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Development of a New Dual Polarity and Viscosity Probe Based on the Foldamer Concept

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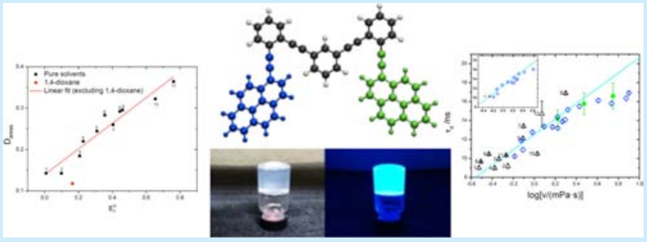
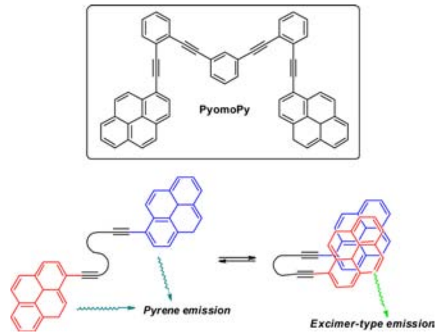
Supporting Information

ABSTRACT: Small molecular probes able to act as sensors are of enormous interest thanks to their multiple applications. Here, we report on the development of a novel supramolecular dual viscosity and polarity probe based on the foldamer concept, which increases the resolution limits of traditional probes at low viscosity values (0−4 mPa·s). The applicability of this new probe has been tested with a supramolecular organogel.



Fluorescent molecular probes are a type of structure whose photophysical properties are mainly influenced by the local surrounding media.[1] Despite their tremendous success as chemosensors and biological imaging, the possibility of using the same probe as a multisensor at the microscale is less common.[2] Interesting examples are fluorescent molecular rotors (FMR), in which changes in the viscosity or polarity of the media have been correlated with spectral shifts and/or variations in the intensity of fluorescence peaks.[3] Although of great utility, these FMRs usually change their emission properties when the viscosity of the surrounding media is higher than 10 mPa·s.[4] Thus, the development of better molecular probes for low viscosity values may require the design of novel structures able to operate by a different mechanism of action. In this sense, in a seminal work, Moore was able to relate the folding process of functionalized *m*-phenylene ethylene oligomers with the solvent polarity (solvent helicogenicity).[5] This result suggests that foldamers could behave as molecular polarity probes. Moreover, the emission process of folded structures could be additionally related to other properties of the surrounding media. Thus, for example, highly emissive species, such as pyrene−pyrene excimer-type structures, may be less easily deactivated in viscous media, thus yielding a new type of molecular viscosity probe.[6]

Scheme 1. Working Hypothesis and Structure of PyomoPy



Within our recent interest in foldamers,[7] we have synthesized a bis-pyrenephenylene ethylene oligomer, **PyomoPy**, a dynamic system with different emissive species, which is able to behave as a new dual viscosity and polarity molecular probe (Scheme 1). **PyomoPy** was selected due to its broad solubility and the absence of excited-state dynamics characterized by fluorescence rise times in time-resolved fluorimetry.[7b] The complete photophysical study of **PyomoPy** allowed the simultaneous detection of the emission derived from folded (excimer-like) and unfolded states (pyrene-type).[8] The recorded UV−vis absorption (Figure S1, Supporting Information) and fluorescence spectra (Figure S2) displayed broad, multiple bands, with differences in the relative intensity depending on the solvent. This behavior had been previously described for *o*-[9] and *m*-oligophenylene ethynylenes[10] (OPEs) lacking the pyrene moiety and is related to the existence of folded and unfolded species with different emission features. In our case, the emission is enhanced by the presence of the pyrene subunit. The corresponding spectra are not concentration dependent, guaranteeing that the observed phenomena have an intramolecular nature and do not come from any aggregation event.

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To understand the relationship between the dynamic behavior of **PyomoPy** and the solvent nature, we explored the complex topology of the ground-state potential energy surface by optimizing representing conformers belonging to any of these four categories, unfolded (type A) or folded (type B−D) species and calculating their relative stabilities (Figure 1, Table S1). The largest energy gap between the unfolded conformers was calculated to an amount up to 2.86 kcal mol^{-1}. Folded species B-D were destabilized by 0.67−1.37 kcal mol^{-1} with respect to the most stable unfolded A. These results imply that the observation of folded states in solution is mediated by the solvent and not by $\pi-\pi$ interactions.

