# Quantitative SERS Using the Sequestration of Small Molecules Inside Precise Plasmonic Nanoconstructs

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Ⓢ Supporting Information

**ABSTRACT:** We show how the macrocyclic host, cucurbit[8]uril (CB[8]), creates precise subnanometer junctions between gold nanoparticles while its cavity simultaneously traps small molecules; this enables their reproducible surface-enhanced Raman spectroscopy (SERS) detection. Explicit shifts in the SERS frequencies of CB[8] on complexation with guest molecules provides a direct strategy for absolute quantification of a range of molecules down to $10^{-11}$ M levels. This provides a new analytical paradigm for quantitative SERS of small molecules.



**KEYWORDS:** *Quantitative SERS, cucurbit[8]uril, host−guest, self-assembled nanoparticles*
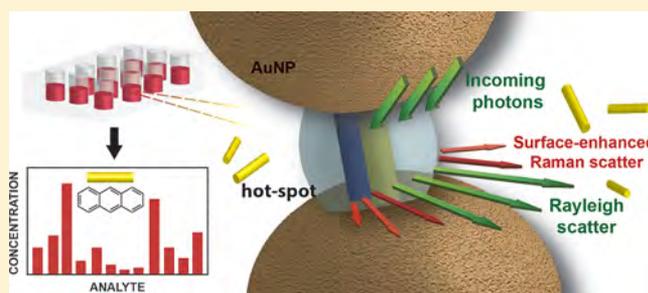
There is a growing demand for reliable small molecule detection techniques that are simple, fast, highly sensitive, require negligible sample preparation and are amenable to high-throughput analyses in various applications spanning from diagnostics in medicine to environmental monitoring.[1] Surface-enhanced Raman spectroscopy (SERS) is an extremely sensitive molecular finger-printing technique that fulfills these technological criteria and detection systems based on it are increasingly being developed.[1−3] SERS relies on the generation of large localized electric fields near nanoscale metallic components such as nanoparticles (NPs) due to optical excitation of their surface plasmon resonances. The low-cost commercial availability of NPs and their ease of synthesis without the need for sophisticated instruments make them a favorable choice as SERS substrates over planar nanostructures and electrodes.[4] The colloidal nature of NPs in solutions means that they can be dispersed through the environmental matrix of interest. On spontaneous aggregation, the constructive coupling between plasmon resonances of adjacent NPs create regions of intense local fields in the gap between NPs, called "hot-spots". As a result of the large electric field enhancements, the normally weak Raman scatter is amplified to generate SERS signals of analyte molecules located in these hot-spots, which can lead to single molecule detection.[5,6] Although the enhancement itself can be tuned by the size and shape of the NPs,[7] it has been realized that regulating the gap distance between NPs is crucial for reproducible signals.[8−10] Control over the gap distance between NPs in colloids, however, has proved challenging. The irreproducibility of the SERS signals arising from the variable gap distances has made quantification of analytes difficult using SERS and has been a subject of intense research.[11−13]

Nevertheless, direct detection techniques, such as SERS,[14] can be useful for monitoring analytes such as polyaromatic hydrocarbons (PAHs). PAHs are a class of pollutants that need to be monitored at ultralow concentrations leading to long sampling times and preconcentration to obtain measurable signals. However, small neutral or hydrophobic molecules usually have a low affinity for SERS substrates and are unable to aggregate NPs, especially at low concentrations. As a result, SERS of hydrophobic molecules in general has proved difficult.[15] SERS analyses of PAHs have been carried out using functionalized NPs for environmental sensing,[16−24] however, the sensitivity of detection needs to be improved for practical applications.

Supramolecular host molecules with a neutral, hydrophobic cavity are an attractive option for molecular recognition based sensors for the characterization and detection of organics, especially water-insoluble analytes, in various environmental matrices. Using a host molecule with a specific vibrational signature would provide an internal standard for quantification and also allow the study of complexation behavior spectroscopically with SERS. This will be hugely beneficial, since current methods like isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) suffer from drawbacks in their requirements for relatively high concentrations and high volumes of analytes or long acquisition times, which restricts their applicability in host−guest kinetic studies. Host−guest SERS has been demonstrated with cyclodextrins[14,25,26] using thiolated cyclodextrin-based hosts for detecting PAHs quantitatively from mixtures,[14] but sensitivity remains a persistent issue while the spacer deformations give gap irreproducibility. Another demonstration of host−guest SERS-

based sensors was reported by Witlicki et al.,[27] however, it relied on resonance enhancement for achieving high sensitivity, which requires a selection of analytes with ideal resonance matched conditions.

