Electronic Supplementary Material (ESI) for Nanoscale Advances.

Optimal centrifugal isolating of liposome-protein complexes from human plasma

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Table S1. Molar percentage of the employed lipids in each liposomal formulation.

Lipoplexes were formed by mixing DOTAP-DOPE-PEG2k liposomes (1 mg/mL) and DNA (1 mg/mL) at a fixed lipid-to-DNA ratio of 10:1 (vol/vol). Final solutions were left 20 min at room temperature prior to perform the experiments.

	DOTAP	DOPE	DOPE-PEG2k
DOTAP-DOPE 1:3	25%	75%	0
DOTAP-DOPE 1:1	50%	50%	0
DOTAP-DOPE 3:1	75%	50%	0
PEGylated DOTAP-DOPE	50%	35%	15%

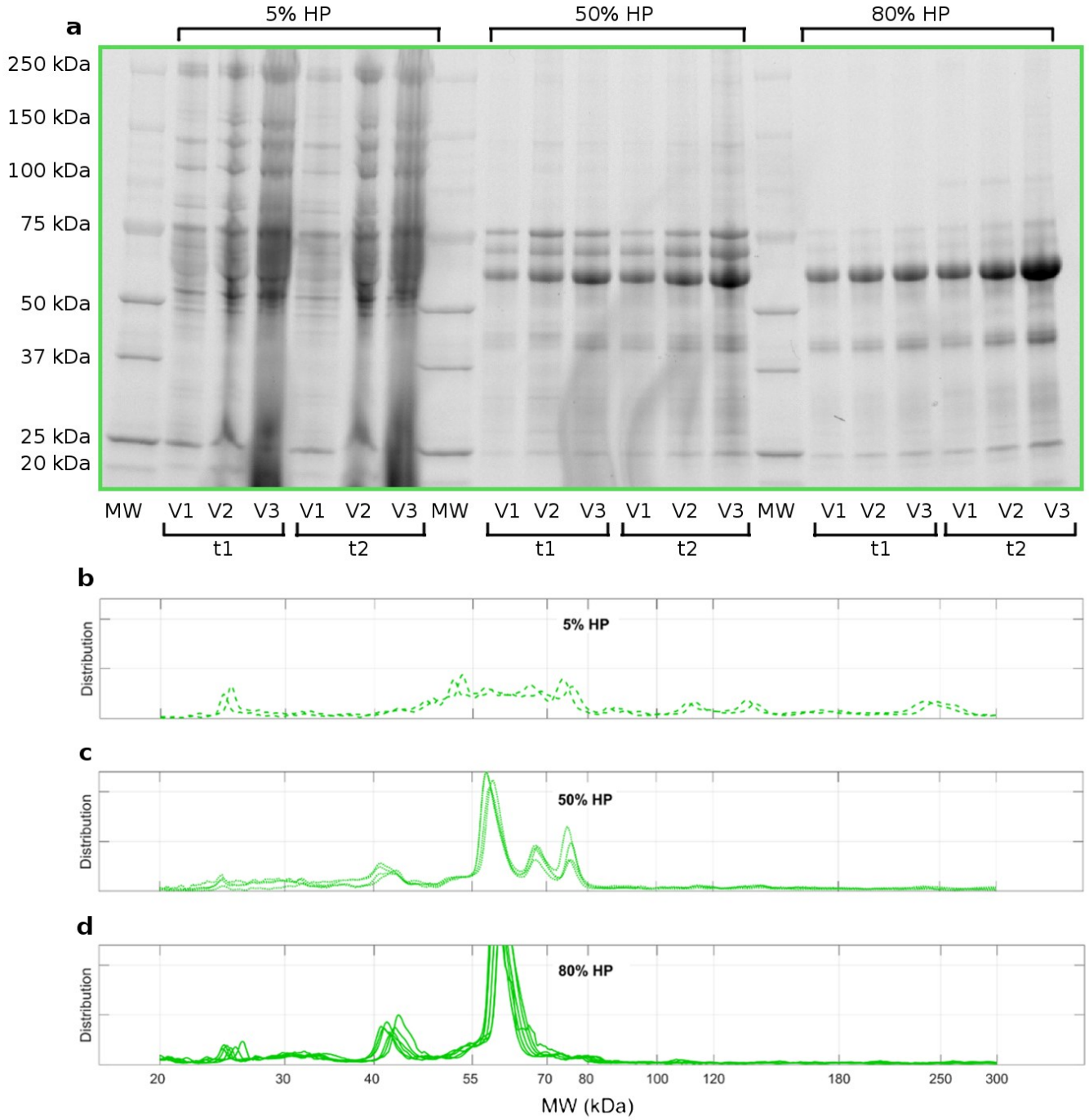

Figure S1. (a) Uncropped 1D SDS-PAGE image corresponding to the protein corona formed on DOTAP-DOPE 1:1 (mol/mol) upon exposure to human plasma under different incubation