Supporting Material

Re-orientation and dimerization of the membrane-bound antimicrobial peptide PGLa from microsecond all-atom MD simulations

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Lipid mismatch. Upon insertion, there is a bilayer density mismatch between the two lipid bilayer leaflets that could affect the orientation of the peptide. For the dimeric T-state, we performed two simulations using the CHARMM force field, where the number of lipids in the peptide containing leaflet was kept unchanged (36 lipids) and where it was reduced to 30 lipids to make room for the peptide. Representative structures are shown in Figure S1. Tilt angle, rotation angle as well as insertion depth did not show major changes between the two simulations.

Loss of helical structure in the 2 μs OPLS simulations. Figure S2 shows snapshots of the main conformations encountered during the unrestrained OPLS simulations, as well as the number of intra-molecular hydrogen bonds and the orientation of key residues with respect to the membrane normal. The overall structure of the S-state is an interfacial α-helix, closely resembling the state described in the NMR experiments. The helix is not entirely stable throughout the 2 μs MD run, with two other perturbed-helical phases observed and an overall ~73% helicity (Figure S2 lower panel). The first phase – a fully formed α-helix (I) – is followed by a phase in which the canonical α-helical structure is lost between residue A8 and I13, and the backbone is stabilized instead by a long-lived π-helix ($i \rightarrow i+5$) A8-I13 hydrogen bond (II). This transition leads to a distinct drop in the number of backbone hydrogen bonds and to an abrupt change of the A8 side chain orientation with respect to the membrane normal. The third phase is characterized by a complete loss of helical structure between residues A8 and A14 (III). This conformation is short-lived (~200 ns), and the peptide quickly reverts to the more helical phase II.

To investigate the effect of the force field on the observables, we performed 3 additional simulations of 2 μs length each: One, where the critical backbone hydrogen bonds at G7 and G11 were restrained to be helical (R-Gly), and two, in which the entire peptide was helically restrained (R-all), run at 35 ºC and 120 ºC. Figure S3 shows the quadrupolar splittings to all OPLS simulations. The unrestrained simulation (Free) exhibits relatively large statistical errors due to the long timescale (~200-700 ns) of the transitions in the backbone. The fit to this and the glycine-restrained simulation (R-Gly) is poor. If quadrupolar splittings are considered as an experimental benchmark of the simulation quality, these results indicate that MD works best with a relatively rigid model of PGLa, while the unrestrained simulations overestimate the flexibility of the peptide in the bilayer.

Dimer insertion protocol. To generate a dimer structure, the first step was the insertion of two folded peptides into the same bilayer leaflet at high temperature, similar to the S-state procedure (Figure S4). This structure was used as the starting point of the unbiased (but ultimately unsuccessful) spontaneous aggregation simulations at 35 ºC and 150 ºC (Figure 4). The alternative strategy to obtain dimers is shown in Figure S5. The peptides

were first pulled into contact using a pulling force and a slow rate of 1 Å/ns to ensure and orderly displacement of the intermediate lipid molecules. The peptides were then allowed to sample dimeric conformations using high-temperature simulations (150 ºC) and a center-of-mass distance restraint with k = 10 kJ/mol*Å^2. From this simulation, a parallel and an anti-parallel dimer with very close contacts were chosen, and used for the subsequent production runs, where the temperature was reduced to 35 ºC and the distance restraint removed. Plots of the orientational angles τ and ρ for the dimer over the course of the stable simulations at 35 ºC using the CHARMM and OPLS force fields are shown in Figure S6.

