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Computational Study of DPAP Molecular Rotor in Various Environments: From Force Field Development to Molecular Dynamics Simulations and Spectroscopic Calculations

Marina Macchiagodena^{*,a}, Gianluca Del Frate^{*,a}, Giuseppe Brancato^{a,b}, Balasubramanian Chandramouli^{a,c}, Giordano Mancini^{a,b}, and Vincenzo Barone^{a,b}

^aScuola Normale Superiore, Piazza dei Cavalieri 7, I-56126 Pisa, Italy

^bIstituto Nazionale di Fisica Nucleare (INFN) sezione di Pisa, Largo Bruno Pontecorvo 3, I-56127 Pisa, Italy

^cCompunet, Istituto Italiano di Tecnologia (IIT), Via Morego 30, I-16163 Genova, Italy

Abstract

Fluorescent molecular rotors (FMRs) belong to an important class of environment-sensitive dyes capable to act as nanoprobes to measure the viscosity and polarity of their microenvironment. FMRs have found widespread applications in various research fields, ranging from analytical to biochemical sciences, for example in intracellular imaging studies or in volatile organic compound detection. Here, a computational investigation of a recently proposed FMR, namely the 4-(diphenylamino)phthalonitrile (DPAP), in various chemical environments is presented. A purposely developed molecular mechanics force field is proposed and then applied to simulate the rotor into a high- and low-polar solvent (i.e., acetonitrile, tetrahydrofuran, *o*-xylene and cyclohexane), a polymer matrix and a lipid membrane. Subtle effects of the molecular interactions with the embedding medium, the structural fluctuations of the rotor and its rotational dynamics are analyzed in some detail. Results correlate with a previous work, thus supporting the reliability of the model, and provide further insights on the environment-specific properties of the dye. In particular, it is shown how molecular diffusion and rotational correlation times of the FMR are affected by the surrounding medium and how the molecular orientation of the dye becomes anisotropic once immersed in the lipid bilayer. Moreover, a qualitative correlation between the FMR rotational dynamics and the fluorescence lifetime is detected, a result in line with the observed viscosity dependence of its emission. Finally, optical absorption spectra are computed and successfully compared with their experimental counterparts.

Keywords

Fluorescent molecular rotor; force field parametrization; molecular dynamics simulations; rotational dynamics; lipid membrane

* marina.macchiagodena@sns.it, gianluca.delfrate@sns.it.

1 Introduction

During the last decades, the chemistry toolbox has been significantly expanded by the development of new organic fluorophores characterized by innovative structural and optical features. In particular, molecular dyes able to modulate their photophysical properties in response to different surrounding environments are nowadays employed in wide areas of chemical research, ranging from environmental to photovoltaics applications,[1] and from biology to medicine.[2–5] In this context, fluorescent molecular rotors (FMR) have gained much attention owing to their simple synthesis and versatility.[6–8] Typically, FMRs are characterized by an electron acceptor moiety and an electron donor unit, which are connected by a flexible spacer with conjugated bonds. Such a chemical linker ensures, upon excitation, an electronic density shift from one unit to the other and confers to FMRs a peculiar sensitivity towards local viscosity and polarity of the environment. In FMRs, emission is finely modulated by intramolecular structural changes occurring in the excited state, in addition to solvent dipolar relaxation. Moreover, in most FMRs the fluorescence signal stems from the competition between a locally (bright) excited state and a twisted intramolecular (either bright or dark) charge-transfer (TICT) state.[6] As a result, FMRs have been successfully employed as intracellular microviscosity detectors for *in vivo* applications.[9,10] This is relevant in view of the connection of plasma and cellular viscosity changes with biochemical processes and diseases.[11,12]

Among the many FMRs reported to date, the recently synthesized 4-(diphenylamino)phthalonitrile (DPAP) turned out to be the prototype of a novel class of FMRs.[13] DPAP chemical structure presents a tertiary amine electron donor and two nitrile groups acting as electron acceptor moieties, embedded in a π -extended conjugated system (Figure 1a). In contrast to most FMRs, which are characterized by a locally excited (LE)/TICT state mechanism, DPAP photophysical behavior is basically modulated by a free rotational motion of its phenyl rings.[13] DPAP spectroscopic response and its polarity and viscosity dependence have been already exploited in several applications.[14–16] Usually the *modus operandi* of FMR photophysical mechanisms, including DPAP, are typically addressed by quantum-mechanical investigations of their electronic excited states and conformational changes. However their detailed structural and dynamical features in complex molecular environments and their specific interactions with the surroundings, which ultimately modulate the FMR spectroscopy, still remain largely elusive. Due to the several interplaying effects that directly and indirectly affect FMRs upon dissolution in condensed-phase systems, a thorough *in silico* investigation may help to properly identify the molecular determinants of the recorded experimental observables and possibly uncover the subtle relationship between molecular dynamics and spectroscopy.[17,18]

