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Electronic Supplementary Information

Lysozyme Adsorption on Carbonaceous Nanoparticles Probed by Second Harmonic Light Scattering

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S1. Synthesis of CNP

CNPs were synthesized from glucose and NaOH by hydrothermal method [1]by taking them in 10:1 ratio (w/w) and dissolving in 15 mL water. The solution was then placedin a 100 mL autoclave which was heated at 250°C for 12h (CNP-1) and 2 h (CNP-2) in an oven. The reaction mixture was then allowed to cool to room temperature to obtain a dark brown solution of CNPs which was then filtered using a syringe filter (0.2μm) followed by 30 min centrifugation at 2500 rpm. The supernatant containing CNPs was collected and characterized.

S2. TEM, UV-Vis, ATR-IR, and zeta potential measurements

For TEM sample preparation, the supernatant solution containing CNPs was sonicated for 5 min and, thereafter, 20 μL of the solution was drop-casted on a carbon coated copper grid (200 mesh). The grid was dried in a desiccator for 24 hr. The TEM histograms were made by taking size distribution of around 600 particles using the digimizer software.

For UV-Vis measurements, diluted solution of CNPs was taken in a 1 mL quartz cuvette and the spectrum was collected in the wavelength range of 200-800 nm. IR measurements were carried out in a Bruker spectrometer in an ATR cell. For IR spectral measurements, freeze dried samples were taken and the spectra recorded from 4000 to 500 cm^{-1}. Zeta potential (ZP) measurements were done in a Horiba SZ-100 nanoparticle analyzer instrument. For ZP measurement, 600 μL of the CNP solution was taken in a carbon electrode cell.

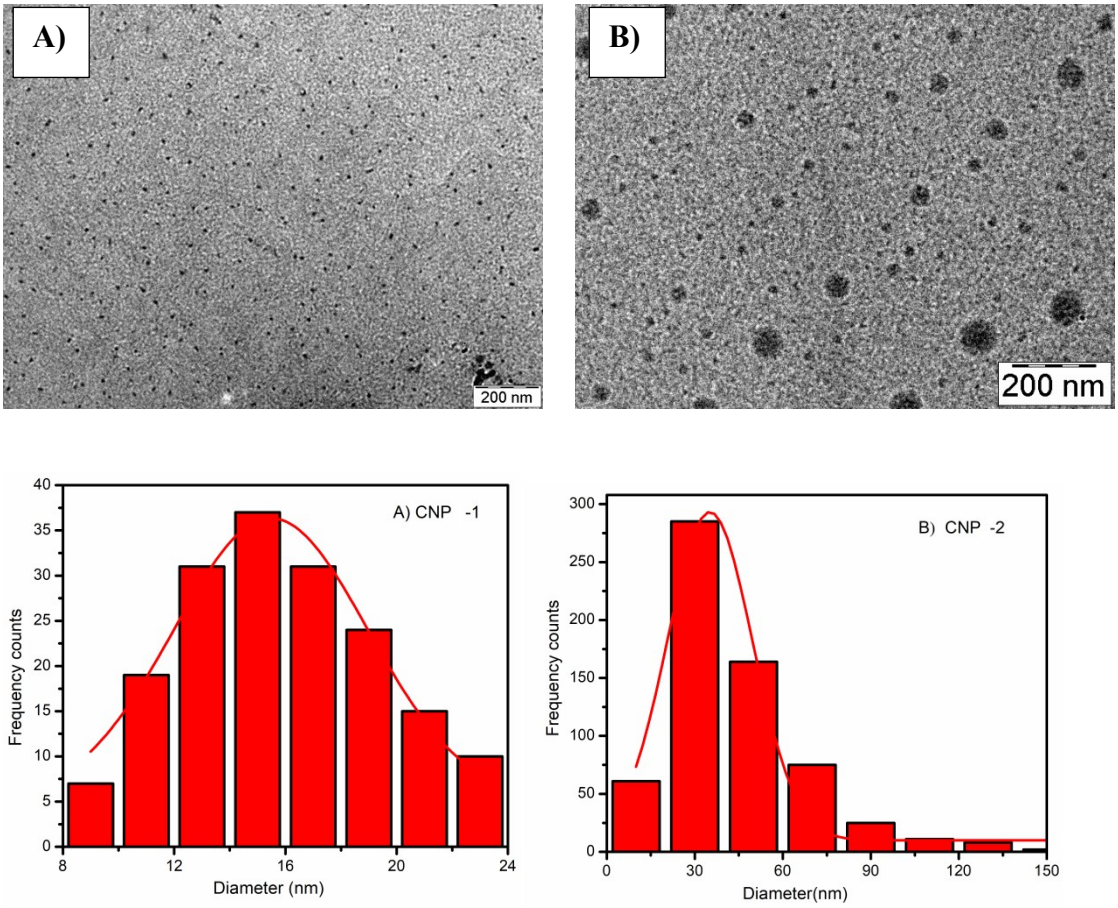

Fig. S1. TEM images and size distribution histograms of A) CNP-1, and B) CNP-2.

