Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

TB drugs permeability through mycolic acid monolayer: A Tale of two force fields

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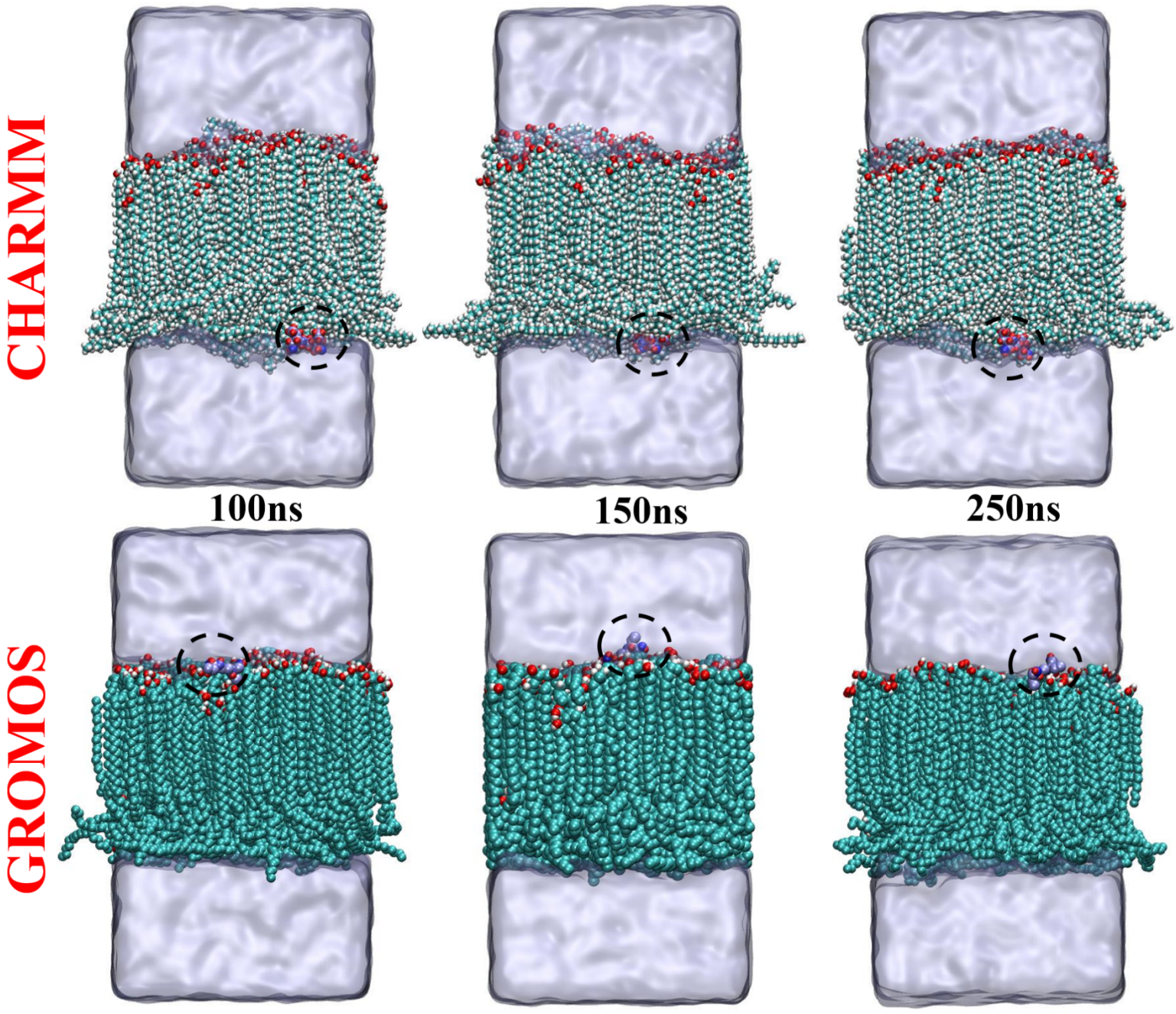

Fig. S1: Instantaneous snapshots of ethambutol-mycolic acid monolayer system at different time points. Color code for mycolic acid: C: Pale Blue, O: Red, H: white. The color code for a drug molecule is based on atomic mass. For ethambutol: H: Red, O: Blue, N: light blue, C: whitish blue. Water is presented using an ice-blue transparent surface. Drug molecules are encircled using a dashed line for clarity.

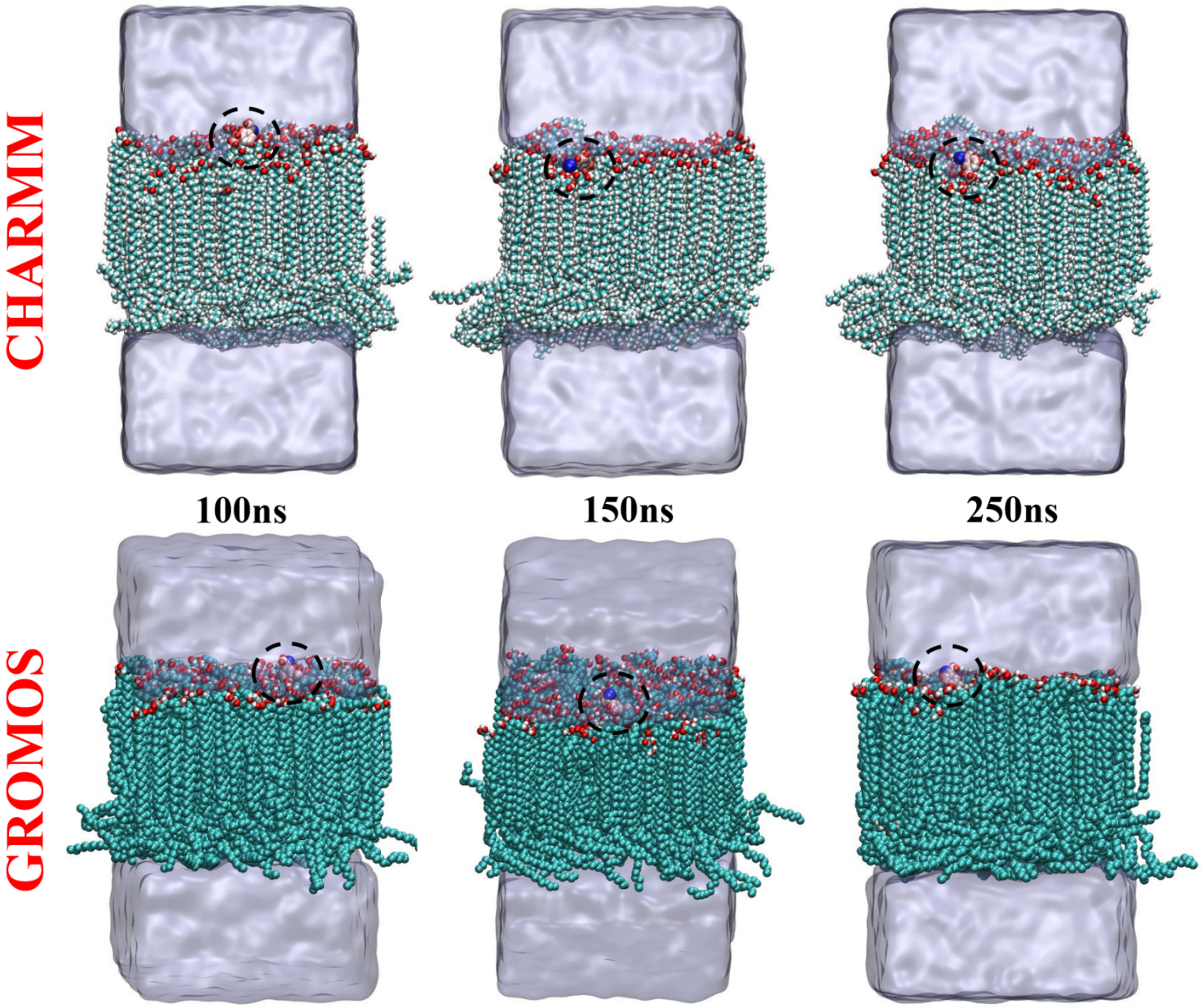

Fig. S2: snapshots of ethionamide-mycolic acid monolayer system at different time points. Color code for mycolic acid: C: Pale Blue, O: Red, H: white. The color code for a Drug molecule is based on atomic mass. For ethionamide: S: Deep Blue, H: red, C: reddish-white, N: white. Water is presented using an ice-blue transparent surface. Drug molecules are encircled using a dashed line for clarity.

