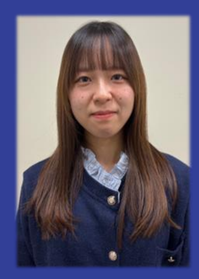
334

https://doi.org/10.17952/38EPS.2026.P334

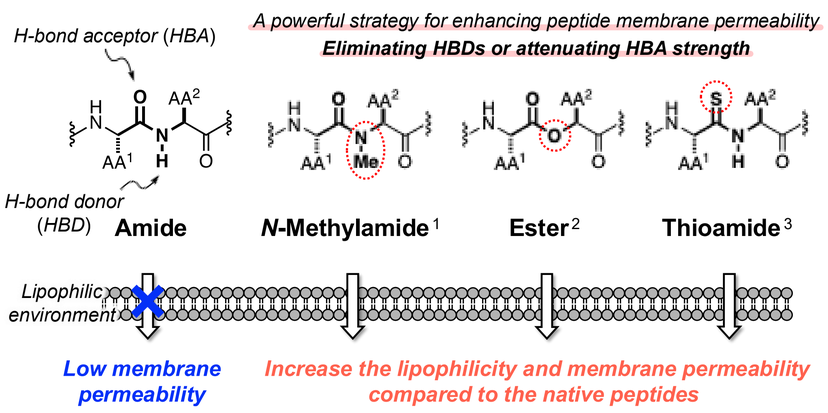
Amide-to-Chloroalkene Substitution as Peptide Backbone Modification for Enhancement of Membrane Permeability

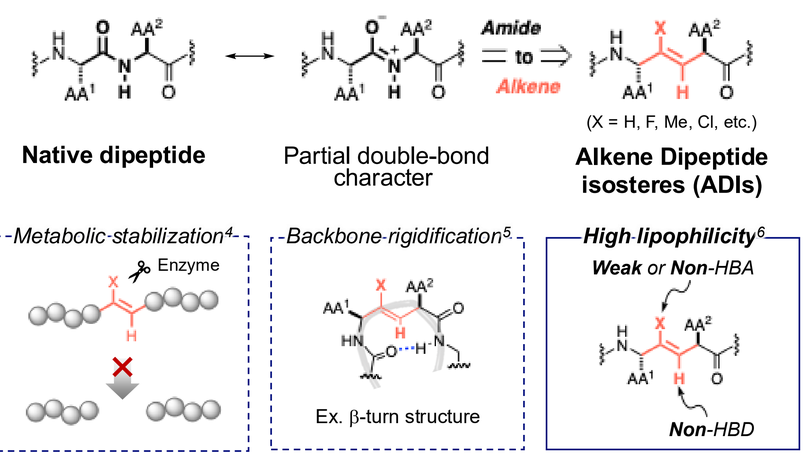
Sayuri Takeo[1†], Mio Takeda[2], Chihiro Iio[1], Ai Sakakibara[2], Showmitra Saha[3], Natsuki Shibata[3], Yuki Yamazaki[4], Takahiro Fujii[5], Ryuhei Harada[6], Kohei Sato[2,7,8], Nobuyuki Mase[2,7,8], Mizuki Watanabe[4], Takanori Oyoshi[3,8], Tetsuo Narumi [1,2,7,8]

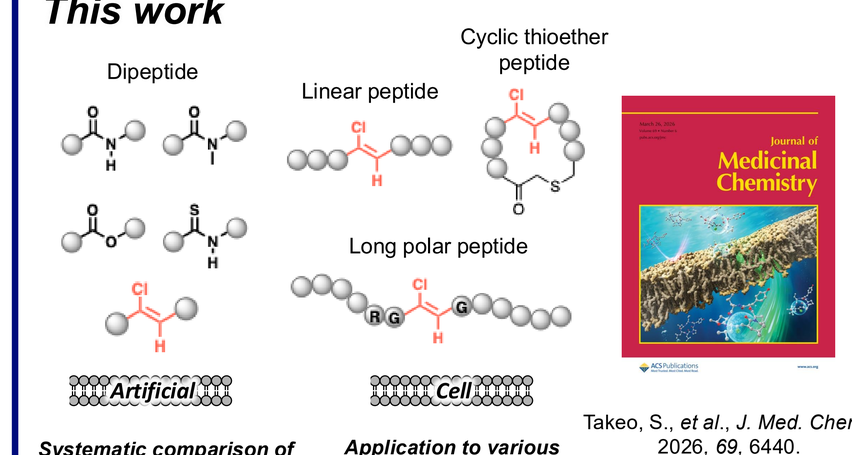
[1]Graduate School of Medical Photonics, Shizuoka University, [2]Department of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, [3]Department of Science, Graduate School of Integrated Science and Technology, Shizuoka University, [4]Faculty of Pharmaceutical Sciences, Hokkaido University, [5]College of Biological Sciences, University of Tsukuba, [6]Center for Computational Sciences, University of Tsukuba, [7]Department of Applied Chemistry and Biochemical Engineering, Faculty of Engineering, Shizuoka University, [8]Research Institute of Green Science and Technology, Shizuoka University, [†]Current address: Faculty of Pharmaceutical Sciences, Kobe Gakuin University



