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Supplemental Information

Influenza A M2 Channel Clustering at High Protein/Lipid Ratios: Viral Budding Implications

Joana Paulino, Xiaodong Pang, Ivan Hung, Huan-Xiang Zhou, and Timothy A. Cross

Supplemental Information

Session 1. ^{2}H NMR line shape analysis for deuterated methyl groups

The anisotropic nature of the ^{2}H quadrupolar interaction results in ^{2}H NMR spectra that are dependent on the angle between quadrupolar interaction tensor (V_{zz}) axis (which for C-^{2}H lies along the C-H bond) and the molecule motional axis. In the situation in which the molecular motions are not able to average the quadrupolar interaction to zero the resulting broad spectrum (named "powder-pattern") contains information on the amplitude and rate of the modes of motion present at the deuterated site. In the case of amino acids bearing methyl groups, such as alanine, the ^{2}H NMR spectrum can report not the local motion of the methyl group but also the global motions of the protein in which the amino acids resides. For the specific case of membrane proteins the motional modes that the methyl group of an alanine residue can report are: the methyl deuteron rotation about its C3 symmetry axis (along the Cα-Cβ bond; Figure S1), the motion of the peptide plane that is equivalent to a wobbling motion of the Cα-Cβ bond (Figure S2), and the overall rotation of the protein within the membrane (Figure S3).

The effect of the fast rotation of methyl deuterons about their C3 symmetry axis (Figure S1) reduces the static quadrupolar coupling constant (QCC) of 171 kHz by a factor of 3 (57 kHz), due to the fact that all 3 sites are equally populated. This motion can be described as 120° jumps between 3 sites about a symmetry axis (C3) that, in the case of tetrahedral geometry, makes an angle (β) of 70.5° with each of the C-^{2}H bonds. The resulting effect is that of an averaged V'_{zz} that is collinear to the C3 symmetric axis. Deviations of the methyl group tetrahedral geometry have been reported to be on the order of ~1° (1) and do not influence the averaged spectra significantly. In short, fast (i.e., ps timescale) methyl rotation alone leads to a quadrupolar coupling of 57 kHz, which is the starting point for further analysis on methyl ^{2}H spectra.

The libration (Figure S2) of the peptide plane also has an effect on the spectra of the methyl side chains. The libration translates into a wobbling of the Cα-Cβ bond, leading to a reduction of V'_{zz} by a factor equal to the order parameter factor, S^2, which depends on the motional amplitude angle θ as given by $S^2 = \frac{1}{2}(3cos^2\theta - 1)$ (2). The wobbling motion of Cα-Cβ bond is modeled as the rotation of this bond about a cone

with solid angle θ. The effect of this type of motion on the ^{2}H spectra is an effective reduction of V'_{zz} to an apparent V'_{zzap}.

Finally, global axisymmetric rotation of the protein within the lipid bilayer (Figure S3) further averages the quadrupolar interaction (V'_{zzap}). Depending on the orientation of the methyl local motional axis (the C3 axis) with respect to the global rotation axis, which for a membrane protein is parallel to the membrane normal, the effect on the line shape can be dramatic, leading to the collapse of the sharp features characteristic of a ^{2}H ssNMR spectrum.

