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Excitonic coupling directly revealed on single chains of polyfluorene by
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Abstract

Molecular aggregates were discovered in 1930's, yet, the forces and excitonic coupling energy associated with the aggregate formation have not been detected so far. We directly measure such force and energy on single chains of the conjugated polymer polyfluorene using atomic force and fluorescence microscopes. The polyfluorene chain is attached on either side to a substrate and an AFM tip, respectively, and mechanically stretched under intense laser irradiation. The force – extension curves show force peaks that are attributed to gradual unfolding of the chain. Upon the irradiation, neighboring conjugated segments interact via excitonic coupling when in contact and experience an attractive force which is detected by the AFM. Analysis of the force curves provides excitonic coupling energy which is of same order as theoretically calculated values for a face-to-face fluorene dimer, and in agreement with the energy obtained from single-chain fluorescence spectra. Apart from contributing an essential piece of knowledge in the field of molecular photophysics, the work demonstrates on molecular scale a novel energy conversion mechanism from light to mechanical energy which could be potentially used, e.g., as a driving mechanism for molecular motors.

The exceptional photophysical properties of molecular aggregates continue to fascinate scientists since their discovery^{1,2}. Upon mixing in solutions under specific conditions, certain organic dyes can form both J- and H-aggregates, characterized by varying color and distinct spectral and photophysical features³⁻⁵. Over the decades, great effort has been spent on synthesis of novel dyes and utilization of the unique properties of molecular aggregates for functional materials^{6,7}. The phenomenon is not restricted to aggregation of small-molecule dyes but has been also observed to cause some of the characteristic properties of light absorbing and emitting conjugated polymers⁸. The signature spectral features of molecular aggregates, the bathochromic (for J-aggregates) and hypsochromic (for H-aggregates) absorption shifts, have been successfully explained by the dye geometrical arrangement in Kasha's theory³. The theory itself has roots in the concept of molecular excitons⁹ and its application in molecular crystals¹⁰. The spectral shifts are explained due to splitting of the excited state of the aggregate caused by dipole-dipole interaction between transition dipole moments of neighboring molecules. The amount of the splitting, which provides the exciton coupling energy, depends on the distance between the molecules, their arrangement and on the magnitude of their transition dipoles. The arrangement of the molecules also determines the allowed or forbidden character of optical transitions into the split states, which in turn determines the H- or J- character of the aggregates. While the Kasha's theory is relatively simple, it has been successfully used to qualitatively describe experimental results on a range of molecular structures over the decades. Still, there has been so far no direct measurement of the energy associated with the exciton coupling, other than by spectroscopic means.

The dipole-dipole interaction between two neighboring molecules is an attractive one, leading to a strongly bound pair in the lower energy level of the split excited state.

Separating such bound state under illumination therefore requires mechanical force that can be, in principle, provided and measured by atomic force microscopy (AFM). The feasibility of such approach has been shown theoretically for force spectroscopy on dimers of perylene and terylene derivatives which were estimated to require forces on the order of pN to tens of pN to break the excitonic bond¹¹. Experimentally, force spectroscopy based on stretching of single macromolecular chains with an AFM cantilever has become an established technique that has been used to study conformational changes, nanorheology, mechanical dynamics and function of DNA, proteins as well as synthetic polymers¹²⁻¹⁸. Further, the single-chain force spectroscopy has been combined with optical microscopy to demonstrate an optomechanical cycle on photochromic dyes¹⁹ or to study and manipulate protein conformations^{20,21}.

Synthetic conjugated polymer chains in compact conformations or in films often exhibit spectral properties that can be explained as a result of interactions between individual conjugated segments of the chain. Conjugated segments take a role of individual chromophores and interaction between their transition dipole moments gives rise to phenomena that are well known for aggregates of small molecules, including J- or H-aggregation or excimer formation²²⁻²⁵. A good example is the conjugated polymer polyfluorene which is known for its outstanding optoelectronic properties including bright blue emission in solution^{26,27}. Under specific conditions the blue emission can be accompanied by a green emission band, the origin of which has been a subject of longstanding debates²⁸, but which has recently been shown, on single-molecule level, to arise from intra-chain aggregates^{29,30}.

