

NMR crystallography with microED

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Three-dimensional electron diffraction crystallography (microED) can solve structures of sub-micrometer crystals, which are too small for single crystal X-ray crystallography. However, R factors for the microED-based structures are generally high (15-30% for small molecules) because of dynamic scattering. Thus, R factor may not be reliable provided that kinetic analysis is used. Consequently, there remains 1) ambiguity to locate hydrogens and 2) assignment of nuclei with close atomic numbers, like carbon, nitrogen, and oxygen. On the other hand, ¹H solid-state NMR is readily available using fast MAS probes and ¹³C, ¹⁴N, ¹⁵N, and ¹⁷O are completely different nuclei for NMR observation. Thus, information from solid-state NMR and microED is complementary.

Herein, we demonstrate combined approach using solid-state NMR and microED to solve crystalline structure. First, well established NMR crystallography approach is employed. Isotropic chemical shifts are very sensitive to local environment, thus crystalline structure, however, there are no intuitive way to predict chemical shifts from structures. GIPAW procedure paves a way to estimates chemical shifts of crystalline materials in a very high accuracy. This makes isotropic chemical shift as a reliable measure for structure validation. While wrong position of ¹H and misassignment of carbon/nitrogen/hydrogen result in poor agreement between experimental and calculated chemical shifts, right structure can be easily chosen among candidates. We show that this approach, which is well established with XRD structures, equally works well with microED. The crystalline structures are determined by microED and validated by isotropic chemical shifts [1]. To further validate the structure, next, we demonstrate another measure using dipolar-based ssNMR experiments in addition to isotropic chemical shifts. In principle, dipolar couplings provide useful information for structure elucidation, as the size of coupling is inversely proportional to the cube of internuclear distances. However, spin dynamics is often complicated due to presence of multiples of intra- and intermolecular couplings for small molecules, making structure elucidation difficult. On the other hand, it is readily calculated for given structures if the spin system is simple enough (< 8 spins). Here we utilize ¹H-¹H selective recoupling of proton (SERP) experiments [2-4] and ¹H-¹⁴N phase-modulated rotational-echo saturation-pulse double-resonance (PM-RESPDOR) as dipolar-based NMR experiments [5, 6]. While the former selects a subset of ¹H-¹H spin systems, the latter simplifies the spin system by decoupling ¹H-¹H interactions. As a result, SERP and RESPDOR probe ¹H-¹H and ¹H-¹⁴N networks, respectively. The structure is solved by microED and then validated by evaluating the agreement between experimental and calculated dipolar-based NMR results [7]. As the measurements are performed on ¹H and ¹⁴N, the method can be employed for natural abundance samples. Furthermore, the whole validation procedure was conducted at 293 K unlike widely used chemical shift calculation at 0 K using the GIPAW method.

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