Gel Trapping Enables Optical Spectroscopy of Single Solvated Conjugated Polymers in Equilibrium

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Supporting Information

ABSTRACT: Single-molecule studies have provided a wealth of insight into the photophysics of conjugated polymers in the solid and desolvated state. Desolvating conjugated chains, e.g., by their embedding in inert solid matrices, invariably leads to chain collapse and the formation of intermolecular aggregates, which have a pronounced effect on their properties. By contrast, the luminescent properties of individual semiconducting polymers in their solvated and thermodynamic state remain largely unexplored. In this paper, we demonstrate a versatile gel trapping technique that enables the chemistry-free immobilization and interrogation of individual conjugated macromolecules, which retain a fully equilibrated conformation by contrast to conventional solid-state immobilization methods. We show how the technique can be used to record full luminescence spectra of single chains, to evaluate their time-resolved fluorescence, and to probe their photodynamics. Finally, we explore how the photophysics of different conjugated polymers is strongly affected by desolvation and chain collapse.

KEYWORDS: conjugated polymers, single-molecule spectroscopy, photodynamics, gel entrapment, single-molecule fluorescent lifetime imaging

Conjugated polymers, macromolecules with a semiconducting backbone, have been studied extensively in the past decades. Until very recently, the main interest in this class of luminescent macromolecules was in the creation of solid-state optoelectronic materials and devices, such as photovoltaics,1−3 light-emitting diodes,4−8 and memories.9−11 More recently, it has become clear that the delicate coupling between chain conformation and photophysical properties of these chains can be exploited in solution to create sensitive optical molecular sensors,12−17 for example to accurately detect enzymatic proteolysis,18 reveal the presence of small quantities of nucleic acid polymers,13,19,20 or illuminate the pathways of protein self-assembly.12,21 Very recently, our group has demonstrated that the coupling of emission patterns to chain conformation can even be used to create ultrasensitive molecular tension sensors, which can reveal sub-picoNewton forces down to the scale of single macromolecular chains.22

Single-molecule luminescence spectroscopy has been an extraordinarily valuable tool to elucidate the complex photophysics of semiconducting polymers.23−28 To interrogate a single semiconducting chain, it needs to be immobilized, to prevent diffusion of the chain away from the interrogation window, and separated from neighboring chains, to ensure that the optical information that is obtained derives from an isolated and single chain only. Conventionally, this is done by embedding the conjugated polymer of interest at very low concentrations in a nonfluorescent and optically transparent solid matrix, e.g., in poly(methyl methacrylate) (PMMA), polystyrene, or Zeonex.26,27,29−31 Samples are typically prepared by drop casting or spin coating, during which a common solvent for matrix and semiconducting polymer evaporates and traps the conjugated chains in the glassy matrix.

Received: August 5, 2019
Accepted: October 24, 2019
Published: October 24, 2019

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is further evidenced by the fact that the photophysics of the chains shows a strong sensitivity to the processing and annealing conditions\textsuperscript{31,37,38} and choice of matrix.\textsuperscript{23,26,36,39}

By contrast, in single-molecule experiments on biomolecules, where solvation is crucial for molecular function, immobilization is often achieved by means of interfacial attachment using methods from surface chemistry.\textsuperscript{40−42} Only very recently has this approach been applied to probe the photodynamics of single conjugated polymer chains of polythiophene.\textsuperscript{43} Yet, this approach is experimentally taxing, as it requires chemical modification of the macromolecular species of interest in a dedicated synthesis and passivization of the surface, which is challenging for semiconducting polymers that have strong interactions with solid surfaces.\textsuperscript{44} Moreover, the coupling of polymer chains to solid substrates is known to affect their conformation and conformational dynamics.\textsuperscript{45−47} These limitations and the growing interest in solution-based diagnostics using semiconducting polymers pose the need for approaches to evaluate the full photophysics of semiconducting polymers in a solvated state. This raises two distinct requirements: first, solvent exchange dynamics must be rapid to mimic the pure solvent surroundings of diagnostic experiments while, second, restricting the center-of-mass motion of chains sufficiently, without the necessity for chemical modification of the chains and free from the potential perturbations caused by solid substrates.

