Published in final edited form as:
Methods Mol Biol. 2013 ; 964: 15–24. doi:10.1007/978-1-62703-251-3_2.

Methods for the Study of Dopamine Receptors Within Lipid Rafts of Kidney Cells

Peiying Yu, **Van Anthony Villar**, and **Pedro A. Jose**

Abstract

There is increasing evidence that G protein-coupled receptor (GPCR) signaling is regulated in lipid raft microdomains. GPCRs and GPCR-signaling molecules, including G proteins and protein kinases, have been reported to compartmentalize in these microdomains. Dopamine D_1-like receptors (D_1R and D_5R) belong to a family of GPCRs that are important in the regulation of renal function. These receptors are not only localized and regulated in caveolae that contains caveolin-1 but are also distributed in non-caveolar lipid rafts which do not contain caveolin-1. This chapter describes detergent- and non-detergent-based methods to obtain lipid raft fractions from renal proximal tubule cells.

Keywords

Lipid rafts; Caveolae; Membrane microdomains; Dopamine receptor

1. Introduction

Dopamine receptors belong to the α group of the rhodopsin-like family of G protein-coupled receptors (GPCRs) and are classified into two subfamilies depending on their effect on adenylyl cyclase activity. The D_1-like receptors (D_1R and D_5R) stimulate while the D_2-like receptors (D_2R, D_3R and D_4R) inhibit adenylyl cyclase activity (1). Dopamine D_1-like receptors have been implicated in the modulation of various neural processes, including learning, memory, reward, and motor activity (2, 3), and in the regulation of blood pressure by actions on the adrenergic nervous system, hormone secretion, and epithelial ion transport (1).

Lipid rafts are membrane microdomains composed of cholesterol, sphingolipids, glycosylphosphatidylinositol-linked (GPI-linked) proteins, and other proteins such as caveolin (4–6). Caveolae and lipid rafts have been implicated to play a role in cellular processes like membrane sorting, receptor trafficking, signal transduction, and cell adhesion. Lipid rafts serve as signaling platforms for several signaling molecules such as G protein subunits, enzymes, and adaptor proteins that play important roles in signal transduction in a variety of mammalian cells (4–7). Lipid rafts are characterized by their relative insolubility in nonionic detergents at 4°C and light buoyant density on sucrose gradient (4, 7). Among the lipid rafts, caveolae are the best characterized, are localized on cell surface

invaginations, and are formed by polymerization of caveolin proteins with cholesterol (5–9). Three caveolin genes encode the caveolin proteins, namely, caveolin-1, caveolin-2, and caveolin-3. Caveolin-1 has been used as a marker protein for caveolae (5–9). There are several other markers for lipid rafts, such as flotillin-1, CD55, and alkaline phosphatase (10, 11). Flotillin-1 has been used as a lipid raft marker protein in cells that do not contain caveolae, i.e., blood cells (11), neural cells (12), and rat kidney tubule cells (13, 14). We have reported that there are non-caveolar lipid rafts in human embryonic kidney cells since these cells are devoid of measurable caveolin-1 (13).

There are several ways to prepare lipid rafts using detergent or detergent-free methods. Detergent-free methods have been developed to isolate lipid rafts (7–9). Schnitzer et al. described a detergent-free method to isolate lipid rafts from rat lung vasculature by perfusion with a suspension of cationic colloidal silica particles, which is a good method for in vivo studies (7). The methods reported by Smart et al. and Song et al. are also detergent-free and have been extensively used to isolate lipid rafts membranes from a variety of cells (8, 9). The method by Smart et al. allows the isolation of a more purified fraction of lipid rafts because it uses purified plasma membranes rather than total cell lysates (8), in contrast to the method by Song et al. which uses total cell lysates (9). The results obtained using non-detergent extracted rafts are more reproducible and generate a greater fraction of inner leaflet-membrane lipids than detergent-extracted rafts (15). The samples obtained by detergent methods have been termed detergent-resistant membranes (DRMs) or detergent-insoluble fraction (7, 10, 15). The nonionic detergents, e.g., Triton X-100, are commonly used to purify lipid raft fractions (7, 10, 16–18). However, different detergents may yield different lipid raft components because different types of raft proteins have varying degrees of resistance to extraction by specific detergents (10, 15). Differences between detergent and non-detergent methods for the preparation of lipid rafts may be responsible for the observed variability in the lipid composition of the isolated rafts (7, 10, 15). We now describe non-detergent and detergent methods to isolate lipid raft membranes.

