Ultrafast FRET at fiber tips: Potential applications in sensitive remote sensing of molecular interaction

Nabarun Polley a, Soumendra Singh a,b, Anupam Giri a, Prasanna Kumar Mondal a, Peter Lemmens c, Samir Kumar Pal a,*

a Department of Chemical, Biological and Macromolecular Sciences, S. N. Bose National Centre for Basic Sciences, Block JD, Sector I1, Salt Lake, Kolkata 700098, India
b Centre for Astroparticle Physics and Space Science, Bose Institute, Salt Lake Campus, Block EN, Sector V, Salt Lake, Kolkata 700091, India
c Institute for Condensed Matter Physics, TU Braunschweig, Mendelsohnstr 3, 38106 Braunschweig, Germany

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Förster resonance energy transfer (FRET) strategy is well adopted in fiber–optics for efficient sensor design. However, resonance type energy transfer from one molecule (donor) to other (acceptor) should meet few key properties including donor to acceptor energy migration in non-radiative way, which is hard to conclude from simply emission quenching of the donor, rather needs careful investigation of excited state lifetime of the donor molecule. In the present study, we have shown that the evanescent field of an optical fiber can be coupled to covalently attached donor (dansyl) molecules at the fiber tip. By using picosecond resolved time correlated single photon counting (TCSPC) we have demonstrated that dansyl at the fiber tip transfers energy to a well known DNA-intercalating dye ethidium upon surface adsorption of DNA at the fiber tip. Our ultrafast detection scheme selectively distinguishes the probe (dansyl) emission from the intrinsic emission of the fiber. The validation of the energy transfer mechanism to be of resonance type (FRET), allows us to estimate the distance between the probe dansyl and the surface adsorbed DNA. We have also used the setup for the remote sensing of the dielectric constant (polarity) of an environment as the excited state lifetime of the probe dansyl heavily depends on the polarity of the immediate host environment. FRET signal from a used fiber tip immediately after adsorption of DNA reveals stepwise surface desorption of the biomolecule in saline solution. The reusability of the fiber tip for sensing has also been demonstrated.

1. Introduction

Immediately after development of the quantitative theory for the resonance energy transfer by Theodor Förster in 1948, the state of Förster resonance energy transfer (FRET) became popular in bio–physical research [1]. However, the use of FRET in fiber optics is evident in 1990s [2,3], which is relatively late given the first development of the field in the mid–20th century [4]. FRET is a photophysical process where the excited state energy from a donor is transferred ‘non-radiatively’ to an acceptor molecule at close distance via dipole–dipole coupling. Till date the reports on the FRET based fiber sensors rely on the fluorescence quenching of the donor (probe) molecule in sensitized fiber [5–9]. However, fluorescence quenching of a donor molecule may result from the radiative energy transfer, which is just a re-absorption of the donor radiation by the acceptor in the medium due to spectral overlap between donor emission and acceptor absorption spectra. The potential danger of concluding resonance type energy transfer has been discussed in a recent literature [10]. The study shows [10] that faster excited state lifetime in the presence of an acceptor is the only way to conclude a resonance type energy transfer in a donor–acceptor system. This issue is addressed pictorially in the upper panel of Fig. 1. Although from the definition of the resonance type energy transfer, the importance of excited state lifetime of donor molecule is clearly evident, no report on the use of lifetime in the FRET based fiber optic sensor is surprisingly evident in contemporary literature.

In a typical development of FRET based fiber optic biosensors for the biomedical diagnostics, a specific fluorophore (energy donor) conjugated antibody is immobilized to the distal end of an optical fiber. Another fluorophore (energy acceptor) in the antibody specific antigen in the medium under test quenches the emission of donor fluorophore. The proximal end of the fiber is connected

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to a fluorometer in order to monitor the quenching as FRET signal concluding the presence of the specific antigen in the medium [6]. Such sensing scheme has been successfully used for the detection of pathogenic microbes [8] in ground pork samples. In all the sensors, evanescent field of the propagating light is used to deliver excitation energy to the donor molecules and eventually collect FRET signal from the distal sensitized end to the proximal end of the fiber. Sensible use of the evanescent field of an optical fiber for the simultaneous use of potential diagnostics and therapy (theranostics) of hyperbilirubinemia is recently reported from our group [11]. In the above mentioned applications, the time resolved fluorescence properties remain unexplored. However, validation of FRET and consequent use of the formulism for the estimation of the molecular distance between the donor and the acceptor demands a careful analysis of the excited state lifetime of the fluorophores with picosecond resolution [12]. In some of the recent studies the usefulness of the picosecond resolved fluorescence measurement for the FRET based sensor (not using an optical fiber) has been recognized [13–15]. In a report using quantum dots linked to DNA have been used in an ultrasensitive nanosensor based on fluorescence resonance energy transfer (FRET) capable of detecting low concentrations of DNA in a separation-free format [13]. Another study demonstrated that fluorescence lifetime data accurately be recorded via miniature fiber endoscopes that can discriminate dichotomous labeled structures and cells [14]. In a recent study FRET between donor nanoparticle and acceptor quantum dots is utilized in protein quantification [15].

