ORIGINAL PAPER

# Low-Temperature Spectral Dynamics of Single TDI Molecules in *n*-Alkane Matrixes

Sebastian Mackowski · Stephan Wörmke · Moritz Ehrl ·
Christoph Bräuchle

**Abstract** We report on studies of the influence of the matrix on the spectral dynamics of the zero-phonon-line (ZPL) emission by means of single molecule spectroscopy at low temperature. The host–guest system combinations consist of terrylenediimide (TDI) molecules embedded in four *n*-alkane matrixes of hexane, heptane, pentadecane, and hexadecane. Excitations into the broad vibronic absorption band and spectrally dispersed detection allows us to monitor fluorescence of single TDI molecules as a function of time. In the case of long-chain *n*-alkanes (pentadecane and hexadecane), the ZPL line is quite stable, showing spectral jumps of moderate frequency of less than $10 \text{ cm}^{-1}$ with an average time between the jumps of 10 s. In a clear contrast, the spectral dynamics of TDI molecules embedded within the short-length *n*-alkane matrixes (heptane and hexane) feature much more frequent spectral jumps that occur on a broader energy scale. The results suggest that matrixes composed of short-chain molecules are more susceptible to translations and/or rotations, which influence the fluorescence of single guest chromophores.

**Keywords** Single molecule spectroscopy ·
Vibronic excitation · Spectral dynamics · *n*-alkanes ·
Zero-phonon-line

S. Mackowski · S. Wörmke · M. Ehrl · C. Bräuchle
Department of Chemistry and Biochemistry,
Ludwig-Maximilian-University Munich,
81377 Munich, Germany

S. Mackowski (✉)
Institute of Physics Nicolaus Copernicus University Torun,
Ul. Grudziadzka 5,
87-100 Torun, Poland
e-mail: mackowski@fizyka.umk.pl

## Introduction

Fluorescence spectroscopy on a single molecule level [1–3] is the most direct way to gain insight into interactions both between the molecule and its immediate surroundings as well as between the molecules themselves. It is therefore not surprising that this approach has been applied to variety of systems [4], including, dye molecules [5–8], polymers [9, 10], chemically synthesized nanocrystals [11, 12], semiconductor quantum dots [13, 14], and light-harvesting complexes [15–18]. The changes in the frequency, intensity and polarization of the fluorescence attributable to an individual emitter have been used for instance to understand the details of the energy transfer in molecular assemblies [19, 20] and to describe the energy landscape of the protein surrounding the chromophore [21].

Single chromophores embedded in various host matrixes have also been used to probe the dynamics and interactions present in host–guest systems [22–24]. Since at low temperatures, due to suppression of electron–phonon interaction, the linewidth of the zero-phonon-line (ZPL) emission can be lifetime-limited [25], even minute changes in the chromophore surroundings can be observed. In most cases such an experiment relies on scanning the excitation laser through the ZPL, thus monitoring frequency changes of the ZPL excitation as a function of time [5]. One of the limitations of this approach is however a relatively narrow region of available frequency changes given by the compromise between the scanning range of the laser and the time resolution of the spectral dynamics. In order to overcome this, Kiraz and coworkers [26] have recently demonstrated that the broadband laser excitation tuned into the vibronic band of the molecule combined with spectrally dispersed fluorescence detection provides a way to easily measure spectral changes larger than 100 wave-numbers on

sub-second scale. On the other hand, the approach based on direct excitation of the ZPL can be applied only to relatively stable chromophores, where the spectral changes occur on timescales slower than the laser scan. Indeed, the vibronic excitation method has been postulated to overcome this important drawback.

