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Fast-SEA ligation: a chemoselective NCL-inspired ligation reaction effective in the nanomolar concentration range

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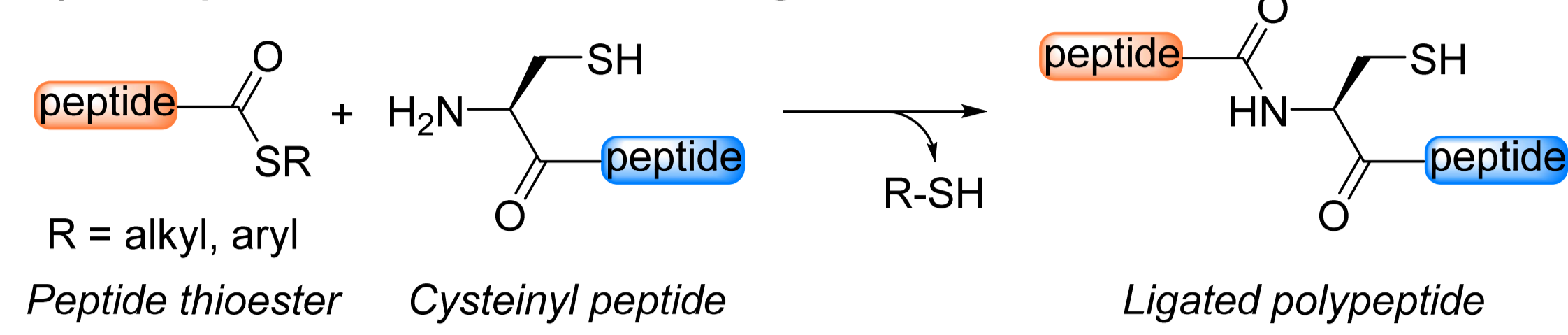
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See: B. Snella, B. Grain, J. Vicogne, F. Capet, B. Wiltschi, O. Melnyk, V. Agouridas. *Angew. Chem. Int. Ed.* **2022**, *asap*

Introduction

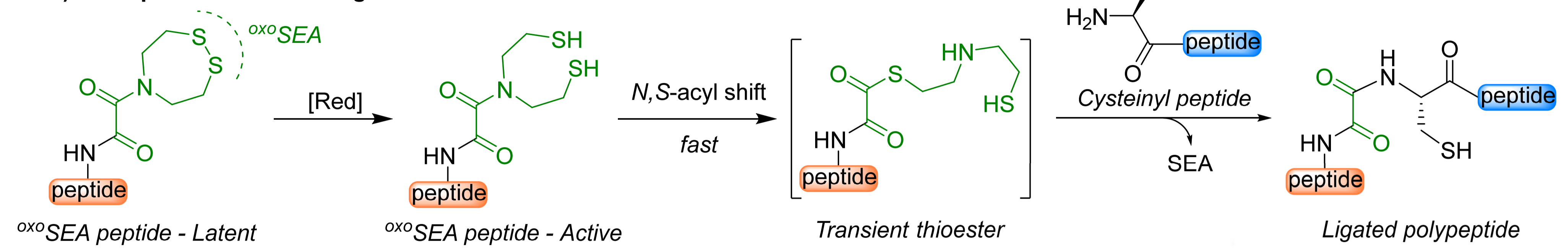
The **Chemical Protein Synthesis** has highly benefitted from the properties of the thioester functional group, in particular with the development of the **Native Chemical Ligation** (NCL) reaction.^{1,2} However, **NCL-based modification of proteins** suffers from the moderate reactivity of classical alkyl and aryl thioesters³, especially in highly dilute media. Seeking for fast reactive thioesters surrogates, we have discovered that **oxalyl amide SEA** (^{oxo}SEA), in the form of a latent thioester precursor,⁴ could, upon activation, act as a powerful acylating agent in chemoselective ligation reactions with β -aminothiols such as the cysteine residue.

