Ultrafast Fluorescence Spectroscopy and Supra-biomolecular Interactions of Organic Dyes

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Abstract

This short article presents a glimpse of the select photochemical research in our group, along with a brief account of the femtosecond fluorescence up-conversion facility, developed in RPCD, that offers a convenient and sensitive ultrafast fluorescence detection, to cater to the photochemical research in the Division. In the present article, the discussion is restricted to typical molecular systems like triphenylmethane (TPM) dyes and thioflavin T, that shows significant structural modulations in the excited state. Understanding the relaxations and modulations of excited state properties on binding to ss-DNA, BSA or with macrocyclic hosts, are the motivations of the present study. The topics covered are (i) intriguing ultrafast excited state relaxation dynamics in malachite green (ii) G-quadruplex formation in ss-DNA in the presence of malachite green (iii) the cooperative interaction of brilliant green with BSA and cucurbit[7]uril and (iv) formation and breakage of a stimulus-responsive supramolecular capsule, formed by thioflavin T, metal ions and cucurbit[7]uril.

Key Words: Fluorescence up-conversion, Triphenylmethane dyes, Thioflavin T, Cucurbit[7]uril, G-quadruplex

Introduction

In photochemical studies, one of the major issues is to understand, the geometric and electronic distribution of the molecule, the surrounding medium, and other extrinsic factors which influence the molecular properties and to what extent can one use this information to control excited-state dynamics. In the event of molecular interaction, physico-chemical processes like, electron transfer, proton transfer, charge transfer, solvation, torsional dynamics etc., occur in the picosecond to femtosecond timescales and a detailed investigation of such processes warrants sensitive and reliable experimental facilities. In general, fluorescence-based techniques are very sensitive to investigate various photochemical processes. In our group, steady-state as well as time-resolved fluorescence with a wide time resolution from nanosecond to femtosecond have been extensively used, to investigate various complex photochemical processes including excited state relaxation, electron transfer processes, modulation of excited state properties via supramolecular interaction and others. This article presents some of the studies carried out using ultrafast fluorescence technique like fluorescence up-conversion with time resolution down to ~100 femtosecond and also the intriguing photophysical properties and their advantageous modulation in some of the potential organic dyes, exploiting their interaction in a supra-biomolecular environment.

Femtosecond fluorescence up-conversion facility

Understanding the details of molecular events in real time, is of utmost importance to execute controlled chemical reactions and to channelize them into various applications.
The concept of obtaining the ultrafast time resolution in the fluorescence measurements, relies on the non-linear effect of generating sum-frequency photons from the mixing of the fluorescence photons with a gate light pulse, which are spatially separated in sequence, to introduce a path difference in steps of 2 micron each, which is equivalent to a 6.6 fs time delay. Depending on the changes in the fluorescence intensity of the sample at different delay periods, the intensity of the up-converted light also changes accordingly and the latter is detected with a sensitive photon counter to generate the kinetic traces. The schematic and the actual arrangement of the femtosecond fluorescence up-conversion setup, developed in our Division is shown in Fig. 1. With this set up, the instrument resolution is ~100 fs [1].

Ultrafast excited-state dynamics of malachite green

Triphenylmethane (TPM) dyes are one of the earliest organic molecules investigated, to understand the ultrafast torsional motions in the excited states and to exploit their advantages in areas ranging from laser dyes to photodynamic therapy. For the TPM dye, Malachite Green (MG), employing the fluorescence up-conversion and other optical measurements and following $S_2$ state excitation, the data revealed a cascade population relaxation along the $S_2$ state, with a time constant of ~130 fs, that is almost independent of solvent viscosity, contrary to the relaxation dynamics of the $S_1$ state (Fig. 2A).

Fig. 1: Schematic (A) and the actual photograph (B) of the femtosecond fluorescence up-conversion setup developed in RPCD during Xth plan period.

Fig. 2: (A) Fluorescence decay traces obtained in an ethanol solution of MG on excitation at 410 nm (a) at 450 nm (b) at 680 nm. (B) Schematic representation of the proposed relaxation pathways in malachite green.
Following wavelength-dependent time-resolved anisotropy measurements along the S₂ and S₁ emission bands, we propose a relaxation pathway along a conical intersection of S₂ and S₁ potential surfaces, supported by the torsional motion of the unsubstituted phenyl ring (Fig. 2B). Present study identifies the major structural changes associated with the excited state of TPM dyes, which are of direct relevance to the application of these dyes in different areas [2,3].