Cucurbit[n]urils (CB[n]) are an important class of macrocyclic receptors, which not only function as precise rigid spacers between NPs, but also act as a host for hydrophobic and/or small cationic molecules due to their unique barrel shaped geometry with carbonyl lined portals.[28−30] CB[n] bridges adjacent NPs through its two electronegative carbonyl portals to form SERS hot-spots[31−33] and this ability has been shown to play a dual role in SERS detection using AuNPs.[9,34,35] While CB[5], CB[6], and CB[7] can usually accommodate only one guest molecule inside their cavity, the larger homologue CB[8] can host more than one guest at a time to form ternary complexes as shown in Figure 1a. Thus, the ability of CB[8] to
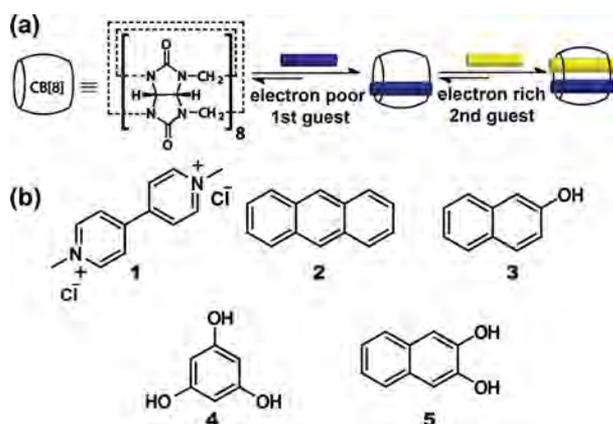
**Figure 1.** (a) Stepwise formation of 1:1:1 ternary complexes of macrocyclic host CB[8]. (b) Chemical structures of dicationic electron-deficient methyl viologen first guest ($G_1$) (**1**) and electron-rich aromatic second guests ($G_2$), anthracene (**2**), 2-naphthol (**3**), phloroglucinol (**4**), and 2,3-naphthalenediol (**5**).

form uniform hot-spots as a result of its rigid geometry and binding affinity to gold in addition to its ternary host−guest chemistry makes CB[8] an excellent choice for developing SERS-based molecular sensors for quantitative analysis. The 1:1:1 CB[8] ternary complexes are usually stabilized in water through hydrophobic forces as well as through $\pi-\pi$ interactions between the two guests.[36−39]

Here we report ultrasensitive SERS-based detection of hydrophobic aromatics and determine their complexation properties using CB[8] as a precise rigid supramolecular spacer. In order to explore the exclusive molecular recognition ability of CB[8], its ternary complexes were investigated with SERS using a fixed first guest ($G_1$) and a number of analytes as second guests ($G_2$) (see Figure 1b). A proportion of the guest molecules present in the solution get trapped inside the host cavity between adjacent AuNPs as a result of their complexation behavior with CB[8] as illustrated in Figure 2a. Enhanced Raman scatter is observed from the ternary complexes ($[G_2 \cdot G_1] \subset CB[8]$) as a result of their localization in the hot-spots during SERS analysis.

