Electronic Supplementary Information (ESI)

# An ultrasensitive colorimetric sensor for efficient detection of $Hg^{2+}$at physiological pH

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## 1. Materials and methods:

### *1.1. Chemicals and stock solutions*

Silver nitrate ($AgNO_3$), trisodium citrate dihydrate ($C_6H_5Na_3O_7.2H_2O$), and sodium borohydride ($NaBH_4$) were procured from SRL Pvt. Ltd (India). Acetylcholinesterase (from *Electrophorus electricus*), and Mercury (II) chloride were purchasedfrom Sigma-Aldrich, India. Tris (hydroxymethyl) aminomethane and Acetylthiocholine Iodide (ATChI) were obtained from Himedia Laboratories Pvt. Ltd (India). The heavy metal salts like $Al^{3+}$, $Ni^{2+}$, $Cr^{3+}$, $Cd^{2+}$, $Mg^{2+}$, and $Zn^{2+}$ were purchased from Sigma-Aldrich, India, whereas$Fe^{2+}$, $Fe^{3+}$, $Cr^{6+}$, $Pb^{2+}$, and $Mn^{2+}$ were from SRL Pvt. Ltd (India). The cleaning of glassware apparatus was done with aqua regia solution, following which they were rinsed withMilli-Q waterat least thrice and dried thoroughly. All experiments were performed with analytical grade chemical reagentswithout additional purification.

The stock solutions and further dilutions were all freshly prepared in Tris buffer (10 mM, pH 7.4). Stock solutions of AChE (400 mU) and ATChI (10 mM) were kept at 4 ºC when not in use. Iodide ions in acetylthiocholine poses as a hindrance for lower level detection of $Hg^{2+}$. [1] Therefore, washing out of iodide ions by $AgNO_3$ and the removal of excess $Ag^+$ ions using NaCl was necessary. After treatment, final ATCh concentration was ~10 mM.

### *1.2. Synthesis of silver nanoparticles (AgNPs)*

Synthesis of silver nanoparticles capped withsodium citrate wasperformed using slight modifications to Creighton's method [2]. 125 µL of 100 mM$AgNO_3$solution was added to 50 mL Milli-Q water under vigorous stirring and ice-cold condition. Trisodium citrate (125 µL, 100 mM) solution was added to this as a stabilizing agent. After an incubation of few minutes, 3 mL

of 5 mM$NaBH_4$ was added. A prominent color change from colorless to pale yellow color was observed as a consequence of the rapid reduction of Ag ions by$NaBH_4$ and the formation of AgNPs. After stirring for 30 min, the colloidal AgNPs were stored for 24 hin dark condition.

*1.3. Instrumentation*

UV-visible absorption spectroscopy was performed for the synthesized AgNPs and reaction mixture using UV-2600 (Shimadzu, Tokyo, Japan) in the spectral range starting from 200 to 800 nm.The particle size distribution ofAgNPsbefore and after$Hg^{2+}$ addition were acquiredusing a particle size analyzer (90 Plus Particle Analyzer, Brookhaven instruments Corporation, NY, USA).

**References**


1. J. Sun, L. Guo, Y. Bao and J. Xie, *Biosens. Bioelectron.*, 2011, **28**, 152–157.
2. M. Elavarasi, A. Rajeshwari, S.A. Alex, D. N. Kumar, N. Chandrasekaran and A. Mukherjee, *Anal. Methods.*2014, **6**, 5161–5167.

