New insights on a divalent cation channel by >100 kHz magic-angle spinning NMR

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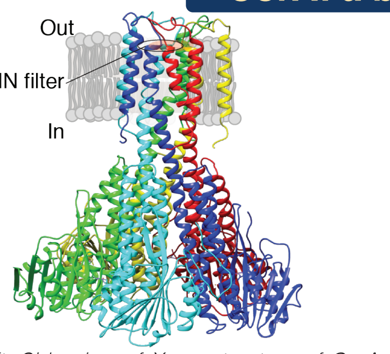
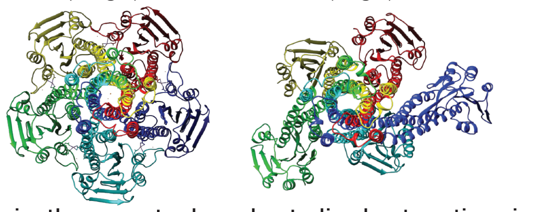
P-MEM-NMR

CorA: a bacterial M^{2+}-channel



4I0U (+Mg^{2+}) 3JCG (-Mg^{2+})



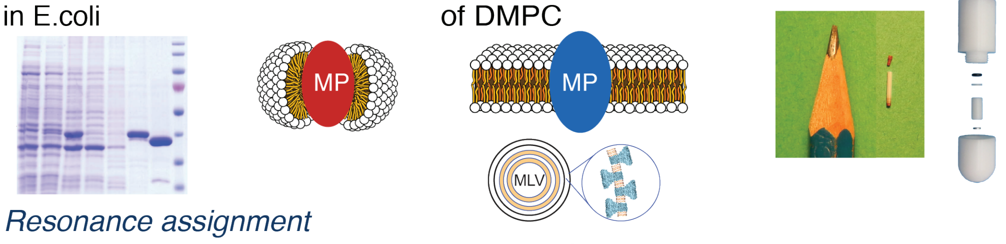
Left: Side view of X-ray structure of CorA (PDB ID: 4I0U) in presence of Mg^{2+}. Right: Top view of the same symmetric state side-by-side to one of the asymmetric states observed by cryo-EM in the absence of Mg^{2+} (PDB ID: 3JCG).

Mg^{2+} is the most abundant divalent cation in the cell. The pentameric channel (5x42 kDa) CorA is the primary uptake system of Mg^{2+} in prokaryotes.
Structural information is available [1,2] but **the mechanism of transport regulation remains elusive.**

Membrane proteins at fast MAS

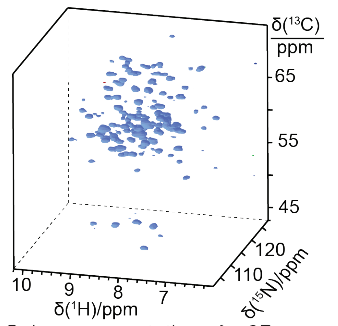
Sample preparation

1. Expression of **[^1H, ^{13}C, ^{15}N]-CorA** in E.coli
2. Purification in detergent
3. Reconstitution in **multilamellar vesicles** of DMPC
4. Direct centrifugation into **0.7 mm rotor**



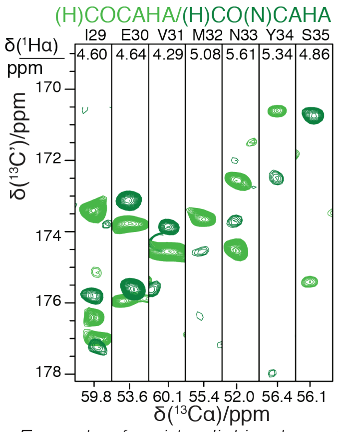
Resonance assignment

- Long coherent lifetimes at **110 kHz MAS and high magnetic fields** (800-1000 MHz) allow the acquisition of ^1H-detected multidimensional experiments with improved sensitivity and resolution.



Cube representation of a 3D ^1H-^{15}N-$^{13}C\alpha$ spectrum of CorA.

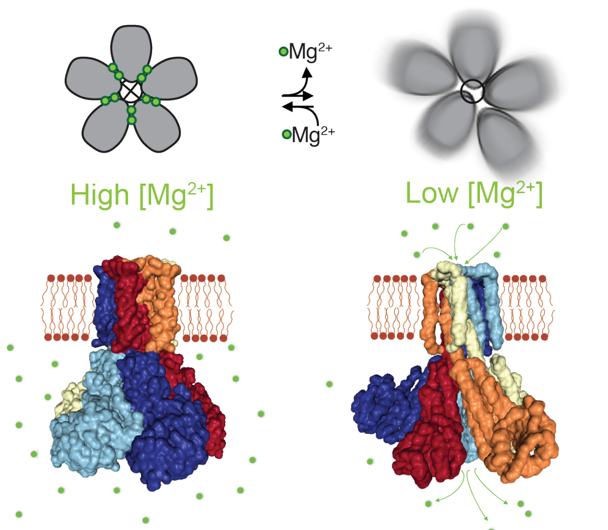
- The acquisition of an **extended dataset of H^N- and Hα-detected 3D experiments** [3,4] combined with automatic assignment by FLYA [5] allows fast, unambiguous resonance assignment.



