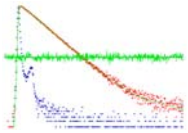


Plasmon enhancement of protein fluorescence by silver nanostructures

The use of metal surfaces in conjunction with fluorescence molecules employing a plasmon effect, sometimes referred to as metal enhanced fluorescence, can be advantageous because of the possible enhancement of photophysical properties. For example, the emission intensity of the fluorophore, can be improved. It is possible to produce metal structures in-situ from silver nitrate via light irradiation, within a host material such as sol-gel derived silica or a polysaccharide film. This approach allows control over position that maybe useful for potential lab on a chip applications and is demonstrated by monitoring the fluorescence from a FITC labelled protein.

Fluorescence close to metal surfaces

The proximity of a conducting metal surface or colloid to a fluorophore is known to affect the fluorescence emission. The metal can change the electric field experienced by the fluorophore leading to the possibility of an increase in the quantum yield, photostability and decrease in the fluorescence lifetime. There is also the potential for an increased efficiency of energy transfer via the Förster mechanism. However, the distance is critical in determining how the phenomenon is manifest, as at short distances (<50 Å) energy transfer between fluorophore and metal can occur leading to a decrease in intensity and lifetime. A slight increase in distance above this (to <100 Å) allows a concentration of the electric field causing an increase in the observed fluorescence intensity (lightning rod effect). At distances greater than this (up to ~150 Å) there can be an apparent enhancement of the radiative decay rate (k_r), which leads to an increase in the fluorescence intensity, but a decrease in the lifetime, as is evident from the equations for quantum yield (ϕ) and lifetime (τ) given below, where k_{nr} is the non radiative decay rate.

$$\phi = \frac{k_r}{k_r + k_{nr}} \qquad \tau = \frac{1}{k_r + k_{nr}}$$

The application of this effect has grown, especially in the sensing field, as it enables an increase in sensitivity.

Laser irradiation of $AgNO_3$

It is possible to perform a light induced reaction on silver nitrate in the presence of water, which enables the formation of silver clusters $(Ag^0)_n$ by aggregation with the following reaction,

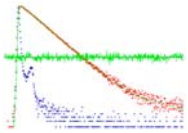
$$Ag^+ + H_2O + h\upsilon \rightarrow Ag^0 + H^+ + OH^\bullet$$

$$nAg^0 \rightarrow \left(Ag^0\right)_n \rightarrow \left(Ag^0\right)^c$$

Silica made using the sol-gel technique is a well suited host in which to perform this reaction. Sol-gel derived silica can be highly porous, robust, with a good optical quality and can retain solvents. This means that a silver salt can be incorporated and the light induced reaction to form silver nanostructure performed in-situ. Sol-gel derived media can be produced as thin films or monoliths and further control over the sample can be exercised by performing the irradiation on a microscope stage to allow for precise positioning of the regions to be exposed. In the work shown here, controlled irradiation of sol-gel derived silica films was performed using a *HORIBA Scientific DynaMyc* equipped with a *DeltaDiode* laser excitation source operating in CW mode. By using the same laser in pulsed mode fluorescence lifetime imaging (FLIM) measurements on labelled proteins adsorbed to the samples could be made.

Monitoring the fluorescence of adsorbed FITC-BSA

Bovine serum albumin (BSA) labelled with fluorescein isothiocyanate (FITC) was adsorbed to the surface of the pre-patterned films. These sol-gel derived films containing silver nitrate had been exposed to controlled *DeltaDiode* irradiation on a *DynaMyc* stage to form a pattern. Analysis of the patterned areas using other techniques had shown them to contain silver nanostructures. Fig. 1, below shows a patterned area of a sol-gel, as viewed using the CCD camera via a beam splitter, prior to protein adsorption and a FLIM measurement of the average lifetime of the protein label.



