

# CHAPTER 6

## SPECTROSCOPIC STUDIES ON METHYLENE BLUE AND NILE BLUE BOUND TO COATED GOLD NANORODS

In this chapter the results of our study on the effect of L-SP electric field of AuNRs on the optical (absorption and fluorescence) properties of two dyes Methylene Blue (MB) and Nile Blue (NB) are presented where the L-SP band was tuned and detuned with respect to the absorption maxima of the dyes. Binding between dyes and nanorods were established by electrostatic interaction by making the nanorods negatively charged after coating them with polystyrene sulphonate (PSS). The effect of dye/rod ratio on the extinction and fluorescence decay properties of the dye-AuNR complex is described.

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*Work discussed in this chapter resulted in the following publication:*

1. 'Spectroscopic investigations on the binding of Methylene Blue and Nile Blue to negatively charged gold nanorods' R. Shrivastava, B. Jain and K. Das, *Journal of Molecular Structure*, Vol. 1020, pp. 56-62 (2012).

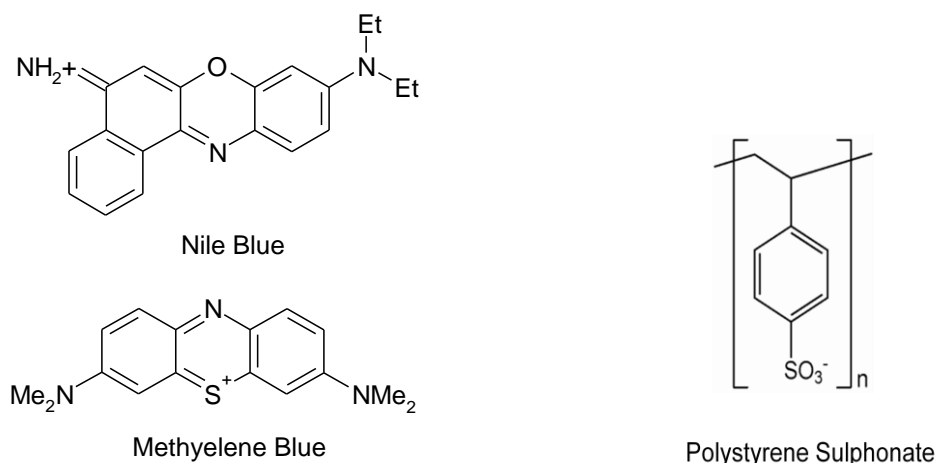
## 6.1 Introduction

The surface plasmon (SP) electric field of metallic nanoparticles (NP) can significantly affect the absorption and emission properties of molecular chromophores located nearby. Usually this results in a quenching of their fluorescence [158–163, 164–167], however, a good overlap between the molecular and plasmonic resonance may also result in resonant coupling between them [168–173, 174–179]. The radiative and nonradiative decay of the excited state of the chromophores is expected to depend critically on size and shape of the nanoparticle, the distance between the chromophore and the nanoparticle, the orientation of the molecular dipole with respect to the dye-nanoparticle axis, and the overlap of the molecule's emission with the nanoparticle's absorption spectrum [180].

In this chapter we have investigated how the SP field of gold nanorods affects the optical (absorption and emission) properties of two adsorbed dyes when their SP absorption is tuned to the absorption maximum of dye or away from it. For this we prepared PSS coated gold nanorods (AuNR) of different aspect ratio having their longitudinal surface plasmon band (L-SP) tuned with the absorption maxima of two dyes NB and MB and L-SP band tuned away from the absorption band of the dyes (the detuned condition). Earlier, the SP fields of different gold nanostructures have been utilized to enhance the Raman scattering from these dyes [181, 182].

## 6.2 Experimental Details

NB and MB from Exciton were used as received. The chemical structure of Nile Blue (NB), Methylene Blue (MB) and the repeating unit of PSS are shown in Fig. 6.1. AuNR samples with ensemble L-SP peaks at 730 nm and 700 nm, were grown in aqueous solutions varying the gold to seed ratio, and ascorbic acid to gold ratio as described in chapter 2 [98]. Shorter rods were prepared by oxidative shortening of an initial rod with L-SP at 700 nm, using  $\text{H}_2\text{O}_2$  (50%) as the oxidizing agent [179].



**Figure 6.1** Chemical structures of the dyes and the polymer PSS used in this study

The ratio between the  $\text{H}_2\text{O}_2$  solution and the as-grown nanorod solution was  $\sim 1:5$ . The oxidation reaction was stopped at controlled periods of time by centrifugation at 12,000 rpm for 2-3 min and washing twice with water and finally dispersing in water. This oxidation process led to the formation of two AuNR samples with L-SPR at 635 nm and 660 nm, matching to the molecular absorption of NB ( $\lambda_{\text{max}} = 635$  nm) and MB ( $\lambda_{\text{max}} = 664$  nm) respectively.

These prepared rods had a layer of CTAB over them which is positively charged. To make them negatively charged, PSS (75 KDa) was coated over these positively charged rods making their overall surface charge negative for binding to the positively charged dyes following the method as described in chapter 2. The excess PSS was removed by centrifuging and resuspending the pellet in Millipore water. The PSS coating changed the zeta potential (measured using a 90 Plus size and zeta potential analyzer) of the rods from  $\sim +50$  mV to  $\sim -45$  mV.

