Supplementary Information for “Optical monitoring of polymerizations in droplets with high temporal dynamic range”

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Solvent and reagent purification for fluorescence microscopy experiments:

Toluene (Optima™ Grade, Fisher) was used as solvent for most reactions. Notably, even this grade of solvent contained fluorescent impurities significant enough to measure on our microscopy setup. To remove these impurities, the toluene was purified by vacuum transfer at room temperature. Liquid N_2 was used to cool the receiving flask and condense toluene from the vapor phase. All glassware used in this process was first rinsed thoroughly with HPLC solvent, dried, and then cleaned in a plasma etcher for 5 minutes at 300W to remove potential organic fluorescent impurities from the glassware as well. Fractions of purified toluene were kept sealed immediately after collection in clean round bottom flasks until use.

Norbornene (bicyclo[2.2.1]hept-2-ene, Sigma Aldrich) was also seen to have significant fluorescent impurities. The monomer was purified by sublimation at room temperature onto a cold finger cooled with dry ice/acetone.

Norbornadiene (bicyclo[2.2.1]hepta-2,5-diene, Sigma Aldrich) was used as received, without further purification.

All other materials were purchased commercially and used as provided, except where noted.

Surface Functionalization of Glass Reaction Chambers

Perfluoroalkylated glass chambers were constructed to facilitate droplet immobilization and imaging over long timescales. Unfunctionalized glass chambers were constructed first by the following procedure: Coverslips (25x25mm, Fisherbrand™) and micro slide rings (Thomas®, 3x15mm and 5x18mm) were rinsed with HPLC grade methanol and ultrapure water, dried under

nitrogen, and then O_2 plasma cleaned (300 W, 5 minutes). The rings were bonded to the coverslips with sodium silicate as described previously.

To functionalize the glass chambers, each chamber or coverslip was first rinsed with HPLC methanol and ultrapure water. The chambers were dried under nitrogen and then O_2 plasma cleaned in a plasma etcher at 300W for 5 min. A 5% solution of 1H,1H,2H,2H-perfluorodecyltriethoxysilane in 2-propanol was prepared and placed in the cleaned chambers for 1 hour to allow the silane to physisorb to the surface. After 1 hour, the solution was removed, and the chambers were again dried under nitrogen before heating at 110 C for 10 minutes in order to create a covalent bond to the surface. They were then allowed to cool down to room temperature before finally rinsing with 2-propanol. Functionalized chambers were made in batches and could be stored and used for droplet immobilization long after their initial preparation.

Sample Preparation:

Stock solutions of **PDI-NB** and **TPE-NB** in purified toluene were prepared in cleaned scintillation vials and used immediately or stored sealed in light-tight containers. Purified norbornene was kept as a saturated solution in either purified toluene (~8 M) or norbornadiene (~6 M). Reaction mixtures were prepared by dilution of the above samples into purified toluene (or norbornadiene, where appropriate), to afford 80% of the saturated concentration of monomer, 2 mM **TPE-NB**, and ~1 uM **PDI-NB** in solution. Stock solutions of Grubbs Generation II catalyst (GG2) were prepared in purified toluene immediately prior to use, as degradation of the catalyst occurs too quickly to store these over long periods of time.

Prior to imaging, the chambers were rinsed, then filled with ultrapure water to act as a continuous phase surrounding the organic droplet phase before being placed on the robotic droplet production platform.

Fluorescence Microscopy setup and Timelapse Imaging:

Microscopy was performed on an inverted microscope (Nikon Ti-U), with excitation provided by a 532 nm laser (Coherent Sapphire FP, 80 mW) for the PDI dye, and excitation provided by a 405 nm laser (Cobolt MLD, 120 mW) for excitation of the TPE dye. Each laser was attenuated with ND filters, then coaligned through a telescope to expand the beams until their diameters were slightly larger than the back aperture of the objective (Nikon 2x, 0.1 NA, Plan Apo). The excitation beam passed through a polarizer (Thorlabs PBS251) and half-wave plate (Thorlabs WPH10M-532) to afford a linearly polarized 532 nm beam for fluorescence polarization anisotropy experiments. Electronic shutters (Uniblitz) at the beginning of each beam path were used to keep the sample from being exposed to excitation light until images were taken (thus preventing excessive photobleaching). Emission from the sample was passed through a multiedge dichroic mirror (Semrock Di01-R405/488/532/635-25x36) and directed to the collection optics. Computer controlled sliding mounts (Thorlabs ELL6K, ELL9K) were used to automatically select the appropriate spectral filters and polarization filters for each image. During excitation with the 532 nm laser, a 532 nm long pass filter (Semrock LP03-532RU-25) was in place, and during 405 nm excitation, the sliding mount switched to a 470/100 band pass filter (Semrock FF02-470/100-25) which was used to isolate the emission of the AIE dye. A second 4 position sliding mount was used to switch between two orthogonally oriented

polarizing filters (Thorlabs LPVISE100-A) for acquisition of the parallel and perpendicular fluorescence polarization images at each timepoint. During acquisition of the TPE emission images, this sliding mount moved to a third empty position to allow all emitted light through regardless of polarization. Emission was collected and recorded on an EM-CCD camera (Andor, Ixon 897). Custom labview code interfaced with the camera and synchronized the shutters, sliding mounts, and camera exposures times to ensure all three images were automatically collected at each timepoint. The shutters were closed in between exposures to minimize photobleaching. Unless stated otherwise, each timelapse experiment used a 100 ms exposure time with 300 EM gain. The CCD was cooled to -80C during data acquisition. Frames were taken 5 minutes apart for up to 300 repetitions maximum (i.e. up to 1500 min or 25 hours).

