

CURRICULUM VITAE



PERSONAL INFORMATION

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EDUCATION

B.S. (2000-2004) Undergraduate school of Industrial Chemistry, Faculty of Engineering, Kyoto University
M.S. (2004-2006) Department of Synthetic Chemistry and Biological Chemistry, Graduate school of Engineering, Kyoto University
Ph.D. (2006-2009) Department of Chemistry, Graduate school of Science, Osaka University

RESEARCH AND PROFESSIONAL EXPERIENCE

posdoc (2009-2011) Research Center for Advanced Science and Technology, The University of Tokyo
posdoc (2011-2012) Department of Biomedical Engineering, Boston University
assistant professor (2012-2013) Research Center for Advanced Science and Technology, The University of Tokyo
assistant professor (2013-2019) Department of Chemistry and Biotechnology, The University of Tokyo
associate professor (2019-current) Department of Biomolecular Engineering, Nagoya University

AWARDS

2021 Research Planning Award in the Society of Synthetic Organic Chemistry, Japan (有)

機合成化学協会研究企画賞)

- 2020 Special Lecture of Young Investigators (若い世代の特別講演会) in the 100th CSJ Annual meeting
- 2019 Award for Young Investigator from the Japanese Peptide Society
- 2019 JST PRESTO Investigator
- 2018 Young Investigator Award of the Japanese Society for Epigenetics
- 2017 Lecture Award in 11th Symposium on Biofunctional Chemistry
- 2017 Lecture Award (academic) in the 97th CSJ Annual meeting
- 2012 Postdoctoral Fellowship for Research Abroad, Japan Society for the Promotion of Science
- 2010 Outstanding Young Researcher Poster Presentation Award in PACIFICHEM 2010, Honolulu, USA
- 2009-2012 Research Fellowship for Young Scientists, Japan Society for the Promotion of Science (PD)
- 2006-2009 Research Fellowship for Young Scientists, Japan Society for the Promotion of Science (DC1)
2005. 9 Poster Award in 4th International Symposium on Nucleic Acids Chemistry

RESEARCH PROJECTS

- 1) Efficient methods for multiple peptide fragments ligation (2015-current)
- 2) Development of caged-Arg and photocontrol of peptide function (2015-current)
- 3) Protein conjugation method using silyl-protected alkynes (2015-2016)
- 4) Total chemical synthesis of histone proteins for epigenetics research (2012-current)
- 5) Chemical analysis of DNA and RNA methylation and hydroxymethylation (2012-current)
- 6) Improvement of nucleic-acid probe for RNA imaging (streptavidin conjugates and CPP (Cell Penetrating Peptide) conjugates) (2012-current)
- 7) Chemical enrichment of hydroxylated Lys and Tyr for proteomics analysis (2012-2014)
- 8) Development of novel peptide antibiotics toward therapy for infectious diseases caused by multidrug resistant bacteria (2011-2012)
- 9) Construction of orthogonal ribosome-tRNA pairs for non-standard peptide production (2009-2011)
- 10) Activation of *in vitro* translation by antisense oligonucleotides (2008-2009)
- 11) Photoswitching of the interaction between azobenzene-containing peptide and its RNA aptamer ~towards development of photoresponsive artificial riboswitches~ (2005-2008)
- 12) Total synthesis of mirror image DNA (L-DNA), and application of L-DNA to molecular biology as a molecular tag (2003-2006)

SKILLS

[1] Chemistry

standard organic synthesis procedure (column chromatography, TLC, extraction, filtration, etc.), Fmoc-solid phase peptide synthesis, protein chemical synthesis (peptide ligation), solid phase DNA synthesis, and etc.

[2] Molecular biology and E. coli handling

in vitro selection procedure (library design and construction, *in vitro* transcription, binding assay, RT-PCR, ethanol (isopropanol) precipitation, cloning, sequencing and etc.), *in vitro* translation experiment (flexizyme preparation, ribosome engineering and optimization of translation system), plasmid amplification and engineering, protein expression and purification, ribosome purification, RNA handling, and etc.

