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Adsorption and Vibrational Study of Folic Acid on Gold Nanopillar Structures Using Surface-enhanced Raman Scattering Spectroscopy

Regular Paper

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Abstract

This paper presents a study of adsorption and vibrational features of folic acid, using surface-enhanced Raman scattering (SERS). A gold-capped silicon nanopillar (Au NP) with a height of 600 nm and a width of 120 nm was utilized to study the vibrational features of FA molecules adsorbed on the nanopillars within the high electromagnetic field areas. The adsorption behaviour of folic acid and the band assignment of the main vibrations together with the optimized geometry of folic acid and folic acid in the presence of a cluster of 10 gold atoms were assessed using the density functional theory (B3LYP(6-31G(d))) and the scalar relativistic effective core potential with a double-zeta basis set (LANL2DZ). The vibrations obtained from the solid-state folic acid and the folic acid on a gold cluster were in accordance with those observed experimentally. The analysis of the main vibrations indicated that the interaction of folic acid with the Au NP occurred primarily through the nitrogen atoms, from their pteridine ring. Finally, the obtained adsorption isotherm for folic acid was deduced from the analysis of the SERS spectra and it followed a negative cooperative binding model.

Keywords Gold Nanopillars, SERS, Folic Acid

1. Introduction

Folic acid (FA), also known as vitamin B, is a compound of low molecular weight (441 g/mol), which is formed by a pteridine ring, p-aminobenzoic acid and glutamic acid. One of the most important biochemical functions of FA is related to its role in the folate-requiring reactions for pyrimidine and purine synthesis, as well as methylation reactions for the fabrication of DNA [1]. Since the early 1990s it has been known that there is a relationship between FA deficiency and an increased risk of birth defects and chronic diseases, as well as congenital heart defects [2]. FA is a high-affinity ligand for the folate receptor (FR) (Kd=1nM), which maintains its strong binding properties

when conjugated to other molecules [3]. As a result, FA coupled to FR has been successfully applied in the therapy and diagnosis of cancer and tropical diseases [1]. Different methods have been developed for FA and FR detection, including cytological testing, fluorescent imaging, electrochemical methods and radio-labelled assays [4]. While the effectiveness of these methods has yet to be challenged, certain issues — like the time-consuming nature of radio-labelled assays — have encouraged us to explore new methods and techniques for FA detection.

Raman spectroscopy is a high-resolution photonic technique that provides chemical and structural information in a relatively short amount of time. Small sample areas or volumes can be used and water solution does not interfere with the spectrum. The most important disadvantage associated with the technique is perhaps the poor sensitivity that arises from the tiny amount of Raman-scattered photons, compared to those that are Rayleigh-scattered. Further disadvantages include the fluorescence displayed by samples in the spectrum and the use of laser irradiation, which in principle can damage the sample. Sensitivity issues were overcome with the development of the surface-enhanced Raman scattering (SERS) technique. The information obtained from SERS spectra contains greater detail and a higher signal than from conventional Raman spectroscopy. SERS has been widely used to obtain information for vibrational analysis and to observe the adsorption of biomolecules on metal substrates. Recent developments in the synthesis and fabrication of new colloid metal nanoparticles and solid support-based SERS substrates have led to the detection at trace levels of different compounds [5]. SERS metal substrates can be divided into two groups: i) colloid metal nanoparticles, and ii) roughened metallic surfaces [6]. The main limitation in using colloid metal nanoparticles is the formation of nanoparticle conglomerates after the addition of the sample, which often leads to poor reproducibility in the the SERS spectra [7]. An ideal SERS substrate must have enough hot spots to guarantee an effective interaction with the analyte, and a homogeneous surface to produce reproducible Raman spectra. Recently, we have developed a simple method to produce flexible free-standing Au NPs for SERS applications [8]. These Au NPs produce a remarkably large enhancement of the Raman scattering signal. Yang et al. [9] have used the same Au NP as a probe in a bioassay for the specific detection of vasopressin. Surface homogeneity and reproducibility in the Raman spectra converted Au NP into a potential SERS substrates for detection and studies of the adsorption behaviour of different biomolecules.

