

Structure Determination of a Bio-Inspired Self-Assembled Light-Harvesting Antenna by Solid-State NMR and Molecular Modeling

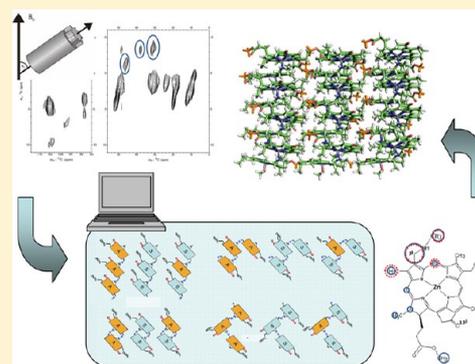
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S Supporting Information

ABSTRACT: The molecular stacking of an artificial light-harvesting antenna self-assembled from 3¹-aminofunctionalized zinc-chlorins was determined by solid-state NMR in combination with quantum-chemical and molecular-mechanics modeling. A library of trial molecular stacking arrangements was generated based on available structural data for natural and semisynthetic homologues of the Zn-chlorins. NMR assignments obtained for the monomer in solution were validated for self-assembled aggregates and refined with ¹H–¹³C heteronuclear correlation spectroscopy data collected from samples with ¹³C at natural abundance. Solid-state ring-current shifts for the ¹H provided spatial constraints to determine the molecular overlap. This procedure allows for a discrimination between different self-assembled structures and a classification of the stacking mode in terms of electric dipole alignment and π – π interactions, parameters that determine the functional properties of light-harvesting assemblies and conducting nanowires. The combination with quantum-mechanical modeling then allowed building a low-resolution packing model *in silico* from molecular stacks. The method allows for moderate disorder and residual polymorphism at the stack or molecular level and is generally applicable to determine molecular packing structures of aromatic molecules with structural asymmetry, such as is commonly provided by functionalized side chains that serve to tune the self-assembly process.



■ INTRODUCTION

Inspired by the biological design of natural chlorosomes, semisynthetic artificial light-harvesting antennae and photosynthetic reaction center units have been made through noncovalent self-assembly of suitably functionalized chlorin dye molecules, controlled by the dynamic self-assembly equilibria that are established by cooperative intermolecular interactions and steric crowding in the side chains.^{1–5} Their capability to harvest light and transfer the excitation energy over long distances critically depends on the collective dielectric properties, alignment of electric dipoles, hydrogen-bonding interactions, and molecular overlap,^{6–8} which can be tuned by the chemical modification of functionalities of natural molecular building blocks.^{9,10} Such semiartificial supramolecular assemblies have been recently shown to not only have highly efficient electronic energy transfer properties but also function also as highly conducting electrical nanowires.¹¹ Efficient methods to determine the molecular packing interactions inside these assemblies are essential for rational, directed design of novel materials for energy conversion; however, the lack of strict long-range order, variability in size and composition, and restricted polymorphism often renders such structures unsuitable for high-resolution diffraction methods to resolve their organization and packing.

Solid-state NMR for structure determination has been steadily developing in recent years as a bottom-up technique. Abundant distance constraints are collected at short range, typically 0.3 to 0.6 nm, and are used to model a high-resolution structure bottom-up without taking into account higher-order top-down constraints. While this approach may work well for proteins and highly ordered crystalline material, it is of limited use for self-assembled supramolecular systems that are subject to moderate heterogeneity due to limited control of a well-defined suprastructural framework over the microstructure. An alternative way is to first determine the supramolecular packing framework and resolve microscopic packing modes in a top-down approach.¹² Recent examples are the computational integration of ultrahigh field CP-MAS NMR data and diffraction results that was used for resolving the packing interactions of various types of chlorosome light-harvesting assemblies.^{13–21} For chlorophyll assemblies, this has provided profound insight into how the self-assembly processes can be

Special Issue: Rienk van Grondelle Festschrift

Received: March 4, 2013

Revised: April 7, 2013

Published: April 8, 2013

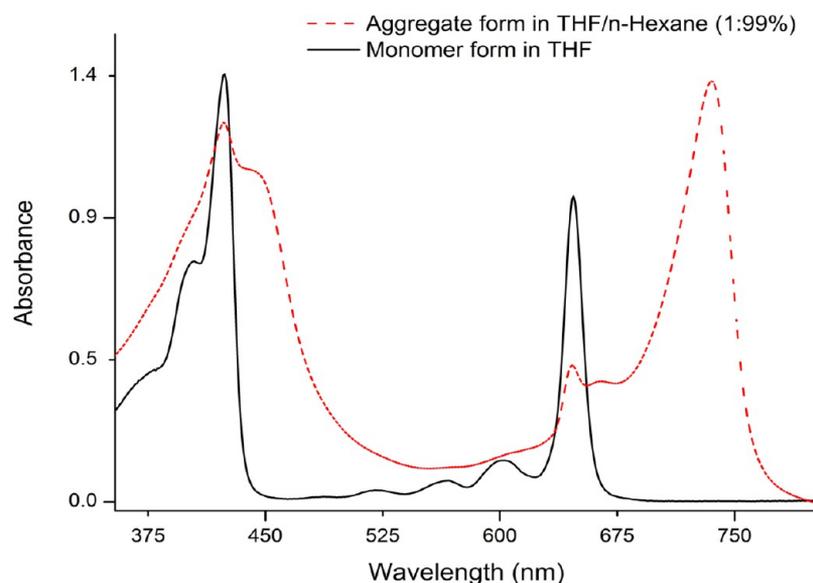


Figure 1. Absorption spectra of **6** in THF (monomer) and in THF/*n*-hexane (1:99%, aggregate form).

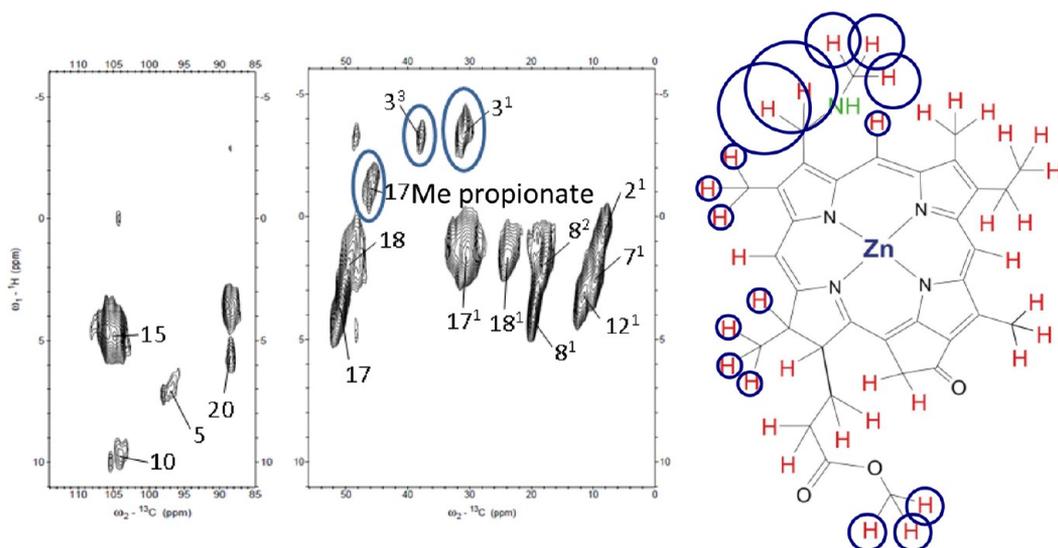


Figure 2. Left: ^1H - ^{13}C HetCor spectrum (mixing time 256 μs) of aggregates of **6**, with large aggregation shifts indicated in the spectrum. Right: the chemical structure of **6**, with aggregation shifts of $\Delta\sigma \geq 2$ ppm indicated as blue circles. The sizes of the circles are proportional to the observed aggregation shifts.

steered by inserting dislocations in a homogeneous packing framework with pseudosymmetry and restricted polymorphism at the molecular level.²¹

In this contribution to this special issue in honor of Rien van Grondelle, we combine sparse magic-angle spinning (MAS) NMR heteronuclear dipolar correlation spectroscopy data with molecular modeling and density functional theory (DFT) calculations as a concept to provide an evidence base for resolving molecular stacking and packing for a new class of amino-functionalized Zn-chlorins that can form self-assembled light-harvesting antennae for possible use in modular artificial photosynthesis device vehicles. A library of trial structures is constructed from prior knowledge collected on related natural and artificial specimens and by an homology approach we propose to circumvent the immediate need for isotope labeling, cryo-EM, or low-resolution diffraction data for every individual sample when determining structure.

