

## Supplementary Information

### Protein Corona of Anionic Fluid-Phase Liposomes Compromises Their Integrity rather than Uptake by Cells

Daria Tretiakova<sup>1</sup>, Maria Kobanenko<sup>1</sup>, Anna Alekseeva<sup>1</sup>, Ivan Boldyrev<sup>1</sup>, Sergey Khaidukov<sup>2</sup>, Viktor Zgoda<sup>3</sup>, Olga Tikhonova<sup>3</sup>, Elena Vodovozova<sup>1</sup> and Natalia Onishchenko<sup>1, a, \*</sup>

<sup>1</sup> Laboratory of lipid chemistry, Department of chemical biology of glycans and lipids, Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow, Russian Federation; mkobanenko@lipids.ibch.ru (M.K.); daria@lipids.ibch.ru (D.T.); anna@lipids.ibch.ru (A.A.); ivan@lipids.ibch.ru (I.B.); elvod.ibch@yandex.ru (E.V.)

<sup>2</sup> Laboratory of carbohydrates, Department of chemical biology of glycans and lipids, Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, 117997 Moscow, Russian Federation

<sup>3</sup> Institute of Biomedical Chemistry, ul. Pogodinskaya 10, 119121, Moscow, Russian Federation; vic@ibmc.msk.ru (V.Z.); ovt.facility@gmail.com (O.T.)

<sup>a</sup> The author's current affiliation is the Center for Soft and Living Matter, Institute for Basic Science, UNIST-gil 50 bldg, 103, 44919 Ulsan, Republic of Korea.

\* Correspondence: natalia.r.onishchenko@gmail.com; Tel.: +8210-5855-5281

Table S1. Liposome diameter before incubation with 50% HP and after incubation followed by separation of the liposome–protein complexes using SEC

Sample ID	$D_H \pm SD, \text{ nm}$	
	Liposomes	Liposome–protein complexes
PC	194.4 $\pm$ 3.8	186.2 $\pm$ 1.8
33CH	199.9 $\pm$ 4.4	190.1 $\pm$ 3.0
CHPG	164.6 $\pm$ 2.6	150.3 $\pm$ 1.4
10PG	153.1 $\pm$ 0.6	160.6 $\pm$ 5.4
40PG	148.7 $\pm$ 1.1	232.2 $\pm$ 9.5

Data obtained for ~200-nm liposomes prepared in 10 mM KCl with 1 mM K<sub>2</sub>HPO<sub>4</sub> and 1 mM KH<sub>2</sub>PO<sub>4</sub> measured using Litesizer 500 (Anton Paar GmbH, Austria).

Calcein release assay

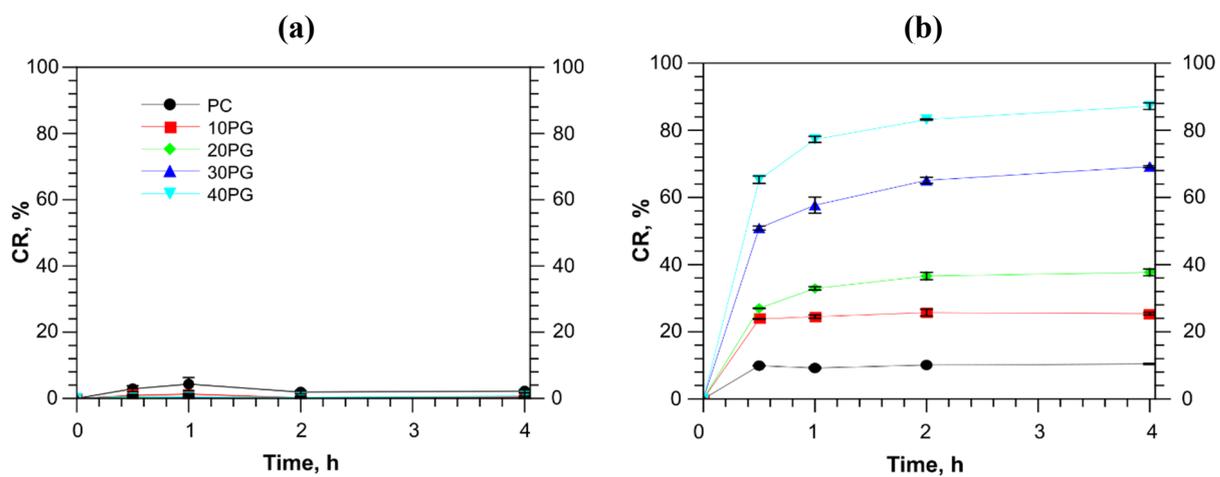


Figure S1. Calcein release assessment in PBS (a) and in 50% human plasma (b) for liposomes with increasing POPG percentage.