The adsorption study of biomolecules on SERS substrates is important for the analysis, identification and quantification of these compounds [10]. Jing et al. [11] have investigated the adsorption behaviour of L-cysteine on the surface of a variety of metals. The vibrational features of adenine-containing microRNA chains adsorbed on roughened gold substrates were studied using SERS [12]. A new gold grass-like nanostructure was used for studying the adsorption of DNA bases on polyvinyl alcohol [13]. To date, every research group has used gold-capped nanopillars for studying the adsorption behaviour of biomolecules.

Several SERS studies have been carried out to detect FA and cells over-expressing folate receptors. Boca-Farcau [14] has used SERS-labelled gold nanotriangles for the multimodal detection of human ovarian cancer cells. Hu et al. developed a cancer diagnostic probe based on reduced graphene oxide and gold nanoparticles, for the detection of FA [15]. In a very interesting work, Kokaislová et al. studied the effect of temperature on the spectral features of FA, using gold and silver SERS substrates [16]. Previous work has focused on SERS studies as a means of detecting FA and there is currently little knowledge about the adsorption and vibrational features of FA on SERS nanostructured gold substrates.

In this work, we present for the first time an adsorption SERS study for the vibrational study of FA on large-area gold-capped silicon nanopillars. The DFT methods have allowed us to calculate the Raman vibrations of FA, helping us to understand better the adsorption of FA on gold-capped nanopillars. Most of the calculated Raman vibrations coincided well with those from experimental SERS spectra. Finally, the adsorption isotherm was fitted to a Hill model exhibiting a negative cooperative binding of FA molecules on the surface of the Au NP.

2. Experimental Set-up

2.1 Measurement Tools and Techniques

Raman scattering and SERS measurements were performed using a Thermo Scientific DXR Raman microscope. An optical microscope was coupled to a single grating spectrometer with 5 cm^{-1} FWHM spectral resolution and ±2 wavenumber accuracy. A frequency-stabilized single diode laser was operated at 532 nm.

The Raman scattering spectrum of FA was collected using a 10x long working distance objective, 20 mW laser power and 5s signal accumulation times. FA SERS spectra were recorded using a 10x long working distance objective, 0.1 mW laser power and 5s signal accumulation times. The power density was kept ~12 kW/cm^2 at the sample to minimize photoinduced and thermal effects [8].

In order to characterize the formation of FA layers adsorbed on the Au NPs, scanning electron microscopy (SEM) analysis was performed using a Quanta FEG SEM (Oregon, USA).

2.2 Fabrication of SERS-active Au NP Substrates

The substrates were fabricated using a three-step process. First, maskless Si RIE (reactive ion etching) was utilized to form Si NP structures. Second, an O_2-plasma process was systematically applied, in order to (i) remove Si RIE

byproducts from the Si surface, and (ii) control the Au NP cluster size. Lastly, an Au metal film was deposited on the Si NPs using e-beam evaporation. As a result, a series of vertical free-standing gold-capped silicon nanopillar structures (Au NP), surrounded by a continuous gold film at the base of the pillars, was obtained (see Fig. 1) [8]. The obtained Si NP density was approximately ~18 pillars/μm^2. The Si NP dimensions were as follows: Si NP height ~600 nm, Si NP width ~50 nm, Au cap height and width ~300 and 120 nm, respectively. All fabricated Au NP structures were utilized within three days to minimize any effects related to oxidation of the Au surface.

2.3 Preparation of FA Solutions

FA was obtained from Sigma-Aldrich Corp. All chemicals used in this study were of analytical grade. FA solutions were prepared as described by Castillo et al. [17]. FA molecules were dissolved in water with an addition of 50 μL of NaOH (1 M), due to the poor solubility of FA. The FA powder (0.029 g) was mixed with H_2O (25 mL) and magnetically stirred until a yellow-to-clear transition was observed.

