Supporting Information

Microfluidic Device for the Determination of Water Chlorination Levels Combining a Fluorescent *meso*-Enamine BODIPY Probe and a Micro-hydrocyclone for Gas Bubble Separation

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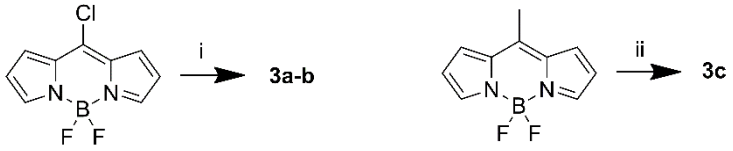
I Synthesis procedures.



1 **2**

Scheme S1. Precursors for the syntheses of the fluorescent molecular probes **3a-c** were prepared as described in the literature.[1,2]

8-chloro-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 2H), 7.41 (d, 2H, J = 4 Hz), 6.57 (s, 2H).

8-methyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 2H), 7.30 (s, 2H), 6.53 (s, 1H), 2.63 (s, 3H).

8-(*E*)-(2-diethylaminovinyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (3a). Triethylamine (0.2 mL, 1.4 mmol) was added in one portion to a solution of 8-chloro BODIPY (**1**) (0.080 g, 0.35 mmol) in CH_2Cl_2 (12 mL). The reaction mixture was vigorously stirred under air at room temperature for 20 h. Afterwards, the organic solution was washed with water, dried over $MgSO_4$ and vaporized to dryness. The crude product was purified by means of column chromatography (SiO_2, CH_2Cl_2/*n*-hexane 2:1 to CH_2Cl_2) to give BODIPY **3a** as a red, crystalline solid (0.026 g, 38% yield). ^{1}H NMR (400 MHz, DMSO-d^6) δ 8.32 (d, J = 12 Hz, 1H), 7.37 (bs, 2H), 7.23 (bs, 2H), 6.44 (d, J = 12 Hz, 1H), 6.37 (m, 2H), 3.71 (m, 4H), 1.29 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d^6) δ 157.7, 145.7, 131.0, 130.1, 120.1, 113.1, 97.4, 51.5, 43.6, 14.2, 12.5. MS (ESI+) m/z found: 312.1446 $[M+Na]^+$ $C_{15}H_{18}BF_2N_3Na$ requires 312.1459 $[M+Na]^+$.

8-(*E*)-(2-piperidinovinyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (3b). 1-Ethylpiperidine (0.25 mL, 1.8 mmol) was added in one portion to a solution of 8-chloro BODIPY (**1**) (0.100 g, 0.44 mmol) in CH_2Cl_2 (12 mL). The reaction mixture was vigorously stirred under air at room temperature for 20 h. Afterwards, the organic solution was washed with water, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by means of column chromatography (SiO_2, CH_2Cl_2) to give BODIPY **3b** as a red, crystalline solid (0.024 g, 18% yield). ^{1}H NMR (400 MHz, DMSO-d^6) δ 8.33 (d, J = 12 Hz, 1H), 7.34 (m, 2H), 7.23 (bs, 2H), 6.80 (m, 2H), 6.55 (d, J = 11.6 Hz, 1H), 3.65 (m, 2H), 3.54 (m, 2H), 1.27 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d^6) δ 158.05, 144.35, 130.56, 129.51, 121.10, 112.03, 97.22, 52.50, 24.45, 20.61. MS (ESI+) m/z found: 302.1612 $[M+H]^+$ $C_{16}H_{19}BF_2N_3$ requires 302.1640 $[M+H]^+$.

8-(*E*)-(2-cyclohexylvinyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (3c). 8-Methyl BODIPY (**2**) (0.090 g, 0.43 mmol) was dissolved in dry toluene (20 mL) containing molecular sieves (4 Å). To the mixture cyclohexanecarboxaldehyde (0.1 mL, 0.87 mmol) was added, followed by glacial acetic acid (1.5 mL, 26 mmol) and piperidine (1.3 mL, 13 mmol). After 2 h of stirring at room temperature, thin layer chromatography showed completion. The suspension was concentrated and filtered a through short SiO_2 plug (CH_2Cl_2). The collected orange fraction was evaporated to dryness and purified by means of column chromatography (SiO_2, AcOEt/cyclohexane 1:5) to give **3b** as a dark-red solid (0.080 g, 61% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.85 (s, 2H), 7.25 (d, J = 4 Hz, 2H), 6.71 (m, 1H), 6.65 (m, 1H), 6.52 (d, J = 3.2 Hz, 2H), 2.32 (m, 1H), 1.91 - 1.72 (m, 5H), 1.39 - 1.23 (m, 5H). ^{13}C NMR (101 MHz, DMSO-d^6) δ 154.67, 145.03, 142.99, 133.99, 128.62, 120.90, 117.79, 42.57, 32.36, 26.05, 25.84. HRMS (ESI-) m/z found: 299.1554 $[M-H]^-$ $C_{17}H_{18}BF_2N_2$ requires 299.1531 $[M-H]^-$.

