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Research Article

Colorimetric Analysis on Flocculation of Bioinspired Au Self-Assembly for Biophotonic Application

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Gold nanoparticles exhibited strong surface plasmon absorption and couplings between neighboring particles within bioactivated self-assembly modified their optical properties. Colorimetric analysis on the optical modification of surface plasmon resoanance (SPR) shift and flocculation parameter functionalized bioinspired gold assembly for biophotonic application. The physical origin of bioinspired gold aggregation-induced shifting, decreasing, or broadening of the plasmon absorption spectra could be explained in terms of dynamic depolarization, collisional damping, and shadowing effects.

1. Introduction

Integrated systems of plasmonic nanometals and biomolecules are of great interest for biophotonic applications because of their optical colorimetric properties and distinctive microscopic structures. It is well known that the extinction spectra of surface plasmon resoanance (SPR) or intraband transitions of monodispersed nanometals are tunable, from visible to near infrared region, by controlling the individual morphologies and structures that confine their dielectric functions. A combination of the optical tunability and bioinspired self-assembly of nanometals through biospecific interactions functionalizes nanometals for photonic applications. Couplings between bioinspired nanometal self-assembly modify optical properties. Mole sensitivity of bioactivators that inspire Au nanometal self-assembly has been introduced down to less than ~10 nM by monitoring SPR modification [1]. The functionalized biosensors could also be coupled with Raman for identifying bio activators. Au nanoparticles for the biophotonic applications were chosen because they have good biocompatibility, strong and tunable SPR, controllable homogeneous morphology, and weak photodegradation [2]. The integrated material systems of plasmonic nanometals and biomolecules for biophotonic applications were avidin-inspired biotin attached-Au assembly and DNA-linked Au-hybridization, because they have high affinity and biospecific interaction [3–6]. Recent investigations on mass sensitivity of bioactivators for Au nanometal self-assembly were conducted mostly based on SPR peak red shift. However, the physical origin of SPR spectral shift, decrease, and broadening of bioinspired nanometal-assembly can be explained in terms of dynamic depolarization, radiation damping, and shadowing effects. Therefore, it is suggested that combinational analysis of both flocculation parameter [7–9] and peak shift of SPR on bioactivated nanometals would closely track any aggregation process [1], and might promote the bioplasmonic material system for photonic applications in the diagnosis of pathogenic and genetic diseases [10–12].

2. Experiment

Gold nanoparticles without any organic surfactants [13–16] and biotin (vitamin H)-linked thiol (BLT)-attached

gold nanometals [8, 17] were prepared by the literature procedures. The BLT was synthesized by simply coupling biotin with 2-mercaptoethylamine after activation of the carboxylic acid group of biotin with pentafluorophenol. Average diameters of near monodispersed gold nanoparticles were from ~4.5 nm to ~22 nm within electrostatic dipole approximation. Complexation of 2-mL gold aqueous solution and 0.6-mL dipotassium bis(*p*-sulfonatophenyl) phenylphosphane dihydrate (BSPP) were shaken gently for 14 hours at room temperature for stabilizing gold colloidal particles [18–20]. Mixture of 0.1 mL of 1 mM BLT with BSPP-capped gold solution was incubated for 4 hours at room temperature for preparing BLT-attached gold solutions. Residual of BSPP and BLT from the BLT-attached gold solution was completely removed. Bovine serum albumin (BSA) (0.05% w/v) was added to the BLT-attached gold nanoparticle solution to minimize nonspecific adsorption of proteins [21–23]. A stock solution of streptavidin (10^{-5} M) was prepared in deionized water and stored at near-freezing temperature. The concentration of streptavidin to cause gold nanoparticle aggregations was similar to Sastry et al.'s report [8]. Addition of 0.1-mL of streptavidin solution (10^{-5} M) at room temperature into 1-mL of BLT (1 mM)-attached mixture of 2-mL gold aqueous solution and 0.6-mL BSPP could lead to their aggregations with biospecific binding. Biotin-streptavidin interaction is biomolecular recognition of Ka ~10^{15} moL^{-1} as a high affinity, and is stable over a wide range of pH and temperature [3–6]. Streptavidin is a tetrameric protein, and coordinates to the biotin ligands from Au colloidal nanoparticles which lead to cross-linking of the particles [8]. The mixture of the induced-aggregation of the gold colloidal nanoparticles was stirred for 10 minutes and stored at room temperature for microscopic and spectroscopic analysis.