**Interaction of malachite green with G-quadruplex**

As discussed, due to the structural relaxation in the excited states, the radiative lifetimes of TPM dyes are, in general, very short (< 1 ps) in low viscosity solvents. The lifetime, however, increases substantially upon binding of the TPM dyes with bio-macromolecules like DNAs and proteins, that possess potential applications in photodynamic therapy, drug delivery, catalysis, site/sequence specificities and therapeutics. Oligonucleotide sequences containing guanine-rich stretches can form unique, mutually hydrogen-bonded, internally-folded, or inter-strand G-quadruplexes (see Fig. 3), which are active in chromosomal telomeres, gene promoters, immunoglobulin switch regions.

Using photochemical methods, we demonstrate the binding interactions of a guanine-rich single strand oligomer sequence d(G₂T)₁₃G with the biologically active chromophoric dye, MG. Convincingly, malachite green supports the formation of G-quadruplex and forms a strong complex with it, resulting in ~100 fold enhancement in its fluorescence yield. This specific interaction as compared to other poly nucleotide sequences, is a convenient method, to detect the G-quadruplex formation in DNAs and RNAs [4].

**Brilliant green with BSA and cucurbit[7]uril: an enhancer strategy**

The advantage of placing dye/drug in a supra-biomolecular environment has immense applications in enhancing the photochemical activity. In our study, we observed a fluorescence enhancement by a factor of ~300 and a largely increased binding affinity, when the TPM dye, Brilliant Green (BG), binds to bovine serum albumin (BSA) in the presence of a macrocyclic host cucurbit[7]uril (CB7). Observed results indicate a cumulative (multiplicative) fluorescence enhancement as compared to the effects observed in the presence of CB7 alone (factor of 6) or BSA alone (factor of 45) (Fig. 4). The present

![Fig 3: (A) Binding curves for MG with the oligomers. ▲ denotes the increase in emission intensity with the addition of NaCl upto 500 mM (5). (B) Representation of the folding patterns in the G-rich oligomer (i) and a schematic representation of the binding of MG on the quadruplex (ii).](image-url)
observation suggests a new supramolecular ‘enhancer strategy’, to improve the binding of selected organic dyes to biomolecules, which could improve their sensitivity in sensor applications and their activity in medicinal applications, prominently photodynamic therapy [5].

Stimulus-responsive fluorescent supramolecular capsule

Non-covalent interaction through host-guest approach, where a guest molecule is encapsulated into the macrocyclic host cavity, can introduce pronounced effects on, as well as, fine-tuning of the physico-chemical properties of the included guest, which find immense importance in photostability, drug delivery, photodynamic therapy, catalysis and sensor applications. In our study, we observed an intriguing non-covalent interaction of Thioflavin T (ThT), a fibril diagnostic dye, with CB7 in the presence of metal cations [6]. In the absence of metal ions ThT forms 2:1 (CB7:ThT) complex with CB7. Incorporation of metal ion to the 2:1 complex solution, leads to an unusual enhancement in the fluorescence emission (~270 fold in the presence of Ca²⁺). Detailed photophysical characterization with support from NMR and anisotropy data has revealed, a novel stimulus-responsive cooperative metal ion, binding to the stoichiometrically selected (CB7)₂•ThT complex, demonstrating a highly fluorescent supramolecular nano-
capsule, which responds to stimulants like adamantyl amine (AD) and provides a controlled release of the components as presented in Fig. 5. Such stimulus-responsive capsular complexes are projected to find application in areas like drug-delivery vehicles, nanoreactors or metalloenzyme models.

Fig. 4: Plots of the fluorescence intensity ratio (I/I₀) for BG with addition of CB7 (◊), BSA (●) or both (○). Inset: Schematic representation of the proposed supra-biomolecular complex and the structure of CB7.

Fig. 5: Schematic representation of the formation and stimulus responsive breakage of the supramolecular capsule.

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References