Supporting Information to: Dynamic Nuclear Polarization Study of Inhibitor Binding to the $M2_{18-60}$ Proton Transporter from Influenza A

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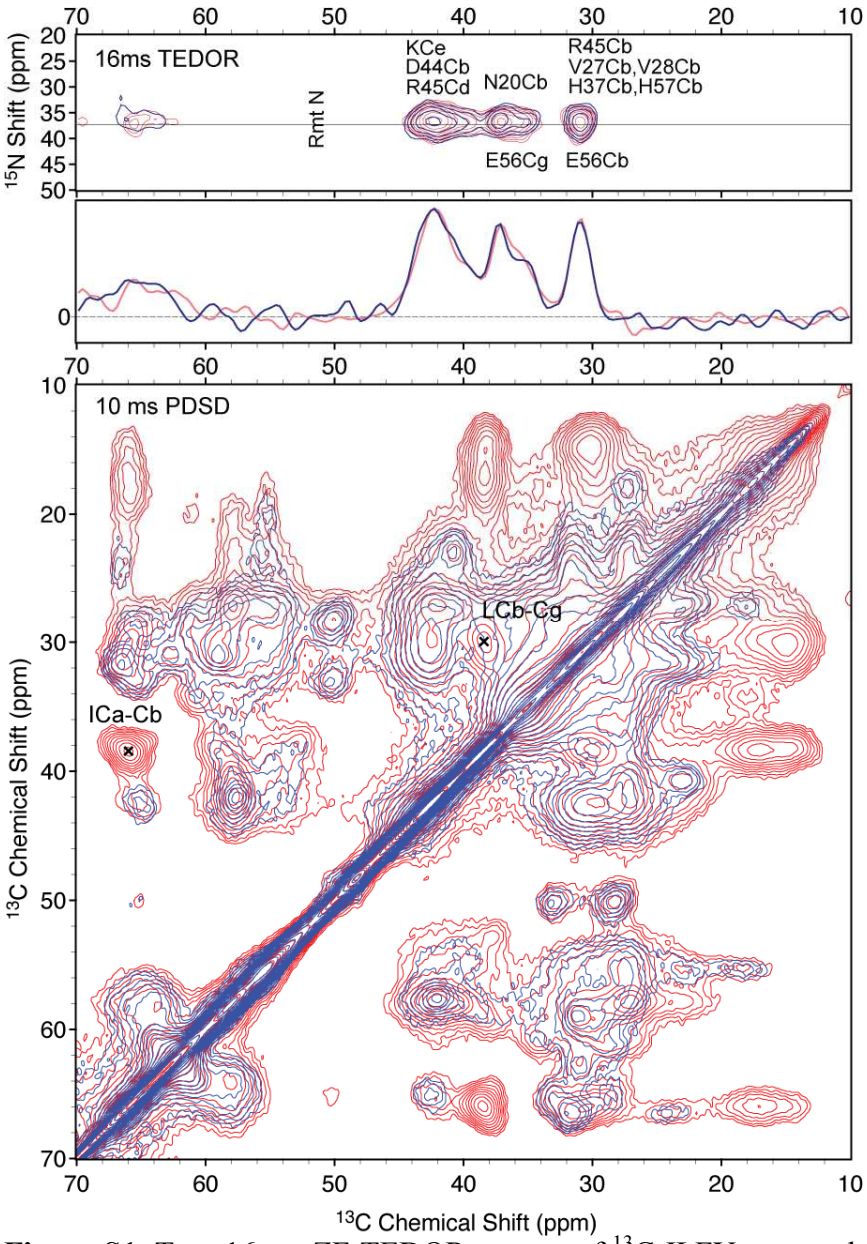

Figure S1. Top: 16 ms ZF-TEDOR spectra of ^{13}C-ILFY reverse labeled M2 (blue) show identical crosspeaks as FY reverse labeled M2 (red). A 1D slice from the 2D spectrum is displayed. Bottom: A 10 ms PDSD spectrum demonstrates successful labeling for these samples since Ile and Leu cross peaks are absent from the blue spectrum. Spectra were

recorded at 400 MHz, 9009 kHz MAS, and a temperature of ~105 K. D21G-D24G M2 was used, and ^{15}N was incorporated into the backbone of the protein with ^{15}N Leucine.

