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Fluorescence enhancement of fluorophores in live cells using metallic nanoparticles

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ABSTRACT

Plasmonic enhancement of fluorescence has been challenging in in vivo imaging applications. We present a study demonstrating the plasmonic enhancement of fluorescent membrane proteins within their native physiological environment using tailored metallic nanoparticles. This work highlights two schemes to influence the distance between the emitting dipoles and the enhancing nanoparticles, namely the addition of nanoparticles in the buffer solution and the incorporation in the polymer matrix at the bottom of the cells. Incorporating biological structures native to the cellular environment offers opportunities for the optimization of in vivo fluorescence imaging methods and the detection of membrane proteins.

Keywords: Fluorescence, Membrane proteins, Plasmonic enhancement, Gold nanoparticles, Light microscopy

1. INTRODUCTION

The coupling of metallic nanoparticles to fluorophores can give rise to a series of effects attributable to plasmonic excitation.[1] One prominent effect is the modification of the local density of states (LDOS), which in turn can modulate the radiative decay rate of the fluorescent molecule.[1,2] For this reason, the fluorescence of fluorophores with a low quantum yield is expected to be significantly enhanced by the coupling with a metallic nanoparticle with the right geometry and resonance properties.[3]

Plasmonic enhancement has been proven successful in several in vitro experiments involving biomolecules.[4–6] However, due to the complex and crowded cellular environment, its application to in vivo imaging has remained elusive. Despite sparse tests in more complex systems,[7,8] physiologically-relevant experiments are still lacking.

In this study, we apply metal nanoparticles to living cells expressing a fluorescent membrane protein (the genetically encoded voltage indicator QuasAr6a)[9] and demonstrate in vivo plasmonic enhancement of fluorescence. The chosen fluorophore is ideal because it has a very low quantum yield and is located at a position expected to be reachable by external nanoparticles.

We chose gold nanostars as the material and geometry of the nanoparticles because of their tunability within the range of emission of fluorescence by QuasAr6a.[10] We tested two different approaches to bring the nanostars close to the cell membrane: the first involved the diffusion of the particles directly in the imaging buffer where the fluorescent cells are immersed, and the second the plating of the fluorescent cells onto a polymer matrix of poly-L-lysine (PLL, suitable for cell adhesion) embedding the nanoparticles. In this way, we aimed to formulate reasonable hypotheses regarding the coupling distance between the fluorophores and the nanostars, and prepare future work on how to optimize this.

The preliminary results showed a difference in the enhancement achievable with the two methods, suggesting that indeed the coupling distance needs to be taken into consideration. Overall, the improvement in the fluorescence emission was prominent enough to motivate us to pursue a better enhancement in future experiments.

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2. RESULTS AND DISCUSSION

We first synthesized colloidal gold nanostars following an established protocol[10] tuned so that the plasmonic resonance would align with the emission peak of QuasAr6a, expected to be close to the one of its template Archon1[11] at 705 nm. The colloid was characterized via UV/Visible spectroscopy (**Fig. 1a**) and via SEM imaging (**Fig. 1b**).

These two tests confirmed the correct formation of the nanostars and the position of the plasmonic peak.

