Supporting information

Bioconjugation of 2-Arachidonoyl glycerol (2-AG) biotinylated antibody with gold nano-flowers toward immunoassay of 2-AG in human plasma samples: A novel immuno-platform for screening of immunomodulatory and neuroprotection

Ahmad Mobed (PhD) [a,b,c1], Fereshteh Kohansal (MSc Student) [d1], Ali. Ahmadalipour (Assistant Prof.) [a,e*], Mohammad Hasanzadeh (Assistant Prof.) [a b**], Flor Zargari (Assistant Prof.) [a f]

[a] Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

[b] Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, 51664, Iran.

[c] Aging Research Institute, Tabriz University of Medical Sciences, Tabriz, Iran.

[d] Department of Biochemistry, Higher Education Institute of Rab-Rashid, Tabriz, Iran.

[e] Research Center of Psychiatry and Behavioral Sciences, Tabriz University of Medical Sciences, Iran.

[f] Department of Medical Sciences, Marand Branch, Islamic Azad University, Marand, Iran

Corresponding author
* Ali Ahmadalipour (Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran)
Email: alipoura@tbzmed.ac.ir
** Mohammad Hasanzadeh (Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, 51664, Iran)
E-mail: hasanzadehm@tbzmed.ac.ir

[1] Co first author

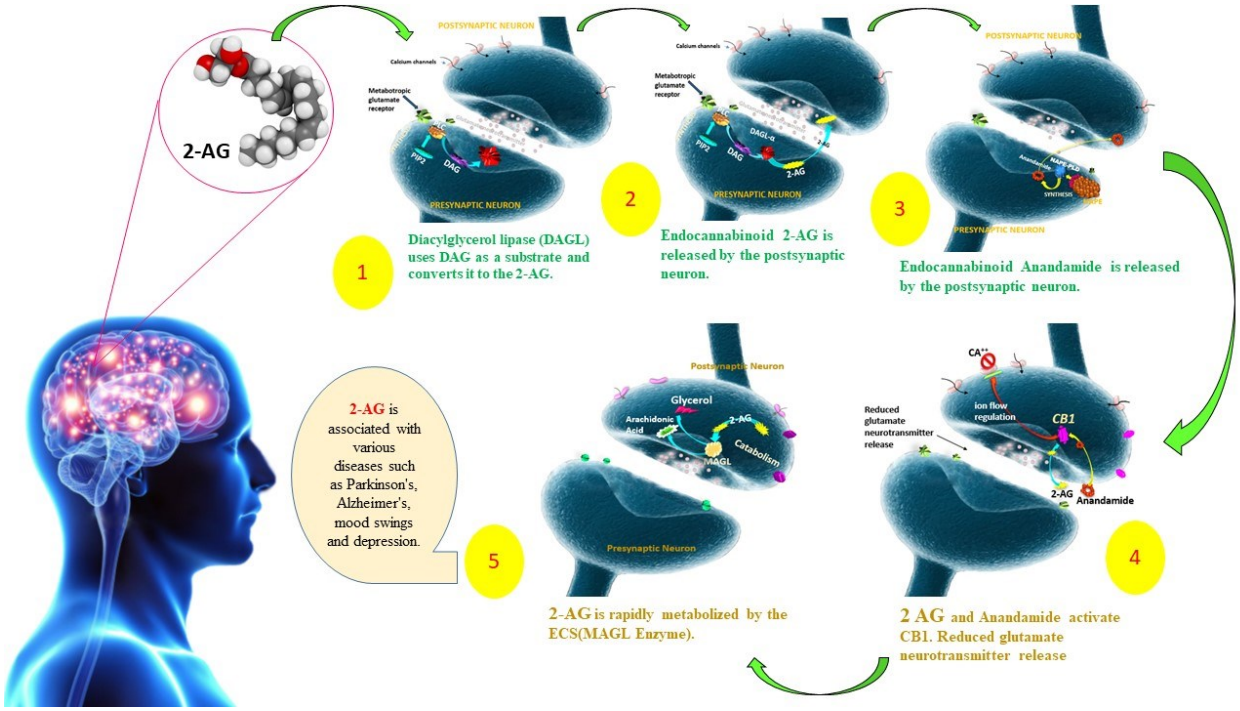


Fig. S1. 2-AG formation pathway and signaling in the human body.

