DNA Spacers
1. Motivation:
Currently ongoing research directed towards the development of point-of-care devices based on the SPCE platform has two limitations: (i) procedure of mounting fluorophore on plasmonic substrates. Current methods involve spin-coating the fluorophore dispersed in a polymer matrix or linker-based monolayer formation of the radiating dipole on the plasmonic substrate. These processes are typically affected by either the solubility behaviour of polymers or long fabrication time. (ii) Necessity of an additional spacer layer to achieve in excess of 10-15 fold fluorescence enhancements.

In conventional SPCE, non-plasmonic organic polymers, Poly (vinyl alcohol) and Poly (methyl methacrylate) have been used to disperse the fluorophores and spacer materials. Recently, DNA has been exploited in various applications in photonics and plasmonics. So far several fascinating photonic properties of DNA have been reported, but there has been no research finding till date on DNA based spacer for SPCE enhancements. In this chapter, we present the first time use of DNA, a highly non-linear bio-polymer, as a dynamic plasmonic spacer material on a SPCE substrate. The chapter details the use of a tunable bio-spacer to achieve modulated fluorescence emission intensities.

2. Experimental details:
Rhodamine 6G (Rh6G), poly vinyl alcohol (PVA), Calf Thymus DNA were procured from Sigma-Aldrich. 50nm silver thin film coated on pyrex with 5 nm silica top layer were purchased from EMF Corp, USA. DNA thin film was obtained by spin-coating aqueous solution of calf thymus DNA. Decoration of silver and carbon dots (DNA-Ag and DNA-CD) on DNA were achieved with suitable modifications to earlier reported procedures. The brief procedure is as follows: DNA (60µg/mL) was prepared in Tris-EDTA buffer with pH = 8.0. The DNA was homogeneously dissolved with mild stirring for 24 hours. A 0.2mL of 50mM solution of AgNO$_3$ was added to 9.8 mL of DNA solution. This solution was placed in a UV reactor for 3 hours. The solution turned red, indicating the formation of DNA-Ag. DNA-CD was prepared by taking DNA (60µg/mL) in millipore water and incubating the solution at 180°C in an electric thermostatic oven for 12 hours. The resulting DNA-CD was centrifuged at 11000g for 30 minutes followed by purification through dialysis (3500 Da) for 48 hours. This DNA-CD was further used to decorate AgNP on the CD by following the method mentioned in chapter 4. The SPCE substrate with each of the bio-spacer was then attached to a hemi-cylindrical prism mounted on a rotating stage. The substrates were
illuminated with a 532 nm c.w. laser (5 mW) and the emission was passed through a 550 nm long-pass filter, before collecting into a fiber coupled to Ocean Optics USB 2000+ fiber optic spectrometer. The angularity and enhancements were measured with the help of a rotating stage. Polarization measurements were carried out by placing a polarizer between the prism and the collection fiber. Commercially available Lumerical FDTD solutions and TFCalc. softwares were used for complimenting the experimental results with the simulation studies. Time Correlated Single Photon Counting (TCSPC) studies were carried out to determine the time resolved fluorescence decay profiles.

3. Results and discussions:

When we replaced PVA with DNA to form Rh6G embedded spacer layer, a p-polarized SPCE emission was observed with a 4.5 fold fluorescence enhancement compared to free space. 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 5.1a). Continuing with our experience with AgNP and CD decorations as in chapter 3, here we decorated the DNA with silver nanoparticles and carbon dots. Use 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. In line with this understanding, we observed a 14-fold and 58-fold emission enhancements in the SPCE region (Figure 5.1b). We also decorated the carbon dots (CD) present on the DNA surface with silver nanoparticles (DNA-AgCD). This innovation in bio-spacer fabrication resulted in 138-fold enhancements in fluorescence emission (Figure 5.1b).
Quenching of Rh6G emission is known on account of its minor groove binding interaction with DNA.\textsuperscript{5,6} 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\textsuperscript{7}, 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 (Figure 5.2). Further, tunable 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.\textsuperscript{7,8} In contrast, this chapter illustrates, site directed bio-spacer fabrication technology, with silver nanoparticles, CD and AgCD decorated in-situ on the DNA framework with a docked fluorophore.
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 on SPCE enhancements in a bio-nanocavity. We have carried out TCSPC studies to experimentally determined PFs of Rh6G present in the bio-nanocavities, with the use of decay times (Figure 5.3a).

We have also carried out FDTD simulations (Figure 5.3b) to theoretically predict the PF. Interestingly, the experimental and theoretical PF were strikingly similar for DNA-CD and DNA-AgCD nanocavities (Figure 5.3c). 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/simulation based PF determination can be used to reliably estimate SPCE enhancements. Further validation of relation between PF and SPCE enhancements has been presented in the ensuing chapter 6.
Figure 5.3. (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.

Table 5.1. Theoretical Purcell factors for different bio-nanocavities

<table>
<thead>
<tr>
<th>Bio-Nanocavity</th>
<th>Site/type of fluorophore binding</th>
<th>Purcell factors (from simulations)</th>
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<tbody>
<tr>
<td>DNA-CD</td>
<td>Intercalation</td>
<td>0.037</td>
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<tr>
<td></td>
<td>Minor groove</td>
<td>4.440</td>
</tr>
<tr>
<td>DNA-AgCD</td>
<td>Intercalation</td>
<td>1.090</td>
</tr>
<tr>
<td></td>
<td>Minor groove</td>
<td>8.060</td>
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4. Conclusions:

We have successfully demonstrated a novel approach to predict SPCE enhancements with the use of experimental or simulation based PF. 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.

5. References: