# Gold Nanoparticle Functionalized Surfaces for Plasmon-Enhanced Fluorescence Detection of Nucleic Acids

Tiago Policarpo[1,2]

1- Student number 87752

2 - Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais,1049-001 Lisboa

**Abstract:** The use of metallic nanostructures as optical antennas to intensify the fluorescence of molecules, has been a topic of interest amongst the scientific community. The phenomenon of metal-enhanced fluorescence promises to greatly improve our current capabilities of fluorescence detection and therefore enable the creation of more sensitive sensors. Dimers of gold nanoparticles are of special interest, as these structures can concentrate light to yield large electric fields in the gap between the two particles. The plasmon nearfield promotes large effects of fluorescence enhancement in dye molecules or other emitters. Therefore, closely packed films of nanoparticles which could induce nearfield enhancements similar to those produced by dimers, but over a larger area, are of great interest. Nanoparticles dimers were prepared, using DNA strands as molecular linkers, with moderate results. Furthermore, experiments of fluorescence enhancement were performed using gold nanorods to amplify the emission from two fluorophores, aluminium tetrasulfonated phthalocyanine and ATTO-647N, obtaining maximum enhancement factors of around 200 and 80 times, respectively. Closely packed gold nanoparticle films were then prepared using an air-liquid interface organization deposition method. These were tested to characterize their fluorescence enhancement abilities using the molecular beacon BF1-BD2-TD and the DNA spacer BF1-BT, labelled with ATTO-647N fluorophore. The results suggest the surface areas of highly-dense organized nanoparticles making them suitable for fluorescence enhancement. Although some improvements in particle organization and surface coverage are required, the films developed here constitute a promising step toward a plasmonic substrate that could be used for increasing sensitivity in fluorescence-based sensor devices.

## Introduction

The advances in molecular fluorescence, allowed for the manufacturing of optical sensors able to probe events at the molecular level. With this technology now being applied in multiple fields, such as environmental pollution and industrial product quality control or disease markers detection in medicine[1]. Furthermore, with the rise in nanotechnology, the combination of these two disciplines could allow the production of miniaturized optical sensors. The main advantage of such technology would be the ability to perform point of care testing. Though, this requires the use of label free detection, increasing the need to be able to detect molecules present in low concentration in the samples. In order to overcome that obstacle, the application of metal-enhanced fluorescence through the use of nano-antennas, is proposed. For this, a nano-antenna strategy may be implemented, taking advantage of a physical phenomenon known as the localised surface plasmon resonance (LSPR), which

gives rise to strong local electric fields optically-induced using metallic nanoparticles. If a fluorophore is present in that area, it is affected by the increased number of photons as well as by an increase in radiative rate, leading to an increase in the detected fluorescence intensity.

However, the morphology of the metallic nanostructure influences greatly its fluorescence enhancing capabilities. One of the most interesting is the gold nanoparticle (AuNP) dimers, due to an intense hotspot created in the interstice between the two AuNPs. In this work, the production of AuNP dimers is tested, and the results characterized using a variety of approachs. Enhancement factors produced by nanostructures like gold nanorods (AuNRs) are then assessed in an effort to compare them to the ones produced by AuNPs films. Closely-packed AuNPs are then produced, using a deposition method allied to the monolayer self-assembly of AuNPs in the air-liquid interface, due to their possible application interest in microsensors. Following, fluorescence enhancement experiments are carried out in those surfaces, as well as the study of the action mechanism of a molecular beacon.

## Materials and methods

### *AuNP dimer protocol and characterization*

The dimers were produced according to the protocol described by Zhang, *et al.*[2]. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP)[2] and Bis(p -Sulfonatophenyl) phenylphosphine (BSPP)[3] were used to improve the stability of the DNA and AuNPs respectively, both at a concentration of 1mM. The different populations created were separated by agarose gel electrophoresis with a concentration of 0,7% agarose, at 110V for 30 minutes.

### *AuNP dimer protocol and characterization*

The protocol was based on the use of mercaptoethanol to remove the DNA strands from the AuNPs, for the measurements a fluorimeter Fluorolog Horiba Jobin Yvon with an excitation energy of 470nm and a 2nm slit was used.

### *Confocal Fluorescence microscopy*

The measurements were performed with MicroTime 200 by PicoQuant GmbH confocal fluorescence microscope, for the obtention of spectra the 482nm laser was used and for the obtention of images and intensity measures the a 639nm laser was used.

### *Scanning/Transmission electron microscopy(SEM/TEM)*

For SEM the JEOL JSM-7001F Field Emission Scanning Microscope was used, operating at high vacuum. The samples were laid in a carbon support and covered by a gold film and measured after it dried. As for the TEM images, a HITACHI H-8100 was used, coupled with a ThermoNoran EDS (Energy Dispersive X-ray Spectroscopy) detector and a digital image acquisition.

### *2-D organized AuNP film*

The films were obtained using the method described by Liebig, *et al.*[4]. Using ethanol and toluene of a spectroscopic grade.

## Results and Discussion

### *Production and characterization of AuNP dimers*

Applying the protocol proposed by Zhang *et al*'s [2], the dimers were constructed using complementary DNA strands as a spacer, by firstly functionalizing the AuNPs with complementary DNA strands and consequently

conjugating both samples, and promoting the DNA hybridization.

The resulting hybridized and functionalized solutions were then separated through agarose gel electrophoresis. While for the functionalized AuNPs, a single band was expected on the gel, for the hybridized at least two bands were expected, one containing functionalized only AuNPs, migrating the same as the functionalized samples, and other which migrated less, containing dimers, also other bands of more complex agglomerates could be obtained. The efficacy of the method used was assessed firstly through confocal fluorescence microscopy, by depositing the samples obtained and gathering emission spectra for the structures present. This data suggested the presence of single and dimerized AuNPs in the samples in the bands which were expected to contain dimers (figure 1)[5]. Since the diffusion coefficients vary with the hydrodynamic radius of the structures it was also studied, for that FCS measures of an observation area inside of drops of different sample solutions, be it functionalized or hybridized AuNPs were obtained (figure 1). The results showed a decrease of diffusion coefficient as the hydrodynamic radius increased and overall the values were similar to the expected, obtained through theoretical calculations. However, the sample thought to contain AuNPs dimers (sample A2-b2) varies drastically from the expected values, probably due to the mixed population of dimers and single AuNPs present as shown later by the TEM images (figure 2), in which the samples thought to contain dimers were only 50% dimers and the remaining single AuNPs, showing that the dimerization process was inefficient.

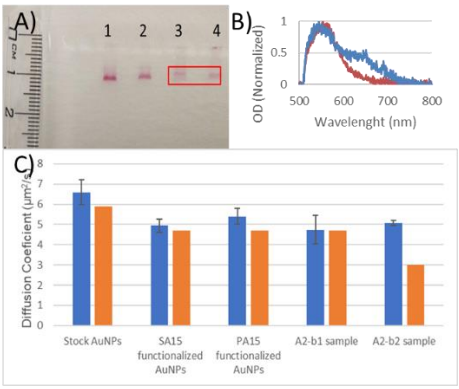


**Figure 1 –** A) Purification of samples through electrophoresis. AuNPs functionalized with the DNA chains PA15 and SA15, present in lane 1 and 2 respectively. Samples of AuNPs with hybridized DNA in lanes 3 and 4. Normalized extinction spectrum for functionalized AuNPs (orange) and dimerized AuNPs (blue) found in a dimerized sample (B). Diffusion coefficients obtained for stock AuNPs, AuNPs functionalized with SA15 or PA15 DNA strands AuNPs, sample from the top (A2-b2) and from the bottom band (A2-b1) of well 4 in image A), obtained experimentally (blue) and estimated diffusion coefficient values for each sample (orange).

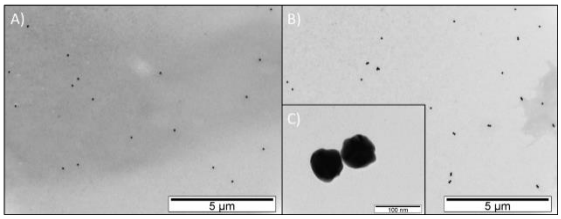


**Figure 2 -** Image obtained by TEM, corresponding to the sample from the top band (A2-b2) and from the bottom band (A2-b1) of well 4 in figure 1 in (A), (B) respectively. Image C) is a magnification of image B).

