Functionalization of multilayer carbon shell-encapsulated gold nanoparticles for surface-enhanced Raman scattering sensing and DNA immobilization

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Abstract:

In this paper, we reported the surface modification of **multilayer carbon shell-encapsulated gold nanoparticles (Carbon Nanoparticles, or CNPs)** *via* an oxygen plasma treatment approach and their subsequent functionalization with extraneous gold (Au) nanoparticles for Surface-enhanced Raman Scattering (SERS) sensing and DNA immobilization. The oxygen plasma treatment process led to purification of the multilayer carbon shell, reduction of the shell thickness, and derivatization of carbon shell with functional carboxylic groups. This further opens potential opportunities for surface functionalization of CNPs with extraneous Au nanoparticles or λ -DNA fragments, which were both achieved through a well-defined carbodiimide based covalent linking chemistry without removing the CNPs from the substrate. The resulting CNP-Au nanoparticle hybrids as well as the CNP-DNA architectures were characterized using various microscopic and spectroscopic techniques. The CNP-Au nanoparticle hybrids were further used as highly-sensitive SERS substrate for the detection of trace amount water-containing organic molecule. Meanwhile, they were also fundamentally simulated for their plasmonic behavior using discrete dipole approximation (DDA) method to understand the basic Raman enhancement principle. On the other hand, highly-ordered assembly of CNP-DNA architectures were also demonstrated in detail for their great potential in future DNA detection/recognition and bio-device applications.

Keywords:

Multilayer carbon shell-encapsulated gold nanoparticle (CNP); Oxygen plasma treatment; Carbodiimide chemistry; SERS; DNA immobilization

1. Introduction

Sensitive, selective, rapid, and cost-effective analysis of biomolecules has become of importance due to the increasing demand for lab-on-chip-type analytical devices [1-3]. Deoxyribonucleic acid (DNA) can be potentially used as a building block for the assembly of nanoscale electronic devices, clinical testing, environmental monitoring, and food safety [4]. However, high-efficiency DNA purification and yield remain a challenge for such applications. In addition, high cost, low efficiency, and poor repeatability of DNA binding substrates or detection devices is still a major limitation. These necessitates detailed study of immobilization of DNA on sensitive and selective substrates. Chemical absorption, covalent binding, electrostatic attraction, copolymerization, and streptavidin-biotin affinity systems are frequently reported methods for DNA immobilization and detection [5] however have not completely overcome the limitations.

The applications of gold (Au) nanoparticles in DNA-based architectures and other chemical sensors are also interesting and promising, allowing the programmability, molecular recognition, and catalytic property of biopolymers to couple to the unique optical, thermal, electric and catalytic properties of Au nanoparticles [6-8]. Numerous practices have since been made including the preparation of ordered nanostructures [9], gene and drug delivery [10] and biosensing [11]. For instance, DNA has been used as a rigid spacer between Au nanoparticles to prepare ordered architectures with distance-dependent optical [12] and physical properties [13,14] in terms of heat, electron, and energy transfer. In ref. 12, the authors reported the preparation of highly uniform DNA-tailorable Au nanoparticle architectures with 1 nm inter-particle gap. Such controllable interior nanogaps highly strengthened the near-field electromagnetic field induced by the localized surface plasmon resonance (LSPR) of Au nanoparticles, and led to significantly improved detection sensitivity of the surface-enhanced Raman scattering (SERS) sensor. These studies open new insights and reliable approaches to the development of bio-nano sensing devices and other bio-

technologies due the well-established thiol-chemistry, bio-compatibility and chemical stability of Au nanoparticles [15-17].

Optical and sensing performances of Au nanoparticles can be significantly improved through their surface-functionalization or heterostructuring [18-20]. The thiol-chemistry was the most used approach to combine Au nanoparticles with extraneous thiolated nanostructures or bio-components. However, this also limited or complicated the widening hybridization of Au nanoparticles with other thiol-absent materials since additional thiolating step has to be involved in the hybridization process. For instance, thiolated tips were normally pre-attached at the end of DNA stretches so that they can be covalently linked to the Au nanoparticles [18,21]. This not only brought extra demand of raw DNA materials and complicated the process, but also increased the toxicity of final composite product due to the low-compatibility of the thiol items in a lot of bio-systems. Thus, development of thiol-free approaches for hybridization or functionalization of Au nanoparticles are feasibly demand and promising.

