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

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Abstract: We demonstrate hybrid MAS NMR and TEM for de novo structure determination of para-crystalline materials with a bioinspired fused naphthalene diimide (NDI) - salphen - phenazine prototype light harvesting compound. Starting from chiral building blocks with $C_2$ molecular symmetry we determine the asymmetric unit by MAS NMR, index low resolution TEM diffraction data, resolve reflection conditions, and show for the first time ability to determine the space group from reciprocal space data using our hybrid approach. Transfer of molecular $C_2$ symmetry into $P2_1/c$ packing symmetry provides a connection across length scales to overcome both lack of long range order and missing diffraction phase information. Refinement with heteronuclear distance constraints confirms the racemic $P2_1/c$ packing that is 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 run orthogonal to the intermolecular electric dipole moment present in the solid. Finally, we determine the orientation of flexible lamellae on an electrode surface.

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 MAS NMR and top-down EM structure determination methodology shown promise to overcome the limitations of either technique. Mas NMR is intrinsically a microscopic method that cannot solve a structure de novo as 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 are used with a conjectured rather than determined space group, making MAS NMR 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 can be diminished by using a 2D TEM periodogram as a band pass filter to resolve sparse regions of nonzero intensity in reciprocal space. 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). 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 excitation 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 dipole, and the Zn-salphen provides a recognition motif for coordination.

The molecular symmetry and asymmetric unit were determined from MAS NMR shifts collected with $^{13}$C at natural abundance. We indexed the TEM diffraction and determined both the unit cell parameters and a genuine space group by analysis of systematic absences in a sparse reciprocal space.

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Figure 1. Chemical structure of anti DATZnS(3′-NMe), a fusion hybrid of NDI (red), Phenazine (green) and Salphen (yellow).
intensity pattern with the help of the molecular symmetry information obtained by MAS NMR. The technologies were then merged at the molecular level and unit cell. As indexing of an unknown structure is not possible by TEM alone, the diffraction phase problem was implicitly overcome by connecting across

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 2010°5 supercell,\textsuperscript{30} and (D) simulated diffraction pattern obtained with the NMR derived geometry in the P2/c space group.\textsuperscript{31} (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-$^1$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.
length scales through transfer of molecular symmetry to packing symmetry. This provides a structural underpinning for engineering supramolecular material in the desired sheets with parallel aligned dipoles. Fourier transform filtering in reciprocal space averages static heterogeneity in real space and enables extrapolating the short and medium range ordering in a para-crystalline lattice to establish a full 3D lattice model which we refined with MAS NMR heteronuclear \(^1\text{H}-^{13}\text{C}\) correlation data. Finally, we determined the orientation of DATZnS(3′-NMe) on a surface to relate to the geometry of biomimetic material in electrode applications.

The symmetric NMR response of the DATZnS(3′-NMe) provided conclusive evidence that the asymmetric unit is half of the molecule (Figure S1, Table S1). Of the two possible conformational pairs, the symmetric pair was chosen. The symmetry correlation and the chemical shift of the 5b 13C in the central part of the NDI motif correlate with the 2 1 J inversion symmetry in the structure. This leads to a P2\(/c\) space group with a racemic packing from the 2\(1/2\) glide plane that explains both the systematic absence of reflections from the density and the diffraction pattern (Figure 4 C,D).\(^9\) The analysis validated the systematic absence of reflections from the glide plane in the P2\(/c\) space group, and showed that -1 0 1 and 1 0 - 1 are quenched (Figure 4B). The strong 0 1 0 and 0 -1 0 are from lamellar spacing and alternating regions of Zn-salphen and NDI. The phenazone dipole are aligned along the surface and are perpendicular to the NDI stacks that run parallel to the surface with the plane of the NDI rings at an angle of 45º (Figures 4E, F). Figure 5 shows how molecular recognition leads to 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 by the nonplanar metal salphen. It allows for packing in an achiral P2\(/c\) 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 future.

With \(C_2\) molecular symmetry preserved, DATZnS(3′-NMe) self-assembles into polar planes without inversion symmetry
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 C₂ symmetry. (B) These self-assemble into enantiomerically pure ∆ and ∆ polar layers with a transfer of molecular C₂ 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 dense a packing with quenching of electric dipoles.

Keywords: NMR spectroscopy; Electron microscopy; Photochemistry; Molecular recognition; Self-assembly; Magic Angle Spinning; Artificial Photosynthesis

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Packing of a fused chromophore light harvesting material is resolved with a four-step hybrid approach involving MAS NMR and cryo-EM. Chemical shift and distance constraints from NMR data are combined with reflection conditions from the Fourier transform of EM images to overcome heterogeneity and problems with indexing the diffraction and determining the spacegroup.