Supporting Information

Effect of Surface-engineered AuNPs on gene expression, bacterial interaction, protein denaturation, and toxicology assay: An *in vitro* and *in vivo* model

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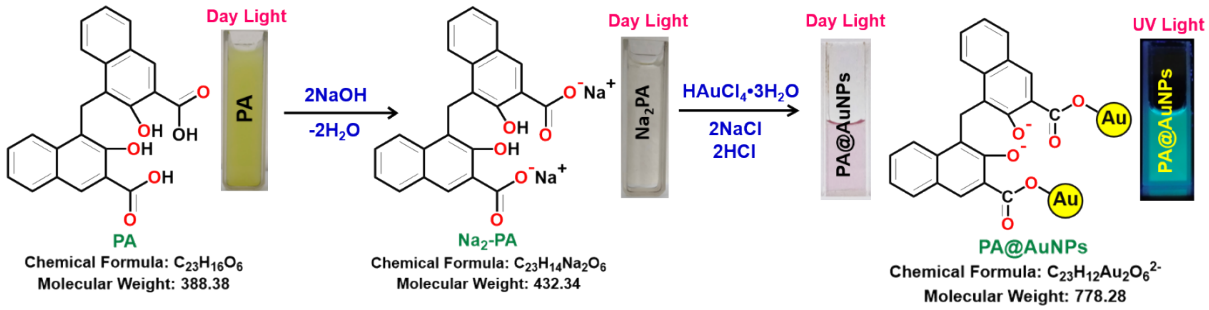
1. Synthesis of PA@AuNPs



Scheme SI1. Synthesis of PA@AuNPs (inset images: corresponding reaction solutions under daylight and UV light).

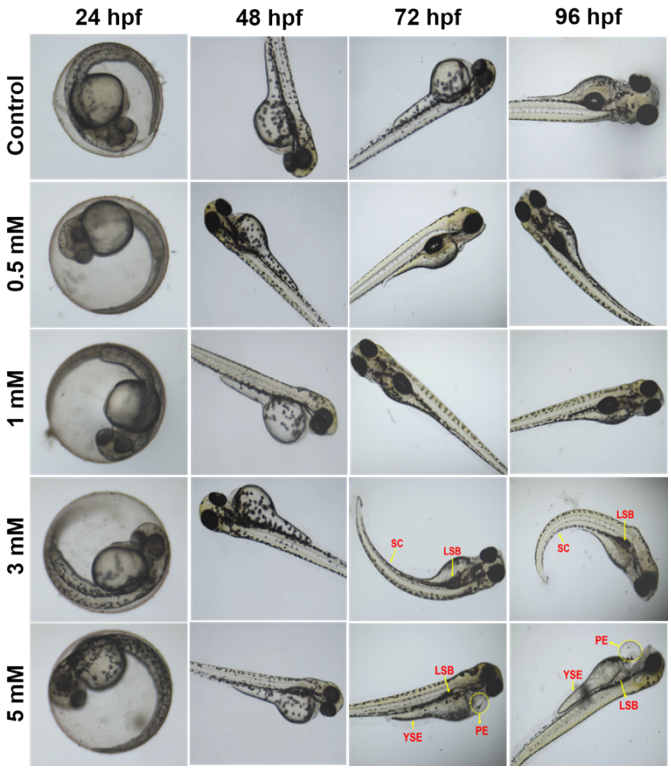


Figure SI1. Morphological changes in zebrafish embryos affected by various concentrations of PA@AuNPs (0.5, 1, 3, and 5 mM). The control showed normal development of an embryo, and the PA@AuNPs exposed to the embryo showed deformities like SC-spinal curvature, LSB-lack of the swim bladder, YSE-yolk solk edema, and PE-pericardial edema.

