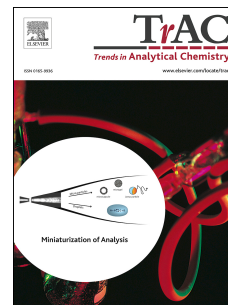


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# SERS biosensors based on cucurbituril-mediated nanoaggregates for wastewater-based epidemiology

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## Abstract

Hierarchical self-assembly of nanoparticles (NPs) mediated by macrocyclic molecules, cucurbiturils (CBs), provides a facile method to fabricate surface-enhanced Raman spectroscopy (SERS) sensors for potential applications in biosensing and environmental monitoring. In contrast to conventional techniques for wastewater-based epidemiology (WBE), CB-NP SERS sensors offer great opportunities for on-site quantification of trace chemical and biological markers due to its high sensitivity, selectivity, reproducibility, multiplexing capability and tolerance against contamination. The working principles of the CB-Au NP nanocomposites including fabrication, sensing mechanisms and structure-property relationships are explained while the design guidelines and selected examples of CB-Au NP SERS sensors are discussed. The review concludes by highlighting recent advances in this area and exploring opportunities in the context of WBE.

## Keywords

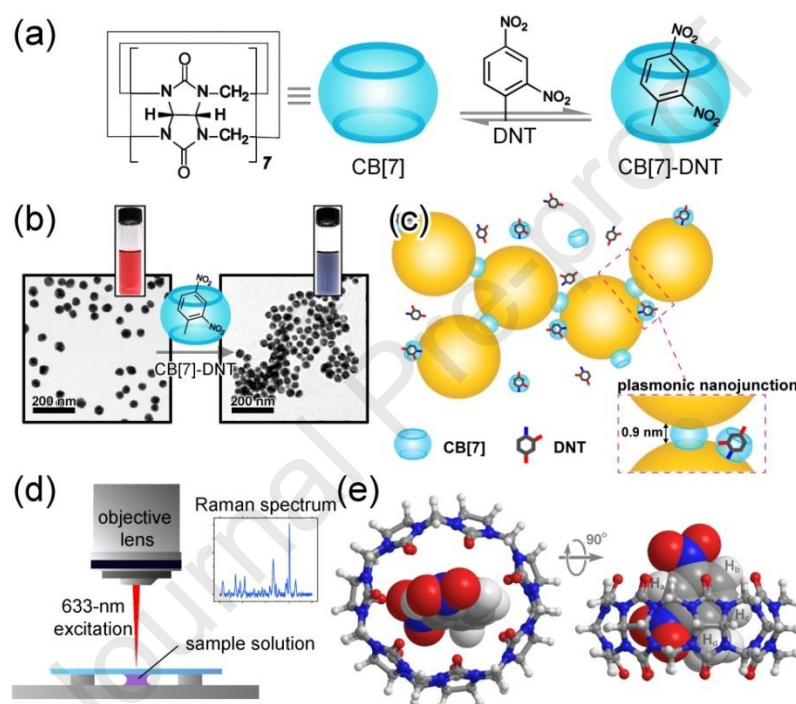
Wastewater-based epidemiology; surface-enhanced Raman spectroscopy; biosensing; environmental monitoring; cucurbituril; gold nanoparticles; self-assembly; host-guest complexation; nanocomposites

## 1. Introduction

Current challenges in quantification of trace chemical and biological markers such as illicit drugs, infectious diseases and hazardous substances in the environment have driven the development of high-performance sensors in response to global concerns in public health and security. Wastewater-based epidemiology (WBE), which refers to systematic sampling and quantification of biomarkers in wastewater,<sup>1</sup> is a powerful tool for population-level monitoring of a wide range of informative target analytes including cocaine, methamphetamine and MDMA (3,4-methylenedioxymethamphetamine)<sup>2-4</sup>. WBE is a non-invasive, near real-time and cost-effective method to probe large-scale information of human activities. For instance, its application has been extended to the early detection of infectious disease outbreak including the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the current COVID-19 pandemic<sup>5-7</sup> which has caused 4.3 million deaths<sup>8</sup> to date. Early detection of infectious diseases at community-level can help the government and the public to take effective measures to minimise viral spread.

While traditional methods for WBE such as liquid chromatography-mass spectrometry and high-resolution mass spectroscopy are sensitive and selective,<sup>9</sup> they are expensive, require trained

personnel and involve complex procedures for sample preparations. In contrast, surface-enhanced Raman spectroscopy (SERS) is a promising technique for WBE.<sup>10</sup> It can be used to simultaneously identify multiple chemicals and biomarkers via their vibrational fingerprints meanwhile offering numerous advantages such as rapid response, portability, high sensitivity, ease of use, limited sample preparation and tolerance to complex matrix. SERS can greatly increase the Raman signals of analyte targets located in close proximity to the plasmonic nanoparticles (e.g. gold nanoparticles, Au NPs) or nanostructured surfaces by 4 to 10 orders of magnitudes<sup>11</sup>. This is attributed to the strong electromagnetic enhancement of the incident light absorbed on or near the surface of the plasmonic materials via localised surface plasmon resonance (LSPR), and the chemical enhancement associated with the increase in polarisability during charge transfer between the plasmonic nanostructures and analyte targets.



