

Supplementary Information for

Rapid determination of vitamin B₁₂ (cobalamin) based on silver nanoclusters capped by polyethyleneimine with different molecular weights and terminal groups

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Experimental section

Chemicals and reagents

Silver nitrate (AgNO_3), hyperbranched polyethylenimine (PEI) (M_w 600, 10000, 70000 and terminated by ethylenediamine (EDA)), agarose, formaldehyde (35 wt %), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), vitamin B_{12} (VB_{12}), nicotinic acid (VB_3), (+)-pantothenic acid calcium salt hydrate (VB_5), D-biotin (VB_7), ascorbic acid (VC), ergocalciferol (VD_2), cholecalciferol (VD_3), vitamin K_1 (VK_1) and menadione (VK_3) were purchased from Aladdin Reagent Co., Ltd. (China). Thiamine hydrochloride (VB_1), pyridoxine hydrochloride (VB_6), (\pm)- α -tocopherol (VE) and PEI terminated by ethoxylated groups (EOD) were obtained from Sigma-aldrich (China). The VB_{12} injections (0.50 mg/mL) and tablets (25 μg) were bought from a local drug store. All reagents used were of at least analytical reagent grade. The water used was purified through a millipore system.

Apparatus

All fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. The photomultiplier tube (PMT) voltage was set at 400 V and the slit width was 10 and 10 nm for excitation and emission respectively. The ultraviolet-visible (UV-vis) absorption spectra were obtained on a Cary 300 Bio UV-vis spectrophotometer. Transmission electron microscopy (TEM) and high-resolution transmission electron microscopy (HRTEM) of Ag nanoclusters (Ag NCs) were carried out on a JEM-2100F electron microscope. The X-ray

photoelectron spectroscopy (XPS) measurements were performed on a Thermo Scientific Escalab 250 photoelectron spectrometer.

Synthesis of Ag NCs

In previous literature, Ag NCs capped by PEI (Ag NC-PEIs) with different molecular weights were successfully synthesized.^{1,2} Herein, we further synthesized two Ag NCs capped by PEI with different terminal groups named as ethylenediamine (EDA) and ethoxylated groups (EOD). The synthetic procedures were similar to that reported before.^{1,2} Firstly, PEI was dissolved in deionized water by stirring for 2 min, and then with the addition of a certain amount of AgNO₃, the solution was stirred for 2 min. Last, the mixtures were reduced with freshly prepared formaldehyde solution by vigorously shaking for approximately 2 min. The color of the mixture changed from colorless to yellow. The final solution was stored in the dark at ambient environment for at least 4 days before its further application. The detailed amounts of AgNO₃, various PEIs, and formaldehyde were shown in Table S1.

VB₁₂ detection

In the quenching studies, different concentrations of VB₁₂ solutions were prepared before detection. First of all, 0.15 μL of as-prepare Ag NC-PEIs, 200 μL of HEPES (pH=7.81) and various concentrations of VB₁₂ were mixed together. Then the mixed solution was diluted to 1 mL with deionized water. The working solution was well shaken and allowed to stand for 5 min before fluorescence measurements. The effect of probe concentration, pH, reaction time and temperature were investigated by

adding 20 μM VB_{12} using Ag NC-PEI-EDA as probe. Finally, the fluorescence intensity of Ag NC-PEIs in the absence of VB_{12} (F_0) and in the presence of VB_{12} (F) were recorded, respectively, and the fluorescence quenching efficiency was expressed as $\Delta F = (F_0 - F) / F_0$.

Selectivity

The process of selectivity was similar to the aforementioned assay of VB_{12} . Briefly, 0.15 μL of as-prepare Ag NC-PEIs, 200 μL of HEPES (pH=7.81) and the various vitamins were mixed together, and then these solutions were diluted to 1 mL with deionized water. Furthermore, due to the different solubility of these vitamins, ethanol was selected to dissolve VD_2 , VD_3 , VE and VK_3 , whereas VK_1 was prepared in dimethyl sulfoxide (DMSO).