EXPERIMENTAL METHODS

For the formation of aggregates of **6**, 100 times excess of *n*-hexane was added to **6** dissolved in tetrahydrofuran (THF). The sample was incubated overnight at 4 °C in the dark to form precipitates. The sediment was transferred in several steps to centrifuge tubes and spun for 7 min at 2000 rpm. The pellet was loaded directly in a 4 mm CRAMPS rotor and dried under vacuum overnight. All handling of the sample was performed in the dark or using green light and under a nitrogen flow to prevent photodamage. 1D ^{13}C and 2D ^1H - ^{13}C frequency-switched Lee-Goldburg (FLSG) heteronuclear correlation experiments were performed with a Bruker AV-750 spectrometer equipped with a 4-mm triple resonance MAS probe head using a ^{13}C radio frequency of 188.6 MHz and a sample temperature of 293 K. Spinning frequencies of 13 kHz were used. The ^1H chemical shift scale was calibrated from a FSLG spectrum of solid tyrosine HCl salt.

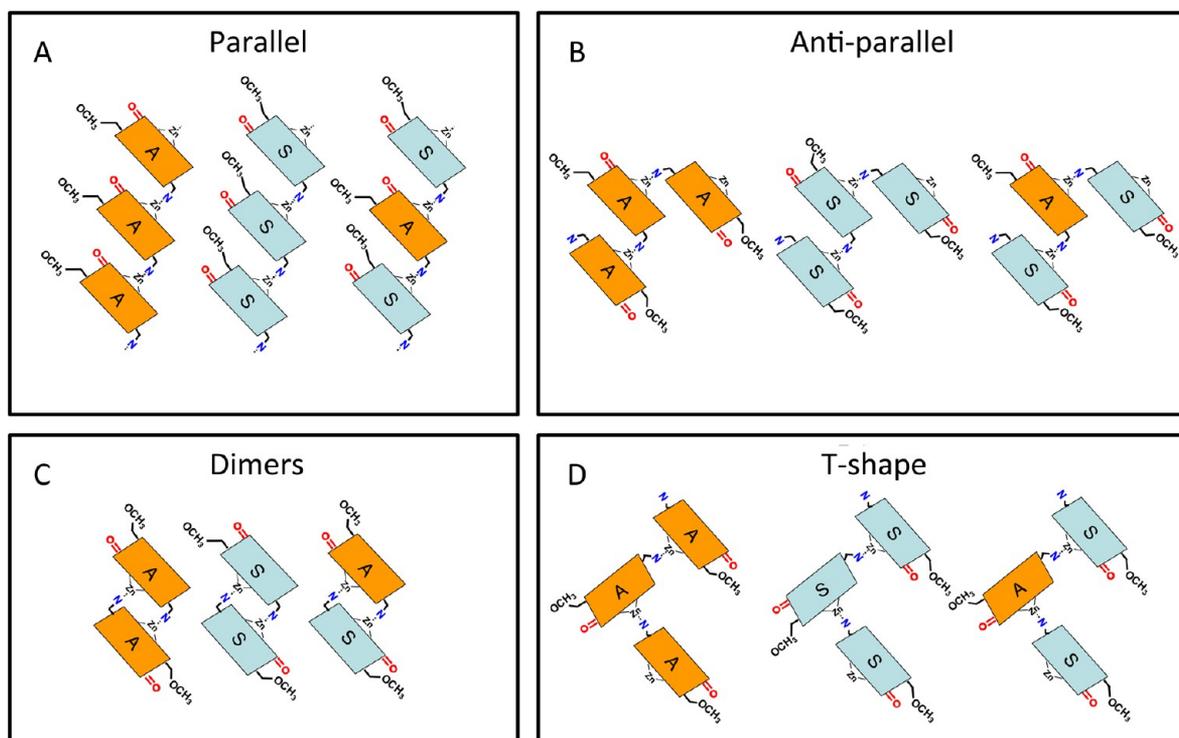


Figure 3. Trial library of stacking modes for **6**. (A) Parallel anti, syn, and syn-anti stacks. (B) Antiparallel anti, syn, and syn-anti stacks. (C) Anti, syn, and syn-anti dimers. (D) T-shaped anti, syn, and syn-anti stacks.

