Supporting information

Peptide-Mediated Controllable Crosslinking of Gold Nanoparticles for Immunoassay with Tunable Detection Range

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EXPERIMENTAL SECTION

Preparation of AuNPs. We prepare the AuNPs with different sizes via citrate reduction. We prepare the 13 nm AuNPs according to previous report.[9] In brief, we heat 100 mL aqueous solution of 50 mg/mL (800 μL) of $HAuCl_4$ hydrate with reflux for 30 min, and quickly add 10 mL of 20 mM trisodium citrate aqueous solution to the refluxed mixture. The change in color occurs immediately from yellow, to purple and finally wine red. We boil the mixture for additional 30 min, and cool them slowly to room temperature. We prepare the larger size of AuNPs (30 nm and 60 nm) through reducing the amount of trisodium citrate.

The preparation of biotin-ALP conjugate and biotin-antibody conjugate. The SA-biotin-ALP conjugate and biotin-antibody conjugate are prepared according to previous report.[1] We prepare the biotin-labeled detection antibody and biotin-labeled ALP. Biotin is diluted to 1 mg/mL using DMF, detection antibody and ALP is diluted to 1 mg/mL using PBS buffer. The two solutions are mixed at a molar ratio of 20:1 and stirred for 1 h. The mixed solution is transferred to a clean centrifugal ultra-filtration unit (10 Kd filter) and centrifuged at 9000 rpm for 10 min at 4 oC to remove excess biotin. After washing 3 times using PBS (pH 7.0, 0.01 M), the biotin-labeled detection antibody and biotin-labeled ALP are resolved in PBS and stored at -20 ℃. We obtain the SA-biotin-ALP conjugate by mixing the solution of SA (5 mg/mL,10 μL) and the biotin-labeled ALP (6.1 mg/mL, 30 μL) for 20 min.

The characterization of AuNPs. We perform the dynamic light scattering (DLS) measurements and zeta potential measurement at 25 ℃ on a Malvern Zetasizer Nano ZS (Malvern, U.K.) with a back scattering detection at 173° and a He–Ne laser (λ = 632.8 nm). We incubate each samples at 25 ℃ for 2 min to reach equilibrium before measurement. We acquire transmission electron microscopy (TEM) images with a Tecnai G2 F20 U-TWIN (FEI, USA), using 200 kV acceleration voltage. We prepare our samples by depositing a drop of solution (5 μL) on copper-supported carbon films and allowing it to dry overnight.

The procedure of PAAI for PCT detection. We modify the 96-well plates with

capture antibody (100 μL) diluted 1:2000 (5 mg/mL) in a carbonate buffer (0.2 M sodium carbonate/bicarbonate, pH 9.6) at 4 ℃ overnight. After three times of washing with PBST (0.01 M PBS, with 0.5% Tween 20), we block these plates with blocking buffer (3% wt BSA in PBS) for 2 h at 37 ℃. Subsequently, we wash the plates three times and add diluted solution of PCT to each well. After 1 h incubation and additional three round of washing, we add the solution of diluted (1:3000) biotin-labeled detection antibody (100 μL) and further incubate for 1 h at 37℃. After three times washing, we add the SA-biotin-ALP conjugate (100 μL) diluted 1:2000 for 1 h at 37℃. After another three rounds of washing, peptide 2 (**CRYR**) solution diluted 1:500 (100 μL) in water is added for 1 h at 37 ℃. We added the solution of AuNPs (100 μL) to each well at room temperature. After 15 min, we collect the spectra of the solutions by UV-vis spectrometry.

The procedure of PAAI for IL-6 detection. We modify the 96-well plates with capture antibody (100 μL) diluted 1:1500 (5 mg/mL) in a carbonate buffer (0.2 M sodium carbonate/bicarbonate, pH 9.6) at 4 ℃ overnight. After three times washing with PBST (0.01 M PBS, with 0.5% Tween-20), we block these plates with blocking buffer (3% wt BSA in PBS) for 2 h at 37 ℃. Subsequently, we wash the plates three times and add diluted solution of IL-6 to each well. After 1 h incubation and additional three round of washing, we add the solution of diluted (1:2000) biotin-labeled detection antibody (100 μL) and further incubate for 1 h at 37 ℃. After three times washing, we add the SA-biotin-ALP conjugate (100 μL) diluted 1:2000 for 1 h at 37 ℃. After another three rounds of washing, peptide 3 (**CYK**) diluted 1:500 (100 μL) in water is added for 1 h at 37 ℃. We added the solution of AuNPs (100 μL) to each well at room temperature. After 15 min, we collect the spectra of the solutions by UV-vis spectrometry.