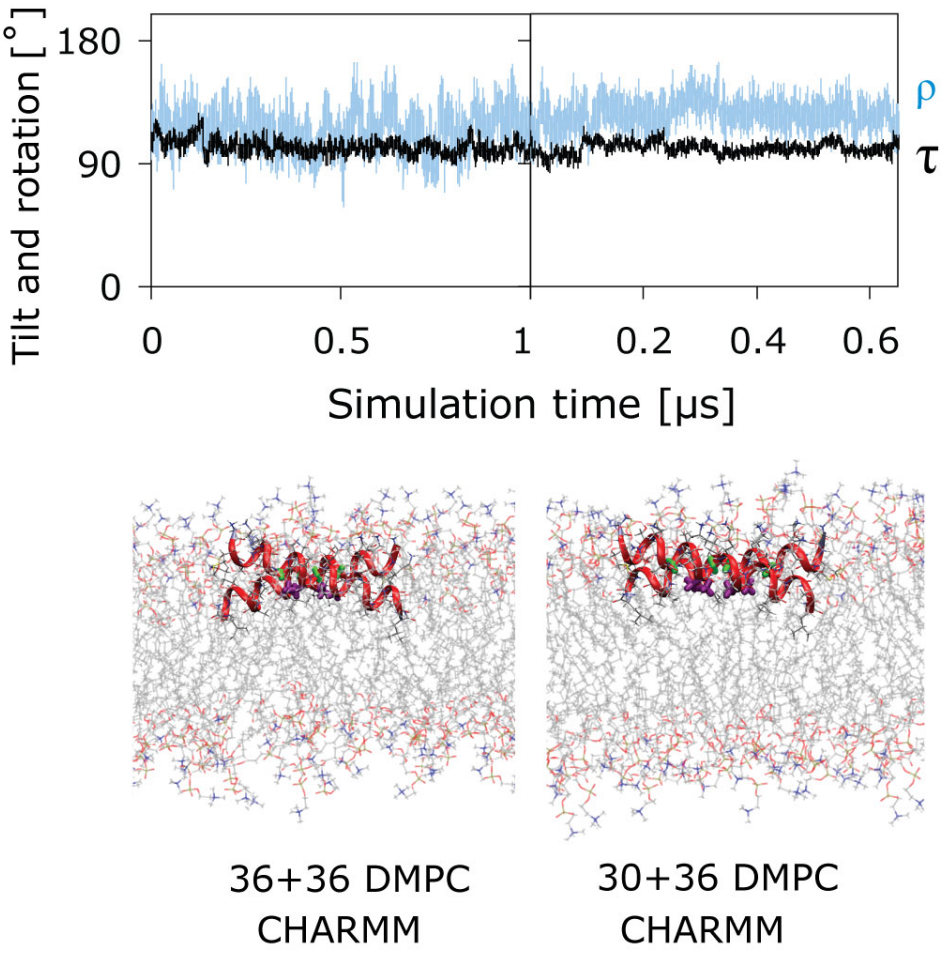

Figure S1. PGLa in the dimeric T-state in a DMPC lipid bilayer at 35ºC using the CHARMM force field. Representative structures of the simulations are shown for symmetric membranes, and for simulations in which the mass imbalance upon insertion was reduced by removal of 6 lipid molecules in the leaflet containing the dimer. There are no significant conformational or orientational changes of the dimer, and the orientational angles τ and ρ are not affected.