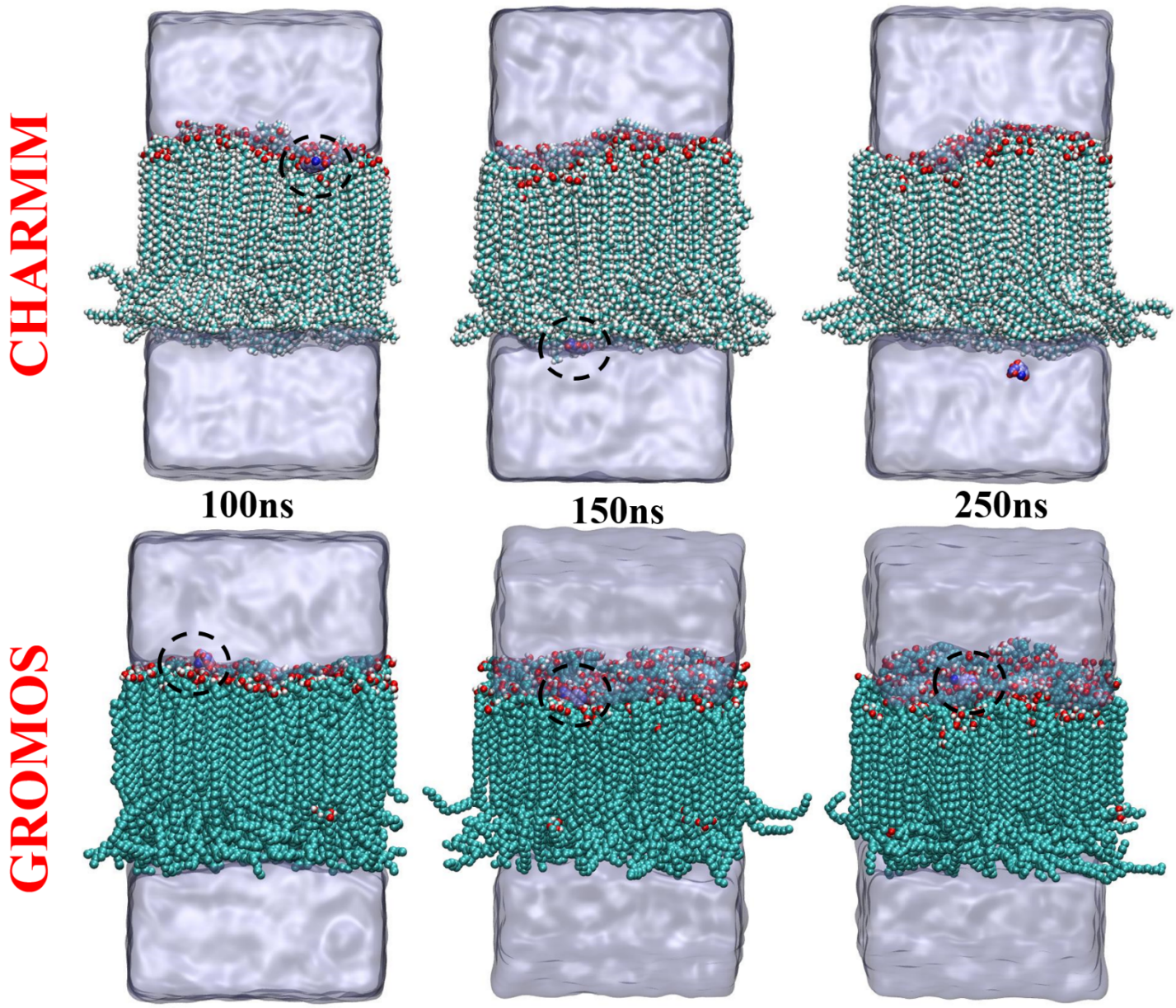

Fig. S3: snapshots of Isoniazid-mycolic acid monolayer system at different time points. Color code for mycolic acid: C: Pale Blue, O: Red, H: white. The color code for a drug molecule is based on atomic mass. For isoniazid: H: Red, O: Blue, N: light blue, C: whitish blue. Water is presented using an ice-blue transparent surface. Drug molecules are encircled using a dashed line for clarity.

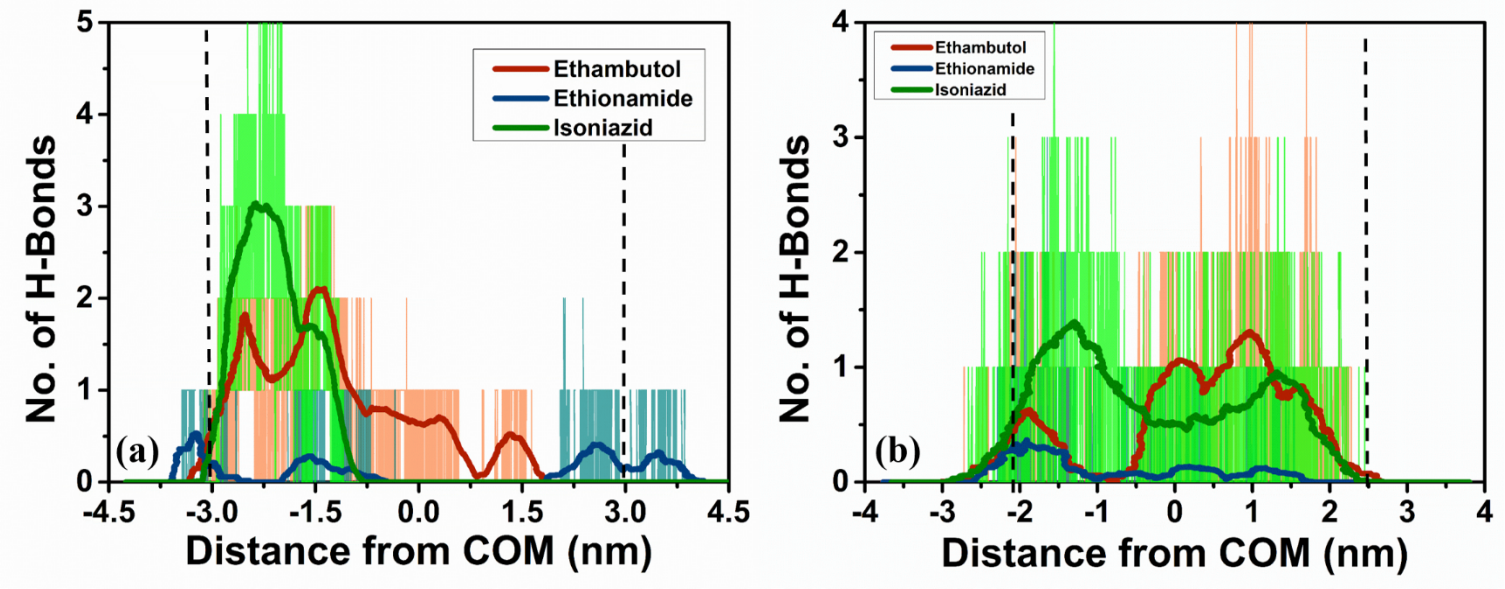

Fig. S4: Spatial variance of the number of hydrogen bonds during the pulling of drug molecules through the MA monolayer for (a) CHARMM and (b) GROMOS FFs.

