Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Controlled Assembly of Gold Nanodiscs via Click Chemistry and DNA Hybridization for Tunable Plasmonic Coupling
『クリックケミストリーおよびDNAハイブリダイゼーションを用いた金ナノディスクの集合制御によるプラズモンカップリング調節』

Plasmonic gold nanoparticles have a unique collective oscillation of their conduction electrons, named as localized surface plasmon resonance (LSPR). When gold nanoparticles assemble at a close distance from each other, their plasmons couple, resulting in plasmonic shift. The plasmonic coupling depends on the assembly size, spatial arrangement, and interparticle gap distance. Uncontrolled assembly negatively affects the optical properties.[1] Therefore, controlled assembly is essential to prevent aggregation, control the assembly size, regulate the assembly structure, and alter the plasmonic coupling. Simple mixing of linker-modified gold nanospheres (AuNPs) allows to produce dynamic self-assembly with external stimuli, whereas isotropic AuNPs tend to assemble into three-dimensional (3D) structure which is difficult to determine the number of particles within the assembly cluster.[2] In contrary, anisotropic gold nanodiscs (AuNDs) formed one-dimensional (1D) assembly via face-to-face interaction.[3] However, the use of a single type of ligand restricted the ability to tune the assembly size and the interparticle gap distance. Therefore, AuNDs were modified with two types of ligands to control the assembly, including the interparticle gap distance and multimerization degree to tune the strength of plasmonic coupling. To achieve this purpose, assembly was driven by two approaches (Fig. 1): (a) cross-linking between alkyne (**Alk**)- and azide (**N3**)-modified AuND and (b) direct hybridization between complementary single-stranded DNA-modified AuND.

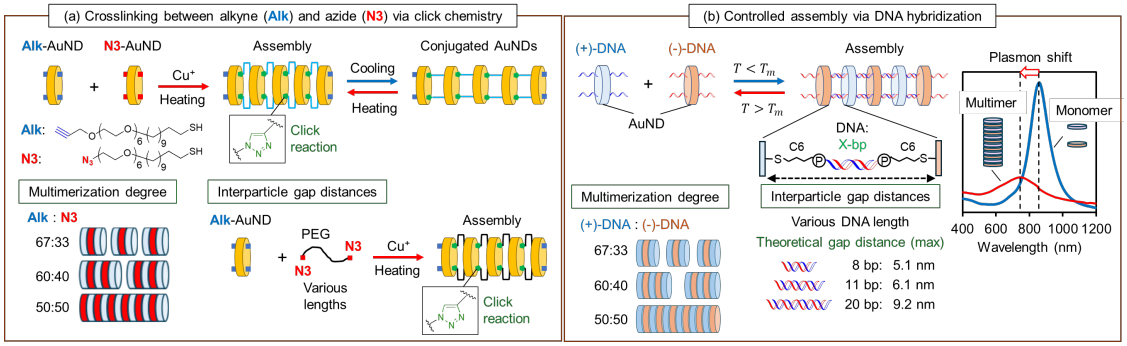

Figure 1. Two approaches used in this study to control the multimerization degree and interparticle gap distances: (a) attempted assembly and crosslinking of AuNDs via click chemistry and (b) controlled assembly of AuNDs via DNA hybridization.

First, AuNDs (91 and 10 nm in diameter and thickness) were modified with thermo-responsive (**C1**) and negatively charge (**COOH**) ligands via ligand exchange reaction, exhibiting reversible assembly and disassembly upon heating and cooling. The addition of **Alk** or **N3** ligand altered the assembly and disassembly behaviors due to the ligand hydrophobicity. Click reaction between **Alk**- and **N3**-modified AuND was carried out upon heating in click solution containing of Cu^{+}. Samples with and without click ligands exhibited irreversible assembly, suggesting that Cu^{+} might influence **COOH** ligand which reduced the electrostatic repulsion forces to facilitate disassembly. Cross-links between **Alk**-modified AuND and **N3**-PEG-**N3** linker were not clearly confirmed due to the strong adhesion—possibly involving hydrophobic interactions and van der Waals forces—which inhibited the dissociation of assembled AuNDs. Besides, the bulkiness of **N3**-PEG-**N3** linker might restrict **N3** groups to access the **Alk** groups on AuND surface, reducing the click reaction efficiency. Although AuNDs were modified with both **Alk** and **N3** ligands, click reactions between AuND-**Alk**-**N3** were not clearly confirmed because click solutions with and without Cu^{+} suppressed the disassembly behavior. As a result, the combination of thermo-responsive and click-reactive ligands was ineffective to

control the assembly and disassembly of AuNDs due to the strong face-to-face interactions driven by hydrophobic effects, van der Waals forces, hydrogen bonding, and ionic interactions.

