Binding properties of SIM/SUMO complexes

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Introduction

The non-covalent interaction between the SUMO protein and the SUMO Interaction Motif (SIM) plays an essential role in several intracellular processes. We aim to understand the nature of these complexes using molecular dynamics simulations and sophisticated free energy methods.

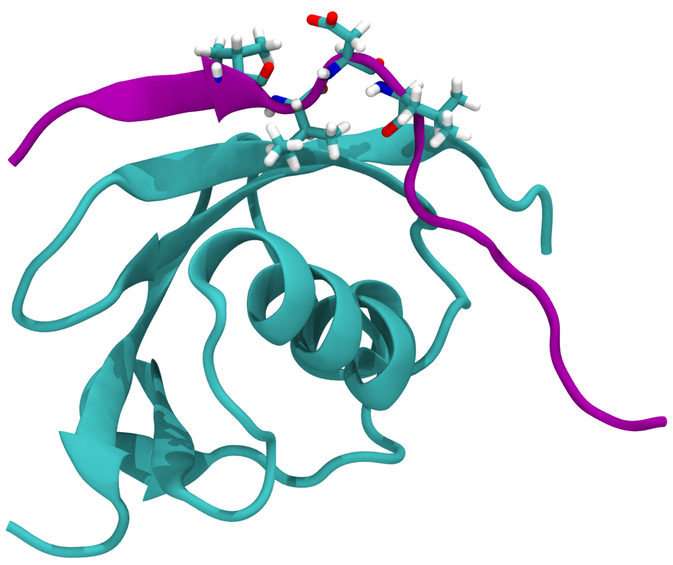

Figure 1: The Snapshot shows a cartoon representation of the SUMO protein (cyan) and peptide chain containing the PIAS SIM (purple). The SIM consits of the residues Val-5, Ile-6, Asp-7 and Leu-8, which are shown in licorice representation.

Here we consider peptide chains containing the SIM of the protein PIAS or SIM2 or SIM3 of the protein RNF4 in complex with SUMO (see also figure 2). Similar peptides are known to be disordered in solution, which complicates the calculation of the free energy. Main questions regarding this complex are:

- Is the standard binding free energy accessible by MD simulations despite the structural flexibility of the ligand?
- How important are residues in the neighborhood of the SIM for high affinity binding?
- What dictates the preferential orientation of the peptide with respect to the SUMO?

PIAS SIM peptide	KKVEVIDLTIDSSSDE
RNF4 SIM2 peptide	DEIVDLTCE
RNF4 SIM3 peptide	EPVVVDLTHND

Figure 2: Chemical composition of the considered peptides. Residues of the SIM core motif are colored blue. Acidic flanking residues are colored red.

Furthermore we are also interested in the complex formed by a peptide containing SIM2 and SIM3 of RNF4 and a SUMO dimer. Open question are:

- What are the bound orientations of the SIMs?
- Is the SIM SUMO bond in the dimer complex the same as in the complexes formed by the monomers?

Conclusion

- Despite the structural flexibility of the ligands, reliable and accurate estimates of the binding free energies could be obtained.
- For the SIM of PIAS and SIM3 of RNF4 the parallel binding mode is highly favored. For SIM2 of RNF4 the antiparallel binding mode is favored.
- Residues flanking the SIM contribute strongly to the binding energy in the parallel binding mode.
- In case of the diSIM/diSUMO complex the SIMs frequently change the bound orientation.

Acknowledgment

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The simulations have been performed on the computing cluster PALMA2 at the University of Münster

References

[1] Gumbart, J. C. et al., J. Chem. Theory Comput., **2012**, 9, 794.

Standard binding free energy: Results

- Binding free energies ΔG^0 are in good agreement with experimental values for similar systems.
- PIAS peptide binds in both directions. In antiparallel orientation the flanking residues are unrestrained by the binding.

	SIM2 (kcal/mol)	SIM3 (kcal/mol)	PIAS (kcal/mol)
ΔG^0_{para}	-1.95	-5.55	-5.05
ΔG^0_{anti}	-5.65	-1.10	-2.91
ΔG^0_{gesamt}	-5.65	-5.55	-5.06
$\Delta G^0_{experiment}$	-6.10	-5.61	-6.54

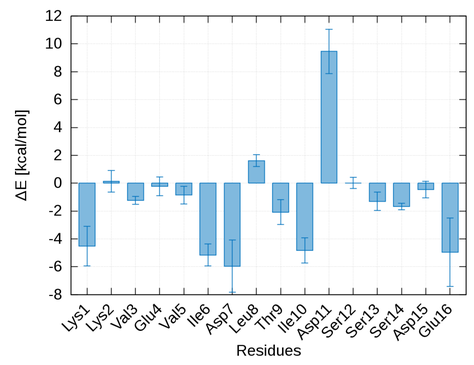

Figure 3: The potential energy differences for each residue of the PIAS peptide between parallel and antiparallel orientation. We include non bonded interactions to SUMO, PIAS residues and solute.

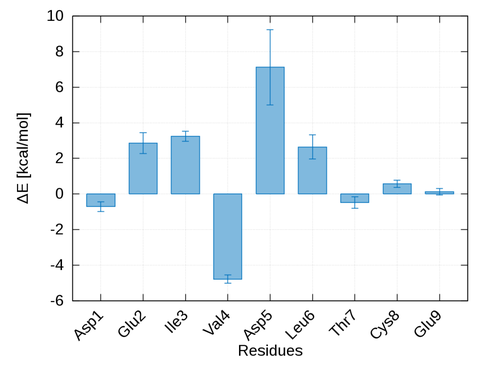

Figure 4: The potential energy differences for each residue of the SIM2 peptide between parallel and antiparallel orientation. We include non bonded interactions to SUMO, PIAS residues and solute.