Introduction







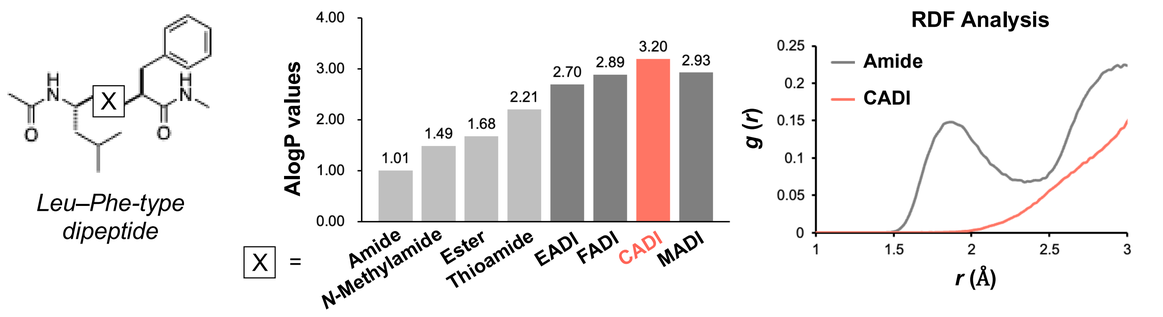
Takeo, S., *et al.*, *J. Med. Chem.* 2026, *69*, 6440.

[1] White T. R., *et al.*, *Nat. Chem. Biol.* **2011**, *7*, 810. [2] Hosono, Y., *et al.*, *Nat. Commun.* **2023**, *14*, 1416. [3] Ghosh, P., *et al.*, *Nat. Commun.* **2023**, *14*, 6050.

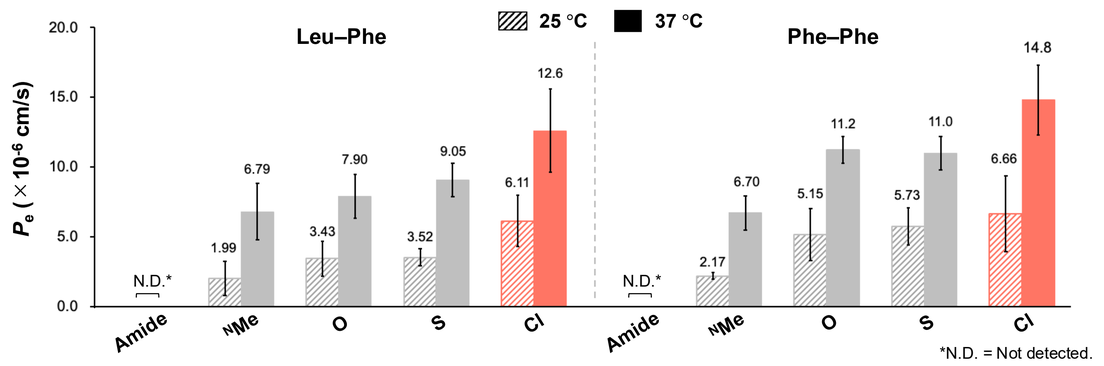
[4] Altman, R. A., *et al.*, *ACS Chem. Neurosci.* **2018**, *9*, 1735. [5] (a) Wipf, P., *et al.*, *J. Org. Chem.* **1998**, *63*, 6088. (b) Kodama, Y., *et al.*, *J. Org. Chem.* **2022**, *87*, 2167. (c) Takeo, S., *et al.*, *J. Org. Chem.* **2026**, *91*, 8588. [6] Takeo, S., *et al.*, *J. Med. Chem.* 2026, *69*, 6440.