a) Principle of the Native Chemical Ligation



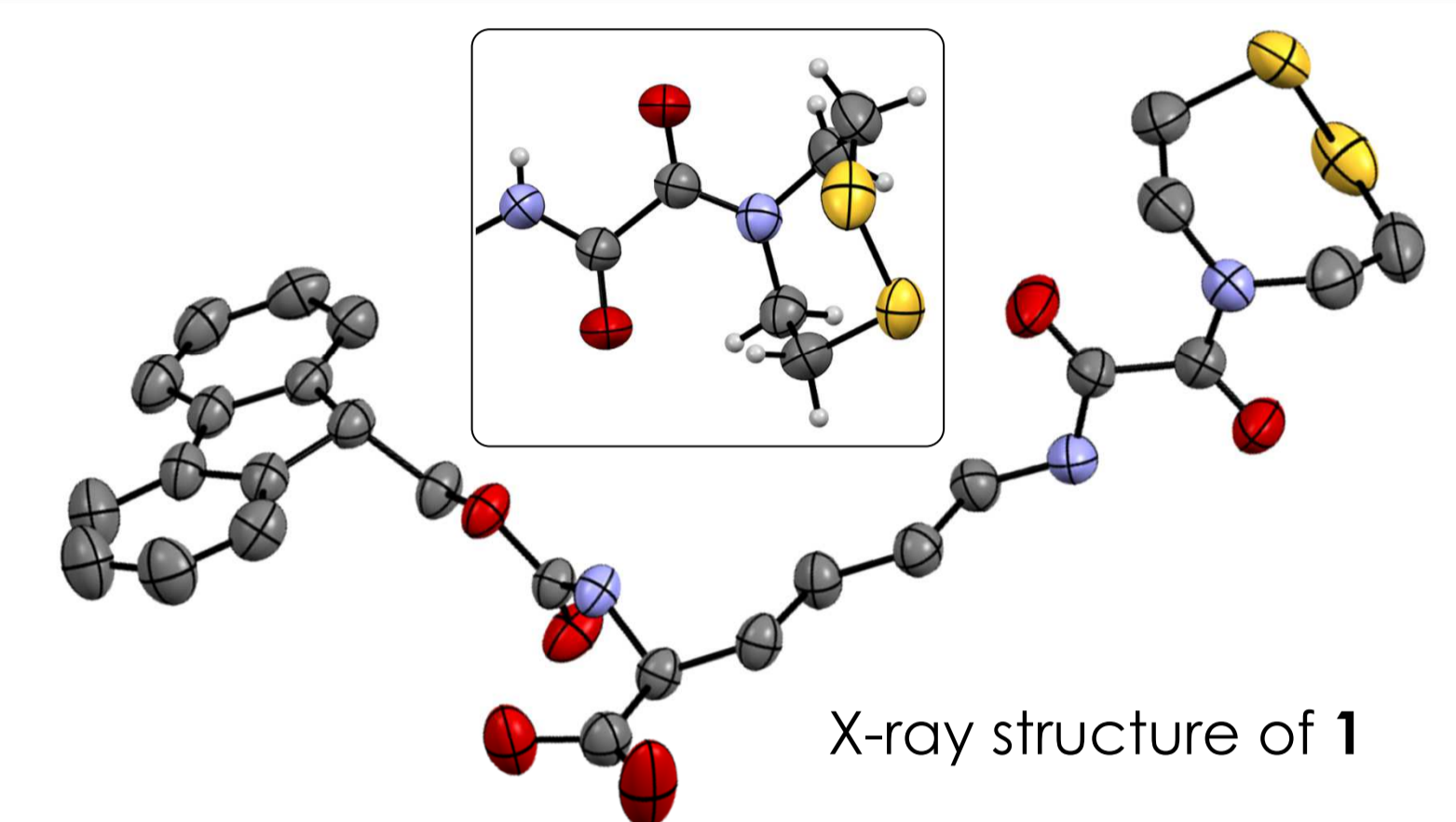
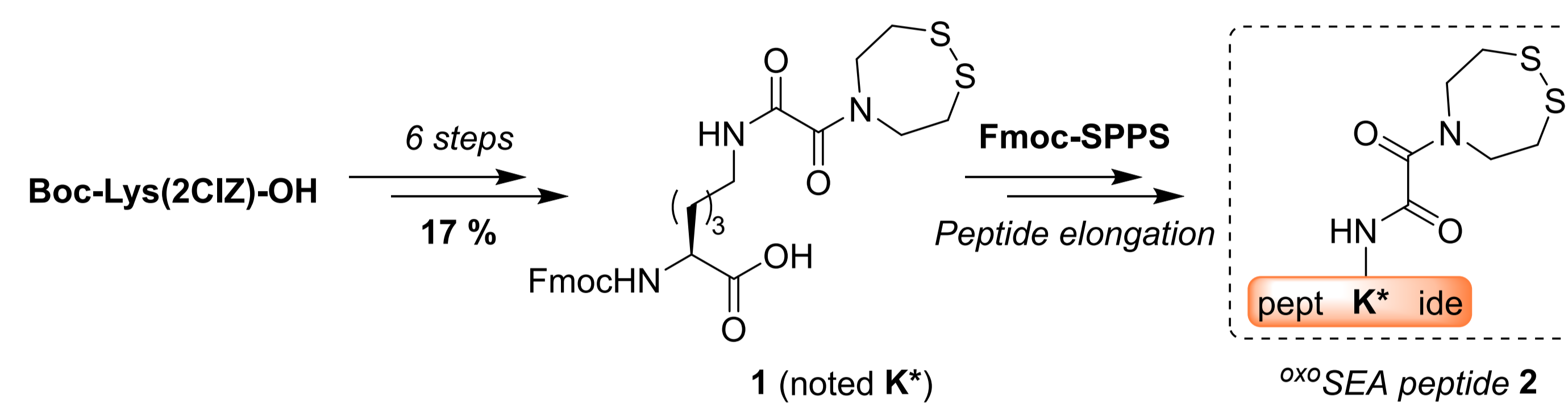
Rate constant of the ligation:
~ 0.3 to 4 M⁻¹ s⁻¹ for NCL
~ 30 M⁻¹ s⁻¹ for ^{oxo}SEA ligation