***DNA Coverage of AuNPs***

To assess if the problems felt during the dimerization protocol were due to deficient DNA functionalization of the AuNPs surface. To test this hypothesis, the number of DNA

strands per AuNP was assessed by fluorescence spectroscopy using fluorescein-labelled oligonucleotides and the values retrieved were compared to the maximum values reported in the literature[6].

To determine the concentration of AuNPs in each sample, their extinction spectrum was measured, and the respective sample concentration was determined according to the specification sheets from the supplier. Then by removing the DNA strands from the AuNPs' surface, which was done using mercaptoetanol[7], the fluorescence of the free DNA could be measured and by comparing it to a calibration curve for the concentration of fluorescein, prepared previously, the concentration of DNA strands can be known. Note that some assumptions are necessary for this to apply, each strand of DNA contains one molecule of the fluorophore, the entirety of DNA strands was removed from the NPs by the mercaptoethanol and also, no free DNA strands are present in the sample previous to the mercaptoethanol treatment. Finally, by dividing the dye-labelled DNA concentration by the NPs concentration, we obtain the number of DNA strands per NP (Table 1). As presented in table 1 the difference between the expected ratio, based on literature, and the experimentally obtained results do not differ significantly. Those differences can be due to the error inherent to the experiment setup, or even to the fact of, as shown previously, the nanoparticles are not perfect spheres and may vary slightly in size and shape, which could lead to the discrepancy in DNA coverage. Based on these results, it is possible to conclude that the difficulty in obtaining nanoparticle dimers is probably not due to the lack of coverage of DNA strands in the NPs. Another interesting result

**Table 1 -** Results for the DNA coverage of various size NPs and $I/I_0$ values for BF1-BT molecules attached to different size AuNPs.

| NP size (nm) | DNA Strand | Expected DNA strands per NP[6] | Obtained DNA strands per NP | I/I0 (%) |
|---|---|---|---|---|
| **20nm** | PAF30 | 180 | 142 | 17,4 |
| | SAF30 | | 142 | 17,9 |
| **40nm** | PAF30 | 430 | 453 | 20,6 |
| | SAF30 | | 543 | 18,8 |
| **80nm** | PAF30 | 1400 | 1408 | 70,1 |
| | SAF30 | | 1310 | 56,1 |

observed in this experiment, is the quenching effect of the nanoparticles have on the fluorescence of fluorescein dye. By comparing the emission spectra of the sample with the DNA strand attached to the NPs and the sample with just the DNA strands (after removal with mercaptoethanol), it was possible to see a significant change in the amount of fluorescence of both samples(Table 1). This is most likely due to a quenching effect of the NPs, since the amount of fluorescein to be measured is equal in both samples, if not, slightly lower in the case of free DNA strands, due to the lack of complete efficiency in the removal of the DNA strands from the NPs. This finding is supported by literature which already showed this phenomenon to occur[8]–[11]. This happens because the fluorophore's quantum yield is decreased by loss of energy to the metallic structure which is not compensated by the increase of excitation and radiative rates from the enhanced electric field of the surface. Moreover, since various sizes of nanoparticles were tested it is also possible to establish a correlation between the nanoparticle size and its quenching effectiveness. As seen in the table above, the smaller the metallic nanostructure,

the higher the quenching effect. This result is also in accordance with the literature[11].

***Fluorescence enhancement by single AuNRs***

Due to the low success in the formation of dimers, gold nanorods (AuNRs) were chosen, since they present a similar model to that of AuNPs dimers, to perform the enhancement experiments on aluminium tetrasulfonated phthalocyanine (AlPcTS) and on BF1-BT.

To enhance the fluorescence intensity, AuNRs should have a photoluminescence spectrum with a longitudinal plasmon resonance band overlapping the absorption and emission spectra of the fluorophore used, in order to enhance its fluorescent capabilities[12]–[14].

