A novel protocol for the design of artificial $(\beta/\alpha)_8$-barrel proteins

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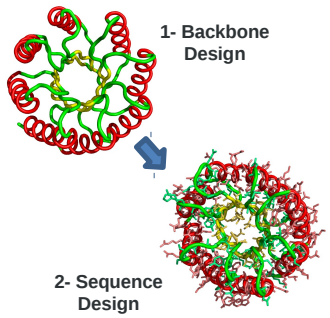
Abstract

Designing *de novo* proteins of more than 100 amino acids is a challenge, and many attempts were made in our group to create artificial $(\beta/\alpha)_8$-barrel proteins (Octarellins) [1–4]. Up to date, in literature there is just one successful example, thank to use of internal spatial symmetry [5]. Here we present a new protocol to design *de novo* $(\beta/\alpha)_8$-barrel proteins without symmetry restriction.

First (Top Figure), a backbone of 240 amino acids was created in 4 steps: (I) Rosetta ParametricDesign produced an highly symmetric polyalanine scaffold with no loops; (II) Rosetta Fixed-Backbone Design used the previous output to substitute the alanines in all the positions; (III) Loops were constructed with Modeller joining the terminus of the secondary structure elements and (IV) RosettaRelax performed relaxation, creating around 4000 different models. 28 backbone models were selected for the next steps of sequence design.

Second (Bottom Figure), 10 cycles of Rosetta Design and Relax were performed, with amino acid restriction based on their position in the 3D structure and the definition of three layers: core, boundaries and surface.

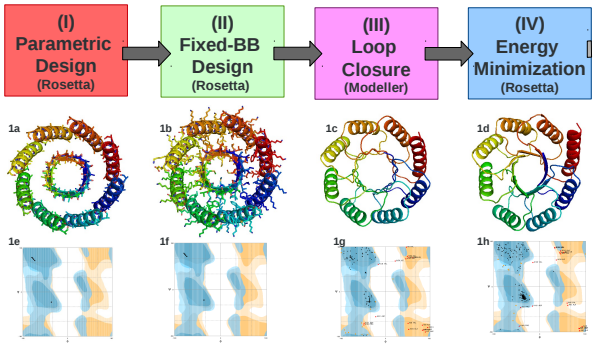
More than 10000 different sequences were created and analyzed in term of amino acid composition, sequence similarity with natural protein, secondary structure prediction, and molecular dynamics simulations. The 30 best candidate sequences have been selected for experimental verification.



Results

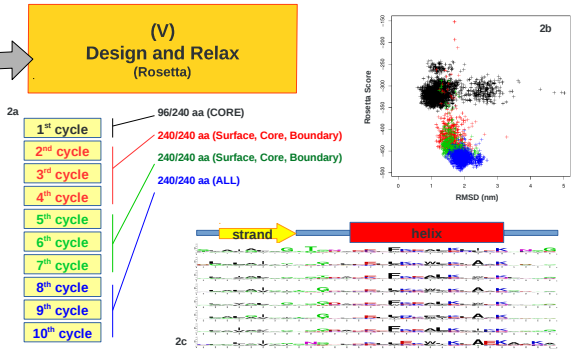
1- Backbone Design (~9500 target backbones)

A *de novo* backbone for the $(\beta/\alpha)_8$-barrel fold is built in 4 steps. (I) A polyalanine scaffold of 240 aa is created (**Fig.1a**); (II) the alanines are then substituted according to their position in the protein: cores, boundaries, loops or surface (**Fig.1b**); (III) loops are created connecting the termini of two neighboring secondary structure elements (**Fig.1c**) and (IV) the chain is relaxed (**Fig.1d**). The Ramachandran plots (**Fig.1e-h**) report the progress of the design starting from an highly geometric scaffold to a more natural-like polypeptide.