b) Principle of the ^{oxo}SEA Ligation



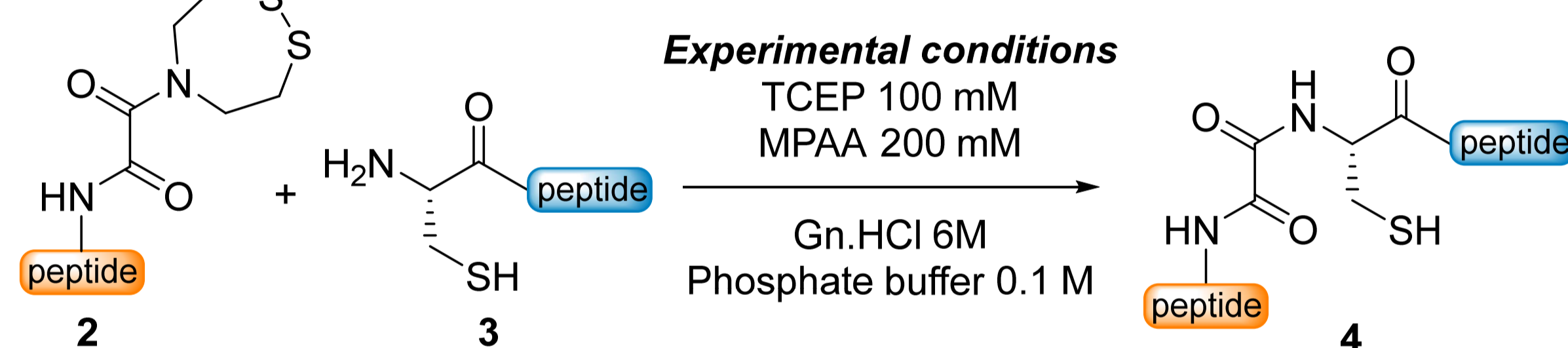
1) Synthesis of ^{oxo}SEA peptides

- Synthesis of protected lysine derivative **1** at the **gram scale** with **17% overall yield**
- Introduction of Fmoc-Lys(^{oxo}SEA)-OH **1** within peptide segments at **any position**, according to standard Fmoc-SPPS protocols



