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Editorial

Historically, most chemistry has been done by mixing elements together and heating them. But the trouble with heating something is that the energy shows up in the molecules as random motion. The energy breaks old chemical bonds, makes new ones, and overcomes barriers to transitions.

The tantalizing question is how to perform more efficient chemistry. Is there a better way to run a chemical reaction? Lasers offer intriguing possibilities. With their ability to deliver small, uniform bundles of energy to tiny targets, lasers have in recent years spurred many chemists to rethink how to trigger reactions.

In theory, photons of just the right energy can drive atoms into excited states, make or break chemical bonds, or become absorbed by some molecules while being deflected by others. In practice, though, such precise control has proved elusive in the laboratory. Only recently have several teams of chemists begun to show how to put theory into practice by controlling some simple molecular reactions with lasers.

Lasers are indispensable in a number of surgical specialties, from dermatology to oncology, and the development of new medical laser technologies and techniques offers tremendous opportunities to improve the practice of medicine further, from developing better sutures to treating osteoporosis where the latter has taken strong roots in the society.

I am grateful to Dr.P.N.Bajaj, Chemistry Division for providing the Focus. I am appreciative of the efforts rendered by Dr.D.K.Palit, RC & CDD, BARC and all the authors for bringing out this bulletin in a short notice.

G.A. Rama Rao

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P.K. Pujari
pujari@magnum.barc.ernet.in

Lasers in Chemistry

Guest Editor

Dipak K. Palit

Radiation and Photochemistry Division
Bhabha Atomic Research Centre
Mumbai 400 085

FOCUS

Dr. P.N. Bajaj

Chemistry has come a long way from the stage of mixing reactants in a reaction vessel under certain conditions, identifying the products, establishing the overall mechanism from macroscopic investigations, and optimizing the macro parameters, such as temperature, pressure, and/or using a catalyst, to get maximum yields of the products of interest. This approach has paid rich dividends – enriched the mankind in terms of materials, technologies, healthcare, etc. From a periodic table of hundred and odd elements, millions of compounds have been prepared to make life comfortable, notwithstanding some abusive aberrations exploited by malevolent individuals. But, chemists of today are not contended with this achievement. They want to control reactions, i.e., steer reactions in the desired direction. Chemists' cherished dream has been to carry out mode, or bond, selective reactions, eventually leading to control of chemical reactions. To achieve this, and develop new technologies, it is necessary to know "what are the various elementary steps involved in a reaction, and what is the detailed dynamics of each elementary step? In order to investigate dynamics of a molecular process, physical or chemical, fast techniques are required. The development of lasers has greatly helped in this endeavour. Laser is playing a multi-dimensional role in chemistry – it can act as a detector, a monitor, an investigator, a facilitator, a selector as well as a controller. Lasers have revolutionized the field of chemistry, especially chemical dynamics.

Laser can identify as well as quantify species of interest, such as an atom, a molecule, a radical, an ion, etc., probe the interior of a species, monitor the inner dynamics, motion of the species as a whole, dynamics of its interaction with another species, or photon, transformation from one species to another, and so on. Optical absorption, laser induced fluorescence (LIF), Raman scattering, coherent anti-stoke Raman scattering (CARS), resonance ionization spectroscopy, optoacoustic spectroscopy, optogalvanic spectroscopy, cavity ringdown absorption spectroscopy, laser induced break down spectroscopy, thermal lensing technique, four-wave mixing technique, etc., are some of the laser-based techniques being employed by chemists for identification and quantification of species of interest, fundamental studies as well as technological exploitations. Interference in LIF due to unwanted species can be eliminated by using spectral window, temporal window, or both, and this has made LIF a very powerful nondestructive analytical technique. Employing optical fibres, laser can be used to analyze samples, as well as monitor processes in inaccessible area, or hazardous environment, radioactive or otherwise. Laser based techniques have armed chemists with the capability of single-atom detection.

Using laser(s), one can prepare the reactants in well-defined quantum states, and study their reaction dynamics. The critical stage in a chemical reaction, the progression from the reactants to the products through the transition state, takes place in less than a picosecond. So, with laser pulses of femtosecond duration, one can monitor the journey of the reactants from the reactant valley to the product valley, i.e., breaking of old bond(s) and formation of new bond(s), by taking snapshots, from the time a chemical act starts, that is to say that one can determine the status of a chemical reaction with time resolution of laser pulse duration.

In 1988, Zewail and his team, at California Institute of Technology, Pasadena, USA, carried out a novel pump-probe experiment to understand dynamics of photodissociation of ICN molecule, using two femtosecond lasers, one to dissociate the molecule, and the other to probe the dynamics. In this experiment, the molecule absorbs a photon from the pump laser, and begins to dissociate to give CN and I^*, excited state of the iodine atom. The second laser pulse, fired a few femtoseconds later, probes dynamics of the dissociation. It has been

found that CN takes about 600 fs to become free of iodine atom. But, based on recoil velocity of the dissociation, CN should be at a distance of 12A^{o} in 600 fs. This shows that a significant part of 600 fs is spent in the transition state. Subsequently, many such state-of-the-art experiments were performed to understand detailed dynamics of chemical reactions. It is now possible to characterize the elusive 'transition state', which determines the course of a chemical reaction.

Laser can exert force, selective as well as brute, to affect desirable changes, both physical and chemical. Using a laser, one can selectively pick a species of interest, from a sea of various other species, ionize it, or engage it in a reaction, unimolecular or bimolecular, and exploit this for ultra-purification and separation of isotopes. One can also deposit energy selectively into an internal degree of freedom that is more effective in increasing the rate of a reaction rate, or the rate of a particular reaction channel, or opens up a new reaction channel. This can be exploited for separation of isotopes, or producing the desired product in good yield, and eliminating or minimizing the unwanted products, a waste. The use of high-power lasers in chemistry has opened new vistas. Using lasers, one can develop novel methods of reaction control, passive as well as active. Control schemes, based on either frequency or coherent characteristics of laser radiation, have been proposed and demonstrated.

A photochemical method, based on selective excitation of zinc isotopes, with isotope shifts within the Doppler profile of the transition, by two-photon absorption from two counter-propagating laser beams, and subsequent decay of the excited atoms, either directly, or through a cascade, to long lived states ($4p^3\ P_{0,1,2}$), and enhanced cross-section of the reaction of the excited atoms with CO_2, has been demonstrated for separation of zinc isotopes. Investigations on single-photon absorption by an atom, or a molecule, as well as a few photon absorption by a molecule, and subsequent reaction with suitable reactants, is being actively pursued from the viewpoint of laser isotope separation (LIS). In 1985, we, too, observed enhancement in the associative ionization of uranium with O_2, by a few orders of magnitude, on excitation of uranium atoms. Photochemical method will be compact and less energy intensive.

When line tunable high-power TEA CO_2 laser became a commercial product in seventies, chemists all over the world got fascinated by this laser to achieve their cherished dream of bond and mode selective chemistry, by exciting vibrational modes of polyatomic molecules. Pulses from CO_2 laser provided, for the first time, a means of exciting suitable molecules to dissociation level in the ground electronic state, by absorbing several IR photons. This phenomenon, commonly called infrared multiple photon dissociation (IRMPD), has been extensively investigated, and exploited to separate isotopes of many elements, such as B, C, S, Cl, Os, etc.

F. Fleming Crim and his team, at University of Wisconsin, Madison, Wisconsin, USA, demonstrated vibrational state control of photodissociation as well as bimolecular reactions. For example, by selective excitation of O-H or O-D mode in water molecule, they showed preferential scission of the corresponding bond upon subsequent electronic excitation. They also demonstrated mode selective reaction of water molecule with H atom. This approach, a passive control, has been employed to control many reactions.

Wave nature of atoms and molecules has made development of quantum control of processes, physical as well as chemical, possible. Quantum control of reaction can be achieved through phase control of the lasers. This coherent control, an active control, can be achieved in many ways, such as interference between two coherent excitation routes, pump-dump technique and optimized laser control to create and control the excited molecular wave packets.

Using two sequential short duration coherent laser pulses, Zewail and his team demonstrated control of reaction of xenon with iodine through excitation of iodine. The first laser pulse, called pump pulse, creates a wave packet in the intermediate state B of iodine. The second laser pulse, called control laser pulse, lifts the wave packet above the reaction threshold. The reaction yield was modulated by varying the delay between the pump and control pulses. R.J. Gordon and his team, at university of Illinois, Chicago, USA, demonstrated phase control of ionization rates of HCl and CO, as well as branching ratio of the two product channels in the case of HI and DI, namely, photoionization and photodissociation, through interference effect. Many interesting experiments on coherent control of chemical reactions have been carried out. Coherence control

has made chemistry very fascinating, and put it on a high pedestral. It has, in fact, given birth to a new area in chemistry, Coherence Chemistry, which is rapidly coming of age.

Laser have been extensively used to study fast and ultrafast photophysical processes, such as intramolecular energy transfer, photoindiced electron transfer, interfacial as well as in bulk, important from the viewpoint of understanding fundamental processes, carrying out selective chemistry, or making devices, such as optical switches, solar cells, etc.

Though the dream of the scientists to realize bond selective chemistry has met with obstacles, andappears to be not as simple as envisaged originally because ofintramolecular energy redistribution, it is partly realized in reactions involving small molecules, and the march towards the goal is still on.

In short, lasers are being employed to understand the mysteries of the chemical act, as well as carry out new chemistry, selective and/or efficient. Chemistry would not have been the same what it is to-day, without the induction of lasers into its weaponry. Lasers are playing increasingly important role in bio-sciences also. Identification, quantification, diagnostic as well as therapy are some of the functions performed by lasers.

IANCAS has been bringing out various theme-based scientific and technological bulletins relevant to the Department as well as society. In the present issue, "Lasers in Chemistry", some of the exploitations of lasers in chemistry and biology related area have been compiled. I am sure that the readers will find the articles of this issue very interesting and thought provoking.

Dr. P.N. Bajaj
Radiation and Photochemistry Division, BARC
pnbajaj@apsara.barc.ernet.in

Guest Editorial

Dr. Dipak K. Palit

Discovery of lasers in 1960 and tremendous development of laser technology since then, have completely revolutionized each and every field of science and engineering. At present lasers are available spanning the electromagnetic spectrum from submillimeter waves to soft X-rays; their output power varies anywhere between a few microwatts to a few petawatt (10^{12} Watt in peak power). Lasers have been developed to operate both in continuous wave (CW) or pulsed mode with durations as short as a few attoseconds (10^{-18} s). Lasers with ultra high resolution (nearly single frequency with bandwidth of as low as a few Hz), have also been developed.

With the advent of these lasers, new areas of research have emerged both in fundamental and applied sciences. Lasers have made possible the development of many powerful spectroscopic techniques, both in time (ultrafast) and frequency (high resolution) domain, which could never be conceived using conventional light sources. These techniques have totally transformed the research in the fields of physics, chemistry and biology. Nuclear science and technology has also not been deprived from the gifted-power of the laser radiation, which has found its applications in fusion, isotope separation as well in the development of particle accelerators. The applications of lasers have been so extensive in science and technology in recent years that a single monograph is not adequate to cover all the aspects of laser applications. However, in this special thematic issue of IANCAS on "Applications of Lasers in Chemistry and Biology', we have made a novel effort to bring out a brief review of the applications of lasers in physical chemistry and biology.

Two most significant and sophisticated developments in the laser technology is the technique of 'mode-locking', which has helped in production of light pulses as short as a few femtoseonds and 'chirped pulse amplification', which has helped to amplify the output of a short laser pulse of a few nano Joule to a few hundreds of Joules. The spectroscopists and physical chemists have always been the pioneer in using these technologies as their tools to develop the more and more sophisticated spectroscopic techniques for investigation of atomic and molecular processes, which are very fast and not possible to study using conventional spectroscopic techniques. In my article, I have discussed the development of the ultrafast spectroscopic techniques and their application in investigation of the microscopic dynamics of laser induced chemical reactions. Sophisticated understanding about the relaxation processes in molecules and evolution of the system along the reaction coordinate have generated the possibility of bond-selected reaction control using laser. The aspects of controlling a chemical reaction using a laser have been elaborated by Dr. S. K. Sarkar. Dr. Sarkar has exemplified how the laser pulses, which are properly shaped with respect to their phase, frequency and polarization, can be used for breaking chemical bonds selectively at one's will. Dr.H. N. Ghosh has reviewed the application of ultrafast spectroscopic technique to study the electron injection and charge recombination dynamics in dye-sensitized nano-particles and emphasized the importance of reducing or eliminating the possibility of charge recombination process, which is responsible for the low efficiency of man-made solar energy devices. Drs. P. Sharma, R. K. Vatsa and S. K. Kulshrestha have explored the different aspects of interaction of high-energy laser pulses with atoms and molecules in the gas phase as well as in clusters. Drs. P. D. Naik and Awadesh Kumar have given a brief but concise account of the different techniques of laser spectroscopy.

Recently, Biologists have also found the lasers as valuable tools for their research. Fruitfulness of applications of lasers in biology and medicine is evident from the articles written by the scientists of three renounced groups, which have been active in this field of research. Prof. G.Krishnamoorthy describes how the time-resolved fluorescence spectroscopic technique has been established to be one of the most revealing windows of dynamics in proteins, DNA and other biomolecules. Dr Sudipta Maiti and Shri K. Garai describe how the lasers are combined with microscopy to create a whole new area called 'Biophotonics'. Traditional limits of light microscopy have been creatively tackled to yield quantitative measurements of molecular size to sub-nanometer scales and to produce fluorescence images of UV fluorescent biomolecules using multiphoton microscopy and fluorescence correlation spectroscopy.

I gratefully acknowledge the dedicated efforts of the authors, who have contributed to this issue of IANCAS Bulletin. On behalf of IANCAS and on my own behalf, I sincerely thank all of them.

Lasers in Chemistry

Dr. Prakash D. Naik *was born in 1959 in Karwar, India. He received his Bachelor of Science in Chemistry in 1980 from Karnataka University, Dharwar, India and Master of Science in Chemistry in 1983 from University of Mumbai, India. He is currently working as Scientific Officer in Radiation & Photochemistry Division of Bhabha Atomic Research Center, Mumbai, India. He received his Ph.D. Degree in Chemistry from University of Mumbai in 1992. He worked for a period of one and half year at Institute of Physical Chemistry, University of Heidelberg, Germany in the area of photodissociation and reaction dynamics with Prof. J. Wolfrum during 1992-1993. His current research interests focus on laser induced dissociation dynamics involving small polyatomic molecules and kinetics of important reactions in gas phase.*

Dr. Awadhesh Kumar *was born in 1964 in Bihar, India. He received his Bachelor of Science in Chemistry in 1985 from University of Delhi, India and Master of Science in Chemistry in 1987 from Indian Institute of Technology, Kanpur, India. He is currently working as Scientific Officer in Radiation and Photochemistry Division of Bhabha Atomic Research Center, Mumbai, India. He received his Ph.D. Degree in Chemistry from University of Mumbai in 1995. He worked for a period of two years at National Tsing Hua University, Hsinchu, Taiwan with Prof. Yuan Pern Lee. His current research interests focus on dynamics of gas phase reactions induced by lasers using resonant four-wave mixing and laser induced fluorescence with special reference to atmospherically important free radical species.*

Introduction

Chemistry that started as an art and wild-guess practices at the alchemist time has been developed as a fully-grown science subject. In this evolution, in recent years, lasers have made a significant contribution. The characteristics of lasers like high power, monochromaticity, small divergence, coherency and pulse nature are extensively being exploited for application in chemistry. In the beginning, chemical scientists felt that the cherished dream of breaking a bond, as they wish, would be fulfilled by lasers. They thought that they just need a right frequency IR-laser photon beam (photon of frequency exactly in tune with local mode frequency of chemical bond) to deposit energy in excess of bond dissociation energy for carrying out the bond selective chemistry. On subsequent development of monochromatic, tunable laser sources in IR region, e.g. CO_2 laser, the above type of experiments i.e. infrared multiphoton excitation (IRMPE) and dissociation (IRMPD) were performed. It was realized from these experiments that the density of states at energy equivalent to chemical bond is so high that in most of the cases, the selectively pumped energy is lost from its site of excitation due to rapid intramolecular vibrational energy distribution.

Although the dream of chemist is yet to be fully realized, it is well known that lasers have contributed immensely to chemical science, be as an analytical tool, as a reactant in synthesis, purification of material, probing the reaction, to name a few. This article deals with the first two applications, which are the most important areas of chemistry and other applications are listed in Table 1.

Dr. Prakash D. Naik and and Dr. Awadhesh Kumar, Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: pdnaik@apsara.barc.ernet.in; awadesh@apsara.barc.ernet.in

TABLE 1. Applications of Lasers in Chemistry

Characteristics	Advantages	Applications
High Power	Multiphoton process Low detector noise High scattering intensity	Nonlinear spectroscopy Laser pyrolysis Laser Induced breakdown Spectroscopy Saturation Spectroscopy Improved sensitivity Raman spectroscopy
Monochromatic	High resolution State selection	Spectroscopy Isotope separation Photochemically precise State-to-state reaction dynamics
Collimated beam	Long path lengths Forward scattering observable	Sensitivity Nonlinear Raman spectroscopy
Coherent	Interference between separate beams	Coherent anti-Stokes Raman Spectroscopy (CARS) Degenerate Four-wave mixing (DFWM) Two-colour Resonant Four-Wave Mixing (TC-RFWM)

Laser as an Analytical Tool

With the advent of the lasers, spectroscopic techniques advanced in two very important ways. For one, the capabilities of conventional techniques have been greatly enhanced. Second, new methods, previously impossible to implement have emerged. The high intensity of laser beam allows one to develop spectroscopic tool based on the weak processes such as Raman scattering and higher order processes, which were not practicable using conventional light sources. The most important techniques in this category are based on the nonlinear phenomena. It is well known that the contribution of higher order terms to induced polarization becomes significant only at very high photon intensities as intra-atomic electric field is very high. The contribution of higher order terms in induced polarization, P, can be approximately estimated from the famous Bloembergen expression,

$$P^{(n+1)}/P^{(n)} = E/E_{at} \quad (1)$$

Where E is the electric field of the incident electromagnetic light wave and E_{at} is the intra-atomic electric field and typically of order $3x10^8$ V/cm. Even at high intensities of $10^9 W/cm^2$, the ratio of succeeding polarization is small, about 10^{-3}. This shows why non-linear processes are negligible with conventional light and why high intensities of laser radiation are required to exploit such processes.

The most widely used spectroscopic techniques in chemistry are based on absorption and emission of electromagnetic radiations.

Laser based Absorption Techniques

Tunable Diode Laser Absorption Spectroscopy (TDLAS)

With the development of tunable dye laser, laser based absorption techniques started emerging but the real up-shoot was observed with development of semiconductor lasers with broad tunability in the infrared spectral range. In recent years semiconductor laser based absorption techniques are extensively being used for qualitative and quantitative analysis of chemical species. A laser-based technique has multi-dimensional advantage over the conventional discharge lamp, which includes compact device size, in-line measurement capability, and large signal-bandwidth. The real advantage is exploited in this area by the marriage of laser devices with sophisticated signal processing techniques.

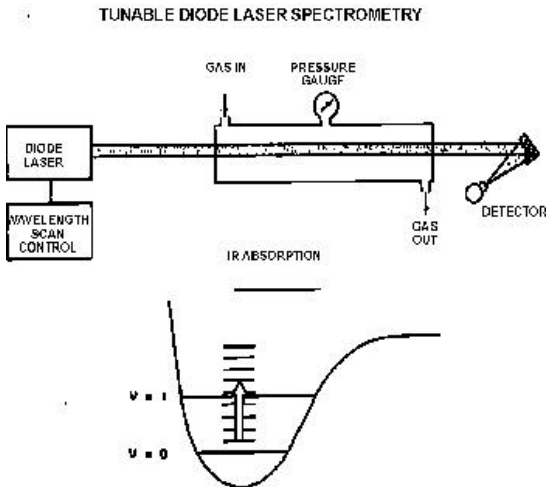

Fig. 1 A schematic representation showing a general setup, which is fundamental to all tunable diode laser systems.

Tunable diode laser absorption spectroscopy (TDLAS) is a highly selective and versatile technique for measuring many trace atmospheric constituents with detection sensitivities in the sub-parts-per-billion (ppbv) concentration range [1]. The heart of this technique is a tunable diode laser source, which emits in the mid-infrared (IR) spectral region between 3 and 30 μm. Many semi-conductor diode lasers are available commercially to access different regions in the mid-IR. In this region these lasers have typical band-width from tens to several hundred cm^{-1}. By adjusting the laser conditions, generally the temperature and/or injection current, the output wavelength of individual devices can be tuned continuously in small intervals of several cm^{-1} throughout the entire tuning width.

Atmospheric gases have moderate to strong absorptions in mid-IR region, while the major constituents, oxygen and nitrogen do not. This makes mid-IR accessed by diode lasers, an extremely attractive spectral region for detecting many atmospheric chemical species with high selectivity. In addition, at low sampling pressure (1 to 50 torr), the absorption lines in this region resulting from vibrational-rotational transitions, are very sharp. Thus the overlap between the transition lines of different chemical species is minimal, especially for small molecules. In other words, the spacing between individual absorption regions generally exceeds typical absorption line-width of 0.001 cm^{-1} for most atmospheric molecules. Tunable diode laser (TDL) linewidths by contrast, are typically in the 10^{-4} to 10^{-5} cm^{-1} range. These conditions result in the high resolution, and hence high selectivity. Thus compared to spectroscopic measurements in the visible and ultraviolet regions, where many atmospheric species exhibit broad and non-structured absorption features, TDLAS has many fold advantages. The schematic of simple and typical TDLAS is shown in the Fig. 1.

A multi-pass absorption cell [2,3] is usually employed as the sampling cell to increase sensitivity. Using base paths of 0.3 to 1.5 m, such cells result in total absorption path-lengths ranging between 10 and 200 m. In addition to direct absorption, TDL measurements are frequently carried out using the technique of harmonic detection. Most frequently, second harmonic detection is employed, which produces a zero baseline signal, thus eliminating the necessity of measuring small differences between two large intensities, I and I_o, as is the case for direct absorption. In this configuration, using total path-lengths around 100m, minimum detectable absorbance ($\ln I_o/I$) of 10^{-5} to 10^{-6} is frequently obtained.

Cavity-Ringdown Laser Absorption Spectroscopy (CRLAS)

Cavity-ringdown laser absorption spectroscopy (CRLAS) is an ultrasensitive method to make quantitative absorption measurements of very low concentrations of analytes [4]. The technique uses a laser pulse that is reflected back and forth between two highly reflecting mirrors, resulting into a very long path length, see Fig. 2.

An optical detector is placed behind one of the mirrors to detect the small amount of the light that passes through it. With no absorbing analyte present, the laser pulse will decrease in intensity after each round trip due to the loss of light through the mirrors and other losses. The intensity of the laser pulse decreases rapidly when an absorbing species is present between the mirrors. The analyte concentration is determined by calibrating this decay time with known concentration of analyte.

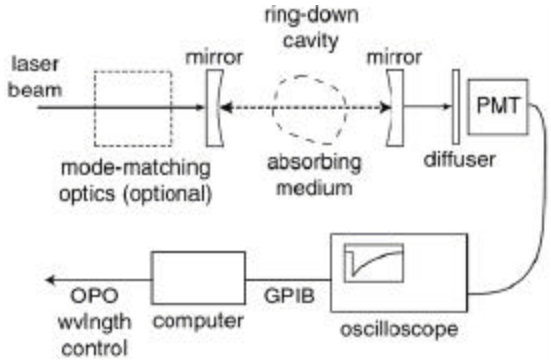

Fig. 2 A schematic of typical Cavity-Ringdown Laser Absorption Spectroscopy (CRLAS)

CRLAS can be used from the near-UV to the mid-IR. The wavelength range is only limited by instrumental constraints, such as the availability of high reflectivity mirrors and suitable pulsed laser sources. Accessing the near and mid-IR is accomplished with frequency-conversion techniques, such as Raman shifting or optical parametric oscillators (OPO). In principle, the analyte can be a solid, liquid, or gas. In practice, CRLAS is primarily used to measure gas-phase species due to scattering losses in solids and liquids.

Photoacoustic Spectroscopy

On illuminating a material with non-stationary (modulated or pulsed) radiation, the absorbed energy manifests into sound, this phenomenon is called photoacoustic (PA). Photoacoustic spectroscopy (PAS) is the application of the PA effect for spectroscopic purpose. When incident photons are absorbed by a molecule, depending on the energy of the photon, rotational, vibrational and electronic energy levels are excited. If condition is maintained such that most of the excited energy is lost due to collisions with similar molecules or buffer gas, the localized heating of the irradiated zone occurs. This results into local thermally-induced pressure rise, which generates pressure wave. The pressure wave (sound wave) when monitored by the microphone, gives an acoustic signal. The amplitude of this signal is proportional to the amount of local heat, and thus to the strength of absorption of molecules at the irradiated wavelength. The absorption spectrum is obtained by acquiring the

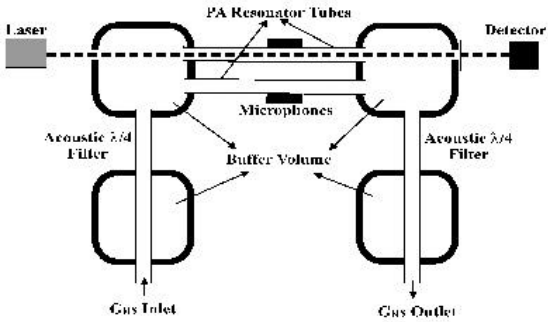

Fig. 3 A typical Differential photo-acoustic cell

acoustic signal as function of the wavelength of laser. Fig. 3 shows the cross section view of the differential photo-acoustic cell with two resonator tubes, buffer volume, and λ/4 filters being used in our laboratory for obtaining third overtone spectra of water molecules [5]. With the development of solid-state diode laser, this technique is progressing as a gas analyzer.

Laser Based Emission Techniques

Laser-Induced Fluorescence

Laser induced fluorescence (LIF) technique involves the absorption of radiation of a very precise wavelength by atomic/molecular species, and monitoring of subsequent emission from the excited species to obtain very specific probes of chemical species and its concentration. It is used in two different modes either by fixing the excitation wavelength and obtaining the dispersed fluorescence spectrum, or by obtaining the total fluorescence intensity as a function of excitation wavelength. Although in principle it can be used for detecting single molecular species, in practice for most of the systems one may obtain the sensitivity of 10^6 particles/cm^3. This technique may be used for in-situ monitoring, and is a well-established laboratory technique for obtaining the real time concentration of many small chemical species. Unlike absorption measurements, a long path length is not required in order to get high sensitivity, since photon counting techniques are very effective at low light levels. The major applications are in the measurements of OH, NO, CN, NO_2, NCO, SO, S_2, CH_3O, Cl, etc.

