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Purcell Factor Based Understanding of Enhancements in Surface Plasmon-Coupled Emission with DNA architectures

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We demonstrate the successful application of DNA thin films as dynamic bio-spacers in surface plasmon-coupled emisison platform. Site-directed DNA modification using silver and carbon nanomaterials resulted in amplified Purcell Factor (PF) and >130-fold fluorescence enhancements. We present unique architectures of DNA as a plasmonic spacer in metal-dielectric-metal substrates.

Purcell factor (PF) has been utilized for understanding the effect of a cavity environment on the spontaneous emission of a radiating dipole.[1] However the use of PF as an experimental metric to evaluate and correlate the performance of different nanomaterials for ultra-amplification of fluorescence enhancements in the surface plasmon-coupled emission (SPCE) platform[2,3] has not been reported so far. SPCE has emerged as an innovative technique for harnessing light-matter interactions towards translational applications[4,5] owing to its distinctive properties: photon sorting, fluorescence enhancements resulting from p-polarized emission and signal collection efficiency.[2,3] However, there are two important caveats in the development of an ideal point-of-care device based on the SPCE platform- (i) methodology of fluorophore mounting onto plasmonic substrates. Current techniques involve spin coating the fluorophore-polymer matrix or linker based monolayer formation on the plasmonic substrate. These processes are typically affected by either the solubility behaviour of polymers or long fabrication time. (ii) requirement of an additional spacer layer to achieve in excess of 10-15 fold fluorescence enhancements. In this communication, we present a tuneable bio-spacer to achieve modulated plasmon-coupled fluorescence emission intensities that is correlated with the PF for bio-nanocavities.

In conventional SPCE, non-plasmonic organic polymers, Poly (vinyl alcohol) and Poly (methyl methacrylate) have been used to disperse the fluorophores and spacer materials.[3,7] Recently, DNA has been exploited in various applications in photonics and plasmonics.[8] However, we report the first time use of DNA, a highly non-linear bio-polymer, as a dynamic plasmonic spacer material in a SPCE environment. Several interesting photonic properties of DNA have been reported, but there has been no research finding till date on DNA based tuning of PF for SPCE enhancements.

An aqueous solution of Rhodamine 6G (Rh6G) in DNA was spin coated on a silver substrate to couple the fluorescence emission. A standard setup with prism coupler, rotating stage and fibre coupled detector was used (Figure 1 top) for all SPCE measurements. In the SPCE region, angular distribution of p-polarized emission was observed, with a 4.5 fold fluorescence enhancement compared to free space (FS). This confirmed the coupling of fluorescence emission from Rh6G/DNA with the surface plasmons. However, the enhancements obtained with DNA spacer were lower when compared to conventional SPCE obtained with PVA thin films (Figure 1 left). As an extension to our ongoing work in spacer engineering, we decorated the DNA with silver nanoparticles and carbon dots.[9] The mounting of this modified spacer on a silver thin film, resulted in the entrapment of the radiating dipole in a bio-nanocavity created between the DNA and the silver film. Plasmonic nanocavities formed between two, high dielectric constant materials have shown augmented fluorescence.[10] In line with this we observed a 14-fold and 58- fold emission enhancements in the SPCE region (Figure 1 right).

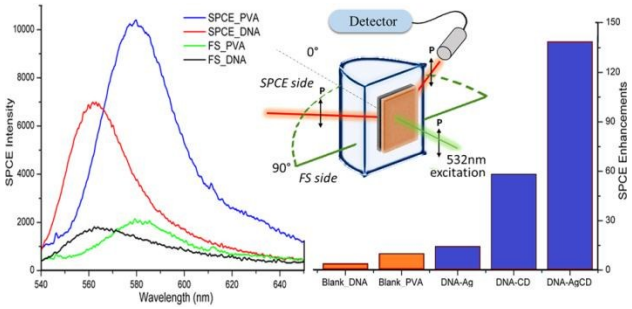

Figure 1. Schematic of SPCE platform (top), Free space and SPCE spectra of Rh6G in PVA and DNA spacers (left), SPCE enhancements obtained with DNA-Ag, DNA-CD and DNA-AgCD compared to unmodified PVA and DNA (right).

