
Supplementary information

Functional and morphological adaptation in DNA protocells via signal processing prompted by artificial metalloenzymes

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Supplementary Information for

Functional and Morphological Adaptation in DNA Protocells via Signal-Processing Prompted by Artificial Metalloenzymes

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Materials and Instrument:

ssDNA oligomers were purchased from Integrated DNA Technologies (IDT) as listed below in Table S1. The enzymes T₄ ligase (low concentration 2 U μL^{-1}), Exonuclease I (40 U/ μL) and Exonuclease III (200 U μL^{-1}) were purchased from Lucigen. Inorganic pyrophosphatase (2 U μL^{-1}) and Φ_{29} polymerase (10 U μL^{-1}) were purchased from New England Biolabs and Promega, respectively. Deoxynucleotide triphosphate (dATP, dTTP, dGTP and dCTP) 100-110 mM 1 mL were purchased from Jena Bioscience. Magnesium acetate tetrahydrate (MgAc_2), Sodium chloride (NaCl), Citric acid, Tris(2-carboxyethyl)phosphine hydrochloride, Tween 80, tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl and Trizma buffer substance pH=8), disodium ethylenediaminetetraacetate dehydrate (EDTA), bovine serum albumin (BSA), Biot-Atto₅₆₅ and acetic acid were purchased (as bioreagent grade if available) from Sigma-Aldrich. Agarose Low EEO was purchased from AppliChem. Oregon Green 488 Neutravidin conjugate, SYBR gold, loading dye, 50 bp and 1 kb ladder were purchased from ThermoFisher Scientific. The base of all solutions for protocell preparation is a TE buffer that consists of 10 mM of Tris(hydroxymethyl)aminomethane (pH=8.0) and 1 mM of EDTA. The buffer exchange was performed according to the specific experiment. DNA sequences were stored frozen at -25 °C in TE buffer. Gel electrophoresis were run using 1-2 wt% Agarose gel in TAE buffer containing 40 mM of TRIS-HCl, 20 mM of acetic acid and 1 mM of EDTA.

Dry solvents were purchased from Sigma Aldrich or Acros Organics and used without further purification. Water used in the catalytic reactions was purified with the Milli-Q Advantage system. Chemicals were purchased from Sigma Aldrich, Acros Organics, Alfa Aesar, Fluorochem, and used without further purification.

Thermal ramps were programmed on a Personal Thermocycler (Jena Analytics), DNA concentration were measured using a ScanDrop (Jena Analytic) spectrophotometer with a standard value of 33 $\mu\text{g}/\text{OD}_{260}$. Confocal laser scanning microscopy (CLSM) was performed on Leica TCS SP8 microscope.

Synthesis of Subs-I and Subs-II were carried out according to the procedures already reported in the literature.^{1,2} All catalytic reactions were carried out with non-degassed solvents under air. Reaction mixtures were stirred using Teflon-coated magnetic stir bars. The abbreviation “rt” refers to reactions carried out approximately at 23 °C. The temperature was maintained using Thermowatch-controlled heating blocks.

Quantitative GC-MS analysis was performed on a Shimadzu GCMS-QP2010S equipped with Agilent HP1-1MS (length: 30 m; Diameter: 0.25 mm; Film: 0.25 μM). GC column flow 2.05 mL/min Helium at linear velocity mode. GC inlet Temperature 250 °C, Split Ratio 80:1 – 99:1, oven Temperature 50 °C, hold for 1.0 min, 20 °C/min to 230 °C, hold 1 min. Runtime is 10.0 min. MS parameters: ion source Temperature 230 °C, interface Temperature 280

°C, Scan range m/z 50-500, scan time 2.5-10 min, Single Ion Mass (ch1 m/z 158.00, ch2 m/z 128). Quantitative parameters (target, m/z 128, retention time 5.700 min; internal standard (1-methoxynaphthalene), m/z 158, retention time 7.550 min). Injection volume: 1 µL.

High resolution mass spectra (HRMS) were acquired using electrospray (ESI) and were recorded at the analytical facility of the Chemistry department of the University of Basel. Fluorescence assays were recorded on Tecan fluorimeter Infinite M1000Pro. Samples were prepared in a 96-well plate (ThermoFischer Scientific Nunclon 96 Flat Black). Measurements settings: increment 1.0 nm, averaging time 0.1 s, excitation slit width 5.0 nm, emission slit width 5.0 nm, PMT voltage 620 V. Excitation 325nm, Emission 450 nm Bandwidth interval 5.0 nm, settle time 0.1 s. Kinetic experiments settings: Temperature 37 °C, Kinetic cycle 14-25 (kinetic interval: 5.00 min).

Abbreviations

PC/PCs	Protocell/protocells
Sav	Streptavidin
WT	Wild-type
Biot-Ru	Biotin-conjugated Hoveyda–Grubbs catalyst (second generation)
ArM	Artificial metalloenzymes
RCM	Ring-closing metathesis
ssDNA	Single-stranded DNA
dsDNA	Double-stranded DNA
Sav⊂PCs	Streptavidin-encapsulated protocells
Biot-Atto ₅₆₅ (Catalyst mimic)	Biotin-conjugated Atto ₅₆₅
(Biot-Ru⊂Sav)⊂PCs	Catalyst-encapsulated protocells
TON _{Ru}	Turnover number of the catalyst
FM	Force-sensing modules
[r.u.]	Concentration of the repeating unit of the ssDNA multiblock polymer
Sav ^{mutant}	Streptavidin mutated at specific position
pA	polyadenine

Supplementary Table 1. Sequences used, with their abbreviations, the sequence codes used for ordering at IDT, the purification grade and modifications.

	Name	Sequence 5'→3'	Purification	Modification
Templates	Temp(A ₂₀ -m)	/5Phos/ATC TAT CCT AAT TTT TTT TTT TTT TTT TTT TGA ACC CGT AT	HPLC	5'-Phosphorylation
	Temp(T ₂₀ -n)	/5Phos/ ATC CTC TAA AAT CAA AAA AAA AAA AAA AAA AAG TAA AAC CAC ACG	HPLC	5'- Phosphorylation
Ligation	m	TTA GGA TAG ATA TAC GGG TTC	HPLC	None
	n	TTT TAG AGG ATC GTG TGG TTT T	HPLC	None
Primers	m-exo	TTA GGA TAG ATA TAC GGG T*T*C	Desalting	Phosphorothioated Twice
	n-exo	TTT TAG AGG ATC GTG TGG TT*T* T	Desalting	Phosphorothioated Twice
Barcode* strands	Atto ₄₈₈ -m*	/5ATTO488N/ TGA ACC CGT ATA TCT ATC CTA A	HPLC	5' Atto 488 (NHS ester)
	Atto ₅₆₅ -n*	/5ATTO565N/ AAA ACC ACA CGA TCC TCT AAA A	HPLC	5' Atto 565 (NHS ester)
	Atto ₆₄₇ -n*	/5ATTO647NN/ TGA ACC CGT ATA TCT ATC CTA A	HPLC	5' Atto 647N (NHS ester)
	Atto ₆₄₇ -m*	/5ATTO647NN/ TGA ACC CGT ATA TCT ATC CTA A	HPLC	5' Atto 647N (NHS ester)
	Biot-m*	/5Biotin/ TGA ACC CGT ATA TCT ATC CTA A	HPLC	5' Biotin (C6 spacer)
	m*	TGA ACC CGT ATA TCT ATC CTA A	Desalting	None
	n*	AAA ACC ACA CGA TCC TCT AAA A	Desalting	None
Force Module strands	m*-y*-x	TGA ACC CGT ATA TCT ATC CTA ATT GAG CCG CGC ACG CCG GTC GAC GAT	Desalting	None
	x*-z*-m*	ATC GTC GAC TCG GCA GCA GCG TGA CTTGAA CCC GTA TAT CTA TCC TAA	Desalting	None
	Cy5-y	/5Cy5/ CGG CGT GCG CGG CTC	HPLC	5' Cy5 (C3 spacer)
	z-Q	AGT CAC GCT GCT GCC GA /3IAbRQ/	HPLC	3' Iowa Black (C3 spacer)

Supplementary Note 1: Expression and purification of Sav mutants.

