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Self-Assembled Nanowires

Fluorescent Nanowires Self-Assembled through Host–Guest Interactions in Modified Calcein

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The preparation of well-defined nanostructures, such as vesicles, nanorods, and nanowires,[1] self-assembled from low-molecular-weight components, has attracted much interest. These noncovalently linked compounds find remarkable applications in material sciences, for example, in bottom-up approaches for the fabrication of optoelectronic devices.[1] To obtain highly fluorescent nanowires after self-assembly is a challenge because of the self-quenching π–π-interacting systems.[2] Additionally, aggregation in aqueous solution, induced by π–π interactions, is a major reason for the formation of unwanted irregular aggregates.[1a] Recently, well-defined self-assembled fluorescent nanomaterials based on porphyrin matrices have been produced by Jayawickramarajah and co-workers.[1d] The expansion of such supramolecular nanowire systems with different fluorescent compounds is of interest. This novel system offers relative to previous systems the advantage of a simple synthetic route.

We have recently reported the formation of supramolecular structures such as rings based on dendrimeric combinations of cyclodextrin and adamantane molecules through click chemistry. Furthermore the preparation of nanocycles comprising β-cylcodextrin (β-CD)-click-ferrocen units was accomplished.[3] Here, we report the successful preparation of novel structures from calcein **1** (Figure 1) through β-CD/adamantane host–guest interactions of their four functional arms. Moreover, we describe the aqueous self-assembly of the β-CD–calcein compound **2** and the adamantane–calcein compound **3** into novel fluorescent wires (Figure 1).

The investigation of these wires was accomplished and their structure was studied by cryogenic transmission electron microscopy (cryo-TEM), tomographic transmission electron microscopy (TOMO), fluorescence spectroscopy, and scanning electron microscopy (SEM). The measurements demonstrated a uniform and straight shape of these fluorescent wires formed in aqueous solution with length of several micrometers (cryo-TEM). This study also shows the systematic self-assembly driven by host–guest interactions without the formation of thermodynamically trapped structures and agglomeration caused by π–π interactions. Additionally, not only the arrangement of fluorescent wires is described but also their remarkable stability. The β-CD–calcein compound **2** was prepared through amidation of calcein **1** with propargylamine followed by a click reaction with β-CD azide.[4] Analogously to the first step in the preparation of **2**, calcein was modified with adamantylamine through amidation to isolate **3**.

Although the calcein compound **3**, on its own, is insoluble in water, the addition of **2** followed by stirring for 12 h resulted in solubilization of **3**. Thus, it can be suggested that supramolecular inclusion complexes are formed wherein the β-CD arms of the calcein compound **2** complex the hydrophobic arms of **3**.

To verify that the starting components, β-CD–calcein compound **2** and adamantane–calcein compound **3,** do not self-aggregate in aqueous solution, dynamic light scattering (DLS) measurements were performed. The β-CD-modified calcein compound **2** did not agglomerate ($D_h = 2$ nm), whereas calcein **1** aggregated in a buffer solution (pH 9) and showed an increased hydrodynamic diameter (D_h) of 180 nm (see Figure S1 in the Supporting Information). The agglomeration of **1** can be ascribed to π–π interactions, which are a major reason for the formation of unwanted irregular aggregates.[5] By functionalization of calcein **1** with four bulky and sterically challenging β-CD units π–π agglomeration is suppressed. DLS measurements of the adamantane–calcein compound **3** also indicated only minor tendency of aggregation ($D_h = 8$ nm). The addition of **2** to **3** in aqueous solution resulted in the formation of big agglomerates of 1 μm in diameter. This result is not in contrast to the greater lengths obtained by electron microscopic methods, because this length is calculated by assuming a spherical shape according to the Stokes–Einstein equation. If a rod-like shape are considered, larger values will be obtained.[6]

Cryo-TEM studies were conducted to analyze the composition and structure of the self-assembly. As shown in Figure 2a, the transmission electron image of the isolated compound **4** displays several micrometer-long wires. Because the wire length reached the mirometer regime we were able to perform SEM on the dried compound. The SEM image (Figure 2b) of the wire sputtered with Pd also showed the formation of a defined wire, which exhibited a rough surface morphology relative to the rather smooth surface observed by cryo-TEM. This phenomenon may be explained by the fact that the wires exist in a swollen state when water is present, whereas removal of the solvent causes their collapse and formation of a rather rough surface. To verify that the wire is not a one-dimensional, flat construct a tomographic trans-

