Spinodal decomposition of chemically fueled polymer solutions Supplementary information

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Materials and Methods

Substances

exo,exo-norbornene dicarboxylic anhydride (95 %. ABCR), KOH (85 %, Roth), ethyl vinyl ether (> 98 %, TCI), dibutylhydroxytoluene (BHT, > 99 %, Sigma Aldrich), trifluoroacetic acid (> 99.9 %, VWR), *N,N*-diisopropylethylamine (DIPEA, > 99 %, TCI), 2-(*N*-Morpholino)ethanesulfonic acid (MES, > 99 %, Sigma Aldrich), Atto 488 amine (95 %, Atto-TEC), 1 M NaOH solution (VWR), 1 M HCl solution (VWR), *N*-hydroxysuccinimide (NHS, 98 %, Sigma Aldrich), *N*-Ethyl-*N*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC hydrochloride, 98 %, ABCR) and third generation Grubbs catalyst (G3, Sigma Aldrich) were used as received. All solvents were analytical or HPLC grade and were used as received unless stated otherwise. For all experiments Milli-Q water with conductivity less than 0.055 mS cm⁻¹ was used.

Instrumentation

High-performance liquid chromatography (HPCL). Analytical HPLC experiments were performed using a Dionex UltiMate 3000 system equipped with a WPS3000 SL autosampler, a VWD-3400 RS Variable Wavelength Detector, and a 10 mm flow cell. Chromatography was performed at 25 °C, using a Kromasil C18 HPLC column (250 mm x 4.6 mm, 5 μ m particle size) as the stationary phase. For the mobile phase acetonitrile/H₂O mixtures containing 0.1 vol% TFA with a flow rate of 1 mL min⁻¹ were used. For kinetic experiments samples of 1 μ L were drawn at regular intervals and chromatography was performed using a gradient from 40/60 to 98/2 acetonitrile/H₂O. EDC concentrations were quantified by measuring absorbance at 220 nm with calibration curves recorded in triplicate. Retention time 2.55 min, calibration constant (5.65 ± 0.05) mAU mM⁻¹.

Nuclear magnetic resonance (NMR) spectroscopy. NMR measurements were performed at RT on a Bruker Ascent spectrometer (Bruker, Germany) operating at 400 MHz. Chemical shifts are reported relative to the residual solvent peak ($\delta = 2.50$ ppm for DMSO- d_6).

Confocal Laser Scanning Microscopy (CLSM). CLSM imaging was conducted using a TCS SP8 microscope (Leica, Germany) operated at an excitation wavelength of 488 nm and detection in the range of 510-600 nm. Images were recorded with a 63x/1.20 water-immersion objective at resolutions of 2048 x 2048, corresponding to an area of 246.15 µm x 246.15 µm. To ensure comparability, the same parameters were used for all images.

Size-exclusion chromatography (SEC). SEC was performed using an Agilent 1200 system equipped with a 1260 Series isocratic pump and a refractive index detector. The stationary phase consisted of a pre-column and three Suprema SDV columns (8×300 mm, Polymer Standards Service) with pore sizes 100, 3000, 3000 Å respectively. Measurements were conducted at RT with a flow rate of 1 mL min⁻¹ in 10 mM aqueous PBS (pH 7.4). Narrowly distributed poly(ethylene oxide) standards were used for calibration (Polymer Standards Service).

Titrations. Titrations were performed with a 907 Titrando auto-titrator (Metrohm, Switzerland) using a glass electrode for potentiometric pH determination and a conductivity module. Standardized buffer solutions with defined pH were used for calibration.

Turbidity measurements. Turbidity measurements were conducted using a Tecan Spark platereader, measuring absorbance at 450 nm in 30 s intervals. All experiments were performed in triplicate at 23 °C. Samples with a volume of 150 μ L were prepared in transparent 96-well plates using the built-in dispensing system and mixed by orbital shaking for 30 s.

Computational Methods

Image Analysis

Quantitative image analysis for the determination of the characteristic length scale L was implemented in Python using standard libraries, and specifically the *NumPy* package for fast Fourier transform¹, a radial-averaging implementation inspired by x-ray scattering analysis^{2, 3}, and the *lmfit* package for curve-fitting.⁴ Source code is available in the form of separate files.