■ *Artificial membrane permeability of dipeptide mimics*

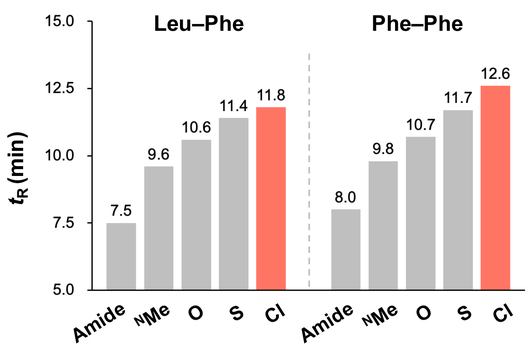
Mechanistic analyses combining AlogP calculations and molecular dynamics simulations



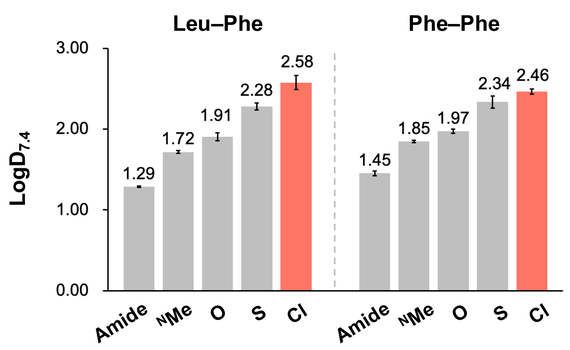
Passive permeability using PAMPA[7]



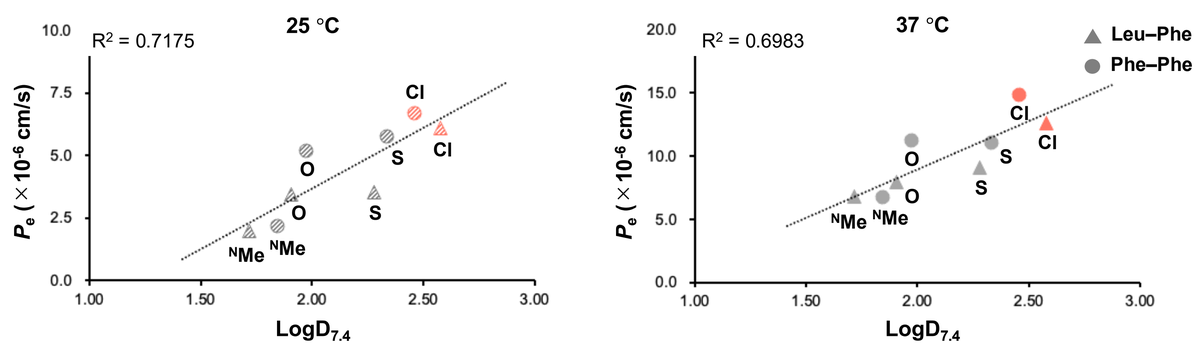
HPLC retention time in C18 column



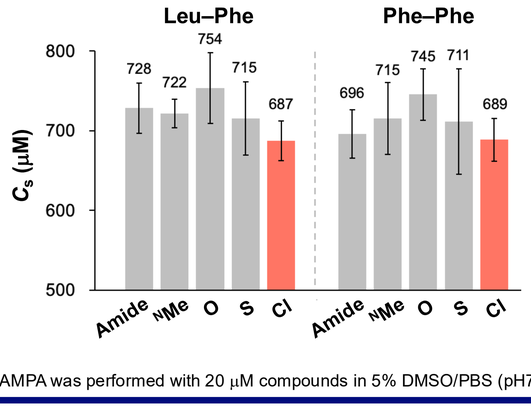
$LogD_{7.4}$ values



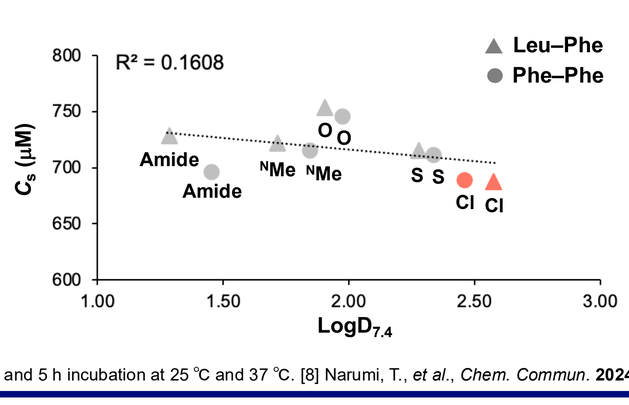
Linear correlation plot of P_e (PAMPA) vs.$logD_{7.4}$