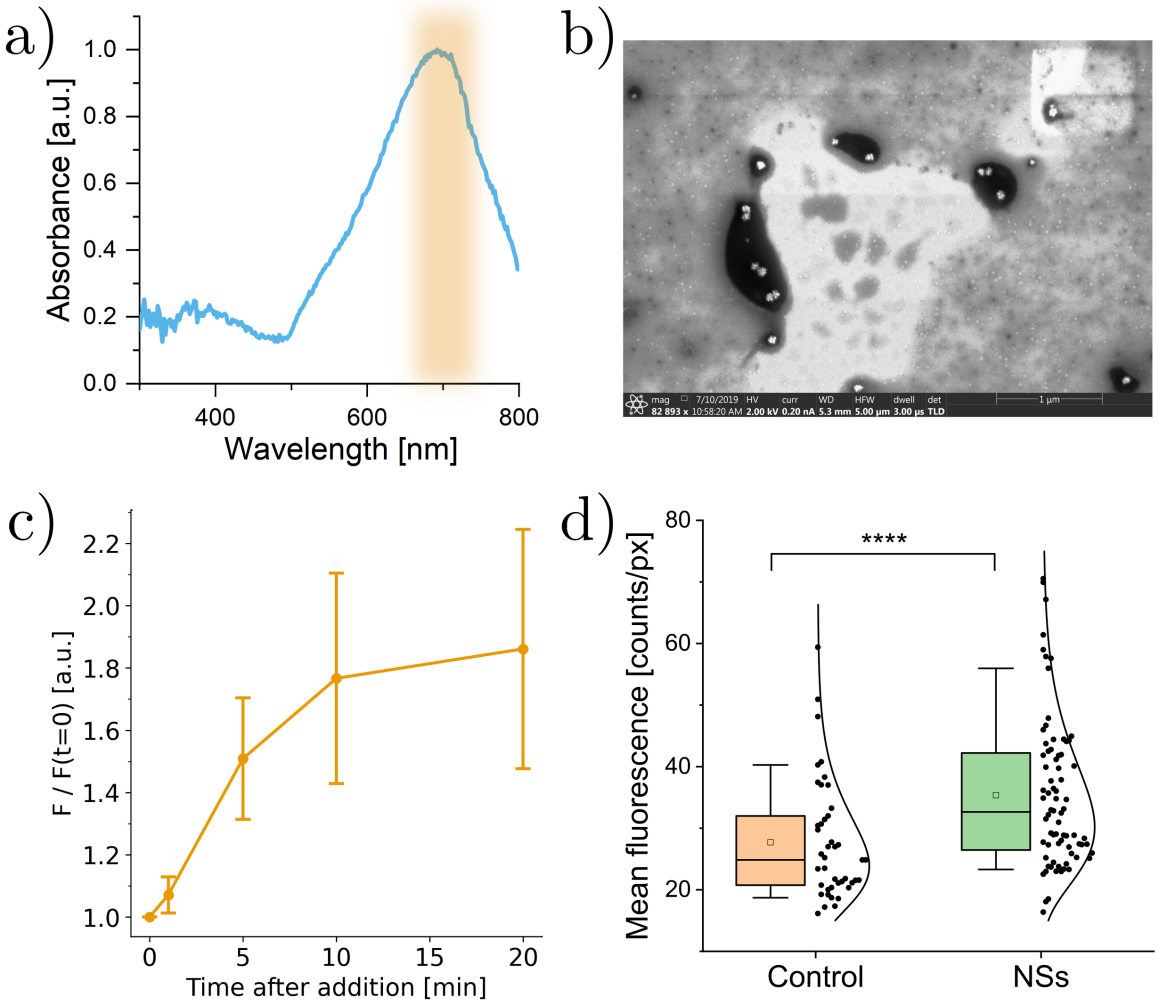

Figure 1. **a.** Normalized absorbance for the gold nanostar colloid after correction for the blank sample (blue line), compared with the emission range of Archon1[11] (orange semi-transparent band). The alignment is expected to maximise the plasmonic enhancement of the fluorescence. **b.** Example of SEM micrograph of a gold nanoparticle colloid sample drop-casted onto a silicon substrate. The nanostars are recognizable due to the charging of the tips, which appear as bright spots. The black halo around the particles is supposed to be an excess of PVP. **c.** Fluorescence of QuasAr6a in cell membrane increases over time after the addition of 100 μL of gold nanostar colloid in the buffer solution, compared to the initial fluorescence. **d.** Screening results of the mean fluorescence in samples where the nanostars (NSs) have been incorporated in the PLL matrix, as compared to a sample without nanostars (Control). Each dot represents one cell. Boxes represent the 25-75% range, whiskers the 10-90% range, squares the means and mid-lines the medians. The increase in intensity is statistically significant according to a Mann-Whitney test.

Application in experiments targeting cellular physiology requires the cell membrane to be in the near field of the nanostars. We were interested in application methods that would be compatible with diagnostic or lab-on-a-chip applications and set out to test the fluorescence enhancement we could achieve under relevant conditions. First, we tested simple addition. We pipetted 100 μL of the gold nanoparticle colloids into the imaging buffer in which the cells are immersed. We expected the gold nanostars to diffuse in the sample and eventually slowly sediment onto the cell surface.[12] We observed the fluorescence signal in the cells over time and represented it as a ratio to the initial signal. We observed an increase that roughly followed a decaying exponential, suggesting that the phenomenon is guided by diffusion and Brownian motion (**Fig. 1c**).[12] We inferred that the coupling distance in this case was sufficient to result in a significant, macroscopic effect. We quantified the enhancement on the mean to 90 $\pm$ 40 % (avg $\pm$ sem). The nanostar concentration and cell membrane composition might also influence the effect.