2) ^{oxo}SEA Ligation study

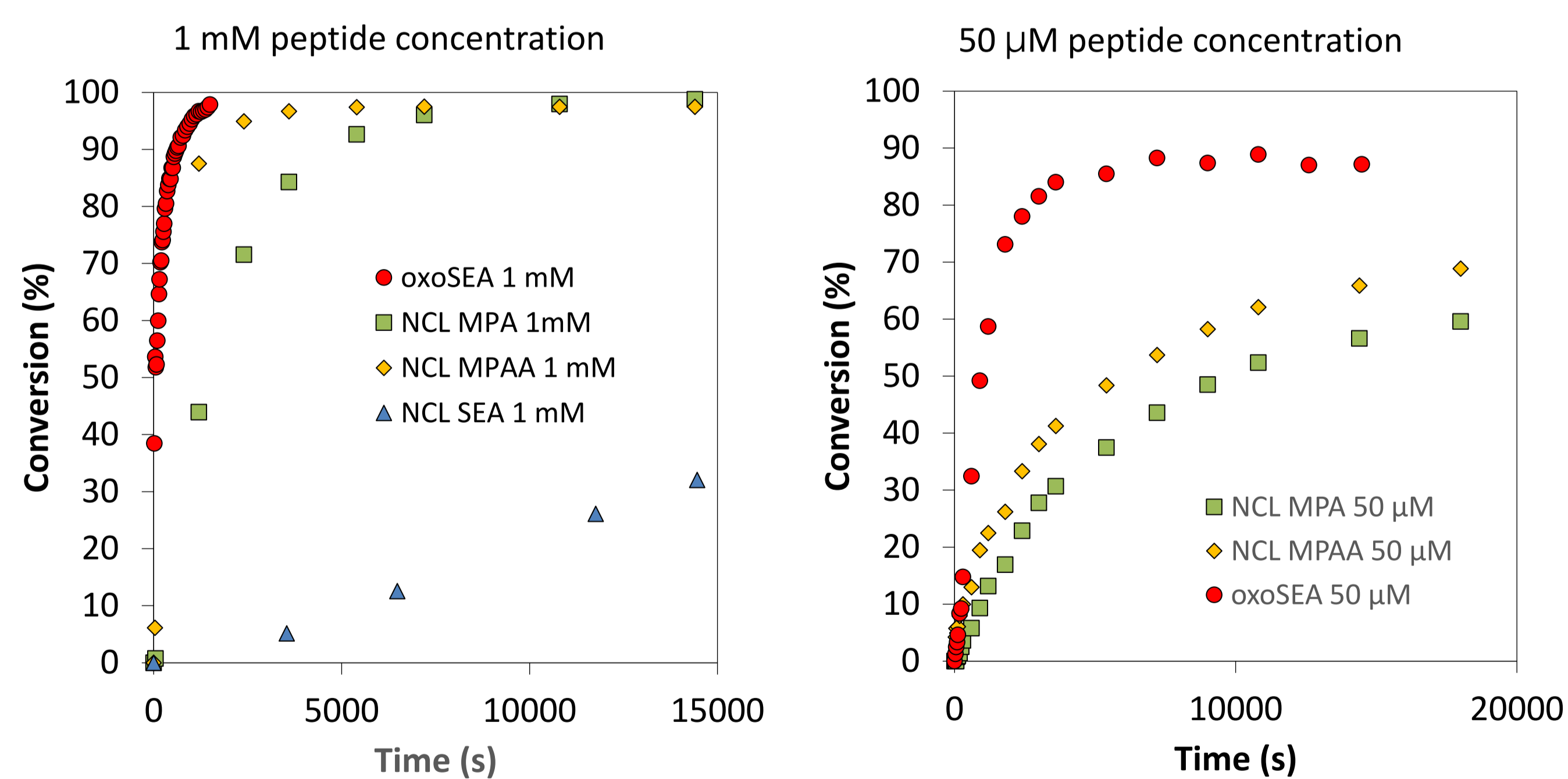
Investigation on the reaction of ^{oxo}SEA peptide **2** with cysteinyl peptide **3**.



