Supplementary Materials

Conformational dynamics and stability of bilayers formed by mycolic acids from the *Mycobacterium tuberculosis* outer membrane

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Section S1. Algorithm for the refinement of conformation identification

The assignment of the structures obtained during MD modeling to one of the seven WUZ conformations was carried out based on the comparison of the distances *ac, ae, ce* and *bd* with the boundary distances given in Table S1.

Table S1. Bounds of intramolecular distances for determining WUZ conformations.

Conformation	Boundary distances, nm			
	ac	*ae*	*ce*	*bd*
W	< 2.3	< 2.8	< 2.0	< 1.3
aZ	> 2.3	> 2.8	< 2.0	< 1.3
eZ	< 2.3	> 2.8	> 2.0	< 1.3
sZ	< 2.3	< 2.8	< 2.0	> 1.8
eU	< 2.3	> 1.5	> 2.0	> 1.8
sU	> 2.3	< 2.8	> 2.5	< 1.6
aU	> 3.0	> 2.8	< 1.8	> 1.8

The use of the boundary distances given in Table S1 makes it possible to definitely classify about 65% of the structures formed during MD evolution. The remaining structures formally belong to the Straight conformation, but in reality, often resemble one of the seven WUZ conformations. Therefore, for their additional analysis and assignment, the *k*-means clustering algorithm was used with a preliminary determination of the cluster centers.

Cluster centers for conformations α (α = W, sZ, eU) were calculated as the average values of the distances $\langle ac\rangle_\alpha$, $\langle ae\rangle_\alpha$, $\langle ce\rangle_\alpha$, $\langle ed\rangle_\alpha$ over all molecules of conformation α that were already classified using boundary distances for sampling structures from the MD trajectory at the simulation time of 100 ns. The choice of time is due to the need to accumulate a sufficient population of structures in different conformations and achieve their structural diversity relative to the initial state.

The number of structures assigned to the types aZ, eZ, sU, aU was usually not enough to find the hypothetical cluster centers in a similar way with the required accuracy. The method of additional extrapolation was used to find the centers of these clusters. To do this, from the coordinates of the centers of clusters W, sZ and eU and the boundaries of intramolecular distances *ac, ae, ce, ed* (Table S1) of conformations W, sZ and eU, three 4-dimensional vectors v_α were formed by the formula:

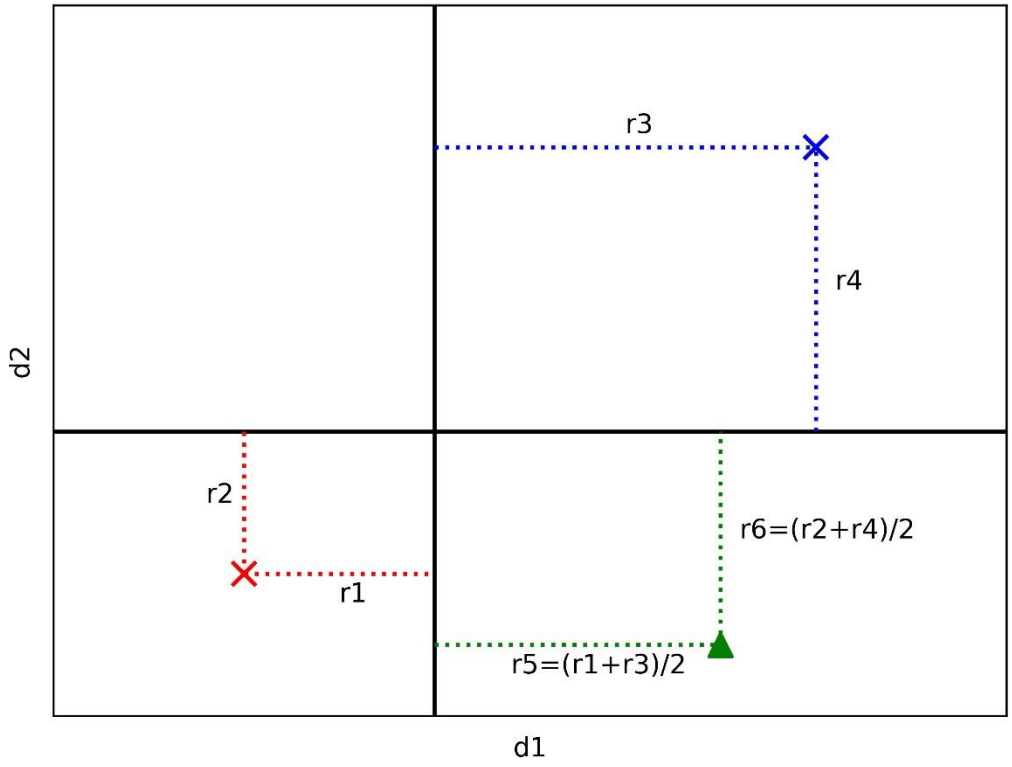
$$v_\alpha = (|\langle ac\rangle_\alpha - ac_\alpha|, |\langle ae\rangle_\alpha - ae_\alpha|, |\langle ce\rangle_\alpha - ce_\alpha|, |\langle bd\rangle_\alpha - bd_\alpha|),$$

which were then averaged over three values of α (α = W, sZ, eU). The components of the averaged vector $\langle v\rangle$ correspond to the approximate margins from the cluster center to the distance boundaries of the corresponding conformations. These margins can be used to find unknown cluster centers.