In this work we use the vibronic excitation approach to observe low-temperature spectral dynamics of single terrylenediimide (TDI) molecules embedded in Shpol'skii matrixes formed by odd and even *n*-alkanes with different lengths of the carbon chain. Four matrixes of *n*-alkanes were studied: hexane, heptane, pentadecane, and hexadecane. Schematic structures of both host and guest molecules are displayed in Fig. 1. The analysis of fluorescence trajectories shows that for long-chain *n*-alkanes (pentadecane and hexadecane), the fluorescence of single TDI molecules is stable, showing only occasional spectral jumps of moderate frequency ($<10$ cm$^{-1}$) between well-defined energy levels. In contrast, the spectral jumps observed for TDI molecules embedded in the short-length *n*-alkane matrixes (heptane and hexane), occur more frequently, with average time between the two jumps being even less than 1 s. We also find significant, three-fold increase in average energy of the jump. Remarkably, no qualitative differences between the two matrixes with either short or long-chain length are observed. The results suggest that the susceptibility of structural changes depend dramatically upon the length of the host molecule, and thus the matrixes built of short-chain molecules are more vulnerable for translations and/or rotations which influence the fluorescence of individual guest chromophores.

## Materials and methods

The experiments were performed using a low-temperature confocal microscope setup (Fig. 2) with the sample and the microscope objective (NA 0.85, Halle, WD 0.4 mm) placed in the cryostat (Janis) and immersed in liquid helium bath at 2 K. The sample holder was mounted to XYZ piezo-actuators (Attocube) that allowed moving the sample in all three directions with an accuracy of about 100 nm, while keeping the microscope objective fixed. The fluorescence was excited at 602 nm with a ring dye laser (Coherent 899, dye Rhodamine 6G) pumped with an argon ion laser (Coherent Innova). The excitation power of 3.5 mW was kept the same for all measurements, unless stated otherwise. After passing a dichroic mirror (HQ 620 LP, AHF Tuebingen), the fluorescence was filtered with a bandpass filter (HQ 670/10, Chroma Technologies) before being focused on a confocal pinhole (100 μm diameter). The signal was dispersed with a monochromator (Jobin-Yvon HR 460) and detected using a slow-scan couple-charged



**Fig. 1** Chemical structure of TDI guest molecule and all four host molecules: hexane, heptane, pentadecane, and hexadecane
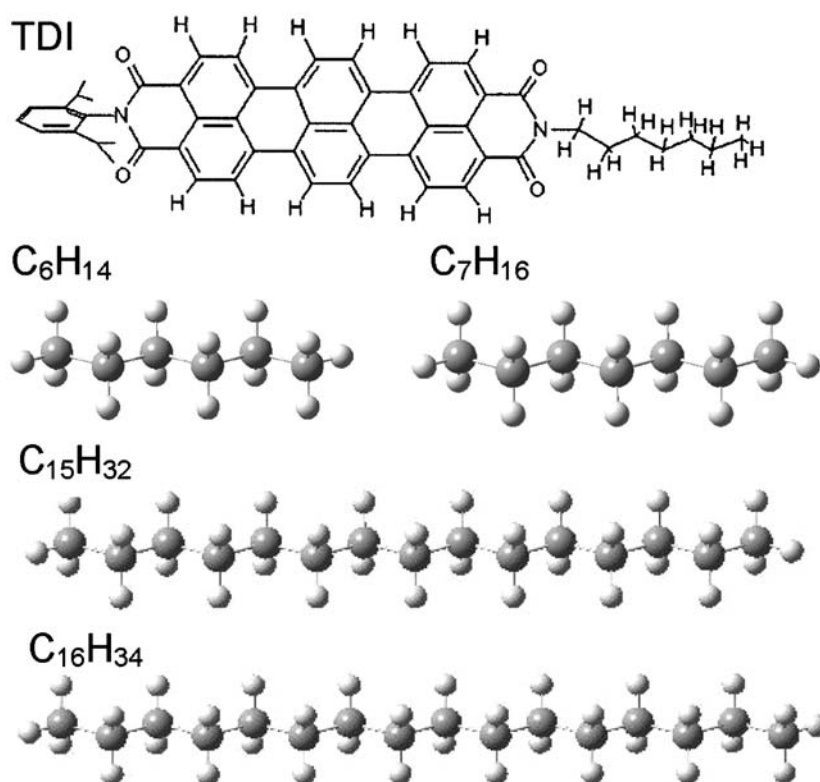
**Fig. 2** Schematics of the experimental setup used for studying spectral dynamics of single TDI molecules at cryogenic temperatures