conditions (i.e. 5% HP, 50% HP and 80% HP) and centrifugation parameters (i.e. total volume V1=50 µL, V2=100 µL, V3=200 µL and t1=15 min, t2=60 min). Some lanes corresponding to 5% HP (i.e. V2, V3 for both t1 and t2) were not evaluable by densitometric analysis, due to the extremely abundant pellets. (b-d) Normalized protein patterns for each condition, grouped by HP concentration. Superimposition of distributions clearly indicates that protein patterns depend on HP concentration, but do not depend on centrifugation protocol.

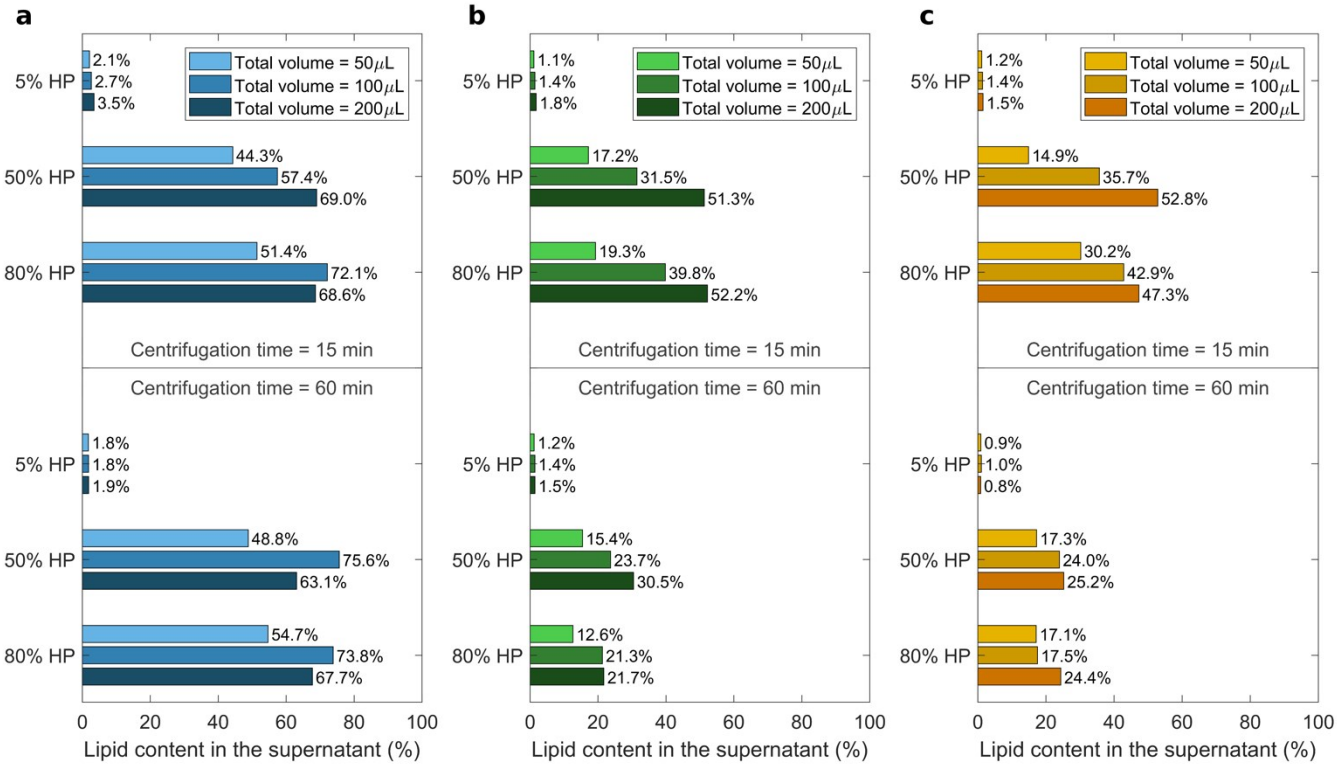

Figure S2. Recovery rates of lipids in the supernatant represented by percentage ratio of the concentration of lipid after precipitation to its initial concentration (before precipitation) for (a) DOTAP-DOPE 1:3 (mol/mol), (b) DOTAP-DOPE 1:1 (mol/mol), (c) DOTAP-DOPE 3:1 (mol/mol).

