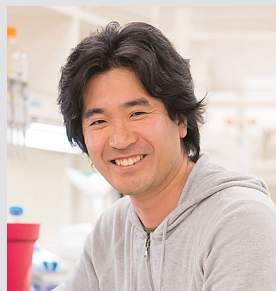


Protein Design Using Computational and Experimental Approaches

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Education

2001 B.S. Kobe University
2006 Ph.D. Kobe University

Professional Employment

2003 JSPS Research Fellow
2006 Postdoctoral Fellow, Kobe University
2007 Postdoctoral Fellow, Kyoto University
2007 JSPS Postdoctoral Fellow for Research Abroad
2009 Postdoctoral Fellow, University of Washington
2014 Associate Professor, Institute for Molecular Science
Associate Professor, The Graduate University for Advanced Studies
2014 JST-PRESTO Researcher (additional post) (–2017)
2018 Associate Professor, Exploratory Research Center on Life and Living Systems (ExCELLS)

Awards

2013 Young Scientist Award, The 13th Annual Meeting of the Protein Science Society of Japan
2013 Young Scientist Award, The 51st Annual Meeting of the Biophysical Society of Japan
2018 Morino Foundation for Molecular Science

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MITSUMOTO, Masaya
KAIDA, Shingo
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Keywords

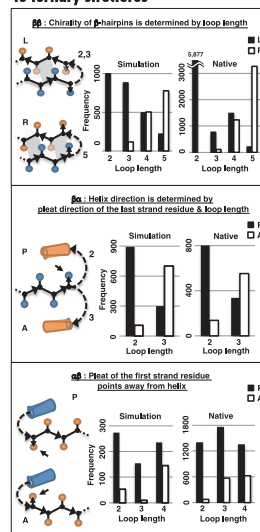
Protein Design for Structure and Function, Protein Folding, Structural Biology

Protein molecules spontaneously fold into unique three-dimensional structures specified by their amino acid sequences from random coils to carry out their functions. Many of protein studies have been performed by analyzing naturally occurring proteins. However, it is difficult to reach fundamental working principles of protein molecules only by analyzing naturally occurring proteins, since they evolved in their particular environments spending billions of years. In our lab, we explore the principles by computationally designing protein molecules completely from scratch and experimentally assessing how they behave.

Protein design holds promise for applications ranging from catalysis to therapeutics. There has been considerable recent progress in computationally designing new proteins. Many of protein design studies have been conducted using naturally occurring protein structures as design scaffolds. However, since naturally occurring proteins have evolutionally optimized their structures for their functions, implementing new functions into the structures of naturally occurring proteins is difficult for most of cases. Rational methods for building any arbitrary protein structures completely from scratch provide us opportunities for creating new functional proteins. In our lab, we tackle to establish theories and tech-

nologies for designing any arbitrary protein structures precisely from scratch. The established methods will open up an avenue of rational design for novel functional proteins that will contribute to industry and therapeutics.

Rules relating local backbone structures to tertiary structures



Selected Publications

- N. Koga, R. Tatsumi-Koga, G. Liu, R. Xiao, T. B. Acton, G. T. Montelione and D. Baker, "Principles for Designing Ideal Protein Structures," *Nature* **491**, 222–227 (2012).
- Y.-R. Lin, N. Koga*, R. Tatsumi-Koga, G. Liu, A. F. Clouser, G. T.

Montelione and D. Baker*, "Control over Overall Shape and Size in De Novo Designed Proteins," *Proc. Natl. Acad. Sci. U. S. A.* **112**, E5478–E5485 (2015).

1. Robust Folding of a De Novo Designed Ideal Protein Even with Most of the Core Mutated to Valine

De novo designed ideal proteins, which are stabilized completely consistent local and non-local interactions, exhibit a remarkable property of extremely high thermal stability, compared with naturally occurring proteins. Whereas non-local interactions such as tight hydrophobic core packing have been traditionally considered to be crucial for protein folding and stability, the rules suggest the importance of local backbone structures in protein folding. We studied the robustness of folding of de novo designed proteins to the reduction of the hydrophobic core, by extensive mutation of large hydrophobic residues (Leu, Ile) to smaller ones (Val) for one of the designs. Surprisingly, even after 10-residue mutations from all of Leu and Ile to Val, a mutant with most of the core filled with Val was found to not be a molten globule and fold into the same backbone structure as the original design, with high stability. These results highlight the significance of local backbone structures for the folding ability and high thermal stability of designed proteins.