**Fig. 1.** Trace chemical detection using SERS mediated by CB-Au NPs, as illustrated by using 2,4-dinitrotoluene (DNT) as a target analyte.<sup>12</sup> (a) Formation of a host-guest complex between CB7 and DNT can be achieved by mixing the individual components under sonication at room temperature. (b) A mixture of CB7-DNT host-guest complexes and empty CB7 can mediate the formation of precise plasmonic nanojunctions via aqueous self-assembly upon mixing with Au NPs, where the encapsulated DNT molecules are positioned in close proximity to the plasmonic hotspots. Panels show ex situ TEM images before and after the addition of CB7-DNT complexes; corresponding photographs of the sample solutions are shown in the inset. (c) Schematic diagram visualising the formation of plasmonic nanojunctions, highlighting the role of CBs as the “supramolecular glue” that binds the Au NPs together. (d) SERS measurement can be performed on a drop of CB7-DNT-Au NPs solution using a Raman microscope or spectrometer with laser excitation. (e) Molecular model of a CB7-DNT complex optimised at CPCM/wB97XD/6-31G\* level of theory.

Despite the strong merits of quantitative SERS detection of trace analytes in wastewater, its practical implementation is hindered by the low reproducibility of the SERS signals. Colloidal NPs such as Au NPs and Ag NPs are easy to synthesise and functionalise for tunable plasmonics. SERS signals can be enhanced by using salt (e.g. NaCl) or molecular linkers (e.g. biotin-streptavidin) that mediate NP

aggregation to form a 3D network of plasmonic hotspots, however, this often results in poor signal reproducibility due to the random nature of uncontrolled aggregation.<sup>13</sup> In the past decade, it has been demonstrated that the issues of poor signal intensity and reproducibility from uncontrolled aggregations can be addressed by the emerging novel SERS substrates based on cucurbit[*n*]urils (CB*n* where *n* = 5–8, 10) and Au NPs.<sup>14</sup> CBs are highly symmetric, rigid and Raman-active which can form host-guest complexes with analyte targets by trapping them inside their hydrophobic cavity<sup>15,16</sup> and bind to the Au NP surface via their electron-rich carbonyl portals.<sup>13</sup> Aqueous self-assembly of Au NPs mediated by CBs allows pristine formation of precise plasmonic nanojunctions with a controlled spacing of ~0.9 nm (i.e. height of CBs) between the adjacent Au NPs,<sup>17</sup> thus localising the target analytes in close proximity to the hotspots with enhanced SERS intensity and reproducibility (Fig. 1). This method offers ease of sample preparation on-site and significantly extend the practical shelf-life of the SERS substrates since pre-formed plasmonic nanojunctions or nanostructures are prone to contamination and degradation over time.

Herein, this review aims to explore the potential application of CB-Au NP SERS in WBE. The structure of the review is as follows. Fabrication of CB-NP nanocomposites via hierarchical aqueous self-assembly will be explained, covering factors that underpin their formation kinetics. Important properties of the precise plasmonic nanojunctions within the CB-NP nanocomposites will be reviewed, supported by fundamental theories and modelling results. Meanwhile host-guest chemistry of CBs that enables trapping of analyte molecules around the plasmonic hotspots will be discussed. Finally, structure-property relationships of the CB-NP sensor will be elaborated in the aspects of sensitivity, selectivity, reproducibility and tolerance against contamination. Practical considerations of designing CB-NP SERS sensors will be discussed and illustrated by selected examples in biosensing and environmental monitoring. Performance of the sensors will also be benchmarked against other sensing schemes (e.g. high-performance liquid chromatography (HPLC), fluorescence, colorimetric methods) and similar materials/chemical systems (e.g. Au NPs, cyclodextrins-modified Au nanorods). The review will conclude by summarising the recent advances in CB-NP SERS sensors, and the resultant opportunities in WBE. Potential pathways for future fundamental and translational research will also be proposed.

## ***2. Working principles of the CB-NPs – fabrication, sensing mechanism and structure-property relationships***

### ***2.1. Facile fabrication via hierarchical self-assembly in aqueous media***

While the possibility of trapping Au NPs inside the CB cavity was suggested by Corma et al.<sup>18</sup> and the first CB-Au interaction was reported by An et al.<sup>19</sup>, we reported the first CB-NP nanocomposites made via aqueous self-assembly in 2010.<sup>17</sup> In particular, the CB-Au NP nanocomposites can be synthesised by NaBH<sub>4</sub> reduction of an aqueous solution of HAuCl<sub>4</sub> in the presence of CB*n* (*n* = 5–8), with the colour of the solution dependent on the molar ratio of CB: Au.<sup>17,20</sup> Alternatively, CB-Au NP nanocomposites can also be produced by simply mixing solutions of CBs and pre-formed Au NPs stabilised by citrate or chloride ions.<sup>17,20,21</sup> The latter approach is considered more robust and versatile because it decouples the Au NP growth from the CB-mediated self-assembly. In particular, CB-Au NPs can be made from commercially available CB samples and colloidal Au NPs of different sizes stabilised in citrate or phosphate buffer.

