

# Single molecule spectroscopy: Spontaneous and light-induced frequency jumps

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**Abstract:** This paper reports on frequency jumps of single molecule excitation lines. The first part deals with spontaneous spectral jumps (spectral diffusion) and gives examples of what can be learned from investigations of spectral diffusion about the low temperature physics of amorphous solids. The second part of this article discusses light-induced frequency jumps in the crystalline system terrylene in p-terphenyl where reversible single molecule hole-burning allows the optical manipulation of single absorbers in a remarkably controlled and reproducible fashion. Furthermore we demonstrate how this system permits investigations of one and the same chromophore over a period of a few weeks at least.

## INTRODUCTION

The ability to optically detect a single fluorescing molecule in the condensed phase has paved the road for novel experiments in various research fields spanning the wide range from quantum optics in solids[1] up to the study of diffusional motion in biological systems[2]. One important feature of experiments at the single molecule level is the absence of ensemble averaging which may mask a number of effects observable only for a single quantum system and which prevents to determine distributions of molecular properties in the optical domain. In the condensed phase such molecular properties may be strongly dependent on the specific - possibly changing - nanoenvironment of the molecule and therefore the single molecule serves as a probe of the surrounding matrix.

In single molecule spectroscopy (SMS) in solids at low temperatures single chromophores are isolated by their specific optical transition frequencies (zero-phonon lines) which can be addressed selectively by a narrow linewidth laser source[3]. The extreme sensitivity of the sharp excitation lines to their local environment renders SMS a superb technique to study host-guest interactions in solids on a nanoscopic scale. A slight change of the local environment or a minute conformational reorientation of the molecule - in both cases the host-guest interaction will be modified - may induce a change of the line shape or even a jump of the excitation line to a new frequency position which can be followed in time. Such processes can occur spontaneously when the surrounding matrix structure is evolving in time, a phenomenon typically observed in disordered hosts[4,5,6,7]. On the other hand frequency jumps of the excitation line can also be triggered by light irradiation[5] and both phenomena will be described by representative examples in this paper.

## SPONTANEOUS SPECTRAL JUMPS OF SINGLE MOLECULES (SPECTRAL DIFFUSION)

The absorption signals of single molecules are highly sensitive to conformational changes in the surrounding matrix. Therefore they can be used to probe the low temperature dynamics of the host. Amorphous materials such as polymers and glasses exhibit rich and manifold dynamics due to their inherent disorder [8]. The low-energy excitation modes characteristic for the amorphous state are modelled as an ensemble of asymmetric double-well potentials. Each of the double-well potentials is dependent on a specific generalized conformational coordinate of the host. Since only the two lowest energy levels of each

double-well contribute to the matrix dynamics at low temperatures, these excitations are referred to as two-level-systems (TLSs)[8].

TLS flips can be monitored via the absorption signal of an impurity center, as TLSs in the vicinity of the chromophore are coupled to its optical transition by dipole-dipole interactions[5,6,7,9,10]. Depending on its rate a given TLS transition can either cause dephasing or spectral diffusion of the optical transition[11]. In this article we will limit our discussion of amorphous systems to slow TLS fluctuations that cause spontaneous frequency shifts of single molecule absorption lines (spectral diffusion) in the time range between milliseconds and seconds. The phenomenon of spectral diffusion was first observed in SMS in a crystalline system, namely pentacene in p-terphenyl[4,12]. Some of the pentacene molecules showed stochastic spectral jumps between different absorption frequencies on a time scale of seconds[4]. In this system the low temperature dynamics is assumed to be induced by the existence of domains of p-terphenyl molecules with different orientational ordering of the central phenyl rings. Flip-flop motions of the inner phenyl ring are thermally accessible at  $T = 1.5$  K for p-terphenyl molecules residing at a domain boundary and the potential energy of this rotational movement can be modelled as a TLS. The TLS transitions shift the absorption line of a nearby impurity center via dipolar coupling and all experimental data could be interpreted with a stochastic model for the phonon-assisted TLS flips[13].

