

UNIVERSITY OF GRONINGEN

Faculty of Science and Engineering

ZERNIKE INSTITUTE FOR ADVANCED MATERIALS

**A Review of the Properties Obtainable using Single
Molecule Spectroscopy**

by Mihai Andrei Frantiu

with the supervision of prof. Richard Hildner

February 26, 2021

Table of Contents

1	Introduction	2
2	Experimental Techniques	2
2.1	Spectroscopy	2
2.2	Single Molecule Spectroscopy	4
2.2.1	Single Molecule Resolution	4
2.2.2	SMS Microscope Configurations	4
2.3	SMS Advantages	5
2.4	SMS Limitations	5
3	Properties Accessible through SMS	6
3.1	Spatial Information	6
3.2	Structural Information	9
3.3	Electronic Information	11
4	Studying the Effects of Guest-Host Interactions	14
4.1	Charge-Transfer Complexes	14
5	Conclusion and Outlook	15
	References	17

1 Introduction

With the advent of opto-electronic materials, the scientific world has been on a race to find the brightest colours and the darkest solar cells. It is only natural then, that the tools with which we investigate these materials would also improve, some even being just discovered. Amongst these investigation techniques, single molecule spectroscopy is one of the most powerful. As the name suggests, this technique concerns the spectral analysis of a single emitting unit (the chromophore). Although having the same base principles, this method offers unprecedented access to the fastest processes occurring at the single molecule level, at the cost of more restrictive experimental conditions.

Non-intrusive in nature, Single Molecule Spectroscopy (SMS) is most often applied to conjugated polymer chains which are able to absorb and emit light. By removing the effect of ensemble averaging present in bulk/solution spectroscopy experiments, one can obtain an accurate description of not only the electronic transitions of the chromophore but also a plethora of spatial and structural characteristics. Subsequently it would be useful to create a catalogue of properties that are obtainable through this technique which is the purpose of this review. Although there are a few reviews on the subject [1] [2], the question of collecting the characterisation possibilities offered by SMS has never been posed.

In the next sections I will offer a brief refresher on the topic of light-matter interactions followed by the restrictions involved in SMS experiments. This is followed by an in-depth treatment of possible experimental methods and their specifics. The advantages and disadvantages posed by this technique will also be compared at the end of the Experimental Method section. The obtainable properties will then be shown, ordered according to the types of information they provide. In the discussion section, an experiment involving the interaction of a charge-transfer complex and its host matrix will be presented and improvements to the method will be given. Finally, the last section will contain a short conclusion and an outlook on the future applicability of this powerful technique.

2 Experimental Techniques

2.1 Spectroscopy

Electromagnetic waves can interact with matter as long as there is a dipole moment on a length-scale comparable to the wavelength of radiation. The possible interactions give rise to the three most common phenomena found in spectroscopy: absorption, emission and scattering.

In this review we are only interested in the absorption, emission and scattering of photons caused by a change in the electron distribution of the molecule, as such the radiation encountered will be in the UV/Vis and X-ray part of the spectrum.

Although the classical description of a dipole moment is enough to describe the spectrum of some of the simpler molecules and their vibrations, a quantum mechanical treatment is necessary for uncovering the finer mechanisms behind electronic transitions that appear in many spectra [4]. Using Schrodinger's equation, the allowed electronic transitions become dependent on the overlap of the electronic wavefunctions which is quantified using the transition dipole moment operator:

$$\mathbf{R}_{nm} = \int \psi_n^* \boldsymbol{\mu} \psi_m d\tau \quad (1)$$

Even with such a complete description of the interaction, it is difficult to obtain exact information about the electronic structure of a single chromophore because of line broadening effects. These effects are mostly caused by ensemble averaging and temperature. Single Molecule Spectroscopy is a technique that removes the former of the the two effects mentioned above.

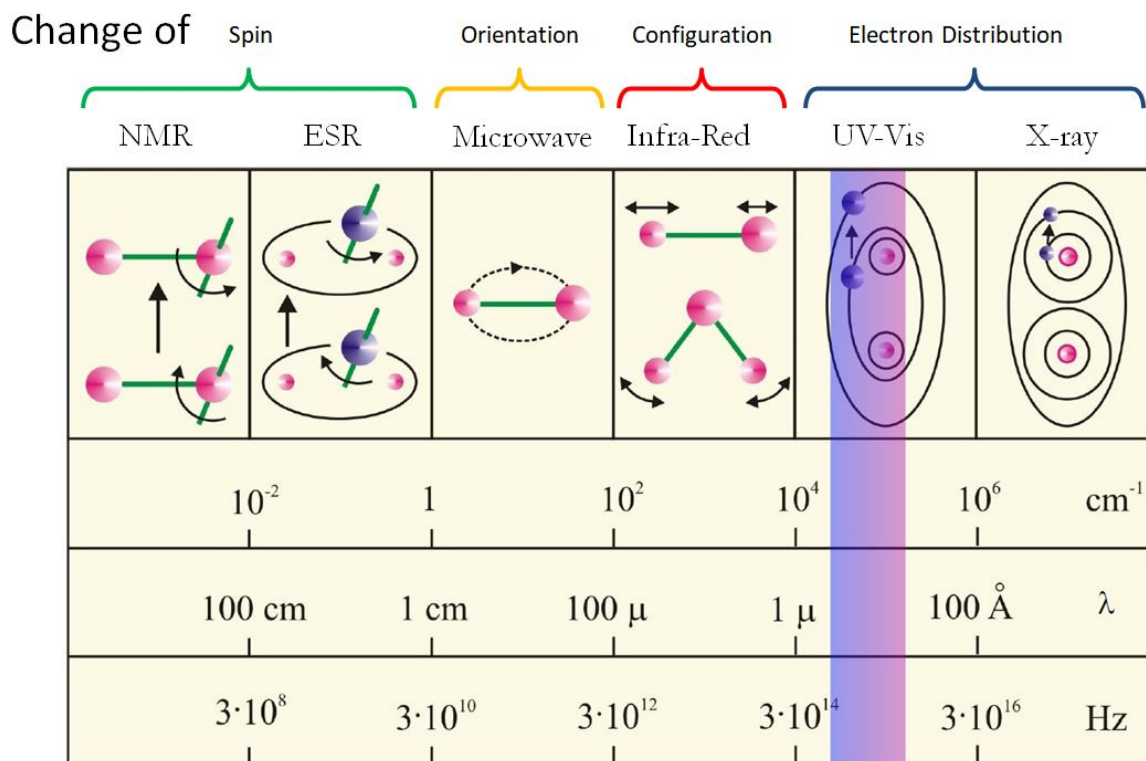


Figure 1: Possible configurations for a change in dipole moment. The energy required to create an excited state increases from left to right.(obtained from [3])

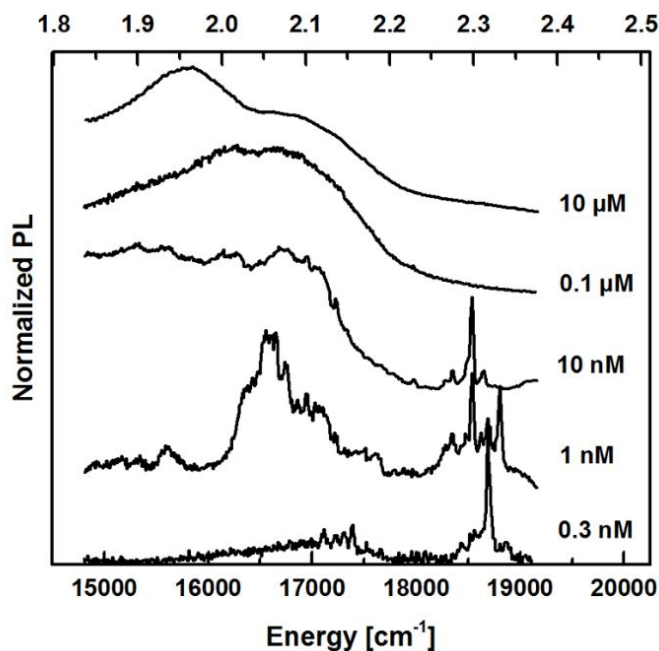


Figure 2: PL spectra of P3HT for five decreasing values of the concentration. This graph illustrates the gradual appearance of the undiffused, single molecule spectrum with decreasing concentration.(obtained from [5])

2.2 Single Molecule Spectroscopy

SMS experiments operate on the same basic principles as any other spectroscopy experiments and the optical components are essentially the same. The main differences are the sample preparation and experimental conditions. As the name suggests, SMS strives to investigate the interactions of light with a single chromophore.

