Real-time monitoring of formation and dynamics of intra- and interchain phases in single molecules of polyfluorene

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Abstract
Poly(9,9-dioctylfluorene) (PFO) is one of the most important conjugated polymer materials, exhibiting outstanding photophysical and electrical properties. PFO is also known for a diversity of morphological phases determined by conformational states of the main chain. Our goal in this work is to address some of the key questions on formation and dynamics of one such conformation, the β-phase, by following in real time the evolution of fluorescence spectra of single PFO chains. The PFO is dispersed in thin polystyrene film and the spectra are monitored during the process of solvent vapor annealing with toluene. We confirm unambiguously that the PFO β-phase is formed on true single-chain level at room temperature in the solvent-softened polystyrene. We further find that the formation of the β-phase is a dynamic and reversible process occurring on the order of seconds, leading to repeated spontaneous transitions between the glassy and β-phases during the annealing. Comparison of PFO with two largely different molecular weights (M_w) shows that chains with lower M_w undergo the transition to the β-phase much faster. For the high M_w PFO β-phase chains, a detailed Franck-Condon analysis shows large distribution of the Huang-Rhys factor S, and even dynamic changes of this factor occurring on a single chain. Such dynamics are likely a manifestation of changing coherence length of the exciton. Further, for the high M_w PFO chains we observe an additional conformational state, a crystalline γ-phase. The γ-phase formation is also a spontaneous reversible process in the solvent-softened matrix. The phase can form both from the β-phase and the glassy phase, and the formation requires high M_w to enable inter-segment interactions in a self-folded chain.
Polyfluorenes, and in particular poly(9,9-dioctyfluorene) (PFO), have attracted considerable attention over the past 20 years because of their outstanding photophysical and electrical properties, including efficient blue emission (both as photoluminescence and electroluminescence), high optical gain and charge transport properties [1-3]. In addition, PFO is thermally and chemically stable, and easily solution-processable, leading to a range of potential applications such as light-emitting diodes and lasers, sensors and photonic devices [4-7].

From the point of conjugated polymer photophysics, as well as potential applications, the diversity of conformational states and of the resulting properties of PFO is another motivating factor in the research. In solutions of good solvents and in films cast from such solutions, the PFO chains find themselves in random coil conformation characterized by a distribution of torsional angles between monomers from 120° to above 140°, with an energy minimum at 135° [8]. This conformation is referred to as glassy or α-phase. Under certain conditions, part of the PFO chain can undergo conformational change resulting in well-ordered segment of the chain with torsional angles of 165° [8]. The increased order results in extended conjugation length which is manifested by red shift of absorption and fluorescence well separated from the glassy phase. This so-called β-phase conformation shows narrow and vibrationally-resolved absorption band and mirror-image fluorescence spectrum [9, 10], features which are unusual in most largely disordered conjugated polymers. The β-phase exhibits higher stability and improved optoelectronic properties, such as color purity or quantum efficiency [11].

Such distinctive β-phase can be induced in as-prepared PFO thin films by annealing in solvent vapor [12], dipping into a solvent [5] or by thermal annealing and cycling [13, 14]. Films containing β-phase can be also prepared during spin-coating by adjusting the solvent quality or boiling point [15], or by using solvent additives [16]. Apart from solid films, formation of β-phase has been also observed upon cooling dilute solutions of PFO in poor solvents [17]. Since the first observations, considerable effort has been spent towards explaining the mechanism of β-phase formation [18-20]. Still, the picture is incomplete and many questions remain. Evidence indicates that at least the first stage of β-phase formation is an intramolecular exothermic process involving planarization of part of the PFO chain. The β-conformation on individual PFO chains appears to be a reversible metastable state which can be stabilized by aggregation between the backbones of neighboring chains [17, 21]. During solvent or temperature annealing of glassy PFO films the β-phase transition is likely driven by mechanical stress originating from different thermal expansion of the substrate and the PFO film, or from different solubility of the backbone and the aliphatic side chains. The PFO chains respond to the stress by planarizing with the sidechains arranged in a zigzag manner [12]. In dilute solutions, the first step of β-phase formation was found to be driven by the side chain interactions, because these interactions change upon cooling due to varying conformational and rotational freedom [22, 23]. Apart from the side-chain length [22], the overall molecular weight of PFO also affects the efficiency of β-phase formation but different approaches provide conflicting results [23-25]. Some of the questions on reversibility of the β-phase or on the role of aggregation can be unveiled by studying individual PFO chains on truly single-molecular level [26-29]. For example, cryogenic temperature experiments on oligofluorenes dispersed and solvent annealed in inert polymer films showed that a minimum of 9 monomer units are necessary to efficiently form β-phase [28]. Very recent
study on PFO immobilized in solution confirmed reversibility of the β-phase upon cycling between good and poor solvents [30].

Apart from the glassy and β-phases, an intermediate γ-phase (also referred to as crystalline phase) is occasionally observed. Spectrally, this form of PFO appears as a long-wavelength shoulder in absorption of the glassy phase [12], its emission is located between the glassy and β-phases [8], and is identifiable in Raman spectra [31-33]. The γ-phase is most readily formed by thermal annealing above the melting point of PFO. In terms of the backbone conformation, calculations show that, similar to the glassy phase, there is a distribution of torsional angles between adjacent monomers, with an energy minimum at 150° [8]. Very recently, γ-phase has been observed in fluorescence of individual aggregates of PFO formed by solvent vapor annealing [34].

Here, we attempt to address some of the key questions on formation mechanism and dynamics of β-phase by following in real time the evolution of fluorescence spectra of single chains of PFO. The PFO is dispersed in thin-film polystyrene (PS) matrix and the spectra are measured during the process of solvent vapor annealing (SVA) with toluene. We confirm unambiguously that β-phase is formed in PFO on true single-chain level at room temperature when dispersed in the solvent-softened PS matrix at concentrations of 10^{-9} M. While there have been indications of the single-chain nature of β-phase formation from previous ensemble studies [12, 17], the ultimate proof can be provided only by studying individual chains since dilute dispersions or solutions do not necessarily prevent aggregation, which can even be promoted by SVA. We further find that the formation of β-phase on single chains is a dynamic and reversible process occurring on the order of seconds. We observe repeated spontaneous transitions between the glassy and β-phases during the SVA, pointing to a metastable character of the PFO chain conformation. Comparison of PFO with two largely different molecular weights (M_w) shows that chains with lower M_w undergo the transition to the β-phase much faster. Only for the high M_w PFO chains we observe the glassy, β and a new intermediate phase spectrally located between the two. Detailed Franck-Condon analysis shows that this third phase can be assigned to the γ-phase (crystalline phase) based on the spectral peak position, Huang-Rhys factor and spectral linewidth. For the high M_w PFO chains we also observe real-time step-wise changes of fluorescence spectra from the β-phase to the γ-phase (but not vice versa) which are interpreted as chain folding and transformation of neighboring β-phase segments into a γ-phase domain. The γ-phase can also form directly from and disintegrate into the glassy phase. Overall, the fraction of PFO chains showing the γ-phase increases with increasing SVA time. For the β-phase chains we find large distribution of the Huang-Rhys factor S, and even dynamic changes of this factor on a single chain. Such changes are uncorrelated with the spectral peak positions, pointing to the dynamic character of exciton coherent delocalization in the β-phase PFO at room temperature.

Results and discussion

The samples under investigation are PFO chains of different molecular weights (with M_w of 68 kDa, PDI of 3.45, and M_w of 500 kDa, respectively) dispersed in thin films of PS at the concentration of 10^{-9} M and subject to SVA on top of the microscope stage with a good solvent toluene. It has been shown previously that SVA of matrix-dispersed
conjugated polymer chains can, under certain conditions (combinations of good and poor solvents), lead to the growth of conjugated polymer aggregates [35]. To investigate such possibility in our samples we measured and analyzed series of fluorescence images of the low Mₘ PFO before and during the SVA, with the SVA times of 15 minutes and 2 hours, respectively. The Fig. S1 shows that even though the SVA leads to diffusion of considerable fraction of the single PFO chains, the average numbers of emitting spots (PFO chains), as well as the emission intensity distributions are similar in all three cases. This result indicates that, unlike the recent study on PFO aggregates [34], we can analyze and interpret our results as phenomena happening on the true single-chain level.