The recombinant expression of streptavidin in *E. coli* cells used in this paper relies on a pET expression system.^{1,2}

Expression in TOP10(DE3) *E. coli* cells. The pre-culture (10 mL LB-medium containing 50 $\mu\text{g mL}^{-1}$ kanamycin) was inoculated from a glycerol stock and incubated overnight (37 °C, 200 rpm shaking). The main culture (1 L LB-rich medium containing: 5.35 g L^{-1} yeast extract, 10.70 g L^{-1} bactotryptone, 1.77 g L^{-1} Na_2HPO_4 , 1.70 g L^{-1} KH_2PO_4 , 1.34 g L^{-1} NH_4Cl , 0.36 g L^{-1} Na_2SO_4 , 0.24 g L^{-1} MgSO_4 , 0.5 g L^{-1} glucose, 6.31 g L^{-1} glycerol and 50 $\mu\text{g mL}^{-1}$ kanamycin) was inoculated with pre-culture to a starting OD₆₀₀ of 0.1 and incubated for 2.5 h (37 °C, 200 rpm shaking). The temperature was set to 20 °C and the culture was incubated for 1 h. At an OD₆₀₀ = 1.0, the main culture was induced with IPTG (final concentration: 40 μM) and incubated for additional 20 h (20 °C, 200 rpm shaking). The cell culture was centrifuged (3200 g, 8 °C, 10 min). The cell pellet was resuspended in 50 mL lysis buffer (PBS buffer (1x, pH 7.4) containing 0.5 mg mL^{-1} DNase, 1.0 mg mL^{-1} Lysozyme and 1 mM PMSF) and incubated for 1 h (37 °C, 200 rpm shaking). Sample was freeze-thawed once and incubated for another 3 h (25 °C, 180 rpm shaking).

Expression in BL21(DE3) *E. coli* cells. The pre-culture (25 mL LB-medium containing 50 $\mu\text{g mL}^{-1}$ kanamycin) was inoculated from a glycerol stock and incubated overnight (37 °C, 200 rpm shaking). The main culture (1 L Auto-induction medium (ZYP-5052)232 containing 50 $\mu\text{g mL}^{-1}$ kanamycin) was inoculated with pre-culture (20 mL) and incubated for 24 h (30°C, 180 rpm shaking). Cells were harvested and treated as described for the expression in *E. coli* TOP10(DE3).

SDS-PAGE analysis. The solubility and oligomeric state of the expressed Sav mutants were analysed by SDS-PAGE. 200 μL cell lysate was centrifuged (21000 g, 8 °C, 10 min), the supernatant (= soluble fraction) was removed and the cell pellet (= insoluble fraction) was dissolved in 200 μL urea (8 M). Both fractions (19 μL each) were mixed with 1 μL B4F (0.04 mM in DMSO) and 10 μL loading buffer (3x) and applied to the polyacrylamide gel.

Purification of cell lysate. The cell lysate was filled into dialysis bags (6-8 kDa MWCO) and dialyzed against guanidinium hydrochloride (6 M, pH set to 1.5 with HCl, 12 h), followed by dialysis in Tris-HCl (20 mM, pH 7.4, 12 h) and iminobiotin-binding buffer (50 mM NaHCO_3 , 500 mM NaCl, pH 9.8, 12 h). The sample was centrifuged (3200 g, 10°C, 45 min) and filtered (0.2 μm filter). The sample was purified by affinity column chromatography (AKTAprime Plus chromatography system equipped with a 2-iminobiotin sepharose column). The eluate was neutralized with Tris-HCl (pH 7.4, final concentration: 20 mM), followed by dialysis in MilliQ-water (4 x 12 h) and lyophilization.

Supplementary Note 2: ICP-MS analysis and activity assay for the RCM with protocells.

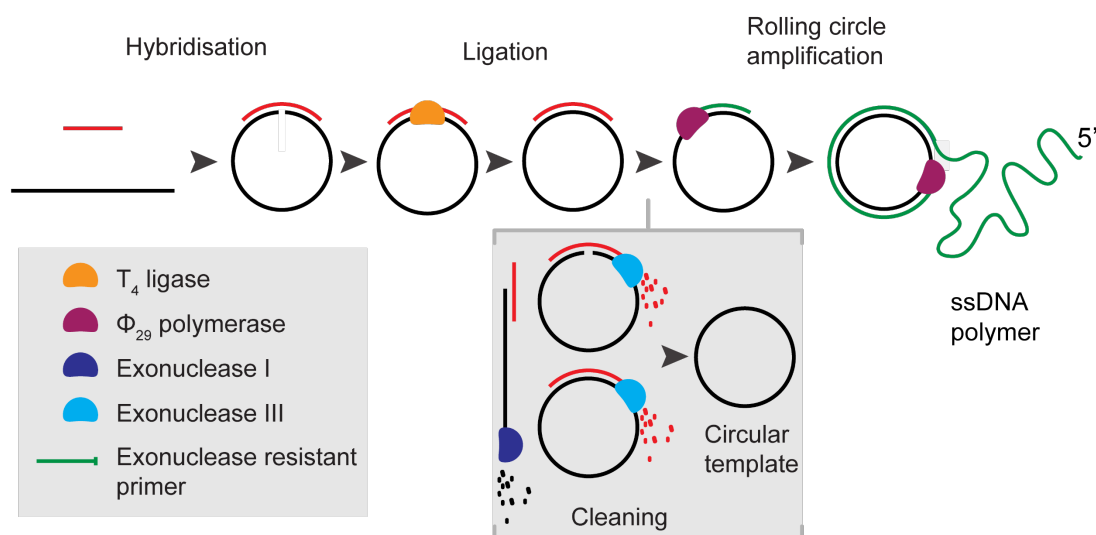
The biotin-loaded protocells (Biot-PCs) (700 μL) were charged with Sav (40 μM , 21 μL of a 1.33 mM stock solution), and incubated for 1 h at room temperature. After incubation, the solution was centrifuged and washed with PBS buffer. The catalyst Biot-Ru was then added to the Sav $\subset$ PCs (25 μM , 3.5 μL of a 5 mM stock solution in DMSO), and the resulting mixture was incubated for 1 h at room temperature. After incubation, the solution was divided in 2 aliquots, and one aliquot was subjected to centrifugation followed by washing with PBS buffer, while the other aliquot was not washed with PBS buffer. The washed and non-washed PCs (20 μL) were then placed into a microplate reader, followed by the addition of a solution of umbelliferone Subs-I (180 μL of a 111.11 μM solution in PBS buffer containing 200 mM MgCl_2 , pH 6). The plate was inserted in a Tecan reader (Infinite M1000Pro) and analysed by fluorescence (temperature 37 $^{\circ}\text{C}$, kinetic cycle: 15, kinetic interval: 5.00 min, excitation 325nm, emission 450 nm, bandwidth interval 5.0 nm, settle time 0.1 s). The remaining volume of the PCs was split into three 100 μL aliquots, diluted to 250 μL with Milli-q water and submitted to ICP-MS analysis.

The ICP-MS measurements were performed on two different samples, one containing washed ArM $\subset$ PCs and the other one contained the protocells with an excess catalyst (Biot-Ru) in solution (Fig. 3d,e). From the results, it was evident that 24% of the added catalyst diffused inside the PCs. The TON_{Ru} (calculated from actual Ru content) of these two scenarios are plotted against the reaction time (Fig. 3d).

