SUPPORTING INFORMATION

Polar/Apolar Interfaces Modulate the Conformational Behavior of Cyclic Peptides with Impact on Their Passive Membrane Permeability

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1 Hydrophobicity Profile of CDPs 2-7

CDP **2** (for which phenylalanine is replaced by alanine) shows an even stronger anisotropic hydrophobicity than CDP **1**. CDPs **3-8** contain a proline. Although prolines can influence the conformational dynamics [1], CDPs **3-7** show the same anisotropic hydrophobicity pattern as CDP **1**. Figure S1 displays CDP **3** as a representative of the proline containing peptides. The highest populated 'open' state has a slightly more circular backbone conformation, but the leucines still point towards the peptide normal. They form a large continuous hydrophobic patch in the 'closed' conformation and smaller patches in the 'open' conformations. Due to the higher circularity in the 'open' conformations of the proline-containing peptides, their leucine side chains at position 1 and 6 are slightly further apart and a backbone N-methylation (shown in yellow) bridges their hydrophobic patches.
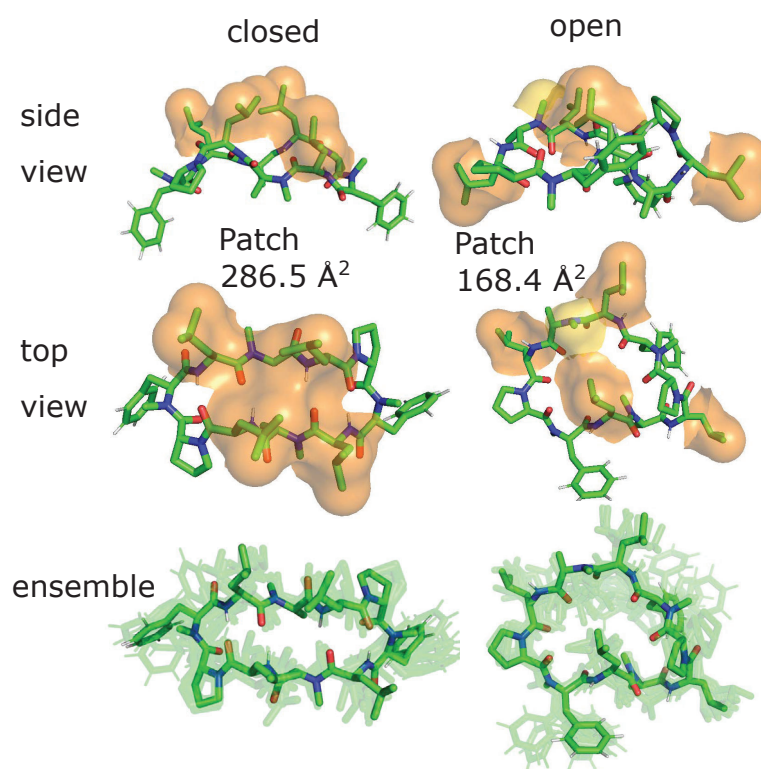
Figure S1: Metastable conformations of proline-containing CDP 3 in water. The 'closed' as well as the highest populated 'open' state are shown. In the two top panels, the solvent-accessible surface of the hydrophobic leucine residues is indicated by orange spheres. For the 'open' conformation, the solvent-accessible surface of a backbone N-methylation, which bridges the hydrophobic leucine patches, is indicated by a yellow sphere. The conformational ensemble of the metastable states is shown at the bottom.
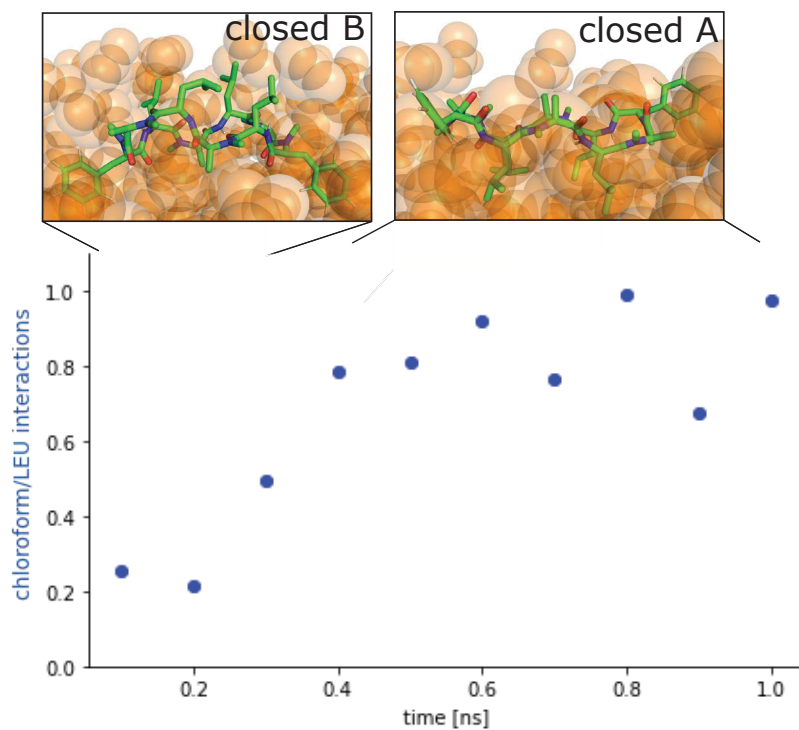
2 Additional Figures

Figure S2: Zoom in of the first nanosecond of the rotation from orientation B to A for CDP **1** in the 'closed' conformation. Blue points show the orientation of the peptide with respect to the membrane measured by the side-chain contacts. In the simulation snapshots, the chloroform molecules are shown as orange spheres. Water molecules are not shown for clarity.