Bulk fluorescence spectra of drop-cast films of PS mixed with ensemble-level concentrations of low Mₘ PFO before SVA and during SVA for different time periods (Fig. S2) show that the β-phase formation proceeds even after a short period SVA of 15 minutes. The spectra are dominated by the β-phase emission peak at 437.3 nm, and the glassy phase peak originally at 423.8 nm remains as a shoulder. This situation is reflected by the single-chain experiments. As shown in an example in Fig. 1, within the same location of the film we observe chains with purely β-phase emission, purely glassy phase emission and occasionally also spectra with mixed glassy and β-phase characteristics. Distribution of the fluorescence 0-0 peak wavelengths obtained from the fitting of 63 single PFO chains shows a peak around 422 nm corresponding to the glassy phase and a peak around 436 nm corresponding to the β-phase, consistent with other recent single-molecule studies [30, 34].

![Image](image_url)

**Figure 1.** a) Fluorescence microscopic image of low Mₘ PFO single chains after 15 min SVA; b) - d) Fluorescence spectra from the PFO chains numbered 1-3 in a), showing b-phase (b), glassy phase (c) and mixed characteristics (d). The spectra are fit with a sum of Gaussian functions; e) Distribution of the 0-0 fluorescence peaks obtained from 63 single PFO chains.

The spectra presented in Fig. 1 are stationary spectra obtained with an exposure time of 0.3 s. In reality, the spectra of most single PFO chains during SVA are highly dynamic on the time scales of seconds to tens of seconds at room temperature. Apart from the
previously reported reversible transitions between the blue glassy phase emission and the aggregation-induced green-band emission [36, 37] (an example of which is shown in Fig. S3), we observe reversible step-wise transitions between the glassy and the β-phases. One such example of a single-step transition from β-phase emission to glassy phase emission is shown in Fig. S4. Such spectral change can be alternatively interpreted as due to photobleaching of a β-segment on the PFO chain. It is well known that due to their smaller bandgap the β-phase segments function as energy traps where the excitons absorbed over the whole chain accumulate and from where the emission takes place [13, 38]. It has been observed that even as little as 7% fraction of β-phase in a sample causes the energy to be emitted solely from the β-phase segments [11]. However, the spectral change in the Fig. S4 is accompanied with very little decrease of overall emission intensity (about 5%, determined from integrated spectral intensities), which points against photobleaching of part of the PFO chain, and allows interpretation of the data as reversible conformational change from the β-phase back to the glassy phase. This interpretation implicitly assumes that the photoluminescence quantum efficiency (PLQY) of the glassy and β-phases are comparable. The PLQY of the β-phase PFO remains an open question. There have been reports on PLQY both decreasing [39] and increasing [40] with increasing β-phase fraction, or peaking at a certain fraction [11]. A temperature dependence study of the PLQY also indicated that at room temperature the glassy and β-phase PLQYs are comparable [41], making the interpretation of our data in terms of the reversible conformational change between the glassy and β-phases plausible.

The spectral measurement presented in Figs. S3 and S4 is carried out by dispersing the emitted light with a monochromator and for such experiment the PFO chain has to stay immobile over the acquisition time. However, as shown in Fig. S1, a considerable fraction of the single PFO chains undergo diffusion during the SVA process. We have verified in separate experiments that for the excitation intensities used there is no observable laser heating of the sample, and the observed diffusion is due to the swelling of the matrix. To follow the spectral changes of the diffusing PFO chains, we split the fluorescence image using a dichroic mirror into short-wavelength (SW) and long-wavelength (LW) parts, as shown in Fig. 2. Signal present in both the SW and LW parts indicates emission from glassy phase chain whereas signal in the LW part only corresponds to the β-phase. Examples of spectral dynamics detected from immobile and diffusing single PFO chains are shown in Fig. 2d and 2e, respectively. A drop in the intensity of the SW channel and a correlated simultaneous increase in the intensity of the LW channel indicates formation of the β-phase. In principle, an alternative explanation could involve reversible thermal rearrangement of the chain that would switch on and off an energy transfer process from the glassy to the β-phase. However, such explanation is less likely as it would require perpendicular orientation of neighboring transition dipoles to completely stop the intramolecular energy migration.
Figure 2. a) Fluorescence spectra from single low M<sub>w</sub> PFO chains in glassy (black) and β-phase (red), together with a transmission spectrum (blue) of the dichroic filter used to split the fluorescence image; b), c) Details of split fluorescence images of a single PFO chain in glassy phase (b) and β-phase (c), with SW and LW indicating the short- and long-wavelength channels, respectively; d), e) Time evolution of the SW (black) and LW (red) channel intensities of an immobile (d) and diffusing (e) PFO chains. The symbols g and β denote the corresponding phases.

