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

Supporting Information

Carbon nanotube-based multicolor fluorescent peptide probes for highly sensitive multiplex detection of cancer-related proteases

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1. Characterization of MWCNTs

Scanning electron microscope (SEM) imaging was used to characterize the MWCNT-based multicolor nanoprobes. Figure S1 shows SEM images of MWCNTs before and after linking with fluorophore-labeled peptides. Before fluorophore-labeled peptides linking, an average MWCNTs diameter of about 65 nm was measured (Figure S1, A). After fluorophore-labeled peptides linking, the diameter of MWCNTs did not change obviously (Figure S1, B), which was attributed to the small size of the fluorophore-labeled peptides.

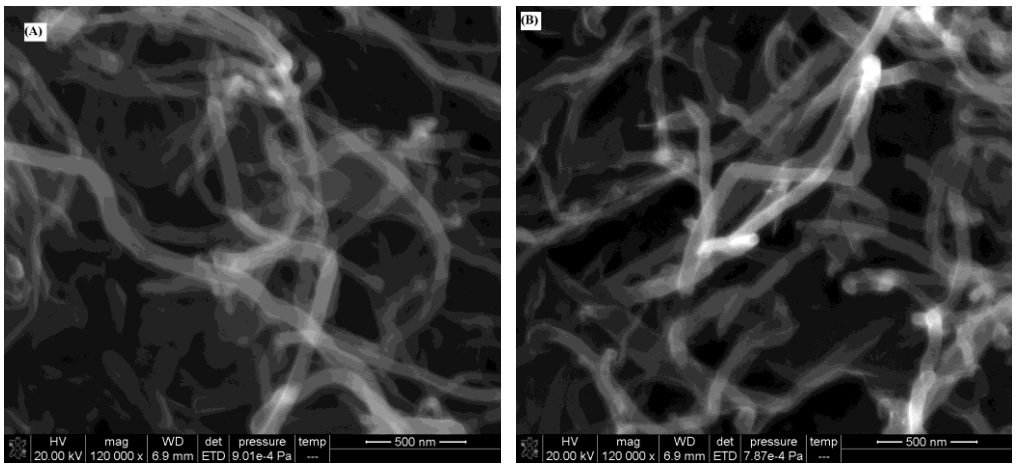

Figure S1. SEM images of MWCNTs and MWCNT-based multicolor nanoprobes. (A) Images of MWCNTs before linking with fluorophore-labeled peptides; (B) images of MWCNT-based multicolor nanoprobes.

2. Assays of two nucleases

The multicolor nanoprobe was used to detect two proteases simultaneously. Upon the simultaneous addition of MMP-7 and MMP-2, short peptide fragments carrying FITC and Cy3 were generated by specific protease-catalyzed peptide cleavage reactions, and then they released from the MWCNT surface, resulting in the enhancement of fluorescence of FITC and Cy3. At the same time, the fluorescence intensity of Cy5 did not change greatly (Figure S2, A). Also, when the multicolor nanoprobe was treated with MMP-7 and uPA, the fluorescence intensities of FITC and Cy5 increased, and the fluorescence intensity of Cy3 remained almost unchanged (Figure S2, B). When the multicolor

nanoprobe was treated with MMP-2 and uPA, only the fluorescence of FITC was quenched by MWCNTs (Figure S2, C). These results indicate that the multicolor nanoprobe can be used to simultaneously detect MMP-7 & MMP-2, MMP-7 & uPa and MMP-2 & uPA pairs.

