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This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1725762> since 2020-07-15T10:32:39Z

Published version:

DOI:10.1016/j.colsurfb.2020.110780

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Supporting Information

Towards the control of the biological identity of nanobiomaterials: impact of the structure of $\{01\bar{1}0\}$ surface terminations of nanohydroxyapatite on the conformation of adsorbed proteins

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Keywords: nanohydroxyapatite; surface structure; adsorbed protein; protein conformation; biological identity; IR spectroscopy; CD-UV spectroscopy; zeta-potential

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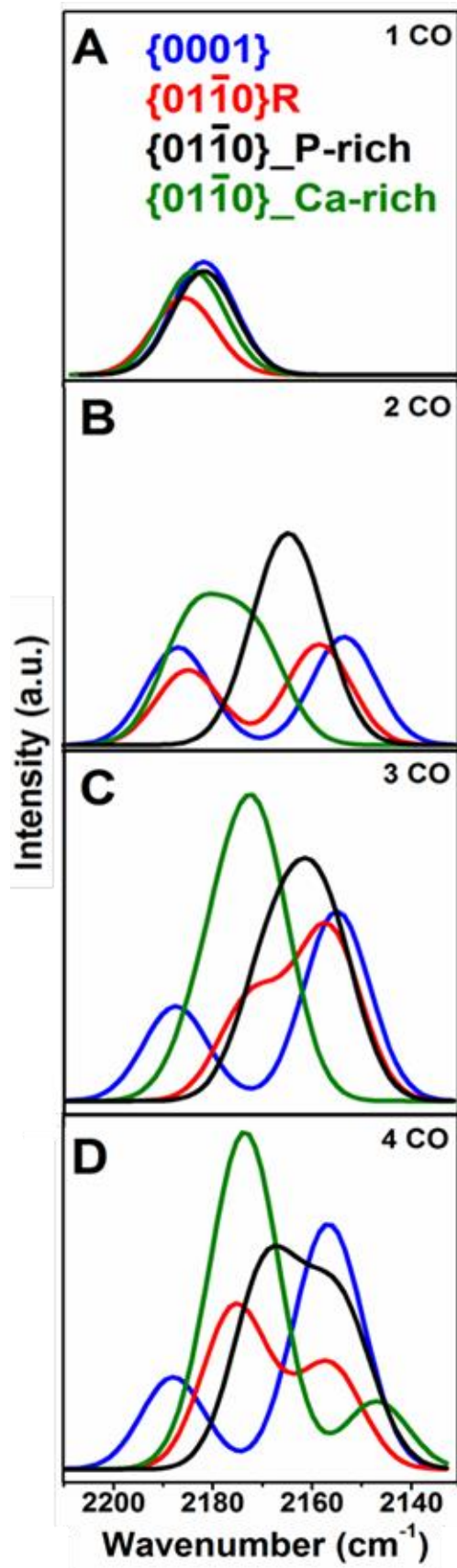


Figure S1. (adapted from the Supporting Information of ref.(1)) Scaled B3LYP harmonic infrared spectra computed for carbonylic adducts on various hexagonal hydroxyapatite surface terminations. In each panel are the spectra calculated for 1, 2, 3 and 4 CO molecules (panels A-D, in the order) for unit cell of each of the four HA surface terminations considered: {0001}, blue curves, stoichiometric {0110}R (R= reacted with water), red curves; {0110}P-rich, black curves and {0110}Ca-rich, green curves. These profiles were used to fit spectra of CO adsorbed on the HA nanoparticles of interest reported in Figure 3 of the main text.

1. Chiatti F, Corno M, Sakhno Y, Martra G, Ugliengo P. Revealing Hydroxyapatite Nanoparticle Surface Structure by CO Adsorption: A Combined B3LYP and Infrared Study. *Journal of Physical Chemistry C* 2013; 117 (48), 25526–34. doi: 10.1021/Jp4086574.