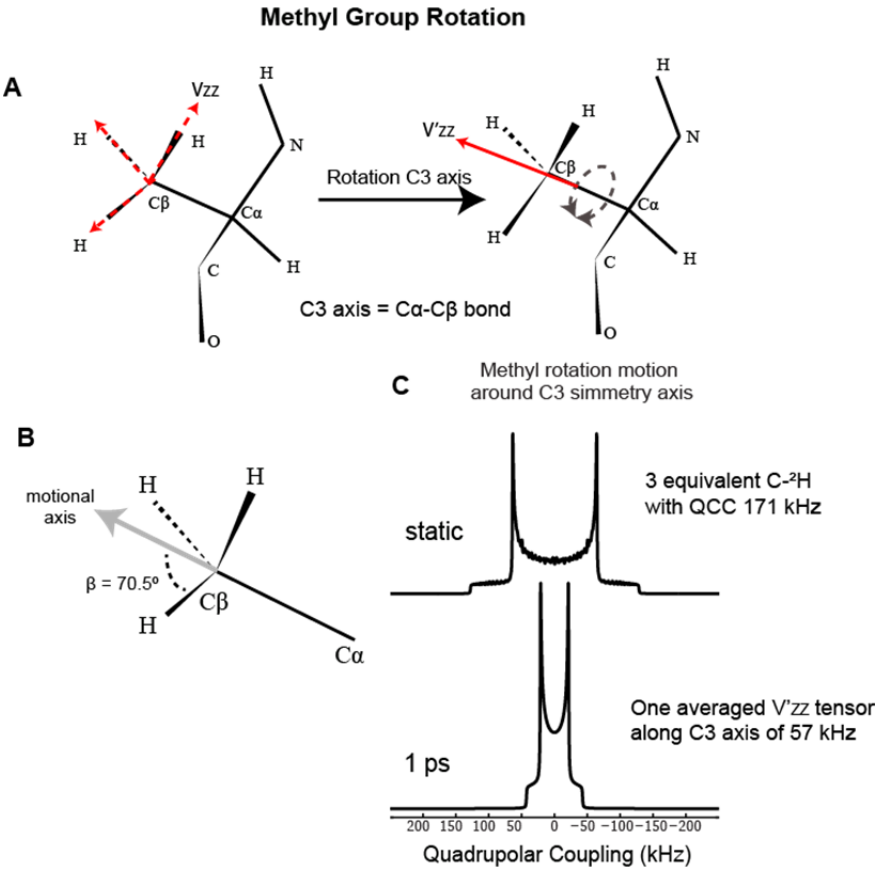

Figure S1. Effect of the fast rotation of a methyl group around its C3 symmetry axis on the ^{2}H spectra. A) The main component of the ^{2}H quadrupolar interaction (V_{ZZ}) is oriented along the C-^{2}H bond. In a methyl group all 3 interaction are equivalent. The ps rotation about the C3 symmetry axis averages the V_{ZZ} interactions to a V'_{ZZ} that is oriented along the C3 symmetry axis (Cα-Cβ bond). B) The Euler angle β between the quadrupolar interaction tensor and motional axis. C) The effect of ps methyl group rotation on the NMR spectra of a methyl deuteron.