Analysis of real samples

In this assay, we selected VB_{12} injections and tablets samples to evaluate the sensor performance. The injections were diluted 15 times for detection. The tablets should be incubated at 100 $^\circ\text{C}$ for two hours to remove sugar; next, they were powdered and dissolved in deionized water; then the mixtures were centrifugated to obtain supernatant for determination. Other steps were completed as described above.

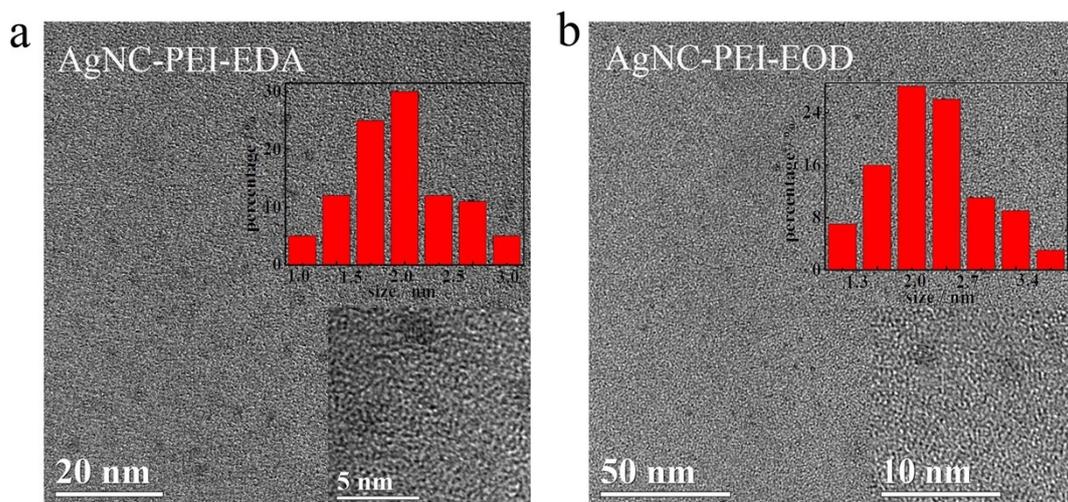


Fig. S1 TEM and HRTEM images of Ag NC-PEI-EDA (a) and Ag NC-PEI-EOD (b).

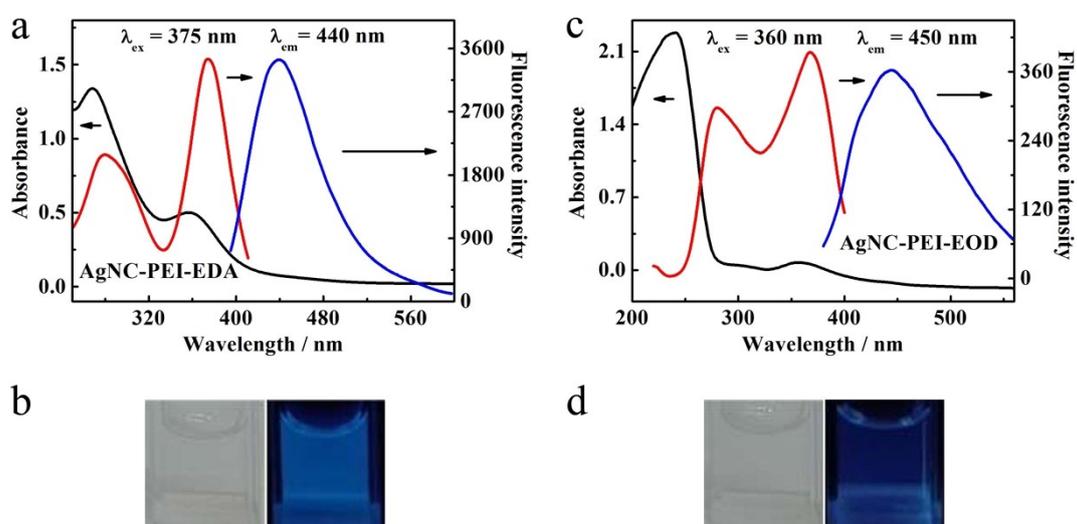


Fig. S2 UV-vis absorption spectrum, excitation spectrum and emission spectrum of Ag NC-PEI-EDA (a) and Ag NC-PEI-EOD (c). The corresponding photographs of Ag NC-PEI-EDA (b) and Ag NC-PEI-EOD (d) under visible light and UV light at 365 nm. The concentration of Ag NC-PEI-EDA was 5 μ L/mL for fluorescence spectra and UV-vis absorption, and for Ag NC-PEI-EOD the concentration was 500 μ L/mL.

