Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Poly(dimethylsiloxane) as a room-temperature solid solvent for photophysics and photochemistry (Electronic Supplementary Information)

John A. Clark,[a] Samantha Robinson,[a] Eli M. Espinoza,[b] Duoduo Bao,[a] James B. Derr,[c] Luca Croft,[a] Omar O'Mari,[a] William H. Grover[a] and Valentine I. Vullev*[a,b,c,d]

Table of Contents:

[a] *Department of Bioengineering, University of California, Riverside, CA 92521, USA.*
[b] *Department of Chemistry, University of California, Riverside, CA 92521, USA.*
[c] *Department of Biochemistry, University of California, Riverside, CA 92521, USA.*
[d] *Materials Science and Engineering Program, University of California, Riverside, CA 92521, USA.*

Materials. Prepolymer of PDMS (Sylgard 184 silicone elastomer base kit) was obtained from Dow Corning Corporation. The dyes, 3,3′-diethylthiacyanine iodide, thioflavin T, Congo red, TWEEN 40, and *tris*(hydroxymethyl)aminomethane were purchased from Millipore Sigma. *N*-phenyl-4-dimethylamino-1,8-naphthalimide was synthesized as previously described.[S1] The solvents were purchased from Fisher Scientific, and the polystyrene cuvettes from VWR International, LLC. The original bacterial cultures were purchased from Carolina Biological Supply Co. and stored at –80 °C.

Preparation of PDMS blocks. The PDMS was prepared by mixing 10 (weight) parts of silicone elastomer base with 1 (weight) part of curing agent in plasticware. The mixture was vigorously stirred for 2 to 5 min and degassed for 30 min under vacuum. The degassed prepolymer mixture was poured in into polystyrene cuvettes, serving as molds, and allowed to cure and solidify for 24 hours at room temperature under vacuum. Periodically, the vacuum was released and the PDMS in the cuvettes was visually examined for inconsistencies and inhomogeneities. The cuvettes with solidified elastomer were broken and the polystyrene pieces removed to obtain the PDMS block (see Movie 1). When needed, the PDMS material is readily cut with a scalpel. Nevertheless, the surfaces obtained after such cuts do not have the smoothness needed for optical measurements.

