

Submission ID #: 70283

Scriptwriter Name: Pallavi Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21436363>

Title: A Technique for Stabilizing Membrane Proteins in Nanodiscs

Authors and Affiliations:

Xiaomin Zeng^{1,*}, Jingyi Yang^{2,*}, Zixue Wang³, Zhenming Jin¹, Zhenhua Ming², Ran Zhang³, Baobin Li³

¹College of Biology, Hunan University

²State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, College of Life Science and Technology, Guangxi Key Laboratory for Sugarcane Biology, Guangxi University

³Jinshan Hospital, Zhongshan Hospital, Institute for Translational Brain Research, State Key Laboratory of Medical Neurobiology, MOE Frontiers Center for Brain Science, Fudan University

*These authors contributed equally

Corresponding Authors:

Baobin Li	libaobin@fudan.edu.cn
Ran Zhang	zhang_ran@fudan.edu.cn

Email Addresses for All Authors:

Xiaomin Zeng	Xiaomin-01@hnu.edu.cn
Jingyi Yang	2308301083@st.gxu.edu.cn
Zixue Wang	23111520057@m.fudan.edu.cn
Zhenming Jin	jinzm@hnu.edu.cn
Zhenhua Ming	zhming@gxu.edu.cn
Baobin Li	libaobin@fudan.edu.cn
Ran Zhang	zhang_ran@fudan.edu.cn

Author Questionnaire

1. **Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

The two locations are within 500 meters of each other.

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 32

Number of Shots: 54

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Jingyi Yang:** This protocol highlights a reproducible nanodisc reconstitution workflow with a general method framework.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Jingyi Yang:** Detergents destabilize membrane proteins, nanodiscs with MSPs and lipids preserve native structure for cryo-EM analysis.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Zixue Wang:** This protocol helps researchers study membrane protein structures using nanodisc reconstitution.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Zixue Wang:** The most challenging part is to optimize the ratio of membrane protein, MSP, and lipids.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Xiaomin Zeng:** Functional assays, surface plasmon resonance, and mass spectrometry can also be performed using this procedure.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Preparation of Lipid Stock for Nanodisc Assembly

Demonstrator: Zixue Wang

2.1. Begin by adding the lipid components prepared in chloroform into a glass test tube to prepare a 100-microliter lipid mixture and mix thoroughly [1][2].

2.1.1. WIDE: Talent adding the required components to a vial with the labelled bottles of individual components in the frame and mixing. *Video editor: Please use split screen to show 2.1.1 to 2.1.2*

2.1.2. TEXT ON A PLAIN BACKGROUND

Lipid mixture components:

1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE): 50 μ L

2-oleoyl-1-palmitoylglycero-3-phosphocholine (POPC): 25 μ L

1-palmitoyl-2-oleoylphosphatidylserine (POPS): 25 μ L

Desired ratio of DOPE:POPC:POPS: 2:1:1

2.2. Evaporate the chloroform under a gentle stream of nitrogen gas until all liquid has been removed [1].

2.2.1. Talent evaporating the chloroform from the glass test tube under a gentle stream of nitrogen gas.

2.3. Add approximately 2 milliliters of n-pentane dropwise to the glass test tube [1] and evaporate the solvent using nitrogen gas to obtain a dry, transparent lipid film [2].

2.3.1. Talent adding approximately 2 milliliters of n-pentane dropwise to the glass test tube.

2.3.2. The liquid is being evaporated using nitrogen gas. **NOTE: The footage from 2.2.1 can be used for step 2.3.2.**

2.4. After loosening the tube's cap, place the tube in the vacuum desiccator. Evacuate the desiccator and maintain it under vacuum to remove any residual solvents [1].

2.4.1. Talent placing the glass test tube in the desiccator and maintaining it under vacuum.

2.5. Then, remove the lipid film from the vacuum desiccator [1] and add 200 microliters of detergent-free low salt buffer to hydrate the lipid film [2-TXT].

2.5.1. Talent removing the glass test tube containing the lipid film from the vacuum

desiccator.