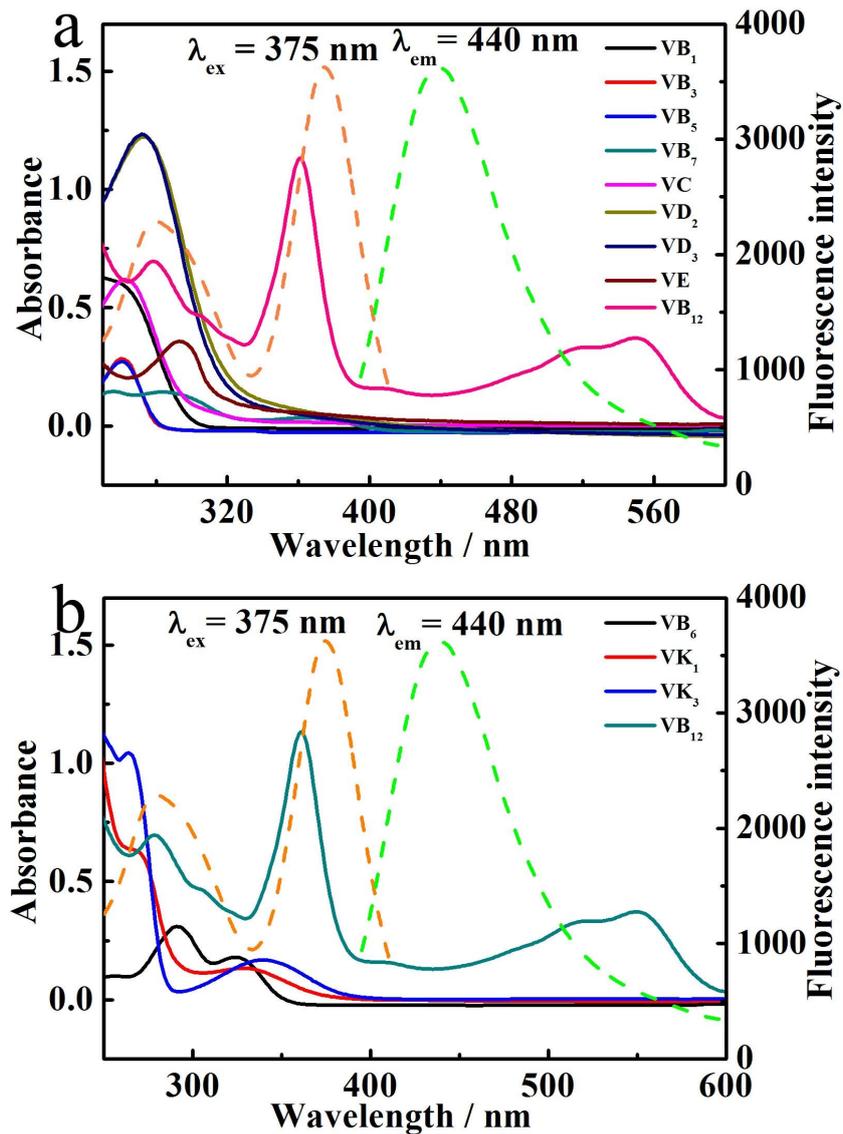


Fig. S3 Fluorescence excitation and emission spectra(dotted line) of 5 μ L/mL Ag NC-PEI-EDA and absorption spectra (solid line) of 60 μ M VB₁, VB₃, VB₅, VB₇, VC, VD₂, VD₃, VE and VB₁₂ (a). Fluorescence excitation and emission spectra (dotted line) of Ag NC-PEI-EDA (5 μ L/mL) and absorption spectra (solid line) of 60 μ M VB₆, VK₁ and VK₃ and VB₁₂ (b).

