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Preparation of samples for Lipid-CLEM imaging

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Visualizing sub-organellar lipid distribution using correlative light and electron microscopy

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We use this protocol and it's working

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Abstract

The Lipid-CLEM method enables room temperature correlative light and electron microscopy visualization of the intracellular localization of lipids. It combines minimally modified (bifunctional) lipid probes and on-section click chemistry labelling. Using Lipid-CLEM, relative lipid densities in membrane nanodomains can be determined. This protocol covers the essential aspects for monitoring lipid localization by CLEM.

Materials

- 200,000 - 240,000 U2OS cells per dish
- 1-palmitoyl-2-oleoyl-glycerol-3-phosphocholine (Avanti, #26853-31-6)
- Bifunctional lipid (Sigma, CAS: 57-88-5, #C8667-5G)
- Cholesterol (Sigma, CAS: 57-88-5, #C8667-5G)
- PBS (0.22 µm, PES filter)
- Alpha-methyl-cyclodextrin (Bio-reagent, #CDeXA-076/BR, CAS: 69902-02-5)
- Avanti Mini-Extruder with 0.1 µm polycarbonate membranes (Avanti, #610005-1Ea)
- 1-Hexadecene (TCI, #H0610-100ML)
- FluoroBrite DMEM (ThermoFisher Scientific, #A1896701)
- Fetal bovine serum (gibco, #10270-106)
- Leica EM-ICE (Leica Microsystems) or a Wohlwend Compact 03 (Wohlwend GmbH)
- Leica AFS2 machine and AFS sample wheels (Leica Microsystems, #16707154) and AFS sample ring holders (Leica Microsystems, #16707157)
- Uranyl acetate (electron microscopy sciences, #22400)
- Lowicryl HM20 (Embedding Kit 15924-1, Polysciences Inc.) or K11M (Embedding Kit 18163-1, Polysciences Inc.)
- Millex 0.22 µm filter (Merck, #SLGP033RS)
- AF-594 picolyl-azide dye (Jena bioscience, CLK-1296-1)
- THPTA (Jena bioscience, CLK-1010-1G)
- 35-degree diamond knife ultra (Diatome, #MT15630)
- Grid maps mats (electron microscopy sciences, #71172)
- Picolyl-azide-biotin (Jena Bioscience, #CLK-1167-5)
- Primary antibody against biotin (Rockland, #100-4198)
- Protein A gold (CMC-Utrecht, #PAG 10NM/S)
- Glutaraldehyde
- Multicolor TetraSpecs™ (ThermoFisher, # T7279)
- 50 nm TetraSpecs™ (ThermoFisher, # C47819)
- Lead citrate (EMS, #17800)

Safety warnings

Handle uranyl acetate with extreme care! Do not proceed with this step without prior training. This solution is radioactive and toxic! Handle with extreme care! Do not proceed with this step without prior training.

Before start

Confirm that all utilized reagents are fresh, and check the structural integrity of bifunctional lipid probes, ideally by NMR. Filter all solutions filtered using a Millex 0.22 µm filter (Merck, #SLGP033RS) to avoid dirt on samples.

Preparation of Sapphire disks and cell seeding

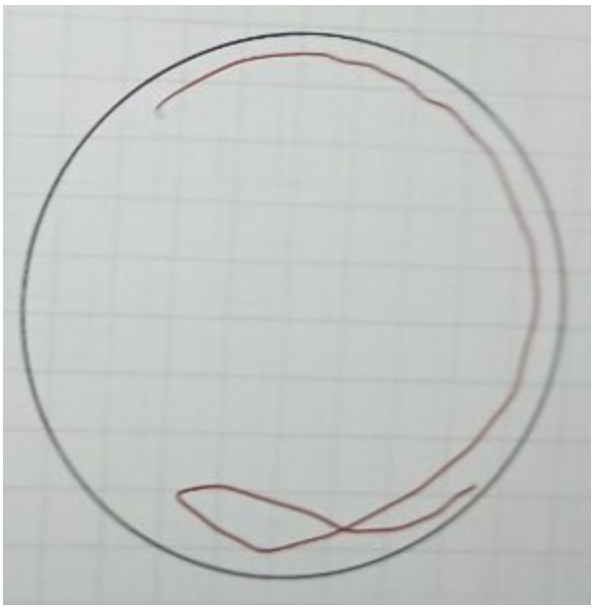
1d 10h 15m

1 Clean 3 mm x 0.05 mm Sapphire disks (Wohlwend GmbH, #405 or Leica, #16702766) by sonicating in soap water twice for 15 min at 60 °C in a scintillation vial. Rinse the sapphires three times with distilled water. Transfer the sapphire disks in a new scintillation vial and sonicate in 70% Ethanol three times at room temperature for 10 min. Store the disks in 100% Ethanol. For step 2., remove the sapphire disks, dry them one by one with a blow dryer, and place them on a clean glas slide.

