

Ultrasensitive system for the real time detection of H₂O₂ based on strong coupling in a bio-plasmonic system

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ABSTRACT

We theoretically investigate the dependence of the different parameters of an optical biosensor for the detection of Hydrogen peroxide (H₂O₂) based on absorption enhancement of Cytochrome *c* molecules near gold nanoparticles. H₂O₂ is a major reactive oxygen species which is involved in signaling pathways and oxidative stress in cells. We use the Green's function approach as well as confirm the corresponding simulation results using the surface integral formulation. Further we show that this technique can be applied for detection of other small molecules, like oxygen and carbon monoxide.

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1. INTRODUCTION

Sensing using plasmonic resonances in thin films, nanoparticles and nanostructures has been a key area of research for scientists since it opens up avenues for detection of various biomolecules and biological species in real time and with high specificity [1-7]. Even though standard localized Plasmon resonance (LSPR) sensing techniques using different plasmonic nanoparticles give highly sensitive specific real time information either *in-vivo* or *in-vitro*, they suffer from the drawback that the analyte to be sensed must cause enough refractive index change to be measurable. Moreover, to specifically detect an analyte by its refractive index, one must know the dimensions of the analyte and the properties of the environment beforehand, which is difficult to determine experimentally. To circumvent these limitations, other techniques which do not rely on the shift of the plasmon resonance, like surface enhanced Raman spectroscopy (SERS), surface enhanced infrared absorption spectroscopy (SIERA), fluorescence modification using plasmonic structures etc... have been utilized [8]. In SERS and SIERA, due to the high field generated in the vicinity of the nanoparticle at Plasmon resonance, the Raman spectrum and the infrared absorption spectrum of the molecules in the hot spot region are enhanced, thus making it possible to measure [8]. Recently, Liu *et al.* [3] showed that the absorption spectrum of free unbound Cytochrome *c* (Cyt *c*) could be measured in the scattering spectrum of the gold nanoparticle (AuNP) when the distance between them was in the order of a few nanometers [9]. The explanation given was that the absorption of Cyt *c* was enhanced by the local field enhancement at the plasmon resonance and thus they named the phenomenon plasmon resonance energy transfer (PRET). It should be noted that in the paper by Liu *et al.* the Cyt *c* was not bound covalently to the surface of the AuNP, thus the distance between gold and Cyt *c* was not fixed, which is a critical parameter in any energy transfer process. The same group also showed that it is possible to detect copper ions using this technique [10]. Recently, it was shown that the absorption spectrum of Cyt *c* could also be seen in the scattering spectrum of a dielectric medium that possessed no plasmonic resonances. Thus it is not immediately clear what exactly is PRET and what are the necessary conditions for having PRET. In the present paper, Cyt *c* was chosen as the absorbing molecule (acceptor in

PRET process) as it exhibits different absorption spectra in the oxidized and reduced forms as shown in Fig. 1. The change between the reduced to the oxidized form of Cyt *c* can be brought about by reaction with H₂O₂.

Thus by monitoring the absorption of Cyt *c* one can detect the level of H₂O₂ in the surrounding. H₂O₂ is an important biomolecule, that belong to the reactive oxygen species, because it is associated with various processes like signaling cascades, protein hydrolysis and maintenance of the redox homeostasis in a cell [11, 12]. An imbalance in the level of H₂O₂ to anti-oxidants is known as oxidative stress. Oxidative stress can be observed in unhealthy and diseased cells and, for example, in diseases like cancer, Parkinson's, Alzheimer's etc..., there is overproduction of H₂O₂. Beyond a certain extent, the cell can no longer maintain the homeostasis, which leads to cell death through different pathways, thus severe oxidative stress is highly detrimental to the cell [13, 14, 15]. Hence detection of H₂O₂ becomes important from a medical perspective. Standard plasmonic sensors cannot be used for detection of H₂O₂ because the refractive index change caused by H₂O₂ is negligibly small and does not produce a measurable shift of the plasmon resonance peak.

The experimental results obtained by Liu *et al.* suggest that PRET originates from near field interactions between the plasmon resonance of the AuNP and the absorption bands of Cyt *c* [9]. However, no extensive theoretical investigations have yet been performed on the parameters affecting the energy transfer between Cyt *c* and AuNP. In this paper, we theoretically study PRET using Green's function formalism and study its dependence on different parameters. Moreover we show that this sensing technique can be extended for the detection of other biologically important molecules, like oxygen and carbon mono-oxide.