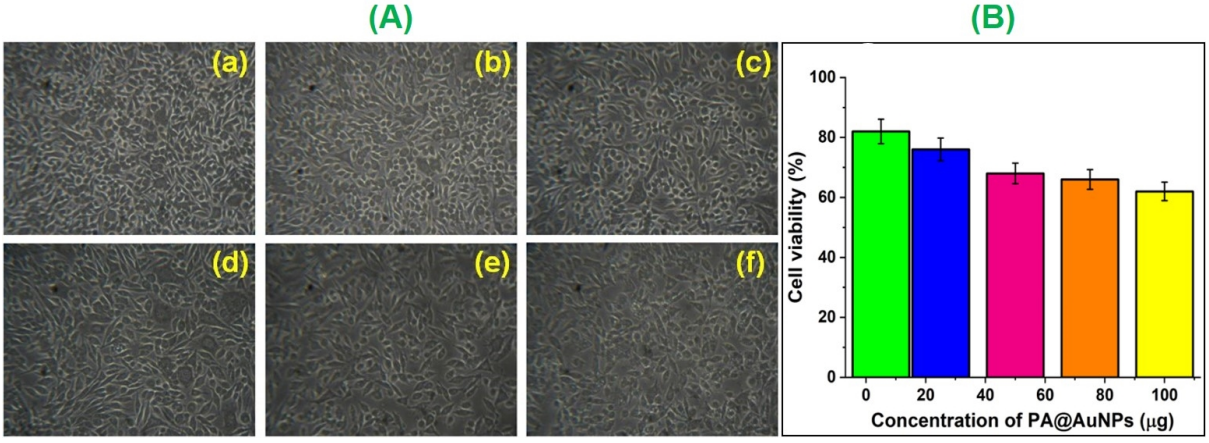


Figure SI2. (A) The cytotoxic effect of PA@AuNPs (0, 5, 25, 50, 75, and 100 µL) with L929 fibroblast cells by the standard MTT viability assay. L929 fibroblast cells were incubated with PA@AuNPs for 24 h (images (a) untreated L929 fibroblast cells, (b) L929 fibroblast cells + 5 µL PA@AuNPs, (c) L929 fibroblast cells + 25 µL PA@AuNPs, (d) L929 fibroblast cells + 50 µL PA@AuNPs; (e) L929 fibroblast cells + 75 µL PA@AuNPs, and L929

fibroblast cells + 100 µL PA@AuNPs, respectively). (B) Evaluation of cell viability of PA@AuNPs (0, 5, 25, 50, 75, and 100 µg) by the standard MTT assay.

2. Method

2.1. Cell culture

The cell line was cultured in a 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and an antibiotic solution containing, Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37 ºC in a humidified 5% CO_2 incubator. The viability of cells was evaluated by direct observation of cells by an inverted phase contrast microscope and followed by the MTT assay method.

2.2 Cells seeding in 96 well plates

A two-day-old confluent monolayer of cells was trypsinized and the cells were suspended in 10% growth medium, 100 µL cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37 ºC in a humidified 5% CO_2 incubator.

2.3. Isolation of total RNA by TRIzol Method

A total RNA sample was isolated following the manufacturer's instructions. By adding TRIzol solution, cells are disrupted and release RNA. As a result of adding isopropanol, RNA precipitates as a white pellet around the tube's sides and bottom. After adding 1 ml of TRIzol reagent, the culture well plate was incubated for 5 min. This mixture was transferred to a new, sterile Eppendorf tube, and 200 µL of chloroform was added. It was then forcefully shaken for 15 sec and allowed to incubate for two to three minutes at ambient temperature. Finally, it was centrifuged for fifteen minutes at 4 ºC at 14000 rpm. After gathering the obtained aqueous layer, 500 µL of 100% isopropanol was added, and the mixture was incubated for 10 minutes at ambient temperature before being centrifuged for 15 min at 4 ºC at 14000 rpm. The pellet was cleaned with 200 µL of 75% ethanol after the supernatant was removed, and it was then centrifuged for five minutes at 4 ºC at 14000 rpm in a cooling centrifuge. After drying the RNA pellet was suspended in TE buffer.