In this work, a molecular dynamics (MD) study of DPAP in multiple environments (Figure 1b-e) has been carried out in order to describe the effect of the embedding medium on the structural and dynamical behaviour of the rotor. Acetonitrile (ACN), tetrahydrofuran (THF), *o*-xylene and cyclohexane were considered as solvents, to include a reasonable range of bulk properties (as static dielectric constant and viscosity). Additionally the study has been extended to include the atactic poly(methyl methacrylate) polymeric matrix (PMMA) and the hydrated 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) lipid bilayer, since

applications of DPAP in both polymeric films[15] and in cell membrane environments[16] have been reported in previous studies. Hence, DPAP has been chosen as an illustrative example for the computational treatment of a FMR in different target environments. A multistep computational protocol has been set out,[17] which involves the development of a reliable ground-state molecular model of the rotor based on quantum-mechanical (QM) calculations, extensive atomistic simulations in all the environments and *a posteriori* spectroscopic calculations of the optical absorption spectra. On the other hand, emission spectra, whose modeling would require the use of the excited-state force field, will be addressed in a future work, following the same computational procedure established in the present study. Special attention was given to the *ad hoc* parameterization route: it is worth noting that a reliable force field is crucial in this context, owing to sensitivity of the dynamical and spectroscopic response to conformational changes of the FMR.[13] Analysis of the MD trajectories has allowed to shed some light on the structural and dynamic properties of the dye within the considered embeddings and concurrent local structural perturbations of the surroundings. Furthermore, the rotor mobility and rotational dynamics were scrutinized in view of the dye-environment specific interactions. Interestingly, in some of the environments a relation between molecular rotational dynamics and fluorescence lifetime has emerged from our analysis. This result may have far-reaching implications for the possible exploitation of spectroscopic techniques to gather detailed molecular information in a large number of materials.

2 Methods

2.1 Force field parameterization

A molecular-mechanics force field for DPAP was developed based upon the following standard form:

$$E_{intra} = E_{stretch} + E_{bend} + E_{tors} + E_{nb} \quad (1)$$

where $E_{stretch}$ and E_{bend} are the classical harmonic potentials for stretching and bending, i.e.

$$E_{stretch} = \frac{1}{2} k_{ij}^s (r - r_{eq})^2 \quad (2)$$

$$E_{bend} = \frac{1}{2} k_{ijk}^\theta (\theta - \theta_{eq})^2 \quad (3)$$

with k_{ij}^s , k_{ijk}^θ , r_{eq} and θ_{eq} the force constants and the equilibrium values of stretching and bending potentials, respectively. E_{tors} in Eq. 1 includes the torsional potential for rigid and improper dihedral angles, described through the standard harmonic form:

$$E_{tors}^R = \frac{1}{2} k_{ijkl}^{\xi} (\xi - \xi_{eq})^2 \quad (4)$$

and for flexible dihedral angles, modeled according to:

$$E_{tors}^F = k_{ijkl}^{\phi} (1 + \cos(n\phi - \gamma)) \quad (5)$$

where k_{ijkl}^{ξ} and k_{ijkl}^{ϕ} are the force constants which govern stiff and flexible torsions, and ξ_{eq} , γ and n are the equilibrium value, the phase and the multiplicity, respectively. The last term in Eq. 1, E_{nb} , is the sum of the non-bonded dispersion and electrostatic contributions, which are modeled by means of standard 6-12 Lennard-Jones and Coulomb potentials. Note that, in the present force field, non-bonded interactions are counted for all pairs of atoms separated by three or more bonds, without applying any scaling factor.

All intramolecular terms were parameterized using the Joyce software[19] by fitting energy gradients and Hessian matrix to corresponding QM data. In particular, bond, angle and stiff torsion terms were fitted to a QM Hessian matrix computed at DPAP optimized geometry, while torsional potentials of flexible dihedral angles were further refined through relaxed potential energy surface (PES) scan calculations. DPAP optimized geometry, PES scan energies and Hessian matrix were computed at the density functional theory (DFT) level, according to the B3LYP exchange-correlation functional and the SNSD basis set.[20,21] Bulk solvent effects were taken into account by means of the Conductor-like Polarizable Continuum Model (C-PCM).[22] Atomic partial charges were computed according to class IV CM5 charges[23] at the minimum energy configuration, whereas Lennard-Jones parameters were transferred from the standard OPLS/AA FF.[24] Acetonitrile and cyclohexane only were considered within the C-PCM to evaluate the influence of solvent polarity on DPAP structural and electronic properties, however FF parameters and atomic charges were finally based on acetonitrile owing to negligible differences in the obtained parameters (i.e., the largest atomic charge deviation was $2.98 \times 10^{-2}e$). All QM calculations were performed using the Gaussian 09 suite of programs.[25]

2.2 Molecular dynamics simulations

Classical MD simulations of DPAP in different environments were performed using the GROMACS (ver. 4.6.5) software package.[26] The OPLS-AA FF[24] was used to describe intramolecular and intermolecular potential of tetrahydrofuran, *o*-xylene and acetonitrile with the exception of ACN atomic charges, which were estimated using the CM5 population analysis[23] and tested in a previous work.[27] For cyclohexane, the general Amber FF (GAFF) for organic molecules[28] has been selected, since preliminary investigations showed that poor results were obtained in reproducing density with OPLS-AA. PMMA coordinates and topology were taken from a previous study,[29] using standard OPLS parameters. The hydrated (by means of TIP3P water molecules[30]) DOPC bilayer was

modeled according to CHARMM FF[31], which is able to reproduce available experimental information on the structure and dynamics of phospholipid bilayers reasonably well.[32–34]