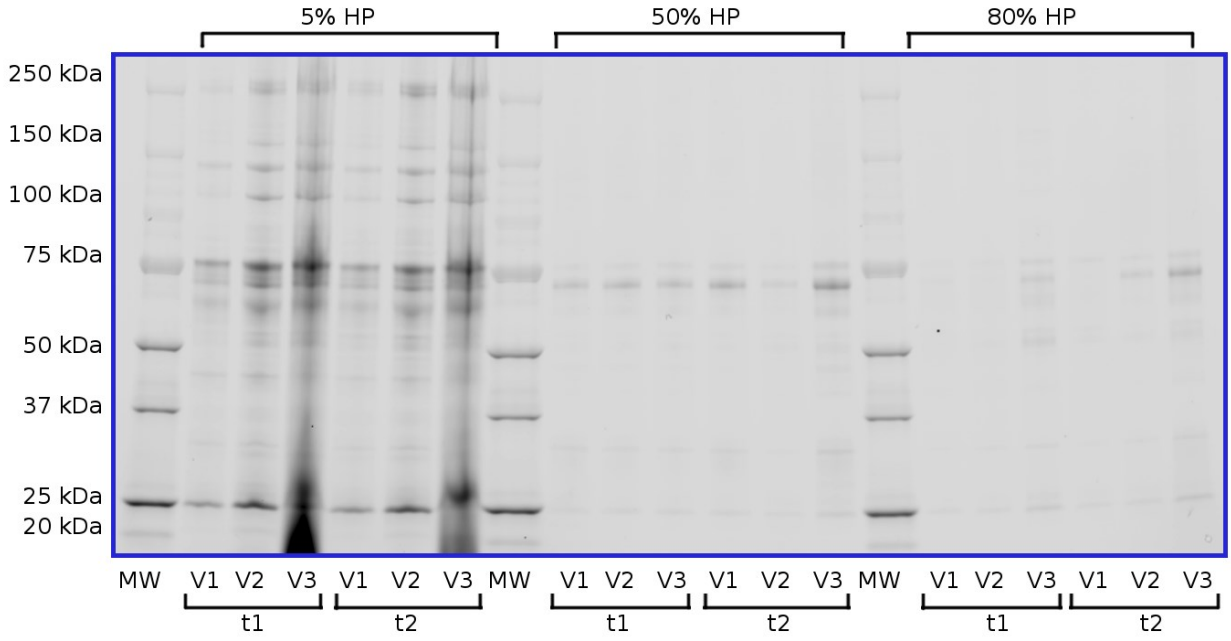

Figure S3. 1D SDS-PAGE image corresponding to the protein corona formed on DOTAP-DOPE 1:3 (mol/mol) upon exposure to human plasma under different incubation conditions (i.e. 5% HP, 50% HP and 80% HP) and centrifugation parameters (i.e. total volume V1=50 µL, V2=100 µL, V3=200 µL and t1=15 min, t2=60 min). Protein patterns depend on HP concentration, by do not depend on centrifugation protocol.