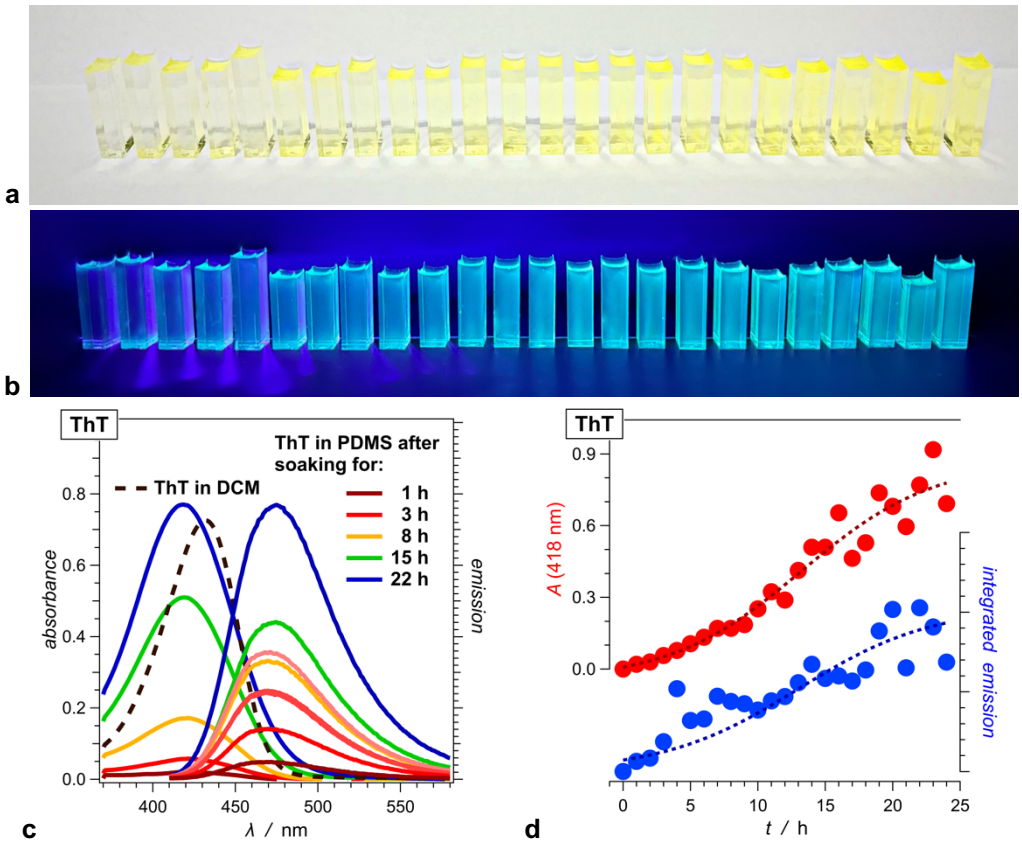

Figure S1. Loading ThT in PDMS blocks by soaking them in 20 μM DCM solution of the dye for different durations of time. (a) Bright-field and (b) fluorescence images of the blocks soaked for 1 to 24 hours (λ_{ex} = 365 nm). The swelling and soaking of all 24 blocks in the same DCM ThT solution was initiated at the same time. Each hour, one block was taken out, washed with DCM, and allowed to shrink to its original size. (c) Absorption and emission spectra of ThT in the PDMS blocks soaked for different durations of time. For comparison, the absorption spectrum of the ThT in the DCM soaking solution is shown. (d) An increase in the absorbance at 418 nm and the emission intensity (obtained from integrating the fluorescence spectra) with the time of soaking.

To remove any small-molecular-weight contaminants left over after the polymerization, the PDMS blocks were immersed in DCM and allowed to swell and soak for 5 to 12 hours. The PDMS blocks were removed from the solvent, washed with DCM, and allowed to shrink by placing them under vacuum for two hours to evaporate the solvent remaining in them. This cleaning procedure was repeated three times. In some of the steps, swelling and soaking in volatile amines and hydrocarbons can also prove helpful.

Loading dyes in PDMS blocks. PDMS blocks are immersed in DCM solutions of the dyes with different concentrations. The concentrations of the DCM dye solutions are usually two-to-five times larger than the desired concentrations in the PDMS samples. For THIA, we kept the PDMS blocks in the DCM dye solutions for about 5 hours (Figure 2). For ThT, the soaking time can extend to more than 5 hours without visible changes in the absorption and emission spectra of the dye (Figure S1). A careful examination, however, reveals a slight bathochromic shift of the ThT fluorescence spectra as the soaking time extends, i.e., this shift is about 1 nm for eight hours of soaking, and increases to about 5 nm for 24 hours of soaking. The PDMS blocks are taken out of the dye solutions, washed with copious amounts of DCM, and allowed to shrink back to their original by evaporating the solvent from the PDMS interior under vacuum. The blocks are clear and transparent, showing the color of the dye that is incorporated in them.

Preparation of SOA samples. SOA is purified by silica column using chloroform as the eluent and recrystallized with ethanal. Due to fragility of quartz and glass cuvettes, we prepare the SOA samples in 6-mm Pyrex test tubes. A small volume of a DCM stock solution of the dye was mixed thoroughly with SOA powder. The mixture was placed into the Pyrex tube and melted using a heat gun. It is essential to allow the molten mixture to homogenize. It may require some stirring before allowing it to cool down and solidify. When the mixture is uniform, allow one hour for cooling before measurements.

Preparation of bacterial samples. The bacterial samples are inoculated in a nutrient rich Luria Broth Agar and allowed to replicate in a shaker at 36 ºC for 16 hours to ensure they are in the exponential growth phase. The bacteria are centrifuged down to pellets and then broth is poured out and instead replaced with 2 mM Tris buffer (pH 8.5). The bacteria cells are then subjected to two additional centrifugation and tris-buffer replacement. The cell density is confirmed prior to studies using a hemocytometer. Stock solutions of the staining dyes are added to the cell suspensions. Final dye concentrations in the cell suspension are 5 to 10 μM. After allowing the cells to uptake the dye for several minutes, the suspensions are ready for imaging and spectroscopy.

The buffer is prepared by adding 242 mg of Tris(hydroxymethyl)aminomethane to 1L of MilliQ water. The pH is adjusted to 8.5 by adding small aliquots of HCl, and 2.55 mL TWEEN 40 is added. The prepared buffer (2 mM Tris, pH 8.5, with 2 mM TWEEN 40) is autoclaved and ready to use for biological samples.

Optical spectroscopy. Steady-state absorption spectra are recorded in a transmission mode using a JASCO V-670 spectrophotometer (Tokyo, Japan). The steady-state emission spectra and the time-correlated single-photon counting (TCSPC) fluorescence decays are measured, using a FluoroLog-3 spectrofluorometer (Horiba-Jobin-Yvon, Edison, NJ, USA), equipped with a pulsed diode laser (λ = 406 nm, 200-ps pulse width). We place the 2-mm cuvettes, the thin PDMS slabs and the round 6-mm test tubes with the SOA samples in a short-path-length cuvette adapter that fits in 1-cm cell holders of the spectrometers.

For the liquid samples in 1-cm cuvettes and in 1-cm PDMS blocks, the emission is recorded at a right-angle configuration. For the liquid samples in 2-mm cuvettes and for the samples in thin PDMS slabs, we use a small-angle (face-fluorescence, FF) configuration. For the flat samples thinner than 1 cm (in PDMS slabs, in 2-mm cuvettes and in 1-mm cuvettes), the measured absorption is converted to the absorbance for 1-cm pathlength by dividing by the thickness of the sample in centimeters. For the round SOA samples, the conversion factor was obtained from absorption and emission measurements of the same liquid sample placed in a test tube and in a 1-cm cuvette.