One DPAP molecule was solvated or embedded into a number of molecules representing the above mentioned environments enforcing periodic boundary conditions (see details in Table 1). In particular, in order to simulate DPAP in the hydrated DOPC bilayer, a rectangular box was chosen. After a steepest descent energy minimization, the systems were slowly heated up from an initial temperature of 150 K to 300 K for about 500 ps using the velocity-rescale thermostat[35] and a coupling constant (τ) equal to 0.1 ps. All systems, except DPAP in lipid bilayer, were equilibrated for 1 ns (with a timestep of 1.0 fs) in a NPT ensemble, using the Berendsen barostat,[36] and the velocity-rescale thermostat with coupling constants of 1.0 ps and 0.1 ps respectively. In the case of lipid bilayer, the equilibration run lasted 6 ns according to a NPT ensemble, using the semi-isotropic pressure coupling. Afterwards, all production runs were carried out in the NVT ensemble, using the velocity-rescale thermostat ($T=298.15$ K and $\tau=0.1$ ps) and increasing the integration timestep from 1.0 to 2.0 fs. Fastest degrees of freedom were constrained with the LINCS algorithm.[37] In the case of cyclohexane, only bonds with hydrogen atoms were kept rigid. The total sampling time was about 130 ns for all the systems. Electrostatic potential was described using the Particle Mesh Ewald (PME) method,[38] using a real-space cutoff of 1.4 nm and spline interpolation of order 4. Van der Waals interactions were computed applying a cutoff of 1.4 nm. OPLS combination rules were used. System coordinates were stored every 500 steps (i.e., each picosecond). Trajectories analysis were performed with the TRAVIS package[39] and homemade scripts.

2.3 Absorption spectra calculations

Vertical transition energies were computed at the CAM-B3LYP/SNSD level of theory on selected configurations sampled during the MD simulations. The obtained energies were convoluted with Gaussian functions in the energy domain using a properly chosen half width at half maximum (HWHM) value, σ_ν [40] according to:

$$\epsilon(\nu) \propto \sum_{i \in \text{states}} \frac{f_i}{\Delta_\nu} \exp \left[-\left(\frac{\nu - \nu_i^0}{\sigma_\nu} \right)^2 \right] \quad (6)$$

where

$$\sigma_\nu = [2\sqrt{2\ln(2)}]^{-1} \Delta_\nu \quad (7)$$

and f_i and ν_i^0 are the oscillator strength and the frequency (in wavenumbers) of the i -th excitation, respectively. Environmental effects were included using the C-PCM.[22] The final spectrum was converted to the wavelength domain ($\epsilon_\lambda(\lambda)$) for a comparison with experiments. Then, single transition energies issuing from 200 snapshots, as extracted from the corresponding MD trajectories, are averaged according to:

$$\bar{\epsilon}(\lambda) = \sum_{c \in \text{confs}} \frac{\epsilon_c(\lambda)}{N_{\text{confs}}} \quad (8)$$

Charge transfer (CT) character of DPAP was computed (CAM-B3LYP/SNSD level) in acetonitrile and cyclohexane by evaluation of the CT index, as described in Ref[41], in order to determine the spatial extent of the electronic rearrangement upon excitation.

3 Results and Discussion

3.1 DPAP force field

DPAP optimized structure adopts a propeller-like conformation in order to minimize steric hindrance among the three phenyl rings (Figure 2). DPAP belongs to the C_1 point group since the presence of cyano substituents on one ring breaks the D_3 symmetry that otherwise characterizes the parent molecule (i.e. triphenylamine). The central NC1C1'C1'' moiety (for atom labeling see Figure 2) adopts a nearly planar geometry. From DFT calculations (Section 2.1 for details), the C1NC1' and C1NC1'' angles are equal to about 121°, whereas C1'NC1'' is 117° in acetonitrile (i.e., the highest polar solvent) and in cyclohexane (lowest polar solvent), the difference between C1NC1' (or C1NC1'') and C1'NC1'' being ascribed to the inductive and resonance effects of the two cyano groups on ring 1.[42] The three main degrees of freedom, characterizing DPAP conformational changes in solution, are the ring torsional angles with respect to the central amine group (i.e. NC1C1'C1''), hereafter referred to as dihedral **1** (C2C1NC1''), dihedral **2** (C1''NC1'C2') and dihedral **3** (C2''C1''NC1') (see Figure 2). Owing to the subtle interplay between structural conformation and photophysical properties, special attention was due to the parametrization of the torsional potentials along such dihedral angles, as described in the following.

Starting from the DFT optimized geometry of the dye, distinct relaxed potential energy scans along the dihedral angles were performed (Figure 3): each torsional angle was modified in multiple steps (at least 25), then the dye structure was relaxed keeping frozen the accounted dihedral angle to avoid spurious distorted conformations due to close interactions between the phenyl rings. Note that potential energy curve (PEC) of dihedral angle **2** and **3** are equivalent, in this case. Solvent (i.e. acetonitrile) effects have been included implicitly in calculations (see Methods section for details). No noticeable differences between acetonitrile and cyclohexane were observed, as shown in Figure S2. The obtained DFT PEC was used to refine torsional potential terms of the DPAP FF. Results are depicted in Figure 3, where DFT and MM PEC profiles show negligible differences. Both PEC are symmetric with respect to the planar geometry (at either 0° or 180°) and show four minima in correspondence to the propeller-like conformations. Overall, the PEC profile is typical of aromatic amines: a similar profile, for example, has been obtained for the triphenylamine.[43] In particular, dihedral angle **2** and **3** show symmetry-related energy minima at $\pm 130^\circ$ and $\pm 50^\circ$, whereas dihedral **1** minima are located at $\pm 25^\circ$ and $\pm 155^\circ$. These results are consistent with DPAP optimized geometry, in which **1**, **2** and **3** are, respectively, 22°, 51° and 125° in acetonitrile and 23°, 53° and 127° in cyclohexane. Two energy barriers characterize the interconversion