Here, we directly measure the excitonic coupling energy associated with molecular aggregates on single polyfluorene chains using a combination of force spectroscopy and

fluorescence microscopy. The polyfluorene chain is mechanically stretched by an AFM cantilever from a self-folded conformation into completely stretched state, and the stretching is accompanied with intense laser irradiation close to the polyfluorene absorption peak. Under such conditions, the force curve of the chain unfolding shows small force peaks that are attributed to excitonic coupling of an intra-chain H-aggregate.

Single PFO chain stretching in dark

Single chain force spectroscopy was performed with poly(9,9-dioctylfluorene) (PFO) terminated at both ends with amino groups. The amino groups react on either ends with epoxy groups on the surface of silane-functionalized quartz substrate and silicon cantilever to form covalent bonds (Fig. 1a). Immobilization of individual amino-terminated PFO chains on the functionalized surface was confirmed by single-chain fluorescence microscopy and spectroscopy (Supplementary Information, Supplementary Fig. 1 and 2). The force experiments are carried out in toluene using an AFM head placed on top of an inverted fluorescence microscope. Typical force spectra (force – extension curves) in Fig. 1b, c show single force peaks with a magnitude of several hundred pN, indicating that the amino-terminated PFO chains are correctly picked up by the functionalized AFM cantilever and extended to full length before the bond breakage. The force spectra are well fit with a modified freely jointed chain model (m-FJC)³¹ which accounts for the chain elasticity. The extension length at the bond breakage, the rupture length, corresponds to the length of a fully stretched polymer chain. The rupture lengths of 51 single PFO chains plotted in a histogram in Fig. 1d have an average value of 80.7 nm, longer than 60 nm of the full length of the PFO chain as estimated from the molecular weight and quantum chemical size of a fluorene monomer.

Fig. 1. Experimental force spectroscopy on single PFO chains under laser illumination. **a**, Chemical structure of the amino-terminated PFO and scheme of the force spectroscopy measurement. **b**, **c**, Force – extension curves of single PFO chains measured without the presence of light. The red lines are fits using the m-FJC model. **d**, Distribution of rupture lengths (lengths of fully stretched chains at bond breakage). **e**, **f**, Same as **b**, **c**, measured under illumination of 375 nm laser with power of 14.2 W.cm⁻²; the fits involve the origin of the stretching curves. **g**, Distribution of the peak values of the small force peaks appearing only under laser illumination, for two different laser powers as indicated in the legend.

The larger average rupture length value indicates that longer polymer chains are more easily picked up by the AFM cantilever because the amine-terminated free end of the polymer chain diffuses in a hemisphere whose diameter corresponds to chain length, and a larger hemisphere increases the reaction probability.

Single PFO chain stretching under illumination

Typical force spectra in the presence of 14.2 W.cm^{-2} of 375 nm laser illumination are shown in Fig. 1e, f. The main force peaks are similar to those obtained without the illumination, including good fits with the m-FJC model. The main difference is the appearance of additional small force peaks in the initial stages of the stretching. These small force peaks appear *only* under the laser illumination, as verified on 55 single PFO chains under illumination vs. 51 single chains stretched in dark. A histogram of the magnitude of the small force peaks in Fig. 1g has an average value of $47 \pm 2 \text{ pN}$, an order of magnitude agreement with the theoretically predicted value for excitonic coupling in perylene dimers¹¹. To further confirm the light-induced origin of the small force peaks, we performed the same experiment with reduced light intensity of 5.6 W.cm^{-2} . The average force value obtained from the histogram of 40 single chains (Fig. 1g) is $29 \pm 2 \text{ pN}$. While not decreasing proportionally with the light intensity, this results still verifies the light-induced nature of the phenomenon. The lack of proportionality is likely caused by the inability to detect small force peaks under noise reaching up to 10 pN.