The examples in Fig. 2 d, e show that on the level of individual isolated PFO chains the β-phase formation is a dynamic and reversible process, and that during the SVA the β-phase conformation is a metastable state, regardless of whether the chain is immobilized by the matrix polymer or diffusing in the voids of the solvent-swelled matrix. In both cases the dynamic rheological nature of the SVA process is the likely driving force for the conformational switching of the PFO chains.

We next turn to the high M<sub>w</sub> PFO (500 kDa). Compared to the low M<sub>w</sub> PFO, 15 min or 30 min SVA does not cause any observable changes in the bulk fluorescence spectra of drop-cast films of PS mixed with ensemble-level PFO concentrations (Fig. S2). The samples have to be annealed for at least 2 hours to achieve similar spectral signature of the β-phase as for the 15 min-annealed low M<sub>w</sub> PFO samples. This observation is reflected on the single-chain level as well. As the Fig. S5 shows, most of the PFO chains are in the glassy state and occasionally show mixed glassy and β-phase characteristics. Similar to the low M<sub>w</sub> PFO, we find frequent reversible transitions between the blue glassy phase emission and the green-band emission (Fig. S5).

After 2 h or longer SVA we observe single PFO chain spectra corresponding to conformations other than the glassy phase. The Fig. 3a, b shows examples of the glassy and β-phase spectra, similar to those found for the low M<sub>w</sub> PFO. In addition, we observe a third type of spectrum growing in between, which we assign to the γ-phase (Fig. 3c). This assignment is based mainly on the results of Franck-Condon analysis [15, 42, 43] of
a statistical sample of single PFO chain spectra. In the analysis we concentrate on the 0-0 and 0-1 vibronic spectral features and determine the Huang-Rhys factor $S$ from the relation between the intensities $I_{0,0}$ and $I_{0,1}$ of the two bands as $S = I_{0,1}/I_{0,0}$ [42]. The spectra were fit with a combination of four Gaussian functions, three of which accounted for the 0-0, 0-1 and 0-2 vibronic bands. The fourth optional component was used to compensate for possible broad background features (such as weak green-band emission) and was not used in the analysis. Examples of the fits are shown in the Fig. 3a-c as red lines. The fitting provided reliable values of the 0-0 and 0-1 energies, intensities and linewidths, and of the Huang-Rhys factor. A histogram of the 0-0 peak energies (converted to wavelengths) of 80 single PFO chains is shown in Fig. 3d. Similar to the low $M_w$ PFO (Fig. 1e) we observe distributions of the glassy phase 0-0 peaks centered around 421 nm (blue) and of the β-phase peaks centered around 434 nm (green). In addition, there is another distribution peak centered around 428 nm (grey) which was absent for the low $M_w$ PFO. The position of this 0-0 energy distribution is one indication of the γ-phase [8, 32]. Very similar distribution observed recently on single PFO aggregates was also assigned to this phase [35].

Another evidence of qualitatively different phases present in the PFO chains is provided by a comparison of 0-0 peak linewidths. The Fig. 3e plots the 0-0 linewidths against the energy of the 0-0 peak. Following the assignment from the histogram in Fig. 3d, the 0-0 energy is divided into β- and γ-phase regions. The Fig. 3e shows that the β-phase 0-0 width is considerably narrower than the γ-phase linewidth, as also indicated in the single PFO spectra (Fig. 3b, c). The average values of the linewidths obtained from the plot are 36 meV for the β-phase region and 54 meV for the γ-phase region, a trend consistent with previous observations [8]. There is also a less obvious but still distinct difference in the values of the Huang-Rhys parameter, plotted in the Fig. 3f against the 0-0 energy. The average values of $S$ are 0.31 for the β-phase region and 0.37 for the γ-phase region. The values for the β-phase are comparable to single-mode Huang-Rhys factor found for PFO at low temperatures [15]. The phonon energy $E_p$ of the 0-1 transition shows a narrow distribution with an average value of 0.168 eV, corresponding in energy to several Raman modes in the 1100 cm$^{-1}$ - 1400 cm$^{-1}$ range [11]. The product $E_pS$ provides the reorganization energy which is distributed within the range of 20 and 100 meV, as shown in Fig. S6.