2.4. cDNA Synthesis

With the TRIzol reagent, total RNA was isolated, and then total RNA's concentration and purity were assessed. With the help of a cDNA preparation kit, the template

complementary DNA was synthesized. About 5 µL of RT easy mix, 0.5 µL of oligodT, and an RNAse-free tube were filled with 2 L of RNA template (0.5 g total RNA). Then the total reaction solution was made up to 10 µL with the addition of sterile distilled water, and then the solution was mixed gently. For, cDNA synthesis by using a thermal cycler. The cycling conditions were applied for 20 min at 42 ºC and 5 min at 85 ºC (Table SI1).

Table SI1. Temperature and time variations in cycling conditions.

Step	**Temperature ºC**	**Time (minutes)**	**No. of cycles**
Synthesis of cDNA	42	20	1
Inactivation	85	5	1

3. Characterization

Table SI2. Statistical analysis of anticancer assay by MTT Method

Cell line - MDA MB 231									
SAMPLE CODE									
	OD1	**OD2**	**OD3**	**% viability 1**	**% viability 2**	**% viability 3**	**Average**	**Stdev**	**Std error**
Control	0.72	0.72	0.71	100	100	100	100	0	0
6.25	0.61	0.62	0.62	84.52	85.50	87.44	85.82	1.48	0.49
12.5	0.51	0.51	0.51	70.03	70.93	71.13	70.70	0.58	0.19
25	0.41	0.40	0.40	57.55	54.94	56.15	56.21	1.30	0.43
50	0.34	0.33	0.33	47.50	45.47	46.25	46.41	1.02	0.34
100	0.23	0.22	0.22	32.80	31.34	31.92	32.02	0.73	0.24

Table SI3. Statistical analysis of Gene expression study

	Experimental Well 1	Experimental Well 2	Experimental Well 3	Control Well 1	Control Well 2	Control Well 3	Average Experimental Ct Value	Average Experimental Ct Value	Average Control Ct Value	Average Control Ct Value	ΔCt Value (Experimental)	ΔCt Value (Control)	Delta Delta Ct Value	Expression Fold Change
	Raw Ct Value	**Raw Ct Value**	**Raw Ct Value**	**Raw Ct Value**	**Raw Ct Value**	**Raw Ct Value**	**TE**	**HE**	**TC**	**HC**	**ΔCTE**	**ΔCTC**	**ΔΔCt**	**2^-ΔΔCt**
Housekeeping Gene	20.43	20.16	20.38	22.29	22.08	22.24	-	20.32	-	22.20	7.25	6.96	0.29	0.816014485
Gene being Tested	27.88	27.59	27.26	29.35	29.06	29.08	27.58	-	29.16	-				

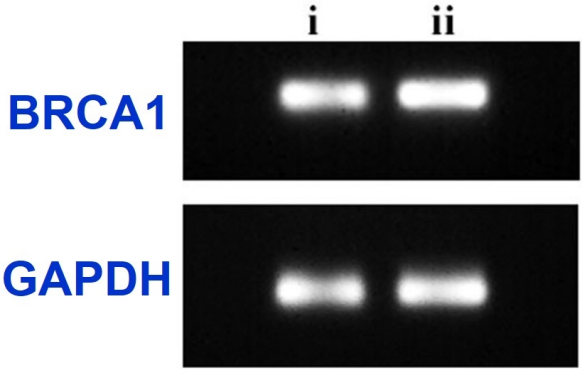


Figure SI3. Agarose gel electrophoresis image of GAPDH and BRCA1.

