Stain Protocol for the Detection of N-Terminal Amino Groups during Solid-Phase Peptide Synthesis

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Solid-phase peptide synthesis (SPPS) – pioneered by the 1984 Nobel Prize winner Robert Bruce Merrifield – is the method of choice for the preparation of polypeptides. This highly versatile technology is used worldwide for the manual as well as automated synthesis of a wide range of peptides. Despite its success, direct monitoring of reactions on resin is not as straightforward as for reactions in solution where samples can be easily collected and directly analysed through a variety of analytical and spectroscopic methods, such TLC, HPLC and NMR. The Kaiser test – based on the reaction between primary amino groups and ninhydrin which develops an intense blue color – is destructive and can easily lead to false positives (for example when Fmoc protecting groups are labile to the pyridine contained in the test cocktail) or negatives (secondary amines such as proline give rise to a rather ambiguous red-brownish color). Besides, the Kaiser test is time-consuming and the test cocktail contains highly toxic reagents such as KCN.

The group of Professor Hiroyuki Konno at Yamagata University (Japan) recently reported a new test protocol to detect *N*-terminal amino groups during Fmoc-SPPS using a reversible and non-destructive reaction. Professor Konno explained: "This is a novel approach attempted for the first time by our research group, since for a hundred years ninhydrin has been used as a detecting reagent for primary amino groups. The Kaiser test with ninhydrin results in blue staining and is definitely useful, but it sacrifices the small amount of resin used for the test and cannot detect most secondary and tertiary amino groups." He continued: "Interestingly, our methodology can detect most primary, secondary and tertiary amino groups, using a reversible process and coloration. Therefore, we do not need to lose any resin."

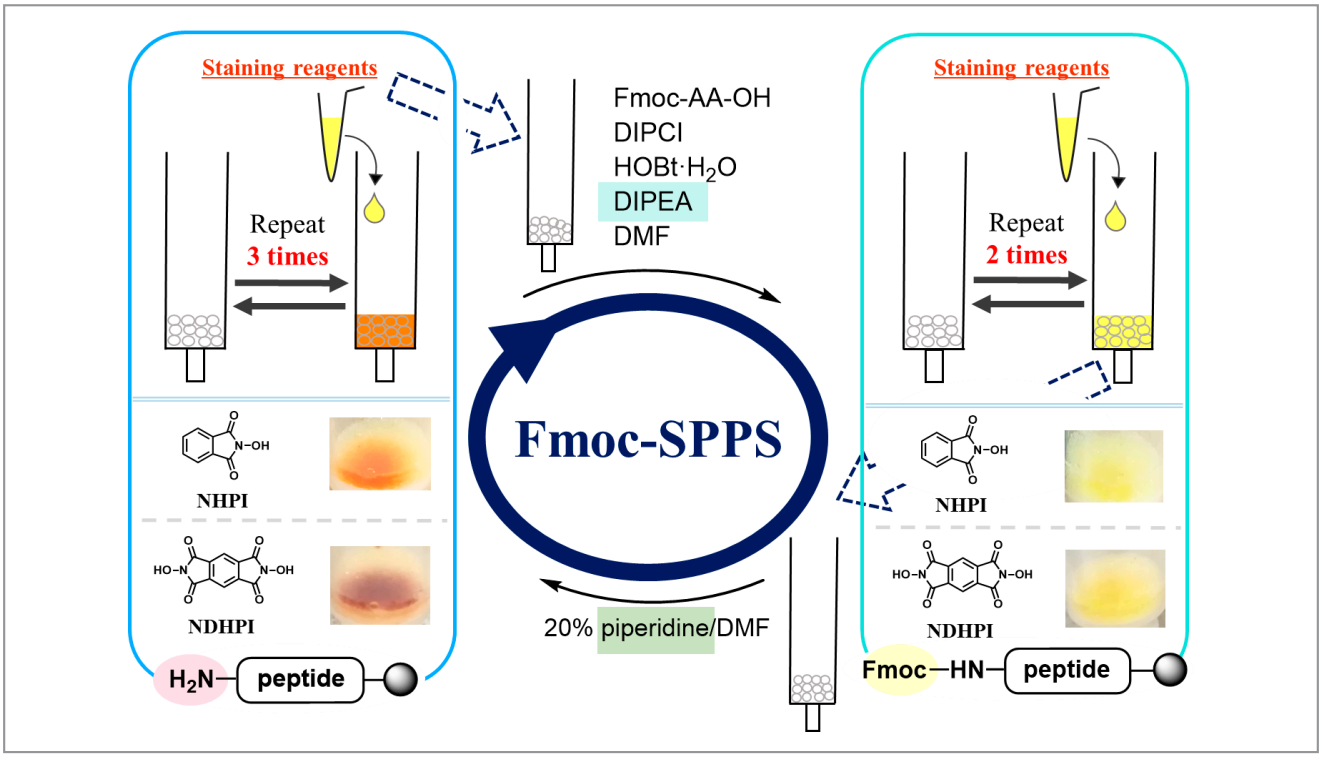


Figure 1 The protocol of staining of free amino groups and protected amino groups.

The group tested all 20 proteogenic amino acids, some *N*-methyl amino acids, and Aib to ensure that all of them could be detected by their new approach. Professor Konno remarked: "Fortunately, no byproducts have ever been shown in HPLC analysis."

The new protocol for staining free amino groups and protected amino groups is shown in Figure 1. "This is a unique method that makes it very easy to check the presence of amino groups," explained Professor Konno. He continued: "In addition, only cheap reagents and no expensive analytical equipment are required. As I saw the red crystal of the complex with *N*-hydroxyphthalimide and dimethylamine, I had a "Eureka moment" concerning this amino group staining. Since the red color disappears as the complex is dissociated, I immediately felt that this phenomenon was valuable."

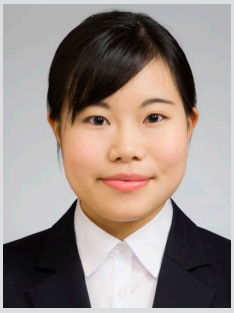
Professor Konno then paid tribute to Rio Suzuki, the sole co-worker, who performed all the experiments and analyzed the data. Professor Konno remarked: "She is an outstanding student and the success of this research is due to her deep insight and sharp observant eye."

Professor Konno concluded by speaking about potential or actual applications for their work, saying: "In the future, one of our most important aims is to reduce the stoichiometric proportions of reagents used by this system in peptide synthesis, not only to reduce costs but also the environmental impact of the protocol."

About the authors

Prof. H. Konno

Hiroyuki Konno is currently a professor at Graduate School of Science and Engineering, Yamagata University (Japan). He received his Ph.D. from the Pharmaceutical Institute, Tohoku University (Japan) in 1999 under the supervision of Prof. Kunio Ogasawara. He conducted postdoctoral research at University of Pennsylvania (USA) with Prof. Amos B. Smith, III as a JSPS fellow. He was an Assistant Professor of Tokushima University (Japan), Kyoto University (Japan) and Kyoto Prefectural University of Medicine (Japan) for eight years. In 2009, he began his independent career at Yamagata University. His research interests mainly focus on enzyme inhibitors, solid-phase synthesis and total synthesis of peptidyl natural products.



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Rio Suzuki obtained her B.Eng. degree from Yamagata University (Japan) in 2018. She then obtained a Master's degree in 2020 under the supervision of Prof. Hiroyuki Konno. She is currently a researcher at DENKA Co. Ltd. in Japan. Her research focuses on bioorganic chemistry (especially solid-phase peptide synthesis).