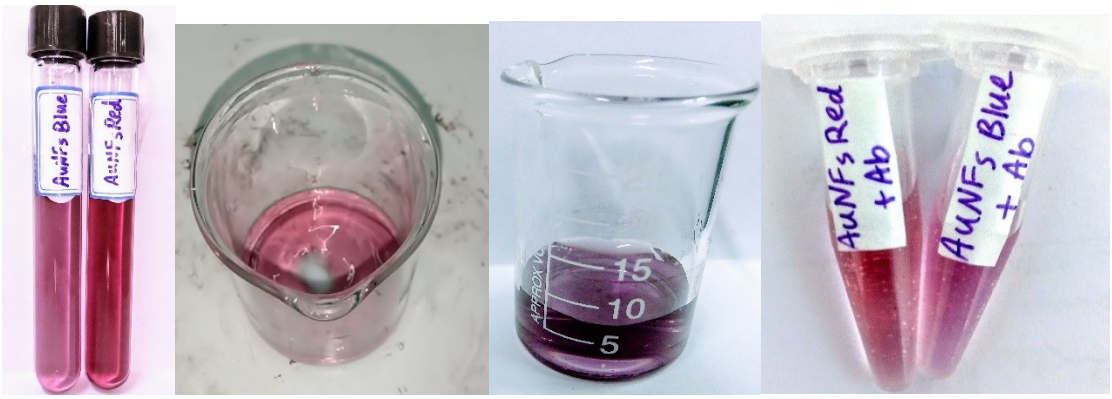


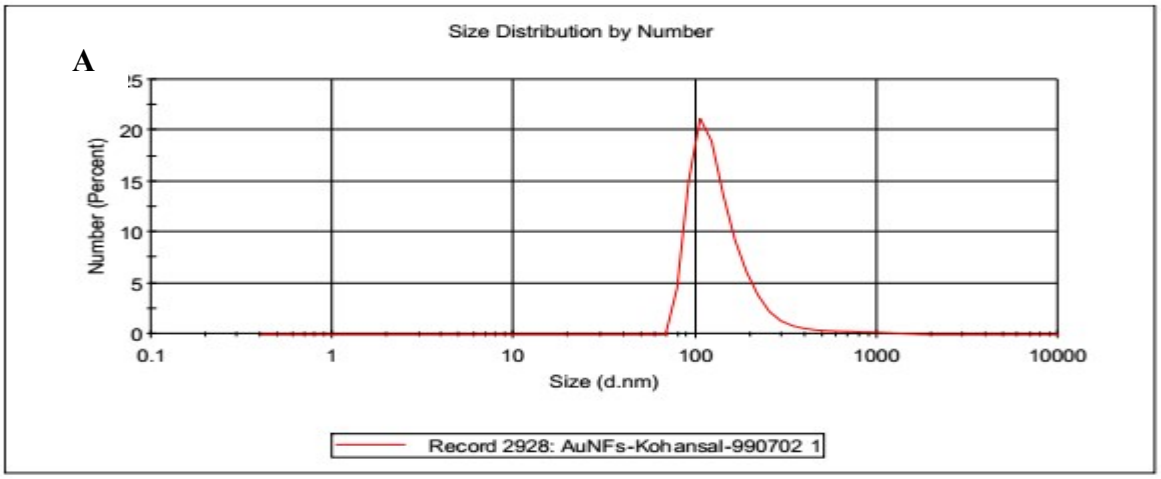
Fig. S2. Photographic images of two synthesized gold nanoparticles in different pH and its conjugation with biotinylated antibody of 2-AG biomarker.

Results

			Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm):	303.2	Peak 1:	150.1	100.0	113.9
PdI:	0.340	Peak 2:	0.000	0.0	0.000
Intercept:	0.768	Peak 3:	0.000	0.0	0.000

Result quality Refer to quality report

A

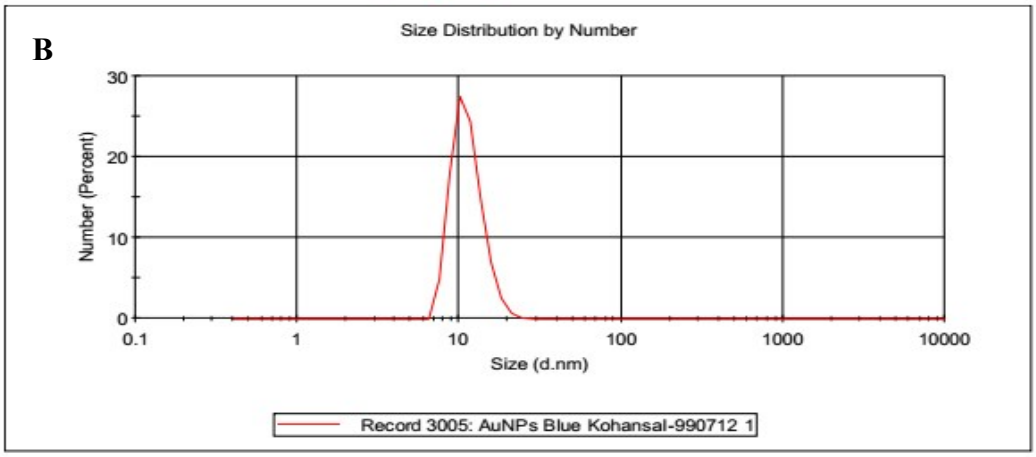


Results

			Size (d.n...	% Number:	St Dev (d.n...
Z-Average (d.nm):	94.31	Peak 1:	71.86	0.0	24.77
PdI:	0.316	Peak 2:	11.33	100.0	2.574
Intercept:	0.820	Peak 3:	0.000	0.0	0.000

