
Supplementary information

**Near-identical macromolecules
spontaneously partition into concentric
circles**

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Supplementary Information for

**Near-identical macromolecules
spontaneously partition into concentric circles**

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1. General and materials for synthesis

1.1. General for synthesis

Preparative thin-layer chromatography (PTLC) was performed using 1 mm–thick preparative layer plates of silica gel 60 F₂₅₄ with a concentrating zone of 20 × 4 cm (Supelco, Merck). A Sephadex™ G-25 desalting column (PD-10 desalting column) was utilized for desalting and sample purification. Ultracentrifugation was carried out with centrifugal filters with cut-off molecules with molecular weight < 3,000 (Amicon® Ultra-0.5 mL or 4 mL 3K) and < 100,000 (Amicon® Ultra-0.5 mL 100K). Aqueous poly(ethylene glycol) (PEG) samples were evaporated to dryness using a lyophilizer (EYELA Co., Ltd., FDS-2000). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF–MS) was performed using a Bruker Daltonics autoflex™ speed MALDI-TOF spectrometry with dithranol as a matrix. Proton nuclear magnetic resonance (¹H NMR, 500 MHz) and carbon (¹³C) NMR (126 MHz) were recorded using a JOEL model JNM-ECA 500II spectrometer, where chemical shifts (δ , ppm) were determined relative to the signal of chloroform (CHCl₃) in deuterated chloroform (CDCl₃) (δ = 7.26 ppm, ¹H NMR; δ = 77.16 ppm, ¹³C NMR).

1.2. Materials for synthesis

Cyanine 3 N-hydroxysuccinimide (NHS) ester and Cyanine 5 NHS ester were obtained from Lumiprobe GmbH, Germany. The syntheses of the dye-appended PEG samples were performed following a publication with some modifications¹. 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS) was prepared according to a literature procedure².

2. Synthesis of dye-appended PEGs

^{Me}PEG_{5K}^{NBD}: A 1-mL acetonitrile solution of *N,N*-Diisopropylethylamine (DIPEA, 0.21 mL, 1.22 mmol) was added dropwise to a 4-mL acetonitrile solution of NBD-chloride (NBD-Cl, 347 mg, 1.74 mmol) and ^{Me}PEG_{5K}^{NH₂} (290 mg, 0.058 mmol) under stirring. The reaction mixture was allowed to stir in the dark at room temperature (~25 °C) for 48 hours followed by filtration. The filtrate was concentrated and dried. The crude product was further purified via PTLC with an eluent of chloroform:methanol = 16:1 (v/v) to yield a yellow solid. The crude product was dissolved in water and eluted through a PD-10 desalting column with milli-Q water to remove low MW compounds and ultrafiltered by an Amicon Ultra 4-mL 3K filter 8 times using milli-Q water. The refined product was freeze dried to obtain a dark yellow powder (90 mg, 30%, NBD-labelling rate, 94% confirmed by ¹H NMR).

¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.49 (d, *J* = 8.5 Hz, 1H, Ph-*H*), 6.21 (d, *J* = 6.5 Hz, 1H, Ph-*H*), 3.65 (m, 444H, CH₂), and 3.38 (s, 3H, CH₃).

^{Me}PEG_{20K}^{NBD}: A 1-mL acetonitrile solution of *N,N*-Diisopropylethylamine (DIPEA, 0.02 mL, 0.12 mmol) was added dropwise to a 2-mL acetonitrile solution of NBD-Cl (2.4 mg, 0.012 mmol) and ^{Me}PEG_{20K}^{NH₂} (116 mg, 0.0058 mmol) under stirring. The reaction mixture was allowed to stir in the dark at room temperature (~25 °C) for 48 hours followed by filtration. The filtrate was concentrated and dried. The crude product was dissolved in water and eluted to a PD-10 desalting column with milli-Q water to remove low MW compounds and ultrafiltered by an Amicon Ultra 4-mL 3K filter 8 times using milli-Q water. The refined product was freeze dried to obtain a yellow powder (92 mg; NBD-labelling rate, 67% confirmed by ¹H NMR).

¹H NMR (500 MHz, CDCl₃): δ (ppm) 8.49 (d, *J* = 8.5 Hz, 1H, Ph-*H*), 6.21 (d, *J* = 6.5 Hz, 1H, Ph-*H*), 3.64 (m, 1800H, CH₂), and 3.38 (s, 3H, CH₃).

^{Me}PEG_{5K}^{RhB}: *N,N'*-Dicyclohexylcarbodiimide (DCC, 173 mg, 0.84 mmol) was added to an 8-mL dichloromethane solution containing rhodamine B (RhB, 201 mg, 0.42 mmol), ^{Me}PEG_{5K}^{OH} (1.07 g, 0.21 mmol), and DPTS (188 mg, 0.42 mmol). The mixture was allowed to stir at room temperature (~25 °C) for 48 hours followed by filtration. The filtrate was concentrated and dried to obtain crude product, which was further purified using a silica gel column with an eluent of chloroform:methanol = 11:1 (v/v) to yield a dark purple solid. The product was dissolved in water and submitted to a PD-10 desalting column with the eluent of milli-Q water to remove low MW compounds and ultrafiltered

by an Amicon Ultra 4-mL 3K filter for 8 times using milli-Q water. The refined product was freeze dried into a dark purple powder (420 mg, 37%, RhB-labelling rate, 99% confirmed by ^1H NMR).

^1H NMR (500 MHz, CDCl_3): δ (ppm) 8.34 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H, RhB), 7.82 (t, 1H, RhB), 7.75 (t, 1H, RhB), 7.32 (m, 1H, RhB), 7.07 (m, 2H, RhB), 6.93 (m, 2H, RhB), 6.84 (d, 2.5 Hz, 2H, RhB), 4.18 (m, 2H, CH_2), 3.64 (m, 444H, CH_2), 3.38 (s, 3H, CH_3), and 1.35–1.25 (m, 15H, CH_2 - CH_3).

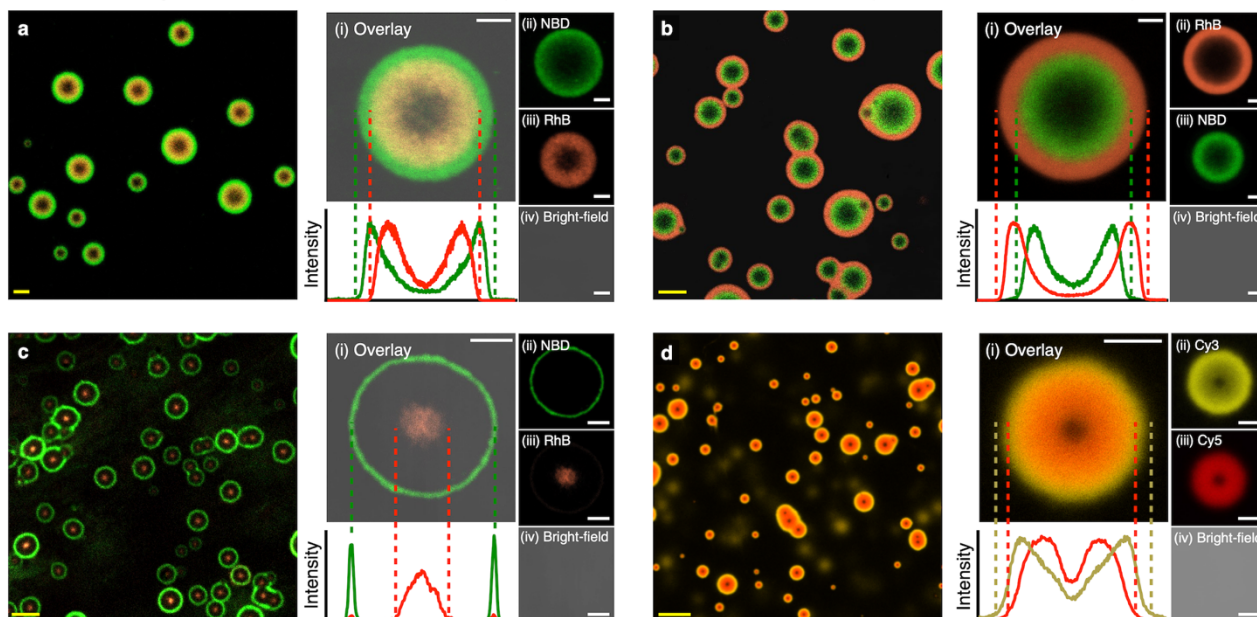
$^{\text{H}}\text{PEG}_{500\text{K}}^{\text{RhB}}$: *N,N'*-Dicyclohexylcarbodiimide (DCC, 82 mg, 0.4 mmol) was added to an 8-mL dichloromethane solution containing rhodamine B (RhB, 20 mg, 0.2 mmol), $^{\text{H}}\text{PEG}_{500\text{K}}^{\text{OH}}$ (200 mg, 0.4 μmol), and DPTS (90 mg, 0.2 mmol). The mixture was allowed to stir at room temperature for 72 hours followed by filtration. The filtrate was concentrated and dried to obtain a crude product. The crude product was dissolved in water and submitted to a PD-10 desalting column with the eluent of milli-Q water to remove low MW compounds and ultrafiltered by an Amicon Ultra 4-mL 100K filter 8 times using milli-Q water. The refined product was freeze dried to obtain a violet powder (390 mg, RhB-labelling rate, 49% confirmed by ^1H NMR).

^1H NMR (500 MHz, CDCl_3): δ (ppm) 8.29 (d, $J = 8.0$ Hz, 1H, RhB), 8.05 (s, 1H, RhB), 7.70 (m, RhB), 7.49 (m, RhB), 7.04 (m, RhB), 6.85–6.80 (m, RhB), and 3.60 (m, 45400H, CH_2).

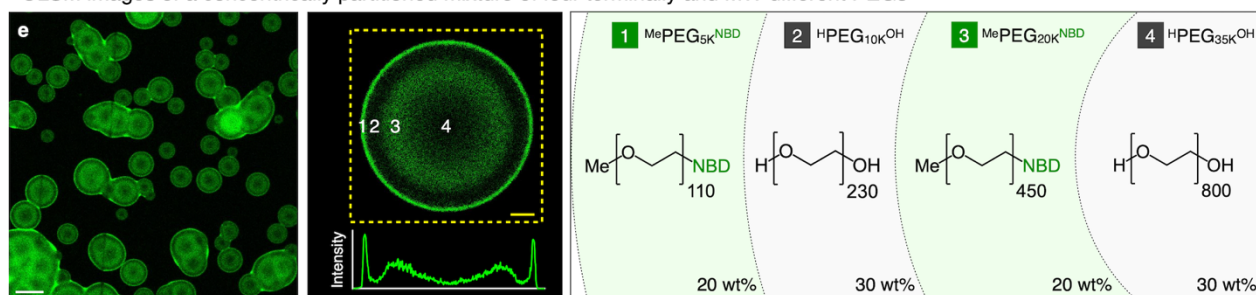
$^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{Cy3}}$ and $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{Cy5}}$: $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NH}_2}$ was dissolved in HKM buffer (25 mM HEPES-KOH, 100 mM KCl, pH 8.3). To this, a dimethyl sulfoxide (DMSO) stock solution of Cyanine 3 NHS ester was added in 100 equivalents, and the mixture was incubated at 37 °C for 6 hours. The reaction mixture was submitted to a PD-10 desalting column with the eluent of milli-Q water to remove low MW compounds and ultrafiltered (8 $\times$) by an Amicon Ultra 0.5-mL 3K filter using milli-Q water. Likewise, $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NH}_2}$ labelled with Cy5 was prepared by using Cyanine 5 NHS ester.