2.5.2. Talent adding 200 microliters of detergent-free low salt buffer to hydrate the lipid film. **TXT: Final Lipid Concentration: 10mg/mL**

2.6. After resuspending the lipid, divide it evenly into four glass tubes [1]. Sonicate the suspensions until they become clear and transparent, indicating successful liposome formation [2].

2.6.1. Talent dividing the resuspended lipid evenly into four glass tubes.

2.6.2. Talent sonicating the glass tubes.

2.7. Then, immediately flush the headspace of each tube with nitrogen gas [1-TXT].

2.7.1. Talent flushing the headspace of each glass tube with nitrogen gas. **TXT: Store the tubes at -80 °C until use**

3. Over-expression and Purification of Membrane Scaffold Proteins (MSPs)

Demonstrator: Jingyi Yang

3.1. Following transformation of Escherichia coli BL21 (*B-L-Twenty-One*) cells with the MSP1D1 (*M-S-P-One-D-One*) plasmid carrying a 7 times His tag and culture expansion [1], induce MSP (*M-S-P*) expression by adding IPTG (*I-P-T-G*) to a final concentration of 0.5–1 millimolar [2].

3.1.1. Talent shows the culture flask containing transformed cells.

3.1.2. Talent adding IPTG to the culture flask. **TXT: IPTG: Isopropyl β -D-1-thiogalactopyranoside; Incubation: 16 °C, 16–20 h, Shaking**

3.2. Centrifuge the bacterial culture at 4 degrees Celsius for 10 minutes [1]. Discard the supernatant and retain the cell pellet [2].

3.2.1. Talent placing the bacterial culture into the centrifuge for centrifugation at 4 degrees Celsius for 10 minutes.

3.2.2. Talent discarding the supernatant from the centrifuged culture and shows the cell pellet in the tube.

3.3. Then, resuspend the pellet derived from 1 liter of culture in 100 to 150 milliliters of lysis buffer [1]. Homogenize thoroughly until a uniform suspension with no visible clumps is achieved [2].

3.3.1. Talent resuspending the cell pellet in 100 to 150 milliliters of lysis buffer.

3.3.2. Talent homogenizes the suspension until no visible clumps remain.

3.4. Now, use a refrigerated ultrasonic cell disruptor to break the bacteria at 35 percent power with cycles of 3-second pulses followed by 6-second pauses [1-TXT].

3.4.1. Talent disrupting the bacterial suspension with the refrigerated ultrasonic cell

disruptor. **TXT: Continue until the lysate becomes clear and light brown in color**

3.5. Centrifuge the lysate at 30,000 *g* for 45 minutes at 4 degrees Celsius [1] and collect the supernatant for subsequent steps [2].

3.5.1. Talent placing the tube in the centrifuge.

3.5.2. Talent collecting the supernatant.

3.6. After purifying the lysate using a Nickel-NTA (*Nickel-N-T-A*) column, elute the target protein with the elution buffer and collect the eluate fractions [1-TXT].

3.6.1. Talent collecting eluate fractions during protein elution. **TXT: Collect fractions until $A_{280} < 0.01$.**

3.7. Now, transfer the pooled eluate into dialysis tubing [1] and dialyze it against dialysis buffer at 4 degrees Celsius with gentle stirring overnight [2-TXT].

3.7.1. Talent transferring the pooled eluate into dialysis tubing.

3.7.2. Talent placing the dialysis tubing into the buffer for overnight dialysis at 4 degrees Celsius with gentle stirring. **TXT: Concentrate dialyzed solution to 2–5 mg/mL MSP using ultrafiltration**

4. Activation of Bio-Beads for Nanodiscs Assembly

Demonstrator: Xiaomin Zeng

4.1. Weigh approximately 250 milligrams of Bio-Beads into a 50-milliliter centrifuge tube [1].

4.1.1. WIDE: Talent weighing approximately 250 milligrams of Bio-Beads and adding into a 50 milliliter centrifuge tube.

4.2. Add 25 to 30 milliliters of methanol to the tube [1]. Thoroughly rotate or vortex the mixture in the dark [2]. After incubating the tube at 4 degrees Celsius for 20 minutes, carefully discard the supernatant [3].