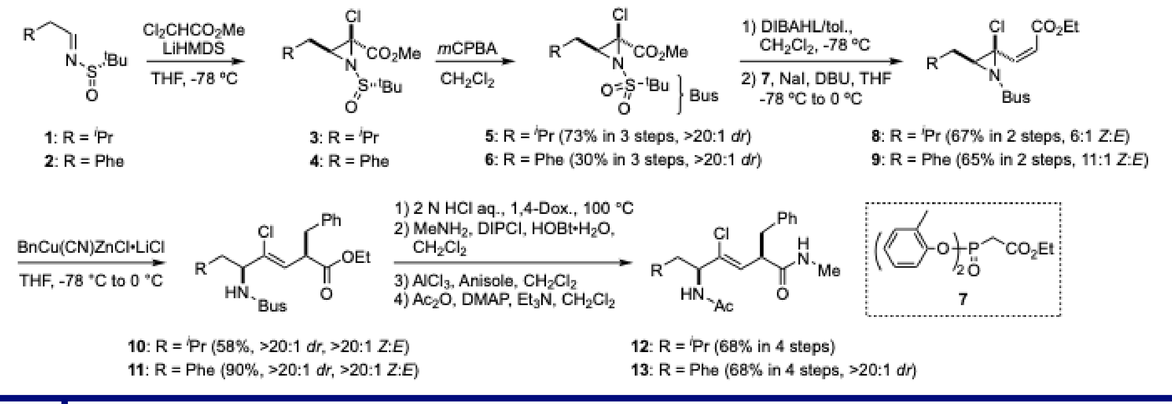
Aqueous solubility of dipeptide mimics



Linear correlation of solubility vs. $logD_{7.4}$



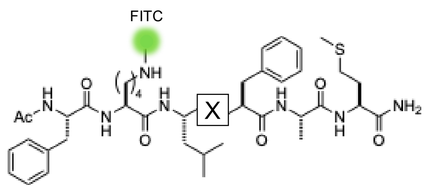
Synthesis of the CADI mimic of Ac-L-Leu-L-Phe-NHMe and Ac-L-Phe-L-Phe-NHMe[8]



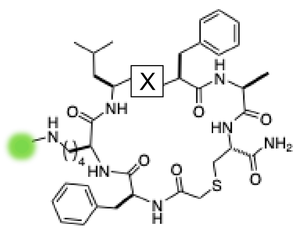
[7] PAMPA was performed with 20 μM compounds in 5% DMSO/PBS (pH7.4) and 5 h incubation at 25 ℃ and 37 ℃. [8] Narumi, T., *et al.*, *Chem. Commun.* **2024**, *60*, 3563.

■ *Cell membrane permeability of the hexapeptide mimics*[9]