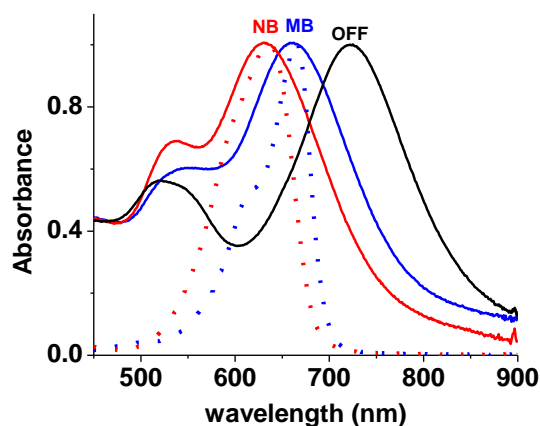
### 6.2.1 Absorption & Fluorescence spectroscopy

Absorption and fluorescence experiments were carried out in a cuvette of 1 cm path length. The absorption spectra of the dye-nanorod complex were recorded after addition

of a concentrated stock solution (typically few micro litres) of dye to a 0.06 nM nanorod solution. Similarly, the fluorescence spectra of the dye-nanorod complex were recorded after addition of a concentrated stock solution (typically few micro litres) of nanorods to a 5  $\mu$ M dye solution. The changes observed in the spectra after addition of dye was immediate and did not change with time during experiments. Steady state absorption and emission and time resolved fluorescence measurements of the rod-dye complex were done as described in chapter 2, section 2.3. NB and MB were excited at 635 and 665 nm respectively for steady state emission measurements. The emission lifetimes of the dyes were obtained by excitation at 635 nm using a picosecond diode laser. The fluorescence quantum yields ( $\Phi_f$ ) of MB and NB in aqueous environment were taken from previous reports [183,184] and the relative quantum yield in the presence of AuNRs were calculated accordingly. The radiative ( $k_r$ ) and non radiative ( $k_{nr}$ ) rates were calculated according to the relation:  $k_r = \Phi_f/\tau_f$  and  $k_{nr} = 1/\tau_f - k_r$ , where  $\tau_f$  is the average fluorescence lifetime.

### 6.3 Results

The normalized absorbance spectra of the AuNRs are shown in Fig. 6.2.

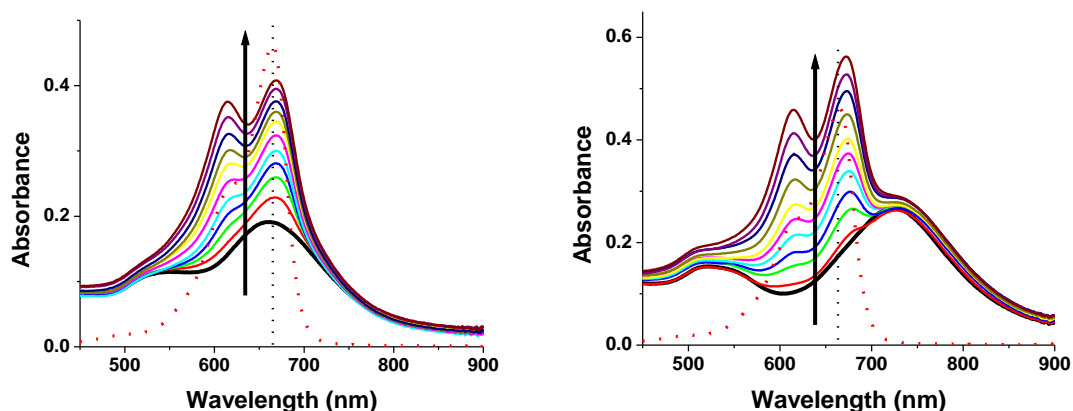


**Figure 6.2** Normalized absorbance spectra of AuNRs used in this study along with the absorbance spectra of MB and NB (as dots).

AuNRs with average aspect ratio of  $3.5 \pm 0.2$ ,  $2.5 \pm 0.2$  and  $2.3 \pm 0.1$  had the L-SP peaks centered at 730, 660 and 630 nm, respectively. The L-SP peak of AuNR at 630 nm, matches with the absorption of NB and the L-SP peak of another set of AuNR at 660 nm, matches with the absorption of MB while AuNRs having L-SPR at 730 nm is detuned from the absorption band of both the dyes.