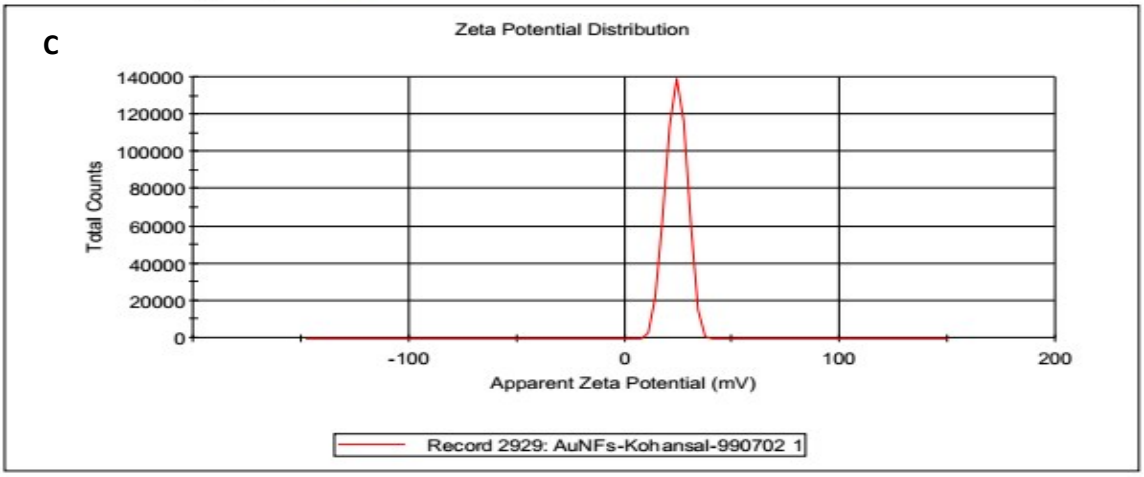
Result quality Refer to quality report

B



		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): 23.9	Peak 1:	23.9	100.0	4.80
Zeta Deviation (mV): 4.80	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.368	Peak 3:	0.00	0.0	0.00
Result quality Good				

C



Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -9.31	Peak 1:	-9.31	100.0	4.65
Zeta Deviation (mV): 4.65	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.492	Peak 3:	0.00	0.0	0.00
Result quality Good				

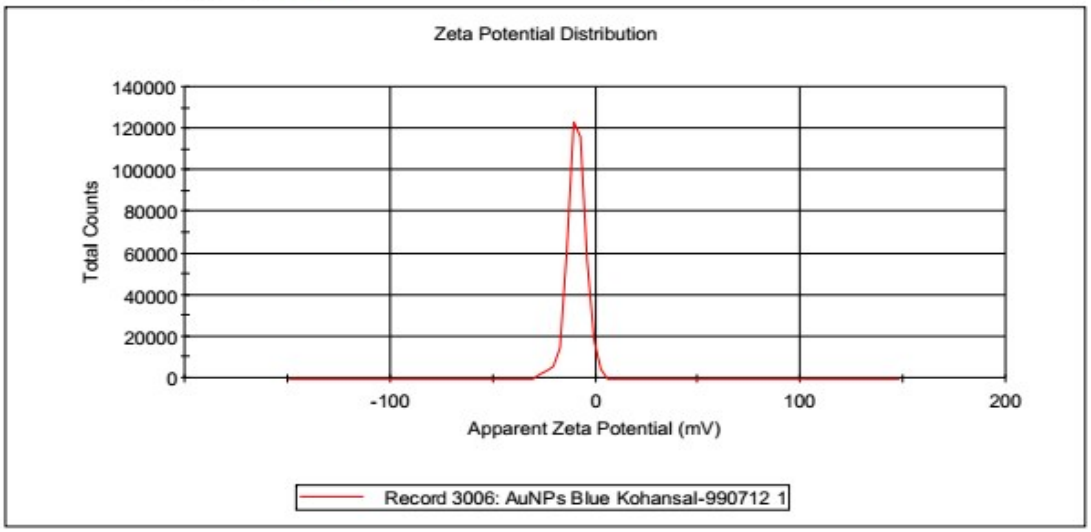


Fig. S3. (**A,C**) DLS and **(B,D**) Zeta potential of AuNPs synthesized in pH=6.14 and 4.19, respectively. Measurement parameters were as follows: a laser wavelength of 633 nm (He–Ne), a scattering angle of 173◦ (fixed-without changing possibility), and measurement temperature of 25◦(D

