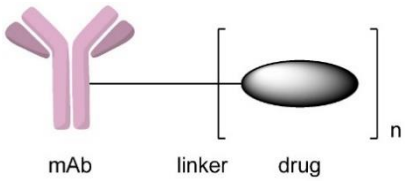
ADC Conjugation by TCEP Reduction with Maleimide Drug Linker

Maleimide-PEG modified drug can be conjugated to the antibody at the pH 7.5 buffer solution contained 50 mM sodium phosphate, 50 mM sodium chloride and 2 mM EDTA The disulfide bridges in the antibody were then reduced with various molar equivalents of TCEP at 30°C for 2 hours to afford different numbers of available sulfhydryl groups on the antibody molecule, then the reduced antibody was then conjugated with various molar equivalents of maleimide-PEG modified to afford a range of different DARs.



Condition: a. TCEP at 30°C for 2 hours; b maleimide-drug, PBS, 7.4, EDTA

Example protocol

Note: the example is for H32 monoclonal antibody -MMAE conjugation. The conjugation of other maleimide-drug to other antibody can follow the protocol accordingly.

MMAE with maleimide-modified VC was conjugated to the H32 monoclonal antibody at pH 6.5-7.5 conjugation buffer solution contained 50 mM sodium phosphate, 50 mM sodium chloride and 2 mM EDTA by Michael addition to form H32-VCMMAE. The disulfide bridges in H32 antibody were then reduced with various molar equivalents of TCEP at 30°C for 2 hours to afford different numbers of available sulfhydryl groups on the antibody molecule, then the reduced antibody was then conjugated with various molar equivalents of VCMMAE to afford a range of different DARs.

Reduction

A solution of antibody (250 µL, 48 mg/mL in 15 mM histidine, 50 mM sucrose, 0.01% polysorbate-20, pH 6) was diluted with 4.2 mM histidine, 50 mM trehalose, pH 6 (750 µL), and EDTA (25 mM, 4% v/v). The pH was adjusted to ~7.4 using Tris-HCl (1 M, pH 8) after which tris(2- carboxyethyl)phosphine hydrochloride (TCEP.HCl, 10 mM, 20 equiv) was added, and the resulting mixture was incubated at room temperature (RT) for 1−3 h. The excess TCEP was removed by a Viva spin centrifugal concentrator (30 kDa cutoff, PES) using 4.2 mM histidine, 50 mM trehalose, pH 6. The pH of the resulting antibody solution was raised to ~7.4 using Tris-HCl (1 M, pH 8) after which dehydroascorbic acid (DHAA, 10 mM, 20 equiv) was added and the resulting mixture was incubated at RT for 2 h.

Conjugation and separation

a solution of linker−drug (10 mM in DMA) was added to the antibody solution above, if needed, DMA was added followed by the final concentration of DMA was 5−10%. The resulting mixture was incubated at rt in the absence of light for 1−2 h. In order to remove the excess of linker drug, activated charcoal was added and the mixture was incubated at rt for at least 0.5

h. The charcoal was removed using a 0.2 µm PES filter, and the resulting ADC was formulated in 4.2 mM histidine, 50 mM trehalose, pH 6, using a Viva spin centrifugal concentrator (30 kDa cutoff, PES).

Analysis of DAR distribution by hydrophobic interaction chromatography

The DAR distributions of the ADCs were analyzed by hydrophobic interaction chromatography (HIC) with a TSKgel Butyl-NPR column (2.5 µm particles and 4.6 mm ID × 3.5 cm, Tosoh Bioscience, Montgomeryville, PA). Mobile phase A, an aqueous solution of 1.8 M ammonium sulfate and 25 mM sodium phosphate at pH 7, and mobile phase B, a mixture of 75% (v/v) 25 mM sodium phosphate aqueous solution at pH 7 and 25% (v/v) isopropyl alcohol, were used to elute the samples. A linear gradient from 100% mobile phase A to 100% mobile phase B over 12 minutes at a flow rate of 1 mL/min was employed to elute the samples. The temperature was set at 25°C. Peaks were monitored by their absorbance at 248 nm. Finally, the drug-to-antibody ratio (DAR) of the samples was calculated by

$$DAR = \sum n \times A_n / \sum A_n$$

where n denotes the number of drugs attached to the antibody (DAR species) and An denotes

the area under each DAR species peak cluster.