Single-Molecule Pump-Probe Detection Resolves Ultrafast Pathways in Individual and Coupled Quantum Systems

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(Received 18 November 2004; published 25 February 2005)

We report the first experimental study of individual molecules with femtosecond time resolution using a novel ultrafast single-molecule pump-probe method. A wide range of relaxation times from below 100 up to 400 fs is found, revealing energy redistribution over different vibrational modes and phonon coupling to the nanoenvironment. Addressing quantum-coupled molecules we find longer decay times, pointing towards inhibited intramolecular decay due to delocalized excitation. Interestingly, each individual system shows discrete jumps in femtosecond response, reflecting sudden breakup of the coupled superradiant state.

DOI: 10.1103/PhysRevLett.94.078302 PACS numbers: 82.37.–j, 34.30.+h, 71.35.–y, 82.53.–k

Ultrafast processes play a crucial role in the functioning of both natural and synthetic molecular assemblies [1]. For example, energy transfer between chromophores in photosynthetic complexes and conjugated polymers typically occurs on a 0.5 to 2.0 ps time scale [2], while intramolecular dynamics is even faster. The energy transfer is mediated by delocalized electronic excitation, in direct competition with disorder in the system, ultrafast decay through intramolecular vibrations, and coupling to the environment. To address the wide range of ultrafast processes, generally advanced spectroscopic methods are invoked. Initially spectral hole burning [3] was developed to probe the underlying dynamics of inhomogeneously broadened bands in solids. With femtosecond photon echo spectroscopy [4] the inhomogeneous linewidth could be eliminated to study ultrafast dephasing times and solvation dynamics. Currently mainly four-wave mixing and pump-probe spectroscopies [5] are applied to address both dephasing and population dynamics, predominantly of vibrational wave packets. Unfortunately, all these approaches are restricted to processes that can be optically synchronized and therefore yield only the spatial average over some conformational subset.

In contrast to this, single-molecule studies [6] commonly reveal how changes in the local environment lead to large variations, in both space and time [7–9]. However, single-molecule detection relies essentially on background-free detection of Stokes shifted fluorescence. The limited photon yield is a bottleneck in dynamic studies, restricting real-time observations typically to the microsecond regime [8]. Only at cryogenic conditions are cross sections large enough to allow resonant detection with a faster response [10]. As a result the femtosecond regime has so far remained untouched in the single-molecule field operating at ambient conditions.

Here, we bridge the gap between ''ultrafast'' and ''single-molecule'' detection and present a novel fluorescence-based pump-probe method that gives direct access to ultrafast phenomena in individual quantum systems. The first results on both single and coupled molecules are presented, where from molecule to molecule a wide range of ultrafast decay times is observed.

Optical observation of single quantum systems is based on detection of fluorescence, i.e., spontaneous emission from an electronically excited state with typically a nanosecond lifetime [6,7]. Ultrafast detection is generally based on stimulated processes, such as transient absorption. Therefore, to extend single-molecule detection into the femtosecond domain, we need to exploit ultrafast stimulated transitions in competition with the detected spontaneous decay. To this end, the optical transition in a molecule is saturated such that the photocycle of stimulated absorption and emission dominates over the spontaneous decay [Fig. 1(a)]. Upon electronic excitation with a femtosecond laser pulse, a set of molecular vibrations will be simultaneously excited, which exhibits ultrafast coupling to other internal vibrations of the molecule [12]. Only when the initial excited state has leaked to modes that are not accessible for the incoming (de)excitation light, the molecule can no longer be stimulated back to the ground state and is bound to relax through spontaneous decay. The competition between stimulated and spontaneous emission is controlled by exciting the molecule of choice with two consecutive pulses and varying the delay between those pulses [Fig. 1(b)]. Large organic molecules at room temperature display wide single-molecule emission spectra ($\Delta\lambda \sim 50$ nm); hence coherent effects are not expected due to very fast optical dephasing ($\tau_d \sim 20$ fs). As a result a single short saturating pump pulse (duration $> \tau_d$) exciting a molecule has an equal chance to leave the molecule in the ground or excited state. This leads to a 50% chance of emitting a fluorescent photon when the quantum efficiency is close to unity. Applying a second probe pulse at zero