Analysis of the coupling energy

The force spectroscopy results are further analyzed and interpreted in terms of excitonic coupling between conjugated segments of a folded PFO chain, as schematically shown in

Fig. 2a. In the initial phases of stretching under illumination, two conjugated segments are coupled face-to-face in H-aggregate manner. This excitonic coupling is broken as the chain is lifted by stretching (Fig. 2b), resulting in the appearance of the small force peak (Fig. 2c). Integration of the force-extension curve provides energy associated with the excitonic coupling. Further, the extension length along the force peak is related to the coupling length (shown in Fig. 1b). Both quantities were analyzed on an ensemble of 55 single PFO chains and are shown in the histograms in Fig. 2d, e. Peak value of the coupling energy distribution is 0.54 ± 0.08 eV. To evaluate the appropriateness of this number and to facilitate comparison with theoretical calculations or with spectroscopic data, this value has to be corrected so that it corresponds to coupling energy between two conjugated segments, one of which is in the excited state at all time. The laser light used in this work is a picosecond pulsed source. The average number of conjugated segments excited by one laser pulse can be estimated using the pulse energy and average absorption cross section to obtain 1.6 conjugated segments per pulse. The absorption can happen anywhere on the chain but the excitation energy is quickly funneled to the lowest energy site which is the aggregate. Further, the laser repetition rate of 80 MHz results in a laser pulse arriving every 12.5 ns. Excited state lifetime of the green band emission of PFO has two components, a shorter one of 1.4 ns which is spectrally centered around 500 nm, and a longer one of 5.1 ns which spectrally corresponds to a region between 520 and 575 nm³⁰. Considering the longer component as representing the weakly emissive H-aggregate, we use the 5.1 ns and calculate that the aggregate excited state is populated on average 41% of the time.

Fig. 2. Analysis of light-induced force peaks from force-extension curves. **a**, Schematic illustration of the initial phases of stretching of a folded PFO chain, with the part of the chain forming an aggregate highlighted in orange. **b**, Schematic illustration of breaking of the aggregate by the chain stretching, depicting the coupling length. **c**, Detail of the light-induced small force peak, showing schematically the coupling energy and length parameters. **d**, Distribution of the excitonic coupling energy obtained under illumination with 14.2 W.cm^{-2} from 55 single PFO chains. The red line is a Gaussian fit of the distribution. **e**, Distribution of coupling lengths from the same 55 PFO chains, with the black line is a Gaussian fit of the distribution. **f**, Correlation between the distributions in **d** and **e**.

Taking the two correction factors into account, we can estimate that the excitonic coupling energy of one conjugated segment is $(0.54 \text{ eV}/1.6)/0.41 = 0.82 \text{ eV}$. This value corresponds to the peak of the coupling energy distribution in the Fig. 2d. The distribution itself is broad with a long tail towards high energy values. Such distribution is caused

mainly by the distribution of the PFO molecular weights (with the polydispersity index of 1.61) which results in larger absorption cross-sections and larger numbers of conjugated segments excited in the longer chains.

Distribution of the coupling lengths (as defined in the Fig. 2c) has a peak at 4.9 ± 0.3 nm. Considering the size of a fluorene monomer, this corresponds to about 6 monomer units. This is a very reasonable number since the conjugated segment length of glassy-phase PFO is about 8 monomers³², and the measured coupling length provides a lower limit of the actual length of the segment that took part in the aggregate, as seen in Fig. 2c. The measured coupling energy and coupling lengths are generally well correlated, as shown in Fig. 2f. Such correlation can be expected, since longer contact length should lead to higher energy. The deviations observed for several points in the longer lengths region are probably caused by geometry factors (distance or orientation) that result in weaker coupling.

