Aggregation of Metal-Nanoparticle-Induced Fluorescence Enhancement and Its Application in Sensing

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ABSTRACT: Fluorescence-based detection methods have been widely utilized in various applications. Materials that display aggregation-induced emission (AIE) are excellent fluorescence probes to offer high contrast ratio. Chromophore-conjugated plasmonic metal nanoparticles (NPs) have been recently found to display significantly enhanced fluorescence emission upon the formation of aggregates. This new type of AIE enhancement has a totally different working mechanism. It is based on aggregation-induced plasmon coupling of metal NPs to enhance the fluorescence intensity of chromophores by increasing both the excitation efficiency and radiative decay rates, instead of reducing nonradiative decay rates as in typical AIE. AIE enhancement of chromophore-conjugated metal NPs results in a dramatic change in fluorescence intensity from severely quenched fluorescence to significantly enhanced fluorescence upon aggregate formation. It offers excellent contrast ratio and is attractive for developing platforms for highly sensitive sensing and imaging applications with reduced background. This mini-review summarizes the basic working principle and recent progress in fluorescence enhancement by coupled metal NPs on the single-molecule level, aggregation-induced plasmon coupling enhanced fluorescence of chromophore-conjugated metal NPs, and their applications in sensing. Perspectives on further utilization of this interesting phenomenon for various biomedical applications have also been discussed.

INTRODUCTION

Fluorescence-based detection methods have been widely utilized in various applications including sensing, imaging, and diagnosis due to their high sensitivity, convenience, good repeatability, and low cost. Integration of fluorescence-based detection with microscopy has further extended the applications to chemical and biological imaging with subwavelength resolution, allowing non invasive visualization of biological events at the subcellular level and detection on the single molecular level. To achieve highly sensitive sensing and imaging outcomes, the use of fluorescence probes with high brightness is required. Tremendous research efforts have been devoted to developing robust fluorescence probes with high brightness such as small organic and inorganic molecules, polymers, and various nanoparticles (NPs). Compared to their molecular counterparts, fluorescent NPs display unique advantages such as significantly improved brightness as well as multifunctional capability through various nanoeengineering techniques, which allow them to be employed for various sophisticated applications such as multiplexed sensing and imaging, targeted bioimaging, and imaging-guided therapy.

The performance of fluorescence methods is largely determined by the contrast ratio of signal strength over the noise level. In addition to improved signal strength by increasing the brightness of fluorescence probes, contrast ratio could also be improved by reducing the noise level from the background fluorescence. A lot of efforts have been devoted to developing assays with a dramatic change in fluorescence responses. In particular, materials that display aggregation-induced emission (AIE) offer high contrast ratio and have attracted vigorous research since its discovery by Tang et al. in 2001, which provides a new concept for lots of potential applications. It breaks the common belief that aggregation of chromophores or nanoparticles will generally result in fluorescence quenching, a long-standing problem for their biomedical applications, as molecules and nanoparticles tend to form aggregates in the biological environments. The mechanism of AIE of organic molecules is generally believed to be due to improved emission quantum yield (QY) as a result of restricting intramolecular motion to minimize the nonradiative decay process in the aggregated state.

Lots of new chromophores with AIE properties have been developed, mostly small molecules. Chromophore-conjugated plasmonic metal NPs have been recently found to display significantly enhanced fluorescence emission upon the formation of plasmonic aggregates which could be considered as another type of AIE. This new type of aggregation induced emission enhancement in chromophore-
conjugated metal NPs has a totally different working mechanism. It is based on aggregation-induced plasmon coupling of metal NPs to enhance the fluorescence intensity of chromophores by increasing both the excitation efficiency and radiative decay rates, instead of reducing nonradiative decay rates as in the case of typical AIE. Aggregation-induced emission enhancement of chromophore-conjugated metal NPs can give an excellent contrast ratio as a result of the dramatic change in fluorescence intensity from severely quenched fluorescence to significantly enhanced fluorescence in response to analyte-induced aggregate formation. This feature will allow the development of a lot of potential sensing and imaging applications. In this mini-review, we will provide a brief introduction on the mechanism of aggregation-induced plasmon-coupling-enhanced fluorescence, summarize the recent progress, and end with a future perspective on challenges and potential applications.