The DNA-linked Au nanoparticles also interact biospecifically and inspire self-hybridization that creates surface plasmon coupling between neighboring particles, and modifies their optical properties. For DNA-linked Au-hybridization [15, 24], Au nanoparticles with ~12-nm average diameter were prepared by the citrate reduction of $HAuCl_4$, and were chemically modified with 5′-or 3′-alkythiol-capped 12-base oligonucleotides (Au-S-5′-DNA or Au-S-3′-DNA). A mixture solution with ~1/220 mole ratio of 14 nM Au nanoparticles (6.1 mL) to 5′- or 3′-DNA containing thiol functional group (1.59 mL) was shaken for 20 hours at room temperature. The Au-S-5′-DNA (0.2 mL) and Au-S-3′-DNA (0.2 mL)- 12-base oligonucleotides were hybridized to a series of 20-μL oligonucleotide linkers ranging from 24 to 72 base pairs in the length of ~8–24 nm. For the DNA-linked gold nanoparticle solutions, each 20-μL of the 36.5-μM DNA linker solution (24, 48, 72 base linker) was added to 790-μL of DNA-modified gold nanoparticle solution in 0.3 M NaCl, 10 mM phosphate buffer (pH 7), and 0.01% azide solution. In the DNA-driven nanometal hybridization process, oligonucleotide-functionalized Au nanoparticles were exposed to free oligonucleotide, one end of which was harmonizing to the DNA on half of the Au nanoparticles, the other end of which was matching to the DNA on the rest of the spherical nanoparticles. The different rates of DNA hybridization were associated with the three oligonucleotide linkers of 24, 48, and 72 base pairs. The DNA hybridization pulled the nanoparticles together and aggregated them each other. The resulting optical spectra of DNA-linked Au-hybridization differed significantly from those of noninteracting and monodispersed particles.

3. Result and Discussion

Schematic diagrams, transmission electron microscopic images, and typical absorption spectra of monodispersed nanoparticles and bioinspired Au aggregations were shown in Figures 1(a) and 1(b). The schematic diagrams displayed the avidin-mediated assembly of biotin-attached gold nanoparticles [1, 17, 25]. Their typical absorption spectra showed SPR shift from 519 nm with bare Au and 526 nm with BLT-attached Au nanoparticle solution to 550 nm by adding 10^{-5}-M (strept)avidin solution. The binding of (strept)avidin to the BLT-attached Au nanoparticles was completed within ~10 minutes at room temperature. The spectral change of SPR for BLT-attached Au nanoparticle is owing to dielectric environment change, and that for self-assembly is because of aggregated size and coupling between the particles. The strong aggregation of avidin-mediated assembly of the BLT-attached Au nanoparticles was additionally confirmed by TEM as shown in Figure 1(c). Similarly, schematic diagram [15, 24, 26], typical absorption spectra, and TEM images of DNA-linked gold nanoparticle assembly were shown in Figures 2(a), 2(b), and 2(c), respectively. The schematic diagram explains three different linker lengths of 24-, 48-, 72-base DNA-conjugated Au assembly through hybridization of each 12-base DNA-conjugated Au nanoparticles [15, 24]. Large spectral changes and assembly conditions were evidenced by their optical absorption and TEM images.

The combinational analysis of both flocculation parameter and peak shift of SPR on bioactivated Au nanometals was shown in Figures 1(d) and 1(e). The flocculation parameter was evaluated by following Mayya et al.'s process [28], which was depicted in the order of extinction normalizations of Au nanoparticles and assembly, subtraction of the spectra of Au nanoparticles from that of bioinspired Au assembly, and extinction integration between flocculation resonance [7, 29] at 600 nm and cutoff wavelength at 800 nm that encountered the longitudinal component up to nanoparticle diameters ~56 nm [7, 8].

Maximum changes of extinction and SPR peaks between Au and Au-biotin-avidin were ~0.3 and ~1.2, and 6 nm and 53 nm, respectively, with particle concentrations of ~1×10^{-5} mol/m^3 and average diameters of ~4 to ~23 nm. Shifting of the SPR peak of colloidal special AU nanoparticles well explained by the Mie theory [30], and that of extinction spectra of self-assemblies was known as a collective nature of the aggregate response [31]. The lowering extinction of Au self-assembly could be attributed to a shadowing effect [31]. In contrast, smaller Au nanoparticle assemblies, for example, D~4.5 nm, did not exhibit the lowering extinction compared with that of bare Au colloidal particles that implied a shortening collisional dephasing rate of Au self-assemblies