[3] Analytical measurements

HPLC, NMR, mass spectrometry (MALDI-TOF-MS and ESI-TOF-MS), fluorescence spectrum, UV-Vis spectrum, CD spectrum, gel-electrophoresis, surface plasmon resonance (SPR), FACS, and etc.

[4] Management of people

Up to 11 people at the same time (including foreign students and posdocs).

PUBLICATIONS

[1] Original papers

1. Kamo, N.; Kujirai, T.; Kurumizaka, H.; Murakami, H.; **Hayashi, G.*** (co-corresponding author); Okamoto, A.* Organoruthenium-Catalyzed Chemical Protein Synthesis to Elucidate the Functions of Epigenetic Modifications on Heterochromatin Factors, *Chemical Science* **2021** *in press*
2. Kondo, T.; Eguchi, M.; Kito, S.; Fujino, T.; **Hayashi, G.**; Murakami, H. cDNA TRAP display for rapid and stable *in vitro* selection of antibody-like proteins, *Chemical Communications* **2021** 57, 2416-2419.
3. Kondo, T.; Iwatani, Y.; Matsuoka, K.; Fujino, T.; Umemoto, S.; Yokomaku, Y.; Ishizaki, K.; Kito, S.; Sezaki, T.; **Hayashi, G.**; Murakami, H. Preparation of monobody mRNA libraries for selection against SARS-CoV-2 targets, *bio-protocols* **2020** prep616.
4. Hao, F.; Murphy, K. J.; Kujirai, T.; Kamo, N.; Kato, J.; Koyama, M.; Okamoto, A.; **Hayashi, G.**;

Kurumizaka, H.; Hayes, J. J. Acetylation-modulated communication between the H3 N-terminal tail domain and the intrinsically disordered H1 C-terminal domain, *Nucleic Acids Research* **2020** 48, 11510-11520.

5. Ogata, M.; **Hayashi, G.**; Ichiu, A.; Okamoto, A. L-DNA-tagged fluorescence in situ hybridization for highly sensitive imaging of RNAs in single cells, *Organic and Biomolecular Chemistry* **2020** 18, 8084-8088.
6. Kondo, T.; Iwatani, Y.; Matsuoka, K.; Fujino, T.; Umemoto, S.; Yokomaku, Y.; Ishizaki, K.; Kito, S.; Sezaki, T.; **Hayashi, G.**; Murakami, H. Antibody-like proteins that capture and neutralize SARS-CoV-2, *Science Advances* **2020** 6, eabd3916.
7. Nakatsu, K.; Yanase, M.; **Hayashi, G.*** (co-corresponding author); Okamoto, A.* Fmoc-Compatible and Sequence-Independent Peptide C-Terminus Alkyl Thioester Formation Using Cysteinylprolyl Imide, *Organic Letters* **2020** 22, 4670-4674.
8. Kamo, N.; **Hayashi, G.*** (co-corresponding author); Okamoto, A.* Chemical Synthesis of Cys-Containing Protein via Chemoselective Deprotection with Different Palladium Complexes, *Organic Letters* **2019** 21, 8378-8382.
9. Yanase, M.; Nakatsu, K.; Cardos, C. J.; Konda, Y.; **Hayashi, G.*** (co-corresponding author); Okamoto, A.* Cysteinylprolyl imide (CPI) peptide: a highly reactive and easily accessible crypto-thioester for chemical protein synthesis, *Chemical Science* **2019** 10, 5967-5975.
10. ***Hayashi, G.**; ***Yanase, M.** (co-first author); Nakatsuka, Y.; Okamoto, A. Simultaneous and Traceless Ligation of Peptide Fragments on DNA Scaffold, *Biomacromolecules* **2019** 20, 1246-1253.
11. Kamo, N.; **Hayashi, G.**; Okamoto, A. Triple Function of 4-Mercaptophenylacetic Acid Promotes One-Pot Multiple Peptide Ligation, *Angewandte Chemie International Edition* **2018** 57, 16533-16537. (Highlighted in SYNFACTS **2019**; 15(02): 0204)
12. Kamo, N.; **Hayashi, G.**; Okamoto, A. Efficient peptide ligation between allyl-protected Asp and Cys followed by palladium-mediated deprotection, *Chemical Communications* **2018** 54, 4337-4340.
13. **Hayashi, G.**; Tamai, M.; Okamoto, A. Hybridization-Sensitive Fluorescent Oligonucleotide Probe Conjugated with Cell-Penetrating Peptides for Enhanced Cellular Uptake, *Chemistry Letters* **2017** 46, 1803-1806.
14. Sueoka, T.; **Hayashi, G.**; Okamoto, A. Regulation of the Stability of the Histone H2A-H2B