2. Materials

2.1. Cell Culture

1. HEK-293 cells heterologously expressing human D_1 receptor (HEK-hD_1) that have been previously characterized (13).
2. Prepare complete medium for cell culture by adding 5 mL of Pen/Strep and 50 mL of FBS to 500 mL of DMEM/F12 medium.
3. Grow cells in 150-mm dishes with complete medium in a humidified incubator in 5% CO_2 and 95% air.

2.2. Sucrose Gradient Centrifugation

All stock solutions are prepared in distilled water at room temperature and stored at 4°C.

1. 250 mM 2-N-morpholino ethanesulfonic acid (Mes) stock solution, pH ≈ h 6.7–6.8.
2. 1.5 M sodium chloride (NaCl) stock solution.

3. Mes-buffered saline (MBS) solution: 25 mM Mes and 150 mM NaCl, pH ≈ 6.7–6.8.
4. 500 mM sodium carbonate, pH 11 (pH need not be adjusted).
5. 5%, 35%, and 80% sucrose solutions in MBS buffer (see Note 1).
6. Add protease inhibitor cocktail to the sodium carbonate and sucrose solutions.
7. Protein assay using BCA kit (Pierce Thermo Scientific (Rockford, IL)).
8. Phosphate-buffered saline (PBS).
9. D_1-like receptor agonist fenoldopam (1 mM) (Sigma-Aldrich, St. Louis, MO) stock solution, aliquoted into small volumes (50 μL/aliquot), protected from light, and stored at −20°C. Antioxidants are needed for prolonged incubation of dopamine and dopamine agonists.
10. Prepare fresh solution of methyl-β-cyclodextrin (βCD) (Sigma) (2%) in DMEM/F12 serum-free medium (SFM) at room temperature.
11. Cholesterol-βCD solution (Sigma) for cholesterol repletion experiment:
    a. Dissolve cholesterol (20 mg/mL) in ethanol by sonication.
    b. Dissolve βCD (2%) in DMEM/F12 SFM.
    c. Prepare cholesterol-βCD solution by adding 20 μL of cholesterol solution to 10 mL cyclodextrin solution, mix by vortexing, and incubating the cholesterol-βCD solution at 40°C for 30 min (see Note 2).
12. 50% OptiPrep stock solution: 45 mL of 60% OptiPrep mixed with 9 mL of OptiPrep diluent.
13. MBSTS buffer: MBS with 0.5% (v/v), Triton X-100 in 10% sucrose, or other nonionic detergents, e.g., β-octyl glucoside, CHAPS, deoxycholate, Lubrol WX, Lubrol PX, Brij 58, Brij 96, Brij 98 (Sigma), as needed (see Note 3).
14. Prepare 5% and 30% gradient OptiPrep solutions according to Table 1 using 50% OptiPrep stock solution and MBSTS buffer.
15. 6× sample buffer: 7.5 mL of 0.5 M Tris–HCl, pH 6.8, 1 g of SDS powder, 3.6 mL of 100% glycerol, 2 mg of bromphenol blue, 1 g of dithiothreitol in a total 10 mL volume with distilled water.

---

[1]The sucrose solutions (5%, 35%, and 80%) are prepared in MBS buffer, pH 6.8 (13) rather then in sodium carbonate solution (pH 11) (9). This results in a sample pH near 7.0 instead of pH 11. This may be beneficial to most of the enzyme proteins.

[2]For cholesterol depletion experiment, the cells are incubated with methyl-β-cyclodextrin (βCD) (2%) for 1 h at 37°C. However, methyl-α-cyclodextrin has been recommended as a negative control (19). For cholesterol repletion experiments, βCD and cholesterol complex is used (Subheading 3.1, step 3). There is a commercially available cholesterol-cyclodextrin complex (SIGMA #C4951). However, the complex can also be prepared as described above (Subheading 2.2, item 11). An inactive analog of cholesterol (cholestane-3β, 5α, 6β-triol) has been suggested as a control (20).