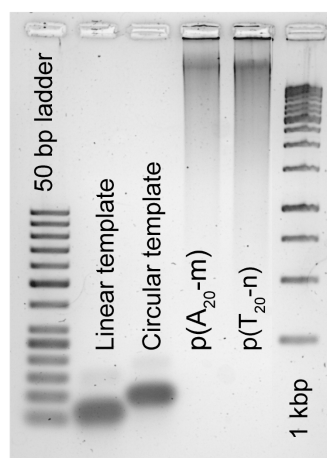
Supplementary Table 2. ICP-MS results of Ru-loaded protocells with and without washing

Sample	Washed_Ru content (mg kg ⁻¹)	Non washed_Ru content (mg kg ⁻¹)	Blank_ Ru content (mg kg ⁻¹)
1	0.012	0.111	—
2	0.022	0.058	—
3	0.016	0.048	—
Average	0.017 $\pm$ 0.005	0.072 $\pm$ 0.03	—

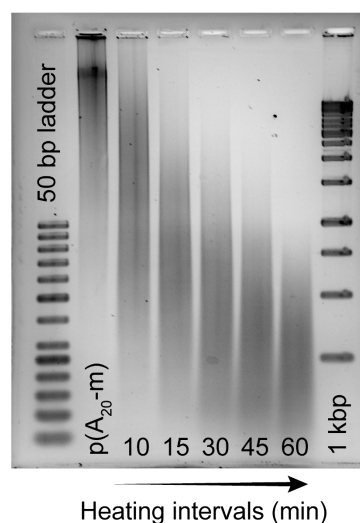
a Synthesis of circular ssDNA template and multiblock ssDNA polymer via RCA



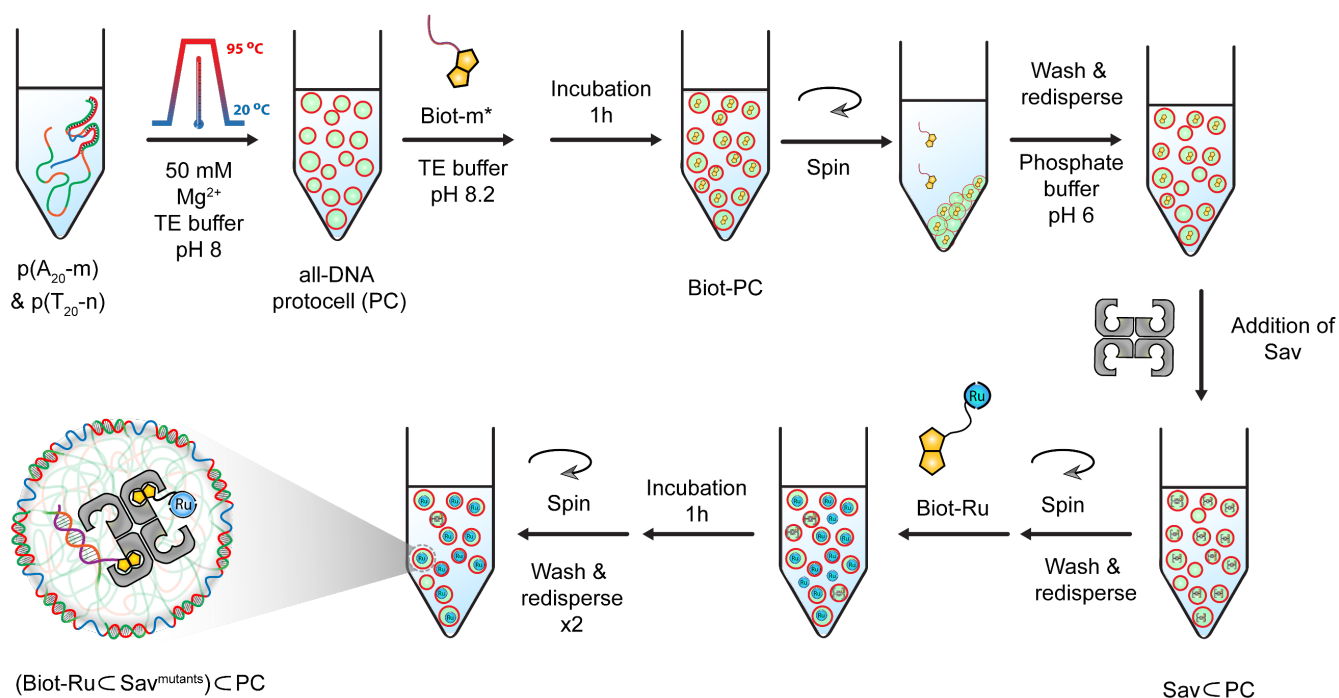
b GEP characterisation of the ssDNAs



c Heat-induced cleavage of the p(A₂₀-m)

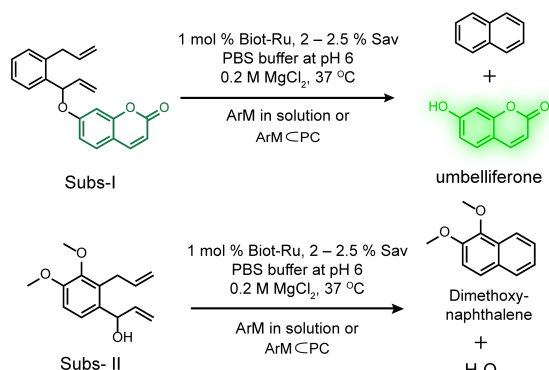


Supplementary Fig. 1: ssDNA synthesis via rolling circle amplification (RCA). (a) Schematic representation of the entire process and (b) corresponding gel electrophoresis. A commercial ssDNA (black) is circularized using a complementary ligation strand (red) and ligated using T_4 ligase. Unligated products and ligation strands are then digested by Exonucleases I and III and the cleaned templates are amplified using a primer Φ_{29} polymerase (maroon). (b) Gel electrophoresis characterization of the linear ssDNA, circular template and the RCA products, namely p(A₂₀-m) and p(T₂₀-n). (c) Gel electrophoresis of the thermal cleavage of p(A₂₀-m) at 95°C in TE buffer together with the linear template p(A₂₀-m). Gel electrophoresis are run in TAE buffer using 1 % agarose gel and 6 V/cm for 90 min with SYBR gold staining. 50 bp Ladder bands correspond to 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 base pairs, and the 1kbp ladder bands correspond to 250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000 and 10000 base pairs from top to bottom.



Supplementary Fig. 2: Encapsulation of the catalyst in the PC's interior. To synthesize all-DNA protocells (PCs), we have combined two polymers, namely $p(A_{20-m})$ and $p(T_{20-n})$ in a stoichiometric ratio of 4/1, in a TE buffered (pH=8) solution. The ssDNA polymer mixture was heated at 95 °C for 5 min for the homogenization and to decrease the molecular weight. Then the solution was charged with 50 mM $MgAc_2$ followed by a heating ramp (20 °C → 95 °C; 3 °C/ min). A stock solution of Biot-m* was added to the PC-medium and left to stand at room temperature for 1 h. The suspension containing biotin-loaded PCs (Biot-PCs) were centrifuged and redispersed before adding a solution of Sav. The buffer was exchanged to phosphate buffer at pH 6 before the addition of the Sav. The Sav<PC solution was incubated for 2 h and centrifuged and redispersed before addition of a Biot-Ru stock solution (5 mM in DMSO). Finally, the catalyst-loaded PCs ((Biot-Ru<Sav<mutants)<PCs) were washed two times and used for further experiments.

