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ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

A switchable peroxidase mimic derived from the reversible co-assembly of cytochrome *c* and carbon dots

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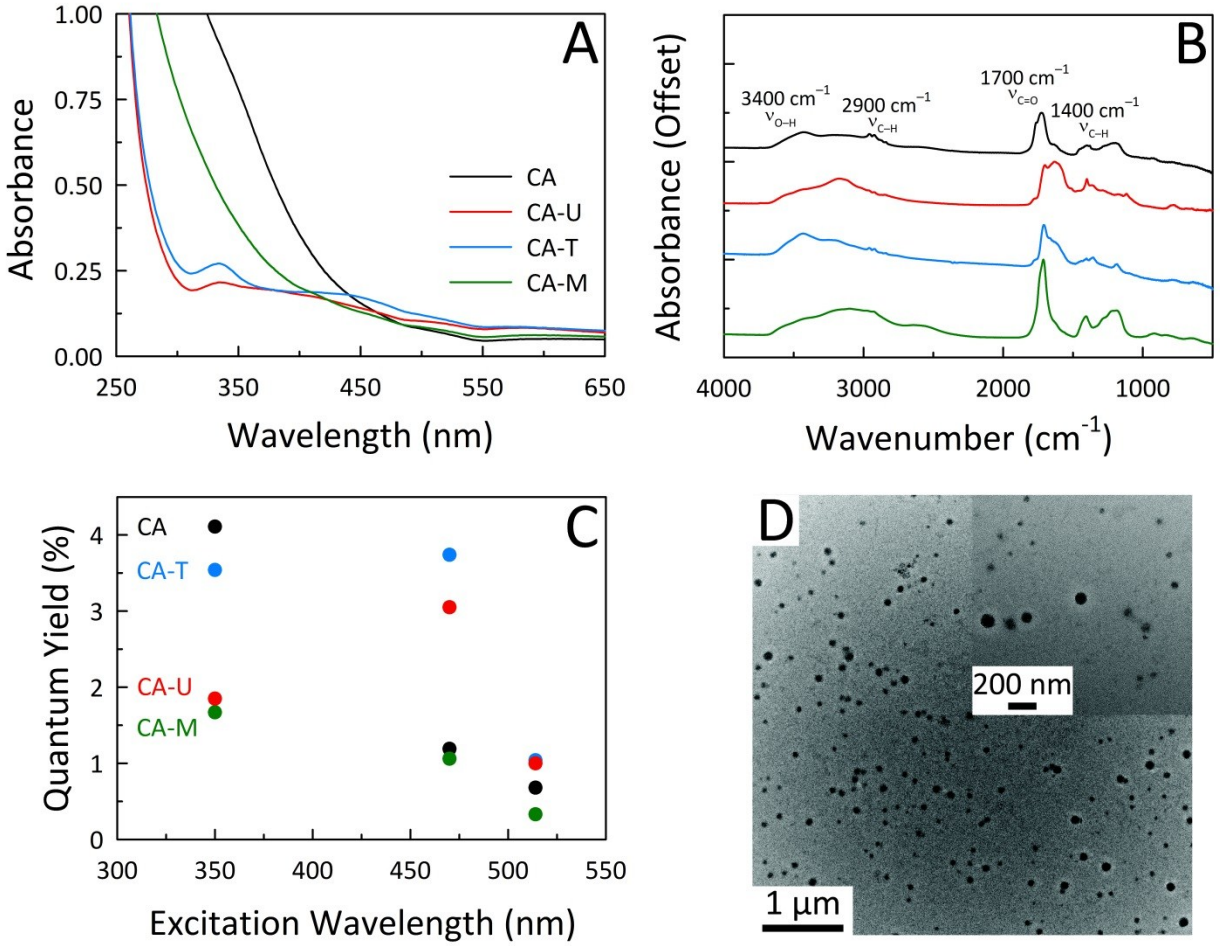