2.2.1 Single Molecule Resolution

Firstly, the probing volume is significantly smaller than other spectroscopy experiments. This is usually done by confocal or wide-field fluorescence microscopy, where the size of the probing volume can be manually adjusted for each sample and experiment [1]. Furthermore, the fluorescence optical set-up can be customized with a coincidence detection system, providing time- or frequency-resolved information on the electronic transitions. The various SMS techniques will be further discussed in the next section.

Secondly, when preparing the sample, a number of restrictions must be considered. The concentration of the target chromophore must be low enough such that molecules can be individually selected using a small probing volume. The value for such a low concentration is usually between 0.01 nM and 1nM [6] [5].

The solvent substance is referred to as the host matrix and must naturally be transparent to the light of the excitation and emission. As the guest molecule is preferred to be stationary in a medium, it is usually embedded in the host solution then the entire sample is cooled. Unless the conducted experiment investigates electron transfer between the chromophore and the host matrix, the two substances must not exchange electrons easily. Especially for low temperature measurements, the coupling of the two can introduce substantial broadening effects to the spectrum [7].

In order to further increase the accuracy of the measurements, only the isolated chromophores of highest absorption cross section are selected. This ensures that the fluorescence is maximum and enhances the signal to noise ratio.

2.2.2 SMS Microscope Configurations

Single Molecule Spectroscopy is an extremely sensitive technique that requires a number of conditions to be met in order to obtain relevant results. For this purpose, two methods for optical microscope configurations can be distinguished: scanning and wide-field [1].

In the first case, the small probing volume condition is met by actually reducing the incident area of the probing light by means of a pinhole (confocal) or small aperture comparable in size to the diffraction limit of incident light (near-field) [8]. This method is advantageous when a higher SNR (signal-to-noise ratio) is required, as much of the sampling volume is occupied by the light emitting species. It also benefits from enhanced temporal resolution compared to the other SMS method because of its more sensitive CCD cameras.

In the second case, the entire sample is illuminated, much like a traditional microscope and the desired spatial resolution is achieved by means of the objective. The greatest advantage this method offers is the simultaneous recording of multiple chromophores and their time evolution. Most commonly, the epifluorescence configuration is used which is the standard for most biological fluorescence experiments. In this case, the light emitted by the sample is directed through the same objective as the excitation light, but the much higher spatial resolution is selected through the NA value. As only the emitted light is incident on the detector (following the selection done by the dichroic mirror and emission filter) this technique has increased SNR.

Another common microscopy configuration used in fluorescence experiments is TIRF (Total Internal Reflection Fluorescence). In this method, the superficial penetration depth of the evanescent waves caused by total internal reflection is used. This ensures that only chromophores located in the first few nanometres under the TIR interface are excited by the laser. Additionally, the intensity of

said evanescent waves is exponentially decaying away from the interface, thus this method is extremely sensitive to the position of the chromophore relative to the interface.

The methods discussed above mostly concern themselves with identifying the electronic configuration of the molecule as well as the possible transitions across the spectrum. In recent years the purpose of SMS techniques has drifted from investigating said electronic structure to obtaining as much information as possible from each emitted photon. Many ingenious ways of taking advantage of light-matter interactions were developed and information such as molecular orientation, macromolecular conformation or even energy transfer phenomena is now accessible.

Polarization Microscopy is a method that takes advantage of the dependence of the transition dipole moment on the alignment of the chromophore with the exciting electronic dipole moment [1]. By rotating the polarization of the exciting beam, a modulation appears in the emission [9]. This method gives information on the spatial orientation of the target molecule.

Fluorescence Resonant Energy Transfer (FRET) requires the presence of two fluorophores of differing transition energies. By exciting the shorter wavelength fluorophore and recording the emission of the other, information can be obtained on the relative positions of the two. Moreover, in cases where the chromophores are on the same macromolecule, the dynamics of conformation and bending can be closely recorded as proximity between the two fluorophores is proportional to the emission intensity.

Fluorescence Lifetime Measurements can be performed for SMS experiments in order to monitor the dynamics of light-matter interactions [10]. This takes advantage of the fact that a single molecule is considered and thus single photon counting can be employed in uncovering the excited state lifetimes [11].

2.3 SMS Advantages

The most important aspect of single molecule spectroscopy is evident in its name. By removing the effect of ensemble averaging, a much more accurate spectrum can be drawn of the considered molecule. It is common to obtain peaks of FWHM (full-width-half-maximum) as small as the resolution of the spectrometer that correspond to specific electronic transitions on a single chromophore.

In such conditions, the interactions between host matrix and guest molecule have substantial effects on the obtained spectrum [12]. This aspect is normally avoided by carefully choosing the appropriate non-interacting, transparent host matrix. This is not always the case, as the interaction between host matrix and guest molecule can be the studied phenomenon itself.

Single molecule spectroscopy also gives access to isolated, single events such as the relative approach of donor and acceptor molecules in FRET microscopy [13]. Additionally, any conformational changes to the chromophore will affect its spectrum and can be recorded with a high temporal resolution. Many dynamic effects, especially in biological processes can be easily investigated using optically active markers.

2.4 SMS Limitations

When considering emissive electronic transitions from a single chromophore, the rate of emitted photons is substantially smaller than the ones responsible for excitation. Moreover, any interaction of the host matrix (containing many times more molecules) with the incident em-wave will affect the spectrum arriving at the detector and drown out the signal of interest. A main limitation of SMS is thus the need for high Signal-to-Noise Ratio (SNR) to obtain any sort of useful information. The experimental set-up must then be fine-tuned such that the SNR is maximised.

Another drawback of the technique is the low-temperature requirement. An appropriate (large absorption cross-section) chromophore must first be found and isolated from external excitation, including temperature. Moreover, the molecule must be stationary for the duration of the experiment. Due to all of the mentioned experimental conditions, the considered fluorophore must be introduced into a transparent solid. Glass would be an excellent candidate due to its optical properties and stability, however the processing temperature makes it impossible to embed an organic molecule. Consequently, most host matrices used in SMS experiments are polymer crystals such as PMMA, P3HT, n-hexadecane or toluene [14] [15] [16].

One of the elements that affect the SNR the most is evidently the host matrix. Not only the choice for host molecule but also the purity of the matrix (number of defects) is important for obtaining a good signal [2]. Most often, the host matrix is polycrystalline upon freezing and an area of high crystalline order must be chosen for conducting experiments. The chromophore must also be present at ultra-low concentrations and a selection process occurs before each experiment. This can make the sample preparation step a laborious process.

3 Properties Accessible through SMS

Considering the many creative and inventive ways in which single molecule spectroscopy has been used to characterize molecules or investigate their interaction with the environment, it would be useful to create a catalogue of obtainable characteristics through this technique. These characteristics can offer information on three main aspects of the investigated molecule.

