Sol–gel entrapped light harvesting antennas: immobilization and stabilization of chlorosomes for energy harvesting†

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The chlorosome is a highly specialized supramolecular light-harvesting antenna complex found in green photosynthetic bacteria and is composed of self-assembled bacteriochlorophyll (BChl) pigments entrapped in a lipid vesicle. These organelles are of interest for development of synthetic devices for solar harvesting and conversion because the organization and packing of BChls in the chlorosome provides a highly efficient light collection and energy funneling mechanism with properties that are superior to similar artificial systems based on self-assembled BChl pigment analogues. In this study, we investigated sol–gel chemistry as an approach to entrap and stabilize chlorosomes isolated from Chloroflexus aurantiacus. Two distinct synthesis approaches that differed in the H2O/Si ratio in the gels were investigated. Spectrophotometric analysis showed that the chlorosomes were intact when encapsulated in sol–gels and did not suffer any deleterious effects during the entrapment process. In addition, the integrity of the chlorosomes was unaffected by methanol levels that can result during the formation of sol–gels. Using small-angle neutron scattering it was not only possible to characterize the properties of the sol–gel matrix but also the size, shape and aggregation state of the entrapped chlorosomes. The sol–gels formed at a higher H2O/Si ratio (FH gels) resulted in a more branched gel structure with a larger pore size compared to the gels formed at lower H2O/Si ratio (PH gels). The chlorosomes entrapped in FH gels had dimensions of \( \frac{1}{24} \times 16.0 \times 51.1 \times 180.1 \) nm which agrees well with the size of chlorosomes previously determined using cryo-transmission electron microscopy, while the chlorosomes in the PH gels appear to be aggregated. The approach described here offers new possibilities for the development of artificial solar-harvesting and energy conversion devices based on naturally occurring photosynthetic systems.

Introduction

Light harvesting is the first step in a complex succession of energy and electron transfer events that enable the conversion of solar energy to chemical energy by photosynthetic organisms. The diversities in habitats and available illumination have resulted in photosynthetic organisms developing numerous solutions for light harvesting through the evolution of different light-harvesting antenna systems. These antennas are highly specialized and are optimized to allow photosynthetic organisms to capture a maximum amount of the energy available in their environment. The exquisite molecular architecture found in photosynthetic organisms has provided inspiration for the development of synthetic analogues for conversion of solar energy to chemical and electrical energy. To date, the structural and functional properties of naturally occurring molecules have yet to be fully emulated by synthetic approaches. Therefore, strategies to immobilize and stabilize intact photosynthetic organisms or isolated components of the photosynthetic apparatus have received considerable attention for the development of biohybrid materials. Biohybrid materials have the advantages of maintaining the superior functional properties of natural biomaterials, stabilizing their catalytic function, increasing their longevity and providing a protective environment through entrapment in a synthetic system.

The sol–gel approach has received considerable attention for the entrapment and stabilization of biomolecules since biocompatible sol–gel processes were reported. One attractive feature
is the diversity in the functionality and architecture of materials that can be achieved by modification of the synthesis conditions, making it possible to produce a wide range of materials including films, gels and powders. Some of the modifications employed to entrap and stabilize biomolecules include careful control of the hydration level of the gels, addition of stabilizing agents such as glycerol\textsuperscript{7–9} and vacuum removal of alcohol produced during precursor hydrolysis.\textsuperscript{3,8} In addition, organically modified sol–gel precursors such as methyltriethoxysilane,\textsuperscript{9} gluconolactone-modified silanes\textsuperscript{10} and polyol-based silanes that release biocompatible alcohols such as glycerol\textsuperscript{11–13} have been used to achieve favorable conditions for biomolecule stabilization.

The high optical clarity and good mechanical and thermal stability achievable using sol–gel chemistry are essential properties for light-harvesting applications. Entrapped photoactive biomaterials including pigments,\textsuperscript{14,15} proteins\textsuperscript{7,16–19} and whole photosynthetic organisms\textsuperscript{20,21} have been reported. Advantageous use of the physical properties of sol-gels and the biochemical stability conferred by the gel microenvironment, have led to the development of photoactive gel materials. A notable example involved the entrapment of proteoliposomes bearing both the light-activated proton pump bacteriorhodopsin and F\textsubscript{0}F\textsubscript{1}-ATP synthase. By maintaining the integrity of the proteoliposome and the native activities of both proteins in hydrated gels, Luo \textit{et al.} were able to demonstrate light-driven phosphorylation of ADP to ATP in the bulk gel.\textsuperscript{22} Sol–gel entrapment not only stabilizes photoactive pigments and proteins but can also modulate native catalytic properties allowing biohybrid systems to carry out photochemical conversions not possible in nature. A recent report of direct coupling between the photodependent reactions of photosystems I and II facilitated by sol–gel entrapment exemplifies this advantage of biohybrid sol–gel materials for light harvesting and energy conversion.\textsuperscript{23}

