Optical Imaging

Introduction

The excellent optical properties of gold nanoparticles make them suitable for a broad range of applications extending from diagnostics to drug delivery and DNA therapy. However, in most cases, spherical gold nanoparticles exhibit absorption peaks at ~580 nm, well below the transmission window (650–900 nm) of most biological entities such as skin, tissue and hemoglobin. This limited wavelength window restricts the applications of spherical gold nanoparticles.

Gold nanorods exhibit many similar characteristics as gold nanoparticles, but they are elongated to optimize absorption and scattering properties. This ability to elongate the nanoparticle asymmetrically allows for tuning of the absorption between 550 nm to 1,400 nm, making gold nanorods attractive for applications in diagnostics. This protocol summarizes the conjugation and purification of non-surface functionalized gold nanorods to carboxyl groups and of carboxyl-functionalized nanorods to Streptavidin or IgG. The conjugation of carboxyl gold nanorods to Streptavidin or IgG essentially involves conjugation to an amine using EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) (Prod. No. E1769), chemistry. This conjugation is important in biological applications including lateral flow diagnostics. Streptavidin and IgG are extensively used for tagging antigens and biotin.

EDC is a carboxyl and amine-reactive zero-length crosslinker. EDC reacts with a carboxyl group first and forms an amine-reactive O-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea byproduct (see Figure 1). The intermediate is unstable in aqueous solutions, and therefore, two-step conjugation procedures require N-hydroxysuccinimide (Prod. No. 130672) for stabilization. Failure to react with an amine results in hydrolysis of the intermediate, regeneration of the carboxyl, and release of an N-substituted urea. A side reaction is the formation of a N-acrylurea, which is usually restricted to carboxyls located in hydrophobic regions of proteins. EDC can be used to conjugate carboxyl to amine groups in peptides, proteins, and DNA labeling through 5’ phosphate groups.

Materials

1. Carboxyl-conjugated gold nanorods, 1.75 mg/mL, OD=50, 1 mL or gold nanorods (Prod. Nos. 716812, 716820, 716839, 776688) or CTAB-capped gold nanorods (Prod. Nos. 900362, 900363, 900364, 900365, 900366, 900367)
2. (+)-α-Lipoic acid (Prod. No. T5625)
3. N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride, 5 mg (Prod. No. E1769)
4. PBS Buffer, pH 7.4, 10 mL (Prod. No. P4417)
5. Streptavidin or IgG, 100 µL, 1 mg/mL solution (Prod. No. 85878, Streptavidin, or specific IgG)
6. Ultra pure water, 10 mL
7. Low binding 2 mL microcentrifuge tube

![Figure 1](image1.png)

**Figure 1.** One-step EDC reaction with carboxyl and amine-containing molecules. EDC reacts with a carboxyl group first and forms an amine-reactive O-acylisourea intermediate that quickly reacts with an amino group to form an amide bond and release of an isourea byproduct.