The fluorescence quantum yields, ϕ_f (Table 1), are determined by comparing the integrated emission intensities of the samples with the integrated fluorescence of a reference sample with a known fluorescence quantum yield, ϕ_{f0}:

$$\phi_f = \phi_{f0} \frac{\int F(\lambda)\, d\lambda}{\int F_0(\lambda)\, d\lambda} \times \frac{1 - 10^{-A_0(\lambda_{ex})}}{1 - 10^{-A(\lambda_{ex})}} \times \left(\frac{n}{n_0}\right)^2$$

The subscript "0" indicates the fluorescence, F, the absorption, A, at the excitation wavelength, λ_{ex}, and the refractive index, n, of the standard with a known quantum yield. For a standard, we use ethanol solution of coumarin 151, $\phi_{f0} = 0.49$. Because the solutions are diluted, n are the refractive indexes of the solvents. For PDMS, we use $n \approx 1.42$,[S2] and for SOA, $n = 1.466$. For samples in 1-cm PDMS block and the 4-mm PDMS slabs, the obtained emission decays with TCSPC were identical. The estimated fluorescence quantum yields, however, show improved reproducibility when the emission is recorded from samples in PDMS slabs at small-angle FF configuration. This trend is ascribed to the attainment of improved homogeneity of the dye distribution in the PDMS slabs, in comparison with the PDMS blocks.

The transient-absorption (TA) data, $\Delta A(\lambda, t)$, are recorded in transmission mode with 2-mm quartz cuvettes using a Helios pump-probe spectrometer (Ultrafast Systems, LLC, Florida, USA) equipped with a delay stage allowing maximum probe delays of 3.2 ns at 7 fs temporal step resolution (Figure S2a,c,e,g). Following chirp correction, we carry out global-fit analysis as implemented by Surface Xplorer (Ultrafast Systems, LLC, Florida, USA) (Figure S2b,d,f,h). The absorbance of the samples at the excitation wavelength, $A(\lambda_{ex})$, is adjusted to about 0.4 to 0.6 in the 2-mm cuvettes. Immediately prior to the measurements, all samples are purged with argon for 5 to 10 min per 1 mL of sample. The laser source for the Helios is a SpitFire Pro 35F regenerative amplifier yielding (Spectra Physics, Newport, CA, USA) generating 800-nm pulses (>35 fs, 4.0 mJ, at 1 kHz). The amplifier is pumped with of an Empower 30 Q-switched laser ran at 20 W at the 2nd harmonic. A MaiTai SP oscillator provided the seed beam (55 nm bandwidth). For the ThT and THIA sample, we use the second harmonic of the SpitFire output for a pump, i.e., $\lambda_{ex} = 400$ nm (Figure S2).

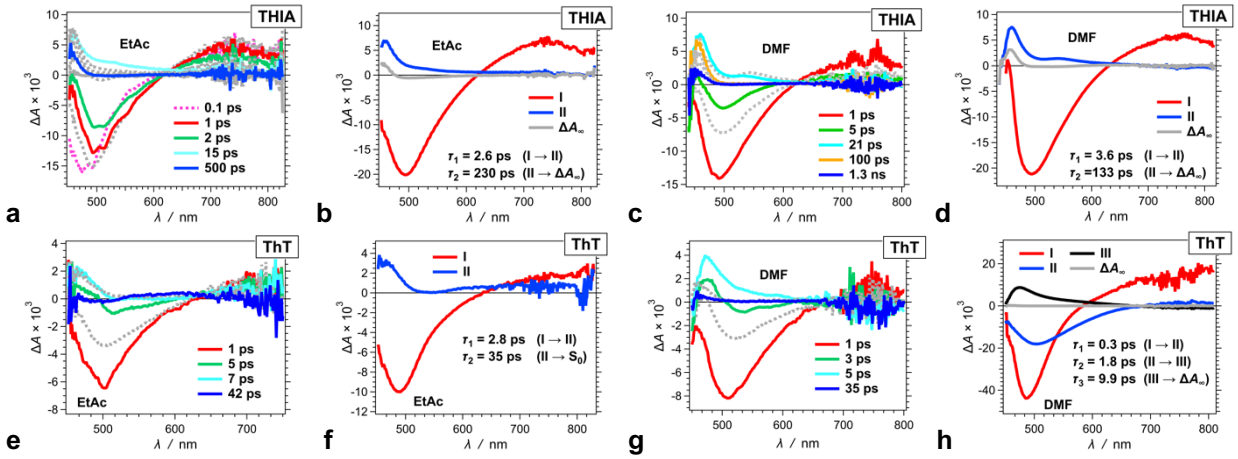

Figure S2. Transient absorption (TA) dynamics of (a-d) THIA and (e-h) ThT in (a,b,e,f) EtAc and (c,d,g,h) DMF. (a,c,e,g) TA spectra recorded at different times ($\lambda_{ex} = 400$ nm, IRF FWHM = 200 fs). (b,d,f,h) Evolution-associated differential spectra (EADS) with the corresponding transition timeconstants extracted from the TA data using global-fit analysis as implemented by Surface Xplorer (Ultrafast Systems, FL, USA).

