Supporting Information

Tuning Plasmons Layer-by-Layer for Quantitative Colloidal Sensing with Surface-enhanced Raman Spectroscopy

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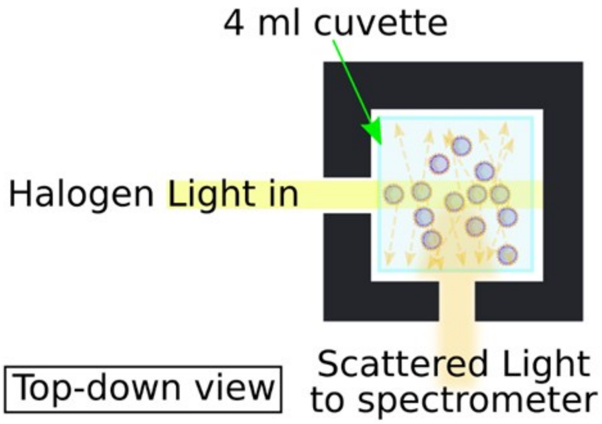

Figure M1: Schematic for the scattering experimental design for measurements made on the sensors. Collimated light from a halogen bulb is directed into a 10 mm path length (4 ml) plastic cuvette. Scattered light is collected at 90° to the incidence beam and directed to the spectrometer.

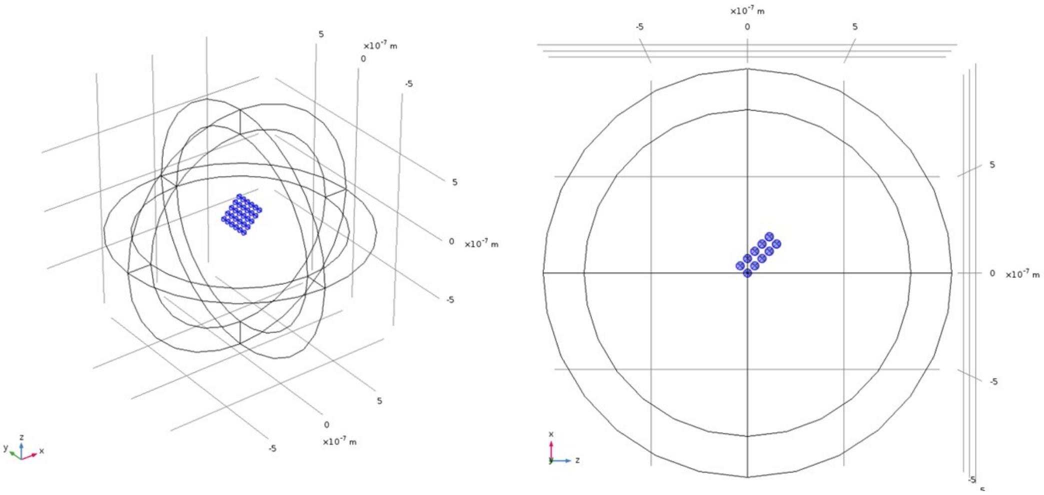

Figure M2: Geometry of the simulation model (a) 3D view (b) 2D view.

The scattering cross-section of the particles was calculated using the following equation:

$$\sigma_{sc} = \frac{1}{I_0}\iint (n \cdot S_{sc})dS$$

where n is the normal vector pointing outwards from the nanoparticle, S_{sc} is the scattered intensity (Poynting) vector and I_0 is the incident intensity. The integral is taken over the closed surface of the nanoparticles.

S1: Scattering spectra with different core sizes of sensors.

