Electronic Supplementary Material (ESI) for Environmental Science: Nano.
This journal is © The Royal Society of Chemistry 2019

Electronic Supplementary Information

Membrane outer leaflet is the primary regulator of membrane damage induced by silica nanoparticles in vesicles and erythrocytes

Saeed Nazemidashtarjandi,[a] Amir M. Farnoud,[b,*]

[a]Department of Chemical and Biomolecular Engineering and [b]Biomedical Engineering Program, Ohio University, Athens, OH 45701

[*]To whom correspondence should be addressed: Amir M. Farnoud, Department of Chemical and Biomolecular Engineering and bBiomedical Engineering Program, Ohio University, Athens, OH 45701, Tel.: (740) 593-1426; Fax: (740) 593-0873; E- mail: farnoud@ohio.edu

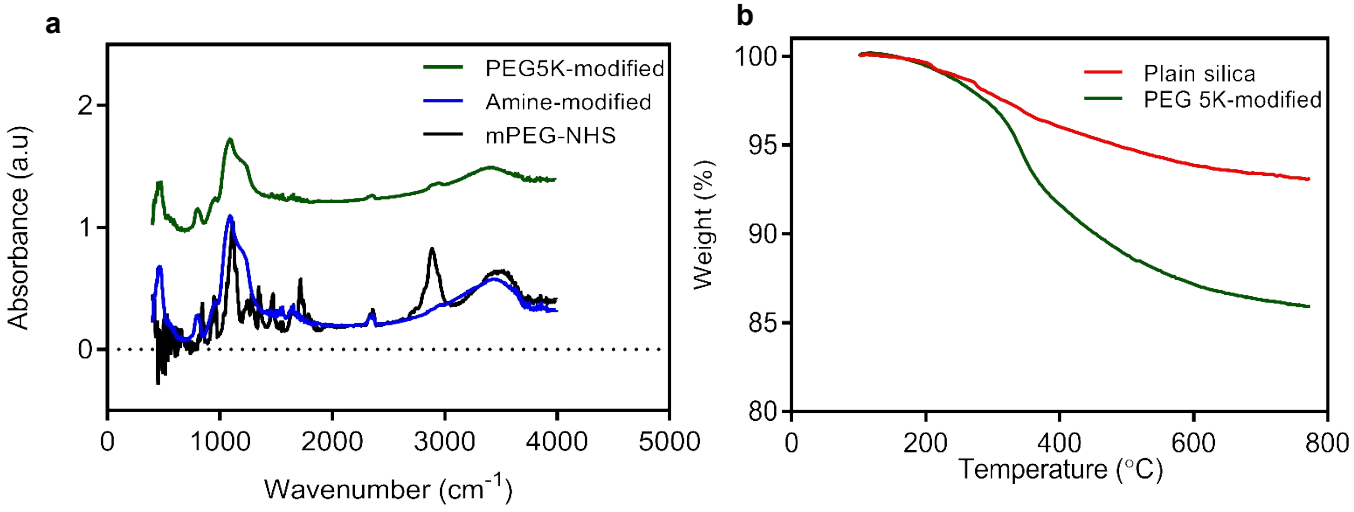


Figure. S1. **a**, Fourier Transform Infrared (FT-IR) spectra of PEG 5K-modified and amine-modified silica nanoparticles, The spectrum for methoxy polyethylene glycol (m-PEG-NHS) is also given for comparison. New absorption peaks at 2886 and 2960 cm^{-1} were observed in PEG 5K-modified silica nanoparticles corresponding to peaks of PEG-NHS[1]. **b**, Thermogravimetric analysis (TGA) of plain and PEG 5K-modified silica nanoparticles. Particle weight loss as a function of temperature was used to estimate the grafting density of PEG molecules using Equation 2. PEG 5K-modified silica particles had an estimated grafting density of 0.33 PEG chains/nm^2.