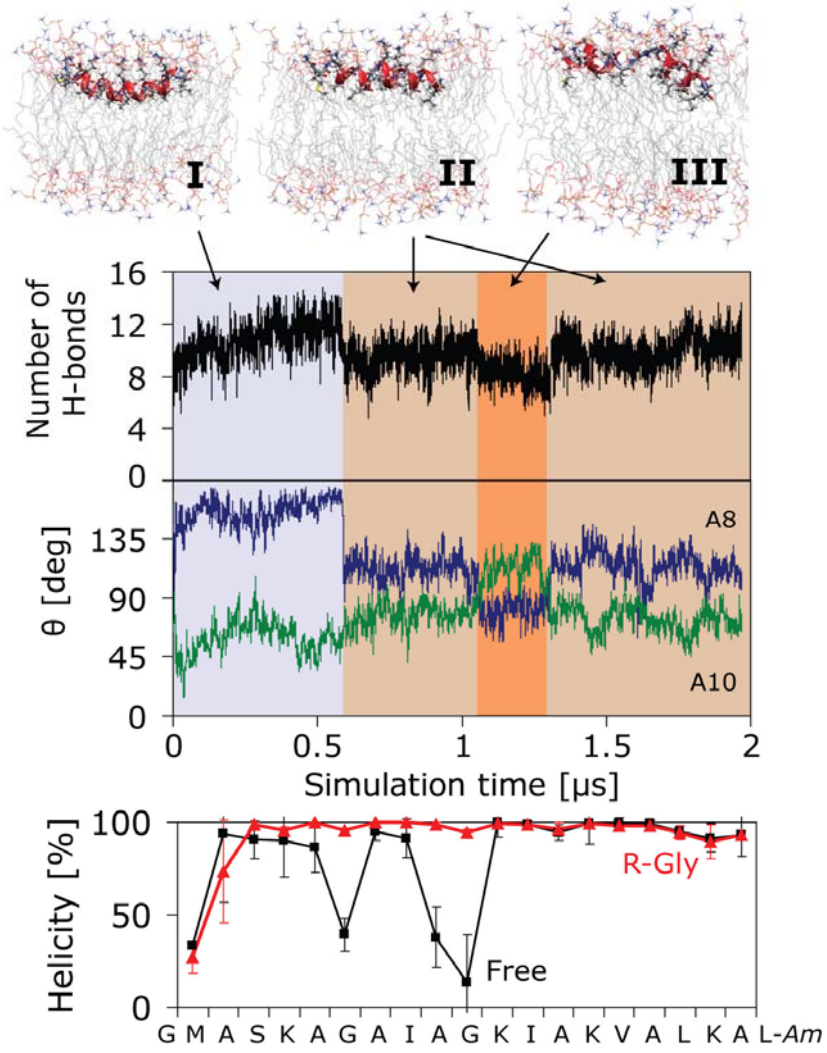

Figure S2. Structural flexibility of the S-state of PGLa during the unrestrained ('Free') 2 μs MD simulation in a DMPC lipid bilayer at 35 ºC. There are three major conformations: A fully formed α-helix (I), a perturbed helix involving an A8-I13 hydrogen bond (II), and a partially unfolded helix in which residues A8-A14 are unstructured (III). The three phases display a characteristic number of backbone hydrogen bonds and the orientation of the residues A8 and A10 (angle θ of the C_α-C_β bond) with respect to the membrane normal. The overall helicity averaged for each residue reveals that the structural flexibility arises largely from the loss of helical structure at the two glycines G7 and G11. PGLa remains entirely stable in a 2 μs simulation when the backbone hydrogen bonds involving G7 and G11 are restrained ('R-Gly').

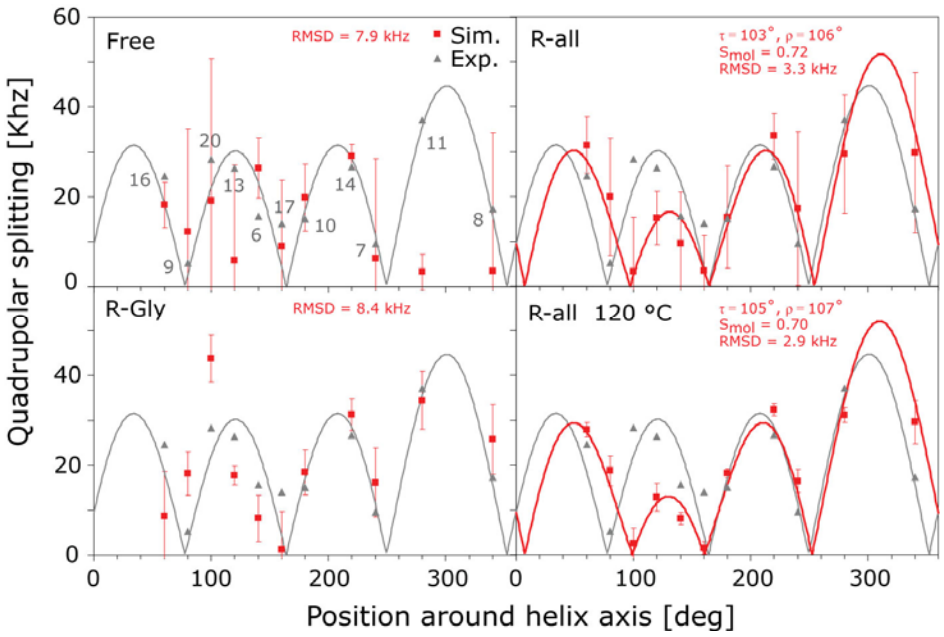

Figure S3. Quadrupolar splittings obtained from the OPLS MD simulation of monomeric PGLa in the S-state in a DMPC lipid bilayer at 35ºC (Free: unrestrained peptide, R-all: helical restraints on all backbone hydrogen bonds, R-Gly: helical restraints for G7 and G11 only). ^{2}H-NMR data from Ala-d_3-labeled PGLa in DMPC at P/L = 1:200 are shown as grey triangles [16], with the number of the labeled residue indicated and the grey line corresponding to the fitted helical wave. Computed splittings are shown in red, with the best fit represented by a red line. The splittings from Free and R-Gly simulations did not give good fits and the fits are not shown.