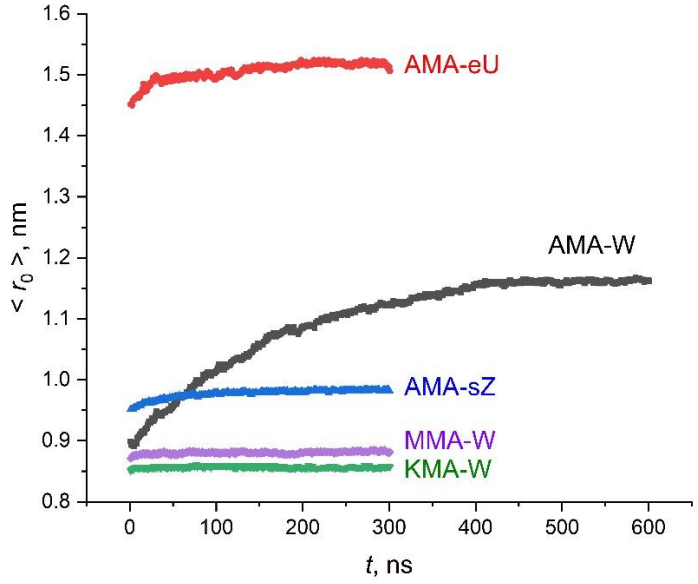
For this purpose, the vector <*v*> was combined with the coordinates of the boundaries of the corresponding conformation β (β = aZ, eZ, sU, aU): if the value of the boundary distance in Table S1 is preceded by the sign <, then the corresponding coordinate of the vector <*v*> was subtracted from this value; if there is a > sign, then it was added to it. The resulting vector c_β determines the approximate coordinates of the β cluster center. These coordinates are then passed to the *k*-means clustering algorithm.

$$c_\beta = (ac_\beta \pm ac_{<v>}, ae_\beta \pm ae_{<v>}, ce_\beta \pm ce_{<v>}, bd_\beta \pm bd_{<v>})$$

This procedure can be illustrated by the following scheme (for simplicity, only two distances d_1, d_2 out of four defining distances *ac*, *ae*, *ce*, *bd* are shown). Solid black lines represent the distance boundaries. Blue and red crosses show the cluster centers for the two out of three abundant conformations (e.g., W, sZ, or eU). For them, the v_α vectors are (r_1, r_2) and (r_3, r_4). The averaged vector components <*v*> = (r_5, r_6) define the offsets of the unknown center c_β (green triangle) for the β cluster from the respective distance boundaries (β is one of aZ, eZ, sU, aU).



(*a*)



(*b*)

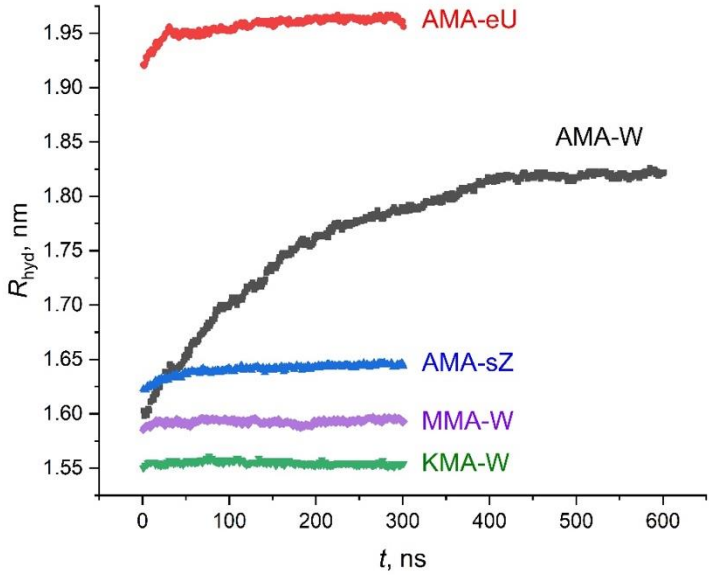
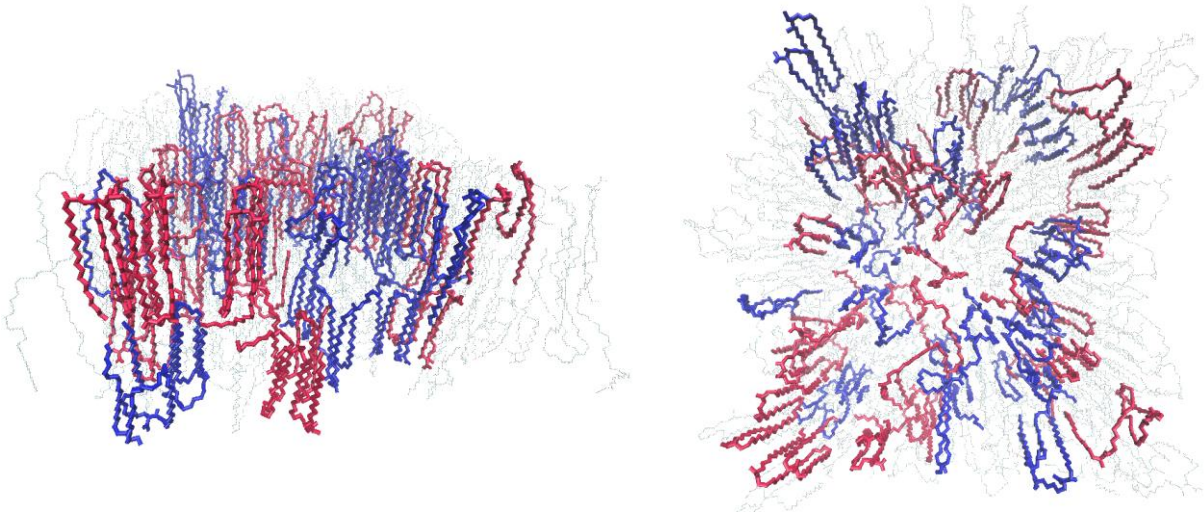


Figure S1. Changes in the average massless gyration radii (*a*) and average hydrodynamic radii (*b*) of AMA, KMA, MMA in the membranes of different initial structure during MD simulation.