While the observation of spontaneous frequency jumps was quite unexpected for a crystalline host, it was anticipated for spectral diffusion to be very common in highly disordered matrices such as polymers and glasses. Indeed single molecule spectral diffusion has been observed in a number of amorphous host-guest systems[5,6,7,9,10,14]. Generally one expects to find a number of TLSs coupling to a given chromophore but one can encounter absorbers whose spectral dynamics is dominated by the interaction with only one TLS[6]. In that case the TLS transitions will cause the molecular absorption line to jump between two distinct spectral positions corresponding to the ground and excited state of that TLS[6,9]. Thus it is possible to deduce the TLS transition rates directly by monitoring the rapid changes of the absorption frequency. The transition rates can then be obtained by a simple kinetic analysis since the absorption frequency of the chromophore indicates which state the TLS is in at any given moment. However, the temporal resolution of this direct approach is limited by the fact that at present a minimum integration time of about 100 ms is needed to scan across the single molecule absorption line.

A higher temporal resolution can be gained by measuring the temporal distribution of fluorescence photons emitted by a single molecule since the strong correlation of the fluorescence photons directly reflects the dynamical processes involved. It is convenient to employ analysis by means of the fluorescence intensity autocorrelation function  $g^{(2)}(\tau)$  which is defined classically by the following expression [15]:

$$g^{(2)}(\tau) = \langle I_f(t) \cdot I_f(t + \tau) \rangle / \langle I_f(t) \rangle^2 \quad (1)$$

where  $I_f$  is the fluorescence intensity.  $g^{(2)}(\tau)$  can be deduced from the distribution of photon pairs separated by times  $\tau$ , provided that the integration time is longer than all characteristic times of the intensity fluctuations. For the time range investigated here ( $\tau \geq 1$  ms)  $g^{(2)}(\tau)$  can be obtained conveniently by a digital logarithmic correlator which directly calculates the fluorescence intensity autocorrelation function from the temporal distribution of the photoelectric pulses registered by a phototube.

The rate parameters of TLS flips coupling to the optical transition can be obtained from the decay of the fluorescence intensity correlation function[7]. Limiting our discussion to molecules that couple strongly to only one TLS makes the analysis of  $g^{(2)}(\tau)$  especially straightforward: When we monitor the total fluorescence with the excitation wavelength held fixed at one of the two spectral positions of a chromophore coupled to one TLS we will observe bright periods separated by dark intervals (background level) as the molecular absorption line is shifted into and out of resonance with the laser irradiation by the spontaneous TLS transitions. It can be shown [7] that this will result in a monoexponential decay of  $g^{(2)}(\tau)$  on the time scale of the TLS transition rates:

$$g^{(2)}(\tau) = 1 + C \exp(-K\tau) \quad (2)$$

The decay parameter  $K$  in Eqn. (2) is the sum of the transition rates into the ground ("down" transition) and excited ("up" transition) state of the TLS:  $K = k_d + k_u$ . We will illustrate the application of this method for the spectral diffusion of terrylene ( $C_{30}H_{16}$ , a higher homologue of perylene) molecules embedded in polyethylene, a system for which the use of correlation spectroscopy to unravel TLS dynamics has first been demonstrated[7]. In Fig. 1 we present the data of a terrylene molecule that is switching its absorption frequency between the frequency positions marked as 1 and 2, respectively, due to interaction with one spontaneously flipping TLS[16] (position 2 corresponds to the TLS being in its ground state).

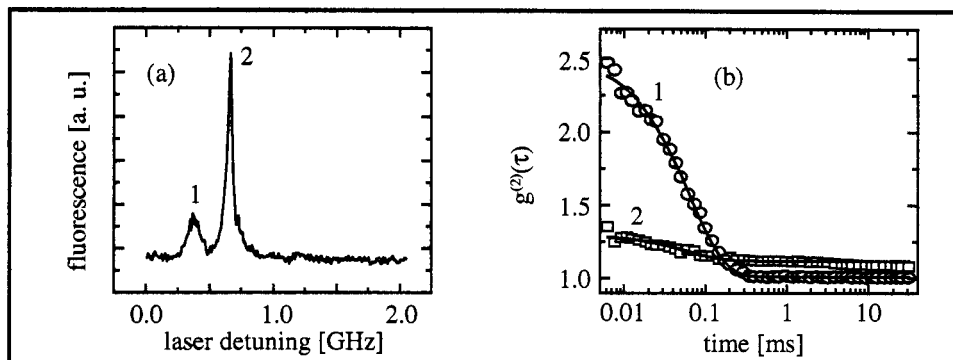


Fig. 1. Spectral Dynamics of a single terrylene molecule in a polyethylene matrix at  $T = 1.4$  K. (a) Coupling to a single TLS causes the molecular absorption line to undergo fast spectral jumps between frequency positions 1 and 2. (b) The fluorescence intensity autocorrelation function  $g^{(2)}(\tau)$  of the same molecule measured on positions 1 and 2, respectively. (adapted from ref. [16])

