

DESIGN OF TEMOPORFIN MOLECULES FUNCTIONALIZED WITH
TWO-PHOTON ABSORBING ANTENNAS AND THEIR POTENTIAL USE IN
PHOTODYNAMIC THERAPY

by

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ABSTRACT

DESIGN OF TEMOPORFIN MOLECULES FUNCTIONALIZED WITH TWO-PHOTON ABSORBING ANTENNAS AND THEIR POTENTIAL USE IN PHOTODYNAMIC THERAPY

Since its approval in 2003 by the European Agency of Medicine (EAM), temoporfin has been clinically used as a photosensitizer for photodynamic therapy. However, Temoporfin's significant limitations such as low solubility in water and high aggregation probability in biological environments have created a window of opportunity for increasing its efficacy through modifications. In this thesis, three new drug molecules are proposed and obtained by modifying the temoporfin molecule with the addition of three different two-photon absorbing (TPA) dye molecules.

To explore the newly proposed molecules' suitability to photodynamic therapy, various theoretical chemistry tools are utilized such as Density Functional Theory (DFT), and Time-Dependent Density Functional (TD-DFT) on various levels of theory for quantum mechanical (QM) calculations. The results shed light on the electronic spectra of candidate drug molecules. Later, molecular dynamic techniques are exploited for further analysis of the novel drug molecules' behavior in settings similar to biological environments.

ÖZET

İKİ FOTON ABSORBE EDEN GRUPLARLA İŞLEVLENDİRİLMİŞ TEMOPORFİN MOLEKÜLLERİNİN TASARIMI VE FOTODİNAMİK TERAPİDE OLASI KULLANIMLARI

Temoporfin, Avrupa Tıp Ajansı (EAM) tarafından 2003 yılında onaylanmasından bu yana bir fotodinamik terapi ilacı olarak kullanılmakta. Ancak, Temoporfin'in bu tedavi yöntemi kapsamında sudaki düşük çözünürlüğü ve yüksek agregasyon ihtimali gibi ciddi sınırlamaları mevcuttur. Bu sınırlamalar, bu molekülün etkinliğini arttırmak için bir fırsat sunmaktadır. Bu tezde, fotodinamik terapi için, Temoporfin molekülünün iki-foton emici boya molekülü ile modifiye edilmesi sonucu oluşturulmuş, üç yeni ilaç molekülü önerilmiştir.

Önerilen üç molekülün fotodinamik tedaviye uygunluğunu araştırmak için hesapsal bir çalışma yapılmıştır. Kuantum mekanik hesaplar çeşitli seviyelerde Yoğunluk Fonksiyonel Teorisi ve Zamana Bağlı Yoğunluk Fonksiyonel Teorisi kullanılarak tamamlanmıştır. Önerilen molekülün fotokimyasal özellikleri, yapılan hesaplamaların sonuçlarıyla aydınlatılmıştır. Moleküllerin biyolojik ortamdaki davranışlarını incelemek içinse Moleküler Dinamik metodundan faydalanılmıştır.

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LIST OF SYMBOLS

E	Energy
E_{xc}	Exchange Correlation Energy Functional
H	Hamiltonian Operator
h	Planck's Constant
$\hbar$	The value of Planck's constant divided by 2π
i	Electron
j	Electron
$J(\rho)$	Coulomb Interaction Energy Between Electrons
m	Mass
N	Number of Electrons
P	Wigner Distribution Function of a Pure State
r	Radius
R_m	Position
T_{ni}	Kinetic Energy of Non-Interacting Electrons
U	Electron–Electron Interaction Energy
V	Potential Energy
V_{eff}	Effective Potential Energy
V_{ni}	Potential Energy of Non-Interacting Electrons
Z_m	Effective Nuclear Charge
ϕ_s	Quantitative Topological Descriptor
ϕ_i	Atomic Orbitals
λ_{max}	Maximum Absorption/Emission Wavelength
∇	Gradient
ρ	Electron Density
σ_{TPA}	Two-Photon Absorption Cross Section
Ψ	Wavefunction

LIST OF ACRONYMS/ABBREVIATIONS

B3LYP	Becke-3-parameter Lee-Yang-Par Functional
CAM-B3LYP	Coulomb Attenuated Method
CD	Cyclodextrin
DFT	Density Functional Theory
GGA	Generalized Gradient Approximation
GM	Göppert-Mayer Unit
GTO	Gaussian Type Orbitals
IC	Internal Conversion
ISC	Intersystem Crossing
LC	Long-Range Corrected
LCAO	Linear Combinations of Atomic Orbitals
LDA	Local Density Approximation
M062X	Empirical Exchange-Correlation Functional
MD	Molecular Dynamics
MM	Molecular Mechanics
mTHPC	5,10,15,20-tetrakis(3-hydroxyphenyl)chlorin
NIR	Near-Infra Red
ns	nanosecond
NTO	Natural Transition Orbitals
OPA	One-Photon Absorption
PCM	Polarizable Continuum Model
PDT	Photodynamic Therapy
PEG	Polyethyleneglycol
PS	Photosensitizer
QM	Quantum Mechanical
RMSD	Root-Mean-Square Deviation
S	Singlet
SOCC	Spin-Orbit Coupling Constant

STO	Slater Type Orbital
T	Triplet
TD-DFT	Time-Dependent Density Functional Theory
TIP3P	Transferable Intermolecular Potential with 3 Points
TPA	Two-Photon Absorption
TPE	Two-Photon Excited
UV/VIS	Ultra Violet/Visible Spectrum
ZPE	Zero-Point Energy

1. INTRODUCTION

1.1. Photodynamic Therapy

The medicinal properties of sunlight have been known since ancient times and people have historically utilized these effects. Various civilizations, including the Egyptians, Chinese, and Greeks have harvested the therapeutic trait of Sunbeams for medicinal purposes for at least 4000 years. [1, 2] Following the invention of the microscope, the antibiotic effect that can be exerted through light was discovered, and the Nobel Prize-winning research by Niels Finsen in 1903 started the grounds for modern-day photodynamic therapy (PDT) [3–5]. In his work, Niels showed that Lupus Vulgaris disease and smallpox -the epidemic of those days- could be treated via the use of specific and intense lights [5]. Advances in medicine and technology since those days have enabled treatment strategies to become more and more targeted and non-invasive and this has pushed researchers to come up with new strategies and drugs for efficient and low-side-effect treatment options [6]. Since its first modern-day debut, photodynamic therapy (PDT) has had several developments in itself.

1.1.1. Mechanism and Advantages of Photodynamic Theory

PDT is a promising form of treatment for various conditions such as age-related macular degeneration, different skin conditions e.g., acne, and terminal cancer types, for instance, skin and non-small cell lung carcinomas [7, 8]. This method of treatment takes advantage of light activation of molecules, offering a non-invasive and more localized treatment option compared to its alternatives. In this method, the PDT agent -called a photosensitizer- is administered into the patient's bloodstream. Then the target tumorous area is irradiated with light of a specific wavelength enabling the activation of the dark-inactive photosensitizer molecule. Upon absorbing the photon, the PS molecule is excited to its singlet excited state, and via internal conversions (ICs) and intersystem crossing (ISC) the first triplet excited state (T_1) is populated. Be-

cause $T_1 \rightarrow S_0$ is a spin-forbidden process, the T_1 states are usually longer-lived [9]. This time interval is enough for the photosensitizer to transfer its energy to molecular oxygen around its environment. Oxygen has a triplet ground state and upon receiving the PS's energy, it converts to singlet oxygen ($^1O_2$) which is very reactive and cytotoxic. The produced $^1O_2$ goes on to react with its environment – in this case tumorous tissue and causes cell death [10–12]. Mechanism of PDT is illustrated with a Jablonski diagram in Figure 1. There are two generations of PS molecules: the first and second generations. Second-generation PSs are improved versions of first-generation PSs with a similar working mechanism. Second-generation PS molecules have narrower absorption bands and shorter half-lives than first-generation photosensitizers [13]. These two characteristics allow the second-generation PS molecules to have a more precise activation wavelength and a shortened time of photosensitivity that will be experienced later by the patient.

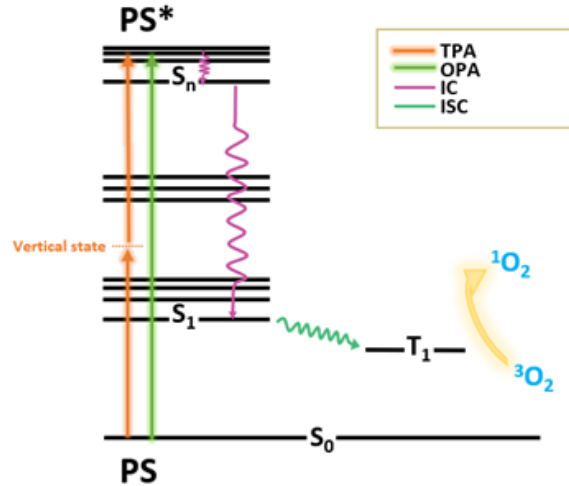


Figure 1.1. Jablonski diagram describing the Photodynamic Therapy (PDT) process.

PDT offers a non-invasive type of treatment path decreasing the recovery time of patients, its repeatability allows an effective treatment process while the tissue targeting property of PDT limits the damage to healthy tissues [14–16]. However, as nothing can be perfect, PDT has its own restrictions. Photodynamic therapy is limited by the tissue penetrating ability of the light that will activate the PS. Generally, the near-infrared (NIR) portion of the biotherapy window of light (650–1350 nm) is known to

penetrate deeper into the skin and therefore can reach deeper positioned tumor cells but the penetration depth a photon can reach depends on the water content of the tissue [17,18]. Also, because the main goal of PDT is generating singlet oxygen, the oxygen content of the target tissue becomes important. Some limitations arise relating to the design of the PS molecules. An ideal PS molecule should have a narrow absorption band with high intensity in the NIR portion of the UV/VIS light spectrum, it should be soluble and non-reactive in biological environments and should be able to populate its T_1 state upon excitation.

1.2. Temoporfin

Temoporfin, also known as mTHPC (meso-Tetrahydroxyphenylchlorin) is a synthetic tetrapyrrole molecule that contains four phenol groups attached from their meta positions to a chlorin cycle. Its structure is showcased in Figure 2. The chlorin cycle is very similar to a porphyrin macrocycle with the single difference of a single bond between two carbon atoms instead of a double. Temoporfin has been used as a PDT agent under the commercial name Foscan[®] since it was approved by the European Medicines Agency in 2003 [19]. mTHPC molecule is a second-generation photosensitizer, which has the desired narrow absorption band with high intensity at 652 nm which, falls into the biotherapy window and is a stable compound in biological environments. But the shortcomings of this PDT agent are its low solubility and tendency to aggregate due to π -stacking [20]. mTHPC already being an approved and commercial drug molecule for various conditions, and scientists have found the motivation to increase the performance of this photosensitizer molecule. Several strategies have been employed to overcome these shortcomings; [19] some of which are: encapsulation of mTHPC with peptides [21], cyclodextrins, [22] and nanoparticles [23] to increase its solubility and tissue penetration abilities, carrying it with liposomes increasing the targeting properties [24,25]. A computational work showed an increase in the fluorescence quantum yield and the formation of aggregates around the lipid bilayer mimicking the cell membrane upon the encapsulation of mTHPC with heptakis(2,3,6-tri-O-methyl)- β -cyclodextrin (TM- β -CD). [22]

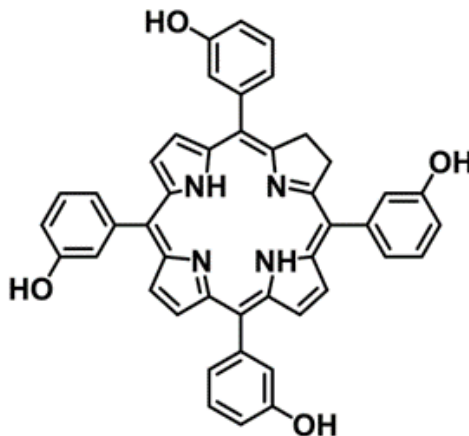


Figure 1.2. Chemical structure of temoporfin (mTHPC).

1.3. Two-photon Excited Photodynamic Therapy

Another approach to increase the efficacy of PDT is using PS molecules with high two-photon absorption (TPA) cross sections (σ_{TPA}). Two-photon absorption was theorized by Maria Goeppert-Mayer back in 1931 and later observed in the 1960s after the invention of lasers [26]. The mechanism involves a molecule simultaneously absorbing two photons of equal or different energies, going through a virtual state to reach an excited state under high-intensity light. Molecules with TPA capacity harvests energy of two photons to reach an excited state. This characteristic enables such molecules to become excited by absorbing lower energy light compared to the traditional one-photon absorption (OPA) path [27]. In the context of PDT, this approach is highly beneficial as longer wavelength light will suffice to activate the PS molecule.

In previous studies, the σ_{TPA} for mTHPC has been calculated as 28 ± 8 GM at 775 nm in DMSO [28] and 18 GM at 800 nm in 20% ethanol, 30% polyethylene glycol, and 50% distilled water [29]. Both these experiments indicated the possibility of using mTHPC in two-photon excited (TPE) PDT. For efficient TPA, however, the cross-section would preferably be higher. For this purpose, Senge et al. published a study in 2021 functionalizing mTHPC molecule with different groups to both increase the σ_{TPA} and compared the TPA increasing patterns of the modifier groups [30]. Similarly,

another group has studied the effect of changing small functional groups on a Ru(II) complex and observed an increase in σ_{TPA} values [31].

Upon analyzing the literature on increasing the σ_{TPA} of large systems, it can be said that increasing the conjugation chain in the molecule, integrating triple bonds, and modifying with groups that already possess a high σ_{TPA} increase the resulting TPA of the system. To this end, in this work, the mTHPC molecule is modified to increase its σ_{TPA} as well as its overall PDT agent features upon functionalizing it with three different dye groups: DTP1, DTP2, and DPP. These molecules are selected due to their high σ_{TPA} and their structures are given in Figure 1.3. DTP derivative dyes are derived from the substitution of a π -chain and an acceptor group to different positions on the 2,5-di thienyl pyrrole molecule [32–34]. They have been analyzed extensively via both experimental [32] and computational [33,34] methods. DTP1 showed two absorption peaks one at 820 nm and one at 566 nm, its TPA cross sections are 422.0 and 1243.6 GM respectively. DTP2 molecule, on the other hand, had the σ_{TPA} of 742 and 6977.7 GM for its peaks at 870 nm and 624 nm, respectively. The polyethylene glycolated (PEG) diketopyrrollopyrrole (DPP) molecule has been tested on a porphyrin derivative that has a zinc (ZnP) molecule located in its core and the modification of the system with DPP has increased the σ_{TPA} for all wavelengths but for the λ_{max} the σ_{TPA} has gone from 20 GM to 1400 GM upon addition of a single DPP molecule [35]. Said dye molecules are attached to the temoporfin molecule by removing a phenol group from mTHPC and attaching the dyes with a triple bond to its place. DTP derivative molecules are attached from the neighboring carbon to sulfur on thiophene rings and the DPP molecule is attached from the para position on one of its benzyl groups.

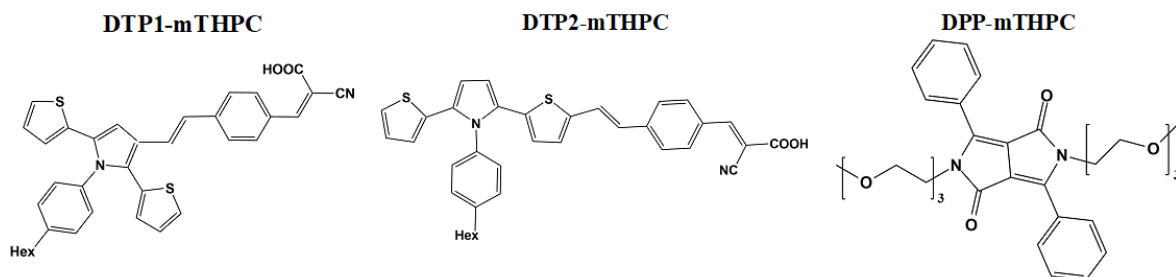


Figure 1.3. 2D structure of dye molecules.

1.4. Aim of the Study

For this fully computational work, our aim was to improve the photophysical properties of mTHPC and make it an overall better PDT agent. To this end, we have designed three molecules by combining three high σ_{TPA} dye compounds with the original mTHPC molecule. Designed molecules are presented in Figure 1.4. A variety of quantum mechanical (QM) analyses were performed to predict the photophysical properties of new candidate molecules such as one-photon absorption (OPA), TPA, spin-orbit coupling constants (SOCC), singlet and triplet energy states of the molecules, and natural transition orbitals (NTO). Subsequently, benefiting from molecular dynamic (MD) simulations, candidate molecules were modeled in various environments that mimic biological conditions such as in bulk water, encapsulated cyclodextrin (CD) units, and in near proximity to the membrane. To address the solubility problem of the mTHPC molecule, the candidate PS molecules were encapsulated in CDs. It is well-known that β -cyclodextrins constitute an encapsulated complex in a 2:1 ratio with mTHPC molecule, therefore this ratio is exploited for DTP1 and DTP2 modified temoporfin molecules. However, for the DPP-mTHPC molecule, assuming the solubility of the overall molecule will be increased due to the two highly soluble PEG groups, only one CD complex is used in the encapsulation of this molecule.

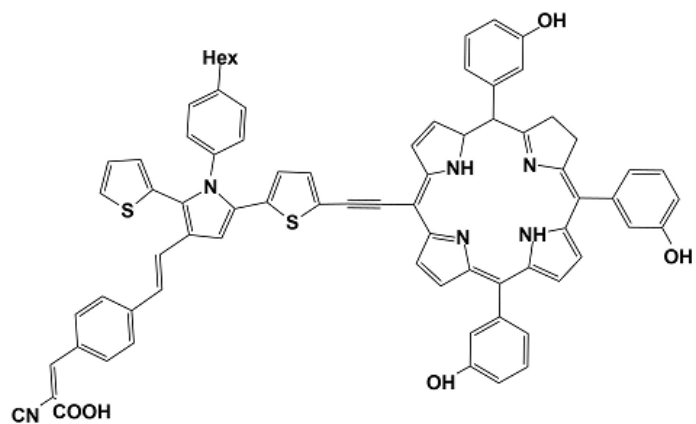
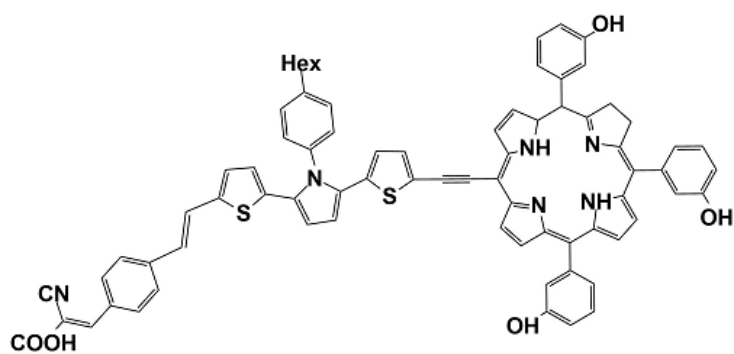
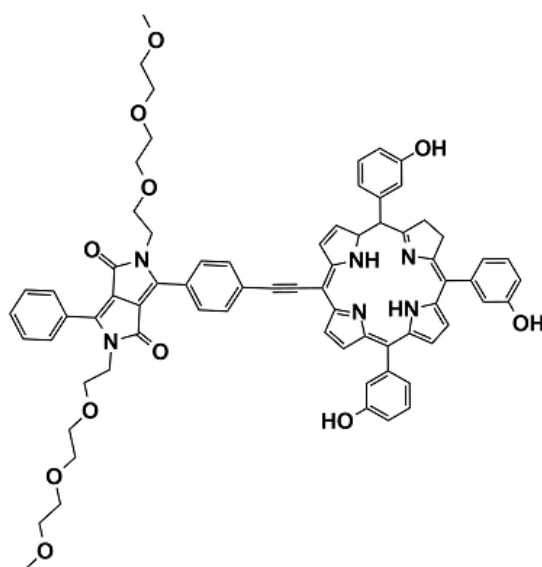
DTP1-mTHPC**DTP2-mTHPC****DPP-mTHPC**

Figure 1.4. 2D structure of proposed PS molecules.

