Preparing and Processing Plasma Membrane Sheets

This protocol has been developed for visualising cell surface microdomains, if this is the aim then it is essential to use antibodies directly conjugated to gold no bigger than 5nm, see Prior 2003a and b for details. The technique gives unrivalled 2D views of the cell surface often several square microns in area and can be applied to studies of cell surface compartmentalisation, sites of endocytosis and viral interactions with the cell surface.

Poly-l-lysine grid preparation.

1. Incubate pioloform-coated grids with 1 mg/ml Poly-L-lysine (Sigma P8920) for 10 min.
2. Rinse twice in 1 ml distilled water for 1 min each wash.
3. Air dry and store in a dust-free environment.

Cell processing.

1. Culture cells on 10-mm glass coverslips; transfect if necessary.

Note: Try to ensure that on the day of the experiment the cells are 70-80% confluent for optimal plasma membrane sheets.

2. Place grids onto filter paper in a group (typically two per coverslip) with poly-L-lysine-coated pioloform side facing up.
3. Immediately before use, wash cells with PBS to remove any debris.
4. Remove the coverslip from the culture dish; touch the edge of the coverslip with filter paper to remove excess solution, and place the coverslip onto grids, cell side down.
5. Place filter paper on top of the coverslip and exert pressure using a silicon bung for 1 to 2 s.

Note: Corks, rubber bungs, or any similar device can be used. The pressure exerted needs to be calibrated for each cell type.

6. Remove the filter paper and turn the coverslip over so that the grids are uppermost.
7. Carefully add sufficient KOAc buffer (25mM Hepes, 115mM KOAc, 2.5mM $MgCl_2$, pH 7.4) around the grids so that they float up onto the top of the drop.

Note: Use 200µl of KOAc for a 13mm round coverslip, the surface tension of the buffer should dislodge the grids, if they don't move once the buffer has been added use forceps to gently lift an edge and encourage the grid pop up.

8. Wash grids by transferring them to another drop of KOAc buffer on Parafilm.
9. Fix for 10 min with fixative. All of the following steps are carried out at room temperature.

Note: we use 4% paraformaldehyde, 0.1% glutaraldehyde in KOAc. We find that

10. Quench free aldehyde groups with 20 mM glycine in KOAc for 15 min (3x).
11. Block non-specific antibody-binding sites with Blocking Solution (0.2% BSA, 0.2% fish skin gelatin in PBS) for 10 min.
12. Immuno-label with gold-conjugated antibody diluted in Blocking Solution for 30 min.

Note: Spin the gold-conjugated antibody for 2 min in a microcentrifuge at maximum speed before use if it hasn't been spun on a glycerol gradient.

13. Wash the labeled grids with Blocking Solution five times for 5 min each.
14. Wash the grids in de-ionized water five times for 1-2 min each.
15. Incubate on ice for 10 min in a mixture of nine parts of 2% methylcellulose to one part of 3% uranyl acetate

Note: Uranyl acetate precipitates in the presence of phosphate ions, ensure that the tweezers used for transferring the grids are also washed in de-ionized water between each water wash.

16. Pick up the grids in 5-mm copper wire loops, drain excess mixture on filter paper, and leave to dry in the loops (20 min).
17. Remove grids from the loop taking care to leave the film over the sections intact; the grids are now ready for viewing.

Finding plasma membrane sheets.

Finding isolated sheets can be difficult because the grid is covered with cell debris including whole cells and cell fragments. Grids are systematically scanned at a magnification of 4-6000x, the illumination should be bright and the microscope optimized for high contrast. A common place to find good areas is next to semi-intact cells where the procedure has successfully disrupted sections of the cell margin. All plasma membrane sheets should have a well-defined edge; where lots of cell debris is present the sheets often stand out as looking clean against the dirty background. On a good grid there can be more than 50 reasonable areas however 10-15 plasma membrane sheets is more typical.

References

Prior IA, Parton RG, and Hancock JF. Observing cell surface signaling domains using electron microscopy. *Science's STKE* (2003) 2003: pl9.

Prior IA, Muncke C, Parton RG and Hancock JF. Direct visualization of Ras proteins in spatially distinct cell surface microdomains. *J. Cell Biol.* (2003) 160: 165-70.