Specificity of PAAI. We also study the specificity of this assay. We prepare 10 μg/mL solution of CRP containing interferences including human IgG (5 μg/mL), PCT (80 ng/mL) and IL-6 (100 pg/mL) respectively and perform immunoassay with aforementioned procedure.

We prepare 20 ng/mL solution of PCT containing interferences including human IgG (5 μg/mL), CRP (20 μg/mL) and IL-6 (100 pg/mL) respectively and perform immunoassay with aforementioned procedure.

We prepare 50 pg/mL solution of IL-6 containing interferences including human IgG (5 μg/mL), PCT (80 ng/mL) and CRP (20 μg/mL) respectively and perform immunoassay with aforementioned procedure.

Clinical sample analysis. We detect the 15 human serum samples (obtained from Beijing Friendship Hospital in accordance with the rules of the local ethical committee (2017-P2-099-01) with the PAAI, Roche-ECL and the conventional ELISA, and each sample is assayed thrice.

(1) Chen, Y.; Zou, M.; Qi, C.; Xie, M.; Wang, D. N.; Wang, Y. ; Xue, Q.; Li, J.; Chen, Y. *Biosens. Bioelectron*. **2013**, *39*, 112-117.

Table S1. The potential of the original peptides and the peptides after enzyme treatment.

Potential	Original peptide (mV)	After enzyme treatment (mV)
Peptide 1	-4.91±1.22	8.34±3.77
Peptide 2	-8.17±3.91	7.76±1.48
Peptide 3	-6.58±1.53	11.38±2.99
Peptide 4	-10.95±2.84	8.57±2.07

Table S2. The comparison of analytical performances of PAAI for detection of CRP, PCT and IL-6 with the published reports.

Name	Methods	LOD	Linear Range	Reference
CRP	PAAI	1.15 μg/mL	3.15 - 100 μg/mL	This work
	SPR biosensor	1.17 μg/mL	0.01 - 20 μg/mL	[53]
	Immunosensor	0.03 μg/mL	1 -30 μg/mL	[54]
	Fluorescence sensor	70 pg/mL	0.1 -10 ng/mL	[50]
PCT	PAAI	0.24 ng/mL	0.2 -25 ng/mL	This work
	Lateral flow test	0.02 pg/mL	1 - 1000 pg/mL	[48]
	Microfluidic immunoassay	48.9 pg/mL	250 - 1×10^5 pg/mL	[49]
	Electrochemical immunosensor	0.43 pg/mL	1 - 2000 pg/mL	[51]
IL-6	PAAI	12.51 pg/mL	50 -1600 pg/mL	This work
	Fluorescence assay	2 pg/mL	15 - 900 pg/mL	[52]
	Microfluidic immunoassay	1 pg/mL	5 -1280 pg/mL	[49]
	Electrochemical sensor	1 pg/mL	1 - 300 pg/mL	[55]

Table S3. The performance variations of the PAAI for the CRP, PCT and IL-6 detection.

	Concentration	Intra CV(%)	Inter CV(%)
CRP	5 μg/mL	8.73	10.99
	10 μg/mL	7.57	13.78
	20 μg/mL	5.77	11.95
	40 μg/mL	6.36	6.58
	80 μg/mL	6.82	9.15
PCT	5 ng/mL	8.14	9.74
	10 ng/mL	7.19	10.67
	20 ng/mL	9.81	10.51
	40 ng/mL	5.44	7.92
	80 ng/mL	6.87	9.38
IL-6	50 pg/mL	7.43	12.43
	100 pg/mL	10.21	13.78
	200 pg/mL	9.86	10.94
	400 pg/mL	9.37	9.67
	800 pg/mL	11.54	12.69

Table S4. The quantitative results of PAAI and Roche-ECL method for detection of PCT and IL-6 in real serum samples, and the quantitative results of PAAI and ELISA method for detection of CRP.