In the present study, we have sensitized the distal end of a silicon fiber tip by covalently tethering the well known biological probe dansyl [16]. An self developed optical setup containing two

![Fig. 1](image-url)
off-axis parabolic mirrors and a dichroic mirror has been used to launch light from a picosecond laser to the proximal end of the fiber tip and to collect fluorescence signal for a 16-channel PMT array connected to picosecond resolved time correlated single photon counting (TCSPC) modules. Ethidium labeled genomic DNA (from calf thymus) [17–19] is used as a model analyte for the sensing application. The absorption spectrum of the well known DNA-label ethidium has strong spectral overlap with the emission spectrum of the probe dansyl at the fiber tip, which is the prerequisite of efficient resonance energy transfer from dansyl (donor) to ethidium (acceptor) in the test DNA. We have observed that the fiber itself is having an intrinsic emission at the wavelength around 460 nm, which is close to the emission (505 nm) of the probe dansyl at the fiber tip. Our picosecond resolved measurement strategy allows us to distinguish the background emission from the fluorescence signal of the probe dansyl. A significant shortening of the donor fluorescence lifetime in presence of ethidium labeled DNA at the sensor tip not only validates the resonance type sensing mechanism (FRET), also estimates the distance between the donor at the fiber surface to the test DNA using dipole–dipole coupling formulism. The advantage of using dansyl as a probe is also evident in our use of the sensitized fiber tip as remote sensor of polarity (dielectric constant) of a liquid mixture of two miscible solvents (water and 1,4-dioxane). A molecular pathway in the surface desorption of DNA from the fiber tip in saline solution is evident during our studies on the reusability of the sensor tip for the repetitive measurement.

2. Materials and methods

2.1. Materials

All the optical components used in our studies were received from Thorlabs Inc., USA. The 16-channel time correlated single photon counting (TCSPC) setup is assembled in our laboratory with all the required components (PML-SPEC multi-wavelength detection assembly consisting of a polychromator and a 16 channel PMT PML-16C, FI100-Bundle, Simple Tau-130EM with SPC-130EM & DCC-100 cards, Express Card 54 and SPCM-64 software) from Becker & Hickl, Germany. A LDH-P-C-375 picosecond pulsed laser source with a PDL-80-D PicQuant laser driver (PicQuant, Germany) were used as the UV light source. The laser driver with the adjustable laser output power can be operated with a repetition frequency of 31.25 kHz–80 MHz (40 MHz in our case). The overall instrumental response of the TCSPC system is found to be 200 ps. For the fabrication of the fiber-sensor, we have used multi-mode silica core fiber FT200UMT (Thorlabs Inc., USA). As per the vendor’s specifications, the core, clad, and outer diameters of the silica fiber are 200 μm, 225 μm and 500 μm respectively. For the high resolution electron microscopy of the fiber tip, we have used Quanta FEG 250 scanning electron microscope (SEM). The dansyl chloride, precursor of the probe dansyl was received from Molecular Probes, USA. The calf thymus DNA, (3-aminopropyl) triethoxysilane (APTES) and ethidium bromide (EtBr) were purchased from Sigma-Aldrich, USA.

