Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supplementary Material

Dual-Sensitive Fluorescent Nanoprobes for detection of matrix metalloproteinases and low pH in 3D Tumor Microenvironment

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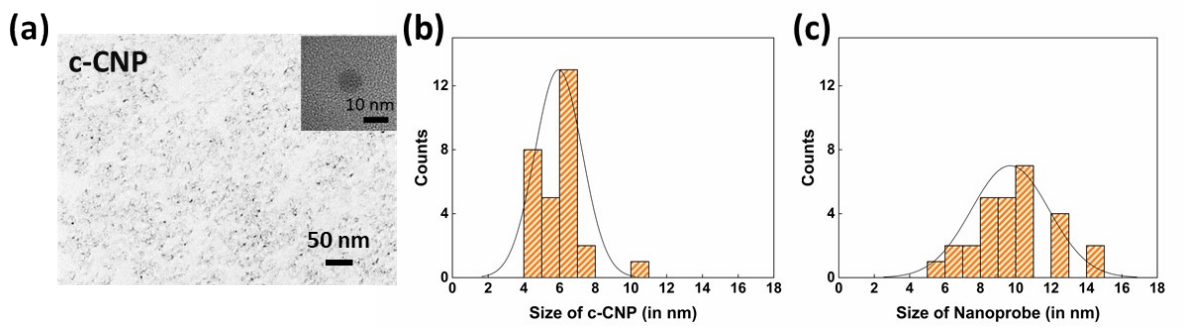


Fig S1: (a) Transmission electron microscope image of c-CNP showing nanoparticles with spherical morphology. The inset shows HRTEM image of the c-CNP; Size distribution histograms for **(b)** c-CNP and **(c)** Nanoprobes.

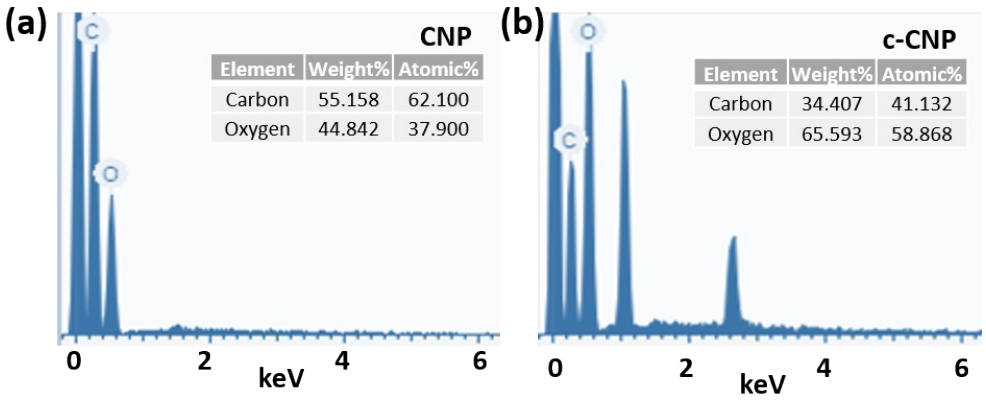


Fig S2: Elemental analysis for **(a)** CNP and **(b)** c-CNP using Energy dispersive X-ray Analysis

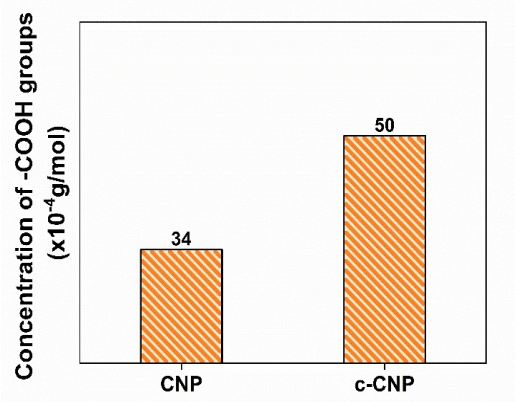


Fig S3: Bar graph showing increase in concentration of –COOH functional groups on nanoparticles' surface post carboxylation as calculated by Boehm's titration method.

