Supplementary Material

Choline-Induced Selective Fluorescence Quenching of Acetylcholinesterase Conjugated Au@BSA Clusters

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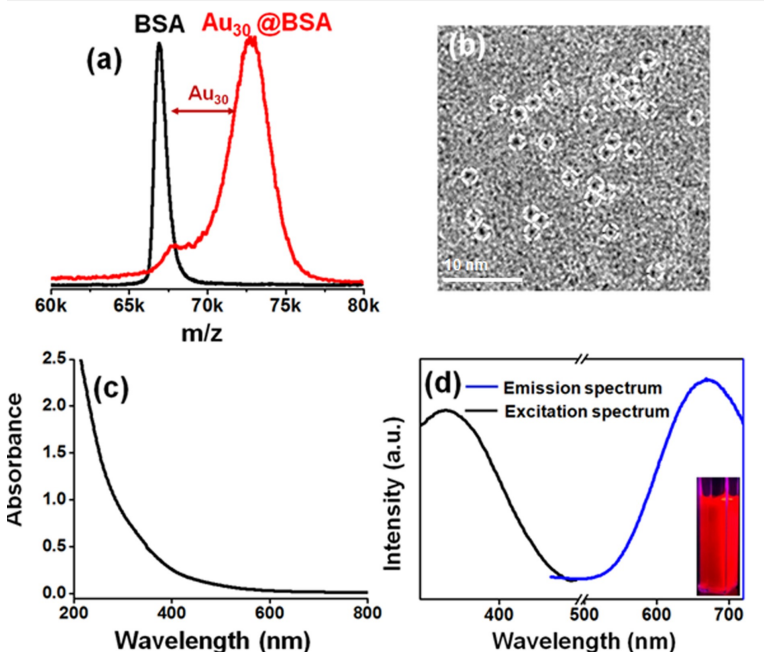
S1. SupplementaryFigure 1

Characterization of Au_{QC}@BSA



FigS1: *(a) MALDI TOF MS of BSA (black trace) and Au_{QC}@BSA (red trace) shows the presence of Au_{30} core in BSA. (b) Transmission electron microscope (TEM) image of Au_{QC}@BSA showing about 1 nm particle size. Some of them are circled. (c) UV –Visible absorption spectrum of Au_{QC}@BSA (d) Fluorescence excitation (black trace) and emission spectrum (blue trace) of Au_{QC}@BSA showing 677 nm emission when excited at 365 nm. Photograph of Au_{QC}@BSA under UV light irradiation (365 excitation) is shown in the inset.*

S2. Supplementary Figure 2

Characterization of AChE conjugated cluster (Au_{QC}@BSA-AChE)

