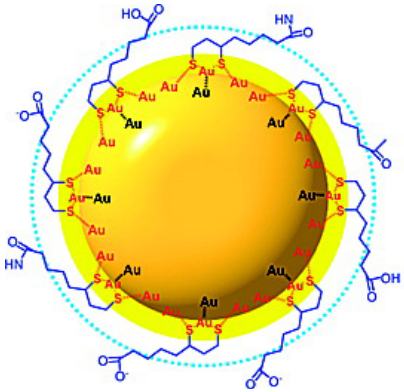
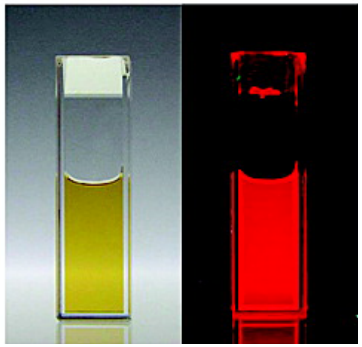
Synthesis, Characterization, and Bioconjugation of Fluorescent Gold Nanoclusters toward Biological Labeling Applications





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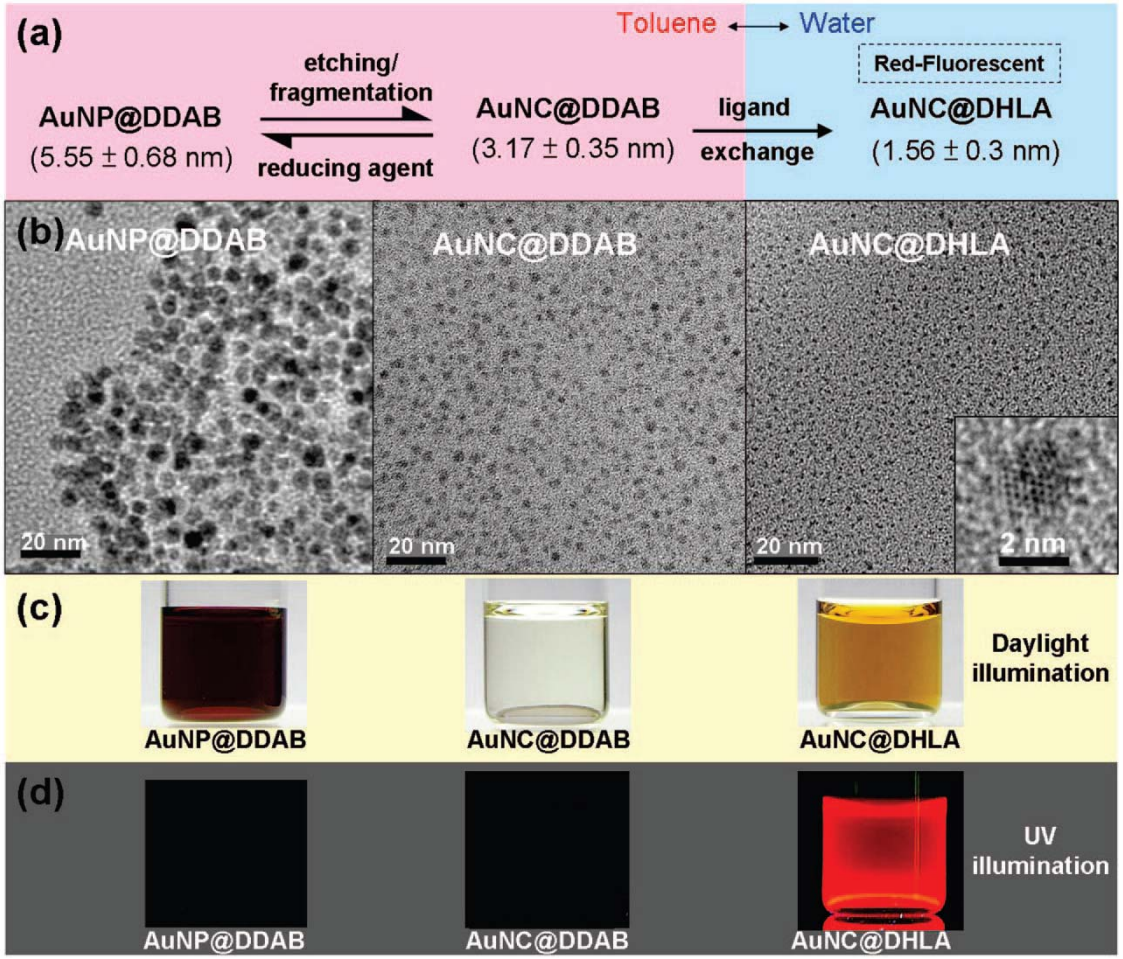
Introduction

