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Broaden the Applications of Peptidyl Asparaginyl Ligases

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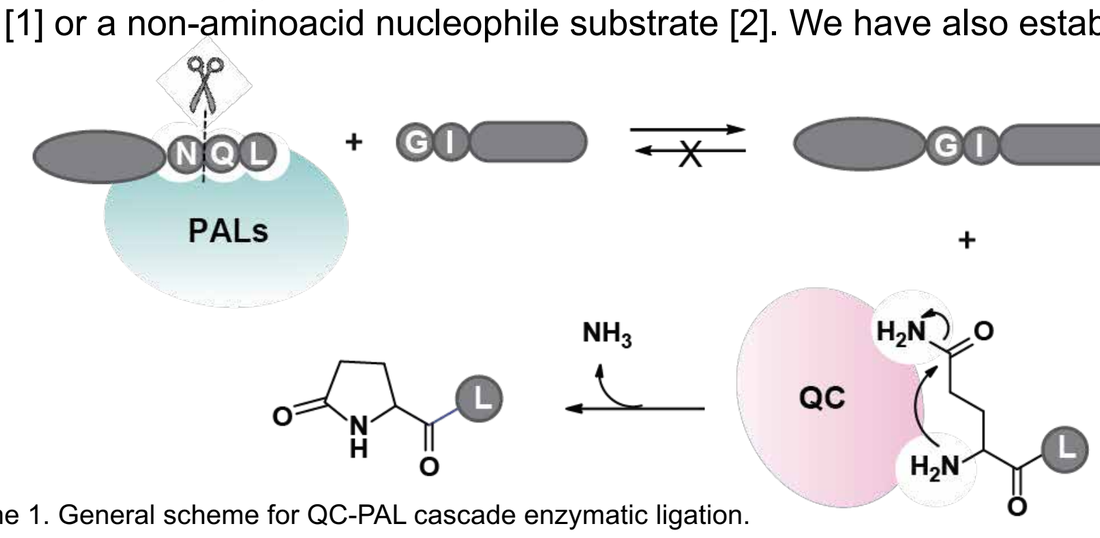
INTRODUCTION

Peptidyl asparaginyl ligases (PALs) are transpeptidases that recognize substrates with an Asx-Xaa-Yaa (P1-P1'-P2') tripeptide motif, where P1-Asx is Asn or Asp. PALs cleave the peptide bond after the Asx residue to form an acyl-enzyme thioester intermediate which is subsequently resolved by the N^{α}-amine of an incoming nucleophile peptide, forming a new Asx-peptide bond. Owning to their short recognition motifs and high catalytic efficiency, PALs have been used in many applications, such as peptide/protein macrocyclization, protein C- or N-terminal conjugation and cell-surface labelling. In our previous work, we have discovered that PALs can accept an unnatural amino acid as the P1 residue [1] or a non-aminoacid nucleophile substrate [2]. We have also established a method that overcomes the reversibility of PAL-mediated ligation – a major shortcoming of the transpeptidation reaction – by coupling it to glutaminyl cyclase (QC)-catalysed pyroglutamyl formation (Scheme 1) [3]. By quenching the nucleophilic α-amine of a P1'-Gln in a lactam, PAL-mediated ligation becomes irreversible and can achieve near-quantitative yields. Thus, this cascade reaction scheme greatly improves the efficiency of PAL-mediated ligation. Recently, we show that the ligation yields of non-aminoacid nucleophiles with antibody can also be improved using QC-PAL cascade reaction scheme. In this poster, we will present various examples of bioconjugates, including antibody-drug conjugates, that are prepared by PAL-mediated ligation. These examples further establish PALs as precision biomanufacturing tools for protein-based biologics.



Scheme 1. General scheme for QC-PAL cascade enzymatic ligation.

RESULTS

1. Protein-peptide ligation

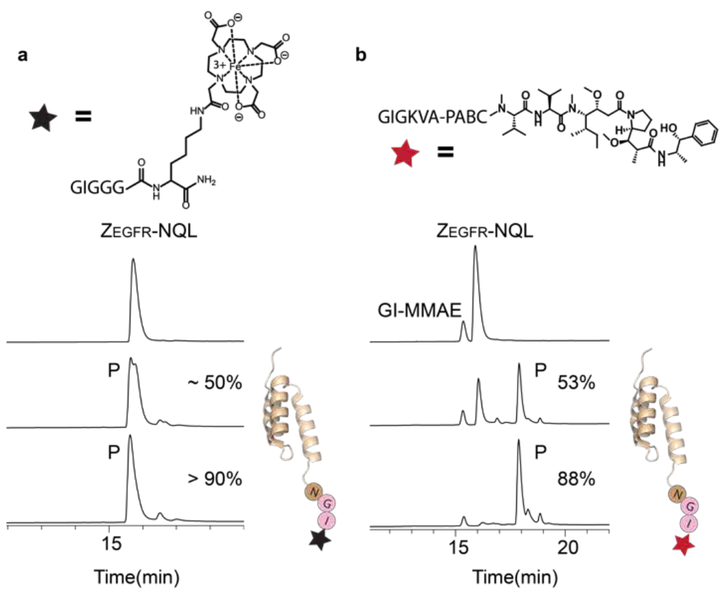


Figure 1. PAL-QC coupled cascade ligation of affibody Z_{EGFR} with DOTA and MMAE peptides. Yields are estimated by HPLC analysis, in the presence or absence of QC. Black and red stars refer to DOTA and MMAE peptides, respectively.

