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Single-Molecule Triplet-State Photon Antibunching at Room Temperature

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We have probed single-molecule metal-to-ligand charge transfer (MLCT) dynamics of a ruthenium complex at room temperature. Using photon antibunching measurements under continuous wave (CW) laser excitation, nonclassical photon statistics, and excitation power dependent measurements, we were able to selectively measure the single-molecule MLCT state lifetime. This work demonstrated, as the first single-molecule photon antibunching measurement of the triplet excited state, a new application of single-molecule spectroscopy on excited-state dynamics and ground-state recovering dynamics of an important class of chemical species that have often been used and studied in energy conversion and electron transfer.

Transition metal complexes such as ruthenium complexes, having metal-to-ligand charge transfer states, are being extensively investigated for their involvement in solar energy conversion,[1] interfacial electron transfer,[2] and electron transfer in biological systems.[3−6] Photophysical behaviors such as excitation, emission, and excited-state dynamics of ruthenium complexes usually involve a metal-to-ligand charge transfer (MLCT) process.[7] The dynamics of this process can be highly complex and inhomogeneous, especially when molecules are involved in interactions and perturbations with heterogeneous local environments such as at interfaces or in covalent interactions with electron-transfer proteins. Single-molecule spectroscopy has been shown to be a powerful approach to studying such complex photophysical dynamics in inhomogeneous systems. However, there are only few studies involving single-molecule detection of transition metal complexes, typically because of low quantum yield and low emission rates.[8,9] In this letter, we demonstrate the use of photon antibunching to measure triplet-state lifetimes at room temperature on a microsecond time scale. We apply this approach to the studies of the MLCT state dynamics of a ruthenium complex, with the potential application of probing MLCT ground-state recovering dynamics and electron transfer rates of ruthenium complexes that may have wide utilization in solar energy conversion systems[1] and fundamental research of protein redox systems.[3−6]

We studied the MLCT triplet-state excitation and relaxation of single molecules of tris(4,7-diphenyl-1,10-phenanthrolin)-ruthenium(II) dichloride ($Ru(dpp)_3$, CAS registry 36309-88-3) (Figure 1A, inset). $Ru(dpp)_3$ has the MLCT absorption band at 450 nm and the emission band at 610 nm (Figure 1A). Upon photon excitation, Ru-to-dpp charge transfer occurred in forming a triplet MLCT state through a fast intersystem crossing from the singlet excited state. The MLCT state relaxation can be measured by the ground-state recovering (i.e., the phosphorescence lifetime of the MLCT state). $Ru(dpp)_3$ has a quantum efficiency[10] of ∼0.3 for the phosphorescence from the MLCT triplet state, and the single-molecule detection of $Ru(dpp)_3$ has been previously demonstrated[8] and used as an oxygen sensor.[8,11]

At the ensemble-averaged level, the excited-state lifetime of $Ru(dpp)_3$ can be measured by time-correlated single-photon counting using pulsed excitation or by a frequency-domain lifetime measurement using modulated excitation. However, these approaches are not applicable for measuring the triplet-state lifetime of $Ru(dpp)_3$ single molecules. The time-correlated single-photon counting is not an effective way of obtaining single-molecule triplet-state lifetimes, because it needs a long measurement time due to the long lifetime. The frequency-domain lifetime method is also ineffective for measuring single-molecule lifetimes, because single molecules do not emit a sufficient number of photons to define a resolvable frequency response spectrum required by this method. In this study, the lifetimes of single $Ru(dpp)_3$ molecules were measured by analyzing the photon statistics under CW laser excitation. Photon antibunching, a type of nonclassical photon statistical behavior, is the signature of emission from a single quantum system and can reveal information about transition rates between the photophysical states.[12,13] Single-molecule photon antibunching has been demonstrated first at low temperature and later at room temperature.[13,14] At room temperature, single-molecule photon antibunching is challenging, because single molecules are prone to photobleaching. We have simulated single dye molecule and single $Ru(dpp)_3$ photon antibunching experiments.[15] The simulation shows that photon antibunching of dye molecules under ambient condition is limited by a poor signal-to-noise ratio, while photon antibunching of $Ru(dpp)_3$ can give a reliable measurement of the lifetime.

