Dynamic Polymer Free Volume Monitored by Single-Molecule Spectroscopy of a Dual Fluorescent Flapping Dopant

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ABSTRACT: Single-molecule spectroscopy (SMS) of a dual fluorescent flapping molecular probe (N-FLAP) enabled real-time nanoscale monitoring of local free volume dynamics in polystyrenes. The SMS study was realized by structural modification of a previously reported flapping molecule by nitrogen substitution, leading to 20 times increased brightness of the probe. In polystyrene thin film at the temperature of 5 K above the glass transition, the spectra of a single N-FLAP molecule undergo frequent jumps between short- and long-wavelength forms, the latter one indicating planarization of the molecule in the excited state. The observed spectral jumps were statistically analyzed to reveal the dynamics of the molecular environment. The analysis together with MD and QM/MM calculations show that the excited-state planarization of the flapping probe occurs only when sufficiently large polymer free volume of more than, at least, 200 Å³ is available close to the molecule, and that such free volume lasts for an average of 1.17 seconds.

Polymer free volume is a practical concept for interpreting polymer properties connected to the fraction of unoccupied volume.

¹ While arbitrary definition of “free volume” often led to controversy, quantitative evaluation of polymer free volume is important for developing membrane materials as well as packing materials for chromatography that adsorb, diffuse, and separate small molecules.² Local free volume moves from moment to moment due to thermal fluctuation of surrounding polymer chains. To evaluate the free volume directly and quantitatively, several approaches have been established such as positron annihilation lifetime spectroscopy (PALS),³ inverse gas chromatography (IGC),⁴ ¹²⁹Xe NMR spectroscopy,⁵ and molecular probe.^{6–8} In recent years, some studies have been reported that compare experimental results with molecular dynamics (MD) simulation.⁹ However, real-time monitoring of the local free-volume dynamics has only been achieved by single-molecule spectroscopy (SMS) analysis of conformationally flexible probes.¹⁰ In this literature, single-molecule fluorescence lifetime has been analyzed because the conformational change of the fluorescent probe, twisted peryleneimide, results in very little spectral difference. This work is an example of the great potential that the SMS technique holds for spatially-resolved dynamic studies of polymers and soft matter in general.¹¹ Apart from free-volume probing, SMS has been used, e.g., to analyze heterogeneity of local relaxation processes of polymers near glass transition temperature (T_g),^{12–14} to characterize diffusion in solutions or melts,^{15,16} or to monitor polymerization reactions on molecular level.^{17,18} In particular, the SMS method could be an attractive alternative to tracking the dynamics of the polymer free volume because of its high location specificity, suitable

dynamic range, non-invasive nature, and relatively affordable and simple optical instrumentation. Here we succeeded in demonstrating such free-volume monitoring by developing a new flexible fluorophore, nitrogen-embedded flapping molecule (N-FLAP), that shows dual fluorescence (FL) spectrum depending on the bent/planar conformations (Figure 1). Compared with the previously reported flapping anthracene,¹⁹ the nitrogen-embedded molecular framework led to much improved FL brightness as well as high photostability, when excited by a visible light. With this unique molecular probe physically doped in polystyrenes, SMS study has been performed to demonstrate that dynamic nanoscale changes of polymer free volume can be monitored in real time by following local environment-dependent spectral changes of a single N-FLAP molecule. This is the first application of a dual emissive molecule²⁰ to the free-volume monitoring in a single-molecule level.

Figure 1. (a) Nitrogen-embedded flapping fluorophore (N-FLAP) as a bright dual-fluorescent probe and (b) real-time analysis of polystyrene free volume using the N-FLAP dopant. λ_F : Fluorescence wavelength.

Synthesis and characterization of the N-FLAP probe

Previously reported flapping anthracene (FLAP in Figure 3a) works as a fluorescent viscosity probe that shows polarity-independent dual FL.¹⁹ The flapping anthracene undergoes a bent-to-planar conformational change in the lowest singlet excited state (S_1), while it instantly returns to the most stable bent form in the singlet ground state (S_0). Since the S_1 planarization is partially suppressed in a viscous media, ratiometric FL analysis is available for quantifying local viscosity. On the other hand, the flapping anthracene has only weak absorption bands in the visible range, and low photostability has been demonstrated when excited by a strong UV light.²¹ This photodegradation behavior is a serious shortcoming for the application to the single-molecule FL analysis, in which continuous FL emission is required for counting photon signals.²² Particularly for the spectroscopic study, bright FL as well as the high photostability is expected for accumulating photons with respective energies. Here we designed a flapping phenazine (N-FLAP) probe, in which reactive 9 and 10 positions of the original anthracene moieties were capped by nitrogen atoms to suppress undesired photoreactions.

N-FLAP was newly prepared by the Pd-catalyzed phenazine synthesis.²³ The coupling reaction of compounds **1**²⁴ and **2** followed by an in-situ oxidation provided N-FLAP in 45% yield (Figure 2a). Single-crystal X-ray diffraction analysis revealed a V-shaped structure of N-FLAP with the cyclooctatetraene (COT) bending angles, defined in Figure 3d, of $\theta = 36.5^\circ$ and 39.2° (Figure 2b).

Figure 2. (a) Synthesis of N-FLAP. SPPhos Pd G2: A commercially available 2nd generation SPPhos precatalyst. (b) Single crystal X-ray structure analysis. Thermal ellipsoids were drawn at the 50% probability level. Hydrogen atoms were omitted for clarity.

Optical properties of the N-FLAP probe

Absorption bands of N-FLAP were red-shifted (415 nm in CH_2Cl_2) compared with the previously reported flapping anthracene (Figure 3a). Remarkably, the molar absorption coefficient (ϵ) in the visible region increased more than 10-fold ($5.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) compared with that of the flapping anthracene ($4.1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ at 398 nm). The difference in the molar absorption coefficients was supported by time-dependent density functional theory (TD-DFT) calculations. A model structure, N-FLAP', was treated for the calculations, in which the terminal OC_6H_{13} substituents of N-FLAP were replaced by OCH_3 groups. Strong visible absorption of an allowed transition ($f \approx 0.5$) is expected for N-FLAP', while only a weak visible absorption with small oscillator strength ($f \approx 0.06$) is assigned for FLAP (Figure S15). In addition, molecular orbital (MO) energy levels originating from the phenazine wings of N-FLAP'

become lower than those of the anthracene wings of FLAP by the nitrogen substitution. The lower LUMO level may lead to the suppression of photo-oxidation in N-FLAP.

N-FLAP showed a green fluorescence peaked at 518 nm with a large Stokes shift of 4800 cm^{-1} in CH_2Cl_2 , indicating a conformational planarization in the lowest singlet excited state (S_1), as seen in other flapping anthracenes (Figure 3a). Absolute fluorescence quantum yield of N-FLAP ($\Phi_F = 0.42$) was higher than that of FLAP ($\Phi_F = 0.27$).²⁵ Thus, the brightness of N-FLAP, defined as $\epsilon \times \Phi_F$, is 20 times higher than that of FLAP (Figure 3a, inset table), overcoming the drawbacks of the previous FLAP probe for the SMS use. Polarity independence in the FL spectra of N-FLAP was confirmed in various solvents (toluene, CH_2Cl_2 , acetone, DMF, and DMSO) covering a wide range of relative permittivity (ϵ_r) from 2.4 (toluene) to 46.7 (DMSO) at room temperature. The peak wavelength of the FL band shifted within a small range of 514–523 nm (Figure 3b), suggesting a negligible charge-transfer character in the excited state. On the other hand, when dispersed in a rigid poly(methyl methacrylate) (PMMA) matrix, N-FLAP emitted a blue FL ($\lambda_F = 460 \text{ nm}$, $\Phi_F = 0.13$) with smaller Stokes shift of 2360 cm^{-1} (Figure 3c). This drastic FL chromism was also observed with increasing viscosity of a 2-methyltetrahydrofuran solution at lower temperatures (Figure S7d). These behaviors indicate that the conformational planarization of N-FLAP in S_1 is suppressed in the rigid (highly viscous) environments.

