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Facilitating the access to repeat proteins by a novel water-compatible oligomerization process

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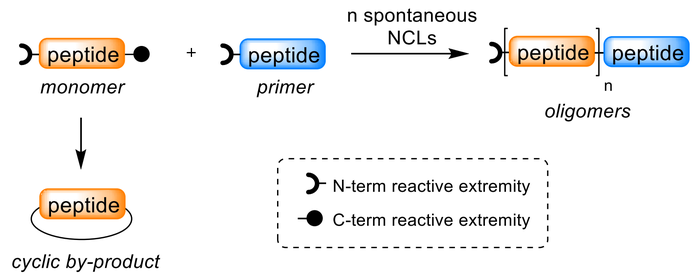
Introduction

In nature, **repetitive motifs** are frequently **contained in proteins**[1] and are known to be associated with important physical or biological functions. Logically, producing such domains is an appealing strategy for engineering minimal protein mimics endowed with desired properties.

In this context, we aim to develop a method enabling the total synthesis of proteins made of repetitive motifs by **spontaneous and chemoselective oligomerization of peptide segments**. Using NCL as an oligomerization method, this strategy is classically complicated by the **intramolecular cyclization of the bifunctional monomer used for polymerization**. To solve this, we exploited the non classical reactivity of the *N*-selenoethyl cysteine (**SetCys**), a chemical device recently discovered by the team,[2] through native chemical ligations (NCL)[3,4] for peptide segments oligomerization.

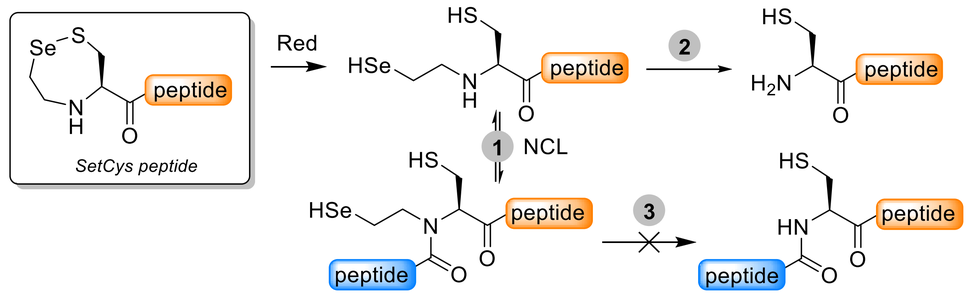
Principle of oligomerization process



1) SetCys properties

The three important SetCys properties for this work:

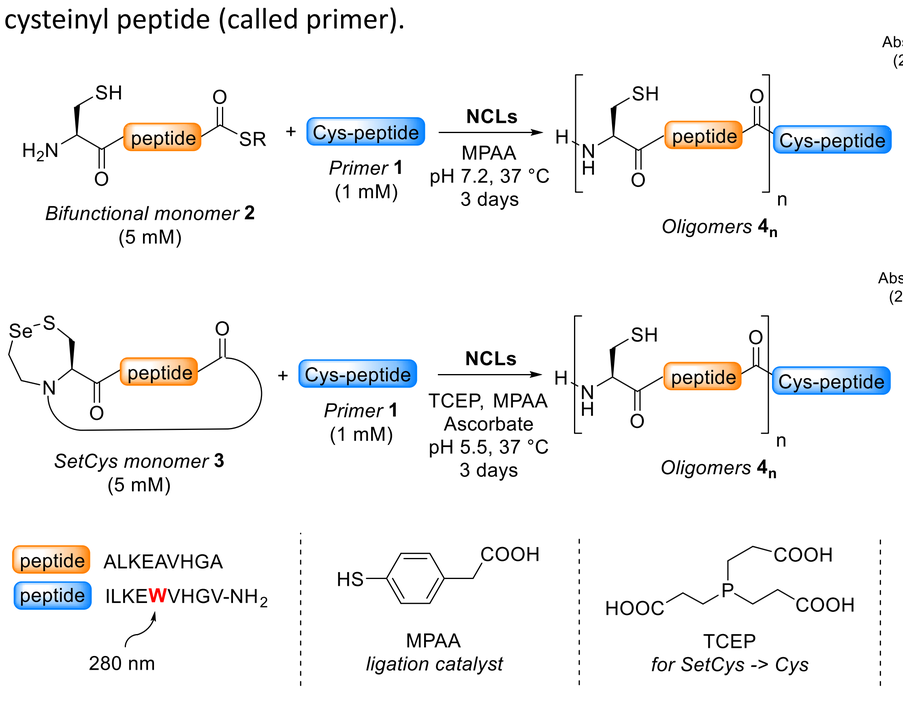
- *Property 1.* ***N*-acylation of the SetCys leads to a reversible amide bond** under NCL conditions
- *Property 2. N*-selenoethyl **arm self-immolation after selenosulfide bond reduction**
- *Property 3. N*-selenoethyl **arm is stable when SetCys α-amino group is acylated**