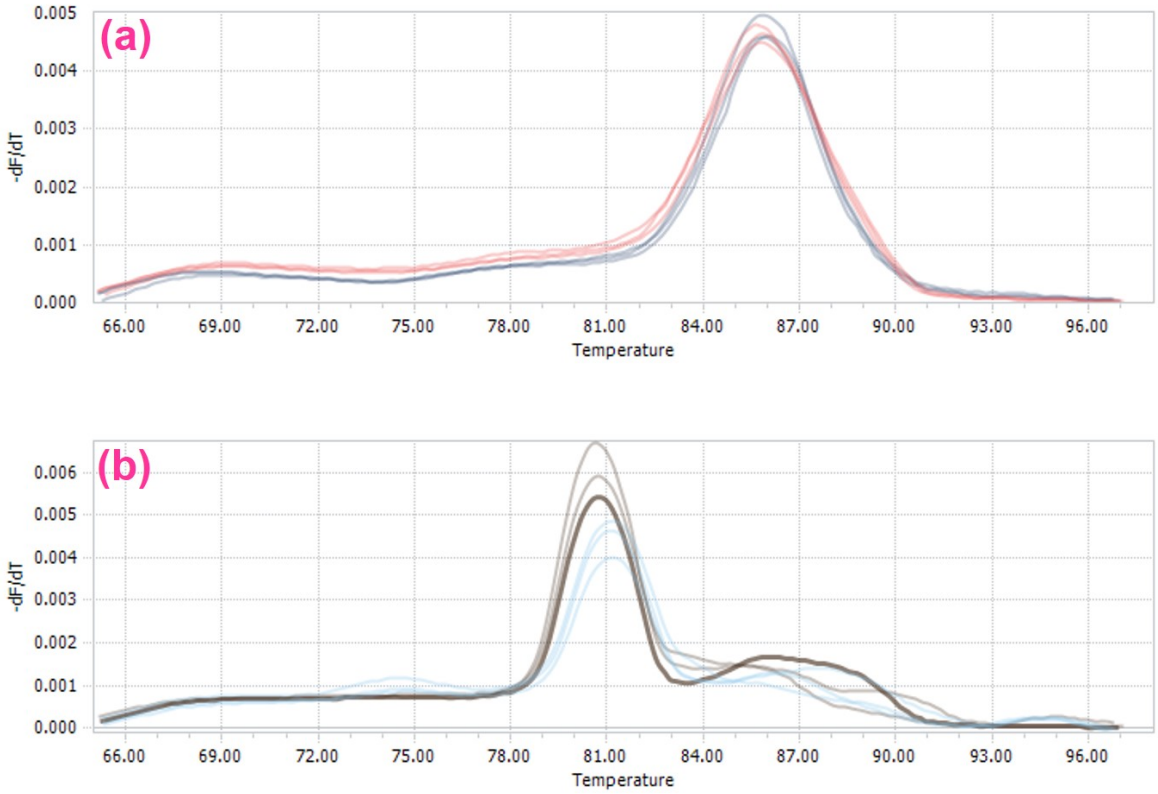


Figure SI4. Real-time melting peaks of PA@AuNPs, (a) GAPDH and (b) BRCA – 1.