[1] Precursor **1**: Y. Zhang, X. Shao, Y. Wang, F. Pan, R. Kang, F. Peng, Z. Huang, W. Zhang, W. Zhao, *Chem. Commun.*, 2015, **51**, 4245.

[2] Precursor **2**: M. Zhang, E. Hao, Y. Xu, S. Zhang, H. Zhu, Q. Wang, C. Yu, L. Jiao, *RSC Adv.*, 2012, **2**, 11215; D.-C. Wang, H.-P. Wang, S. Gao, T.-Y. Zhang, X.-J. Peng, *Acta Crystallogr. Sect. E Struct. Rep. Online*, 2007, **63**, o2238.

II Oxidation products.

Upon addition of 1 equiv. of sodium hypochlorite to **3a**, an immediate disappearance of the proton shifts at 6.31 and 8.18 ppm belonging to the double bond was observed by ^{1}H NMR. After addition of sodium hypochlorite, a broad peak at 4.28 ppm could be observed corresponding to a hydroxyl group. This signal disappeared from the spectra after further addition of reactant, indicating that only the intermediate product bears a hydroxyl moiety. The signals corresponding to the ethyl groups and the BODIPY core were shifted to two different values, validating the formation of two reaction products.

Precise structure determination was nonetheless difficult due to the presence of water (NaOCl aqueous solution) in the range of potential alkyl proton shifts from 2.5 to 3.2 ppm.

One of the structures seems to be asymmetric, as the BODIPY core proton shifts are different for each.

It shall be noted that transient (and thermally labile) BODIPY oxidation products have been reported before, e.g., for 3-styryl-BODIPY upon reaction with permanganate or hydrogen peroxide which also partly escaped rigorous identification by common analytical methods.[3,4]

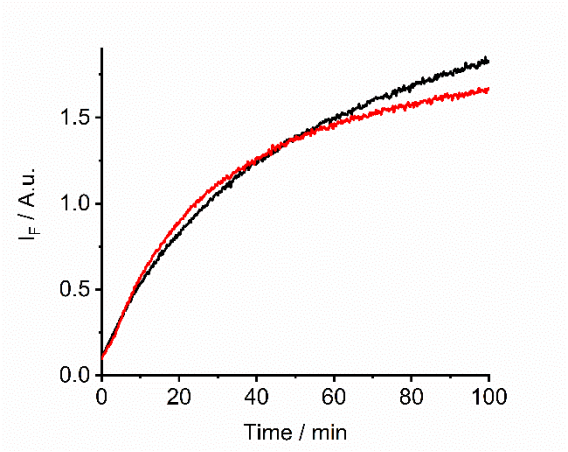
Table S1. ^{1}H NMR shifts (CD_3CN) of the molecular probe **3a** and of the products obtained after addition of 1 equivalent (P1) and an excess of sodium hypochlorite (P2) to the solution

	3a	**P1**	**P2**
	Shifts / ppm		
-N-CH_2-**CH_3**	1.29	1.32	1.08
-N-**CH_2**-CH_3	3.62	3.28	3.25
-**CH**=**CH**-	6.31, 8.18	-	-
-**OH**	-	4.28	-
BDP-**CH_x**-**CH_x**-N	-	*nd*	*nd*
BDP	6.38, 7.1, 7.38	6,41, 7.22, 7.35, 7.49, 7.82, 7.94	6.68, 7.77, 7.99

[3] G. Jung, A. Schmitt, M. Jacob, B. Hinkeldey, *Ann. N. Y. Acad. Sci.*, 2008, **1130**, 131.
[4] A. Schmitt, B. Hinkeldey, B. Hötzer, G. Jung, *J. Phys. Org. Chem.* 2009, **22**, 1233.

III Additional spectroscopic properties.

a)



b)

Parameters	3a	3b
τ_1 (min)	22 ± 2	13 ± 1
τ_2 (min)	138 ± 43	50 ± 3

Fig. S1 a) Time *vs* fluorescence intensity plot, recorded at 542 nm, of molecular probes **3a** (black line) and **3b** (red line) in methanol upon addition of 0.5 equiv. of NaOCl. ($c_{\mathbf{3a,b}}$ = 10 µM, λ_{exc} = 440 nm, room temperature). b) Kinetic parameters obtained from second order fitting.

