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Optically induced angular motion of single-molecules

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Abstract – Optically induced angular motion of individual molecules exhibiting both fluorescence
and photo-isomerization properties is investigated in polymer films, by means of a polarized resonant optical pump and probe scheme. The single-molecule dynamics is analyzed using a kinetic model accounting for both photo-isomerization and thermal diffusion phenomena. This study shows that the process is strongly diffusion driven and reveals the relevant time scales that can lead to a pure light-induced molecular-rotation control.

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Single-molecule detection has triggered a wide range of investigations on matter at the nanometric scale. Various seminal works [1–5] have used successfully single molecular probes to report local interactions and heterogeneity of their nearby environment in polymer matrices. In this work we attempt to address a further step in single-molecule physics investigation, which is to influence the dynamics of a single molecule.

It has been known since decades that orienting molecules can be achieved by the polarized optical excitation of trans-cis photo-isomerizable systems (fig. 1(a)) [6,7]. This effect relies on cycles of angular randomization events originating from the cis-trans relaxation pathway, which favor the molecular rotational motion towards a direction perpendicular to the optical excitation polarization. Photo-induced rotation has been exploited in polymer media for holographic optical storage using dichroism, birefringence or polarization gratings [6–8]. The behavior of the reorientation process remains however incompletely elucidated due to the lack of experimental access at the relevant microscopic scale. In particular, the contribution of thermal diffusion and the interaction of the molecules with their nearby environment is averaged and thus only partially accessible through ensemble measurements [6,7,9]. Such questions can be addressed by single-molecule investigations, for which rotational kinetics can be directly measured with the benefit of a statistical approach. Ultimately the optical manipulation of the orientation of isolated chromophores is of major interest for numerous studies in biology and physics [10,11].

In this work, we down-scale photo-induced rotation with the goal of steering individual molecules by use of the mechanical modifications [12] induced by photo-isomerization. Observing single molecules requires a high fluorescent quantum yield, which is antagonistic with an efficient photo-isomerization radiationless relaxation pathway [13]. A reasonable compromise can be found in DCM (4-(dicyano methylene)-2-methyl-6-(p-dimethyl aminostyril)-4H-pyran) (fig. 1(a)), which is being used in this work, with fluorescence and photo-isomerization quantum yields of respectively 0.15 and 0.3 in CH2Cl2 [14]. To vary the environment conditions, DCM molecules were diluted (10−9M) in two polymers of different glass transition temperatures: poly(methyl acrylate) (PMA) (Tg = 8°C) and poly(methyl methacrylate) (PMMA) (Tg = 120°C). Thin films of 250 nm thickness were obtained by spin coating. The PMMA films were cured at 120°C during 2 hours before being used. The experiments are performed at room temperature on an inverted confocal microscope configuration using a high-numerical-aperture objective (60×, N.A. = 1.4) with a continuous laser excitation at 473 nm, leading to about 300nm spatial resolution. A constant nitrogen flow over the sample surface is

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used to reduce photo-bleaching. An orientational pump and probe scheme is performed by way of a Pockels cell placed at the entrance of the microscope: the pump step is a linear polarization, whose purpose is to induce a molecular-orientation change, whereas the probe step is a circular polarization which reads out the orientational dynamics (fig. 1(b)). The incident intensity is chosen so as to allow an observation time before photo-bleaching larger than the photo-orientation time observed in ensemble measurements (typically 5 to 10 s). The fluorescence intensities $I_{/}/$ and $I_{\bot}$ are recorded along two reference perpendicular analysis directions // and $\bot$ on two avalanche photodiodes using a polarizing beamsplitter.

The linear dichroism factor $A = (I_{/}/ - I_{\bot})/(I_{/}/ + I_{\bot})$ is used to monitor the orientational dynamics of single molecule dipoles, their projection angle in the sample plane $\theta(t)$ can be written as $A(t) = \cos^2 \varphi(t) - \sin^2 \varphi(t) = \cos 2\theta(t)$ (see footnote 1). Single molecules are localized as isolated diffraction-limited bright spots on a 10 $\mu$m $\times$ 10 $\mu$m fluorescence image.

First, free thermal rotational motion of single DCM molecules is investigated under circularly polarized probe excitation. A typical rotational behavior of a single DCM molecule in PMA is shown in fig. 1(c) up to the photo-bleaching time (here at 12.2 s). The time dependence of $A(t)$ displays large angular fluctuations on a time scale of a few ms to a few seconds, driven by thermally induced motion in the polymer pores. Within the time resolution of 50 ms, faster time dynamics might be averaged-out [3]. This motion is not of pure Brownian nature due to complex local interactions with the polymer environment, occurring at different time scales. As a comparison fig. 1(g) shows a single DCM molecule in the PMMA polymer exhibiting slow and small fluctuations. In addition to this in-plane projection behavior, the total emitted intensity $(I_{/}/ + I_{\bot})$ decreases with strong fluctuations, showing out-of-plane re-orientation effect under circular pumping (fig. 1(e)). Contrary to PMA the angular motion in PMMA (fig. 1(i)) is seen to be confined within a small angular range of a few degrees, which is a signature of higher constraints provoked by the polymer environment. Within a few seconds, the maximum-deduced-fluctuation angular ranges are 25° in PMA and 5° in PMMA. Such values exceed the angular error margin related to detection noise, whose upper limit is estimated to be 1°.