Dimer by H2A Tyr57 Phosphorylation, *Biochemistry* **2017** 56, 4767-4772.

15. **Hayashi, G.**; Kamo, N.; Okamoto, A. Chemical synthesis of dual labeled protein via differently protected alkynes enables intramolecular FRET analysis, *Chemical Communications* **2017** 53, 5918-5921.
16. Yamaguchi, S.; Matsushita, T.; Izuta, S.; Katada, S.; Ura, M.; Ikeda, T.; **Hayashi, G.**; Suzuki, Y.; Kobayashi, K.; Tokunaga, K.; Ozeki, Y.; Okamoto, A. Chemically-activatable alkyne-tagged probe for imaging microdomains in lipid bilayer membranes, *Scientific Reports* **2017** 7, 41077.
17. **Hayashi, G.**; Koyama, K.; Shibata, H.; Kamio, A.; Umeda, T.; Nagae, G.; Aburatani, H.; Okamoto, A. Base-Resolution Analysis of 5-Hydroxymethylcytosine by One-Pot Bisulfite-Free Chemical Conversion with Peroxotungstate, *Journal of American Chemical Society* **2016** 138, 14178-14181.
18. **Hayashi, G.**; Sueoka, T.; Okamoto, A. In vitro and in cell analysis of chemically synthesized histone H2A with multiple modifications, *Chemical Communications* **2016** 52, 4999-5002.
19. Yamamoto, K.; Chikaoka, Y.; **Hayashi, G.**; Sakamoto, R.; Yamamoto, R.; Sugiyama, A.; Kodama, T.; Okamoto, A.; Kawamura, T. Middle-down and chemical proteomic approaches to reveal histone H4 modification dynamics in cell cycle, *Mass Spectrometry* **2015** 4, A0039
20. **Hayashi, G.**; Sakamoto, R.; Okamoto, A. 2-oxazoline formation for selective chemical labeling of 5-hydroxylysine, *Chemistry – An Asian Journal* **2015** 10, 1138-1141.
21. Jeong, HS.; **Hayashi, G.**; Okamoto, A. Diazirine Photocrosslinking Recruits Activated FTO Demethylase Complexes for Specific N⁶-methyladenosine Recognition, *ACS Chemical Biology* **2015** 10, 1450-1455.
22. **Hayashi, G.**; Yanase, M.; Takeda, K.; Sakakibara, D.; Sakamoto, R.; Wang, DO.; Okamoto, A. Hybridization-sensitive fluorescent oligonucleotide probe conjugated with a bulky module for compartment-specific mRNA monitoring in a living cell, *Bioconjugate Chemistry* **2015** 26, 412-417.
23. *Terasaka, N.; ***Hayashi, G.** (co-first author); Katoh, T; Suga, H. An orthogonal ribosome-tRNAs pair via engineering of the peptidyl transferase center, *Nature Chemical Biology* **2014** 10, 555-557.
24. Okamoto, A.; Sugizaki, K.; Yuki, M.; Yanagisawa, H.; Ikeda, S.; Sueoka, T.; **Hayashi, G.**; Wang, DO. A nucleic acid probe labeled with desmethyl thiazole orange: a new type of hybridization-sensitive fluorescent oligonucleotide for live-cell imaging, *Organic and Biomolecular Chemistry*

2013 11, 362-371.

