***Japan Academy Prize to:***

Hiroaki Suga
Professor, Graduate School of Science,
The University of Tokyo
Professor, Research Center of Advanced Science,
The University of Tokyo

for "Pioneering Works for the Development of De Novo Peptide Therapeutics"

***Outline of the work:***

Dr. Hiroaki Suga developed three highly innovative technologies, "flexizymes (for tRNA aminoacylation)", "a flexible in-vitro translation system (for synthesis of nonstandard peptides)", and "RaPID platform system (for drug screening)". The combination of these three technologies enables for the one-pot synthesis of libraries of "nonstandard macrocyclic peptides" from mRNA templates and effectively discover potent and selective binders against protein targets of interest, which has cultivated the area of "nonstandard peptide therapeutics" as a new modality of molecules with about 2,500 Dalton (Da).

Although drug discovery and development historically have relied on small molecules with about 500 Da, in this century protein drugs, referred to as biologics, have become another line in drug development. Particularly, antibody drugs with about 150,000 Da have given tremendous successes, even becoming the mainstream of drug industry. In such a trend, Dr. Suga has made efforts on developing novel concept, nonstandard macrocyclic peptide therapeutics, with 2,000–3,000 Da, containing nonproteinogenic amino acids.

To realize this concept, he first devised the "flexizyme technology", consisting of a series of artificial RNA enzymes (ribozymes) capable of catalyzing aminoacylation onto tRNAs. The promiscuous catalytic ability of flexizymes enables the preparation of a wide range of aminoacyl-tRNAs charged with nonstandard building blocks, which can be utilized in translation. The genetic code in translation consists of only 20 proteinogenic amino acids, which are strictly controlled by protein factors, such as aminoacyl-tRNA synthetases, initiation, elongation, termination, and recycling factors. A reconstituted in-vitro translation system allows researchers to control the contents and concentrations of such protein factors, referred to as "flexible in-vitro translation (FIT)" system, and effectively to replace designated proteinogenic amino acids with arbitrary nonproteinogenic amino acids. Thus, the flexizyme and FIT technologies rewrite the genetic code and realize the genetic code reprogramming to express various nonstandard peptides containing multiple nonproteinogenic amino acids. Particularly, N-methyl-amino acids, D-amino acids, and b/g-amino acids were used to be nearly impossible to incorporate into peptide nascent chains, but Dr. Suga has demonstrated effective incorporation of such exotic ones into not only linear but also macrocyclic scaffolds, opening a new path for constructing novel peptide libraries.

Moreover, he has integrated this method with a messenger RNA display technique, enabling for displaying macrocyclic nonstandard peptides on mRNA templates via a puromycin linker, referred to as RaPID (random nonstandard peptides integrated discovery) system. This system is able to screen mass libraries containing as high as 10 trillion of molecules against a protein target of interest, and iterative cycles of enrichment of binding species lead to the discovery of de novo potent and selective binders in a week. This rapidness of discovery process and high success rate have given a strong impact on academic and industrial research in the drug discovery field. Indeed, he has established many collaborations with national and international researchers who have expertise in various therapeutic areas, giving tremendous successes for the discovery of desired drug candidates.

His achievements have been well recognized by the communities of science, resulting in many awards, including the Award of The Chemical Society of Japan for Creative Work (2013), the Akabori Memorial Award, Japanese Peptide Society (2014), the Max-Bergmann Medal, Germany (2016), the Japan Innovator Award (2016), the Vincent du Vigneaud Award, American Peptide Society (2019), the Prelog Lecture, ETH Zurich, Switzerland (2022), the Research Award of the Alexander von Humboldt Foundation, Germany (2022), and the Wolf Prize in Chemistry, Israel (2023), and more. Moreover, a startup company established by his technologies, called PeptiDream Inc., became a public trading company at the Tokyo Stock Exchange Market in 2006 (currently at the Prime Market), and globally set collaborations with pharmaceutical companies for the discovery of macrocyclic nonstandard peptides, and the technologies have been also licensed to more than 10 of them.

