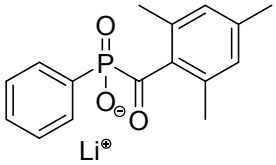
Electronic Supplementary Information

One-step photostructuring of multiple hydrogel arrays for compartmentalized enzyme reactions in microfluidic devices

1 Characterization of the photoinitiator LAP



NMR:

^{1}H-NMR (500 MHz, D_2O, δ) [ppm]: 7.68 (t, J = 7.5 Hz, 2H, Ph), 7.45 (t; J = 10.0 Hz, 1H, Ph), 7.36 (t; J = 7.5 Hz, 2H, Ph), 6.75 (s, 2H, Ph), 2.11 (s, 3H, Ph-CH3), 1.96 (s, 6H, Ph-CH3).

^{13}C-NMR (125 MHz, D_2O, δ) [ppm]: 231.5 (d, *CO*-Ph) 139.4 (s, *Ph*-CH_3), 137.8 (d, *Ph*-CO), 137.5 (s, *Ph*-CH_3), 133.3 (s, *Ph*-CH_3), 132.0 (s, Ph), 131.9 (d, Ph), 131.6 (d, Ph), 128.0 (d, Ph), 127.7 (s, Ph), 19.8 (s, Ph-*CH_3*)., 18.2 (s, Ph-*CH_3*).

^{31}P-NMR (200 MHz, D_2O, δ) [ppm]: 12.20 (s, Ph-*PO_2*-CO).

2 Preparation of the quantum dots[1]

Synthesis of AIS/ZnS QDs. Aqueous colloidal solutions of AIS quantum dots (QD) were prepared by a reaction between sodium sulfide and a mixture of silver(I) and indium(III) complexes with anions of thioglycolic acid (TGA). The starting molar ratio of silver to indium to sulfur was adjusted to 1:7:10.

In a typical synthesis, 1.0 mL aqueous 0.1 M $AgNO_3$ solution and 2 mL aqueous 1.0 M TGA solution were added to 96 mL deionized (DI) water under magnetic stirring and ambient conditions. The resulting turbid yellowish suspension becomes transparent after the addition of 0.45 mL aqueous 5.0 M NH_4OH solution and colorless – after the addition of 0.7 mL aqueous 1.0 M $InCl_3$ solution containing 0.2 M HNO_3. Then, 1.0 mL aqueous 1.0 M Na_2S solution is added at intense stirring and the resulting solution in heated on a water bath at 90–95 °C for 30 min.

The AIS QD were covered with a ZnS shell before the rotary evaporation via the decom-position of Zn^{II}–TGA complex. For this, 1.0 mL aqueous 1.0 M TGA solution and 1.0 mL aqueous 1.0 M $Zn(CH_3COO)_2$ solution (the latter containing 0.01 M HNO_3) were added at intense stirring and the solution was additionally heated for 30 min to let the shell form.

Before the rotary evaporation, 0.5 mL aqueous 1.0 M TGA solution was added again to prevent the agglomeration of colloidal QDs during the solvent extraction. The as-prepared AIS/ZnS QD colloids were concentrated by a factor of ~15 on a rotary evaporator at around 40 °C. The concentrated AIS/ZnS QD colloids are further denoted as “crude colloids”.

Size selection procedure. In a typical size-selection procedure, to 10 mL of crude colloid 2.5 mL of 2-propanol were added to initiate aggregation of the QD with subsequent centrifugation at 4500 rpm for 5 min. The precipitate was separated and designated further as fraction #1. Then, a fresh smaller portion of 2-propanol (0.5 mL) was added resulting again in the formation of turbid solution which also was centrifuged at 4500 rpm for 5 min and the precipitate was collected. This procedure was repeated 6 times with 0.5 mL of 2-propanol to produce precipitates that were designated as fractions #2–7. The following two fractions (#8 and #9) of the QD precipitate were obtained by a similar procedure using 1.0 mL 2-propanol and finally the two last fractions, #10 and #11 were selected by adding 2 mL of 2-propanol. The collected precipitates #1–11 were dissolved in 1 mL of DI water and stored in the dark at room temperature. For absorption and photoluminescence measurements the AIS/ZnS QD solutions were diluted by a factor of 100–500 (depending on the fraction number) by DI water.

Sizes. As recently reported by Eychmüller et al., the size varies in a range from 3.5-4 nm to less than 2 nm as the fraction number is increased from 1 to 11.[1] Therefore, for evaluations, the QD sizes in fractions 4, 7, 10 can be adopted as being 3-3.5 nm (“orange” QDs, fraction 21.2.4), 2.5-3.0 nm (“yellow” QDs, fraction 21.2.7), and 2 nm and smaller (“green” QDs, fraction 21.2.10), respectively.