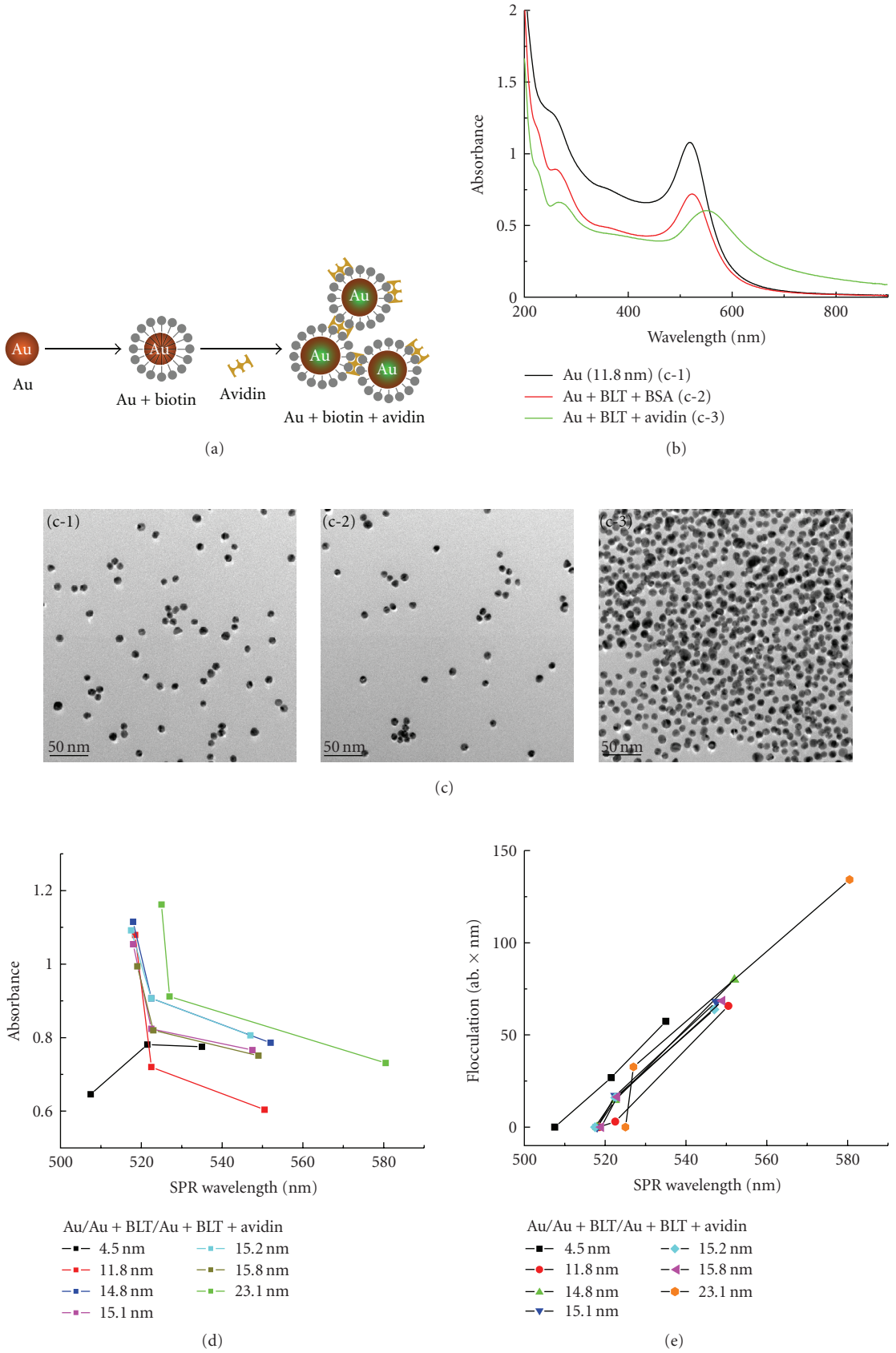
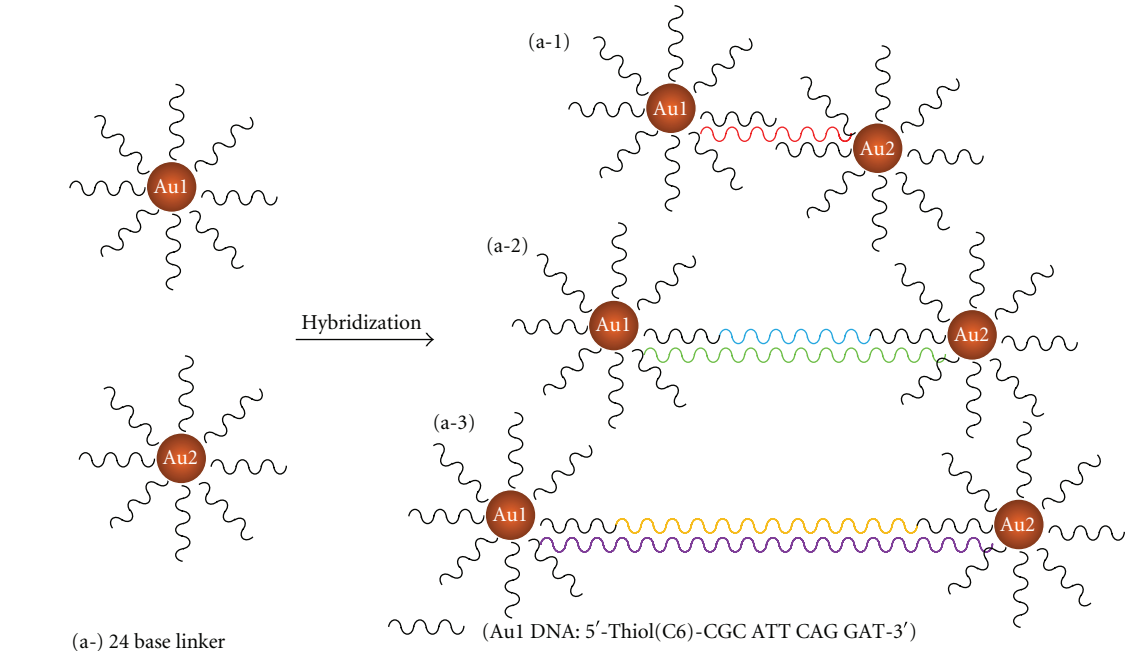
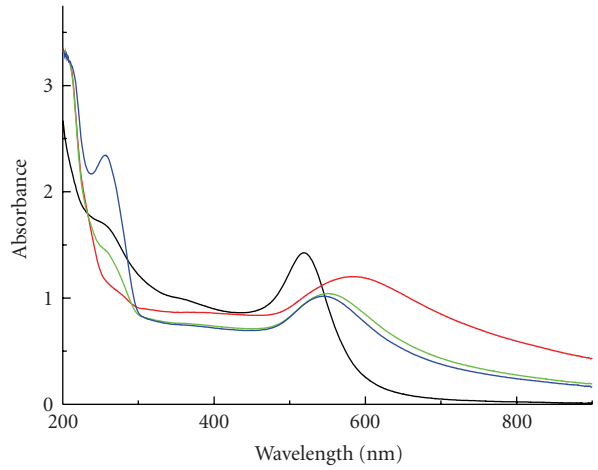