Table S1. Zeta potential (ZP) data of CNP in water.

CNP	Zeta potential (mV)
CNP-1	-27.3 ± 2.8
CNP-2	-20.3 ± 1.9

Table S2. Peak assignments of ATR-IR spectra of CNP-1 and CNP-2

CNP-1		CNP-2	
Peak wavenumber (cm^{-1})	Assignment	Peak wavenumber (cm^{-1})	assignment
3264	O-H stretch	3264	O-H stretch
2976	C-H stretch	2942	C-H stretch
2942	C-H stretch	1779	C=O stretch
1695	C=O stretch	1716	C=O stretch
1573	C=C stretch	1589	C=C stretch
1413	C-O-H bend	1417	C-O-H bend
1325	C-O stretch	1357	H-C-H bend
1075	C-O-C bend /C-O stretch	1071	C-O-C bend /C-O stretch
924-650	H-C-H out-of-plane bends	772-650	H-C-H out-of-plane bends

S3. Elemental analysis

Elemental analysis was done on a Thermo Scientific Flash 2000 organic elemental analyser. The percent composition of each element obtain from elemental analysis is given in the following table.

Table S3. Elemental analysis of CNP

CNP	% C	% H	% O (Calculated)
CNP-1	37	5	58
CNP-2	39	5	56

S4. Concentration calculation of CNP

The concentration calculations for CNP were done assuming that CNP consist of graphitic unit cell as reported previously [2,3]. One graphitic unit cell consists of 4 carbon atoms and unit cell dimensions are a=2.46 and h=6.71 Å.

$$\text{Concentration in mol } L^{-1} \text{ of CNP} = \frac{Number\ of\ moles\ of\ CNP}{Volume\ \ in\ L}$$

Number of moles of CNP in given weight of lyophilized powder

$$= \frac{Number\ of\ CNPs\ in\ the\ given\ weight\ of\ lyophilized\ powder}{Avogadro\ Number}$$

Number of CNP in given weight of lyophilized powder

$$= \frac{Number\ of\ carbon\ atoms\ in\ the\ given\ weight\ of\ lyophilized\ powder}{Number\ of\ carbon\ atoms\ in\ one\ CNP}$$

Number of carbon atoms in the given weight of lyophilized powder

$$= \frac{(Weight\ of\ lyophilized\ powder)(Avogadro\ Number)}{Average\ Molecular\ weight}$$

The CNP consists of C, H, O and not purely carbon, however it is difficult to determine the arrangement of the C, H and O in the CNP. The elemental analysis details the percentages of C, H and O in the CNP. Thus, we used percentage composition of C, H and O in CNPto obtain an average molecular weight value including contributions from C, H and O.

$$\text{Average molecular weight} = \frac{[(\%C)(12) + (\%O)(16) + (\%H)(1)]}{100}$$

It was assumed that unit cell consists of entity with the average molecular weight calculated above instead of pure carbon. Thus, calculated average molecular weight value was used instead of atomic weight of C for calculating number of C atoms in given weight of CNP.

Number of C atoms in one CNP (n) = (4) (n_g)

Where, number of C atoms present in each graphitic unit cell = 4

and n_g is number of graphitic unit cell present in one CNP

Number of graphitic unit cell present in one CNP (n_g) = $\frac{Volume\ of\ one\ CNP}{Volume\ of\ graphitic\ unit\ cell}$

Volume of one CNP = $\frac{\pi d^3}{6}$ where, d = average diameter of CNP determined from TEM

Volume of graphitic unit cell[4] = $\frac{\sqrt{3}ha^2}{2}$ where, a = 2.46 and h = 6.71 Å

The CNP solutions with known concentrations were used to make a calibration plot and the molar extinction coefficient (ε) for the CNP was determined using equation.

