Plasmonic enhancement of one-photon- and two-photon-excited single-molecule fluorescence by single gold nanorods

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Conclusions and outlook

This thesis is a collection of experimental attempts to enhance photoluminescence of fluorescent molecules and quantum dots with single gold nanorods (GNRs) and relevant applications. Special attention is focused on the interactions between *single* emitters and GNRs. Investigation of single emitters provides direct information from each individual emitter, and thus reveals the characters and detailed processes of the microscopic components that are covered under the macroscopic behavior of the entirety in a conventional ensemble measurement. The first three chapters report enhancement of one-photon-excited fluorescence of single molecules. In the last two chapters, we investigate enhancement of two-photon-excited fluorescence/luminescence, which is much less studied in the literature. Every chapter includes its own conclusion. Here we summarize the important results of each chapter and discuss about open questions and prospects for future research.

Single-molecule fluorescence enhancement with DNA transient binding

Fluorescent dyes emitting at wavelengths in the near-infrared region are of particular interest for *in vivo* applications due to the absence of autofluorescence and deeper penetration depth. Unfortunately, however, most biocompatible near-infrared fluorescent dyes are weak emitters with fairly low quantum yield. It is thus quite challenging to image near-infrared dyes at the single-molecule level with conventional single-molecule fluorescence microscopy. In Chapter 2, we used gold nanorods with localized surface plasmon resonance (LSPR) in the near-infrared region to enhance the fluorescence of single molecules of a near-infrared dye. Nanorods with the LSPR in the near-infrared region often give rise to higher enhancement factors compared to nanorods resonating in the visible region because of stronger plasmon resonances and lower loss in the metal. In this chapter, we made use of the reversible hybridization of molecule-carrying short DNA oligomers to their complementary docking strands (transient binding) to visualize single-molecule enhancement events near individual GNRs. Complementary DNA strands offer the ability to bind the desired target molecule at the nanorod hot spot. When the fluorescence intensity of the dye molecules near a nanorod is monitored over time such events can be seen as signal bursts in the intensity trajectory.

Additionally, we found that hot electrons generated on the surface of plasmonic structures under focused continuous-wave (CW) laser irradiation are able to break the Au-S bond connecting the DNA oligomers and gold. This hot-electron effect was previously only observed with pulsed lasers.

DNA oligomer-based transient binding is a robust chemical approach that allows one to control the distance and binding time efficiently. Combined with plasmonic enhancement, a rich variety of single-molecule studies can be envisioned. We demonstrated observing fluorescence from refreshing molecules emitting at a fixed point near a nanorod. In such a situation, one can study fluorescence enhancement without the randomness caused by the inhomogeneity of the local electric field. For example, one can study the enhancement for molecules with different quantum efficiencies but placed at the same position with respect to the plasmonic nanoantenna.

Plasmonic hot electrons deteriorate the photostability of our enhancement experiments. However, interesting applications utilizing hot electrons have been demonstrated for controlled cargo release [1], to control the surface chemistry of nanoantennas with subdiffraction precision [2], and photocatalysis, to name a few. These possibilities were demonstrated mostly with the use of a femtosecond pulsed laser. If CW lasers can also generate hot electrons, as we demonstrated here, the application of hot electrons can be largely extended.

Single-molecule electrochemistry enabled by fluorescence enhancement

Chapter 3 shows that it is possible to observe the redox cycles of a single molecule in real time using fluorescence as the readout. We use single GNRs to enhance the fluorescence signal from single redox-sensitive Methylene Blue molecules so that the electrochemical properties of single molecules can be studied. By fixing a single molecule on the substrate near a GNR, we were able to record and quantify the redox-induced fluorescence blinking of the same molecule at different electrochemical potentials and thus determine the mid-point potential of the molecule. The observation time of a molecule is limited only by photobleaching.

Single molecules are local reporters inside a complex system. Our single-molecule electrochemical technique could be extended to chemical and biological systems to measure the local redox potential. At present, measurement should be performed in a system with a low pH value of 2 in order to match the mid-point potentials of the electron mediator and Methylene Blue. Future work would be to optimize the experimental design so that it works at physiological conditions before it can be applied in biological systems.