Obviously the jumping dynamics are faster than the time it takes to scan the laser across the investigated spectral region as the lines on positions 1 and 2 appear to be static in all scans. To investigate the underlying TLS transitions we recorded the fluorescence intensity autocorrelation function  $g^{(2)}(\tau)$  on both positions. The two correlation functions are characterized by a single exponential step as is anticipated from Eqn. (2). The decay parameter is the same at both positions - this shows that the lines originate from the same molecule - and equals the sum of the individual TLS transition rates ( $K = 1.7 \times 10^4 \text{ s}^{-1}$ ). We also find the contrasts  $C$  of the correlation function at both positions to be inversely proportional to the intensity of the spectral lines as is predicted by theory[7]. It is possible to calculate the energy asymmetry (splitting) of the TLS involved by assuming thermal equilibrium and fitting a Boltzmann factor to the relative intensities of the lines ( $\Delta E = 1.5 \text{ cm}^{-1}$ ). As the ratio of these intensities is also proportional to the ratio of the TLS transition rates  $k_u/k_d$ , we can easily determine these rates by using the known value of  $K$ . That way we obtain  $k_d = 1.4 \times 10^4 \text{ s}^{-1}$  (this rate corresponds to the  $1 \rightarrow 2$  transition) and  $k_u = 3.1 \times 10^3 \text{ s}^{-1}$  ( $2 \rightarrow 1$  transition). This example demonstrates how characteristic TLS parameters can be obtained from single molecule correlation measurements with a simple analysis. The opportunity to investigate isolated TLSs seems especially attractive since this in principle enables us to determine distributions of TLS parameters that cannot be obtained from measurements on large ensembles and that are important for the explanation of low temperature properties of glasses.

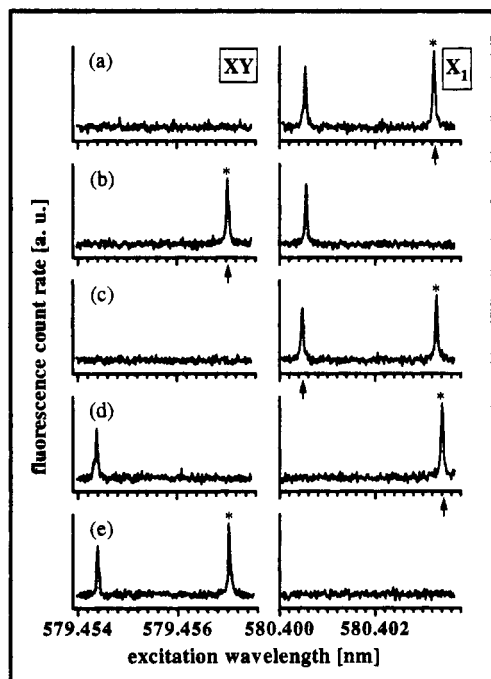
Information about the transition mechanism of a TLS can be gained from temperature dependent measurements of the fluctuation rates of this TLS. As a thorough discussion of this method is beyond the scope of this paper, we have to refer the reader to the literature[7,17].

## LIGHT INDUCED SPECTRAL JUMPS

Another interesting feature of SMS is the observation of spectral dynamics triggered by laser irradiation because this offers the possibility to directly manipulate single absorbers by optical excitation. A light-induced, discrete frequency jump is the single molecule analogue of spectral hole-burning. The first example of single molecule hole-burning was reported for perylene in polyethylene [4,5,9]. Here, molecules can be found that change their absorption frequency when excited resonantly and these molecules will sometimes return spontaneously to their original spectral position. Such behaviour makes it

seem likely that these molecules visited other well defined spectral positions in the meantime, but these were never identified for perylene in polyethylene. Light-driven jumps between two distinct spectral positions have first been observed for terrylene molecules embedded in the Shpol'skii matrix hexadecane[18]. Some molecules exhibited a clearly light driven nature of these transitions albeit the jump width of 100 - 200 MHz was comparatively small and left the homogeneous lines at the two different positions almost overlapping.

