Inter-subunit coupling in PyrR pyrimidine synthase attenuator protein oligomers

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1. Introduction

Many proteins form oligomers under physiological conditions, which can confer thermal stability, greater complexity in structural and functional activity. Understanding the mechanisms of assembly provide insight into protein evolution, providing a framework for how protein structures adapt to gain new function. Previously, due to computational limits, intrinsic dynamics, or vibrational signatures, have typically been modelled implicitly by considering the conformation of a participating protein subunit in isolation. Generally, it is assumed the isolated subunit is in a conformation which captures the implicit effect of the binding partner on the intrinsic dynamics, suggesting the influence of a partnering subunit is already integrated. However, this description lacks detailed information on the influence of critical contacts at the protein-protein interface. Since the binding of many proteins to their protein partners is tightly regulated via control of their relative intrinsic dynamics, investigation of the intrinsic dynamics of proteins is necessary for the comprehensive understanding of function. In this study, we examine the case of a protein family, pyrimidine synthesis attenuator PyrR[1], to understand the effect of the binding partners in the stability of the tetramer vs. the dimer, and to uncover signals that link to their modulation via allostery. By partitioning the covariance matrices from elastic network models to obtain normal modes[2], we found that explicitly modelling the partnering subunits revealed the influence of perturbations that extend from the tetrameric interface, that is not captured by modelling the subunits in isolation. We want to confirm this effect with greater detail with molecular dynamics (MD) simulations.

2. Simulation preparation

Table 1 Systems prepare for MD simulations, 5GP - Guanosine-5'-Monophosphate

PDB ID.	Monomer	Dimer	Tetramer
4p82	No 5GP	No 5GP	N.A.
4p86	5GP	5GP	5GP
	without 5GP	without 5GP	without 5GP

Initial structures were obtained from the PDB for PyrR dimer (4p82) and PyrR tetramer (4p86 with 5GP). Missing residues were modeled using Modeller[3], and the subunits were extracted as monomer (4p82, 4p86) and dimer (4p86) with and without allosteric ligands, 5GP. Explicit solvent MD simulations were prepared using the Amber99sb-ildn-ions forcefield[4], in GROMACS[5] v2021 on the local computing cluster before performing the production run for 300 ns on SQUID GPUs, for four replicas per system.

3. Overall rigidity of 4p86 dimer without 5GP vs. 4p82 dimer with no 5GP

Preliminary analysis shows the persistence of lower root mean squared fluctuations in the dimer from the 4p86 tetramer X-ray crystal structure, despite the removal of the allosteric ligand, 5GP. In Figure 1, we

see that the regions with larger RMSF differences tend to be closer to the dimeric interface. When selecting residues that interact with 5GP in the initial PDB file (4p86) shows an asymmetry in the number of residues within 5Å between chains. More residues in chain B are in contact with the ligand, and this affects the apparent rigidity of chain B in the Dimer from 4p86, even when the ligand is removed.

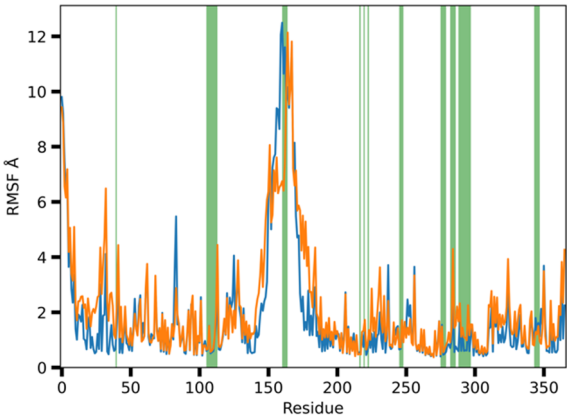

Figure 1: Root-mean-square fluctuations of Dimer from PDB 4p82 (with no 5GP) in orange vs. Dimer (with 5GP removed) from PDB 4p86 in blue. The residues that interact with 5GP in the initial structure are marked in green panels.

4. Future direction

As part of our investigation, we will be exploring the pairwise correlation within each protein system to understand the changes in dynamics conferred by allosteric ligand-binding and the influence of coupling from partnering protein subunits. We will differentiate the stabilizing effects of allosteric ligand-binding via a systematic comparison with the ligand-free structure, at different orders of oligomerization.

5. Conclusion

Our results show that the MD simulations were effective in capturing crucial changes to the flexibility of PyrR from different starting conformations. With our analysis of coupling using the MD simulations trajectories, we expect to uncover the interplay between allosteric ligand-binding and oligomerization on the stability of the PyrR protein.

References

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