Individual tubular J-aggregates stabilized and stiffened by silica encapsulation

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Abstract
Amphiphilic cyanine dyes in aqueous solution self-assemble into J-aggregates with diverse structures. In particular, the dye 3,3′-bis(3-sulfopropyl)-5,5′,6,6′-tetrachloro-1,1′-dioctyldibenzimidazo-carbo-cyanine (C8S3) forms micrometer long double walled tubular J-aggregates with a uniform outer diameter of 13 ± 0.5 nm. Interestingly, these J-aggregates exhibit strong exciton delocalization and migration, similar to natural light harvesting systems. However, their structural integrity and hence their optical properties are very sensitive to their chemical environment as well as to mechanical deformation, rendering detailed studies on individual tubular J-aggregates difficult. We addressed this issue and examined a previously published route for their chemical and mechanical stabilization by in situ synthesis of a silica coating that leaves their absorbance and emission unaltered in solution. Here, we demonstrate that the silica shell with a thickness of a few nanometers is able to stabilize the tubular J-aggregates of C8S3 against changes of pH of solutions down to values where pure aggregates are oxidized, against drying under ambient conditions, and even against the vacuum conditions within an electron microscope. Dried silica-covered aggregates are brittle, as demonstrated by manipulation with a scanning force microscope on a surface. Transmission electron microscope images confirm that the thickness of the coatings is homogeneous and uniform with a thickness of less than 5 nm; scanning TEM energy dispersive X-ray spectroscopy confirms the chemical composition of the shell as SiO₂; and electron energy loss spectra could be recorded across a single freely suspended aggregate. Such a silica shell may not only serve for stabilization but also could be the base for further functionalization of the aggregates by either chemical attachment of other units on top of the shell or by inclusion during the synthesis.

Keywords Amphiphilic cyanine · J-aggregates · Silica-covered aggregates · APTES · TEOS · Aqueous silanization · TEM · EELS

Introduction
Tubular aggregates of amphiphilic cyanine dyes [1] are a special type of J-aggregates [2] and have recently attracted attention [3–6] because they might serve as model systems for chlorosomal light harvesting complexes [7]. The main questions that can be addressed using these systems relate to their optical properties such as light absorption, excitation energy transfer, and energy migration properties, and how these properties depend on the structural arrangement of the molecules. While these model systems share characteristics with natural chlorosomes, in some respects, the model systems can have superior characteristics due to a high degree of order and strong intermolecular exciton coupling in these systems [3].

The tubular aggregates spontaneously self-assemble in aqueous solution by means of hydrophobic interactions between the surfactant dyes if the concentration exceeds the critical aggregation concentration, which is generally on the order of 10⁻⁴ mol/L [8]. The tubular structures typically consist of a double wall of dye molecules with an arrangement reminiscent of a lipid double layer [1]. The tubes have an outer diameter of typically 10 to 20 nm, depending on the
molecule used, and lengths exceeding microns. For a given choice of dye, the aggregates are very homogeneous and the diameter is uniform [5]. They exhibit the characteristic optical properties of J-aggregates, i.e., strong and narrow absorption band that is strongly red shifted relative to the monomer and a nearly resonant narrowband fluorescence emission. The J-absorption band here splits into several bands with two pronounced and characteristic peaks due to the tube-like arrangement of the dyes [9]. These optical properties are a clear signature for strong exciton coupling with effective exciton migration [4, 10].

Charge and energy transfer processes within these aggregates or between aggregates and other species depend strongly on the mutual distance and orientation of the molecules within the aggregate or on the separation and mutual orientation of the second species units and the dye molecules within the aggregates [11–13]. Due to the high concentration of aggregates in solution, mutual contact that leads to bundling effects [6] is inevitable, which affects the optical properties. Lowering the concentration is not an option, because it leads to dissolution of the aggregates. Therefore, a method that allows isolating and immobilizing the aggregates is needed. The latter is also important for orientation-dependent measurements with given polarization. To avoid statistical averaging over ensembles of aggregates, it furthermore is very desirable to access single aggregates by microspectroscopy methods. Additionally, the structures should be stabilized and protected against dry environments, because a fundamental prerequisite for their structure is the presence of water and respective hydrophobic forces.

Several methods for isolation and immobilization have been explored. Each has been successful but also has its drawbacks. One method is direct deposition of the aggregates on hydrophilic surfaces [5, 14]. However, in that case, the structure depends very sensitively on moisture conditions, and photodegradation is an issue. Another method is embedding in a matrix of a transparent solid, e.g., sugar [4] or polyglycol at low temperature [15] or embedding in polymer layers [16]. Most methods, however, are bulk methods that result in immobilization and stabilization, but do not permit the study of isolated aggregates.

