Electronic Supplementary Material (ESI)

for

Probing local pH-based co-precipitation processes in self-assembled silica-carbonate hybrid materials

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S1. Materials and Methods.

Barium chloride dihydrate (min. 99%) was purchased from Riedel-de Haën and used as received. The silica source was a commercial sodium silicate solution (so-called water glass) containing about 12.5 wt% SiO_2 and 13.8 wt% Na, as supplied by Sigma-Aldrich (reagent grade). All solvents employed for the spectroscopic measurements were of UV spectroscopic grade (Aldrich). Stock solutions (0.1 and 1 M) of hydrochloric acid, hypochlorite acid, potassium hydroxide and sodium hydroxide, required for pH adjustments, were obtained from Merck (p.a. grade). All solutions and dilutions were prepared with water of MilliQ quality, sourced from a Milli-Q Synthesis A10 system equipped with a Quantum EX Ultrapure Organex cartridge (Millipore). All air- and moisture-sensitive reactions were carried out under argon atmosphere in oven-dried glassware.

Thin layer chromatography (TLC) was performed on Merck Silica Gel 60 F254 TLC plates with a fluorescent indicator for 254 nm excitation. Compounds were visualised under UV light at 254 nm. Column chromatography was carried out with Merck Silica Gel 60 (0.040-0.063 mm) using the specified eluents. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV 400 and Bruker AVANCE III 500 MHz spectrometers at 27°C, using residual protonated solvent signals as internal standard (^{1}H: $\delta[CDCl_3]$ = 7.26 ppm and ^{13}C: $\delta[CDCl_3]$ = 77.16 ppm). Assignments are based on chemical shifts and/or DEPT spectra (where Ar is used as abbreviation to indicate aromatic moieties). High-resolution mass spectrometry (HR-MS) was performed with a Thermo Scientific Exactive Orbitrap in the positive ion mode using the Thermo Xcalibur operating and data acquisition software, or on a Waters LCT Premier XE instrument. Ultrahigh-performance liquid chromatography mass spectrometry (UPLC-MS) was performed with a Waters Acquity UPLC equipped with a Waters LCT Premier XE mass detector for high-resolution MS (ESI ionisation), and with Waters Alliance systems (consisting of a Waters Separations Module 2695, a Waters Diode Array Detector 996 and a Waters Mass Detector ZQ 2000) equipped with an Acquity BEH C18 (2.1x50 mm) column.

UV/Vis absorption spectra were recorded on an Analytik Jena Specord 210 Plus spectrophotometer. Steady-state fluorescence measurements were carried out on Horiba Jobin-Yvon FluoroMax-4P and Spectronics Instrument 8100 spectrofluorometers, using standard 10 mm path length quartz cuvettes or PMMA cells. Fluorescence lifetimes were determined with a unique customised laser impulse fluorometer with picosecond time resolution, as described elsewhere.[S1,S2] The fluorescence lifetime profiles were analysed using the Horiba Scientific software package DAS 6.

pH values were monitored with a digital pH meter (pH lab 827, Metrohm) equipped with a glass electrode (Biotrode). For comparison, pH measurements were also performed with a second digital pH meter (WTW pH 537), equipped with a different glass electrode (InLab 423, Mettler-Toledo). Calibration of the instruments was performed with standard aqueous buffer solutions of pH 4.00, 7.00

and 9.00 from Metrohm. The measurement uncertainties of the pH electrodes are estimated to ≤ 0.03 pH units.

Polarised optical microscopy (POM) studies were carried out on a Zeiss Imager.M2m microscope equipped with EC Epiplan-Neofluar 5x/10x/20x and LD Epiplan 50x objectives, a lambda plate, and a Zeiss AxioCam MRc 5 CCD camera for imaging. For scanning electron microscopy (SEM), specimens were mounted on aluminium stubs by means of double-sided adhesive tape and subsequently investigated on a Hitachi TM3000 tabletop microscope at acceleration voltages ranging from 3 to 10 kV (without previous sputtering). Confocal laser scanning microscopy (CLSM) was performed using a Zeiss LSM 510 Meta laser scanning microscope and a Zeiss Axiovert 200M inverted microscope, equipped with Plan-Neofluar 10x/0.3 and Plan-Neofluar 20x/0.5 objectives and PMT detectors for the transmission and fluorescence channels. All studies were carried out utilising an Argon laser with a wavelength of 488 nm at a laser power of 15% to excite dye **2**. For confocal observation, the pinhole was set to 1 au (area unit).