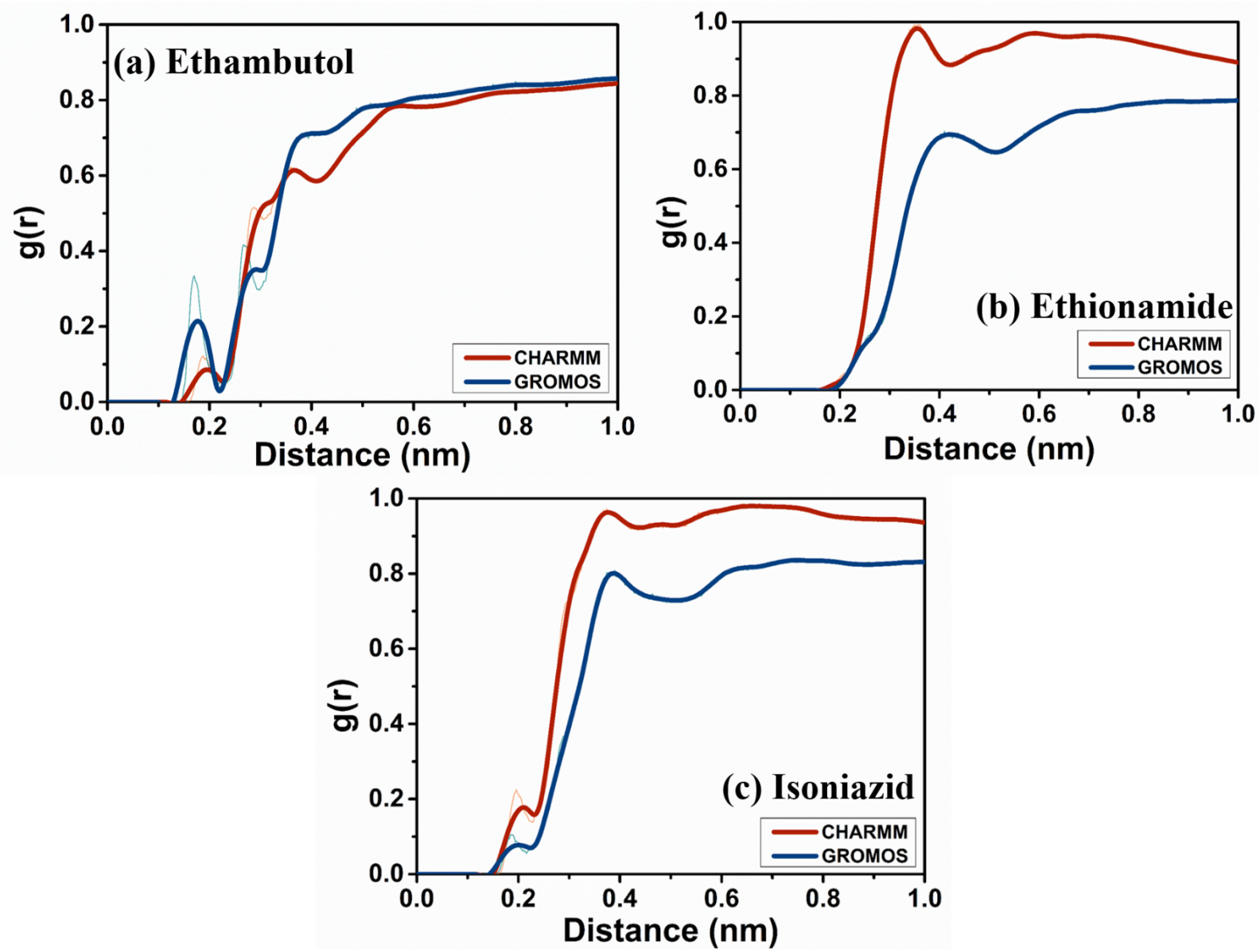

Fig. S5: Radial distribution function (g(r)) of water oxygen atoms with respect to the center of mass of (a) Ethambutol, (b) Ethionamide, and (c) Isoniazid for different FFs.

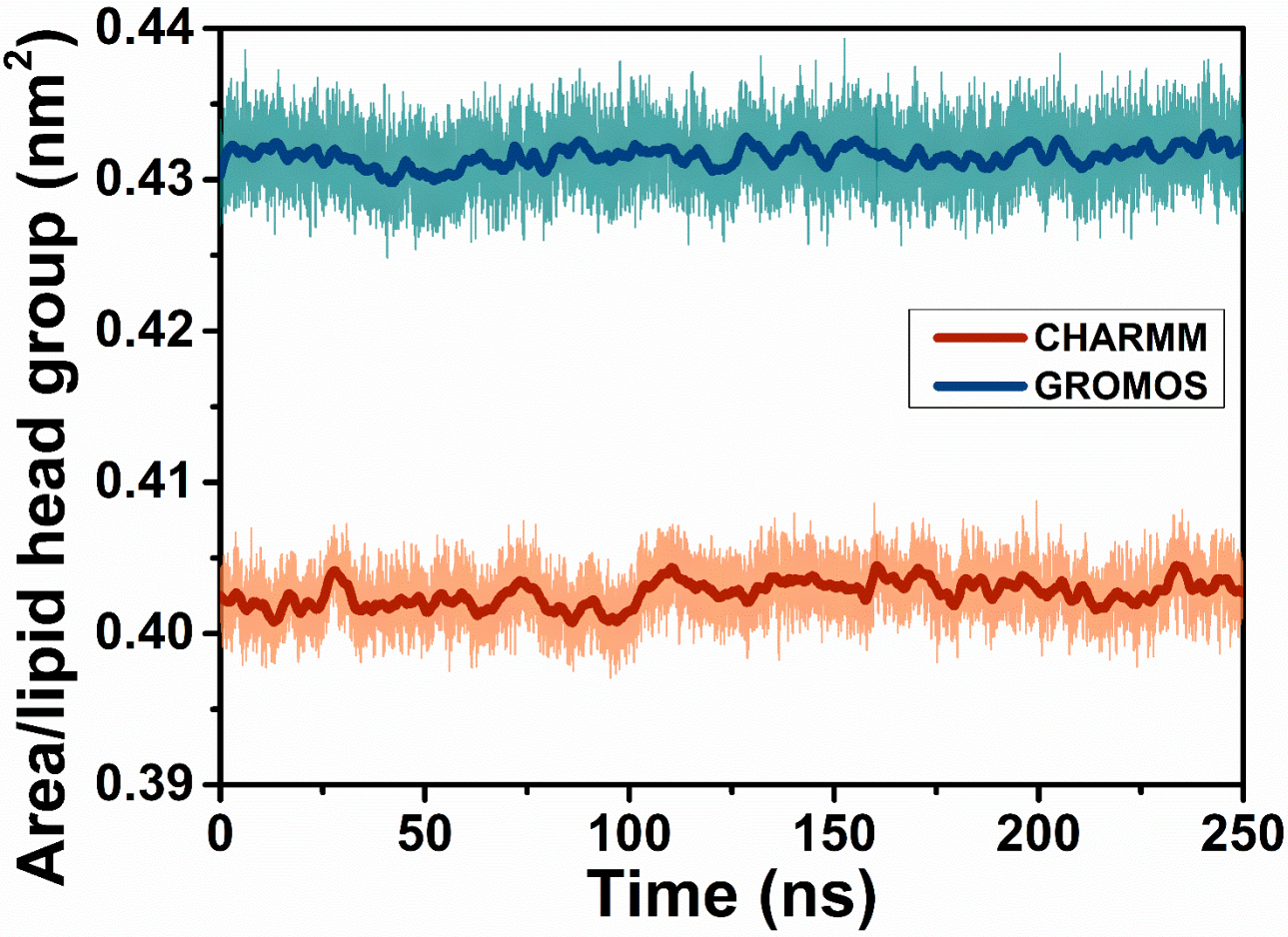

Fig. S6: Area per lipid (Area/lipid) of the mycolic acid monolayer for two different FFs.