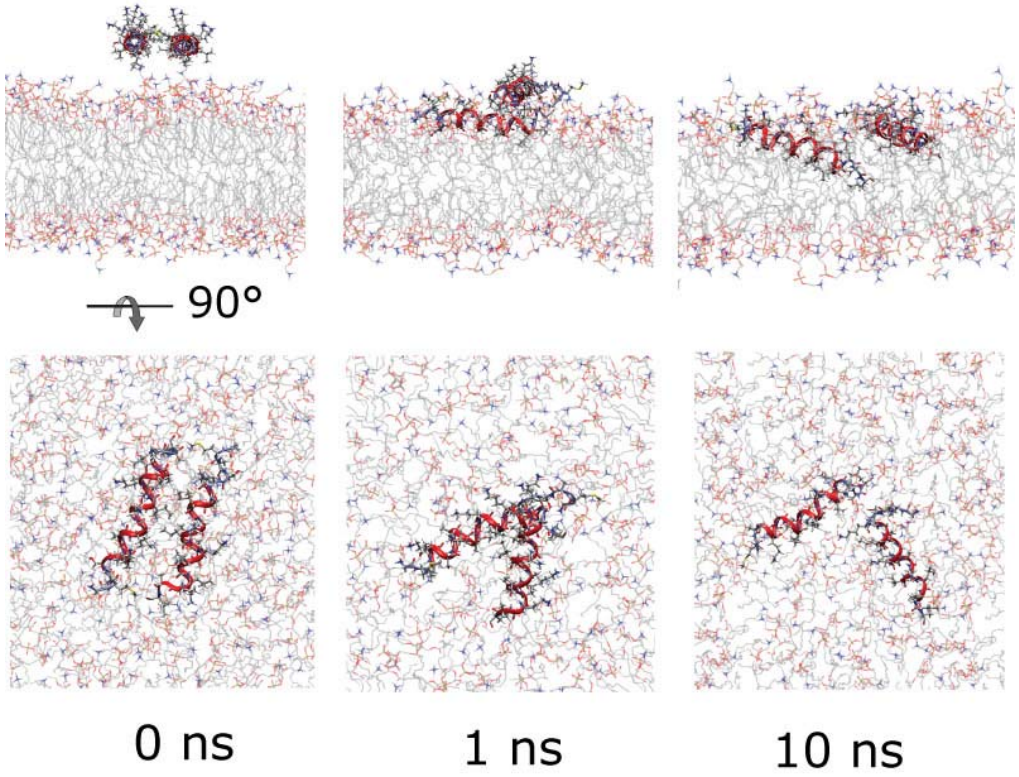

Figure S4. Membrane insertion protocol for two PGLa peptides. The simulations proceeded identical to the monomer insertion runs. The two peptides were place into the solvent ~10 Å away from the membrane. Helical restraints were applied and the temperature raised to 217 ºC. To ensure both peptides inserted into the same bilayer leaflet, a harmonic distance restraint was applied between the two peptides. After 10ns, the peptides were completely embedded in the membrane, in a position similar to that observed for the single S-state.

