Supporting Information

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In Vivo Retargeting of Poly(beta aminoester) (OM-PBAE)
Nanoparticles is Influenced by Protein Corona

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SUPPLEMENTARY INFORMATION

Table S1: KHret NPs physicochemical characterization encapsulating mRNA.

Nanoparticle formulation	Hydrodynamic diameter (nm; by DLS)	Hydrodynamic diameter (nm; by NTA)	Mode of the hydrodynamic diameter (nm; by NTA)	PDI (by DLS)	Surface charge (mV, by DLS)
KHret _{fresh}	153,66 ± 19,07	121,17 ± 3,49	110	0,221 ± 0,052	---
KHret _{lyophilized}	166,46 ± 6,81	133,10 ± 8,22	125	0,152 ± 0,030	29,92 ± 6,66

mRNA-loaded KHret nanoparticles were also characterized to confirm that changing the nucleic acid loaded, physicochemical properties of nanoparticles did not change. Data from KH mRNA-loaded nanoparticles were previously published in Fornaguera et al., [1].

Table S2: BCA quantification of proteins of each fraction.

Sample	Fraction	Concentration (μg/mL)
KH	SN1	7243
	SN2	191
	SN3	185
	P	57
KHret	SN1	7030
	SN2	185
	SN3	172
	P	42

BCA kit was used to quantified each protein fraction. As shown in Table S2, most proteins were found in the first supernatant, independently on the use of KH or KHret nanoparticles, which corresponds to those proteins not attached or slightly attached to nanoparticle surface (soft corona). Since we are only interested in those proteins strongly attached (hard corona), this was the fraction analyzed by proteomics, the last supernatant (SN3), after the de-attachment using a detergent.

Table S3: Top 5 most abundant proteins found by MALDI/TOF protein identification in either KH or KHret nanoparticles.

A KH NPs

Accession	Protein	MW [kDa]	pI	#Alt. Proteins	Score	#Peptides	SC [%]	RMS90 [ppm]	# Spec Counts
ALBU_BOVIN	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4	69,2	5,8	1	121,2	3	4,6	3,3	5
BOJYN6_BOVIN	Alpha-2-HS-glycoprotein OS=Bos taurus GN=AHSG PE=1 SV=1	38,4	5,2	2	51,4	2	4,7	1,42	2
TRFE_BOVIN	Serotransferrin OS=Bos taurus GN=TF PE=2 SV=1	77,7	6,9	2	47,9	2	4,1	0,85	2
A2MG_BOVIN	Alpha-2-macroglobulin OS=Bos taurus GN=A2M PE=1 SV=2	167,5	5,7	1	54,1	1	0,7	1,68	1
HBA_BOVIN	Hemoglobin subunit alpha OS=Bos taurus GN=HBA PE=1 SV=2	15,2	9,1	1	28,3	1	6,3	0,55	1

B KHret NPs

Accession	Protein	MW [kDa]	pI	#Alt. Proteins	Score	#Peptides	SC [%]	RMS90 [ppm]	# Spec Counts
ALBU_BOVIN	Serum albumin OS=Bos taurus GN=ALB PE=1 SV=4	69,2	5,8	1	142,5	4	6,9	0,97	4
APOD_BOVIN	Apolipoprotein D OS=Bos taurus GN=APOD PE=2 SV=1	21,4	4,6	2	32,5	2	6,9	1,34	2
A1AT_BOVIN	Alpha-1-antitrypsin OS=Bos taurus GN=SERPINA1 PE=1 SV=1	46,1	6,1	1	25,6	1	1,7	0,99	1
APOA2_BOVIN	Apolipoprotein A-II OS=Bos taurus GN=APOA2 PE=1 SV=2	11,2	9,1	1	47,1	1	9	1,23	1
BOJYN6_BOVIN	Alpha-2-HS-glycoprotein OS=Bos taurus GN=AHSG PE=1 SV=1	38,4	5,2	2	36,2	1	3,3	3,92	1

KH and KHret NPs were incubated with FBS for 18h and, after this time, they were cleaned to remove the soft corona. In here, only hard corona (those proteins strongly attached to nanoparticles, removed through detergent treatment) is analyzed. Five most abundant proteins found are presented. It is worth noting that these proteins do not correspond to most abundant plasma proteins, [2] so if they have been detected, this means that they play a role in corona. As it can be seen in the plots, the amount of proteins forming hard corona is markedly low, which indicates that only a small strong corona is formed. Nevertheless, protein types are different in both types of nanoparticles analyzing, being this the explanation of the differential accumulation when retinol is added, since apolipoproteins, which favor liver targeting, are only found in those nanoparticles containing retinol.

Figure S2: GPC of retinol-PBAE, to calculate its molecular weight, in reference to polystyrene standards.

Figure S3: TEM and AFM micrographies of KH nanoparticles to demonstrate their rounded shape.

Figure S4: Examples of the cell fluorescence after 24h transfection with mRNA eGFP, as a function of cell line and nanoparticles incubated.

Figure S5: SDS-PAGE electrophoresis of different fractions of the nanoparticles previously incubated with FBS.

SDS-PAGE electrophoresis, experimentally performed as described in Fornaguera doctoral thesis [3], was used here to demonstrate that soft coronas are mainly formed by albumin, as expected, due to the high concentration of this protein in the whole protein fraction, that enables its soft attachment to nanoparticle surface thanks to its prevalence in comparison to other blood proteins. The samples correspond to the fractions obtained by performing the same procedure than for proteomics analysis, where, in this gel, the supernatant of the first cleaning step (SN1 – soft corona), the cleaning of the second cleaning step (SN2 – soft corona), the pellet from the denaturalization step (P3 – non-detached proteins) and the supernatant after denaturalization (SN3 – hard corona) are shown.

Explanation S1: Buffer composition used for livers digestion.

Hanks I

200 mL Hanks-Hepes buffer

0.0456 g EDTA

0.2 g BSA

0.7U Heparin 5% (0.7mL; 0.1g/2mL)

Hanks II

300 mL Hanks-Hepes buffer

0.1755 mg $\text{CaCl}_2 \cdot \text{H}_2\text{O}$

Hanks-Hepes

50 mL Hanks 10x

1.048 g NaHCO_3

450 mL milliQ water

Gasify with 95/5 CO_2/O_2 (or all CO_2 if O_2 is not available)

Add 1.5 g Hepes

Adjust pH to 7.4

Hanks 10x

27.6 g NaCl

1.44 g KCl

0.52 g KH_2PO_4

0.72 g MgSO_4

8 g NaHCO_3

1 L milliQ water

Autoclaved