Efficient TEV tag-assisted one-pot protein semisynthesis

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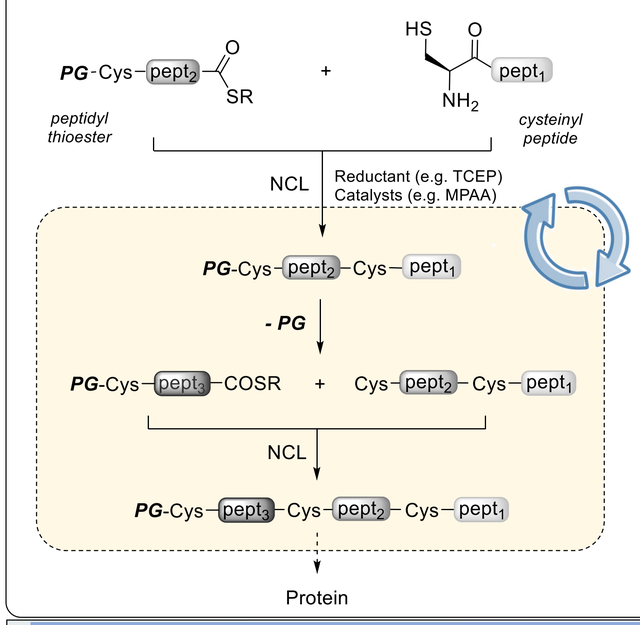
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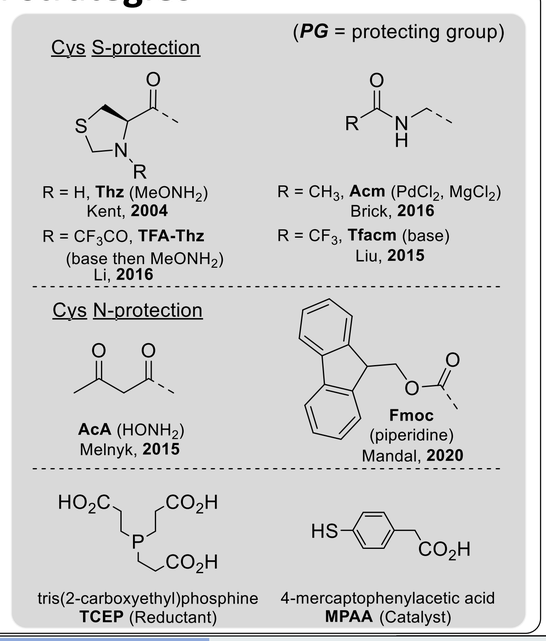
Introduction

Peptide and protein drugs are attracting significant interest due to their high specificity and efficacy.[1] Significant efforts have been made to develop efficient chemical and biochemical processes for the synthesis of these biomolecules, with the repurposing of enzymes for synthetic applications representing an attractive approach.[2,3]

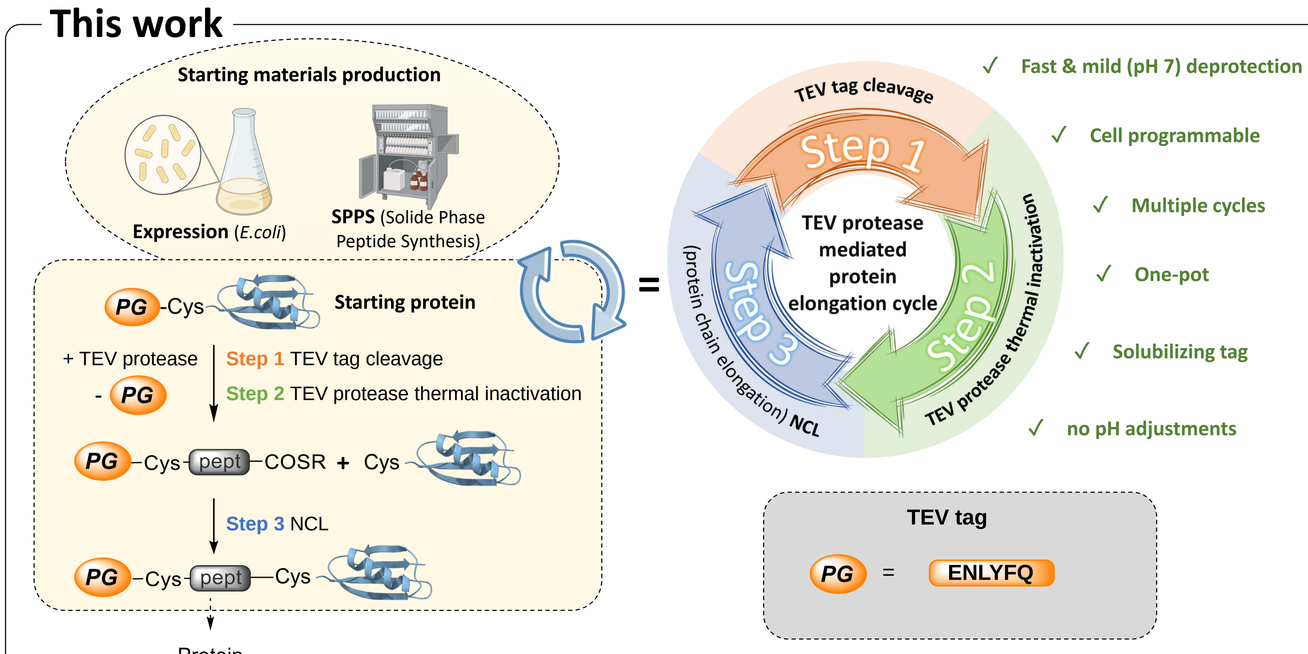
In this work, we designed an innovative and efficient method for protein semisynthesis. This protein elongation strategy exploits the Native Chemical Ligation (NCL) to form the peptide bonds and the Tobacco Etch Virus (TEV) tag as a protecting group (*PG*) for cysteine (Cys) to control the sequential concatenation of synthetic or recombinant peptide segments in water.[4,5] The TEV tag is removed under mild conditions by the addition of TEV protease, an enzyme with high specificity and cleavage efficiency, thereby minimizing the formation of by-products. After cleavage, the enzyme is thermally inactivated in a few minutes, allowing the elongation process to continue in one pot with a new TEV-protected peptide.[6] Iterative cycles afford long polypeptides with high purity and isolated yields.