Optical imaging. Bright field and fluorescence images of bacterial samples were acquired using a Nikon Ti-U inverted microscope (Nikon Inc., Melville, NY) equipped with a 40× Nikon objective. A 100-watt Xenon arc lamp (LUDL Electronic products, Hawthorne, NY), in combination with a FITC filter set (lex = 482, lem = 536 nm, band widths = 25 nm, Chroma Technology, Bellows Falls, VT), was used for fluorescence imaging. The images were recorded with a Hamamatsu EM- CCD digital camera (model C9100-13, Hamamatsu Corp., Bridgewater, NJ) controlled by HCImage application software. For the fluorescence images, a DAPI filter set allows excitation in the near UV region and transmits emission in the blue spectral region, which matches the absorption and fluorescence of THIA and ThT.

Evaluating sample concentrations. The dye concertation in the PDMS samples were estimated directly from the absorption maxima, assuming $\varepsilon \approx 9\times10^4$ M^{-1} cm^{-1} for THIA and $\varepsilon \approx 3.6\times10^4$ M^{-1} cm^{-1} for ThT.[S3,S4] Nevertheless, cyanine dyes, such as THIA, are prone to aggregation that, while might not shift the absorption maxima (e.g., for THIA), can induce some changes in the molar extinction coefficients.[S5]

Indirect estimation of the dye concentration in PDMS allows avoiding the assumption that the molar extinction coefficient is invariant to the solvating media. Recording the absorption of the DCM dye solutions and their volumes before and after the soaking of the elastomer offers a means for estimating the sample concentration in the PDMS blocks from the moles of dye, n_{dye}, transferred into them, i.e., $n_{dye} = (V_0 A_0 - V_A A_A)\ (\varepsilon\ L)^{-1}$. The subscripts "0" and "A" designate before and after the soaking process (Figure S3). In a 20-mL scintillation vials, a 1×1×4-cm PDMS block is usually covered with about 16 mL of DCM dye solution, and after the soaking is completed, about 10 mL solution is poured out of the vial.

The direct and indirect estimates of dye concentration in the doped PDMS blocks can differ by up to 50% (Figure S3). Differences in the molar extinction coefficient of THIA in different solvating media and heterogeneity of dye distribution in the PDMS blocks can affect the direct evaluation of the dye concentration. The indirect evaluation, on the other hand, yields average dye concentrations and may not represent the optical paths through the PDMS blocks during measurements. In that respect, the direct estimates provide information about the dye concertation within the volume of the passing optical beam in the spectrophotometer. While it matters for photophysical analysis, it warrants caution about the assumed values of the dye extinction coefficients when evaluating dye concertation.

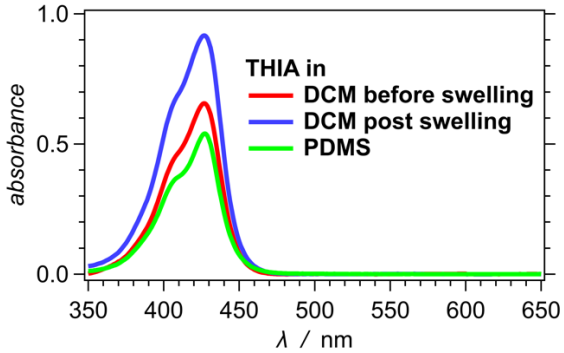

Figure S3. Absorption spectra of (1) DCM solution of THIA (7 μM) used for swelling a 1×1×4-cm PDMS bock, (2) the DCM dye solution after the 12-hour soaking, and (3) the PDMS block after it shrunk to its original size. Assuming that the molar extinction coefficient of THIA in the three samples is the same (~ 9×10^4 M^{-1} cm^{-1} at the absorption maximum), the dye concentration in the PDMS can be calculated directly from the absorption, i.e., $C = A_{PDMS}\ \varepsilon^{-1}\ L^{-1} = 6$ μM. Conversely, the THIA concertation in the doped PDMS can be estimated from the volumes of the DCM solutions before and after the soaking, V_0 and V_A, respectively, the volume of the PDMS block, V_{PDMS}, and the absorption of the dye solutions before and after the soaking, A_0 and A_A, respectively, i.e., $C = (V_0 A_0 - V_A A_A)\ (\varepsilon\ L\ V_{PDMS})^{-1} \approx 4$ μM, which is about 30% less from what the direct estimate from A_{PDMS} yields.

