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Probing a Microviscosity Change at the Nematic-Isotropic Liquid Crystal Phase Transition by a Ratiometric Flapping Fluorophore

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Understanding the microviscosity of soft condensed matter is important to clarify the mechanisms of chemical, physical or biological events occurring at the nanoscale. Here, we report that flapping fluorophores (FLAP) can serve as microviscosity probes capable of detecting small changes. By the ratiometric fluorescence analysis, one of the FLAP probes detects a macroscopic viscosity change of a few cP, occurring at the thermal phase transition of a nematic liquid crystal. We discuss the impact of the chemical structure on the detection capability, and the orientation of the FLAP molecules in the ground and excited states. This work contributes to experimentally providing a molecular picture of liquid crystals, which are often viewed as a continuum.

Analysis of the viscosity of liquid crystals is important not only in basic science^[1] but also in applications to displays and microfluidic devices^[2]. Since low-viscosity nematic liquid crystals have a fast response time to external fields, molecular theory to describe the viscosity of nematic liquid crystals have been widely discussed.^[3] 5CB (4-cyano-4'-pentylbiphenyl) is one of the most-studied nematic liquid crystal, which shows a thermal phase transition at the clearing point around 35 °C. A discontinuous decrease in shear viscosity (only by a few cP change around 25 cP) during the isotropic-to-nematic phase transition of 5CB was reported using a rheometer (Fig. 1a) and discussed along with a small discontinuous increase in density.^[4] However, it is challenging to monitor "microviscosity" in a phase transition of LC materials by using fluorescent molecular viscosity probes. Although one example was reported for a fluorogenic molecular rotor to detect a phase transition of a liquid crystal "80B4100", the structure, phase and viscosity value of this liquid crystal were undisclosed.^[5] Microviscosity is a different measure from shear viscosity obtained from

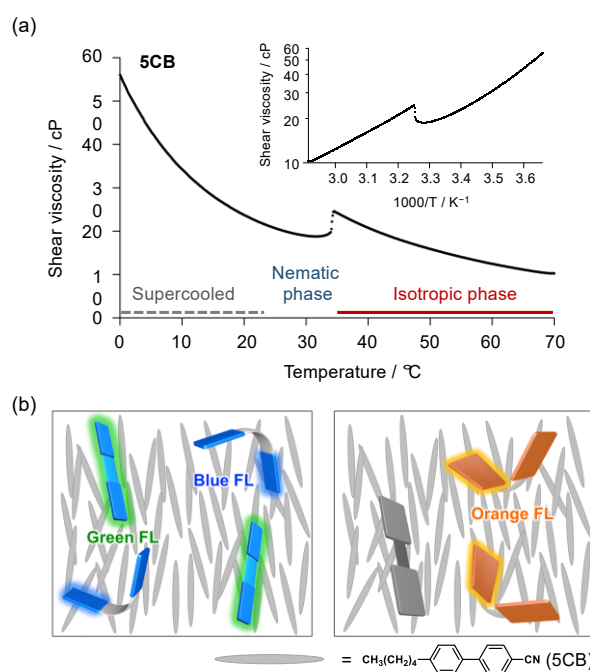


Fig. 1. (a) Temperature-dependent shear viscosity of 5CB, measured in a cooling process (6 °C/min) by a rheometer. (inset) Andrade plot (b) Schematic representation of a nematic liquid crystal doped with viscosity-probing flapping fluorophores **FLAPO** (ratiometric probe; left) and **FLAP1** (fluorogenic probe; right), in which photoexcited molecules are partially planarized.

rheometers. Microviscosity reflects the degree to which ultrafast conformational dynamics of photoexcited molecules is suppressed in a nanoscale environment. While molecular viscosity probes represented by molecular rotors have been widely used for bioimaging technique,^[6] applications to monitoring nano-environments of condensed matter have been also demonstrated.^[7,8a]

With this background, we have previously developed flapping fluorophores (FLAP) as a new category of molecular viscosity probe.^[8] While conventional molecular rotors probe

