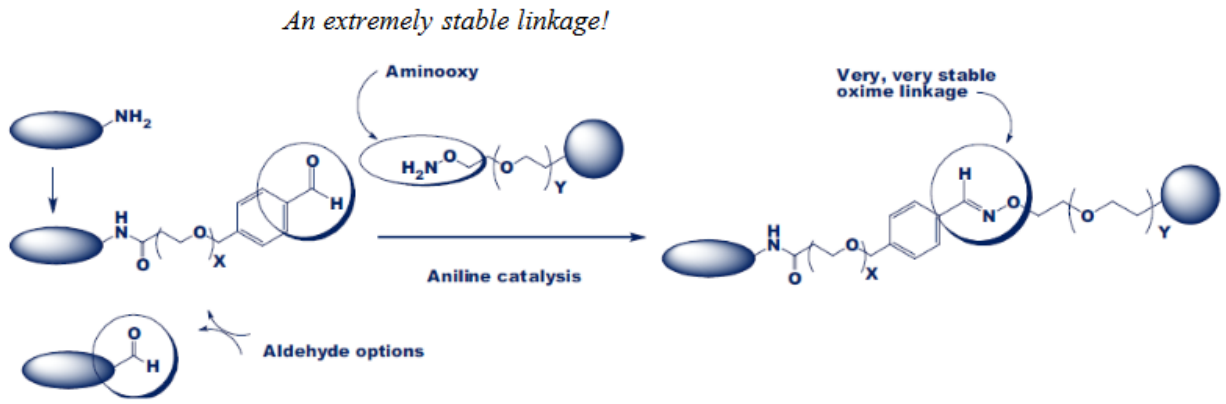
PEG Aminooxy (PEG-ONH2)

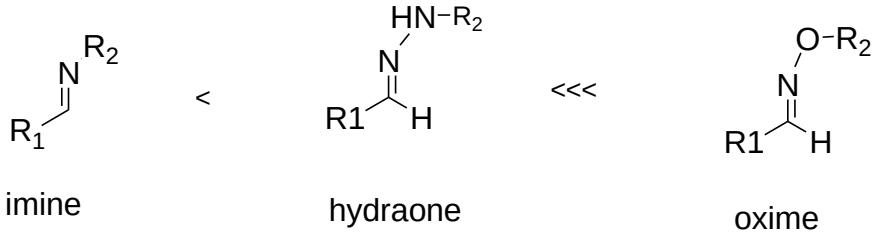
Introduction

Aminooxy PEG Linkers can be used in bioconjugation through the reaction of the aldehyde group with aminooxy to form aldoxime. It is much more stable than hydrazone and imine. In most of cases, it can be used directly without reduction while hydrazine and imines bond normally need be reduced to form stable C-N bond.

Alkoxyamines react with carbonyls most efficiently in amine-free, neutral conditions (pH 6.5-7.5). Carbonyls may exist at the reducing end of polysaccharides. To create additional carbonyls, oxidize sugar groups using either a specific oxidase, such as galactose oxidase, or 1-10mM sodium meta-periodate or use Ald-PEG-NHS ester to introduce an aldehyde group. Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1M sodium acetate, pH 5.5), although neutral buffers such as phosphate- buffered saline can be used. If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration into neutral buffer might be necessary to obtain the optimal alkoxyamine reaction. sometimes, aniline can be used to accelerate the coupling rate of hydrazide and alkoxyamine moieties with reactive aldehydes / ketones (carbonyls).



b. stability



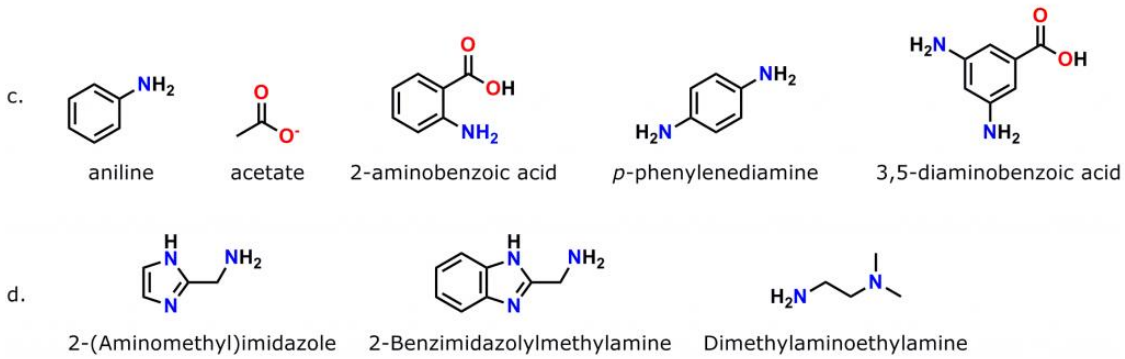


Figure 1: Oxime bond formation is a specific, bioorthogonal reaction that can be greatly accelerated by the use of suitable catalysts.

a. Scheme for the formation of aldoximes, when an aminooxy compound reacts with an aldehyde.
b. Stability comparison of imine, hydrazone, and oxime.
c. Common catalysts used to accelerate oxime bond formation.
d. Recently discovered low-toxicity catalysts for oxime bond formation.

Example protocol for labeling glycoproteins with an alkoxyamine-biotin reagent

Note: The optimal alkoxyamine-biotin concentration and reaction conditions depend on the specific protein and downstream application. For best results, empirically optimize the molar ratio of reagent and glycoprotein.

A. Materials required

- Alkoxyamine-biotin Solution: 50mM alkoxyamine-biotin reagent in DMSO. Prepare a volume sufficient to achieve the desired final concentration in step B.3.
  Note: Alkoxyamine biotin reagents are hygroscopic solids that are difficult to weigh and dispense. To facilitate handling, make a 250mM stock solution by dissolving the entire contents of the vial (50mg) in a dry (anhydrous, molecular sieve-treated) organic solvent such as DMSO. Store the stock solution at -20°C for up to 1 month; warm the vial to room temperature before opening to prevent moisture condensation.
- Oxidation Buffer: 0.1M sodium acetate, pH 5.5
- Sodium meta-periodate at 20mM in Oxidation Buffer: Prepare solution immediately before use in an amber vial or other light-protecting vessel.
- Coupling Buffer: 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (PBS) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein at 2mg/mL in Oxidation Buffer
- Dialysis cassette or desalting column

B. Procedure

1. Add 1mL of cold sodium meta-periodate solution to 1mL of cold glycoprotein solution and mix well. Incubate mixture for 30 minutes on ice or at 4°C protected from light.
   Note: To oxidize only sialic acid groups, add 50µL of sodium meta-periodate instead of 1mL, which results in 1mM periodate final concentration rather than 10mM).
2. Remove excess sodium meta-periodate and exchange the buffer by dialysis against coupling buffer or with a desalting column equilibrated with coupling buffer.
3. Add 1 part of Alkoxyamine-biotin Solution to 9 parts oxidized protein post buffer-exchange (results in 5mM alkoxyamine biotin). Mix the reaction for 2 hours at room temperature.
4. Separate the biotinylated protein from non-reacted material by dialysis or desalting. Store the biotinylated protein using the same conditions as for the non-biotinylated sample.