S2. Synthesis and Characterisation of BODIPY Dyes.

8-(4-hydroxyphenyl)-1,3,5,7-tetramethyl-2,6-diethyl-4,4-bis(3,6,9,12-tetraoxaheptadec-16-ynyl)-4-bora-3a,4a-diaza-s-indacene (dye **2**): The synthesis of **2** was carried out according to a procedure adopted from ref.[S3] To a solution of 2.10 g 2,5,8,11,14-pentaoxaheptadec-16-yne (8.0 mmol, 10.0 eq., synthesised as described elsewhere[S4]) in 20 mL anhydrous tetrahydrofuran (THF), 9.50 mL ethylmagnesium bromide (9.5 mmol, 1.0 M solution in THF, 12.0 eq.) was added. The reaction was heated at 60°C overnight. After cooling down to room temperature, a solution of 317.0 mg **1** (0.8 mmol, 1.0 eq., synthesised as described elsewhere[S5]) in 15 mL anhydrous THF was added. The resulting mixture was stirred at 60°C for additional 5 h to achieve complete consumption of the starting material. Subsequently, the solution was concentrated under reduced pressure to remove parts of the THF. Then 100 mL dichloromethane (CH_2Cl_2) was added and the organic phase was washed with 100 mL brine. After extraction of the aqueous phase for three times with CH_2Cl_2, the combined organic solutions were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica using ethyl acetate/methanol as eluent to give compound **2** as purple-brown oil (0.407 g, 60%).

Analytical data:

HR-MS (ESI+): m/z calculated for $C_{47}H_{69}BN_2O_{11}Na$ $[M+Na]^+$: 870.4923, found 870.4908.

^{1}H-NMR (400MHz, $CDCl_3$) [ppm]: δ = 0.97 (t, 6H, *J* = 7.5 Hz, 2 × CH_2-C$\underline{H}_3$), 1.32 (s, 6H, 2 × C-C$\underline{H}_3$), 2.30 (q, 4H, *J* =7.5 Hz, 2 × C$\underline{H}_2$-CH_3), 2.67 (s, 6H, 2 × N-C-C$\underline{H}_3$), 3.37 (s, 6H, 2 × C$\underline{H}_3$), 3.54 (m, 4H, 2 × C$\underline{H}_2$), 3.64 (m, 28H, 2 × C$\underline{H}_2$), 4.18 (d, *J* = 2.4 Hz, 4H, 2 × C$\underline{H}_2$-C), 6.92 (d, 2H, *J* = 8.6, 2 × C$\underline{H}_{ar}$), 7.06 (d, 2H, *J* = 8.6, 2 × C$\underline{H}_{ar}$).

Absorption and emission spectra of dyes **1** and **2** in aqueous solution are shown in Fig. S1, while Figs. S2 and S3 summarise the pH dependence of the absorption and emission behaviour of **1** and **2**, respectively, for a change from near-neutral to alkaline conditions. Table S1 compiles the relevant spectroscopic data, including the fluorescence lifetime (τ_f).

The protonation constant of **2** was determined to be $pK_A = 9.31 \pm 0.01$ in water by Boltzmann curve-fitting as a function of pH using the fluorescence intensity at 527 nm (cf. Fig. S3). Although the pK_A shifts by ca. 0.5 units compared to **1**, the modification with two amphiphilic PEG moieties increases the solubility in water significantly while retaining the spectroscopic properties of the parent dye **1**.