(a) (b)

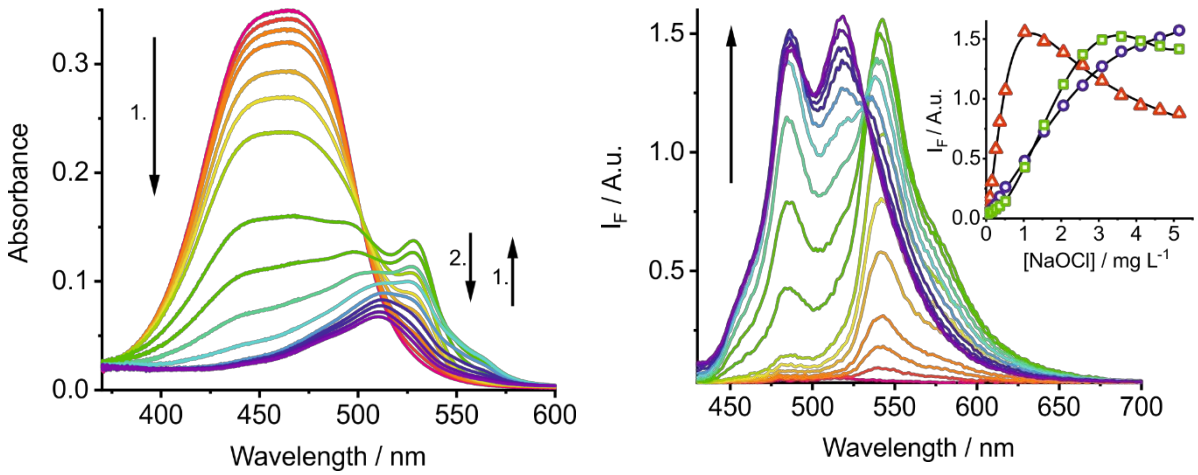


Fig. S2 (a) Absorption and (b) emission spectra of molecular probe **3b** in methanol upon increasing concentrations of NaOCl. Inset (b): Emission intensities at 486 (green squares), 518 (blue circles) and 542 nm (red triangles). ($c_{\mathbf{3b}}$ = 10 µM, λ_{exc} = 420 nm).