Figure 3. (a) Absorption and FL spectra of N-FLAP, compared with the previously reported flapping anthracene (FLAP) in CH_2Cl_2 . Relative area of the FL spectra is proportional to the brightness of the probes. (b) Solvent dependence (ϵ_r ; relative permittivity) and (c) environment dependence in the FL spectra of N-FLAP. Excitation wavelength $\lambda_{\text{ex}} = 365 \text{ nm}$. (d) Calculated energy profiles of N-FLAP' in the excited state (S_1) and the ground state (S_0). With the fixed COT bending angle θ (see inset), structural optimizations were performed by (TD-)DFT calculations at the TPSSH/6-31+G(d) level.

To support the explanation of conformational dynamics in S_1 , (TD-)DFT calculations were conducted. Although the calculation results are very sensitive to the selected levels of theory (Supporting Information, Section 4.1), here we chose the TPSSH/6-31+G(d) level for better interpreting the experimental results. Potential energy profiles of N-FLAP' were delineated with changing the COT bending angle θ by 2 degrees in the ground state (S_0) and the excited state (S_1) (Figure 3d). In S_0 , the optimized structure of N-FLAP' takes a V-shaped form ($\theta = 38.8^\circ$), while the planar structure gives a transition state. In contrast, the most stable geometry in S_1 is the planar conformation ($\theta = 0^\circ$), at which the S_0 - S_1 transition is allowed ($f \approx 0.3$). The calculation results rationalize the observed green bright FL with a large Stokes shift (Figure S13e). In addition, an electronic configuration switch was predicted during the conformational planarization in S_1 , as the case with reported FLAP.^{19,25}

Thermal and viscoelastic properties of the host polymers

High molecular-weight polystyrene (high MW PS; $M_w \approx 192,000$) and low MW PS ($M_w \approx 850$) were selected for this study. Glass transition temperatures (T_g) of high MW PS and low MW PS were determined to be 102°C and 18°C , respectively, based on inflection points of the DSC profiles in the cooling process. Dynamic viscoelasticity measurements showed that the polystyrenes hardened at around T_g , and the storage modulus (G') was 0.9×10^9 Pa (65°C and below) for high MW PS and 7×10^7 Pa (25°C) for low MW PS.

Figure 4. (a) DSC profiles and (b) temperature-dependent dynamic viscoelasticity of the host polymers, high MW PS and low MW PS. Scan rate: $5^\circ\text{C}/\text{min}$ for both measurements. G' : storage modulus. G'' : loss modulus.

Methods of single-molecule fluorescence spectroscopy

For single-molecule FL studies, toluene solutions of the host PS polymers and a trace of N-FLAP were prepared (the concentration of N-FLAP: 10^{-9} M) and then spin-coated to form the sample films on glass substrates. After annealing the PS films, the SMS experiments were carried out at 23°C in N_2 atmosphere on top of an inverted FL microscope equipped with an imaging spectrograph (Figure 5a). Switching to the spectral

mode of the spectrograph enables wavelength dispersion of FL signal from individual molecules, the dynamics of which can be then monitored for extended periods of time. Figures 5b and 5c show a typical FL image of the dispersed N-FLAP molecules and an example of a single-molecule FL spectrum, respectively.

Figure 5. (a) Scheme of the experimental setup. (b) An example of microscopic FL image, in which the bright spots correspond to the N-FLAP single molecules dispersed in a spin-coated polystyrene thin film. (c) An example of single-molecule FL spectrum of the N-FLAP probe.

Results of the single-molecule fluorescence spectroscopy

SMS of N-FLAP in the high MW PS was performed first. As mentioned above, the high MW PS has its T_g at 102°C (Figure 4) and represents rigid environment for the N-FLAP molecule. An example of time evolution of single-molecule FL spectrum in the high MW PS is shown in Figure S23. At the measurement temperature of 23°C (much lower than T_g), the molecule showed a spectrally stable emission with a FL peak (0-0 vibrational band) at 445 nm . The single-molecule spectrum has a clearly resolved vibronic structure, while ensembles of N-FLAP molecules in bulk PS films provided only averaged broad structureless spectrum mainly in a blue region (420 – 520 nm) (Figure S23). This behavior is typical for most of the N-FLAP molecules trapped in the high MW PS, indicating almost complete suppression of the conformational planarization of N-FLAP in S_1 .

Next, SMS in the low MW PS was conducted. Since the measurement temperature of 23°C is slightly above its T_g (18°C), the PS chains start undergoing segmental relaxation in the form of micro-Brownian motion. The dynamic nature of the polymer matrix is reflected in the spectroscopic behavior of the individual N-FLAP molecular probes. A typical example of the time evolution plot is shown in Figure 6a. This molecule stayed in the blue form most of the time with the peak position at 464 nm , but occasionally undergoes a spectral jump to the green form, reminiscent of the solution-phase FL spectra with a peak top around 520 nm (Figure 3b). In other N-FLAP molecules (Figure S24), the spectral jumps occurred with varying frequency. There is a wide distribution of times for which the molecule stays in the green form. The observed FL peak wavelengths also showed a large variance in the green range (490 – 540 nm). Such variety of the green FL spectra is evident even for the same N-FLAP molecule in different time ranges (see the spectra 1, 3 and 6 in Figure 6b). The spectral distribution is characterized by plotting a histogram of the FL peak positions based on the analysis of 105 spectra (Figure 6c). When overlaid with ensemble

FL spectra of N-FLAP molecules dispersed in a rigid high MW PS matrix and in a toluene solution of PS, there is a good correspondence between the single-molecule and ensemble spectral regions both for the blue and green FL bands.

Figure 6. (a) Time evolution for the FL spectra of a single N-FLAP molecule dispersed in the low MW PS matrix. The vertical axis indicates measurement time (from top to bottom). Each horizontal line represents a FL spectrum with the signal intensity shown in grey scale. Numbers (1–6) are assigned for each time range and (b) the corresponding FL spectra, fitted with a sum of three Gaussian functions. (c) Histogram of the single-molecule FL peak wavelengths analyzed from 105 individual spectra. FL peaks at shorter wavelengths are shown as blue bars, while longer ones counted as green bars. Ensemble FL spectra of N-FLAP in a drop-cast high MW PS film (blue solid line) and in a PS-containing toluene solution (green solid line) were also shown.