Figure 1: (a) Schematic diagram [1, 17, 25], (b) typical absorption spectra, (c) typical TEM images of bare Au, Au+Biotin, and Au+Biotin+Avidin, (d) absorbance as a function of SPR peak wavelength, and (e) flocculation as a function of SPR peak wavelength of bare Au, Au+Biotin, and Au+Biotin+Avidin.

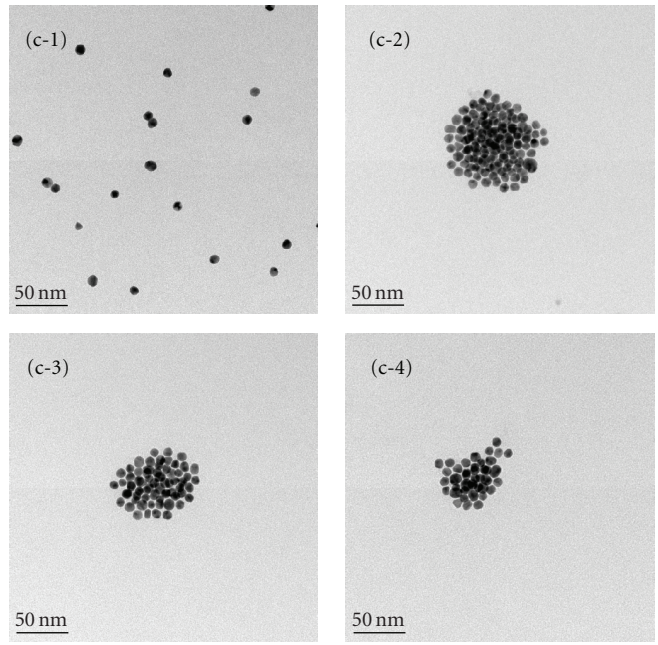


(a)



