number of different applications, such as surface plasmon resonance (SPR), electron microscopy, or NMR.

To facilitate subsequent experiments, we recommend to use scaffold proteins which have an affinity tag different from the target protein. E.g. if the target protein is rho1D4 or strep-tagged, the scaffold proteins can be his-tagged, which is convenient for purification of the complex, antibody detection, and immobilization to surfaces. For his-tagged target proteins, we recommend to use untagged scaffold proteins, or those carrying a different tag. Cube Biotech offers a range of his-tagged and untagged proteins in lyophilized format. Available scaffold proteins are mainly different in size, which in turn influences the diameter of the resulting nanodiscs, and which should be adapted to the expected size of the membrane-spanning part of the membrane protein.

Because of the diversity of proteins, this protocol can only give first guidelines. Optimization factors include, but are not limited to:
- concentration of target protein (This protocol: 2.5 mg/mL, but up to 20 mg/mL may be used)
- ratio of scaffold protein to target protein (This protocol: 1:20, but ratios from 1:10 to 1:150 may be used)
- ratio of lipid to scaffold protein (This protocol: 1:55, as described in (1,2))
- nature of phospholipid (This protocol: POPC, but other phospholipids and mixtures may be used)

In this protocol, target protein, scaffold protein, phospholipid, and cholate are mixed together. The detergent is removed by the addition of absorbant, and the remaining components spontaneously assemble into nanodiscs with integrated membrane protein. Size-exclusion chromatography separates fully assembled nanodiscs from unassembled components to yield a homogeneous fraction.

This protocol applies both for human and mouse MSP1D1-His proteins.

Alternatively, empty nanodiscs can be assembled and used in cell-free expression reactions, as described in a separate protocol. All our protocols are available for free download at www.cube-biotech.com/protocols.
Please contact us at contact@cube-biotech.com if you have questions or need assistance in adapting the protocols for your target protein.

Equipment

- ☐ 37°C incubator
- ☐ 4°C incubator
- ☐ End-over-end shaker
- ☐ Micropipettor
- ☐ Micropipetting tips
- ☐ Gel filtration column (e.g. HiLoad 16/600 Superdex 200 pg, GE Healthcare #28-9893-35)
- ☐ FPLC instrument (e.g. Äkta or BioRad) with integrated UV detector and fraction collector
- ☐ Magnetic stirrer
- ☐ Centrifuge for 2 mL microtubes (e.g. Eppendorf)
- ☐ 2 mL microtubes
- ☐ SDS PAGE equipment
- ☐ Optional: Western Blot equipment

Materials

- ☐ MSP1D1-His protein (e.g. 5 x 2 mg, Cube Biotech #26116) or
  mouse MSP1D1-His protein (e.g. 5 x 2 mg, Cube Biotech #26516)
- ☐ Palmitoyl-oleoyl-phosphatidylcholine (POPC)
- ☐ Sodium cholate
- ☐ Tris base
- ☐ Hydrochloric acid (HCl)
- ☐ Biobeads SM-2 (e.g. Bio-Rad 152-3920)
- ☐ Single use syringe (e.g. 1 mL)
- ☐ Single use needle (e.g. 0.45 x 25 mm)
- ☐ 0.45 micron filter
- ☐ Protein concentrator (e.g. Amicon Ultra 10 kDa)
- ☐ SDS-PAGE buffers and solutions
- ☐ Optional: PentaHis Antibody (Cube Biotech #40040)

Solutions and buffers

ND Protein Buffer (100 mL)

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris base, pH 7.4	20 mM	121.14	1 M	60.57 g/ 500 mL Set pH to 7.4 using HCl	2 mL
Instructions: Use ND Protein Buffer to prepare ND Lipid Buffer.					

ND Lipid Buffer II (50 mL)

Component	Final concentra-tion	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Na cholate	200 mM	430.55	-	-	4.3 g
ND Protein Buffer	1x				50 mL
Instructions: Dissolve sodium cholate in ND Protein Buffer. Always prepare fresh.					

Procedure

1. Prepare a membrane protein solution containing your target protein of interest. As a starting point, use 200 µL of the protein solution at a concentration of 2-5 mg/mL.

Note: Depending on the target protein, required protein concentrations might be up to 20 mg/mL.

2. Determine the molar concentration of target protein solution, using the molecular weight and extinction coefficient e.g. at 280 nm.
3. Calculate the required amount of MSP protein to be 20 times the molar quantity of the target protein.
4. Calculate the required amount of POPC to be 55 times the molar quantitiy of the MSP protein (or 1,100 times the molar quantity of the target protein).

Example: Target protein has a MW of 45,000 Da. Therefore, 500 µg protein in a 200 µL solution corresponds to 11 nmol of target protein in the 1 ml reaction mix.
To obtain a 20 fold excess of MSP protein, use 220 nmol of MSP, i.e. 5.57 mg (MW 25.309 g/mol).
For a 55 fold excess of phospholipid, use 12.1 µmol or 9.2 mg of POPC (MW 760 g/mol)

5. Resuspend the POPC in 500 µL ND Lipid Buffer II and incubate for 20 min at 37°C to fully solubilize the phospholipid.
6. Resuspend the MSP protein in 500 µL ND Protein Buffer in a 2 mL microtube. Add the target protein, solubilized in detergent solution.

Note: The protein was lyophilized from a solution containing 4 mg/ml protein in 20 mM Tris pH 7.4, 100 mM NaCl, 0.5 mM EDTA.

7. Add the POPC solution to the MSP and membrane protein solution, and incubate the entire mix at 4°C for 2 h.
8. During the incubation, equilibrate 2.5 g biobeads or similar adsorbant in ND Protein Buffer according to the manufacturer's instructions. Degas the solution by ultrasound to remove any oxygen solubilized in the biobead solution.

Note: It is important to degas the adsorbant solution for an efficient detergent removal.

9. Add the protein/POPC mix to 750 µL of the biobead solution and incubate at 4°C on an end over end shaker for 8-12 h.
10. Spin the solution at 10-12,000 x g for 2 min, and transfer the supernatant to a fresh tube. Add 750 µL of fresh equilibrated biobead solution and incubate at 4°C on an end over end shaker for 8-12 h.
11. Repeat step 10 at least one more time to ensure complete detergent removal.
12. Remove the supernatant, and filter the nanodisc mix through a 0.45 micron filter to remove precipitates that might have formed during incubation.
13. Apply the nanodisc mix on a gel filtration column. Apply the mix in several portions if necessary. Monitor absorbance at 280 nm.

Note: Optionally, analysis of fractions in the western blot can be done e.g. with Penta His antibodies which recognize the his-tagged MSP1D1 protein.

14. Collect fractions of ca. 500 µL size, and analyze the samples by SDS PAGE. MSP proteins have an apparent molecular mass of around 20 kDa.
15. Concentrate the elution fractions which contain the nanodiscs with inserted membrane protein using protein concentrators.
16. Freeze the nanodiscs containing membrane proteins at -80°C in a solution containing 10% glycerol for future use, or use them directly in the desired experiment.

References:
1. Bayburt, T. H., Grinkova, Y. V., & Sligar, S. G., 2002, Self-assembly of discoidal phospholipid bilayer nanoparticles with membrane scaffold proteins. Nanoletters, 2: 853-856.

2. Denisov, I. G., Grinkova, Y. V., Lazarides, A. A., & Sligar, S. G., 2004, Directed Self-Assembly of Monodisperse Phospholipid Bilayer Nanodiscs with Controlled Size. J. Am. Chem. Soc., 126: 3477-3487.