Carbon nanostructures such as carbon nanotubes and graphene have been widely used as the backbone for DNA immobilization [22-25] due to their interesting properties and broad surface chemistries. Recently, the authors reported a facile and scalable chemical vapor deposition (CVD) approach for the growth of **multilayer carbon shell on Au nanoparticle** [26-28]. This process led to the fabrication of multilayer carbon shell encapsulated Au nanoparticles (**referred as carbon nanoparticles, CNPs**), which preserve the unique optical properties from Au nanoparticle [29,30], and at the same time present the excellent surface chemical properties of **carbon shell** [31], which make CNPs of promise for future chemical sensor and bio-medicine applications. Nevertheless, for the as-produced CNPs, the surface of carbon shell is highly hydrophobic due to the presence of amorphous carbon (a normal by-product from CVD) and lack of surface functionalities such as carboxyl (-COOH) and hydroxyl (-COH) groups [32]. Meanwhile, the as-produced CNPs show a

relatively thick carbon shell (~10-15 layers), which may slightly shadow the plasmonic properties of the Au nanoparticle core [29]. As a result, further surface modification of CNPs is necessary for the purpose of removing extra amorphous carbon, reducing the shell thickness and introducing more surface functionalities (-COOH and -COH).

Accordingly, in this paper we conducted an oxygen plasma treatment to the as-produced CNPs. Various plasma conditions were used to evaluate their influence on the shell structure, thickness and surface functionality content. The best treatment condition were proposed and the CNPs treated with this condition were further used for the covalent linking of extraneous Au nanoparticles or for the immobilization of λ -DNA fragments. The plasmonic properties of the resulting extraneous Au nanoparticle-attached CNPs (referred as CNP-AuNP hybrids) were simulated using the discrete dipole approximation (DDA) method. These CNP-AuNP hybrids were further used as the SERS substrate for chemical sensing organic dyes. Meanwhile, the highly-ordered assembly of CNP-DNA architectures immobilized on the silicon substrate were characterized by various microscopic and spectroscopic methods.

2. Experimental details

2.1. Materials and methods

N-type silicon wafers (100) were purchased from IWS (Colfax, CA). Commercial gold colloids (~10 nm) was purchased from Sigma-Aldrich (St. Louis, MO). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), and 2-(N-morpholino) ethanesulfonic acid (MES) were purchased from THERMO scientific (Rockford, IL). TrackIt™ λ -DNA/Hind III Fragments were purchased from Invitrogen (Grand Island, NY). DI water (18.1 M Ω -cm) was obtained using a Barnstead International DI water system (E-pure D4641). All chemicals were used

without further purification. The growth of multilayer carbon shell on Au nanoparticles were carried out in GSL-1100X Tube Furnace (MTI Corporation) with a quartz tube from ChemGlass (Vineland, NJ). Oxygen plasma treatment was conducted in a Nordson March Jupiter III Reactive Ion Etcher (Concord, CA).

2.2. Fabrication and plasma treatment of CNPs

The fabrication and patterning of CNPs on the silicon substrate were carried out as previously reported by the authors [26-31]. Briefly, the silicon substrate was first treated in the piranha solution ($\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 4:1$) at 100 °C for 30 min to create surface -OH groups. Siloxane functionalization of the substrate was carried out in 2 mM MPTMS/ethanol solution for 12 h. The functionalized substrate were then rinsed with ethanol, dried in N_2 and further placed in vacuum oven at 80 °C for 1 h to complete Si-O bond formation. In the following, this substrate was put in the diluted commercial gold colloid (~1 mM) and slightly stirred overnight for the attachment of Au nanoparticles on the silicon substrate. Prior to the growth of multilayer carbon shell, the Au nanoparticles decorated silicon substrate was treated with oxygen plasma for 15 min at 160 W. This process led to the formation of surface gold oxide, which acted as the catalyst for the initial growth of carbon shell on Au nanoparticles in the following CVD process. The CVD growth was conducted at 675 °C. Xylene was used as the carbon source with a feeding rate of 10 mL/h. A mixture of 10% H_2/Ar with a total flow rate of 1.15 SLM was used as the carrier gas. The growth process was continued for 1 h, resulting in the as-produced CNPs. Finally, plasma treatment of the as-produced CNPs were conducted at various conditions including different duration, plasma power and oxygen pressure. Detailed experimental parameters were listed in Table S1 (Supplementary Data).