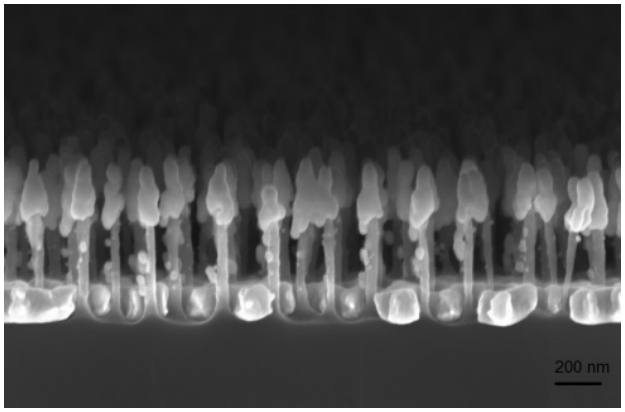


Figure 1. SEM image of gold-capped silicon nanopillars

2.4 FA Isotherms

Serial dilutions of the FA solutions in the range of 0.1–100 μM were prepared. Single droplets (~1 μL) of various FA concentrations were then dispersed on the SERS active substrates and left to dry. The droplets were then allowed to spread over the whole Au NP surface area (5 x 5 mm^2) for several minutes, to ensure homogeneity in the surface coverage and to achieve a suitable time of adsorption equilibrium, and thus avoid any change due to the desorption of FA. Before every SERS measurement, the FA-Au NP was gently rinsed with Milli-Q water and dried with nitrogen flow to remove the non-adsorbed FA. The Raman scattering signal was recorded over an area of ~0.05 mm^2, with 17 μm and 11 μm x and y step sizes, respectively.

For each concentration, an exposure time of 5s/spectra was used and 238 spectra were obtained. The 689 cm^{-1} peak intensity averaged from the 238 spectra was used for the construction of the isotherm plot.

2.5 Computational Methods

In order to identify the main vibrations of FA Raman scattering spectra, their vibrational frequencies were calculated using the density functional theory (DFT) method, using B3LYP theory combined with the standard 6-31G(d) basis set. In order to correct for systematic errors, all calculated frequencies were scaled by a factor of 0.9614, in accordance with the results reported in [18]. A cluster of Au atoms was chosen as a model system for the Au NP structures [19, 20]. In order to decrease the high computational demands for larger clusters, a cluster of 10 Au atoms (Au10) was selected and proven to be a good candidate for simulating the FA-Au SERS spectrum. The Au10 cluster was planar and large enough to cover a representative part of the FA (see Figure 4). The interaction between the FA and Au10, the FA-Au10 geometry optimization and the Raman spectra were computed using the scalar relativistic effective core potential (ECP) with double-zeta basis sets (LANL2DZ) [21]. All the geometries of the model system were fully optimized. In addition, the HOMO-LUMO energy gap and the chemical hardness [22] of the FA molecule were also calculated (see supporting information and Figure S1). The calculations were carried out using the Gaussian 09 software package [19].

3. Results and Discussion

3.1 Raman Spectra of Folic Acid

To study the vibrational frequencies and amplitudes of state solid FA, a DFT-RB3LYP(6-31G(d)) basis set was used to calculated its Raman spectra.

Table 1 and Figure 2 show the calculated and experimental Raman spectra of solid-state FA and FA adsorbed on gold-capped silicon nanopillars, respectively. Most of the vibrational bands from the solid-state FA are in good concordance with previous similar works [16]. The Raman spectra in Figure 2(b) show vibration bands mainly in the range between 1700 and 600 cm^{-1}. The most intense band was located at 1606 cm^{-1} and can be related to the stretching vibration of NH from the pteridine ring (pt) of the FA. Medium-strong-intensity Raman bands were vibrating at 1570, 1359, 1249 and 682 cm^{-1}. According to our theoretical assignment (Fig. 2a andTable 1), these observed bands are dominated by the asymmetric vibration of the C=N from the pt, the rocking vibration of the CH from the p-aminobenzoic acid moiety (paba), the rocking vibration of the C=N from the pt, and the asymmetric vibration of the C=C from paba, respectively.

The experimental and theoretical Raman vibrations were in good agreement, as is shown in Figures 2(a) and 2(b).Some of the relative positions of the Raman peaks do not correspond well with certain bands, despite the fact that

we corrected the calculated frequencies by a factor of 0.9614. However, the computational calculations were helpful for the assignment of the Raman modes of the FA in the experimental spectrum. Thus, the most intense bands present in the calculated FA Raman spectrum are also present in its experimental spectrum.