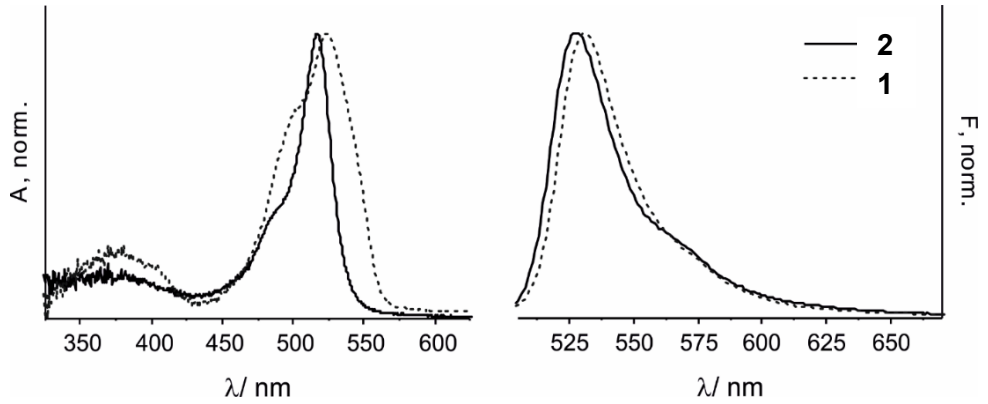

Figure S1. Normalized absorption (left) and emission spectra (right) of dyes **1** and **2** in water.

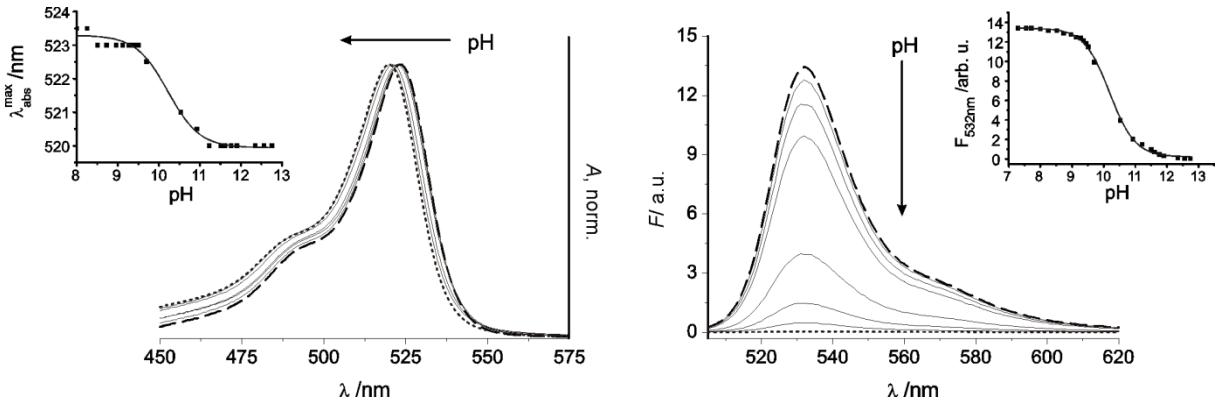

Figure S2. Dependence of the absorption and fluorescence of dye **1** on a change in pH from 7.3 to 12.8 upon addition of aqueous KOH solutions (λ_{ex} = 495 nm, $c_1 = 1.39 \cdot 10^{-6}$ M, EtOH/H_2O); pH 7.3 (dashed line), pH 12.8 (dotted line); selected intermediate steps (solid lines). Insets: corresponding titration curves, given by the shift of the absorption maximum (left) and the fluorescence intensity at the emission maximum (right) as a function of pH.