DFT calculations were carried out with the ADF program package (ADF2010, SCM, Theoretical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands, <http://www.scm.com>), and NMR chemical shift calculations were performed with the Becke–Perdew (BP86) functional and a triple- ζ polarized (TZP) Slater-type orbitals (STOs) basis set.^{22,23}

RESULTS AND DISCUSSION

3^1 -Methylamino-functionalized Zn-chlorins aggregates were prepared by diluting the 3^1 -methylamino Zn-chlorin-*d* (**6**) in THF with a large amount of excess hexane (99:1 THF/hexane). Aggregation resulted in a decrease in the Q_y band of the monomer at 647 nm and an increase in the aggregate band at 735 nm within ~ 1 h (Figure 1). From the optical data, it is obvious that aggregation takes place, but nothing conclusive about the packing can be derived.

Figure 2 shows the 2D heteronuclear ^1H – ^{13}C spectrum of the supramolecular aggregates of **6** with the NMR assignments indicated. Strongly upfield shifted proton resonances with $\sigma^{\text{H}}_i < 0$ ppm are from ^1H nuclei that show pronounced ring current shifts and are highlighted by the blue circles. A strong cross peak around 106 (^{13}C) and 5 ppm (^1H) is observed, of which the shift in the carbon matches the NMR chemical shift of the C15, which does not have a directly attached proton but apparently acquires a substantial signal from nearby protons that are not directly attached to ^{13}C nuclei. While the response of the C15, without a direct proton attached, appears very strong in the solid-state HETCOR response at 102 ppm, it can be unambiguously assigned in the solution ^{13}C APT spectrum shown in the supplement to a unique methine bridge response at 105 ppm from an unprotonated ^{13}C (Figure S10, Supporting Information). We attribute the strength of this signal in the solid state to multiple nearby protons in the structure in the solid aggregate that can transfer their polarization to the C15. It

is unlikely that this signal represents, for example, the C5 ^{13}C response by signal reversal in the solid state relative to the monomer in solution. The APT data show a characteristic C5 signal at 97.6 ppm that is apparently reproduced at 96.6 ppm in the solid state, whereas a reversal of C15 signals across a large shift range of ~ 9 ppm has not been encountered across the library of semisynthetic systems that we have studied before. The aggregation shift of 3.1 ppm for the C15 is considerable, and in depth investigations will have to be performed in the future to find out the details of the molecular mechanisms behind this shift, which may also lead to a further refinement of the stacking model for the aggregate.

Similar cross-correlation signals have been observed in ^1H – ^{13}C heteronuclear dipolar correlation spectra of Chl aggregates.^{19,24} For most protons, including the 3^1 and the amino-methyl ^1H that experience large ring current shifts, a single ^1H NMR response is detected, providing strong evidence that the Zn-chlorins form a uniform extended supramolecular framework with a similar packing environment for all monomers. However, within this uniform scaffolding environment, restricted polymorphism is observed: The cross-correlation peaks involving the C10 and C5 show a splitting of the signal in both the ^1H and the ^{13}C dimensions, indicative of two slightly different conformers of the Zn-chlorin molecules in the assembly. This makes an approach aiming for a unique high-resolution structure essentially obsolete.

To quantify the aggregation-induced ring current shifts $\Delta\sigma^{\text{H}}_i$, we compared the solid-state chemical shifts (σ^{H}_i) of the amino Zn-chlorin aggregates with the NMR chemical shifts for monomers in CD_2Cl_2 solution ($\sigma^{\text{H}}_{\text{liq}}$), and the most significant upfield shifts ($\Delta\sigma^{\text{H}}_i < -2$ ppm) are drawn in the chemical structure in the right panel of Figure 2. Tables S1 and S2 in the Supporting Information list the ^1H assignments and aggregation shifts and the ^{13}C assignments and aggregation shifts. The

