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Zinc chlorins for artificial light-harvesting self-assembly into antiparallel stacks forming a microcrystalline solid-state material

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We introduce a concept to solve the structure of a microcrystalline material in the solid-state at natural abundance without access to distance constraints, using magic angle spinning (MAS) NMR spectroscopy in conjunction with X-ray powder diffraction and DFT calculations. The method is applied to a novel class of materials that form (semi)conductive 1D wires for supramolecular electronics and artificial light-harvesting. The zinc chlorins 3-devinyl-3'-hydroxyethyl-132-demethoxycarbonylphoophoribide a (3',5'-bis-dodecylxoy)benzyl ester zinc complex 1 and 3-devinyl-3'-methoxymethyl-132-demethoxycarbonylphoophoribide a (3',5'-bis-dodecylxoy)benzyl ester zinc complex 2, self-assemble into extended excitonically coupled chromophore stacks. 1H-13C heteronuclear dipolar correlation MAS NMR experiments provided the 1H resonance assignment of the chlorin rings that allowed accurate probing of ring currents related to the stacking of macrocycles. DFT ring-current shift calculations revealed that both chlorins self-assemble in antiparallel π-stacks in planar layers in the solid-state. Concomitantly, X-ray powder diffraction measurements for chlorin 2 at 80 °C revealed a 3D lattice for the mesoscale packing that matches molecular mechanics optimized aggregate models. For chlorin 2 the stacks alternate with a periodicity of 0.68 nm and a 3D unit cell with an approximate volume of 6.28 nm3 containing 4 molecules, which is consistent with space group P21221.

artificial antenna | density functional theory | microcrystalline structure | solid state NMR | X-ray diffraction

Green sulfur bacteria and the green nonsulfur bacteria are found in anoxic environments where the light intensities can be extremely low; with single chlorophyll photon absorption rates <1 photon per hour. To survive under such low light intensities, green bacteria such as Chlorobium tepidum evolved chlorosomes, very efficient extramembrane antenna systems that consist of 1–2 × 103 (1–3) self-organized bacteriochlorophyll (BChl) molecules in highly ordered suprastructures. Chlorosomes accomplish ultrafast energy transfer via pigment-pigment interactions, instead of pigment-protein interactions. They have the longest exciton diffusion lengths of any known pigment assembly at room temperature (4–6). The extremely fast exciton mobility leads to favorable light-harvesting efficiencies of these systems, which recommends these aggregates for possible applications in supramolecular photonic, electronic or photocatalytic devices for molecular electronics or solar energy conversion (7, 8).

It is known that the self-assembly of BChl c pigments observed in chlorosomes is replicated by BChl c aggregates in nonpolar solvents like hexane (9–11). Tamiaki and coworkers developed a model zinc chlorin compound, which is a BChl d analogue that forms extended dye aggregates and replicates the aggregation behavior of BChl c in nonpolar solvents (12). In recent years, some of us have demonstrated that the self-assembly process of BChl c, d, and e into a tubular structure can be mimicked by using semisynthetic zinc chlorin 1 (Fig. 1) molecules that are preprogrammed for this mode of self-assembly, i.e., possessing the 3'-OH group, a central metal ion, and 13Cl–O moieties along the Qy axis, and containing at least 2 extended alkyl tails at the 172 carboxylic acid substituent (13). In addition, self-assembly of highly ordered 1-dimensional (1D) π-stacks of zinc chlorin 2 (Fig. 1), with the OH substituted by a methoxy functionality, was observed by scanning tunneling microscopy (STM) on conductive and atomically flat highly oriented pyrolytic graphite (HOPG) surfaces (14).

Hence, detailed spectroscopic and microscopic investigations have already provided convincing evidence for the formation of extended π-stacks of these chromophores in solution and on surfaces (14). However, a detailed local crystal structure to elucidate the packing of the zinc chlorins (hereafter chlorin) 1 and 2 in bulk is hitherto unknown. The packing behavior of these dyes in the solid state is of importance considering the potential for such closely spaced 1D π-stacks and layered assemblies in organic electronics and photovoltaics. Suitable single crystals for a complete X-ray crystal structure analysis are not available for these microcrystalline materials, which led us to explore the utilization of solid-state NMR in combination with powder diffraction, molecular modeling and DFT calculations to provide information about the 3D packing of the chromophores in the bulk.