Optical properties. Below are the absorption and photoluminescence spectra of samples of “orange”, “yellow” and “green” QD. For photoluminescence measurements the solutions were diluted to obtain the same optical density on the excitation wavelength (420 nm). Absorption and photoluminescence spectra were recorded using a UV-Vis spectrophotometer Cary 60 and a fluorescence spectrometer Fluoromax 4, respectively, in standard 1.0 cm optical quartz cuvettes. Photoluminescence quantum yields are 41% (“orange”), 46% (“yellow”), and 34% (“green”).

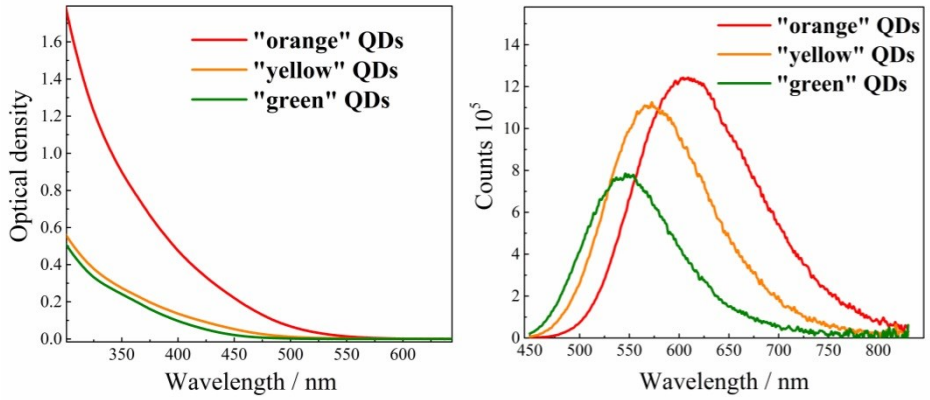


Figure S1: Absorption spectra (left) and photoluminescence spectra (right) of the quantum dots.

3 Fluorescence spectra of the hydrogel-entrapped quantum dots

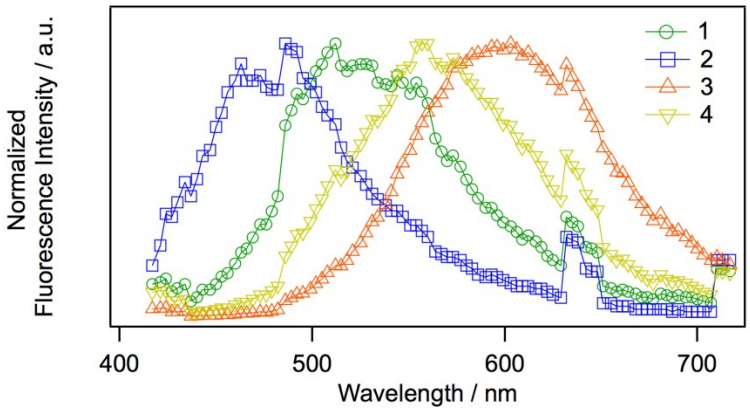


Fig. S2: Fluorescence spectra of the hydrogel-entrapped quantum dots. Numbering corresponds to Fig. 4 in the main text. 1: “green”, 2: non-stained, 3: “orange”, 4: “yellow”. Fluorescence of 2 is attributed to some autofluorescence of the hydrogel.

4 Photomasks

Figure S3: Photomasks for the Structuring of Hydrogel dots. Left: Mask for the formation of hydrogels stained with quantum dots. Right: Mask for the production of double-chamber microfluidic chips. Size of the masks: 7.6 x 2.6 cm². Diameter of the Dots: 350 µm

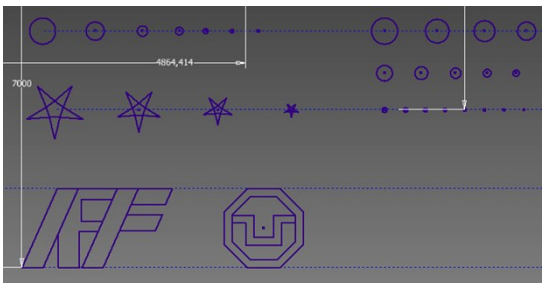


Figure S4: Template for the photomasks (drawn with Autodesk Inventor) for the Structuring of Hydrogel test patterns. Diameter of the Dots: 500, 350, 150, 100, 75, 50 µm (left); 500, 450, 400, 350, 300, 250, 200, 150, 125, 100, 90, 80, 70, 60, 50, 40, 30 µm (right). Diameter of the Stars: 1064, 798, 533, 266 µm. Height of the logos: 1500 µm

5 POM moulds

The manufacturing accuracy i.e. the depths of the chambers and the planarity of the whole mould was confirmed by confocal microscopy (µsurf explorer, Nano Focus, Germany) and height profiles of the moulds were obtained.

