Electronic Supplementary Information

Computational Design and Clinical Demonstration of an Affordable Copper Nanocluster based Universal immunosensor for Sensitive Diagnostics

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Characterization of copper nanoclusters

Transmission Electron Microscopy

The sample for TEM imaging was prepared by drop casting diluted solution of CuNCs on to a carbon coated copper grid and dried under vacuum before the imaging procedure. The analysis of the TEM images of the copper nanoclusters was performed. Based on the size measurements of particles using ImageJ, the size of the clusters was confirmed to be 2-5 nm as seen in figure S1. The particles were found to be moderately monodispersed.

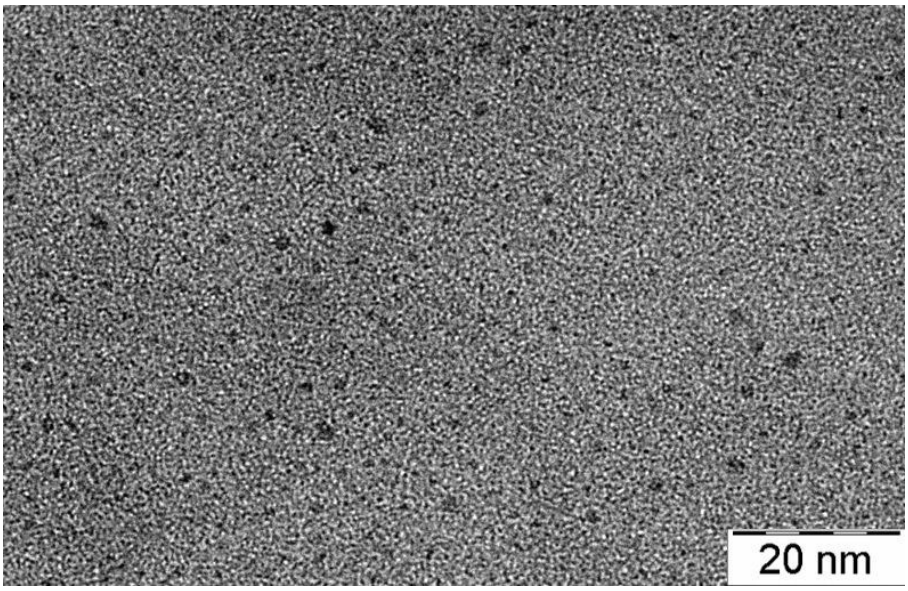

Figure S1 TEM image of Copper nanoclusters which indicates the size of the CuNCs to be around 2-3 nm.

UV-Visible absorption spectroscopy

Absorbance spectrum was obtained (Shimadzu UV-2450) by using UV-Vis absorption spectrophotometer. Figure S2 shows the UV-visible absorbance spectra of the glutathione capped CuNCs. The luminescent CuNCs have a unique UV−Vis absorption, which shows no characteristic peaks unlike the larger particles. The characteristic absorption peak at 507 nm, arising from the surface plasmon resonance of Cu nanoparticles was not observed in UV-Vis spectra[20]. A peak like feature observed at 350 nm can be attributed to UV absorption of glutathione conjugated to copper as observed in many other nanoparticles[21]. This indicated the formation of CuNCs.

Photoluminescence Spectroscopy

Photoluminescence excitation and emission spectra were recorded on Spectramax M5 spectrophotometer. The copper nanoclusters exhibited broad emission which peaked at 598 nm when excited with 394 nm as shown in the figure S2. The observed orange luminescence originates from the quantum confinement effects due to extra small size of these nanoclusters[22]. The addition of glutathione also lead to the creation of new energy states which resulted in the enhancement of fluorescence[23]. The fluorescence emission from CuNIS remained unchanged for more than 3 months exhibiting their good colloidal stability which is highly needed for the immunoassay applications