Image processing, registration, and data analysis

Images acquired with the above setup were analyzed using custom matlab scripts. Images taken through each polarization filter were initially slightly offset from each other, meaning the location of a droplet in one image channel was at a different set of pixel locations in the other. To correct for this, calibration images were taken of light transmitted through a TEM grid (Ted Pella) in each channel. A thresholded version of this image then provided a set of locations that could be related to the same set of locations in the 2nd channel. These pixel positions were used to calculate an affine transformation matrix which can transform any pixel position in the first image to the corresponding pixel in the second image. In this way, each location in real space as represented in the first image is given a pixel address that corresponds to it in the second image, and our calculations can be made even if intervening optics deflect or distort the image so that objects of interest are not at the exact same pixels of the camera sensor in each channel.

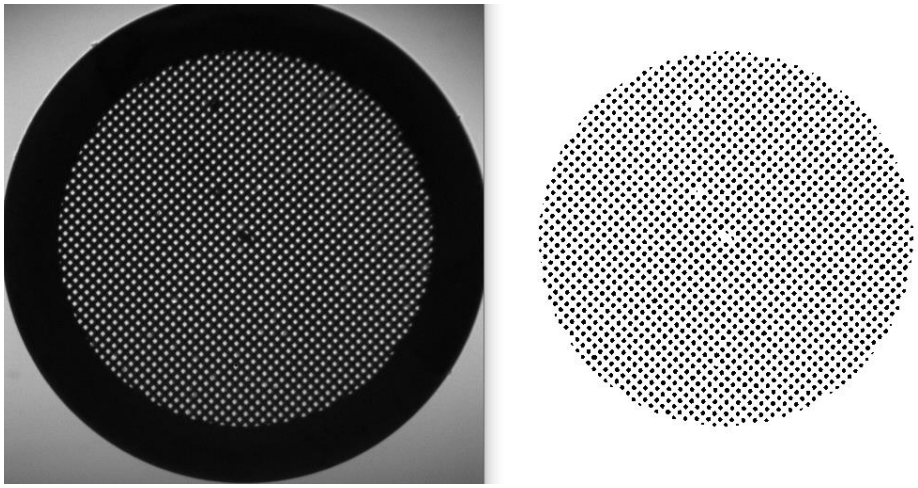


Figure 1: left, TEM grid image, bright field. right, thresholded image used for affine transform calculation (parallel channel). A nearly identical image is taken in the perpendicular channel to allow for affine transformation calculation.

Background subtracted fluorescence images of droplet arrays were then processed by a custom matlab script. Briefly, a representative frame from the timelapse video was chosen to identify the droplets (such as the right image in Figure 2). This image was smoothed and thresholded to identify the bright areas of the image corresponding to droplets. The thresholded

image becomes a list of pixels of interest, which is fed into a DBSCAN-based clustering algorithm which associates all pixel positions within one droplet (figure 3). A map of these same positions in the parallel image channel is created by transforming the points in the parallel pixel position list using the previously calculated affine transformation matrix.

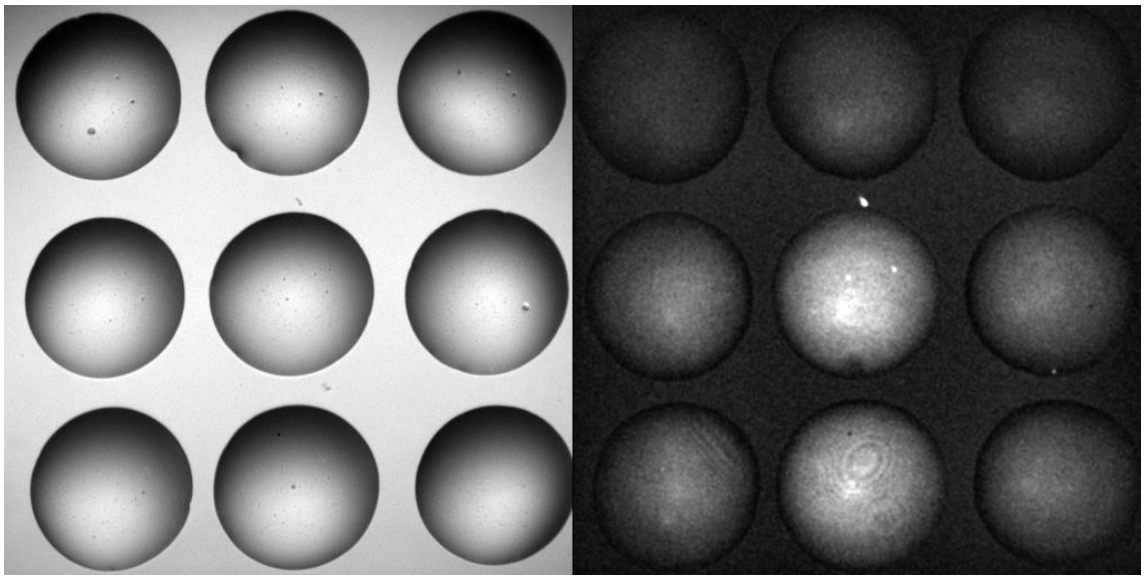


Figure 2: left, bright field image of droplet array. right, fluorescence emission image, parallel polarization channel