Comparison with quantum chemical calculations and single-chain fluorescence

Feedback on the values obtained from the force spectroscopy can be provided by quantum chemical calculations and by spectroscopic data. The Fig. 3a shows the molecular aggregate energy scheme as introduced in the Kasha's model. Splitting of the S_1 energy level provides the excitonic coupling energy which can be also obtained from a spectral shift between monomer and aggregate fluorescence bands. An example of a fluorescence spectrum of a single PFO chain measured in softened polystyrene matrix during solvent vapor annealing³³ is shown in Fig. 3b. Under such conditions, the PFO spectrum shows dynamic changes between the monomer spectrum peaked around 420 nm and the green band between 530 and 580 nm, due to the chain conformational dynamics³³.

Fig. 3. Spectroscopic and quantum chemical determination of excitonic coupling. **a**, Schematic illustration of energy level splitting upon aggregation showing the exciton coupling energy. **b**, Fluorescence spectrum of a single PFO chain measured in polystyrene matrix during solvent-vapor annealing with toluene. **c**, **d**, Geometrical arrangement of face-to-face stacked fluorene dimer used in the coupling energy calculations. **e**, Coupling energy as a function of the distance d calculated for the dimer in **c**, **d** using the atomic transition charges method.

The particular spectrum in Fig. 3b shows contribution from both forms, and enables an estimation of the excitonic coupling energy as the difference between the maxima of the two peaks at 420.4 and 563.5 nm, respectively. Such coupling energy is 0.74 eV, and given the broad range of the green band, the energy can vary between 0.6 and 0.8 eV. These values are in very good agreement with the 0.82 eV energy obtained from the force spectroscopy.

Theoretically, the excitonic coupling in molecular aggregates can be separated into Coulomb interactions which are the dominant contributions at large and intermediate intermolecular distances and short-range interactions related to molecular orbitals overlap³⁴. For large distances the Coulombic contribution can be approximated by interaction between point transition dipoles. For distances comparable to the molecular size the contribution is more precisely calculated considering interactions between individual charges located on atoms that contain the π electrons involved in the optical transitions³⁵ (atomic transition charges, ATC). Since, generally, the excitonic coupling is sufficiently well described by the Coulombic contribution³⁴, we follow a previous approach¹¹ and use the ATC method to calculate the excitonic coupling energy of a face-to-face stacked fluorene dimer as a function of the intermolecular distance. The geometrical arrangement is shown in Fig. 3c, d, and results of the calculation for the S_0 - S_1 transition using DFT and TD-DFT at the B3LYP/6-31G++(d,p) level are shown in Fig. 3e. The calculated coupling energy of a dimer is multiplied by 8 in the Fig. 3e to account for the average length of the PFO conjugated segment³². For a realistic range of intermolecular distances between 2 Å and 8 Å the calculated coupling energy ranges from 0.46 eV to 0.023 eV. For the shorter distances in this range, these values are of the same order of magnitude as the excitonic coupling of 0.82 eV obtained from the force

spectroscopy. Deviations from the geometry shown in Fig. 3c, d lead to reduced coupling energy, as shown in the calculation results in Supplementary Fig. 3 and Supplementary Fig. 4. Such effects could be responsible for the deviation from linearity observed in Fig. 2f. A lack of still better agreement between the above calculated and experimental results could be caused by a non-negligible contribution of the short-range interactions or by the size of conjugated segments which could be larger than the 8 monomer units³⁶.

Conclusions and outlook

Strong light-induced coupling between molecules in molecular aggregates resulting in an attractive force has been known for decades but direct demonstration of the coupling strength by mechanical means has not been attempted or achieved so far. We succeeded in such demonstration not only qualitatively using atomic force microscopy but to a large extent also quantitatively using single-molecule spectroscopy and quantum chemical calculations as a reference. The results contribute an important piece of knowledge in the field of molecular photophysics, and will have an impact in related areas as well. In addition, the work demonstrates on molecular scale a novel energy conversion mechanism from light to mechanical energy, and this fact could have implications, e.g., in development of driving mechanisms of future molecular motors.