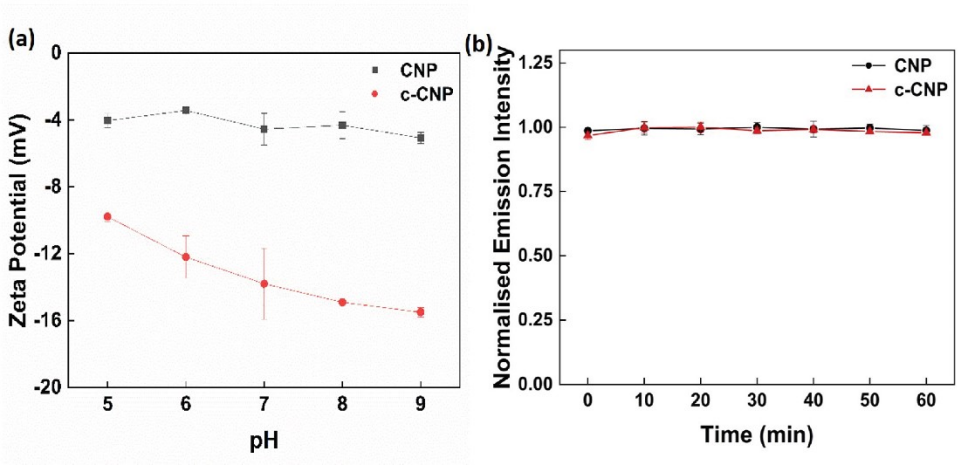


Fig S4: (a) Zeta potential curves for CNP (black solid line) and c-CNP (red solid line) showing change in zeta potential values with varying pH (pH 5 to pH 9). **(b)** Plot showing photostability of CNP and c-CNP (λ_{ex}= 365 nm and λ_{em}= 450 nm) exposed to UV lamp for 60 min.

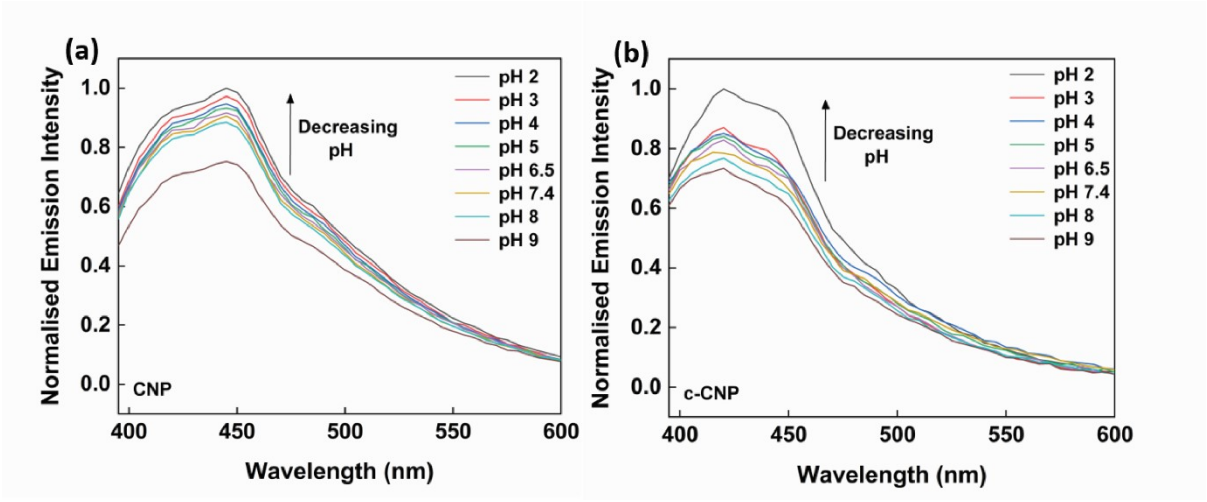
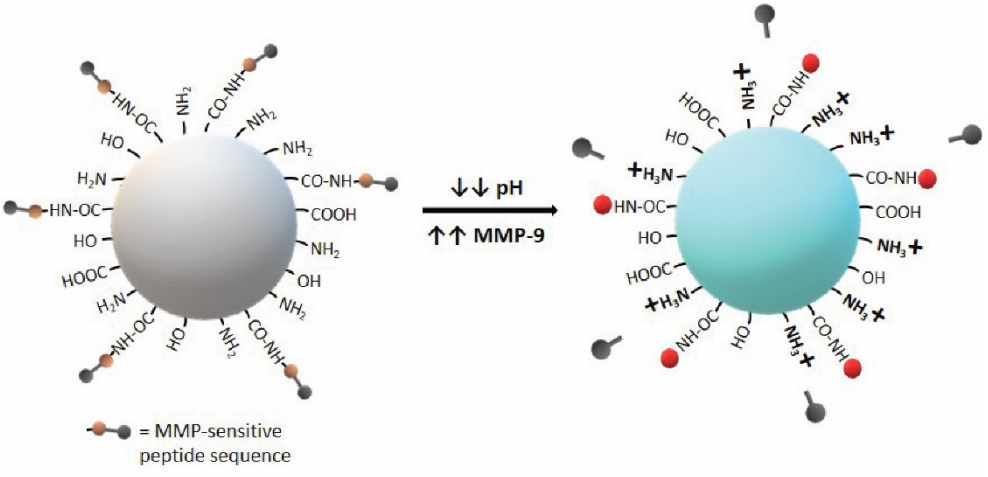