- Energetically, one orientation is favorable for almost all residues. Specifically, also residues not part of the SIM core motif contribute strongly to the binding energy.

Standard binding free energy: Convergence

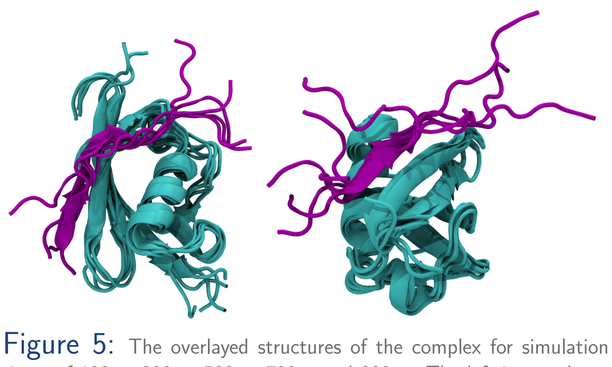

Figure 5: The overlayed structures of the complex for simulation times of 100ns, 300ns, 500ns, 700ns and 900ns. The left image shows the complex in parallel, the right image shows the complex in antiparallel orientation.

- Despite being bound to SUMO the SIM peptides are very flexible, as the example of the PIAS peptide shows in figure 5.
- Figure 6 shows the convergence of different contributions to the binding free energy.
- In case of the PIAS system 5 ns of simulation time require one day of simulation time on three nodes (= 216 cores) on average.

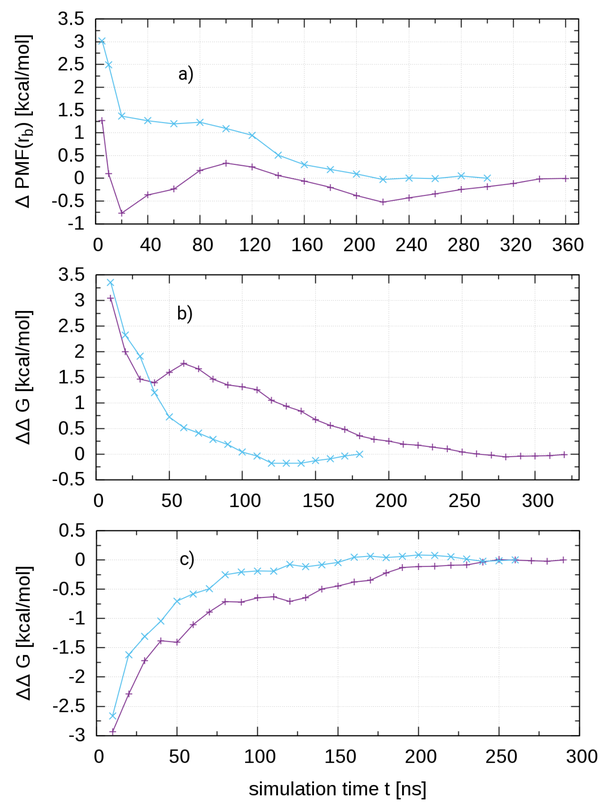

Figure 6: Part a) shows the difference between the potential of mean force at distance r_b and time t and the potential of mean force after the full simulation time $\Delta\mathrm{PMF}(r_b)$ in $kcal/mol$ for the parallel (purple) and antiparallel (blue) orientation. Part b) and c) show the difference $\Delta\Delta G$ between the contribution of restraining the RMSD in bound and free state after simulation time t and after full simulation time, respectively. Again, the parallel orientation is shown in purple and the antiparallel orientation is shown in blue.

Properties of the diSIM/diSUMO complex

- No consensus on structural properties of diSIM/diSUMO complexes, for example bound orientation of the SIMs.
- Sampling the configuration space of this complex is demanding since the complex is very flexible.
- Parallel simulation at different temperatures: 200 ns on 30 nodes ($\approx$ 2100 cores) per week.

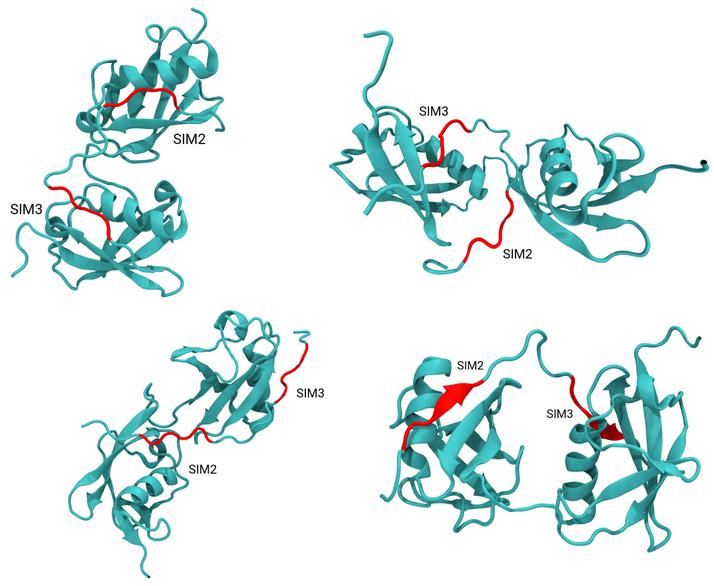

Figure 7: Different structures of the diSIM/diSUMO complex from a 1200 ns parallel tempering run. During the simulation the SIMs change the binding orientation frequently, this shows the high flexibility of the complex.