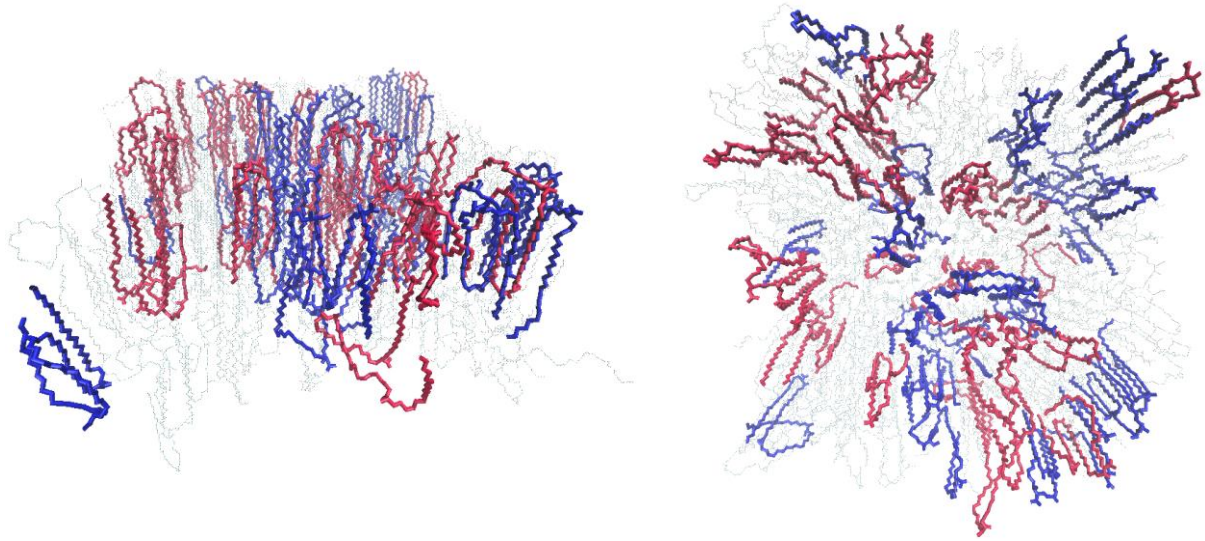
Side view

Top view

0 ns



100 ns



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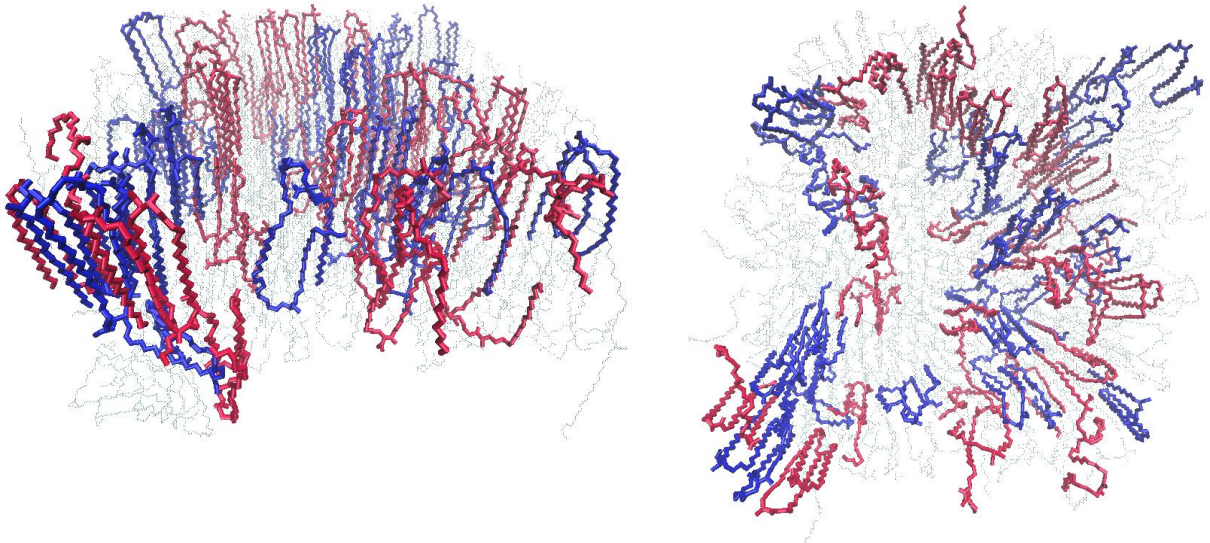


300 ns

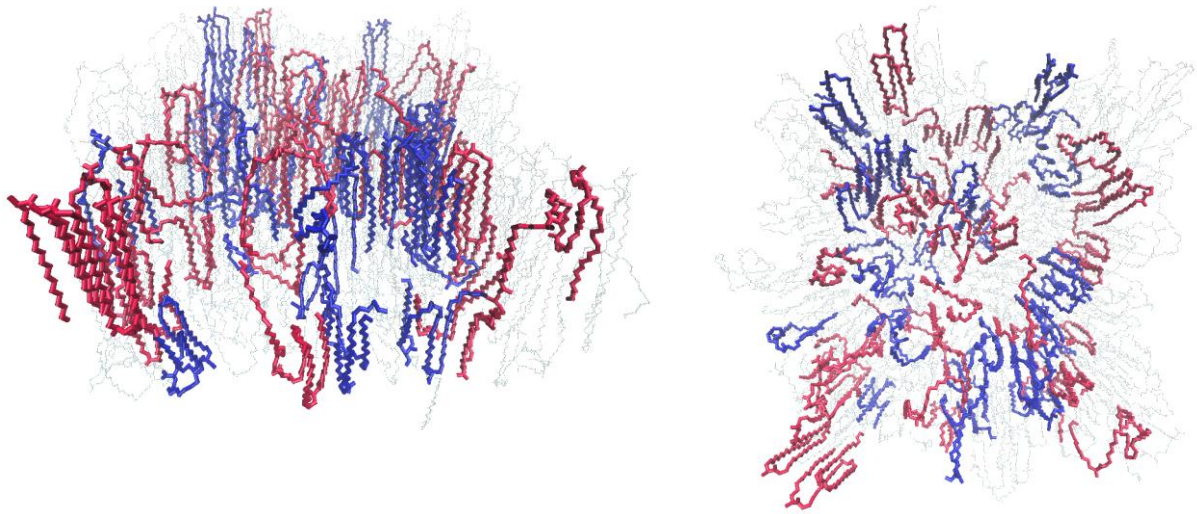


Figure S2. Mutual arrangement of MMA and KMA in a mixed membrane 140AMA(W):30MMA:30KMA for the initial configuration (0), 100, 200 and 300 ns of molecular dynamics simulation. MMA molecules are highlighted in blue, KMA – in red. Weak halftone background represents the AMA molecules with W conformation.