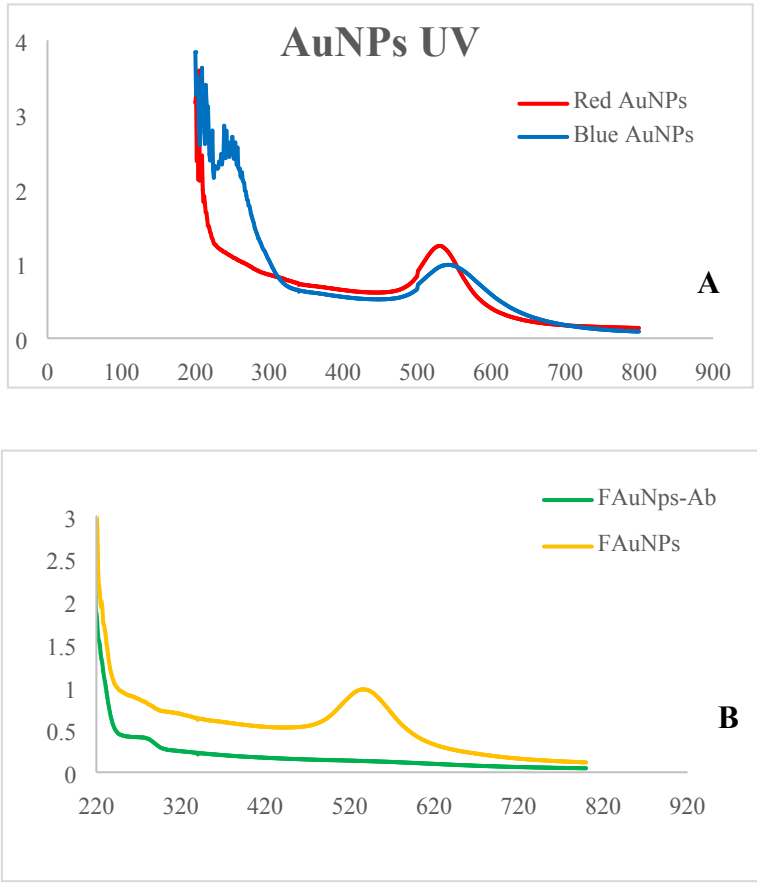
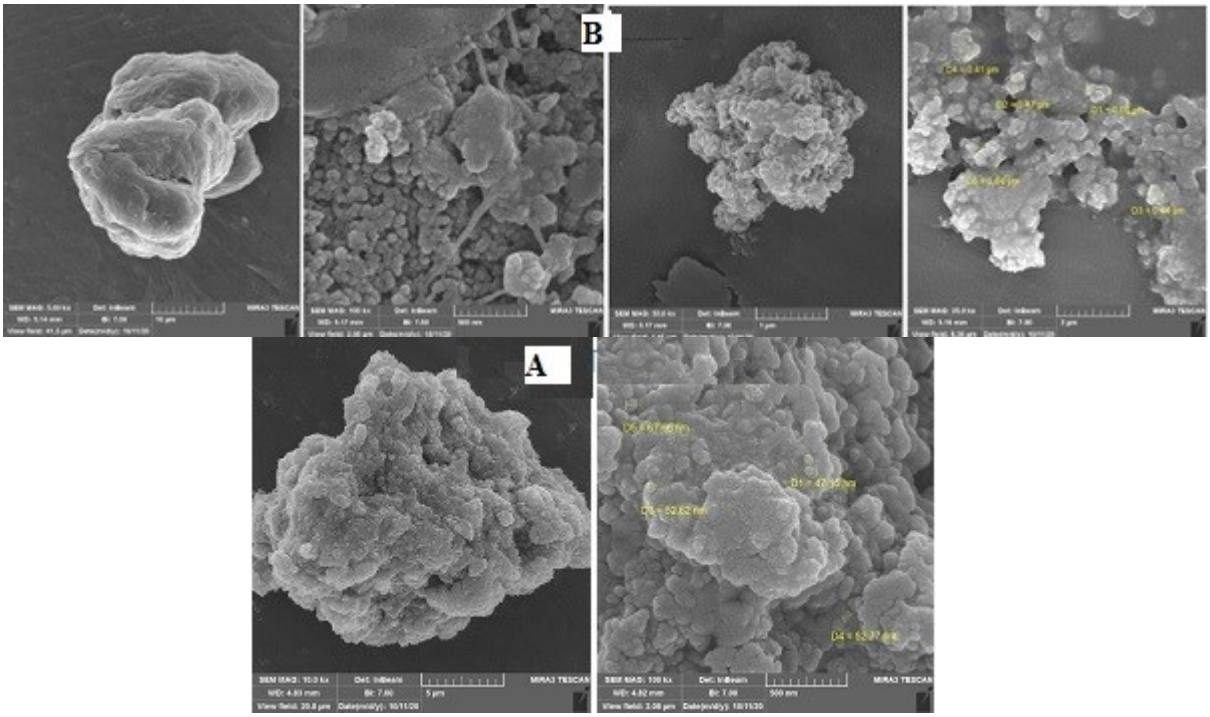


Fig. S4. (A) Uv/Vis absorbance spectrum of AuNPs sythesised in pH =6.15 and pH= 4.19. **B)** Uv/Vis absorbance spectrum of AuNPs befor and after conjugation with biotinalyated Ab.


