Physicochemical Trapping of Neurotransmitters in Polymer-Mediated Gold Nanoparticle Aggregates for Surface-Enhanced Raman Spectroscopy

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ABSTRACT: Because of the sharp distance dependence of surface-enhanced Raman spectroscopy (SERS), analyte molecules that do not exhibit strong affinity for Au/Ag often elude detection. New methods of integrating such analytes with SERS substrates are required to circumvent this limitation and expand the sensitivity of SERS to new molecules and applications. We communicate here a solution-phase, capture agent-free method of aggregating Au nanospheres in the presence of five neurotransmitters (dopamine, epinephrine, noradrenaline, serotonin, and histamine) and preventing sedimentation by encapsulating the aggregated nanospheres with polyvinylpyrrolidone, thereby trapping the neurotransmitters in close proximity to the Au nanospheres and enabling SER detection. The primary advantages of this physicochemical trapping method, which is generalizable to analytes beyond the scope of this work, are the high signal-to-noise ratio and spectral consistency down to nM levels. Normal Raman spectra and density functional theory calculations corroborate the accuracy of the spectra. Spectra collected over a wide range of concentrations were used to construct adsorption isotherms for all molecules that do not exhibit strong affinity for Au/Ag. This method demonstrates the sensitivity of SERS was accomplished using molecules that are ideal for SERS detection. Therefore, many challenges remain in the quest to make SERS a generalizable analytical technique applicable to a wide variety of molecules. How well a molecule performs in a SERS experiment depends primarily on three things: (1) the magnitude of the electromagnetic field generated by excitation of the plasmonic noble metal (Au or Ag) nanostructures that enhances the Raman scattering signal ("SERS substrate"), (2) the proximity of the molecule to the SERS substrate's electromagnetic field, and (3) the molecule's Raman cross-section, or inherent polarizability derivative, which influences the likelihood of the molecule to Raman scatter photons.

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Remarkable sensitivity limits of SERS have been defined using well-designed combinations of highly enhancing SERS substrates with highly polarizable molecules (and therefore large Raman cross-sections) that bind directly to the SERS substrate (e.g., the dye molecule, Rhodamine 6G). A large body of work has focused on fabricating highly enhancing SERS substrates, resulting in enhancement factors of up to $10^{12}$. In applying SERS to biosensing, and indeed to many other applications in which the molecule being probed does not readily adsorb to Au/Ag, the main challenge in using SERS is bringing the molecule in close enough proximity to the SERS substrate to benefit from that signal enhancement. A recent study of the distance dependence of SERS shows that when the distance between the substrate and the molecule exceeds 1 nm, there is an 80% loss in signal enhancement. As a consequence, beyond a few nm from the substrate, virtually no signal enhancement is observed, leading to failed SERS experiments.

The primary strategy to bridge this gap and bring biomarkers and other molecules of interest in close proximity with the Au or Ag surface has been to employ capture agents. Capture agents are molecules that readily bind to Ag or Au (often through an Ag/Au-thiolate bond), and also bind the molecule of interest (ideally only the molecule of interest), therefore acting as middlemen between the surface and the molecule of interest. Although this strategy can succeed, for it to enable direct sensing, or detection of vibrational modes of the molecule, the capture agent must be either very small or capture the molecule very close to the surface. This presents an ongoing challenge in the SERS biosensing community because as a capture agent gains specificity, it also typically increases in size (e.g., an affinitive small molecule partition layer vs a DNA/RNA aptamer). As a consequence, SERS detection using capture agents often relies on correlating a binding event with peak shifts or intensity changes in the SER spectrum of the capture agent. This indirect method of detection via capture agent-enabled SERS is vulnerable in our experience to false positives, often relies on multivariate statistics whose significance has been underreported in the literature, and suffers from limited options for specific capture agents, the design of which is a field still in development.

Thus, it is of interest to circumvent the need for a capture agent and find ways to directly sense molecules that are weakly Ag/Au-affinitive. The direct detection of neurotransmitters has been demonstrated with templated Au nanoparticles, aggregated Au and Ag nanoparticles (dried or concentrated via centrifugation), and with electrochemical SERS, but the spectra from these different studies are insufficiently consistent when compared to each other to conclude that direct sensing of neurotransmitters cannot be improved upon. We view solution-phase physicochemical trapping as an alternative to other SERS substrates and to capturing a molecule with a capture agent as an internally consistent and reproducible method. By this, we mean trapping molecules of interest within the sensing volume (≤ 1 nm from the surface) of a SERS substrate through physical and chemical interactions between the substrate, surrounding media, and molecules.