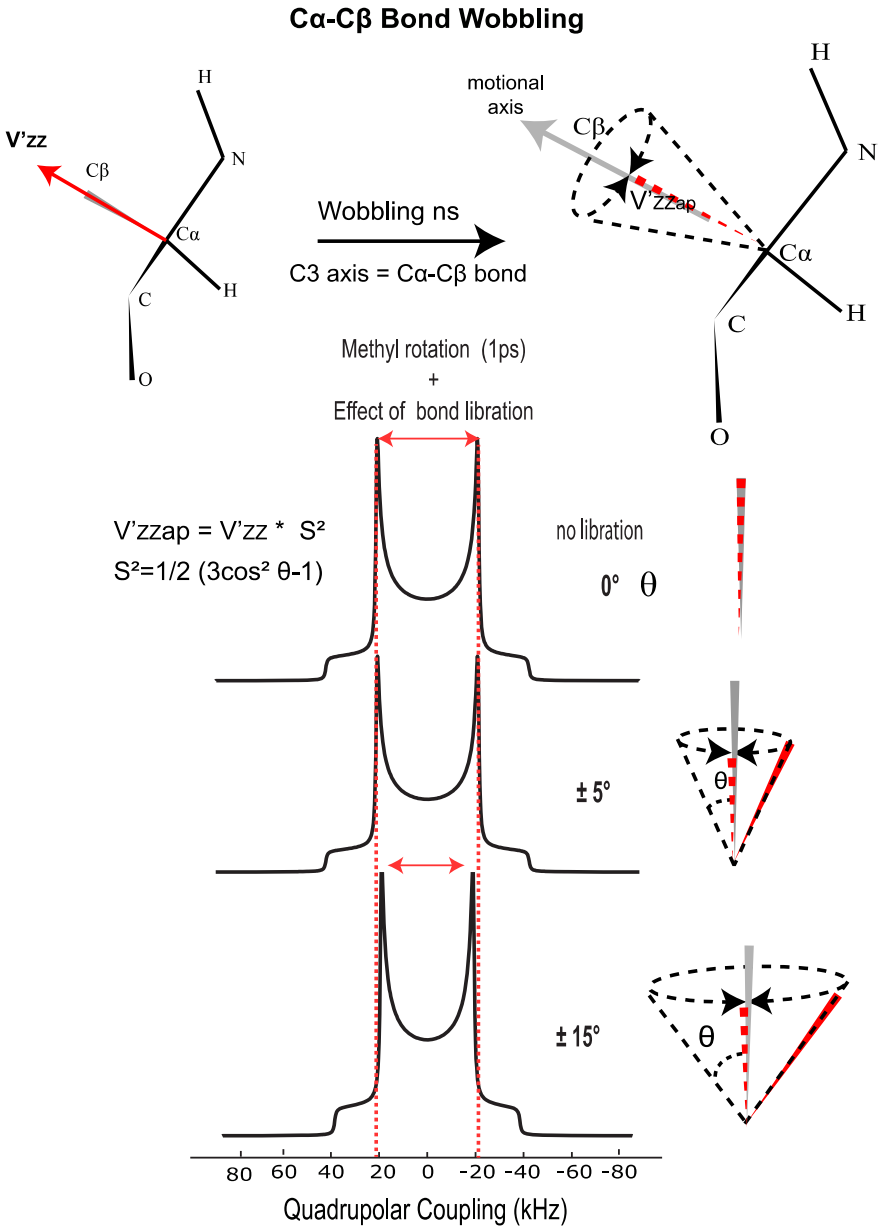

Figure S2. Effect of peptide plane libration on the ^{2}H spectra of a methyl deuteron undergoing fast rotational motion about its C3 symmetry axis. Top: model of the wobbling motion as a rotation around a cone of amplitude angle θ. The V'_{ZZ} is further averaged by a factor S^2 giving a reduced value, V'_{ZZap}. Bottom: effect of the wobbling motion amplitude on the ^{2}H NMR powder spectra of a methyl group. The larger the value of θ the more averaged (smaller value) is V'_{ZZap}. The effect of the wobbling motion on the overall width of the powder pattern is less evident (smaller) than the effects of methyl rotation (Fig 1S) and global rotation (Fig S3).