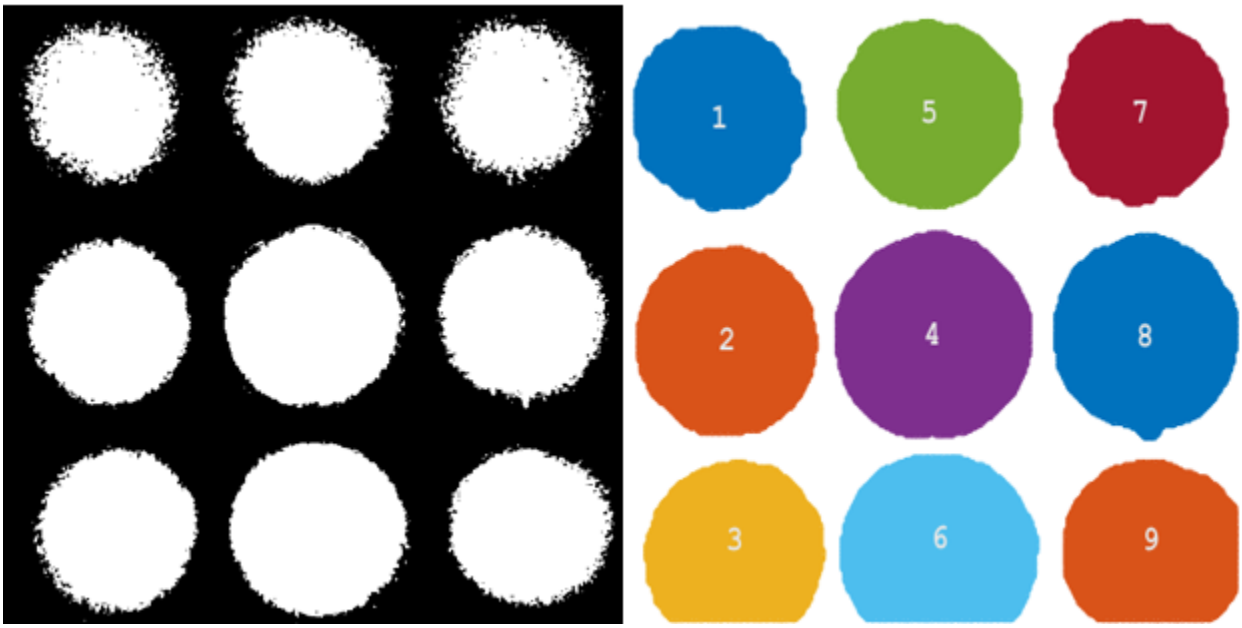


Figure 3: left, thresholded version of the fluorescence image from Figure 2. right, clusters output by DBSCAN-based clustering algorithm

With the list of pixels of interest generated, the code then accesses the intensities of each pixel of interest in each frame for both videos, and calculates the anisotropy at each point of interest for every frame (details of g factor correction are in the following section):

$$r = \frac{I_{\parallel} - gI_{\perp}}{I_{\parallel} + 2gI_{\perp}}$$

These values were stored in order to generate videos of the changing anisotropy over time (as shown in the color-scaled images in the main text). The clusters are used to associate pixels which are part of the same droplet, to allow for averaging across each droplet at each time point. This averaging was used to generate the anisotropy response curves (plots of r vs time) shown in the main text.

The same clustering strategy was used for the AIE channel videos collected with 405 nm excitation in order to isolate droplets automatically and plot their emission intensity over time (here, with only one channel to account for, no image registration or correction was required).

Calculation of g factor

The correction factor g used in the anisotropy calculation was determined using images taken of a standard solution of oxazine 170 perchlorate, following a previously published method[1]. Using the known rotational correlation time, fluorescence lifetime, and steady-state anisotropy value of oxazine in water, we determined the g factor necessary at each pixel in our image to produce the proper measured anisotropy[2]. The g factor correction for each pixel is calculated using the position mapping strategy described above and kept in a matrix which is generated at the same time as the affine transform, so that both are loaded and applied together for data taken with a given alignment of the optical setup.

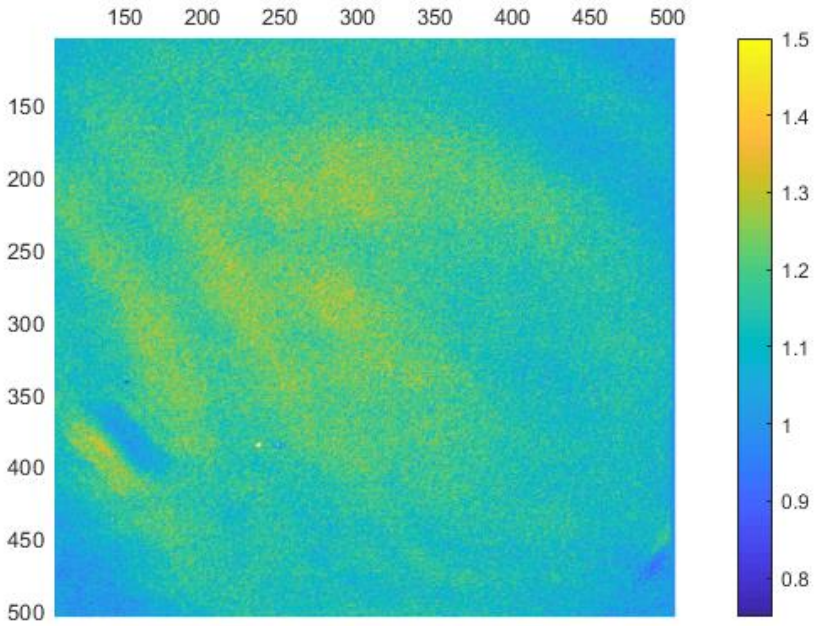
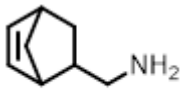


