Covalent Coupling Procedure on beadBALL-Amine via Mannich Condensation

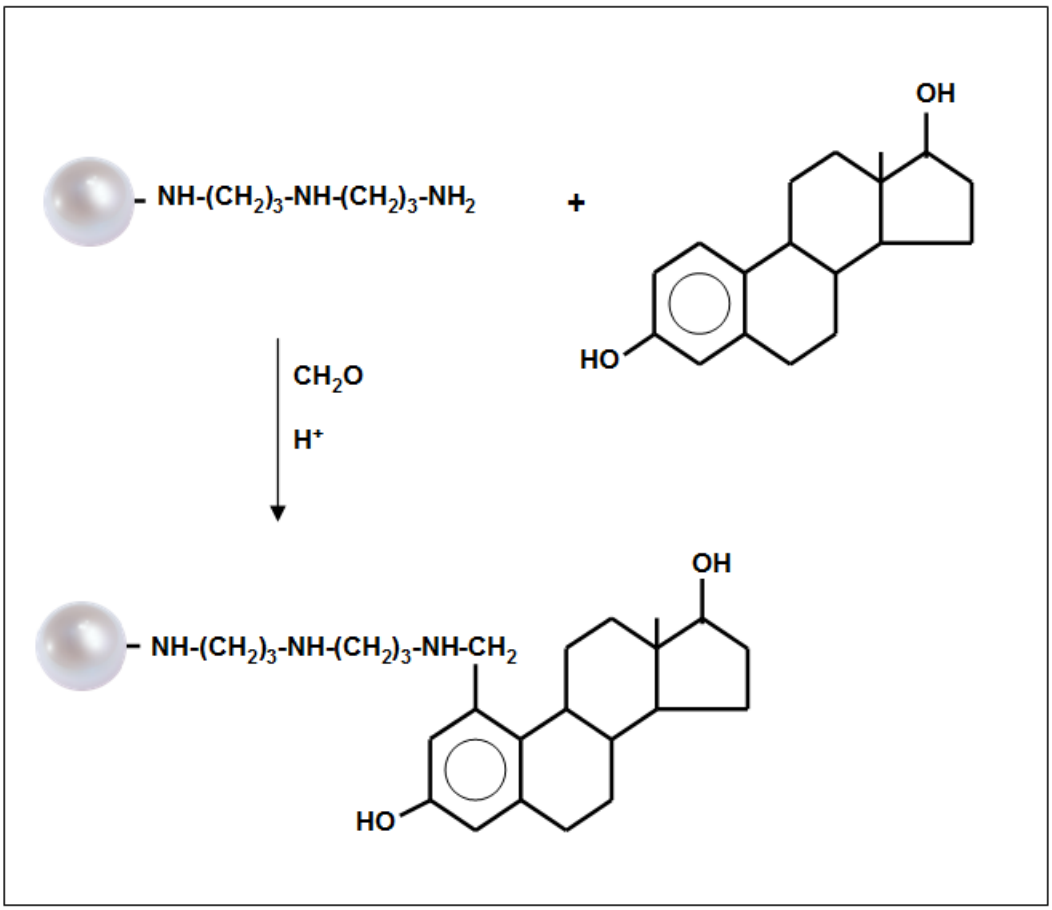
Introduction:

This procedure describes the immobilization of compounds via aromatic ring to **beadBALL-Amine** by the Mannich reaction.

The Mannich reaction is useful for the coupling of compounds such as drugs, steroidal compounds, dyes, or other organic molecules which do not contain reactive components, e.g. primary amines, carboxylic acids, aldehydes or sulfhydryl groups.

The ligands are coupled by condensation with formaldehyde to terminal amine groups of the microspheres.

The coupling via Mannich reaction forms very stable covalent bonds which are useful for most affinity separations.



Equipment and reagents:

- **beadBALL-Amine**
- **Formaldehyde 37 %**
- **MES buffer:**
  0.1 M 2-(N-Morpholino)ethanesulfonic acid (MES), pH 4.7
- **Blocking & Storage buffer:**
  PBS, 0.1 % BSA, 0.05 % sodium azide
- **Microcentrifuge**

Technical note:

- If the ligand is not soluble in plain water or aqueous buffer, the reaction can be carried out in solutions containing up to 50 % ethanol.
- For antibodies or proteins we recommend to use a minimum amount of 50 µg antibody / protein per 10 mg **beadBALL-Amine**. In general, the higher the amount of antibody/protein per milligram of **beadBALL-Amine**, the higher will be the degree of microsphere surface coating with the protein.
- **Prepare the EDC solution immediately before use and transfer the needed volume rapidly into the reaction tube.**

Protocol:

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

1. Transfer 10 mg **beadBALL-Amine** microspheres in a 2 ml microcentrifuge tube, add 1 ml **MES buffer** and centrifuge for 1 minute at 500 x g. Remove the supernatant, add 1 ml **MES buffer**, resuspend the pellet completely by thoroughly vortexing, centrifuge and remove the supernatant.
2. Add 0.25 ml **MES buffer** and resuspend the microspheres. Add 0.2 ml Formaldehyde and mix by vortexing.
3. Add ligands (e.g. 50 µg proteins dissolved in ddH_2O) and incubate by a shaker for two hours at room temperature.
4. Wash the particles 3 x with 1 ml PBS as described in position 1.
5. Resuspend the microspheres in an appropriate volume of **Blocking & Storage buffer.**