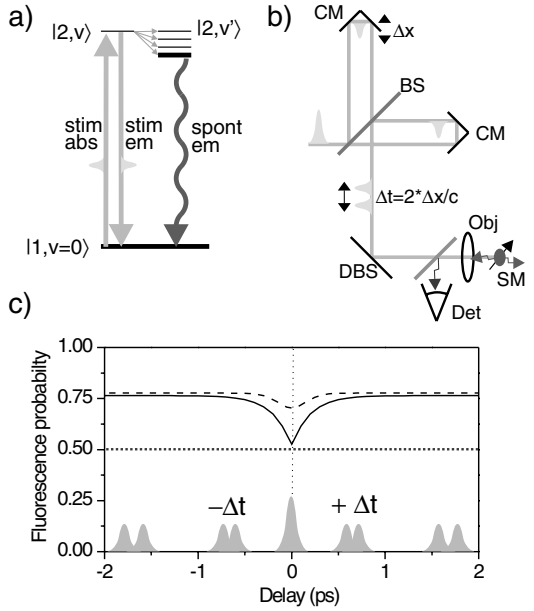

FIG. 1. (a) Ground state $|1, v = 0\rangle$ is coupled to excited state ($|2, v\rangle$) by saturation with a laser pulse. The balance between stimulated processes gives an equal state probability for both states. However, the excited state $|2, v\rangle$ couples to other vibrational states $|2, v'\rangle$ in fs-ps time and the $|2, v'\rangle$ states ultimately decay by spontaneous emission. The states $|2, v'\rangle$ have reduced coupling constants for stimulated emission by the laser pulse to the ground state: a new equilibrium between level $|1, v = 0\rangle$ and $|2, v\rangle$ is reached. Employing two equal pulses with a controllable delay the stimulated photocycle can be manipulated resulting in enhanced spontaneous decay. (b) 280 fs pulses (circularly polarized) are split by a beam splitter (BS) and recombined. One corner mirror (CM). is translated to provide a variable delay (Δt) between the two beams. The "double pulse" excites the single molecules (SM). Fluorescence is collected by a high numerical aperture objective (Obj) and passed via a dichroic beam splitter (DBS) onto a photon counting detector (Det). (c) The fluorescence probability for a single saturating pulse is 0.5 (dotted line). For two pulses the fluorescence probability increases from 0.5 at zero delay to 0.75 for infinite delay, resulting in a dip in the fluorescence intensity (solid line). For a realistic longer pulse duration (280 fs, dashed line) the dip becomes convoluted with the pulse width and the femtosecond decay time is retrieved by deconvolution [11].

delay does not enhance the chance on a fluorescent photon since the transition is already saturated by the first pump pulse. However, the balance is broken when the probe pulse arrives after the molecule had some time to decay from the excited state to a vibronic state, out of resonance for stimulated emission. As a result, stimulated absorption dominates and the total chance of a ground state molecule to end in an excited state increases, leading to an enhanced chance on the emission of a fluorescent photon. In fact, the fluorescence enhancement is the counterpart of suppression by stimulated emission depletion of all vibronic states using a second longer wavelength pulse [13]. Most importantly, the additional fluorescence signal we gain by the probe pulse will increase for longer delay [Fig. 1(c)], where the typical time over which the increase takes place is a direct measure for the time the initially excited state needs to relax. As a result, our single-molecule pump-probe (SM2P) method reveals the very first decay, the energy redistribution time (τ_{er}), of a single quantum system.