4.2.1. Talent adding 25 to 30 milliliters of methanol to the centrifuge tube containing Bio-Beads.

4.2.2. Talent rotating or vortexing the tube to mix the beads in methanol.

4.2.3. Talent discarding the supernatant from the tube. **TXT: Repeat methanol wash 3x followed by aqueous washes 3x**

4.3. Then, add 25 to 30 milliliters of detergent-free low salt buffer to the beads [1]. Shake or rotate the tube on a rotating table to mix and incubate as demonstrated earlier [2].

4.3.1. Talent adding detergent-free low salt buffer to the Bio-Beads.

4.3.2. Talent placing the tube on a rotating table to mix. **NOTE: The footage from 4.2.2 can be used for step 4.3.2.**

4.4. Discard the supernatant and repeat the low salt buffer equilibration step three times to

fully condition the beads [1].

4.4.1. Talent discarding the supernatant from the centrifuge tube. **NOTE: The footage from 4.2.3 can be used for step 4.4.1.**

4.5. Divide the activated Bio-Beads equally into three 1.5-milliliter microcentrifuge tubes for further use [1].

4.5.1. Talent dividing the Bio-Beads equally into three 1.5-milliliter microcentrifuge tubes.

5. Nanodisc Assembly

5.1. Retrieve one aliquot of the pre-prepared lipid stock and thaw it [1]. Immediately centrifuge the tube at 7000 *g* for 5 seconds at 4 degrees Celsius to collect the contents at the bottom [2].

5.1.1. Talent retrieving and thawing one aliquot of the lipid stock.

5.1.2. Talent placing the tube in the centrifuge for centrifugation at 7000 *g* for 5 seconds at 4 degrees Celsius.

5.2. Ultrasonicate the lipid stock in a water bath at 60 percent amplitude [1-TXT]. Continue sonication until the lipid stock becomes clear and transparent [2].

5.2.1. Talent ultrasonication of the lipid stock in a water bath. **TXT: Check clarity and temperature every 5–10 min to avoid overheating**

5.2.2. Shot of the lipid stock appearing clear and transparent.

5.3. After thawing the prepared MSP, determine the protein concentration using a NanoDrop [1].

5.3.1. Talent measuring protein concentration using a NanoDrop.

5.4. Calculate the molar amount of the target membrane protein using the presented equation [1].

5.4.1. TEXT ON A PLAIN BACKGROUND

$$n = C \times V$$

C = molar concentration, V = Volume

5.5. Then, calculate the required volumes of MSP and lipid stock solutions based on a molar stoichiometric ratio of target protein to MSP to lipid of 1 to 5 to 250 [1].

5.5.1. Talent performing the calculation of volumes based on the 1 to 5 to 250 molar ratio. **NOTE: Slated as 5.6.1**

5.6. Combine the corresponding volumes of lipid and MSP to prepare a 200-microliter master mix [1-TXT]. Then, slowly add 50 microliters of the mix to the concentrated

protein solution while gently pipetting to ensure homogeneity [2].

5.6.1. Talent combining lipid and membrane scaffold protein to prepare the master mix. **TXT: Incubate on ice for 10 min** **NOTE: Slated as 5.5.1**

5.6.2. Talent adding 50 microliters of the mixture to the protein solution and gently pipetting.

5.7. Then, place the combined mixture on ice for 1 hour and gently invert the tube every 15 minutes to mix thoroughly [1].

5.7.1. Talent placing the tube on ice and periodically inverting it to mix.

5.8. Now, take one aliquot of the pre-activated Bio-Beads and aspirate the residual low salt buffer [1]. Add the protein solution to the beads [2] and incubate with rotation in the dark for 1 hour at 4 degrees Celsius [3].

5.8.1. Talent aspirating residual low salt buffer from the pre-activated Bio-Beads.

5.8.2. Talent adding the protein solution to the Bio-Beads.

5.8.3. Talent placing the tube on a rotating device for incubation in the dark.

5.9. Centrifuge the mixture at 7000 g for 5 seconds at 4 degrees Celsius [1]. Carefully transfer the supernatant to a second aliquot of Bio-Beads from which the low salt buffer has been aspirated [2]. After mixing the content well by gentle inversion, incubate in the dark for 4 to 8 hours or overnight [3].