	Sample	SPR (nm)	Peak ab.	Floc. (A × λ)	
—	Au (~ 12 nm)	~ 519	~ 1.43	~ 0	(c-1)
—	Au-DNA 24	~ 584	~ 1.2	~ 135	(c-2)
—	Au-DNA 48	~ 550	~ 1.04	~ 81	(c-3)
—	Au-DNA 72	~ 545	~ 1.02	~ 72	(c-4)

(b)



(c)

Figure 2: (a) Schematic diagram [24, 26, 27], (b) typical absorption spectra, and (c) typical TEM images of bare Au, 24-base, 48-base, and 72-base DNA-linked gold nanoparticle assembly.

[32–36]. However, the flocculation factors of bioinspired Au assemblies for various sizes of ~4 to ~23 nm were just increased comparing to extinction integration of bare Au colloidal nanoparticles. It indicates that spectral red shift and broadening can be major contributions to increase the flocculation factors for larger particle sizes, and damping rate decrease, and red shift are possible contributions to flocculation factor increase with smaller sizes.

The plasmon frequency shift of the DNA-linked aggregates was inversely dependent on the lengths of oligonucleotide linkers, and was directly related to the interparticle distance as well as the aggregate size. The optical properties of DNA-linked Au nanoparticle aggregates could be controlled through choice of DNA linker length within these novel structures [31]. The changes of SPR peaks between Au and Au hybridizations were ~64, 34, and 28 nm for the three oligonucleotide linkers of 24, 48, and 72 base pairs, respectively. Weak red shifts and lower extinctions are probably due to the weak coupling effect between neighboring particles and less unit volume density of Au particles within hybridization as the base pair of oligonucleotide linker is increased. The flocculation of SPR spectra of Au aggregates was inversely proportional to the base pairs of oligonucleotide linker, which possibly could be due to weak red shift and less density instead of a shadowing effect.

4. Concluding Remarks

In summary, Au-biotin-avidin and DNA-linked Au nanoparticles interact biospecifically and inspire self-hybridization that creates surface plasmon coupling between neighboring particles and modifies their optical properties. Highly selective SPR properties and their flocculations of bioinspired Au-assembly or Au-hybridizations have important implications for the development of colorimetric biological detection or treatment. Therefore, a combination of plasmonic optical tunability and DNA-linked Au-hybridization provides an excellent colorimetric analysis for biophotonic applications.

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The idea of magnetoelectricity has been around for decades, and several materials and concepts have been studied; however, recent discoveries of compounds in which the magnetoelectric (ME) coupling is strong have sparked a renaissance. ME symmetry allows magnetization (resp., polarization) to be induced by the application of an electric (resp., magnetic) field. The initial impetus driving ME materials was the prospect of employing a single field excitation, for example, voltage gradient, to tune both polarization and magnetization characteristics. Significant progress in ME nanostructures has been made in recent years, mainly driven by materialist use in new class of multifunctional device applications, for example, electric field-controlled magnetic data storage, antenna miniaturization using passive substrates, and field control for spintronics applications. Manipulation of electric polarizations by external magnetic fields (and vice versa) has been demonstrated in some of single-phase multiferroic materials, for example, $BiFeO_3$. In this case, the intrinsic ME effect arises from the coupling between electric and magnetic order parameters. Intrinsic ME coupling occurs in compounds in which time-reversal and space-inversion symmetries are absent. A significant ME effect is also observed in the case of two-phase (thin films) composite multiferroics made by combining ferroelectric and ferromagnetic substances together, for example, $BaTiO_3/CoFe_2O_4$, and lead-zirconate-titanate (PZT)/ferrite (or Terfenol-D). In these previous studies, it is commonly believed that elastic coupling amongst the ferroelectric and magnetic order parameters is at the origin of the ME effect, that is, an electric field E induces strain in the ferroelectric; this strain is passed on to the ferromagnet, where it causes a net magnetization M in the sample. A basic understanding of the distributions of electric, magnetic, and internal stress fields within the material allows one to manipulate the relative stability of domain structures. One would expect that the ME effect is large if the coupling at the interface is large; thus, composites with large surface area (such as multilayer systems and nanogranular mixtures) and strongly ferroelastic components are especially effective. The theoretical understanding of the microscopic mechanism that causes the strong coupling between the electric and magnetic degrees of freedom is quite limited. We therefore anticipate that ME nanostructures may open up a whole new dimension for a wide range of materials having cost effective, small size planar, and broadband multifunctional device applications.

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