Here we focus the SM2P experiment [Fig. 1(b)] on a characteristic system: perylene-diimide molecules [572 (602) nm absorption (emission) maximum] embedded in a polymethyl-metacrylate (PMMA) film. Single molecules were detected using a scanning confocal microscope [14]. Typically an energy of $\sim$2 pJ per laser pulse (280 fs) on a diffraction limited (Ø 250 nm) spot ($I_{avg} = 4$ kW/cm^2) was enough to saturate a single molecule, such that on average one photon is emitted for every two laser pulses. A single perylene-diimide molecule emits in general 10^7–10^8 photons before irreversible photodegradation occurs; therefore, a pulse repetition rate of 1 MHz was chosen to allow for an observation time up to a minute. In all experiments, the fluorescence intensity is recorded while sweeping the delay line symmetrically through negative and positive delay [Fig. 1(c)]. Moreover, before and after each delay scan the single pulse excitation signal is measured to provide a reference value. To complement the femtosecond decay data also the nanosecond fluorescence lifetime (τ_f) is measured simultaneously using time correlated single photon counting [14].

Figure 2(a) shows some typical single-molecule delay traces for three perylene molecules in the same sample. Clearly, a dip is visible for all traces, with its minimum at zero delay, while more fluorescence is observed for longer delay times. For molecule *A* two consecutive scans are shown (*A*1 and *A*2), showing a similar width of the dip feature. Wider dips are observed for molecules *B* and *C*. The decay time τ_{er} is determined by fitting with a three level model and taking into account the convolution with the finite femtosecond pulse length. We find times from 400 to below 100 fs for molecules in the same polymer film. It should be noted that the recovered redistribution time depends solely on the width of the dip and is independent of the degree of saturation induced by a single pulse. The depth of the SM2P dip is related to the redistribution time (shorter times lead to shallower dips); however, it is also influenced by the degree of saturation. The noise observed in the SM2P scans is purely determined by photon counting statistics, which sets a fundamental limit to the accuracy of τ_{er}. Under the experimental conditions used, we find that the accuracy is at best 50 fs for τ_{er} shorter than 150 fs and $\sim$35 fs for longer decay times.

The ultrashort times are attributed to two dominant processes. First, through *intramolecular* vibrational redistribution, the initially excited vibrations are coupled to other vibrational modes that can no longer be probed by the laser light [11]. A second process is the transfer of the excess vibrational energy to the surrounding matrix. In a polymer glass only inertial, *intermolecular* phonon coupling gives rise to ultrafast energy relaxation [15]. Further

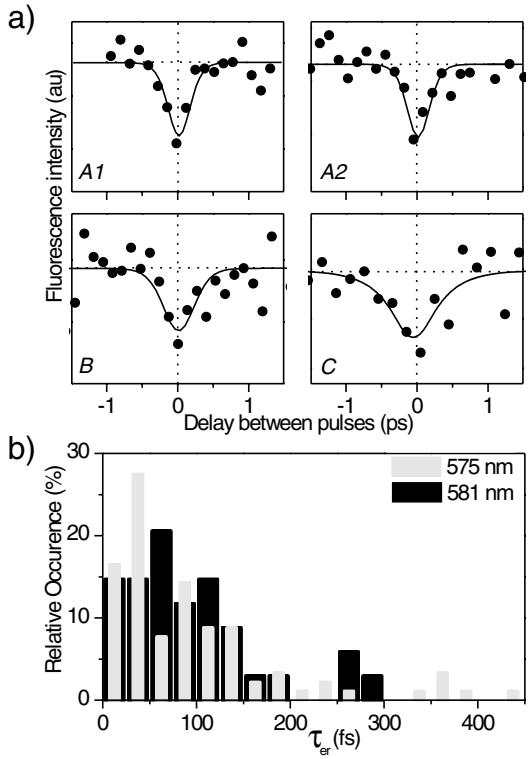

FIG. 2. (a) Delay traces on different single perylene molecules in PMMA. Every molecule exhibits a fluorescence dip around zero delay. Traces $A1$ and $A2$ show two consecutive measurements (12 s interval) on the same molecule. The solid curves show fits with decay times of 97 and 94 ± 50 fs, respectively. Longer times (130 ± 50 fs and 400 ± 30 fs) are recovered for two other molecules B and C in the same sample. The dashed lines indicate the signal for longer delay times. (b) Relative occurrence of decay times (energy redistribution time τ_{er}) when exciting at 575 nm (gray bars) and 581 nm (black bars).