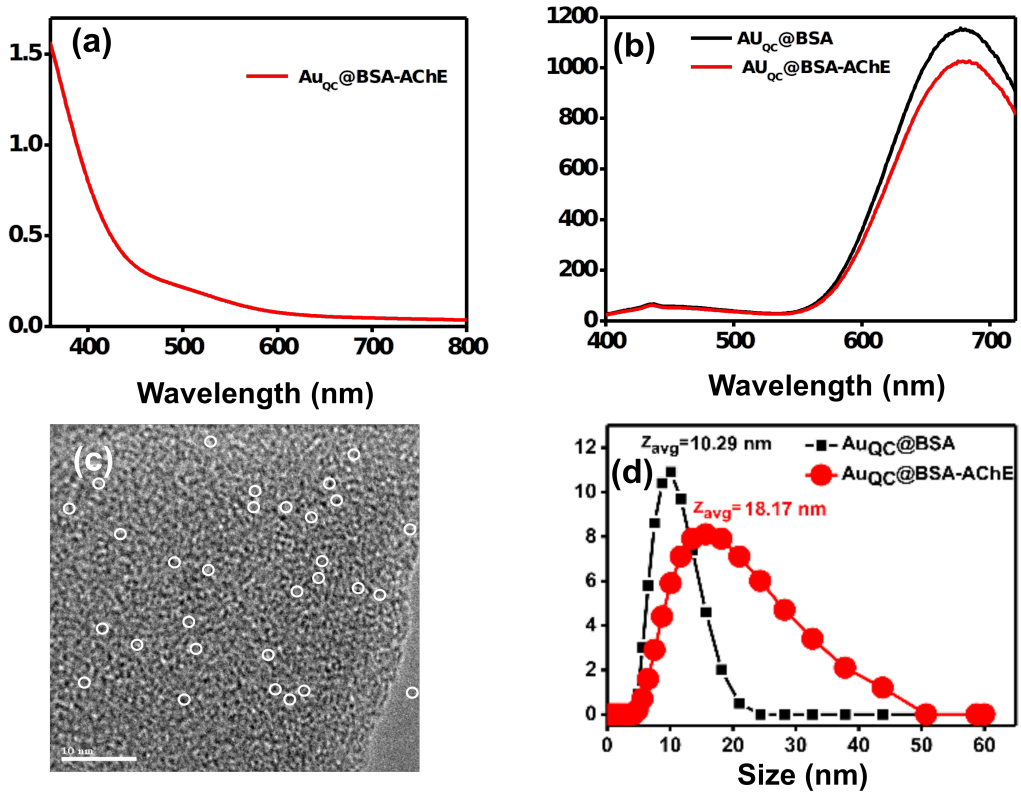


Fig S2:*(a)UV-visible absorption spectrum of Au_{QC}@BSA-AChE.(b)Fluorescence emission spectra of BSA protected cluster (Au_{QC}@BSA)(black) and acetylcholinesterase conjugated cluster (Au_{QC}@BSA-AChE) (red). (c) TEM image of AChE conjugated cluster (Au_{QC}@BSA-AChE)with cluster core of 1 nm. Some of them are circled.(d) Particle size distribution of Au_{QC}@BSA and Au_{QC}@BSA-AChE showing the size difference after conjugation of enzyme.*

S3. Supplementary Figure 3

Stern-Volmer plot

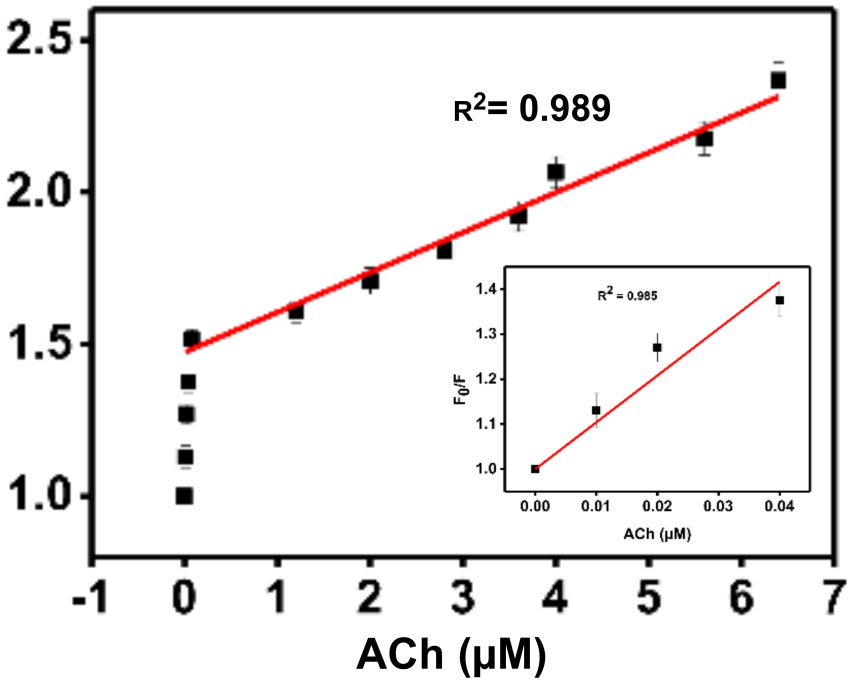
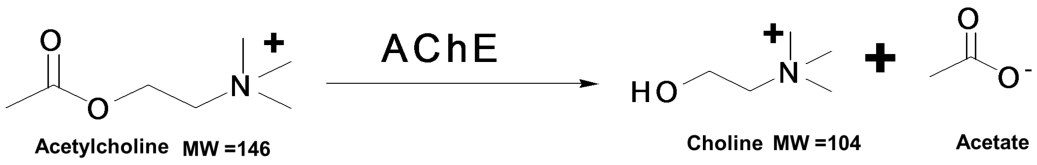


