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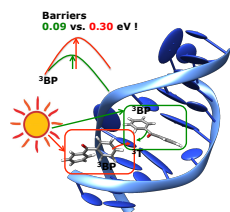
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Resolving the Benzophenone DNA-Photosensitization Mechanism at QM/MM Level

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ABSTRACT: Benzophenone, the parent of the diarylketone family, is a versatile compound commonly used as a UV blocker. It may also trigger triplet-based DNA photosensitization. Therefore, benzophenone is involved in DNA photo-damage induction. In absence of experimentally-resolved structure, the mechanism of DNA damage production remains elusive. Employing a hybrid quantum mechanics/molecular mechanics approach, here we address the spin transfer mechanism between this drug and proximal thymine, i.e. the DNA nucleobase most prone to suffer triplet damages.



Keywords: DNA Photosensitization, Benzophenone, Energy Transfer, QM/MM

Benzophenone (BP) is a versatile molecule, with a remarkable photochemistry¹. Notably a well-recognized feature of BP is a relatively long-lived lowest triplet state² lying at long wavelengths (> 320 nm). For this reason BP covers a large range of applications notably as a photoinitiator³, and its properties are largely exploited⁴. Yet it also induces DNA degradation⁵, leading to photolesions following the most efficient triplet photosensitization process.^{6,7} Indeed, triplet states of benzophenone (noted ^3BP hereafter), as well as of other aromatic ketones selectively induce energy transfer to proximal thymines, since the latter is the nucleobase featuring the lowest-lying triplet states (noted ^3T hereafter). Photosensitization process represents an efficient and most deleterious mechanism for ^3T formation. The nature of ^3T has been intensively investigated⁸⁻¹¹, yet often on isolated entity (nucleobase or nucleoside monophosphate). In presence of a vicinal thymine, ^3T may fol-

low competitive cycloaddition pathways. The latter may lead to DNA mutagenic adducts (triplet photodamage¹²), such as cyclobutane pyrimidine dimers¹³. Because of its relevance in DNA lesions induction, it becomes crucial to gain insights into the photosensitization mechanisms, in particular assessing the efficiency of the $^3\text{BP} \rightarrow ^3\text{T}$ energy transfer, which results in the population of the ^3T state. Whereas the feasibility, and even the efficiency, of this process have been clearly established experimentally⁶, a detailed view of its mechanism at the atomistic and electronic level is still lacking.

Photosensitization efficiency depends crucially on two main factors: the intrinsic efficiency of the photosensitizers to populate its triplet manifold, high for arylketones¹⁴⁻¹⁷, and the efficiency of the subsequent energy-transfer, that is strongly dependent on the coupling with the environment, and specifically with the DNA nucleobases. In the case of benzophenone time-resolved spectroscopy has evidenced, in gas phase, solution and crystal a fast intersystem crossing (~ 10 ps) followed by a relatively long-lived phosphorescence.¹⁷

Drugs may non-covalently bind DNA at its minor or major groove, but they may also intercalate or insert in the double-stranded DNA (see the seminal works by Barton¹⁸). By employing classical molecular mechanics, two of us recently probed¹⁹ two stable adducts (Figure 1) for BP interacting with a poly(dA-dT) decamer: the minor groove binding (MGB) and the double-insertion (DI). The latter is a more specific interaction mode characterized by the ejection of an adenine-thymine base pair. These two interaction modes are different enough to evidence different spectroscopic properties (most notably in induced circular dichroism)¹⁹. This difference suggests that the coupling with DNA nucleobases, responsible for energy-transfer process, may be quite different as well between the two modes. Hence, one interaction mode may play a more important role in the sensitization and population of ^3T . In the lead of the different computational studies having unraveled the intrinsic photophysical properties of isolated ^3BP and ^3T ,⁸⁻¹⁰ in this letter we monitor the induction of a triplet-triplet transfer

