Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Interaction of Spin-Labeled HPMA-based Nanoparticles with Human Blood Plasma Proteins – Introduction of protein-corona free polymer nanomedicines

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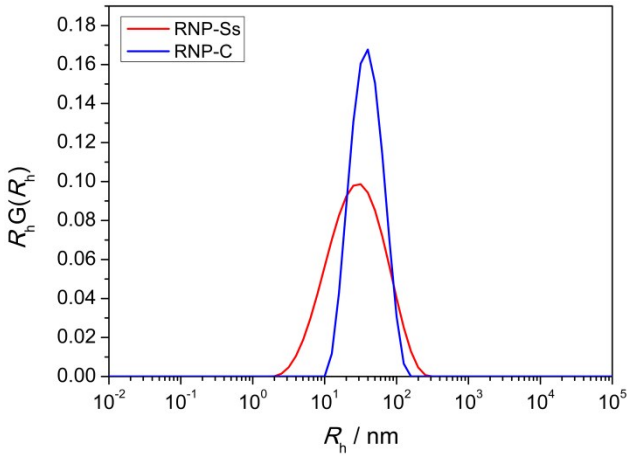

Fig. S1 Distribution functions of hydrodynamic radii for RNP-C and RNP-Ss in PBS buffer at 37 °C.

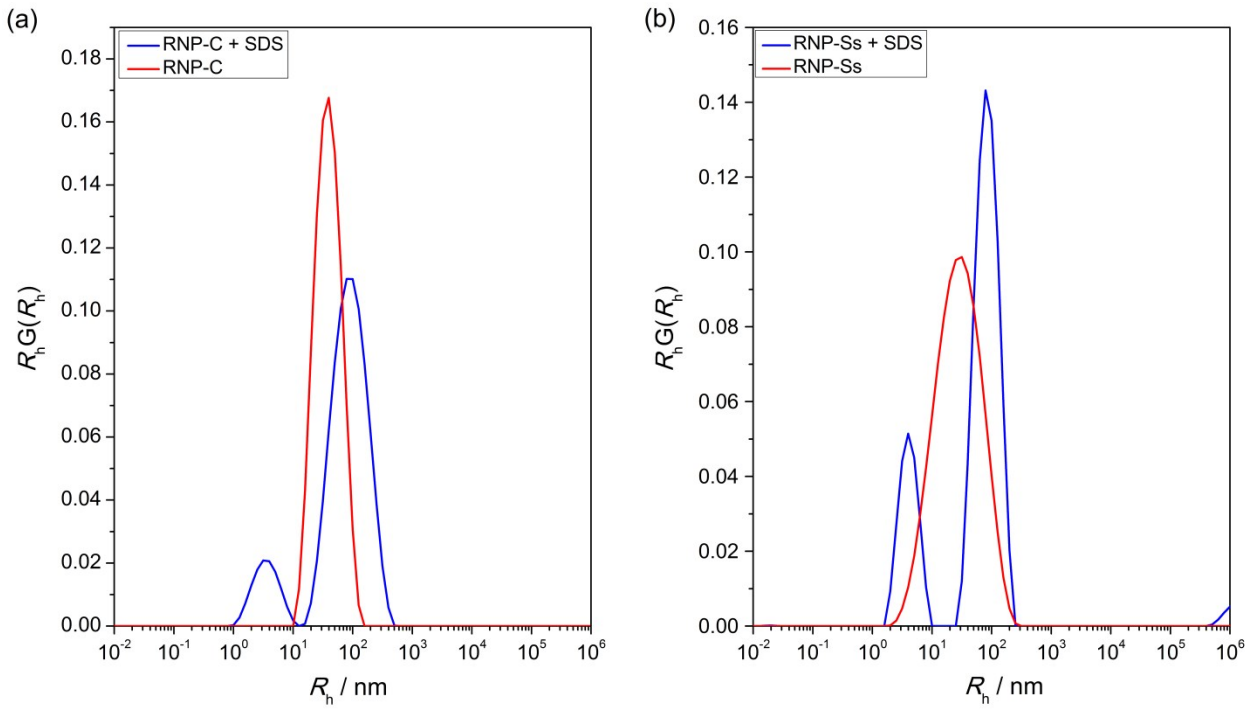

Fig. S2 Distribution functions of hydrodynamic radii for (a) RNP-C and (b) RNP-Ss in PBS buffer with and without SDS (c = 2 mg mL^{-1}) at 37 °C.

