Accelerated Linker-Free Solid-Supported Native Chemical Ligation

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Introduction

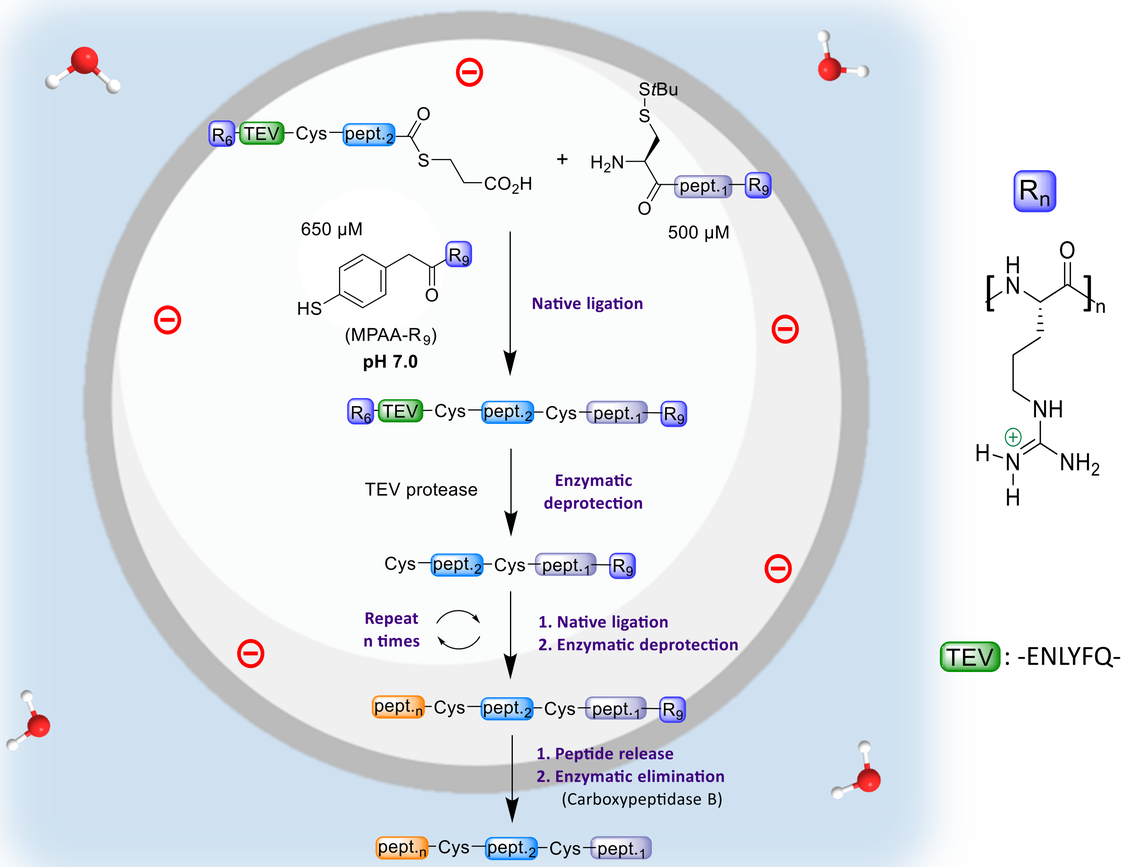
Native chemical ligation (NCL) involves the reaction of a C-terminal thioester peptide with a N-terminal cysteinyl peptide to form a native peptide bond in water. This reaction enables the assembly of unprotected peptide segments either in solution or on a solid support. Combined with the solid phase peptide synthesis (SPPS), NCL allows the synthesis of longer peptide sequences in greater quantities[1,2].

However, chemical protein synthesis by chemoselective concatenation of unprotected peptide segments remains limited. Major challenges include the poor solubility of certain peptides and intermediates in aqueous environments, the need for intermediate purification steps, and the relatively slow kinetics of ligation reactions. To overcome these limits, the solid-phase chemical ligation (SPCL) was developed, but this approach often requires the attachment of the growing peptide chain to the solid support. SPCL methods also face several challenges, including the need for specific attachment chemistry, difficulties to monitor the reaction and slow diffusion which can lead to reduced yields[3].

In this study, we present an alternative strategy to existing SPCL approaches, based on the incorporation of the species into the solid support through electrostatic interactions.