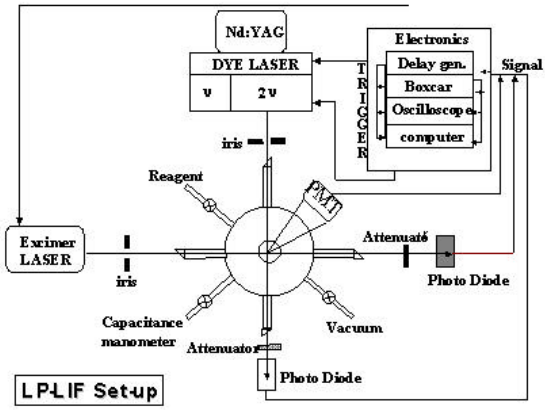

Fig. 4 Schematic of Laser Photolysis Laser Induced Fluorescence Setup

The laser-induced fluorescence spectrum of OH was utilized for many years in laboratory measurements of rate coefficients, before it was used for field measurements. The absorption spectrum of the OH radical lies in the ultraviolet near 300 nm. The generation of laser radiation in this region usually involves frequency doubling of the visible output of a tunable pulsed laser with high repetition rates. A state-of-the-art system usually uses a copper vapour laser, excimer laser (308 nm) or Nd:YAG pumped dye laser to pump a dye laser, the output of which is doubled to 300 nm. The laser radiation is tuned to a single rotational line in the OH spectrum, to enable specific detection of OH. The schematic of the LIF setup [6], which has been coupled with the laser photolysis is given in Fig. 4.

Laser Induced Breakdown Spectroscopy

Laser Induced Breakdown Spectroscopy (LIBS), often referred to as Laser Induced Plasma Spectroscopy (LIPS), is a type of atomic emission spectroscopy, which utilises a highly energetic laser pulse as the excitation source. LIBS can analyse any matter regardless of its physical state, be it solid, liquid or gas [7]. Even slurries, aerosols, and gels can be readily investigated. At high temperatures all elements emit light, similarly at sufficiently high laser power we can get emission from all the elements. Thus, the elements detected by laser and their sensitivities depend on power of the laser, energy levels and the transition probability of an element, resolution and wavelength range of the spectrograph, sensitivity of the detector and solid angle subtended by it at the emission volume. Operationally LIBS is very similar to arc/spark emission spectroscopy. Elements that can be sensitively measured using LIBS include Al, Ba, Be, Ca, Cd, Cr, Cs, Fe, Mg, Mn, Na, Ni, Pb, Se, Ti, and V, among others.

A typical LIBS system consists of a Nd:YAG solid-state laser, a spectrometer with a wide spectral range and with sufficient resolution, and a time gated detector with high sensitivity and fast response rate, see Fig. 5. This is coupled to a computer, which can rapidly process and interpret the acquired data. As such LIBS is one of the most experimentally simple spectroscopic analytical techniques, making it one of the cheapest to purchase and to operate. Apart from Nd:YAG laser, other lasers used for LIBS are mainly excimer laser generating energy in the visible and ultraviolet regions. In specimens with a complex matrix, i.e., containing a large number of different elements, it is necessary to use the spectrometer with high resolution so that it can resolve spectral emission lines in close proximity.

In LIBS the laser is focused onto a small area at the surface of the specimen. On firing the laser a small amount of material is ablated, ~ 1 µg, which instantaneously superheats generating a plasma plume with temperatures of ~10,000°C. At these temperatures the ablated material dissociates (breaks down) into excited ionic and atomic species. At the initial stage, plasma emits a continuum of radiation due to population of many excited states, and, thus, it is difficult to obtain any useful information about the species present. Subsequently, this plasma expands at supersonic velocities and cools down within a timeframe of a few µs. At this point, the characteristic atomic emission lines of the elements can be observed. The delay between the emission of continuum radiation and characteristic radiation is in the order of 10 µs, this is why it is necessary to temporally gate the detector.

A small amount of material is consumed during the LIBS process, hence the technique is considered essentially non-destructive or minimally-destructive. In addition with an average power density of <1W used, there is almost no specimen heating surrounding the ablation site. Due to the

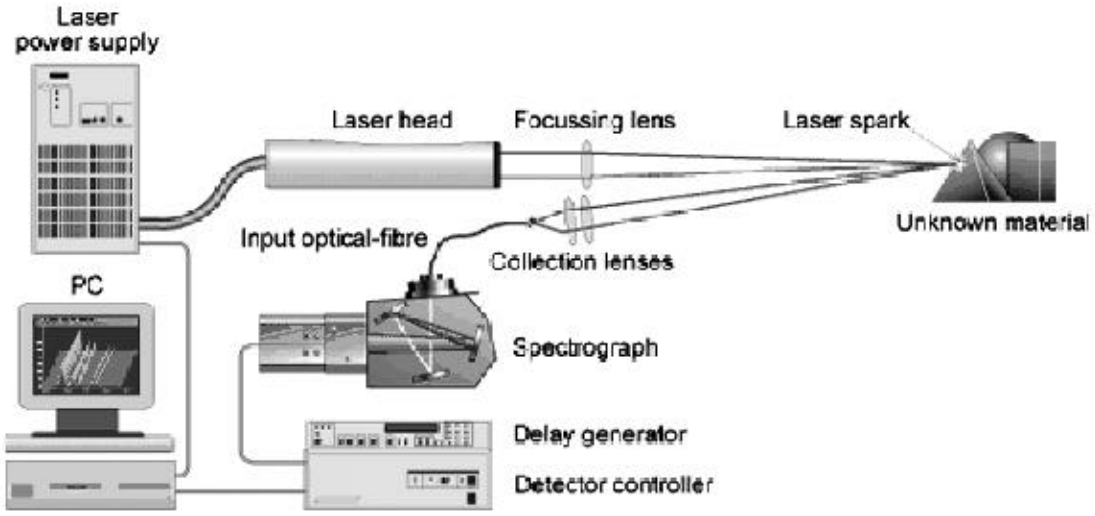

Fig. 5 A typical Schematic of Laser Induced Breakdown Spectroscopy

nature of this technique, sample preparation is typically minimised to homogenisation or is often unnecessary where heterogeneity is to be investigated or where a specimen is known to be sufficiently homogenious. Thus, the possibility of contamination during chemical preparation steps is reduced. One of the major advantages of the LIBS technique is its ability to depth profile a specimen by repeatedly discharging the laser in the same position, effectively going deeper into the specimen with each shot. This can also be applied to the removal of surface contamination, where the laser is discharged a number of times prior to the analysing shot. LIBS is also a very rapid technique giving results within seconds, making it particularly useful for high volume analyses or on-line industrial monitoring.

LIBS is an entirely optical technique, therefore it requires only optical access to the specimen. This is of major significance as fibre-optics can be employed for remote analysis. It should be noted that the future of LIBS lies on the miniaturisation of the components and the development of compact, low power, portable systems. If this is achieved then it may replace portable X-ray fluorescence, in particular to detect the light elements, for which LIBS is more sensitive than X-ray fluorescence.

LIBS, like all the other analytical techniques is not without limitations. It is subject to the matrix effects, which can be minimised by good specimen preparation and the use of accurate calibration standards. It is also subject to variation in the laser spark and resultant plasma, which often limits reproducibility. The accuracy of LIBS measurements is typically better than 10% and precision is often better than 5%. The detection limits for LIBS vary from one element to the other depending on the specimen type and the experimental apparatus used. Even so, detection limits of 1 to 30 ppm are not uncommon, but can range from >100 ppm to <1 ppm.

Laser Synthesis

Laser photons being quite costly, it may be used profitably in the synthesis of high valued products. The two areas where it has shown some promises are (1) synthesis of thermodynamically less stable isomer and (2) synthesis of chiral substances in high enantiomeric purity. The former strategy has been utilized in the conversion of 7-dehydrocholesterol to pre-vitamin D3. It has been shown that by using two-step laser photolysis with KrF (248 nm) and N_2 (337 nm) laser radiation, it is possible to eliminate the possible side reaction to the great extent, which is encountered in the normal photolytic method. This two-step laser photolysis route is shown to be a profitable method for vitamin D3 synthesis [8]. It is quite known that the medicinal

property of a chiral substance is very much dependent on its enantiomeric purity. In some cases one enantiomer acts as a best medicine, while other reduces its activity and may act as poisonous substance. Thus in future laser based synthesis may find its application in preparation of enantiomerically pure chiral compounds for high valued medical products.

Apart from synthesis of medicine, laser photon provides organic and medical chemists with a very powerful and versatile tool to selectively trigger reactions of target molecules or groups giving the possibility of selectively activating or deactivating drugs or enzymes. In many cases a disease that requires treatment with antibiotics, that is cytotoxic compounds, is localized in one particular organ or tissue. Systemic application of conventional chemotherapeutic agents, however, lacks selectivity and every tissue in the body suffers some damage, known as general toxicity. To improve selectivity of cytotoxic drugs, the work on the development of photo-activated antibiotics is actively pursued in many laboratories [9]. These compounds are virtually harmless in the dark, but are converted into the active forms in the desired location by laser irradiation. Such approach permits dual selectivity since the simultaneous presence of both photo-activated drug and light is required, so ideally the healthy regions of the system will not be affected. The wavelength region of interest for this purpose is in the range 650 to 950 nm, as both water and oxyhamoglobin are transparent in this wavelength region.

Laser Isotope Separation

One of the toughest scientific challenges has been an effective and inexpensive separation of a desired isotope of a chemical element from the remaining isotopes for applications ranging from medicine to energy to weapons [10]. Traditionally, isotope separation has been performed through gaseous diffusion and gas centrifuge. Over the past two decades, scientists and engineers have developed another technique, fundamentally different and much more efficient, called laser isotope separation (LIS). The technique is based on the fact that different isotopes of the same element, while chemically identical, absorb different colours of laser light. Therefore, a laser with narrow linewidth can be precisely tuned to ionize only atoms of the desired isotope, which are then drawn to electrically charged collector plates. This bulletin carries a separate article on this technique.

Conclusion

After all the development in chemistry, the focus is now on for better and more economical method of synthesis with increased yield and obtaining the substance in the environmental friendly way. Although lasers have not directly contributed much in chemistry in its primary goal, it has definitely contributed immensely by providing the faster and better sensitive analytical tools both for probing the reaction in real time scale and for the qualitative and quantitative analysis of chemical species. It will not be an exaggeration if one says that the most of the conventional analytical techniques are getting replaced by the laser based techniques. In years to come, laser will definitely bring a revolution in chemical analysis and in synthesis of several new and high valued products.

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Laser Steering of Chemical Dynamics

Dr. Sisir K. Sarkar *joined Multidisciplinary Research Scheme (MDRS), BARC in 1974 and is presently the Head, Radiation & Photochemistry Division, BARC. He has been involved from the very outset in Laser Isotope Separation programme of DAE and has established the necessary infra-structure and a dedicated research group for addressing various chemical problems using lasers. Many of these works have won international recognition from active laser chemistry laboratories around the world. After receiving Ph.D fromMumbai University, Dr. Sarkar did his post-doctoral work at Columbia University, New York with Prof. J.W.Flynn in the area of chemical dynamics using high-resolution semiconductor diode laser. He has worked extensively as visiting scientist at the P.N.Lebedev Physical Institute & Institute of Spectroscopy, Russia; Kyoto Institute of Technology, Japan and Institute of Chemical Process Fundamentals, Czech Republic with various laser systems including Free Electron Laser. He has published more than 150 papers and several review articles in reputed journals and conferences. His research interest includes laser selective photophysics and photochemistry, chemical dynamics, spectroscopy and laser development.*

Introduction

Chemistry is A + B $\rightarrow$ C + D, substances & their transformations. Change symbolized by the arrow ($\rightarrow$) in the equation, is always of real value, whether it is the chemistries of food preparation, explosives, dyes or metallurgy. The hold is psychological as well as economic; it is fascinating, frightening, thrilling – all of these - to see color changes, healing & explosions. But all along it has been easy to focus on the before and after, on the reactants and products, on the noun and not on the verb. But there is a change in the chemistry today, for the arrow is now well on its way to being understood. But why should we wish to do so many things with the arrow? Because we desire that which is not easily given unto us. Or we may profit from this capability.

In the laser's bright light, we are seeing signposts for a NEW DYNAMICS, where we direct the bonds to follow particular pathways leading to desired products – helping them to break their chains of bondage -a happy ending.

Exciting the Reaction Coordinate

Chemistry is traditionally performed by 'shake and bake' - by putting things together and applying heat. This random and wasteful deposition of heat energy into a large number of possible pathways often results in a product mixture rich in unwanted species. It is the coupling efficiency of the driving force (heat, pressure, etc.) with the reacting molecule that determines the efficiency of the reaction in terms of the desired product yields. The view of a chemical transformation as motion along the reaction coordinate, driven by specific molecular vibrations, points to a conceptually simple means of controlling the rate and path of the reaction; i.e., preparing a special vibration in a reactant molecule using IR laser photons, that excites motion along the desired reaction coordinate. In this manner laser light can be made to preferentially drive a particular pathway by matching the desired energy requirements, consequently forming specific products.

The implementation of bond-selected reaction control requires a sophisticated understanding of the relationship between vibrational excitation and the

Dr. Sisir K. Sarkar, Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085;
E-mail: sksarkar@apsara.barc.ernet.in

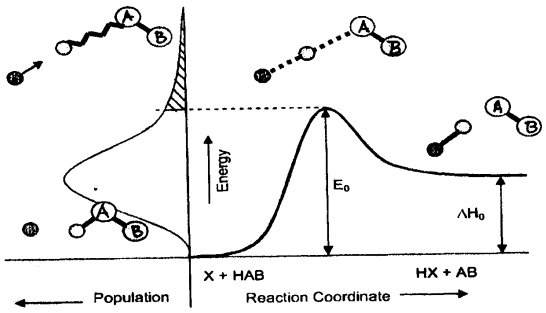


Fig. 1 Schematic diagram of the energy profile along the reaction coordinate for a bimolecular reaction. The shaded area of the distribution shows the HAB molecules with sufficient energy to react

evolution of the system along the reaction coordinate. The reaction coordinate is an unbound vibrational motion of imaginary frequency that carries the system across the energy landscape of the reacting species. Specific vibrational modes in reactants will have greater or lesser components along the reaction coordinate and choosing the correct vibration to excite is the key to control. In practice intuitive concepts (e.g. bending mode excitation favours isomerisation) relating the initial and final states of the system may be invoked. For example, in the hydrogen atom abstraction reaction the H-A stretching vibration has a large component along the reaction coordinate since that bond lengthens in passing through the transition state (Fig. 1). Obviously coupling of the initial state to the "wrong" modes would be detrimental, and thus the big challenge is "to prevent the system from going the wrong way".

The majority of chemical reactions possess activation energies in the region of 3 eV. The energy barriers to chemical reactions are therefore generally higher than the energy contained in a single vibrational quantum. For example, a 10.6 μm CO_2 laser photon has 0.117 eV of energy. The pioneering experiments on laser isotope separation conducted in the 1970s pointed the way to Multiphoton chemistry. Multiphoton absorption is now an established process for depositing sufficient energy in reactants.

Multiphoton Excitation - Isotope Separation

Probably none of the phenomena in laser photochemistry discovered in the past is as exciting as infrared multiphoton excitation and dissociation (IRMPE and IRMPD). In 1973 Russian chemists Ambartsumian and Letokhov announced the first successful laser-induced unimolecular dissociation of isotope specific SF_6. The energetics of the process were quite astounding, requiring approximately 35 photons per molecule to break the S-F bond!

$$SF_6 + nh\nu \rightarrow SF_5 + F \ (n \sim 35, CO_2 \text{ laser})$$

This pioneering isotope separation experiment was made possible by the relatively large isotopic shift in the absorption spectrum of sulphur. A high-energy CO_2 laser was focused into a gas cell containing 32-SF_6 and 34-SF_6 at low pressure (~0.1 torr). The laser was tuned to the 32-SF_6 (ν_3) vibrational mode. After a few thousand laser pulses analyses by IR spectroscopy and mass spectrometry of the contents of the cell reveals the almost total destruction of the irradiated species (32-SF_6). Thus the initial gas mixture was enriched in 34-SF_6. Subsequent experiments have indicated that for successful multiphoton dissociation a rather stringent set of conditions must apply. The laser excitation must be of sufficient energy to cause bond fission and it must be delivered in a short time if the dissociation is not to be that favoured by statistical thermodynamics. Some high laser fluence (J cm^{-2}) threshold is generally observed to achieve the desired rate of photon absorption. The isotope selectivity is lost exponentially with increasing pressure as collisional-induced relaxation processes disrupt the selectivity of excitation.

The following qualitative picture has emerged out of the numerous experimental and theoretical studies in this subject. The molecular energy levels are separated into three regions. In the lowest energy range (region I), the density of molecular states is very low and laser field interacts with isolated discrete molecular states. Several mechanisms to overcome the anharmonic detuning have been considered viz. (a) power broadening (b) rotational anharmonicity compensation (c) multiphoton transitions etc. Once the molecule has absorbed a few quanta, the density of molecular states become very large and time evolution can no longer be

described in terms of a few isolated molecular states. This region is denoted as quasi-continuum (region II) where restrictions for absorption are less severe and the energy leaks from the driven mode to the other modes, building up until it enters a true continuum (region III) from where dissociation or predissociation or isomerisation channels open up. Recognition of this method as a means by which selective photochemistry may be realised, has whetted the appetite of photochemists who are excited by its possible applications in dissociation, isomerisation, control of chemical equilibria, synthesis of complex molecules and finally in bond selective chemistry. However, the SF_6 experiment was not in fact an example of bond-selected reaction. Similar experiments exciting the nearly identical stretching vibrationof S-F in SF_5Cl demonstrated that excitation energy was shared by the entire molecular framework and this case the weakest (S-Cl) bond was broken.

Changing Reaction Rates

Monitoring the products of reactants prepared in vibrationally excited states allows the extent of rate changes to be observed. Low activation energy or exothermic reactions possess fast rates even at room temperature so experimentally it might be difficult to observe any rate increase due to laser excitation. Endothermic reactions or those with high activation energies are much better suited to the study of reaction rate control. An example of an endothermic reaction that has been accelerated by vibrational excitation is that between photolytically produced Cl atoms and water. A series of O-H stretching states were prepared by depositing different number of quanta of O-H stretching excitation in one bond of the H_2O molecule. Three different vibrational states were produced, |02>, |03> and |04>, corresponding to the absorption of 2, 3 or 4 photons into the same bond. The reaction rate was monitored by looking at the production of OH spectroscopically.

$$Cl + H_2O \rightarrow OH + HCl$$

Two quanta of excitation place the system very near the top of the activation energy for the reaction (72 kJ mol^{-1}). Very low reaction rates were observed in this case. The deposition of another quanta of vibrational excitation (corresponding to the |03> state) enhanced the reaction rate by a factor of 8, consistent with overcoming the activation energy. Adding another quanta of excitation to the same bond, the reaction rate was further doubled (Fig. 2). Further experiments showed that on producing the |04> state a non-reactive water molecule is transformed into one that reacts on roughly half of its collisions with a Cl atom. This clearly demonstrate that vibrational excitation accelerated this reaction far beyond the thermal rate.

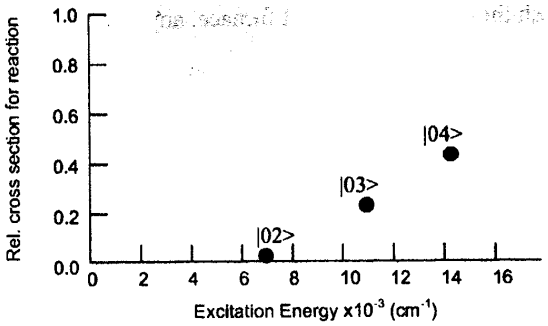


Fig. 2 Relative reaction rates for different vibrational states of H_2O

Reaction Control - Bond Selective Chemistry

The drive for the growth in the field of laser control was the dream of chemists to effect bond (mode) selective chemistry. Laser light has the unique ability to be absorbed by specific bonds in a molecule, creating relatively large and instantaneous nonequilibrium situations. Translating such specific excitation into specific reactivity has been the focus of much research but success has come only in the last decade.

Early indications were that as a general rule lasers should not be anticipated to break specific chemical bonds. Most bonds don't vibrate separately, like guitar strings or springs, but "couple" so that energy pumped into one bond almost instantly distributes itself through all the bonds in the molecule. Only a few molecules, such as water and ammonia have bonds sufficiently "uncoupled" for a nanosecond laser to influence the course of a chemical reaction. The bond coupling process is known as intramolecular vibrational energy redistribution (IVR). It is the microscopic,

intramolecular analogue of the macroscopic intermolecular process that brings a system to thermal equilibrium. The conclusion from these early experiments was that chaos rules out selective processes in unimolecular reactions. Chaos is a long time phenomenon and in systems with many degrees of freedom energy redistribution occurs in a sequential fashion with different stages occurring on different time-scales. The hope is that new femtosecond lasers will provide access to the time domain where energy redistribution into unwanted modes (IVR) is at least partially frozen, where the molecular motion is modified by the laser field. Unlike cw or nanosecond excitation, femtosecond lasers can in principle almost "pluck" a particular atom, exciting much localised regions of molecules.

Passive Control

The idea here is to use IR laser photons to excite specific bond vibrations (modes) in reactant molecules before reaction. The reaction is then left to evolve along the selected reaction coordinate, forming the products of choice. Successful passive control has been demonstrated for reactions involving simple molecules such as water, hydrogen cyanide, acetylene and ammonia. These systems contain bonds of rather different frequencies and it is possible to excite a specific bond that has a large component along the reaction coordinate, for example the H-A stretching vibration in the H-abstraction reaction. Successful examples of mode selective reaction control include: Cl + HOD, Cl + HCN, H + HOD, H + D_2O.

The key to initiating a bond-selected chemical reaction is depositing the energy in a motion that preferentially carries the system along the reaction coordinate. Fast, direct reactions fulfill this criterion best, because the reaction occurs before the excitation energy becomes statistically distributed among the various modes through IVR, in which case vibrational excitation is no more effective than heat.

The first bond-selected bimolecular reaction was demonstrated in 1990. It involved gas phase, vibrationally excited, isotopically substituted water molecules (HOD) and hydrogen atoms. The isotopic substitution differentiates the bonds so as to investigate their selective reaction. The overtone vibration (4 ν_{OH}) is isolated from the rest of the molecule because there are no other resonances in this region, so is a likely candidate for retaining its initial excitation. The excitation of the O-H bond leads to its preferential cleavage, influencing the product branching ratio. Laser-induced fluorescence of the products reveals a 200-fold excess of OD over OH. Further work has shown that exciting the O-D bond has the opposite effect, despite the HOD (4 ν_{OH}) and HOD (5 ν_{OD}) states having very similar energies.

$$H + HOD\ (4\ \nu_{OH}) \rightarrow H_2 + OD$$

$$H + HOD\ (5\ \nu_{OD}) \rightarrow HD + OH$$

Preparing a well-characterized vibrational state is typically performed using a highly monochromatic (bandwidth <0.1 cm^{-1}), IR wavelength (1-3 μm), millijoule energy laser pulse. Reactants are held in a low-pressure cell or formed into a supersonic jet expansion. The reactants must not collide more than once between excitation and detection. Collisions involving unexcited reactants or products with other species will lead to non-specific energy transfer distorting the observation of products. Reactive atoms (X) can be prepared by laser photolysis or microwave discharge of precursor molecules (e.g. X_2). The IR laser pulse selectively excites the reactant molecules in the presence of the reactive atoms. After a delay of about 1 μs to allow reaction, the products are detected using a spectroscopic technique such as laser-induced fluorescence (LIF) or resonantly enhanced multiphoton ionisation (REMPI) (Fig. 3). Such measurement also allows one to the determine the relative populations of individual energy states in the products.

Quantum (Active) Control

The passive control strategy is probably limited to a handful of simple reactions. In large molecules where the vibrational coupling is strong it may be impossible to identify a specific vibration with a significant component along the reaction coordinate. A potentially more powerful approach is to actively intervene during the course of the reaction to 'guide' the evolution of the reactants into products by controlling the phase of their motions.

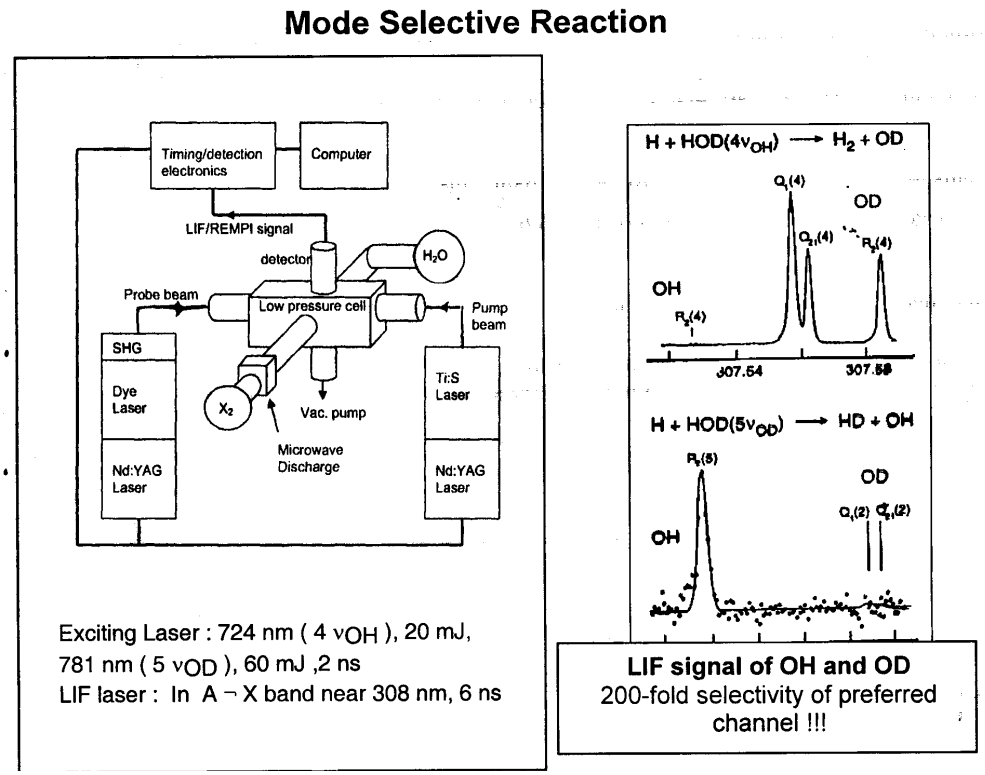


Fig. 3 Experimental set up for mode selective reaction and typical LIF signals of the products

A few laser chemists are trying to develop 'quantum control' (also known as active control), in which the laser pulse is delivered during the reaction itself and 'chaperones' it along the desired path by making molecules move in new ways. This approach requires a series of laser pulses whose spectral and temporal characteristics are continuously tuned to match the split second bends and stretches of individual bonds. The energy supplied by this complex series of laser pulses is thus tuned to the requirements of the chosen reaction co-ordinate. Laser chemists can now predict these requirements. The availability of high intensities from today's lasers makes this a tempting solution and with this experimental results have started pouring in.

Several control schemes have been proposed, which are based on either frequency or coherence characteristics of laser sources (Fig. 4). Brumer and Shapiro have proposed the coherent control scheme, which basically makes use of coherence nature of laser source. The control is achieved by making use of interference effect between two coherent excitation routes; the optimization parameter is the phase difference between two pumping sources. The other scheme is pump and dump scheme. In this scheme, the set of eigen-states are excited by a femtosecond laser source (pump laser). As the excited states evolve with time and when matches with the requirement of the desired product state, they are dumped into a lower repulsive state by another femtosecond laser (dump laser). The second scheme can easily be adapted in isotope selective manner and since the process is independent of isotope shifts, it can be more versatile.

