Electronic Supplementary Material (ESI) for Nanoscale.


# SUPPORTING INFORMATION

## Simple Spectroscopic Determination of the Hard Protein Corona Composition in AuNPs: Albumin at the 75 %

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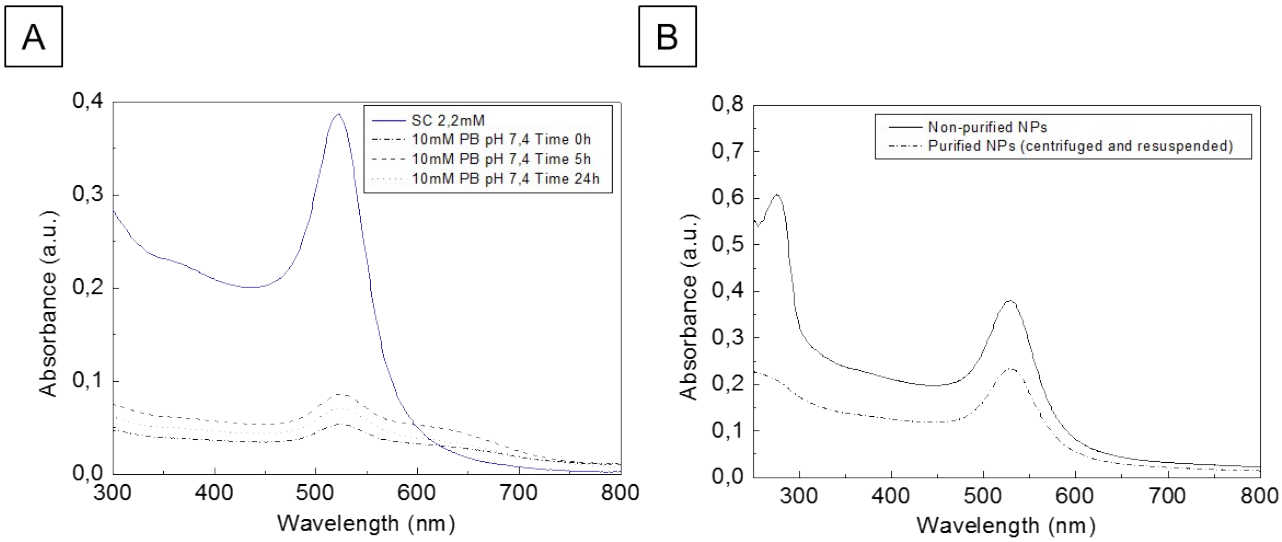
**Figure 1.** UV-Visible spectra of AuNPs after purification in physiological media (A) in the absence of proteins at different time points and (B) in the presence of protein excess.




AuNPs were synthesized in 2.2 mM Sodium Citrate, pH ~ 8. Citrate negative atoms cover the surface of the NPs and this anion allows the stabilization of the colloidal material, preventing aggregation by electrostatic repulsion. When introduced in the physiological buffer, the 10 mM Phosphate Buffer pH 7.4, NPs are destabilized due to electrostatic screening of the stabilizing anions. Under these conditions, NPs aggregates when undergoes purification process, changing their colloidal dispersion (Panel (A)).

The Panel (B) reports the depletion of the protein excess during purification through the *stress test*. The disappearance of the protein's peak at 280 nm is consistent with the expected depletion of the in-solution protein excess.

**Figure 2.** Evolution of the hydrodynamic size by number of AuNPs exposed to the different protein solution analyzed by DLS at different time points. Representative DLS by number.