^{oxo}SEA ligation with a peptide concentration of 1 mM is:

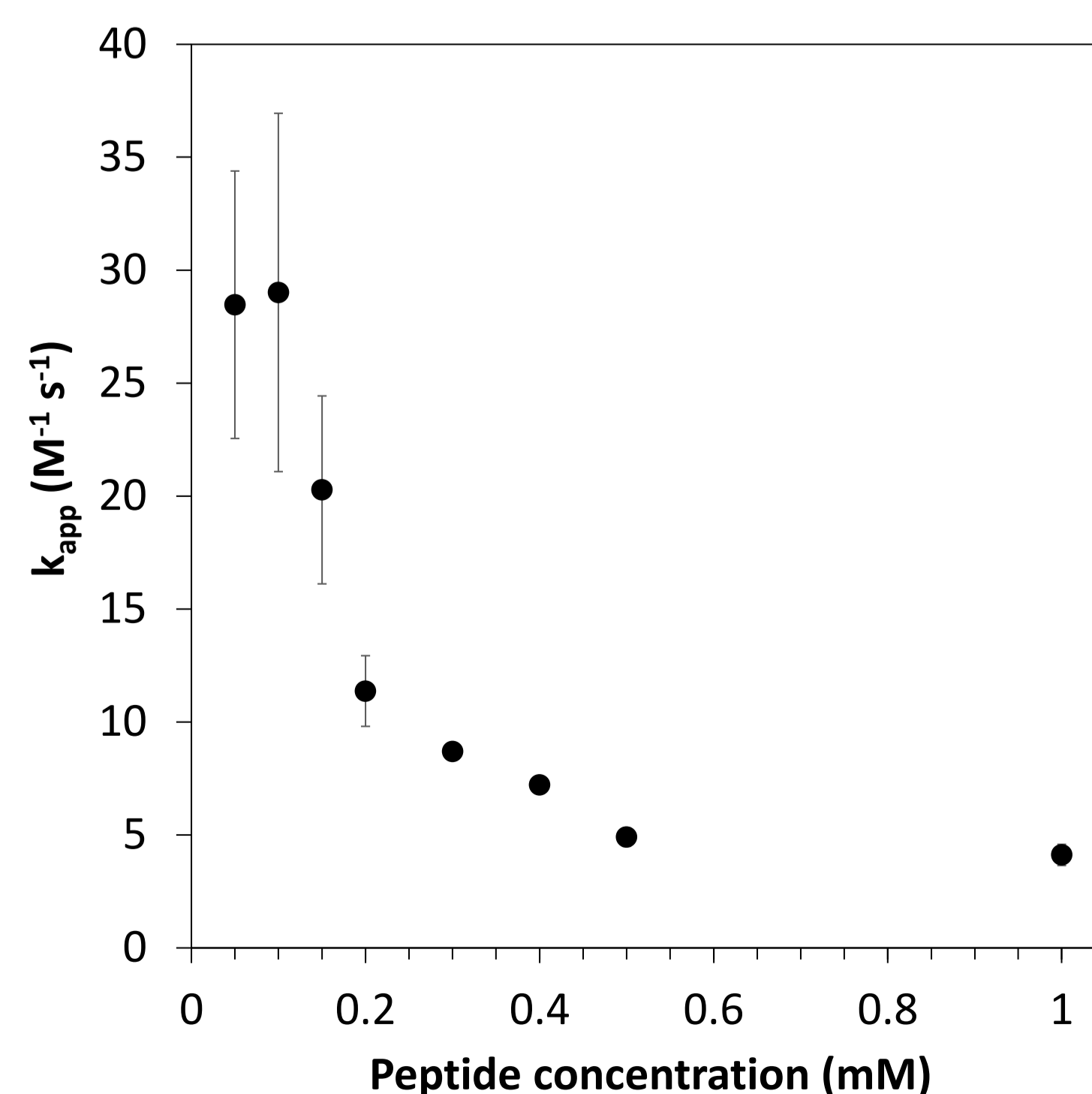
- **Efficient** (HPLC conversions to polypeptide **4** beyond 95%)
- **Fast** (Half-reaction time of 200 s)
- Optimal at a **working pH = 5,5**
- **Triggered by** reductants (TCEP)