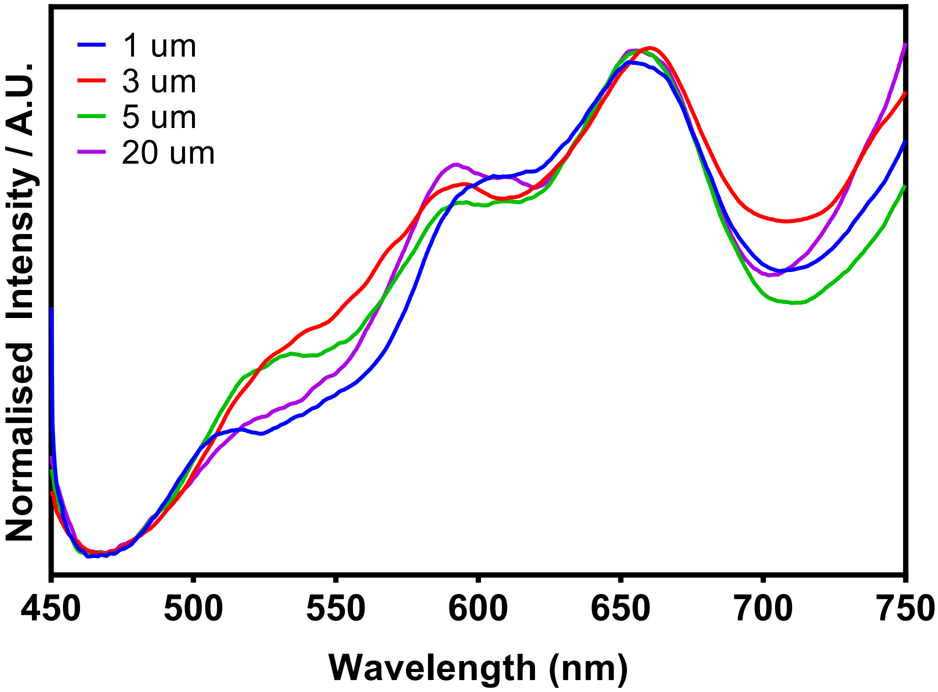

Figure S1: Normalised scattering spectra for 4 differently sized polystyrene cores coated under identical conditions with an AuNP (40 nm diameter) layer. The spectra are very similar with no large shift in peak positions observed between the different sizes demonstrating that the localized surface plasmon resonance is largely unaffected. In this case only a PEI cushion layer was coated on the polystyrene bead cores before deposition of the AuNP layer.

S2: Simulation results for coupling between layers.