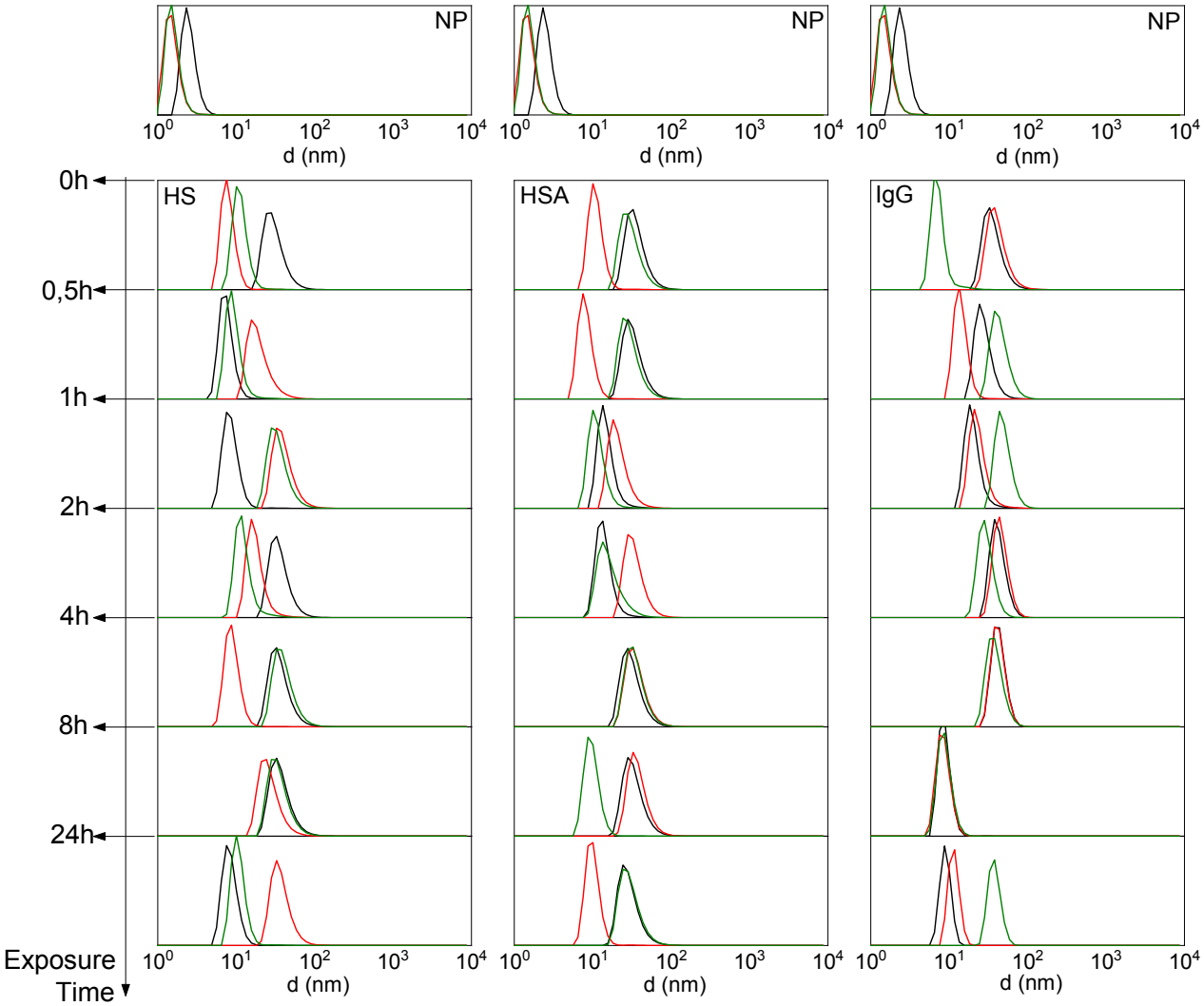


**Figure 3.** Evolution of the hydrodynamic size by number of purified AuNPs after exposure to the different protein solution analyzed by DLS at different purification time points. Representative DLS by number.