The 3D reconstruction of z-stack CLSM images was performed using UCSF Chimera (Version 1.14, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311).⁵

Kinetic Modeling

Kinetic models were implemented in Python, using standard libraries, and specifically the *odeint* function implemented in the *SciPy* package for the solution of ordinary differential equations⁶, the *lmfit* package for curve-fitting⁴, and the *uncertainpy* package for the uncertainty analysis and calculation of confidence intervals⁷. For details, see Supporting Notes S2, S3. Source code is available in the form of separate files.

logP SA⁻¹ Calculations

Octanol-water partition coefficients (log*P*) and solvent accessible surface area (SA) were calculated with Materials Studio 2019.⁸ log*P* values for oligomeric models represent an atom-based method.⁹ SA values were calculated within the Forcite Molecular Dynamics (MD) module after Geometry Optimization and Anneal cycles.

Experimental

Synthesis of exo, exo-norbornene dicarboxylic acid (NDAc)



NDAc was synthesized according to a modified literature procedure¹⁰. *exo,exo*-norbornene dicarboxylic anhydride (10.0 g, 60.9 mmol) was dissolved in a solution of KOH (10.0 g, 151.5 mmol, 2.5 equiv.) in H₂O (100 mL). The reaction mixture was stirred at RT for 2 h and was washed with ethyl acetate (200 mL). The title compound precipitated from the aqueous phase as a colorless solid upon acidification with conc. HCl (pH 2). The solids were filtered, washed with H₂O, recrystallized from 1 M HCl to remove remaining inorganic salt impurities, and dried in vacuo. Yield: 6.43 g (58 %).

¹H-NMR (300 MHz, DMSO-*d*₆) δ = 12.12 (s, 2 H, 9,10-*H*), 6.22 (m, 2 H, 1,6-*H*), 2.94 (m, 2 H, 3,4-*H*), 2.45 (d, 2 H, 2,5-*H*), 2.04 (d, 1 H, 7-*H*), 1.30 (d, 1 H, 7-*H*) ppm.

Synthesis of poly(exo, exo-norbornene dicarboxylic acid) (PNDAc)



PNDAc was synthesized via ring-opening metathesis polymerization (ROMP) according to a modified literature procedure¹⁰ in an inert N₂ atmosphere using standard Schlenk techniques. Prior to use, THF was stirred over basic alumina (20 vol%) for 1 h to remove peroxides, followed by the addition of BHT (5 g L⁻¹) as a stabilizer. A Schlenk flask equipped with a stir bar was charged with NDAc (911 mg, 5.00 mmol, 100 equiv.) and THF (10 mL). In a separate Schlenk flask, a solution of third generation Grubbs catalyst (G3, 44.2 mg, 50.0 µmol) in THF was prepared. The polymerization (M:I 100:1, monomer concentration 0.25 M) was initiated by rapid addition of the initiator to the monomer solution. The reaction mixture was stirred at RT for 5 min, after which the polymerization was terminated by the addition ethyl vinyl ether (0.50 mL, 0.38 g, > 500 equiv.) and stirred for an additional 30 min. The polymer was purified by three cycles of precipitation from diethyl ether (200 mL) and dissolution in DMSO (20 mL), after which it was dried in vacuo. Even after prolonged drying, not all DMSO could be removed, and 32 wt% DMSO (determined by ¹H-NMR) remained in the polymer. This fact was accounted for in all experiments using the polymer.

¹H-NMR (400 MHz, DMSO- d_6) δ = 12.06 (2 H, 9,10-*H*), 5.43 and 5.25 (2 H, *trans* and *cis* 1,6-*H*), 3.20 and 2.78 (2 H, *cis* and *trans* 2,5-*H*), 2.73 (2 H, 3,4-*H*), 1.98 (1 H, 7-*H*), 1.19 (1 H, 7-*H*) ppm. Contains impurities of DMSO (δ = 2.55 ppm). Backbone double-bonds have a *trans/cis* ratio of 45/55 as determined from the ratio of the signals at 5.43 and 5.25 ppm.

SEC (10 mM aqueous PBS pH 7.4) D = 1.10, $M_{n,SEC} = 51$ kg mol⁻¹



Figure S1. Characterization of poly(*exo*,*exo*-norbornene dicarboxylic acid) (PNDAc) with theoretical degree of polymerization DP = 100, $M_{n,calc} = 18.2 \text{ kg mol}^{-1}$. (a) ¹H NMR spectrum (400 MHz, DMSO-*d*₆). The ratio of *trans/cis* double bonds was determined as 45/55 from the ratio of the *trans*-1,6 and *cis*-1,6 signals. (b) SEC trace (10 mM aqueous PBS pH 7.4), D = 1.10, $M_{n,SEC} = 51$ kg mol⁻¹.

