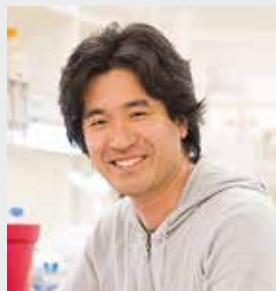


# Protein Design Using Computational and Experimental Approaches

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### Education

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2006 Ph.D. Kobe University

### Professional Employment

2003 JSPS Research Fellow  
2006 Postdoctoral Fellow, Kobe University  
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2007 JSPS Postdoctoral Fellow for Research Abroad  
2009 Postdoctoral Fellow, University of Washington  
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2014 JST-PRESTO Researcher (additional post) (–2017)

### Awards

2013 Young Scientist Award, The 13<sup>th</sup> Annual Meeting of the Protein Science Society of Japan  
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### Keywords

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

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).
- J. Fang, A. Mehlich, N. Koga, J. Huang, R. Koga, M. Rief, J. Kast, D. Baker and H. Li, "Forced Protein Unfolding Leads to Highly Elastic and Tough Protein Hydrogels," *Nat. Commun.* **4**:2974 (2013).

## 1. Principles for Designing Ideal Protein Structures

Understanding the principles for protein folding is complicated by energetically unfavorable non-ideal features—for example kinked  $\alpha$ -helices, bulged  $\beta$ -strands, strained loops and buried polar groups—that arise in proteins from evolutionary selection for biological function or from neutral drift. Here, we uncovered the principles for protein folding by designing “ideal” protein structures, which are stabilized by 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 left), which were identified using a combination of folding simulations and analyses of naturally occurring proteins. Building backbone structures according to the rules (Figure 1 top right) and placing side chains stabilizing the backbone structures, we can readily design the proteins that have funnel-shaped folding energy landscapes leading into the target folded state.

Using this approach, we designed sequences predicted to fold into ideal protein structures consisting of  $\alpha$ -helices,  $\beta$ -strands and minimal loops, using the Rosetta program. Designs for five different topologies were found to be monomeric and very stable and to adopt structures in solution nearly identical to the computational models (Figure 1 bottom right). These results suggest that the local backbone structures determine the tertiary folded structures rather than the details of amino acid sequences.

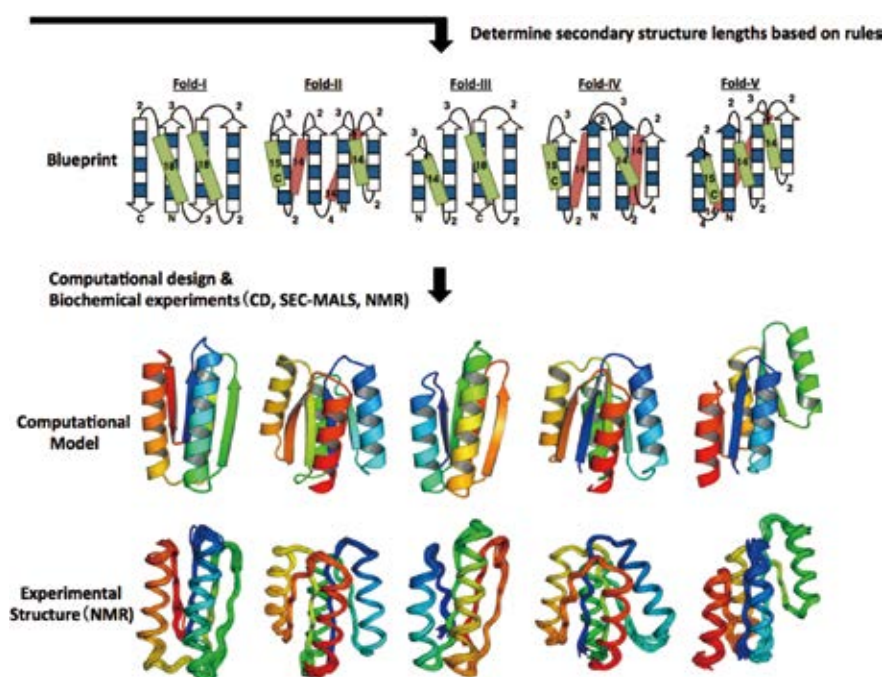
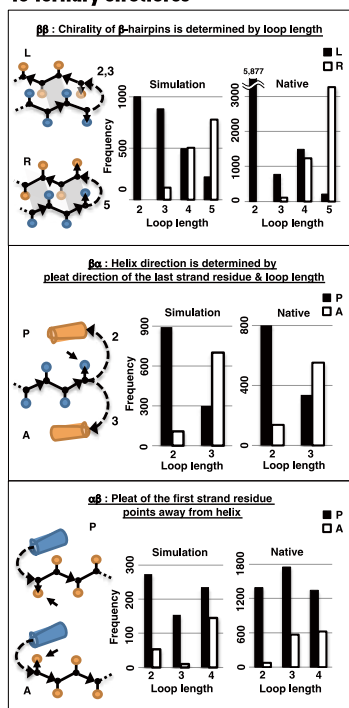
## 2. Control over Overall Shape and Size in De Novo Designed Proteins

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 demonstrate the control afforded by the resulting extended rule set by designing a series of protein with the same fold but considerable variation in secondary structure length, loop geometry,  $\beta$ -strand registry, and overall shape. Solution NMR structures of four designed proteins for two different folds show that protein shape and size can be precisely controlled within a given fold. These extended design principles provide the foundation for custom design of protein structures performing desired functions.

### References

- 1) S. J. Fleishman, S. D. Khare, N. Koga and D. Baker\*, *Protein Sci.* **20**, 753–757 (2011).
- 2) H. Kenzaki, N. Koga, N. Hori, R. Kanada, W. Li, K. Okazaki, X.-Q. Yao and S. Takada\*, *J. Chem. Theory Comput.* **7**, 1979–1989 (2011).
- 3) N. Koga, R. Tatsumi-Koga, G. Liu, R. Xiao, T. B. Acton, G. T. Montelione and D. Baker, *Nature* **491**, 222–227 (2012).
- 4) J. Fang, A. Mehlich, N. Koga, J. Huang, R. Koga, M. Rief, J. Kast, D. Baker and H. Li\*, *Nat. Commun.* **4**:2974 (2013).
- 5) Y.-R. Lin, N. Koga\*, R. Tatsumi-Koga, G. Liu, A. F. Clouser, G. T. Montelione and D. Baker\*, *Proc. Natl. Acad. Sci. U. S. A.* **112**, E5478–E5485 (2015).

### Rules relating local backbone structures to tertiary structures



**Figure 1.** Left: Rules relating local backbone structures to tertiary motifs. Right: De novo designed protein structures.