The best method would be to encapsulate the aggregates in situ in solution with a shell that protects them against mechanical and chemical damage, is optically transparent, does not disturb the molecular arrangement of the dyes, and is accessible for attachment of further functional groups. In a series of previous publications, we have demonstrated that the tubular aggregates of the amphiphilic TDBC dyes can be covered by various materials in situ due to electrostatic forces and hydrogen bonding. For example, polyvinyl alcohol was used for carboxylic-substituted dyes resulting in a homogeneous coverage by a shell of few nanometer thickness [17]. Linear polyelectrolytes were used [18, 19] to at least partially coat sulfonyle-substituted aggregates with a coating thickness of a few nanometers. A major disadvantage of these polymer coatings was their effect on the structure and homogeneity of the aggregates, at least for some of the PEs.

The most promising coating was achieved with the dye 3,3′-bis(3-sulfopropyl)-5,5′,6,6′-tetrachloro-1,1′-diocetylbenzimidace-carbo-cyanine (C8S3) and a shell of amorphous silica, synthesized via a sol-gel-like process [19, 20]. The procedure is based on the classical Stöber procedure [21] for silica synthesis in alcoholic solutions. It was modified for aqueous solution, and a procedure was developed based on successive addition of (3-aminopropyl)triethoxysilane (APTES) and tetraethyl orthosilicate (TEOS) as precursors to the aggregate solution as indicated in the sketch of Fig. 1. This two-step addition is necessary due to the requirement of using an aqueous solution and having a negative surface charge on the C8S3 aggregates, a consequence of the sulfonate end groups. The amine-functionalized silane (APTES) is added first to the aggregate solution to function as an anchor group on the aggregate surface. It is thought to adsorb by electrostatic attraction at the negatively charged sulfonate

![Fig. 1 Sketch of chemical structure of the amphiphilic carbocyanine C8S3 and the two-step protocol [19] used to synthesize a closed shell of silica on top of the aggregates of the anionic carbocyanine C8S3](image-url)
groups (Scheme 1). Then, in a second step, the desired amount of TEOS is added to grow the silica shell. In order to form silica, the silica precursors must undergo hydrolysis, which is base catalyzed with the addition of ammonium (Scheme 2). The formation of the amorphous SiO₂ shell is, in principle, then a cross-linking between the silanes with loss of the ethyl groups resulting in ethanol as a byproduct (Scheme 3). The major challenge is to make this process to occur primarily on the aggregate surface to avoid unwanted gelation.

The most problematic step is the addition of APTES. If APTES hydrolyzes before associating with the aggregates, it can form nanoparticles with positive surface charge due to the amine groups. These positively charged particles can cause rapid flocculation of the negatively charged aggregates and sticking of aggregates to the container walls. Furthermore, small APTES particles may lead to inhomogeneous coverage of the aggregate surface. Therefore, it has to be added at neutral pH to avoid catalyzing its hydrolysis but hydrolyzed later.

For TEOS, the rate of hydrolysis is slow enough to favor deposition on the aggregate surface instead of direct gelation [22, 23]. However, it is barely soluble in water and it is prone to forming silica clusters with surface hydroxyl groups (e.g., on the glass surface of the vials). It could be possible for hydrolyzed TEOS to associate directly with C8S3 by hydrogen bonding to the sulfonate groups, but attempts to prepare C8S3 aggregates only with TEOS simply resulted in aggregates embedded in silica films when dried on the transmission electron microscope (TEM) grids. Coating of isolated aggregates was only obtained with APTES as an anchor group.

Nevertheless, we demonstrated the practicability of this process in our previous work [19] and showed by UV-vis spectroscopy that the resulting silica shells on tubular aggregates of C8S3 leave the molecular structure of the aggregates mostly unaffected. In addition to some structural characterization, we also claimed chemical stabilization of the aggregates and some enhancement of photostability. Despite the problems involved in this method, we discarded other methods for silica encapsulation under mild conditions, such as catalytic shell formation from silicic acid on cationic surfaces [24], since they are not compatible with the negative surface charge of the aggregates and charge reversal due to the adsorption of polycations did not produce a homogeneous covering [18].

In this publication, we describe the synthesis protocol in more detail than in the previous work [19] in order to clarify and discuss the implications of various parameter changes. This allows us to vary parameters in a targeted manner, such as the pH value or the preparation conditions, to achieve optimum results. As an advancement, we characterize the obtained structures with respect to their mechanical and chemical stability. It will be shown that these silica-covered structures are mechanically rigid and even appear to be brittle. We will further demonstrate that the silica shell protects the aggregates against changes in different solvent conditions, especially change of pH. We show that the structures are stable enough to be dried on solid substrates without considerable structural distortion and even can be investigated with an electron microscope under ultra-high vacuum conditions at room temperature, without further embedding into a matrix as is usually needed for cryo-TEM. Hence, a major improvement, with respect to the previous publication [19], is the optimization of the preparation protocol which leads to silica-covered tubular aggregates that are stabilized against deformation and environmental changes, including vacuum conditions. It is shown that this silica shell coverage leads to efficient stabilization and protection with all properties that are required for investigation of isolated, immobilized structures in dry and low gas pressure environments.