Comparison with classical NCL rates highlighted the tremendous reactivity of the ^{oxo}SEA group, even more when decreasing peptide concentration to 50 μ M.



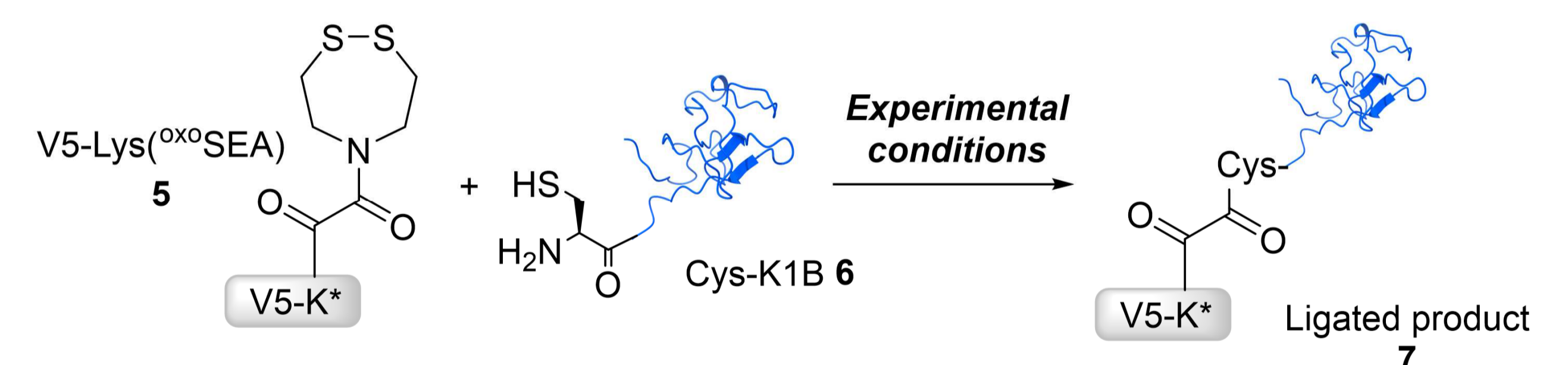
Determination of apparent second-order rate constant for the ^{oxo}SEA ligation in the micromolar concentration range:

- Diminution of the peptide concentration led to the increase of the rate constant
- Rate break at 150 μ M, and below this concentration value, apparent second-order rate constant thrives around 30 M⁻¹ s⁻¹

