Supporting Information

Oximes and Hydrazones in Bioconjugation: Mechanism and Catalysis

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Selected protocols for oxime/hydrazone bioconjugation

Protocol 1: Labeling of sialic acids on cell surfaces (reference 1)

Step 1: Oxidation of sialic acids with sodium periodate

The cells (BJA-B K20 or K88) were washed with Dulbecco's phosphate buffered saline (pH 7.4, PBS, Invitrogen) and suspended to 1×10^6 cells/mL in PBS containing 1 mM sodium periodate. The cells were treated with sodium periodate for 30 min at 4 °C and the reaction was subsequently quenched with 1 mM glycerol before washing in PBS.

Step 2: Aniline-catalyzed oxime formation

The periodate treated cells were washed in PBS and suspended to 1×10^6 cells/mL in PBS/5% fetal bovine serum (pH 6.7) containing 100 μM of the respective alkoxyamine and 10 mM aniline. The reaction was carried out at 4 °C for 90 min with gentle agitation on an end-over-end rotator or a rocking tray. Subsequently, the cells were washed in PBS.

Protocol 2: Labeling of aldehyde-containing DNA (reference 2)

Aldehyde-containing DNA (100 μM) was reacted with the respective α-effect nucleophile (alkoxyamine or hydrazide, 0.4 mM and 10 mM, respectively) in the presence of *p*-anisidine (100 mM) in sodium phosphate buffer (40 mM, pH 5-6). The reaction was carried out at room temperature for 24 h. The obtained yields were typically >90%.

Protocol 3: *p*-Phenylenediamine-catalyzed one-pot oxime-bioconjugation with proteins that contain N-terminal serine (reference 3)

The protein (600 μM) was reacted with sodium periodate (600 μM) in 105 mM sodium phosphate buffer (pH 7.0, including 125 mM NaCl). The mixture was allowed to incubate for 15 min at room temperature with mild agitation. The oxidation was terminated by the addition of serine to a final concentration of 6 mM. After addition of the quencher, the solution was mixed and incubated for 1 h at room temperature. Subsequent oxime coupling was performed in the same reaction mixture by addition of the respective alkoxyamine stock solution in sodium phosphate (pH 7.0, 100 mM) and *p*-phenylenediamine, which resulted in final concentrations of 200 μM protein, 1 mM alkoxyamine, and 10 mM *p*-phenylenediamine. The reaction tube was covered with foil and incubated for 22 h at room temperature. The obtained yields were typically >80%.

Protocol 4: *m*-Phenylenediamine-catalyzed oxime-bioconjugation with aldehyde-containing proteins (reference 4)

The respective alkoxyamine (250 μM) was reacted with an aldehyde-containing protein (50 μM) in the presence of *m*-phenylenediamine (80 mM) in Tris•HCl buffer (50 mM, pH 7.5). After 5 min at room temperature, the mixture was analyzed via SDS-PAGE. The labeling of the protein was almost quantitative.

Protocol 5: Fast catalyst-free hydrazone-bioconjugation with hydrazide-containing proteins and *ortho*-chlorobenzaldehydes (reference 5)

The protein hydrazide (30 µM) was reacted with *ortho*-chlorobenzaldehyde (or a derivative thereof, 200 µM) in aqueous PME buffer (100 mM PIPES, 1 mM $MgSO_4$, 2 mM EGTA at pH 7.0). The mixture was stirred at room temperature for 30 min and subsequently analyzed by RP-HPLC. The respective hydrazone was typically obtained with >90% yield.

Protocol 6: Doxorubicin-labeling of hydrazide-containing peptides in methanol (reference 6)

The respective peptide (10 µmol) and doxorubicin hydrochloride (20 µmol, 2 equiv.) were dissolved in anhydrous methanol (3 mL). Trifluoroacetic acid (6 µL) was added and the mixture was stirred in the dark at room temperature for 24 h. Subsequently, the product was purified via semi-preparative HPLC (solvent A: NH_4OAc buffer, pH 7; solvent B: acetonitrile). The conjugate was obtained in 62% yield.

References

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