Scheme 1 Principal sketch of the chemical reactions leading to the formation of the SiO₂ shell by the sol-gel process from APTES and TEOS. (i) Hydrolysis of APTES (and TEOS) triggered by the addition of ammonium. (ii) APTES adsorbs electrostatically with the positive amine group to the negatively charged sulfonate groups of the aggregate surface. Addition of ammonium catalyzes the hydrolysis of these compounds and hence leads to the silica formation by cross-linking of hydrolyzed silanes.
Experimental

Preparation of C8S3 J-aggregates

The dye 3,3′-bis(3-sulfopropyl)-5,5′,6,6′-tetrachloro-1,1′-dioctylbenzimidacarbocyanine (FEW Chemicals, Wolfen, Germany) was used as received. Isolated tubular J-aggregates of C8S3 were prepared by a two-step dilution of a 2.92 mM methanol stock solution with deionized water. Methanol was purchased from Sigma Aldrich with a purity > 99.9% (GC grade). For a typical preparation, 130 μL of the methanol stock solution was added to 500 μL of deionized water in a clear glass vial and mixed with a vortexer by pulsing it three times for about a second. The solution immediately becomes bright pink upon adding the stock solution to water, indicating the formation of aggregates. The aggregate solution was stored in the dark for 24 h, after which an additional 500 μL of deionized water was added to slow the development of bundled aggregates. The dye concentration of the resulting solution is then 0.34 mM. The solution was stored in darkness and used within 3 days of preparation.

Silica coating of C8S3 J-aggregates

The general procedure used here is closely following the protocol given by Qiao et al. [19] The silica coated J-aggregates were prepared in disposable 1.5-μL Eppendorf tubes. (3-aminopropyl)triethoxysilane (APTES) with 99% purity was purchased from Sigma Aldrich and used without further purification. About 100 μL of both APTES and TEOS were transferred into disposable 1.5-μL Eppendorf tubes inside a nitrogen flushed glovebox where the relative humidity was 5% or less. The APTES and TEOS in the Eppendorf tubes were removed from the glovebox and the rest of the preparation was completed within 15 min under ambient conditions. Outside of the glovebox, the APTES was then diluted 1:49 v/v in methanol. TEOS was used with or without dilution according to the desired shell thickness. For every 250 μL of 0.34 mM C8S3 J-aggregate solution, 2 μL of freshly diluted APTES was added, and the solution was mixed either by tilting three times or pulse vortexing three times. As soon as the mixing was completed, TEOS and aqueous ammonia were added, repeating the mixing between the additions. Typically, for every 250 μL of C8S3 aggregate solution, 0.6 μL of neat TEOS, and 20 μL of 0.25% aqueous ammonia were added. This is in contrast to the previously published protocol, which was carried out without any adjustment of the solution pH. The samples were stored in the dark for at least 2 h (typically up to 24 h) before further use. The solution was diluted by a factor of 10 before depositing on a TEM grid.

Characterization methods

Absorption spectra of the solutions were collected by a Shimadzu RFPC 1201 photoparameter in transmission mode. Emission spectra were measured on a Jasco 6500 fluorescence spectrometer with a Xenon lamp. To avoid inner filter effects, 0.2 mm quartz sandwich cuvettes (Hellma) were used for measurements in solution. Samples for dry spectra were prepared by letting 30 μL of solution dry overnight on mica cut to fit into a cuvette adaptor. Sample and reference pieces of mica were cut from the same piece of freshly cleaved mica to ensure similar thickness for probe and reference sample in order to correct the spectra for absorption of mica.

Standard TEM images were collected with a JEOL JEM 2100 TEM operated at 200 kV. Images were collected using the TVIPS F416 CMOS camera and the EMMenu4 software. The scanning TEM (STEM) energy dispersive X-ray spectroscopy (EDXS) and electron energy loss spectroscopy (EELS) investigations were performed with JEOL JEM2200FS instrument set at acceleration voltage of 200 kV. The probe diameter for STEM high angle annular dark field (HAADF) imaging as well as EDX and EEL spectroscopies was 1 nm. Standard TEM grids with 6–10-nm thick amorphous carbon and holey-amorphous carbon, both on Cu 300-mesh supports, were purchased from Plano GmbH (S160-3-V and S147-3, respectively). Silica coated J-aggregates were deposited on the TEM grids anywhere from 2 h to 2 weeks after solution preparation. Grids without holes were plasma-treated 1 min with 30 W power and 0.2 mbar atmospheric air immediately before use (Electronic Diener Zepto plasma treater). Holey carbon film was chosen to allow for investigation of free-standing sections of individual aggregates spanning the holes. A typical sample was prepared by depositing a 6 μL droplet on the TEM grid, allowing it to sit for 30 s, and then carefully removing the excess liquid by blotting with a filter paper.