Discussion for the dynamic single-molecule fluorescence

The appearance of the green FL band of the flapping probe in solution is a consequence of the low viscosity environment which enables the planarization of the chromophore in S_1 .¹⁹ On the other hand, in the solid state the flapping molecule requires certain free space for the planarization to happen.²⁶ In polymers, this free space should be closely related to the concept of free volume,¹ although there is a non-negligible difference between the “free volume” values estimated by molecular probes and by PALS.¹⁰ (PALS studies generally report smaller volumes than those reported by molecular probe studies.) In a polymer film above the glass transition temperature T_g , segmental relaxation causes dynamical changes to the free volume which can repeatedly form and disappear around the N-FLAP probe. This temporary appearance of the free volume would enable the excited-state planarization of N-FLAP and emission of the green band, and its disappearance would cause a return to the blue emission (Figure 7a). Alternatively, the segmental micro-Brownian motion of the surrounding polymer chains itself could also cause mechanical planarization of N-FLAP in the ground state, which would also result in the green emission from the planarized N-FLAP in a confined space (Figure 7b). To distinguish between

these two possible mechanisms, i.e., existence of a free volume vs. segmental motion-induced planarization, we carried out control experiments in both high and low MW PS using another flapping dye, which has been reported as a flexible mechanophore that does not undergo spontaneous excited-state planarization even in solution.²⁷ The flexible mechanophore (A-FLAP) has bulky triisopropylsilyl (TIPS) ethynyl groups introduced on the anthracene moieties (Figure S25), and when mechanically compressed in a crystalline phase it attains the planar conformation and emits red-shifted fluorescence. Calculated energy barrier for the mechanical planarization of A-FLAP in S_0 is practically same as that of N-FLAP. The SMS results of A-FLAP in both high and low MW PS showed that, irrespective of the matrix, the single-molecule spectra are of the short-wavelength form, namely, the bent conformation of A-FLAP is preserved (Figure S25). This result means that even in the rubbery-state PS film above T_g the segmental motion does not cause mechanical planarization of the A-FLAP or N-FLAP dyes, and that the observed green FL of N-FLAP is emitted via the excited-state planarization during the temporal appearance of the local free volume in the low MW PS film (Figure 7a).

Figure 7. Two possible mechanisms for the single-molecule spectral dynamics in Figure 6. (a) One possibility is that the excited-state planarization of N-FLAP is allowed only when the free volume temporarily appeared around the N-FLAP probe. (b) The other is that compulsory planarization is mechanically induced by dynamic segmental motions of the surrounding polymer chains.

To get an insight into the dynamics of free volume formation and disappearance, we further analyzed the spectral time traces of single N-FLAP molecules in the low MW PS. Specifically, we evaluated the time intervals for which the molecule stayed in the blue form before jumping to the green form, and separately the intervals for which the molecule stayed in the green form before reversing back to the blue one. Histograms of both intervals (Figure 8) show exponential distributions which, when fitted with single-exponential functions, provide the characteristic duration times of the blue form (τ_b) of 10.4 s and of the green form (τ_g) of 1.17 s. In the picture of polymer free volume, these results show that it takes an average of 10.4 s for a sufficiently large free volume to form around the N-FLAP molecule, and that this volume lasts for an average of 1.17 s before it disappears. It is only during the presence of the sufficient free volume that the N-FLAP molecule can undergo repeated planarization after each excitation and emit the green fluorescence continuously for several seconds. We have estimated that the minimum free volume required for the N-FLAP planarization is on the order of 200–300 Å³ (Figure 8e). This estimate is comparable with upper limits of distributions of free volume in PS

measured by positron annihilation²⁸ or fluorescence photochromic probes.⁶ The large difference between the two characteristic times τ_b and τ_g reflects the formation and disappearance mechanisms of the large free volume. The formation is associated with cooperative motion of several segments of chains surrounding the molecule. However, once the free volume is formed, a motion of a single segment is sufficient for its destruction. The τ_g should therefore reflect the segmental relaxation time (α -relaxation) whereas the τ_b is longer due to the cooperative nature of the formation process. The τ_g of 1.17 s is well within the range of α -relaxation times measured by dielectric spectroscopy for PS with a varying range of molecular weight around the T_g temperature.²⁹

Figure 8. (a) Time evolution of single-molecule FL spectra of N-FLAP in the low MW PS; the shaded areas indicate the short (blue) and long (green) wavelength forms. (b) Histogram of time duration of the blue spectral form before switching to the green one, constructed from 90 data points and fitted with an exponential. (c) Histogram of time duration of the green spectral form before switching to the blue one, constructed from 84 data points and fitted with an exponential. (d) Histogram of reorientation times of single PDI molecules in the low MW PS constructed from 955 events and fitted with an exponential. Inset shows an example of defocused FL image. Overall, 73 out of 130 PDI molecules measured in the low MW PS showed the rotational motion. PDI: *N,N*-di-*n*-octyl-3,4,9,10-perylenetetracarboxylic diimide. (e) Estimated minimum volume required for the excited-state planarization of N-FLAP'. In the space-filling models, terminal alkyl chains were not considered because conformationally flexible moieties generally do not disturb dynamic motions of rigid chromophores.³⁰

There is also a way of directly measuring the local relaxation times by monitoring reorientation of single-molecule fluorescent probes doped in the film.^{10,13,14} As a reference, we have carried out such measurement using a perylene diimide (PDI) dye and the technique of defocused imaging (Figure 8d).

However, rather than analyzing the reorientation process by plotting the autocorrelation function and fitting it with the stretched exponential (KWW) function,^{10,13} we chose to plot a distribution of reorientation times for a statistical sample of single molecules. For this purpose, reorientation time is defined as the time for which the molecule stays in a certain orientation before reorientation by more than 30° occurs. Since such reorientation supposedly reflects a segmental motion of a nearby PS chain, this analysis enables direct comparison with the distribution of the τ_g times obtained for the N-FLAP molecules. The results (presented also in Figure S26) show that the distribution of reorientation times is also exponential, and fitting with a single exponential function provides a characteristic reorientation time τ_r of 0.75 s. This value is generally in very good agreement with the 1.17 s of τ_g , and indicates that the lifetime of the free volume is indeed determined by the segmental motion.

While both τ_b and τ_g show overall exponential distributions, there is an interesting trend observed from traces of different individual molecules in Figure S24. At least within the observation time of 160 s, there appears to be a kind of local memory effect in spectral traces, i.e., a molecule which tends to have short τ_g and long τ_b shows this behavior throughout the observation time, and the same is true for long τ_g and moderate τ_b (Figures S24b and S24d, respectively). This observation points to spatial heterogeneity of the α -relaxation process that has been observed before in poly(*n*-butyl methacrylate) (PnBMA) near T_g ¹⁰ as well as in supercooled liquids³¹ and that persists for minutes of the measurement times.

We note that, in principle, the observed spectral dynamics of the N-FLAP dye could be alternatively explained by assuming that the dye-surrounding polymer becomes temporarily liquid-like, enabling the excited-state planarization in the same way as in a solution. However, such local liquid-like state is not very probable at or near T_g . In addition, it should also be observable in the PDI reorientation experiment where it would lead to periods of fast rotation, none of which has been observed. This mechanism of green-emission formation would be more relevant at higher temperatures close to the melting point.