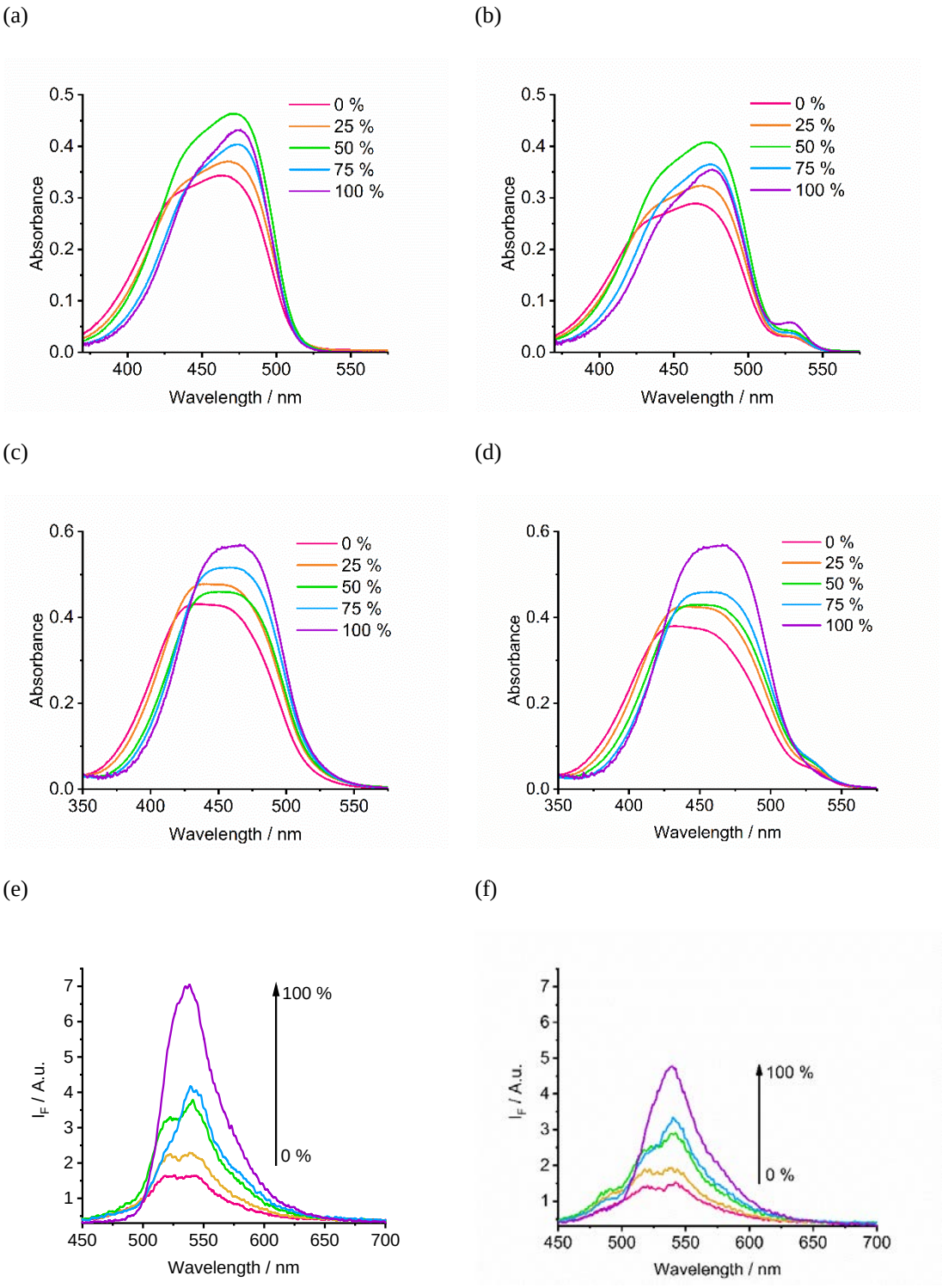


Fig. S3 Photophysical properties of the molecular probes in water-methanol mixtures: 0, 25, 50, 75 and 100 % (pure) methanol. Absorption spectra of molecular probe **3a** before (a) and after (b) addition of 0.25 mg L^{-1} of NaOCl. Absorption spectra of molecular probe **3b** before (c) and after (d) addition of 0.25 mg L^{-1} of NaOCl. Emission spectra of molecular probe **3a** (e) and **3b** (f) after addition of 0.25 mg L^{-1} of NaOCl. ($c_{\mathbf{3a\text{-}b}}$ = 10 µM, λ_{exc} = 440 nm).