Recently, MAS NMR has been successfully developed and used for structure determination of uniformly enriched compounds or multiple spin clusters in solid state biological systems, disordered systems, and polypeptides. It has been used in 13C and 15N enriched samples to study the self-organization of BChls in chlorosomal light harvesting antennae (11, 15). Labeling of the semisynthetic chlorins containing isotope atoms is possible using 13C and 15N enriched starting materials to study the self-organization of BChls in chlorosomal light harvesting antennae (11, 15). Labeling of the semisynthetic chlorins containing isotope atoms is possible using 13C and 15N enriched starting materials. However, materials research critically depends on the preparation and screening of multiple compounds for realizing artificial systems with the desired properties. Considering the high costs that labeled starting materials demand, this would be an expensive endeavor, because a large fraction of the labeled material is lost in a multistep synthesis or derivatization process. In such cases it is more practical and economical to have a method to screen...
resolved 1H-13C cross peaks in the 2D spectra. Quantum unsaturation leads to a favorable dispersion of NMR signals and of macrocycles, via the chemical structure of the molecule. The probing of ring-current effects that can be related to the stacking proton chemical shifts of aggregated chlorins allows accurate sized, unsaturated molecules of low symmetry. Access to the environments for every molecule. The 31-OH and 31-OCH3 which implies a local packing order with unique structural peaks without significant broadening is detected for compounds 

resonances corresponding to the chlorin ring unambiguously for compounds 1 and 2 as depicted in Fig. 2 A–D. A single set of peaks without significant broadening is detected for 1 and 2, which implies a local packing order with unique structural environments for every molecule. The 31-OH and 31-OCH3 resonances for chlorins 1 and 2 respectively, were not observed even at lower contour levels, possibly because of some form of dynamics and inefficient cross-polarization (11, 17). The solid-state proton shift values have been listed in Table 1 as \( \sigma^H_1 \) and \( \sigma^H_2 \) respectively.

Aggregation shifts are defined as chemical shifts in the solid relative to the monomer shifts in solution. To obtain these monomer shifts, chlorin dyes 1 and 2 were dissolved in the coordinating solvents tetrahydrofuran-d8 (THF-d8) and pyridine-d5, respectively. The 1H monomer shifts for 1 and 2 are listed in Table 1 as \( \sigma^H_{1, \text{liq}} \) and \( \sigma^H_{2, \text{liq}} \) respectively. The aggregation shifts for 1 and 2 given by \( \Delta \sigma^H_i = \sigma^H_i - \sigma^H_{i, \text{liq}} \) are listed in Table 1. Significant upfield aggregation shifts are observed, both for chlorins 1 and 2 and these have been depicted as circles for values less than −2 ppm in Fig. 1, where the circles have a radius proportional to the magnitude of the shift.

For the assignment of the 1H shifts, the FSLG technique was applied to solid samples of chlorins 1 and 2 (16). Fig. 2 shows the 2D heteronuclear 1H-13C spectrum where the assignment is indicated. It was possible to assign almost all of the proton correlation spectra of chlorins 1 (A and B) and 2 (C and D) recorded at a field of 17.6 T employing a spinning rate of 13 kHz and sample temperatures of 298 K. The assignments of the chlorin rings are indicated.

