Mesoscopic quantum emitters from deterministic aggregates of conjugated polymers

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An appealing definition of the term “molecule” arises from consideration of the nature of fluorescence, with discrete molecular entities emitting a stream of single photons. We address the question of how large a molecular object may become by growing deterministic aggregates from single conjugated polymer chains. Even particles containing dozens of individual chains still behave as single quantum emitters due to efficient excitation energy transfer, whereas the brightness is raised due to the increased absorption cross-section of the suprastructure. Excitation energy can delocalize between individual polymer chromophores in these aggregates by both coherent and incoherent coupling, which are differentiated by their distinct spectroscopic fingerprints. Coherent coupling is identified by a 10-fold increase in excited-state lifetime and a corresponding spectral red shift. Exciton quenching due to incoherent FRET becomes evident from the ratio of the radiative to nonradiative decay processes.

The challenge lies in obtaining small, morphologically well-defined aggregates of conjugated polymers of comparable molecular weight. Because the ease of deterministic synthesis of ultralarge \(\pi\)-conjugated complexes deteriorates rapidly with size, one may consider instead growing molecule-like objects by van der Waals bonding to small aggregates—the “mesoscopic” approach. Such aggregates can be grown in a controlled way by single-molecule solvent vapor annealing, raising the question of what the fundamental size scale is for which a transition from molecular to bulk behavior occurs.

Fluorescent molecules in an aggregate can interact by coherent (8, 9) or by incoherent excitation energy transfer (EET) (10). Both processes can lead to a change in fluorescence lifetime and spectrum and are therefore hard to distinguish in ensemble measurements. On the single-chain to single-aggregate level, differentiation is much easier: coherent interchromophoric coupling between parallel chromophores leads to a delocalization of excitation energy, resulting, in the simplest case, in an \(H\)-aggregate-like spectral shift: the excited-state energy level splits in two, with a redistribution of oscillator strength to the higher-lying state (11). In a molecule in the solid state, with inhibited motion and diffusion, emission becomes excimer-like, broadening and shifting to the red (12). Because oscillator strength is lost from the lowest-energy transition, an increase in fluorescence lifetime is observed—provided, however, that there is no incoherent EET (i.e., FRET) to molecular quenching sites which induce nonradiative decay (13, 14).

We recently approached the investigation of intermolecular interactions on the subensemble level by designing model systems with parallel chromophores within a single molecule (13). Excimer-like emission evolves for parallel oligomers spaced fewer than 5 Å apart (14). However, long-range interactions over mesoscopic distances, such as incoherent EET which can persist over tens of nanometers (15), or the coherent coupling of multiple chromophoric units at once, remain inaccessible in these small model systems. We therefore aim to isolate highly ordered interchain aggregates to study and compare their electronic properties with those of single chains by using single-aggregate and single-molecule spectroscopy. Fig. 1 illustrates the basic approach pursued to building molecular aggregates from the bottom up. We use poly(\(\pi\)-phenylene-ethynylene-butadiynylene) (PPEB), because this material class is well known to give rise to excimer-like emission in the solid state (16) as seen in a strong spectral shift to the red from solution phase to solid film. In a bulk film, both ordered (red) and disordered (green) domains exist. Dissolving the bulk to the level of single molecules, i.e., single polymer chains, gives rise to spatially discrete objects which can contain multiple chromophores. The challenge lies in obtaining small, morphologically well-defined and spatially isolated aggregates of a particular size: this is the

Significance

Bright and stable single-photon sources, based on molecular objects, have contributed to exploring the foundations of quantum mechanics and measurement theory. Because the photon emission rate scales with molecular size, the most direct approach to increasing brightness is to enlarge the molecular object itself. But how big can it become while still retaining molecular characteristics of a deterministic photon source? We tackle this question by growing defined aggregates out of single chains of a conjugated polymer. Multichain aggregates show discrete single-photon emission, even when the individual chains display multiphoton emission. Single-aggregate spectroscopy reveals how coherent and incoherent excitonic intermolecular coupling mechanisms, known from the bulk, evolve with aggregate size.