Both the above schemes seems to have a limited application, as they have only one optimization parameter, phase in coherent control scheme and delay between pump and dump laser pulses in pump-dump scheme. To make use of full potential of laser source, Rabitz and co-workershave proposed the inverse quantum mechanical control scheme. Here, depending upon the choice of

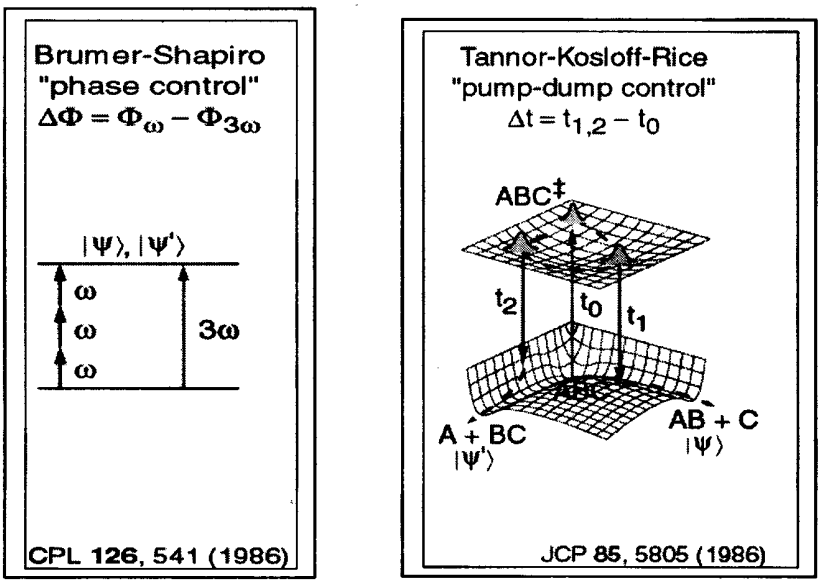


Fig. 4 Two schemes of Single parameter Coherent control

products, the shape of the electric field of the laser is worked out inversely, and made use to obtain the desired product. The desired electric field requirements for a given product choice is obtained using optimal control theory. There are two bottlenecks in this approach. Firstly, the accurate potential energy surface of the molecular systems is not available, and it is difficult to evaluate even with the present-day computation systems. Secondly, the required laser field turns out to be so complex that it is a Herculean task to generate it.

Given the general goal of steering the dynamics of the molecular system, the next consideration is how to identify the appropriate laser fields to meet the posed objectives. There are two major experimental aspects: first is a femtosecond pulse shaping system and the second is a control methodology based on a learning genetic algorithm. The pulse shaping system is based on modulating the amplitude and/or phase of the pulse in the spectral domain by use of a computer controlled programmable spatial light modulator. In this method the computer that controls the pulse shaping devise, also analyzes the output of the experiment and makes use of the learning algorithm capable of recognizing patterns in the input-output measurement relationship, thus guiding an iterative sequence of new experiments such that the desired outcome is maximized (Fig 5).

Pulse-shaping is the key component for coherent control. The shaped pulse was produced by first dispersing the frequencies comprising the short pulse with a grating transforming the pulse from time domain to frequency domain. The light was collimated with a cylindrical lens to provide a ribbon of light (the Fourier plane), where the frequencies are addressed spatially. The core of the pulse shaper is an externally addressable pair of liquid crystal arrays sandwiched between two parallel polarizers. Voltage to each individual strip of this structure is supplied through a matching pattern of indium-tin oxide (ITO) electrodes bonded to the surfaces of the nematic-crystal arrays. The SLM permits independent control of phase and amplitude for each of its n= 1…128 pixels. After recombining the modulated frequency components with a second lens grating pair, appropriately shaped pulse, typically 1mJ, 800 nm, 80 fs, 1 kHz rep rate is used for such experiments. Using this scheme, a few interesting systems investigated and their control experimental results are shown in Figs. 6-8.

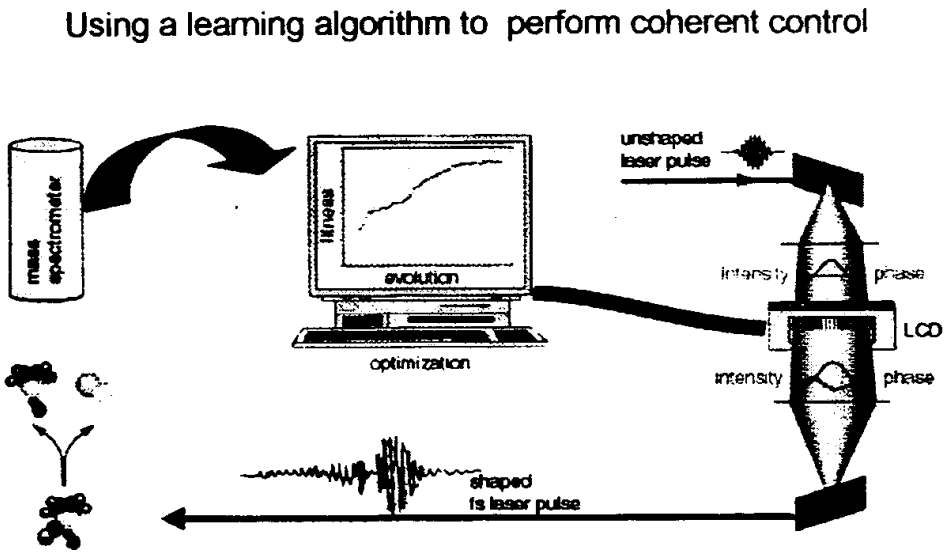


Fig. 5 Experimental scheme for optimal coherent control

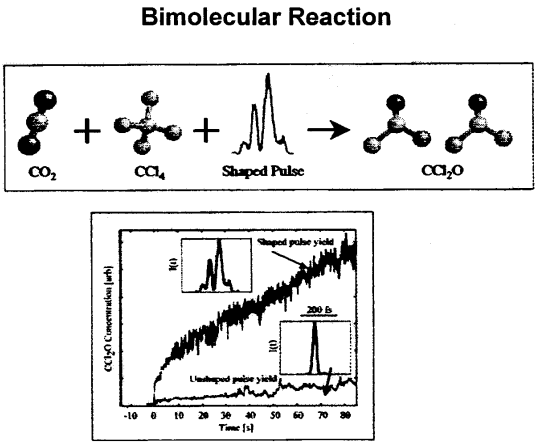
Challenges and Future directions

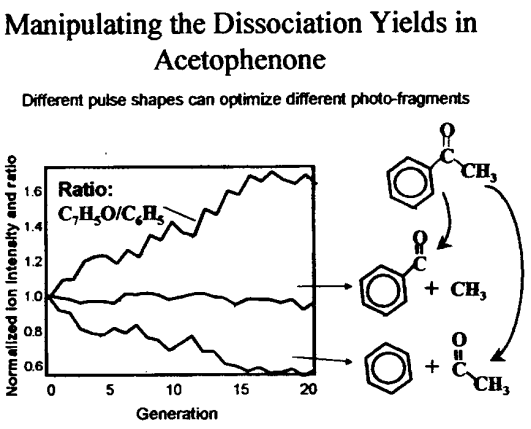
However, physical systems such as molecules are three-dimensional. One can therefore ask if it is possible to make use of the vectorial properties of light as well and to additionally "pull" along the correct spatial directions. This requires controlling the polarization state of light on a femtosecond time scale. Only recently such a technique called femtosecond polarization pulse shaping has been realized. For the first time, temporal intensity, momentary frequency, degree of ellipticity and orientation of elliptical principal axes can be varied in a complex manner within a single laser pulse using a 128-pixel, two layer LCD inside a zero-dispersion compressor.

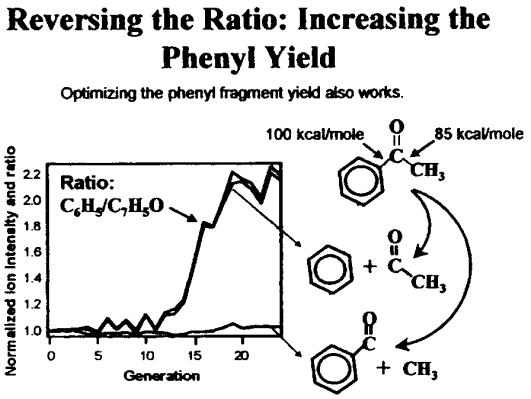
Fig. 9 shows the automated optimization of different fragmentation / ionization processes in the organometallic compound dicarbonyl (cyclopentadienyl) iron chloride, $CpFe(CO)_2Cl$, in the gas phase. Femtosecond polarization pulse shaping can be considered to be a novel spectroscopic technique, because the temporal as well as three-dimensional spatial properties of quantum wave functions can potentially be addressed and controlled. Potential control of stereochemistry i.e., photoisomerization reaction which has relevance in vision, chiral selectivity i.e., selectively generate different enantiomers (D or L) in drug design and door to many other possibilities has just been opened.

Lasers can now routinely be used to nudge, trap, and stir atoms to perform Nobel-prize winning feats, but so far molecules have avoided being subject to the same level of control. Now short laser pulses can be used to create large rotational forces on molecules, causing them to rapidly spin around at speeds that can be extremely well controlled. A molecule placed in a polarized laser field will align itself along the direction of polarization. If the polarization of the field were made to slowly rotate, the molecule would follow. Finally, if the rotation of the polarization is accelerated, the molecule would experience centrifugal forces that could either distort or even break some of its bonds. Now by placing a cloud of chlorine gas in such an 'optical centrifuge', the molecules begin to spin about 6 trillion times a second. At such a high speed the centrifugal forces are enough to snap the bonds holding each molecule together and the molecules shatter into a shower of chlorine atoms.

Potential uses of such optical centrifuge include separating gases of different molecules, or even isotopes of the same moleculesince heavier molecules break apart at slower spin rates because of







Figs. 6, 7 & 8 Results of typical bimolecular & unimolecular control experiments

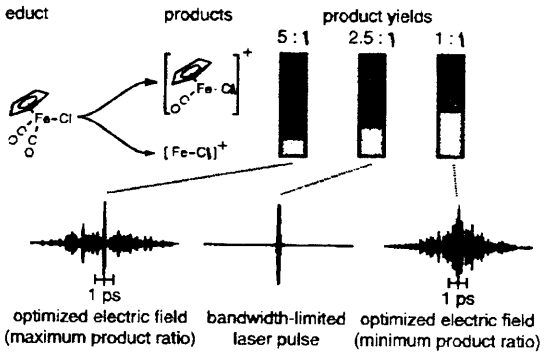


Fig. 9 Using specifically shaped femtosecond laser pulses, the $CpFeCOCl^+$ / $FeCl^+$ product ratio can be maximized as well as minimized with respect to bandwidth-limited laser pulses

a tiny difference in their moments of inertia which measures how they respond to rotation. This could also be used to break only selected bonds in a molecule, leaving bonds away from the centre of rotation intact, and thus opening the door to direct 'bond-by-bond control' of molecular chemistry.

Such control works, not only in the gas phase, where dephasing times are long, but also in the liquid phase and most recently demonstrated on surfaces. It has been shown to be robust and should occur for essentially any system. By determining the precise field that optimizes the desired product, we also learn about the molecule. By 2005, more than hundred systems have been successfully controlled and it can be envisaged that coherent control has applications far beyond Chemistry, Physics and Biology.

Acknowledgement

The author thanks his many colleagues in the control community upon whose work this article is written and apologize to those whose work could not be discussed.

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Femtochemistry: A Technique to Study Ultrafast Dynamics of Laser Induced Chemical Reactions

Dr. Dipak K. Palit *received his undergraduate training in Chemistry at Burdwan University, West Bengal. After a brief stint at the Kolkata University, he joined the 26th Batch of BARC Training School in 1982 and then Chemistry Division, BARC, after one year. Currently he is the head of Radiation and Ultrafast Chemistry Section in Radiation and Photochemistry Division. He obtained his Ph.D. Degree of Bombay University in 1989 working with Dr. J. P. Mittal. He was awarded the 'BOYSCAST Fellowship (1991- 92) of DST for post-doctoral research in the University of Pennsylvania, USA to work in the field ofultrafast spectroscopy with Prof. Robin M. Hochstrasser. He continues to work in the same field to study the microscopic dynamics of laser and high-energy radiation induced chemical reactions in condensed phase. In recognition of his contribution to the field of ultrafast dynamics, he has been awarded Rev. Fr. L. M. Yeddanapalli Memorial Award (1999) by the Indian Chemical Society, Bronze Medal (for the year 2001) of Chemical Research Society of India and Homi Bhabha Science & Technology Award of the Department of Atomic Energy, 2002.*

Introduction

Until very recently, the actual atomic motions involved in chemical reactions had never been observed in real time despite the rich history of chemistry over two millennia. Chemical reactions involve breaking of bonds in molecules or forming of bonds to form new molecules or change of geometrical shape of molecules. All these processes occur with awesome rapidity. Whether in isolation or in any other phase, this ultrafast transformation is a dynamic process involving the mechanical motion of electrons and atomic nuclei. The speed of atomic motion is ~1 km/second and, hence, to record atomic-scale dynamics over a distance of an angström, the average time required is ~100 femtosecond (fs). The very act of such atomic motions as reactions unfold and pass through their transition states is the focus of the field of femtochemistry. With fs time resolution, we can "freeze" structures far from equilibrium and prior to their vibrational and rotational motions, or reactivity. In femtochemistry, studies of physical, chemical, or biological changes are at the fundamental timescale of molecular vibrations: the actual nuclear motions (Fig. 1). The ephemeral transition states, denoted in the past by a bracket $[TS]^{\ddagger}$ for their elusiveness, can now be clocked as a molecular species, $TS^{\ddagger}$. In over a century of development, ultrafast pulsed-laser techniques have made direct exploration of this temporal realm a reality. A femtosecond laser probe pulse provides the shutter speed for freezing nuclear motion with the necessary spatial resolution. The pulse probes the motion by stroboscopy, i.e., by pulsed illumination of the molecule in motion and recording the particular snapshot. A full sequence of the motion is achieved by using an accurately timed series of these probe pulses, defining the number of frames per second. The individual snapshots combine to produce a complete record of the continuous time evolution—a motion picture, or a movie.

Moreover, the fs timescale is unique for the creation of coherent molecular wave packets on the atomic scale of length, a basic problem rooted in the development of quantum mechanics and the duality of matter. Molecular wave functions are spatially diffuse and exhibit no motion. Superposition of a number of separate wave functions of appropriately chosen phases can produce the spatially localized and moving coherent wave packet. Laser pulses of duration of a few tens of 'fs' are shorter than the oscillation period of a low frequency vibrational motion and the bandwidth of the laser pulse is

Dr. Dipak K. Palit, Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085;
E-mail: dkpalit@apsara.barc.ernet.in

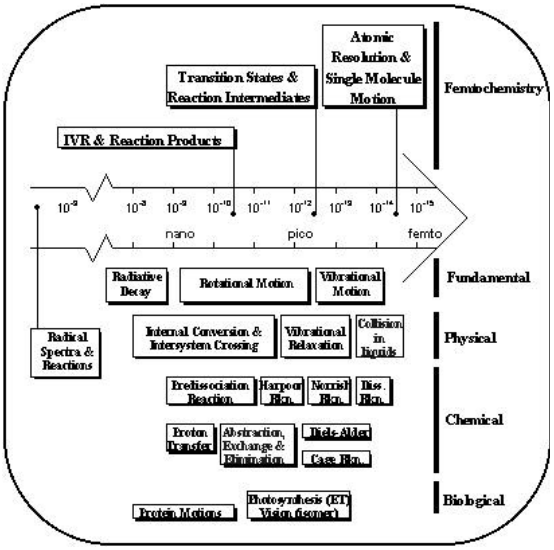


Fig. 1 Timescales: The relevance to physical, chemical, and biological changes. The fundamental limit of the vibrational motion defines the regime for femtochemistry. Examples are given for each change and scale.

sufficient for simultaneous excitation of a few vibrational levels coherently. These properties have a direct consequence of creating a coherent vibrational motion or wave packet in molecules following photoexcitation using fs laser pulses. The packet has a well-defined (group) velocity and position which now makes it analogous to a moving classical marble, but at atomic resolution, and without violation of the uncertainty principle. As long as the wave packet (typical width ~0.05 Å) is sufficiently localized on the scale of all accessible space (~0.5 Å or more), a description in terms of the classical concepts of particle position and momentum is entirely appropriate. In this way, localization in time and in space is simultaneously achievable for reactive and nonreactive systems.

This article deals with ultrafast spectroscopic techniques that have helped to unravel the microscopic dynamics of laser induced chemical reactions in condensed phase.

Time-Resolved Techniques for Ultrafast Spectroscopy

Invention of Flash Photolysis Technique

The method of investigation of photoinduced chemical reactions was first developed by Norrish and Porter a few years after World War II. They produced an intense burst of light (flash lamp) and created radicals in the sample, and, using another source of continuous light, they recorded the spectra of these radicals with microsecond time-resolution. They called this technique as 'Flash Photolysis'. For their contributions to fast kinetic spectroscopy, Norrish & Porter were awarded the 1967 Nobel Prize. During 1960's, the invention of the laser changed the picture. Discovery of Q-switched nanosecond lasers made possible the generation of giant and short duration laser pulses, which were used as the excitation source instead of the microsecond flash lamps improving the time-resolution of the flash photolysis technique to a few nanoseconds.

Pump-probe Technique

Subsequently there was a quick development of a new technique for generation of shorter pulses. Laser pulses of duration of a few picosecond were possible to generate by mode-locking in 1966. But the limitation of the response of the electronic devices (synchronization units) and the optical detectors, which were available that time, to less than a few nanoseconds, posed the problem to improve the time-resolution to picosecond time-scale using the picosecond lasers as the excitation source in conventional flash photolysis technique. However, the physical chemists could overcome the limitation of the electronics and optical detectors very intelligently by devising the new version of the flash photolysis technique, now known as the 'pump-probe technique'. The principle of this technique has been schematically presented in Fig. 2.

In this technique, the excitation (pump) and the probe pulses are generated from the same laser (for pumping and probing at the same wavelength) using a suitable beam splitter. It has also been possible to generate tunable probe pulses because of the very large peak power of the ultrashort laser pulses (20 ps laser pulse of 20 mJ energy has the peak power of 1

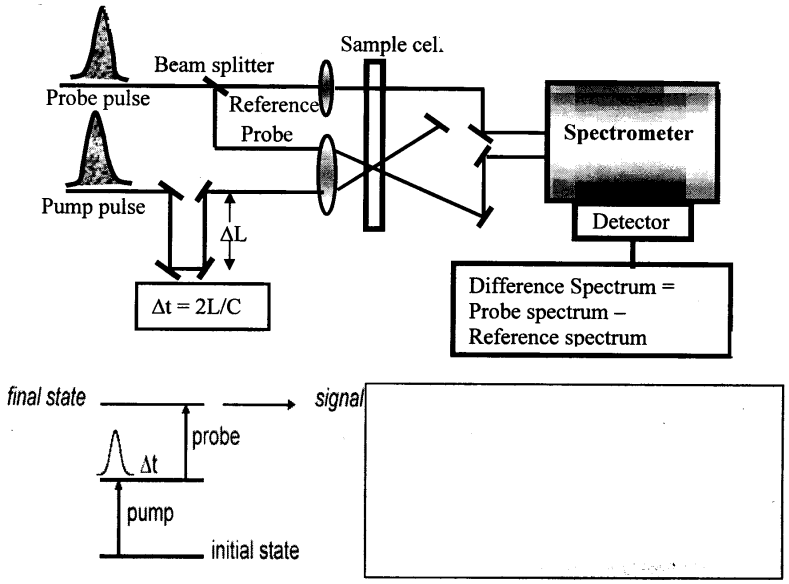
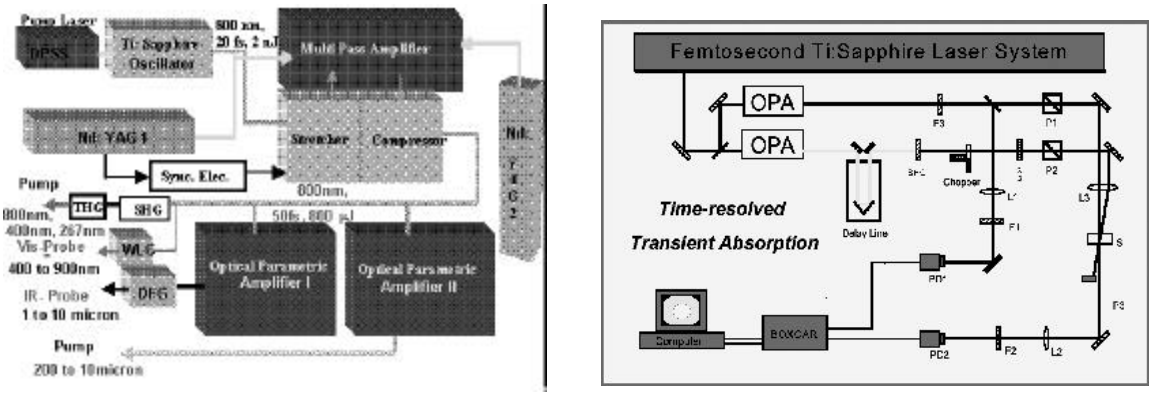


Fig. 2 Schematic diagram of a pump-probe set-up.

Giga Watt (10^9 Watt) power). This large amount of peak power when focussed onto a small region can create a large non-linear effect in any kind of medium (water or glass plates) and generate a 'continuum' light pulse of same duration of the original laser pulse but having a broad spectrum covering the entire visible and near IR region (400 – 1000 nm). This 'continuum' pulse can be used for probing the absorption of transient species created by the other part of the laser pulse. In a transient absorption experiment, the change in absorbance due to photoexcitation of the sample is measured in double-beam spectrophotometric method, by splitting the probe beam into two, one of which sees the excited sample and the other the unexcited one. Information regarding the excited state absorption (ESA), which occurs as positive absorption, or stimulated emission (SE) or bleaching, both of which are occurring as negative absorption, can be obtained by comparing the integrated intensities of the two probe pulses detected by integrating photodiodes (slow detectors) and boxcar averagers. The time-resolved measurements are performed by varying the relative lengths of the optical paths of the pump and probe beams using a 'single axis linear motion stage', which should have sufficient resolution and accuracy to obtain the desired time resolution (considering the fact that the light travels 1 mm distance in 3.3 ps or 1 μm in 3.3 fs). The 'zero-time' of the measurement is fixed when the pump and probe pulses reach at the same, i.e. the length of the paths traveled by the pump and probe pulses measured from the beam splitter are exactly same within a few microns accuracy. Once the resolution of the delay rail is sufficiently small, the time resolution of the technique is limited only by the duration of the laser pulse, not on the detector response.

Hence, the time-resolution of this technique could be further improved by the discovery of sub-picosecond pulses from dye lasers in 1974 and a 6 fs pulse in 1987. In 1991, with the generation of fs pulses from solid-state Ti-sapphire lasers by Sibbett and colleagues, dye lasers have been replaced, and fs pulse generation has become a standard laboratory tool; the state of the art laser generating ~4 fs has made it into the Guinness Book of World Records (Douwe Wiersma's group). The wide tunability has been mastered using continuum generation as well as optical parametric amplification (OPA). The latter coupled with the difference frequency generation (DFG) technique has made possible generation of tunable femtosecond pump and probe pulses in the region 300 nm - 10,000 nm region and the probe pulses even in the terahertz region (1 – 100



(A) (B)

Fig. 3 Femtosecond Ti:Sapphire laser system (A) and the pump-probe set-up (B)

cm^{-1}). The wide tenability of femtosecond lasers has made possible monitoring the excited electronic as well as vibrational states of all kinds of molecules. The time-resolved vibrational spectroscopy with a less than 100 fs time-resolution has been proved to be a powerful technique for investigation of evolution of the molecular structure of the transient species or the transition states. At present, the transient absorption detection is not the only technique used in the pump-probe experiment for transient studies but also many other detection techniques, such as laser-induced fluorescence, time-of-flight mass spectrometry, laser multiphoton ionization, photoelectron spectroscopy, electron diffraction as well as nonlinear spectroscopic techniques (coherent antistokes Raman (CARS) and degenerate four-wave mixing (DFWM)) have been used for studying the dynamics of laser induced chemical reactions. Coulomb explosion is the most recent powerful probe developed by Will Castleman for arresting reactive intermediates. The schematic diagram of the femtosecond transient absorption spectrometer is shown in Fig. 3.

Dynamics of Chemical Bond-Breaking Process

Photochemists have dreamt for a long time to break a particular bond of a molecule selectively by using light to produce a useful fragment out of it. However, following photoexcitation of molecules, the excitation energy is quickly dissipated among all the vibrational modes and the bond breaking is seen to be controlled by the laws of statistical thermodynamics and selectivity is lost! Hence the questions have been frequently asked if it is possible to by-pass the laws of statistical thermodynamics by using intense ultrashort laser pulses and break the molecules at our will. The question of selectivity opened the door to the following fundamental questions: what is the time required to break a chemical bond in a photo-dissociation process and how the energy supplied to the molecule is partitioned between the fragments and the different kinds of motions of the fragments, such as kinetic, rotational and vibrational. Based on the concept of coherence and intramolecular vibrational-energy redistribution (IVR), Prof. Ahmed Zewail of California Instititue of Technology, USA, designed in 1987 an experiment to monitor the process of bond breakage ($ICN^* \rightarrow I + CN$) with ~40 fs time resolution, resolving, for the first time, the elementary process of a chemical bond and observing its transition states [1]. This gave birth of 'Femtochemistry'. One year later in 1988, they reported their results on the photodissociation dynamics of NaI and HgI_2 [2-4], which represented a paradigm for the field of femtochemistry. In recognition of his pioneering work in femtochemistry, Prof. Zewail was awarded the Nobel Prize in chemistry in 1999. In his Nobel Lecture, he emphasized the importance of femtochemistry: '… Knowledge of the mechanisms of chemical reactions is also important for our ability

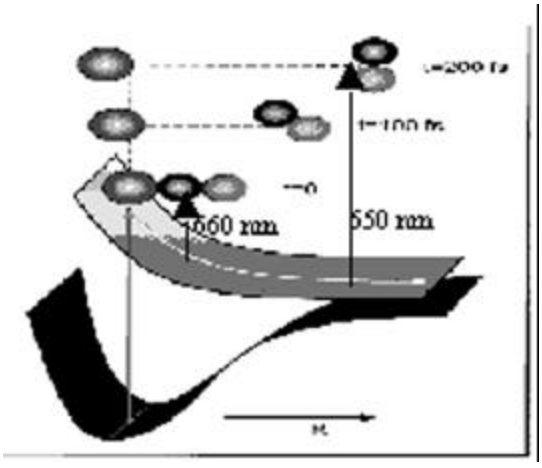


Fig. 4 Schematic potential energy diagram to show the photodissociation of HgI_2 and monitoring the transition states using probe lights of different wavelengths

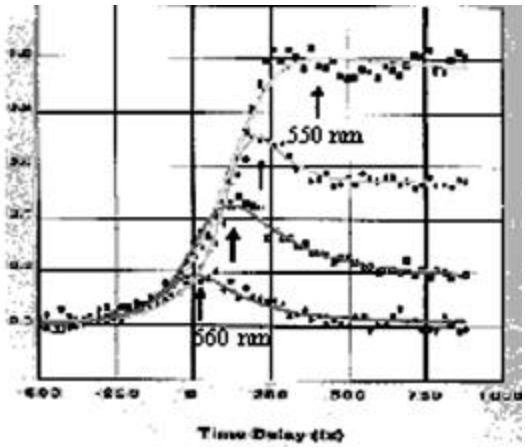


Fig. 5 Transient absorption signal at different monitoring wavelengths. Probe of 550 nm monitors the vibrationally hot HgI photoproduct and shows that the bond dissociation time is about 250 fs.

to control the reactions. A desired chemical reaction is often accompanied by a series of unwanted competing reactions that lead to a mixture of products and hence the need for separation and cleansing. If the reaction can be controlled by initiating reactivity in selected bonds, this could be avoided….".