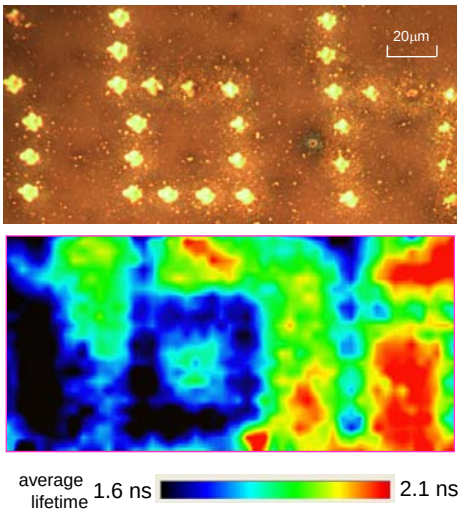


Fig. 1. Beam splitter image (top) and FLIM measurement (bottom). The influence of the irradiated areas (containing silver nanostructures) are clearly seen.

The difference in intensity and lifetime is illustrated in Fig. 2, where an enhancement in intensity accompanied by a decrease in fluorescence lifetime is observed in the vicinity of the irradiated area.

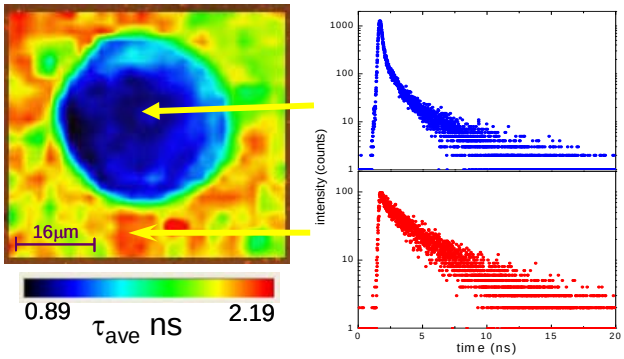


Fig. 2. FLIM measurement (left, showing average lifetime) and representative decay profiles (right) from FITC-BSA in and outside an irradiated area.

FRET enhancement

The effect of the presence of silver nanostructures extends to the enhancement of Förster energy transfer. Making use of a donor (Rhodamine 6G) within a sol-gel derived film and acceptor (Texas red) linked by different length spacers attached to the film surface, see Fig. 3.

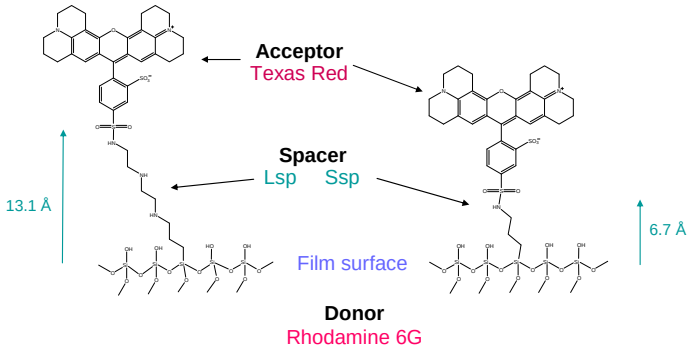


Fig. 3. Illustrative figure of donor-acceptor system

From fluorescence lifetime measurements the presence of silver nanostructures produced in-situ was found to produce an enhancement in the rate of energy transfer for both lengths of spacers. Further details are in the references, as is a demonstration of substituting the silica host for a polysaccharide one.

Summary

The use of FLIM measurements made on a microscope system enable the fluorescence lifetimes of spatially different regions, with and without the influence of silver nanostructures to be obtained.

This note acknowledges the following papers;

G. Hungerford, M. Toury, D. McLoskey, S. Finnigan, S. Gellie and A.S. Holmes-Smith, **2010**. *In situ formation of silver nanostructures produced via laser irradiation within sol-gel derived films and their interaction with a fluorescence tagged protein*. Phys. Chem. Chem. Phys. 12, 14720.

A.S. Holmes-Smith, G.R. McDowell, M. Toury, D. McLoskey and G. Hungerford, **2012**. *The influence of silver nanostructures formed in-situ in silica sol-gel derived films on the rate of Förster resonance energy transfer*. Chem. Phys. Chem., 13, 535.

G. Hungerford, M. Toury, D. McLoskey, N. Donaldson, A.S. Holmes-Smith, **2012**. *In-situ formation of silver nanostructures within a polysaccharide film and its application as a potential biocompatible fluorescence sensing medium*. Soft Matter, 8, 653.