# Results and discussion
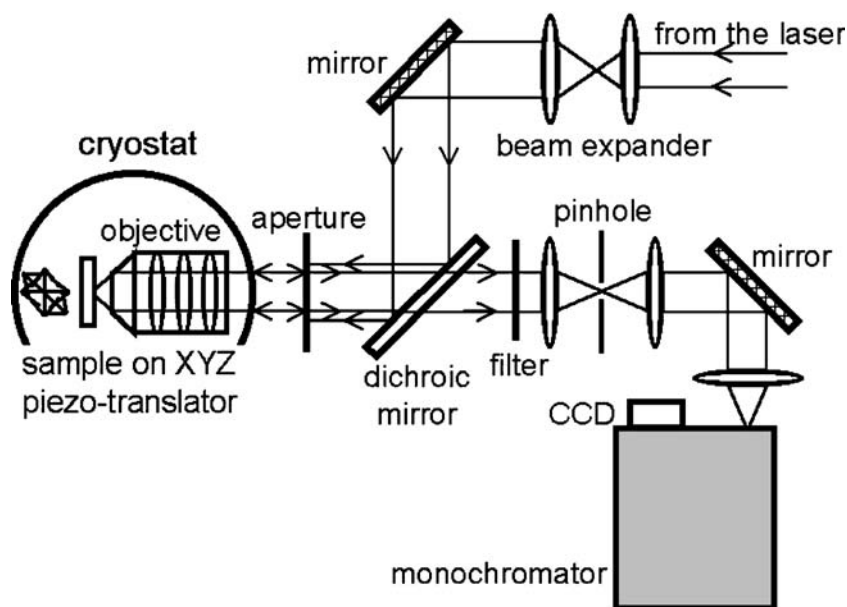
device camera (Acton Research HB128) equipped with a 1,200 g/cm grating. The spectral resolution of the setup was about 1 cm$^{-1}$ and in all cases an integration time of 1 s was used. The molecules were found by moving the piezo-actuators and simultaneously monitoring the emission. After finding a molecule a sequence of 600 spectra was collected.

The TDI solutions in pentadecane and hexadecane were prepared by adding TDI into the respective *n*-alkane and diluting the mixture to single molecule concentrations (approximately one molecule per 1 μm$^2$). The final concentration was determined using single molecule measurement at room temperature. Atmospheric oxygen was removed from the solution by several thaw and freeze cycles with liquid nitrogen and intermediate evacuation. Shortly before inserting the sample into the helium bath, 20 μL of the solution was placed in a cuvette (Hellma) and covered with clean coverslip with both steps carried out in saturated Ar atmosphere. For TDI solutions in hexane and heptane the procedure was identical except that the solution was placed in small, thin-walled tubes, which afterwards were melted in order to prevent evaporation. After freezing, all four samples showed white, snowy color. For every *n*-alkane matrix two samples were prepared and about 30 traces were measured.

## Results and discussion

Initial experiments were performed on TDI in hexadecane in order to compare the performance of the present setup with the previously used one based on a galvanometer scanner and telecentric optical system [26] and determine the excitation power suitable for further measurements. In Fig. 3 we show the spectral traces measured at $T=2$ K for the same single TDI molecule. The vertical axis corresponds to the energy scale in wave-numbers while the horizontal axis represents the time. The excitation power was 2, 4 and 10 mW and was increased subsequently after completing the measurement for lower power. At the lowest excitation power, the ZPL is very stable, showing clearly detectable spectral jumps after approximately 7 min. The observation of spectral jumps is a clear indication that only a single molecule is studied. As soon as the excitation power increases to 4 mW, the spectral jumps occur more frequently, however their energy range does not change substantially compared to the previous measurement. Further increase of the excitation power to 10 mW results, in addition to yet more frequent spectral jumps, also in an increase of their spectral range. The power dependence of spectral dynamics observed for this TDI molecule suggests that, similar to previous experiments [26], an increase of the excitation power leads through heating to rapid activation of local degrees of freedom and this interaction is responsible for observed spectral jumps of the ZPL.

