Supporting information

A Novel Fluorescent Detection Strategy for Lysozyme in Tears Based on Glycoside Bond Hydrolysis

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Experiments conditions optimization of HS quenching NR

The conditions of heparin sodium (HS) quenching fluorescence of neutral red (NR) were investigated, including the pH value of BR buffer solution, the amount of buffer solution, the concentration of NR, and reaction time.

Initially, the pH of the BR buffer was evaluated. A series of BR buffer solutions with different pH (2-7) was added to the NR-HS system and reacted for some time. When the pH value is 3, the difference in fluorescence intensity between NR and the NR-HS system is the largest (Fig. S1A). Therefore, a BR buffer solution with pH 3 was selected as the optimal pH for the experiment.

Then, the influence of the dosage of BR buffer solution on the NR-HS system was studied. In the two groups, different amounts of BR buffer solution (pH=3) (30 μL, 40 μL, 50 μL, 60 μL, 70 μL) were added and reacted at room temperature. The fluorescence intensity ΔF of the system reached maximum when the amount of buffer solution was 50 μL (Fig. S1B). Therefore, 50 μL was selected as the optimal amount of buffer in the system.

To investigate the effect of NR concentration on NR-HS, different concentrations of NR (2 μM, 3 μM, 5 μM, 7.5 μM, 10 μM) were added to react with HS (Fig. S1C). With the increase of the NR concentration, ΔF first increased and then decreased. When the concentration of NR was 5 μM, the fluorescence changed the most. Therefore, 5 μM of NR solution was selected as the optimal concentration.

Lastly, the reaction time between HS and NR was analyzed. As shown in Fig. S1D, ΔF reached its maximum when the incubation time was 30 min. Therefore, 30 min reaction at room temperature (25 ℃) is selected as the best reaction time for HS detection.

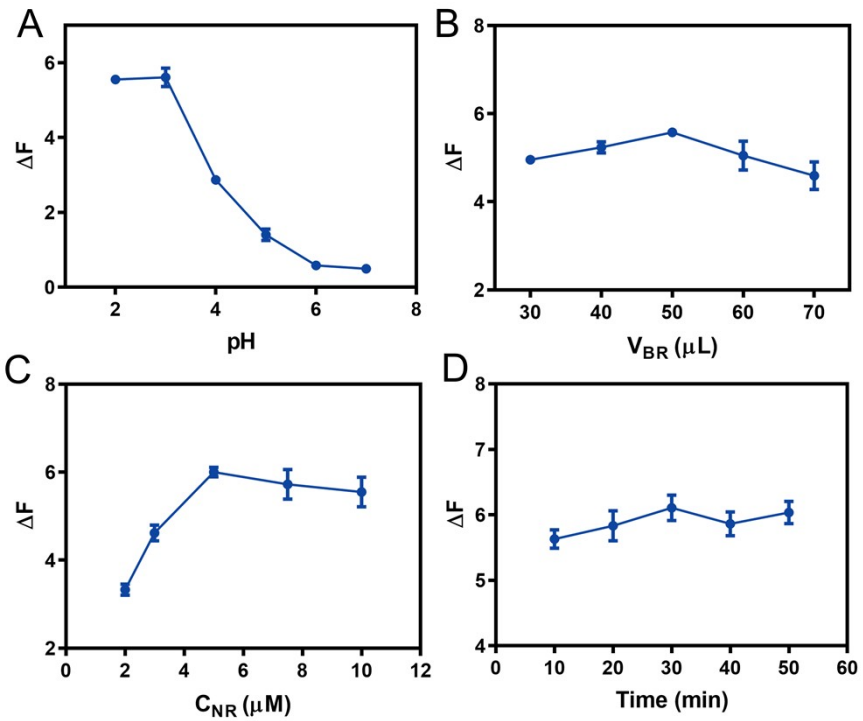

Fig. S1 Optimization of the conditions. The effects of (A), the pH value of the BR buffer solution, (B) the amount of BR buffer, (C) the NR concentration, and (D) the reaction time between HS and NR on the detection of LYZ.

Table S1. Fluorescence lifetime.

sample	A_1	τ_1	A_2	τ_2	A_3	τ_3	τ
NR	0.07221	1.84148	0.29528	1.84129	0.34402	1.84139	1.84 ns
NR/0.2 μg/mL HS	0.18643	1.7014	0.44907	1.70128	0.45032	1.70151	1.70 ns
NR/0.8 μg/mL HS	0.28805	1.81809	0.24653	1.81807	0.22106	1.81807	1.82 ns

Table S2. Comparison with other assays for detecting LYZ.

Sensors	Linear range	LOD	Detection time	Ref
Colorimetric method	100 nM-1 mM	60 nM	95 min	1
Plasmon resonance light-scattering	5-1600 nM	2.32 nM	90 min	2
Electrochemical detection	0-30 μM	862 nM	-	3
Silver nanocluster-based aptasensor	2-25 nM	5.6 nM	4 h	4
Fluorimetric method	0.5-10 μg/mL (35.6-713 nM)	0.42 μg/mL (29.9 nM)	30 min	This work

References

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