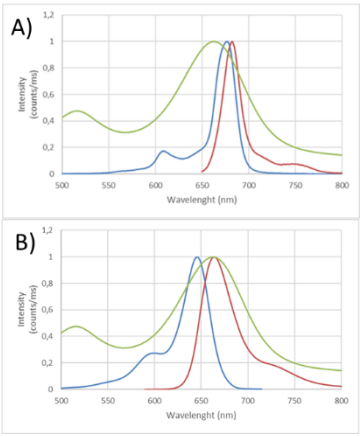


**Figure 3 –** Absorbance (blue) and emission (red) spectra of AlPcTS(A) and BF1-BT (B) overlapping with the extinction spectrum of stock nanorods (green).

Furthermore, it is reported that the maximum enhancement of fluorescence of a molecule with a metallic structure, occurs when its peak of emission is slightly shifted toward lower energy (longer wavelength) than the peak for the surface plasmon band. However, it is still required an overlap between the LSPR band and the fluorophore's emission spectra[12], [13]. This was observed o be the case for the fluorophores used (see figure 3).

***Fluorescence enhancement of AlPcTS***

Previously to the experiment the AuNRs were functionalized with amino-undecano-thiol (AUT), to serve as a spacer between the AuNR and the fluorophore, thus preventing quenching by excessive proximity. Using a 639 nm laser as excitation source, FLIM images of a surface area were observed. From this, particles were chosen, and FCS measurements were performed both, before and after the addition of AlPcTS. In opposition to the control, in the sample with the fluorophore, intense bursts of fluorescence were recorded, correspondent to the transit of a fluorophore molecule through the AuNR's intensification hotspot (figure 4). Upon analysis of the intensity time traces obtained, the values for enhancement factors and $FOM_{enh}$ can be calculated, by selecting the maximum intensity of the fluorescence intensity bursts and comparing it to the brightness of a single AlPcTS molecule, of 0,24 counts-per-molecule/m. For the $FOM_{enh}$ calculation, the quantum-yield used was of 34%[15].

These results demonstrate the enhancement potential of a gold NR antenna on the fluorescence of AlPcTS, which is about 200 times increase in the detected emission when compared to the same molecule alone.

As mentioned previously, the overlap between the fluorophore's absorption and emission spectra with AuNR's is a key factor for enhancement. It was then of interest to collect spectra of the AuNRs selected, however this was not possible, nonetheless, the FLIM images showed that the AuNRs chosen presented a very similar intensity and size between themselves, also being the most

abundant type of signal. All this suggest that they were single AuNRs.

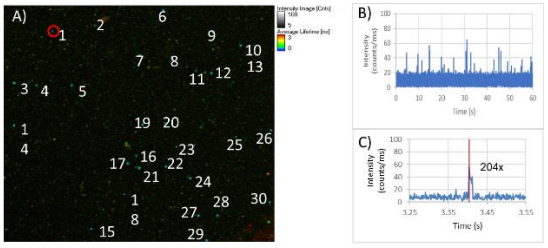


**Figure 4 –** (A) FLIM image of the surface studied. (B) time intensity trace obtained for the AuNR identified as 1 in image A) and its maximum intensity peak (C) with correspondent enhancement factor, for the fluorescent enhancement experiment with AlPcTS.

***Fluorescence enhancement of BF1-BT***

The experiment described here is similar to that used in the previous section, but the fluorescent molecule was changed from AlPcTS to a thiolated DNA spacer BF1-BT that is 10-bp long and is labelled with ATTO-647N dye.

Emission spectra were taken using the 482nm laser, to confirm that indeed single AuNPs are being measured, and not particle aggregates. Furthermore, it was also confirmed the overlap between the longitudinal surface plasmon band and the absorbance or emission spectra of the fluorophore, which is crucial for fluorescence enhancement to occur (figure 3). An excitation laser at 639nm was then used, to perform the FCS measurements, before and after the addition of BF1-BT. As before, the bursts of fluorescence intensity were only observed after the addition of the fluorophore. The data was then analysed by two methods, the one used before with AlPcTS and by extrapolating the maximum intensity through the intensity frequency histograms. Knowing the brightness of an individual fluorophore molecule, which in this case is 2,46 counts-per-molecule/ms and its quantum-yield, 65%[16], the enhancement factors and respective $FOM_{enh}$ can be calculated. Which corresponded on average to a 81 and 56 times increase in the detected emission when compared to the same molecule alone, for the "cherry-picking" and the extrapolation method respectively. As it is observable the two methods of analysis provide similar results, which is concordant to the literature[17]. Also, the method that provides the higher enhancement factors the cherry-picking", which was expected since it relies on the maximum measure alone instead of taking into account the intensity of other more frequent but lower intensity bursts.