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solution and diluted to a concentration of $\sim 10^{-11}$ M, above typical concentrations for single-molecule experiments (14). The solution was spin-coated to obtain a 200–250-nm-thick PMMA film with single molecules uniformly distributed. During SVA, the PPEB/PMMA film is in a heterogeneous mixture of solid- and liquid-like phases in which single chains undergo diffusion, as illustrated in Fig. 1. Diffusion of single chains leads to aggregation. After 30 min of SVA, the sample was dried with nitrogen, immobilizing molecules and aggregates.

The films were scanned by a confocal microscope to identify the positions of single molecules and aggregates. This information was used to localize one single molecule, or aggregate, at a time in the diffraction-limited excitation spot at 405 nm. A dichroic mirror split the PL into two detection channels above and below 532 nm, as shown in Fig. 24, to separate emission from aggregates (orange) and isolated chains (blue-green) remaining in the film. Fig. 2B–E shows a series of confocal images for samples with different solvent mixtures used for SVA. Green denotes PL detected below 532 nm, originating from isolated chains, and red the aggregate PL above 532 nm. Fig. 2B shows an image of 20 × 20-μm² area of single chains in PMMA at single-molecule concentration (10⁻¹² M). Exclusively green emission from isolated chains is seen in diffraction-limited spots. Aggregates can be formed by selectively dissolving the PMMA matrix in acetone, in which the conjugated polymer is insoluble. Addition of a suitable polymer solvent (chloroform) to acetone promotes solubility in the swollen PMMA matrix, driving the formation of larger aggregates. Fig. 2C–E shows corresponding images of samples after 30 min of SVA with varying acetone to chloroform vapor ratios denoted in brackets (see Methods for details on sample preparation). A second population of orange to red diffraction-limited spots evolves after SVA with 100% acetone vapor (Fig. 2C), indicating that these spots emit significantly above 532 nm. After SVA with a vapor of 90:10 acetone:chloroform ratio (Fig. 2D), the overall number of spots decreases whereas the brightness of the red spots increases. This effect is more pronounced after SVA with an 80:20 ratio (Fig. 2E) (7). The increasing aggregate size with increasing solvent–polymer miscibility can be rationalized in terms of Ostwald ripening (18), which describes the particle size up to which stable aggregates can be formed. All images are shown on the same intensity scale, which leads to a blurring of the brightest spots for the largest aggregates. In all cases, the individual spots are diffraction-limited.

The fraction of red PL, $F_{\text{red}}$, is calculated as $I_{\text{red}}/(I_{\text{red}} + I_{\text{green}})$ and shown for each particle in the histograms. Before SVA, only $F_{\text{red}} < 0.3$ values are found, whereas a significant number of particles exhibit values above 0.3 after SVA. Based on this observation, we count the number of single chains (green) and aggregates (red) per image before and after SVA, providing the average number of chains in an aggregate as stated in each image. Using this preparation method for isolated aggregates of different sizes, we can now compare the spectroscopic properties of single chains and aggregates.

**Spectroscopic Properties of Individual Polymer Chains vs. Single Aggregates**

First, we compare the morphology of isolated chains and aggregates using excitation polarization spectroscopy, which reports on the overall anisotropy in absorption (19). The excitation beam is linearly polarized in the sample plane and the polarization is rotated while recording the PL intensity as depicted in Fig. 34. The excitation polarization modulation depth, $M$, is obtained by fitting the PL intensity, $I$, as a function of polarization angle, $\theta$, to Malus’ law,

$$I(\theta) \propto I + M \cos^2(\theta - \Phi),$$

where $\Phi$ is the orientation angle of the molecular transition dipole moment for maximal PL. For each single spot, $M$ was acquired, yielding a histogram as shown in Fig. 3B. Because molecular weight
affects chain morphology (20–22) we compared two different weights of PPEB. The histogram shaded in dark green shows the distribution of modulation depth values for short chains (Mn ~ 40 kDa). The light-shaded histogram reports on long chains with Mn ~ 210 kDa. The anisotropy decreases as chain length increases because longer chains fold more (20). This behavior is in contrast with that of isolated aggregates containing, on average, 12 chains, shown in Fig. 3C. Only spots with PL emission above 532 nm were taken into account to separate the aggregates from remaining single chains. The resulting modulation depth histogram has a maximum at τ ∼ 0.8. We conclude that PPEB undergoes aggregation-induced ordering during SVA, leading to the first building blocks of crystalline structures which characterize the bulk film (23, 24).

