Insights into structure and function of an ion channel by ultrafast magic angle spinning NMR

Tobias Schubeis, Andrea Bertarello, Marta Bonaccorsi, Tanguy Le Marchand, Jan Stanek, Guido Pintacuda

Centre de RMN à Très Hauts Champs, Institute of Analytical Sciences, University of Lyon, 69100 Villeurbanne, France

A milestone towards understanding of transmembrane channel functionality is the site specific characterization of minimal changes in structure and dynamics due to the presence (or absence) of metal ions. NMR spectroscopy of proteins in solution provides the resolution and sensitivity to observe these effects but is inherently limited by the protein size. This size limitation is in principle absent for solid-state NMR under magic-angle spinning (MAS), for which new state-of-the-art equipment and new concepts allow nowadays the rapid acquisition of well-resolved, atomic-level fingerprint spectra of membrane proteins in lipid bilayers (1).

We challenge the capacity of this technique at high magnetic fields and ultrafast (> 100 kHz) MAS on the bacterial divalent cation channel CorA (2), a pentamer of 5 x 42 kDa, comprised of two transmembrane helices and a large cytoplasmic domain, hosting various metal binding sites (usually for Mg^{2+} or Co^{2+}). We present strategies to obtain sequence specific chemical shift information (3), which gives insight into the secondary structure, as well as experiments that report on motions of different time scales (4). We finally discuss how this unique residue-specific information can be used to describe the transport of ions through the CorA channel. The data presented is complementary to already available crystal and cryo-EM structures, and represents a powerful example of integrated structural biology.

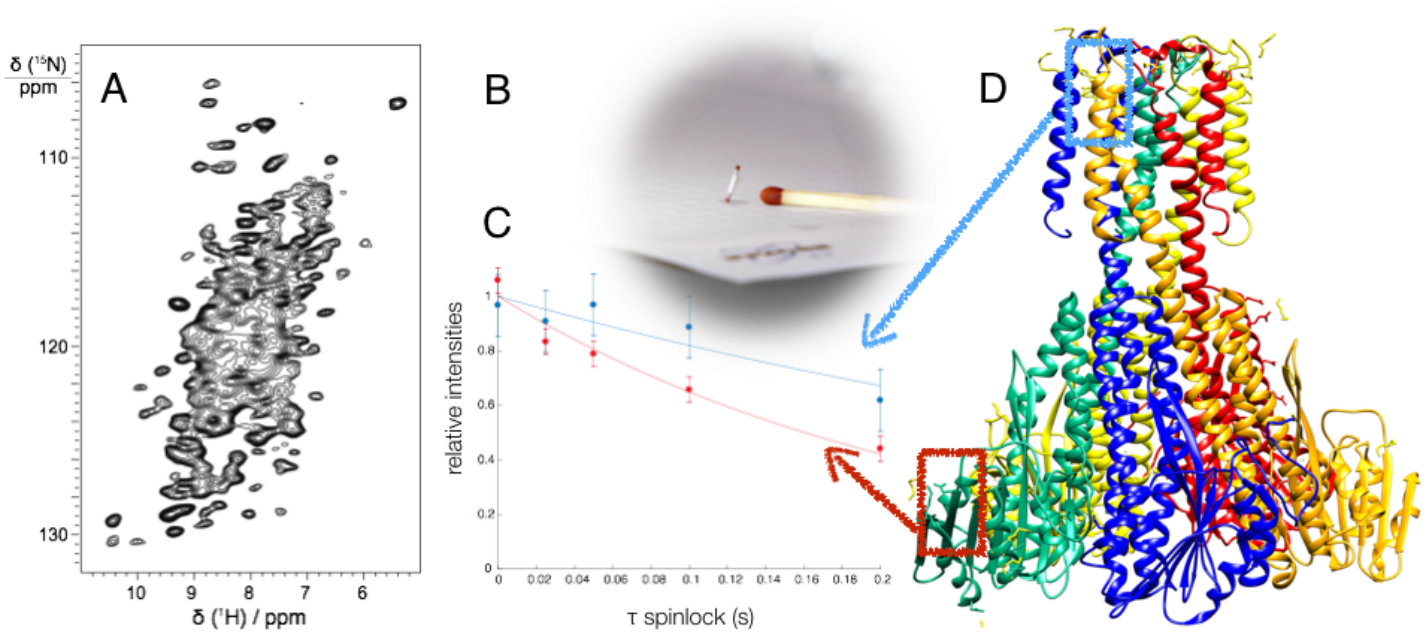

Figure 1. (A) 2D $^{1}H,^{15}N$ dipolar correlation spectrum of Mg^{2+}-bound $^{15}N,^{13}C$-CorA in DMPC bilayers, obtained on a 1 GHz spectrometer at 110 kHz MAS. (B) The sample in a 0.7 mm Bruker rotor. (C) Representative ^{15}N R_{1rho} relaxation decays for two Gly residues in the transmembrane and in the cytoplasmic domains of the channel. (D) Ribbon diagram of the CorA cryo-EM structure.

References. (1) T. Schubeis, T. Le Marchand, L. B. Andreas and G. Pintacuda "^{1}H NMR in solids evolves as a powerful new tool for membrane proteins", J. Magn. Reson. 2018, in press. (2) D. Matthies, O. Dalmas, M. J. Borgnia, … , A. Bartesaghi, E. Perozo, S. Subramaniam "Cryo-EM structures of the magnesium channel CorA reveal symmetry break upon gating", Cell, 2016, 164, 747-756; (3) J. Stanek, L. B. Andreas, K. Jaudzems, D. Cala, D. Lalli, A. Bertarello, T. Schubeis, I. Akopjana, S. Kotelovica, K. Tars, A. Pica, S. Leone, D. Picone, Z.-Q. Xu, N. E. Dixon, D. Martinez, M. Berbon, N. El Mammeri, A. Noubhani, S. Saupe, B. Habenstein, A. Loquet, G. Pintacuda, "Backbone and side-chain proton NMR assignment in fully protonated proteins: microcrystals, sedimented assemblies, and amyloid fibrils", Angew. Chem. Int. Ed. Engl. 2016, 55, 15504-15509; (4) J. R. Lewandowski "Advances in solid-state relaxation methodology for probing site-specific protein dynamics", Acc. Chem. Res. 2013, 46, 2018-2027.