Thus, his research accomplishment has given great impact on not only science in academia but also Japanese economy. In consideration of his achievements, he deserves the recognition of the Japan Academy Prize.

## List of Main Publications


1. "An in vitro evolved precursor tRNA with aminoacylation activity." H. Saito, D. Kourouklis, H. Suga; **EMBO. J.** 20, 1797–1806 (2001).
2. "A highly flexible tRNA acylation method for nonnatural polypeptide synthesis." H. Murakami, A. Ohta, H. Ashigai, H. Suga; **Nat. Methods** 3, 357–359 (2006).
3. "Strcutural basis of specific tRNA aminoacylation by a small in vitro selected ribozyme." H. Xiao, H. Murakami, H. Suga, A.R. Ferre-D'Amare; **Nature** 454, 358–361 (2008).
4. "Translation initiation with initiator tRNA charged with exotic peptides." Y. Goto, H. Suga; **J. Am. Chem. Soc.** 131, 5040–5041 (2009).
5. "Diverse backbone-cyclized peptides via codon reprogramming." T. Kawakami, A. Ohta, M. Ohuchi, H. Ashigai, H. Murakami, H. Suga; **Nat. Chem. Biol.** 5, 888–890 (2009).
6. "Apolar surface area determines the efficiency of translocon-mediated membrane-protein integration into the endoplasmic reticulum." K. Öjemalm, T. Higuchi, Y. Jiang, Ü. Langel, I. Nilsson, S.H. White, H. Suga, G. von Heijne; **Proc. Natl. Acad. Sci. U.S.A.** 108, E359–364 (2011).

7. “Flexizymes for genetic code reprogramming.” Y. Goto, T. Katoh, H. Suga; **Nat. Protocols** 6, 779–790 (2011).
8. “Discovery of macrocyclic peptides armed with a mechanism-based warhead: isoform-selective inhibition of human deacetylase SIRT2.” J. Morimoto, Y. Hayashi, H. Suga; **Angew. Chem. Int. Ed. Engl.** 51, 3423–3427 (2012).
9. “Structural basis for the drug extrusion mechanism by a MATE multidrug transporter.” Y. Tanaka, C.J. Hipolito, A.D. Maturana, K. Ito, T. Kuroda, T. Higuchi, T. Katoh, H.E. Kato, M. Hattori, K. Kumazaki, T, Tsukazaki, R. Ishitani, H. Suga, O. Nureki; **Nature** 496, 247–251 (2013).
10. “An orthogonal ribosome-tRNA pair via engineering of the peptidyl transferase center.” N. Terasaka, G. Hayashi, T. Katoh, H. Suga; **Nat. Chem. Biol.** 10, 555–557 (2014).
11. “Artificial human Met agonists based on macrocycle scaffolds.” K. Ito, K. Sakai, Y. Suzuki, N. Ozawa, T. Hatta, T. Natsume, K. Matsumoto, H. Suga; **Nat. Commun.** 6, 6373 (2015).
12. “Expanding the amino acid repertoire of ribosomal polypeptide synthesis via the artificial division of codon boxes.” Y. Iwane, A. Hitomi, H. Murakami, T. Katoh, Y. Goto, H. Suga; **Nat. Chem.** 8, 317–325 (2016).
13. “Promiscuous enzymes cooperate at the substrate level en route to lactazole A.” A.A. Vinogradov, M. Shimomura, N. Kano, Y. Goto, H. Onaka, H. Suga; **J. Am. Chem. Soc.** 142, 13886–13897 (2020).
14. “Macrocycle peptides delineate locked-open inhibition mechanism for microorganism phosphoglycerate mutases.” H. Yu, P. Dranchak, Z. Li, R. MacArthur, M.S. Munson, N. Mehzabeen, N.J. Baird, K.P. Battalie, D. Ross, S. Lovell, C.K. Carlow, H. Suga, J. Inglese; **Nat. Commun.** 8, 14932 (2017).
15. “Ribosomal synthesis and de novo discovery of bioactive foldamer peptides containing cyclic $\beta$-amino acids.” T. Katoh, T. Sengoku, K. Hirata, K. Ogata, H. Suga; **Nat. Chem.** 12, 1081–1088 (2020).
16. “Development of cyclic peptides with potent in vivo osteogenic activity through RaPID-based affinity maturation.” N.K. Bashiruddin, M. Hayashi, M. Nagano, Y. Wu, Y. Matsunaga, J. Takagi, T. Nakashima, H. Suga; **Proc. Natl. Acad. Sci. U.S.A.** 117, 31070–31077 (2020).
17. “GTP-state-selective cyclic peptide ligands of K-Ras (G12D) block its interaction with Raf.” Z. Zhang, R. Gao, Q. Hu, H. Peacock, D.M. Peacock, S. Dai, K.M. Shokat, H. Suga; **ACS Cent. Sci.** 6, 1753–1761 (2020).
18. “Ribosomal elongation of aminobenzoic acid derivatives.” T. Katoh, H. Suga; **J. Am. Chem. Soc.** 142, 16518–16522 (2020).
19. “Accurate broadcasting of substrate fitness for lactazole biosynthetic pathway from reactivity-profiling mRNA display.” A.A. Vinogradov, E. Nagai, J.S. Chang, K. Narumi, H. Onaka, Y. Goto, H. Suga; **J. Am. Chem. Soc.** 117, 26728–26738 (2020).
20. “Posttranslational chemical installation of azoles into translated peptides.” H. Tsutsumi, T. Kuroda, H. Kimura, Y. Goto, H. Suga; **Nat. Commun.** 12, 696 (2021).
21. “Lasso-grafting of macrocyclic peptide pharmacophores yields multi-functional proteins.” E. Mihara, S. Watanabe, N.K. Bashiruddin, N. Nakamura, K. Matoba, Y. Sano, R. Maini,