We then tested whether we enhancement in a structured sample. 200 μL of colloidal nanostars were deposited by drop-casting onto the imaging dishes, then covered in PLL for incorporation in the polymeric matrix. On top of this layer, the fluorescent cells were seeded. We expected the layer to be more uniform compared to the diffusion-guided process of sedimentation, but we had no way of controlling its thickness and therefore the coupling distance.

The screening experiments involved comparing control samples with nanostars-coated samples. Despite the broad distribution of the fluorescence signal (due to many biological factors, among which expression levels and cell health), the results showed a clear statistically significant difference between the two samples (**Fig. 1d**). The enhancement on the median amounted to 31 $\pm$ 10 % (uncertainty propagated from the standard error). This value is considerably lower than the one measured in the previous scheme. We attributed this difference to a higher distance between the fluorophores and the nanostars caused by the PLL layer, which weakened the plasmonic coupling.

In this work, we presented a first set of experiments on fluorescence enhancement in live cells. The future outlook on this work would necessarily involve control of the coupling distance, the use of a thinner polymeric layer for cell adhesion, and a more efficient method to fix the nanostars to the glass substrate.

3. MATERIALS AND METHODS

Colloidal synthesis of gold nanostars

The method for creating PVP (polyvinylpyrrolidone) coated gold nanostars follows a process inspired by the work of Barbosa et al.[10] In their approach, they utilized gold spherical seeds measuring 15 nm in radius. We carried out a double purification step to reduce the cytotoxicity of DMF (dimethylformamide). To initiate the synthesis, 273 μL of gold seed solution (with an estimated concentration of 0.5 mM) were combined with a solution consisting of 15 mL of 10 mM PVP (obtained from Sigma-Aldrich, PVP10) dissolved in DMF (obtained from Sigma-Aldrich, D4551).

UV/Visible Spectroscopy

We utilized a Perkin Elmer's Lambda 900 UV/VIS/NIR spectrophotometer equipped with PMT and PbS detectors, covering a wide range from 200 to 2500 nm. The instrument ensures high accuracy with 0.08 nm precision in the UV/Visible range and 0.3 nm in the NIR range. An integrating sphere was employed to accommodate samples with high optical density. From the absorbance of the sample, we subtracted the contribution of a blank sample, consisting of MilliQ water within a quartz cell.

Scanning Electron Microscopy (SEM) inspection

The sample for SEM inspection was prepared by depositing the colloidal nanoparticles onto ITO coated glass coated using drop-casting. Micrographs were obtained using a Thermo Fisher Scientific's FEI Verios 460. The sample landing energy ranged from 50V to 30 kV. Secondary electrons were captured using a "Through the Lens Detector" while in immersion mode (high-resolution mode). The chamber maintained a stable pressure below 10^{-5} mbar. The images were taken at a consistent working distance of 5.3 mm.

Cell culture and fluorescence microscopy

HEK293T cells (Catalog number ATCC® CRL-3216™) with passage number ¡30 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (VWR Seradigm Premium Grade), 50 units/mL Penicillin (Life Technologies), 50 μg/mL Streptomycin (Life Technologies), and 1% GlutaMAX (Life Technologies) at 37 °C under 10% CO_2. The cells were transfected two days prior imaging via Mirus transfection, employing 400 ng of plasmid. Then, the day after, they were plated on poly-L-lysine (PLL) coated imaging dishes. For the experiment in Fig. 1c, 100 μL of gold colloid has been added to the imaging medium. For experiments in Fig. 1d, 200 μL of nanostar colloid was let dry overnight directly onto the imaging dish, then coated with PLL for incorporation in the polymeric matrix. The imaging buffer is an extracellular (EC) solution composed of 125 mM NaCl, 2.5 mM KCl, 3 mM CaCl2, 1 mM MgCl2, 15 mM HEPES, and 30 mM glucose.[13] pH of EC buffer is adjusted to 7.3 with NaOH. The osmolarity of the EC buffer is adjusted to 300 mOsm with sucrose. We employed our custom-built widefield microscope[14] to investigate the fluorescence of the membrane protein QuasAr6a, either with a simple frame recording or automatic screening.

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