In this paper, we present a simple and versatile gel trapping method that enables the study of single semiconducting polymers in their native solvated state, without requiring chemical modification. We exemplify that it can be used to record complete luminescence spectra of individual semiconducting chains and measure their excited-state lifetime and photodynamics. We find that desolvation has a drastic effect on both the mean photophysical properties and the population heterogeneity, which is attributed to the non-equilibrium conformations that emerge during chain collapse. Finally, we employ this method to explore the origin of large-intensity jumps often observed in the emission of a prototypical organic emitter, poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylenevinylene] (MEH-PPV). These intermittent photodynamics are sometimes attributed to emergent quenching chromophores that form during chain aggregation.\textsuperscript{23,26} Our results show that there is no strong apparent correlation between solvation quality and the extent of these intermittent intensity fluctuations. Our approach offers a simple yet versatile platform for the immobilization and optical interrogation of luminescent macromolecules in a thermodynamically equilibrated state.

**RESULTS AND DISCUSSION**

In our search for a facile method to immobilize semiconducting polymers while allowing continuous solvent exchange, we study two different semiconducting polymers. The first is MEH-PPV, whose absorption and emission spectra are shown in Figure 1a, a well-studied and prototypic organic semiconductor.\textsuperscript{23,26,36} The second is an acceptor-doped polyfluorene, which we have previously shown to be suited as a molecular tension sensor,\textsuperscript{22} which exhibits both donor emission in the blue and acceptor emission in the red (Figure 1b). To immobilize these chains while allowing them to retain a solvated state and rapid solvent exchange, we trap the semiconducting polymers in a solvent-swollen gel made from...
polydimethylsiloxane (PDMS). These trapping gels are optically transparent, exhibit a pore size that can be tuned by the cross-linking density, and can be prepared and swollen in a variety of common solvents for both PDMS and semiconducting polymers, such as toluene, chloroform, and tetrahydrofuran (THF).

The liquid precursor of the PDMS gel and a minute amount of conjugated polymer are dissolved together in a common solvent of choice; this liquid is loaded into a glass sample chamber, which is hermetically sealed to avoid solvent evaporation; within a few hours, the gel network polymerizes around the semiconducting polymers, forming a porous solvogel as the trapping matrix. These gels contain 50 wt % solvent and 50 wt % PDMS chains with an estimated network mesh size of ∼10 nm, based on the density of cross-links formed. To verify the suppression of center-of-mass diffusion of the chains, we perform a fluorescence recovery after photobleaching (FRAP) experiment on a solvogel with a large concentration of conjugated polymers; indeed, no recovery of the luminescence occurs in the bleached zone, even after 30 min (Figure 1c). This indicates the effective suppression of macromolecular diffusion within the gel. By contrast, the same experiment repeated with a low molecular weight fluorophore, with dimensions of the order-of-magnitude of the solvent, reveals a very rapid recovery of fluorescence within several seconds (Figure 1d). Thus, while the gel traps the larger macromolecular chains, small molecules, such as solvent, can freely diffuse throughout the matrix (see SI for control measurements in a solid matrix). This is in line with previous observation that solvent dynamics in gels with sufficiently large pore sizes is virtually unhindered.

Since each semiconducting macromolecule contains multiple chromophores, we use the established approach of a linear scaling between number of spots and concentration to establish the concentration limit in which the chains are well-dispersed and can be observed in isolation. We record diffraction-limited images (Figure 1e,f) of the two different conjugated polymers embedded in the gel matrix on a home-built hyperspectral confocal laser scanning microscope (see SI). We find a linear relationship in the number of spots counted per frame and the concentration when the conjugated polymer concentration in the gel remains <50 pM; in our experiments we work well within this regime at 10 pM (see Figure 1g).