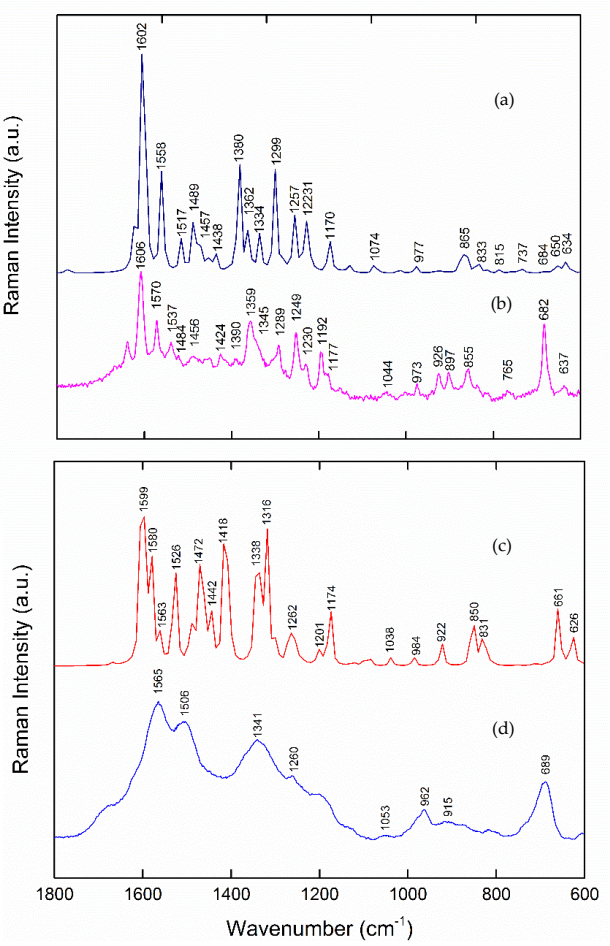


Figure 2. DFT-calculated (a) and experimental (b) Raman spectra of solid-state FA; DFT calculated (c) and experimental (d) SERS spectra of FA adsorbed on gold-capped silicon nanopillars

3.2 SERS Spectra of Folic Acid

The calculated and experimental SERS spectra of FA on gold-capped silicon nanopillars are shown in Figures 2(c) and 2(d), respectively. Figures 3(a) and 3(b) show a comparison between the calculated main vibrations of solid-state FA and FA in the presence of an Au10 cluster, respectively.

The presence of new Raman bands, as well as the shifting of the main vibrations and increasing of the signal intensity, clearly indicates the effect of gold on the geometry and orientation of the FA. For example, the vibrations at 1338, 1174, 964 and 661 cm^{-1} are Raman modes that are producing by the enhancement of the electromagnetic field, caused by the FA molecules located close to the metal silicon nanopillars. A similar Raman spectrum is obtained when FA is adsorbed on the surface of gold-silicon nanopillars (Fig. 2(d)). Most of the SERS vibrations of FA adsorbed on Au NPs are consistent with previous studies using different

Free FA (cm^{-1})		FA-Au NP (cm^{-1})		
Calc.	**Exp.**	**Calc.**	**Exp.**	**Assignment**
1602	1606	1599	1565	$s(NH_2)+\nu_{as}(C=N)(pt)$; $\nu_s(C=C)$ $+\varrho(CH)(paba)$
1558	1570	1580	1506	$s(NH_2)+\nu_{as}(C=N)(pt)+\varrho(CH)$; $\nu_s(C=C)+\varrho(NH)(paba)$
1489	1484			$\varrho(NH)(OH)+\nu_s(C=C)(GA)$
1334				$\omega(CH_2)(paba)$; $\varrho(CH)(OH)(GA)$
1257	1249			$\varrho(NH)(OH)+\omega(CH_2)(GA)$
1231	1230			$\varrho(NH)(OH)+\omega(CH_2)(GA)$
1074	1044	1038		$\varrho(NH)(OH)+\omega(CH_2)(GA)$
977	973	984	962	$\varrho(CH_2)(paba)$
650	682	661	689	$\nu_s(C=N)(pt)$; $\nu_{as}(C=C)(paba)$

[a]symbols: ν, stretching; δ, bending; ϱ, rocking; ω, wagging; *s*, scissoring; ar, aromatic; GA, glutamic acid; pt, pteridine; paba, p-aminobenzoic acid

Table 1. Assignment of the experimental and calculated Raman and SERS bands of free FA and FA adsorbed on Au NPs