The enhancement factors of the different AuNRs were plotted against the position of their longitudinal plasmon determined from the single-particle

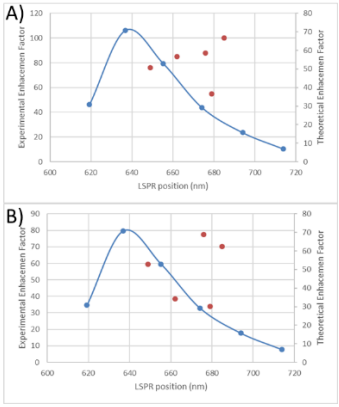


**Figure 5 –** Correlation of the top enhancement factors obtained experimentally with the "cherry-picking" (A) or the extrapolation analysis method (B) and calculated values (red) with the position of the AuNR longitudinal peak, for the BF1-BT fluorescence enhancement assay with AuNRs.

emission spectra as demonstrated in figure 5. In the same plot, the experimental enhancement factor are compared to theoretical values that

had been previously calculated, via discrete dipole approximation, by the research group that hosted this project [16].

***2-D organized AuNP film***

The AuNP films were constructed according to the protocol described in Liebig, *et al*[4], which described the self-assembly of a AuNP monolayer in the air-liquid interface of a droplet by ethanol-toluene addition. Three surfaces were constructed using this protocol with AuNPs of 20, 40 and 80 nm in diameter, to test the applicability of this protocol using different sized nanoparticles. Early characterization of the surfaces was carried out by optical and confocal fluorescence microscopy. The images obtained through optical microscopy and FLIM images were further supported by the emission spectra taken of various points of the surfaces. These observations pointed to the presence of 3 main types of structures, single, closely-packed and agglomerated AuNPs. Note that the closely-packed AuNPs, is composed of isolated dimers and higher aggregates or even closely-packed AuNPs which exhibit spectra similar to

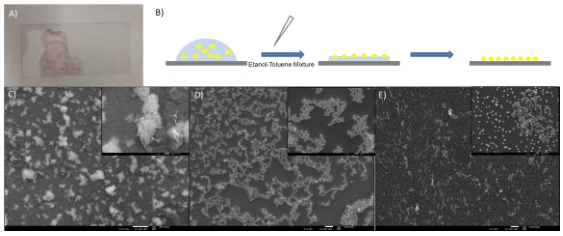


**Figure 6 –** AuNP film deposition. A) image of the glass slide after deposition of 80nm AuNPs. B) scheme of the protocol used. C), D) and E) TEM images and zoomed of the surfaces with 20, 40 and 80nm AuNPs respectively.

those of dimers. These findings were then corroborated by TEM images (figure 6).

***Fluorescence enhancement by the prepared 2-D organized AuNP film***

Fluorescence enhancement experiences were then carried out in the 80nm AuNP surface previously prepared, using BF1-BT and BF1-BD2-TD.

***Fluorescence enhancement of BF1-BT by the prepared 2-D organized AuNP film***

This experiment aimed to assess the enhancement factors produced on BF1-BT, by the films prepared, and so, consequently being able to establish a comparison between the values obtained for the AuNRs in previous experiments and the films. However, this experimental data showed to be unusable, due to contaminations on the surface. This was discovered since the intensity time traces obtained as control, of points on the surface without the presence of the fluorophore, showed high fluorescence intensity bursts, meaning that some molecules with

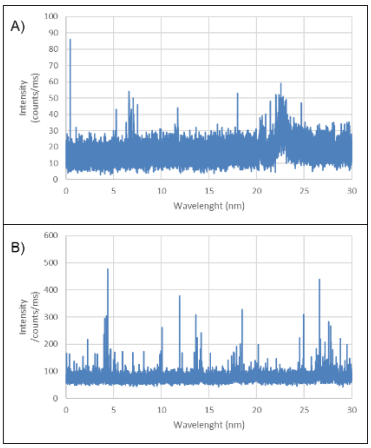


**Figure 7 –** Variation of the fluorescence intensity through time of point 2, prior (A) and after (B) the addition of BF1-BT.

fluorescence were present already in the surface (figure 7). Knowing this, it becomes impossible to distinguish the bursts obtained due to the presence of BF1-BT from the ones

caused by contaminations. It is then impossible to withdraw any conclusion from this experiment, even though as mentioned previously the fluorescence intensities obtained in this surface were much higher than those from the AuNRs, which indicated it could have higher fluorescence enhancing capabilities, as expected.

