Sensitization of an Endogenous Photosensitizer: Electronic Spectroscopy of Riboflavin in the Proximity of Semiconductor, Insulator, and Metal Nanoparticles

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ABSTRACT: Riboflavin (Rf) is a class of important vitamins (Vitamin B2) and a well-known antioxidant. Here we have synthesized nanohybrids of Rf with a number of inorganic nanoparticles (NPs); namely zinc oxide (ZnO), titanium oxide (TiO2), aluminum oxide (Al2O3) and gold NPs of similar sizes. While high resolution transmission electron microscopy (HRTEM) confirms integrity and sizes of the NPs, intactness of the molecular structure of the drug Rf is revealed from absorption and steady-state emission spectra of the drug in the nanohybrid. Raman spectroscopy on the nanohybrids shows the nature of molecular complexation of the drug with the inorganic NPs. For the semiconductor and insulator NPs, the complexation is found to be noncovalent, however, a covalent attachment of the drug with the dangling bonds of metal atoms at the surface is observed. In order to investigate antioxidant activity of the nanohybrids, we have performed 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay of the nanohybrids in dark as well as under blue light irradiation. Whereas change of the antioxidant activity of the nanohybrids with respect to free riboflavin in the absence of light is observed to be insignificant, a drastic change in the activity in the case of TiO2 and ZnO in the presence of light is evident. No change in the case of Al2O3 and a significant decrease in the antioxidant activity for gold nanohybrids are also remarkable. Picosecond-resolved fluorescence studies on the nanohybrids reveal a molecular picture of the differential antioxidant activities. An ultrafast photoinduced electron transfer from Rf to ZnO and TiO2 are clearly evident from the corresponding fluorescence transients. We have compared the picosecond-resolved transients with that of Rf in the presence of a well-known electron acceptor benzoquinone (BQ) and found similar time scales. No temporal change in the fluorescence transient of riboflavin in Al2O3 nanohybrids compared to that of free Rf is observed indicating uneventful excited state relaxation of the nanohybrids. Nanosurface energy transfer (NSET) over Förster resonance energy transfer (FRET) is found to be the prevailing de-excitation mechanism in the case of gold nanohybrids, because of the strong spectral overlap between Rf emission and surface plasmon absorption of the gold NPs. Different excited state mechanisms as revealed from our studies are expected to be useful for the design of NP-sensitized drugs, which are reported sparsely in the literature.

1. INTRODUCTION

Riboflavin (Rf) or vitamin B2 is an essential micronutrient and substantially present in dietary products such as vegetables, mushrooms, yeast, milk, cheese, eggs, and meat.1–3 Although remarkable medicinal use of the vitamin as a food supplement followed immediately after the Nobel-winning (in chemistry, 1938) structural determination of the micronutrient, alternative use of the vitamin as a potential photodynamic therapy (PDT) agent is also evident in early4 as well as in contemporary5 literature. Upon light irradiation of a specific wavelength, a photosensitizer absorbs photon and elevated from ground state to an excited singlet state which in due course decays to the triplet excited state via intersystem crossing (ISC). Then the triplet excited state energy is transferred to molecular oxygen at ground state to produce singlet oxygen, which is a central intermediate for cytotoxic action in PDT. It is well established that Rf upon visible light irradiation generates reactive oxygen species (ROS) such as singlet oxygen and superoxide anion.6,7 Riboflavin photosensitization has been used as a therapy for the treatment of keratosis, where the vitamin is an endogenous photosensitizer.8,9