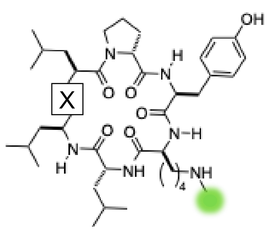
Linear hexapeptide **14**

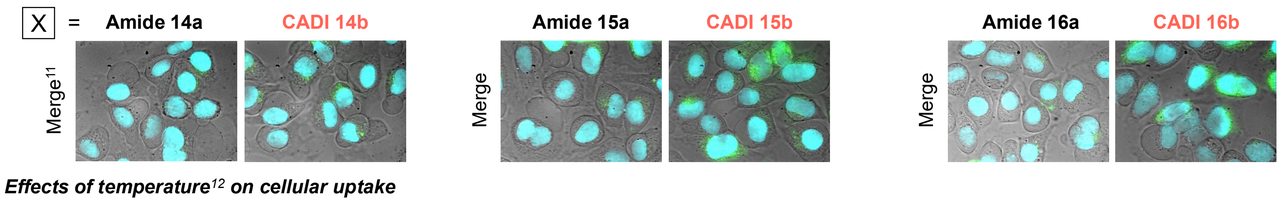


Cyclic thioether hexapeptide **15**

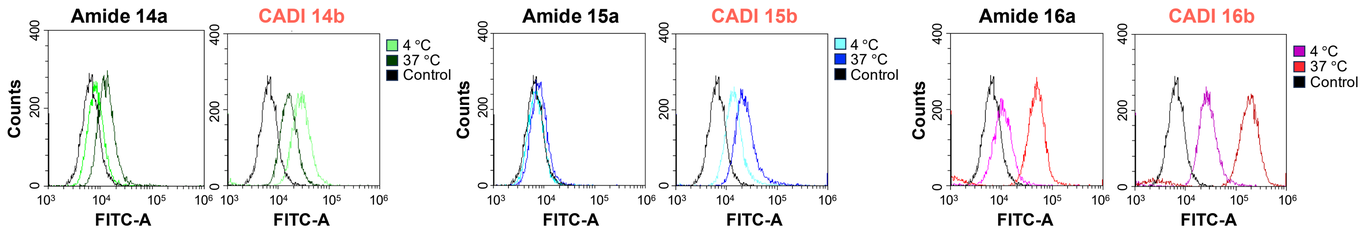


Cyclic hexapeptide[10] **16**

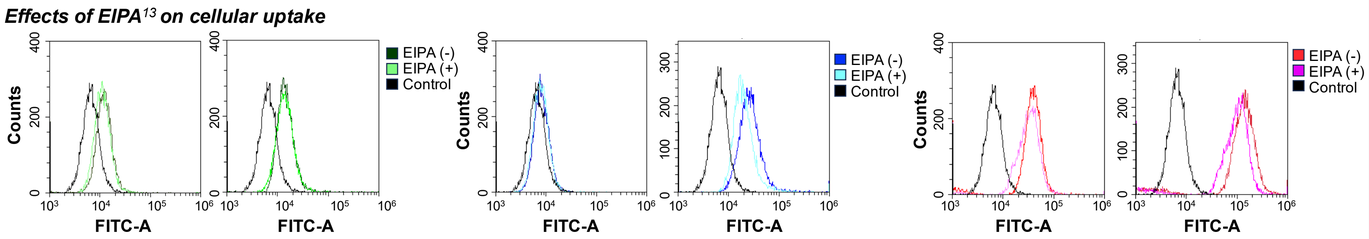




Effects of temperature[12] on cellular uptake

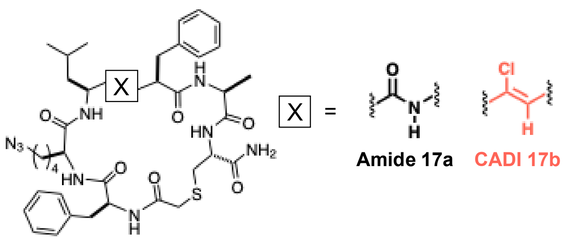


Effects of EIPA[13] on cellular uptake

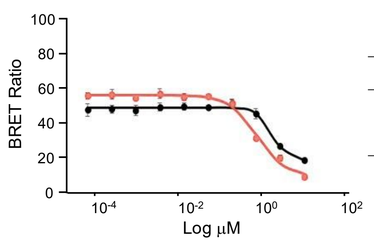


CADI-enhanced uptake mainly occurs via an energy-dependent endocytic pathway.

Quantitatively compare the membrane permeability



Concentration response curves obtained from the NanoClick assay

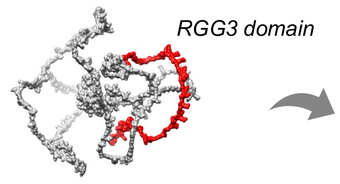


Comp.	EC_{50}
Amide 17a	1.85 ± 0.21 μM
CADI 17b	0.67 ± 0.04 μM

CADI enhances cytosolic delivery by 2.8-fold.

