Optical Single Molecule Spectroscopy for Monitoring Conformational Dynamics of a Single Biomacromolecule

Guangcun Shan† and Wei Huang*,†
Institute of Advanced Materials, Fudan University, Shanghai 200433, P.R. China

*Email: wei-huang@fudan.edu.cn, 041055004@fudan.edu.cn

Abstract. Dynamic structural changes of biomacromolecules undergoing biochemical reactions can be studied using novel single-pair fluorescence resonance energy transfer experiments (sp-FRET), where the time-stamped photon sequence represents the data. We have built a diffusive model of single biomacromolecule, in which a single FRET pair resides on a biomacromolecule that undergoes diffusion in solution, with the goal to understand how the dynamics of the biomacromolecule is reflected in the observed photoemission statistics. And then we utilize the information carried by detected photons and use the time-correlation analysis technique to understand the underlying complex dynamical process of a single biomacromolecule. Our study establishes potentially useful technique and guidelines for the experimental study of following the dynamical processes in real time and revealing the mechanism of complex biochemical reactions.

1. Introduction

In the final decades of last century, much scientific research has turned toward the great dream and imagination proposed by Richard Feynman in 1959 to manipulate and control matter on an atomic and molecular scale. As a distinguished example, optical probing of single molecule, atom or ion in condensed matter environments, offers the advantages of operation at a distance, which can result in significant challenges as well as opportunities. Furthermore, recently the fluorescence resonance energy transfer (FRET) technique is undergoing an increasingly popular method of probing the complex dynamics of single biomacromolecules and measuring distances in the 2 to 10 nm range [1,2,5-10]. The ability to watch one macromolecule of interest at a time helps us to obtain unique information on relevant observables and record asynchronous time trajectories of observables that would otherwise be hidden during biochemical reactions.

Near-field scanning optical microscopy (NSOM) [3,4,14] is based on a probe consisting of a very small (sub-wavelength) aperture that is placed in close proximity (in the near field; <10 nm) to the sample under study. By using the probe as an excitation source, fluorescence of a single molecule has been detected. The emission spectra [5] and excited state lifetime [12] of a single molecule have also been measured. The marriage between FRET and NSOM offers many potential advantages when distance and orientation information is required on a molecular level. The sp-FRET technique is useful for studying intramolecular conformational changes (donor and acceptor are attached to two different sites on the same macromolecule) and intermolecular interactions (donor is attached to one macromolecule and the acceptor is attached to a second).
Furthermore, it is likely that future dynamic structural studies of biomolecules will rely heavily on sp-FRET technique. However, several challenges need to be overcome before sp-FRET is accepted as a mainstream tool. In particular, improvements in fluorophore attachment techniques, photophysical properties of fluorophores and photon data analysis technique will greatly benefit this new field [10].

The fluctuations of the single-molecule lifetime contain detailed dynamic information. In this light, single-molecule fluorescence lifetime holds the promise of serving as a measure for conformational dynamics. In a sp-FRET experiment, we record in real time both \( t \) and the chronological time \( t_i \) of each detected photon. The sequence of such “time stamped” photons, \( \{ t_1(t_1), t_2(t_2), \ldots, t_n(t_n) \} \), is then subjected to statistical analysis. Such a scheme allows for measurements of fast dynamics or for measurements of dynamics over a broad range of time scales. Photon statistics, however, demands that a number of photons be obtained, or “binned”, in order to reach a statistically reasonable estimate of the lifetime [4]. Therefore, single-molecule lifetime measurement using time-correlated single-photon counting (TCSPC) is oblivious to events that occur on a time scale faster than or comparable to the bin time (2ms). To improve the time resolution and statistics for single-molecule studies, we have recently introduced a new analysis strategy for studying biomolecular conformational dynamics [10,16].

The approach analyzes experimental data with higher-order correlation functions [16]. In the case of three-time correlation functions, one calculates the correlation in the fluctuation of observables that are collected at three different times. The correlation function depends on two variables: \( t_1 \), the time separation between the first and the second event; and \( t_2 \) the time separation between the second and the third event. Since there are two variables in this three-time correlation function, one can construct a two-dimensional (2D) correlation surface based on the three-time correlation analysis [2]. As will be discussed, the 1D correlation function cannot determine whether a simulated trajectory is based upon a continuous model or a discrete model, whereas the three-time correlation method demonstrates a superior discrimination power over the 1D approach.