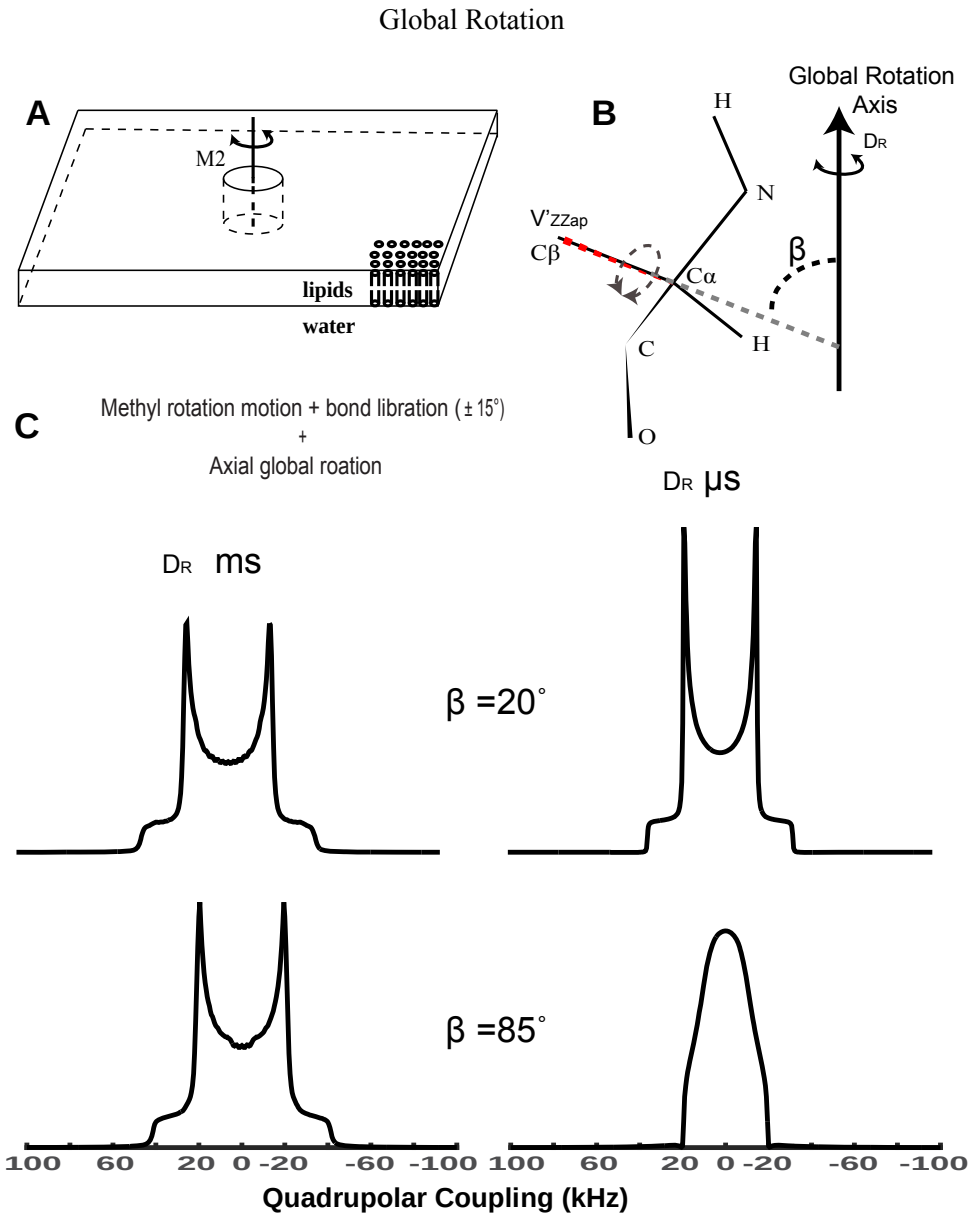

Figure S3. Effect of global axial rotation on the ^{2}H NMR spectra of a methyl group. A) Model of an M2 channel (cylinder) global axial rotation within the lipid bilayer, according to Saffman and Delbrück (2). B) The Euler angle β between the averaged quadrupolar interaction tensor (V'_{ZZap}) and the global rotation axis; D_R is the rotational diffusion constant. C) Effect of global rotation with $1/D_R$ on the ms to μs timescale on the spectra of methyl deuterons oriented with β at 20° or 85°.

Session 2. Saffman-Delbruk Calculation of Rotational Correlation Time

According to the Saffman-Delbruck model (3), the rotational diffusion constant of an integral membrane protein can be approximated as that of a cylinder rotating in viscous environment (which represents the lipid bilayer), given by

$$D_R = \frac{k_B T}{4\pi\mu r^2 h} \qquad \text{Equation 1.}$$

Here k_B is the Boltzmann constant (1.38064852×10^{-23} J/K), T is the temperature in Kelvin, r is the radius of the protein, h is the thickness of the lipid bilayer, and μ is the lipid bilayer viscosity. The radius of M2TM can be estimated as 12 Å, and the thickness of lipid bilayers is typically around 40 Å. The viscosity for most unsaturated lipids above their phase transition temperature is around 0.1 to 0.2 Pa·s (4). With these numbers, we can estimate a rotational diffusion constant of 0.37 x 10^6 s^{-1} to 0.75 x 10^6 s^{-1}, corresponding to a rotational correlation time of 0.7 to 1.3 μs, which is in reasonable agreement with the value of 0.55 μs obtained from line shape simulations for M2TM.

Session 3. Supplementary Figures

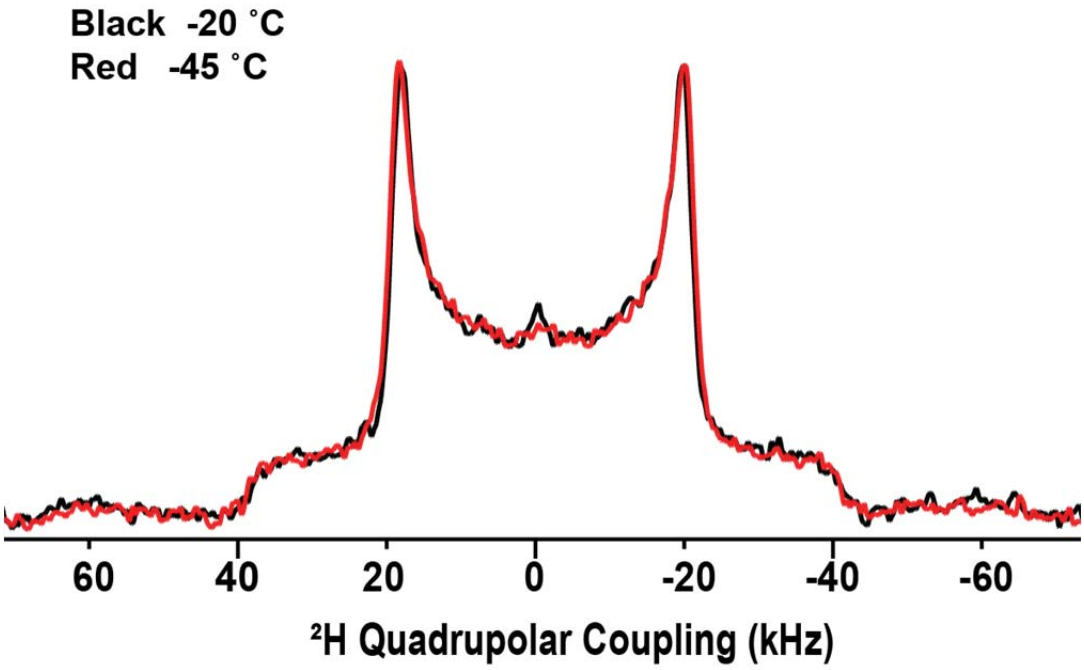

Figure S4. ^{2}H NMR spectra of M2TM d4Ala$_{29}$ in DOPC:DOPE (4:1) at pH 7.5 and L:T = 80:1. Acquired in a 21.1T magnet with 30 μs echo time at -20 °C (black) and at -45 °C (red).

