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COMMUNICATION

Low-dimensional Carbon Spacers in Surface Plasmon-Coupled Emission with Femtomolar Sensitivity and 1000-fold Fluorescence Enhancements

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We have engineered the use of 2D, 1D and 0D carbon allotropes as spacers to achieve in excess of 1000-fold fluorescence enhancements in Surface Plasmon-Coupled Emission (SPCE) platform. We also have demonstrated the femtomolar sensitivity of silver decorated carbon dots (AgCD) in the detection of a radiating dipole.

Following the first report by Lakowicz¹ in 2004 on the unique emission traits of a fluorophore near a metal thin film, SPCE has attracted much attention on account of its photon-sorting ability² and enhanced sensitivity directed towards numerous applications³.

The use of SPCE to realize a 1000-fold postulated¹ fluorescence enhancement has however been elusive. In conventional SPCE, the surface plasmons couple only with the perpendicularly oriented dipoles, resulting in a limited, 3-10 fold fluorescence enhancements¹. Earlier reports from our lab, have worked towards an increase in these enhancements by adopting two strategies that involve: i) coupling of non-perpendicularly oriented dipoles with the use of a spacer material and ii) amplifying the fluorescence of radiating dipoles with the aid of metal nanoparticles⁴. Our previous work on the use of silver and gold nanoparticles as a spacer was hence explored to boost the coupling of the radiating dipole with the surface plasmons of a metal thin film⁴. In the same line, for the first time, we have investigated the use of low-dimensional carbon allotropes, 2D-graphene, Graphene oxide (GO); 1D-Carbon nanotubes (CNT) and 0D-Nano-diamonds (ND), Fullerenes (C60, C70) and Carbon dots (CD) as spacers in SPCE substrates to enhance the fluorescence emission. Our technique with femtomolar sensitivity, has found application in the observation of bio-tagged fungi, weakly fluorescent aggregates of Rhodamine 6G (Rh6G) and labeled antibody detection. In the case of nanoparticles on silver thin film, only the surface plasmon resonance (SPR) of the nanoparticles is a factor affecting the SPCE enhancements⁴. The use of 0D, 1D and 2D carbon spacers enabled us to explore the additional factors, namely π - π , cation- π and columbic interactions⁵ that influence SPCE properties.

In conventional SPCE studies, the dye is dispersed in a polymer and spin-coated on a 50nm silver/gold thin film vapor deposited on a glass slide. The spacer layer is an additional layer that is introduced

between the dye layer and the metal thin film to prevent non-radiative deactivation¹. In the present work, we studied the plasmonic coupling properties of low-dimensional carbon allotropes in two formats namely: 1) Rh6G-Carbon allotrope double layer (RC2): Carbon allotropes were dispersed in poly vinyl alcohol (PVA) and spin coated as the spacer layer on top of a SPCE substrate (see details in ESI†), followed by a 30nm layer of Rh6G and 2) Rh6G-Carbon allotrope single layer (RC1): Carbon allotropes were admixed with Rh6G in PVA and spin coated on a silver thin film (Fig.1). In RC2, the enhancements have been attributed to the plasmonic coupling property of the material alone, on account of an increase/decrease in the fluorescence coupling with the surface plasmon modes of the silver thin film. This strategy helped in distinguishing and exploiting the π - π , cation- π and columbic interactions for higher SPCE enhancements, angularity and polarization.