In this study we investigated the sol–gel entrapment and stabilization of chlorosomes extracted from the phototrophic green filamentous bacterium \textit{Chloroflexus aurantiacus}. The majority of the light-harvesting antennas found in nature are protein–pigment complexes such as the chlorophyll-based light-harvesting complexes of eukaryotes and purple bacteria and the phycobilisomes of cyanobacteria.\textsuperscript{1} In contrast, chlorosomes are highly specialized large ellipsoidal organelles that are thought to be comprised of a lipid monolayer encapsulating an estimated 125 000 to 300 000 molecules of bacteriochlorophyll c (BChl c) and carotenoids that assembl in the absence of a protein scaffold.\textsuperscript{24,25} The transfer of excitonic energy to the reaction centers is thought to be facilitated by the lamellar arrangement of the stacked BChl c dimers in the chlorosomes.\textsuperscript{26–28} The chlorosomes are attached to the cytoplasmic cell membrane by a baseplate protein complex that binds BChl a pigments and serves as an intermediary in the energy transfer between the chlorosome and the B808–866 complex. The light absorbed by the chlorosome is ultimately transferred to a reaction center in the inner cytoplasmic membrane that facilitates electron transfer and generates a proton gradient across the membrane. Chlorosomes have drawn attention for the development of artificial photosystems because of the unique organization of BChls, which provides a highly efficient light collection and energy funneling mechanism desirable in synthetic devices.\textsuperscript{29–32}

We report here the entrapment and stabilization of purified chlorosomes using sol–gel chemistry. Small-angle neutron scattering (SANS) was used to investigate the structural properties of the resulting biohybrid materials. Using the contrast matching technique it was possible to observe the scattering of the silica matrices and entrapped chlorosomes independently of each other by varying the scattering length density of the buffer in the wet gels. Spectrophotometric characterization of the entrapped chlorosomes and the effect of methanol, a by-product of sol–gel formation, was also investigated.

**Experimental**

**Chlorosome preparation**

\textit{Chloroflexus aurantiacus} J-10-fl cells were grown anaerobically at 48 °C under 60 ± 10 \( \mu \)mol photons m\textsuperscript{2} s\textsuperscript{−1} as described previously.\textsuperscript{33,34} Intact chlorosomes were isolated by the approach of Feick \textit{et al.}\textsuperscript{35} with minor modifications. After sonication and removal of the cell debris by centrifugation at 20 000 \( \times \) g for 30 minutes, the membrane fraction was separated by ultracentrifugation at 200 000 \( \times \) g for 2 hours. The membrane fraction was then mixed with 2 M NaI in 20 mM Tris–HCl, pH = 8.0 and concentrated into floating pellets by ultracentrifugation at 135 000 \( \times \) g for 16 hours. Pellets were pooled and resuspended in 20 mM Tris–HCl, pH = 8.0, and then fractionated on a 15–45% sucrose density gradient.\textsuperscript{34} Purified chlorosomes with the baseplate attached were used in this study.

**Sol–gel synthesis**

Chlorosomes were entrapped in gels formed according to two different approaches after the method of Ellerby \textit{et al.}\textsuperscript{4} A fully hydrolyzed (FH) sol solution was prepared by mixing 5.0 g (33 mmol) of tetramethylorthosilicate (TMOS), 0.958 mL of 40 mM HCl (0.4 mmol) and 0.35 mL H\textsubscript{2}O (19.4 mmol) followed by sonication for \( \sim \)10 minutes in a bath sonicator cooled with ice. The PH sol solution was prepared by mixing 1.52 g (9.99 mmol) TMOS, 0.02 mL 40 mM HCl (0.4 \( \mu \)mol) and 0.35 mL H\textsubscript{2}O (19.4 mmol) followed by sonication for 24 hours to remove methanol produced by the hydrolysis of TMOS. In this study we investigated the sol–gel entrapment and stabilization of chlorosomes extracted from the phototrophic green filamentous bacterium \textit{Chloroflexus aurantiacus}. The majority of the light-harvesting antennas found in nature are protein–pigment complexes such as the chlorophyll-based light-harvesting complexes of eukaryotes and purple bacteria and the phycobilisomes of cyanobacteria.\textsuperscript{1} In contrast, chlorosomes are highly specialized large ellipsoidal organelles that are thought to be comprised of a lipid monolayer encapsulating an estimated 125 000 to 300 000 molecules of bacteriochlorophyll c (BChl c) and carotenoids that assembl in the absence of a protein scaffold.\textsuperscript{24,25} The transfer of excitonic energy to the reaction centers is thought to be facilitated by the lamellar arrangement of the stacked BChl c dimers in the chlorosomes.\textsuperscript{26–28} The chlorosomes are attached to the cytoplasmic cell membrane by a baseplate protein complex that binds BChl a pigments and serves as an intermediary in the energy transfer between the chlorosome and the B808–866 complex. The light absorbed by the chlorosome is ultimately transferred to a reaction center in the inner cytoplasmic membrane that facilitates electron transfer and generates a proton gradient across the membrane. Chlorosomes have drawn attention for the development of artificial photosystems because of the unique organization of BChls, which provides a highly efficient light collection and energy funneling mechanism desirable in synthetic devices.\textsuperscript{29–32}

Circular dichroism (CD) spectra were recorded on a Jasco 810 CD spectropolarimeter from 350 to 900 nm at 25 °C.