- Fluorescent noble metal nanoparticles –potential labels for biologically motivated experiments
- Less cytotoxicity compared to semi conductor quantum dots
- Water soluble-Bioconjugation

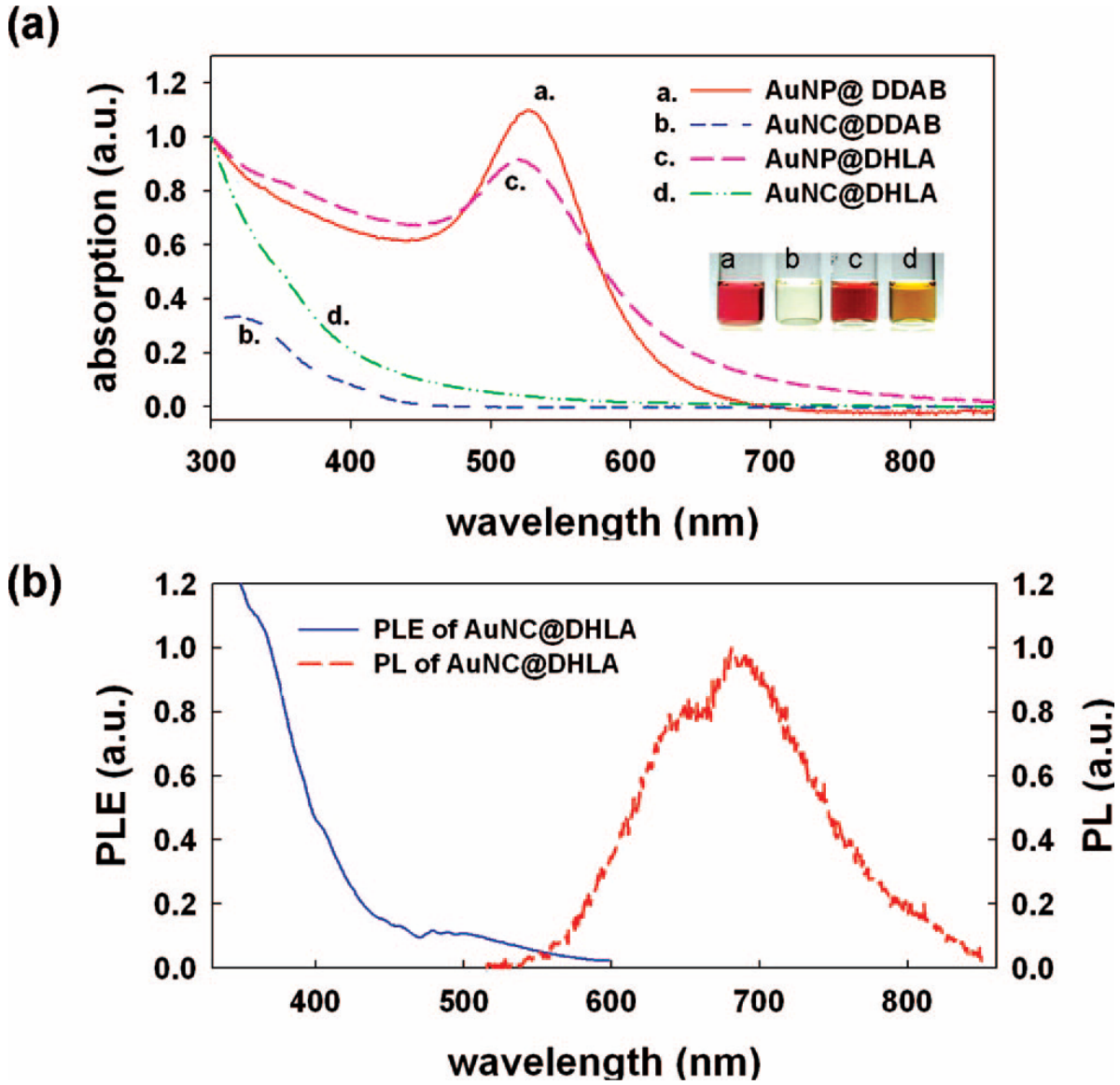
Experimental



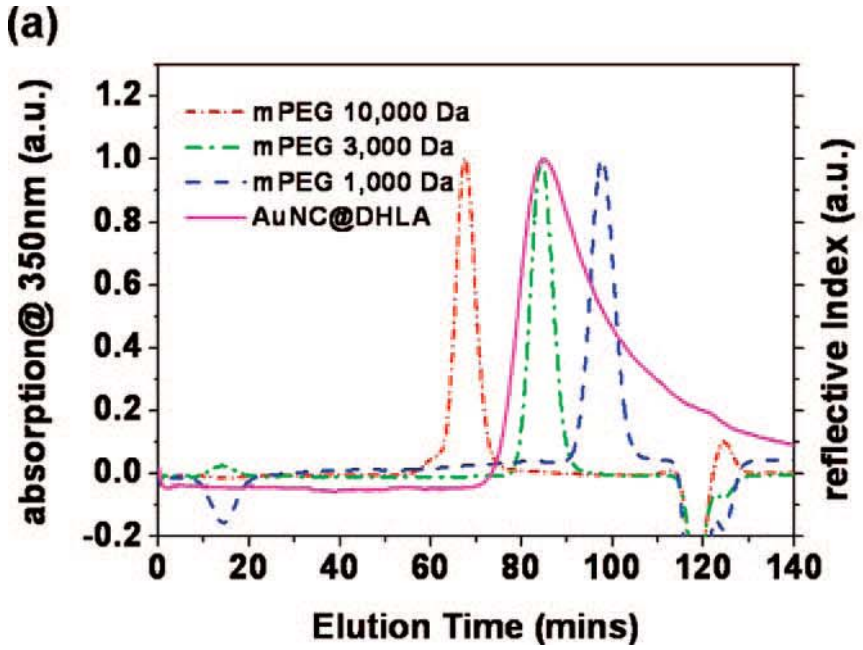
DDAB
Didodecyldimethylammonium bromide

