

Imidazole-imidazole hydrogen bonding in the pH sensing Histidine sidechains of Influenza A M2

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Materials and Methods

The protein (residues 18-60) was expressed as a fusion to TrpLE, cleaved with cyanogen bromide, and purified by HPLC, as described previously.¹ The serine at position 50 was changed from the native cysteine, resulting in the sequence, RSN²⁰ DSSDPLVVAA³⁰ SIIGILHLIL⁴⁰ WILDRLFFKS⁵⁰ IYRFFEHLK⁶⁰. Lyophilized protein was resuspended in octyl glucoside detergent and reconstituted in perdeuterated (95% d78-phytanoyl 50% d at the alpha position, methyl-d9-choline) DPhPC lipids (FBReagents.com) at a lipid to protein ratio of 1 by mass. The drug-containing sample was equilibrated with a 40 mM solution of rimantadine after reconstitution. All spectra were acquired on a 950 MHz Bruker Avance III spectrometer using a Bruker 0.7 mm HCDN probe. Unless otherwise indicated, the spinning frequency was 100 kHz and the gas flow was set to 500 liters per hour to maintain a sample temperature of about 20 °C, (260 K thermocouple temperature) as determined using the chemical shift of KBr. The proton spectrum was referenced by setting the chemical shift of water to 4.75 ppm. The nitrogen spectrum is reported on the liquid ammonia scale, using the IUPAC relative frequency ratios.

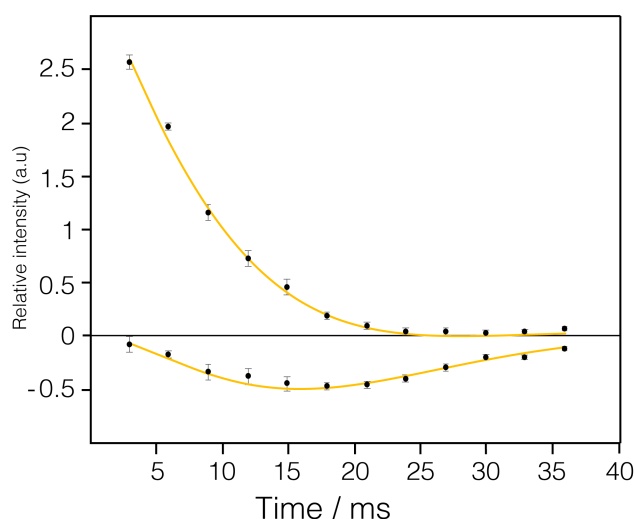


Figure S1. Fit of the starting signal on N₂₂ (positive curve) and the buildup of the antiphase term detected at the chemical shift of N₅₁ (negative curve). The curve was fit with the 8.9 Hz J coupling, and a T₂ of relaxation time of 44 ms applied to both curves.

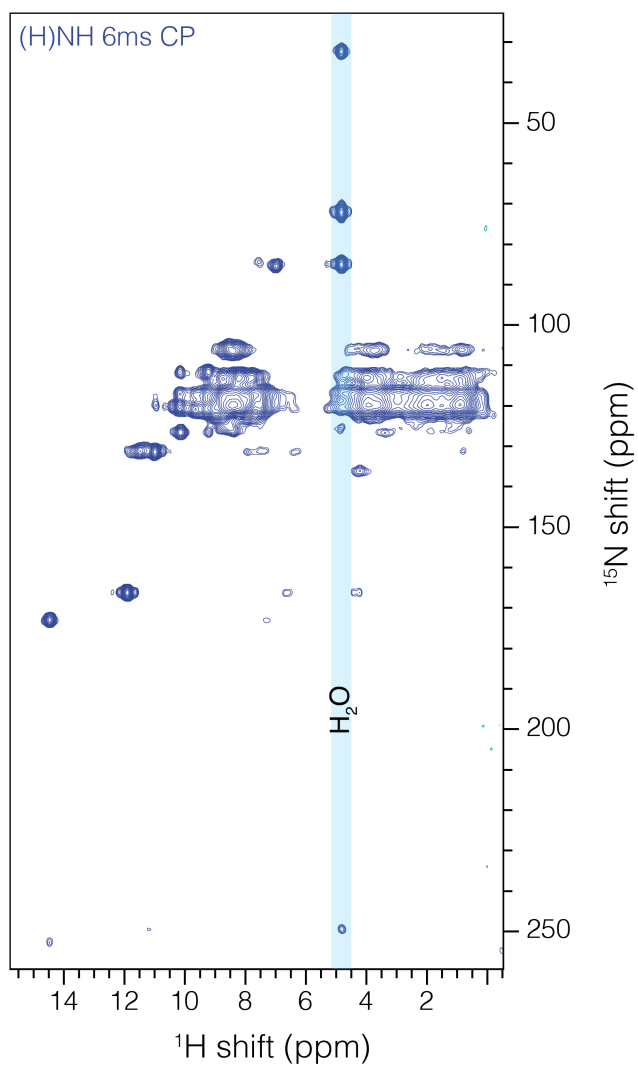


Figure S2. Full long CP spectrum from Figure 3 showing the same water chemical shift for the contacts to side-chain nitrogen of R and K in the amphipathic helix and the residue H37 in the channel. This peak at 4.85 ppm is separated from the bulk water at 4.75 ppm.

1. Schnell, J. R.; Chou, J. J., Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* **2008**, *451* (7178), 591-5.