The emergence of well-ordered aggregates is further supported by comparing typical PL spectra and transient PL decays of an individual aggregate and a single chain in Fig. 3D and E. Whereas the PL spectrum of single chains is well structured with a 0–0 peak at 465 nm and a vibronic progression reaching up to 520 nm (Fig. 3D, green spectrum), the spectrum of the aggregate is less structured with a suppressed 0–0 transition around 500 nm (Fig. 3D, red spectrum). Simultaneously, the PL lifetime is increased 10-fold from 0.5 to 5.3 ns in going from single chains to aggregates. These observations are explained within the framework of excimer-like luminescence from H-aggregates, i.e., coherent interchromophoric coupling (12, 14, 25–27). Coupling of adjacent chromophores oriented parallel to one another in the excited state leads to an energetic splitting of the excited state, where the transition dipole moment vanishes in the lower-lying energy level as sketched in Fig. 3D, Inset. The PL therefore shifts to the red concomitant with a decrease in radiative rate, leading to an increase in PL lifetime provided that the fluorescence quantum yield does not change (11, 28).

**Role of Coherent and Incoherent Interchromophoric Coupling in Different Aggregate Sizes.** To substantiate the correlation between red-shifted PL and increased PL lifetime, we recorded the Fred value and the PL lifetime, τPL, for each spot in the microscope image. Fig. 4 shows scatter plots between Fred and τPL for the different samples: for 326 isolated chains in Fig. 4A, Fred is narrowly distributed between 0.07 and 0.25 with 0.4 ns < τPL < 1.2 ns. This distribution changes dramatically upon SVA. For the smallest aggregates with ~12 chains, the Fred values for 583 aggregates scatter between 0.1 and 0.85 (Fig. 4B), with a strong correlation between Fred and τPL, which can be as large as 6.3 ns. Annealing with a solvent mixture (90:10 acetone:chloroform) leads to an average size of 18 chains per aggregate. For these particles, the scatter plot of 529 spots (Fig. 4C) indicates a bimodal distribution. The first distribution shows Fred ∼ 0.2 and small τPL values from 0.4 to 2 ns, similar to the isolated chain (the monomer). The second distribution is characterized by Fred ∼ 0.8, with τPL values scattering strongly between 0.8 and 6 ns. Even larger aggregates with, on average, 54 chains can be formed by annealing with an 80:20 solvent ratio; the corresponding scatter plot of 299 particles (Fig. 4D) shows Fred values grouped almost exclusively around 0.8, again accompanied by a wide range of τPL values between 1 and 5 ns.

We conclude that the sample with the smallest aggregate size of, on average, 12 chains consists of isolated chains (green spots in Fig. 4B), small or loosely bound aggregates (yellow spots), and large or strongly bound aggregates (red spots). The strong correlation between PL red shift and increased PL lifetime implies the emergence of a coherently coupled interchromophoric excited state within polymer aggregates. Small or loosely bound aggregates vanish in samples with increasing aggregate size. However, the scatter of PL lifetimes increases with increasing aggregate size. This effect can be explained by interchain EET and luminescence quenching (10). In an aggregate, the probability of generating a fluorescence quencher such as a hole polaron (10) is greater than in an isolated chain because more molecular units are involved in absorption and longer-range charge transfer can occur, thus raising the susceptibility to exciton quenching (29, 30).