### 6.3.1 Absorption properties of dye-AuNR systems

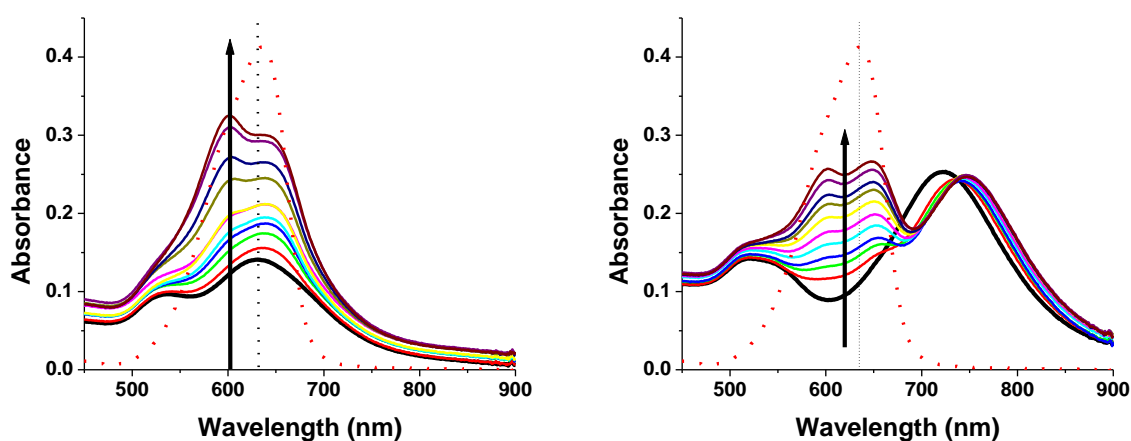
The changes observed in the absorbance spectra of the PSS coated AuNRs upon gradual addition of MB and NB is shown in Fig. 6.3 and 6.4 respectively for the tuned and detuned conditions.



**Figure 6.3** Changes in the absorbance spectrum of PSS coated AuNR (60 pM thick black curve) upon addition of MB. The arrow indicates increasing amount of the dye (in 0.5  $\mu\text{M}$  steps). For comparison the spectra of 5  $\mu\text{M}$  MB (dotted red curve) is also shown. The dashed line represents the absorption maxima of the free dye. Left: Tuned condition; right: detuned condition.

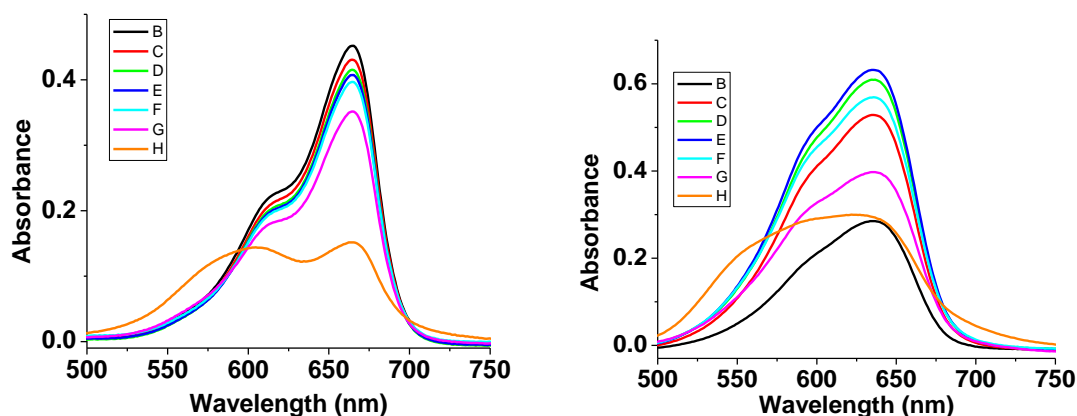
For comparison, the absorption spectra of 5  $\mu\text{M}$  dye (the highest dye concentration used) are also shown in the figures. From these figures it is clear that with increasing amount of dye the resulting absorbance spectra becomes significantly different from the dye or nanorod absorbance spectra. The observed changes are likely to result from an electrostatic interaction between the positively charged dye and the negatively charged PSS coated AuNRs. The effect of addition of MB to an AuNR solution (0.06

nM) is described in Fig. 6.3. For the tuned condition, where the L-SP band of the AuNR matches with the absorption maxima of the dye, the gradual addition of the dye results in the appearance of two bands centred at 615 and 669 nm. For the detuned condition, where the L-SP band of the AuNRs were located at 730 nm, gradual addition of the dye again results in the appearance of two peaks located at 615 and 670 nm while the shoulder at 730 nm represents that of the L-SP band of the AuNRs. The effect of addition of NB to an AuNR solution (0.06 nM) is described in Fig. 6.4.



**Figure 6.4** Changes in the absorbance spectrum of PSS coated AuNR (60 pM, thick black curve) upon addition of NB. The arrow indicates increasing amount of the dye (in 0.5  $\mu\text{M}$  steps). For comparison the spectra of 5  $\mu\text{M}$  NB (dotted red curve) is also shown. The dashed line represents the absorption maxima of the free dye. Left: Tuned condition; right: detuned condition.

For the tuned condition, the gradual addition of the dye results in the appearance of two bands centred at 603 and 640 nm. For the detuned condition, the gradual addition of the dye now results in the appearance of two peaks located at 600 and 645 nm with a shoulder at 525 nm (T-SP) and at  $\sim 730$  nm (L-SP) representing the SP band of the rods. In addition the L-SP band of the AuNR shows a distinct red shift (from  $\sim 730$  nm to  $\sim 750$  nm) in the presence of the dye. The effect of only PSS on the absorption spectra of the dyes was also investigated as shown in Fig. 6.5. The absorption of MB decreases monotonically with increase in PSS concentration and at 10  $\mu\text{M}$  concentration of the polymer it splits into two bands.