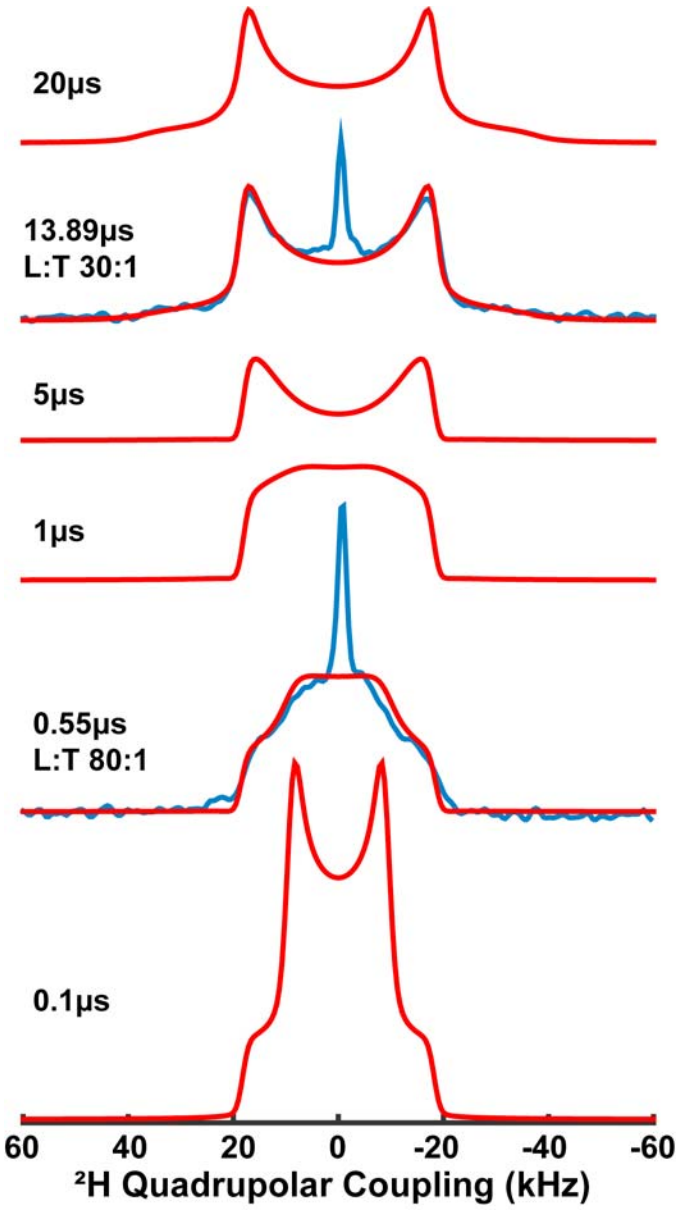

Figure S5. RCT dependence of calculated ^{2}H NMR line shape of M2TM Ala$_{29}$ methyl deuteron from 0.1 μs to 20 μs. In red, ^{2}H NMR line shape calculated with parameters: fast ps methyl rotation, Cα-Cβ bond wobbling angle = 15°, Cα-Cβ bond angle with respect to the channel main axis = 85°. In blue, experimental ^{2}H NMR spectra of deuterated Ala$_{29}$ M2TM at L:T 30:1 and L:T 80:1.