We commence our exploration by studying the effect of solvation on the photophysics of the well-studied conjugated polymer MEH-PPV (Figure 1a). It is well-known that MEH-PPV emission is sensitive to the local environment. For single diffraction-limited images of an individual MEH-PPV chain, we record a full luminescence spectrum with a spectral resolution of ∼1 nm (see Experimental Details and SI). We find that the luminescence spectra of single chains trapped in their gel matrix (example shown in Figure 2a, additional spectra shown in the SI) are very comparable to ensemble-averaged emission spectra recorded in toluene solutions on a fluorescence spectrometer. They exhibit peak emission at ∼540 nm and show distinct vibronic transitions, which are also visible in the single-molecule spectra as a shoulder at ∼600 nm.

To exemplify the effect of desolvation on MEH-PPV photophysics, we take these same samples and remove the toluene from the solvogel to result in desolvated conjugated polymers trapped in an inert matrix. This results in two distinct changes in the single-molecule spectra (Figure 2b; for additional spectra see SI, including a comparison to MEH-PPV in solid-state PMMA). First, we observe that the emission maximum is blue-shifted by approximately 30−40 nm; as the solvent is removed, the chains undergo intramolecular collapse, which introduces additional curvature in the backbone and
reduces their conjugation length (blue shaded area in Figure 2c).32,35,50 Second, we observe the appearance of a red emission band (red-shaded area in Figure 2c). This has been extensively described in the literature, in particular at low temperatures, in both experiments and simulations.50−52 The appearance of this red emission band is ascribed to increased ordering within the chain, which increases the conjugation length. Köhler et al. describe these phenomena as a second-order phase transition that occurs within MEH-PPV chains.50 A threshold local segment density of MEH-PPV is necessary to trigger this transition,50−52 which could explain the emergence of the red emission in our experiments. Removal of toluene from our solvogel while MEH-PPV remains trapped in the PDMS mesh will lead to chain collapse and the growth of unfavorable interactions with the PDMS matrix. Since this collapse leads to an increase in the local segmental density within the coil, this could trigger the formation of a more ordered state from the disordered equilibrium conformation of the polymer in solution. We observe both increased blue emission and increased red emission within single chains, suggesting that both sharp turns within the polymer occur and longer, ordered regions are formed due to collapse, all in an effort to minimize the exposed polymer contact area.50

To quantify these changes, we record single-molecule spectra for a large number of individual chains (N = 201 for solvated and N = 138 for desolvated chains) and extract the wavelength of maximum emission. Histograms of the emission maximum show a clear shift of 40 nm in the emission maximum as a result of solvent removal (Figure 2d,e). To quantify the emergent red emission, we measure the integrated red emission between 650 and 700 nm, normalized to the overall emission intensity. Also here, we see a substantial increase in emission as a result of desolvation, comprising as
much as 50% of the total emitted intensity (Figure 2f,g). These results indicate not only that desolvation has a drastic effect on the photophysics of the semiconducting chains but that intramolecular ordering and collapse are effectively suppressed in our gel trapping approach.

Since our gel trapping approach is suitable to any semiconducting polymer that has a common solvent with our PDMS solvogel and that is large enough to become trapped in the network pores, we can readily apply the same approach to different chemistries, without the need for any chemical modifications of the polymers under study to enable surface immobilization. The effect of solvation on chain conformation becomes even more apparent in acceptor-doped semiconducting polymers, previously used as a molecular tension sensor. Our design consists of a donor backbone of dialkylfluorene, doped with a minority fraction of the red-emitting acceptor dithienyl benzothiadiazole (DTBT) (Figure 1b).

In a swollen and solvated state, these semiflexible polymers adopt a coiled conformation, in which the local segmental density is relatively low (Figure 3a) and should exhibit a Gaussian distribution. This should lead to a low energy transfer efficiency between fluorene donors and DTBT acceptors with a normal distribution. As the chain collapses, the local segmental density increases and becomes more inhomogeneous, thereby increasing the efficiency of energy transfer and population heterogeneity (Figure 3b,c). These molecular sensors probe changes in chain conformation at the scale of the energy transfer radius $R_0$. As such, these doped conjugated polymers are very sensitive to not only mechanical strain but also the solvency of their local environment. This should provide additional proof that our gel trapping method allows us to interrogate single conjugated polymers in truly solvated conditions.