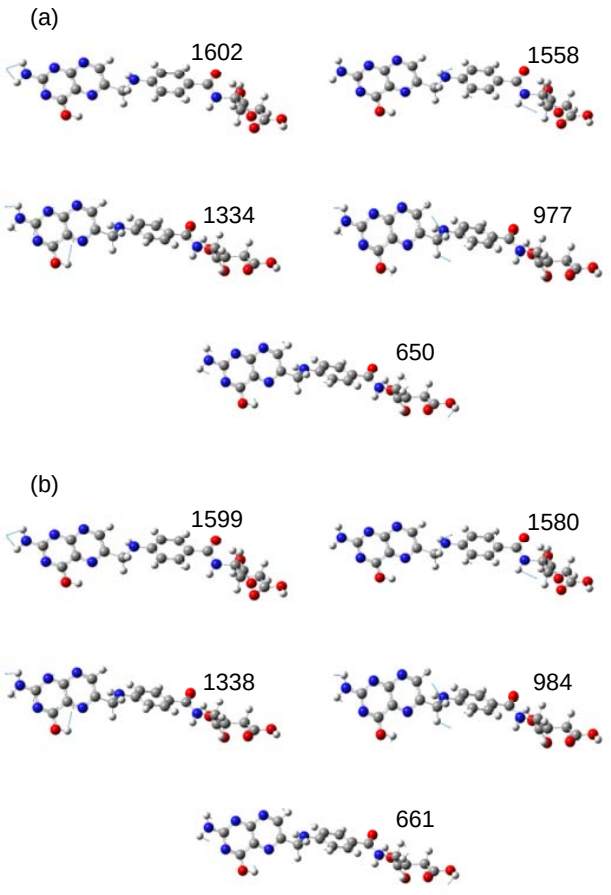


Figure 3. Comparison of the calculated main vibrations (cm^{-1}) for the theoretical Raman-active solid-state FA and FA-Au10 modes, (a) and (b) respectively

nanoparticles colloids and metal substrates [23]. The broad bands at 1565 and 1341 cm^{-1}, from the stretching vibrations of NH and C=N from the pt, indicate a strong and direct interaction of the nitrogen with the gold-capped silicon nanopillars. The weak interaction between the benzene ring of the paba and the Au NP is further confirmed by the presence of the Raman mode at 661 cm^{-1}. The absence of bands at 1489, 1249 and 1231 cm^{-1} — which are the vibration modes of the C-C, OH and O=N-C (Fig. 2(a)) from the GA fragment of the FA — suggests that the GA is not in direct contact with the Au NP. This remarkable feature leads us to conclude that the pteridine ring is probably the main part of the FA adsorbed on the surface of the Au NP.

Such changes in the SERS FA spectrum are corroborated by DFT calculations of the optimized geometry of FA molecule interacting with Au10 clusters. To decrease the high computational demands of larger clusters, an Au10 cluster has been selected and proven to be a good candidate for SERS and adsorption simulations. Our theoretical study suggests an adsorption model in which the FA interacts with the gold atoms primarily through the nitrogen atoms from the pteridine ring, as shown in Figure 4.

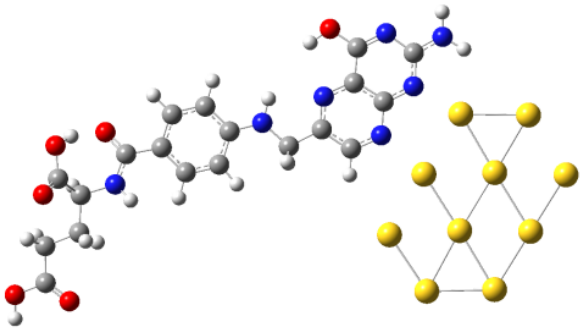
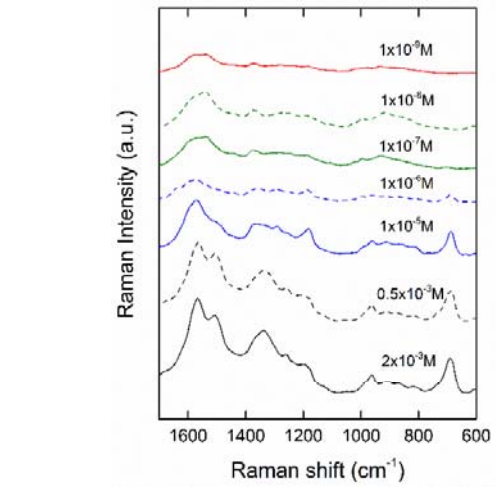