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Methods

1. Synthesis of the amino-terminated PFO

1.1. Synthesis of 2-(7-bromo-9,9-dioctyl-9H-fluorene-2-yl)-4,4,5,5-tetra-methyl-1,3,2-dioxaborolane

Under N₂ atmosphere, 2,7-dibromo-9,9-dioctyl-9H-fluorene (2.02 g, 3.7 mmol) was dissolved in 20 mL THF and cooled to -78 °C. BuLi (2.4 mL, 6.0 mmol) was added dropwise to the solution and stirred for 1 hour. Then, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.8 mL, 3.9 mmol) was added dropwise, the reaction mixture was heated to room temperature and kept for 24 hours. Subsequently, the organic layer was washed with saturated NH₄Cl aqueous solution and dried with MgSO₄. The solvent was evaporated and the crude product was purified by column chromatography using a mixed solvent of hexane/CH₂Cl₂ (v/v = 4/1). 0.90 g colorless oil (**1**) was obtained as the product with yield of 41 %.

¹H-NMR (CDCl₃, 300 MHz): 7.43-7.81 (m, 6H), 1.85-2.02 (m, 4H), 1.39 (s, 12H), 0.94-1.28 (m, 20H), 0.82 (t, J = 6.8 Hz, 6H), 0.49-0.62 (m, 4H). MS: m/z 594.50 (calc. 595.495).

1.2. Synthesis of nitro-terminated PFO (NO₂-PFO-NO₂)

Pd(dba)₂ (10 mg, 0.01 mmol), tert-Bu₃P (26 mg, 0.06 mmol) and 1-bromo-4-nitrobenzene (18 mg, 0.045 mmol) were dissolved in THF. The solution was stirred for 2 h in N₂ and used as an initiator (**2**). In another flask, **1** (0.28 g, 0.47 mmol) was dissolved in THF (5.4 mL) and 2 M aqueous solution of K₃PO₄ (0.5 mL) in N₂. Initiator solution was added to this mixture at 0 °C to initiate polymerization. After 1 h, 4-nitrophenylboronic acid (82.4 mg, 0.49 mmol) was added to the reaction mixture for end capping. The product was extracted 3 times with CH₂Cl₂. Extracted organic layers were combined, washed with brine and dried with Na₂SO₄. Na₂SO₄ was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was dissolved in a minimum amount of THF and added dropwise to methanol with stirring. The precipitate was collected by filtration (0.1 g, yield: 36 %). The product (**3**) was further purified by HPLC (0.05 g, yield: 18 %).

¹H-NMR (CDCl₃, 400 MHz): 0.81 (m, 10H), 1.14 (m, 20H), 2.12 (br, 4H), 7.68-7.70 (m, 4H), 7.83-7.85 (d, 2H). GPC: M_n = 27650, M_w / M_n = 1.61.

1.3. Synthesis of amino-terminated PFO (NH₂-PFO-NH₂)

Compound **3** (20 mg, 0.7 μmol) was dissolved in THF and cooled to 0 $^{\circ}\text{C}$. Excessive amount of NaBH_4 was added to this solution. Then, the reaction mixture was heated to room temperature and stirred for 6 h. After filtration, organic layer was extracted with CH_2Cl_2 and dried with Na_2SO_4 . The organic solvent was removed under reduced pressure and the residue was purified by the reprecipitation method with CH_2Cl_2 /methanol (15 mg, yield: 75 %).

2. Sample preparation

2.1. Functionalization of quartz substrate

First, quartz substrate was sonicated two times in ethanol for 20 min and transferred into alkaline solution ($\text{NH}_4\text{OH}/\text{H}_2\text{O}_2/\text{H}_2\text{O}$, 1:1:5 v/v) for 1 h. To obtain -OH rich surface, quartz substrate was soaked in piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, 7:3 v/v) for 30 minutes. The piranha solution was then completely removed by rinsing with water. To functionalize the surface with epoxy groups, the cleaned quartz substrate was incubated overnight in 3-glycidyloxypropyltrimethoxysilane (TCI) and thoroughly rinsed with ethanol and toluene.