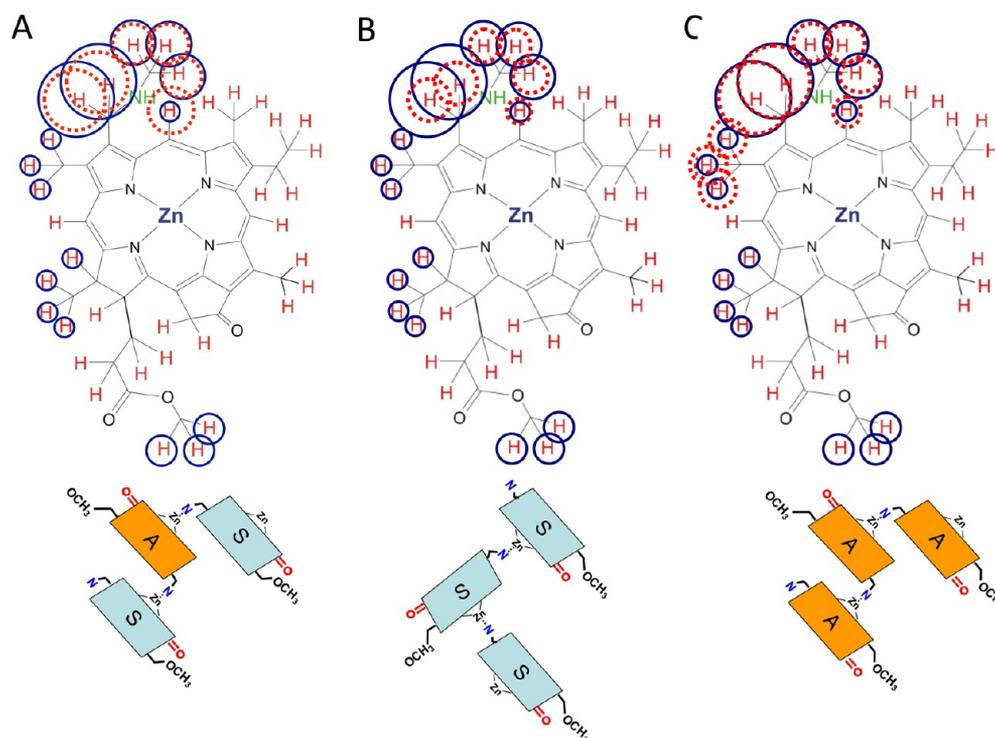


Figure 4. DFT-calculated NMR ring-current shifts (red dashes, the sizes are proportional to the calculated shifts) and experimental NMR aggregation shifts (blue solid lines) for (A) antiparallel syn-anti stacks, (B) syn T-shape stacks, and (C) antiparallel anti stacks.