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Ultraviolet-visible (UV-vis) spectra were recorded from 300 to 900 nm using a Varian Cary 50 Bio spectrophotometer. The effect of methanol on the stability of the chlorosomes was investigated by suspending chlorosome samples in 10 mM sodium phosphate buffer containing 0 to 50% (v/v) methanol and recording their UV-visible absorption spectra at intervals during a 14 day time period. The samples were stored at 22 °C between measurements. The chlorosome sample in 10% (v/v) methanol was inadvertently destroyed before the measurement at day 14 was obtained.

SANS measurements

Single sol–gels either containing or lacking chlorosomes were measured in the presence of humidifying phosphate buffers at pH = 7.0 prepared from 20%, 60% or 100% deuterium oxide (D2O). This was accomplished by dividing each gel into three equal sections (~2 × 1 × 0.1 cm3) to ensure structural similarity in the contrast series. The gel fragments were then placed in 1 × 1 × 4.5 cm3 quartz cuvettes and equilibrated in the appropriate D2O-containing buffer prior to the SANS measurements. The samples were humidified during measurement by adding 0.1 mL of buffer to the cuvettes before sealing.

SANS measurements were made using the Bio-SANS instrument at the High Flux Isotope Reactor, Oak Ridge National Laboratory (Oak Ridge, TN). Bio-SANS was configured with an incident neutron wavelength of 6.0 ± 0.15 Å and a moveable 1 × 1 m2 position sensitive detector (Ordela, Oak Ridge, TN). Scattering from each sample was recorded at sample-to-detector distances of 1.1 m, 6.8 m and 15.3 m yielding data over 0.003 Å−1 < q < 0.7 Å−1 (q = (4π/λ)sinθ, λ is the incident neutron wavelength and 2θ is the scattering angle). The scattering intensity curves, I(q), were obtained by azimuthally averaging the processed two-dimensional detector images, which were normalized to the incident beam monitor counts and corrected for detector dark current, pixel sensitivity and solid angle geometry. For samples measured in 20% D2O and 100% D2O buffer, the scattering from the quartz cell was subtracted before the scattering from the appropriate buffer was scaled to 95% of the gel scattering intensity and subtracted. For the samples measured in 60% D2O, the scattering from the quartz cell was subtracted from all samples before the control gel was scaled to 95% of the chlorosome gel scattering intensity at q = 0.5 and subtracted to yield the scattering profile of only the entrapped chlorosomes.

SANS data analysis

Scattering intensity profiles were modeled using Igor Pro routines made available by the NIST Center for Neutron Research. The structures of silica-based sol–gel materials can be modeled as aggregates composed of fundamental (SiO2)4 units. A useful approach to calculating the small-angle scattering of aggregate structures has been proposed by Beaucage and applied extensively in various materials exhibiting hierarchical structure/assembly. By this approach, a unified model function can be constructed to include scattering from any number of “structural levels”. The scattering from each level is taken to be the sum of two functions—a Guinier exponential and a power-law that is “structurally” limited to zero scattering intensity at low and high values of q. Eqn (1) is a general form of the unified model for n distinct structural levels

\[
I(q) = \sum_{i=1}^{n} \left( G_i \exp \left( -\frac{q^2 R_{gi}^2}{3} \right) + B_i \exp \left( -\frac{q^2 R_{gi+1}^2}{3} \right) \left( \frac{6}{q} \frac{\text{erf} \left( q R_{gi} / R_{gi+1} \right)}{R_{gi+1}} \right)^p \right)
\]

where \( G_i \) and \( B_i \) are the Guinier and power-law prefactors, \( R_{gi} \) is the radius of gyration of the fundamental unit in the \( i \)th structural level, and \( P_i \) is the power-law exponent of the \( i \)th level.

An ellipsoid form factor was chosen for modeling the chlorosome scattering. The small-angle scattering arising from a tri-axial (semi-axes \( a \neq b \neq c \)) ellipsoid can be described by eqn (2)

\[
I(q) = I_B + \frac{I_d \rho^2}{V_{ell}} \int_{0}^{1} \phi^2(q) \left( q^2 \sin^2 \left( \frac{\pi X}{2} \right) \right) \frac{2}{X} \cos \left( q X \right) \, dq
\]

and \( I_B \) the constant incoherent background intensity, \( I_d \) a constant intensity scaling factor, \( R_{g} \) the ellipsoid radius of gyration and \( a, b, c \) the ellipsoid semi-axes. The Chimera modeling and visualization package was used to compare the model ellipsoid with a volume reconstructed from the electron micro-tomograph of a single C. aurantiacus chlorosome reported by Psenčík et al. and deposited in the Electron Microscopy Data Bank under accession number 1642.