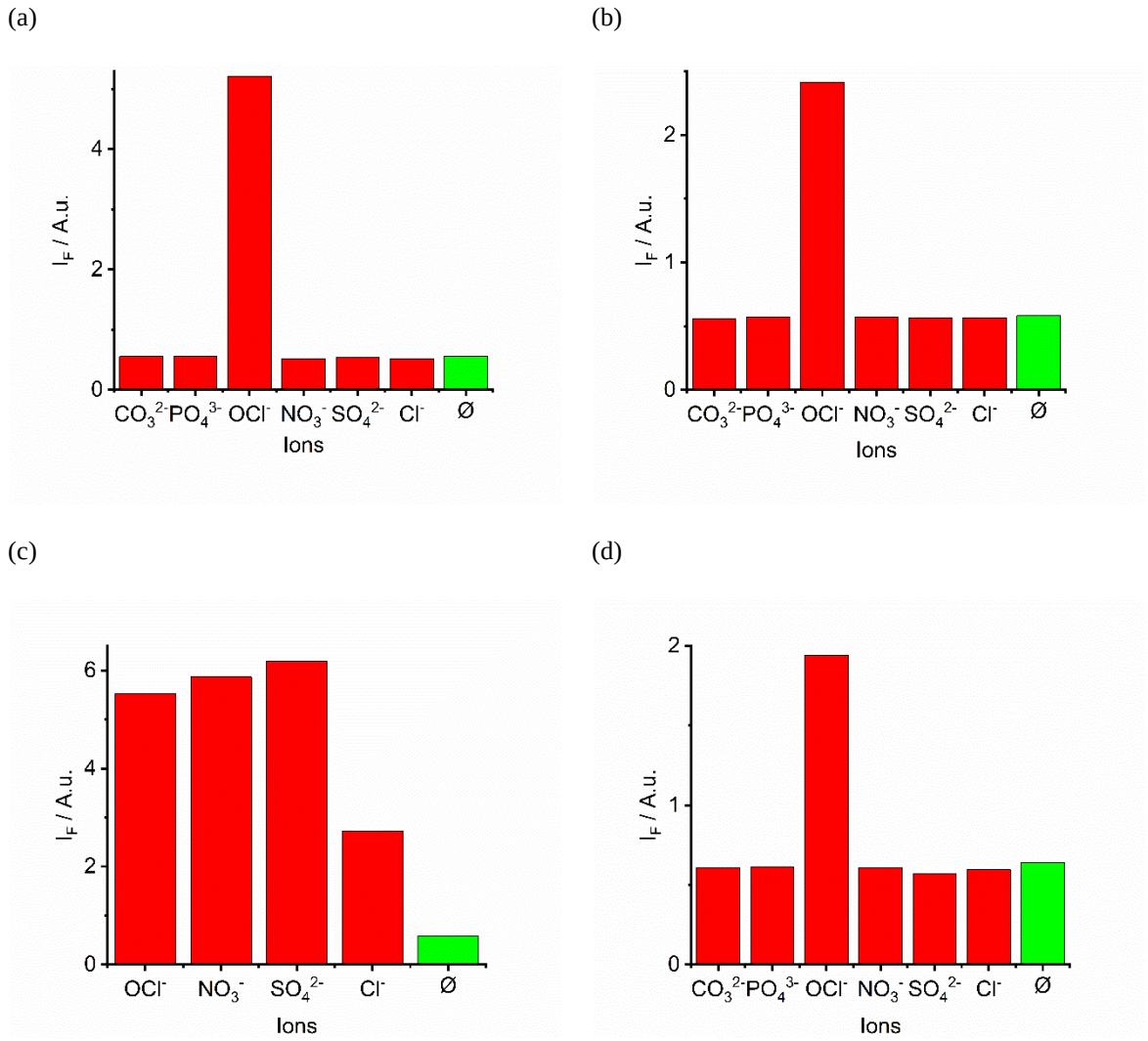


Fig. S4 Emission intensity recorded at 542 nm of molecular probe **3a** in (a) methanol and (b) water/methanol 1/1 mixture and molecular probe **3b** in (c) methanol and (d) water/methanol 1/1 mixture upon addition of 5 µM of Na_2CO_3, Na_3PO_4, KNO_3, Na_2SO_4, NaCl, NaOCl ($c_{\mathbf{3a}}$ = 10 µM, λ_{exc} = 440 nm).

IV Microfluidic systems.

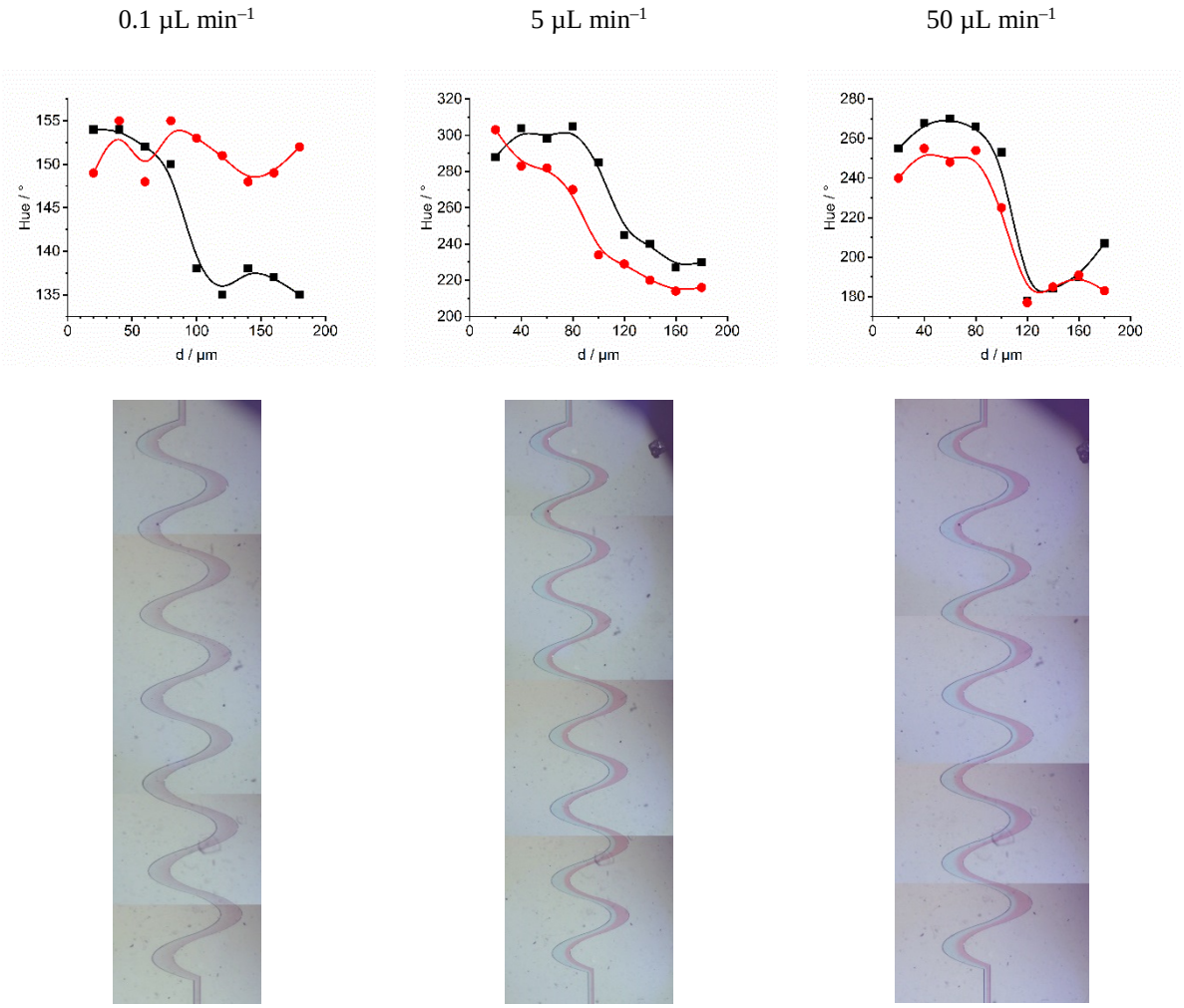
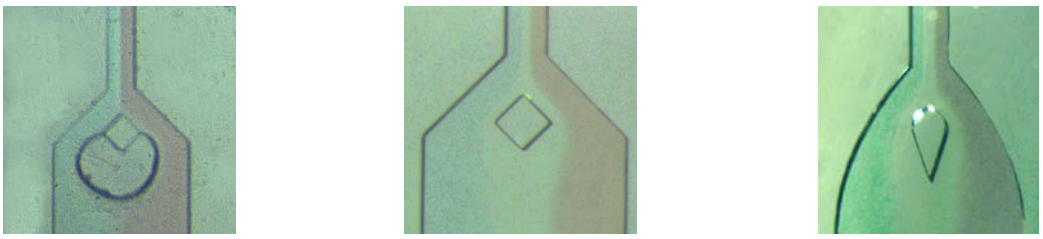