Existing C to N peptide elongation strategies





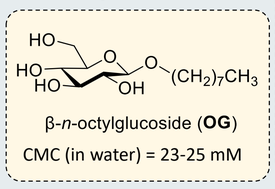
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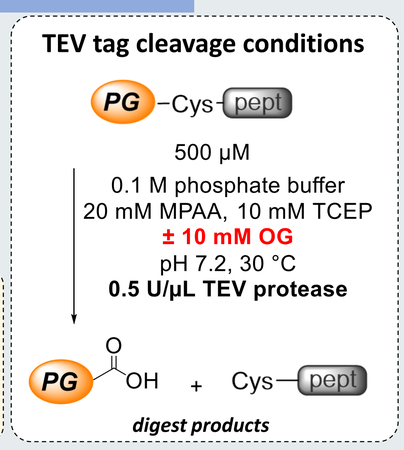


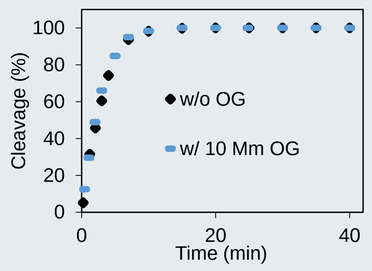
1. NCL and TEV protease buffer conditions

We used OG (10 mM) in the NCL reaction buffer to improve peptide solubility. OG is a non-ionic detergent with a critical micelle concentration (CMC) of 23–25 mM in water. However, several studies have shown that TEV protease activity can be affected by detergents.[6]

We therefore investigated the impact of OG on TEV protease activity.



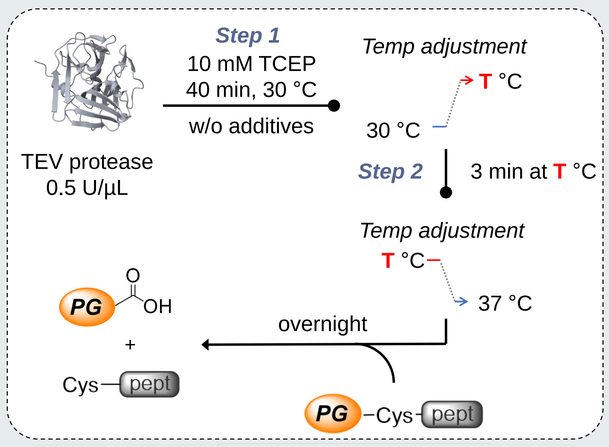




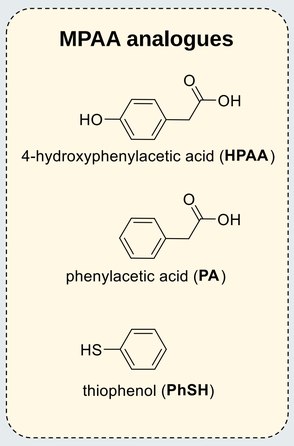
No effect of 10 mM OG on TEV protease activity