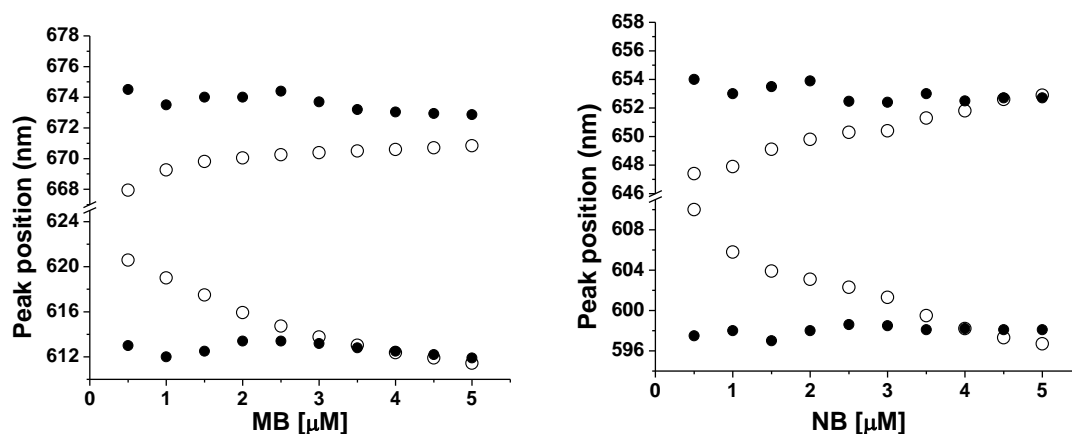


**Figure 6.5** Absorption spectra of MB (left) and NB (right) in the presence of varying amount of PSS. B: No PSS; C: 0.1 nM PSS; D: 1 nM PSS; E: 10 nM PSS; F: 100 nM PSS; G: 1  $\mu$ M PSS and H: 10  $\mu$ M PSS

The absorption of NB, however, increases up to a PSS concentration of 10 nM and from there it starts to decrease and at 10  $\mu$ M concentration of the polymer the absorption band gets considerably broader. As stated in the introduction section, when the molecular and plasmonic resonances are overlapping, resonance coupling between them is a possibility, which is characterized by a splitting of the plasmon band. In this study, resonant coupling is expected to occur between-dyes and PSS coated AuNRs for the tuned condition because the absorption maximum of dyes matches with that of the L-SP band. In order to validate this, the absorption spectra of the dye-AuNR complex (in the range of 590-750 nm for MB-AuNR and 550-710 nm range for NB-AuNR) were fitted to two lorentzian line shapes (with an additional baseline).

Fig. 6.6 describes the changes of the absorption band maxima with increasing dye concentration. For the tuned condition, the separation between the high and low energy band increases with increasing dye concentration and seems to saturate when the dye concentration reaches 5  $\mu$ M. For the detuned condition, where the resonant coupling is expected to be weak, a similar Lorentzian fitting is done (in the range of 550-700 nm for MB-AuNR and 550-710 nm range for NB-AuNR; where the dye absorption is

dominating) to see how the band maxima evolve with increasing dye concentration. It is observed that the splitting of the high and low energy band occurs immediately after addition of 0.5  $\mu\text{M}$  dye and thereafter stays same.



**Figure 6.6** Changes in the absorption peak positions upon increasing amount of dye (in 0.5  $\mu\text{M}$  steps). Hollow and solid circles represent the tuned and detuned conditions respectively. The spectra were fitted in the 590-750 nm (550-700 nm for detuned) and 550-710 nm (580-680 nm for detuned) range for MB and NB respectively by a pair of lorentzian line shapes along with a baseline.

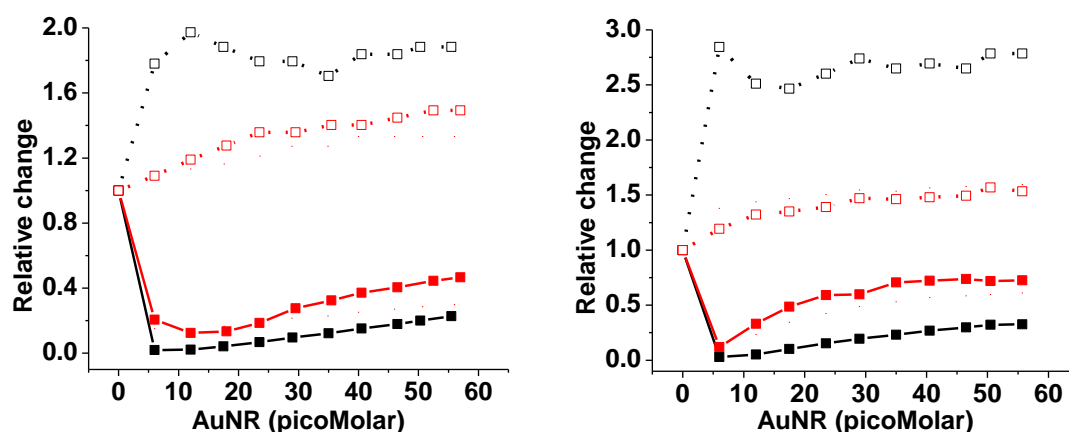
### 6.3.2 Fluorescence properties of the dye-AuNR systems

In order to investigate how the SP field of the AuNRs affects the fluorescence properties of MB and NB, the fluorescence spectra of dyes were studied in the presence of increasing amount of AuNRs. For both tuned and detuned conditions, the fluorescence intensities of dyes were observed to be decreased in the presence of AuNRs (data not shown). The relative changes in the fluorescence quantum yield and the average lifetime for dyes upon sequential addition of PSS coated AuNRs are shown in Fig. 6.6.