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microviscosity by internal rotation dynamics, the flapping fluorophores sense nano-environments by a bent-to-planar conformational change in the excited state. The flapping fluorophores show high sensitivity particularly in a low viscosity range below 100 cP.^[8a,8c-e] Very recently, a modified flapping probe even realized real-time monitoring of dynamic polymer free volume by single-molecule fluorescence spectroscopy.^[8f] Here, we report the attempts to monitor the microviscosity change in the thermal phase transition of 5CB, using flapping fluorophores (Fig. 1b) as well as a commonly-used molecular rotor, **BODIPY-C₁₂**.^[6a,9] Furthermore, we have analyzed anisotropy in the absorption/fluorescence of the FLAP-doped 5CB with uniaxial alignment to gain insight into the orientation of the FLAP molecules in a liquid crystal.

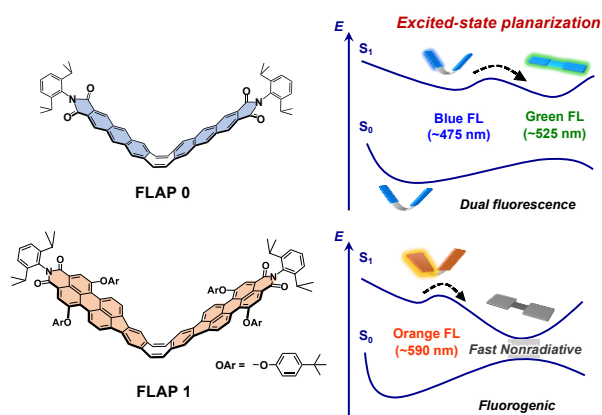


Fig. 2. Flapping anthraceneimide **FLAP0** and flapping peryleneimide **FLAP1** (left). Dual fluorescence properties of **FLAP0** and fluorogenic properties of **FLAP1** are demonstrated with their energy profiles of the ground state (S_0) and the excited state (S_1) (right).

FLAP0^[8a-8c] and **FLAP1**^[8d,8e] were synthesized according to the reported protocols. Since their planarization dynamics and an accompanying electronic configurational switch in S_1 are sensitive to local environments, both **FLAP0** and **FLAP1** work as a viscosity probe in a low viscosity range (below 100 cP). However, a subtle difference in their S_1 excited state profiles leads to the distinct spectroscopic responses. **FLAP0** shows a bright green FL emitted from the planar geometry (Fig. S4-5) in low-viscosity media, while the intensity of the blue fluorescence (FL) derived from the V-shaped geometry (Fig. S4-4) increases in high-viscosity media due to the suppression of the planarization in the excited state.^[8a] Polarity-independence has also been confirmed in the ratiometric FL of **FLAP0**. On the contrary, **FLAP1** does not show FL from the planar geometry due to fast nonradiative decay. However, the S_1 conformational planarization of **FLAP1** is significantly suppressed by increasing viscosity, and it works as a fluorogenic viscosity probe. A pronounced FL enhancement and a prolonged FL lifetime are observed in a viscous media even less than 3 cP.^[8d,8e] **BODIPY-C₁₂** is categorized as a fluorogenic molecular rotor. Since the dynamic internal rotation in S_1 is suppressed in highly viscous media, FL quantum yield increases with bright green FL and FL lifetime prolongs.^[6a,9]

We started experiments from FL measurements of the three molecular viscosity probes in 5CB (*ca.* 20-cP viscosity at 25 °C) and in benzonitrile (PhCN; 1.2-cP viscosity at 25 °C), which is a reference solvent bearing a similar dipole moment to 5CB (Fig. 3, See Chapter 1 in the SI for details). The ratiometric probe