We communicate here a generalizable, Raman reporter label-free, capture-agent-free method for aggregating Au nanoparticles (AuNPs) with a salty buffer in the presence of an analyte molecule to the point of SERS activity and then halting the aggregation with polyvinylpyrrolidone (PVP), preventing overaggregation and eventual sedimentation that is detrimental for SERS activity. This method was inspired by a recently published protocol for fabricating AuNP oligomers from colloidal AuNPs for ultrafast spectroscopy. Transmission electron microscopy of a dimer formed by aggregation induced by an adsorbing analyte, revealed a thin PVP layer encapsulating the dimer. This layer of encapsulating PVP is believed to be what halts aggregation, and we hypothesized that it also had the potential to trap a weakly adsorbing analyte within the sensing volume of a nanoparticle.

We demonstrate that our AuNP/PVP method is simple, rapid, and effective for solution-phase direct SERS detection of five neurotransmitters (dopamine, epinephrine, norepinephrine, serotonin, and histamine) with spectral accuracy down to nM levels as supported by comparison to normal Raman spectra and density functional theory (DFT)-calculated spectra of each neurotransmitter. The primary advantages of this method include the clean subtraction of any contribution to the SER spectrum arising from the AuNPs or PVP, which allows the SER contribution from only the molecule of interest to be isolated, and the consistency of the SER spectra down to the limits of detection, which provides a high degree of confidence in the spectra obtained through this method even at low concentration. Additionally, the solution-phase nature of the method results in SER spectra that are averaged over all orientations and may allow for environmental tuning (e.g., pH changes to which biological molecules are often sensitive).

Because the spectra are consistent with concentration and the SER intensity depends on the neurotransmitter concentration, we are able to construct adsorption isotherms from which we can obtain the Au adsorption dissociation constant ($K_a$) for each neurotransmitter. This information is relevant not only to applying this method to other molecules (i.e., estimating for which molecules this method will be effective) but also to the design and multiplexing potential of any Au plasmonic sensor for these five neurotransmitters. Our results indicate that physicochemical trapping may be a route forward for directly sensing weakly Au-affinitive molecules with the chemical specificity of SERS. We also demonstrate that spectra collected with our method can offer some insight into the surface interactions of these neurotransmitters.

**EXPERIMENTAL SECTION**

**Materials.** All buffers and chemicals were purchased from Sigma-Aldrich in the highest purity grade available (at least 95%) and were used as received without further purification. Colloidal citrate-reduced AuNPs (90 nm spheres, 0.25 mg Au/mL) were obtained from STA Technologies and were used as received without further purification.

**Neurotransmitter Sample Preparation.** All samples were prepared in the exact same way. Stock solutions (10 mM or 1 mM) were freshly prepared in 1xDPBS (pH 7.4, no Ca or Mg) from the salts of the neurotransmitters and serially diluted. Dopamine, epinephrine, norepinephrine, and serotonin spontaneously autocopolymerize in water. At mM concentrations, such solutions turn brown within minutes and result in a loss in SER signal. To prevent autopolymerization and accurately measure the SER signal intensity of a given sample, 100 mM ascorbic acid (AA) was added to the 1xDPBS (“100 mM AA 1xDPBS”) used to make the stock solutions and dilutions of these four neurotransmitters. AA acts as a reducing agent and prevents the autopolymerization from occurring on
the time scale of our SERS measurements. The concentration is low enough that no normal Raman (NR) detection of AA was observed at the power levels used for SERS (200–300 μW). AA is completely nonaffinitive for Au and so does not contribute to the SER signal.

Further dilutions of the analytes were prepared in 400 μL aliquots in either 1xDPBS or 100 mM AA 1xDPBS in 15 mL plastic falcon tubes and stored for up to 3 h at room temperature prior to SERS. Final concentrations of the neurotransmitters were calculated with 1.4 mL volume (total volume of the aliquot and the AuNPs).

AuNP Aggregation (“AuNP/PVP Method”). PVP powder (Average MW ~55 000) was dissolved in 18.2 MΩ cm water via sonication (10 min) and vortex mixing (2 min) to form a ~13 wt % solution (~1.3 g per 10 mL water, room temp).