Steady state spectroscopic studies [5] reveal that when HgI_2 is excited by a photon of 330 nm wavelength to one of its dissociative electronic excited state (HgI*), the molecule is dissociated into two fragments, mercurus iodide ($HgI^{\#}$) and iodine atom (I). In this process, the $HgI^{\#}$ fragment is produced in the ground electronic state (X^2S^+), but with a large amount of excess vibrational energy. The iodine atom is also produced in the ground electronic state. Using the ultrafast transient absorption technique, it is possible to follow the dynamics of the bond breaking and energy flow process in the photodissociation reaction of HgI_2 in ethanol solution.

$$HgI_2 \xrightarrow{hv,330\ nm} HgI^* \rightarrow HgI^{\#} + I \qquad (1)$$

HgI_2, being a tri-atomic linear molecule, has four fundamental modes - one symmetric stretch, one asymmetric stretch and two degenerate bending modes corresponding to the frequencies 155, 237 and 33 cm^{-1}, respectively. Symmetric stretch and bending modes are bound motions but asymmetric stretch can provide the dissociative motion. This mode has the fundamental frequency, $\nu_0 = 237\ cm^{-1}$ and its time period of oscillation is 141 fs. On excitation of HgI_2 molecule onto one of the excited electronic dissociative state, the HgI_2* system evolves along the dissociative potential energy surface (Fig. 4). This motion can be described as the separation of the HgI and I fragments due to stretching of one of the Hg-I bond due to asymmetric stretch motion. When the separation between the two fragments reaches to about 5Å, they become free from each other's influence and the molecule is considered to be dissociated into two fragments. Different molecular configurations of IHg — I, which have different internuclear separations between the I and HgI fragments existing at different times between photoexcitation and dissociation, can be designated as the $TS^{\#}$'s. After creation of HgI_2* state on photoexcitation, it is possible to probe the transition states as well as the dissociated fragment or the product state, $HgI^{\#}$, using probe light of appropriate colour. In an experiment, a 330nm pump was used to dissociate the molecule and probe pulses in the wavelength range 400 to 700 nm to monitor the $TS^{\#}$ and the nascent photoproduct, $HgI^{\#}$ (Fig. 5). Both the laser pulses have 50 fs duration. The results obtained from the experiments using 330 nm pump

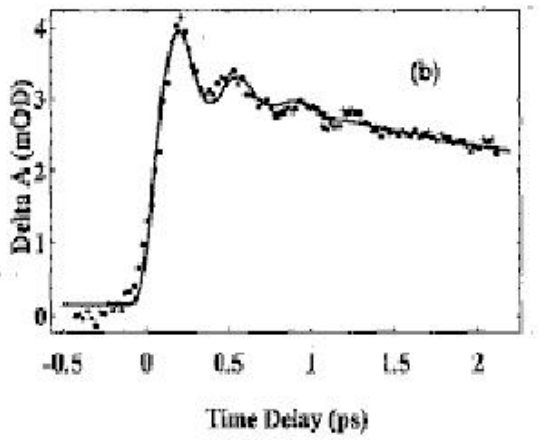


Fig. 6 Photolysis of HgI_2 in ethanol

and 550 nm probe pulses are presented here to explain the photodissociation and energy flow dynamics in the said process.

Let us make a simple calculation to illustrate the fact that the photodissociation process is really very fast and have a rough estimate of the time required to break the I-HgI bond in the photodissociation process. The excitation energy due to a single photon of 330 nm wavelength is 33,303 cm^{-1} and the energy required to photodissociate the I-HgI bond is 21,000 cm^{-1}. Hence the energy of recoil with which the fragments are moving away from each other is 9303 cm^{-1}. Hence the velocity of the fragments, with which they are getting separated is, $v = (2E_{recoil}/\mu)^{1/2} = 2$ Km/sec, where μ is the reduced mass of the separated fragments. If the bond is said to have broken when the fragments travel away by about 5Å or 5 x 10^{-13} Km apart from each other, the bond dissociation time is calculated to be about 250 fs.

The temporal dynamics of the transient absorption recorded at 550 nm due to photolysis of HgI_2 in ethanol is shown in Fig. 6. The transient signal recorded is due to $HgI^{\#}$ species, which absorbs in the visible region (400 – 700 nm). The time required to achieve the maximum absorbance in the signal due to the photodissociated fragment HgI is about 250 fs. This gives the bond dissociation time and agrees well with the value calculated.

The most important and interesting feature of the temporal dynamics of $HgI^{\#}$, as shown in Fig. 6, is an oscillatory component of the signal superimposed on the decay of $HgI^{\#}$. The oscillatory component of the signal persists for a few hundred fs. The total decay dynamics of $HgI^{\#}$ has been modeled with the response function,

$$OD(t) = A \exp(-t/\tau_A) \cos(\omega t + \pi) + B \exp(-t/\tau_B) \quad (2)$$

A and B are amplitude factors, τ_A the damping time constant for the oscillation amplitude, ω and π are frequency and phase of the oscillatory component and τ_B is the population decay time of the transient species. The best-fit parameters obtained are: A/B = 1.71; $\tau_A = 330 \pm 33$ fs; $\omega/2\pi c = 89.5 \pm 15$ cm^{-1}; $\tau_B = 2.75 \pm 0.27$ ps. The features observed in Fig. 6 arise from the modulated absorption of $HgI^{\#}$. Following photodissociation of HgI_2, $HgI^{\#}$ is created in a coherent superposition of vibrational states, which is popularlry known as 'Wave packet'. The wave packet then oscillates between the classical turning points of the potential energy surface of the ground electronic state of the $HgI^{\#}$ species causing the 550 nm absorption to be tuned in and out of resonance between the ground electronic state and one of its excited state (Fig. 7).

The fundamental frequency and anharmonicity constant of the stretching vibration, the only mode of Hg — I, are 126 and 1.3 cm^{-1}, respectively. Calculation shows that the beats correspond to a vibrational frequency of ca v = 15 and that the observed signal originates mainly from a fragment which is born with ca 1700 cm^{-1} of excess (mean) vibrational energy (Fig. 7). The remaining photolysis energy (ca 7600 cm^{-1}) must then be distributed among the translational and rotational motions of the fragments as well as the associated solvent motions. Finally, the overall signal decay with lifetime τ_B, which is 2.7 ps, should be due to solvent induced vibrational relaxation (EVR) process undergone by $HgI^{\#}$. The phase of the wave packet has been found to be π radian. This result suggests that when Hg—I bond is compressed, the molecule does not absorb at 550 nm. If the Hg — I bond in the nascent product HgI is compressed, such as would occur in a dissociation reaction producing I

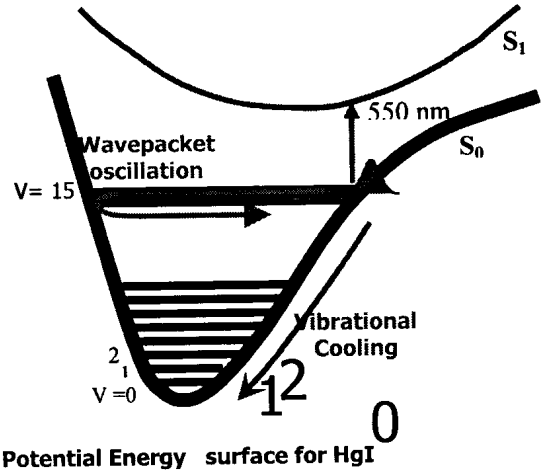


Fig. 7 Wavepacket Oscillation in the ground state of HgI. HgI is produced with high vibrational energy equivalent to that of v=15. While oscillating the wavepacket damps out because of vibrational cooling.

and HgI from HgI_2 through the asymmetric stretching coordinate, the resulting wave packet would not be detected until it would move to the attractive turning point of the ground state PES. Hence this would yield a phase shift of π for the absorption signal, as observed in Fig. 7.

In conclusion, the femtosecond time-resolved absorption technique could resolve that in photolysis of HgI_2 by 330 nm laser pulses of 50 fs duration, HgI is produced in a coherent superposition of vibrational levels on the ground electronic state. The mean excess vibrational energy corresponds to about 1700 cm^{-1}. This excess vibrational energy is dissipated to the solvent with an average lifetime of about 2.7 ps.

Ultrafast Intermolecular Hydrogen Bond Dynamics In the Excited States of Molecules

Intermolecular hydrogen bonding is a site-specific local interaction between hydrogen donor and acceptor molecules. Hydrogen bonding is a fundamental element of chemical structure and reactivity of water, proteins, and the DNA building blocks of life. The nature of hydrogen bond in solution is of particular interest and has been probed by diverse experimental and theoretical methods. However, not much information on structural and relaxation dynamics of hydrogen bond after electronic excitation of molecules that are part of an intermolecular hydrogen bond is available. Many organic molecules with a suitable functional group, such as, C=O, are known to be good hydrogen bond acceptors and even forms hydrogen bonded complexes with hydrogen bond donating solvents, such as alcohols and primary amines. Upon photoexcitaion, as a consequence of significant difference in charge distribution in the higher-energy electronic states of the solute molecules, the solute and the solvent molecules, engaged in the formation of hydrogen bonds, need to reorganize themselves. In this process, since the said solvents are associated liquids and the particular solvent molecule, which is engaged in formation of the hydrogen bond with the solute molecule, also is a part of the hydrogen-bonded network structure of the solvent, reorganization between the solute and solvent will disturb the hydrogen bond network structure of the solvent too and will be needing some kind of relaxation. This process is defined as the hydrogen bond dynamics and this controls the excited state dynamics of the solute molecule in a significant way. Dynamics of hydrogen bond occur on ultrafast time scales mainly set by vibrational motions of the hydrogen donor and acceptor groups. Experiments in femtosecond time regime have shown the potential to monitor the microscopic features of the hydrogen bond dynamics in real time [6].

Hydrogen bond dynamics in the excited state of fluorenone in alcohols using visible probe.

Fluorenone has been selected as an ideal probe molecule for studying the hydrogen bond dynamics because it is known to form intermolecular hydrogen-bonded complex with alcohols in the ground and excited states. Fluorenone is a planar molecule with a rigid frame-work and hence, no other relaxation process, such as conformational or configurational relaxation, than the relaxation process arising due to solvent motions are important in the excited singlet (S_1) state. It is of interest to see how the femtosecond spectroscopic technique has been applied to investigate the ultrafast relaxation dynamics in the S_1 state of fluorenone in different kinds of solvents and establish that the hydrogen

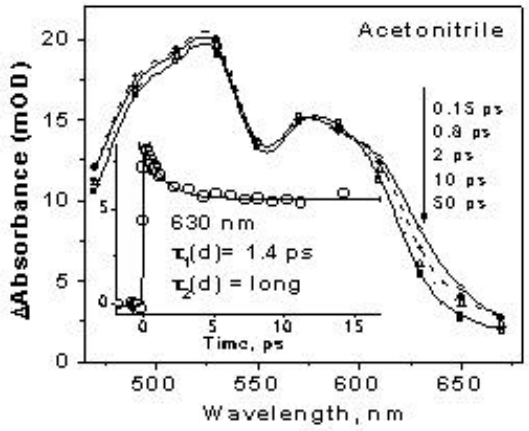


Fig. 8 Time-resolved spectra in acetonitrile

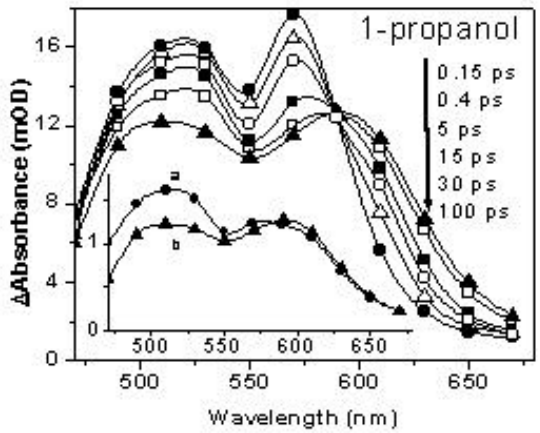


Fig. 9 Time-resolved spectra in 1-propanol.

bond dynamics does really play an important role in the relaxation process of the S_1 state of fluorenone in alcoholic solvents [6].

Figures 8 and 9 compare the time-resolved absorption spectra of the transient species formed upon photoexcitation of fluorenone in acetonitrile and 1-propanol using 400 nm laser pulses of 70 fs duration. In acetonitrile, the time resolved transient absorption spectra recorded reveal a slight decrease of absorbance in the 570 – 700 nm. The temporal profile recorded at 630 nm consists of an ultrafast decay component, followed by another very long-lived component, which arises as a residual absorption in sub-500 ps time-domain. The lifetime of this short component are 1.4 ± 0.2 ps. The lifetime of the S_1 state of fluorenone in acetonitrile is about 19 ns. Hence, considering a rigid and simple chemical structure of fluorenone, the ultrafast time-constants τ_1(d) can only be correlated to a process, in which the vibrationally hot molecules in the S_1 state transfer the excess vibrational energy to the surrounding solvent molecules.

On the other hand, the time-resolved spectra of fluorenone in 1-propanol solvent has shown much larger evolution than that in acetonitrile (Fig. 9). The transient spectrum constructed for 0.15 ps delay-time, i.e. immediately after photoexcitation, consists of two ESA bands with maxima at ca 520 and 590 nm. These features are very similar to those of the transient spectrum, recorded in acetonitrile (inset of Fig. 9), although, the relative intensities of these two bands in these spectra are somewhat different. The features of the transient spectra evolve with increase in delay-time because of decrease in absorbance in the 590 - 670 nm region and a concomitant increase in absorbance in the 490-590 nm region. A few of the temporal profiles recorded at different wavelengths following photoexcitation of fluorenone in 1-propanol are presented in Figure 10, along with the multiexponential best-fit functions. Each of the temporal profiles recorded in the 490 – 570 nm region shows an initial rise of transient absorption with the instrument response time (~120 fs) followed by another slower growth. The growth lifetimes, τ_1(g), which have been obtained by analyzing the temporal profiles monitored at 510 and 530 nm, are seen to have nearly equal values (~ $15 \pm$ 0.5 ps), but are shorter than those obtained from the analyses of the profiles at 550 and 570 nm (45.4 ± 1 ps). The temporal profiles recorded in the 610 – 700 nm are nonexponential and the lifetimes of are wavelength dependent. This suggests the occurrance of a non-equilibrium dynamical process because of hydrogen-bonding interaction in the excited state of fluorenone.

Similarities in the features of the transient spectrum recorded at 0.15 ps delay-time in 1-propanol and acetonitrile (inset of Fig. 9) suggest that upon photoexcitation, the hydrogen-bonded complex in 1-propanol dissociates easily and rapidly and the excited state of the free fluorenone molecule is produced in ultrafast time scale (which is faster than the time resolution of spectrometer). However,

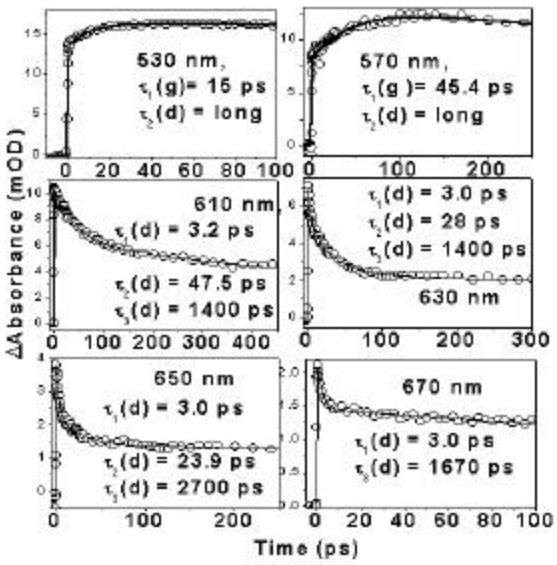


Fig. 10 Temporal absorption profiles recorded at a few wavelengths following photoexcitation of fluorenone in 1-propanol.

the solvent molecules cannot reorganize rapidly to incorporate the newly released alcohol molecule into its two-dimensional hydrogen bond net-work structure, i.e. there is a 'dangling' hydrogen bond still present. This non-equilibrated state of the excited fluorenone molecule is associated with a completely non-hydrogen-bonded solvent molecule, or a solvent molecule bonded into a chain to form a branch point, or some other unfavorable hydrogen bond configuration. This is a poorly solvated state, which is sufficiently unstable and tends to undergo geminate reformation of fluorenone – alcohol hydrogen bond with high probability. This reformation process is accompanied by the requisite reorganization of the hydrogen bond structure of the solvent to fully equilibrate and incorporate the dangling hydrogen bond into the hydrogen bond net-work structure of the solvent. With increase in delay-time, the evolution of the time-resolved absorption spectra, which is associated with the rapid decrease of transient absorption in the 590 – 700 nm region and concomitant increase in absorption in the 510 – 590 nm region, can be assigned to the geminate reformation of the hydrogen bond possibly with a new equilibrium geometry, accompanied by equilibration of the hydrogen bond net-work structure of the solvent.

Development of two ESA bands with maxima at 515 and 570nm with different growth lifetimes, as well as the wavelength dependent decay of the excited state of the non-hydrogen-bonded form measured in the 610 - 650nm region, are possibly the consequences of formation of two different conformers of the hydrogen-bonded complex in the excited state (Fig. 10). To delineate the aspects of hydrogen-bond dynamics in fluoreneone-alcohol systems investigated here, the optimized geometries of the fluorenone-methanol hydrogen-bonded complexes both in the ground and excited states are theoretically calculated. In both the ground and excited states, two distinct conformations for the solute-solvent hydrogen-bonded complexes for the fluorenone-methanol systems could be found. The structures of these conformers have been presented in Fig. 11. Two different conformations have arisen because of the different orientations of the methyl or the trifluroethyl group with respect to the plane on which the hydrogen bond (i.e. the [>C=O···H-O] moiety) exists. Considerable changes in the geometrical conformations of the hydrogen-bonded complexes in the ground and excited states, dictates the requirement of the reorganization of the hydrogen bond in the excited state of fluorenone.

Time-resolved IR absorption spectroscopic technique to study Hydrogen-Bond Dynamics in the Excited States of Hydrogen-bonded Complexes:

The example presented above reveals that in spite of the broad nature of the absorption spectra of transient species, valuable information can be obtained regarding the hydrogen bond dynamics by the detailed anslysis of the trnasient spectra and the dynamics monitored at different wavelengths using ultrafast visible spectroscopic technique. It was shown that time-resolved vibrational spectroscopic technique can be a more useful technique to monitor the hydrogen-bond dynamics in real time [7]. The vibrational spectroscopic technique has an added advantage over the one, which has the detection in the visible region that with this technique it is possible to observe changes in distinct functional groups involved in the hydrogen bond formation, which provided site-specific insight into local

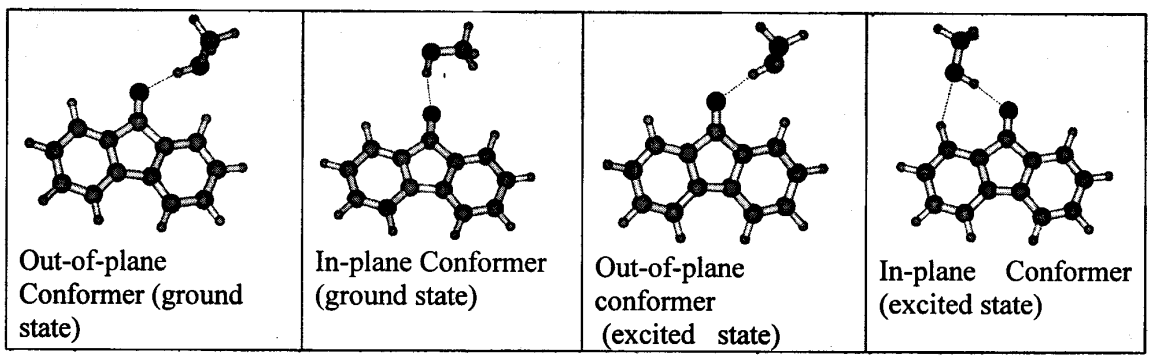
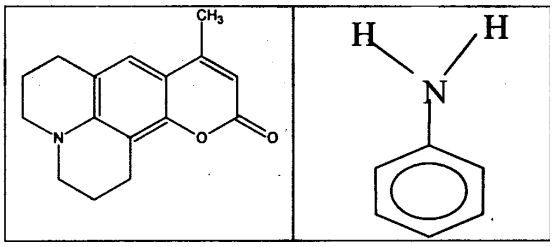


Fig. 11 Optimized structures of the fluorenone-methyl alcohol hydrogen-bonded complexes

dynamics. Coumarin-102 (C-102) was selected as the probe to study the hydrogen bonding interaction with aniline solvent (Scheme I). C-102 is shown to form hydrogen-bonded complex with aniline in the ground state. C=O group has a strong and broad absorption band in the 1710 – 1745 cm^{-1} region with an maxima at ca 1738 cm^{-1}, which is free from overlapping of any other bands related to O-H or N- H stretching or bending vibrations and hence structural changes in the C=O group can be easily monitored without any interference due to changes in the solvents hydrogen-bonded network structure. However, the changes in the hydrogen bonded network structure of the solvent on the C=O group following photoexcitation of the molecule can also be monitored by monitioning the changes in C=O absorption.



Scheme I Chemical structure of the Coumarin –102 molecule and aniline.

Curve 'a' in Fig. 12 shows the steady state FTIR spectrum of a solution of C-102 (15 x 10^{-3} mol dm^{-3}) in tetrachloroethylene (TCE). It shows a very strong absorption band having the maximum at ca 1738 cm^{-1} due to stretching vibration of the free C=O group. Vibrational spectrum of C-102 recorded in neat aniline has the absorption maximum due to C=O group at 1698 cm^{-1} (curve 'b'). The appearance of the new absorption band indicates the formation of an association complex between C-102 and aniline via formation of hydrogen bond between the C=O group of C-102 and H-N group of aniline (C=O…H-N).

Time resolved infrared absorption spectroscopic studies have been performed with the solutions containing C-102 in neat aniline. Upon photo-excitation of coumarin – amine systems by ultra-short (duration of about 150 fs) laser pulses of 400 nm, which is resonant to the electronic $S_1 \leftarrow S_0$ transition of C-102, only the C-102 chromophore is excited. Aniline molecules do not absorb at 400 nm and hence they stay in their ground electronic states. Figure 13 presents the temporal profile of the transient absorption monitored at 1742 cm^{-1}. The hydrogen-bonded complex has no absorption at 1742 cm^{-1} but the free C=O group absorbs strongly at this frequency. The probe is resonant to C=O stretching and causes the v=0 to v=1 transition in the S_1 state of C-102. The instrument response in terms of time-limited rise of transient absorption followed by a tri-exponential decay is observed. The lifetimes of the two components decaying in the early time domain were determined to be 0.5± 0.1 and 6.8± 0.4 ps and the third component is very long and lifetime of about a few nanosecond.

Since the lifetime of the S_1 state of C-102 in neat aniline is 1.4 nanoseconds, the time dependent absorbance changes, as shown in Fig. 13, indicates the role of hydrogen bond in the excited state

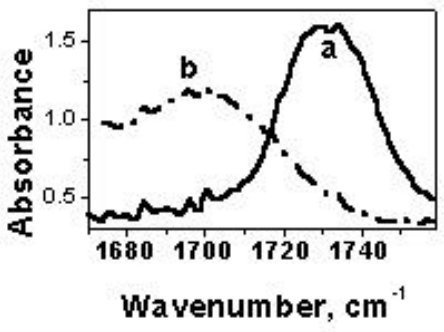


Fig. 12 Steady state FTIR spectra of C-102 (1.5 x 10^{-2} mol dm^{-3}) in tetrachloroethylene (TCE) and in neat aniline (curve 'b').

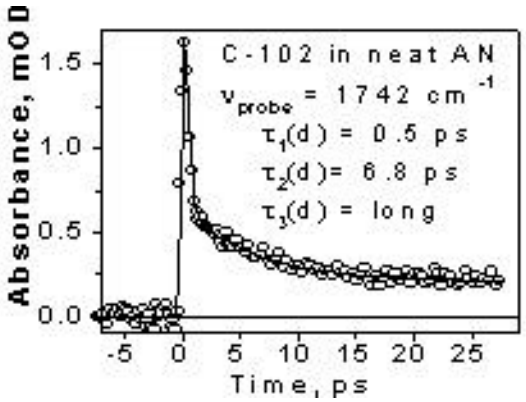


Fig. 13 Time-resolved change of vibrational absorption monitored at 1742 cm^{-1} in neat aniline (C and D). The lifetimes obtained by two exponential fittings of the data are given in the insets.

dynamics of C-102 in aniline. The appearance of transient absorption signal at 1742 cm^{-1}, which indicates the formation of free C=O group, immediately after the electronic excitation of the C-102 chromophore in C-102-aniline hydrogen-bonded complex indicates the instantaneous dissociation (<250fs) of the hydrogen bond between C-102 and aniline. The cleavage of the hydrogen bond is driven by the changes of the local charge distribution in the excited state of C-102. The impulsive enhancement of the transient absorption following optical excitation represents a non-equilibrium geometry of the cleaved hydrogen bond. Since 85% of the transient infrared absorption signal, which is characteristic of the C=O group, decays within a few tens of a picosecond (this is much shorter than the lifetime of the S_1 state of C-102 in aniline), it could be correlated with the process of reformation of hydrogen bond after its cleavage in the S_1 state. However, this new hydrogen bond, reformed between C-102 in the S_1 state and aniline molecules in the ground state, have equilibrium geometry and electronic structure, which are different from those formed when both are in the ground state.

Based on the arguments presented earlier, a model is proposed in which hydrogen bond breaking is followed by solvent reorganization on two time scales. After initial bond breaking, the fragments are rapidly, but incompletely, solvated to leave a 'dangling' hydrogen-bond. The component with 0.6 ps lifetime associated with the temporal profiles shown in Fig. 13, which represent the vibrational dynamics of the C=O group of C-102 in neat aniline, possibly have been arisen due to this rapid non-diffusive component of solvation. To complete the equilibration of the product state, the dangling bond must be fully incorporated into the hydrogen-bond structure of the solvent. This process occurs on a longer time scale related to the rate of hydrogen-bond reorganization in the bulk solvent. In hydrogen –bonding solvents, the longest component of the Debye dielectric relaxation is generally assumed to be connected with the rate of hydrogen-bond reorganization in the solvent. Thus the slower decay process having lifetime of about 6.8 ps could be correlated to the diffusive restructuring of the first solvation shell around the C=O group of C-102 in the S_1 state. The longitudinal relaxation time (τ_L) in liquid aniline, which is predicted to be the solvation time by the simplest continuum theory, has been determined to be 8.1 ps.