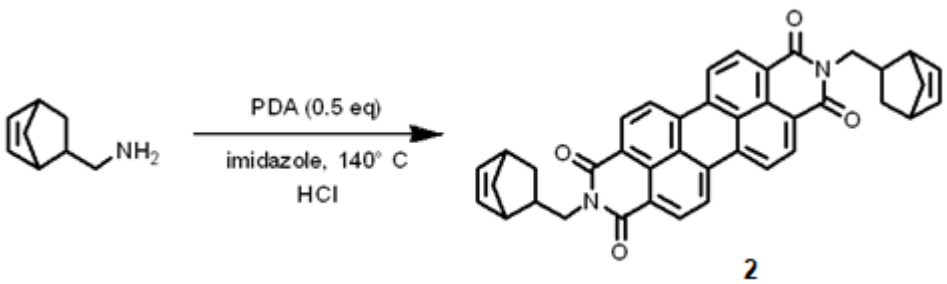
Figure 4: Visualization of a g factor matrix - x and y axes correspond to image pixel positions. Color axis is g factor value for a given pixel.

Synthesis of 5-norbornene-2-methylamine (1)



1

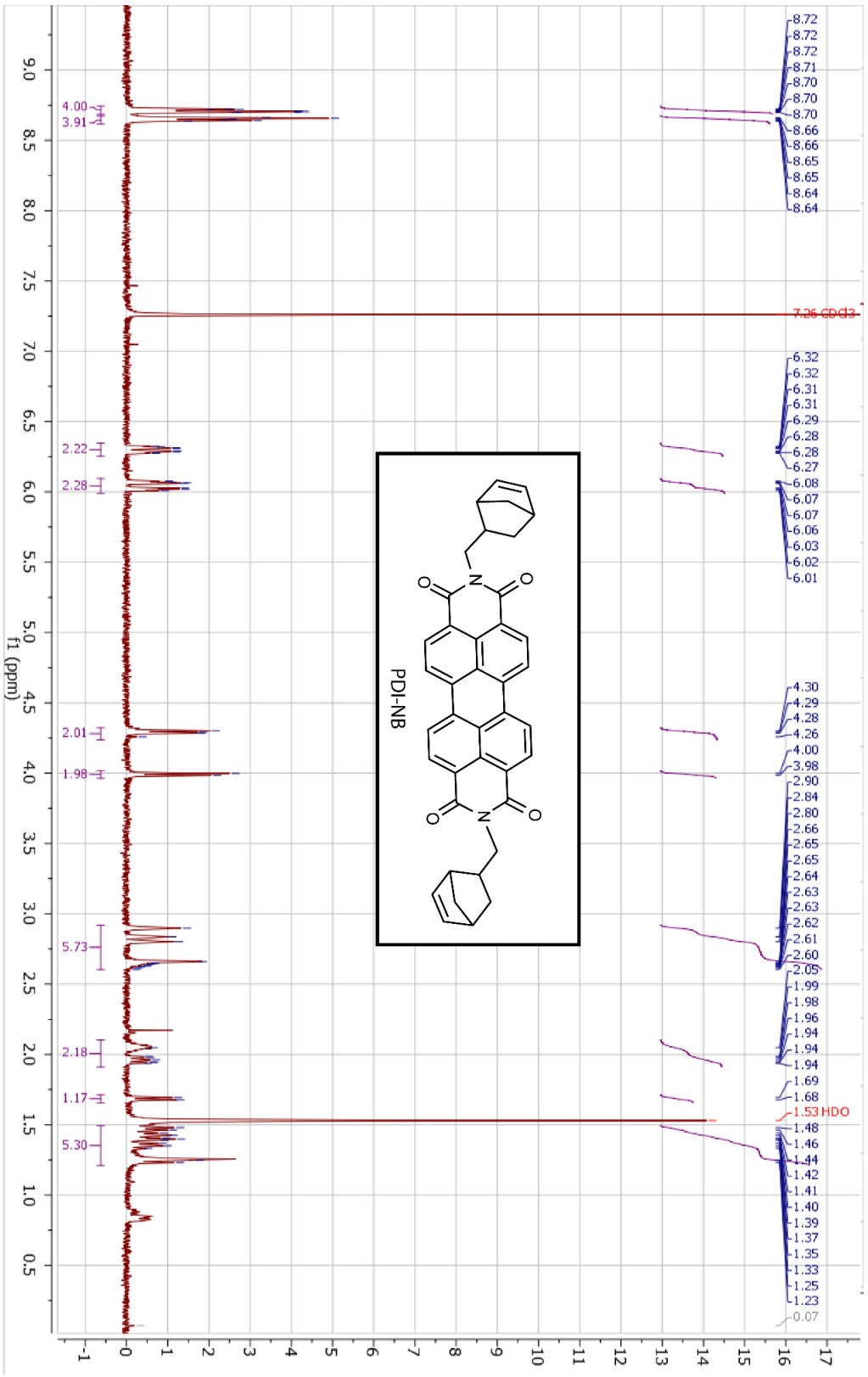
Compound **1** (5-norbornene-2-methylamine) was synthesized according to literature procedure[3]. 5-norbornene-2-carbonitrile (3.00 g, 25.2 mmol) was dissolved in 10 mL of diethyl ether and degassed with nitrogen. This solution was added under air-free conditions to a suspension of lithium aluminum hydride (1.878 g, 41.5 mmol) in 20 mL diethyl ether at 0° C. The reaction mixture was allowed to warm to room temperature and then stirred for 1 hour, followed by reflux for 30 minutes. The mixture was then cooled to 0° C and quenched by slowly adding 15 mL water. The ether layer was washed with 30 mL water, 30 mL 10% NaOH, and 30 mL brine solution, then dried over $MgSO_4$ and evaporated to yield 2.16 g of clear, almost colorless liquid **1** (70% yield). NMR spectra matched reported values[3].