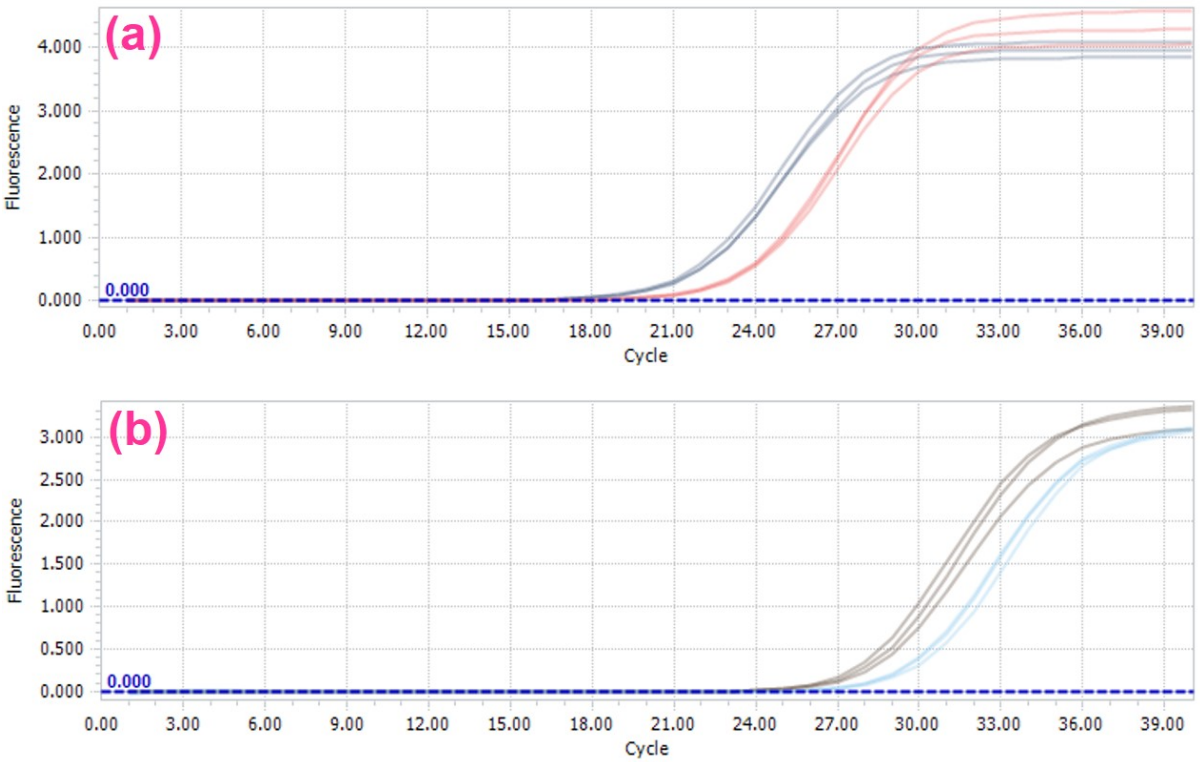


Figure SI5. Real-time amplification curves of PA@AuNPs, (a) GAPDH and (b) BRCA – 1.

