

Supporting Information

Direct Surface Enhanced Raman Spectroscopic Detection of Cortisol at Physiological Concentrations

T. Joshua Moore and Bhavya Sharma*

Department of Chemistry, The University of Tennessee Knoxville, 1420 Circle Drive, Knoxville, Tennessee 37996, United States

Corresponding author email: bhavya.sharma@utk.edu

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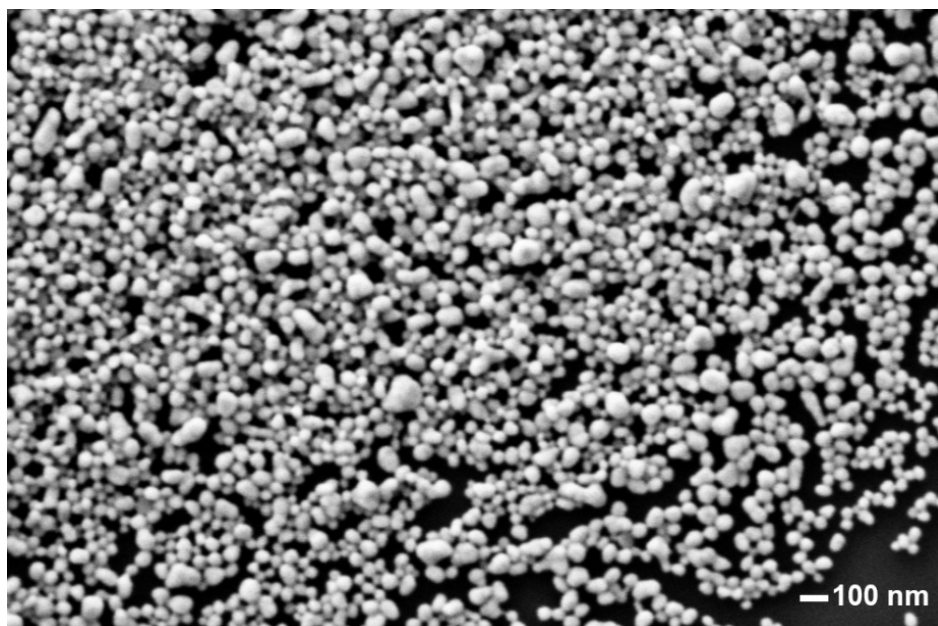


Figure S1. SEM image of the prepared hydroxylamine -reduced silver nanoparticles (HA-AgNP), showing good monodispersity and an average diameter of ~ 30 nm.

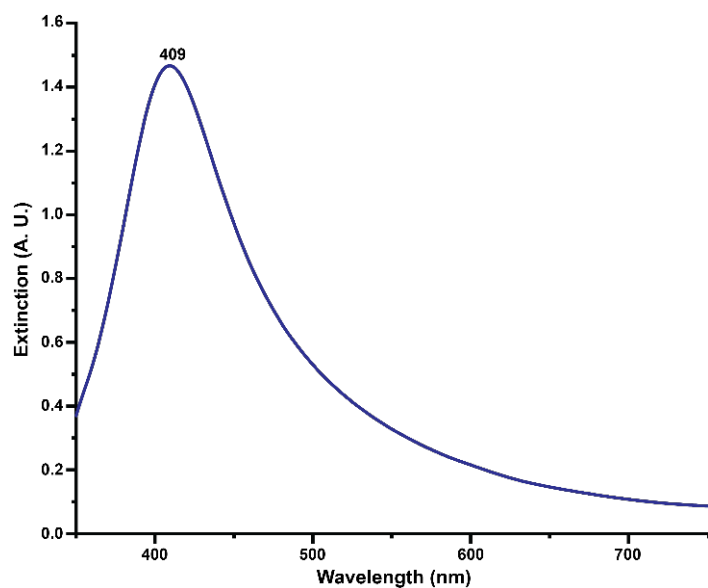


Figure S2. Extinction spectrum of synthesized HA-AgNP, showing an LSPR maximum located at 409 nm.