The feasibility of forming the CB-Au NP nanocomposites both before and after the reduction of HAuCl<sub>4</sub> indicates that the formation mechanism is thermodynamically driven instead of kinetically trapped. Increasing the molar ratio of CB: Au causes a decrease in the ratio of doubly capped (bridging) to singly capped (non-bridging) CB and thus a decrease in the size of CB-Au NP nanocomposites. The presence of

CB stabilises the initially formed Au NPs and prevents them from clustering in the reduction step. The fabricated CB-Au NP nanocomposites are stable in aqueous solution for up to three months with no precipitation due to the presence of  $\text{Na}^+$  cations on the vacant carbonyl portals of the singly capped CBs, stabilising the nanoaggregates electrostatically.<sup>20</sup>

### 2.2. Robust sensing mechanism for reproducible SERS signals

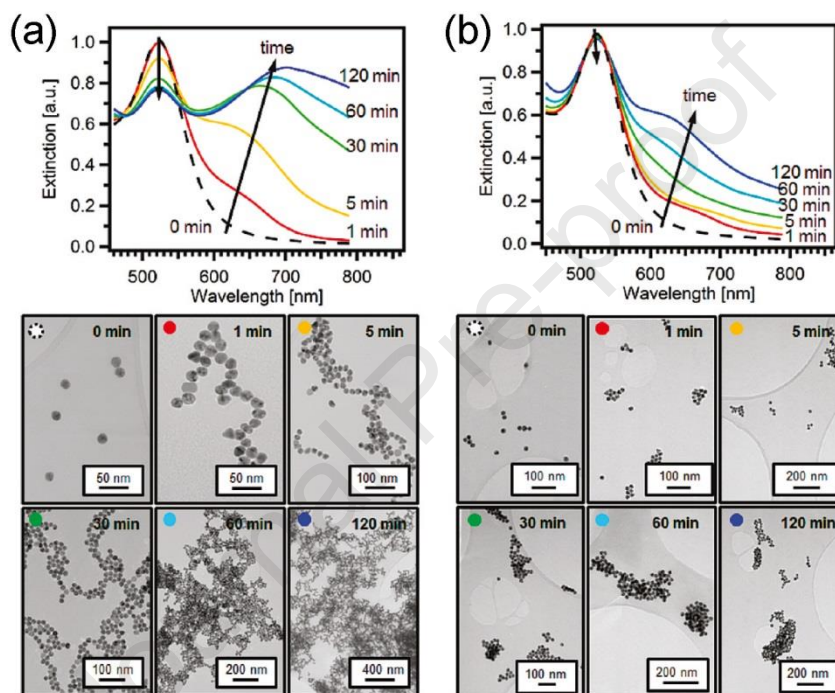
Detection of molecular analytes using CB-Au NP nanocomposites as SERS substrates can be achieved by encapsulating target analytes inside the CB cavity prior to in situ formation of the nanocomposites (Fig. 1a). CBs are a family of supramolecular host molecules that can form host-guest inclusion complexes via aqueous self-assembly driven by hydrophobic and electrostatic interactions. The resultant host-guest complexes typically retain the ability to bind to the surface of Au NPs and thus mediate their controlled aggregation, in case both portals of the CB are not sterically obstructed by the guest. If one of the portals is sterically hindered, then aggregation can be mediated by empty CBs in the system which are in dynamic equilibrium with the host-guest complexes.<sup>12,22</sup> Precise plasmonic nanojunction can be generated by simply adding the aqueous solution of the host-guest complexes to a solution of plasmonic NPs, where CBs will rapidly mediate the formation of the nanojunctions while simultaneously position the encapsulated analyte molecules at the centre of or in close proximity to the plasmonic hotspots (Fig. 1b,c). This configuration allows strong coupling between LSPR of adjacent Au NPs and therefore enable gigantic electric field enhancement at the nanojunction. As a result, upon photo-excitation in a Raman spectrometer, analyte molecules are exposed to intense optical near-field at the nanojunctions between two plasmonic NPs, giving rise to strong and reproducible SERS signals. The measurements can be performed on a small drop of sample solution or on liquid samples in a cuvette (Fig. 1d). Molecular modelling can be used to visualise the binding geometry of a complex, estimate the binding strength, as well as to aid interpreting the resultant SERS spectra (Fig. 1e). Identification of target analytes can be performed by matching the characteristics Raman peaks against databases, while quantification can be performed by plotting calibration curves using Raman intensity of the analyte main peak and/or by using machine learning algorithms.

### 2.3. Established structure-property relationships allowing rational design of bespoke sensing systems

As a critical parameter that determines the strength of near-field enhancement, the interparticle spacing between adjacent Au NPs can be precisely controlled at  $\sim 1.0$  nm, as evident by transmission electron microscopy (TEM) data, which is consistent with the CB portal-to-portal van de Waals dimension of 0.91 nm. The sub-nanometer spacing allows strong coupling between LSPR of adjacent Au NPs, which leads to gigantic electric field enhancement within the nanojunction and thus superior SERS enhancement. As in the case of attaching CBs onto a flat Au surface,<sup>19</sup> CBs are capped by Au NPs via their electron rich carbonyl portals where electron density are polarised towards the Au NP surface upon binding and weaken the C=O bond.<sup>17</sup> The interaction between CBs and Au NPs is stronger than that of other unfunctionalised macrocycles due to multivalent binding interactions.<sup>19,23</sup> The aqueous self-assembly process is mainly driven by the enthalpic gain from the interaction between carbonyl and Au, and the entropic gain from the release of surrounding water molecules.

