Supporting Information

Recombinant protein polymers for colloidal stabilization and improvement of cellular uptake of diamond nanosensors

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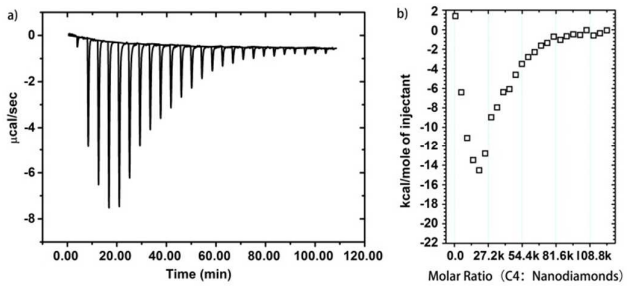


Figure SI-1. Characterization of binding of C_4 protein polymer to nanodiamonds using ITC. Nanodiamonds suspended in MilliQ water at a concentration of CND = 1 mg mL-1, were titrated (at 30°C) with a 1 mM C_4 in MQ water. a) Heat flow versus time during the injections. b) Heat released per mole of added C_4 versus the molar protein to nanodiamond ratio.

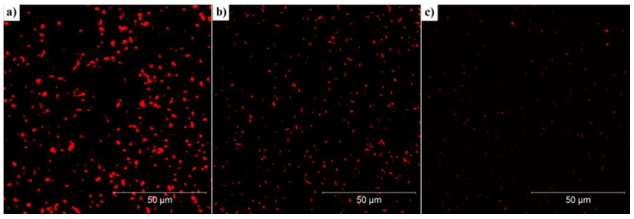


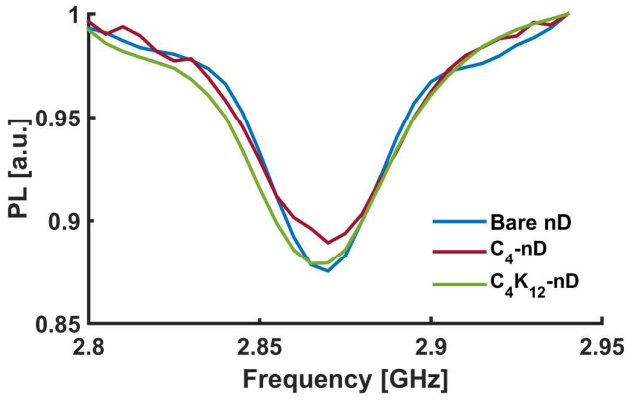
Figure SI-2. During the uptake experiments clusters of nanodiamonds deposited over the coverslip. Those clusters were imaged with a confocal microscope. (a) The cluster formed by bare nanodiamonds have bigger size (average area: 1.7 μm^2) in comparison with both coated cases, (b) C_4 (average area: 0.9 μm^2) and (c) C_4-K_{12} (average area: 0.3 μm^2) conjugated nanodiamonds. In all the cases, bare and conjugated nanodiamods were

mixed with growth medium (DMEM-HG complete), at concentration of 10 µg mL^{-1} of nanodiamonds, 630 µg mL^{-1} of C_4 and 662.4 µg mL^{-1} of C_4-K_{12} polymer protein, and let it incubate for two hours with HT29* cells.

Magnetooptical properties

Method: For magnetic resonance measurements, 10 µL of dispersions of coated and uncoated nanodiamonds were applied on clean microscope slides and dried. The dispersions were prepared by suspending 10 µL of the 120 nm nanodiamond stock solution in 90 µl of water, C_4 or C_4-K_{12} protein polymers at concentrations of, respectively, 7 mg mL^{-1} and 7.36 mg mL^{-1}. A home built diamond magnetometer was used, similar to those previously used by others[39,40]. The magnetometer is essentially a confocal microscope with built in microwave electronics. A laser power of 1 mW was used for illumination at a wavelength of 532 nm. After scanning the sample with adsorbed diamond nanoparticles, we focused on individual diamond particles and recorded an optically detected magnetic resonance. A frequency sweep was performed for the microwave, for frequencies around the expected resonance frequency of the NV center at 2,87 GHz. This microwave signal was produced with a microwave synthesizer (Hittite HMC-T2100) that sent its signal (power of 27 dBm) to a homemade antenna (a short circuit of a copper wire at the end of a coaxial cable) wish was located few micrometer from the sample. Simultaneously with the electromagnetic irradiation, the intensity of the fluorescence was collected using an Olympus UPLSAP40x NA=0.95 objective and an Avalanche photodiode (SPCM-AQRF- 15-FC) in single photon counting mode.