[3]Extraction using nonionic detergents. In general, Triton X-100 or CHAPS can solubilize the membranes that are extremely enriched in cholesterol and sphingolipids (15). Different raft proteins have different sensitivities to the different detergents. For example, even in the same cell type, different GPI-anchored proteins which associate with lipid rafts can be distinguished based on their sensitivity to solubilization in nonionic detergents. A good example of this is the prion protein, a GPI-linked protein, which was found only in non-raft fractions after solubilization in 0.5%Brij 96, but was distributed evenly between the raft and non-raft fractions when 0.5% Triton X-100 was used (21).

2.3. Western Blot for Lipid Raft Proteins

1. Nitrocellulose membranes (0.2 μm pore size) (Invitrogen Life Technologies, Grand Island, NY).
2. Pre-stained molecular weight markers (Invitrogen Life Technologies).
3. Vertical midi-format electrophoresis cell, which should include a buffer tank and lid with power cables.
4. Criterion Precast Gels: 4–20% polyacrylamide gel, 26-well gel (Bio-Rad, Hercules, CA) or 8–16% polyacrylamide gel, 15-well gel (Invitrogen).
5. 10× Tris/Glycine/SDS stock buffer, to make 1× running buffer.
6. 10× Tris/Glycine buffer, to make 1× transfer buffer containing 20% methanol.
7. 10× PBS-tween-20 buffer, to make 1× washing buffer.
8. 0.1% Amido Black, 45% MEOH, 10% acetic acid in distilled water.
9. 0.1% Ponceau S solution in 5% acetic acid (remove the dye from the membrane by several washes with distilled water).
10. Primary antibodies and secondary antibodies conjugated to horseradish peroxidase.
11. Enhanced chemiluminescence (ECL) Western blotting detection reagents (GE Healthcare, Waukesha, WI).

3. Methods

3.1. Preparation of Lipid Raft Fraction with Non-detergent Method

Caveolae and lipid raft proteins are resistant to the solubilizing actions of detergents and some non-detergent reagents, such as sodium carbonate. Therefore, the raft proteins and membranes can be prepared using these detergents or reagents for sucrose gradient centrifugation. To prepare caveolar-enriched or non-caveolar lipid rafts, one can use the detergent-free sucrose gradient centrifugation protocol according to Song et al. (9) with slight modifications (13). This method can be adopted for all mammalian cells and tissues, including those that do not express caveolin-1 (13, 14), i.e., HEK-293 cells. For example, rat renal proximal tubule cells, used as an example, do not express caveolin-1 and therefore do not have caveolae (13, 14). We suggest using at least two 150-mm dishes for a single preparation. All experiments are carried out at 4°C except for cell culture and cell treatments.

1. Collect cell pellets. Culture cells in 150-mm dishes with DMEM/F12 complete medium at 37°C until the cells reach 95% confluence. Remove the cell culture medium and wash the cells twice with PBS. Then, starve the cells in DMEM/F12-SFM for 1–2 h at 37°C. Treat the cells with vehicle or drugs (e.g., fenoldopam, 2% βCD, cholesterol–cyclodextrin solution) at 37°C for 1 h. Wash the cells once with cold PBS or cold DMEM/F12-SFM. Scrape the cells into a 15 mL tube containing cold PBS. Pellet the cells by centrifugation at 2,000 × *g* for 5 min. Discard the supernatant to obtain the cell pellet.

2. Prepare cell homogenates. Add 1.5 mL of 500 mM sodium carbonate to the cell pellet and mix by vortexing. Place the 15 mL tube containing the cells on ice and homogenize the cell suspension using a Dounce homogenizer (10 strokes), a Teflon polytron (three 10-s bursts), and a tip sonicator (three 30-s bursts). The homogenization steps are carried out on ice (see Note 4). Add 1.5 mL of 80% sucrose (final volume 3 mL, sucrose concentration, 40%) and mix the homogenate by vortexing (three 30 s bursts) and sonicating (three 30 s bursts) on ice. Determine the protein concentrations by BCA kit (OD 570).

3. Prepare a discontinuous sucrose gradient. Place equal amounts of cell homogenates (3 mL) into the bottom of each precooled 12 mL ultracentrifuge tubes and overlay 4.5 mL of 35% sucrose and 4.5 mL of 5% sucrose to each tube. The ultracentrifuge tubes should be balanced when placed and positioned in SW-41 buckets.

4. Centrifuge the tubes containing the cell homogenates at 180,000 × *g* (38,000 rpm) for 16 h at 4°C in a Beckman SW41 rotor (see Note 5).