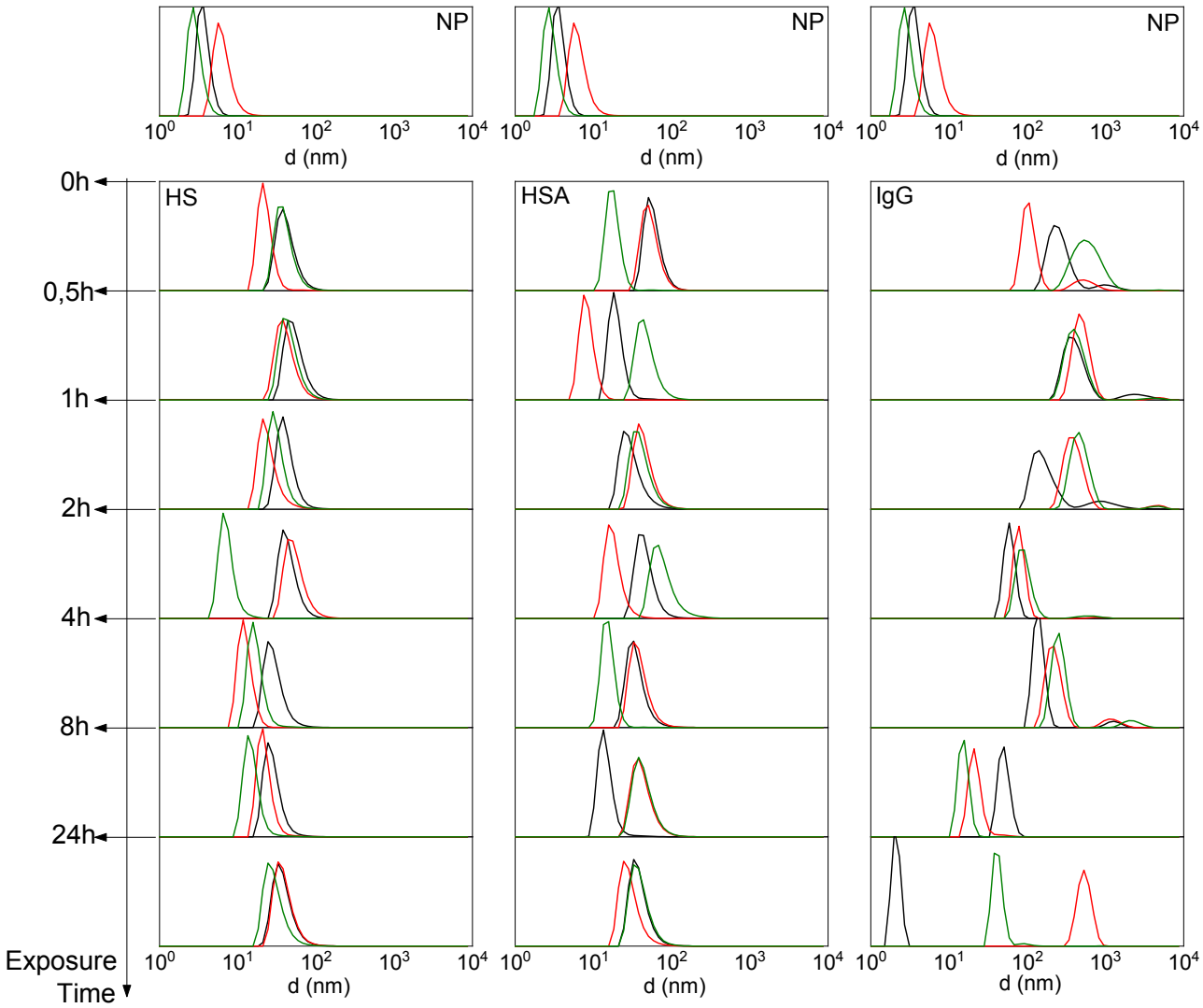


**Figure 4.** Representative Z-potential measurements at different incubation times of the AuNPs exposed to the different protein solution (A) and of the purified AuNPs after exposure to the different protein solution (B). The dashed line represents protein's reference value in 10mm Phosphate Buffer.