B
A

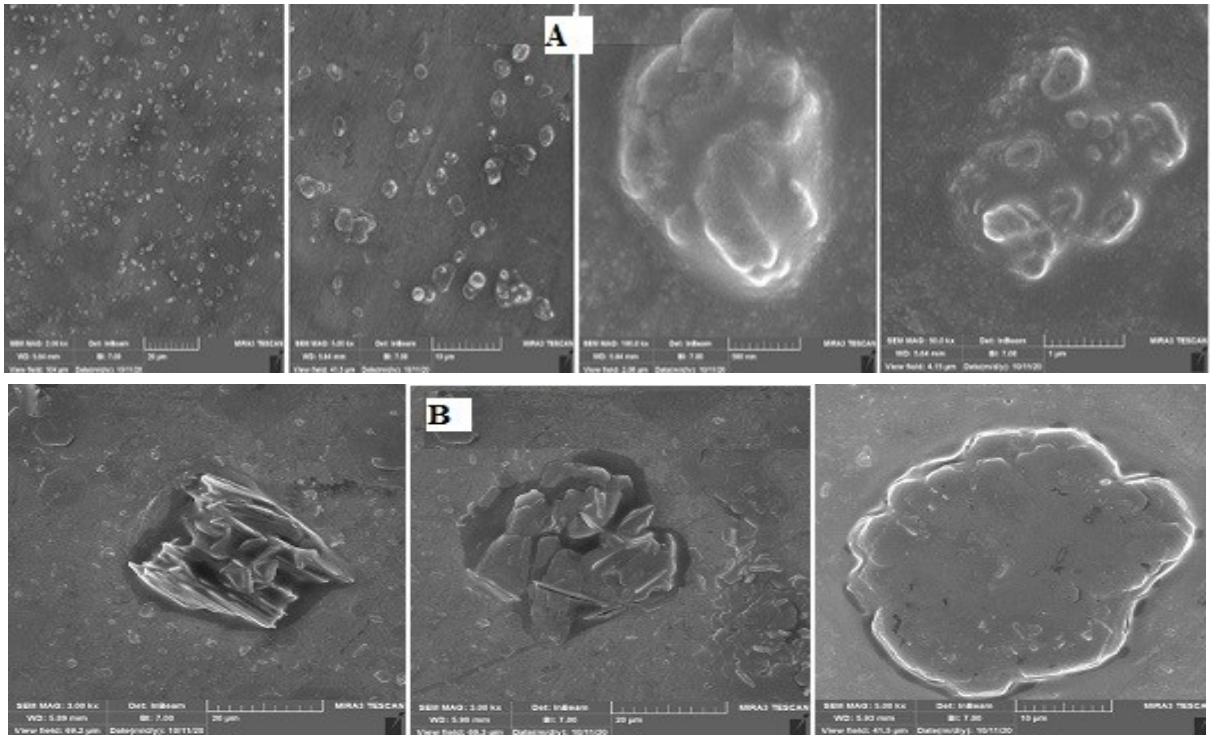
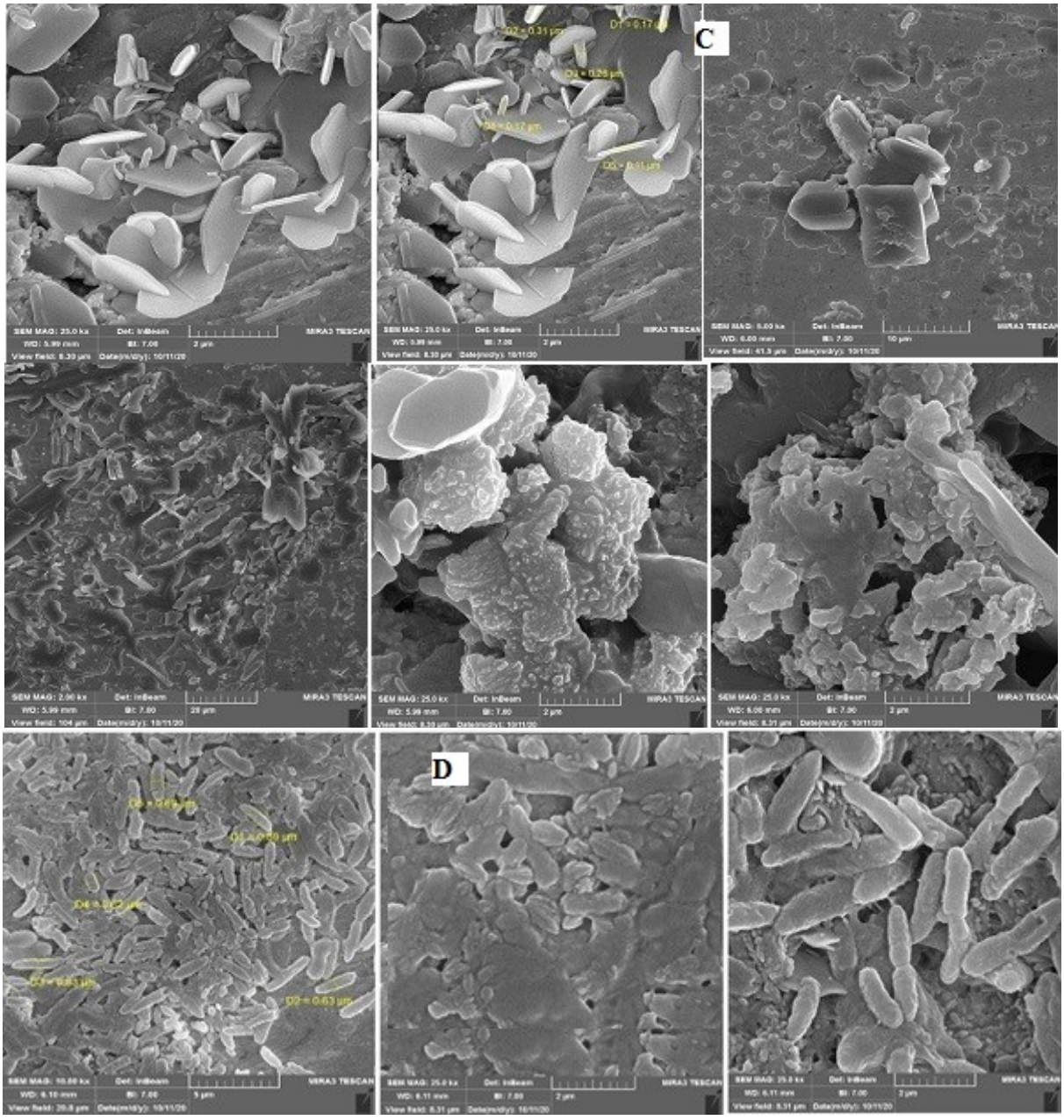


