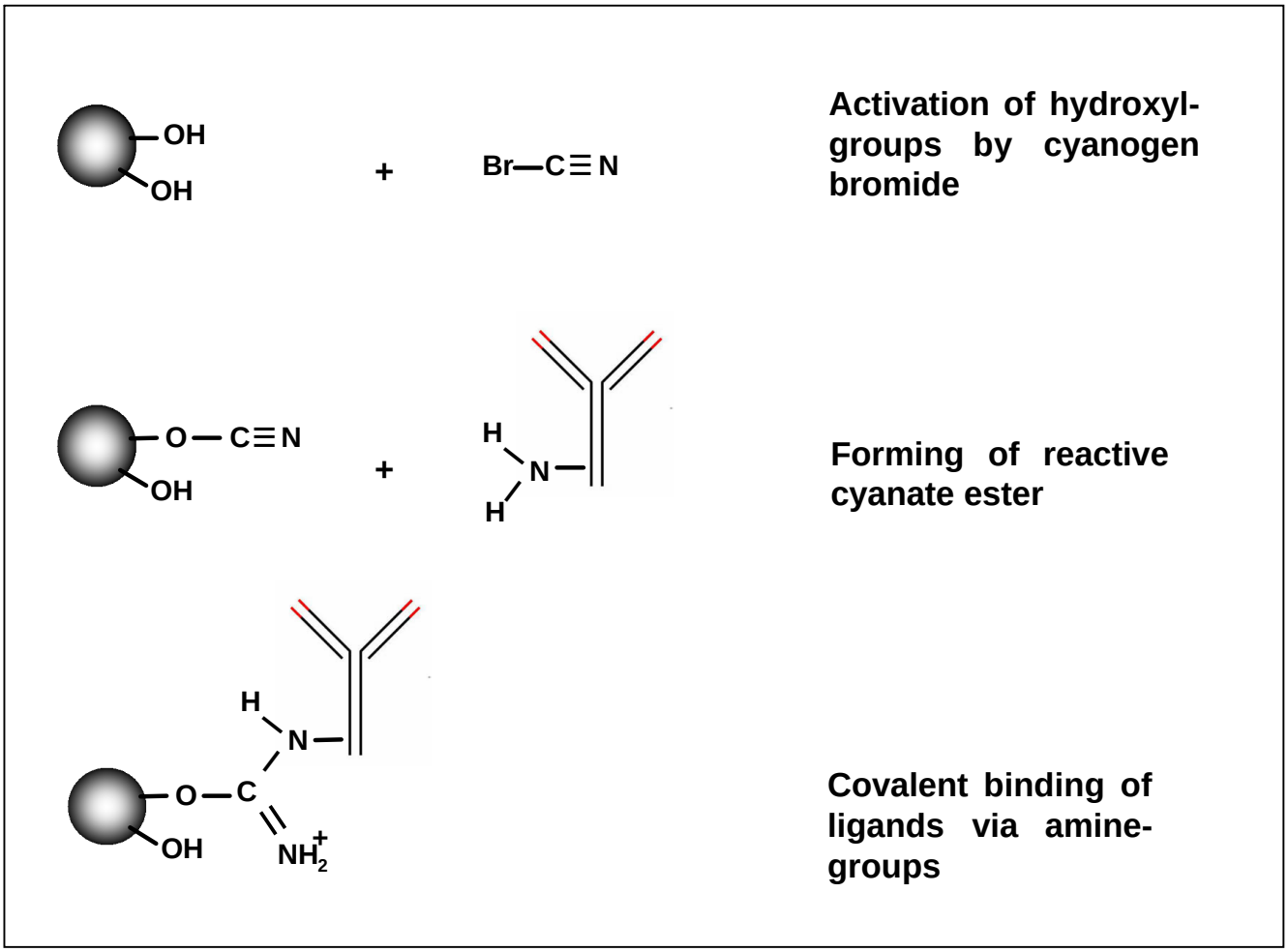
Covalent Coupling Procedure on SiMAG-Hydroxyl and screenMAG-Hydroxyl by Cyanogen Bromide (CNBr) Activation

Introduction:

This procedure describes covalent coupling of amino-group containing ligands such as antibodies, proteins or low molecular weight substances to **SiMAG-Hydroxyl** and **screenMAG-Hydroxyl** by the CNBr activation.

The CNBr activation is relatively simple to carry out and is very reproducible. The method is especially mild for coupling of sensitive biomolecules such as enzymes and antibodies.

The CNBr react with terminal hydroxyl groups from the beads to very reactive cyanate esters and imidocarbonates.



Equipment and reagents:

- **SiMAG-Hydroxyl or screenMAG-Hydroxyl**
- **Cyanogen Bromide**

  CAUTION: CNBr activation procedure should be carried out in a well ventilated hood since CNBr is highly toxic.

  5 M cyanogen bromide in acetonitrile
- **Activation & Wash Buffer:**

  0.2 M Borate buffer pH 8.5
  PBS pH 7.4
- **Blocking & Storage Buffer:**

  PBS, 0.1 % BSA, 0.05 % sodium azide
- **Magnetic Separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Technical Note:

- All buffers used for activation or coupling may not contain buffer ingredients with amino groups or proteins.
- For antibodies or proteins we recommend to use a minimum amount of 50 µg antibody/protein per 10 mg **SiMAG-Hydroxyl** (or **screenMAG-Hydroxyl**). In general, the higher the amount of antibody/protein per milligram of SiMAG-Hydroxyl, the higher will be the degree of magnetic particle surface coating with the protein.

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

Protocol:

1. Wash the **SiMAG-Hydroxyl** (or **screenMAG-Hydroxyl**) particles 1 x with 1 ml Borate Buffer using the magnetic separator and resuspend the particles in 0.25 ml Borate Buffer.

2. Add 0.1 ml 5 M CNBr to the particles and mix by vortexing. Place the tube in ice cold water for 10 minutes.

3. After incubation quickly wash the particles 2 x with 1 ml PBS and resuspend the activated particles in 0.25 ml PBS.

4. Add amino group containing ligands (e.g. 50 µg protein dissolved in ddH_2O) to the activated particles and mix the suspension on a shaker for 2 hours at room temperature.

5. Wash the particles 3 x with 1 ml PBS.

6. Resuspend the particles in Blocking/Storage buffer.