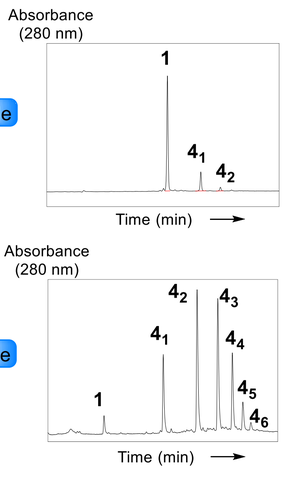


2) SetCys enables peptide oligomerization using NCL

➢ Preliminary results

As a proof of concept, we compared the NCL-mediated oligomerization of peptide segments using monomers equipped with either a Cys or SetCys residue. The reaction is initiated by a cysteinyl peptide (called primer).





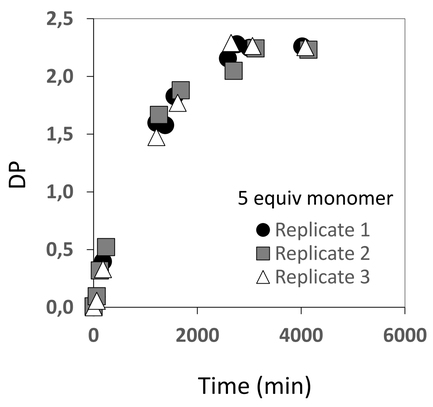
In the case of SetCys monomer, TCEP is added to reduce the selenosulfide bond and hence induce the loss of *N*-selenoethyl arm. The latter process displays the fastest rate at pH 5.5. Sodium ascorbate is used as radical inhibitor to avoid deselenization of SetCys by TCEP. MPAA is used as a catalyst for NCL reactions.[5]

This experiment shows that **using cyclic SetCys monomers enabled peptide oligomerization by spontaneous NCL** reactions whereas Cys bifunctional monomers mainly led to the intramolecular cyclized by-product.

➢ Optimization of the process

The optimization aims to **increase the degree of polymerization (DP)** of the process, i.e. the number of successive ligations.

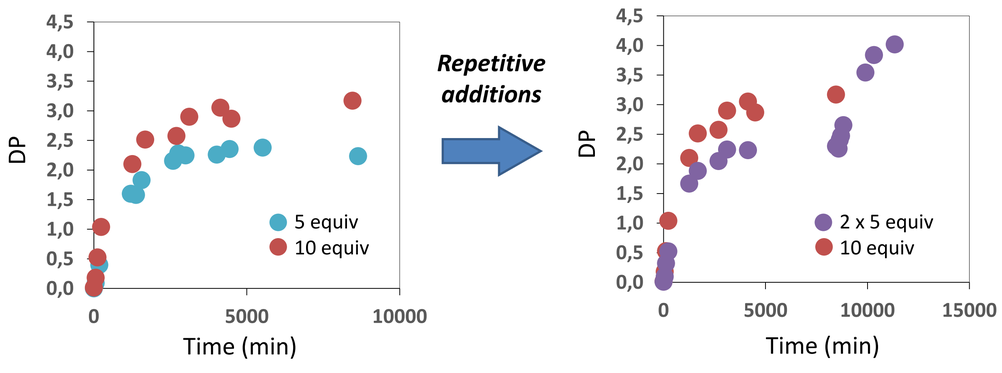
After screening of pH, temperatures, monomer nature and various additives, the optimal reaction conditions resulted in a **DP of 2.4 and 55% conversion of monomer into oligomers** using 5 equivalents of monomer.