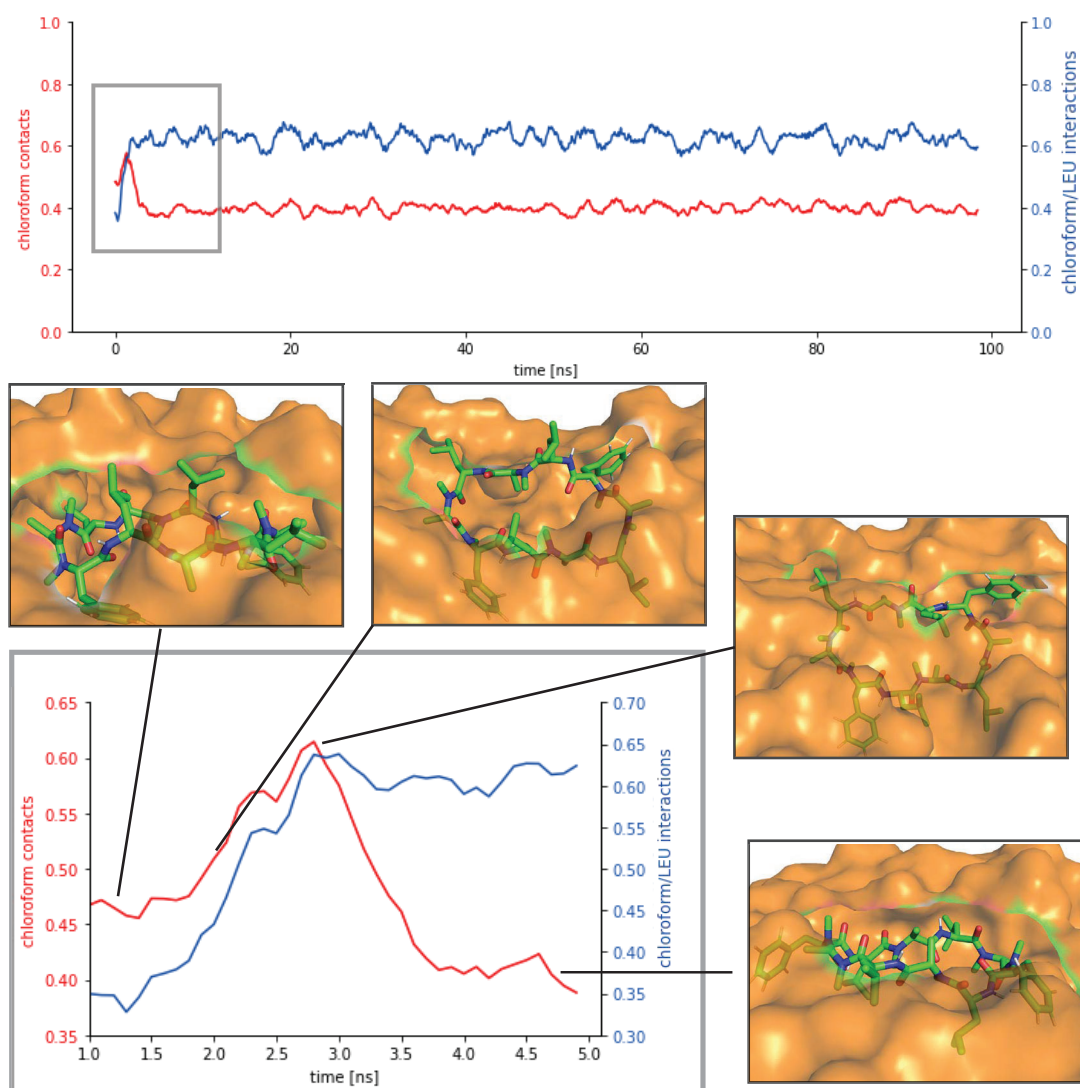
Figure S3: Representative simulation and zoom into the first 5 ns of the rotation from orientation B to A for CDP **1** in the 'open' conformation. Blue lines show the orientation of the peptide with respect to the membrane measured by the side-chain contacts. Red lines show the fraction of chloroform contacts with respect to all solvent contacts of the peptide. Simulation snapshots are shown at specific points of the simulation indicated by the black lines. Chloroform molecules are colored orange and shown in surface representation. Water molecules are not shown for clarity.
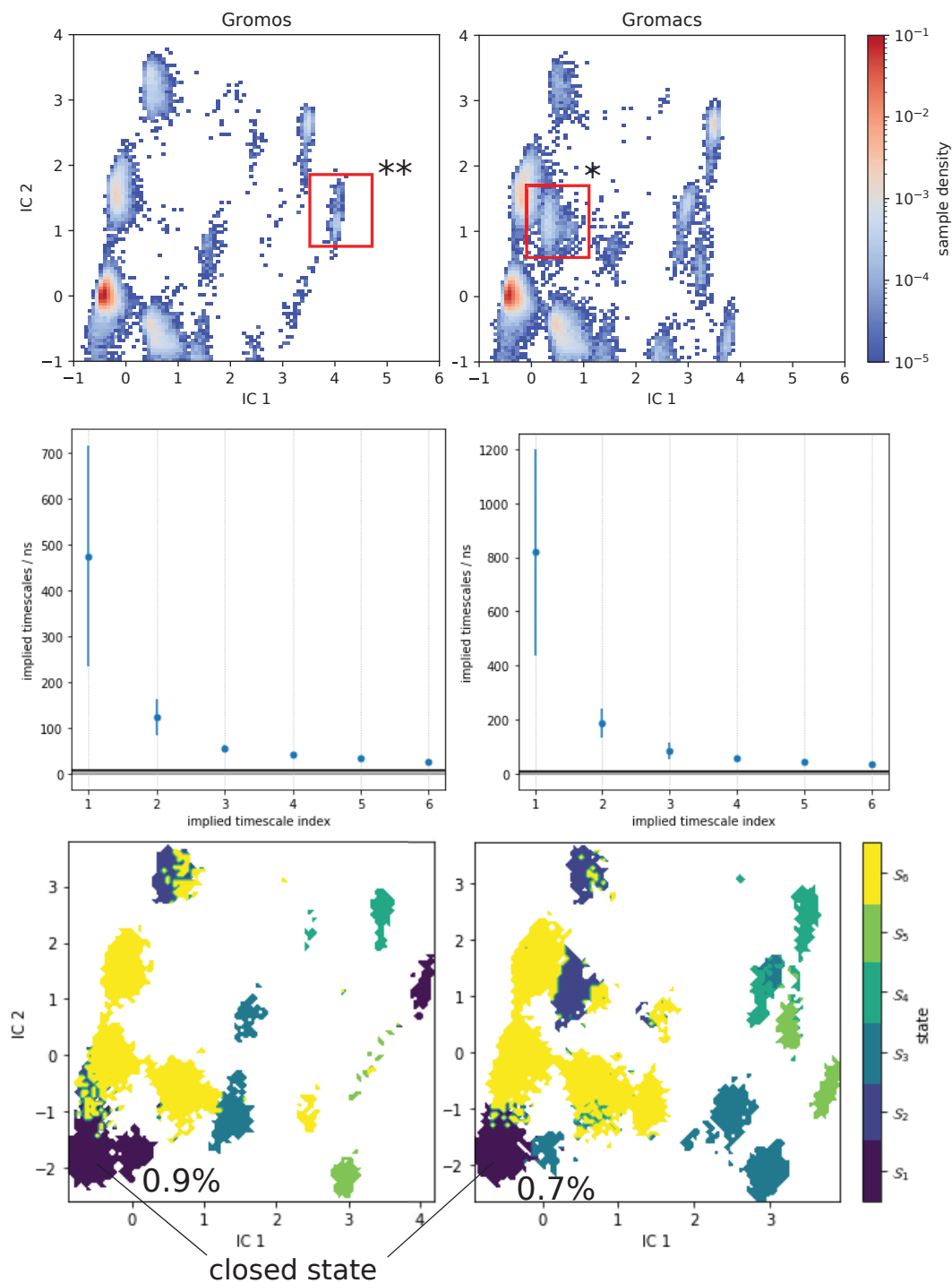
Figure S4: Comparison of Markov state model (MSM) results of CDP **1** in water with the GROMOS and GROMACS simulation software packages. (Top): Stationary distributions according to the MSM in the TICA subspace. The distribution overlap, except for two minor conformational states marked with red boxes. (Middle): Implied timescales of the MSMs. (Bottom): State assignment of the PCCA+ algorithm. The 'closed' state and its population is highlighted.