2. METHODOLOGY

2.1. Density Functional Theory

Computational chemistry is intimately related to Schrödinger’s equation. From this equation, all information about the system can be derived, but not always simultaneously. This equation contains all information about a particle or a system. This equation serves as a starting point in many computational simulations and can be expressed as

$$\hat{H}\Psi = E\Psi, \quad (2.1)$$

where $\hat{H}$ is the Hamiltonian of the system and ψ is the wavefunction. The Hamiltonian is the energy (E) operator. But, there are some approximations in order to make this equation less computationally taxing; such as the Born-Oppenheimer approximation. This approximation assumes because the nucleus is much heavier and much slower than electrons, it is stationary. So, the Born-Oppenheimer implemented version of the $\hat{H}$ is depicted in Equation (2.2),

$$\hat{H} = [\hat{T} + \hat{V} + \hat{U}] = \left[\sum_{i=1}^N \left(-\frac{\hbar^2}{2m_i} \nabla_i^2 \right) + \sum_{i=1}^N V(\mathbf{r}_i) + \sum_{i<j}^N U(\mathbf{r}_i, \mathbf{r}_j) \right]. \quad (2.2)$$

In Equation (2.2) i and j are electrons, $\hbar$ is $h/2\pi$ where h is the Planck’s constant, r is the coordinates of the electrons, m is the mass, N is the number of electrons of the system, T , V and U are the kinetic, potential, and electron-electron interaction energies of the system. Now, a widely used approach to solving the Schrödinger equation is called Density Functional Theory (DFT). This method was proposed by Hohenberg and Kohn in 1964 [36]. According to this theory, the electron density of a system ($\rho(r)$) is unique to its given potential $v(r)$, and this statement goes both ways. The external potential of a given molecule is defined by the attractions from the nuclei around it, as

$$v(r) = - \sum_m \frac{Z_m}{|r - R_m|}. \quad (2.3)$$

In this statement, m , R_m , and Z_m are the mass, position, and effective charge of the nucleus, respectively. The ground state energy and other properties that depend on the Hamiltonian can be determined by the electron density. The reason is that, by integrating the electronic density, one can obtain information on the number of electrons, the position and charges of nuclei, and therefore the Hamiltonian. Hence, the energy can be expressed in terms of a function of electron density. This is where the 'functional' comes from in the name of this method, DFT. Energy, as a function of electron density is given in Equation (2.3) as

$$E[\rho] = \int \hat{\rho}(r)v(r) dr + T[\rho] + V[\rho]. \quad (2.4)$$

In this equation, the two unknown functionals are $T(\rho)$ and $V(\rho)$. Kohn and Sham [37] proposed to insert two new functionals in place of the two unknown. So, the new energy equation expressed in terms of ρ became this:

$$E[\rho] = V_{ne}[\rho] + T_s[\rho] + J[\rho] + E_{xc}[\rho], \quad (2.5)$$

where $T_{ni}(\rho)$ is the kinetic energy of non-interacting electrons, $V_{ni}(\rho)$ is the potential energy of non-interacting electrons, and $J(\rho)$ is the Coulomb interaction energy between electrons. The newly introduced $E_{xc}(\rho)$ term is called the exchange-correlation energy functional. Conceptually, one can calculate the density by minimizing this newly introduced exchange-correlation energy functional. For this purpose, Kohn and Sham described orbitals (ϕ_i) [37], and the density is given as

$$\rho(r) = \sum_i^N \phi_i^2(\mathbf{r}). \quad (2.6)$$

The Hamiltonian of a system with non-interacting electrons can be denoted as:

$$\hat{H} = - \sum_i^N \frac{1}{2} \nabla_i^2 + \sum_i^N v_{\text{eff}}(r_i), \quad (2.7)$$

where $v_{\text{eff}}(\mathbf{r}_i)$ is the effective external potential. Then, the solutions of Kohn-Sham equations that are one-electron orbitals are used to construct exact wavefunctions.

$$f_i \phi_i(r) = \epsilon_i \phi_i(r), \quad (2.8)$$

where f_i is the Kohn-Sham operator

$$f_i = -\frac{1}{2} \nabla_i^2 + v_{\text{eff}}(r_i). \quad (2.9)$$

Then, the effective potential can be represented as:

$$v_{\text{eff}}(r) = v(r) + \frac{\partial J[\rho]}{\partial \rho(r)} + \frac{\partial E_{xc}[\rho]}{\partial \rho(r)}. \quad (2.10)$$

2.2. Functionals

DFT offers a computationally less burdensome solution to the Schrödinger equation by relating the energy of a system to its electron density. However, the DFT method does not offer a direct and single solution, as the E_{xc} term is still unknown. It is currently not possible to obtain an exact exchange-correlation functional, but approximations can be used to get an E_{xc} value as close to accuracy as possible.

The first approximation of E_{xc} was proposed by Kohn and Sham and it is known as local density approximation (LDA). LDA perfectly describes a uniform electron gas. But it is common knowledge that the electron densities of systems significantly deviate from uniformity. Hence, for quantum chemical calculations, the LDA method is usually not sufficient.

A more accurate approach is the generalized gradient approximation (GGA) [38]. GGA methods considered the varying nature of the electron density and correlated the exchange and correlation energies to both the density and the gradient of density. The energy expression for GGA methods is given as

$$E_{\text{XC}}^{\text{GGA}}[n] = \int n(r) \varepsilon_{\text{XC}}(n(r), |\nabla n(r)|) dr. \quad (2.11)$$

Yet another approach was to include some exact Hartree-Fock exchange into the E_{xc} functional. In computational chemistry, the most widely used hybrid functional is B3LYP [39]. This functional is a hybrid of LDA and GGA functionals and how it treats the exchange-correlation functional can be expressed as

$$E_{\text{XC}}^{\text{B3LYP}} = (1 - a)E_{\text{X}}^{\text{LSDA}} + aE_{\text{X}}^{\text{HF}} + b\Delta E_{\text{X}}^{\text{B}} + (1 - c)E_{\text{C}}^{\text{LSDA}} + cE_{\text{C}}^{\text{LYP}}. \quad (2.12)$$

The reason B3LYP is the most widely used functional, ever, is that it had perform very well. The functionals developed after B3LYP had the goal of outperforming it. The long-range correction (LC) method can be said to reach this goal. Especially,

the long-range corrected hybrid density functional approximation method. How these methods describe the exchange-correlation functional is described as:

$$E_{\text{XC}}^{\text{LCDF}} = E_{\text{X}}^{\text{SR-DF}}(\omega) + E_{\text{X}}^{\text{LR-HF}}(\omega) + E_{\text{C}}^{\text{DFA}}. \quad (2.13)$$

The long-range correction feature improves the calculations on long-chain polarizability, charge transfer excitations, and optical properties. LC-BLYP [40], CAM-B3LYP [41] and ω B97XD [42] functionals use this approximation.

2.3. Basis Sets

In computational chemistry, basis sets represent electronic wave functions. A finite number of basis functions come together to accurately represent the electronic orbitals of the system. In DFT calculations that do not include transition metals, generally, Slater-type orbitals (STOs) [43] and Gaussian-Type orbitals (GTOs) [44] are enough to describe the orbitals of the system as linear combinations of atomic orbitals (LCAO). Both types of orbitals use coefficients to accurately describe each element's atomic orbitals. Normally, Slater-type orbitals are sufficient to accurately describe an orbital system. But, in these types of bases, the probability of an electron being at position r decreases sharply and results in a cusp in the plot when $r=0$ as can be seen in Figure 2.1. This creates a computational problem, as the first derivative of a cusp is undefined due to the definition of a derivative. When Frank Boys realized this problem of STOs, he figured that the linear combinations of GTOs can be used to express STOs without the computational cusp problem.

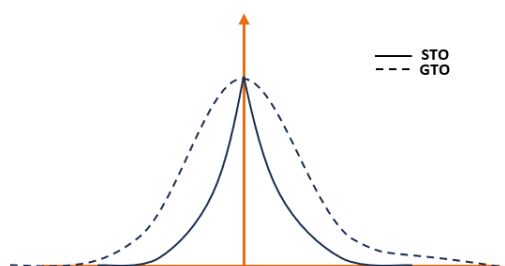


Figure 2.1. STO vs GTO.

To reduce the computational cost, split valance basis sets were developed by John Pople [45]. These types of bases treat the core and valence orbitals separately, describing the core orbitals with a minimalist basis set and using more computational power to accurately describe the valance orbitals. The reason valance shells are treated with more detail is that they are more susceptible to the chemical environment than the core orbitals. Some popular split valance basis sets are 6-31G*, 6-311+G, and 3-21G.

The first number in these expressions is the number of primitive functions describing the core or inner shell orbitals. The numbers following the hyphen refer to the number of Gaussian functions used to describe valance orbitals. The “+” in the representation indicates diffuse functions. Diffuse functions are especially necessary when modeling systems with high electron densities. “+” means heavier atoms are treated with diffuse functions, “++” indicates hydrogens are also treated with diffuse functions. the asterisk “*” represents polarization functions. These functions add a set of higher angular momentum orbital to atoms. Single “*” adds higher angular momentum orbitals only to heavy atoms, whereas, “**” adds them to hydrogen and helium atoms. The asterisk denotation can also be as (d) or (d,p).

2.4. Polarizable Continuum Model

Solvent environments are created by the use of solvation models in computational chemistry. These models aid researchers achieve more accurate results in their studies. The solvation models can be categorized into two: implicit solvation models and explicit solvation models. Explicit models describe each solute molecule individually, which makes the simulation more accurate but the computational cost of these models are usually too high. On the other hand, implicit solvation models describe the solvent environment as a polarizable continuum with a static dielectric constant. These models are usually preferred among the solvation model categories as they are more efficient. The solvation free energy definition of implicit solvation models is given as

$$\Delta G_{\text{solvation}} = \Delta G_{\text{cavity}} + \Delta G_{\text{dispersion}} + \Delta G_{\text{electrostatic}} + \Delta G_{\text{repulsion}}. \quad (2.14)$$

This equation depicts the change in the Gibbs free energy of solvation ($\Delta G_{\text{solvation}}$) as the sum of ΔG_{cavity} meaning the energy required to form cavity inside the solute, $\Delta G_{\text{dispersion}}$ representing the solute-solvent dispersion interactions, electrostatic interactions between solute and solvent ($\Delta G_{\text{electrostatic}}$), and $\Delta G_{\text{repulsion}}$ describing exchange solute-solvent interactions. One of the widely used implicit solvation models is Polarizable Continuum Model (PCM) [46]. It considers all the energies given in Equation (2.14). In this model, cavities are described by van der Waals spheres positioned on individual solute atoms. The reaction field is defined by the polarization charges that are placed on the cavity surface. There are three main approaches to PCM: Dielectric PCM, Conductor-like PCM, and Integral Equation PCM.

2.5. Time-Dependent Density Functional Theory

Time-Dependent Density Functional Theory (TD-DFT) is a way of calculating the excited electronic states. Previously described Hohenberg-Kohn theorems are good for ground-state energies but not excited states [47]. TD-DFT method is based on the theorem proposed by Runge and Gross. This theorem states that for every time-dependent external potential, there exists a unique time-dependent electronic density, while introducing a time-independent constant that affects the mentioned connection.

A system, $|t\rangle$, exposed to an outside potential of $\hat{V}_t$ can be depicted as

$$\left(\hat{H}(t) + \hat{V}_t \right) |(t)\rangle = i \frac{\partial}{\partial t} |t\rangle, \quad (2.15)$$

and the time-dependent KS Hamiltonian is:

$$\hat{H}(t) = \sum_{pq} f_{pq}(t) \hat{E}_{pq}. \quad (2.16)$$

$f_{pq}(t)$ is the Kohn-Sham operator, previously defined in Equation (2.8) but this time because the density is time-dependent, its dependents j_{pq} and $v_{xc,pq}$ terms are also time-dependent. Considering this time dependency, the time-dependent KS operator is depicted as

$$f_{pq}(t) = h_{pq} + j_{pq}(t) + v_{xc,pq}(t). \quad (2.17)$$

In equation (2.17), h_{pq} is the one-electron Hamiltonian integrals, and j_{pq} is the complex

term, which also existed in DFT equations, adding many-body effects into the equation.

2.6. Wigner Distribution Model

Proposed by Eugene Wigner in 1932 [48], the Wigner distribution connects Schrödinger’s wavefunction to the probabilistic nature of quantum systems. In this function, the position and momentum of particles are described in terms of ψ . The Wigner distribution $W(x, p)$ of a pure state is

$$P(x, p) = \frac{1}{\pi\hbar} \int_{-\infty}^{\infty} \psi^*(x+y)\Psi(x-y)e^{2ipy/\hbar} dy, \quad (2.18)$$

where ψ is the wave function, x , and p are the position and momentum of particles, respectively. It should be noted that these terms are exchangeable with any conjugate pair. This equation is symmetric in x and p

$$P(x, p) = \frac{1}{\pi\hbar} \int_{-\infty}^{\infty} \varphi^*(p+q)\Psi(p-q)e^{2ipy/\hbar} dy, \quad (2.19)$$

where φ is the normalized momentum-space function that is proportional to the Fourier transform of ψ . The Wigner distribution becomes:

$$P(\vec{r}, \vec{p}) = \frac{1}{(2\pi)^3} \int \Psi^*(\vec{r} + \frac{\hbar\vec{s}}{2})\Psi(\vec{r} - \frac{\hbar\vec{s}}{2})e^{i\vec{p}\cdot\vec{s}} d^3s \quad (2.20)$$

in 3D. Lastly, the density matrix Wigner distribution in mixed states is:

$$P(x, p) = \frac{1}{\pi\hbar} \int_{-\infty}^{\infty} \langle x+y|\hat{\rho}|x-y\rangle e^{-2ipy/\hbar} dy. \quad (2.21)$$

2.7. Natural Transition Orbitals and ϕ_s Index

When analyzing the transitions to excited states by using methods such as TD-DFT, sometimes it is hard to interpret the exact nature of the transition of electrons from occupied molecular orbitals to virtual molecular orbitals. This is because these transitions are contributed by multiple combinations of occupied and virtual orbitals. One tool to get over this cumbersome analysis is the natural transition orbitals (NTOs). When the rectangular Transition Density Matrix $\mathbf{T}$ is used to express the molecular orbital basis together with the unitary matrices $\mathbf{O}$ and $\mathbf{V}$

$$W = O^\dagger T, \quad V(W)_{ij} = w_i \delta_{ij}. \quad (2.22)$$

Upon performing a rotation over canonical occupied and virtual spaces, the Occupied and Virtual NTOs are obtained as

$$\psi_{\text{ok}} = \sum_{i=1}^N (O)_{ik} c_i \psi_{\text{vk}} = \sum_{k=1}^N \left(\sum_{i=N+1}^K (V)_{ik} c_s \right) \quad (k = 1, \dots, N). \quad (2.23)$$

Usually, one couple of NTOs significantly prevails which makes their interpretation easy and straightforward.

One quantum mechanical descriptor is enough to quantitatively assess the charge-transfer ability of a chromophore. This descriptor is called the ϕ_s index and it quantifies how much the attachment and detachment densities overlap with each other in 3D space.

$$\begin{aligned} \phi_S &= \theta^{-1} \int_{R^3} d^3\xi \, p \sqrt{\varrho\Gamma(\xi)\varrho\Lambda(\xi)} \\ \theta &\equiv \frac{1}{2} \left[\int_{R^3} d^3\xi \sum_{\tau=\Gamma,\Lambda} \varrho_\tau(\xi) \right] \end{aligned} \quad (2.24)$$

$$\phi_S \in [0; 1].$$

Doing an NTO analysis also yields this ϕ_s index. ϕ_s index can have values between 0 and 1. A value close to 0 indicates a local charge transfer while a value near 1 depicts a transition of long-range charge transfer nature.

2.8. Molecular Dynamics Simulations

Molecular dynamics (MD) is a widely used method to observe the behavior of systems for a desired amount of time. During this time, the atoms and molecules in the system are allowed to interact with each other with each having a trajectory. Newton's equations of motion are used to create these trajectories.

In MD simulations, a way to imitate the bulk effect is by using periodic boundary conditions (PBC). The finite box selected for the simulation is infinitely reiterated in all directions throughout space. The use of PBC, therefore, creates an infinite lattice. PBC assumes that a particle in a simulation cell interacts with the particles inside the same simulation cell as well as the adjacent periodic image cells. One limitation of

PBC is its incapability to account for long-range interactions. One way of overcoming this issue of PBC is the particle mesh Ewald method. This method utilizes Fourier transforms to transform the charge distributions from real space to reciprocal space and consequently calculates the long-range electrostatic interactions efficiently.

Solvation models for MD are the same as quantum mechanical solvation models: implicit and explicit. Implicit models increase the accuracy of solvating effects but increase the computation cost exponentially. The explicit solvent model is the more widely used type. Correctly modeling water as a solvent is important in MD; hence there are a variety of different water models. The rigid water models rely on non-bonded interactions, the flexible water model allows the stretching of bonds of water molecules, and polarizable models account for the explicit polarization of water. TIP3P is the most widely used water model due to its high computational efficiency, and it is also used throughout in water-environment-containing simulations described in this dissertation.

3. RESULTS AND DISCUSSION

In pursuit of proposing new PDT photosensitizers, the designed molecules were thoroughly investigated by the tools computational chemistry has to offer. To gain insight into the photochemistry of the PS molecules, various theoretic methods were employed.

3.1. Computational Protocol

The ground state geometry optimizations were carried out with DFT and at the M06-2X/6-31+G** level of theory for each molecule. To model vertical excitations TD-DFT was employed. A benchmark was performed to determine the best functional to describe the systems with CAM-B3LYP, LC-BLYP, and ω B97X-D functionals. Previous studies suggested ω B97X-D was the most favorable functional to describe excited states of chlorin and porphyrin derivatives [22, 34, 49] and the results obtained from our benchmark did not yield any contrary results. Therefore, all the later excited-state calculations were performed with it except the TPA calculation. All DFT and TD-DFT calculations were executed with Gaussian 16 software [50]. The solvent effects were mimicked using the integral equation formalism polarizable continuum model [51](IEF-PCM). Fluorescence spectra were simulated by optimizing the S_1 geometries at ω B97X-D/6-31+G** level of theory.

Nexton-X software package [52] was used to create 100 geometries around the zero-point energy of the PSs' ground states. Amsterdam Modeling Suite AMS/2022.103 [53, 54] was employed for energy state and SOCC calculations. These calculations were conducted on a set of 20 geometries that were randomly selected out of 100 initial conditions. ω B97X-D/DZP level of theory was used for the calculations performed with AMS package. Natural Transition Orbital Analysis was performed to figure out the charge transfer characters of the transitions to the excited states by employing the NANCYEX2.0 code [55, 56].

To calculate the two-photon absorption strengths, the publicly available Dalton20 code was utilized [57]. Due to the lack of ω B97X-D functional in this program, CAM-B3LYP functional was used in calculations together with 6-31+G* basis set.

Finally, for the MD simulations, Amber20 program package was used [58]. The Antechamber program [59] and Generalized Force Field (GAFF) [60] was employed to create customized force fields for the candidate PS molecules. Lipid17 [61] and GLYCAM06j [62] force fields were used to describe the lipid membrane and cyclodextrin molecules respectively. The water environments were created with TIP3P [63] water model. For root-mean-square deviation (RMSD) and distance analyses Cpptraj feature of Amber package was used [64].

3.2. Geometry Optimizations

Geometry optimizations of candidate photosensitizers are performed using the DFT method. The ground states were optimized both in the gas phase and in water (PCM) at the M06-2X/6-31+G** level of theory. The results are reported in Figures 3.1 and 3.2, respectively. In all geometry optimizations, the chlorin core of the temoporfin molecule kept its planarity, while the dihedral angle at which the phenol groups on the chlorin ring changed depending on the molecule, environment, and excitation state.

The rotation of the electron-rich phenolic groups on the temoporfin molecule affects its overall molecular structure and they contribute to its interactions with the biological environments. The non-substituted temoporfin molecule has its phenolic groups almost orthogonal to its chlorin ring. This trend is continued in these PS molecules as well. However, the influence of solvent presence and energy state influences these molecules at different rates. For example, as can be seen in Figures 3.1 and 3.2E, the DPP antenna group is more rigid in gas and water environments compared to DTP derivatives. This rigidity leads to stable dihedral angles of the phenol groups; both in S_0 and S_1 states. For DTP2-mTHPC molecule, solvation does not significantly impact the positions of neither the phenol groups nor the DTP2 group. Contrarily, the S_0 and

S_1 geometries have a significant difference in terms of the positions of these groups and these can be observed in Figures 3.2 E and F. This transition results in the thiophene moiety and its attached chain on DTP2 group approaches orthogonality with respect to temoporfin. DTP1-mTHPC undergoes the most significant changes in geometry upon changes in solvent or energy state. Dihedral angles showcased in Figures 3.1 and 3.2A present a 10 degree difference when the environment changes from vacuum to water. Figures 3.2 A and B depict the change in geometry as the dihedral angle deviates from orthogonality, transitioning from the S_0 to S_1 geometry. This transition also introduces a bend in the $R-C\equiv C-R'$ group which connects the antenna group to temoporfin. Also, when considering this transition, the change in proximity of the hexyl chain on DTP1 to temoporfin's phenolic group can be noted.