To probe the effects of desolvation on the chain conformations, we compare three different scenarios: (i) embedding in a polystyrene matrix, spin-coated from toluene, (ii) embedding in a desolvated gel, prepared by evaporating the toluene after preparation, and (iii) embedding in the solvated PDMS–toluene gel. Example single-molecule spectra for each of these matrices are shown in Figure 3d–i. We note that all spectra recorded in a PDMS matrix, either solvated or free from toluene, contain a sharp Raman scattering peak around 450 nm, corresponding to the Raman shift of the poly(dimethylsiloxane) chains in response to the excitation laser. We interpolate our data across this sharp Raman peak for further data analysis. As in our previous work, we integrate the donor and acceptor emission intensity, $I_D$ and $I_A$, to obtain a ratiometric measure for the intrachain energy transfer efficiency, expressed as $E = I_A/I_D + I_A$. We extract these data...
from single-molecule spectra for \( N \approx 200 \) individual measurements for each of the three matrices. Energy transfer histograms reveal exactly what we speculated to occur above. Desolvation leads to a substantially higher intrachain energy transfer efficiency, due to chain collapse and the associated increase in local segmental density. Interestingly, the non-thermodynamic state of the desolvated versus the thermodynamic conformations of the solvated chains becomes apparent when considering the shape of the distributions. For solvated samples, the segmental density should be Gaussian, reflected as a Gaussian distribution of the energy transfer probability \( P(E) \) (Figure 3m,n). By contrast, chain collapse is a probabilistic phenomenon characterized by the emergence of kinetically trapped aggregated states, which lead to a wide distribution of segmental densities, and thus a broad distribution in \( P(E) \) (Figure 3m,n). To further study the effect of solvency on single-chain conformation, we compare our data on PF8-DTBT solvated in a toluene/PDMS matrix, which represents good solvent conditions, with measurements of this same polymer in a cyclohexane/toluene/PDMS matrix, at a 1:4 ratio of poor (cyclohexane) and good solvent (toluene) (see SI for details). Changing the solvency of the swelling fluid for the conjugated chains leads to a shift of \( E \) to much larger values, as expected for chains that approach their theta-point and deswelling (see Figure S6) while retaining a narrow distribution that signals an equilibrium state. Interestingly, we note that this scenario is still distinctly different from truly collapsed chains in a solid matrix, where the population heterogeneity is substantially enlarged (as evidenced in Figure 3m,n).

These two experiments highlight that our approach is capable of immobilizing chains without affecting their thermodynamic state and that this has a pronounced effect on the polymer photophysics as compared to studies in solid matrices. To further substantiate this claim, we also perform time-resolved fluorescence experiments on MEH-PPV chains.

We measure the fluorescence lifetime of individual chains using fluorescence lifetime imaging (FLIM, see Experimental Details for details) on MEH-PPV chains either spin-coated in PMMA from toluene, as is conventionally done to prepare samples for single-molecule spectroscopy, or fully solvated in our gel trapping system. We chose to compare lifetimes of our solvated system to lifetimes of MEH-PPV embedded in PMMA, as this allows for a more direct comparison to literature values. In solution, ensemble-averaged measurements of the excited-state lifetime of conjugated polymers tend to show very short lifetimes (0.2–0.5 ns for MEH-PPV), while these can be substantially larger in a desolvated state, embedded in a solid and glassy matrix. Measured lifetimes in these solid matrices can range from sub-nanosecond (0.5 ns on average) to lifetimes of 1 ns and larger, both in ensemble-averaged and single-molecule studies.