2. Protein-protein ligation

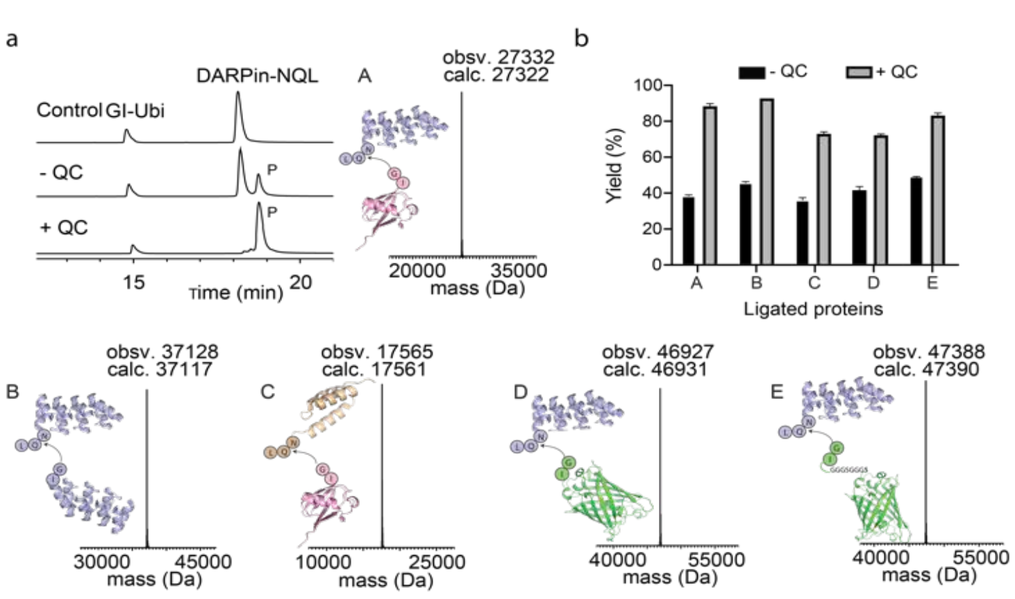


Figure 2. PAL-QC coupled cascade protein-protein ligation. (a) HPLC monitoring of VyPAL2-mediated ligation between DARPin and ubiquitin. (b) Yields of various protein−protein ligations in the presence or absence of QC [DARPin (purple), ubiquitin (pink), Z_{EGFR} (yellow), GFP (green)].

3. Large protein ligation

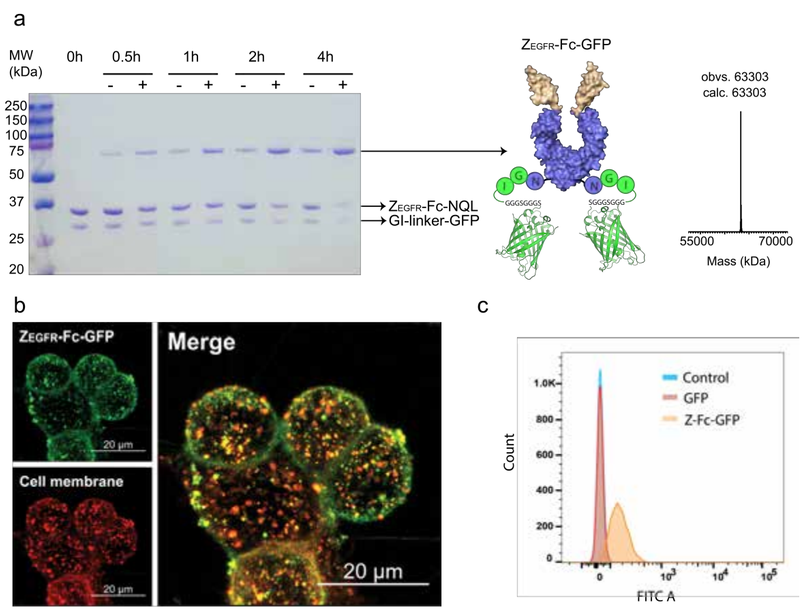


Figure 3. PAL-QC coupled cascade ligation of large proteins. a) SDS-PAGE of the ligation reaction. b). Confocal microscopy image of ZEGFR-Fc-GFP binding to A431 cells c). Flow cytometry analysis of ZEGFR-Fc-GFP targeting A431.