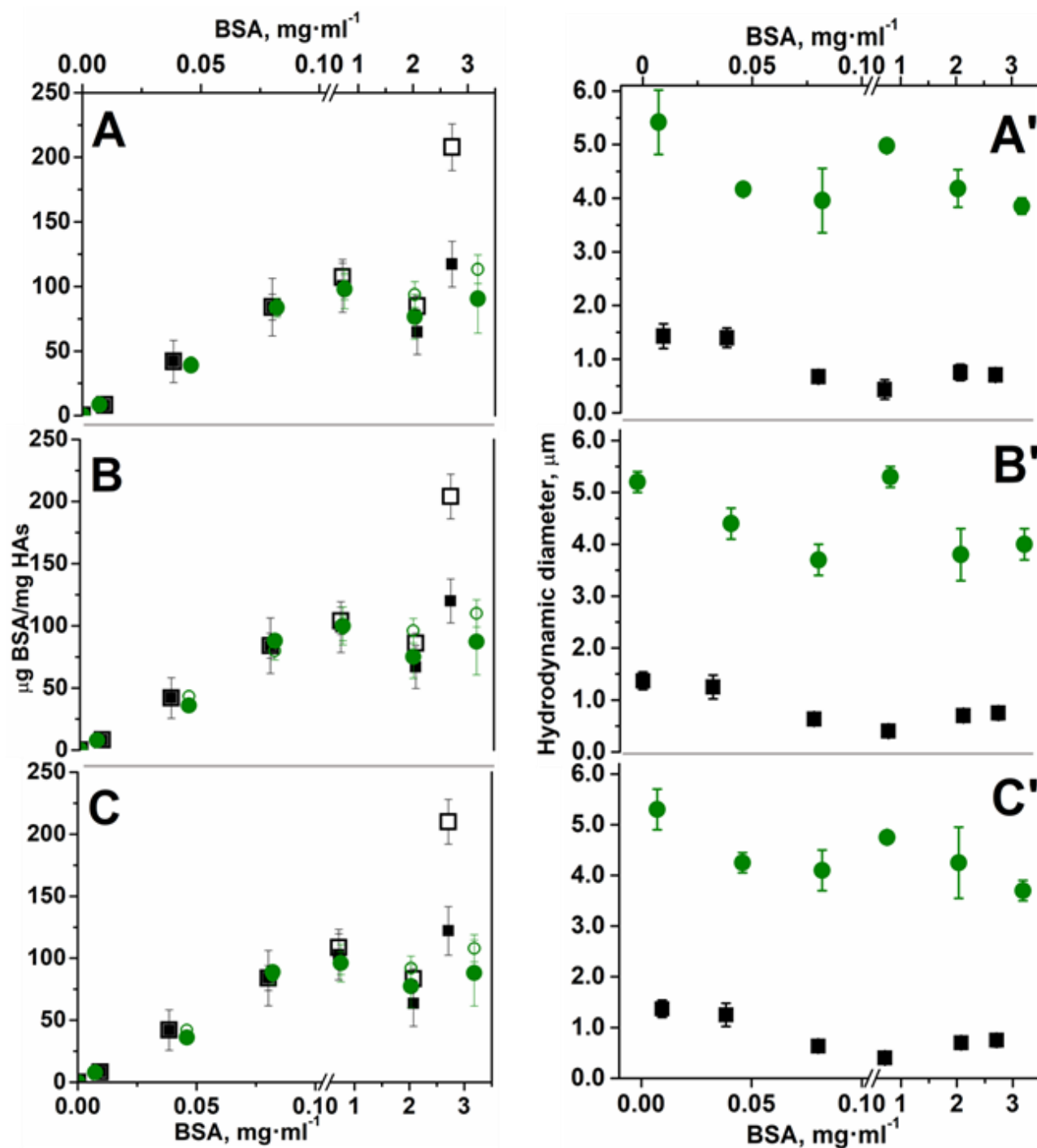


Figure S2. BSA adsorption isotherms (left panels) and related hydrodynamic diameters measured by DLS for incubation times of: A.A') 15 min; B,B') 30 min; C.C') 60 min. Values reported on the X-axis: concentration of BSA in solution in equilibrium with the adsorbed one at the end of incubation.