Fig. S5. (A&B) FESEM AuNPs synthesised in variuos pH (6.15 and 4.19) in different magnification, respectively.


C
D

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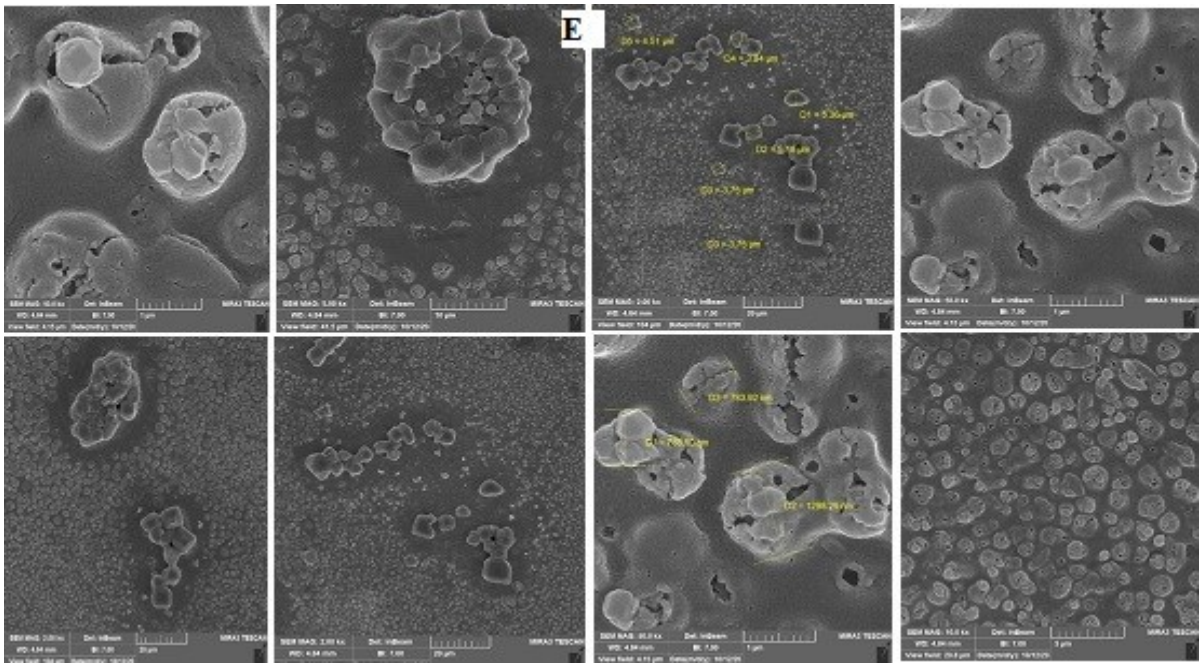