To overcome the strong face-to-face interactions, AuNDs were modified with DNA ligands using three methods: (i) click reaction between DBCO-DNA and **N3**-functionalized AuNDs, (ii) salt aging, and (iii) butanol dehydration. Among these methods, butanol dehydration was adopted as a simple and effective strategy to prepare DNA-modified AuNDs. The grafting density of 20 base DNA on AuND surface was calculated as 0.013 strands/nm^2, which is approximately four times lower than that obtained by salt-aging method in the previous report. AuNDs modified with complementary short DNA ligands ((+)-DNA-AuND and (-)-DNA-AuND) without spacers were assembled into 1D cylindrical structures with varying interparticle gap distances and degree of multimerization to tune the plasmonic coupling. Assemblies with short interparticle gap distances (< 10 nm) exhibited a significant blue shift in the extinction spectra, indicating a strong plasmonic coupling. The DNA-hybridization-driven assembly was confirmed by introducing blocking DNA strands, which suppressed hybridization, and by performing multiple heating and cooling cycles that demonstrated reversible assembly and disassembly under specific salt concentration. The plasmonic coupling was regulated by controlling the multimerization degree or interparticle gap distances using three methods: (i) adjusting salt concentrations, (ii) adjusting the molar mixing ratios of (+)-DNA-AuND to (-)-DNA-AuND, and (iii) changing the lengths of DNA ligands (8, 11, and 20 base pair) of comparable assembly sizes.

Salt concentration provided a simple means to control the assembly sizes by modulating the strength of the charge repulsion between AuNDs, enabling the tunable plasmonic coupling. Besides, the assembly sizes were regulated by adjusting the molar mixing ratios of (+)-DNA-AuND to (-)-DNA-AuND, where excess of (+)-DNA-AuND terminated the multimerization reaction, following the principles of condensation polymerization. The plasmonic shift enhanced by reducing DNA length due to the stronger plasmonic coupling of shorter interparticle gap distance, whereas the decrease in the length of DNA ligands was limited by the colloidal stability of AuNDs. In addition, assembly (T_A) and disassembly (T_D) temperatures could be tuned by changing salt concentrations and DNA lengths. T_D gradually increased with increasing salt concentration, consistent with general property of free DNA. Interestingly, T_A was lower than T_D in the lower salt concentrations or longer DNA lengths, defined as hysteresis which is associated to the strong electrostatic repulsion between phosphates of the DNA ligands or the increased probability of the DNA base pairings during partially mismatched hybridization. Importantly, the observed T_D values matched the corresponding T_m values of free DNA owing to the spacer-free DNA ligand design which optimally balances suppression factors (e.g., steric hindrance, limited hybridization zones) and promotion factors (e.g., flat surfaces, moderate DNA ligand density to improve accessibility).[4]

Although tuning the molar ratios successfully modulated the degree of multimerization, the estimated degree of polymerization was not precise with inconsistence of the values and broader distributions. Salt-induced assembly also proceeded further association of assembled structure by increasing salt concentration upon drying which limited the accurate evaluation of multimerization degree. As a result, both methods did not achieve defined-number assemblies due to the limited kinetic control. Then, gel electrophoresis and centrifugal separation were applied to separate AuNDs assemblies according to the sizes. However, assembled AuNDs dissociated into monomers during electrophoresis because DNA hybridization was sensitive to salt concentration and temperature. The centrifugation-based approach could not separate the individual multimers, preventing homogeneous assembly sizes. Therefore, psoralen molecule and dithiol-PEG were applied to crosslink the assembled AuNDs. Nevertheless, further optimization is required to improve the static assembly formation. In the future, combining the crosslinking and gel electrophoresis will open the possibility to isolate discrete AuND oligomers, resulting in defined-number assemblies with well-defined plasmonic properties.

References

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