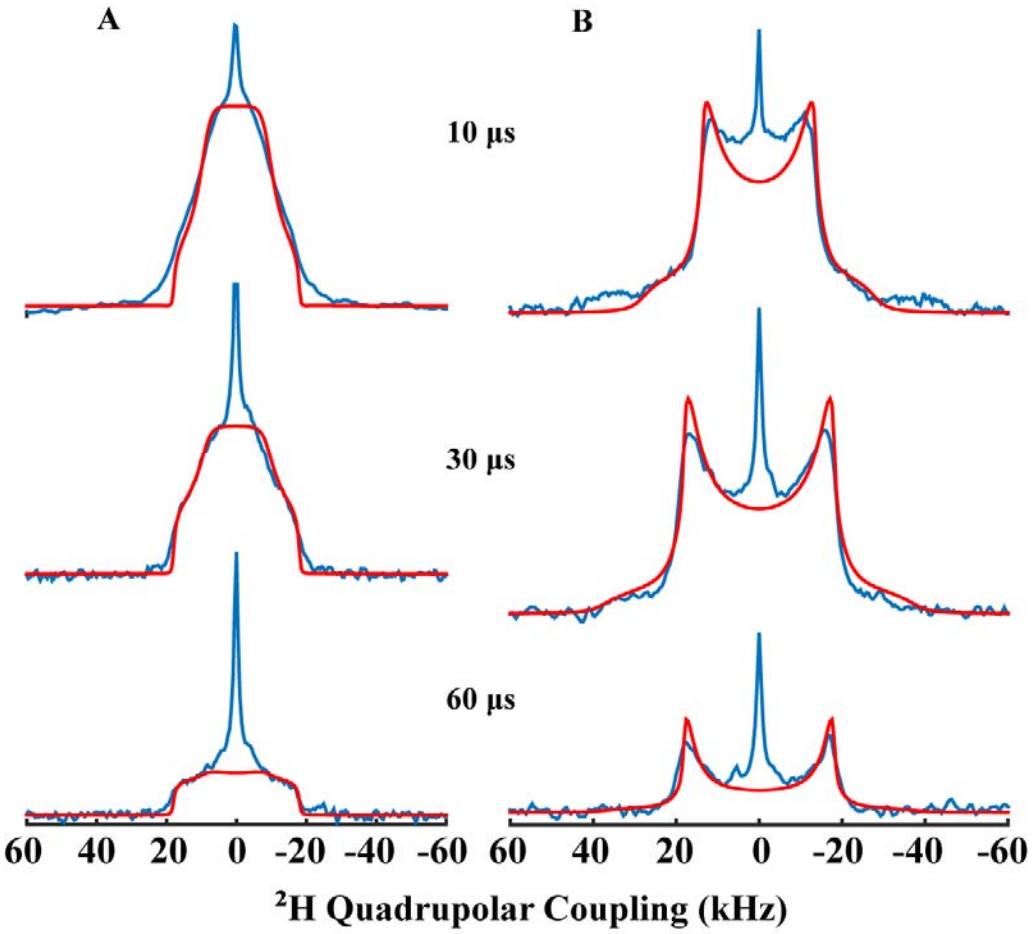

Figure S6. ^{2}H spectra of M2TM d4Ala$_{29}$ in DOPC:DOPE (4:1) at pH 7.5 and 20 °C in a 19.6 T magnet. A) L:T = 80:1; B) L:T = 30:1. Blue: spectra acquired with 10, 30, and 60 µs echo times; red: line shapes calculated with parameters: fast ps methyl rotation, Cα-Cβ bond wobbling angle = 15°, Cα-Cβ bond angle with respect to the channel main axis = 85°, and axial rotation motion with RCT = 0.55 ± 0.1 µs (A) and RCT = 13.89 ± 2 µs (B).

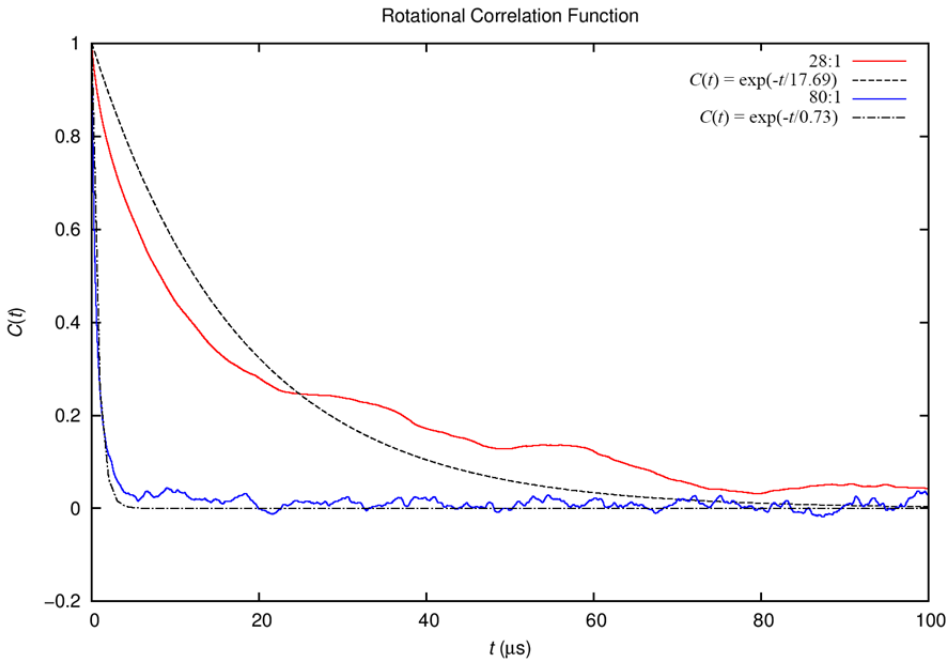

Figure S7. Exponential fitting of the time-correlation function for the axial orientation in the mixed orientation CG simulations. The data at L:T = 80:1 and 28:1 (blue and red) are fit with RCTs of 0.73 and 17.69 μs (black), respectively.