4. Antibody-drug conjugation

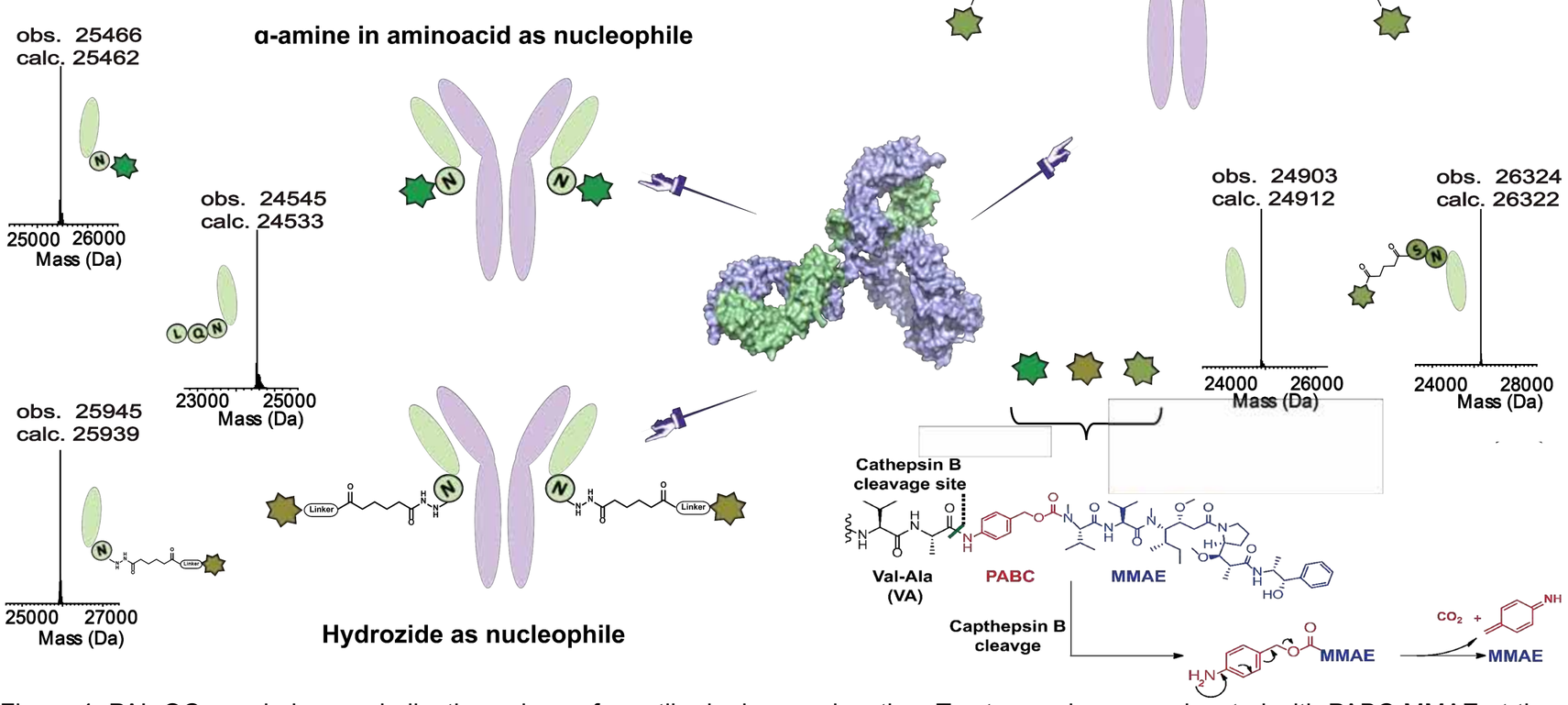


Figure 4. PAL-QC coupled cascade ligation scheme for antibody-drug conjugation. Trastuzumab was conjugated with PABC-MMAE at the N or C terminal of the light chains of Trastuzumab.

5. Cytotoxicity of protein-drug conjugates

Compound	IC_{50} (nM) BT-474	MCF7
MMAE	0.05 ± 0.01	0.08 ± 0.02
Trastuzumab	> 100	> 100
MMAE-PABC-Trastuzumab (DAR2)	2.49 ± 0.05	> 100
Trastuzumab-PABC-MMAE (DAR2)	0.16 ± 0.01	> 100
Compound	**A431**	**MCF7**
Z_{EGFR}-Fc-PABC-MMAE	69.53 ± 28.9	> 100
Z_{EGFR}-PABC-MMAE	12.89 ± 3.02	> 100

Table 1. IC_{50} of MMAE-labelled proteins on A431 (EGFR+), BT-474 (HER2+) and MCF7 (EGFR-/HER2-) cells. IC_{50} values were presented as mean ± SEM.

CONCLUSION

To sum up, we successfully used the PAL and QC enzymes for ADC preparation. The synthetic ADCs showed high selectivity towards HER2+ cell lines (BT-474). These results show that the PAL-QC cascade ligation scheme is a powerful tool for ADC generation.

ACKNOWLEDGEMENTS

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