Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary information

Protein denaturation caused by heat inactivation detrimentally affects biomolecular corona formation and cellular uptake

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Content

1. Physico-chemical properties of nanoparticles

Table S 1. Physico-chemical properties of all investigated nanoparticle systems. Measurements were performed in technical triplicates. Values are given as mean ± SD.

	Diameter (nm)	**Zeta Potential (mV)**
PS-PEG$_{NC}$	120 ± 12 nm	-7 ± 1 mV
HES	220 ± 22 nm	-19 ± 2 mV
PS-PEG$_{C}$	128 ± 12 nm	+18 ± 2 mV
PS-COOH	116 ± 11 nm	-7 ± 1 mV
PS-NH$_2$	126 ± 12 nm	+4 ± 1 mV

2. Protein quantification

Table S 2. Protein quantification of the absolute amount of protein bound to PS-PEG_{NC} after incubation in the respective protein source via Pierce Assay. Values are given as mean ± SD from two independent experiment (*n* = 2) with two technical replicates each. The amount of protein in mg per m^2 nanoparticles surface area is presented.

PS-PEG_{NC}	**Native**	**Heat inactivated**
Serum	1.18 ± 0.04 mg/m^2	0.96 ± 0.03 mg/m^2
Plasma	1.04 ± 0.10 mg/m^2	1.51 ± 0.08 mg/m^2

Table S 3. Protein quantification of the absolute amount of protein bound to HES nanocapsules after incubation in the respective protein source via Pierce Assay. Values are given as mean ± SD from two technical replicates. The amount of protein in mg per m^2 nanocapsules surface area is presented.

HES	**Native**	**Heat inactivated**
Serum	0.53 ± 0.10 mg/m^2	0.49 ± 0.01 mg/m^2
Plasma	0.52 ± 0.12 mg/m^2	0.65 ± 0.04 mg/m^2

3. Circular dichroism spectroscopy

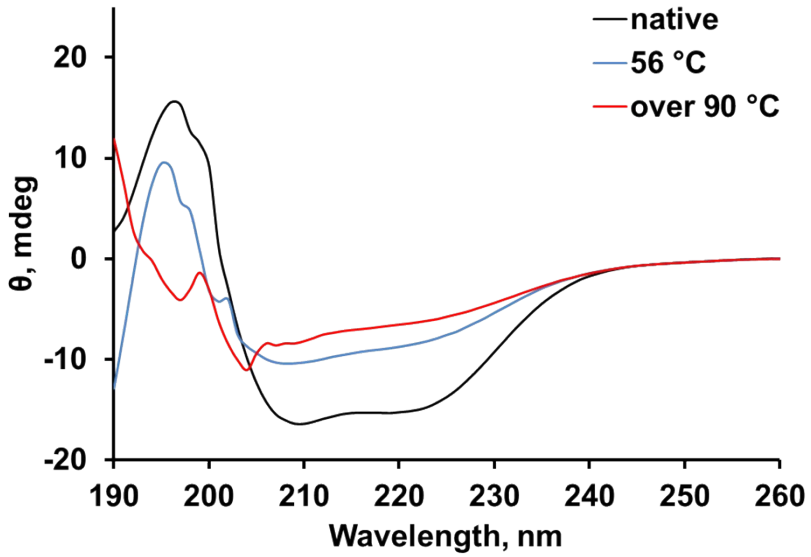

Figure S 1. CD spectra of native and heat inactivated clusterin. All CD spectra were recorded at wavelengths ranging from 190 nm to 260 nm. The ellipticity θ (mdeg) is plotted against the wavelength λ (nm). One representative measurement is shown. The experiment was repeated two independent times (n = 2) yielding similar results.