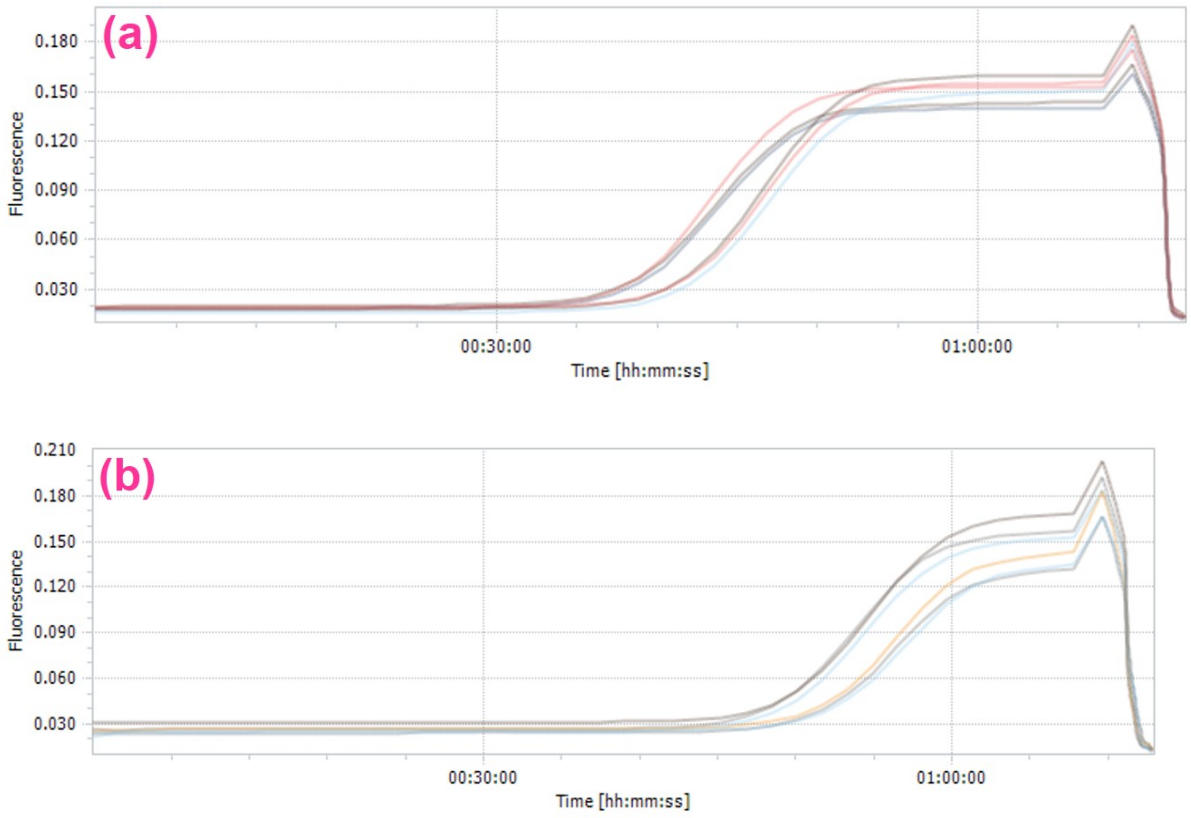


Figure SI6. Real-time fluorescence curves of PA@AuNPs, (a) GAPDH and (b) BRCA – 1.

Table SI4. The various steps involved, the time taken, and the temperature of each step.

Steps	**Time required**	**Temperature**
Initial activation step	2 min	95 ºC
3 step cycling		
Denaturation	10 sec	95 ºC
Annealing	1 min	58 ºC
Extension	1 min/kb	72 ºC
Number of cycles	40 cycles	68 ºC
End of PCR cycling	Indefinite	4 ºC

Table SI5. Gross morphological changes in vital organs after acute toxicity testing of PA@AuNPs in Sprague-Dawley rats

Parameters	**Groups**					
	Control			**PA@AuNPs**		
	Heart	**Liver**	**Kidney**	**Heart**	**Liver**	**Kidney**
Organ Weight (gms)	1.06 ± 0.24	11.07 ± 1.02	1.08 ± 0.12	1.04 ± 0.12	11.69 ± 1.19	1.08 ± 0.04
Discoloration	Nil	Nil	Nil	Nil	Nil	Nil
Lesion	Nil	Nil	Nil	Nil	Nil	Nil
Structural change	Nil	Nil	Nil	Nil	Nil	Nil

Table SI6. Histological scoring of stained organ sections after acute toxicity testing

Sl. No.	**Group**	**Organs**		
		Heart	**Liver**	**Kidney**
1	Control	0	0	0
2	PA@AuNPs	0	0	0

Table SI7. Parameters under consideration during acute toxicity study.

Sl. No.	**Parameters**	**Control (Out of 5**	**PA@AuNPs (Out of 5**

		animals)	animals)
1	Mortality	0	0
2	Convulsions	0	0
3	Weight loss (≥ 2g)	0	0
4	Salivation	0	0
5	Piloerection	0	0
6	Abnormal Reflexes	0	0
7	Gastro-intestinal abnormality	0	0
8	Muscular tone	0	0
9	Motor Control	0	0
10	Analgesia	0	0

Table SI8. Gross morphology surrounding PA@AuNPs after subcutaneous implantation.

Sl. No.	Parameter	PA@AuNPs	
		Week 2	Week 4
1	Edema	Nil	Nil
2	Hemorrhage	Nil	Nil
3	Pus or discharge	Nil	Nil
4	Lesion or ulceration	Nil	Nil
5	Necrosis of adjacent tissue	Nil	Nil
6	Vascularization	moderate	excellent

Table SI9. Comparative table of AuNPs for *in vitro* and *in vivo* analysis.

S. No.	Materials used	*In vitro* and *in vivo* models	Ref.
1	AuNPs	TNBC – MDA-MB-231	1
2	AuNPs	TNBC – MDA-MB-231	2
3	Polyethylene Modified Au-based SiRNA	TNBC – MDA-MB-231	3