2. SIMULATION TECHNIQUE

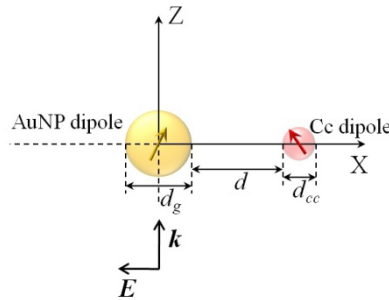


Figure 1. Geometry of the system: the Cyt *c* (Cc) is placed at a short distance *d* from a gold nanoparticle.

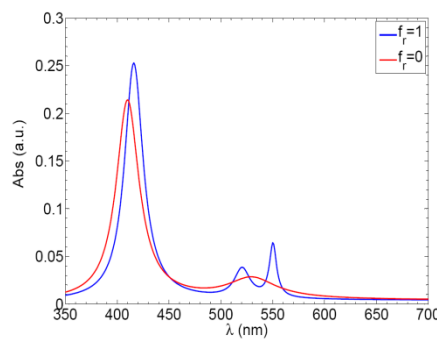


Figure 2. Absorption spectrum of Cyt *c* in the reduced and oxidized states.

The green's function method was used for studying the interaction between AuNP and Cyt *c* [16]. We approximate Cyt *c* and AuNP as point dipoles (see Fig. 1) with realistic polarizabilities. The AuNP can be approximated as a for small particles since higher multipole orders do not play a significant role in the energy transfer . Under these assumptions the field at any point in space can be given by the following equation,

$$\vec{E}_i = \vec{E}_i^0 + \sum_{j=1, j \neq i}^n \Delta \epsilon_j \vec{G}_{ij} V_j k_0^2 \vec{E}_j + \Delta \epsilon_i \vec{M}_i k_0^2 \vec{E}_i - \frac{\Delta \epsilon_i}{\epsilon_b} \vec{L} \vec{E}_i \quad (1)$$

where G_{ij} is the green's tensor, $\Delta \epsilon_j$ the dielectric constant contrast between element j and the background (ϵ_b), V_j the volume of the j^{th} element and E_i^0 the electric field at the i^{th} element due to the incident plane wave. In the simulation results presented in this paper, the size of the AuNP and Cyt c is taken to be 50 nm and 3.4 nm, respectively.

The scattering spectrum of AuNP is calculated by evaluating the integral of the intensity of the scattered electric far-field on a spherical surface with radius 10 microns. The dielectric function of Cyt c is modeled using the following equation,

$$\epsilon_i = \epsilon_w + \sum_{k=1}^3 \frac{f_{pk}^2}{f_{0k}^2 - f^2 - i\gamma_{0k}f}$$

$$\epsilon_{\text{eff}} = f_r \epsilon_{\text{red}} + (1 - f_r) \epsilon_{\text{red}} \quad (2)$$

where the parameters f_{pi} , f_{oi} and γ_i represent the resonance strength, frequency and damping, respectively, of the different resonances; f is the frequency and ϵ_w is the dielectric constant of water ($\epsilon_w=1.7689$; since Cyt c is generally dissolved in water). For reduced (oxidized) Cyt c the Q-band(s) are at 550 nm and 520 nm (530 nm). For a partly oxidized solution of Cyt c we use a linear combination of the dielectric functions of reduced and oxidized Cyt c as given in Eq. (2), where f_r is the fraction of reduced Cyt c . If f_r is equal to 1 then the solution is completely oxidized and when f_r is equal to 0 then it is completely reduced. It should be noted that the parameter f_{pi} is dependent on the concentration of Cyt c present. Figure 2 shows the absorption spectrum of Cyt c for the two different oxidation states. These absorption data fit well with the experimentally measured absorption spectra of thin Cyt c film (data not shown).