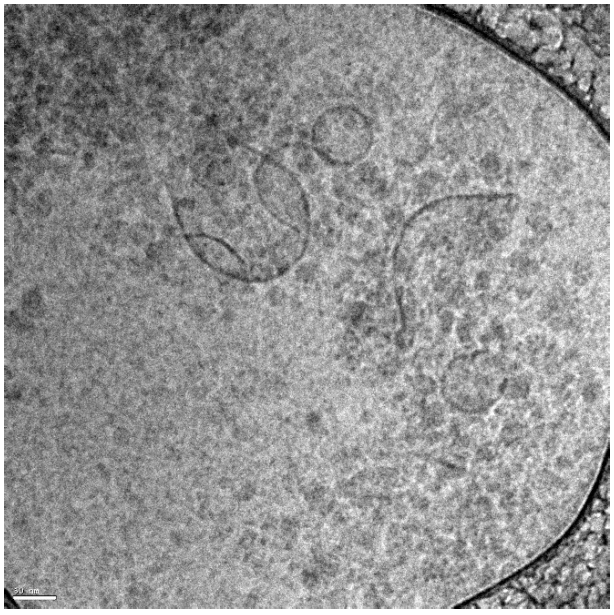

Fig. S3 Cryo-TEM image of RNP-Ss in the presence of human plasma.

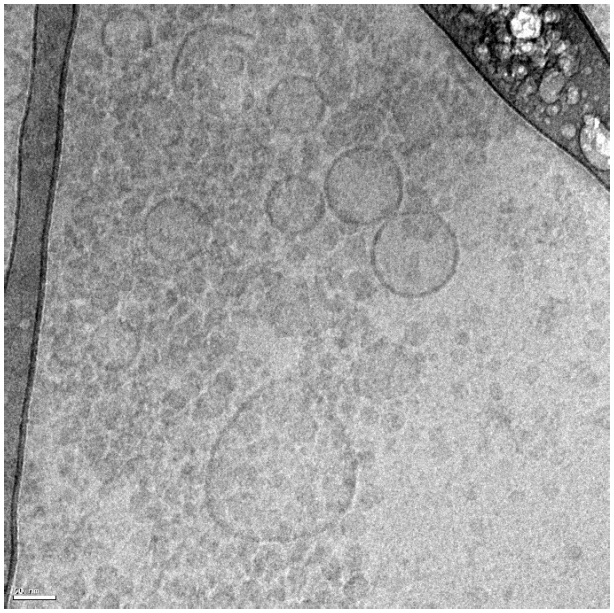

Fig. S4 Cryo-TEM image of RNP-Ss in the presence of LDL.

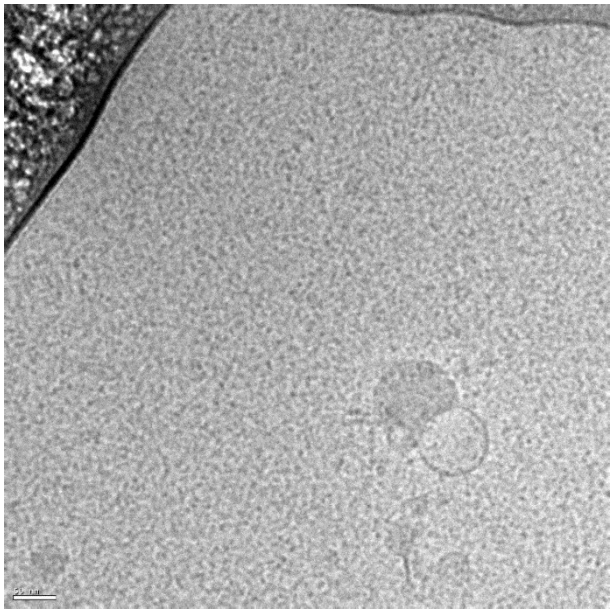

Fig. S5 Cryo-TEM image of RNP-Ss in the presence of HSA.

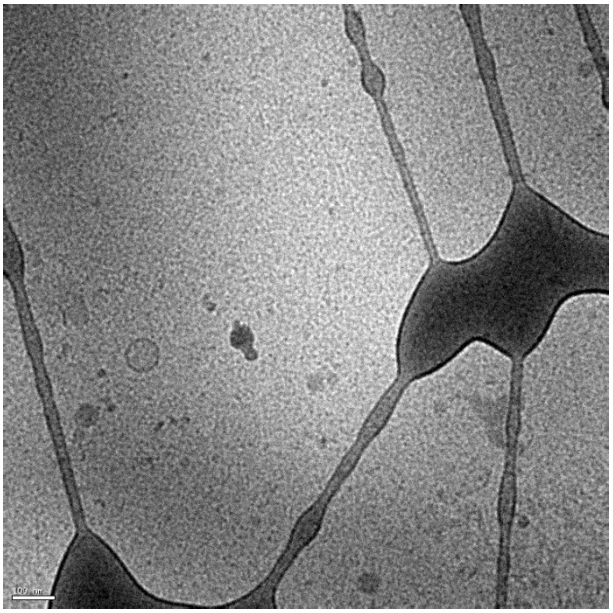

Fig. S6 Cryo-TEM image of RNP-C in the presence of HSA.