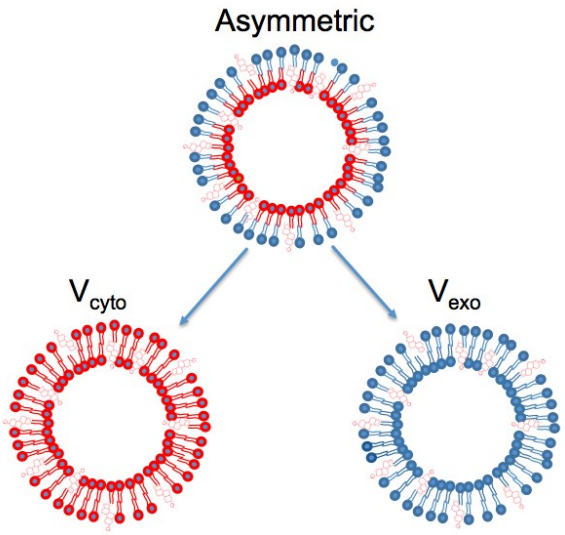


Figure. S2. Vesicles mimicking the exofacial (V_{exo}) and cytofacial (V_{cyto}) leaflet of erythrocyte plasma membranes were synthetized based on the lipid composition of the erythrocyte cell membrane[2–4]. V_{exo} vesicles had a composition of 44% cholesterol (Chol), 26% dioleoylphosphatidylcholine (DOPC), 24% sphingomyelin (SM), and 6% dioleoylphosphatidylethanolamine (DOPE) and V_{cyto} vesicles had a composition of 44% Chol, 8% DOPC, 5% SM, 25% DOPE, and 18% dioleoylphosphatidylserine (DOPS).