Differences in spectral linewidths are often explained in terms of energetic disorder in ensembles of emitters. However, in the case of single conjugated polymer chains, there is likely only one emitting site on each chain and the emission spectra are free of inhomogeneous broadening. The observed significant narrowing from 54 meV in the γ-phase to 36 meV in the β-phase is similar to what was observed, e.g., between the glassy and β-phases in ensembles of PFO at low temperatures [15]. The difference is still more striking for single PFO molecules at low temperatures [27, 28].
Figure 3. a) - c) Examples of fluorescence spectra of single chains of high M_w PFO, showing the glassy (a), β- (b) and γ-phases (c). The red lines are fits to a combination of Gaussian functions. d) Distribution of the 0-0 fluorescence peaks obtained from 80 single PFO chains. For the γ- and β-phases, the dark shades correspond to SVA for 2 hours, the light shades to SVA for 4 hours. e) 0-0 fluorescence linewidth plotted against the 0-0 peak energy. Black symbols - SVA for 2 hours, red symbols - SVA for 4 hours; f) Huang-Rhys factor S plotted against the 0-0 peak energy. Black symbols - SVA for 2 hours, red symbols - SVA for 4 hours.

Despite the long history of the PFO morphology research, there is surprisingly no consistent explanation of the line narrowing effect in the β-phase. Here, we do not attempt to achieve that, but our analysis provides another parameter relevant to the discussion, i.e., the width of the 0-1 emission line. A plot in Fig. S7 of the linewidths of both the 0-0 and 0-1 fluorescence peaks against the 0-0 peak energy in the β-phase spectral region shows that the 0-1 line is much broader. Its average value of 0.55 meV is comparable to the 0.54 meV 0-0 linewidth of the γ-phase. This difference could be a signature of line narrowing due to extended exciton coherence length, observed often in J-aggregates [44]. With increasing coherence length, the oscillator strength of 0-n emission lines is
Theoretical approach would be necessary to describe these results. The observed changes in the spectra 1 and 2 is only by a factor of 1. The factor of ~1.7 for theory predicts spectral narrowing by the factor of 37 meV. The qualitative trend follows the change in the 0-0 emission linewidth. For the spectra 1-4 in Fig. 4b (with S values of 0.41, 0.14, 0.35 and 0.14, respectively) we observe corresponding linewidths of 40, 36, 42 and 37 meV. The qualitative trend follows the S values and appears to confirm the changing exciton coherence as the origin of the differences in the spectra. Quantitatively, however, theory predicts spectral narrowing by the factor of 1/√Ncoh [44] which would correspond to a factor of ~1.7 for S going, e.g., from 0.41 to 0.14, whereas the narrowing observed in the spectra 1 and 2 is only by a factor of 1.1. Evidently, more rigorous theoretical approach would be necessary to describe these results.