[9] Cell: Hela, Hoechst 0.5 μg/ml for 5 min at 37 °C. [10] Rezai, T., *et al.*, *J. Am. Chem. Soc.* **2006**, *128*, 2510. [11] sample: 5 μM for 3 h at 37 °C. [12] sample: 5 μM for 1 h at 4 °C or 3 h at 37 °C. [13] Nakase, I., *et al.*, *Molecular Therapy* **2004**, *10*, 1011. EIPA: 100 μM for 30 min at 37 °C, sample: 5 μM for 30 min at 37 °C.

■ *Application of ADIs to G4-binding RGG peptides*[14]



STK5-1[15] (Amide-type) K_d = 1.47 μM

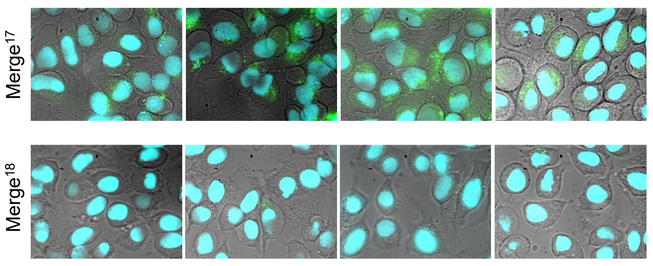
H-GDDRRGGRGGYDR-G-X-G-YRGRGGK

YTK-11 (CADI-type) K_d = 14.6 μM

RGG peptide **18**



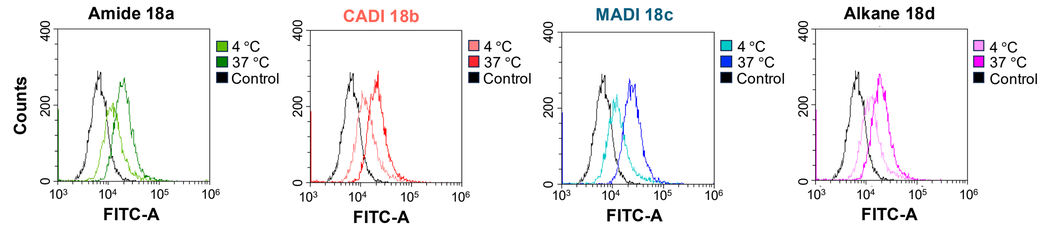
X = Amide 18a, CADI 18b, MADI 18c, Alkane 18d



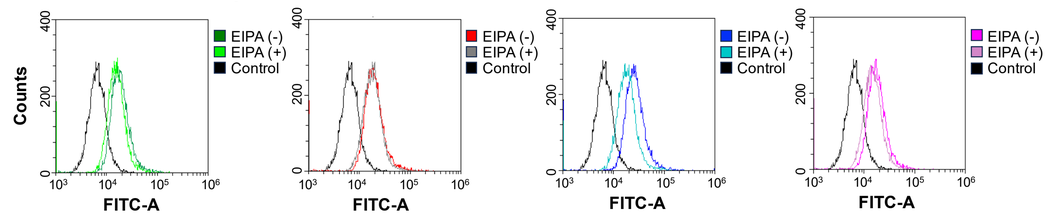
G4-binding affinity of RGG peptide mimics

	Binding constants: K_d (μM)		Selectivity Index (SI)
	G4 (TERRA)	Single strand	
18a	33 ± 2.7	52 ± 5.1	1.6
18b	36 ± 8.3	174 ± 41	**4.8**
18c	21 ± 2.0	133 ± 25	**6.3**
18d	> 500	162 ± 7.8	-

Effects of temperature[19] on cellular uptake



Effects of EIPA[20] on cellular uptake



Amide-to-alkene substitution in RGG peptides can enhance not only membrane permeability but also biologically relevant properties.

[14] Cell: Hela, Hoechst 0.5 μg/ml for 5 min at 37 °C. [15] Takeo, S., *et al.*, *Chem. Pharm. Bull.* **2025**, *73*, 938. [16] Nakase, I., *et al.*, *Molecular Therapy* **2004**, *10*, 1011. [17] sample: 5 μM for 3 h at 37 °C. [18] Cytochalasin D: 10 mM for 30 min, sample: 5 μM for 3 h at 37 °C. [19] sample: 5 μM for 1 h at 4 °C or for 3 h at 37 °C. [20] EIPA: 100 μM for 30 min at 37 °C, sample: 5 μM for 30 min at 37 °C.