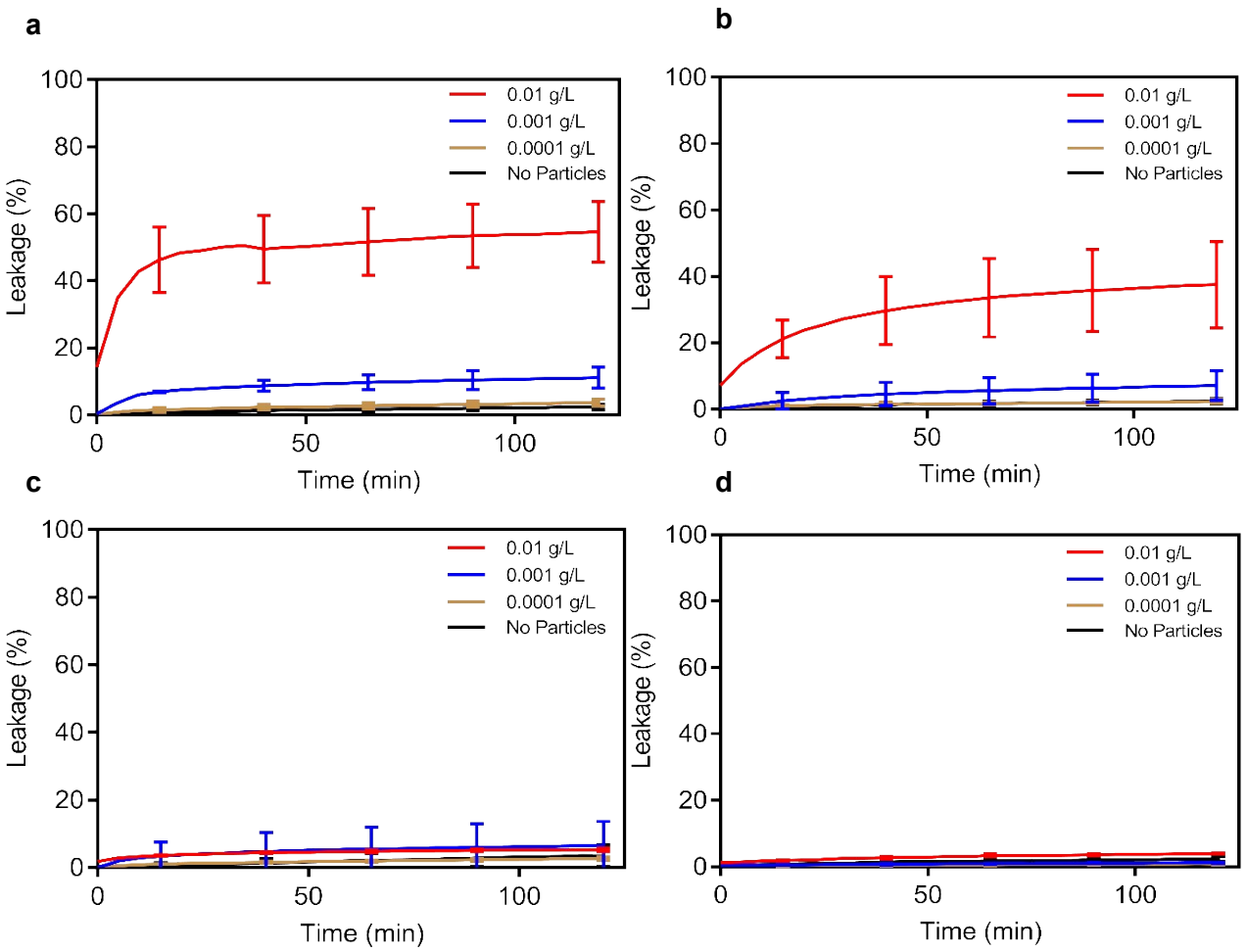


Figure. S3. Leakage of CF from V_{exo} vesicles induced by various concentrations of **a**, Plain, **b**, Amine-modified, **c**, Carboxyl-modified, and **d**, PEG 5K-modified engineered silica nanoparticles (50 nm) following incubation at 37 °C in PBS. Error bars demonstrate the standard deviation from at least three independent experiments. **A**, One-way ANOVA with Dunnett's post-hoc compared to control (i.e. no particles), after two hours of incubation: plain silica P(0.0001 g/L)=0.9930, P (0.001 g/L)=0.1520, and ****P (0.01 g/L)<0.0001. **B**, One-way ANOVA with Dunnett's post-hoc compared to control (i.e. no particles), after two hours of incubation: amine-modified P(0.0001 g/L)>0.9999, P(0.001 g/L)=0.7923, and ****P(0.01 g/L)<0.0001. **c**, One-way ANOVA with Dunnett's post-hoc did not reveal a significant difference compared to control (p>0.05), **d,** One-way ANOVA with Dunnett's post-hoc did not reveal a significant difference compared to control (p>0.05).