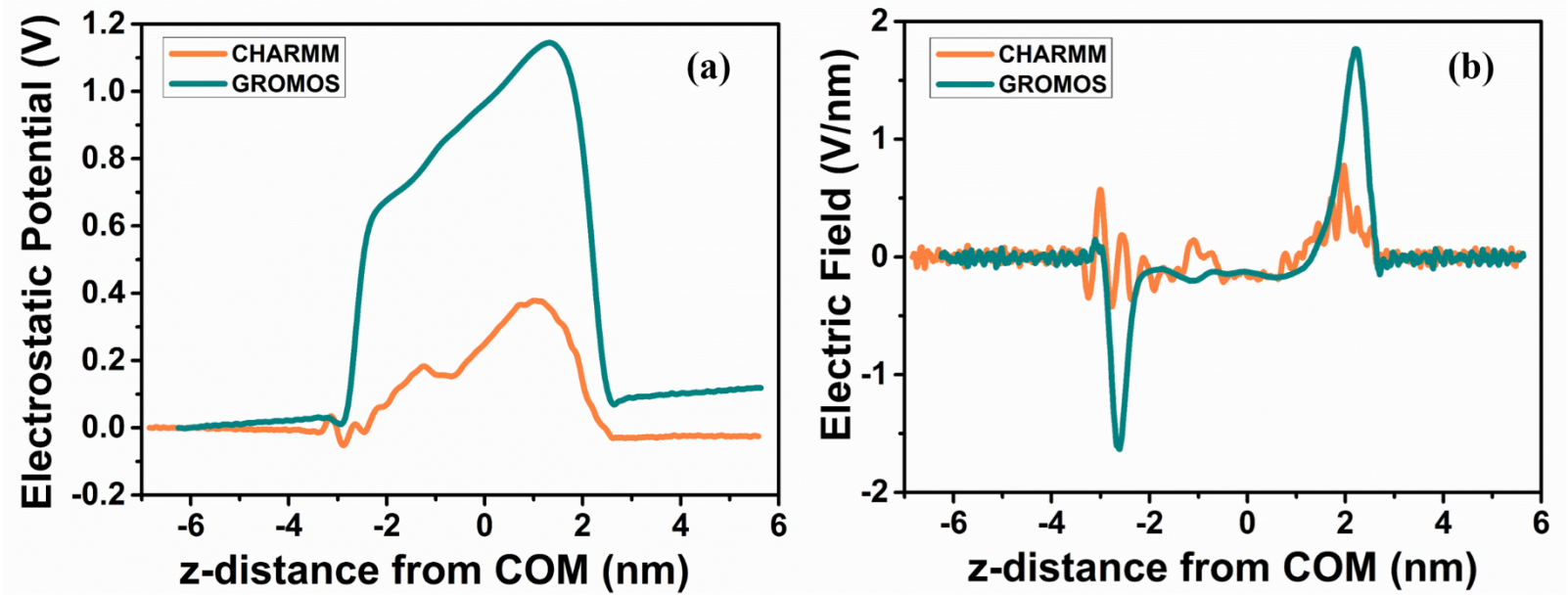

Fig. S7: (a) Electrostatic potential and (b) electric field intensity across the z-direction of the simulation box for two different FFs.

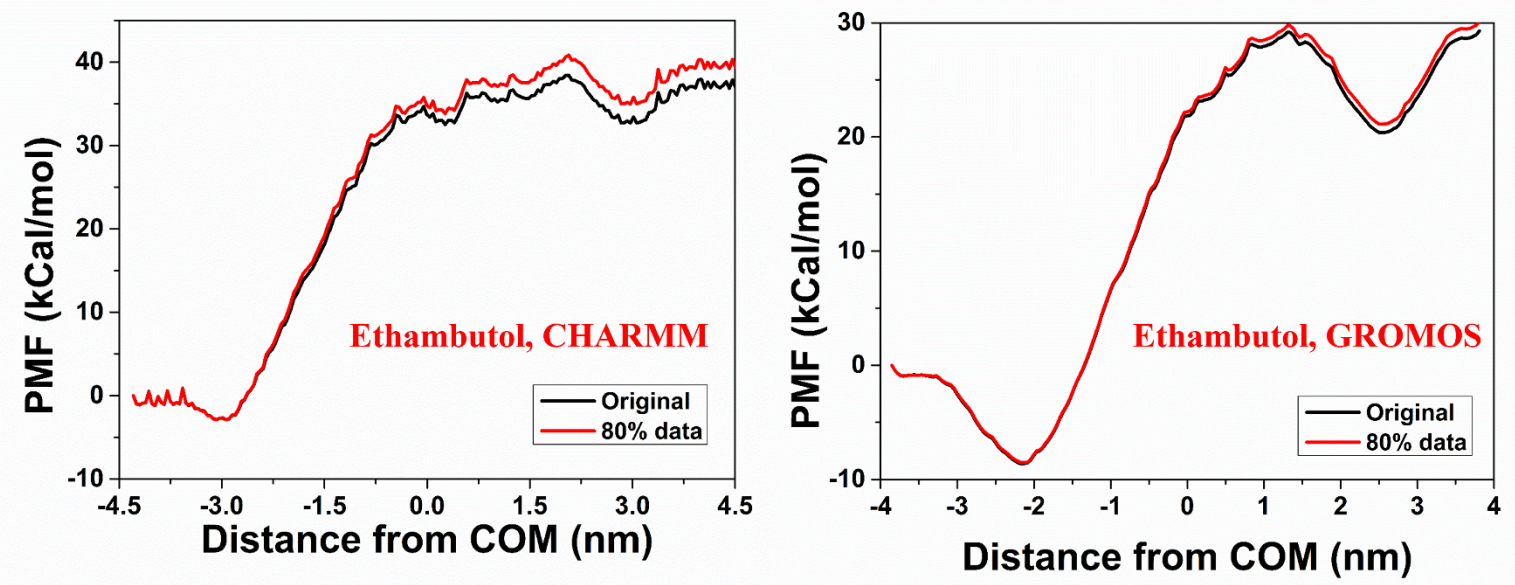

Fig. S8: Representative PMF plots demonstrate the convergence of umbrella sampling. After calculating PMF, the last 20% of data from every US window was discarded and PMF was calculated again to illustrate the difference of the PMF curves. As the curves have not changed significantly (within the error bar of Fig. 8 of the main text), the sampling was sufficient. The protocol is taken from:

https://ambermd.org/tutorials/advanced/tutorial17/section2.php.