To investigate the PL quenching mechanism in the aggregates, we examined the correlation of PL intensity and lifetime for the largest aggregates (54 chains on average). To ensure complete aggregation of the chains, only spots with Fred > 0.7 were selected, marked in blue in Fig. 4D. The corresponding scatter plot is shown in Fig. 5A. Short PL lifetimes correspond to low PL intensities. In contrast, long lifetimes arise for both high and low PL intensities, corresponding to unquenched and quenched aggregate PL. Because there is an inherent distribution in aggregate size, a direct correlation between PL lifetime and intensity is masked in the statistical analysis of many single aggregates. However, this averaging is overcome by considering the temporal

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**Fig. 2.** Controlled growth of conjugated polymer aggregates from isolated single chains under different processing conditions. (A) Schematic illustrating the splitting of single-spot PL into two detection channels, for photons with λ > 532 nm (denoted Ired) and λ < 532 nm (denoted Igreen). The fraction of red emission for each single spot is defined as Fred. The acetone to chloroform ratio used for SVA of single chains is given in parentheses above each confocal scanning microscope image (B–E). The corresponding Fred values are shown in histograms below each image alongside the average number of polymer chains per aggregate.
dynamics in PL lifetime and intensity of a single aggregate, as demonstrated in Fig. 5B: a reduction in PL intensity correlates directly with a drop in PL lifetime, implying a decrease in quantum yield due to increased nonradiative rate. A large aggregate from the sample containing, on average, 54 chains was placed in the confocal excitation area, and the PL intensity, lifetime, and \( F_{\text{red}} \) values were recorded simultaneously. The PL intensity shows strong fluctuations between discrete intensity levels over timescales of seconds. Quenching events are as strong as 80% of the maximum PL intensity, and are correlated with PL lifetime, which fluctuates between \(~4.1\) ns (at maximum intensity) and \(~1\) ns (at minimum intensity). At the same time, \( F_{\text{red}} \) remains constant at \(~0.8\), implying that the spectrum and thus the coherent interchromophoric coupling does not change during dynamic PL quenching events (30–33).

The correlation between PL intensity and lifetime with increasing aggregate size implies that the fast PL decay generally seen in bulk films of PPEB-based materials (34), where coherent interchromophoric coupling induces a red shift, arises from photochemical quenchers (34, 35), which have a strong effect on fluorescence over a large area surrounding the quencher. The strong blinking observed here in multichain aggregates implies long-range interchain EET (7), which should also result in efficient singlet–singlet annihilation with subsequent single-photon emission (36). Such behavior was previously reported for highly ordered single P3HT chains in conjunction with efficient singlet–triplet annihilation (21), single cyano-substituted polyphenylene vinylene chains (37), and synthetic and natural multichromophoric light-harvesting systems (5, 36, 38–41), but has not been observed for large multichain aggregates. Interchain EET can be resolved through the photon statistics in fluorescence and quantified by the degree of photon antibunching relative to isolated chains.

**Single-Photon Emission from Multichain Aggregates.** A single chain can generally be described by a series of more-or-less strongly interacting chromophores (42, 43). Upon excitation with light, multiple chromophores can enter the excited state at once. If the chromophores are independent of each other—that is, there is no dipolar coupling or electron tunneling—multiple photons are emitted simultaneously. However, even in an unfolded chain, energy transfer between chromophores will usually occur so that one chromophore can transfer its energy to an excited state of another, even if the latter is already excited. Because double excitation of a molecular unit changes configuration coordinates, subsequent excitation above the fundamental gap dissipates energy nonradiatively. Such singlet–singlet annihilation, driven by energy transfer, leads to photon antibunching from multichromophoric systems (36, 38). The quality of photon antibunching as a function of molecular size is therefore directly related to energy transfer within the multichromophoric aggregate (21).