In the past two decades, nanoparticle- (NP-) based therapeutic products have flourished for the enhancement of drug action.10–13 Nanomaterials are attributed with distinguished physicochemical properties, such as very small size, greater surface area to mass ratio, and high reactivity, which is
different from that of bulk materials of similar composition.11 Nanomaterials as drug carriers can overcome some of the constraints such as low solubility and diffusivity, shortened blood circulation half-life, high immunogenicity and low bioavailability found in conventional therapeutic and diagnostic agents.11,14 Because of a higher surface area to volume ratio, nanocarriers show upgraded pharmacokinetics and bio distribution of therapeutic agents and reduce toxicity by their accumulation at the target site.15 The solubility of hydrophobic drugs increases by the formation of nanoconjugates and render them convenient for parenteral administration. Moreover, the stability of various therapeutic agents such as peptides and oligonucleotides increases which can be utilized to conduct the drug to central nervous system due to their limited size and greater barrier permeability.15,16 However, reports on sensitization of drugs by NPs through their electronic coupling are sparse in the literature. On the other hand, formation of nanohybrid of a drug with an inorganic nanoparticle is the key for the modulation of drug activity in the nanohybrid. In one of our earlier reports, we have explored the photoinduced ultrafast dynamics in a well-known cancer drug, protoporphyrin IX (PP) compared to that of only PP. Zinc oxide NPs act as drug carriers of approximately 30 nm sizes. We confirmed the sizes of the NPs using high resolution transmission electron microscopy (HRTEM). Raman spectroscopic studies provide an insight on the nature of the attachment of riboflavin to the surfaces of the semiconductor (TiO2 and ZnO), the insulator (Al2O3) NPs and metal (gold NPs). In order to measure the photoantioxidant activity of the nanohybrids, we have performed the well-known 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay under blue light irradiation (λmax = 450 nm). Time-resolved fluorescence studies on the nanohybrids were carried out to understand the electron transfer from photoexcited Rf to ZnO or TiO2, which eventually intensifies the fluorescence.22

In the present study, we have synthesized nanohybrids of vitamin B2 with various inorganic NPs such as zinc oxide (ZnO), titanium oxide (TiO2), aluminum oxide (Al2O3), and gold NPs of approximately 30 nm sizes. We confirmed the sizes of the NPs using HRTEM. Raman spectroscopic studies provide an insight on the nature of the attachment of riboflavin to the surfaces of the semiconductor (TiO2 and ZnO), the insulator (Al2O3) NPs and metal (gold NPs). In order to measure the photoantioxidant activity of the nanohybrids, we have performed the well-known 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay under blue light irradiation (λmax = 450 nm). Time-resolved fluorescence studies on the nanohybrids were carried out to understand the electron transfer from photoexcited Rf to ZnO or TiO2, which eventually intensifies the ROS activity in the Rf–semiconductor nanostructures. The phenomenon of nanoscale surface energy transfer (NSET) is predominant in the nanohybrids of riboflavin with gold. Our studies unravel a mechanistic pathway of drug sensitization with various inorganic NPs to determine the photoinduced antioxidant property of this important vitamin.

2. MATERIAL AND METHODS

Chemicals used for synthesis in this study are of analytical grade and without further purification. Approximately 30 nm ZnO NPs, TiO2 NPs, Al2O3 NPs, riboflavin, gold (III) chloride, and sodium citrate were purchased from Sigma-Aldrich. The suitable solvent for p-benzoquinone (BQ, Fluka) was ethanol (Merck). Millipore water was used as aqueous solution.

2.1. Synthesis of Riboflavin Nanohybrids with ZnO NPs, TiO2 NPs, and Al2O3 NPs. A 20 μM Rf solution was prepared in alcohol. Then 0.5 mg/mL of solid 30 nm ZnO, TiO2, and Al2O3 were added to prepared Rf solution and were stirred constantly for 12 h. The sensitization of ZnO NPs, TiO2 NPs, and Al2O3 NPs with Rf dye was performed at room temperature in the dark. After the sensitization process, the solution was centrifuged for a while and the clear supernatant solution of unattached dyes was decanted. Then the nanohybrids were washed with alcohol 3–4 times. Then the samples were dried in a water bath. The Rf concentration in the nanohybrids was 3 μM.

2.2. Synthesis of Riboflavin–Gold Nanohybrids. A 10 mL 80 μM Rf solution was prepared in water. Then 100 mM stock solution of AuCl3 was prepared, and 50 μL of Au (III) chloride solution was mixed with 10 mL of the stock riboflavin solution. The mixture was kept under UV-light (λmax = 365 nm) for 15 min until the characteristic surface plasmon resonance band was observed at 540 nm.23 Here, a different technique is used to remove free riboflavin from Rf–AuNPs. Since it is difficult to retrieve Rf–AuNPs after centrifugation, the excess dye was driven out by dialysis prior to the experiments. The Rf and AuNPs concentrations in the nanohybrids were 40 μM and 0.1 nM, respectively.

