Electronic Supplementary Material (ESI)
for
Functionalisation of Silica-Carbonate Biomorphs

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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 used solvents were of UV spectroscopic grade (Aldrich). Stock solutions (0.1 and 1 M) of hydrochloric acid and sodium hydroxide, required for pH adjustments, were obtained from Merck (p.a. grade), while borax/HCl buffer solution (pH 9) was received from Fluka. 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). The used triethoxysilanes (3-MPTES: 92%; 3-APTES: 98%; DNPTES: 92%; 3-MPA: 99%) and TEOS (min. 99%) were purchased from ACR. 10,12-Pentacosadiynoic acid (PCDA, 97%), Ellman reagent (5,5'-dithiobis(2-nitrobenzoic acid), min. 98%), 4-nitrophenol (spectrophotometric grade, min. 99.5%), resazurin sodium salt (BioReagent, dye content: 80%), fluorescein isothiocyanate (FITC, isomer I, min. 90%) and hydroxylamine solution (50% in water) were obtained from Sigma-Aldrich. Sodium borohydride (98%) was purchased from Merck.

Polarised optical microscopy 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. The used confocal laser scanning microscope was a Zeiss LSM780. All CLSM measurements were done with the PlanApo 63x / 1.40 objective, using an Ar+ laser diode (458, 488, 514 nm), a DPSS laser (561 nm) or a HeNe laser (633 nm) as light source. The microscope was equipped with three fluorescence detectors (one GaAsP array and two PMTs) and another PMT for transmission images. For scanning electron microscopy and EDX analysis, specimens were mounted on aluminium stubs by means of double-sided adhesive tape and were subsequently investigated on a Hitachi TM3000 tabletop microscope at acceleration voltages ranging from 3 to 10 kV (without previous sputtering). High-resolution SEM images were acquired with a Zeiss Crossbeam 1540XB microscope with the SE2 detector at 5-15 kV. TEM studies were carried out on a Zeiss Libra 120 microscope at 120 kV. UV-Vis and fluorescence spectra were recorded using an Agilent Cary 50 spectrophotometer and a Perkin Elmer LS 50B fluorescence spectrometer, respectively. Measurements were performed in quartz cuvettes with a path length of 10 mm from Hellma Analytics. A Perkin Elmer Spektrum 100 ATR-FTIR instrument was used for IR spectroscopy, while Raman microspectroscopy was performed by a custom-built setup installed with a confocal Leica microscope with two lasers (488 and 633 nm).

S2. Growth and Characterisation of Native and Functionalised Biomorphs.

Synthesis of native biomorphs: Silica biomorphs were grown from aqueous solutions containing 5.0 mM BaCl$_2$ and 9 mM SiO$_2$, the latter being introduced either directly as sodium silicate (water glass) or through hydrolysis of TEOS at pH 11.3 (adjusted by NaOH) prior to mixing with the barium
solution. 10 mL of the reaction mixture were filled into cylindrical wells of standard polystyrene plates (VWR Nunclon 6-well plates, volume: 17 mL, area: 9.6 cm², 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 indiffusion of CO₂ from the atmosphere into the alkaline solution (starting pH: 11.0), where it was converted to HCO₃⁻ and finally CO₃²⁻ 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.

One-pot synthesis of fluorescent biomorphs: In-situ functionalisation was achieved by replacing 5 vol% of the TEOS with 3-(2,4-dinitrophenylamino)propyltriethoxysilane (DNPTES) in the above-described procedure (i.e. 8.6 mM TEOS and 0.4 mM DNPTES). After 12-24 hours of growth, the glass substrates were removed from the mother liquor and rinsed intensively with water and ethanol.

Post-silanisation of biomorphs: Freshly synthesised biomorphs (stuck on glass coverslips) were immersed into a 1 vol% solution of functional triethoxysilane (3-MPTES, 3-APTES or DNPTES) in a 95:5 mixture of ethanol and water. After 12 h, the substrates were removed and washed intensively with water and ethanol.

Labeling of amine-functionalised biomorphs: Coverslips carrying aminosilane-functionalised biomorphs were incubated in a FITC solution at pH 9 for 2 h. The FITC solution was prepared by mixing 1 mL of borax buffer with 100 µL of 1 mM FITC in ethanol. After incubation, the coverslips were washed with water and ethanol and then directly observed under the CLSM.

Labeling of thiol-functionalised biomorphs with QDs: Coverslips carrying thiol-modified biomorphs were immersed into 1 mL of a 20 nM solution of CdSe@ZnS quantum dots (d$_{ave}$ = 9 nm) in toluene for 6 h. After the incubation step, the structures were removed, washed several times with water and ethanol, and then directly investigated under the CLSM.

Labeling of thiol-functionalised biomorphs with AuNPs: Coverslips carrying thiol-modified biomorphs were immersed into 1 mL of a 10 nM aqueous gold colloid solution (d$_{ave}$ = 14 nm) for 24 h. During incubation, the colour of the solution changed from red to purple (cf. UV-Vis spectra in Figure S1b). Afterwards, the coverslips were removed and washed several times with water and ethanol before inspection under the CLSM.