from BP drug to the most vicinal thymine, explicitly modeling its complex inhomogeneous embedding through hybrid quantum mechanics/molecular mechanics (QM/MM) techniques. Simple models of energy-transfer have been inferred from quantum mechanics (QM) calculations on symmetric and isolated systems,²⁰ and the dependence on the distance between the two transferring monomers has been particularly captured²¹. Also studies where a DNA subfragment has been employed have contributed to shed light on some of the photophysical properties²². However, the B-DNA solvated double strand may play a crucial role in tuning and controlling this process. One can already surmise the role of mechanical embedding, the relative orientations of benzophenone and the interacting thymine (here noted T4) being very different in the two binding modes (see Figure 1). Even more importantly, the drug and thymine nucleobase will experience a strong anisotropic electrostatic embedding in particular due to the phosphate moieties. These environment effects have also been recently observed in triplet-triplet exciton migrations in a poly(dA).poly(dT)²³. The ease of energy-transfer is strongly confirmed, as well as the role of the DNA environment in stabilizing ³T (which is in agreement with experimental evidences)⁶. Most notably and for the first time, we explicitly monitor the triplet-triplet transfer mechanism at atomistic and electronic level, and this DNA damage initiating process is found to be almost spontaneous. Hence the high photo-toxicity of benzophenone and similar exogenous DNA sensitizers is firmly rationalized.

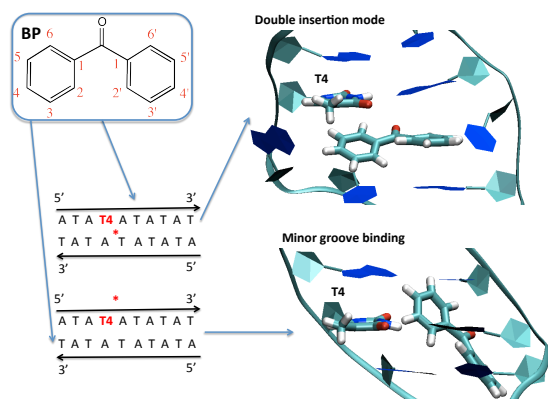


Figure 1. Scheme and cartoon representations for BP interacting with a poly(dA-dT) decamer in minor groove or via double-insertion. In both cases, T4 is the BP most proximal thymine (water molecules are omitted for the sake of clarity).

Representative snapshots of minor-groove bound and double-inserted BP interacting with a poly(dA-dT) decamer were considered¹⁹ (see SI for details). Equilibrium geometries for thymine and benzophenone ground singlet states, $Q(^1T)$ and $Q(^1BP)$, as well as for the corresponding triplet states, $Q(^3T)$ and $Q(^3BP)$ were obtained. QM/MM geometry optimizations were performed with the COBRAMM²⁴ interface, coupling Gaussian09²⁵ and AMBER²⁶ packages. The hybrid *meta* GGA M06-2X²⁷ functional and am-

berff99bsc0 force field²⁸ were chosen after careful calibration (see SI). To locate the energy-transfer path between the equilibrium triplet benzophenone ($Q(^3BP)$) and the triplet thymine ($Q(^3T)$), we use a simplified coordinate $Q(\xi)$, with ξ ranging from 0 to 1:

$$Q(\xi) = \xi Q(^3T + ^1BP) + (1 - \xi) Q(^3BP + ^1T) \quad (1)$$

The triplet excited states have been obtained using Time Dependent DFT (TD-DFT) and *ab initio* CAS-PT2 and RAS-PT2 (See SI for details). In the case of TD-DFT, the excited states nature has been analyzed in terms of Natural Transition Orbitals (NTO)^{30,31}.