**Results and Discussion**

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![Fig. 1. Chemical structure of 31-hydroxy and 31-methoxy zinc chlorins 1 and 2. Detected 1H upfield aggregation shifts of aggregated chlorins 1 and 2 relative to their monomers in THF-d8 and pyridine-d5, respectively, are shown as solid circles. Calculated ring-current shifts for 1 and 2 in the antiparallel stack model are shown as dashed circles. Values less than −2 ppm are depicted. The circles have a radius proportional to the magnitude of the shift.](image1)

![Fig. 2. Contour plot sections of 1H-13C heteronuclear MAS NMR dipolar correlation spectra of chlorins 1 (A and B) and 2 (C and D) recorded at a field of 17.6 T employing a spinning rate of 13 kHz and sample temperatures of 298 K. The assignments of the chlorin rings are indicated.](image2)
From a correlation plot comparing the solid state chemical shifts of 1 and 2 with their monomer shifts in solution, it is clear how pronounced the $^1$H aggregation shifts are within the $^1$H chemical shift range (Fig. S1). The $^1$H aggregation shifts are dominated by ring-current effects, in line with observations in other chlorin aggregates and are more useful than the $^{13}$C shifts for resolving the structure (Fig. 1) (18, 19). They are an accurate probe of the secondary fields induced by the aromatic rings. The magnitude of the aggregation shifts for chlorins 1 and 2 (cf. Fig. 1 and Table 1) are very similar, which indicates that the pattern of stacking and the stacking motif is similar for both 1 and 2 in the solid, despite a pronounced difference at the 31 side chain, OH with H-bonding capability for compound 1, and a methoxy functionality for compound 2. The importance of the hydrogen bond has been questioned before, because extended stacks were found for simplified artificial BChl mimics due to packing by hydrophobic interactions without hydrogen bonding (20). The absence of the $–$OH resonance in the data of compound 1 is well in line with studies on Mg-chlorin aggregates of biological origin and semisynthetic Cd-chlorin derivatives. Apparently, the $–$OCH$_3$ behaves in a similar way, due to unfavorable cross-polarization (CP)-MAS properties, corroborating a similar role for the $–$OCH$_3$ in compound 2 compared with the $–$OH moiety in 1.

To explain the aggregation shift patterns, structural models of coordinating chlorins for 1 and 2 were built based on previously published AFM and STM data in Hyperchem 7 (13, 14). The first is the parallel stack model in which the arrangement of the chlorin cores is directed by the coordination of hydroxyl or methoxy groups to the zinc ions, and the alkyl chains of one stack are all arranged in the same direction (Fig. 3A), whereas the second is the antiparallel monomer stack model in which the alkyl chains of neighboring chlorins are oriented in opposite directions (Fig. 3B) (21). These models have been selected because compound 1 is shown to form rod like aggregates, which are thought to be built from the parallel stack motif. Compound 2, however, has been shown to aggregate with either the antiparallel stack motif or the parallel stack motif. To simplify the quantum mechanical calculations, the 17–3,5-bis-dodecylxybenzylalcohol tail has been replaced by a 17-methyl group and the 8-ethyl is replaced by an 8-methyl group.

For chlorins 1 and 2, an initial parallel stack model with 6 molecules each was constructed and optimized using molecular mechanics with an MM+ force field. To investigate the possibility of H-bonding between stacks, larger aggregates of 1 were built by optimizing multiple stacks placed together with their 31-OH group of one stack within H-bonding distance of the 13$^3$C=O of the adjacent stack. In this way a twenty four molecule aggregate of 4 hexamer stacks was optimized for chlorin 1. The interstack H-bond network remained intact on optimization. Due to the presence of the methoxy functionality at 31 in chlorin 2, H-bonding is not possible and a single parallel stack of 12 chlorin 2 molecules was optimized. This single parallel stack for compound 2 was energetically stable. Likewise, to optimize the antiparallel stack model for 1 and 2, a single stack of 6 molecules each was constructed. Optimization revealed that these structures are unstable as single stacks. A second 6 molecule stack was added for both 1 and 2 in the interstack H-bonding arrangement for compound 1 and with the methoxy functionalities pointing toward the 13$^3$C=O of the adjacent stack for compound 2. On optimization, energetically stable aggregates were obtained for both compounds. This confirms that a stabilization mechanism is in operation to promote aggregation. To determine the factor leading to this stabilization, various contributions to the total energy for a single stack and 2 stack aggregate of compounds 1 and 2 are listed in Table S1. It was found that although the electrostatic contribution to the total energy is the most dominant, it increases linearly with aggregation. Hence, it does not contribute to the stabilization of the aggregates. There is no contribution at all from H-bonding to the stabilization of compound 2. This is to be expected because there is no H-bonding functionality present at 31. Interestingly, for compound 1 there is also no contribution from H-bonding to the total energy. However, both for compounds 1 and 2, the contribution from the nonbonding interactions or the van der Waals interaction increases nonlinearly with aggregation i.e., its contribution increases 4 to 5 times on going from a single stack to a 2 stack aggregate. This nonbonding energy corresponds to the stabilization from increased π–π stacking on going from a single to multiple stacks (can be seen in Fig. 4A). As a result, for the antiparallel stack model both for compounds 1 and 2, the increased π–π overlap on increased aggregate size leads to an energetically cooperative stabilization mechanism, in an extended space filling structure. Under different sample