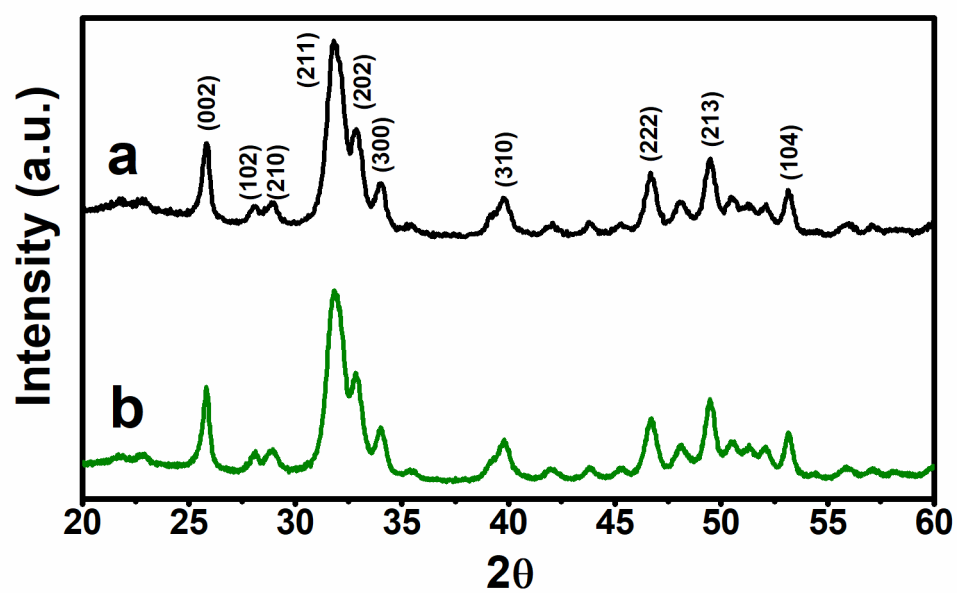


Figure S3. XRD patterns of: (a) HA-HT and (b) HA-LT.

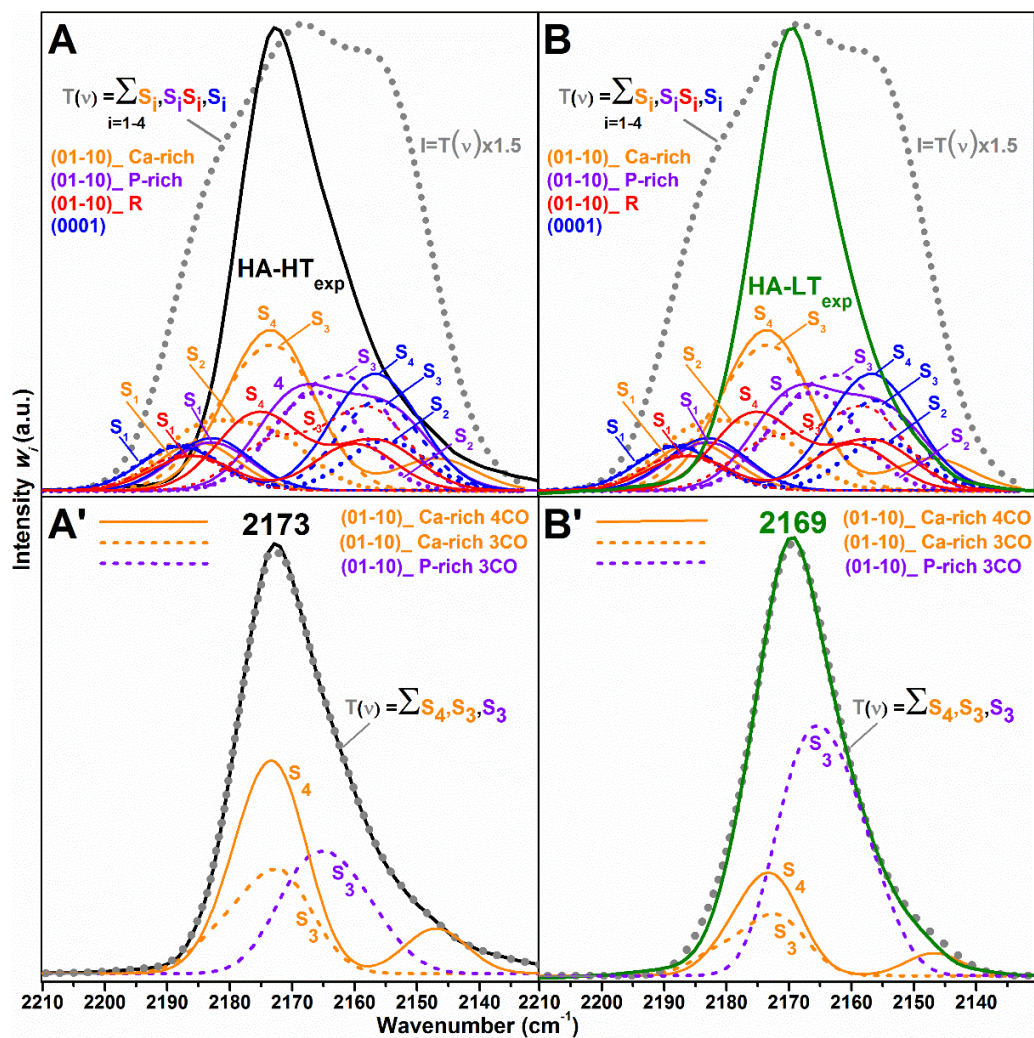


Figure S4. IR spectra (black and green solid lines) of 25 mbar of CO adsorbed at ca. 100 K on: (a), (a') HA-HT, (b), (b') HA-LT.

