



## 35T SCH NMR $^{17}\text{O}$ Spectroscopy Reveals Long-Term Water Stability in a Transmembrane Pore

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### Introduction

Gramicidin A (gA) is dimeric ion channel with a 20 Å long pore supporting a single water file across liquid crystalline bilayers [1,2]. We have performed  $^{17}\text{O}$  NMR of gA using the SCH Magnet at 35.2T and report here that the electric dipole orientation of the water wire within the pore is orders of magnitude more stable than had been predicted by molecular dynamics (MD) simulations [2].

### Experimental

gA  $^{17}\text{O}$  single site labeled at Gly<sub>2</sub>, Leu<sub>4</sub>, Leu<sub>10</sub>, Leu<sub>12</sub> or Leu<sub>14</sub> in DMPC bilayers were aligned as described previously [3ab]. Oriented sample solid-state NMR (OSsNMR) spectra were acquired at the SCH magnet at the NHMFL in Tallahassee. The SCH was set at 35.2T, 203 MHz  $^{17}\text{O}$  field. An NMR probe optimized for static samples developed for the SCH magnet by the RF group in the NMR facilities of the NHMFL was used. The spectra was acquired with a DFS-echo pulse sequence with a 90° solid pulse of 1.5 μs and 20 μs echo time in the presence of 30 kHz  $^1\text{H}$  decoupling.

### Results and Discussion

The OSsNMR spectra of gA  $^{17}\text{O}$  Gly<sub>2</sub>, Leu<sub>4</sub> and Leu<sub>10</sub> show two well-resolved signals (Fig. 1). The same was not true for Leu<sub>12</sub> and Leu<sub>14</sub> where a single narrow resonance was observed for each site. While Gly<sub>2</sub>, Leu<sub>4</sub> and Leu<sub>10</sub> are exposed to the single file water wire, Leu<sub>12</sub> and Leu<sub>14</sub> carbonyls are fully exposed to the aqueous environment. Density functional theory (DFT) simulations in good agreement, reproduce the experimental peak distances observed in the NMR spectra (Table I). This confirms that the peak doubling is due to asymmetrical hydrogen bonding between residues in each monomer and the water wire, which is stable on the millisecond time scale. MD simulations that show the waters in the channel flipping at the subnanosecond time scale cannot correctly model this result.

### Conclusions

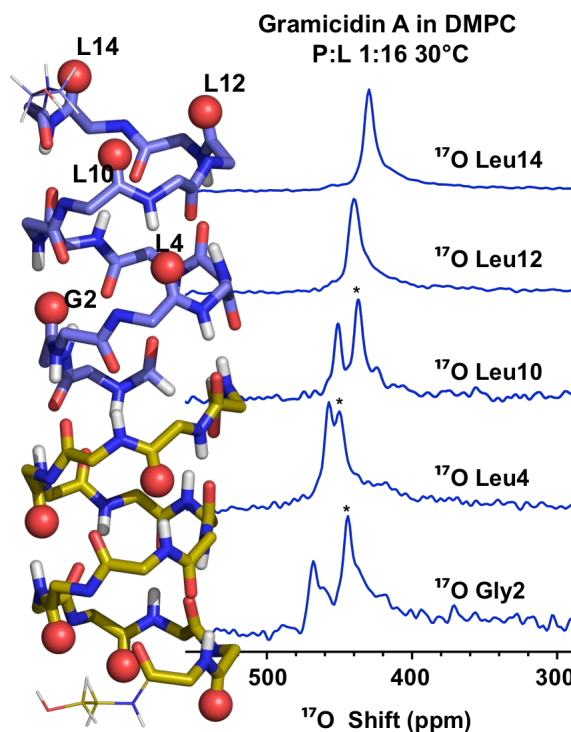
The asymmetric distribution of water hydrogen bonding to the pore lining carbonyls of the two monomers of the gA backbone causes doubling of the resonances, breaking the antiparallel dimeric symmetry of gA. This is confirmed by DFT calculations. The stability of the water wire orientation is on the millisecond timescale in that, if the water wire flipped on the sub msec timescale, there would be averaging of the resonance pairs and only a single peak would be observed in the spectra. This is the first time that long-term water stability has been reported within a channel.

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### References

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**Figure 1.** Gly<sub>2</sub>, Leu<sub>4</sub>, Leu<sub>10</sub>, Leu<sub>12</sub> and Leu<sub>14</sub> carbonyl oxygens were individually labeled with  $^{17}\text{O}$  such that each sample was labeled in both gA monomers with a single isotopic label. Peaks corresponding to residues where a stable hydrogen bond with water take place (DFT assignment) are marked with a \*.

**Table I.** Distance (in ppm) between peaks in NMR spectra and resonances calculated from DFT simulation.

Residue	NMR	DFT
Gly <sub>2</sub>	23.2 ± 2.5	25.9
Leu <sub>4</sub>	6.9 ± 2	4.8
Leu <sub>10</sub>	14 ± 2.8	8.7