Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supplementary Information

A DNAzyme-mediated signal amplification biosensor for ultrasensitive detection of lead ions based on SERS tags

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Contents:

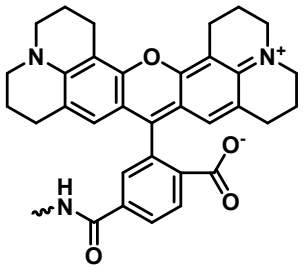
S1 Experimental section

S1.1 Chemicals

Table S1. DNA Sequences used in this work.

Note	Sequence
17E-2(9+9) DNAzyme	5'-CAT CTC TTC TCC GAG CCG GTC GAA ATA GTT GGT- 3'
Hairpin DNA	5'-NH_2-T15-GTG TTC AGT GTG TGG TGC ACC AAC TAT /RA/ GGA AGA GAT GGA AGT AGA CCC TGA ACA G- 3'
Capture DNA	5'-CCC AAC CCG CCC TAC CCT TTT TT -SH - 3'
Rox-DNA	5'-ROX-TTT TTT CCT AGC GAC -SH- 3'
Padlock DNA	5'-ACT GAA CAC CCC AAC CCG CCC TAC CCA AAA CCC AAC CCG CCC TAC CCG CAC CAC AC- 3'

The structure of Rox



S2. Optimization of the experimental conditions

S2.1 Optimization of system pH and RCA reaction temperature

The effect of system pH and reaction temperature on the variation of SERS intensity generated by the system was explored at a lead ion concentration of 1×10^{-14} M and other experimental conditions were the same. In Fig. S1A, the smallest Raman intensity is shown when the pH is 6.0, the Raman intensity at pH 7.0 increases

compared to pH 6.0 and the SERS intensity reached a maximum at pH 7.4. Therefore, pH 7.4 was chosen as the optimum condition. Fig. S1B shows the variation of SERS intensity produced by the system at different RCA reaction temperatures. From the graph, it can be seen that the system produced the maximum Raman signal intensity at 37°C, so 37°C was selected as the experimental optimum temperature.

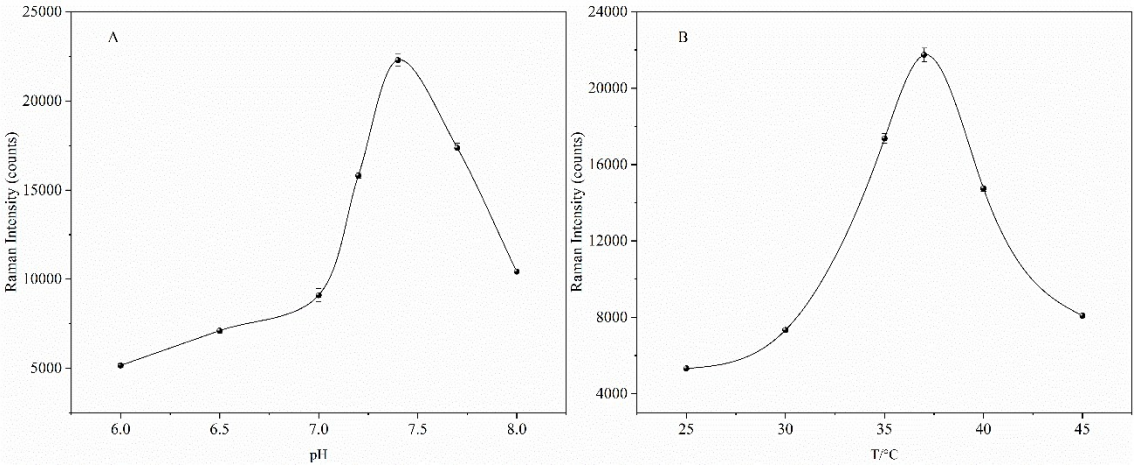


Fig. S1 (A) Effect of pH on the SERS intensity for 1×10^{-14} M Pb^{2+} (B) Effect of the reaction temperature on the SERS intensity for 1×10^{-14}M Pb^{2+}.