25. **Hayashi, G.**; Hong, C.; Hagihara, M.; Nakatani, K. Activation of prokaryotic translation by antisense oligonucleotides binding to coding region of mRNA, *Biochemical and Biophysical Research Communications* **2012** 429, 105-110.
26. **Hayashi, G.**; Hagihara, M.; Nakatani, K. RNA aptamers that reversibly bind photoresponsive azobenzene-containing peptides, *Chemistry – A European Journal* **2009**, 15, 424-432.
27. **Hayashi, G.**; Hagihara, M.; Nakatani, K. Genotyping by allele-specific L-DNA-tagged PCR, *Journal of Biotechnology* **2008**, 135, 157-160.
28. **Hayashi, G.**; Hagihara, M.; Dohno, C.; Nakatani, K. Photoregulation of a peptide-RNA interaction on a gold surface, *Journal of American Chemical Society* **2007**, 129, 8678-8679.
29. **Hayashi, G.**; Hagihara, M.; Kobori, A.; Nakatani, K. Detection of L-DNA-tagged PCR products by surface Plasmon resonance imaging, *ChemBiochem* **2007**, 8, 169-171.
30. Nakatani, K.; Hagihara, S.; Goto, Y.; Kobori, A.; Hagihara, M.; **Hayashi, G.**; Kyo, M.; Nomura, M.; Mishima M.; Kojima C. Small-molecule ligand induces nucleotide flipping in (CAG)_n trinucleotide repeats, *Nature Chemical Biology* **2005**, 1, 39-43.
31. Suda, H.; Kobori, A.; Zhang, J.; **Hayashi, G.**; Nakatani, K. *N,N'*-Bis(3-aminopropyl) -2,7-diamino-1,8-naphthyridine stabilized a single pyrimidine bulge in duplex DNA, *Bioorganic and Medicinal Chemistry* **2005**, 13, 4507-4512.
32. Hagihara, S.; Kumasawa, H.; Goto, Y.; **Hayashi, G.**; Kobori, A.; Saito, I.; Nakatani, K. Detection of Guanine-Adenine Mismatches by Surface Plasmon Resonance Sensor carrying Naphthyridine-Azaquinolone Hybrid on the Surface, *Nucleic Acids Research* **2004**, 32, 278-286.

[2] Reviews, Perspectives and Book chapters

33. **林剛介** 「タンパク質を化学で創る時代へ向けて」, 名大鏡友会会報「若手教員の研究教育」 **2021**, 3, 21.
34. **林剛介** 「タンパク質化学合成を加速させるペプチド連結反応の化学 ～タンパク質を化学で創る時代を目指して～」, 化学と工業 ～飛翔する若手研究者～ **2020**, 73, 956-957.
35. Nakatsu, K.; **Hayashi, G.*** (co-corresponding author); Okamoto, A.* Toolbox for Chemically Synthesized Histone Proteins, *Current Opinion in Chemical Biology* **2020** 58, 10-19.