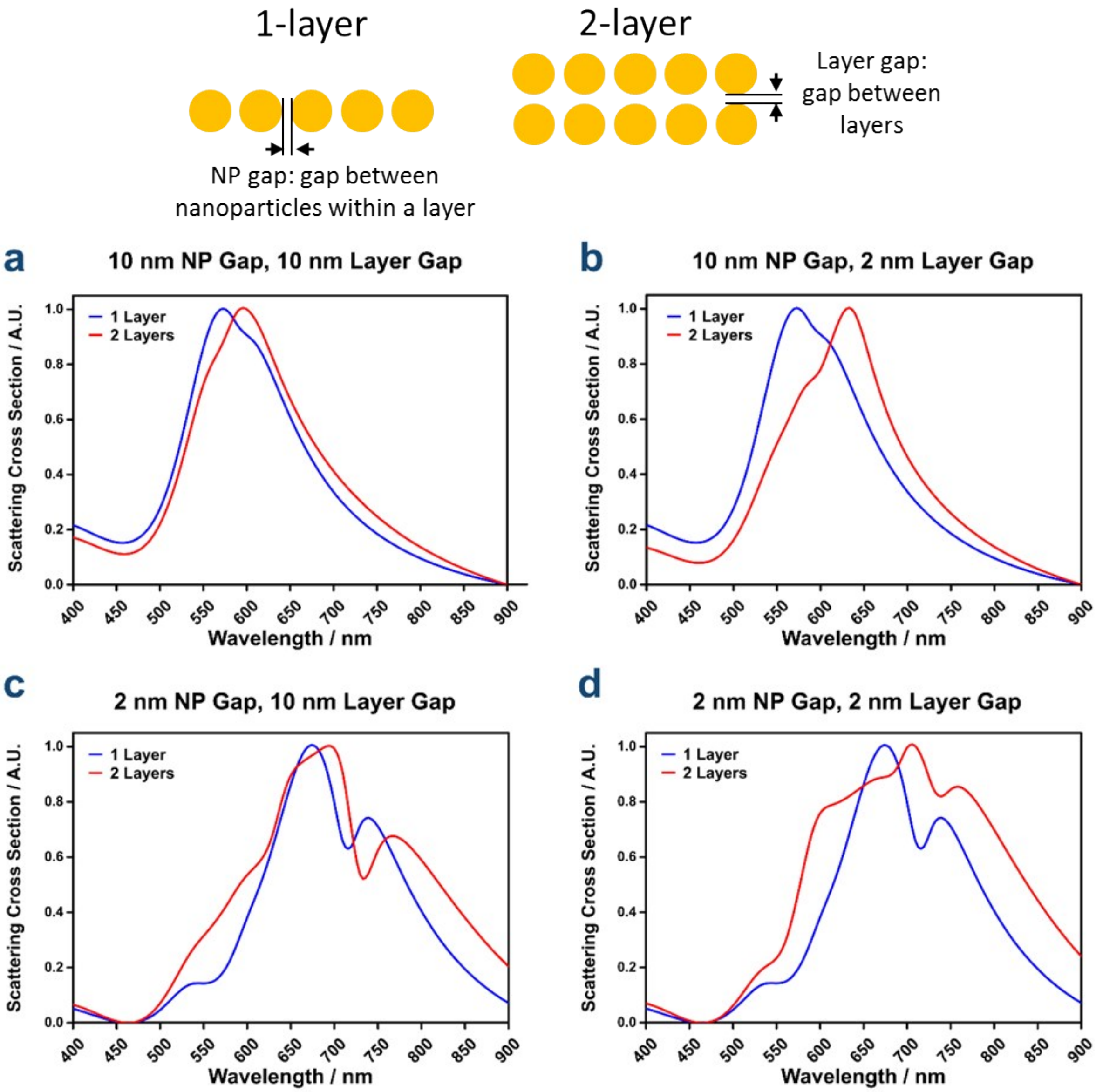

Figure S2: Simulations to show the effect of spacing between layers of nanoparticles. Finite-element simulations were performed using COMSOL Multiphysics v5.3. 1-layer corresponds to a 2D array of 5x5x1 while 2-layers correspond to a 3D array of 5x5x2 nanoparticles (40 nm diameter each) in water. The array was oriented 45^{o} to the incident optical field to mimic the situation on sensors where multiple polarisations would interact with the layered nanoparticles on the colloid surface. Results are shown for various gaps between nanoaparticles within a layer and between layer 1 and layer 2 (a) intra-layer (NP gap):10 nm, inter-layer (Layer gap): 10 nm; (b) intra-layer:10 nm, inter-layer: 2 nm; (b) (a) intra-layer: 2 nm, inter-layer: 10 nm; (b) (a) intra-layer: 2 nm, inter-layer: 2 nm. 2 nm gap was chosen because we have an intervening polyelectrolyte layer, which at uniform monolayer coverage is expected to be approx. 2 nm. Figure S2d most likely represents the case of our sensors where the intra-layer and inter-layer coupling is at a similar length scale. With decreasing gap the scattering peaks show a red-shift with the highest red-shift being observed in Fig. S2d for the 2-layer sensor.