2.2. Sensitization of fiber tip

For the sensitization, we have etched the clad (1 cm) manually at the distal end of a 1 m long silicon fiber following the methodology reported in the literature [20,21]. The SEM images of the fiber tips before and after the etching are represented in the Fig. 1a and b, respectively (lower panel of Fig. 1). The fiber diameters (core and clad) are found to be consistent with the supplier’s specification. After etching the fiber tips were cleaned carefully. For cleaning, first the fiber tips were cleaned by bath sonication in acetone for 30 min to remove any residual clad material from the fiber core. Then another cleaning cycle with water–ethanol mixture in bath sonicator was run for the next 30 min. A typical fiber surface after the cleaning process is represented in Fig. 1c. In order to start the sensitization process, as reported in literature [21–23], the fiber tips were then immersed into H2SO4 solution maintaining a constant temperature at 80 °C using a hot plate. After H2SO4 was added into the H2SO4 solution with a concentration ratio of H2SO4:H2O2 = 3:1 (also known as the piranha solution) the fiber tip was kept for another 20 min. This solution is a strong oxidizing agent that can remove the residual clad and organic constituents from the fiber tip surface. At the same time, the solution also serves as hydroxylation agent revealing the surface extremely hydrophilic as shown in Fig. 1d. After thoroughly rinsing with millipore water several times, the fiber tips were immersed in APTES solution for 40 min at 45 °C to conjugate the APTES molecules with the surface hydroxyl groups of the fiber through dehydroxylation reaction (Fig. 1e). Next, we have covalently functionalized the terminal amine functional groups of the conjugated APTES molecules with a fluorescent dye (dansyl chloride) by exploiting the nucleophilic reactivity of the amine groups. After thoroughly rinsing the fiber tips with water (to remove any free APTES molecule from the surface), for the attachment of dansyl group (dansylation), the tips were immersed in an aqueous solution of pH ~ 10. Then, dansyl chloride solution in acetonitrile was added drop wise into the aqueous solution under continuous stirring. This dansylation process was performed in dark, at low temperature (4°C), and after complete addition of dansyl chloride the system was kept overnight for proper dansylation. The fiber tips were finally taken out from the aqueous solution and properly rinsed with acetonitrile to remove the excess and unreacted dansyl chloride from the fiber surface. In this study, we have used these dansylated fiber tips (Fig. 1f) as an efficient FRET based sensor and tool for monitoring the polarity (dielectric constant) of a test environment.

2.3. Instrumentation design

The instrumentation with TCSPC is designed to monitor the change in the excited state lifetime of the dansyl probe due to Förster resonance energy transfer (FRET) from the sensitized fiber tip. The schematic representation of the setup is shown in Fig. 2. A picosecond (pulse width of 70 ps) laser beam passes through L1 (an aspheric condenser lens of 30 mm focal length), M1 (a dichroic mirror, which reflects <400 nm and passes wavelengths in the visible light) and a pair of off-axis parabolic mirrors (M2 and M3 of focal lengths 132 mm and 25 mm respectively), and eventually enters into the proximal end of the sensitized fiber. For the collection of the fluorescence signal from the sensitized fiber tip, the fiber bundle (F-100) is placed at the focal point of M2, which carries the signal to the polychromator–PMT array and finally to the TCSPC module. The 16 channel PMT was used to measure simultaneous time resolved decays corresponding to 16 different wavelengths. From maximum counts of the channels, in a given acquisition time, the steady state emission spectrum of the probe dansyl (fluorescence intensity versus wavelength) can be generated. All the experiments were performed in dark condition to avoid any ambient light interference. The schematic of the sensitized fiber tip and the sensing strategy is shown in the inset of Fig. 2.

2.4. Formalism of Förster resonance energy transfer (FRET)

The observed fluorescence transients were fitted by using a nonlinear least square fitting procedure to a function \( X(t) = \int_0^t E(t')R(t'-t')dt' \) comprising of a convolution of the instrument response function (IRF) \( E(t) \) with a sum of exponentials \( R(t) = A + \sum_{i=1}^{N} B_i e^{-t/\tau_i} \) with pre-exponential factors \( (B_i) \).
characteristic lifetimes ($\tau_1$) and a background ($A$). The quality of the curve fitting was evaluated by reduced Chi-square and residual data. From the best fitted parameters the relative contribution in a multi exponential decay was finally expressed as,

$$c_n = \left( B_n/\sum_{i=1}^{N} B_i \right) \times 100.$$ 

To estimate the FRET efficiency of the donor and hence to determine the distance between the donor–acceptor pair, we followed the methodology described in chapter 13 of Ref. [12]. The Förster distance ($R_0$) is given by,

$$R_0 = 0.211 k^2 n^4 \lambda_0^3 Q_0 \left( \int_0^\infty \frac{F_0(\lambda) e(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_0(\lambda) d\lambda} \right)^{1/6} \text{ (in } \text{Å}),$$