3.1 Spatial Information

This category includes characteristics that derive from the absolute or relative position and orientation of the molecule, with the most basic experiment being Single Molecule Detection (SMD) [17], [18].

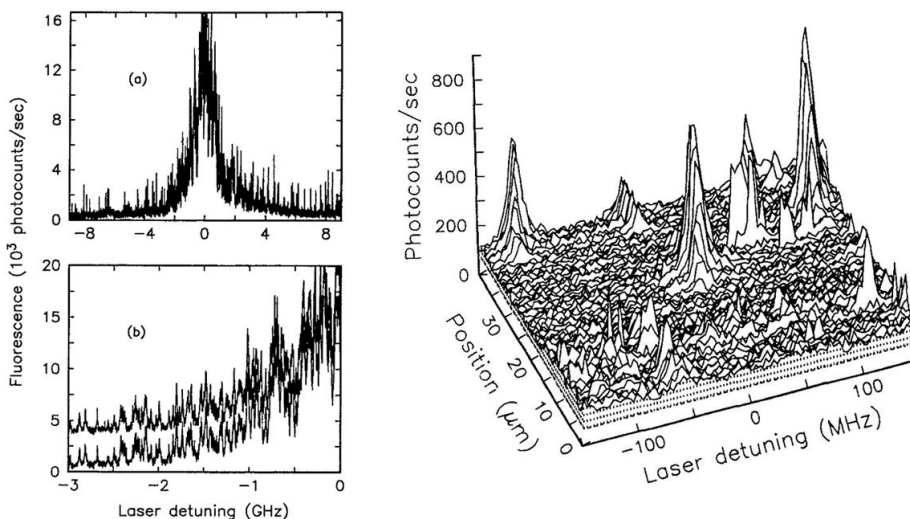


Figure 3: Fluorescence Excitation Spectrum of a) $10 \mu\text{m}$ thick, low concentration pentacene in p-terphenyl. In b) two of the same spectra are compared side by side on an expanded scale. It is important to note the coincidence of peaks between the two spectra, signifying the presence of a single defect (pentacene molecule). In the landscape on the right, the probe was swept in one direction over the sample and a spectrum was recorded at each position. Each "cluster" of peaks represents a pentacene molecule. (obtained from [16])

One of the first single-molecule spectroscopy experiments was the detection and spectroscopy of pentacene molecules in a p-terphenyl crystal [16]. In this experiment a $5 \mu\text{m}$ focal spot was used for a 2 nW exciting frequency modulated (FM) laser [19], [20]. In order to obtain an excitation spectrum, the laser frequency was swept over 18GHz. By raster scanning of the sample, one can map with relative accuracy the location of the impurities (pentacene molecules) in the sample. Once chosen, a chromophore was analyzed and its excitation spectrum was obtained. Moreover, the emission was recorded over a period of time to also investigate the spectral dynamics of the molecule.

In the example above the chromophore coincides with the impurity molecule, however that is not always the case. A molecule can contain multiple chromophores that may or may not be coupled in any way [21]. SMS can be used to investigate the orientation and position of each chromophore individually as well as relative to each other. In an experiment performed by Lupton *et al* [2], the degree of order in the orientation of chromophores on the same P3HT chain was investigated. By changing the host matrix material, the guest polymer has a tendency to form chains of different shapes. In one host material, the polymer is well solvated and forms random coil like structures that absorb light of any polarization. In less solvated samples however, the polymer collapses into anisotropic, rod-like structures that can only absorb light polarized in the direction of the dipole moment of the molecule.

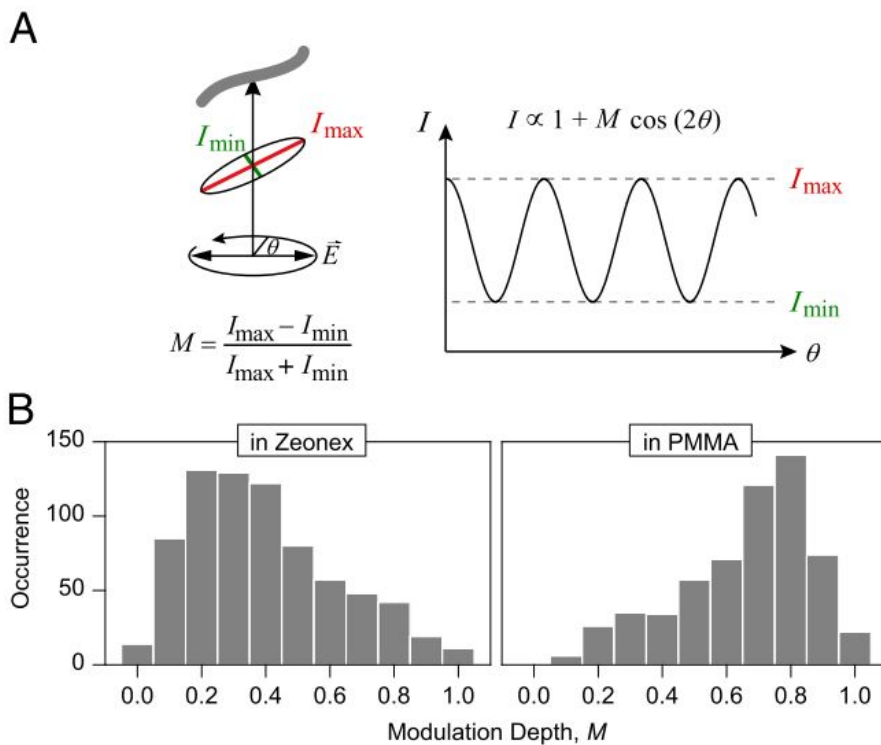


Figure 4: A) Defining the modulation depth which quantifies the degree of chain extension, order and anisotropy. B) Histogram of M values for P3HT chains in Zeonex and in PMMA. The purpose of this graph is to show the shape dependency of the solute on the host matrix.(obtained from [22])

The orientation of the chromophore can also be obtained from polarization modulation experiments. In an experiment by Moerner *et al* [1] the fluorophore is excited with light of variable polarization in two orthogonal directions in the plane of the sample. The emission is recorded and a polar plot can be made showing the angles that the dipole moment of the molecule aligns with.

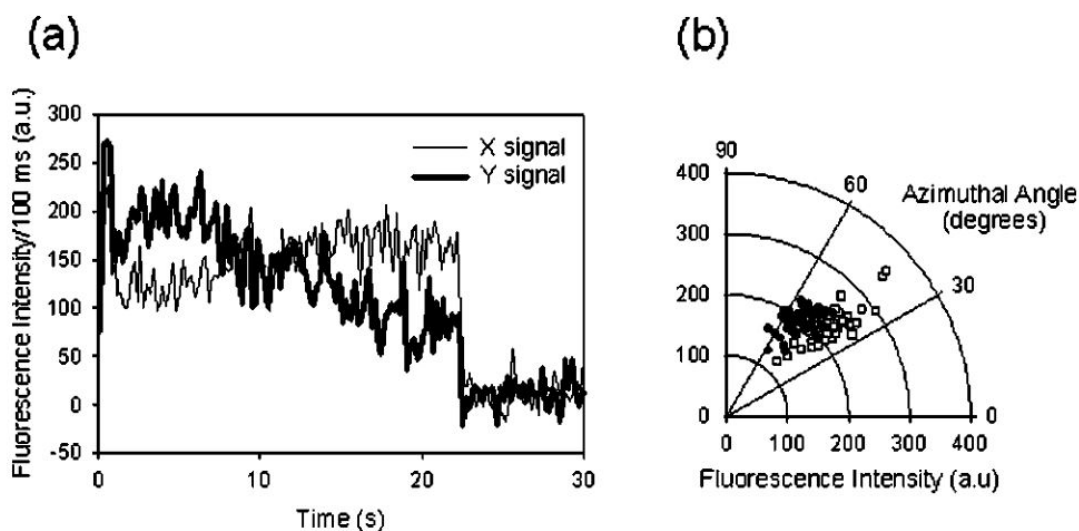


Figure 5: Plot obtained by recording the emission from an excitation light of varying polarization. a) Emission caused by light on two orthogonal directions which essentially follow the rotation of the sample. b) The trajectory of the in-plane dipole moment of the fluorophore and its respective intensity. (obtained from [1])

In addition to locating single chromophores in a host matrix, this technique also allows for their counting. In Fig. 6, it is shown that increasing the length of a conjugated polymer chain will eventually cause a discontinuity in the conjugated π system. The emission spectrum will then show the presence of at least another peak, corresponding to a chromophore of different colour. By counting the peaks, one can count the number of chromophores present in the considered molecule.