With FLIM, we record lifetime images containing tens to hundreds of diffraction-limited single-molecule spots (Figure 4e). We use object identification algorithms to find the centroid of each single spot and extract its time-resolved fluorescence trace. We observe strong differences between fluorescence decay curves recorded in a desolvated PMMA matrix (Figure 4a,b) and those recorded for single polymers in their solvated state (Figure 4c,d). We note that the differences in photon counts between the desolvated and solvated matrices arise from the strong decrease in quantum yield of MEH-PPV upon embedding in a solid matrix. The quantum yield for MEH-PPV in PMMA is 0.1, \(^{50}\) while the quantum yield of MEH-PPV in solution is 0.55. \(^{58}\) With constant integration time and excitation flux, such a reduction in quantum yield would result in substantially lower photon count. For desolvated chains, we find a single-exponential decay of a few nanoseconds, while for solvated chains the decay is more rapid and has a more complex multiexponential shape. We find that for solvated chains these curves are well described by a double-exponential decay. By fitting the decay curves for thousands of measurements on single molecules, we can reconstruct lifetime histograms with high statistical certainty. The solvated chains show two distinct populations in their lifetime distribution \( P(\tau) \), which is well-described by a double Gaussian, with characteristic decay times of \( \langle \tau \rangle = 0.3 \) ns and \( \langle \tau \rangle = 0.5 \) ns (\( N = 2025 \) chains). These values correspond well to those reported for ensemble-averaged measurements on MEH-PPV in solution. \(^{35,56,59}\)

The bimodal lifetime distribution that we observe is unexpected based on previous experiments in the solid state, but as these are lifetimes for single solvated chains, there is no reference data to benchmark these against. We note that both populations are below the mean fluorescence lifetime of single chains in PMMA. \(^{60}\) This implies that the secondary peak in the lifetime distribution for the solvated chains is most likely not due to single-chain aggregates that have remained in solution (which is further confirmed by Figure 2). Thus, the bimodal lifetime distribution we observe, of spots that all feature an MEH-PPV characteristic emission spectrum, is most likely not aggregates. Rather, we may speculate that it originates in the fact that single solvated chains exhibit multiple decay pathways of the excited state; this is also manifest in the shape of the single-molecule intensity decay curves (Figure 4c,d), which exhibits two exponential decays at the same characteristic lifetimes. We believe these may be due to the much larger conformational and vibrational freedom of chains in a solvated state, where not only small-scale molecular fluctuations can occur but conformational relaxation of the chains as a whole is also possible. These shape fluctuations not only drastically reduce the mean lifetime but also give rise to stronger effects of polydispersity since the conformation relaxation of chains is strongly length-dependent. However, we cannot fully exclude the effect of nanoaggregation of small and rodlike chains in the polydisperse mixture, of which the secondary lifetime peak could be a result. Based on work by Wang et al. \(^{61}\) it could be possible that no good solvent for MEH-PPV exists, and that as such, nanoaggregation cannot be suppressed even in highly dilute solutions. By contrast, we find a single broad distribution of lifetimes for chains in a static PMMA matrix, with \( \langle \tau \rangle = 2.1 \) ns (\( N = 2371 \) chains). While the average value we find is slightly higher than what is usually reported in the literature, \(^{62,55}\) it is established that low molecular weight MEH-PPV oligomers can have lifetimes of up to 5 ns \(^{52}\) as the result of nanoaggregation. Our sample is highly polydisperse and thus contains a substantial amount of low molecular weight oligomers. During spin coating and film solidification, long and short chains of MEH-PPV exhibit vastly different transport rates, and it is thus possible that even at the very low concentrations we employ aggregation occurs for short chains of MEH-PPV. The presence of nanoaggregates of short chains would explain the skew in our lifetime data for MEH-PPV in solid PMMA. To further substantiate this hypothesis, we perform a correlation analysis by plotting the excited-state lifetime as a function of intensity for each diffraction-limited spot in our experiments. For the solid-state sample, the resulting correlation plot, displayed as an intensity...
Our data show no apparent effect of solvation quality, and the emergence of intermittent intensity jumps under continuous excitation. This could be interpreted in one of two ways. Either, there is no correlation between the aggregation state of the chains and these unusual photophysics, as has been suggested in some cases previously, or aggregates remain present in all solvents, as work by Wang et al. has shown that there might be no good solvent possible for MEH-PPV. This is in line with the hypothesis above of the presence of small nanoaggregates resulting in a two-population behavior in the lifetime distributions. This would suggest that any differences we observe between toluene, chloroform, and THF (see Figure S1) could still be caused by aggregation effects, as there is no nonaggregation state to benchmark against. On the basis of the work by Wang et al. we would expect MEH-PPV in chloroform or toluene to be in very similar conformations (with hydrodynamic radius versus molecular weight scaling exponents of 0.41 and 0.4, respectively, below $\Theta = 0.5$ expected from Flory-Huggins theory for good solvents). For THF we would expect even worse solvency, but such an effect does not present itself in our data (see Figure S1). Clearly, the link between conformation of MEH-PPV and solvent quality is one that requires further study to clarify fully.