To a 400 μL aliquot of diluted neurotransmitter was added 1 mL of colloidal 90 nm citrate-reduced AuNPs, which have a rusty pink color. This mixture was immediately shaken four times, upon which the color of the colloid changes from pink to purple. Immediately after shaking the mixture four times (about 3 s), 200 μL of 13 wt % PVP was added, the mixture was pipetted up and down four times, and the mixture was again vigorously shaken four times. It is important to work rapidly once the AuNPs are added; the aggregation is swift, and failure to follow the steps in rapid succession results in nanoparticle overaggregation and loss of signal enhancement. Overaggregation will be easily identifiable by an icy blue tinge and loss of opacity. Following the addition of PVP and observation that the sample had the desired purple color, 1 mL of the final mixture was added to a 2 mL aluminum-capped flat-bottomed glass vial for SERS. 

SERS. All SER spectra were collected at room temperature using a home-built Raman microscope system. Light from a 785 nm diode laser was directed to a Nikon Eclipse-Ti inverted microscope equipped with a Nikon CFI Plan Fluor 10× objective. Samples were positioned above the microscope objective at the same focus depth, and Raman scattering was collected in backscattering geometry through the same objective. The backscattered light was passed through a 785 nm RazorEdge ultraspread long-pass edge filter to remove Rayleigh scattering and focused onto a 1/3 m imaging spectrograph (SP2300, Princeton Instruments), dispersed (600 groove/mm grating), and focused onto a liquid nitrogen-cooled CCD detector (Spec10:400BR, Princeton Instruments). Laser power at the sample varied between 200 and 300 μW, and the cumulative acquisition time was 180 s (5 s per exposure with 36 exposures). Spectra were collected over multiple days for each neurotransmitter with each day of collection spanning both nonconsecutive and consecutive concentrations.

NR. All NR spectra were collected using the same Raman microscope system as for SERS. The laser power at the sample varied from 6.2 to 18 mW and the cumulative acquisition time was either 60 s (5 s per exposure with 12 exposures) or 180 s (5 s per exposure with 36 exposures) for liquid samples. The laser power at the sample varied from 2.4 to 3.2 mW, and the cumulative acquisition time varied from 30 to 180 s for solid samples. Multiple frames were collected for solid samples (all of them white crystalline powders) to check that no degradation caused by laser exposure had occurred.

Density Functional Theory. Electronic structure calculations in this work were performed using the Amsterdam density functional (ADF) computational chemistry package.29 Geometry optimization, frequency, and Raman polarizability calculations were completed for neutral gas-phase dopamine, epinephrine, norepinephrine, serotonin, and histamine using the Becke-Perdew (BP86) GGA exchange–correlation functional and a triple-ζ polarized (TZP) basis set. Raman polarizability derivatives were calculated using the frequency-dependent AORESPONSE module of the ADF package with the lifetime parameter set to 0.004. Differential Raman scattering cross-sections were then calculated as

$$\frac{\partial \sigma_i}{\partial \Omega} = \frac{\pi^2}{\omega^2} \omega (\omega - \omega_i)^4 \frac{h}{8\pi^2c \omega_j} S_j \frac{1}{4S_j (1 - \exp(-\frac{\hbar\omega}{k_B T}))}$$

where ω and ω_i are the frequencies of the 785 nm incident field and of the jth vibrational mode, respectively.30 The scattering factor S_j is equal to 4S_jω_j^2 + 7γ_j^2, where ω_j^2 is the isotropic and γ_j^2 the anisotropic polarizability derivative.30 Raman scattering intensities calculated using this method were broadened via convolution with a Lorentzian distribution characterized by a full-width half-maximum of 20 cm^-1, similar to the experimentally measured linewidths.

To rationalize the concentration-dependent variation observed in the experimentally measured histamine SER spectra (details below), both neutral and protonated histamine species were considered. The additional proton was placed on the aliphatic amine group in the charged species, as this functional group has the lowest pK_a and is expected to be the first group to undergo a protonation change. The conductor-like screening model (COSMO) was used to account for solvation effects present in the aqueous environment, which are expected to affect normal-mode frequencies of charged species as the solvent partially screens Coulomb interactions. All calculated Raman spectra are presented without additional scaling or frequency offsets.