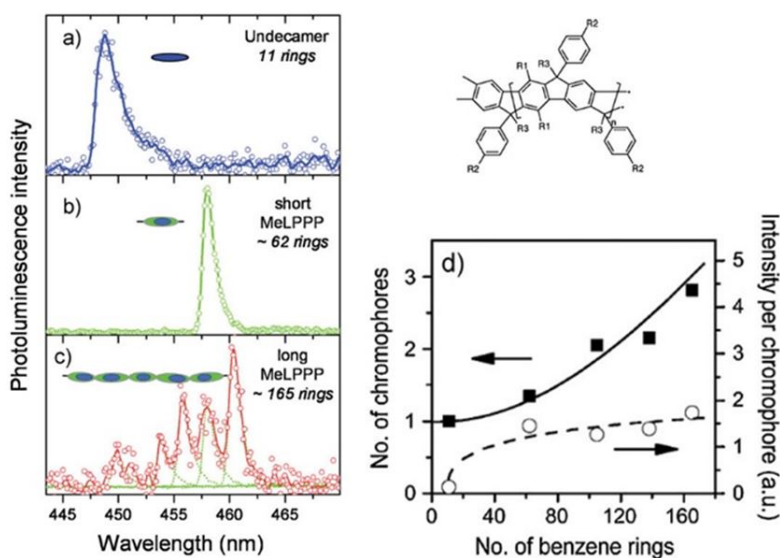


Figure 6: Single molecule emission spectra of the conjugated polymer MeLPPP. a) The undecamer. b) Short chain with 62 rings. c) Long chain with 165 rings. In d) the information is collected and shows an increase in the number of chromophores with the number of benzene rings. (obtained from [2])

3.2 Structural Information

One of the main sources of chromophoric disorder in long conjugated polymer chains is the shape [23] and torsion angle [7] of the backbone. It is along the backbone of a polymer that the conjugated π system extends, and knowing that a chromophore is defined by the longest, continuous conjugated π system it is natural that the shape of the backbone affects the fluorescence of the polymer. In the paper by Thiessen *et al*, the shape and degree of torsion in the backbone has a direct effect on position of the Zero-Phonon Line (ZPL, corresponds to the 0-0 transition).

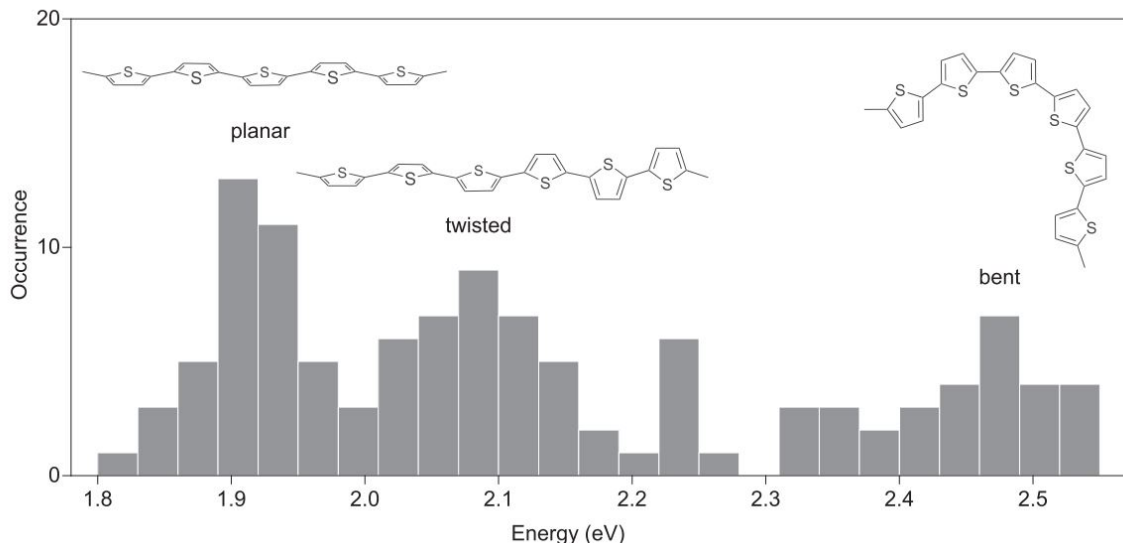


Figure 7: Histogram of the 0-0 transition energy for the same single-chromophore polymer, P3HT. A trimodal distribution can be observed, with each peak corresponding to one of three most common conformations. (obtained from [22])

Following a different line of reasoning, another group [24] investigated the effects of slightly altering the chemical structure of the polymer. By adding fluorine to the monomer, the polymer chain morphology changes significantly and consequently, the spectrum changes as well. Initially, the considered polymer P3EHT shows a diffuse spectrum due to conformational and torsional freedom in the chain. The group discovered that backbone fluorination promotes the formation of aggregates with extended, rod-like structure.

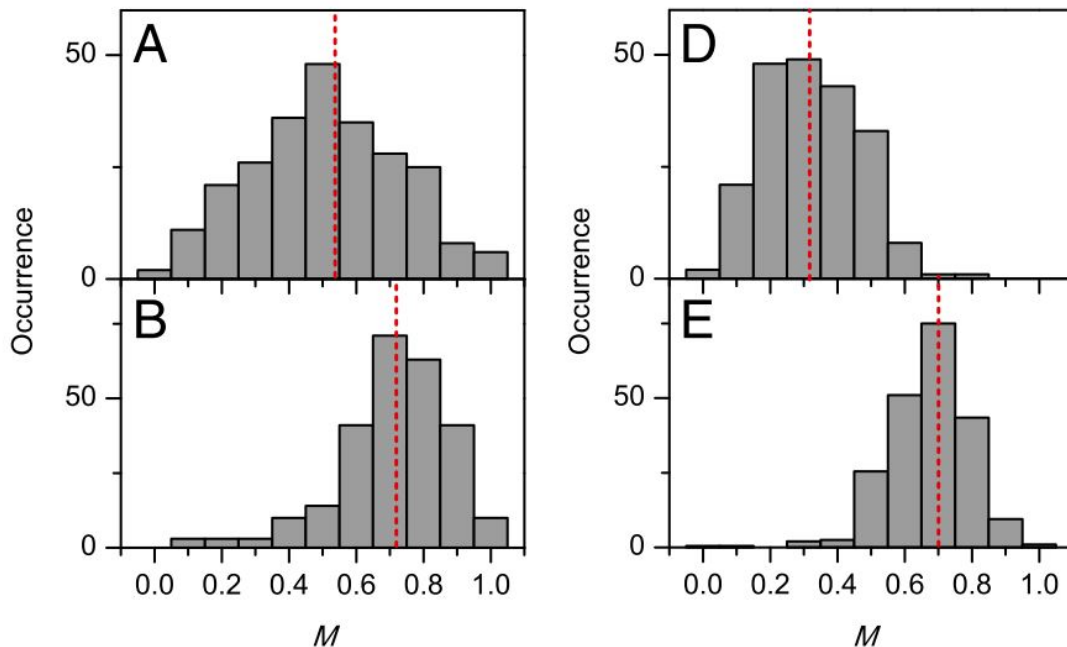


Figure 8: Histograms of the fluorescence excitation modulation depth M (describes degree of chain ordering) for single chains of A) P3EHT and B) F-P3EHT and aggregates D-E of P3EHT and F-P3EHT respectively (obtained from [24])