Fig. S1 (A) UV-Vis spectra of the four C-dot samples. The CA and CA-M derived C-dots presented rapidly increasing absorbance in the near UV region which is characteristic of an aromatic sp^2 domain's π-π* transitions. Conversely, CA-U and CA-T derived C-dots displayed small peaks at approximately 340 nm, likely due to nitrogen incorporation within the C-dot. In addition, the CA-T derived C-dots displayed an additional peak centred around 450 nm. (B) FTIR spectra of the four C-dot samples with the following peak assignments (line colors correspond to the legend in (A)): 3400 cm^{-1}, stretching vibrations of O–H (ν_{O-H}) and/or N–H (ν_{N-H}); 1700 and 1650 cm^{-1}, stretching vibrations of C=O ($\nu_{C=O}$); 1650-1550 cm^{-1}, NH/NH_2 deformation (ν_{N-H}) and skeletal vibrations of aromatic groups ($\nu_{C=C}$); 1300-1000 cm^{-1}, stretching vibrations of C-O-C and C-OH (ν_{C-O-C} and ν_{C-OH}); 2900 and 1400 cm^{-1}, aliphatic stretches (ν_{C-H}). (C) Excitation wavelength-dependent quantum yield values of the various C-dots. For all of the C-dots, the quantum yield values decreased as the excitation wavelength increased with the CA and CA-T derived C-dots both exhibiting the highest quantum yields across all wavelengths. (D) Representative TEM images of the CA-derived C-dots showing that the C-dot diameters ranged from 30 to 60 nm with some dots reaching 100 nm in size. The C-dots derived from the other precursors were of a similar size regime.