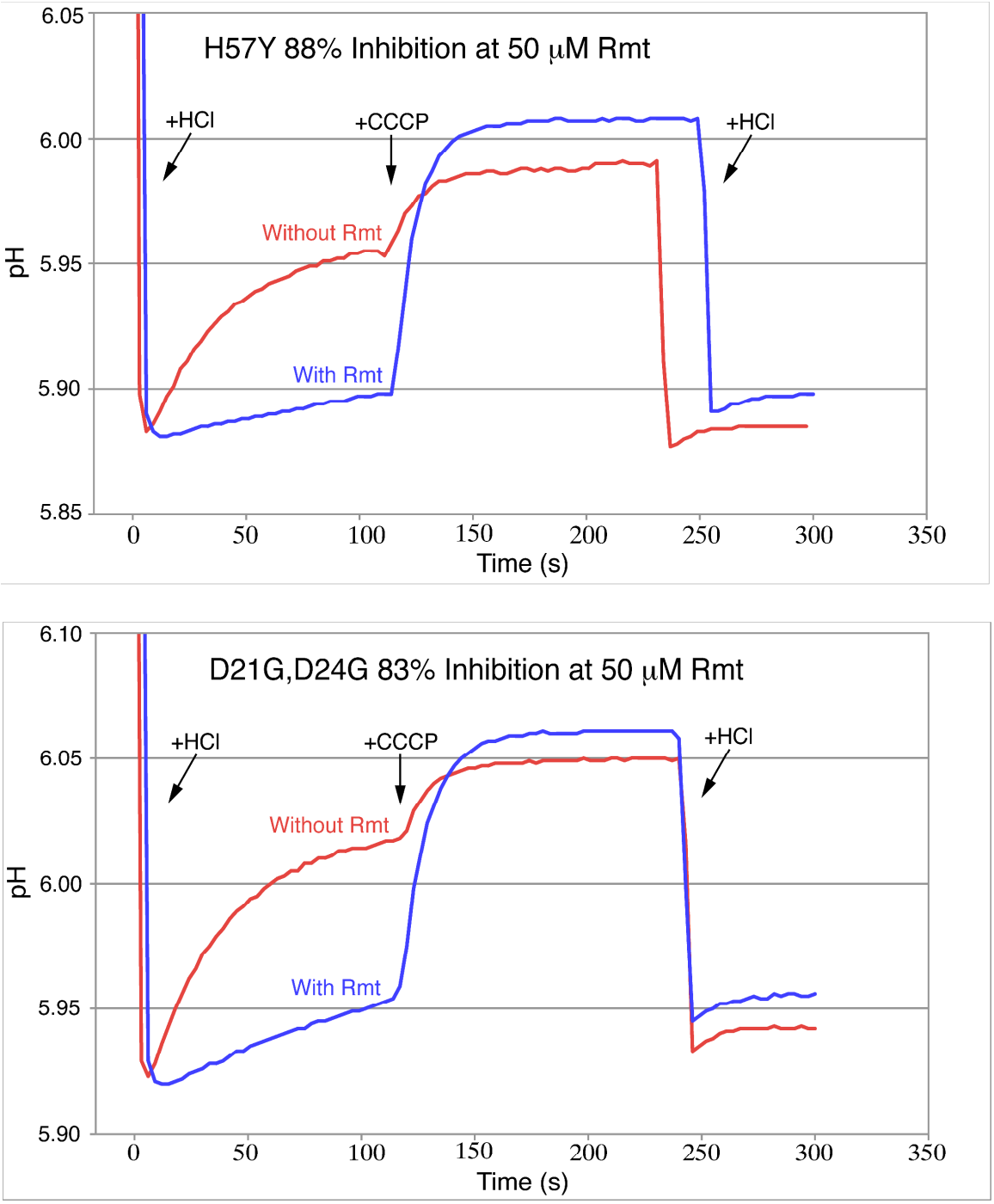

Figure S2. Channel current is measured indirectly by pH in a liposome assay described previously.(*1*) Briefly, M2 is prepared in liposomes, and the pH outside the liposomes is