Another interesting phenomenon observed from the dynamic changes in the single
PFO spectra in Fig. 4 is the process of formation and dissociation of the \( \gamma \)-phase. These all appear to be single-step processes, similar to the glassy – \( \beta \)-phase transitions observed for the low M\textsubscript{w} PFO. The \( \gamma \)-phase can be formed both from the \( \beta \)-phase (as shown in Fig. 4c, d) or from the glassy phase (as shown in Fig. 4e, f). It dissociates into the glassy phase (Fig. 4g, h), and we have also observed on a single chain the whole sequence from \( \beta \)- via \( \gamma \)- to the glassy phase. However, we have not found a transition from the \( \gamma \)-phase back to the \( \beta \)-phase confirming the conformational rigidity of the \( \beta \)-phase. Further information is extracted from Fig. 3. In both the histogram in Fig. 3d and the linewidth and Huang-Rhys plots in Fig. 3e, f we distinguished between data points obtained for 2h SVA and points obtained for 4h SVA. Even though the statistics is limited, we observe that after 2h SVA, 11 out of 32, or 34%, single PFO chains transformed into the \( \gamma \)-phase. This fraction increased to 67% (14 out of 21 PFO chains) after 4h SVA. We can, therefore, summarize that the \( \gamma \)-phase can be formed on single chains of PFO, but requires large M\textsubscript{w}. The \( \gamma \)-phase formation is a spontaneous reversible process in the solvent-softened polymer matrix at room temperature, this phase can form from both the \( \beta \)-phase and the glassy phase, and the formation process requires longer time than the \( \beta \)-phase. Apart from a chain conformation characterized by inter-monomer torsion angle of 150\(^\circ\), the \( \gamma \)-phase has been alternatively associated with a crystalline form of PFO [12]. The dependence of its formation on M\textsubscript{w} and time would suggest that even on the level of single PFO chains the phase is a crystalline-like form involving interchain interactions of a self-folded chain only present in high M\textsubscript{w} PFO. We note that since the \( \beta \)-phase has the lowest bandgap of the three conformation forms considered here, observation of a \( \gamma \)-phase spectrum on a single PFO chain implies that there are no \( \beta \)-phase segments present on such chain that would form energy traps and that would be populated by energy transfer.

Conclusions
Observing complex systems such as amorphous polymers on the level of single molecules reveals phenomena and properties that are otherwise hidden or averaged out in polymer films or solutions. This work brings several examples of such novel observations, including the metastable character of \( \beta \)-phase formation on single PFO chains, formation and dissociation of \( \gamma \)-phase in single chains, and the dynamic nature of the exciton coherence length on single chains. The results will be important from the point of fundamental photophysics of conjugated polymers and of polyfluorenes in particular, but will also have impact in novel material design with advanced functionalities.
Figure 4. a), c), e), g) Two-dimensional plots (time vs. wavelength) of time evolution of fluorescence spectra from single PFO chains. The fluorescence intensity is shown by the grey scale. The red arrows and the corresponding numbers denote the regions from which the 1D spectra were plotted. Each line (spectrum) in the plot was measured with an integration time of 0.3 s; b), d), f), h) One-dimensional fluorescence spectra taken from the regions of the 2D plots denoted by the corresponding numbers.

Materials and methods

Sample preparation
Low molecular weight poly(9,9-di-n-octylfluorenyl-2,7-diyl) (PFO, Mw 67,642 g/mol, polydispersity index PDI 3.45) was purchased from Sigma Aldrich. High molecular weight PFO (Mw 500,000 g/mol) was obtained CDT Ltd. Polystyrene (PS, M_n 106,751 g/mol, PDI 2.45, Sigma Aldrich) was used as a matrix. Samples for fluorescence microscopy measurements were prepared by dropping 150 µL of 1 wt% PS toluene solution mixed with appropriate amount of PFO onto cleaned quartz substrate and spin coating (3000 rpm, 30 s). The PFO concentration in the resulting thin films was on the order of 10^{-9} M.

Fluorescence microscopy setup
Fluorescence from single PFO chains was measured using an inverted microscope (IX 71, Olympus). The excitation light of 360 nm was provided by a continuous wave laser (UV-FN-360, 100mW, CNI). The excitation power measured at the microscope stage was on
the order of $1 \sim 5 \text{ W/cm}^2$. Fluorescence was collected by an oil immersion objective lens (UplanFLN 100×, N.A. 1.3, Olympus) and passed through a dichroic mirror (Dichro 375, Chroma) and a long-pass filter (LP 377, Edmund). For the spectral measurements the signal was further dispersed using an imaging spectrograph (CLP-50LD, Bunkou Keiki). Fluorescence was detected with an electron-multiplying (EM) CCD camera (iXon, Andor Technology) with an exposure time of ranging from 100 to 300 ms. To monitor the diffusing PFO chains, the emission was divided spectrally by an image splitter (Optosplit II, Cairn) equipped with a dichroic mirror (Dichro 430, Chroma), into short-wavelength (below 430 nm) and long-wavelength (above 430 nm) parts which were imaged side-by-side on the EM CCD. The solvent vapor annealing was carried out on top of the microscope stage by placing a small petri dish filled with toluene next to the sample and covering both the sample and the solvent dish with a larger petri dish.
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