Spectral Processing. All SER intensity counts were divided by the power and acquisition time. Spectral intensity, where included, is reported as power- and acquisition time-normalized analog-to-digital units (ADU mW^-1 s^-1). Cosmic rays were removed. The background and SER contribution of PVP was removed by subtracting from the neurotransmitter SER spectrum the spectrum of a “blank” PVP sample (400 μL 1xDPBS or 100 mM AA 1xDPBS + 1 mL AuNPs + 200 μL 13 wt % PVP) obtained on the same day and under the same conditions as the respective neurotransmitter SER spectrum. For all spectra shown, no smoothing or background subtraction (other than of the blank sample) was performed on the spectra unless otherwise stated. All spectral processing (intensity counts normalization, background subtractions, Savitzky-Golay smoothing) was performed in Matlab R2018b.

Raman Peak Fitting. To observe the change in SER intensity with concentration over the widest range, the most intense and persistent peak (present even at low concentration) was identified and was baseline subtracted, fit with a Lorentzian function, and the area under the curve was integrated. In some instances, two Lorentzian functions were used and summed together to obtain the most accurate fit. Raman peak fitting was performed in Origin 2017.

K_d Determination. To determine the K_d of the neurotransmitters to Au, we selected a modified form of the Langmuir isotherm, the Hill equation (linearized):
\[
\log \left( \frac{L}{f} \right) = \alpha \log(L) - \log(K_d)
\]

This equation was selected because it includes a parameter, \(\alpha\), that allows for deviations from an ideal Langmuir isotherm arising from adsorbed molecules perturbing the environment around them and making subsequent binding events more (\(\alpha > 1\), “cooperative”) or less (\(\alpha < 1\), “inhibitive”) favorable. An \(\alpha = 1\) would indicate that there is no effect. The fractional occupancy, \(f\), is taken to be the ratio \((A/A_{\text{max}})\) between the integrated persistent SER peak area of an individual sample \((A)\) and the integrated persistent SER peak area of a concentrated sample in the signal saturation plateau with the highest integrated peak area \((A_{\text{max}})\). \(L\) is the free neurotransmitter concentration, which is taken to be the neurotransmitter concentration of the sample in 1.4 mL volume. This equation is used to construct a linear Hill plot by plotting \(\log \left( \frac{L}{1-f} \right)\) versus \(\log(L)\). The Hill plot can then be fit with a linear regression, from which both the value of \(\alpha\) and \(K_d\) can be derived: \(\alpha\) is the slope of the line, and \(K_d\) is the antilogarithm of the negative of the \(y\)-intercept.

As \(f\) approaches 0 or 1, the term \(\left( \frac{L}{1-f} \right)\) approaches an asymptote, meaning that this equation is most useful for the linear range of the isotherm. Thus, we fit only the linear range around the 50% saturation point to exclude the upper and lower plateaus where \(f\) approaches 0 and 1. Data points for concentrated samples that extended far into the saturation plateau or for which the sample had overaggregated (resulting in decreased signal intensity) and outliers were first excluded.

Following these exclusions, the interquartile range of the data (25% to 75%, the range between the first and third quartiles, or the middle 50% of the data) was taken to construct a Hill plot that captures only the linear range of the isotherm and excludes both the signal saturation and depletions plateaus. The only exception to this approach is for histamine, for which the adsorption isotherm deviated significantly in its shape from a Langmuir isotherm in that there was a middle plateau around the 50% saturation point. In this case, if the entire isotherm was fit (after excluding data points for which \(f \geq 0.95\) and data points that extended into the saturation plateau to approximate the linear range), the residuals of the fit returned a U-shape, which indicated that the data were not linear. In response, and in accordance with qualitative changes in the SER spectra that coincide with the two linear ranges in the adsorption isotherm separated by the plateau, we estimated the infection points in the data and separated them into three distinct regions (Region 1, the middle plateau, and Region 2). We then fit Hill plots constructed from Region 1 and Region 2 separately, which resulted in two \(K_d\) and \(\alpha\) values \((K_{d1} \text{ and } K_{d2}, \text{ and } \alpha_1 \text{ and } \alpha_2\) respectively) for histamine, which more accurately captured the data than would a single fit for the entire isotherm. This is supported by qualitative changes in the SER spectra, the smaller norms of residuals, and the more randomly dispersed residuals for the two regions (see Figures S-11.1–S-15.7 for full details on all five neurotransmitter \(K_d\) fits).

Hill plot construction was performed in both Microsoft Excel Version 16.19 and Matlab R2018b. Linear regression fits, significances, and residuals were obtained using Matlab R2018b.