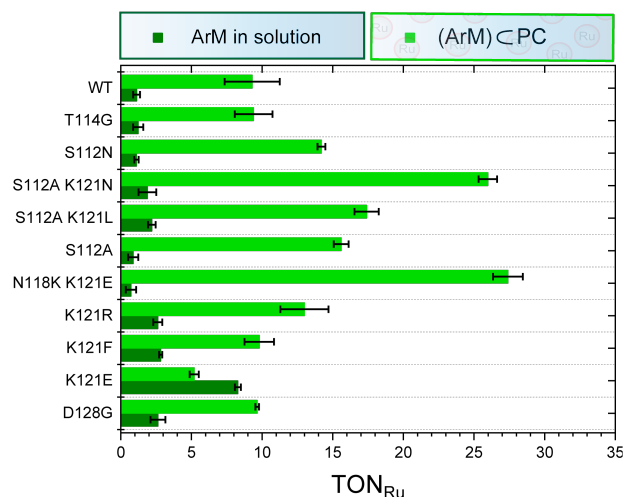
a ArM-catalysed RCM (Subs-I and Subs-II)



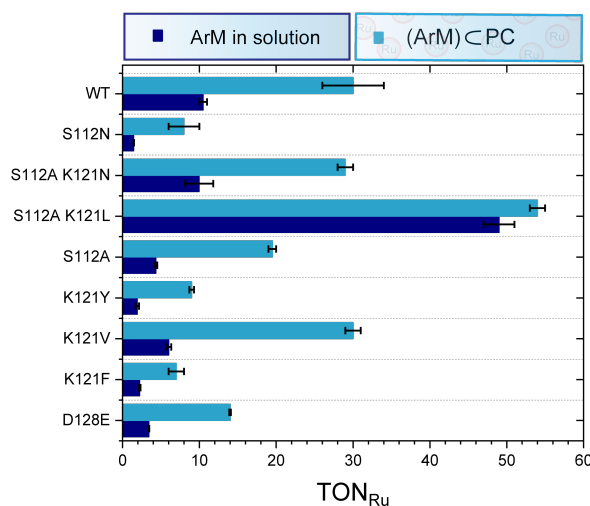
c Effect of MgCl₂ on RCM (Subs-I) for Biot-Ru < Sav^{N118K K121E}

	0.2 M MgCl ₂	1 M MgCl ₂
TON _{Ru}	1.78 ± 0.3	1.88 ± 0.53

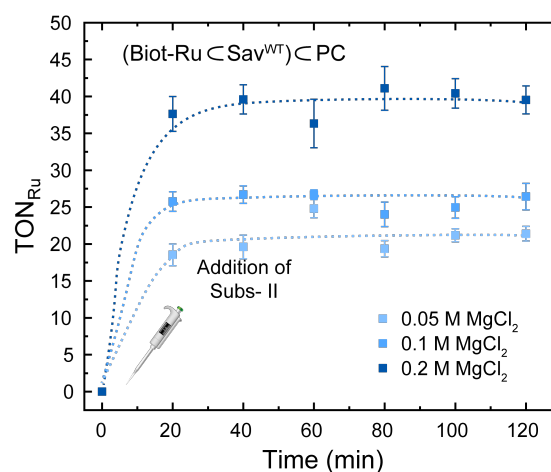
b Screening of Sav^{mutant} on RCM (Subs-I) inside and outside the PCs



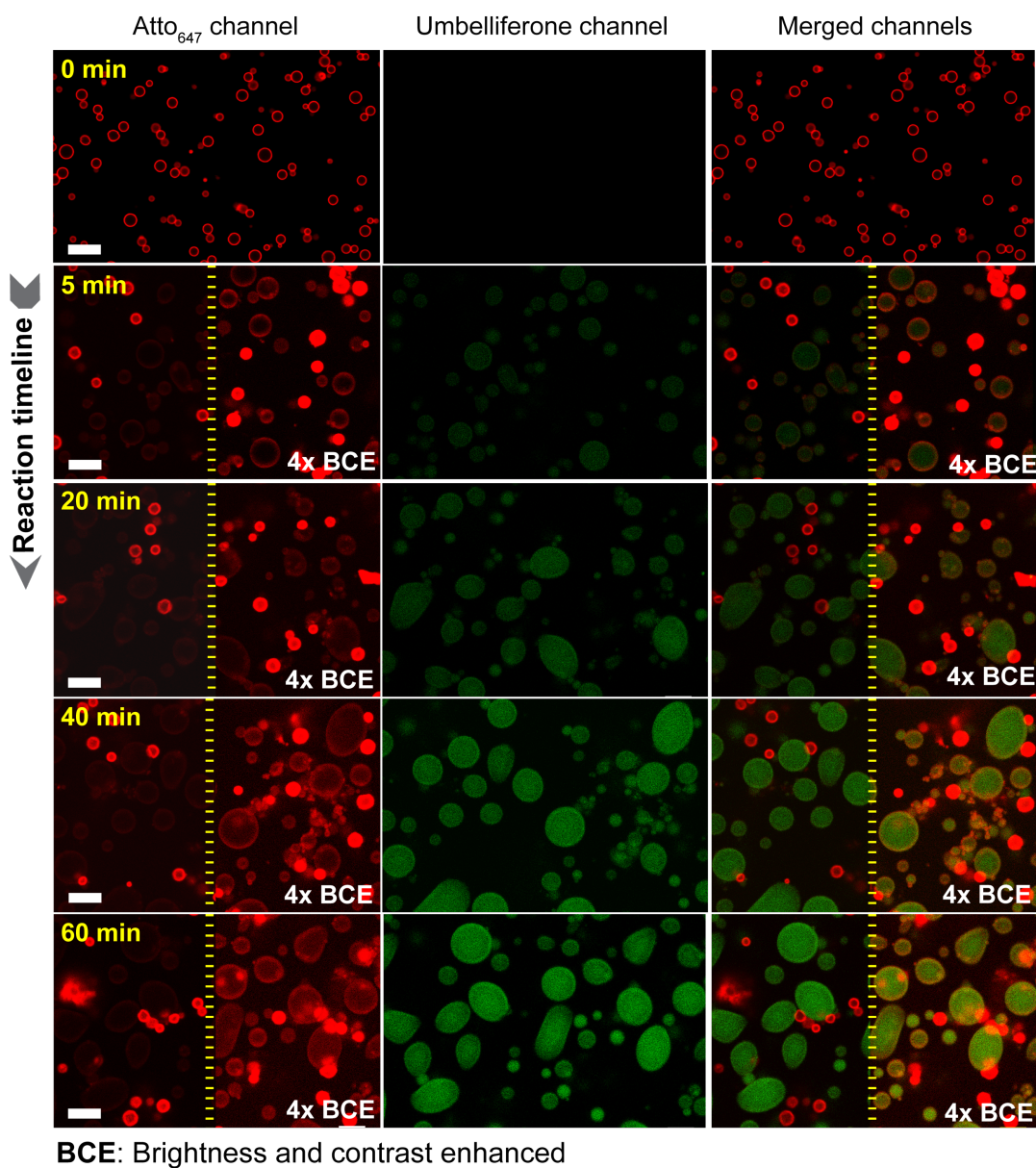
d Screening of Sav^{mutant} on RCM (Subs-II) inside and outside the PCs



