J Fluoresc (2012) 22:1415–1419
DOI 10.1007/s10895-012-1104-7

RAPID COMMUNICATION

Spontaneous Emission of Singlet Oxygen Near Dielectric Nano-objects and Radiative Diagnostics of Bio-Objects

D. Mogilevtsev · A. Maloshtan · S. V. Lepeshkevich · B. M. Dzhagarov

Received: 3 May 2012 / Accepted: 27 June 2012 / Published online: 3 August 2012

Abstract We investigate modifications of a spontaneous emission rate near the surface of the hemoglobin-like dielectric structure in the long-wavelength limit. We demonstrate that notwithstanding the small size of the dielectric structure, an influence of its shape cannot be neglected. Even for moderate dielectric contrasts one can expect having significant and detectable enhancement or suppression of the spontaneous emission rate near the surface of the biological macromolecule.

Keywords Singlet oxygen · Spontaneous emission · Human hemoglobin

It is well known that dielectric objects much smaller than the wavelength of an emitter radiation can, nevertheless, modify a spontaneous emission rate of an emitter placed near them [1, 2]. Generally, this modification is not so large as for metallic nano-antennas, where due to plasmonic resonances there is an enhancement or suppression of a spontaneous emission rate on the scale of several orders of magnitude. Nevertheless, it is significant and it can be exploited. Detecting modification of spontaneous emission, one can infer an information about dynamics of emitters motion near nano-objects. Such an information can be highly relevant for practical applications (especially, in photomedicine).

Here we address a particular problem of this kind, namely, a spontaneous emission of the molecular singlet oxygen in the vicinity of the dielectric object structurally similar to human hemoglobin. For a long time the molecular oxygen (O_2) is object of the intense studies due to its prominent role in a multitude of biological and physico-chemical reactions. Singlet oxygen is highly reactive agent. Therefore, generation of singlet oxygen is a key process in photoinduced selective destruction of unhealthy cells of human body. Production of singlet oxygen is ultimately important for photomedicine, in particular, for photodynamic therapy of cancer, skin and infectious illnesses [3–5]. Thus, studying mechanisms of possible modifications of singlet oxygen radiative spontaneous emission is really important for devising ways of using radiative dynamics of singlet oxygen for practical purposes.

We should specially emphasize that the very process of singlet oxygen radiative spontaneous emission in realistic surroundings is quite non-trivial. There are two possible excited singlet states of the molecular oxygen, $a^1\Delta_g$ and $b^1\Sigma_g^+$. The state $b^1\Sigma_g^+$ is very short-living and relaxes quickly to the lower-lying singlet state $a^1\Delta_g$. But this latter state in the isolated molecular oxygen is extremely long-living. Radiative transition to the ground triplet state ($X^3\Sigma_g^-$) has a magnetic dipole character and is strictly forbidden by symmetry, spin and parity selection rules, making it one of nature's most forbidden transitions [3–6]. Actually, an estimated lifetime (inverse decay rate) of $a^1\Delta_g$ state is 72 min for the isolated O_2 molecule [7].

D. Mogilevtsev (✉)
Centro de Ciências Naturais e Humanas,
Universidade Federal do ABC,
Santo André, SP 09210-170, Brazil
e-mail: d.mogilevtsev@ifanbel.bas-net.by

D. Mogilevtsev · A. Maloshtan · S. V. Lepeshkevich · B. M. Dzhagarov
Institute of Physics, Belarus National Academy of Sciences,
F.Skarina Ave. 68, Minsk, 220072, Belarus

However, in realistic sufficiently dense surroundings (especially in liquids) both radiative and non-radiative decay rates are greatly enhanced due to collisions with other molecules. The radiative decay rate correlates well with polarizability of the surrounding medium and its index of refraction [4, 7]. One should notice that due to interaction of molecular oxygen with molecules of the surrounding medium (for example, solvents, soft matter, etc.) the symmetry of the molecular oxygen is changed and the transition from the singlet exited state, $a^1\Delta_g$, has no more the purely magnetic dipole character. The radiative rate constant of a→X transition is determined by the electronic orbital angular momentum contribution to the magnetic dipole transition moment [3]. This radiative rate is extremely sensitive to intermolecular collisions and increases by three-four orders in magnitude upon collisions in solvents [4]. The interaction of oxygen and solvent molecules leads to the reduction of singlet oxygen symmetry. As a result, the a→X transition borrows electric dipole transition from the b→a transition. More detailed explanation of this phenomenon can be found in Ref. [3]. Since we are interested in spontaneous emission modification caused by inhomogeneities of the non-magnetic dielectric medium, we shall treat the transition in question as the dipole electric one.