3. Multi-component concentric partitioning

CLSM images of four examples of concentrically partitioned mixtures of three terminally and MW-different PEGs by DLLPS

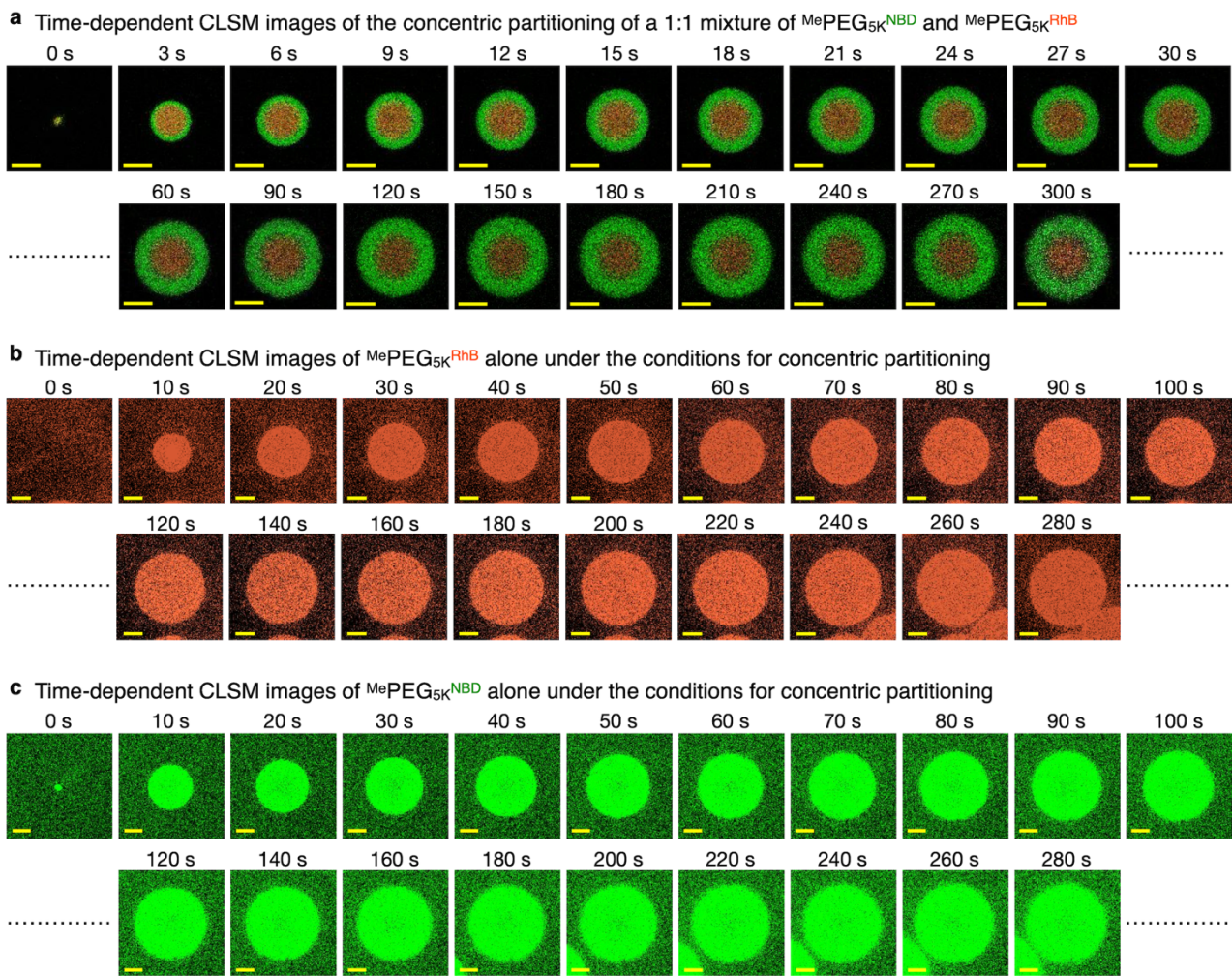


CLSM images of a concentrically partitioned mixture of four terminally and MW-different PEGs



Supplementary Fig. 1. a–d, Confocal laser scanning microscopy (CLSM) images (left) of concentrically partitioned mixtures of three terminally and molecular weight (MW)-different poly(ethylene glycol)s (PEGs). Mixtures of PEGs: **a**, $\text{MePEG}_{5\text{K}}^{\text{NBD}}$, $\text{MePEG}_{5\text{K}}^{\text{RhB}}$, and $\text{MePEG}_{5\text{K}}^{\text{NH}_2}$, **b**, $\text{MePEG}_{5\text{K}}^{\text{RhB}}$, $\text{MePEG}_{20\text{K}}^{\text{NBD}}$, and $\text{MePEG}_{20\text{K}}^{\text{NH}_2}$, **c**, $\text{MePEG}_{5\text{K}}^{\text{NBD}}$, $\text{HPEG}_{500\text{K}}^{\text{OH}}$, and $\text{HPEG}_{500\text{K}}^{\text{RhB}}$, **d**, $\text{MePEG}_{5\text{K}}^{\text{Cy3}}$, $\text{MePEG}_{5\text{K}}^{\text{Cy5}}$, and $\text{MePEG}_{5\text{K}}^{\text{NH}_2}$. Right insets: (i) magnified overlay images, (ii)–(iii) fluorescence images [$\lambda_{\text{ext}} = 488 \text{ nm}$ (NBD and Cy3); $\lambda_{\text{ext}} = 552 \text{ nm}$ (RhB); $\lambda_{\text{ext}} = 638 \text{ nm}$ (Cy5)], and (iv) bright-field images, together with their normalized fluorescence intensity distributions along a line across the image center. White scale bars, $2.5 \mu\text{m}$. Yellow scale bars, $3 \mu\text{m}$. **e**, CLSM image [$\lambda_{\text{ext}} = 488 \text{ nm}$ (NBD)] of a concentrically partitioned mixture of $\text{MePEG}_{5\text{K}}^{\text{NBD}}$, $\text{HPEG}_{10\text{K}}^{\text{OH}}$, $\text{MePEG}_{20\text{K}}^{\text{NBD}}$, and $\text{HPEG}_{35\text{K}}^{\text{OH}}$ (right), together with a magnified CLSM image and its normalized fluorescence intensity distributions along a line across the image center (middle). White scale bars, $10 \mu\text{m}$. Yellow scale bars, $2 \mu\text{m}$.

4. Dynamics of concentric partitioning

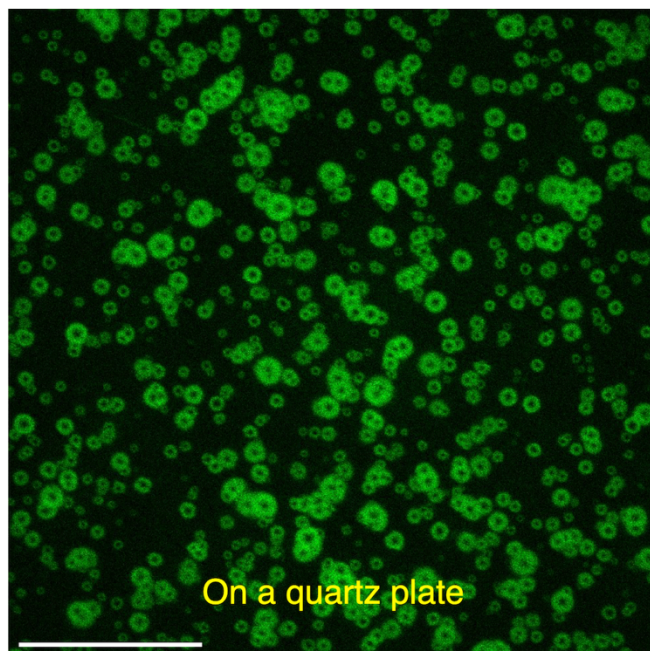


Supplementary Fig. 2. **a**, CLSM images [$\lambda_{\text{ext}} = 488$ nm (nitrobenzoxadiazole, NBD); $\lambda_{\text{ext}} = 552$ nm (Rhodamine B, RhB)] of time-dependent concentric partitioning of a 1:1 mixture of MePEG_{5K}^{NBD} and MePEG_{5K}^{RhB}. Scale bars, 5 μm . **b**, CLSM images [$\lambda_{\text{ext}} = 552$ nm (RhB)] of time-dependent changes in MePEG_{5K}^{RhB} alone under the conditions for concentric partitioning. **c**, Confocal laser scanning microscopy (CLSM) images [$\lambda_{\text{ext}} = 488$ nm (NBD)] of time-dependent changes in MePEG_{5K}^{NBD} alone under the conditions for concentric partitioning.

5. Studies of the spontaneously formed $(\text{NH}_4)_2\text{SO}_4$ layer

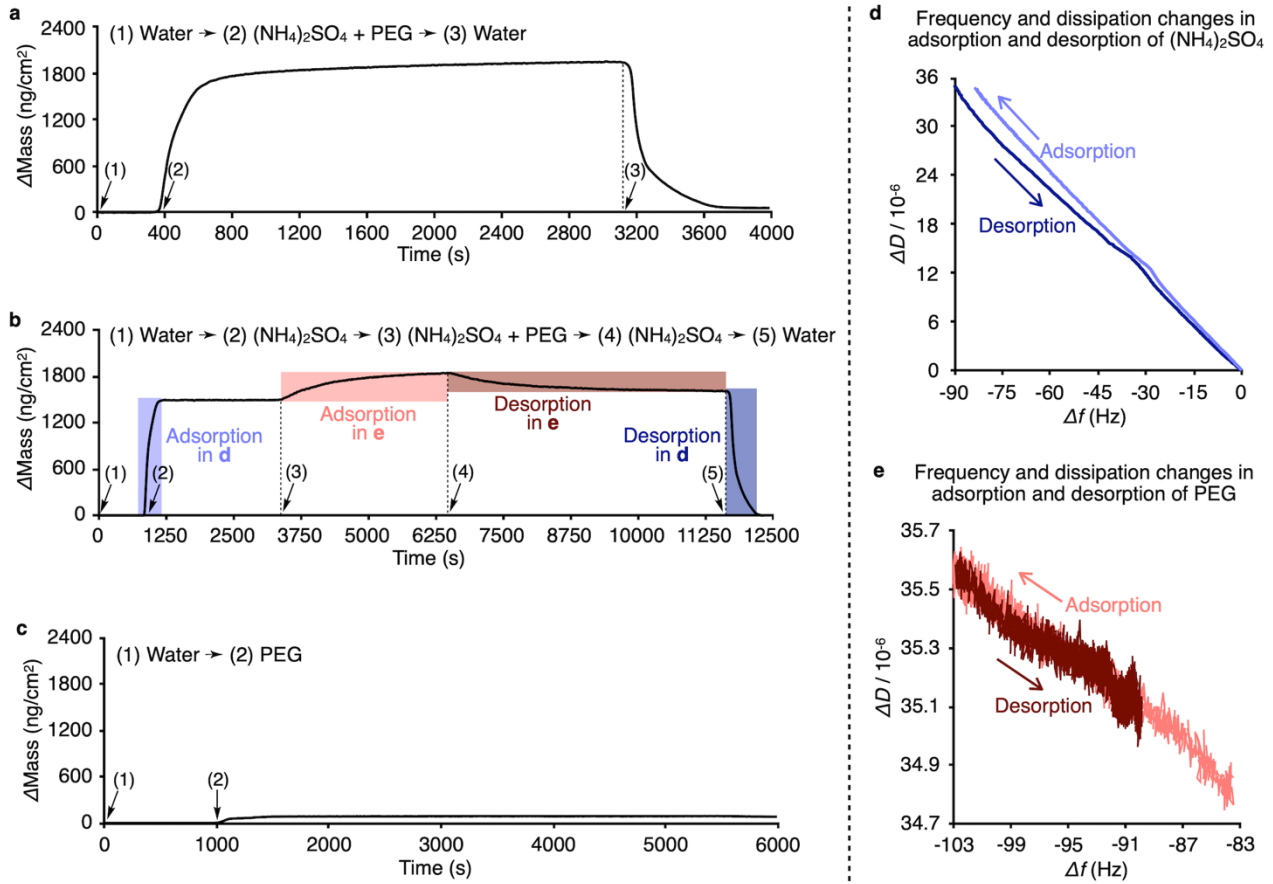
5.1. Concentric partitioning on a quartz plate

CLSM image of a concentrically partitioned 1:1 mixture of $\text{MePEG}_{5\text{K}}^{\text{NBD}}$ and $\text{MePEG}_{5\text{K}}^{\text{NH}_2}$



Supplementary Fig. 3. Confocal laser scanning microscopy image [$\lambda_{\text{ext}} = 488 \text{ nm}$ (nitrobenzoxadiazole, NBD)] of a concentrically partitioned 1:1 mixture of $\text{MePEG}_{5\text{K}}^{\text{NBD}}$ and $\text{MePEG}_{5\text{K}}^{\text{NH}_2}$ on a quartz plate. Inset: bright-field image. Scale bars, $50 \mu\text{m}$.