CB[8] induces AuNP aggregation (see Supporting Information) and shows intense SERS signals at 437 and 832 cm$^{-1}$ as seen in Figure 2b (i), which have been assigned to ring scissor and ring deformation modes respectively.[40] These signals can be observed within a few seconds of CB[8] addition to the 60
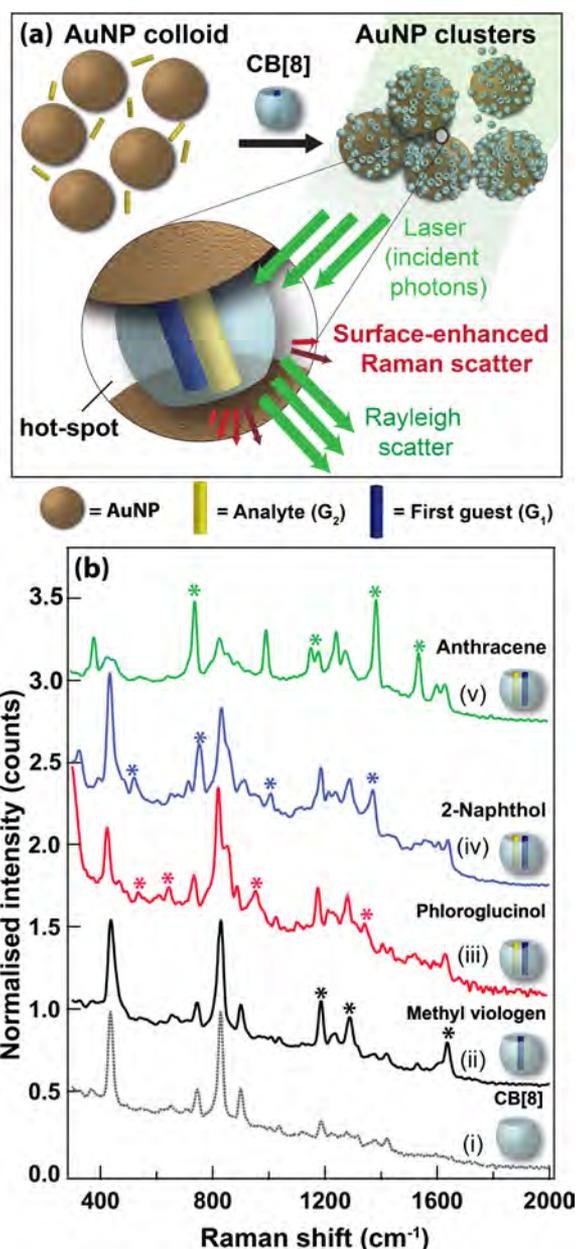
**Figure 2.** (a) Schematic of host−guest SERS analysis using ternary complexation: CB[8] aggregates AuNPs and localizes the analyte ($G_2$) in the hot-spot for SERS analysis. (Note: The AuNPs are not drawn to scale with the analytes.) (b) SERS spectra of (i) CB[8] (5 $\mu$M); (ii)−(v) CB[8] complexes. SERS signals from both CB[8] and guest molecules are observed in the CB[8] complex spectra. (Note: spectra are stacked for clarity.)

nm gold colloidal aqueous solution. The resulting AuNP clusters remain stable for at least 60 min. Since CB[8] has a well-defined Raman signature, it can be used as an internal standard enabling analyte signal quantification. The affinity of second guest molecules toward CB[8] can be significantly controlled by limiting spatial availability in the cavity or tuning charge interactions by using a suitable first guest.[41,42] Methyl viologen ($MV^{2+}$) (**1**), the chosen first guest for this study, is a doubly charged electron-deficient molecule, which is known to form a strong 1:1 host−guest complex with CB[8] ($K_a = 8.5 \pm 0.3 \times 10^5$ M$^{-1}$ at 27 °C).[43] When CB[8] is added to the gold colloidal solutions containing **1** below a concentration of

10 $\mu$M, the signature $MV^{2+}$ peaks at 1195, 1297, and 1650 cm$^{-1}$ are clearly observed in the SERS spectrum as highlighted in Figure 2b (ii) (see Supporting Information for Raman spectra of **1**). This is a result of the localization of **1** in the hot-spots due to complexation with CB[8]. It is noteworthy that **1** is unable to aggregate AuNPs in the absence of CB[8] below 50 $\mu$M levels and therefore, Raman or SERS signals from **1** alone cannot be observed at such low concentrations (see Supporting Information). This demonstrates the role of CB[8] in hot-spot formation, which is not achievable with the probe molecule alone.