In our experiments, $Ru(dpp)_3$ (Aldrich) was diluted to 3×10^{-10} M in methanol and was spin-coated on poly(methyl methacrylate) (PMMA)-coated glass coverslips. The single-molecule sample was under constant nitrogen gas purge during the experiments, and all experiments were conducted at room temperature. A CW HeCd laser with 442-nm wavelength was used for photoexcitation. The intensity was modulated by a

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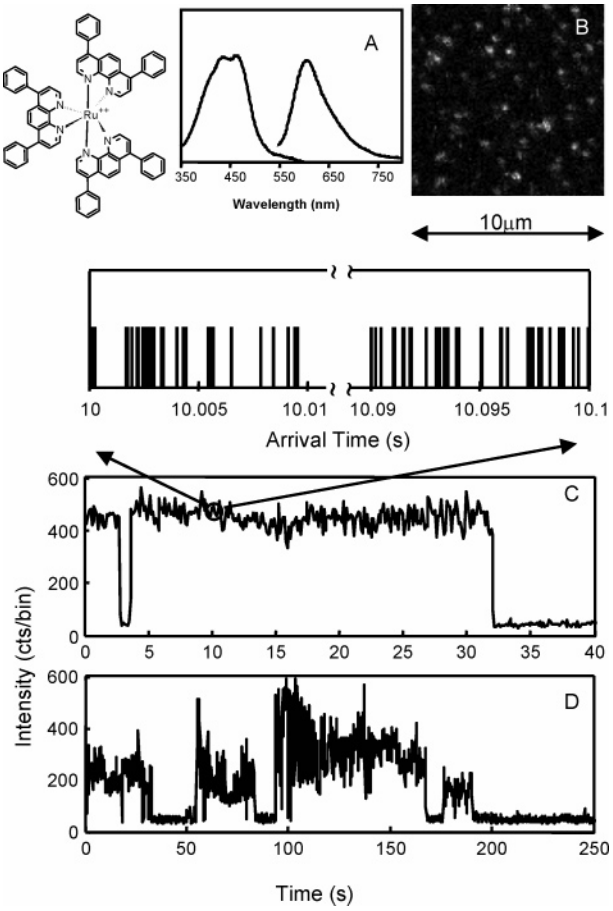

Figure 1. (A) Absorption (left) and emission (right) spectra of Ru(dpp)$_3$ in ethanol. Inset: the molecular structure of Ru(dpp)$_3$. (B) The phosphorescence intensity image of single Ru(dpp)$_3$ molecules with 100-nm pixel size and integration time of 4 ms per pixel. The excitation laser has 0.4-μW power and uncontrolled polarization. (C) and (D) are the typical phosphorescence intensity trajectories of two Ru(dpp)$_3$ molecules. The bin time of the trajectories is 0.1 s. The arrival times of a fraction of the detected photons from 10.0 s to 10.1 s of a trajectory (C) is also shown as the raw experimental data. The total number of photons in this time period converts into one data point in the intensity trajectory.