Fig S3: *Stern-Volmer plot constructed using the complete data taken from Fig 2B (0-6.4 μM). Inset shows the Stern-Volmer plot at lower concentration of ACh (0-0.04μM)*



Scheme S1: *Hydrolysis of acetylcholine on presence of AChE to give choline.*

S4. Supplementary Figure 4

Selectivity towards coexisting substances

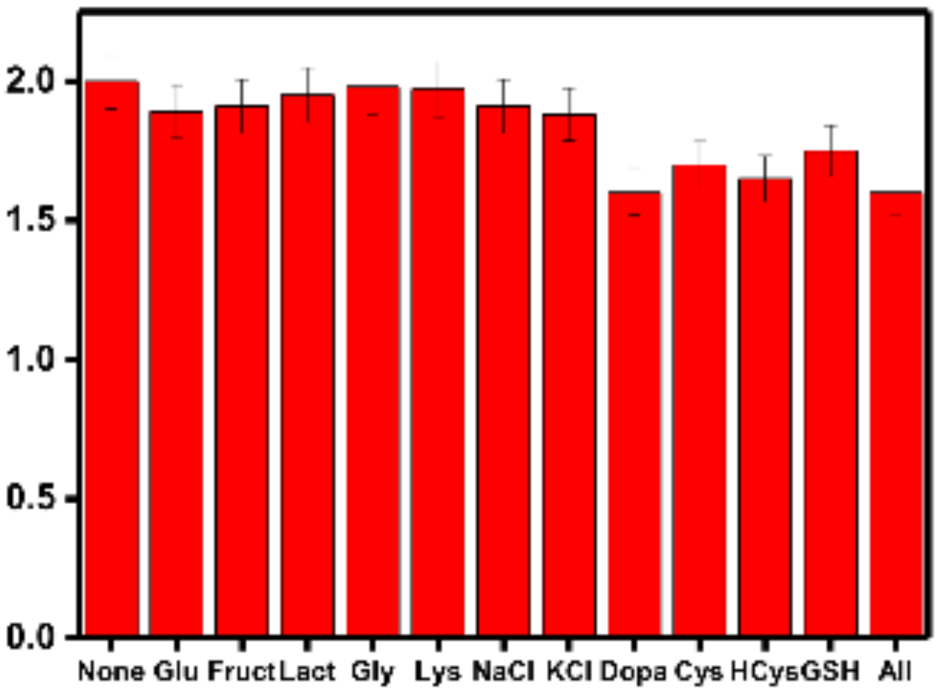


Fig S4:*Selectivity of the Au_{QC}@BSA-AChE for the detection of ACh over otherAnalytes such as Glucose (Glu), Fructose (Fruct), Lactose (Lact), Glycine (Gly), Lysine (Lys), NaCl, KCl, Dopamine(Dopa), cysteine(Cys), glutathione(GSH) and presence all analytes (All).Concentration of all analyte were maintained as 6.4 µM.The error bar represent standard deviations based on three independent measurements.*

