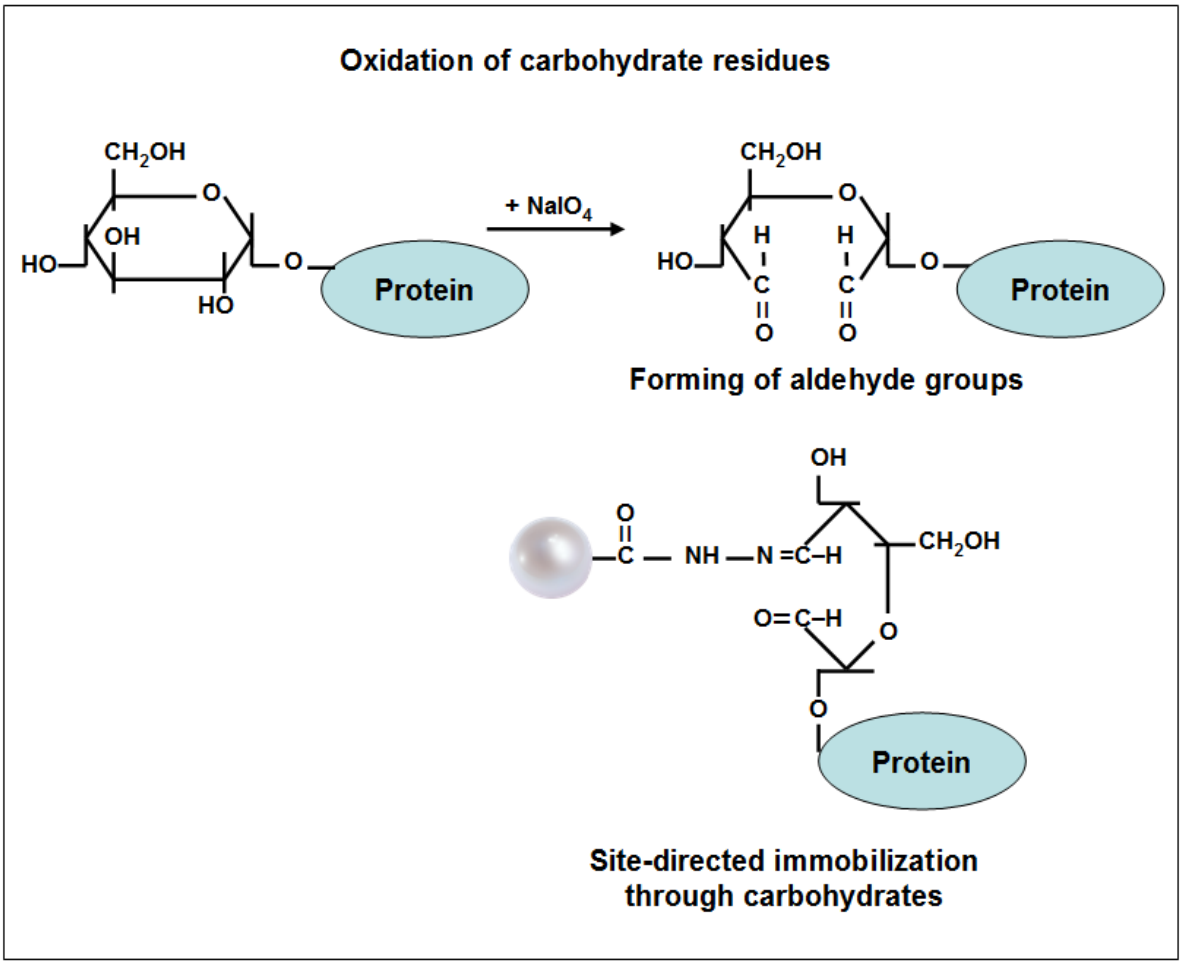
# Covalent Coupling Procedure on beadBALL-Hydrazide

## Introduction:

This procedure describes the covalent coupling of aldehyde or ketone group containing ligands by the formation of stable hydrazone linkages on **beadBALL-Hydrazide**.

Glycoproteins can be immobilized by oxidation with sodium periodate to generate formyl groups on their carbohydrate chains.

This coupling method is a powerful way to immobilize proteins and leave critical active sites free.



## Equipment and reagents:

- **beadBALL-Hydrazide**

- **Wash & Coupling buffer:**
  0.1 M sodium phosphate buffer, pH 7.0

- **Oxidation Reagent:**
  sodium meta-periodate ($NaIO_4$)

- **Blocking buffer:**
  0.1 M D-glyceraldehyde in **Wash & Coupling buffer**

- **Storage buffer:**
  PBS, 0.1 % BSA, 0.05 % sodium azide

- **Microcentrifuge**

## Technical note:

- The reaction is light sensitive and should be performed in the dark.

- Coupling efficiencies hydrazide modified microspheres depend on the structure and the size of the target glycoprotein. The user should empirically optimize the concentration of the protein. We recommend starting with ~1 mg oxidized protein for 10 mg **beadBALL-Hydrazide**.

**The following protocol describes the coupling of glycoproteins on *10 mg* microspheres. The procedure can be scaled up by adjusting volumes of required reagents.**

## Protocol:

### Oxidation:

1. Dissolve 5 -10 mg glycoprotein in 1 ml 0.1 M **Wash & Coupling buffer.**

2. Add 1 ml glycoprotein solution to an opaque vial containing 5 mg **Oxidation Reagent** ($NaIO_4$) and swirl gently to dissolve the oxidizing agent.

3. Incubate the sample in the dark at room temperature for 30 minutes.

4. Stop the reaction and remove unreacted **Oxidation Reagent** by desalting and buffer exchange through Sephadex G-25 column.

   Equilibrate a 5 ml Sephadex G-25 column with **Wash & Coupling buffer.** Apply the oxidized sample to the column and allow it to enter the gel bed. Apply a 0.5 ml rinse of **Wash & Coupling buffer** and allow it to enter the gel bed. Finally apply 2 ml **Wash & Coupling buffer** and collect the eluent, which contains ideally 2.5 – 5.0 mg/ml oxidized glycoprotein.

### Coupling:

1. Transfer 10 mg **beadBALL-Hydrazide** microspheres in a 2 ml microcentrifuge tube, add 1 ml **Wash & Coupling buffer** and centrifuge for 1 minute at 500 x g. Remove the supernatant, add 1 ml **Wash & Coupling buffer**, resuspend the pellet completely by thoroughly vortexing, centrifuge and remove the supernatant.

2. Resuspend the microspheres in 0.75 ml **Wash & Coupling buffer.** Mix the microspheres with 0.25 ml oxidized protein solution (2.5 – 5.0 mg/ml). This 1 ml suspension contains 0.625 – 1.25 mg oxidized protein and 10 mg **beadBALL-Hydrazide.** Incubate for a minimum of six hours at room temperature.

3. After incubation wash the microspheres 3 x with 1 ml **Wash & Coupling buffer** as described in position 1.

4. Add 0.2 ml **Blocking buffer** and mix gently for 30 - 60 minutes.

5. Wash the microspheres 3 x with 1 ml **Storage buffer** as described in position 1.

6. Resuspend the microspheres in an appropriate volume of **Storage buffer**.