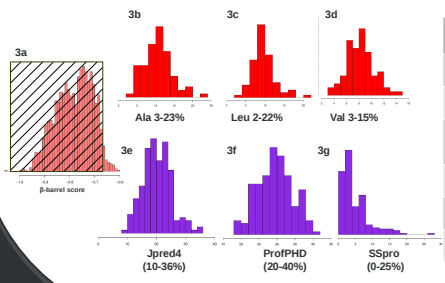
2- Sequence Design (~10300 models)

54 backbones out of ~9500 were chosen and subjected to 10 cycles of sequence design and relax with different restrictions in the allowed amino acid substitution (**Fig.2a**). The RMSD against the target backbone vs the Rosetta energy, is reported (**Fig.2b**), colored according to the cycle step. The sequence logo of ~1700 models is reported (**Fig.2c**); each line represent a 30 aa unit composed by strand-loop-helix-loop.



3- *In silico* Validation (~750 models)

The 56 groups of target backbones containing >10k sequences were tested to select the best models for protein production, and natural $(\beta/\alpha)_8$-barrels were used as comparison against the models. First was analyzed the redundancy between models and the sequence alignment against natural protein (ID < 40%). H-Bond content in the β-barrel was analyzed (**Fig.3a**). Amino acids content was analyzed (**Fig.3b-d**). Secondary structure prediction was tested with Jpred4, ProfPHD and SSpro (**Fig.3e-g**). A resume of all the steps is reported (**Fig.3h**)

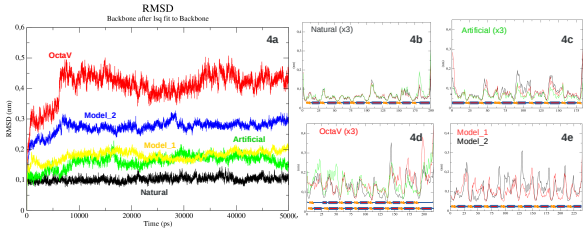


3h

	Models
Total Created	10300
Considered	6955
H-Bond Content	4527
AA Content	1370
SS Prediction	1066
Final Models	764

4- Molecular Dynamics (in progress)

Molecular Dynamic simulations of 50 ns were performed on at least one model for each of the remaining 44 groups. Control simulations were performed in triplicate with 2 natural and one artificial $(\beta/\alpha)_8$-barrels (positive controls) and with the OctarellinV, known to be an αβα-sandwich (negative control). The RMSD vs simulation time is reported for 2 models and 3 controls (**Fig.4a**). The rms fluctuation per residue is reported for the 3 controls (**Fig.4b-d**) and for the 2 models (**Fig.4e**). The secondary structure content is reported on the bottom of each graph; for the OctarellinV is reported for both the model (bottom) and the structure (top).



Conclusions

Here we report a new protocol for the design of artificial $(\beta/\alpha)_8$-barrel proteins, that differs from previous attempts in both construction of the backbone targets (Step 1) and post-design validation performed *in silico* (Steps 3 and 4). Molecular Dynamics seems to be a powerful tool in discriminating between models. Moreover, this protocol, which can be easily modified and adapted to the design of different protein folds/targets, appears to be flexible and reliable.

1. Goraj, K. et al. Synthesis, purification and initial structural characterization of octarellin, a de novo polypeptide modelled on the α/β-barrel proteins. Protein Eng. 1990, 3, 259–266.
2. Houbrechts, A. et al. Second-generation octarellins: two new de novo (β/α)8 polypeptides designed for investigating the influence of β-residue packing on the α/β-barrel structure stability. Protein Eng. 1995, 8, 249–259.
3. Offredi, F. et al. De novo backbone and sequence design of an idealized α/β-barrel protein: evidence of stable tertiary structure. J. Mol. Biol. 2003, 325, 163–174.
4. Figueroa, M. et al. Octarellin VI: Using Rosetta to Design a Putative Artificial (β/α)8 Protein. PLoS ONE 2013, 8, e71858.
5. Huang, P.-S. et al. De novo design of a four-fold symmetric TIM-barrel protein with atomic-level accuracy. Nat. Chem. Biol. 2015, advance online publication.

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