MD simulations and QM/MM calculations

To validate the proposed mechanism, we also performed calculations considering the host polymer (500 entangled chains of the low MW PS). First, classical MD simulations of the low MW PS with a single N-FLAP' molecule were conducted to obtain a PS geometry that contains the sufficient void space for the flapping motion of N-FLAP'. While such the geometry was not obtained in the time range of 1000 ns when simulated at 25°C, those geometries were generated by the simulation at high temperature (see SI for details). Then, the N-FLAP' molecule with the fixed COT bending angle was placed in the void space, and additional MD calculations were conducted only for the PS chains that were spatially overlapped with N-FLAP'. After that, constraint geometry optimization was performed with the QM/MM^{32,33} (quantum mechanics/molecular mechanics) calculations (Figure 9a). The PS geometry was fixed and treated with the MM model, while the electronic structure of N-FLAP' was considered with the QM model. After several trials at different calculation levels, it was found that the ONIOM (TD-CAM-B3LYP/6-31+G(d):MM) calculation provided a reasonable interpretation of the observed single-molecule FL behavior of N-FLAP in the low MW PS. When the total energy of N-FLAP'(S₁)@Low MW PS was plotted by changing the COT bending angle θ of N-FLAP' every 5 degree, local energy

minima were obtained at which N-FLAP' takes an almost planar geometry ($\theta = 5^\circ$) and a bent geometry ($\theta = 30^\circ$) (Figure 9b). Due to the confined space in the host polymer, completely planar N-FLAP'(S₁) geometry (most relaxed in the gas phase) did not give an energy minimum of N-FLAP'(S₁)@Low MW PS. This result suggested that the relaxed N-FLAP'(S₁) geometry depends on the specific shape of the temporarily appeared void space, and therefore the spectral form of the green FL band have a structural variety (Figure 6 and Figure S24). The predicted energy barrier between bent and planar conformations of N-FLAP'(S₁) in the polymer is also consistent with the clear FL switch between the blue and green forms, rather than a gradual shift of the FL peak.

Figure 9. (a) Structural model for the QM/MM calculations, in which N-FLAP' is placed at the center of the low MW PS (500 polymer chains). Green void (calculated by POVME 2.0³⁴) corresponds to the free volume around the N-FLAP' probe. (b) Relative energy of N-FLAP'(S₁)@Low MW PS in the ONIOM (TD-CAM-B3LYP/6-31+G(d):MM) calculations.

Conclusions

Nitrogen-embedded flapping molecule has been developed as a superior environment-sensitive dual fluorescent probe for single-molecule fluorescence spectroscopy. Nitrogen substitution on the flapping wings enabled efficient visible excitation, leading to 20-times high brightness of the probe. Suppression of photo-oxidation process also results in long-term observation in the single molecule study. The probe in the low molecular weight polystyrene ($T_g = 18^\circ\text{C}$) provided dynamic single-molecule fluorescence spectrum at 23°C that frequently jumps to the long-wavelength form, originating from the temporarily permitted excited-state planarization dynamics of the flapping probe in sufficiently large polymer free volume. In this way, the probe is capable of visualizing local free volume changes on the order of $200\text{ \AA}^3$ on millisecond time scales for extended periods of time. Statistic analysis of the duration time in the short- and long-wavelength spectra demonstrated that it takes 10.4 ± 0.5 seconds for the large free volume to generate, while it lasts for 1.17 ± 0.06 seconds. MD simulations and the following QM/MM calculations, in which the probe molecule was treated as the QM model while polystyrene chains were treated as the MM model, supported the dynamic spectral behaviors and the rough estimation of the free volume. The use of single-molecule imaging techniques can, in principle, also provide 3-dimensional localization of the probe and enable position-dependent studies of the free volume dynamics.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental and theoretical details (PDF)

Accession Codes

CCDC 1987186 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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Supporting Information

Dynamic Polymer Free Volume Monitored by Single-Molecule Spectroscopy of a Dual Fluorescent Flapping Dopant

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Contents

1. General information.....	S2
2. Synthesis of compounds	S5
3. Photophysical properties.....	S11
4. Density functional theory (DFT) calculations	S14
5. Single-molecule fluorescence spectroscopy	S25
6. MD simulations and QM/MM calculations	S29
7. Supporting references	S30

1. General information

Synthesis

All reagents and solvents were of commercial grade and were used without further purification unless otherwise noted. Thin-layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F₂₅₄ (Merck). Column chromatography was performed on Wakogel® C-300 or C-400. ¹H and ¹³C NMR spectra were recorded on a JEOL ECA-600 (600 MHz for ¹H and 151 MHz for ¹³C) spectrometer. Chemical shifts were reported in ppm relative to the residual solvents as internal standards (CHCl₃: δ = 7.26 ppm for ¹H and δ = 77.16 ppm for ¹³C). High-resolution atmospheric-pressure-chemical-ionization time-of-flight mass-spectrometry (HR-APCI-TOF-MS) was recorded on a Bruker micrOTOF spectrometer.

Photophysical measurements

UV-visible absorption spectra were recorded on a Shimadzu UV-3600 spectrometer. Steady-state fluorescence (FL) and excitation spectra were recorded on a JASCO FP-8500 spectrofluorometer. Absolute fluorescence quantum yields were determined on a Hamamatsu Photonics C9920-02S. FL lifetimes were measured on a Hamamatsu Photonics QuantaTaurus-Tau C11367. Low-temperature measurements were performed using a Unisoku CoolSpeK UV USP-203-B cryostat. All measurements denoted in this section were conducted using a 10-mm square quartz cell unless otherwise noted.

Single-crystal X-ray diffractometry

X-ray crystallographic data of N-FLAP was collected at −180 °C with a Rigaku XtaLAB P200 diffractometer equipped with a copper MicroMax007HF generator, VariMax-Cu optics, and a PILATUS 200K two-dimensional detector. The structure was solved by direct method SHELXT^[S1] and refined by SHELXL^[S2] programs. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed using AFIX instructions. Crystallographic data of N-FLAP have been deposited in the Cambridge Crystallographic Data Center with the CCDC numbers of 1987186.

Thermal and rheological measurements

Differential scanning calorimetry (DSC) was conducted on a Hitachi High-Tech Science DSC7000X. Rheological measurements were performed on an Anton Paar MCR702 rheometer.

Sample preparation for single molecule experiments

The samples were prepared by mixing dilute spectral grade toluene solution of either N-FLAP, A-FLAP or *N,N'*-dioctyl-3,4,9,10-perylenedicarboximide (PDI, Aldrich) with 2 wt% toluene solution of either high MW or low MW polystyrene in the ratio of 1:99. 200 mL of the mixed solution were spin-coated on pre-cleaned quartz cover glass at 3000 rpm for 30 s to obtain approximately 100-nm thick films with final concentration of the dye on the order of 10^{-9} M.

Single molecule fluorescence spectroscopy

Fluorescence from single N-FLAP molecules was measured using an inverted fluorescence microscope (IX 71, Olympus). The excitation light was provided either by super-continuum laser (NKT Photonics Fianium) at 410 nm or by He-Cd laser at 442 nm. Fluorescence was collected by an oil immersion objective lens (UplanFLN 100 $\times$, N.A. 1.3, Olympus) and passed through appropriate dichroic and a long-pass filters. For the spectral measurements the signal was further dispersed using an imaging spectrograph (CLP-50LD, Bunkou Keiki). The spectrograph uses a flipping mirror (in front of the grating) which, when used, relays the microscopic image to the CCD camera. In this imaging mode a single molecule is brought into the center of the image, the spectrograph slit is closed and the mirror is removed. The fluorescence signal falling into the vertical slit is spectrally dispersed, and at the vertical position of the molecule provides the molecular fluorescence spectrum. Fluorescence was detected with an electron-multiplying (EM) CCD camera (iXon, Andor Technology) with an exposure time of ranging from 100 to 500 ms. The samples on the microscope stage were under continuous stream of dry nitrogen gas to minimize photobleaching.

