Supplementary Information

Colorimetric and Optical Hg(II) Ions Sensor Developed with Conjugates of M13-Bacteriophage and Silver Nanoparticles

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M13-bacteriophage growth, purification and amplification process:

$M13_{wild}$ was grown and purified following standard biochemical protocols described in literature.[1-4] Briefly, one colony of E. coli XL-1 blue was grown in 3 ml of LB media to mid-log phase (E. coli XL-1 blue culture) and infected with 10 µL of $M13_{wild}$. The culture was incubated at 37°C with shaking for 12 h, and then centrifuged to remove E. coli. The $M13_{wild}$ was collected by PEG/NaCl (20% PEG and 2.5 mol/L NaCl) precipitation and reconstituted in Tris-bufferred saline (10 mM). The typical yield was ~ 20 mg of $M13_{wild}$ per litter. The final concentration was determined spectrophotometrically using an extinction coefficient of 3.84 cm^2/mg at 269 nm.[3,5]

Attachment of cation binding peptides at the major coat protein (Gene VIII) of M13 is reported previously.[6] Briefly, two primers were designed to insert 4E into the gene VIII protein: 5′-ATATATCTGCAGNKGAAGAGGAAGAGCCCGCAAAAGCGGCCTTTAA CTCCC-3′ (4E), and 5′-GGAAGCTGCAGCGAAAGACAGCATCGGAACGAGG-3′ (linearization primer). To collect $M13_{4E}$ phages, the inverse polymerase chain reaction (PCR) cloning method was performed using the above mentioned primers (the linearization primer with $M13_{4E}$ primer). The PCR product was purified then the amplified plasmid, and phage plagues were verified by DNA sequencing. Furthermore, we have amplified the $M13_{4E}$ phage for our experiments, and the methods were same to the $M13_{wild}$ as mentioned above.[3, 5]

References:

1. S. Manivannan, I. Kang, Y. Seo, H. E. Jin, S. W. Lee and K. Kim, *ACS Appl. Mater. Interfaces*, 2017, **9**, 32965-32976.