measured as a function of time. Addition of HCl initiates channel conduction of protons into the liposomes, the initial slope of which is used as a measure of proton flux. The proton flux per channel was calculated using CCCP to equalize the pH inside and outside the liposome, followed by addition of a known amount of HCl to calculate the total buffering capacity. The D21G,D24G double mutant and the H57Y mutant are drug sensitive, with ~80-90% reduction in proton current in the presence of 50 uM Rimantadine, similar to wild type protein.

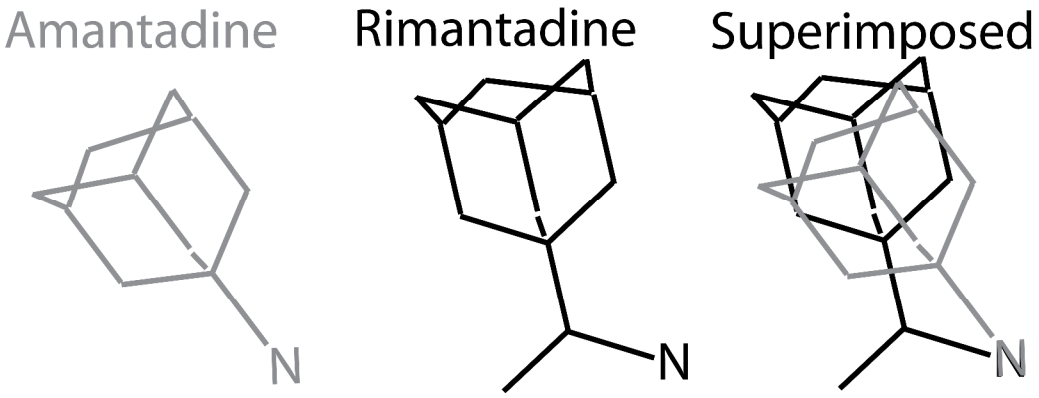

Figure S3. An illustration of the tilt angle measured by Hong and coworkers for Amantadine and Rimantadine(*2, 3*). Amantadine is tilted 37° from the bilayer normal, while Rimantadine is tilted by only 13°. In both cases, the amine and adamantyl ends of the molecule occupy similar positions relative to the membrane normal. The illustration was generated using a single internuclear distance and ideal angles of 120° for the projection of tetrahedral geometry in 2 dimensions.

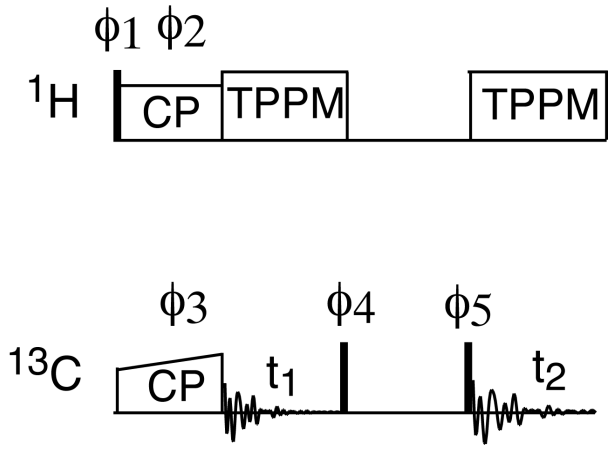

Figure S4. The Proton Driven Spin Diffusion (PDSD) pulse program used in Figure 1 is depicted as previously described(*4, 5*). Narrow rectangles represent 90º pulses. A ^{1}H flip and decoupling power level of 83 kHz was used. Two pulse phase modulation(*6*) (TPPM) was optimized with phases of 18 and 0 degrees, and a pulse length of 5.8 μs. 83 kHz of ^{13}C was used for flip pulses. Cross polarization (CP) was applied for 1.5 to 2 ms with constant irradiation at ~70 kHz on ^{1}H and with an optimized ramp on ^{13}C centered at one rotor frequency below the proton nutation frequency. The phase cycle was: ϕ_1 = 13, ϕ_2 = 2, ϕ_3 = 1133, ϕ_4 = 2, ϕ_5 = 1111 3333 2222 4444, $\phi_{receiver}$ = 2442 4224 3113 1331, where x=1, y=2, -x=3, -y=4.