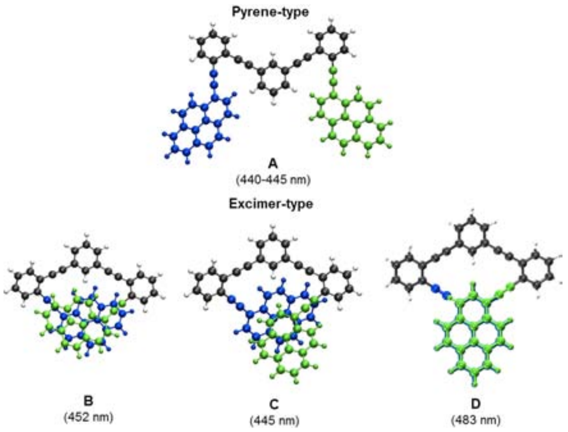

Figure 1. Unfolded (A) and folded (B−D) conformers of PyomoPy and their TD-CAM-B3LYP/6-311+G** calculated emission energies in hexane.

Samples of **PyomoPy** in different solvents were excited at 405 nm with a ~90 psfwhm pulsed diode laser (Edinburgh EPL405). The fluorescence decay traces exhibited four different decay times, and consequently four main transitions, which were responsible for the shape of the fluorescence spectra. The time-resolved emission spectroscopy (TRES) analysis, in the 420−570 nm range ($\Delta\lambda$ = 3 nm, Figure 2 and Figure S4, Supporting Information) confirmed the presence of four emissive species (Table S4). Taking into account the magnitude of the experimental lifetimes, we assigned the shortest decay time (τ_1) to the unfolded type-A structures and the largest three decay times (τ_2, τ_3, and τ_4) to the excimer-like, folded structures B−D. The areas of the species-associated emission spectra (SAEMS) (Figure 2 and Figure S5) provide a simple manner to quantify the relative amount of each one of these species (see below).

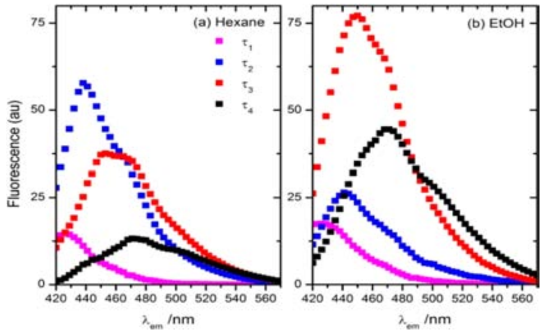

Figure 2. TRES deconvolution of the SAEMS associated with the shortest (τ_1, magenta), short intermediate (τ_2, blue), long intermediate (τ_3, red), and the longest (τ_4, black) decay times of PyomoPy in (a) hexane and (b) EtOH.

The assignation of the emission bands with the corresponding S_1 excited-states TD-DFT calculations using CAM-B3LYP functional[11] were performed. Taking into account the relevance of the solvent effects in our experimental study, the spectra were recomputed considering the chromophores immersed in a continuum dielectric medium (hexane and MeOH).

The results were essentially the same and any observed difference in the emission spectra was attributed to the intrinsic photophysical properties of the system and not to direct solvent effects in the emission properties of A-D (Table S2). Calculations showed the existence of three structures: unfolded type-A rotamers (emission at 440−445 nm, and a vibrational band at 400 nm) and folded B and C (B transition at 452 nm; C at 445 nm) with very close vertical emission wavelengths. Folded structure D showed calculated emission wavelength at 483 nm, which is clearly different from the others. These results are in excellent agreement with those obtained in the TRES experiments (Figure 3). Hence, the experimental band centered at 475 nm with a shoulder at 510 nm is assigned to the folded species D emitting at 483 nm according to TD-DFT. Interestingly, this structure was calculated to be 1.37 kcal mol^{-1} intrinsically less stable than the most stable unfolded species type-A (Table S1). Therefore, detection of D suggests that its formation is favored by the presence of solvent. Calculations also allowed knowing the relative intensity of the bands from the oscillator strength f (Table S2). For pyrene type-A emissions the factor ranges in hexane from 0.72 to 1.32. The excimer-type emission of B and C showed similar values: B, 0.52, and C, 0.72. Excimer-type emission of D was much less favorable with an f value of 0.1. The significant intensity of the emission band attributed to D in the emission spectra with such low f value suggests that this folded conformation is dominating the population of the conformational equilibrium. As expected, the calculated f values in MeOH were the same as in hexane with differences of less than 4%, allowing the comparison of the results between the different solvents (Table S2). Thus, we focused our attention in the areas of the SAEMS from the TRES experiments, which were solvent dependent, to quantify the conformational equilibrium. In particular, we employed the area under the D-band for three main reasons: (a) the transition is well separated; (b) the D band is almost totally confined in the range of wavelengths examined;