Scanning force microscopy (SFM) experiments are performed with a JPK NanoWizard III. For the intermittent contact mode, tips with resonance frequency of 300 kHz were used (Olympus OMCL-AC160TS 300 kHz 26 N/m). The samples were prepared by spin coating 30 μL solution, without any additional dilution, on a freshly exposed 1 × 1 cm HOPG surface with rotation speed of 30 rps.

Results and discussion

Optimization of shell synthesis

The type of structures formed by silica, and the rate at which they form, is known to be sensitive to pH, catalyst, the presence of salts, temperature, the solution composition, and the choice and concentration of precursors [23]. Due to the
sensitivity of the J-aggregates to solution conditions [18], the pH range that could be used was limited. The J-aggregates are oxidized under acid conditions, resulting in a loss of absorbance and fluorescence. This makes acidic conditions unattractive. However, the J-aggregates are stable under basic conditions up to about pH 10 [18].

When added at neutral pH, the positively charged amine groups on APTES associate with the sulfonate groups of C8S3 and can start to hydrolyze within the few minutes before TEOS is added. The hydrolyzed APTES can then cause condensation of TEOS directly on the aggregate surface without gel formation. However, APTES is also highly reactive with water. It is necessary to avoid even atmospheric moisture before addition to the J-aggregate solution to avoid hydrolysis of APTES and the formation of dimer and trimer oligomers before adding to the aggregate solution [25]. If oligomers of APTES do form, this can cause rapid flocculation and gelation of the aggregate solution, similar to the addition of a bivalent salt. Silanization around pH 9, with ammonia used as the base, is known to generally result in isolated particles instead of networks [21, 26]. Silica surfaces formed under basic conditions tend to have a negative surface charge, which stabilizes them as colloids [26].

In order to find the optimum pH range for the preparation of a homogeneous silica shell without gelation or precipitation, we varied the pH in a range acceptable for the aggregates. Samples were prepared by adding APTES to the aggregate solution with near neutral pH, then adding TEOS and finally ammonia to adjust the pH. This was carried out for pH 7, 8, 9, and 10. Sample preparation with a final pH of 9 was found to be the most reliable for avoiding gelation or flocculation. Samples prepared under all four pH conditions were imaged in standard TEM, and typical images are shown in Fig. 2. Only short tubule segments were found for samples prepared at pH 7 and 10, and these short segments appear to be connected by silica forming network structures. Samples prepared at pH 8 still had longer intact tubular structures, and the structures tended to be more isolated. Samples prepared at pH 9 typically had isolated, long, uniform looking tubules. If a sample prepared at pH 9 was stable after a few hours, then it would not gel or show serious changes in TEM images for weeks after preparation. Otherwise, the silica and aggregates would form a gel at the interface between the container wall and the solvent within a few hours.

The network formation at pH 10 is likely due to TEOS hydrolyzing too rapidly. If a silica precursor molecule, such as TEOS, undergoes hydrolysis multiple times before condensation, then it has multiple points available for condensation at the same time and can easily form networks. Networks could form directly in solution or starting from the molecules associated with the aggregates. The end result is a loosely networked structure with sections of aggregates embedded.
The similar formation of a network of short aggregate bundles at pH 7 is probably due to TEOS hydrolyzing too slowly. Since unhydrolyzed TEOS is very poorly soluble in water, if the hydrolysis step is slow, then the TEOS phase separates from the aqueous solution. This would then leave the aggregates with a partial coating of APTES. Unhydrolyzed APTES molecules encourage bundling by partially masking the aggregate surface charge, and APTES can rapidly dimerize with molecules associated with other aggregates. Cross-linking between aggregates that are not stiffened by a full silica coating could result in short pieces being broken off if there is any solution agitation. As TEOS gradually hydrolyzes, it can react with the APTES on the surfaces of these bundles and cross-linked aggregate segments.

The samples prepared at pH 8 and 9 probably have long, isolated, coated aggregates because the rate of TEOS hydrolysis is neither too fast nor too slow. TEOS can hydrolyze rapidly enough to be available for reaction with APTES before it causes bundling. With an intermediate rate of hydrolysis, only one or two active sites may be available on a TEOS molecule at any time, leading to more linear growth instead of networks. Sufficiently rapid growth of the TEOS shell may stiffen the aggregates so that short regions do not break off as frequently. Apparently, the condensation of TEOS is slower than that of APTES, allowing hydrolyzed TEOS to keep the surface charge negative without resulting in cross-linking when two growing shells come near each other. The results for pH 8 and pH 9 were quite similar, but samples prepared at pH 9 generally had fewer short segments.