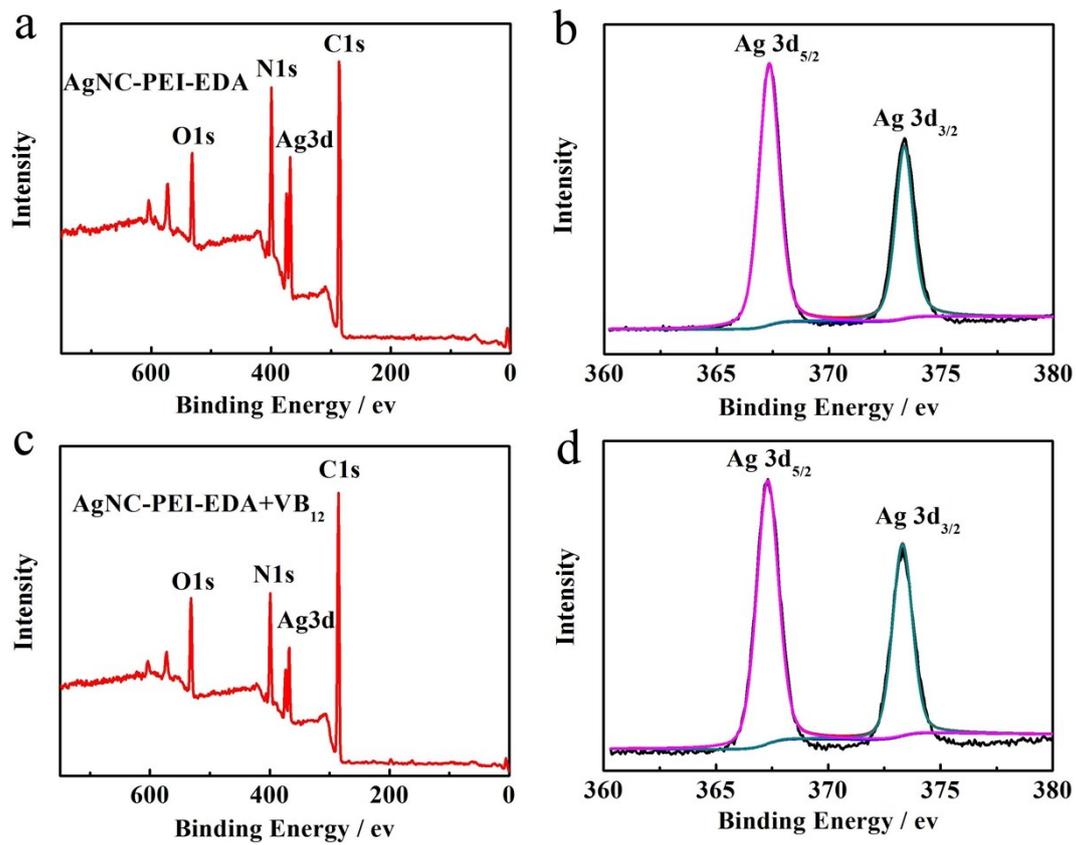


Fig. S4 XPS spectra of Ag NC-PEI-EDA in the absence and presence of VB₁₂ and the corresponding Ag3d spectra (a and b, free Ag NC-PEI-EDA; c and d Ag NC-PEI-EDA in the presence of VB₁₂)