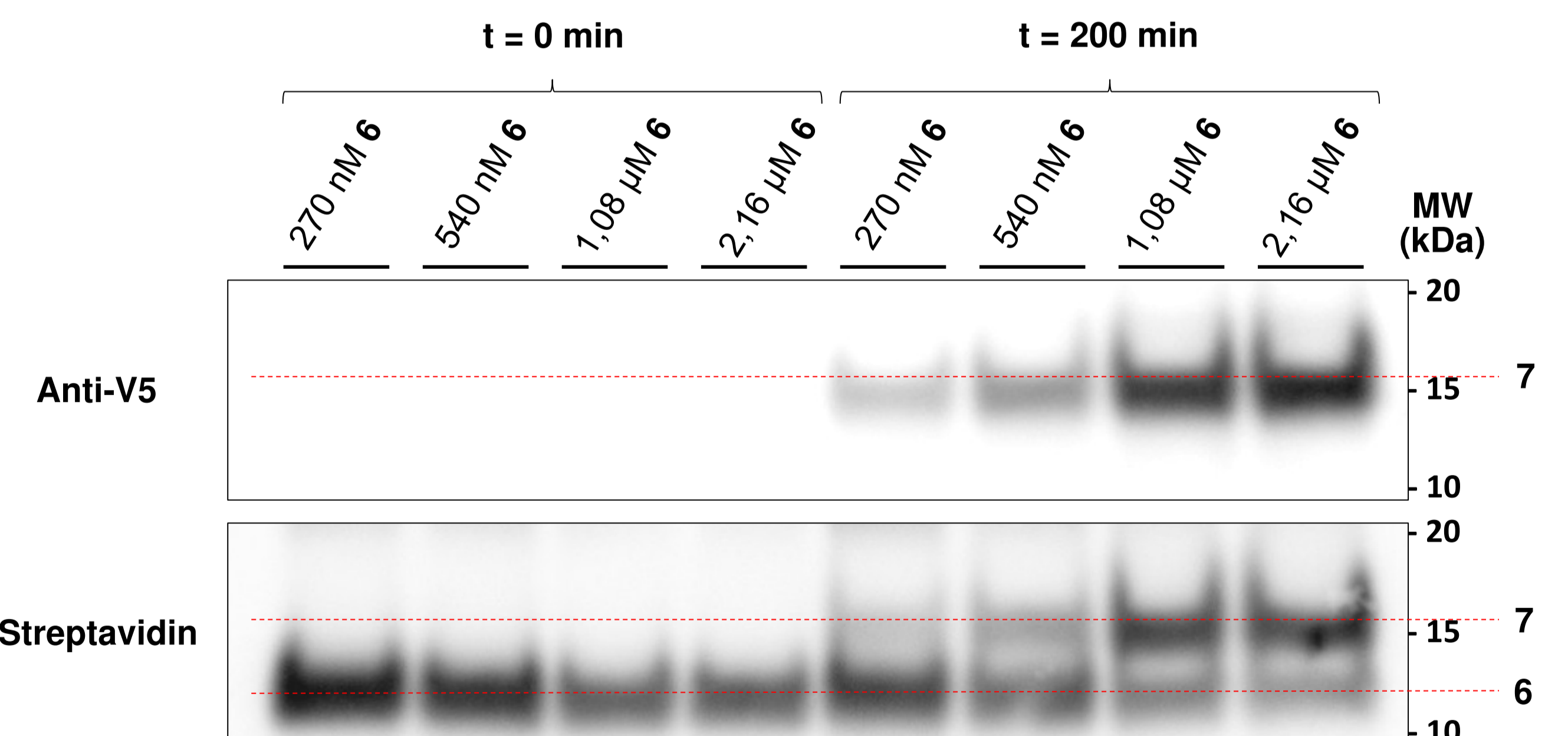


3) Nanomolar concentration range

To further explore the performance of the ^{oxo}SEA-mediated ligation in the **nanomolar concentration range**, we opted for SDS-PAGE separation followed by Western Blot as the analysis tool. This allowed us to work at the protein level by monitoring the reaction between the ^{oxo}SEA peptide derived from V5 peptidic tag **5** and the C-terminally biotinylated kringle 1 domain of the hepatocyte growth factor (K1/HGF) equipped with a N-terminal cysteine **6**.



- Detection of ligated product **7** after 200 minutes of reaction
- ^{oxo}SEA ligation effective in highly dilute solution, with peptide concentration of **270 nM**



Moreover, this ligation reaction is also efficient and chemoselective in a **complex protein medium**, as it has been successfully performed in a crude protein extract of a THP-1 whole cell lysate supplemented with 100 nM of **6** in the presence of a 15-fold excess of **5** (see article).

Conclusion

The groundbreaking reactivity of this **activatable oxalyl thioester surrogate** allows us to perform peptide and protein modification through a **chemoselective ligation reaction**. The latter proceeds extremely fast in aqueous media, under mild conditions and in **highly dilute medium** which can be as complex as a cell lysate. By being easy to introduce within peptide segments in the form of a lysine derivative, oxalyl thioesters might be of significant importance for the development of novel **crosslinking strategies** or the **polycondensation of water-soluble (bio)molecules**.