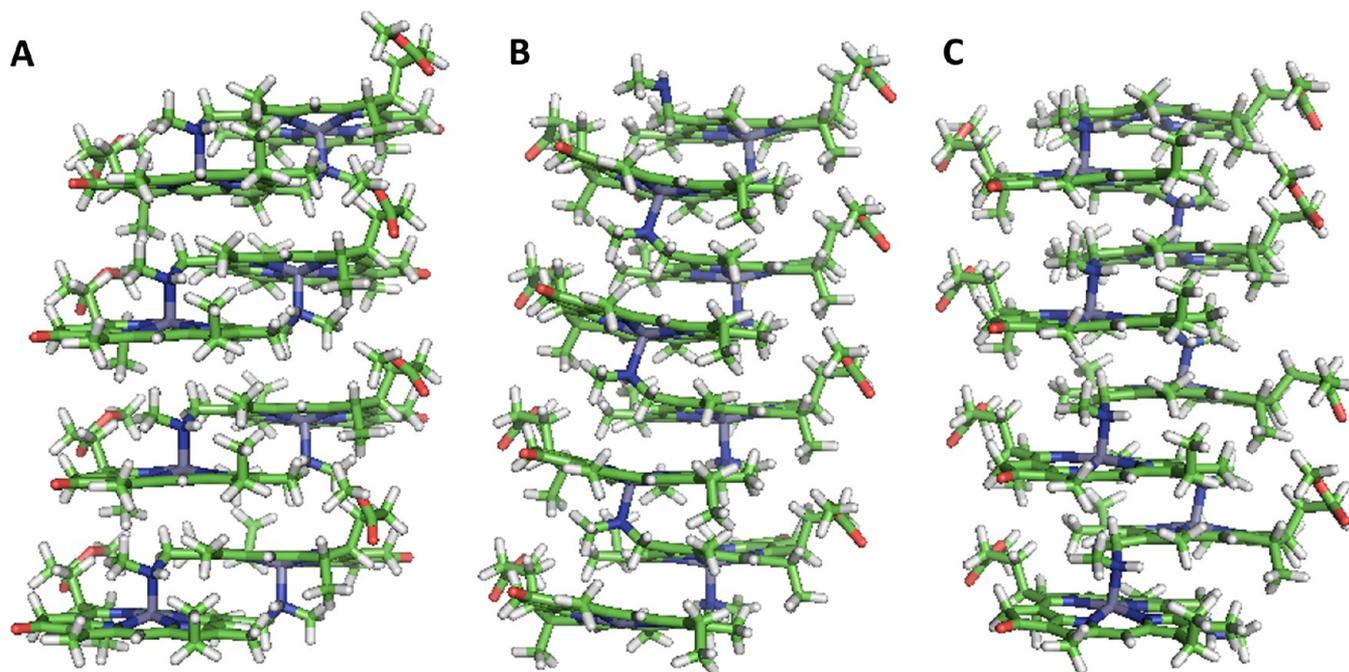


Figure 5. Geometry-optimized vertical stack models of (A) syn-anti dimers, (B) antiparallel anti, and (C) antiparallel syn stacks.

possible scaffolding modes of the amino Zn-chlorins are schematically drawn in the cartoon pictures in Figure 3A–D. They are primarily constrained by steric crowding between the building blocks and are identified as: (A) the anti, syn, and syn-anti oblique-running parallel stack, (B) the anti, syn, and syn-anti staggered running antiparallel stack, (C) the anti, syn, and syn-anti dimer stack, and (D) the anti, syn, and syn-anti T-shaped stacks, adding up to 12 modes in total. Here we follow the nomenclature in Ganapathy et al.¹⁴ to define the spatial

relationship between the magnesium ligand and the 17-propionic-methyl side chain. In the syn conformation, they are on the same side, while in the anti conformation they are on opposite sides of the macrocycle. The corresponding IUPAC naming would be α for the syn and β for the anti form.²⁵

In contrast with the Zn-chlorin ester complexes studied by Ganapathy et al.,¹³ there is little evidence of a ring current shift for the H12¹ nuclei of **6**. This effectively excludes the possibility of the three oblique parallel stack modes (Figure 3A) because