among such energy minima, whose positions correspond to the planar and orthogonal geometry of the considered ring with respect to the central amine moiety: a small one of less than 1 kcal/mol and a larger one of about 3-5 kcal/mol. Interestingly, there is an apparent swap of such barriers in the two type of torsional angles: the highest energy barrier corresponds to the orthogonal configuration in case of dihedral **1** and to the planar configuration in case of dihedral **2** and **3**. As a consequence, the torsional potentials of the three dihedral angles cannot be treated equivalently. This peculiar observation is ultimately due to the resonance effect of the cyano groups on ring **1**, which confer to an extra stabilization energy to the planar geometry. For the unsubstituted phenyl rings, the orthogonal conformation is energetically more favourable with respect to the planar one because the steric hindrance is minimized. The situation is reversed in case of the di-substituted ring (i.e., ring **1**). Such a resonance effect has been highlighted by a Natural Bond Orbital (NBO) analysis performed on the four DPAP conformers with **1** and **2/3** dihedral angles set to either 0° or 90°; in particular in correspondence of the planar ring **1** conformer there is a strong intra-molecular hyper conjugation interaction between the N lone pair and anti-bonding C1 orbitals (Figure S3). The whole set of FF parameters is reported in the ESI (Table S1 to Table S6).

3.2 DPAP in solutions

MD simulations of the dye in solution, i.e. acetonitrile, tetrahydrofuran, *o*-xylene and cyclohexane, were carried out at normal conditions. DPAP intermolecular interactions and solvation have been analyzed in terms of the radial distribution functions (RDF). Figure 4 shows the RDF issuing from the center of mass (COM) of solvent molecules (acetonitrile, tetrahydrofuran, *o*-xylene or cyclohexane) and the center of each of the three DPAP aromatic rings (RC). Noticeable differences are detected for ring **1** compared to ring **2/3**. In the case of acetonitrile (Figure 4, blue line), the first main peak located at approximately 5 Å is clearly higher for the unsubstituted aromatic rings. Such peak takes a height of 1.25 and 0.9 for ring **2/3** and **1**, respectively. A second less pronounced peak appears at 10 Å. A similar, but more structured, profile is found in cyclohexane (Figure 4, green line), where ring **1** is again less interacting with surrounding solvent molecules. The first peak is located at 6 Å of COM...RC distance. Three more peaks are observed at 11, 15 and 20 Å. The four peaks are smooth and well resolved, thus indicating a well-defined solvent structure, as already pointed out by previous theoretical and experimental studies on cyclohexane liquid solution. [44,45] The difference between ring **1** and **2/3** can be ascribed to the two cyano substituents, which turned away solvent molecules from ring **1** center. In case of THF and *o*-xylene solvents, the RDF profile is intermediate between the two more structurally different solvents, acetonitrile and cyclohexane, as can be admired in Figure 4. Ring **1** in particular, appears to be less solvated, compared to the cyclohexane case, with a peak height of 1.2 in both solvents. Looking at the other rings (**2** and **3**), the distribution is closer to the case of cyclohexane, though a lower interaction (of height 1.6 approximately) between ring center and THF COMs is detected.

To highlight specific interactions between the cyano groups and the solvent, the RDF between the cyano nitrogens and the solvent hydrogen atoms (i.e. the methyl hydrogen atoms of acetonitrile and the cyclohexane hydrogen atoms) has been considered. The

corresponding RDFs are depicted in Figure S4. The N...H intermolecular interactions are well established in acetonitrile, with a well-defined peak (height of 1.3) located at approximately 2.6 Å, with an integral value computed at the end of the peak of 11. These results indicate that nitrogens are well disposed to interact with up to four acetonitrile molecules. A second peak is present at 4 Å. For cyclohexane, no specific interactions have been found which is consistent with its molecular symmetry. In Figure S5 the same analysis has been reported for *o*-xylene and tetrahydrofuran. The methyl hydrogen atoms of *o*-xylene show the same profile already found for the acetonitrile, since both corresponding structures present at least a CH₃ group.

The acetonitrile solution was also analyzed to observe the opposite interaction, i.e. the one that involves DPAP aromatic ring H atoms and acetonitrile N atoms. In this case, acetonitrile acts as a hydrogen bonding acceptor. Figure S6 shows individual RDF for each H atom belonging to DPAP. Note that DPAP H atoms are considered equivalent under the assumption that the three rings may undergo free rotations. Steric obstruction and ring oscillations prevent solvent molecules from approaching H atoms in ortho position with respect to the tertiary amine nitrogen. This is the reason why the corresponding RDF are the lowest (see red, blue and green lines in Figure S6). The other hydrogen atoms instead are easily accessible and can interact with acetonitrile. In Figure S6 the hydrogen atom colored in black is the most disposed to interact with acetonitrile, being assisted by the nearby cyano groups. Indeed, cyano substituents act as hydrogen bonding acceptors, thus facilitating the interaction with the ring H atom. This concurrent interaction has been proved by the correlation map of combined RDFs, i.e. the N(DPAP)...H(ACN) and the H(DPAP)...N(ACN) RDF (ESI Figure S7).