## 2. Supporting figures:

**Figure S1.** UV-visible spectra for AgNPs with $Hg^{2+}$ ($10^{-9}$to $10^{-12}$M) added in the presence of ATCh (80 µM) and AChE (400 mU $mL^{-1}$)

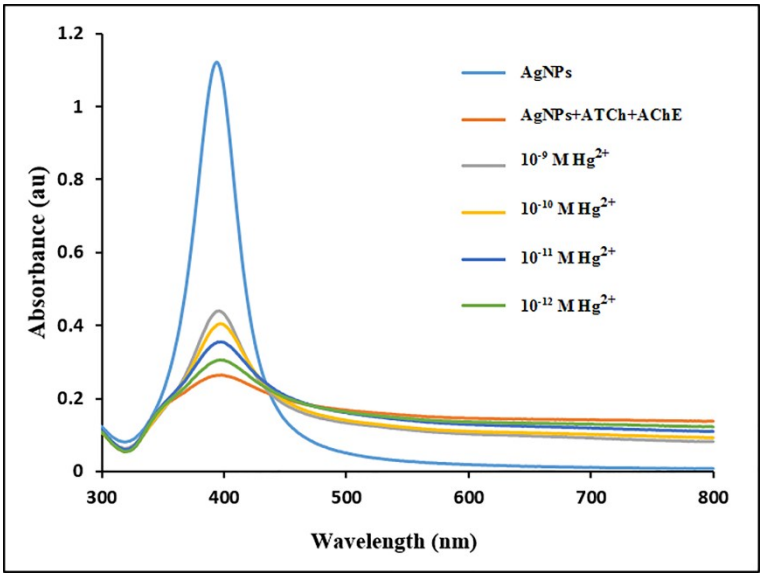

**Figure S2.** Hydrodynamic size distribution for (A) synthesized AgNPs and (B) addition of AChE (400 mU $mL^{-1}$) and ATCh (80 µM) to the AgNPs.

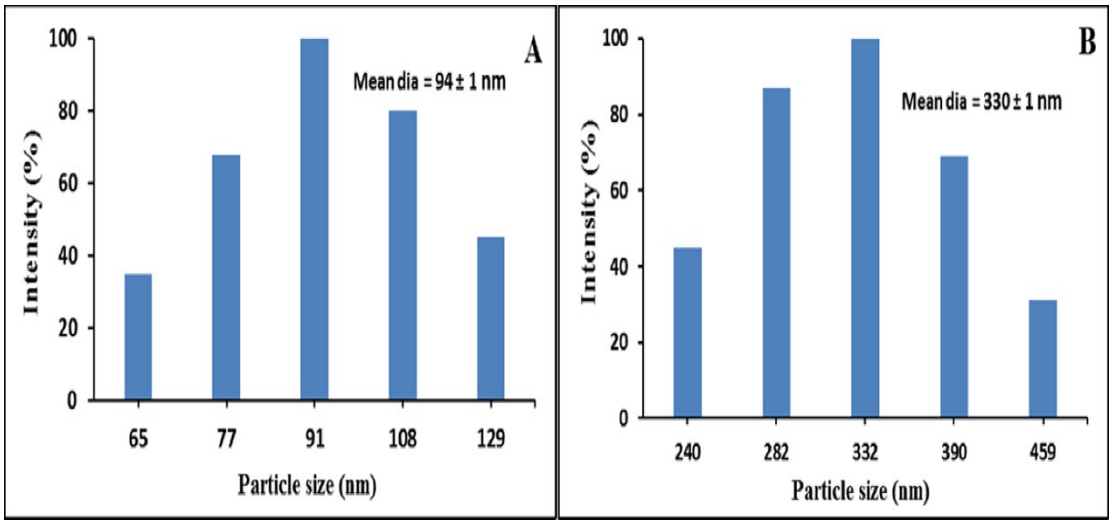

**Figure S3.** UV-visible spectral changes for AgNPs in the presence of ATCh (80 μM), AChE (400 mU $mL^{-1}$) and $Hg^{2+}$ (1 nM) alone and in presence of AgNPs, AChE and ATCh.