Total peptide assay

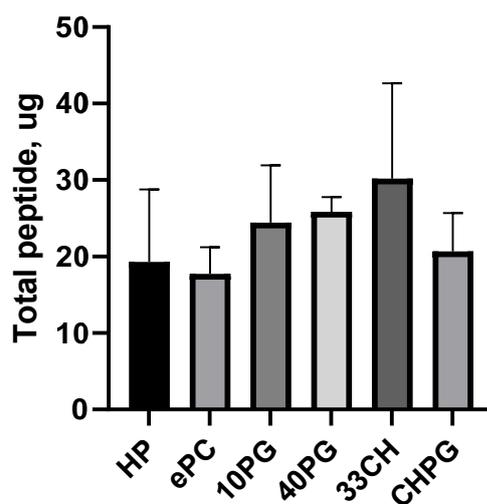


Figure S2. Peptide content in samples after proteolysis and filtration on STrap filters measured using the Pierce™ Quantitative Colorimetric Peptide Assay kit (Thermo Scientific, Waltham, MA, USA). No statistically significant differences according to Welch's ANOVA,  $p < 0.05$ . Note that HP sample represents combined material of three SEC runs (one SEC run was sufficient for other samples).

Western blotting

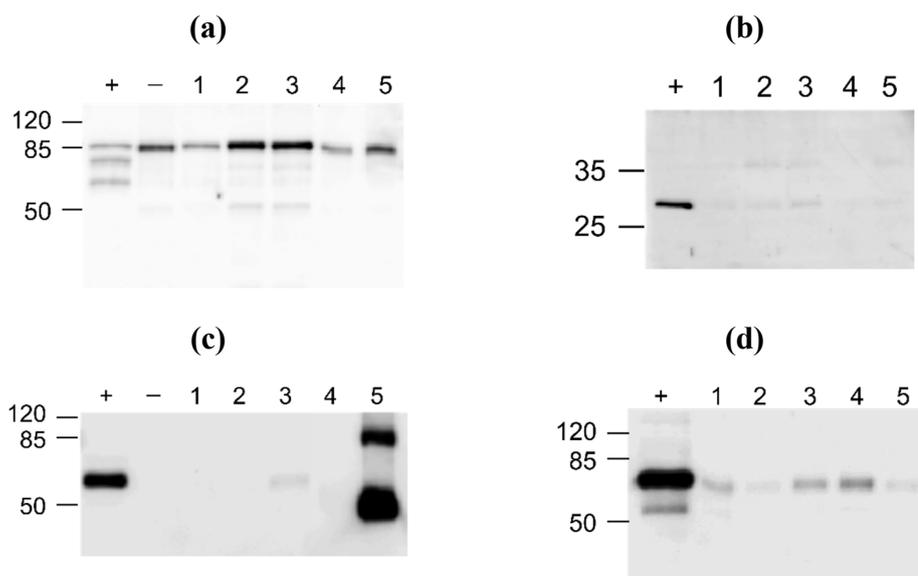


Figure S3. Western blotting results with (a) anti-IgM, (b) anti-ApoA1, (c) anti-beta-2-glycoprotein 1, and (d) anti-HSA antibodies. Designations: +, diluted HP; -, negative control HP; 1, PC; 2, 33CH; 3, CHPG; 4, 10PG; 5, 40PG

Western blotting has given us the reassurance of mass spectroscopy data, as we have observed the same patterns of protein adsorption for IgM, ApoA1, beta-2-glycoprotein 1, and albumin (Figs. S2a-d).

*Liposome uptake by EA.hy926 cells*

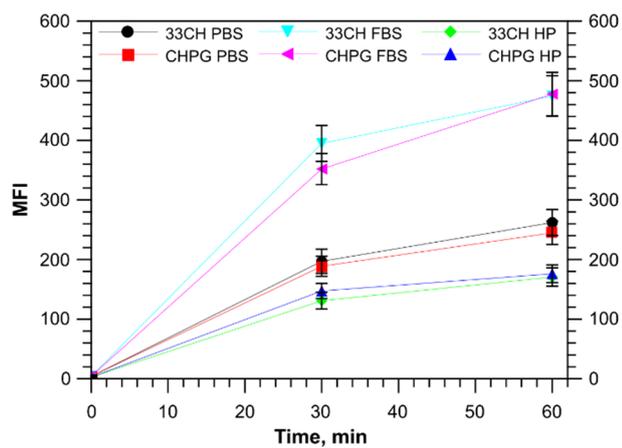


Figure S4. Liposome (33CH and CHPG samples) uptake by EA.hy926 cells without protein corona, with protein corona formed in FBS and with protein corona formed in HP.