The protocol with pH adjusted to 9 that most consistently produced stable solutions with what appear to be long, intact, isolated J-aggregate tubules in the TEM images was used for all the further characterizations described in this paper, if not mentioned otherwise. The details of this protocol are given in the experimental section. The samples prepared according to the optimized protocol will be referred to as “coated” for convenience. Dry samples were prepared by allowing 30 μL to dry in the dark overnight on a roughly 2 × 2 cm² piece of freshly cleaved mica.

While it was clear from the TEM images that the morphology and general shape of the aggregates was not disturbed, absorption and emission spectra were taken, because the shape of the spectra is very sensitive to any changes of molecular packing and hence the internal structure of the aggregates [27–29]. The spectra quasi serve as a “fingerprint” to probe the integrity of the aggregate structure. The optical spectra of aggregate solutions (see Fig. 3, the spectra of bare aggregates) can be explained reasonably well by a structural model where the aggregate tube is formed by a double layer of dyes [27, 29], as it was confirmed also by molecular dynamics simulations [30]. In each dye layer, also called tube wall, the dyes are assumed to be arranged in a herringbone structure that wraps helically up to form the cylindrical wall. The excitonic coupling gives rise to at least two Davidov split bands in each wall, one polarized along the aggregate axis, the other polarized perpendicular [27]. The transitions with parallel polarization appear as the absorption peaks at 589 nm for the outer and at 599 nm for the inner wall. The perpendicular polarized transitions are among the other peaks and shoulders between 520 and 580 nm (see Fig. 3a) that carry much less oscillator strength and will not be considered further here. The emission spectrum (see Fig. 3b, bare aggregates) essentially shows emission from the two intense exciton peaks with almost zero stokes shift. The intensity at the peak at 589 nm however is much less than the intensity at 599 nm and appears merely as a shoulder which is the result of

![Fig. 3](image-url) Absorption (a) and fluorescence (b) spectra of C8S3 aggregates with and without silica coating in solution and after drying on mica. Dry aggregates were prepared on mica, and for absorbance, a sheet of mica with identical thickness was used for the sample and the reference beam.
energetic relaxation due to excitonic coupling between these two states [31]. It is noteworthy to mention that the observation of a clearly recognizable shoulder at 589 nm in emission is an unambiguous signature for structural integrity [5] and more easy to detect for small amount of aggregates than an absorption signal. It is therefore a significant observation that for solutions of silica coated aggregates, not only the absorption but also the emission spectra are very similar compared with the bare aggregate solutions. Especially, the two strong absorption peaks at 589 nm and 598 nm are still present with almost unchanged spectral width and peak position. Only a very small red shift of less or approximately equal to 1 nm is observed for the peak intensity at 599 nm, see Fig. 3 a.

Spectra were also taken of samples allowed to dry on mica for at least 12 h, see dotted lines in Fig. 3. For the absorption measurements, a bare piece of mica with the same thickness was used as a reference. The absorption spectrum of the bare aggregate then has only a single broad peak centered at 591 nm. In contrast, the coated aggregate samples still had the two separate peaks centered at 588 nm and 599 nm in the absorbance spectra, though they were significantly broadened which is taken as an indication for disorder of the molecular packing [32].

The emission spectra are presented in Fig. 3 b for the solutions and the dried aggregates as well. For silica coated aggregates the two peaks at 589 and 599 nm are well recognizable in the dried state, albeit somewhat broadened. For bare dried aggregates, the broadening of the peaks is so large that the two distinct peaks cannot be resolved any more. This is in accordance with the broad absorption band in this case. The excitonic band structure is mostly destroyed, although excitonic coupling between the dyes is retained, which causes the red shift. In general, the spectra indicate that the arrangement of the dye molecules within the aggregates was not significantly disturbed, and that the coated aggregates retain their structure when dried.

It is remarkable that these aggregates can be imaged by standard transmission electron microscopy at room temperature. Typical images had already been shown in Fig. 2. In Fig. 4, the TEM image of bare aggregates (a) and silica-covered ones (b) are shown in a magnified view. The outer diameter of the coated aggregates was typically 22 ± 2 nm. The images demonstrate the possibility to investigate single, isolated aggregates outside the aqueous environment (as used for cryo-TEM) and in vacuum.