The experimental results clearly demonstrate the ability of the ultrafast time resolved vibrational spectroscopy to reveal the dynamics of hydrogen-bond not only involving the solvent molecule directly linked to the initially excited hydrogen-bonded acceptor solute molecule, but also the dynamics in hydrogen-bonding net work further away from it.

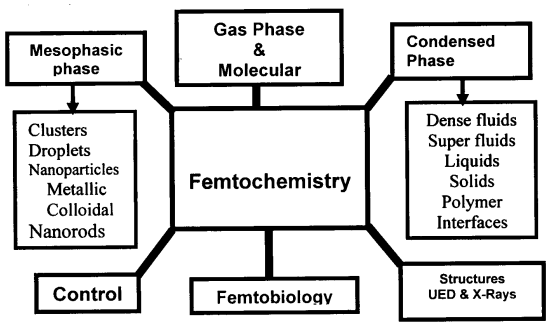


Fig. 14 Areas of Study in Femtochemistry

Epilogue

This article deals briefly how the femtosecond absorption spectroscopic technique helps to investigate the ultrafast dynamics of photodissociation and hydrogen bond. This technique has also been extensively used to study the ultrafast dynamics of intramolecular energy and electron transfer as well as conformational relaxation dynamics in suitably selected or synthesized model molecules, which have shown potential applications in photonics and solar energy conversion [7-10]. In a large molecule, electron and energy transfer processes, which are also in competition in many cases, have been shown always accompanied by large changes in conformation. In addition, the article of Dr. Ghosh in this issue presents an account how the ultrafast spectsocopic technique has been used in investigation of electron injection and charge recombination dynamics in dye-sensitized nano-particles. Presently, a laser driven picosecond accelerator based pulse radiolysis instrument is being developed. This system will use a femtosecond terawatt laser, to generate the picosecond electron beam using the principle of photo-electric effect as well as monitoring the transient species generated by the electron beam.

Applications of femtochemistry have continued to address the varying complexity of molecular systems, from diatomics to proteins and DNA. As the ability to explore shorter and shorter timescales has progressed from the millisecond to the present stage of widely exploited femtosecond capabilities, each step along the way has provided surprising discoveries, new understanding, and new mysteries. In their editorial on the 10th anniversary of Femtochemistry, Will Castleman and Villy Sundström put this advance in a historical perspective [11]. The Nobel report addresses with details the field and its position in over a century of developments. Fig. 14 summarizes areas of study and the scope of applications. Developments will continue, and new directions of research will be pursued. Surely, studies of transition states and their structures in chemistry and biology will remain active for exploration in new directions, from simple systems to complex enzymes and proteins, and from probing to controlling of matter—femtochemistry, femtobiology, and femtophysics. Since the current femtosecond lasers (4 fs) are now providing the limit of time resolution for phenomena involving nuclear motion, one may ask: Is there another domain in which the race against time can continue to be pushed? Sub-fs or attosecond resolution may one day allow for the direct observation of the coherent motion of electrons. In the coming decades, it may be possible to view electron rearrangement, say, in the benzene molecule, in real time.

Acknowledgement

I gratefully acknowledge the active contribution of my coworkers, Drs. H. N. Ghosh, A. K. Singh, G. Ramakrsihna, Shri J. A. Mondal and Ms. V. Samant. I am also grateful to Drs. J. P. Mittal and T. Mukherjee, for their constant encouragement.

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Interaction of Laser Radiation with Matter in Gas Phase: from Multiphoton Excitation to Coulomb Explosion

Dr. S.K. Kulshreshtha *graduated from Agra University in 1966 and joined the BARC Training School for the one year Orientation Course in Nuclear Sciences with specialization in Chemistry. After completion of this training he joined the Chemistry Division of BARC and started his research carrier with the study of diamagnetically substituted spinal ferrites using Mossbauer spectroscopy for which he was awarded Ph.D. degree in Physics by Bombay University in 1974. He did his post doctoral work in Germany at University of Erlangen – Nurnberg. Since last 38 years he has been working in the Chemistry Group of BARC in different capacities and is currently Associate Director, Chemistry Group and Head, Chemistry Division in BARC. During his long tenure of research, he has been associated with a variety of investigation and has published 135 research papers in reputed international journals. He is the recipient of a number of Scientific Awards and has been actively associated with a number of evaluation committees of the Department of Atomic Energy. His current research interest involves Material Science & Structural Chemistry with special emphasis on molecular electronics, surface science and catalysis, glass & ceramics, metal clusters and hydrogen storage and hydrogen energy.*

Dr. R.K. Vatsa *passed M. Sc. (Physical Chemistry) from Delhi University in 1986 securing first position. He joined Chemistry Division, BARC in 1987 after graduating from the 30 batch of the training school. Since then he has been involved with the gas phase photodissociation/ionisation of atoms, molecules and radicals. He obtained Ph.D (Chemistry) in 1994 from Bombay University under the guidance of Dr. J.P. Mittal. He did his post-doctoral work in Heidelberg University with Prof. J. Wolfrum on H atom detection using vacuum ultraviolet laser induced fluorescence. Along with Prof. Wolfrum's student, he completed the fabrication of high temperature vacuum ultraviolet laser induced fluorescence setup at Physical Chemistry Institute which is the only facility to study high temperature reactions with VUV detection of atoms.He along with other coworkers indigenously developed the Time of Flight Mass Spectrometer (TOFMS) system in the Chemistry Division. He has been a visiting scientist to Germany, France, UK and Czech Republic. He has 45 publications to his credit in various international/national journals. His current interests include photochemistry of molecular clusters and Coulomb explosion.*

Shri Pramod Sharma *obtained his M. Sc (Organic Chemistry) from University of Rajasthan in 1998. He joined NM&SC Division of BARC after completion of 43 batch of Training school in 1999-2000. Since then he has been actively involved in the study of laser induced (single and multiphoton) gas-phase photochemical studies of organic molecules using time of flight mass spectrometry. He has designed many components of TOFMS system. He is currently working on Coulomb explosion of clusters.*

Shri P. Sharma, Dr. R.K. Vatsa and Dr. S. K. Kulshreshtha, Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: mrsi04@apsara.barc.ernet.in

Light-matter interaction is a topic of research interest since the early days of electromagnetic theory. Light may be regarded as an electromagnetic wave, like any other wave, that has an amplitude, a frequency and a phase. The advent of laser in 1960 completely revolutionized the control over all three of these factors. The amplitude of the electromagnetic wave is related to its intensity and current laser facilities allow intensities up to about 10^{20} W/cm^2, fifteen orders of magnitude higher than the pre-laser era. This advancement in laser technology has revolutionized the understanding of primary photophysical and photochemical processes. The ever increasing spectral and time resolution in addition to power and range of wavelengths available have made it possible to excite molecules in different regions of electromagnetic spectrum with higher efficiency. Another great advantage with lasers is that they are pulsed sources providing optical pulses ranging from nanosecond to femtoseconds. This allows the study of phenomenon, which occurs on moderately fast to ultrafast timescale.

One of the most significant accomplishment in the field of lasers over the past decade has been the development of laboratory-scale table–top lasers which are capable of generating optical pulses with powers of 10^{12} Watt. Pulses from such lasers, when focused to small focal spot sizes, can give rise to intensities exceeding 10^{20} W/cm^2, which can produce novel and unforeseen effects in atoms, molecules and condensed matter (clusters and solids) such as generation of higher order VUV and X-rays, Coulomb explosion-leading to generation of few keV electrons, multiply charged energetic ions and even neutrons [1]. Laser induced Coulomb explosion is analogue to Coulomb explosion induced in glasses used for waste disposal by internal α-radiations. This phenomenon is of great concern in waste disposal as the total atomic displacement produced in the way of Coulomb explosion from each α-particle track is ~ 40000 to 80000, which exceeds the radiation damage from α-particles and heavy recoil nuclei altogether (~3500) by more than one order. This leads to degradation of materials undergoing self-irradiation due to internal radioactive agents. Studies have shown that degradation due to Coulomb explosion is very important for materials having low ionization energy - such as glasses which are often used for nuclear waste immobilization [2].

This article brings out the features of the interaction of laser with matter (atoms, molecules, and clusters), their intensity dependent ionization mechanisms and focus on the Coulomb explosion of van der Waal clusters.

Intensity Dependent Ionization Mechanisms: Multiphoton and Optical Field Ionization

Interaction of laser radiation, having photon energy much smaller than the ionization potential, with atoms and molecules primarily leads to their excitation by absorption of one or more photons provided certain optical selection rules are satisfied. These excited atoms or molecules would normally decay back to ground state by emission of radiation or undergo dissociation. However before decaying, if the laser intensity is sufficiently high, then these excited atoms/molecules can absorb additional photons from the laser pulse and reach higher excited states or may even get ionized. This process is called nonlinear ionization since the ionization of atoms/molecules shows a strong nonlinear dependency on laser intensity. Based on the intensity regime (see table 1), these nonlinear ionization processes have been loosely divided into multiphoton ionization (MPI) and optical field ionization (OFI) regime. The latter is a general term for different types of ionization mechanisms observed at very high laser intensity namely tunneling ionization, above threshold ionization and barrier suppression which are induced by the electric field of the laser pulse. A question which normally arises is that which mechanism is responsible for ionization for a given set of experimental conditions. A useful parameter γ, called the Keldysh parameter [3] helps the experimentalist to separate these nonlinear multiphoton processes into two regimes, the multiphoton and optical field ionization. γ is basically the ratio of atomic (molecular) electronic energy and field induced energies, $\gamma = (I_p/2U_p)^{1/2}$ [where I_p is ionization potential of atom or molecule and U_p is ponderomotive energy given in eV by 9.33×10^{-14} I (W/cm^2)(λ(microns))^2]. When $\gamma > 1$, the ionization is attributed to MPI processes, while for $\gamma < 1$ it is attributed to optical field ionization. In other words if ionization potential, intensity and

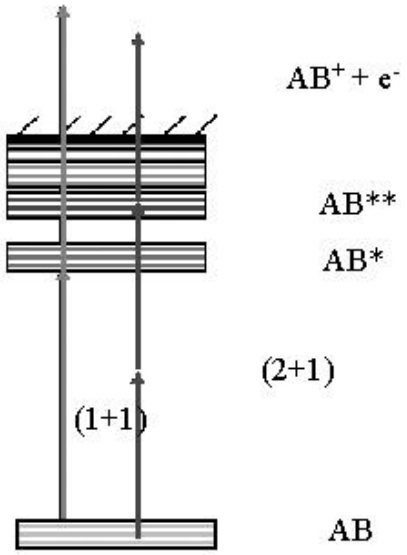

Fig. 1 (1+1) and (2+1) REMPI schemes for laser ionization

wavelength are known, it is rather easy to say whether the given species will ionize by MPI or OFI process. The qualitative difference between multiphoton and optical field ionization lies in the frequency dependency of the rate of these processes. The dependency is significant for multiphoton ionization and zero for optical field ionization.

In MPI, sometimes one encounters a special case where either the single photon or multiple photons of the laser light are resonant with a specific energy level of the atom/molecule. Ionization in these cases is called Resonance Enhanced Multi Photon Ionization (REMPI). In such cases the onset of ionization begins at ~ 10^6 W/cm^2 for (1+1) process which implies that first photon is resonant with certain energy level while the second photon ionizes the molecule. There could be other possibilities such as (2+1) or (3+1) REMPI processes (Fig.1) which requires simultaneous absorption of two or three photons to reach the intermediate resonant state. Since the cross section for two/three photon processes are much lower, these processes will require a higher intensity threshold (~ 10^9 W/cm^2) for onset of ionization as compared to (1+1) process. How does one know about the no. of photons that are resonant with an energy level?. Here, for a (2+1) and (3+1) process, the plot of log integrated ion signal vs log laser intensity shows a slope of ~ 2 and 3 respectively (and not 3 and 4). REMPI is very effective for studying the excited states of radicals and transient species and in detecting atoms/molecules with high sensitivity. It must be mentioned here that a (2+1) REMPI process could be very different from 3 photon MPI process, though the total no. of photons involved are same.

There are other cases in which multiple photons are absorbed by atoms/molecules without the involvement of real excited state. This phenomena is called Non-resonant multiphoton ionization and starts appearing ~ 10^{11} W/cm^2. Here the excitation is from initial ground state via intermediate virtual level to the ionization continuum. In the intensity region of 10^{13} W/cm^2, non resonant MPI becomes a dominant process for most of the atoms/molecules. Some qualitative peculiarities of multiphoton ionization process are given below.

(i) If an intermediate resonant state exists, the ionization rate is always much greater than direct ionization. The value of radiation frequency at which resonance ionization can occur are well defined.

(ii) If threshold ionization and above threshold ionization occur simultaneously, then the rate of above threshold ionization can be of same order of magnitude as the threshold ionization. It is difficult to detect above threshold ionization from ion detection only. However, one of the consequences of above threshold ionization is that electrons are ejected at energy greater than threshold ionization.

(iii) Difference between direct ionization and resonance ionization vanish at very high laser field strengths. The resonance states are shifted and spread by the radiation fields as strong as atomic field, so that resonance maxima in the excitation curves vanish.

Optical field ionization processes (involving above threshold ionization, barrier suppression ionization and tunneling ionization) takes place at intensities above ~ 10^{14} W/cm^2 where the electric field associated with the laser pulse approaches atomic field [4]. For a laser pulse with intensity I (W cm^{-2}), the associated electric field is given by:

Table 1. An approximate classification of laser intensities

Classification*	Intensity (W/cm^2)	Associated Electrical field (V/cm^{-1})
Weak	10^{-3}- 10^4	1-2.7X 10^3
Moderate	10^4-10^9	2.7X 10^3-1X10^6
Strong	10^9-10^{12}	1X 10^6- 2.7X10^7
Intense	10^{12}-10^{15}	2.7X 10^7-1X10^9
Super intense	10^{15}-10^{20}	1X10^9- 2.7X10^{11}

* the boundaries between different regions are not well defined

$$E\ (Vcm^{-1}) = 27.45\ (I)^{1/2}$$

Briefly, above threshold ionization occurs when an electron is excited to a Rydberg state located above the ionization potential. The Rydberg state then autoionizes creating a charged ion. Autoionization may lead to an ion which may be able to absorb additional photons, resulting in multiple charging of the ion. The processes of barrier suppression and tunneling ionization are often operative in high intensity laser fields of the order of 10^{15} W/cm^2 or higher. When the electronic states of the chromophore interacts with strong electric fields of laser pulse, the electronic states are Stark shifted to lower energies. The resulting potential surface allows ionization to occur more readily.

Interaction with Atoms

Atoms have very narrow absorption bands. As a result, on interaction with radiation from a resonant laser, they absorb and simultaneously emit frequencies characteristic of the element under investigation. This principle is commonly utilised for their detection in atomic absorption as well as emission spectroscopies.

Photoionization is one of the most fundamental and rich phenomena in atomic physics. The ionization of a quantum system is called nonlinear if the condition $h\nu < E_i$ is fulfilled, where $h\nu$ is the photon energy of the radiation and E_i is the binding energy of the outermost electron in the system. Such an ionization of an atom contradicts the Einstein's relation for the atomic photoelectric effect which is given by the opposite relation i.e. $h\nu > E_i$. However, multiphoton ionization $nh\nu > E_i$ is in agreement with Einsteins relation. At laser intensities in the range of 10^8-10^{12} W/cm^2 atoms undergo photoionization by multiphoton processes.

Resonance ionization of atoms coupled with suitable detection method such as time of flight mass spectrometer has been used for selective excitation and ionization of atoms with very high sensitivity (10^6 atoms or so). The selectivity arises mainly because of well characterized levels which are used for excitation and ionization. Use of narrow line-width lasers provides isotope selectivity and at the same time ensures that no undesirable species undergo excitation/ionization, thus suppressing the background. Due to these unique features resonance ionization is well suited and widely used for trace determination of actinides and other radioisotopes occurring in the environment. For ^{239}Pu, a three step excitation/ionization scheme using electronic and Rydberg transitions which employs lasers with wavelength λ_1= 420.76 nm, λ_2= 847.28 nm and λ_3 = 767.53 nm has been shown to be very efficient with a detection limit of 1x10^6, which is two orders of magnitude better than conventional α-spectroscopy of ^{239}Pu.

For intensities $>10^{14}$ W/cm^2 , the most prominent interaction channel for atoms is above threshold ionization (ATI) in which ionization takes place by the absorption of a number photons which is greater than the minimum required to ionize the atoms. At still higher laser intensities (~ 10^{15} W/cm^2) the predominant ionization mechanism is by tunneling through the Coulomb potential, which is distorted by the applied electric field associated with the laser pulse.

Interaction with Molecules

Molecules interacting with nano, pico and femtoseconds lasers undergo, Emission (fluorescence and phosphorescence), dissociation and ionization, the latter processes becoming dominant at the shorter pulse duration. Fluorescence and phosphorescence are simply re-emission of the absorbed laser energy from excited singlet and triplet states respectively. Fluorescence is observed when the total energy of the laser excited state is less than the energy required for lowest dissociation channel. Decrease in fluorescence intensity indicates operation of other channels which are mostly nonradiative. This method is used to determine the threshold bond dissociation energy as well as life time of the excited state. A typical example is acetone which shows high fluorescence yield beyond 307 nm. However, a sharp decrease in fluorescence intensity was observed for wavelengths lower than 307 nm suggesting an onset of hemolytic dissociation into CH_3+ CH_3CO radicals.

In studies with visible or UV radiations under weak or moderate laser intensity (10^3-10^6 W/cm^2), a molecule absorbs a single photon and dissociates on the lowest potential energy surface accessible within the Frank-Condon window. As the photon energy increases from UV to VUV, dissociation as well as ionization can take place. The dissociation mechanism in the UV and VUV region could be completely different. For example, absorption of a photon from Lyman alpha (121.6 nm, 10.2 eV) can excite a molecule AB to a neutral superexcited state AB**, which can then decay either by predissociation (to form A+B* or A*+ B) or by autoionization (to form $AB^+ + e^-$). Dissociative ionization (to form $A^+ + B + e^-$) occurs if the ionic state is unbound. Ion pair formation resulting in formation of $A^+ + B^-$ is also observed in some cases. This competition between photoionization and photodissociation is inherently interesting because it is typically a multielectron and multicontinuum process.

Much more complex dynamics becomes possible with intense coherent radiation. Irradiation with visible or UV lasers at intensities sufficient to induce multiphoton absorption opens the possibility of ladder switching and ladder climbing mechanism. In ladder switching process (dissociation followed by ionization), there is excessive fragmentation of the molecule which leads to dominance of low mass fragments in the spectrum with the parent peak often being missing entirely. On the other hand in ladder climbing mechanism (ionization followed by dissociation) the molecules first photoionizes, which then undergoes photofragmentation, leading to presence of parent ion along with photofragments in the mass spectrum. Here the fragmentation and subsequent photon absorption by the excited state neutral or parent ion occur within the same laser pulse. In Fig. 2, time of flight mass spectra of dimethyl disulphide (DMDS, CH_3SSCH_3) and dimethyl sulphide (DMS, CH_3SCH_3) recorded at 355 nm are presented. For DMDS, absence of molecular ion at m/e=94 suggested the dominance of ladder switching mechanism at 355nm. While in case of DMS presence of parent ion at m/e= 62 suggested the dominance of ladder climbing mechanism at this wavelength [5, 6].

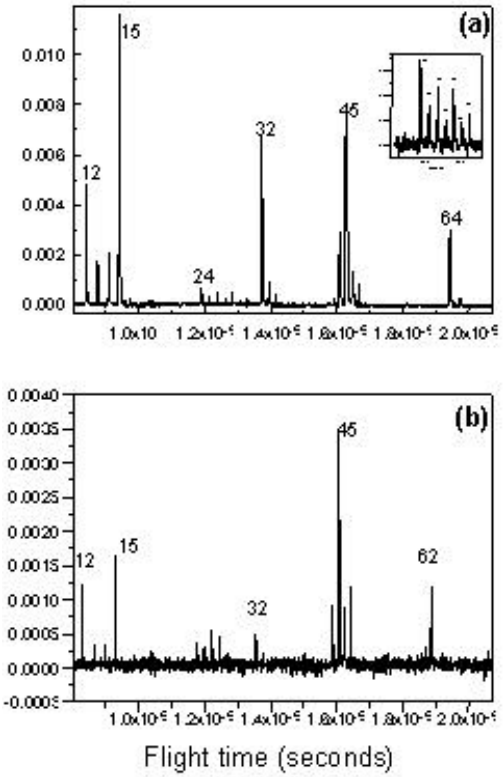

Fig. 2 Time of flight mass spectra of (a) DMDS and (b) DMS recorded at 355 nm

Even though absorption of a single photon may be sufficient to dissociate the molecule, above-threshold absorption of additional photons can lead to ionization and dissociation on higher potential energy surfaces. For polyatomic molecules, the competition between dissociation and above-threshold absorption opens the door to population of vibrational degrees of freedom that might not normally be accessible from the ground state. Still richer behaviour becomes possible in fields strong enough to alter the potential energy that binds the molecules. For example high-intensity non-resonant laser fields from ultrashort laser pulses can cause very large electric field gradient. Under such field's molecules can experience a large torque along the polarization vector of the field due to their anisotropic polarizability. Higher peak intensity fields ~ 10^{14} W/cm^2, produce an electric field strength that is comparable to that experienced by valence electrons in molecules (~ 1V/Å), and cause structural deformation. At these electric field strengths, ionization takes place and at higher laser intensities multiple ionization and Coulomb explosion are observed.

The field intensities for the tightly focused pico and femtosecond laser pulses are high enough that they cause alignment of the molecule prior to its dissociative photoionization. The strong laser field induces a dipole moment, which then tends to trap the molecule in pendular states, causing them to liberate about the electric field direction (laser polarization direction), leading to photofragment angular distribution. If a number of electrons are removed from the constituent atoms of the molecule, the resulting repulsive electrostatic force leads to Coulomb explosion, in which the molecule dissociates yielding ionic fragments with energies, which are determined in part by the internuclear separation when ionization took place.

Interaction with Clusters

Clusters can be defined as aggregate of atoms or molecules. These clusters have been studied by chemists and physicists because of the unique position that clusters hold as an intermediate state between molecules and solids. Numerous studies have shown the change in properties of cluster with increasing size and substitution. Photodissociation studies coupled with theoretical calculations have been very useful in revealing the stabilization energy and mode of decay of a cluster with given no. of atoms and molecules.

On one hand MPI studies on atomic and molecular clusters (van der Waals clusters) have been carried out using intense nanosecond laser pulses with the desire to understand the structure and bonding that occurs as the clusters grow in size, while on other hand using intense picosecond-femtosecond laser pulses the phenomenon of OFI and Coulomb Explosion (CE), which involves generation of multiply charged atomic and molecular species with large kinetic energy due to removal of several electrons, has been explored. The understanding of these processes (OFI and CE) is of great significance because of their fundamental importance and their close relation with processes such as coherent control, molecular alignment, higher harmonic generation, etc. Ionization of clusters is very different as compared to that of atoms and molecules. Clusters undergo two types of ionization. The first is ionization of the constitutent atoms/molecules in which an ion and electron is created. The electron is not able to leave the cluster and in this respect this phenomena is called inner ionization. This gives rise to a nano plasma. The second is removal of the ionized electron from the cluster field. This is called outer ionization. It is the inner ionized electron which couples the incident laser energy to the cluster plasma. In this way a large amount of laser energy is transered to the cluster which then displays unforeseen effects.

Clusters have high local electron density due to which they efficiently interact with laser radiation. Due to this strong interaction, clusters are attractive targets for studying the phenomenon of CE and for generation of highly charged species even at low laser intensity as compared to atoms and molecules. To date, several studies dealing with CE process in molecules and clusters, induced by picosecond-femtosecond pulses in the intensity regime of 10^{14} - 10^{18} W/cm^2 have been carried out giving rise to highly charged atomic species e.g. I^{n+}, $n \leq 15$ for CH_3I clusters, Xe^{m+}, $m \leq 40$ for Xe clusters, with kinetic energies upto 1MeV. The intensity threshold for the formation of these multiply charged

species can be predicted by the simple formula of Auguste et al. [7]

$$I(W/cm^2) = 4X10^9 E_i^4/Z^2$$

Where E_i is ionization potential of the multiply charged ion (eV) and Z is charge on the ion. Using the above equation, the intensity threshold for appearance of multiply charged species is found to be greater than $10^{13} W/cm^2$. However, it was possible to observe occurrence of Coulomb explosion at intensity as low as 10^9 W/cm^2 using nanosecond laser. Though multiphoton ionization of molecular clusters with intense nanosecond laser has been investigated for many years, for the first time occurrence of Coulomb explosion at such low intensity has been observed.

What is Coulomb Explosion ?

Interaction of intense laser pulses with molecular clusters has led to several interesting phenomenon including Coulomb explosion, leading to the generation of higher order harmonics, energetic electrons, multiply charged ions and even neutrons [8]. Coulomb explosion in clusters occurs due to the stripping of a large number of electrons by intense laser field and the high positive charge on the cluster leads to its disintegration due to Coulomb repulsion, which leads to creation of multiply charged ions with significantly large kinetic energy. In the past, laser induced Coulomb explosion of methyl iodide monomers and its clusters, has been studied using pico and femto-second lasers, having intensity in the range of 10^{14}-10^{16} W/cm^2 and the formation of highly charged carbon (C^{4+}) and iodine (I^{15+}) ions with keV kinetic energies was reported.

Methyl iodide clusters were irradiated using nanosecond dye laser pulses of 557-567 nm wavelength with an average intensity of 10^9 W/cm^2 and the ions formed were detected using linear time of flight mass spectrometer. In the mass spectra signals due to multiply charged atomic ions up to C^{4+} and I^{5+} with kinetic energies in the range of 0.3-2 keV and a series of cluster fragments of the type $[(CH_3)(CH_3I)_n]^+$ with $1 \leq n \leq 6$ were observed. The charge states and kinetic energies associated with different ionic species in the present study using nanosecond laser, were found to be comparable to those of femtosecond lasers with intensities in the

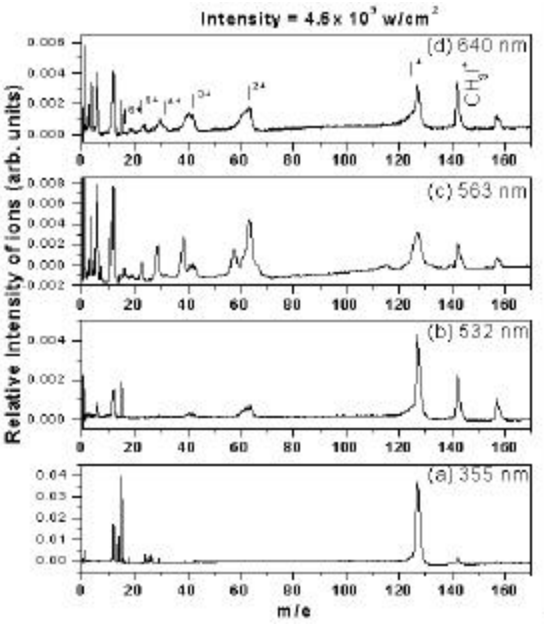

Fig. 3 Wavelength dependency of Coulomb explosion phenomena in methyl iodide clusters.

range of 10^{14}-10^{16} W/cm^2 [9,10]. Further, with a view to understand the wavelength dependence of Coulomb explosion phenomena, additional experiments were carried out over few selective wavelengths in the region of 355 to 643 nm and clear evidence for the enhancement of Coulomb explosion process with increasing wavelength was observed (Fig. 3). Experiments were also carried out as a function of laser intensity. It was found that with increase in laser intensity there was an increase in the yield of higher charged atomic ion. Fig.4 shows intensity dependent mass spectra of multiply charged carbon and iodine ions.