Single-molecule enhancement of hot-band absorption induced luminescence

In Chapter 4, we show that GNRs are able to enhance luminescence from hot-band absorption. Hot-band absorption induced luminescence shows an anti-Stokes shift with respect to the excitation wavelength. The anti-Stokes emission appears to be two-photon-excited fluorescence at the first glance considering the large two-photon absorption cross-section of the studied squaraine fluorophore [3, 4], but it is an one-photon process with a linear relation between emission intensity and excitation power. The anti-Stokes emission can be

excited by both CW and femtosecond lasers, and the emission intensity is the same under the same excitation power. Moreover, we observed the exponential temperature-dependence of hot-band absorption.

Hot-band absorption is usually a process with low efficiency as the optical excitation has to start from a high vibration level at the ground state, which is not energetically favorable. In this chapter, we demonstrate the feasibility of enhancing anti-Stokes luminescence with plasmonic nanorods. A femtosecond laser was used in our study, but it should also work with a CW laser, and the same enhancement is expected. This is potentially valuable for fluorescence imaging as the low background benefiting from anti-Stokes emission can be obtained without implementation of costly two-photon microscopy instruments.

Two-photon-excited luminescence enhancement of single quantum dots

Two-photon-excited luminescence is particularly advantageous for imaging biological specimens since it offers reduced scattering, deep sample penetration, and intrinsic confocality. Two-photon excitation should lead to a higher excitation enhancement factor than one-photon excitation due to the quadratic dependence of the fluorescence intensity on the excitation intensity. However, it is more difficult to demonstrate (single-molecule) two-photon-excited fluorescence/luminescence enhancement as shape instability of GNRs upon ultrafast laser irradiation limits the excitation power, and thus the emission signal of fluorophores. This was a problem because if a nanorod is reshaped, the enhancement properties are weakened due to the strong dependence of the position of the LSPR on the shape of the nanorod. In Chapter 5, we successfully demonstrated 10^4 fold enhancement of two-photon-excited luminescence of *single* quantum dots. This was possible thanks to the larger two-photon absorption cross-sections of quantum dots, which are typically 10^3 times higher than organic molecules. The fair agreement between the experimentally obtained enhancement factor and numerical simulations using electromagnetic theory confirms that the transient broadening of the plasmonic resonance by femtosecond excitation is not a limiting factor for two-photon-excited luminescence enhancement by individual GNRs and an electromagnetic consideration is sufficient to describe the luminescence enhancement.

The study in this chapter paves the way for future studies of single-molecule-single-particle plasmonic enhancement of two-photon-excited luminescence. In perspective, GNRs can be used to enhance the two-photon-excited luminescence of I-III-VI_2 ternary quantum dots, whose quantum yields are often lower than 10%. I-III-VI_2 ternary quantum dots have attracted increasing attention as promising alternatives for toxic cadmium based II-IV quantum dots for their low toxicity and unique optical properties [5, 6]. Increasing the luminescence of cadmium-free quantum dots will extend their applications in many fields.

Two-photon-excited fluorescence enhancement of fluorescent molecules

Apart from enhancing quantum dots, it is also valuable to enhance the two-photon-excited fluorescence of organic dye molecules because of their small size as labeling probes. In Chapter 6, we circumvented the problem of nanorod reshaping by increasing the concentration of molecules, thus studied the enhancement effect of an ensemble of molecules near individual GNRs. Single-nanorod measurements allowed us to study systematically the de-

pendence of fluorescence enhancement on the LSPRs of nanorods and the excitation power. The results further support that, at powers low enough to avoid reshaping, two-photon fluorescence enhancement is not notably affected by the plasmon broadening by femtosecond pulses.

Future work will be to push the current experiments to single molecules. To this end, due to the weak two-photon emission, a single molecule has to be tethered near a GNR to allow enough time to collect emission photons from the single molecule. A promising option is the DNA transient binding technique described in Chapter 2. The background from the luminescence of the GNR can be filtered out spectrally (by a band-pass filter) and temporally (luminescence of GNRs is much shorter-lived than fluorescent molecules). Improving the photothermal stability of GNRs by, for instance, silica coating [7, 8], could increase the reshaping power threshold and thus the photon counts of single molecules, allowing easier observation of two-photon-excited fluorescence enhancement.

This thesis shows the potential of GNRs for fluorescence enhancement. The next goal of the project is to extend plasmonic enhancement to even weaker emitters, such as lanthanide ions [9]. Two-photon excitation will be used to get access to fluorophores absorbing in the near UV, between 300 and 400 nm. We will use plasmon resonances at red wavelengths for the excitation enhancement, while maintaining the emission enhancement for the red luminescence. Plasmonic enhancement will potentially generalize single-molecule spectroscopy to even weaker emitters.

References

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