S3: Scatter plots of SERS intensities from different sensors

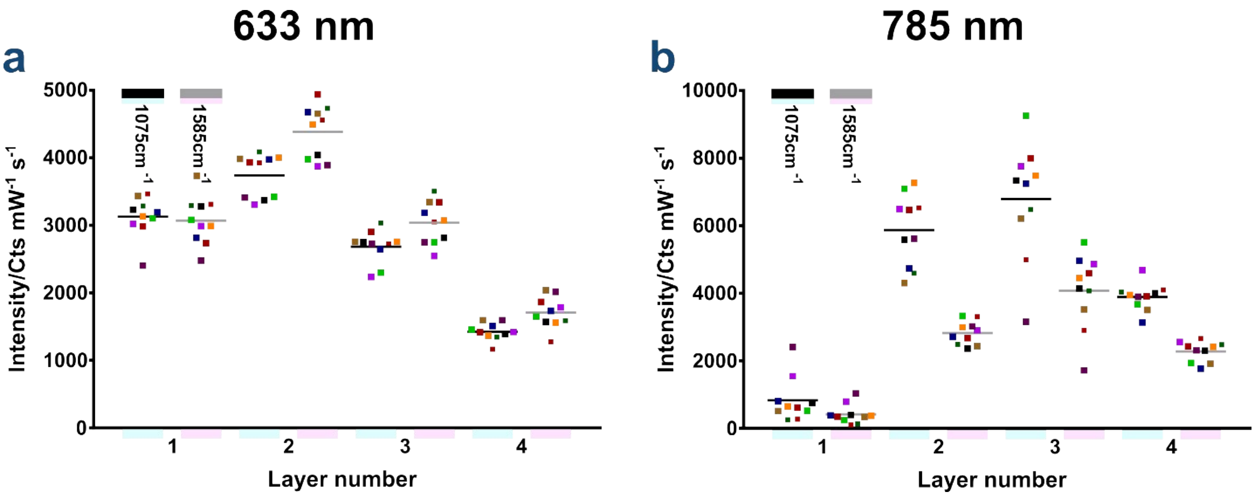

Figure S3: Individual intensities of the 1075 cm^{-1} and 1585 cm^{-1} peaks for the 10 sensors interrogated from each sample with each laser wavelength. The corresponding coloured dots for each sample demonstrate peaks from a single sensor.

S4: Calculation of the enhancement factors for SERS

SERS enhancement factor (EF) can be determined by:

$$\frac{I_{SERS}/N_{SERS}}{I_{Raman}/N_{Raman}}$$

Where I_{SERS} and I_{Raman} are the SERS and Raman intensities (normalised to laser power and integration time) respectively. In this case, N_{SERS} is the number of 4-MBA molecules on the sensor surface that are interrogated by the laser. N_{Raman} is the number of 4-MBA molecules probed in a crystal. N_{SERS}, for a 2 AuNP layered sensor, and N_{Raman} are calculated as follows:

N_{SERS} calculation:

The area of the sensor probed by the laser is determined by:

$$Area = \pi r^2$$

Where r is equal to the lateral resolution under diffraction limited conditions:

$$R_{lat} = \frac{0.61\lambda}{NA}$$

Where λ is equal to the wavelength (633 nm) and NA is the numerical aperture of the objective (0.4).

$=2.93 \times 10^6$ nm^2

The number of 40 nm AuNPs that can maximally coat this area (based on their cross-sectional area) is, therefore, 2325. The scattering data (figure 2d) would suggest that there is primarily long distance coupling in a single layer. We therefore determine that the coverage is most likely to be nearer 50% and therefore 1162.5 AuNPs. Long distance coupling reduces with addition of a second layer, suggesting that there could now be near to 100% coverage, and thus 2325 AuNPs in 2 layers. Polyelectrolyte binding on the first layer will limit binding sites for 4-MBA and the underside of the second layer will also be bound to polyelectrolyte. We therefore estimate that 25% of the total AuNP surface area is available for binding although the actual area for hot-spots might be considerably less. This would present a total 'active' surface area of 2.92×10^6 nm^2 ($=2.92 \times 10^{-8}$ cm^2).

Binding density of 4-MBA in a self-assembled monolayer = 0.5 nmol/cm^2.[1]

□ **Moles of 4-MBA = 1.46×10^{-17} Moles**

$1.46 \times 10^{-17} \times 6.022 \times 10^{23}$

=8.79×10^7 Molecules of 4-MBA probed by the laser.

N_{Raman} calculation:

The focal volume for a confocal Raman spectrum of the 4-MBA crystal is determined by**:**

Volume of a cylinder (to determine focal volume):

$$v = \pi r^2 \cdot l$$

Where r is equal to the lateral resolution and l is equal to the axial resolution:

$$R_{lat} = \frac{0.61\lambda}{NA} \qquad R_{axial} = \frac{2n\lambda}{NA^2}$$

Where λ is equal to the wavelength (633 nm), NA is the numerical aperture of the objective (0.4) and n is the refractive index of the medium (1.552 for crystalline 4-MBA).