Catalytic reduction of pNP: 3.4 mL water and 4 µL of a 50 mM aqueous solution of 4-nitrophenol were given into a quartz cuvette with a stirrer. Subsequently, 1 mg of the AuNP-functionalised biomorph were dispersed in the solution. After addition of 1 mg NaBH₄, the reaction was monitored over time with UV/Vis spectroscopy.

Catalytic reduction of resazurin: First, AuNP-functionalised biomorph were prepared in a µ-dish for fluorescence microscopy (ibidi GmbH, diameter: 35 mm) according to the protocol described above. After the dish had been placed on the specimen stage of the CLSM, 2 mL of a freshly prepared aqueous solution of 16 µM resazurin and 160 µM hydroxylamine were added and the progress of the conversion to resorufin was followed in the fluorescence channel of the CLSM.

Synthesis of PCDA-functionalised biomorphs: First, 20 mg of native biomorphs were treated with 1 M sodium hydroxide solution for 12 h to remove the outer silica skin. After repeated washing with water, the biomorphs were re-dispersed in 4 mL 1 M NaOH and 100 mg PCDA in 1 mL THF were added through a syringe filter. After 12 h incubation (adsorption of PCDA), the structures were washed
several times with water to remove non-adsorbed monomers, followed by exposure to UV light ($\lambda = 365$ nm) for 5 min to trigger polymerisation.

**S3. Quantification of the Degree of Thiol Functionalisation with the Ellman Reaction**

The amount of thiol groups anchored on the biomorphs via silanisation was determined with the help of the Ellman reagent (5,5’-dithiobiis(2-nitrobenzoic acid)). In the presence of free thiol groups, this molecule splits and releases nitrothiobenzoate (NTB) in a quantitative reaction, as illustrated in Figure S1c for the case of a thiol-bearing biomorph. NTB exhibits significant absorption at 408 nm in alkaline solution and thus the reaction can readily be monitored by UV-Vis spectroscopy. Figure S1a shows a time-dependent series of corresponding spectra for a 8.8 µM solution of the Ellman reagent in borax buffer (pH 9) containing 18 mg of thiol-functionalised biomorphs, along with a plot of the area of the peak at 408 nm (red dots and line). In order to convert this data into an actual number of free thiol groups on the biomorphs, we performed reference experiments in which defined amounts of 3-mercaptopropionic acid (3-MPA) (0-7 µM) were added to 8.8 µM Ellman reagent solution buffered at pH 9. The resulting spectra after a reaction time of 15 min are shown in Figure S1b. Plotting the area of the peak as a function of concentration (red dots) gives a calibration curve that can be approximated by a linear fit (thick red line), which was used to calculate the actual number of thiol groups on the biomorphs after completion of the reaction shown in Figure S1a. This yields an effective thiol loading of 947 ± 75 nmol per g biomorphs.

**Figure S1.** a) UV/Vis spectra showing the progress of the Ellman reaction as a function of time in the presence of 18 mg thiol-modified biomorphs at pH 9. b) UV/Vis spectra of solutions in which the Ellman reagent was converted with different amounts of 3-MPA. The thick red line is a linear fit to the experimental peak area-concentration data, which was used as a calibration curve. c) Schematic drawing of the Ellman reaction on thiol-functionalised biomorphs.
S4. Estimation of the AuNP Loading on Functionalised Biomorphs.

The amount of gold nanoparticles immobilised on the biomorphic structures was estimated in different ways. First, the catalytic activity – as derived from resazurin-to-resorufin reaction rates determined by UV/Vis spectroscopy with and without Au – was used as a measure. Here we assumed that the catalytic activity of the AuNPs bound on the surface of biomorphs is comparable to that of the same particles in a colloidal dispersion (reference experiment). Under these conditions, measured reaction rates can easily be converted to apparent AuNP loadings on biomorphs (in mol per g) if the used absolute mass of AuNP@Biomorph catalyst is known. Following this approach, it needs to be considered that the obtained value will represent a lower limit for the true loading, because the net catalytic activity of surface-bound particles is most likely lower than that of the same amount of dispersed particles, due to the fact that reagents need to be transported to/from the surface by diffusion and that some of surface sites may not readily be accessible.

The second used method was EDX spectroscopy on intact AuNP-bearing biomorphs. Corresponding measurements gave an atomic ratio of Ba:Si:Au = 94:100:1. With the known density of solid gold (50 atoms per nm³) and mean diameter of the used AuNPs (14 nm), the loading of AuNPs on the biomorphs can be calculated from the EDX data. The problem of this approach is that EDX is a surface-sensitive technique with a typical penetration depth of a few microns. Consequently, the contribution of elements that are enriched at the surface of the functionalised biomorph (Si and Au) will be disproportionately higher than that of the bulk material below (mainly BaCO₃). Therefore, the resulting value for the loading should be regarded as an upper limit.