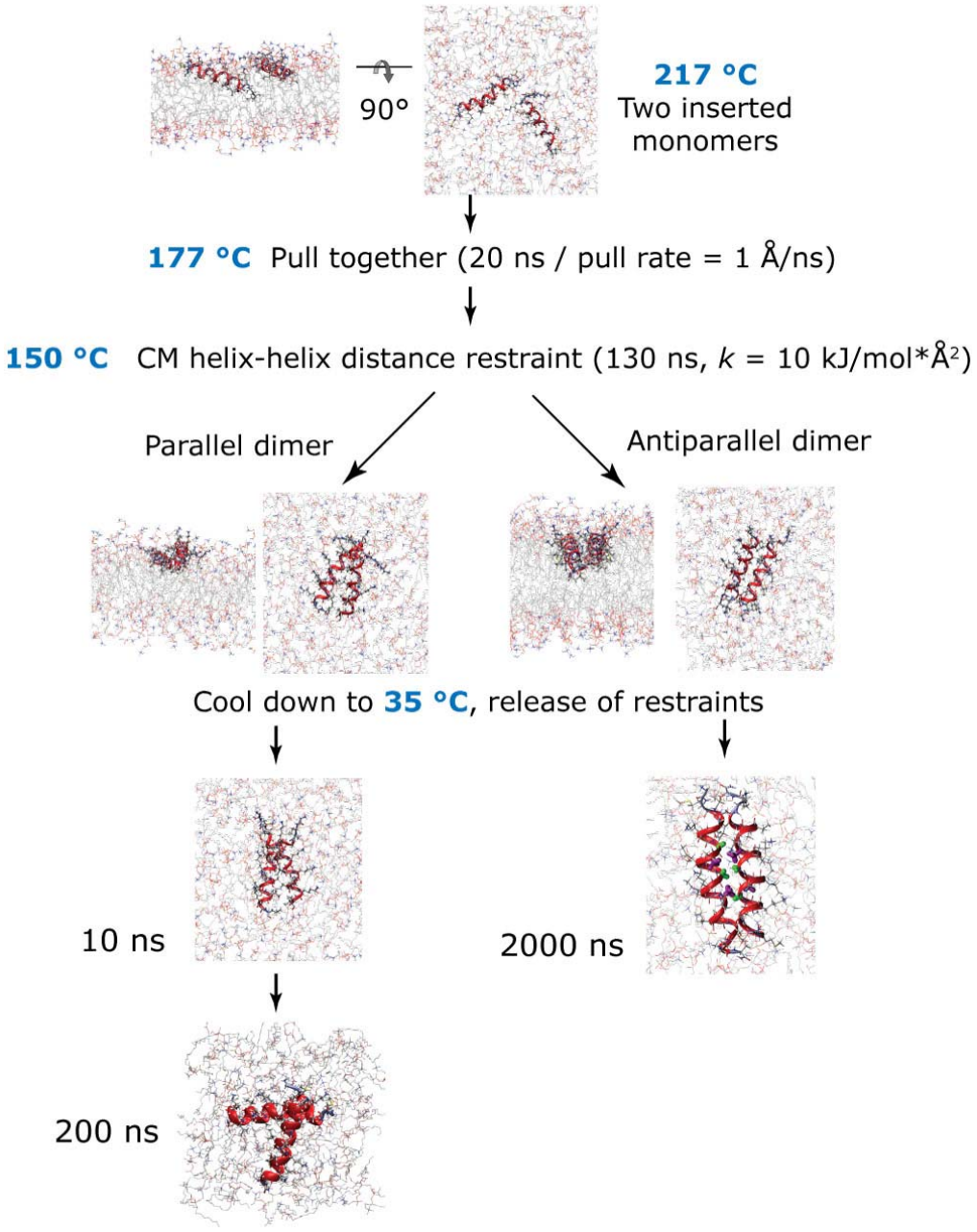

Figure S5. Protocol for the dimer generating simulations. After bilayer insertion, the two PGLa peptides were pulled together at 177 ºC over 20 ns using a harmonic helix center-of-mass (CM) distance restraint (k = 10 kJ/mol*Å^2), with a slow pulling rate of 1 Å/ns. Both peptides were helically restrained. Once a contact minimum was formed, the pull force was switched off and the two helices were allowed to rearrange rapidly while retaining high temperature (150 ºC) and the CM distance restraint. This simulation sampled both parallel as well as anti-parallel dimers. Two initial structures were selected from dimers with very close contacts. These systems were subsequently cooled to 35 ºC and the distance restraint removed. In the production runs, a very slow drift away from the dimer structure was observed for the parallel dimer (Figure 4), but not for the anti-parallel dimer.