The orientational pump-probe scheme is next implemented applying sequences of 1 s pump periods in the // linear polarization direction, alternated with 50 ms of circularly polarized read-out periods using a Pockels cell as a fast polarization switch (pump and probe intensities remain identical). This probing time is chosen so as to be negligible compared to the pump duration, in order to limit out-of-plane reorientation. Figure 1(d) (respectively, (h)) displays a characteristic evolution of a single DCM molecule in PMA (respectively, PMMA). In both polymers $A(t)$ decreases toward negative values representative of the $\bot$ direction with visible fluctuations in PMA and smaller amplitude in PMMA. After the pump periods have been switched off, the molecule recovers its free thermal motion. The corresponding out-of-plane motion for single molecules is seen to be not necessarily correlated with their in-plane rotation (fig. 1(f) for PMA and fig. 1(j) for PMMA).

Although the photo-induced reorientation of single molecules exhibits visible fluctuations, averaging over more than 100 molecules before photo-bleaching,
nevertheless, shows that in both polymers an average reorientation takes place perpendicularly to the pump polarization direction (fig. 2(a)). In addition, an intensity decrease was seen to occur, as expected from an out-of-plane motion. Although the limited observation time does not allow to give evidence for the complete equilibration, the averaged $A(t)$ dynamics shows that the reorientation process takes place within a few seconds in both polymers, with averaged re-orientation angles of 3° to 5°. In a control experiment, no rotation effect is observed for the non-photo-isomerizable fluorophore Nile Red (fig. 2(a)). This relatively low rotational efficiency, also observed in concentrated polymers\(^2\), is partly due to the modest photo-isomerization quantum yield of DCM in comparison to non-fluorescent azo-benzene molecules. In addition, the occurrence histograms of $\theta(t)$ (taken at different times (fig. 2(b, c)) exhibit only weak modifications which suggest the dominant role of thermal diffusion in both polymers. This is also observed in fig. 2(a, iii) for the free thermal random orientation within a shorter equilibrium time of 5 s.

Although the reorientation behavior of observed molecules may look similar in both polymers, a dynamic representation points out additional information on the ability of this system to achieve molecular optical steering. Figure 3 displays the concurrence diagrams of $A(t)$ between the initial stage of the measurement ($t = 0$) and different times in the pump-probe sequence, showing static and dynamical heterogeneities which are not distinguishable in the averaged data. In both polymers after a few seconds of pumping, the dichroism values of hundreds of molecules are seen to deviate towards negative values, which is consistent with the averaged behavior. In PMA (fig. 3(a)), the angular motion mechanism is seen to evolve within a large heterogeneity, with angles variations up to 85°. This heterogeneity is dynamic, as illustrated by the few marked molecules, which explains the low efficiency of the ensemble rotation towards the direction in PMA. In PMMA on the contrary, the heterogeneity is reduced with a molecular reorientation taking place within a smaller angular range with a better stability (fig. 3(b)). A better orientational control is thus expected in PMMA where the local environment decreases the thermal rotational freedom.

The increase of single molecule photo-induced rotation efficiency is crucial for further attempts towards an optimized rotational control. Experiment shows large fluctuations of efficiency $A(t)$ for a single molecule. The orientational movement of a single molecule has a probabilistic nature and is described by an unknown stochastic process. At time $t$ the in-plane orientation of the molecule $\theta$ is a random variable with probability density function (PDF) $\rho(\theta, t)$. The degree of control of single molecule's angular motion depends on the shape of $\rho(\theta, t)$, closely related to the still unsolved problem of the nature of a single dipole relaxation in a polymer matrix. If the distributions are sufficiently narrow, the steering is statistically reliable. In the following description, we develop a model which gives a description of both the dynamical observed behavior, and the reliability of obtaining a molecular steering in the present configuration.

We calculate $\rho(\theta, t)$ using a kinetic model with the coefficients which have straightforward correspondence to microscopic parameters of the dyes and host polymer matrix. The model describes the evolution of the in-plane component of the transition dipole and constitutes a simple generalization of an exactly solvable two-state model [15] for a study of processes accompanying the holographic inscription of data in azo-polymers in the degenerated two-wave mixing experiment [16]. In spite of its in-plane character, the model is seen to be not sensitive to small out-of-plane rotations. The dynamics

\(^2\)Ensemble measurements are more delicate to analyze since they are also affected by angularly selective photo-bleaching (data will be presented in a forthcoming paper).
of molecular orientation is studied using single-particle trajectories rather than solving master equations for ensemble-averaged quantities.