5. Remove the tubes from the bucket at the end of the ultracentrifugation step. A light-scattering band that contains caveolae-enriched lipid raft membranes is seen at the interface of the 5–35% sucrose gradient. Carefully collect twelve 1 mL fractions by pipetting 1 mL starting from the top of the ultracentrifuge tube and transfer the fractions into the pre-labeled 1.5 mL microcentrifuge tubes (see Note 6). The light-scattering band is located at the 3rd to 5th fractions from the top, with the peak at the 4th fraction.

6. Prepare samples for immunoblotting. Transfer 0.5 mL aliquots from each fraction into other pre-labeled 1.5 mL microcentrifuge tubes. Add 0.1 mL of 6× sample buffer to each sample. Vortex each tube until dye and samples are mixed well and put the tubes in boiling water for 5 min. The samples for immunoblotting can be saved at −20°C until use. The rest of the fractionated samples not mixed with the 6× sample buffer are saved at −80°C (see Note 7).

3.2. Preparation of Lipid Raft Fraction with Detergent Method

Detergent resistance or detergent insolubility results from the segregation of integral or membrane-associated proteins into cholesterol- and glycosphingolipid-enriched membrane microdomains termed lipid rafts. The nonionic detergents such as Triton X-100, β-octyl glucoside, CHAPS, deoxycholate, Lubrol WX, Lubrol PX, Brij 58, Brij 96 and Brij 98 have been used to purify lipid raft fractions (7, 10, 16–18). However, different detergents may

[4]To avoid loss of cell samples during homogenization, we use a tip sonicator (five 20-s bursts, with a 2-min interval after each burst) instead of using Dounce homogenizer and Teflon polytron. All sonication steps should be performed with the test tubes on ice. This homogenization procedure can be used for cells but not for tissues.

[5]The SW40 or SW41 rotors can be used for sucrose gradient centrifugation. The speed of the centrifugation is specific for each rotor. In general, the rotor speeds are 38,000 rpm (18,000 × *g*)/16 h for SW41 rotor and 36,000 rpm (16,000 × *g*)/18 h for SW40 rotor.

[6]Collect twelve 1 mL fractions starting from the bottom by inserting a fine plastic tube in to bottom of the centrifuge tube and withdrawing one 1 mL each time using a 2 mL syringe, or a peristaltic pump.

[7]The sucrose gradient samples with pH 6.8–7.0 can be stored at −80°C for enzyme assays, e.g., adenylyl cyclase assay. There are many ways to concentrate the fraction samples such as speedvac concentrator or by precipitation with 10% trichloroacetic acid (TCA). The membranes from lower sucrose gradient fractions can also be concentrated by three-fold dilution of the samples with MBS and pelleted by centrifugation at 20,000 × *g* for 30 min.

yield different lipid raft components because different types of raft proteins have varying degrees of resistance to different detergents (10, 15).

1. Collect cell pellets (see Subheading 3.1, step 1) (One 150-mm dish for one preparation).
2. Prepare cell extract on ice for 30 min in 0.3 mL cold MBSTS (0.5% Triton X-100 and protease inhibitors) by pushing the cell suspension through a 25-gauge needle, ten times (cell pellet volume is about 0.1 mL/dish and the total cell lysate volume is about 0.4 mL). Adjust the cell extract (0.4 mL) to 40% OptiPrep by adding 0.8 mL of cold 60% OptiPrep, mix the cell extract by vortexing. Determine the protein concentrations using a BCA kit (OD 570). The total protein amount should be the same for all centrifuge tubes with the same volume (1 mL).
3. Prepare a discontinuous OptiPrep gradient. Load 1 mL of the cell extract into the bottom of precooled 5 mL ultracentrifuge tubes. Overlay with 1 mL of each 30%, 25%, 20%, and 0% OptiPrep solutions in MBSTS buffer, as prepared in Table 1 (see Note 8).
4. Ultracentrifuge the OptiPrep gradient solutions at 175,000 × *g* (42,000 rpm) at 4°C for 4 h in Beckman SW 50.1 rotor. Other rotors can be used such as SW 55 (4 h at 170,000 × *g*), TLS55 rotor (2.5 h at 250,000 × *g*). However, the equivalent *g*-force and centrifugation time should be adjusted according to the rotor type. Label 1.5 mL microcentrifuge tubes for the next step.
5. Carefully remove the ultracentrifuge tubes. Collect 0.5 mL fractions from top to bottom and prepare for immunoblotting, as in Subheading 3.1.