S2.2 Optimization of the amounts of Phi29 DNA polymerase and T4 Ligase

Based on the fact that all other conditions were the same, the amounts of T4 ligase and phi29 DNA polymerase were adjusted in order to achieve the best results from the experiment. As shown in Figure S2A, the SERS intensity increased rapidly when the amount of phi29 DNA polymerase was increased from 0.2 to 0.5 $U\mu L^{-1}$. However, after 0.5 $U\mu L^{-1}$, the SERS intensity showed a slight downward trend. Therefore, 0.5 $U\mu L^{-1}$ of phi29 DNA polymerase is the optimum concentration. Similarly, Figure S2B shows the effect of T4 ligase concentration. As can be seen from the graph 5 $U\mu L^{-1}$ of T4 ligase was chosen as the optimal amount.

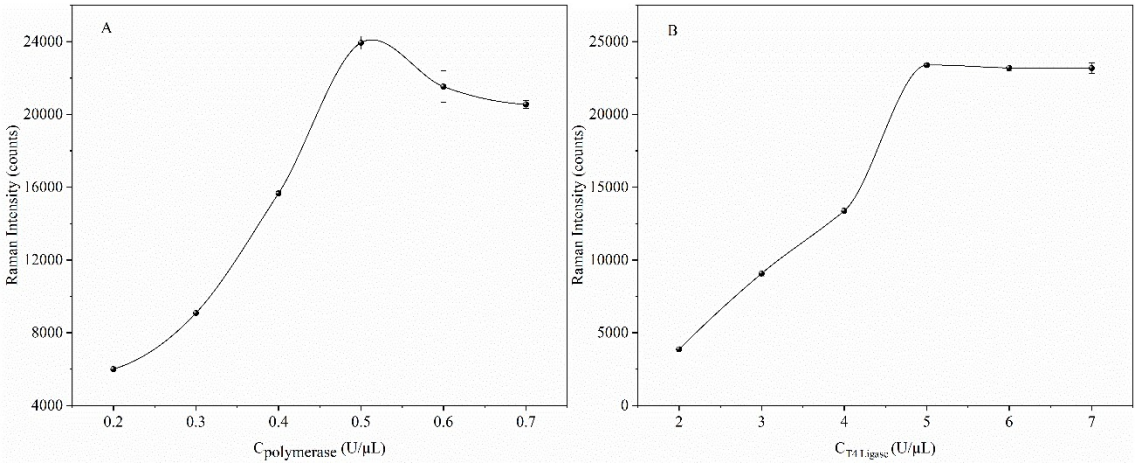


Fig. S2 (A) Effect of the amounts of Phi29 DNA polymerase on the SERS intensity for 1×10^{-14} M Pb^{2+} (B) Effect of the amounts of T4 Ligase on the SERS intensity for 1×10^{-14} M Pb^{2+}.

S2.3 Optimization results for recycling reaction time

Fig. S3 shows the variation in SERS intensity produced by recycling reaction at different time intervals. The results show that the SERS intensity enhanced with increasing reaction time, with a significant increase in Raman signal occurring at 120 min. And when the time exceeds 120 min the Raman intensity tends to a steady state. Therefore, a recycling reaction time of 2 hour was chosen.

