

Supplementary Information

CdSe/ZnS-labeled carboxymethyl chitosan as a bioprobe for live cell imaging

Min Xie,^a Hui-Hui Liu,^b Ping Chen,^a Zhi-Ling Zhang,^a Xiao-Hui Wang,^c Zhi-Xiong Xie,^b Yu-Min Du,^c Bo-Qun Pan^d and Dai-Wen Pang^{*a}

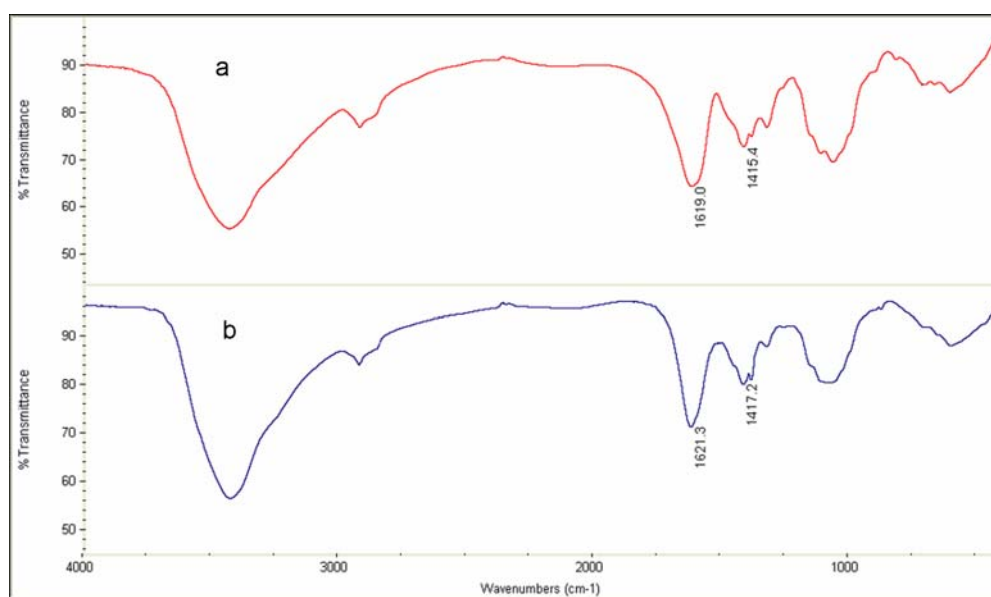


Figure SI-1. FTIR spectra of carboxymethyl chitosan (a) and CdSe/ZnS-labeled carboxymethyl chitosan (b). Bands at 1619.0 and 1415.4 cm⁻¹ are assigned to asymmetric and symmetric stretching vibrations of carboxyl group, respectively, both of which shifted to higher wavenumbers, 1621.3 and 1417.2 cm⁻¹ after CdSe/ZnS labeling, indicating that carboxyl group of carboxymethyl chitosan coordinates with metal ions on the surface of CdSe/ZnS.

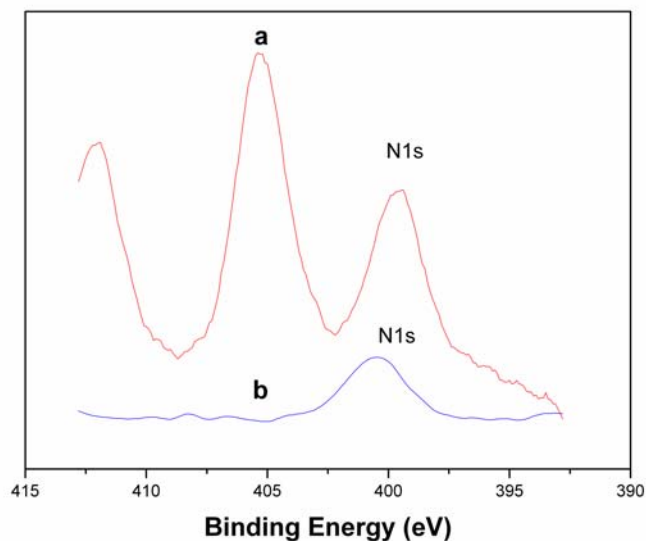


Figure SI-2. XPS spectra of N1s of CdSe/ZnS-labeled carboxymethyl chitosan (a) and carboxymethyl chitosan (b). It is well-known that the binding energy of N1s will shift to lower value when nitrogen is bound to metal ions, due to a transfer of electron from nitrogen to metals. The peak positions of N1s of carboxymethyl chitosan and CdSe/ZnS-labeled carboxymethyl chitosan centered at 400.5 eV and 399.8 eV, respectively, indicating that the nitrogen of the amino group in carboxymethyl chitosan coordinates with the metal ions on the surface of CdSe/ZnS.

