Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Influence of Graphene Oxide On the Bile Salts-Ligand Interaction: A Spectroscopy Study

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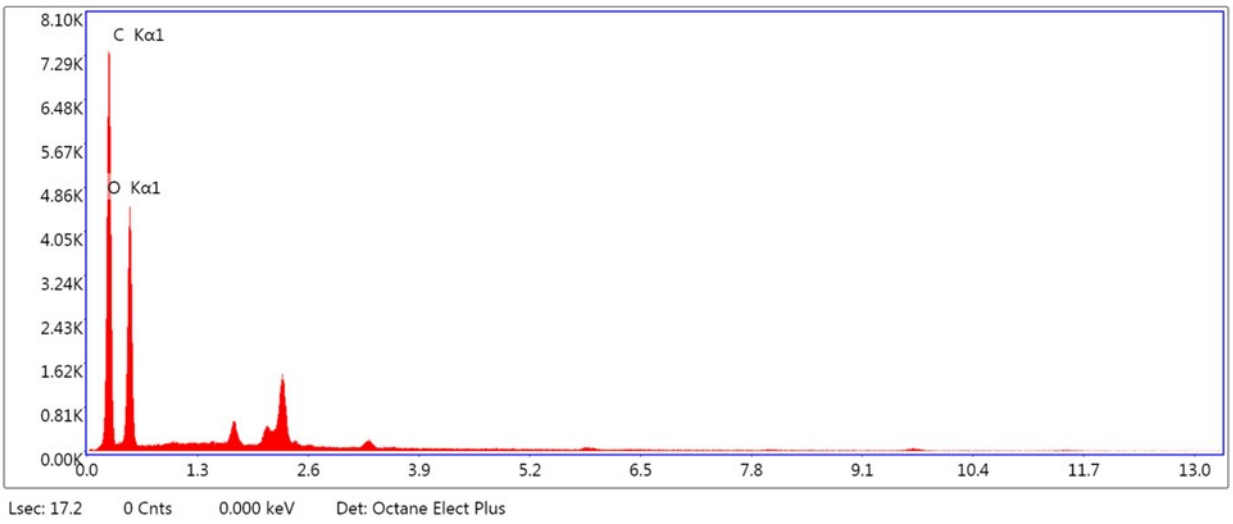
S1. Elemental composition of GO using energy dispersive X-ray (EDX) composition analysis



Element	Weight %	Atomic %	Error %
C K	54.20	61.18	5.16
O K	45.80	38.82	9.77

S2. Absorption studies of NB with bile salts (50 mM) and varying the concentration of GO:

NB shows absorption maximum in water at 635 nm as shown in fig S1 (a). For 50 mM NaDC, absorption intensity increases accompanied by a red shift of 7 nm, i.e. absorption maximum appears at 642 nm. Since NaDC forms micelles at concentration of 6 mM, it thus highlights the interaction of NaDC micelles and the monomeric form of NB. This is due to the strong electrostatic attraction between the anionic NaDC micelles and the cationic NB molecule. On initial addition of 3 μg/ml GO, absorption intensity value initially decreases. For 92 μg/ml GO, the intensity increases as well as the absorption peak appears at 650 nm. This shows that there is interaction between GO and NB-NaDC system.

On addition of 50 mM NaGC to NB in water, the absorption not only increases but the absorption maximum experiences a bathochromic shift by 7 nm which corresponds to the peak at 642 nm. This may be due to the ion-pair interaction between the anionic NaGC micelles and cationic NB dye molecules. Here too on addition of GO 7 μg/ml, the absorption intensity decreases with no change in the absorption maximum position but on final addition of 76 μg/ml GO the absorption intensity increases accompanied by generation of a new absorption maximum at 650 nm. This explains the interaction between GO and NB-NaGC system.

In case of NaTC, the absorption maximum at 635 nm of NB molecules experiences a bathochromic shift by 10 nm, i.e. peak at 645 nm is generated followed by an increase in the intensity on addition of 50 mM NaTC. This is due to the electrostatic force of attraction between cationic NB and anionic NaTC molecules which are in the micelle form. On initial addition 3 μg/ml GO, the peak position remains the same however, the intensity slightly

increases. For final addition of 61 µg/ml GO, the absorption intensity increases followed by a new absorption peak appearing at 651 nm, cementing the fact that there is interaction between GO and NB-NaTC system.

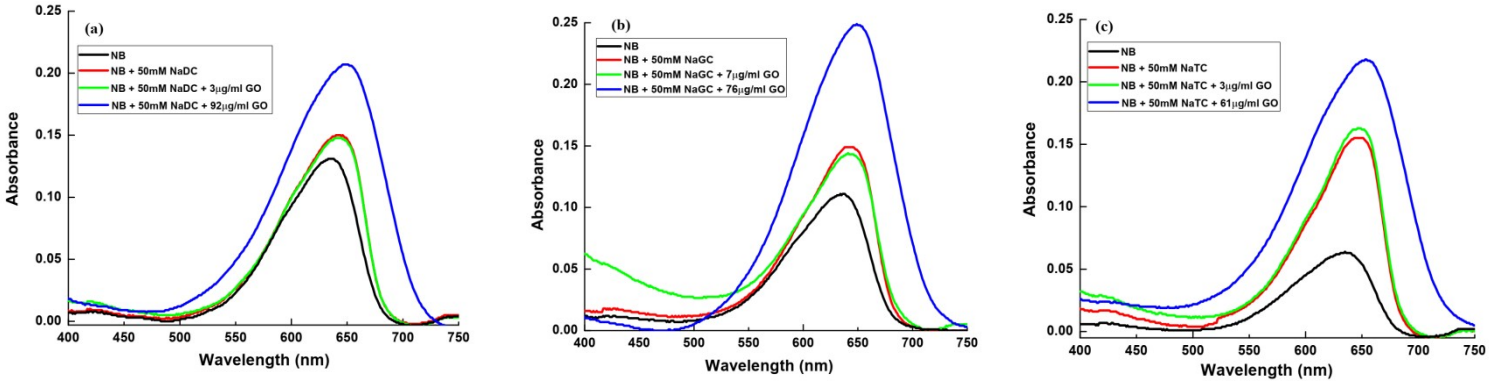


Figure S1. The absorption spectra of NB in presence of bile salt (a) NaDC (50 mM), (b) NaGC (50 mM), and (c) NaTC (50 mM) with varying the concentration of GO.

S3. Zeta potential measurements.

