2D Electronic Spectroscopy of Biological Systems

Motivation

The dynamics of photoactive biological systems, including many light-harvesting complexes, rely on very fast and efficient transfers of energy between absorptive chromophore antenna complexes and other cofactors. The energy pathways are difficult to describe theoretically given the complexity of many of these systems, and the dynamics occur on femtosecond to picosecond time scales. Therefore, ultrafast spectroscopic methods are required to effectively probe the energy transfer in these systems.

Photosynthetic complexes are of particular interest. In these systems, visible light is absorbed by light-harvesting antennae, which then transfer the energy to a reaction center to allow stable charge separation which can be used to fuel metabolic processes in the organism. Two-dimensional electronic spectroscopy (2DES) has recently been shown to be a powerful tool for revealing energy transfer pathways in the Fenna-Matthews-Olson photosynthetic complex. [2,3] 2DES in the near infrared regime was made possible by an experimental innovation that provided passive phase stability necessary for the experiment. [1] This innovation has been demonstrated to work in the visible regime, [1] which will allow us to extend the technique to study other photosynthetic light-harvesting proteins, including the LH2 complex from purple bacteria. Another system of interest is the photoactive protein DNA photolyase, which is found in a wide range of different organisms and is responsible for repair of UV-induced damage to DNA. [4]

Methods

An optical analog of 2D nuclear magnetic resonance (NMR) spectroscopy, 2DES allows us to probe the electronic transitions of a system and to directly observe their coupling with high time resolution. Through this technique, we gain direct information about transition frequencies, separate measurements of homogeneous and inhomogeneous broadening, coupling strength between chromophores, and transition dipoles. In addition, the 2D spectrum is complex valued to give both the real (absorptive)

and imaginary (dispersive) parts of the spectrum so that we can obtain the full third order nonlinear susceptibility of the sample in question. Polarization-dependent experiments can also give information on the dipole angles in the complex by showing anisotropies in the spectra. While much of this information can be obtained by a combination of other nonlinear spectrocopies, 2DES provides this in a single experiment. [5]

Two-dimensional electronic spectroscopy is a four-wave mixing technique which examines the third order polarization properties of the sample. The sample, initially held in the ground state, is excited by a pump pulse into a coherence between ground and excited states, allowing individual dipoles to oscillate at their natural frequencies. A second pump pulse, arriving at a time τ later transfers the system into a population. After this, the sample is allowed to relax for a time T, during which time many interesting dynamics can occur, including energy and charge transfer and conformational changes. A third "probe" pulse induces the sample to radiate a signal field as a function of time t after the probe pulse. The three exciting pulses and a low-intensity reference are arranged in the four corners of a "boxcar" geometry so that the signal is phase matched to be collinear with the reference pulse, from which the complete electric field (that is, the amplitude and phase) of the signal can be recovered in the frequency domain (ω_t). Figure 1A shows the timing of the pulses and Figure 1B the boxcar geometry used.