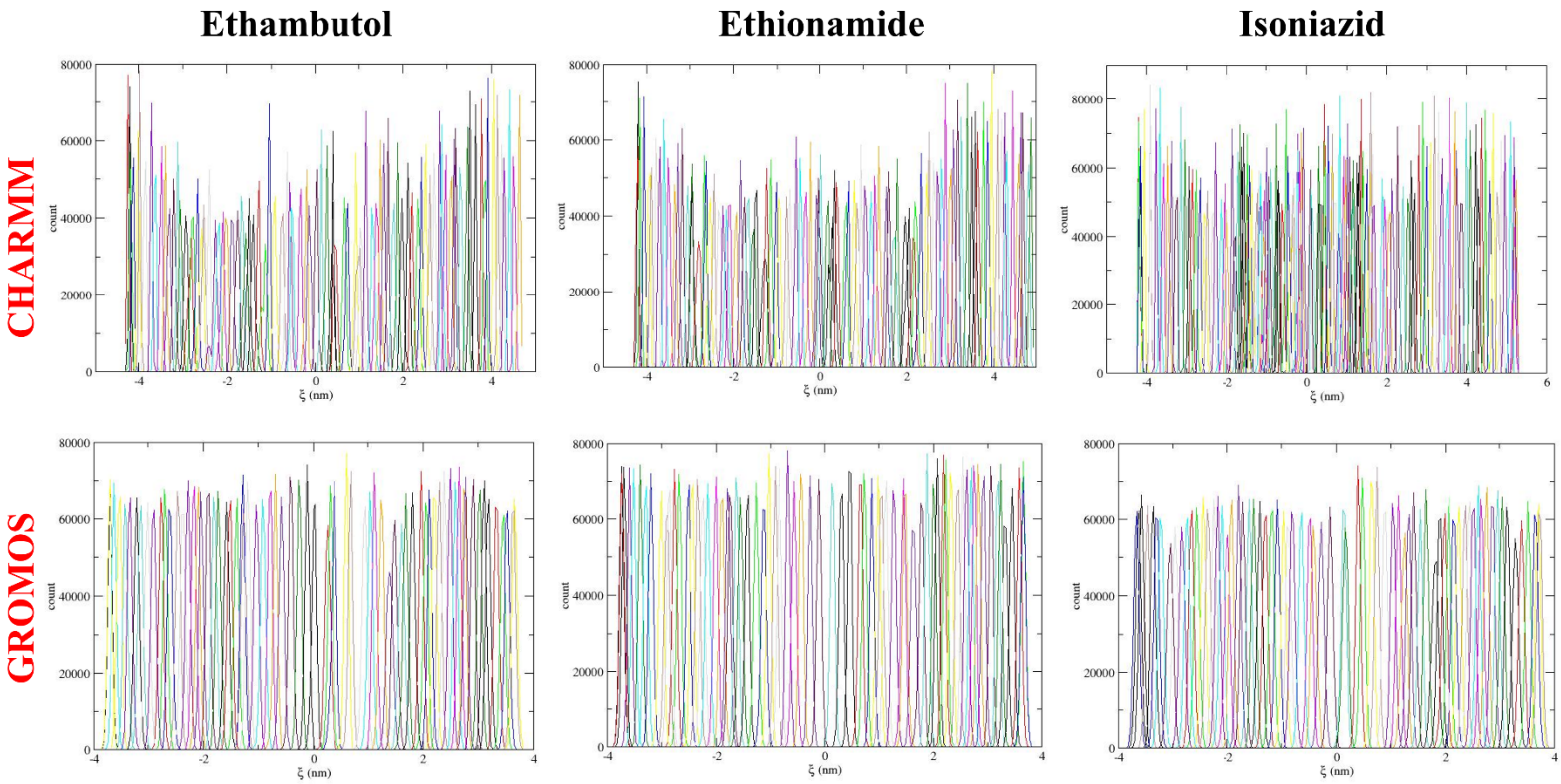

Fig. S9: Umbrella sampling (US) histograms for different drugs under two different FFs.

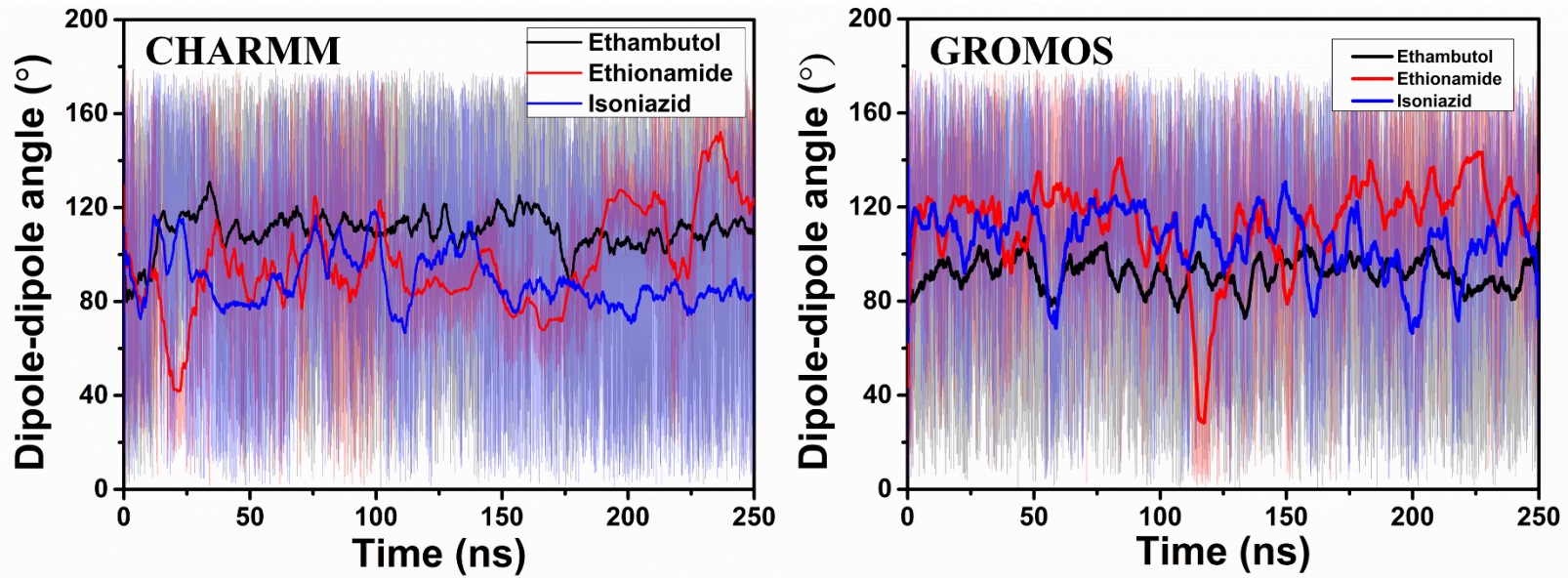

Fig. S10: Angle between the dipole of a TB drug molecule and that of mycolic acid monolayer. This angle provides insight into the orientation of drug molecules on the MA monolayer surface.

Table S1: Properties of TB drug molecules used for the study (MIC data obtained from Ref.[1] Other data obtained from: https://www.drugbank.com/)

Drug name	**LogP**	**Solubility**	**MIC (µg/ml)***	**Number of Hydrogen bond doner atoms**	**Number of Hydrogen bond acceptor atoms**
Ethambutol	0.4	1000 mg/ml	8	4	4
Ethionamide	0.5	Practically insoluble	2	1	1
Isoniazid	-0.7	1.4E+005	0.5	3	2

		mg/L (25°C)			

*** MIC Against M.tb strain H37rv**

S.1 Membrane-drug distance and number of contacts

Minimum distances between the different drug molecules and the mycolic acid (MA) monolayer have been presented in Fig. S11 for both the CHARMM and GROMOS FFs together with the snapshots of the systems at different time points (Snapshots of all the drug-monolayer systems at different time points have been presented in Fig. S1-S3 in SI). The minimum distance is defined as the minimum of all atomic-pair distances between the heavy atoms of drug molecules and those of the MA monolayer. For the combination of CHARMM FF parameters and ethambutol, the minimum distance first increased and, at ~20 ns, the distance decreased to 0.2 nm and remained stable for the rest of the 250 ns simulation time (Fig. S11(a)). This happened because of the preferential attachment of ethambutol to the tail of the mycolic acid chain. In the case of the GROMOS FF, the ethambutol molecule was attached to the monolayer surface within the first ~300 ps and remained there for the rest of the simulation window (Fig. S11(a)). Unlike CHARMM FF, ethambutol did not exhibit any preferential affinity toward the tail of the mycolic acid molecules, in the case of the GROMOS FF. More importantly, ethambutol never penetrated inside the mycolic acid monolayer but rather preferred to remain on the surface, for both CHARMM and GROMOS FFs.

A similar trend has been observed in the case of ethionamide. When CHARMM FF parameters were employed, the drug molecule attached to the mycolic acid monolayer within 5 ns and subsequently periodically detached and again reattached to the membrane surface for the rest of the 250 ns long simulation (Fig. S11(b)). For the GROMOS FF, ethionamide was attached to the

mycolic acid monolayer surface at ~ 2 ns and remained stable on the surface for the entire simulation window (Fig. S11(b)). For isoniazid as well, periodic adsorption and desorption of the drug molecule were recorded for CHARMM FF (Fig. S11(c)). When GROMOS FF parameters were used, isoniazid quickly got adsorbed stably on the surface of the monolayer (Fig. S11(c)). From the observed behavior, it can be commented that the GROMOS FF provides better stability of the drug molecules on the mycolic acid monolayer surface, compared to CHARMM FF.

We have also computed the number of contacts formed between the heavy atoms of the drug molecules and those in the mycolic acid monolayers. Contact is formed when any heavy atom of the drug molecule is within a distance of 0.4 nm of any heavy atoms of MA. The number of contacts for various drug molecules and different force field parameters is presented in Fig. S12. The number of contacts was generally noticed to be higher in the case of GROMOS FF, for all of the drug molecules (Fig. S12). This reflects the fact that the drug molecules are more stable on the monolayer under GROMOS FF.

