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

Veronica Delsoglio*, Gustav Oberdorfer
Graz University of Technology, Institute of Biochemistry, Austria

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 proteins 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-type hemes via a bis-his coordination.

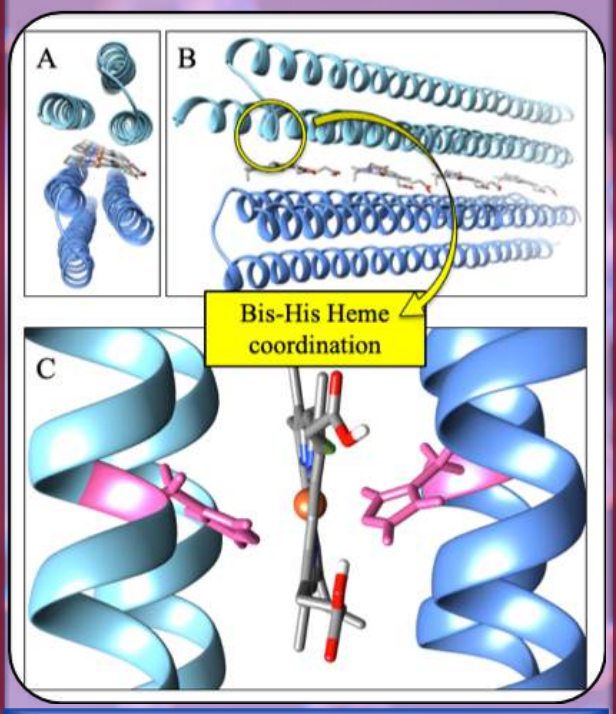
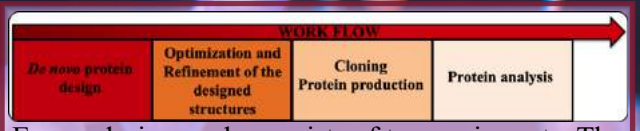


Figure 1. Overall fold of the three homodimeric protein structures H 3H5L_2 A, H 3H5L_2 B, H 3H5L_2 C. *(A) Side view of the homodimer.* ***(B)*** *Front view of the homodimer.* ***(C)*** *Heme moiety bis-coordination by two different histidine residues located on each first helix of the respective bundles.*

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 coordinated the heme cofactors (***Figure 1***).

MATERIALS AND METHODS



Every design cycle consists of 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 refimenent 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 one – ***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

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

A process of protein design and optimization (***Figure 3***) is simplified starting from a truncated alpha helical bundle structure (***Figure 2***). Thousands of redesigned protein sequences are generated using PackRotamers mover. Comparing the sequences in terms of residual Rosetta Energy Units (REU) per residue, allows the best residue at each sequence position to be selected.

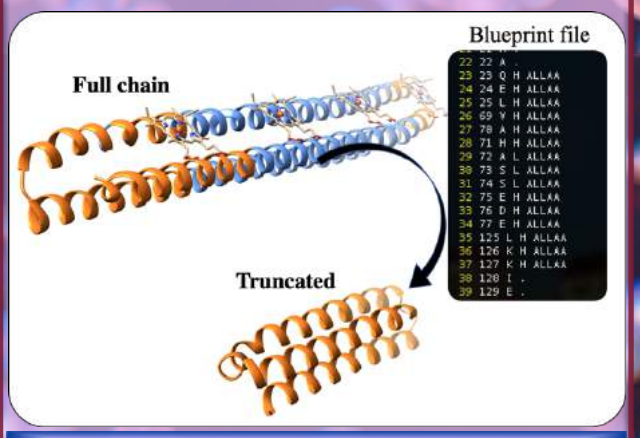


Figure 2. Truncation of the full length α-helices bundles
The initial monomeric α-helical bundle's length is reduced using a remodel: it considers the task informations contained in a blueprint file which describes what will be done to the input structure.

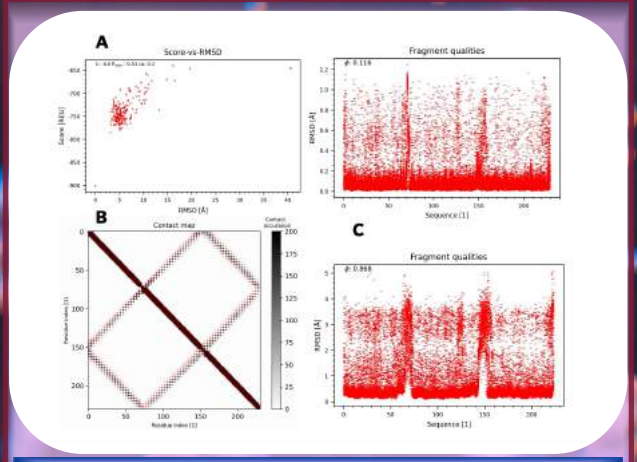


Figure 3. Design and Optimization of the truncated α-helical monomeric bundle
Ab initio calculation graph; ***(B)*** *Contact maps;* ***(C)*** *Fragment Quality map; all these analysis reveal a good and stable protein sequence.*

Second Cycle: Cloning and Protein production, Protein Analysis

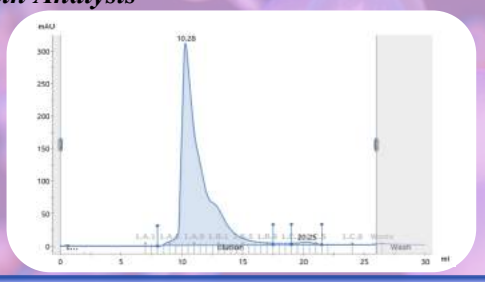


Figure 4. SEC chromatography on FPLC
SEC column used: Superdex 75 Increase 10/300 GL. The peak at elution volume 10.28 ml corresponds to a protein molecular weight range of ***13***.*700 Da – ***29***.*000 Da (homodimeric state).*

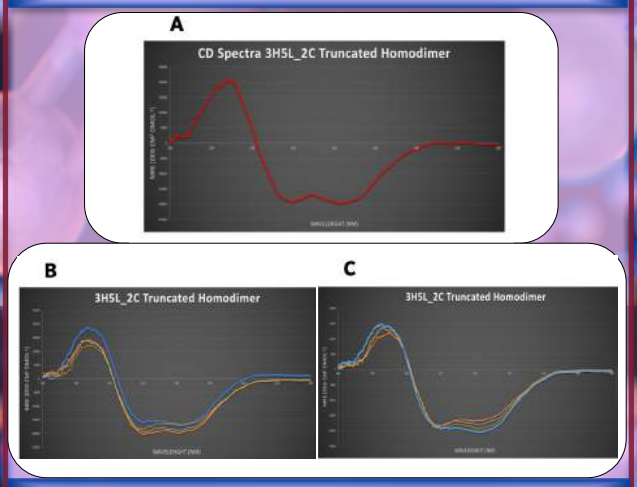


Figure 5. CD spectra and Protein Thermostability analysis
(A) *The CD spectra show two negative peaks (208 nm and 222 nm) and a positive one (around 190 nm) which are typical of alpha-helices secondary structures.* ***(B-C)*** *Both the graphs show the CD spectra profiles at specific temperatures in a set range (initial temperature in blue 25°C and final temperature in orange 95°C). Graph B: temprature increased from 25°C to 95°C. Graph C: temperature decreased from 95°C to 25°C.*

FUTURE PERSPECTIVES

- Design and optimization of the heme binding pocket region in order to concretely favour the probability of the heme binding process;

CONTACTS:
veronica.delsoglio@tugraz.at
Slack AG-PROTEIN Design (veronica)

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