OptiPrep™ Mini-Review MM02

Proteo- and DNA-liposomes – methodology and bibliography

- This OptiPrep™ Mini-Review contains a brief summary of the methodology for the separation of proteoliposomes from soluble proteins (Section 1); Table 2.1 in Section 2 contains a comprehensive 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.

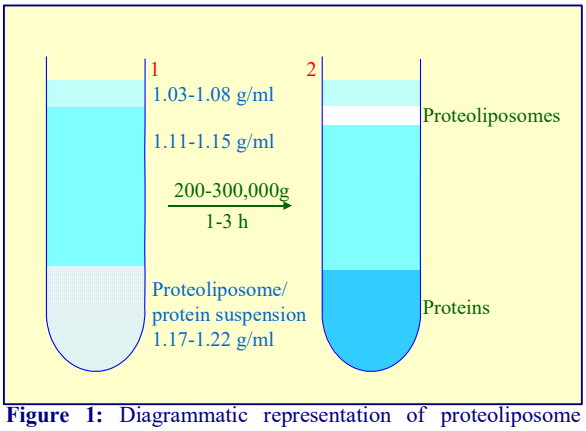

Figure 1: Diagrammatic representation of proteoliposome flotation strategy, before (1) and after (2) centrifugation

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. Bibliography

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

1. Christis, C., and Munro, S. (2012) *The small G protein Arl1 directs the trans-Golgi– specific targeting of the Arf1 exchange factors IG1 and BIG2* J. Cell Biol., **196**, 327–335
2. Ling, Y., Stefan, C.J., MacGurn, J.A., Audhya, A. and Emr, S.D. (2012) *The dual PH domain protein Opy1 functions as a sensor and modulator of PtdIns(4,5)P2 synthesis* EMBO J., **31**, 2882–2894
3. Jin, H., Roehl White, S., Shida, T., Schulz, S., Aguiar, M., Gygi, S.P., Bazan, J.F. and Nachury, M.V. (2010) *The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia* Cell **141**, 1208–1219
4. Huang, W., Choi, W., Hu, W., Mi, N., Guo, Q., Ma, M., Liu, M., Tian, Y., Lu, P., Wang, F-L., Deng, H., Liu, L., Gao, N., Yu, L. and Shi, Y. (2012) *Crystal structure and biochemical analyses reveal Beclin 1 as a novel membrane binding protein* Cell Res., **22**, 473-489
5. Bonander, N., Jamshad, M., Oberthur, D., Clare, M., Barwell, J., Hu, K., Farquhar, M.J., Stamataki, Z., Harris, H.J., Dierks, K., Dafforn, T.R., Betzel, C., McKeating, J.A. and Bill, R.M. (2013) *Production, purification and characterization of recombinant, full-length human claudin-1* PloS One, **8**: e64517
6. Perez, T.D., Nelson, W.J., Boxer, S.G. and Kam, L. (2005) *E-Cadherin tethered to micropatterned supported lipid bilayers as a model for cell adhesion* Langmuir, **21**, 11963-11968
7. Volmer, R., van der Ploeg, K. and Ron, D. (2103)*Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains* Proc. Natl. Acad. Sci. USA, **110**, 4628–4633
8. Doan, T., Coleman, J., Marquis, K.A., Meeske, A.J., Burton, B.M., Karatekin, E. and Rudner, D.Z. (2013) *FisB mediates membrane fission during sporulation in Bacillus subtilis* Genes Dev., **27**, 322–334
9. Eroglu, C.A., Cronet, P., Panneels, V., Beaufils, P. and Sinning, I. (2002) *Functional reconstitution of purified metatropic glutamate receptor expressed in the fly eye* EMBO Rep., **3**, 491-496