Results: We characterize the NV center's magnetooptical properties using Electron Spin Resonance ESR for the bare and the protein-polymer coated nanodiamonds. Results for the ESR experiment are shown in Figure 6. As expected, for bare nanodiamonds, the NV centers in Figure 6 show a decrease of their fluorescence intensity when exposed to an external electromagnetic field at frequency near 2.87 GHz. As for the photoluminescence, we find that the protein polymer coating hardly affects the magnetooptical properties of the nanodiamonds. The magnitude of the ESR signal for bare nanodiamonds and protein polymer coated nanodiamonds is almost the same.



SI-3. The magneto-optical properties of the nanodoamods (nD) remain almost unalterable after being coated with the C_4 and C_4-K_{12} protein polymers, as it is shown by ESR measurements of bare nanodiamonds (blue line), C_4 (red line) and C_4-K_{12} (green line) coated nanodiamonds. Nanodiamonds at 100 µg mL^{-1} in MilliQ water, C_4 protein at 6.3 mg mL^{-1} and C_4-K_{12} at 6.624 mg mL^{-1} in MilliQ water. The fluorescence intensity (y

axis) of the NV center drops when it is excited with an external electromagnetic field at frequency near to 2.87 [GHz] (x-axis).

Protein polymer binding stability at different pH

Considering the fact that the conjugation of the protein polymers and the surface of the nanodiamonds is made merely by physical absorption, we were interested in to evaluate the robustness of these bonds when the coated particles are exposed to an alkaline or acidic environment. The experiments consisted in measuring the hydrodynamic diameter of the coated particles after dispersing them in media at pH 4.5, 5.5, 6.8, 7.9 and 8.9. It was assumed that the desorption of protein polymers from the nanodiamonds surface would be reflected in a reduction of the hydrodynamic diameter (HD) of the particles when they are measured by DLS.

Method: Media at pH 4.5 and 5.5 was prepared by diluting hydrogen chloride (HCl) in MilliQ water (pH 5.31) until the desired pH values were reached. The media at pH 6.8, 7.9 and 8.9 were prepared similarly but by adding sodium hydroxide (NaOH) instead. The samples were prepared by dispersing 1 µL of C_4 coated- or C_4-K_{12} coated nanodiamonds (100 µg L^{-1}), as appropriate, in 999 µL of the media previously made. The hydrodynamic diameter was measured and analysed following the same procedure explained previously in the method section of the main article.

Results: The measures of hydrodynamic diameter showed low variability between different pH conditions. Especially, the C_4 coated nanodiamonds reported more consistent results across different samples. On the other hand, the average of the HD of the C_4-K_{12} coated particles shows a small increase as the pH turns more basic. Neither of these situations suggests the occurrence of desorption of the protein polymers from the nanodiamonds surface. On the contrary, the slight increment in size could be an indication of the increasing in the thickness of the ions layer surrounding the particles, or very slight aggregation.

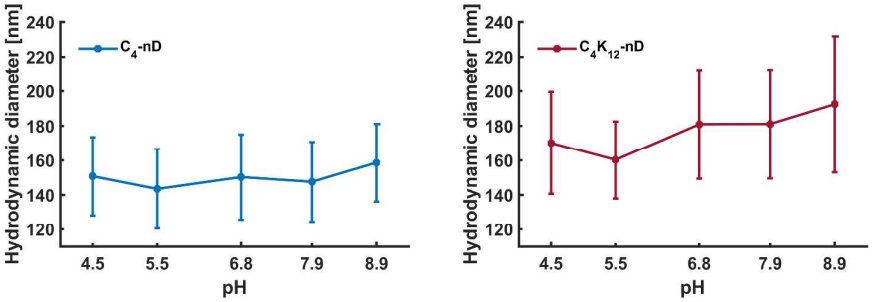


Figure SI-4. At pH 6.8, the average HD is 150.3 and 180.9 of the C_4- (blue) and C_4-K_{12}- (red) coated nanodiamonds (nD) respectively. The comparison of this value with the one from samples in alkaline and acidic medium, and considering the wide distribution of the results, doesn't suggest a considerable reduction of the particle's size that could be attributable to the desorption of the protein polymers. Instead, the small changes of size can be explained by a change in the thickness of the electric dipole layer that surrounds the nanoparticles.