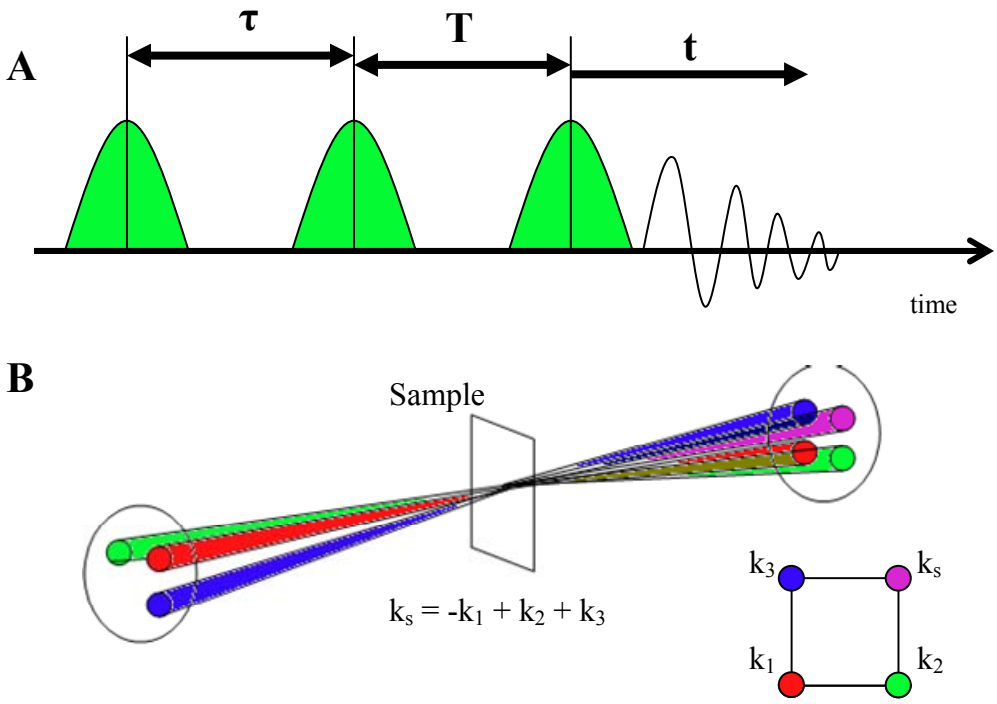

Figure 1. (A) This shows the pulse sequence in the 2DES experiments. The first two pulses are our "pump" pulses, while the third pulse is the "probe". The reference pulse which interferes with the signal is not shown but occurs before the relevant exciting pulse train. (B) The boxcar geometry of the pulses with the phase matching condition for a positive τ scan. Pulses 1 and 2 are the first and second pump pulses, respectively, and pulse 3 is the probe.

By maintaining a constant relaxation time T and scanning over both positive and negative values for the time delay τ, we obtain multiple line spectra, each for a given τ value. We then take an inverse Fourier transform over time τ to produce a 2D spectrum with axes ω_τ vs. ω_t. The spectrum can then be easily read as follows: ω_τ is the excitation frequency, and ω_t is the detected frequency, as measured in the spectrometer. Upon separating the real and imaginary components, the resulting absorptive 2D spectrum shows features as depicted in Figure 2. Diagonal peaks amplitudes show the transition dipole for a given species, while the shape of the peaks show the degree of inhomogeneity. Cross peak amplitude shows the coupling between electronic transitions. By taking a series of 2D spectra for various relaxation times T, we can see the evolution of these dynamics over femtosecond or picosecond time scales.

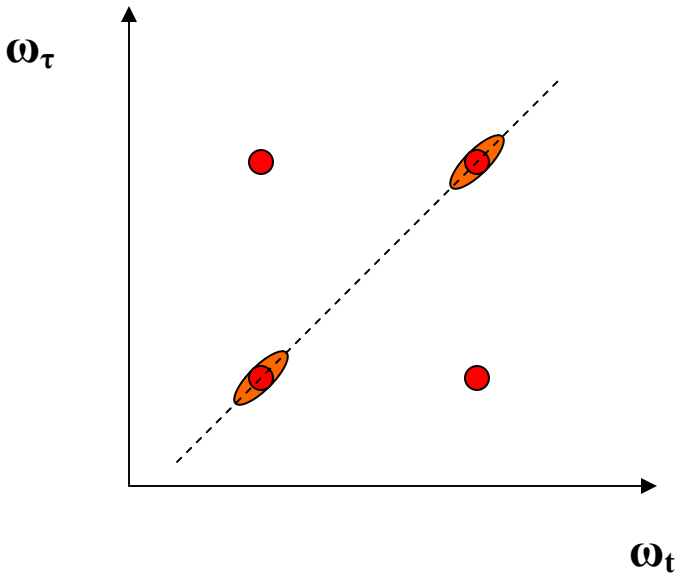

Figure 2. An idealized depiction of a 2D spectrum for a simple two-state system. The y-axis is the excitation frequency, and the x-axis is the detection frequency. Note that spreading of the diagonal peaks along the diagonal gives a measurement of inhomogeneous broadening, while the peak width perpendicular to the diagonal is homogeneous broadening only. The smaller cross peaks indicate coupling.