**Protection against chemicals**

The rough appearance of the silica structure on the TEM images (see Fig. 4) gives rise to the suspicion that the coating is porous. It is therefore important to check if the coating can protect the aggregates against changes in solution composition. For this, we make use of the fact that the aggregates lose optical activity when the pH is lowered below 4 due to protonation of the dye molecules [18]. Absorption spectra were taken of solutions of bare and coated aggregates before and after adding aqueous HCl to lower the pH to 2. For this, 45 μL of 21 mM HCl was added to 100 μL of bare or coated aggregate solution. The resulting spectra are shown in Fig. 5. The bare aggregate solution immediately became colorless and, as expected [18], the solution shows no absorbance at this acidic pH. The coated aggregate solution did not have any visible color change and was allowed to sit for 10 min before a spectrum was taken. The absorption spectrum of the coated aggregates at pH 2 is corrected to account for the change in concentration after addition of HCl. It exhibits the typical absorption spectrum of the aggregates, but with slightly reduced intensity of the sharp exciton peaks at 589 nm and 599 nm, which is accompanied by a slight broadening of these bands. This implies that the silica shell can protect most of the dye molecules from protonation and preserves the aggregate structure in acidic conditions.

Fig. 4 Transmission electron microscopy (TEM) images of bare (a) and silica-covered (b) aggregates. Images were taken at room temperature on samples dried on standard TEM grids with 6–10-nm thick amorphous carbon film. The typical outer diameter of the silica tubes appeared to be 22 ± 2 nm for the standard protocol as described in the experimental section.
Mechanical stability

The preservation of the typical aggregate spectra upon drying (Fig. 3) implies that the coating protects the aggregates from structural deformations. But it is also apparent from the TEM images that the silica-covered structures are quite straight with almost no curvature. In other words, the persistence length and hence the stiffness of the initially flexible tubular aggregates appears to be increased. However, from the TEM images, one cannot conclude about the strength of the silica shell. It could not be determined from the TEM images.

**Fig. 5** Absorption spectra of bare (a) and silica-covered (b) aggregates as prepared with a pH of solution 9 (blue and black curves) and after changing the pH of the solution to 2 by addition of hydrochloric acid, HCl (red curves). The absorbance of the coated aggregate solution at pH 2 is corrected for the change in concentration. The absorbance of the bare aggregates completely vanishes at acidic solution and can be identified in the graph by the small noise signal around zero.

**Fig. 6** SFM images and measured heights for (a) bare aggregates and (b) silica coated aggregates spin coated on HOPG (Tapping Mode™ height images). The height profiles are measured along the (green) line markers across the images. The structures with the lowest height also have the most uniform dimensions and therefore are identified as single tubes. Thicker structures are bundles of several tubes.
be gel- or rubber-like, but it also could be brittle like glass. Therefore, attempts were made to break individual units, with and without silica shell for comparison. For this purpose, bare and coated aggregates were deposited on HOPG surfaces by spin coating at very low rotation frequencies (about 600 to 1200 rpm). SFM was used to first image the aggregates in Tapping Mode™ and then also to manipulate individual aggregates in contact mode.

Typical SFM images recorded in Tapping Mode are shown in Fig. 6. Aggregate structures can be found for both bare and coated aggregates after depositing on HOPG and are easily identified by the thread-like structures. While the structures can vary in width and height, there are many structures with identical width and height. This common width and height are also smaller than for any of the variably sized structures. Structures with the minimum width and height are identified to be single aggregates. The larger objects are bundles, which have been previously observed in solutions of this dye [6]. Besides that, there are islands of flat objects with constant and regular height visible. Those are probably due to adsorption of monomers or remaining non-adsorbed silica. In case of the bare aggregates, large areas (dimensions of hundreds of nanometers) of a homogeneous background film were observed. It seems that in this case, either more monomers are remaining in the solution or aggregates also disintegrate upon adsorption at the HOPG surface—a possibility we do not investigate further here.

The measured height for bare aggregates is 8 nm, which is smaller than the $13 \pm 0.5$ nm diameter measured for bare aggregates in solution. Such a low height, less than the real tube diameter, was also observed in previous SFM investigations on aggregates dried on quartz substrates [5]. It is reasonable to assume that the aggregates are structurally destroyed upon drying. It could be that the double layer tubes simply collapse with the walls lying flat on top of each other. The width of one double layer wall is about 4 nm, so a collapsed structure would be in the order of 8 nm. Such a structural destruction could explain the changes of the absorption and emission spectra as shown in Fig. 3, where only an overall red shift of spectra is seen but no features of the exciton band structure of the double

