Geometric modeling of coiled-coils using Isambard : the case of the RM6 variant of the Repressor of Primer protein.

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Abstract

Retro-proteins are molecules with reversed amino acid sequences compared to their parents exhibiting unknown foldability. Recent experiments on the retro-RM6 (ROP deletion variant, PDB ID: 1ROP) protein suggested that rRM6 is stable and with a similar fold to its parent. In this preliminary report, we examine the ability of geometric modeling using ISAMBARD to determine the structure of the RM6 protein (PDB ID: 1QX8) and show that we can obtain refined models. The models exhibited RMSD values (from the crystallographic structure) of only ~1.15Å for 196 residues of the bundle. These results suggested that geometric modeling via the ISAMBARD tool could be employed to generate potentially useful models for the retro-isomer of RM6.

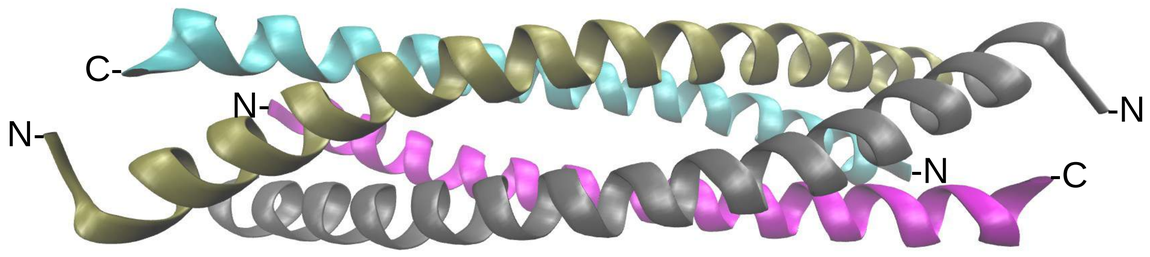

Figure 1: RM6 protein (PDB ID: 1QX8). RM6 is a stable and regular homotetrameric left-anti-parallel helix bundle with a five residue deletion (DADEQ) in the turn region which restores the heptad motif. Chains A, B, C, D are colored cyan, magenta, gray and tan respectively.

Introduction

Structural studies suggested that rRM6 exhibited high similarity to its parent on a secondary structure and oligomerization level, in addition to being slightly less compact compared to RM6 (Figure 1) [1]. Molecular replacement attempts on crystallographic data obtained from the rRM6 crystals [2] failed to allow a complete structure determination, implying potential differences between the retro-isomer and its parent. In order to obtain possibly useful models for molecular replacement calculations, geometric modeling could be employed that utilizes our prior knowledge on coiled coil geometry in order to *de novo* build protein backbones. This method does not require an available resolved structure or sequence homology which proves useful when modeling a retro-protein. On this note, the tools CCBuilder and ISAMBARD [3,4] offer the parameters and the metaheuristic approaches necessary to solve the optimization problem of coiled coil parameterization (Table 1).

Table 1: The Coiled Coil parameters, their mean values and ranges applied.

	Parameter	Mean value	Value range
Dynamic	Superhelical radius	7.0	2.0
	Superhelical pitch	200	150
	φCα	ideal_phica	27
	Z -shift	0.0	20
Static	Helix length	Based on the orientation	-
	Major handedness	Left or right	-
	Helix orientations	Parallel or antiparallel	-

*Delta = 360/7, ideal_phicas = [n * delta - (delta/2) for n in range(1, 8)]

Methods

- ISAMBARD requires Coiled Coil specifications, parameter ranges (Table 1) [4] and amino acid sequences.
- The metaheuristic methods employed were the CMAES, GA, DE and PSO.
- BUFF which is an empirical free-energy force field is integrated to ISAMBARD to evaluate the generated models [4].
- Sequence selection was tailored on helix orientations.
- For model evaluation, the RMSD values for all the generated models were calculated before and after refinement with the GalaxyRefineComplex web server [5].

Results

- The results indicated the presence of one and only one pronounced energy minimum for the d register which is only present when the (correct) antiparallel helical arrangement is used (Figure 2 A).
- Geometric modeling gave a clear solution corresponding to the correct d-antiparallel-left-handed among models with different superhelical twists (Figure 2 B).
- For the d-antiparallel-left-handed RMSD over 192 Cα atoms was only 1.8Å, which dropped to an impressive RMSD of only 1.15Å after energy minimization with Galaxy[5] (Figure 2 C).
- Geometric modeling is powerful enough to be able to find convincing solutions even when initiated from completely wrong initial parameters (data not shown).