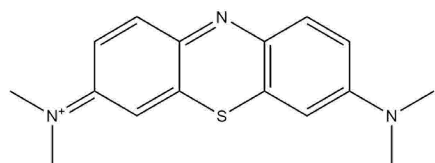


Figure S3. Structure of methylene blue

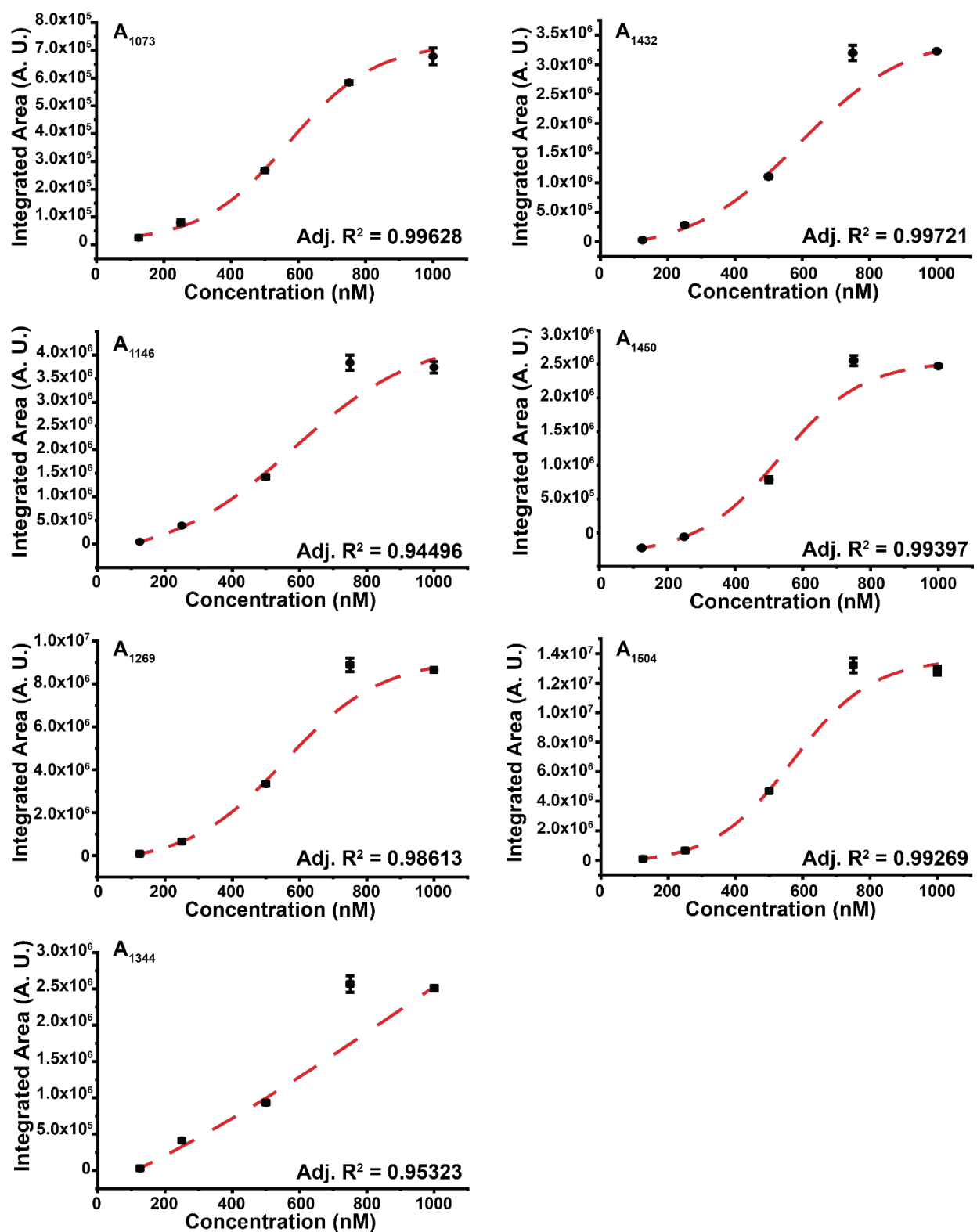


Figure S4. Sigmoidal concentration response plots for the observed peaks in SERS spectra of cortisol obtained from samples in the physiological range (125-1000 nM). Error bars are present on all data points, but the small magnitude of some error bars is obscured by the data marker in several instances.