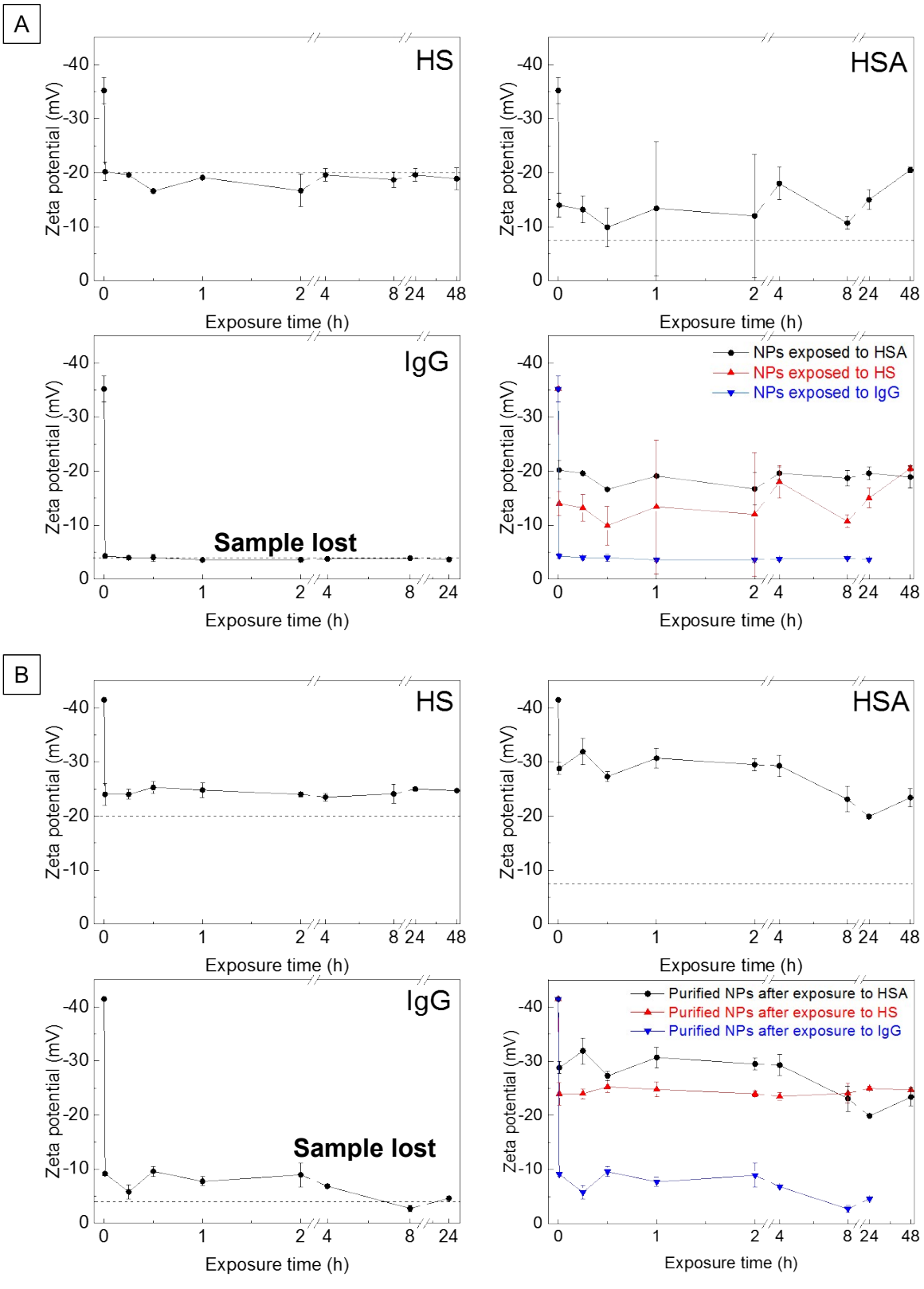


**Figure 5.** (A) Z-potential and (B) SPR peak position ($\lambda_{max}$) evolution up to 48h of exposure time for purified NPs after exposure to HS (red triangle) and HSA (black circle) compared to the NP control.

