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Review

Novel physical chemistry approaches in biophysical researches with advanced application of lasers: Detection and manipulation☆

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ABSTRACT

Novel methodologies utilizing pulsed or intense CW irradiation obtained from lasers have a major impact on biological sciences. In this article, recent development in biophysical researches fully utilizing the laser irradiation is described for three topics, time-resolved fluorescence spectroscopy, time-resolved thermodynamics, and manipulation of the biological assemblies by intense laser irradiation. First, experimental techniques for time-resolved fluorescence spectroscopy are concisely explained in Section 2. As an example of the recent application of time-resolved fluorescence spectroscopy to biological systems, evaluation of the viscosity of lipid bilayer membranes is described. The results of the spectroscopic experiments strongly suggest the presence of heterogeneous membrane structure with two different viscosity values in liposomes formed by a single phospholipid. Section 3 covers the time-resolved thermodynamics. Thermodynamical properties are important to characterize biomolecules. However, measurement of these quantities for short-lived intermediate species has been impossible by traditional thermodynamical techniques. Recently, development of a spectroscopic method based on the transient grating method enables us to measure these quantities and also to elucidate reaction kinetics which cannot be detected by other spectroscopic methods. The principle of the measurements and applications to some protein reactions are reviewed. Manipulation and fabrication of supramolecues, amino acids, proteins, and living cells by intense laser irradiation are described in Section 4. Unconventional assembly, crystallization and growth, amyloid fibril formation, and living cell manipulation are achieved by CW laser trapping and femtosecond laser-induced cavitation bubbling. Their spatio-temporal controllability is opening a new avenue in the relevant molecular and bioscience research fields. This article is part of a Special Issue entitled "Biophysical Exploration of Dynamical Ordering of Biomolecular Systems" edited by Dr. Koichi Kato.

1. Introduction

In this review article, we describe the novel methodologies that fully utilize the characteristics of lasers for studying biological assemblies. Recent development of laser technologies has a strong impact on various fields of experimental sciences, including physics, chemistry, and biology. Novel methodologies utilizing the advanced laser systems have been applied to biological sciences intensively and extensively. The irradiation obtained from the lasers has two characteristics that are particularly advantageous for studying biophysical systems: coherence in time and in space. It is relatively easy for preparing short light pulses with duration of 100 fs or shorter, by using a mode-locking technique which requires radiation coherent in time. Time evolution of various events is directly observed with time-resolved spectroscopies using the pulsed laser as the light source. It is now possible to use a table-top laser that produces femtosecond light pulses tunable from the mid-infrared to ultraviolet spectral regions, or 10 μm to 250 nm in wavelength. Manipulation of biological assemblies including the crystals, amyloid fibrils, or living cells under a microscope has become possible, partially because of the spatial coherence of the laser radiation. When two angled laser beams cross at a position where a sample is placed, they form an interference pattern in space within the sample. The transient grating method records the formation and decay of this interference pattern or "grating". Basic thermodynamic quantities are evaluated in a time-resolved fashion with this method.

The short light pulses have two properties which allow us to design various experiments: short duration and high peak power. A direct application of the short temporal width of the light pulse is the

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development of "time-resolved" measurements. In the time-resolved measurements, time-resolved spectroscopy for example, time-dependent events are recorded as they evolve. Historically, it started with the "flash photolysis" method which combined the detection of the absorption spectroscopy in the visible [1] or mid-infrared [2] region with the photoirradiation of a sample with a light pulse prepared by a flash lamp. Reaction intermediates with a lifetime of microseconds or longer were successfully detected with the flash photolysis experiments. After the 1960s, then, pulsed lasers have replaced the flash lamps as the actinic light source in the flash photolysis. Techniques for the flash photolysis have been developed rapidly and continuously since the introduction of the pulsed laser, especially in the visible spectral region, following the rapid development of the laser technology. Introduction of the mode-locked Ti:sapphire laser, or the Ti:sapphire oscillator, in around 1990 and the development of the Ti:sapphire regenerative amplifier [3] allowed an easier access to a stable pulsed laser output of 100 fs duration. Time-resolved detection schemes have been combined with a number of measurements including various absorption and emission spectroscopies in the spectral region of deep UV to THz, and diffraction measurement by X-ray and electrons.

High peak power is another important property of the short light pulse. Because a short pulse has the light intensity only for a short period of time, the light intensity when the light is on can be large even if the average power is modest. The energy of a single light pulse in a 1 kHz laser output with an average power of 1 W, for example, is as small as 1 mJ. The peak power of this light pulse, however, is 10 GW, if the laser pulse has 100 fs duration. The high peak power of the pulsed laser is advantageous for nonlinear optical processes [4–6]. It is not difficult to convert the wavelength of a pulsed laser output when nonlinear polarization, or polarization whose amplitude is not proportional to the amplitude of the applied electric field, is efficiently induced by its high peak power. Second harmonic generation, SHG, and optical parametric amplification, OPA, both dependent on the second-order polarization, are techniques frequently used for converting the laser wavelength.