S5. Supplementary Figure 5

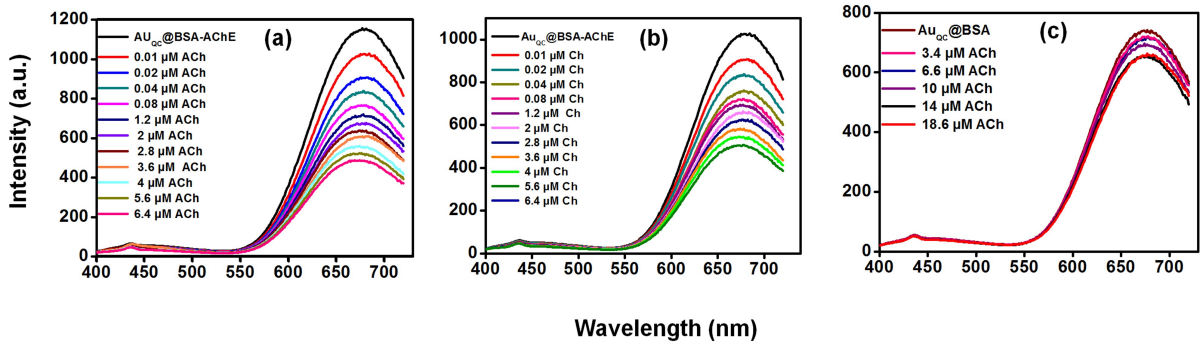


Fig S5:*(a) and (b) are showing the variation of the fluorescence emission spectrum of Au_{QC}@BSA-AChE with different concentrations of acetylcholine (ACh)(Repeat of Fig 1a),*

and choline (Ch), respectevly. (c) Emission intensity does not change much when different concentration of Ach was added to Au_{QC}@BSA solution as shown in c.

S6. Supplementary Figure 6

Fluorescence study with tetramethylammonium bromide

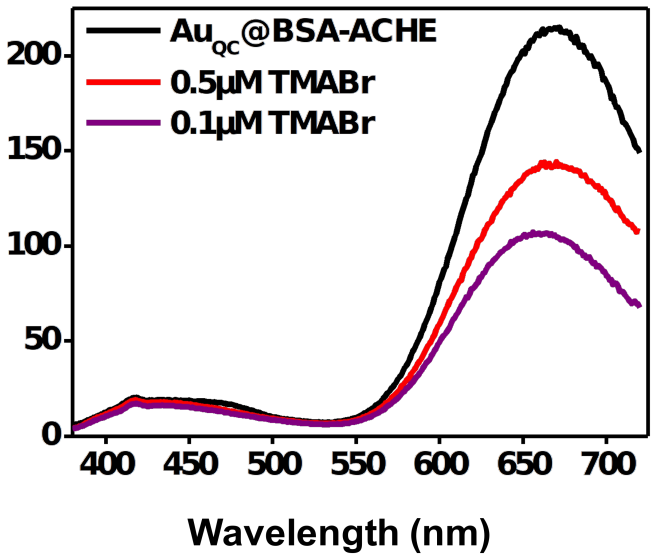


Fig S6:*Flourescence emission spectra of Au_{QC}@BSA-AChE with different concentrations of tetramethylammonium bromide (TMABr).*

