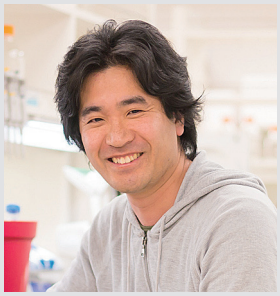
Protein Design Using Computational and Experimental Approaches

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)
2022 Professor, Osaka University

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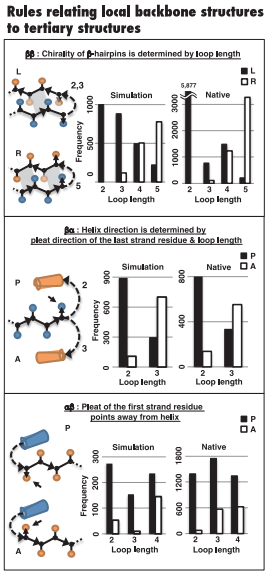
Keywords Protein Design for Structure and Function, Protein Folding, Structural Biology

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 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

- R. Koga*, M. Yamamoto, T. Kosugi, N. Kobayashi, T. Sugiki, T. Fujiwara and N. Koga*, "Robust Folding of a De Novo Designed Ideal Protein Even with Most of the Core Mutated to Valine," *Proc. Natl. Acad. Sci. U. S. A.* **117(49)**, 31149–31156 (2020).
- N. Koga*, R. Koga, G. Liu, J. Castellanos, G. T. Montelione and D. Baker*, "Role of Backbone Strain in De Novo Design of Complex α/β Protein Structures," *Nat. Commun.* **12**, 3921 (12 pages) (2021).

1. State-Targeting Stabilization of Adenosine A_{2A} Receptor by Fusing a Custom-Made De Novo Designed α-Helical Protein

G-protein coupled receptors (GPCRs) are known for their low stability and large conformational changes upon transitions between multiple states. A widely used method for stabilizing these receptors is to make chimeric receptors by fusing soluble proteins (*i.e.*, fusion partner proteins) into the intracellular loop 3 (ICL3) connecting the transmembrane helices 5 and 6 (TM5 and TM6). However, this fusion approach requires experimental trial and error to identify appropriate soluble proteins, residue positions, and linker lengths for making the fusion. Moreover, this approach has not provided state-targeting stabilization of GPCRs.

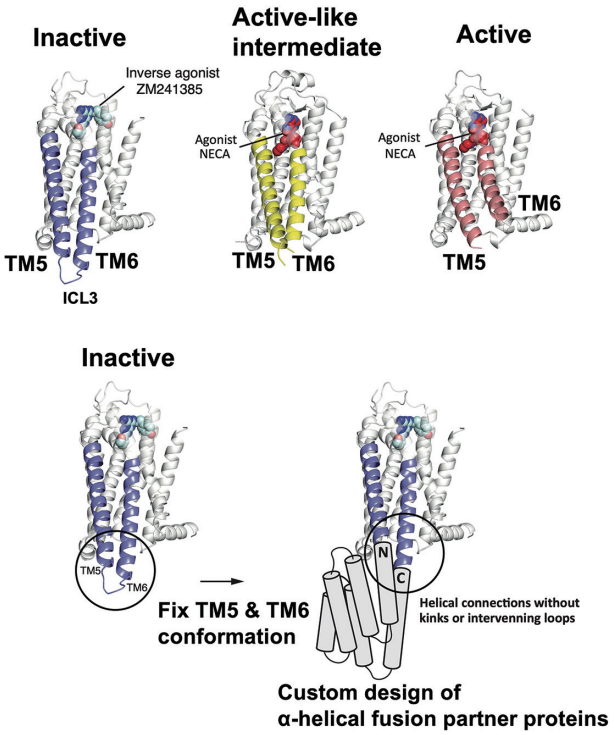


Figure 1. Strategy for state-targeting stabilization of GPCR, using de novo designed fusion partner proteins.

We designed fusion partner proteins customized for stabilizing one of the class A GPCRs, adenosine A_{2A} receptor (A_{2A}R), in an inactive state. Class A GPCRs are the largest subfamily of GPCRs, and the receptors in the class have been suggested to undergo large conformational changes in TM6 associated with TM5 upon the state transitions. We assumed that the TM5 and TM6 conformation could be fixed in a specific state through straight helical connections between a fusion partner protein and A_{2A}R. Therefore, we sought to design α-helical protein structures de novo, of which the N- and C-terminal helices are, respectively, connected to TM5 and TM6 of an inactive state A_{2A}R structure without any kinks or intervening loops.

The chimeric A_{2A}R fused with one of the designs (FiX1) exhibited increased thermal stability. We studied the stability of A_{2A}R–FiX1 by measuring the apparent melting temperatures in the clear-native polyacrylamide gel electrophoresis (CN–PAGE) method. The melting temperature was found to be significantly increased. For comparison, the melting temperature for A_{2A}R–BRIL (BRIL is one of the major fusion partner proteins) was also measured; the value was comparable to those of A_{2A}R–FiX1. Moreover, compared with the wild type, the binding affinity of the chimera against the agonist NECA was significantly decreased, whereas that against the inverse agonist ZM241385 was similar, indicating that the inactive state was selectively stabilized. Our strategy contributes to the rational state-targeting stabilization of GPCRs.

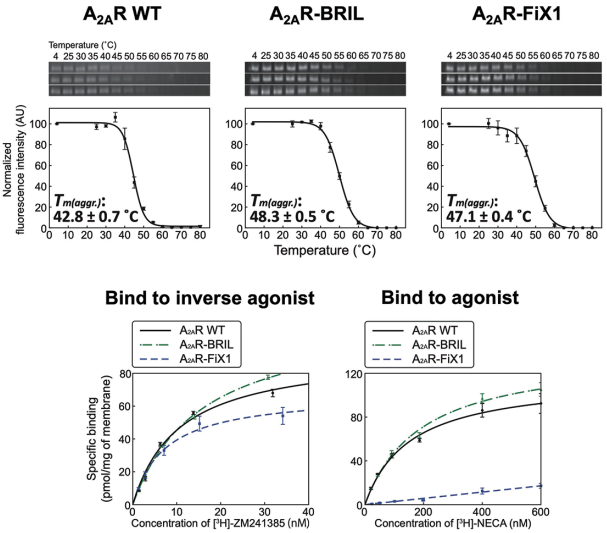


Figure 2. Experimental characteristics of A_{2A}R fused with or without fusion partner proteins.

Reference

1) M. Mitsumoto, K. Sugaya, K. Kazama, R. Nakano, T. Kosugi, T. Murata and N. Koga*, *Int. J. Mol. Sci.* **22**, 12906, 13 pages (2022).

Awards

MITSUMOTO, Masaya; The Student Presentation Award of Biophysical Society of Japan (BSJ) (2021).
KAIDA, Shingo; The Student Presentation Award of Biophysical Society of Japan (BSJ) (2021).
MITSUMOTO, Masaya; The SOKENDAI Award from Dean of School of Physical Sciences (2022).
KAIDA, Shingo; The Chemical Society of Japan Tokai Branch Award (2022).