Figure 4. Optimized geometry of FA adsorbed on a cluster of 10 gold atoms

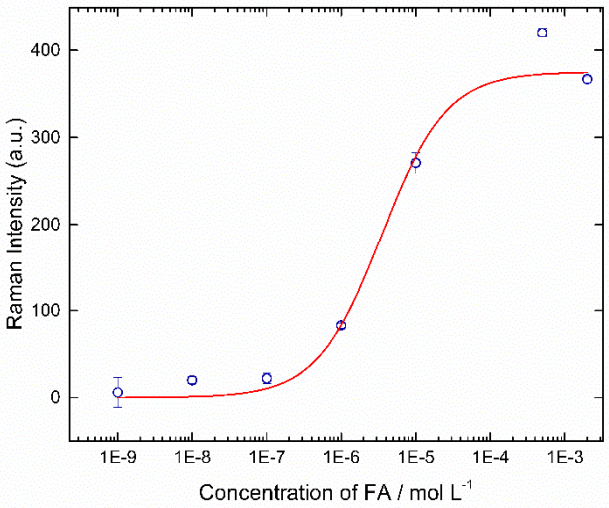
3.3 FA Binding to Au Surface and Adsorption Isotherm

The adsorption of FA on Au NPs was studied as a function of concentration, using the intensity of the symmetric stretching mode C=N of the pteridine ring at 689 cm^{-1}. The SERS spectra of the adsorbed FA are shown in Fig. 5 (a) for seven different solution concentrations of FA, ranging between $1x10^{-9}$ and $2x10^{-3}$ M. It was observed that the maximal SERS intensity occurs at a concentration of $0.5x10^{-3}$ M (Raman band at 689 cm^{-1}, Fig. 5(a)). Concentrations below $1x10^{-5}$ M exhibited a reduced Raman signal, and at concentration $1x10^{-9}$ M a weak Raman band was observed.

The intensity of the SERS band was related to the amount of FA adsorbed on the nanopillars. Therefore, to obtain more detailed information about the adsorption behaviour of FA on Au NPs, we plotted the SERS intensity versus the FA concentration. For this purpose, a SERS peak had to be selected. In the case of FA, the intensity of the symmetric stretching mode C=N of the pteridine ring at 689 cm^{-1} was chosen as a marker peak.



(a)



(b)

Figure 5. SERS spectra of different concentrations of FA adsorbed on gold-capped nanopillars: (a) the spectra were recorded for 5 s at a laser power of 0.1 mW; (b) SERS and adsorption isotherm of FA on Au NPs. The intensity measured at 689 cm^{-1} was plotted as a function of log FA concentration.

Fig. 5(b) shows the adsorption isotherm obtained for FA at different concentrations. The equilibrium SERS intensities of FA adsorbed on silver nanopillars were fitted using nonlinear curve analysis to the Hill equation [24] of the form

$$I_{SERS} = \frac{I_{\max} \times FA^n}{K_{ads} + FA^n}$$

where $I_{\max}$ is the maximal SERS intensity, FA is the concentration of the folic acid solutions, K_{ads} is the equilibrium constant for dissociation, and n is a cooperative constant.

When n>1 or n<1, attraction and repulsion between the adsorbate molecules will take place, respectively. Note that, when n=1, the Hill equation behaves as a Langmuir isotherm. We obtained K_{ads}= $4.2x10^{-6}$ M^{-1} and n= 0.90, which

indicates that the adsorption of FA to the nanopillars decreased the affinity of the new incoming FA molecules to the surface, clearly suggesting a negative cooperative binding.

Importantly, the present study, aside from offering an adsorption and the vibrational analysis study of FA on Au NPs, is a preliminary step toward the fabrication of a sensing device based on a SERS platform, in order to detect FA, or cancer cells over-expressing folate receptors at < nM concentrations.

4. Conclusions

The adsorption of FA on Au NPs was carried out using SERS spectroscopy and DFT calculations. The comparison between SERS and Raman scattering spectra shows that the interaction of the FA with the Au NP surface mainly occurs through the nitrogen in the pteridine ring. This fact was further confirmed by the calculation of the optimized geometry of FA adsorbed to a cluster of 10 gold atoms. In addition, we demonstrated that FA molecules adsorbed on Au NPs follow a negative cooperative binding mechanism, from which the adsorption constant and n-index were calculated, being $4.2x10^{-6}$ M^{-1} and 0.90, respectively. The present study serves as a starting point for the fabrication of a gold nanopillar-based detection system for sensing FA, or cancer cells over-expressing folate receptors.

5. Acknowledgements

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