relaxation through the structural, diffusive reorientational motion of the matrix molecules occurs only on a much longer time scale [16]. To investigate the influence of specific molecular vibrations, the excitation wavelength was varied from the absorption maximum at 575 to 581 nm, corresponding to 355 and 175 cm^{-1}, respectively, above the origin of the excited electronic state. The occurrence of observed relaxation times for a total of 126 molecules is plotted in Fig. 2(b). Clearly, a wide time range is observed for both wavelengths. The lack of any significant dependence on excitation energy indicates that a set of vibrations is involved varying from molecule to molecule. In fact, the spectrum can vary up to ~10 nm (~300 cm^{-1}) from molecule to molecule and different conformations can occur in the matrix depending on the nanoenvironment [7,17]. Apparently, variations in the local surroundings of the molecule affect the redistribution of excitation energy, either through induced conformational changes that modify the coupling between intramolecular vibrational modes or by differences in the intermolecular phonon coupling. As a result, the spatiotemporal heterogeneity is even reflected in the ultrafast dynamics of a single molecule. A similar wide range in τ_{er} values, from below 100 fs up to even 1.3 ps, was measured for a variety of dyes (both in air and matrices), such as the polyisocyanine DiD, the biolabel Cy3.5, and the water soluble dye Atto590.

Having demonstrated the concept and applicability of SM2P, the main strength of our approach is towards ultrafast processes in extended molecular assemblies (conjugated polymers and photosynthetic systems) in a complex environment. As a first step in this direction, we have studied an excitonically coupled system consisting of two rigidly linked perylene-diimide units in a head to tail configuration. The coupled system shows redshifted emission (608 nm) and reduced excited state lifetime (4.54 ns) compared to the monomer (6.15 ns). Both spectral and lifetime effects are consistent with strong excitonic dipole-dipole coupling (~300 cm^{-1}) between the chromophores, forming a new superradiant quantum system that exhibits collective emission properties [18–20].

In Fig. 3, a 32 s selection of a SM2P time trace of the response of a selected single perylene dimer is shown. The fluorescence intensity is recorded continuously while the delay between the pulses is swept up and down. In addition to the repetitive delayed pulse pattern several revealing details are observed. Clearly, after ~10 s the overall fluorescence signal jumps to a lower level. This is caused by one of the chromophores undergoing an irreversible change, which disrupts the electronic coupling and leaves only one uncoupled active chromophore. Simultaneously the excited state lifetime τ_f increases from 4.5 to 5.5 ns due to the suddenly localized excitation energy, i.e., abrupt loss of superradiance. Most importantly, we find that the femtosecond decay time τ_{er} drops significantly from ~140 to ~50 fs for this particular pair. Such a systematic reduction of τ_{er} upon photobleaching of one of the chromophores was found for *all* investigated coupled molecules. In Fig. 4, a histogram of the femtosecond decay time τ_{er} is plotted for both coupled and uncoupled cases, together with the correlation between τ_{er} and the fluorescence life-

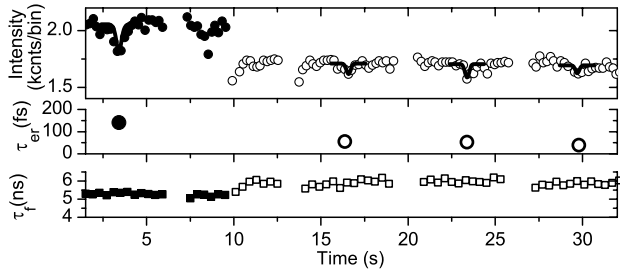

FIG. 3. Time trace of the fluorescence signal (top), femtosecond decay time τ_{er} (middle), and fluorescence lifetime τ_f (bottom) for the coupled system. The pulse delay scan was repeated 5 times and τ_{er} determined, where the fits are indicated (solid lines). After 10 s (top trace), the fluorescence intensity suddenly reduces due to photodegradation of one of the two chromophores. The abrupt decoupling is accompanied by a considerable decrease in the femtosecond decay time (140 to 50 fs) and an increase of the fluorescence lifetime (4.9 to 5.7 ns).