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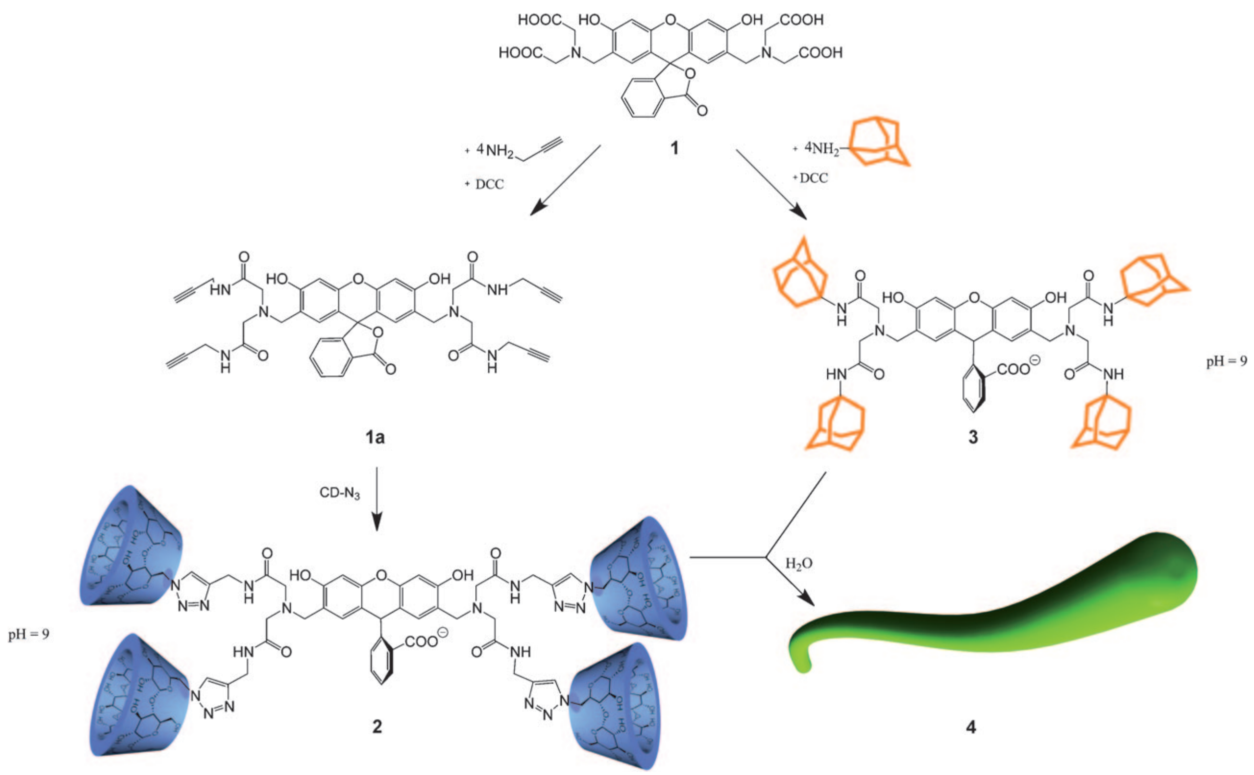

Figure 1. Chemical structures of calceins **1**, **1a**, **2,** and **3** and the aqueous self-assembly of the nanowires **4** (strongly idealized structure).

mission electron microscopic image recorded with a tilt angle of 135° in steps of 1.5° was conducted (see the movie in the Supporting Information). The movie impressively shows the three-dimensional shape of the wires. The movie and the pictures only describe the self-assembly as a phenomenon because the mechanism of formation has yet not been fully described. We are currently investigating the mechanism with a library of fluorescent dyes.