DHLA-Dihydrolipoicacid

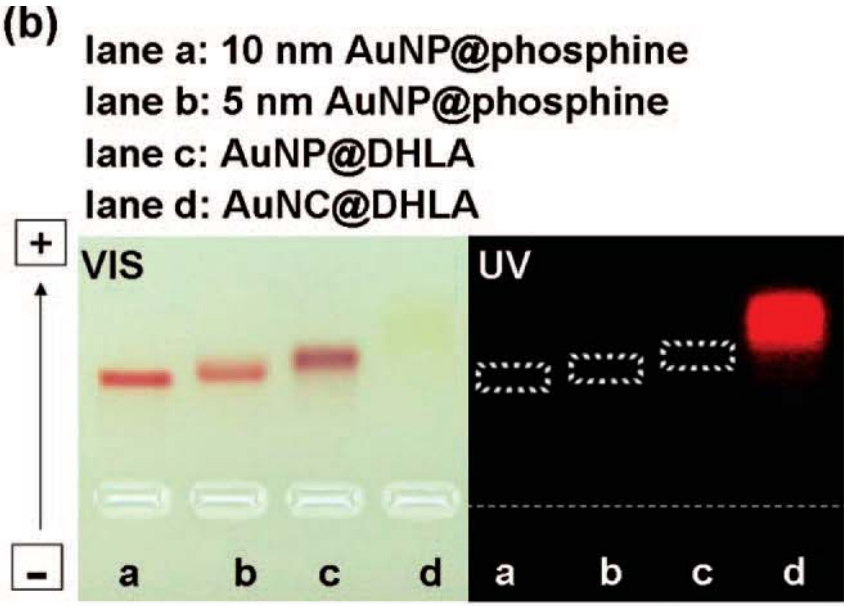
Purification-Size exclusion Chromatography and gel electrophoresis


(a)
absorption (a.u.)
wavelength (nm)
a. AuNP@ DDAB
b. AuNC@DDAB
c. AuNP@DHLA
d. AuNC@DHLA
a
b
c
d
(b)
PLE (a.u.)
PL (a.u.)
wavelength (nm)
PLE of AuNC@DHLA
PL of AuNC@DHLA