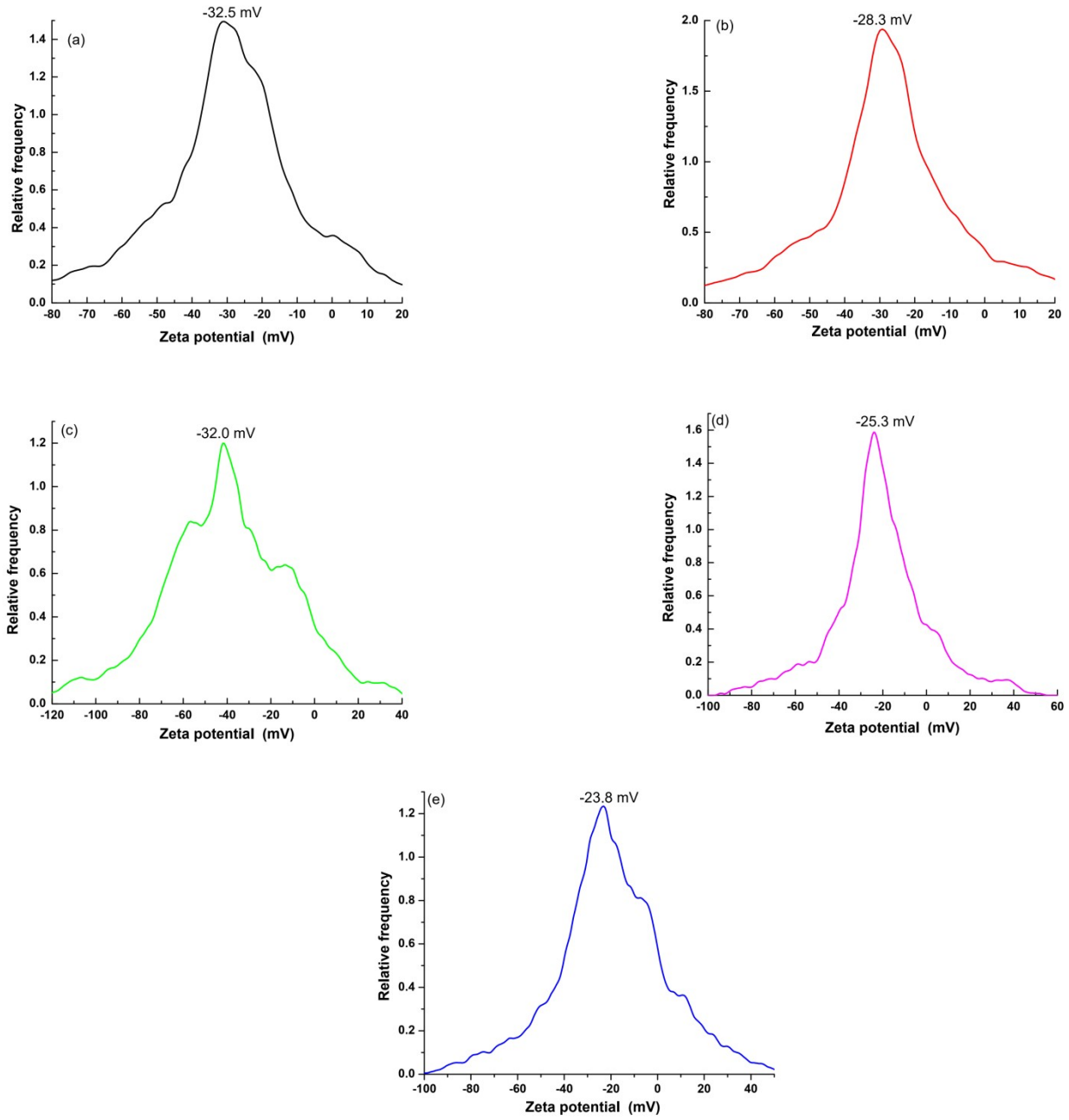


Figure S2. (a) Zeta potential measurement in the presence of 90 µg/ml GO. Zeta potential measurement in the presence of 90 µg/ml GO, NB and 50 mM bile salts (b) NaC, (c) NaDC, (d) NaGC and (e) NaTC.

S4. Absorption studies of NB with bile salts:

The interaction of NB with NaGC and NaTC is not documented in the literature, the change in the photophysical properties of NB in the presence of NaGC and NaTC must be presented to the readers in a detailed manner. In case of NaGC, when the latter is added to NB in water, initially there is slight increase in the absorption intensity with no change in the absorption maximum position. However, when 5 mM of NaGC is added, NB experiences a red shift in the absorption maximum by almost 8 nm i.e. absorption maximum appears at 643 nm. Also a shoulder band appears at 607 nm as shown in fig S3. The latter is due to the formation of H-dimers of NB which is promoted by NaGC. For the final addition of 52 mM NaGC, the peak appeared at 643 nm followed by an enhancement in intensity value. This clearly indicates that NaGC induces the formation of J-dimers of NB dye. This is due to the ion-pair interaction between the anionic bile salt and cationic dye molecule leading to interaction between the involved moieties in the system. Thus in the presence of NaGC, the monomeric, H-dimers as well the J dimers of NB coexist in the solution.

Similarly, for NaTC, on addition of 1 mM NaTC, a shoulder band appears at 607 nm which corresponds to the formation of H-dimers of NB with increase in the absorption intensity. However, on addition of 5 mM of NaTC till the final concentration of 55 mM NaTC, along with increase in the intensity value, the absorption peak of NB experiences a bathochromic shift by 8 nm generating a new absorption peak at 643 nm. This corresponds to the J-dimers of NB induced by NaTC in the medium. The electrostatic attraction is exist between the oppositely charged moieties cationic dye and anionic bile salt. Hence here too, the monomeric, H-dimers as well the J dimers of NB coexist in the solution in presence of NaTC.

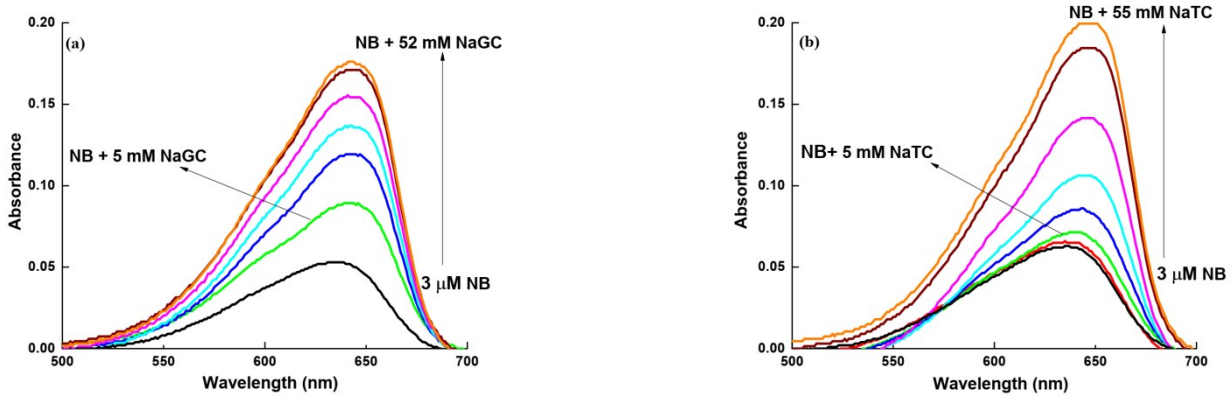


Figure S3. The absorption spectra of NB with varying the concentration of bile salts (a) NaGC and (b) NaTC.

S5. Absorption studies of NB with GO (7.5 µg/ml) and varying the concentration of bile salts:

In case of NaDC as shown in fig S4 (a), when the latter is added to NB-GO system, there is formation of both H-dimer as well as J-dimer of NB in the solution. This occurs when the concentration of NaDC is gradually increased. However, on addition of 7 mM NaDC, and above, the H-dimer disappears whereas the J-dimer is predominant in the solution. This is followed by increase in the intensity value. For 61 mM NaDC, the J-dimer is predominant in the medium. Thus it showcases an interaction between NB-GO and NaDC system. Similarly, for NaGC, the equilibrium between H-dimer and J-dimer form of NB is undisturbed till the addition of 5 mM NaGC in NB-GO system. However, on increasing the concentration of NaGC above 5 mM, i.e. till 52 mM of NaGC in the system, the J-dimer form is dominant and the H-dimer form disappears along with an increase in the absorption intensity.

Just like NaC, NaDC and NaGC, the same phenomenon was observed for NaTC. When the latter's concentration is 7 mM, H-dimer and J-dimer forms of NB is in equilibrium. On

increasing the concentration beyond 7 mM, the equilibrium is disturbed, and only the J-dimer form prevails as evident for 55 mM NaC followed by an enhancement in the absorption intensity.

