Supporting Information for

A universal fluorometric assay strategy for glycosidases based on functional carbon quantum dots: β-galactosidase activity detection in vitro and in living cells

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1. Figure S1. TEM image (A) and size distribution in diameter (B) of β -CD-CQDs nanoprobe, and TEM image (C) and size distribution in diameter (D) of pure CQDs.

2. Figure S2. XPS wide spectra of pure CQDs (A) and β -CD-CQDs nanoprobe (B).

3. Figure S3. UV-visible spectra of CQDs and β -CD-CQDs.

4. Figure S4. (A) The fluorescence spectra of CQDs as a function of *p*-NP concentration in the range of 0.0 - 52.0 mM. (B) Linear fitting curve between quenching efficiency I₀/I and the concentration of *p*-NP according to Stern-Volmer equation.

5. Figure S5. (A) Comparison of time-resolved fluorescence decay curves of CQDs in the presence of varying amount of *p*-NP. (B) Linear fitting curve between τ_0/τ and the concentration of *p*-NP according to Stern-Volmer equation. The lifetime of CQDs decreases as the addition of *p*-NP.

6. Figure S6 Linear fitting curve between quenching efficiency I_0/I for β -CD-CQDs and the concentration of *p*-NP according to Perrin's model.

7. Figure S7. (A) Comparison of time-resolved fluorescence decay curves of

 β -CD-CQDs in the presence of varying amount of *p*-NP. (B) The relationship between τ_0/τ and the concentration of *p*-NP. The lifetime of β -CD-CQDs is not changed as the addition of *p*-NP.

8. Figure S8 Comparison between the UV-visible spectrum of *p*-NP and the fluorescence emission spectrum of β -CD-CQDs nanoprobe.

9. Figure S9 The fluorescence intensity of β -CD-CQDs nanoprobe as a function of incubation time in the presence of NPGal (200.0 μ M) and varying level of β -Gal (50.0, 100.0. 150.0 and 200.0 U/L).

10. Figure S10 (A) Fluorescence spectra of the mixture containing β -CD-CQDs nanoprobe and NPGal (150.0 μ M) as increasing level of β -Gal from 0.0 to 100.0 U/L. (B) The fluorescence intensity of the nanoprobe versus β -Gal activity. Inset: the fitting curve between fluorescence intensity and β -Gal level.

11. Figure S11 (A) Fluorescence spectra of the mixture containing β -CD-CQDs nanoprobe and NPGal (300.0 μ M) as increasing level of β -Gal from 0.0 to 100.0 U/L. (B) The fluorescence intensity of the nanoprobe versus β -Gal activity. Inset: the fitting curve between fluorescence intensity and β -Gal level.

12. Figure S12 (A) Fluorescence spectra of the mixture containing β -CD-CQDs nanoprobe, NPGal (200.0 μ M) and β -Gal (100.0 U/L) in the presence of different concentrations of D-galactal (from bottom to top): 0.0, 50.0, 100.0, 200.0, 300.0, 400.0, 500.0 and 600.0 μ M, respectively. (B) The fitting curve between inhibition efficiency and concentration of D-galactal.

13. Figure S13 Effect of β -CD-CQDs with various concentrations (10.0 – 200.0 µg/mL) on the OVCAR3 cells.



Figure S1. TEM image (A) and size distribution in diameter (B) of β -CD-CQDs nanoprobe, and TEM image (C) and size distribution in diameter (D) of pure CQDs.



Figure S2. XPS wide spectra of pure CQDs (A) and β -CD-CQDs nanoprobe (B).



Figure S3. UV-visible spectra of CQDs and β -CD-CQDs nanoprobe.



Figure S4. (A) The fluorescence spectra of CQDs as a function of *p*-NP concentration in the range of 0.0 - 52.0 mM. (B) Linear fitting curve between quenching efficiency I₀/I and the concentration of *p*-NP according to Stern-Volmer equation.



Figure S5. (A) Comparison of time-resolved fluorescence decay curves of CQDs in the presence of varying amount of *p*-NP. (B) Linear fitting curve between τ_0/τ and the concentration of *p*-NP according to Stern-Volmer equation. The lifetime of CQDs decreases as the addition of *p*-NP.



Figure S6 Linear fitting curve between quenching efficiency I_0/I for β -CD-CQDs and the concentration of *p*-NP according to Perrin's model.



Figure S7. (A) Comparison of time-resolved fluorescence decay curves of β -CD-CQDs in the presence of varying amount of *p*-NP. (B) The relationship between τ_0/τ and the concentration of *p*-NP. The lifetime of β -CD-CQDs is not changed as the addition of *p*-NP.



Figure S8 Comparison between the UV-visible spectrum of *p*-NP and the fluorescence emission spectrum of β -CD-CQDs nanoprobe in PBS solution (pH 7.4).



Figure S9 The fluorescence intensity of β -CD-CQDs nanoprobe as a function of incubation time in the presence of NPGal (200.0 μ M) and varying level of β -Gal (50.0, 100.0. 150.0 and 200.0 U/L).



Figure S10 (A) Fluorescence spectra of the mixture containing β -CD-CQDs nanoprobe and NPGal (150.0 μ M) as increasing level of β -Gal from 0.0 to 100.0 U/L. (B) The fluorescence intensity of the nanoprobe versus β -Gal activity. Inset: the fitting curve between fluorescence intensity and β -Gal level. The detection limit was calculated as 0.6 U/L, and the linear range is 2.0 – 70.0 U/L.



Figure S11 (A) Fluorescence spectra of the mixture containing β -CD-CQDs nanoprobe and NPGal (300.0 μ M) as increasing level of β -Gal from 0.0 to 100.0 U/L. (B) The fluorescence intensity of the nanoprobe versus β -Gal activity. Inset: the fitting curve between fluorescence intensity and β -Gal level. The detection limit was calculated as 0.7 U/L, and the linear range is 2.3 – 70.0 U/L.



Figure S12 (A) Fluorescence spectra of the mixture containing β -CD-CQDs nanoprobe, NPGal (200.0 μ M) and β -Gal (100.0 U/L) in the presence of different concentrations of D-galactal (from bottom to top): 0.0, 50.0, 100.0, 200.0, 300.0, 400.0, 500.0 and 600.0 μ M, respectively. (B) The fitting curve between inhibition efficiency and concentration of D-galactal.



Figure S13 Effect of β -CD-CQDs with various concentrations (10.0 – 200.0 µg/mL) on the OVCAR3 cells. The viability of cells without is defined as 100%. The results are the mean \pm standard deviation of five separate measurements.