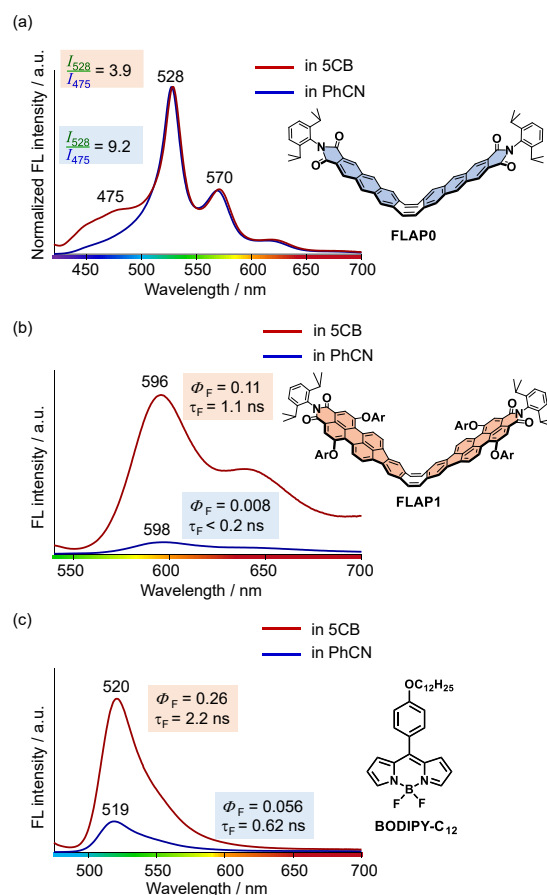


Fig. 3. FL properties of (a) **FLAP0** ($\lambda_{\text{ex}} = 405$ nm), (b) **FLAP1** ($\lambda_{\text{ex}} = 520$ nm), and (c) **BODIPY-C₁₂** ($\lambda_{\text{ex}} = 460$ nm) in 5CB and in PhCN. Concentration of the viscosity probes $< 10^{-4}$ M. The FL intensities in (b) and (c) are presented to account for the relative FL quantum yields. Non-polarized excitation with no analyzer in a standard quartz cuvette (1.0 mm).

FLAP0 shows a smaller FL ratio of I_{528}/I_{475} ($= 3.9$) in 5CB than in PhCN (9.2). This indicates that the planarization in S_1 is partially suppressed due to higher viscosity of 5CB and the blue fluorescence from the V-shaped conformer was observed more significantly. On the other hand, the fluorogenic probe **FLAP1** display a large difference in FL quantum yield and FL lifetime. In PhCN, the FL quantum yield (Φ_F) of **FLAP1** was 0.008 and the fluorescence lifetime (τ_F) was approximately 0.1–0.2 ns, whereas, in 5CB, Φ_F increased more than 10 times to 0.11 and τ_F also prolonged to 1.1 ns. The similar tendency was observed for **BODIPY-C₁₂** ($\Phi_F = 0.056$, $\tau_F = 0.62$ ns in PhCN and $\Phi_F = 0.26$, $\tau_F = 2.2$ ns in 5CB). These results also indicate that the planarization of **FLAP1** as well as the internal bond rotation of **BODIPY-C₁₂** is partially suppressed in viscous 5CB. In other words, the difference of microviscosity between PhCN and 5CB was detectable with all three probes.

Next, temperature dependence of the FL properties was studied in 5CB (Fig. 4). The concentration of the fluorescent probes is in the order of 10^{-5} M (less than 10^{-2} wt%), which has no influence on the thermal phase transition of 5CB (Fig. S2-11). As the temperature increased over the clearing point of 5CB