For both tuned and detuned conditions, the quantum yield drops down significantly with the first addition of AuNRs (6 pM) and then increases slowly and starts to level off at higher rod concentrations. The quantum yield of fluorescence for MB decreases by 50% for the tuned condition whereas it decreases by 25% for the detuned



condition. In case of NB, the amount of decrease is 77% and 67% for the tuned and detuned condition respectively.

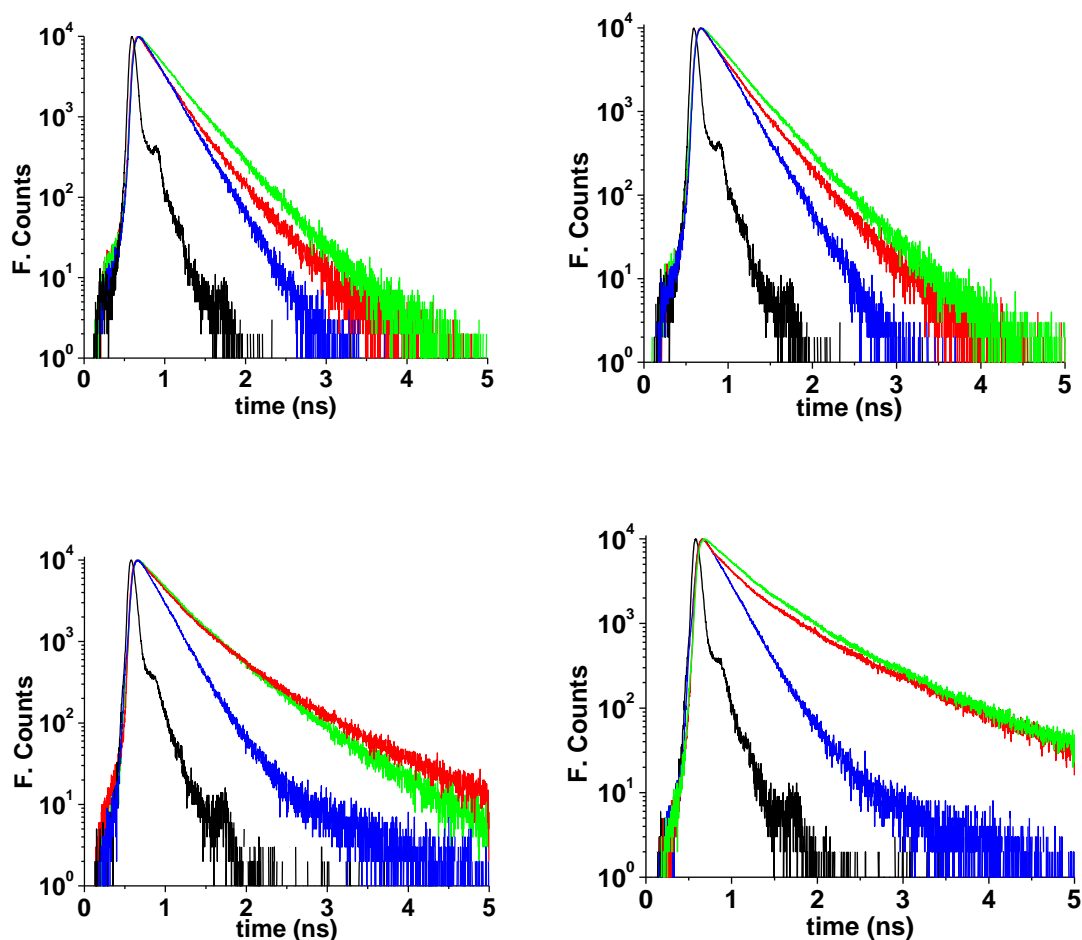


**Figure 6.7** Relative changes in the fluorescence quantum yield (filled squares) and lifetime (hollow squares) of the two dyes (5  $\mu\text{M}$ ) in the presence of varying amount of PSS coated AuNRs. Left: tuned and right: detuned condition. NB: black; and MB: red.

The observed fluorescence decays of the two dyes in the absence of AuNRs can be well fitted by a sum of two exponentials. For MB and NB, the average lifetimes (in the absence of AuNRs) are observed to be similar as 220 ps. Since the fluorescence of dyes was significantly decreased in the presence of AuNRs, it is expected that their fluorescence lifetimes should also get decreased in the presence of AuNRs. However, the fluorescence lifetimes of dyes were observed to increase in the presence of AuNRs as shown in Fig. 6.8. Although the margin of increase is only modest for MB, it is quite significant for NB. At a rod concentration of 12 pM, the average lifetime of MB increases by 1.2 and 1.4 times for the tuned and detuned conditions respectively whereas for NB this amounts to 2 and 2.5 times. However, increasing the rod concentration further does not affect the lifetimes any more.