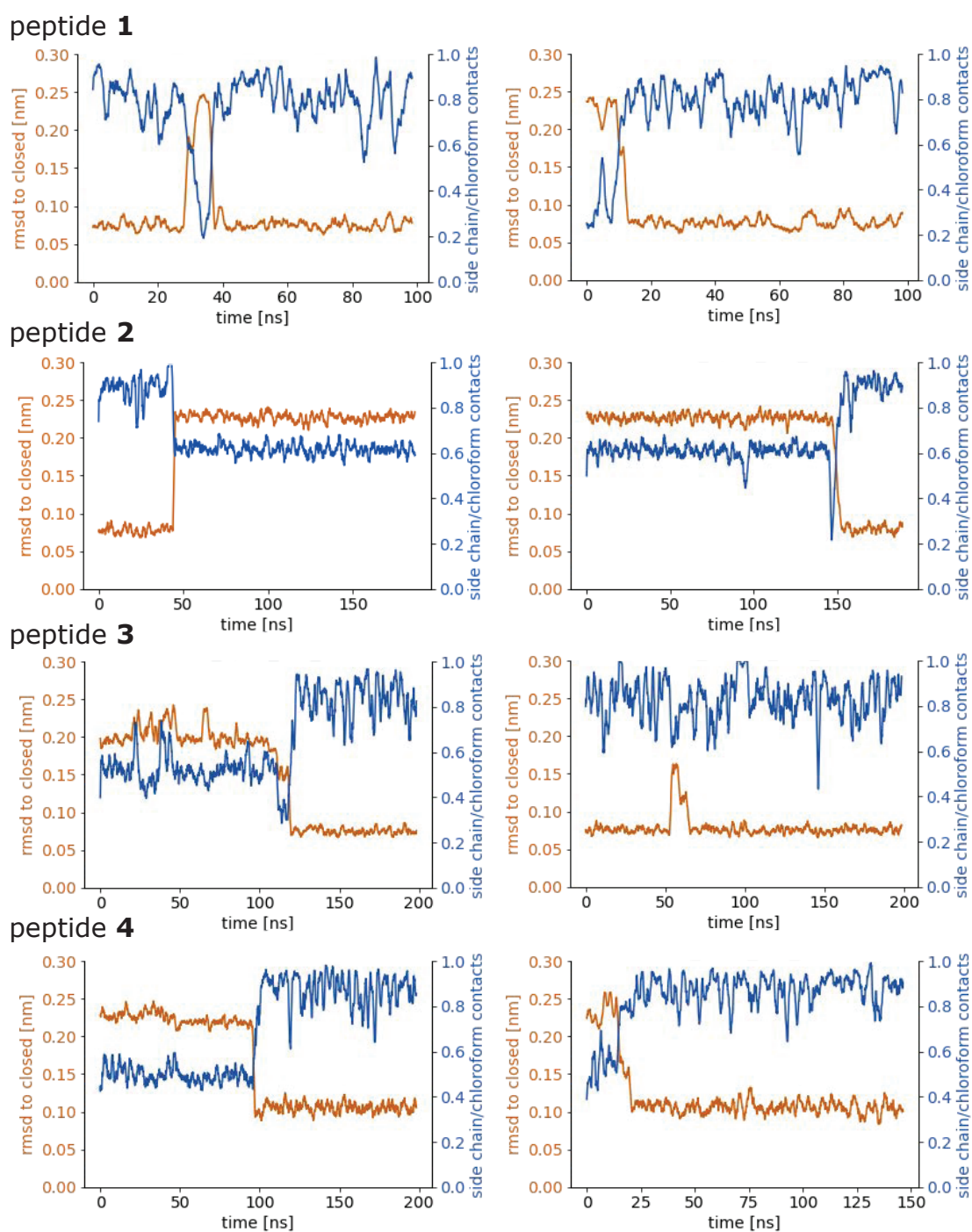
Figure S5: Trajectories with closing events for CDPs **1**-**4** at the interface. The orange line represents the RMSD with respect to the 'closed' NMR solution structure, and the blue line gives the orientation of the peptide measured by the side-chain contacts.
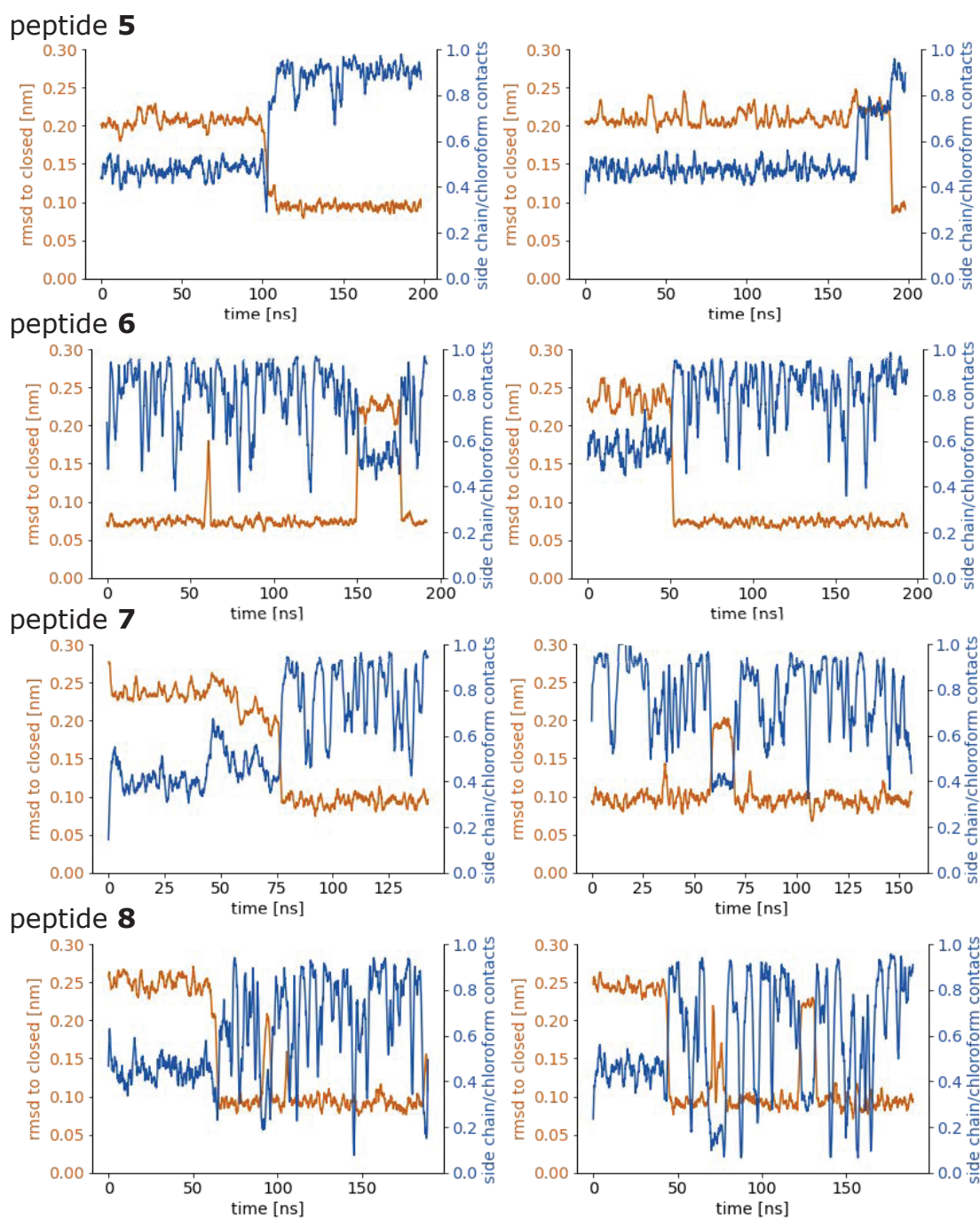
Figure S6: Trajectories with closing events for CDPs **5-8** at the interface. The orange line represents the RMSD with respect to the 'closed' NMR solution structure, and the blue line gives the orientation of the peptide measured by the side-chain contacts.