3.3 DPAP in polymeric matrix and lipid bilayer

DPAP was docked into the cavity of a pre-equilibrated atactic PMMA matrix following a similar protocol of a previous study.[29] The DPAP structure was embedded into the polymer matrix avoiding close contacts with the surrounding polymer chains and the system was minimized via the steepest descent algorithm until a energy threshold of 0.5 kJ/mol was reached. Within the simulated time interval, DPAP remained trapped into the PMMA cavity, displaying no translational motion and little reorientational freedom (*vide infra*).

The main structural features characterizing the rotor within the PMMA matrix were described by evaluating the RDF between DPAP cyano N atoms and PMMA methyl H atoms, and between DPAP H atoms and PMMA carbonyl O atoms. Inspection of Figure S8 reveals a noticeable structure arising from the interaction of the polymer hydrogen atoms and the nitrogen atoms of the cyano substituents, with an average distance evaluated from the last 2 ns of the MD simulation of 3.15 Å. Other distinguishable RDF peaks, due to the tangled structure of the polymer bundle, are located at 4.4, 6.4 and 9.2 Å. By comparison, a labile interaction takes place between the polymer carbonyl O and DPAP aromatic H atoms with a first low peak at about 3 Å.

On the other hand, in the study of the DPAP/DOPC membrane system, an initial configuration was obtained from a previous equilibrated membrane configuration containing one cholesterol unit, in which cholesterol was replaced by DPAP. In the starting

configuration, DPAP was embedded within the lipid membrane at 2 Å depth from the hydrophilic interface. To characterize DPAP molecular dynamics in the hydrated DOPC bilayer, the lateral displacement and in-depth distance of the rotor from the lipid polar surface, as a function of time, were monitored (Figure 5). The lateral displacement (along the XY plane) shows a slow diffusive regime ≈ 15 Å in 100 ns of simulation. During the simulation, DPAP sequesters itself well within the dense membrane up to 13.43 Å from the lipid surface, which also accounts for its hindered rotations (see below). The average immersion distance was 7.05 Å which is less than half of the average bilayer thickness (38 Å). This result is consistent with the experimental evidence from a previous bioimaging study[46] in which DPAP was shown to localize preferentially within the cell membrane and into lipid vesicles according to its hydrophobic nature. A complete permeation was not observed within the present sampling, since it would require much longer timescales.

To analyze DPAP orientation within the lipid membrane during the MD simulation, the time evolution of the angle formed between the normal to the lipid bilayer and the normal to the DPAP NC1C1' C1'' moiety (see Figure 6) was evaluated. As can be noted in Figure 6, most of the time DPAP molecular plane was oriented at an angle around 30° (or equivalently 150°) with respect to the lipid bilayer orthogonal axis, undergoing only two rotational transitions during the 100 ns time interval. The present analysis provided evidence of the anisotropic effect of the lipid environment, which, coupled to the intrinsic viscosity of the lipid alkyl chains, has severely hindered DPAP rotational dynamics. This result is not surprising since it appears consistent with what it is known for other organic compounds once embedded into lipid membranes. This is the case of cholesterol[47] and polarity-sensitive probes as Prodan and Laurdan,[48] which have been studied using a free energy and an integrated QM/PCM and Molecular Field Theory approach, respectively. Another subtle structural effect due to the presence of DPAP into the DOPC membrane is the change of the lipid order parameter evaluated considering alternatively the first shell of lipids around DPAP or those not in close contact with the dye. Results are reported in Figure 7, where the deuterium order parameter for each of the two DOPC hydrophobic chain has been computed. The deuterium order parameter is a measure of the motional anisotropy of a particular C-D bond investigated and yields its time-averaged orientation during the MD simulation. It allows to obtain information about the structural orientation and flexibility of lipid chains in a bilayer. From inspection of Figure 7, it is apparent the structural effect caused by the introduction of the FMR within the membrane which especially acts on the chain 1 and on lipids closed to DPAP. Indeed a peak between carbon atoms 10 and 14 appears.

3.4 Comparison of the structural and dynamic features of DPAP in multiple environments

The chemical environments considered in this work cover a broad spectrum of complex molecular embeddings, ranging from apolar/hydrophobic to polar/high-permittivity solvents and from low-density to highly viscous environments, including also a non-homogeneous and anisotropic system (i.e. the hydrated lipid membrane). Furthermore, the structural complexity and molecular weight of the embedding molecules increase considerably from acetonitrile to PMMA. In this section, the influence of the surrounding medium on the dynamic and structural properties of DPAP was scrutinized in some detail.

First, DPAP mobility was evaluated in terms of its self-diffusion constant in all embeddings. With the exception of PMMA, noticeable translational motions were observed. The obtained diffusion constants, D , spanned several orders of magnitude (Table 2) as a result of medium viscosity and intermolecular interactions (as evidenced in the previous sections). In particular, a qualitative agreement with viscosity is apparent, as reported in Table 2. However, a quantitative relation, as predicted by the Stokes-Einstein equation[49] (which correlates the orientational diffusivity with viscosity) could not be obtained, likely due the formation of environment-specific interactions.

The distribution of the three flexible dihedral angles of DPAP (i.e. dihedral **1**, **2** and **3**, see description above), was evaluated as issuing from the MD simulations in all the considered environments. The angle distributions reflected the corresponding periodic torsional potentials based upon which the FF was derived, as shown in Figure S9. In all six MD simulations, DPAP selectively populated the three different torsional angles, with the highest occurrence falling within the minimum-energy configurations, while other geometries were progressively disfavoured according to the QM energy scan profile reported in Figure 3. In all cases except PMMA, the three aromatic rings were able to undergo a complete rotation, thus populating all minima predicted by the QM analysis. At this point, it is worth noting that DPAP rings may oscillate around their corresponding free energy minima but cannot rotate independently. Rather, structural transitions of the three dihedral angles may occur only in a concerted way, as shown in Figure S11 where the time evolution of the DPAP dihedral angles in cyclohexane is reported during 1 ns time interval. Such a coupled rotational motion is another sign of the steric hindrance among the aromatic rings.