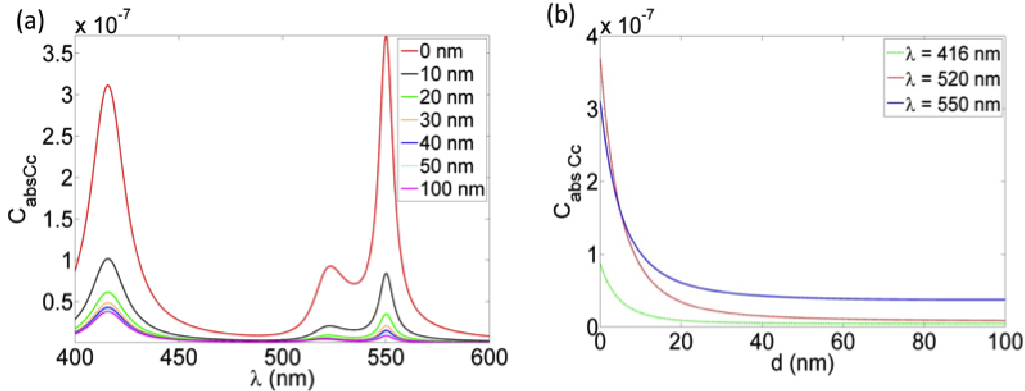


Figure 3. (a) Absorption spectrum of reduced Cyt c as a function of spacing between the two dipoles and (b) the absorption at the resonances of reduced Cyt c as a function of distance.

3. RESULTS AND DISCUSSIONS

In this section we present the main results of this paper and carry out a small discussion of how this absorption enhancement process is helpful for the detection of H_2O_2 . One of the main parameters affecting the energy transfer or the absorption enhancement process is the separation distance d between the acceptor and the donor. It can be seen from Fig. 3 that when d decreases the absorption of reduced Cyt c increases. It should be noted that the absorption of reduced Cyt c is enhanced in the near field of the nanoparticle, i.e. when $d < 40$ nm (we obtain analogous results for oxidized Cyt c , not shown here). It can be seen that when the molecule is placed directly on the surface of the gold nanoparticle i.e. $d = 0$ nm, the absorption of reduced Cyt c is enhanced by a factor of almost 20. Another interesting observation is that when the separation distance is smaller than 7 nm, the Q-band of reduced Cyt c at 550 nm exhibits higher absorption than its Soret band at 416 nm, as shown in Fig. 3(b). In most measurements, the absorption coefficients are measured at the Soret

bands as opposed to the Q-bands because of their higher intensity, but through the absorption enhancement due to the gold nanoparticle it is viable to record the absorption coefficient at the Q-band's wavelength. In experiments dealing with the coupling of nanoparticles and molecules, keeping the number of molecules constant between two different experiments is extremely difficult. Therefore it may be possible to infer the absorption enhancement due to the AuNP directly taking the ratio between the Q-band and the Soret band. This gives a direct way of quantifying the absorption enhancement in the system. Secondly, considering the effect of the relative orientation of Cyt *c* and AuNP, we observe that the spatial map of the absorption enhancement of the Q-band is very similar to that of the electric field distribution at the localized plasmon resonance itself.

Since we are actually interested in measuring the change of oxidation state of Cyt *c* molecules for sensing H₂O₂, we now investigate how the previously discussed absorption enhancement can improve the sensitivity if this measurement. Figure 4(a) shows the absorption spectra of both oxidized and reduced Cyt *c* for both enhanced ($d = 0$ nm) and non enhanced cases ($d = 1000$ nm). It can be clearly seen that the absorption of both oxidized and reduced Cyt *c* is enhanced by the plasmon resonance. In most experiments we actually measure the scattering spectrum of the AuNP. By taking the differential scattering spectrum of AuNP in the presence of reduced and oxidized Cyt *c* we observe the signature of the change in the oxidation state (Fig. 4(b)). Moreover, it is seen that this signature is inexistent when there is no AuNP. It should be noted that for experimentally validating the absorption enhancement, as well as the detection of change of oxidation state of Cyt *c*, it is necessary to bind it to the surface of the AuNP. Chemical routes are ideal for conjugating Cyt *c* and AuNP, since the separation distance can be controlled within angstroms by using different linker molecules, which is not possible using a standard nano-fabrication technique.