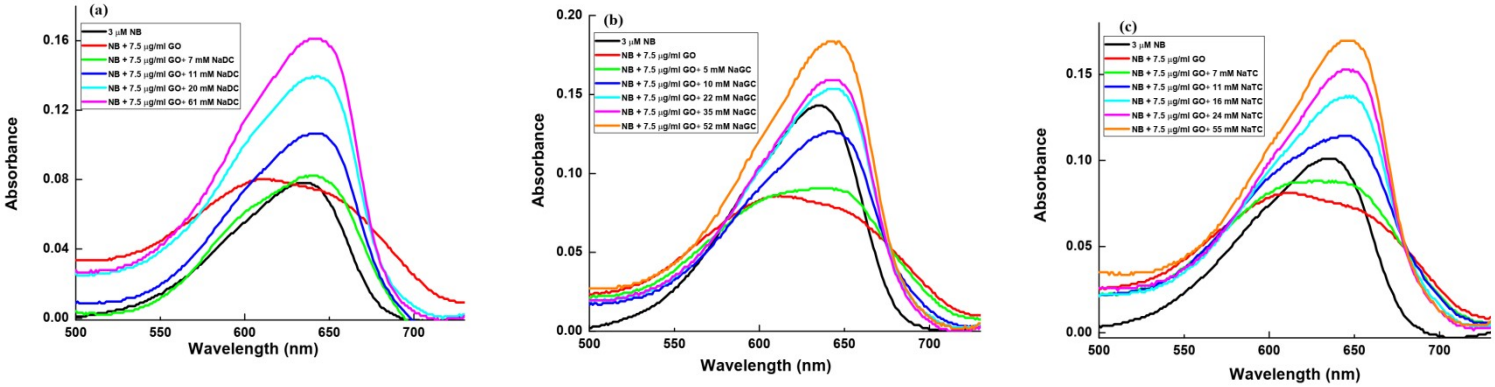


Figure S4. The absorption spectra of NB in the presence of 7.5 μg/ml GO, and varying the concentration of bile salts (a) NaDC, (b) NaGC and (c) NaTC.

S6. Emission studies of NB with bile salts (50 mM) and varying the concentration of GO:

Just like NaC, when 50 mM NaDC as shown in fig S5 (a) is added to NB in water, the latter experiences a slight blue shift by almost 5 nm, and the emission maximum is generated at 670 nm followed by a huge increase in the fluorescence intensity. This proves the complexation between NB and NaDC micelles in the system. On gradual addition of GO, till 92 μg/ml GO, the fluorescence intensity decreases completely. This justifies the interaction of GO with NB via hydrogen bonding and π–π stacking interactions.

For 50 mM NaGC, the fluorescence intensity of NB increases accompanied by a slight blue shift in the emission maximum of NB by 4 nm, and hence the emission maximum appears at 671 nm. This supports the fact that NaGC micelles do indeed interacts with the cationic NB molecules i.e. the latter experiences a hydrophobic environment provided by NaGC micelles.

The addition of GO, till the final concentration of 76 µg/ml, showcases the decrease in the fluorescence intensity of NB completely with no change in the emission maximum. This justifies the interaction of NB between GO in the presence of NaGC micelles.

Likewise, for NaTC, the trend observed here too is also the same. NB experiences a slight blue shift by 4 nm as well as enhancement in the fluorescence intensity, and thus proves the NB interacts with NaTC micelles. On gradually adding GO, here too the cationic NB experiences a steady decrease in the intensity value with no change in the emission maximum, and the intensity value completely decreases for the final addition of 61µg/ml GO. Like the other bile salts, we assume the mode of interaction of GO is due to hydrogen bonding and π–π stacking interactions.

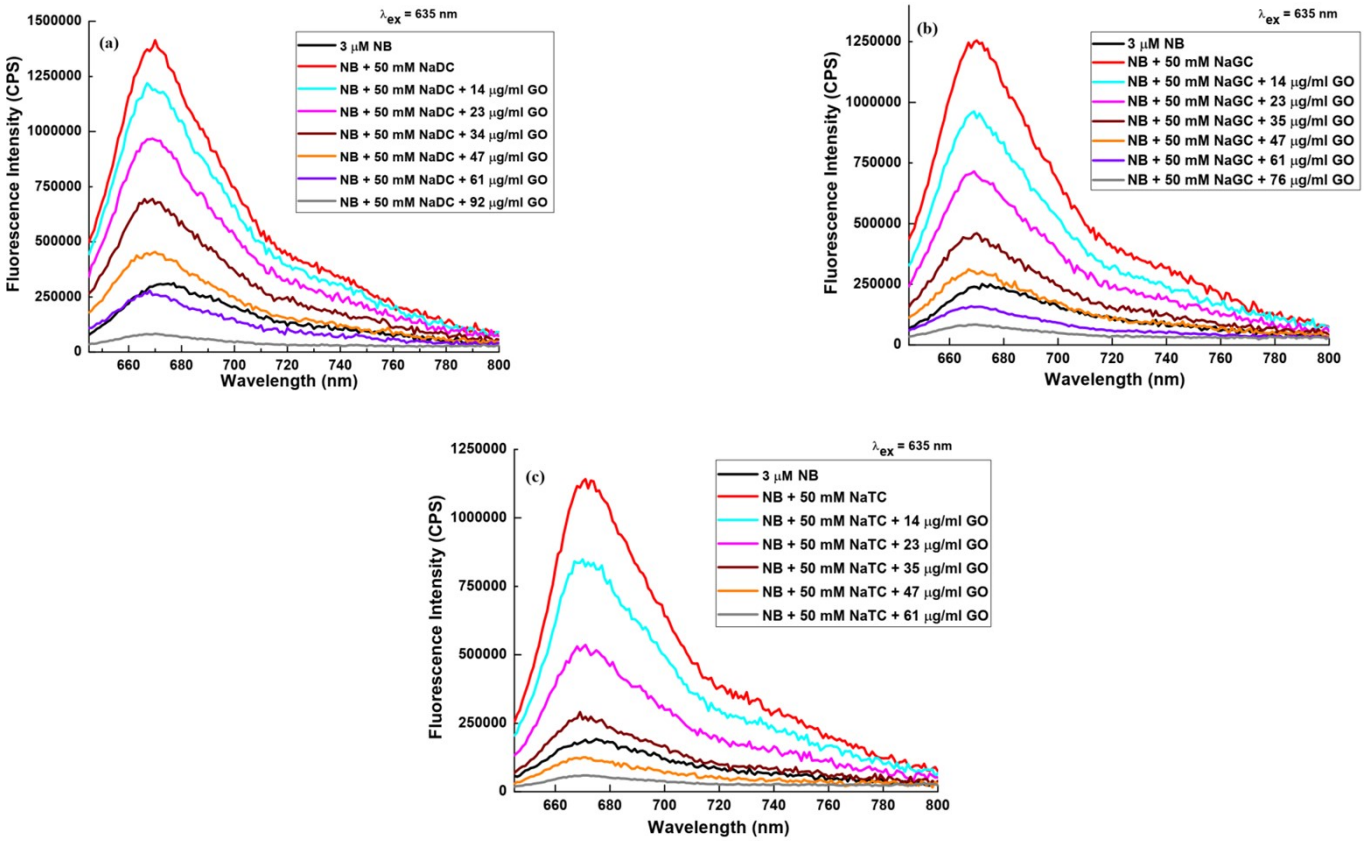


Figure S5. The emission spectra of NB in the presence of bile salts (50 mM) and varying the concentration of GO in (a) NaDC, (b) NaGC and (c) NaTC.

S7. Emission studies of NB with bile salts:

The fluorescence study of NB in the presence of NaGC and NaTC is not reported to the best of our knowledge. The emission spectra of NB by varying the concentration of NaGC shows that on adding 0.5 mM NaGC, the intensity value slightly increases with no change in the emission maximum position. However, on gradual addition of NaGC, not only does the intensity value increases but also NB experiences a blue shift by almost 5 nm i.e. emission maximum is formed at 670 nm as shown in fig S6. For 52 mM NaGC, the intensity reaches a constant value accompanied by emission maximum at 670 nm. This implies that NaGC micelle not only provides a hydrophobic microenvironment to NB but also NaGC micelles interacts with the NB molecules. Hence, as a result the rate of non-radiative transition decreases which eventually increases the intensity value.

