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Reconstitution of circularized nanodiscs using SpyCatcher-SpyTag

Shangwen Zhang
Qian Ren
Huan Bao (hbao@scripps.edu)
The Scripps Research Institute

Method Article

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Abstract

Circularized nanodiscs (cNDs) with diameters over 20 nm are useful tools for biochemical reconstitutions of membrane protein complexes[1]. However, the application of these large cNDs is limited by low yields and time-consuming procedures. To address this challenge, we herein present a protocol to ease the reconstitution of large cNDs using the SpyCatcher-SpyTag technology.

Introduction

Reagents

LB Broth (Teknova, cat. no. L9145)

Kanamycin monosulfate (Sigma, cat. no. BP861)

LB agar Kanamycin plates (Sigma, cat. no. L0543)

1,2-dioleoyl-sn-glycero-3-phosphocholine (PC) (Avanti Polar Lipids, cat. no. 850375)

Nitrilotriacetic acid (Ni^{2+}-NTA)-chelating Sepharose (GE healthcare, cat. no. 17-5247-01)

Superose 6 increase 10/300 GL (GE Healthcare, cat. no. 29091597)

spMSP plasmids (Addgene, spMSP1D1 (ID: 173482), spNW15 (ID: 173483), spNW25 (ID: 173484), spNW30 (ID: 173485), spNW50 (ID: 173486), spNW80 (ID: 173487), spNW100 (ID: 173488))

BL21 STAR™ (DE3) cells (Thermo Fisher, cat. no. C601003)

PD MiDiTrap G-25 (Cytiva, cat. no. 28918008)

cOmplete, Mini, EDTA-free Protease Inhibitor Cocktail (Sigma, cat. no. 4693159001)

Formvar/carbon-coated copper grids (Ted Pella, Inc., cat. no. 01754-F)

Uranyl formate (Fisher, cat. no. NC0782276)

Equipment

Branson cell disrupter (Fisher, cat. no. 22-309782)

5920 R Centrifuge (Eppendorf, cat. no. 2231010061)

Avanti JXN26 centrifuge (Beckman, cat. no. B34183/01750276)

AKTA pure 25 L (GE Healthcare, cat. no. 29018224)

PELCO easiGlow™ (Ted Pella, Inc., cat. no. 91000S).

ThermoFisher Science Tecnai G2 TEM

Procedure

Transformation of spMSP plasmids into BL21 cells.

1. Thaw 1 vial of BL21 STAR™ (DE3) cells on ice.
2. Add 1 µL of spMSP plasmids (10-50 ng/ µL) into the vial and keep it on ice for 10 minutes.
3. Heat shock at 42 °C for 30 seconds and place the vial on ice for 2 minutes.

4. Add 500 µL LB broth into the vial and shake it at 37 °C for 1 hour.

5. Spread the cells on LB agar Kanamycin plates.

6. Incubate the plates overnight at 37 °C.

Expression of spMSPs in BL21 cells.

1. A single colony from the plate was picked into 10 ml LB media supplemented with 50 µg/ml kanamycin. Shake the culture at 37 °C overnight (200 rpm).

2. Transfer the 10 ml culture into 1 L LB supplemented with 50 µg/ml kanamycin. Incubate the 1L culture at 37 °C with shaking at 200 rpm until OD_{600} = 0.7.

3. Add IPTG (0.2 mM) into the culture and continue incubation with shaking at 120 rpm for 20h at 16 °C.

4. Collect the cells by centrifugation at 3450 x g for 20 minutes using 5920 R.

Purification of spMSPs.

1. Cells were resuspended in buffer A (50 mM Tris-HCl (pH 8),100 mM NaCl, 5% glycerol, 2 mM β-mercaptoethanol) plus one cOmplete tablet.

2. Cells were lysed on ice using a Branson cell disrupter (60% duty cycle, 45 secs).

3. Cell lysate was clarified by centrifugation at 12, 000 x g for 45 mins using JXN26.

4. The supernatant was loaded onto a 1 ml Ni^{2+}-NTA column, followed by extensive wash (20 column volume) using buffer B (50 mM Tris-HCl (pH 8), 20 mM Imidazole, 400 mM NaCl, 5% glycerol, 2 mM β-mercaptoethanol). Proteins were eluted in buffer C (50 mM Tris-HCl (pH 8), 500 mM Imidazole, 400 mM NaCl, 5% glycerol, 2 mM β-mercaptoethanol), desalted in buffer A using PD MiDiTrap G-25 (GE Healthcare), and stored at -80 °C.

Nanodiscs reconstitution

1. Purified spMSPs were incubated with PC lipids at different ratios in buffer A containing 0.05% DDM. Samples were kept on ice for 30 min, and detergents were slowly removed with BioBeads (1/3 volume) and gentle shaking (4 °C, overnight).

2. cNDs were fractionated by size exclusion chromatography (SEC) using the Superose 6 10/300 in buffer A. Fractions corresponding to cNDs were kept and subjected to analysis by negative stain electron microscopy.

Negative stain electron microscopy

1. Formvar/carbon-coated copper grids were glow discharged (15 mA, 25 secs) using PELCO easiGlow™.

2. cNDs (10 µg/ml) were applied onto the grids for 30 secs, followed by staining with 0.75% uranyl formate for 1 minute.

3. Images were collected using a ThermoFisher Science Tecnai G2 TEM (100 kV) equipped with a Veleta CCD camera (Olympus).

Troubleshooting

If polydisperse particles are formed from SEC, protein: lipid ratios need to be optimized.

Time Taken

Day-1, Preparation of LB plates and media.

Day 0, Transformation of spMSP plasmids into BL21 cells.

Day 1-2, Protein expression in BL21 cells.

Day 3, spMSP purification and cND reconstitution.

Day 4, Fractionation of cNDs by SEC.

Day 5, Characterization of purified cNDs by negative stain electron microscopy.

Anticipated Results

The described protocol is able to generate highly monodisperse cNDs.

cNDs should show the expected size by negative stain EM.

References

1. Nasr, M.L. et al. Covalently circularized nanodiscs for studying membrane proteins and viral entry. *Nat Methods* **14**, 49-52 (2017).

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