In addition to offering control over the near-field enhancement via the formation of plasmonic nanojunctions, CB-mediated aggregation also allows control over the far-field LSPR extinction via the formation of aggregates with different morphologies, which can be achieved by carefully selecting the CB:NP ratio. In this context, matching the wavelength of the far-field LSPR extinction with that of the excitation laser can maximise the efficiency of the SERS phenomenon, generating strong SERS signals. Meanwhile coupling of LSPR will result in red-shift in extinction towards the near infrared (NIR) range where optical excitation generally causes less intense fluorescence background, and

exhibits a larger penetration depth.<sup>24</sup> In particular, the two main kinetic growth regimes for aqueous CB-Au NP assemblies were investigated as a function of CB concentration in a time-resolved manner shortly after the initial report on CB-Au NP nanocomposites (Fig. 2).<sup>13</sup> At higher molar ratio of CB5: Au NP (80:1), the sticking probability of Au NPs was high and successful coagulation from NP collisions were likely to occur. Open and elongated chain-like structures were initially formed as the colliding particles could not reach the very centre of the nanoclusters but were more likely to collide with the outermost structures. Quasi-fractal networks of CB5-Au NPs were formed as a result, following the diffusion-limited colloidal growth regime (DLCA) as shown in Fig. 2a. In contrast, slow growth of tight compact nanoclusters was observed at lower molar ratio of CB5: Au NP (60:1), following the reaction-limited colloidal growth regime (RLCA) as shown in Fig. 2b. The growth rate is strongly determined by the concentration of CB in both regimes.



**Fig. 2.** (a) Top: Extinction spectra of a CB5: Au NP (80:1) mixture measured up to 2 hours after mixing. Bottom: Representative TEM images of the corresponding time points in the extinction spectra, consistent with the morphologies formed via the DLCA kinetics. (b) Top: Extinction spectra of a CB5: Au NP (60:1) mixture measured up to 2 hours after mixing. Bottom: Representative TEM images of the corresponding time points in the extinction spectra, consistent with the morphologies formed via the RLCA kinetics.<sup>13</sup>

Since  $CB_n$  is Raman-active, its major homologues ( $CB_5$ –8) can be identified and differentiated by using Raman spectroscopy (RS).<sup>25</sup> Meanwhile, the Raman signals of CBs can potentially be used as internal standard to calibrate a SERS spectrum though it has not been demonstrated in the literature yet. CB has two major characteristic peaks at  $\sim 450\text{ cm}^{-1}$  and  $\sim 830\text{ cm}^{-1}$  in the Raman spectra, which corresponds to the ring scissor mode and ring deformation mode. Notably, the CB peaks show systematic shifts in vibrational frequencies as the number ( $n$ ) of glycoluril units of  $CB_n$  changes in both experiments and computational simulations due to steric and ring strain factors. For each additional glycoluril unit, the  $450\text{ cm}^{-1}$  peak shifts negatively by  $4\text{ cm}^{-1}$  as it is a scissor vibration of the N-C-N bonds and becomes less stained when the ring size increases. In contrast, the  $830\text{ cm}^{-1}$  peak shifts positively by  $1\text{--}2\text{ cm}^{-1}$  following an increase in the regular  $sp^3$  bond angle and thus strain with

the increase in number of glycoluril unit. Although it is possible to differentiate the  $CB_n$  homologues from their peak positions in bulk solid or liquid samples using RS, it is not suitable for trace level detection due to its inherently weak signals. SERS is then required to enhance the Raman signals of CBs by means of plasmonic NPs or nanostructures such as Au NPs, though the SERS peaks are broader than the Raman peaks in solution due to the weak interaction between the molecular orbitals and Au NP surface. The precise plasmonic hotspots in the CB-Au NP nanocomposites allows SERS detection of trace CB in solution, with a similar trend in the shift of the  $450\text{ cm}^{-1}$  and  $830\text{ cm}^{-1}$  peaks as the ring size of CB increases.

Host-guest chemistry of CBs enables trapping of target analytes within its cavity in solution. CBs outperform classical supramolecular hosts such as cyclodextrins and calixarenes as they exhibit stronger binding and higher selectivity towards shape and charge of guest molecules. Among all, CB7 stands out for its remarkably high binding affinity for cationic and neutral guests in water, whilst similar binding affinities can only be achieved for oppositely charged host-guest complexes in other supramolecular systems<sup>26</sup>. For instance, CB7 has ultrahigh binding affinities towards a wide variety of guest molecules ( $\sim 10^{15}$ – $10^{17}\text{ M}^{-1}$ ) including ferrocene, adamantanes and diamantanes derivatives, reaching and even surpassing those of biotin-avidin pairs.<sup>27-30</sup> This is attributed to optimal size, shape and multipolar complementarity between rigid CB cavity and the guest molecules. The capability of CBs to trap small molecules inside its hydrophobic cavity via host-guest complexation meanwhile mediating the formation of precise nanojunctions within the CB-Au NP nanocomposites allows detection of a wide range of target analytes with superior sensitivity, which is particularly important for the detection of molecules with poor affinities to Au surface.