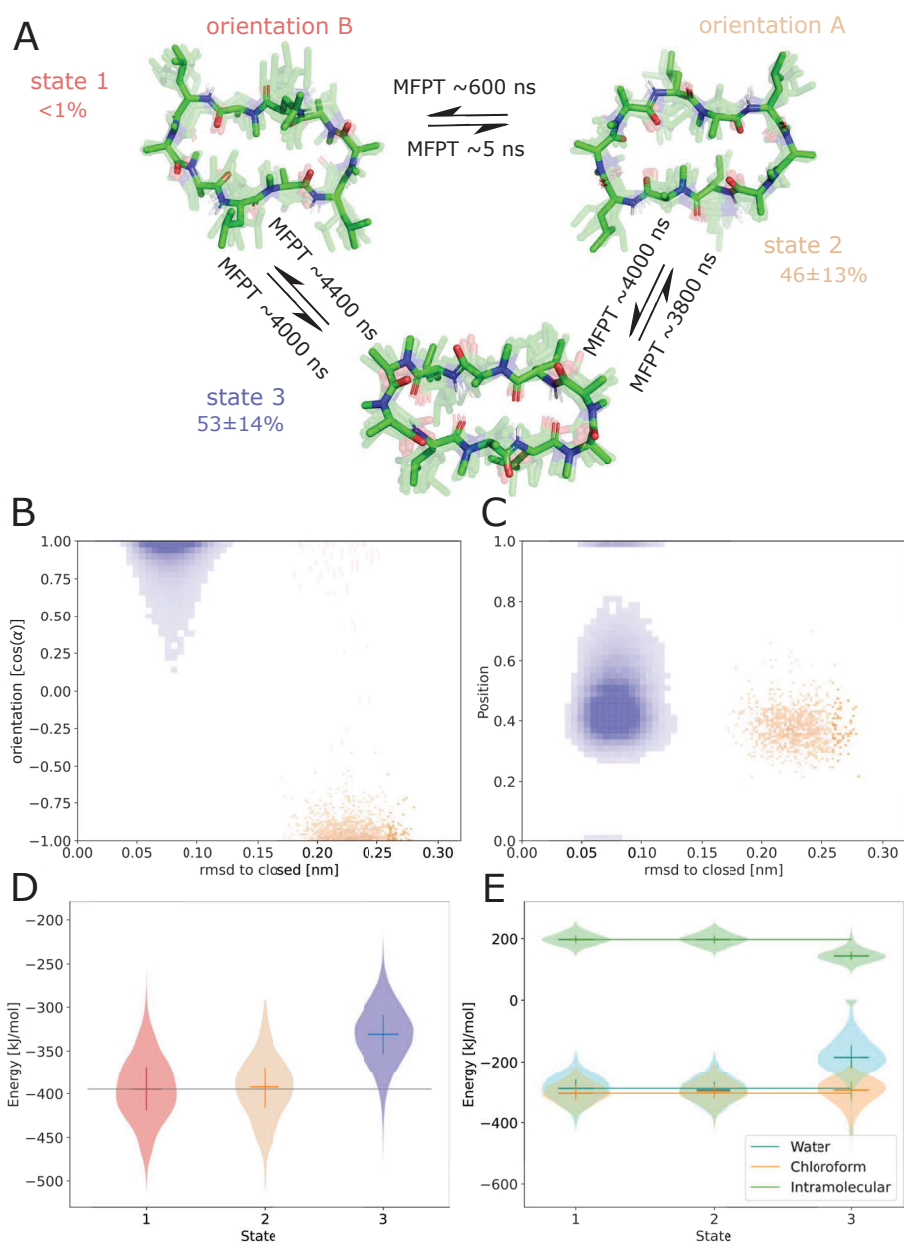
Figure S7: MSM of CDP **2** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
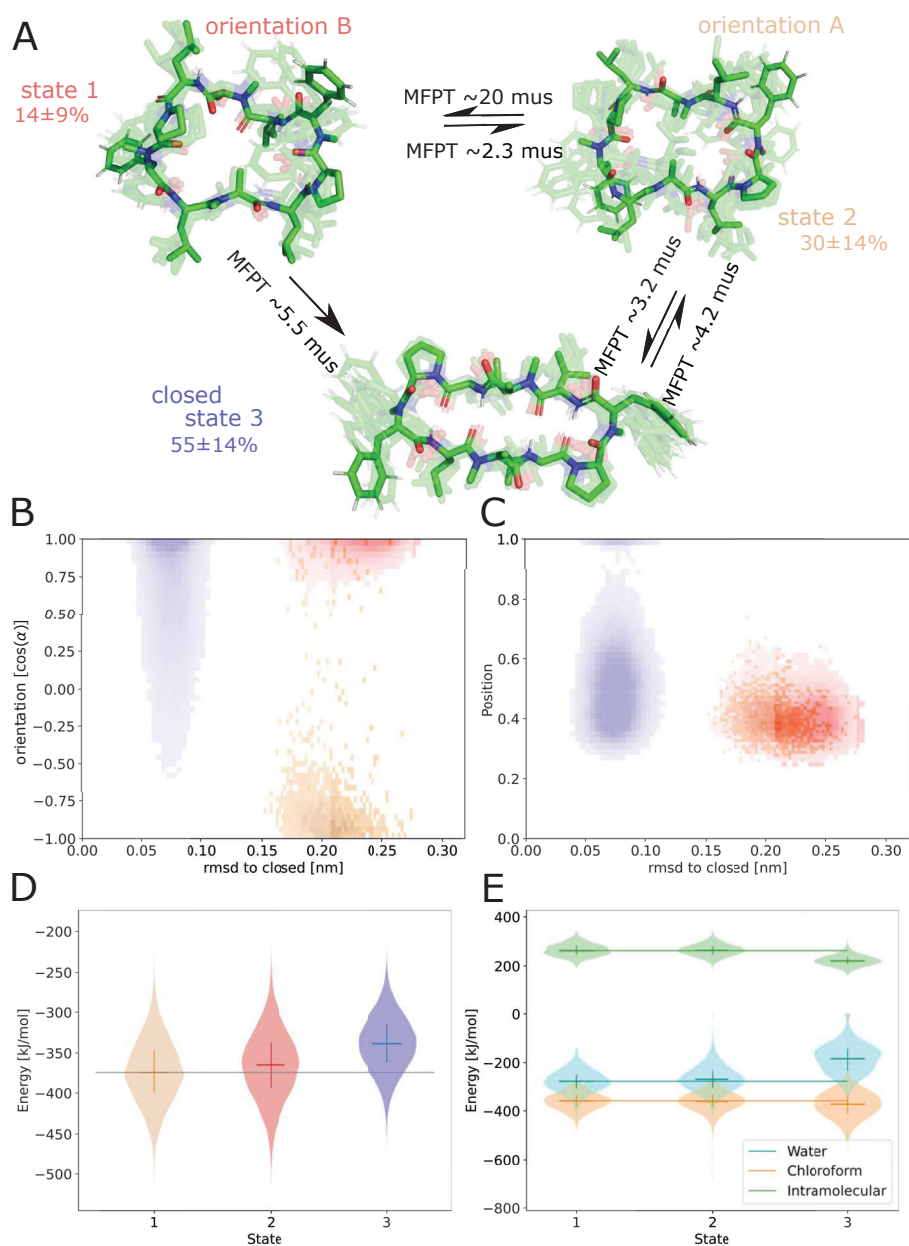
Figure S8: MSM of CDP **3** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
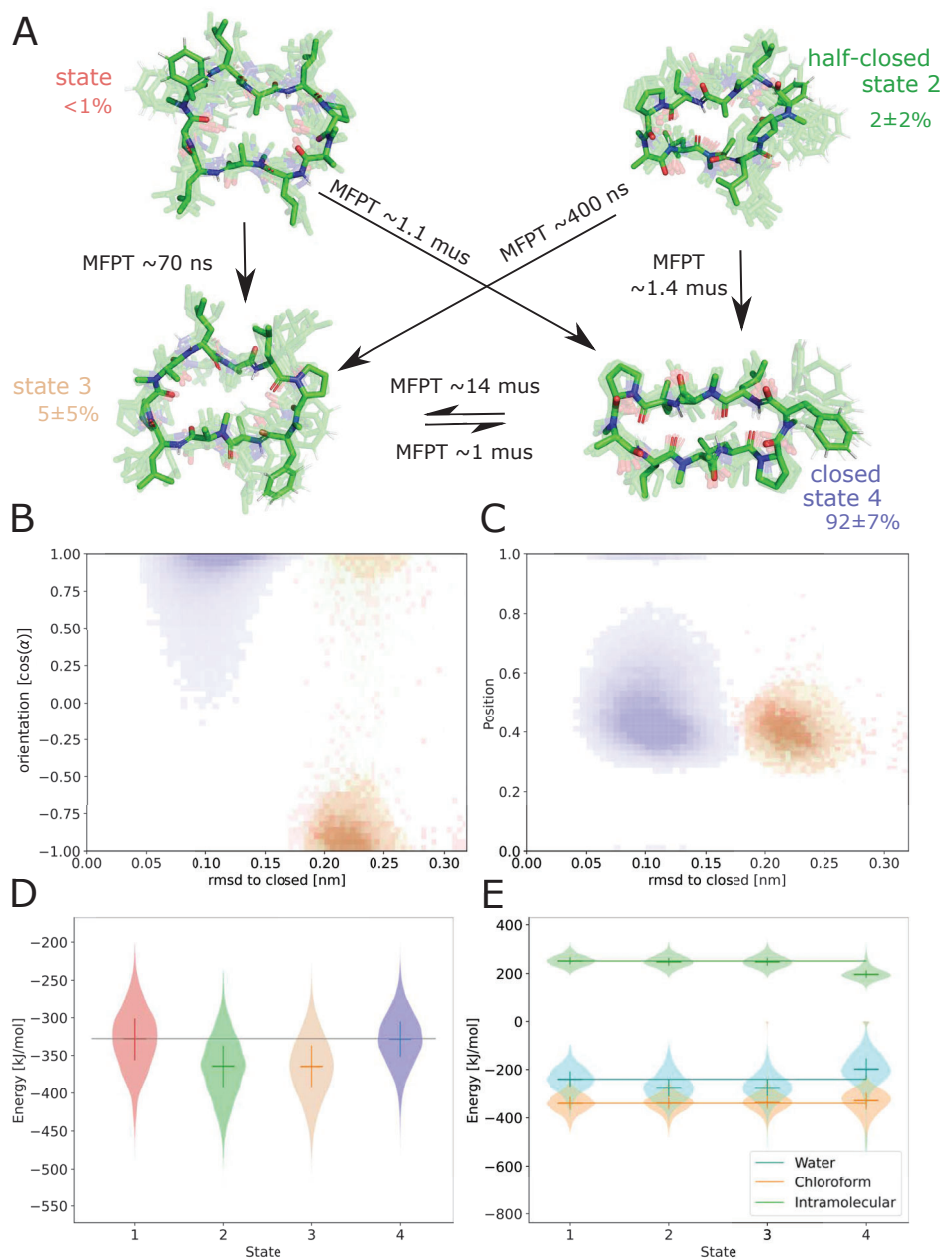
Figure S9: MSM of CDP **4** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
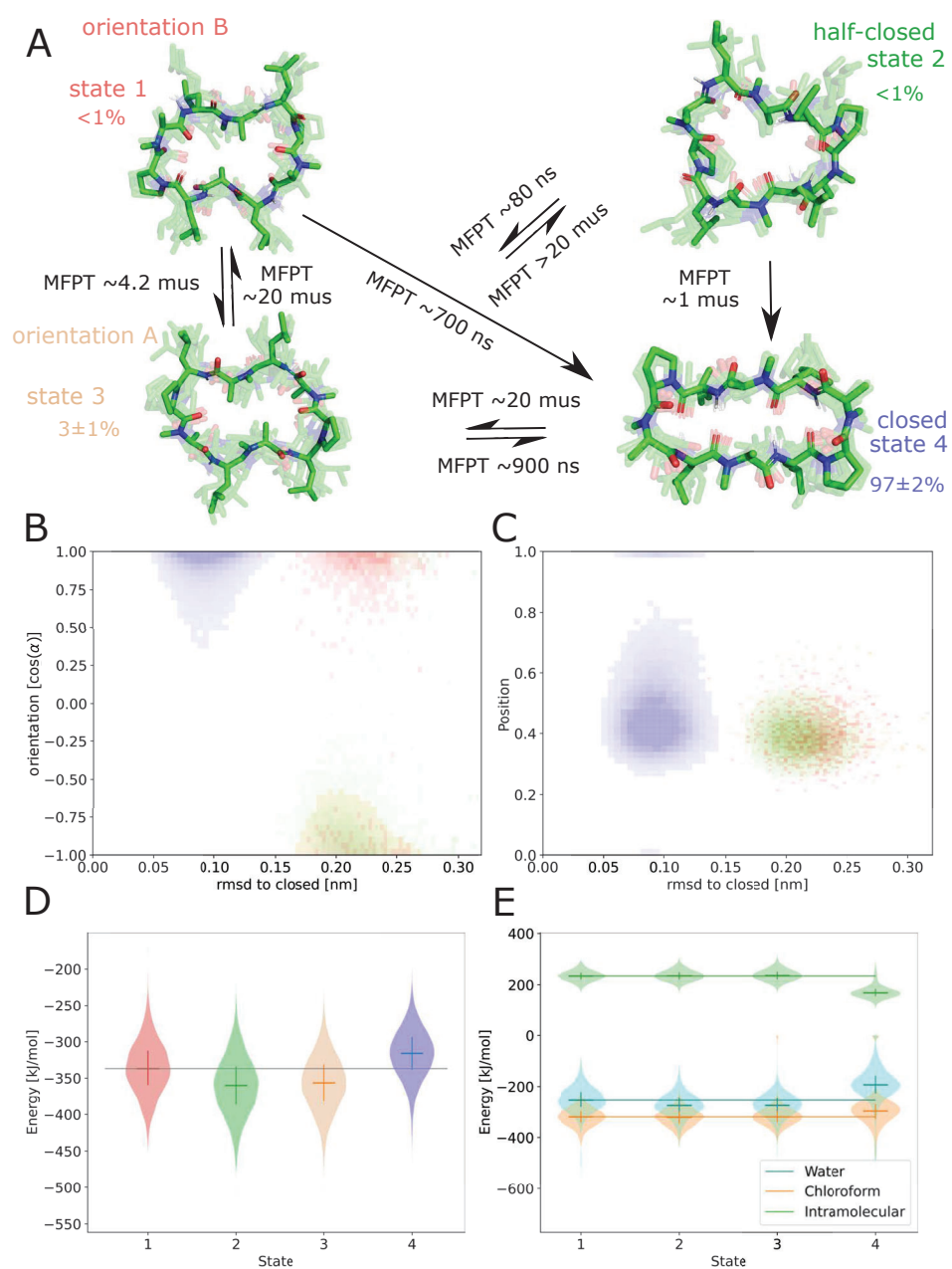
Figure S10: MSM of CDP **5** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
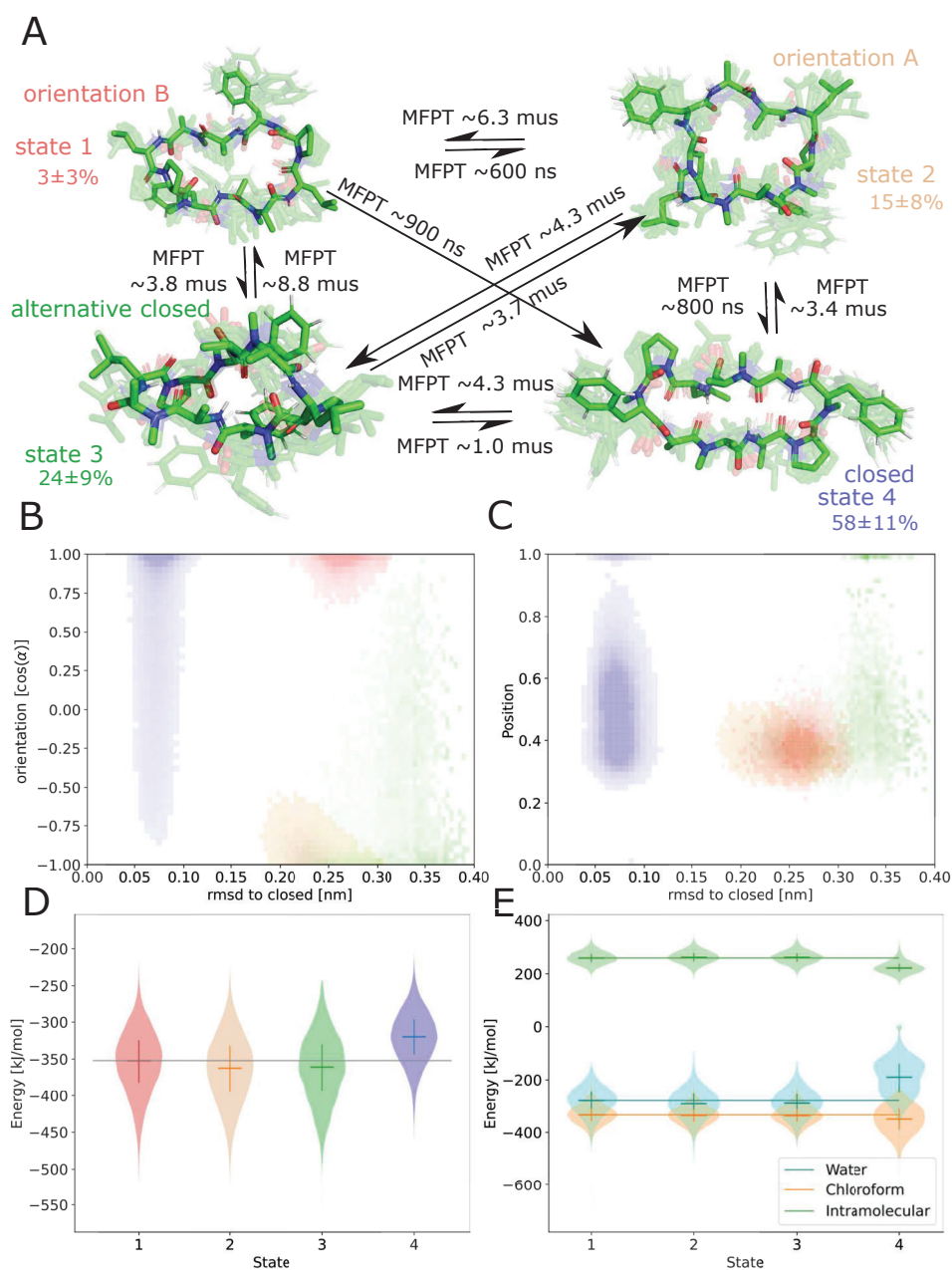