Here it should be especially stressed that despite the obviously strong influence of the surrounding, it is possible to distinguish modifications of the transition rate from the state $a^1\Delta_g$ to the ground triplet state, $X^3\Sigma_g^-$, caused by changes in optical characteristics of the surrounding media. It demonstrates obvious dependence on the refractive index of the surrounding dielectric media. So, it is reasonable to expect strong dependence of the transition rate on inhomogeneities of the refractive index distribution of the surrounding medium present in the O_2 vicinity (just as it is for less exotic electric dipole emitters in such case). One has good reasons to expect significant modification of the spontaneous emission in optically inhomogeneous biological tissues in dependence of O_2 position within the tissue. In particular, significant modifications are to be expected in the vicinity of large complex biomolecules.

In the present work, adult human hemoglobin (HbA) was chosen as an object of study for reasons of both being very important for medical applications and ability to represent good general example of biopolymers proclivity toward modification of spontaneous emission rate. HbA is an allosteric protein that carries O_2 in blood and tissues [8]. It is a tetramer consisting of four subunits, each containing one heme group to which one molecular oxygen binds reversibly. A key role in the O_2 rebinding is played by protein cavities that describe paths from the surrounding medium to the heme group [9, 10]. The migration routes of O_2 in both the ground triplet state, $X^3\Sigma_g^-$, and the state $a^1\Delta_g$ can be considered to be the same. The results obtained in the present work hold for rather general class of dipole emitters (for example, fluorescent markers) in the vicinity and inside different bio-polymers. Here we show that the spontaneous emission rate of the singlet oxygen can be quite different when this molecule approaches HbA from different directions or penetrates the protein matrix through a hydrophobic pocket or other hollows on HbA surface.

When the emitter is placed inside a dielectric medium, spontaneous emission is modified via two different mechanism: the first one is the already mentioned influence of inhomogeneities of the refractive index distribution, and the second one is connected with local field effects. The modification via the local field effects occurs even in absence of any refractive index inhomogeneities. Influence of local field effects on spontaneous emission is well studied and understood for cases considered here, and can be easily accounted for (see, for example, the review work [11] and recent works [12, 13]; we should add that we do not really need considering them here, since the emitting molecule is nearly always considered to be inside the same liquid surrounding, and we are studying respective changes in the spontaneous emission). The case of the singlet oxygen moving inside the hydrophobic pocket will be discussed specially. So, for illustrating effects of inhomogeneities (which we term as "geometric effects"), we assume for the time being that our emitter is a structural part of the dielectric object in question, so it is not necessary to consider the local field effects. This suggestion is not unrealistic, since it is exactly the case, for example, for color centers in nano-diamond crystallites [14–17]. Color centers cannot be defined independently of the vacancy in the diamond lattice, so one can hardly speak about an influence of the local field in the case.

Now let us demonstrate how significant could be a "geometric" modification of the spontaneous emission. For our "structure part" dipole emitter in a homogeneous dielectric media with the refractive index n the emission rate, Γ is

$$\Gamma = n\Gamma_1,$$

where Γ_1 would be an emission rate of our dipole in the hypothetic homogeneous dielectric media with the refractive index equal to unity (but able to influence the O_2 molecule as to produce modifications of the

transition rate from the state $a^1\Delta_g$ to the ground triplet state, $X^3\Sigma_g^-$, similar to as it is in realistic media). Thus, for a medium with the refractive index, say, $n = 1.46$ (the typical refractive index for the silica glass), the emission would be 46 % enhanced. But if we consider our emitter inside a glass sphere with the diameter much smaller than a wavelength of the emitted radiation, we have the following [18, 19]:

$$\Gamma = \left(\frac{3}{n^2+2}\right)^2 \Gamma_1.$$

Thus, instead of enhancement, for a silica glass sphere in vacuum one has nearly 53 % suppression of the spontaneous emission rate. For a diamond nano-crystallite ($n = 2.4$) the rate, Γ, of the spontaneous emission of the dipole source inside the sphere is only 15 % of the rate Γ_1. Due to such dielectric screening, which is strongly affected by a shape of the small object, one has rather noticeable change in the rate of spontaneous emission.