The 1:1 $[MV^{2+}]{\subset}CB[8]$ complexes serve as substrates for binding analytes in a SERS assay, where the electron deficient nature of **1** makes the subsequent inclusion of electron rich aromatic compounds in CB[8] energetically more favorable.[44] In order to evaluate ternary complexes of $[G_2{\cdot}MV^{2+}]{\subset}CB[8]$ for SERS detection, where both hydrophobicity and charge interactions are factors governing overall stability of the complexes, a variety of aromatic compounds were studied as second guests (see Figure 1b). The chosen $G_2$ have different aqueous solubilites ranging from highly hydrophobic molecules such as anthracene (**2**) and naphthalene to more water-soluble molecules like 2-naphthol (**3**), phloroglucinol (**4**), and 2,3-naphthalenediol (**5**). SERS signals from the second guests were clearly identified in all the ternary complex spectral measurements (see Figure 2b (iii)–(v) for representative results and Supporting Information for Raman spectra of **2**, **3** and **4**). For example, SERS signals from **2** at 744 and 1395 cm$^{-1}$ are seen in the ternary complex spectra (Figure 2b (v)). The peaks at 1000 and 1549 cm$^{-1}$ are stronger in SERS compared to Raman as a result of imposition of surface selection rules[45] in the former process. Other analytes (**3** and **4**) can similarly be seen and are highlighted in the ternary complex spectra in Figure 2b (iv) and (iii). Analogous to the observations made with **1**, SERS signals from the second guests were only observed upon addition of CB[8] to the NP colloids containing the guest probes at concentrations below 50 $\mu$M. This indicates CB[8] ternary complex formation in the hot-spots that can be readily detected by SERS.

Molecular vibrations are extremely sensitive to the electronic environment and hence formation of ternary complexes would be expected to manifest as spectral peak shifts. A close inspection of the ternary complex SERS spectra show emergence of new peaks in addition to signals from $G_2$, **1** and free CB[8], which are distinctly absent in their individual SERS spectra. The most prominent new peaks are seen at approximately 470 and 865 cm$^{-1}$ (i.e., 30 cm$^{-1}$ higher than the signals for uncomplexed CB[8]) as seen in Figure 3 (see Supporting Information for $[MV^{2+}]{\subset}CB[8]$ spectra). We propose that the additional signals are a result of the alterations in the ring vibrational modes of complexed CB[8], which arise from inclusion of guests inside the cavity and have therefore been assigned to complexed CB[8]. The observed SERS signals for complexed CB[8] are in reasonable agreement with calculated Raman shifts (HF/3-21G level of theory) for CB[8] complexed with **1** (Table 1). The calculated Raman frequencies are expected to be observed in gas phase while the SERS measurements were made in aqueous AuNP solution. Therefore, the disparities in the Raman shifts are assumed to be caused by differences in the media and by the restriction to Hartree–Fock methods on account of computational costs. As further evidence, the smaller homologues, CB[5] and CB[7] were used as controls in this study. The cavity volume of CB[5]
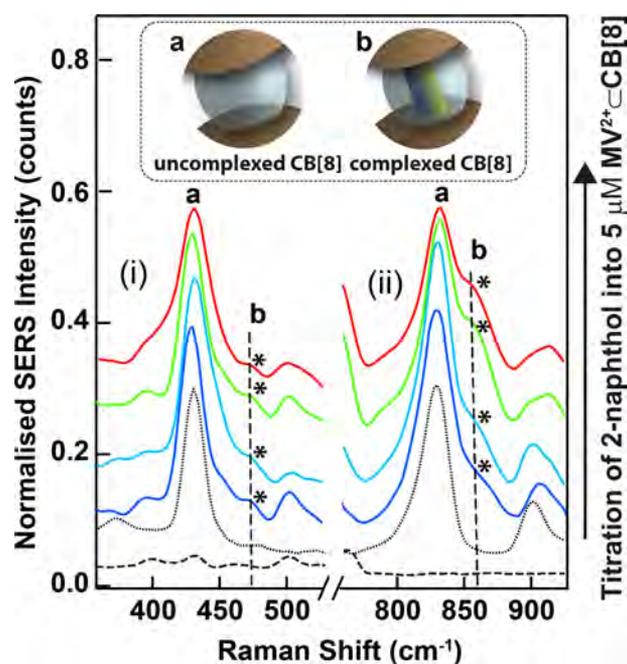