2. Theoretical Analysis Model
Consider a biomacromolecule that undergoes conformational changes among different states with different fluorescence lifetimes. Given a kinetic scheme, all the dynamic information is contained in the time-dependent state propagator \( G(f, t_f; i, t_i) \), which describes the conditional probability of the system arriving at state \( f \) at time \( t_f \) given the system being at state \( i \) at time \( t_i \). The structure of \( G(f, t_f; i, t_i) \) is determined by the rate constants that characterize the kinetic scheme. One goal of statistical analyses of single molecule trajectories is therefore to determine the interconversion rates from experimental observables – which, in the present case, are the time stamped photon sequences from TCSPC. This section discusses the underlying analysis scheme.

This approach introduced here utilizes correlation analyses of the observables. Specifically, we define a three-time fluctuation-correlation function of three events separated by periods \( t_1 \) and \( t_2 \)

\[
C_3(t_1, t_2) = \left\langle \delta \gamma(t_1 + t_2)^{-1} \delta \gamma(t_1)^{-1} \delta \gamma(0)^{-1} \right\rangle = \left\langle \delta \gamma^{-1} \right\rangle \tilde{C}_3(t)
\]

where the normalized three-time correlation function is
\[ \hat{C}_3(t_2, t_1) = \lim_{{t_2 \to 0}} \lim_{{t_1 \to 0}} \langle \delta \gamma(t_1 + t_2) \delta \gamma(t_1) \delta \gamma(0) \rangle \] (2)

where

\[ \langle \delta \gamma(t_1 + t_2) \delta \gamma(t_1) \delta \gamma(0) \rangle = \langle \gamma(t_1 + t_2) \gamma(t_1) \gamma(0) \rangle - \left( \langle \gamma(t_2) \gamma(0) \rangle + \langle \gamma(t_1) \gamma(0) \rangle + \langle \gamma(t_1 + t_2) \gamma(0) \rangle \right) \langle \gamma^{-1} \rangle + 2 \langle \gamma^{-1} \rangle^3 \] (3)

The temporal correlation comes from either the \( \langle \gamma(t) \gamma(0) \rangle \) term or the \( \langle \gamma(t_1 + t_2) \gamma(t_1) \gamma(0) \rangle \) term. They can be expressed in terms of the propagator \( G(f, t; i, t) \) as

\[ \langle \gamma(t) \gamma(0) \rangle = \sum_{\beta} \gamma_{\beta}^{-1} G_{\beta}(t) \gamma_i^{-1} \rho_{\beta}^{i,eq} \] (4)

and

\[ \langle \gamma(t_1 + t_2) \gamma(t_1) \gamma(0) \rangle = \sum_{\beta, \delta} \gamma_{\beta}^{-1} G_{\beta}(t_2) \gamma_{\delta}^{-1} G_{\delta}(t_1) \gamma_i^{-1} \rho_{\beta}^{i,eq} \] (5)

where \( G_{\beta}(t) = G(f, t; i, t_2) \), \( t = t_f - t \), and \( \gamma_i^{-1} \) and \( \rho_{\beta}^{i,eq} \) are the fluorescence lifetime and equilibrium probability for finding the system at state \( i \), respectively. In practice, the correlation function is calculated from experimental data utilizing the general expressions for higher lifetime moments \([9]\):

\[ \langle \gamma(t) \rangle = \frac{1}{TA} \sum_{p} \frac{\tau_p^{z-1}}{\Gamma(z)} \] (6)

\[ \langle \gamma(t)^{-z} \gamma(0)^{-z} \rangle = \frac{1}{(T-t)A} \sum_{\{\gamma > p\}, \{\rho\}} \frac{\tau_\gamma^{z-1}}{\Gamma(z)} \frac{u(t_q - t_p - t)}{\Gamma(z_2)} \] (7)