![Fig. 3. Schematic representation of the models used for ring-current shift calculations. (A) is the parallel-stack model and (B) is the antiparallel stack model.](image-url)
preparation conditions, other arrangements could be feasible that also involve hydrogen-bonding between the stacks. Such phenomena of polymorphism are well-known for organic pigments, e.g., in the structurally related phthalocyanine pigments (22). These aggregates were further extended to 4 stacks as shown in Fig. 4A for chlorin 2. 3H ring-current shifts were calculated for chlorins 1 and 2 in the parallel (Fig. S2) and antiparallel (Fig. 1) stack models and are listed in Table 2.

From the differences between the experimental and the calculated ring current shifts for the 4 models (Table S2) it is clear that the aggregation shifts of chlorin 2 are in very good quantitative agreement [Table S2, $\Delta \sigma_{H}^{\text{calc}} - \Delta \sigma_{H}^{\text{calc}}$ (A) values] with the calculated ring-current shifts for the antiparallel stack arrangement. In contrast, the calculated ring-current shifts for 1 are in semiquantitative agreement with the observed aggregation shifts for the antiparallel stack model although they reproduce the essential features, in particular the combination of very strong aggregation shifts in the 3-CH2 region and significant 12-CH3 shifts. We also modeled aggregates of 1 in the closed-dimer model (Fig. S3), which has been discussed in the literature (15, 21). Ring-current shift calculations revealed a substantial mismatch (Fig. S4 and Table S3) allowing us to eliminate this model. It is also known that compounds 1 and 2 in non polar solvents (14, 23) show an aggregate bathochromic shift of the $Q_y$ bands of $\approx$80–100 nm, and such shifts are not possible for dimers. Because similar solvent combinations are used to obtain solid aggregates, closed-dimers are unlikely.

The magnitude of the aggregation shifts observed for the protons 121 for both compounds 1 and 2 precludes their existence as individual 1-dimensional $\pi$-stacks in the solid-state. This is because in a single antiparallel stack there is no significant ring overlap in the region of 121-CH3 that could cause such a strong upfield shift. Overlap with chlorins from adjacent stacks is thus essential.

The alkyl tails constitute a factor that is important for the supramolecular organization of the 1D $\pi$-stacks. Chlorin 2 forms extensive lamellae of $\pi$-stacks on an atomically flat HOPG surface, as has been shown by the STM technique. On HOPG this arrangement is most probably favored by the strong intermolecular interaction of alkyl chains with graphite. Probably the interdigitation of tails between adjoining stacks plays an important role in stabilizing the extended lamellar structure that is observed on surfaces, in contrast with the extended H-bonding in the natural BCHl stacks.