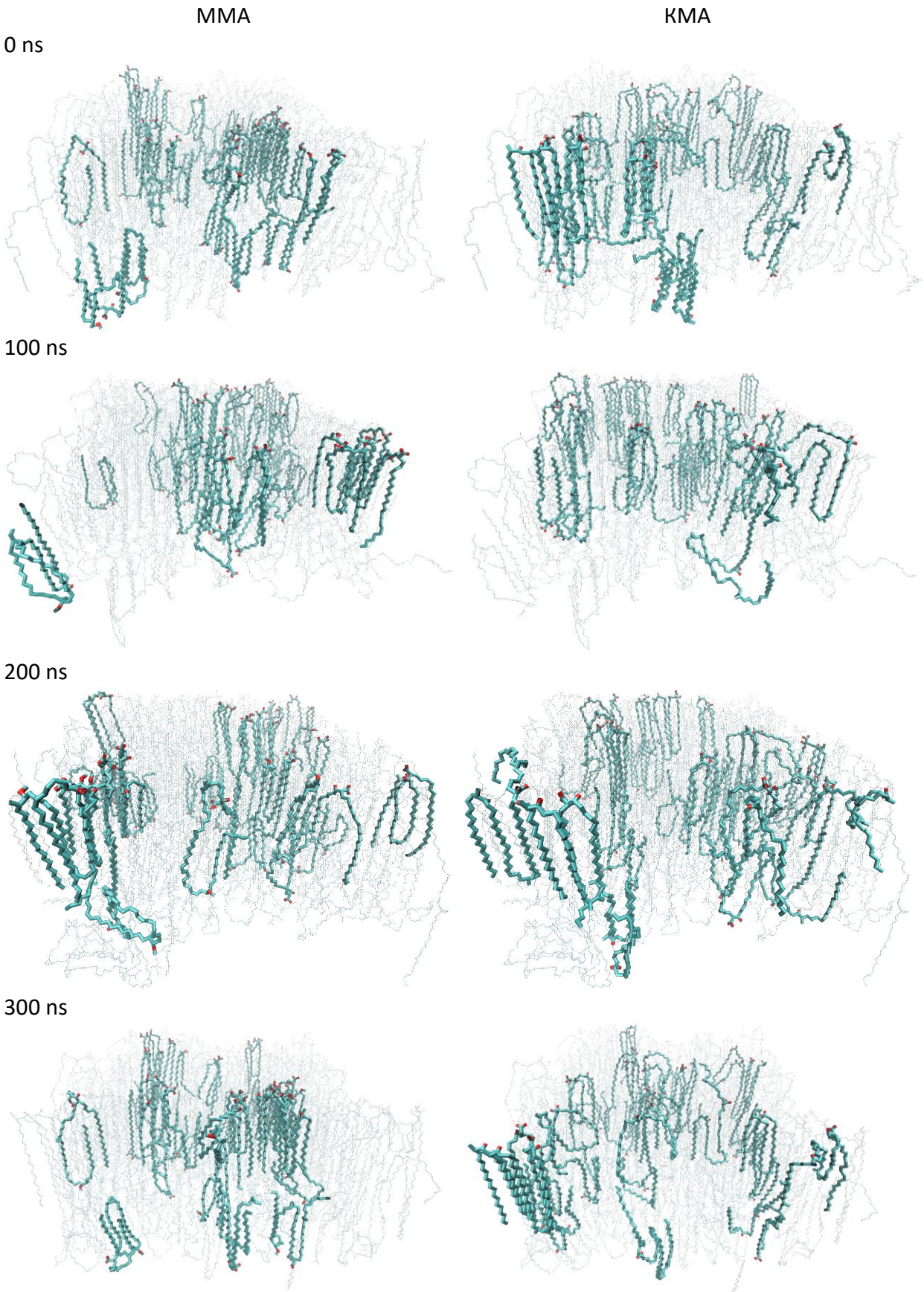


Figure S3. Conformations of MMA and KMA in a mixed membrane 140AMA(W):30MMA:30KMA for the initial configuration (0), 100, 200 and 300 ns of molecular dynamics simulation. Weak halftone background represents the AMA molecules with W conformation. Side view.