The third way to calculate the loading is based on in-situ UV-Vis experiments during the functionalisation step, i.e. when thiol-modified biomorphs were incubated in a dispersion of gold colloids. In the absence of biomorphs, the dispersion showed significant absorbance at 514 nm due to surface plasmon resonance, in good agreement with the literature. However, upon exposure to the thiol-bearing biomorphs, the intensity of the band at 514 nm decreased and the colour of the solution changed from red to a dark violet, accompanied by the emergence of an additional absorption peak at ca. 595 nm (Figure S2c). This second band indicates the formation of one-dimensional superstructures (chains) of the gold colloids in dispersion and in fact, such aggregated structures could also be observed by TEM (Figure S2b). Interestingly, this effect only occurred in the presence of biomorphs, while neither BaCO₃ nor silica particles alone did have any noticeable influence on the absorption spectra. In turn, when as-grown biomorphs were used (no thiol modification), the new band at 595 nm formed, but the peak at 514 nm did not decrease in intensity (cf. Figure S2d). While we cannot explain these findings in detail at the moment, a comparison of the absorbance at 514 nm (peak area) after exposure to i) unmodified biomorphs (Figure S2d) and ii) thiol-bearing biomorphs (blue curve in Figure S2c) can be taken as a measure for how many Au nanoparticles were bound on the thiol-modified biomorphs during AuNP functionalisation. Values resulting for the loading from this approach are also considered to be upper limits, because the formed aggregates of AuNPs have limited colloidal stability and hence some of them may have precipitated in the course of the experiment (thus reducing the detected absorption without actually binding to biomorphs).
Figure S2. a) TEM image of thiol-modified biomorphs after incubation in AuNP dispersion, rinsing with water and crushing for TEM preparation. The remaining fragments consist of three components: carbonate nanocrystals (ca. 200 nm long and 50 nm wide, not shown here), silica spheres (50-200 nm in diameter, indicated by the blue arrow), and small gold colloids (14 nm) with high contrast (highlighted by red arrows) that decorate some of the silica particles. Corresponding electron diffraction (ED) patterns show strong reflections that can be assigned to crystalline gold (note that the shown area of the sample mainly contains (amorphous) silica spheres and AuNPs). b) TEM micrograph of AuNP networks that were isolated from gold dispersions after exposure to thiol-modified biomorphs. The ED pattern proves that the aggregated particles consist of Au. c) UV/Vis spectra of the colloidal AuNP dispersion before (red) and after (blue) incubation with thiol-modified biomorphs (blue). The inserted sketch illustrates how the colour of the solution changed. d) UV/Vis spectra of the AuNP dispersion at different times during incubation with unfunctionalised biomorphs (no thiol modification).

Values resulting for the AuNP loading from the above-described approaches are compiled in Table S1. It is obvious that the agreement between the estimated loadings is not good, but still the data give an idea about the order of magnitude.

<table>
<thead>
<tr>
<th>Method</th>
<th>Calculated Loading [nmol/g]</th>
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<tbody>
<tr>
<td>Catalytic Reaction</td>
<td>0.03 ± 0.01</td>
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<tr>
<td>EDX</td>
<td>0.50 ± 0.05</td>
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<tr>
<td>UV-Vis Spectroscopy</td>
<td>0.74 ± 0.01</td>
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Table S1. Loadings of AuNPs on biomorphs after functionalisation, as estimated by different methods.
S5. Raman and IR Analyses of Polymer-Coated Biomorphs.

In addition to optical microscopy, SEM and CLSM, the composition of the biomorphs after functionalisation with PCDA was also investigated by means of Raman and IR spectroscopy. Corresponding results are summarised in Figure S3. Local Raman spectra (Figure S3a) collected from native aggregates before polymerisation show characteristic signals of BaCO$_3$ at 135, 153 and 1063 cm$^{-1}$, along with a strong band at 523 cm$^{-1}$ that can be assigned to silica (blue spectrum Figure S3a). Upon treatment with 1 M NaOH, this latter signal has vanished (red spectrum in Figure S3a), indicating successful removal of the outer silica skin. After adsorption of PCDA and UV illumination, the obtained Raman spectrum is completely different and displays bands that can exclusively be attributed to poly(PCDA) (black spectrum in Figure S3a). The presence of the polymer is also reflected in the curved baseline of the spectrum, which results from the fluorescence of poly(PCDA). In turn, the absence of distinct carbonate and silica modes suggests that the newly formed polymer coating is rather thick and hence the Raman signal can no longer be obtained from the inner part of the structure.

The above findings are further confirmed by IR data (Figure S3b). While the spectrum measured for a biomorph after removal of the silica skin (black curve in Figure S3b) shows only bands typical for barium carbonate (693, 855 and 1412 cm$^{-1}$), new signals emerge after the polymerisation step, namely CO modes at 1511 and 1556 cm$^{-1}$ as well as CH modes at 2748 and 2982 cm$^{-1}$, both being characteristic for poly(PCDA).

Figure S3. a) Local Raman spectra collected from a native biomorph (blue), a biomorph after removal of the outer silica skin (red), and a PCDA-modified biomorph (black). b) IR spectra of biomorphs after leaching in 1 M NaOH (black) and after functionalisation with PCDA (red).

References.