Examining homogeneity of dye distribution in PDMS. Visual evaluation of the PDMS blocks provides a facile and expedient way to examine if the dyes are homogeneously distributed through the bulk of the elastomer. After 8 hours of soaking with THIA and ThT, the PDMS blocks show clear transparent yellow(ish) consistency (left blocks on Figure S4a,c). In the case of THIA, some cloudiness forms on bottom of the block, which spreads upwards and becomes quite apparent after 22 hours of soaking (left and central blocks on Figure S4a). Aggregation that leads to formation of solid particles of the dye salt inside the PDMS block can account for this loss of transparency. Extending the soaking times of the PDMS block in the THIA DCM solution leads to: (1) a growth of a 405-nm shoulder on the absorption band of THIA in PDMS (Figure 2c), which is consistent with aggregate formation; and (2) baseline upward shift with shortening the wavelength (Figure 2c), which is consistent with formation of particles with sizes comparable with the wavelength of visible light. In this sample preparation, we observe baseline shift when the soaking times extend beyond 10 hours. Nevertheless, the THIA-doped PDMS block after 8-hours of soaking does not show baseline shift (Figure 2c) even though it appears to have a cloudy region (Figure S4a). The beam of the spectrophotometer, however, does not pass close enough to the bottom of the PSMS block to be affected by this scattering deposit of THIA particles.

The PDMS blocks doped with ThT is clear even after extended soaking times. After 22-hour of soaking, however, greenish glow appears in the center of the PDMS block (Figure S4c). It is not cloudy, and the green glow is from the ThT fluorescence under the room-lights illumination. This observation suggests that the ThT concentration in the center is higher than throughout the rest of the block. Also, the ability to see fluorescence from ThT under illumination from ambient light is consistent with the enormous increase in its emission quantum yield when placed in PDMS (Table 1).

The 4-mm PDMS slabs are not intensely colored (the dye absorption is around 0.15), but the dye distribution in them appears homogeneous (Figure S4a,c).

The fluorescence band ThT and THIA (when excited at about 400 nm) overlap and extend to about 550 nm (Figure 3a,b), which is consistent with the greenish fluorescence that is apparent for the THIA-doped PDMS (Figure S4b). Nevertheless, the ThT-doped PDMS samples show bluish fluorescence (Figure S4d), most likely from the LE emission. The excitation for the fluorescence images is at 365 nm.

The fluorescence images of these PDMS samples give further insight about the homogeneity of the dye distribution in them (Figure S4b,d). The THIA-doped PDMS block, soaked for 8 hours, shows increased florescence signals from the middle that extends along the height of the whole block (left block on Figure S4b). Even though the PDMS block is clear and transparent, this observation indicates an increase in the dye concentration toward the center of the block. Indeed, extending the soaking periods leads to formation of particulate aggregates responsible for the cloudiness observed on the bright-field images.

The fluorescence images of the ThT samples show two types of heterogeneity of dye distribution. The block soaked for 8 hours shows enhanced fluorescence at the walls and the edges, especially on the top and on the bottom (left block on Figure S4d). As the soaking time increases, the region with enhanced fluorescence signal moves to the center of the block (middle block on Figure S4d). The soaking times do not always allow reaching equilibrium. The solvent permeates into the PDMS faster than dyes. Thus, the dye resides longer at the surface of the PSMS blocks and gets collected there before it migrates to the center. Conversely, during drying, the solvent evaporates from the outside to the center and enhances the dye concertation in the DCM solution the polymer matrix near the walls of the PDMS blocks. Even if the dye is homogeneously distributed in the swollen PDMS, the solvent evaporation generates a concertation gradient that can drive the dye toward the center, which leads to this type of heterogeneity observed in some of the 1-cm blocks. The properties of the dye and the solvent affect the interplay between these two effects.

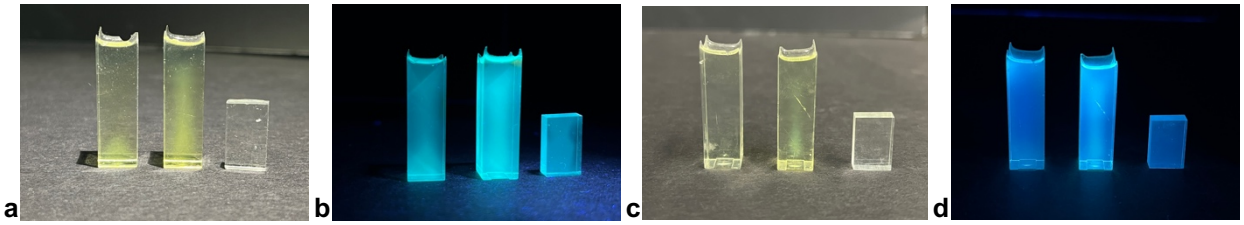

Figure S4. (a,c) Bright-field and (b,d) fluorescence images of PDMS blocks loaded with (a,b) THIA and (c,d) ThT. On each image: left – 1×1-cm block soaked with 20 μM dye solution in DCM for 8 hours; centre – 1×1-cm block soaked with 20 μM dye solution in DCM for 22 hours; and right – 0.4×1-cm slab soaked with 10 μM dye solution in DCM for 12 hours. The fluorescence images are obtained by illumination with 365-nm UV light.

