A Hybrid Solid-State NMR and Electron Microscopy Structure-Determination Protocol for Engineering Advanced para-Crystalline Optical Materials


Abstract: Hybrid magic-angle spinning (MAS) NMR spectroscopy and TEM were demonstrated for de novo structure determination of para-crystalline materials with a bio-inspired fused naphthalene diimide (NDI)-salphen-phenazine prototype light-harvesting compound. Starting from chiral building blocks with $C_2$ molecular symmetry, the asymmetric unit was determined by MAS NMR spectroscopy, index low-resolution TEM diffraction data, and resolve reflection conditions, and for the first time the ability to determine the space group from reciprocal space data using this hybrid approach was shown. Transfer of molecular $C_2$ symmetry into P2/c packing symmetry provided a connection across length scales to overcome both lack of long-range order and missing diffraction-phase information. Refinement with heteronuclear distance constraints confirmed the racemic P2/c packing that was scaffolded by molecular recognition of salphen zinc in a pseudo-octahedral environment with bromide and with alkyl chains folding along the phenazine. The NDI light-harvesting stacks ran orthogonal to the intermolecular electric dipole moment present in the solid. Finally, the orientation of flexible lamellae on an electrode surface was determined.

Chemical self-assembly to bridge the gap from dead to living matter is a challenging field. Controlling complexity, flexibility, and functionality of synthetic and biomimetic material requires engineering soft para-crystalline compounds that lack crystalline long-range order in at least one dimension. However, resolving their microscopic and mesoscopic order at atomic resolution from the anisotropic background heterogeneity has proven to be quite difficult. Only recently has the hybrid bottom-up magic-angle spinning (MAS) NMR spectroscopy and top-down electron microscopy (EM) structure-determination methodology shown promise to overcome the limitations of either technique.[2] MAS NMR spectroscopy is intrinsically a microscopic method[3] that cannot solve a structure de novo because packing order is determined by minimizing steric hindrance with screw axes or glide planes at higher levels in the structural hierarchy. For small organic molecules forming microcrystals, modeling protocols with a conjectured rather than determined space group are used, making MAS NMR spectroscopy a chemical shift filter for selection and validation.

Earlier, we showed for the largest biological (protein-free and grossly heterogeneous) light antennae that the limitations of MAS NMR spectroscopy can be diminished by using a 2D TEM periodogram as a band-pass filter to resolve sparse regions of nonzero intensity in reciprocal space.[4] Here we present the important next step by showing that genuine space-group information can be extracted from TEM data in reciprocal space.

To demonstrate this, we use a prototypical engineered biomimetic chromophore light-harvesting material that consists of fused Br-substituted naphthalene diimide (NDI), phenazine, and Zn-salphen building blocks (Figure 1).[5] This system, denoted as DATZnS(3−NMe), models the chlorosome that is built from parallel stacks of bacteriochlorophyll (BChl) connected by metal coordination in a recognition motif. The BChl stacks form polarizable curved sheets and tubes that are thought to yield a dielectric response upon excitation with crossing of energy levels and coherent mixing of exciton states for energy transport. Although the model is chemically unrelated to BChl, we show that it self-assembles into stacks that form extended polar curved sheets like the natural paradigm. The NDI of the model is capable of overlap, the phenazine carries the electric

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The symmetric NMR response of the DATZnS(3'-NMe) and two from the NDI motif (Figure 3). Transfer involving 3'-NMe and 4, 5's protons of salphen and the 4, 5 13C nuclei in the NDI motif (Figure 3). Transfer involving 3'-NMe and 4, 5 13C nuclei from Figure S4 in the Supporting Information. Both the 3'-NMe and 4, 5 13C nuclei in the central part of the NDI motif correlated with the 21 and 71 CH3 protons. This revealed the formation of slipped J-aggregates for the NDI, with the alkyl tail above the plane of a neighbouring molecule. It implied that the correlations and buildup from protons at the alkyl chain to 13a, 9a 13C nuclei were also intermolecular. Similar motifs were observed in the supramolecular structure of the NDI, which we refined with MAS NMR spectroscopy. The technologies were indexed to TEM data collected for a short mixing time (Figure S3 in the Supporting Information) and observed between the 3', 3', 3', and 3'-protons of salphen and the 4, 5 13C nuclei in the NDI motif (Figure 3). Transfer involving 3'-NMe and 4, 5 13C nuclei from Figure S4 in the Supporting Information. Both the 3'-NMe and 4, 5 13C nuclei in the central part of the NDI motif correlated with the 21 and 71 CH3 protons. This revealed the formation of slipped J-aggregates for the NDI, with the alkyl tail above the plane of a neighbouring molecule. It implied that the correlations and buildup from protons at the alkyl chain to 13a, 9a 13C nuclei were also intermolecular. Simi-
larly, buildup of CP intensity from the alkyl CH to the quaternary \(^{13}\)C on the phenazine core could be considered intermolecular from the abundant cloud of protons on the alkyl chain. Strong \(\pi-\pi\) stacking interactions and aligned electric dipoles explained why the material had a high density, a low energy, and was insoluble. The aliphatic tails were oriented in the same direction as the salphen wings and were situated in voids between the phenazine moieties. Tight packing with the alkyl chains folded along the phenazine bridge of a neighboring molecule explained the observation of strong heteronu-

**Figure 2.** Integration of TEM and MAS NMR. (A) TEM image of the DATZnS(3'-NMe) on a carbon-coated grid, revealing the curved lamellar character of the compound; (B) Fourier transform of the selected region showing the TEM diffraction pattern with systematic absences; (C) projection of the electron-density map with a 20 \(\times\) 10 \(\times\) 5 supercell;\(^{28}\) (D) simulated diffraction pattern obtained with the NMR-derived geometry in the \(P2_1/c\) space group;\(^{29}\) (E) orientation of DATZnS(3'-NMe) on the carbon grid (orange color); (F) viewed along the b axis; and (G) along the a axis.