Dye-functionalization of PNDAc

To a solution of PNDAc (0.10 g, 0.55 mmol monomer units, 1.10 mmol carboxylic acids) in DMSO (10 mL), NHS (12.7 mg, 0.11 mmol, 0.1 equiv. with respect to carboxylic acids) and EDC hydrochloride (2.10 mg, 11.0 μ mol, 0.01 equiv., from 5 mg ml⁻¹ stock solution) were added, and the mixture was stirred at RT for 15 min. Atto-488 amine (94.6 μ g, 0.11 μ mol, 10⁻⁴ equiv., from 0.5 g L⁻¹ stock solution in DMSO) and DIPEA (287 μ L, 213 mg, 1.65 mmol, 1.5 equiv.) were added, and the reaction mixture was stirred overnight. The polymer was purified by dialysis (regenerated cellulose tubing, MW cut-off 14 kg mol⁻¹) against H₂O for 5 d and then freeze-dried.

Fueling experiments (CLSM)

For the investigation of investigation of phase separation via CLSM, fueling experiments were conducted at PNDAc concentrations of 3.64 g L⁻¹, corresponding to 20 mM monomer units. For sample preparation, 10 g L⁻¹ stock solutions of fluorescently labeled PNDAc in 100 mM MES buffer at the respective pH (5.3, 6.1, 7.0) were prepared and stirred at RT for 24 h to ensure homogenization. Samples were prepared by diluting the stock solution with a solution of 100 mM NaCl in 100 mM MES buffer at the respective pH and the fueling cycle was started by the addition of an appropriate volume of a freshly prepared 100 mM solution of EDC·HCl in 100 mM MES buffer. The volumes of dilution buffer and EDC solution were calculated to yield the desired final PNDAc and EDC concentrations. NaCl was added to the buffer used for dilution to exclude effects due to variations in salt concentration at different EDC concentrations, keeping the total salt concentration at 178 mM for all experiments. Immediately after the addition of EDC, samples were mixed by repeated (three times) rapid uptake and dispensing using Eppendorf air displacement pipettes. All samples were prepared with a total volume of 150 µL in glass-bottom, 96-well plates directly on the CLSM microscope stage. Time-dependent imaging was started immediately after mixing of the sample, leading to an initial delay of approximately 2 s before image acquisition.

Fueling experiments (HPLC and turbidity measurements)

Fueling experiments for HPLC analysis and turbidity measurements were conducted at PNDAc concentrations of 0.36 g L^{-1,} corresponding to 2 mM monomer units. For sample preparation, 3 g L⁻¹ stock solutions of PNDAc in 100 mM MES buffer at the respective pH (5.3, 6.1, 7.0) were prepared and stirred at RT for 24 h to ensure homogenization. Samples were prepared by diluting the PNDAc stock solution with a solution of 100 mM NaCl in 100 mM MES buffer at the respective pH and the fueling cycle was started by the addition of an appropriate volume of a freshly prepared 100 mM solution of EDC·HCl in 100 mM MES buffer. The volumes of dilution buffer and EDC solution were calculated to yield the desired final PNDAc and EDC concentrations. NaCl was added to the buffer used for dilution to exclude effects due to variations in salt concentration at different EDC concentrations, keeping the total salt concentration at 144 mM for all experiments.

For HPLC analysis, samples were prepared with a total volume of $150 \ \mu$ L in glass vials fitted with a micro-insert. Immediately after the addition of EDC, samples were mixed by repeated (three times) rapid uptake and dispensing using Eppendorf air displacement pipettes. Samples for HPLC analysis were taken in 15.7 min intervals directly from the reaction mixture.

For turbidity measurements, samples were prepared as described above with total volumes of $150 \,\mu$ L, in clear-bottom 96-well-plates. Mixing was achieved via orbital shaking in the plate-reader for 30 s, after which absorbance measurements were immediately started.

Supplementary Note 1: Determination of pKa

The solubility of PNDAc and derived species with a degree of anhydride formation $x_{anhydride} < 1$ is dictated by the degree of deprotonation of the carboxylic acid moieties.