Fig. S5 Mixing efficiency of the sinusoidal passive mixer (ratio 1:1) from the 2D microfluidic system at various flow rates. Red and blue food dyes dissolved in water were used as mixing indicators and images were acquired under an Olympus SXZ16 microscope. The hue values of the inlet (black squares) and outlet (red circles) sections (9 points) were measured using CorelDraw software.

(a) (b) (c)



d / µm	*blue*	*yellow*	*red*
0	0,33	0,29	0,38
100	0,39	0,24	0,37
200	0,31	0,34	0,35
300	0,37	0,19	0,44
400	0,29	0,37	0,35
500	0,32	0,29	0,39
600	0,29	0,35	0,35
700	0,32	0,29	0,39

d / µm	*blue*	*yellow*	*red*
0	0,35	0,30	0,36
100	0,36	0,27	0,37
200	0,33	0,31	0,36
300	0,36	0,21	0,43
400	0,29	0,41	0,30
500	0,31	0,37	0,32
600	0,30	0,35	0,35
700	0,34	0,31	0,35

Fig. S6 (a) Example of gases accumulating in the low-pressure area at the rhombus' backside. Dispatching efficiency of the rhombus- (b) or wing- (c) shaped channels from the 2D microfluidic system at 5 µL min^{-1}. Red, yellow and blue food dyes dissolved in water were used as indicators and images were acquired under an Olympus SXZ16 microscope. The width of each flow indicates homogeneity at various distances from the top of the images were measured using CorelDraw software.

(a) (b) (c) (d) (e)

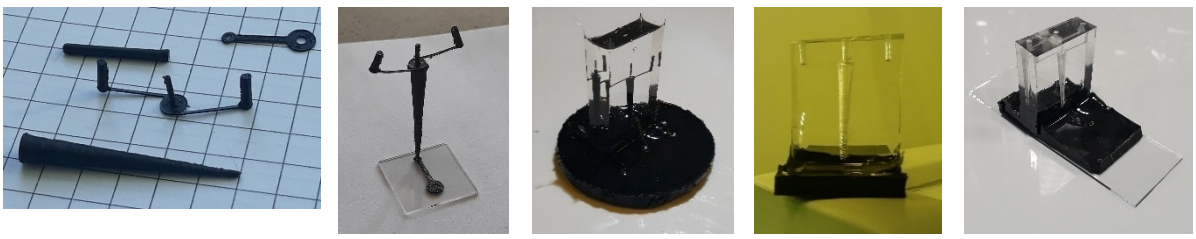


Fig. S7 Manufacturing steps of the 3D microfluidic chip: (a) 3D-printing of the ABS-scaffold, (b) mounting the 3D mold, (c) Curing PDMS around the scaffold, (d) Removing the ABS by bathing in acetone and (e) Sticking on a glass substrate and passivation of the PDMS wall.