Similarly, for NaTC, the emission spectra of NB, highlights that on adding of 5 mM NaTC, the intensity slightly increases without any change in the emission peak position. For the final addition of 55 mM NaTC, the emission maximum of NB shifts to 670 nm and the intensity value soars high which implies the fact that NaTC micelles interacts with NB molecules. Thus by providing hydrophobic microenvironment to NB, the rate of non-radiative transition decreases as result of which the intensity of NB enhances. Since in case of both NaGC and NaTC micelles a prominent hypsochromic shift is observed this clearly conveys the fact that the NaGC and NaC clearly perturb the conjugated pi system of NB i.e. the latter clearly possess a less conjugated pi system in the presence of NaGC and NaTC micelles. Excitation spectra analysis clearly reconciles with the data obtained from steady-state absorption and fluorescence measurements.

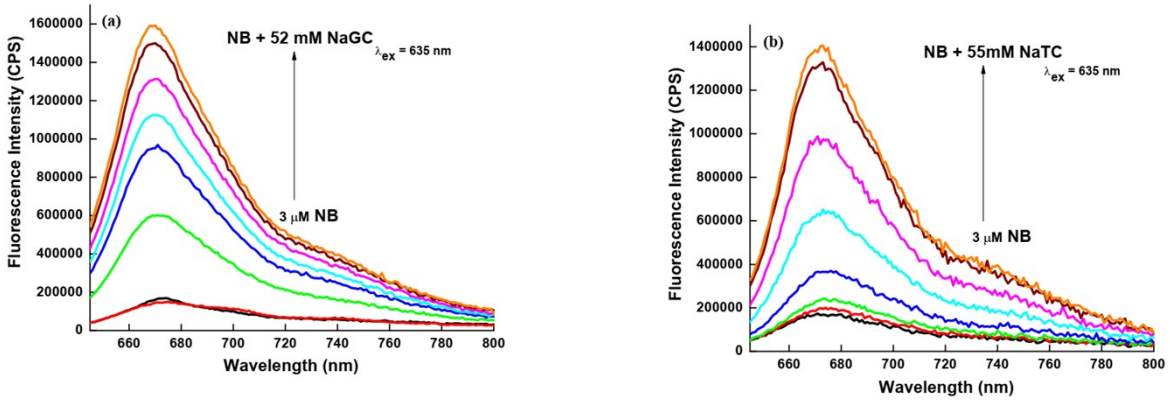


Figure S6. The emission spectra of NB in presence varying the concentration of bile salts (a) NaGC and (b) NaTC.

S8. Emission studies of NB with GO (7.5 µg/ml) and varying the concentration of bile salts:

On gradual addition of NaDC in NB-GO system as shown in fig S7 (a), on addition of 1 mM NaDC, fluorescence intensity increases. However, for 61 mM of NaDC, NB's fluorescence intensity not only enhances, but emission maximum shifts to the blue end by 7 nm i.e. the emission peak is generated at 668 nm. Thus here too, NaDC provides a strong hydrophobic environment to NB in the presence of GO as a result of which NaGC micelles interacts with NB molecules which are already adsorbed on GO surface. Hence, by decreasing the rate of non-radiative transition huge increment in the fluorescence intensity can be justified. When NaGC was added to NB-GO system just like in case of previous systems, the fluorescence intensity slightly increases. On the final addition of 52 mM NaGC, emission maximum of NB shifts to 670 nm and the intensity also drastically increases due to the decrease in the rate of non-radiative transition. Thus this reflects the strong interaction between NB molecules with NaGC micelles which in turn provides a strong hydrophobic environment to NB molecules. Similarly, for NaTC, on initial addition, slight enhancement in the intensity is observed. For 55 mM NaTC, the intensity increases due to the decrease in the rate of non-radiative transition accompanied by a blue shift in the emission maximum of NB by 5 nm. This too

demonstrates that a hydrophobic environment is provided to NB molecules due to interaction of NB molecules with NaTC micelles.

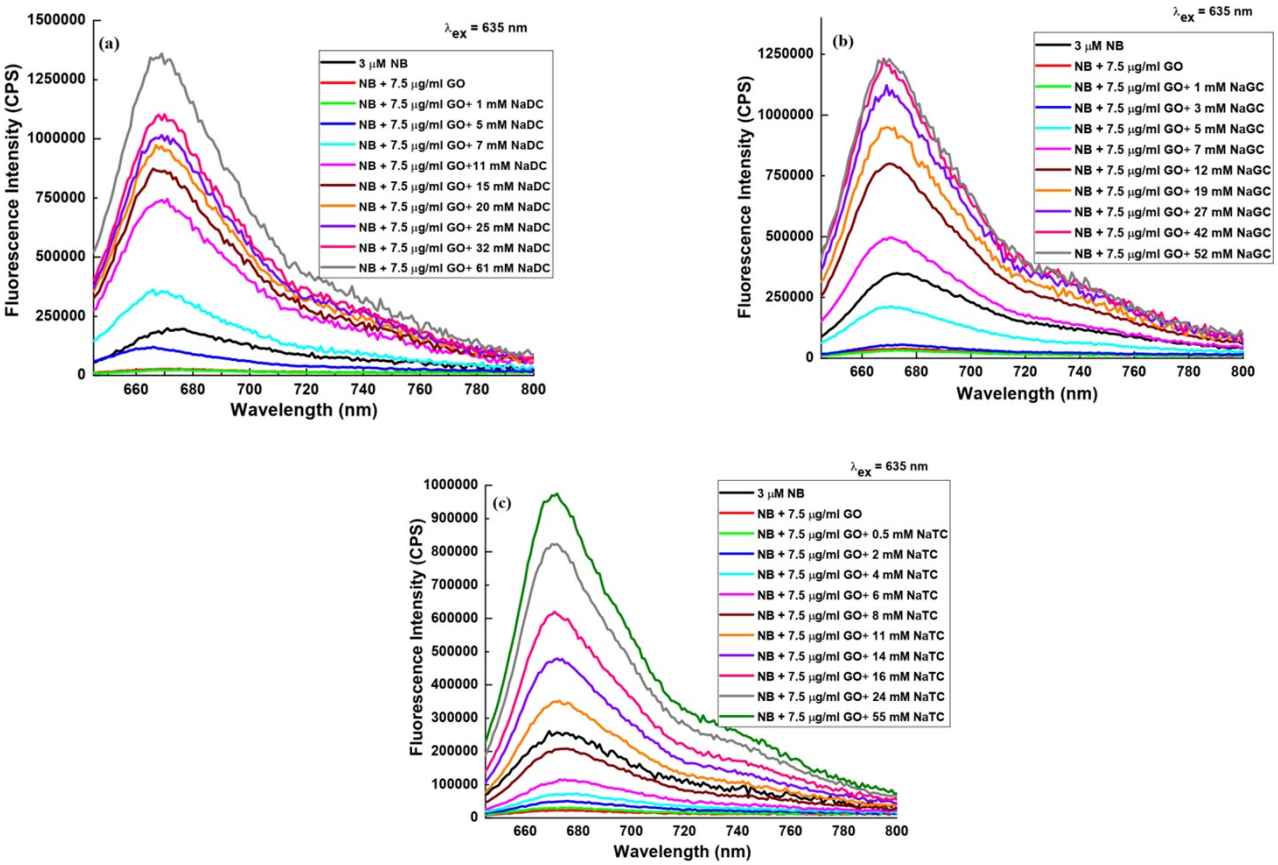


Figure S7. The emission spectra of NB in the presence of 7.5 μg/ml GO, and varying the concentration of bile salts (a) NaDC, (b) NaGC and (c) NaTC.

S9. Excitation spectral studies.

The presence of multiple emissive species of NB in the presence of GO, bile salts and GO-bile salt system were investigated by monitoring the excitation spectra. We have chosen the emission wavelength of 700 nm to collect the excitation spectra as shown in Fig S8. In water, the maximum of NB appears at 635 nm as observed in the normalized fluorescence excitation spectra. On addition of 7.5 μg/ml GO, the maximum undergoes a slight red shift by 3 nm. This implies that on addition of GO, NB exhibits its native state in water. In case of 7.5 μg/ml GO and 50 mM of respective bile salts, the maximum of NB appears at 645 nm, 640 nm, 640

nm, and 645 nm for NaC, NaDC, NaGC, and NaTC respectively. Hence this resonates with the data obtained from absorption spectra as discussed earlier. Since the maximum value of NB undergoes a red shift by 5 to 10 nm, it clearly states the fact that there is interaction between NB with GO and the respective bile salts in the medium.