Figure S11: MSM of CDP **6** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
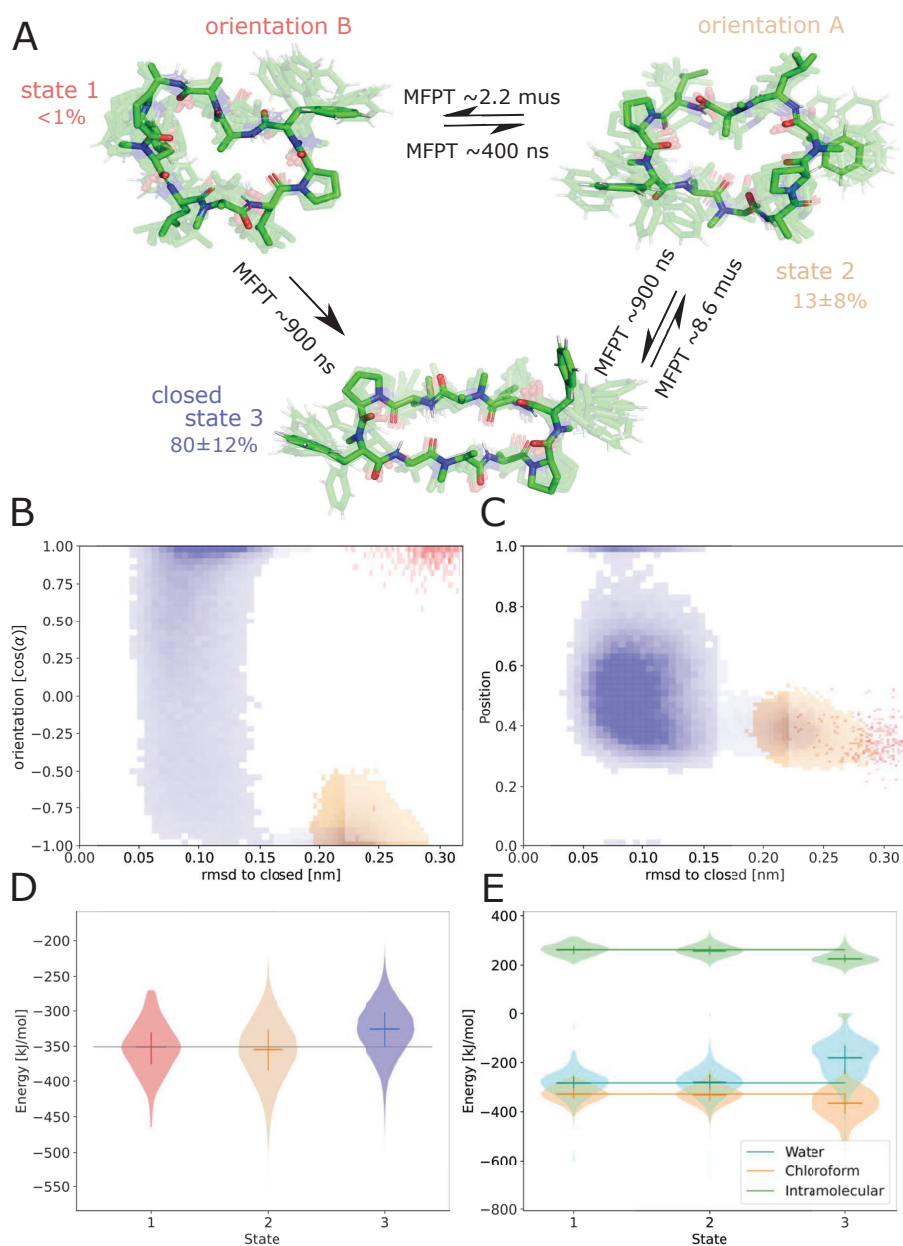
Figure S12: MSM of CDP **7** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
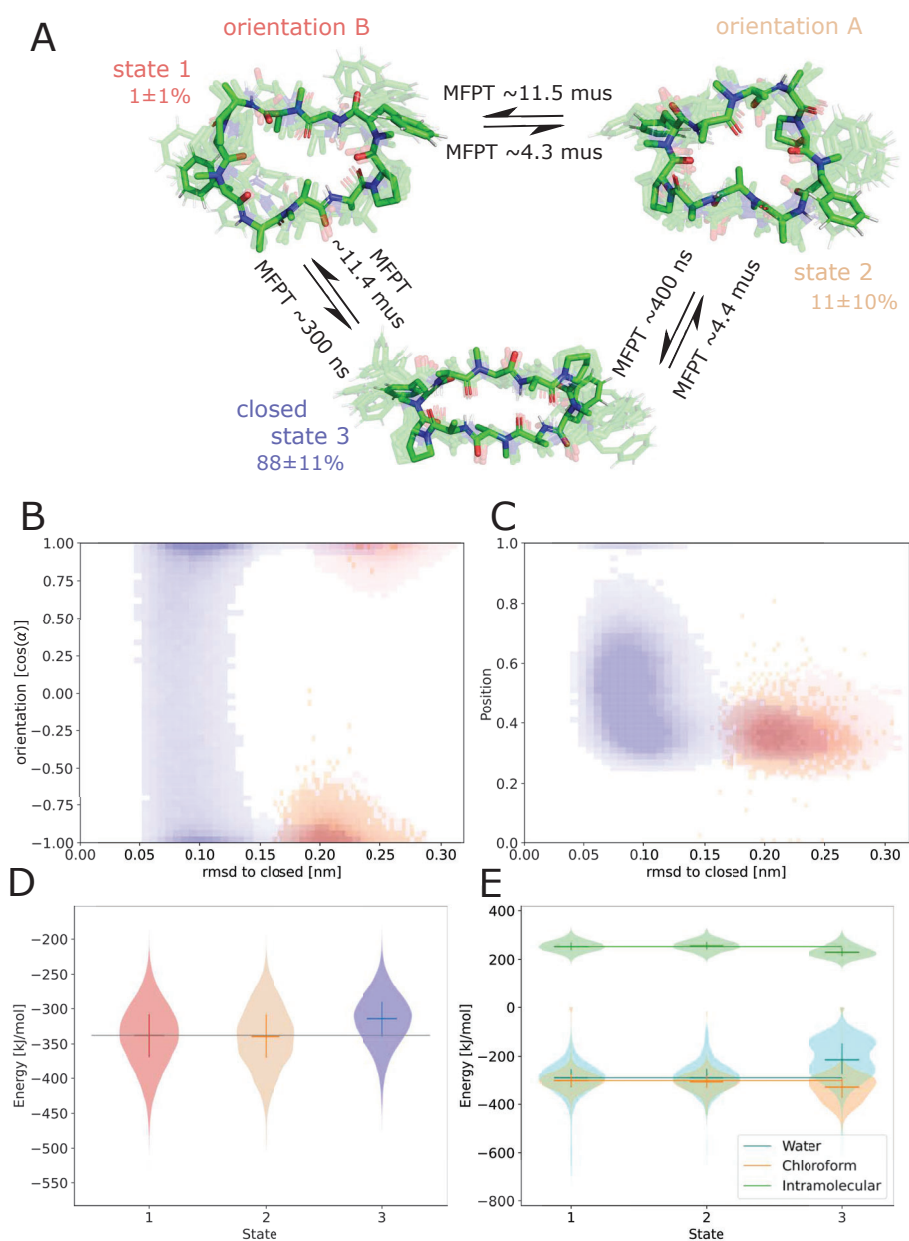
Figure S13: MSM of CDP **8** in the water/chloroform system. **A**: Representative members of the metastable states, their equilibrium populations and MFPTs between them. **B**: Metastable state assignment mapped to the orientation feature and the RMSD with respect to the 'closed' state. **C**: Metastable state assignment mapped to the