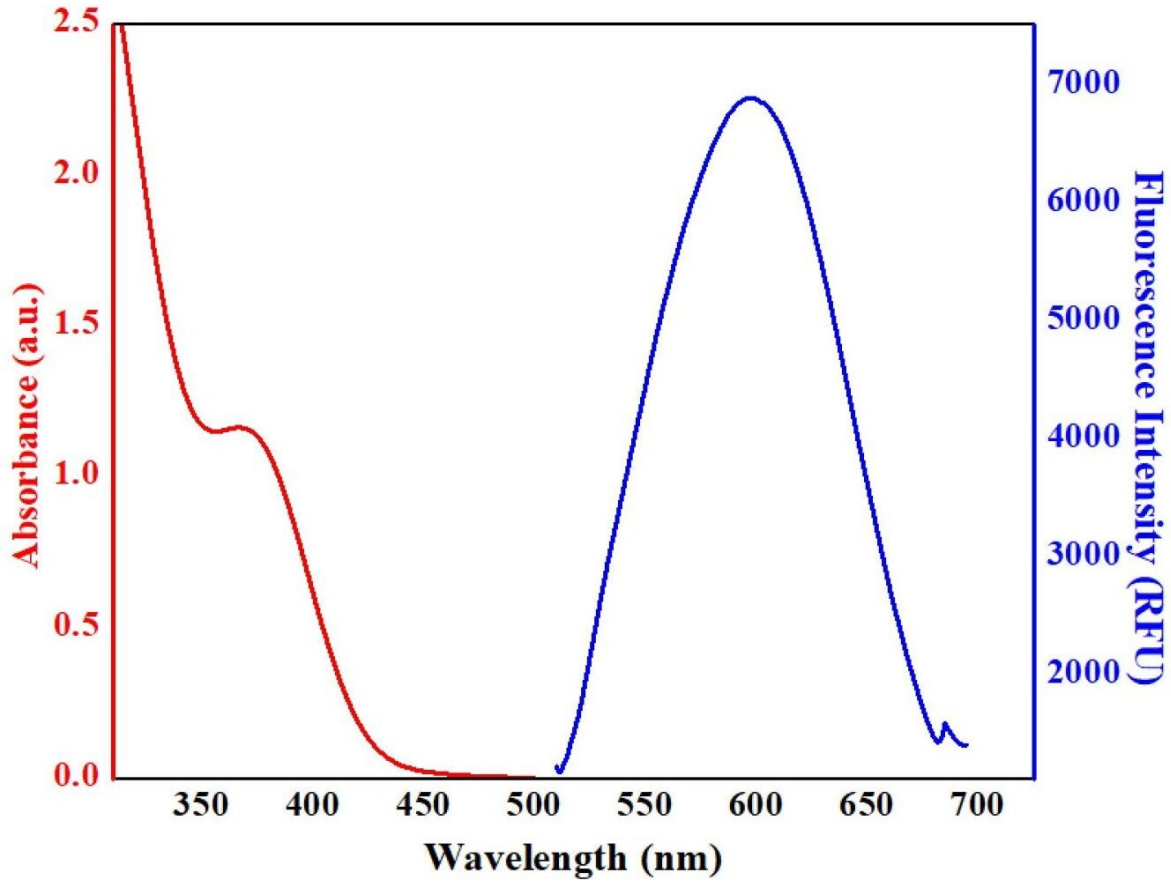

Figure S2 The absorbance and photoluminescence spectra of glutathione functionalized copper Nanocluster

Optimization of CuNIS concentration in HIV-1 p24 immunoassay

Concentration of CuNIS greatly affects the performance of the assay. Six concentrations of CuNIS, 1 µg/ml, 2 µg/ml, 2.5 µg/ml, 3.0 µg/ml, 3.5 µg/ml, 4 µg/ml and 5 µg/ml were taken to study its effect on the Signal to blank ratio (S/B ratio). The blank measurements were performed by performing the immunoassay in absence of the antigen. The optimization of CuNIS was carried out by measuring and calculating the S/B ratio for concentration of 250 pg/mL of p24 antigen. It is clear from the figure S3 that no linear relation exists between the CuNIS concentration and the S/B ratio. At a concentration 3.0 µg/ml of CuNIS the highest S/B ratio was observed. With an increase in CuNIS concentration beyond the optimal concentration the blank intensity was also observed to have increased rapidly when compared to the signal intensity. This increase of blank intensity could be attributed to the non-specific absorption of CuNIS.