All the above experiments suggest that methyl iodide clusters absorb laser energy very efficiently. However, the mechanism by which these clusters absorb energy is still unclear. Although various theoretical models have been proposed to explain the explosion dynamics of clusters under intense field irradiation, none can adequately explain the experimentally observed features that presently drive research in this area. Under the nanosecond

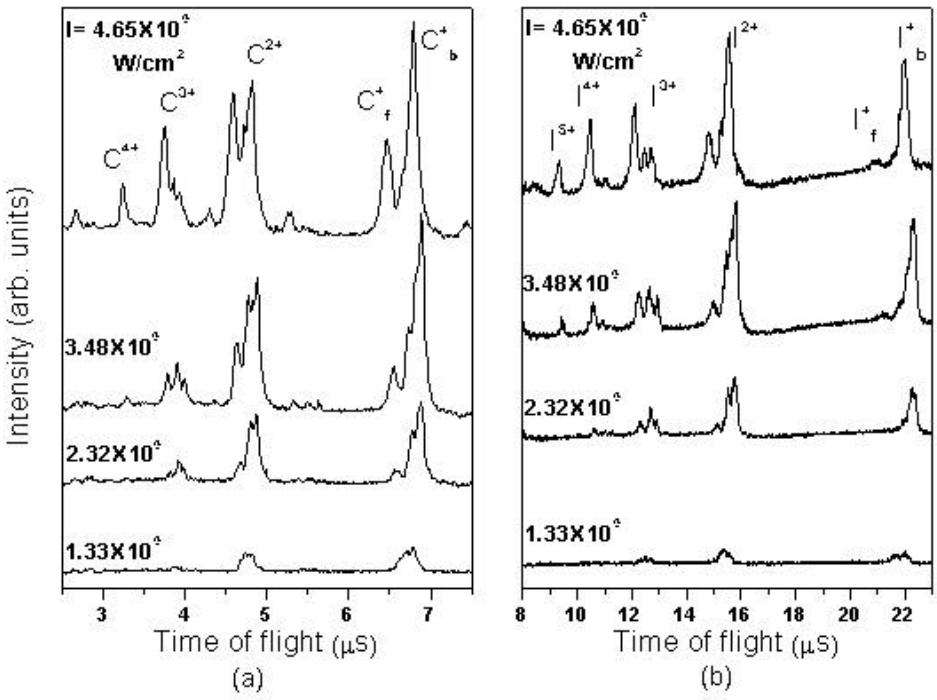

Fig. 4 Variation in signal of multiply charged atomic ions as a function of laser intensity

conditions, it is clear that most molecules in the cluster are multiphoton ionized at the leading edge of the incident laser pulse as the Keldysh parameter for the experimental conditions is ~ 180 (γ >1). Thus, under these conditions, ionization process in the methyl iodide cluster is initiated by MPI resulting into formation of several ion cores within the cluster. As mentioned earlier, some of the liberated electrons are confined inside the ionized cluster forming a nano-plasma [11]. These confined electrons which are under the influence of Coulomb field inside the cluster, keep extracting energy from the laser pulse via inverse Bremsstrahlung process during electron-ion and electron-neutral collisions. Once the electron energy exceeds the ionization potential, further ionization can occur via electron impact ionization i.e. (e,2e) type of reaction leading to increased charged states of the cluster. The newly ejected electron will start a new cascading sequence of collisional heating and ionization processes by extracting energy via above mentioned process. This, sequence of events spreads very fast inside the cluster nano-plasma which finally results in the formation of multiply charged cluster ions in a step by step process via inelastic collisions. A stage comes when Coulomb repulsion overcomes the total cohesive energy of the cluster and the multiply charged cluster explodes resulting in formation of multi-charged atomic ions with large kinetic energy.

Ditmire et al have shown occurrence of D-D nuclear fusion using super-intense laser cluster interaction [12, 13]. In their studies, these authors have shown that excitation of deuterium clusters with high intensity (> 10^{16} W/cm^2) from femtosecond laser can produce a superheated microplasma that ejects deuterium ions with large kinetic energies. The fast deuterium ions ejected from the exploding clusters can then collide with ions ejected from other clusters in the plasma. If the ion energy is high enough (greater than a few keV), D+D nuclear fusion can occur with high probability. The well known signature of this process arises from one channel of the fusion reaction, i.e. D+D ^{3}He+n, in which a neutron is released with 2.45 MeV of energy. An efficiency of about 10^5 fusion neutrons per joule of incident laser energy has been reported for this process [12,13]. As a result, the research in

laser-cluster interaction area continues and many more new phenomena/effects are expected to be unraveled.

Conclusion

Development of high intensity lasers has allowed novel areas of atomic, molecular and plasma physics to be explored. Interaction of high intensity laser pulses with matter in the gas phase has allowed the study of basic physics in regimes relevant to astrophysics, atomic physics or in strongly correlated plasmas. This has produced new insights into the nature of light-matter interaction and generated new sources of coherent short-wavelength radiations by higher harmonic generation. Today, a high-intensity femtosecond laser beam can be converted to radiation sources of white coherent continuum, VUV coherent-light, X-rays, electrons, highly-charged ions and even neutrons, all of the beams will have the same order of pulse width as the irradiation laser. New chemical applications will be possible with these new types of beams. The interaction with clusters represents an important transition in the dynamics of intense laser-matter interaction from molecules to solids.

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Ultrafast Interfacial Electron Transfer Dynamics in Dye-Sensitized Semiconductor Nanoparticle Surface

Dr. Hirendra N. Ghosh *obtained M. Sc. In Chemistry from IIT, Kharagpur and joined Chemistry Division, BARC, in 1990, through BARC Training School Course (33rd batch, 1989-90). Dr. Ghosh obtained his Ph.D. degree in 1996 from Bombay University for his work on fast photochemical processes in liquid and microheterogenious media. He did his Post-doctoral studies during 1997-1998, with Prof. Tim Lian at the Chemistry Department of Emory University, Atlanta,.USA, in femtosecond infrared spectroscopy. Dr. Ghosh was awarded the "INSA Young Scientist" Medal for the year of 1998 for his outstanding Ph.D. work. He has also received the prestigious "Anil Kumar Bose Memorial Award" in 2000 for the best output during the year after INSA Young Scientist Award by the Indian National Science Academy. New Delhi. Dr. Ghosh has also received APA-Prize for Young Scientists award in the year 2004 from the Asian and Oceanian Photochemistry Association for his excellent contribution in the field of interfacial electron transfer dynamics in dye-sensitized semiconductor nanoparticles. He has developed Femtosecond laser spectrometer detecting both in visible and infrared region. His current research interest includes ultrafast interfacial electron transfer and charge carrier relaxation dynamics in semiconductor nanoparticles and quantum dot materials also development of Femtosecond terahertz spectrometer.*

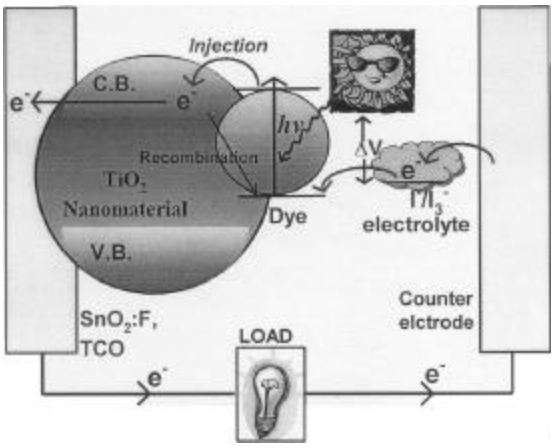
Introduction

Interfacial electron transfer between a discrete molecular state and conducting surface of semiconductor nanoparticle is the simplest of all surface reactions: it involves only the exchange of electron without breaking the bond. This simplicity offers the best opportunity to understand of reaction coordinate. Research in this area is strongly motivated by both its fundamental importance and the large number of practical applications, such as solar energy conversion, waste water treatment, nano electric devices, surface catalytic mechanisms and almost all modern imaging (photography and xerography) [1-7]. The application of solar energy conversion is primarily due to much more charge separation in these solid state materials relative to an all molecular approach. Despite the numerous applications of this process, the understanding of charge transfer at surfaces is lagging well behind due to greater inherent experimental difficulties in studying surfaces.

To understand the interfacial charge transfer processes, one needs total information on the electronic coupling between the two resonant electronic states undergoing electron exchange, on the nuclear activation barrier to attend the condition of electronic resonance and on the barrier crossing dynamics. These features are common to all charge transfer reactions, both heterogeneous and homogeneous, and each plays a pivotal role in the rate determining reaction.

For majority of the electron transfer reactions, the kinetics is dominated by the energetics of the reaction coordinate. Because of its dominant role, finding a regime where barrier-crossing dynamics can be accessed is a distinct challenge. Here the barrier crossing rate is just the rate of nuclear passage along the reaction coordinate and is controlled by nuclear relaxation of the intermolecular and intramolecular modes of the acceptor and the solvent. At this level of generality, the dependence of charge transfer on the energetics and barrier crossing dynamics is the same for both the homogeneous and heterogeneous charge transfer reactions.

Hirendra N. Ghosh, Radiation & Photo Chemistry Division, Bhabha Atomic Research Center, Trombay, Mumbai 400 085;
E-mail: hhghosh@magnum.barc.ernet.in



Scheme 1 Kinetic model for electron transfer from a molecular adsorbate to semiconductor nanomaterials in Dye-sensitized solar cell.

In contrast, a marked distinction between the homogeneous and heterogeneous cases is expected for the electronic coupling. The effect of solid-state band on the coupling is the fundamental issue in the current topic. The degree of electronic coupling determines the level of theoretical approximation is required to model correctly the current problem. In addition, the most unique feature of electron transfer processes at surfaces is one of few reaction mechanisms in which there is a dense manifold of electronic levels intimately connected to the reaction coordinate. In this regard, the conduction band of the solid state represents a large number of source terms for electron donor or acceptor levels in the reaction coordinate. This effect is expected to significantly enhance the degree of mixing between states relative to the discrete two states coupling in the homogeneous electron transfer problem.

Most of the work on interfacial charge transfer has been conducted using electrochemical approaches, which are steady state in nature and therefore, determine the rate-limiting step in the reaction mechanism. To access information on the fastest dynamical processes occurring at the surfaces, a "jump" experiment of some kind is required. These experiments entail a rapid change in one of the reaction variables (temperature, pressure, electronic state etc.) on a time scale faster than the dynamics of interest. By probing the subsequent relaxation of the system from the non-eqilibrium point, information on the fastest processes involved in a particular reaction mechanism can be obtained. In order to access information on the barrier crossing dynamics and issues of electronic coupling for the surface electron transfer, it is necessary to study the reaction dynamics on the time scales shorter than nuclear relaxation (approximately 100 fs). This time scale requires an all-optical approach

This review will focus on the use of time domain optical spectroscopy to directly probe the dynamics of charge transfer at semiconductor surfaces. Here the semiconductor serves as the optical switch for turning on interfacial charge transfer processes with high quantum yield (~1). The impulsive non-equilibrium condition (the "jump") is the generation of electron and hole pairs in the surface region. With help of current laser technology, the surface can be optically prepared near or at the adiabatic crossing point in the electron transfer coordinate on time scales faster than all the relevant relaxation dynamics. The electron distribution across the interface can give the information on the degree of electronic coupling and the barrier crossing dynamics.

The interfacial charge transfer processes will be explored for two different t = 0 boundary conditions: (1) charge carriers will be optically prepared in the solid state and allowed to cross the interface to a molecular acceptor (electron emission case) and (2) optical excitation of a molecular species at the surface in which electron transfer occurs into the electronic band of the solid (electron injection case)

In the case of electron emission the key photophysical processes governing the electron transfer step for these systems are shown in Fig. 1. The magnitude of the space charge field is enormous (10^5 - 10^6 V/cm) and acts to very efficiently separate the electron-hole pairs, which are photogenerated, migrate into the space charge region. The large coulombically driven separation prevents recombination of the electron and hole carrier wave function. In addition, the field polarity is such that the minority carrier is selectively confined to the surface reaction plane where the wave function overlaps with the molecular acceptor distribution at the surface. The overall dynamics of the charge carrier lifetimes at the surface are related to the

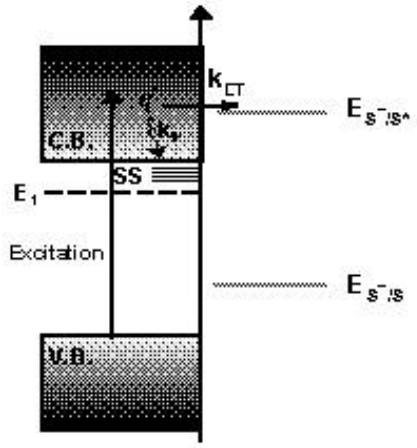


Fig. 1 Electron Emission Case Mechanistic scheme for electron transfer from the electronically excited Semiconductor nanoparticle to adsorbed Dye. Here S/S⁻ is the excited sensitized dye/anion radical couple, K_{ET} is the forward electron transfer, K_{tr} is the trapping dynamics, E_f is Fermi energy level of the semiconductor, SS the surface state*

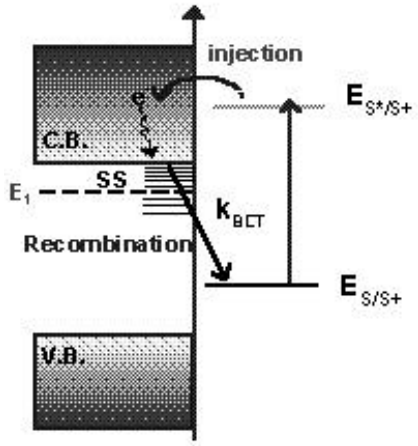


Fig. 2 Electron Injection Case Mechanistic scheme for electron transfer from the electronically excited dye to Semiconductor nanoparticle. S/S⁺ is the excited sensitized dye/cation radical couple, Injection is the forward electron transfer, k_{BET} is the back electron transfer, K_{tr} is the trapping dynamics, E_f is Fermi energy level of the semiconductor, SS the surface state*

electronic coupling across the interface and the relaxation dynamics of both the solid and liquid state side of the surface.

In the case of electron injection, it involves the optical preparation of molecular excited states resonant with the conduction band states at dye sensitized semiconductor surfaces. For the energetics shown in Fig 2, the electron transfer processes is barrierless, and the reaction coordinate involves coupling of the discrete state to the field-assisted k states. The propagation of the electron away from the initial site and into the solid state does not require nuclear relaxation. In this case, the time evolution of the excited state (S_1) to the free carrier state provides a direct probe of the electronic coupling or wave function overlap between a discrete molecular state and the delocalised solid state electronic levels. The information gained from these studies provides the closest analogy to the time evolution of transition states at electrode surfaces. A fundamental understanding of electron transfer at this surface will provide the general principles for maximizing the efficiency of charge separation at interfaces using dye sensitization.

Models for Surface Electron Transfer

A. Electron Emission Case: Weak Electronic Coupling Limit

The emphasis in electron transfer theory correlates the electron transfer rate to nuclear and electronic factors of the reaction coordinate. Early works by Marcus [8], Levich [9], Gerischer [10] give simple expression which is amenable to experimental tests. The rate equation is formulated for the problem of electron transfer at electrode surfaces, assuming harmonic displacements for the solvent modes involved in solvent reorganization along the reaction coordinate. For a semiconductor surface, the problem of charge transfer simplified by assuming it involves thermalized charge carriers at the valence or conduction band edge. With these assumptions, the factors controlling the electron transfer rate constant (k) for an electron energy E at the surface can be written as

$$k = \nu_{eff}\, k(r) \exp\left[\frac{-(E - E_{redox} - \lambda)^2}{4kT\lambda}\right] \qquad (1)$$

where λ is the medium reorganization energy; E_{redox} is the redox potential of the acceptor/donor redox couple in solution, ν_{eff} is the effective frequency of the nuclear coordinate and k(r) is the transmission coefficient which is related to the square of the electronic coupling. This is essentially a Fermi golden rule expression where the transition probability is weighted by Franck-Condon factors contained in the Boltzmann statics of attaining resonance.

After achieving electronic resonance, the nuclear activation barrier dominates the reaction kinetics. This barrier can be estimated from the above equation assuming the energy scales quadratically with displacement along the nuclear coordinates. This aspect of analytical treatments of electron transfer rate and the understanding of it gained from the solution phase studies of homogeneous electron transfer.

B. Electron Emission Case: Strong Coupling Limit and the Hot carrier Model

To achieve the maximum rate of charge transfer from the semiconductor to a surface acceptor, it is desirable to have as small a nuclear activation barrier as possible, this can be achieved by proper choice of redox potentials. Again it depends on the degree of electronic coupling between the molecular states and extended band states, i.e. degree of adaibaticity. The electronic coupling between the molecular potential and the periodic lattice potential is the fundamental issue. At surfaces, the coupling is complicated by any intervening solvent layer, which would act as an insulating barrier to the electronic overlap. Considering the solvent barrier Boudreaux et al found the electronic tunneling time is ~100fs using one dimension model, which demonstrate appreciable electronic coupling. This one-dimensional analysis ignores the mismatch in the electronic density of states between molecular acceptor and the semiconductor, which would act to statistically reduce the transmission probability to the molecular acceptor. Thus, the estimates of the electron transfer dynamics based on this model should be considered upper limits. However, the estimates are consistent within the adiabatic limit of charge transfer. This work is conceptually very important as it pointed out the possibility of using hot electron channels for energy storage.

The hot carrier model is described within the framework of the Marcus-Gerischer model for weakly adaibatic conditions. Schmickler [11] has recently reworked the problem for variable electronic coupling at an electrode surface in which associated Hamiltonian is treated to higher order and explicitly includes the bath phonons. Using typical electronic couplings observed for homogeneous electron transfer, solutions were found in which the electronic coupling is so enhanced at the surface that it falls in the strong coupling limit. In comparison to homogeneous electron transfer, a molecule at a surface has many more electronic levels acting as electron sources and the process becomes activation less.

C. Electron Injection case: Incorporating the Electronic Continuum

For charge Injection from a localized molecular state into a semiconductor band, the process is the complementary problem to the charge transfer discussed above. There is, however a distinction in that the huge phase space of the acceptor semiconductor states creates the possibility of localizing the charge in the acceptor half-space without any need to invoke nuclear relaxation.

Traditionally, electron transfer theory follows the Marcus-Levich [8-9] formulation, which postulates that the electronic levels of the donor and acceptor can be modeled as single discrete levels in a potential well. The nuclear potentials of the reactants and the products are assumed to be parabolic with a splitting due to coupling between sites. Relaxation within this nuclear continuum is necessary to stabilize the charge transfer, and rate of this relaxation provides the upper limit for the electron transfer rate.

For electron transfer reactions between molecular species, which have a simple electronic structure this treatment, is quite successful. In the case of semiconductor interfaces, the charge

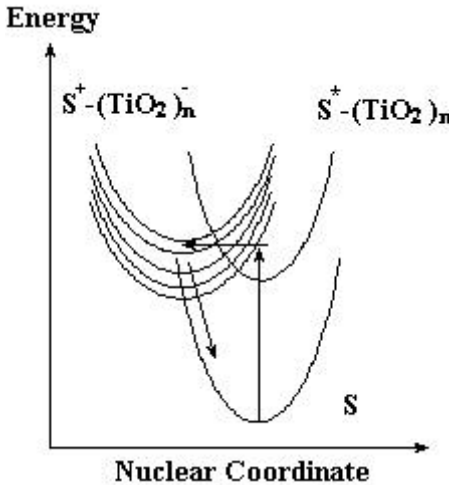


Fig. 3 A Marcus-type potential energy diagram for charge transfer processes at semiconductor interfaces showing the interaction between the reaction coordinate and the manifold of k states of semiconductor. $(TiO_2)_n$ refers to the semiconductor surface, and S is the molecular state.

donor/acceptor at the surface is still a molecular species; however, the semiconductor has a quasi-continuum of electronic states with considerable delocalization of the wave function over many electronic states. A schematic representation of the potential energy surface and electronic degeneracy at the adiabatic crossing point of the reaction coordinate is shown in Fig.3.

In this model, the electron resonantly tunnels across the interface to a single electronic state of the semiconductor, which is mixed with the entire quasi-continuum of conduction band states. This electron transfer rate depends only on the electronic density of states and the electronic coupling. The electron now exists in a mixed state of coupled band states. The very large number large number of these states prevents any recurrence on the donor site before scattering and relaxation in the solid phase (electronic de-phasing and carrier thermalization) processes break the coherence. These conditions are sufficient for the propagation of the electron into the semiconductor and separation from the initial molecular state. In a normal Marcus approach, the relaxation of the nuclear degrees of freedom, both intramolecular and intermolecular, is the rate limiting step in an electron event - on the order of 100fs for most molecular species. The delocalization/relaxation model described above allows electron transfer to occur on the same time scale as the electron de-phasing in the semiconductor (as fast as 10fs), and it is not necessary to invoke the bath mode (ν_{eff}).

Experimental Approaches to a Real Time View of Electron Transfer

In terms of dynamics, the effect of the activation barrier is well described by Boltzmann statics. Thus, from an experimental point of view, one would like to determine the spatial propagation of an electron across an interface under zero barrier conditions, i.e. at the adiabatic crossing point of the reaction surface. At this point, the electron transfer time is determined by barrier crossing dynamics and the degree of electronic coupling. This condition defines the upper limits to the electron transfer rate for a given surface. Optical preparation of nonequilibrium electrons that are resonant with both band and molecular states access this condition. The band-gap of semiconductors prevents complete thermalization and the large electric fields present within the surface space charge region act to spatially direct the electron into reaction channel.

The model problem must be studied under low electron densities to avoid excessive electron-electron scattering and Coulombic effects between carriers, which have nothing to do with reaction coordinate, but would complicate the dynamics. The condition will be referred to as the one electron limit. The high sensitivity and time resolution needed to study these transition state processes at surfaces have only recently become available with the development of proper laser sources and new optical spectroscopy developed specifically to address this problem.

Solid State Donor Condition

The sensitivity and time resolution problems have been overcome by using transient grating spectroscopy to follow the electron dynamics at the surfaces. Transient grating studies of carrier dynamics have been conducted to follow nonradiative carrier recombination. In these

experiments it has been observed that the minority carriers photogenerated within the space charge region to the surface on 100 fs time scales. The separation of the electron-hole pairs and motion of the minority carrier in the surface region was monitored optically through changes in polarization of either reflected or transmitted probes via the intrinsic electrooptic effect in GaAs(100).

In the present context, the most relevent work is the insitu grating studies of charge transfer at n-GaAs(100)/$Se^{2-/1-})_{aq}$ liquid junctions [12]. The interface is known to be stabilised against photooxidation and degradation in the presence of high concentration in the presence of high concentration of Se^{2-} which acts as an efficient hole acceptor. The quenching of the hole carrier component to the signal was demonstrated by an applied bias dependence. This earlier work was only able to place an upper limit of 30ps for the effective hole carrier transfer dynamics of the surface distribution. For the technical reacsons, the time resolution was limited by the long infrared pulses used for the probe.

The dynamics of charge carrier trapping and recombination and hole transfer reactions in opaque, aqueous suspension of Degussa P-25 TiO_2 are probed by Bowman et al [13] using femtosecond time-resolved diffuse reflectance spectroscopy. Appearance of photogenerated electrons were observed in TiO_2 nanoparticles when it was excited by a femtosecond UV pulse, and the appearance time was found to be <100 fs (pulse limited). Trapping of the photogenerated conduction band electrons occurs in less than 500 fs. Interfacial hole transfer dynamics of the P-25 TiO_2/SCN^- complex are probed as a function of thiocyanate ion concentration. A dramatic increase in the population of trapped charge carriers is observed within the first few picoseconds, demonstrating that interfacial charge transfer of an electron from the SCN^- to a hole on the photoexcited TiO_2 effectively competes with electron-hole recombination on an ultrafast time scale. A hole transfer reaction between a series of catechols and photoexcited quantum size ZnO nanoparticles was reported [14] and demonstrated the reactivity of the particles with changing size.

Excited State Donor Condition

In recent years, electron transfer between semiconductor nanoparticles and dye sensitizers has been intensely studied by fast and ultrafast laser spectroscopy [1,7,12, 15]. By measuring the excited state dynamics of the sensitizer through transient absorption or fluorescence decay, the electron injection rates from various adsorbed dye molecules into various semiconductor nanoparticles have been inferred, ranging from sub-picoseconds to tens of picoseconds and even nanoseconds. Subpicosecond transient absorption spectroscopy was used by Tachibana et.al.[15] to syudy the rate of electron injection following optical excitation of the ruthenium dye [Ru(4,4"-dicarboxy-2-2"-bipyridine $)_2(NCS)_2](N_3)$ adsorbed on to the surface of nanocrystalline titanium dioxide (TiO_2) films. Dynamics of the electron transfer processes was observed by monitoring the dye excited state and cation states in solution. Electron injection time was found to be biphasic, with 50% occuring in <150 fs (instrument limited) and 50% in 1.2±0.2 ps.

Most transient absorption studies in the visible and near-IR region are hindered by spectral overlap of absorption in various electronic states, such as the excited states, cationic state and ground state as well as stimulated emission. Fluorescence quenching studies are often complicated by non-ET-related quenching pathways, such as energy transfer among sensitizer molecules and the dynamic fluorescence stoke shift. Because of these complexities, there have been many conflicting reports of electron transfer rates has been observed.

To systematically study ET dynamics in the solid-liquid interface, new in situ techniques that are capable of assigning the Et process unambiguously and that complement the existing visible/near-IR transient absorption and fluorescence quenching techniques are needed. Femtosecond mid-IR spectroscopy provides such an approach for these interfacial problems because it can directly study the dynamics of electrons in the semiconductor in addition to adsorbates. As demonstrated in bulk and quantum well semiconductor materials, valence band holes and conduction band electrons in semiconductors have strong absorptions in the

infrared region. These absorptions consist of free carrier absorption, which is often broad and increases with wavelength, intraband transitions between different valleys (or sub-bands) within the conduction or the valence bands, and absorptions of trap states. Since these IR absorptions of electrons are direct evidence for the arrival of electrons inside semiconductors, they provide an unambiguous spectroscopic probe for studding interfacial electron transfer between semiconductor and adsorbates. Lian and co-workers [16,17] have carried out experiments to study the electron injection from photo-excited dye molecules to TiO_2 nanoparticles and thin films using sub-picosecond infrared spectroscopy in the mid-IR region. They have determined electron injection and charge recombination dynamics by directly monitoring the electron in the nanoparticles with out any ambiguity.

Conclusion

Time domain spectroscopic studies of surface reaction dynamics are giving a fairly extensive real time view of the electron trajectory across an interface. The spatial distribution of charge carriers, surface populations of donors and acceptors, and the solvent coordinate for the reaction field are now accessible through time domain spectroscopy.