**PRINCIPLES OF METAL-ENHANCED FLUORESCENCE (MEF)**

Noble metal NPs, such as Au, Ag, and Cu, have been known to display unique phenomena of localized surface plasmon resonance (LSPR) as a result of collective oscillation of conduction band electrons induced by interactions with light radiation (Figure 1). LSPR of noble metal NPs results in a significantly enhanced local electric field near the metal NPs, which is responsible for lots of interesting phenomena such as surface-enhanced Raman scattering (SERS), surface-enhanced fluorescence (SEIRA), and metal-enhanced fluorescence (MEF). The LSPR frequency depends on the size, shape, and composition of metal NPs and their surrounding dielectric environment as well as interactions between adjacent metal NPs (Figure 1b).

Plasmon coupling interaction between adjacent metal NPs in nanoassembly or aggregates will result in the appearance of a new SPR band at the longer wavelength range. The formation of metal NP aggregates will be accompanied by an obvious solution color change, which can be easily visualized by the naked eyes. This phenomenon has been widely utilized in the development of various detection schemes known as colorimetric detection. In addition to the change in the extinction spectra, more importantly, plasmon coupling interactions will result in a giant local electric field in the gap region of metal NPs, which has been known as a “hot spot”. Previous studies revealed that local electric field enhancement (IEF) for Ag NP dimers can reach up to ca. 106, much larger than that of isolated Ag NPs (102). The giant enhancement of local electric field has been known to be responsible for a huge change in optical responses such as SERS and two-photon photoluminescence. Various optical assays based on aggregation of metal NPs have been developed for a wide range of applications to take advantage of the aggregation-induced dramatic change in optical responses such as colorimetric detection, SERS, two-photon photoluminescence, and hyper-Rayleigh scattering.

The photophysical processes involved in fluorescence are illustrated in Figure 2a. After absorption of light (the excitation process, with its corresponding rate denoted as $\gamma_{\text{EX}}$) to promote molecules from the ground state ($S_0$) to their singlet excited state ($S_1$), the excited molecules will eventually relax back to the $S_0$ state through radiative decay (with the rate of $k_R$) by dissipating the energy as fluorescence emission or nonradiative decay (with the rate of $k_{NR}$) by dissipating the energy as thermal energy. Fluorescence quantum yield is determined by the competition between the radiative and nonradiative decay processes, i.e., $\eta = k_R/(k_R + k_{NR})$. The overall fluorescence intensity is proportional to the product of excitation rate and emission quantum yield. The corresponding fluorescence lifetime ($\tau$) is inversely proportional to the sum of the radiative and nonradiative decay rates, i.e., $\tau = 1/(k_R + k_{NR})$.

The principle of MEF is illustrated in Figure 2b. When a chromophore is brought to the proximity of plasmonic metal NPs, metal–chromophore interactions will modify the rates of all three relevant photophysical processes: excitation, radiative and nonradiative decay processes. First, the excitation rate is proportional to the square of the electric field and will increase significantly as a result of amplified local electric field. Second, the radiative decay rate ($k_R'$) of the chromophore will be

**Figure 1.** (a) Schematic illustration of localized surface plasmon resonance of metal NPs. (b) Normalized extinction spectra of nanospheres (A), nanocubes (B), and nanorods with different aspect ratios (C–E), respectively. Electric fields of silver nanosphere monomer (c) and dimer (d). Reprinted with kind permission from refs 6 and 10. Copyright 2007 by Annual Reviews, 2008 American Chemical Society.

**Figure 2.** Working principle of metal-enhanced fluorescence. (a,b) Effects of metal–chromophore interactions on excitation and radiative and nonradiative decay processes of chromophores and (c) separation distance dependent metal-enhanced fluorescence. $\gamma_{\text{EX}}$, $\gamma'_{\text{EX}}$ are excitation rates in the absence and presence of plasmonic metal NPs.
enhanced as a result of Purcell effects, which is favorable for improving quantum yield.\(^\text{15}\) Third, energy transfer from the chromophore to metal NPs \((k_{\text{ET}})\) will introduce an additional nonradiative deactivation pathway \((k_{\text{NR}} = k_{\text{NR}}' + k_{\text{ET}}),\) which is unfavorable for improving quantum yield as \(QY' = \frac{k_{\text{ET}}}{k_{\text{ET}} + k_{\text{NR}}'}\). As \(k_{\text{ET}}', k_{\text{NR}}',\) and \(k_{\text{ET}}\) are all dependent on the metal–chromophore distance, the overall fluorescence intensity is strongly dependent on the metal–chromophore distance (Figure 2c). Direct contact of chromophores with metal NPs will generally result in fluorescence quenching. Au and Ag NPs have been demonstrated to exhibit superquenching to fluorescence of various chromophores.\(^\text{16}\) As the metal–chromophore distance increases, a transition from fluorescence quenching to enhancement could be obtained (Figure 2c).\(^\text{17}\) Even longer separation distance will result in no influence of metal NPs on the fluorescence intensity of the chromophore. The optimum fluorescence enhancement generally occurred at a separation distance of 5–30 nm, depending on the nature of the chromophore and metal NPs.\(^\text{9,18}\)