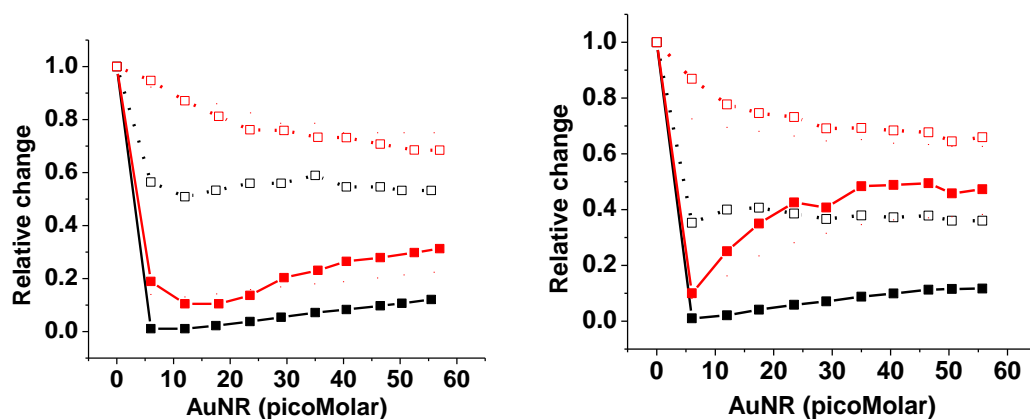
At the highest rod concentrations used in this study ( $\sim 56$  pM) the average lifetime of MB increases by 1.5 times for both tuned and detuned conditions whereas for NB this amounts to 2 and 2.8 times respectively. Since changes in the quantum yield and

lifetimes are governed by the radiative and the nonradiative processes in the excited state, how they change in the presence of the AuNRs is shown in Fig. 6.9.



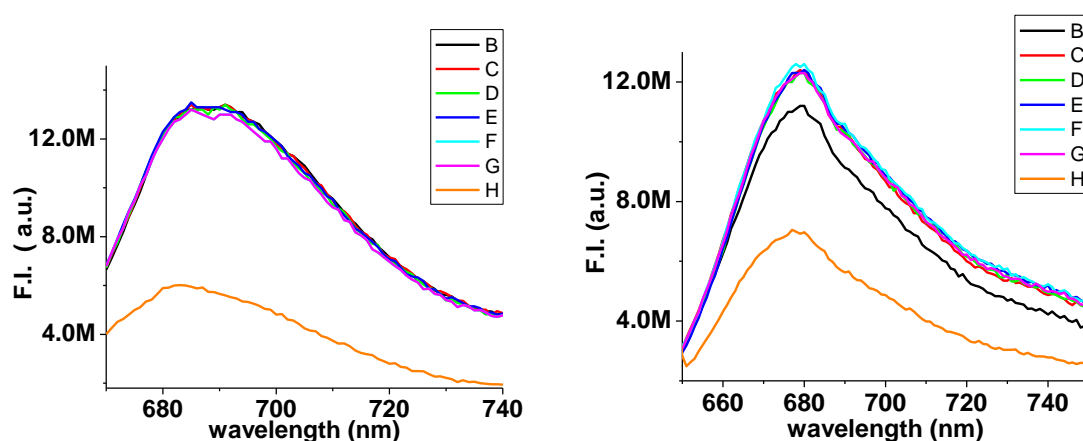
**Figure 6.8** Changes in the fluorescence lifetimes of the two dyes ( $5 \mu\text{M}$ ) in the presence of various amounts of AuNRs. Black: only dye; Red: dye +  $12 \text{ pM}$  AuNR and Green: dye +  $60 \text{ pM}$  AuNR. Top: MB and bottom: NB. The left panel shows the decays for the tuned and the right panel shows the decays for the detuned conditions.

For both tuned and detuned conditions, the radiative rate ( $k_r$ ) decreases significantly ( $> 80\%$ ) with the initial addition of AuNRs. After that, for the tuned condition,  $k_r$  increases only modestly for both the dyes. However, for the detuned condition, against increasing rod concentration, the observed trend of  $k_r$  is quite different for MB and NB. The nonradiative rates for both the dyes were observed to decrease in the presence of increasing amount of AuNRs.

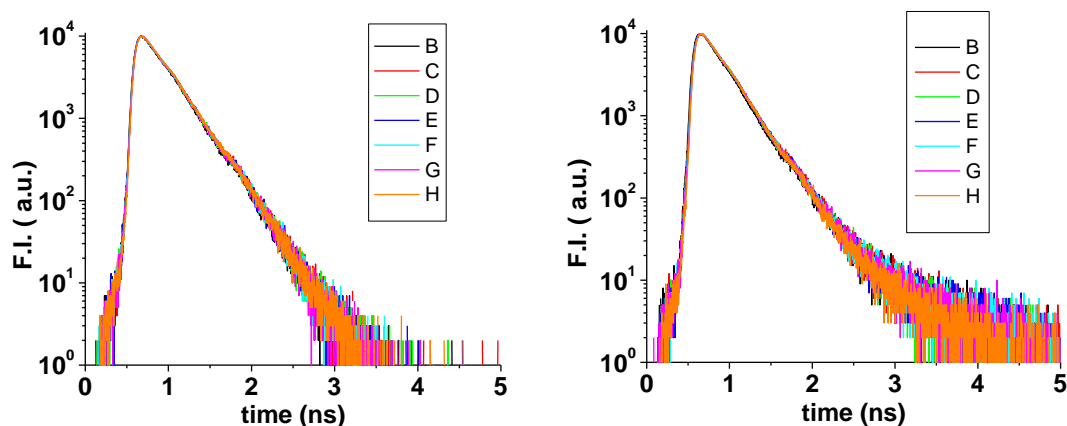


**Figure 6.9** Relative changes in the  $k_r$  (filled squares) and  $k_{nr}$  (hollow squares) of the two dyes in the presence of varying amount of PSS coated AuNRs. Left: tuned and right: detuned condition. NB: black; and MB: red.