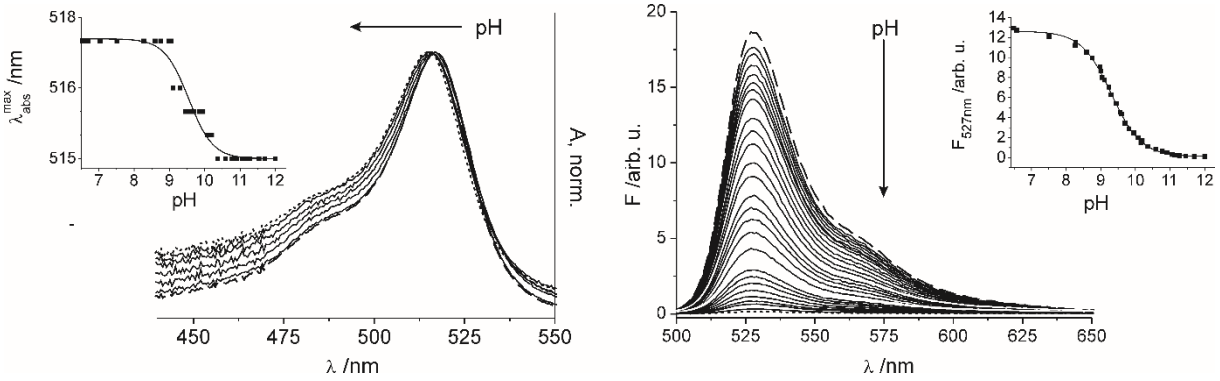

Figure S3. Dependence of the absorption and fluorescence of dye **2** on a change in pH from 6.5 to 12.0 in neat water (λ_{ex} = 495 nm, $c_2 = 1.39 \cdot 10^{-6}$ M, H_2O); pH 6.5 (dashed line), pH 12.0 (dotted line), intermediate steps (solid lines). Insets: corresponding titration curves, given by the shift of the absorption maximum (left) and the fluorescence intensity at the emission maximum (right) as a function of pH.

Table S1. Spectroscopic data of dyes **1** and **2** in selected solvents at 298 K.

Compound	Solvent	λ_{abs} / nm	λ_{em} / nm	τ_f / ns
2	MeCN	517	526	n.d.
	H_2O/EtOH	518	527	4.72
	H_2O	517	527	3.42
1	MeCN	521	531	4.72
	H_2O/EtOH	523	532	5.57
	H_2O	523	532	2.67[a]

[a] Longest lifetime component of a multiexponential fit.

S3. Growth and Characterisation of Biomorphs.

Silica biomorphs were grown from aqueous solutions containing 5.0 mM $BaCl_2$, 8.4 mM SiO_2, and 8.9 mM Na^+, prepared as described in detail elsewhere.[S6] Portions of this mixture (typically 10 mL) were filled into cylindrical wells of standard polystyrene plates (VWR Nunclon 6-well plates, volume: 17 mL, area: 9.6 cm^2, depth: 1.7 cm). Subsequently, glass coverslips (15x15 mm) were placed on the bottom of the wells as substrates for growth, and the well plate was covered loosely with a lid. Crystallisation of barium carbonate occurred upon gradual in-diffusion of CO_2 from the atmosphere into the alkaline solution (starting pH: 11.0), where it was converted to HCO_3^- and finally CO_3^{2-} ions needed for precipitation. In this way, complex structures are formed spontaneously on interfaces over periods of several hours. Their isolation is quite straightforward, as the glass substrates can simply be removed from the mother liquor with a pair of tweezers and only need to be rinsed with water and ethanol.

In order to be able to trace local variations of pH in situ during the formation of biomorphs, growth was carried out directly underneath a confocal microscope. For this purpose, we used special microdishes that had a flat glass bottom (ibidi GmbH, µ-dish, diameter: 35 mm) and thus were ideally suited for imaging the front of sheets evolving along this interface. Initially, 4 mL of growth solution (prepared as described above) were filled into the dishes and left open to the atmosphere to absorb CO_2 over time. After 2 h (when the first structures had started to form), 0.1 mL of a 100 µM solution of **2** in water:acetone 9:1 were added (note that the small amounts of acetone introduced in this way did not affect the growth behavior of biomorphs to any noticeable extent). The final concentration of the dye in the mother liquor thus was 2.44 µM.

After a promising (and still growing) aggregate had been identified in the microscope and fluorescent dye was added, the development of the structure was monitored in situ over time (as in many previous studies[S6,S7] with regular optical microscopy), while continuously collecting transmission and fluorescence data.

References.

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