# **Complementary Analysis of the Hard and Soft Protein Corona: Sample Preparation Critically Effects Corona Composition**

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## SUPPLEMENTARY INFORMATION

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- 1. Pierce 660 nm protein assay



**Figure S1.** Adsorbed protein masses on HES nanocapsules with different functionalities. Quantification was performed via a Pierce 660 nm Assay (Thermo Scientific, Rockford, USA). Mean values (n = 3), error bars ( $\pm$  SEM).

#### 2. Isothermal titration calorimetry - Data evaluation

Data evaluation of the performed ITC measurements was performed using the NanoAnalyze<sup>TM</sup> Data Analysis Software Version 2.3.6 from TA instruments. All obtained heat changes were analyzed with a fit according to an independent binding model<sup>1, 2</sup> (equation S1). This model is based on the assumption that a ligand L independently binds to one site of a macromolecule M without any cooperativity effects.

$$\Delta q = \left(\frac{\left(N[M]K_a + [L]K_a + 1\right) - \sqrt{\left(N[M]K_a + [L]K_a + 1\right)^2 - 4NK_a^2[M][L]}}{2K_a}\right) - [ML]_{n-1}\Delta H\Delta V_{cell}$$
(S1)

From the fit the parameters N,  $K_a$  and  $\Delta H$  are obtained, whereas [M] is the concentration of the macromolecule, [L] the concentration of the ligand, [ML] the concentration of the formed complex and  $\Delta V_{cell}$  the change of the total cell volume during the titration. To calculate the entropy change  $\Delta S$  of the reaction, the reaction isotherm equation (equation S2) was combined with the Gibbs-Helmholtz equation (equation S3) and solved for  $\Delta S$  (equation S4).

$$\Delta G = -RT \cdot \ln K_a \tag{S2}$$

$$\Delta G = \Delta H - T \cdot \Delta S \tag{S3}$$

$$\Delta S = R \cdot \ln K_a + \frac{\Delta H}{T} \tag{S4}$$

Here  $\Delta G$  is the Gibbs free energy, R is the universal gas constant and T the temperature, so that for known  $K_a$  and  $\Delta H$  the entropy change can be calculated.

## 3. DLS analysis of plasma samples

The obtained independent size fractions can be attributed to different protein fractions present in human plasma.<sup>3</sup>



Figure S2. Distribution of relaxation times  $H(\ln \tau)$  for plasma at a scattering angle of  $\theta = 90^{\circ}$  obtained by a CONTIN<sup>4, 5</sup> data analysis. The sample shows three independently diffusing species corresponding to the hydrodynamic radii indicated by the red arrows.

### 4. DLS analysis of functionalized HES nanocapsules



**Figure S3.** A) Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of non-functionalized HES capsules mixed with HSA solution at  $\theta = 64^\circ$ . The blue line (-) represents the forced fit composed of the sum of the individual components whereas the red line (-) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits. B) Hydrodynamic radii of pure non-functionalized HES capsules (black squares •) and of the aggregate formed in HSA solution (red squares •). Striped columns represent the aggregate fraction relative to the capsule fraction.



**Figure S4.** Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of non-functionalized HES capsules mixed with ApoA1 solution at  $\theta = 64^{\circ}$ . The blue line (–) represents the forced fit composed of the sum of the individual components whereas the red line (–) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits.



**Figure S5.** A) Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of COOH-functionalized HES capsules mixed with plasma at  $\theta = 64^\circ$ . The blue line (-) represents the forced fit composed of the sum of the individual components whereas the red line (-) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits. B) Hydrodynamic radii of pure COOH-functionalized HES capsules (black squares •) and of the aggregate formed in plasma (red squares •). Striped columns represent the aggregate fraction relative to the capsule fraction.



**Figure S6.** A) Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of COOH-functionalized HES capsules mixed with HSA solution at  $\theta = 64^\circ$ . The blue line (-) represents the forced fit composed of the sum of the individual components whereas the red line (-) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits. B) Hydrodynamic radii of pure COOH-functionalized HES capsules (black squares •) and of the aggregate formed in HSA solution (red squares •). Striped columns represent the aggregate fraction relative to the capsule fraction.



**Figure S7.** Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of COOH-functionalized HES capsules mixed with ApoA1 solution at  $\theta = 64^{\circ}$ . The blue line (-) represents the forced fit composed of the sum of the individual components whereas the red line (-) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits.



**Figure S8.** Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of NH<sub>2</sub>-functionalized HES capsules mixed with plasma at  $\theta = 64^\circ$ . The blue line (–) represents the forced fit composed of the sum of the individual components whereas the red line (–) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits.



**Figure S9.** Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of NH<sub>2</sub>-functionalized HES capsules mixed with HSA solution at  $\theta = 64^\circ$ . The blue line (–) represents the forced fit composed of the sum of the individual components whereas the red line (–) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits.



**Figure S10.** Upper graph: Autocorrelation function  $g_1(t)$  (black circles •) of NH<sub>2</sub>-functionalized HES capsules mixed with ApoA1 solution at  $\theta = 64^\circ$ . The blue line (–) represents the forced fit composed of the sum of the individual components whereas the red line (–) represents the fit with an additional aggregation function. Lower graph: Corresponding residuals resulting from the difference between the data and the two fits.

## 5. Additional references

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