In a similar manner, Hildner *et al* [14] investigated the degree of planarization of the backbone conjugated π -system. The three species are chosen for the difference in their side-groups and difference in backbone length. Comparing the two PDOPT samples one can observe the presence of more than one ZPL in the more extended one. This is a telltale sign of a multichromophoric structure with multiple emitters per polymer chain. Comparing PDOPT with P3HT on the other hand, one can immediately notice the difference in ZPL peak width which indicate a larger freedom of movement for the side chains, the further they are from the backbone. Additionally, the ZPL position for PDOPT is significantly red-shifted compared to the P3HT sample. This is thought to originate from the increased planarity of the conjugated backbone facilitated by non-covalent interactions with the side-groups.

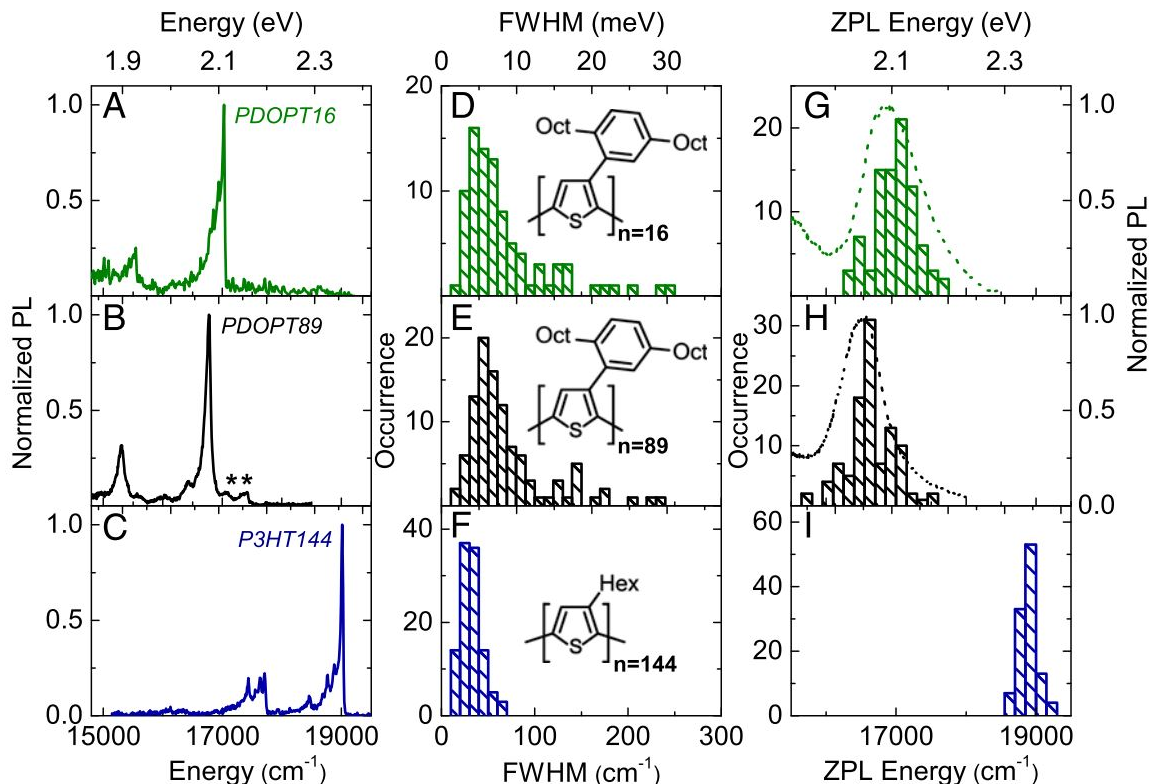


Figure 9: A-C single chain spectra of PDOPT16, PDOPT89, P3HT144; D-F histogram of peak widths at the FWHM; G-I histogram of recorded ZPL energies.(obtained from [14])

3.3 Electronic Information

Finally, single molecule spectroscopy is a powerful tool for investigating the electronic transitions of a conjugated polymer for use in opto-electronic applications such as photo-voltaics. While bulk(solution) measurements offer some initial insight into the expected colour of a conjugated polymer, it is only through single molecule techniques that the intra- and intermolecular excitation dynamics are uncovered [25]. Additionally, only by obtaining single-chain spectra can the effect of aggregation or higher solute concentration be analyzed and the effects of spectral diffusion [26] are much easier to study when ensemble averaging is removed.

The absence of ensemble averaging offers unprecedented access to high resolution spectra around the ZPL. This is only possible at very low temperatures as shown in Fig.11 where the two high resolution spectra recorded at 20K do not show the same narrow peak as the one performed at 2 K.

In conjunction with Raman Spectroscopy, one can more accurately investigate the vibrational modes and their coupling with the electronic transitions (fluorescence vibrations) [28] [Pleaseinsert“PrerenderUnicode–”int]. This is only possible due to the reduced inter-coupling between chromophores on adjacent conjugated polymers. The good agreement between the fluorescence and Raman spectrum allows for a better understanding of the fluorescence peaks and unambiguous assignment of the vibrational modes.

Under single molecule conditions the fluorescence lifetime of a chromophore can be obtained [29]. Using a dichroic mirror and two avalanche photo detectors, one can take advantage of the two-photon technique to calculate the times of absorption and emission by the molecule and thus calculate the lifetime of the excited state.

Another time-dependent experiment that can be performed through SMS involves the phenomenon of electron transfer. A telltale sign of this phenomenon is the fluorescent blinking of the chromophore

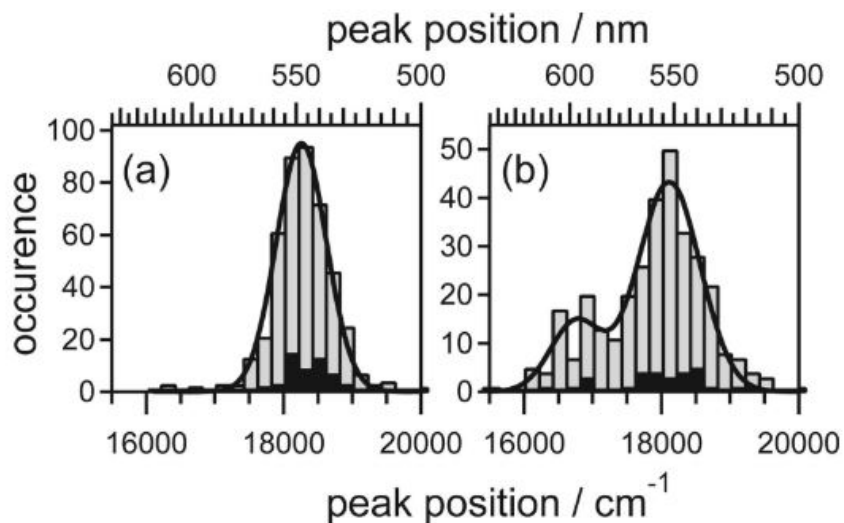


Figure 10: Distribution of the emission peaks of MEH-PPV single chains of (a) high and (b) low molecular weight at 1.2 K. The black bars show the number of single chains considered for which a ZPL could be identified. (obtained from [27])