3.3. Immunoblotting to Analyze Lipid Raft Proteins

Western blot allows the identification and analysis of the lipid raft proteins. In general, one should first identify where the peak of lipid raft fractions is located using lipid raft marker proteins such as caveolin-1, caveolin-3, or flotillin-1. To compare the effect of drugs on lipid raft protein expression, 4–20% Criterion Precast Gradient Gel with 26 wells per gel is recommended. All the steps are carried out at room temperature.

1. Run gels. Pre-warm the sucrose gradient samples in a water bath at 37°C. Mix the samples completely by vortexing (there should be no precipitate at the bottom of the tubes). Load the samples and molecular weight marker into a 4–20% Criterion Precast gradient gel. Run the gel with running buffer at 120 V for about 2 h. Stop the electrophoresis when the dye migrates to 0.5–1.0 cm above the bottom edge of the gel.
2. Transfer the proteins from gels onto nitrocellulose membranes. Prepare the sandwich of gels and nitrocellulose membranes in transfer buffer and place the

[8]The OptiPrep discontinuous gradient can be made by overlaying 3 mL of 30% and 0.5 mL of 5% Optiprep solutions (16), or by overlaying 1 mL of 30%, 1 mL of 25%, 1 mL of 20%, and 1 mL of 0% OptiPrep solutions (17). However, it is best to prepare an OptiPrep continuous gradient using a machine for preparing gradients (Bio-Rad) or by overlaying 0.8 mL of each 30%, 25%, 20%, 15%, and 0% OptiPrep solutions and precentrifugation at 175,000 × *g* (42,000 rpm) at 4°C for 2 h in Beckman SW 50.1 rotor. Subsequently, load the protein samples at the bottom of the continuous OptiPrep gradient tube.

sandwich into semidry transfer equipment and start the transfer at 0.24 mA at constant current for 60–90 min.

3. Block the membranes. Rinse the membranes twice with distilled water after transfer. Verify the protein loading by staining the membranes with 0.1% Ponceau S solution or 0.1% Amido Black solution for 10 s and washing the sheets with distilled water. The stained sheets can be scanned to record the protein loading information. Block the membranes for 1 h in blocking buffer (5% nonfat dry milk in PBST washing buffer).

4. Perform the immunoblotting. Incubate the blocked membranes overnight at 4°C with primary antibody diluted in blocking buffer (see Note 9). Remove the primary antibody following the overnight incubation and wash the membranes 3× with wash buffer. Incubate the membranes for 1 h with secondary antibody diluted in blocking buffer.

5. Develop the film. Incubate the membranes for 1 min with ECL reagent after washing with wash buffer 3×. Visualize the immunoreactive bands by autoradiography (see Note 10).

Acknowledgments

These studies were supported in part by grants from the National Institutes of Health (HL023081, HL074940, DK039308, HL068686, and HL092196).

References

1. Jose PA, Eisner GM, Felder RA. Role of dopamine receptors in the kidney in the regulation of blood pressure. Curr Opin Nephrol Hypertens. 2002; 11:87–92. [PubMed: 11753092]
2. Holmes A, Lachowicz JE, Sibley DR. Phenotypic analysis of dopamine receptor knockout mice; recent insights into the functional specificity of dopamine receptor subtypes. Neuropharmacology. 2004; 47:1117–1134. [PubMed: 15567422]
3. Kong MMC, Hasbi A, Mattocks M, Fan T, O'Dowd BF, George SR. Regulation of D1 dopamine receptor trafficking and signaling by caveolin-1. Mol Pharmacol. 2007; 72:1157–1170. [PubMed: 17699686]
4. Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997; 387:569–572. [PubMed: 9177342]
5. Insel PA, Head BP, Ostrom RS, Patel HH, Swaney JS, Tang CM, Roth DM. Caveolae and lipid rafts: G protein-coupled receptor signaling microdomains in cardiac myocytes. Ann N Y Acad Sci. 2005; 1047:166–172. [PubMed: 16093494]
6. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science. 2010; 327:46–50. [PubMed: 20044567]
7. Schnitzer JE, McIntosh DP, Dvorak AM, Liu J, Oh P. Separation of caveolae from associated microdomains of GPI-anchored proteins. Science. 1995; 269:1435–1439. [PubMed: 7660128]