where $k^2$ is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor. We assumed that the orientation factor $k^2$ is equal to 2/3. The refractive index ($n'$) of the medium was assumed to be 1.4 [12]. In the above equation $Q_0$ represents the quantum yield of the donor in absence of acceptor. The overlap integral $I(\lambda)$ expresses the degree of spectral overlap between the donor emission and the acceptor absorption, is given by,

$$I(\lambda) = \frac{\int_0^\infty F_0(\lambda) e(\lambda) \lambda^4 d\lambda}{\int_0^\infty F_0(\lambda) d\lambda}$$

where, $F_0(\lambda)$ is the fluorescence intensity of the donor in the wavelength range of $\lambda$ to $(\lambda + d\lambda)$ and $e(\lambda)$ is the extinction coefficient (in $M^{-1} cm^{-1}$) of the acceptor at the wavelength $\lambda$.

Once the value of $R_0$ is known, the donor–acceptor distance ($R$) can easily be calculated using the formula,

$$R^6 = \left( \frac{R_0^6 (1 - E)}{E} \right),$$

where $E$ is the FRET efficiency, measured by using the lifetime of the donor in the absence ($\tau_D$) and presence ($\tau_{DA}$) of acceptor, defined as,

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \quad (4)$$

It has to be noted that Eq. (4) holds rigorously only for a homogeneous system (i.e. identical donor–acceptor complexes) in which the donor and the donor–acceptor complex have single exponential decays. However, for donor–acceptor systems with multi-exponential decay lifetimes, the FRET efficiency ($E$) is calculated from the amplitude weighted lifetime ($\langle \tau \rangle = \sum \alpha_i \tau_i$), where $\alpha_i$ is the relative amplitude contribution to the lifetime $\tau_i$. We have used the amplitude weighted time constants for $\tau_D$ and $\tau_{DA}$ to evaluate $E$ using Eq. (4).

3. Results and discussion

3.1. Optimization of fiber length for the sensor application

We have observed that bare fiber without sensitization shows a fluorescence signal (peak around 460 nm) under 375 nm excitation, which is close to the emission from the probe dansyl (peak at 505 nm) as shown in the inset of Fig. 3a. Coupling of laser beam and eventually collecting signal from fiber without sensitization also reveal fluorescence transient. For the optimization of the fiber length in our application, first we have used a 30 cm long optical fiber with one end sensitized with dansyl. The sensitized fiber reveals two peaks in the fluorescence transient measurement (Fig. 3a). Our control experiment with the fiber of similar length without sensitization shows only one peak (peak 1) with lifetime of 2.65 ns, revealing the contribution of the background emission from the bare fiber. 2.65 ns lifetime value is found to be consistent with that of the clad only, which is made of technology enhanced clad silica (TECS) polymer (details of the spectroscopic properties are not available from the vendor) upon UV excitation (data not shown). Upon sensitization of the fiber tip with dansyl we have observed two peaks in the time resolved studies as shown in Fig. 3a. While numerical fitting of peak 1 reveals time constant of 2.65 ns, peak 2 shows a lifetime of 3.83 ns (Table 1). The lifetime of peak 2 is also found to be consistent with that of the fully dansylated short fiber, essentially confirming the signal from the fiber tip. In order to further confirm the origin of the peak 2 to be from the fiber tip we have performed the time resolved studies with optical fiber of different lengths. For the fiber of 30 cm length the time interval between two peaks (peaks 1 and 2 in Fig. 3a) is measured to be 2.58 ns, which is consistent with the estimated time interval ($t = (2 \times n \times \text{Fiber length})/c$, where $n$ and $c$ are the refractive index of fiber core and the speed of light in vacuum respectively) of 2.82 ns. For fibers of lengths 1 meter (Fig. 3b) and 11 cm (inset of Fig. 3b) the measured time intervals of 9.17 ns and 0.82 ns respectively are in agreement with the estimated values of 9.74 ns and 0.97 ns. The...
observation clearly justifies the optimization of the length of the optical fiber for the sensing application and confirms peak 2 in the Fig. 3 to be the signal from sensitized fiber tip in the distal end. For example, our studies on a fiber of length around 11 cm shows that background emission is very close to the signal from the sensitized fiber tip (Fig. 3b inset). Here, we have used a fiber length of 1 m with one end sensitized with dansyl probe and transient signal obtained is shown in Fig. 3b.