2.3. Covalent linking of extraneous Au nanoparticles

The silicon substrate decorated with plasma-treated CNPs (at the best condition) were put into 50 mM MES buffer (pH = 4.7) solution containing 2 mM EDC and stirred for 10 min to form *o*-acylisourea ester, then 4 mM of NHS was added into the solution and continuously stirred for 1 h for the formation of stable amine-reactive NHS ester. The substrate was then slightly washed with ethanol and further immersed in the diluted Au colloid/ethanol solution (~1 mM) for 24 h. This process led to the covalent attachment of extraneous Au nanoparticles on the plasma-treated CNPs (or the formation of CNP-AuNP hybrids) [31,33].

2.4. Immobilization of λ -DNA fragments

The well-defined carbodiimide chemistry [31,34] was used for the attachment or immobilization of λ -DNA fragments on the CNPs decorated silicon substrate. Briefly, plasma-treated CNPs (at the best condition) on silicon substrate were first put into 50 mM MES buffer (pH = 4.7) with 2 mM EDC and stirred for 10 min, then 4 mM NHS was added into the solution and further stirred for 1 h. The substrate was then successively rinsed with MES buffer and PBS buffer (pH=7.4). The substrate was further put in 5 mL PBS buffer containing 4 mM λ -DNA, 1 mM EDC and 2 mM NHS for DNA immobilization. The DNA-anchoring process was continued for 24 h, leading to the formation of CNP-DNA architectures patterned on the silicon substrate.

2.5. Characterization methods and simulation details

Scanning Electron Microscopy (SEM, JOEL-7000), Transmission Electron Microscopy (TEM, Tecnai F-20) and/or Atomic Force Microscopy (AFM) were used for characterizing the morphology and structure of various nanostructures including CNPs (before and after plasma treatment), CNP-AuNP hybrids and CNP-DNA architectures. A Bruker Senterra Raman system was used to identify the vibrational modes in CNPs (before and after plasma treatment). The chemical/element analysis was conducted *via* X-ray Photoelectron Spectra (XPS, Kratos Axis 165).

Fourier Transform Infrared spectroscopy (FTIR, JASCO 4100) was used to characterize the surface functional group of CNPs and CNP-DNA architectures. The SERS sensitivity of the CNP-AuNP hybrids were demonstrated by immersing the substrate in the aqueous solution containing 10^{-6} M Rhodamine 6G (R6G) or Methylene Blue (MB). The Raman signals of the absorbed dyes on such SERS substrate were collected using the same Bruker Senterra Raman system.

To further understand the SERS effect, discrete dipole approximation (DDA) method was used for simulating the surface plasmonic properties of the as-produced CNPs and CNP-QD hybrids [29,35]. The DDA method is based on the solution of the 3D Maxwell equation *via* the DDSCAT code developed by Draine and Flatau [35]. Detailed simulation procedures have been demonstrated earlier by the authors [28,36]. Briefly, DDSCAT 7.2 was utilized to calculate the extinction (including absorption and scattering) and further estimate the normalized electric field or surface plasmon generation of the targets created according to the experimental CNPs and CNP-AuNP hybrids. The normalized electric field intensity is defined as the strength ratio between the electric field generated near the target surface and the incident electric field ($|E|/|E_0|$). The geometrical targets were generated using 3D max software. The effective radius of the dipoles was calculated using: $r_{\text{eff}} = (3V/4\pi)^{1/3}$, where V is the total volume of the target and is calculated with $V = N \cdot d^3$ (N is the number of dipoles and d is the lattice spacing in cubic array).

3. Results and discussion

3.1. Growth of multilayer carbon shell on Au nanoparticles

The growth of multilayer carbon shell on the Au nanoparticles was achieved *via* the xylene-based chemical vapor deposition process [27]. Commercial Au nanoparticles was patterned on the surface-oxidized silicon substrate through the MPTMS-functionalization as described above [37].

Gold oxide was further created on the surface of Au nanoparticles *via* the oxygen plasma treatment. This oxide layer has been found to be of importance for the catalytic growth of initial carbon layers on the Au nanoparticles (Fig. 1a) and the detailed mechanism was reported in our previous studies [26]. The following growth was completed by continuous decomposition of CH₄ in a H₂/Ar environment and further deposition of carbon atoms on the initial carbon shell. TEM image in Fig. 1b shows the as-production multilayer carbon shell-encapsulated Au nanoparticles (CNPs) dispersed on the lacy carbon TEM grid. The average diameter of CNPs was increased to 25.4 ± 3.9 nm as compared with the pristine commercial Au nanoparticles (~ 10 nm) due to their inevitable aggregation during the high-temperature CVD process. Representative high-resolution TEM images in Fig. 1c and d illustrate that thickness of the as-produced carbon shell is $\sim 3.2 \pm 0.6$ nm, with an inter-lattice spacing of $\sim 0.38 \pm 0.03$ nm. This value is slightly larger than the natural c-axis spacing of graphite (~ 0.34 nm) due to the curvature structure of carbon shell [27]. The TEM image in Fig. 1d was converted by Fast Fourier transform (FFT) method. The resulting FFT image (Fig. 1e) shows the well-defined six-fold symmetry expected for graphite structure [38,39] and the other two symmetric spots are corresponding to the (111) plane of Au nanoparticles. More TEM images of the as-produced CNPs obtained in the repeating growth were shown in Fig. S1 (Supplementary Data).

