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# Gold nanorod-based fluorometric ELISA for the sensitive detection of a cancer biomarker

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## Supplementary Information

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**Methodology for ELISA and nanoELISA**

The protocol followed for ELISA and nanoELISA was similar to ELIFA and nanoELIFA, respectively, till the addition of HRP-conjugated streptavidin. After this, instead of flourogenic substrate, the conventional chromogenic substrate (100 µL of TMB solution) provided in the kit was added and incubated for 30 min (5 min for nanoELISA) at room temperature, and the reaction was stopped using 50 µL of 2N $H_2SO_4$ (stop solution). The absorbance measurements for the ELISA plate were made at a wavelength of 450 nm using an ELISA plate reader (BioTek, PowerWave XS2, USA).

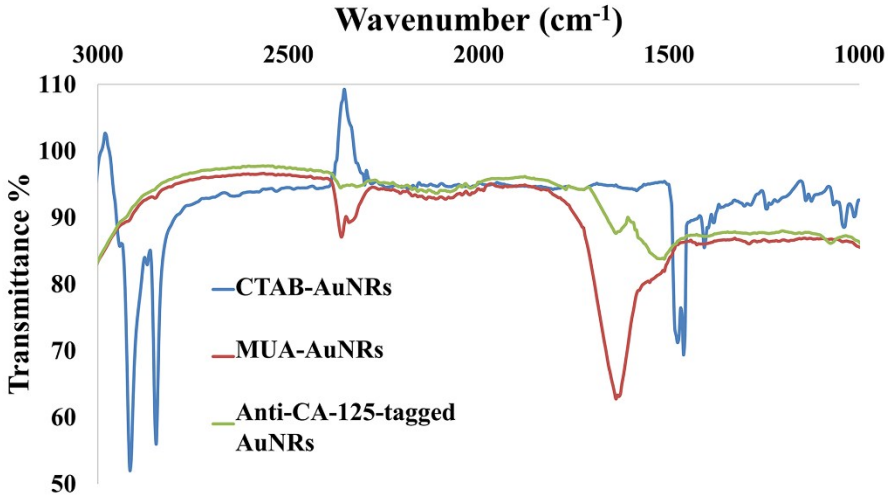


**Fig. S1.** FTIR spectral analysis of CTAB-AuNRs, MUA-AuNRs, and anti-CA125-tagged-AuNRs.

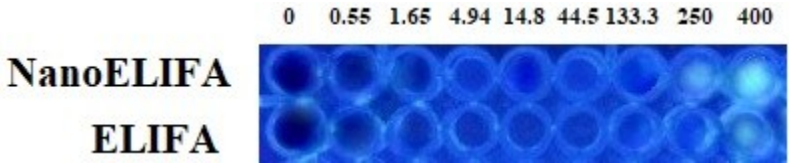


**Fig. S2.** Photographic image of 96-well plate taken under UV illumination for nanoELIFA and ELIFA towards different concentrations of CA125 (0–400 U/mL).

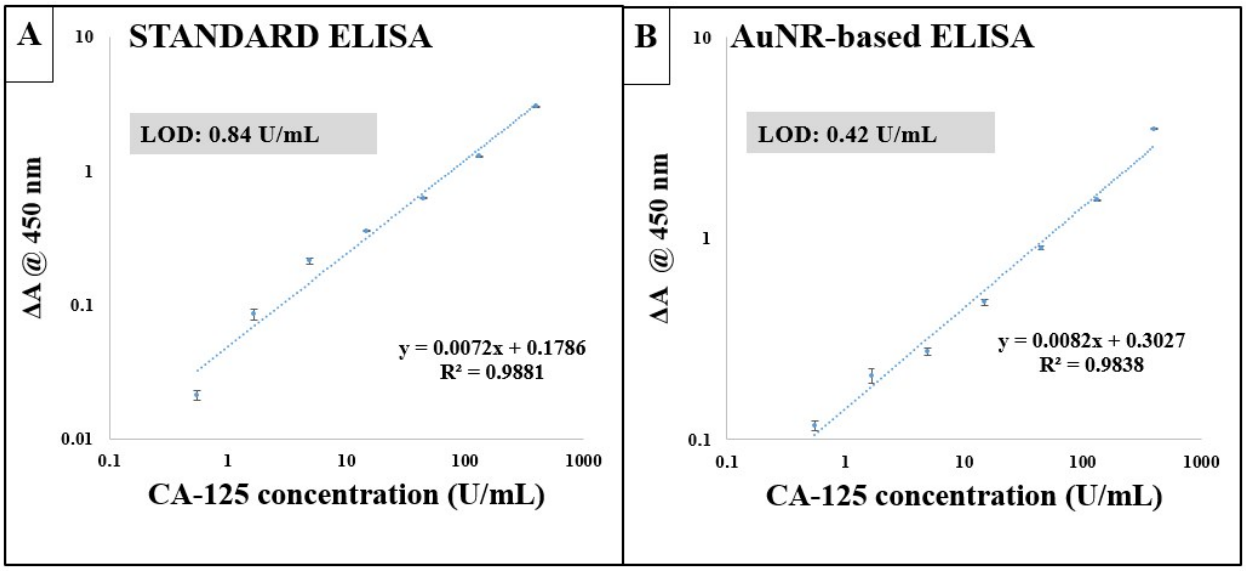


**Fig. S3.** Linear calibration curve showing sensitivity of ELISA and NanoELISA towards different concentrations of CA125.