We report light driven spectral dynamics of terrylene molecules embedded in the crystalline matrix p-terphenyl. This system exhibits four distinct purely electronic origins, denoted sites  $X_1$  to  $X_4$ [19]. These four origins are attributed to four different insertion sites of unknown structure. Terrylene molecules belonging to site  $X_1$  ( $\lambda_{\text{abs}} = 580.4$  nm) do not show any signs of spontaneous spectral diffusion when scanned rapidly with low excitation intensities but their signals do all change their spectral positions upon



**Fig. 2.** Light driven, reversible frequency jumps of two terrylene molecules of site  $X_1$ . The absorption signals of one molecule have all been marked with an asterisk for identification purposes. An arrow indicates resonant excitation that will be followed by a spectral jump. (adapted from ref. [20])

resonant excitation[20]. This behaviour is illustrated in Fig. 2: In (a) we see the fluorescence excitation spectra of two molecules from site  $X_1$ . The molecule whose absorption line is marked with an asterisk is then excited resonantly until a sudden drop of the fluorescence signal to background level indicates spectral hole-burning (the burn-off traces are not shown here). We then (b) find the other molecule still at its place in  $X_1$  while the burned-off molecule has increased its absorption frequency by 843 GHz and resides at a new spectral position denoted the XY site. The spectral jump is reversible as resonant excitation in XY forces the molecule back to its old spectral position in  $X_1$  (c) - this can be demonstrated accurately since the other molecule serves as a frequency marker. The marker molecule exhibits an identical jump behaviour upon excitation (d) which is reversible as well (not shown). Finally (e), when we coerce both molecules to their XY spectral position we find that their former spectral pattern in  $X_1$  is very well reproduced in XY. This means that both molecules show the same jump width.

What has been demonstrated here for two absorbers is an intrinsic property of the  $X_1$  site: All molecules from that site undergo identical spectral jumps that are light driven and reversible, provided that the total concentration of absorbers is kept low enough. So far we have investigated about 20 terrylene molecules in six different crystals taken from three sublimation runs.

The variation in jump width was only  $\pm 2$  GHz from sample to sample and the deviation for molecules in one and the same crystal was 500 MHz (0.6 % of the jump width) at most. The high reproducibility of the jump width is especially remarkable because spectral jumps of 843 GHz (more than 10 thousand times the homogeneous line width of terrylene in p-terphenyl) are unparalleled in single molecule spectroscopy up to now. A striking difference to SMS in polymers is the well-behaved manner in which the terrylene molecules in this host react to outside optical manipulation: The chromophores do not exhibit any tendencies towards spontaneous spectral jumps but can be forced to undergo light driven changes in absorption frequency in a very controlled and reproducible fashion.

The reversible  $X_1$  - XY jumps just described constitute the characteristic property of the  $X_1$  site, but the full spectral dynamics of  $X_1$  molecules is a little more complicated. Though molecules in XY will normally return to their  $X_1$  spectral position when excited resonantly, there is about a 15 percent chance that they will jump to a third spectral position instead. Contrary to the well defined  $X_1$  and XY sites we find that this

third spectral position is different from molecule to molecule and for most molecules we did not take the time to identify it. But we always could "reset" the investigated molecules to their original spectral positions in  $X_1$  by raising the temperature to 40 K for about a minute. This indicates that the insertion geometry corresponding to the  $X_1$  spectral position is the most stable one and explains why we never found molecules in XY without prior hole-burning in  $X_1$  when investigating low concentration samples. We want to stress once more the fact that contrary to the problems in amorphous hosts we never "lost" the signal of a terylene molecule because of irreversible hole-burning; this is the main advantage of this system.