**Figure 3.** SERS signals in the region (i) 450–550 cm$^{-1}$ and (ii) 750–950 cm$^{-1}$ from $[2\text{-naphthol}{\cdot}MV^{2+}]{\subset}CB[8]$. 2-naphthol (250 nM–10 $\mu$M) was titrated into 1:1 $[MV^{2+}]{\subset}CB[8]$ (5 $\mu$M). Complexed CB[8] vibrations at 470 and 860 cm$^{-1}$ are absent from the solid Raman spectra of 2-naphthol (dashed line) and SERS spectra of $[MV^{2+}]{\subset}CB[8]$ (dotted line). (Note: spectra are stacked for clarity.)

**Table 1. Raman Shifts (cm$^{-1}$) for Uncomplexed CB[8] and Complexed CB[8]**

| uncomplexed CB[8] | | complexed CB[8] | |
| --- | --- | --- | --- |
| observed | calculated | observed | calculated |
| 432 | 437 | 450 | 446 |
| 827 | 834 | 860 | 840 |
| 880 | 888 | 900 | 902 |

is unable to accommodate **1** and titration of **1** into a 60 nm AuNP solution containing CB[5] during SERS analysis does not show changes in the vibrational signature of CB[5]. On the contrary, CB[7] forms a strong 1:1 complex with **1** and SERS spectra of $[MV^{2+}]{\subset}CB[7]$ shows complexed CB[7] signals as similarly observed with CB[8] complexes (see Supporting Information). Although changes in the immediate environment of probe molecules are known to cause shifts in their Raman signals, this phenomenon has not been reported previously for supramolecular host–guest complexes to our knowledge.

The peaks of complexed CB[8] are readily observed and can form the basis of a supramolecular binding assay. The molar ratio of complexed CB[8] ($\theta$) is obtained from the SERS signal intensities for the complexed CB[8] and uncomplexed CB[8] peaks, where $\theta$ = complexed CB[8]/(complexed CB[8] + uncomplexed CB[8]). The value of $\theta$ as a function of increasing $G_2$ concentration fits well to a simple Langmuir model and representative plots are shown for **2**, **3**, and **4** in Figure 4a (also see Supporting Information). This simple SERS-based approach can directly determine the binding constant, $K_a$ for $G_2$ with $[G_1]{\subset}CB[8]$. The $K_a$ values obtained for different second guests are in good agreement with those previously reported in literature[44] (see Table 2) but can be obtained within 30 min using this simple SERS approach. It is notable
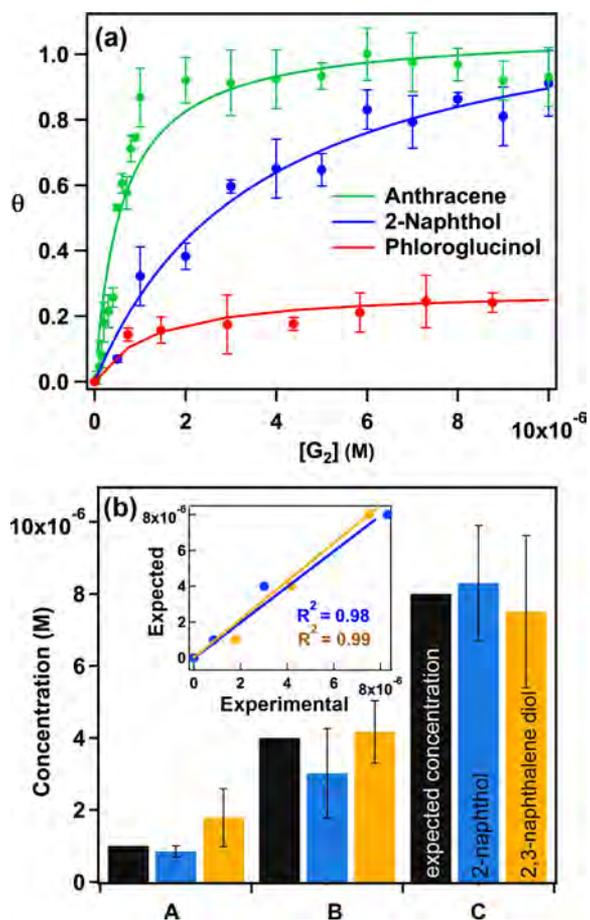
**Figure 4.** (a) Representative plots of molar ratio of complexed CB[8] ($\theta$) as a function of increasing $G_2$ concentration fitted to a simple Langmuir model for anthracene, 2-naphthol and phloroglucinol. (b) Blind study results for quantitative determination of 2-naphthol (blue) and 2,3-naphthalenediol (yellow) at different concentrations: $A = 1 \times 10^{-6}$ M, $B = 4 \times 10^{-6}$ M, and $C = 8 \times 10^{-6}$ M (black bars represent expected concentrations). Inset shows a linear correlation between expected and experimentally determined concentrations.