S7. Supplementary Figure 7

Fluorescence study with alkyl alcohol

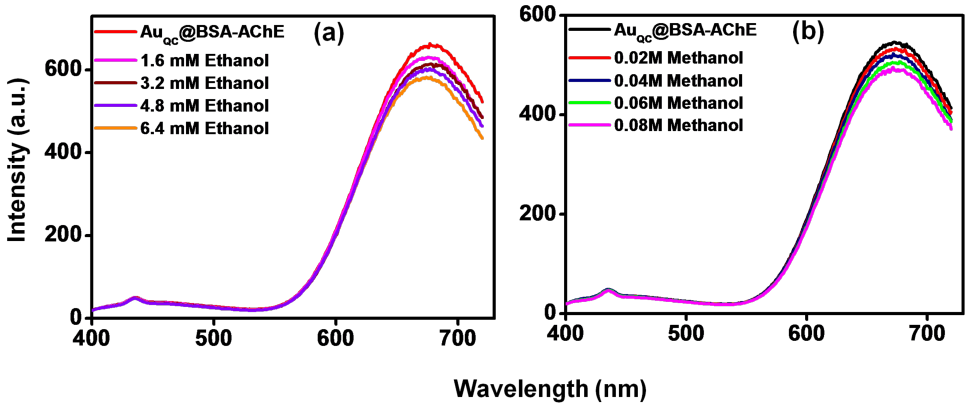


Fig S7: *(a) Emission spectra of Au_{QC}@BSA-AChE with different concentrations of ethanol and (b) emission spectra of Au_{QC}@BSA-AChE with different concentrations of methanol.*

S8. Supplementary Figure 8

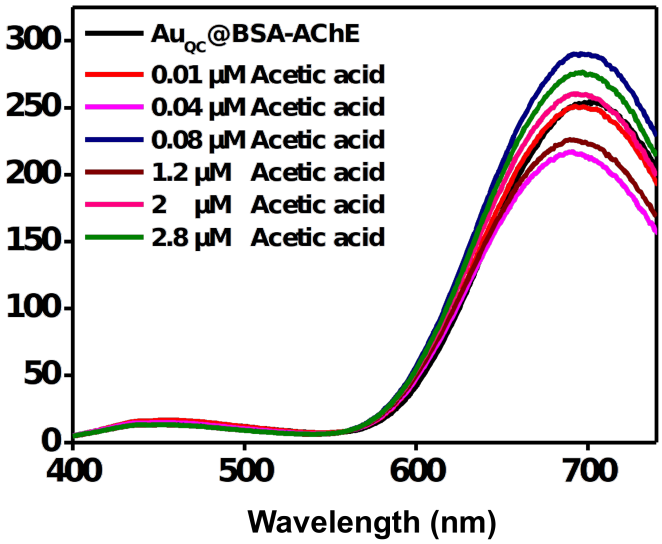


Fig S8:*Emission spectra of Au$_{QC}$@BSA-AChEwith different concentrations of acetic acid.*

S9. Supplementary Figure 9

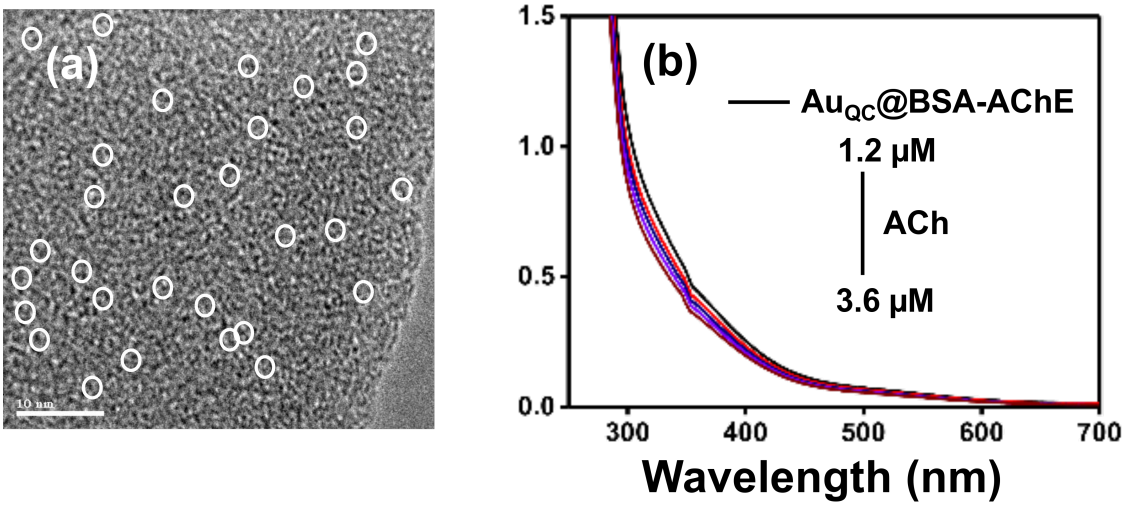


Fig S9:**(a)** *TEM image of Au$_{QC}$@BSA-AChE) after the treatment of ACh(6.4 µM) with cluster core of 1 nm. Some of them are circled. (b)UV -Visible absorption spectrum showing change in absorbance of Au$_{QC}$@BSA-AChE after addition of different concentration of ACh.*