For BP, as expected, the first triplet excited state (T_1) corresponds to a $n-\pi^*$ state. Note that a second triplet state, T_2 ($\pi-\pi^*$), lies slightly higher in energy both at Franck-Condon and T_1 optimized geometry. The vertical transition for the ³BP state is found at 3.21 eV, in satisfactory agreement with the experimental estimate of 3.01 eV.⁶ For thymine, the first triplet state is unsurprisingly a $\pi-\pi^*$ state. Two higher triplet states of thymine³² correspond to $n-\pi^*$ localized on the two carbonyl groups and are usually involved in the Paternó-Büchi reaction.³³ The nature of the lowest triplet states of thymine and benzophenone are not qualitatively affected by interaction with DNA. Energetically ³T is stabilized by DNA embedding mainly due to dispersion interactions, as already evidenced by *ab initio* calculations on thymine dimers.³³ The ³T adiabatic excitation energy in DNA is found at 3.15 eV, the same order of magnitude than the 2.90 eV estimate reported by Miranda and coworkers.^{6,34}

We report in Figure 3-A the low-lying triplet excited electronic states, along the interpolation coordinate $Q(\xi)$ for the DI mode calculated at TD-DFT and CAS-PT2 level. At both level of theories the T_1 state is initially centered on benzophenone (³BP) and rapidly ($\xi=0.375$ both for RAS-PT2 and TD-DFT) crosses with the T_2 state, which corresponds to the first thymine-centered triplet (³T). The crossing point can be reached overcoming an extremely low energy barrier (0.07 eV at RAS-PT2 and 0.09 eV at TD-DFT), therefore denoting a very facile process. This value represents an upper bound to the exact barrier since we used an approximate coordinate instead of the minimum energy reaction path. Both the value of the energy barrier and the general evolution of the potential energy curves are quite similar between *ab-initio* and TD-DFT values. The rapid stabilization of the ³T state after the crossing constitutes an additional confirmation of the favored nature of the triplet-transfer both from an energetic and kinetic point of view. Indeed the ³T triplet state at $Q(^3T)$ is significantly more stable than the corresponding ³BP triplet at $Q(^3BP)$. Another strong indication that the spin transfer is a very efficient process is the fact that the second ³BP state ($\pi-\pi^*$) crosses already at $\xi=0.0$ with ³T thus opening a possible second channel for an even more efficient transfer process. This latter aspect may be even more important since the population of the first triplet state ($n-\pi^*$) in BP should happen through the second ($\pi-\pi^*$) triplet state, because of

the El Sayed rule¹. In Figure 3-B we report the energetic profile for the MGB, because of the previous validation of TD-DFT only the latter values are reported here. A $^3\text{BP}/^3\text{T}$ crossing is found at $\xi=0.50$ with an increased barrier of 0.30 eV. Even if in this case the sensitization happening from T_1 will need to overcome a non-negligible barrier, once again a second crossing occur at $\xi=0.25$ that can be reached barrierless via the second $\pi-\pi^*$ ^3BP triplet state, hence leading to efficient ^3T population.

In the case of the model systems (i.e. gas-phase interacting BP and T) the profile given in SI reveals a much more inefficient crossing between the first ^3BP and ^3T triplet states. Even if interestingly the model system scenario looks quite similar to the minor-groove binding, the coupling with the complex B-DNA environment appears crucial in allowing an easy and fast energy-transfer process. Considering the proximity between the donor (BP) and the acceptor (T) laying at about 3.2 and 7.5 Å for DI and MGB, respectively, the short-range Dexter energy transfer process should dominate. However due to the exponential decay with the distance the transfer rate is found 100 times higher for double insertion than for minor groove binding. This trend is also confirmed by the overlap between the wavefunctions of the two chromophores, estimated at 0.08 and 0.01 electrons for DI and MGB, respectively (see SI for details).