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In our earlier work[3], we achieved in excess of 1000-fold enhancements in SPCE intensity with the use of silver decorated carbon dots as spacer materials. Here we adopt similar methodology to decorate the carbon dots (CD) present on the DNA surface with silver nanoparticles (DNA-AgCD). This novelty in bio-spacer fabrication resulted in 138-fold enhancements (four times greater than that with c60 spacer[4]) in fluorescence emission (Figure 1 right). Quenching of Rh6G emission is known on account of its minor groove binding interaction with DNA.[11,12] Due to which we observed a shift in emission maxima to lower wavelength as the Rh6G aggregation is minimized, similar to our earlier observation with graphene[3], leading to lower SPCE enhancements with the DNA spacer when compared to PVA. We believe that SPCE has the potential to differentiate the interaction of intercalators and groove-binding agents with DNA, owing to the radiating dipole distance and coupling efficiency with the DNA modified spacers at different orientation angles to the nanocavity (see ESI Figure S6). Further, tuneable fluorescence enhancements can be achieved with the use of hybrid synergy spacers: DNA-Ag, DNA-CD and DNA-AgCD. It is important to note that nanomaterials based fluorescence enhancements in a conventional SPCE platform are as a result of a conditional probability for finding a fluorophore near the hot-spot zone.[3,13] In contrast, the current work illustrates, site directed bio-spacer fabrication technology, with silver nanoparticles, CD and AgCD decorated in-situ on the DNA framework with a docked fluorophore.

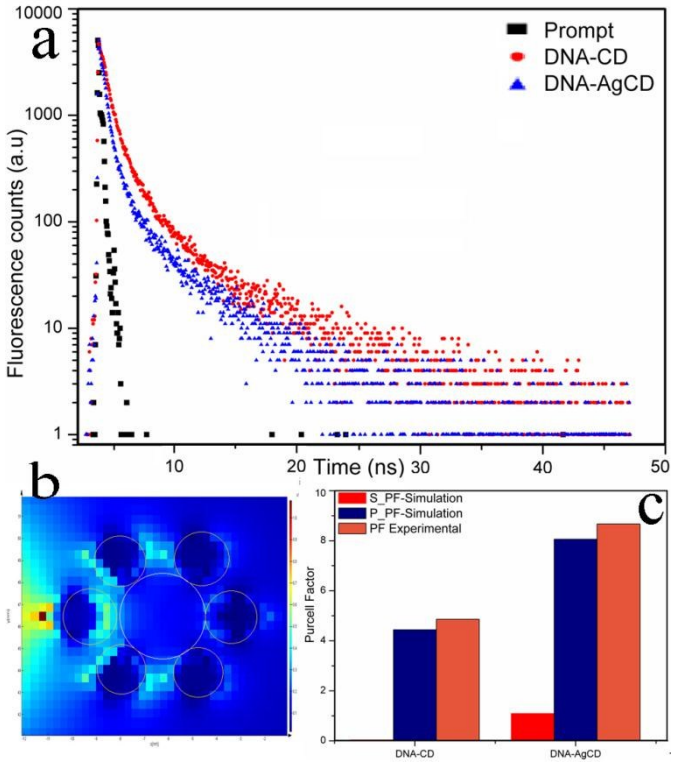

Figure 2. (a) Fluorescence decay of Rh6G in DNA-CD and DNA-AgCD; (b) FDTD simulation of diverse electric field intensity around DNA-AgCD; (c) Correlation of theoretical (perpendicular and parallel oriented radiating dipole) and experimental PF values.

It is worthy to mention here the absence of metric models to validate, predict, compare and understand fluorescence enhancements obtained from experimental SPCE studies. We present the first time understanding of Purcell effect[1] on SPCE enhancements in a bio-nanocavity. We have carried out time-correlated single-photon counting (TCSPC) studies to experimentally determined PFs of Rh6G present in the bio-nanocavities, with the use of decay times (Figure 2a). The presence of Rh6G in different cavity environments accounts for the multi-exponential decay profile. Those Rh6G molecules that are in the cavity environment have a short life-time component vis-à-vis the Rh6G molecules that at a distance from the cavity, accounting for the heterogeneous decay curve.[14]

