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

*Yuma Goto,¹ Shun Omagari,¹ Ryuma Sato,² Takuya Yamakado,³ Ryo Achiwa,³ Nilanjan Dey,³
Kensuke Suga,³ Martin Vacha^{1,*} and Shohei Saito^{3,*}*

¹Department of Materials Science and Engineering, School of Materials and Chemical Engineering,
Tokyo Institute of Technology, Ookayama 2-12-1-S8-44, Meguro-ku, Tokyo 152-8552, Japan

²Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced
Industrial Science and Technology (AIST)

³Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

KEYWORDS. polymer free volume, single-molecule spectroscopy, dual fluorescence, viscosity probe.

Introduction: Polymer free volume is a rational 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 report, 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. 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 environment-induced spectral changes of a single N-FLAP molecule. In this way, the probe is capable of visualizing local free volume changes on the order of 200 Å³ on millisecond time scales for extended periods of time. The use of single-molecule imaging techniques can, in principle, provide also 3-dimensional localization of the probe and enable position-dependent studies of the free volume dynamics.

Fig. 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.

Synthesis and characterization of the flapping molecular probe

Previously reported flapping anthracene (FLAP in Fig. 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 strong UV light.²⁰ 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.²¹ Particularly for the spectroscopic study, bright FL as well as the high photostability is expected for accumulating photons with respective energies. Here we designed and synthesized flapping phenazine (N-FLAP), 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.²² The coupling reaction of 2,3,8,9-tetrabromodibenzo[*a,e*]COT **1**²³ and 4,5-bis(hexyloxy)benzene-1,2-diamine **2** followed by an in-situ oxidation provided N-FLAP in 45% yield (Fig. 2a). Single crystals of N-FLAP were obtained by slow diffusion of methanol vapor into a toluene solution, and single-crystal X-ray diffraction analysis revealed a V-shaped structure of N-FLAP with the COT bending angle, defined in Fig. 3d, of $\theta = 36.5^\circ$ and 39.2° (Fig. 2b). The crystal packing showed a characteristic columnar structure, in which the phenazine wings are π -stacked on the both wings of N-FLAP with an interplanar distance of 3.3–3.4 Å. This multifold columnar π -stacking is unique for the flapping molecules bearing the central COT ring without bulky substituents.^{22,23}

Fig. 2. (a) Synthesis of N-FLAP. SPhos Pd G2: 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). (b) the 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 flapping molecular probe

Absorption bands of N-FLAP were red-shifted (415 nm in CH₂Cl₂) compared with the previously reported flapping anthracene (Fig. 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 ($4.1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$, 398 nm). The difference in the molar absorption coefficients can be rationalized by the analysis of time-dependent density functional theory (TD-DFT) calculation results. The 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 (Fig. S16). As a result, the strong visible absorption of the allowed transition ($f \sim 0.5$) is expected for N-FLAP, while only a weak visible absorption with small oscillator strength ($f \sim 0.06$) is assigned for FLAP (see Supporting Information, Section 4.2 for details).

N-FLAP showed a green fluorescence peaked at 518 nm with a large Stokes shift of 4800 cm⁻¹ in CH₂Cl₂, indicating a conformational planarization in the lowest singlet excited state (S₁), as seen in other flapping anthracenes (Fig. 3a). Absolute fluorescence quantum yield of N-FLAP ($\Phi_F = 0.42$) was higher than that of FLAP ($\Phi_F = 0.27$).²⁶ Thus, the brightness of N-FLAP, defined as $\epsilon \times \Phi_F$, is 22 times higher than that of FLAP (Fig. 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, CH₂Cl₂, acetone, DMF, and DMSO) covering a wide range of relative dielectric constants (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 (Fig. 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 ($\lambda = 460 \text{ nm}$, $\Phi_F = 0.13$) with smaller Stokes shift of 2360 cm⁻¹ (Fig. 3c). This drastic FL chromism was also observed with increasing the viscosity of a 2-methyltetrahydrofuran solution at lower temperatures (Fig. S7d). These behaviors indicate that the conformational planarization of N-FLAP in S₁ is suppressed in the rigid (highly viscous) environments.

To support the explanation of conformational dynamics in S₁, we conducted (TD-)DFT calculations of a model structure, N-FLAP', in which the terminal OC₅H₁₃ substituents of N-FLAP were replaced by OCH₃ groups. 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 (S₀) and the excited state (S₁) (Fig. 3d). In S₀, the optimized structure of N-FLAP' takes a V-shaped form ($\theta = 38.8^\circ$), while the planar structure ($\theta = 0^\circ$) gives a transition state. In contrast, the most stable geometry in S₁ is the planar conformation ($\theta = 0^\circ$), at which the S₀–S₁ transition is allowed ($f \sim 0.3$). The calculation results rationalize the observed green bright FL with a large Stokes shift (Fig. S13d,e). In addition, an electronic configuration switch was predicted during the conformational planarization in S₁, as the case with reported FLAP.^{19,26}

Fig. 3. (a) Absorption and FL spectra of N-FLAP, compared with the previously reported flapping anthracene (FLAP) in CH_2Cl_2 . The relative area of FL spectra is proportional to the brightness of the flapping molecules. (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.

Thermal and viscoelastic properties of the host polymers

High molecular-weight polystyrene (high MW PS; $M_w = 192,000$) and low MW PS ($M_w = 850$) 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. Temperature-dependent 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.

Fig. 4. (a) DSC profiles and (b) temperature-dependent viscoelasticity of the host polymers, high MW PS and low MW PS. Scan rate: 5 °C/min for both measurements.

Single-molecule fluorescence spectroscopy of the flapping probe in the host polystyrenes

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 (Fig. 5a). Switching to a 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. Figs. 5b and 5c show a typical FL image of the dispersed N-FLAP molecules and an example of a single-molecule FL spectrum, respectively.

Fig. 5 a) Scheme of the experimental setup. b) An example of microscopic fluorescence 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 spectrum of the N-FLAP probe.

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 (Fig. 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 Fig. 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 integrated signals from ensembles of N-FLAP molecules in bulk PS films provided only averaged broad structureless spectrum mainly in a blue region (420-550 nm) (Fig. 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 .

Fig. 6 a) Time evolution for the fluorescence 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 fluorescence spectrum with the FL 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. Spectral data of 105 molecules were analyzed. FL peaks at shorter wavelengths are shown as blue bars, while longer ones counted as green bars. Fluorescence spectra of N-FLAP in a drop-cast PS film (blue solid line) and in a PS-containing toluene solution (green solid line) were also shown. d) Two possible mechanisms for the single-molecule spectral dynamics. 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 (top). The other is that compulsory planarization is mechanically induced by dynamic segmental motions of the surrounding polymer chains (bottom).

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 Fig. 6a. This molecule stayed most of the time in the blue form 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 between 514–523 nm (Fig. 3b). In other N-FLAP

molecules (Fig. 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 one N-FLAP molecule in different time ranges (see the spectra 1, 3 and 6 in Fig. 6b). The spectral distribution is characterized by plotting a histogram of the FL peak positions based on the analysis of 105 spectra (Fig. 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 their integrated spectral regions both for the blue and green FL bands.

The appearance of the green FL band of the flapping system 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.²⁷ 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 (Fig. 6d, top). Alternatively, the segmental micro-Brownian motion of the matrix chains itself could also cause mechanical planarization of the N-FLAP in the ground state and this would also result in the green emission from the planarized N-FLAP in a confined space (Fig. 6d, bottom). 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.²⁸ The flexible mechanophore (A-FLAP) has bulky triisopropylsilyl (TIPS) ethynyl groups introduced on the anthracene moieties (Fig. 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 results of single-molecule detection 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 (Fig. S25). This result means that even in the rubbery-state PS film above T_g the segmental motion is not causing 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 surrounding free volume in the polymer film (Fig. 6d, top).

Fig. 7 a) 2D plot of time evolution of fluorescence spectra of a single N-FLAP molecule in low MW PS matrix; 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. Inset shows the fitted τ_b value. 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. Inset shows the fitted τ_g value. d) Histogram of reorientation times of single PDI molecules in low MW PS constructed from 955 events and fitted with an exponential. Inset shows the fitted value of τ_r , together with an example of defocused fluorescence image. Overall, 73 out of 130 PDI molecules measured in low MW PS showed rotational motion. e) Estimated volume required for the excited-state planarization of N-FLAP (see SI for details). In the space-filling models, terminal alkyl chains were not considered because conformationally flexible moieties generally do not disturb dynamic motions of rigid chromophores.²⁹

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 (Fig. 7) 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 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 continuously the green-form spectrum 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 (Fig. 7e). This estimate is comparable with upper limits of distributions of free volume in PS measured by positron annihilation³⁰ or fluorescence photochromic probes⁶. 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.³¹

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 perylene diimide (PDI) dyes and the technique of defocused imaging. 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 Fig. 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 Fig. S24. At least within the observation time of 160 s, there appears to be a kind of local memory effect^{32,33} 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 (molecules 2 and 4 in Fig. S24). This observation points to spatial heterogeneity of the α -relaxation process that has been observed before in the PnBMA polymers near T_g ¹⁰ as well as in supercooled liquids³⁴ 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 were conducted without N-FLAP in order 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 we placed the N-FLAP' molecule in the void space and performed QM/MM^{35,36} (quantum mechanics/molecular mechanics) calculations (Fig. 8a). The PS geometry was fixed and treated with MM, while the electronic structure of N-FLAP' was considered with QM. 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)@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 ($\theta = 30^\circ$) (Fig. 8b). 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)@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 lot of structural variety (Fig. 6 and Fig. S24). The predicted energy barrier between bent and planar conformations of N-FLAP is also consistent with the clear FL switch between the blue and the green forms, rather than a gradual shift of the FL peak.