Most of the previous MEF studies focus on fluorescence enhancement of chromophores by isolated metal NPs.\(^\text{15}\) As discussed above, coupled metal nanostructures give much stronger local electric field than isolated metal NPs, which is responsible for giant SERS signals and strong two-photon photoluminescence of aggregated metal NPs.\(^\text{3,14}\) When chromophore molecules are placed at the gap region with a giant local electric field, stronger metal–chromophore interaction is expected to result in larger fluorescence enhancement. It has been demonstrated on the substrate and in the colloid solution\(^\text{19}\) as well as on the single-particle level\(^\text{20–22}\) that coupled metal nanostructures displayed much larger emission enhancement of chromophores compared to the monodispersed metal NPs.

■ ENHANCED FLUORESCENCE OF SINGLE MOLECULES BY COUPLED METAL NANOSTRUCTURES

Smart design of coupled metal nanostructures is of vital importance for achieving large plasmon coupling enhanced fluorescence. The local electric field amplification is highly sensitive to the interparticle separation distance.\(^\text{23}\) Numerical simulation showed that the electric field in the gap junction of an Au nanosphere (NS) dimer increases with the reducing gap size with an optimal distance of 0.5 nm. Further decrease in the interparticle distance will result in electron tunneling between two nanoparticles, leading to reduced surface charge densities and consequently decreased local electric field. Therefore, it is crucial to control the gap size of coupled metal nanostructures for the highest MEF enhancement. Various assembly methods such as electrostatic interactions, molecular interactions, DNA-assisted assembly, and lithography have been utilized to prepare various coupled metal nanostructures to study plasmon coupling enhanced fluorescence on the single particle level.\(^\text{20–22}\)

In 2009, Kinkhabwala et al. demonstrated large single-molecule fluorescence enhancement by using lithographically fabricated bowtie nanoantennas (Figure 3a).\(^\text{20}\) The Au bowtie nanoantenna was composed of a pair of tip-to-tip Au nanoantennas with controllable gap size ranging from 14 to 80 nm. Near-infrared emitting dyes (quantum yield of ~2.5%) TPQDI, were embedded in a thin layer of PMMA covering the Au bowtie nanoantennas. The fluorescence of single TPQDI molecules was monitored by a confocal scan of the film. The fluorescence of TPQDI at the gap region experienced giant enhancement as a result of enhanced excitation efficiency and fluorescence quantum yield. The enhancement increased with the decreasing gap size. Single-molecule fluorescence enhancement of up to 1340-fold was observed at the smallest gap of 14 nm. Fluorescence lifetime was observed to be as short as 10 ps.

In 2014, Zhang et al. studied single-molecule fluorescence enhancement by Au nanorod (NR) dimers with a tip-to-tip orientation (Figure 4).\(^\text{21}\) The Au NR dimers with tip-to-tip orientation were prepared by the DNA origami technique, which allows gap size of the Au NR dimer to be precisely controlled down to 6.1 nm. A commercial dye, ATTO-655, with a relatively high fluorescence QY of ~30% was introduced to the gap region of the Au NR dimer through a homemade flow cell. The enhanced fluorescence of a single ATTO-655 molecule corresponded to a series of temporally separated

Figure 3. (a) Finite-difference time-domain calculation of local electric field enhancement in the Au nanobowtie and (b) fluorescence enhancement of single dye molecules in the Au nanobowtie. Scale bar: 100 nm. Reprinted with kind permission from ref 20. Copyright 2009 American Chemical Society.