To ascertain the strength of the carboxylic acid moieties, we conducted potentiometric and conductimetric titrations of PNDAc at different NaCl concentrations. The latter is necessary, as fueling experiments require the use of buffer to ensure constant pH, and EDC is used in the form of its HCl salt, which is a weak acid with $pK_a = 11^{11}$, both of which contribute to a finite ionic strength in the system. To ensure complete dissolution of the polymer, PNDAc was fully deprotonated by the addition of NaOH, and titrations were performed from high to low pH. Despite the spatial proximity of the carboxylic acids, we only find a single, relatively pronounced protonation step (Figure S2a), indicating that individual carboxylic acid moieties are relatively insensitive to the overall degree of deprotonation. We therefore assume that, similarly, the acid dissociation will be relatively independent of the degree of anhydride formation in the fueling process. With increasing salt concentration pK_a' – the apparent pK_a – decreases from 6.8 in the absence of added salt, and asymptotically approaches $pK_a' = 6.0$ at [NaCl] = 150 mM, which is the relevant salt concentration for fueling experiments (Figure S2b).



Figure S2. Determination of apparent pK_a values pK_a' for PNDAc as a function of salt concentration. Salt concentration was adjusted by the addition of NaCl (a) Potentiometric and conductimetric titrations without added salt. Conditions: 1.82 g L⁻¹ polymer/5 mM monomer units, pH adjusted to 11 with NaOH prior to titration with HCl. (b) pK_a' as a function of [NaCl].

Supplementary Note 2: Kinetic Model

We developed a simple kinetic model for the CRN that assumes homogeneous distribution of the components and comprises the following reactions:

Reaction 1: Hydration of EDC



Direct deactivation of EDC by water is pseudo-first order side reaction that consumes EDC, but does not contribute to the fueling process.

Reaction 2: Activation/Anhydride Formation



Second order reaction of a dicarboxylic acid monomer unit with EDC to form a carboxylic anhydride and the EDC-derived urea (EDU). For simplicity, we treat this reaction as independent of the degree of anhydride formation. In contrast to the prevalent model for EDC-fueled systems in literature, we opted to treat the activation step as a single reaction, rather than a two-step process consisting of ratedetermining formation of the *O*-acylisourea, followed by anhydride formation.¹² Due to the presence of a rate-determining step, and the unavailability of experimental data for the *O*-acylisourea intermediate, this is a reasonable assumption that helps to reduce the complexity of the network and facilitate data fitting.

Reaction 3: Deactivation/Anhydride hydrolysis



Hydrolysis of one anhydride moiety by water. For simplicity, we assume this reaction to be of pseudofirst order and independent on the degree of anhydride formation.

In mathematical terms, the concentrations of all species in the model are described by the following set of ordinary differential equations (ODEs):

$$\frac{\partial [diacid]}{\partial t} = -k_{activation} [diacid] [EDC] + k_{deactivation} [anhydride]$$
$$\frac{\partial c [anhydride]}{\partial t} = k_{activation} [diacid] [EDC] - k_{deactivation} [anhydride]$$

$$\frac{\partial [EDC]}{\partial t} = -k_{activation} [diacid] [EDC] - k_{side} [EDC]$$
$$\frac{\partial [EDU]}{\partial t} = k_{activation} [diacid] [EDC] + k_{side} [EDC]$$

We implemented this model in Python using the *odeint* module implemented in the *Scipy*-package to solve the ODEs.¹ The rate constant for EDC hydration k_{side} was determined directly by from HPLC-measurements of EDC concentration over time, while $k_{activation}$ and $k_{deactivation}$ and their corresponding uncertainties were obtained by fitting experimental datasets to the ODEs using the *lmfit*-package (see Supplementary Note 3)⁴. Using the obtained rate constants then allows us to predict time-dependent concentrations of all species of the CRN for arbitrary initial conditions. Additionally, we performed an uncertainty analysis using the *uncertainpy*⁷-package to calculate 90 % confidence intervals (CIs) for the concentration profiles. This is achieved by treating the rate constants as normal distributed with standard deviations equal to the uncertainty obtained from the fits and sampling the joint probability distribution using a quasi-Monte Carlo method.

Supplementary Note 3: Determination of rate constants

Direct deactivation of EDC: k_{side}

To account for EDC hydration as a non-fueling pathway for fuel consumption, we monitored the concentration profiles of 35 mM EDC solutions in 100 mM MES buffer at pH 5.3, 6.1 and 7.0 via HPLC (Figure S3). We found rate constants $k_{side} = 3.4 \cdot 10^{-3} \text{ s}^{-1}$, $4.5 \cdot 10^{-4} \text{ s}^{-1}$, and $1.5 \cdot 10^{-4} \text{ s}^{-1}$ at pH = 5.3, 6.1, and 7.0 respectively. This is in good agreement with values reported in literature for similar conditions¹³ and indicates that direct deactivation of EDC is slow and thus negligible in most experiments, but can play a role at low pH and large EDC excess.