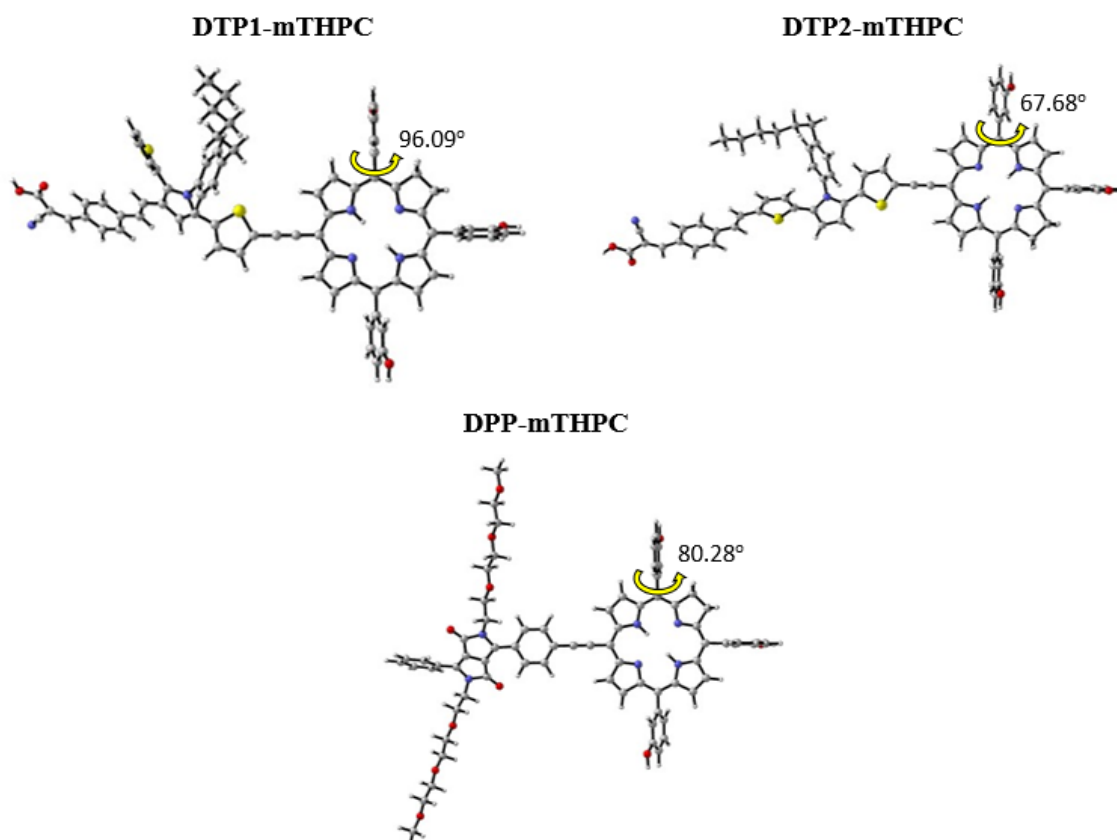


Figure 3.1. Vacuum optimized geometries of the dye - antenna attached temoporfin molecules in their ground state (S_0 .)

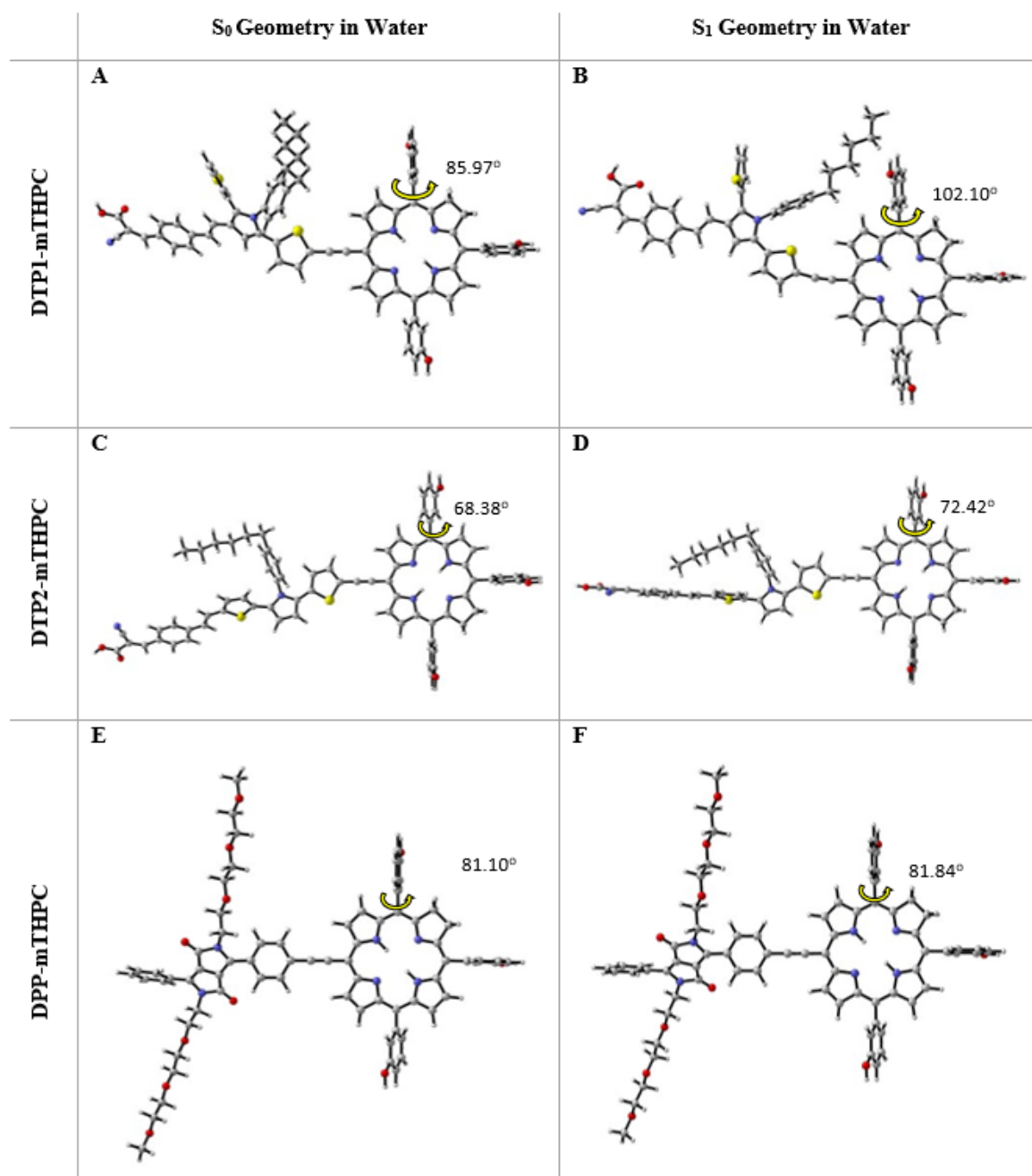


Figure 3.2. Optimized geometries of the dye - antenna attached temoporfin molecules in their ground state (S_0) and first excited singlet state (S_1) in water (PCM).

3.3. Vertical Excitations

To evaluate the effects of how modifying temoporfin molecule had altered its photophysical properties, one and two-photon absorption spectra of proposed molecules are explored. A dynamic approach by Wigner distribution was achieved, and considering several geometries situated around ZPE has increased the precision of the produced results. With this dynamic approach, a benchmark of functionals was carried out. 40 geometries of each molecule were randomly sampled from 100 initial conditions created on the ground state energy surface and three functionals were used to create absorption spectra of each molecule. The benchmark involved CAM-B3LYP, LC-BLYP and ω -B97X-D functionals and its results are reported in Figure 3.3.

In these benchmarks, it was observed that CAM-B3LYP and ω -B97X-D functionals created similar absorption spectra while LC-BLYP functional created blue-shifted spectra for every molecule. Upon assessing the results of this benchmark, it was decided to remove the LC-BLYP functional from consideration as it could not reproduce the signature absorption peak of temoporfin 652 nm with the desired resolution. Between the other two functionals, due to its wide use in literature to describe the excited state of molecules, it was decided to carry on later calculations with ω -B97X-D functional. Also, previous computational work analyzing temoporfin [22] and DTP derivative molecules [33, 34] both had used this functional and were able to recreate experimental results.

The electronic structure program Dalton2020 was used to calculate the TPA spectra. This program does not contain the ω -B97X-D. A literature search was conducted to decide the computational method for this calculation. Benchmarks from previous work [65, 66] indicate that CAM-B3LYP is the best compromise between computational cost and ability to recreate experimental results among the available DFT functionals in Dalton2020 program. Based upon these benchmarks, and the similar results for OPA spectra yielded by CAM-B3LYP and ω -B97X-D functionals the TPA calculations were carried out using CAM-B3LYP functional.

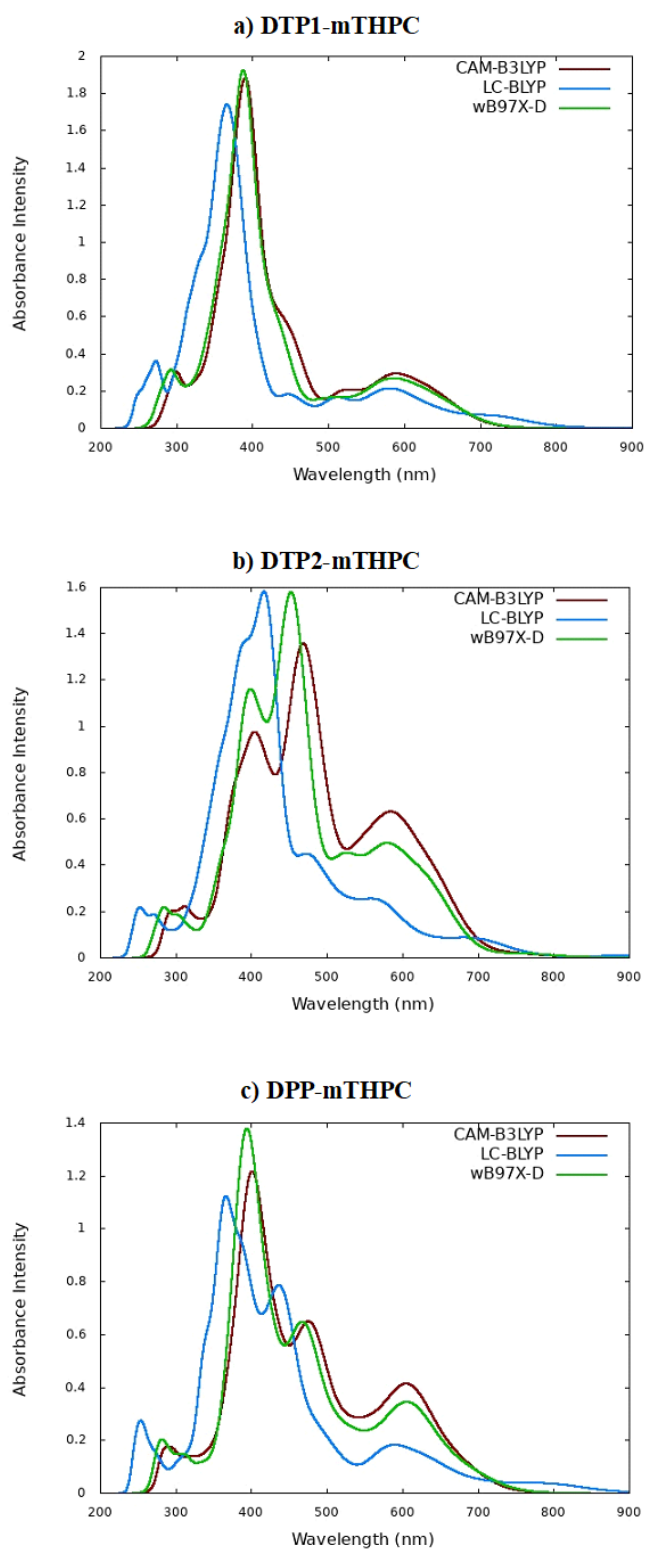


Figure 3.3. TD-DFT functional benchmark with CAM-B3LYP, LC-BLYP and ω -B97X-D functionals for a) DTP1-mTHPC, b) DTP2-mTHPC and c) DPP-mTHPC molecules.

Moving on to the calculations, a bargain between computational cost and precision must be made. Clearly, the larger the sample size -in this case, the number of initial conditions considered- the more precise the results. A benchmark was carried out to compare how decreasing the sample size affected the results so that there could be an agreement between precision and computational cost. The results were reported in Figure 3.4, and it was seen that decreasing the number of geometries did not change the overall pattern for absorption spectra, despite some differences. 20 and 10 initial conditions were found sufficient to reproduce a credible electronic spectrum, henceforth OPA and TPA calculations are carried out using 20 and 10 initial conditions, respectively.

In Figure 3.5, the single-point calculation results of OPA spectra in vacuum and in water are demonstrated. This comparison is made to observe the effect of solvation on the absorption spectra. The environment did not induce a significant shape or position change in molecules' spectra. The OPA strength seems to be quenched by the presence of water in DTP derivatives. Conversely, for the DPP-mTHPC molecule, water environment was found to enhance the OPA strength.

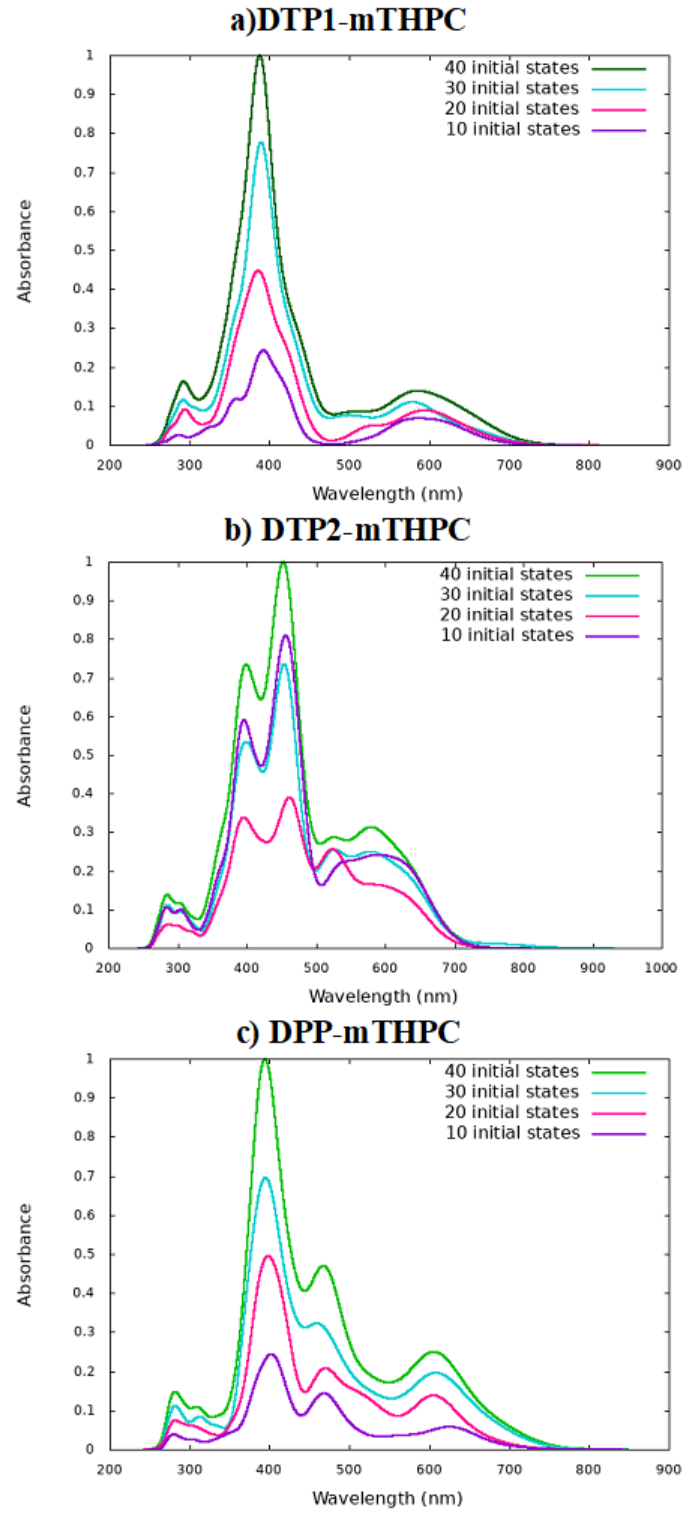


Figure 3.4. Benchmark of initial conditions at the ω B97X-D/6-31+G(d,p) level of theory.

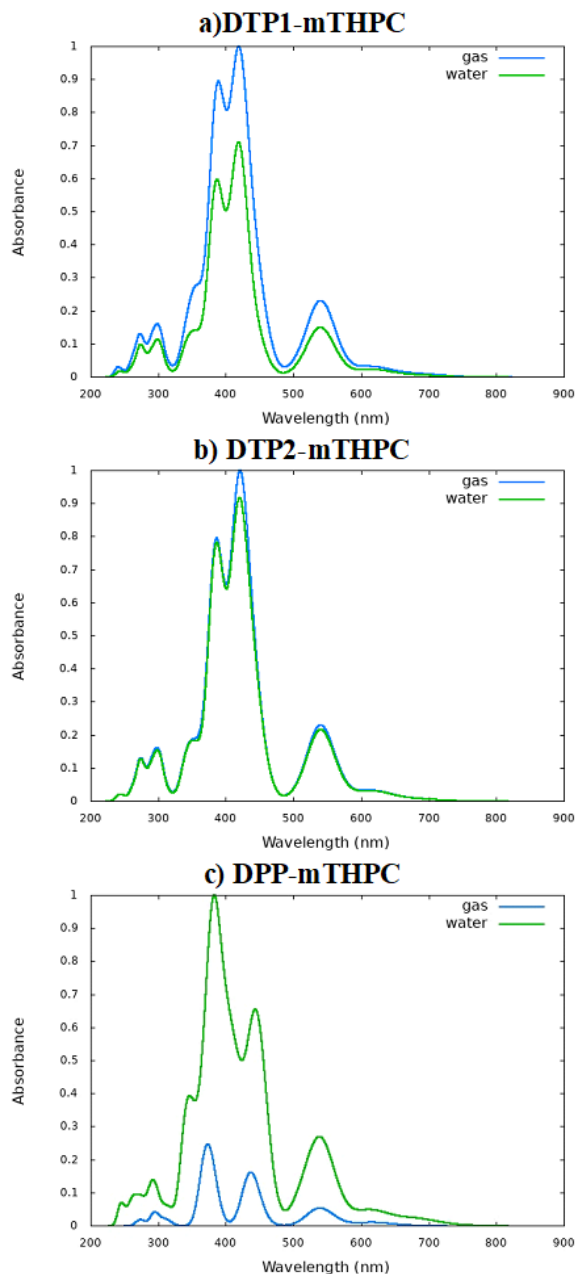


Figure 3.5. Proposed molecules' OPA spectra in vacuum and in PCM water environment. Single point calculations at the ω B97X-D/6-31+G(d,p) level of theory.

The superimposed OPA spectra of all three PS molecules in water environment and, for comparison, the OPA spectrum of unmodified temoporfin molecule calculated at the ω B97X-D level are reported in Figure 3.6. In Table 3.1, The $\lambda_{\max}$ values for each molecule are reported. The values reported for mTHPC are experimental values [67]

and their corresponding σ_{TPA} 's are reported in Table 3.1. Upon calculation with 40 initial conformations, the results show that modifying the temoporfin molecule with dye groups has caused a blue shift to the Q-band around 24.8 eV for each molecule. This may be due to the redistribution of electron density throughout the molecules resulting from the extended chain conjugation.