The fluorescence spectra of the adamantane–calcein compound **2** (Figure 2d) exhibit a slightly increased emission maximum at 550 nm relative to the fluorescence maximum observed for calcein **1**. This fact can be explained by intramolecular interactions of the phenolate oxygen atom forming a hydrogen bond with the CO unit of the amide group in the adamantyl moiety. The β-CD calcein compound **3** shows an emission maximum which is slightly bathocromically shifted to a higher value of 575 nm. This shift can be explained by intramolecular hydrogen-bonding interactions between the OH groups of the cyclodextrin moieties and the CO groups of the propylamide units. These hydrogen bonds can form because CD does not deprotonate at $\mathrm{pH} < 12$, which was shown by NMR spectroscopy by Gaidamauskas et al.[7] The wires exhibit two significant emission maxima which are in close proximity so that a peak with an apparent shoulder appears. These maxima are due to the combination of the two different calcein components **2** and **3**. From the original fluorescence spectra it was apparent that the CD–calcein compound **3** shows an intensified fluorescence, which is a well-described phenomenon because the quenching process is restricted by the β-CD units in aqueous solution.[8] Addition of the adamantane–calcein compound **2** to **3** does not cause any difference in the fluorescence intensity and a quenching process did not occur (see Figure 2d). Thus, these suprastructures are highly fluorescent and simple to synthesize. The green-fluorescent wires were shown by light scanning microscopy (Figure 2c).

Defined supramolecular structures formed which were further analyzed by scanning electron microscopy (SEM). The SEM image also clearly displays the wirelike morphology with a length of a micrometer (Figure 2b). To test the thermal stability of the wire **4** differential scanning calorimetry was conducted (see Figure S3 in the Supporting Information). Interestingly, a significant change in the structure of the wires is first observed at a temperature of 75 °C, which indicates a substantial stability of the wires even under rather harsh conditions. This stability can most likely be ascribed to the multivalent properties of the self-assembly.

These experiments show that supramolecular self-assembled wires can be formed in aqueous solution from cyclodextrin- and adamantane-modified calcein with remarkable stability even after evaporation of the solvent. The fluorescence measurement also confirmed the successful formation of these self-agglomerated structures.

In conclusion, we have described a concept for the self-assembly between CD- and adamantane-modified calcein forming well-defined wires in aqueous solution because of host–guest interactions. Detailed results display the homomeric structure of the wires, their water solubilization, and their exceptional resistance towards disassembly. This approach also successfully led to highly fluorescent wires showing no self-quenching. This novel system offers the advantage of a simple synthesis for highly fluorescent nano-

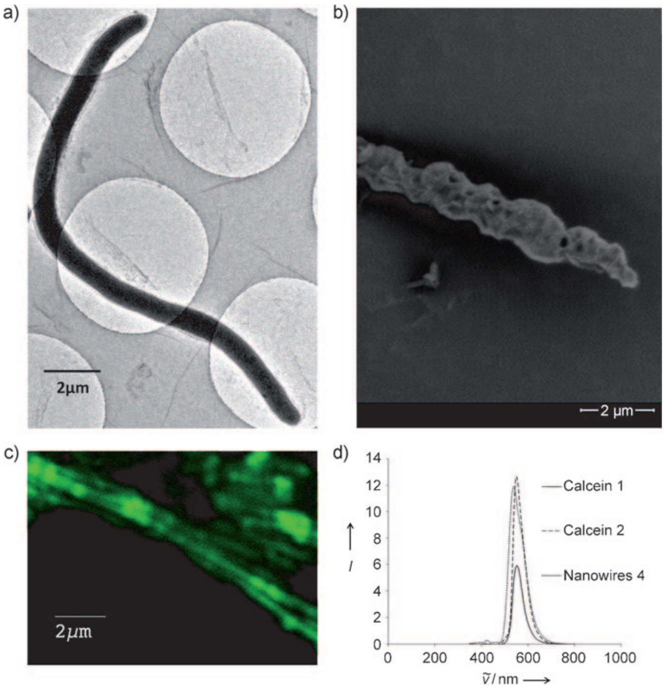

Figure 2. a) Cryo-TEM image of a calcein wire, b) SEM image of a dried wire, c) light scanning microscopy of the green-fluorescent wires, d) fluorescence spectra of calcein **1** (——), the β-CD–calcein compound **2** (-----), and the nanowires **4** (•••••; all spectra are measured in aqueous solution).

wires, which will find interesting applications as fluorescent dyes in microbiology.