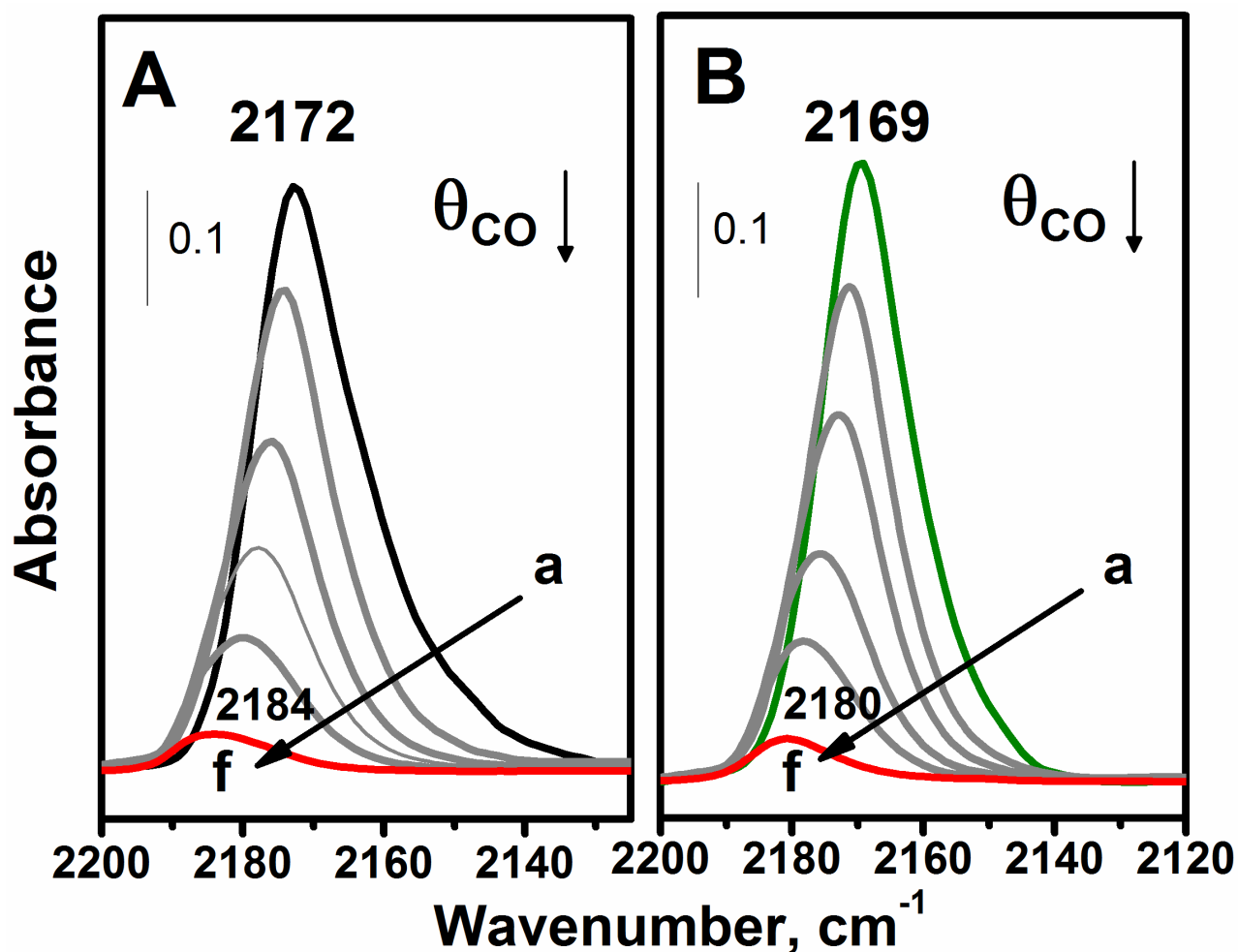


Figure S5. IR spectra of HA-HT and HA-LT (panels A and B, respectively) pre-outgassed at 433 K and then contacted at 100 K with CO, from 25 mbar (a) complete outgassing (f). Spectra resulted from i) the subtraction of the spectra of the samples before CO adsorption, and ii) the subtraction of the spectrum of gaseous CO (for data collected with samples in equilibrium with a CO pressure ranging from 25 to 4 mbar), with a proper adjustment of the intensity using the interactive spectrum subtraction utility of the OPUS 5.0 software by Bruker. For CO pressure < 4 mbar, the contribution of gaseous CO was negligible with respect signals due to adsorbed probe molecules.

Comment to Figure S5:

The contact with 25 mbar of CO resulted in a band with maximum at 2172 and 2169 cm^{-1} for HA-HT and HA-LT, respectively, asymmetric towards the low frequency side. A decrease of the band intensity occurred while decreasing CO coverage, with a shift of the maxima towards 2184 and 2180 cm^{-1} .