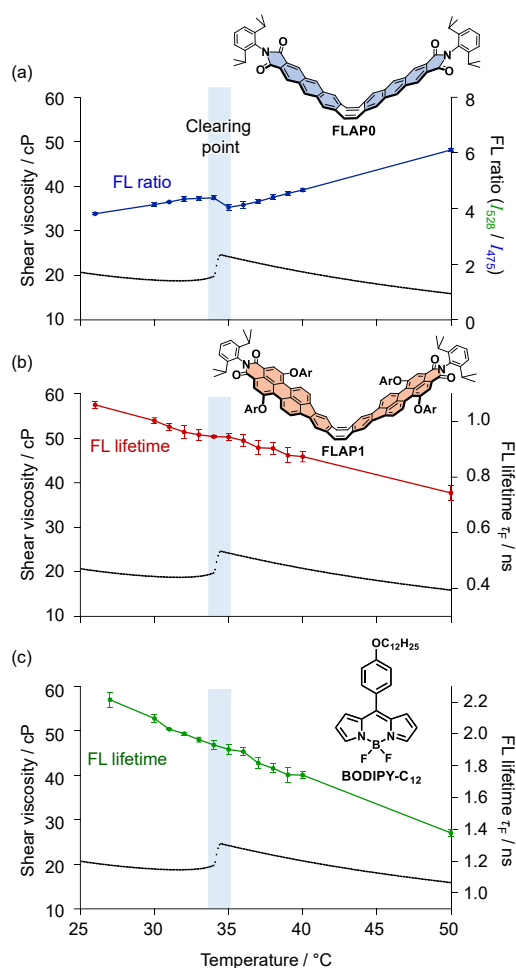


Fig. 4. Temperature-dependent (a) FL ratio of **FLAP0**, (b) FL lifetime of **FLAP1**, and (c) FL lifetime of **BODIPY-C₁₂** doped in 5CB. Each measurement was performed when the temperature remains constant for 5–10 min after the gradual heating. Measurements were performed 3 times to display sample standard deviation.

(isotropic phase), the FL ratio of I_{528}/I_{475} continuously increased in **FLAP0**, and the fluorescence lifetime became shorter in **FLAP1**. On the other hand, a rigid peryleneimide fluorophore **Wing1**, which is a reference compound of flexible **FLAP1**, showed almost constant FL lifetime in the higher temperature range (Fig. S2-8). These results clearly indicate that the observed FL responses of FLAP are derived from the flexible motion, and that the planarization dynamics in S_1 is promoted in higher temperature. **BODIPY-C₁₂** also showed the similar tendency with **FLAP1**. Interestingly, these tendencies of the three probes are also consistent in the temperature range below the clearing point (nematic phase). However, more importantly, only **FLAP0** displayed a characteristic discontinuous change in the I_{528}/I_{475} FL ratio at the nematic-to-isotropic phase transition of 5CB, whereas **FLAP1** and **BODIPY-C₁₂** did not detect the phase transition by the FL lifetime measurement. The values of the FL ratio (I_{528}/I_{475}) using **FLAP0** was 4.4 ± 0.1 at 34 °C and 4.0 ± 0.1 at 35 °C. The similar tendency was observed at different heating rates (Fig. S2-4. and Table S2-3). The significant decrease of the FL ratio over the narrow temperature range can be attributed to the detection of

a nano-environmental change by the phase transition. Since **FLAP0** has the polarity independence in a wide range of dielectric permittivities,^[8a,8c] the decrease of the FL ratio can be mainly assigned to the increase in "microviscosity" around the clearing point in the nematic-to-isotropic phase transition. Namely, the planarization dynamics of **FLAP0** in the excited state become slightly more suppressed when the nematic 5CB turns isotropic at the clearing point. It is noteworthy that the shear viscosity measured by a rheometer also increased in the nematic-to-isotropic phase transition. The agreement supports the close relationship between the macroscopic and microscopic (local) viscosities, which has been recognized as Förster–Hoffman (F-H) equation (Fig. S2-6),^[6a,6b,9] particularly in Newtonian fluids. The efficacy of **FLAP0** over **BODIPY-C₁₂** for the detection of the phase transition would originate from the different motions for the viscosity sensing (flapping vs rotation) and the gap of the required free volumes for the dynamic conformational change.^[8f] On the other hand, the unsuccessful detection by **FLAP1** would be simply due to the saturated viscosity response below 20 cP.^[8e]