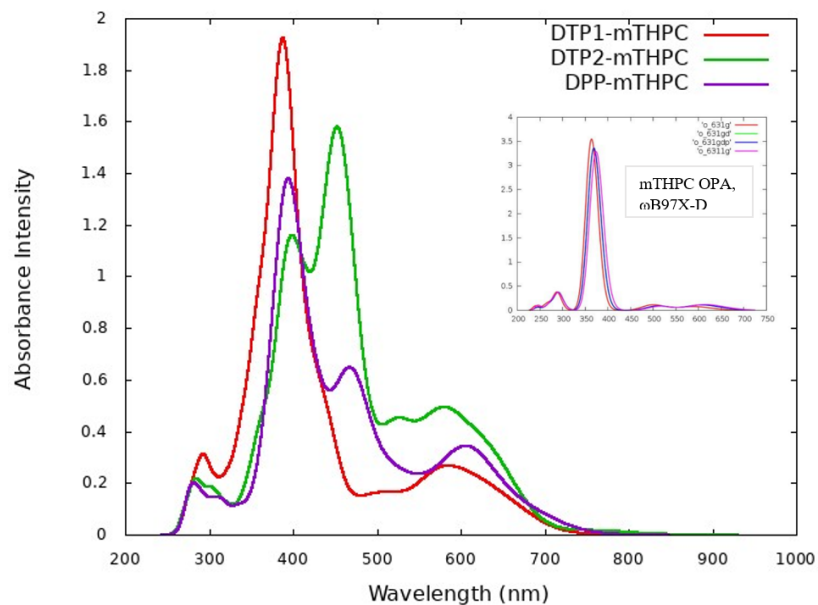


Figure 3.6. OPA calculated in water and from 40 initial conditions. TD-DFT, ω B97X-D/6-31+G(d,p) level of theory. OPA spectrum of unmodified mTHPC is also provided for comparison.

Table 3.1. The wavelengths in nanometers at which the molecules' absorbance of one photon and emission is strongest.

$\lambda_{\text{max}}(\text{nm})$	mTHPC	DTP1-mTHPC	DTP2-mTHPC	DPP-mTHPC
OPA	420	388	453	394
	650	586	580	605

The superimposed TPA graphs are presented in Figure 3.7 and in Table 3.2 maximum values of σ_{TPA} in Göppert-Mayer ($1 \text{ GM} = 10^{-50} \text{ cm}^4 \text{ photon}^{-1}$) for the modeled molecules are summarized. In Table 3.2, experimentally found TPA cross section of unmodified temoporfin is also included. [28] Prior studies which focused on increasing

σ_{TPA} of systems showed that modifications done on the parent molecule, such as the addition of carbonyl groups, alkyne linkers, and high two-photon absorbing molecules had a significant effect on increasing the TPA absorption [31, 35, 68, 69]. Findings of this work show that this case is no different. Our results show remarkable increases of σ_{TPA} for each antenna-mTHPC system compared to the unmodified mTHPC. The addition of DTP1, DTP2, and DPP groups has not only increased the σ_{TPA} values immensely but also λ_{max} value of TPA is seen in the therapeutic window, perfect for efficient and deep skin penetration.

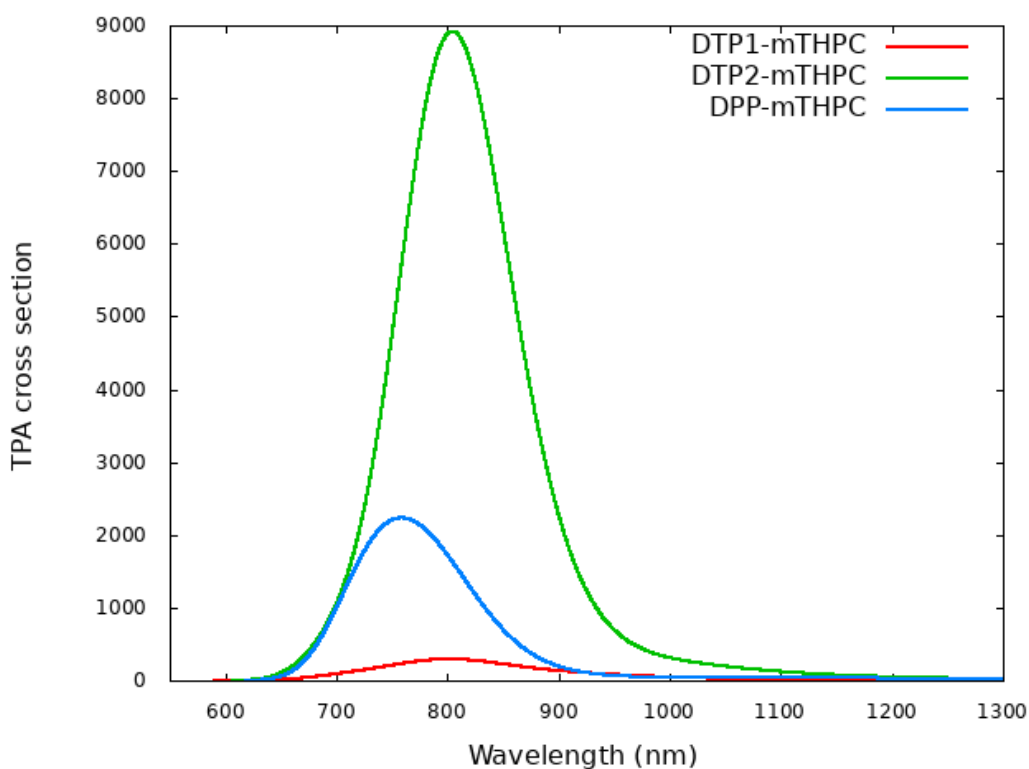


Figure 3.7. Superimposed two-photon absorption spectra of molecules calculated at the CAM-B3LYP/6-31+G(d) level of theory.

Table 3.2. λ_{max} and the corresponding TPA cross section values of molecules.

	$\lambda_{max}(\text{nm})$	$\sigma_{TPA}(\text{GM})$
mTHPC	775	28 ± 8
DTP1-mTHPC	801	295
DTP2-mTHPC	804	8917
DPP-mTHPC	759	2235

3.4. Energy States and Intersystem Crossing Pathways

To obtain insight into the nature of the transition from ground states to singlet excited states, NTOs of $S_0 \rightarrow S_n$ transitions for the first four singlet excited states were calculated. Occupied NTOs (oNTO) and Virtual NTOs (vNTO) of DTP1-mTHPC, DTP2-mTHPC, and DPP-mTHPC molecules are reported together with the corresponding Φ_s and oscillator strength values in Tables 3.3, 3.4 and 3.5, respectively. In short, the calculation yielded the results that were expected. The charge transfer nature of these transitions was found to be local $\pi \rightarrow \pi^*$ which is apparent from the near 1 value of Φ_s values. The Φ_s value quantifies the overlap between electron density transferred from the ground state and repositioned in the excited state. Near 1 Φ_s value indicates that the electron density has not dramatically changed positions. This can also be seen in the figures describing NTOs in the respective figures. Also, the non-zero oscillation values signify that these first four singlet excited states can all be populated, some more efficiently than others, and there are no dark states. An anecdotal point can be made such that the orthogonal phenolic groups of the mTHPC molecule and p-(hexyl)phenyl groups do not contribute to the electronic transitions for simply they are positioned orthogonally to the rest of the π -conjugated chromophore.

Table 3.3. Natural Transition Orbitals (NTOs), Φ_s values, oscillation strengths of DTP1-mTHPC from ground state to the first four singlet excited states.

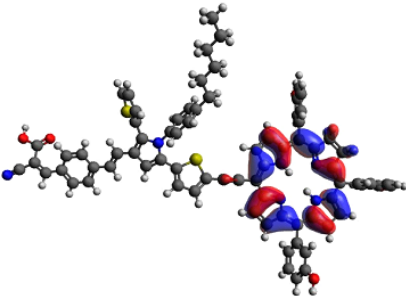
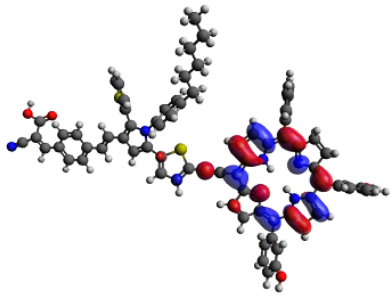
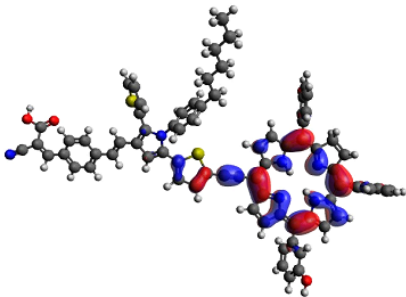
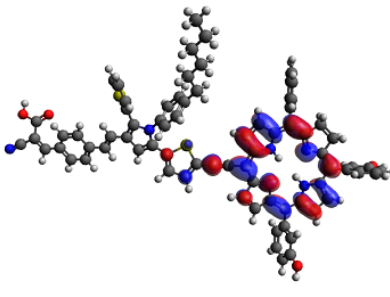
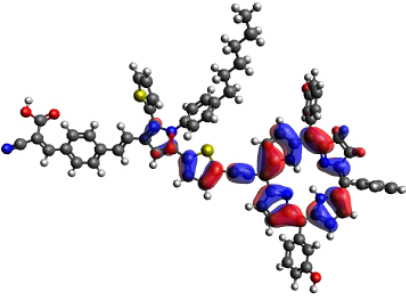
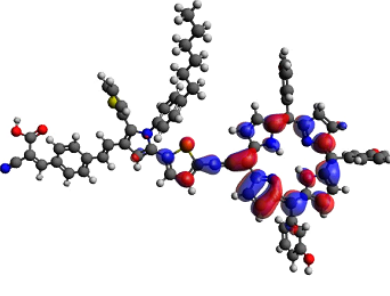
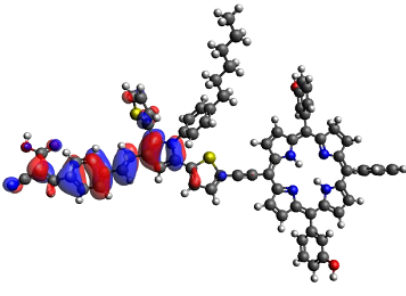
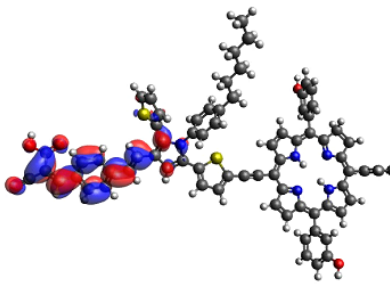
DTP1-mTHPC	oNTO	vNTO
$S_0 \rightarrow S_1$ $\Phi_s \text{ Lowdin} = 0.9635$ $\Phi_s \text{ Mulliken} = 1.0127$ $f = 0.0687$ 1.9988 eV		
$S_0 \rightarrow S_2$ $\Phi_s \text{ Lowdin} = 0.9541$ $\Phi_s \text{ Mulliken} = 1.0204$ $f = 0.4969$ 2.2934 eV		
$S_0 \rightarrow S_3$ $\Phi_s \text{ Lowdin} = 0.9117$ $\Phi_s \text{ Mulliken} = 0.9669$ $f = 3.0233$ 2.9554 eV		
$S_0 \rightarrow S_4$ $\Phi_s \text{ Lowdin} = 0.8523$ $\Phi_s \text{ Mulliken} = 0.9065$ $f = 0.5730$ 3.1714 eV		

Table 3.4. Natural Transition Orbitals (NTOs), Φ_s values, oscillation strengths of DTP2-mTHPC from ground state to the first four singlet excited states.

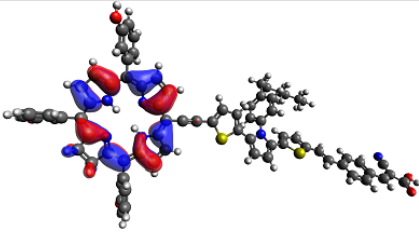
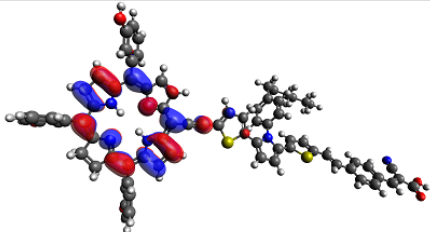
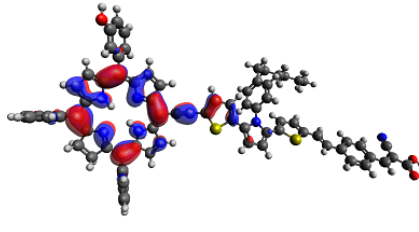
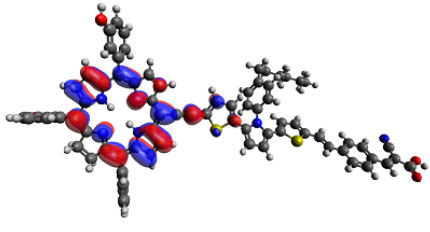
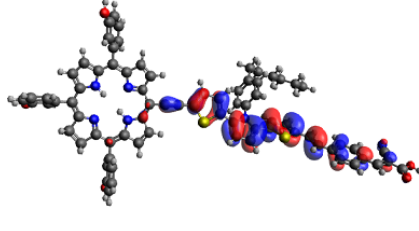
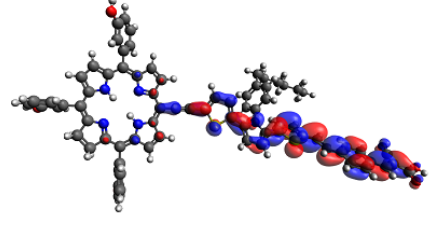
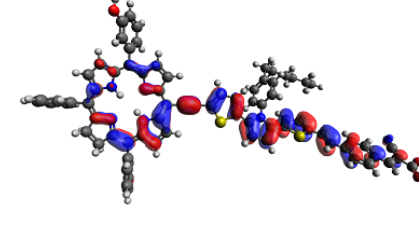
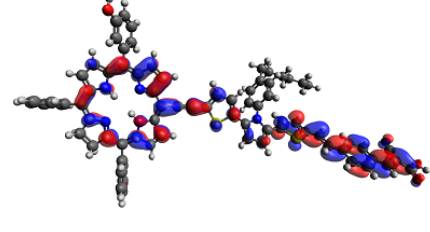
DTP2-mTHPC	oNTO	vNTO
$S_0 \rightarrow S_1$ $\Phi_{S\text{Lowdin}} = 0.9652$ $\Phi_{S\text{Mulliken}} = 1.0115$ $f = 0.0556$ 2.01eV		
$S_0 \rightarrow S_2$ $\Phi_{S\text{Lowdin}} = 0.9572$ $\Phi_{S\text{Mulliken}} = 1.0205$ $f = 0.5528$ 2.30 eV		
$S_0 \rightarrow S_3$ $\Phi_{S\text{Lowdin}} = 0.9035$ $\Phi_{S\text{Mulliken}} = 0.9468$ $f = 3.5579$ 2.91eV		
$S_0 \rightarrow S_4$ $\Phi_{S\text{Lowdin}} = 0.9204$ $\Phi_{S\text{Mulliken}} = 0.9545$ $f = 0.2093$ 3.20eV		

Table 3.5. Natural Transition Orbitals (NTOs), Φ_s values, oscillation strengths of DPP-mTHPC from ground state to the first four singlet excited states.

DPP-mTHPC	oNTO	vNTO
$S_0 \rightarrow S_1$ $\Phi_S \text{ Lowdin} = 0.9593$ $\Phi_S \text{ Mulliken} = 1.0132$ $f = 0.1086$ 1.9967 eV		
$S_0 \rightarrow S_2$ $\Phi_S \text{ Lowdin} = 0.9583$ $\Phi_S \text{ Mulliken} = 1.0213$ $f = 0.4989$ 2.2880 eV		
$S_0 \rightarrow S_3$ $\Phi_S \text{ Lowdin} = 0.9291$ $\Phi_S \text{ Mulliken} = 0.960$ $f = 1.4831$ 2.7816 eV		
$S_0 \rightarrow S_4$ $\Phi_S \text{ Lowdin} = 0.9399$ $\Phi_S \text{ Mulliken} = 0.9881$ $f = 1.1988$ 3.1419 eV		

The photodynamic therapy mechanism requires the production of cytotoxic singlet oxygen in order to destroy the tumorous area. The energy needed to create singlet oxygen from its triplet molecular form is 0.98 eV. This is why any proposed photosensitizer molecule must have a populatable T_1 state that is of higher energy than 0.98 eV. To ensure our candidate molecules meet this requirement, energy levels of the first 10 singlet and 10 triplet states are calculated for all three PSs. To be able to comment on the possibility of $S \rightarrow T$ transition path, SOCCs are also calculated for each molecule. Both these calculations are carried out from 20 starting conditions sampled around the ZPE of S_0 state. In Figure 3.8, and Tables 6-11; detailed energy level diagrams, S-T energy gap values, and SOCC values are reported, respectively.

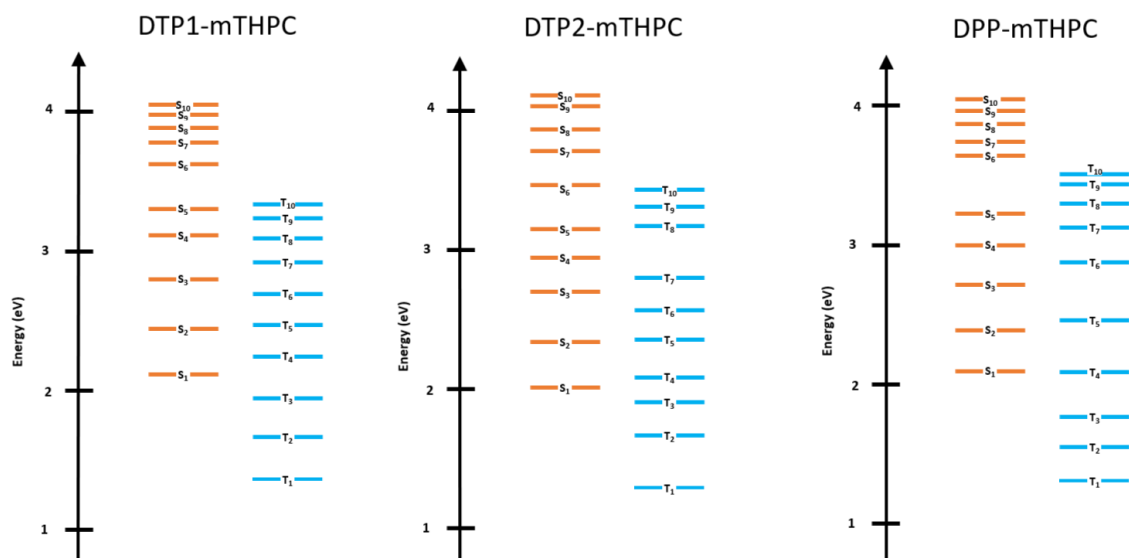


Figure 3.8. To scale energy level diagrams of a) DTP1-mTHPC, b) DTP2-mTHPC, and c) DPP-mTHPC molecules in water at ω B97X-D/ DZP level of theory calculated from S_0

Table 3.6. S-T energy gaps calculated from S_0 level for DTP1-mTHPC molecule at ω B97X-D/DZP level of theory.

ST Energy Gap from S ₀ Geometry											
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	
DTP1-mTHPC	T ₁	0.756 ± 0.19	1.078 ± 0.21	1.439 ± 0.32	1.753 ± 0.20	1.946 ± 0.22	2.263 ± 0.30	2.421 ± 0.30	2.523 ± 0.28	2.620 ± 0.28	2.688 ± 0.27
	T ₂	0.450 ± 0.21	0.773 ± 0.23	1.133 ± 0.34	1.447 ± 0.22	1.640 ± 0.24	1.958 ± 0.31	2.116 ± 0.32	2.218 ± 0.30	2.314 ± 0.29	2.382 ± 0.28
	T ₃	0.170 ± 0.25	0.496 ± 0.27	0.853 ± 0.36	1.167 ± 0.26	1.360 ± 0.28	1.678 ± 0.34	1.835 ± 0.35	1.938 ± 0.33	2.034 ± 0.33	2.102 ± 0.32
	T ₄	-0.127 ± 0.29	0.196 ± 0.25	0.556 ± 0.35	0.870 ± 0.24	1.063 ± 0.26	1.380 ± 0.32	1.538 ± 0.33	1.640 ± 0.31	1.737 ± 0.31	1.805 ± 0.30
	T ₅	-0.351 ± 0.17	-0.028 ± 0.20	0.332 ± 0.31	0.646 ± 0.19	0.839 ± 0.21	1.156 ± 0.29	1.314 ± 0.30	1.416 ± 0.28	1.513 ± 0.27	1.581 ± 0.27
	T ₆	-0.581 ± 0.25	-0.258 ± 0.27	0.102 ± 0.36	0.416 ± 0.26	0.609 ± 0.28	0.927 ± 0.34	1.085 ± 0.35	1.187 ± 0.33	1.284 ± 0.33	1.351 ± 0.32
	T ₇	-0.803 ± 0.28	-0.481 ± 0.29	-0.120 ± 0.38	0.194 ± 0.28	0.386 ± 0.30	0.704 ± 0.36	0.862 ± 0.36	0.964 ± 0.35	1.061 ± 0.34	1.129 ± 0.33
	T ₈	-0.971 ± 0.26	-0.649 ± 0.28	-0.288 ± 0.37	0.026 ± 0.27	0.218 ± 0.28	0.536 ± 0.35	0.694 ± 0.35	0.796 ± 0.34	0.893 ± 0.33	0.961 ± 0.20
	T ₉	-1.118 ± 0.22	-0.795 ± 0.24	-0.434 ± 0.34	-0.120 ± 0.23	0.072 ± 0.25	0.390 ± 0.32	0.548 ± 0.32	0.650 ± 0.31	0.747 ± 0.30	0.814 ± 0.29
	T ₁₀	-1.218 ± 0.23	-0.895 ± 0.24	-0.535 ± 0.351	-0.221 ± 0.24	-0.028 ± 0.25	0.290 ± 0.32	0.448 ± 0.33	0.550 ± 0.31	0.646 ± 0.30	0.714 ± 0.29

Table 3.7. S-T energy gaps calculated from S_0 level for DTP2-mTHPC molecule at ω B97X-D/DZP level of theory.