$=3.57 \text{ x } 10^{10}$ nm^3
$=3.57 \text{ x } 10^{-11}$ cm^3

The number of MBA molecules within this focal volume can be determined with:
Volume = $3.57 \text{ x } 10^{-11}$ cm^3
4-MBA density = 1.346 g/cm^3
 Mass of 4-MBA = $4.81 \text{ x } 10^{-11}$ g

Molecular weight of 4-MBA = 154.19
 Moles of 4-MBA = $3.12 \text{ x } 10^{-13}$ Moles
$3.12 \text{ x } 10^{-13} \text{ x } 6.022 \text{ x } 10^{23}$
$=1.89 \text{ x } 10^{11}$ Molecules of 4-MBA probed by the laser.

EF calculation:
The normalised intensities for I_{SERS} and I_{Raman} are 4317 and 103.5 respectively. As shown above, N_{SERS} and N_{Raman} are $8.79 \text{ x } 10^7$ and $1.89 \text{ x } 10^{11}$ respectively:

$$\frac{4317/8.79\;x\;107}{103.5\;/1.89\;x\;1011}$$

 EF = $9 \text{ x } 10^5$

References

1 P. N. Sisco and C. J. Murphy, *J.Phys.Chem.*, 2009, **113**, 3973–3978.

S5: Scattering spectra of internal standard sensors with different spacer layers

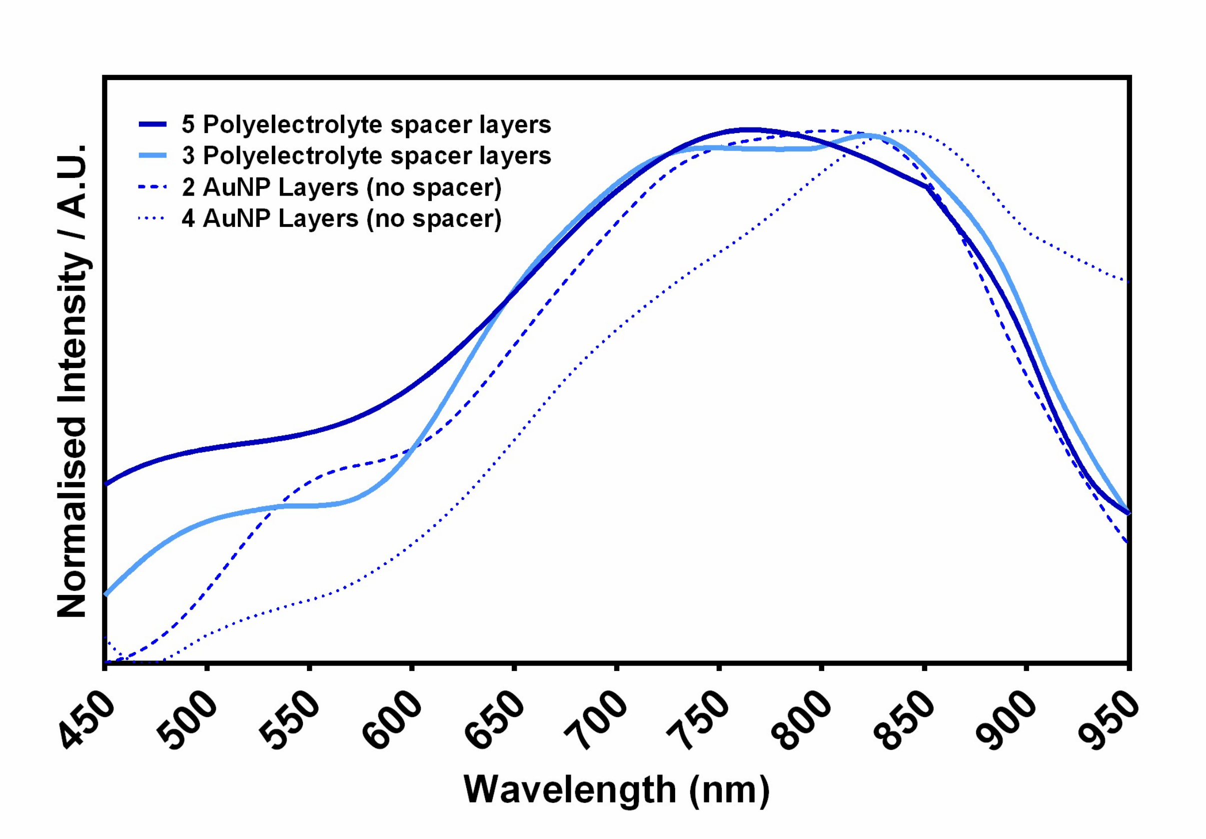

Figure S5. Scattering spectra of internal standard sensors (with 3 (PEI/PSS/PEI) and 5 polyelectrolyte layers ($(PEI/PSS)_2PEI$) as spacer), compared to sensors with two and four AuNP layers. The LSPR of the internal standard sensors closely matches the spectrum of two layer sensors. However, sensors with 3 spacer layers are slightly more red-shifted and present a second peak, indicating that there is incomplete optical separation. 5 spacer layers are sufficiently thick to prevent plasmon coupling between the two SERS-active zones.

Table S6. Mean and relative standard deviations for internal standard sensors with 3 and 5 spacer layers after application of 10 µM adenine (*n* = 10). The peak ratio RSDs (most notably that of the 4-MBA peaks) are considerably lower for the 5-layer spacer. This demonstrates that they have a uniform LSPR, compared with that of the 3-layer spacer. Intensity values are in counts s^{-1} mW^{-1}.

	3 Spacer Layers		**5 Spacer Layers**	
	Mean	**RSD**	**Mean**	**RSD**
735 cm^{-1} Intensity	1763.53	13.49%	5093.11	9.19%
1075 cm^{-1} Intensity	3980.82	10.28%	6690.29	13.45%
1585 cm^{-1} Intensity	5133.66	13.59%	9590.58	10.93%
1075:1585	0.78	10.11%	0.7	3.80%
735:1075	0.45	22.35%	0.77	12.81%
735:1585	0.35	26.75%	0.53	10.43%