A= εCl

A = absorbance values at 268 nm for CNP-1 and 2 respectively

C = concentration of the solution

l = path length of the sample

Table S4. Molar extinction coefficients of CNPs at 268 nm.

CNP	Size of CNP (nm)	Extinction coefficient ($M^{-1}cm^{-1}$)
CNP-1	15 ± 4	1.72 x 10^7
CNP-2	35 ± 17	3.23 x 10^8

The calculated molar extinction coefficient values were used to determine the concentration of CNP solution obtained after hydrothermal reaction. The CNP solution obtained after hydrothermal reaction was used as a stock solution to prepare solutions with different concentrations.

S5. Incident laser power dependence of the SH signal

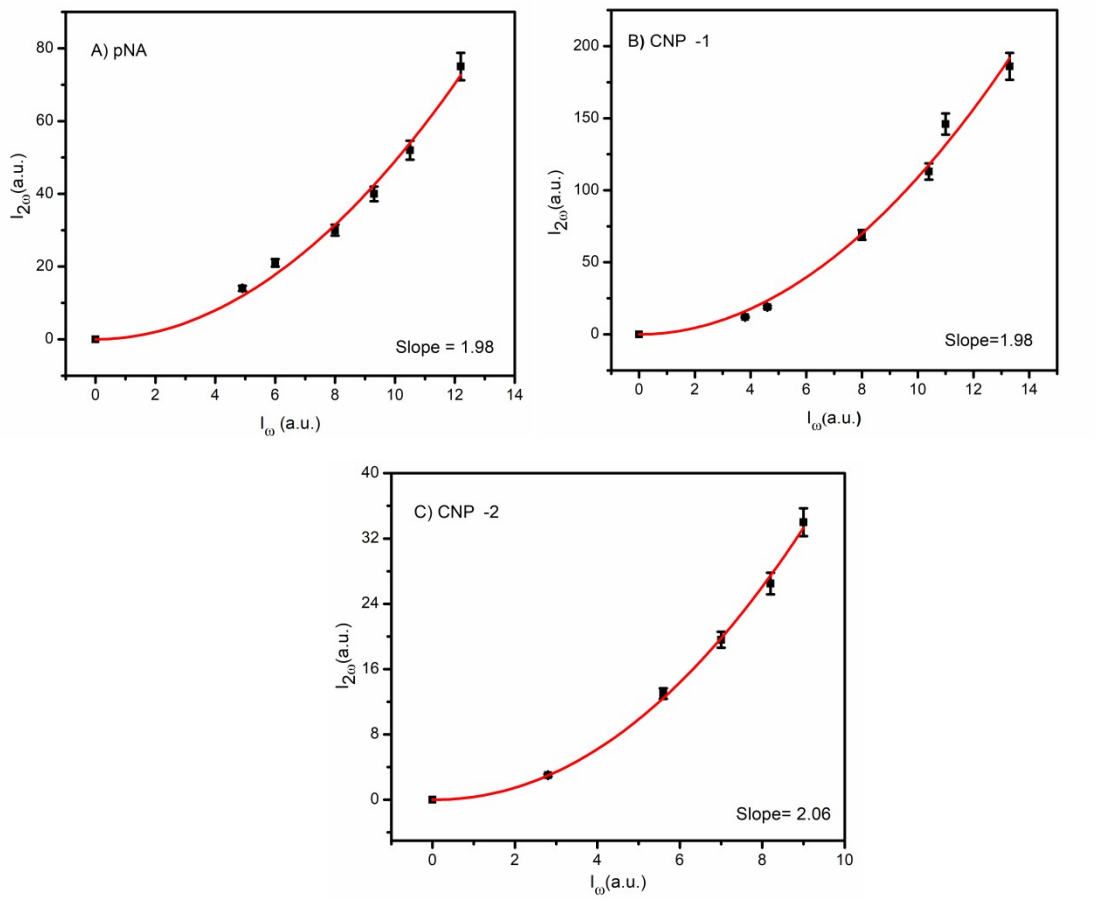

Fig. S2. Power dependence plots of A) pNA, B) CNP-1, and C) CNP-2. The plots show the quadratic dependence of SHLS signal with respect to incident laser intensity.