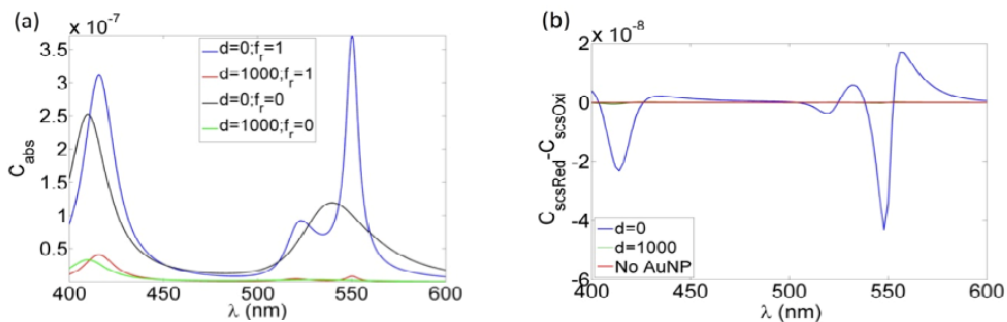


Figure 4. (a) Absorption spectrum of reduced and oxidized Cyt *c* in the presence and absence of the absorption enhancement; (b) The differential scattering spectrum of a AuNP-reduced Cyt *c* system and AuNP-oxidized Cyt *c* system in the presence and absence of absorption enhancement.

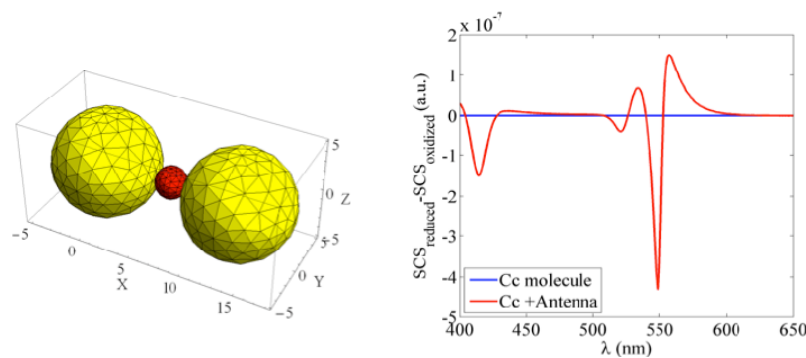


Figure 5. (left) Geometry used for SIE simulation; (right) Differential spectrum between reduced and oxidized Cyt *c* obtained by the SIE method.

Figure 5 shows the differential scattering spectrum using surface integral method. It can be seen that we also obtain similar results with SIE method which includes a complete discretization of the AuNP, and hence includes all multipoles orders. This demonstrates that the simple two dipole model can be used to accurately model this kind of interactions.

Let us finally mention that this approach can be applied to other analytes. For example, by choosing Hemoglobin as the donor molecule instead of Cyt *c*, it is possible to detect oxygen and carbon mono-oxide. Indeed Hemoglobin shows an analogous absorption spectrum change as compared to Cyt *c* on reaction with either CO or oxygen. Figure 6 shows the dielectric function of oxygenated (with oxygen) and deoxygenated (without oxygen) Hemoglobin [17].

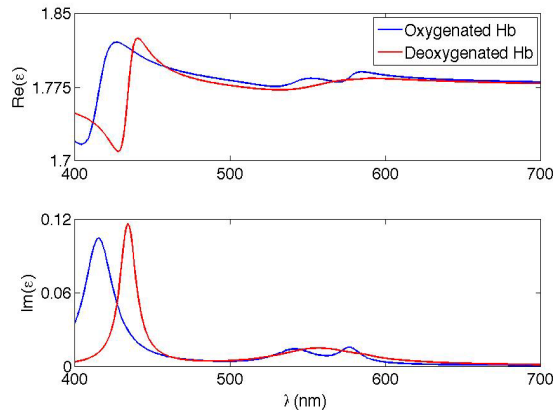


Figure 6. Dielectric functions of Hemoglobin in the oxygenated (blue) and deoxygenated (red) states.

4. CONCLUSIONS

We have theoretically studied the effect of different geometrical parameters on the energy transfer between gold nanoparticle and Cyt *c* molecule. Green's function technique is used for the simulations and it is shown that the PRET process can be modeled using a two dipole system and the results agree with the results of the surface integral method. Furthermore, it is shown that the absorption enhancement via the near field of the nanoparticle can be used for sensitive detection of H₂O₂.

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