ST Energy Gap from S ₀ Geometry											
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	
DTP2-mTHPC	T ₁	0.722 ± 0.37	1.049 ± 1.30	1.407 ± 0.37	1.652 ± 0.31	1.860 ± 0.32	2.145 ± 0.33	2.419 ± 0.34	2.576 ± 0.34	2.738 ± 0.33	2.818 ± 0.33
	T ₂	0.344 ± 0.29	0.672 ± 1.68	1.030 ± 0.29	1.275 ± 0.21	1.483 ± 0.23	1.768 ± 0.24	2.042 ± 0.24	2.199 ± 0.24	2.361 ± 0.24	2.441 ± 0.24
	T ₃	0.108 ± 0.28	0.436 ± 1.91	0.794 ± 0.28	1.039 ± 0.20	1.247 ± 0.22	1.532 ± 0.23	1.808 ± 0.23	1.963 ± 0.23	2.125 ± 0.23	2.205 ± 0.23
	T ₄	-0.075 ± 0.24	0.252 ± 2.10	0.611 ± 0.24	0.856 ± 0.14	1.064 ± 0.16	1.349 ± 0.18	1.622 ± 0.18	1.779 ± 0.18	1.941 ± 0.18	2.022 ± 0.18
	T ₅	-0.343 ± 0.24	-0.015 ± 2.36	0.343 ± 0.24	0.588 ± 0.14	0.796 ± 0.16	1.081 ± 0.18	1.354 ± 0.18	1.512 ± 0.18	1.674 ± 0.18	1.754 ± 0.18
	T ₆	-0.553 ± 0.26	-0.226 ± 2.57	0.132 ± 0.26	0.377 ± 0.17	0.585 ± 0.19	0.870 ± 0.20	1.144 ± 0.21	1.301 ± 0.21	1.463 ± 0.21	1.543 ± 0.20
	T ₇	-0.788 ± 0.25	-0.461 ± 2.81	-0.103 ± 0.26	0.142 ± 0.16	0.350 ± 0.18	0.635 ± 0.20	0.909 ± 0.20	1.066 ± 0.20	1.228 ± 0.20	1.308 ± 0.20
	T ₈	-1.155 ± 0.26	-0.828 ± 3.17	-0.470 ± 0.26	-0.225 ± 0.17	-0.017 ± 0.19	0.268 ± 0.20	0.541 ± 0.20	0.699 ± 0.21	0.861 ± 0.20	0.941 ± 0.20
	T ₉	-1.299 ± 0.24	-0.971 ± 3.32	-0.613 ± 0.24	-0.368 ± 0.14	-0.160 ± 0.17	0.125 ± 0.19	0.399 ± 0.19	0.556 ± 0.19	0.718 ± 0.19	0.798 ± 0.18
	T ₁₀	-1.420 ± 0.25	-1.092 ± 3.44	-0.734 ± 0.25	-0.489 ± 0.15	-0.281 ± 0.18	0.004 ± 0.19	0.278 ± 0.19	0.435 ± 0.19	0.597 ± 0.19	0.677 ± 0.19

Table 3.8. S-T energy gaps calculated from S_0 level for DPP-mTHPC molecule at ω B97X-D/DZP level of theory.

ST Energy Gap from S ₀ Geometry											
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇	S ₈	S ₉	S ₁₀	
DPP-mTHPC	T ₁	0.788 ± 0.15	1.082 ± 0.18	1.412 ± 0.19	1.693 ± 0.15	1.922 ± 0.17	2.333 ± 0.17	2.435 ± 0.19	2.565 ± 0.19	2.658 ± 0.19	2.742 ± 0.19
	T ₂	0.544 ± 0.17	0.838 ± 0.20	1.167 ± 0.21	1.448 ± 0.17	1.678 ± 0.19	2.088 ± 0.19	2.191 ± 0.20	2.320 ± 0.20	2.413 ± 0.21	2.497 ± 0.21
	T ₃	0.329 ± 0.15	0.623 ± 0.18	0.952 ± 0.19	1.234 ± 0.15	1.463 ± 0.17	1.874 ± 0.17	1.976 ± 0.18	2.106 ± 0.18	2.199 ± 0.19	2.283 ± 0.19
	T ₄	0.010 ± 0.15	0.304 ± 0.18	0.634 ± 0.19	0.915 ± 0.14	1.144 ± 0.16	1.56 ± 0.16	1.657 ± 0.18	1.787 ± 0.18	1.880 ± 0.19	1.964 ± 0.19
	T ₅	-0.364 ± 0.15	-0.070 ± 0.18	0.259 ± 0.19	0.540 ± 0.15	0.770 ± 0.17	1.180 ± 0.17	1.283 ± 0.19	1.412 ± 0.19	1.505 ± 0.19	1.589 ± 0.20
	T ₆	-0.783 ± 0.16	-0.489 ± 0.19	-0.160 ± 0.20	0.122 ± 0.15	0.351 ± 0.17	0.762 ± 0.17	0.864 ± 0.19	0.993 ± 0.19	1.086 ± 0.20	1.170 ± 0.20
	T ₇	-1.033 ± 0.17	-0.739 ± 0.20	-0.410 ± 0.21	-0.128 ± 0.17	0.100 ± 0.19	0.511 ± 0.19	0.614 ± 0.20	0.743 ± 0.20	0.836 ± 0.21	0.920 ± 0.21
	T ₈	-1.206 ± 0.14	-0.912 ± 0.17	-0.583 ± 0.18	-0.301 ± 0.14	-0.072 ± 0.16	0.339 ± 0.16	0.441 ± 0.18	0.570 ± 0.18	0.663 ± 0.18	0.748 ± 0.18
	T ₉	-1.343 ± 0.13	-1.049 ± 0.17	-0.720 ± 0.18	-0.438 ± 0.13	-0.209 ± 0.15	0.202 ± 0.15	0.304 ± 0.17	0.434 ± 0.17	0.527 ± 0.18	0.611 ± 0.18
	T ₁₀	0.788 ± 0.15	1.082 ± 0.18	1.412 ± 0.19	1.693 ± 0.15	1.922 ± 0.17	2.333 ± 0.17	2.435 ± 0.19	2.565 ± 0.19	2.658 ± 0.19	2.742 ± 0.19

Table 3.9. Spin-orbit coupling constants values calculated from S_0 level for DTP1-mTHPC molecule at ω B97X-D/DZP level of theory.

SOCC from S ₀ Geometry											
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	
DTP1-mTHPC	S ₁	0.128 ± 0.09	0.210 ± 0.28	0.136 ± 0.17	0.204 ± 0.15	0.091 ± 0.13	0.111 ± 0.15	0.064 ± 0.08	0.097 ± 0.14	0.182 ± 0.26	0.249 ± 0.36
	S ₂	0.263 ± 0.20	0.128 ± 0.11	0.126 ± 0.16	0.134 ± 0.14	0.158 ± 0.21	0.216 ± 0.25	0.111 ± 0.14	0.166 ± 0.19	0.222 ± 0.21	0.188 ± 0.17
	S ₃	0.164 ± 0.21	0.129 ± 0.17	0.086 ± 0.07	0.108 ± 0.14	0.116 ± 0.19	0.200 ± 0.45	0.354 ± 0.66	0.490 ± 0.80	0.250 ± 0.58	0.222 ± 0.18
	S ₄	0.174 ± 0.10	0.112 ± 0.10	0.077 ± 0.08	0.132 ± 0.14	0.166 ± 0.15	0.156 ± 0.15	0.182 ± 0.18	0.264 ± 0.24	0.170 ± 0.14	0.256 ± 0.32
	S ₅	0.217 ± 0.20	0.164 ± 0.20	0.116 ± 0.14	0.193 ± 0.18	0.291 ± 0.42	0.240 ± 0.32	0.390 ± 0.87	0.252 ± 0.78	0.278 ± 0.38	0.342 ± 0.74
	S ₆	0.705 ± 0.88	0.572 ± 0.97	0.330 ± 0.56	0.555 ± 1.05	0.494 ± 1.0	0.956 ± 2.01	0.284 ± 0.31	0.520 ± 0.86	0.382 ± 0.58	0.411 ± 0.87
	S ₇	0.588 ± 0.51	0.856 ± 1.01	0.422 ± 0.61	0.480 ± 0.46	0.534 ± 0.86	0.649 ± 1.10	0.840 ± 1.26	1.241 ± 1.61	0.548 ± 1.21	0.620 ± 0.74
	S ₈	0.610 ± 0.69	0.853 ± 1.29	0.464 ± 0.67	0.434 ± 0.59	0.463 ± 0.63	0.584 ± 1.09	0.532 ± 0.89	0.465 ± 0.91	0.596 ± 0.60	0.366 ± 0.35
	S ₉	0.981 ± 1.75	0.806 ± 0.90	0.466 ± 0.48	1.122 ± 2.36	0.380 ± 0.43	0.913 ± 1.16	0.800 ± 1.59	0.748 ± 1.29	0.589 ± 0.92	0.590 ± 1.11
	S ₁₀	0.908 ± 0.80	0.611 ± 0.71	0.634 ± 0.91	1.108 ± 1.50	0.470 ± 0.81	0.272 ± 0.31	0.694 ± 0.67	1.689 ± 2.21	0.780 ± 1.22	0.660 ± 1.05

Table 3.10. Spin-orbit coupling constants values calculated from S_0 level for DTP2-mTHPC molecule at ω B97X-D/DZP level of theory.

DTP2-mTHPC	SOCC from S ₀ Geometry										
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	
	S ₁	0.110 ± 0.11	0.252 ± 0.19	0.136 ± 0.14	0.170 ± 0.11	0.196 ± 0.15	0.170 ± 0.15	0.210 ± 0.17	0.316 ± 0.19	0.232 ± 0.18	0.386 ± 0.36
	S ₂	0.278 ± 0.15	0.093 ± 0.08	0.128 ± 0.07	0.112 ± 0.10	0.230 ± 0.18	0.252 ± 0.17	0.289 ± 0.20	0.350 ± 0.28	0.368 ± 0.29	0.646 ± 0.61
	S ₃	0.125 ± 0.11	0.136 ± 0.10	0.207 ± 0.12	0.202 ± 0.18	0.411 ± 0.40	0.416 ± 0.33	0.850 ± 0.47	0.465 ± 0.29	0.650 ± 0.68	0.636 ± 0.55
	S ₄	0.194 ± 0.12	0.176 ± 0.11	0.226 ± 0.18	0.220 ± 0.13	0.396 ± 0.32	0.318 ± 0.27	0.508 ± 0.39	0.416 ± 0.30	0.384 ± 0.33	0.488 ± 0.35
	S ₅	0.202 ± 0.14	0.205 ± 0.13	0.317 ± 0.40	0.260 ± 0.24	0.260 ± 0.25	0.216 ± 0.15	0.368 ± 0.29	0.375 ± 0.37	0.360 ± 0.27	0.372 ± 0.35
	S ₆	0.292 ± 0.24	0.268 ± 0.23	0.424 ± 0.29	0.419 ± 0.31	0.346 ± 0.22	0.422 ± 0.20	0.773 ± 0.43	0.646 ± 0.56	0.801 ± 0.92	0.492 ± 0.27
	S ₇	0.490 ± 0.27	0.488 ± 0.25	0.474 ± 0.39	0.502 ± 0.40	0.916 ± 0.63	0.688 ± 0.54	0.804 ± 0.52	0.631 ± 0.32	0.660 ± 0.42	0.584 ± 0.48
	S ₈	0.928 ± 1.12	0.945 ± 1.00	0.506 ± 0.82	0.391 ± 0.38	0.534 ± 0.40	0.662 ± 0.71	0.534 ± 0.42	0.650 ± 0.55	0.816 ± 0.58	0.780 ± 0.96
	S ₉	1.357 ± 1.53	1.457 ± 1.78	0.916 ± 0.90	0.708 ± 0.57	1.010 ± 0.81	0.758 ± 0.64	0.924 ± 0.82	1.026 ± 1.29	0.822 ± 0.76	0.798 ± 0.83
	S ₁₀	0.886 ± 0.94	0.913 ± 0.48	0.868 ± 0.60	0.716 ± 0.77	0.900 ± 0.52	1.123 ± 1.44	0.584 ± 0.56	1.006 ± 1.23	0.604 ± 0.47	0.554 ± 0.52

Table 3.11. Spin-orbit coupling constants values calculated from S_0 level for DPP-mTHPC molecule at ω B97X-D/DZP level of theory.

DPP-mTHPC	SOCC from S ₀ Geometry										
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	
	S ₁	0.126 ± 0.10	0.087 ± 0.06	0.270 ± 0.20	0.260 ± 0.14	0.182 ± 0.11	0.154 ± 0.11	0.174 ± 0.11	0.344 ± 0.29	0.228 ± 0.17	0.269 ± 0.21
	S ₂	0.244 ± 0.17	0.180 ± 0.14	0.076 ± 0.06	0.135 ± 0.11	0.260 ± 0.12	0.224 ± 0.10	0.345 ± 0.21	0.648 ± 0.78	0.508 ± 0.58	0.300 ± 0.20
	S ₃	0.160 ± 0.10	0.332 ± 0.44	0.156 ± 0.14	0.088 ± 0.06	0.180 ± 0.09	0.554 ± 0.60	0.811 ± 1.10	2.523 ± 5.90	0.823 ± 1.05	0.626 ± 0.70
	S ₄	0.180 ± 0.12	0.360 ± 0.68	0.174 ± 0.12	0.102 ± 0.07	0.178 ± 0.08	0.380 ± 0.46	0.574 ± 1.04	2.122 ± 5.23	0.675 ± 0.62	0.506 ± 0.49
	S ₅	0.208 ± 0.17	0.275 ± 0.32	0.206 ± 0.12	0.226 ± 0.10	0.128 ± 0.07	0.156 ± 0.10	0.288 ± 0.23	0.552 ± 0.96	0.424 ± 0.40	0.358 ± 0.22
	S ₆	0.834 ± 0.82	3.199 ± 7.58	0.415 ± 0.49	0.174 ± 0.15	0.273 ± 0.27	0.736 ± 0.90	0.986 ± 1.60	0.670 ± 0.84	0.670 ± 0.75	0.748 ± 1.29
	S ₇	1.203 ± 1.85	2.283 ± 5.18	0.518 ± 0.62	0.194 ± 0.12	0.270 ± 0.22	0.850 ± 1.29	0.576 ± 0.59	0.812 ± 1.00	0.846 ± 1.08	0.501 ± 0.71
	S ₈	1.737 ± 2.00	2.864 ± 6.10	0.446 ± 0.54	0.318 ± 0.19	0.375 ± 0.38	1.058 ± 1.40	0.673 ± 0.68	2.125 ± 4.45	0.568 ± 0.57	0.410 ± 0.38
	S ₉	5.992 ± 9.21	1.457 ± 1.74	1.417 ± 1.92	0.546 ± 0.58	0.932 ± 1.80	1.528 ± 2.09	0.995 ± 1.47	1.045 ± 1.31	1.162 ± 2.00	0.506 ± 0.52
	S ₁₀	2.451 ± 3.85	4.444 ± 7.88	3.435 ± 7.17	0.923 ± 1.36	1.010 ± 1.45	1.949 ± 2.13	1.155 ± 1.58	1.110 ± 1.02	0.894 ± 1.13	0.834 ± 1.74

Results obtained show that all three of our proposed molecules meet the requirement of having the T_1 state of higher energy than 0.98 eV. For PDT mechanism purposes, PS molecules must have an efficient process of intersystem crossing (ISC). For ISC to occur the energy gap between the relevant singlet and triplet excited states

must be small. A large S-T gap may result in the promotion of fluorescence rather than non-radiative decay paths. Also, the two energy states that are subject to ISC should have high enough SOCCs. A SOCC value of 0.2 cm^{-1} is considered high enough for these S $\rightarrow$ T transitions to occur. All plausible $S \rightarrow T$ paths are reported in Table 3.12. In Figure 3.9, the most favorable paths from S_0 to T_1 level are reported for each molecule. Constructing these paths, information obtained from both ground state and excited state geometries are considered together with the NTO calculation results. Detailed explanations for the proposed paths are also given right after Figure 3.9.

Table 3.12. All plausible pathways going from ground state to T_1 state. Based on 20 initial geometries, performed at ω B97X-D/DZP level.

	Possible $S_0 \rightarrow T_1$ paths	ΔE_{ST} in eV	SOCC (cm^{-1})
DTP1-mTHPC	$S_0 \rightarrow S_3 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.102	0.20
	$S_0 \rightarrow S_4 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.194	0.18
	$S_0 \rightarrow S_5 \rightarrow T_9 \rightarrow T_8 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.072	0.28
DTP2-mTHPC	$S_0 \rightarrow S_3 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.144	0.42
	$S_0 \rightarrow S_4 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.160	0.38
	$S_0 \rightarrow S_5 \rightarrow T_8 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.050	0.32
	$S_0 \rightarrow S_6 \rightarrow T_{10} \rightarrow T_9 \rightarrow T_8 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.087	0.99
DPP-mTHPC	$S_0 \rightarrow S_4 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.102	0.47
	$S_0 \rightarrow S_5 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.056	0.29
	$S_0 \rightarrow S_6 \rightarrow T_{10} \rightarrow T_9 \rightarrow T_8 \rightarrow T_7 \rightarrow T_6 \rightarrow T_5 \rightarrow T_4 \rightarrow T_3 \rightarrow T_2 \rightarrow T_1$	0.080	0.36

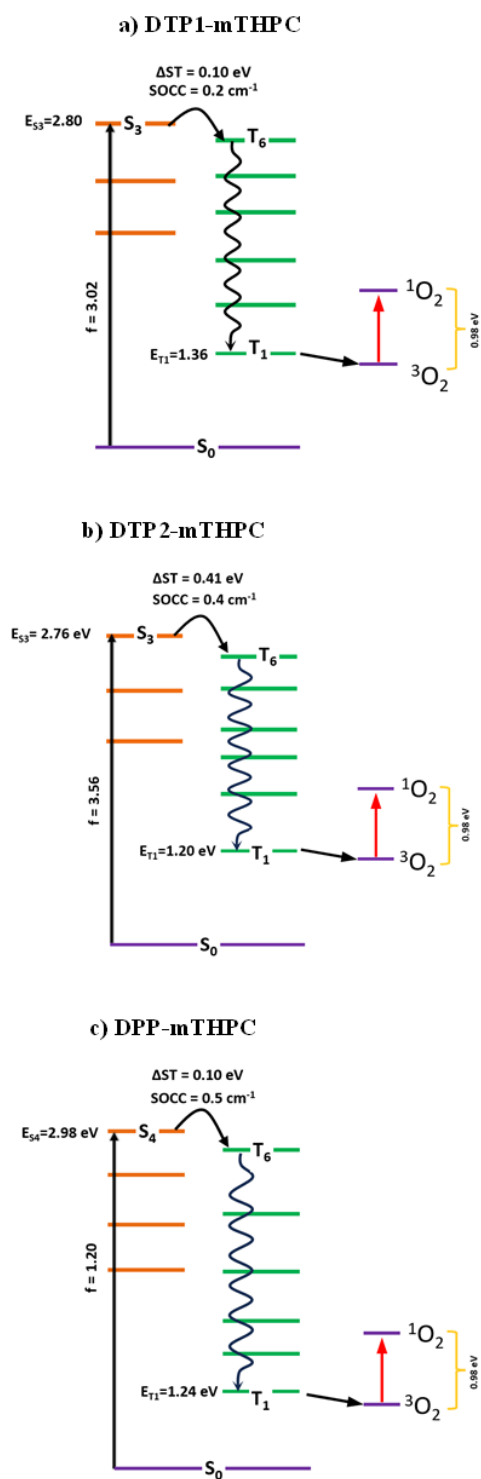


Figure 3.9. To scale energy state diagrams of the most plausible ISC pathways from S_0 state to T_1 state of each molecule. Based on 20 initial geometries and performed at $\omega B97X-D/DZP$ level of theory.