2.2. Immobilization of $\text{NH}_2\text{-PFO-NH}_2$ on a quartz substrate

$10^{-6} \sim 10^{-8}$ M of amino terminated PFO ($\text{NH}_2\text{-PFO-NH}_2$) in THF solution was

dropped onto the functionalized substrate. After THF was completely dried, the substrate was rinsed with toluene to remove the unreacted polymer chains and dried with N₂ gas.

2.3. Functionalization of AFM cantilever

AFM cantilever was functionalized in almost the same way as the quartz substrate. First, the cantilever was immersed in ethanol for 1h and transferred into alkaline solution (NH₄OH/H₂O₂/H₂O, 1:1:5 v/v) for 1 h. Then, the cantilever was soaked in piranha solution (H₂SO₄/H₂O₂, 7:3 v/v) for 30 minutes and thoroughly rinsed with water. Finally, the cleaned cantilever was incubated overnight in 3-Glycidyloxypropyltrimethoxysilane (TCI) and thoroughly rinsed with ethanol and toluene.

3. Combined confocal and atomic force microscopic setup

The single molecule and AFM measurements were carried out using a home-built setup that combines an inverted fluorescence microscope (IX 73, Olympus) with an AFM head (MFP-3D-SA, Asylum Research). The AFM cantilever used was HQ:CSC38/Al BS (MikroMasch). Nanofishing experiments were performed in toluene. The sample was excited with a 375 nm laser (LDH-D-C375, Pico Quant). Fluorescence from the sample 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). The objective lens was mounted on a three-axis piezo stage (PS3L60-030U, NC3311, Nanocontrol) to accurately align its center position with respect to the AFM tip. The fluorescence signal was detected by an electron-multiplying charge-

coupled device (EMCCD) camera (iXon, Andor Technology) equipped with an imaging spectrograph (CLP-50LD, Bunkou Keiki) to measure fluorescence spectra. The integration time and gain of EMCCD were 0.2 s and 300, respectively.

4. Theoretical calculation of excitonic coupling between two PFO segments

Excitonic coupling J is calculated following the same way as reported recently for simulation of force spectroscopy of PDI molecule dimer¹¹. The Coulombic interaction is calculated as the interaction between atomic transition charges (ATCs). ATCs for p -th atom in the k -th excited state was expressed as

$$q_p^k = \sqrt{2} \sum_b^{N_{AO,p}} \sum_c^{N_{AO}} \sum_j^{unocc} \sum_i^{occ} A_{ij} C_i^{pb} C_j^c S_{bc} \quad (4-1)$$

where i is the index of occupied molecular orbital (MO), j is the index of virtual unoccupied MOs, b and c are indexes of atomic orbital basis function, N_{AO} is the total number of atomic orbitals, $N_{AO,p}$ is the total number of atomic orbitals of p -th atom, A_{ij} is the CI coefficient, C_i^{pb} is the atomic orbital coefficient b of molecular orbital i of p -th atom, C_j^c is the atomic orbital coefficient b of MO j and S_{bc} is the overlap matrix between the AO basis functions b and c . The excitonic coupling J_c was calculated using the equation (4-2)

$$J_c = J_c^J = \sum_{IJ} \frac{q_i q_j}{4 \pi \epsilon_0 r_{ij}} \quad (4-2)$$

where r_{ij} is the distance between atom i and j of molecule I and J and ϵ_0 is the vacuum permittivity. The ATCs of each atom were calculated from isolated fluorene monomer for S_0 - S_1 transition using DFT and TD-DFT at B3LYP / 6-31G++(d,p) level. Because the effective conjugation length of PFO is 8 monomer units³², the calculated J_c between the two fluorenes was multiplied by the factor of 8.

Data availability

Data available on request from the authors.

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Author contributions

M.V. and K.N. conceived the idea, designed the research and discussed the results. T.N. synthesized the polymer and performed the optical and AFM experiments. X.L. assisted in the AFM experiments and discussed the results. S.O. and T.N. carried out the quantum chemical calculations. T.N. analyzed the results. T.N. and M.V. wrote the paper.

Competing interest

The authors declare no competing interests.

Additional information

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