Sample	CRP(μg/mL)		PCT(ng/mL)		IL-6(pg/mL)	
	PAAI	ELISA	PAAI	Roche-ECL	PAAI	Roche-ECL
1	14.6±1.32	8.60±0.70	4.37±0.84	2.13±0.21	20.9±2.15	15.8±0.94
2	7.15±0.83	11.1±1.02	6.76±0.80	1.08±0.24	38.3±3.68	29.4±1.05
3	17.9±1.64	16.3±1.16	5.02±1.24	2.03±0.20	37.6±2.27	38.3±1.24
4	20.6±1.35	15.6±0.97	6.67±0.31	3.92±0.21	42.1±2.94	30.7±1.12
5	20.9±1.97	22.1±1.53	2.11±0.43	1.46±0.23	18.5±2.92	12.8±0.84
6	23.3±1.01	19.6±0.73	8.72±0.52	2.43±0.24	28.9±2.71	18.4±1.17
7	15.6±0.80	9.34±0.87	5.43±0.61	6.11±0.27	33.7±3.29	25.0±1.13
8	19.5±1.96	10.8±1.32	6.41±0.62	8.99±0.58	35.2±1.74	30.8±0.92
9	10.7±0.72	8.44±0.52	0.94±0.12	0.16±0.03	26.1±1.84	19.3±1.31
10	5.67±0.61	7.14±0.41	0.67±0.13	0.08±0.01	35.1±3.53	26.3±0.97
11	28.6±1.26	20.3±1.09	22.3±1.34	26.6±1.37	70.8±3.98	64.6±2.64
12	65.4±2.98	43.8±2.61	70.4±3.77	59.7±1.87	62.5±3.44	65.4±2.28
13	7.39±1.22	10.6±0.94	0.61±0.13	0.09±0.02	27.5±2.20	25.3±0.97
14	20.3±3.80	19.3±1.67	2.20±0.30	3.35±0.12	29.4±2.33	21.5±0.84
15	53.7±2.71	45.3±1.72	30.7±2.56	35.2±1.46	46.7±2.41	53.8±1.97

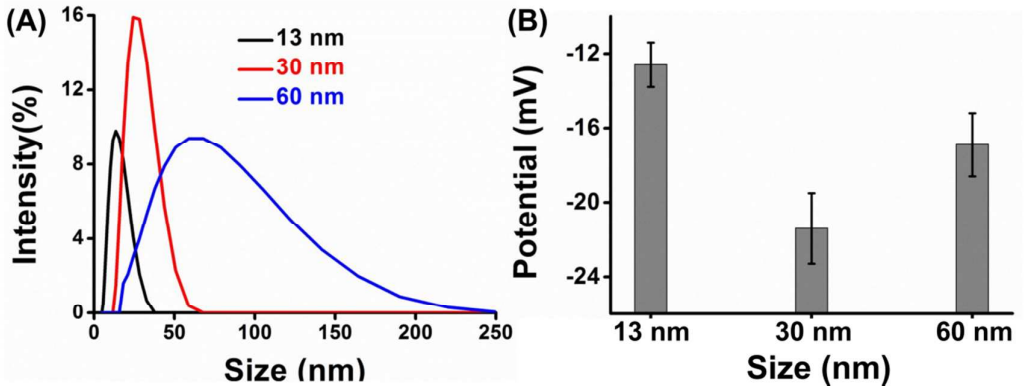

Figure S1 The size distribution and potential of the AuNPs (13 nm, 30 nm, 60 nm).