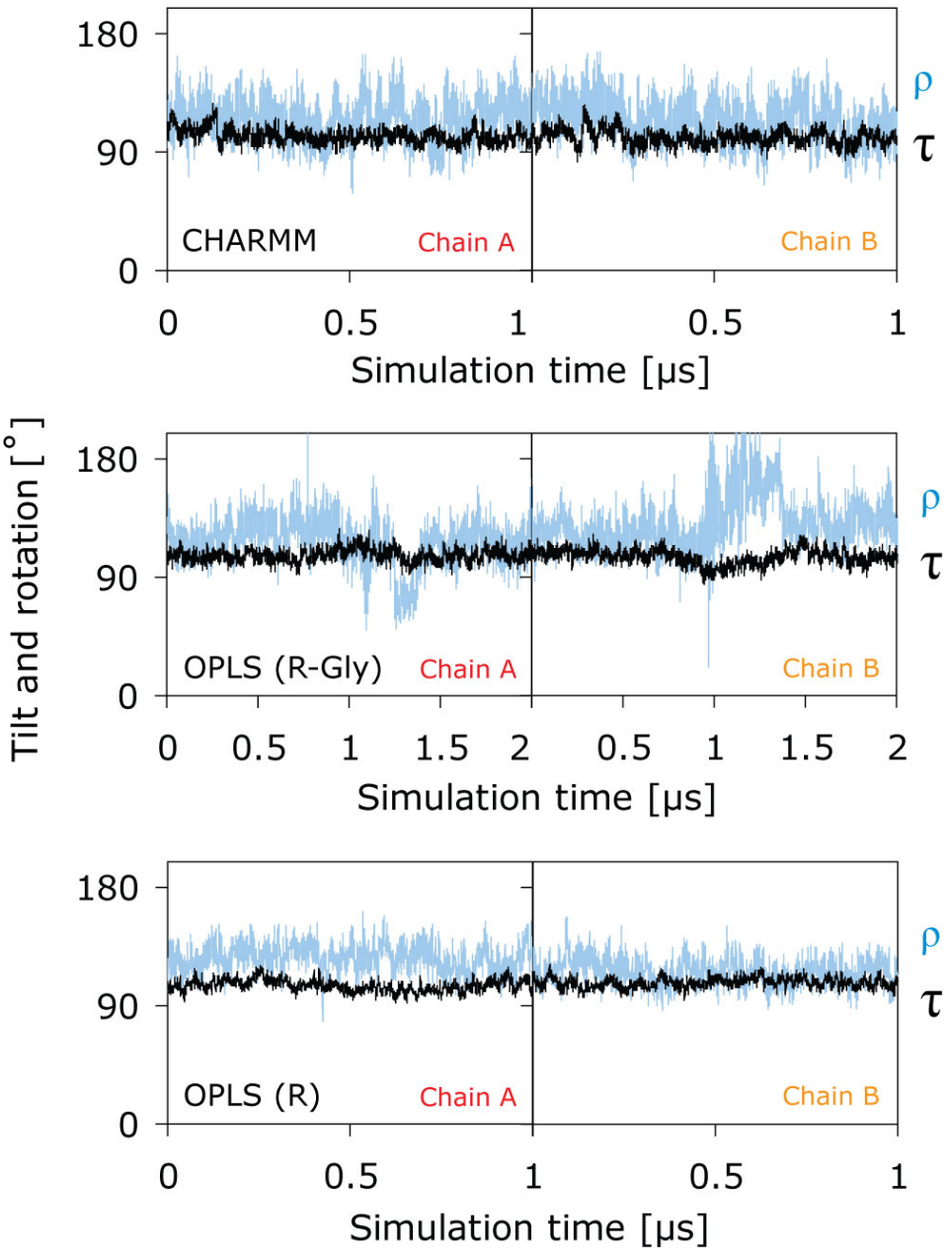

Figure S6. Summary of the simulations of PGLa in the dimeric T-state in a DMPC lipid bilayer at 35ºC using the CHARMM and OPLS force fields. The plots show the orientational angles τ and ρ for both peptides in the dimer over the course of the simulation time. All simulations resulted in a stable dimerization interface. The OPLS simulation with restrained hydrogen bonds on the Gly positions (OPLS-R-Gly) exhibit a short phase (1 - 1.3 μs) where several backbone hydrogen bonds were broken, resulting in a transient perturbation of the overall tilt and rotation vector.