5.2. Quartz crystal microbalance profiles



Supplementary Fig. 4. **a–c**, Quartz crystal microbalance with dissipation (QCM-D) profiles of estimated ΔMass on a quartz sensor in response to water, aqueous $(\text{NH}_4)_2\text{SO}_4$ solution (26.4 wt%), aqueous $\text{MePEG}_{5K}^{\text{OH}}$ solution (1 $\mu\text{wt}\%$), and aqueous two-phase dispersion of PEG-enriched droplets [$(\text{NH}_4)_2\text{SO}_4$ (26.4 wt%) and $\text{MePEG}_{5K}^{\text{OH}}$ (1 $\mu\text{wt}\%$)] in different sequential flow orders. **d–e**, Plots of frequency against dissipation (overtone number of 7) in adsorption–desorption of $(\text{NH}_4)_2\text{SO}_4$ (**e**) and PEG (**f**) in **b**.

The amount of adsorbed $(\text{NH}_4)_2\text{SO}_4$ for forming the crystalline salt layer from the total amount of $(\text{NH}_4)_2\text{SO}_4$ was estimated using the Sauerbrey equation^{3,4}:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q\mu_q}}\Delta\text{Mass}$$

$$\frac{\Delta M}{M} = \frac{\Delta\text{Mass} \times A}{F \times t \times \rho \times [\text{Salt}]} = 0.0018\%$$

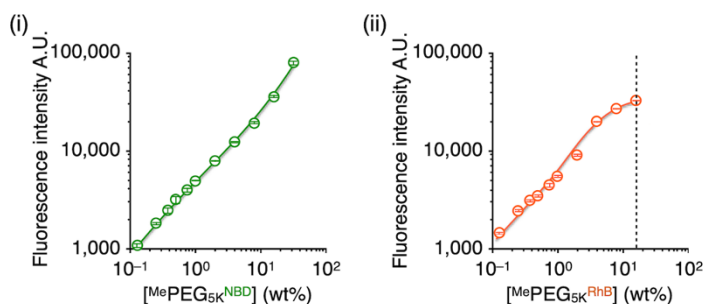
ΔMass : mass change, Δf : frequency change with an overtone number of 7,

f_0 : fundamental resonant frequency, A : piezoelectrically active area, ρ_q : quartz density,

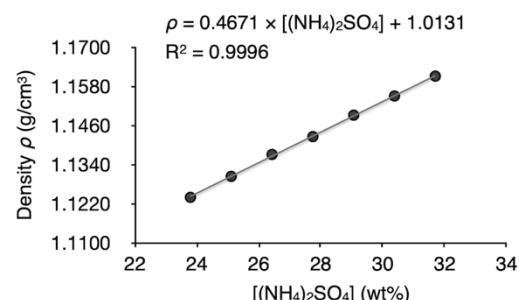
μ_q : shear modulus of quartz, F : flow rate, t : time, ρ : salt density

6. Phase diagram profiles

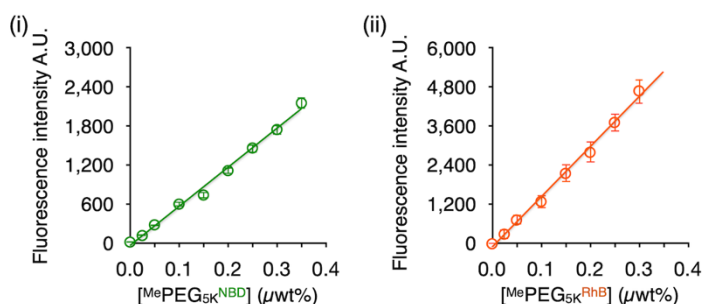
a Standard curves for determining [PEGs] in PEG-enriched phases



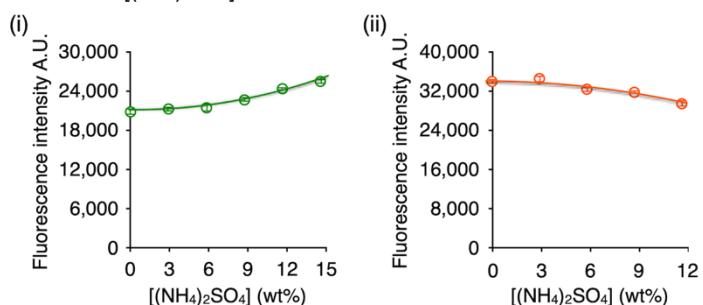
b Standard curves for determining [(NH₄)₂SO₄] in (NH₄)₂SO₄-enriched phases



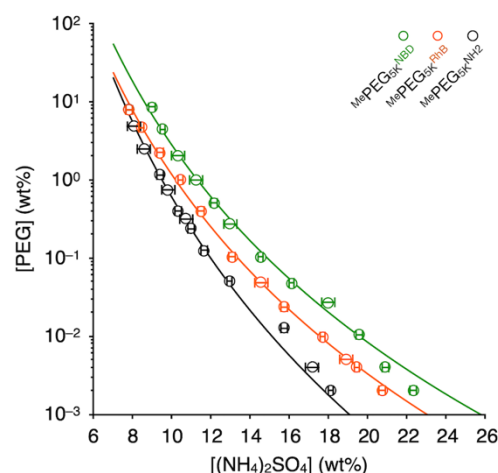
c Standard curves for determining [PEGs] in (NH₄)₂SO₄-enriched phases



d Effects of [(NH₄)₂SO₄] on the emissions of NBD- and RhB-labelled PEG



e Binodal curves (semi-log scale) of terminally different PEGs in an aqueous solution of (NH₄)₂SO₄ at 20 °C



Supplementary Fig. 5. **a**, Standard curves for estimating (i) [MePEG_{5K}^{NBD}] and (ii) [MePEG_{5K}^{RhB}] in their PEG-enriched phases, where the fluorescence intensities of 30- μ L standard PEG solutions were analyzed by confocal laser scanning microscopy (CLSM) in a 96-well plate. The standard PEG solutions were prepared by dissolving different amounts of PEG in a (NH₄)₂SO₄ solution (9.3 wt%) to achieve different concentrations (0.1–2 wt%). The high PEG concentrations in the range of 2–32 wt% were achieved by dissolving PEG compounds in water. **b**, Standard density curve for estimating [(NH₄)₂SO₄] in the (NH₄)₂SO₄-enriched phases using a densitometer. Standard solutions (1 mL) were prepared by dissolving different amounts of (NH₄)₂SO₄ in water to achieve different concentrations. **c**, Standard curves for estimating (i) [MePEG_{5K}^{NBD}] and (ii) [MePEG_{5K}^{RhB}] in the (NH₄)₂SO₄-enriched phases, where the fluorescence intensities of 30- μ L standard PEG solutions were analyzed by CLSM in a 96-well plate. The standard PEG solutions were prepared by dissolving different amount of PEG in in a (NH₄)₂SO₄ solution (9.3 wt%) to achieve different concentrations (0–0.4 μ wt%). **d**, Effects

of $[(\text{NH}_4)_2\text{SO}_4]$ on the emissions of (i) $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NBD}}$ and (ii) $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{RhB}}$ analyzed by CLSM. PEG samples: $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NBD}}$ (0.1 wt%) and $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{RhB}}$ (0.1 wt%). **e**, Binodal curves of aqueous $(\text{NH}_4)_2\text{SO}_4$ solutions of terminally different PEG samples ($^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NBD}}$; green, $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{RhB}}$; orange, $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NH}_2}$; black) at 20 °C plotted at the semi-logarithmic scale in the range of 1 $\mu\text{wt}\%$ –100 wt%. The solid curves are nonlinear fitted curves. All error bars represent the standard deviations of three samples.

Supplementary Table 1. Summary of tie line data of $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NBD}}$ (upper), $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{RhB}}$ (middle) and $^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NH}_2}$ (lower) in aqueous $(\text{NH}_4)_2\text{SO}_4$ at 20 °C

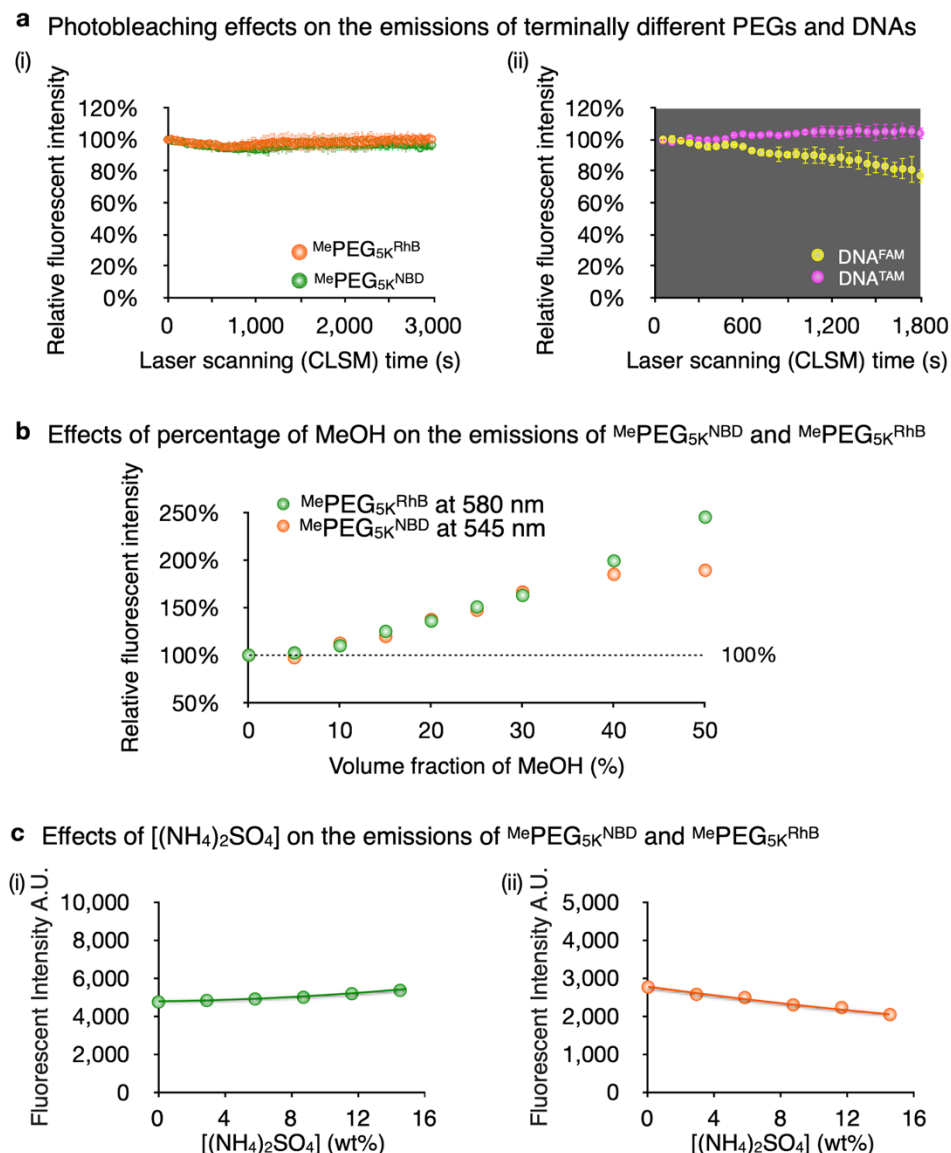
	Initial conditions		Composition after primary LLPS			
	$[(\text{NH}_4)_2\text{SO}_4]$ (wt%)	[PEG] ($\mu\text{wt}\%$)	Composition in $(\text{NH}_4)_2\text{SO}_4$ -enriched phase		Composition in PEG-enriched phase	
			$[(\text{NH}_4)_2\text{SO}_4]$ (wt%)	[PEG] ($\mu\text{wt}\%$)	$[(\text{NH}_4)_2\text{SO}_4]$ (wt%)	[PEG] (wt%)
$^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NBD}}$	26.428	5.00	26.548 ± 0.008	0.150 ± 0.004	$11.62 \pm 0.05^{**}$	0.78 ± 0.03
$^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{RhB}}$	26.428	5.00	26.531 ± 0.003	0.322 ± 0.007	$10.31 \pm 0.07^{**}$	0.89 ± 0.05
$^{\text{Me}}\text{PEG}_{5\text{K}}^{\text{NH}_2}$	26.428	5.00	26.053 ± 0.006	0.097^*	$9.36 \pm 0.08^{**}$	$1.12 \pm 0.10^{**}$