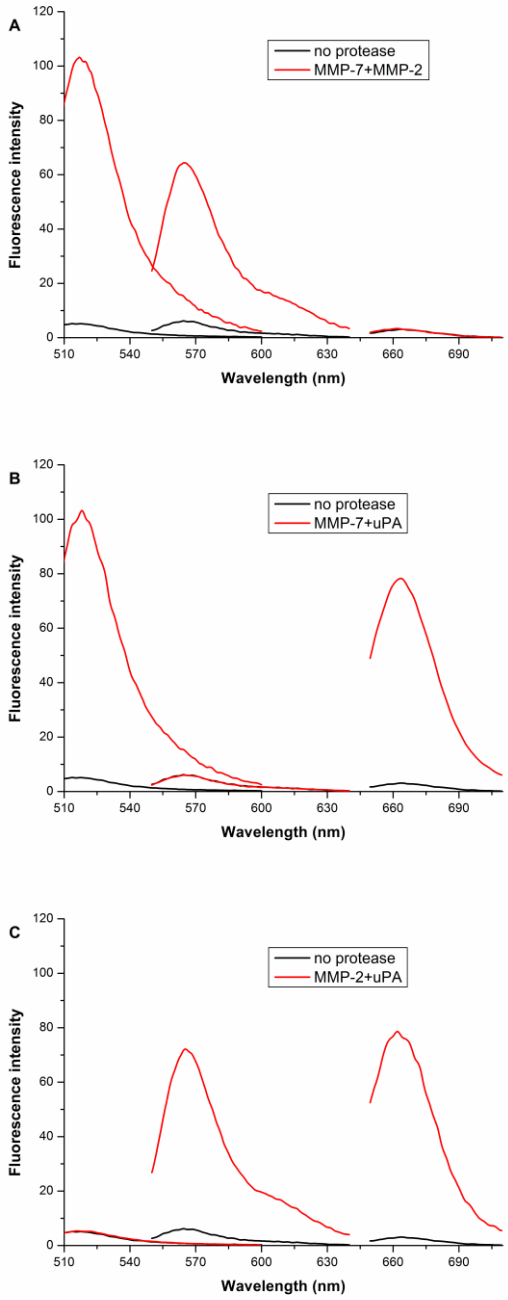

Figure S2. Fluorescence emission spectra of the multicolor nanoprobe after mixing with after treatment with two of the three proteases (A-C). Concentrations of proteases: 200 pg/mL MMP-7, 400 pg/mL MMP-2, and 200 ng/mL uPA. The fluorescence spectra of the three dyes involves were measured and then these three spectra were combined into a single figure. The fluorescence measurement of FITC, Cy3 and Cy5 were excitation at 490 nm, 535 nm, and 645 nm (corresponding to MMP-7, MMP-2, and uPA, respectively), respectively.

3. Assays of three nucleases

The multicolor nanoprobe was also applied to detect the three proteases simultaneously. In the absence of proteases, the fluorescence of all three dyes involved was quenched. When the multicolor nanoprobe was mixed with the three proteases, specific peptide cleavage reactions occurred and short peptide fragments carrying FITC, Cy3 or Cy5 were released from the surface of MWCNTS, which led to the recovery of fluorescence of these three dyes (Figure S3). Therefore, by monitoring the dyes' fluorescence change before and after the addition of the proteases, it was easy to use the multicolor nanoprobe for the simultaneous detection of three proteases.

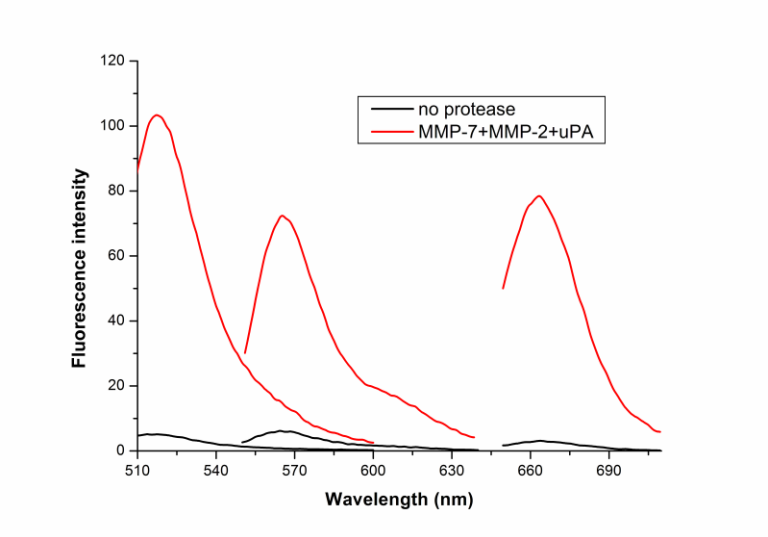

Figure S3. Fluorescence emission spectra of the multicolor nanoprobe after treatment with three proteases simultaneously (200 pg/mL MMP-7, 400 pg/mL MMP-2, and 200 ng/mL uPA), with excitation wavelengths of 490 nm for FITC, 535 nm for Cy3 and 645 nm for Cy5 (corresponding to MMP-7, MMP-2, and uPA, respectively). The fluorescence spectra of the three dyes involved were measured and then these three spectra were combined into a single figure.