3) Repetitive additions of monomer

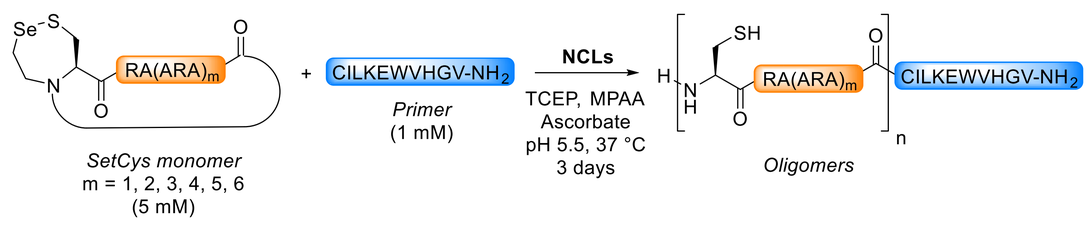
Then, the impact of monomer excess on the length of oligomers was then explored:

- Surprisingly, increasing the **monomer equivalents from 5 to 10** resulted in a small **increase in DP, from 2.4 to 3.0**
- In contrast, **repetitive additions** of 2 x 5 equiv of the monomer led to a further **increase in DP, reaching 4.0**

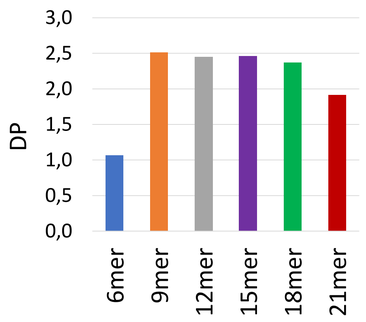


The slow continuous addition of monomer was also performed but the experiments displayed the same results than the one batch addition.

4) Impact of the size of the monomer on DP



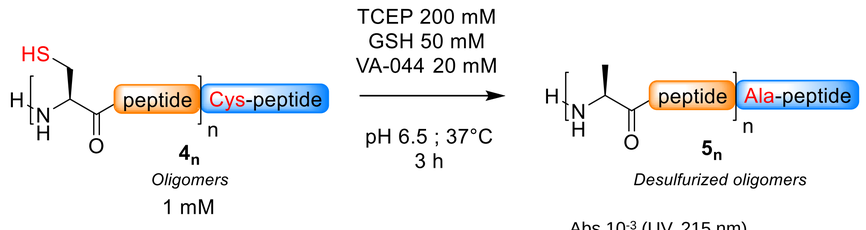
Number of AAs	6	9	12	15	18	21
DP	1.46	2.42	2.33	2.35	2.26	1.86
Isolated yield (%)	23	36	30	36	38	38



The process achieves **optimal DPs** using a monomer containing **between 9 and 18 AAs**.

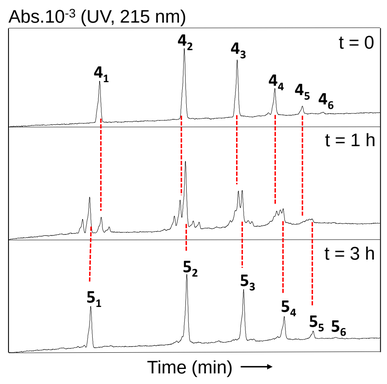
5) Post-oligomerization modification

Due to the high frequency of Ala in proteins, the **combination of NCL with a Cys desulfurization** step is a **powerful synthesis tool** for biological applications.[6,7,8]



To initiate the chemical synthesis of the target proteins, the **compatibility between the oligomerization process and Cys desulfurization** was assessed.

HPLC purified oligomers were engaged in a desulfurization reaction, which was **completed after 3 h** even for the largest oligomers which contain more cysteines.



Conclusion

In literature, the only described strategy to avoid intramolecular cyclization of monomers in oligomerization processes is the conformational rigidification of monomers.[9] However, this strategy does not enable the oligomerization of most of the small peptides due to their conformational flexibility.

We **achieved and optimized an innovative oligomerization process** using **SetCys residue.** The fact that the process favours the formation of oligomers over of intramolecular cyclization is ascribed to the **reversibility of the Xaa-SetCys peptide bond under NCL conditions.**

Moreover, the compatibility of the process with post-oligomerization modifications has been established. This **enlarges the scope of the study** and will **enable the synthesis of bio-inspired and functional repeat protein mimics**.

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