Y. Yin, K. Sakai, T. Arimori, K. Matsumoto, H. Suga, J. Takagi; **Nat. Commun.** 12, 1543 (2021).

22. "One-pot in vitro ribosomal synthesis of macrocyclic depsipeptides." M. Nagano, Y. Huang, R. Obexer, H. Suga; **J. Am. Chem. Soc.** 143, 4741–4750 (2021).
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25. "Macrocyclic peptides exhibit antiviral effects against influenza virus HA and prevent pneumonia in animal models." M. Saito, Y. Itoh, F. Yasui, T. Munakata, D. Yamane, M. Ozawa, R. Ito, T. Katoh, H. Ishigaki, M. Nakayama, S. Shichinohe, K. Yamaji, N. Yamamoto, A. Ikejiri, T. Honda, T. Sanada, Y. Sakoda, H. Kida, T.Q.M. Le, Y. Kawaoka, K. Ogasawara, K. Tsukiyama-Kohara, H. Suga, M. Kohara; **Nat. Commun.** 12, 2654 (2021).
26. "Site-specific nonenzymatic peptide S/O-glutamylation reveals the extent of substrate promiscuity in glutamate elimination domains." A.A. Vinogradov, M. Nagano, Y. Goto, H. Suga; **J. Am. Chem. Soc.** 143, 13358–13369 (2021).
27. "The RaPID platform for the discovery of pseudo-natural macrocyclic peptides." Y. Goto, H. Suga; **Acc. Chem. Res.** 54, 3604–3617 (2021).
28. "An ultrapotent and selective cyclic peptide inhibitor of human beta-factor XIIa in a cyclotide scaffold." W. Liu, S.J. de Veer, Y.H. Huang, T. Sengoku, C. Okada, K. Ogata, C.N. Zdenek, B.G. Fry, J.E. Swedberg, T. Passioura, D.J. Craik, H. Suga; **J. Am. Chem. Soc.** 143, 18481–18489 (2021).
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30. "In vitro selection of foldamer-like macrocyclic peptides containing 2-aminobenzoic acid and 3-aminothiophene-2-carboxylic acid." T. Katoh, H. Suga; **J. Am. Chem. Soc.** 144, 2069–2072 (2022).
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34. "Accurate models of substrate preferences of post-translational modification enzymes from a combination of mRNA display and deep learning." A.A. Vinogradov, J.S. Chang, H. Onaka, Y. Goto, H. Suga; **ACS Cent. Sci.** 8, 814–824 (2022).
35. "Accurate models of substrate preferences of post-translational modification enzymes from a combination of mRNA display and deep learning." A.A. Vinogradov, J.S. Chang, H. Onaka,