S6.Monochromator scans of the SH signal

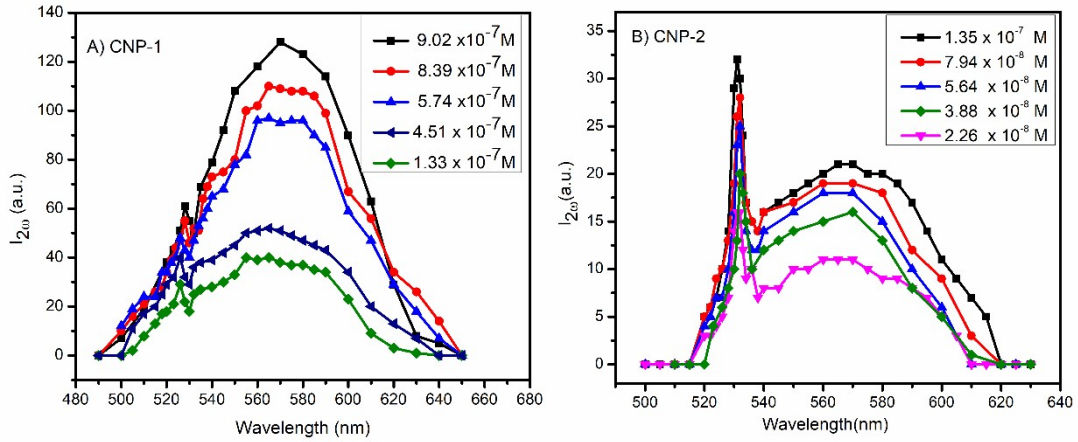

Fig. S3. Monochromator scans at different concentrations of A) CNP-1, and B) CNP-2

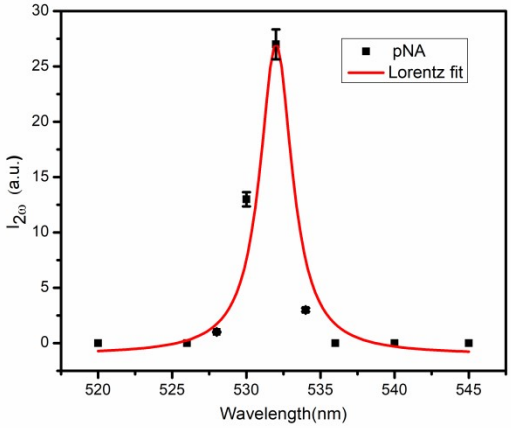

Fig. S4. Monochromator scan of pNA in water-methanol mixture

S7. Temperature dependent SHLS data with MLM fitting

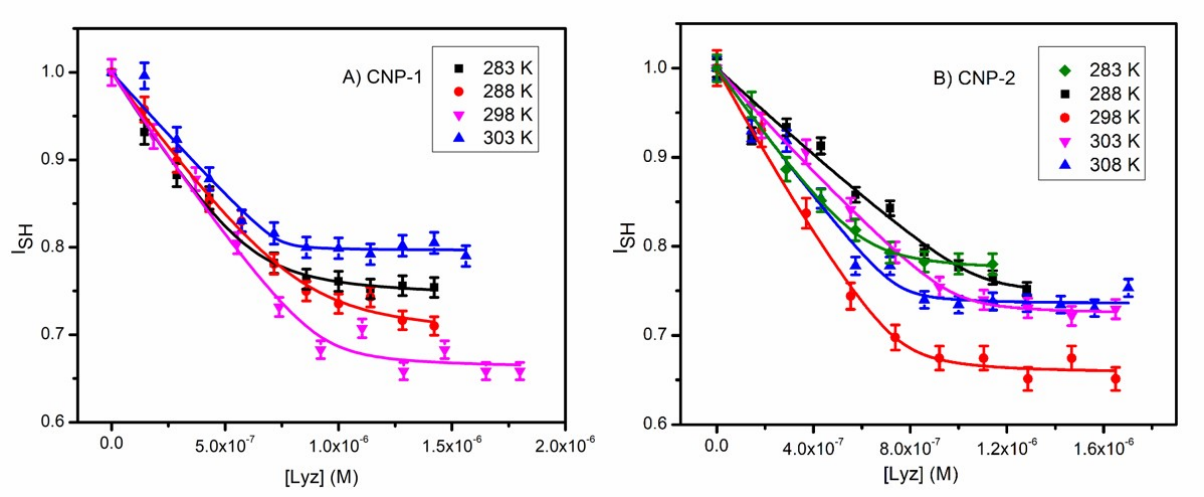

Fig. S5. Temperature dependent SHLS data of Lyz adsorption on CNPs. Lines through the data points are MLM fits.

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