Figure 8. (a) Structural model for the QM/MM calculations. Terminal substituents (b) Relative energy of N-FLAP(S₁)@PS in the ONIOM (TD-CAM-B3LYP/6-31+G(d):MM) calculations.

Corresponding Authors

* s_saito@kuchem.kyoto-u.ac.jp

* vacha.m.aa@m.titech.ac.jp

REFERENCES

1. White, R. P.; Lipson, J. E. G. Polymer Free Volume and Its Connection to the Glass Transition. *Macromolecules* **2016**, *49*, 3987–4007.
2. Low, Z.-X.; Budd, P. M.; McKeown, N. B.; Patterson, D. A. Gas Permeation Properties, Physical Aging, and Its Mitigation in High Free Volume Glassy Polymers. *Chem. Rev.* **2018**, *118*, 5871–5911.
3. Dlubek, G.; Pointeck, J.; Bondarenko, V.; Pompe, G.; Taesler, C.; Petters, K.; Krause-Rehberg, R. Positron Annihilation Lifetime Spectroscopy (PALS) for Interdiffusion Studies in Disperse Blends of Compatible Polymers: A Quantitative Analysis. *Macromolecules* **2002**, *35*, 6313–6323.

4. Yampolskii, Y. P.; Kaliuzhnyi, N. E.; Durgarjan, S. G. Thermodynamics of Sorption in Glassy Poly(vinyltrimethylsilane). *Macromolecules* **1986**, *19*, 846–850.
5. Golemme, G.; Nagy, J. B.; Fonseca, A.; Algieri, C.; Yampolskii, Y. ^{129}Xe -NMR study of free volume in amorphous perfluorinated polymers: comparison with other methods. *Polymer* **2003**, *44*, 5039–5045.
6. **Molecular Probe:** Victor, J. G.; Torkelson, J. M.; On Measuring the Distribution of Local Free Volume in Glassy Polymers by Photochromic and Fluorescence Techniques. *Macromolecules* **1987**, *20*, 2241–2250.
7. **Molecular Probe:** Itagaki, H.; Horie, K.; Mita, I. Luminescent probe studies of the microstructure and mobility of solid polymers. *Prog. Polym. Sci.*, **1990**, *15*, 361–424.
8. **Molecular Probe:** Strehmel, B.; Strehmel, V.; Younes, M. Fluorescence Probes for Investigation of Epoxy Systems and Monitoring of Crosslinking Processes. *J. Polym. Sci., Part B: Polym. Phys.*, **1999**, *37*, 1367–1386.
9. **MD simulation:** Jansen, J. C.; Macchione, M.; Tocci, E.; De Lorenzo, L.; Yampolskii, Y. P.; Sanfirova, O.; Shantarovich, V. P.; Heuchel, M.; Hofmann, D.; Drioli, E. Comparative Study of Different Probing Techniques for the Analysis of the Free Volume Distribution in Amorphous Glassy Perfluoropolymers. *Macromolecules* **2009**, *42*, 7589–7604.
10. **sm-FL lifetime:** Vallée, R. A. L.; Cotlet, M.; Van Der Auweraer, M.; Hofkens, J.; Müllen, K.; De Schryver, F. C. Fluorescence Lifetimes and Emission Patterns Probe the 3D Orientation of the Emitting Chromophore in a Multichromophoric System. *J. Am. Chem. Soc.* **2004**, *126*, 2296–2297.
11. Wöll, D.; Braeken, E.; Deres, A.; De Schryver, F. C.; Uji-I, H.; Hofkens, J. Polymers and Single Molecule Fluorescence Spectroscopy, What Can We Learn? *Chem. Soc. Rev.* **2009**, *38*, 313–328.
12. **sm-FL anisotropy:** Deres, A.; Floudas, G. A.; Müllen, K.; Van der Auweraer, M.; De Schryver, F. C.; Enderlein, J.; Uji-i, H.; Hofkens, J. The Origin of Heterogeneity of Polymer Dynamics near the Glass Temperature as Probed by Defocused Imaging. *Macromolecules* **2011**, *44*, 9703–9709.
13. Paeng, K.; Kaufman, L. J. Single Molecule Experiments Reveal the Dynamic Heterogeneity and Exchange Time Scales of Polystyrene near the Glass Transition. *Macromolecules* **2016**, *49*, 2876–2885.
14. Oba, T; Vacha, M. Relaxation in Thin Polymer Films Mapped across the Film Thickness by Astigmatic Single-Molecule Imaging. *ACS Macro Lett.* **2012**, *1*, 784–788.