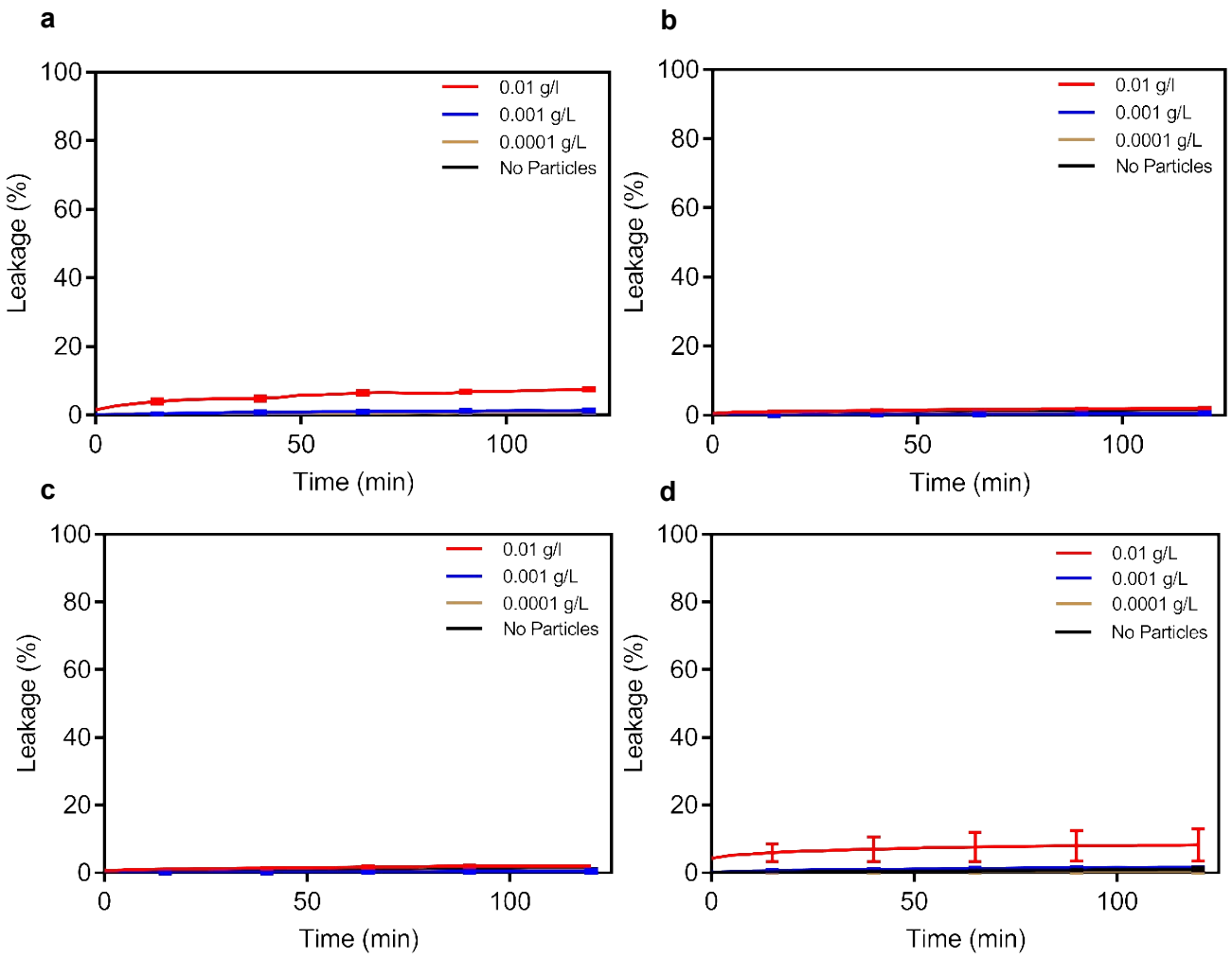
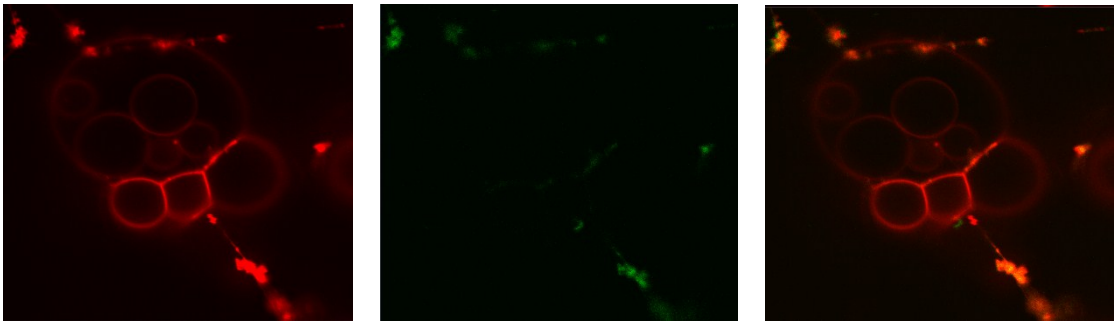


Figure. S4. Leakage of CF from V_{cyto} vesicles induced by various concentrations of **a**, Plain, **b**, Amine-modified, **c**, Carboxyl-modified, and **d**, PEG 5K-modified silica nanoparticles (50 nm) following two hours of incubation at 37 °C in PBS. Error bars demonstrate the standard deviation from at least three independent experiments. **A**, One-way ANOVA with Dunnett's post-hoc compared to control (i.e. no particles) after two hours of incubation: P(0.0001 g/L)=0.9999, P (0.001 g/L)=0.9912, and *P(0.01 g/L)=0.0207. **B**, One-way ANOVA with Dunnett's post-hoc compared to control (i.e. no particles) after two hours of incubation: amine-modified P(0.0001 g/L)=0.5378, P(0.001)=0.6777, and *P(0.01 g/L)=0.0208. **c**, One-way ANOVA with Dunnett's post-hoc did not reveal a significant difference compared to control (p>0.05). **d**, One-way ANOVA with Dunnett's post-hoc did not reveal a significant difference compared to control (p>0.05).