In order to highlight the different intramolecular dynamics of DPAP in the selected environments, the time evolution and distribution of the dihedral angle **1** was evaluated and depicted along 1 ns in Figure 8 and Figure S12. In simple organic liquids (acetonitrile, cyclohexane, *o*-xylene and tetrahydrofuran) several complete rotations of the dicyano substituted ring were observed with ACN and cyclohexane yielding the fastest and lowest rotation rate, respectively. On the other hand, in the DOPC bilayer only a small amplitude oscillation (from -30° to $+30^\circ$) was noticed and in the PMMA matrix no transitions were observed. A more quantitative analysis along the entire MD trajectories was carried out by evaluating the time autocorrelation function (ACF) of ring **1** torsional angle (Figure S13). Overall, the same trend discussed above was observed. The corresponding rotational correlation times ($\tau_{\text{rot}}^{\text{dih}}$) suggested the slowest dynamics to occur in the PMMA matrix (see results in Table 2). Note that the transition frequency of DPAP torsional angle is to be considered a direct consequence of the interaction with the environment, in addition to the intrinsic viscosity of the medium.

Furthermore, to characterize DPAP molecular rotations, the ACF of the axis perpendicular to the NC1C1'C1'' group (i.e., three *ipso* carbon atoms marked in yellow in inset of Figure 9b) was evaluated as a function of time. The ACF in acetonitrile decays more rapidly than in all other systems, showing a rotational correlation time (τ_{rot}) of about 6.55 ps (Table 2), followed by THF ($\tau_{\text{rot}} = 13.5$ ps), cyclohexane ($\tau_{\text{rot}} = 62$ ps) and *o*-xylene ($\tau_{\text{rot}} = 84$ ps). This result appears to be consistent with the interactions between investigated solvent

molecules and DPAP rings highlighted in Figure 4. The rotational motions appeared highly retarded in the more viscous systems as the membrane or the polymeric embedding. Here, it is worth noting that DPAP maintained a higher degree of rotational freedom into the lipid bilayer than into the polymeric matrix (both intramolecular ring rotations and whole-molecule rotations, see Table 2). However, rotational (and translational) motions of DPAP in PMMA could not be sampled satisfactorily within the simulated time interval, owing to the high inertial mass of the polymeric matrix. Therefore, the estimated correlation times (i.e., $\tau_{\text{rot}}^{\text{dih}}$ and τ_{rot}) have to be considered qualitatively more than quantitatively.

Previously, DPAP fluorescence quantum yield was observed to follow approximately a Förster-Hoffmann relation[50] when plotted against viscosity in a set of low-dielectric and increasingly viscous solvents.[13] Here, excluding the DPAP/PMMA and DPAP/THF systems, a good degree of correlation between DPAP rotational dynamics (τ_{rot}) and the observed fluorescence lifetime (τ_{fl}) was noted in the same environments under investigation, as reported in Table 2 and depicted in Figure S14. This finding is consistent with the view that non-radiative processes are disfavored in more viscous and less interacting embeddings, thus enhancing the half-life time of the corresponding excited states. As a matter of fact, entrapping fluorescent dyes into nanoparticles, such as silica-based particles, is a fruitful strategy exploited in imaging applications to achieve extended emission lifetimes. In our study, the role of the environment in modulating DPAP fluorescence signal emerged as connected with the capability to hinder or enhance DPAP rotational dynamics.

3.5 Optical absorption spectra of DPAP

Theoretical absorption spectra of DPAP in all environments were evaluated by carrying out spectroscopic calculations, at the CAM-B3LYP/SNSD level of theory, on 200 molecular configurations extracted from MD trajectories. Spectra were generated from the convolution of vertical excitation energy calculations on the first few excited states using an empirical half-width-half-maximum (HWHM) parameter to better match experiments. The four liquids (for which an experimental counterpart is available) were modeled with the PCM and using a HWHM (σ in formula 7) of 0.2 or 0.1 eV. The simulated absorption spectra are depicted in Figure 10 and compared to the experimental counterparts taken from ref[13] (spectra are normalized for comparison). The main broad peak, located at 329 nm and 327 nm in acetonitrile and cyclohexane, respectively, corresponds essentially to the $S_1 \leftarrow S_0$ (see Figure S15) and $S_2 \leftarrow S_0$ transitions. The same transition accounts for the main peaks at 327 nm and 324 nm in the case of *o*-xylene and THF. In all solvents, the main band is well reproduced by our calculations: in acetonitrile, the theoretical spectrum is slightly redshifted by about 8 nm, while in cyclohexane deviation is only 3 nm. Similar deviations of 2 nm and 7 nm are observed for the other two low-dielectric solvents. A second peak, which has been assigned to the $S_3 \leftarrow S_0$ transition, appears at smaller wavelengths. The peak maximum is well defined in cyclohexane (292 nm) and *o*-xylene, and fairly reproduced by present computations (299 nm for cyclohexane; 298 nm for *o*-xylene). The same transition is not appreciable in acetonitrile as well as in THF, and the corresponding peak is merged in the first wide band. Finally, in the four solvents another optical band at shorter wavelengths resulted from calculations did not match an experimental counterpart in the considered region of the electromagnetic spectrum. Results on the maximum absorption peaks are