2

A norbornene-functionalized PDI monomer **PDI-NB** was synthesized. This synthesis was adapted from another PDI synthesis in the literature[4]. A solution of 0.5 g (4.07 mmol) **1** and 0.705 g (1.8 mmol) perylene-3,4,9,10-tetracarboxylic dianhydride (PDA) in imidazole (10 g) was stirred at 140° C for 4 hours, then allowed to cool to room temperature. Ethanol was added to dissolve the imidazole as it solidified. This mixture was treated with 2 M HCl and let sit for 1 hour. A precipitate formed and was collected via vacuum filtration and washed with DI water, then dried under vacuum overnight to yield 0.9 g of the red solid PDI monomer **2** (83% yield). Portions of this were purified by column chromatography (1.2% ethanol in chloroform, silica gel). Purified solid was triturated with pentane x3 before use in experiments ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 – 8.69 (m, 4H, perylene core CH), 8.65 (m, 4H, perylene core CH), 6.30 (m, 2H, norbornene CH), 6.10 – 5.99 (m, 2H, norbornene CH), 4.32 – 4.24 (m, 2H), 3.99 (d, J = 7.2 Hz, 2H), 2.92 – 2.60 (m, 6H), 2.10 – 1.91 (m, 2H), 1.68 (d, J = 8.4 Hz, 1H), 1.49 – 1.21 (m, 5H); ^{13}C NMR (CDCl3) δ 163.97, 163.75 (C=O), 137.80, 137.19 (norbornene C=C), 136.39, 133.33, 131.75, 131.66, 123.33, 123.29 (perylene C), 49.71, 45.53, 45.46, 44.87, 44.78, 44.48, 42.67, 41.98, 38.17, 37.89, 30.98, 30.88 (norbornene C, mix of endo/exo isomers). UV/Vis: $\lambda_{max}(\varepsilon)$ = 521 (78720). MS (MALDI): *m/z* expected = 602.2 *m/z* found = 602.2.

NMR Spectrum of PDI-NB



UV/Vis Absorption Spectrum of PDI-NB dye:

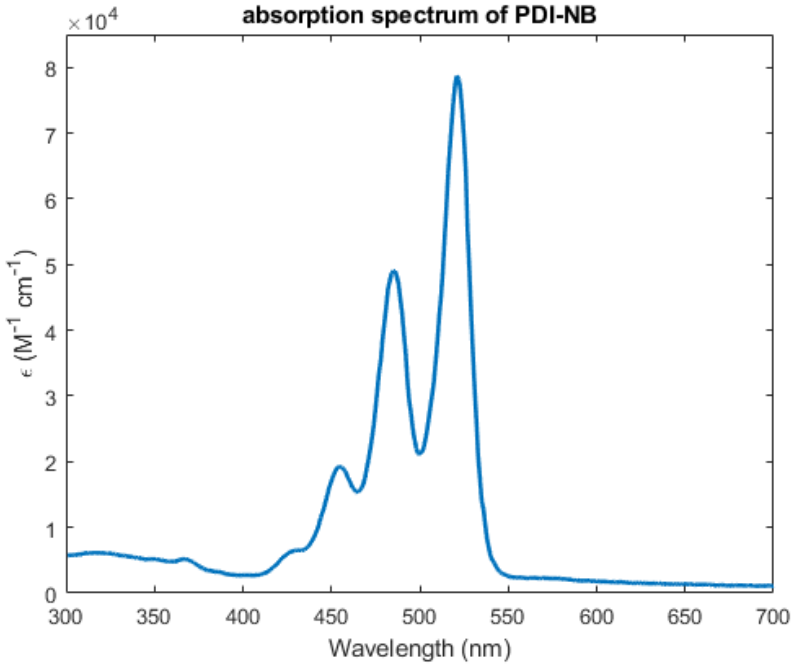


Figure 5: Absorption spectrum of PDI-NB. UV-vis spectra were recorded on a Varian CARY 50 SCAN spectrophotometer. λ_{max} = 521, ε = 78720 M^{-1} cm^{-1}

Fluorescence Spectrum of PDI-NB dye:

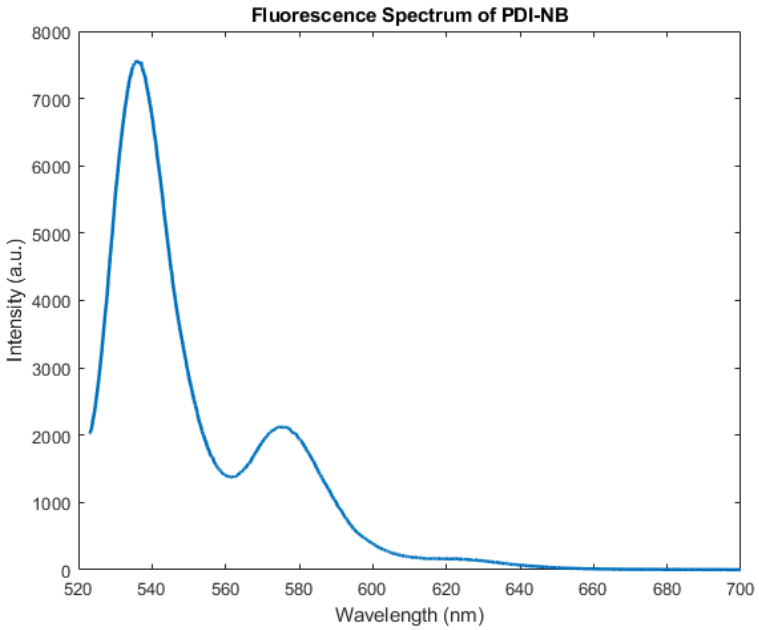


Figure 6: Fluorescence spectrum of PDI-NB in toluene. λ_{exc} = 521.

Fluorescence spectra were recorded on a F-4500 Fluorescence Spectrophotometer using Xe light source.

Contact Angle Measurements:

To confirm that functionalization of the glass coverslips with perfluorosilanes was successful, contact angle measurements were taken using a DataPhysics Instruments OCA series Contact Angle System. 10 uL DI H_2O was dispensed at the “slow” rate setting. Unfunctionalized coverslips were used as a control, giving contact angles in the range 39.8-53.4. Fluorinated coverslips gave values in the range of 70.3 – 91.1, indicating a higher degree of hydrophobicity than the unfunctionalized glass.

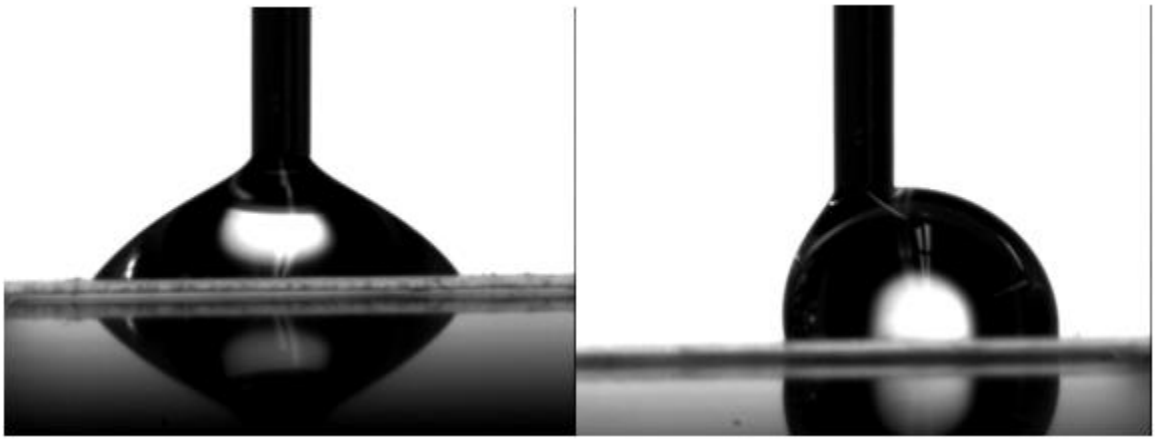


Figure 7: Contact angle measurements. Left, water on unfunctionalized coverslip. Right, water on fluorinated coverslip.

Droplet Stabilization and Surfactant tests

As toluene droplets are unstable in aqueous environment, we sought to stabilize them using typical surfactants (listed below). In our hands, any of the various surfactants tried had either no discernible benefit or accelerated droplet contraction and disappearance (Figure 8). We hypothesize that this is due to a solubilizing effect of the surfactants on the toluene/monomer organic phase. Switching back to only ultrapure water as the continuous phase resulted in longer droplet stability (see figures 9 and 10). All droplets shown in Figures 8-10 contain norbornene monomer in toluene, but with no catalyst added.

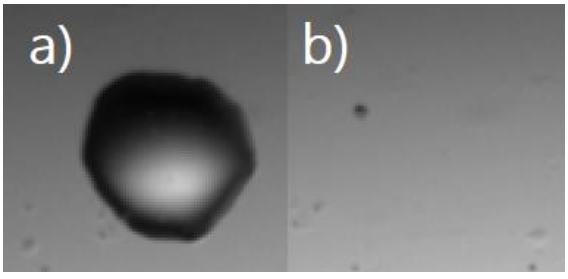


Figure 8: Droplet surrounded by aqueous continuous phase with TWEEN surfactant. Bright field image. a) t = 0 minutes b) t = 890 minutes

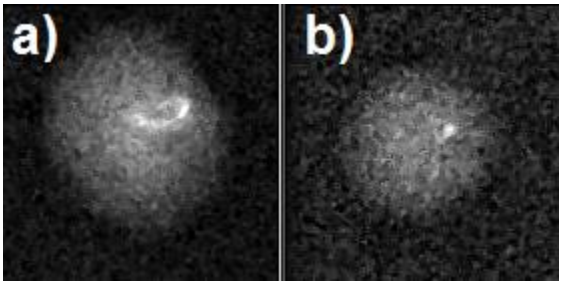


Figure 9: Droplet surrounded by pure water (fluorescence emission).
a) t = 0 minutes b) t = 890 minutes

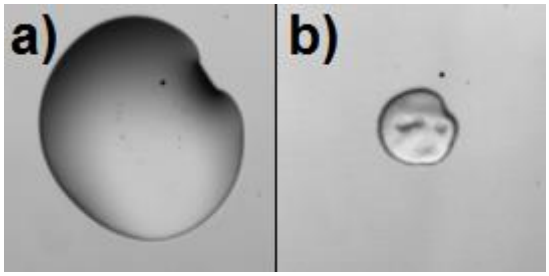


Figure 10: Even after 24 hours, the droplet has contracted but not disappeared.
a) t = 0 hours. b) t = 24 hours

Surfactants tested include TWEEN 20, TWEEN 80, Sodium dodecyl sulfate, Triton X-100, MTAB, CTAB, CA 720, and CO 720. Interestingly, the production of polymer itself seems to slow droplet contraction, regardless of the contents of the surrounding continuous phase (See Figure 11).