15. Habuchi, S.; Sato, N.; Yamamoto, T.; Tezuka, Y.; Vacha, M. Multimode Diffusion of Ring Polymer Molecules Revealed by a Single-Molecule Study. *Angew. Chem. Int. Ed.* **2010**, *49*, 1418–1421.
16. Ito, S.; Itoh, K.; Pramanik, S.; Takatsugu, K.; Takei, S.; Miyasaka, H. Evaluation of Diffusion Coefficient in a Dextrin-Based Photo-Curable Material by Single Molecule Tracking. *Appl. Phys. Express* **2009**, *2*, 075004.
17. Wöll, D.; Uji-i, H.; Schnitzler, T.; Hotta, J.; Dedecker, P.; Herrmann, A.; De Schryver, F. C.; Müllen, K.; Hofkens, J. Radical Polymerization Tracked by Single Molecule Spectroscopy. *Angew. Chem. Int. Ed.* **2008**, *47*, 783–787.
18. Ueda, K.; Aizawa, M.; Shishido, A.; Vacha, M. Real-Time Molecular-Level Visualization of Mass Flow during Patterned Photopolymerization of Liquid-Crystalline Monomers. *NPG Asia Mater.* **2021**, *13*, 25.
19. **Flapping anthracene:** Kotani, R.; Sotome, H.; Okajima, H.; Yokoyama, S.; Nakaike, Y.; Kashiwagi, A.; Mori, C.; Nakada, Y.; Yamaguchi, S.; Osuka, A.; Sakamoto, A.; Miyasaka, H.; Saito, S. Flapping Viscosity Probe That Shows Polarity-Independent Ratiometric Fluorescence. *J. Mater. Chem. C* **2017**, *5*, 5248–5256.
20. **Photodegradation:** Kimura, R.; Kitakado, H.; Osuka, A.; Saito, S. Flapping Peryleneimide as a Fluorescent Viscosity Probe: Comparison with BODIPY and DCVJ Molecular Rotors. *Bull. Chem. Soc. Jpn.* **2020**, *93*, 1102–1106.
21. **Photostability required for SMS:** Yang, S. K.; Shi, X.; Park, S.; Ha, T.; Zimmerman, S. C. A Dendritic Single-Molecule Fluorescent Probe That Is Monovalent, Photostable and Minimally Blinking. *Nat. Chem.* **2013**, *5*, 692–697.
22. **Pd-catalyzed coupling:** Laha, J. K.; Tummalapalli, K. S. S.; Gupta, A. Palladium-Catalyzed Domino Double *N*-Arylations (Inter- and Intramolecular) of 1,2-Diamino(hetero)arenes with *o,o'*-Dihalo(hetero)arenes for the Synthesis of Phenazines and Pyridoquinoxalines *Eur. J. Org. Chem.* **2013**, 8330–8335.
23. **COT-Br₄ precursor:** Kimura, R.; Kuramochi, H.; Liu, P.; Yamakado, T.; Osuka, A.; Tahara, T.; Saito, S. Flapping Peryleneimide as a Fluorogenic Dye with High Photostability and Strong Visible-Light Absorption. *Angew. Chem. Int. Ed.* **2020**, *59*, 16430–16435.
24. **Two-fold pi-staking:** Yuan, C.; Saito, S.; Camacho, C.; Kowalczyk, T.; Irle, S.; Yamaguchi, S. Hybridization of a Flexible Cyclooctatetraene Core and Rigid Aceneimide Wings for Multiluminescent Flapping π Systems. *Chem. Eur. J.* **2014**, *20*, 2193–2200.