Figure 4. (a–c) Plasmon coupling enhanced fluorescence as a function of gap distance by using the Au nanorod dimer using the DNA origami method, and the average length of Au NRs is 43.5 nm. (d–f) Schematic illustration of fluorescence enhancement by the Au nanosheet dimer and trimer. (f) Fluorescence enhancement vs hot spot volume. Reprinted with kind permission from refs 21 and 22. Copyright 2015 American Chemical Society.
The performance of these optical antennas in the blue and green regions. Inspired by the observations of larger single-particle level due to average results of the ensemble enhancement factors are relatively smaller than those on the counterparts, high spectral range for dyes with high QY. In the case of the Au enhancements of more than 100 times throughout the visible molecule enhancement was experimentally characterized with single-antennas consisting of Ag NPs displayed as in Figure 4d−f.22 A fluorescence enhancement of 600-fold was achieved by using an 80 nm Au NP dimer with 6 nm gap as nanoantennas accompanied by a detection volume isolated down to 70 zL. Integration of self-assembled nanoantenna-induced fluorescence enhancement with fluorescence correlation spectroscopy allows a wide range of applications for biosensing and single-molecule detections.

Later in 2015, Punj et al. utilized self-assembled Au NPs to enhance single-molecule fluorescence detection at high micromolar concentrations (Figure 4d−f).22 A fluorescence enhancement of 600-fold was achieved by using an 80 nm Au NP dimer with 6 nm gap as nanoantennas accompanied by a detection volume isolated down to 70 zL. Integration of self-assembled nanoantenna-induced fluorescence enhancement with fluorescence correlation spectroscopy allows a wide range of applications for biosensing and single-molecule detections.

In 2017, Vietz et al. demonstrated broadband fluorescence enhancement by self-assembled Au and Ag NPs throughout the visible spectrum (Figure 5).20 The optical antennas were fabricated by using the DNA origami method to self-assemble two 80 nm Ag or Au NPs. Three different dyes (Alexa488, Atto542, and Atto647N) were utilized as the model fluorophores, covering the spectrum from the blue to red. The performance of these optical antennas in fluorescence enhancement was experimentally characterized with single-molecule fluorescence measurements. This work showed that antennas consisting of Ag NPs displayed fluorescence enhancements of more than 100 times throughout the visible spectral range for dyes with high QY. In the case of the Au counterparts, high fluorescence enhancements were observed in the red to near-infrared region. The results indicate that Ag-based antennas strongly outperform their Au counterparts in the blue and green regions.

### Aggregation-Induced Plasmon Coupling Enhanced Fluorescence

In addition to single-particle spectroscopy, large fluorescence enhancement by coupled metal nanostructures has also been demonstrated on the substrate and colloid solution.19 The enhancement factors are relatively smaller than those on the single-particle level due to average results of the ensemble measurements. Inspired by the observations of larger fluorescence enhancement by coupled metal nanostructures compared to monodispersed metal NPs13 and fluorescence quenching of chromophores in direct contact with metal NPs,16 Li et al. designed a fluorescence turn-on scheme by utilizing silica-coated Ag NPs to light-up the prequenched fluorescence of chromophores that were directly linked to Au NPs (Figure 6a).3 Plasmon coupling interactions between Au NPs and Ag NPs resulted in a transition from fluorescence quenching to enhancement with emission intensity significantly higher than that of unquenched free Rhodamine B isothiocyanate (RiTC). Here RiTC was chosen as a model chromophore, which has reasonable fluorescence quantum yield (70% in methanol and 13% in H2O). Upon directly attaching to the surface of 13 nm Au NPs (with ~600 RiTC molecules attached to each Au NP), the fluorescence of RiTC was quenched by about 23-fold compared to that of the same amount of free RiTC in solution. SiO2-coated Ag NPs (80 nm) were utilized to light up the quenched fluorescence of RiTC. SiO2-coated Ag NPs were surface modified with thiol groups to allow coupling with Au NPs via thiol−Au interactions to form coupled nanostructures of Ag@SiO2→Au-RiTC NPs. The gap distance between outside Au NPs and core Ag NPs was tuned from 4 to 50 nm by controlling the thickness of the SiO2 shell to explore the optimal distance for fluorescence enhancement.

![Figure 5](image-url)

**Figure 5.** (a) Schematic illustration of optical antenna consisting of two 80 nm Ag or Au NPs attached to a DNA origami; (b−d) experimental fluorescence enhancement (b), simulated electric-field enhancement (c), and relative change in quantum yield (d) for different dyes by Ag (gray) or Au (yellow) NP dimers. Reprinted with kind permission from ref 24. Copyright 2017 American Chemical Society.