***Fluorescence enhancement of BF1_BD2-BT by the prepared 2-D organized AuNP film***

The molecular beacons action was also assessed in the same surface previously used. As mentioned previously, the molecular beacon BF1-BD2 is composed by a DNA hairpin probe that in the closed conformation (i.e. absence of target TD) has the fluorophore (Atto-647N) positioned close to a quencher (QSY-21). Upon hybridization of the loop region of the DNA hairpin in BF1-BD2 with the target TD sequence, the hairpin opens and fluorescence is recovered. This mechanism was assessed by comparing the average maximum intensity of intensity-lifetime histograms of FLIM images of the surface taken, before the addition of BF1-BD2, after it, after the addition of the target TD and lastly, after 30 minutes of the TD addition, see table 2. In the control measurement, the intensity determined results from the emission of AuNPs and any impurities that might be present. When the molecular beacon BF1-BD2 was added, it was observed an increase in fluorescence due to its interaction with the nanoparticles present on the surface. The emission from BF1-BD2 is detectable because the fluorophore's emission is not completely quenched in the closed configuration. After the addition of the target sequence TD, another increase in intensity was observed. This turn, the intensity increase was attributed to the beacon's conformational change from closed to open hairpin upon hybridization with the target. After 30 minutes passed from the addition of TD, the analysis showed an increase in fluorescence intensity. This result suggests that more target molecules had time to hybridize, thus increasing the fluorescence signalling response. These findings are in agreement with the fluorescence sensor design and with data previously obtained by the research group hosting this project. This experiment demonstrated the potential of the plasmonic substrates developed here for the enhancement of fluorescence signalling in sensors for nucleic acid detection. However, in order to truly assess the influence of the metallic nanostructures on the fluorescence signals of the molecular beacon tested, it would be desirable to perform enhancement experiments as the ones previously reported in this document, i.e. experiments combining single-particle and single-molecule fluorescence detection.

**Table 2 -** Average of the fluorescence intensities (with SD) obtained for each step of the experiment.

| Measurements | Frequency (kcounts) (with SD) |
|---|---|
| **Control** | 225 |
| **BF1-BD2 added** | 318 ± 12 |
| **TD added** | 340 ± 22 |
| **TD (After 30 minutes)** | 371 ± 15 |

## Conclusions

In this work it was shown that the production of AuNP dimers with defined spacing is possible using DNA strands, although the efficiency of the method was subpar. To analyse the cause

an assay to test the functionalization of AuNPs was performed, which indicated that this was not the issue, but rather some aggregation or instability of the stock nanoparticles. Furthermore, throughout this work, the fluorescence enhancing properties of metallic nanostructures interacting with fluorophore molecules were assessed, producing results which are comparable to those predicted through theoretical simulations.

A method for producing closely packed organized AuNPs films was implemented with some degree of success. Even though, the film produced was not homogeneous, some areas presented the desired morphology. These films were then used to conduct fluorescence enhancement assays, either with only the fluorophore component of the molecular beacon or with the entire beacon structure. Despite, the contamination problems encountered in the first case, the results showed that large fluorescence signals were obtained from the fluorophore, but contaminations present precluded any further conclusions. The latter assay, using the entire beacon structure, also showed that it is possible to distinguish the presence of a target nucleic acid for the DNA hairpin probe used, by showing different fluorescent signal intensities when the target was either absent or present.

Despite this, there is still much room for improvement, either by optimizing the processes used or by applying different methods. The repetition of fluorescence enhancement assays using the nanoparticle films needs to be carried out, in order to properly assess the enhancement capabilities of such structures. Another step to be taken in the future is the implementation of AuNPs films as plasmonic substrates into microchannels. This would allow testing the fluorescence enhancement effect under experimental conditions closer to the application envisioned for plasmonic substrates, which would be the demonstration of more sensitive lab-on-a-chip fluorescence-based sensors.

