Optimization of assay conditions

Impact of reaction time

Kinetics, as a basic aspect of chemical processes, provides a way to better understand the rate, equilibrium and mechanism of chemical processes. Conclusion of kinetics study should reveal the optimal time of measurement to obtain the constant signal ratio. Therefore, the aggregation kinetics of unmodified Au NPs in the presence of different nereistoxin concentrations was well studied by measuring the temporal evolution of $A_{660}/A_{519}$ at an interval of 1 min with time up to 10 min. Four typical concentrations of nereistoxin (100, 200, 300 and 400 $\mu$g kg$^{-1}$) were used to evaluate the aggregation kinetics (Fig. S1 A). It can be seen that $A_{660}/A_{519}$ increases very rapidly in the first 1 min and slight growth to the maximum during 3 min, then almost remains at a small constant value, which means the aggregation and spectral variation of Au NPs could almost complete within 3 min. In addition, the sedimentation was obviously visible after 12 hr. Although the signal ratio was not affected by the sedimentation, the loss of extinction made the color of the colloid less distinguishable. Therefore, 3 min is selected as the optimum measurement time for both visual and UV-visible spectroscopic measurement.

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Impact of media pH

Media pH could influence the performance of colorimetric sensor [1], especially for Au NPs-based-colorimetric assay because pH of the solution has a direct effect on the stability of Au NPs solution. The effect of media pH on the sensibility of present assay was investigated from pH 3.0 to 9.0. The strong acidic media and strong basic media (pH < 3.0 and pH > 9.0) were excluded because citrate modified Au NPs were not stable at those rigorous conditions. The change of the absorption ratio ($A_{660}/A_{519}$) versus different pH values was recorded in Fig. S1 B. It could be easily observed that the absorption ratio ($A_{660}/A_{519}$) increased with the decreasing of media pH level, and the highest absorption ratio ($A_{660}/A_{519}$) was obtained at pH 3–4. It could be attributed to the higher concentration of H$^+$ promoting the protonation of amine group and subsequently enhancing the electrostatic attraction between Au NPs and nereistoxin [2]. Consequently, pH 4.0 of media is chosen for the colorimetric assay.

Impact of salt

As the force of electrostatic repulsion diminishes significantly at high salt concentrations, citrate-capped Au NPs undergo aggregation; salts are often used to modulate Au NP stability and aggregation [3]. However, Au NP cannot assemble when insufficient salt is present. For that reason, the proper salt type and concentration should be used to achieve optimal assay performance. NaHSO$_4$ is well-known to destabilize the Au NPs [4], and as we presented above, acidic media could improve detection sensitivity of our system, the acidity of HSO$_4^-$ salt would further promote destabilization of Au NPs [5]. Thus, Au NPs were premixed with different concentrations of NaHSO$_4$ and the resulting mixtures were used to detect nereistoxin. As shown in Fig. S1 C, different sensitivities and sensing ranges were obtained for the Au NPs solution containing different amounts of NaHSO$_4$. For example, $A_{660}/A_{519}$ is 0.089, 0.109 and 0.179 at 50 μg kg$^{-1}$ for Au NPs with 0.3, 0.6 and 0.9 mmol L$^{-1}$ NaHSO$_4$, respectively, whereas $A_{660}/A_{519}$ is 0.080 for Au NPs
without NaHSO₄. It indicates that the developed colorimetric assay exhibited high sensitivity for nereistoxin detection when 0.9 mM of NaHSO₄ is added. Besides, when more than 0.9 mM of NaHSO₄ was contained in Au NPs solution, the wine-red color of Au NPs could not be kept, which would narrow the range of color change. So, 0.9 mmol L⁻¹ of NaHSO₄ was chosen to obtain the high sensitivity and wide sensing range.

Fig. S1. (A) Plots of A₆₆₀/A₅₁₉ versus time at different nereistoxin concentrations; (B) Plots of A₆₆₀/A₅₁₉ of Au NPs after addition of 250 μg kg⁻¹ nereistoxin with different pH; (C) Plots of A₆₆₀/A₅₁₉ versus different nereistoxin concentrations in the present of NaHSO₄.
References


