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HKU Chemists Developed a Clickable Tryptophan Modification Strategy for Late-Stage Diversification of Native Peptides

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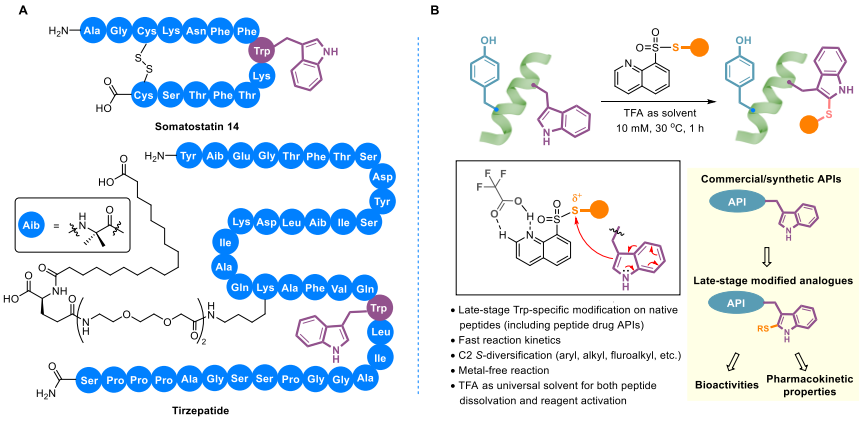

Figure 1. (a) Tryptophan residues in peptide drugs; (b) Late-stage tryptophan-selective *S*-diversification of native peptides developed in this research project. Image adapted from 'Clickable tryptophan modification for late-stage diversification of native peptides', *Science Advances* 2024. DOI: 10.1126/sciadv.adp995.

Peptides are gradually emerging as middle-sized therapeutic agents in addressing unmet medical needs. Compared to small-molecule drugs, peptides can target complex biological processes more precisely while generally less complex and more a cost-effective than large biological drugs like antibodies. Over 100 FDA-approved peptide drugs have been on the market since the first peptide hormone insulin was developed in 1923, among which about 40 drugs for treating a wide range of diseases (e.g., cancer, cardiovascular, and metabolic diseases) contain at least one tryptophan (Trp) residue, which is a key amino acid.

Modifying Trp residues in peptide molecules can help regulate the drug-target interactions and improve drug stability, bioavailability, and pharmacokinetics properties. However, enabling transformations on these functionally dense molecules requires a high level of selectivity, such as chemoselectivity, regioselectivity, and stereoselectivity. Additionally, peptides' nucleophilic functionalities make them sensitive to redox conditions, further complicating modifications. The limited solvents for dissolving unprotected peptides also pose challenges. As a result, developing site-specific late-stage peptide modifications is a daunting task.

Recently, a team led by Professor Xuechen LI from the Department of Chemistry of The University of Hong Kong (HKU) developed a clickable tryptophan modification strategy. This strategy allows for the easy modification of a specific part of a peptide molecule even at the late-stage of drug development process. This late-stage diversification technique allows them to fine-tune peptides even after the core structure is established. Their findings have recently been published in *Science Advances*.

This approach involves a late-stage catalyst-free C2-sulfenylation reaction using S-modified quinoline-containing thiosulfonate reagents. Through this method, the researchers efficiently install a variety of functional groups onto the tryptophan (Trp) residues within the native peptide structures. The introduced groups include trifluoromethylthio, difluoromethylthio, (ethoxycarbonyl) difluoromethylthio, alkylthio, and arylthiol.

In this transformation, trifluoroacetic acid (TFA) was used as the optimal solvent and played an important role in the activation of the reagents via hydrogen bond interaction. Moreover, the super dissolving capability of TFA for hydrophobic and aggregation-prone peptides ensures the applicability of this method to challenging molecules like lipopeptides and self-assembling peptides, even at relatively high concentrations.

This method was successfully applied to the late-stage modification of several on-market peptide drugs, such as somatostatin, octreotide, lanreotide, setmelanotide, daptomycin and semaglutide, as well as the bioactive glycopeptide hAdn-WM6877, showcasing the applicability of this method to the diversity-oriented modification of peptide-based active pharmaceutical ingredients.

Professor Li's team found that the bioactivity and serum stability of modified melittin analogs were improved, which demonstrated the great potential of this method in drug development. Because Trp widely exists in RiPP natural products like darobactin and chloropeptin I, and drug leads screened from phage display and mRNA display, this method will also be usable to the natural product late-stage diversification for making molecular libraries and functional probes.

The team also believed this single-step clickable late-stage Trp modification method would provide a robust platform for generating structural analogues in cost-efficient manner, satisfying the demand for optimising drug activities and pharmacokinetic properties, and will become a precious tool for medicinal chemists, peptide chemists, and chemical biologists.

About the research team

This research was accomplished independently by Xuechen Li's team in the Department of Chemistry (Faculty of Science, HKU). Postdoctoral fellow Dr Yisa XIAO from Professor Li's group at HKU Chemistry is the first author of this paper. Other researchers include Dr Han LIU, Ms Haiyan ZHOU and Dr Pengfei SHI from HKU, as well as Dr Xueqian ZHAO of The Hong Kong Polytechnic University, who also contributed greatly to this research project. This work was supported by the Research Grants Council of Hong Kong (17312022, 17306521, AoE/P-705/16, T11-104/22-R), the National Natural Science Foundation of China (22177097) and the Laboratory for Synthetic Chemistry and Chemical Biology under the Health@InnoHK Program by the Innovation and Technology Commission. Professor Xuechen Li is the recipient of the Research Grants Council-Senior Research Fellow Scheme (SPFS2324-7S01).

More information about Professor Xuechen Li's research group can be found at https://chemistry.hku.hk/staff/xcli/XCLiGroup/index.html

About the research paper:

Journal title: "Xiao, Y., Zhou, H., Shi, P., Zhao, X., Liu, H., and Li, X.* Clickable tryptophan modification for late-stage diversification of native peptides. *Science Advances* 2024".

The journal paper can be accessed from here: https://www.science.org/doi/10.1126/sciadv.adp9958

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