Energy transfer between nearby chromophores is often mediated by Fluorescence Resonance Energy Transfer (FRET), a dipole-dipole interaction by which the excitation of one molecule is transferred to a nearby molecule without an emission/absorption event. This is demonstrated by the decrease of excited donor lifetimes in the presence of acceptor molecules with overlapping dipole frequencies, which indicates an additional decay pathway independent of normal photon emission. [6]. For biological macromolecules on the size scale of proteins, FRET is one of the major methods of energy transfer between chromophores and is therefore very important in the systems we will be studying.

One of the most interesting and useful features of FRET is its sensitive dependence on the distance between chromophores. The rate of energy transfer is given by

$$k = \frac{\phi_D}{\tau_D}\left(\frac{8.785\times10^{-25} I}{n^4 R^6}\right) \qquad (1.1)$$

Where ϕ_D is the fluorescence quantum yield, τ_D is the donor lifetime in the absence of the acceptor, *n* is the refractive index of the medium, *R* is the center-to-center distance between donor and acceptor, and *I* is a spectral overlap factor between the donor emission and acceptor absorption. [7]

As Eq. (1.1) suggests, FRET efficiency drops off very quickly at large separations. Between about 0.5 and 10 nm, the FRET rate is large enough to be easily measured and highly sensitive to small changes in distance. In a way, precision FRET intensity/rate measurements can be used as a sort of spectroscopic ruler. [12]

As a first experiment and a simple system to help calibrate our setup, we have obtained samples of DNA with a donor and acceptor pair attached to the 3' and 5' ends of the DNA strand, respectively. The DNA can be synthesized to a specific number of base pairs and thus offers a very well defined separation between donor and acceptor. By obtaining 2D spectra of the FRET paired DNA in solution for various DNA lengths and directly analyzing the rate of energy transfer from the cross peaks, we can study the FRET system experimentally. This will also allow us to study the limits at which the rate equation approximations break down. For instance, the rate equation given above is derived with the assumption that the chromophore size is small compared to their separation, but that is not always the case for large molecules. [9] Studies of these simple systems serve as a prelude to the study of photosynthetic complexes, which function primarily by energy transfer along a network of chromophores. Many of these dynamics are poorly understood at the current time and will make excellent systems of study under 2DES.

Experimental Setup

Our laser source consists of a Titanium Sapphire oscillator which seeds a regenerative amplifier. From here, the 800 nm beam is split and fed into two separate non-collinear optical parametric amplifiers (NOPAs). [8] A diffractive optic splits each NOPA pulse into two beams, and a series of three variable delays separates the pulses temporally so that we can obtain the scanable time delays τ and T for our 2DES experiments. The use of a pair of parabolic mirrors allows us to arrange the four beams precisely in a boxcar geometry. The beams focus and converge at a sample cell, and the

signal pulse is spatially selected and directed into a spectrometer, where the spectral scans are taken by a CCD. Before the sample, a neutral density filter attenuates the reference pulse so as to maximize interference with the signal.

One of the major advantages of using two NOPAs is that it allows us to separately tune our pump beams and our probe beam over a wide spectral range of ~ 475 – 1000 nm. In this way, we can probe the dynamics of complex biological systems with a wide range of chromophores and large frequency shifts over multi-step energy transfer pathways. Another major strength of the setup is the aforementioned diffractive optic, which allows for automatic alignment of the reference pulse along the same path as the signal and excellent passive phase stability. [1]

Our setup is currently complete, and we are in the process of optimizing the beam spot size and calibrating the time delay motors. We have already obtained a simple 2DES spectrum of Rhodamine-6G in ethanol. With the use of our tunable NOPAs, we will be able to access a wide range of electronic transfers in first DNA-bound FRET pairs and shortly thereafter in the photosynthetic LH2 complex and the DNA repair protein photolyase. Using 2DES, we aim to obtain new information about the ultrafast dynamics of a wide array of biological systems.

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