2. N. Korkmaz, *Colloids Surf., B*, 2013, **112**, 219-228.

3. G.P. Smith and V.A. Petrenko, Phage display, *Chem. Rev.*, 1997, **972**, 391-410.

4. Y. Seo, S. Manivannan, I. Kang, S. W. Lee and K. Kim, *Biosens. Bioelectron.*, 2017, **94**, 87-93.

5. J. Sambrook and D.W. Russell, Cold Spring Harbor Laboratory Press 2001.

6. J. P. Park, M. Do, H. E. Jin, S. W. Lee and H. Lee, *ACS Appl. Mater. Interfaces*, 2014, **6**, 18653-18660.

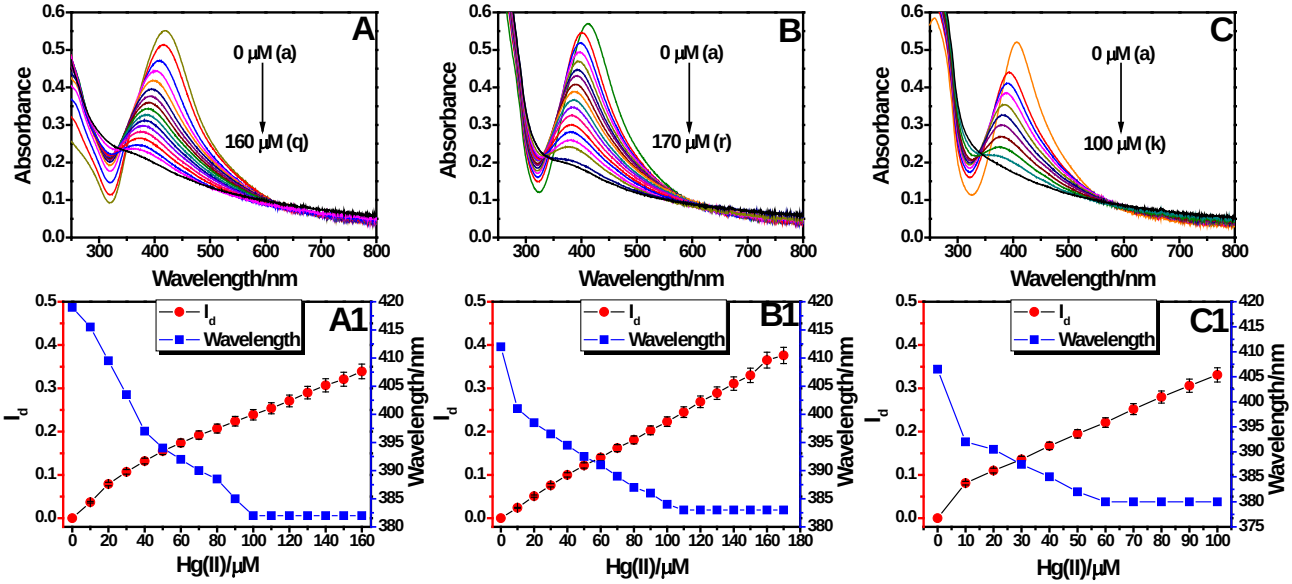


Figure. S1 (**A-C**) SPR absorption spectral changes and (**A1-C1**) plots of changes in the absorption intensity and wavelength observed for (**A** and **A1**) SSG–Ag NPs, (**B** and **B1**) SSG–Ag_{wild} NCBs, and (**C** and **C1**) SSG–Ag_{4E} NCBs upon each addition of 10 μM of Hg(II) ions.

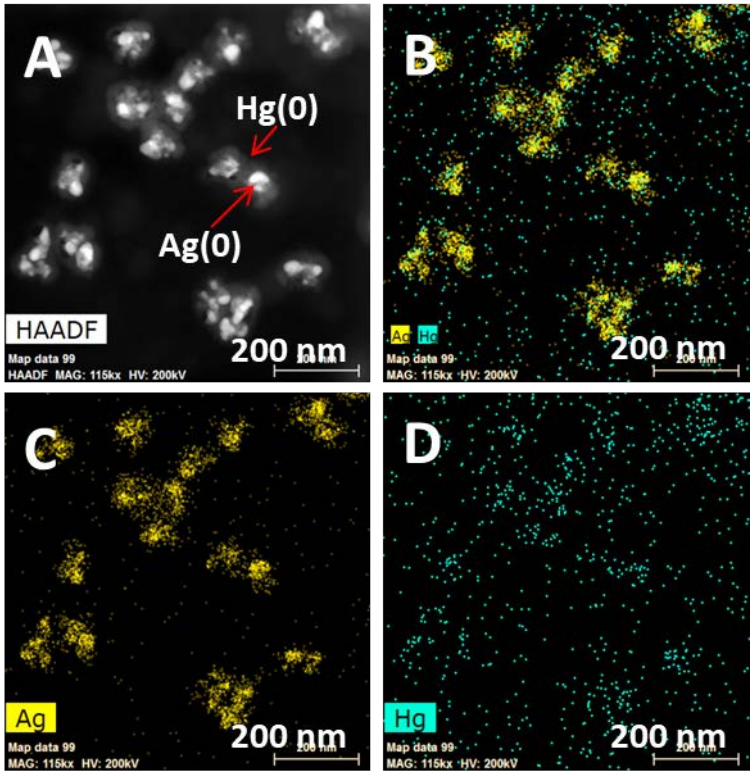
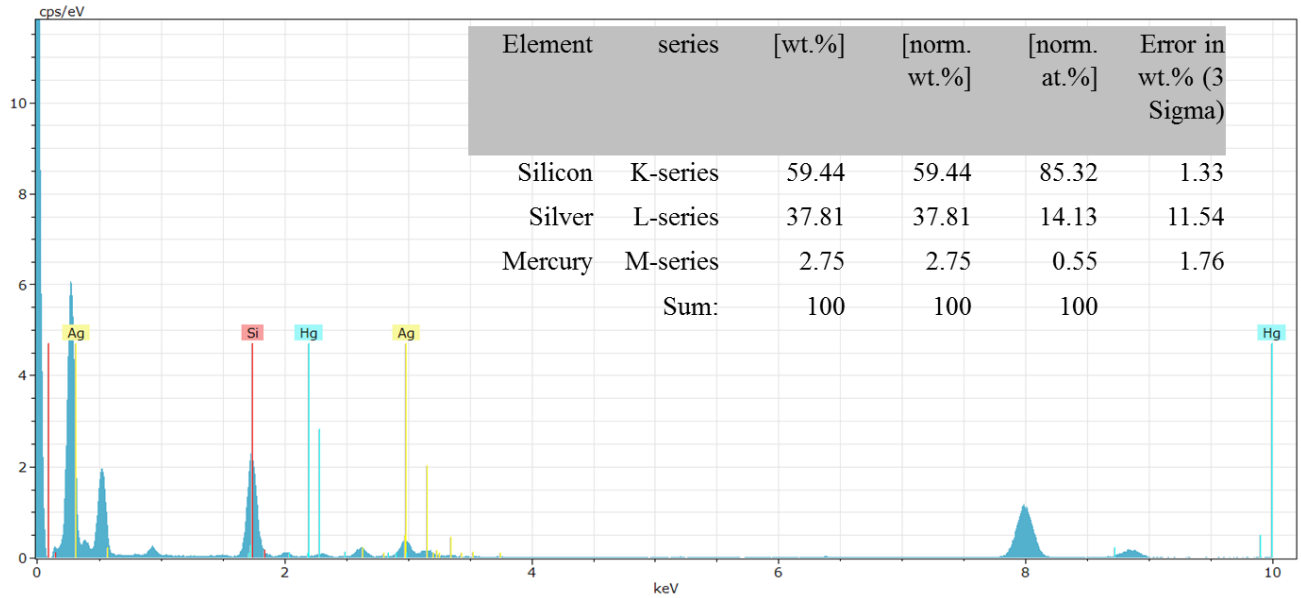