S.2 Drug-monolayer interactions

The interactions of drug molecules with the MA monolayers are of utmost importance because they dictate the behavior of drug molecules in the membrane environment. In the present section, the VdW/electrostatic interactions between the drug molecules and the MA monolayer will be described. It should be iterated here that the drug-MA interactions also depend on the FF used for the study and can differ significantly from FF to FF. Moreover, the distinction of interaction energies into VdW/electrostatic components is a manifestation of the FF parameters used. Experimentally it is very difficult to distinguish between these two components of interaction

energy. In the present study, ethambutol was found to be interacting with the mycolic acid dominantly through VdW interactions, both for CHARMM and GROMOS FF (Fig. S13). Moreover, the VdW interaction energies were found to be of similar magnitudes for both sets of force field parameters (Fig. S13). The coulombic contribution was noticed to be minor compared to VdW interactions, for both sets of force field parameters (Fig. S13).

For ethionamide, when CHARMM FF was used, the drug molecule interacted with the monolayer via both electrostatic and VdW interactions, and the electrostatic interactions became dominant between 150 ns and 200 ns (Fig. S13(b) and S14(b)). For GROMOS FF, VdW interaction energy was noted to be the major contributor toward drug-membrane interactions, with negligible coulombic interactions (Fig. S13(b) and Fig. S14(b)). Although a weaker drug-membrane VdW interaction for CHARMM FF was observed for up to 125 ns; similar magnitudes were recorded afterward for both the FF (Fig. S13(b)). However, because of the difference in the magnitudes of the electrostatic interactions, the overall interactions between ethionamide and the MA membrane significantly differ for CHARMM and GROMOS FF parameter sets.

When isoniazid is considered, again the VdW interactions were found to be the dominant contributor to the drug-membrane interactions for both the CHARMM and GROMOS FF (Fig. S13(c) and Fig. S14(c)). The VdW interactions were observed to be slightly greater for GROMOS FF throughout the simulation time (Fig. S13(c)), while the greater coulombic interaction was found for GROMOS force field from 150 ns onward (Fig. S14(c)). From the above-mentioned behavior, it can be easily concluded that the GROMOS FF better stabilized the drug molecules on the MA monolayer membrane, compared to CHARMM FF. Our simulation study suggests that for both CHARMM and GROMOS FF, the strength of the drug-membrane

VdW interactions for ethambutol was slightly greater than isoniazid (Fig. S13(a) and S13(c)), whereas the coulombic interaction for isoniazid was slightly higher for GROMOS FF (Fig. S14(a) and S14(c)).

Another important mode of interaction is the formation of hydrogen bonds between the drug molecules and the mycolic acids. Because the oxygen atoms (receptors in the hydrogen bond formation) of ethambutol were exposed to the solvent side and there are only donor atoms present in the tail region of mycolic acids, this particular drug did not form any hydrogen bonds with the membrane, when CHARMM FF was used (Fig. S15(a)). The other two drug molecules (ethionamide and isoniazid) formed hydrogen bonds with the MA monolayer (Fig. S15(b) and 6(c)). It is noteworthy that isoniazid did not form H-bonds with monolayer after 150 ns onward because, after 150 ns, isoniazid was attached to the tail side of the MA monolayer (see Fig. S11(c)), where donor/receptor atoms necessary for H-bond formation was absent (Fig. S15(c)). For GROMOS FF, all drug molecules formed hydrogen bonds with the monolayer, and no significant differences were noted (Fig. S15).

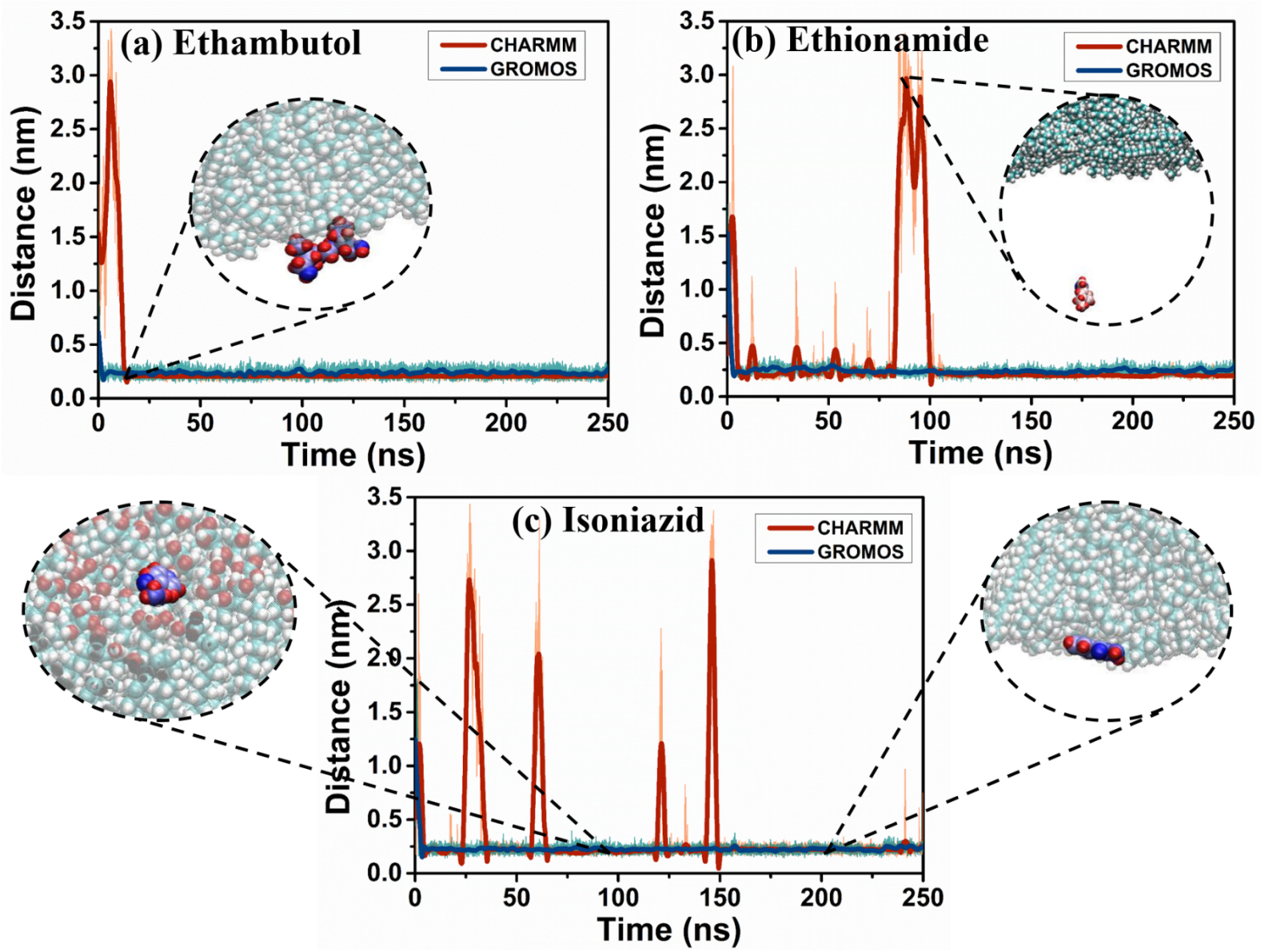

Fig. S11: Minimum of the atomic pair distance or minimum distance is a signature of the drug-membrane interaction. Temporal evolution of the minimum distance between mycolic acid monolayer and (a) Ethambutol, (b) Ethionamide, and (c) Isoniazid for different FFs. Under

GROMOS FF, the drug molecules are stably bound to the membrane surface. Snapshots of the drug-monolayer systems have been shown at different time points for the CHARMM FF with color codes for mycolic acid: C: Pale Blue, O: Red, H: white. The color code for a drug molecule is based on atomic mass. For ethambutol/isoniazid: H: Red, O: Blue, N: light blue, C: whitish blue. For ethionamide: S: Deep Blue, H: red, C: reddish-white, N: white.