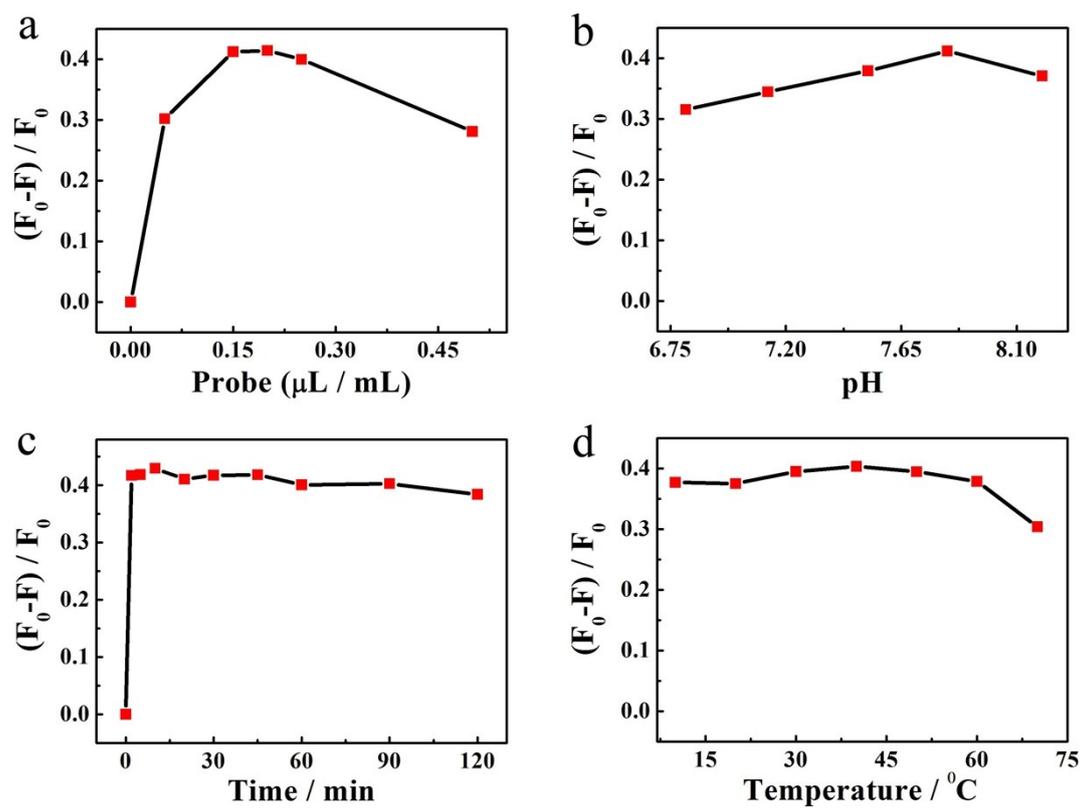


Fig. S5 Optimization of reaction conditions for detecting VB₁₂ based on Ag NC-PEI-EDA (a, probe concentrations; b, pH values; c, reaction time; d, temperature).