We model our biological nano-object with four conjuncted ellipsoids (see Fig. 1). With this model we represent qualitatively main geometric features and structure of the hemoglobin. Notice that actual size of the structure does not affect a modification of the emission rate, provided that this size is much smaller that the emission wavelength. Also, in the model we have included for the sake of comparison two types of hollows near the surface of the object: a hydrophobic pocket (i.e. filled with air) and the pocket filled with the liquid surrounding HbA (i.e. water with the refractive index 1.33). The realistic refractive index value typical for protein matrix was taken for the simulation, $n = 1.6$ [20–22].

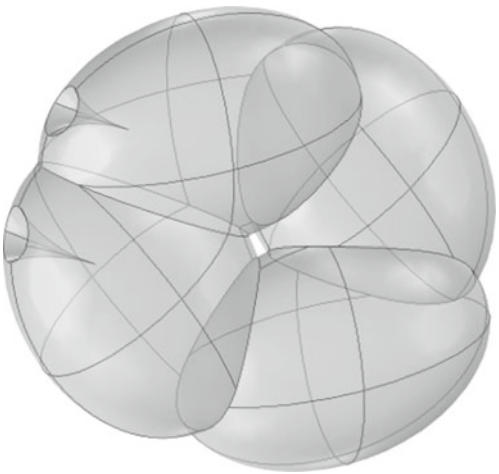

Fig. 1 The sketch of the modelled geometry. The hemoglobin-like dielectric structure is modelled by four chained ellipsoids with semiaxes 8, 6, and 4 *nm*, with two hyperbolically shaped pockets. One of the pocket is filled with surrounded medium (water, $n = 1.33$), another with air (n=1). The refractive index of the protein molecule is taken to be $n = 1.6$

To estimate the emission rate modifications we use the reciprocity theorem. It can be formulated in the following way: in the electrostatic limit (the limit of infinitely large wavelengths of the emitted radiation) a detected field amplitude does not change upon exchange of the emitter and the detector registering the emitted field [19]. Hence, the incident plane wave propagating along some direction given by the vector **e** will produce in the given point the same field as the detector situated in this very point radiated in the direction $-\mathbf{e}$ in the close vicinity of this point (actually, in the far-field zone as it is in the electrostatic limit). So, to estimate a modification of the emission rate it is sufficient to calculate a respective change of the field intensity in the given point excited by a impinging plane wave.

This estimation we have done for different orientation of the nano-object using the software package "COMSOL multiphysics" [23]. The nano-object was positioned near the coordinate system origin, and was embedded within a spherical computational domain bounded by a perfectly matched layer to prevent unwanted reflections. The system was excited by a y-polarized plane wave travelling in z-direction. This corresponds to the y-polarized emitter placed near HbA and the emission detection in z-direction in the far-field zone. The wavelength of the excitation is $\lambda = 3\mu m$, which provides the static scattering regime. Only the scattered electric field is considered. Results of our simulation are presented in Fig. 2. Also, it is useful to notice that the radiative dipole field distribution can be directly modelled by the method described in Ref. [24].

Based on Fig. 2, one can make a number of conclusions about an influence of geometrical features on the spontaneous decay rate. There are two distinct regions where the emission is modified differently. The first one is the closely spaced (less than the molecular size) external vicinity of our "hemoglobin" model. The second region corresponds to the situation, when the molecular oxygen penetrates inside the hemoglobin through openings, or pockets (schematically depicted in Fig. 1).

In the first region (outer vicinity of HbA) O_2 can experience moderate enhancement (less than 50 %) of the spontaneous emission rate in comparison with the spontaneous rate in the surrounding medium. Here one should emphasize an important feature potentially useful for diagnostics of the singlet oxygen transport near HbA: modification of the spontaneous emission rate depends strongly on the O_2 position with respect to HbA (as it could be guessed looking on highly asymmetric molecular shape Fig. 1). So, a registered