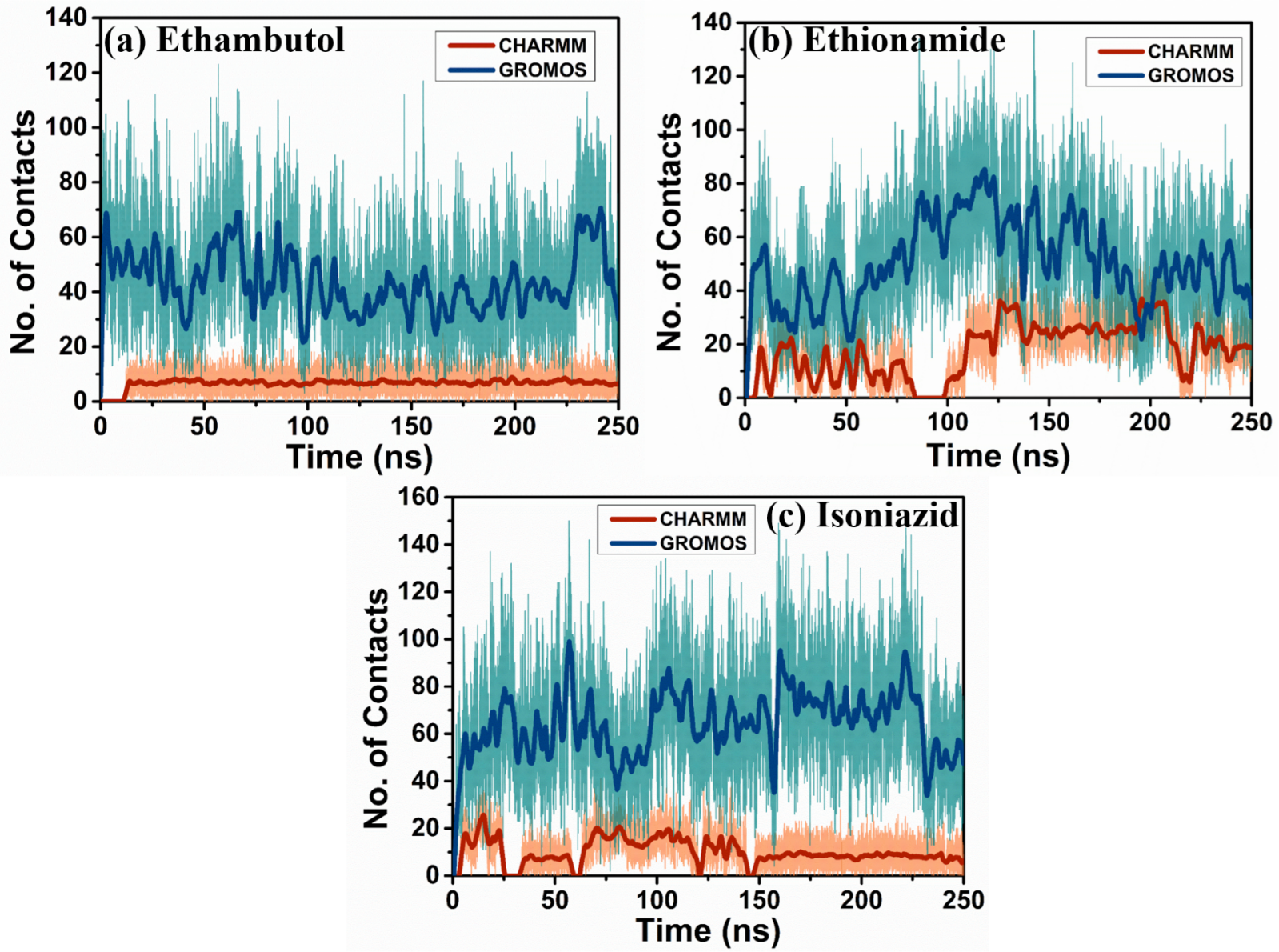

Fig. S12: Number of contacts can be used to quantitatively assess the affinity of drug to membrane. Number of contacts formed between heavy atoms of mycolic acid monolayer and (a) Ethambutol, (b) Ethionamide, and (c) Isoniazid for different FFs. Number of contacts was found to be higher for GROMOS FF, confirming better stability of the drug molecules on monolayer surface.

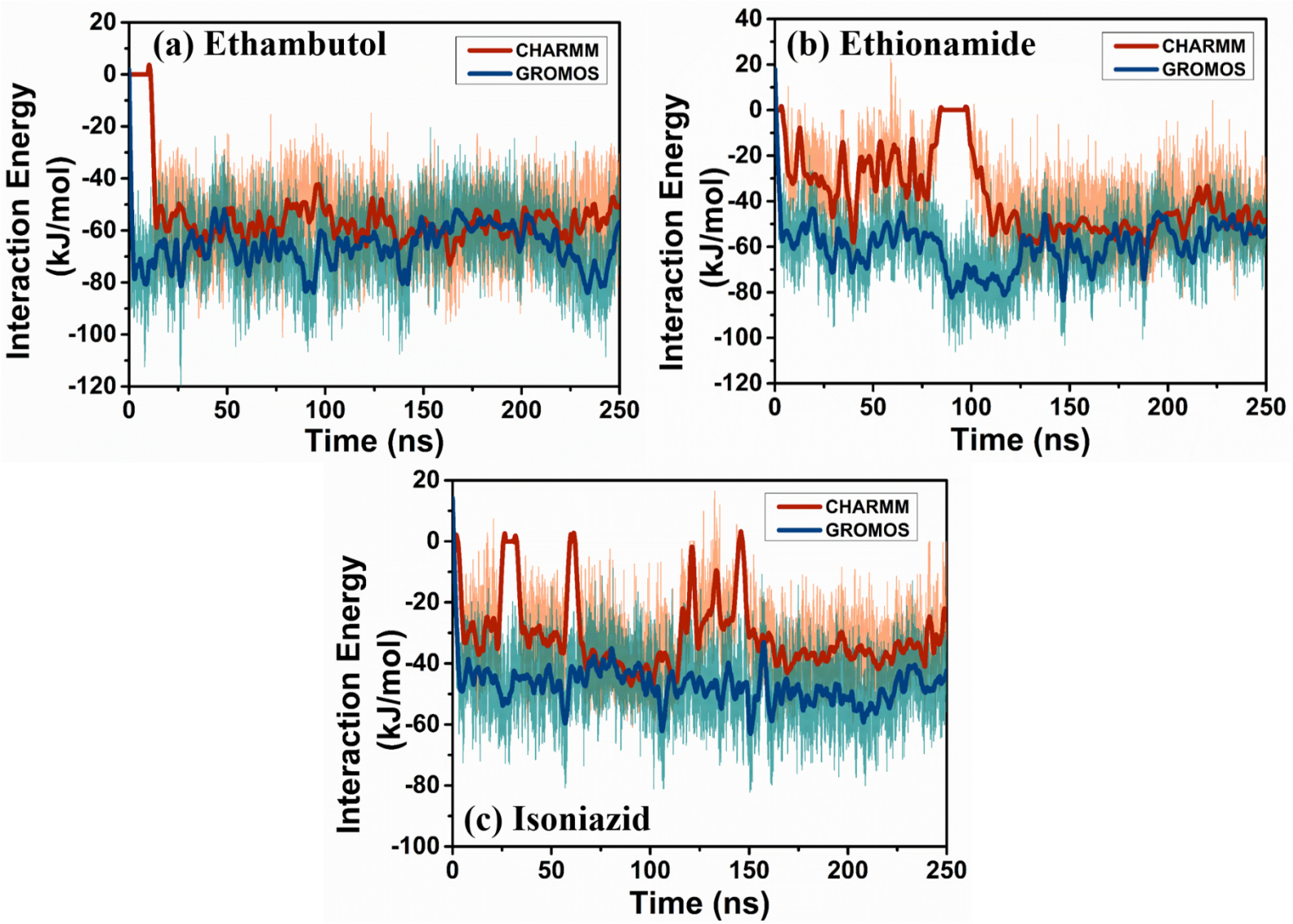

Fig. S13: Different modes of interaction between drugs and monolayer dictate the system dynamics. Drug-monolayer VdW interaction energy as a function of time for (a) Ethambutol, (b) Ethionamide, and (c) Isoniazid. VdW interaction was found to be attractive in nature for all of the cases.

