New Frontiers of Single-Molecule sensing by Plasmon-Enhanced Fluorescence

A. Colombelli[1], M. Cesaria[1], S. Scarano[2], , M. Minunni[2], A. Creti[1], M. Lomascolo[1], R. Rella1, M. G. Manera[1*],

1 CNR-IMM Lecce section, strada prov.le Lecce-Monteroni, Lecce, Italy
2 Dipartimento di chimica, Università di Firenze, via della Lastruccia, Firenze, Italy

**mariagrazia.manera@cnr.it*

Plasmon-enhanced fluorescence (PEF) is a remarkable tool with ultra-high sensitivity for detection and imaging down to the single-molecule level offering enhanced emissions and decreased lifetimes, with resolutions significantly better than the diffraction limit.
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1. Introduction

The effect of coupling a molecule to resonantly excited plasmonic nanoparticles (NPs) is an active area of research.
The resonant charge oscillations associated with the excitation of Localised Surface Plasmon Resonances (LSPR) in metallic nanoparticles (NPs), give rise to a large local electric field enhancement near their surface. Molecules placed in the NP near field can couple to this enhanced electric field, yielding several important effects [1]. One of them in a fluorescence enhancement effect.
The fluorescence technique has become popular in biological research, including single molecule detection, cellular imaging, gene profiling, proteomics, drug discovery and disease diagnostic. Fluorescent markers are very efficient for labelling and cell imaging. However, this technique is applicable only for molecules with inherent fluorescence, which often suffers from two major drawbacks: weak fluorescence signal from a low concentration of fluorophores and low photostability of the molecular fluorophores.
In the presence of a metal surface, the excitation as well as emission characteristics of the fluorophore can be changed in terms of modification in the molecular cross-section. Metallic NPs have been shown to enhance the fluorescence emission and decrease the molecular excited state fluorescence lifetimes. The fluorescence enhancement is attributable to a combination of processes including enhanced absorption by the molecule in the local electromagnetic "hot spots," modification of the radiative decay rate of the molecule, and an enhanced coupling efficiency of the fluorescent emission to the far field [4]. Plasmonic enhancement on gold or silver nanostructures has been widely applied as an emerging tool in biotechnology, such as fluorescence sensing, multiphoton excitation, and planar immunoassays [5].
These effect are extremely sensitive to excitation and emission frequencies, as well as to the NPs shape, size, distribution on a planar substrate and to the distance between the NPs and fluorophore.

In this study, large area short-range ordered array of metal nanodisks are realized on a flat substrate by hole mask lithography. A proper surface engineering allowed the possibility of sweeping between different spectral features in the Visible range. Supported by a proper theoretical modelling tool, a comprehensive understanding of the specific interaction NP-fluorophore system, resulting from the overlap between absorption/emission band of fluorophore system and plasmonic band of metal NPs is achieved. Time-resolved fluorescence spectroscopy is employed to study the size and distance effects on plasmon enhanced fluorescence. The decay behaviour is demonstrated to be sensitive to the interaction between the fluorophore and nanoparticle and can provide detailed information on the coupling mechanism.
The method pave the way towards the development of novel single-molecule detection schemes.

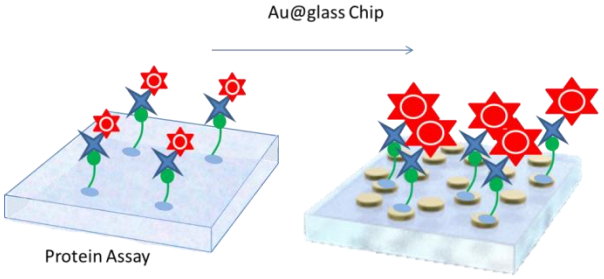

Fig. 1 Representative scheme of the realized short-range metal nanodisks array on glass before and after monolayer fluorophore-coating.

References

1. Zhang, F.; Gotz, G.; Winkler, H. D. F.; et al.. *Angew. Chem. Int. Ed*. **48**, 6632 (2009).
2. Scholes, G. D.; Fleming, G. R. *J. Phys. Chem. B* **104**, 1854 (2000).
3. Ming Li, Scott K. Cushinga, Nianqiang Wu, *Analyst* **140,** 386, (2015).
4. Lakowicz J.R. *Anal. Biochem.* **337** 171–194 (2005).
5. Li, J.-F., Li C.-Y. , Aroca R., *Chem. Soc. Rev.* **46,** 3962 (2017).