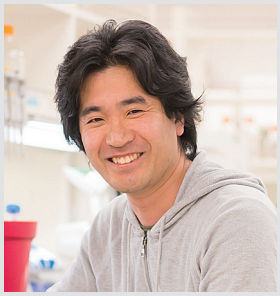
Protein Design Using Computational and Experimental Approaches

Research Center of Integrative Molecular Systems
Division of Trans-Hierarchical Molecular Systems



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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 Universtiy
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
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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 proteins with new functions. 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 technologies 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.

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).
- P. Nordenfelt, T. Moore, S. Mehta, J. Kalappurakkal, V. S. Swaminathan, N. Koga, T. Lambert, D. Baker, J. Waters, R. Oldenbourg, T. Tani, S. Mayor, C. M. Waterman and T. Springer, "Direction of Actin Flow Dictates Integrin LFA-1 Orientation during Leukocyte Migration," *Nat. Commun.* **8**, 2047 (2017).
- Y. Lin, N. Koga, S. M. Vorobiev and D. Baker, "Cyclic Oligomer Design with De Novo αβ-Proteins," *Protein Sci.* **26**, 2187–2194 (2017).
- S. Basak, R. P. Nobrega, D. Tavella, L. M. Deveau, N. Koga, R. Koga, D. Baker, F. Massi and C. R. Matthews, "Networks of Electrostatic and Hydrophobic Interactions Modulate the Complex Folding Free Energy Surface of a Designed βα Protein," *Proc. Natl. Acad. Sci. U. S. A.* **116**, 6806–6811 (2019).

1. Principles for Designing Ideal Protein Structures

We uncovered the principles for protein folding by designing "ideal" protein structures, which are stabilized completely consistent local and non-local interactions. We discovered a set of rules relating local backbone structures (secondary structure patterns) to tertiary motifs (Figure 1), which were identified using a combination of folding simulations and analyses of naturally occurring proteins. Building backbone structures according to the rules, and placing side chains stabilizing the backbone structures, we can readily design the proteins which have funnel-shaped folding energy landscapes leading into the target folded state. Using this approach, we have succeeded in designing ideal protein structures for five different topologies. These results suggest that the local backbone structures determine the tertiary folded structures rather than the details of amino acid sequences.

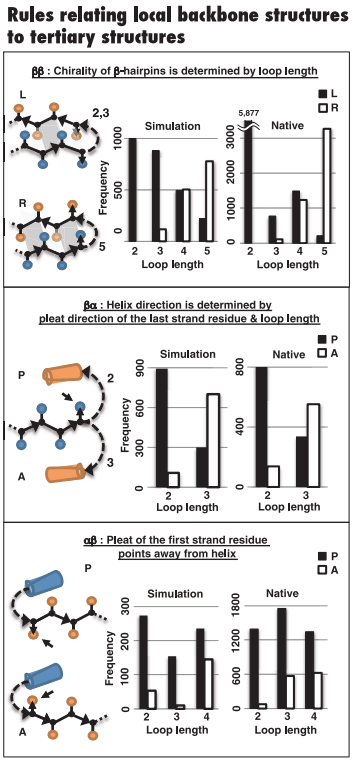

Figure 1. Rules relating local backbone structures to tertiary motifs.

2. Control over Overall Shape and Size in De Novo Designed Proteins

To achieve fine control over protein shape and size within a particular topology, we have extended the design rules by systematically analyzing the codependences between the lengths and packing geometry of successive secondary structure elements and the backbone torsion angles of the loop linking them. We demonstrated that the control is afforded by the resulting extended rule set by designing a series of proteins within the same fold but considerable variation in secondary structure length, loop geometry, β-strand registry, and overall shape. These extended design principles would provide the foundation for custom design of protein structures performing desired functions.

3. Robust Folding of De Novo Designed Ideal Protein Even with Most of the Core Filled with 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.

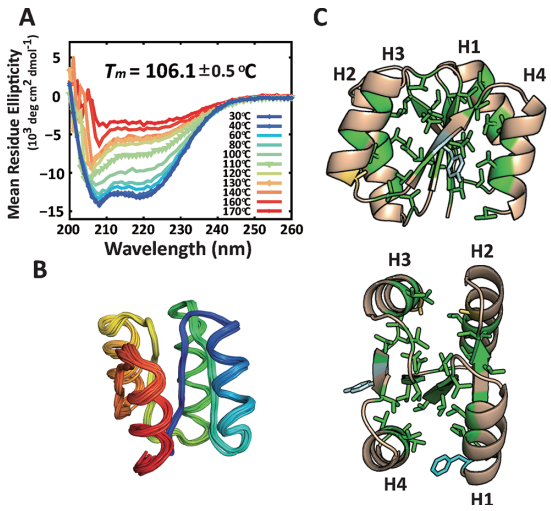

Figure 2. Experimental characterizations of the design with most of the core filled with Val.

References

1) R. Koga* and N. Koga*, *Biophys. Physicobiol.* **16**, 304–309 (2019).
2) 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).