2.3. Optical Studies. Shimadzu UV-2600 spectrophotometer was used to measure absorption spectra using a quartz cell of 1 cm path length. The extinction coefficient values of 12 200 M−1 cm−1 at 450 nm for riboflavin (Rf)20 and 2.93 × 104 for citrate-capped AuNPs22 were used to calculate the concentration of the samples. The extinction coefficient of riboflavin and its nanoconjugates were measured to be same and thus the similar value was used for calculation purpose. The degradation kinetics under blue light was measured with spectroscopic precision using an indigenously developed fiber-optic based system which is generally used for measuring sensitive optical measurements.22 DPPH has a characteristic absorbance maximum at 520 nm. This characteristic absorption peak was chosen to monitor the DPPH degradation under blue light irradiation at room temperature. Spectra suite software from Ocean Optics detected the absorbance of DPPH at 30 s intervals and recorded them. Finally, the DPPH absorbance at 520 nm was plotted as a function of time. The experiments on steady-state fluorescence measurements were performed using JobinYvon Fluorolog fluorometer. The emission of Rf along with its conjugates were taken upon excitation at the wavelength of 409 nm. The reference are OD and ODR; and the refractive indices of Rf and DPPH concentrations were 0.1 μM and 10 μM respectively. The fluorescence measurements were performed with the excitation and emission bandwidth slits of 2 nm. The equation used to calculate the quantum yield is $Q = \left( \frac{Q_R}{I_R} \right) \left( \frac{OD_R}{OD} \right) \left( \frac{n^2}{n_R^2} \right)$, where the quantum yields of the reference and the Rf are Q_R and Q, the integrated fluorescence intensities of Rf and the reference are I_R and I, the excitation wavelength, the absorbance of Rf and the reference are OD and OD_R, and the refractive indices of Rf and the reference solutions are n and n_R.23 The reported absolute quantum yield of Rf in ethanol is 0.3.24 The Raman measurements were performed in a micro Raman set up which consists of spectrometer (Lab RAM HR, Jovin Yvon) with a Peltier-cooled CCD detector. A He–Ne laser with wavelength 633 nm, objective 10X, integration time 20 s and a power of 6 mW was used as excitation. A time correlated single photon counting (TCSPC) from Edinburgh Instruments was used in performing the time-resolved fluorescence spectroscopic experiments. A picosecond pulsed laser diode having instrument
response function (IRF) of 80 ps was used for excitation of the sample at 409 nm. The emission was monitored through a polarizer oriented at 55° from the vertical position and the excitation was vertically polarized. The multiexponential (n) function, \( \sum A_i \exp(-t/\tau_i) \) is used to fit the fluorescence transients, where, \( A_i \)'s are weighted percentages with time constants of \( \tau_i \). The equation \( \tau = \sum A_i \tau_i \) when \( \sum A_i = 1 \) expresses the average lifetime at excited state.

### 2.4. Förster’s Resonance Energy Transfer (FRET) Technique

The technique is discussed in our previous studies.\(^{3,17} \) In brief, the donor–acceptor distances were derived using the equation, \( R_0 = 0.211 \times \left[ \kappa n^2 \Phi_{D}\eta(\lambda) \right]^{1/6} \) in Å. \( R_0 \) is assigned as the donor–acceptor distance at 50% energy transfer efficiency, \( \kappa^2 \) is the relative orientation of the donor–acceptor transition dipoles in space. The magnitude of \( \kappa^2 \) is 0.66 for random orientation of donor–acceptor. The refractive index \( n \) of the medium is 1.33. \( J(\lambda) \) is the spectral overlap between the donor emission and the acceptor absorption. It is denoted by,

\[
J(\lambda) = \int_0^\infty \frac{F_D(\lambda) \varepsilon_A(\lambda) \lambda^4}{\int_0^\infty F_D(\lambda) \lambda^4} d\lambda
\]

(1)

where, \( F_D(\lambda) \) is the donor fluorescence intensity in the wavelength range of \( \lambda \) to \( \lambda + d\lambda \) and is dimensionless. \( \varepsilon_A(\lambda) \) is the acceptor extinction coefficient (in \( M^{-1} \text{ cm}^{-1} \)) at \( \lambda \). After knowing the \( R_0 \) value, the \( \tau \) is determined from the equation

\[
r^6 = \left[ R_0^6 \varepsilon(1 - E) \right]/E
\]

(2)

where, \( E \) is the efficiency of energy transfer, which can be deduced as

\[
E = 1 - \frac{\tau_{DA}}{\tau_D}
\]

(3)

where, \( \tau_{DA} \) and \( \tau_D \) are donor lifetimes with and without acceptor.\(^{27} \)

### 2.5. Nanosurface Energy Transfer (NSET) Technique

The technique is explained in our previous studies.\(^{25} \) This technique is based on the model of Persson and Lang.\(^{26} \) The distances of donor–acceptor pair were calculated using the following equations.