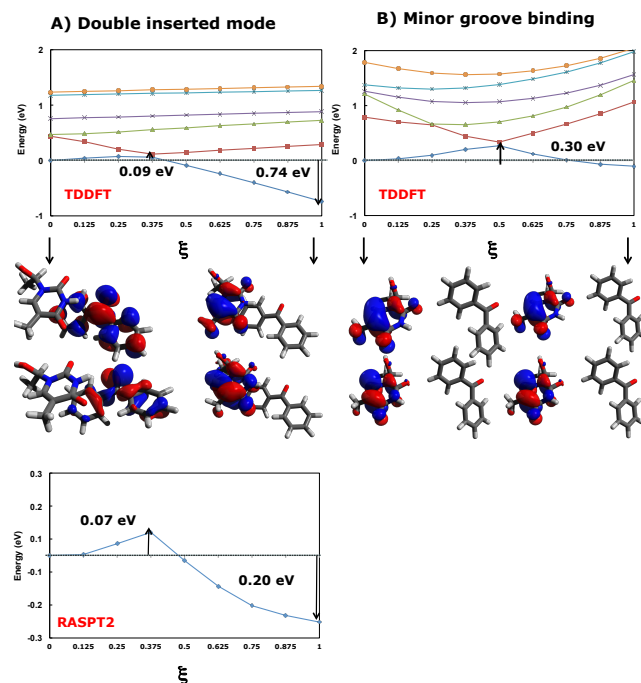


Figure 3. Adiabatic potential energy evolution of the six first triplet excited states for $^3\text{BP} \rightarrow ^3\text{T}$ transfer in B-DNA for double-insertion (A) and minor groove binding mode (B). The QM/MM geometries at $\text{Q}(^3\text{BP})$ and $\text{Q}(^3\text{T})$ are reported in Figure S2. “Occupied” (top) and “virtual” (bottom) NTOs are also reported for $\xi=0.0$ and $\xi=1.0$.

Indeed, even if our study does not allow to directly quantifying the energy-transfer characteristic times and rates, we prove unambiguously that the ^3T population happens quite rapidly. No significant barrier will hint the efficient spin-

transfer from the first triplet state in double-insertion mode. Furthermore, a second barrierless pathway from the ^3BP $\pi-\pi^*$ state is evidenced both for DI and MGB. Still a full characterization of the transfer process will necessitate the estimation of the reorganization energy, which in principle can be achieved by using molecular dynamics umbrella sampling. However, considering the almost-barrierless transfer profile, and the relative rigidity of DNA is in our opinion it is quite improbable that this reorganization energy should result in a kinetic block hindering the process.

The crucial role of a complex and realistic B-DNA environment in tuning the energy transfer from benzophenone triplet state to DNA has been modeled at QM/MM level of theory. In particular starting from stable interaction modes we have unraveled, for the first time, the mechanism of the triplet-triplet energy transfer from benzophenone to the closest thymine nucleobase. The former process is indeed the key step in DNA UVA photodegradation, since ^3T may evolve toward dangerous mutagenic photoproducts. From the one hand we have concluded that the DNA environment makes the energy-transfer energetically favored lowering the energy of the ^3T state compared to the gas-phase. On the other hand the transfer process is also kinetically favored since we found that the triplet-triplet transfer can be achieved efficiently following an almost barrierless pathway. It is noteworthy that the two stable interaction modes give rise to non-equivalent transfer pathways. Indeed if DI shows a barrierless path involving the first $n-\pi^*$ ^3BP state, MGB may proceed barrierless only from the second ^3BP ($\pi-\pi^*$), the latter state however could be populated during the BP photophysical evolution because of the El Sayed rule.¹ Furthermore the almost barrierless potential energy profile, in $n-\pi^*$ or $\pi-\pi^*$, implies that the transfer process should be fast and should happen within the first vibrational movements. This fact is also quite significant since the minor-groove binding is metastable and experiences a residence time close to DNA of about 10 ns. In the forthcoming we plan to extend this study exploring more carefully the sequence effect on the transfer, in particularly assessing the preference for 3' or 5' thymine. Furthermore, explicit non-adiabatic QM/MM molecular dynamics may help in obtaining a sound estimation of the characteristic transfer time and reorganization energy. Eventually, the future design of novel phototherapeutic drugs³⁵ with increased efficiency and selectivity may greatly benefit by similar QM/MM studies that have proved to be accurate enough in delivering a reliable and sounding picture of the transfer process.

ASSOCIATED CONTENT

Scheme of the QM/MM partition and methodological details. Energy diagrams for DNA interacting BP and diabatic potential energy surfaces for a model BP+T p-stacked system. Details on the Dexter transfer. Cartoon representations for Figure 3. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Funding Sources

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