As informative the images of the blocks are about the homogeneity of the dye distribution within their bulk, they do not provide the best means for gaining quantitative estimations about the extent of heterogeneity. A form of imaging tomography, such as confocal microscopy using objectives with a board field of view and long working distance, can prove beneficial for non-distractive quantification of the distribution of the fluorescent dye in the PDMS samples. While a destructive approach, cutting slices from the PDMS blocks allows facile and expedient imaging of the cross sections at different heights using regular broad-field cameras (Figure S5). Traces of the fluorescence images of the slices provide information about the relative distribution of the dyes across the various cross sections (Figure S6). Uniform thickness of the slices is key for relating the fluctuating of the relative intensity along the traces to variations in the dye concentration. Nevertheless, uneven distribution of the illumination presents challenges with relating the measured pixel intensity on the image with the dye concentration. The UV lamps used for illuminating the PDMS samples generate scattering patterns from the non-fluorescence surfaces and materials in the visible spectral range (not from autofluorescence). Additional low-pass filters in front of the UV lamp could help eliminating these background patterns, but they actually prove beneficial for the image analysis. First, they improved the means for focusing the camera, and second, they generate background for signal correction. Taking advantage of the red-green-blue (RGB) composition of the images, consideration the fluorescence spectra of THIA and ThT, and assuming that Mie scattering (i.e., wavelength invariant) dominates the background, suggest that the scattering patterns dominate in the red components of the images, while the dye fluorescence contribute mostly to the green and the blue components. Subtracting the red component from the green and blue components removes many of the patterns originating from the rough surfaces of the slices and from the background in general (Figure S7). Furthermore, the ThT blue components in some of the image regions tend to be saturated. Therefore, we focus on the green components (with the red components subtracted from them) for extracting information about the distribution of the dye fluorescence intensity (Figure S5b,d, S6).

The fluorescence images of the cross sections and the corresponding traces show trends that the visual examination of the blocks does not quite reveal. In the top of the THIA-doped block soaked for 8 hours, the dye appears more or less homogeneously distributed (Figure S5a,b, S6a,b); in the middle, it is quite heterogeneously spread with largest concentration in the center of the block; and on the bottom, the dye signal is intense with some heterogeneity. (The spikes on the traces originate from the dark and bright lines around the slice on the images.) Soaking the 1-cm PDMS blocks for 22 hour enhances these patterns (Figure S6a,b). The THIA-doped 4-mm PDMS slabs show homogeneously distributed dye across the short axis of the cross section used for spectroscopy analysis (Figure S6a).

The slices of the ThT-doped PDMS show different patterns of heterogeneity than those of the THIA samples. Even in the 4-mm slab and in the 1-cm block after 8-hour soaking, ThT tends to accumulate near the surface and show smaller concentration in the center (Figure 6c,d). Extended soaking of the 1-cm block changes this pattern and an results in higher concentration of the dye in the center of the block (22-h, Figure

6c,d). Overall, the 4-mm slab shows least heterogeneity even for ThT, which makes it preferred for the spectroscopic analysis.