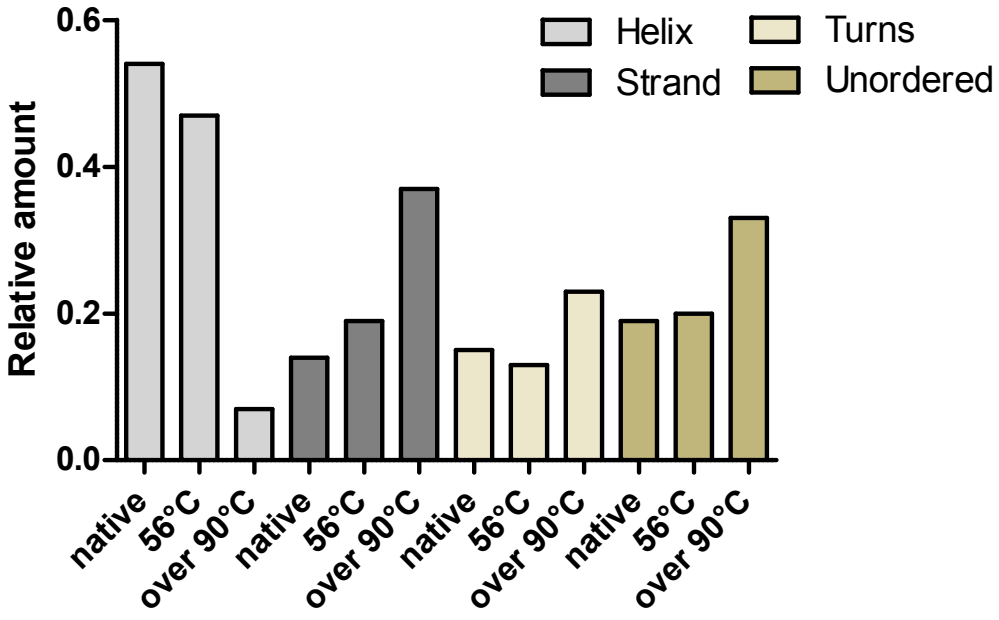

Figure S 2. Calculation of the structural alterations after heat inactivation of clusterin using DichroWeb[1-2] . Calculated values from one representative measurements are shown. The experiment was repeated two independent times (n = 2) yielding similar results.

4. Isothermal titration calorimetry

Table S 4. Native or heat inactivated (90 °C) clusterin was titrated towards PS-PEG_{nc} nanoparticles via isothermal titration calorimetry. The resulting integrated heats together with fits corresponding to an independent binding model are shown in Figure 3D. The obtained parameters by the fit are given ± an average technical error of 10%.

Clusterin	**Native**	**90 °C**
N	346 ± 35	412 ± 41
Δ*H* (kJ mol^{-1})	364 ± 36	455 ± 46
Δ*S* (J $mol^{-1}K^{-1}$)	-1075 ± 108	-1372 ± 137
K_a (L mol^{-1})	$5.5*10^7 \pm 5*10^6$	$1.2*10^7 \pm 1*10^6$

5. LC-MS and SDS-PAGE

Table S5. All identified corona proteins are summarized in a separate Excel File giving their relative abundance in % and the absolute amount in fmol.