With the use of optical methods and semiconductor interfaces, we must pay attention to surface space charge fields. The surface field affects the electronic states coupled to the reaction coordinate and plays a critical role in reaction dynamics. For charge injection, it assists in charge migration away from the surface. In the case of charge emission from a continuum, space charge field breaks up the electronic continuum and localizes the electron (or hole) within 10 A^0 of the surface. The wave function is now more like a molecular state, and the process of crossing the interface approximates that of homogeneous electron transfer with nuclear relaxation as the upper limit for the rate.

For aqueous interfaces, the nuclear relaxation time scale at the barrier crossing point should correspond to the ~100 fs solvent relaxation dynamics determined by the coupling of the reaction field to the hindered rotational and translation modes of water. This motion represents the fastest rate of nuclear passage, and the associated time scale is competitive with nonradiative relaxation and surface state trapping dynamics in the solid state half-space. As a result of which this model in the case of interfacial charge transfer problem reduces the degree of coupling between a particular molecular acceptor and the contributing states of the solid state.

The extension of this work to other systems, along with more detailed structural and energetic information about the surface region will be the challenging task. In addition, there are certain dynamical pathways that have been extrapolated from the bulk studies that need to be established at the interface. Notably, the non-radiative carrier relaxation and solvent rexation dynamics are inferred from bulk dynamics. This information is needed to identify which states are involved in the interfacial charge transfer step and properly identify the degree of adaibaticity of the reaction coordinate. More detailed theoretical treatments of the surface reaction dynamics are also required to understand the interfacial phenomena. Perhaps most importantly, an ab initio level calculation of the electronic coupling between the delocalised band states and a discrete molecular state near the saddle point need to be conducted. Finally, one of the most important future applications of this research is to exploit the use of hot electron reaction channels for surface processing and solar energy collection and to understand and control the simplest surface reactions.

Acknowledgment

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Multiphoton Excited Fluorescence as a Probe of Biological Systems

Dr. Sudipta Maiti *received his undergraduate training in Physics at IIT Kanpur. After a brief stint at the University of Rochester, he obtained his Ph.D. at the University of Pennsylvania working with Prof. R. M. Hochstarsser and Prof. P. L. Dutton in the area of femtosecond infrared spectroscopy. He then moved to Cornell University to work as a post doctoral fellow with Prof. W. W. Webb on the emerging field of laser assisted nonlinear microscopy. He currently heads the Biophotonics laboratory at the Tata Institute of Fundamental Research, where his group has built various biophotonic instruments including the multiphoton microscope and the FCS spectrometer. He is currently interested in gaining a physical understanding of neurobiological problems using optoelectronic devices. He is a Wellcome Trust Senior Overseas Research Fellow.*

Shri Kanchan Garai *received his undergraduate training in Physics at Kolkata University, Kolkata. He is currently a PhD student at the Tata Institute of Fundamental Research, working under supervision of Dr. Sudipta Maiti. He is now working with Alzheimer's amyloid beta aggregation using Fluorescence Correlation Spectroscopy and other optical techniques.*

Introduction

Light Microscopy in Biology

For four centuries since Zacharias Janssen built one, the light microscope has remained an indispensable tool of the biologist. No other tool lets him look at his subject with so much detail but with so little damage. However, fundamental physics has set limits to the resolution achievable with a given wavelength. No matter how good the microscope is, we are forever doomed to stay on the larger side of 200 nm as long as we insist on using visible radiation. However, with a molecular view of biology emerging in the last half a century, this limitation has become a real hurdle in understanding how life works.

A separate problem with microscopes has been that of contrast - the inability to see just the molecular species of interest in a specimen. The molecule of interest is always crowded in a live cell with everything that absorbs or scatters light in a similar wavelength. Development of the fluorescent proteins of the GFP family over the last few years has been a boon, and now we can see the species of our interest, as long as we are able to fuse it with an appropriate fluorescent protein. What happens when we cannot do it, or when doing so would severely impair the functioning of that molecule? One possibility is to use the native fluorescence of the molecule. Many of the body's constituents are indeed fluorescent, albeit in the ultraviolet. That robs us of the critical ability to study our specimens with minimal damage, as UV irradiation is incompatible with live biology.

Combining lasers with microscopy has opened up new possibilities for biologists, and a whole new area called 'Biophotonics' has emerged. Traditional limits of light microscopy have been creatively tackled to yield quantitative measurements of molecular size to sub-nanometer scales, or to

Dr. Sudipta Maiti, Tata Institute of Fundamental Research, Colaba, Mumbai; E-mail: maiti@tifr.res.in

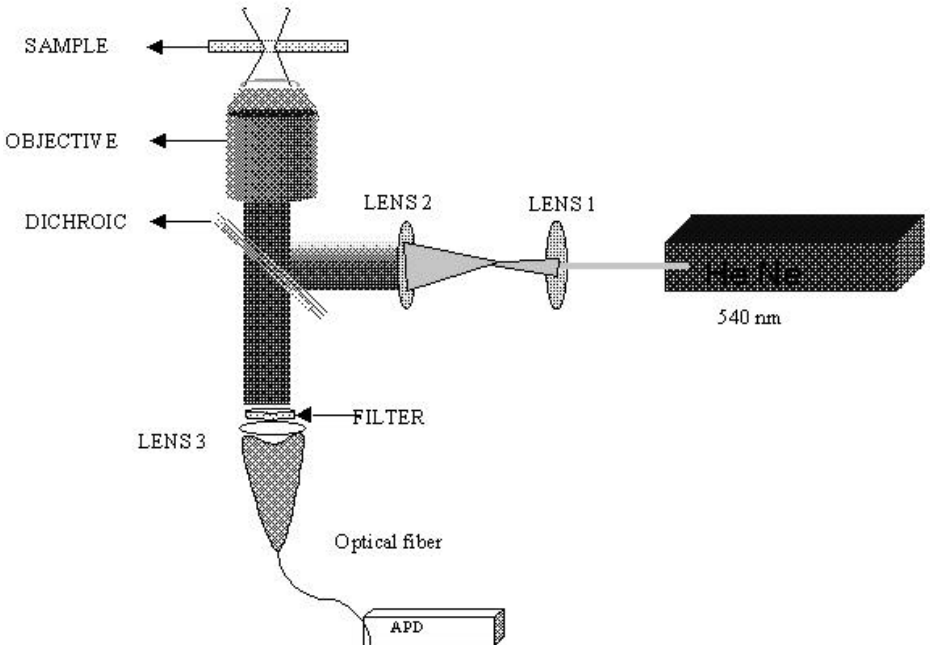

Fig. 1 FCS Setup: A green HeNe laser (540nm) is used for FCS. The fluorescence and the excitation is separated by using a dichroic (560DCLP). The excitation is focused with a water immersion objective. A filter (DF605/75) is used to separate the fluorescence from back scattered excitation light.

produce fluorescence images of UV fluorescent biomolecules using only benign infrared radiation. Two of these techniques are multiphoton microscopy and fluorescence correlation spectroscopy.

The Out-of-reach Problems

A large number of very significant problems remain out of reach due to these limitations of microscopy. We will deal with two specific challenges here – studying protein aggregation, and visualizing neurotransmitter molecules in the brain.

Protein aggregation in one hand is a biophysical curiosity: a large number of normally soluble proteins under different circumstances form insoluble aggregates, for reasons not yet understood. However, it is also a great health concern – it appears that some of the native proteins aggregate under physiological conditions as the body grows old, giving rise to such well known diseases such as Alzheimer's and Parkinson's. The challenge here is to understand the initial phases of the growth of these aggregates. Since the monomers and much of the multimeric population is well below the resolution limit, it becomes imperative to find a way of making a simultaneous size measurement of a population of different sized particles, as a function of time.

Neurotransmitters are small molecules used by one neuron to communicate with another neuron across the synaptic junction. They are packaged into small intracellular vesicles at a high concentration. A Ca^{++} mediated signal induces these vesicles to fuse with the plasma membrane and unload their content into the extracellular space. Since none of the neurotransmitter molecules are fluorescent in the visible region, and since it is not feasible to fuse them with fluorescent proteins while keeping their function intact, no neurotransmitter has ever been directly imaged in a live cell. In this context, the monoamine neurotransmitters are of particular interest as they are related to various psychopathologies. Two of these are serotonin and dopamine. These molecules are structurally related to the amino acids tryptophan and tyrosine

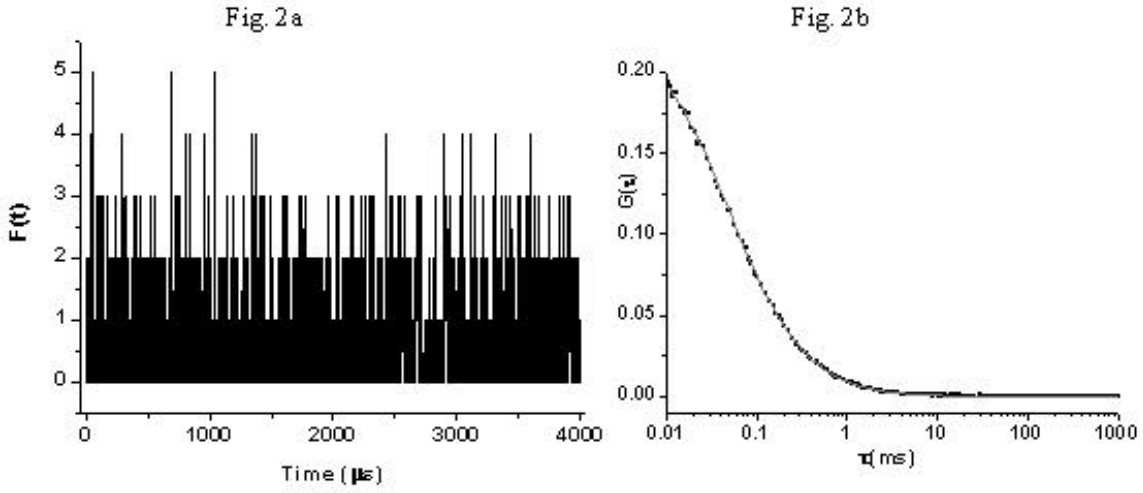

Fig. 2 Fluorescence and Autocorrelation trace from FCS: Figure 2A is the no. of photons F(t) detected by the detector in each 2 microseconds as a function of time. In figure 2B the black dots represent the experimental autocorrelation G(τ) calculated from F(t) using Eq. 1 and the thin line is the fit of the experimental data using Eq. 3.

respectively, and are consequently fluorescent, though only under ultraviolet excitation. Multiphoton microscopy uses femtosecond pulses of longer wavelength (visible or infrared) photons to achieve ultraviolet excitation, and it is possible to record the first images of serotonin vesicles in live neurons.

Fluorescence Correlation Spectroscopy (FCS)

The Idea of FCS

Fluorescence correlation spectroscopy (FCS), invented by Magde, Elson and Webb [1] can measure the molecular size by measuring the diffusion time of the molecules in solution. FCS monitors the total fluorescence F(t), which is proportional to the number of molecules N(t), present in a tiny probe volume (~ 0.2 μm^3) continuously as a function of time. F(t) fluctuates with time due to fluctuation of N(t) as a result of diffusion of molecules in and out of the probe volume. The fluctuation time scale is proportional to the residence time of the molecule inside the probe volume (called diffusion time τ_D) and can be extracted from the autocorrelation function G(τ) of F(t).

$$G(\tau) = \frac{\langle F(t)F(t+\tau)\rangle}{\langle F(t)\rangle^2} \qquad \text{(eq. 1)}$$

A typical fluorescence trace F(t) and autocorrelation curve G(τ) can be seen from Figs. 2A and 2B respectively. The characteristic decay time of G(τ) indicates the τ_D of the particle.

A small sub-femtolitre probe volume can be achieved by focusing a laser beam with an aberration free high numerical aperature (NA) objective lens and confocal detection of the fluorescence from the sample (the details of the set up will be discussed in the methods section). In this set up the G(τ) can be calculated as

$$G(\tau) = \frac{1/N}{(1+\tau/\tau_D)\left(1+\frac{w_0^2\tau}{z_0^2\tau_D}\right)^{1/2}} \qquad \text{(eq. 2)}$$

where w_0 and z_0 are radial and axial extents of the Gaussian probe volume respectively from the centre of the focus. Hence the diffusion time τ_D can be obtained by calculating the autocorrelation of F(t) and then from the fitting with eq. 2. A typical fit of G(τ) is shown in Fig. 2B. If the sample contains

multiple species (say, m) of different sizes then the eq. 2 can generalized as

$$G(\tau) = \sum_{i=1}^{m} \frac{1/N_i}{(1+\tau/\tau_{Di})\left(1+\frac{w_0^2\tau}{z_0^2\tau_{Di}}\right)^{1/2}} \qquad \text{(eq. 3)}$$

The diffusion coefficient D, can be calculated from τ_D by using the formula

$$6\,D\,\tau_D = r_0^2 \qquad \text{(eq. 4)}$$

The hydrodynamic size of the molecule can be determined from D by using Einstein formula

$$R_h = kT/6\pi\eta D \qquad \text{(eq. 5)}$$

where k is the Boltzmann constant, T is the temperature and η is the viscosity of the solution.

FCS can also be used to study the kinetics of any other process which involves spontaneous fluctuations of the fluorescence of a molecule, e.g. chemical reactions in equilibrium.

The Challenge: Protein Aggregation

Aggregation and deposition of amyloid beta peptide (Aβ) in the brain is a pathological hallmark of Alzheimer's disease (AD) [2,3]. Aβ is a small peptide with 39-43 residues and two major variants, viz., $A\beta_{1-40}$ and $A\beta_{1-42,}$ are soluble in normal brains but aggregate to form amyloid fibrils and deposit in the diseased brain. Notably, there is a class of diseases, viz, Parkinson's disease, Creutzfeldt - Jakob disease (CJD), typeII diabetes etc. which happen due to aggregation of different proteins. However, the cause and a detailed mechanism of protein aggregation are not well understood.

Various techniques such as dynamic light scattering (DLS), small angle neutron scattering (SANS), electron microscopy (EM) etc. have been used to study protein aggregation [4-7]. But DLS or SANS are not very sensitive and cannot be used at small concentrations of the Aβ peptide. EM, on the other hand, cannot be used in aqueous solution. However, FCS measurements can be done in aqueous solutions and is very sensitive at low concentrations, which is necessary to detect the early events of aggregation of Aβ.

The Technique

FCS setup

FCS experiments require a very small optical probe volume inside the sample and then efficient detection of fluorescence from the probe volume [8,9]. This is achieved by using a laser beam as an excitation source and an aberration free high numerical aperture (NA) objective lens and a confocal detection set up (fig. 1). In a confocal setup the fluorescence from the sample is collected by the same objective and then focused onto a pin hole (which is a 25 micron optical fiber in our case), placed before the detector. An avalanche photodiode (APD) is used as a single photon detector and the signal from the APD is then fed to the computer where the data is processed either with a data processing card (ALV 5000, ALV Laser GmbH) or with a generic I/O card and conventional programming software. This home-built instrument has a sensitivity that far exceeds the specifications of commercially available machines, and can detect 240,000 photons/second from a single molecule of rhodamine-B.

Data Analysis Algorithm

For samples containing only one kind of molecule, eq. 2 describes the mathematical from of the autocorrelation, $G(\tau)$. The experimental data (obtained using eq. 1) can be fitted with a least square fitting routine to extract the diffusion time, τ_D. In an aggregating solution there are multiple species of different sizes present and the conventional fitting routines cannot give a faithful description of the particle distribution. A bias free fitting routine based on the maximum entropy method which fits the FCS data with a quasi-continuous distribution of τ_D `s [10] was developed.

It was found that Aβ has a well defined thermodynamic saturation concentration (C_{sat}) [11]. The C_{sat} of Abeta1-40 to be 15 µM at physiological pH was measured and noted that the peptide does not form precipitates below C_{sat} but it will aggregate and form precipitates above C_{sat} if the sample is allowed enough time to reach equilibrium. The whole size distribution of Aβ particles from a 135 μM solution which is well above saturation was followed continuously with time and plotted in Fig. 2, where

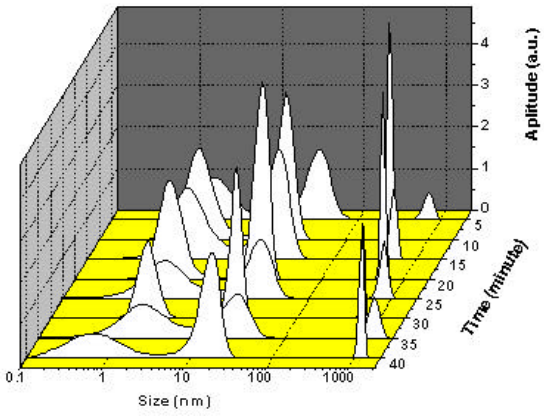

Fig. 3 *Kintics of Aβ aggregation: The three dimensional graph shows the development of the size distribution of the Aβ particles in solution as a function of time. The size axis is calibrated using the hydrodynamic size of Rhodamine (0.78 nm).*

the y-axis is the hydrodynamic size of the species, x-axis is time and the z-axis is the population of the species multiplied by the square of its brightness. The figure shows primarily three kinds of particles: monomers (~1.6 nm) and low molecular weight multimers (upto 3 nm), larger oligomers (10-40 nm) and very big particles. The larger oligomers have been found to be toxic to neurons and have drawn a lot of attention [4,12]. It is possible to characterize them with FCS and study their effect on cultured cells with optical microscopy. Various metal ions such zinc and copper promote aggregation of Aβ and have been found abundantly in the amyloid deposits in the brain [13, 14]. The role of these metal ions on Aβ aggregation and their implications on the disease is of current interest.

Multiphoton Microscopy

The idea

If a fluorophore absorbs in the ultraviolet, but not at lower wavelengths, we understand that its first excited state is separated from the ground state by an energy equivalent to that available from an ultraviolet photon. Normally, a photon having a longer wavelength (say twice) would not have enough energy and would not be absorbed by this molecule. However, if somehow the energies of two of such lower energy photons could be combined, the molecule in principle can be excited, and can subsequently emit a fluorescent photon from this state by a normal mechanism (see Fig. 4). Multiphoton excitation achieves exactly that. Soon after the birth of modern quantum mechanics, Maria Goppert-Meyer recognized that perturbation theory allows such a quasi-simultaneous interaction of a molecule with two photons, in effect helping it to end up in the excited state. However, such second order effects would have very small probability of occurrence, unless the impinging lightfield were very intense. It took three decades and the invention of the laser to verify this remarkable prediction [15]. Another three decades later, Watt Webb and coworkers focused a femtosecond laser source (to keep the instantaneous intensity high while the average intensity low) through a microscope objective lens into a cell, scanned each point of the cell and collected the resultant fluorescence from each point to generate a multiphoton fluorescent image of the cell [16]. This constituted the invention of the multiphoton microscope.

The Challenge: Visualizing Neurotransmitters

It was recognized a few years later that the technique of two-photon microscopy can be extended to perform three-photon microscopy and to image ultraviolet molecules in live cells [17]. The specific challenge was to observe the neurotransmitter molecules in live cells. Neurotransmitter synthesis, sequestration into vesicles, and subsequentexocytosis form the basis of chemical neurotransmission in all higher organisms [18]. Very little quantitative information exists about the intracellular events controlling these processes. Neurotransmitters come in many varieties, but serotonin is a special one. Serotonin containing neurons in the brain have been implicated in a wide range of phenomena such as cognition, affect, pain, emesis , sex, neuroendocrine functions, learning and memory tasks, regulation of sleep, blood pressure, sensory responsiveness and suicidal behavior. Psychiatric disorders involving the 5-HT system result from an alteration in the content, distribution and flux of the neurotransmitter. Drugs acting on the serotonergic system have been found to be effective in the treatment of depression.

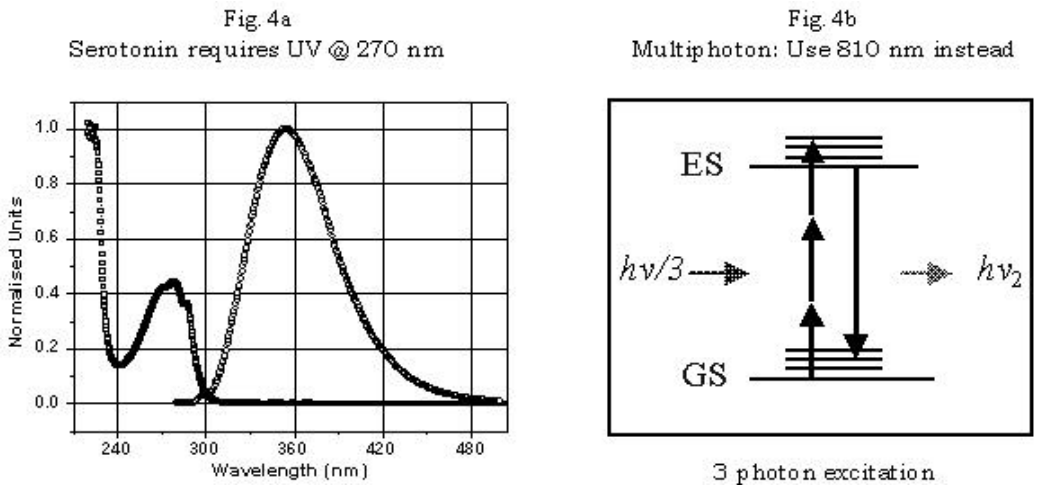

Fig. 4 The necessity for multiphoton excitation (a) The absorption and emission spectra of serotonin. The appropriate peak for single-photon excitation is at 270 nm. (b) Three-photon excitation at 810 nm can effectively excite the same UV transition

Quantitative characterization of the serotonin vesicles is critical for understanding these processes. We need to know how many vesicles are there in each cell, where they are located, how much neurotransmitter is contained in each vesicle, and finally how this pool responds to signals for exocytosis and to drugs of abuse such as amphetamines. In short, we need to have a detailed single neuron view of many of the processes that are understood at the level of the organism. It is difficult to quantify serotonin distribution in a living neuron in a microscopic scale, as most methods of identifying serotonin, such as PET/SPECT do not quantify the level of endogenous serotonin. Direct imaging of the autofluorescence of the neurotransmitters can potentially determine all these quantities. While it is possible to image the autofluorescence from serotonergic and dopaminergic neurons with ultraviolet excitation, these images have not resolved individual neurotransmitter vesicles [19], and appear to be toxic to the cells. A better alternative appears to be multiphoton excitation, which uses infrared [17] to probe ultraviolet fluorophores with high three-dimensional resolution. It was shown that three-photon microscopy can simultaneously image all the serotonergic vesicles inside a cell and quantify their content. The first success came in imaging large serotonin vesicles in a model cell line [17], but the smaller neuronal vesicles could not be resolved. However, now the efforts have been successful in imaging endogenous neurotransmitter vesicles in live neuronal cells.

The Technique

A multiphoton microscope with a design that optimizes the performance in the ultraviolet region uses both infrared (for three-photon UV excitation) and visible (for two-photon UV excitation) femtosecond laser sources. For three-photon excitation, a 76 MHz pulse-train of ~100 fs pulses from a modelocked titanium:sapphire laser (MIRA 900, Coherent., USA) operating at 740 nm is sent into a multiphoton microscope constructed in-house using a confocal scan box (MRC 600, BioRad, USA) and an inverted microscope (TE 300, Nikon, Japan). A water immersion objective (Apochromat, 60 X, 1.2 NA, Nikon, Japan) focuses and collects the fluorescence from the sample. A dichroic mirror mounted on a custom made mount near the base of the objective lens reflects the fluorescence onto a PMT (Thorn EMI, UK). A 400 nm longpass dichroic (Chroma Technology Corp., USA) is used in all of our measurements except for obtaining the ratio of the emission characteristics, for which a 675 dcxruv dichroic (Chroma Technology Corp., USA) is used. A saturated solution of copper sulfate with 1 cm path length is placed in front of the PMT to filter out

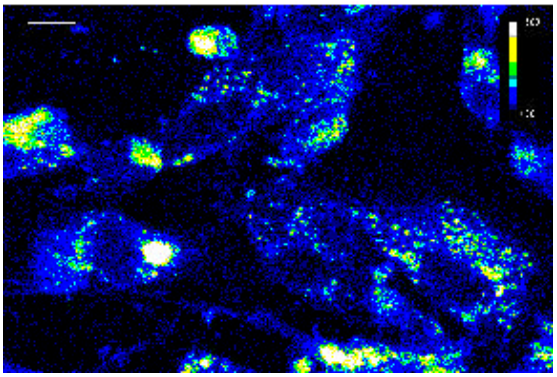

Fig. 5 A three-photon fluorescence image of a group of serotonergic RN46A cells. The dot-like structures are the serotonin vesicles. The hollow regions inside the cells are the nuclei.

back-scattered infrared light. The PMT signal is then fed to the external inputs of the scan box. For two-photon excitation with visible radiation, an analogous scheme is used, except the laser source is a ring-cavity optical parametric oscillator with intra-cavity doubling (MIRA OPO), which provides tunable femtosecond light between 540 and 640 nm, at 76 MHz.

Conclusion

Biophotonics depends on a combination of modern laser technology and fruitful ideas for tackling biological problems. The quick commercialization of these new ideas is testament to the potential of this field (both FCS and MPM have become commercial products within the last few years). The ability to build one's own instrument is also crucial, as neither the protein aggregation study nor the neurotransmitter imaging described here would have been possible with commercial instruments. In addition to the techniques of FCS and MPM described here, other significant technologies such as optical tweezers, laser microsurgery, single molecule imaging and near-field scanning optical microscopy have emerged in the last few years. As long as photonics experts keep interacting with problems of modern biology, this field will keep flourishing with novel inventions.

Acknowledgements

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Time-Resolved Fluorescence Reveals the Dynamics in Proteins and Protein-DNA Complexes

***Prof. G. Krishnamoorthy**, did M.Sc. in Chemistry from Madras Christian College, 1973, and Ph. D. from TIFR in 1980 on T-Jump Relaxation Kinetics of enzyme-ligand binding supervisor: Prof. S.Prabhananda and Post-doctoral research at Cornell University, SA, 1981-84; Bioenergetics, Membrane transport; electron transport in proteins. 1974-present: TIFR (Currently Professor in Chemical Sciences). Current Research Interests: Dynamics of proteins, nucleic acids and membranes; Application of time-resolved fluorescence techniques to solve complex problems in biomolecular dynamics and function. Some of the outstanding problems solved in recent years include the following: Showed that the transition between folded and unfolded proteins follows continuous structural changes even in elementary systems; Presence of nanometer size functional domains on cell surfaces was demonstrated in a most direct way; Correlation of protein dynamics and proton transfer was shown; Fluorescence dynamics was used to get structural information on protein fibrils.*

Introduction

The concept that dynamics along with structure form the basis of activity of biomolecular systems has gained experimental support in recent years [1]. By virtue of its sensitivity, selectivity and large temporal range, fluorescence spectroscopy has become one of the most revealing windows of biomolecular dynamics [2]. Of the several experimental methods available for studying macromolecular dynamics, fluorescence-based methods have the following advantages: (i) dynamics of a specific group or a segment of a massive macromolecular system can be observed without interference from the rest of the system; (ii) the timescale of observable dynamics covers a wide range of femtoseconds to seconds; and (iii) observations can be made under sparse levels of samples.