To reveal interchromophoric interactions, we measured the statistics of fluorescence photons by splitting the detection path onto two detectors, which yields the number of correlation events, \( N_C \), in dependence on the difference in photon arrival times, \( \Delta t \), between the two detectors, as sketched in Fig. 6B. Inset. Fig. 6A shows a typical PL transient of a short PPEB chain (40-kDa sample) with strong blinking accompanied by a gradually decaying PL intensity. Fig. 6B plots the correlation events acquired with laser pulses separated by 50 ns. The ratio of the magnitude of the central peak at \( \Delta t = 0 \) to that of the lateral peaks, \( N_C/N_L \), provides a measure for the degree of photon antibunching (depicted as blue dashed lines in Fig. 6B, E, and H). A ratio of \( N_C/N_L = 1 \) implies an infinite number of independently emitting chromophores in the excitation spot, whereas \( N_C/N_L = 0 \) corresponds to a single effective chromophore (44); in a multichromophoric aggregate such an observation translates to near-unify EET efficiency within the particle (36, 38). For the example shown in Fig. 6A, a ratio of \( N_C/N_L = 0.44 \) is determined, which approximately corresponds to two
independently emitting chromophores averaged over the entire acquisition time of 15 s (44). The NC/NL ratio for 160 single chains is plotted in Fig. 6C. The distribution is broad with a maximum around 0.3. The scatter most likely reflects the molecular weight distribution. A higher NC/NL is found for longer PPEB chains (210-kDa sample), implying more active chromophores. An intensity trace for such a molecule (Fig. 6D) leads to a ratio of NC/NL = 0.71 (Fig. 6E), with the distribution for 203 chains showing a maximum around 0.7 (Fig. 6F).

PPEB aggregates yield more surprising results. Fig. 6G shows a typical PL transient of a PPEB aggregate (~12 40-kDa polymers/aggregate), with the corresponding cross-correlation shown in Fig. 6H. Strong photon antibunching is found with an NC/NL ratio of 0.16; the object therefore closely resembles a single-photon source, although it consists of multiple chains, which by themselves do not show strong photon antibunching. The distribution of NC/NL values between particles (Fig. 6I) retains the breadth seen in isolated chains (Fig. 6C), but the absolute values are significantly reduced, with a maximum around 0.1, implying virtually perfect photon antibunching from single-chain aggregates.

### Fig. 4. Evolution of spectral characteristics from single chains to deterministic aggregates of increasing size. (A) Scatter plot of PL lifetime, τPL, and fraction of red emission, Fred, for single chains and (B–D) isolated PPEB aggregates formed by SVA with different acetone to chloroform vapor ratios, denoted in brackets. The average number of chains per aggregate is stated in each panel. The colors scale from green (isolated chain) over yellow/orange (weakly aggregated chains) to red (ordered aggregates). The red-most spots in the light blue shaded area in D were used for the further analysis in Fig. 5A.

### Conclusions

Based on these observations, we draw the following conclusions: (i) Slow aggregation by SVA leads to highly ordered aggregates in which coherent coupling between single chains evolves (Figs. 2 and 3). (ii) This coupling can best be described in the context of the formation of an excited state involving multiple chromophores with excimer-like emission of substantial oscillator strength, and leads to a strong red shift in PL and a decrease in radiative rate (Figs. 3 and 4). (iii) The coherent coupling between at least two chromophores along with the high degree of structural ordering in the multichain aggregates promotes effective EET, which does not occur at the single-molecule level (Figs. 4 and 5). (iv) EET is so effective that tens of chains couple together to behave as a single quantum emitter (Fig. 6). (v) The formation of quenchers becomes more likely with increasing aggregate size, opening up additional nonradiative decay channels observed by a reduction in the PL lifetime (Fig. 5). This effect is the likely reason why the long PL lifetime, reported here for single aggregates, is not observable in bulk PPEB-based films (34) even though the emission spectra are very similar. Single-aggregate spectroscopy of conjugated polymers can therefore bridge the gap between isolated chains and bulk films, revealing mesoscopic interactions which are not apparent in both extreme states of the material. Unexpected phenomena such as deterministic single-photon emission evolve in this mesoscopic size regime, provided chain ordering is well controlled. The strong spectroscopic differences between single chains and aggregates provide a unique observable for studying nucleation and crystallization pathways of conjugated polymers in situ, opening new experimental routes to polymer physics in general. Finally, we stress that our approach to controlling morphology of single emitters in situ is applicable to any form of emitter, be it a colloidal quantum dot or a phosphorescent molecule. The recent interest in the surprising morphology and counterintuitive orientational anisotropy of triplet emitters in organic light-emitting diodes (45), which controls light out-coupling efficiency, will provide a rich environment for applying the techniques presented here.
Fig. 6. Photon antibunching from single multichain aggregates due to efficient interchain energy transfer and singlet–singlet annihilation. Typical PL intensity transients (blinking) are shown on the left for short (~40 kDa, A) and long (~210 kDa, D) single PPEB chains, and aggregates consisting, on average, of 12 polymers (G). (B, E, and H) The corresponding photon statistics in emission are shown in terms of the correlation events, N, of two photodetectors in the emission pathway. The molecules were excited by laser pulses (20-MHz repetition rate), allowing for the difference Δτ in photon arrival times between the two detectors to be controlled. The ratio between the signal of the center peak, Nc, and lateral peaks, Nl, is stated in each panel, which specifies the contrast of photon antibunching. (C, F, and I) Histograms of Nc/Nl values for the three samples measured over 160, 203, and 176 spots, respectively.