2h

2 Sketch of coated Sapphire disks with carbon with a total of approximately 15 nm carbon film using the safematic CCU-010 at 10 A current at 5.0-5 mbar pressure. This includes a 2-step coating procedure: First, coat the sapphires on the glas slide with 10 nm carbon. Carefully scratch a “2” (as indicated below) into the carbon coat, leaving as much space as possible for cells to grow. Remove any carbon dust with a blow dryer. Finally coat the sapphires with an additional 5 nm carbon.

1h



3 Bake coated Sapphire disks overnight at 180 °C in a suitable heat stable glass petri dish. Note: Place the carbon coated sapphires in the oven at room temperature and let them heat up to 180 °C; in the morning turn off the oven but only remove the sapphires after they slowly cooled down to room temperature to avoid crack formation in the carbon coat. The sapphire disks are now sterile. To ensure the sapphire disks remain sterile handle the petri dish only with gloves, and keep it closed. Use coated Sapphire disks within one week. Sapphires can be baked again for re-sterilization.

12h

4 Immediatly before cell seeding: Glow-discharge coated Sapphire disks at 15 mA negative, under 0.24 mbar for 90 s using a Pelco easiGlow (model 91000). Handle carefully with sterile gloves. Note: these settings were used for seeding of U2OS cells, but settings might have to be adapted for other cell types. 15m

5 Under the cell culture hood: Place Sapphire disks in 35 mm glass bottom dishes (Cellvis, #D35-14-1.5-N) and pre-incubate with cell culture medium for 30 min at 37 °C.
Seed 200.000 - 240.000 U2OS cells per dish. Distribute evenly. If cells do not attach evenly on the sapphire disks, carefully agitate the dishes to re-distribute cells. This should be repeated 3 - 4 times every 10 - 20 min. 1h

6 Grow cells for 12-18 hours prior to lipid loading, crosslinking and high-pressure freezing. Final cell density should be ~80 – 90 %, but this might vary with cell type. 18h

Liposome preparation and lipid loading 1h 50m

7 Mix 11.5 µL (25 mg/ml in chloroform) of 1-palmitoyl-2-oleolyl-glycerol-3-phosphocholine (Avanti, #26853-31-6) with 100 µL of bifunctional lipid (0.75 µmol/100 µl in chloroform) and 14.5 µL of cholesterol (Sigma, CAS: 57-88-5, #C8667-5G) (10 mg/ml in chloroform). This gives a final solution of 0.75 mM POPC, 1.5 mM PC and 0.75 mM of cholesterol (total lipid: 3 mM). 15m

8 Dry the lipid solution using Ar or N2, or under vacuum over night in a desiccator. Samples can be further dried by heating to 37°C for 5-10 minutes. 30m

9 Resuspend the dry lipid film using 500 µL of filtered PBS (0.22 µm. PES filter). Vortex and sonicate the solution until no lipid clumps or lipid films are visible on the tube. 15m

10 Prepare liposomes from the lipid suspension using an Avanti Mini-Extruder with 0.1 µm polycarbonate membranes (Avanti, #610005-1Ea), extruding at least 21 times. 10m

11 Dissolve the liposomes and alpha-methyl-cyclodextrin (Bio-reagent, #CDexA-076/BR, CAS: 699020-02-5) in serum-free medium to final concentrations of 0.5 mM liposomes and 4 mM alpha-methyl-cyclodextrin to generate the lipid loading solution for delivering phospholipids. 5m

12 Incubate the liposome/alpha-methyl-cyclodextrin lipid loading solution for at least 30 min at 37 °C before proceeding. 30m

13 Optional: When using bifunctional fatty acids for metabolic labelling, dissolve the fatty acid in 100% ethanol and dilute this solution with full medium without cyclodextrin to reach a final concentration of 5 µM to obtain the fatty acid loading solution.

14 Before lipid loading, gently wash the cells three times with serum free medium. 1m

15 For phospholipid loading, remove medium and gently place the liposome/alpha-methyl-cyclodextrin lipid loading solution onto the cells for 4 minutes at 37 °C, unless otherwise stated. 4m

16 Optional: Fluorescent cargo such as transferrin or LDL can be loaded before, in parallel or after lipid loading, depending on the loading requirements.