25. **Four-fold pi-staking:** Mouri, K.; Saito, S.; Yamaguchi, S. Highly Flexible π -Expanded Cyclooctatetraenes: Cyclic Thiazole Tetramers with Head-to-Tail Connection. *Angew. Chem. Int. Ed.* **2012**, *51*, 5971–5975.
26. **FL quantum yield of FLAP:** Yamakado, T.; Takahashi, S.; Watanabe, K.; Matsumoto, Y.; Osuka, A.; Saito, S. Conformational Planarization versus Singlet Fission: Distinct Excited-State Dynamics of Cyclooctatetraene-Fused Acene Dimers. *Angew. Chem. Int. Ed.* **2018**, *57*, 5438–5443.
27. **Free space for the planarization:** Kotani, R.; Liu, L.; Kumar, P.; Kuramochi, H.; Tahara, T.; Liu, P.; Osuka, A.; Karadakov, P. B.; Saito, S. Controlling the S₁ Energy Profile by Tuning Excited-State Aromaticity. *J. Am. Chem. Soc.* **2020**, *142*, 14985–14992.
28. **Acetylene FLAP:** Yamakado, T.; Osubo, K.; Osuka, A.; Saito, S. Compression of a Flapping Mechanophore Accompanied by Thermal Void Collapse in a Crystalline Phase. *J. Am. Chem. Soc.* **2018**, *140*, 6245–6248.
29. Chen, J.; Kistemaker, J. C. M.; Robertus, J.; Feringa, B. L. Molecular Stirrers in Action. *J. Am. Chem. Soc.* **2014**, *136*, 14924–14932.
30. Liu, J.; Deng, Q.; Jean, Y. C. Free-Volume Distributions of Polystyrene Probed by Positron Annihilation: Comparison with Free-Volume Theories. *Macromolecules* **1993**, *26*, 7149–7155.
31. Roland, C. M.; Casalini, R. Temperature Dependence of Local Segmental Motion in Polystyrene and Its Variation with Molecular Weight. *J. Chem. Phys.* **2003**, *119*, 1838–1842.
32. Fukaminato, T.; Umemoto, T.; Iwata, Y.; Yokojima, S.; Yoneyama, M.; Nakamura, S.; Irie, M. Photochromism of Diarylethene Single Molecules in Polymer Matrices. *J. Am. Chem. Soc.* **2007**, *129*, 5932–5938.
33. Irie, M. Photochromism of diarylethene single molecules and single crystals. *Photochem. Photobiol. Sci.* **2010**, *9*, 1535–1542.
34. R. Zondervan, F. Kulzer, G. C. G. Berkhout and M. Orrit, Local viscosity of supercooled glycerol near T_g probed by rotational diffusion of ensembles and single dye molecules, *Proc. Natl. Acad. Sci. USA* **104**, 2007, 12628–12633.
35. Lin, H.; Truhlar, D. G. QM/MM: what have we learned, where are we, and where do we go from here? *Theor. Chem. Acc.* **2007**, *117*, 185–199
36. Senn, H.; Thiel, W. QM/MM methods for biomolecular systems. *Angew. Chem. Int. Ed.* **2009**, *48*, 1198–1229.

Supporting Information

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

Yuma Goto,¹ Shun Omagari,¹ Ryuma Sato,² Takuya Yamakado,³ Ryo Achiwa,³ Nilanjan Dey,³ Kensuke Suga,³ Martin Vacha^{1,} and Shohei Saito^{3,*}*

¹Department of Materials Science and Engineering, School of Materials and Chemical Engineering, Tokyo Institute of Technology, Ookayama 2-12-1-S8-44, Meguro-ku, Tokyo 152-8552, Japan

²Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)

³Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

Contents

1. General information.....	S2
2. Synthesis of compounds	S5
3. Photophysical properties.....	S11
4. Density functional theory (DFT) calculation.....	S14
5. Single-molecule fluorescence spectroscopy	S22
6. Supporting references	S26

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 nonhydrogen 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 analyses and rheological measurement

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

Classical molecular dynamics simulations

To investigate the behavior of PS with N-FLAP, we conducted molecular dynamics (MD) simulations for the system including one N-FLAP, in which replace alkyl chain with methyl group, and 500 PS molecules. The low MW PS is modeled using winmostar^[S3] and 500 PS molecules are randomly replicated within the box size of $170 \times 170 \times 170 \text{ \AA}^3$. Here N-FLAP located to the center. The energy minimization is performed for this system during 5000 steps and the temperature is then heated from 0 K to 5 K at NVT ensemble ($T = 5 \text{ K}$) for 10 ps, subsequently the temperature is heated from 5 K to 600 K at NPT ensemble ($P = 1 \text{ bar}$ and $T = 600 \text{ K}$) for 100 ps. Next, the system is equilibrated at NPT ensemble ($P = 1 \text{ bar}$ and $T = 600 \text{ K}$) for 10 ns. Here the box size is $94 \times 93 \times 95 \text{ \AA}^3$. Finally, the production run is performed at NPT ensemble ($P = 1 \text{ bar}$ and $T = 600 \text{ K}$) for 100 ns. The force fields of N-FLAP and PS are created using Gaussian 16^[S4] and antechamber module of AMBER 16 program suite^[S5]. In MD simulations, the linear constraint solver algorithm,^[S6] Nosé-Hoover thermostat,^[S7,S8] and the Berendsen barostat^[S9] are applied for the constraints, for the temperature regulation, and for pressure regulation, respectively. The periodic boundary conditions are applied with the particle mesh Ewald method^[S10] and the simulation time step is 2 fs. In this study, MD simulations were performed by the GROMACS version 5.1.4 program suite.^[S11]

Quantum mechanics / molecular mechanics calculation

For the structures obtained from MD simulations, we used the quantum mechanics/molecular mechanics (QM/MM) methods to estimate the energy of N-FLAP in PS molecules and our Own N-layer Integrated molecular Orbital molecular Mechanics^[S12] is adopted. In QM/MM calculations, N-FLAP and PS molecules are divided into QM region and MM region, respectively, and the structure of N-FLAP obtained from MD simulations is replaced by X-ray structure. We considered eleven COT bending angles for N-FLAP, namely 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 degrees and the lowest singlet excited state geometry optimization is performed by time-dependent (TD) density functional theory (ONIOM(TD-CAM-B3LYP/6-31+G(d):MM) and ONIOM(TD-TPSSH/6-31+G(d):MM)) using Gaussian 16. Note that the dihedral angle of N-FLAP is constrained during the geometry optimization and the force fields for MM region are the same with MD simulations.