Classical molecular dynamics (MD) simulations

Molecular dynamics (MD) simulations were performed for 500 polymer chains of the low molecular-weight (MW) polystyrene (PS) containing an N-FLAP' molecule. The low MW PS was modeled using winmostar^[S3] and the 500 PS chains were randomly replicated within the box size of $170 \times 170 \times 170 \text{ \AA}^3$. Here, N-FLAP' was placed in the center of the entangled PS chains. Energy minimization was performed for this system during 5000 steps, and then the temperature was increased from 0 K to 5 K at the NVT ensemble ($T = 5 \text{ K}$) for 10 ps. Subsequently, the temperature was increased from 5 K to 600 K at the NPT ensemble ($P = 1 \text{ bar}$ and $T = 600 \text{ K}$) for 100 ps. Next, the system was equilibrated at the NPT ensemble ($P = 1 \text{ bar}$ and $T = 600 \text{ K}$) for 10 ns. Here the box size was set to $94 \times 93 \times 95 \text{ \AA}^3$. Finally, the production run was performed at the NPT ensemble ($P = 1 \text{ bar}$ and $T = 600 \text{ K}$) for 100 ns. To obtain force fields of N-FLAP' and PS, geometry optimization at the B3LYP/6-31G(d) level and the following single-point electronic structure calculation at the MP2/6-311++G(d,p) level were performed using Gaussian 16.^[S4] The force fields of N-FLAP' and PS were created by antechamber module of the AMBER 16 program suite.^[S5] In the MD simulations, the linear constraint solver algorithm,^[S6] the Nosé-

Hoover thermostat,^[S7,S8] and the Berendsen barostat^[S9] were applied for the constraints, for the temperature regulation, and for the pressure regulation, respectively. The long-range electrostatic interactions were considered by the particle mesh Ewald method^[S10] and the direct space cut-off distance was set to 10 Å. The van der Waals interactions were calculated using a switched cut-off between 8 and 10 Å. The simulation time step was set to 2 fs. In this study, the MD simulations were performed by the GROMACS version 5.1.4 program suite.^[S11]

Quantum mechanics / molecular mechanics (QM/MM) calculation

N-FLAP' structures with fixed COT bending angles (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 degrees) were considered. The corresponding initial geometries of the N-FLAP'@Low MW PS system for the QM/MM calculation were obtained by constraint MD simulations, in which the energy minimization was performed only for the PS chains that were spatially overlapped with N-FLAP', during 1000 steps using GROMACS version 5.1.4. The QM/MM calculation was performed with the ONIOM^[S12] (our own N-layered integrated molecular orbital and molecular mechanics) method to estimate the energy of N-FLAP'@Low MW PS. The single N-FLAP' and the 500 PS molecules were divided into a QM region and an MM region, respectively. With the fixed COT bending angles, the constraint geometry optimization of N-FLAP' in the lowest singlet excited state (S_1) was performed by the time-dependent (TD) density functional theory (ONIOM(TD-PBE0/6-31+G(d):MM), ONIOM(TD-TPSSH/6-31+G(d):MM) and ONIOM(TD-CAM-B3LYP/6-31+G(d):MM)) using Gaussian 16. Note that the force fields for the MM region are the same as those in the MD simulations.

Computational visualization of the polymer free volume

Polymer free volume (green void space in Figure 9a) was calculated by POVME ver 2.0.1 using the default settings (GridSpacing was set to 1 Å).^[S13] The void space was visualized only in the sphere with a radius of 10 Å from the center of mass of N-FLAP', using the QuickSurf representation of the Visual Molecular Dynamics (VMD)^[S14] program.

2. Synthesis of compounds

Figure S1. Synthesis of the flapping phenazine (N-FLAP).

1,2-Bis(hexyloxy)benzene (S1): Catechol (1.5 g, 14 mmol) and potassium carbonate (7.5 g, 55 mmol) were placed in a Schlenk flask, and the flask was purged with argon. Anhydrous DMF (20 mL) and 1-bromohexane (7.7 mL, 55 mmol) were added to the flask, and the solution was stirred for 24 h at 100 °C. The mixture was poured into water and extracted with diethyl ether three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 , and the solvent was removed in vacuo. The crude mixture was purified by silica-gel column chromatography (hexane/dichloromethane, 4:1) to give S1 (5.3 g) as a colorless oil, which was used for the next step without further purification. All characterization data for S1 matched with those previously reported.^[S15]

4,5-Dinitro-1,2-hexyloxybenzene (S2): Compound S1 (5.3 g) was dried under vacuum in a two-necked flask and dissolved in AcOH (30 mL). HNO_3 (30 mL, 69%) and fuming HNO_3 (30 mL) were added to the solution and stirred over 2 h at 0 °C. The resulting orange solution was stirred for another 2 h at room temperature. The mixture was poured onto ice, and the precipitation was collected by vacuum filtration and washed with H_2O . Recrystallization from ethanol afforded S2 (4.3 g, 12 mmol, 86% over 2 steps) as a yellow solid. All characterization data for S2 matched with those previously reported.^[S17]

4,5-Bis(hexyloxy)benzene-1,2-diamine (2): Hydrazine monohydrate (0.41 mL, 8.4 mmol) was added to a suspension of the palladium on activated carbon (39 mg, 10% Pd basis) and **S2** (150 mg, 0.42 mmol) in EtOH (9.7 mL) in a Schlenk flask equipped with a reflux condenser under argon atmosphere. After the reflux for 16 h, the hot solution was filtered through Celite, and the solvent was removed under vacuum to give the product as a white solid. The product **2** was unstable under air and used to the following reaction immediately without any purification.

2,3,12,13-Tetrakis(hexyloxy)cycloocta[1,2-*b*:5,6-*b'*]diphenazine (N-FLAP): Compound **2** obtained from 0.42 mmol of **S2** was dried under vacuum in a Schlenk flask. The flask was filled with argon, and 2,3,8,9-tetrabromodibenzo[*a,e*]cyclooctene (**1**) (44 mg, 84 μ mol), SPhos Pd G2 (7.2 mg, 10 μ mol), SPhos (3.8 mg, 9.3 μ mol) and Cs₂CO₃ (180 mg, 0.56 mmol) were placed in the flask. Here, SPhos Pd G2 is a commercially available 2nd generation SPhos precatalyst, that is, chloro(2-dicyclohexylphosphino-2',6'-dimethoxy-1,1'-biphenyl)[2-(2'-amino-1,1'-biphenyl)]palladium(II). Anhydrous toluene (1.5 mL) was poured to the flask, and the mixture was refluxed for 39 h. The reaction mixture was cooled to room temperature and then diluted with an excess amount of CHCl₃. The obtained suspension was filtered through a Celite pad, and the filtrate was evaporated to obtain the black residue. The residue was purified by silica-gel column chromatography (CH₂Cl₂/AcOEt, 4:1) to obtain crude product as a brown solid. Subsequent precipitation from CH₂Cl₂/MeOH and filtration provided a pure sample of **N-FLAP** as a yellow solid (30 mg, 38 μ mol, 45% based on the amount of **1**). Slow diffusion of MeOH into a toluene solution of **N-FLAP** gave needle-shaped yellow crystals for the single crystal X-ray diffractometry.

