Gold Nanoparticle Photoaffinity Labels for Electron Microscopy

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The conventional methods for steric stabilization of transition metal and semiconductor nanoparticles (NPs) involve the use of organic or natural polymers, surfactants, lipid bilayers and silica coatings. These methods significantly increase overall size of the NPs [1], and that could be problematic for some applications because of dielectric nature of the coating versus the conducting or semiconducting properties of the metal core may affect the properties and performance of the stabilized NPs in the desired end-application. To reduce overall size of the stabilized NPs we have developed a self-assembling coating comprising a hydrophobic metal chelating domain that seals the metal surface from aqueous buffers and a variable length hydrophilic terminal groups that stabilize and biocompatible the NPs (Figure 1). The terminal groups also provide means to further functionalize and cross-link NPs to specific groups when a small proportion (10-30%) of activatable terminal groups, e.g., -NH2 or -COOH, are included on the NP surface. We have used this strategy to develop metal NP labels for microscopic localization and imaging [2,3]. We now report gold nanoparticle (AuNP) photo-affinity labels (PALs) prepared following a similar strategy. PALs enable direct verification of the spatial proximity of macromolecular components of proteins that are not amenable to crystallography or NMR [4]; the smaller-sized heavy metal NP labels will enable unambiguous localization macromolecular components by electron microscopy and tomography at higher resolution.

The AuNP-PALs in the size range 1.5-8 nm were prepared by direct reduction of a gold salt in the presence of thiol ligands (Figure 1A and B) by optimizing gold salt-to-ligand ratio and addition rate of the reducing agent [5]. The surface photo-reactive groups on the AuNPs was varied by using a mixture of thiol ligands (100% to 25% photophore- and 0% to 75% -OH or -CH3 terminated ligands). Alternatively, the photophore-terminated ligands were introduced onto AuNPs by the place of exchange method (up to 30%) [6], or by reacting anhydride or N-hydroxysuccinimide ester (NHS-) derivatives of photophores with -NH2 functionalized AuNPs [3]. Ligand coatings with dihydriopipoyl, p-mercaptobenzoyl, and mecapto-octanoyl- to -undecanoyl cores produced photo- and thermally-stable AuNPs.

Photoaffinity labeling was performed by irradiating (100 W long-range UV lamp with a <365 nm cut-off filter; photon flux 3.6 x 10^4 Einstein/min-L (ferrioxalate actinometry)) a stirred mixture of AuNP-PAL and protein (1-10 nanomoles of albumin, ferritin, or thyroglobulin; 60, 450 and 650 kDa respectively) in oxygenated 0.1 M PBS, pH 7.4, buffer at 5 ±1°C for 30 to 60 min; the ratio of gold-to-protein was varied from 1:1 to 2.5:1 and the controls, with and without photophores, were stored in dark under similar conditions. The reaction mixture was purified by gel permeation chromatography (Superdex-200, Superose-6 or Biosep $4000$ following irradiation, AuNP-labeled fractions were identified by UV-Vis spectroscopy and labeling was confirmed by electron microscopy. AuNPs functionalized with 100% photo-affinity ligands (Figure 1B) lead to very low labeling and protein aggregation (Figure 2), while AuNPs with 25-30% surface photoaffinity ligands gave up to 14% percent labeling (Figure 3 TEM of
purified protein fraction). The low photo-labeling can be explained based on inherent low PAL efficiencies [7] and slow/less efficient reaction rates at AuNP-monolayer interface [8] [9].

References:

[9] The authors acknowledge funding from NIH (R43EB008621, R01GM085802 & R43GM112374).

Figure 1: A) Representative self-assembling ligand coatings for noble metal nanoparticle stabilization; B) Ligands that yielded gold-photoaffinity-labeled proteins.

Figure 2. TEM of Apoferritin-5 nm AuNP aggregates; 100% lipoyl-benzophenone surface ligands.

Figure 3. Photaffinity labeling of thyroglobulin by 5nm AuNP stabilized with 25% benzyol-PEG-benzophenone and 75% benzyol-PEG. Left: TEM of chromatographically purified labeled fraction with many-gold labeled molecules; Right: control, AuNPs incubated with thyroglobulin, stored in dark and purified. Grids were negatively stained with NanoVan. Bars = 100nm.