3.2. Oxygen plasma treatment of CNPs

With the purpose of creating surface oxygen-containing functionalities (-COH and -COOH), removing amorphous carbon and further adjusting the carbon shell thickness, oxygen plasma treatment was conducted for the as-produced CNPs. Three sets of experimental conditions including duration, plasma power and oxygen pressure (Table S1, Supplementary Data) were studied to understand the oxidation mechanism and further obtain the best condition for the plasma

treatment. Representative TEM images of the CNPs treated with various conditions were shown in Fig. 2a-l and more proof images were further detailed in Fig. S2-S4 (Supplementary Data). Significant structure changes on the CNPs were observed after the plasma treatment, as schematically summarized in Fig. 2m. The variation of carbon shell thickness according to the plasma condition was also shown in Fig. 2n. One can observe that the oxygen plasma treatment, even with short duration of 5 s (Fig. 2b), has a significant etching effect on the carbon shell (Fig. 2n). When the treatment was continued for 60 s, carbon shell in some regions of the nanoparticle surface were completely removed, resulting in partially covered carbon shell on the Au nanoparticles (Fig. 2d). Meanwhile, at constant duration (15 s, plasma power set), plasma treatment with low power (30 W or 60 W, for instance), rather than etched away the carbon shell, converted the shell into an amorphous structure (diamond-like carbon [40,41]) and resulted in an increase of shell thickness (Fig. 2o). This is probably due to the gentle incorporation of oxygen atoms in the carbon shell without the formation of carbon oxides (CO_2 or CO), which led to the distortion of the crystallized carbon shell [40]. However, when the plasma power exceeds 90 W, significant etching effect was observed. The carbon shell were partially etched away but retained their graphite crystalline structure. In addition, varying the oxygen pressure led to a relatively weak effect on the carbon shell structure. The shell thickness was slightly increased at high pressure. This is probably because of the shortening of the mean free path of oxygen atoms due to the increased possibility of self-collision at high pressure [42], and resulted in the decrease of collision speed of oxygen atoms to the carbon shell.

Since above TEM analysis indicates that the oxygen plasma process is powerful in the etching of carbon shell, it is necessary to understand its influence on the quality of carbon. This was characterized in detail *via* Raman spectroscopy and the results were shown in Fig. 3. All of these samples before and after plasma treatment show the well-defined D-band at $\sim 1310\text{ cm}^{-1}$ and G-

band at $\sim 1584\text{ cm}^{-1}$ [43-45]. In addition, the G-band was split and an additional D'-band was observed at $\sim 1595\text{ cm}^{-1}$ for all of these samples. The emergence of this D'-band was normally considered to be attributed to the randomly distributed impurities and the surface charges in carbon shells [46,47]. For CNPs, the observed D'-band is due to the interaction between the localized vibration mode of surface plasma from Au nanoparticles and the extended phonon mode of carbon shell [48-47]. Meanwhile, G-band for carbon materials is due to the E_{2g} mode at the Γ -point, arising from the stretching of C-C bond in graphite materials, and is common for all sp² carbon systems [44]. G-band is highly sensitive to strain effects in sp² hybridized atoms and thus is a significant indication for the quality of graphite structure. The D-band is caused by the disordered sp² hybrid structure of carbon and sp³ carbon system such as amorphous carbon [48,49]. D-band is a reflection of the content of defects and impurities in carbon shells. As a result, the intensity ratio of D-band and G-band (I_D/I_G) is critical for identifying the crystallizing quality of carbon materials.