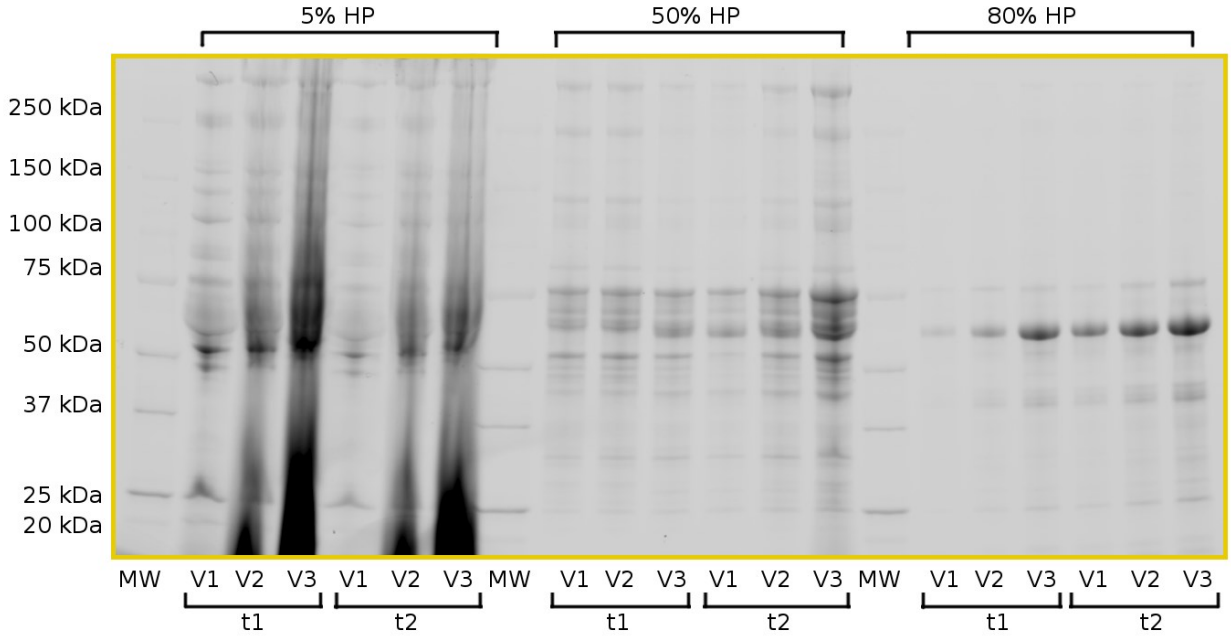

Figure S4. 1D SDS-PAGE image corresponding to the protein corona formed on DOTAP-DOPE 3:1 (mol/mol) upon exposure to human plasma under different incubation conditions (i.e. 5% HP, 50% HP and 80% HP) and centrifugation parameters (i.e. total volume V1=50 µL, V2=100 µL, V3=200 µL and t1=15 min, t2=60 min). Protein patterns depend on HP concentration, by do not depend on centrifugation protocol.

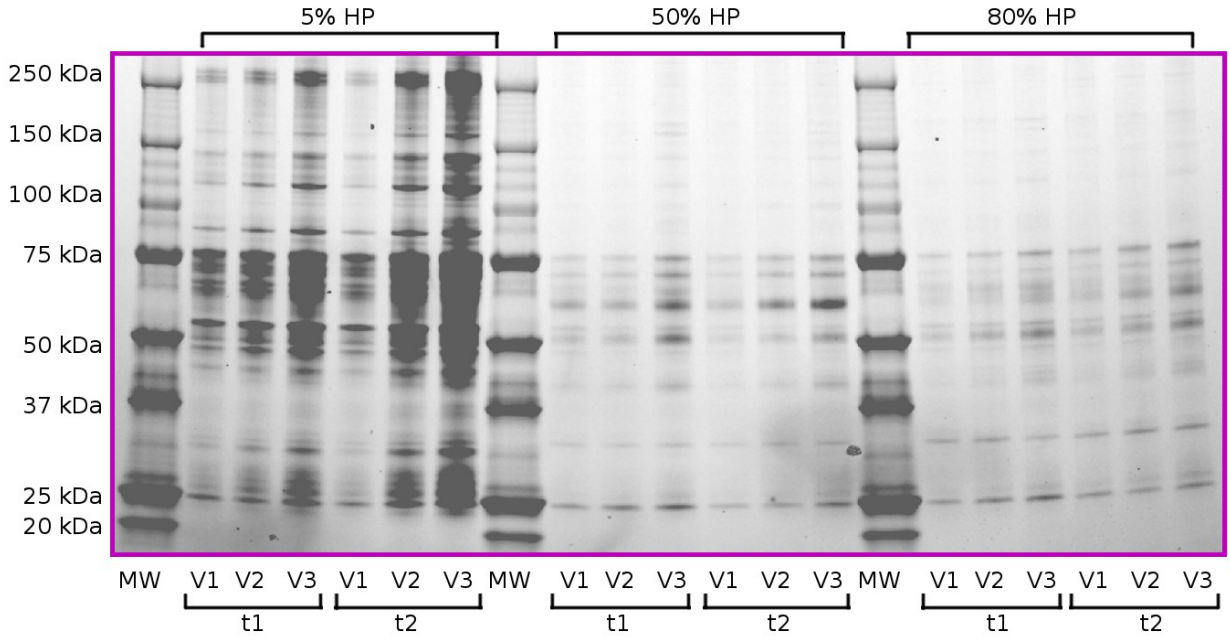

Figure S5. 1D SDS-PAGE image corresponding to the protein corona formed on PEGylated DOTAP-DOPE upon exposure to human plasma under different incubation conditions (i.e. 5% HP, 50% HP and 80% HP) and centrifugation parameters (i.e. total volume V1=50 µL, V2=100 µL, V3=200 µL and t1=15 min, t2=60 min). Protein patterns depend on HP concentration, by do not depend on centrifugation protocol.