\[
k_{21} = 0.3 \left( \frac{\mu^2 \omega_{D} \epsilon_{D}}{\eta \omega_{D} \kappa \epsilon_{D} d^4} \right)
\]

(4)

which can be expressed in more measurable parameters through the use of the Einstein \( A_{21} \) coefficient.\(^{27} \)

\[
A_{21} = \frac{a_{D} \epsilon_{D}^3}{3 \epsilon_{D} \eta \pi \epsilon_{D} d^4 \mu^2}
\]

(5)

The rate of energy transfer in accordance with Coulomb’s law ((1/4)\( \pi \varepsilon_0 \)) is expressed as

\[
k_{21} = 0.225 \frac{c^3 \varepsilon_{D}^{\omega_{D}}}{a_{D}^2 \omega_{D} \kappa \epsilon_{D} d^4 \mu_{D}^{\omega_{D}}}
\]

(6)

where \( c \) denotes the speed of light and the quantum yield \( (\Phi_{D}) \) of the donor is 0.26. The angular frequency \( (\omega_{D}) \) for the donor is \( 4.1 \times 10^{15} \text{ s}^{-1} \) and the angular frequency \( (\omega_{D}) \) for bulk gold is \( 8.4 \times 10^{15} \text{ s}^{-1} \). \( d \) is the donor–acceptor separation. The average lifetime of the dye (\( \tau_{D} \)) is 4760 (ps), and the Fermi wavevector (\( k_f \)) for bulk gold is \( 1.2 \times 10^8 \text{ cm}^{-1} \). The \( d_0 \) value is the distance at which a dye exhibits equal probabilities for energy transfer and spontaneous emission. The \( d_0 \) value is given by,

\[
d_0 = \left( 0.225 \frac{c^3 \varepsilon_{D}^{\omega_{D}}}{a_{D}^2 \omega_{D} \kappa \epsilon_{D} d^4 \mu_{D}^{\omega_{D}}} \right)^{1/4}
\]

(7)

Here, we have used \( k_{\text{time-resolved}} \) as \( k_{\text{time-resolved}} = \frac{1}{\tau_{\text{donor–acceptor}}} - \frac{1}{\tau_{D}} \)

(8)

where \( \tau_{\text{donor–acceptor}} \) depicts the average donor–acceptor lifetime.

### 3. RESULTS AND DISCUSSION

Figure 1a shows a high-resolution transmission electron microscopic (HR-TEM) image of ZnO NP. Lattice fringes of ZnO NP is found to be \( \sim 0.26 \text{ nm} \) which corresponds to the distance between two (002) planes.\(^{17} \) The average particle size is found to be \( \sim 28.5 \pm 0.4 \text{ nm} \) from experimental TEM data. Figure 1b and 1c show lattice fringes of TiO2 and Al2O3 with

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**Figure 1.** (a–d) HRTEM images of various nanoparticles. The right panel shows the corresponding particle size distribution of the NPs.
interplanar distances 0.36 nm between two (101) planes and 0.42 nm between two (110) planes, respectively. The average particle size is estimated to be \( \sim 24.4 \pm 0.1 \) nm for TiO\(_2\) and \( \sim 41.4 \pm 0.1 \) nm for Al\(_2\)O\(_3\). From the HRTEM images, TiO\(_2\) NPs seem to be faceted with different crystal planes. However, careful investigation on the interfringe distances, for a number of NPs reveals a distance of 0.36 nm which is consistent with (101) plane of TiO\(_2\) crystal. Thus, surface roughness of the TiO\(_2\) NPs is concluded to be responsible for the faceted appearance of the NPs. The lattice fringes of RF–AuNP (Figure 1d) distinctly show an interplanar distance of \( \sim 0.31 \) nm, corresponding to the interspace between two (222) planes. The average particle size for RF–AuNP is found to be \( \sim 29.2 \pm 0.1 \) nm.