[9]The primary antibody can be diluted in an antibody diluting solution (Invitrogen), and the diluted primary antibody can be collected and saved at −20°C for subsequent usage. The primary antibody diluted in 5% milk buffer is not recommended for storage.
[10]To visualize the immunoreactive bands, the use of Licor (Odyssey) is recommended. When using Licor, the membranes should be blocked using a special blocking solution, such as casein or BSA blocking buffers (Bio-Rad), and the appropriate secondary antibody conjugated to IRDye® infrared dyes (in PBS casein buffer). The immunoreactive bands are visualized by scanning the membrane using Licor.

8. Smart EJ, Ying YS, Mineo C, Anderson RG. A detergent-free method for purifying caveolae membrane from tissue culture cells. Proc Natl Acad Sci U S A. 1995; 92:10104–10108. [PubMed: 7479734]
9. Song KS, Li S, Okamoto T, Quilliam LA, Sargiacomo M, Lisanti MP. Co-purification and direct interaction of Ras with caveolin, an integral membrane protein of caveolae microdomains. Detergent-free purification of caveolae microdomains. J Biol Chem. 1996; 271:9690–9697. [PubMed: 8621645]
10. Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci U S A. 2003; 100:5813–5818. [PubMed: 12724530]
11. Salzer U, Prohaska R. Stomatin, flotillin-1, and flotillin-2 are major integral proteins of erythrocyte lipid rafts. Blood. 2001; 97:1141–1143. [PubMed: 11159550]
12. Huang P, Xu W, Yoon SI, Chen C, Chong PL, Liu-Chen LY. Cholesterol reduction by methyl-beta-cyclodextrin attenuates the delta opioid receptor-mediated signaling in neuronal cells but enhances it in non-neuronal cells. Biochem Pharmacol. 2007; 73:534–549. [PubMed: 17141202]
13. Yu P, Yang Z, Jones JE, Wang Z, Owens SA, Mueller SC, Felder RA, Jose PA. D1 dopamine receptor signaling involves caveolin-2 in HEK-293 cells. Kidney Int. 2004; 66:2167–2180. [PubMed: 15569306]
14. Breton S, Lisanti MP, Tyszkowski R, McLaughlin M, Brown D. Basolateral distribution of caveolin-1 in the kidney. Absence from H^+-ATPase-coated endocytic vesicles in intercalated cells. J Histochem Cytochem. 1998; 46:205–214. [PubMed: 9446827]
15. Pike LJ. Lipid rafts: heterogeneity on the high seas. Biochem J. 2004; 378:281–292. [PubMed: 14662007]
16. Waheed AA, Jones TL. Hsp90 interactions and acylation target the G protein Gα12 but not Gα13 to lipid rafts. J Biol Chem. 2002; 277:32409–32412. [PubMed: 12117999]
17. Verkade P, Harder T, Lafont F, Simons K. Induction of caveolae in the apical plasma membrane of Madin-Darby canine kidney cells. J Cell Biol. 2000; 148:727–739. [PubMed: 10684254]
18. Macdonald JL, Pike LJ. A simplified method for the preparation of detergent-free lipid rafts. J Lipid Res. 2005; 46:1061–1067. [PubMed: 15722565]
19. Vial C, Evans RJ. Disruption of lipid rafts inhibits P2X1 receptor-mediated currents and arterial vasoconstriction. J Biol Chem. 2005; 280:30705–30711. [PubMed: 16006561]
20. Murtazina R, Kovbasnjuk O, Donowitz M, Li X. Na^+/H^+ exchanger NHE3 activity and trafficking are lipid raft-dependent. J Biol Chem. 2006; 281:17845–17855. [PubMed: 16648141]
21. Madre N, Smith KL, Graham CH, Jen A, Brady K, Hall S, Morris R. Functionally different GPI proteins are organized in different domains on the neuronal surface. EMBO J. 1999; 18:6917–6926. [PubMed: 10601014]

Table 1
Prepare varying concentrations of OptiPrep solutions

Solutions (total 5 mL)	30%	20%	10%	5%
50% OptiPrep (mL)	3.0	2.0	1.0	0.5
MBSTS (mL)	2.0	3.0	4.0	4.5