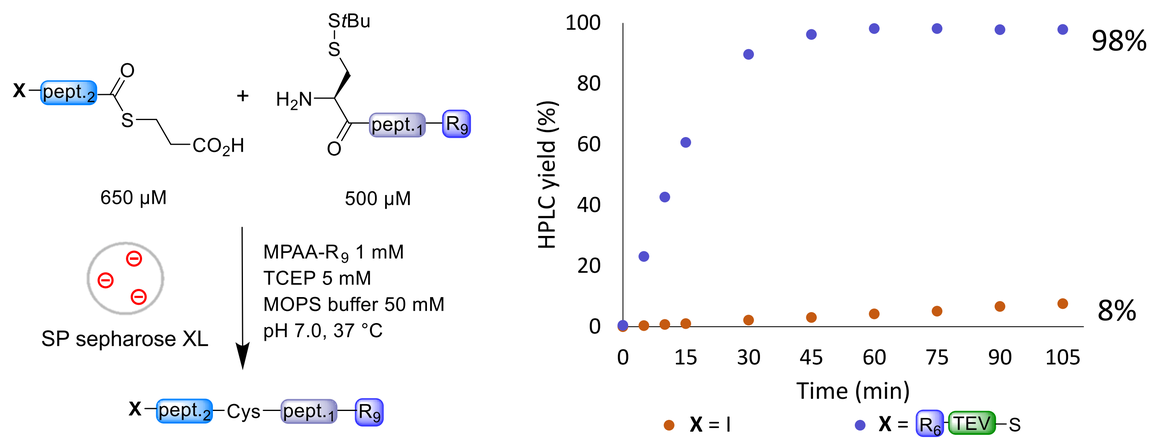
1. Electrostatically-assisted ligation with reversible peptide binding

The SPCL process we develop takes place in an anionic solid support (SP Sepharose XL), enabling the peptide segments and the catalyst (MPAA-R_9) tagged with poly-Arg to be incorporated into the solid support and retained within it at the working pH. Through these interactions, the catalyst only needs to be added once, remains throughout the process and can be recycled at the end.



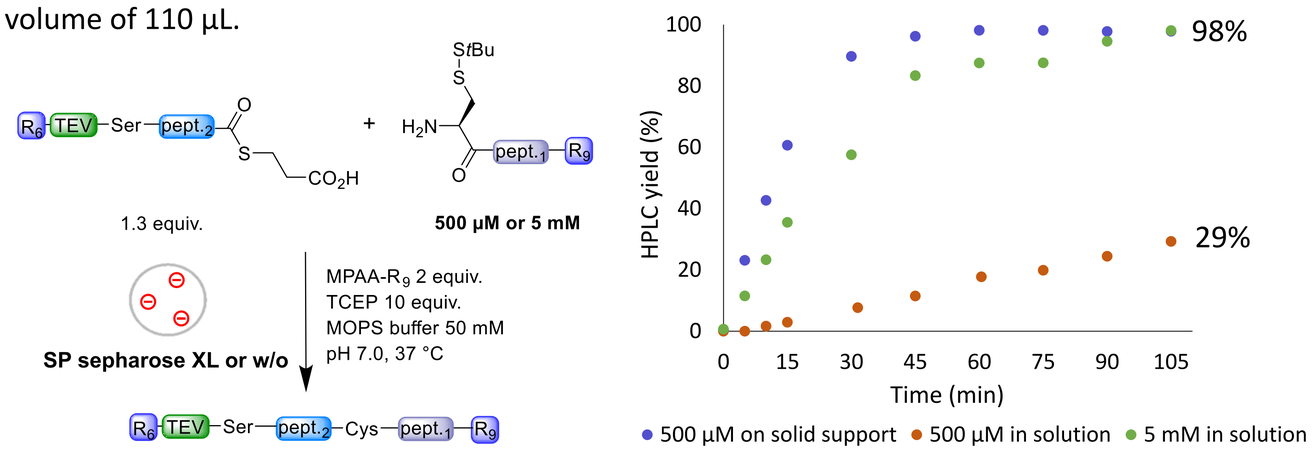
The thioester peptide carries a TEV tag at the N-terminal position, which can be removed by TEV protease. The resulting free cysteine then reacts with another thioester peptide to form a new native peptide bond. Finally, the poly-Arg tag is enzymatically cleaved by carboxypeptidase B after release into solution.

2. Exploring the impact of key parameters on ligation efficiency



The presence of the poly-Arg tag is essential to allow the concentration of all species into the support and enable the acceleration of ligation rate.

The experiment with SP sepharose XL was performed with 10 µL of beads for a total reaction volume of 110 µL.



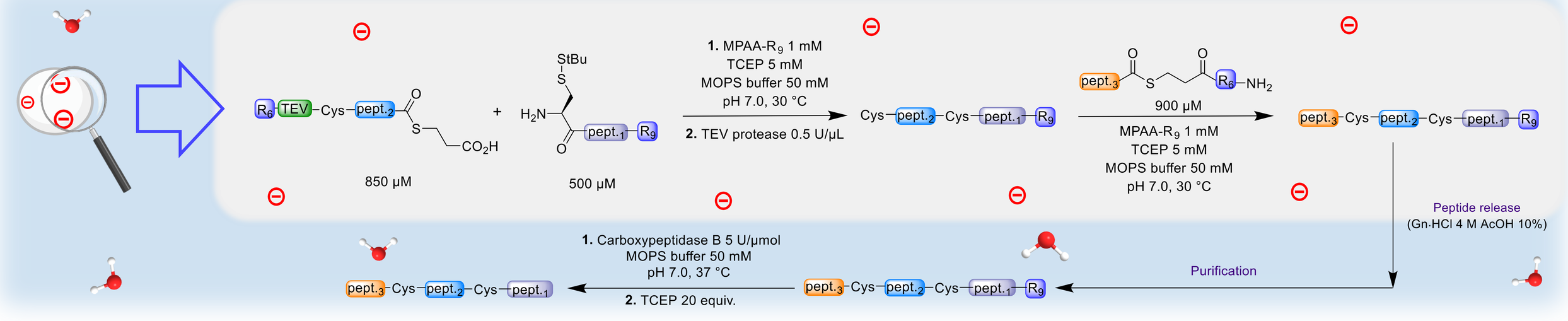
In the absence of the solid support, the reaction is significantly slower despite identical species concentrations (500 µM). Even with tenfold higher concentrations (5 mM) in solution to mimic those within the support, the reaction remains slower than on the solid support. This suggests that mechanisms beyond the concentration effect contribute to the SPCL reaction.