![Figure 6](image-url)

**Figure 6.** (a) Scheme of lighting up Au NPs quenched fluorescence by using Ag NPs; (b) fluorescence spectra of Au-RiTC NPs upon gradual addition of Ag@SiO2; and (c) optimum emission enhancements by SiO2-coated Ag NPs with different SiO2 shell thicknesses. Reproduced from ref 3 with permission. Copyright 2016 The Royal Society of Chemistry.
At the optimum SiO$_2$ shell thickness of 13 nm, the coupled metal nanostructures displayed up to 4.4 times enhancement in fluorescence intensity compared to isolated free RiTC and 101 times enhancement compared to prequenched RiTC in RiTC-Au NPs.

Following the work by Li et al., Zhu et al. further investigated the influence of different factors on plasmon coupling enhanced fluorescence by utilizing DNA-assembled nanostructures (Figure 7). The effects of shape and size of metal NPs, dye distribution, and separation distance have been investigated by using Cyanine 5 (Cy5) as the model fluorescence probe. Similar to the approach by Li et al., the fluorescence of Cy5 was prequenched by attaching DNA-linked Cy5 to the surface of Au NSs. The quenched fluorescence of Cy5 was turned on by forming assembled nanostructures with different NPs through DNA hybridization. Among the three different morphologies studied (Au NSs, Au NRs, and Au@Ag core–shell NSs), Au@Ag NSs gave the best performance in plasmon coupling enhanced fluorescence. However, the absolute fluorescence intensity is still far below that of the same amount of free Cy5 in solution due to random distribution of Cy5 in the coupled nanostructures of the initial scheme (Figure 7a). In the initial design, only a small fraction of Cy5 molecules are located at the gap region to experience the fluorescence enhancement. An improved scheme as shown in Figure 7d was proposed to optimize the distribution of dyes, which resulted in performance improvement by 2.5 times (fluorescence enhancement of 20-fold vs 7.9-fold). Between two different sizes of Au@Ag NSs (65 and 30 nm), 65 nm Au@Ag NSs were found to display five times better fluorescence enhancement factors compared to 30 nm ones (Figure 7c). The separation distance between Au NSs and Au@Ag NSs was adjusted by controlling the strand length of DNA. Optimum fluorescence enhancement of 80-fold was obtained at 8.2 nm separation distance by using 65 nm Au@Ag NSs as the enhancing substrate.

As prequenched fluorescence offers reduced background, this plasmon coupling-enhanced fluorescence phenomenon was further utilized to develop a simple fluorescence turn-on platform for highly sensitive and selective detection of the DNA sequence. Compared to conventional fluorescence turn-on methods based on fluorescence recovery, the change in fluorescence intensity as a result of plasmon coupled enhanced fluorescence can go significantly beyond the extent of fluorescence recovery, which is expected to give improved sensitivity. The detection limit of this method was estimated to be 3.1 pM. Due to its high sensitivity to the subtle change in the structures of nanoassembly, this method also displayed exceptional selectivity to allow detection of single-base mismatch at room temperature. The totally matched DNA displayed fluorescence enhancement of 80-fold versus 8.1-, 3.2-, and 1.5-fold for one-, two-, and three-base mismatched DNA sequences, respectively (Figure 7d).