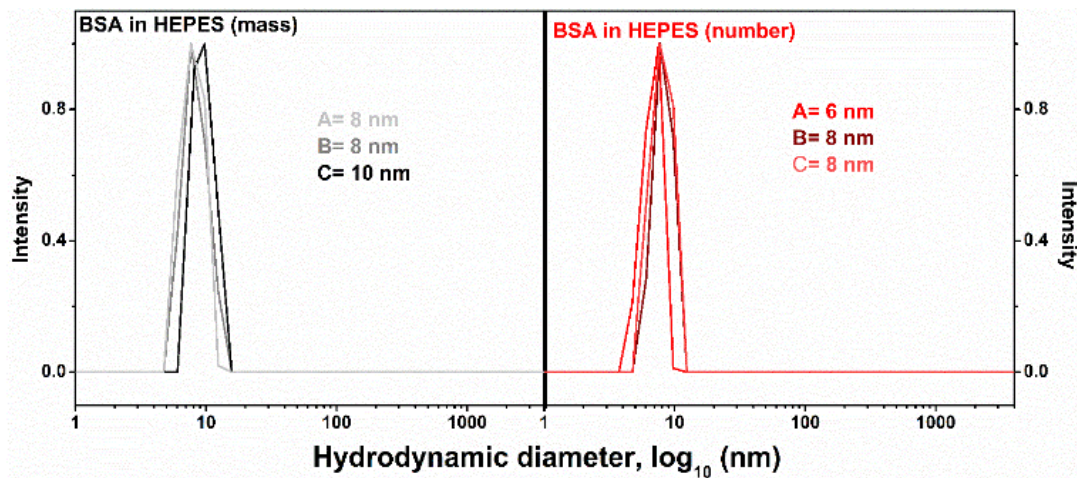


Figure S6. Raw data of DLS measurements of BSA solutions in HEPES ($0.1 \text{ mg} \cdot \text{ml}^{-1}$). Each spectrum of the triplicate is reported both in mass (left panel) and number (right panel) distributions.

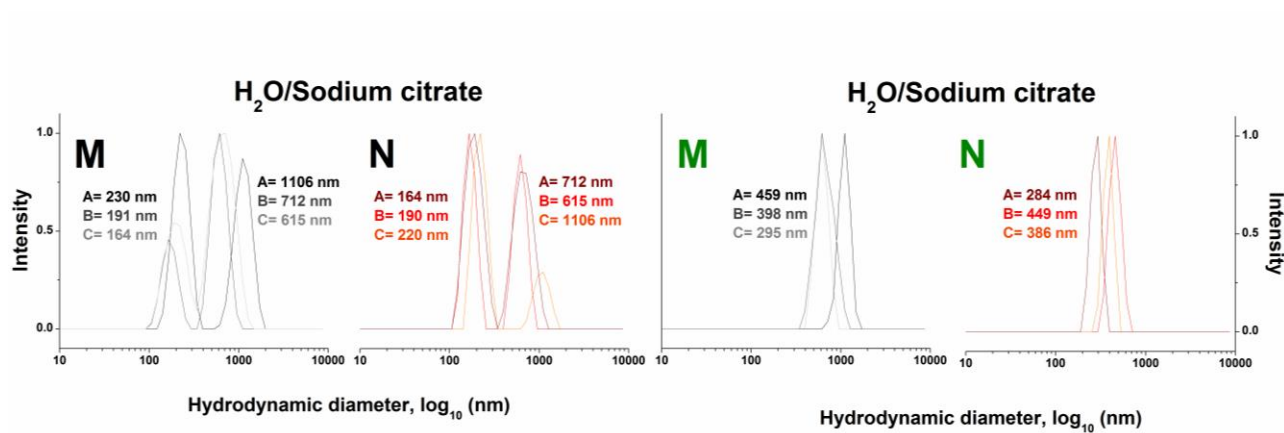


Figure S7. Raw data of DLS measurements (in triplicate for each condition) related to dispersion of HA-HT (first and second columns) and HA-LT (third and fourth columns) suspended in watery solution of sodium citrate 0.1 M for 1 day; pH adjusted at 6.5 by NaOH addition. Data are presented both in mass (black lines, in sub-panels M) and number (red lines, sub-panels N) distributions.