The excellent day-to-day stability of the terylene signals in p-terphenyl also enabled us to conduct long-time investigations of specific molecules. We will illustrate this for the two chromophores described in Fig. 2. As their signals were well isolated - both spectrally and spatially - from any other fluorescing feature in the sample, we could unambiguously identify them from day to day and monitor their spectral behaviour for about three weeks. Mostly we focussed on the molecule whose absorption lines are marked with an asterisk in Fig. 2, using the other absorber mainly as a frequency marker and for identification purposes. All information about this molecule's absorption frequencies and spectral jumps is summarized in Table 1 which is the "spectral diary" of this chromophore.

TABLE 1 The spectral diary of a terylene molecule

Date	spectral position ( $\lambda_{\text{abs}}$ in $X_1$ )	position (nm) in XY	number of $X_1$ - XY jump cycles	additional positions ( $\lambda_{\text{abs}}$ [nm])
11/28/95	580.400	579.456	1	none
11/29	580.399	579.455	1	none
11/30	580.400	579.457	4	none
12/01	580.400	579.456	3	579.158
12/04	580.398	579.453	3	none
12/05	580.400	579.453	3	579.154
12/08	580.397	579.451	4	none
12/11 12/12	580.398	579.453	8	579.153 578.828 578.556
12/13	580.403	579.457	1	none
12/14	580.403	579.457	2	none
12/19	580.399	not visited	0	none

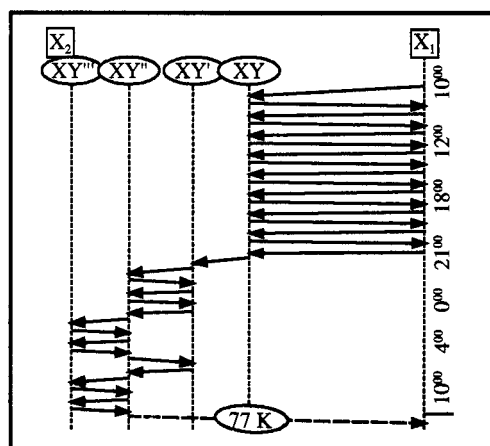


Fig. 3. 24 hour spectral trace of a terylene molecule

From the data in Table 1 we see that the absorption wavelength (est. uncertainty  $\pm 0.001$  nm) of the molecule in  $X_1$  and XY basically remained constant during the three week observation period, albeit we notice a slight reshuffling of  $\pm 0.003$  nm; the other molecule from Fig. 2 showed similar frequency shifts (Fig. 2 was recorded as a part of the diary on 12/14/95). These changes are probably caused by annealing processes of the host (the cryostat was allowed to reach liquid nitrogen temperatures every night). In the course of this investigation we identified three additional spectral positions of this molecule which we denoted  $XY'$  ( $\lambda_{\text{abs}} = 579.153-8$  nm),  $XY''$  ( $\lambda_{\text{abs}} = 579.828$  nm) and  $XY'''$  ( $\lambda_{\text{abs}} = 579.556$  nm). Figure 3 shows a 24 hour spectral trace of the molecule that was recorded on the 11<sup>th</sup> and 12<sup>th</sup> of December. We see that the molecule underwent eight  $X_1$  - XY jump cycles before it burned off to  $XY'$ . From then on we find similar light induced jump cycles between  $XY' - XY''$  and  $XY'' - XY'''$ . No direct transitions  $XY' - XY'''$  were observed, also no light driven jumps back to XY or  $X_1$  occurred. The molecule was "reset" to  $X_1$ , however, when the cryostat reached 77 K the following night. Such a reproducibility of spectral positions and long-time stability of molecular absorption lines are characteristic advantages of the system terylene in p-terphenyl.

The reproducibility of the  $X_1$  - XY transitions and the long time stability of the molecules makes it seem likely that the mechanism underlying these changes in absorption frequency can be investigated at the single molecule level. It is tempting to postulate a mechanism similar to that of the spectral diffusion of pentacene in the same matrix[13], namely a flip of the central phenyl ring of a p-terphenyl molecule in the

immediate vicinity of the investigated chromophore, but a change of the absorber geometry itself has to be considered as well. With the data available so far we cannot conclusively argue in favour of either possibility, but a first tentative discussion can be found in ref. [20].