* Estimated value from the binodal curves based on $[(\text{NH}_4)_2\text{SO}_4]$ in $(\text{NH}_4)_2\text{SO}_4$ -enriched phase

** Estimated values from the binodal curves based on [PEG] in PEG-enriched phases

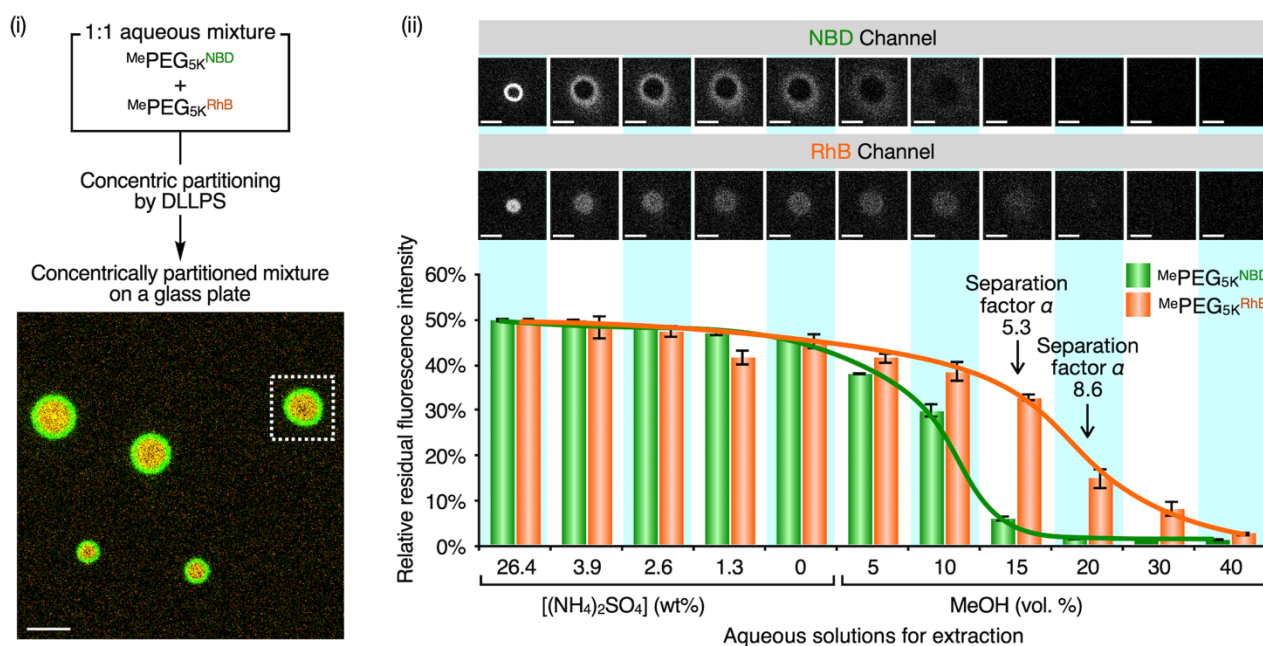
7. Selective extraction of terminally different PEG and DNA samples

7.1. Calibration curves for quantitative fluorescence imaging



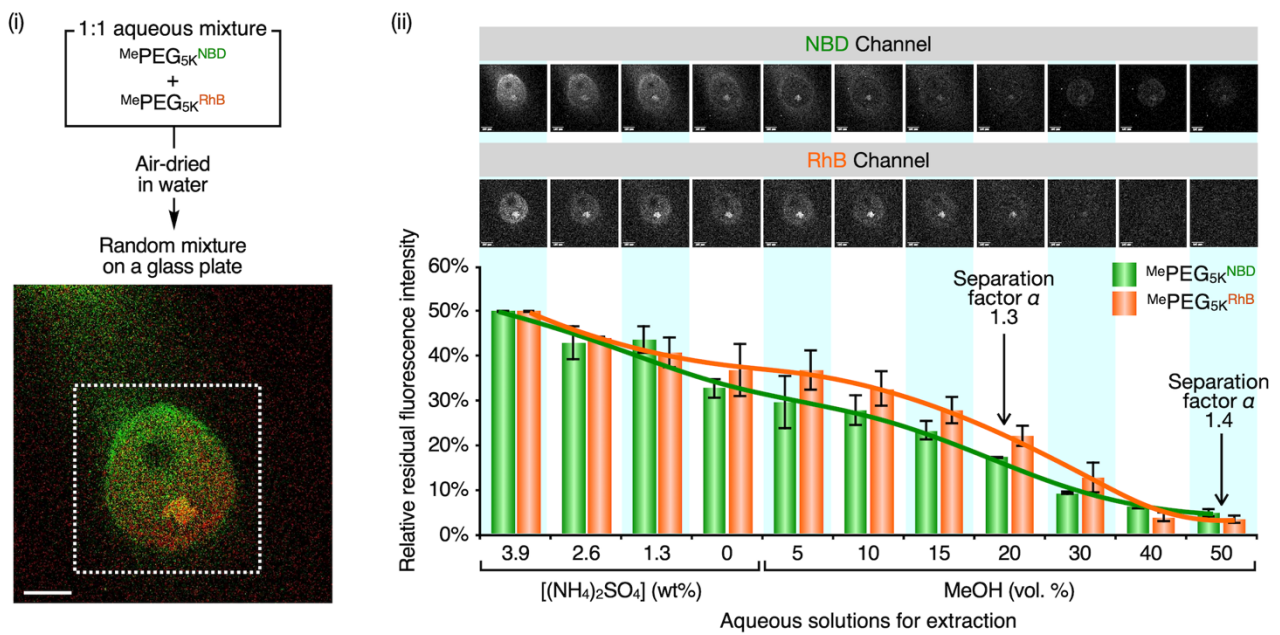
Supplementary Fig. 6. **a**, Calibration curves of fluorescence intensities of terminally different (i) PEG samples and (ii) DNA samples upon confocal laser irradiation with confocal laser scanning microscopy (CLSM) conditions of $[\lambda_{\text{ext}} = 488 \text{ nm}$ (nitrobenzoxadiazole, NBD); $\lambda_{\text{ext}} = 552 \text{ nm}$ (rhodamine B, RhB); $\lambda_{\text{ext}} = 488 \text{ nm}$ (fluorescein amidite, FAM); $\lambda_{\text{ext}} = 561 \text{ nm}$ (5-carboxy-tetramethylrhodamine, TAM)]. The error bars represent the standard deviations of three samples. **b**, Calibration curves of fluorescence intensities of $\text{MePEG}_{5K}^{\text{NBD}}$ (10 $\mu\text{wt}\%$, green) and $\text{MePEG}_{5K}^{\text{RhB}}$ (10 $\mu\text{wt}\%$, orange) solutions in different volume fractions of methanol (MeOH) obtained using a spectrofluorometer. **c**, Calibration curves of fluorescence intensities of $\text{MePEG}_{5K}^{\text{NBD}}$ (10 $\mu\text{wt}\%$, i) and $\text{MePEG}_{5K}^{\text{RhB}}$ (10 $\mu\text{wt}\%$, ii) solutions (50 μL) at different $(\text{NH}_4)_2\text{SO}_4$ concentrations via CLSM in a 96-well plate.

7.2. Selective extraction of the concentrically partitioned PEG mixtures (Step-wise)



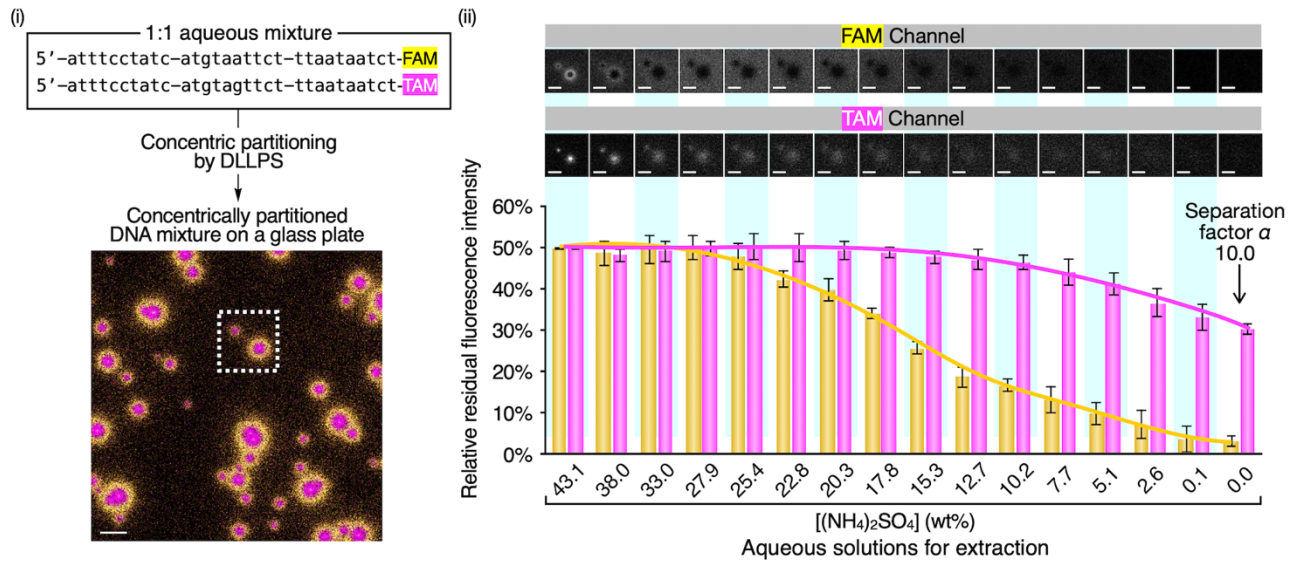
Supplementary Fig. 7. (i) Confocal laser scanning microscopy (CLSM) images [$\lambda_{\text{ext}} = 488$ nm (nitrobenzoxadiazole, NBD); $\lambda_{\text{ext}} = 552$ nm (rhodamine B, RhB)] of a concentrically partitioned 1:1 mixture of terminally different poly(ethylene glycol)s (PEGs), MePEG_{5K}^{NBD} and MePEG_{5K}^{RhB}, and (ii) residual fluorescent intensity changes on the glass plate upon stepwise extraction with different aqueous solutions. Scale bars, 10 μm . The error bars represent the standard deviations of three patterns.

7.3. Extraction of the random PEG mixtures (Step-wise)



Supplementary Fig. 8. (i) Confocal laser scanning microscopy (CLSM) images [$\lambda_{\text{ext}} = 488$ nm (NBD); $\lambda_{\text{ext}} = 552$ nm (RhB)] of a 1:1 random mixed terminally different PEGs (MePEG_{5K}^{NBD} and MePEG_{5K}^{RhB}) and (ii) residual fluorescent intensity changes on the glass plate upon stepwise extraction with different aqueous solutions. Scale bars, 100 μm . The error bars represent the standard deviations of three patterns.