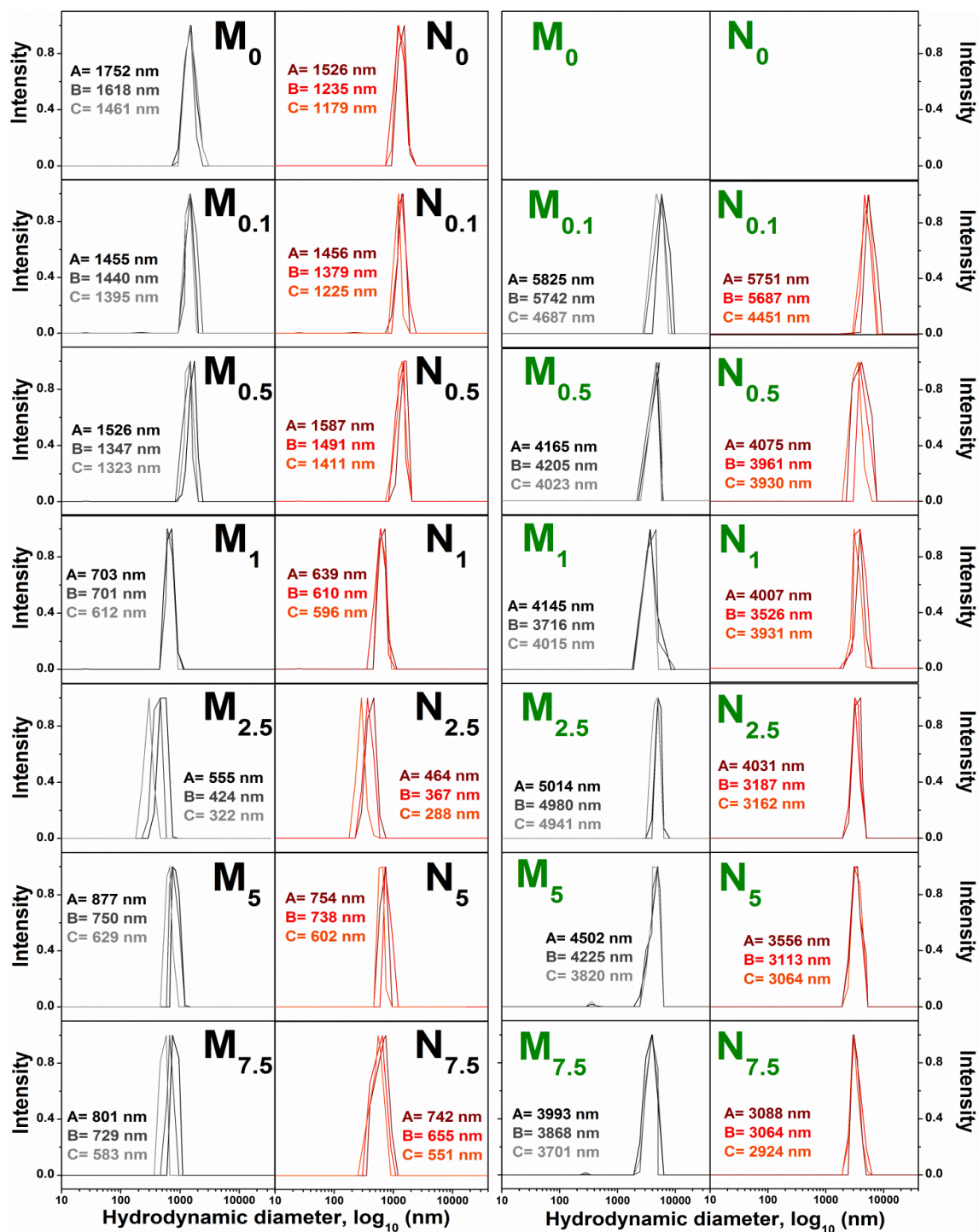


Figure S7, continued. Raw data of DLS measurements (in triplicate for each condition) summarized in Figure 2, panel A in the main text. All data are reported both in mass ($M_{n=(0-7.5)}$, black lines) and number ($N_{n=(0-7.5)}$, red lines) distributions: first and second columns sample = HA-HT; third and fourth columns sample = HA-LT. Conditions were: bare HA NPs suspended in HEPES (first row; HA-LT formed agglomerates with size outside the maximum of the detection range), and HA NPs with irreversibly adsorbed BSA, after incubation in protein solutions at different initial concentrations (0.1-7.5 mg·ml⁻¹).