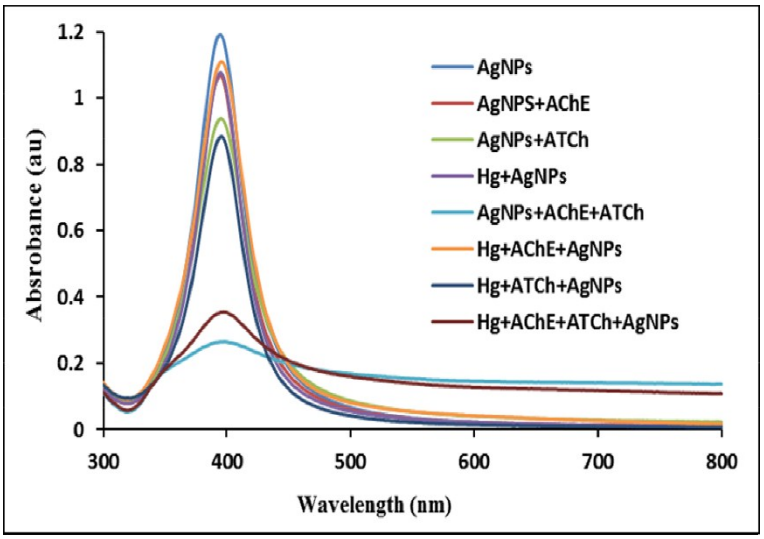

**Figure S4.** Hydrodynamic size distribution for AgNPs after addition of AChE(400 mU $mL^{-1}$), ATCh(80 μM) and various concentrations of $Hg^{2+}$ **(A)**$10^{-12}$M **(B)**, $10^{-11}$M **(C)**, $10^{-10}$M and**(D)**$10^{-9}$M.

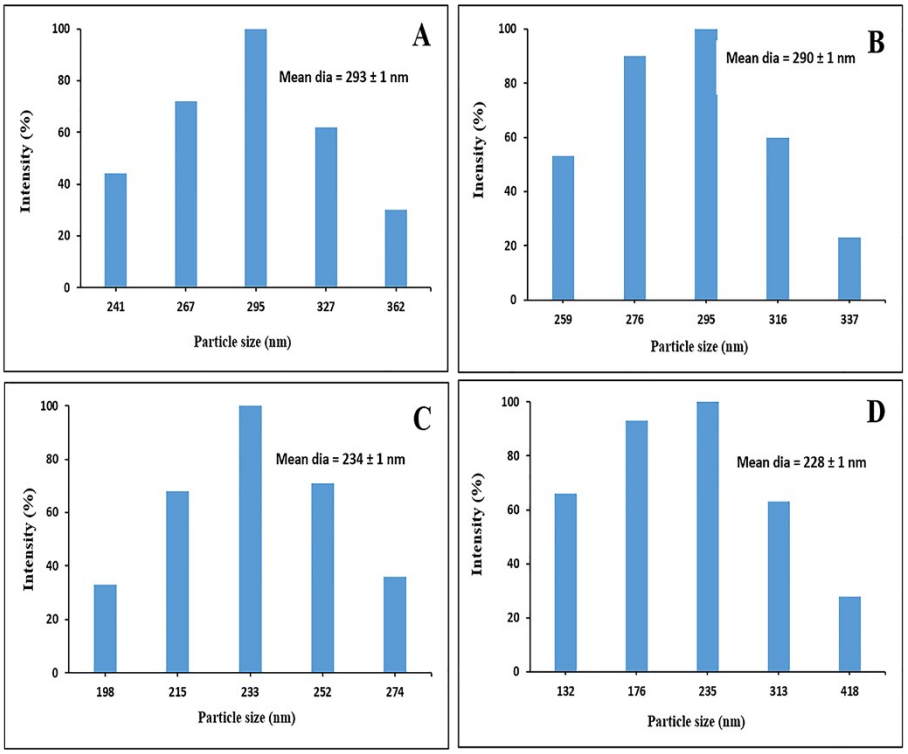

**Figure S5.** UV-visible spectra for AgNPs in presence of stimulated body fluid alone.