Limit of Detection (LOD) Determination

The linearity of the concentration response of the SERS spectra was investigated by peak fitting the individual 30 s exposures for each concentration of cortisol. The peaks were fit using GRAMS/AI software (ThermoScientific), and the resultant area values were analyzed statistically. Each peak's mean area was plotted (Origin software) as a function of concentration, and error bars were established as ± 1 standard deviation from the mean. To determine the limit of detection (LOD) for cortisol, the 1504 cm^{-1} peak was selected as the peak of interest since it has an R^2 value closer to unity in the linear regression analysis. Three frames at each of the concentrations were collected and the peak area was integrated for each spectrum. The resulting concentration and integrated area data were analyzed using ANOVA, and the standard error of the intercept was calculated. The LOD ($3.3 \times \text{standard error/slope}$) was calculated to be 177 nM, which agrees with visual LOD analysis of ~ 200 nM.

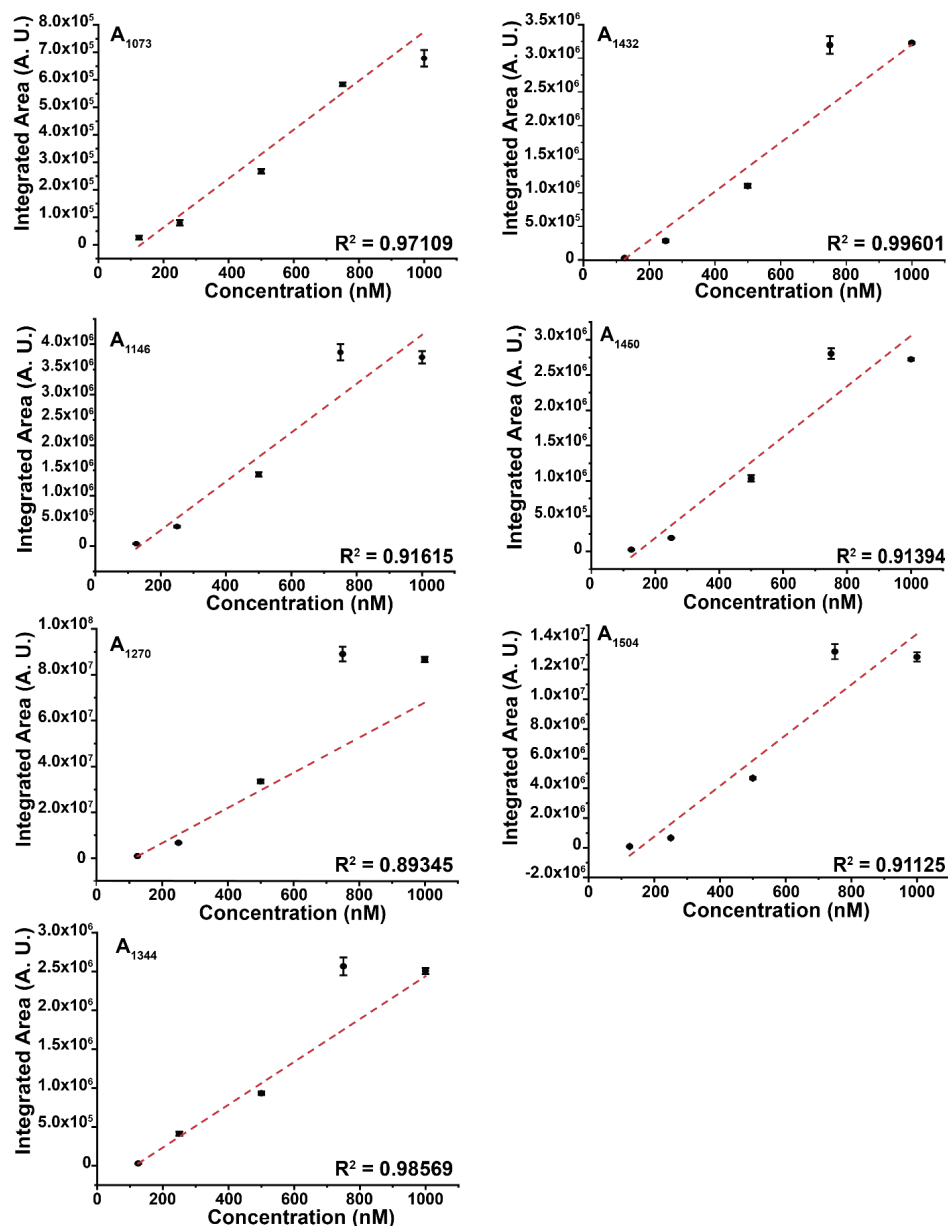


Figure S5. Linear regression plots for the observed peaks in SERS spectra of cortisol obtained from samples in the physiological range (125-1000 nM). Error bars are present on all data points, but the small magnitude of some error bars is obscured by the data marker in several instances.