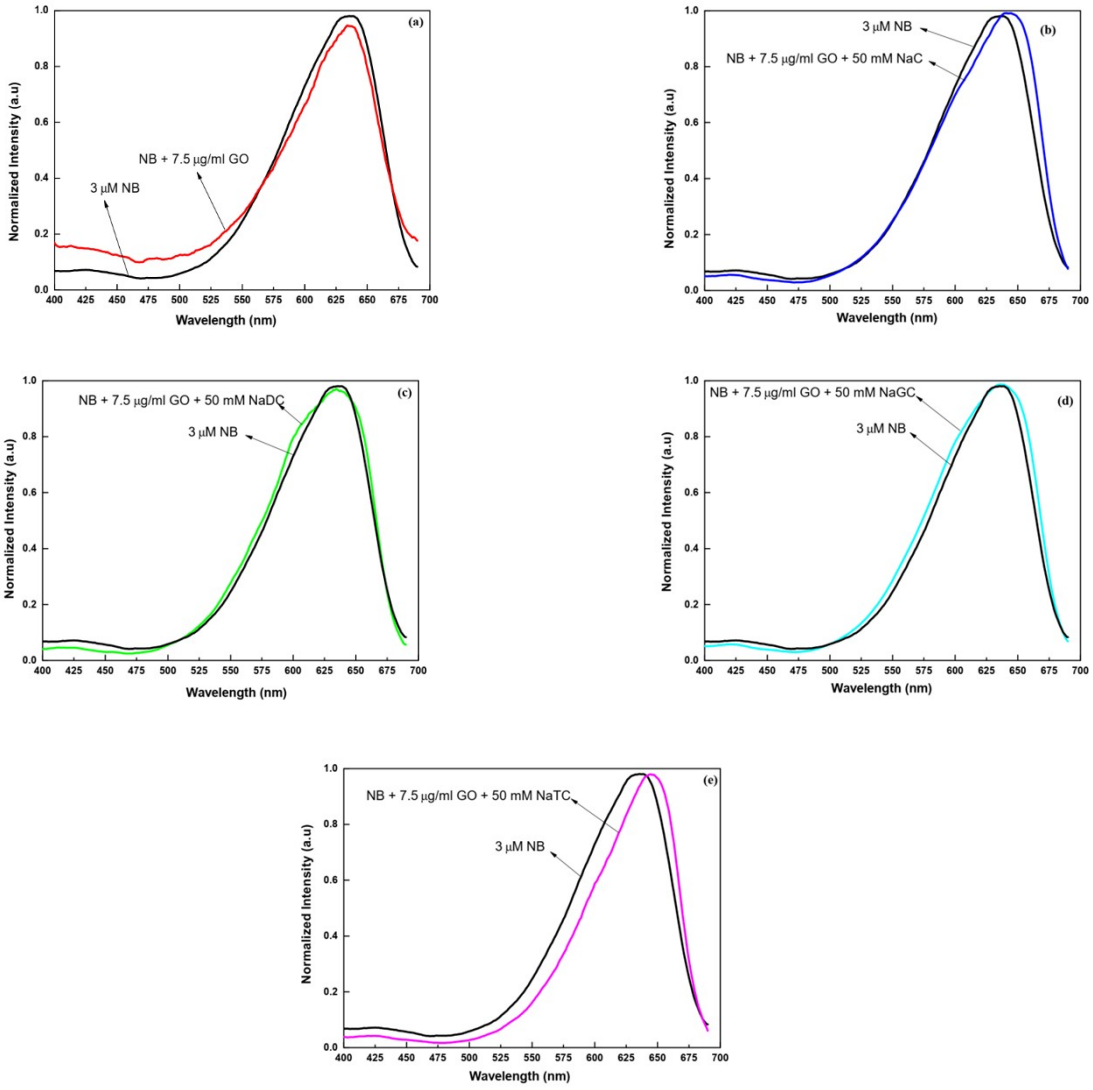


Figure S8 : The normalized fluorescence excitation spectral profile of NB in a) 7.5 µg/ml GO, b) 7.5 µg/ml GO + 50 mM NaC, c) 7.5 µg/ml GO + 50 mM NaDC, d) 7.5 µg/ml GO + 50 mM NaGC and e) 7.5 µg/ml GO + 50 mM NaTC. In all figures excitation spectra of NB in water was also shown.

In case of 50 mM bile salts and by varying the concentration of GO in the medium it was observed that the excitation spectra undergo a prominent red shift as shown in Fig S9. For 50 mM NaC and 90 µg/ml GO, the maximum appears at 640 nm, for 50 mM NaDC and 92 µg/ml GO, the maximum appears at 640 nm, for 50 mM NaGC and 76 µg/ml GO, the maximum appears at 640 nm and for 50 mM NaTC and 61 µg/ml GO, the maximum appears at 640 nm. This too cements the fact that there is interaction between NB, bile salts and GO.

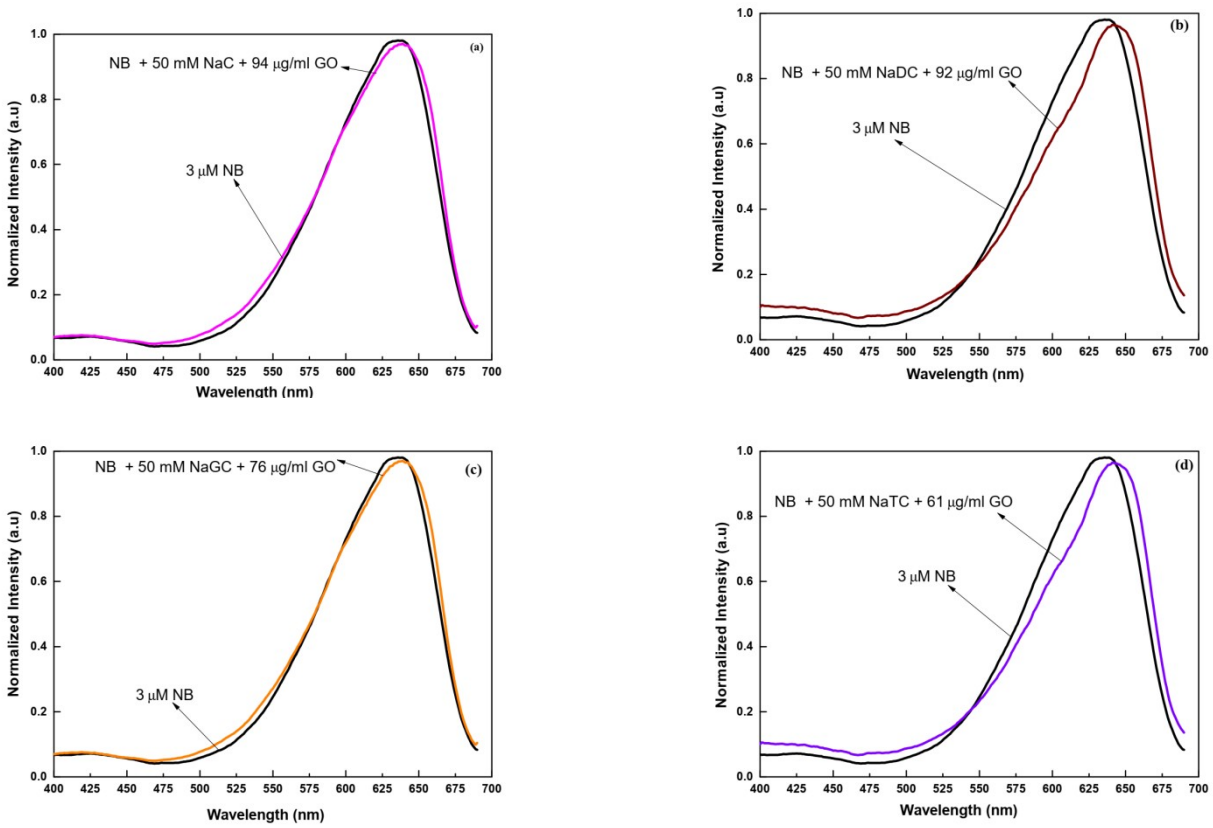


Figure S9: The normalized fluorescence excitation spectral profile of NB in a) 50 mM NaC + 94 µg/ml GO, b) 50 mM NaDC + 92 µg/ml GO, c) 50 mM NaGC + 76 µg/ml GO and d) 50 mM NaTC + 61 µg/ml GO.