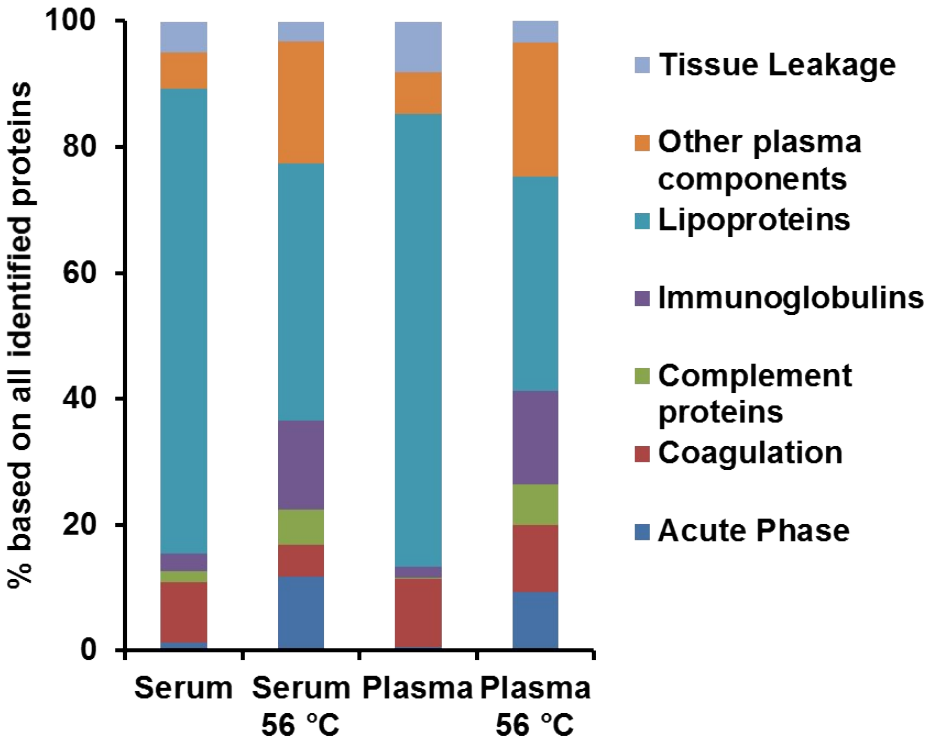

Figure S 3. Protein corona analysis of PS-PEG$_{NC}$. All identified proteins were classified into seven different classed based on their biological properties.

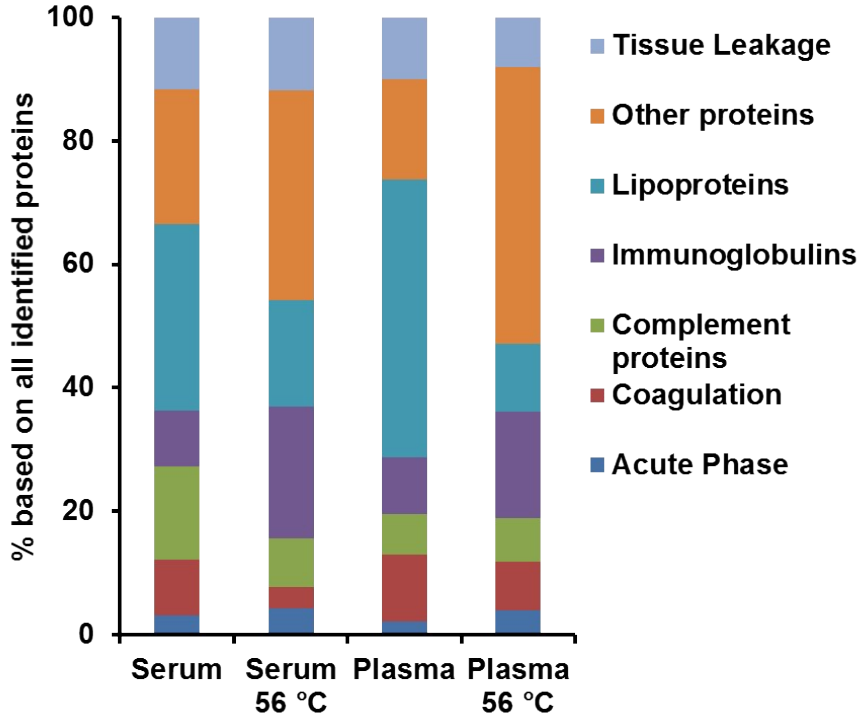

Figure S 4. Protein corona analysis of HES nanocapsules. All identified proteins were classified into seven different classed based on their biological properties.