In addition to boosting the sensitivity via surface-enrichment, host-guest complexation also improves the selectivity of the sensors via molecular recognition. The first example of molecular recognition using CB-Au NP nanocomposites via SERS was demonstrated with the dye molecule, rhodamine 6G (R6G).<sup>13</sup> The relatively larger water-soluble CB homologue, CB7, forms a strong 1:1 host-guest complex with R6G whereas CB5 is too small to accommodate it, illustrating the specificity of CB host-guest binding via in situ SERS measurements. CB7 can simultaneously control the precise interparticle spacing of Au NPs while capturing R6G inside its cavity, thus localising the analyte targets in close proximity to the plasmonic hotspots with enhanced sensitivity and reproducibility due to the strong field enhancement. Nevertheless, CB5 signals can be observed clearly in the presence of R6G, illustrating the tolerance of the CB-Au NP SERS system against contamination. The CB-Au NP nanocomposites are promising candidates for powerful SERS sensing and their recent applications will be discussed in the next section.

### ***3. Design guidelines and selected examples of CB-NP SERS sensors in biosensing and environmental monitoring***

While the emerging CB-Au NP nanocomposites are promising SERS sensors, a good combination of merits, including sensitivity, selectivity and multiplexing ability, is required for practical applications in biosensing and environmental monitoring. Among all, sensitivity is a major concern for WBE as the target analytes are highly diluted in wastewater stream and thus their concentrations are typically very low ( $\sim 0.01$ – $3.60\text{ ng mL}^{-1}$ )<sup>31</sup>. Primary concentration methods (e.g. two-phase separation, adsorption-elution and ultrafiltration) are usually required before subsequent quantification of the analytes using lab-based instruments.<sup>32</sup>

#### ***3.1. Sensitivity combined with reproducibility from precise plasmonic nanojunction***

For on-site quantification, SERS offers an excellent sensitivity comparable to HPLC-MS<sup>33</sup>. However, the ultrasensitivity is often accompanied by large signal variation and poor reproducibility due to formation of random plasmonic hotspots. This problem can be overcome by the CB-Au NP SERS sensors in which CB is used to control the interparticle spacing between NPs and generate precise plasmonic hotspots. The target analytes are thus localised at the centre of or in close proximity to the hotspots, resulting in highly sensitive and reproducible SERS signals. For instance, an important biomarker creatinine (CRN) of clinically relevant concentrations (300–3000  $\mu\text{g mL}^{-1}$  (2.65–26.5 mM), from normal to excess level in human) can be quantified in water and highly diluted synthetic urine using the CB7-Au NP nanocomposites formed by a combination of CB7-mediated aggregation and CRN-mediated electrostatic self-assembly.<sup>22</sup> The CB7-Au NP sensor has reached a sub- $\mu\text{M}$  detection limit of 12.5  $\text{ng mL}^{-1}$  (111 nM) for CRN via host-guest complexation between CB7 and CRN. This detection limit is lower than the current state-of-the-art methods using similar materials (e.g. Au NPs, Ag NPs and BN-Au nanocomposites), while the SERS signals of CRN using the CB7-Au NP substrates are also highly reproducible and tolerant against the presence of other biomolecules in the complex matrix. In addition, the CB7-Au NP sensor overcomes the issue of non-specificity of the standard clinical method for CRN qualification, a colorimetric technique based on the Jaffe reaction<sup>34</sup>, as picric acid forms reddish-orange complexes with numerous biomarkers besides CRN in basic solution. Similarly, CB-Au NP SERS sensors can detect drug molecules theophylline (TPH) and theobromine (TBR), which are metabolites of caffeine, down to  $\sim 9 \text{ ng mL}^{-1}$  (50 nM) with highly reproducible signals.<sup>35</sup> The detection limit of MeX using CB-Au NP nanocomposites is an order of magnitude better than similar SERS techniques. The SERS sensors allow real-time quantification as opposed to conventional technique such as HPLC.

In another example, trace amount of pollutants polyaromatic hydrocarbons (PAHs), including anthracene, 2-naphthol, phloroglucinol and 2,3-naphthalenediol, can be quantified using CB8-Au NP SERS sensors for environmental monitoring applications.<sup>36</sup> Notably, the relatively larger CB8 is able to trap two guests, an electron-deficient first guest (i.e. methyl viologen,  $\text{MV}^{2+}$ ) and an electron-rich second guest (i.e. PAHs), inside its hydrophobic cavity to form ternary complexes. This is particularly useful as hydrophobic molecules such as PAHs have low affinity for SERS substrates. The CB8-Au NP SERS sensors can detect PAHs down to  $\sim 130\text{--}180 \text{ ng mL}^{-1}$  ( $\sim 1 \mu\text{M}$ ), which is much lower than similar systems based on cyclodextrins-modified Au nanorods ( $9.6 \mu\text{g mL}^{-1}$  ( $50 \mu\text{M}$ ))<sup>37</sup>, giving thanks to the rigid and highly symmetric structure of CBs and thus the formation of precise plasmonic hotspots.