As shown in Fig. 3a, the as-produced CNPs show large D-band due to the curvature-induced disorder in multilayer carbon shell and the presence of amorphous carbon [26]. The plasma treatment resulted in significant intensity decrease for both D-band and G-band due to the etching of carbon shell. Plasma treatment for 5 s and 15 s led to a decrease of I_D/I_G from ~ 1.52 for the as-produced CNPs to ~ 1.34 and ~ 0.97 , respectively. However, when the treatment duration exceeded 40 s, I_D/I_G was increased compared with the as-produced CNPs. The influence of such plasma treatment on I_D/I_G is a comprehensive effect. On the one hand, the plasma treatment removes amorphous carbon on CNPs as well as on the substrate, which improves quality of carbon shells on Au nanoparticles and leads to an increase of I_D/I_G ; On the other hand, oxygen plasma creates new surface functionalities (-COOH and -COH) as well as other defects in the crystalline structure of carbon shell [50], this will lead to a decrease of I_D/I_G . As a result, at the initial stages of the treatment (5 s and 15 s), we observed a decrease in I_D/I_G . However, when the treatment continued

(> 30 s), more defects were created in the carbon shell, leading to the increase of I_D/I_G . In addition, it is worth mentioning that when the treatment was lasted for 90 s or 120 s, both D-band and G-band became very weak due to the lack of carbon shell.

Fig. 3c and d show the Raman spectra and corresponding I_D/I_G of CNPs after plasma treatment at different power. For this set of study, new-prepared fresh CNPs were used and it is necessary to mention that the I_D/I_G of the as-produced CNPs is slightly different for various sets. As described above, plasma treatment at lower powers induced the formation of amorphous carbon shell on the Au nanoparticles (Fig. 2e and f). Thus, obvious decrease of I_D/I_G was not observed at 30 W and 60 W. Further increasing the plasma power resulted in the decrease of I_D/I_G . However, when the power increased to 150 W, I_D/I_G was slightly increased due to the severe damage of carbon shell. The influence of oxygen pressure on the Raman spectra and I_D/I_G was shown in Fig. 3e and f. Oxygen plasma exhibited high mean free path at lower oxygen pressure and thus they interacted with the carbon shell with a higher speed. This led to more damage on the carbon shell and thus the I_D/I_G obtained at 200 mTorr and 250 mTorr were largely increased (Fig. 3f). However, no significant change on I_D/I_G was observed at high pressure due to the weak interaction between oxygen plasma and the carbon shell.

Quantitative analysis of the surface functionalities on CNPs was conducted using XPS, which gave signals for various elements including Au, C, O and Si for the as-produced CNPs (Fig. S5a, Supplementary Data). The Si peaks arise due to the substrate. Pure element Au peaks for intact Au nanoparticle cores were observed at ~ 84.59 eV and ~ 88.27 eV, which are consistent with the binding energy of Au $4f_{7/2}$ and Au $4f_{5/2}$ respectively [26]. For O 1s peaks, there could be several origins, such as absorbed molecules and native oxide (SiO_2) from the substrate. Thus, O 1s peaks should not be utilized for analyzing the surface functionalities of CNPs. High-resolution spectra were collected for C 1s peaks (Fig. S5b, Supplementary Data) and were further deconvoluted into

several carbon groups including C-C, -COH and -COOH. The molar fraction of carboxyl and hydroxyl radicals in the carbon shell was obtained from their area percentage in the total C 1s spectrum area.

XPS spectra were also collected for CNPs after plasma treatment at various conditions. The variation of hydroxyl and carboxyl content in the carbon shell as a function of the duration, plasma power and oxygen pressure were shown in Fig. 4a-c. Several representative deconvoluted XPS spectra of C 1s were shown in Fig. 4d-i. The detailed full range spectra and deconvoluted C 1s spectra corresponding to all the plasma conditions (Table S1, Supplementary Data) were further shown in Fig. S5-S10 (Supplementary Data). The as-produced CNPs show a carboxyl content of 4.82% and hydroxyl content of 6.53% due to the absorbed water moisture and the inevitable oxidation in air. Plasma treatment of 5 s and 15 s resulted an increase of carboxyl content to 10.00% and 16.79%, and an increase of hydroxyl content to 16.51% and 21.62%, respectively (Fig. 4a). However, when the plasma treatment exceeded 30 s, the carboxyl and hydroxyl content significantly decreased. This is consistent with the change of I_D/I_G observed in Fig. 3b and due to the over-etching of carbon shell. With respect to the plasma power, large powers (e.g., 120 W, 150 W) led to high content of carboxyl and hydroxyl groups in the carbon shell because of the intensive interaction between oxygen plasma and carbon (Fig. 4b). Moreover, in the pressure study, it was observed that the CNPs treated with low pressure (e.g., 200 mTorr, 250 mTorr) show relatively low content of carboxyl groups due to the lack of oxygen atoms. However, when the pressure increased to 600 mTorr or 700 mTorr, the generated plasma becomes weak as described above, resulting decreased content of carboxyl and hydroxyl groups (Fig. 4c). Thus, only proper oxygen pressure (300~400 mTorr) can lead to high content of carboxyl and hydroxyl functionalities.