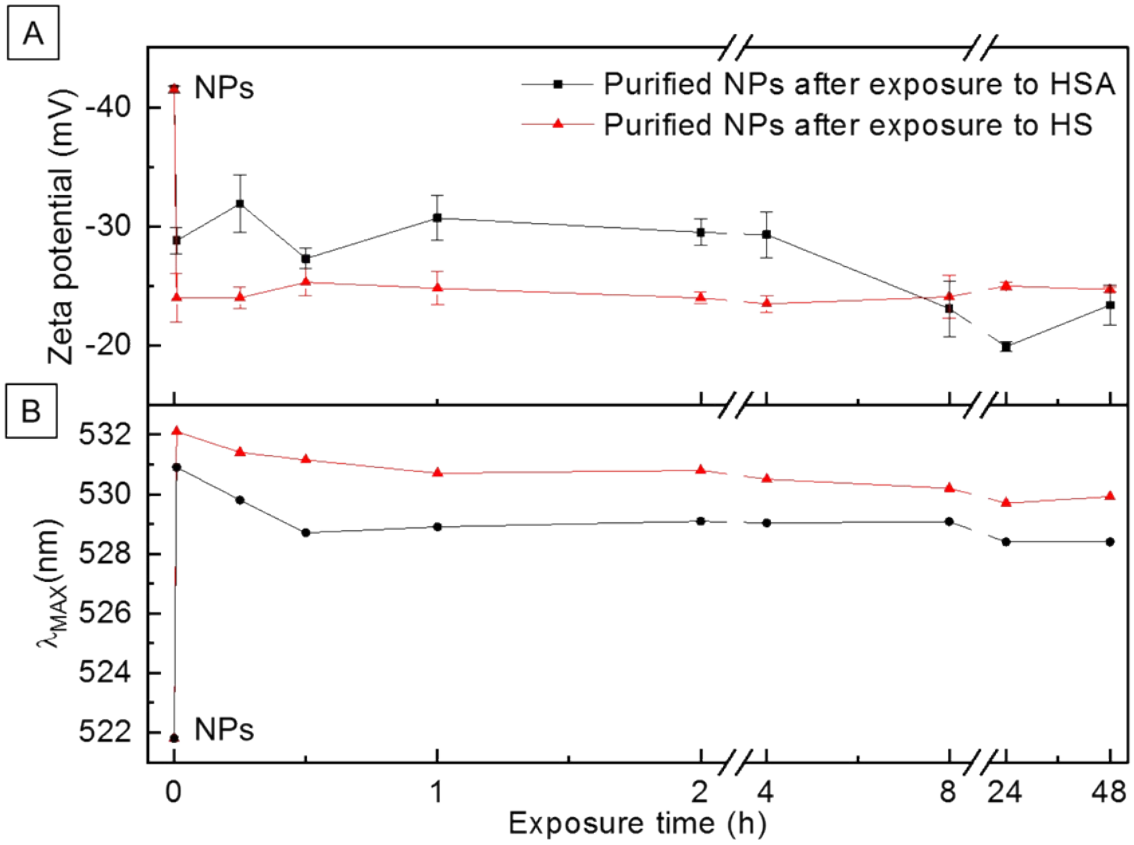


**Figure 6.** Desorption of proteins forming the hard-PC. (A) normalized SPR absorbance of the HS hard-PC and the HSA hard-PC for either after the first (solid black line) and the second (dashed black line) purification cycle compared to the NP control (blue dash-dotted line); (B) normalized SPR absorbance spectra of the as-purified HS hard-PC and HSA hard-PC (solid black line) and the 72-hours-HS and HS hard-PC (dashed black line) compared to NP control (blue dash-dotted line) and the correspondent Zeta potential values over time.