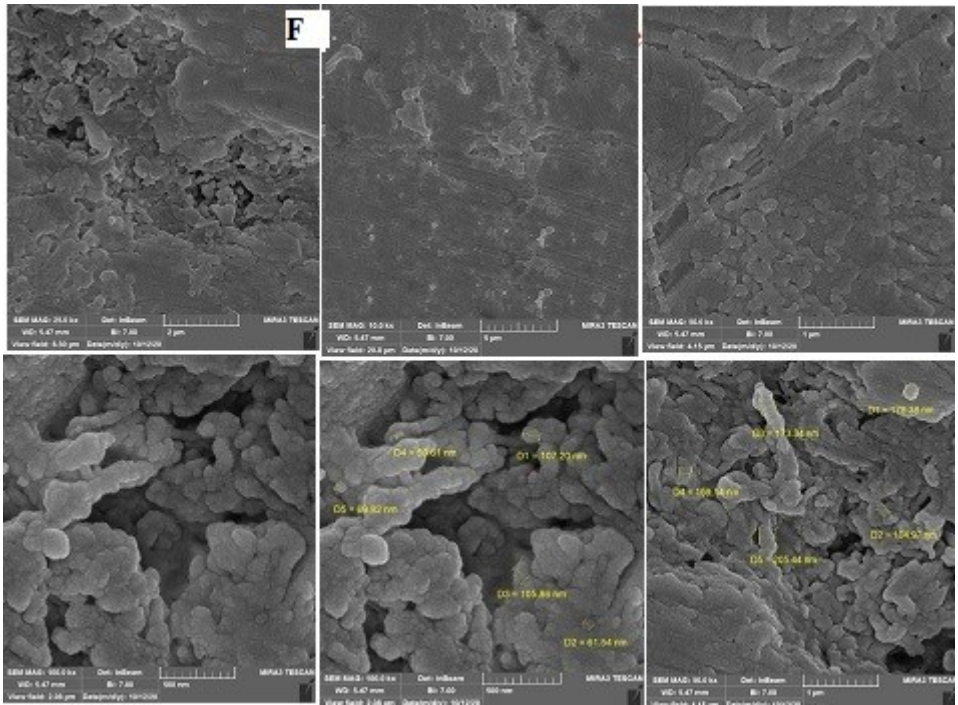

Fig. S6. (C-F) FE-SEM of

AuNPs-biotinylated Ab, biotinylated Ab-Ag-2(Antigenn) on the surface of gold electrode in different magnification, respectively.

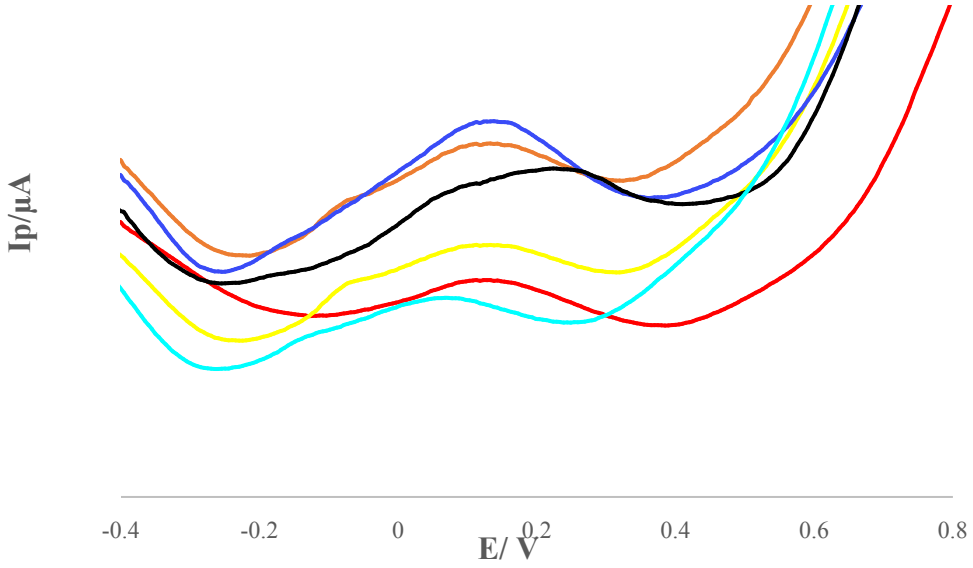

Fig. S7. SWVs of the immunosensor in the presences of 2-AG with various concentration in human plasma samples. (RSD=1.56%, n=3)