17 Optional: If lipid transport pulse chase experiments are to be carried out, the lipid loading solution is removed, cells are washed gently with medium and placed in full medium for the appropriate chase time at 37 °C.

18 Optional: For metabolic labelling place the fatty acid loading solution onto cells and incubate for 17 h at 37 °C.

UV irradiation and high-pressure freezing 6h 0m 13s

19 Precoat planchettes (Wohlwend GmbH, #389 and #242) with 1-Hexadecene (TCI, #H0610-100ML) and blot excess Hexadecene immediately before use.

20 Remove the Sapphire disk from the 35 mm glass bottom dish and place in a fresh 35 mm glass bottom dish charged with FluoroBrite DMEM (Thermofisher Scientific, #A1896701) supplemented with 20 % fetal bovine serum (gibco, #10270-106) as a cryo preservative.

21 Place a spacer ring in the dish around the Sapphire disk to ensure a minimum distance between cells and UV lamp. Irradiate samples for 3 seconds using a custom-built hand-held LED device (violumas, VC2X2C45L9-365), with the LED placed exactly on top of the spacer ring. 3s

22 Immediately after UV exposure, place the sapphire disk on the flat side of a 0.3 mm planchette (Wohlwend GmbH, #242), add a drop of cryopreserving on top, and cover the disks using a 0.025/0.275 mm planchette (Wohlwend GmbH, #389) with the 0.025 mm side facing towards the cells.Ensure that there are no air bubbles in the sandwich. Blot excess cryopreservant. 10s

23 Perform high-pressure freezing (HPF) with a Leica EM-ICE (Leica Microsystems) or a Wohlwend Compact 03 (Wohlwend GmbH) system. Frozen sapphires can be directly processed continuing with step 26, or can be stored long term under liquid nitrogen. 6h

Automatic freeze substitution 5d 22h 8m

24 Pre-cool the Leica AFS2 machine to -90 °C. Pre-cool 1) an aluminum bath, 2) EM grade acetone, 3) freeze substitution medium, and the 4) HM20/ K11M mix as mentioned below. 5h

25 Assemble AFS sample wheels (Leica Microsystems, #16707154) and AFS sample ring holders (Leica Microsystems, #16707157) as indicated by the manufacturer.

26 Add 4 ml freeze substitution medium containing 0.1% uranyl acetate (electron microscopy sciences, #22400) in acetone to each AFS sample wheel. This solution is radioactive and toxic! Handle with extreme care! Do not proceed with this step without prior training.

27 Transfer frozen sapphire disks from the liquid nitrogen to an aluminum bath filled with EM grade acetone. By using the "2" that was scratched into the carbon coat as a reference, flip all sapphires with the cells facing up. Place the sapphires into the AFS wheel, with the cells facing up. 2h

28 Use either Lowicryl HM20 (Embedding Kit 15924-1, Polysciences Inc.) (for unpolar samples and better membrane ultrastructure preservation) or K11M (Embedding Kit 18163-1, Polysciences Inc.) (for polar samples and dye penetration into the section) according to manufacturer instructions. 1h

29 Use a Leica AFS2 machine and robot embedding according to manufacturer instructions (Leica Microsystems). Follow the following step-wise protocoll for freeze substitution: 5d 14h 8m

Step	T_{start} (°C)	T_{end}(°C)	Slope	Time	Reagent	%	Transfer	Agitation	UV
1	-90	-90	0	0:08	FS medium	100%	stay	on	
2	-90	-90	0	2:00	FS medium	100%	stay	on	
3	-90	-90	0	2:00	FS medium	100%	stay	on	
4	-90	-90	0	2:00	FS medium	100%	stay	on	
5	-90	-45	5	9:00	FS medium	100%	stay	off	
6	-45	-45	0	7:00	FS medium	100%	stay	off	
7	-45	-45	0	1:00	Acetone	100%	exch/fill	on	
8	-45	-45	0	1:00	Acetone	100%	exch/fill	on	
9	-45	-45	0	1:00	Acetone	100%	exch/fill	on	
10	-45	-45	0	2:00	HM20/ K11M	10%	mix	on	
11	-45	-45	0	2:00	HM20/ K11M	25%	mix	on	
12	-45	-35	5	2:00	HM20/ K11M	50%	mix	on	
13	-35	-25	2.5	4:00	HM20/ K11M	75%	mix	on	
14	-25	-25	0	10:00	HM20/ K11M	100%	exch/fill	off	
15	-25	-25	0	10:00	HM20/ K11M	100%	exch/fill	off	
16	-25	-25	0	10:00	HM20/ K11M	100%	exch/fill	off	
17	-25	-25	0	48:00	HM20/ K11M	100%	stay	off	X
18	-25	20	5	9:00	HM20/ K11M	100%		off	X
19	20	20	0	12:00	HM20/ K11M	100%		off	X

Sectioning

2w 0d 4h

30 Blocks obtained fresh from the AFS should be stored for an additional 2 weeks minimum in the dark under ventilation in a chemical hood. 2w

31 Section fully cured resin blocks using Leica EM UC6 and a 35-degree diamond knife ultra (Diatome, #MT15630). 4h

32 Cut sections to a thickness of 500 nm for tomography and 100 nm for standard TEM unless stated otherwise. Other section thicknesses work as well, but we suggest not to stain sections thicker than 500 nm.

