FLUORESCENCE ENHANCEMENT WITH PLASMONIC NANOSTRUCTURES

Sharmistha Dutta Choudhury
Radiation & Photochemistry Division

Abstract

The interactions of fluorophores with plasmonic nanostructures lead to several favorable effects such as increased brightness, better photostabilities and reduced excited-state lifetimes that can be exploited to improve fluorescence technology. This article describes the development of novel silver-gold nanocomposite structures for metal-enhanced fluorescence and silver nanostructures for efficient fluorescence correlation spectroscopy, with reduced detection volumes and increased signal intensities.

Keywords: Metal-Enhanced Fluorescence, Silver-Gold Nanocomposites, Surface-Plasmon, Fluorescence Correlation Spectroscopy, Single Molecule Fluorescence

Introduction

Fluorescence detection is a versatile and widely used research tool due to its high sensitivity, ease of detection and rapid response. Today fluorescence spectroscopy and microscopy require minimal sample volumes and extremely low concentration of fluorophores for single molecule detection. The coupling of fluorescence with surface-plasmon oscillations in metal nanostructures provides us the opportunity to create new kinds of ultrabright fluorescent probes or develop alternative methodologies that can improve the capabilities of modern fluorescence technology.\(^1\)

A fluorophore that is placed at a certain optimal distance from metal nanoparticles, can exhibit increased fluorescence intensities, decreased fluorescence lifetimes and improved photostabilities. This interesting phenomenon, known as metal-enhanced fluorescence (MEF), arises due to two effects. First, is the creation of an intense excitation field around the metal nanoparticle that provides increased excitation rates for a fluorophore that is placed in its vicinity. Secondly and more importantly, the near-field coupling of fluorescence with the localized surface-plasmon oscillations in the metal nanoparticle, increases the radiative decay rate of the fluorophore. The coupled plasmon-fluorophore system eventually radiates into the far-field with increased emission intensity.\(^1\) Since fluorophore-plasmon interactions depend on the properties of metal nanostructures, it is essential to construct robust and reproducible metallic nano substrates with controlled geometry and tunable optical features, in an easy and cost effective manner, to realize the full potential of MEF. We have demonstrated the facile fabrication of silver-gold nanocomposite (Ag-Au-NC) substrates by galvanic replacement reaction of silver by gold. Our studies indicate that these substrates are not only easy to prepare but also provide excellent fluorescence enhancements for widely used fluorescent dyes.\(^2,3\) Another important area where plasmonic
nanostructures can play a major role in fluorescence correlation spectroscopy (FCS). With typical diffraction limited observation volumes obtained with conventional confocal microscopy systems, it is possible to perform FCS measurements only at low fluorophore concentrations (pico- to nanomolar). We have shown that simple silver nanostructures (AgNS), can be used to increase the upper concentration limit for FCS measurements and also provide increased fluorescence intensities for better detection efficiency.

**Fluorescence enhancement using silver-gold nanocomposite substrates**

The galvanic replacement reaction of silver with gold is an elegant approach for preparing novel metal nanostructures, that is driven by the difference in the reduction potentials of \( \text{AuCl}_4^-/\text{Au} \) and \( \text{Ag}^+/\text{Ag} \). Immersion of silver coated glass slides into the \( \text{HAuCl}_4 \) solution causes spontaneous oxidation of elemental silver and the deposition of nanoscale Au particles on the surface of the sacrificial silver substrate, leading to formation of the nanocomposite substrate, Ag-Au-NC. The fabricated substrates with surface deposited gold nanoparticles provide a robust surface, while at the same time the residual silver provides favorable metal-fluorophore interactions for better fluorescence enhancement. Fig. 1 shows a schematic of the substrate fabrication and the large intensity enhancement observed for the fluorophore, ATTO655, immobilized on Ag-Au-NC substrate. Most interestingly, the intensity enhancement is accompanied by decrease in the fluorescence lifetime of ATTO655, which is a clear signature of fluorophore-plasmon coupling effect. Scanning confocal microscopy images (Fig. 1) show much brighter fluorescence spots on the nanocomposite surface compared to a control glass slide.