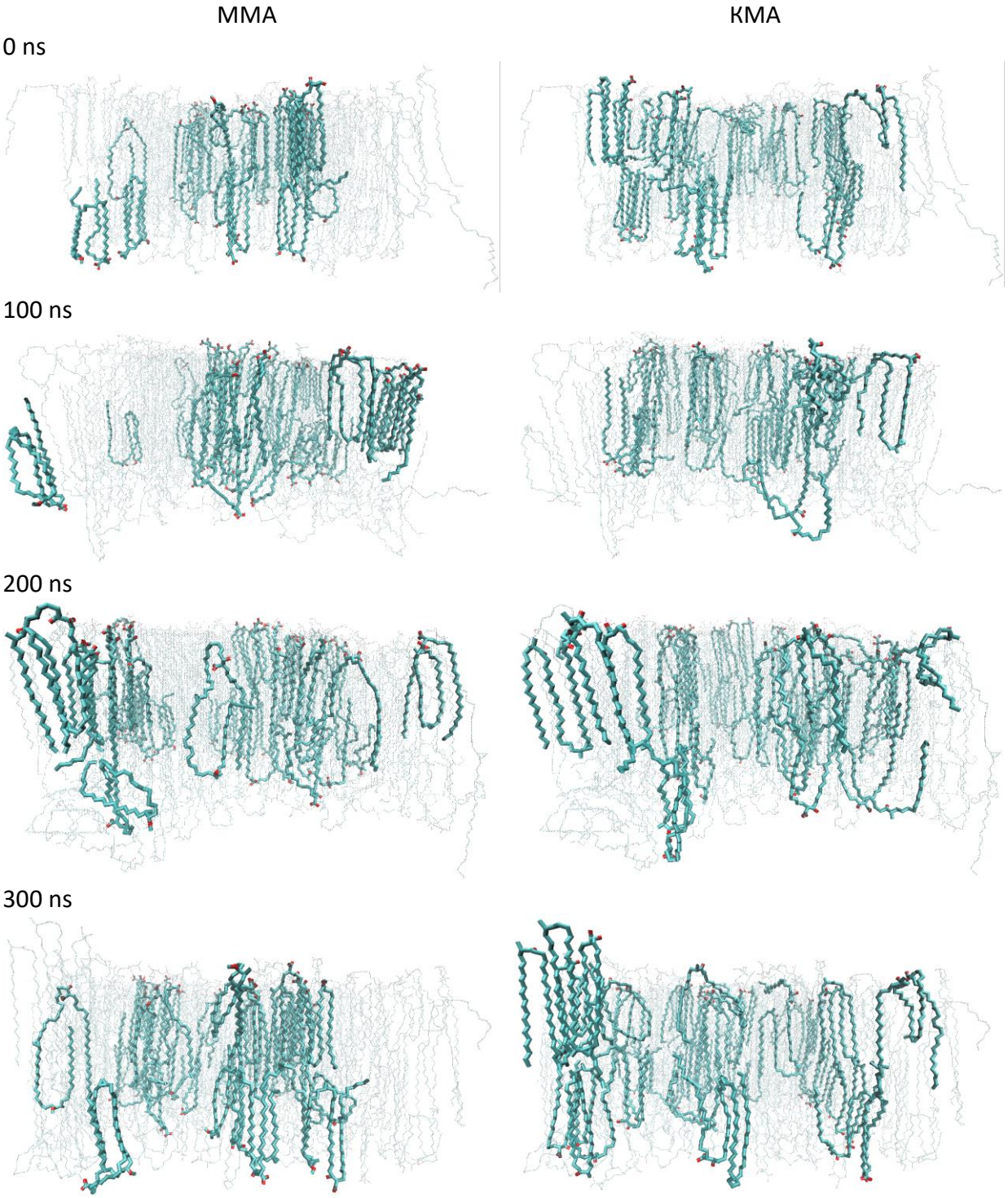
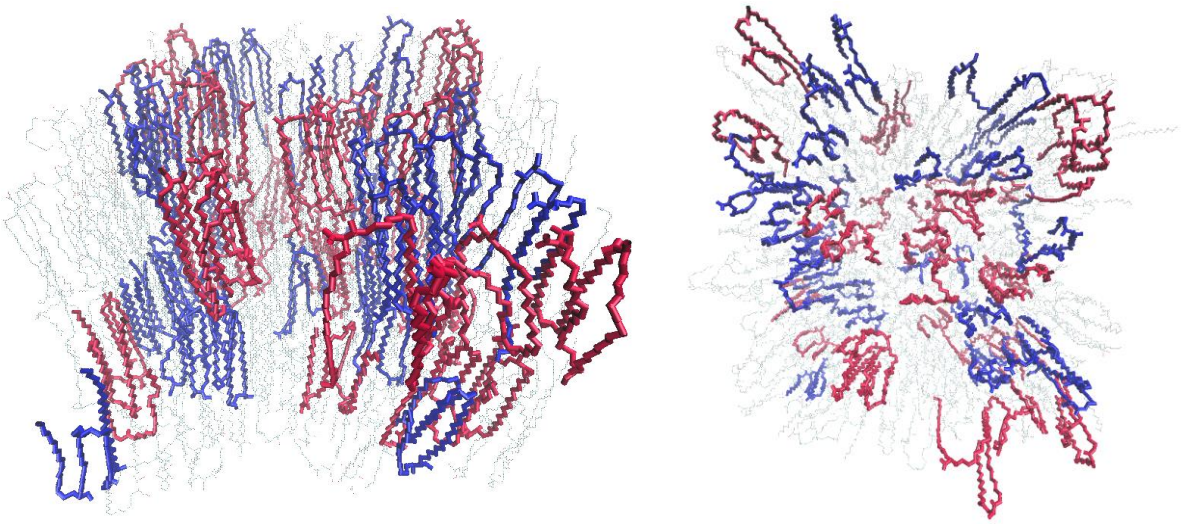


Figure S4. Conformations of MMA and KMA in a mixed membrane 140AMA(W):30MMA:30KMA for the initial configuration (0), 100, 200 and 300 ns of molecular dynamics simulation. Weak halftone background represents the AMA molecules with W conformation. The observation point passes through the XOY plane.

