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New method for in situ assay of α-glucosidase activity and the inhibitor screening through enzyme substrate mediated DNA hybridization chain reaction

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1 Experimental section

1.1 DNA sequences

Table S1 DNA sequences designed in the experiment

Name	Sequences
CPBA-IC	5'-TTAACCCACGCCGAATCCTAGACTCAAAGT-Amidophenylboronic acid-3'
H1	5'-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG-3'
H2	5'-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG-3'

1.2 Recovery experiment

For recovery experiment, α-Glu at different concentrations were added into serum to give samples. Enzyme concentrations in the obtained serum samples were determined by using the established method and recovery ratios were calculated.

2 Results and discussions

2.1 Optimization of experimental conditions

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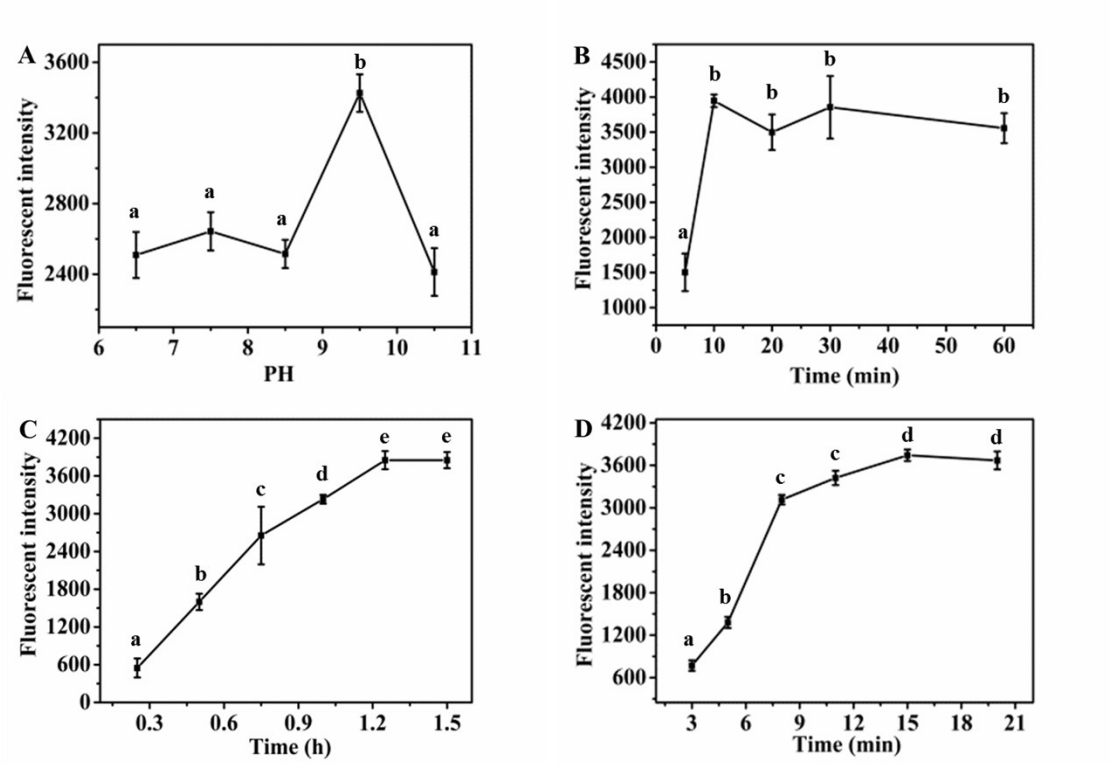

Figure S1 The fluorescence intensities versus (A) pH values for CPBA-IC binding with AMNSs-pAPG, the time for (B) CPBA-IC binding with pAPG/AMNSs, (C) HCR, and (D) SGI dying. Columns with different letters are significantly different ($p \leq 0.05$).

The extent of formation of boronate ester is crucial for HCR around AMNSs-pAPG and fluorescence signal output. To improve the performance of the established method, the experiment conditions including pH values and time for the binding of CPBA-IC with AMNSs-pAPG have been optimized. The optimal pH value and time are 9.4 and 8 min, respectively (Figure S1 (A) and (B)). Furthermore, time for HCR and SGI dying also influence the performance of the method. 1.2 h and 10 min have been separately chosen as optimal time for HCR and SGI dying in our study.

2.2 Recovery experiment

Table S2 α-Glu concentrations detected and the comparison with the given concentrations in serum samples.

Samples	α-Glu concentrations detected (U/mL)	Standard concentrations (U/mL)	Recovery ratio (%)	Relative error (%)
1	0.45	0.5	90.1	3.5
2	0.57	0.6	95.3	2.1
3	0.67	0.7	95.7	3.8
4	0.77	0.8	96.2	3.6
5	0.99	0.9	110.1	2.8
6	1.10	1.0	110.1	2.6

We further verify the applicability of the established method in complicated samples with the usage of serum as complex matrix. The different amounts of α-Glu were added into bovine serum and the concentrations were further analyzed through our method. As illustrated in Table S2, the recovery ratios are 90% ~ 110% with the relative error within 4%. These results well signify the constructed method can be applied for enzyme activity analysis in complex matrix.

2.3 Detection of inhibitors at cellular level

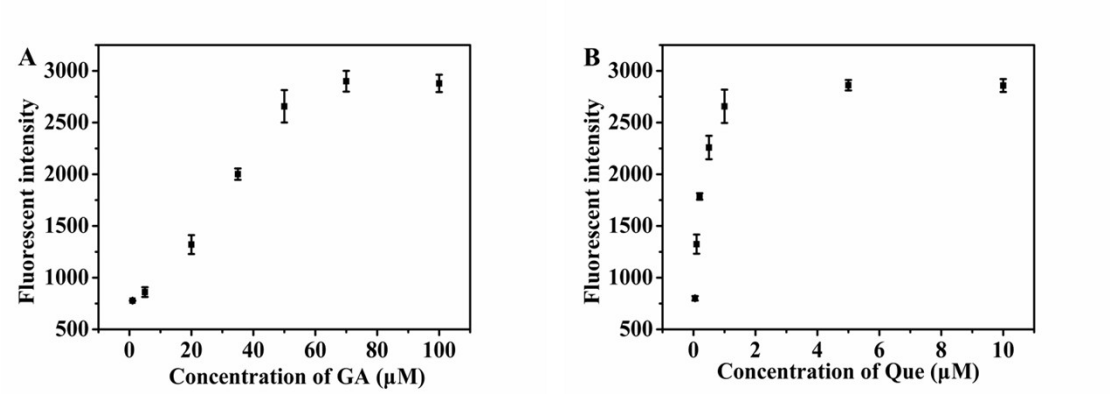

Figure S2 (A) Fluorescence intensities with the addition of various concentrations of GA (1, 5, 20, 35, 50, 70 and 100 μm) into Caco-2 cell. (B) Fluorescence intensities with the addition of various concentrations of Que (0.05, 0.1, 0.2, 0.5, 1, 5 and 10 μM) into Caco-2 cell.