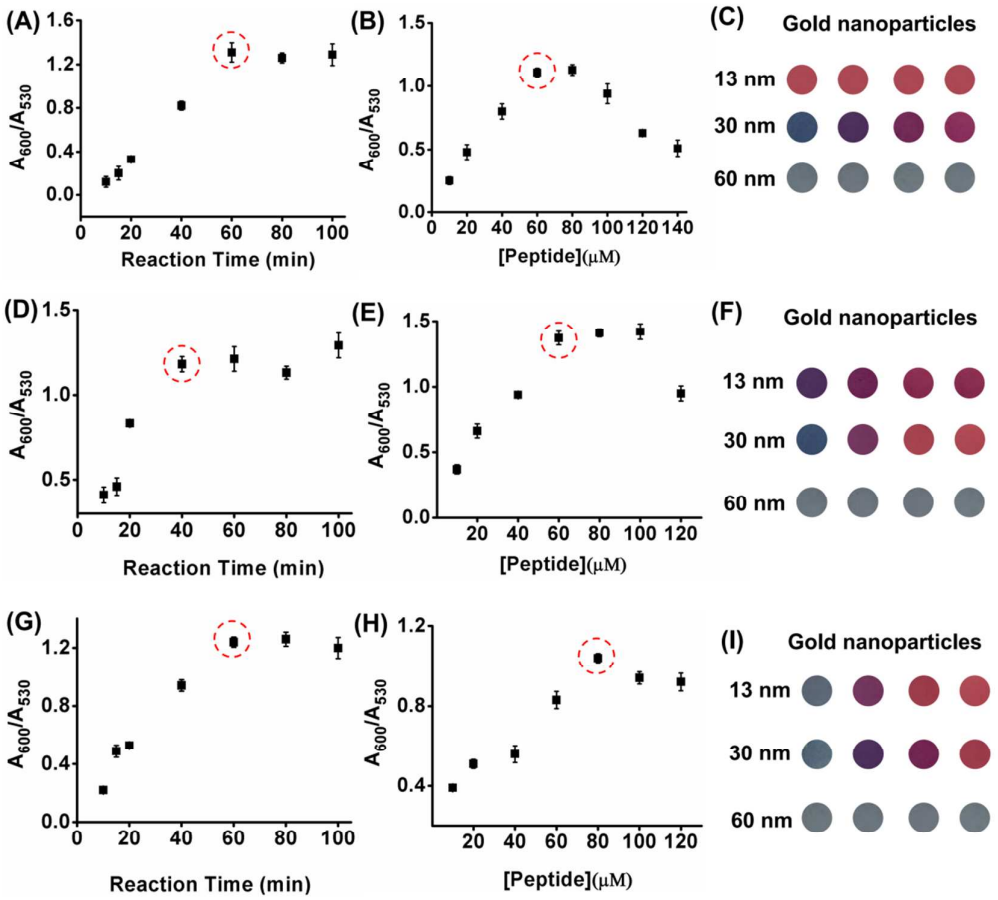

Figure S2. The optimization of reaction conditions for ALP sensing. (A-C) The optimization of **CSKSK** (**CSKSK**-ALP-AuNPs) reaction conditions. (D-F) The optimization of **CYR** (**CYR**-ALP-AuNPs) reaction conditions. (G-I) The optimization of **CRYR** (**CRYR**-ALP-AuNPs) reaction conditions.