Scattering from the FH and PH control gel samples in 100% D2O buffer was analyzed to characterize the surface properties of the solid silica matrices. Systems with a sharp two-phase interface, such as the silica–pore interface in these systems, generally exhibit characteristic power-law decay in scattering intensity as \( q \) approaches the upper limits of the small-angle scattering regime. Where the decay is proportional to \( q^{-4} \) Porod’s law can be applied to the measured scatter to determine the surface area of the interface, \( S_{SAS} \), and the mean chord lengths, \( L_{cv} \), or mean spatial extents of each phase measured along infinite random intercepts through the bulk two-phase system. Application of this analysis to other sol–gel materials has determined similar surface characteristics as those found from nitrogen-adsorption isotherm studies of those systems.

For the FH and PH gel systems, Porod’s law can be applied as

\[
\frac{S_{SAS}}{V_{ph}} = \lim_{q \to 0} \frac{I(q)q^d}{\pi \varphi_1 \varphi_2} \quad (3)
\]

and

\[
L_{cv} = 4 \varphi_2 \frac{V_{ph}}{S_{SAS}} \quad (4)
\]

where \( V_{ph} \) is the pore volume per unit mass of the solid phase, \( \varphi_1 \) and \( \varphi_2 \) are the volume fractions of the solid and pore phases,
respectively, and $\bar{l}_o$ is the mean chord length of the pore phase. The numerator of eqn (3) is known as the Porod constant that was determined as the intercept of the linear region of the scattering data plotted as $I(q)/q^4$ vs. $q^4$ for each sample. The denominator of eqn (3) is known as the scattering invariant which was determined by numerical integration after extrapolating the scattering patterns beyond the low- and high-$q$ limits of the small-angle regime ($10^{-5}$ Å$^{-1} < q < 10^1$ Å$^{-1}$) using the NIST NCNR analysis package. The incoherent background intensities modeled in the unified model for the FH and PH 100% D$_2$O control gel scattering were subtracted from each curve prior to determining the Porod constants and invariants. Volume fractions and pore volumes per unit mass of the solid phase for both gel types were determined using the bulk density of wet gel samples, the mass of solid silica remaining after lyophilizing the samples, and an assumed condensed silica density of 2.25 g cm$^{-3}$.

Results and discussion

The characteristics and properties of a sol–gel material are dependent on a number of factors that affect the rate of the hydrolysis and condensation reactions. These include pH, temperature, reagent concentrations, catalyst, and H$_2$O/Si mol ratio. In this study, the sol–gel syntheses were initiated by hydrolysis of TMOS in H$_2$O at a fixed TMOS/H$_2$O mol ratio with a catalytic amount of HCl. The resulting sol solution is then mixed with the chlorosome preparation in a phosphate buffer at pH = 7.0 which promotes condensation of the nascent silica particles to form optically clear silica gels. The chlorosomes become encapsulated in the interstitial pores of gel as the silica network forms. Two different syntheses, which differed in the H$_2$O/Si ratio in the hydrolysis reaction and the final sol–gel, were investigated to determine how the difference in synthesis condition affects the properties of the entrapped chlorosomes. In the first case, the H$_2$O/Si ratio was 8 in the hydrolysis reaction and 49 in the final gel. Under this synthesis condition, it is expected that the hydrolysis reaction goes to completion before the condensation reaction proceeds (termed FH gels), which results in the formation of Si clusters followed by agglomeration of SiO$_2$ to form the final gel structure. In the second approach, the H$_2$O/Si mol ratio in the hydrolysis reaction was 2 (termed PH gels) and 17 in the final gel resulting in a synthesis condition where gelation proceeds by the formation of linear or branched SiO$_2$ polymers that entangle to give the bulk structure. Based on the final H$_2$O/Si ratio in the gels, the PH gels are expected to form a denser SiO$_2$ matrix compared to the FH gels. This conclusion is supported by the pore volume fractions observed for the FH and PH control gels and by the surface areas and mean pore chord lengths determined from the small-angle scattering data, as summarized in Table 1. It is clear that, while both materials had similar surface areas per gram of silica, a greater silica volume fraction and a smaller mean pore chord length were determined for the PH gel matrix demonstrating its relatively higher SiO$_2$ matrix density.