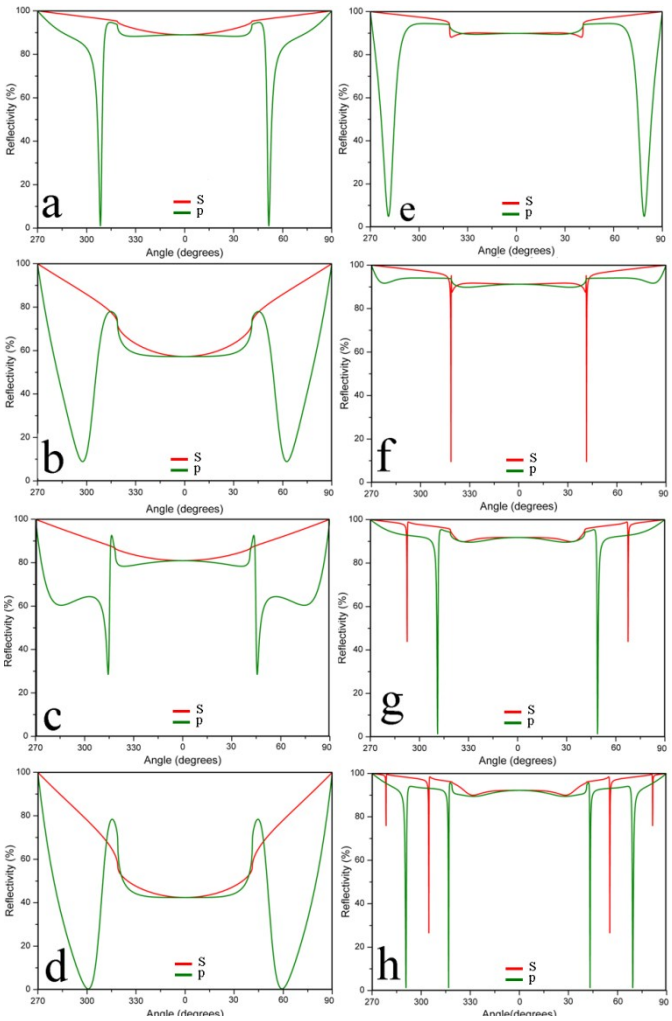

Figure 3. Simulation of angle dependent reflectivity for 30nm DNA bio-spacer on (a) silver, (b) gold, (c) aluminium and (d) copper; Tuning of reflectivity minima with DNA film thickness (e) 80nm, (f) 100nm, (g) 300nm and (h) 500nm.

We have also carried out finite-difference time-domain (FDTD) simulations (Figure 2b) to theoretically predict the PF. Interestingly, the experimental and theoretical PF were strikingly similar for DNA-CD and DNA-AgCD nanocavities (Figure 2c). It is also important to note that the nanocavity with a greater PF showed superior SPCE enhancement. In short, using this method we explicitly demonstrate that

experimental or simulation based PF determination can be used to reliably estimate SPCE enhancements.

SPCE studies have been carried out with different metal thin films, aluminium[15], copper[16] and gold[17], to name a few. Hence, to demonstrate the application of DNA thin films as dynamic plasmonic spacers, we have carried out simulation studies by replacing the conventional polymers used in SPCE platform, with DNA as the spacer layer on these metal thin films. The minimum reflectivity calculations were carried out using TfCalc. software. The surface plasmon modes appear at a particular angle of excitation, through which the plasmon-coupled radiation is emitted.[2,3] Figure 3 a-d presents the reflectivity minimum with 30nm DNA as the spacer on silver, gold, aluminium and copper.

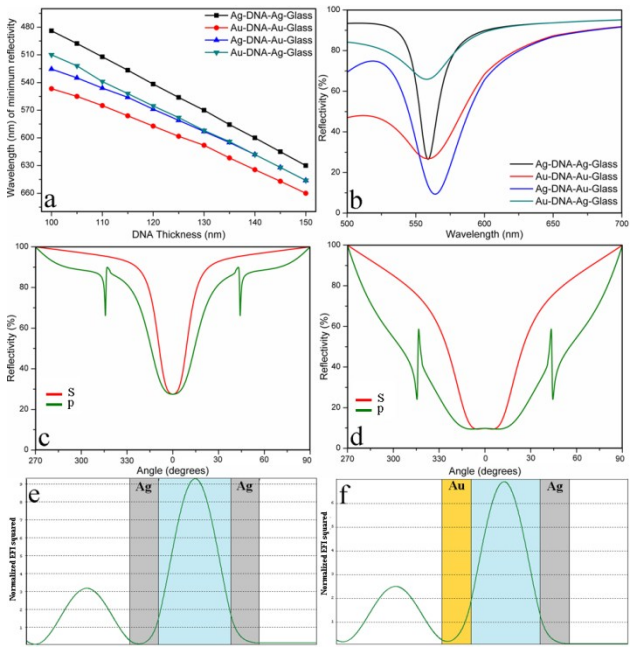

Figure 4. (a) Modulation in the beaming wavelength observed at 0° with DNA film thickness in different MDM substrates; (b) Wavelengths with reflectivity minima at 0° for different MDM substrates with 140nm DNA bio-spacer; Simulated reflectivity curves showing modes at normal incidence for MDM stacks- (c) Ag-DNA-Ag (symmetric), (d) Au-DNA-Ag (asymmetric); Calculated electric field intensities for 560nm light at 0° for MDM stacks-(e) Ag-DNA-Ag, (f) Au-DNA-Ag;