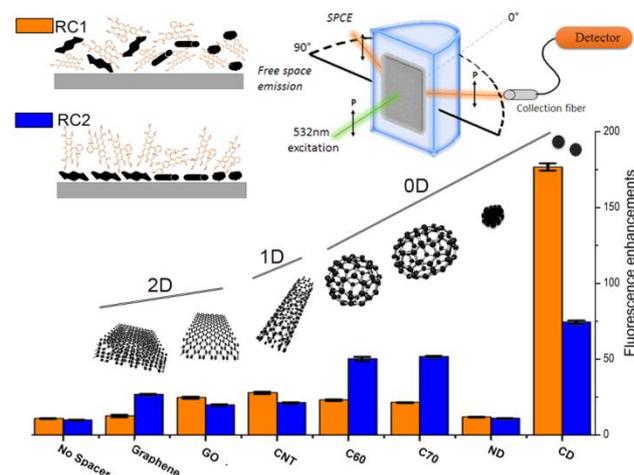


Fig. 1. Schematic of the SPCE platform (top right), Rh6G-Carbon allotrope in double layer format (RC2) and single layer format (RC1) (top left). Fluorescence enhancements achieved with 2D, 1D and 0D carbon spacers in RC1 and RC2 formats (bottom).

Previous reports⁶ have suggested that the π - π and cation- π interactions between the Rhodamine dyes and the π -conjugated systems like graphene and CNT lead to a decrease in the fluorescence emission of Rh6G, as the excited dye molecules decay through non-radiative pathways. Likewise, in the case of graphene, CNT, C60, C70 and GO in the RC1 format, a reduction in the free space emission was observed. Graphene, C60 and C70 showed smaller fluorescence enhancement also in the case of RC1 compared to RC2 owing to the π - π and cation- π interactions in RC1 format.

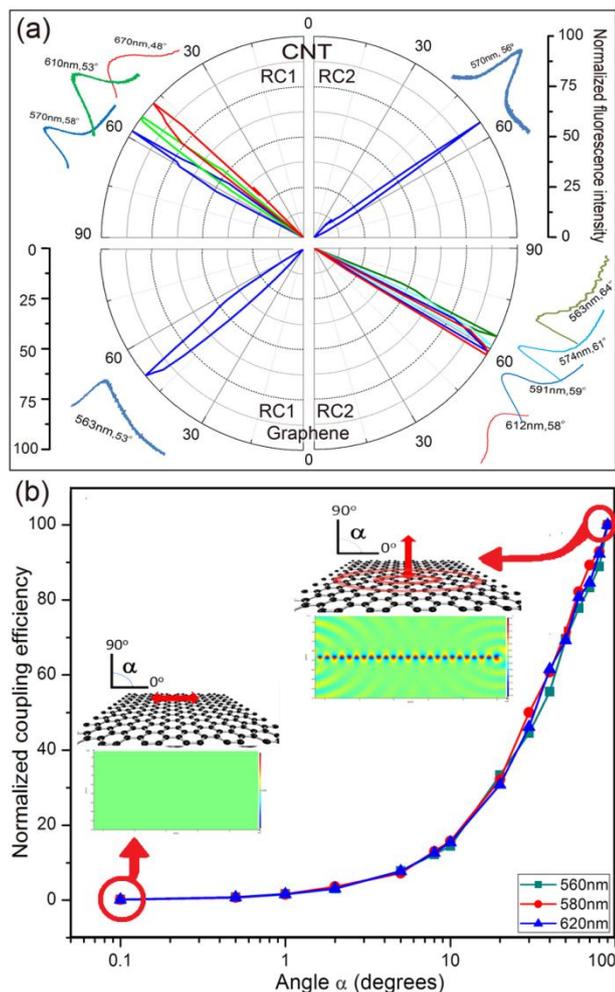


Fig. 2. (a) Angular distribution of fluorescence emission with CNT and graphene in RC1 and RC2. Deconvolution of higher-order aggregates with CNT in RC1 (top left). High-resolution of Rh6G, H and J-dimers with graphene in RC2 (bottom right). (b) FDTD simulations of Rh6G emission coupling efficiency with graphene at different orientation angles (α) of the dipole.

However, CNT and GO in RC1 showed larger SPCE enhancements than that in RC2. It was interesting to note that CNT and GO were O-functionalized while graphene, C60 and C70 were not. Consequently, the coulombic interactions between the positively charged Rh6G and the negatively charged CNT and GO directed the aggregation of the dye molecules to yield dimers and trimers. These higher-order aggregates of Rh6G emit at longer wavelengths than Rh6G monomers (Fig.2a) and were observed in the SPCE region at dissimilar angles as predicted by TFCalc simulations (ESI). In RC2, the emissions corresponding to these molecular multiplexes were not

observed, substantiating that the dye molecules aggregated in RC1 on account of coulombic interactions.