pept.1 : -ILKEPVHGAW- pept.2 : -LKEPVHGA- TEV : -ENLYFQ- R_9 : -RRRRRRRRR R_6 : RRRRRR-

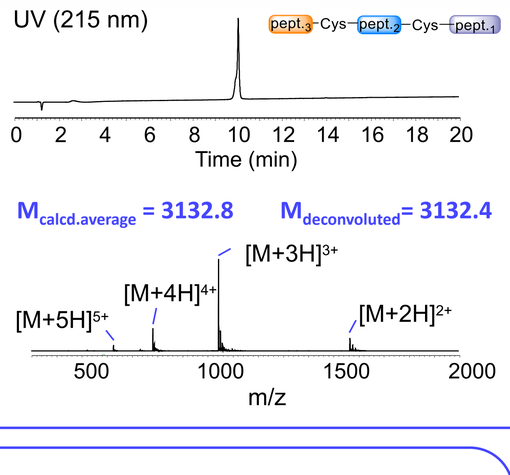
3. Synthetic pathway toward a native polypeptide

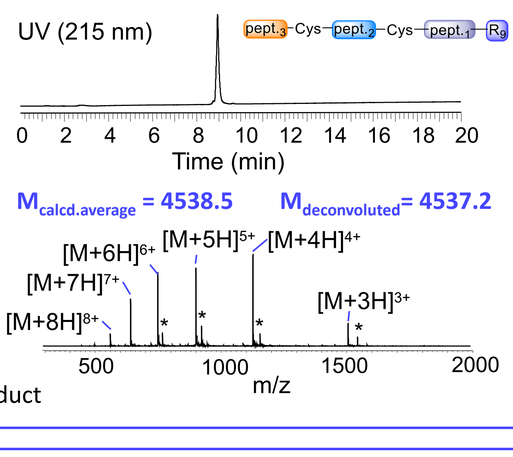
First, the peptide segments and catalyst accumulate within the support, allowing the ligation reactions to take place into it. Electrostatic binding to the support enables rapid release upon increasing the ionic strength, allowing the reaction to be easily monitored at any stage.

pept.1 : -ILKEPVHGAW- TEV : -ENLYFQ-
pept.2 : -LKEPVHGA- R_6 : RRRRRR-
pept.3 : ILKEPVHGA- R_9 : -RRRRRRRRR

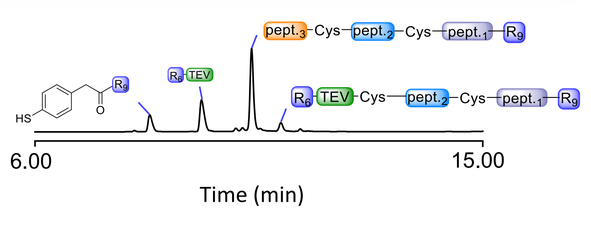


Finally, the R_9-tag was removed in solution to form the native peptide sequence in presence of carboxypeptidase B. The native polypeptide was obtained with a yield of 67%.





* : TFA adduct



Once all ligation steps were completed, the R_9-tagged polypeptide was released from the support and isolated with a yield of 65%.

Conclusion

Electrostatic interactions between the negatively charged solid support and the positively charged poly-Arg tags on the peptide segments and catalyst enhance the ligation rate. The reaction proceeds significantly faster on the solid support than in solution, even at tenfold higher concentrations. Combined with enzymatic deprotection and C-terminal poly-Arg tag removal, this strategy enabled the successful synthesis of a native polypeptide from three peptide segments.

The next objective is to extend this strategy to the synthesis of a full-length protein in its native form.

References

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(2) Agouridas, V.; El Mahdi, O.; Diemer, V.; Cargoet, M.; Monbaliu, J.-C.; Melnyk, O. *Chem. Rev.* **2019**, *12*, 7328-7443.
(3) Agouridas, V., Ollivier, N. ; Vicogne, J. ; Diemer, V. ; Melnyk, O. *Acc. Chem. Res.* **2022**, *55*, 2685-97.

Acknowledgments

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