Experimental Section

Synthesis of the precursor calcein **1a**: To a solution of **1** (0.50 g, 1.00 mmol) and propargylamine (0.28 g, 5 mmol; 1.25 equiv per carboxyl group in **1**) in DMF (3 mL), was added dicyclohexylcarbodiimide (DCC; 1.03 g, 5 mmol) in DMF (7 mL). The reaction solution was heated to 60 °C and left to stir for 24 h. Then the reaction mixture was filtered and the crude product precipitated in acetone. The product was then dissolved and dehydrated by a freeze-drying process to afford **1a** as a purple solid (686 mg, 95 %). FT-IR (cm^{-1}): 3288, 3238 (CONH, NH); 3024 (C=CH); 2928, 2853 (CH_2, CH_3); 2116 (C≡CH); 1657, 1524 (CONH); 1580 (COO^-); 1448 (CH_3, CH_2); 1147, 1090 (COH); 1043 (COC); 968 (C≡CH); 891,828, 806, 785, 749 (aromatic); HRMS (ESI): *m*/*z*: calcd for $C_{42}H_{38}N_6O_9$ [M^+]: 770.27; found: 770.29.

Reaction of **1a** with CD-N_3: To a solution of **1a** (0.15 g, 0.21 mmol) and cyclodextrin azide (CD-N_3)[1] (1.00 g, 0.84 mmol; 4 equiv per alkyne group in **1a**) in DMF (2 mL), was added sodium ascrobate (17 mg, 0.04 mmol) and $CuSO_4$ (7 mg, 0.08 mmol). The reaction vessel was then placed in a microwave and irradiated at 90 °C for 1 h (30–70 W). The crude product was precipitated in acetone, afterwards dissolved in water and dehydrated by a freeze-drying process to obtain **2** (855 mg, 74 %). IR: $\tilde{\nu} = 3321$ (OH); 2927 (CH_2, CH_3); 1657; 1584 (CONH); 1387 (CH_3); 1152 (CO); 1078 (COH); 1027 (COC); 1002, 944, 844, 753 (aromatic); ^{1}H NMR (300 MHz, [D_6]DMSO): $\delta = 7.82$ (br, 1 H), 7.50 (br, 1 H), 7.32 (br, 2 H), 7.10 (br, 2 H), 6.57 (br, 2 H), 5.75 (br, 14 H), 5.10 (br, 2 H), 4.84 (d, 6 H), 4.30–4.67 (br, 6 H), 3.93 (br, 4 H), 3.45 (br, 28 H), 3.21–3.47 ppm (br, 14 H); MALDI-TOF: *m*/*z*: calcd. for $C_{210}H_{314}N_{18}O_{145}$: 5409.78 [$M^+$]; found: 5409.80.

Reaction of **1** with adamantylamine: To a solution of **1** (0.50 g, 1 mmol) and adamantylamine (0.61 g, 4 mmol) in DMF (10 mL) was added a solution of dicyclohexylcarbodiimide (DCC) in DMF (7 mL). The reaction mixture was heated to 60 °C and left to stir for 24 h. Then the reaction mixture was filtered and the crude product precipitated in acetone. The product was then dissolved and dehydrated by a freeze-drying process to afford **3** as a purple solid (958 mg, 86 %). FT-IR (cm^{-1}): 3322, 3265 (CONH, NH); 3030 (C=CH); 2924, 2907, 2850 (CH_2, CH_3); 1703 (CO); 1640, 1526 (CONH); 1579 (COO^-); 1450 (CH_3, CH_2); 1146, 1089 (COH); 1001, 952, 891, 828, 813, 787 (aromatic); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.66$ (m, 1 H), 7.69 (m, 1 H), 7.35 (m, 2 H), 7.04 (d, 2 H), 6.53 (m, 2 H), 5.29 (s, 2 H), 4.0 (s, 4 H), 3.29 (s, 4 H), 1.20–2.20 ppm (m, 60 H); ESI: *m*/*z*: calcd for $C_{70}H_{86}N_6O_9$: 1154.65 [M^+]; found: 1154.6.

Preparation of the nanowires **4**: The calcein compound **3** (10 mg) was added to a solution of **2** (30 mg) in water (10 mL). The resulting heterogeneous mixture was stirred at room temperature for 24 h. The originally water-insoluble calcein compound **3** became soluble to form a homogenous mixture. After dehydration by a freeze-drying process the nanowires **4** were isolated as a purple solid. The solid was afterwards dissolved in distilled water and filtered (pore diameter of the filter: 0.45 μm).

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