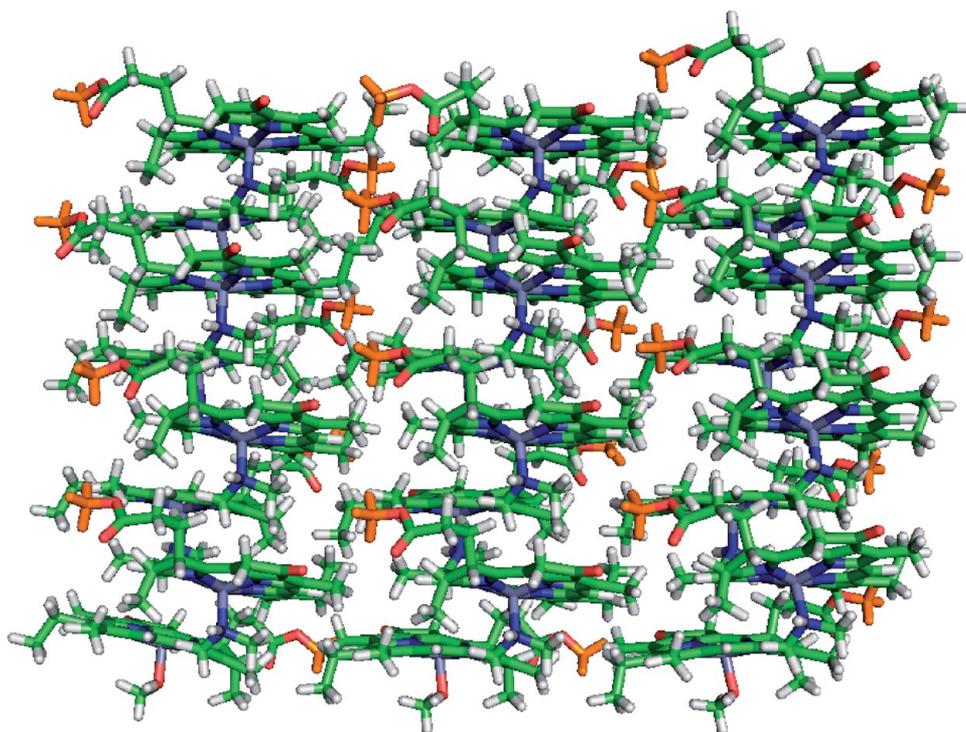


Figure 6. Geometry-optimized lateral associates of the antiparallel anti vertical stacks stabilized by packing interactions of the 17 propionate-methyl (drawn in orange).

for parallel arrangements each chlorin is subject to ring-current shifts of comparable magnitude at the 12^1 nuclei on one side and at the 2^1 , 3^1 and amino-methyl nuclei on the other side of the molecule, according to ring-current shift calculations for natural and artificial BChl oblique parallel-stack assemblies.^{13,14}

Packing models were created in silico from T-shape, antiparallel, and dimer amino Zn-chlorin stack modes using HyperChem (HyperCube) and optimized by the molecular-mechanics MM⁺ force field. To make all chlorins five-coordinated, the chlorin molecules at the edges of the aggregate structures were capped with a MeOH functionality. NMR ring current shifts were calculated for the various models by quantum mechanical DFT methods. Figure 4 shows the calculated aggregation shifts for the antiparallel syn-anti, T-shape syn, and antiparallel anti models to illustrate the selectivity of the models with respect to their produced ring-current shifts. ^1H shift calculations for the dimer anti and antiparallel syn-anti stacks both indicated large ring current effects for the H5 nuclei (~ -5 ppm) and very small contributions for the H2¹ response. The calculated ring current effects are in line with previous calculations for the piggyback stack models of the hydroxyl and methoxy Zn-chlorin analogues and contrast with the NMR analyses of compound 6.^{13,26} Hence, the anti dimer stacks and the vertical syn-anti antiparallel stacks can be rejected as viable packing models. In addition, the large negative ring current shifts of ~ -8 ppm for the H3¹ are reproduced only when the H3¹ is positioned between two adjacent macrocycle molecules and the 3^1 protons experience ring current effects from both sides. Thereby the T-shape stack modes can be discarded as well because all T-shape models produce ring-current shifts only up to ~ -4 ppm. The molecules in the T-shape model are also packed with significantly higher energies, ~ 90 kcal/molecule, than in the

antiparallel models, ~ 60 – 70 kcal/molecule (Table S3, Supporting Information).

On the basis of the patterns of the NMR ring current shifts, we conclude that the amino Zn-chlorins preferably stack in anti or syn antiparallel modes or in syn or syn-anti vertical stacked dimers. Of these four modes, the syn dimers were discarded because they did not form stable vertical stacks in silico. The three remaining modes (anti and syn antiparallel and syn-anti dimers) formed vertical stacks with comparable orientation of the Zn-chlorins, hence, with very similar molecular packing and ring current shifts and are presented in Figure 5. The syn antiparallel stacks were stabilized at somewhat higher energies (~ 70 kcal/mol) than the anti antiparallel and syn-anti dimer stacks (~ 60 kcal/mol), and based on its higher stabilization energy, this mode appears less preferred. The resulting anti antiparallel and syn-anti dimer structures differ only in their coordination through staggered vertical association versus intradimer coordination, and both show a very good match with the experimental NMR shifts, except for the proton chemical shift of the 17 propionate-methyl side chain, which has a significant aggregation shift of -3.7 ppm in the NMR heteronuclear correlation spectrum, whereas very minor aggregation shifts are predicted by the vertical stack models.

We used the antiparallel anti model to build lateral stacks that were stabilized through packing interactions of the 17 propionate-methyl side chains (Figure 6), which reduced the free energy of the MM⁺ geometry-optimized structures by ~ 5 kcal/mol. (See Table S3 in the Supporting Information.) Quantum-mechanical DFT chemical shift calculations on the embedded propionate-methyl side chain in the lateral stacks validated that lateral stacking can induce an upfield shift of the 17 propionate-methyl proton. The magnitude of the calculated shift (-1.7 ppm) is somewhat less than is observed experimentally (-3.7 ppm); however, in contrast with ring

current shifts, the effects of conformational distortions and the associated dynamics on the NMR chemical shifts are complex and more difficult to predict in a quantitative way than the ^1H ring current shifts. This shows that the stacking determined by the NMR can lead to 3-D self-assembly as well. Determination of the 3-D structure at higher resolution will probably require complementary diffraction data from reasonably ordered sample preparations, which may be encountered in the future for other members of the Zn-chlorin class.

