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Plasmonic enhancement of single-molecule fluorescence under one- and two-photon excitation

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Summary

This thesis provides a general strategy to handle the major obstacle in single-molecule detection, that the optical signals from common molecules are normally too weak compared to the background. Based on fluorescence, we can suppress the background efficiently by spectral filtering as the photons are emitted at longer wavelength than excitation light (Stokes-shifted); based on the plasmonic enhancement, we are able to enhance the brightnesses of the emitters, hence to improve the signal-to-noise ratio in the single-molecule detection. We exploit the single-crystalline gold nanorods (GNRs) as our basic frameworks in the studies because of their strong near-field enhancement and because of their tunable plasmon resonance. In the whole study, we investigate theoretically and experimentally single-molecule detection with the fluorescence enhancement by a single GNR or by a GNR dimer for the ultra-weak emitters, which can either be molecules with extremely low quantum yield ($\sim 10^{-4}$), or molecules under two-photon excitation.

One/two-photon-excited fluorescence enhancement: single GNR vs GNR dimer

We first theoretically investigate single-molecule fluorescence enhancement by a single GNR and by a GNR dimer. As an example, we kept the diameters of all GNRs constant 40 nm, and their plasmon resonances were tuned to the same wavelength of 765 nm. In our study, three dyes with very different absorption and emission bands were examined. Under weak excitation, the fluorescence enhancement can be considered as the product of excitation enhancement and emission enhancement. Simulations showed that the GNR dimer exhibits better performance on enhancing both the excitation and radiative rates of an emitter, hence provides stronger fluorescence enhancement compared to the single GNR. Most interestingly, a GNR dimer offers much stronger enhancement for the nonlinear optical processes, e.g two-photon-excited fluorescence. As is shown in chapter 2, our simulations reveal that, for a GNR dimer with gap of 5 nm, the enhancement factor for two-photon-excited fluorescence can be as high as $\sim 10^8$. The influence of plasmon resonances on the fluorescence lifetime and on the spectral shape is also investigated for all dyes. At last, we show that at

high excitation intensity, the enhancement factor for both one- or two-photon-excited fluorescence will be saturated to a limited value of the radiative enhancement by the plasmonic structures.

Quantum yield limits for the detection of single molecule enhanced by a single gold nanrod

Single-molecule techniques based on fluorescence depend strongly on the emission from the target molecules against the background noise, therefore suffer from the limitations imposed by weak emitting properties of most molecules. By enhancing the fluorescence with plasmonic nanostructures, such as GNRs, we can extend the application of these techniques to a wider range of species. Theoretical analysis indicates that emitter with lower quantum yield will experience stronger fluorescence enhancement because of larger emission enhancement, yet, the emitted photons will be less. In this work, we explore the lower limit of fluorescence quantum yield for single-molecule detection with the fluorescence enhanced by a single GNR. We specifically designed an infrared dye with the extremely low quantum yield of 10^{-4} and a comparatively large Stokes shift of $3,000\text{ cm}^{-1}$ to demonstrate single-molecule detection by fluorescence enhancement. For molecule with such large Stokes-shifts, both the excitation rate and the overall fluorescence enhancement need to be considered to get best single-to-noise ratio for single molecule detection. Based on the theoretical optimized conditions, we confirm experimentally the detection of single-molecule fluorescence with an enhancement factor of 3 orders of magnitude for the quantum yield 10^{-4} . Theoretical simulations indicate that single-molecule signals should be detectable for molecules with quantum yield as low as 10^{-6} , provided the dwell time of the molecules in the plasmonic hot spot is long enough.

Controlled synthesis of gold nanorod dimers with end-to-end configurations

Plasmonic coupling between metallic nanoparticles can strengthen the near-field enhancement inside the interparticle gaps, as well as the local density of photon states, compared to the individual nanoparticles. As a consequence, metal nanoparticle aggregates can be applied to enhance ultra-weak signals, such as two-photon-excited fluorescence, the signals of which are normally too weak to detect at the single-molecule level. Nanoparticle dimers provide single plasmonic hotspots without reducing the enhancement factors for both excitation and emission rates. Therefore nanoparticle dimer structures can be considered as the best plasmon-coupling system for single-molecule fluorescence enhancement in the sense that i) they provide single sites for fluorescence enhancement; ii) the photoluminescence backgrounds from the nanoparticles can be reduced compared to multimer aggregates.

Among all kinds of nanoparticles, the elongated GNRs may be among the best building blocks for the plasmonic dimer nanostructures, simply because of their narrow, intense, and tunable plasmon resonance with light. Here, we applied two strategies to synthesize the end-to-end GNR dimers with the aid of biotinylated streptavidin as the molecular linkers. Firstly, we illustrate the assembly in bulk of GNRs in the presence of the biotinylated streptavidin. The biotinylated streptavidin can bind specifically to the tips of the GNRs in the presence of

the surfactant cetyltrimethylammonium bromide (CTAB), hence can ensure the end-to-end assembly of the GNRs. The assembly was monitored by recording the extinction spectra of the assembly solution. The constructs were later deposited and kept to dry between glass slides. Secondly, we illustrate the GNR dimer assembly directly on glass slides. We first immobilized the single nanorods on a clean glass slide, and functionalized them with the biotinylated streptavidin specifically on the tips of the each GNR. The molecular linkers functionalized on the immobilized gold nanorods have the chance of absorbing a second GNR in the solution, hence forming dimer structures. During the assembly, based on the bottom-up flipped strategy, we can separate the inevitable multimer aggregates. Therefore, we can get purified dimer structures on the glass. More details can be seen in chapter 4.

Two-photon-excited single-molecule fluorescence enhancement

Two-photon-excited fluorescence refers to the nonlinear optical process where the emission of a fluorophore is due to the absorption of two photons of identical frequency. Compared to one-photon excitation, two-photon-excited spectroscopy offers reduced scattering, deep sample penetration and intrinsic optical sectioning. Single-molecule detection of two-photon-excited fluorescence, however, is challenging because of the extremely low two-photon absorption cross section of most molecules. Here we applied the end-to-end GNR assemblies to enhance the single-molecule fluorescence under two-photon excitation. Simulations show that for an end-to-end GNR dimer with interparticle separation of 5 nm, the overall fluorescence enhancement can reach 10^7 in the gap between the GNRs. With such high enhancement factors, we successfully detected enhanced two-photon-excited fluorescence from single ATTO 610 molecules.