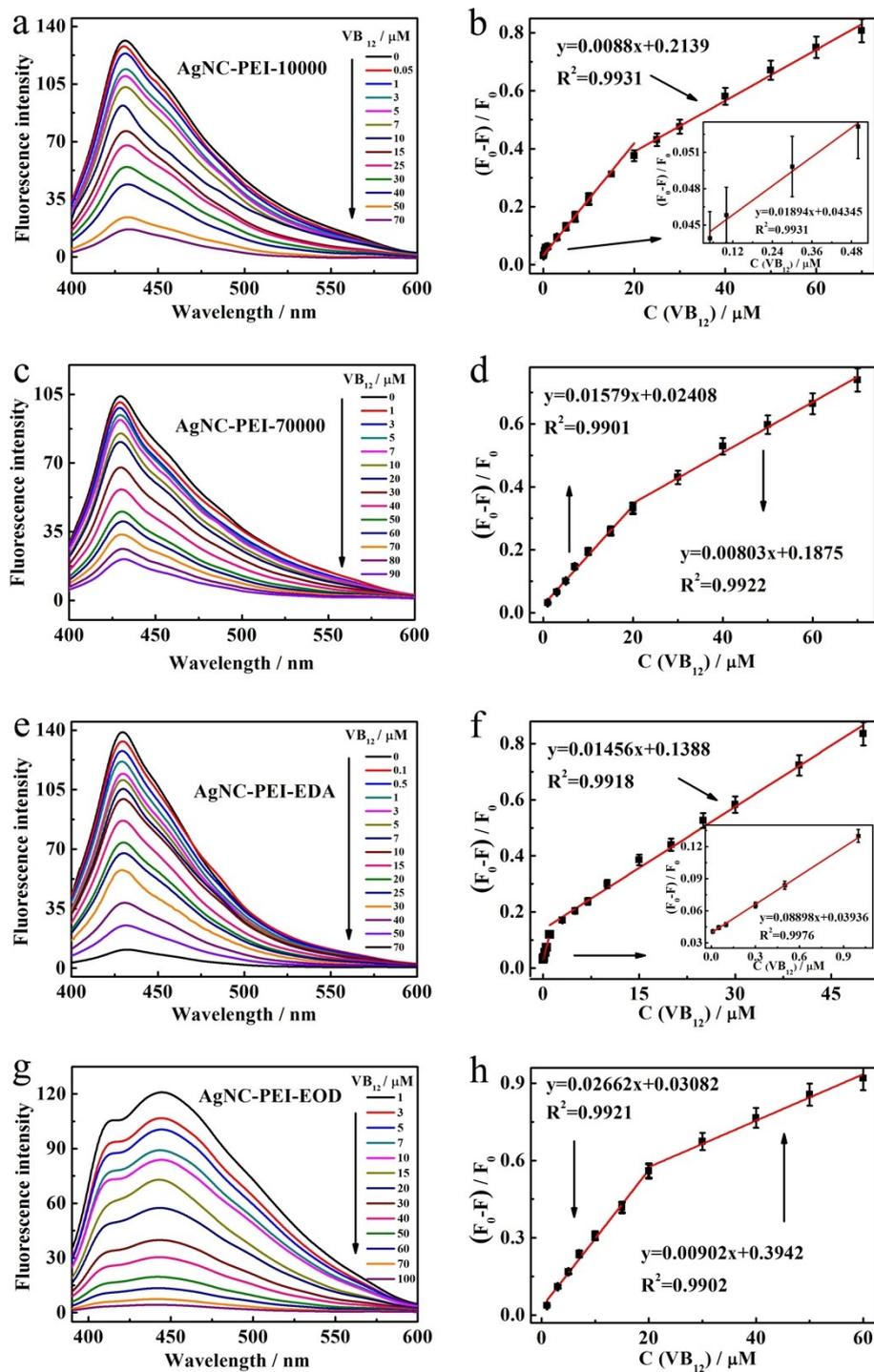


Fig. S6 Fluorescence spectra of Ag NC-PEIs in the addition of different concentrations of VB₁₂ and the corresponding linear ranges (a and b, Ag NC-PEI-10000; c and d, Ag NC-PEI-70000; e and f, AgNC-PEI-EDA; g and h, Ag NC-PEI-EOD; the inserts are the linear responses below 1 μM).

Table S1. The detailed amounts of AgNO₃, PEIs, and formaldehyde used in the synthesis of Ag NC-PEIs.

Ag NC-PEIs	PEI (g)	Water (μ L)	AgNO ₃ (mol)	HCHO (mol)
Mw 600	0.0094	209	1.5×10^{-5}	9.3×10^{-5}
Mw 10000	0.0094	70	2.5×10^{-5}	8.0×10^{-5}
Mw 70000	0.0147	125	2.0×10^{-5}	7.0×10^{-5}
Terminated EDA (Mw 800)	0.0094	200	1.5×10^{-5}	1.0×10^{-4}
Terminated EOD (Mw 70000)	0.1167	2620	2.0×10^{-5}	1.8×10^{-4}

Table S2. The influence of templates on the detection of VB₁₂ based on Ag NC-PEIs.

Ag NC-PEIs	Linear equation	Linear range	LOD
Mw 600	$y=0.02640x+0.05090$	5 nm-70 μ M	2.62 nM
	$y=0.007930x+0.2958$		
Mw 10000	$y=0.01894x+0.04345$	50 nm-70 μ M	27 nM
	$y=0.008800x+0.2139$		
Mw 70000	$y=0.01579x+0.02408$	1 μ M -70 μ M	745 nM
	$y=0.008030x+0.1875$		
Terminated EDA (Mw 800)	$y=0.08898x+0.03936$	10 nm-50 μ M	7.31 nM
	$y=0.01456x+0.1388$		
Terminated EOD (Mw 70000)	$y=0.02662x+0.03082$	1 μ M -60 μ M	433 nM
	$y=0.009020x+0.3942$		

References

- 1 F. Qu, N. B. Li and H. Q. Luo, *Anal. Chem.*, 2012, **84**, 10373-10379.
- 2 F. Qu, X. Zou, R. Kong and J. You, *Talanta*., 2016, **146**, 549-555.