DTP1-mTHPC: $S_0 \rightarrow S_3 \rightarrow T_6 \rightarrow \dots \rightarrow T_1$. $S_0 \rightarrow S_3$ transition has the highest oscillation strength and, therefore, the most probable transition among the first four $S_0 \rightarrow S_n$ transitions, as presented in Figure 3.9. This means that this transition has the highest probability of populating the S state densely with electrons. Furthermore, as can be derived from Figures 3.14 and 3.17, $\Delta E_{(S_3-T_6)}$ is 0.194 eV together with SOCC of 0.20 cm^{-1} .

DTP2-mTHPC: $S_0 \rightarrow S_3 \rightarrow T_6 \rightarrow \dots \rightarrow T_1$. As reported in Figure 3.10, the highest oscillation having transition among the first four $S_0 \rightarrow S_n$ transitions, $S_0 \rightarrow S_3$ transition and the $\Delta E_{(S_3-T_6)}$ gap is 0.132 eV as shown in Figure 3.15. The SOCC value was found as 0.42 cm^{-1} and presented in Figure 3.18.

DPP-mTHPC: $S_0 \rightarrow S_4 \rightarrow T_6 \rightarrow \dots \rightarrow T_1$. Though lower than the highest oscillation values of the other two proposed molecules, in itself, the highest oscillation strength in the first four $S_0 \rightarrow S_n$ transitions is found to be $S_1 \rightarrow S_4$. Figure 3.16 presents the energy gap of $S_4 - T_6$ is calculated to be 0.122 eV. The SOCC value for this transition is 0.38 cm^{-1} as can be seen in Figure 3.19.

3.5. Molecular Dynamics (MD) Simulations in Different Environments

After confirming that the photophysics of unaltered mTHPC has been enhanced upon modification with TPA antenna units, simulations of these PS molecules in environments that mimic biological systems were carried out. The computational details of these simulations are provided in Tables 3.13-3.15. Snippets of systems at their starting point are visualized in Figures 3.10-3.12. Throughout this portion of our work, the solvation environment is chosen as water as it is the most biologically relevant solvent.

Table 3.13. Molecular dynamics simulation details for DTP1-mTHPC.

a) DTP1-mTHPC	In vacuo	In water	β-CD complex in water (2:1)	Near POPC in water
Force Field	GAFF	GAFF	GLYCAM_06j-1, GAFF	GAFF, Lipid17
Box Size	-	20 Å x20 Å x20 Å		104.3 Å x 104.2 Å x 126.8 Å
Time Step (fs)	0.2			
Ensemble	NPT			
Water Model	-	TIP3P		
Minimization step	1 → no restrictions	1 →waters restricted 2→no restrictions	1 →waters restricted 2→no restrictions	1 →PS+POPC restricted 2→PS restricted 3→ no restrictions
Heating	0K→310K			
Simulation time	1100ns	173ns	225ns	46ns

Table 3.14. Molecular dynamics simulation details for DTP2-mTHPC.

b) DTP2-mTHPC	In vacuo	In water	β-CD complex in water (2:1)	Near POPC in water
Force Field	GAFF	GAFF	GLYCAM_06j-1, GAFF	GAFF, Lipid17
Box Size	-	20 Å x20 Å x20 Å		104.3 Å x 104.2 Å x 126.8 Å
Time Step (fs)	0.2			
Ensemble	NPT			
Water Model	-	TIP3P		
Minimization step	1 → no restrictions	1 →waters restricted 2→no restrictions	1 →waters restricted 2→no restrictions	1 →PS+POPC restricted 2→PS restricted 3→ no restrictions
Heating	0K→310K			
Simulation time	1100ns	130ns	310ns	77ns

Table 3.15. Molecular dynamics simulation details for DPP-mTHPC.

c) DPP- mTHPC	In vacuo	In water	β-CD complex in water(1:1)	Near POPC in water
Force Field	GAFF	GAFF	GLYCAM_06j- 1, GAFF	GAFF, Lipid17
Box Size	-	20 Å x20 Å x20 Å		104.3 Å x 104.2 Å x 126.8 Å
Time Step (fs)	0.2			
Ensemble	NPT			
Water Model	-	TIP3P		
Minimization step	1 → no restrictions	1 →waters restricted 2→no restrictions	1 →waters restricted 2→no restrictions	1 →PS+POPC restricted 2→PS restricted 3→ no restrictions
Heating	0K→310K			
Simulation time	1100ns	213ns	305ns	46ns

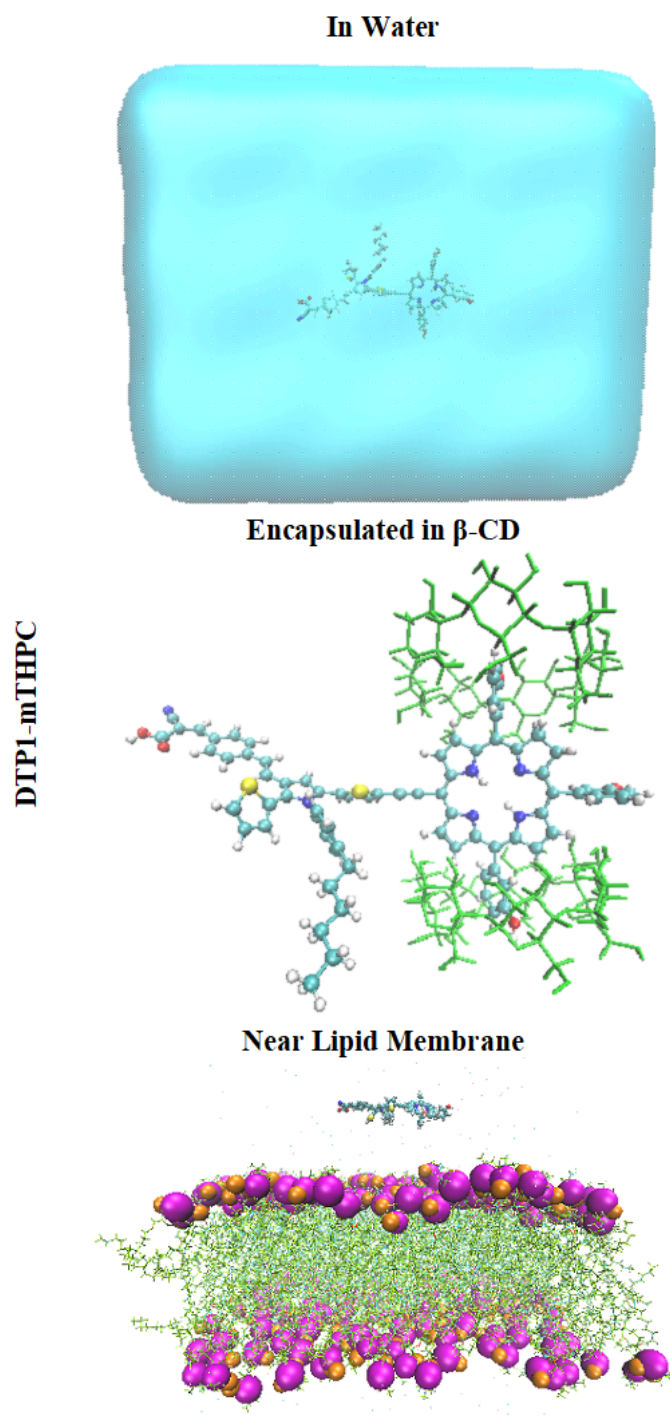


Figure 3.10. DTP1-mTHPC molecule in water, encapsulated in β -cyclodextrin and near the lipid membrane.

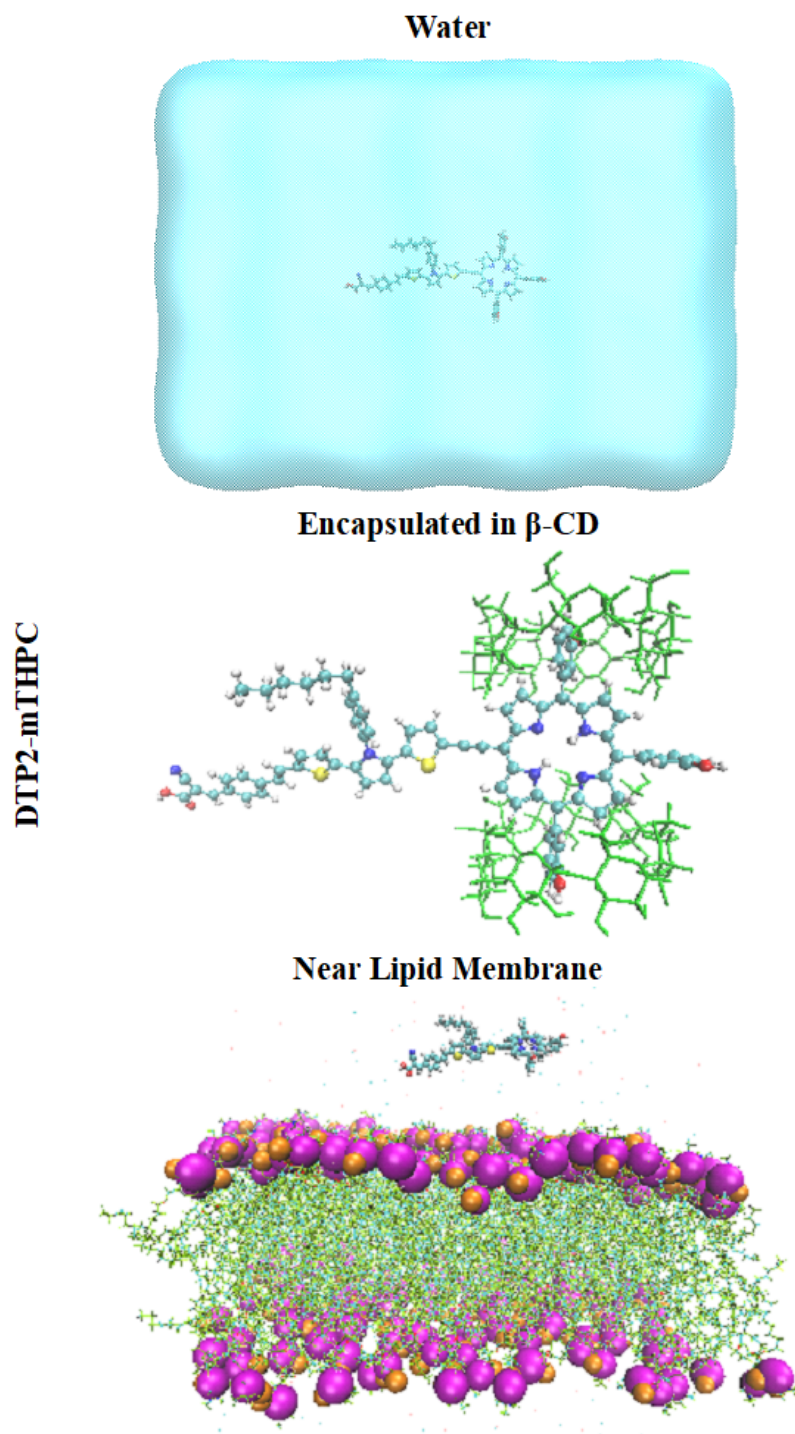


Figure 3.11. DTP2-mTHPC molecule in water, encapsulated in β -cyclodextrin and near the lipid membrane.

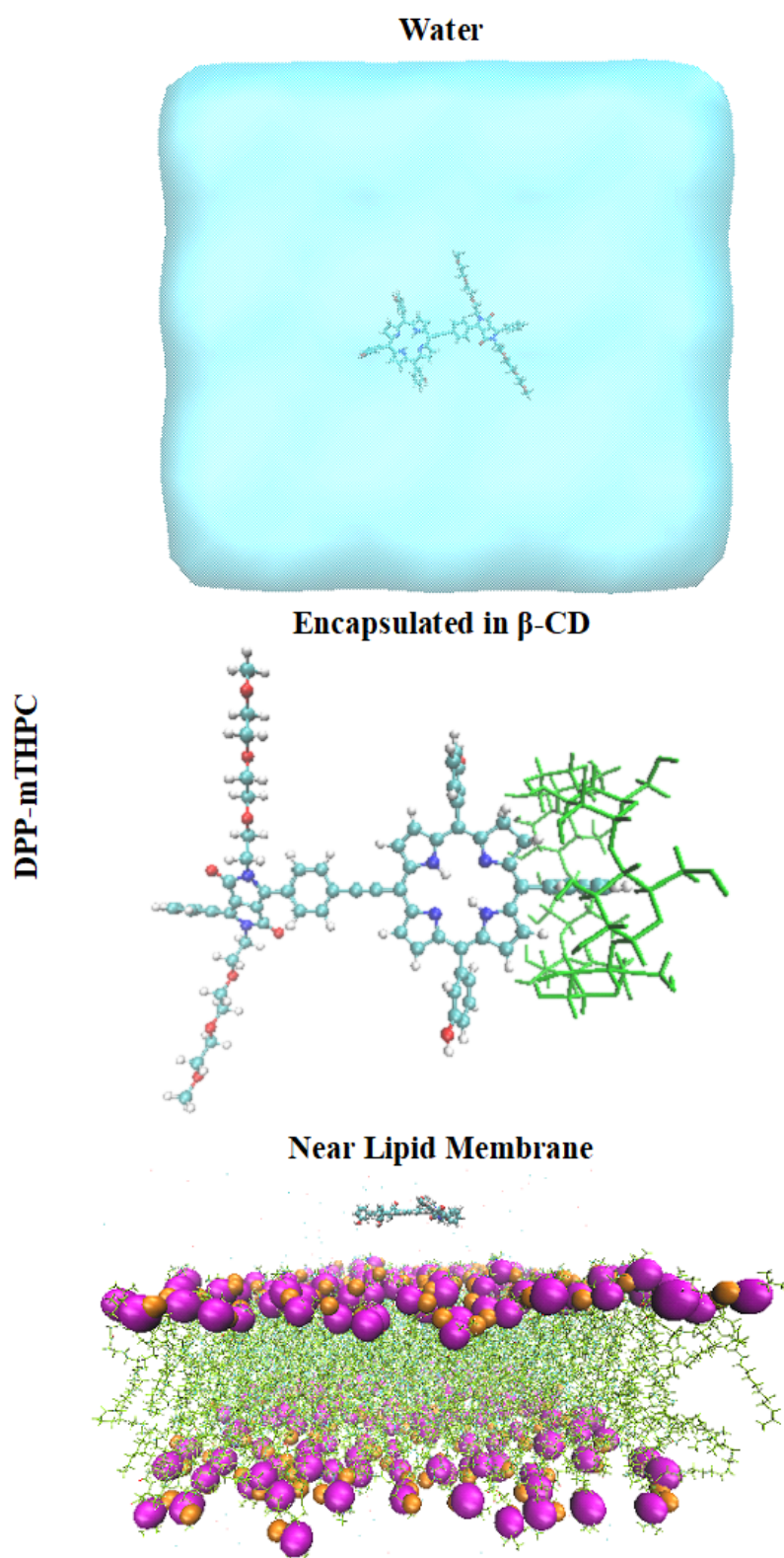


Figure 3.12. DPP-mTHPC molecule in water, encapsulated in β -cyclodextrin and near the lipid membrane.

Figures 3.13-3.15 presents the root-mean-square deviation (RMSD) plot of simulations in vacuum, in water, in water while encapsulated with β -cyclodextrin and near the lipid bilayer. The figures contain RMSD plots for the chlorin and temoporfin structures in these molecules together with the RMSD plot of the entire PS molecules.

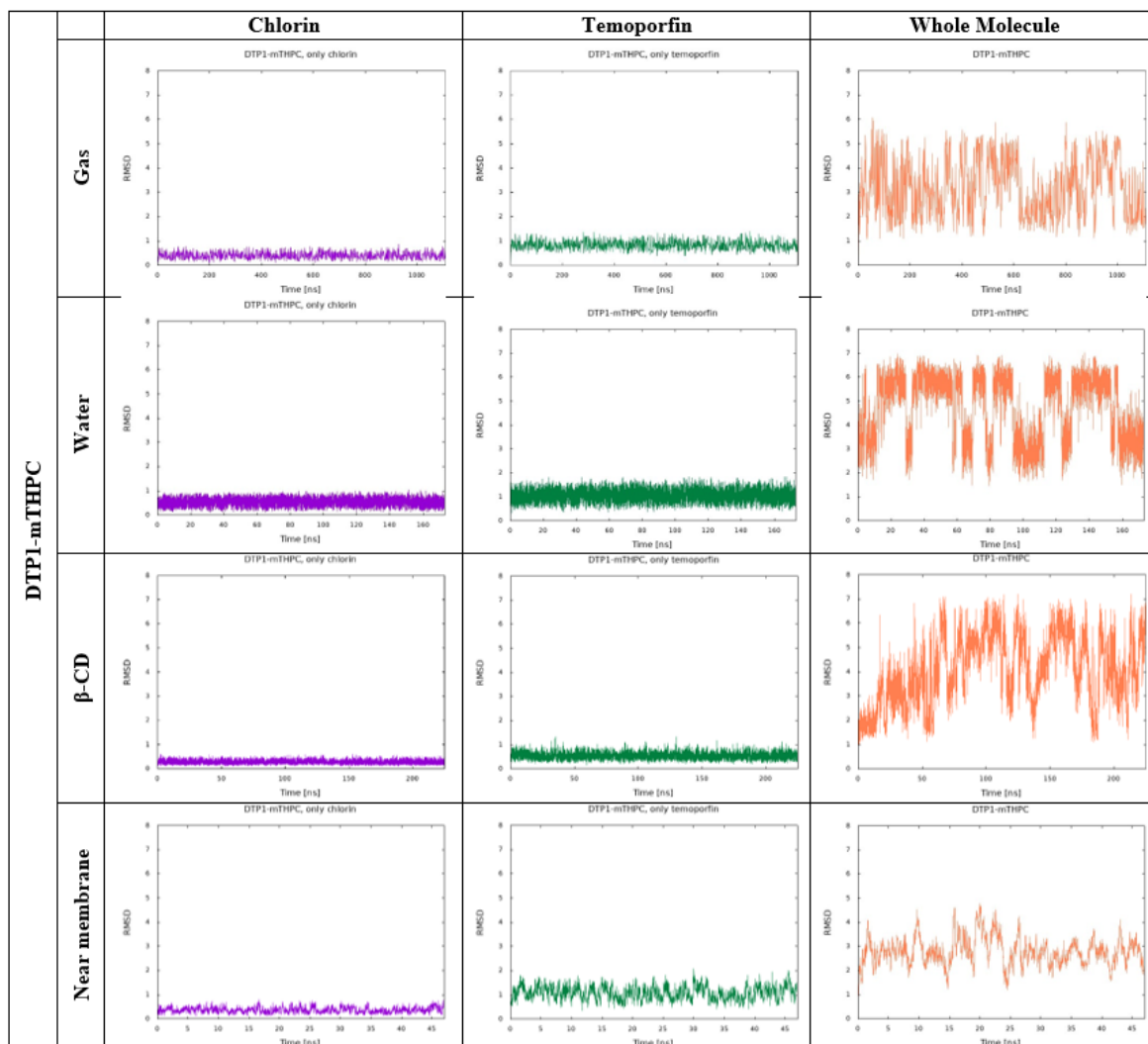


Figure 3.13. Root-mean-square deviation diagrams DTP1-mTHPC molecule from its simulations in a vacuum, in bulk water, encapsulated in β -cyclodextrin and near lipid bilayer.