Y. Goto, H. Suga; **ACS Cent. Sci.** 8, 814–824 (2022).

36. "In vitro selection of macrocyclic alpha/beta(3)-peptides against human EGFR." R. Wakabayashi, M. Kawai, T. Katoh, H. Suga; **J. Am. Chem. Soc.** 144, 18504–18510 (2022).
37. "State-selective modulation of heterotrimeric Galphas signaling with macrocyclic peptides." S.A. Dai, Q. Hu, R. Gao, E.E. Blythe, K.K. Touhara, H. Peacock, Z. Zhang, M. von Zastrow, H. Suga, K.M. Shokat; **Cell** (2022) 185, 3950–3965.
38. "Selective macrocyclic peptide modulators of Lys63-linked ubiquitin chains disrupt DNA damage repair." G.B. Vamisetti, A. Saha, Y.J. Huang, R. Vanjari, G. Mann, J. Gutbrod, N. Ayoub, H. Suga, A. Brik; **Nat. Commun.** 13, 6174 (2022).
39. "De novo discovery of thiopeptide pseudo-natural products acting as potent and selective TNIK kinase inhibitors." A.A. Vinogradov, Y. Zhang, K. Hamada, J.S. Chang, C. Okada, H. Nishimura, N. Terasaka, Y. Goto, K. Ogata, T. Sengoku, H. Onaka, H. Suga; **J. Am. Chem. Soc.** 144, 20332–20341 (2022).
40. "Post-translational backbone-acyl shift yields natural product-like peptides bearing hydroxyhydrocarbon units."; T. Kuroda, Y. Huang, S. Nishio, Y. Goto, H. Suga; **Nat. Chem.** 14, 1413–1420 (2022).
41. "A macrocyclic peptide inhibitor traps MRP1 in a catalytically incompetent conformation." H.L. Pietz, A. Abbas, Z.L. Johnson, M.L. Oldham, H. Suga, J. Chen; **Proc. Natl. Acad. Sci. U.S.A.** 120, e2220012120 (2023).
42. "Designing receptor agonists with enhanced pharmacokinetics by grafting macrocyclic peptides into fragment crystallizable regions." K. Sakai, N. Sugano-Nakamura, E. Mihara, N.M. Rojas-Chaverra, S. Watanabe, H. Sato, R. Imamura, D.C. Voon, I. Sakai, C. Yamasaki, C. Tateno, M. Shibata, H. Suga, J. Takagi, K. Matsumoto; **Nat. Biomed. Eng.** 7, 164–176 (2023).
43. "Potent de novo macrocyclic peptides that inhibit O-GlcNAc transferase through an allosteric mechanism." M.G. Alteen, H. Peacock, R.W. Meek, J.A. Busmann, S. Zhu, G.J. Davies, H. Suga, D.J. Vocadlo; **Angew. Chem. Int. Ed. Engl.** 62, e202215671 (2023).
44. "In vitro selection of macrocyclic peptide inhibitors containing cyclic gamma(2,4)-amino acids targeting the SARS-CoV-2 main protease." T. Miura, T.R. Malla, C.D. Owen, A. Tumber, L. Brewitz, M.A. McDonough, E. Salah, N. Terasaka, T. Katoh, P. Lukacik, C. Strain-Damerell, H. Mikolajek, M.A. Walsh, A. Kawamura, C.J. Schofield, H. Suga; **Nat. Chem.** 5, 998–1005 (2023).