To gain further insight into how these alkyl chains influence the 3D packing of $\pi$-stacked chlorin 2 in the solid state, X-ray powder diffraction was measured at ambient temperature and at 80 °C and the results are shown in Fig. 5. Both diffraction patterns show nonequidistant and very sharp reflections in the small and wide angle regions, which confirm a highly ordered 3-dimensional arrangement of the dyes in these phases. In addition, the wide angle scattering at 80 °C shows a diffuse part with its maximum at $\approx$4.5 Å ($2\theta \approx 20^\circ$), which could be

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Fig. 4. Proposed 3-dimensional packing of chlorin 2 in an alternating antiparallel $\pi$-stack arrangement: (A) Antiparallel stack model of 2 from MM+ calculations with approximate unit cell. (B) Schematic representation of a possible packing matching the model and the X-ray data (see Table 3 and Results and Discussion) in projections along b (Upper) and c (Lower) with unit cell axes a–c. The arrows and +/- signs, respectively, show the direction of the O—Zn coordinating bond within an individual $\pi$-stack. Additionally, the symmetry elements for this hypothetical packing (space group $P2_12_12_1$) are shown. The orientation of the chains is arbitrary, but consistent with the proposed symmetry. The dashed line indicates a lattice plane parallel to (211), which is comparatively densely occupied by Zn atoms in this model.

Fig. 5. X-ray diffraction pattern of 2 at ambient temperature (gray lines) and at 80 °C on heating (black lines). Inset shows an enlarged plot of the wide angle range; numbers indicate the reflection numbers for the pattern at 80 °C used in Table 3.
attributed to a partially liquid-like packing of the chains. The small-angle reflections at 80 °C may be indexed with satisfactory accuracy on a primitive 2-dimensional rectangular grid with lattice constants \( a = 28.6 \text{ Å} \) and \( b = 32.3 \text{ Å} \) (cf. experimental and calculated \( d \) spacings in Table 3). The 3 strongest reflections in the wide-angle region are also numbered (reflections 9–11). The sharp peak at \( 2\theta = 26° \) (reflection 11) indicates a definite long-range order in the structure of the chlorin \( 2 \) with intervals of 3.4 Å. This value is characteristic of densely \( \pi \)-stacked dyes and delivers the interplanar distance of the chlorins in the direction perpendicular to the 2-dimensional cell. This assumption is supported by the literature known \( \pi \) - \( \pi \) distances in crystals of stacked zinc phorphyrins (3.46 Å) (24, 25).

A comparison with the dimensions measured for the packing obtained from the MM + optimized antiparallel stack model shows, that twice the distance between the chlorin \( 2 \) rings along the stacking direction is a periodic one and can be considered as the third dimension \( h = d_{\text{calc}} \) (reflection 11) = 6.8 Å in an orthorhombic 3D unit cell with a volume of \( V = a \times b \times h = 6.28 \text{ nm}^3 \). However, 1 molecule of \( 2 \) in the crystalline state has an approximate volume of 1.47 nm\(^3\) estimated according to the volume increment method of Immirzi and Perini (26). Thus, the unit cell should contain 4 molecules of \( 2 \) with a molecular volume of 1.57 nm\(^3\), a reasonable value for the less dense phase at 80 °C, which is also consistent with the packing model. The 3D interpretation is further supported by the strong intensity of reflection 9. Using the lattice parameters from the NMR models, it may be indexed 201, and in the packing model, the \( \pi \) atoms occupy this lattice plane comparatively densely. A possible space group consistent with these considerations would be \( P2_1 2_1 2_1 \).

Fig. 4 shows the antiparallel stack model as derived from NMR studies and MM + calculations and a schematic depiction of the corresponding 3D packing mode that was derived from the XRD data collected at 80°. It is not possible to uniquely index the XRD pattern at ambient temperature. As can be seen in Fig. 5, however, the strongest reflections are only slightly changed in position and intensity (reflections 1, 2, 3, 9, 10, 11). Thus, even though the space group symmetry may be different at room temperature (most probably monoclinic or triclinic) the principal packing mode of the \( \pi \)-stacks appears the same as observed at 80 °C where the alkyl chains are partially molten and the material transforms into a less ordered soft crystalline phase.