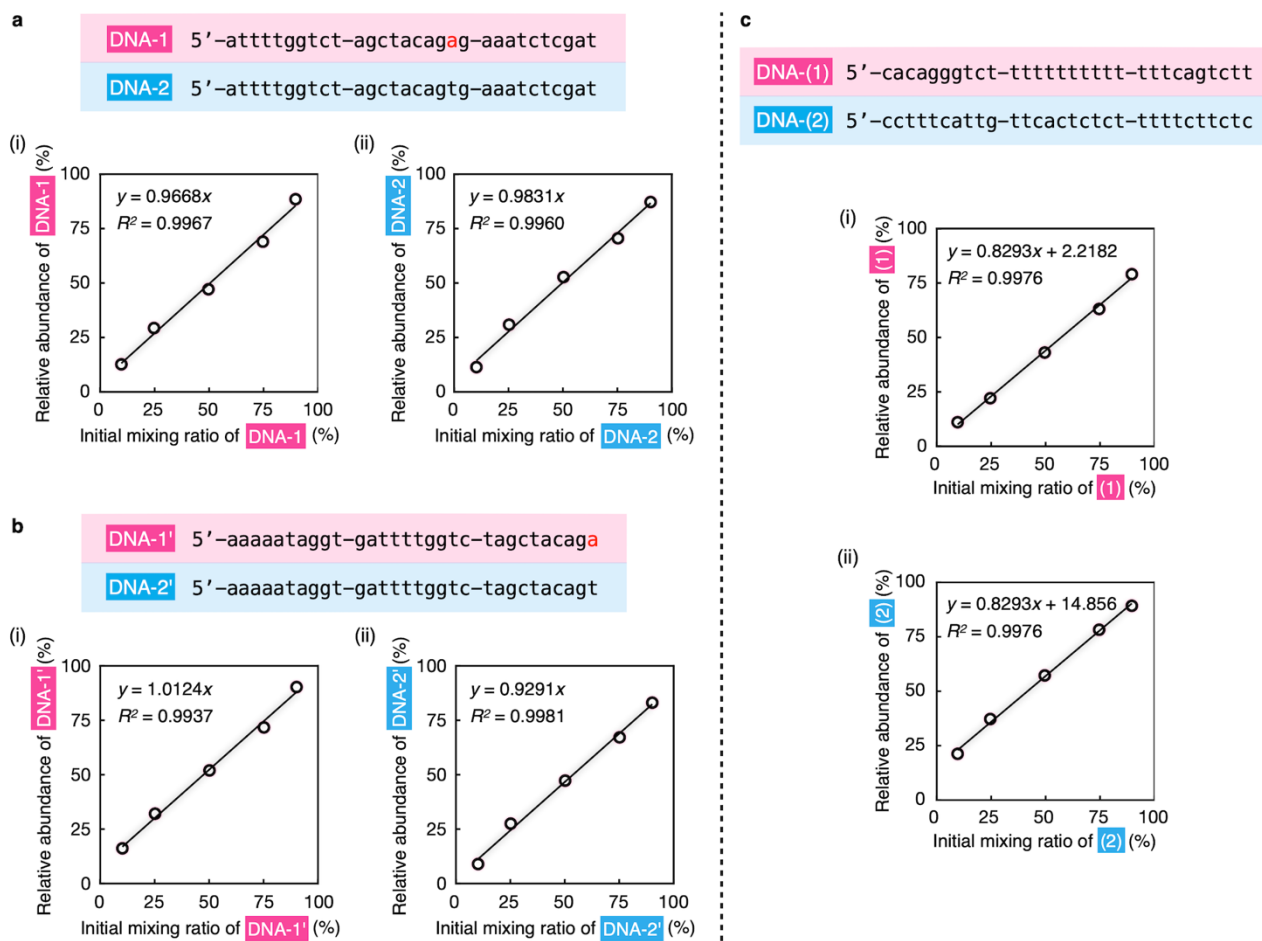
7.4. Selective extraction of the concentrically partitioned DNA mixtures



Supplementary Fig. 9. (i) Confocal laser scanning microscopy (CLSM) images [$\lambda_{\text{ext}} = 488$ nm (fluorescein amidite, FAM); $\lambda_{\text{ext}} = 561$ nm (5-carboxy-tetramethylrhodamine, TAM)] of a concentrically partitioned 1:1 mixture of terminally different single-stranded DNAs and (ii) residual fluorescent intensity changes on the glass surface upon stepwise extraction with different aqueous solutions. Scale bars, 5 μm . The error bars represent the standard deviations of three patterns.

8. Quantitative DNA analysis

8.1. Standard curves for quantitative DNA analysis

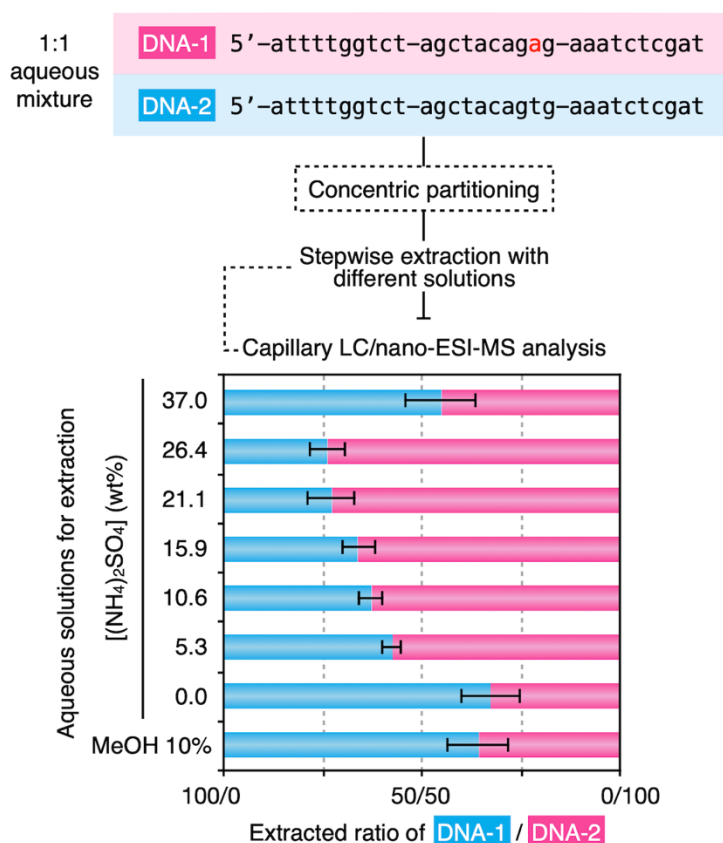


Supplementary Fig. 10. a–c, Standard curves for the quantitative DNA analysis of DNA mixtures at different mixing ratios via capillary liquid chromatography (LC)/nano-electrospray ionization mass spectrometry (nano-ESI-MS). DNAs in **a**: (i) DNA-1, 5'-atTTTggtctagctacag(t)gaaatctcgat, (ii) DNA-2, 5'-atTTTggtctagctacag(a)gaaatctcgat (single nucleotide variant). DNAs in **b**: (i) DNA-1', 5'-aaaaataggtgattttggtctagctacag(t), (ii) DNA-2', 5'-aaaaataggtgattttggtctagctacag(a) (single nucleotide variant). DNAs in **c**: (i) DNA-(1), 5'-cacagggtcttttttttttttcagtc**t**, (ii) DNA-(2), 5'-cctttcattgttcactctctttttcttct**c** (a: adenine, t: thymine, g: guanine, c: cytosine).

Supplementary Table 2. List of m/z for extracted ion chromatograms

Name	Charge	$z = -14$	$z = -13$	$z = -12$
DNA-1	5'-atTTTggtctagctacagagaaatctcgat	657.42–657.65	708.07–708.32	767.18–767.41
DNA-2	5'-atTTTggtctagctacagtgaatctcgat	656.78–657.00	707.37–707.61	766.42–766.65
Name	Charge	$z = -14$	$z = -13$	$z = -12$
DNA-1'	5'-aaaaataggtgattttggtctagctacaga	662.64–662.86	713.69–713.90	773.23–773.50
DNA-2'	5'-aaaaataggtgattttggtctagctacagt	662.00–662.22	712.98–713.24	772.50–772.76
Name	Charge	$z = -15$	$z = -14$	$z = -13$
DNA-(1)	5'-cacagggtctTTTTTTTTTTTcagtcctt	606.53–606.72	649.91–650.13	699.99–700.22
DNA-(2)	5'-cctttcattgttcactctctttttcttctc	595.93–596.13	638.56–638.78	687.75–687.98

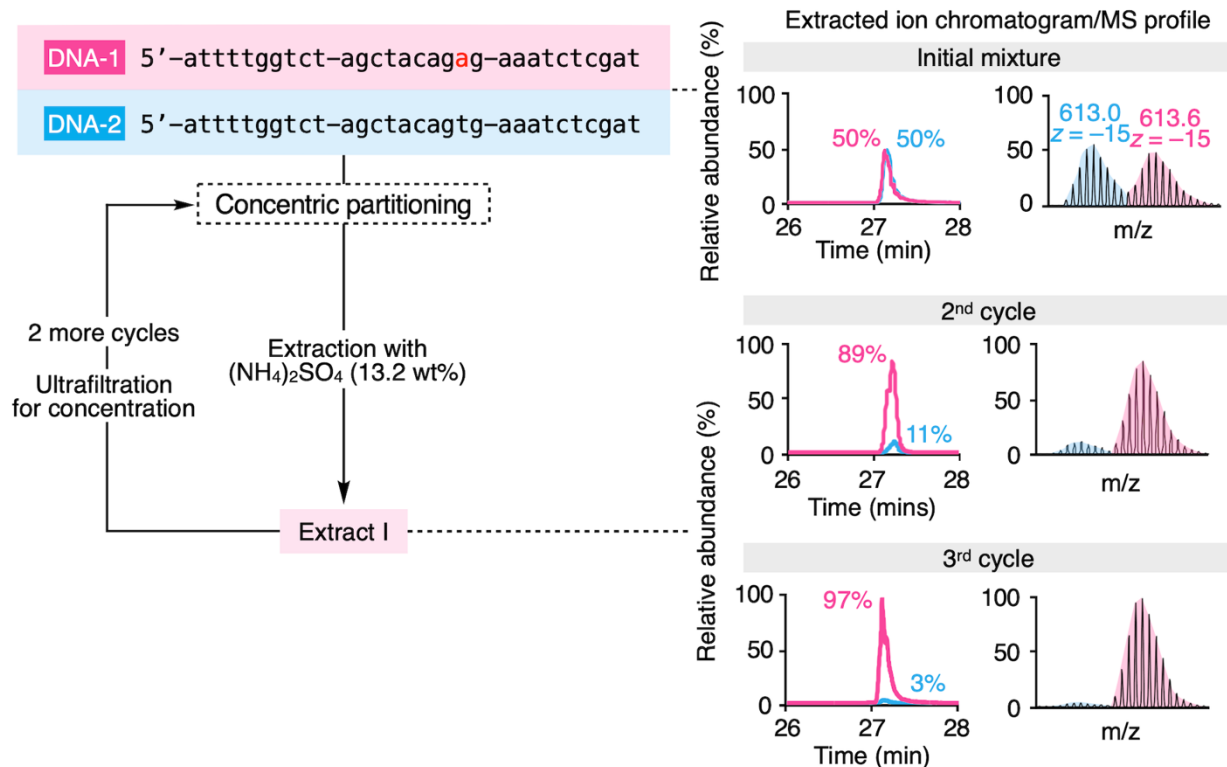
8.2. Stepwise extraction



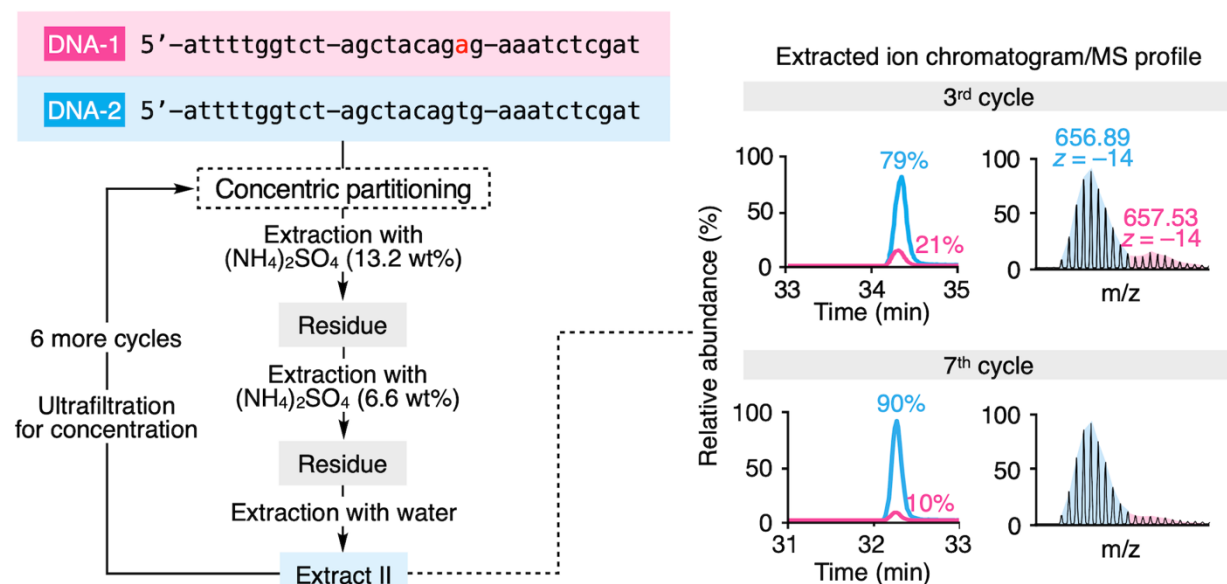
Supplementary Fig. 11. Quantitative DNA analysis of a concentrically partitioned 1:1 mixture of DNA-1 and DNA-2 upon stepwise extraction with different solutions via capillary liquid chromatography (LC)/nano-electrospray ionization mass spectrometry (nano-ESI-MS). DNA-1: "5'-at t t t g g t c t a g c t a c a g (t) g a a a t c t c g a t", DNA-2: "5'-at t t t g g t c t a g c t a c a g (a) g a a a t c t c g a t" (a: adenine, t: thymine, g: guanine, c: cytosine). The error bars represent the standard deviations of three samples.