Colloidal Characterization



Size exclusion chromatography
Size sensitive technique



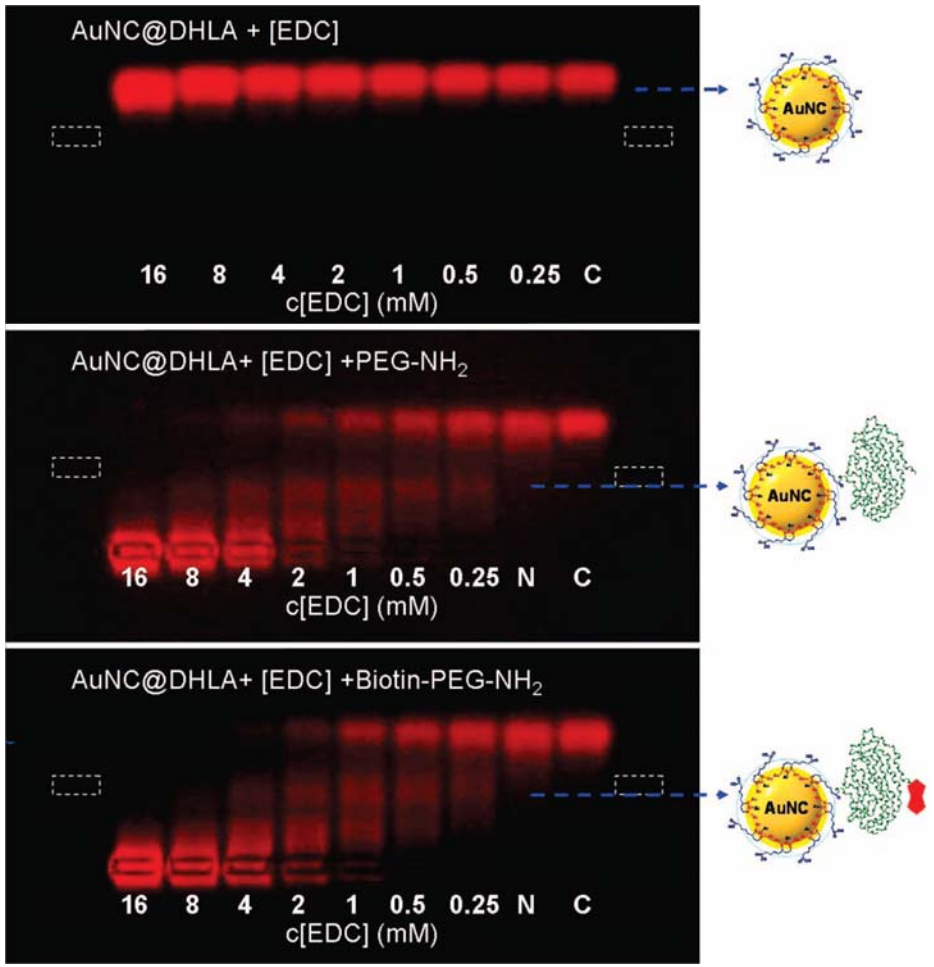
Gel Electrophoresis -Size and charge sensitive technique

The hydrodynamic diameter *d*eff of AuNC@DHLA nanoclusters was determined with size exclusion chromatography to be 1.3 *d*eff 3.4 nm using 1-3 kDa polyethylene glycol (PEG) molecules as size standard. Hydrodynamic diameter of AuNC@DHLA was determined with gel electrophoresis to be smaller than 5 nm, using bis(p-sulfonatophenyl)- phenylphosphine capped Au NPs of different size as standard. The size increment compared to the TEM results which refer to the diameter of only the inorganic Au core is attributed to the thickness of the organic capping of the particle surface with DHLA and adsorbed counterions.