**Table S1** Optimization of HRP–HPPA reaction.

| HPPA mg/mL | $H_2O_2$ % | ΔFI (nm)* | | |
|---|---|---|---|---|
| | | HRP 10 nM | HRP 50 nM | HRP 100 nM |
| 5 | 0.03 | 83.33 | 127.61 | 215.9 |
| | 0.06 | 44.74 | 229.46 | 399.53 |
| | 0.1 | 19.99 | 52.11 | 199.23 |
| | 0.3 | 15 | 44.11 | 190.29 |
| | 0.6 | 12.1 | 25.24 | 46.1 |
| 7.5 | 0.03 | 15.1 | 28.99 | 54.99 |
| | 0.06 | 26.45 | 106.56 | 63.37 |
| | 0.1 | 20.02 | 36.07 | 189.78 |
| | 0.3 | 71.23 | 139.36 | 243.83 |
| | 0.6 | 84.7 | 202.4 | 334.14 |

* : Difference in fluorescence intensity with respect to control; ΔFI= FI for test HRP concentration– FI for control.

**Table S2** Optimization of volume ratio of AuNRs: blocking buffer and AuNRs: anti-CA125 for improved nanoELIFA response.

| Volume ratio optimization | | Fluorescence intensity | | ΔFI* |
|---|---|---|---|---|
| | | 0 U/mL | 100 U/mL | |
| AuNRs: blocking buffer | 1:1 | 19.29 | 56.34 | 37.05 |
| | 2:1 | 20.49 | 72.11 | 51.62 |
| | 4:1 | 23.21 | 80.23 | 57.02 |
| | 20:1 | 25.35 | 131.34 | 105.99 |
| | 40:1 | 37.23 | 129.3 | 92.07 |
| AuNRs: anti-CA125 | 10:1 | 45.32 | 134.46 | 89.14 |
| | 20:1 | 25.35 | 131.34 | 105.99 |
| | 40:1 | 22.84 | 81.32 | 58.48 |
| | 60:1 | 20.54 | 63.47 | 42.93 |

* : Difference in fluorescence intensity with respect to control; ΔFI= FI for 100 U/mL– FI for 0 U/mL.

**Table S3** Optimization of AuNR concentration for nanoELIFA

| AuNR concentration (μM) | Fluorescence intensity | | ΔFI* |
|---|---|---|---|
| | 0 U/mL | 100 U/mL | |
| Conjugate 1[a] | 23.42 | 74.22 | 50.8 |
| Conjugate 2[b] | 25.43 | 131.21 | 105.78 |
| Conjugate 3[c] | 60.23 | 134.25 | 74.02 |
| Conjugate 4[d] | 86.43 | 138.8 | 52.37 |

a : AuNR concentration = 3.8 μM; b : AuNR concentration = 7.6 μM; c : AuNR concentration = 15.2 μM; d : AuNR concentration = 30.4 μM

* : Difference in fluorescence intensity with respect to control; ΔFI= FI for 100 U/mL– FI for 0 U/mL.

**Table S4** Optimization of incubation time (min) after addition of HPPA and $H_2O_2$.

| Incubation time (min) | Fluorescence intensity | | ΔFI* |
|---|---|---|---|
| | 0 U/mL | 100 U/mL | |
| **0** | 20.22 | 27.24 | 7.02 |
| **1** | 22.45 | 56.74 | 34.29 |
| **3** | 24.32 | 81.32 | 57 |
| **5** | 25.15 | 130.34 | 105.19 |
| **10** | 26.32 | 131.35 | 105.03 |
| **20** | 27.23 | 132.35 | 105.12 |
| **30** | 28.11 | 134.32 | 106.21 |

* : Difference in fluorescence intensity with respect to control; ΔFI= FI for 100 U/mL– FI for 0 U/mL.

**Table S5** Statistical performance of the ELIFA and nanoELIFA for CA125 detection.

| CA125 (U/mL) | RSD % | | | | | |
|---|---|---|---|---|---|---|
| | Run-to-Run | | Day-to-Day | | Batch-to-Batch | |
| | ELIFA | Nano-ELIFA | ELIFA | Nano-ELIFA | ELIFA | Nano-ELIFA |
| 50 | 1.3 | 1.7 | 2.7 | 2.6 | 3.6 | 3.4 |
| 100 | 1.8 | 1.5 | 1.9 | 1.5 | 3.3 | 3.7 |
| 200 | 1.2 | 1.6 | 1.8 | 2.2 | 3.1 | 2.2 |

a : Run-to-run RSDs estimated from three parallel analyses for each concentration.

b : Day-to-day RSDs estimated from three parallel analyses for each concentration.

c : Batch-to-batch RSDs estimated from three parallel analyses for each concentration.