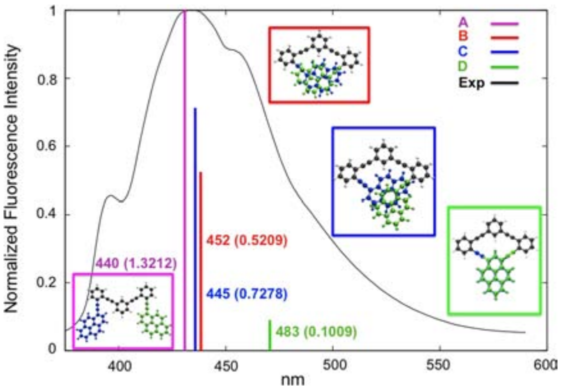

Figure 3. Calculated stick spectrum for conformers A−D superimposed on the experimental emission spectra of PyomoPy. The height of the sticks centered at the position of the vertical emission wavelength is scaled according to the computed relative oscillator strengths.

and (c) on the basis of the *f* value and the area of this band, this was the main folded species in solution.

As initially suggested by Moore for functionalized *m*-OPEs,[5] the amount of folded species could be efficiently correlated with the normalized empirical solvent polarity parameter (E_T^N).[12] The representation of the contribution of the folded D form versus the E_T^N parameter shows a clear linear relationship (Figure 4a, R^2 = 0.93) for practically all the studied solvents with polarities ranging from 0 to 0.8, including MeOH, EtOH, halogenated (CH_2Cl_2), apolar alkanes (hexane), and aromatic solvents (toluene).[13] The only exception was 1,4-dioxane for which the experimental result is slightly lower to the expected one owing to its polarity. Changes in the conformation of this solvent in the proximity of the probe could explain the change in its local polarity. In any case, the inclusion of such solvent in the relationship diminishes the adjustment to a remarkable R^2 = 0.88. It is worth noting that exceptions observed by Moore in his previous work (CH_2Cl_2 and polar protic solvents) notably lie in the linear trend in our case. Therefore, an estimation of the polarity of a solvent can be determined using a TRES analysis of this compound, which represent a new approach to this kind of measurements.[1]

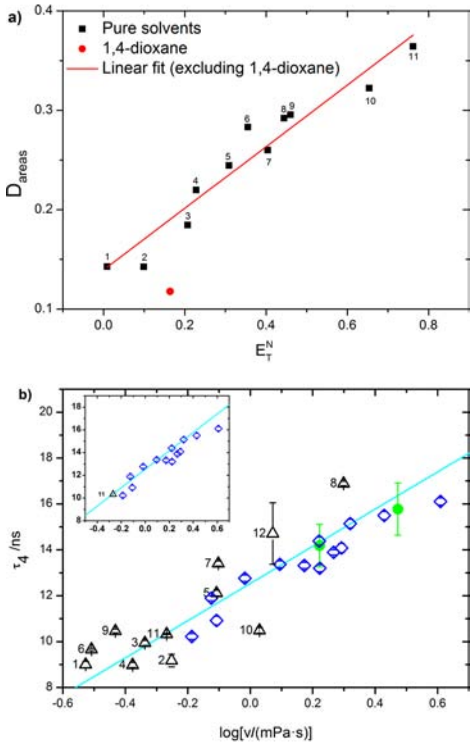

Figure 4. (a) Relative area of folded D forms (D_{areas}) vs E_T^N parameter. Solvents used (black squares) and red symbol: 1,4-dioxane. (b) τ_4 vs log (viscosity) of **PyomoPy** in pure solvents (black triangles), MeOH/glycerin mixtures (blue diamonds), and EtOH/glycerin mixtures (green circles). The linear fit (blue line) excludes ethanol. Inset shows MeOH/glycerin mixtures. Solvents: (1) *n*-hexane, (2) toluene, (3) THF, (4) AcOEt, (5) CH_2Cl_2, (6) acetone, (7) DMF, (8) DMSO, (9) MeCN, (10) EtOH, (11) MeOH, (12) Dioxane.