Finally, the NMR results of compound **6** were compared with results obtained from two types of 3^1 -hydroxy-methyl-Zn-chlorin ester complexes¹³ and two types of 3^1 -hydroxy-methyl cadmium (Cd) chlorines.²⁷ The NMR line widths of the 1D CP MAS spectra obtained from aggregates of **6** are similar to the widths of the signals for the 1D CP MAS data collected from 3^1 -hydroxy-methyl-Zn-chlorin ester complexes, which are confirmed to form microcrystalline materials and also stack in an antiparallel mode. Considerable line broadening occurs in the NMR spectra of glassy or amorphous solids; hence, the narrow signals for the amino Zn-chlorins point to structures that are well ordered on the mesoscale, as opposed to, for example, fiber-type structures. The ring-current shift pattern of the amino Zn-chlorin assemblies is very similar to the pattern of the aggregation shifts in two Cd-chlorin assemblies that were tentatively modeled based on parallel stacking modes only. When the full library of packing modes according to Figure 3 is considered, the better match is obtained for antiparallel stacks, and here we propose that the Cd-chlorins also adopt an antiparallel arrangement, thereby using the methodology developed in the present work to refine the models described in de Boer et al.²⁷

Apparently, antiparallel stacking, starting from a dimer building block or from an antiparallel motif at the basis, appears as a common denominator across various artificial semisynthetic chlorin structures. Semisynthetic chlorins without long phytol tails at the C17 position lack the amphiphilic character to promote phase separation and the formation of micellar structures. This contrasts with the stacking modes of natural chlorosomes, in which the BChl phytol chains have thermodynamic control over the supramolecular structures. For these natural systems, a variety of almost isoenergetic parallel stacking configurations is probed, and the BChls self-assemble into domains of parallel syn-anti stacks or alternating parallel syn and anti stacks with correlation lengths up to ~ 40 monomers.²¹ The different stacking modes of natural BChls versus BChl mimics is of particular interest for their functioning because in the parallel stacking modes the electric dipoles are aligned to form ferroelectric-type extended planar structures, while for the antiparallel stacking modes electric dipoles are largely compensated at the molecular level.

In conclusion, we demonstrate that solid-state NMR in combination with quantum-chemical and molecular-mechanics modeling can resolve and selectively compare molecular structures of chromophore nanoassemblies for functional chemical programming of their supramolecular structure. This method is not limited to chlorin-based assemblies but can be applied to assemblies of any type of aromatic molecules that induce ring-current shifts, provided that they have some form of structural asymmetry, which is a requirement for unambiguous assignment of their NMR chemical shifts. Such structural asymmetry is usually supplied by functionalized side chains that serve to program and steer the self-assembled structures by chemical modification. The models provide a basis for further

investigation with complementary techniques, although care has to be taken that the structures are the same. For instance, we performed preliminary AFM experiments for molecules deposited on a surface and found that this most likely leads to a packing that is different from the one that is observed with NMR in the bulk.

■ ASSOCIATED CONTENT

📄 Supporting Information

Synthesis and preparation of **6**, solution NMR spectra of **6** and tables with the ^1H and ^{13}C chemical shift assignments, aggregation shifts, and calculated stabilization energies per molecule. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The use of supercomputer facilities was sponsored by NWO Physical Sciences, with financial support from The Netherlands Organization for Scientific Research (NWO). This work was supported by the European Science Foundation through the grant Tubitak-110M803 and by the program "Complex Materials: Cooperative Projects of the Natural, Engineering and Biosciences" of the Volkswagen Foundation (funding to A.R.H. and H.J.M.d.G.). This research is financed in part by the BioSolar Cells open innovation consortium, supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. This research was partially supported by the Eurosolartandem collaborative research project, which is part of the Eurosolartandem Eurocores program of the European Science Foundation.

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