\[ \langle \gamma(t_1 + t_2)^{-z} \gamma(t_1)^{-z_2} \gamma(0)^{-z_2} \rangle = \frac{1}{(T-t)A} \sum_{\{\gamma > p\}, \{\rho\}} \frac{\tau_\gamma^{z-1}}{\Gamma(z)} \frac{u(t_q - t_p - t)}{\Gamma(z_2)} \] (8)

where \( T \) is the total length of the single-molecule trajectory; \( A_0 = \bar{I} / \bar{\gamma}^{-1} \) is the proportionality constant that compensates for lifetime-dependent fluorescence intensity \( I \), or sampling rate; \( \Gamma(z) = \int_0^\infty y^{z-1} e^{-y} dy \); \( \Delta \) is the chronological time increment along a single-molecule trajectory such that there is no more than one photon within each \( \Delta \) interval; and \( u \) is defined as:

\[ u(t_q - t_p) = 1 \text{ for } |t_q - t_p| \leq \Delta / 2 \text{ and } u(t_q - t_p) = 0 \text{ otherwise.} \]

Eqs.(6)–(8) allow direct calculation of the dynamical quantities on the left-hand of the equation via the experimentally realizable expressions on the right-hand side.
3. Diffusive Model of a Single Biomacromolecule

In a typical sp-FRET experiment, one uses a pair of chromophores attached on a biomacromolecule under study. If the donor is excited by an incident laser, the excitation energy can be transferred from the donor to the acceptor, provided that the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor. This leads to a reduction in the donor’s fluorescence intensity and excited state lifetime, and an increase in the acceptor’s emission intensity. The fluorescence signal from the acceptor then depends on the FRET rate[4,7,8,10]

\[ \Gamma_F = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6 \]

where \( R_0 \) is the Förster radius, \( \tau_D \) is the donor emission lifetime, and \( r \) the distance between the donor and the acceptor. The FRET rate is thus very sensitive to \( r \), which could be used as a “spectroscopic ruler” that allows one to reveal the distance changes of the probed macromolecule to monitor the conformational changes. Thus, the energy transfer rate, in turn, depends sensitively on the donor and acceptor distance \( r \). In the discussion that follows, we assume that the donor and acceptor separation distance \( r \) is a stochastic variable, which undergoes a diffusive model and can be described by the Langevin equation. As the biomacromolecular conformation fluctuates, the effective radiative decay rate fluctuates continuously through the dependence \( r(t) \).

The dynamics of \( r(t) \) then follows the Langevin equation of Brownian motion [11]

\[ \frac{d}{dt} r(t) = -\mu r(t) + f_r(t) \quad (10) \]

where \( \mu \) is the drift coefficient that characterizes the correlation time of the stochastic variable \( r(t) \). In Eq. (14), \( f_r(t) \) is a Gaussian random variable with mean \( \langle f_r(t) \rangle = 0 \) and variance \( \langle f_r(t)f_r(t_0) \rangle = -2\mu D \delta(t-t_0) \) where \( \lambda \) characterizes the magnitude of the fluctuation. The conditional probability density \( G(r_t,t_r;\tau_D) \) that gives the probability of finding \( r(t_1) = r_1 \) given \( r(t_0) = r_0 \), can be shown to follow the well-studied Fokker–Planck equation

\[ \frac{\partial}{\partial t} G(r,t;\tau_D) = \frac{\partial}{\partial r} \left[ \mu r G(r,t;\tau_D) \right] + \mu D \frac{\partial^2}{\partial r^2} \left[ G(r,t;\tau_D) \right] \]

The solution to Eq. (11) is

\[ G(r,t;\tau_D) = \left[ 2\pi D \left( 1 - e^{-2\mu(t-t_0)} \right) \right]^{-1/2} \exp \left[ -\frac{(x-x_0e^{-\mu(t-t_0)})^2}{2\lambda \left( 1 - e^{-2\mu(t-t_0)} \right)} \right] \]

and the equilibrium distribution of \( r \) is

\[ \rho_{eq}^r = \frac{1}{\sqrt{2\pi D}} e^{-r^2/2D} \]