Fig S5: Emission spectra (λ_{ex}= 365 nm) for **(a)** CNP and **(b)** c-CNP in pH buffer solutions ranging from pH 2 to pH 9 indicating that fluorescence intensity reduces as pH value increases.



Scheme S1: Schematic showing dual-sensing in nanoprobes. When placed in low pH environment, protonation and deprotonation of surface functional groups & transfer of protons from protonated amine groups to conjugated carbon structure lead to enhancement in blue fluorescence. The presence of hydroxyl and carboxyl functional groups may also cause change in surface state of the carbon nanoparticles and lead to pH-sensitive fluorescence emission. Moreover, in presence of MMP-9, the peptide linking fluorophore-quencher (6' TAMRA and TQ3) pair gets cleaved resulting in activation of the fluorophore and hence, red emission is generated.

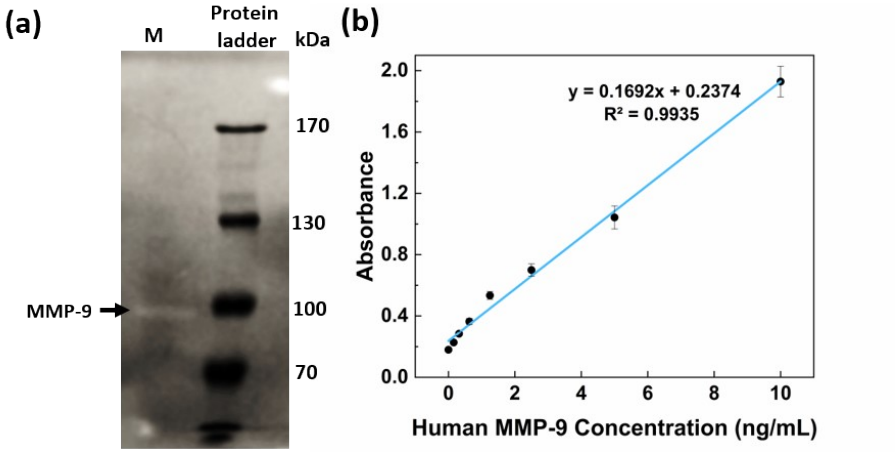


Fig S6: (a) Gelatin zymogram for conditioned media collected post-PMA treatment to the U2OS cells (M = conditioned media) and **(b)** Standard for Human MMP-9 ELISA.