### 3.2. Validation of FRET at the fiber tip: measurement of proximity of DNA

After the optimization of the required length of the fiber we use the sensitized fiber tip as FRET based sensor for the detection of DNA. A strong spectral overall between the donor emission with that of the absorption spectrum of the acceptor is the prerequisite of FRET [12] and is evident from Fig. 4a. From Fig. 4a we have estimated the overlap integral of dansyl (donor) emission spectrum with that of the ethidium (acceptor) labeled DNA absorption (Eq. (2)) to be $1.36 \times 10^{13} \text{ M}^{-1} \text{ cm}^{-1} \text{ nm}^{-4}$. A significant steady state quenching of dansyl emission at the fiber tip is evident from Fig. 4b, where the emission peaking at 610 nm is the energy acceptor ethidium in the DNA. In a controlled experiment the magnitude of the steady state quenching of the dansyl emission upon surface adsorption of EB-labeled DNA the concentration of DNA in the solution could be measured. An earlier report from this group shows that the concentration of a model analyte bilirubin in an aqueous solution can be measured from the surface adsorption of the analyte on a sensing optical fiber dipped in the solution [11]. A direct evidence of the resonance type energy transfer is evident from both the steady state quenching (Fig. 4b) and time resolved fluorescence transients of the donor dansyl at the fiber tip as shown in Fig. 4c. The faster fluorescence decay of the dansyl in ethidium labeled DNA solution is evident from Fig. 4c and Table 1, which confirms the proximity of the biomolecule to the fiber surface. Inset of Fig. 4c shows similar fluorescence decay transients of dansylated fiber tip in absence and presence of unlabelled DNA in water, monitored at $\lambda_{\text{em}} = 505 \text{ nm}$ eliminating the possibility of the steady state and temporal quenching due to electron transfer [24]. On taking a quantum yield of dansylated fiber tips in absence of acceptor ethidium to be 0.7 [25], we have estimated a FRET efficiency of 49% using Eq. (4) which is found to be reproducible within 5% error limit. The estimated Förster distance, $R_0$, for the FRET pair is found to be 26.4 Å. The donor–acceptor distance ($R$) using Eq. (3) is calculated to be 26.8 Å, indicating a very close proximity of the DNA molecules to the fiber tip.

### 3.3. Remote sensing of a medium with different dielectric constants

Monitoring the polarity in a hazardous environment including petroleum processing column is reported to be important for the quality control of the petroleum product [26]. However, the remote sensing of the polarity in the reaction chamber (column) is unavoidable for the very hazardous nature of the reaction
chamber. The remote sensing ability of the sensitized fiber tip using the picosecond resolved TCSPC strategy is evident from Fig. 5a and Table 1. Here, we have used a liquid mixture of two different miscible solvents 1,4-dioxane and water with different proportions. It has been reported earlier [27] that a solution of different dielectric constants can easily be prepared by mixing various proportions of 1,4-dioxane (dielectric constant = 4) and water (dielectric constant = 80). We have used the liquid mixture as model environment for the remote sensing studies. As shown in Fig. 5a (numerical fitting is shown in Table 1), a distinct change in the lifetime of the probe dansyl at the fiber tip is evident with the change in dielectric constant of the liquid mixture. While longer lifetime in environment with lower dielectric constant is evident from the Fig. 5a, the inset of the figure shows gradual red-shifting of the steady state emission spectrum of the dansyl probe at the fiber tip with the increase in the water content of the medium (higher dielectric
constant). The observation is consistent with the fact that the excited state of the probe is heavily dependent on the dielectric constant of the host medium (polarity) due to the nonradiative twisted intra-molecular charge transfer (TICT) events upon photoexcitation [16]. In Fig. 5b we have plotted the dielectric constant of the medium with the nonradiative TICT rate ($k_m$) estimated in the following way [24]: $k_m = \left(1/\tau_s\right) - \left(1/\tau_{\text{dioxan}}\right)$, where $\tau_s$ is the average lifetime of the probe dansyl in any medium and $\tau_{\text{dioxan}}$ is that in pure dioxane. As evident in Fig. 5b, the rate constants follow an exponential rise function with the increase in dielectric constant of the medium. The dielectric constant of the medium can be estimated by using the empirical formula: $k_m = 0.016 \times D^{1/15.94}$, where $D$ is the dielectric constant of the test medium.