![Fig. 7 SFM manipulation of bare and silica-covered aggregates of C8S3 dispersed on HOPG by spin coating. (a) Image of bare aggregates taken in Tapping Mode™. (b) Result of dragging the tip along the surface in manipulation mode, i.e., in contact mode with increased lateral force, at several places crossing aggregates. The manipulation paths are indicated by the (green) dotted arrows. The tip appears to cut through the aggregates and to remove material not only from the aggregates but also from the surrounding surface implying that there is actually a layer of dye molecules on top of the HOPG. The material is collected by the tip and released at the end of the manipulation path as a big lump upon retraction of the tip. (c) Image of coated aggregates taken in tapping mode. (d) Result of dragging the tip along the surface. The manipulation path is indicated by the (green) dotted arrow. One part of the coated aggregate is clearly broken off (part above the arrow); another part is kinked (part below the arrow). Broken pieces can be pushed around the surface, as is demonstrated by further images in the Supporting Information.](image-url)
wall tubular structure are recognizable. However, one also has to bear in mind that the height of the aggregates in Fig. 6 is only the relative height between the top of an aggregate and the surrounding substrate. Since the substrate is covered by a background film of molecular monomers, as mentioned above, it could also be that the aggregate is partially immersed into this layer, and the actual diameter of the aggregates would be larger than the measured height. Furthermore, the different interaction between the SFM tip and the aggregate or the substrate may cause height anomalies in this intermittent contact mode [33].

For the silica coated aggregates, the measured height is around 25 nm, in good agreement with the typical diameter found from TEM imaging. As seen from the height profile given in Fig. 6, also the larger object in Fig. 6 b has the same height. Apparently, this is a double stranded aggregate with both tubes lying parallel. The surface structure of the silica coated aggregates looks much rougher than in the case of the bare aggregates. This is in accordance with the TEM images.

Manipulation of single aggregates with the tip of an SFM is demonstrated in Fig. 7. Tapping Mode images of bare and coated aggregate samples before and after manipulation mode are shown. The manipulation consists of dragging the tip in contact mode with increased vertical force along the line marked with a dotted arrow. This manipulation of bare aggregates results in a gap in the aggregate structure and the appearance of a large deposit of material at the end of the manipulation path. This lump of material was collected by the tip during the manipulation and deposited there upon retraction of the tip. The remaining aggregate does not seem to have any significant plastic deformation or change in position. Additionally, the path of the tip can be clearly identified by deep (black) scratches crossing the aggregates. These scratches are removals of the surrounding layer of monomers or other loosely adhered material. The removed material contributes to the lump at the end of the manipulation path. Overall, one notices a decrease of the size of bare aggregates with time which becomes obvious from comparison of the images before and after manipulation. Based on our experience, we attribute this behavior to progressive drying out of the structures.

In contrast, the manipulation of silica coated aggregates shows a very different behavior. Dragging the tip across coated
aggregates causes segments of the aggregates to break off. No bending or plastic deformation is seen, as reported for other semi-flexible materials such as DNA [34] or dendronized polymers [35]. The aggregates are apparently brittle, and the segments are sufficiently rigid to be pushed around on the surface. This is further demonstrated in the Supporting Information. No loose material is removed from the surface along the tip path. This implies that the coated aggregates are truly laying on the HOPG surface rather than a bed of other dye molecules. The small islands of material that can be seen between the aggregates may be due to free dye molecules or silica precursors. These manipulation experiments provide strong evidence for enhanced mechanical stability of the coated aggregates. The mechanical rigidity combined with the fact that they can also span free-standing holes make these structures excellent candidates for further measurements of the mechanical elasticity by means of bending tests on individual aggregates similar to experiments performed by Kis et al. [36].

**TEM characterization of freely suspended aggregates in vacuum**

The enhanced mechanical stability of the coated aggregates provides new opportunities for studying these aggregates with minimal background signal from the surroundings. One interesting approach is using TEM grids with holey carbon films as a substrate. Aggregates suspended across the holes can then be studied in vacuum without any interference from the substrate. As can be seen in Fig. 8, the silica-covered aggregates are able to span holes. This was never observed for bare aggregates.

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**Fig. 9** Chemical analysis of silica-covered aggregates of C8S3. (a) STEM-HAADF image with some isolated single aggregates in the field of view, (b) integral EDX spectrum, (c) and (d) elemental map of silicon and oxygen of the field of view of (a). See text for more details.
because they pull back to the edges of the holes due to the drying process, see Fig. 8 a and b. This behavior is further demonstrated using fluorescence microscopy in the Supporting Information.