e Effect of MgCl₂ on RCM (Subs-II) inside the PCs

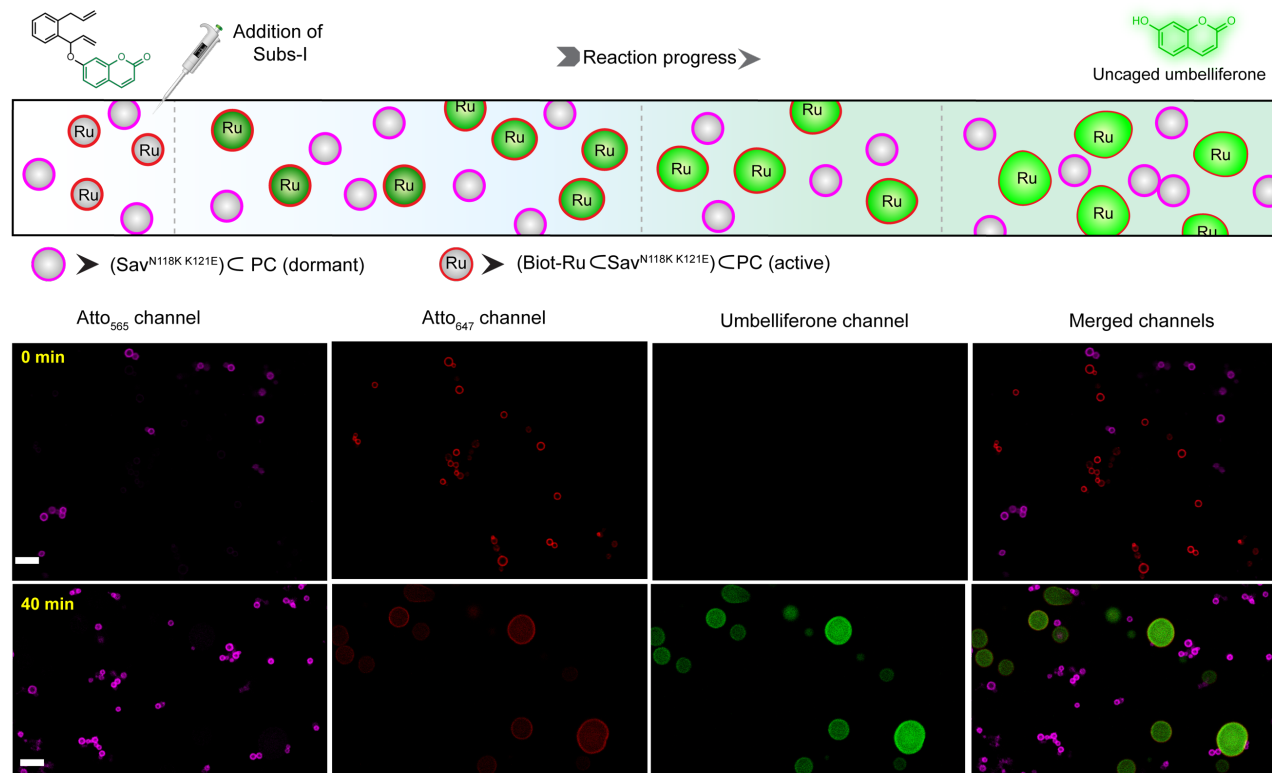


Supplementary Fig. 3: Screening and comparison of the Sav^{mutants} on RCM for Subs-I and Subs-II inside the PCs and in solution and effect of MgCl₂. a) Schematic representation of ArM-catalysed RCM for Subs-I and Subs-II. b) Genetic optimization and comparison of ArM for the uncaging of umbelliferone from Subs-I in the core of the ArM<PCs and in solution. c) Effect of very high MgCl₂ concentration on TON_{Ru} for the RCM of Subs-I using Biot-Ru<Sav^{N118K K121E}. Higher Mg²⁺ concentrations do not increase TON when going beyond 0.2 M, hence any Mg²⁺ enrichment as counterions in the PCs is not decisive for increased TON in the PC. See main MS (Figure 3h) for the effect crowding. d) Genetic optimization and comparison of ArM for RCM of the dimethoxynaphthalene precursor (Subs-II) in the core of the ArM<PCs and in solution. e) Effect of MgCl₂ concentration on TON_{Ru} for the RCM of Subs-II inside ArM<PCs. A higher MgCl₂ concentration (up to 0.2 M) has a beneficial impact on TON_{Ru}. The mutant screening inside the PCs and in solution were performed using 1% Biot-Ru loading (1 μM) and the TONs were calculated with the Ru content obtained from the ICP-MS values (Supplementary Table S2). Conditions: PCs are prepared in TE buffer with 50 mM MgAc₂, and before assembling the ArM within the PC, the buffer medium is exchanged to PBS (pH 6) with 0.2 M MgCl₂, [Subs-I] = 100 μM.

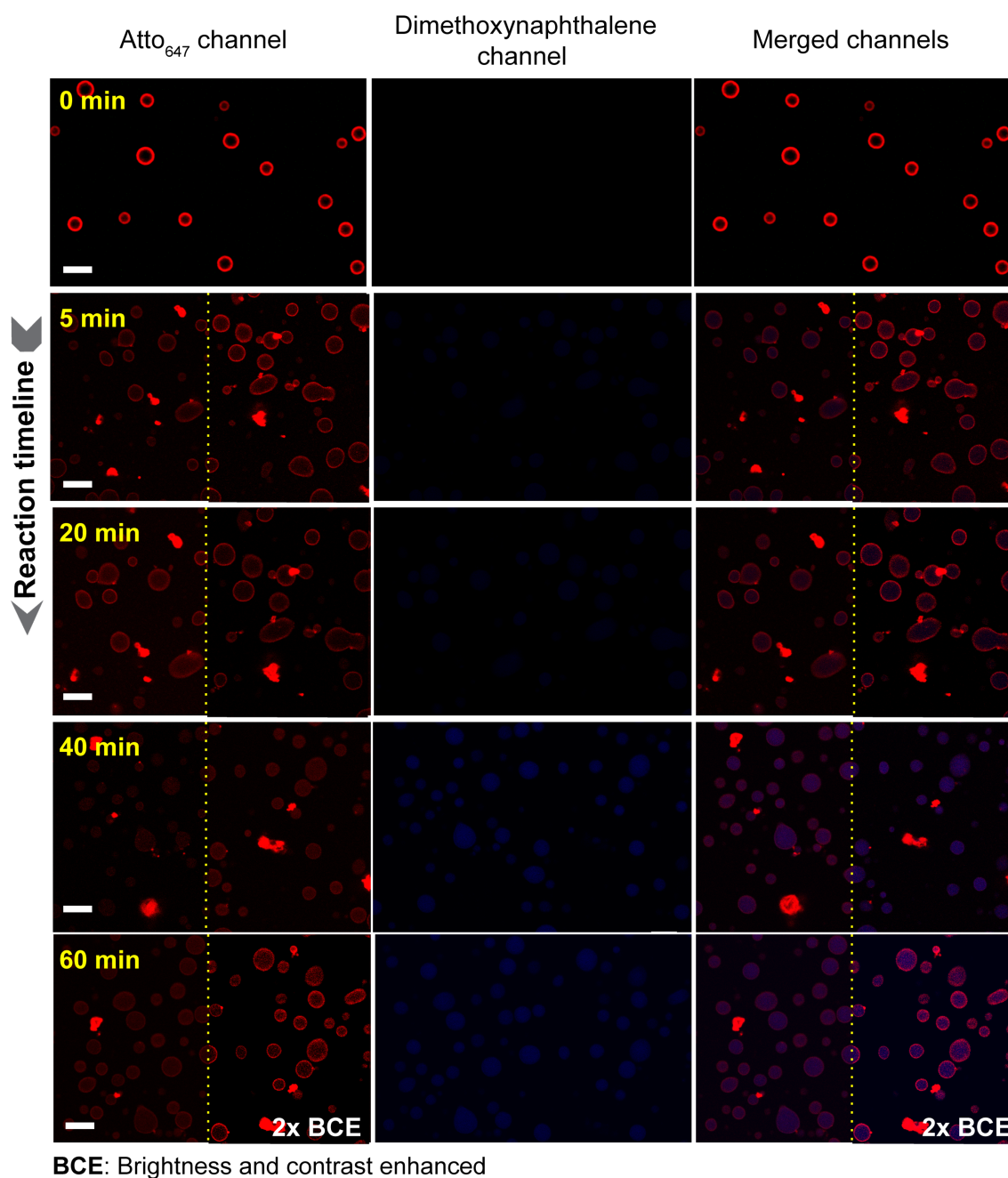


Supplementary Fig. 4: CLSM monitoring of ArM-catalysed RCM (Subs-I) inside PCs. The CLSM images of the RCM-induced *in-situ* umbelliferone uncaging inside the PCs (before the addition of Subs-I and at 5, 20, 40 and 60-min time intervals) in a mixed PC system (active-PC and dormant-PC). The uncaged-umbelliferone channel (green) reveals the formation and accumulation of the product inside the PC core and the shell. All the PCs were tagged with Atto₆₄₇-n* to visualize the shells. At the beginning, both active and dormant PCs are indistinguishable. Growth of active PCs is evident. The brightness and the contrast of parts of the images (for red and merged channels) are enhanced to visualize the shell of the protocells clearly. Scale bar = 5 μ m.