UV–visible spectroscopy is a useful technique to understand the complexation between RF and the NPs.\(^{30}\) The absorption maxima of RF is at 360 and 445 nm in water and ethanol, respectively. The absorption, emission and excitation spectra of RF with its nanohybrids are shown in Figure 2a–c, respectively, in water for gold nanohybrids and in ethanol for the rest. The RF absorption in the presence of different NPs are shown in Figure 2a. The change in absorbance peak at 445 nm is due to complex formation when RF attaches to surfaces of ZnO, TiO\(_2\), and Al\(_2\)O\(_3\) NPs. The observation is well documented in the literature.\(^{17,28}\) No distinct peak of RF is observed in these nanohybrids due to the very low riboflavin concentration and large scattering of the nanoparticles. However, the presence of riboflavin is evident in emission and excitation spectrum of Figure 2, parts b and c, respectively. The concentration of riboflavin is estimated by differential spectra method (data not shown). However, a surface plasmon band (SPB) exists in RF–AuNP due to the contribution of AuNPs. The SPB exists as a result of collective oscillations of the electron at the NP surface (6s electrons for AuNPs) which is correlated with the electromagnetic field of the incoming light.\(^{31}\) From the normalized steady-state emission data of RF with its various nanohybrids (RF–ZnO, RF–TiO\(_2\), and RF–Al\(_2\)O\(_3\) in ethanol and RF–AuNPs in water), no change in peak position is observed. Figure 2c shows the corresponding excitation spectra.

The Raman spectra of RF–nanohybrids with ZnO, TiO\(_2\), and Al\(_2\)O\(_3\) NPs show similar vibrational modes as that of the corresponding NPs (as shown in Figure 3b) which proves the good retention of crystal structure of the NPs after the complex formation. These studies reveal no change in the peak positions of the NPs. Additionally, the characteristic Raman peaks for RF in the nanohybrids show insignificant perturbation which indicates the absence of strong chemical interaction of RF with

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**Figure 2.** (a) Absorption, (b) emission and (c) excitation spectra of RF in ethanol and RF–nanocomposites (RF–ZnO, RF–TiO\(_2\), and RF–Al\(_2\)O\(_3\) in ethanol and RF–AuNP in water). The excitation spectra of RF along with its various nanohybrids show no relative change in the peak position. For the clarity three representative spectra were shown.

**Figure 3.** Raman spectra of (a) RF and RF–AuNPs in solid phase and (b) different nanoparticles and their nanohybrids with RF in solid phase.
the NPs. The observation is consistent with the reported literature. The invariance of the peak positions of Rf–nanohybrids suggests that the complexation is mainly due to physisorption.30 However, when Rf is attached to gold NP the characteristic Rf peak is shifted from 1346 to 1358 cm$^{-1}$ and also broadened (Figure 3a). Moreover, the peak at 1563 cm$^{-1}$ for AuNPs is moved to 1540 cm$^{-1}$ in the presence of Rf molecules. This suggests a covalent bond formation between Rf and Au NP.32