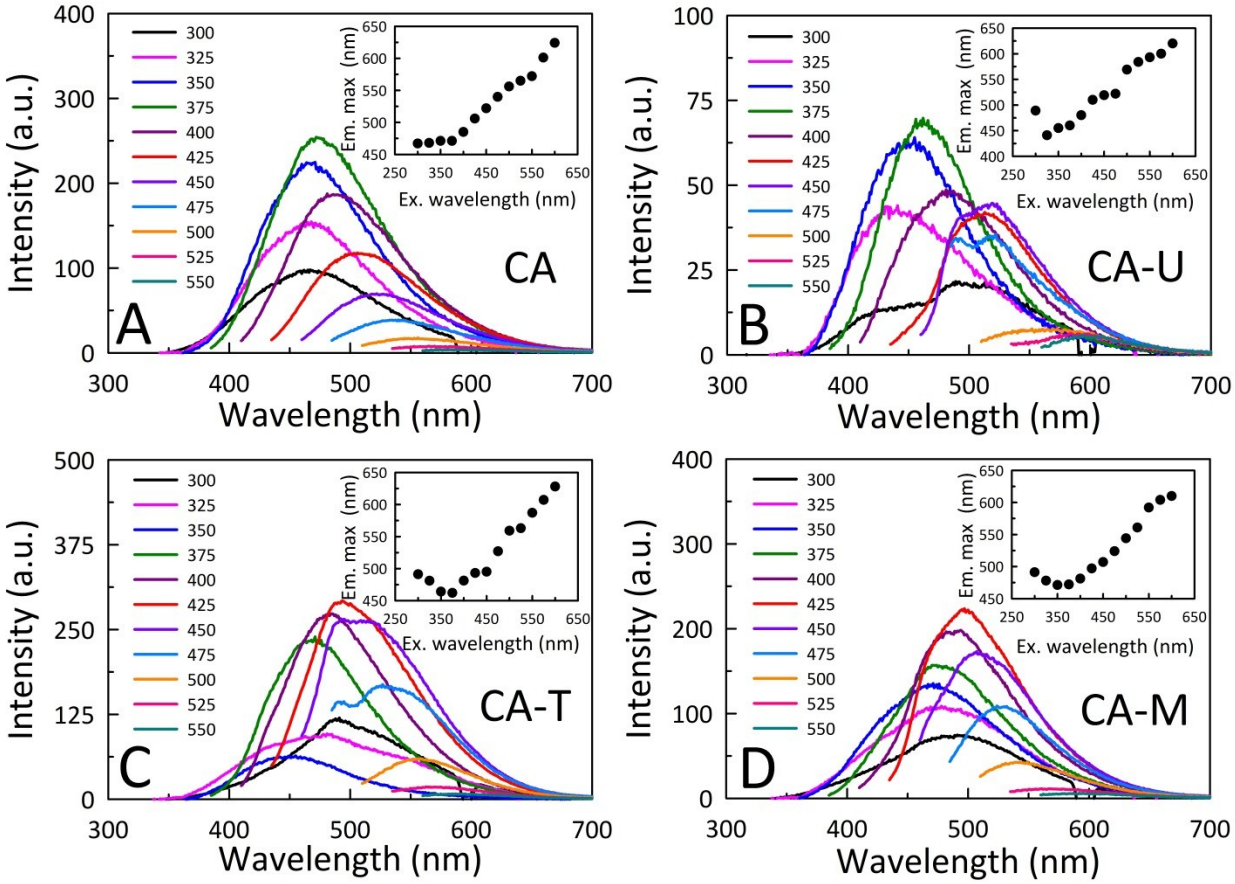

Fig. S2 Fluorescent emission spectra of the (A) CA, (B) CA-U, (C) CA-T, and (D) CA-M derived C-dots. In general, upon increasing the excitation wavelength, the fluorescent emission maxima displayed a slight hypsochromic shift, followed by a larger bathchromic shift, which is further highlighted within the inset plots.

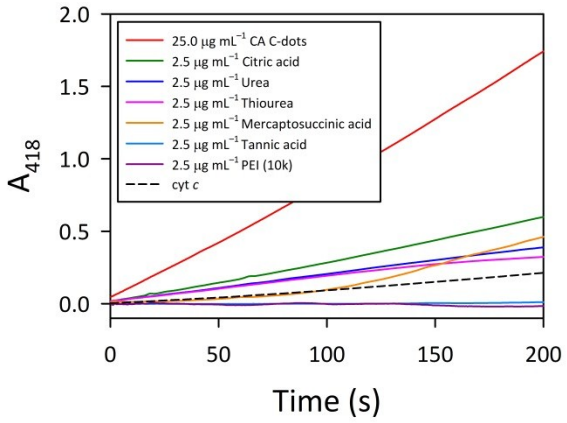

Fig. S3 Catalytic control studies substituting the various C-dots with their precursors as small 'model' molecules. In addition, larger molecules such as tannic acid and 10 kDa PEI were also tested. The concentration of these model molecules was 10 times lower than that of the C-dots since this was a fairer comparison in regards to surface groups available for interaction (operating under the reasonable assumption that only ~10% of the total C-dot comprise surface moieties). The model molecules produced little to no enhancement over the activity of native cyt *c* indicating that electrostatics wasn't the only factor at play here; the co-assembly of C-dots and cyt *c* was indeed crucial for the enhancement.