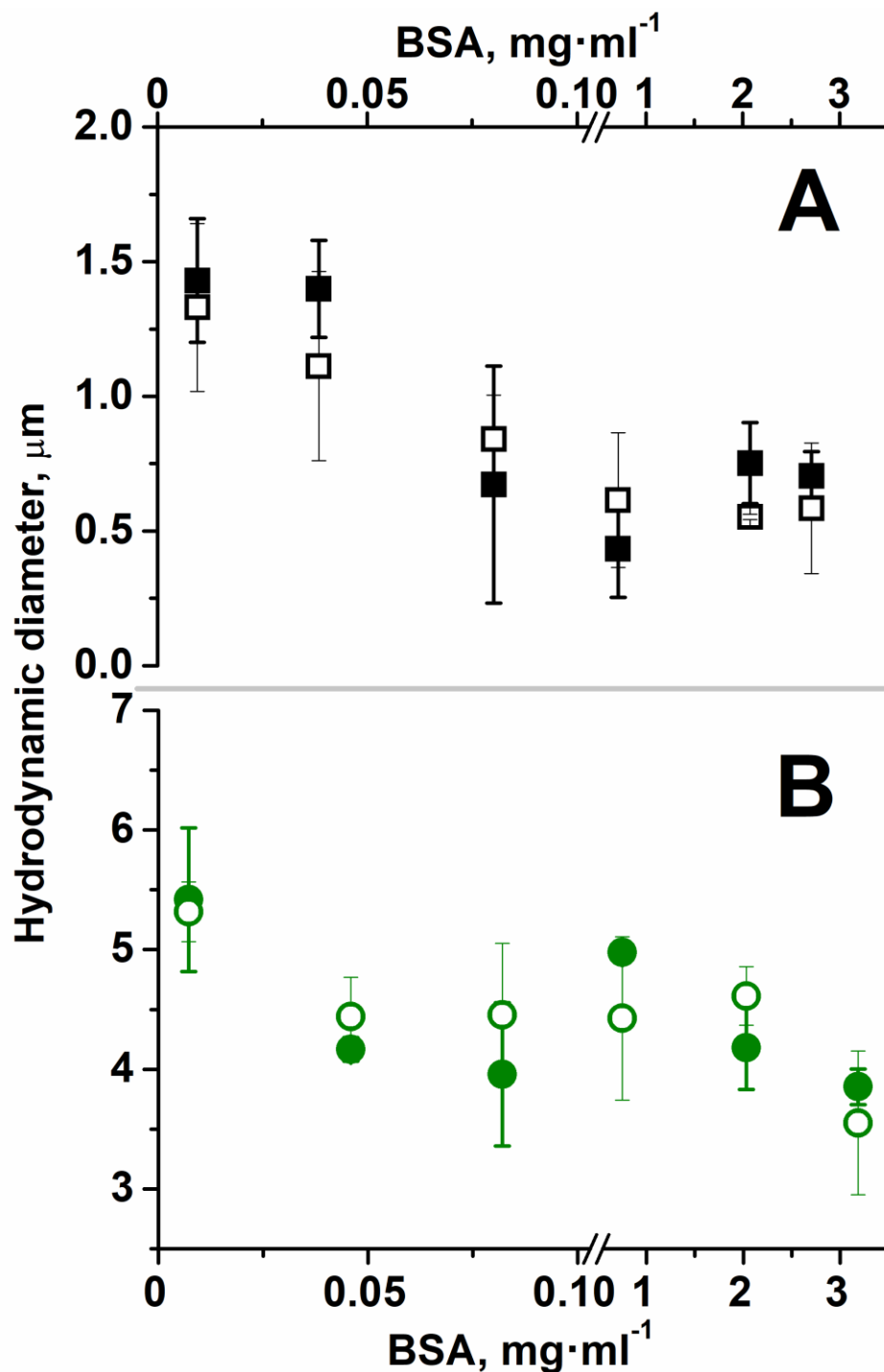


Figure S8. Hydrodynamic diameters of HA-HT and HA-LT (squares and circles in panel A and B, respectively) in incubation with both total amount (empty symbols) and irreversible fraction (full symbols) of adsorbed BSA. Values reported on the X-axis: concentration of BSA in solution in equilibrium with the adsorbed one at the end of incubation. Data are reported as mean values of at least triplicate measurements \pm standard deviation.