Three experimental methods are accounted for in the following sections of this review article. The pulsed laser plays an essential role in these methods for the investigation of the biomolecules and their assemblies. In Section 2, experimental techniques for time-resolved fluorescence measurements with pulsed excitation are explained. Technical advantages of the time-resolved fluorescence detection with a streak camera are described, together with its application for evaluating the viscosity in lipid bilayer membranes. Transient grating method is explained in Section 3. Time dependence of the refractive index induced by the photoirradiation, which reflects temperature change, molecular diffusion, and volume change, is traced with this method. Thermodynamical quantities for metastable states of proteins are determined with this method. Manipulation of biological systems including supramolecules, proteins, and living cells by intense laser irradiation is described in Section 4. A single assembly, a single crystal, and a sphere of amyloid fibrils are prepared at the focus for supramolecules, amino acids, and proteins, respectively, by the intense CW laser irradiation under a microscope. The fabrication of protein crystals and amyloid fibrils, as well as the manipulation of single living cells, are also made possible with the femtosecond laser irradiation.

2. Time-resolved fluorescence spectroscopy and evaluation of viscosity in lipid bilayer membranes

2.1. Fluorescence lifetime

Fluorescence detection is an experimental method commonly used in biological researches. One of the reasons for the popularity of the fluorescence detection is the high efficiency of the fluorescence process. A fluorescent molecule or a fluorophore (a fluorescent group in a molecule) is excited to an electronically excited state when it absorbs a photon whose energy matches the energy for an optical absorption of the molecule. The molecule in the excited state then emits a photon as fluorescence. The quantum yield of fluorescence, defined as the probability of a fluorescence event after the absorption of an incoming photon, is close to one for a strongly fluorescent molecule. The cross section for the fluorescence emission will be in the same order of the absorption cross section, which is typically 10^{-17} cm^2/molecule. The absorption cross section of 10^{-17} cm^2/molecule corresponds to a molar extinction coefficient ε of 6×10^3 dm^3 mol^{-1} cm^{-1}. If this molecule is irradiated with a cw laser whose wavelength is 500 nm, in resonance with an absorption band of this molecule, with an average power of 10 μW focused into an area of 1 μm^2, then this molecule will statistically be excited to its excited state 3×10^4 times in a second if the excited state lifetime is shorter than $(3 \times 10^4)^{-1}$ s. If the quantum yield of fluorescence is one, this molecule emits 3×10^4 photons every second. If a lens that collects the light within the incident angle of 45 degrees or numerical aperture of 0.71 is used for the collection optics, 21% of the emitted fluorescence is delivered to a monochromator. Because the maximum quantum efficiency expected for a typical charge-coupled device (CCD) detector commercially available is as high as 0.9 and because the readout noise of a CCD detector is a few counts/pixel, detection of 6×10^3 photons during one second from a single fluorescence probe is technically quite possible. The estimation for a fluorescence experiment with pulsed excitation gives the same result as long as the detector responses linearly to a large number of photons that reaches the detector in the short pulse duration and observation of the fluorescence event is not affected by possible multi-photon processes caused by the pulsed excitation.

In a steady-state fluorescence measurement, fluorescence intensity is recorded as a function of excitation wavelength and emission wavelength. Fluorescence intensity is plotted against the emission wavelength, or emission photon energy, in an ordinary fluorescence spectrum. For a fluorescence excitation spectrum, the excitation wavelength (or excitation photon energy) is the major parameter. In time-resolved fluorescence spectroscopy, however, the time interval, or time delay, between the fluorescence excitation and fluorescence detection is a major parameter as well. Here, we express the fluorescence intensity simply as a function of time as I(t). Time dependence of the fluorescence intensity is often expressed as a single exponential decay function,

$$I(t) = I_0 \exp(-k_f t) \tag{3}$$

where k_f is the fluorescence decay rate constant and I_0 is the fluorescence intensity at $t = 0$. The fluorescence decay rate constant is a sum of the radiative decay rate constant k_r and the non-radiative decay rate constant k_{nr} [7–9],

$$k_f = k_r + k_{nr} \tag{4}$$

The fluorescence lifetime τ_f is therefore expressed with the radiative decay lifetime τ_r and the non-radiative decay lifetime τ_{nr} as

$$\frac{1}{\tau_f} = \frac{1}{\tau_r} + \frac{1}{\tau_{nr}} \tag{5}$$

The rate of the radiative decay is determined by the characters of the initial and final states of a fluorescence transition, while the non-radiative decay rate can change largely depending on the chemical environments surrounding the fluorescence probe. It is therefore possible to evaluate the surrounding environments, such as viscosity and polarity, by measuring the fluorescence lifetime if the radiative lifetime is known.

Intensity calibration for a fluorescence measurement is not trivial. The recorded fluorescence intensity is easily affected by the different spectral response of the detector and monochromator, off-axis signal collection, or re-absorption of the fluorescence signal by the sample. Careful and sometimes tedious calibration procedure is necessary when the fluorescence signals recorded for more than one wavelengths or