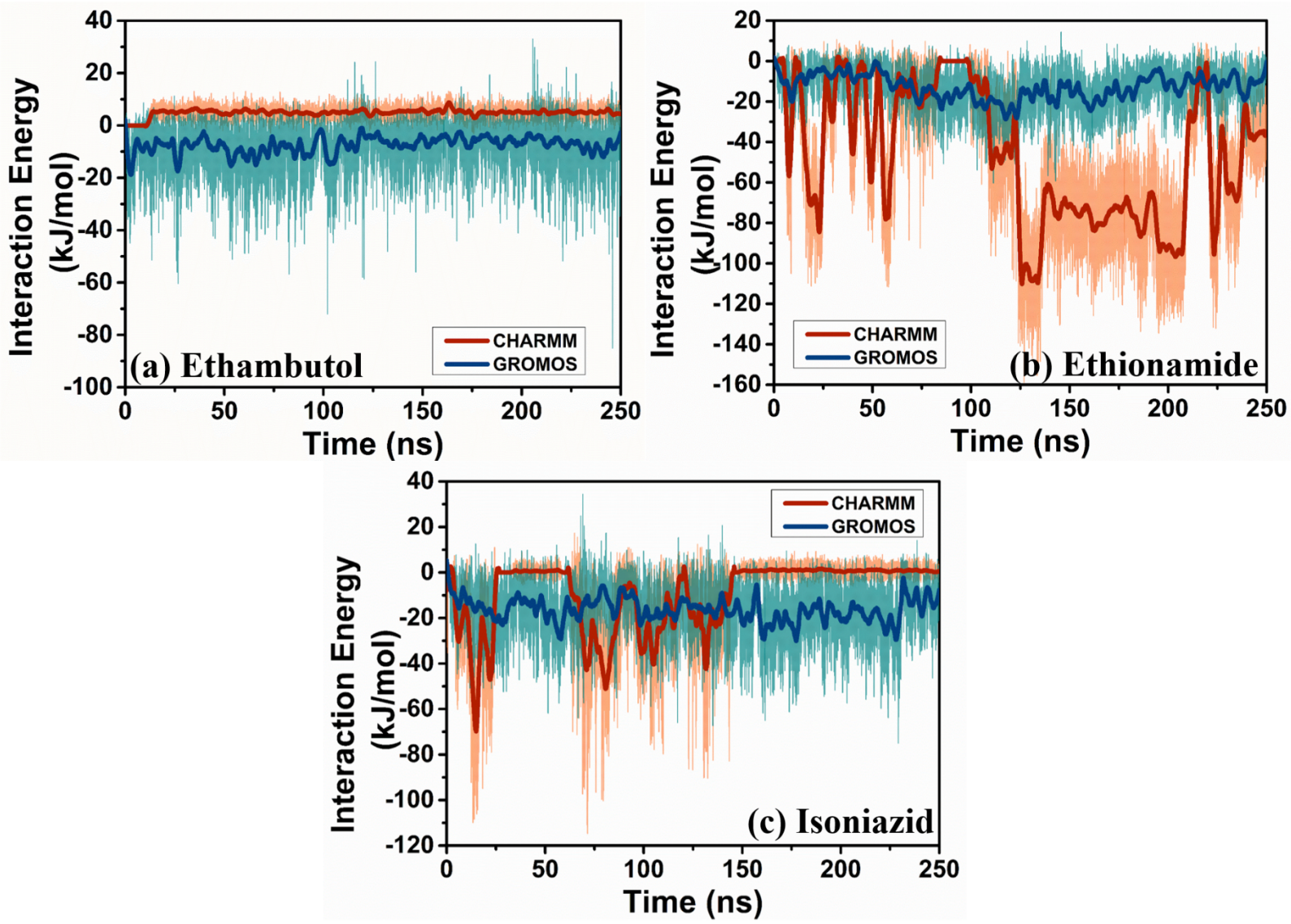

Fig. S14: Coulombic interaction also influences behaviors of drug molecules. Drug-monolayer electrostatic interaction energy as a function of time for (a) Ethambutol, (b) Ethionamide, and (c) Isoniazid.

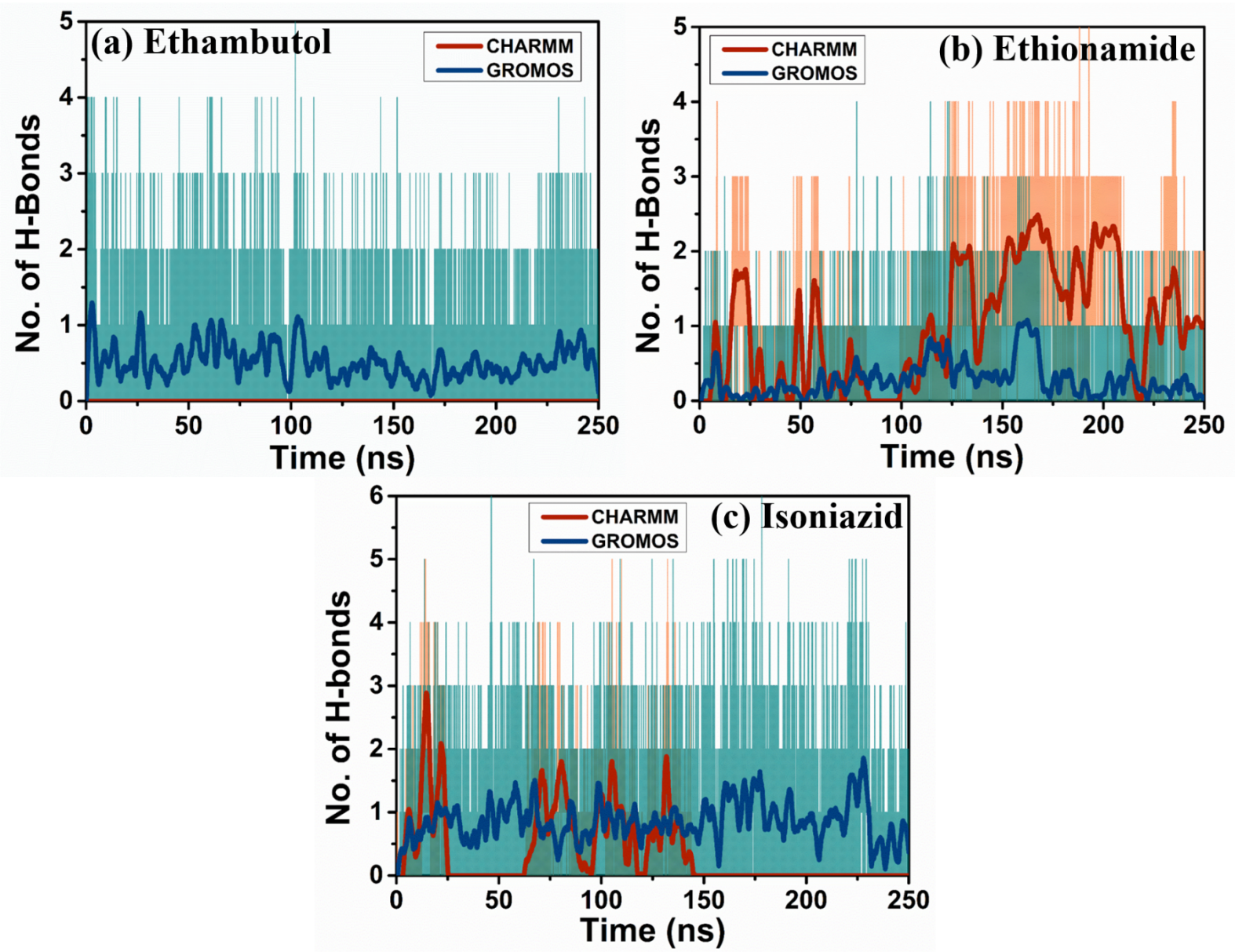

Fig. S15: Hydrogen bond formed between drugs and monolayer surface affects the adsorption kinetics. Temporal behavior of the number of hydrogen bonds formed between the mycolic acid monolayer and (a) Ethambutol, (b) Ethionamide, and (c) Isoniazid, as a function of two force fields.

References:

1 M. T. Heinrichs, R. J. May, F. Heider, T. Reimers, S. K. B. Sy, C. A. Peloquin and H. Derendorf, Mycobacterium tuberculosis Strains H37ra and H37rv have equivalent minimum inhibitory concentrations to most antituberculosis drugs, *The International Journal of Mycobacteriology*, 2018, **7**, 156–161.