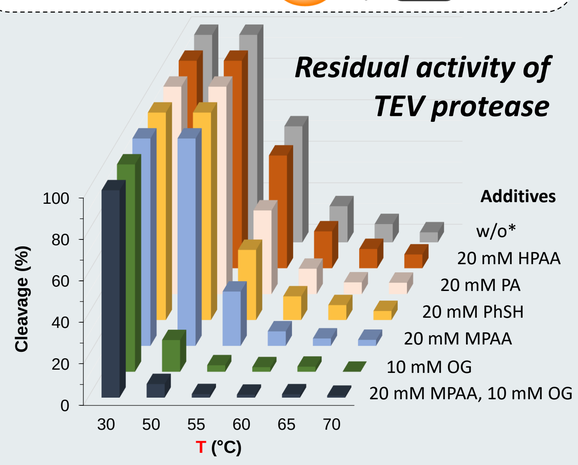
2. Irreversible thermal inactivation and TEV protease melting temperature (T_m): the impact of OG and MPAA

Heat-induced TEV protease inactivation was assessed after 3 min in 10 mM TCEP buffer in the presence of different additives.[7] Interestingly, we observed a significant reduction in residual enzyme activity when MPAA and OG were used, with a pronounced effect when used in combination. MPAA analogues were also tested, revealing the importance of the thiophenol group in MPAA in promoting TEV protease inactivation. We performed complementary studies using nano differential scanning fluorimetry (NanoDSF) that showed a significant reduction of the melting temperature (T_m) of TEV protease when OG and MPAA were used in combination.



MPAA analogues





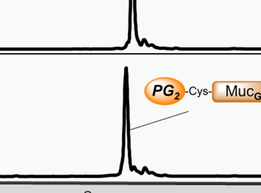
Buffer	TEV protease T_m (°C)
w/o*	54.3
20 mM MPAA	51.2
10 mM OG	49.0
20 mM MPAA, 10 mM OG	40.9

Best inactivation condition: 20 mM MPAA, 10 mM OG, 55 °C

* 10 mM TCEP, 0.1 M phosphate buffer, pH 7.2

3. TEV-assisted elongation cycle

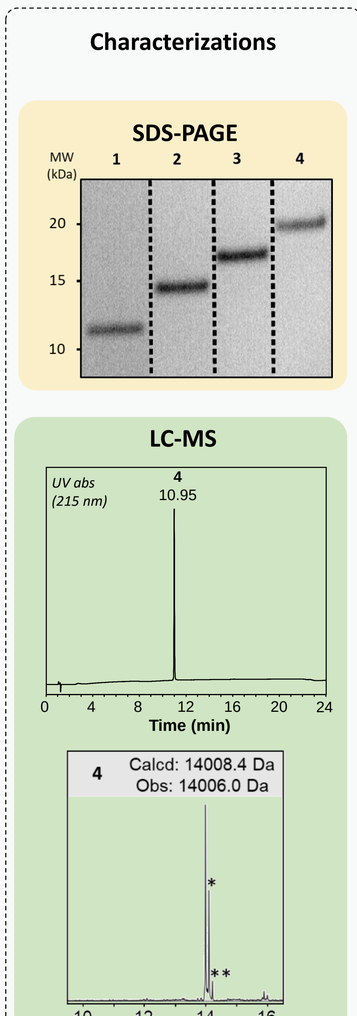
Thus, we designed a TEV-assisted elongation cycle in a buffer containing 10 mM TCEP, 20 mM MPAA, 10 mM OG in 0.1 M phosphate buffer, pH 7.2 as follows:



- Step 1: **TEV tag cleavage** (30 °C, 40 min)
  in situ production of cysteinyl protein
- Step 2: **TEV protease inactivation**
  (55 °C, 3 min)
- Step 3: **NCL**, addition of peptide thioester
  (1 equivalent, 37 °C, overnight)

4. Application of TEV-assisted elongation cycle to protein semisynthesis

The elongation cycle was repeated three times in one pot. We used this method to elongate a recombinant TEV-tagged protein (PG_1-Cys-GB1, 30 nmol, produced in *Escherichia coli* cells) using different mucin1-derived (PG_2-Cys-Muc) peptide thioesters.[8] *PG*s differ by the amino acid sequence preceding the tag and can incorporate charged residues to enhance the solubility of the segments.



Isolated yields: 27% (2), 31% (3), and 31% (4)

Conclusion

The TEV protease-mediated one-pot elongation cycle was successfully demonstrated over three consecutive steps, yielding a 14 kDa polyMuc-GB1 protein.

The combination of OG and MPAA enabled a fast and efficient TEV protease inactivation at a mild temperature of 55 °C, providing suitable conditions for iterative protein elongation.

Acknowledgment We gratefully acknowledge the EPS for funding my conference registration.

References

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