Side view

Top view

0 ns



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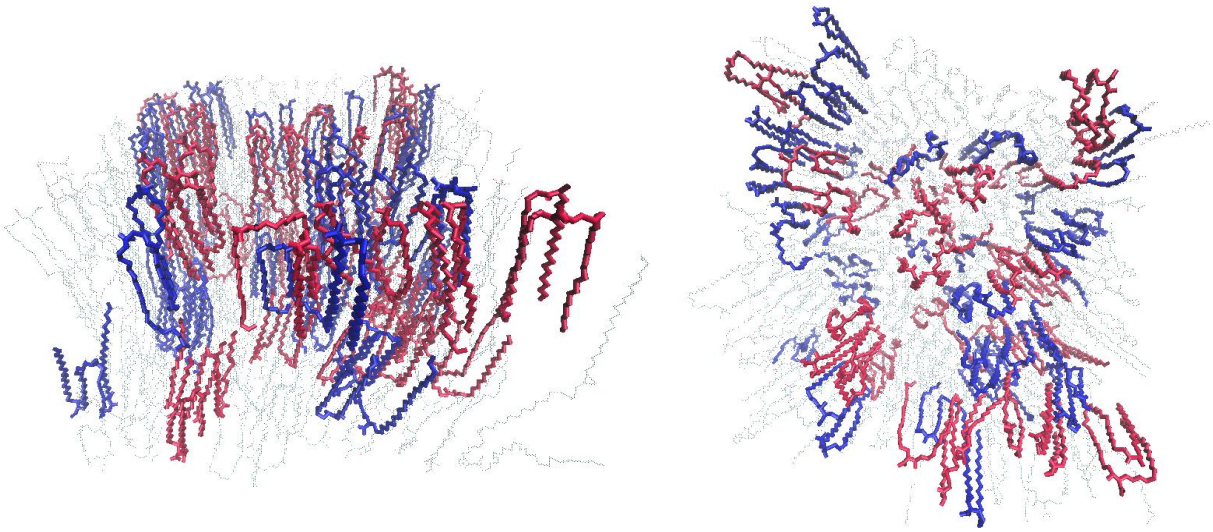
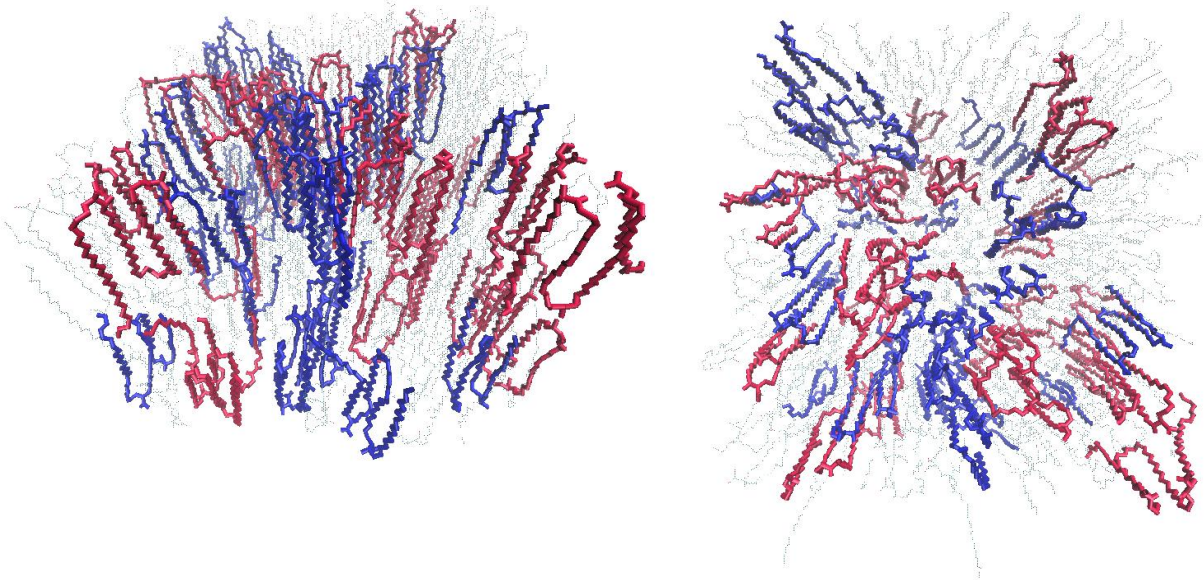


200 ns



300 ns

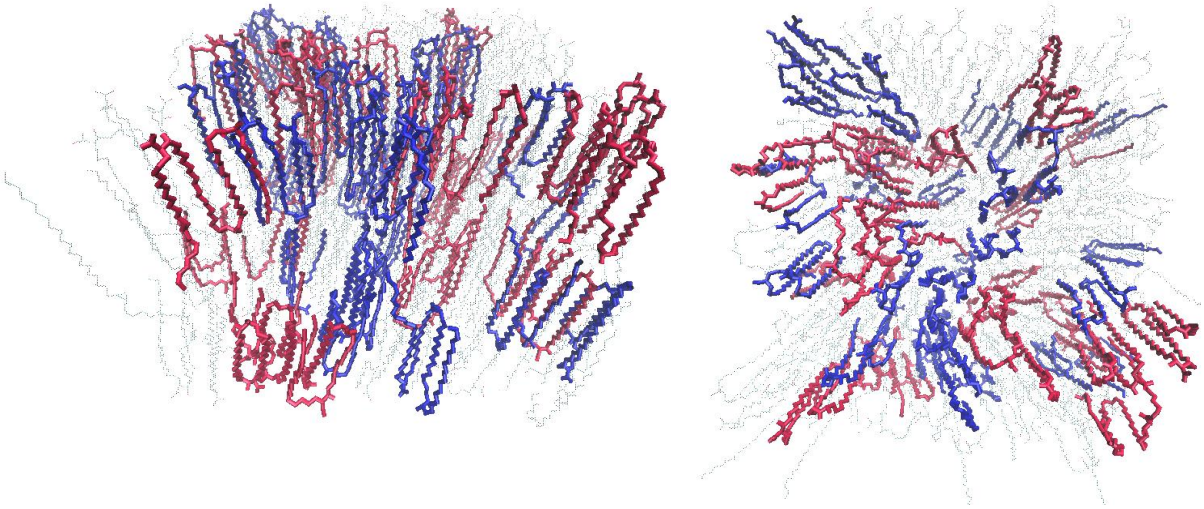
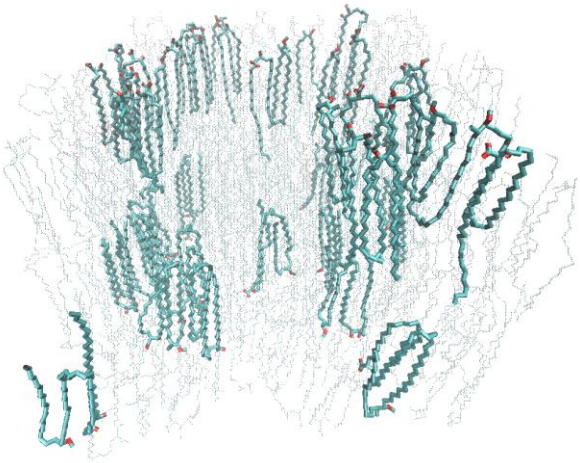
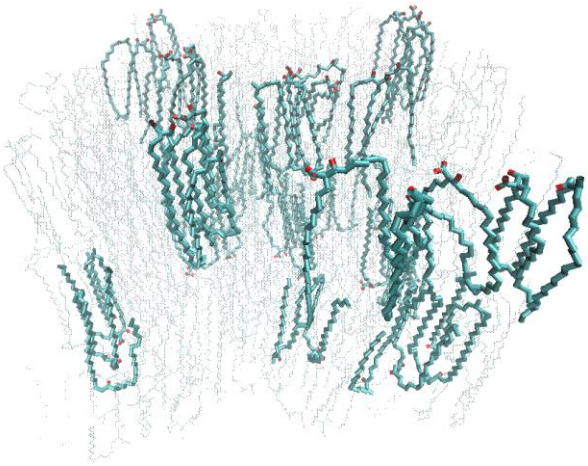


Figure S5. Mutual arrangement of MMA and KMA in a mixed membrane 140AMA(eU):30MMA:30KMA for the initial configuration (0), 100, 200 and 300 ns of molecular dynamics simulation. MMA molecules are highlighted in blue, KMA – in red. Weak halftone background represents the AMA molecules with eU conformation.