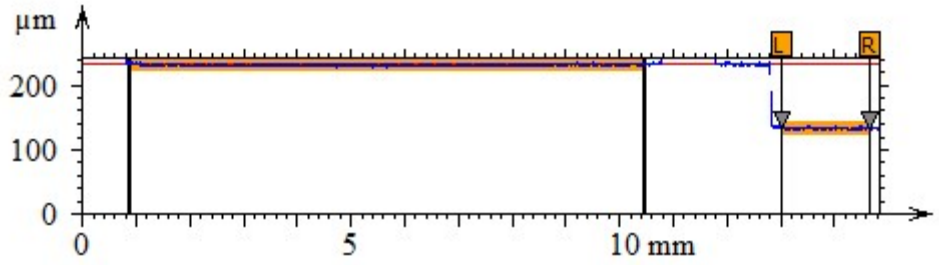


Figure S5: Confocal Microscopy image for a POM mould with an envisaged chamber depth of 100 µm. The average height of the step is 98.5 µm.

Table S1: Envisaged chamber depth and actual chamber depth (according to confocal microscopy) of the POM moulds used for the photo polymerization of hydrogel structures.

POM mould no.	Envisaged chamber depth [µm]	Actual chamber depth [µm]
1	20	18
2	50	45
3	75	75
4	100	99
5	130	130
6	160	160

6 Height of hydrogel dots in the swollen state

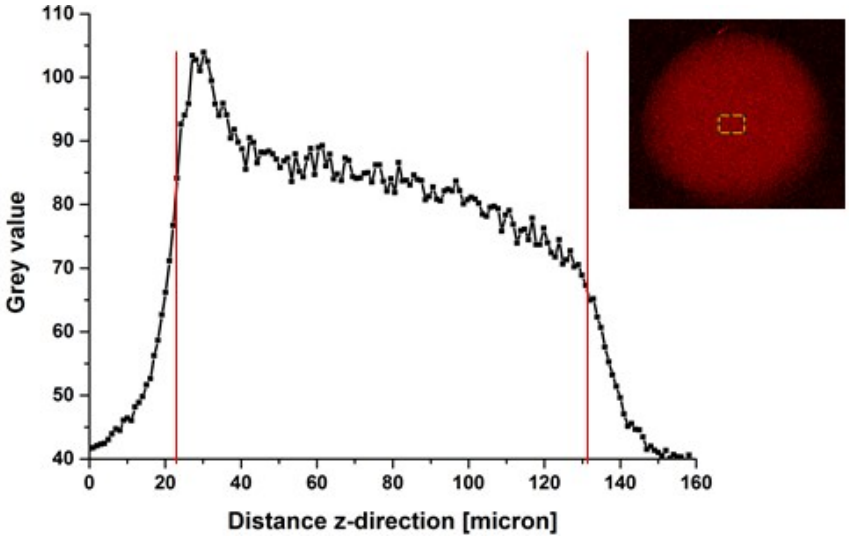


Fig. S6: Exemplary result of fluorescence microscopy of a fluorescently stained hydrogel dot in the swollen state obtained by photopolymerization with a photomask diameter of 350 µm and a POM mould with a 100 µm deep curvature. The threshold of the grey value for the height determination is derived from the steepest parts of the curve. Upon measuring six individual hydrogel dots an average dot height of 104 ± 6 µm was determined.