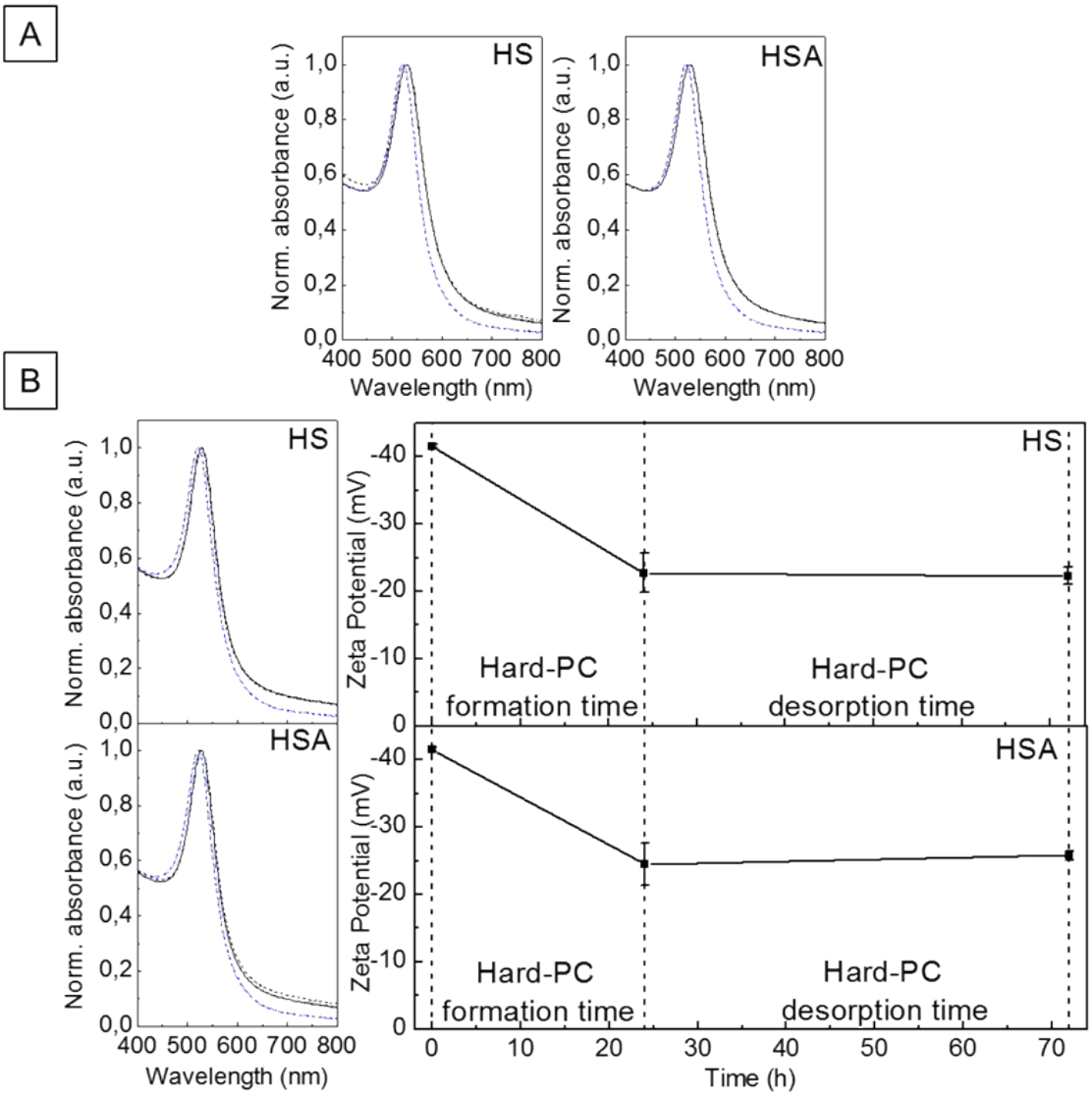


**Table 1**. LC-MS analysis after tryptic digestion of the HS hard-PC.

| Accession | Description | Σ Coverage | Σ# Proteins | Σ# Unique Peptides | Σ# Peptides | Σ# PSMs | Score | Coverage | # Peptides | # PSM | # AAs | MW [kDa] | Calc. pI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P02768 | Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 - [ALBU_HUMAN] | 7,39 | 1 | 3 | 3 | 8 | 185,88 | 7,39 | 3 | 8 | 609 | 69,3 | 6,28 |

| | Sequence | # PSMs | # Proteins | # Protein Groups | Protein Group Accessions | Modifications | MH+ [Da] | A2 | IonScore A2 | Exp Value A2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | LVAASQAALGL | 1 | 1 | 1 | P02768 | | 1013,60 | High | 79 | 6,79E-08 |
| | ALVLIAFAQYLQQCPFEDHVK | 3 | 1 | 1 | P02768 | C14 | 2490,28 | High | 48 | 1,78E-04 |
| | DVFLGMFLYEYAR | 4 | 1 | 1 | P02768 | | 1623,79 | High | 47 | 1,77E-04 |

| Accession | Description | ΣCoverage | Σ# Proteins | Σ# Unique Peptides | Σ# Peptides | Σ# PSMs | Score | Coverage | # Peptides | # PSM | # AAs | MW [kDa] | calc. pI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P01860 | Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2 - [IGHG3_HUMAN] | 7,96 | 1 | 1 | 1 | 1 | 56,07 | 7,96 | 1 | 1 | 377 | 41,3 | 7,90 |

| | Sequence | # PSMs | # Proteins | # Protein Groups | Protein Group Accessions | Modifications | MH+ [Da] | A2 | IonScore A2 | Exp Value A2 |
|---|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SCDTPPPCPR | 1 | 1 | 1 | P01860 | C2, C8 | 1186,50 | High | 56 | 5,68E-06 |

C14, C2, C8 Carbamidomethyl