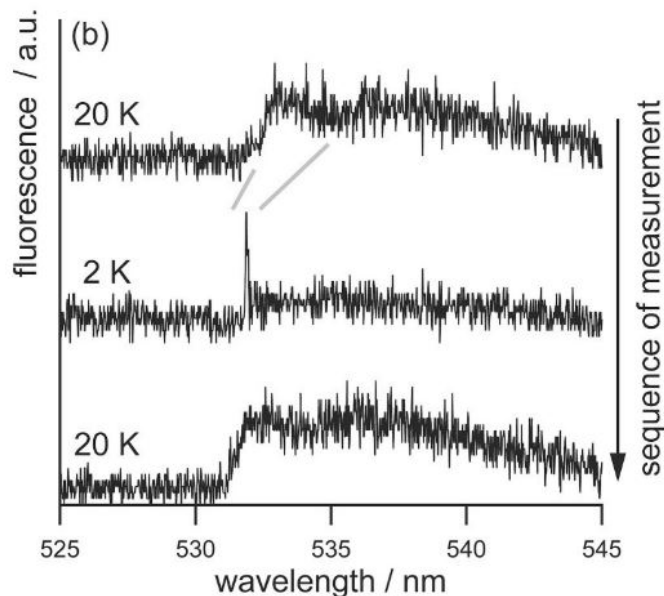


Figure 11: Three high-resolution single-chain spectra at 2 K and 20 K. Note the disappearance of the ZPL at higher temperatures. (obtained from [27])

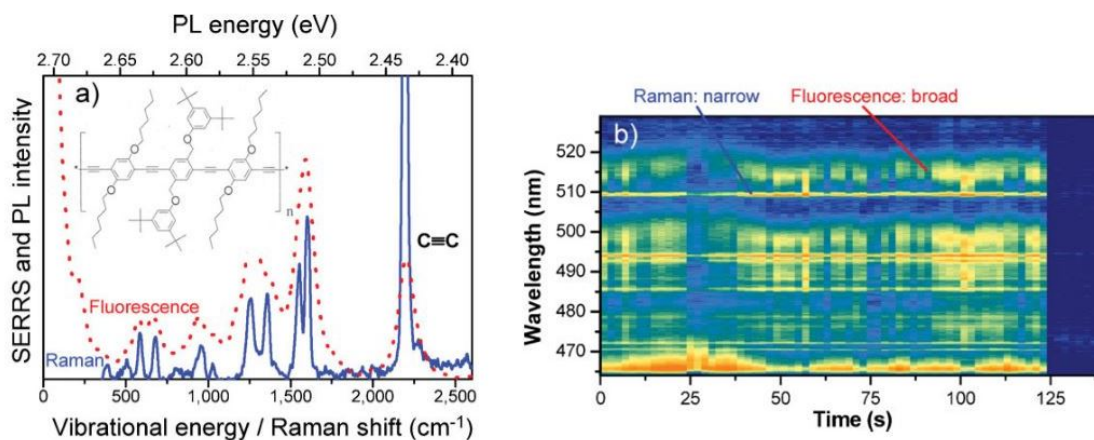


Figure 12: a) Single chain fluorescence (red) against the Raman spectrum (blue) b) Simultaneous fluorescence and Raman vibrations from a single polymer chain. (obtained from [2])

[30]. Upon defining the "on" and "off" states, one can create a switch duration histogram. If a single electron transfer event is responsible for the switching, the aforementioned histogram should follow an exponential curve.

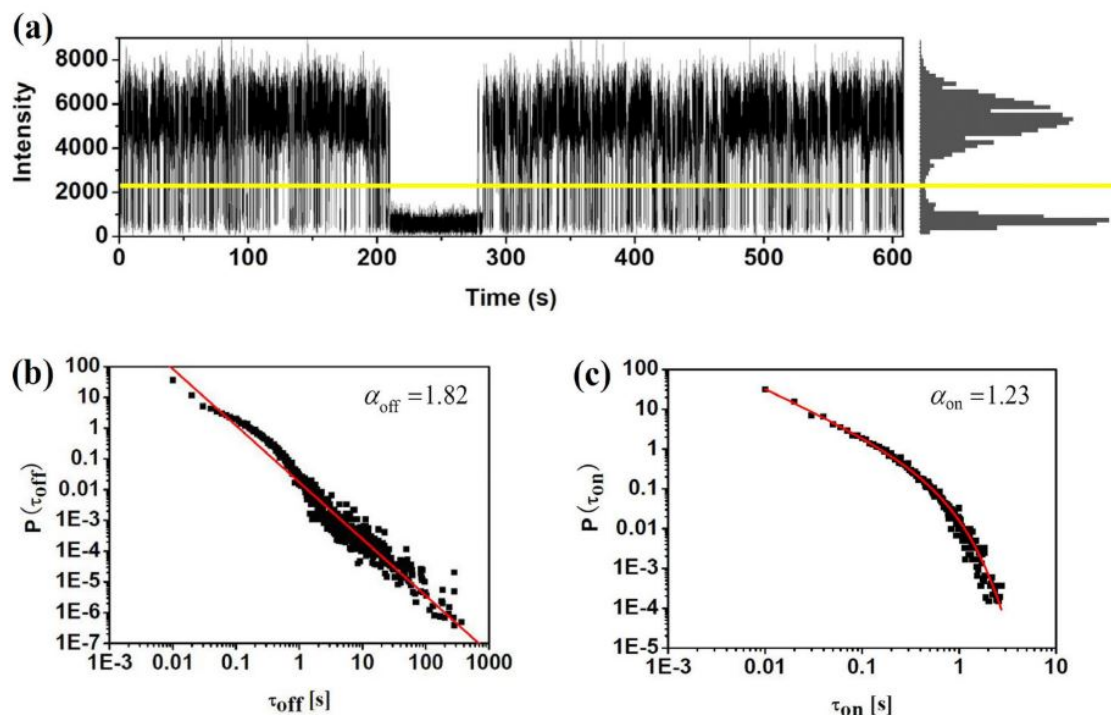


Figure 13: a) Fluorescence trajectory of a single fluorophore exhibiting clear differences between the "on" and "off" states. b)-c) Histograms of the "off" and "on" durations on a log-log plot; the exponential decay trend is obvious. (obtained from [31])

4 Studying the Effects of Guest-Host Interactions

Much progress has been made on investigating the physical properties of single-chain conjugated polymers using SMS, however no experiment truly considers a completely isolated molecule. The presence of the host matrix always affects the spectral characteristics of the guest molecule to some extent. Experimentalists strive to remove such effects, yet studying their consequences can also be of value.

Typically, a considered sample is frozen solid and the host matrix thus attains a poly-crystalline structure. This means that the environment each guest molecule experiences is different and the interactions between the two can vary from one location in the crystal to another. Furthermore, the environment is not completely static and external factors such as electromagnetic radiation, pressure or stress applied in the sample can alter the local conditions.

The information we can directly obtain from SMS experiments on a typical sample is radiation intensity (of excitation, absorption or emission experiments) and their time-dependent evolution. From the latter, one can obtain lifetime measurements of excited states of the considered conjugated compound.

One of the less investigated phenomena is charge transfer in donor-acceptor complexes [32]. While highly dependent on the local conditions, CT in such material systems is important for organic photovoltaic devices [33].

4.1 Charge-Transfer Complexes

Upon the association of two or more organic molecules, in some cases a salt-like complex can be formed where the electronic charge is partially transferred from one of the molecules (the donor) to the other (the acceptor). The rearrangement of electronic charge causes an electrostatic potential that stabilizes the complex. The relative freedom that the electric charge has to move around the complex makes these compounds a good candidate for organic electronics and circuitry. Furthermore, these complexes can also be used in opto-electronic applications where the excitation and emission spectra can be very different. In these applications, the donor molecule absorbs a photon of appropriate energy (HOMO-LUMO gap) which creates an excited state over the whole complex. The radiative relaxation occurs at the acceptor molecule which has a lower HOMO-LUMO gap.