With the equilibrium distribution of Eq. (13) and the conditional probability of Eq. (12), the three-time correlation function of Eqs. (1), (2) and (3) for the continuous-diffusive model are, respectively

\[ C_3(t) = \left( \frac{R_0}{\tau_D} \right)^6 \exp(3/2D) \left[ 2 + \exp(D(1+e^{\mu t_1}+e^{\mu t_2})) \times e^{-\mu(t_1+t_2)} \right] \]

\[ -\exp(De^{-\mu t_1}) - \exp(De^{-\mu t_2}) - \exp(De^{-\mu(t_1+t_2)}) \]

(14)
It is possible to determine $\mu$ and $D$ constants in the continuous-diffusive model from the initial condition and/or the normalized autocorrelation function [2,10].

Now a continuous-diffusive model of a single biomacromolecule combined with the 2D three-time correlation analysis method have been introduced. Through detailed study on single-pair fluorescence resonance energy transfer spectroscopy [10], we have found that the three-time correlation analysis shows superior performance over the autocorrelation approach. Besides, we have shown, in our forthcoming paper [10], that three-time correlation analysis and higher order correlation function analysis is capable of handling models of degenerate state observables and especially handling conformational dynamics of a single biomacromolecule.

4. Conclusions

It is likely that future dynamic structural studies of biomacromolecules and bio-molecular machines will rely heavily on optical single molecule spectroscopy, and in particular on sp-FRET. However, several challenges need to be overcome before sp-FRET are accepted as a mainstream tool. The theoretical study that we presented here mainly focused on new statistical methods and diffusive model that utilize the recently developed photon-by-photon strategy in single-molecule spectroscopy. In this detection scheme, the delay time $t$ of each detected photon relative to its excitation pulse in TCSPC is recorded in real time to give a delay time sequence $\{\tau_p, (\tau_p)\}$. The feasibility of this approach to resolving conformational dynamics models has been examined via statistical analysis. In some cases, the 1D two-point autocorrelation approach is unable to distinguish one kinetic model from another. To improve the discriminative power of the correlation approach, a 2D three-time correlation analysis method have been introduced. The three-time correlation analysis shows superior performance over the autocorrelation approach. We have shown in this paper that three-time correlation analysis is especially capable of handling conformational dynamics of a single biomacromolecule. Furthermore, our study establishes potentially useful technique and guidelines for the experimental study of following the dynamical processes in real time, observing the reaction intermediates and revealing the mechanism of complex biochemical reactions.

Acknowledgement. This work is financially supported by the National Natural Science Foundation of China (Grant No.: 60325412, 90406021, and 50428303).

References

[1] Ha, T. et al. Chem. Phys. 247, 107–118 (1999).
[2] Eigen, M., Rigler, R. Proc. Natl. Acad. Sci. USA 91, 5740–5747 (1994).
[3] Pohl, D. W., Denk, W., Lanz, M. Appl. Phys. Lett. 42, 651–653 (1984).
[4] Deniz, A.A. et al. Proc. Natl. Acad. Sci. USA 96, 3670–3675 (1999).
[5] Trautman, J. K., Macklin, J. J., Brus, L. E., Betzig, E. Nature 369, 40–42 (1994).
[6] Schmidt, T., et al. Proc. Natl. Acad. Sci. USA 93, 2926–2929 (1996).
[7] Ha, T., Enderle, T., Ogletree, D. F., Chemla, D. S., Selvin, P. R., Weiss, S. Proc. Natl. Acad. Sci. USA 93, 6264–6268 (1996).
[8] Ha, T., Enderle, T., Chemla, D. S., Selvin, P. R. Weiss, S. Phys. Rev. Lett. 77, 3979–3982.
[9] Dahan, M. et al. Chem. Phys. 247, 85–106 (1999).
[10] Guangcun Shan, Wei Huang, Phys. Rev. Lett., in press (2005)
[11] Ishii, Y. et al. Chem. Phys. 247, 163–173 (1999).
[12] Bian, R. X., Dunn, R. C., Xie, X. S., Leung, P. T. Phys. Rev. Lett.75, 4772–4775 (1995).
[13] Betzig, E., Trautman, J. K. Science 257, 189–191 (1992).