For NB, the magnitude of decrease in  $k_{nr}$  was observed to be significantly higher than MB. The values of fluorescence quantum yields, average lifetimes, radiative and nonradiative rates for the two dyes in the presence of varying amount of PSS coated AuNRs are given in Table 6.1.



**Figure 6.10** Fluorescence spectra of MB (left) and NB (right) in the presence of varying amount of PSS. B: No PSS; C: 0.1 nM PSS; D: 1 nM PSS; E: 10 nM PSS; F: 100 nM PSS; G: 1 μM PSS and H: 10 μM PSS.



**Figure 6.11** Fluorescence decay traces of MB (left) and NB (right) in the presence of varying amount of PSS. B: No PSS; C: 0.1 nM PSS; D: 1 nM PSS; E: 10 nM PSS; F: 100 nM PSS; G: 1  $\mu$ M PSS and H: 10  $\mu$ M PSS

We also studied the effect of PSS on the fluorescence properties of the dyes as shown in Fig. 6.10 and Fig.6.11. For PSS concentration range of 0.1 nM to 1  $\mu$ M, the changes in the fluorescence intensities of the dyes were modest and only at a polymer concentration of 10  $\mu$ M, it were observed to decrease significantly. However, lifetimes of the two dyes remains unchanged at all the concentrations (0.1 nM- 1 $\mu$ M) of the polymer.

## 6.4 Discussion

The objective of this study was to investigate how the optical properties of two dyes (MB and NB) are affected by the SP electric field of the AuNRs for the tuned and detuned conditions. The absorption spectrum of the dye-AuNR complex is significantly different from the free dye or the free AuNR for both tuned and detuned conditions. It has been reported that in aqueous solution both MB and NB can form aggregates (dimers) upon addition of different aggregating agents [185, 186, 187]. For example, electrostatic interaction between NB and silica colloids results in the formation of the dimeric species [187] while negatively charged polymers like PSS has been shown to favor the formation of dimeric MB [186]. The formation of dimeric species will reduce the concentration of the monomeric species and in absorption spectrum this would reflect in an increase in the

**Table 6.1** Fluorescence parameters of MB and NB in the presence of various amounts of PSS coated AuNRs

Tuned					Detuned				
AuNR (pM)	$\Phi_f$	$\tau_f$ (ns) (average)	$k_r$ ( $\times 10^8 s^{-1}$ )	$k_{nr}$ ( $\times 10^8 s^{-1}$ )	AuNR (pM)	$\Phi_f$	$\tau_f$ (ns) (average)	$k_r$ ( $\times 10^8 s^{-1}$ )	$k_{nr}$ ( $\times 10^8 s^{-1}$ )
<b>Methylene Blue</b>									
<b>0</b>	0.040	0.22	1.80	43.0	0	0.040	0.22	1.80	43.0
<b>6</b>	0.008	0.24	0.18	37.4	6	0.005	0.27	0.34	41.2
<b>12</b>	0.005	0.26	0.45	33.4	12	0.013	0.30	0.19	37.8
<b>18</b>	0.005	0.28	--	--	17.5	0.019	--	0.19	35.3
<b>23.5</b>	0.007	0.30	0.76	31.5	23.5	0.024	0.31	0.25	33.1
<b>29.5</b>	0.011	0.30	0.73	29.8	29	0.024	0.33	0.37	33.0
<b>35.5</b>	0.013	0.31	0.87	29.8	35	0.028	0.33	0.42	31.8
<b>40.5</b>	0.015	0.31	0.86	29.4	40.5	0.029	0.33	0.48	31.8
<b>46.5</b>	0.016	0.32	0.89	29.1	46.5	0.030	0.33	0.51	30.7
<b>52.5</b>	0.018	0.33	0.82	27.8	50.5	0.029	0.35	0.54	29.8
<b>57</b>	0.019	0.33	0.85	28.4	55.7	0.029	0.34	0.57	29.7
<b>Nile Blue</b>									
<b>0</b>	400E-5	0.22	0.179	44.7	0	400E-5	0.22	0.179	44.7
<b>6</b>	7.6E-5	0.40	0.002	25.2	6	11.6E-5	0.62	0.002	16.0
<b>12</b>	8.7E-5	0.44	0.002	22.7	12	21.1E-5	0.55	0.004	18.2
<b>17.5</b>	16.7E-5	0.42	0.004	23.8	17.5	40.7E-5	0.54	0.008	18.5
<b>23.5</b>	27.2E-5	0.40	0.007	25.0	23.5	60.9E-5	0.57	0.010	17.5
<b>29</b>	38.6E-5	0.40	0.010	25.0	29	77.8E-5	0.60	0.013	16.7
<b>35</b>	48.9E-5	0.38	0.013	26.3	35	93.0E-5	0.58	0.016	17.2
<b>40.5</b>	60.9E-5	0.41	0.015	24.4	40.5	107.0E-5	0.59	0.018	16.9
<b>46.5</b>	71.7E-5	0.41	0.017	24.4	46.5	119.0E-5	0.58	0.021	17.2
<b>50.3</b>	80.3E-5	0.42	0.019	23.8	50.5	129.0E-5	0.61	0.021	16.4
<b>55.5</b>	91.3E-5	0.42	0.022	23.8	55.7	130.0E-5	0.61	0.021	16.4

intensity of the dimer band (usually located on the higher energy side of the monomer band) and a decrease for the monomer band. This is indeed seen in the presence of 10  $\mu\text{M}$  PSS (Fig. 6.5).