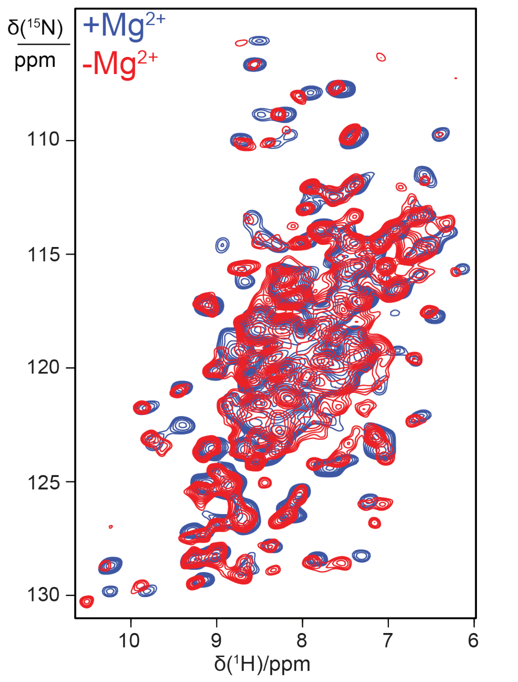
Example of residue linking based on $^{13}C'$, $^{13}C\alpha$, Hα correlations.

Is an "on-off" model sufficient?

The «on-off» model vs « fingerprint» spectra by magic-angle spinning NMR



Schematic representation of the symmetry-break based gating model derived from X-ray and cryo-EM structures [1].

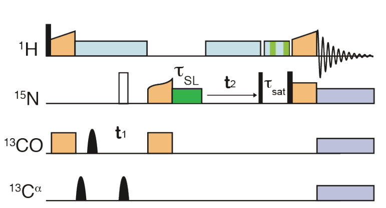


2D ^1H-^{15}N dipolar correlation spectra by MAS NMR of CorA embedded in hydrated DMPC bilayers recorded at 1 GHz ^1H Larmor frequency and 107 kHz MAS.

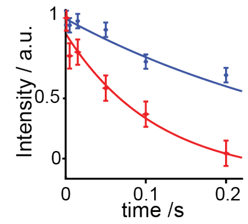
Highly similar room-temperature MAS NMR spectra obtained for CorA in lipid bilayers with and without Mg^{2+}.

A simple two-state model is not enough to describe Mg^{2+} transport regulation in CorA.

Site-specific backbone dynamics



(H)CONH-based pulse sequence for the measurement of ^{15}N relaxation rates.

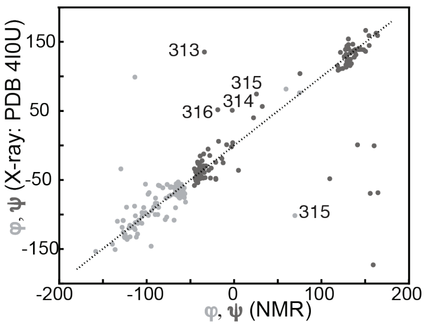


Examples of ^{15}N $R_{1\rho}$ relaxation decays together with the corresponding mono-exponential fits.

Site-specific ^{15}N $R_{1\rho}$ rates, reporting on **ns-μs dynamics**, are measured introducing a relaxation filter in 2D/3D dipolar correlation modules and monitoring the signal decay of each amide pairs in a series of experiments with increasing relaxation delays.

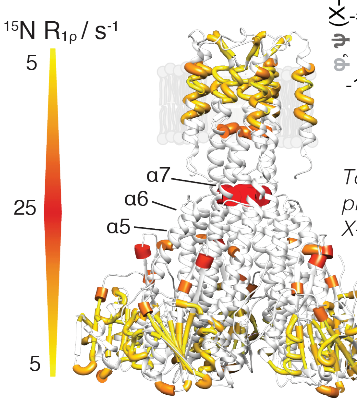
A dynamic hydrated closed channel

At high [Mg^{2+}] **good agreement was found with secondary structure from X-ray and cryo-EM,** with small local variations. The lipid environment stabilizes the structure of the periplasmic loop (residues 313-316).

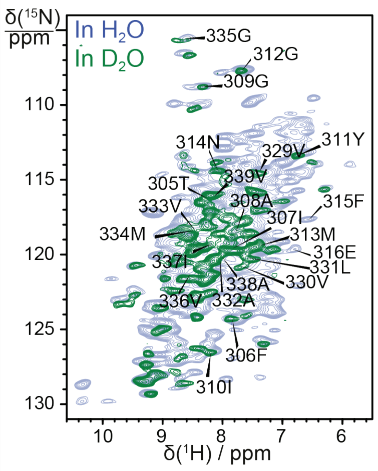


Top: Correlation between dihedral angles predicted from chemical shifts and from X-ray structure.