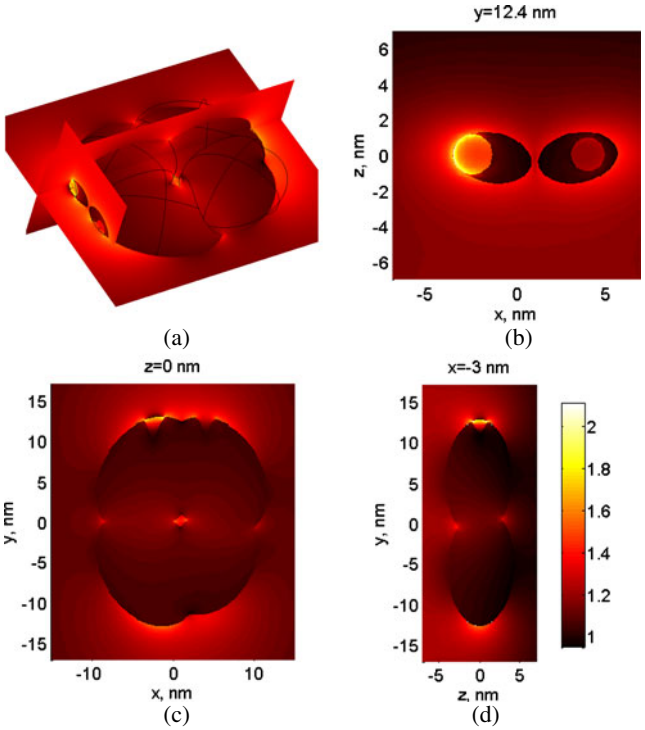

Fig. 2 Cross-sections of the scattered electric field pattern. Figure (**a**) shows relative positions of cross-section planes in 3-dimensions. (**b**, **c**, **d**) Y,Z,X-plane cross-sections. Colors depict the enhancement of the field relative to the field in water. The field enhancement up to 2 times occurs at the water-air boundary at the top of the left (hydrophobic) pocket in the panel (**b**). The right pocket in the panel (**b**) is filled with water

narrow distribution of spontaneous emission rates will inevitably point out to the localization of the singlet oxygen (or to the presence of some preferential direction of approach).

Modifications in the second region (the pockets) are more interesting. Near the edges of the water-filled pocket there are regions of sharply enhanced emission (up to two times). However, they are rather narrow. Inside the pocket the enhancement quickly diminishes; for narrow deep pockets it is possible to have even a suppression of the emission due to dielectric screening. However, this suppression is slight (less than 5 %) due to weak contrast between the molecular refractive index and the surrounding medium (the ratio of refractive indices is about 1.2). The picture is quite different for the hydrophobic, air-filled pocket. Near the entrance, i.e. near the water-air interface over the whole entrance to the pocket one has rather sharp modifications (strong enhancement and some suppression) of the spontaneous emission (see Fig. 2). Here one can have more than 100 % enhancement in comparison with the emission in water inside quite an extended region.

Deep inside the hydrophobic pocket spontaneous emission is suppressed in comparison with the emission in water. This suppression is slight, however, it is pronouncedly stronger than in the water-filled pocket. It is natural to be expected, since the spontaneous emission of dipole emitter in water is enhanced in comparison with emission in medium with refractive index equaled to unity. For example, adopting a spherical real cavity model appropriate for the case [11–13, 25], one has

$$\Gamma_{\text{water}} = \Gamma_1 n \left(\frac{3n^2}{2n^2 + 1} \right)^2$$

where n is the respective refractive index at the emitter's location. So, according to this model, the rate of emission in water is 81 % larger than the rate of emission in air.

Here we should point out that in case of penetration of the singlet oxygen deep inside the hydrophobic pocket the actual observed changes in spontaneous emission rate might be much stronger. Indeed, deep inside the pocket changes in O_2 symmetry allowing the rapid transition rate from the singlet state $a^1\Delta_g$ to the ground triplet state, $X^3\Sigma_g^-$, can take place in a way different to as it was occurring in liquid surrounding media. In this case the oxygen molecule collides not with water molecules but with amino acid residues of "pocket protein walls". However, this goes beyond the scope of our current consideration.

Concluding: we have shown that the spontaneous emission rate of the singlet oxygen can be markedly enhanced near the surface of the bio-molecule, in particular, near the human hemoglobin. Enhancement is more pronounced near the entrance to the hydrophobic pocket of HbA, whereas one can expect some suppression of the spontaneous emission deep inside the hydrophobic pocket. Generally, it seems quite feasible to infer character of O_2 dynamics near HbA from measurement of spontaneous emission rates. We believe that such a possibility can be highly relevant for practical applications, especially, in photomedicine.

This work was supported by the Foundation of Basic Research of the Republic of Belarus and by the National Academy of Sciences of Belarus through the Program "Convergence" (Project 3.1.04), and the Brazilian Agency FAPESP (project 2011/19696-0) (D.M.).

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