5.9.1. Talent placing the tube in the centrifuge for centrifugation at 7000 g for 5 seconds at 4 degrees Celsius.

5.9.2. Talent transferring the supernatant to a second aliquot of Bio-Beads.

5.9.3. Talent placing the tube for incubation in the dark. **NOTE: The footage from 5.8.3 take 2 (A0043) can be used for step 5.9.3**

5.10. Once the protein solution is centrifuged briefly, transfer the supernatant to the third aliquot of pre-activated Bio-Beads [1]. Incubate with rotation at 4 degrees Celsius in the dark for 1 hour [2].

5.10.1. Talent transferring the supernatant to the third aliquot of Bio-Beads.

5.10.2. Talent placing the tube on a rotating device for incubation at 4 degrees Celsius in the dark. **NOTE: The footage from 5.8.3 take 2 can be used for step 5.10.2.**

5.11. Following a brief centrifugation, transfer the supernatant to a clean microcentrifuge tube [1]. Centrifuge at 17000 g at 4 degrees Celsius for 10 minutes to remove aggregates [2].

5.11.1. Talent transferring the supernatant to a clean microcentrifuge tube.

5.11.2. Talent placing the tube in the centrifuge for centrifugation at 17000 g at 4 degrees Celsius for 10 minutes.

5.12. After purifying the nanodisc Task2 complexes by size-exclusion chromatography, pool the peak fractions elution [1]. Concentrate them to a final nanodisc Task2 concentration of 1.2 to 1.5 milligrams per milliliter [2].

5.12.1. Talent collecting and pooling peak fractions from the chromatography elution.

5.12.2. Talent concentrating the pooled fractions using concentrator tube.

5.13. To verify the nanodisc assembly, analyze the samples using 12 percent SDS-PAGE and confirm the presence of MSP and target membrane protein [1].

5.13.1. Talent at the gel doc analysing the samples using SDS-PAGE.

Results

6. Results

- 6.1. The size exclusion chromatography profile displayed two major peaks corresponding to the assembled nanodisc complex at 15.1 milliliters [1] and free MSP at 18.0 milliliters [2].
 - 6.1.1. LAB MEDIA: Figure 2. *Video editor: Highlight the peak labeled “Nanodisc” at approximately 15.1 milliliters*
 - 6.1.2. LAB MEDIA: Figure 2. *Video editor: Highlight the peak labeled “MSP” at approximately 18.0 milliliters*
- 6.2. Quantification of peak areas showed that the assembled nanodisc peak had an area of 147.2 [1]. In contrast, the free MSP peak had an area of 31.5, corresponding to an assembly efficiency of 82.4 percent [2].
 - 6.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the larger peak labeled “Nanodisc”*
 - 6.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the smaller peak labeled “MSP”*
- 6.3. Co-migration of the target protein and MSP in SDS-PAGE confirmed their incorporation into a single nanodisc complex [1].
 - 6.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bands indicated by arrows for “Task2” and “MSP” that appear aligned across the lanes*

1. Dioleoyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/dioleoyl>
IPA: /daɪˈoʊliəl/
Phonetic Spelling: dye·oh·lee·oyl
2. Glycero
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycero>
IPA: /ˈɡlɪsəˌroʊ/
Phonetic Spelling: glih·suh·roh
3. Phosphoethanolamine
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphoethanolamine>