**Figure 3.** (A,B) Contour plot sections of \(^1\)H-\(^{13}\)C correlation spectra collected from DATZnS(3'-NMe) with a contact time of 4 ms; (C) the intermolecular interaction (green) between 4/5 \(^{13}\)C and dimethyl amine and the molecular recognition motif; (D) the folding of the tails is obtained from the transfer (violet) between phenazine \(^{13}\)C nuclei and the aliphatic \(^1\)H of the tails.
clear correlation signals between the abundant aliphatic $^1$H and rare phenazine $^{13}$C spins, providing efficient pathways for polarization transfer (Figure 3). The molecular recognition and distorted octahedral surrounding of the Zn$^{2+}$ ion put the $3\text{-}N\text{Me}$ of salphen at 0.45 nm from the 4, 5 $^{13}$C nuclei in NDI, which was in quantitative agreement with the LGCP buildup kinetics and its simulation. This refinement represents the third step in our approach.

In our final and fourth step we determined the orientation of the material on the surface by simulating the diffraction pattern, thereby validating the indexing and space group. A view along the 0.69, 0, 0.69 lattice vector yielded the best match for the density and the diffraction pattern (Figure 2C, D). The analysis validated the systematic absence of reflections from the c-glide plane in the P2$_1$/c space group and showed that $-101$ and $10-1$ were quenched (Figure 2B). The strong 010 and 0$-10$ originated from lamellar spacing and alternating regions of Zn-salphen and NDI. The phenazine dipoles were aligned along the surface and were perpendicular to the NDI stacks that ran parallel to the surface with the plane of the NDI rings at an angle of 45° (Figures 2E, F). Figure 5 shows how molecular recognition leads to a transfer of molecular symmetry for scaffolding. Whereas steric hindrance favors screw axes or glide planes (symmetry operations with a translational component) to allow for interpenetration of symmetry-related molecules, the screw axis is apparently suppressed in DATZnS(3′-NMe) in favour of a twofold axis to accommodate intramolecular C$_2$ symmetry (Figure 5A). This is possible because of the rich structural variability introduced with the nonplanar metal salphen. It allows for packing in an achiral P2$_1$/c space group with a racemic mixture of the two enantiomeric species, thereby circumventing the need for a screw axis in favour of a c-glide plane with inversion symmetry in the structure.

With C$_2$ molecular symmetry preserved, DATZnS(3′-NMe) self-assembles into polar planes without inversion symmetry elements, thereby mimicking the parallel stacking in the chlorosome antenna (Figure 5B), for which the selectivity induced by chirality emerges at the salphen motif. The electric dipoles align and form extended arrays with a positive and a negative side to support charge separation following light absorption in the NDI columns running perpendicular to the electric-field direction (Figure 5). DATZnS(3′-NMe) forms extended chiral layers in the proposed 3D model, arising from planar arrangements of individual C$_2$ motifs (known as organizational chirality), in which the net dipole moment is canceled owing to antiparallel layers.

In conclusion, we have demonstrated hybrid MAS NMR spectroscopy and TEM for de novo structure determination of a bio-inspired para-crystalline material. The concept can be further developed with, for example, pattern recognition across TEM and NMR datasets to facilitate the applicability and broaden the scope. This paves the way for structure determination of advanced organic supramolecular materials that bridge the gap from dead to living matter and are inaccessible to high-resolution diffraction methods.

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**Figure 4.** LGCP buildup curves of the polarization transfer to 4/5 (red) and 13a/9a (green) carbon nuclei compared with a simulated buildup for a heteronuclear $^1$H–$^{13}$C spin pair separated by approximately 4 Å (blue). The buildup curves representing 3 Å (black solid lines) and 5 Å (black dotted lines) are also shown.

**Figure 5.** High-resolution 3D lattice model for para-crystalline DATZnS(3′-NMe); (A) Molecular recognition for steering the packing starts from chiral building blocks with C$_2$ symmetry. (B) These self-assemble into enantiomERICally pure Δ and Λ polar layers with a transfer of molecular C$_2$ symmetry into supramolecular P2 symmetry. The layers comprise arrays of aligned dipoles with a positively charged salphen and negatively charged Br. (C) Alternating layers with opposite chirality self-assemble with a c-glide plane to release steric hindrance and establish a dense packing with quenching of electric dipoles.
culture and Innovation. The microscopy was performed in the NECEN cryo-EM facility in Leiden.

**Conflict of interest**

The authors declare no conflict of interest.

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