According to this plasma treatment study on CNPs, we summarized that the best conditions for obtaining high content of surface -COOH functionalities, appropriate shell thickness as well as

good purification effect are duration of 15 s, plasma power of 120 W, and pressure of 300 mTorr. This was further confirmed by the hydrophilicity test showing in Fig. 5. The 10 μ l DI-water droplet shows a contact angle of $\sim 55.71^\circ$ on clean silicon wafer (Fig. 5a). The contact angle of the same droplet on the as-produced CNP substrate is $\sim 71.40^\circ$ (Fig. 5b). This value was decreased to $\sim 31.86^\circ$ on the plasma-treated CNP substrate (Fig. 5c). The improvement of hydrophilicity on the plasma-treated CNP substrate was demonstrated in Fig. 5d. The as-produced CNPs were highly hydrophobic due to the presence of amorphous carbon and lack of surface functionalities [51]. However, after the plasma treatment, the surface of CNPs become hydrophilic due to the removing of amorphous carbon and the generation of carboxyl and hydroxyl groups, which are favorable for the formation of hydrogen bonds with water molecules. This hydrophilicity test indicates that the obtained best conditions are feasible for above purposes and can be further used for the subsequent surface functionalization of CNPs.

3.3. Hybridization of CNPs with extraneous Au nanoparticles for SERS

In this study, surface functionalization of plasma-treated CNPs includes heterostructuring attachment of extraneous Au nanoparticles and immobilization of λ -DNA fragments. The well-defined carbodiimide chemistry was used for the covalent conjunction in both processes [52-54]. As schematically illustrated in Fig. 6, the carboxyl radicals (-COOH) on the plasma-treated CNPs was first reacted with EDC to form an amine-reactive *o*-acylisourea intermediate. This intermediate was further reacted with NHS to form a semi-stable ester, which may then be reacted with primary amines (-NH₂)-terminated DNA or cysteamine to form the amide crosslinks [53]. Further attachment of extraneous Au nanoparticles on CNPs were completed *via* the thiol chemistry between cysteamine and Au nanoparticles. The presence of NHS in the reaction system enhanced the coupling efficiency [54].

TEM images of the obtained CNP-AuNP hybrids were shown in Fig. 7a-c. One can observe that extraneous Au nanoparticles with a diameter of ~ 10 nm were distributed on the surface of CNPs, resulting an approximate surface coverage of $32 \pm 15\%$. The arrows indicate the presence of carbon shell on CNPs. Such CNP-AuNP hybrids were further used as the SERS substrate (see inset in Fig. 7d) to detect trace-amount dyes (10^{-6} M) containing in DI-water. Various substrates including blank silicon wafer, the as-produced CNPs, and the CNP-AuNP hybrids were immersed in 10^{-6} M R6G or MB solution overnight to anchor the dye molecules. Fig. 7d and e show the Raman signal of R6G and MB obtained on these substrates. We observed that the signal intensity of both R6G and MB obtained on the CNP-AuNP hybrids substrate were significantly enhanced as compared with that on the plasma-treated CNP substrate or the blank substrate. Such Raman enhancement is attributed to (1) the interaction of localized surface plasmon resonance (LSPR) between the attached Au nanoparticles and CNPs, resulting in significantly increased or overlapped electric field at the interface regions (electromagnetic enhancement mechanism) [55], (2) such graphite-like carbon exhibits a hexagonal structure, which is quite similar with the molecule structure of R6G and MB, and thus these CNP-present substrates are more favorable than naked Au nanoparticles for absorbing the target molecules (chemical enhancement mechanism) [56].

To further understand the surface plasmon induced Raman enhancement mechanism, the plasmonic properties of the plasma-treated CNPs and the CNP-AuNP hybrids were simulated using the DDA method. The modeling target was shown in Fig. 8a. The CNPs was considered to be 22 nm in diameter with a carbon shell thickness of 1 nm (corresponding to CNPs after plasma treatment at the best condition). Au nanoparticles with a diameter of 10 nm were closely-packed around the CNPs. The simulation for the plasma-treated CNPs with no surrounding Au nanoparticles was also conducted as comparison. The obtained extinction efficiency as a function of incident wavelength was shown in Fig. 8b. The extinction of the CNP-AuNP hybrids was significantly enhanced as

