Electronic Supporting Information

Rapid fluorescent detection of neurogenin 3 by CdTe quantum dot aggregation

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1. Experimental section

1.1. Reagents and Apparatus

The synthetic peptide fragment of ngn3 (SKQRRSRRKKANDRERNRMH) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The monoclonal anti-ngn3 antibody was obtained from Leagene Co., Ltd. (Beijing, China). The synthetic peptide
fragment of ngn1 (AQDDEQERRRRRGRTR) was obtained from Saierbio Co., Ltd. (Tianjin, China). Sodium borohydride (NaBH$_4$) and DL-mercaptosuccinic acid (MSA) were purchased from Acros Organics (USA). Sodium tellurite (Na$_2$TeO$_3$) and Cadmium chloride (CdCl$_2$) was brought from Sigma-Aldrich Co. (USA). All the other chemicals were analytical grade and used without further purification. Phosphate buffer solutions (PBS) with varied pH value were prepared by varying the ratio of 0.01 M Na$_2$HPO$_4$ to 0.01 M NaH$_2$PO$_4$. Milli-Q water (18.2 MΩcm) was used throughout the experiment. Unless specified, the experiment was conducted at ambient temperature (20 ± 1 °C). Fluorescence emission spectra were measured by a Pekin-Elmer LS 50B spectrometer at an excitation wavelength of 390 nm. UV–vis absorption spectra were recorded by a Cary 50 UV/vis spectrophotometer (Varian, USA). TEM images were obtained from a FEI Tecnai G$^2$F20 transmission electron microscope with an acceleration voltage of 200 kV. Dynamic light scattering (DLS) measurements were performed by a Malvern Zetasizer nano instrument (Malvern, UK).

1.2. Preparation of MSA modified CdTe QDs

CdTe QDs were prepared according to the reported method.$^1$ CdCl$_2$ (0.04 M, 4 mL) was added into 46 mL of water in a one-necked flask, and trisodium citrate dihydrate (0.1 g), Na$_2$TeO$_3$ (0.01 M, 2 mL), MSA (0.1 g), and NaBH$_4$ (50 mg in 1 mL H$_2$O) were added successively with stirring. After the addition of NaBH$_4$ for about 15 min, the flask was attached to a condenser and refluxed at 110 °C under open-air conditions for a time span. The resulting CdTe QDs were washed with ethanol by two cycles of centrifugation/wash
procedure. Finally, the purified CdTe QDs were dispersed in water and stored at 4 °C for further application.

1.3. Calculation of the size and concentration of CdTe QDs

By recording the absorption spectra of the above prepared CdTe QDs, the wavelength of the absorption peak (λ nm) was obtained as 620 nm. According to the method proposed by Peng’s group,\(^2\) the size of the QDs (D) can be calculated by the following empirical formula:

\[
D \text{ (nm)} = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + (1.0064)\lambda - (194.84)
\]

Therefore, the size of QDs is approximately 3.86 nm. The extinction coefficient of CdTe QDs can be obtained from the following equation:

\[
\varepsilon = 10043 (D)^{2.12}
\]

Finally, the concentration of the QDs (C) can be calculated upon the measurement of the absorbance of the QDs solution (A) using Lambert-Beer’s law:

\[
A = \varepsilon b C
\]

The resulting concentration of QDs was 5.2 µM.

1.4. Preparation of the QD-Ab conjugate

1 mL of diluted QDs solution (0.52 µM) was incubated with different concentrations of the anti-ngn3 antibody for 36 h at 4 °C in PBS media. We did not note any aggregation of QDs as examined by the fluorescence emission spectra and TEM. Before each spectral recording, the probe solutions were withdrawn from the refrigerator for at least 2 h to
ensure ambient temperature.

1.5. The detection of ngn3 by the QD-Ab probe

Different volumes of ngn3 (0.02 g/L) corresponding to different concentrations (0, 0.02, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2, and 1.4 µg/mL) were added into the QD-Ab probe solution ([QD] = 52 nM) before the fluorescence emission spectra were recorded. Each measurement was performed three times to evaluate the standard deviation.

1.6. Selectivity test

L-lysine (Lys, 6 µg/mL), L-arginine (Arg, 6 µg/mL), lysozyme (6 µg/mL), and a synthetic fragment of neurogenin1 (ngn1, AQDDEQERRRRGRTR, 6 µg/mL) were each added to the QD-Ab solution in the absence and presence of ngn3 (0.6 µg/mL), then the fluorescence intensity were measured. The results were obtained according to three parallel experiments.

1.7. Detection of ngn3 in human serum

Different volumes of ngn3 (0.02 g/L) corresponding to different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 µg/mL) were mixed with 10 µL human serum before they were added into the QD-Ab solution, and then the fluorescence emission spectra were recorded. Each measurement was performed three times to evaluate the standard deviation.
2. Supporting Figures

**Fig. S1** The pH-dependent fluorescence emission intensity at P1 as a function of ngn3 concentration. The concentration of antibody was 750 ng/mL, the incubation time was 1 min, and the spectra were measured at ambient temperature.

**Fig. S2** The optical spectra of bare QDs, QD-Ab conjugate, and mixture of QD-Ab and 0.6 µg/mL ngn3, respectively.
Table S1 Detection of ngn3 in diluted human serum under the optimal conditions

<table>
<thead>
<tr>
<th>The concentration of added ngn3 (µg/mL)</th>
<th>Mean found (µg/mL)</th>
<th>Mean recovery(^a) (%)</th>
<th>RSD(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.216</td>
<td>108</td>
<td>2.24</td>
</tr>
<tr>
<td>0.4</td>
<td>0.425</td>
<td>106</td>
<td>1.76</td>
</tr>
<tr>
<td>0.6</td>
<td>0.628</td>
<td>105</td>
<td>1.44</td>
</tr>
<tr>
<td>0.8</td>
<td>0.807</td>
<td>101</td>
<td>0.56</td>
</tr>
<tr>
<td>1.0</td>
<td>0.983</td>
<td>98</td>
<td>1.10</td>
</tr>
</tbody>
</table>

\(^a\) Mean recovery (%) = 100 × ([mean found] / [added]). \(^b\) Relative standard deviation of three determinations.

3. References