On the cytotoxicity of CdSe/ZnS-labeled carboxymethyl chitosan

Methylene blue-staining experiments:

Yeast cells were incubated with CdSe/ZnS-labeled CMC and CdSe/ZnS-labeled mercaptoacetic acid, respectively, for 24h, then stained with methylene blue to confirm whether the cells were dead or not. In principle, dead cells will be stained blue, while live cells will not be. In our methylene blue-staining experiments, after incubated with CdSe/ZnS-labeled CMC, almost all of the yeast cells were alive, consistent with the control yeast cells having not been incubated with any water-soluble QDs. However, after incubated with CdSe/ZnS-labeled mercaptoacetic acid, the number of dead yeast cells (stained cells) increased (Figure SI-3). These results suggest that the CdSe/ZnS-labeled CMC is scarcely cytotoxic, but CdSe/ZnS-labeled mercaptoacetic acid is cytotoxic.

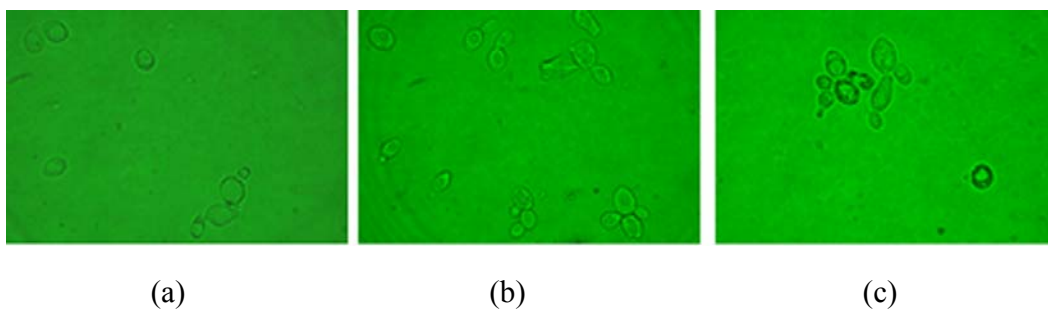


Figure SI-3 Methylene blue staining experiments. (a) Control: yeast cells only; (b) yeast cells having been incubated with CdSe/ZnS-labeled CMC; (c) yeast cells having been incubated with CdSe/ZnS-labeled mercaptoacetic acid.

Colony counting experiments:

Yeast cells were centrifugated at 8000rpm for 5 min and washed with 0.85% NaCl solution, then incubated with CdSe/ZnS-labeled CMC and CdSe/ZnS-labeled mercaptoacetic acid, respectively. Following 1 h incubation at 30 °C, yeast cells were diluted and spread on YPD agar plates. The colonies were countable in 2-3 days. The results from colony counting experiments also confirm that the CdSe/ZnS-labeled CMC is scarcely cytotoxic, and CdSe/ZnS-labeled mercaptoacetic acid is cytotoxic (Figure SI-4). Fewer colonies than those in the control experiment were found in the sample pretreated with CdSe/ZnS-labeled mercaptoacetic acid. However, more colonies than those in the control were observed in the sample pretreated with CdSe/ZnS-labeled CMC, probably due to CMC as a nutritional ingredient.

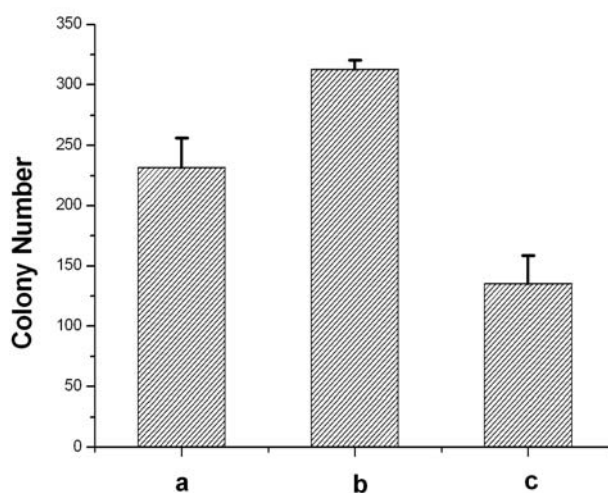


Figure SI-4. Colony counting of live cells. (a) Control: yeast cells only; (b) yeast cells pretreated with CdSe/ZnS-labeled CMC for 1 h; (c) yeast cells pretreated with CdSe/ZnS-labeled mercaptoacetic acid for 1 h.

