Metal Nanostructure-Enhanced Fluorescence and its Applications in Bioassays and Bioimaging

By

Wei Deng

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Except where acknowledged in the customary manner, the material presented in this thesis is, to the best of my knowledge, original and has not been submitted in whole or part for a degree in any university.

Wei Deng

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List of Publications

- Wei Deng, Dayong Jin, Krystyna Drozdowicz-Tomsia, Jingli Yuan, Jing Wu, Ewa M Goldys, *Ultrabright Eu-doped Plasmonic Ag@SiO_2 Nanostructures: Time-gated Bioprobes with Single Particle Sensitivity and Negligible Background*. Adv. Mater**.** 23, 4649–4654 (2011).

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- Wei Deng, Dayong Jin, Krystyna Drozdowicz-Tomsia, Jingli Yuan, Ewa M Goldys, *Metal Nanostructure Enhanced Fluorescence of Europium Chelate BHHCT-Eu^{3+} Applied to Bioassays and Time-gated Bioimaging*. Langmuir, **26**, 10036-10043(2010).

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- Varun K. A. Sreenivasan, Ekaterina A. Ivukina, Wei Deng, Timothy A. Kelf, Tatyana A. Zdobnova, Sergey V. Lukash, Boris V. Veryugin, Oleg A. Stremovskiy, Andrei V. Zvyagin, Sergey M. Deyev, *Barstar: Barnase-a Versatile Platform for Colloidal Diamond Bioconjugation.* J. Mater. Chem., **21**, 65-68 (2011).

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- Ewa M. Goldys, Wei Deng, Nils P. Calander, Krystyna Drozdowicz-Tomsia, Dayong Jin, *Nanoscale Plasmonic Resonators with High Purcell Factor: Spontaneous and Stimulated Emission.* SPIE Proceedings (accepted).

- Wei Deng, Dayong Jin, Krystyna Drozdowicz-Tomsia, Jingli Yuan, Jing Wu, Ewa M Goldys, *Plasmonic Ag/SiO_2 Composite Nanoparticles Doped with Europium Chelate and Their Metal Enhanced Fluorescence.* Proceedings of SPIE, **7909**, 79090Z (2011).

Abstract

Over the last two decades, fluorescence based detection has become one of the leading sensing technologies in biomedical, biological and related sciences. Its sensitivity makes it possible to detect a single biomolecule through labeling with a suitable fluorophore. Two principal fluorophore properties, brightness and photostability, are fundamentally important to achieve a high level of sensitivity and in many conventional fluorophores these often fall short of the requirements. Among the methods used to improve the sensitivity of fluorescence detection, the metal-enhanced fluorescence (MEF) technique has been recently actively developed. The MEF phenomenon occurs when an excited fluorophore is located in close proximity to metals, and it is particularly pronounced near noble metal nanostructures. Electrons in such metal nanostructures exhibit strong resonances often located in the visible part of the spectrum (also known as surface plasmon resonance). They can interact with proximal fluorophores modifying their optical properties and producing increased quantum yield (fluorescence efficiency) and improved photostability. It has been experimentally demonstrated that the MEF technique can increase fluorescence intensity up to several hundreds times. The work through my PhD project mainly focus on siver nanostructures and its potential in fluorescence-based applications requiring very high sensitivity because their strong surface plasmon resonance in the visible matches the absorption and emission bands of most fluorophores.

I began with synthesis for Ag nanostructure-coated silica beads in the solution-based

platform as a new MEF substrate. My first study employed these nanostructures to enhance the fluorescence readout on individual silica beads. These Ag nanostructure were deposited on micrometer size silica beads. The fluorescence enhancement was investigated using a model AlexaFluor 430 IgG immunoassay and AlexaFluor 430 labeling. Approximately 8.5-fold and 10.1-fold higher fluorescence intensities at 430 nm excitation were, respectively, observed from silvered 400 nm and 5 μm silica beads deposited on glass as compared to the control sample. This achievement allowed us to demonstrate for the first time MEF immunoassays on silica beads by using high-throughput flow cytometry. Furthermore, we discovered that these Ag nanostructure-coated silica beads are able to modify the luminescence decay lifetime of lanthanide fluorophores ($\mathrm{BHHCT} - \mathrm{Eu}^{3+}$) for time-gated luminescence bioimaging applications. The fluorescence enhancement factor achieved about 11 times, while the simultaneously measured fluorescence lifetime was reduced twofold. The fluorophore stability was also improved by a factor of three. We applied such bead substrates to time-gated fluorescence imaging of Giardia lamblia cells stained by $\mathrm{BHHCT} - \mathrm{Eu}^{3+}$ with improvement in brightness by a factor of two. This will open up a broad range of opportunities for ultrasensitive and low background fluorescence detection using lanthanide fluorophores. Additionally, I applied these Ag nanostructures to another type of luminophor, upconversion (UC) nanoparticles via a simple bioassy. Specifically, streptavdin (SA)-labeled UC nanoparticles were bound to the biotinlyated anti-mouse IgG antibody which was attached to Ag nanostructure-coated silica beads in advance. These Ag nanostructures produced the strong luminescence enhancement ($\sim$4.4-fold for the green mission and $\sim$3.5-fold for the red emission) from $\mathrm{NaYF}_4 : \mathrm{Yb}, \mathrm{Er}$ nanoparticles, while the simultaneously measured luminescence lifetime was reduced 2-fold for the green mission and 2.2-fold for the red emission. These findings open a new pathway to rationally modulate the UC emission, and broadly impact areas such as bioimageing and bioassays, as well as enable new opportunities for energy harvesting and conversion.

In addition, I developed another kind of MEF substrate in this project, Eu chelate-doped $\mathrm{Ag@SiO}_2$ nanocomposites, and systematically study the interaction between Ag core and Eu chelate (BHHCT-Eu-DPBT) by tuning the distance between them and

Ag core sizes. At high excitation intensities, these nanocomposites showed greatly increased fluorescence enhancement factors of up to 145, due to significantly increased radiative rates in samples with metal cores, from about 700 s^{-1} in control samples to over 5000 s^{-1}. They are bright enough to be observed as single particles and are compatible with low background time-gating by using simple detection systems. A simple bioassay using avidin-biotin binding system was also carried out with the luminescence enhancement factor of $\sim$120, demonstrating the potential of these bioconjugated nanocomposites to be used in a range of biological applications.

Based on these experimental results, we also presented the theoretical analysis for the high fluorescence enhancement factor. This phenomenon observed on the nanostructured silver surfaces is a result of two effects: an increase of a local electromagnetic field near silver nanostructures, leading to increased excitation rate of fluorophores and an increase of the radiative decay rate Γ of fluorophores close to silver nanostructures, reflected both in the fluorescence lifetime and quantum yield. The local electromagnetic field enhancement produces a higher excitation rate but it does not change the lifetime of the fluorophore; this effect is referred as excitation enhancement (E_{ex}). The second effect referred as emission enhancement (E_{em}), increases the quantum yield and reduces the lifetime of the fluorophore. The detailed mechanism was systematically analysed in the following chapters.

In summary, this work mainly covered the fabrication of nanoscaled silver with different geometries and the MEF effect induced by them in biological applications such as bioassays, immunoassays and bioimaging.

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