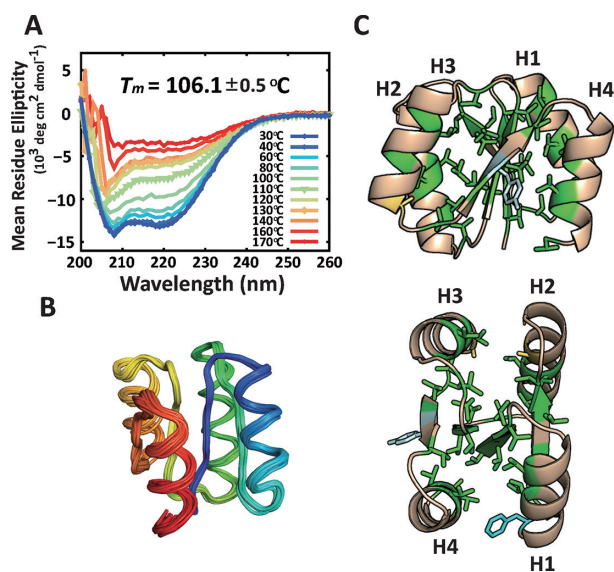


Figure 1. Experimental characterization of the designed protein with most of the core mutated to Val. (A) The far-UV CD spectra at various temperatures. (B) NMR structure. (C) Hydrophobic core side chains are shown in stick. Residues colored in green are valine.

2. Role of Backbone Strain in De Novo Design of Complex α/β Protein Structures

We have elucidated principles for designing ideal proteins with completely consistent local and non-local interactions which have enabled the design of a wide range of new $\alpha\beta$ -proteins with four or fewer β -strands. The principles relate local backbone structures to supersecondary-structure packing arrangements of α -helices and β -strands. Here, we test the generality of the principles by employing them to design larger proteins with five- and six-stranded β -sheets flanked by α -helices. The designs are monomeric in solution with high thermal stability, and the nuclear magnetic resonance (NMR) structure of one was close to the design model, but for two others the order of strands in the β -sheet was swapped. Investigation into the origins of this strand swapping suggests that the global structures of the design models are more strained than the NMR structures. We incorporated explicit consideration of global backbone strain into our design methodology, and succeeded in designing proteins with the original unswapped strand arrangements. These results illustrate the value of experimental structure determination in guiding improvement of de novo design, and the importance of consistency between local, supersecondary, and global tertiary interactions in determining protein topology. The augmented set of principles should inform the design of larger functional proteins.

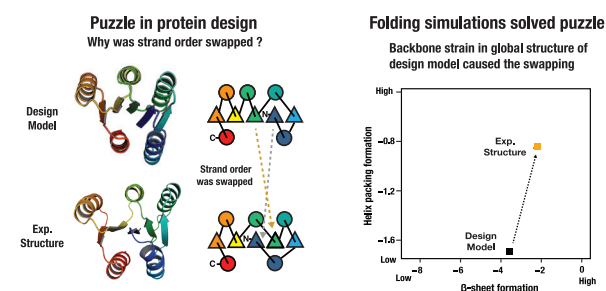


Figure 2. (left) The strand order swapping in de novo design of larger $\alpha\beta$ -proteins has been a long-standing problem for the research team. (right) Backbone ensembles generated from folding simulations identified that backbone strain caused the strand swapping.

References

- 1) R. Koga*, M. Yamamoto, T. Kosugi, N. Kobayashi, T. Sugiki, T. Fujiwara and N. Koga*, *Proc. Natl. Acad. Sci. U. S. A.* **117**, 31149–31156 (2020).
- 2) N. Koga*, R. Koga, G. Liu, J. Castellanos, G. T. Montelione and D. Baker*, *Nat. Commun.* **12**, 3921 (12 pages) (2021).

Awards

KOSUGI, Takahiro; The Young Scientist Excellence Award of Protein Science Society of Japan (PSSJ) (2021).
 MITSUMOTO, Masaya; The Poster Excellence Award of the 1st Molecular Engine Workshop (2021).
 MITSUMOTO, Masaya; The Poster Award of Protein Science Society of Japan (PSSJ) (2021).
 KAIDA, Shingo; The Poster Award of Protein Science Society of Japan (PSSJ) (2021).