Graphene, C60 and C70 in RC1 did not show the presence of Rh6G aggregates. Interestingly, graphene in RC2 exhibited a shift in the emission wavelength maxima from 563nm to 612nm, when observed at different SPCE angles. These emission wavelengths correspond to the weakly fluorescent dimers⁷ of Rh6G at different orientations of the monomer units that vary between H-type ($\alpha=0^\circ$) and J-type ($\alpha=90^\circ$) dimers. Unlike the conventional SPCE, where only the emission from a perpendicularly oriented dipole could couple with silver surface plasmons, we found from our finite-difference time-domain (FDTD) studies that a Rh6G emission could couple with graphene plasmons in all angles except when it is “perfectly parallel” to graphene. Hence when compared to normal SPCE, graphene in RC2 showed more fluorescence enhancement as it helped in coupling the non-perpendicular dipoles with the silver plasmons. Further simulations showed that the coupling efficiency was maximum when the radiating dipole was oriented 90° (perpendicular) to the π -plasmons of the spacer and reduced with a decrease in this orientation angle (Fig.2b). There was no coupling when the dipole was oriented 0° (parallel) to the π -plasmons. This brought about the first time convergence of amplified enhancements and high-resolution in the SPCE platform in the current study.

Earlier reports with 100 and 500 fold fluorescence enhancements were obtained with external modification in the SPCE platform such as electric field assistance and complex optics⁸ that were useful in photon collection. In this work, we report photon funneling to achieve 177 fold enhancements in SPCE with a modified spacer layer - CD in the RC1 format. We attribute this amplification in the fluorescence emission of Rh6G molecules on account of the radiating dipole's presence in the “hot-spots” between CD and the silver thin film. As expected, in the case of CD in the RC2 format, >100 fold enhancement was not observed as the radiating dipoles were not present in the “hot-spot” zone.

Extending this work, SPCE was used as a tool to study the bio-tagging of different microfungi species. The addition of CD in the nutrient broth containing two fungi, *Trichoderma harzianum rifai* (endophytic fungi) and *Aspergillus oryzae* (epiphytic fungi) resulted in its uptake and subsequent use as a bio-tagged spacer in the SPCE substrate. The diverse CD uptake by each of these fungi, in turn related to their varied nutrient uptake mechanism, resulted in dissimilar SPCE enhancements of 39 and 18 fold with *Trichoderma harzianum rifai* and *Aspergillus oryzae* (ESI). This could pave way to understand the nature of nutrition uptake in different microorganisms.

To further improve upon the fluorescence enhancements, we synthesized silver and gold nanoparticle decorated CD (AgCD and AuCD) by UV irradiating CD in the presence of silver or gold ions⁹. These materials were in turn used as spacers in SPCE substrates. AuCD exhibited fluorescence enhancement larger than conventional SPCE but smaller than that of undecorated CD. We however observed an increase in the SPCE enhancements with increasing concentrations of the silver ions (Fig.3a). The use of the AgCD spacer layer resulted in an unprecedented 1178 fold fluorescence signal enhancement attributed to the additional areas of enhanced electromagnetic field intensity that are created at the point of contact between AgNPs and CD⁹, resulting in an increased hot-spot density. It is worthy to mention here that this has been the maximum signal enhancement achieved in SPCE studies so far.