**RESULTS AND DISCUSSION**

Spectrally Isolating Neurotransmitter SER Signal from PVP SER Signal. The PVP contribution to a SER spectrum collected using the AuNP/PVP method can be completely and cleanly removed through simple spectral subtraction (the result of which is referred to as a difference spectrum). As demonstrated in Figure 1, the SER spectrum of the PVP component can be isolated by collecting a SER spectrum from a “blank” sample that is identical in composition to a neurotransmitter sample except that it does not contain any neurotransmitter (Figure 1, “Blank PVP”). By subtracting the Blank PVP spectrum from a neurotransmitter SER spectrum collected under the same power and acquisition parameters (Figure 1, “(70 nM Dopamine) + PVP”), the background and all of the PVP SER peaks are removed, leaving only the SER peaks that come from the neurotransmitter (Figure 1, “(70 nM Dopamine) – Blank PVP”) with minimal background intensity. This critical result suggests that the AuNP/PVP method can be used to confidently identify the SER contribution of any sufficiently Au-adsorbed analyte molecule. The blank PVP sample is designed so that there is a single variable difference between the blank sample and the analyte sample: the analyte itself. That is to say, a blank PVP sample is one in which the 90 nm AuNPs are aggregated with 400 \(\mu\)L of 1xDPBS rather than 400 \(\mu\)L of 1xDPBS with some concentration of neurotransmitter dissolved in it. The clean subtraction of PVP SER peaks is especially important for measuring analytes in low concentrations, at which the PVP SER contribution could obscure or overshadow analyte SER peaks if they were not able to be subtracted out.

**PVP Interactions with AuNPs and Neurotransmitters.**

To investigate whether detection of the neurotransmitters was facilitated, as hypothesized, by the PVP layer around the nanoparticles trapping neurotransmitters in close proximity to the nanoparticles, we reversed the order of addition and introduced the neurotransmitter to an already aggregated and PVP-halted blank sample (Figure S-17). No neurotransmitter
was detected, which indicated that the PVP shell around the nanoparticles did indeed act as a barrier.

In overaggregated samples, no PVP is detected, which suggests that the concentration of PVP used in these experiments is too low for NR detection at the powers used for SERS. The neurotransmitters measured in this work have no apparent effect on the intensity of the PVP SER contribution compared to a blank sample. In other words, the PVP SER intensity is independent of neurotransmitter concentration. One possible explanation for this independence is that the majority of the neurotransmitter signal comes from neurotransmitters trapped in the narrow spaces between AuNPs where perhaps PVP does not infiltrate and where the signal enhancement is highest, whereas the PVP SER signal mainly comes from PVP adsorbed to the outside of many nanoparticle aggregates.

**Accuracy and Resolvability of SER Spectra.** Representative high-concentration SER spectra of each neurotransmitter are shown in Figure 2. Although the clean subtraction of PVP contributions from the neurotransmitter SER spectra provides confidence in labeling those spectra as being highly representative of the neurotransmitters, because direct SERS results for these molecules vary in the literature (dopamine,23,24,31–35 epinephrine,34 norepinephrine,6,34 serotonin,24,25,36–38 histamine39,40), additional steps were taken to confirm their identity and gauge the robustness of the AuNP/PVP method. NR spectra (in solution and of the solid salts of the neurotransmitters) and neutral species gas-phase DFT-calculated Raman spectra of the neurotransmitters were collected for comparison. Some differences in Raman peak location (meaning at what cm⁻¹ the peak is centered) and relative intensity are always expected to occur when comparing SER with NR spectra (solid crystal structure, solvation effects, surface adsorption, and interactions with neighboring surface-bound molecules can all alter the frequency of vibrational modes).1 Gas-phase DFT-calculated spectra likewise vary from experimental spectra but still offer an additional degree of confidence in identification. For each neurotransmitter, the NR and DFT-calculated spectra show good agreement with the SER spectra. A high-concentration serotonin SER spectrum is compared to liquid NR, solid NR, and DFT-calculated spectra in Figure 3. The other comparisons are available in Figures S1–S4. All together, these spectra demonstrate that the AuNP/PVP method is a reliable way to measure an accurate SER spectrum of an analyte molecule in a given set of solution-phase conditions (ionic strength, pH, etc.).