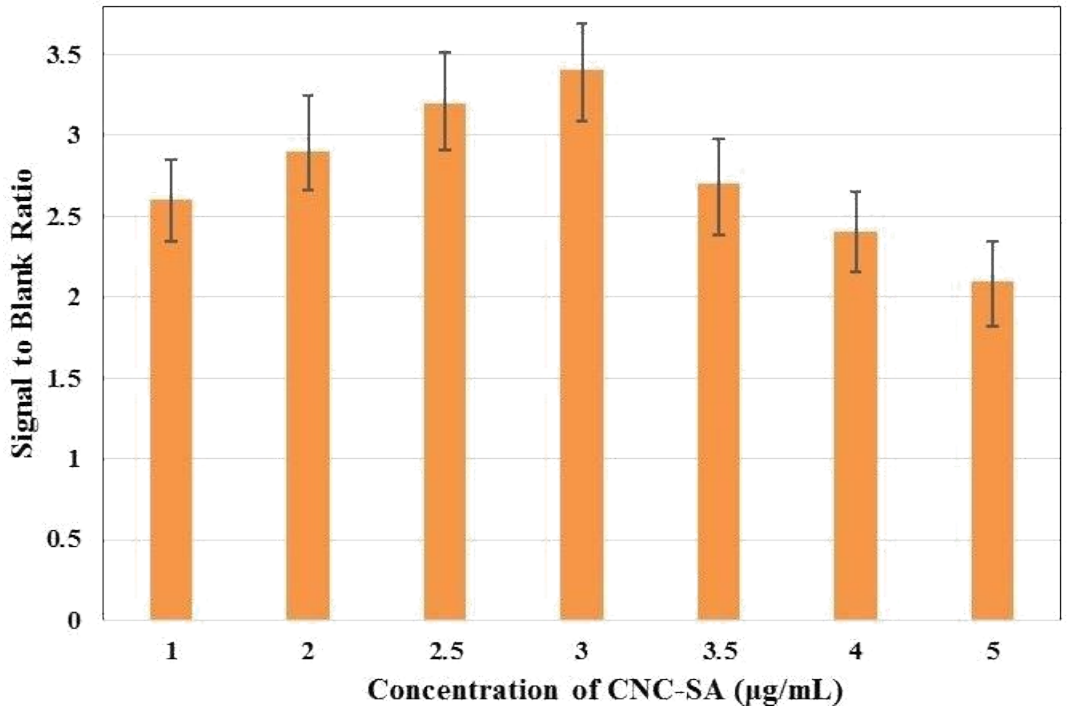

Figure S3- Effect of varying concentration of CuNIS on signal to blank ratio

Effect of presence of an interfering protein

The presence of various proteins and other biomolecules in the assay samples and reagents necessitated the study of interference from other proteins on the immunoassay. BSA was chosen specifically as it is inherently adhesive, thus causing a possible interference. However, it was observed that the BSA has no effect on the signal intensities in the immunoassay. The figure S4(a) clearly illustrates that there is barely any alteration in the signal strength with increasing BSA concentration. This further reinforces the stability of the immunoassay protocol.

Effect of coexistent virions

To further analyze the extent of specificity and cross reactivity of CuNIS, HCV positive plasma samples were spiked in HIV-1 p24 antigen for a fixed concentration of 500 pg/mL. The results as depicted in figure S4(b) indicate that there is no perceivable effect on the fluorescence signal from the HCV virions suggesting that CuNIS is an exceptionally selective and stable sensor.

