High-Resolution Colorimetric Detection of Lipase Activity
Based on Enzyme-Controlled Reshaping of Gold Nanorods

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1. Optimization of the assay condition

The experimental parameters including HAuCl₄ concentration, Tween 80 concentration, PB concentration pH and reaction time were optimized for further experiments. The blue shift of the longitudinal LSPR peak (Δλₖ) was selected as standard for the performance of Au deposition reaction.

The effect of HAuCl₄ concentration was first investigated and optimized. The Δλₖ increased with the increase of HAuCl₄ concentration and tended to level off after 20 mM, at the same time the longitudinal LSPR peak near 800nm disappeared at all and a strong Au nanocrystal plasmon resonance peak about 600nm was observed (Figure S1 a,b). This suggested that the Au atoms have deposited on the AuNRs to form the Au nanocrystals. A high concentration of Au ions can accelerate the reduction reaction,
resulting in a thicker Au shell on the surface of AuNRs, but it also caused loss of overall nanorod structure. Therefore a HAuCl$_4$ concentration at 20 mM was selected as optimal for gold reduction reaction.

Figure S1 c showed that the $\Delta \lambda_b$ increased with the increase of Tween concentration and stabilized at a concentration of 30% (v/v). Therefore a Tween 80 concentration of 30% (v/v) was adopted for following reactions. Investigations were carried out to optimize the concentration of phosphate buffer (PB) solution and the pH. As seen in the Figure S1 d, e, the maximum $\Delta \lambda_b$ occurred at 10 mM PB and the pH7.5, so these results were chosen as the optimal concentration and pH.

We also optimized the response time of the system. As is shown in figure S1 f, $\Delta \lambda_b$ increased with the increase of response time and stabilized at a time of 5 minutes. It indicates that this reaction only takes 5 minutes.
Figure S1. The experimental conditions for Au deposition reaction. The effects of Au-depositing AuNRs (a-b) Au concentration, (c) Tween concentration, (d) pH, (e) PB concentration and (f) reaction time. Error bars represent the standard deviation of three replicates.

2. The Zeta Potential of The AuNRs Modified with Poly (4-styrenesulfonic acid)

![Zeta Potential Chart]

Figure S2. The zeta potential of AuNRs-CTAB (light) and the zeta potential of AuNRs-PSS (right).

3. Absorbance of The AuNRs Modified with Poly (4-styrenesulfonic acid)

![Absorbance Chart]

Figure S3. The UV-vis absorbance spectra of the AuNRs-CTAB in MQ water (black), AuNRs-PSS in MQ water (red), AuNRs-CTAB in PB water (blue) and AuNRs-PSS in PB (green).

4. pH-stat method
Our research (Δλ_b) 45 82 96 105

Table S1. pH-stat method

5. The comparison of Tweens’ type

We used four types of Tween to test their effect on the LSPR of gold nanorods. As is shown in figure S4, Tween 80 has the greatest influence on the Δλ_b of gold nanorods. So we choose the Tween 80 as the best.

Figure S4. The comparison of four Tween (Tween 20, Tween 40, Tween 60 and Tween 80)