SANS with contrast matching

SANS was used to investigate the structure of the chlorosome-free sol–gels, the effects of chlorosome entrapment on gel structure and also the shape of the entrapped chlorosomes. Small-angle scattering is routinely used to measure structures on

Table 1  Silica matrix characteristics for FH and PH control gels

<table>
<thead>
<tr>
<th>Sample</th>
<th>Matrix density (g cm$^{-3}$)</th>
<th>$\phi_{pore}$</th>
<th>Matrix surface area (m$^2$ g$^{-1}$)</th>
<th>Mean pore chord length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH control</td>
<td>0.093 ± 0.003</td>
<td>0.959 ± 0.04</td>
<td>699 ± 19</td>
<td>563 ± 94</td>
</tr>
<tr>
<td>PH control</td>
<td>0.201 ± 0.003</td>
<td>0.911 ± 0.02</td>
<td>797 ± 11</td>
<td>208 ± 18</td>
</tr>
</tbody>
</table>

Fig. 1  Scattering profiles from FH (A) and PH (B) gels hydrated with different D$_2$O-containing buffers. Profiles from gels hydrated with 100%, 60% and 20% D$_2$O buffer are shown in red, green and blue, respectively. In each case, data from chlorosome-containing gels are shown as solid circles while data from control gels are shown as open circles. The scattering profiles of control gels in 60% D$_2$O have been vertically offset for clarity.
the 10–5000 Å length scales, and SANS is uniquely capable of examining individual components of complex biological and biohybrid systems through the contrast matching technique. When the scattering length density (SLD) of a component in a sample “matches” that of the surrounding solvent or buffer, the scattering from that component is effectively masked and only scattering from the remaining components is observed. Since the difference between the neutron SLDs of water and deuterium oxide (D₂O) is relatively large, many components of biomolecule-containing samples such as proteins, lipids and nucleic acids can be selectively matched by simply dissolving or suspending the sample with an H₂O/D₂O buffer having a specific H/D ratio. Chlorosome-containing and control sol–gels were measured in buffers composed of 20% D₂O/80% H₂O (chlorosome contrast match point, Fig. S1†), 60% D₂O/40% H₂O (silica contrast match point†) and 100% D₂O to measure scattering from the gel matrix, the entrapped chlorosomes and the composite system, respectively. Gels for these experiments were cast in single pieces, aged for ~24 hours and then divided into equal pieces for incubation in buffers containing different H₂O/D₂O ratios to ensure structural consistency in each contrast series.

Fig. 1 shows the scattering profiles measured for the FH and PH gels in each buffer composition. In 100% D₂O, where all components contribute to the observed scattering, the scattering profiles of the FH chlorosome and control gels almost overlay each other. While the volume fraction of the silica matrix in the sample is much greater than that of the entrapped chlorosomes, the similarity between the profiles still suggests that chlorosome entrapment did not disrupt or change the gel structure when compared with the FH control. In contrast, the 100% D₂O profile of the PH chlorosome gel deviates greatly from the corresponding PH control at the smallest q values. Given the lower chlorosome content of the PH chlorosome gel compared with the FH gel, it is unlikely that entrapment of dispersed chlorosomes disrupted the PH gel structure. The absence of this increase in the 20% D₂O data, which show scattering from silica only, further supports this interpretation. The increase in scattering intensity at low q is most likely caused by aggregation of the chlorosomes inside the gel structure. The circular dichroism spectra discussed later also suggest that the PH gel entrapped chlorosomes were aggregated.

The effect of contrast matching is most obvious in the scattering observed from the FH and PH control (silica only) gels equilibrated with 60% D₂O. In both cases, the scattering intensity is q-independent and nearly constant giving good confidence that scattering from silica structure is largely absent from the profiles measured for the FH and PH chlorosome gels in 60% D₂O. An analogous extinction of chlorosome scattering occurred in the gels equilibrated in 20% D₂O. The successful contrast matching of the silica and chlorosome components, respectively, allowed for the independent analyses of FH and PH gel structure and entrapped chlorosome shape discussed below.

Sol–gel structure

The unified model described in the Materials and methods section was used to interpret the structural details of the FH and PH sol–gels. Gel structure was examined in control samples in 20% and 100% D₂O and chlorosome-containing samples in 20% D₂O since scattering from each arose entirely from the silica matrix. A model with three structural levels was found to best reproduce the experimental data for both gel types. Fig. 2 shows the fits of the SANS data for the chlorosome containing FH and PH gels at the chlorosome contrast match point (solid black line), and the models agree well with the experimental data (open circles). The dotted gray lines represent the fits to the SANS data at the three different length scales and are interpreted as the structural levels in the material for a fundamental SiO₂ cluster, an aggregate of such clusters, and the association of these aggregates to form the bulk sol–gel matrix. The fitted curves for the control gels in 20% and 100% D₂O are presented in the ESI (Fig. S2–S5†). The two structural parameters obtained for each structural level are the radius of gyration of the scattering unit, Rg, and the power law exponent, P. In this study the Rg values are interpreted as the sizes of the silica structures that dominate the scattering profile in

![Fig. 2](https://example.com/fig2.png)