S7: Representative raw spectra

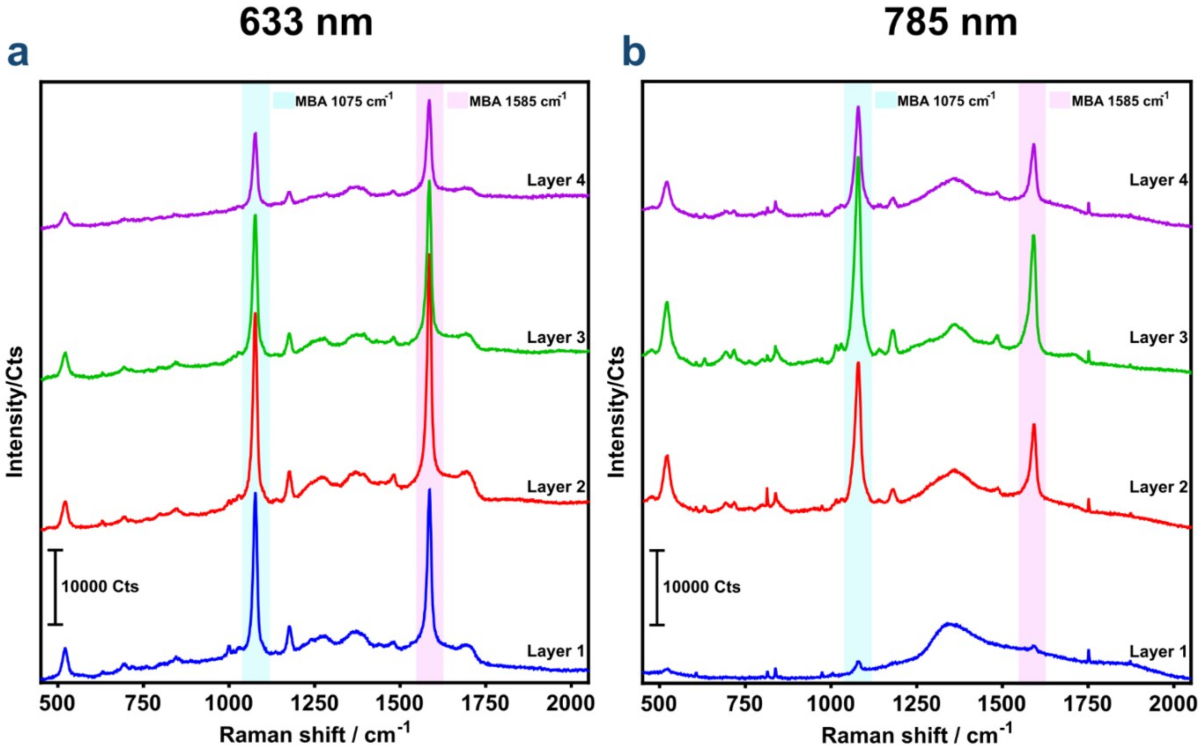

Figure S7. Unprocessed raw spectra of 1-4 layer sensors exposed to 10 µM 4-MBA using 633 nm (a) and 785 nm (b) incidence lasers. Spectra were acquired using 10 second extended scans and laser powers at the objective of 610 µW (633 nm) and 250 µW (785 nm).

S8: Comparison of spectra between pure analyte (MBA) Raman, SERS of MBA on sensor and blank SERS (sensor without analyte)