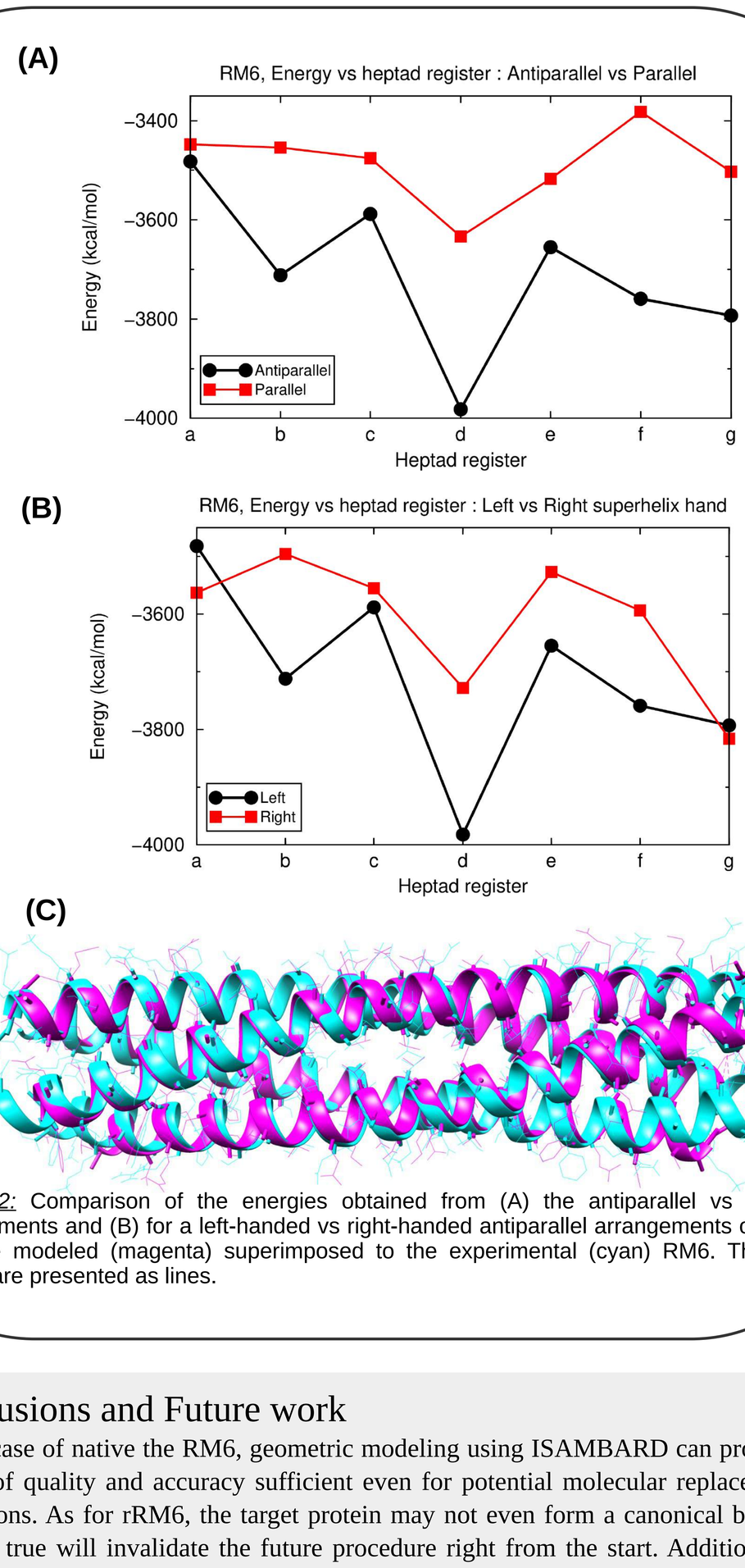

Figure 2: Comparison of the energies obtained from (A) the antiparallel vs parallel arrangements and (B) for a left-handed vs right-handed antiparallel arrangements of RM6. (C) The modeled (magenta) superimposed to the experimental (cyan) RM6. The side chains are presented as lines.

Conclusions and Future work

For the case of native the RM6, geometric modeling using ISAMBARD can produce models of quality and accuracy sufficient even for potential molecular replacement calculations. As for rRM6, the target protein may not even form a canonical bundle which if true will invalidate the future procedure right from the start. Additionally, geometric modeling is sufficiently powerful to be able to produce structures with reasonable packing, but with an offset register of the helices, which further complicates the identification of a putative correct solution. Given these limitations, one possible approach would be to produce and test a large number of rRM6 models for their ability to allow crystallographic structure determination to proceed to completion.

References

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