Monitoring RCM (Subs-I) in a multicoloured PC system

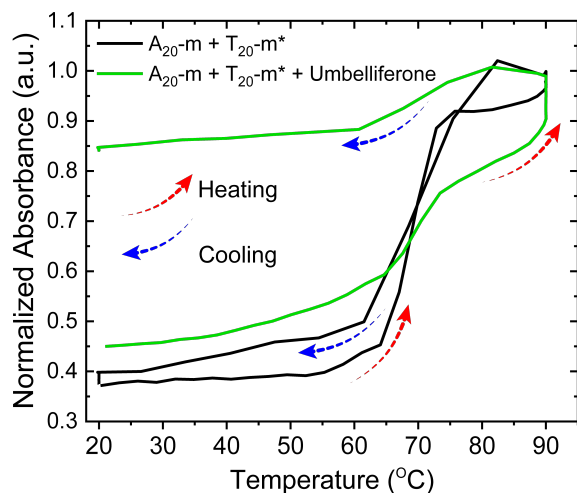


Supplementary Fig. 5: CLSM monitoring of ArM-catalysed RCM in a bicoloured active/dormant PC system. Catalyst-loaded active PCs are dyed with a red shell Atto₆₄₇-n* (red channel) and dormant PCs without catalyst are dyed with a magenta shell Atto₅₆₅-n* (magenta channel). Growth of active-PCs is evident from the red and merged channel. The umbelliferone product selectively accumulates in the active PCs as highlighted by the emerging green fluorescence. The dormant-PCs remain unchanged in colour and size. Scale bar = 5 μm .

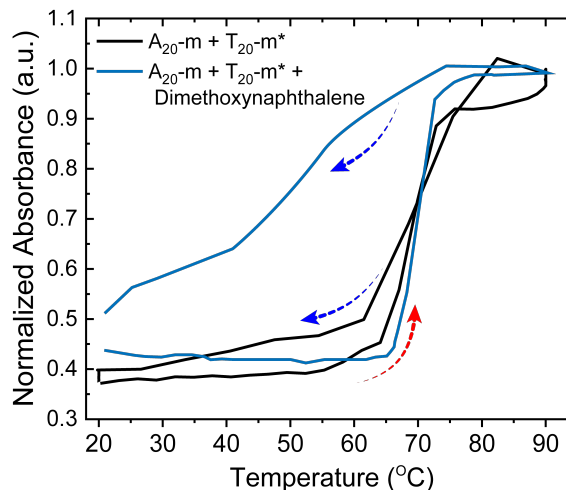


Supplementary Fig. 6: CLSM monitoring of ArM-catalysed RCM (Subs-II) inside PCs. The CLSM images of the RCM-induced dimethoxynaphthalene formation inside the PCs (before the addition of Subs-II and at 5, 20, 40 and 60-min time intervals). The dimethoxynaphthalene channel (blue) reveals the formation and accumulation of the product inside the PCs cores. All PCs were tagged with Atto₆₄₇-n* to visualize the shells. The brightness and the contrast of parts of the images (for red and merged channels) are enhanced to visualize the shell of the protocells clearly. Scale bar = 5 μ m.

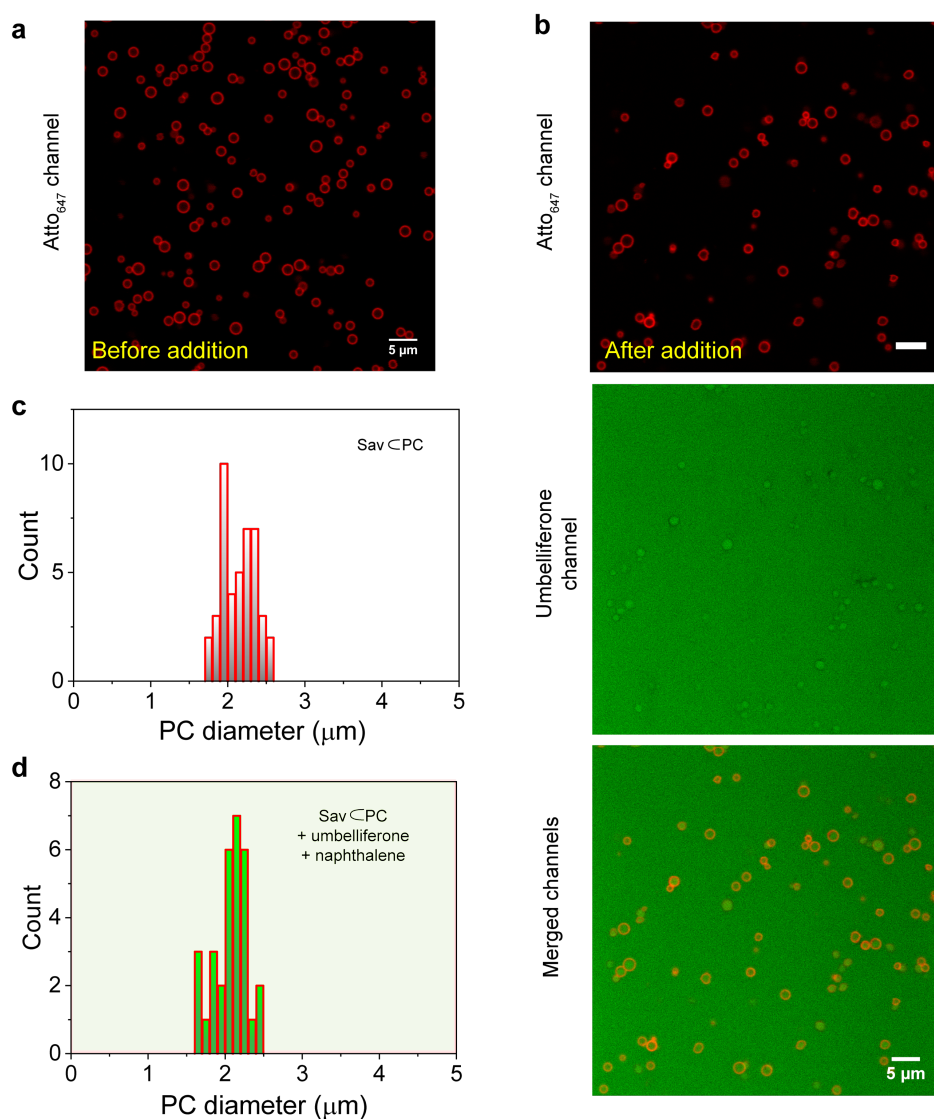
a Effect of umbelliferone on duplex melting



b Effect of dimethoxynaphthalene on duplex melting

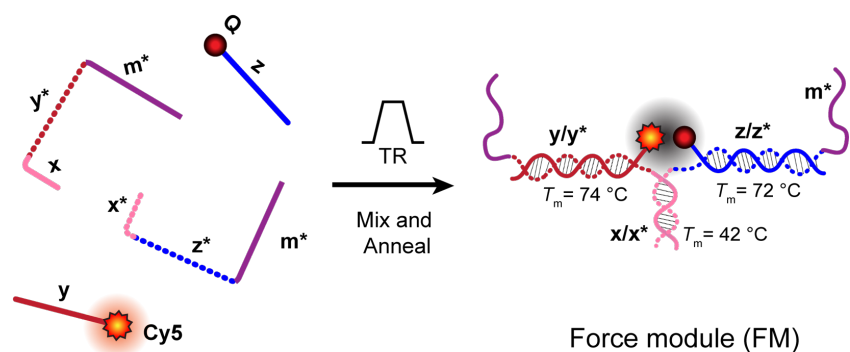


Supplementary Fig. 7: Temperature-induced duplex melting and re-hybridization in the presence of the intercalators. a) The normalized absorbances at 260 nm of a solution containing duplex (A_{20-m}/T_{20-m^*}) (2 μM) were plotted against the temperature (black trace). Heating ramps were performed on 60 μL of solution introduced in a 45 μL Quartz cuvette (Hellma Analytics) and heated at 1 $^{\circ}\text{C}/\text{min}$, the absorbance at 260 nm was recorded every 60 seconds. The duplexes were annealed (20 $^{\circ}\text{C} \rightarrow 95$ $^{\circ}\text{C}$; heating: 3 $^{\circ}\text{C}/\text{min}$ and 95 $^{\circ}\text{C} \rightarrow 20$ $^{\circ}\text{C}$; cooling: 0.01 $^{\circ}\text{C}/\text{min}$) before the experiment to assure complete hybridization. A stock solution of umbelliferone (5 mM in DMSO) was added to attain the final concentration of 100 μM and the same heating ramp was repeated (green trace). b) The effect of dimethoxynaphthalene on the duplex melting behaviour. The red and blue arrows represent the subsequent heating and cooling curves, respectively.