This simple compound has confirmed the seminal idea of Moore that polarity of the solvent is responsible for the folding process in abiotic OPE-based foldamers. Nevertheless, the stabilization does not come from favoring $\pi-\pi$ interactions as previously suggested. On the basis of our results, the stabilizing effect derives from the capability of this molecule to adopt a shape able to diminish its hydrophobic surface, as suggested by Iverson.[14] In that sense, a pure solvophobic effect is able to control the thermodynamics of the system even when $\pi-\pi$ interactions are unfavorable. The magnitude of the longest decay time, τ_4, which corresponds to the folded D form, is solvent dependent but does not correlate with the polarity. In a simple approximation, the increase of the lifetime can be related with the rigidity of this folded species[15] in the corresponding media, which could be correlated with the viscosity. Accordingly with this hypothesis, τ_4 showed a general linear dependency with the solvent viscosity in log scale up to 4 mPa·s (Figure 4b, R^2 = 0.85). Beyond this viscosity value, τ_4 plateaus, showing a saturation effect (Figure S6 and Tables S5 and S6). In this low viscosity range, the decay time varies from 9 to 17 ns, which is a wider range than the reported ones in FMRs (usually 0.01 to 0.04 ns).[3] The only exception was ethanol (solvent 10 in Figure 4b), in which rising times were detected in the fluorescence decay traces. When the viscosity was slightly increased in ethanol:glycerin mixtures the dynamic situation disappears and the expected decay times were recovered. Especially interesting is the exceptional linear relationship showed in methanol:glycerin mixtures of different viscosity (Figure 4b inset, R^2 = 0.99).This mixture has been previously used as a mimic of intracellular media and points out the potential utility of this kind of viscosity probe in biological applications. It is worth noting that the viscosity of cytosol has been estimated to be 1−3 mPa·s.[16] Remarkably, the correlation is mantained between 20 and 40 °C (Figure S7). The values of τ_4 at higher temperatures were slightly smaller than at 20 °C, due to the enhancement of nonradiative deactivation pathways. This demonstrates that the use of τ_4 is a robust approach to measure microviscosity values, even at different temperatures.

The applicability of **PyomoPy** as microviscosity probe was tested with a supramolecular organogel. Gels are solid-like liquids in which their macroscopic properties (e.g., viscosity) are clearly different from the confined liquid. Recently, supramolecular gels have flourished thanks to their multitude of anticipated applications.[17] Although the macroscopic properties of these materials have been studied in depth, their behavior at the microscopic level is less well-known. This knowledge is very useful taking into account their described applications derived from the stabilization and diffusion of key molecules in their interior.[17] For example, the process of crystal growth in gels is affected by the diffusion of the compounds in the gel matrix,[18] which is directly related with the viscosity of the media at local scale. Bearing this idea in mind, we selected an urea-based organogelator (Figure 5a) able to form homogeneous gels in organic solvents, such as toluene, and used as media to grown organic crystals.[19] **PyomoPy** was incorporated to the organogel mixture, composed of 12 mg of organogelator and 2 mL of toluene (Figure 5b), heated, and left for slow cooling. The fluorescence decay of **PyomoPy** in the organogel showed four

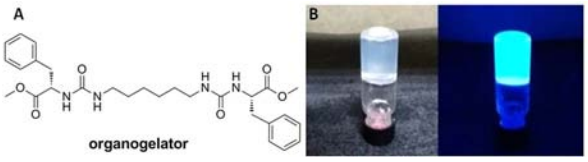

Figure 5. (A) Structure of the urea-based organogelator and (B) organogel with PyomoPy under white light (left) and UV light.

different decay times. The longest decay time was 9.9 ± 0.3 ns. This value did not change versus pure toluene, showing that the local viscosity of the gel is not affected by the fibrous aggregates. Interpolating the τ_4 value in the linear fit equation provided an estimation of the microviscosity of the medium to be of 0.473 ± 0.014 mPa·s. The understanding of the properties of the confined liquids in these gels or other materials is relevant in the development of composite materials in which the liquid component has an active role, such as hydrogels for biological applications. Our method provides a tool for the characterization of such gels.

In summary, a novel dual viscosity and polarity molecular probe based on a new mechanism of action has been presented, studied and compared with traditional FMR probes. We have found that the solvent-dependent folding dynamics of compound **PyomoPy** actually depends on the polarity (E_T^N) of the solvent ($R^2 = 0.93$) and its emission behavior (fluorescence lifetime) can be efficiently correlated to viscosity with an exceptional fluorometric response in the low viscosity regime (0−4 mPa·s). Additionally, we have determined the microviscosity of an organogel composed of an urea-based organogelator and toluene. The compatibility of this probe with novel supramolecular gels opens the possibility to monitor the liquid component of these interesting materials.This novel approach based on the foldamer concept lays the groundwork for a new family of molecular probes.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General experimental details. Synthesis of all new substrates. ^{1}H NMR and ^{13}C NMR spectra of new compounds. Photophysical and theoretical data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01275.

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Notes

The authors declare no competing financial interest.

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