Bio Conjugation

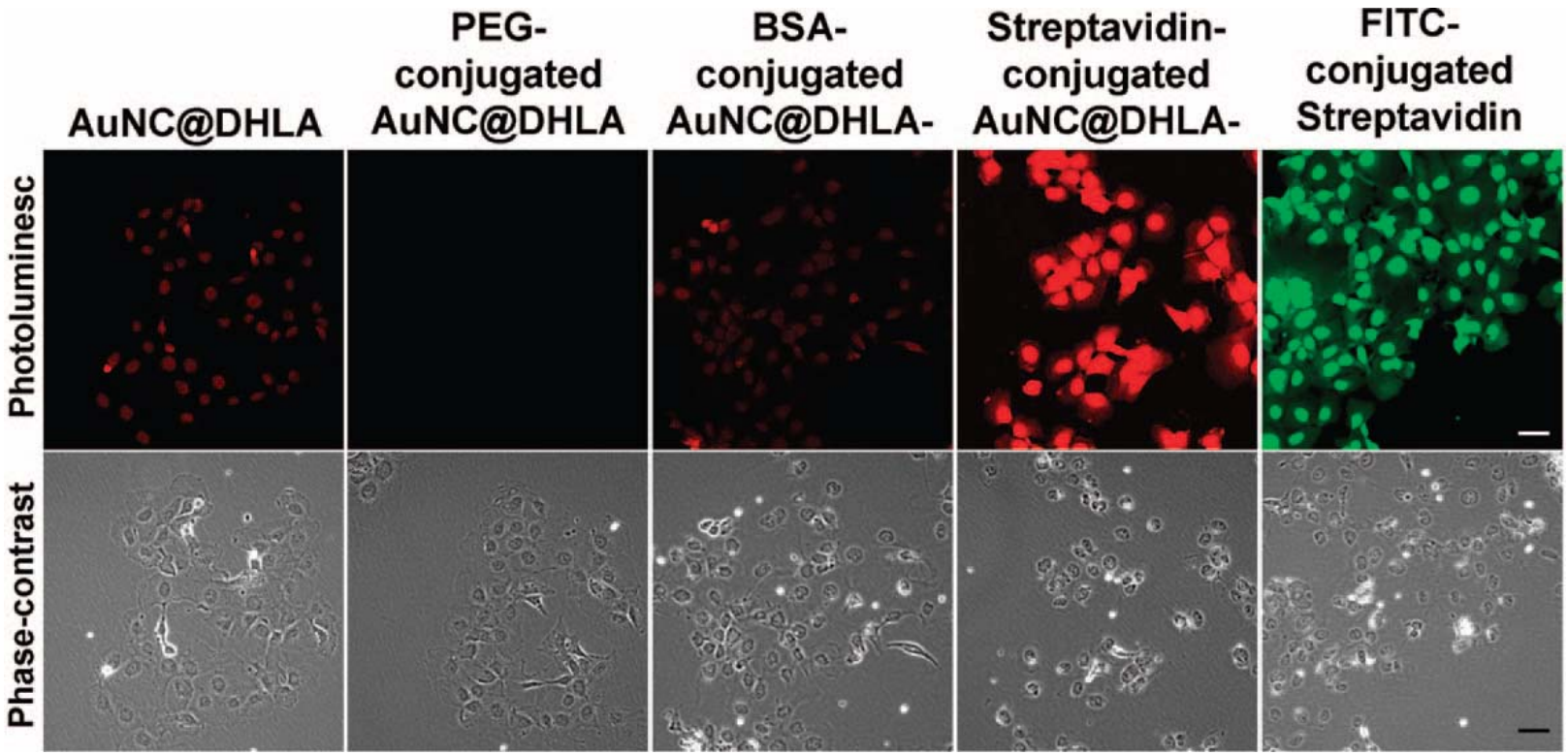
1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)-coupling

The COOH groups present on the surface of AuNC@DHLA due to the DHLA capping allow for covalent linkage of NH2-modified PEG molecules *via* EDC activation: (a) addition of EDC only to AuNC@DHLA serves as negative control without major effect in mobility; (b) addition of EDC under the presence of PEGNH2 retards the particle bands; (c) addition of EDC under the presence of biotinPEGNH2 retards the particle bands. The terminal biotin groups are anchor points for further attachment of biological molecules *via* streptavidin. In both cases retardation is caused by linkage of PEG to the Au particles and the corresponding increment in size. The more the PEG is attached to AuNC@DHLA the larger the conjugates and thus the retardation become. The first discrete band is ascribed to Au particles with exactly one PEG linked per particle. The negative control (lane "N") represent AuNC@DHLA with PEG in the absence of EDC, and lane "C" is a control with AuNC@DHLA only.