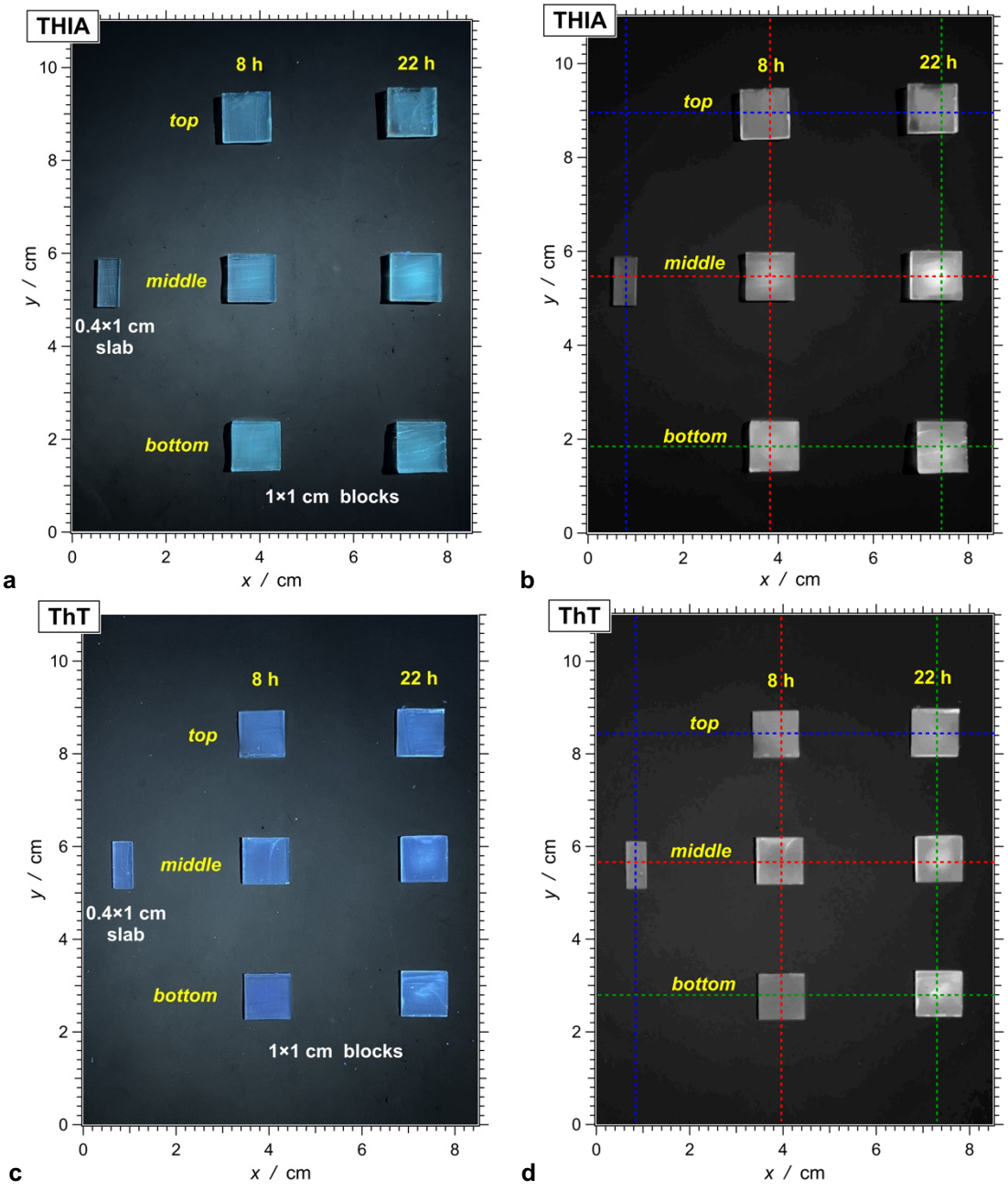

Figure S5. Fluorescence images of cross sections of the PDMS blocks from Figure S4 loaded with (a,b) THIA and (c,d) ThT. In each image, top row shows 2-mm slices were cut from the top sections; middle row – from the middle sections; and the bottom row – from the bottom section of the PDMS blocks. From the 0.4×1-cm slabs, slices only from their middle sections were collected (left column in each image). The middle columns in the images show slices from 1×1-cm PDMS blocks soaked in the dye solutions for 8 hours; and the right columns – from 1×1-cm PDMS blocks soaked for 22 hours. (a,c) Fluorescence images of the arranged slices recorded under 365-nm UV illumination. (b,d) The green components of the same fluorescence images with the red components subtracted from them. The dashed lines show the traces in the images plotted on Figure S5.