**Table 2. Binding Constants ($K_a$) of Ternary Complexes**

| $G_2$ | reported $(M^{-1})^{44}$ | experimental $(M^{-1})$ |
|---|---|---|
| anthracene | | $(1.6 \pm 0.3) \times 10^6$ |
| perylene | | $(6.2 \pm 0.7) \times 10^5$ |
| 2-naphthol | $(6.1 \pm 0.5) \times 10^5$ | $(8.9 \pm 0.6) \times 10^5$ |
| 2,3-naphthalenediol | $(3.2 \pm 0.5) \times 10^5$ | $(1.9 \pm 0.7) \times 10^5$ |
| 2,7-naphthalenediol | $(1.6 \pm 0.5) \times 10^5$ | $(4.8 \pm 0.8) \times 10^5$ |
| 1,5-naphthalenediol | $(1.3 \pm 0.5) \times 10^5$ | $(1.7 \pm 0.5) \times 10^5$ |
| naphthalene | | $(3.9 \pm 0.2) \times 10^4$ |
| phloroglucinol | $(3.7 \pm 0.7) \times 10^2$ | $(5.1 \pm 0.3) \times 10^2$ |

that the complexed CB[8] signals arise from complexed CB[8] molecules only and therefore, the measured $K_a$ values are unaffected by the proportion of direct guest interactions with the AuNP surface, if any. Direct determination of binding constants for water insoluble PAH guests, such as anthracene and naphthalene, by standard techniques is difficult and has not yet been reported. Conversely, this straightforward SERS method is generic and not limited by requirements of high concentrations, additional labels or sophisticated equipment.

Having determined the $K_a$ values for the ternary complexes, the quantitative potential of the method was evaluated in a

blind study. The obtained binding isotherms were used as calibration curves through $G_2 = \theta/(K_a - K_a\theta)$. In the study, unknown single concentrations of **3** and **5** were analyzed below 10 $\mu$M levels. The SERS-based calculated values of analyte concentrations concur with actual analyte concentrations within $\pm 40\%$ (see Figure 4b and Supporting Information). This demonstrates the applicability of our SERS-based binding assay for reproducible quantitative analyses of unknown amounts of analytes at very low concentrations, below 1 ppb.

In summary, a SERS-based method has been developed by exploiting the heterogeneous guest inclusion capability of CB[8] to obtain binding constants even for hydrophobic nonfluorescent molecules at low concentrations in aqueous solutions, normally not achievable with standard techniques. This approach provides an improved method for ultrasensitive quantitative analysis of small aromatic molecules. In this initial study, we have demonstrated the concept using PAHs, but the applicability of this facile and robust method can be easily extended to sensing and diagnostic assays with a variety of other analytes. The detection limits of this system for PAHs is an improvement over existing SERS methods by at least 3 orders of magnitude ($10^{-11}$ M) and requires minimal sample preparation.[46] Therefore, these self-assembled gold colloids aggregated by CB[8] in a controlled and reproducible manner provide a convenient platform for the detection of analytes in aqueous solution and offer major advantages over conventional sensing systems.

## ■ ASSOCIATED CONTENT

**ⓢ Supporting Information**

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

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