MMA KMA

0 ns

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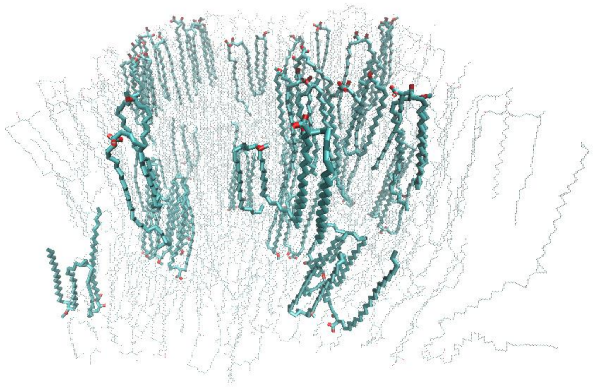
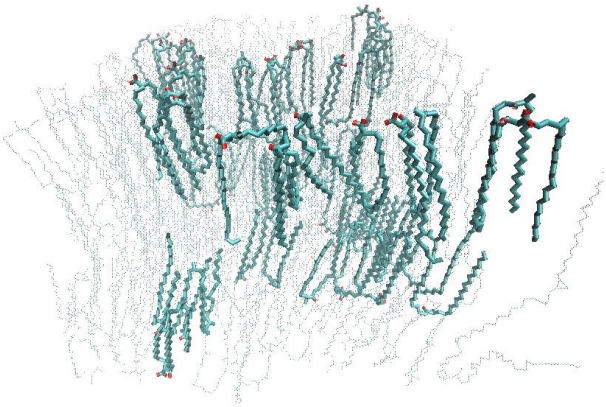

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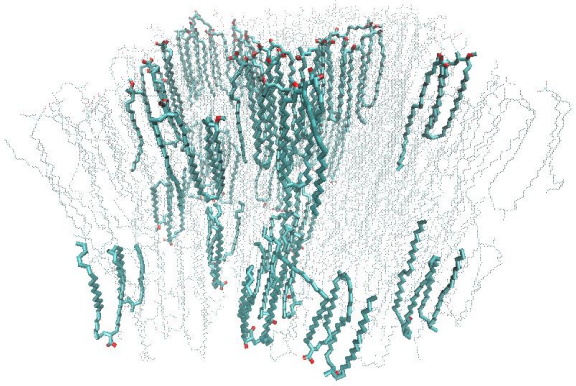
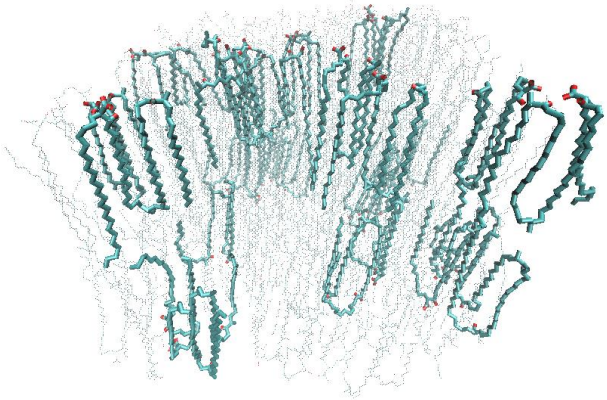



300 ns

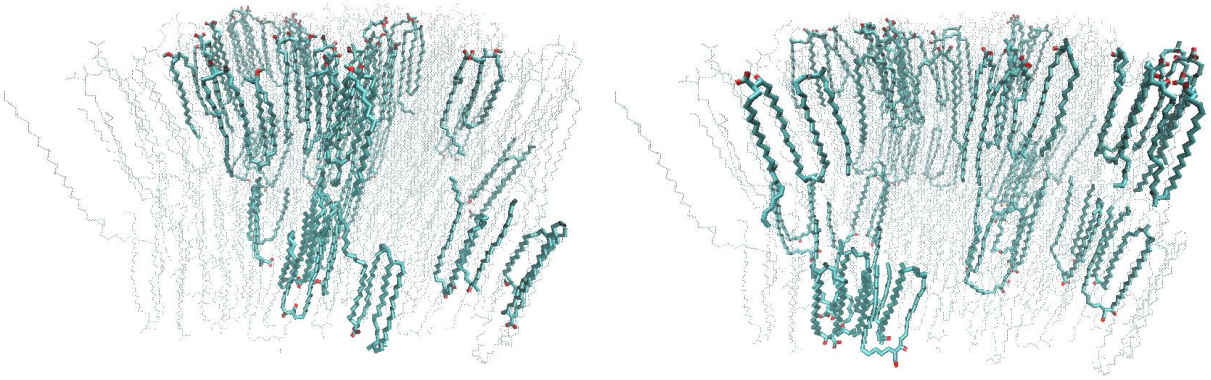
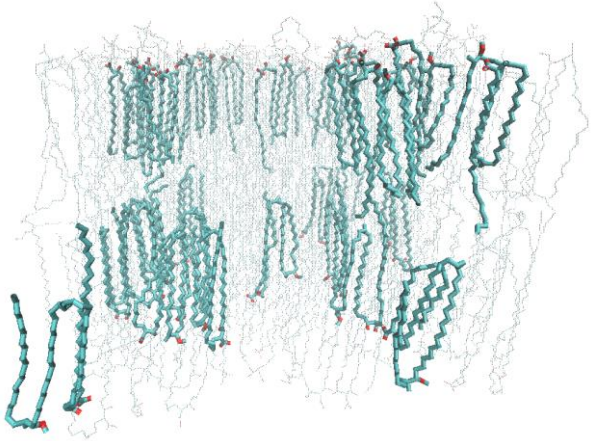
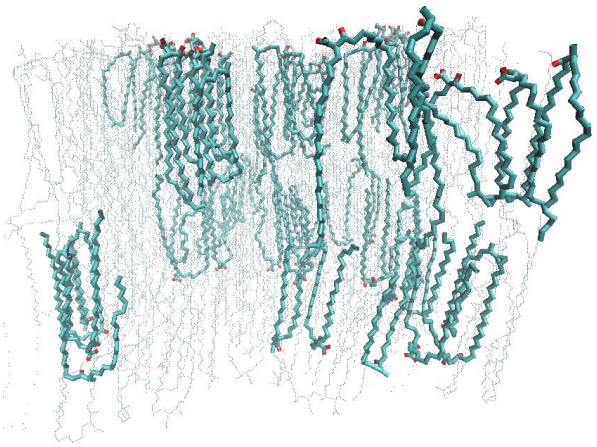