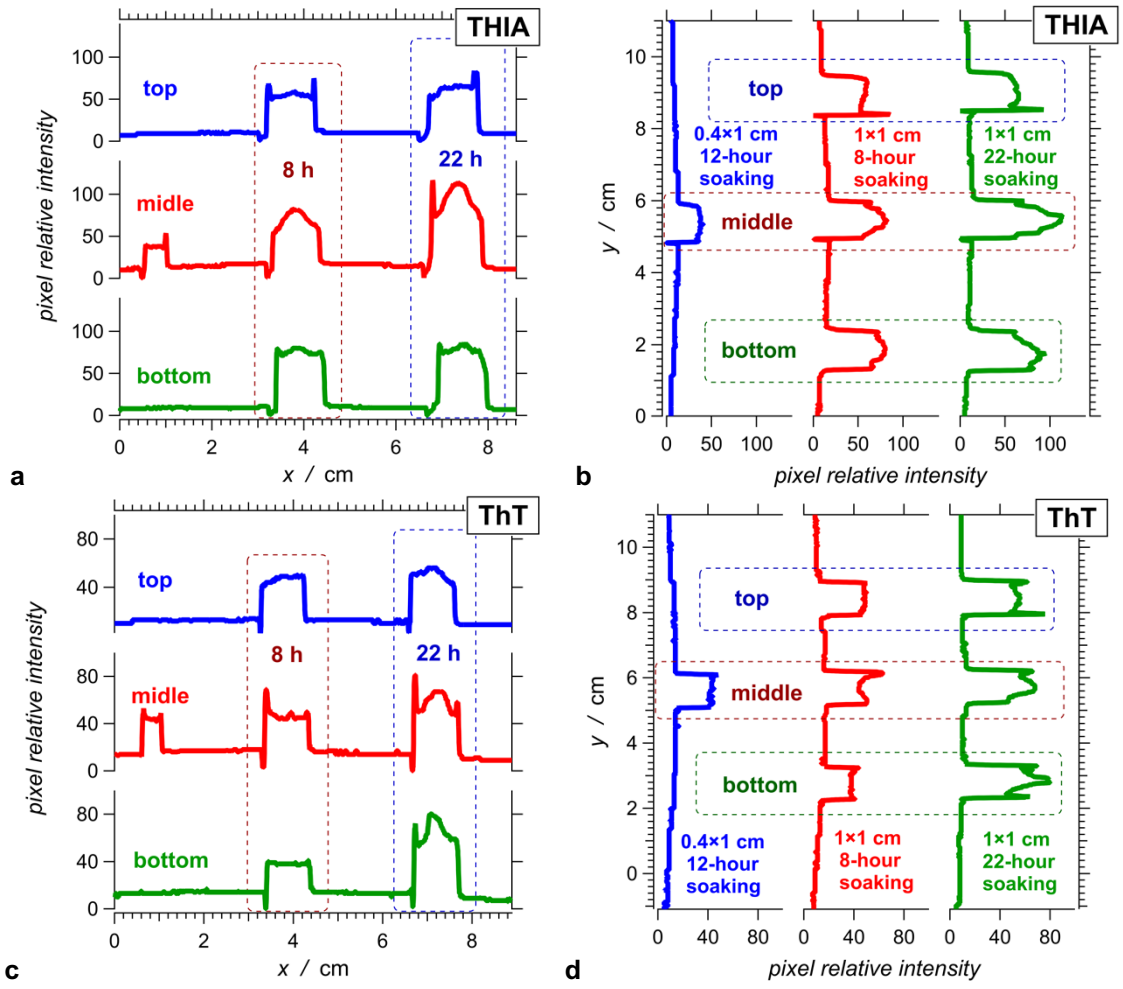

Figure S6. (a,c) Vertical and (b,d) horizontal traces of the relative emission intensity (represented as pixel intensity) from the green-minus-red components of the images of the arranged slices from the PDMS blocks loaded with (a,b) THIA and (c,d) ThT. All traces are shown on Figure S4b and d.

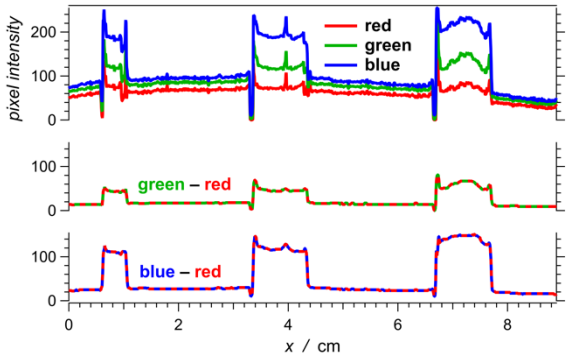

Figure S7. Example of traces of the red, green, and blue component of an image (middle, Figure S5c), along with the differences between the green and red, and between the blue and red components. Because the slices have finite thicknesses, and the illumination and the camera are not perfectly above them, dark and bright lines mark the edges of the slices, which translates in sharp spikes on the traces of the different components of these images.

References:

S1. D. Bao, B. Millare, W. Xia, B. G. Steyer, A. A. Gerasimenko, A. Ferreira, A. Contreras and V. I. Vullev, "Electrochemical oxidation of ferrocene: A strong dependence on the concentration of the supporting electrolyte for nonpolar solvents," *J. Phys. Chem. A*, 2009, **113**, 1259–1267.

S2. C. Meichner, A. E. Schedl, C. Neuber, K. Kreger, H.-W. Schmidt and L. Kador, "Refractive-index determination of solids from first- and second-order critical diffraction angles of periodic surface patterns," *AIP Advances*, 2015, **5**, 087135.

S3. M. Mohankumar, M. Unnikrishnan, G. N. Naidu, S. M. Somasundaran, M. P. Ajaykumar, R. S. Swathi and K. G. Thomas, "Finding the needle in a haystack: Capturing veiled plexcitonic coupling through differential spectroscopy," *J. Phys. Chem. C*, 2020, **124**, 26387–26395.

S4. M. Groenning, "Binding mode of Thioflavin T and other molecular probes in the context of amyloid fibrils—current status," *J. Chem. Biol.*, 2010 **3**, 1–18.

S5. W. West and S. Pearce, "The dimeric state of cyanine dyes," *J. Phys. Chem.*, 1965, **69**, 1894–1903.