compared with the plasma-treated CNPs. The extinction spectra were further deconvoluted into absorption and scattering components (Fig. S12, Supplementary Data), which indicate that the extinction for both targets is dominantly contributed by the absorption. The plasma-treated CNPs show the two extinction maximum peaks at ~ 210 nm and ~ 518 nm, corresponding to the $\pi \rightarrow \pi^*$ transition of aromatic C-C bonds from carbon shell [57] and the surface plasmon resonance of Au nanoparticle cores [58], respectively. After hybridizing with extraneous Au nanoparticles, an obvious red-shift of ~ 27 nm and ~ 22 nm was observed respectively for these two peaks. This is due to the increase of effective radius for the CNP-AuNP target and the interaction of LSPR between the extraneous Au nanoparticles and CNPs.

The strength and distribution of the electromagnetic field around both targets were further simulated by considering the incident wavelength at the extinction maximum (~ 518 nm for plasma-treated CNPs and ~ 540 nm for the CNP-AuNP hybrids). As shown in Fig. 8d and e, the CNP-AuNP hybrids exhibited significantly enhanced electric field all over the target and the strongest electric field regions (“hot spots”) are mainly accumulated at the interface between CNP and various Au nanoparticles. This was further confirmed by the $|E|/|E_0|$ vs. position plots (Fig. 8c) across the center of the targets (A-A line in Fig. 8a). These “hot spots” have been considered to play a critical role in SERS sensing and well-explained the observed Raman enhancement in Fig. 7 d and e.

3.4. Immobilization of DNA on the CNPs decorated substrate

The carbodiimide chemistry was used for the immobilization of λ -DNA fragments onto the plasma-treated CNPs (at the best condition) decorated silicon substrate. The Hind III cleaving technique resulted in λ -DNA fragments with amine ($-\text{NH}_2$) terminations at both ends (Fig. 9a), which open

the possibility for attaching λ -DNA to the (-COOH)-terminated CNPs *via* the formation of amide bonds. FTIR spectra for the as-produced CNPs, plasma-treated CNPs, the pristine λ -DNA and the covalently linked CNP-DNA architectures were shown in Fig. 9b. For the pristine λ -DNA, the band at $\sim 1023\text{ cm}^{-1}$ indicates Thymine (CH_3), $\sim 1080\text{ cm}^{-1}$ indicates the PO_2^- stretch, $\sim 1176\text{ cm}^{-1}$ indicates thymidine (C5-CH_3 bond and in-plane ring stretching), $\sim 1376\text{ cm}^{-1}$ indicates thymidine (C5-CH_3 stretching, C6-H in-plane bending), and $\sim 1480\text{ cm}^{-1}$ indicates deoxyguanosine/deoxyadenosine (N7-C8 stretching) [59-62]. For the as-produced CNPs and plasma-treated CNPs, appearance of C=C and C=O stretches were observed at $\sim 1530\text{ cm}^{-1}$ and $\sim 1650\text{ cm}^{-1}$. The weak signal of C=O is due to the natural oxidation of carbon shell in air, which was also observed in above XPS study. After plasma treatment, the C=O signal was enhanced, allowing for further covalent linking of ($-\text{NH}_2$)-terminated λ -DNA fragments as illustrated in Fig. 6. FTIR spectrum of the CNP-DNA architectures shows several main peaks same as that obtained on the pristine λ -DNA (Fig. 9b), indicating the presence of DNA structures on the plasma-treated CNP substrate. Moreover, the appearance of amide I ($1650\sim 1670\text{ cm}^{-1}$, C=O stretch), amide II and amide III stretches (1550 cm^{-1} and 1240 cm^{-1} , respectively, stretching of C-N and/or blending of N-H) provide further evidence for the formation of amide bonds between plasma-treated CNPs and λ -DNA fragments [63-65].

Representative SEM/TEM images of the CNP-DNA architectures were shown in Fig. 10a-d. One end or both ends of the λ -DNA fragments were observed to be linked on the CNPs. AFM was further used for characterizing large-area of CNP-DNA architectures decorated silicon substrate. As shown in Fig. 10e, most of the DNA fragments were wrapped on CNPs due to the natural van der Waals attraction force [66]. To further stretch the DNA structure and align the architectures in a highly-ordered pattern, the substrate was spin-stretched using 1 mM MgCl_2 aqueous solution [67]. This resulted in highly-ordered alignment of CNP-DNA architectures uniformly dispersed on the

silicon substrate (Fig. 10f). More proof microscopic images of the CNP-DNA architectures before and after spin-stretch were shown in the Fig. S13 (Supplementary Data). Such ordered assembly of CNP-DNA architectures will be of use and interest in future bio-nano electronic devices, sensors and biopolymer molecule analysis.