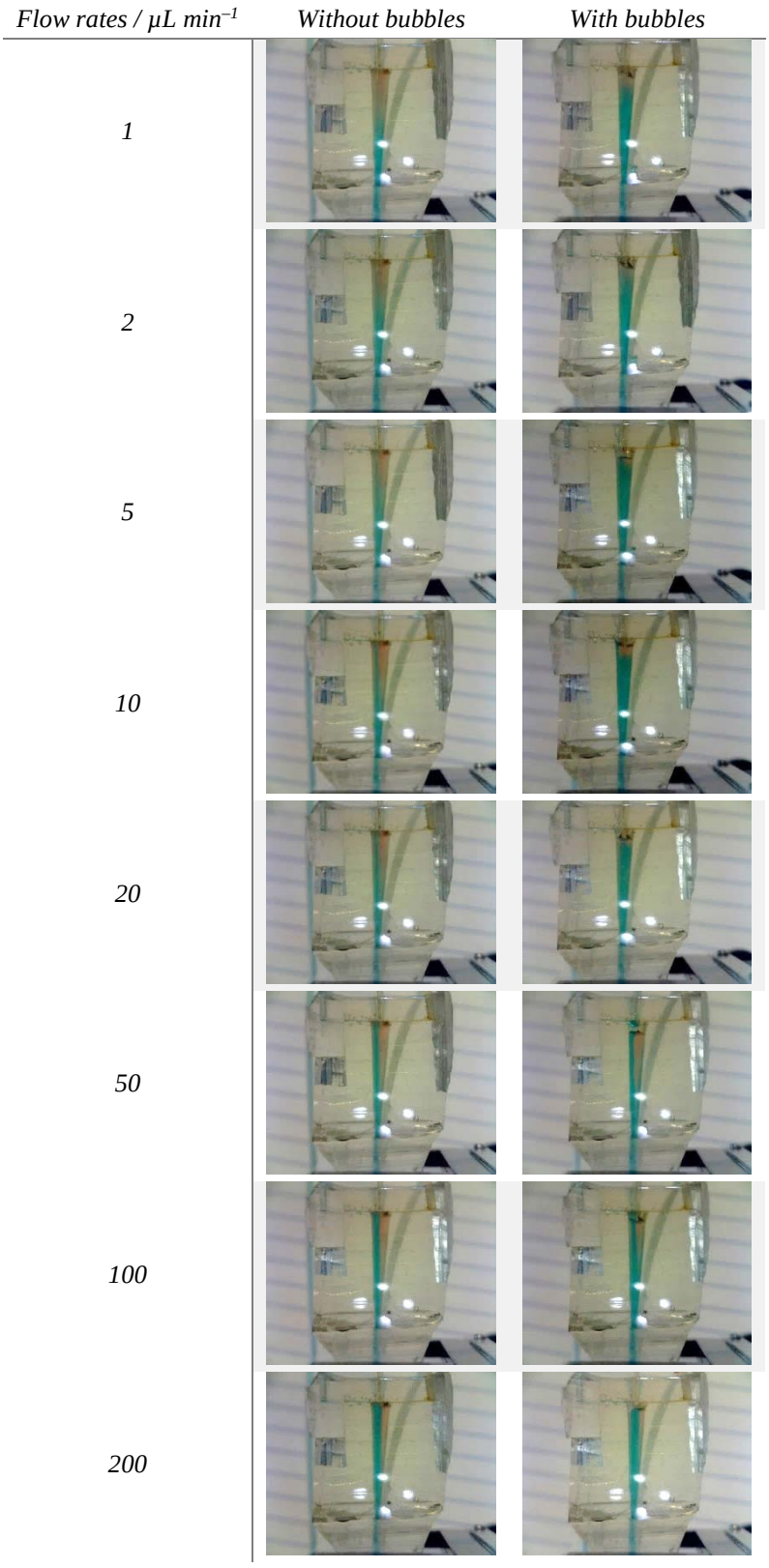


Fig. S8 Mixing efficiency and gas bubble elimination of the conical micro-hydrocyclone from the 3D microfluidic system at various flow rates. Red and blue food dyes dissolved in water were used as mixing indicators and images were acquired with a USB camera.

V Background and gas bubble elimination.