2. Synthesis of compounds

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

1,2-Bis(hexyloxy)benzene (S1): Catechol (1.51 g, 13.7 mmol) and potassium carbonate (7.53 g, 54.5 mmol) were placed in a Schlenk flask, and the flask was purged with argon. Anhydrous DMF (20 mL) and 1-bromohexane (7.7 mL, 55.0 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** (5.25 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.^[S13]

4,5-Dinitro-1,2-hexyloxybenzene (S2): **S1** (5.25 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** (4.32 g, 11.7 mmol, 86% over 2 steps) as a yellow solid. All characterization data for **S2** matched with those previously reported.^[S14]

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 (38.5 mg, 10% Pd basis) and **S2** (154 mg, 417 μ mol) 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. All characterization data for **2** matched with those previously reported.^[S14]

2,3,12,13-Tetrakis(hexyloxy)cycloocta[1,2-*b*:5,6*b'*]diphenazine (N-FLAP): 2 synthesized by the above procedure was dried under vacuum in a Schlenk flask. The flask was filled with argon, and 2,3,8,9-tetrabromo-dibenzo[*a,e*]cyclooctene (**1**) (43.8 mg, 83.5 μ mol), SPhos Pd G2 (7.21 mg, 10.0 μ mol), SPhos (3.80 mg, 9.26 μ mol) and Cs₂CO₃ (182 mg, 559 μ mol) were added to the flask. Then anhydrous toluene (1.5 mL) was added 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. Then the residue was purified by silica-gel column chromatography (CH₂Cl₂/EtOAc, 4:1) to obtain crude product as a brown solid. Through subsequent precipitation from CH₂Cl₂/MeOH and filtration, the pure compound was obtained as a yellow solid (30.4 mg, 45%). Finally, yellow-colored, needle-shaped crystals of the pure compound were obtained via slow diffusion of MeOH into the toluene layer.

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, 101 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 = 2.4 ns (λ_{obs} = 518 nm).

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

Phenazine-2,3-diol (S5): *o*-Phenylenediamine (**S3**) (1.00 g, 9.3 mmol) and 2,5-dihydroxy-1,4-benzoquinone (**S4**) (1.42 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 (1.98 g, quant.), which was used for the next step without further purification. All characterization data for **S5** matched with those previously reported.^[S15]

2,3-Bis(hexyloxy)phenazine (N-Ref): S5 (127 mg, 0.60 mmol) and potassium carbonate (1.66 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 precipitates were collected by filtration. The precipitates were purified by silica-gel column chromatography (gradient elution of 0–10% ethyl acetate 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 (CDCl_3 , 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); $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 101 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 carbons according to the reference spectrum of 2,3-dimethoxyphenazine^[S16]). HRMS (APCI/TOF) m/z : $[M]^-$ Calcd for $\text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_2$ 380.2469; Found 380.2463. UV-visible absorption (in CH_2Cl_2): λ_{max} ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) = 392 (2.4×10^4), and 375 nm (2.0×10^4). Fluorescence (in CH_2Cl_2 , λ_{ex} = 365 nm): λ_{max} = 428 nm; Φ_{f} < 0.01; τ < 0.1 ns (λ_{obs} = 428 nm).

Fig. 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.

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

Fig. 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

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

Fig. 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 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 a single- or double-exponential function. The goodness of fit was evaluated by χ^2 , and a double-exponential model provided better results for **N-FLAP**. Short-lived components ($\tau_1 < 0.1$ ns) might originate from planarization dynamics in the excited state, although further measurements using ultrafast laser spectroscopy are expected.

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

$\lambda_{\text{FL}} / \text{nm}$	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

	$\lambda_{\text{FL}} / \text{nm}$	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

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

4. Density functional theory (DFT) calculation

DFT calculations of the isolated molecules were performed using the Gaussian 16 program,^[S4] and Kohn–Sham orbitals are displayed by the Avogadro program.^[S17] Model structures **N-FLAP'** and **N-Ref'** were calculated, which have methyl groups in place of the hexyl groups of the corresponding molecules (Fig. S10). In constrained geometry optimization with fixed bending angles, the dihedral angle between the C1–C2–C3 and C2–C3–C4 planes in Fig. S11 was fixed using the ModRedundant keyword. The coordinate system for the consideration of configuration symmetry was defined as Fig. S12.

