

Azide Functionalization to Alkyne dyes

Part Numbers:

C - Azide

Introduction

The C- azide family of products include spheres in rods that have been encased in a polymer with azide terminal groups. The proprietary polymer coating protects the gold nanoparticles from salt and variations in pH, and helps reduce non-specific binding and aggregation in in vitro environments.

Materials

- Azide-functionalized gold nanoparticles (AuNPs)
- Alkyne dye (e.g., Alexa Fluor 488-alkyne)
- Copper(II) sulfate pentahydrate (CuSO₄·5H₂O)
- Sodium ascorbate
- Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) or another suitable ligand for Cu(I)
- Deionized water (DI water)
- Dimethyl sulfoxide (DMSO)
- Reaction vessel (e.g., glass vial)
- Magnetic stir bar
- Centrifuge
- Centrifuge tubes
- Dialysis tubing or ultrafiltration device
- UV-Vis spectrophotometer (optional, for analysis)

Procedure

1. ****Preparation of Reaction Mixture****:
 - In a reaction vessel, combine the azide-functionalized gold nanoparticles with an appropriate volume of DI water to make a colloidal suspension.
 - Dissolve the alkyne dye in a small amount of DMSO. The concentration should be enough to achieve a final alkyne-to-azide molar ratio of 1:1.5.
2. ****Addition of Copper Catalyst and Ligand****:
 - Add 1-2 mM CuSO₄·5H₂O to the reaction mixture.
 - Add 2-4 mM sodium ascorbate to the mixture. Sodium ascorbate reduces Cu(II) to Cu(I), which is the active catalyst for the click reaction.
 - Optionally, add a ligand such as THPTA to stabilize the Cu(I) ions. Use a ligand-to-copper ratio of about 2:1.
3. ****Reaction Conditions****:

- Stir the reaction mixture using a magnetic stir bar. The reaction is typically carried out at room temperature.

- Allow the reaction to proceed for 2-4 hours. You can monitor the progress by UV-Vis spectroscopy if desired.

4. **Purification**:

- After the reaction is complete, remove unreacted dye and copper ions. This can be done by centrifugation at speed shown below for 5 minutes.

- Discard the supernatant and resuspend the gold nanoparticles in DI water. Repeat the centrifugation and resuspension steps 3-4 times to thoroughly wash the nanoparticles.

5. **Dialysis or Ultrafiltration**:

- Further purify the dye-conjugated gold nanoparticles using dialysis tubing (MWCO ~12-14 kDa) or an ultrafiltration device to remove small molecular impurities.

- Dialyze against DI water for 24-48 hours, changing the water at regular intervals.

6. Alternate sonicating and shaking of the microcentrifuge tube to resuspend gold nanoparticles and bound alkyne dye into solution.

7. To facilitate binding of the alkyne dye to the gold nanoparticles, vortex for 30 minutes at room temperature, and up to 30C for more efficient conjugations.

Purification is completed by centrifugation, removal of the supernatant, and resuspension using a diluted PBS mixture. These steps are repeated three times.

8. Centrifugation speeds depend on centrifuge but in general, speeds from 8500 to 12000 rcf for 5 minutes are used for nanorods, 1500-15000 for spheres.

Product	rcf
10nm rods	12000
25nm rods	9000
Spheres 100nm	3500
Spheres 50nm	6000
Spheres 10nm	15000

6. **Characterization (Optional)**:

- Characterize the final product using UV-Vis spectroscopy to confirm the presence of the dye on the nanoparticles.

- Dynamic light scattering (DLS) and zeta potential measurements can be used to assess the size distribution and surface charge of the functionalized nanoparticles.

- Transmission electron microscopy (TEM) can be used to visualize the morphology of the nanoparticles.

Conjugation efficiency may be estimated by electrophoretic separation and subsequent protein staining.

Certainly! Here is a chemical procedure to attach an alkyne dye to azide-functionalized gold nanoparticles using the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction, also known as the "click" reaction.

Notes:

- Ensure all glassware and materials are clean and free from contaminants.
- Handle all chemicals, especially the copper catalyst and alkyne dye, with care and use appropriate personal protective equipment (PPE).
- The choice of alkyne dye and azide-functionalized gold nanoparticles can vary depending on the specific application and desired properties.

By following this procedure, you should be able to successfully attach an alkyne dye to azide-functionalized gold nanoparticles through the CuAAC click reaction.