The replacement of the aspheric lens (NA=0.55) with the microscope objective (NA=0.85) results in dramatic improvement of the collection efficiency of the setup. In Fig. 4a we show three examples of fluorescence spectra of TDI molecules detected with the excitation power of 1 mW and in Fig. 4b the histogram summarizing the fluorescence intensity measured for 80 single TDI molecules in hexadecane matrix. The ZPLs are very narrow (linewidth ~ a few cm$^{-1}$) and the spectra show very good signal to noise ratio. The fluorescence intensity of most of the molecules is about 200 counts per second, which is approximately a
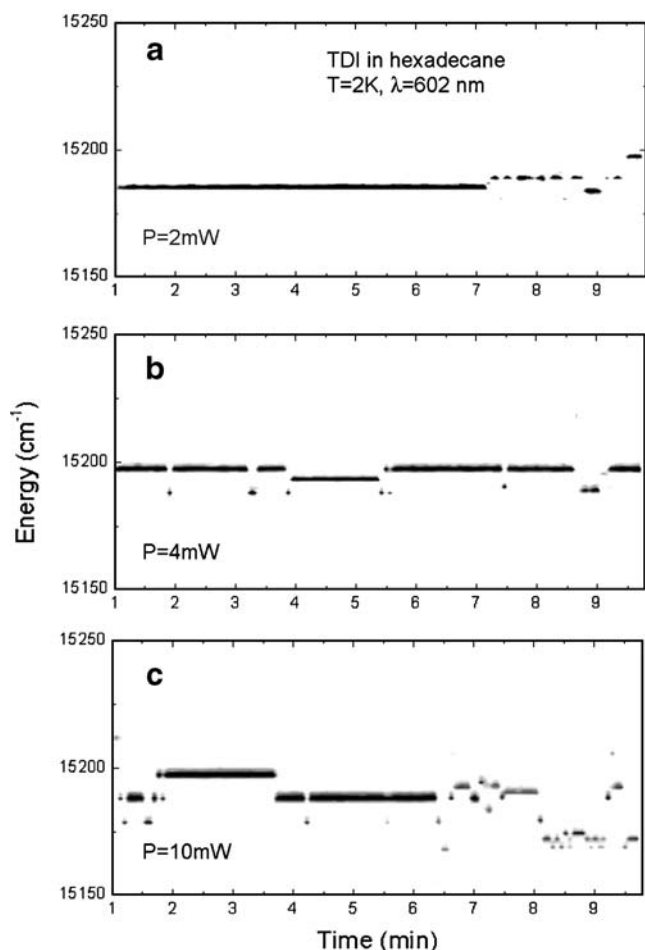
Fig. 3 Spectral trajectories measured at 2 K for single TDI molecule embedded in hexadecane matrix: (**a**) excitation power 2 mW, (**b**) excitation power 4 mW, (**c**) excitation power 10 mW. The excitation wavelength of 602 nm was used and the acquisition time was 1 s

factor of 4 higher than measured previously by Kiraz and coworkers [26] using comparable excitation power density.

The focus of this work is to study the influence of the length of the carbon chain in odd and even n-alkanes on the spectral dynamics of single molecules in cryogenic temperatures. Figure 5 shows the typical behavior observed for single TDI molecules embedded in hexadecane (Fig. 5a) pentadecane (Fig. 5b), heptane (Fig. 5c), and hexane (Fig. 5d). The spectral traces were collected over 10 min with the acquisition time of 1 s. The excitation power was 3.5 mW.

