UNIVERSITÀ DEGLI STUDI FIRENZE

DICUS DIPARTIMENTO DI CHIMICA "UGO SCHIFF" ECCELLENZA 2023-2027

Da un secolo, oltre.

UNIVERSITÀ DEGLI STUDI FIRENZE

PhD Chemical Sciences

Prof IKUO FUJII

Osaka Metropolitan University, Japan

06.03.2024

9:00

Aula Speroni

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Link per partecipare online: https://meet.google.com/ray-daws-nfy

Terrà una conferenza dal titolo

Post-Antibody Drugs: Generation of a novel class of drug modalities based on molecular-targeting helix-loop-helix (HLH) peptides

per il Dottorato di ricerca in Scienze Chimiche

la S. V. è invitata a partecipare

Prof.ssa Anna Maria Papini
Coordinatore del Dottorato

Prof.ssa Anna Maria Papini
Organizzatore

Ikuo Fujii is Specially Appointed Professor at the Department of Biological Science, Graduate School of Science of the Osaka Metropolitan University.

Research Interests

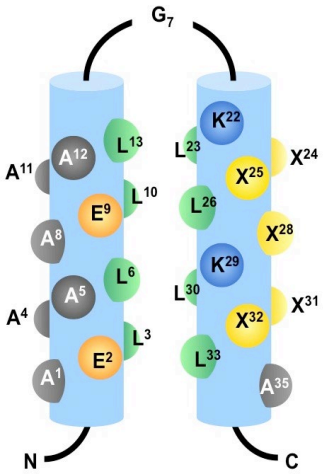
Directed Evolution of Biofunctional Molecules: The aim of his study is to investigate molecular design relying on evolutionary processes, called as “directed evolution”, to generate a novel class of biofunctional molecules.

Post-Antibody Drugs: Generation of a novel class of drug modalities based on molecular-targeting helix-loop-helix (HLH) peptides

Antibodies are indisputably the most successful reagents in molecular-targeting therapy. However, the use of antibodies has been limited due to the biophysical properties and the cost to manufacture. To enable new applications where antibodies show some limitations, we have developed an alternative-binding molecule with non-immunoglobulin domain.

Design of helix-loop-helix

- A helix-loop-helix is 35 amino acid residues
- *N*-terminal α-helix, *C*-terminal α-helix, Glycine linker
- 8 Leucines on the inside faces make a hydrophobic core to stabilize the structure.
- Glutamic acids and lysines on the side faces make a intrachain salt bridge to stabilize the structure and disturb the oligomerization.
- Outside amino acids (**X**) have no contribution for the structure stability, so then, they can be randomized to give a library of helix-loop-helix peptides.



The molecule is a helix-loop-helix (HLH) peptide, which is stable against enzyme degradations in vivo and is too small to show immunogenicity. Here, we introduce our HLH molecular-targeting peptides that show antibody-like functions, high affinity and high specificity for the targeted proteins. Since the HLH peptide folds by virtue of hydrophobic and electrostatic interactions between the amino acid residues positioned inside the molecule, the outside solvent-exposed residues are possible to be mutated with a variety of amino acids to give a combinatorial library of the HLH peptides. Based on our technology of phage-displayed libraries for antibodies, we constructed a phage-displayed library of the HLH peptides. The library was screened against G-CSF receptor to give a binding peptide, which was cyclized by a thioether linkage between the N- and C-termini. The cyclic peptide showed a strong binding affinity (Kd of 4 nM) to the receptor and a long half-life (>2 weeks) in mouse sera, proving an enzyme-resistant property1

Immunization of the HLH peptide to mice showed no induction of the antibody production (non-immunogenic). We have applied our HLH peptide libraries for CTLA42, VEGF3,4, kinases5, HSA6 to obtain their molecular-targeting HLH peptides. In addition, we used the HLH peptide as a scaffold for generating cell permeable targeting peptides through bi-functional grafting: epitope grafting to provide binding activity and arginine grafting to endow cell-permeability7. The HLH peptides provide insights into de novo peptide-based drug discovery and then would be a new therapeutic modality.

Selected publications

1. Fujiwara, D. and Fujii, I. (2013), Phage Selection of Peptide "Microantibodies". *Current Protocols in Chemical Biology*, 5: 171-194. https://doi.org/10.1002/9780470559277.ch130039

2. Tharanga M.R. Ramanayake Mudiyanselage, Masataka Michigami, Zhengmao Ye, Atsuko Uyeda, Norimitsu Inoue, Kikuya Sugiura, Ikuo Fujii, and Daisuke Fujiwara (2020) An Immune-Stimulatory Helix–Loop–Helix Peptide: Selective Inhibition of CTLA-4–B7 Interaction *ACS Chemical Biology 15* (2), 360-368. DOI: 10.1021/acschembio.9b00743

3. Michigami, M; Takahashi, K.; Yamashita, H.; Ye, Z.; Nakase, I.; Fujii, I., (2021) A "ligand-targeting" peptide-drug conjugate: Targeted intracellular drug delivery by VEGF-binding helix-loop-helix peptides via receptor-mediated endocytosis PLoS ONE, 16(2): e0247045. https://doi.org/10.1371/journal.pone.0247045

4. Michigami, M.; Ramanayake Mudiyanselage, T. M. R.; Suzuki, M.; Ishizako, H.;Notsu, K.; Sugiura, K.;Fujii, I. (2022) New Class of Drug Modalities: Directed Evolution of a De Novo Designed Helix–Loop–Helix Peptide to Bind VEGF for Tumor Growth Inhibition *ACS Chemical Biology* 17 (3), 647-653. DOI: 10.1021/acschembio.1c00940

5. D. Fujiwara, K. Mihara, R. Takayama, Y. Nakamura, M. Ueda, T. Tsumuraya, I. Fujii, (2021), Chemical Modification of Phage-Displayed Helix-Loop-Helix Peptides to Construct Kinase-Focused Libraries *ChemBioChem 22*, 3406. https://doi.org/10.1002/cbic.202100450

6. Nakatani, Y.; Ye, Z.; Ishizue, Y.;Higashi, T.; Imai, T.; Fujii, I.; Michigami, M. (2022) "Human and Mouse Cross-Reactive" Albumin-Binding Helix–Loop–Helix Peptide Tag for Prolonged Bioactivity of Therapeutic Proteins *Molecular Pharmaceutics 19* (7), 2279-2286. DOI: 10.1021/acs.molpharmaceut.2c00106

7. Fujiwara, D.; Kitada, H.; Oguri, M.; Nishihara, T.; Michigami, M.; Shiraishi, K.; Yuba, E.; Nakase, I;. Im, H.; Cho, S.; Joung, J. Y.; Kodama, S.; Kono, K.; Ham, S.; Fujii, I. (2016) A Cyclized Helix-Loop-Helix Peptide as a Molecular Scaffold for the Design of Inhibitors of Intracellular Protein–Protein Interactions by Epitope and Arginine Grafting *Angew. Chem. Int. Ed.* 55, 10612. https://doi.org/10.1002/anie.201603230