The above approaches by Li et al. and Zhu et al. rely on using another metal NP to light up the fluorescence of chromophores that was prequenched by Au NPs via forming a nanoassembly, which involved the use of two different metal NPs. This method was recently further simplified and improved by He et al. In the new scheme as shown in Figure 8a, the same type of metal NPs were utilized to act as both quencher and enhancing substrates. Au@Ag NPs were chosen as they have been demonstrated to display good performance in fluorescence enhancement in the previous work. RlTC was chosen as the model chromophore. Conjugation of RiTC with Au@Ag NPs led to fluorescence quenching. Cysteine was chosen as the coupling agent to induce aggregation of Au@Ag NPs to light up the prequenched fluorescence. Cysteine is an amino acid containing a thiol group (–SH), which can bind to the surface of Au or Ag NPs. In an acidic environment, the carboxyl and amino groups of cysteine are ionized to form a zwitterionic structure. Addition of cysteine will lead to aggregation of Au@Ag-RiTC NPs and consequently enhanced fluorescence. A series of Au@Ag NPs with different Ag shell thickness were prepared to optimize the performance. Upon attaching to the surface of metal NPs, the fluorescence of RiTC was quenched by 4.6- to 8.1-fold by Au NPs and Au@Ag NPs, respectively. The quenched fluorescence was subsequently enhanced upon addition of cysteine to induce aggregation of metal-RiTC NPs. The optimum fluorescence of coupled Au-RiTC NPs was 24.3 times that of uncoupled Au–RiTC NPs. For Au@Ag-RiTC NPs, the enhancement factors increased with increasing Ag shell thickness and became saturated as the Ag shell thickness reached ~5.6 nm. Coupled Au@Ag-RiTC NPs with the thickest Ag shell thickness of 5.6 nm displayed...
Figure 8. (a) Scheme of aggregation-induced plasmon coupling enhanced fluorescence of prequenched fluorophores. (b) Emission intensity of coupled Au@Ag-RiTC NPs (red), free RiTC (green), and isolated Au@Ag-RiTC NPs (black). (c) Fluorescence enhancement factors of coupled Au@Ag-RiTC vs isolated Au@Ag-RiTC (black) and free RiTC (green). (d) Emission spectra of Au@Ag(5.6 nm)-RiTC NPs upon addition of various amounts of cysteine. (e) Enhancement factors of coupled Au@Ag-RiTC NPs with various amino acids (10 μM) in tap water. Reproduced with permission from ref 5. 2019 The Royal Society of Chemistry.

optimum fluorescence enhancement: 44.8-fold versus prequenched RiTC and 7.6-fold versus free RiTC. As cysteine is an important amino acid for many physiological processes, cysteine-induced aggregation-enhanced fluorescence of metal-RiTC NPs could be utilized to develop a platform for the detection of cysteine. This method gave a detection limit of 3.8 pM in the tap water, which is more sensitive than most other common methods. This method was also highly selective. Among 21 common amino acids, only cysteine and glutathione molecules displayed aggregation-induced fluorescence enhancement.

As many chemically and biologically important species can cause aggregation of metal NPs, this simple method can be extended for detection of many other analytes. A wide range of analytes could be detected based on this sensing strategy upon appropriate modification of metal NPs with proper recognition moiety. Controlled assembly of plasmonic metal NPs, resulting in red-shifted LSPR spectra in response of analytes, has been utilized in developing colorimetric detection of a wide variety of targets including amino acids, proteins, nucleic acids, DNA sequences, small organic molecules, heavy metal ions, and cancer cells.11 Targets or analytes that induce assembly or aggregation of plasmonic metal NPs to display colorimetric responses could be similarly extended to develop sensing platforms that display aggregation-induced plasmon coupling enhanced fluorescence by using proper chromophores and careful nanoengineering. In this approach, fluorescence was quenched first (OFF state) and subsequently lightened up by aggregation-induced emission enhancement (ON state) to the level many times stronger than that of original unquenched fluorescence. Compared with the conventional fluorescence “turn-on” platform in which the quenched fluorescence just recovers to the level of original fluorescence intensity, this fluorescence “quenching to enhancement” is expected to give much larger contrast ratio and consequently better sensitivity.

This AIE enhancement of chromophore-modified metal NPs is similar to AIE of organic molecules. However, their working mechanisms are totally different. The mechanism of typical AIE of organic molecules generally arises from improved quantum yield due to restriction of intramolecular motion to reduce nonradiative decay rates, which is accompanied by longer fluorescence lifetime. Here AIE of chromophore-conjugated metal NPs is based on aggregation-induced plasmon coupling of metal NPs to simultaneously enhance the excitation efficiency and radiative decay rate of chromophores. Consequently, fluorescence intensity enhancement is accompanied by significantly shortened fluorescence lifetime, which helps to improve the photostability of chromophores for long-term applications.