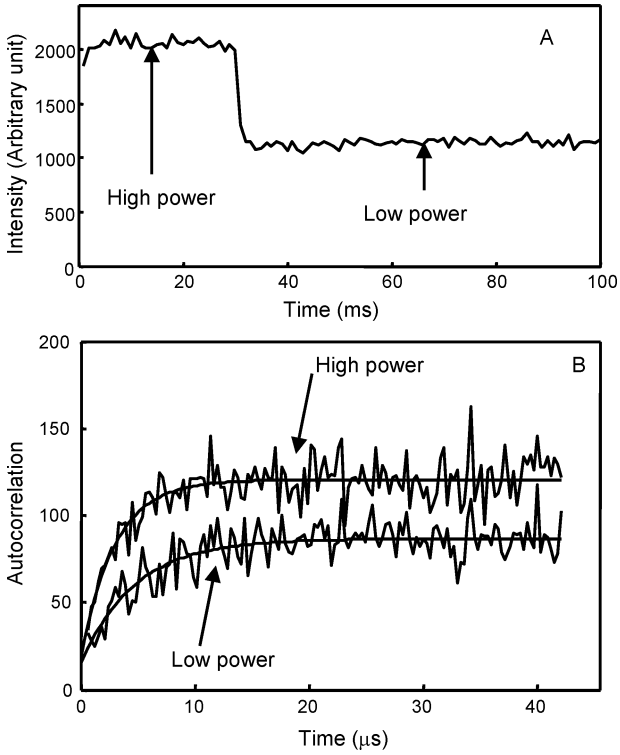

Figure 2. The analysis of the photons emitted from a single molecule, the same set of photons as Figure 1C. (A) The trajectory is wrapped and averaged on the basis of the laser excitation power modulation period of 0.1 s. The bin time of the trajectory is 1 ms. (B) The autocorrelation of the photon trajectory. The upper one is the molecule under high excitation power, and the lower is the same molecule under low excitation power. They are both fitted by single-exponential curves.

Pockel cell (Lasermetrics 3079) with a specific frequency and waveform controlled by a function generator. The laser was coupled through single-mode optical fiber and brought to the epi-port of an inverted microscope (Zeiss Axiovert 200). The laser was reflected by a dichroic beam splitter (Chroma 460DCLP) and focused by an objective (Fluar, 100X, NA1.3). The phosphorescence from a single molecule of Ru(dpp)$_3$ was collected by the same objective and passed through an emission filter (Chroma E470LP). The emission photons were detected by an avalanche photodiode (APD, Perkin-Elmer SPCM-AQR-15). The detected photon time was recorded by a home-built time-stamping device based on the National Instrument data acquisition card (PCI-DIO-32HS). The arrival time of every photon was digitized with the increment of 0.1 μs. The timing error of the measurement system is 0.2 μs. Our experimental detection configuration of antibunching photons is different from that of previous studies.[13,14] Since the lifetime of Ru(dpp)$_3$ is on the microsecond time scale, which is much longer than the detector dead time of ~50 ns, only one detector is needed. Both the optical detection path and the electronic recording device are, therefore, effectively simplified. However, APD has an "after-pulsing" effect which may distort the photon statistics within 1 μs. We have calibrated this effect by carefully studying the autocorrelation of the photons from lamp light and use it as a calibration curve.[15]

A single Ru(dpp)$_3$ molecule emission image is shown in Figure 1B. The single molecules give near diffraction limited spots and reasonable signal-to-background ratio (>10:1). The typical phosphorescence intensity trajectories of two single molecules with 0.1-s bin time are shown in Figure 1C,D. Single Ru(dpp)$_3$ molecules typically produce a kilohertz-level detected photon flux and several hundreds of thousands of photons in total before irreversible photobleaching. We also observed that some molecules showed blinking behavior, which agrees with the previous reports in the literature.[8] The excitation laser intensity was modulated by a square wave function. Within every 0.1 s, there is a 0.03-s period of high power and a 0.07-s period of low power. The power ratio is 3.0 (high at 1.2 μW and low at 0.4 μW). The detected photons were separated into two groups according to the power level at their arrival time. We analyzed the photon arrival time by autocorrelation. The autocorrelation function is defined as

$$g(\tau) = \sum_t I(t)I(t+\tau)$$

The autocorrelation function is calculated by the histogram of photon pair time, not necessarily the pairs from consecutive photons. The autocorrelation of the photon arrival time trajectory reveals the antibunching phenomena by showing the decreased probability of two photons arriving within the MLCT state lifetime. The autocorrelation was calculated for each group of photons under both high and low excitation powers. The results from the same single molecule in Figure 1C are shown in Figure 2B. Both autocorrelation curves show a dip near time zero,

suggesting that there is less probability of two photons arriving at the same time or within the excited-state lifetime. This is a signature of photon antibunching, a characteristic property of emission photons from a single molecule.