summarized in Table 3. Similitudes between experimental spectra are well preserved in the theoretical ones, confirming the DPAP solvent-independent features of this kind of spectroscopy within the accounted wavelength region.[51] To verify if further improvements could be achieved in modeling absorptions, the electrostatic embedding[52,53] (EE) was considered to describe solvent molecules during vertical energies computations. Within such paradigm, solvent residues with at least one atom within 20 Å from DPAP have been replaced by the respective atomic charge (according to the corresponding force field) during the QM computation. The EE wavelengths at maximum absorption for acetonitrile and cyclohexane were located at 341 and 327 nm, respectively. This result is in agreement with the PCM case for cyclohexane. Also for acetonitrile, anyway, the peak is in line with the previous investigation, since it is within the corresponding statistical error reported in Table 3. Despite the absorption spectra of DPAP recorded in various solvents were found not to change substantially, in contrast to the emission ones, their theoretical reproduction was not expected to be trivial, since excitation energies are quite sensitive to the dihedral angle **1** both in polar and apolar solvents.[13] Hence, it was largely important to base spectroscopic calculations on reliable molecular structures which also provide a representative sampling of the FMR configurational space.

Charge transfer index was computed for the FMR to check how the spatial extent of the excitation is influenced by the surrounding environment. CT index is based only on the computed electron density for both ground and excited states.[41] The extent of the electronic rearrangement is defined as the distance between the barycenters of the density increment and depletion regions upon electronic excitation. Attention was focused on acetonitrile and cyclohexane, as they are the extremes in the investigated polarity scale. In acetonitrile, the computed CT length is 2.728 Å whereas in cyclohexane, i.e. lower dielectric medium, the same parameter is 2.173 Å. The obtained charges are 0.66e and 0.62e for acetonitrile and cyclohexane, respectively. A graphical representation of the charge centroids is reported in ESI (Figure S16).

Absorption spectra were also computed in the PMMA polymeric matrix and DOPC membrane (HWHM value of 0.2 eV), as depicted in Figure S17, even if no experimental counterparts were available in this case. These two environments are characterized by low dielectric permittivity: PMMA is about 2.8-3 and a similar value can be predicted for the membrane, since DPAP is embedded in the lipophilic layer throughout the MD simulation (Figure 5). In both cases, the dielectric medium was simulated by adopting the butanoic acid ($\epsilon = 2.9931$) within the PCM formalism. Note that PCM was already shown to well reproduce the electrostatic effects of PMMA environment in a previous work.[29] Maximum absorption wavelength was found at about 321 nm for both systems, in line with the calculations for the solvents reported above. The other two peaks take place at about 298 and 268 nm and appear as shoulders of the main first absorption band. However, in the membrane case, the decay at lower wavelength values is faster, and the $S_3 \leftarrow S_0$ transition peak is better defined. It is worth noting that such subtle differences are only due to the DPAP configurations extracted from MD simulations, since the description of the environment (i.e. dielectric continuum) is the same.

4 Conclusions

A molecular model of DPAP, a recently proposed FMR fruitfully employed in various imaging and detection applications, has been presented and investigated through extensive MD simulations in different environments (acetonitrile, tetrahydrofuran, *o*-xylene and cyclohexane solutions, a hydrated DOPC lipid bilayer and a PMMA polymeric matrix). In each case, DPAP has shown peculiar structural and dynamical features, as well as specific interactions with the environment. In the lipid membrane, DPAP has displayed an anisotropic molecular orientation and a jump-diffusion rotational dynamics. This seems consistent with the observed alignment of cholesterol and other organic compounds, once embedded into lipid bilayers. Moreover, the rotor has induced local deviation in the lipid order parameter, a result that may suggest the use of DPAP for detecting membrane structural rearrangements, a rather hot topic in cell membrane biophysics. The reliability of the present DPAP model was further tested by simulating the absorption optical spectra including the effect of the embedding, obtaining an overall good agreement with available spectroscopic data.

The subtle effects issuing from medium viscosity and environment-specific interactions on the dynamic properties of the rotor have been especially highlighted and discussed in view of DPAP molecular mobility. Self-diffusion constant and rotational correlation time of the present FMR are, indeed, strongly modulated by the environment to the extent that they may vary by several orders of magnitude. This is of particular interest since the rotational relaxation of fluorescent dyes can be related to various optical properties, such as fluorescence lifetime, emission intensity, fluorescence depolarization, etc., as well as to properties of the micro-environment, such as the viscosity. Here, a simple quantitative relation between the viscosity of the embedding medium and the FMR dynamics could not be obtained. This result comes as no surprise since the existence of specific dye-environment interactions, the non-continuous rotational dynamics and the shape of the rotor are all factors contributing to appreciable deviations from the ideal Stokes-Einstein-Debye model, as already noted in previous studies (see, e.g., [54–57] where the influence of molecular structure on both viscosity and rotational relaxation times is discussed).