4. Conclusions

In summary, oxygen plasma treatment of CNPs is feasible for purifying the multilayer carbon shell, reducing its shell thickness and further creating surface functionalities. The molar fraction of -COOH on CNPs was observed to be **increased from ~4.8% of the as-produced CNPs to 16.79% after plasma treatment**. The plasma-treated CNPs were further used for the covalent linking of extraneous Au nanoparticles or immobilization of λ -DNA fragments *via* the carbodiimide chemistry. **As compared with the plasma-treated CNPs, CNP-AuNP hybrids** exhibited significantly improved Raman sensitivity for the detection of water-containing organic dyes. Further, plasmonic modeling results indicate that the CNP-AuNP hybrids exhibit much stronger electric field or “hot spots” at the interface areas, which well-explained the observed Raman enhancement. With the same carbodiimide chemistry, the immobilization of highly-ordered assembly of CNP-DNA architectures were also successfully achieved. Such controlled patterning technique will be of great promise for future DNA detection/recognition devices and other biochemical applications.

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Fig. 1 - (a) Schematic showing the growth of multilayer carbon shell on Au nanoparticles. (b-d) TEM images and (e) FFT corresponding to (d) showing the as-produced CNPs.

Fig. 2 - (a-l) TEM images (scale bar: 5 nm) showing the influence of plasma treatment conditions on the morphology of CNPs: (a-d) duration of 5 s, 15 s, 60 s, and 90 s; (e-h) power of 30 W, 60 W, 90 W, and 120 W; (i-l) pressure of 200 mTorr, 250 mTorr, 400 mTorr, and 600 mTorr. (m) Schematic illustration of the morphology of CNPs at various conditions. (n-p) Variation of carbon shell thickness as a function of duration (n), plasma power (o), and oxygen pressure (p).

Fig. 3 - (a, c and e) Raman spectra and (b, d and f) corresponding I_D/I_G of CNPs before and after plasma treatment at different (a,b) duration, (c,d) plasma power, and (e,f) oxygen pressure.

Fig. 4 - (a-c) Variation of -COOH and -COH molar fraction as a function of various durations (a), plasma powers (b), and oxygen pressure (c). (d-i) Deconvoluted XPS spectra of C 1s corresponding to various samples marked in Fig. 4a-c.

Fig. 5 - Hydrophilicity test for the CNP substrate before and after plasma treatment. Digital images of 10 μ L DI-water droplet on (a) the blank silicon wafer, (b) the as-produced CNP substrate, (c) the plasma-treated CNP substrate (at the best conditions). (d) Schematic illustrating the interactions between water and the CNP substrates.

Fig. 6 - Schematic showing the carbodiimide chemistry used for the covalent linking of extraneous Au nanoparticles and λ -DNA fragments onto the plasma-treated CNPs.

Fig. 7 - (a-c) TEM images of the CNP-AuNP hybrids. *The inset in Fig. 7a illustrates the ideal structure of CNP-AuNP hybrids. The arrows show the carbon shell after plasma treatment.* (d, e) Raman spectra R6G (d) and MB (e) that anchored on (0) blank Si substrate, (2) plasma-treated CNPs and (3) CNP-AuNP hybrids. *The inset schematic showing the SERS-based organic sensing on the CNP-AuNP hybrids.*

Fig. 8 - (a) Target for the CNP-AuNP hybrids used in the DDA modeling. (b) Extinction spectra of (1) plasma-treated CNPs and (2) CNP-AuNP hybrids. (c) Plots of normalized electric field vs. position across the center of (1) CNP target and (2) CNP-AuNP hybrid target (the A-A line). (d) Distribution of electromagnetic field near the surface of the plasma-treated CNPs (left, under incident of 518 nm) and the CNP-AuNP hybrids (right, under incident of 540 nm).

Fig. 9 - (a) Schematic showing the Hind III technique for cleaving λ -DNA into fragments. (b) FTIR spectra of (1) as-produced CNPs, (2) plasma-treated CNPs, (3) covalently-linked CNP-DNA architectures, and (4) Pristine λ -DNA fragments.

Fig. 10 - (a,b) SEM images and (c,d) HRTEM images of the covalently-linked CNP-DNA architectures. (e,f) AFM images of the CNP-DNA architectures (e) before and (f) after spin-stretching with assistant of 1 mM MgCl_2 .