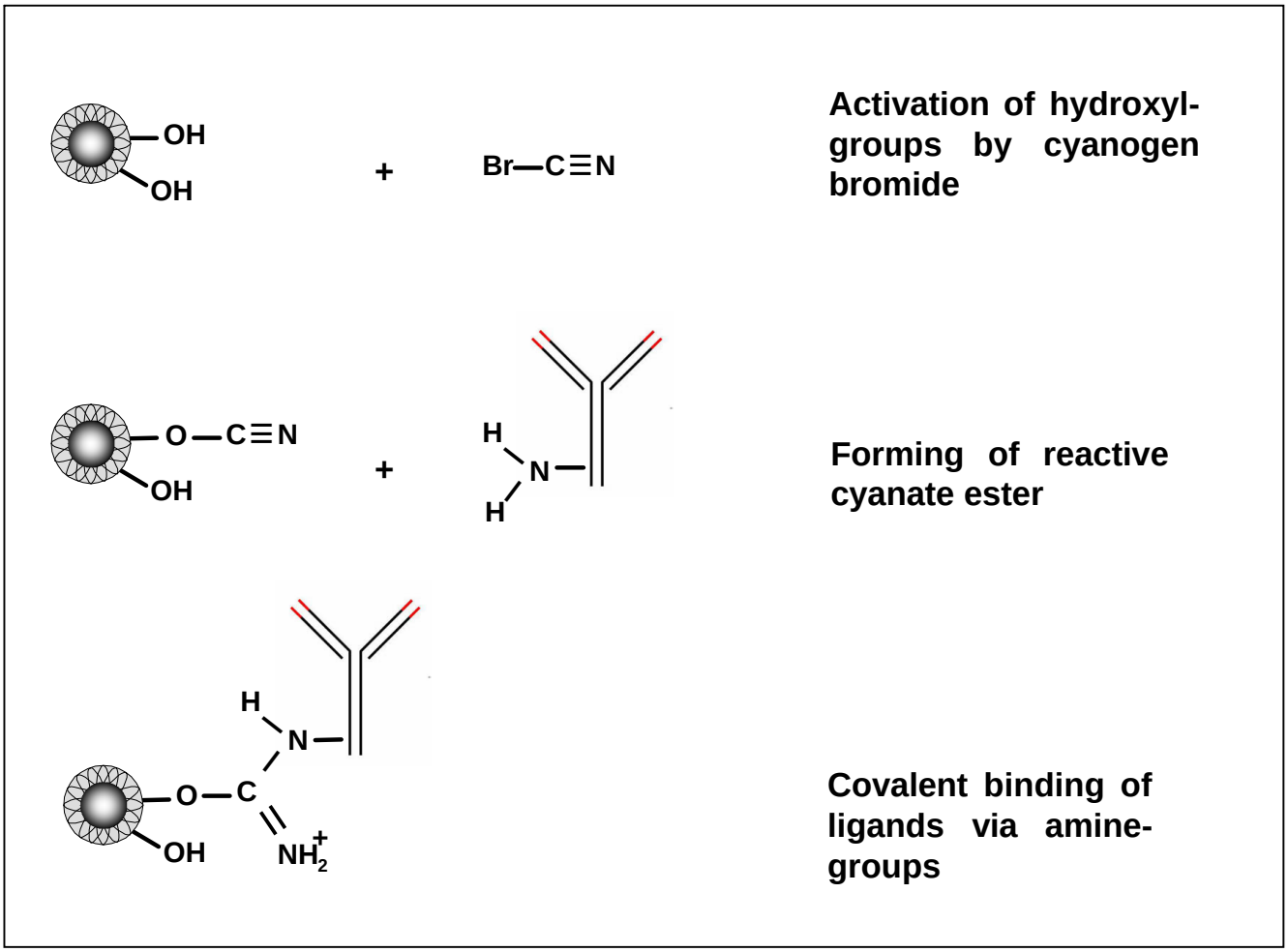
Covalent Coupling Procedure on fluidMAG-D by Cyanogen Bromide (CNBr) Activation

Introduction:

This procedure describes covalent coupling of amine-group containing ligands such as antibodies, proteins or low molecular weight substances to **fluidMAG-D** by the CNBr Activation.

The CNBr Activation is relatively simple to carry out and is very rebroducible. The method is especially mild for coupling of sensitives biomolecules such as enzymes and antibodies.

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



Equipment and reagents:

- **fluidMAG-D**
- **Cyanogen Bromide in 5 M Acetonitrile (e.g. Sigma-Aldrich Cat. No.: 261610)**

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

- **Activation Buffer:**

  0.2 M Sodium hydrogen carbonate buffer (no pH adjustment necessary)
  pH range 8.4 – 8.7.

- **Wash Buffer:**

  PBS pH 7.4 (ice cold)

- **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 amine-groups or proteins.
- For antibodies or proteins we recommend to use a minimum amount of 50 µg antibody/protein per 10 mg **fluidMAG-D**. In general, the higher the amount of antibody/protein per milligram of **fluidMAG-D**, 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 **fluidMAG-D** particles 1 x with 1 ml Activation Buffer using the magnetic separator and resuspend the particles in 0.25 ml Activation Buffer.

2. Add 0.05 ml CNBr to the particles and mix by vortex. Place the tube in ice cold water for 10 minutes.

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

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

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

6. Resuspend the particles in Blocking/Storage buffer by vortexing.