

## Supporting Information

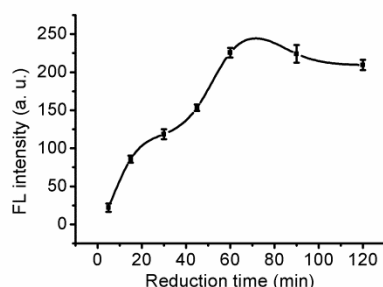
# Enzymatic Synthesis of DNA-templated Alloy Nanocluster and Its Application in Fluorescence Immunoassay

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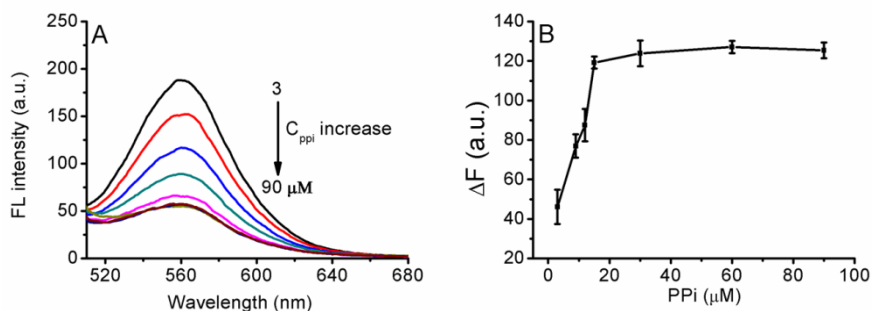
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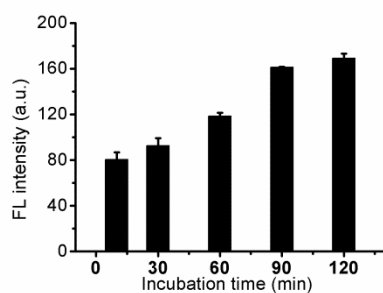
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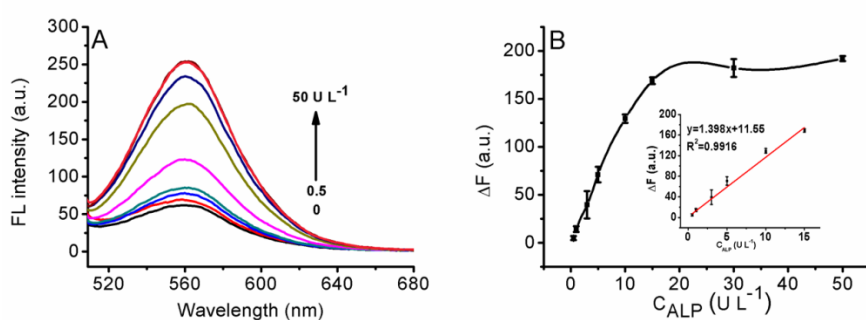
**Figure S1.** The fluorescence of the Cu/Ag NCs with various reduction time.



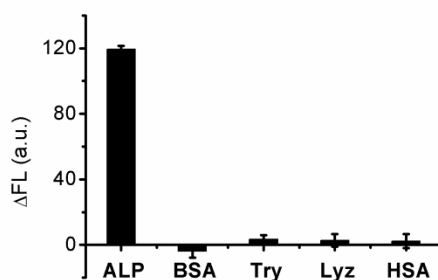
**Figure S2.** (A) Typical fluorescence spectral responses of obtained Cu/Ag NCs under various PPI concentrations at a fixed reduction time of 60 min. (B) Plot of peak intensity of obtained Cu/Ag NCs with respect to PPI concentrations.



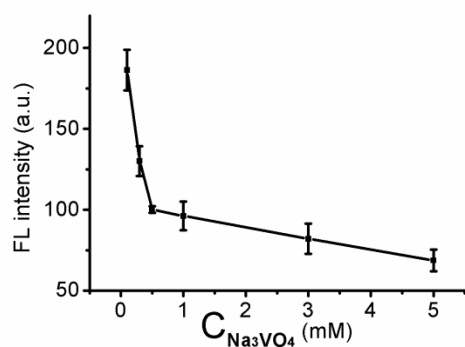
**Figure S3** The effect of incubation time on the assay performance. The concentration of ALP was  $10 \text{ U L}^{-1}$ .



**Figure S4** (A) Fluorescence spectra of obtained Cu/Ag NCs in the presence of ALP with different concentrations. (B) Calibration curve for ALP detection. Inset: the linearity of peak intensity with respect to lower ALP concentrations. Error bars were estimated from three replicate measurements.



**Figure S5** The selectivity of the Cu/Ag NCs based method for ALP assay. The concentration was  $20 \text{ pM}$  for ALP and  $2 \text{ nM}$  for each other interfering proteins.



**Figure S6** The inhibition effect of  $Na_3VO_4$  on ALP activity.

**Table S1.** Comparison of different assays for ALP detection.

| Technique   | Analytical Method                   | Dynamic Range  | LOD     | Time of Incubation |
|---|-------------------------------------|----------------|---------|--------------------|
| Fluorescence <sup>1</sup>                           | CdTe/CdS QDs                        | 3~1000 U/L     | 3 U/L   | 10 min             |
| Fluorescence Resonance Energy Transfer <sup>2</sup> | $\beta$ -cyclodextrin-modified QDs  | 0~800 U/L      | 10 U/L  | 10 min             |
| Fluorescence <sup>3</sup>                           | Enzymatic Formation of QDs          | 0~800 U/L      | 0.5 U/L | 50 min             |
| Colorimetry <sup>4</sup>                            | Gold Nanoparticle                   | 32~100000 U/L  | 3 U/L   | 65 min             |
| Fluorescence <sup>5</sup>                           | Carbon QDs                          | 16.7~782.6 U/L | 1.1 U/L | 30 min             |
| Colorimetry <sup>6</sup>                            | $Cu^{2+}$ -catalyzed ABTS– $H_2O_2$ | 30~400 U/L     | 27 U/L  | 30 min             |
| Fluorescence (This work)                            | Enzymatic Formation of Nanocluster  | 0.5~15 U/L     | 0.3 U/L | 60 min             |

**Table S2.** Determination of AFP added in normal human serum with this proposed immune strategy.

| Samples | Add<br>(ng mL <sup>-1</sup> ) | Found<br>(ng mL <sup>-1</sup> ) | Recovery<br>(%) |
|---------|-------------------------------|---------------------------------|-----------------|
| 1       | 12                            | 12.71                           | 105.9           |
| 2       | 30                            | 29.37                           | 97.9            |
| 3       | 45                            | 40.39                           | 89.8            |
| 4       | 50                            | 51.32                           | 102.6           |

## Reference

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