Figure 4a demonstrates antioxidant activity of the Rf nanohybrids with colloidal ZnO and TiO$_2$ NPs in alcohol without and with light exposure, respectively. Antioxidant activities of the nanohybrids as well as that of the vitamin are usually monitored by the decolourization kinetics of a stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The vitamin or nanohybrids donate a proton/electron to the violet-colored radical DPPH which reduces the radical to DPPH$_2$ form which is yellow in color.33 The free radical quenching kinetics of Rf, Rf–ZnO, and Rf–TiO$_2$ were obtained upon excitation at 409 nm in ethanol and monitored at 520 nm as shown in Figure 5a. The time constants of the fluorescence transients at 520 nm for the singlet excited-state of Rf in ethanol solution showed a lifetime of 3.4 ns as shown in Table 1. The quenching of the lifetime transients is observed in Rf–ZnO and Rf–TiO$_2$ and no change in lifetime is observed in Rf–Al$_2$O$_3$. The observation is consistent with the fact that the excited vitamin transfers electrons to the semiconductor NPs.34−36 The lifetime components of the transients are tabulated in Table 1. In order to confirm the electron migration from Rf, the well-known electron acceptor benzoquinone (BQ) is bound to Rf and the possible excited-state interactions in Rf–BQ composite is investigated with maximum BQ concentration of 10 μM (Figure 5b). The electron transfer time constant (∼100 ps) from Rf to BQ are found to be similar to that of Rf–ZnO and Rf–TiO$_2$ (Table 1). This corroborates with the photoinduced antioxidant activity data (Figure 4a). The enhancement in the constants of the fluorescence transients at 520 nm for the singlet excited-state of Rf in ethanol solution showed a lifetime of 3.4 ns as shown in Table 1. The quenching of the lifetime transients is observed in Rf–ZnO and Rf–TiO$_2$ and no change in lifetime is observed in Rf–Al$_2$O$_3$. The observation is consistent with the fact that the excited vitamin transfers electrons to the semiconductor NPs.34−36 The lifetime components of the transients are tabulated in Table 1. In order to confirm the electron migration from Rf, the well-known electron acceptor benzoquinone (BQ) is bound to Rf and the possible excited-state interactions in Rf–BQ composite is investigated with maximum BQ concentration of 10 μM (Figure 5b). The electron transfer time constant (∼100 ps) from Rf to BQ are found to be similar to that of Rf–ZnO and Rf–TiO$_2$ (Table 1). This corroborates with the photoinduced antioxidant activity data (Figure 4a). The enhancement in the
The emission transient of Rf in water is exponential with the average lifetime value of 4.76 ns. However, the decay time of the Rf in water without AuNP is given by a single exponential decay of time constant 4.76 ns. The emission of Rf–AuNPs in water is quenched compared to that of the Rf. The associated fluorescence transient of Rf–AuNPs is fitted biexponentially with one faster component of 0.4 ns (48%) due to the energy transfer from Rf to AuNPs and a longer component of 4.65 ns (52%) consistent with the decay of Rf only. The scheme of Förster’s Resonance Energy-Transfer (FRET) is employed for the determination of the donor–acceptor distance using eq 2. The energy transfer efficiency and the overlap integral \([f(\lambda)]\) are calculated to be 53.7% from eq 3 and 1.36 × 10^{20} \text{M}^{-1} \text{cm}^{-1} \text{nm}^{-4} from eq 1, respectively. The Förster radius \((r_0)\) is found to be 34 nm. From eq 2, the calculated donor–acceptor separation \((r)\) is 28 nm. As the estimated distance between the donor–acceptor exceeds 100 Å, the phenomenon of Au NPs based surface energy transfer (SET) process is a convenient spectroscopic ruler for long-distance measurement, which follows 1/d^4 distance dependence. NP-induced lifetime modification serves as a ruler to unravel the distance range well beyond 10 nm.

This kind of long distance SET from fluorescent dyes to Au NPs is well documented in the literature. In order to approve the NSET formulism, the distance between donor Rf and acceptor Au NP is determined to be 16.71 nm \((d_0 = 17.36 \text{ nm})\) from eqs 6 and 7, respectively. Herein, we propose nanosurface energy transfer (NSET) from the donor Rf to the acceptor AuNPs as the calculated donor–acceptor distance is in consonance with the size of the gold NPs (radius 15 nm). Hence, it is worth emphasizing that the energy transfers from Rf to Au NP results in the reduced antioxidant activity of the Rf–AuNP nanohybrids as shown in Figure 4b.

### 4. CONCLUSION

We have conducted an in-depth investigation of the crucial photoinduced dynamics in riboflavin upon complexation with various inorganic nanoparticles (NPs) such as zinc oxide (ZnO), titanium oxide (TiO₂), aluminum oxide (Al₂O₃), and gold NPs of approximately 30 nm sizes. Raman spectroscopic studies reveal that riboflavin is adsorbed on the surface of the semiconductor (TiO₂ and ZnO) and the insulator (Al₂O₃) NPs whereas a covalent attachment is observed with metal (gold).

2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay under visible light irradiation was conducted to prove that semiconductors with a wide band gap show a more enhanced photoinduced antioxidant activity than insulators. Furthermore, the antioxidant activity is decreased in the gold nanohybrids as the electron is not shuttled to the model oxidant (DPPH). Time-resolved fluorescence studies on Rf–ZnO and Rf–TiO₂ nanohybrids unravel the efficient electron transfer from photoexcited Rf to ZnO and TiO₂. To confirm the energy transfer of the riboflavin to AuNPs, we have employed picosecond-resolved fluorescence studies and found that nano surface energy transfer (NSET) is predominant over Förster’s
resonance energy-transfer (FRET). NSET from RF to Au NPs occur due to strong spectral overlap of RF emission and surface plasmon absorption of the gold NP which is responsible for low antioxidant activity. The result of the study of photoinduced dynamics in the RF–nanohybrids could be incorporated in designing more effective photodynamic therapeutic agents.

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**Notes**

The authors declare no competing financial interest.

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**REFERENCES**


(33) Chatt, O. A.; Najar, M. H.; Dar, A. A. Evaluation of Reduction Kinetics of 2,2-Diphenyl-1-Picrylhydrazyl Radical by Flavonoid