8.3. Multiple consecutive extraction

a Consecutive selective extraction of the single nucleotide variant from a 1:1 mixture with its normal form

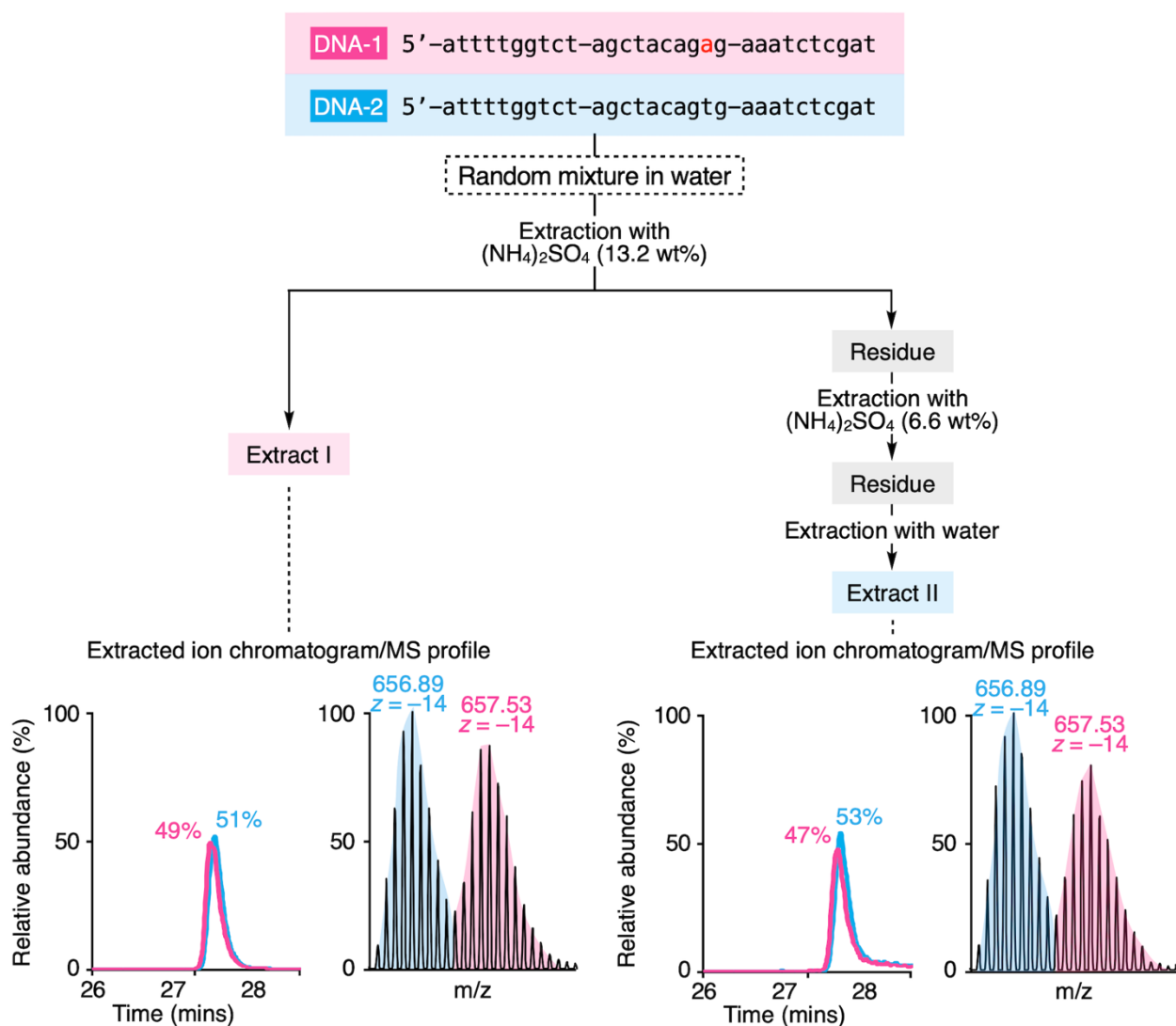


b Consecutive selective extraction of normal DNA from a 1:1 mixture with its single nucleotide variant



Supplementary Fig. 12. a–b, Quantitative DNA analysis of Extract I from three- and Extract II from seven-consecutive partitioning/extraction processes of a concentrically partitioned 1:1 mixture of DNA-1 and DNA-2 via capillary liquid chromatography (LC)/nano-electrospray ionization mass spectrometry (nano-ESI-MS). DNA-1: "5'-atTTTggtctagctacag(t)gaaatctcgat", DNA-2: "5'-atTTTggtctagctacag(a)gaaatctcgat" (a: adenine, t: thymine, g: guanine, c: cytosine).

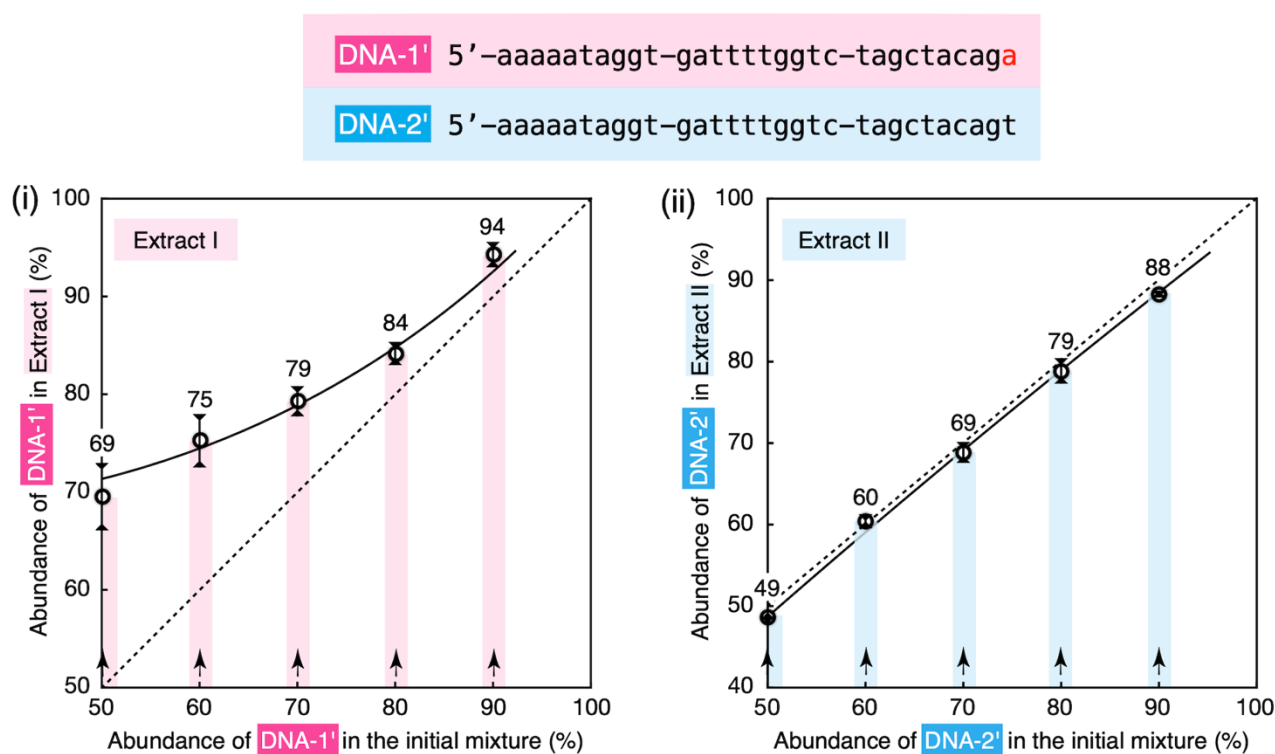
8.4. One-time extraction of a random mixture of DNAs



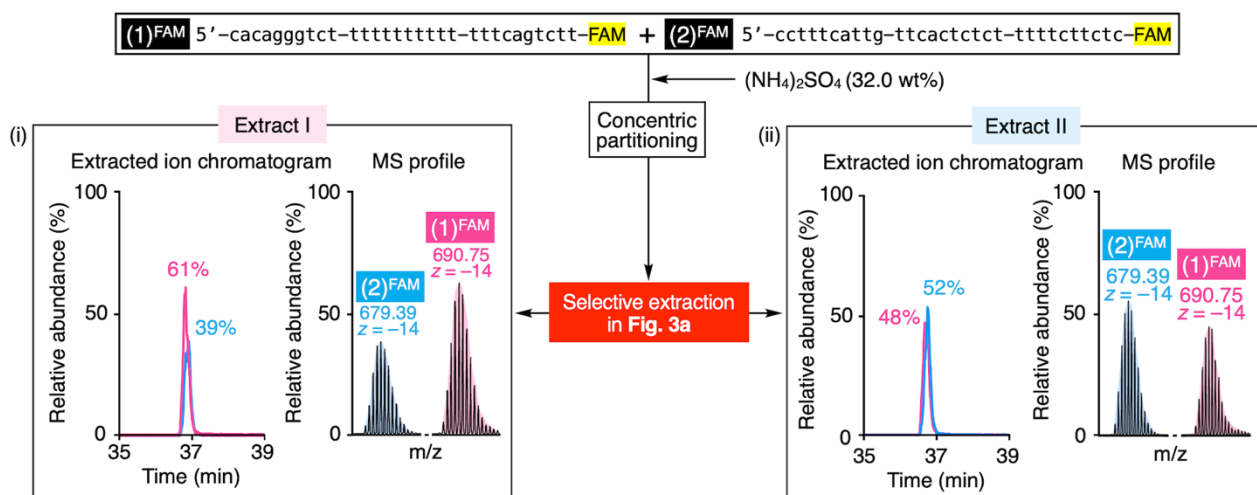
Supplementary Fig. 13. Quantitative DNA analysis of (i) Extract I and (ii) Extract II from one-time extraction of a random 1:1 mixture of DNA-1 and DNA-2 via capillary liquid chromatography (LC)/nano-electrospray ionization mass spectrometry (nano-ESI-MS). DNA-1: "5'-atTTTggtctagctacag(t)gaaatctcgat", DNA-2 "5'-atTTTggtctagctacag(a)gaaatctcgat" (a: adenine, t: thymine, g: guanine, c: cytosine).