Table S1 The fitting parameters used for the EPR spectra simulations and the calculated rotational correlation times for TEMPONE, RNP-Sl, RNP-Ss, and RNP-C in PBS buffer at 37 °C

Parameter	TEMPONE	RNP-Sl	RNP-Ss	RNP-C
g_{xx}	2.008784	2.008784	2.008784	2.008784
g_{yy}	2.006100	2.005900	2.005900	2.005900
g_{zz}	2.002330	2.002330	2.002330	2.002330
A_{xx}	5.54	6.58	8.87	8.23
A_{yy}	5.90	4.00	4.00	3.95
A_{zz}	36.90	38.27	38.27	35.71
W_1	0.368	0.460	1.250	2.202
β_D		66.08	61.80	63.75
R_{prp} / s^{-1}	8.23×10^9	4.16×10^8	1.03×10^8	1.11×10^7
R_{pll} / s^{-1}	3.60×10^9	3.18×10^9	1.32×10^9	6.59×10^8
τ_R / s	2.67×10^{-11}	2.03×10^{-10}	6.90×10^{-10}	3.84×10^{-9}

g_{xx}, g_{yy} and g_{zz} are the Cartesian components of the **g** tensor for the electronic Zeeman interaction, A_{xx}, A_{yy} and A_{zz} are the Cartesian components of the electron/nuclear hyperfine tensor in gauss, W_1 represents the isotropic spherical component of the inhomogeneous line-broadening tensor, R_{prp} and R_{pll} are the axial components of the rotational diffusion tensor in s^{-1}, and β_D is the diffusion tilt angle in degrees.

Table S2 The fitting parameters used for the EPR spectra simulations and the calculated rotational correlation times for RNP-C and RNP-Ss in PBS buffer with and without SDS (c = 2 mg mL^{-1}) at 37 °C

Parameter	RNP-C	RNP-C + SDS	RNP-Ss	RNP-Ss + SDS
g_{xx}	2.008784	2.008784	2.008784	2.008784
g_{yy}	2.005900	2.005900	2.005900	2.005900
g_{zz}	2.002330	2.002330	2.002330	2.002330
A_{xx}	8.23	8.23	8.87	8.87
A_{yy}	3.95	5.30	4.00	4.00
A_{zz}	35.71	35.71	38.27	38.27
W_1	2.202	1.019	1.250	1.130
β_D	63.75	65.08	61.80	66.73
R_{prp} / s^{-1}	1.11×10^7	3.30×10^8	1.03×10^8	9.41×10^7
R_{pll} / s^{-1}	6.59×10^8	7.08×10^8	1.32×10^9	1.09×10^9
τ_R / s	3.84×10^{-9}	3.91×10^{-10}	6.90×10^{-10}	7.82×10^{-10}

Table S3 The fitting parameters used for the EPR spectra simulations and the calculated rotational correlation times for RNP-C in PBS buffer as a function of SDS concentration at 37 °C

	SDS concentration (mg mL^{-1})								
Parameter	0.00	0.05	0.10	0.20	0.30	0.40	0.50	1.00	2.00
g_{xx}	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784
g_{yy}	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900
g_{zz}	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330
A_{xx}	8.23	8.23	8.23	8.23	8.23	8.23	7.29	8.23	8.23
A_{yy}	3.95	3.95	3.95	3.95	3.95	3.95	5.35	5.30	5.30
A_{zz}	35.71	35.71	35.71	35.71	35.71	35.71	35.71	35.71	35.71
W_1	2.202	1.496	1.548	1.496	1.519	1.480	1.322	1.113	1.019
β_D	63.75	63.06	65.12	66.45	68.63	69.75	63.17	65.39	65.08
R_{prp} / s^{-1}	1.11×10^7	1.32×10^7	1.98×10^7	2.63×10^7	3.54×10^7	4.31×10^7	6.06×10^7	1.24×10^8	3.30×10^8
R_{pll} / s^{-1}	6.59×10^8	7.07×10^8	8.30×10^8	1.11×10^9	1.46×10^9	1.81×10^9	1.03×10^9	1.26×10^9	7.08×10^8
τ_R / s	3.84×10^{-9}	3.36×10^{-9}	2.42×10^{-9}	1.82×10^{-9}	1.36×10^{-9}	1.11×10^{-9}	1.07×10^{-9}	6.20×10^{-10}	3.91×10^{-10}

Table S4 The fitting parameters used for the EPR spectra simulations and the calculated rotational correlation times for RNP-C in PBS buffer and in the presence of different proteins at 37 °C, PBS