## CONCLUSION

In this article we wanted to illustrate two typical spectral shifting phenomena that can be observed in single molecule spectroscopy and discuss some aspects of low temperature solid state physics that can be investigated by SMS techniques. Our first point was to demonstrate the potential of SMS to improve our understanding of the low temperature properties of disordered solids. As a refinement of methods such as spectral hole-burning and fluorescence line-narrowing, SMS can probe truly local host-guest interactions on a nanoscopic scale. By investigation of the absorption lines of impurity centers we can gain insight into the energetical topography of single two-level-systems without the ensemble averaging inherent in most other techniques. From that it is basically possible to obtain the exact distribution of the TLS parameters in a polymer, a knowledge that is important for the explanation of its macroscopic properties, e. g. the low temperature heat capacities.

On the other hand we wanted to show the potential of crystalline host-guest systems in SMS, here on the example of terrylene in p-terphenyl. This systems exhibits well-defined light driven spectral jumps, highly reproducible from sample to sample, that make possible the optical manipulation of single absorbers in a very controlled fashion. Especially convenient is the fact that contrary to the situation in amorphous hosts no spontaneous spectral dynamics is observed. Furthermore, the width of the light induced jumps is enormous as compared to the homogeneous line width and thus the two main spectral positions are well separated from each other. This makes terrylene in p-terphenyl a very promising system for investigations aimed to improve our understanding of molecular hole-burning mechanisms that might one day be interesting for the design of optical data storage materials at the single molecule level.

## REFERENCES

- [1] Th. Basché, W. E. Moerner, M. Orrit and H. Talon, *Phys. Rev. Lett.* **69**, 1516 (1992).
- [2] Th. Schmidt, G. J. Schütt, W. Baumgartner, H. J. Gruber and H. Schindler, *J. Phys. Chem.* **99**, 17662 (1995).
- [3] Th. Basché and W. E. Moerner, *Angew. Chem. Int. Ed. Engl.* **32/4**, 457 (1993).
- [4] W. P. Ambrose, Th. Basché and W. E. Moerner, *J. Chem. Phys.* **95**, 7150 (1991).
- [5] Th. Basché and W. E. Moerner, *Nature* **355**, 335 (1992).
- [6] A. Zumbusch, L. Fleury, R. Brown, J. Bernard and M. Orrit, *Phys. Rev. Lett.* **70**, 3584 (1992).
- [7] L. Fleury, A. Zumbusch, M. Orrit, R. Brown and J. Bernard, *J. Lumin.* **56**, 15 (1993).
- [8] *Amorphous Solids: Low Temperature Properties*, (Ed. W. A. Philips), Springer, Berlin, 1981.
- [9] Th. Basché, W. P. Ambrose and W. E. Moerner, *J. Opt. Soc. Am. B* **9**, 829 (1992).
- [10] P. Tchénio, A. B. Myers and W. E. Moerner, *J. Lumin.* **56**, 1 (1993).
- [11] G. J. Small in *Spectroscopy and Excitation Dynamics in Condensed Matter Systems*, (Eds. V. M. Agranovitch and R. M. Hochstrasser), North-Holland, Amsterdam, 1983.
- [12] W. P. Ambrose and W. E. Moerner, *Nature* **349**, 225 (1991).
- [13] P. D. Reilly and J. L. Skinner, *J. Chem Phys.* **102**, 1540 (1995).
- [14] J. Tittel, R. Kettner, Th. Basché, C. Bräuchle, H. Quante and K. Müllen, *J. Lumin.* **64**, 1 (1995).
- [15] R. Loudon, *The Quantum Theory of Light*, Oxford University Press, Oxford, 1983.
- [16] Th. Basché, R. Kettner, J. Tittel and S. Kummer, *Exp. Tech. Phys.* **41**, 197 (1995).
- [17] R. Kettner, Th. Basché and C. Bräuchle, *J. Phys. Chem.*, in preparation.
- [18] W. E. Moerner, T. Plakhotnik, T. Irngartinger, M. Croci, V. Palm and U. P. Wild, *J. Phys. Chem.* **98**, 7382 (1994).
- [19] S. Kummer, Th. Basché and C. Bräuchle, *Chem. Phys. Lett.* **229**, 309 (1994), *Chem. Phys. Lett.* **232**, 414 (1995).
- [20] F. Kulzer, S. Kummer and Th. Basché, *J. Phys. Chem.*, in preparation.