The SER spectra of dopamine, epinephrine, and norepinephrine are, as expected, qualitatively similar owing to their closely related chemical structures (Table 1). Their distinguishing SER features are predominantly in the 400–1000 cm⁻¹ range, where vibrational modes tend to be more delocalized and, therefore, sensitive to structural differences among molecules containing similar functional groups. Dopamine is easily resolvable by eye from the other two owing to its distinctive peaks in this region. Epinephrine and norepinephrine are less easily distinguishable but are distinct enough that they can be resolved by principle components analysis (see Figure S-5), which implies that identification of an unknown SER spectrum could be possible even for structurally similar analytes. This is an important result for real sensing applications, as there is no specificity to the

![Figure 2](image1.png)

**Figure 2.** Representative high-concentration SER spectra of each of the five neurotransmitters: 40 μM dopamine (blue trace, top), 37 μM epinephrine (red trace), 40 μM norepinephrine (green trace), 90 nM serotonin (purple trace), and 3 μM histamine (orange trace, bottom).

![Figure 3](image2.png)

**Figure 3.** Comparison of 90 nM serotonin SER spectrum (black trace) with NR of 100 mM serotonin dissolved in 0.1 N HCl (green trace, top), NR of solid serotonin (red trace, middle), and DFT calculated gas-phase Raman (blue trace, bottom). Dashed lines are included to emphasize spectral differences and similarities.
AuNP/PVP method beyond the inherent chemical specificity of SERS.

Spectral Consistency with Concentration. Unlike some other methods, the positions of Raman peaks in the SERS spectra obtained using the AuNP/PVP method do not shift as the concentration of a neurotransmitter decreases, as shown for serotonin in Figure 4 and in Figures S-6–S-10 for the others. This provides confidence in and easy identification of a molecule when analyzing a low-concentration spectrum, as it can be expected to be the same, excepting intensity, as a high-concentration spectrum.

Histamine exhibits an interesting phenomenon in that while the peak positions remain constant, the relative intensities of some peaks change with concentration, as shown in Figure 5 (black traces). At lower concentrations, the intensities of the peaks centered at 1308 cm⁻¹, 1318 cm⁻¹, and 1338 cm⁻¹ are

Table 1. 2D Molecular Structures, $K_d$, and $\alpha$ Values for All Five Neurotransmitters with 95% Confidence Intervals (95% CI), Degrees of Freedom-Adjusted $R^2$, and SSE

<table>
<thead>
<tr>
<th>Neurotransmitter</th>
<th>2-D Molecular Structure</th>
<th>$K_d$ (95% CI: high to low)</th>
<th>$\alpha$ ± 95% CI</th>
<th>Adjusted $R^2$</th>
<th>SSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td><img src="image1" alt="Dopamine Structure" /></td>
<td>5.7x10⁻⁴ M (95% CI: 9.9x10⁻⁴ to 3.3x10⁻⁴ M)</td>
<td>0.57 ± 0.042</td>
<td>0.96</td>
<td>0.20</td>
</tr>
<tr>
<td>Epinephrine</td>
<td><img src="image2" alt="Epinephrine Structure" /></td>
<td>2.2x10⁻⁵ M (95% CI: 7.6x10⁻⁶ to 6.4x10⁻⁵ M)</td>
<td>0.86 ± 0.083</td>
<td>0.97</td>
<td>0.089</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td><img src="image3" alt="Norepinephrine Structure" /></td>
<td>3.1x10⁻⁶ M (95% CI: 1.0x10⁻⁶ to 9.5x10⁻⁷ M)</td>
<td>1.0 ± 0.086</td>
<td>0.99</td>
<td>0.029</td>
</tr>
<tr>
<td>Serotonin</td>
<td><img src="image4" alt="Serotonin Structure" /></td>
<td>1.7x10⁻¹⁰ M (95% CI: 1.1x10⁻¹¹ to 2.6x10⁻⁹ M)</td>
<td>1.5 ± 0.18</td>
<td>0.98</td>
<td>0.052</td>
</tr>
<tr>
<td>Histamine</td>
<td><img src="image5" alt="Histamine Structure" /></td>
<td>$K_d$: 4.1x10⁻⁶ M (95% CI: 1.8x10⁻⁷ to 9.3x10⁻⁷ M) $K_d$: 8.3x10⁻⁸ M (95% CI: 1.9x10⁻⁷ to 3.6x10⁻⁷ M)</td>
<td>$\alpha_1$: 0.73 ± 0.18 $\alpha_2$: 1.1 ± 0.27</td>
<td>Region 1: 0.92 Region 2: 0.90</td>
<td>Region 1: 0.031 Region 2: 0.19</td>
</tr>
</tbody>
</table>

Figure 4. SER spectra of serotonin showing that as the concentration decreases, the signal intensity decreases, but the peak locations remain constant.