**Fig. 2** Fits of SANS data from FH (A) and PH (B) chlorosome-containing sol–gels at the chlorosome contrast match point (20% D₂O). Scattering data are shown as open circles. The broken lines are the intensities calculated for each level in the unified model which when added to the incoherent background (thin solid line) sum to give the total calculated intensity (thick solid line).
each of the three structural levels. The power-law exponents correspond to the fractal dimensions of the structural levels and provide insight into the organization of the gel structure. Structures composed of linear or branched polymeric chains have fractal dimensions of 1–2 and 2–3, respectively. Exponents of 3–4 are obtained for surface fractals, and values > 4 indicate that the solvent interface is not well-defined as might occur if there is solvent penetration into the material.\textsuperscript{52}

A simultaneous fitting approach was chosen for modeling the structures of the FH and PH gels. By this approach, structural parameters derived from each scattering profile could be constrained to be equal because the samples were from fragments of the same gel or from gels synthesized from the same sol solution. For example, in fitting the profiles from the FH gels, the fundamental unit radius of gyration, \(R_g\), and power-law exponent, \(P\), of each of the three structural levels were correlated as equivalent for the 20\% and 100\% \(\text{D}_2\text{O}\) control gels since these samples were prepared from the same gel and, therefore, had identical structures. Additionally, the values of \(R_g\) and \(P\) derived from the “smallest” structural level of the 20\% chlorosome gel were also correlated as equal to those from the 20\% and 100\% controls. The FH chlorosome and control gels were prepared using the same sol solution, and the smallest silica particles are expected to form entirely during the sol hydrolysis prior to the addition of chlorosomes for entrapment.\textsuperscript{48} These physically relevant correlations of structural properties also served to reduce the number of free fitting parameters relative to the available data. A summary of the structural parameters obtained from fitting profiles from the FH and PH gels is shown in Table 2.

Across the three FH gels, the fundamental unit was found to have a \(R_g\) of \(\sim 21.2\) \(\text{Å}\), a value similar to that found for other silica-based sol–gel structures.\textsuperscript{53,54} The fitted \(R_g\) values of the intermediate level in the control and chlorosome gels were similar at \(\sim 84.3\) \(\text{Å}\) and \(\sim 91.4\) \(\text{Å}\), respectively, and the power-law exponent for the largest structural levels were found to be \(\sim 2.3\) and \(\sim 2.4\), respectively. While a Guinier term was included for the second structural level, an accurate determination of \(R_g\) was not possible due to the absence of a Guinier region. However, we can estimate that the \(R_g\) must be \(\sim 500\) \(\text{Å}\) or larger because a smaller \(R_g\) would influence the curve shape and diminish the quality of the fit. The similarities of the intermediate unit size and power law exponent for the two gels suggests that the entrapped chlorosomes did not affect the aggregation of silica clusters or the mass-fractal packing of these aggregates and, therefore, had only a small effect on the final gel structure on the length-scales observed by SANS.

In the case of the PH gels it was possible to obtain a single set of structural parameters from the three-level model that describe both the chlorosome-containing and control gels which suggests that the gel structure is essentially unaffected by the presence of chlorosomes. This is expected given that the \(\text{SiO}_2\) content of PH gels is approximately 2.2 times greater than its FH gel counterpart and the structural parameters of the FH gels are also similar with and without chlorosomes. Furthermore, using a single structural model further reduced the number of free parameters relative to the available data without compromising the goodness of the fit as is apparent from Fig. 2B. The fundamental unit of the PH gels was found to have a \(R_g\) of \(\sim 12.9\) \(\text{Å}\) which is somewhat smaller than that of the FH gels. In the PH gels, the condensation reaction proceeds before complete hydrolysis of TMOS which may result in the formation of smaller silica particles compared to the FH gels (\(\sim 21.4\) \(\text{Å}\)). The smaller values of \(R_g\) along with the reduced power law exponents for the second and third structural levels, indicate that the \(\text{SiO}_2\) matrix of the PH gels is dominated by entangled linear (\(\text{SiO}_2\)) chains compared to the branched structures found in the FH gels.

**Spectrophotometric analysis of the entrapped chlorosomes**

The UV-vis absorption spectra of the chlorosome sol–gel samples and the control gels without chlorosomes prepared for SANS measurements are shown in Fig. 3. The absorption spectrum of chlorosomes has a characteristic peak at \(\sim 740\) \(\text{nm}\) (\(Q_y\) band), which is assigned to assembled BChl c oligomers and a peak in the Soret region at \(464\) \(\text{nm}\) which is due to the porphyrin ring of BChl. The presence of a shoulder in the spectrum at \(792\) \(\text{nm}\) is due to BChl a and indicates that the baseplate protein is associated with the chlorosome.\textsuperscript{55} Disruption of the chlorosomes results in a decrease in the intensity of the \(Q_y\) band and a concomitant appearance of a peak at \(\sim 666\) \(\text{nm}\) due to the presence of free BChl c monomers. These spectral features can be used to distinguish between intact and disrupted chlorosomes. All gels have good optical properties which is an important feature for potential roles in solar energy conversion. Overall, the spectra are consistent with previously published spectra of chlorosomes isolated from *C. aurantiacus*\textsuperscript{56,57} and display the characteristics of intact chlorosomes with the baseplate protein attached, as described above. The absorption spectra of the gels at \(\sim 2\) weeks after synthesis show that the entrapped chlorosomes maintain their viability in the gels.