Lakowicz *et al* reported that SPCE is predominantly p-polarized, as the surface plasmons selectively couple with p-polarized light in the conventional method.[2] However, this can be modified by varying the thickness of the PVA thin film.[18] We found that the DNA bio-spacer also shows similar property to couple with s-polarized light (see ESI Figure S3). Figure 3 e-h depicts the reflectivity minimum for both p and s polarized light with different thickness of DNA spacer. 100nm thick DNA film suggests selective coupling of predominantly s-polarized light (Figure 3f) while greater thicknesses show polarization selective coupling at various angles.

Recent off-shoot of SPCE has been the steering of fluorescence emission with metal-dielectric-metal (MDM) structures[19] where the excitation of surface plasmons and observation of fluorescence emission are normal to the metal surface. We explored the use of DNA thin films for symmetric and asymmetric MDM structures with different combinations of silver, gold, copper and aluminium thin films. Figure 4a captures the decrease in resonance wavelength of minimum reflectivity at 0° for silver-gold MDM combinations (see ESI Figure S4 and S5 for other MDM architectures) with increase in thickness of DNA spacer and its dependence on the MDM composition. This is indicative of the DNA film thickness required to achieve normal emission angle from the fluorophore present in the MDM. Figure 4b presents the change in resonance wavelength with the composition of the MDM. In this communication, the DNA plasmonic spacer has been simulated for beaming emission normal to the surface by varying the metal composition and spacer thickness.

Figure 5a is a compilation of the reflectivity simulations for several thicknesses of DNA bio-spacer showing the change in the reflectivity minimum of s-polarized and p-polarized cavity modes. These results demonstrate that in comparison with conventional SPCE, DNA spacer in MDM substrates could be used to couple both p- and s- polarized light with cavity modes. This enables polarization-resolved observation of emission, achieved by altering either the angle of observation or by varying the DNA spacer thickness. The variation in electric field intensities is indicative of the enhancements in s and p-polarized light when excited at appropriate resonance dip angles (figure 5 c,d). The plasmonic properties of the DNA spacer material is highlighted by its ability to assist the formation of cavity modes and surface plasmon modes in MDM architectures, enabling further steering of fluorescence.

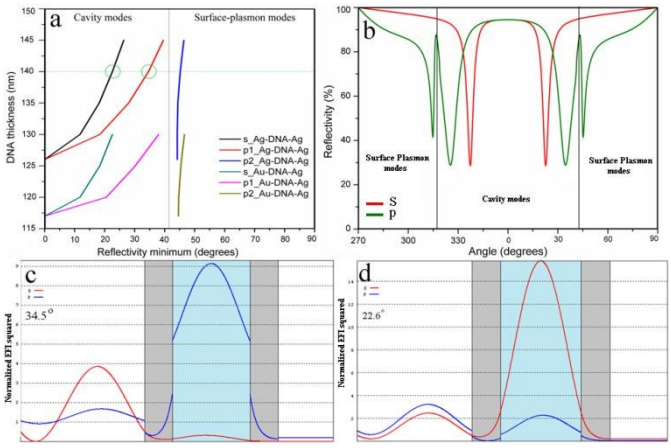

Figure 5. (a) Angle dependence of cavity and surface plasmon modes on DNA bio-spacer thickness for Ag-DNA-Ag (symmetric) and Au-DNA-Ag (asymmetric) MDM stacks; (b) Angle-dependent reflectivity with 140nm DNA bio-spacer for Ag-DNA-Ag MDM stack showing two cavity modes and one plasmon mode; Simulated electric field intensities corresponding to (c) s- polarized and (d) p- polarized cavity modes for Ag-DNA-Ag MDM stack.

Conclusions

To summarize, we demonstrate a novel approach to predict SPCE enhancements with the use of experimental or simulation based PF. This extends the use of PF in bio-

nanotechnology. In addition to this, FDTD simulations and TCSPC studies of Rh6G in bio-nanocavities were used to understand the tunability of SPCE signal enhancements. Based on our analysis, we believe that SPCE has the potential to evaluate fluorophore-DNA interactions based on orientation and distance of the radiating dipole in the bio-nanocavity. From a larger perspective, the unique DNA spacer based MDM architectures will result in remarkable spectral resolution capabilities with applications in optical telecommunication systems.

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