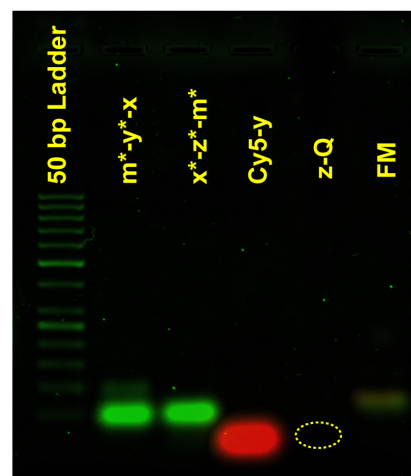


Supplementary Fig. 8: Effect of umbelliferone and naphthalene on the Sav-loaded protocells. A mixture of umbelliferone (500 μM) and naphthalene (500 μM) was added to a medium containing Sav^{N118K K121E}-PCs and incubated (60 min) at room temperature. The red channel represents the Atto₆₄₇-tagged shell of the PCs. a) CLSM image of Sav^{N118K K121E}-PCs before the addition of the mixture of umbelliferone and naphthalene. b) CLSM images of Sav^{N118K K121E}-PCs after the addition of umbelliferone and naphthalene (after 60 min). The umbelliferone and naphthalene (invisible in CLSM) accumulate at the shell and in the core of the protocells via intercalating into the duplexes. The green medium represents excess umbelliferone in the environment. No swelling of the PCs is observed, which proves that the production of the uncaged products inside the PCs triggers the morphological transformations. c) The size distribution of the PCs before the addition of the products. d) The size distribution of the PCs after the addition of the products. The scale bar = 5 μm.

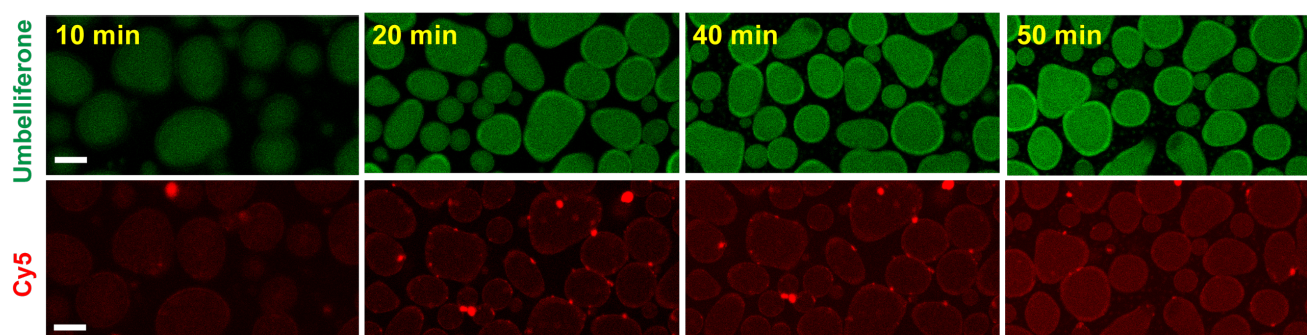
a Assembly of the mechanofluorescent reporter (FM)



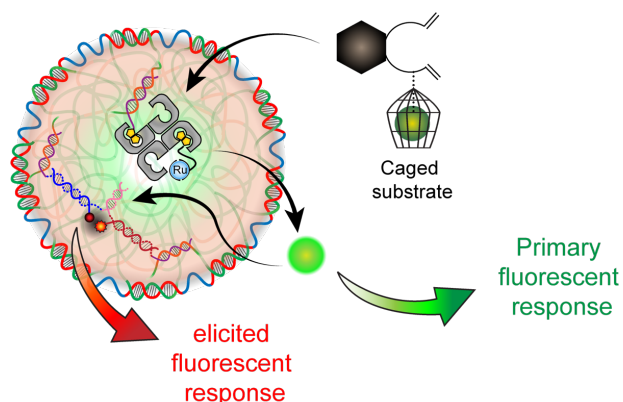
b GEP characterisation



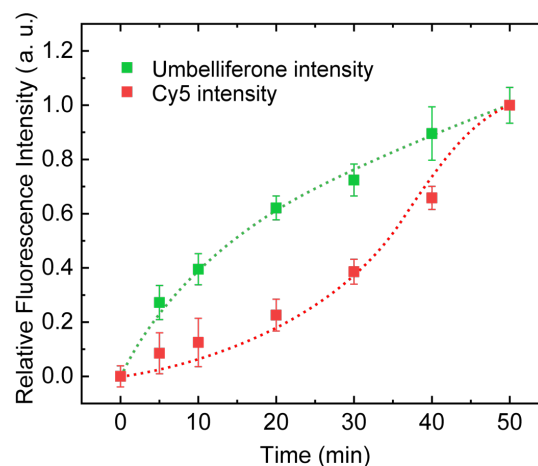
c RCM-induced self-reporting protocellular adaptation



d Downstream signal output



e Primary and elicited fluorescence



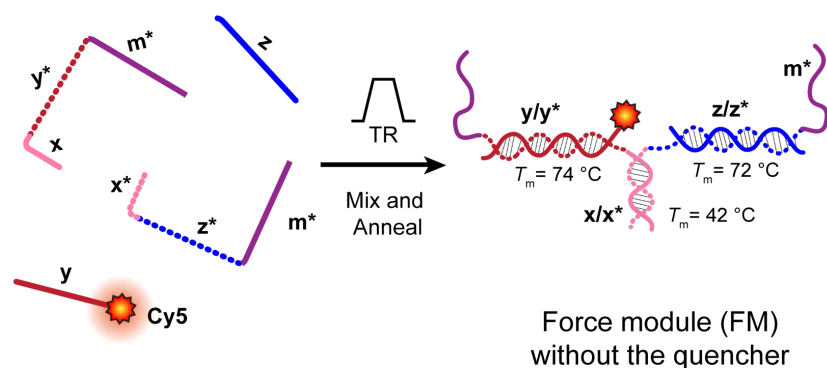
Supplementary Fig. 9: Preparation and operational principle of RCM-triggered self-reporting protocellular adaptation.

a) Self-assembly of a typical mechanofluorescent force-sensing module (FM). The red-emitting fluorophore (Cy5) and the quencher (Iowa Black quencher, Q) are placed in close proximity, and FRET prevents the emission of red fluorescence.

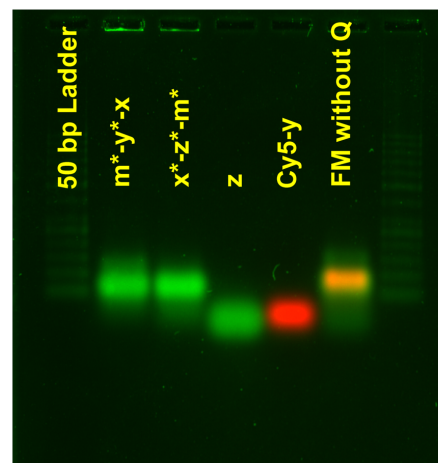
b) Bicolour overlapped GEP of various partial or complete mechanosensing modules. The gel is imaged first without SYBRgold staining, where only fluorescent constructs are visible (black-red colour scale) and after SYBR-gold staining (black and green scale). GEP of the module FM components. Lane 1: m^*-y^*-x , lane 2: $x^*-z^*-m^*$, lane 3: Cy5-y, lane 4: z-IowaRQ (invisible because of the quencher), lane 5: FM (full module poorly visible because of the quencher). The GEP was run in 2 wt% agarose, TAE buffer for 90 min at 90 V. The 50 bp ladder bands correspond to 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900

and 1000 bp. c) Time-dependent CLSM imaging of RCM-induced umbelliferone uncaging catalysed by (Biot-RuCSav^{N118K K121E})₂PCs partly functionalized with FM. The green channel reports on the uncaging of umbelliferone inside the PC matrix, and the red channel reveals the elicited fluorescent output from the dissociated FM modules in which FRET is substantially reduced. d) Schematic representation of the FM-loaded active PCs in which 70% of the barcode domain (m) inside the PCs are used for catalyst loading and the rest of the barcodes are used to introduce the FM. The RCM-induced uncaging of umbelliferone triggers the swelling of the PCs by destabilizing the duplexes present at the matrix and the shell of the PCs. The uncaged umbelliferone additionally destabilizes the reporter duplex of the FM (shown in pink, $T_m = 42^\circ\text{C}$), leading to a separation of the red-emitting fluorophore and the quencher thus decreasing FRET and giving rise to the second fluorescence (red, Cy5) output. e) Normalized (or relative) time-dependent fluorescence intensity of the RCM product (green trace, umbelliferone) and normalized Cy5 fluorescent intensity from the dissociated FM (red trace) inside the protocells. The scale bar = 5 μm