7 Assembly of the microfluidic device

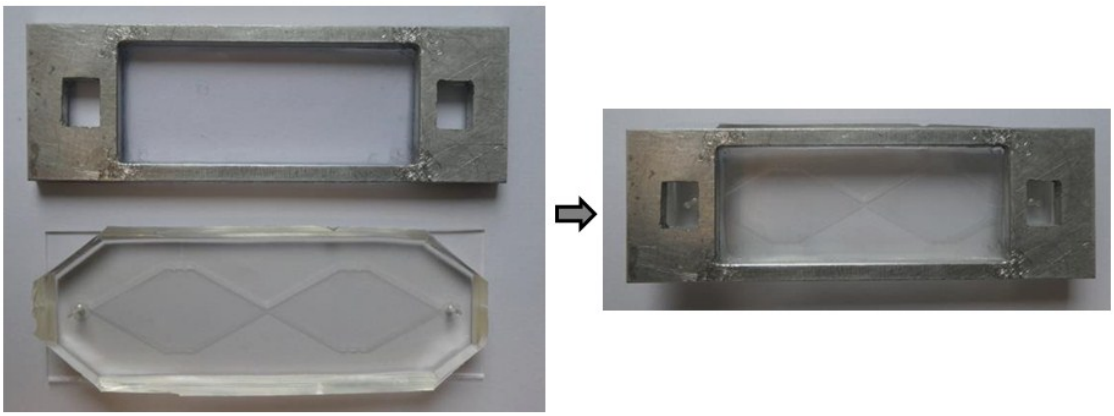


Figure S7: PDMS-on-glass microfluidic device and aluminum frame with acrylic glass window (left) and alignment of the frame on top of the PDMS-on-glass device (right).

8 Calculation of the relative enzymatic activity within the microfluidic device

The amount of GOx immobilized in the microfluidic devices was calculated from the enzyme concentration in the hydrogel precursor and the volume of the hydrogel dots. Within this calculation, a complete entrapment of the GOx within the hydrogel network was assumed and leakage was neglected. Based on the specific activity of GOx the maximum possible activity within the microfluidic device was calculated. Applying Lambert-Beers law, the concentration of $[ABTS^*]^+$ was calculated and

thereby the reaction conversion within the microfluidic device was determined. To compare the microfluidic devices with different enzyme amounts, the measured substrate conversion was related to the maximum possible substrate conversion with the enzyme amount immobilised in the microfluidic device. The parameters and formulas applied for the activity calculation are listed in Tab. S2.

Table S2: Parameters and formulas applied for the calculation of the concentration-dependent relative GOx activity in the microfluidic device.

1 Geometry of the microfluidic device			
Amount of hydrogel dots	n_{dot}	195	
Radius of hydrogel dots	r_{dot}	0,175 mm	Defined by photomask (diameter: 350 µm)
Height of hydrogel dots	h_{dot}	0,10 mm	Defined by POM mould (depth: 100 µm)
Volume of n hydrogel dots	V_{dots}	1,875 µL	$V = \pi r^2 h$
2 Enzymes in the microfluidic chip			
Specific enzyme activity	A_{spez} (GOx) A_{spez} (HRP)	14,3 U/mg 76,2 U/mg	Determined in ABTS assay
Enzyme concentration in hydrogel precursor	c (GOx) c (HRP)	5 up to 45 U/mL 10 up to 90 U/mL	Adjusted in device fabrication
Maximum enzyme activity in microfluidic device	A_{max} (GOx) A_{max} (HRP)	U	$A_{max} = c\, V_{\mathrm{dots}}$
3 UV-Vis measurement			
Extinction coefficient of $[ABTS^*]^+$	ε_{ABTS}	24,1 L/mmol cm	Determined by calibration[2]
Substrate concentration (glucose and ABTS)	$c_{subs.}$	5 mmol/L	Applied in microfluidic experiments
Substrate flux (volume)	$Fv_{subs.}$	20 µL/min	Applied in microfluidic experiments
Substrate flux (substance quantity)	$Fn_{subs.}$	0,0001 mmol/min	$Fn_{subs.} = Fv_{subs.}\, c_{subs.}$
Pathlength of cuvette	d	0,15 cm	Applied in microfluidic experiments
Absorbance	A	[a.U.]	Measured in microfluidic experiments
Concentration of $[ABTS^*]^+$	$c_{[ABTS^*]+}$	[mmol/mL]	$c = \frac{E}{\varepsilon * d}$
Product flux (volume)	$Fv_{Product}$	20 µL/min	Defined by substrate flux
Product flux (substance quantity)	$Fn_{Prod.}$	[mmol/min]	$Fn_{Prod.} = Fv_{Prod.}\, c_{[ABTS*]+}$
Enzyme activity	A_{exp} (GOx)	[µmol/min] = [U]	$A_{exp} = Fn_{Prod.}\, 1000$
Relative enzyme activity	A_{rel} (GOx)	[%]	$A_{rel} = 100\, \frac{A_{exp}}{A_{max}}$

9 Literature

1. A. Raevskaya, V. Lesnyak, D. Haubold, V. Dzhagan, O. Stroyuk, N. Gaponik, D. R. T. Zahn and A. Eychmüller, *J. Phys. Chem. C*, 2017, **121**, 9032-9042.
2. D. Simon, F. Obst, S. Haefner, T. Heroldt, M. Peiter, F. Simon, A. Richter, B. Voit and D. Appelhans, *Reaction Chemistry & Engineering*, 2019, **4**, 67-77.