¹H NMR (CDCl₃, 600 MHz): δ / ppm = 7.91 (s, 4H), 7.23 (s, 4H), 7.22 (s, 4H), 4.17 (t, J = 6.6 Hz, 8H), 1.92 (tt, J_1 = 6.7 Hz, J_2 = 6.7 Hz, 8H), 1.51 (m, 8H), 1.34–1.38 (m, 16H), and 0.90 (t, J = 7.0 Hz, 12H); ¹³C {¹H} NMR (CDCl₃, 151 MHz): δ / ppm = 154.6, 142.5, 140.9, 137.9, 133.3, 128.4, 105.6, 69.4, 31.6, 28.8, 25.8, 22.7, and 14.2. HRMS (APCI/TOF) m/z : [M]⁺ Calcd for C₅₂H₆₄N₄O₄ 809.4933; Found 809.494. UV-visible absorption (in CH₂Cl₂): λ_{max} (ϵ / M⁻¹ cm⁻¹) = 279 (6.6×10^4) and 415 nm (5.3×10^4). Fluorescence (in CH₂Cl₂, λ_{ex} = 365 nm): λ_{max} = 518 and 555 nm; Φ_F = 0.42; τ_F = 2.4 ns (λ_{obs} = 518 nm).

Figure S2. Synthesis of the reference compound (**N-Ref**).

Phenazine-2,3-diol (S5): *o*-Phenylenediamine (**S3**) (1.0 g, 9.3 mmol) and 2,5-dihydroxy-1,4-benzoquinone (**S4**) (1.4 g, 10 mmol) were placed in a two-necked flask, and the flask was purged with argon. Water (260 mL) was added to the flask, and the solution was stirred for 12 h at 110 °C. After cooling down to the room temperature, the resulting mixture was filtered and dried under reduced pressure to give **S5** as a dark brown solid (2.0 g, quant.), which was used for the next step without further purification. All characterization data for **S5** matched with those previously reported.^[S16]

2,3-Bis(hexyloxy)phenazine (N-Ref): Compound **S5** (130 mg, 0.60 mmol) and potassium carbonate (1.7 g, 12 mmol) were placed in a Schlenk flask, and the flask was purged with argon. Anhydrous DMF (6.0 mL) and 1-bromohexane (0.34 mL, 2.4 mmol) were added to the flask, and the solution was stirred for 3.5 h at 80 °C. Water was added to the mixture, and the resulting precipitate was collected by filtration. The precipitate was purified by silica-gel column chromatography (gradient elution of 0–10% AcOEt in hexane) to afford a crude product as a red solid. The crude product was further purified by recrystallization from hot hexane to afford **N-Ref** as a white solid (94.5 mg, 41%).

¹H NMR (CDCl₃, 600 MHz): δ / ppm = 8.13 (m, 2H), 7.73 (m, 2H), 7.34 (s, 2H), 4.22 (t, J = 6.6 Hz, 4H), 1.95 (tt, J_1 = 7.5 Hz, J_2 = 6.9 Hz, 4H), 1.55 (m, 4H), 1.34–1.42 (m, 8H), and 0.92 (t, J = 6.9 Hz, 6H); ¹³C{¹H} NMR (CDCl₃, 151 MHz): δ / ppm = 154.6, 142.2, 142.0, 128.9 (two peaks are overlapped), 105.7, 69.4, 31.7, 28.8, 25.8, 22.7, and 14.2 (Note: 11 peaks were observed for the 12 unique carbons. The peak at 128.9 ppm is probably derived from two carbon signals according to the reference spectrum of 2,3-dimethoxyphenazine^[S18]). HRMS (APCI/TOF) m/z : $[M]^+$ Calcd for C₂₄H₃₂N₂O₂ 380.2469; Found 380.2463. UV-visible absorption (in CH₂Cl₂): λ_{max} (ϵ / M⁻¹ cm⁻¹) = 392 (2.4×10^4) and 375 nm (2.0×10^4). Fluorescence (in CH₂Cl₂, λ_{ex} = 365 nm): λ_{max} = 428 nm; Φ_F < 0.01; τ_F < 0.1 ns (λ_{obs} = 428 nm).

Figure S4. (a) ^1H NMR and (b) $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of **N-Ref** in CDCl_3 at room temperature. Peaks marked with asterisk indicate residual solvents or impurities.

Figure S5. HR-APCI-TOF mass spectra of **N-FLAP** (top: observed, bottom: simulated).

Figure S6. HR-APCI-TOF mass spectra of **N-Ref** (top: observed, bottom: simulated).

3. Photophysical properties in solution

3.1 Absorption, fluorescence (FL) and excitation spectra

Figure S7. Photophysical properties of N-FLAP in solution. (a) Concentration dependence of the absorption spectra in CH_2Cl_2 . (b) Solvent dependence of the absorption spectra. (c) Excitation spectra monitored at different FL wavelengths in CH_2Cl_2 . (d) Temperature dependence of the FL spectra ($\lambda_{\text{ex}} = 365 \text{ nm}$) in 2-methyltetrahydrofuran (melting point: 137 K).

Figure S8. (a) Absorption and FL spectra ($\lambda_{\text{ex}} = 365 \text{ nm}$) of **N-Ref**, compared with **N-FLAP** in CH_2Cl_2 . (a) Concentration dependence of the absorption spectra of **N-Ref** in CH_2Cl_2 . (b) Excitation spectra of **N-Ref** monitored at 428 nm in CH_2Cl_2 .

3.2 Fluorescence lifetime

The fluorescence lifetime of **N-FLAP** and **N-Ref** was measured by fitting FL decay profiles ($\lambda_{\text{ex}} = 365$ nm) with single- or double-exponential functions. The goodness of fit was evaluated by χ^2 , and a double-exponential model provided better results for **N-FLAP** (Table S1) than a single-exponential function (Table S2). Short-lived components ($\tau_1 < 0.1$ ns) may originate from planarization dynamics in the excited state.

Table S1. FL lifetime of **N-FLAP** in CH_2Cl_2 fitted with a double-exponential function^[a]

λ_F / nm	χ^2	τ_1 / ns	τ_2 / ns	A_1	A_2
440	1.16	< 0.1	2.4	5740	400
465	1.20	< 0.1	2.3	2760	600
518	1.11	< 0.1	2.4	−1250	790
558	1.09	< 0.1	2.4	−970	760
608	1.25	< 0.1	2.4	−1250	790

[a] $f(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$

Table S2. FL lifetime of **N-FLAP** and **N-Ref** in CH_2Cl_2 fitted with a single-exponential function

	λ_F / nm	χ^2	τ / ns
N-FLAP	440	(60.0)	(2.0)
	465	(9.80)	(2.2)
	518	1.21	2.4
	558	1.92	2.3
	608	2.28	2.4
N-Ref	428	1.04	< 0.1

Figure S9. FL decay profiles of (a) **N-FLAP** and (b) **N-Ref** in CH_2Cl_2 .