These bright spots correspond to emission from single fluorescent molecules and indicate that the emission intensities are actually enhanced on a molecule by molecule basis. Statistical analysis reveals a large distribution in the intensities of individual ATTO655 molecules on Ag-Au-NC in comparison to glass. This is attributed to differences in metal-fluorophore interactions at different sites on the fabricated substrate. Single molecule studies, therefore, help in gaining additional information on the heterogeneity of the Ag-Au-NC substrate, which is masked in ensemble fluorescence measurements. Very high intensities (~ 80 fold enhancement) can be achieved for single molecules that are suitably located in the “hot spots” of the plasmonic substrate.

These results are expected to have a large implication for biological studies. For example, MEF can improve the fluorescence intensities and hence the detection of fluorophore labeled biomolecules. By proper choice and design of the nano metallic substrate it is possible to obtain intensity enhancements for the intrinsically weak fluorescence from biomolecules that emit in the UV region, like proteins or DNA. Thus, fluorophore-plasmon coupling provides the opportunity for label-free studies of biomolecules.

Fig. 1: Metal-enhanced fluorescence with Ag-Au-NC substrates.
Further, since MEF is accompanied by reduction in the fluorescence lifetimes, the photostability of the probe molecule can be significantly increased. This leads to dramatic improvements especially for single molecule fluorescence studies of biological molecules, for high-throughput bioassays and medical diagnostics. The metal proximity-induced fluorescence enhancement can also be used to design novel experiments for selective biomolecular binding and recognition.

**Fluorescence correlation spectroscopy with silver nanostructures: reduced detection volumes and increased intensities**

Fluorescence correlation spectroscopy (FCS) is a widely used technique to investigate the interactions and dynamics of molecules, below micromolar concentrations. We have shown that silver nanostructure (AgNS) substrates that are conveniently prepared in the laboratory (by wet chemical synthesis or thermal vapour deposition) without any elaborate nanofabrication, can extend the applicability of FCS to higher concentrations by reducing the effective detection volume.\(^5\) In addition to reduced detection volumes, the plasmonic nanostructures also allow tuning of the fluorescence properties of molecules. Compared to open volume, fluorescence bursts with very high signal intensities are observed on the AgNS substrate. This interesting behaviour is attributed to fluorophore-plasmon coupling effects. Moreover, the introduction of plasmonic nanostructures has the distinct advantage of making FCS studies feasible at high concentrations without any modification of the conventional optical set-up. Figure 2 shows a schematic of the FCS set-up in open volume and on AgNS substrates. The autocorrelation functions, obtained for the fluorescence intensity fluctuations due to diffusion of the fluorescent probe molecule, ATTO655, show several notable features, particularly an increase in the amplitude and a decrease in the correlation time, on AgNS compared to the open volume. Most interestingly, at 9 µM fluorophore concentration, no time correlation can be observed in the open volume whereas a distinct correlation is observed on AgNS (Figure 2). These results suggest that there is a reduction in the effective fluorescence detection volume on the AgNS substrates. From a statistical analysis of several independent measurements at different positions on the AgNS, the effective detection volume is estimated to be reduced by about a factor of 18±10 on the AgNS substrate in comparison to open volume.\(^5\) We propose that two effects are responsible for the reduction in the detection volumes in the present substrates. First, is the physical confinement of the molecules within the nanospaces between the silver nanoparticles and second is the modification of the near-fields in the vicinity of the plasmonic nanoparticles. Since the plasmon coupled near-field effect exists within a small region (upto ~200 nm) around the metal nanoparticles, a very small and bright fluorescence volume is created in the immediate vicinity of these particles. This effect also increases the fluorescence count rates and improves the detectability of single diffusing molecules.

This study highlights the simplicity with which plasmonic nanostructures can be incorporated on
a standard confocal microscopy set-up to augment its measurement capabilities. We believe that the plasmonic AgNS substrates will be a widely used platform for performing FCS studies and addressing many biological problems that demand observation volumes below the classical diffraction limit.

Acknowledgements

I would like to thank Prof. J. R. Lakowicz, University of Maryland Baltimore, for introducing me to this interesting subject area and also the co-authors of our published papers. I thank all colleagues of RPCD and CG for their encouragement and support.

References