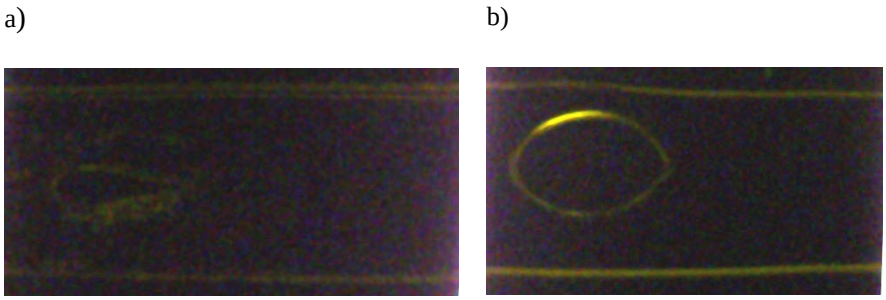


Fig. S9 Residual fluorescence inside the transparent 2D-microfluidic chip detection chamber after flowing probe **3a** in a) 50/50; v/v; water – methanol mixture and in b) water. Images were acquired under Olympus SXZ16 microscope using a GFP filters set ($c_{\mathbf{3a}}$ = 10 µM).

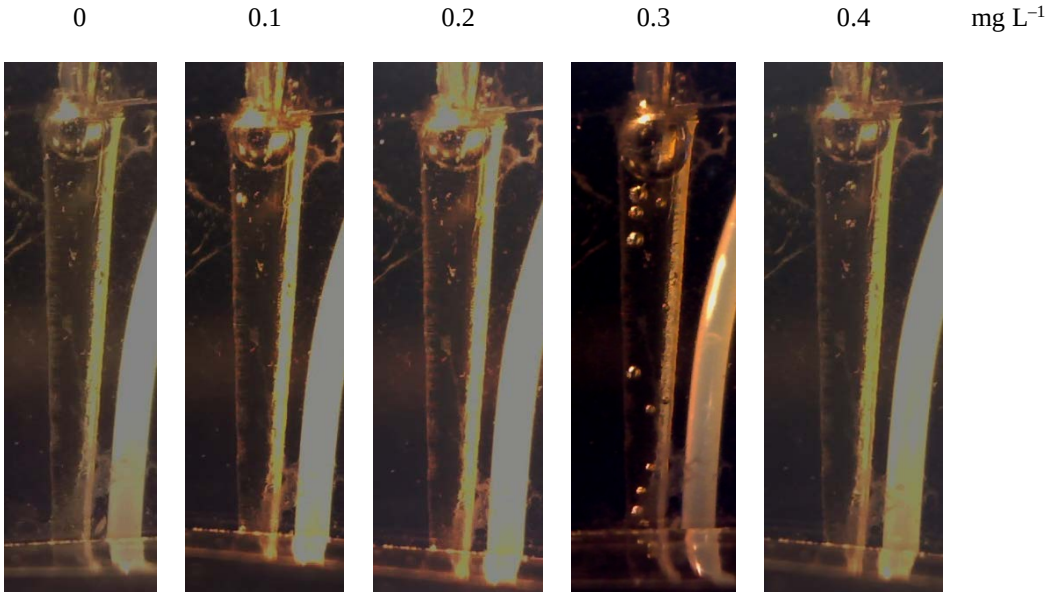


Fig. S10 Monitoring the accumulation and elimination of the gas bubbles in the micro-hydrocyclone upon increasing concentrations of chlorine (50/50 (v/v) water – methanol mixture, $c_{\mathbf{3a}}$ = 10 µM).

VI Calibration of the commercial chlorine test.

The standard procedure for free and total chlorine determination using Macherey-Nagel Nanocolor (Chlore / Ozone 2, Test 0-17 (06.17), Ref.: 985017) was followed.

A QC1277 Residual chlorine reference material (Sigma-Aldrich, lot LRAB2542, total residual chlorine: 1.73 ± 0.0751 mg L^{-1}, residual free chlorine: 1.66 ± 0.00617 mg L^{-1}) was used to calibrate the Nanocolor test together with a Analytik Jena Specord 210 Plus spectrophotometer.

The factors calculated for 1 cm cuvettes on this spectrometer with 1 nm slit were of 28.66 L mg^{-1} for the residual free chlorine and 30.10 L mg^{-1} for the total residual chlorine.

Table S2. Absorbance and calculated concentration of free and total chlorine using the Macherey-Nagel test 0-17 coupled to a laboratory spectrometer and 1 cm quartz cuvettes.

Samples	***Absorbance***		***Concentration / mg L^{-1}***	
	Free chlorine	**Total chlorine**	**Free chlorine**	**Total chlorine**
QC1277 reference diluted by 4	0.19	0.21	1.66	1.73
Pool 1 - sample 1	0.01	0.08	0.02	0.17
Pool 1 - sample 2	0.01	0.09	0.02	0.19
Pool 1 - sample 3	0.11	0.13	0.24	0.27
Pool 2 - sample 1	0.01	0.02	0.02	0.04
Pool 2 - sample 2	0.01	0.02	0.02	0.04
Pool 2 - sample 3	0.00	0.02	0.00	0.04