Nevertheless, the present study has unraveled a possible correlation between DPAP rotational correlation time and its fluorescence lifetime in all considered environments (except PMMA and THF): the more retarded the rotational relaxation, the longer the emission life-time. Intriguingly, this finding may provide a molecular insight on the effective control exerted by the FMR rotational dynamics towards the competition between radiative and non-radiative decay processes, which ultimately modulates the dye fluorescence signal. Accordingly, specific intermolecular interactions more suited to interfere with molecular rotations seem to be the key factor modulating the emission response of the present FMR. While this point would necessitate further investigation and validation, for example by modeling the dye excited state and emission dynamics, if confirmed it could be used to gather detailed molecular dynamics information on the dye, as well as on its interactions with the environment, through standard spectroscopic techniques.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) 4-(diphenylamino)phthalonitrile (DPAP) structure. Configurations of DPAP in some of the studied environments; only acetonitrile (b), cyclohexane (c), hydrated 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (d) and poly(methyl methacrylate) polymeric matrix (e) are shown.

Figure 2. DPAP propeller-like conformation is indicated with green vectors. The rings centers (**1**, **2** and **3**), the *ipso* (C1, C1' and C1'') and the *ortho* (C2, C2' and C2'') carbon atoms are labeling in red and black respectively.

Figure 3. Potential energy curve of dihedral angle **1** (panel (a)) and **2/3** (panel (b)). QM data, black points; MM data, continuous lines.

Figure 4. Radial distribution functions between DPAP ring centers (i.e., **1**, panel (a); **2**, panel (b); **3**, panel (c)) and acetonitrile (blue), *o*-xylene (magenta), tetrahydrofuran (cyan) or cyclohexane (green) center of mass.

Figure 5.

Displacement in XY plane (a) and Z dimension (b) of DPAP within the membrane. DPAP permeation within the membrane was estimated based on the distance between DPAP centroid (blue circle in b) and centroid of upper-layer phosphates (red circle in b).

Figure 6.

Evolution of the angle between the normal to the plane of lipid bilayer hydrophilic interface (vector in blue) and the axis perpendicular (vector in orange) to the plane defined by the three *ipso* carbon atoms (in yellow in insert) during 100 ns of simulation.

Figure 7.

DOPC deuterium order parameter of the alkyl chain 1 (a) and alkyl chain 2 (b). The nomenclature is indicated in insert of panel (a). Black: lipid molecules far from DPAP; red: lipid molecules close to DPAP.

Figure 8.

Time dependent dihedral distribution function for dihedral angle **1** for the first ns of simulation in ACN (a), cyclohexane (b), hydrated DOPC lipid bilayer (c) and PMMA polymeric matrix (d).

Figure 9.

(a) Mean square displacement (nm²) of DPAP in acetonitrile (blue line), tetrahydrofuran (cyan line), *o*-xylene (magenta dashed line), cyclohexane (green line), DOPC bilayer (red line) and PMMA (violet line). **(b)** Autocorrelation function of the vector (computed as reported in Section 1 of the ESI) perpendicular to the plane defined by the three *ipso* carbon atoms (in yellow), shown in the insert.

Figure 10.

Comparison between theoretical (a) and (c) and experimental (b) and (d) absorption spectra of DPAP in acetonitrile (continuous blue line), cyclohexane (dashed green line), *o*-xylene (dashed magenta line) and tetrahydrofuran (continuous cyan line).

Table 1

Technical details of the performed Molecular Dynamics simulations.

Environment	N. of molecules	Box edge (nm)	Force Field	Density (kg/m ³) ^a
Acetonitrile	3053	6.62	OPLS-AA[24]	750.6±0.1 (776.0[58])
THF	962	5.24	OPLS-AA[24]	756.2±0.1 (778.1[58])
<i>o</i> -Xylene	556	4.82	OPLS-AA[24]	849.2±0.1 (880.0[59])
Cyclohexane	997	5.69	GAFF[28]	887.2±0.1 (875.8[60])
DOPC bilayer	200 DOPC 5791 H ₂ O	8.27 x 8.27 x 6.26	CHARMM[31]	-
PMMA	2 chains of 2920 monomers	9.45	OPLS-AA[24,29]	-

^aIn parenthesis are reported the experimental values.

Table 2

Dynamical and spectroscopic properties of DPAP.

Environment	D ($10^{-5} \text{ cm}^2 \text{ s}^{-1}$)	τ_{rot} (ps)	$\tau_{\text{rot}}^{\text{dih}}$ (ps)	μ (mPa s)	τ_{fl} (ns)[13]
Acetonitrile	3.68 ± 0.02	6.55 ± 0.06	5.91 ± 0.07	0.344[61]	2.61
THF	1.54 ± 0.03	13.5 ± 0.9	12.8 ± 0.2	0.47[62]	12.9
o-Xylene	0.18 ± 0.02	84 ± 7	80 ± 5	0.81[63]	12.5
Cyclohexane	0.17 ± 0.03	62 ± 4	62 ± 4	0.887[64]	9.16
DOPC bilayer	0.024 ± 0.006	173 ± 67	151 ± 66	134-195[65]	14.3
PMMA	-	28600 ± 8100	11000 ± 7000	$\sim a$	12.3

^aThe PMMA viscosity has not been reported since the corresponding value strictly depends on the chain lengths.[66] In general, polymer viscosity is determined upon dissolution in a solvent.[67–69]

Table 3

Maximum absorption peak wavelength (nm)

Environment	Absorption peak (nm)	
	Theory	Experiment[13]
Acetonitrile	329 ± 20	321
THF	331 ± 18	324
o-Xylene	325 ± 17	327
Cyclohexane	327 ± 15	324
DPOC	323 ± 12	-
PMMA	321 ± 13	-