36. 林剛介; 岡本晃充 「タンパク質化学合成を加速するペプチドライゲーシンの新技術」, *有機合成化学協会誌 ～総合論文～* **2020** 78, 130-139.
37. 林剛介; 岡本晃充 「エピジェネティック修飾の謎を解き明かす核酸およびタンパク質化学」, *CSJ カレントレビュー ～生体分子反応を制御する：制御分子、方法、反応場～* **2020**
38. 林剛介 「令和元年度ペプチド学会奨励賞を受賞して」, *日本ペプチド学会ニュースレター* **2020**, 115, 13-16.
39. 林剛介 「タンパク質化学合成を活用した翻訳後修飾研究」, *ファルマシア ～最前線～* **2020**, 56-1, 46-50.
40. Sueoka, T.; Koyama, K.; Hayashi, G.; Okamoto, A. Chemistry-driven Epigenetic Investigation of Histone and DNA Modifications, *The Chemical Record* **2018** 18, 1727-1744
41. 林剛介 「研究会・国際会議から」, *日本化学会バイオテクノロジー部会ニュースレター* **2017**, 21, 2, 40-41.
42. 林剛介 「タンパク質化学合成法を武器に多細胞生物の謎に挑む」, *日本化学会生体機能関連化学部会ニュースレター 研究紹介* **2017**, 32-3, 17-20.
43. 林剛介 「タンパク質を化学の力でいかに作るか?」, *日本化学会生体機能関連化学部会ニュースレター 研究紹介* **2017**, 32-2, 10-13.
44. 林剛介 「若手研究者からのメッセージ」, *日本化学会バイオテクノロジー部会ニュースレター* **2017**, 21, 1, 18-32.
45. 林剛介 「年会に参加して」, *日本エピジェネティクス研究会ニュースレター* **2017**, 46.
46. 林剛介; 岡本晃充 「タングステンが細胞初期化の秘密を解く！～DNA 修飾を一塩基レベルで解析～」, *月刊化学「解説」* **2017** 4, 35-40.
47. Hayashi, G.; Nakatani, K. Development of photoswitchable ligand-aptamer complexes, *Methods in Molecular Biology* **2014** 1111, 29-40.
48. 林剛介; 岡本晃充 「生命現象を司る DNA メチル化～エピジェネティック因子を検出する化学～」, *月刊化学「最新のトピックス」* **2014** 3, 68-70.
49. Hayashi, G.; Okamoto, A. Probe design for the effective fluorescence imaging of intracellular RNA, *The Chemical Record* **2013** 13, 209-217.
50. 林剛介 「ボストン大学留学体験記」, *生命化学研究レター* **2012**, 10, 29-32.

51. Suga, H.; **Hayashi, G.**; Terasaka, N. The RNA origin of transfer RNA aminoacylation and beyond, *Philosophical Transactions of the Royal Society B* **2011**, 366, 2959-2964.
52. **Hayashi, G.**; Goto, Y.; Suga, H. Ribosome evolution for two artificial amino acids in E. coli, *Chemistry and Biology* **2010**, 17, 320-321.
53. **Hayashi, G.**; Ohshiro, Y.; Suga, H. Ribosome synthesis of nonstandard cyclic peptides and its application to drug discovery (特殊環状ペプチドの翻訳合成と医薬品探索への展開), *Seikagaku (生化学)* **2010**, 82, 505-514.

[3] Proceedings

54. **Hayashi, G.** Novel Strategies for Peptide Ligation toward Epigenetic Research, *Peptide Science* **2019**
55. **Hayashi, G.**; Sueoka, T.; Okamoto, A. Epigenetic Analysis of Histone H2A-H2B Dimer Powered by Protein Chemical Synthesis, *Peptide Science* **2018**
56. **Hayashi, G.**; Hagihara, M.; Nakatani, K. RNA aptamers that reversibly bind photoresponsive peptide, *Nucleic Acids Symposium Series* **2008**, 52, 703-704.
57. **Hayashi, G.**; Hagihara, M.; Dohno, C.; Nakatani, K. Reversible regulation of binding between a photoresponsive peptide and its RNA aptamer, *Nucleic Acids Symposium Series* **2007**, 51, 93-94.
58. **Hayashi, G.**; Hagihara, M.; Nakatani, K. Application of L-DNA as a molecular tag, *Nucleic Acids Symposium Series* **2005**, 49, 261-262.
59. Kobori, A.; Peng, T.; **Hayashi, G.**; Nakatani, K. SPR fingerprinting of mismatched base pair, *Nucleic Acids Symposium Series* **2004**, 48, 129-130.