33 Section HM20 samples at a cutting speed of 0.8 mm/s and K11M samples at 1.4 mm/s.

34 Catch sections on copper 200-mesh copper grids with carbon support film (electron microscopy sciences, CF200-CU-50).

Click Chemistry labelling with fluorophores

2h 20m

35 Prepare a click mix solution of 2 µM AF-594 picolyl-azide dye (Jena bioscience, CLK-1296-1), 0.1 mM CuSO4, 5 mM ascorbic acid and 0.5 mM THPTA (Jena bioscience, CLK-1010-1G) in 100 mM HEPES at pH 7.3. 5m

36 Stain sections on grid maps mats (electron microscopy sciences, #71172) twice for 30 min at 37 °C. 1h

37 Wash for 1 h at 37 °C in 100 mM HEPES and 10 times in deionized water by blotting at room temperature. 1h 15m

Optional: Nanogold labeling

38 Stain sections twice with picolyl-azide-biotin (Jena Bioscience, #CLK-1167-5) for 30 min at 37 °C using the following click mix solution: 10 µM picolyl-azide-biotin, 0.1 mM CuSO4, 5 mM ascorbic acid and 0.5 mM THPTA (Jena bioscience, CLK-1010-1G) in 100 mM HEPES at pH 7.3.

39 Wash for 30 min at 37 °C in 100 mM HEPES and 10 times in deionized water by blotting.

40 Block sections using filtered blocking buffer (2% bovine serum albumin in PBS (BSA, Sigma, #A7030-10G)) for 10 min.

41 Incubate section with the primary antibody against biotin (Rockland, #100-4198) at a 1:5,000 dilution in blocking buffer for 1 h.

42 Wash sections in blocking buffer four times for 2 min each.

43 Incubate sections with 10 nm protein A gold (CMC-Utrecht, #PAG 10NM/S) in blocking buffer for 20 min at dilutions as indicated by the manufacturer.

44 Wash sections with 0.1% blocking buffer (0.1% 2% bovine serum albumin in PBS) four times for 2 min each.

45 Fix sections using 1% glutaraldehyde in PBS for 5 min.

46 Rinse sections in excess water.

47 Repeat antibody and gold bead labeling three times in total.

Section post-processing

15m

48 Stain sections on one side with multicolor TetraSpecs™ by incubating grids for 5 min on a drop of freshly sonicated fiducials in PBS at 1:100 dilution for 100 nm sized TetraSpecs™ (ThermoFisher, # T7279) for K11M sections and 1: 25.000 dilution for custom ordered 50 nm TetraSpecs™ (ThermoFisher, # C47819) for HM20 sections.

15m

49 Image samples by widefield microscopy using LEDs. Ensure suitable filtercubes are used.

50 After light microscopy imaging, coat sections with freshly sonicated 15 nm gold beads on both sides at dilutions indicated by the supplier in PBS (CMC-Utrecht, #PAG 15NM/S).

51 For K11M sections: Stain sections with 1 % uranyl acetate solution (electron microscopy sciences, #22400) in deionized water for 7 min at room temperature, wash thoroughly in

deionized water, and stain with a 0.04% (m/v) lead citrate (EMS, #17800) aqueous solution for 2 min at room temperature.

52 Wash sections thoroughly in deionized water and dry before imaging by electron microscopy.

53 Proceed with electron microscopy and correlation.

Protocol references

Ori Avinoam *et al.*, Endocytic sites mature by continuous bending and remodeling of the clathrin coat. *Science* **348**,1369-1372(2015).DOI:**10.1126/science.aaa9555**

Wanda Kukulski, Martin Schorb, Sonja Welsch, Andrea Picco, Marko Kaksonen, John A.G. Briggs; Correlated fluorescence and 3D electron microscopy with high sensitivity and spatial precision. *J Cell Biol* 10 January 2011; 192 (1): 111–119. doi: https://doi.org/10.1083/jcb.201009037

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