4. Density functional theory (DFT) calculations

DFT calculations of the isolated molecules were performed using the Gaussian 16 program,^[S4] and Kohn–Sham orbitals are displayed by the Avogadro program.^[S19] Model structures **N-FLAP'** and **N-Ref'** were calculated, which have OCH₃ groups in place of the OC₆H₁₃ groups of the corresponding molecules (Figure S10). The COT bending angle was defined as the dihedral angle between the C1–C2–C3 and C2–C3–C4 planes in Figure S11. Constrained geometry optimization was performed with the fixed COT bending angles using the ModRedundant keyword. The coordinate system for the consideration of configuration symmetry was defined as Figure S12.

Figure S10. Structures of **N-FLAP'** and **N-Ref'**.

Figure S11. Definition of the COT bending angle θ used in the constrained geometry optimization.

Figure S12. Definition of the coordinate system for **N-FLAP'** geometries bearing a bent C_{2v} or a planar D_{2h} symmetry. The symmetries of excited electronic states are shown in parentheses, whose transition dipole is oriented in each axis.

4.1. Selecting a suitable calculation level for the interpretation of the observed FL properties

To select the appropriate level of theory, calculated S_1 energy profiles of **N-FLAP'** were compared in several conditions. We first conducted TD-DFT calculations at the PBE0/6-31+G(d) level, which was selected in the previous work on flapping anthracene.^[S20] However, this level was not suitable for the **N-FLAP'** case because the most stable S_1 planar geometry gave a non-emissive A_2 symmetry ($f = 0$) when using the PBE0 functional (Figure S13a). Since an emissive B_2 state ($f \sim 0.3$), which could rationalize bright FL in solution, was found at a higher energy level than that of the A_2 state, we continued to search for the other optimal condition. Then, calculations with various basis sets, such as def2-SVP, def2-SVPD, aug-cc-pVDZ, and D95+(d) were attempted without changing the PBE0 functional, but the energy level of the dark A_2 state was still lower than the emissive B_2 state. Therefore, calculations with the B3LYP and CAM-B3LYP functionals were also examined with the 6-31+G(d) basis set, but these attempts yielded the same results (Figure S13b,c). Since the B3LYP functional, whose percentage of Hartree–Fock (HF) exchange is 20%, afforded a smaller energy gap between the A_2 and B_2 states than that of the PBE0 functional (HF exchange = 25%), we next tried the TPSSh functional with the smaller HF exchange (10%).

Figure S13d shows an S_1 energy profile delineated at the TPSSh/6-31+G(d) level. The emissive B_2 state at the planar geometry gave a global minimum in S_1 , which agrees with the observed bright green FL with the large Stokes shift. To get a deeper insight, we compared electronic configurations of the three energetically close states at the same COT bending angle of 15° (Figure S13e). The Kohn–Sham molecular orbitals (MOs) of **N-FLAP'** are categorized into two groups, MO_{PHZ} and MO_{COT} . The former is a MO group in which the electronic contribution of the phenazine (PHZ) moieties is significant. The latter MO group is more contributed by the central cyclooctatetraene (COT) ring. While both of transitions to the A_2 state marked in red (dominantly contributed by $HOMO_{COT} \rightarrow LUMO+1_{COT}$) and to the A_2 state marked in blue ($\pi\pi^*$ transition of phenazine, that is, $HOMO_{PHZ} \rightarrow LUMO_{PHZ}$) are forbidden ($f = 0$), the transition to the B_2 state (mainly contributed by $HOMO_{COT} \rightarrow LUMO_{PHZ}$) is allowed ($f = 0.34$). As a result, it was suggested that the interplay of COT and phenazine is essential for the bright FL.

Note that the planar B_{2u} state with the D_{2h} symmetry (analogous to the B_2 state with the bent C_{2v} symmetry) has an imaginary frequency. Then, we found a B_2 state with a planar C_{2v} symmetry as a global minimum in S_1 with no imaginary frequency, although the energy difference between the two states is only less than 0.1 kcal/mol, and the corresponding MO energy levels are almost the same (Figure S14). Thus, we did not consider the symmetry breaking in this paper.

Figure S13. (a-d) S_1 energy profiles of N-FLAP' in C_{2v} symmetry using four different functionals. (e) S_0 - S_1 transitions in slightly different geometries with the same COT bending angle of 15° . The calculation was performed at the TD-TPSSH/6-31+G(d) level.

Figure S14. (a) Energetically close excited states calculated for S_1 . (b) Kohn–Sham orbitals and their energy levels of the S_1 planar **N-FLAP'** geometries (see Figure S20–S22). The calculation was performed at the TD-TPSSH/6-31+G(d) level.

4.2. Discussion of the absorption spectra based on the S_0 optimized geometries

Optimized geometries of **FLAP**, **N-FLAP'**, and **N-Ref'** in S_0 at the TPSSh/6-31+G(d) level of theory were used in the following calculations, in which **FLAP** and **N-FLAP'** take $\theta = 38.0^\circ$ and 38.8° , respectively.

Table S3. Excitation energies and oscillator strengths of **FLAP** calculated at the TD-TPSSh/6-31+G(d) level.

Transition	Excitation energy	Configurations	f
$S_0 \rightarrow S_1 (A_2)$	2.53 eV (491 nm)	HOMO $\rightarrow$ LUMO (93%)	0
		HOMO-1 $\rightarrow$ LUMO+1 (7%)	
$S_0 \rightarrow S_2 (B_1)$	2.64 eV (470 nm)	HOMO $\rightarrow$ LUMO+1 (68%)	0.0022
		HOMO-1 $\rightarrow$ LUMO (32%)	
$S_0 \rightarrow S_3 (B_1)$	2.94 eV (422 nm)	HOMO-1 $\rightarrow$ LUMO (67%)	0.0582
		HOMO $\rightarrow$ LUMO+1 (31%)	
$S_0 \rightarrow S_4 (B_2)$	3.02 eV (410 nm)	HOMO $\rightarrow$ LUMO+2 (53%)	0.0037
		HOMO-2 $\rightarrow$ LUMO (46%)	
$S_0 \rightarrow S_5 (A_2)$	3.06 eV (405 nm)	HOMO-1 $\rightarrow$ LUMO+1 (92%)	0
		HOMO $\rightarrow$ LUMO (6%)	

Table S4. Excitation energies and oscillator strengths of **N-FLAP'** calculated at the TD-TPSSh/6-31+G(d) level.

Transition	Excitation energy	Configurations	f
$S_0 \rightarrow S_1 (A_2)$	2.58 eV (481 nm)	HOMO $\rightarrow$ LUMO (96%)	0
		HOMO-2 $\rightarrow$ LUMO+1 (3%)	
$S_0 \rightarrow S_2 (B_1)$	2.70 eV (459 nm)	HOMO $\rightarrow$ LUMO+1 (85%)	0.0022
		HOMO-2 $\rightarrow$ LUMO (15%)	
$S_0 \rightarrow S_3 (B_2)$	2.76 eV (450 nm)	HOMO-1 $\rightarrow$ LUMO (94%)	0.4786
		HOMO $\rightarrow$ LUMO+2 (4%)	
$S_0 \rightarrow S_4 (A_1)$	2.91 eV (426 nm)	HOMO-1 $\rightarrow$ LUMO+1 (80%)	0.0228
		HOMO-3 $\rightarrow$ LUMO (17%)	
$S_0 \rightarrow S_5 (B_1)$	2.94 eV (422 nm)	HOMO-2 $\rightarrow$ LUMO (84%)	0.0126
		HOMO $\rightarrow$ LUMO+1 (14%)	

Table S5. Excitation energies and oscillator strengths of **N-Ref'** calculated at the TD-TPSSH/6-31+G(d) level.