For TDI molecules embedded in long-chain n-alkane matrixes (hexadecane and pentadecane), the ZPL emission is very stable and features only occasional spectral jumps between clearly-resolved energy levels. The frequency of these spectral jumps is often less than 10 cm$^{-1}$. On the other hand, the spectral dynamics of the ZPL emission measured for single TDI molecules embedded in short-

chain n-alkanes (hexane and heptane) features a very different pattern. The spectral jumps occur much more frequently, very often on a timescale shorter than 1 s and in addition, the average energy of the jumps seems to be significantly larger. Such an extremely short time between the spectral jumps resulted that some fluorescence spectra feature more than just a single line. This indicates that during the acquisition time the molecule changes its frequency due to interaction with its surroundings. Moreover, in most cases it is not possible to distinguish in the trajectory any well-resolved energy levels between which the jumps take place. In fact, the spectral trajectories observed for TDI molecules in heptane and hexane are rather similar to the data measured recently for TDI in amorphous PMMA matrix [26]. We note that out of approximately 30 TDI molecules measured for each host matrix, the behavior of only a few (less than 10%) molecules have not followed the general pattern shown in Fig. 5. Remarkably, no qualitative difference between
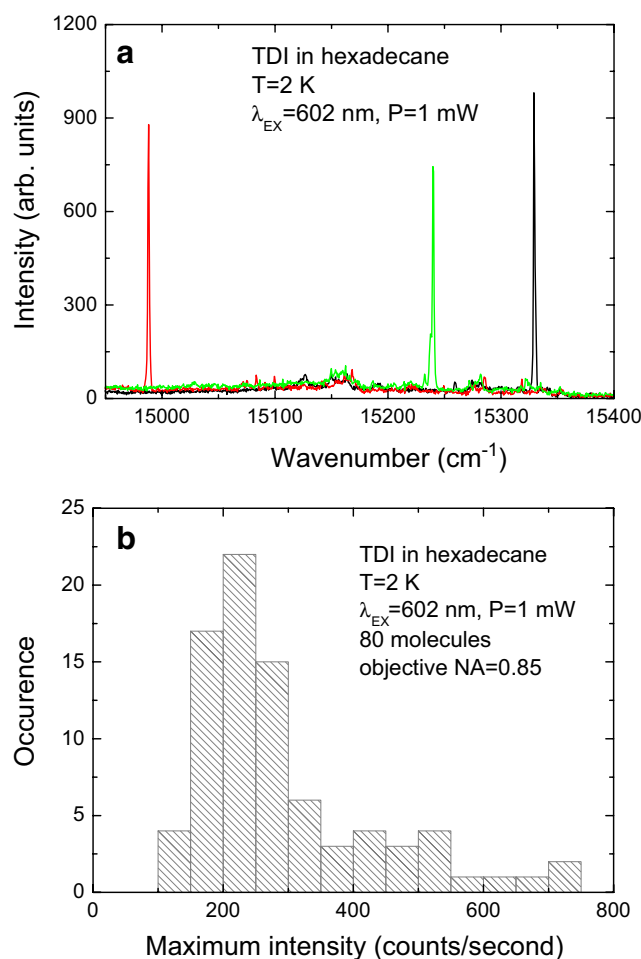


Fig. 4 **a** Typical low-temperature fluorescence spectra of single TDI molecules in hexadecane matrix. **b** Distribution of ZPL intensities measured for 80 single TDI molecules. The excitation wavelength and acquisition time of 602 nm and 1 s was used, respectively