Circular dichroism spectroscopy also provides information about the structural integrity of the chlorosome. The CD signal of chlorosomes arises from excitonic interactions of the self-assembled BChl c as has been observed in the CD spectra of chlorophyll–protein complexes.\textsuperscript{58} Disruption of the BChl c assembly results in a loss of CD signal, and can, therefore, be used to determine the structural integrity of entrapped chlorosomes. The CD spectra of the chlorosomes entrapped in the FH and PH gels are shown in Fig. 4. The spectrum from chlorosomes

<table>
<thead>
<tr>
<th>Gel composition</th>
<th>Structural level</th>
<th>(R_g) (Å)</th>
<th>Power law exponent ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH – control</td>
<td>1</td>
<td>53.0 ± 11.9</td>
<td>2.34 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>84.3 ± 2.7</td>
<td>3.91 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>21.2 ± 0.7\textsuperscript{b}</td>
<td>4.88 ± 0.18\textsuperscript{b}</td>
</tr>
<tr>
<td>FH – chlorosome</td>
<td>1</td>
<td>481.8 ± 19.5</td>
<td>2.36 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>91.2 ± 6.7</td>
<td>3.72 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>21.2 ± 0.7\textsuperscript{b}</td>
<td>4.88 ± 0.18\textsuperscript{b}</td>
</tr>
<tr>
<td>PH – control</td>
<td>1</td>
<td>185.9 ± 1.8</td>
<td>1.56 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43.4 ± 1.9</td>
<td>2.52 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.9 ± 0.4</td>
<td>3.14 ± 0.80</td>
</tr>
<tr>
<td>PH – chlorosome</td>
<td>1</td>
<td>185.9 ± 1.8</td>
<td>1.56 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>43.4 ± 1.9</td>
<td>2.52 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.9 ± 0.4</td>
<td>3.14 ± 0.80</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All parameters were correlated in fitting the PH gel scattering.

\textsuperscript{b} Correlated as equal in fitting the FH gel scattering data.
in the FH gel displays an “S”-shaped signal with a positive maximum at 755 nm, a negative maximum at 725 nm and a zero-crossing at 740 nm. This is similar to spectra previously reported for bacteria grown under low light conditions\textsuperscript{56,57} and provides further evidence that the chlorosome structure remained intact after entrapment.

The CD spectrum of chlorosomes entrapped in the PH gel is similar to that from the FH gel except that the positive maximum has a threefold greater rotation for chlorosomes in the PH gel. Such an increase in the rotational strength of the CD signal has been observed from chlorosomes previously\textsuperscript{56,58} and is thought to be due to a change in the aggregation or the oligomeric geometry of the BChl c. Similar increases (so-called PSI-induced CD) have been reported in chirally organized systems such as DNA, chromatins and viruses in response to polymer or salt induced aggregation.\textsuperscript{59} According to Keller and Bustamante\textsuperscript{60} there exists a correlation between rotational strength and the particle size of an object. Furthermore, larger chiral objects with dimensions similar to the wavelength of incident light have a greater effect on the absorption of circularly polarized light than do their smaller counterparts.\textsuperscript{60} Given these prior interpretations of chlorosome CD spectra it is logical to conclude that the chlorosomes entrapped in the PH sol–gel were aggregated instead of being evenly dispersed throughout the silica matrix.

**Effect of methanol on chlorosome stability**

When TMOS undergoes full hydrolysis four equivalents of methanol are released into the sol solution resulting in methanol concentrations $\leq 10\%$ (v/v) in the final FH and PH sol–gel mixtures. The stability of the chlorosomes in methanol-containing solutions is of interest since methanol is commonly used to extract and solubilize BChl c from isolated chlorosomes and is known to react with various free chlorophylls yielding allo-merized or trans-esterified porphyrins.\textsuperscript{61–64} Therefore, it was necessary to determine if the amount of methanol released during TMOS hydrolysis could adversely affect the chlorosomes. Chlorosomes were suspended in buffered methanolic solutions (0–50\% (v/v) methanol), and their absorption spectra were recorded at intervals for a 14 day period. The characteristic peak at $\sim$740 nm (the $Q_y$ band) in the absorption spectrum arises from structured BChl c oligomers, which are expected in an intact chlorosome. A decrease in the magnitude of this peak occurs when the chlorosome is ruptured and the organization of the BChl c oligomers is disrupted.\textsuperscript{65} Fig. 5 summarizes the change in absorbance of the $Q_y$ band over time. Loss of $Q_y$ absorbance occurs for all methanol-containing solutions and the methanol-free control, and it is evident from the data that there is no significant effect due to methanol at concentrations $\approx 20\%$ (v/v) when compared with the control. Therefore, the methanol formed during the entrapment process should not have had a negative impact on chlorosome stability in agreement with the UV-vis spectra of the gel-entrapped chlorosomes.