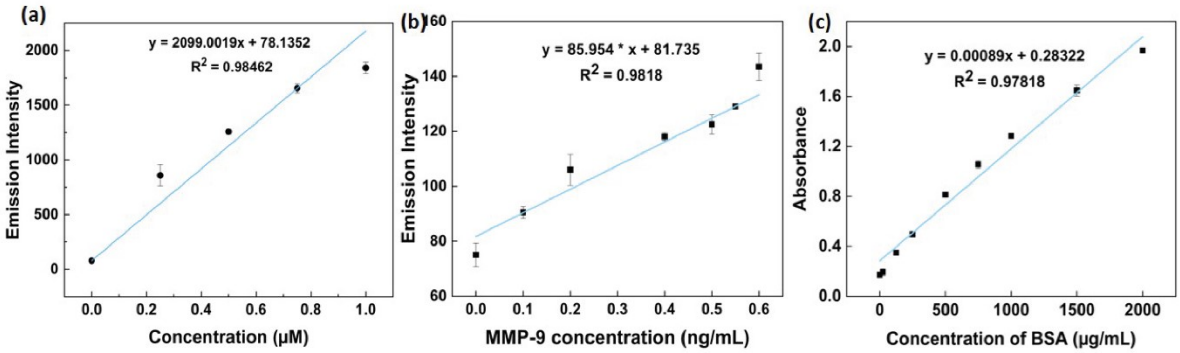


Fig S7: (a) Fluorescence emission curve for known concentrations (in μM) of MMP-9 sensitive peptide incubated for 2 hr with PMA-treated conditioned media of U2OS cells (λ_{ex}= 545 nm and λ_{em}= 576 nm); **(b)** Fluorescence emission plot for nanoprobes' response to presence of MMP-9 at series of concentrations ranging from 0.1 ng/mL to 0.6 ng/mL (λ_{ex}= 545 nm and λ_{em}= 576 nm). The detection limit was calculated as 0.29 ng/mL; and **(c)** Standard for known concentrations of Bovine serum albumin (BSA).

Sample	Absorbance @ 340 nm	Integrated fluorescence	Quantum yield	Quantum yield (%)
Quinine hemisulphate	0.089	92237.5	0.54	54
CNP	0.09	27392.5	0.137	13.7
c-CNP	0.081	18827.5	0.105	10.5

Table S1: Quantum yields of CNP and c-CNP for blue emission

Sample	Absorbance @ 546 nm	Integrated fluorescence	Quantum yield	Quantum yield (%)
Rhodamine B	0.052	222640	0.65	65
Nanoprobe	0.07	19005	0.039	3.9

Table S2: Quantum yield of nanoprobe for red emission

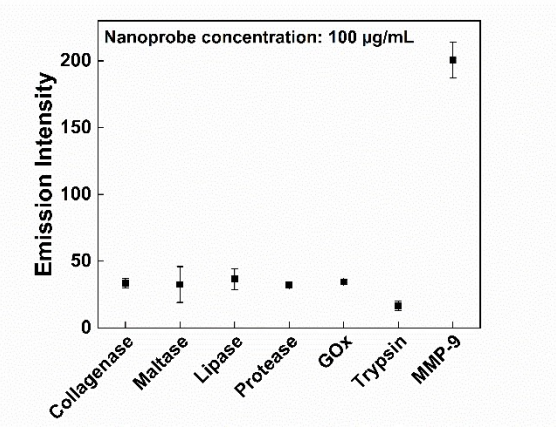


Fig S8: Emission spectra for nanoprobe (λ_{ex}= 545 nm and λ_{em}= 576 nm) incubated with different enzymes for 2 hr to check its specificity towards MMP-9 enzyme detection.