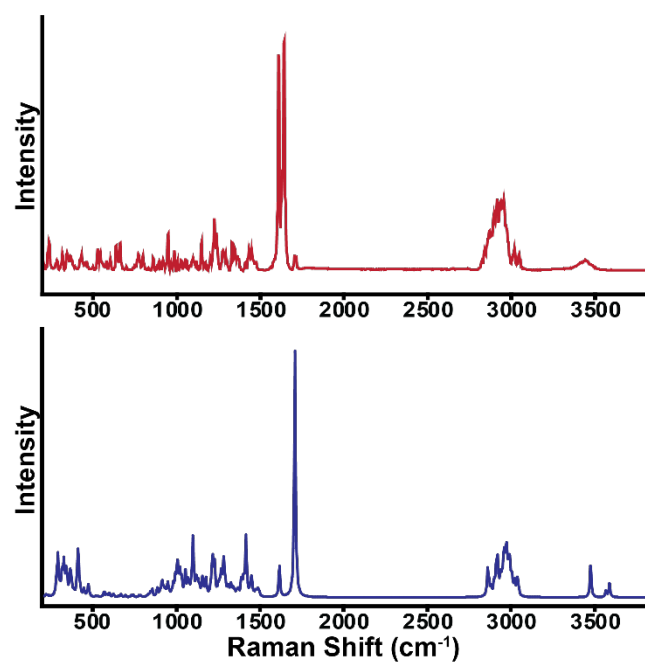


Figure S6. Experimental (top, red) and theoretical (bottom, blue) normal Raman spectra for cortisol in the region from 200 – 4000 cm^{-1} . For the experimental spectrum, $\lambda_{\text{ex}} = 532 \text{ nm}$, $P = 10 \text{ mW}$, and $t = 10 \text{ s}$.

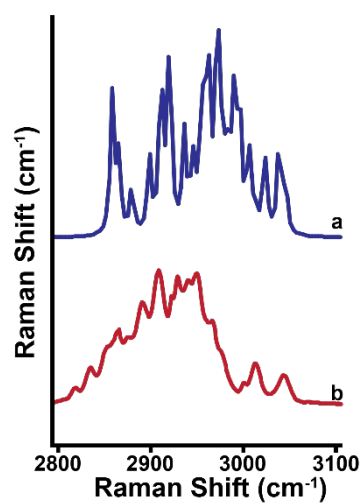


Figure S7. Theoretical (a) and experimental (b) normal Raman spectra for cortisol in the high wavenumber region.

Table S1. Theoretical normal Raman, experimental normal Raman, and SERS peak positions and vibrational modes for cortisol.

Theoretical, scaled (cm ⁻¹)	Normal Raman, solid (cm ⁻¹)	SERS (cm ⁻¹)	Vibrational Modes
1078	1077	1073	Asymmetric ring breathing (C ring)
1148	1149	1146	CH ₂ twist + CH bend + OH bend
1221	1224	-	C-C-C asymmetric stretch (D ring)
1222			C-C-C asymmetric stretch (A ring)
1242	1239	-	C-C-C asymmetric stretch (C ring)
1270	1275	1269	CH bend + CH ₂ bend + OH bend
1332	1330	-	C-C-C asymmetric stretch A ring
1341	1346	1344	C-C-C symmetric stretch (A ring)
1342			
1381	1382	1378	CH ₃ symmetric bend + CH wag + OH wag (C ring)
1385	1390		
1435	1432	1432	CH ₂ scissor (A, B rings)
1446	1448	1450	CH ₂ scissor (D ring + pendant)
1611	1609	-	C=C stretch
-	-	1504	C=C stretch ¹ OR C-C-C asymmetric ring deform. ²
1704	1641	-	C=O stretch

REFERENCES

1. Patterson, M. L.; Weaver, M. J., Surface-enhanced Raman spectroscopy as a probe of adsorbate-surface bonding: simple alkenes and alkynes adsorbed at gold electrodes. *The Journal of Physical Chemistry* **1985**, *89* (23), 5046-5051.
2. Quester, K.; Avalos-Borja, M.; Vilchis-Nestor, A. R.; Camacho-López, M. A.; Castro-Longoria, E., SERS Properties of Different Sized and Shaped Gold Nanoparticles Biosynthesized under Different Environmental Conditions by *Neurospora crassa* Extract. *PLoS ONE* **2013**, *8* (10).