a



b

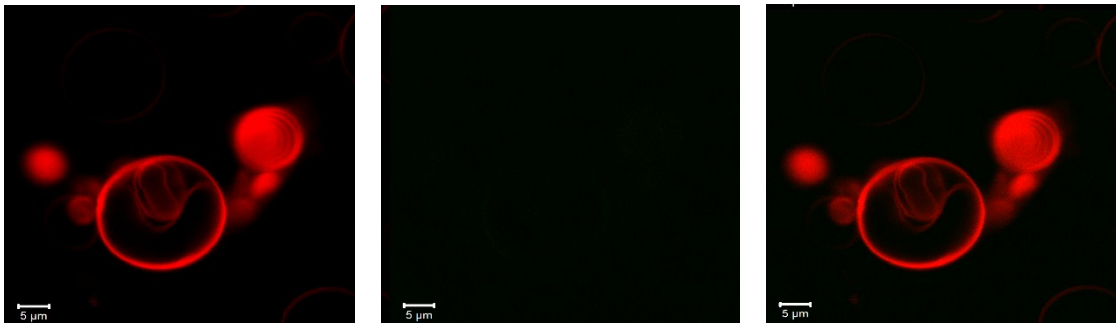


Figure. S5. Confocal microscopy of V_{exo} vesicles, labeled with 0.2 mole% Rho-DOPE (red), after incubation with fluorescent engineered silica nanoparticles (green) at a concentration of 0.01 g/L for 2 hours. **a**, V_{exo} vesicles exposed to plain nanoparticles, **b**, V_{exo} vesicles exposed to amine-modified nanoparticles.

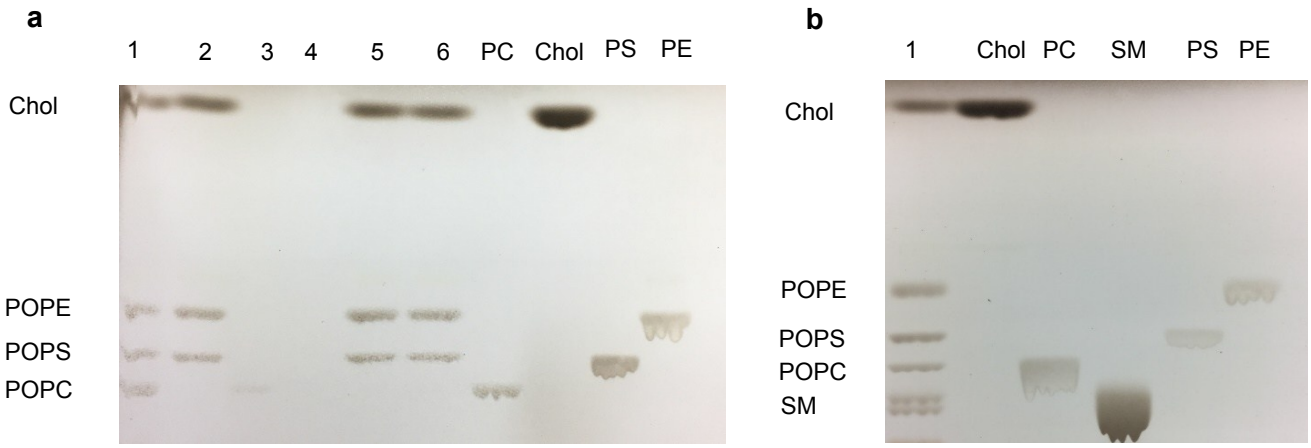


Figure. S6. Lipid transfer using HPαCD for the synthesis of asymmetric vesicles following the methods of Lin and London[5]. The vesicle pellet following ultracentrifugation (please see the Experimental section) in each case were analyzed using thin layer chromatography. **A**, Incubation of POPC MLVs with HPαCD in presence of 3:3:4 POPE: POPS: cholesterol LUVs yielded asymmetric vesicles (lane 1 of the thin layer chromatography plate from left side). Removing cyclodextrin (lane 2), acceptor vesicles (lane 3), acceptor vesicles and cyclodextrin (lane 4), donor vesicles (lane 5), and donor vesicles and cyclodextrin (lane 6) inhibited the synthesis of asymmetric vesicle. The rest of lanes are only lipid bands as controls. **b**, Incubation of POPC/SM MLVs with HPαCD in presence of 3:3:4 POPE: POPS: cholesterol LUVs yielded asymmetric vesicles (lane 1 of the thin layer chromatography plate from left side), the rest of lanes are only lipid bands as controls.