CONCLUSIONS

In this paper we have presented a simple yet versatile approach to enable the optical interrogation of single conjugated polymers in their thermodynamic state. We have shown how chains trapped in a gel matrix, which restricts center-of-mass motion of larger macromolecules but leaves solvent transport unaffected, exhibit more homogeneous photophysical populations and lack the signs of intramolecular aggregation, which complicates a detailed analysis of such studies in the solid state. The trapping method enables a wide variety of different single-molecule methods to be employed, including emission spectroscopy, fluorescence lifetime analysis, and single-chain photodynamics. Our method could also be extended to study single-molecule behavior under the influence of external fields, which could shed light on the effect of chain morphology on charge transport and mobility. By embedding the entire sample in a sample chamber consisting of at least one optically transparent electrode would in principle allow the simultaneous read-out using methods detailed in our paper and the application of electric fields. It is also possible to extend our
method of gel trapping to completely different matrix compositions. For instance, for the entrapment of water-soluble fluorescent polymers or proteins, a polyacrylamide matrix could be employed using the same principles as described in this paper.

We have adopted our approach to shed light on the debate regarding the origins of intermittent intensity jumps in the emission of MEH-PPV. While it was previously assumed that this intermittency must originate from intrachain aggregates, we find no correlation between aggregation state and the abundance of emitters that exhibit such large fluctuations. Our results thus hint that the origins of this photophysical intermittency must lie elsewhere. The study of single-molecule photophysics of MEH-PPV in this study is performed on highly polydisperse samples obtained commercially; while not uncommon,\textsuperscript{65,66} multiple studies on MEH-PPV and other polymers have shown chain length dependent differences when these conjugated polymers are further fractionated into more monodisperse fractions using GPC or other fractionation methods.\textsuperscript{67,68} These results could indicate that chain length will also influence this intermittency. For chain lengths greater than 200 kDa, no substantial length effects have been observed,\textsuperscript{69} but commercial samples also contain a large fraction of much shorter oligomers that approach the conjugation length, whose role is important but not fully clarified.\textsuperscript{70} It is also conceivable that intrinsic chemical defects in the chains, thus being a property of their chemical architecture and not only their conformation, play a role in these features.\textsuperscript{71} Only very recently has single-chain defect analysis become experimentally tractable, which could potentially aid in resolving this problem.\textsuperscript{72}

**EXPERIMENTAL DETAILS**

A detailed description of data analysis routines, including additional data, can be found in the SI. Analysis routines, implemented in Matlab, are available on request from the authors.

**Conjugated Polymers.** MEH-PPV was purchased from Sigma-Aldrich, with a molecular weight of 40–70 kDa (PDI ≈ 6).

Polyfluorene-co-DTBT was prepared by standard Pd-catalyzed Suzuki–Miyaura polycoumadation. In short, 4224 mg (7.7 mmol) of 9,9-si-(2-ethylhexyl)-2,7-dibromofluorene, 4915 mg (8.8 mmol) of 9,9-dicyanofluorene-2,7-diboronic acid-bis(1,3-propanediol)ester, and 504 mg (1.1 mmol) of 7-bis(5-bromo-2-thienyl)-2,1,3-benzothiadiazole were dissolved in 125 mL of toluene, to which was added 75 mL of spectroscopy-grade toluene. This solution was degassed by bubbling with nitrogen under vacuum/nitrogen cycles. A 250 mg of divinyl-terminated PDMS, and another 2 g of silicone hydride (Gelest). We load this liquid solution, and add 2.3 g of divinyl-terminated polydimethylsiloxane (Gelest, $M_w = 62.7$ kDa), 0.2 g of a multifunctional cross-linker, consisting of PDMS chains, carrying $\sim$8% of silicone hydride functional groups (Gelest, $M_w = 5.8$ kDa), and another 2 g of spectroscopy-grade toluene. This yields a final conjugated polymer concentration of 10 pM. We thoroughly mix this solution before adding a platinum catalyst (5 µL, Gelest). We load this liquid precursor mixture into a glass sample chamber, with a #1 coverslip top surface. We seal the sample chamber with PTFE grease to prevent any solvent evaporation. We allow the gel to cure for 3 h, while we store the sample at room temperature in the dark.