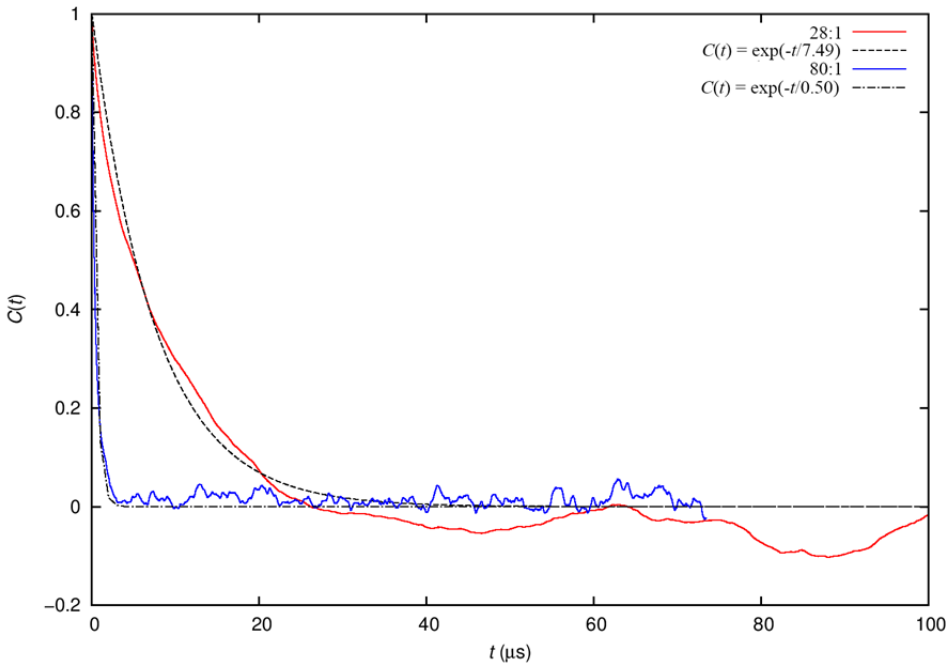

Figure S8. Exponential fitting of the time-correlation function for the axial orientation in the parallel orientation CG simulations. The data at L:T = 80:1 and 28:1 (blue and red) are fit with RCTs of 0.50 and 7.49 μs (black), respectively.