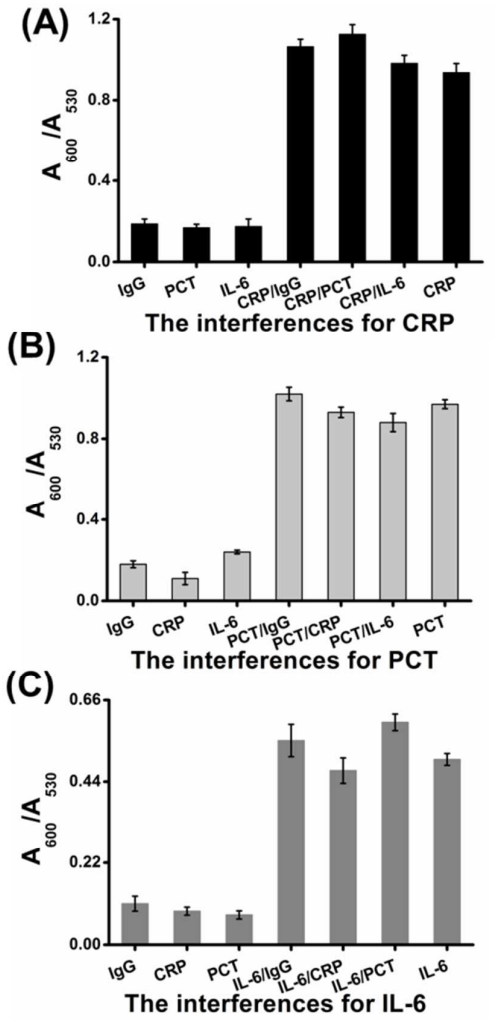

Figure S3. The selectivity of the PAAI for PCT, CRP and IL-6 detection. (A) The selectivity of the PAAI for CRP detection. Human IgG (5 μg/mL), PCT (80 ng/mL) and IL-6 (100 pg/mL) are used as the interferences to evaluate the selectivity. The concentration of CRP is 10 μg/mL. (B) The selectivity of the PAAI for PCT detection. Human IgG (5 μg/mL), CRP (20 μg/mL) and IL-6 (100 pg/mL) are used as the interferences to evaluate the selectivity. The concentration of PCT is 20 ng/mL. (C) The selectivity of the PAAI for IL-6 detection. Human IgG (5 μg/mL), CRP (20 μg/mL) and PCT (80 ng/mL) are used as the interferences to evaluate the selectivity. The concentration of IL-6 is 50 pg/mL.

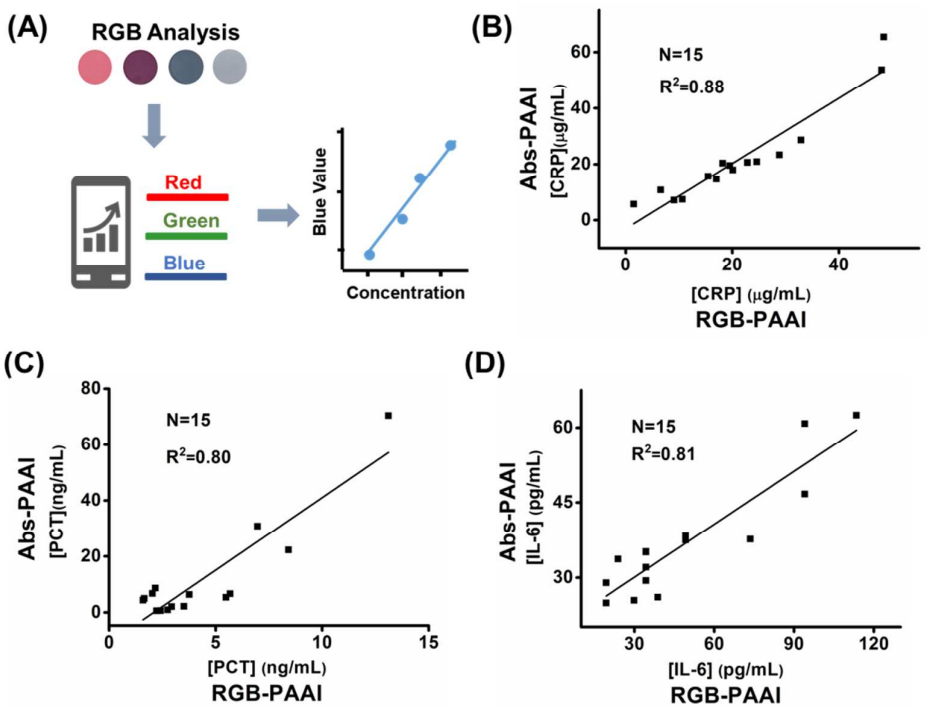

Figure S4. The RGB analysis-mediated PAAI for detection of CRP, PCT, and IL-6 using the smartphone. (A) The mechanism of RGB analysis-mediated PAAI. (B) The comparison between RGB analysis-mediated PAAI and absorbance analysis-mediated PAAI for quantification of CRP. (C) The comparison between RGB analysis-mediated PAAI and absorbance analysis-mediated PAAI for quantification of PCT. (D) The comparison between RGB analysis-mediated PAAI and absorbance analysis-mediated PAAI for quantification of IL-6.

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