position feature and the RMSD with respect to the 'closed' state. **D**: Violin plot with the interaction energies of the metastable state members. **E**: Same as **D**, but split into water, chloroform, and intramolecular contributions. Horizontal lines are added to guide the eye.
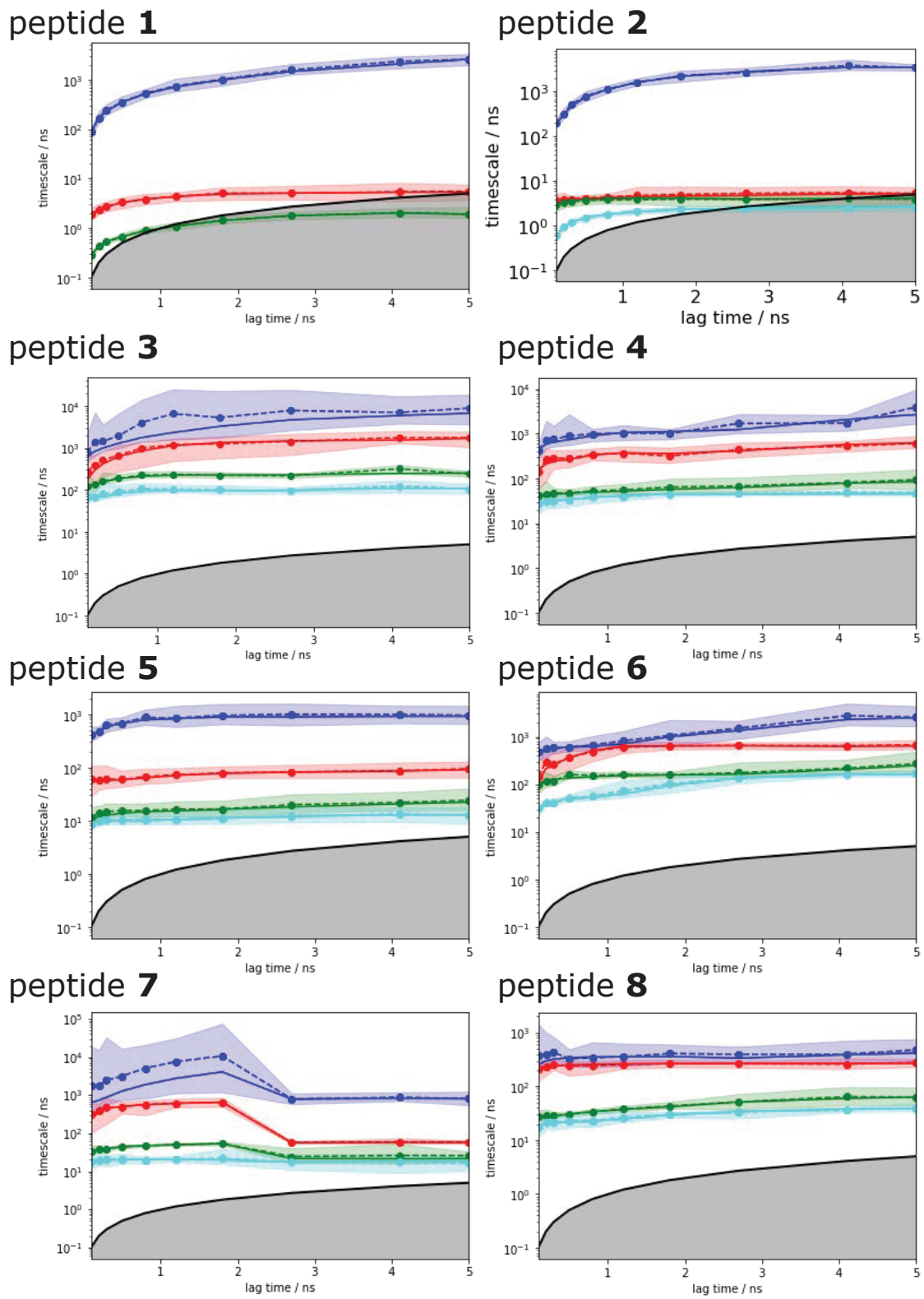
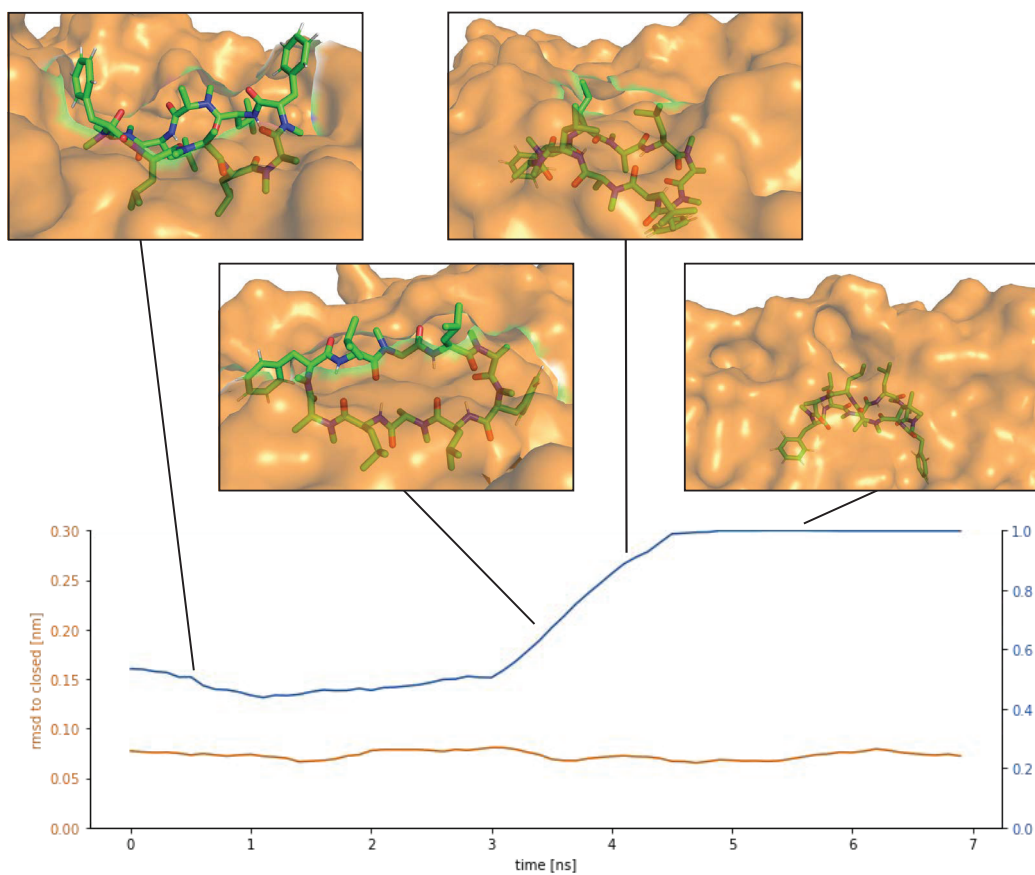**Figure S14:** Implied timescales of the MSMs of CDPs **1**-**8**.

Figure S15: Representative apolar diffusion trajectory of CDP **1**. The orange line shows that the peptide remains in the 