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Direct Observation of Dynamic Behavior of Single Molecules of a Linear π-Conjugated Polymer with a Total Internal Reflection Fluorescence Microscope

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We have synthesized a novel linear π-conjugated rigid-rod polymer *via* cross-coupling reaction of 9,10-dibromoanthracene and 1,4-diethynylbenzene with [Pd]-CuI catalyst system. And we have measured the fluorescence images of the single molecules of novel π-conjugated polymer on slide glass surface using a total internal reflection fluorescence microscope (TIRFM). The intensity of a fluorescence spot of single polymer molecule dropped to baseline in a single-step manner due to desorption, confirming that each single spot is arising from a single fluorophore. It is the first observation of such dynamic behavior of rigid-rod linear π-conjugated single polymer chains. The TIRFM is convenient and powerful tool for characterization of π-conjugated polymers.

KEYWORDS: Single Molecule Detection, π-Conjugated Polymer, Near-field, Fluorescence Image, Optical Microscopy

Introduction

During the past decade the number of π-conjugated polymers investigated as advanced materials for electronic and photonic applications has developed rapidly, and has caused an over increasing interest from both academic and industrial research laboratories.[1] The inherent synthetic flexibility, potential ease of processing, and the possibility of tailoring characteristic properties to accomplish a desired function makes them promising candidates for manifold applications in materials science. Thus, they are used as laser dyes,[2] scintillators,[2] light-emitting diodes,[3] piezoelectric and pyroelectric materials,[4] photoconductors,[5] and are investigated for optical data storage,[6] optical switching and signal processing,[7] as well as in nonlinear optical applications.[8-10] Although π-conjugated polymer is very interesting material as stated above, the mechanism of these electro- and photo-function are still not clear owing to complex structure of the polymer.

Therefore, we thought that if direct observation of single molecule of π-conjugated polymer were achieved, the relationship of polymer structure and the function would be clear.

Recently, imaging of single protein molecules using fluorescence microscopy has made remarkable progress after development of single fluorophore imaging methods which were used for specimen on an air-dried surface.[11] Single fluorophores in aqueous solution have been imaged using a total internal reflection fluorescence microscope, in which the background fluorescence is greatly reduced;[12] when a laser beam is totally reflected in the interface between the glass surface and solution, illumination is limited to only the vicinity near the glass surface.

Experimental Section

Materials

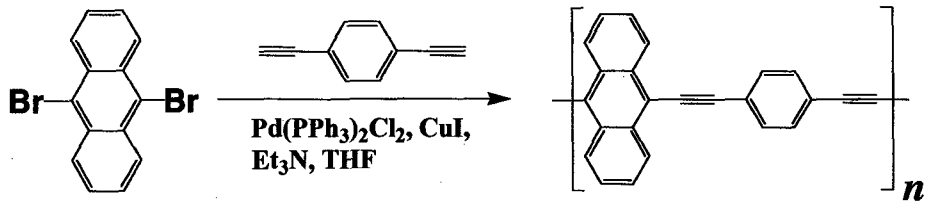
Triethylamine was distilled over calcium hydride. 1,4-Diethynylbenzene (Tokyo Kasei), 9,10-dibromoanthracene (Tokyo Kasei), and the catalyst system were commercially available.

Synthesis

The following reaction procedures were conducted under dry nitrogen according to the reaction conditions of ref. 13.

Polycondensation [Scheme 1]

1,4-Diethynylbenzene (582mg, 4.61mmol) and 9,10-dibromoanthracene (1.55g,



Poly(AEPE)

Scheme 1. Synthesis of Poly(9,10-anthracenediyl-ethynylene-1,4-phenylene-ethynylene) [Poly(AEPE)].