**Fig. 4** Circular dichroism spectra of chlorosomes entrapped in FH (solid line) and PH (dashed line) sol–gels. Spectra are normalized to the OD$_{740}$ of each sample.
SANS analysis of entrapped chlorosomes

The scattering profile for the entrapped chlorosomes in the FH and PH gels obtained at the silica contrast match point is shown in Fig. 6A. The scattering curves were fit with a triaxial ellipsoid form factor as described in Materials and methods. The results of the fitting are shown in Table 3.

The dimensions of the chlorosomes in the FH gel are similar to previously reported values obtained using transmission electron microscopy providing good confidence that the chlorosomes are not distorted or aggregated in this gel. Fig. 6B shows a graphical comparison of the ellipsoid form calculated from the FH-entrapped chlorosome scattering data with a volume-reconstruction of a single *C. aurantiacus* chlorosome from previously reported cryo-electron microscopy tomography data. The remarkable similarity of the model form to the dimensions and general shape reported for these chlorosomes in solution further indicates that sol–gel entrapment does not result in significant changes in chlorosome structure. In contrast to the chlorosomes entrapped in the FH gels, the values obtained from fitting the chlorosomes entrapped in the PH gels are larger than expected and support the CD data which indicates that the chlorosomes are aggregated in the gels. The ability to distinguish between aggregation states of the chlorosomes in the different sol–gels using SANS is important for designing, constructing and characterization of future devices based on this technology.

Conclusions

In this study we report, for the first time, the entrapment and stabilization of chlorosomes in a sol–gel matrix. These organelles are of interest for development of synthetic devices for solar harvesting and conversion because the organization and packing of BCHls in the chlorosome provides a highly efficient light collection and energy funneling mechanism with properties that are superior to similar artificial systems based on self-assembled BCHl pigment analogues. Spectrophotometric analysis showed that the chlorosomes are intact when encapsulated in sol–gels and do not suffer any deleterious effects during the entrapment process. Using small-angle neutron scattering it was not only possible to characterize the properties of the sol–gel matrix but also the size, shape and aggregation state of the entrapped chlorosomes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Diameter (nm)</th>
</tr>
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<tbody>
<tr>
<td>FH-gel entrapped</td>
<td>SANS</td>
<td>16.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180.1 ± 29.5</td>
</tr>
<tr>
<td>PH-gel entrapped</td>
<td>SANS</td>
<td>19.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71.5 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>247.4 ± 197.9</td>
</tr>
<tr>
<td>Psencik et al.28*</td>
<td>Electron</td>
<td>10–20</td>
</tr>
<tr>
<td></td>
<td>microscopy</td>
<td>30–60</td>
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<tr>
<td></td>
<td></td>
<td>140–220</td>
</tr>
</tbody>
</table>

*Fig. 5* The effect of methanol on chlorosome stability. Methanol concentrations were 0% (white), 10% (light gray), 20% (medium gray), 30% (dark gray) and 50% (black) (v/v).

*Fig. 6* Small-angle scattering from gel-entrapped chlorosomes. (A) Data from chlorosomes entrapped in the FH and PH gels are shown as open circles and open squares, respectively, and the calculated scattering profiles from model ellipsoid forms are shown as the solid lines. (B) Comparison of *C. aurantiacus* chlorosome (solid) and model ellipsoid form (mesh) calculated from fitting the scattering of chlorosome entrapped in the FH gel. The chlorosome volume was reconstructed from the electron density map reported by Psencik et al.28
chlorosomes. This study demonstrates the great potential that neutron scattering methods hold for characterizing the structure of complex biobrad systems and for studying the effects of entrapment on the structural properties and distribution of an encapsulated biomolecule or supra-molecular complex.

As described in the introduction, electron transfer from chlorosomes to semiconductor materials has been demonstrated. Sol-gel encapsulation offers new possibilities for entrapment and stabilization of complex photoactive biomolecules that are of central importance for the development of artificial solar harvesting and energy conversion devices. This approach produces optically clear materials which can be molded, cast or deposited onto substrates as thin films. In addition, the entrapment of the chlorosomes in a rigid matrix provides a protective environment against external factors and will increase device performance and longevity. Future directions will include investigating approaches to interface thin films of the encapsulated chlorosomes directly with electrode surfaces and catalysts for electricity and fuel production.

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References