Figure S6. Conformations of MMA and KMA in a mixed membrane 140AMA(eU):30MMA:30KMA for the initial configuration (0), 100, 200 and 300 ns of molecular dynamics simulation. Weak halftone background represents the AMA molecules with eU conformation. Side view.

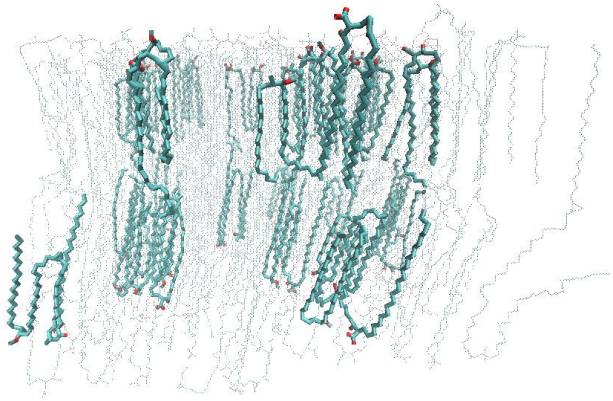
MMA KMA

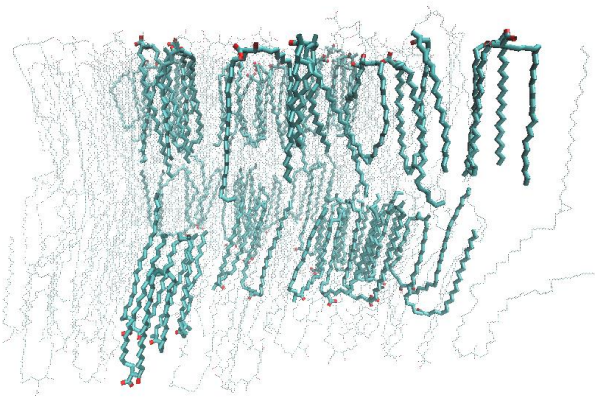
0 ns



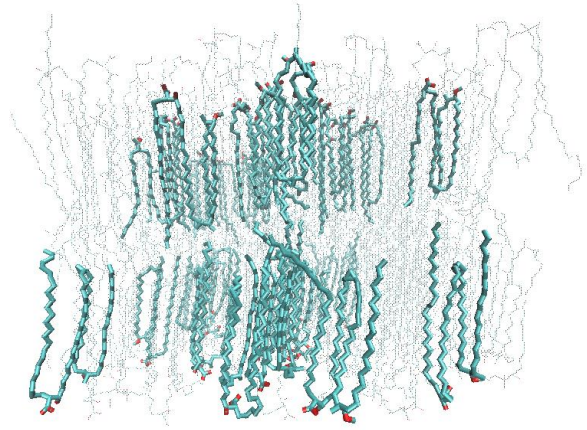


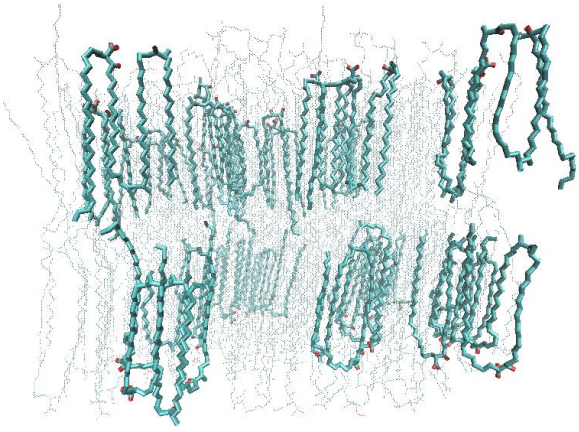
100 ns



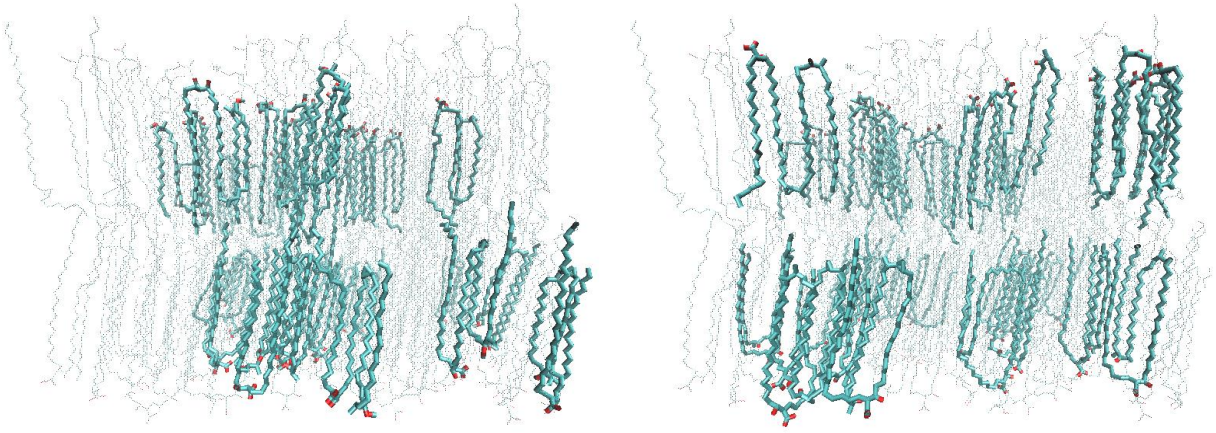


200 ns





300 ns



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