Anisotropy in the absorption/FL of **FLAP0** in a uniaxially aligned 5CB was investigated by micro-spectroscopy to gain insight into the molecular orientation of **FLAP0** in the liquid crystal host (see Fig. S3-1 for details of the measurement). Here, **FLAP0** was doped in 5CB at a weight ratio of 0.5 wt% or 1 wt%, and the mixture was filled in a glass sandwich cell with uniaxial rubbing treatment. Measurement of polarized absorbance spectra showed a small anisotropy along the LC director that disappeared in the isotropic phase (Fig. S3-2), indicating that the orientation of the V-shaped **FLAP0** molecules is not isotropic in the ground state (See the transition dipoles in Fig. S4-2). Upon non-polarized excitation at approx. 380 nm, the emission was anisotropic with stronger intensity along the director, and interestingly, the intensity ratio of the emission peaks (FL ratio) varied depending on polarization (Fig. 5, Fig. S3-3). Whereas the blue emission peak at 475 nm, attributed to the V-shaped **FLAP0**, showed a fluorescent dichroic ratio of $I_{//}/I_{\perp} = 1.6$, the green emission peak at 528 nm, attributed to the planarized **FLAP0**, showed a larger value of $I_{//}/I_{\perp} = 2.5$, causing the I_{528}/I_{475} intensity ratio to vary between 1.8 and 1.2. The anisotropy disappeared in the isotropic phase (Fig. S3-4), suggesting that the nematic order causes the planarization of **FLAP0** to occur preferentially along the nematic director. In fact, it is well known that the viscosity of liquid crystals is anisotropic and depends on the relative directions of the director^[3]; it is therefore natural for the "microviscosity" to also be anisotropic and depend on the relative orientations of the fluorescent probe and the director. Measurement of the non-polarized fluorescence spectrum upon polarized excitation also supported the anisotropy in planarization probability, giving a larger I_{528}/I_{475} ratio for excitation polarized along the director (Fig. S3-5). Since the planarization rate of **FLAP0** is much faster (subnanoseconds in its time constant) than the spectral acquisition rate in these measurements, further investigation employing ultrafast pump-probe experiments^[10] as well as molecular dynamics (MD) simulations would be necessary to fully understand the orientation dynamics of **FLAP0** in a liquid crystal. Nevertheless,

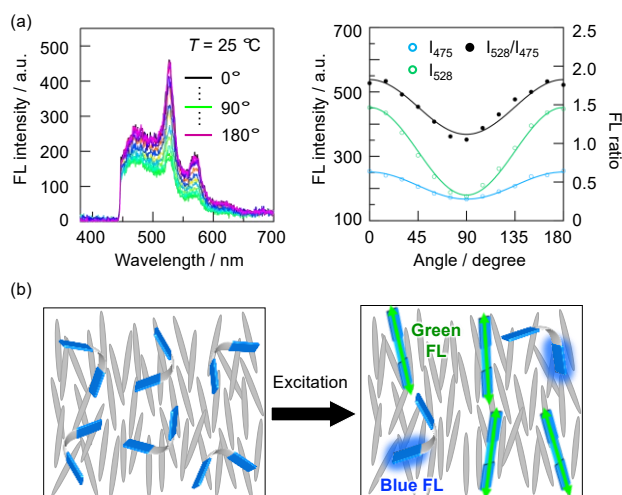


Fig. 5. (a) Analyzer-angle dependence in the FL spectra of **FLAPO** doped in 5CB at 25 °C (nematic phase, uniaxial alignment) (left). Non-polarized excitation: 380 nm. The FL intensities at 475 nm and 528 nm, and the FL ratio of I_{528}/I_{475} plotted with relative directions to the 5CB director (right). (b) Schematic representation of the anisotropic green fluorescence from the planarized excited species and the less anisotropic blue fluorescence from the V-shaped excited species.

their capability for ratiometric detection of small viscosity changes at the nematic-isotropic phase transition, which was also confirmed from microscopic measurements (Fig. S3-6), make flapping fluorophores promising candidates where other probes fall short.

Conclusions

Three molecular viscosity probes, a ratiometric flapping fluorophore **FLAPO**, a fluorogenic flapping fluorophore **FLAP1**, and a fluorogenic molecular motor **BODIPY-C₁₂** were doped in a nematic liquid crystal 5CB, and their fluorescence properties were analyzed at different temperatures. As a result, only by the ratiometric fluorescence spectroscopy using **FLAPO**, a small discontinuous viscosity gap (corresponding to a few cP change in shear viscosity around 20 cP) during the nematic-to-isotropic phase transition of 5CB was successfully detected. Moreover, planarized emissive species of **FLAPO** tend to be parallel to the director of 5CB. These results provide a deeper insight into the molecular theory of the liquid crystal viscosity and will lead to real-time 2D imaging technique of microviscosity during the phase transition of liquid crystals.

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Conflicts of interest

There are no conflicts to declare.

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