**Single-Molecule Experiments.** We perform various single-molecule experiments to characterize different aspects of these molecules in their solvated state, here described in short; for full details see the SI. We collect full spectral information at the single-molecule level using a home-built confocal microscope (ThorLabs, excitation at 405 nm) equipped with a spectrrograph (Andor iDus 416). Single-molecule spectra were deconvolved using serial Gaussian fitting (custom MatLab code, available upon request). The spectra are obtained at a spectral resolution of $\sim$1 nm, and the baseline was subtracted by taking the average signal between 290 and 380 nm.

Single-molecule lifetime measurements are performed on a Leica SP8 confocal, equipped with a FLIM module, consisting of a pulsed, white-light laser source (excitation at 488 nm, 40 MHz pulse rate), equipped with a TCSPC module. Emission was collected between 500 and 750 nm. These data are analyzed by digital analysis of the FLIM data sets, using home-coded software. The centroid positions of the diffraction-limited spots in the intensity image are identified using established particle tracking algorithms implemented in MatLab.\textsuperscript{73} We then use these centroids to reconstruct the intensity decay traces for each single molecule and subsequently fitted these to a multipexponential fitting model. Photodynamics are measured on a Nikon C1 confocal (488 nm CW excitation). We record an image area of $24 \times 12 \, \mu$m, with a 60X oil immersion objective, at an acquisition rate of 12 images/second, giving a time resolution of $\sim$80 ms. Also here we use automated object identification to find diffraction-limited spots. We plot the intensity within these spots, corrected for the dark counts on the background, to obtain the intensity traces. Categorization of these data is inspired by Tenopala-Carmona.\textsuperscript{63} Each trace is assigned to fall in one of two categories, either following an exponential decrease or exhibiting strong intensity jumps and thus deviations from the exponential decay. We fit each trace to an exponential decay function and determine if the fit is good, thus allowing classification into the first category. We use a Gaussian mixture model\textsuperscript{74} to determine what class each trace falls in, utilizing various parameters related to the goodness of fit and the number of detected peaks in the raw intensity data. Our Gaussian mixture model determines to what category each trace belongs in this 2D space. This algorithm is available on request from the authors (see SI for details).

**ASSOCIATED CONTENT**

\* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsnano.9b06164.

Additional FRAP data for MEH-PPV and the small BoDiPy dye in a solid polystyrene matrix; additional single-molecule fluorescence emission spectra for MEH-PPV in solid PMMA matrix, dried PDMS, and PDMS/toluene trapping gel; single-molecule fluorescence emission spectra of P88-DTBT in solid polystyrene, dried PDMS, and PDMS/toluene trapping gel; single-molecule lifetime decay curves for MEH-PPV in solid PMMA and PDMS/toluene trapping gel; single-molecule fluorescence intensity traces of MEH-PPV in solid PMMA spin-coated from chloroform, solid PMMA.
spin-coated from toluene, PDMS/THF trapping gel, and PDMS/toluene trapping gel; details and schematic representation of the single-molecule fluorescence emission setup and a detailed explanation of the data analysis routine to classify the measured intensity traces as either exponential or intermittent (PDF)

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Author Contributions
T.v.d.L. and J.S. conceived and designed the experiment. T.v.d.L., E.H., and R.H. constructed the experimental setup. T.v.d.L. and E.H. performed the experiments and data analysis. P.v.d.S. synthesized the materials. All authors interpreted the data and cowrote the manuscript.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
This work is part of the VIDI research program of J.S. with project number 723.016.001, which is financed by The Netherlands Organisation for Scientific Research (NWO).

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