4.61mmol) were dissolved in triethylamine (200ml) and tetrahydrofuran (20ml), and triphenylphosphine (550mg, 2.10mmol), cuprous iodide (275mg, 1.45mmol), and bis(triphenylphosphine)palladium (II) chloride (298mg, 0.425mmol) were added to this solution. After stirring for 24 h at the reflux temperature, the precipitated salt, triethylammonium iodide, was filtered at room temperature, and was rinsed with ethyl ether. The filtrates and the ether were combined and the solvent was evaporated to dryness. The residue was dissolved in a minimum amount of chloroform and the solution was poured into methanol (200 ml) to form a precipitate which was separated by centrifugation. The resulting polymer was dried under vacuum to give Poly(AEPE) as a red-brown solid. Yield: 95.0%; GPC: M_w=6.75x10^3, M_w/M_n=3.45 (as Polystyrene Standard). Fluorescence spectrum is shown in Figure 1.

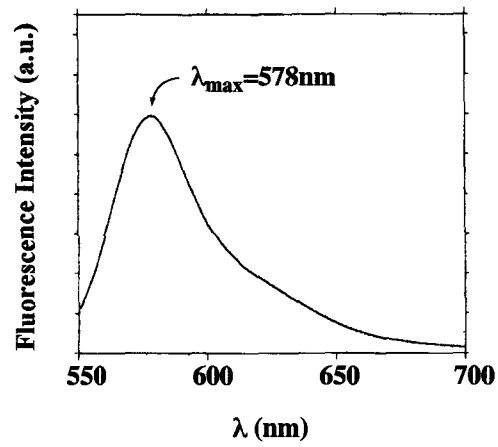


Figure 1. Fluorescence Spectrum of Poly(AEPE) in THF at r.t. λex=532nm.

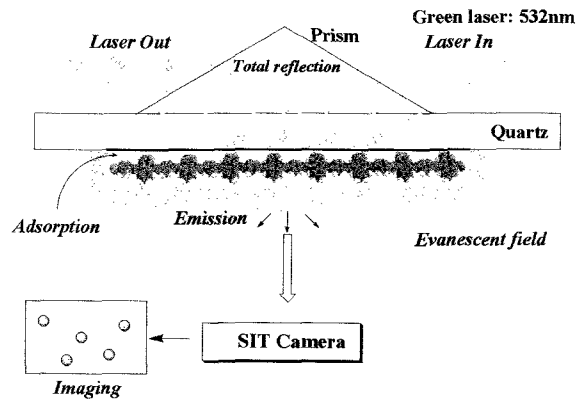


Figure 2. Outline of a Total Internal Reflection Fluorescence Microscopy.

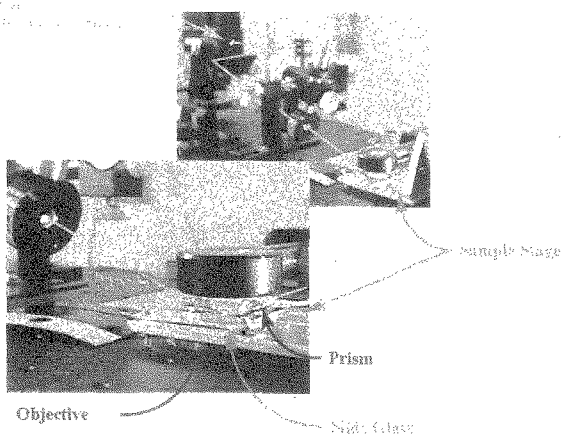


Figure 3. A Part of a Total Internal Reflection Fluorescence Microscope (TIRFM).

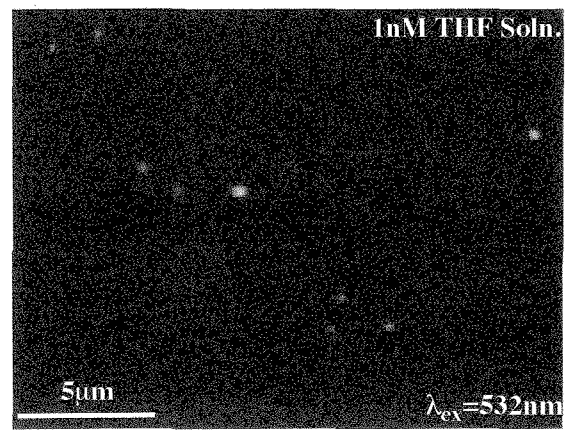


Figure 4. Fluorescence Image of Individual Poly(AEPE).

