Computational design of dimeric *de novo* heme-binding helical bundle proteins

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INTRODUCTION

The comprehension of protein biochemistry, biophysics and folding is important in order to create new protein structures which could tackle important challenges in biotechnology. Indeed the amino acid sequence specifies the protein fold and the protein function. The design of protein sequences with altered or novel functions is challenging. In the last years, the computational methodology has advanced considerably, reaching atomic-level accuracy. With *de novo* protein design it is now possible to simulate protein sequences and predict which ones exhibit specific properties and desired functions.[1] Here I show my efforts towards the design of dimeric helix bundles, which bind b-types hemes via a bis-his coordination.

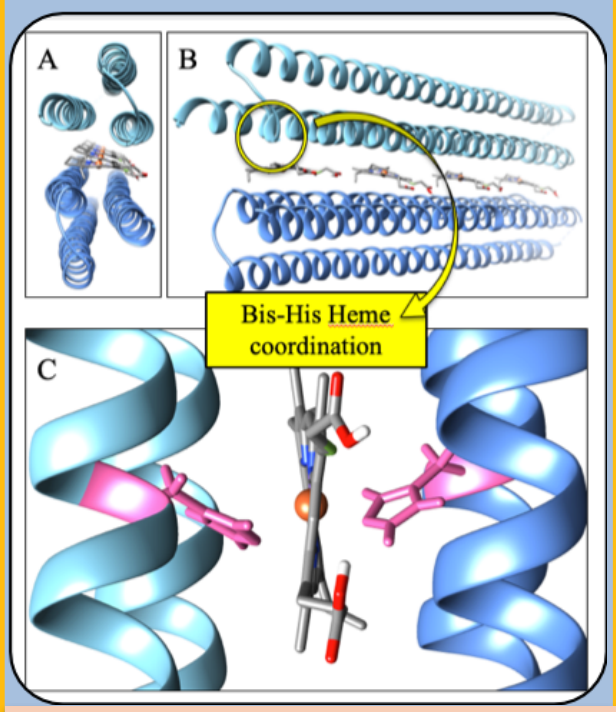
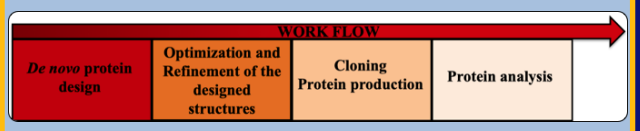


Figure 1. Overall fold of the three homodimeric protein structures H 3H5L_2 A, H 3H5L_2 B, H 3H5L_2 C. (A) *Side view of the homodimer.* ***(B)*** *Front view of the homodimer.* ***(C)*** *Heme rings bis-coordination with four different histidine residues located on each first helix of the respective bundles.*

My work is based on three different homodimeric α-helices bundles - H 3H5L_2 **A**, H 3H5L_2 **B**, H 3H5L_2 **C**. These structures have different amino acid sequences, but are all constituted by two antiparallel untwisted three-helix bundles of 80-residue helices and a 18-residue repeating unit (5 full circles, or 'layers', until they reach identity) [2]. Moreover, they exhibit four histidine residues which are placed to coordinate the heme cofactors (***Figure 1***).

MATERIALS AND METHODS



The work flow can be divided in two main parts. The first one – ***De novo protein design, Optimization and Refinement of the designed structures*** – encompasses the parametric design followed by sequence optimization and refinement of the designed structures. All design steps are carried out in RosettaScripts. For sequence design both, FastDesign and vanilla Pack Rotamers are used. The second part – ***Cloning and Protein production, Protein Analysis*** - concerns the experimental characterization of the designs, which usually consists of cloning, protein expression in E.coli and purification via affinity and size exclusion chromatography. All soluble and purified designs are checked for heme binding via UV/Vis spectroscopy.

RESULTS

The first round of designs showed all but one protein form various oligomeric states in solution (***Figure 2***). Because of that, a second round of design and optimization is currently performed, using a monomeric helix bundle as starting point. In order to simplify the design processes the α-helical bundles are truncated to get a shorter final length (***Figure 3***). After a process of structure optimization (***Figure 4***) one b-type heme ligand is kept for coordination with the histidine residue. After that, symmetry is applied to generate the homodimeric form (***Figure 5***).