8.5. One-time extraction of concentrically partitioned mixtures of DNAs

Selective extraction of a 30-nucleotide DNA (from a human cancer related gene) and its single nucleotide variant (3' end) from their concentrically partitioned mixtures

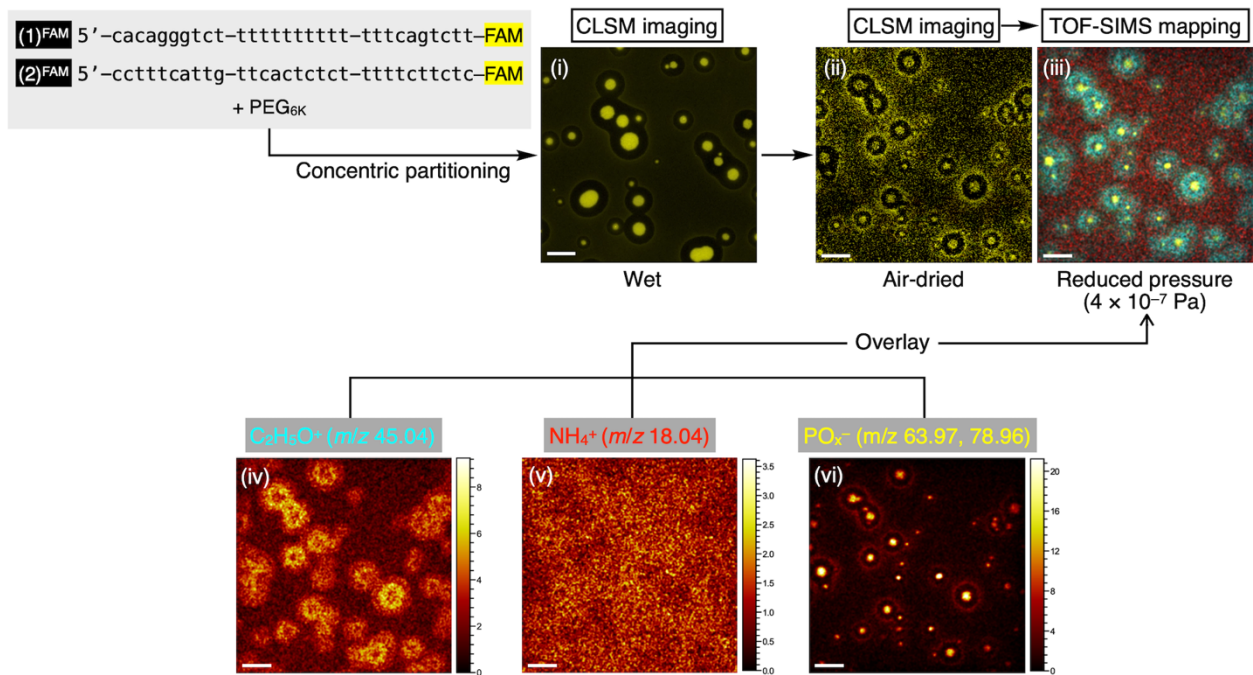


Supplementary Fig. 14. Quantitative DNA analysis of (i) Extract I and (ii) Extract II from one-time partitioning/extraction process of the concentrically partitioned mixtures of DNA-1' and DNA-2' via capillary liquid chromatography (LC)/nano-electrospray ionization mass spectrometry (nano-ESI-MS). DNA-1', "5'-aaaaataggtgattttggctagctacag(t)", is a human cancer-causing single nucleotide variant (3' end) of DNA-2', "5'-aaaaataggtgattttggctagctacag(a)" (a: adenine, t: thymine, g: guanine, c: cytosine). The error bars represent the standard deviations of three samples.



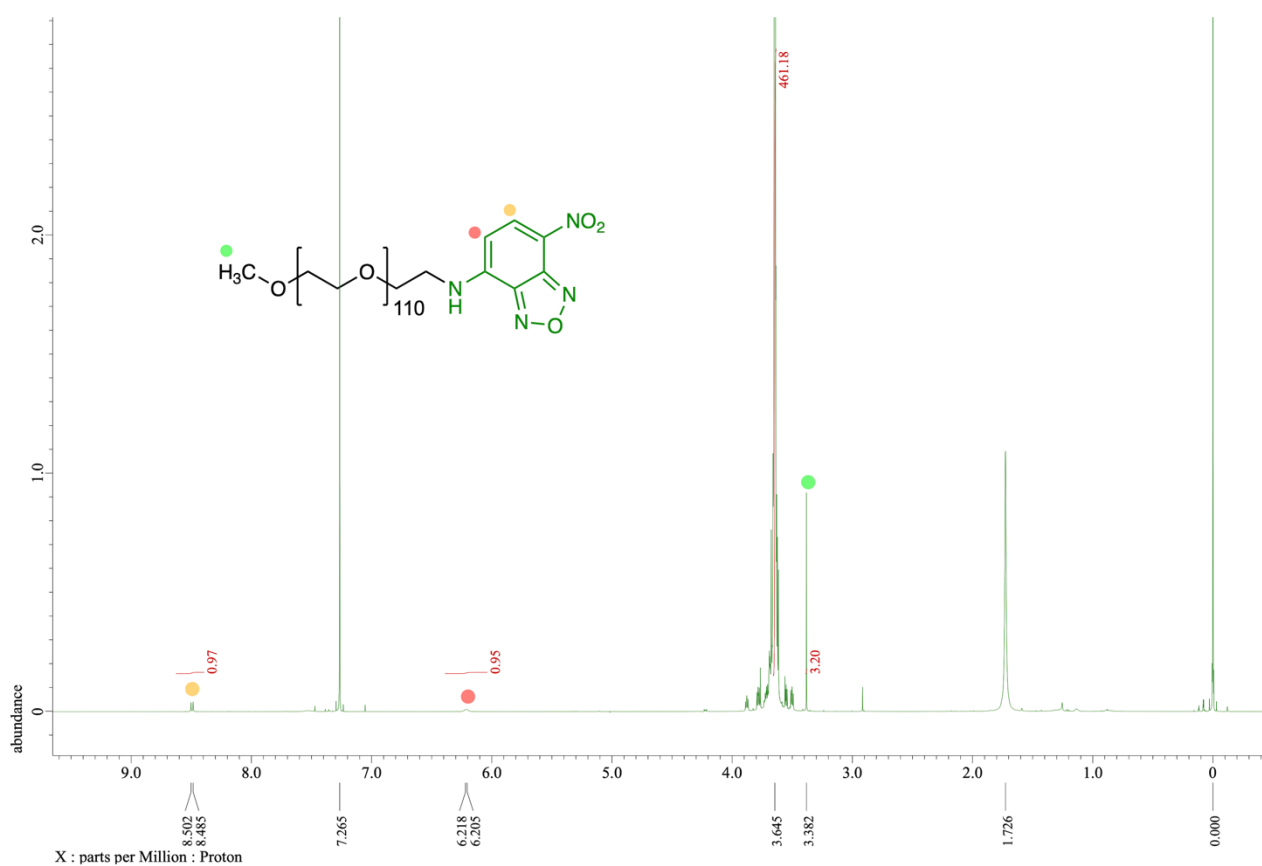
Supplementary Fig. 15. Quantitative DNA analysis of (i) Extract I and (ii) Extract II from one-time partitioning/extraction process of a concentrically partitioned 1:1 mixtures of DNA-(1)^{FAM} and DNA-(2)^{FAM} via capillary liquid chromatography (LC)/nano-electrospray ionization mass spectrometry (nano-ESI-MS). DNA-(1)^{FAM}: "5'-cacagggctctttttttttttcagtcctt-FAM", DNA-(2)^{FAM}: "5'-cctttcattgttcactctcttttcttctc-FAM" (a: adenine, t: thymine, g: guanine, c: cytosine, FAM: fluorescein amidite). (i) and (ii) present extracted ion chromatograms (left) and MS profiles (right).

9. TOF-SIMS profiles for post-hybridization experiment

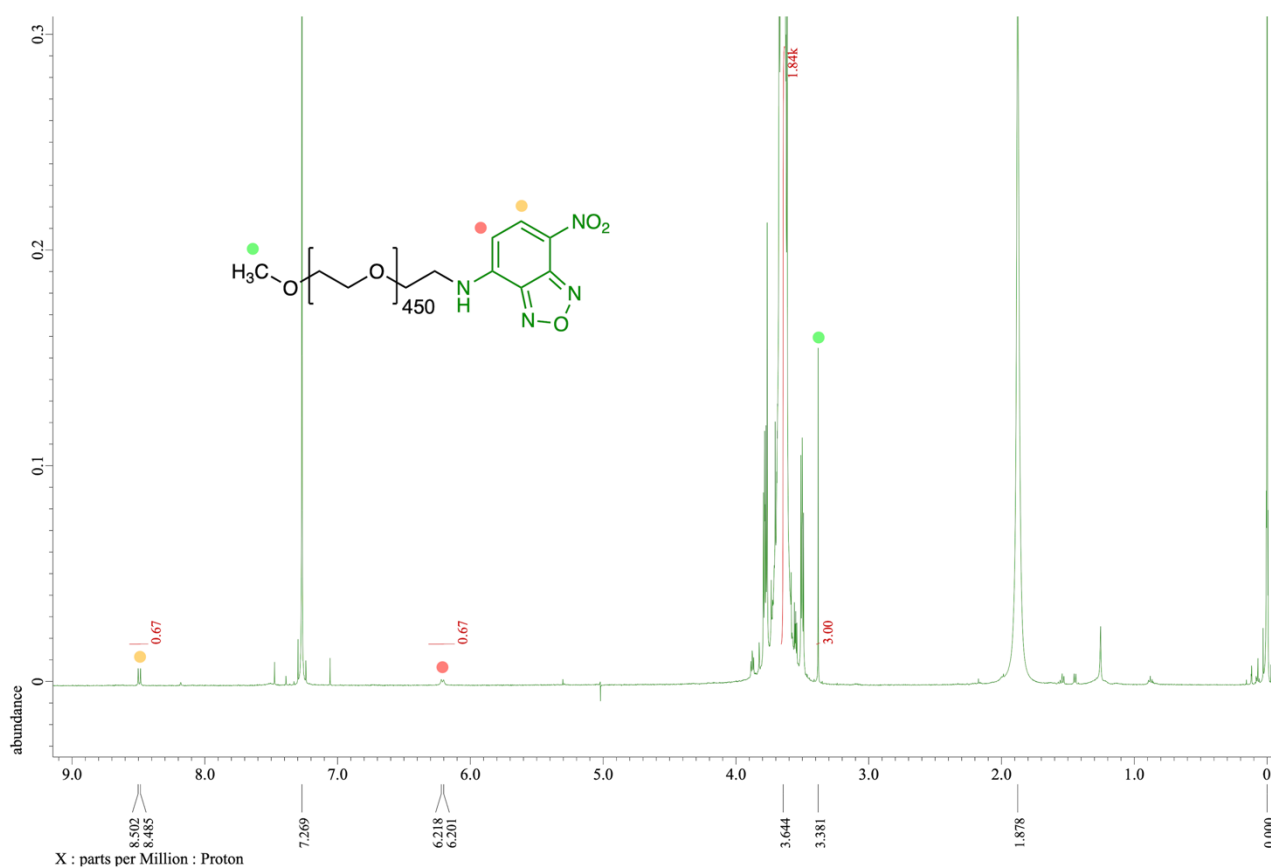


Supplementary Fig. 16. (i)–(ii) Confocal laser scanning microscopy (CLSM) images [$\lambda_{\text{ext}} = 488$ nm (fluorescein amidite, FAM)] and (iii)–(vi) time-of-flight secondary ion mass spectrometry (TOF-SIMS) maps of a concentrically partitioned 1:1:1 mixture of DNA-(1)^{FAM}, DNA-(2)^{FAM}, and PEG_{6K}. DNA-(1)^{FAM}: "5'-cacagggtcttttttttttttcagtctt-FAM", DNA-(2)^{FAM}: "5'-cctttcattgttcactctctttttctctc-FAM" (a: adenine, t: thymine, g: guanine, c: cytosine). (iii)–(vi) TOF-SIMS maps based on overlay, C₂H₅O⁺ (m/z 45.04), NH₄⁺ (m/z 18.04), and PO₂⁻, PO₃⁻ (m/z 63.97, 78.96). Scale bars, 20 μm.