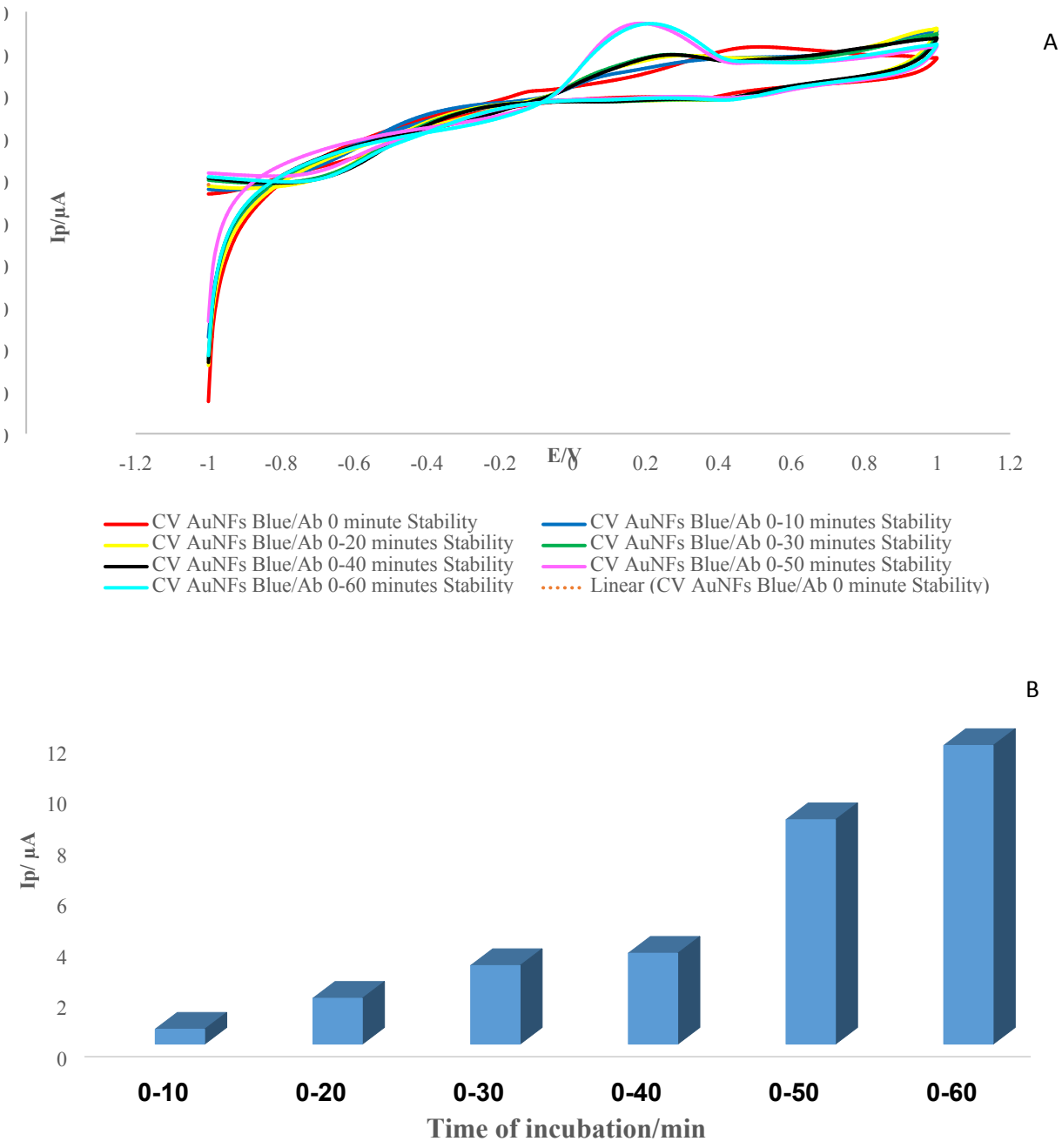

Fig. S8. (A) CV of the gold electrode modified by AuNPs-Ab in different storage time, E_{being}=-1V, E_{step}=0.01V, and scan rate=0.1 V/s in the solution of $K_4Fe(CN)_6/K_3Fe(CN)_6$. **(B)** Histogram of the peak currents *versus* storage time. (RSD=2.11%, n=3)

Table S1. pH responsive behavior of the nanoparticles in some studies.

Nanoparticles	Comments and significances	Ref
AuNPs	Solution pH values play key role in reversibly aggregated or disassembled of particles based on their electrostatic interaction.	[47]
Gold Nanorod	Morphology of GNRs is dependent on the pH of the growth solution. Additionally, pH affected CTAB micelle's stability and reduced its absorption ability on gold surface, leading to morphology variation of the final products.	[53]
AuNPs	Changing the citrate charge regulatory its stabilizing effect during the growth of the particles and affecting the morphology and size.	[45]
AuNPs	Minor pH variations in the gold nanoparticle synthesis methods influence the performance of the citrate oxidation/reduction process and altered size and morphology.	[46]
AuNPs	Changing of the nanoparticles charges subsequently, changing the pH, alterd the color, morphology and size of the AuNPs particle.	This work

Table S2. EDX analyses report in different steps of immunosensor preparation.

El	A		B		C		D		E		F	
C	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%	W%	A%
N	17.57	29.03	21.14	32.19	14.25	22.51	22.14	37.77	26.00	40.27	25.05	42.51
O	9.63	13.64	11.38	14.86	10.03	13.59	12.58	18.40	12.35	16.41	12.55	18.26
P	32.85	40.75	34.24	39.14	40.84	48.44	19.77	25.33	24.71	28.73	16.72	21.31
S	10.82	6.93	7.05	4.16	8.35	5.12	9.22	6.10	7.97	4.79	9.63	6.34
Cl	5.21	3.23	7.88	4.49	9.60	5.68	7.39	4.72	4.31	2.50	5.52	3.51
K	4.31	2.41	3.46	1.79	3.13	1.68	4.00	2.31	7.23	3.79	4.96	2.85
Au	4.98	2.53	5.32	2.49	4.27	2.07	6.60	3.46	4.87	2.32	6.14	3.20
Elt	14.63	1.47	9.52	0.88	9.53	0.92	18.31	1.90	12.56	1.19	19.43	2.01
%	100	100	100	100	100	100	100	100	100	100	100	100.

(A) AuNPs (pH=6.19), (B) AuNPs (pH=4.15), (C) AuNPs-Ab (pH=6.15), (D) AuNPs-Ab (pH=4.19), (E) AuNPs-Ab-Antigen (pH=6.19), (F) AuNPs-Ab-Antigen (pH=4.15).