S10. Supplementary Figure 10

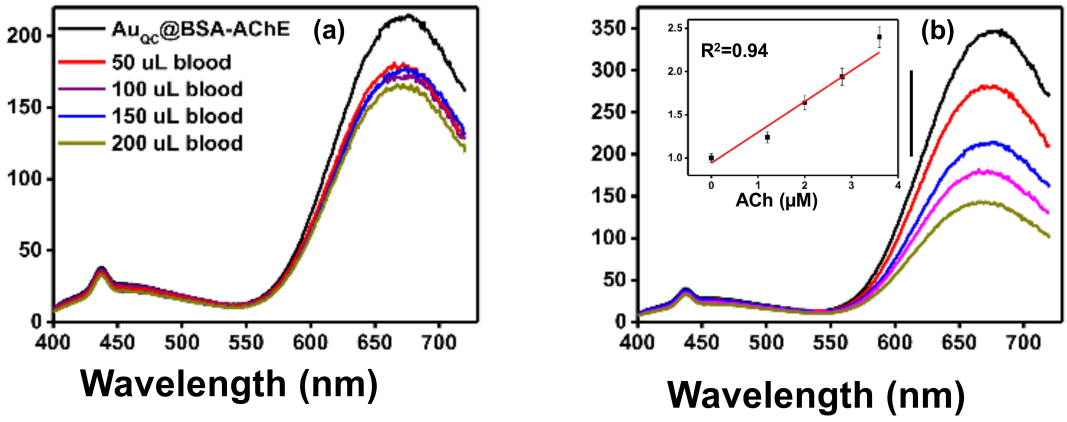


***Fig S10:*(a)** Emission spectra of *Au_{QC}@BSA-AChE with different amount of blood, (b) Emission spectra of Au_{QC}@BSA-AChE with different concentration of ACh spiked blood sample.The arrows indicate the signal changes as increases in ACh concentrations (1.2, 2, 2.8 and 3.6 μM). Inset: a plot of the values of F_0/F at 677 nm versus the concentrations of ACh.*

S1. Supplementary Table 1

Methods	LOD	Linearity	Reference
Amperometry	1.0 μM	0.005–0.4 mM	(Hou et al. 2012)
Electrochemical	26.7 μM	0.25–5.88 mM	(Sattarahmady et al. 2010)
Potentiometriy	10 μM	10- 100 μM	(Barsoum et al. 2004)
Chemiluminescence	0.05 μM	0.05 -100 μM	(Korbakov et al. 2008)
Fluorescence	0.5 μM	0.5-60 μM	(Wei et al. 2014)
Fluorescence	10 nM	0.08-6.4 μM	This Study

Table S1: Comparison of analytical performance of present method with other methods for the detection of ACh.

Reference:

Barsoum, B.N., Watson, W.M., Mahdi, I.M., Khalid, E., 2004. Journal of Electroanalytical Chemistry 567(2), 277-281.

Hou, S., Ou, Z., Chen, Q., Wu, B., 2012. Biosensors and Bioelectronics 33(1), 44-49.

Korbakov, N., Timmerman, P., Lidich, N., Urbach, B., Sa'ar, A., Yitzchaik, S., 2008. Langmuir 24(6), 2580-2587.

Sattarahmady, N., Heli, H., Moosavi-Movahedi, A.A., 2010. Biosensors and Bioelectronics 25(10), 2329-2335.

Wei, J., Ren, J., Liu, J., Meng, X., Ren, X., Chen, Z., Tang, F., 2014. Biosensors and Bioelectronics 52, 304-309.