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

Fig. S11. Definition of a bending angle θ used in the constrained geometry optimization.

Fig. S12. Definition of the coordinate system for geometries bearing a bent C_{2v} or a planar D_{2h} symmetry. Configuration symmetries when the transition dipole is oriented in each axis are shown in the parentheses.

4.1. Optimization of calculation conditions and optical properties based on S_1 energy profiles

Calculation conditions were selected on the basis of S_1 energy profiles of **N-FLAP'**. We first conducted TD-DFT calculation at the PBE0/6-31+G(d) level, which previously provided a S_1 energy profile with an emissive planar geometry for the flapping anthracene.^[S18] However, the most stable S_1 planar geometry of **N-FLAP'** had a non-emissive A_2 symmetry ($f=0$) when using PBE0 (Fig. S13a). Since 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 A_2 state, we continued to search for other optimal conditions. Various basis sets, including def2-SVP, def2-SVPD, aug-cc-pVDZ, and D95+(d) were used with the PBE0 functional, but the energy of the dark A_2 state was still lower than the emissive B_2 state. B3LYP and CAM-B3LYP were also examined with the 6-31+G(d) basis set, but the relative energy levels of the A_2 and B_2 state at the planar geometry was not reversed (Fig. S13b,c). Since B3LYP, 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 PBE0 (HF exchange = 25%), we next tried the TPSSH functional on the basis of the smaller HF exchange (10%).

Fig. S13d shows S_1 energy profiles delineated at the TPSSH/6-31+G(d) level. The lowest S_1 optimized state at the planar geometry has the emissive B_2 symmetry, which agrees with the observed bright green FL with a large Stokes shift. To get further insight into the B_2 state, we compared electronic configurations of the three energetically close S_1 states with different geometries and configurations (Fig. S13e). The Kohn–Sham molecular orbitals (MOs) of **N-FLAP'** are categorized into two groups, MO_{Pz} and MO_{COT} . The former is a MO group in which the electronic contribution of the phenazine (Pz) moieties is significant. The latter group is more contributed by the central cyclooctatetraene (COT) ring. While the dark A_2 state marked in red is mainly contributed only from COT ($HOMO_{COT}\rightarrow LUMO+1_{COT}$), the major transition character of the emissive B_2 state is $HOMO_{COT}\rightarrow LUMO_{Pz}$, indicating the interplay correlation between COT and phenazine is essential for the bright FL. The A_2 state marked in blue originates from the $\pi\pi^*$ transition of phenazine ($HOMO_{Pz}\rightarrow LUMO_{Pz}$) with a negligible effect of COT. Due to the molecular symmetry, the oscillator strength of this state was formally zero. We currently expect that the blue FL of **N-FLAP** observed in rigid environments originates from a bent emissive $\pi\pi^*$ state locating close to the bent A_2 state, as demonstrated in our previous studies.^[S19]

Note that the planar B_{2u} state with the D_{2h} symmetry (analogous to the B_2 state with the bent C_{2v} symmetry) had one imaginary frequency. Thus, we broke the molecular symmetry along the imaginary vibration mode to find a global minimum. Namely, another B_2 state with a planar C_{2v} symmetry was found as a global minimum in S_1 with no imaginary frequency. However, the energy difference between the two states is less than 0.1 kcal/mol, and the corresponding orbital energies were almost the same (Fig. S14). Thus, we do not take into account the symmetry breaking in this paper.

Fig. S13. (a-d) S₁ energy profiles of N-FLAP' in C_{2v} symmetry using four different functionals. (e) Three S₁ states of N-FLAP' bearing different configurations and geometries in which COT bending angles θ are constrained to 15° (TD-TPSSh/6-31+G(d)).

Fig. S14. (a) Energetically close S_1 states and (b) Kohn–Sham orbitals and their energy levels of the planar N-FLAP' bearing different geometries (TD-TPSSH/6-31+G(d)).

4.2. Discussion of optical properties based on S_0 energy profiles

Fig. S15. (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 fixed COT bending angle θ .

Fig. S16. (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 orbitals of **FLAP** and **N-FLAP'** optimized in S_0 and representative transition modes calculated at the TD-TPSSH/6-31+G(d) level.

Fig. S17. (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 orbitals of **N-Ref** and **N-FLAP'** optimized in S_0 and representative transition modes calculated at the TD-TPSSH/6-31+G(d) level.

4.3. Optimized geometries in S_0 and S_1

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

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

Fig. S20. S_1 optimized geometry, representative bond length ($\text{\AA}$), 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.

Fig. 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.