Figure. S2 (A) STEM-HAADF images and (**B-D**) STEM-EDX mapping analyses of AgHg-amalgam crystals.



Element	series	[wt.%]	[norm. wt.%]	[norm. at.%]	Error in wt.% (3 Sigma)
Silicon	K-series	59.44	59.44	85.32	1.33
Silver	L-series	37.81	37.81	14.13	11.54
Mercury	M-series	2.75	2.75	0.55	1.76
	Sum:	100	100	100	

Figure. S3 STEM-EDX point ID analysis of AgHg-amalgam crystals.

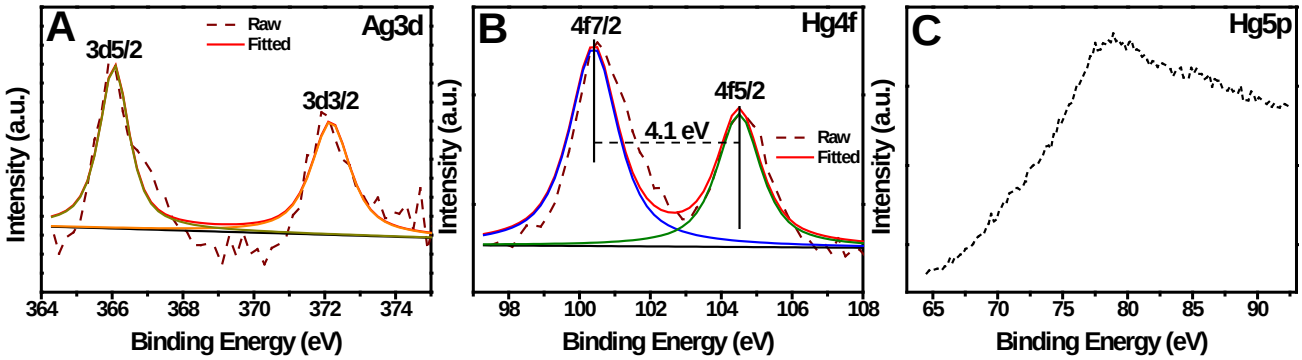


Figure. S4 XPS spectra obtained for the AgHg-amalgam crystals and their corresponding (**A**) Ag3d, (**B**) Hg4f, and (**C**) Hg5p regions of core-level spectra.

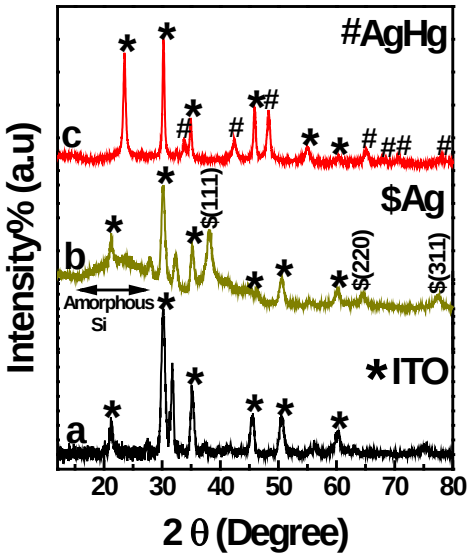


Figure S5. XRD analysis of (a) Bare ITO substrate, (b) SSG–Ag_{4E} CGs, (c) AgHg-amalgam crystals.

Table. S1 Quantification of Hg(II) ions in different water samples by using SSG–Ag_{4E} CGs and ICP-MS.

			Hg(II) Ions (µM)				
			SSG–Ag_{4E} NCBs			**ICP-MS**	
Samples	**Added (µM)**	**Found (µM)**	**Recovery (%)**	**RSD (%)**	**Found (µM)**	**Recovery (%)**	**RSD (%)**
Tab water	0	0	–	–	0	–	–
	25	24.93	99.73	2.02	24.57	98.28	0.74
	50	50.36	100.73	2.06	49.22	98.44	0.51
Pond water	0	0	–	–	0	–	–
	25	26.34	105.36	0.66	28.10	112.40	1.76
	50	52.85	105.71	1.79	54.20	108.4	0.99