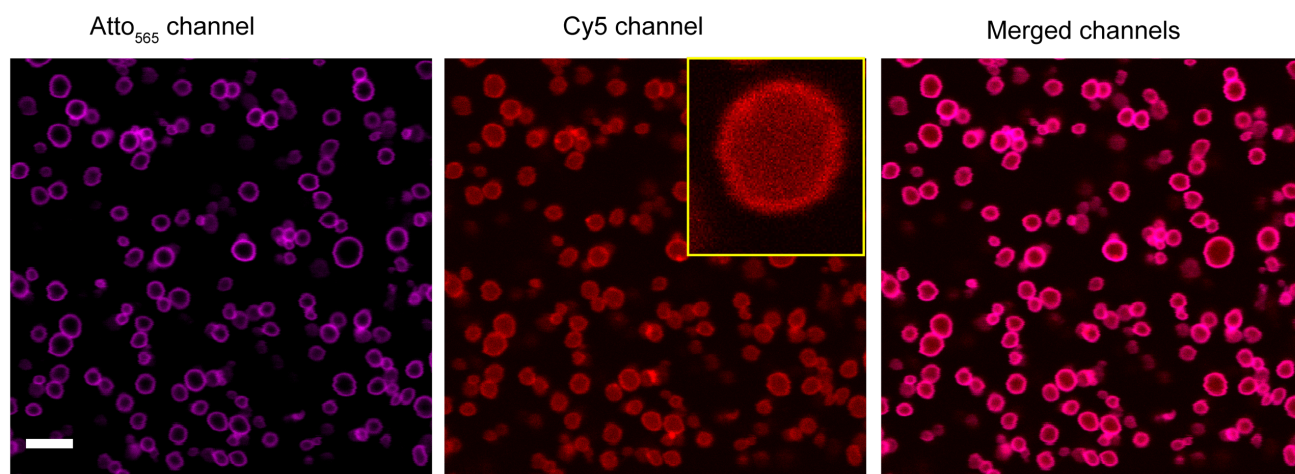
a Assembly of the FM without the quencher



b GEP characterisation

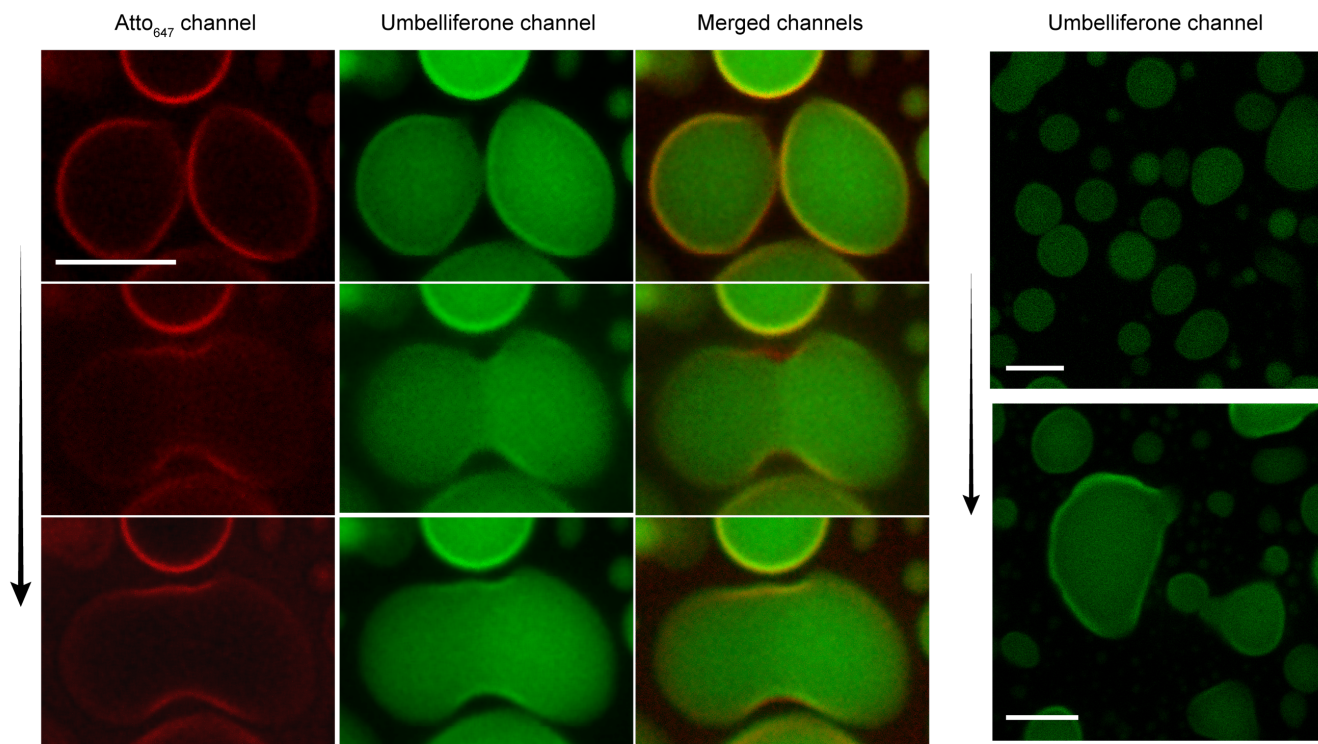


c Distribution of the nonquenched FM inside the PC



Supplementary Fig. 10: The non-quenched force module can be integrated homogeneously in the PC core. a) Self-assembly of a fluorescent force-sensing module without quencher. b) Bicolour GEP of the fluorescent module and its components. The gel is imaged first without the SYBRgold staining, where only fluorescent constructs are visible (black-red colour scale) and after the SYBRgold staining (black-green scale), where all the non-fluorescent constructs are visible. The GEP was run in 2 wt% agarose, TAE buffer for 60 min at 90 V. The 50 bp ladder bands correspond to 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 bp. c) CLSM image of the non-quenched FM loaded PCs. The shells of the PCs are hybridized with Atto₅₆₅-n* (magenta channel) and 35-40% of the core barcodes (m) are hybridized with the fluorescent FM (red channel). The red fluorescence is homogeneous across the full interior. The shell experiences cross talk from the magenta dye (Atto₅₆₅-n*). The scale bar = 5 μm.

RCM-induced inter-particle fusion between growing PCs



Supplementary Fig. 11: Additional CLSM images of inter-particle fusion events during catalysis. Left: Individual channels for the selection shown in the main MS. Right: A larger section at lower magnification. The arrows indicate progress in time. The scale bar: 5 μm .

References

- 1 Sabatino, V., Rebelein, J. G. & Ward, T. R. "Close-to-release": spontaneous bioorthogonal uncaging resulting from ring-closing metathesis. *J. Am. Chem. Soc.* **141**, 17048-17052 (2019).
- 2 Huang, K.-S., Wang, E.-C. & Chen, H.-M. Syntheses of Substituted Naphthalenes and Naphthols. *J. Chin. Chem. Soc.* **51**, 585-605 (2004).
- 3 Studier, F. W. & Moffatt, B. A. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**, 113-130 (1986).
- 4 Studier, F. W. Protein production by auto-induction in high-density shaking cultures. *Protein Expr. Purif.* **41**, 207-234 (2005).