As one application of the robustness against vacuum drying in a TEM, we characterized the aggregates’ chemical composition by energy dispersive X-ray spectroscopy and related STEM imaging. In Fig. 9, a HAADF-STEM image (Fig. 9a) is shown together with respective EDXS elemental maps for silicon (Fig. 9c) and oxygen (Fig. 9d). The full EDX spectrum is also shown (Fig. 9b). In the EDX spectrum, signals not only for Si and O are found, but also for sulfur (S), chlorine (Cl), and carbon (C). These peaks result from the dyes and clearly show the presence of the molecules within the silica tubes. Additionally, copper (Cu) signal is visible due to the copper TEM grids. A quantitative analysis of the signals is difficult, since the molar ratio of the silica to dye is unknown. However, the intensity ratio of silicon to oxygen can be compared with a signal obtained from a thick film of SiO2. The data are shown in the Supporting Information. If both spectra are normalized to the peak intensity of the Si signal, it is found that the silica-covered aggregates have a slightly higher oxygen signal. This higher amount can be explained by oxygen present either from the fact that there may be remaining silanes that have not reacted according to Scheme 2 or from the sulfonate groups of the dyes. Overall, the excess of oxygen compared with a solid SiO2 film is less than 20%, and this is just triple the intensity of the sulfur peak. Although intensities of different elements cannot be compared directly, it tells that the oxygen excess is in the order of magnitude of what is expected for the oxygen present in the dye.

The high stability of freely suspended silica-covered aggregates makes them promising candidates for spectroscopic investigations using electron beams in a scanning TEM. As an example, we demonstrate the measurement of energy electron loss spectra (STEM-EELS) on single aggregates spanning holes of the carbon film. EELS measures energy loss of inelastically scattered electrons due to excitations of electrons of the material that is investigated. In the case here, we looked for inner shell ionizations (K and L edge) of relevant elements, such as silicon, oxygen, and carbon. For future times, it would be desirable to extend this technique to low energy electrons for the detection of electronic transitions in the visible range of few electron volts [37].

The main results for inner shell silicon and oxygen excitation are summarized in Fig. 10. An aggregate spanning a hole of the carbon film was identified by HAADF imaging, and EELS spectra were recorded while scanning the beam across the aggregate. The path of the beam is indicated in Fig. 10 a by the dotted arrow. The EELS spectra were deconvoluted using multivariate analysis calculation to produce element-specific intensity scan profiles [38]. Two of these intensity profiles, the one for the L2,3-edge of silicon and the other for the K-edge of oxygen, are plotted in Fig. 10 b. More information about the spectra and data collection is given in the Supporting Information. One can see that the profiles for silicon and the oxygen signals have the same extension and a similar shape. The intensities are not quantitatively comparable due to the different reaction cross sections. The shape can be explained assuming a simple cylindrical model similar to the one described in detail in the Supporting Information of Ref. [19]. Under the assumption that the signal strength is proportional to the thickness of the structure transmitted by the electron beam (which is justified, since this is proportional to the reaction volume of the beam), the profile is obtained from simple
thickness calculation along the electron beam path. Assuming the tubular aggregate is only covered by a homogeneous silica shell, the calculated EELS profile for the silicon signal looks like the profile that is indicated in Fig. 10b by the solid line. It has the typical peaks at the boundaries of the inner diameter and a circular trough in the center. The depth of this trough depends only on the thickness of the shell and is material-independent. For the fit in Fig. 10b, an inner diameter of 13 nm and an outer diameter of the shell of 25 nm was assumed. The intensity was adjusted manually for approximate fit of the data. If the inner space of the tube is filled with a homogeneous solid silicon cylinder of same density with diameter of 6 nm, then the dotted curve shown in Fig. 10b appears. The data points are between the empty and filled tube, which at least indicates that there is silica filling. This finding is in accordance with previous observations on accidentally found pieces visible from the front end of the tubes [19]. Overall, as with EDX, the silicon and oxygen profiles correlate well to the original tube thickness. However, additionally one can conclude from the EELS scans that the aggregates are not only covered by a silica shell but also are somehow filled in the inner core with silica.

Conclusions

An optimized protocol was elaborated to achieve a homogeneous and uniform coating of the tubular J-aggregates of C8S3 with a shell of silica with thickness of a few nanometers. These silica coatings improve the mechanical stability of the aggregates and decrease their sensitivity to changes in solutions. However, the nature of the products resulting from hydrolysis and condensation of silica precursors in water is very sensitive to reaction conditions and generally difficult to control in water. Preparations with small amounts of APTES are included in the solution is prepared quickly and without pre-exposing APTES to any water source.

The enhanced stability of the coated aggregates permits the study of isolated aggregates in vacuum. This provides the opportunity to investigate the aggregates with vacuum-based methods such as electron spectroscopy. Furthermore, the silica shell should provide a base for further functionalization of the aggregates that allows the investigation of optical properties that are due to distance-dependent interactions with a substrate, such as energy transfer, similar to studies of plasmon effects along metal nanowires [39, 40].

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Compliance with ethical standards

Conflict of interest  The authors declare that they have no conflict of interest.

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