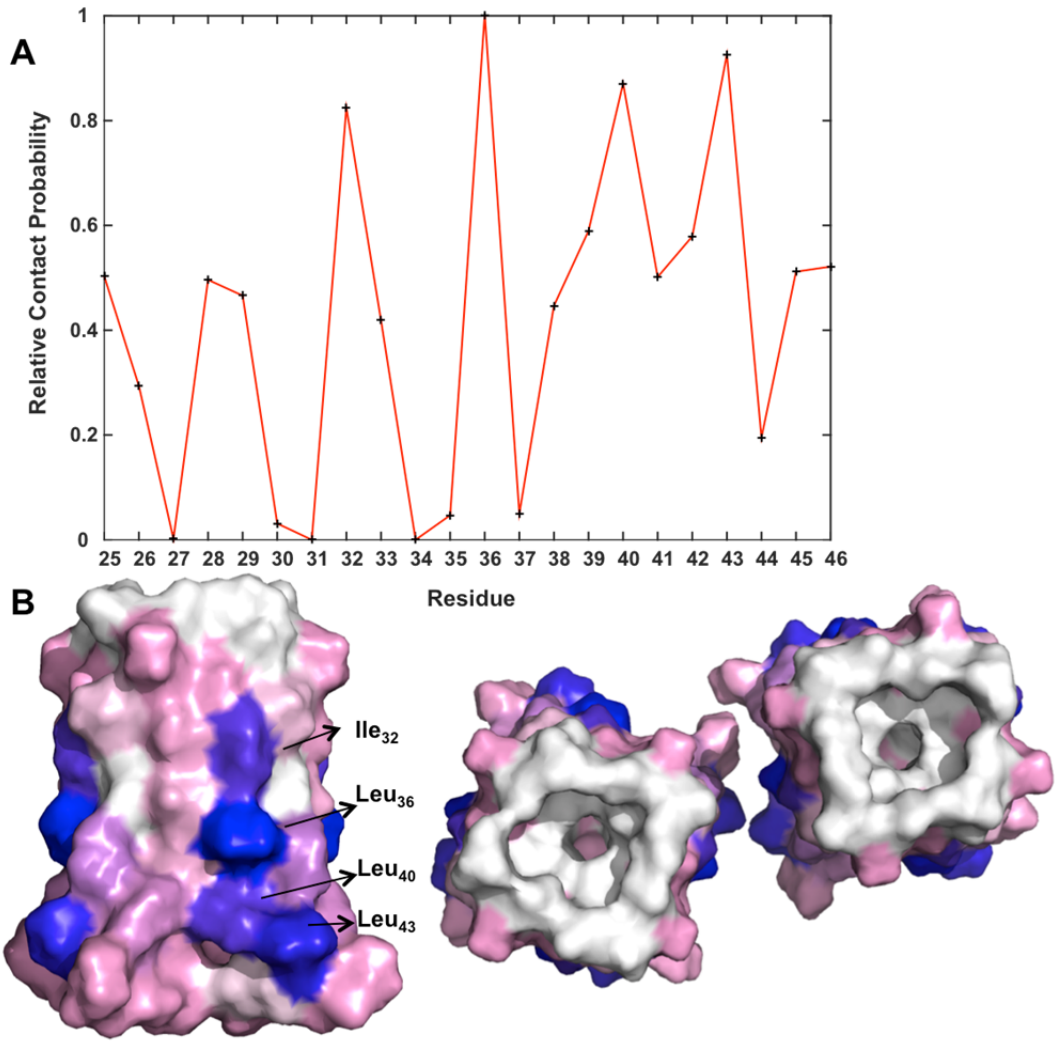

Figure S9. Relative contact frequencies of individual residues in the parallel orientation CG simulations at L:T = 28:1. A) Contact frequency was calculated as the fraction of snapshots where a residue was involved in inter-tetramer contact, and then normalized by the highest value of all residues. B) The M2TM surface colored according to residue contact frequency, from the maximum 1 (blue) to pink (0.5) to 0 (white). In the side view on the left, four residues with the highest contact frequencies are labeled. In the top view on the right, two tetramers are shown in a corner-to-face pose illustrating shape complementarity.

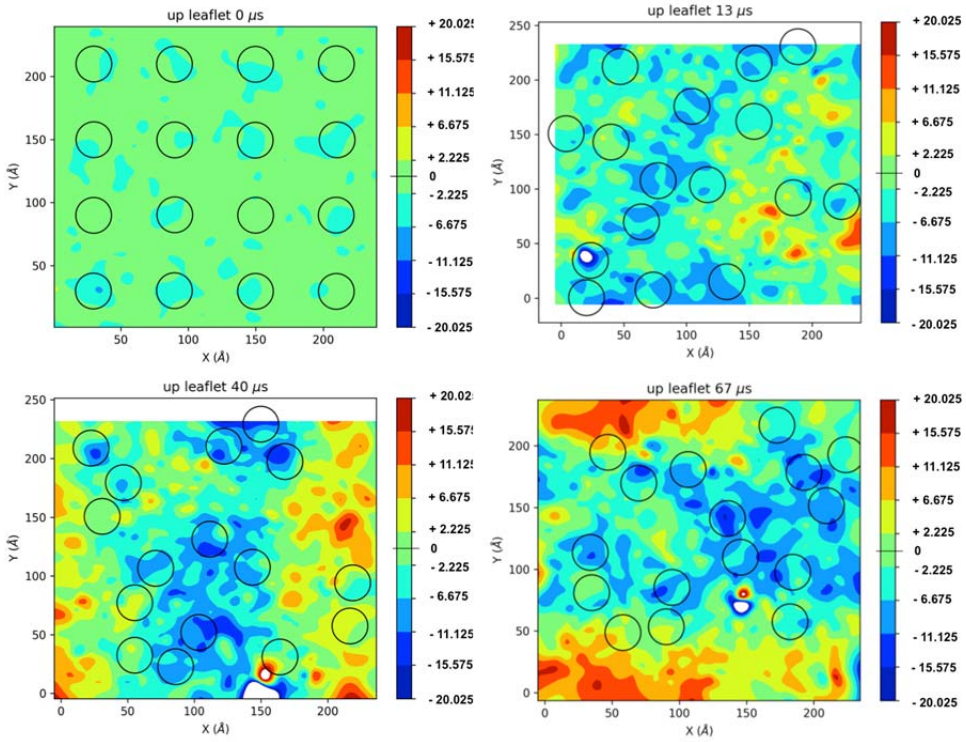

Figure S10. Position of lipid head groups along simulation Z-axis at different simulation times. The position of the nitrogen atom of the lipid head groups was measured at different times in the simulation and plotted relative to the initial position Z=0 at t=0 μs. The blue to red color scale indicates when the head group has moved down (negative) or up (positive) relative to the position at the starting point. Black circles represent where M2CD channels are located in the snapshot.

Supporting References

1. Ottiger, M., A. Bax. 1999. How Tetrahedral Are Methyl Groups in Proteins? A Liquid Crystal NMR Study. J Am Chem Soc, 121:4690-4695.

2. Duer, M. (2002). Solid State NMR Spectroscopy: Principles and Applications. . Blackwell Science Ltd. London, England, 267-268.

3. Saffman, P. G., M. Delbrück. 1975. Brownian motion in biological membranes. P Natl Acad Sci USA, 72: 3111-3113.

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