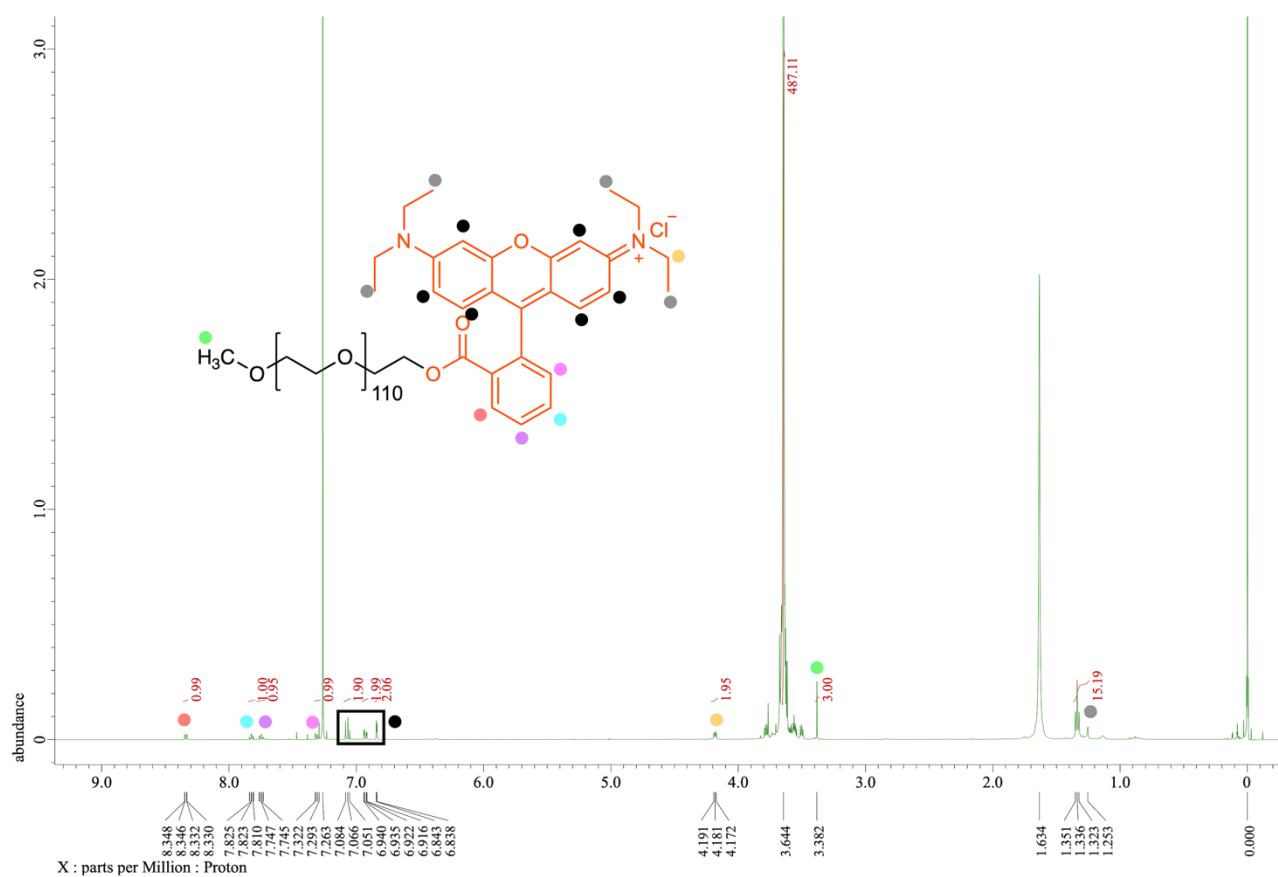
10. Nuclear magnetic resonance spectra



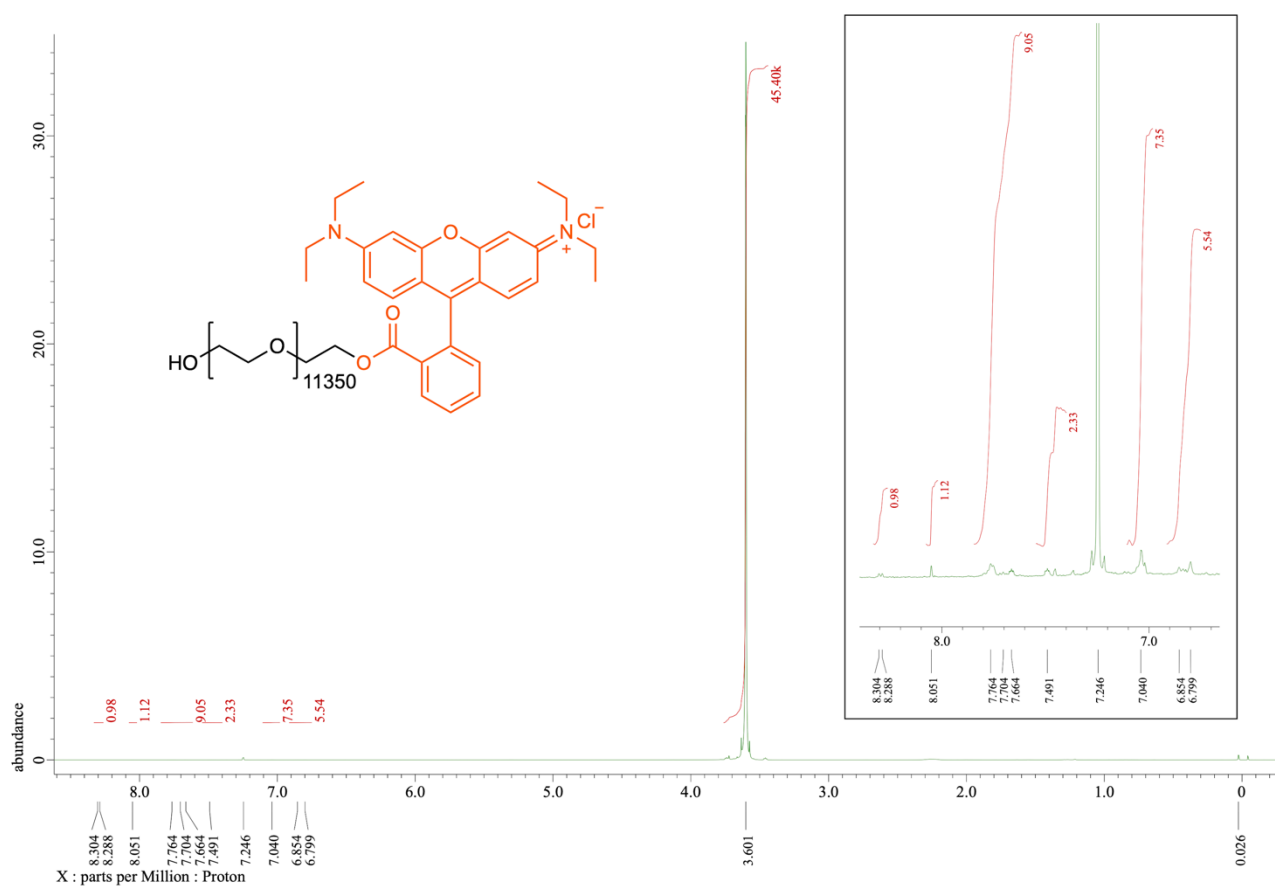
Supplementary Fig. 17. ^1H nuclear magnetic resonance spectrum of $\text{MePEG}_{5\text{K}}^{\text{NBD}}$ in CDCl_3 at 25°C .



Supplementary Fig. 18. ^1H nuclear magnetic resonance spectrum of $\text{MePEG}_{20\text{K}}^{\text{NBD}}$ in CDCl_3 at 25°C .

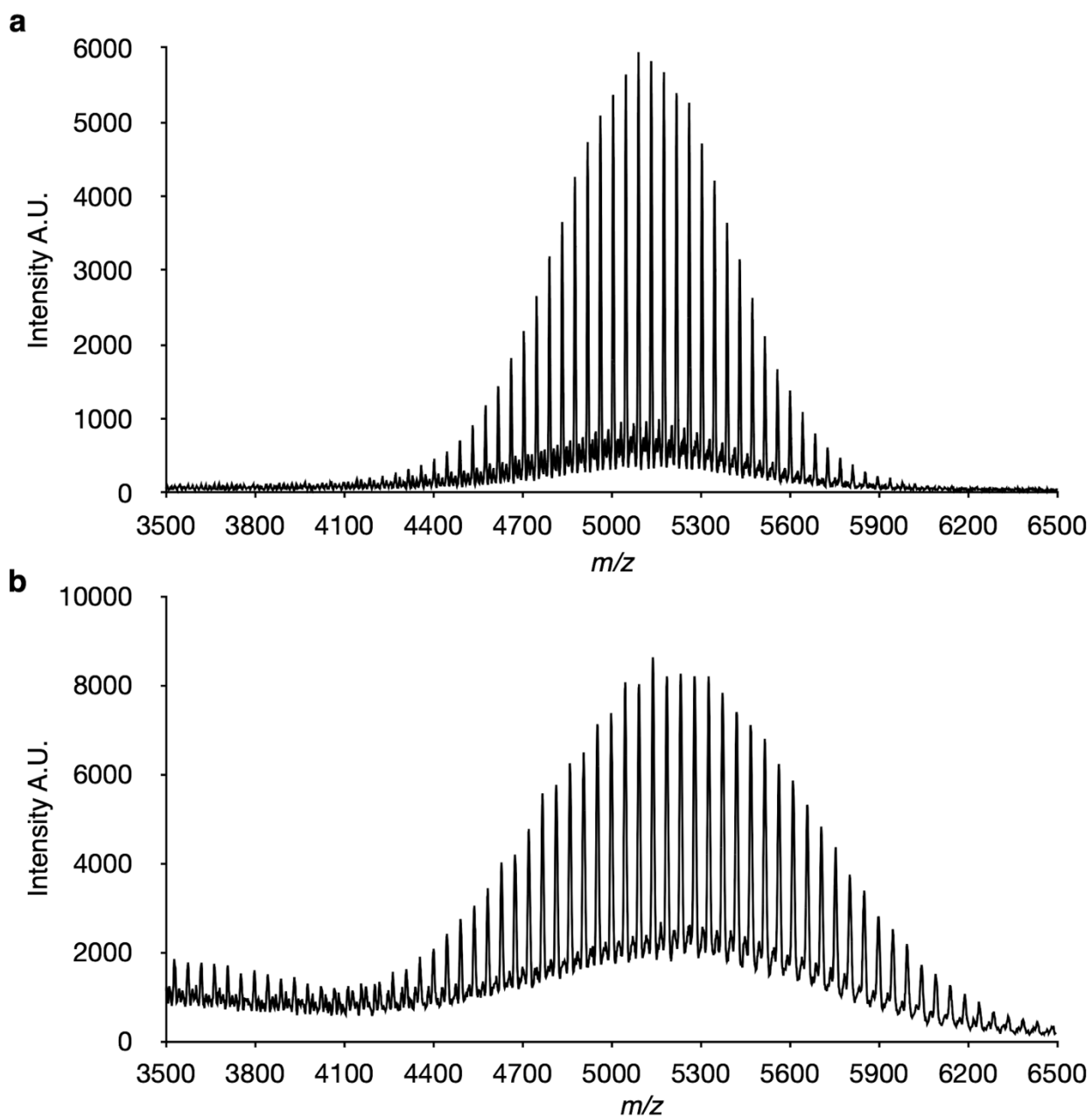


Supplementary Fig. 19. ^1H nuclear magnetic resonance spectrum of $\text{MePEG}_{5\text{K}}\text{-RhB}$ in CDCl_3 at 25°C .



Supplementary Fig. 20. ^1H nuclear magnetic resonance spectrum of $\text{MePEG}_{500\text{K}}\text{-RhB}$ in CDCl_3 at 25°C .

11. Mass spectrometry profiles



Supplementary Fig. 21. a–b, MALDI-TOF-MS of $McPEG_{5K}^{NBD}$ (a) and $McPEG_{5K}^{RhB}$ (b).

12. References

- [1] Xiao, Q., Rubien, J. D., Wang, Z., Reed, E. H., Hammer, D. A., Sahoo, D., Heiney, P. A., Yadavalli, S. S., Goulian, M., Wilner, S. E., Baumgart, T., Vinogradov, S. A., Klein, M. L. & Percec, V. *J. Am. Chem. Soc.* **138**, 12655–12663 (2016).
- [2] Moore, J. S. & Stupp, S. I. *Macromolecules* **23**, 65–70 (1990).
- [3] Sauerbrey, G. J. Z. P. The use of quartz oscillators for weighing thin layers and for microweighing. *Z. Fur. Phys.* **155**, 206–222 (1959).
- [4] Kankare, J. Sauerbrey equation of quartz crystal microbalance in liquid medium. *Langmuir* **18**, 7092–7094 (2002).
- [5] Karczewski, J., Miruszewski, T., Bochentyn, B. & Kusz, B. Determination of ionic conductivity in the Bi-Si-O and Pb-Si-O glasses. *Materials Science-Poland* **35**, 681–686 (2017).