CONCLUSIONS AND PERSPECTIVES

Owing to unique LSPR properties of metal NPs, profound metal–chromophore interactions result in significant modulation in fluorescence intensity of chromophores, which strongly depends on the nature of metal NPs and chromophores as well as on the separation distance between them. Aggregated metal NPs can give rise to a giant local electric field at the gap region and are thus expected to display much larger fluorescence enhancement compared to isolated metal NPs. Coupled metal nanostructures prepared by different assembly methods have been demonstrated to display fluorescence enhancement by thousands of times on the single-molecule level and hundreds of times in the colloid solution. On the other hand, direct contact of the chromophore generally resulted in severe fluorescence quenching. The quenched fluorescence of chromophore-conjugated metal NPs could be lightened up by interacting with another metal NP or forming the aggregates to enable plasmon coupling to significantly enhance their excitation efficiency. Aggregation of chromophore-conjugated metal NP induced emission enhancement gives a large contrast ratio from severely quenched fluorescence to significantly enhanced fluorescence (much higher than the original fluorescence intensity), which is superior to the conventional turn-on methods based on fluorescence recovery. This method is therefore attractive for developing platforms with reduced background for sensing and imaging applications. The design concept of this fluorescence turn-on method is similar to the well-known AIE phenomenon of organic molecules, but with a totally different working mechanism.

This simple method can be easily extended for detection of many other analytes which can induce the aggregation of metal NPs through various assembly methods. Surface modification of metal NPs with proper recognition moiety and coupling agents is critical for selective interactions and effective assembly to ensure strong plasmon coupling interactions to achieve large fluorescence enhancement. This method involves a transition from fluorescence quenching to enhancement by metal NPs of the same type but different assembly states. Careful balance between two is critical to achieve a large contrast ratio. Different coupling interactions such as covalent bonding, π–π interactions, and host–guest interactions will result in different separation distances (metal–metal and metal–chromophore) and the dielectric environment, which will affect plasmon coupling interactions between metal NPs and metal–chromophore interactions. Lots of further efforts...
are required to design proper metal–chromophore pairs and proper nanostructures to achieve optimum performance. In addition to fluorescence enhancement, aggregation of chromophore-conjugated metal NPs will result in significantly enhanced photothermal effects and 2PPL of metal NPs and two-photon excitation fluorescence, which could be utilized for multimodal imaging as well as imaging-guided photother-apy.4,23 Further surface modification of the metal NPs with targeting groups or therapeutic agents will allow development of a lot of biomedical applications based on aggregation of chromophore-conjugated metal NPs.

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**REFERENCES**

(1) Wolfbeis, O. S. An overview of nanoparticles commonly used in fluorescent bioimaging. *Chem. Soc. Rev.* 2015, 44, 4743–4768.
(2) Hong, Y.; Lam, J. W. Y.; Tang, B. Z. Aggregation-induced emission. *Chem. Soc. Rev.* 2011, 40, 5361–5388.
(3) Li, S.; Zhang, T. S.; Zhu, Z. J.; Gao, N. Y.; Xu, Q.-H. Lighting up the gold nanoparticles quenched fluorescence by silver nanoparticles: a separation distance study. *RSC Adv.* 2016, 6, 58566–58572.
(4) Zhu, Z.; Yuan, P.; Li, S.; Garai, M.; Hong, M.; Xu, Q.-H. Plasmon-Enhanced Fluorescence in Coupled Nanostructures and Applications in DNA Detection. *ACS Appl. Bio Mater.* 2018, 1, 118–124.
(5) He, J.; Li, S.; Lyu, D.; Zhang, D.-F.; Wu, X.; Xu, Q.-H. Aggregation Induced Emission Enhancement by Plasmon Coupling of Noble Metal Nanoparticles. *Mater. Chem. Front.* 2019, 3, 2421–2427.
(6) Willets, K. A.; Van Duyne, R. P. Localized Surface Plasmon Resonance Spectroscopy and Sensing. *Annu. Rev. Phys. Chem.* 2007, 58, 267–297.
(7) Le, R. E. C.; Etchegoin, P. G. Principles of surface-enhanced Raman spectroscopy: and related plasmonic effects, 1st ed.; Elsevier: Amsterdam; Boston, 2009; pp 1–663.
(8) Li, N.; Yin, H.; Zhuo, X.; Yang, B.; Zhu, X.-M.; Wang, J. Infrared-Responsive Colloidal Silver Nanorods for Surface-Enhanced Infrared Absorption. *Adv. Opt. Mater.* 2018, 6, 1800436.
(9) Li, J.-F.; Li, C.-Y.; Aroca, R. F. Plasmon-enhanced fluorescence spectroscopy. *Chem. Soc. Rev.* 2017, 46, 3962–3979.
(10) Chen, H.; Kou, X.; Yang, Z.; Ni, W.; Wang, J. Shape- and Size-Dependent Refractive Index Sensitivity of Gold Nanoparticles. *Langmuir* 2008, 24, 5233–5237.
(11) Zhao, W.; Brook, M. A.; Li, Y. *ChemBioChem* 2008, 9, 2363–2371.
(12) Zhang, J.; Fu, Y.; Chowdhury, M. H.; Lakowicz, J. R. Metal-enhanced single-molecule fluorescence on silver particle monomer
and dimer: Coupling effect between metal particles. *Nano Lett.* 2007, 7, 2101−2107.