In the case of single molecule spectroscopy, the sensitivity of the spectrum (emission or absorption) or its intensity to the local environment can be utilized to study said environment. In a paper by Masuo *et al* [12] a charge-transfer complex is investigated in a variety of host matrices. The five chosen host matrix substances were chosen for their increasing free volume. The observed fluorescence shows a fluctuating trend in some instances, while blinking was observed in other with "off" states of over 1 s. The lifetime of the excited state could be obtained in each case by fitting the "on" to "off" time differences to an exponential curve.

By calculating the average lifetime for each set of measurements, as well as the deviation in lifetime from one set of measurements to the next it is possible to quantify the degree of fluctuation through their ratio $\Delta\tau/\tau_{av}$. It is observed that for host matrices with a larger free volume, the degree of fluctuation is also large. This implies that in matrices with a larger free volume, the CT complex has more possibility to dissociate or form geometries that involve non-radiative processes [34], thus yielding the fluctuations in intensity or blinking behaviour.

This experiment although successful in offering an explanation to the fluctuating fluorescence intensity, did suffer from some shortcomings. The CT complex was known not to form at concentration required for SMS and thus a much larger concentration was used (10^{-4} M). Furthermore, the absorption coefficient of this material was low and thus the fluorescence intensities were only up to four times larger than the background signal. In future experiments investigating the effects of the host matrix on a charge transfer compound, the material should be chosen differently with the technique's restrictions in mind such as the more stable TTF:TCNQ.

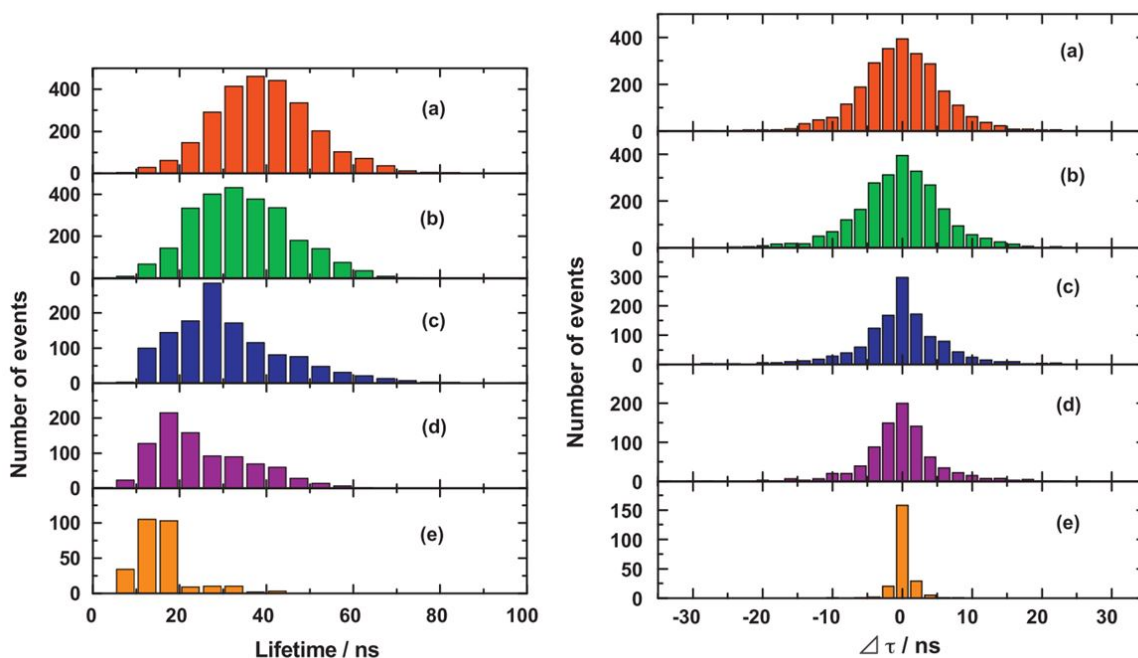


Figure 14: Fluorescence lifetime and lifetime difference histograms of ECz:TCNB complexes in a)PMMA b)PEMA c)PnPrMA d)PC e)Dextrin (obtained from [12])

It would be interesting to observe the effects of polarizability of host-matrix materials on the charge transfer complex. The later is known to have a high degree of polarizability , hence the effect of the host species on the CT complex would be substantial.

5 Conclusion and Outlook

The science of organic opto-electronic devices has made considerable advances not in small part due to the improvement of detection and characterization techniques. One such technique with unexpected applicability beyond its original field is single-molecule-spectroscopy. Spectra obtained using this technique show more detailed features and sharper peaks by virtue of lack of ensemble averaging. In molecular biology this technique offers access to single events while for condensed matter physics it allows for the investigation of the host matrix interactions. Information about the spatial position and orientation of a chromophore as well as its electronic and even vibronic transitions can be easily extracted from single-molecule spectra.

While presently a very powerful investigation tool, this technique still has some limitations and drawbacks, mainly the low-temperature experimental conditions which require the host matrix to be a solid. An experimental method of following the single molecule across a fluid host medium would allow for measurements at higher temperatures and eventually room-temperature measurements. Anchoring the molecule of interest at one end and introducing unidirectional flow could potentially solve the problem of keeping the probing volume on the chromophore. For biologists, the possibility of performing SMS experiments in fluids at higher temperatures could pave the way for in-vivo experiments of biomolecular processes.

Yet another improvement to the measuring system would be increasing temporal resolution that allows access to the fastest processes involved in light absorption or emission. The resolution of the spectrometer can also be increased in order to reach the spectral widths dictated by the time-energy

uncertainty principle [35].

While in most experiments, the host matrix is chosen to have a minimum effect on the single chain spectrum, there is a wealth of guest-host interactions that can be probed by SMS through careful selection of the material system [36] [34]. This could give a better understanding on the electron-transfer in donor-acceptor co-polymer photo-voltaic devices.

Finally, a complete understanding of the electronic and spatial dynamics of a single polymer chain could serve as switches or actuators in molecular machines. Using light as an indirect control mechanism, one can fine-tune the state of a molecule to purposefully alter the system.