'closed' conformation throughout the diffusion process. The blue line indicates the fraction of peptide-chloroform contacts of the total peptide-solvent contacts. The diffusion event starts in orientation A. Next, the peptide rotates along its long axis until the leucine side chains point towards the aqueous phase. Then, it diffuses into the apolar phase until it is fully surrounded by chloroform molecules.
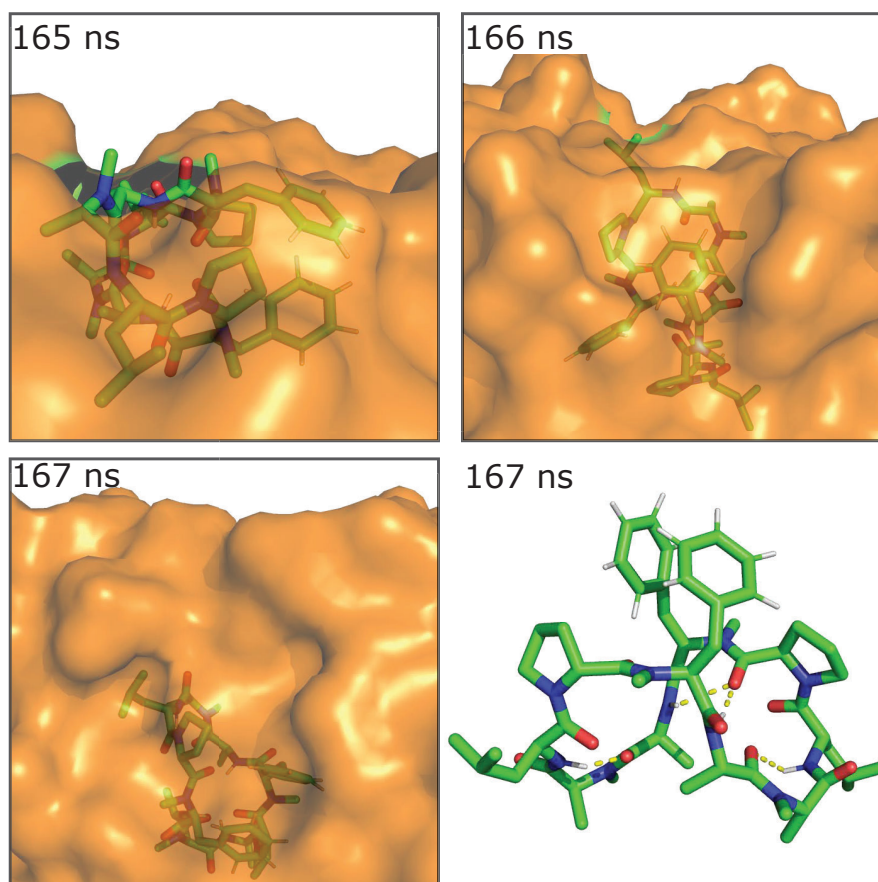
Figure S16: Apolar diffusion process of the 'alternative closed' state of CDP **6**. In this 'alternative closed' state, the phenylalanine and proline side chains build a cage-like structure. The backbone conformation resembles a twisted 'eight'. Four intramolecular hydrogen bonds stabilize the structure with the center carbonyl oxygen contributing to two hydrogen bonds. The peptide diffuses into the apolar phase in a side orientation with one leucine residue pointing upwards.

References

[1] J. Witek, S. Wang, B. Schroeder, R. Lingwood, A. Dounas, H.-J. Roth, M. Fouché, M. Blatter, O. Lemke, B. Keller and S. Riniker, *J. Chem. Inf. Model.*, 2019, **59**, 294–308.