**Fig. 5** Typical spectral trajectories measured for individual TDI molecules embedded in **a** hexadecane, **b** pentadecane, **c** heptane, and **d** hexane
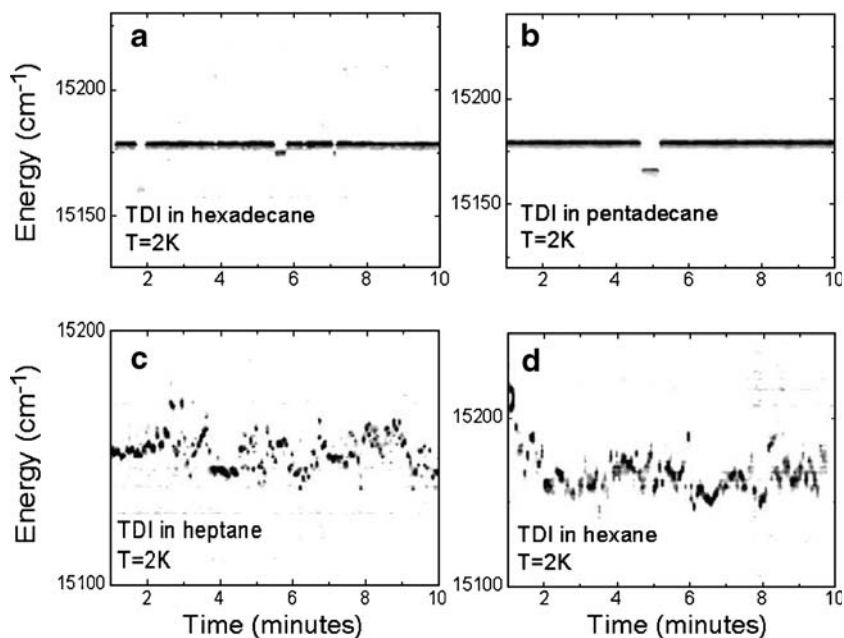


pentadecane and hexadecane as well as between hexane and heptane can be seen. This suggests that the length of the carbon chain plays dominant role in determining the structural changes of the matrix and thus the spectral dynamics of the ZPL.

In order to analyze the experimental results we determine the frequencies and the time between the spectral jumps for TDI molecules in the matrixes with odd number of carbon atoms (heptane and pentadecane). For the trajectories measured in heptane when more than a single ZPL emission line was present only the one with the highest intensity was included. In such cases the time between the spectral jumps was estimated by dividing the acquisition time of 1 s over the number of emission lines present in a single spectrum. For every molecule the average frequency was determined and the spread of frequencies were calculated by subtracting the average value from the measured ones. The summary plots are given in Fig. 6. As already expected from the results shown in Fig. 5, the distribution of jump frequencies for TDI molecules in long-chain pentadecane matrix is very narrow, with the average value of the spectral jump equal to $11$ $cm^{-1}$. The average time between the jumps obtained for this host–guest system is $11$ s. The characteristic values extracted for TDI molecules in heptane are $28$ $cm^{-1}$ and $1$ s, respectively.

**Fig. 6** Summary of the time interval between the jumps and the jump frequency measured for 30 TDI molecules in pentadecane (*upper row*) and heptane (*lower row*) matrixes
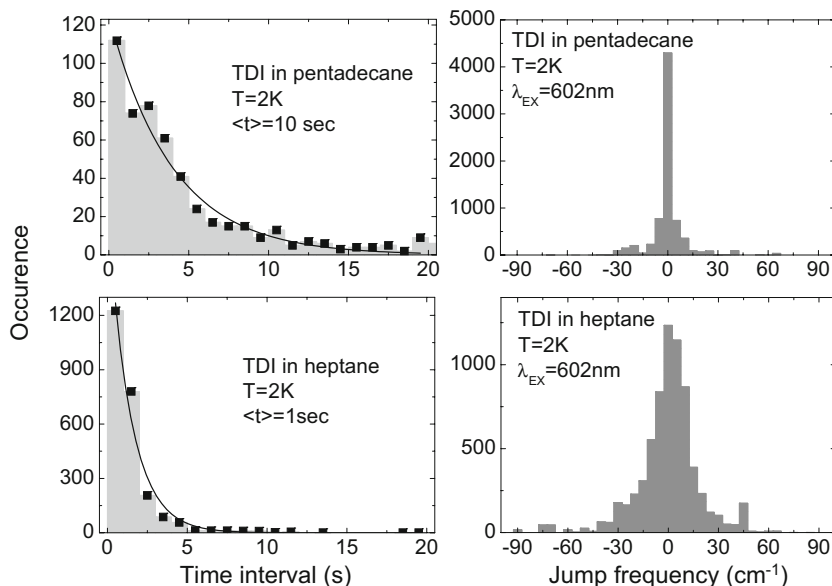
The spectroscopic results obtained for TDI molecules embedded in *n*-alkane matrixes can be qualitatively understood by taking into account the length of both guest and host molecules. Namely, the length of TDI (1.1 nm) is very similar to the long-chain *n*-alkanes, hexadecane and pentadecane. On the other hand, the short-chain *n*-alkanes (hexane and heptane) are much shorter. As a result, in the latter case, the TDI molecule is surrounded by more that two times larger number of host molecules. This leads to dramatic increase of number of conformational states that affect the fluorescence emission of the TDI molecule, and may explain the fact, that in the case of short-chain *n*-alkanes we do not observe any well-defined energy levels between which the spectral jumps take place. The number of host molecules surrounding a TDI molecule can explain the larger average jump frequency observed for TDI embedded in short-chain *n*-alkane matrixes. In addition, it is reasonable to assume that any conformational change (rotation or translation [27]) of the host molecule characterized with shorter length requires less energy than in the case of host molecules having longer carbon chains. Since, as shown in Fig. 3, the spectral jumps are, at least to some degree, photoactivated, the same laser excitation power should induce more conformational changes in the matrix composed of short-chain *n*-alkanes, in agreement with experiment.