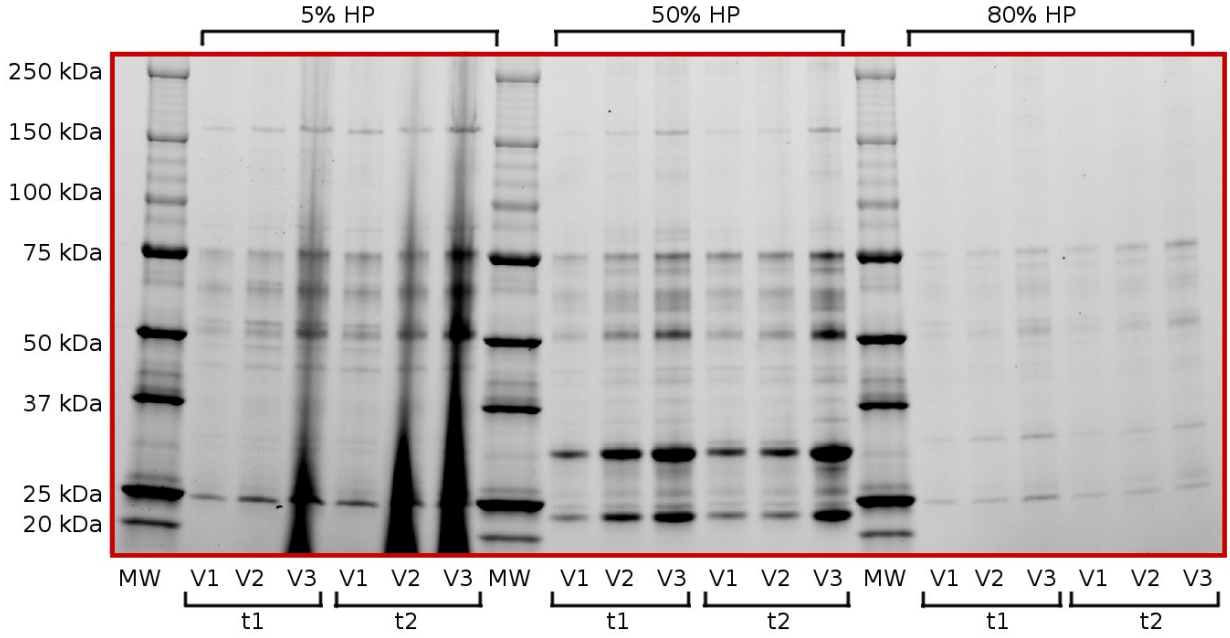

Figure S6. 1D SDS-PAGE image corresponding to the protein corona formed on PEGylated DOTAP-DOPE-DNA upon exposure to human plasma under different incubation conditions (i.e. 5% HP, 50% HP and 80% HP) and centrifugation parameters (i.e. total volume V1=50 µL, V2=100 µL, V3=200 µL and t1=15 min, t2=60 min). Protein patterns depend on HP concentration, by do not depend on centrifugation protocol.