We also conducted two control experiments under the same experimental condition. In one, the detected photons from individual fluorescent spheres of 200-nm diameter showed no antibunching in either autocorrelation or interphoton time histogram, because the fluorescent sphere had multiple emitters, not a single quantum system. In the second control experiment, coumarin 343 single molecules showed no antibunching, which is in the nanosecond time scale that is too fast to be resolved by our instrument setup of ~0.2-μs time resolution.

The photophysical process of $Ru(dpp)_3$ can be considered the hopping of a two-level system, because the intersystem crossing in an excited state is much faster than the excitation and excited-state relaxation because of the heavy metal effect on spin−orbit coupling in our experiments. The analytical relations between the transition rates of the photophysical states and the autocorrelation for a two-level system are presented in eq 1 and 2. The autocorrelation $g(t)$ is an exponential rising curve[12,14]

$$g(t) = A[1 - \exp(-kt)] \tag{1}$$

where the exponential decay rate constant k is the sum of the excitation rate k_{ex} and excited-state relaxation rate k_{re}

$$k = k_{ex} + k_{re} \tag{2}$$

To determine the individual rates of k_{ex} and k_{re}, we analyzed the results of the excitation power modulation experiment, assuming that k_{re} is not affected by the excitation power while k_{ex} is proportional to the excitation power. The autocorrelation rates at high and low excitation power were measured, with k_{ex} and k_{re} then calculated.[14] Both autocorrelation curves of $Ru(dpp)_3$ in Figure 2B were fitted by a single exponential. The rate constants are $k_{(high)} = 2.96 \times 10^5\ s^{-1}$ and $k_{(low)} = 1.80 \times 10^5\ s^{-1}$ for high power and low power excitations, respectively. By assuming an excitation rate ratio of 3.0, we obtained the excitation rate and excited-state relaxation rate by solving linear equations (eq 2) at high and low power excitations. The results were $k_{re} = 1.22 \times 10^5\ s^{-1}$, $k_{ex(low)} = 5.8 \times 10^4\ s^{-1}$, and $k_{ex(high)} = 1.74 \times 10^5\ s^{-1}$. Thus, we derived the MLCT excited-state lifetime from photon statistics under CW laser excitation, an approach unlike the time-correlated single-photon counting method in which pulsed lasers were used.[16]

For a two-level system, the emission rate can be estimated by the excitation rate and excited-state relaxation rate, which provides a rigorous confirmation of our calculated rates. The emission intensity can be calculated as follows:

$$I = \frac{Q_e Q_D}{1/k_{ex} + 1/k_{re}} \tag{3}$$

where Q_e is the quantum efficiency of the single molecule and Q_D is the detection efficiency of the microscope. It is reasonable to assume that Q_e and Q_D are invariant with excitation laser power. Although Q_e and Q_D are not exactly known, the ratio of the emission intensities under high and low excitation powers can be calculated. From the above rate constants of k_{re}, $k_{ex(low)}$, and $k_{ex(high)}$, the emission intensity ratio is 1.8:1, which approximately agrees with the experimental observation (Figure 2A). Thus, the rates calculated from the antibunching photon statistics are reasonable values for the single $Ru(dpp)_3$ complexes.

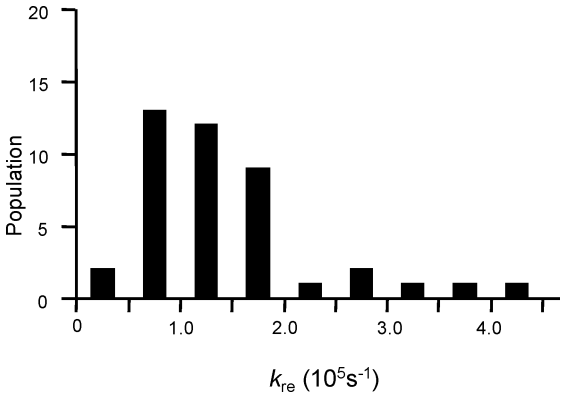

Figure 3. The histogram of the excited-state relaxation rate constants of 42 single $Ru(dpp)_3$ molecules.