References

- [1] W. E. Moerner and David P. Fromm. Methods of single-molecule fluorescence spectroscopy and microscopy. *Review of Scientific Instruments*, 74(8):3597–3619, 2003.
- [2] John M. Lupton. 1689-1721 Single-molecule spectroscopy for plastic electronics: Materials analysis from the bottom-up. *Advanced Materials*, 22(15):1689–1721, 2010.
- [3] C. N. Banwell. *Fundamentals of molecular spectroscopy [by] C. N. Banwell*. McGraw-Hill London, New York, 2nd ed. edition, 1972.
- [4] J. C. Bergquist, Randall G. Hulet, Wayne M. Itano, and D. J. Wineland. Observation of quantum jumps in a single atom. *Physical Review Letters*, 57(14):1699–1702, 1986.
- [5] Dominic Raithel, Sebastian Baderschneider, Thiago B. De Queiroz, Ruth Lohwasser, Jürgen Köhler, Mukundan Thelakkat, Stephan Kümmel, and Richard Hildner. Emitting Species of Poly(3-hexylthiophene): From Single, Isolated Chains to Bulk. *Macromolecules*, 49(24):9553–9560, 2016.
- [6] Dominic Raithel, Sebastian Baderschneider, Thiago B. De Queiroz, Ruth Lohwasser, Jürgen Köhler, Mukundan Thelakkat, Stephan Kümmel, and Richard Hildner. Emitting Species of Poly(3-hexylthiophene): From Single, Isolated Chains to Bulk. *Macromolecules*, 49(24):9553–9560, 2016.
- [7] Hiroyuki Kobayashi, Kousuke Tsuchiya, Kenji Ogino, and Martin Vacha. Spectral multiplicity and spectral dynamics reflect changing conjugation length in single molecules of oligophenylenevinylenes. *Physical Chemistry Chemical Physics*, 14(29):10114–10118, 2012.
- [8] Florian Kulzer and Michel Orrit. Single-molecule optics. *Annual Review of Physical Chemistry*, 55(1):585–611, 2004.
- [9] M. Forster, D. Thomsson, P. R. Hania, and I. G. Scheblykin. Redistribution of emitting state population in conjugated polymers probed by single-molecule fluorescence polarization spectroscopy. *Physical Chemistry Chemical Physics*, 9(6):761–766, 2007.
- [10] C. Eggeling, J. R. Fries, L. Brand, R. Günther, and C. A. M. Seidel. Monitoring conformational dynamics of a single molecule by selective fluorescence spectroscopy. *Proceedings of the National Academy of Sciences*, 95(4):1556–1561, 1998.
- [11] Marc Lor, Jan Thielemans, Lucien Viaene, Mircea Cotlet, Johan Hofkens, Tanja Weil, Christine Hampel, Klaus Müllen, Jan W. Verhoeven, Mark Van Der Auweraer, and et al. Photoinduced electron transfer in a rigid first generation triphenylamine core dendrimer substituted with a peryleneimide acceptor. *Journal of the American Chemical Society*, 124(33):9918–9925, 2002.
- [12] Sadahiro Masuo, Yasumasa Yamane, Shinjiro MacHida, and Akira Itaya. Fluorescence behavior of individual charge-transfer complexes revealed by single-molecule fluorescence spectroscopy: Influence of the host polymer matrix. *Journal of Photochemistry and Photobiology A: Chemistry*, 227(1):65–70, jan 2012.
- [13] Rahul Roy, Sungchul Hohng, and Taekjip Ha. A practical guide to single-molecule fret. *Nature Methods*, 5(6):507–516, 2008.
- [14] Dominic Raithel, Lena Simine, Sebastian Pickel, Konstantin Schötz, Fabian Panzer, Sebastian Baderschneider, Daniel Schiefer, Ruth Lohwasser, Jürgen Köhler, Mukundan Thelakkat, Michael Sommer, Anna Köhler, Peter J. Rossky, and Richard Hildner. Direct observation of backbone planarization via side-chain alignment in single bulky-substituted polythiophenes. *Proceedings of the National Academy of Sciences of the United States of America*, 115(11):2699–2704, 2018.

- [15] Ruiyun Chen, Ruixiang Wu, Guofeng Zhang, Yan Gao, Liantuan Xiao, and Suotang Jia. Electron Transfer-Based Single Molecule Fluorescence as a Probe for Nano-Environment Dynamics. *Sensors*, 14:2449–2467, 2014.
- [16] W. P. Ambrose, Th Basché, and W. E. Moerner. Detection and spectroscopy of single pentacene molecules in a p-terphenyl crystal by means of fluorescence excitation. *The Journal of Chemical Physics*, 95(10):7150–7163, 1991.
- [17] M. Orrit and J. Bernard. Single pentacene molecules detected by fluorescence excitation in a p-terphenyl crystal. *Phys. Rev. Lett.*, 65:2716–2719, Nov 1990.
- [18] Satoshi Habuchi, Suguru Onda, and Martin Vacha. Mapping the emitting sites within a single conjugated polymer molecule. *Chemical Communications*, (32):4868, 2009.
- [19] W. E. Moerner and L. Kador. Optical detection and spectroscopy of single molecules in a solid. *Physical Review Letters*, 62(21):2535–2538, 1989.
- [20] G. C. Bjorklund, M. D. Levenson, W. Lenth, and C. Ortiz. Frequency modulation (fm) spectroscopy. *Applied Physics B Photophysics and Laser Chemistry*, 32(3):145–152, 1983.
- [21] J. Hernando, J. P. Hoogenboom, E. M. H. P. van Dijk, J. J. García-López, M. Crego-Calama, D. N. Reinhoudt, N. F. van Hulst, and M. F. García-Parajó. Single molecule photobleaching probes the exciton wave function in a multichromophoric system. *Phys. Rev. Lett.*, 93:236404, Dec 2004.
- [22] Alexander Thiessen, Jan Vogelsang, Takuji Adachi, Florian Steiner, David Vanden Bout, and John M. Lupton. Unraveling the chromophoric disorder of poly(3-hexylthiophene). *Proceedings of the National Academy of Sciences of the United States of America*, 110(38), 2013.
- [23] S. Tretiak, A. Saxena, R. L. Martin, and A. R. Bishop. Conformational Dynamics of Photoexcited Conjugated Molecules. *Physical Review Letters*, 89(9):1–4, 2002.
- [24] Zhongjian Hu, Ryan T. Haws, Zhuping Fei, Pierre Boufflet, Martin Heeney, Peter J. Rossky, and David A. Vanden Bout. Impact of backbone fluorination on nanoscale morphology and excitonic coupling in polythiophenes. *Proceedings of the National Academy of Sciences of the United States of America*, 114(20):5113–5118, 2017.
- [25]
- [26] R. Hirschmann and J. Friedrich. A hole burning study of excitonic states of chain molecules in glasses. *The Journal of Chemical Physics*, 91(12):7988–7993, 1989.
- [27] Florian A. Feist and Thomas Basché. Fluorescence excitation and emission spectroscopy on single MEH-PPV chains at low temperature. *Journal of Physical Chemistry B*, 112(32):9700–9708, 2008.
- [28] W. E. Moerner. A dozen years of single-molecule spectroscopy in physics, chemistry, and biophysics. *The Journal of Physical Chemistry B*, 106(5):910–927, 2002.
- [29] Takuji Adachi, Jan Vogelsang, and John M. Lupton. Chromophore bending controls fluorescence lifetime in single conjugated polymer chains. *Journal of Physical Chemistry Letters*, 5(12):2165–2170, 2014.
- [30] W. E. Moerner. Those blinking single molecules. *Science*, 277(5329):1059–1060, 1997.
- [31] Rémi Métivier, Fabian Nolde, Klaus Müllen, and Thomas Basché. Electronic excitation energy transfer between two single molecules embedded in a polymer host. *Physical Review Letters*, 98(4):1–4, 2007.

- [32] Jean-Luc Brédas, David Beljonne, Veaceslav Coropceanu, and Jérôme Cornil. Charge-transfer and energy-transfer processes in π -conjugated oligomers and polymers: A molecular picture. *Chemical Reviews*, 104(11):4971–5004, 2004.
- [33] Michael W. Holman, Ruchuan Liu, and David M. Adams. Single-molecule spectroscopy of interfacial electron transfer. *Journal of the American Chemical Society*, 125(41):12649–12654, 2003.
- [34] Renaud A. L. Vallée, Mircea Cotlet, Mark Van Der Auweraer, Johan Hofkens, K. Müllen, and Frans C. De Schryver. Single-molecule conformations probe free volume in polymers. *Journal of the American Chemical Society*, 126(8):2296–2297, 2004.
- [35] Florian A. Feist, Giovanni Tommaseo, and Thomas Basché. Single-molecule spectroscopy of MEH-PPV polymer molecules in different host matrices'. *Journal of Physical Chemistry C*, 113(27):11484–11490, 2009.
- [36] Young Jong Lee, Doo Young Kim, and Paul F. Barbara. Effect of sample preparation and excitation conditions on the single molecule spectroscopy of conjugated polymers. *The Journal of Physical Chemistry B*, 110(20):9739–9742, 2006.