Parametric design of alpha-helical barrels and pore-like assemblies with very high thermodynamic stabilities

Gustav Oberdorfer

University of Washington, Seattle, USA

Gustav Oberdorfer(1,2,7), Po-Ssu Huang(1,7), Chunfu Xu(1,7), Verena Kohler(2), Xue Y. Pei(3), Brent L. Nannenga(4), Joseph M. Rogers(5), Frank DiMaio(1), Tamir Gonen(4), Ben Luisi(3), Karl Gruber(2) and David Baker(1,6,7)

Affiliations:

1. Department of Biochemistry, University of Washington, Seattle, WA, 98195, USA
2. Institute of Molecular Biosciences, University of Graz, Humboldtstrasse 50/3, 8010-Graz, Austria
3. Department of Biochemistry, University of Cambridge, CB2 1GA, United Kingdom
4. Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147, USA
5. Department of Chemistry, University of Cambridge, CB2 1EW, United Kingdom
6. Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, USA
7. Institute for Protein Design, University of Washington, Seattle, WA 98195, USA

Computational design of novel protein structures and enzymes is a promising tool to create superior biological materials with tailor-made properties, new pharmaceuticals, complex fine chemicals or renewable fuels. Here we present a procedure for designing proteins with backbones produced by varying the parameters in the Crick coiled-coil generating equations [1]. Combinatorial design calculations using the software suite Rosetta identify low energy sequences for alternative helix supercoil arrangements. After that, loop modeling is applied to connect the designs with lowest energy. The extent to which the designed sequences encode the designed structures is evaluated using large-scale structure prediction calculations, as well as symmetric and asymmetric protein-protein docking calculations. Subsequently, synthetic genes are generated for sequences that converge strongly on the designed structure for experimental characterization. We applied this approach to monomeric three- and four-helical bundle structures as well as a pentameric five-helix bundle structure using idealized coiled-coil geometries [2]. Recently we expanded this approach to higher complexity backbones, which resulted in the de-novo design of monomeric, antiparallel six-helix bundles with untwisted, left- and right-handed geometries. Initial biophysical characterization of these designs suggests that they fold into the designed structures. In addition, we used Rosetta protein-protein interface design functionality to computationally design self-assembling pore-like structures for their potential use as channels or transporters. We are currently undertaking further structural investigation of all these designs by X-ray crystallography. Ultimately, the designs described above can act as templates for protein or small molecule binding, holding a catalytic machinery or for scaffolding enzymes in reaction cascades. Some of these applications are currently under investigation, including binding of heme-moieties as a prosthetic group and tailoring the pore-like geometries to be used in nanopore sequencing.

[1] F. H. Crick. (1953) The Fourier Transform of a Coiled Coil, Acta Cryst., 6: 685

[2] *Huang, P-S., *Oberdorfer, G., *Xu, C., et al. (2014) High thermodynamic stability of parametrically designed helical bundles. Science, 24 October 2014: 481-485 *equal contribution

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Contact: Dietz Hendrik, dietz@tum.de, phone: 089-289-11615