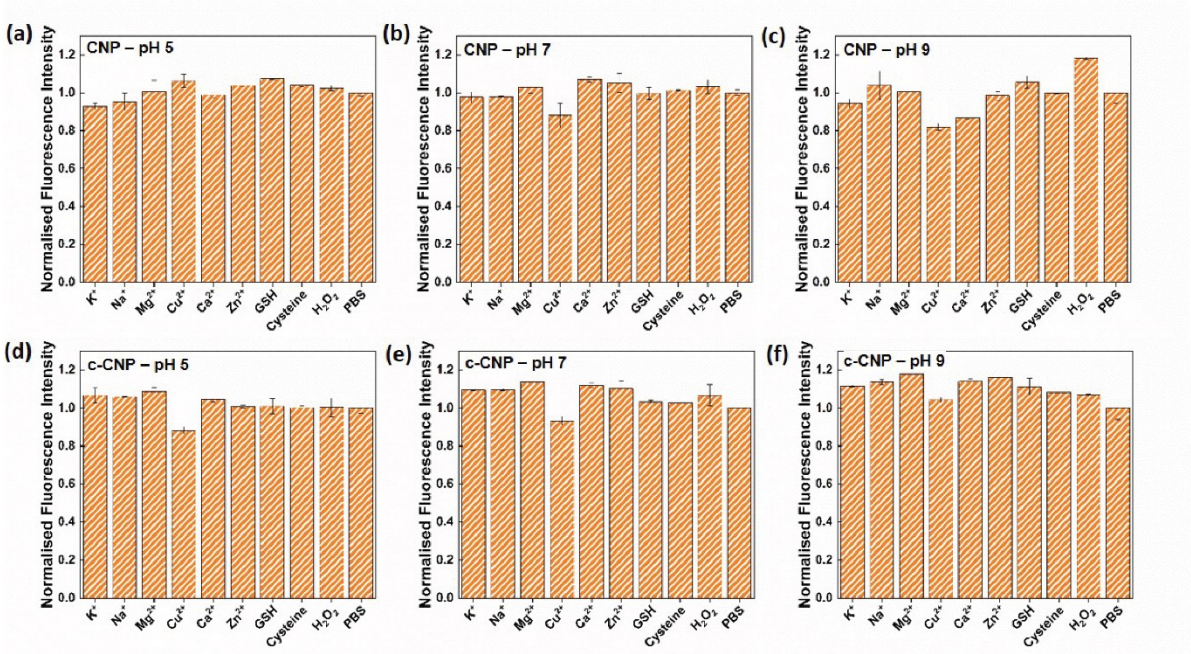


Fig S9: Normalised fluorescence intensity of CNP **(a-c)** and c-CNP **(d-f)** at pH 5, 7 and 9 in presence of various metal ions, biothiols and ROS.

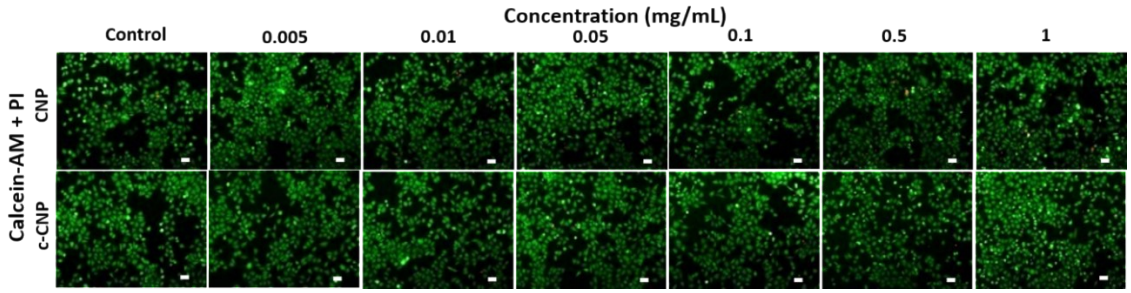


Fig S10: Live dead assay using Calcein-AM and PI for various concentrations (0.005 to 1 mg/mL) of CNP and c-CNP incubated with U2OS cells for 24 hr. (Scale bar: 50 µm)