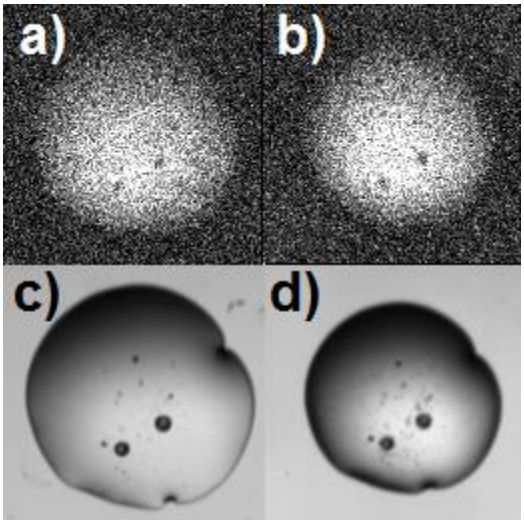


Figure 11: Droplets containing catalyst (and thus active polymerizations) shrink much less over the course of 24 hours. a) Fluorescence image at t = 0 min. b) Fluorescence image at t= 890 min. c) Bright field image at t= 0 min. d) Bright Field image at t= 24 hours.

PDI only control:

To see what effect the presence of monomer in solution has on fluorescence anisotropy readings even when no catalyst is present, a control measurement was performed with a droplet of only PDI-NB in toluene (at ~1 uM), with no monomer present. The same LabView program that is used in acquisition of time-lapse videos was used to take several frames focusing on the PDI-only droplet. A mean value of r = 0.0534 was obtained, indicating a relatively high degree of freedom for the PDI dye in solution, as expected. Notably, this is significantly lower than the typical values for free PDI in our high concentration monomer solutions (where r is typically 0.1), due to differences in viscosity between the two droplet compositions.

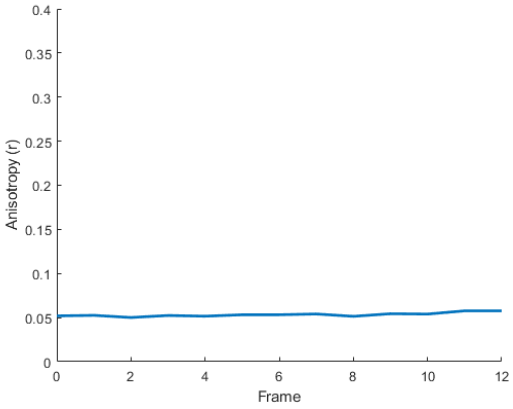


Figure 12: Plot of measured anisotropy values from a toluene droplet containing only PDI-NB dye (with no other monomer or catalyst around)

Unaveraged Traces of NB/NBD reactions in a 3x3 array:

Typically, we track at least 3 droplets with the same conditions in each experiment. In the main text, we average the responses of the droplets together for clarity to produce the plots shown. In figure 13, we show the un-averaged traces (Each trace shown is still the average of many points within each droplet). Despite some inter-droplet variability, droplet behavior and response times are largely the same. Likely sources of this slight variability are imperfections in g-factor and differences in background and noise coming from inhomogeneous excitation.

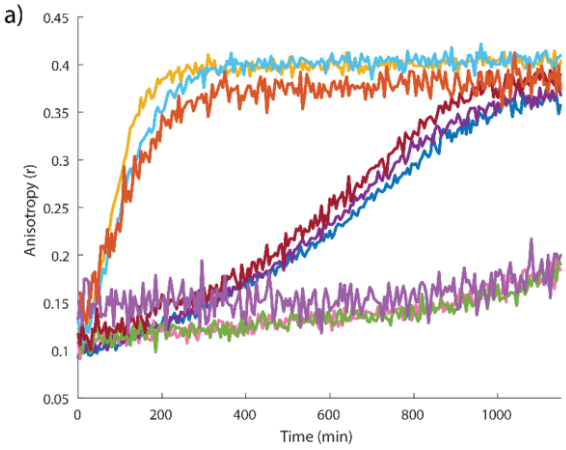


Figure 13: Norbornene/norbornadiene reaction mixtures. Each trace is from a single droplet in a 3x3 array (three replicates per condition). This data was used for the NB/NBD figure in the main text, with each set of 3 droplets averaged together for clarity. Some small variation is seen between droplets, due most likely to their differing positions in the beam and an imperfect g factor correction.

Control reaction without averaging droplets:

In analogy to the above plot, Figure 14 shows 2 sets of 3 droplets where one row contains catalyst and one row does not. Again, the behavior and timescale are similar between all 3 droplets in each set. The long scale fluctuations seen centered around 200 frames are due to changes in background.