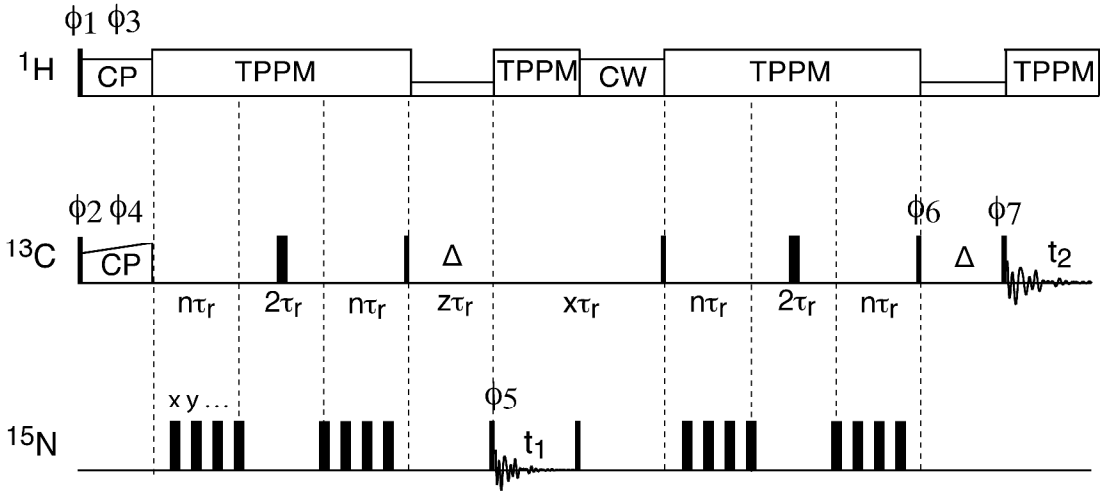

Figure S5. The z-filtered transfered echo double resonance (ZF-TEDOR) pulse program used in Figures 2-4 is depicted as previously described(*7*). Narrow rectangles represent 90º pulses, and broad rectangles represent 180° pulses. A ^{1}H flip and decoupling power level of 83 to 100 kHz was used during evolution, and ~100 kHz was applied during mixing. Two pulse phase modulation (TPPM) was optimized with phases of 18 and 0 degrees, and a pulse length of 5.8 μs (83 kHz) or 4.8 μs (100 kHz). 50 to 83 kHz of ^{13}C was used for flip pulses. A power level of 20 to 40 kHz was typical for flip pulses on the ^{15}N channel. At 278 K, cross polarization (CP) was applied for 1.5 to 2 ms with constant irradiation at ~70 kHz on ^{1}H and with an optimized ramp on ^{13}C centered at one rotor frequency below the proton nutation frequency. At low temperature, 0.9 ms of CP was used. The phase cycle was: ϕ_1 = 16x(1) 16x(3), ϕ_2 = 16x(4) 16x(2), ϕ_3 = 2, ϕ_4 = 1, ϕ_5 = 13, ϕ_6 = 2244, ϕ_7 = 1111 2222 3333 4444, $\phi_{receiver}$ = 4224 1331 2442 3113 2442 3113 4224 1331, where x=1, y=2, -x=3, -y=4. The initial flip pulse on the ^{13}C channel was omitted for spectra acquired at ~100 K. Rotor synchronization is indicated under the ^{13}C channel in the figure. Mixing times (4nτr) are indicated in the manuscript. Z-filters of 2 to 4 rotor periods were sufficient to suppress unwanted coherences.

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