10. Lingwood, D., McTamney, P.M., Yassine, H.M., Whittle, J.R.R., Guo, X., Boyington, J.C., Wei, C-J. and Nabel, G.J. (2012) *Structural and genetic basis for development of broadly neutralizing influenza antibodies* Nature, **489**, 566-570
11. Weeke-Klimp, A.H., Bartsch, M., Morselt, H.W.M., van Veen-Hof, I., Meijer, D.K.F., Scherphof, G.L. and Kamps, J.A.A.M. (2007) *Targeting of stabilized plasmid lipid particles to hepatocytes in vivo by means of coupled lactoferrin* J. Drug Target., **15**, 585-594
12. Sangani, D., Venien-Bryan, C. and Harder, T. (2009) *Phosphotyrosine-dependent in vitro reconstitution of recombinant LAT-nucleated multiprotein signalling complexes on liposomes* Mol Membr. Biol., **26**, 159-170
13. Brugger, B., Nickel, W., Weber, T., Parlati, F., McNew, J.A., Rothman, J.E. and Sollner, T. (2000) *Putative fusogenic activity of NSF is restricted to a lipid mixture whose coalescence is also triggered by other factors* The EMBO J., **19**, 1272-1278
14. Bartsch, M., Weeke-Klimp, A.H., Meijer, D.K.F., Scherphof, G.L. and Kamps, J.A.A.M. (2002) *Massive and selective delivery of lipid-coated cationic lipoplexes of oligonucleotides targeted in vivo to hepatic endothelial cclls* Pharm. Res., **19**, 676-680
15. Bartsch, M., Weeke-Klimp, A.H., Morselt, H.W.M., Kimpfler, A., Asgeirsdottir, S.A., Schubert, R., Meijer, D.K.F., Scherphof, G.L. and Kamps, J.A.A.M. (2005) *Optimized targeting of polyethylene glycol-stabilized anti-intercellular adhesion molecule 1 oligonucleotide/lipid particles to liver sinusoidal endothelial cells* Mol. Pharmacol., **67**, 883-890
16. Eto, K., Noda, Y., Horikawa, S., Uchida, S. and Sasaki, S. (2010) *Phosphorylation of aquaporin-2 regulates its water permeability* J. Biol. Chem., **285**, 40777-40784
17. Wang, F., Yin, S., Wang, X., Zha, L., Sy, M-S. and Ma, J. (2010) *Role of the highly conserved middle region of prion protein (PrP) in PrP-lipid interaction* Biochemistry, **49**, 8169–8176
18. Karatekin, E. and Rothman, J.E. (2012) *Fusion of single proteoliposomes with planar, cushioned bilayers in microfluidic flow cells* Nat. Protoc., **7**, 903-920
19. Moll, R.G. (2003) *Protein-protein, protein-RNA and protein-lipid interactions of signal-recognition particle components in the hyperthermoacidophilic archeon Arcidianus ambivalens* Biochem. J., **374**, 247-254
20. Hu, K., Carroll, J., Fedorovich, S., Rickman, C., Sukhodub, A. and Davletov, B. (2002) *Vesicular restriction of synaptobrevin suggests a role for calcium in membrane fusion* Nature, **415**, 646-650
21. Hu, K., Rickman, C., Carroll. J. and Davletov, B. (2004) *A common mechanism for the regulation of vesicular SNAREs on phospholipid membranes* Biochem. J., **377**, 781-765
22. Di Giovanni, J., Iborra, C., Maulet, Y., Lévêque, C., El Far, O. and Seagar, M. (2010) *Calcium-dependent regulation of SNARE-mediated membrane fusion by calmodulin* J. Biol. Chem., **285**, 23665–23675
23. Geiger, R., Andritschke, D., Friebe, S., Herzog, F., Luisoni, S., Heger, T. and Helenius, A. (2011) *BAP31 and BiP are essential for dislocation of SV40 from the endoplasmic reticulum to the cytosol* Nat. Cell Biol., **13**, 1305-1314
24. Volles, M.J., Lee, S-L., Rochet, J-C., Shtilerman, M.D., Ding, T.T., Kessler, J.C. and Lansbury, P.T. (2001) *Vesicle permeabilization by protofibrillar α-synuclein: implications for the pathogenesis and treatment of Parkinson's disease* Biochemistry, **40**, 7812-7819
25. Myers, S.A., Han, J.W., Lee, Y., Firtel, R.A. and Chung, C.Y.A. (2005) *Dictyostelium homologue of WASP is required for polarized F-actin assembly during chemotaxis* Mol. Biol. Cell, **16**, 2191-2206
26. Davies, K.M., Strauss, M., Daum, B., Kief, J.H., Osiewacz, H.D., Rycovska, A., Zickermann, V. and Kühlbrandt, W. (2011) *Macromolecular organization of ATP synthase and complex I in whole mitochondria* Proc. Natl. Acad. Sci. USA, **108**, 14121-14126

3. Papers published mid 2014 – September 2017

Titles are listed alphabetically by first author and include DNA-liposomes, highlighted in blue

Ref 5 describes the separation of particles on the basis of size.