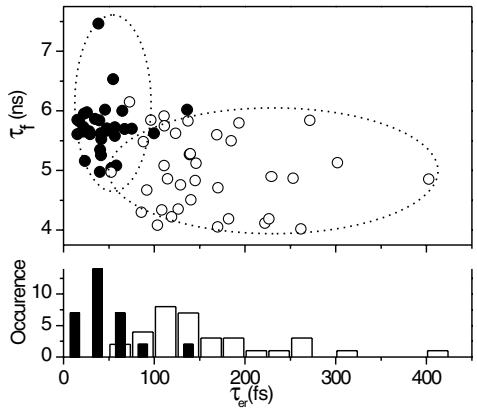

FIG. 4. Femtosecond decay time and corresponding fluorescence lifetime for 29 molecules. Clearly two decay time populations are distinguished: the excitonic system is characterized by a long femtosecond decay time and short excited state lifetime (open dots), while the uncoupled system shows the reverse with a shorter femtosecond decay time and longer excited state lifetime (closed dots). Bottom: τ_{er} for the two different cases with average values of 150 and 45 fs, respectively.

time τ_f. Again, the energy redistribution times are spread over a wide range, yet the decrease in femtosecond decay time upon uncoupling is an even stronger effect. Two separate τ_{er} distributions appear, where the average τ_{er} differs by about a factor of 3. Likewise the τ_{er}-τ_f correlation displays two distinct clusters: electronically coupled and uncoupled.

The longer redistribution times measured for the coupled system are a direct indication of a reduced coupling between the delocalized electronic transition and the nuclear motion upon excitonic interaction of two chromophores [21–23]. This reduction is one of the fingerprints for coherent Coulombic coupling in dye aggregates, now for the first time observed on a single bichromophoric unit. When the conjugated path of one of the chromophores is suddenly disrupted, the excitonic coupling is lost and the electronic excitation energy is relocalized on the remaining chromophore. The concomitant recovery of the fast redistribution times indicates that the molecule starts to ''feel'' the electronic excitation in its vibrational response. Obviously, we have an exquisite method to track the ultrafast pathways of a single excitonic system during real-time variation of its coupling strength.

Interestingly, a significantly wider range of redistribution time values is measured for the coupled perylene dimer than for the uncoupled monomer species. This is indicative of disorder in the polymer matrix at room temperature, due to variations in energy levels, contributions of several vibrational states, and environment. Disorder partially breaks down exciton delocalization [24] and results in exciton coupling strength variation from molecule to molecule, as recently reported for individual perylene dimers [20]. The effect of disorder is confirmed by the spread in dimer fluorescence lifetimes and moderate lifetime reduction (6.15 to 4.54 ns) compared to the factor of 2 expected for a strongly coupled dimer in the absence of disorder.

In conclusion, our new SM2P technique allows for the first time the study of ultrafast pathways in single quantum systems. The operation at ambient conditions and limited number of photons required ($\sim 10^5$ emitted photons) brings the exploration of ultrafast energy transfer and excitonic interactions in a considerable amount of important molecular photonic systems, such as photosynthetic complexes, conjugated polymers, etc., within the range of SM2P.

We thank Rudo Bouwhuis, Nan van Wijngaarden, and Jeroen Korterik for experimental support and Herman Offerhaus, Klaus Boller, and Huib Bakker for fruitful discussions. This work is financed by the Dutch Foundation for Fundamental Research on Matter (FOM).

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