Methods

Sample Fabrication. PPEB was synthesized as described in detail elsewhere (20), and purified using a gel-permeation chromatograph to obtain two samples with a number average molecular weight Mn = 40 kDa with a PDI of 1.46 and Mn = 210 kDa with a PDI of 1.47. PMMA (Mn = 46 kDa, PDI = 2.2) was purchased from Sigma-Aldrich. Isolated chains of PPEB molecules were embedded in a PMMA host matrix by dynamically spin-coating from toluene on glass coverslips, which were cleaned according to a published procedure (4). The PMMA film thickness was 200–250 nm, and the concentration of PPEB in solution before spin-coating was ~10−12 mol l−1 and ~10−11 mol l−1 for the single-molecule and aggregate samples, respectively. The samples were incorporated into a gas flow cell and annealed under solvent vapor with different acetone to chloroform ratios for 30 min to prepare differently sized aggregates. Details of the SVA process for aggregation can be found in ref. 7.

Scanning Confocal Microscope. The samples were investigated with a scanning confocal microscope based on an Olympus IX71 (14). Excitation was carried out by a fiber-coupled diode laser (PicoQuant, LDH-C-D-405) at 405 nm under pulsed excitation with a repetition rate of 20 MHz for photon statistics measurements or 40 MHz for PL lifetime measurements. The excitation light was passed through a clean-up filter (AHF Analysentechnik, HC Laser Clean-up MaxDiode 405/10) and expanded and collimated via a lens system to a beam diameter of ~1 cm and coupled into an oil-immersion objective (Olympus, UPLSAPO 60XO, N.A. = 1.35) through the back port of the microscope and a dichroic mirror (AHF Analysentechnik, z532rdc) and detected time-correlation single-photon counting module (TCSPC, PicoQuant GmbH, HydraHarP 400) for separating single chain and aggregate emission (Fig. 2). The images were evaluated by a home-written LabVIEW software capable of automatically detecting single spots for which the fraction of red emission, Fred, was calculated and simultaneously the PL lifetime was extracted (Fig. 4). Alternatively, the fluorescence signal was split by a 70/30 beam splitter to simultaneously detect 30% of the PL on an APD (Micro Photon Devices S.r.l., PDM Series) connected to the TCSPC unit and 70% on a spectrometer (Andor Technology plc., SR-303I-B) coupled with a CCD camera (Andor Technology plc., DU401A-BV) to obtain PL decays and spectra (Fig. 3 D and E) from spots which were subsequently placed inside the excitation focus. For photon statistics measurements the fluorescence signal was split by a 50/50 beam splitter and detected by two APDs both connected to the TCSPC unit to record time-tagged photon arrival times, which were further analyzed by a home-written LabVIEW program.

Excitation Polarization Spectroscopy. The same microscope was used in wide-field excitation mode for the excitation polarization measurements, which are shown in Fig. 3 A–C. Details can be found in ref. 14. The fluorescence signal passed through an additional filter (AHF Analysentechnik, Edge Basic LP 532 long-pass filter) to select only aggregates emitting above 532 nm for the histogram shown in Fig. 3C.

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