In case of 50 mM bile salts, NB undergoes a red shift in its maximum value as seen from the normalized excitation spectra in Fig S10. For NaC, NaDC, NaGC and NaTC the maximum appears at 642 nm, 639 nm, 640 nm and 645 nm respectively.

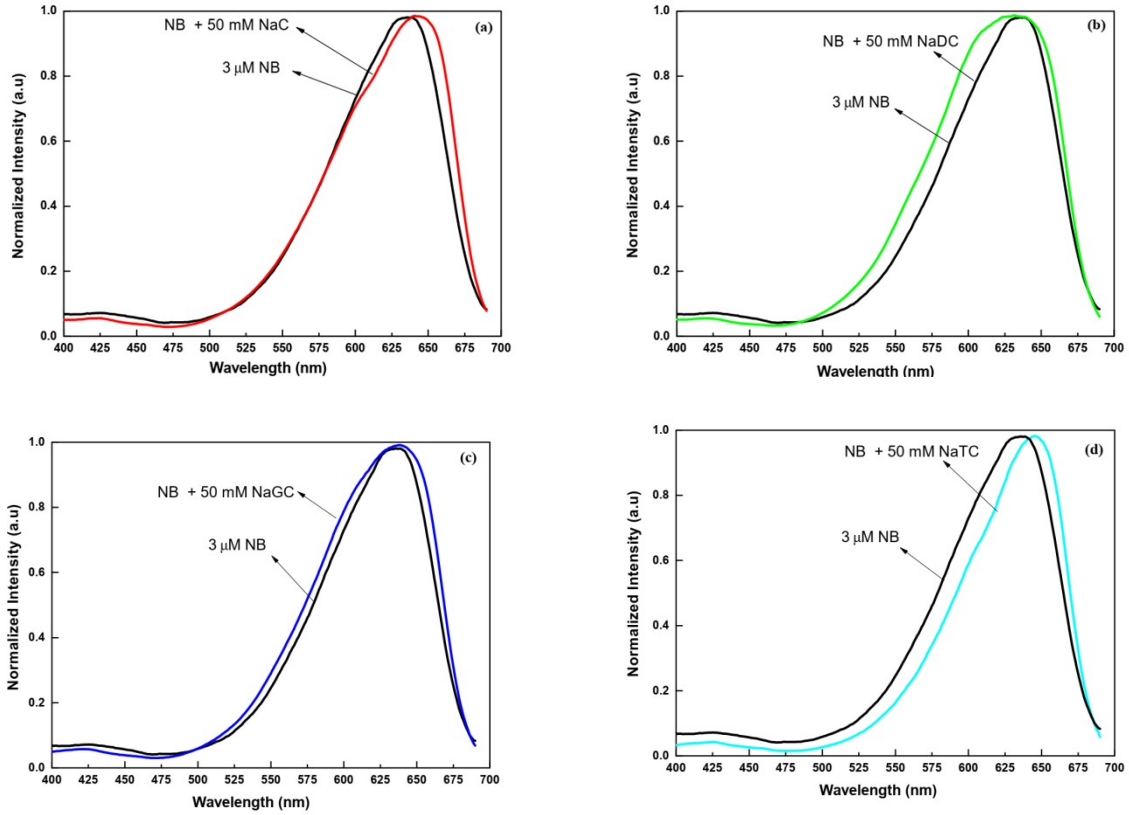


Figure S10: The normalized fluorescence excitation spectral profile of NB in a) 50 mM NaC, b) 50 mM NaDC, c) 50 mM NaGC and d) 50 mM NaTC.

S10. Time-resolved fluorescence emission studies of NB with bile salts (50 mM) and varying the concentration of GO:

For 50 mM NaDC, NB shows biexponential decay with 1.00 ns (58%) and 2.20 ns (42%) as the lifetime components. The faster component is due to the presence of NB molecules in water-micellar interface and the slower component is attributed to the hydrophobic core domain of bile salt aggregates i.e. due to the binding of NB-molecules with NaDC micelles. Average lifetime value also increases to 1.50 ns. On addition of 92 μg/ml GO, the lifetime

components almost remain same. The first component and second component are 0.98 ns (59%) and 2.23 ns (41%) respectively. The average lifetime value turns out to be 1.49 ns. For 50 mM NaGC, the lifetime components of NB are 1.02 ns (63%) and 2.15 (37%) and the average lifetime become 1.43 ns. For 76 μg/ml GO, the lifetime components experience a minimal change i.e. the first component is 0.97 ns (63%) and the second component is 2.14 ns (37%) with 1.40 ns being the average lifetime value. This too justifies the interaction between NB and NaGC micelles in the presence of GO. Finally, in case of 50 mM NaTC, the lifetime components are 0.75 ns (41%) and 1.45 ns (59%). On adding 61 μg/ml GO, the emission decay components experience a little change since the first component and second component are 0.66 ns (42%) and 1.41 ns (58%) respectively. The average lifetime value turns out to be 1.09 ns. This too signifies the fact that on addition of GO, there is interaction between NB and NaTC micelles in the presence of GO. From the obtained data as shown in Fig S11, it is clear that since NB is cationic in nature and the bile salts are anionic in nature, stable NB-bile salts aggregates was formed via strong electrostatic force of interaction.