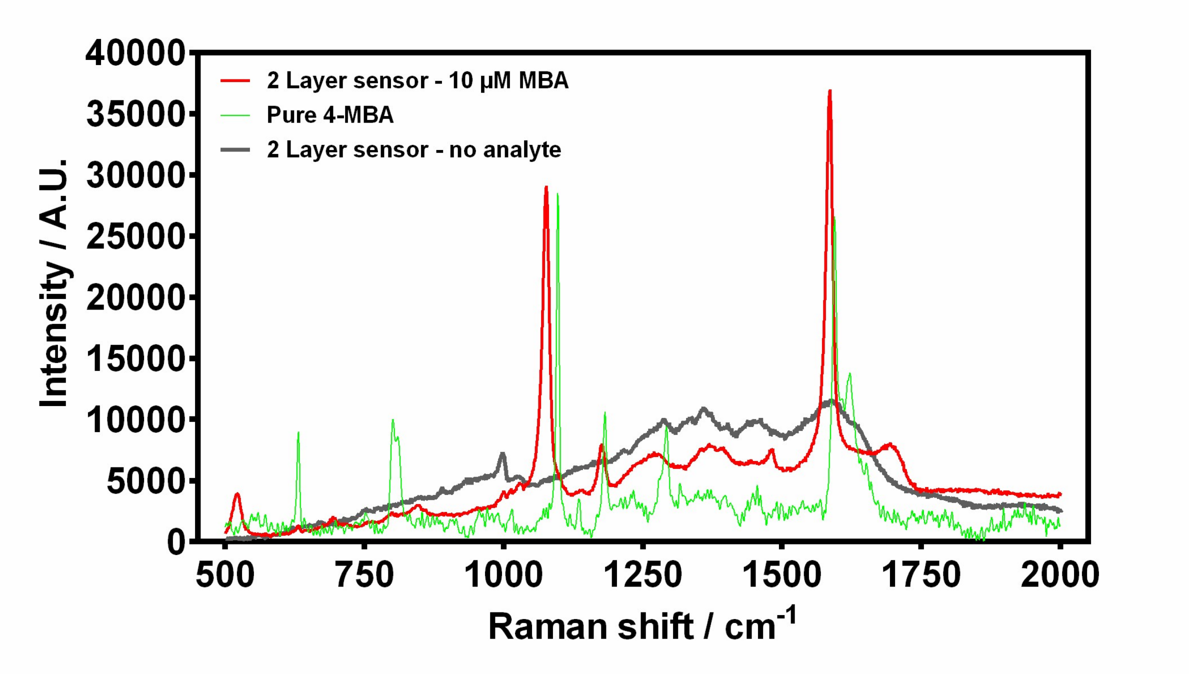

Figure S8. Spectra of a 2 Layer sensor exposed to 10 µM MBA, a 4-MBA crystal, and a sensor exposed to no analyte (control). As expected a shift can be observed in the ring-breathing peaks (1075 cm^{-1} and 1585 cm^{-1}) between the SERS spectrum and the Raman spectrum of a pure 4-MBA sample. No dominant peaks are observed in the control sample.