Second Cycle: Cloning and Protein production, Protein Analysis

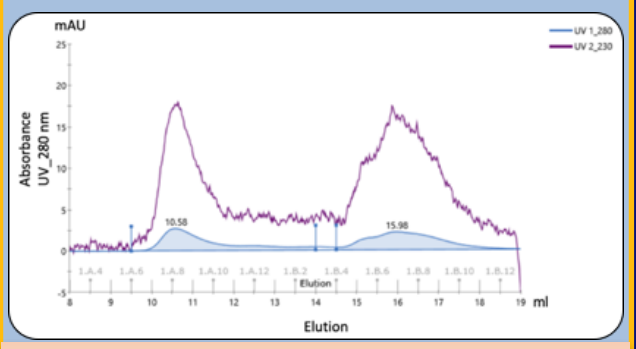


Figure 2. SEC chromatography on FPLC
SEC column used: Superdex 200 Increase 10/300 GL. The peak at elution volume 15.98 ml corresponds to a protein molecular weight range of 29.000 Da – 44.000 Da (homodimeric state).

First Cycle: De novo protein design, Optimization and Refinement of the designed structures

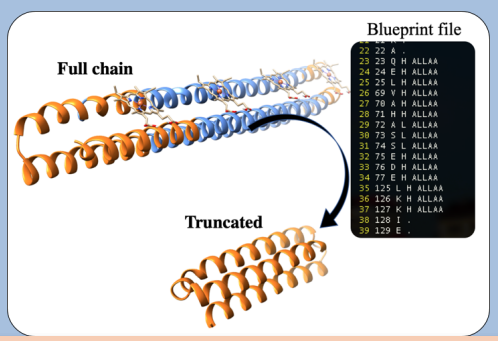


Figure 3. Truncation of the full length α-helices bundles
The initial α-helices monomeric bundle´s length is reduced applying a remodel: it considers the task informations contained on a blueprint file which describes what will be done to the input structure.

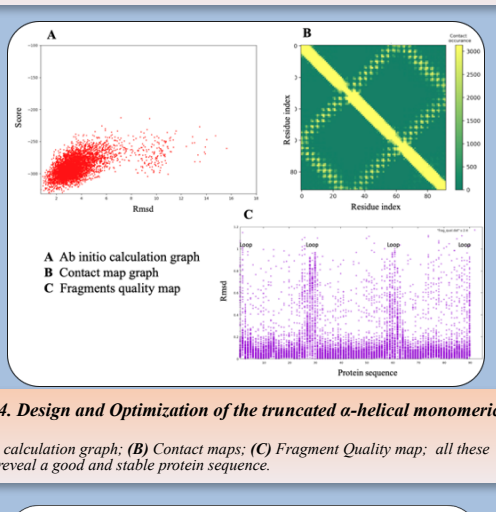


Figure 4. Design and Optimization of the truncated α-helical monomeric bundle
Ab initio calculation graph; (B) *Contact maps;* ***(C)*** *Fragment Quality map; all these analysis reveal a good and stable protein sequence.*

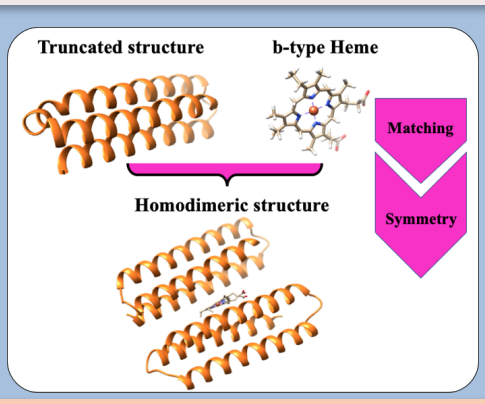


Figure 5. Heme insertion and symmetrization of the structure
Starting from the monomeric bundle a matching a symmetry and a final design steps must be performed in order to insert the heme ligand and to form again a homodimeric final structure.

FUTURE PERSPECTIVES

- Expression, purification and analysis of the designed and optimized homodimeric bundles structures;
- Design of new homodimeric 3H5L_2 protein structures;

REFERENCES

[1] P.-S. Huang, G. Oberdorfer, C. Xu, X. Y. Pei, B. L. Nannenga, J. M. Rogers, F. DiMaio, T. Gonen, B. Luisi, D. Baker, High thermodynamic stability of parametrically designed helical bundles, *Science* **346**, 481-484 (2014).
[2] https://www.rosettacommons.org

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