Supporting Information

Light-triggered nitric oxide release and targeted fluorescence imaging in tumor cells developed from folic acid-graft-carboxymethyl chitosan nanospheres

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Part S1 Synthesis of PEI-stabilized CQD

Briefly, 200 mg of glucose was added into 10 mL solution of glycol/water (1/1, v/v) to form clear mixed solution under stirring and ultrasound. The mixed solution was added to a high pressure nitrifying pot, and heated at 180 °C in a constant-temperature drying oven for 3 h to generate bare CQD. Then, 1.0 mL of PEI solution (20%, v/v) was mixed with 10 mL of the bared CQD solution and rapidly stirred for 3 h at 80 °C. Under ultraviolet lamp excitation, strong fluorescence emission of the final reaction solution indicated the formation of PEI modified or stabilized CQD (i.e. PEI-CQD, 1 wt.%).

Part S2 Determination of NO concentrations

To determine the NO release from CMC-FA-RBS hybrid nanospheres in aqueous solution (PBS, 10 mM, pH 7.4), the colorimetric Griess reaction was employed to measure nitrite or nitrate content in the PBS of CMC-FA-RBS. The details are available as follows: aliquots of PBS of CMC-FA-RBS (1.0 mg·mL⁻¹, 5 mL) was taken and stirred in a centrifugal tube at 37 °C in a water bath. At appropriate time intervals, the solution was centrifuged for 10 min, and the supernatant (0.5 mL) was extracted, replenished with the PBS and combined with Griess reagent (I) (0.1 wt.% of β-naphthylethylenediamine dihydrochloride aqueous solution, 1 mL) and (II) (1 wt.% of sulfanilamide in 5 wt.% of phosphoric acid aqueous solution, 1 mL). The resultant mixed solution was incubated for 15 min at room temperature, protected from light (i.e., in the dark). A purple/magenta color appeared immediately. The maximum absorbance was recorded (at 540 nm) by using UV-vis spectrophotometer, and the standard curve was determined by measuring sodium nitrite (0-100 μM) in PBS. The total release of NO was calculated according to the following equations:

\[ R_1 = C_1 \times 0.005; \]
\[ R_2 = C_2 \times 0.005 + C_1 \times 0.001; \]
\[ R_3 = C_3 \times 0.005 + (C_2 + C_1) \times 0.001; \]
\[ \ldots \]
\[ R_n = C_n \times 0.005 + (C_{n-1} + C_{n-2} + \ldots + C_1) \times 0.001 \]

where \( R_n \) is the release amount of NO measured at each time point, and \( C_n \) is the concentration of NO.

Part S3 PL stability of CMC-FA-CQD hybrid nanospheres
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**Fig. S1** Relative PL intensity of 1.0 mg·mL⁻¹ of CMC-FA-CQD hybrid nanospheres stored in 10 mM of PBS (pH 7.4) for different times at room temperature (~20 °C).

**Part S4** light-triggered NO release from CMC-FA-RBS nanospheres

**Fig. S2** Schematic illustration of the chemistry structure, spherical model and light-triggered NO release of the designed CMC-FA-RBS hybrid nanosphere system.
Fig. S3 Fluorescence imaging of (a) L02, (b) A549, (c) HepG2, and (d) HeLa cells after 24 h incubation with the CMC-FA-CQD hybrid nanospheres (1 mg·mL\(^{-1}\)), excited at 440 nm. The scale bar is 100 μm.

Part S5 Fluorescence imaging from CMC-FA-CQD nanospheres

Fig. S4 Schematic illustration of the chemistry structure, spherical model and fluorescence emission of the designed CMC-FA-CQD hybrid nanosphere system.