In conventional SPCE platform, Rh6G molecules above nanomolar concentrations can alone be detected (Fig.3b). In the current study, we extend the amplified plasmonic enhancement ability of AgCD to assess the limit of detection (LOD) of a regular organic dye (Rh6G) and an LpPLA2 antibody (LpPLA2 Ab) labeled with a quantum-dot

(QD). LpPLA2 has been used as a biomarker for coronary heart disease and stroke¹⁰. With the use of AgCD spacer, we could detect the presence of QD-LpPLA2 Ab and Rh6G at 3.5 picomolar and 100 femtomolar concentration respectively (Fig.3b). The remarkable enhancements and improved LOD for both an organic dye and a quantum dot labeled antibody was achieved on account of the unique spacer material AgCD that is a blend of metallic (Ag) and non-metallic entities (CD). In earlier reports¹¹, metal nanoparticles were shown to enhance the fluorescence of quantum dots. Hence the AgCD spacer can be utilized in SPCE studies to amplify the emission from any radiating dipole (organic dyes, biological fluorophores or quantum dots) linked to an analyte of interest. We believe that a combination of our spacer layer engineering technique with the earlier reported optical approach⁸ in a conventional SPCE platform will result in 50,000 fold enhancement of the free space fluorescence signal. Further studies to synergize these technologies are underway.

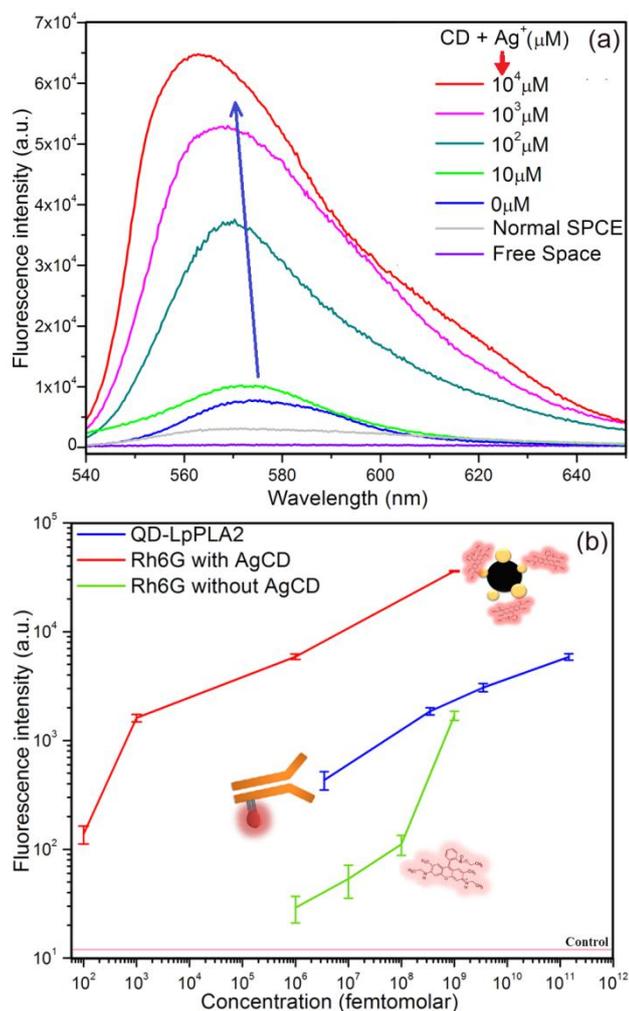


Fig. 3. (a) Silver ion concentration dependent fluorescence enhancements by AgCD (b) Femtomolar sensitivity of the SPCE platform: Double-logarithmic plot of fluorescence intensity at different concentrations of radiating dipoles.

In summary, we demonstrate neoteric advancements in SPCE spacer engineering technology with femtomolar sensitivity and attaining >1000-fold fluorescence signal enhancements. The

use of this platform for bio-tagging and the detection of QD-LpPLA2 Ab at ultra-low concentrations could help from a larger perspective, in screening of therapeutic targets at femtomolar LOD and create point-of-care devices for safety monitoring. We believe that this low-cost, home-built technology will result in real-time, assorted applications in SPCE with remarkable spectral resolution capabilities.

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† Electronic supplementary information (ESI) available: substrate preparation and experimental methods. See DOI: [10.1039/x0xx000000x](https://doi.org/10.1039/x0xx000000x).

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