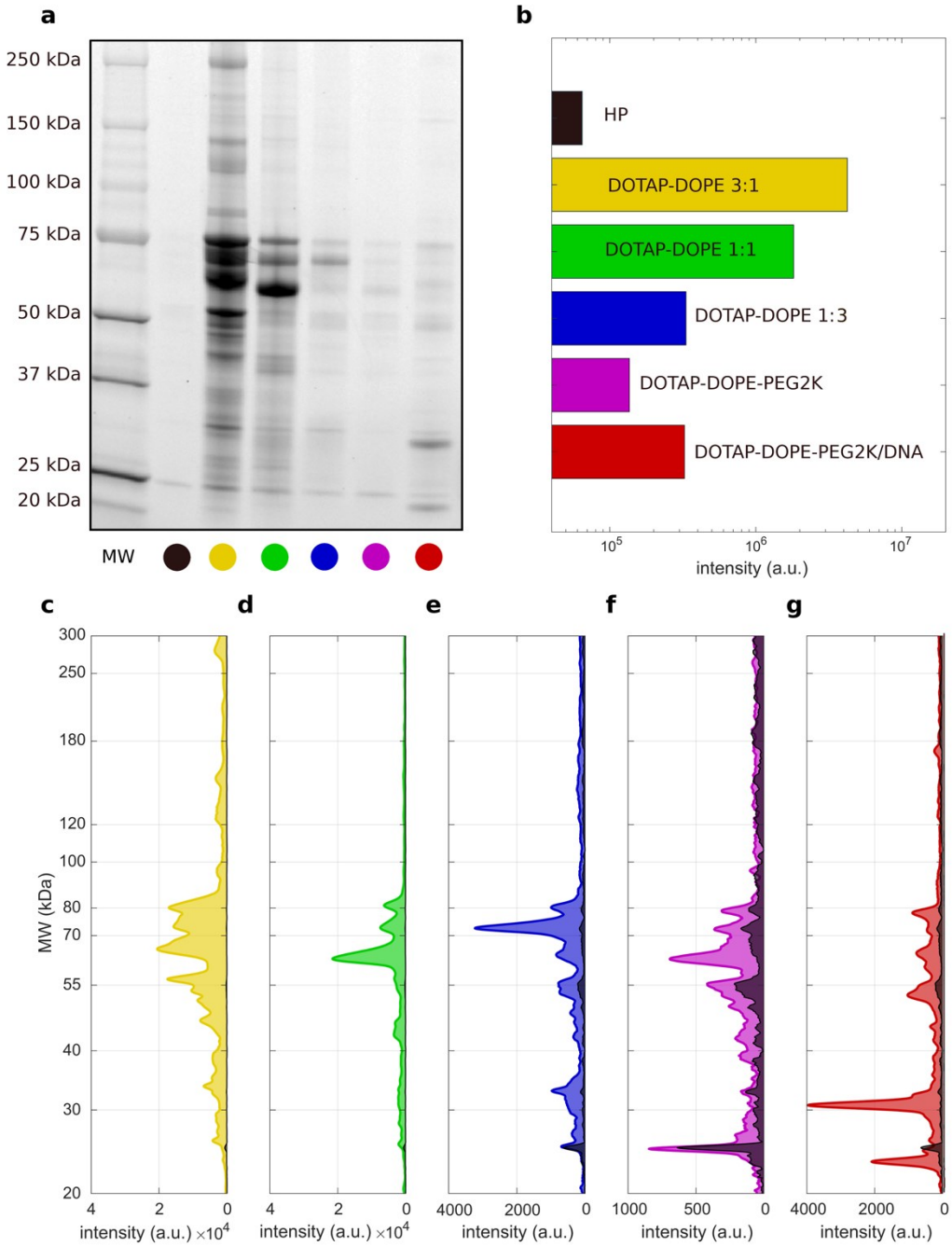

Figure S7. (a) 1D SDS-PAGE image of PC for human plasma (HP) and liposomal formulations upon incubation with HP (50%), total volume = 50 µL and centrifugation time = 15 min. (b)Total lane intensity for each lane. (c-g) Corresponding absolute intensity profiles for the liposomal formulations, whereas the absolute profile of HP is superimposed as black shaded area.

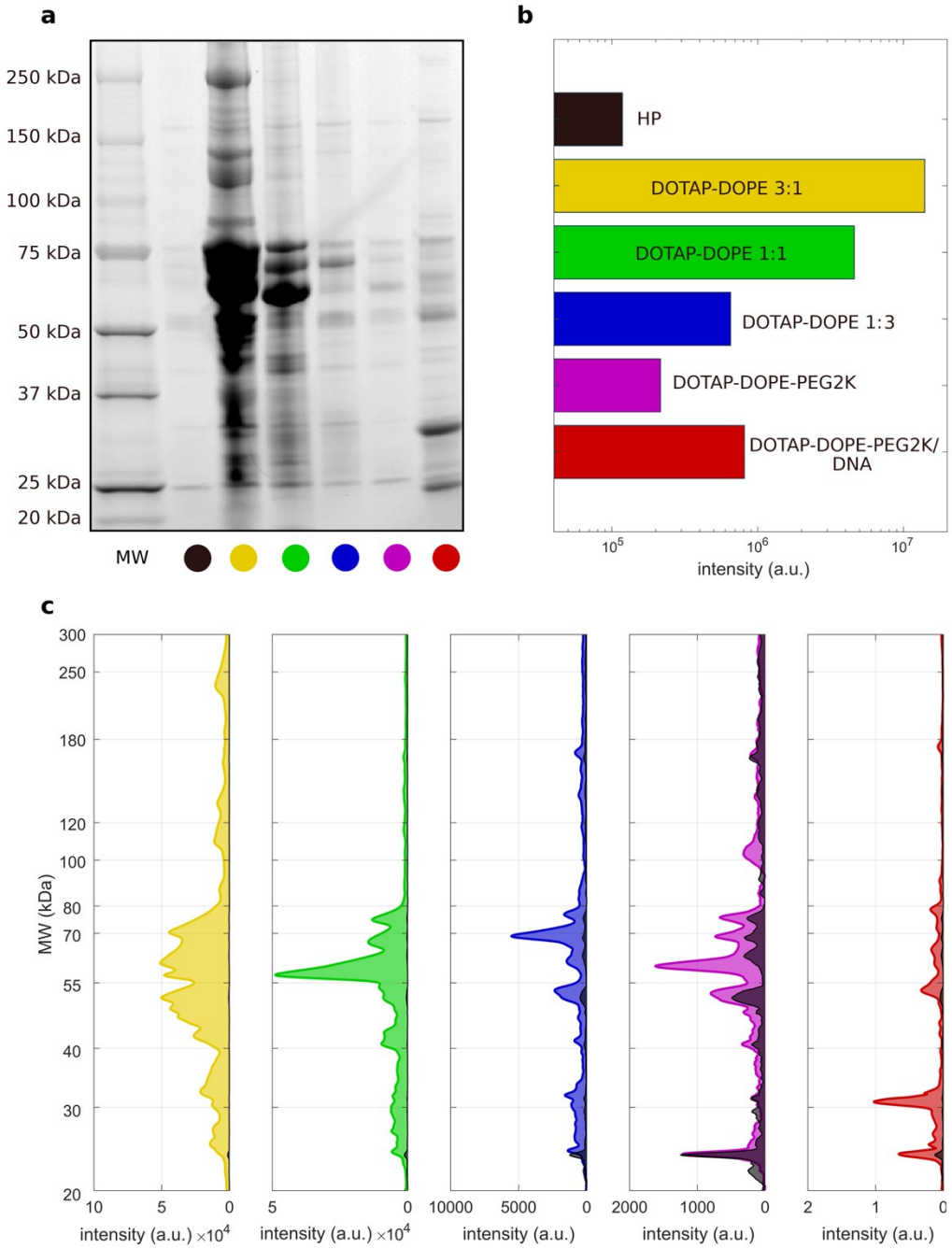

Figure S8. (a) 1D SDS-PAGE image of PC for human plasma (HP) and liposomal formulations upon incubation with HP (50% vol), total volume = 200 µL and centrifugation time = 15 min. (b)Total lane intensity for each lane. (c-g) Corresponding absolute intensity profiles for the liposomal formulations, whereas the absolute profile of HP is superimposed as black shaded area.