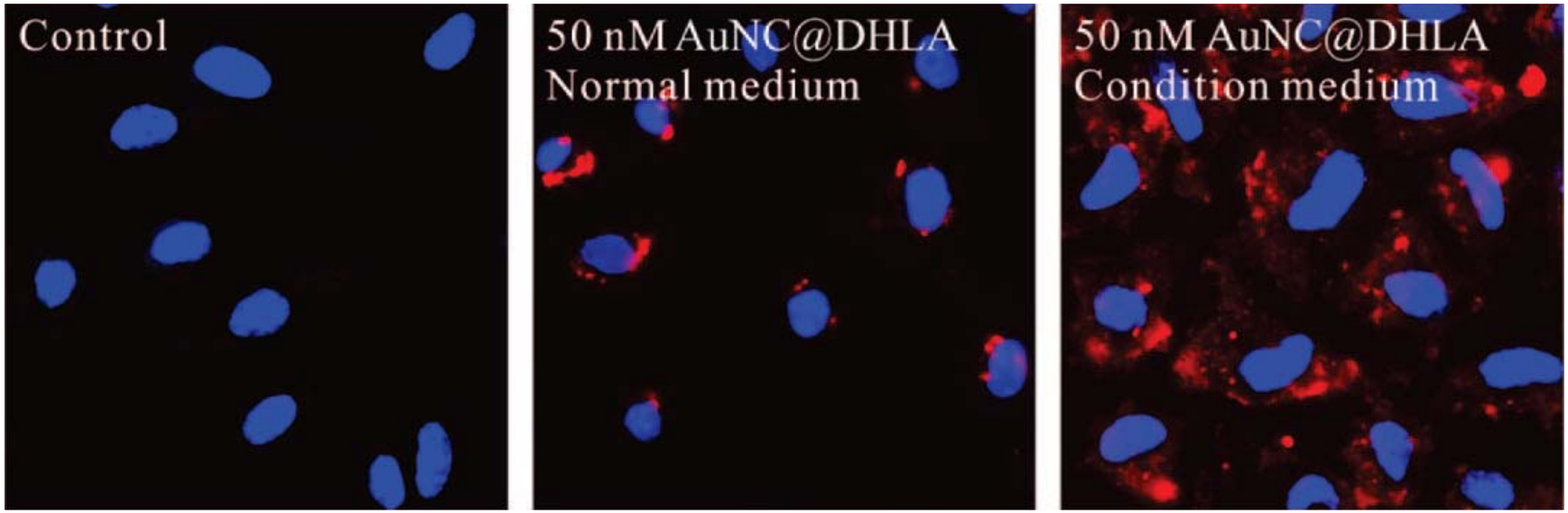
PEGylation extraordinarily improved the stability of AuNC@DHLA in salt-containing buffer (1 M NaCl) as well as in acidic buffer (pH 7).

Specific Labeling of Fixed Cells



Intracellular distribution of endogenous biotin within liver cells was probed with streptavidin conjugated AuNC@DHLA .

Non-specific Uptake of Un conjugated AuNC@DHLA



Nonspecific uptake of unconjugated fluorescent Au nanoclusters (AuNC@DHLA) by human aortic endothelial cells. Cell nuclei were stained with (Hochest 33258) to yield blue fluorescence. The red fluorescence corresponds to the Au NCs: (a) control without Au NCs; (b) serum-supplemented cell medium with 50 nM AuNC@DHLA added; (c) serum-free cell medium with 50 nM AuNC@DHLA added for 5 h.

Conclusion

In this report the synthesis of water-soluble fluorescent gold nanoclusters capped with dihydrolipoic acid (DHLA) was reported. The resulting AuNC@DHLA particles have a quantum yield of around 1-3%, reduced photobleaching compared to organic fluorophores, and very good colloidal stability. AuNC@DHLA can be conjugated with EDC chemistry to biologically relevant molecules such as PEG,BSA, avidin, and streptavidin. Uptake of AuNC@DHLA by cells did not cause acute toxicity. By their small hydrodynamic diameter (5 nm) and inert nature fluorescent Au NCs might become an interesting alternative to colloidal quantum dots, in particular for applications in which the size and biocompatibility of the label is critical. The weakest point so far remains the relatively low quantum yield. On the basis of the current report as well as on previous breakthrough findings it is believed that fluorescent gold nanoclusters have a great potential for applications to biomedical research to offer.