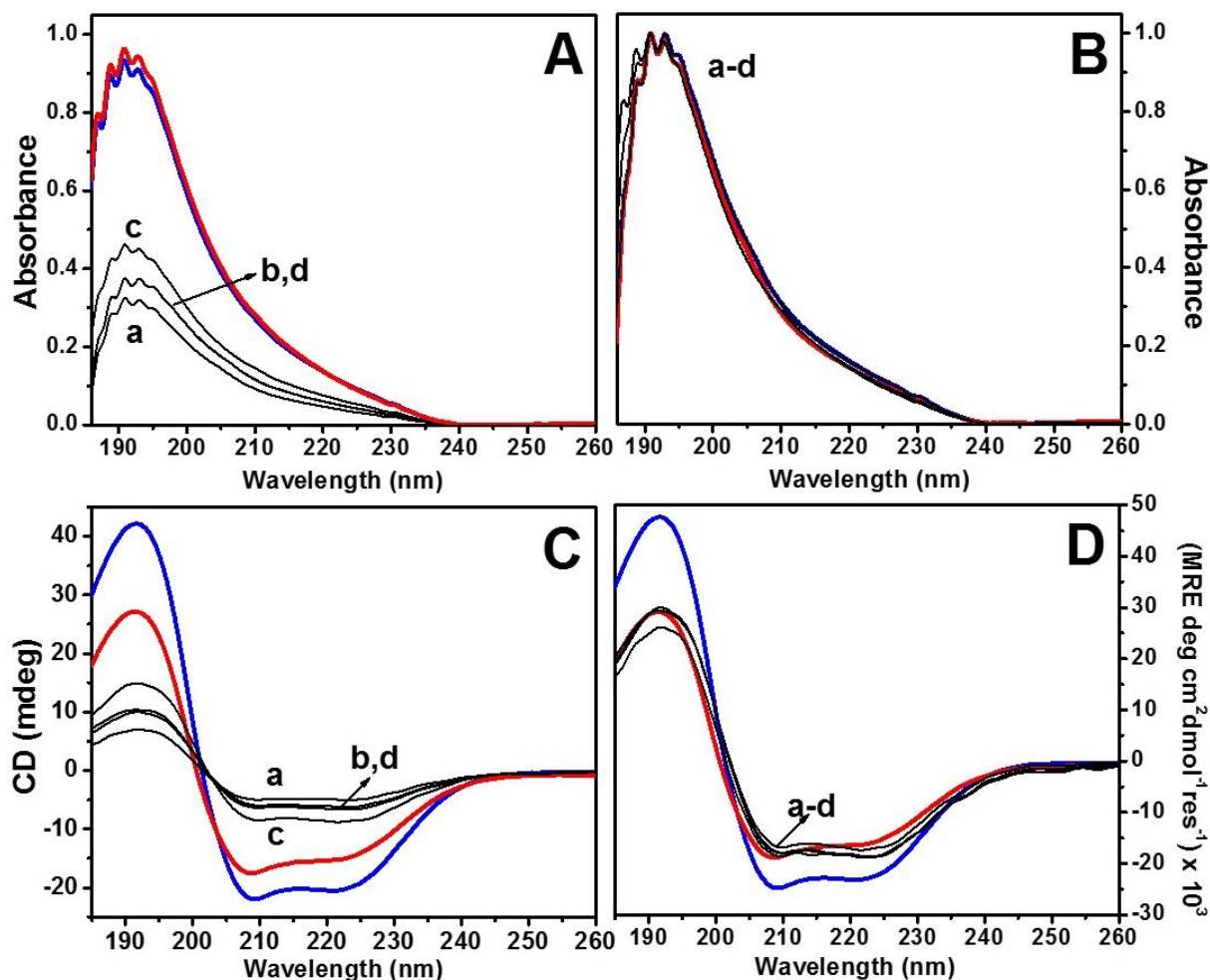


Figure S9. Absorbance and CD-UV spectra (panels A-B and C-D, respectively) of native and thermally treated (373 K, 15 min) BSA water solutions (blue and red lines, respectively) and BSA irreversibly adsorbed on HA-HT after incubation in BSA solutions with initial concentration 0.1, 0.5, 2.5 and 7.5 mg·ml⁻¹ (a, b, c and d black curves, respectively). Black spectra were acquired after resuspension of nanoparticles carrying the protein corona in MilliQ water, because HEPES buffer is not transparent in the 180-200 nm spectral range.

Comment to Figure. S9: The amount of HA powders carrying the irreversible fraction of adsorbed proteins were controlled in order to attain the same nominal concentration of proteins in unit volume of the samples, using as reference values the amount of adsorbed proteins per mass of solid material obtained by UV measurements. Despite the nominal equivalency of the amount of BSA present in such samples, significant differences were obtained in the intensity of the absorption signals in the 180-260 nm range due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the carbonylic groups in the polypeptide backbone (panel A), due to the inhomogeneity and instability of the suspension of the samples during the measurements. Thus, the Absorbance spectra were normalized with respect to the intensity value of native BSA (panel B), and the normalization factors were used for the normalization of the corresponding CD-UV spectra (panel C: original; panel D: normalized). Data elaboration did not affect the shape of the CD-UV lines, as well as the information contained in the change of the relative intensity of the negative bands at 208 and 222 nm with respect native and thermally treated BSA in solution.