So, there is still a lot of work to be done before the implementation of this technology in miniaturized biosensors. However, the scientific community is working towards that goal, as shown by the significant research increase in this area throughout the years and the advancements made during that time.

## References


[1] Darvill, Daniel, Anthony Centeno, and Fang Xie. "Plasmonic fluorescence enhancement by metal nanostructures: shaping the future of bionanotechnology." Physical Chemistry Chemical Physics 15.38 (2013): 15709-15726.

[2] Zhang, Xu, et al. "Toward fast and quantitative modification of large gold nanoparticles by thiolated DNA: Scaling of nanoscale forces, kinetics, and the need for thiol reduction." The Journal of Physical Chemistry C 117.30 (2013): 15677-15684.

[3] Jin, Ping, Zhao Dai, and Tianrui Chang. "The Role of Bis (p-Sulfonatophenyl) Phenylphosphine in Stabilizing Gold Nanoparticles." AASRI International Conference on Industrial Electronics and Applications. 2015.

[4] Liebig, Ferenc, et al. "Deposition of gold nanotriangles in large scale close-packed monolayers for X-ray-based temperature calibration and sers monitoring of plasmon-driven catalytic reactions." ACS applied

materials & interfaces 9.23 (2017): 20247-20253.

[5] Paulo, Pedro MR, et al. "Enhanced Fluorescence of a Dye on DNA-Assembled Gold Nanodimers Discriminated by Lifetime Correlation Spectroscopy." The Journal of Physical Chemistry C 122.20 (2018): 10971-10980.

[6] Hill, Haley D., et al. "The role radius of curvature plays in thiolated oligonucleotide loading on gold nanoparticles." ACS nano 3.2 (2009): 418-424.

[7] Demers, Linette M., et al. "A fluorescence-based method for determining the surface coverage and hybridization efficiency of thiol-capped oligonucleotides bound to gold thin films and nanoparticles." Analytical chemistry 72.22 (2000): 5535-5541.

[8] Dulkeith, E., et al. "Gold nanoparticles quench fluorescence by phase induced radiative rate suppression." Nano letters 5.4 (2005): 585-589.

[9] Schneider, Grégory, et al. "Distance-dependent fluorescence quenching on gold nanoparticles ensheathed with layer-by-layer assembled polyelectrolytes." Nano letters 6.3 (2006): 530-536.

[10] Dulkeith, E., et al. "Fluorescence quenching of dye molecules near gold nanoparticles: radiative and nonradiative effects." Physical review letters 89.20 (2002): 203002.

[11] Anger, Pascal, Palash Bharadwaj, and Lukas Novotny. "Enhancement and quenching of single-molecule fluorescence." Physical review letters 96.11 (2006): 113002.

[12] Munechika, Keiko, et al. "Spectral control of plasmonic emission enhancement from quantum dots near single silver nanoprisms." Nano letters 10.7 (2010): 2598-2603.

[13] Chen, Yeechi, Keiko Munechika, and David S. Ginger. "Dependence of fluorescence intensity on the spectral overlap between fluorophores and plasmon resonant single silver nanoparticles." Nano letters 7.3 (2007): 690-696.

[14] Teixeira, Raquel, et al. "Plasmon-enhanced emission of a phthalocyanine in polyelectrolyte films induced by gold nanoparticles." The Journal of Physical Chemistry C 115.50 (2011): 24674-24680.

[15] Laia, Cesar AT, et al. "Electron-transfer kinetics in sulfonated aluminum phthalocyanines/cytochrome c complexes." The Journal of Physical Chemistry B 108.22 (2004): 7506-7514.

[16] D. Botequim, P. M. R. Paulo, D. M. F. Prazeres, and S. M. B. Costa, “Gold Nanorods Conjugated with Fluorescently-Labeled DNA’s: Highly Loaded Dye-Nanoparticles,” in Gordon Research Conference, From Fundamentals to Applications of Collective Excitations in Nanostructured Materials- Massachusetts, EUA (2018).

[17] Khatua, Saumyakanti, et al. "Resonant plasmonic enhancement of single-molecule fluorescence by individual gold nanorods." ACS nano 8.5 (2014): 4440-4449.