Extended resonance assignment was not accessible in helices 5-7 due to incomplete spectral appearance.
Measurement of ^{15}N $R_{1\rho}$ rates revealed increased dynamics in this region: **Mg^{2+}-bound CorA is a fluctuating structure.**

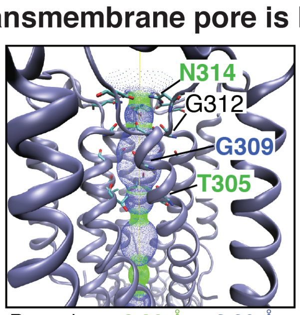


Left: ^{15}N $R_{1\rho}$ rates measured with a 15 kHz spin-lock field shown on CorA structure according to the colour legend on the left. White colour indicates unassigned residues.

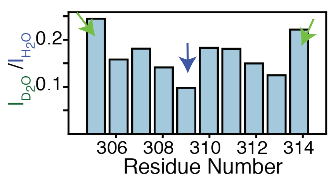


2D ^1H-^{15}N dipolar correlation spectra of fully protonated CorA acquired in H_2O and in D_2O. Residues in transmembrane regions are labelled.

Spectra of [^1H, ^{13}C, ^{15}N]-CorA in DMPC washed with D_2O revealed that the transmembrane portion does not exchange with water. Nonetheless, a slow passage of water is permitted and affects signal intensity.
The transmembrane pore is hydrated in Mg^{2+}-bound state.



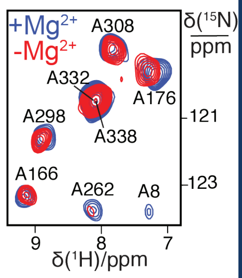
Pore size: < 2.30 Å, > 2.30 Å

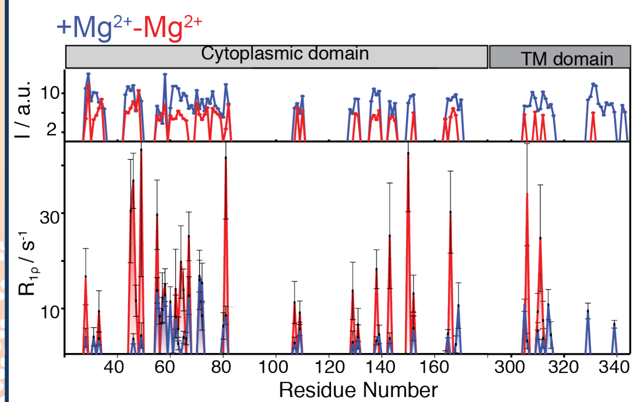


Relative peak intensities in D_2O and H_2O in the pore-lining transmembrane helix (top) correlate with pore size calculated with HOLE (left).

Mg^{2+}- dependent conformational equilibria

The comparison with fingerprint spectra of the Mg^{2+}-free form, together with Small Angle Neutron Scattering and Meta-Dynamics simulations, revealed a conservation of the average structural features and the **existence of asymmetric states independently from Mg^{2+} binding** [6].

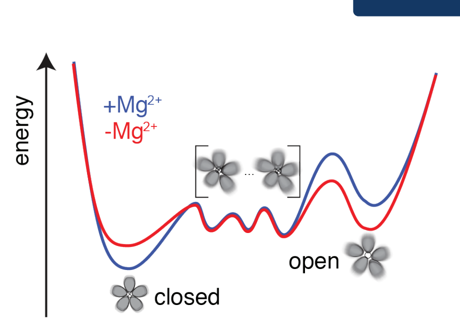




Top: ^1H-^{15}N dipolar correlation spectra of ^{15}N- Ala CorA at 60 kHz MAS, with assignment of all the alanine residues. Amino acid-specific isotopic enrichment was used as a strategy to reduce spectral crowding.
Left: Comparison of peak intensities in 3D ^1H-^{15}N-$^{13}C\alpha$ spectra (top) and of site-specific backbone ^{15}N $R_{1\rho}$ rates measured with a 15 kHz spin-lock field (bottom) plotted along CorA sequence.

The overall reduction in spectral intensity and increase in ^{15}N $R_{1\rho}$ rates indicate that **release of Mg^{2+} induces an overall increase in CorA backbone dynamics**.

Conclusions



The investigation of CorA by MAS NMR allowed to extend the previous two-state model. We find that CorA is able to explore a wide conformational landscape both in the presence and absence of Mg^{2+}. The determining factor for CorA to visit conducting states is the increase in backbone flexibility upon release of Mg^{2+}.

References

[1] Nordin et al. *Biochem. Journ.* 2013, 451, 365
[2] Matthies et al. *Cell* 2016, 164, 747
[3] Barbet-Massin et al., *J. Am. Chem. Soc.* 2014, 136, 12489
[4] Stanek et al. *Angew. Chem.* 2016,55,15504
[5] Schmidt and Güntert *J. Am. Chem. Soc.* 2012,134,12817
[6] Johansen, Bonaccorsi et al. 2021 *submitted*