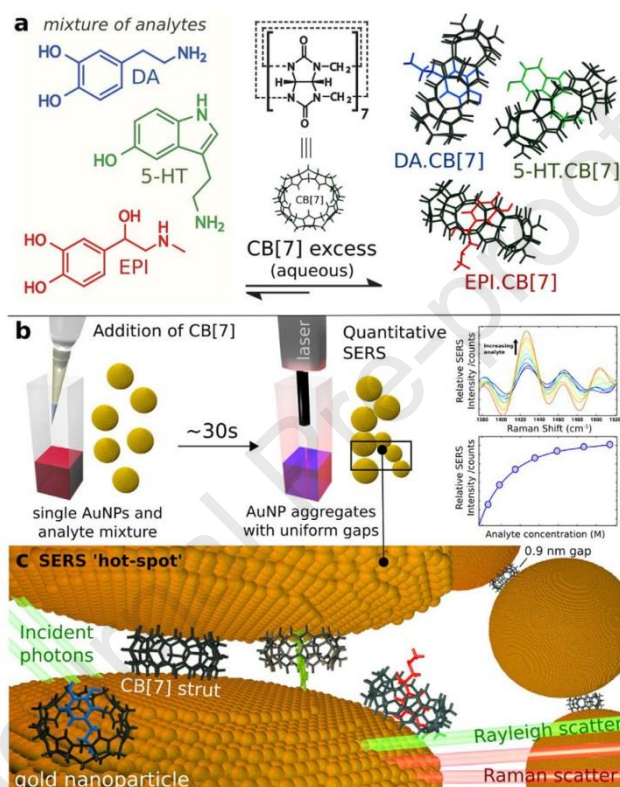
### 3.2. Selectivity underpinned by molecular recognition of CB

Despite the fact that sensitivity is a primary concern for WBE, it may not be sufficient in practical cases, since wastewater is a complex matrix which contains a wide range of background molecules from domestic and industrial waste and hospital discharge<sup>38</sup>. Routine sample preparation and separation process such as HPLC is typically used to address this problem. Although HPLC is highly sensitive, it is time-consuming and requires expensive instrument and trained personnel. Moreover, chemical separations may not be working effectively in some cases, especially when there are molecules with similar structures or chemical functional groups. This issue can be addressed by coupling CB to plasmonic NPs as to incorporate intrinsic selectivity into the sensor. For example, an explosive marker 2,4-dinitrotoluene (DNT), which is present as contaminants in surface water and groundwater, can be quantified using the CB-Au NP SERS sensors via formation of host-guest complexes between CB and DNT and thus localisation of DNT in close proximity to the plasmonic hotspots.<sup>12</sup> In contrast, a structurally similar nitroaromatic explosive, picric acid, cannot be detected by the same system as it is too bulky to be encapsulated by the CB cavity, thus illustrating the selectivity of the CB-Au NP SERS sensors. Common sensors for DNT are based on techniques (e.g. colorimetry, ion mobility spectrometry



and X-ray) that are prone to false positive or require radioactive source while the CB-Au NP SERS sensors are highly specific and relatively safe.

The specificity of the CB-Au NP SERS sensor can also be demonstrated with the phototransformation of trans-diaminostilbene (trans-DAS) inside the CB cavity.<sup>39</sup> In particular, trans-DAS can form a binary (1:1) complex with CB7 but a ternary (2:1) complex with CB8 which has a larger cavity. Upon UV excitation, trans-DAS undergoes photoisomerisation into cis-DAS inside CB7 whereas it undergoes photodimerisation into syn-1a,2a,3b,4b-tetrakis(4-aminophenyl)cyclobutane (syn-TCB) inside CB8, as identified from their characteristic SERS signals during real-time monitoring.



**Fig. 3.** (a) Formation of host-guest complexes between CB7 and epinephrine (EPI), dopamine (DA) and serotonin (5HT). (b) Schematic diagram showing the formation of precise plasmonic nanojunctions between adjacent Au NPs upon addition of CB7, thus allowing SERS quantification of analyte targets. (c) Schematic diagram visualising the localisation of neurotransmitters near the plasmonic nanojunctions via CB7 host-guest complexation.<sup>40</sup>

### 3.3. Multiplexing capability via reproducible quantification and machine learning

In the context of WBE, it is often advantageous to simultaneously detect a number of analyte molecules to obtain a chemical marker profile of the wastewater sample, which allows multi-dimensional correlation to be drawn using advanced data analysis for more robust and reliable insights. Hence, detecting multiple analytes in the same measurement run offers practical advantages such as high throughput sample preparation and measurement, as well as consistent data set from the same sample. The CB-Au NP SERS systems allow multiplexed quantification of target analytes with highly reproducible signals, which are essential to reliable machine learning models, via formation of host-guest complexes and precise plasmonic hotspots, as evidenced by the recent examples of SERS detection of neurotransmitters<sup>40</sup>, methylxanthines (MeX)<sup>35</sup>, nucleobases<sup>41</sup> and nitroexplosives<sup>12</sup>.

The feasibility of quantifying multiple biomarkers using CB7-Au NP SERS sensors was first demonstrated with monoamine neurotransmitters including dopamine (DA), epinephrine (EPI) and serotonin (5HT), as illustrated in Fig. 3.<sup>40</sup> The neurotransmitters can be simultaneously quantified in water and synthetic urine by resolving the contribution from the individual neurotransmitter in the SERS spectra, with a detection limit of  $\sim 77\text{--}92\text{ ng mL}^{-1}$  ( $\sim 0.5\text{ }\mu\text{M}$ ). While electrochemical methods, mass spectroscopy and fluorescence are current state-of-the-art methods used to quantify neurotransmitters, their applications in biosensing and environmental monitoring are limited by the low capability to detect multiple target analytes in complex matrix as additional separation methods are required. Thus, CB7-Au NP SERS sensors are more promising as they can quantify multiple neurotransmitters with fast response.