In summary, we apply the vibronic excitation scheme to study low-temperature spectral dynamics of the ZPL of single TDI molecules embedded in *n*-alkane matrixes with different carbon chain length: hexane, heptane, pentadecane, and hexadecane. For long-chain *n*-alkanes (pentadecane and hexadecane), the ZPL line is quite stable, and only moderate frequency (less than $10 \text{ cm}^{-1}$) spectral jumps are occasionally observed. The average time between the jumps is about 10 s. On the other hand, the spectral dynamics of TDI molecules embedded within the short-length *n*-alkane matrixes (heptane and hexane) feature much more frequent spectral jumps that occur on a much broader energy scale. The results indicate that matrixes composed of short molecules are more susceptible to translations and/or rotations, which influence the spectral stability of single guest chromophore fluorescence.

## References

 1. Tamarat Ph, Maali A, Lounis B, Orrit M (2000) Ten years of single-molecule spectroscopy. J Phys Chem A 104:1–16

 2. Kulzer F, Orrit M (2004) Single-molecule optics. Ann Rev Phys Chem 55:585–611

 3. Weiss S (1999) Fluorescence spectroscopy of single biomolecules. Science 283:1676–1683

 4. Moerner WE (2002) A dozen years of single-molecule spectroscopy in physics, chemistry, and biophysics. J Phys Chem B 106:910–927

 5. Moerner WE, Kador L (1989) Optical detection and spectroscopy of single molecules in a solid. Phys Rev Lett 62:2535–2538

 6. Orrit M, Bernard J (1990) Single pentacene molecules detected by fluorescence excitation in a p-terphenyl crystal. Phys Rev Lett 65:2716–2719

 7. Blum C, Stracke F, Becker S, Mullen K, Meixner AJ (2001) Discrimination and interpretation of spectral phenomena by room-temperature single-molecule spectroscopy. J Phys Chem A 1:05:6983–6990

 8. Hernando J, van Dijk EM, Hoogenboom JP, García-López J-J, Reinhoudt DN, Crego-Calama M, García-Parajó MF, van Hulst NF (2006) Effect of disorder on ultrafast exciton dynamics probed by single molecule spectroscopy. Phys Rev Lett 97:216403

 9. Métivier R, Nolde F, Müllen K, Basché T (2007) Electronic excitation energy transfer between two single molecules embedded in a polymer host. Phys Rev Lett 98:047802

10. Schindler F, Lupton JM, Feldmann J, Scherf U (2004) A universal picture of chromophores in pi-conjugated polymers derived from single molecule spectroscopy. Proc Natl Acad Sci U S A 101: 14695–14700