4	Thioglucose-bound AuNPs	TNBC – MDA-MB-231	4
5	AuNPs	BC – MCF-7	5
6	AuNPs	TNBC – MDA-MB-231	6
7	Citrate-capped-AuNPs	TNBC – MDA-MB-231	7
8	Au-Curcumin (Au-C), Au-Paclitaxel (Au-P), Au-Curcumin-Paclitaxel (Au-CP)	TNBC – MDA-MB-231	8
9	AuNPs	foodborne bacterial pathogens	9
10	A-AuNPs and M-AuNPs	*E. coli*, MDR *E. coli*, *P. a*, MDR *P. a*, MDR *K. p*	10
11	Polyelectrolyte-wrapped AuNPs	Shewanella oneidensis and Bacillus subtilis	11
12	AuNPs (1000 mg /kg)	Mice and Rats	12
13	Pectin-mediated-AuNPs 500 – 1000 µg	Sprague Dawley Rats	13
14	AuNPs 0.9, 9, 90 µg	Sprague Dawley Rats	14
15	PA@AuNPs	TNBC – MDA-MB-231, Sprague Dawley Rats, Bacterial species, and Protein denaturation	Current work

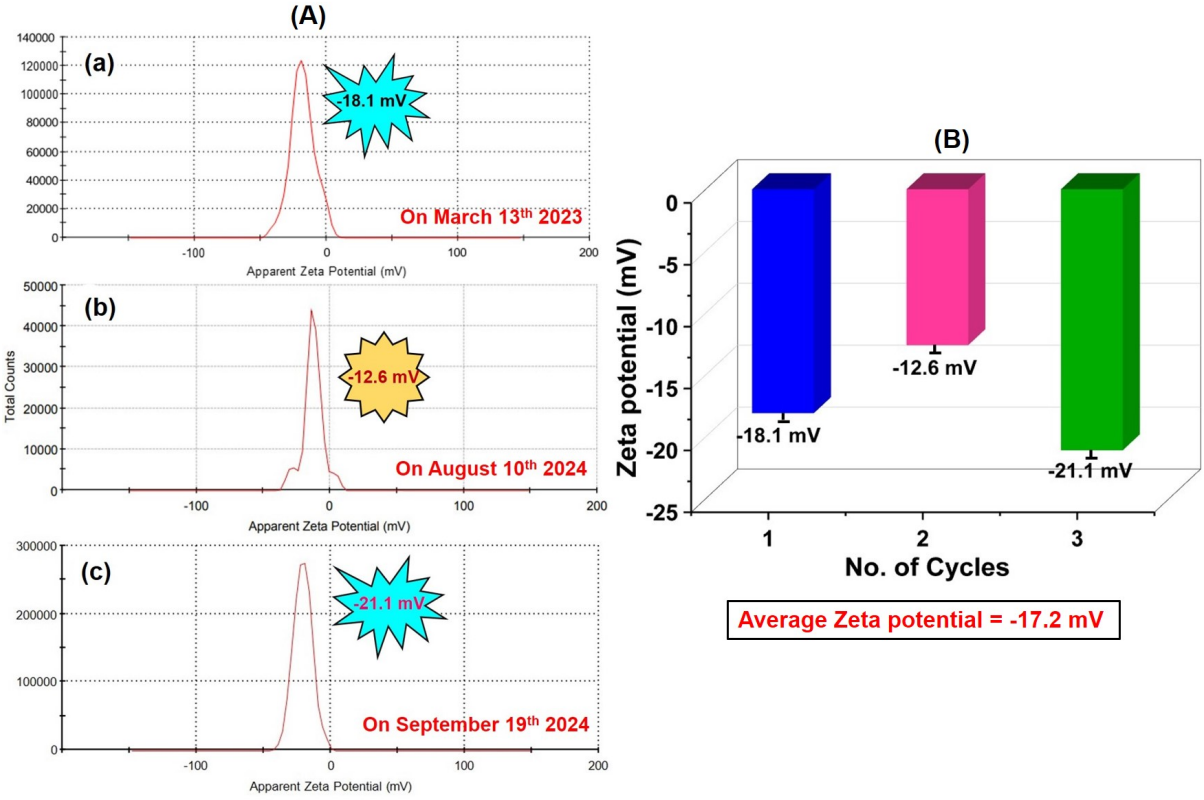


Figure SI7. (A) Different time interval measurements of zeta potential of PA@AuNPs (Average zeta potential = -17.2 mV), (B) Corresponding bar graph (n = 3%).

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