- IPA: /ˌfɑːsfʊʊ,ɛθəˈnoʊlə,miːn/
Phonetic Spelling: fos·foh·eh·thuh·noh·luh·meen
4. Phosphocholine
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphocholine>
IPA: /ˌfɑːsfʊʊˈkoʊliːn/
Phonetic Spelling: fos·foh·koh·leen
 5. Phosphatidylserine
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphatidylserine>
IPA: /ˌfɑːsfəˈtɪdɪlˈsɪriːn/
Phonetic Spelling: fos·fuh·tih·dil·seer·een
 6. Chloroform
Pronunciation link: <https://www.merriam-webster.com/dictionary/chloroform>
IPA: /ˈklɔːrəˌfɔːrm/
Phonetic Spelling: klor·uh·form
 7. n-pentane
Pronunciation link: <https://www.merriam-webster.com/dictionary/pentane>
IPA: /ˈpɛnˌteɪn/
Phonetic Spelling: pen·tayn
 8. Desiccator
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccator>
IPA: /ˈdɛsɪˌkɛtər/
Phonetic Spelling: dess·ih·kay·ter
 9. Sonicate
Pronunciation link: <https://www.merriam-webster.com/dictionary/sonicate>
IPA: /ˈsɑːnɪˌkɛt/
Phonetic Spelling: sah·nih·kayt
 10. Liposome
Pronunciation link: <https://www.merriam-webster.com/dictionary/liposome>
IPA: /ˈlɪpəˌsoʊm/
Phonetic Spelling: li·puh·sohm
 11. Escherichia
Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia>
IPA: /ˌɛʃəˈrɪkiə/
Phonetic Spelling: esh·uh·rih·kee·uh
 12. Plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /ˈplæzˌmɪd/
Phonetic Spelling: plæz·mid
 13. Thiogalactopyranoside
Pronunciation link: <https://www.merriam-webster.com/dictionary/thiogalactopyranoside>
IPA: /ˌθaɪoʊɡəˌlæktəʊpaɪˈrænəˌsaɪd/
Phonetic Spelling: thye·oh·guh·lak·toh·pye·ran·uh·syde
 14. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

- IPA: /'sentrɪˌfjuːdʒ/
Phonetic Spelling: sen·trih·fyooj
15. Homogenize
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /hə'mɑːdʒəˌnaɪz/
Phonetic Spelling: huh·mah·juh·nyze
16. Ultrasonic
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasonic>
IPA: /ˌʌltrə'sɑːnɪk/
Phonetic Spelling: ul·truh·sah·nik
17. Lysate
Pronunciation link: <https://www.merriam-webster.com/dictionary/lysate>
IPA: /'laɪsɪt/
Phonetic Spelling: lye·sayt
18. Nickel-NTA
Pronunciation link: No confirmed link found
IPA: /'nɪkəl en tiː eɪ/
Phonetic Spelling: nih·kuhl en·tee·ay
19. Eluate
Pronunciation link: <https://www.merriam-webster.com/dictionary/eluate>
IPA: /'ɛljuːˌeɪt/
Phonetic Spelling: el·yoo·ayt
20. Dialysis
Pronunciation link: <https://www.merriam-webster.com/dictionary/dialysis>
IPA: /daɪ'æləsis/
Phonetic Spelling: dye·al·uh·suhs
21. Methanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/methanol>
IPA: /'mɛθəˌnɔːl/
Phonetic Spelling: meth·uh·nol
22. Vortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔːrˌtɛks/
Phonetic Spelling: vor·teks
23. Stoichiometric
Pronunciation link: <https://www.merriam-webster.com/dictionary/stoichiometric>
IPA: /ˌstɔɪkiə'mɛtrɪk/
Phonetic Spelling: stoy·kee·uh·me·trik
24. Ultrasonicate
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasonicate>
IPA: /ˌʌltrə'sɑːnɪˌkeɪt/
Phonetic Spelling: ul·truh·sah·nih·kayt
25. NanoDrop
Pronunciation link: No confirmed link found

IPA: /'nænou ,dra:p/

Phonetic Spelling: nan·oh·drop

26. Chromatography

Pronunciation link: <https://www.merriam-webster.com/dictionary/chromatography>

IPA: / ,kroʊmə 'tɑ:grəfi/

Phonetic Spelling: kroh·muh·tog·ruh·fee

27. Nanodisc

Pronunciation link: No confirmed link found

IPA: /'nænou ,disk/

Phonetic Spelling: nan·oh·disk

28. SDS-PAGE

Pronunciation link: No confirmed link found

IPA: / ,ɛs di: ɛs 'peɪdʒ/

Phonetic Spelling: es·dee·es·payj