3.4. Surface desorption of DNA: reusability of the sensor

In order to investigate the efficacy of the sensitized fiber tip for repetitive usage, we have investigated the surface desorption of ethidium labeled DNA from the fiber tip. The detachment of the DNA from the fiber tip is achieved by dipping the used dansylated fiber tip (sensor) in 1 M sodium chloride (NaCl) solution for 10 min [16]. DNA initially attached to the fiber tip by electrostatic interaction between the positively charged amine (NH$_3^+$) groups of APTES and negatively charged phosphate (PO$_4^{-}$) backbone of DNA itself. NaCl detaches the DNA molecules from the dansylated fiber surface and interestingly the average excited state lifetime of the sensor also recovered subsequently from 1.97 ns to 2.46 ns (tending toward its original value of 3.83 ns in absence of DNA), as shown in Fig. 5c. Surface desorption of DNA from the sensor tip is evident from the observation. Involvement of multiple steps in the desorption revealing different DNA distances from the fiber surface in presence of NaCl is also clear from the studies. We have estimated that the intermediate distance of the test DNA from the surface (Eq. (3)) is 30 Å in 10 min, before the biomolecule goes 80–100 Å from the surface, which is beyond the scope of a FRET based sensor [12]. The exploration of such molecular structures can only be achieved using picosecond resolved FRET sensors.

4. Conclusion

In conclusion, we have validated resonance type energy transfer scheme in a model FRET based fiber optic sensor for the first time using picosecond resolved time correlated single photon counting (TCSPC) technique. The ultrafast time domain measurement strategy also avoids possible interference from the background emission of the bare fiber. Confirmation of the FRET mechanism allows us to use dipole–dipole coupling formalism for the estimation of proximity of ethidium labeled DNA with respect to the sensitized fiber tip in molecular resolution. The efficacy of the designed fiber sensor for the detection of various dielectric constants of a liquid medium has also been established. The reusability of the sensor tip for repetitive application is confirmed. Stepwise surface desorption of DNA from the fiber tip is also evident from our studies. In future our study is expected to find the relevance in the sensitive FRET based optical sensor development.

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References


Biographies

Nabarun Polley was born (1989) in Howrah, India. He graduated in Physics (B.Sc.) in 2009 and received Masters Degree in Biomedical Instrumentation in 2011 from University of Calcutta, India. Currently he is persuing Ph.D. under the supervision of Prof. Samir Kumar Pal at S. N. Bose National Centre for Basic Sciences, Kolkata, India. The main focus of his work is to develop and design new biomedical tools using spectroscopic techniques.

Soumendra Singh is a part time Ph.D. student at Department of Chemical Biologi- cal and Macromolecular Sciences, S. N. Bose National Centre for Basic Sciences and a project Scientist C in Center for Astroparticle Physics and Space Science, Bose Institute, India. He received his M.Sc. degree in Electronics from Vidyasagar University, India and M.Tech. degree in Computer Sc. and Application from University of
Calcutta, India. His interest includes design and realization of instrumentation in atmospheric sciences and high frequency wave propagation.

**Anupam Giri** received his M.Sc. in Chemistry from University of Calcutta, India (2009), and he completed his Ph.D. under the supervision of Professor Samir Kumar Pal in the Department of Chemical, Biological and Macromolecular Sciences of S. N. Bose National Centre for Basic Sciences, Kolkata, India (2014). He is currently working as a postdoctoral research associate in Yonsei University, Seoul, South Korea. His research interests include the synthesis, characterization and applications of two dimensional nanomaterials.

**Prasanna Kumar Mondal** is presently working as a Research Associate in the Department of Chemical, Biological and Macromolecular Sciences of S. N. Bose National Centre for Basic Sciences, Kolkata, India. He received his M.Sc. degree in Physics in 2004 from University of Calcutta, India, and he completed his Ph.D. in 2011 under the supervision of Professor Barun Kumar Chatterjee in the Department of Physics of Bose Institute, India. He has more than 11 research papers published in various international peer-reviewed journals.

**Prof. Peter Lemmens** is presently professor in the Institute for Condensed Matter Physics, Institut für Physik der Kondensierten Materie, Braunschweig, Germany. His work relates to the interplay of photons with electronic correlation effects, spin orbit interaction, and nanoscales. He also investigates nanosystems, energy transfer, transition metal oxides and topological systems.

**Prof. Samir Kumar Pal** is presently professor in the Department of Chemical Biology and Macromolecular Sciences, S. N. Bose National Centre for Basic Sciences, Kolkata, India. His field of interest include experimental biophysics in molecular recognition, bio-nano interface, biomedical instrumentation and environmental pollution. He has more than 170 research papers published in various international peer-reviewed journals and 14 patents.