Figure 5. SER spectra (Savitzky-Golay smoothed and polynomial baseline subtracted to facilitate stacking) of histamine (black traces), ranging from 6 nM to 30 μM, compared to DFT-calculated optimized geometry spectra of the protonated (red trace) and neutral (blue trace) forms of histamine. Highlighted in red are the peaks that trend in relative intensity toward the protonated DFT spectrum. Highlighted in blue are the peaks that trend in relative intensity toward the neutral form of histamine.
The sensitivities expected for Au of both epinephrine and norepinephrine compared to dopamine may be explained by the additional aliphatic alcohol group present in the former two molecules, which might facilitate surface packing through hydrogen bonding with adjacent citrate molecules or neurotransmitters. Primary aliphatic amines (pK_a: 9–11) are expected to be protonated at pH 7.4. Thus, in the absence of an alcohol group that can hydrogen bond with its neighbors, the protonated amine might introduce electrostatic repulsion. The lower affinity and pKa value for epinephrine compared to norepinephrine can be explained by its secondary, rather than primary, aliphatic amine, which might introduce steric repulsion between epinephrine molecules. These hypotheses are supported by the relative values of pKa: 0.57 ± 0.042 for dopamine with a primary amine and no aliphatic alcohol, 0.86 ± 0.083 for epinephrine with a secondary amine and aliphatic alcohol, and 1.0 ± 0.086 for norepinephrine with a primary amine and aliphatic alcohol.

Serotonin was expected to exhibit the highest affinity for Au due to its indol amine in addition to a primary aliphatic amine. The a of 1.5 ± 0.18 suggests that serotonin has a cooperative effect, making the Au surface more favorable for additional serotonin molecules to adsorb. This coupled with the comparatively lower affinity of histamine to Au, which also has multiple Au-affinitive nitrogen-containing functional groups, suggests that the surface perturbation and packing orientation of the adsorbing molecules have significant effects on the overall adsorption affinity of a molecule to Au. Thus, affinity involves factors that are not as simple as a linear correlation between functional groups and affinity.

Limits of Detection and Potential for Biosensing. The estimated limits of detection for each neurotransmitter are 20 nM (dopamine), 250 nM (epinephrine), 200 nM (norepinephrine), 90 nM (serotonin), and 10 nM (histamine). These limits of detection could potentially be improved upon with neurotransmitter-specific protocol optimization (i.e., pH, ionic strength, nanoparticle concentration, laser power, and exposure time). With the current protocol, only serotonin has a limit of
detection that is physiologically relevant in serum. In looking to the future and applying this method to biosensing applications, this method would be most likely to work with blood, in which concentrations are typically highest, following preprocessing by filtration or chromatography to remove interfering components (i.e., proteins).

## CONCLUSIONS

We demonstrate that the AuNP/PVP method described herein can be used to detect five neurotransmitters that are otherwise difficult to directly detect by SERS. The quality of the spectra that this method produces makes it a potentially highly useful method for identifying new analytes and obtaining definitive SER spectra. Our results indicate that this method could be used for a wide variety of molecules including those that are weakly ($K_d \sim 500 \mu M$) affinitive for Au. This solution-phase method could be used to quantify analyte concentrations in pursuit of a true biosensor, measure adsorption affinity, or study surface–analyte interactions as a function of solvation environment and concentration.

## ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b00773.

SER, NR, and DFT-calculated spectra; principal components analysis; representative spectra; plots of all adsorption isotherms; results of experiment attempting to detect two neurotransmitters within the same sample; results of experiment testing whether trapping method works when the order of analyte and polymer addition is reversed (PDF)

Video of experimental methods (MP4)

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**Author Contributions**

E.V.E. planned and executed all of the experimental work, analyzed the data, and wrote the manuscript in consultation with all authors. M.B. designed and executed all DFT calculations and simulations in consultation with E.V.E. A.I.H. mentored and encouraged the development of this work. J.L.C. and R.K. encouraged the investigation of neurotransmitters and the development of this work. G.C.S. supervised the computational components of the manuscript. R.P.V.D. supervised the experimental components and direction of the project. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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