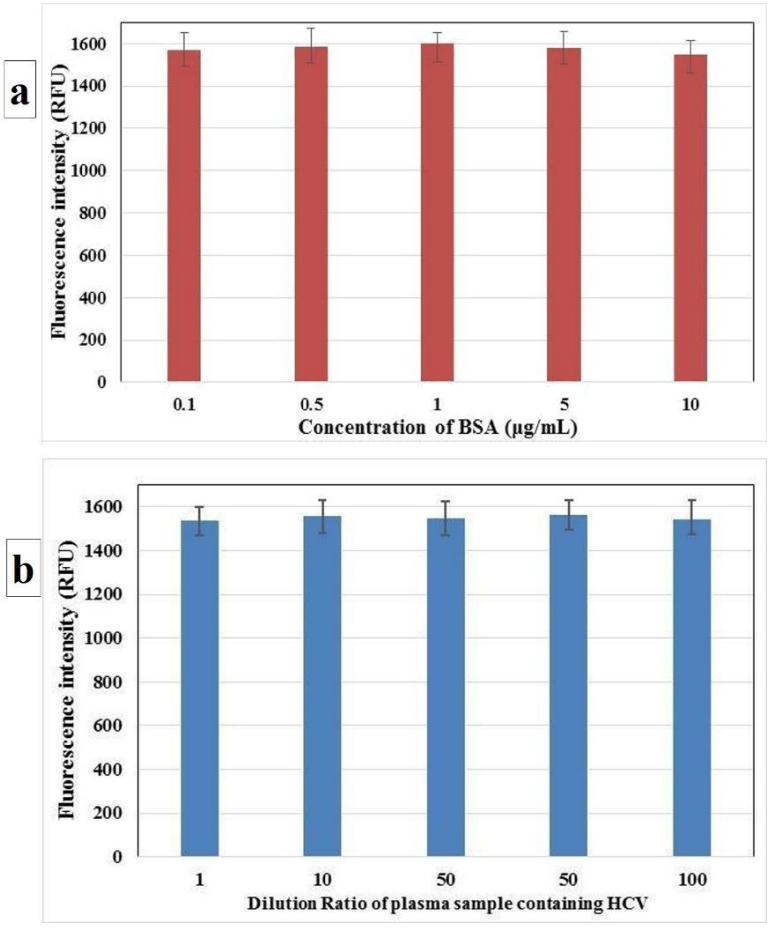

Figure S4 (a) Plot of fluorescence intensities for 500 pg/mL p24 antigen in samples with varying concentration of BSA (b) Effect of dilution of HIV-1 p24 spiked HCV sample on fluorescence intensity from CuNIS.

Recovery rate of the CuNIS based assay

Further evaluation of the analytical sensitivity of the assay was done by taking known concentrations of p24 antigen and spiking the HIV negative plasma samples collected from healthy individuals (HIV negative sample). The plasma samples were diluted 100 times which in turn reduced the sample amount required for analysis. To the diluted plasma sample, purified p24 antigen concentrations of 31, 62, 125, 250, 500 and 1000 pg/mL were added. All experiments were conducted in triplicate and the concentration was calculated by taking the average signal response and comparing it with the standard curve. It is clear from table 8.4 that the concentrations of p24 antigen calculated from the results of CuNCIA are comparable to the actual amounts spiked into sample. Thus, CuNIS can quantitatively determine the concentration of HIV-1 p24 antigen in clinical plasma samples.

Table S1: The recovered concentration of HIV-1 p24 using CuNIS based Immunoassay

S. No	Concentration spiked (pg/mL)	p24 detected(pg/mL)
1	31	27.2 ± 2.1
2	62	56.3 ± 3.2
3	125	114.7 ± 7.2
4	250	233.3 ± 19.2
5	500	476.9 ± 31.2
6	1000	967.2 ± 49.1