1. Chandrasekar, S. and Shan, S-O. (2017) *Anionic phospholipids and the albino3 translocase activate signal recognition particle-receptor interaction during light-harvesting chlorophyll a/b-binding protein targeting* J. Biol. Chem., **292**, 397–406
2. Chu, N.K., Shabbir, W., Bove-Fenderson, E., Araman, C., Lemmens-Gruber, R., Harris, D.A. and Becker, C.F.W. (2014) *A C-terminal membrane anchor affects the interactions of prion proteins with lipid membranes* J. Biol. Chem., **289**, 30144–30160
3. Cortesio, C.L., Lewellyn, E.B. and Drubin, D.G. (2015) *Control of lipid organization and actin assembly during clathrin-mediated endocytosis by the cytoplasmic tail of the rhomboid protein Rbd2* Mol. Biol. Cell, **26**, 1509-1522

4. Fogeron, M-L., Jirasko, V., Penzel, S., Paul, D., Montserret, R., Danis, C., Lacabanne, D., Badillo, A. et al (2016) *Cell-free expression, purification, and membrane reconstitution for NMR studies of the non-structural protein 4B from hepatitis C virus* J. Biomol. NMR, **65**, 87–98
5. Garcia-Diez, R., Gollwitzer, C., Krumrey, M. and Varga, Z. (2016) *Size determination of a liposomal drug by small-angle X-ray scattering using continuous contrast variation* Langmuir, **32**, 772−778
6. Luisoni, S., Suomalainen, M., Boucke, K., Tanner, L.B., Wenk, M.R., Guan, X.L., Grzybek, M., Coskun, U. and Greber, U.F. (2015) *Co-option of membrane wounding enables virus penetration into cells* Cell Host Microbe, **18**, 75–85
7. Rouvinski, A., Dejnirattisai, W., Guardado-Calvo, P., Vaney, M-C., Sharma, A., Duquerroy, S., Supasa, P., Wongwiwat, W. (2017) *Covalently linked dengue virus envelope glycoprotein dimers reduce exposure of the immunodominant fusion loop epitope* Nat. Comm., **8**: 15411
8. Schuhmacher, J.S., Rossmann, F., Dempwolff, F., Knauer, C., Altegoer, F., Steinchen, W., Dörrich, A.K. et al (2015) *MinD-like ATPase FlhG effects location and number of bacterial flagella during C-ring assembly* Proc. Natl. Acad. Sci. USA, **112**, 3092-3097
9. Springer, S., Malkus, P., Borchert, B., Wellbrock, U., Duden, R. and Schekman, R. (2014) *Regulated oligomerization induces uptake of a membrane protein into COPII vesicles independent of its cytosolic tail* Traffic, **15**, 531–545
10. Waldhart, A.N., Dykstra, H., Peck, A.S., Boguslawski, E.A., Madaj, Z.B., Wen, J., Veldkamp, K. et al (2017) *Phosphorylation of TXNIP by AKT mediates acute influx of glucose in response to insulin* Cell Rep., **19**, 2005–2013
11. Weaver, G.C., Villar, R.F., Kanekiyo, M., Nabel, G.J., Mascola, J.R. and Lingwood, D. (2016*) In vitro reconstitution of B cell receptor–antigen interactions to evaluate potential vaccine candidates* Nat. Protoc., 11**, 193-213**
12. Xu, W., Nathwani, B., Lin, C., Wang, J., Karatekin, E., Pincet, F., Shih, W. and Rothman, J.E. (2016) *A programmable DNA origami platform to organize SNAREs for membrane fusion* J. Am. Chem. Soc. **138**, 4439−4447
13. Ysselstein, D., Joshi, M., Mishra, V., Griggs, A.M., Asiago, J.M., McCabe, G.P., Stanciu, L.A., Post, C.B. and Rochet, J-C. (2015) *Effects of impaired membrane interactions on α-synuclein aggregation and neurotoxicity* Neurobiol. Dis., **79**, 150–163
14. Yang, Y., Wang, J., Shigematsu, H., Xu, W., Shih, W.M., Rothman, J.E. and Lin, C. (2016) *Self-assembly of size-controlled liposomes on DNA nanotemplates* Nature Chem., **8**, 476-483
15. Zhang, Z., Yang, Y., Pincet, F., Llaguno, M.C. and Lin, C. (2017) *Placing and shaping liposomes with reconfigurable DNA nanocages* Nat. Chem., **9**, 653-659
16. Xu, W., Nathwani, B., Lin, C., Wang, J., Karatekin, E., Pincet, F., Shih, W. and Rothman, J.E. (2016) *A programmable DNA origami platform to organize SNAREs for membrane fusion* J. Am. Chem. Soc. **138**, 4439-4447

Mini-Review MM02: 4th edition, November 2017