PATENTS

60. NCL 法による高分子の製造方法, 出願番号: 特願 2017-157751, 出願日: 2017/8/18, 発明者: 岡本晃充、林剛介、加茂直己, 出願者: 東京大学
61. PCR primer, PCR method utilizing the same, and PCR amplified product, and device and DNA-protein complex utilizing PCR amplified product (PCR プライマー、それを利用した PCR 法及び PCR 増幅産物、並びに PCR 増幅産物を利用するデバイス及び DNA-タンパク複合体), Jpn. Kokai Tokkyo Koho (2005), JP 2005061429, PCT Int. Appl. (2006), WO/2006/095550. 出願番号: 特願 2006095550, 特願 2007-507025, 出願日: 2006/2/16, 公開番号: 特開 WO2006-095550, 公開日: 2008/8/14, International Application No.:

INVITED LECTURES

62. 第 93 回日本生化学会大会 (2020 年 9 月 オンライン) 「タンパク質化学合成技術を駆使した修飾タンパク質の創製と生化学研究への展開」
63. 第 568 回東北大学薬学研究科セミナー (2020 年 2 月 宮城県仙台市 東北大学) 「修飾タンパク質作製を可能にする最先端技術 ～生合成 vs 化学合成～」
64. Seminar hosted by Dr. Vincent Aucagne (2020 年 1 月 Orléans, France CBM, CNRS Orléans Campus) 「Chemical Protein Synthesis with Novel Ligation Strategies」
65. Seminar hosted by Prof. Mélanie Etheve-Quellejeu (2020 年 1 月 Paris, France University of Paris) 「Novel Peptide Ligation Strategies for Chemical Protein Synthesis」
66. 第 42 回分子生物学会年会 (2019 年 12 月 福岡県福岡市 福岡国際会議場) 「Analysis of nucleosome modifications and dynamics based on chemical protein synthesis」
67. 大阪大学蛋白質研究所セミナー (2019 年 2 月 静岡県浜松市 静岡大学浜松キャンパス) 「エピジェネティクス研究とタンパク質化学合成」
68. 第 91 回日本生化学会大会 (2018 年 9 月 京都府京都市 国立京都国際会館) 「有機金属化学を用いた効率的ヒストン合成と修飾ヒストンを用いたヌクレオソーム解析」
69. 第 165 回東海高分子研究会講演会 (2018 年 8 月 愛知県蒲郡市 鈴岡旅館) 「アミノ酸の精密重合体であるタンパク質の化学的全合成とその効率化」
70. 技術情報協会セミナー ～ペプチド医薬品の透過性・吸収性・安定性向上技術～ (2017 年 4 月 東京都品川区 日幸五反田ビル) 「特殊ペプチドの合成及びスクリーニングと細胞膜透過性における課題」
71. RIKEN Symposium on Chemistry and Biochemistry of Epigenetic Regulation (2017 年 1 月 埼玉県和光市 理化学研究所 和光研究所) 「Epigenetic Analysis by Chemically Synthesized Histone H2A and H2B with PTMs and Fluorophors」
72. 第 16 回日本蛋白質科学会年会 (2016 年 6 月 福岡県福岡市 福岡国際会議場) 「化学合成ヒストンを用いたクロマチン修飾解析プラットフォームの構築」
73. 技術情報協会セミナー ～ペプチド創薬における最新のスクリーニング技術および合成方法の開発～ (2016 年 12 月 東京都品川区 日幸五反田ビル) 「特殊ペプチドの合成手法と医薬シーズの探索」

74. BMB2015 (2015年12月 兵庫県神戸市 神戸ポートアイランド)「化学合成による機能性人工ヒストンの創出」
75. 技術情報協会セミナー ～ペプチド医薬品の市場予測・事業化戦略と製剤化技術の開発～ (2016年9月 東京都品川区 日幸五反田ビル)「医薬シーズとしての特殊ペプチドの創製と細胞膜透過性における課題」
76. 第67回日本細胞生物学会大会 (2015年6月 東京都江戸川区 タワーホール船堀)「化学合成ヒストンを用いた細胞内エピジェネティクス情報取得への試み」
77. 細胞を創る研究会 7.0 (2014年9月 東京都文京区 東京大学弥生キャンパス)「Orthogonal genetic codes by engineered ribosome-tRNA pairs」
78. 理研セミナー (2009年6月 埼玉県和光市 理化学研究所 和光研究所)「L-DNAによる新規PCR産物標識法とリボソームによる翻訳効率制御法の開発」

REFERENCES

- 1) Professor Hiroshi Murakami Ph.D.
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