**Materials and Methods**

Zinc chlorins \( 1 \) and \( 2 \) were synthesized starting from Chl \( a \) extracted from *Spirulina platensis* cyanobacteria (13, 14, 23). The intermediate compounds and the final zinc chlorin dyes \( 1 \) and \( 2 \) were purified by silica-gel column chromatography and subsequently by reverse-phase HPLC, and were characterized by solution-state \( ^1 \text{H} \) NMR and high resolution mass spectrometry (HRMS). Analytical HPLC was performed on a system (PU 2080 PLUS) with a diode array detector (MD 2015) from Jasco equipped with a ternary gradient unit (LG 2080–02) and line degasser (DG-2080–533). Semi-preparative HPLC was performed on a system (PU 2080 PLUS) with a diode array detector (UV 2077 PLUS) from Jasco. HPLC grade solvents (Rectapur) from VWR were used. Reversed phase columns obtained from Macherey-Nagel (analytical: EC 250/4, 6 NUCLEODUR 100–5 C18 ec, precolumn: CC 8/4 NUCLEODUR 100–5 C18 ec; semipreparative: SP 250/21 NUCLEODUR 100–7 C18 ec, precolumn semi-preparative: SP 50/21 NUCLEODUR 100–7 C18 ec) were used. Zinc chlorin \( 1 \) was eluted at 6.1 min with a flow rate of 1 ml/min with methanol/CH\(_2\)Cl\(_2\)(7:3) as eluent whereas zinc chlorin \( 2 \) was eluted at 8.9 min with a flow rate of 1 ml/min with methanol/CH\(_2\)Cl\(_2\)(7:3). The components eluting before the characteristic retention times for \( 1 \) and \( 2 \) were discarded.

The \( ^1 \text{H} \) NMR solution spectra were recorded at 25 °C with a 400-MHz spectrometer (Bruker). Chemical shifts (\( \delta \)) are relative to TMS. The monomer \( ^1 \text{H} \) shifts were determined in coordinating solvents, for zinc chlorin \( 1 \) \((\sigma_\text{ch})\) THF-\( d_8 \) and for zinc chlorin \( 2 \) \((\sigma_\text{ch})\) pyridine-\( d_5 \). Chlorin \( 2 \) has enhanced solubility in pyridine-\( d_5 \) and we have confirmed that the chemical shifts exhibit negligible changes compared with those in THF-\( d_8 \). Additionally, \( ^1 \text{H} \) solution NMR spectra were obtained in freeze-dried samples obtained from MeOH. The \( ^1 \text{H} \) solution NMR measurements were performed at 298 K on a Bruker AV-400 spectrometer. The \( 1 \)H spins were decoupled during acquisition using the 2-pulse phase modulation (TPPM) scheme (27). Two-dimensional \( ^1 \text{H}-^1 \text{C} \) heteronuclear correlation experiments. The \( ^1 \text{H} \) spins were decoupled during acquisition using the 2-pulse phase modulation (TPPM) scheme (27). Two-dimensional \( ^1 \text{H}-^1 \text{C} \) heteronuclear correlation experiments were obtained by using the frequency-switched Lee-Goldburg (FSLG) experiment, by using a short CP time of 256 ms and a \( ^1 \text{H} \) 90° pulse of 3.1 ms (16).

All geometry optimizations were done using molecular mechanics in Hyperchem 7 with an MM + force field and used a Polak-Ribiere conjugate gradient method.
BChl molecules that are significant in causing ring-current effects. The structural model used for the DFT calculations included the surrounding chemical shift (NICS) quantum mechanical calculations were performed (34). Field induced by the ring current effect in the BChls, nucleus-independent shifts were calculated using the Gauge-Independent Atomic Orbital (GIAO) method (32, 33). To calculate the magnetic shielding due to the local magnetic field induced by the ring current effect in the BChls, nucleus-independent chemical shift (NICS) quantum mechanical calculations were performed (34). The structural model used for the DFT calculations included the surrounding BChl molecules that are significant in causing ring-current effects.


X-ray diffraction measurements on a powder-analogue sample kept on a beryllium plate in a temperature controlled heating stage were carried out with a Guinier goniometer (Huber GmbH and Co. KG), using quartz-monochromatized CuKα radiation.

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