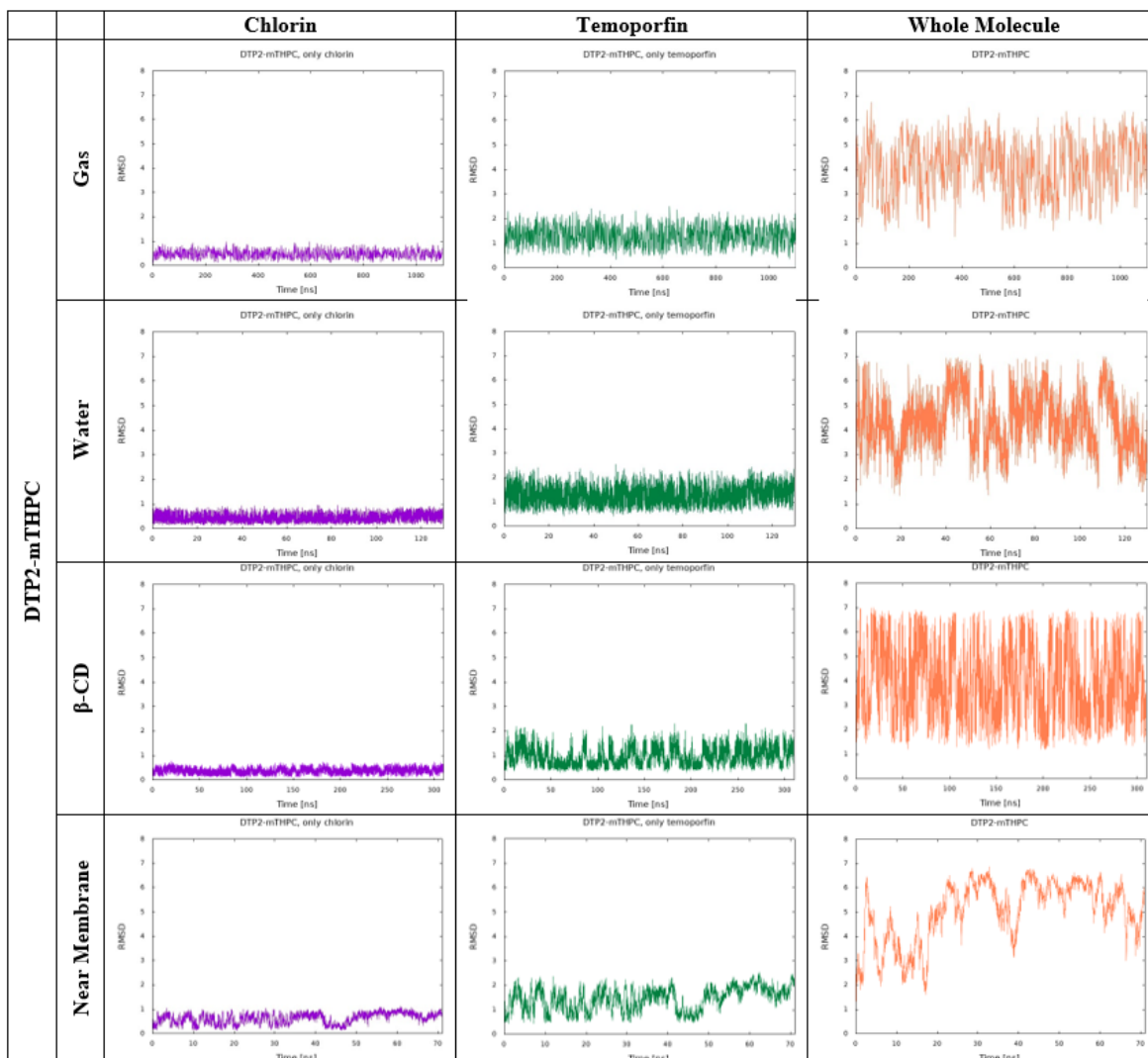


Figure 3.14. Root-mean-square deviation diagrams DTP2-mTHPC molecule from its simulations in a vacuum, in bulk water, encapsulated in β -cyclodextrin and near lipid bilayer.

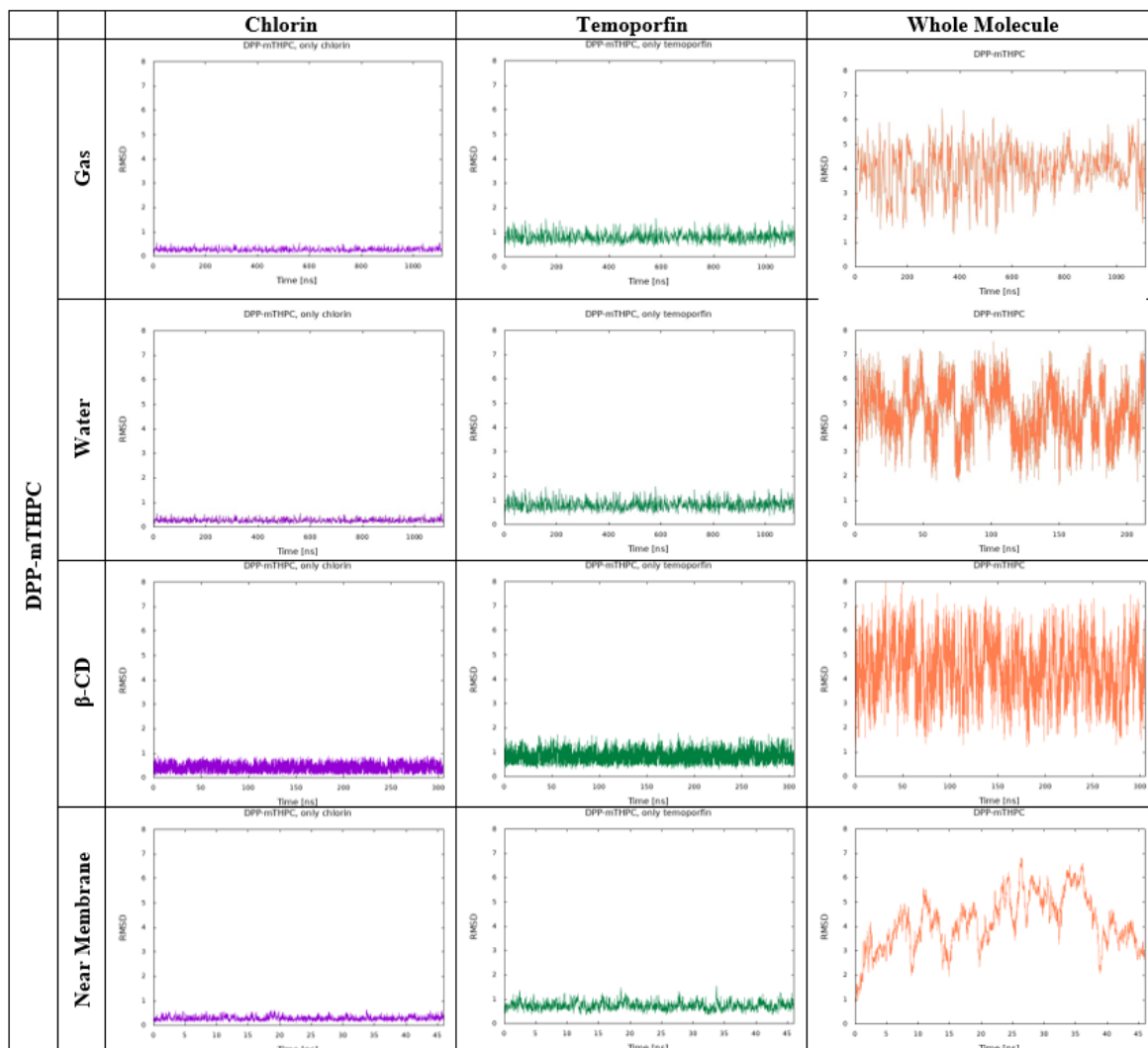


Figure 3.15. Root-mean-square deviation diagrams DPP-mTHPC molecule from its simulations in a vacuum, in bulk water, encapsulated in β -cyclodextrin and near lipid bilayer.

The stability of chlorin and temoporfin plots for each molecule suggests that these PSs are not prone to rotate and go through the radiationless-decay route from these groups. However, due to their long carbon-chained structures, the modified PS molecules are inclined to lose their energy via rotation. This, though, displays a risk of molecules deviating from the proposed ISC pathways that are required in PDT, resulting in the loss of their energy through radiationless decay. This problem can be solved by the encapsulation of the TPA groups together with the temoporfin

group to limit the free motion of the molecules completely. In our work, we solely encapsulated the mTHPC groups to address their water-insoluble nature. The choice of β -CD is justified with previous studies that experimentally confirm the affinity of the phenolic groups on mTHPC to CD's hydrophobic cavity. [70] As suggested by our group's previous computational study, another advantage of using β -CD to encapsulate is the decrease in the likelihood of losing energy via rotation by restricting the free rotation of phenolic groups on temoporfin. [22] This encourages the excited molecule to decay through the ISC and IC pathways, as desired. The addition of DTP groups is not presumed to have a great effect on the solubility parameters of the temoporfin molecule, therefore the common β -CD:mTHPC ratio of 2:1 is not tampered with, for the boost of solubility. However, in contrast to DTP derivatives, the addition of the DPP group is expected to increase the solubility of the original temoporfin molecule, credited to its highly soluble polyethylene glycol (PEG) groups. Subsequently, a 1:1 ratio of β -CD:mTHPC complex is seen fit to continue with simulations. The RMSD plots obtained from the encapsulated simulations of PSs shown in Figure 3.13-15 display no break-off of the CD complexes. This high stability of the complex can be attributed to dispersion forces together with the H-bond formation between the hydroxyl groups on the CD complex and the hydroxyl groups of phenolic groups of mTHPC.

For an efficient PDT mechanism, the photosensitizer is expected to come in proximity to tumorous cells and disrupt their membrane. The efficient accumulation of the PS around the cell membrane leads to increased oxidative stress that results in irreparable damage to cancerous cells. [71] The lipid membrane used for these simulations is obtained through CHARMM-GUI [72] and it consists of 2 lipid layers, each containing 150 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) units, converted into a biological membrane form. The membrane was solvated using the TIP3P water model and with K⁺ and Cl⁻ counterions.

In Figure 3.16, the distance plots of PS molecules and the polar groups (phosphocholine molecules) are presented for each molecule together with the snapshot of the closest contact of them. Position 0 signifies the cross-sectional middle point of the lipid

bilayer. During the simulations, only DTP2-mTHPC molecule showed a high affinity to the lipid membrane. From the starting point of the simulation, the designed PS molecule gradually got closer to the membrane and it positioned itself around the polar heads. Throughout the simulation it persistently kept its position. This persistence can be observed from the distance graph presented in Figure 3.16. Unfortunately, the other two molecules, did not show the same affinity to the lipid structures. Comparing the plots depicting the distance between the PS molecules and the phosphocholine units, it is obvious that DPP-mTHPC and DTP1-mTHPC does not have very high affinities for the lipid structures because they are not attracted to them. This was expected for the DPP-mTHPC molecule. It contains two highly soluble PEG groups and these PEGs favor water environment to a lipid one. DTP1-mTHPC molecules is slightly more in favor of being in proximity to the lipid membrane, however it might not be sufficient enough to be an effective PDT drug. On the other hand, the persistence of DTP2-mTHPC being near the membrane can be explained by the favorable interactions between the hydrophobic PS structures and the lipid membrane such as dispersion forces and the interaction of the negatively charged phosphate groups with phenolic groups of temoporfin and cyano and hydroxyl group of DTP2 molecule.

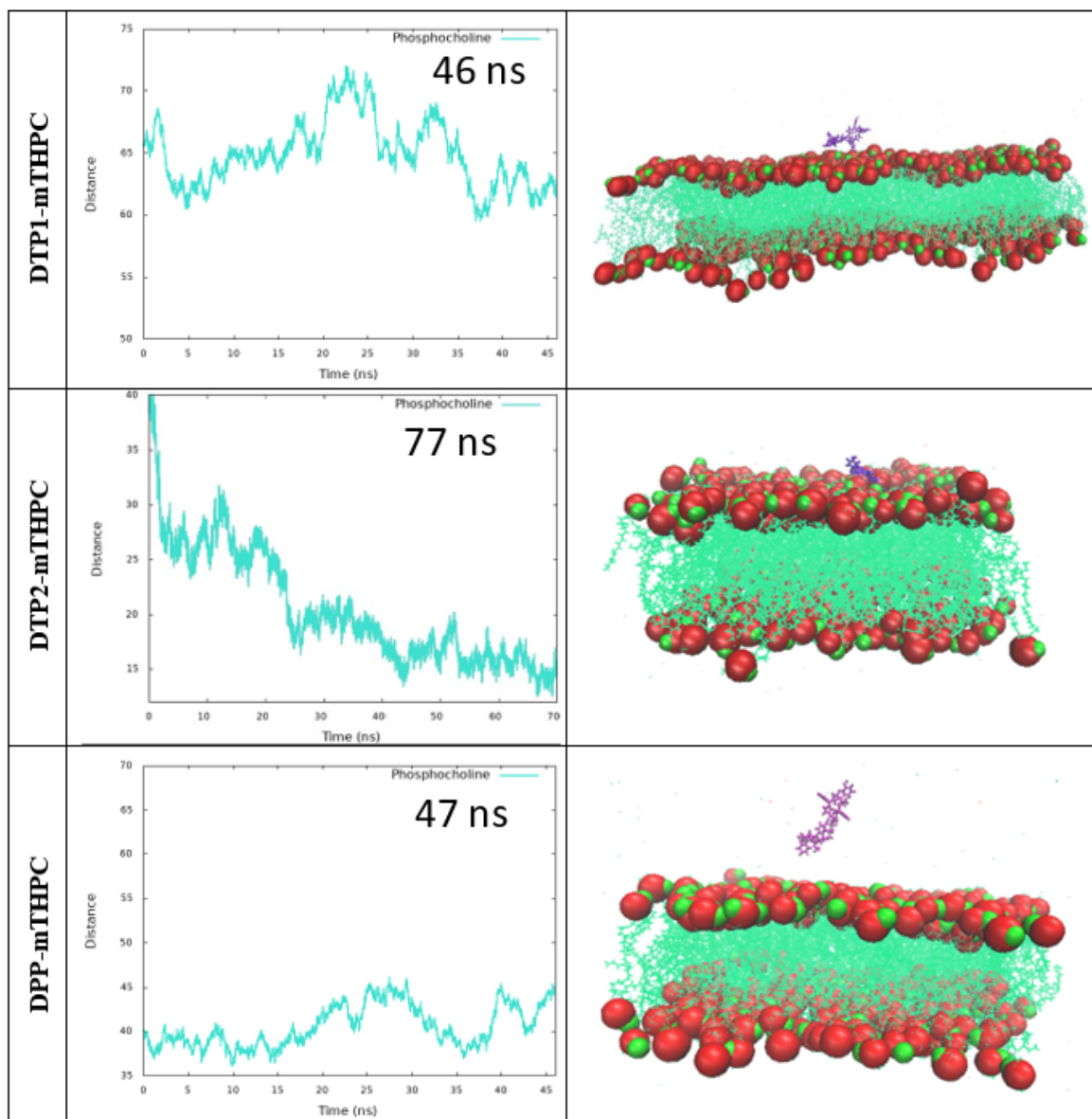


Figure 3.16. The distance graphs of molecules from the lipid-bilayer and their snapshots in their closest positions.

4. CONCLUSION

This thesis proposed three newly designed temoporfin derivatives decorated with TPA antenna moieties as candidate PDT photosensitizers. In-depth quantum mechanical analyses were performed by means of DTF and TD-DFT to enlighten the photophysical properties of these molecules. The yielded results of this study provided valuable insight into the geometries of the proposed PS molecules along with their OPA and TPA spectra and an understanding of their ISC pathways.

The calculated OPA spectra exhibit a blue shift in the Q-band compared to the unmodified mTHPC molecules. Nevertheless, the produced TPA results demonstrate a significant increase in the σ_{TPA} of each proposed molecule. The most increase in TPA cross section value is observed for the DTP2-mTHPC molecule, with an impressive σ_{TPA} of 8917 GM. The second remarkable increase in σ_{TPA} is observed for DPP-mTHPC with a value of 2235 GM. DTP1-mTHPC molecules increase is more humble compared to the other two molecules, but it is still significantly high, nonetheless. It is worth noting that for vertical excitation calculations, a dynamic approach was adopted and the OPA calculations were based on 40 initial conditions, while TPA calculations were based on 10 initial conditions. From this TPA analysis, it can be said that the two of the three proposed PS molecules are excellent candidates for Two-Photon Excited PDT.

For figuring out the excitation energy transfer pathways of the PSs, their NTOs, SOCC values, and energy levels were investigated. As a result of the single-point NTO calculations it was confirmed that in the first four singlet states, there are no dark states and at least one singlet state can be populated densely when PS molecules become excited from the ground state. The energy level and SOCC calculation provided the possible pathways the PS molecules can go through to reach T_1 from S_0 level.

To make this work wholesome, the molecules were simulated in vacuum, and in environments that mimic biological environments, such as water and near membrane. Also, to offer a solution to the water-solubility problem of these molecules simulations were carried out in which the temoporfin group of PS molecules is encapsulated with β -cyclodextrin. The simulation analyses reveal that the temoporfin group of molecules is not prone to lose their energy via non-radiative IC. But, the antenna groups are very mobile and their movements may restrict the molecules' transition to T_1 , and cause them to lose energy via rotation. This is an unwanted scenario for PDT purposes, and we suggest the encapsulation of both the temoporfin and the antenna groups for the mobility hindrance of the molecule, for future works. The DPP molecule, due to its very soluble PEG groups, chose to stay in bulk water and positioned itself in between two lipid membranes created by PBC. This unfavoring of the lipid bilayer puts DPP-mTHPC molecule in a controversial place for PDT. Similarly, DTP1-mTHPC molecule does not favor the lipid bilayer as an ideal PS molecule would. Conversely, in the lipid membrane simulations, DTP2-mTHPC molecule had no problem being attracted to the membrane's polar heads and it preferred to stay on the surface of the membrane. It remained there during the simulation and did not go into the bulk water again.

Considering all our results, DTP2-mTHPC molecule stands out between our three proposed TPA-functionalized molecules. Its high affinity towards the lipid membrane confirms that, as a PDT drug, it has the capacity to travel all the way near a tumorous cell and produce singlet oxygen next to its target. This feature combined with its remarkably high two-photon cross section makes DTP2-mTHPC molecule an excellent candidate for TPE-PDT applications.

REFERENCES

1. Abdel-Kader, M. H., *History of Photodynamic Therapy*, Springer, Berlin, Heidelberg, 2014.
2. Moghissi, K. and R. R. Allison, “A Narrative History of Photodynamic Therapy”, *Nanomaterials for Photodynamic Therapy*, Vol. 133, pp. 1–39, 2013.
3. Hönigsmann, H., “Phototherapy”, *Journal of Investigative Dermatology*, Vol. 133, pp. E18–E20, 2013.
4. Møller, K. I., B. Kongshøj, P. A. Philipsen, V. O. Thomsen and H. C. Wulf, “How Finsen’s Light Cured Lupus Vulgaris”, *Photodermatology, Photoimmunology Photomedicine*, Vol. 21, pp. 118–124, 2005.
5. Grzybowski, A. and K. Pietrzak, “From Patient to Discoverer–Niels Ryberg Finsen (1860–1904) –the Founder of Phototherapy in Dermatology”, *Clinics in Dermatology*, Vol. 30, pp. 451–455.
6. Foo, M. A., M. You, S. L. Chan, G. Sethi, G. K. Bonney, W. P. Yong, E. K. H. Chow, E. L. S. Fong, L. Wang and B. C. Goh, “Clinical Translation of Patient-Derived Tumour Organoids- Bottlenecks and Strategies”, *Biomarker Research*, Vol. 10, pp. 1–18, 2022.
7. Cacaccio, J. C., F. A. Durrani, J. R. Missert and R. K. Pandey, “Photodynamic Therapy in Combination with Doxorubicin Is Superior to Monotherapy for the Treatment of Lung Cancer”, *Biomedicines*, Vol. 10, p. 857, 2022.
8. Kim, H., M. Yang, N. Kwon, M. Cho, J. Han, R. Wang, S. Qi, H. Li, V. N. Nguyen, X. Li, H. B. Cheng and J. Yoon, “Recent Progress on Photodynamic Therapy and Photothermal Therapy”, *Bulletin of the Korean Chemical Society*, Vol. 44, pp. 236–255, 2022.