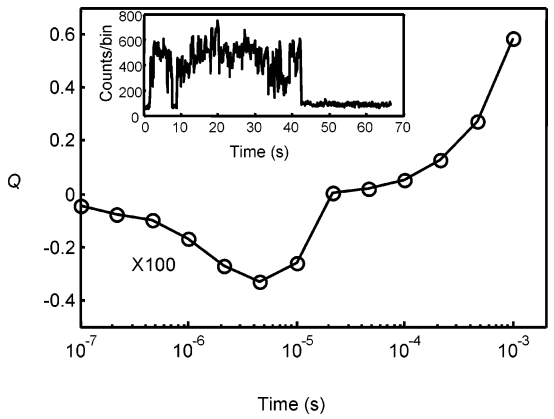

Figure 4. The Mandel parameter of a single molecule. The negative values are magnified by a factor of 100 to enhance the view. This single molecule's phosphorescence intensity trajectory in the 0.1-s bin is shown in the inset.

The relaxation rate constants of 42 single molecules of $Ru(dpp)_3$ were found to be inhomogeneous. Figure 3 shows a histogram of the rates. The overall averaged lifetime is on the same order of magnitude as that reported in the literature (i.e., a 5-μs lifetime in ethanol).[10] The inhomogeneity is likely the result of the heterogeneous environment of the PMMA polymer surface.[17] The error of the lifetime measurement is estimated within 10%, and it is not a major contribution to the broad distribution of the lifetimes. Another factor to introduce inhomogeneity is oxygen. Although we purge the sample with nitrogen, there is still a trace amount of oxygen inevitably, which can reduce the lifetime of $Ru(dpp)_3$.[8]

For further confirmation and characterization of the antibunching photons,[12,14] we have calculated the photon statistics using Mandel's parameter[18] to verify the sub-Poisson or super-Poisson statistics of the detected photons on different time scales. The time-dependent Mandel parameter $Q(T)$ is defined as

$$Q(T) = \frac{\langle I^2 \rangle - \langle I \rangle^2}{\langle I \rangle} - 1$$

where I is the intensity counts at the bin time T. A negative Q indicates sub-Poisson statistics (antibunching), while a positive Q indicates super-Poisson statistics (bunching). We have analyzed the Mandel parameter of a single $Ru(dpp)_3$ molecule's phosphorescence. The Mandel parameter result of a typical molecule is illustrated in Figure 4. The excitation power is at a constant level without modulation. In the time scale of less than 10 μs, the Mandel parameter is negative, which indicates photon antibunching dynamics. In contrast, at the longer time scale of

milliseconds, the Mandel parameter is positive, indicating photon bunching due to the "blinking" of the single-molecule emission intensity.[19]

Transition metal complexes have been widely used as electron donors or acceptors in studying solar energy conversion systems[1] and conformational gated electron transfer in redox electron transfer proteins.[3−6] Single-molecule photon antibunching studies will be particularly effective in resolving the electron transfer dynamics in such complex systems. In this single-molecule photon antibunching study of MLCT state excitation and relaxation dynamics of $Ru(dpp)_3$ at room temperature, we provide a new approach of probing single-molecule triplet-state dynamics. Using an excitation modulation measurement, we were able to obtain both single-molecule excitation and emission rate constants associated with MLCT dynamics. It is promising that the application of single-molecule antibunching measurements will contribute to the fundamental understanding of inhomogeneous electron transfer dynamics in the condensed phase and at interfaces.

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Supporting Information Available: (1) A detailed description and data of the calibration of the detector after-pulsing effect, and (2) a computational simulation of the feasibility of a room-temperature, single-molecule, antibunching experiment for typical dye molecules (nanosecond emission lifetime) and the tris(4,7-diphenyl-1,10-phenanthrolin)ruthenium(II) dichloride ($Ru(dpp)_3$, CAS registry 36309-88-3) reported in this letter. This material is available free of charge via the Internet at http://pubs.acs.org.

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