The dynamic nature of biomolecular structures, which is essential for their function, leads to structural heterogeneity. Due to its origin on dynamics, the apparent level of heterogeneity depends upon the time window used for observation. Large time windows result in an averaging of the structural parameters, whereas shorter windows produce an instantaneous snap shot of the structural variants populating a distribution. The time window set by fluorescence-based methods is linked to the excited state lifetime of the fluorophore, which lies generally in the range of 10 ps to 10 ns. Since the timescale of large scale and high amplitude dynamics in macromolecules is generally in the range of nanoseconds and beyond [3], fluorescence methods capture essentially a snap shot of various structural forms present. The ensemble of structural forms of biomacromolecules could vary in its level of heterogeneity. While the spread in structural parameters is expected to be quite small for native and stable structures, partially structured intermediates encountered in situations such as protein folding pathways are expected to have broader distributions of their structural characteristics. Furthermore, the amplitudes of local and segmental dynamics are expected to be non-uniform throughout the structure and probably related to functional domains of macromolecules. Hence the level of structural heterogeneity could also follow a non-uniform pattern. Such considerations demand that observations be made on fluorescence probes located at specific locations guided by information on the function of the system. The following sections shall deal with examples where time-resolved fluorescence of both intrinsic

Prof. G. Krishnamoorthy,, Tata Institute of Fundamental Research, Colaba, Mumbai; E-mail: gk@tifr.res.in

and extrinsic fluorophores have been used effectively to shed light on protein dynamics, protein folding dynamics and dynamics of DNA-protein complexes.

Protein Dynamics

Dynamic Fluorescence of Tryptophan

The indole chromophore of the aminoacid tryptophan is the natural intrinsic fluorophore for probing protein dynamics. The main parameters derivable from time-domain fluorescence measurements, fluorescence lifetime and fluorescence depolarization time, have information on the microenvironment around the fluorophore [2]. While both these parameters could, in principle, reflect structural heterogeneity, fluorescence lifetime is the preferred parameter due to relatively higher accuracy with which it can be determined. Very high signal-to-noise ratios (S/N) achievable in intensity decay measurements enable reliable recovery of multiple and distributed lifetimes [4]. However, this benefit is often offset by the ultrasensitivity of fluorescence lifetime to a variety of environmental factors which makes the interpretation of lifetimes and changes in them ambiguous. However, when the dominant source of non-radiative decay can be identified with a factor, such as fluorescence resonance energy transfer (FRET) [5], then excited state lifetime becomes a very useful structural signature. In contrast to fluorescence lifetime, fluorescence depolarization time, albeit its low accuracy, is a direct indicator of molecular dynamics [2] and hence could offer relatively less ambiguous conclusions.

While the high sensitivity of the excited state lifetime of tryptophan in proteins has resulted in a wealth of information on protein dynamics, the complex intensity decay kinetics remains as a subject of continuing interest. Although there have been a variety of models for explaining the multiexponential nature of decay kinetics, the rotamer-based ground state heterogeneity model [6] seems to explain most of the observations. A simple demonstration of this model is provided by the data shown in Fig. 1. Fluorescence lifetime distributions obtained by analyzing intensity decay curves by the Maximum Entropy Method (MEM) clearly show that N-acetyltryptophanamide (NATA), a model compound for tryptophan, is associated with a single exponential decay kinetics. In contrast, the di- and tri-peptides showed multiple peaks in their lifetime distributions. The monoexponential nature of decay of NATA could be explained by the fact that the distance between the indole chromophore and the carbonyl carbon, which is presumed to be the quenching group, is similar in all the three rotamers along the C_α-C_β bond. In contrast, any peptide is associated with dissimilar distances between the indole and the quenching groups in the rotamers. Multiple lifetimes have been observed in many proteins having single tryptophan. However there have been situations where either single or nearly single lifetimes are seen such as in the case of the single tryptophan (Trp53) mutant of the protein barstar [7]. This unique situation is associated with the rigidity of the trp side chain as shown by the lack of internal motion in the native (N) state of this protein. The same protein shows three lifetimes along with the presence of internal rotational dynamics of the tryptophan (trp) in molten-globule like states. These observations constitute strong support for the ground state heterogeneity model based on rotamers, to account for the origin of multiexponential nature of tryptophan decay kinetics in general.

Information on Various Structural Forms of a Protein from Fluorescence Dynamics

Barstar, an 89 residue protein, has served as an effective model system in folding and unfolding studies [5, 7-9]. Motional dynamics of tryptophan 53, which is located at the core of the protein, are very sensitive to the overall structural characteristics of the protein. In the native (N) state of the protein, the decay of fluorescence anisotropy of Trp53 follows a single correlation time (φ) of ~5.1 ns [8]. This corresponds to the overall tumbling of the protein which has a mol. wt. of ~10 kD. The absence of any local dynamics within the observable time resolution of ~20 ps is an indication of high rigidity of the protein core where Trp53 is located. As mentioned earlier, this rigidity is probably the origin of the unique single exponential decay of fluorescence intensity. In contrast, when the protein is unfolded (U), Trp53 is associated with two φ values, ~3.5 ns and ~0.7 ns with almost equal amplitudes. Since the model compound NATA

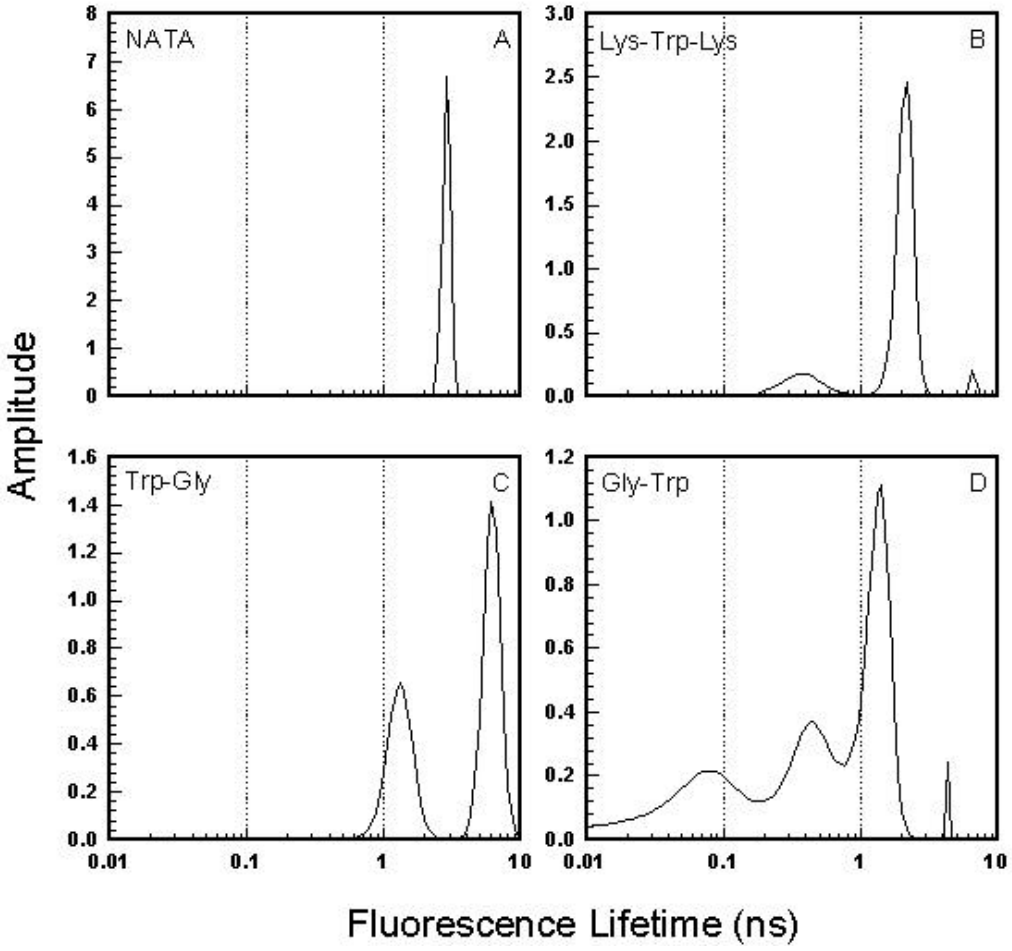

Fig. 1 Fluorescence lifetime distributions analyzed by MEM. A. N-Acetyl Tryptophanamide (NATA); B. Lys-Trp-Lys; C. Trp-Gly; D. Gly-Trp.

shows a value of $\varphi < 0.1$ ns in aqueous solutions, the two correlation times seen in the U form should reflect segmental dynamics of the polypeptide. In some of the models proposed for protein folding [10], the initial step is nucleation of folding in a specific segment of the chain. Thus, the segmental dynamics seen in the U form could be used as a locator of the nucleation site. The two correlation times observed in U form could also have arisen due to the two forms of U differentiated by the cis-trans isomer status of proline 48 .

Apart from N and U forms, barstar is known to exist in several partially folded states. The A form seen at pH 3 [11] has properties similar to that of a molten globule which is a generic form of folding intermediate [12]. Rotational dynamics of Trp53 in the A form shows two φ values, ~1ns and >50 ns (Table 1). This indicates that the core is flexible ($\varphi \sim$ 1 ns) and the protein is extensively aggregated ($\varphi >$ 50 ns) in the A form. Another molten globule-like structural form was observed in salt-stabilized high pH (12) denatured barstar [8]. Rotational dynamics of Trp53 indicated flexible interior and enhanced overall volume of the protein as hallmarks of this form.

Titration of barstar with chemical denaturants such as urea or GdnHCl, when monitored by steady-state probes such as fluorescence intensity or

TABLE 1. Rotational correlation times associated with the core Trp53 in various stable structural forms of the protein barstar

	Struc tural form of barstar	Rotational correlation times, Φ (ns)	(amplitude)
1.	Native (N) state	6.1 ns (1.0)	——
2.	Unfolded (U) state	0.76 ns (0.51)	3.7 ns (0.49)
3.	Low pH molten globule-like form (A-form)	1.1 ns (0.26)	>50 ns (0.74)
4.	High (12) pH denatured form (D- state)	0.26 ns (0.50)	2.6 ns (0.50)
5.	High (12) pH salt-stabilized compact form (P-state)	7.4 ns (1.0)	——
6.	Denaturation transition zone form	1.5 ns (0.53)	11.9 ns (0.47)

circular dichroism (CD), could be fitted to 2-state models similar to observations for many single domain small proteins [13]. However, folding intermediates have been observed during kinetic studies [14], thus creating an apparent contradiction. A likely scenario is that either such intermediates are present at very low concentrations or these intermediates are invisible through the probes normally used during equilibrium titration. Studies of the dynamics of Trp53 come in handy for clarifying the picture. In the transition zone (between the N and U states) the fluorescence anisotropy decay of Trp53 showed $\varphi \sim 12$ ns which is different from that of either the N or U forms [7]. This long correlation time indicates partially folded and subcompact intermediate structure(s). Table 1 which summarizes the φ values obtained in various structural forms of barstar demonstrates the use of rotational dynamics of tryptophan in gaining structural information of proteins.

Time-Resolved Fluorescnce 'Double Kinetics' in Protein Folding

Studies of the motional dynamics of tryptophan observed through time-resolved fluorescence anisotropy measurements provide a revealing insight into the complexities of protein folding dynamics [15]. The ability to observe the dynamics in an ensemble of heterogeneous molecules gives fluorescence-based methods an edge over high resolution techniques such as NMR and X-ray crystallography. Protein folding and unfolding processes generally occur in the timescale of microseconds to seconds [16] and can be monitored through measurements of fluorescence intensity of appropriately located probes. Although integrated fluorescence intensity could be collected with sufficiently high S/N with a time resolution of a few μs, collection of picosecond time-resolved fluorescence usually requires data aquisition windows of several tens of seconds. The experimental set-up for monitoring the time-evolution of time-resolved fluorescence (`double kinetics') during folding or unfolding of proteins is given in Fig. 2. The set-up consists of three main parts: (i) Picosecond high repetition laser system; (ii) Stopped-flow sample handling system and (iii) A dual channel high repetition rate Time-Correlated Single Photon Counting (TCSPC) system. The two detection channels can be set either at two polarizations (for fluorescence anisotropy measurements) or at two wavelengths when needed.

The necessity to collect only a single photon after every pulse of excitation in time-correlated single photon counting (TCSPC) method [17], and time-spreading of the collected photons, are the main causes of the normal requirement of long (~10 s) acquisition times. (Although a part of the problem is overcome in streak camera based measurements [18], these measurements suffer from low dynamic range when compared to the TCSPC method.) Nevertheless, a time resolution of ~20 ms in such 'double kinetics' experimental studies of protein folding reactions monitored by picosecond-resolved rotational dynamics was demonstrated in a specialized TCSPC method [15]. Similar time resolution can be achieved with the help of newer generation TCSPC cards which operate in the

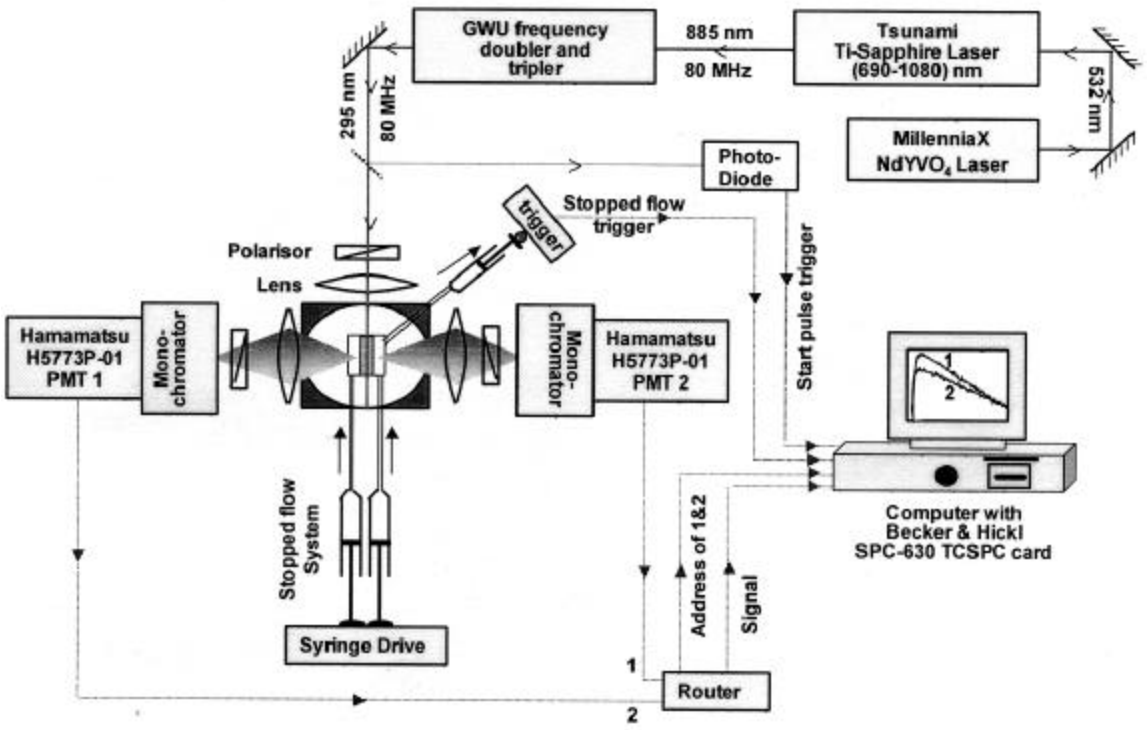

Fig. 2 The experimental set-up for monitoring the time-evolution of time-resolved fluorescence (`double kinetics') during folding or unfolding of proteins. The set-up consists of three main parts: (i) Picosecond high repetition laser system; (ii) Stopped-flow sample handling system and (iii) A dual channel high repetition rate Time-Correlated Single Photon Counting (TCSPC) system. The two detection channels can be set either at two polarizations (for fluorescence anisotropy measurements) or at two wavelengths when needed.

reverse start-stop mode at the full repetition rate (~80 MHz) of the pulsed laser.

Time-Resolved Fluorescence Resonance Energy Transfer (tr-FRET) in Protein Folding

The fluorescence lifetime of tryptophan is too sensitive to environment and this results in the inability to interpret changes in it in a unique way. However, when energy transfer from tryptophan to an intramolecular acceptor occurs, by FRET [19], in a predominant manner, the fluorescence lifetime of the tryptophan becomes a sensitive monitor of intramolecular distances. Furthermore, in complex situations, such as encountered even in small proteins, intramolecular distances could have distributions due to heterogeneity of structure. Such a distribution of intramolecular distances can be obtained when the fluorescence intensity decay profiles are analyzed to generate a distribution of lifetimes. Although there are a number of ways by which distribution of lifetimes can be obtained [20], Maximum Entropy Method (MEM) provides the most unbiased distributions [5]. Information on distance distribution is quite useful as it provides a quantitative estimate of the level of molecular level heterogeneity which is a hallmark parameter in the energy landscape model of protein folding. The first use of this (tr-FRET) method to study protein folding was demonstrated in equilibrium denaturation of barstar [5]. Subsequently, the technique has been applied to the folding kinetics of cytochrome c [18] and barstar. The results from these experiments are described below. The only alternative to tr-FRET in

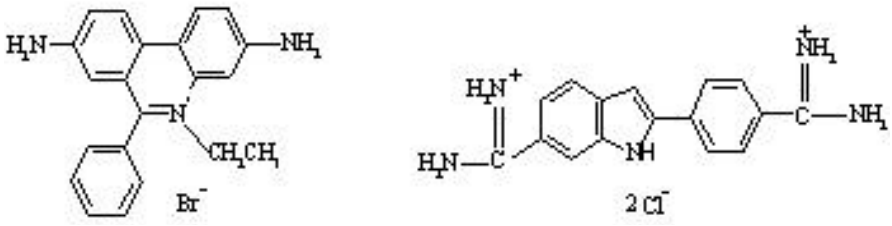

Fig. 3A Chemical structures of some of the non-specific fluorescence probes used for monitoring DNA dynamics. (i) Ethidium bromide, (ii) DAPI, (iii) PicoGreen (PG), (iv) Propidium iodide, (v) YOYO-1.

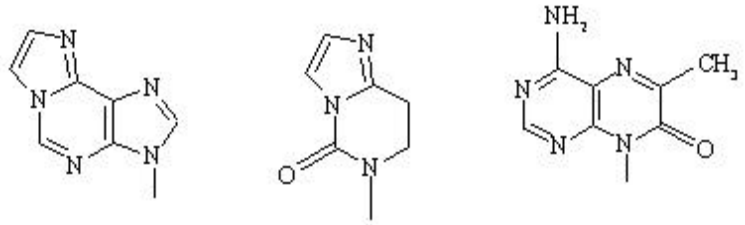

Fig. 3B Chemical structures of some of the site-specific fluorescence probes used for monitoring DNA dynamics. (i) Etheno-A, (ii) Etheno-C, (iii) 6-MAP, (iv) 2-Aminopurine (2-AP), (v) 8-Vinyladenine (8-VA), (vi) Pyrrolo-dC (PdC).

quantifying the level of heterogeneity is single molecule FRET studies [21]. It should be noted that the information (i.e., population heterogeneity) content from both the types of measurements are similar to each other. TCSPC-based fluorescence decay curves are in fact generated by watching a single photon, and hence a single molecule, at a time. Furthermore, typical TCSPC curves are generated from total photon counts in the range of several tens of millions. In contrast, single molecule FRET observations [21] rely on only a few hundreds of events to construct fluorescence intensity histograms.

Evolution of Population Heterogeneity During Protein Folding - Demonstration of 'Folding Funnel'

The non-fluorescent acceptor group, thionitrobenzoate (TNB) is a very efficient quencher of tryptophan fluorescence, by FRET [22]. When TNB is covalently linked to Cysteine-82 of barstar, the fluorescence of Trp53 gets quenched dramatically (~ 95 %) by FRET [5]. The quenching gets substantially relieved when the protein is denatured. When FRET occurs between Trp53 and TNB group covalently attached to a cystein side chain located at various positions in the protein barstar, the lifetime distribution of Trp53 can be translated to provide intramolecular distance and distance distributions. This methodology offers the unique possibility of monitoring the time evolution of various intramolecular distances during folding process. The ability of the tr-FRET technique to differentiate and quantitate various sub-populations within an ensemble has been exploited to follow the structural composition of folding intermediates.

DNA Dynamics

Fluorescence Probes for DNA Dynamics

The fluorescent quantum yield of native DNA which is due to its bases is too small ($\Phi_f \sim 4x10^{-5}$) [23]. However, there have been several attempts to use the intrinsic fluorescence of DNA to gain information on its dynamics. Furthermore, due to its non-specific nature, its use is limited to study only overall dynamics of DNA such as internal motion of DNA bases.

Several extrinsic probes have been developed over the years to make DNA fluorescently visible. They fall into two categories, namely specific and non-specific. Non-specific probes such as ethidium

bromide [24], DAPI [25], propydium iodide [26], YOYO [27], and PicoGreen (PG) [28] as given in Fig.3A and their large range of analogs were originally developed as DNA stains to visualize DNA in fluorescence microscopic images. However, probes such as ethidium [29] and YOYO-1[30] have found use in studying complex dynamic modes of DNA and as condensation indicators. Most of these probes are nearly non-fluorescent in their free form and become highly fluorescent when bound to DNA. Although many of these probes are either mono orbis intercalators, but DAPI [25] work as groove binders. Probe such as PicoGreen [31] has been used to assess the relative population of single-stranded regions and single-strand nicks in ds-DNA. This capability arises presumably due to the modulation of fluorescence quantum yield by the various dynamic modes of DNA.

Owing to its non-specific probing properties, the probes mentioned above are not useful in studying environmental change in a specific location of DNA. Hence site-specific fluorescent probes (Figure 3B) which are mainly nucleotide base analogues are most appropriate in studying the dynamics in a specific region of DNA. Selection of these nucleotide analogs is generally based on the criteria that the fluorescent analog mimics the parent nucleotide in its base-pair hydrogen bonding and other interaction potentials. 2-aminopurine (2-AP) which is being widely used as an analog of adenine forms hydrogen bonds with thymine very similar to those of adenine. Although the quantum yield of 2-AP gets substantially reduced ($\Phi_f \sim 0.1$) on incorporation into DNA, it is still sufficient for monitoring subtle changes in the structure and dynamics [32].

Very recently, 8-vinyl-deoxyadanine (8-VA) has been proposed as an improved substitute of 2-AP [33]. The quantum yield of this new adenine analog is significantly higher than that of 2-AP, when inserted in DNA. In addition, 8-VA is able to adopt an anti conformation that preserves the Watson-Crick hydrogen bonding. Another fluorescent analog pyrrolo-dC (PdC) was introduced few years back which can pair with Guanine. The quantum yield sensitivity of pyrrolo-dC is quite similar to that of 2-AP [34]. All the three probes 2-AP, 8-VA and PdC show significant reduction in their quantum yield on base-pair formation and thus serve as indicators of local hybridization. However, PdC is likely to have greater potential since its excitation wavelength (347 nm) overlaps less with that of proteins. Thus PdC is better suited for studies on DNA-protein complexes.

YOYO-1 as a fluorescent indicator of DNA condensation

Condensation of DNA, whereby DNA transforms from an open and fluctuating architecture into a compact and relatively static form is encountered in a variety of situations both natural and artificial. The process of DNA condensation has to achieve a fine balance between efficient compaction to economize the size while preserving the function such as gene expression in chromosomes. This makes studies on the structure and dynamics of condensed forms of DNA quite rewarding.

The motional dynamics of condensed DNA was studied by the use of YOYO-1. The concentration of YOYO-1 was reduced significantly (D: P, ~ 1: 2000) such that the probability of formation of H-dimers is negligible. In this situation, the fluorescence signal would be controlled mainly by the dynamics of DNA. Motional dynamics of DNA-bound YOYO-1 inferred through picosecond time-resolved fluorescence anisotropy was used in revealing various dynamic modes of extended and condensed forms of DNA. By monitoring the motional dynamics of DNA-bound YOYO-1 under a variety of condensation-inducing conditions such as the presence of cationic polymers and detergents [30], molecular crowding agents such as polyethyleneglycol and cationic peptides such as the nucleocapsid protein NCp7 of HIV-1 virus [35] significant and interesting features were observed on the mechanism of condensation of DNA.

Conclusions

One of the recent themes in modern biology, namely, dynamics along with structure the basis of function of biomolecular systems is getting increasing level of support from various experimental and molecular dynamics simulation techniques. In this article, the power of

time-resolved fluorescence spectroscopy in bringing to light various intricate dynamic aspects of proteins and protein-DNA complexes and, in some cases, their correlation with function has been shown. We believe that this is a very fruitful approach in seeking explanations for complex activity of biomolecular systems especially in the light of the enormous level of structural information being gathered in recent times.

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NUCLEUS

Room-temperature transistor laser is step closer to commercialization

Researchers at the University of Illinois at Urbana-Champaign have demonstrated the room-temperature operation of a heterojunction bipolar transistor laser, moving it an important step closer to commercialization. The scientists describe their work in the Sept. 26 issue of the journal Applied Physics Letters.

"We have shown that the transistor laser, even in its early state of development, is capable of room-temperature operation at a speed of 3 gigahertz," said Nick Holonyak Jr., a John Bardeen Chair Professor of Electrical and Computer Engineering and Physics at Illinois. "We expect the device will operate at much higher speeds when it is more fully developed, as well as play an important role in electronic-photonic integrated circuits."

Room-temperature transistor lasers "could facilitate faster signal processing, large capacity seamless communications, and higher performance electrical and optical integrated circuits," said Milton Feng, the Holonyak Chair Professor of Electrical and Computer Engineering at Illinois. Feng's research on heterojunction bipolar transistors has produced the world's fastest bipolar transistor, a device that operates at a frequency of 600 gigahertz or more, and is a natural platform on which to develop a transistor laser.

The Illinois researchers first reported the demonstration of a light-emitting transistor in the Jan. 5, 2004, issue of Applied Physics Letters. They described the first laser operation of the light-emitting transistor in the Nov. 15, 2004, issue of the same journal. At that time, the transistor laser had to be chilled with liquid nitrogen to minus 73 degrees Celsius.

Room-temperature operation is ultimately required for large-scale commercial applications, said Holonyak, who also is a professor in the university's Center for Advanced Study, one of the highest forms of campus recognition. "If this device operated only at low temperature, nobody would want it, except as a laboratory curiosity or for very limited applications."

After the demonstration of the first semiconductor laser (as well as the first practical light-emitting diode) in 1962, "it took the effort of many people eight years to get the diode laser to operate at room temperature," Holonyak said. "Then it took an additional two years to make it reliable. But the big payoff has only now just begun, after more than 40 years of additional work."

In comparison, it has taken the Illinois researchers less than a year to move the transistor laser from cold operation to room-temperature operation. "Who knows where this new transistor laser technology will be in another 40 years," Holonyak said. "The payoff part of scientific and technological advances never occurs rapidly, at least not the 'big payoff.'

"The transistor laser is still a primitive, laboratory device that will require a lot more work," Holonyak said. "Eventually, optimizing the design and fabrication will result in higher speed laser operation and improved performance, as well as a naturally advantageous way to realize electronic-photonic integrated circuits."

(University of Illinois at Urbana-Champaign-26 Sep-2005)

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