Proteo- and DNA-liposomes – methodology and bibliography

- This **OptiPrep™ Reference List** contains a brief summary of the methodology for the separation of proteoliposomes from soluble proteins (**Section 1**); **Section 2** contains a list of references (up to **mid-2014**) that report the use of OptiPrep™; it serves to highlight some of the significant practical variations that have been reported. A list of more recent papers is given in **Section 3**.

1. Methodological summary

After protein has been incorporated into some form of liposome, it is usually necessary to resolve the newly formed proteoliposomes from any unincorporated protein. The most widely used strategy is described in Figure 1: the sample is adjusted to a density of 1.17-1.22 g/ml (depending on the OptiPrep™ diluent this is approx. equivalent to 30-40% w/v iodixanol) by mixing with a high-density stock solution and layered beneath two lower density layers. The topmost layer is sometimes the isolation buffer rather than a low density iodixanol solution. During centrifugation the proteoliposomes float up through the 1.11-1.15 g/ml barrier to band at the top interface. The big advantage of this strategy is that the unincorporated protein remains in the sample zone and will even tend to sediment in the opposite direction. If the sample is layered on top of a density barrier the proteoliposomes and the free proteins sediment in the same direction. Depending on the details of the gradient and the centrifugation conditions, the separation may be based either on the difference in density between the proteins and proteoliposomes or the more rapid movement of the larger proteoliposomes.

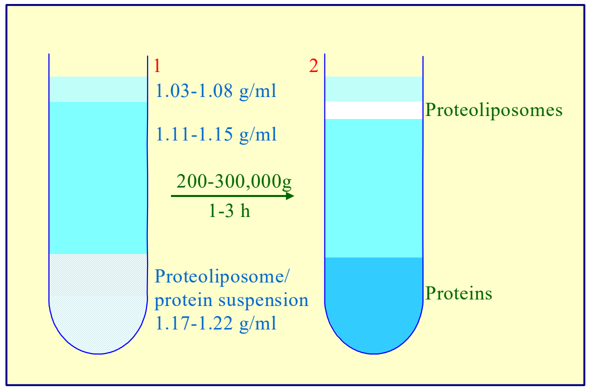

Figure 1: Diagrammatic representation of proteoliposome flotation strategy, before (1) and after (2) centrifugation

There are some significant variants to this general strategy, for example the omission of a low-density layer entirely. The reported *g*-forces also vary quite widely, most are in the 200-300,000 *g* range but both higher 500,000 *g* for 1 h and lower 150,000 *g* for 18 h have been reported. Some of the variations in methodology are listed in the Table in Section 2.

2. Methodological variations

2.1 Table of published papers

The entries are listed alphabetically according to principal area of investigation. All of the papers report the use of flotation rather than sedimentation for purifying the proteoliposomes (the table continues on the next page).

Research topic	%w/v iodix.[1]	x1000*g*/hr[2]	ref #
Arf family - small G-protein effectors –Arf-bound liposome affinity purification	30/20/0	250/0.5	1
Bacterial fusion proteins – domains for phosphatidylinositol-containing liposome binding	30/15/0	250/1	2
Bardet-Biedl syndrome protein complex; recruited to liposomes by $Arl6^{GTP}$	ns	ns	3
Beclin-1, evolutionary conserved domain, association to cardiolipin-containing liposomes	36/31/4	199/3	4
Claudin-1 in proteoliposome – unpredictable effects on viral infectivity	ns	ns	5
COPII vesicles; packaging of fusion protein influenced by dimerization	23/18/0	436/0.33	27
E-cadherin (GP2 modified) in cell adhesion studies	30	200/2	6
ER stress sensor protein (PERK) integrated into liposome via trans-membrane domain (influenced by acyl chain saturation	50/30/5	250/3	7

Research topic	%w/v iodix.[1]	x1000*g*/hr[2]	ref #
Fission protein B incorp. into cardiolipin-containing liposomes – remodelling of lipid membranes formed specifically from *B. subtilis*	40/20/0	300/1.5	8
G-protein coupled metabotropic glutamate receptor (glu binding)	40/30/5	ns	9
Influenza HA (proteoliposome-bound) – Tyr phosphorylation of BCR effectors	15/10/2.5/0	200/2	10
Lactoferrrin-bound liposomes targeted to parenchymal cells	33/0	150/2	11
Linker for Activation of T cells (phosphotyrosine variant)	45/30/0	175/3	12
N-ethylmaleimide-sensitive fusion ATPase (vesicle stability)	30/20/10	ns	13
Oligonucleotide delivery to endothelial cells (gene therapy application)	ns	ns	14
	ns	ns	15
PKA phosphorylation raises water permeability of aquaporin2-liposomes	40/30/0	180/4	16
Prion protein-lipid bilayer interaction essential for pathogenesis	ns	ns	17
Proteoliposome fusion with lipid bilayers (PEG-covered)- microfluidic analysis	30/12/0	280/4-5	18
Signal recognition peptide-receptor association (archaeal cells)	40/30/0	100/5 (fa)	19
SNARE protein incorporation	ns	ns	20
	ns	ns	21
SNARE-dependent liposome fusion, Ca^{2+}/calmodulin regulation	Ns	250/4	22
SV-40 capsid-liposome binding; need for pancreatic microsome luminal extract	40/30/0	250/2	23
α-Synuclein protofibril/fibril interaction with liposomes	30/25/5	200/2	24
Wiskot-Aldrich protein; GST-YFP-BG protein; effect of phosphatidylinositol	40/30/15/5	150/18	25
Y.lipolytica mitochondrial complex I – ATP synthase organization	24/20	80/0.5	26

[1] Discontinuous gradients are described as % (w/v) iodixanol; ns = not stated in text
[2] Centrifugation conditions in thousands of *g*/time in hours; all rotors are swinging-bucket, unless indicated (fa = fixed-angle); ns = not stated in text

2.2 References

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3. Papers published since mid 2014

Titles are listed alphabetically by first author and include papers on DNA-liposomes
Ref 6 describes the separation of particles on the basis of size.

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