Parameter	PBS	HSA	BSA	HDL	LDL	IgG	plasma
g_{xx}	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784
g_{yy}	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900
g_{zz}	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330
A_{xx}	8.23	8.23	8.23	8.23	8.23	8.23	8.23
A_{yy}	3.95	3.95	3.95	3.95	3.95	3.95	3.95
A_{zz}	35.71	35.71	35.71	35.71	35.71	35.71	35.71
W_1	2.202	2.263	2.035	2.114	2.180	2.005	2.157
β_D	63.75	65.41	64.85	65.31	64.87	65.16	64.60
R_{prp} / s^{-1}	1.11×10^7	1.39×10^7	1.28×10^7	1.27×10^7	1.28×10^7	1.29×10^7	1.17×10^7
R_{pll} / s^{-1}	6.59×10^8	5.03×10^8	5.51×10^8	5.40×10^8	5.62×10^8	5.10×10^8	5.76×10^8
τ_R / s	3.84×10^{-9}	3.63×10^{-9}	3.72×10^{-9}	3.76×10^{-9}	3.68×10^{-9}	3.79×10^{-9}	3.88×10^{-9}

Table S5 The fitting parameters used for the EPR spectra simulations and the calculated rotational correlation times for RNP-Ss in PBS buffer and in the presence of different proteins at 37 °C, PBS

Parameter	PBS	HSA	BSA	HDL	LDL	IgG	plasma
g_{xx}	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784	2.008784
g_{yy}	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900	2.005900
g_{zz}	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330	2.002330
A_{xx}	8.87	8.87	8.87	8.87	8.87	8.87	8.87
A_{yy}	4.00	4.00	4.00	4.00	4.00	4.00	4.00
A_{zz}	38.27	38.27	38.27	38.27	38.27	38.27	38.27
W_1	1.250	1.166	1.201	1.163	1.249	1.260	1.161
β_D	61.80	62.74	63.71	63.98	65.20	65.74	62.13
R_{prp} / s^{-1}	1.03×10^8	9.75×10^7	1.04×10^8	1.18×10^8	8.59×10^7	1.07×10^8	8.87×10^7
R_{pll} / s^{-1}	1.32×10^9	1.26×10^9	1.28×10^9	1.17×10^9	1.57×10^9	1.36×10^9	1.35×10^9
τ_R / s	6.90×10^{-10}	7.28×10^{-10}	6.93×10^{-10}	6.57×10^{-10}	7.37×10^{-10}	6.67×10^{-10}	7.58×10^{-10}

Basic characterization techniques:

Determination of molecular weights: The molecular weights of HPMA copolymers bearing cholesterol were determined using a Shimadzu HPLC system equipped with a gel permeation chromatography (GPC) column (TSKgel G3000SWxl, 300 × 7.8 mm; 5 μm), PDA, refractive index (RI) Optilab-rEX and multiangle light scattering (MALS) (DAWN HELEOS II, Wyatt Technology Co., USA) detectors using a methanol:sodium acetate buffer (0.3 M; pH 6.5) mixture (80:20 vol%; flow rate 0.5 mL·min^{-1}).

The molecular weight and polydispersity index of synthesized PCL-*b*-PHPMA diblock copolymers were determined by GPC. The analyses were performed using an SDS 150 pump (Watrex, USA), an autosampler Midas (Spark Holland) evaporative light scattering PL ELS 1000 (Polymer Laboratories), and UV (Watrex UVD 250) detectors. The separation system consisted of two PLgel MIXED-BLS columns (Polymer Laboratories) and was calibrated based on polystyrene (PSS, Germany). DMF was used as a mobile phase at a flow rate of 0.5 mL min^{-1} at 25 °C. Data collection and processing were performed using TriSEC (Viscotek Comp) software.

UV-Vis spectrophotometry: The content of the TT groups in the polymer precursor was determined by UV/Vis spectrophotometry in methanol using the molar absorption coefficient ε_{305} = 10300 $L \cdot mol^{-1} \cdot cm^{-1}$ estimated for MA-βA-TT. The content of hydrazide-terminated side chains in the polymer precursor was determined by a modified TNBSA assay, as previously described.[1] The molar absorption coefficient ε_{500} = 17200 $L \cdot mol^{-1} \cdot cm^{-1}$ (λ = 500 nm) estimated for the model reaction of MA-Ahx-$NHNH_2$ with TNBSA was used.

Nuclear magnetic resonance (NMR): The content of cholesterol bound to polymer carriers was determined by ^{1}H-NMR (Bruker spectrometer, 300 MHz). Integral intensities from ^{1}H-NMR spectrum in $(CD_3)_2SO$ were compared: δ 5.32 t, 1H (C=CH); δ 3.68 br, 1H (C<u>H</u>-OH) or δ 4.70 br, 1H (CH-O<u>H</u>).

REFERENCES

1 T. Etrych, M. Jelínková, B. Říhová and K. Ulbrich, *J. Controlled Release*, 2001, **73**, 89–102.