Figure S3 Direct deactivation of 35 mM solutions of EDC in 100 mM MES buffer at (a) pH = 5.3, (b) pH = 6.1, and (c) pH = 7.0. Error bars represent the standard deviation of triplicate experiments. The solid linear represent linear fits corresponding to rate constants $k_{side} = 3.4 \cdot 10^{-3} \text{ s}^{-1}$ (pH 5.3), $4.5 \cdot 10^{-4} \text{ s}^{-1}$ (pH 6.1), and $1.5 \cdot 10^{-4} \text{ s}^{-1}$ (pH 7.0).

Activation and deactivation reactions: kactivation, kdeactivation

To estimate the rate constants for activation and $(k_{activation} \text{ and } k_{deactivation})$ we conducted fueling experiments at 0.36 g L⁻¹ (2 mM monomer units) with 1 equiv. and 2 equiv. EDC, monitoring the consumption of EDC via HPLC (Figure S4). The rate constants were then estimated by fitting the experimental data to the model described in Supplementary Note 2 via least-squares optimization using the Python package *lmfit.*⁴ All experiments were performed in quintuplicate and all datasets for 1 equiv. and 2 equiv. EDC at a given pH were fitted simultaneously. In accordance with literature reports^{11, 14} we found that activation was fast, but slowed down with increasing pH. while deactivation was slow, but accelerates with increasing pH (see Table 1 in the main manuscript).



Figure S4. Determination of rate constants for anhydride formation and hydrolysis, $k_{activation}$ and $k_{deactivation}$ at (a) pH 5.3, (b) pH 6.1, and (c) pH 7.0 determined from the consumption of EDC monitored via HPLC by simultaneous least-squares optimization of quintuplicate experiments.

Supplementary Note 4: Validation of kinetic model

To validate the kinetic model, we conducted fueling experiments at 0.36 g L⁻¹ (2 mM monomer units) with 4-18 equiv. EDC, and monitored the fuel consumption via HPLC. Both at pH 6.1 and pH 7.0, the observed decrease in EDC matches the predictions of our kinetic model reasonably well at all investigated fuel concentrations, although experimental values are not always within the calculated 90 % confidence intervals (Figures S5, S6). This indicates that in spite of its simplifications, the model describes the fueling process with sufficient accuracy for the prediction of time-dependent concentration profiles. At pH 5.3 and > 2 equiv. EDC, activation and phase separation proceed on a similar time scale as the mixing protocol, which relies on rapid uptake and dispensing with an air displacement pipette (see experimental section). Due to the applied shear stress, this leads the formation of macroscopically visible polymer clusters that interfere with sample collection for HPLC. Additionally, the formation of large polymer-rich domains introduces significant negative feedback for the deactivation reaction, making comparable HPLC experiments at pH 5.3 with higher fuel concentrations impossible.



Figure S5. Validation of the kinetic model at pH 6.1, 0.36 g L⁻¹ PNDAc and 4-15 equiv. EDC



Figure S6. Validation of the kinetic model at pH 7.0, 0.36 g L⁻¹ PNDAc and 4-18 equiv. EDC

Supplementary Note 5: Computational prediction of surface-area normalized octanol/water partition coefficients

A variety of oligomeric models was constructed to simulate EDC fueling of PNDAc. To distinguish necessary parameters of each model, attributes were denoted by composition (*i.e.* NDAc) and a series of numbers $(n_x_y_z)$ describing oligomer length (n), anhydride mole fraction $x_{anhydride}$ in % (x), targeted degree of ionization of carboxylic acids in % (y), and difference (z) between pH and pKa. For example, NDAc_15_60_91_1 indicated a 15-mer oligomer with 60% of monomer units converted to anhydride, 91% ionization of carboxylic acids, and pH – pKa = 1 (see Table S1).