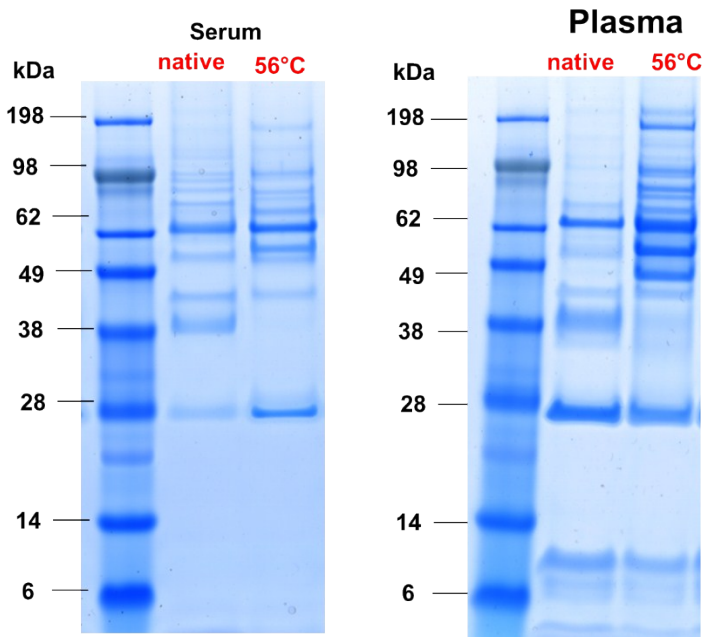

Figure S 5. Protein corona analysis of PS-PEG_C visualized by SDS PAGE. Significant differences depending on the protein source are marked with a red star.

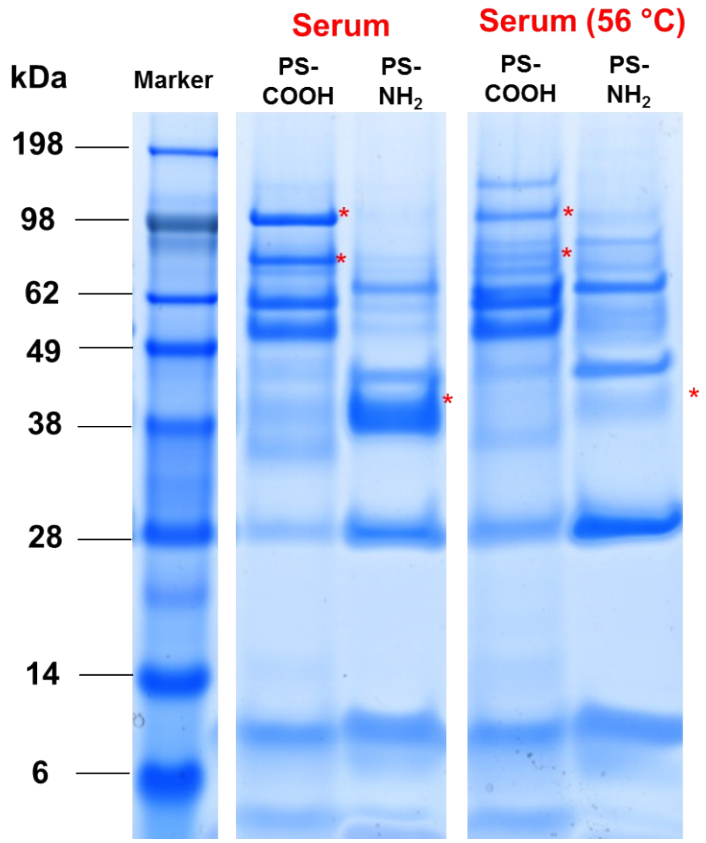

Figure S 6. Protein corona analysis of functionalized PS-NP (PS-COOH and PS-NH_2) visualized by SDS PAGE. Significant differences depending on the protein source are marked with a red star.

7. Literature

1. Whitmore, L.; Wallace, B. A., Protein secondary structure analyses from circular dichroism spectroscopy: methods and reference databases. *Biopolymers* **2008,** *89* (5), 392-400.

2. Whitmore, L.; Wallace, B., DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic acids research* **2004,** *32* (suppl_2), W668-W673.