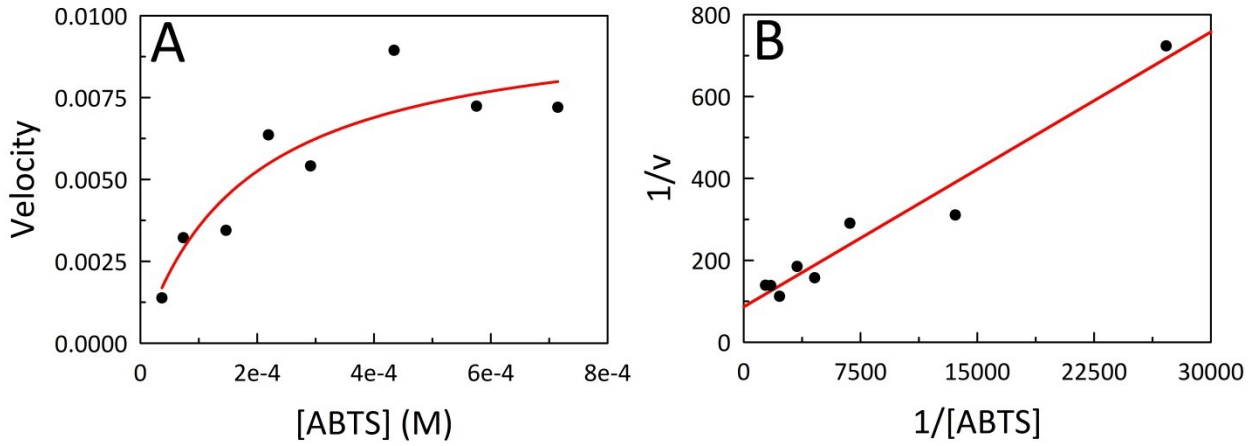

Fig. S4 (A) Michaelis-Menten kinetic plot of cytochrome *c* peroxidase-like activity in the presence of CA derived C-dots. (B) Converting the Michaelis-Menten data in (A) to a Lineweaver-Burke plot afforded the calculation of the characteristic catalytic parameters, V_{max}, K_m, K_{cat}, and K_{cat}/K_m. These parameters were found to be: V_{max} 0.010 ± 0.002 s^{-1}, K_m 0.245 ± 0.021 mM, K_{cat} 1.97 (± 0.39) x 10^4 s^{-1}, and K_{cat}/K_m 8.04 ± (1.74) x 10^7 $M^{-1}s^{-1}$.

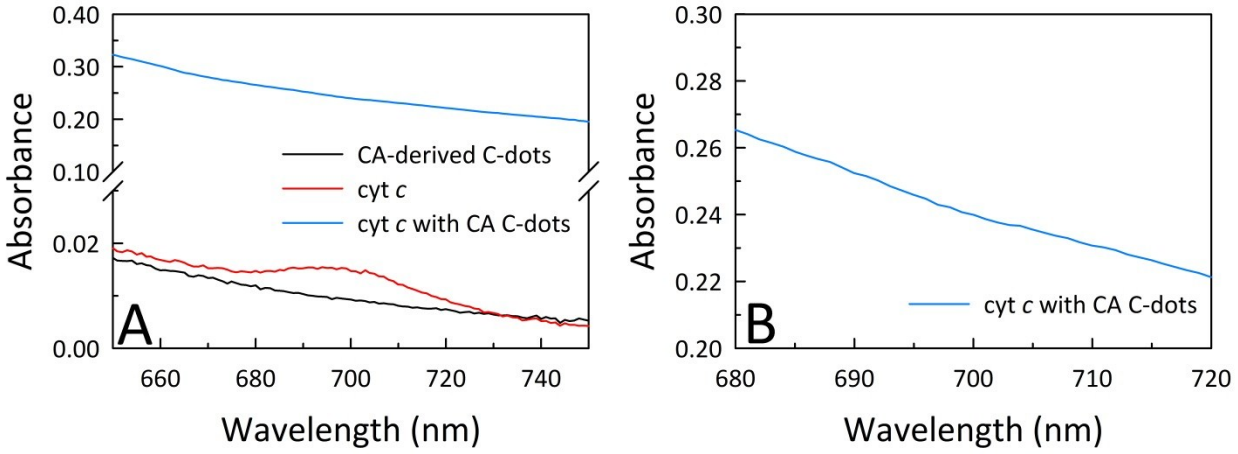

Fig. S5 (A) UV-Vis spectra of neat CA-derived C-dots, native cyt *c*, and C-dots added to cyt *c*. The loss of the characteristic 695 nm band for cyt *c* (associated with the Met80-Fe^{3+} bond) indicates that the microenvironment of the protein's heme group was disrupted, opening it up to allow for more efficient peroxidase-like activity. (B) Zoomed in view of the UV-Vis spectrum of C-dots co-mingled with cyt *c* showing that there is no characteristic Met80-Fe^{3+} feature present near 695 nm, further highlighting the disruption of the protein's heme center.