Initially, the energy of an oligomer was minimized in the Forcite Molecular Dynamics (MD) module using a Geometry Optimization procedure with a COMPASS II force field. The force field contains information on important parameters, like preferred bond lengths, bond angles, torsion angles, partial charges, and van der Waals radius to find the optimal conformation.^{15,16} These simulations ran until the energy of the oligomer decreased below convergence criteria $(1 \times 10^{-4} \text{ kcal mol}^{-1} \text{ energy convergence}, 0.005 \text{ kcal mol}^{-1} \text{ Å}^{-1}$ force convergence, and a $5 \times 10^{-5} \text{ Å}$ displacement convergence). To further minimize conformational energy, oligomers were annealed for 200 cycles with five heating ramps per cycle using a sinusoidal temperature ramp ranging from 300-700 K. Then, conformations from annealing cycles 100, 150, and 200 were selected and these oligomers underwent an additional Forcite Geometry Optimization procedure before calculation of SA. The sequence of Geometry Optimization, annealing, and Geometry Optimization helped access a range of conformations.

SA values were calculated using an algorithm that rolls a ball over the surface of the oligomer to determine solvent accessible surface area. To ensure the SA values are relevant for CRN in water, the ball (1.4 Å radius) matched the size of a water molecule. After averaging SA values for annealing cycles 100, 150, and 200, the standard deviation was < 1.5 %. To calculate log*P* SA⁻¹ values, log*P* values were extracted from the QSAR menu in Materials Studio 2019 using the ALogP98 option.

Model	Xanhydride %	ionization /%	pH-pK _a	log <i>P</i> SA ⁻¹ /10 ³
NDAc_15_100_*_*	100	all	all	4.84
NDAc_15_80_99_2	80	100	2	-0.13
NDAc_15_60_99_2	60	100	2	-4.64
NDAc_15_40_99_2	40	100	2	-8.85
NDAc_15_60_91_1	60	92	1	-3.94
NDAc_15_40_91_1	40	89	1	-7.49
NDAc_15_73_76_0.5	73	75	0.5	-0.24
NDAc_15_60_76_0.5	60	75	0.5	-2.56
NDAc_15_40_76_0.5	40	77	0.5	-6.32
NDAc_15_80_50_0	80	50	0	2.07
NDAc_15_60_50_0	60	50	0	-0.43
NDAc_15_40_50_0	40	50	0	-2.85
NDAc_15_73_240.5	73	25	-0.5	2.67
NDAc_15_60_240.5	60	25	-0.5	1.75
NDAc_15_40_240.5	40	22	-0.5	0.67
NDAc_15_80_12	80	0	-2	4.29
NDAc_15_60_12	60	0	-2	3.88

Table S1. Oligomeric models created for $\log P$ SA⁻¹ calculations

Supplementary Note 6: Theoretical prediction and experimental determination of lifetimes

To predict lifetimes of the phase separated state, we calculated the anhydride concentration profiles and corresponding 90 % confidence intervals using the kinetic model (Figure S7a). Applying log*P* SA⁻¹ as a predictor for phase behavior, we expect phase separation at positive values of log*P* SA⁻¹ and a single stable phase at negative values. We therefore define the lifetime τ as the time after which the anhydride concentration has decreased sufficiently to reach a mole fraction $x_{anhydride}$ with log*P* SA⁻¹ = 0. Additionally, we estimate the uncertainty of the predicted lifetimes by calculating minimal and maximal lifetimes τ_{min} and τ_{max} as the minimal and maximal time for which $x_{anhydride}$ with log*P* SA⁻¹ = 0 is within the 90 % confidence interval.

To determine experimental lifetimes, we conducted fueling experiments at 0.36 g L⁻¹ (2 mM) PNDAc and monitored phase separation via absorbance measurements (Figure S7b). The obtained profiles showed a sharp in increase in absorbance upon the addition of fuel due to the onset of phase separation, followed by a steady, but increasingly slower growth as the polymer-rich domains grow. When the fuel runs out, and anhydride moieties hydrolyze sufficiently to resolubilize the polymer, a pronounced decrease in absorbance occurs. We define the lifetime τ as the time at maximal absorbance, just before the latter strongly decreases.



Figure S7. Computational prediction and experimental determination of lifetimes for the phase-separated state. (a) Exemplary predicted anhydride concentration as a function of time at pH 7 and 8 equiv. EDC. The lifetime τ is defined as the time after the initial anhydride build-up at which the anhydride mole fraction $x_{anhydride}$ with log*P* SA⁻¹ = 0 is reached. (b) Experimental absorbance profile at conditions matching those in (a). We define τ as the time at which absorbance is maximal.

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