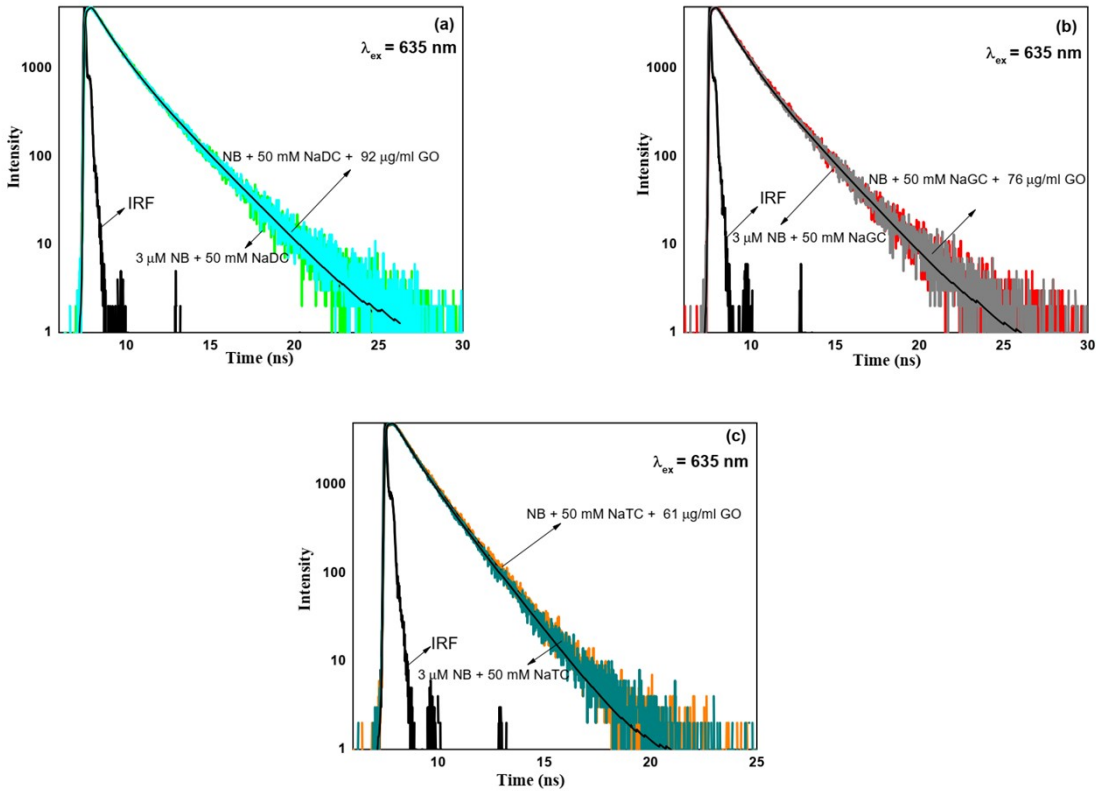


Figure S11. The time-resolved emission spectra of NB in the presence of fixed concentration of bile salts (50 mM) and varying concentration of GO a) NaDC + 92 µg/ml GO, a) NaGC + 76 µg/ml GO and a) NaTC + 61 µg/ml GO.

S11. Time-resolved emission studies of NB with GO (7.5 µg/ml) and varying the concentration of bile salts:

When 3 mM NaDC is added to NB-GO system, the emission decay is fitted triexponentially as shown in Fig S12. The 0.61 ns (32%) component corresponds to the NB molecules located in the NaDC-GO interface whereas, the longer component of 1.85 ns (15%) corresponds to the NB-NaDC complex in the presence of GO. The average lifetime value also increases to 0.52 ns. For the final addition of 61 mM NaDC, the average lifetime becomes 1.50 ns. The shorter component value becomes 1.00 ns (58%) and the longer component value is 2.21 ns (42%). This solidifies the fact that the first component is due to NB molecules in water-

micellar interface whereas, the second component is basically due to the formation of NB-NaDC micelle aggregates.

When 2 mM NaGC is added to NB in the presence of 7.5 μg/ml GO, the average lifetime value of NB slightly increases to 0.43 ns. The shorter component value is 0.37 ns (91%) and the longer component value is 1.12 ns (9%). For the final addition of 52 mM NaGC, the emission decay of NB still exhibits biexponential character. The shorter component value is 1.08 ns (66%) and the longer lifetime component value is 2.22 ns (34%). The average lifetime value also increases to 1.46 ns which thus states the fact that of NB interacts with NaGC micelles in the presence of GO. This clearly shows that increase in the lifetime value of the first component corresponds to presence of NB molecules in water-micellar interface whereas the second component is due to the NB molecules in NaC micelle aggregates in the presence of GO.

Just like the previous bile salts, on addition of 1 mM NaTC to NB in presence of 7.5 μg/ml GO, the trend observed is same. The emission decay of NB exhibits biexponential character. The lifetime value of shorter component is 0.37 ns (93%) and the longer component value is 0.95 ns (7%). The average lifetime value also slightly increases to 0.41 ns. Just like in case of the previous bile salts used the first component corresponds to presence of NB molecules in water-micellar interface and the second component is due to the interaction of NB with NaTC in presence of GO. For the final addition of 55 mM NaTC, the lifetime parameters become 0.84 ns (48%) and 1.50 ns (52%). The average lifetime value increases to 1.18 ns. This explains that NB does interacts with the NaTC micelle aggregates in the presence of GO.

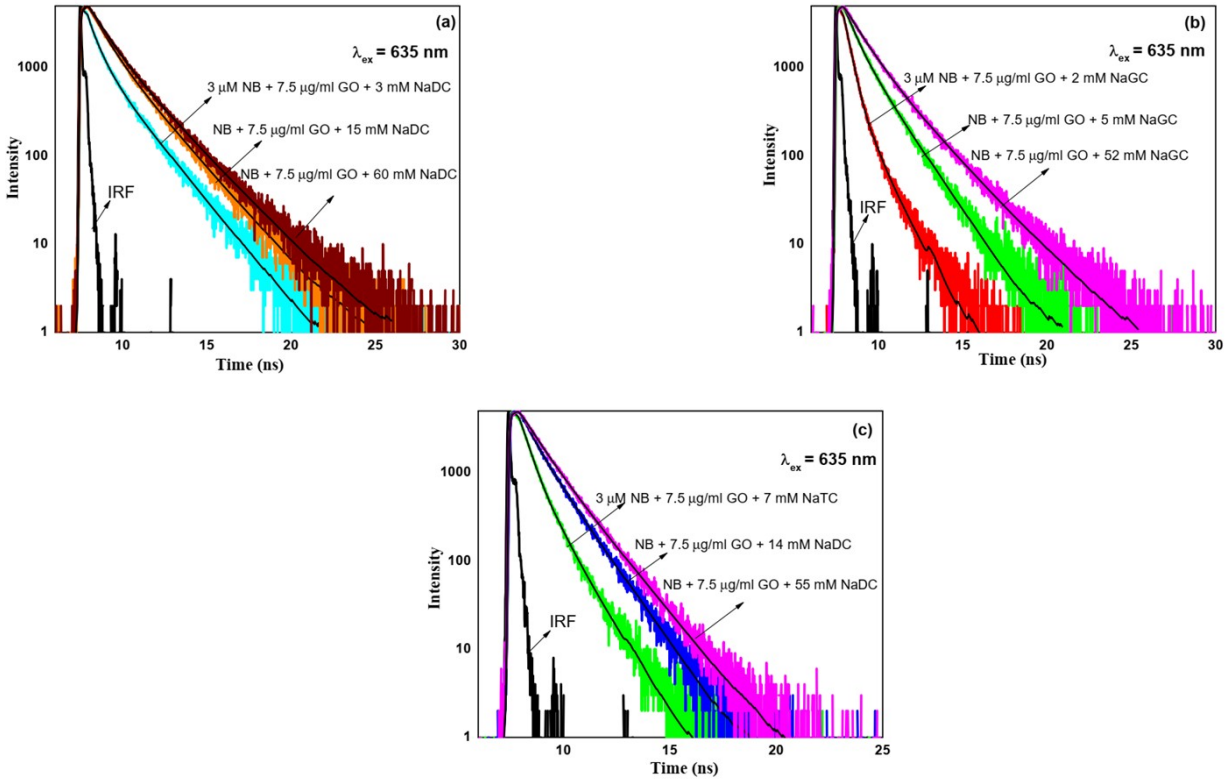


Figure S12. The time-resolved emission spectra of NB in the presence of fixed concentration of GO and varying concentration of bile salts a) 7.5 μg/ml GO + NaDC, b) 7.5 μg/ml GO + NaGC and c) 7.5 μg/ml GO + NaTC.

S12. Time-resolved emission studies of NB with bile salts:

In case of NaDC as shown in Fig S13, on addition of 3 mM NaDC, the emission decay of NB is fitted triexponentially and the lifetime components are 0.10 ns (45%), 0.48 ns (47%) and 1.41 ns (8%). The average lifetime value turns out to be 0.38 ns. For 62 mM NaDC, the lifetime components become 1.03 ns (59%) and 2.23 ns (41%) and the average lifetime value is 1.52 ns. The former component corresponds to presence of NB molecules in water-micellar interface whereas the latter component corresponds to the NB molecules in presence of NaTC micelle aggregates as a result of which the average lifetime value increases.

Similarly, for NaGC and NaTC bile salts, on their gradual addition to NB, the emission decay of the former exhibits biexponential character as shown in table S1. For addition of 2 mM of NaGC and NaTC, the average lifetime value hardly changes as shown in table S1. For the final addition of 52 mM NaGC and 55 mM NaTC, the lifetime value of NB increases to 1.44 ns and 1.14 ns respectively. The decay is fitted biexponentially, and the first shorter lifetime component corresponds to the presence of NB molecules in water-micellar interface, whereas the second component corresponds to the NB molecules incorporated into the NaGC and NaTC micelle aggregates in their respective system, and hence the average lifetime value increases.