Fig. 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

Fig. S23 a), c) 2D plots of time evolution of fluorescence spectra of single N-FLAP dyes in high Mw polystyrene matrix; b), d), e) Typical fluorescence spectra taken from the 2D plots and showing the short and long wavelengths forms; f) Histogram of single-molecule peak wavelengths (bars, obtained from 24 spectra) and bulk fluorescence spectrum of N-FLAP in drop-cast 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 (Fig. 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 in Fig. S23c-e. After a 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 Fig. 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 550 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 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.

Fig. S24 Examples of time evolution for single-molecule fluorescence spectra of the N-FLAP molecules in the low MW PS matrix.

Fig. S25 Single-molecule spectroscopy of A-FLAP molecules. 2D plots of time evolution of fluorescence spectra in a) the high MW PS and b) the low MW PS; c), d) Typical fluorescence spectra taken from the 2D plots in the high MW PS (c) and the low MW PS (d); the spectra are fitted with a sum of Gaussians (red lines), the blue lines are bulk fluorescence spectra of A-FLAP in toluene solution.

Fig. 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.

Supporting references

- [S1] Sheldrick, G. M. *Acta Crystallogr.* **2015**, *A71*, 3.
- [S2] Sheldrick, G. M. *Acta Crystallogr.* **2015**, *C71*, 3.
- [S3] Winmostar V10, X-Ability Co. Ltd., Tokyo, Japan, **2019**
- [S4] Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian 16, Revision B.01, Gaussian, Inc., Wallingford, CT, **2016**.
- [S5] Case, D. A.; Betz, R. M.; Cerutti, D. S.; Cheatham III, T. E.; T. A. Darden, T. A.; Duke, R. E.; Giese, T. J.; Gohlke, H.; Goetz, A. W.; Homeyer, N.; Izadi, S.; Janowski, P.; Kaus, J.; Kovalenko, A.; Lee, T. S.; LeGrand, S.; Li, P.; Lin, C.; Luchko, T.; Luo, R.; Madej, B.; Mermelstein, D.; Merz, K. M.; Monard, G. Nguyen, H.; Nguyen, H. T.; Omelyan, I.; Onufriev, A.; Roe, D. R.; Roitberg, A.; Sagui, C.; Simmerling, C. L.; Botello-Smith, W. M.; Swails, J.; Walker, R. C.; Wang, J.; Wolf, R. M.; Wu, X.; Xiao, L.; Kollman, P. A. AMBER 2016 (University of California, San Francisco, **2016**).
- [S6] Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. G. E. M. *J. Comput. Chem.* **1997**, *18*, 1463–1472.
- [S7] Hoover, W. G. *Phys. Rev. A* **1985**, *31*, 1695–1967.
- [S8] Hoover, W. G. *Phys. Rev. A* **1986**, *34*, 2499–2450.
- [S9] Berendsen, H. J.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J.; *J. Chem. Phys.* **1984**, *81*, 3684–3690.
- [S10] Essmann, U.; Darden, T. A.; Perera, L.; Berkowitz, M. L.; Lee, H.; Pedersen, L. G. *J. Chem. Phys.* **1995**, *103*, 8577–8593.
- [S11] Gromacs User Manual version 5.1.4, www.gromacs.org
- [S12] Dapprich, S.; Komáromi, I.; Byun, K. S.; Morokuma, K.; Frisch, M. J. *J. Mol. Struct. (Theochem)* **1999**, *462*, 1–21.
- [S13] Zhao, B.; Liu, B.; Png, R. Q.; Zhang, K.; Lim, K. A.; Luo, J.; Shao, J.; Ho, P. K. H.; Chi, C.; Wu, J. *Chem. Mater.* **2010**, *22*, 435–449.
- [S14] Howard, M. J.; Heirtzler, F. R.; Dias, S. I. G. *J. Org. Chem.* **2008**, *73*, 2548–2553.
- [S15] Seillan, C.; Brisset, H.; Siri, O. *Org. Lett.* **2008**, *10*, 4013–4016.

- [S16] Conda-Sheridan, M.; Marler, L.; Park, E.-J., Kondratyuk, T. P.; Jermihov, K.; Mesecar, A. D.; Pezzuto, J. M.; Asolkar, R. N.; Fenical, W.; Cushman, M. *J. Med. Chem.* **2010**, *53*, 8688–8699.
- [S17] Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. *J. Cheminfomatics* **2012**, *4*, 17.
- [S18] Yamakado, T.; Osubo, K.; Osuka, A.; Saito, S. *J. Am. Chem. Soc.* **2018**, *140*, 6245–6248.
- [S19] Kimura, R.; Kuramochi, H.; Liu, P.; Yamakado, T.; Osuka, A.; Tahara, T.; Saito, S. *Angew. Chem. Int. Ed.* **2020**, *59*, 16430–16435.