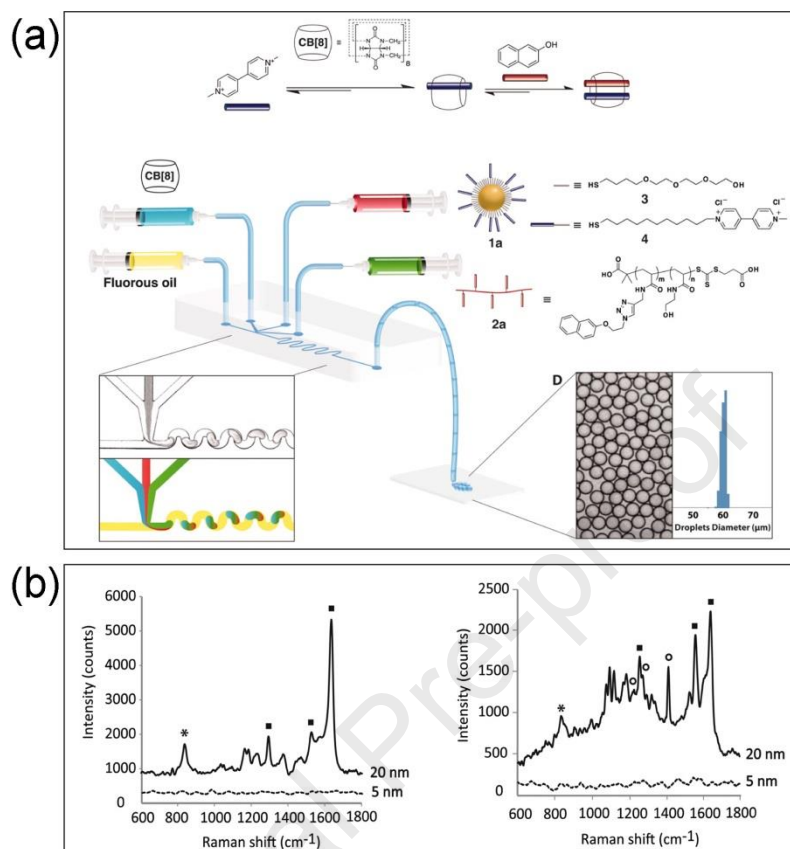
Notably, multiplexed quantification of MeX drug isomers, TPH and TBR, has also been demonstrated with the CB8-Au NP SERS sensors at sub- $\mu\text{M}$  levels.<sup>35</sup> The dynamic range of the sensors can be extended by using machine learning algorithms, which is particularly useful for quantifying multiple analytes in complex matrix such as wastewater. Meanwhile, DNT can be detected in the presence of model organic contaminants such as 4-mercaptobenzoic acid and 4-mercaptophenylboronic acid which bind strongly to Au NPs, implying the potential of the CB-Au NP SERS sensors towards detecting multiple target analytes in environmental monitoring.<sup>12</sup>

#### *3.4. Lab-on-a-chip applications through implementing CB-Au NP nanocomposites in microfluidic technology*

By isolating the CB-Au NP nanocomposites in microdroplets, it enables SERS measurements in a flow with millisecond time resolution. Salmon et al.<sup>42</sup> investigated the relationship between the SERS response and the formation of plasmonic nanoarchitectures (CB5-Au NP nanocomposites) within microfluidic droplets by dark-field scattering spectroscopy. Microdroplets with CB5-Au NP nanocomposites are generated by double flow-focus geometry, where CB5 and Au NPs first form a mixed solution through the first junction, and are immediately sheared into droplets by the oil phase at the second junction. Notably, in the initial regime, the formation of dimer is critical for the optical properties, resulting in a near-linear relationship between the SERS intensities and dark-field scattering. This technique paves the way for application of SERS in WBE, which requires real-time, large-scale and cost-effective measurements, as it provides a simple, fast, high-yield and robust method for preparation of isolated test spots for SERS.

The CB-Au NP nanocomposites can also be embedded into the shell of microcapsules which allows the detection of analytes that are selectively encapsulated within the cavity of microcapsules<sup>43,44</sup>. The formation of such kind of CB-mediated microcapsules benefits from the synergetic effect of formation of supramolecular CB8 ternary complex<sup>45-47</sup> and high detachment energy of NPs from the liquid-liquid interface<sup>48-51</sup>. Zhang et al.<sup>43</sup> first reported the fabrication of CB8-Au NPs supramolecular microcapsule via a single-step approach using a droplet microfluidics technique. The droplet microfluidic device is developed based on a simple T-junction geometry (Fig. 4a), where the oil continuous phase is introduced perpendicularly to the aqueous dispersed phase which has three inlets for Au NPs functionalised with MV moieties, a naphthol-functionalised copolymer and CB8, separately. Highly monodispersed water/oil droplets are generated when the oil phase shears off the aqueous phase. The functionalised Au NPs are arrested at the droplet interface because of the high detachment energy. Consequently, CB8 and the naphthol-functionalised copolymer are dragged onto the droplet interfaces via the formation of CB8 ternary complex with MV groups on the arrested Au NPs. Microcapsules with CB-Au NP nanocomposites on the shell are easily formed after evaporation of water from the initial droplet, which can be used as substrates for SERS detection of encapsulated analytes. A fluorescein isothiocyanate-labelled dextran (FITC-dextran) is loaded into the

microcapsules to investigate the efficiency of encapsulated materials by SERS. The results showed a remarkable SERS enhancement of the FITC signals (Fig .4b)<sup>43</sup>.