11. Nirmal M, Dabbousi BO, Bawendi MG, Macklin JJ, Trautman JK, Harris TD, Brus LE (1996) Fluorescence intermittency in single cadmium selenide nanocrystals. Nature 383:802–804

12. Verberk R, van Oijen AM, Orrit M (2002) Simple model for the power-law blinking of single semiconductor nanocrystals. Phys Rev B 66:233202

13. Bayer M, Hawrylak P, Hinzer K, Fafard S, Korkusinski M, Wasilewski ZR, Stern O, Forchel A (2001) Coupling and entangling of quantum states in quantum dot molecules. Science 291:451–453

14. Nguyen TA, Mackowski S, Jackson HE, Smith LM, Dobrowolska M, Furdyna J, Fronc K, Wrobel J, Kossut J, Karczewski G (2004) Resonant spectroscopy of II-VI self-assembled quantum dots: excited states and exciton-LO phonon coupling. Phys Rev B 70:125306

15. Rutkauskas D, Novoderezhkin V, Cogdell RJ, van Grondelle R (2005) Fluorescence spectroscopy of conformational changes of single LH2 complexes. Biophys J 88:422–435

16. van Oijen AM, Ketelaars M, Köhler J, Aartsma TJ, Schmidt J (1999) Unraveling the electronic structure of individual photosynthetic pigment–protein complexes. Science 285:400–402

17. Mackowski S, Wörmke S, Brotosudarmo THP, Jung C, Hiller RG, Scheer H, Bräuchle C (2007) Energy transfer in reconstituted peridinin–chlorophyll–protein complexes: ensemble and single molecule spectroscopy studies. Biophys J 93:3249–3258

18. Wörmke S, Mackowski S, Jung C, Ehrl M, Zumbusch A, Brotosudarmo THP, Scheer H, Hofmann E, Hiller RG, Bräuchle C (2007) Monitoring fluorescence of individual chromophores in peridinin–chlorophyll–protein complex using single molecule spectroscopy. Biochim Biophys Acta - Bioenergetics 1767:956–964

19. Becker K, Lupton JM, Müller J, Rogach AL, Talapin DV, Weller H, Feldmann J (2006) Electrical control of Förster energy transfer. Nature Mat 5:777–781

20. Cotlet M, Vosch T, Habuchi S, Weil T, Mullen K, Hofkens J, De Schryver F (2005) Probing intramolecular Forster resonance energy transfer in a naphthaleneimide-peryleneimide-terrylenediimide-based dendrimer by ensemble and single-molecule fluorescence spectroscopy. J Am Chem Soc 127:9760–9768

21. Hofmann C, Aartsma TJ, Michel H, Köhler J (2003) Direct observation of tiers in the energy landscape of a chromoprotein: a single-molecule study. Proc Natl Acad Sci USA 100:15534–15538

22. Jung C, Hellriegel C, Platschek B, Wöhrle D, Bein T, Michaelis J, Bräuchle C (2007) Simultaneous measurement of orientational and spectral dynamics of single molecules in nanostructured host–guest materials. J Am Chem Soc 129:5570–5579

23. Lippitz M, Kulzer F, Orrit M (2005) Statistical evaluation of single nano-object fluorescence. Chem Phys Phys Chem 6:770–789

24. Vallée RAL, Van Der Auweraer M, De Schryver FC, Beljonne D, Orrit M (2005) A microscopic model for the fluctuations of local field and spontaneous emission of single molecules in disordered media. Chem Phys Phys Chem 6:81–91

25. Kiraz A, Ehrl M, Bräuchle C, Zumbusch A (2004) Ultralong coherence times in the purely electronic zero-phonon line emission of single molecules. Appl Phys Lett 85:920–922

26. Kiraz A, Ehrl M, Bräuchle C, Zumbusch A (2003) Low temperature single molecule spectroscopy using vibronic excitation and dispersed fluorescence detection. J Chem Phys 11:8:10821–10824

27. Marbeuf A, Brown R (2006) Molecular dynamics in n-alkanes: premelting phenomena and rotator phases. J Chem Phys 124:054901