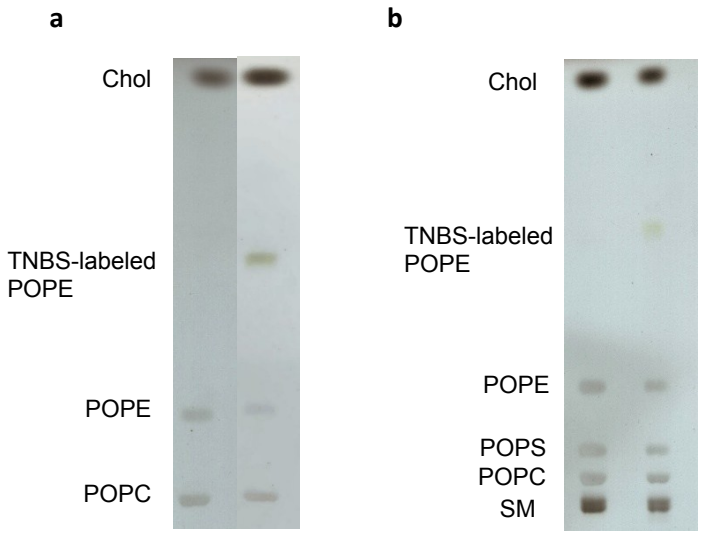


Figure. S7. TNBS labeling of POPE in symmetric and asymmetric vesicles. **a**, symmetric vesicles with a lipid composition of POPE/POPC/Chol (3:3:4) with TNBS labeling (left lane, note the additional band from TNBS-labeled POPE) or without TNBS labeling (right lane). **b**, asymmetric vesicles with a lipid composition of SM:POPCo/POPE:POPSi/Chol without TNBS labeling (left lane) or with TNBS labeling (right lane, note the additional band from labeled POPE).

Table S1. Hydrodynamic diameter and zeta potential (in PBS at physiological pH) of vesicles used in the current study.

Membrane model	Extruded vesicles size (nm)	Zeta potential (mV)
Exofacial vesicles (V_{exo})	354.9 ± 42.8	-4.9 ± 3.2
Cytofacial vesicles (V_{cyto})	205.7 ± 159.4	-25.9 ± 1.0

Table S2. Surface functional group, hydrodynamic diameter, and zeta potential (in PBS at physiological pH) of silica nanoparticles used in the current study. In addition to silica, polystyrene particles (size: 234.1 ± 54.8 nm and zeta potential: 27.4 ± 2.6 mV) were also used.

Non-fluorescent, nominal diameter=100 nm			Fluorescent, nominal diameter= 50 nm		
Surface functional group	Hydrodynamic diameter (nm)	Zeta potential (mV)	Surface functional group	Hydrodynamic diameter (nm)	Zeta potential (mV)
Plain	154.7 ± 5.4	-43.6 ± 3.5	Plain	51.1 ± 22.0	-11.1 ± 1.9
Amine	160.4 ± 6.6	-13.6 ± 2.5	Amine	49.4 ± 19.0	-8.0 ± 0.9
PEG 2K*	170.6 ± 6.6	-19.2 ± 3.1	Carboxyl	53.1 ± 22.9	-11.2 ± 1.1
PEG 5K	182.3 ± 8.5	-32.1 ± 2.3	PEG 5K	253.0 ± 127.5	-12.7 ± 0.5
PEG 20K	184.3 ± 2.6	-33.5 ± 1.1			

* PEG 2K: Poly(ethyleneglycol) with a molecular weight of 2K Daltons.

References

1 H. Xu, F. Yan, E. E. Monson and R. Kopelman, Room-temperature preparation and characterization of poly (ethylene glycol)-coated silica nanoparticles for biomedical applications, *Journal of Biomedical Materials Research*, 2003, **66A**, 870–879.

2 J. A. Virtanen, K. H. Cheng and P. Somerharju, Phospholipid composition of the mammalian red cell membrane can be rationalized by a superlattice model, *Proceedings of the National Academy of Sciences*, 1998, **95**, 4964–4969.

3 M. Garnier, J. R. Attali, P. Valensi, E. Delatour-Hanss, F. Gaudey and D. Koutsouris, Erythrocyte deformability in diabetes and erythrocyte membrane lipid composition, *Metabolism*, 1990, **39**, 794–798.

4 K. Leidl, G. Liebisch, D. Richter and G. Schmitz, Mass spectrometric analysis of lipid species of human circulating blood cells, *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 2008, **1781**, 655–664.

5 Q. Lin and E. London, Preparation of Artificial Plasma Membrane Mimicking Vesicles with Lipid Asymmetry, *PLoS ONE*, 2014, **9**, e87903.