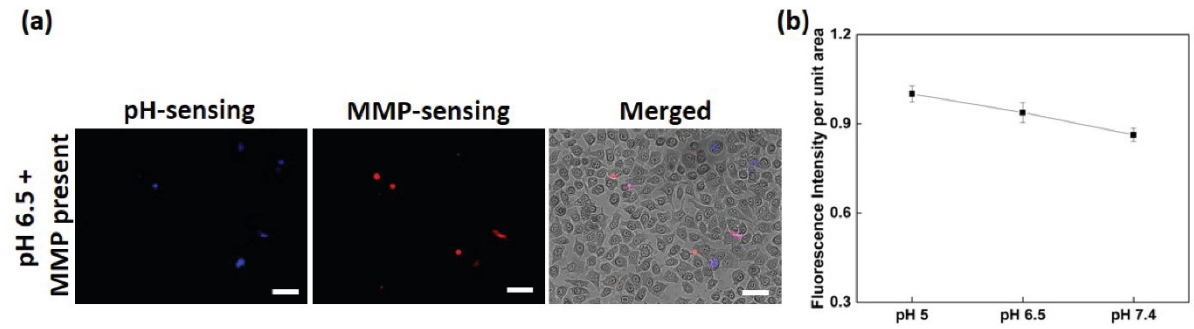


Fig S11: (a) Fluorescence microscopy images of U2OS cells incubated with nanoprobes for 2 hr in conditioned media at slightly acidic pH (pH 6.5) and upregulated MMP enzyme (mimicking tumor microenvironment) showing bright blue and red fluorescence. Scale bar: 50 µm. **(b)** Normalized fluorescence intensity per unit area versus pH plot. The fluorescence intensities were obtained from imaging done on U2OS cells incubated with nanoprobes in conditioned media with pH 5, 6.5 and 7.

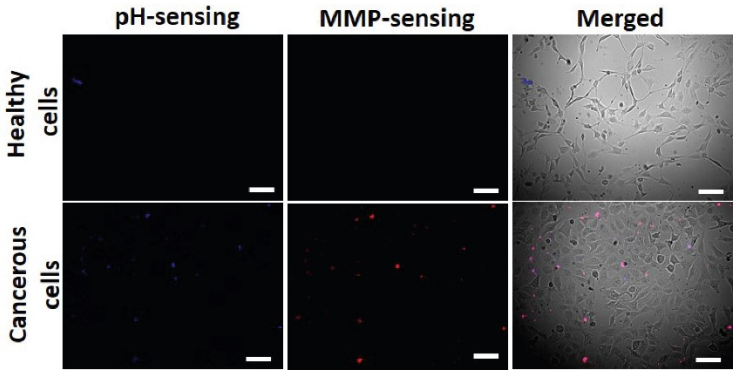


Fig S12: Fluorescence microscopy images of nanoprobes incubated with healthy and cancerous cells for 2 hr showing generation of blue (pH-sensing) as well as red fluorescence (MMP-sensing) in cancerous cells (HT1080); and negligible blue fluorescence and no red fluorescence due to absence of MMP enzyme in healthy cells (NIH-3T3). Scale bar: 100 µm

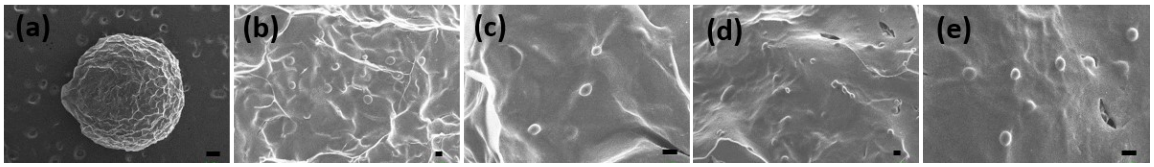


Fig S13: Scanning electron micrographs of **(a)** blank microscaffolds (Scale bar: 300 µm); and microscaffolds with U2OS cells cultured for 24 hr **(b)**, **(c)** without and **(d)**, **(e)** with c-CNP. (Scale bar: 20 µm)