STRUCTURE REFINEMENT OF 6F-TRP-M2-TMD

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Fluorine is not usually found in living organisms and can thus be used as a selective marker when incorporated in biomolecules. Because of its sensitivity and 100% natural abundance, ^{19}F is well suited for NMR investigations on oriented and powder samples to study the structure, interaction and molecular dynamics of peptides and proteins, especially in biomembranes.

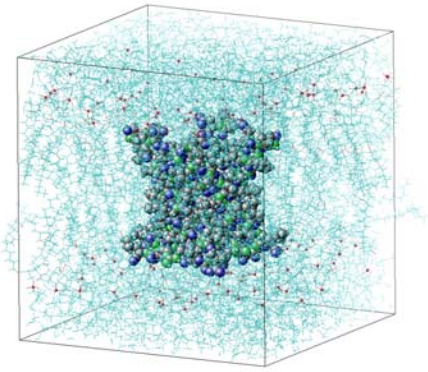

Figure 1: Computer model of M2-TMD in lipid bilayer.

Extremely fast calculations of ^{19}F chemical shift tensors in large molecular systems were carried out. Using the COSMOS-NMR force field with semi-empirical NPA charges (natural population atomic charges), the calculations are efficient enough to be performed in every step of MD simulation or geometry optimization. This technique is applied to molecular dynamics simulations at different pH values on the trans-membrane domain of ^{19}F-labeled (TRP_{41}) M2-channel from influenza A virus embedded in a lipid bilayer, see Figure 1. All NPA charges were calculated once in the beginning. For the force field calculations all atoms were fixed, except those of the TRP-HIS complex and nearby side chains. Only the charges on the TRP-HIS complex and nearby environment were re-calculated in every step of MD and optimization. The global motion of the channel (rotational motion about the bilayer normal) is considered.

For the closed state at pH > 7.0 the following constraints were used for direct structure refinement:
- Heteronuclear distances N_{π}His37-C_{γ}Trp41 = 3.9 ⇒
- Homonuclear distances between opposite 6F-Trp-6F-Trp = 4.0 ⇒
- Rotational averaged ^{19}F-CSA-tensor: δ_{11}=-125 ppm, δ_{22}=-125 ppm, δ_{33}=-110 ppm

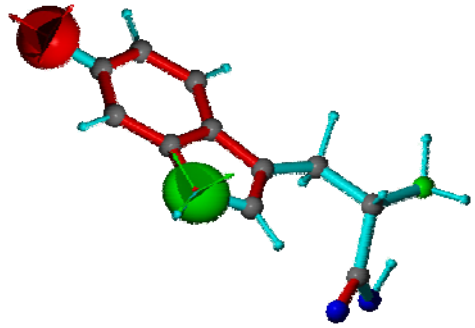

Figure 2: ^{19}F and ^{15}N CSA tenors of 6F-Trp have very similar orientation. Results are obtained from MP2 calculations.

As a starting structure we used the model by Nishimura *et al.*, and it turned out that the optimized structure is very close to the initial coordinates. Small variations of angle and position are suggested by the calculations. Still other structures might be possible, which is under investigation.

Additional *ab initio* MP2 (Møller–Plesset second order correction) calculations results imply that the ^{19}F and ^{15}N CSA tensors have similar directions, see Figure 2. From this it follows that the alignment experimental results for ^{15}N should be very close to the ^{19}F results. This is in agreement with the preliminary experimental data. The ^{15}N and ^{19}F CSA tensors are similarly averaged by the global rotation of M2-TMD within the lipid bilayer.

References

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