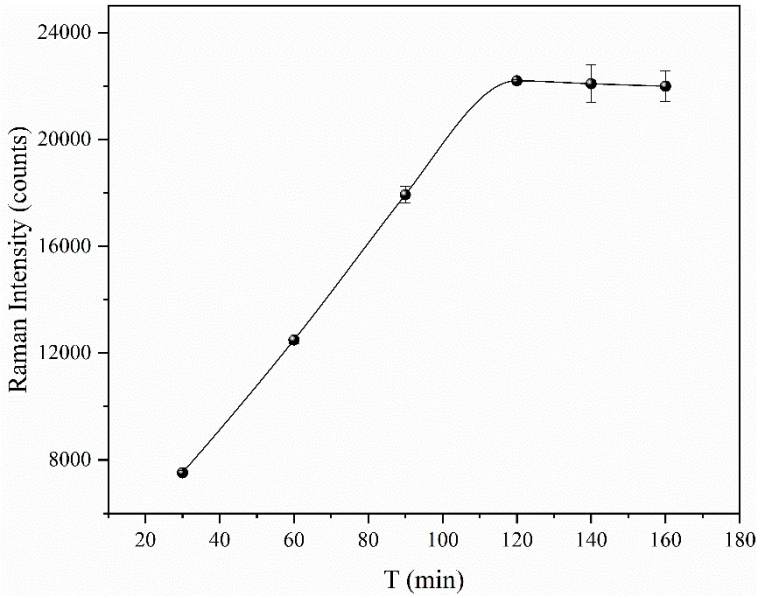


Fig. S3 Effect of recycling reaction time on Raman intensity

S2.4 Optimization of RCA reaction time

This is shown in Figure S4. The Raman intensity increased rapidly with RCA reaction time from 20 min to 1 h which phi29 polymerase and substrate all in the optimal concentrations. And when the time exceeds 1 hour the Raman intensity tends to a slight downward trend. Therefore, 1 h was chosen as the optimal time for the RCA reaction. The above results suggest that a reaction time of 1 h is suitable for the RCA reaction and for subsequent work.

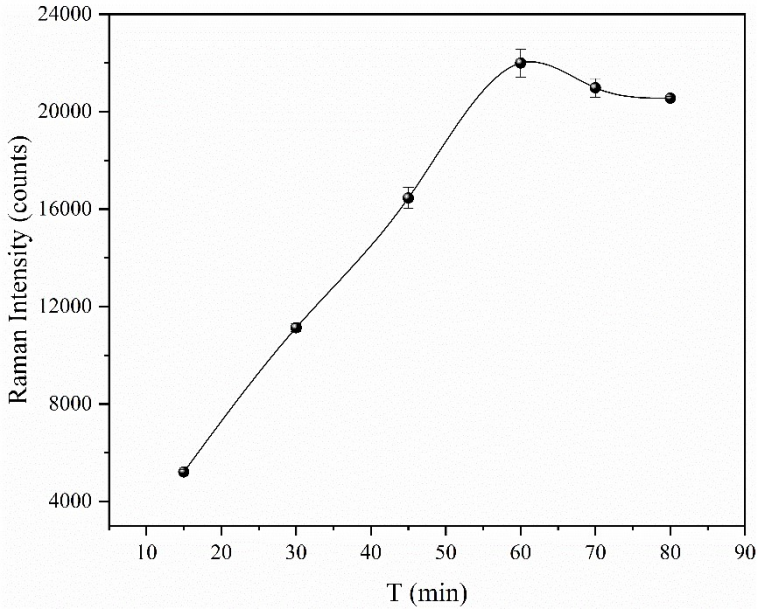


Fig. S4 Effect of the RCA reaction time on the SERS intensity

S3. Characterization of Magnetic bead (MB)

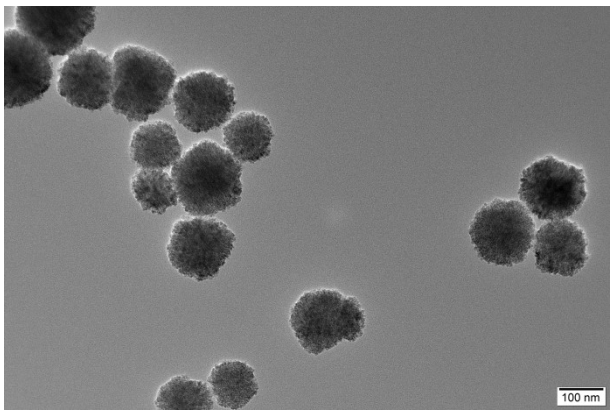


Fig. S5 TEM image of Magnetic Beads