Transition	Excitation energy	Configurations	f
$S_0 \rightarrow S_1$	3.09 eV (401 nm)	HOMO $\rightarrow$ LUMO (97%)	0.0143
$S_0 \rightarrow S_2$	3.15 eV (394 nm)	HOMO-2 $\rightarrow$ LUMO (100%)	0.0011
$S_0 \rightarrow S_3$	3.44 eV (360 nm)	HOMO-1 $\rightarrow$ LUMO (86%) HOMO $\rightarrow$ LUMO+1 (13%)	0.2848
$S_0 \rightarrow S_4$	3.95 eV (314 nm)	HOMO-3 $\rightarrow$ LUMO (96%) HOMO $\rightarrow$ LUMO+2 (3%) HOMO-4 $\rightarrow$ LUMO (56%)	0.0054
$S_0 \rightarrow S_5$	4.72 eV (263 nm)	HOMO $\rightarrow$ LUMO+1 (32%) HOMO-1 $\rightarrow$ LUMO (4%) HOMO-1 $\rightarrow$ LUMO+2 (3%)	0.2758

Figure S15. (a) Calculated oscillator strengths (bars) and observed UV-visible absorption spectra (line) of **FLAP** (black) and **N-FLAP'** (red) in CH_2Cl_2 . (b) Kohn-Sham molecular orbitals of **FLAP** and **N-FLAP'** in the S_0 optimized geometries and selected transition characters with non-zero oscillator strengths, calculated at the (TD-)TPSSH/6-31+G(d) level. MO_{LP} represents MOs with a large contribution of the lone-pair electrons.

Figure S16. (a) Calculated oscillator strengths (bars) and observed UV-visible absorption spectra (line) of **N-Ref** (black) and **N-FLAP'** (red) in CH_2Cl_2 . (b) Kohn–Sham molecular orbitals of **N-Ref** and **N-FLAP'** in the S_0 optimized geometries and selected transition characters with non-zero oscillator strengths, calculated at the (TD-)TPSSH/6-31+G(d) level. MO_{LP} represents MOs with a large contribution of the lone-pair electrons.

Figure S17. (a) Energy profiles of S_0 and vertically excited states (S_a – S_f) of N-FLAP' calculated at the (TD-)TPSSH/6-31+G(d) level. Geometries are optimized in S_0 with the fixed COT bending angle θ . (b) Change in transition characters for the lowest-energy excitation during the ground-state planarization.

4.3. Optimized geometries in S_0 and S_1

Figure S18. S_0 optimized geometry, representative bond length (Å), and bond angles ($^\circ$) of N-FLAP' with bent C_{2v} symmetry ($\theta = 38.8^\circ$) calculated at the TPSSh/6-31+G(d) level.

Figure S19. Constrained S_0 optimized geometry, representative bond length (Å), and bond angles ($^\circ$) of N-FLAP' with planar D_{2h} symmetry ($\theta = 0^\circ$) calculated at the TPSSh/6-31+G(d) level.

Figure S20. S_1 optimized geometry, representative bond length (Å), and bond angles ($^\circ$) of N-FLAP' with planar D_{2h} symmetry ($\theta = 0^\circ$) that provides a A_2 state calculated at the TD-TPSSh/6-31+G(d) level.

Figure S21. S_1 optimized geometry, representative bond length (Å), and bond angles ($^\circ$) of N-FLAP' with planar D_{2h} symmetry ($\theta = 0^\circ$) that provides a B_2 state calculated at the TD-TPSSH/6-31+G(d) level.

Figure S22. S_1 optimized geometry, representative bond length (Å), and bond angles ($^\circ$) of N-FLAP' with planar C_{2v} symmetry ($\theta = 0^\circ$) that provides a B_2 state calculated at the TD-TPSSH/6-31+G(d) level.

5. Single-molecule fluorescence spectroscopy

Figure S23. (a), (c) Time evolution of single-molecule fluorescence spectra of **N-FLAP** in the high MW PS matrix. (b), (d), (e) Typical single-molecule fluorescence spectra taken from the time evolution plots. (f) Histogram of single-molecule peak wavelengths (bars, obtained from 24 spectra) and ensemble FL spectra of **N-FLAP** molecules dispersed in drop-cast the high MW PS film (solid line).

At the measurement temperature of 23 °C, the **N-FLAP** molecule in the high MW PS showed spectrally stable and vibrationally resolved emission with a 0-0 peak at 445 nm (Figure S23). This behavior is typical for most of the **N-FLAP** molecules studied, while only about 30% of the molecules rarely showed a short-term spectral jump towards partially red-shifted region (around 480 nm), as shown in the example of Figure S23c-e. After the spectrally stable emission at 440 nm over a minute, the spectral jump to 476 nm was observed only for several seconds. A histogram of the FL peak positions based on the Gaussian analysis of the spectra is shown in Figure S23f. Here, the FL spectra measured on the level of single molecules should be located within the spectral region of the broad blue FL band of the corresponding sample film, i.e., mainly between 420 and 520 nm. In fact, both single-molecule FL peak spectral regions, i.e., 440–450 nm and 480–490 nm fall within the range of the broad blue FL band of the sample film. Temporarily red-shifted FL band around 480 nm would originate from a partially relaxed form of **N-FLAP** in S_1 before the process of the conformational planarization, caused by small local relaxation of the high MW PS matrix.

Figure S24. Examples of time evolution for single-molecule fluorescence spectra of the **N-FLAP** molecules in the low MW PS matrix. (a) Spectrally stable molecule. (b) Molecule with very short periods of the long-wavelength form. (c) Typical molecule. (d) Molecule with very long periods of the long-wavelength form.

Figure S25. Single-molecule spectroscopy of the **A-FLAP** molecules. Time evolution of fluorescence spectra in (a) the high MW PS and (b) the low MW PS. Although a blinking behavior was observed, the spectral shape in the emissive time region was almost constant. Typical fluorescence spectra taken from the time evolution plots in (c) the high MW PS and (d) the low MW PS. These spectra are fitted with a sum of Gaussians (red lines), in which the blue lines are ensemble spectra of the **A-FLAP** molecules in toluene solution.

Figure S26. (a) Monitoring the rotational dynamics of the PDI molecule in the low MW PS matrix at room temperature. (b) Averaged time for the significant rotation (more than 30°) of the PDI molecule in the low MW PS matrix was estimated.

6. MD simulations and QM/MM calculations

Figure S27. Optimized structures of N-FLAP'(S₁)@Low MW PS with different COT bending angles, obtained by the QM/MM calculations using the ONIOM (TD-CAM-B3LYP/6-31+G(d):MM) method. Initial geometries for the QM/MM calculations were obtained by classical MD simulations (see General information). Green void space was calculated by POVME 2.0^[S13] as the free volume around the N-FLAP' probe.

7. Supporting references

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