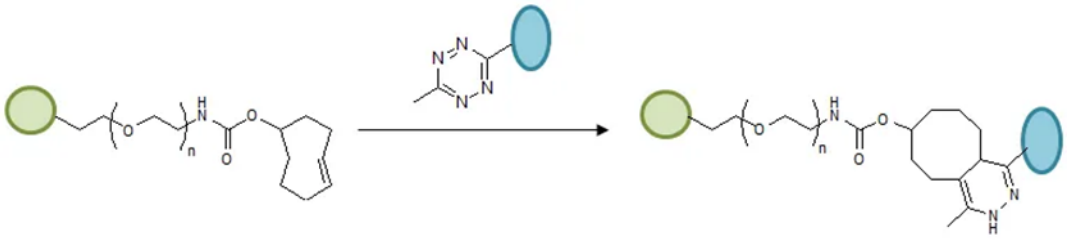
Tetrazine-TCO Ligation

Introduction

This ligation chemistry is based on an inverse–demand Diels–Alder cycloaddition reaction between a trans–cyclooctene and tetrazine reaction pair, forming a dihydropyridazine bond. The Tz and TCO pair shows very high reaction specificity. Its exceptional fast kinetics are reported 1~1x10^6 M^{-1} s^{-1} and is so far the most efficient bioorthogonal reaction reported in the literature. This irreversible process leads to the release of N2 gas, as the only side product during the reaction. The reaction between Tz and TCO can be monitored spectroscopically by following the disappearance of the absorption band between 510 and 550 nm.



Reaction condition: PBS buffer, pH: 6-9; room temperature

The reaction can be set in organic solvents, water, as well as biological media, and does not require activation by a catalyst. Moreover, reactants can be used at very low concentration for their conjugation to large biomolecules due to the high chemoselectivity of the reaction. Trans-cyclooctene (TCO) is seven-fold more reactive than the cis-cyclooctene (CCO) in IEDDA reaction. and they are not reactive toward thiols, amines and other potential nucleophiles present in the biological system.

The combination of ultrafast kinetics, selectivity, and long–term aqueous stability makes TCO–Tz the ideal pair in low concentration applications such as protein–protein conjugations.

Features:

- Biocompatibility – no catalyst required – good for in-vivo applications.
- Mild conditions – conjugation in aqueous buffered media and at low temperature,
- Stability – TCO and Tz moieties are long term stable
- Efficiency – ultrafast kinetics, formation of a stable triazole in quantitative yield, under highly dilute conditions,
- Specificity and Bioorthogonality: TCO reacts only with Tz in the presence of -NH2, -SH, -COOH and other protein functional group

Example Protocol (protein-protein conjugation)

Protein 1 activation by TCO-NHS

100 µg of protein 1 is mixed with 5 µl of 1M NaHCO3 with 100 µl of the PBS-based solution.

20 nmol of TCO-PEG12-NHS ester was added to the mixture. Those reaction mixtures were kept at room temperature for 60 minutes. Desalting procedure is followed by using spin desalting column. The recovery protein amount after desalting was calculated as ~75 µg.

Protein 2 activation by methyl-tetrazine

100 µg of protein 2 is mixed with 5 µl of 1M NaHCO3 with 100 µl of the PBS-based solution.

20 nmol of methyl-tetrazine-PEG8-NHS ester was added to the mixture. Those reaction mixtures were kept at room temperature for 60 minutes. Desalting procedure is followed by using spin desalting columns (Thermo Fisher, Carlsbad, CA). The recovery protein amount after desalting was calculated as ~75 µg.

IEDDA Conjugation

Cross-linking reaction was initiated by mixing the two reaction mixtures. TCO-Protein1 was mixed with Tz-protein2 in 1:1 molar ratio and rotate for 1 hour. The conjugates are ready to use.

Reference

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3. Braun, Klaus et al., International Journal of Medical Sciences, 7(6), 326-339; 2010.