**Fig. 4.** (a) Schematic diagram visualising formation of microcapsules with CB8-Au NP nanocomposites in the shell using a microfluidic device. (b) SERS spectra of empty microcapsules (left) and FITC-dextran encapsulated microcapsules consisting of CB8-Au NP nanocomposites (right).<sup>43</sup>

#### 4. Conclusion and outlook

WBE is a robust and non-invasive tool used to probe information on population health by tracking trace chemical and biological markers in wastewater. As opposed to the traditional methods for WBE, SERS is a powerful technique that offers numerous advantages for biosensing and environmental monitoring. In particular, the potential application of SERS sensors based on CB-Au NP nanocomposites for WBE was explored in this review (Table 1). Compared to other existing SERS approaches,<sup>53,54</sup> SERS using CB-NPs offers advantages including ease of fabrication, high reproducibility, good sensitivity and intrinsic selectivity. Fabrication of CB-Au NP nanocomposites via hierarchical aqueous self-assembly was explained, covering factors that underpin their formation kinetics such as the molar ratio of CB: Au NP. Important properties of the precise plasmonic nanojunctions within the CB-Au NP nanocomposites were reviewed and supported by fundamental theories and modelling results. Supramolecular chemistry of CBs which enables trapping of informative target analytes localised at the centre of or in close proximity to the plasmonic hotspots was discussed. Structure-property relationships of the CB-Au NP sensor was elaborated in the aspects of sensitivity, selectivity, reproducibility and tolerance against contamination. Practical considerations of designing CB-Au NP SERS sensors for WBE were discussed by summarising recent advances in biosensing and environmental monitoring. Performance of the SERS sensors has also been benchmarked against other sensing schemes such as

HPLC, fluorescence and colorimetric methods, as well as similar materials/chemical systems such as citrate-capped Au NPs and cyclodextrins-modified Au nanorods.

**Table 1.** State-of-the-art SERS sensors for WBE and potential candidates based on CB-Au NP nanocomposites. Sample preparation techniques can be employed to increase or decrease the concentration of the analyte to match the suitable detection range. Please refer to ref. 31 for other detection schemes.

Substrate	Analyte	Limit of detection (ng mL <sup>-1</sup> )	Ref.
Au@Ag on glass nanofibrous paper	Methamphetamine	0.1	10
Au nanocylinder-molecularly imprinted polymers	Paracetamol	45	55
CB-Au NP nanocomposites	Creatinine	12.5	22
	Methylxanthines	9	35
	Neurotransmitters	77–92	39
	Nitroaromatics	180	12
	Polyaromatic hydrocarbons and derivatives	130–180	36
	Uric acid	34	21

Whilst CB-Au NP SERS sensors are promising for quantifying trace chemical and biological markers, the ability of the SERS sensors to simultaneously detect and identify tens of target analytes in wastewater in real-time with high sensitivity and reproducibility remains the major issues for WBE. Further efforts are thus required to bridge the gap between the state-of-the-art research and commercially viable sensors for WBE. For instance, the molecular volume of a suitable target analyte is limited by the cavity volume of CB. If the analyte molecule is too large, it cannot fit into the CB cavity, or it can only bind partially into the CB cavity. In the latter scenario, the CB portal will be partially blocked by the analyte molecule, and thus hindering the formation of plasmonic nanojunctions. This limitation can be tackled by adopting a sideways binding orientation of CB at the plasmonic nanojunction, which can be achieved via perthiolation of CB at the equatorial positions<sup>56</sup>. Functionalisation of CB also offers other advantages such as improved aqueous solubility and availability of additional chemistry at the plasmonic nanojunctions. In addition, novel plasmonic NPs such as core-shell NPs and nanotriangles could be incorporated into the CB-NP SERS sensors to enhance the sensitivity further as anisotropic NPs possess intrinsically more plasmonic hotspots than spherical NPs. Also, more work is required in device-level development by means of integrated sample preparation or separation using lateral flow chromatography microfluidics or other similar techniques as to reduce labour-intensive procedures. In order to further enhance the reproducibility, microfluidics should be used to control the aggregation kinetics while standard operating procedure (SOP) and other independent techniques (e.g. UV-Vis spectroscopy) should be developed to validate the measurement results. Along this line, it might be useful to combine SERS with electrochemical techniques, as CBs are known to form host-guest complexes with redox probes<sup>52</sup>, such as MV. Last but not least, data analysis based on cloud-based big data library and machine learning algorithms such as artificial neural network should be adopted to aid real-time quantification of numerous analyte targets in complex matrix, thus paving the way for the CB-NP SERS sensors towards practical application in WBE.

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- Surface-enhanced Raman spectroscopy (SERS) can quantify multiple chemicals and biomarkers with rapid response, high sensitivity, selectivity and tolerance to contamination.
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- Potential pathways for future fundamental and translational research are proposed.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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