The in-plane orientation $\theta$ of a dye undergoes changes due to i) cis $\rightarrow$ trans photo-isomerization cycles, ii) angular redistribution, and iii) Gaussian diffusion, as described below. The angle $\theta$ calculated relative to the light polarization direction takes discrete values: $\theta = \theta_i, \ i = 0, \ldots , N$, with $\theta_0 = 0, \theta_N = \pi/2$. The kinetic model uses transition probabilities per step (time interval $\Delta t$, as described below) and per molecule, characterizing trans-cis photo-isomerization transitions [16,17]: $p(t \rightarrow c) = p_{tc} \cos^2 \theta$ and $p(c \rightarrow t) = p_{ct}$. The former has its origin from quantum-mechanical formula $p(t \rightarrow c) = \sigma I/(h\nu)\cos^2 \theta \Phi$, and is a product of the photo-excitation rate $P(\theta) = \sigma I/(h\nu)\cos^2 \theta$ and the quantum yield $\Phi$, which encompasses the photo-isomerization quantum yield as well as environmental factors such as matrix rigidity. Here $I$ denotes the incident light intensity, $\sigma$ the molecular absorption cross-section, and $h\nu$ the incident photon energy. The $p(c \rightarrow t)$ transition probability has a similar definition, but is angle independent. In the absence of diffusion the time evolution depends solely on the ratio $p_{tc}/p_{ct}$ [16].

Trans molecules perform a random orientational walk at each step (time interval $\Delta t$) with an elementary rotation $\delta = \frac{\pi}{2} N^{-1}$, leading (on a sufficiently large time scale) to a Gaussian diffusion with diffusion constant $D = \delta^2/\Delta t$.

The angular-redistribution process is determined by steric-induced memory around a parental trans orientation of a molecule after a cis $\rightarrow$ trans transition. We assume that this new trans orientation is random in an interval $\delta \theta = N_\delta \delta$ around the parental trans orientation. In simulations we put $N_\delta = 50$.

The relevant parameter affecting the photo-induced orientation effect is thus the ratio $r$ between the characteristic times related to photo-isomerization and orientational diffusion, indicating the competition between the light-induced motion and the thermal diffusion. In our model $r \simeq 10^{-3} \ll 1$, so the system is evolving in an orientational diffusion dominated regime, as suggested by the results from fig. 3.

Our first goal is to show that the kinetic model reproduces the characteristic features of experimentally observed time evolution of dichroism $A(t)$. To this end we study two cases, arbitrary referred to as “strong” ($N = 500$) and “weak” ($N = 1000$) diffusion, with $D_{\text{strong}}/D_{\text{weak}} = 4$. The coefficients $p_{tc}$ and $p_{ct}$ have the same value $p$. In the strong- and weak-diffusion regimes we use $p = p_{\text{strong}} = 0.0047$ and $p = p_{\text{weak}} = 0.0050$. We study 250 molecules in the strong-diffusion regime and 100 molecules in the weak-diffusion regime, in order to use parameters similar to those of the experimental data previously described. Figure 4(a) shows the evolution of linear dichroism factor $A(t)$. This evolution, originally expressed in terms of model time unit (step) $\Delta t$, compared with its experimental counterpart yields $\Delta t = 3.3 \text{ ms}$.

Figures 4(b), (c) show the concurrence diagrams $A(t)$ vs. $A(0)$ for $t = 1s, 3s$ and 6$s$. 250 single molecules, strong diffusion (b); 100 single molecules, weak diffusion (c).

\[^3\] A more detailed description of the model and its parameters is in preparation.
times, the PDFs remain practically unchanged. The fluxes of molecules towards \( \pi/2 \) (photo-isomerization driven) and away from it (angular reorientation) compensate each other. Figure 5(b) shows \( \rho(\theta, t) \) together with the cumulative distribution \( \Theta(\theta, t) = \int_0^\theta \rho(\theta', t) d\theta' \) for \( t = 20 T_0 \). Those plots fully characterize the probabilistic reliability of single-molecule steering efficiency.

Those results show that even though the process is dominated by thermal diffusion, it is potentially efficient for optical steering of such single molecules at longer time scales, providing that a better fluorescence stability is achieved. Additional studies show, as expected, that an increase of a photo-isomerization rate leads to a higher reliability of molecular orientational control, or, conversely, to a decrease of an operational time.

In conclusion, experiments and models on the optically induced rotation of photo-isomerizable single molecules in polymers have both revealed the important role of thermal diffusion, which drives the degree of control of the photo-induced reorientation. The observed effect, a precursor for single-molecule optical steering, can potentially reach higher efficiency and reliability as shown from a simple kinetic model. Although a lot of efforts still need to be done on the matrix and molecules optimization, such an experiment opens possible prospective studies towards the realization of molecular tweezers or molecular optical gates where orientational control of nano-objects is required.

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