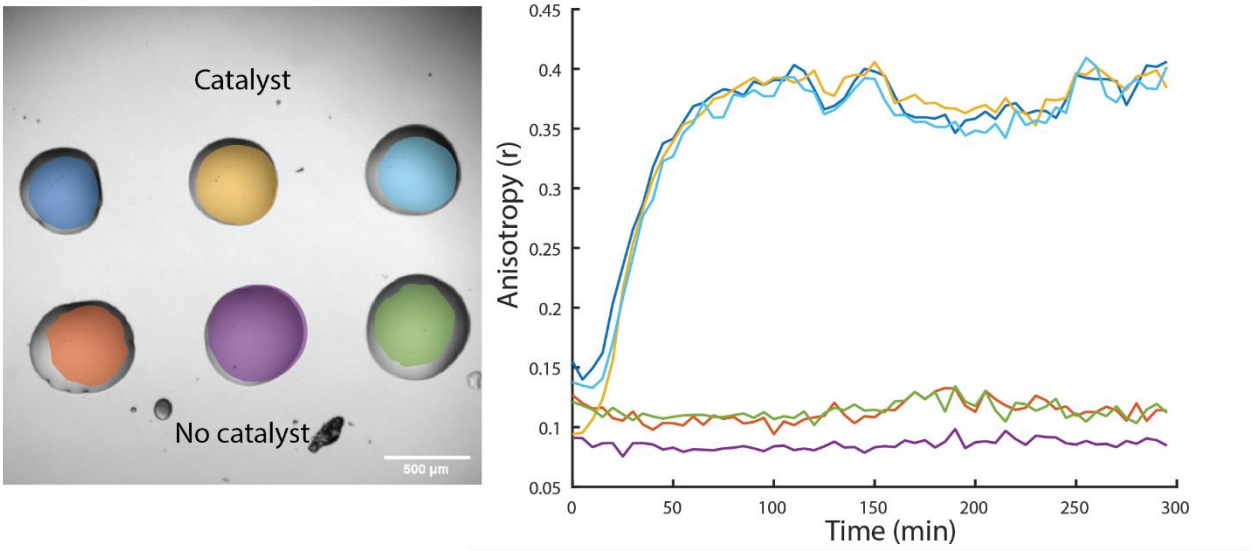


Figure 14: Control reaction comparing droplets with and without catalyst present. A rise in anisotropy is seen concurrent with polymerization in the droplets containing catalyst (top row, false color overlay matched to traces on right). Droplets without catalyst do not display any increase on this timescale, instead maintaining their initial values (bottom row).

Molecular Weight Measurements:

To quench the reaction in the large (20x20) arrays, first the water continuous phase was removed, and an excess of ethyl vinyl ether was layered on the droplets to quench the reaction. The ethyl vinyl ether was allowed to evaporate before the droplets were removed from the coverslip surface using tweezers and collected in a vial. The vial was placed under vacuum overnight (16-18 hours) to remove residual ethyl vinyl ether, solvent, and monomer.

Molecular weights were determined via Gel Permeation Chromatography Multi-Angle Light Scattering (GPC-MALS) using a Jordi Gel DVB 1000A column (500mm x 10mm) at room temperature in chloroform at 0.5ml/min on a system consisting of a ChromTech Series 1500 pump equipped with a multi-angle light scattering detector (DAWN-HELIOS II, Wyatt Technology) and a refractive index detector (Wyatt Optilab T-rEX) normalized to a 30,000 MW polystyrene standard**.**

Comparison of PDI spectra in toluene with and without norbornene:

In order to determine if there is any aggregation of PDI dyes in solution under reaction conditions, a spectrum of the dye was taken at a concentration of 10 μm in toluene and then at a concentration of 10 μm in 7 M norbornene in toluene. This concentration of dye corresponds to ~10x the concentration used in our droplets. Though there is a slight shift in λ_{max} between them, neither spectrum shows evidence for the presence of the aggregated form of PDI, which manifest itself as a distortion of the vibronic progression due to exciton coupling[5]. Since our experiments run at a concentration one order of magnitude less than this, we don't expect PDI aggregation to be significant in any of the polymerization reactions under study in this work.

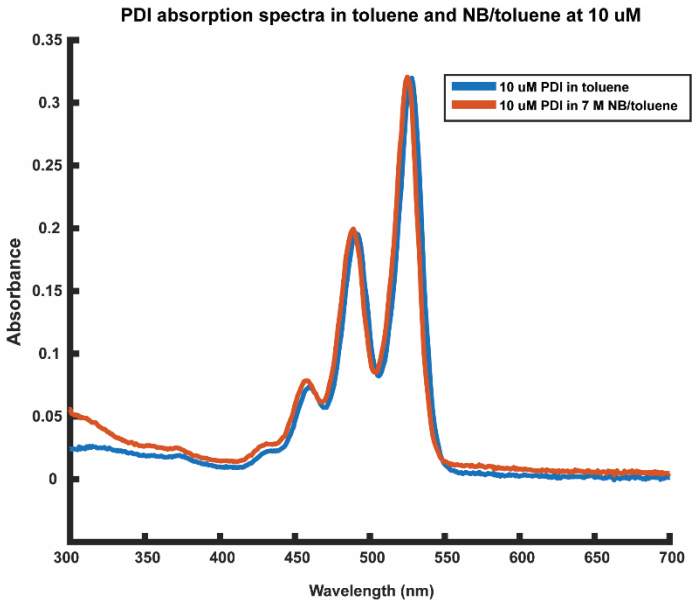


Figure 15: Comparison of absorption spectrum of 10 μM PDI-NB in toluene (blue trace) and in 7 M norbornene in toluene (orange trace).

References

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