9. Su, Y., M. Wu, G. Wang, J. Li, X. Chen, X. Li, G. Wang and K. Zhang, “Benzophenone-Containing Phosphors with an Unprecedented Long Lifetime of 1.8 s Under Ambient Conditions”, *Chemical Communications*, Vol. 59, p. 1525, 2023.
10. Dougherty, T. J., C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan and Q. Peng, “Photodynamic Therapy”, *Journal of the National Cancer Institute*, Vol. 90, pp. 889–905, 1998.
11. Maharjan, P. S. and H. K. Bhattarai, “Singlet Oxygen, Photodynamic Therapy, and Mechanisms of Cancer Cell Death”, *Journal of Oncology*, Vol. 2022, 2022.
12. Luksiene, Z., “Photodynamic Therapy: Mechanism of Action and Ways to Improve the Efficiency of Treatment.”, *Medicina (Kaunas, Lithuania)*, Vol. 39, pp. 1137–1150, 2003.
13. Baptista, M. S., J. Cadet, P. D. Mascio, A. A. Ghogare, A. Greer, M. R. Hamblin, C. Lorente, S. C. Nunez, M. S. Ribeiro, A. H. Thomas, M. Vignoni and T. M. Yoshimura, “Type I and Type II Photosensitized Oxidation Reactions: Guidelines and Mechanistic Pathways”, *Photochemistry and Photobiology*, Vol. 93, pp. 912–919, 2017.
14. Zhang, Y., Y. Wan, Y. Chen, N. T. Blum, J. Lin and P. Huang, “Ultrasound-Enhanced Chemo-Photodynamic Combination Therapy by Using Albumin “nanoglue”-Based Nanotheranostics”, *ACS Nano*, Vol. 14, pp. 5560–5569, 2020.
15. Singh, A. K., R. Malviya, A. Moharana, V. K. Prajapati and A. K. Nirmal, “Photodynamic Therapy of Cancer: Quality and Prospective of Therapy based on Photosensitizer”, *Current Cancer Therapy Reviews*, Vol. 19, pp. 223–236, 2023.
16. Li, X., Y. H. Jeon, N. Kwon, J. G. Park, T. Guo, H. R. Kim, J. D. Huang, D. S. Lee and J. Yoon, “In Vivo-Assembled Phthalocyanine/Albumin Supramolecular Com-

- plexes Combined with a Hypoxia-Activated Prodrug for Enhanced Photodynamic Immunotherapy of Cancer”, *Biomaterials*, Vol. 266, p. 120430, 2021.
17. Wachter, E., D. K. Heidary, B. S. Howerton, S. Parkin and E. C. Glazer, “Light-Activated Ruthenium Complexes Photobind DNA and are Cytotoxic in the Photodynamic Therapy Window”, *Chemical Communications*, Vol. 48, pp. 9649–9651, 2012.
 18. Peng, Q., A. Juzeniene, J. Chen, L. O. Svaasand, T. Warloe, K. E. Giercksky and J. Moan, “Lasers in Medicine”, *Reports on Progress in Physics*, Vol. 71, p. 056701, 2008.
 19. Wiehe, A. and M. O. Senge, “The Photosensitizer Temoporfin (mTHPC) – Chemical, Pre-clinical and Clinical Developments in the Last Decade”, *Photochemistry and Photobiology*, Vol. 99, pp. 356–419, 2023.
 20. Mattioli, E. J., L. Ulfo, A. Marconi, V. Pellicioni, P. E. Costantini, T. D. Marforio, M. D. Giosia, A. Danielli, C. Fimognari, E. Turrini and M. Calvaresi, “Carrying Temoporfin with Human Serum Albumin: A New Perspective for Photodynamic Application in Head and Neck Cancer”, *Biomolecules*, Vol. 13, p. 68, 2023.
 21. Cheng, Y., Q. Chen, Z. Qian, T. Shan, L. Bai, X. Jiang, C. Li, Y. Wang, Y. Cheng, Q. Chen, Z. Qian, T. Shan, L. Bai, X. Jiang, Y. Wang and C. Li, “Versatile Red Blood Cells for Triple-Negative Breast Cancer Treatment via Stepwise Photoactivations”, *Advanced Healthcare Materials*, Vol. 12, p. 2201690, 2023.
 22. Aslanoglu, B., I. Yakavets, V. Zorin, H. P. Lassalle, F. Ingrosso, A. Monari and S. Catak, “Optical Properties of Photodynamic Therapy Drugs in Different Environments: The Paradigmatic Case of Temoporfin”, *Physical Chemistry Chemical Physics*, Vol. 22, pp. 16956–16964, 2020.
 23. Haimov-Talmoud, E., Y. Harel, H. Schori, M. Motiei, A. Atkins, R. Popovtzer, J. P.

- Lellouche and O. Shefi, “Magnetic Targeting of mTHPC to Improve the Selectivity and Efficiency of Photodynamic Therapy”, *ACS Applied Materials and Interfaces*, Vol. 11, pp. 45368–45380, 2019.
24. Yakavets, I., M. Millard, L. Lamy, A. Francois, D. Scheglmann, A. Wiehe, H. P. Lassalle, V. Zorin and L. Bezdetnaya, “Matryoshka-Type Liposomes Offer the Improved Delivery of Temoporfin to Tumor Spheroids”, *Cancers*, Vol. 11, pp. 1366–1382, 2019.
 25. Elberskirch, L., R. L. Harzic, D. Scheglmann, G. Wieland, A. Wiehe, M. Mathieu-Gaedke, H. R. Golf, H. von Briesen and S. Wagner, “A HET-CAM Based Vascularized Intestine Tumor Model as a Screening Platform for Nano-Formulated Photosensitizers.”, *European Journal of Pharmaceutical Sciences*, Vol. 168, pp. 106046–106058, 2021.
 26. Cronstrand, P., Y. Luo and H. Ågren, “Multi-Photon Absorption of Molecules”, *Advances in Quantum Chemistry*, Vol. 50, pp. 1–21, 2005.
 27. Salem, M. A., M. Gedik and A. Brown, “Two Photon Absorption in Biological Molecules”, *Handbook of Computational Chemistry*, pp. 1–19, 2015.
 28. Hamed, B., T. V. Haimberger, V. Kozich, A. Wiehe and K. Heyne, “Two-Photon Cross-sections of the Photosensitizers m-THPC and m-THPP in the 1.05–1.45 μm Range”, *Journal of Photochemistry and Photobiology A: Chemistry*, Vol. 295, pp. 53–56, 2014.
 29. Atif, M., P. E. Dyer, T. A. Paget, H. V. Snelling and M. R. Stringer, “Two-Photon Excitation Studies of m-THPC Photosensitizer and Photodynamic Activity in an Epithelial Cell Line”, *Photodiagnosis and Photodynamic Therapy*, Vol. 4, pp. 106–111, 2007.
 30. Gierlich, P., S. G. Mucha, E. Robbins, L. C. G. da Silva, K. Matczyszyn and M. O.

- Senge, “One Photon and Two Photon Photophysical Properties of Tetrafunctionalized Temoporfin (mTHPC) Derivatives as Potential Agents for Two Photon Induced Photodynamic Therapy”, *ChemPhotoChem*, Vol. 6, pp. 249–261, 2021.
31. Wei, X., X.-H. H. Guo, J.-F. F. Guo, T.-F. F. He, G.-Y. Y. Qin, L.-Y. Y. Zou and A.-M. M. Ren, “Photophysical Exploration of Zn(II) Polypyridine Photosensitizers in Two-Photon Photodynamic Therapy: Insights from Theory”, *Inorganic Chemistry*, Vol. 61, pp. 18729–18742, 2022.
 32. Sharmoukh, W., A. Attanzio, E. Busatto, T. Etienne, S. Carli, A. Monari, X. Assfeld, M. Beley, S. Caramori and P. C. Gros, “2,5-Dithienylpyrrole (DTP) as a Donor Component in DTP–A Organic Sensitizers: Photophysical and Photovoltaic Properties”, *RSC Advances*, Vol. 5, pp. 4041–4050, 2015.
 33. Sengul, O., E. Birsen, B. Mariachiara and W. Sharmouk, “Probing Optical Properties of Thiophene Derivatives for Two - Photon Absorption”, *Theoretical Chemistry Accounts*, Vol. 136, pp. 1–9, 2017.
 34. Sengul, O., M. Marazzi, A. Monari and S. Catak, “Photophysical Properties of Novel Two-Photon Absorbing Dyes: Assessing Their Possible Use for Singlet Oxygen Generation”, *Journal of Physical Chemistry C*, Vol. 122, pp. 16315–16324, 2018.
 35. Schmitt, J., V. Heitz, A. Sour, F. Bolze, H. Ftouni, J. F. Nicoud, L. Flamigni and B. Ventura, “Diketopyrrolopyrrole-Porphyrin Conjugates with High Two-Photon Absorption and Singlet Oxygen Generation for Two-Photon Photodynamic Therapy”, *Angewandte Chemie - International Edition*, Vol. 54, pp. 169–173, 2015.
 36. Hohenberg, P. and W. Kohn, “Inhomogeneous Electron Gas”, *Physical Review*, Vol. 136, p. B864, 1964.
 37. Kohn, W. and L. J. Sham, “Self-Consistent Equations Including Exchange and

- Correlation Effects”, *Physical Review*, Vol. 140, pp. A1133–A1138, 1965.
38. Perdew, J. P., K. Burke and M. Ernzerhof, “Generalized Gradient Approximation Made Simple”, *Physical Review Letters*, Vol. 78, pp. 1396–1396, 1997.
39. Becke, A. D., “Density [U+2010]Functional Thermochemistry. III. The Role of Exact Exchange”, *The Journal of Chemical Physics*, Vol. 98, pp. 5648–5652, 1993.
40. Tawada, Y., T. Tsuneda, S. Yanagisawa, T. Yanai and K. Hirao, “A Long-Range-Corrected Time-Dependent Density Functional Theory”, *The Journal of Chemical Physics*, Vol. 120, pp. 8425–8433, 2004.
41. Yanai, T., D. P. Tew and N. C. Handy, “A New Hybrid Exchange-Correlation Functional Using the Coulomb-Attenuating Method (CAM-B3LYP)”, *Chemical Physics Letters*, Vol. 393, pp. 51–57, 2004.
42. Chai, J. D. and M. Head-Gordon, “Long-Range Corrected Hybrid Density Functionals with Damped Atom–Atom Dispersion Corrections”, *Physical Chemistry Chemical Physics*, Vol. 10, pp. 6615–6620, 2008.
43. Slater, J. C., “Atomic Shielding Constants”, *Physical Review*, Vol. 36, pp. 57–64, 1930.
44. Boys, S. F., “Electronic Wave Functions - I. A General Method of Calculation for the Stationary States of any Molecular System”, *Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences*, Vol. 200, pp. 542–554, 1950.
45. Ditchfield, R., W. J. Hehre and J. A. Pople, “Self-Consistent Molecular-Orbital Methods. IX. An Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules”, *The Journal of Chemical Physics*, Vol. 54, No. 2, pp. 724–728, 1971.

46. Miertuš, S., E. Scrocco and J. Tomasi, “Electrostatic Interaction of a Solute with a Continuum. A direct Utilization of AB Initio Molecular Potentials for the Prevision of Solvent Effects”, *Chemical Physics*, Vol. 55, pp. 117–129, 1981.
47. Gaudoin, R. and K. Burke, “Lack of Hohenberg-Kohn Theorem for Excited States”, *Physical Review Letters*, Vol. 93, p. 173001, 2004.
48. Wigner, E., “On the Quantum Correction For Thermodynamic Equilibrium”, *Physical Review*, Vol. 40, pp. 749–759, 1932.
49. Koca, B., E. Hamuryudan, S. Catak, A. Erdogmus, A. Monari and V. Aviyente, “Exploring the Photophysics of Polyfluorinated Phthalocyanine Derivatives as Potential Theranostic Agents”, *The Journal of Physical Chemistry C*, Vol. 123, No. 40, pp. 24417–24425, 2019.
50. “Gaussian 16 Revision C.01”, Gaussian Inc. Wallingford CT, 2016.
51. Tomasi, J. and M. Persico, “Molecular Interactions in Solution: An Overview of Methods Based on Continuous Distributions of the Solvent”, *Chemical Reviews*, Vol. 94, pp. 2027–2094, 1994.
52. Barbatti, M., M. Ruckebauer, F. Plasser, J. Pittner, G. Granucci, M. Persico and H. Lischka, “Newton-X: A Surface-Hopping Program for Nonadiabatic Molecular Dynamics”, *Wiley Interdisciplinary Reviews: Computational Molecular Science*, Vol. 4, pp. 26–33, 2014.
53. te Velde, G., F. M. Bickelhaupt, E. J. Baerends, C. Fonseca Guerra, S. J. A. van Gisbergen, J. G. Snijders and T. Ziegler, “Chemistry with ADF”, *Journal of Computational Chemistry*, Vol. 22, No. 9, pp. 931–967, 2001.
54. van Gisbergen, S., J. Snijders and E. Baerends, “Implementation of Time-Dependent Density Functional Response Equations”, *Computer Physics Communications*, Vol. 118, No. 2, pp. 119–138, 1999.

55. Etienne, T., X. Assfeld and A. Monari, “New Insight into the Topology of Excited States through Detachment/Attachment Density Matrices-Based Centroids of Charge”, *Journal of Chemical Theory and Computation*, Vol. 10, No. 9, pp. 3906–3914, 2014.
56. Etienne, T., X. Assfeld and A. Monari, “Toward a Quantitative Assessment of Electronic Transitions” Charge-Transfer Character”, *Journal of Chemical Theory and Computation*, Vol. 10, pp. 3896–3905, 2014.
57. Aidas, K., C. Angeli, K. L. Bak, V. Bakken, R. Bast, L. Boman, O. Christiansen, R. Cimiraglia, S. Coriani, P. Dahle, E. K. Dalskov, U. Ekström, T. Enevoldsen, J. J. Eriksen, P. Ettenhuber, B. Fernández, L. Ferrighi, H. Fliegl, L. Frediani, K. Hald, A. Halkier, C. Hättig, H. Heiberg, T. Helgaker, A. C. Hennum, H. Hettema, E. Hjertenæs, S. Høst, I. M. Høyvik, M. F. Iozzi, B. Jansík, H. J. A. Jensen, D. Jonsson, P. Jørgensen, J. Kauczor, S. Kirpekar, T. Kjærgaard, W. Klopper, S. Knecht, R. Kobayashi, H. Koch, J. Kongsted, A. Krapp, K. Kristensen, A. Ligabue, O. B. Lutnæs, J. I. Melo, K. V. Mikkelsen, R. H. Myhre, C. Neiss, C. B. Nielsen, P. Norman, J. Olsen, J. M. H. Olsen, A. Osted, M. J. Packer, F. Pawłowski, T. B. Pedersen, P. F. Provasi, S. Reine, Z. Rinkevicius, T. A. Ruden, K. Ruud, V. V. Rybkin, P. Salek, C. C. Samson, A. S. de Merás, T. Saue, S. P. Sauer, B. Schimmelpfennig, K. Sneskov, A. H. Steindal, K. O. Sylvester-Hvid, P. R. Taylor, A. M. Teale, E. I. Tellgren, D. P. Tew, A. J. Thorvaldsen, L. Thøgersen, O. Vahtras, M. A. Watson, D. J. Wilson, M. Ziolkowski and H. Ågren, “The Dalton Quantum Chemistry Program System”, *Wiley Interdisciplinary Reviews: Computational Molecular Science*, Vol. 4, pp. 269–284, 2014.
58. Salomon-Ferrer, R., D. A. Case and R. C. Walker, “An Overview of the Amber Biomolecular Simulation Package”, *WIREs Computational Molecular Science*, Vol. 3, pp. 198–210.
59. Wang, J., W. Wang, P. A. Kollman and D. A. Case, “Antechamber, An Accessory Software Package For Molecular Mechanical Calculations”, *Journal of Chemical*

Information and Computer Sciences, Vol. 222, pp. 1–41, 2000.

60. Wang, J., R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, “Development and Testing of a General Amber Force Field”, *Journal of Computational Chemistry*, Vol. 25, pp. 1157–1174, 2004.
61. Gould, I. R., A. A. Skjevik, C. J. Dickson, B. D. Madej and R. C. Walker, “Lipid17: A Comprehensive AMBER Force Field for the Simulation of Zwitterionic and Anionic Lipids”, 2018.
62. Kirschner, K. N., A. B. Yongye, S. M. Tschampel, J. Gonza, C. R. Daniels, B. L. Foley and R. J. Woods, “GLYCAM06 : A Generalizable Biomolecular Force Field”, *Journal of Computational Chemistry*, Vol. 29, pp. 622–655, 2007.
63. Mark, P. and L. Nilsson, “Structure and Dynamics of the TIP3P, SPC, and SPC/E Water Models at 298 K”, *Journal of Physical Chemistry A*, Vol. 105, pp. 9954–9960, 2001.
64. Roe, D. R. and T. E. Cheatham, “PTRAJ and CPPTRAJ: Software for Processing and Analysis of Molecular Dynamics Trajectory Data”, *Journal of Chemical Theory and Computation*, Vol. 9, pp. 3084–3095, 2013.
65. Chołuj, M., M. M. Alam, M. T. Beerepoot, S. P. Sitkiewicz, E. Matito, K. Ruud and R. Zaleśny, “Choosing Bad versus Worse: Predictions of Two-Photon-Absorption Strengths Based on Popular Density Functional Approximations”, *Journal of Chemical Theory and Computation*, Vol. 18, pp. 1046–1060, 2022.
66. Beerepoot, M. T., M. M. Alam, J. Bednarska, W. Bartkowiak, K. Ruud and R. Zaleśny, “Benchmarking the Performance of Exchange-Correlation Functionals for Predicting Two-Photon Absorption Strengths”, *Journal of Chemical Theory and Computation*, Vol. 14, pp. 3677–3685, 2018.
67. Grahn, M. F., A. McGuinness, R. Benzie, R. Boyle, M. L. D. Jode, M. G.

- Dilkes, B. Abbas and N. S. Williams, "Intracellular Uptake, Absorption Spectrum and Stability of the Bacteriochlorin Photosensitizer 5,10,15, 20-tetrakis (m-hydroxyphenyl) Bacteriochlorin (mTHPBC). Comparison with 5,10,15,20-tetrakis (m-hydroxyphenyl) Chlorin (mTHPC)", *Journal of Photochemistry and Photobiology B: Biology*, Vol. 37, pp. 261–266, 1997.
68. Gierlich, P., S. G. Mucha, E. Robbins, L. C. G. da Silva, K. Matczyszyn and M. O. Senge, "One-Photon and Two-Photon Photophysical Properties of Tetrafunctionalized 5,10,15,20-tetrakis(m-hydroxyphenyl)chlorin (Temoporfin) Derivatives as Potential Two-Photon-Induced Photodynamic Therapy Agents", *ChemPhotoChem*, Vol. 6, pp. 1–11, 2022.
69. Wei, X., W. B. Cui, G. Y. Qin, X. E. Zhang, F. Y. Sun, H. Li, J. F. Guo and A. M. Ren, "Theoretical Investigation of Ru(II) Complexes with Long Lifetime and a Large Two-Photon Absorption Cross-Section in Photodynamic Therapy", *Journal of Medicinal Chemistry*, Vol. 66, p. 4167–4178, 2023.
70. Yakavets, I., H. Lassalle, I. Yankovsky, F. Ingrosso, A. Monari, L. Bezdetnaya and V. Zorin, "Evaluation of Temoporgin Affinity to β -Cyclodextrins Assuming Self-Aggregation", *Journal of Photochemistry Photobiology, A: Chemistry*, Vol. 367, pp. 13–21, 2018.
71. Lu, Y., W. Sun, J. Du, J. Fan and X. Peng, "Immuno-photodynamic Therapy (IPDT): Organic Photosensitizers and Their Application in Cancer Ablation", *Journal of American Chemical Society Au*, Vol. 3, pp. 682–699, 2022.
72. Jo, S., T. Kim, V. G. Iyer and W. Im, "CHARMM-GUI: A Web-Based Graphical User Interface for CHARMM", *Journal of Computational Chemistry*, Vol. 29, pp. 1859–1865, 2008.
73. Zhao, Y. and D. G. Truhlar, "The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited

- States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06-Class Functionals and 12 Other Fun”, *Theoretical Chemistry Accounts*, Vol. 120, pp. 215–241, 2008.
74. Lee, C., W. Yang and R. G. Parr, “Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density”, *Physical Review. B, Condensed Matter*, Vol. 37, pp. 785–789, 1988.
 75. Choe, S. J., “Comparison of Different Theory Models and Basis Sets in DFT Comparison of Different Theory Models and Basis Sets in Calculations of TPOP24N-Oxide Geometry and Geometries of meso-Tetraphenyl Chlorin N-Oxide Regioisomers”, *Korean Chemical Society*, Vol. 33, p. 2861, 2012.
 76. Hanwell, M. D., D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek and G. R. Hutchison, “Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform”, *Journal of Cheminformatics*, Vol. 4, pp. 1–17, 2012.
 77. Case, D. A., T. E. Cheatham, T. Darden, H. Gohlke, R. Luo, K. M. Merz, A. Onufriev, C. Simmerling, B. Wang and R. J. Woods, “The Amber Biomolecular Simulation Programs”, *Journal of Computational Chemistry*, Vol. 26, pp. 1668–1688, 2005.
 78. Schmidt, A. K., S. Cottaz, H. Driguez and G. E. Schulz, “Structure of Cyclodextrin Glycosyltransferase Complexed with a Derivative of its Main Product -cyclodextrin”, *Biochemistry*, Vol. 37, pp. 5909–5915, 1998.
 79. Humphrey, W., A. Dalke and K. Schulten, “VMD : Visual Molecular Dynamics”, *Journal of Molecular Graphics*, Vol. 7855, pp. 33–38, 1996.
 80. Isborn, C. M., A. W. Götz, M. A. Clark, R. C. Walker and T. J. Martínez, “Electronic Absorption Spectra from MM and Ab Initio QM/MM Molecular Dynamics: Environmental effects on the absorption spectrum of photoactive yellow protein”,

Journal of Chemical Theory and Computation, Vol. 8, pp. 5092–5106, 2012.