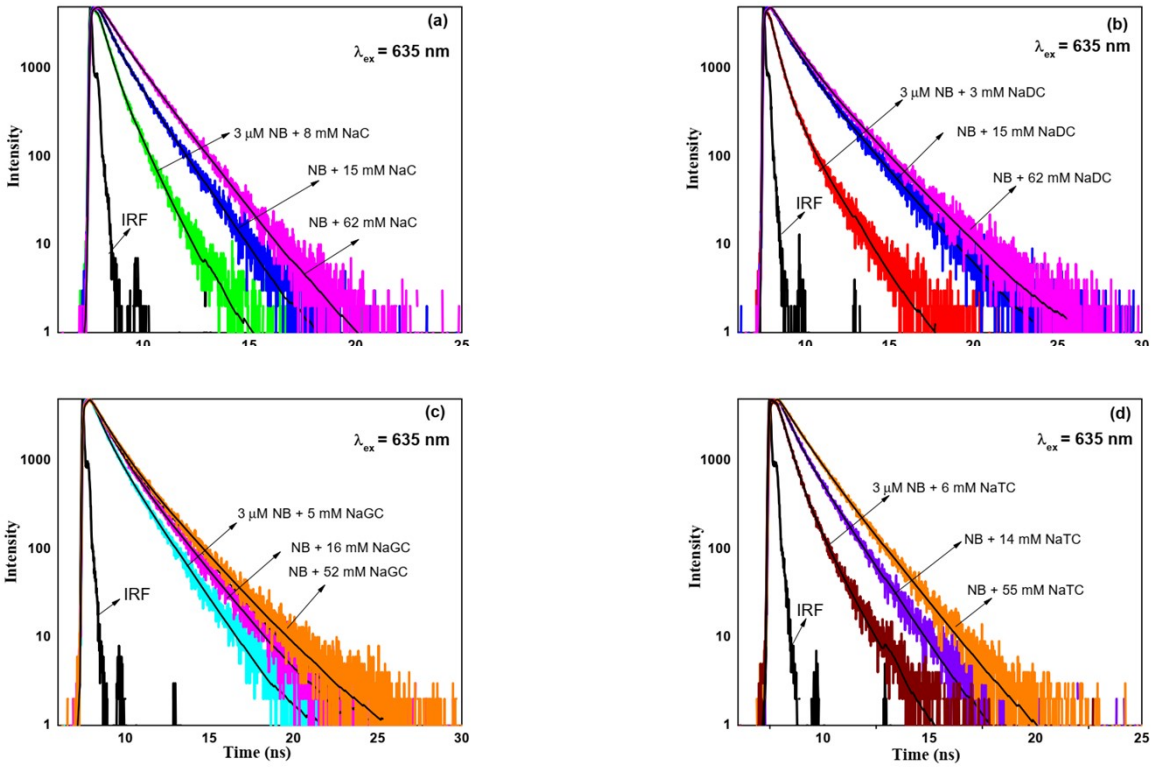


Figure S13. The time-resolved emission spectra of NB in the presence of varying concentration of bile salts a) NaC, b) NaDC, c) NaGC and d) NaTC.

Table S1: Fluorescence lifetime values of NB in the presence of GO and bile salts aggregates.

S. No	Sample	τ_1 (ns)	a_1	τ_2 (ns)	a_2	τ_3 (ns)	a_3	$\langle\tau\rangle^{\#}$ (ns)	$\chi 2$
1.	NB	0.36	100%	-	-	-	-	0.36	1.071
2.	NB+50 mM NaC + 94 µg/ml GO	0.66	38%	1.42	62%	-	-	1.13	1.095
3.	NB+50 mM NaDC + 92 µg/ml GO	0.98	59%	2.23	41%	-	-	1.49	1.103
4.	NB+50 mM NaGC + 76 µg/ml GO	0.97	63%	2.14	37%	-	-	1.40	1.135
5.	NB+50 mM NaTC + 61 µg/ml GO	0.66	42%	1.41	58%	-	-	1.09	1.010
6.	NB +7.5 µg/ml GO + 3 mM NaC	0.36	86%	0.74	14%	-	-	0.41	1.195
7.	NB +7.5 µg/ml GO + 61 mM NaC	0.73	36%	1.42	64%	-	-	1.17	1.097
8.	NB +7.5 µg/ml GO + 3 mM NaDC	0.10	53%	0.61	32%	1.85	15%	0.52	1.131
9.	NB +7.5 µg/ml GO + 61mM NaDC	1.00	58%	2.21	42%	-	-	1.50	1.166
10.	NB +7.5 µg/ml l GO + 2 mM NaGC	0.37	91%	1.12	9%	-	-	0.43	1.017
11.	NB +7.5 µg/ml l GO + 52 mM NaGC	1.08	66%	2.22	34%	-	-	1.46	1.036
12.	NB +7.5 µg/ml GO + 1 mM NaTC	0.37	93%	0.95	7%	-	-	0.41	0.944
13.	NB +7.5 µg/ml GO + 55 mM NaTC	0.84	48%	1.50	52%	-	-	1.18	0.939
14.	NB+3 mM NaC	0.27	48%	0.51	52%	-	-	0.39	0.947
15.	NB+ 62 mM NaC	0.67	34%	1.40	66%	-	-	1.15	1.072
16.	NB+ 3 mM NaDC	0.10	45%	0.48	47%	1.41	8%	0.38	0.963
17.	NB+ 62 mM NaDC	1.03	59%	2.23	41%	-	-	1.52	1.083
18.	NB+ 2 mM NaGC	0.30	72%	0.62	28%	-	-	0.39	1.075
19.	NB+ 52 mM NaGC	0.99	61%	2.15	39%	-	-	1.44	1.038
20.	NB+ 2 mM NaTC	0.30	51%	0.47	49%	-	-	0.38	0.919
21.	NB+ 55 mM NaTC	0.77	45%	1.45	55%	-	-	1.14	0.966
22.	NB +50 mM NaC	0.77	38%	1.44	62%	-	-	1.18	0.944
23.	NB +50 mM NaDC	1.00	58%	2.20	42%	-	-	1.50	1.083
24.	NB +50 mM NaGC	1.02	63%	2.15	37%	-	-	1.43	1.015
25.	NB +50 mM NaTC	0.75	41%	1.45	59%	-	-	1.16	1.013
26.	NB+7.5 µg/ml GO	0.12	13%	0.37	86%	1.11	1%	0.34	1.195

$^{\#}\langle\tau\rangle = a_1*\tau_1 + a_2*\tau_2 + a_3*\tau_3$

Table S2: Rotational relaxation time of NB in the presence of GO and bile salts aggregates.

S. No	Sample	r_0	τ_{rot} (ns)	$\chi 2$
1.	NB + 7.5 µg/ml GO	0.30	0.24	1.156
2.	NB + 7.5 µg/ml GO + 61 mM NaC	0.35	1.44	0.908
3.	NB + 7.5 µg/ml GO + 61 mM NaDC	0.35	2.36	0.916
4.	NB + 7.5 µg/ml GO + 52 mM NaGC	0.35	2.64	1.089
5.	NB + 7.5 µg/ml GO + 55 mM NaTC	0.35	1.65	0.900
6.	NB + 50 mM NaC	0.38	1.31	0.921
7.	NB + 50 mM NaDC	0.38	2.28	0.984
8.	NB + 50 mM NaGC	0.38	2.97	0.900
9.	NB + 50 mM NaTC	0.38	1.66	0.903
10.	NB + 61 mM NaC	0.35	1.41	0.944
11.	NB + 61 mM NaDC	0.35	2.47	0.909
12.	NB + 52 mM NaGC	0.35	2.71	0.920
13.	NB + 55 mM NaTC	0.35	1.68	0.900