(13) Xu, H.; Aizpurua, J.; Käll, M.; Apell, P. Electromagnetic contributions to single-molecule sensitivity in surface-enhanced Raman scattering. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* 2000, 62, 4318−4324.

(14) Guan, Z.; Gao, N.; Jiang, X.-F.; Yuan, P.; Han, F.; Xu, Q.-H. Huge enhancement in two-photon photoluminescence of Au nanoparticle clusters revealed by single-particle spectroscopy. *J. Am. Chem. Soc.* 2013, 135, 7272−7277.

(15) Giannini, V.; Fernández-Domínguez, A. I.; Heck, S. C.; Maier, S. A. Plasmonic Nanoantennas: Fundamentals and Their Use in Controlling the Radiative Properties of Naneomitters. *Chem. Rev.* 2011, 111, 3888−3912.

(16) Fan, C.; Wang, S.; Hong, J. W.; Bazan, G. C.; Plaxco, K. W.; Heeger, A. J. Beyond superquenching: hyper-efficient energy transfer from conjugated polymers to gold nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* 2003, 100, 6297−6301.

(17) Zhang, T.; Yuan, P.; Zhao, T.; Xu, Q.-H. Plasmon-Enhanced Two-Photon Excitation Fluorescence and Biomedical Applications. *Surface Plasmon Enhanced, Coupled and Controlled Fluorescence* 2017, 211−225.

(18) Zong, H.; Mu, X.; Sun, M. Physical principle and advances in plasmon-enhanced upconversion luminescence. *Appl. Mater. Today* 2019, 15, 43−57.

(19) Osorio-Roman, I. O.; Guerrero, A. R.; Albella, P.; Aroca, R. F. Plasmon enhanced fluorescence with aggregated shell-isolated nanoparticles. *Anal. Chem.* 2014, 86, 10246−10251.

(20) Kinkhabwala, A.; Yu, Z.; Fan, S.; Avlasevich, Y.; Muellen, K.; Moerner, W. E. Large single-molecule fluorescence enhancements produced by a bowtie nanoantenna. *Nat. Photonics* 2009, 3, 654−657.

(21) Zhang, T.; Gao, N.; Li, S.; Lang, M. J.; Xu, Q.-H. Single-Particle Spectroscopic Study on Fluorescence Enhancement by Plasmon Coupled Gold Nanorod Dimers Assembled on DNA Origami. *J. Phys. Chem. Lett.* 2015, 6, 2043−2049.

(22) Panj, D.; Regmi, R.; Devilez, A.; Plauchu, R.; Moparthi, S. B.; Stout, B.; Bonod, N.; Rigneault, H.; Wenger, J. Self-Assembled Nanoparticle Dimer Antennas for Plasmonic-Enhanced Single-Molecule Fluorescence Detection at Micromolar Concentrations. *ACS Photonics* 2015, 2, 1099−1107.

(23) Marinica, D. C.; Kazansky, A. K.; Nordlander, P.; Aizpurua, J.; Borisov, A. G. Quantum Plasmonics: Nonlinear Effects in the Field Enhancement of a Plasmonic Nanoparticle Dimer. *Nano Lett.* 2012, 12, 1333−1339.

(24) Vietz, C.; Kaminska, I.; Sanz Paz, M.; Timnefeld, P.; Acuna, G. P. Broadband Fluorescence Enhancement with Self-Assembled Silver Nanoparticle Optical Antennas. *ACS Nano* 2017, 11, 4969−4975.

(25) Guan, Z.; Zhang, T.; Zhu, H.; Lyu, D.; Sarangapani, S.; Xu, Q.-H.; Lang, M. J. Simultaneous Imaging and Selective Photothermal Therapy through Aptamer-Driven Au Nanosphere Clustering. *J. Phys. Chem. Lett.* 2019, 10, 183−188.