Cell concentration (biomass) experiments:

Cell concentration determination results also strongly support the conclusion of CdSe/ZnS-labeled CMC scarcely having cytotoxicity and CdSe/ZnS-labeled mercaptoacetic acid with cytotoxicity. Yeast cells were co-cultivated with CdSe/ZnS-labeled CMC and CdSe/ZnS-labeled mercaptoacetic acid for 18 h, respectively. Then, cell solutions were transferred to 96-well plates and the responding optical density values at 630 nm were recorded. Growth curves of yeast cells for different samples were recorded. The most obvious difference in the growth was found after 18 h cultivation. Figure SI-5 suggests an inhibited growth of the cells when co-cultivated with CdSe/ZnS-labeled mercaptoacetic acid. However, the cell concentration was high for the yeast cells co-cultivated with CdSe/ZnS-labeled CMC.

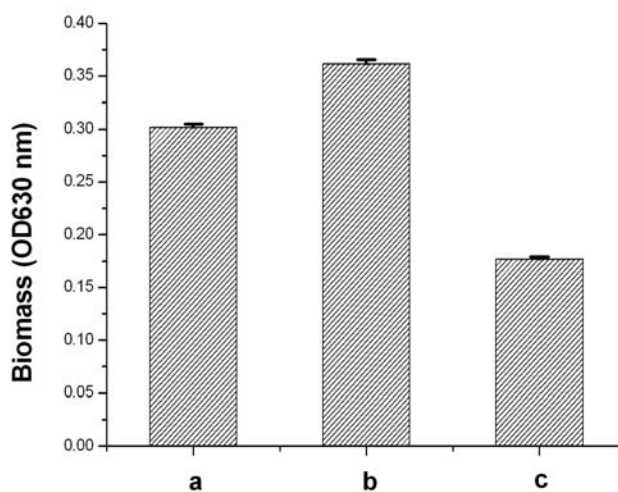


Figure SI-5. Cell concentrations under different cultivation conditions. (a) Control: yeast cells only; (b) yeast cells co-cultivated with CdSe/ZnS-labeled CMC; (c) yeast cells co-cultivated with CdSe/ZnS-labeled mercaptoacetic acid.

Synthesis of CdSe/ZnS:

CdSe/ZnS were prepared (Chinese Patent No. ZL 02 1 39152.1, 2002) as follows¹: (1) Nearly monodisperse CdSe QDs were synthesized according to the scheme reported by Peng² in flask A; a portion of product was taken out and was reheated to 200 °C. (2) A precursor of S was prepared by dissolving 65 μL of hexamethyldisilathiane in 2.5mL of trioctylphosphine and 1mL of dioctylamine in an inert atmosphere glovebox. The precursor of Zn was obtained by mixing 0.0322g of Zn(Ac)₂, 2.65g of trioctylphosphine oxide, and 2.45g of hexadecylamine in flask B, hested to 200 °C, and kept for at least 30 min. (3) These two precursor solutions were transferred dropwise to the vigorously stirring reaction mixture in flask A via a funnel over a period of 10-20 min. Then the mixture was cooled to 90 °C, stirred for an hour, and cooled to room temperature. Finally the product was recovered by precipitating with methanol and redispersed in hexane.

1. H.-Y. Xie, J.-G. Liang, Y. Liu, Z.-L. Zhang, D.-W. Pang, Z.-K. He, Z.-X. Lu and W.-H. Huang, *Journal of Nanoscience and Nanotechnology*, 2005, **5**, 880.
2. L. Qu and X. Peng, *J. Am. Chem. Soc.* 2002, **124**, 2049.

Preparation of carboxymethyl chitosan (CMC):

Carboxymethyl chitosan was prepared according literature¹. Chitosan suspended in 50% (wt.%) NaOH solution was kept in a refrigerator at -18°C for alkalization. Alkali chitosan and isopropyl alcohol (10.0ml) were added into a 100ml reactor and stirred for 1h at 40°C, then 10.0ml chloroacetic acid was added dropwise and refluxed for 24h at 60°C. After dialysed in deionized water for 2 days the resultant solution was filtered, repeatedly washed with acetone and alcohol, then dried under vacuum at 60°C for 48h to obtain CMC.

1. L. Chen, Z. Tian, Y. Du, *Biomaterials*, 2004, **25**, 3725