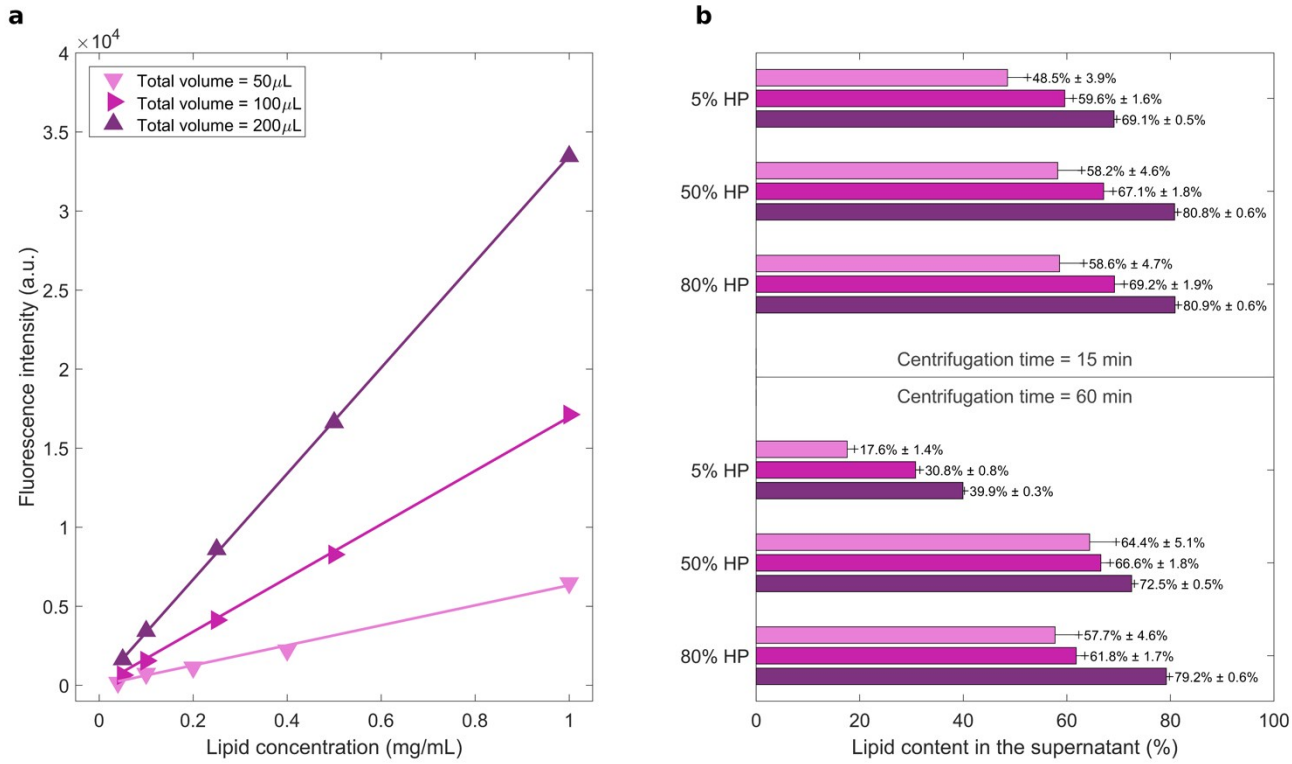

Figure S9. (a) Calibration curves for the measurements of lipid concentration by fluorescence experiments on the PEGylated liposomes. (b) Measured recovery rates of lipids in the supernatant represented by percentage ratio of the lipid amount after precipitation to its initial amount (before precipitation).

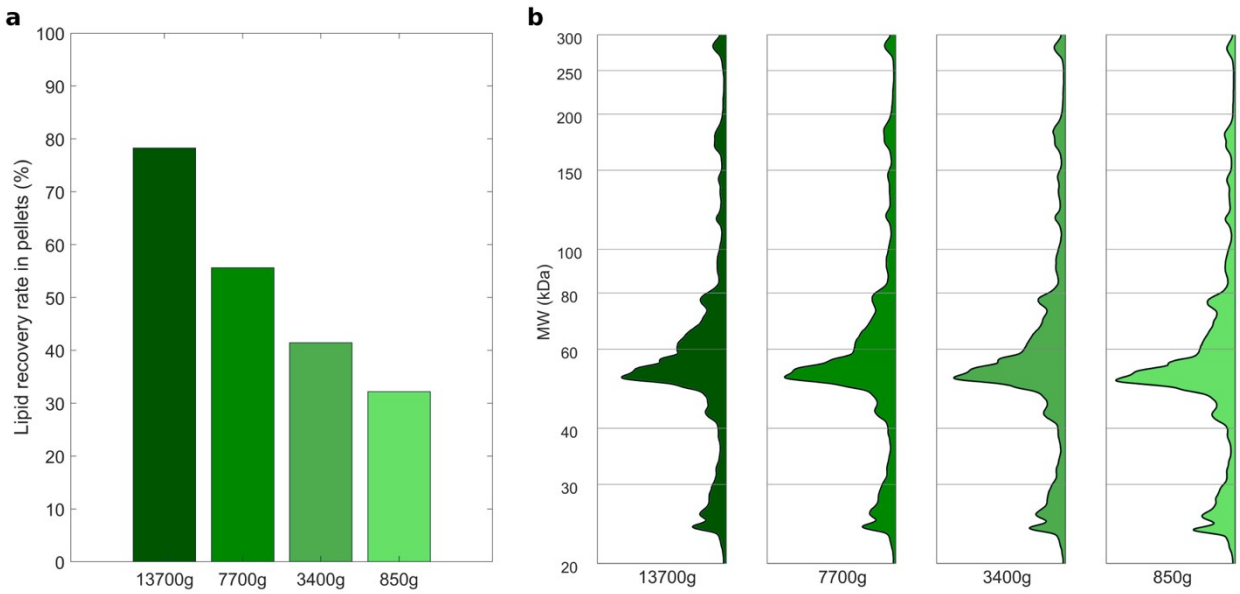

Figure S10. (a) Recovery rates of lipids in the PC pelletized from DOTAP-DOPE 1:1 (mol/mol) after incubation with HP (50% vol) (total sample volume= 100 ml) as a function of centrifugation speed from 13,700 g to 850 g. Recovery rates are given as percentages with respect to the centrifuge speed used to perform all the experiments reported in the work (i.e. 21,400 g). (b) Normalized one-dimensional protein patterns.