For MB and NB, the higher energy band (located at 615 and 600 nm respectively) is likely to represent the dimeric species while the monomeric species absorb at 670 and 640 nm, respectively. However, the relative intensities of the band maxima for the dimeric and monomeric species are observed to be different for MB and NB. For MB, the intensity of the band corresponding to the monomeric species is observed to be higher than that of the dimeric species for both tuned and detuned conditions (Fig. 6.3). In case of NB they are reversed for the tuned condition and nearly equal for the detuned condition (Fig. 6.4). The absorption spectra of the dye-nanorod complex may result from aggregation of the dyes induced by nanorods or from resonance coupling between the dye and the nanorods.

As observed in earlier works [168-179] plasmon-molecular resonance coupling results in a splitting of the plasmon peak whose magnitude increases with increasing dye concentration and then saturates when full monolayer coverage of dye is approached. This is indeed observed for the tuned condition (Fig. 6.6). It is interesting to note that the magnitude of splitting observed at the highest dye concentration was observed to be  $\sim 20$  nm for the NB-AuNR complex, double of that observed for MB-AuNR complex. In an earlier work, the coupling between MB and PSS coated AuNR having an L-SP band at 680 nm was studied [179]. The plasmon shift, defined as the difference between the lower-energy peak wavelength of the hybrid nanostructure and the L-SP peak of the PSS-coated AuNR was observed to be  $\sim 40$  nm for dye:AuNR ratio of  $\sim 130 \times 10^3$ . The plasmon shift observed in our study corresponds to about  $\sim 10$  nm for dye:AuNR ratio of  $\sim 80 \times 10^3$ . The observed spectral features of the dye-AuNR complexes are also different from the earlier study. This is probably due to significant differences in the dye:AuNR

ratio. For the detuned condition the observed trend of the peak splitting is significantly different which we attribute to nanorod induced aggregation of the dyes.

The fluorescence intensities of dyes in the presence of increasing amount of PSS coated AuNRs were observed to be decreased. This might arise due to: formation of dimeric species which are nonfluorescent in nature and/or quenching of fluorescence due to presence of SP electric field. However, the increase in the fluorescence lifetime of the dyes in the presence of increasing amount of PSS coated AuNRs is surprising because as shown in earlier reports, SP electric field generally quenches the lifetime of the chromophores near its vicinity [158-167]. Because of the good overlap between the fluorescence and L-SP band for the tuned condition, this effect is expected to be more pronounced due to energy transfer process. A look at Fig. 6.9 and Table 6.1, shows that both  $k_r$  and  $k_{nr}$  of dyes decreases in the presence of AuNRs. The magnitude of decrease for  $k_r$  is much greater than that for  $k_{nr}$ . For NB,  $k_r$  decreases by ~90% whereas  $k_{nr}$  decreases by 50-60% for both tuned and detuned conditions. For MB,  $k_r$  decreases by ~75-60% whereas  $k_{nr}$  decreases by 30% for tuned and detuned conditions, respectively. The much higher decrease of the radiative rates can be attributed to the combined effect of formation of the non-fluorescent dimeric species of dyes on the surface of the AuNRs and the presence of the L-SPR field. The decrease, in the nonradiative rates is most likely to be due to the binding of the monomeric species of the dye to the PSS coated AuNR surface, which reduces the nonradiative pathways for these dyes (such as, intramolecular proton transfer for NB [188, 189]).

It is interesting to note that the magnitude of the increase in lifetime is indeed affected by the spectral position of the L-SP electric field. From Fig. 6.7 and 6.8 and data presented in Table 6.1 it can be seen that for detuned condition the magnitude of the increase in lifetime is higher compared to the tuned condition. Thus, the observed changes in the optical (absorption and fluorescence) properties of these two dyes upon binding to AuNRs depends upon various factors like, binding geometry (monomer-

dimer), changes in the excited state decay channels and presence of L-SP field of the AuNRs.

## 6.5 Conclusion

We have studied how the spectroscopic (absorption and fluorescence) properties of MB and NB are affected by the L-SP electric field of PSS coated AuNRs. The electrostatic interaction between the negatively charged AuNRs and the positively charged dyes results in significant changes in the absorption and emission properties of the dyes. For both tuned and detuned cases, there are two absorption bands in the 550-700 nm regions of the dye-AuNR complex. For the detuned condition, the resulting changes in the absorption spectra of the dye are attributed to nanorod induced aggregation. However, for the tuned condition the formation of high and low energy bands are attributed to the resonance coupling between the L-SP of the AuNR and dye absorption. This is further supported by the observation that the splitting of the absorption bands increases with increasing dye concentration and then saturates. Although, the fluorescence intensity of the dyes in the presence of increasing amount of PSS coated AuNRs were observed to be decreased, the corresponding lifetimes were observed to increase. This is explained on the basis of the formation of nonfluorescent dimeric species, suppression of the excited state nonradiative decay channels and the influence of the L-SP.