Instrumentation of TIRFM

A spectroscopic device was incorporated into a total internal reflection fluorescence microscope (TIRFM, Figure 2 & 3).[12] The linearly-polarized 532-nm-line output of a CW laser was used. After passing through a focusing lens and a cubic prism, the laser beam was totally reflected. An evanescent field was produced with a penetration depth of about 100nm. The illumination area was 250 x 110 μm^2 ($1/e^2$ value) at the specimen plane. The fluorescence emission from the specimen was collected with an oil-immersion microscope objetive (1.40 NA, 60x, PlanApo; Nikon, Tokyo, Japan).

For imaging, the fluorescence was filtered by a barrier filter (570DF30; Omega Optical, Brattleboro, VT, USA) and then focused by a relay lens onto the faceplate of image intensifier (Model VS4-1845; Video Scope International, Sterling, VA, USA) coupled to a SIT camera (C2400-08; Hamamatsu Photonics, Shizuoka, Japan). The images were recorded on a video cassette recorder.

Results and Discussion

Fluorescence images for Single Poly(AEPE) Molecules.

After poly(AEPE) was adsorbed onto the surface of a slide glass, the fluorescence images were obtained with the total internal reflection fluorescence microscope. Single

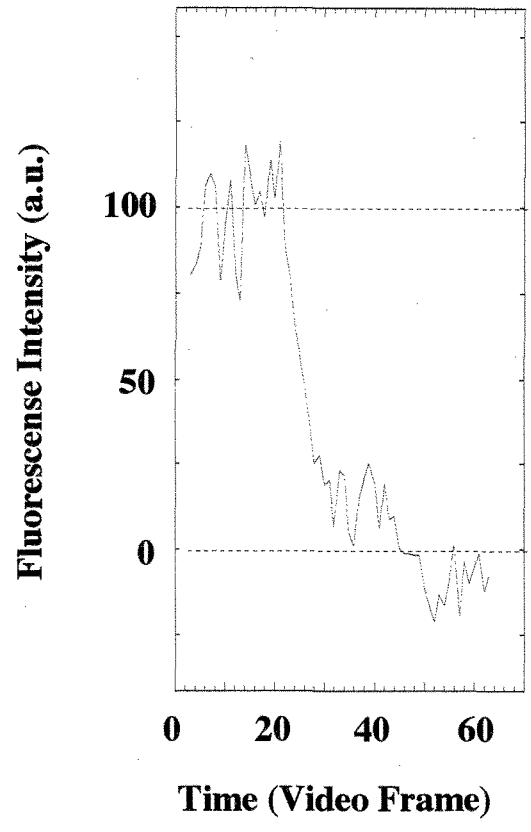


Figure 5. Dynamic Behavior of a Fluorescence Image of Poly(AEPE) Single Molecule under THF observed at the Video Rate (1 Frame=1/30 sec).

poly(AEPE) molecules were observed as fluorescence spots (Figure 4). A typical time course of the fluorescence intensity of an individual spot is shown in Figure 5. After the irradiation of the laser beam for a certain period of time, the fluorescence intensity dropped to baseline in a single-step manner due to desorption, confirming that each single spot (Figure 5) is arising from a single fluorophore.[12)]

In this study, we demonstrated that in organic solution the fluorescence emission image of a single fluorophore can be measured using a total internal reflection fluorescence microscope. With single molecule measurements, we were able to determine the unique behavior of single polymer molecules, such as fluorescence spot image, which were not apparent in conventional bulk measurements.

Acknowledgement

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