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Description	

Single Molecule of a π -Conjugated Polymer Slowly Twinkles in Solution at Room Temperature

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Running head:

Single Molecule of a π -Conjugated Polymer Twinkles in Solution

Abstract:

A phenylacetylene monomer, 4-(decyloxycarbonyl)phenylacetylene (DecCPA), was synthesized and polymerized by using the combined catalyst system, i.e., [Rh(norbornadiene)Cl]₂ and triethylamine, to give a yellow solid π -conjugated polymer [poly(DecCPA)] having the high molecular weight and the cis-transoidal stereo-regular main chain. Poly(DecCPA) was measured using total internal reflection fluorescence microscopy, which is a single molecule detection technique that is carried out in tetrahydrofuran solution at room temperature, and the light-twinkling of a single molecule was observed. This twinkling is the dynamic photon emission phenomenon of single poly(DecCPA) molecule that we mention specifically as resultant from the time scale –changing very slowly in the time scale of milliseconds and seconds– with changed fluorescent intensity associated with single polymer molecule conformational changing caused by the micro-Brownian motion. Although a molecular dynamics was at the picosecond time scale generally, this poly(DecCPA) molecule had the mode of the molecular motion of π -conjugated main chain did this very slowly in a second time scale was shown.

Introduction.

What function does a synthetic polymer have in the single-molecular-level? Does it have such a dynamic multi-functions as protein which a kind of biopolymer? Mankind does not understand the fundamental properties of synthetic polymers yet and still cannot bring the maximum of capabilities out of them.

Polymer is highly capable, useful and indispensable material. Especially, conjugated polymers investigated as advanced materials for photonic and electronic applications have made progress, and has caused an ever-increasing interest from both academic research and industrial development.¹⁾ The conjugated polymers are used as laser dyes,²⁾ organic light-emitting diodes,³⁾ and so on. In addition recently, there are some applications as an optical resolution membrane materials.⁴⁻⁶⁾

Although conjugated polymers are very interesting and important materials, the mechanism in single-molecular-level of these photonic and electronic function are still not clear owing to the various and complicated structure of the conjugated system in the polymer molecules. Thus, the polymers are the complex system of molecules. Therefore, we thought that if direct observation of single molecule of conjugated polymer were achieved, the polymer function would be clear in molecular level toward the polymer

functioning at single molecule unit, that is the single-molecule device.

In the other, 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.⁷⁾ Single fluorophores in aqueous solution have been imaged using a total internal reflection fluorescence microscope (TIRFM), in which the background fluorescence is greatly reduced,⁸⁾ 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. In our previous study, this single molecules imaging method was expanded to the organic polymer solution system for the direct observation of single molecules of photofunctional rigid-rod conjugated polymer that is a poly(aryleneethynylene) having anthracene units in the main chain.⁹⁾

In this study, we report that single molecule imaging of a stereo-regular π -conjugated polymer and detection of single molecule slow-twinkling of the photoluminescence in tetrahydrofuran solution at room temperature for the first time.

Experimental Section.

Measurements.

^1H NMR spectra were measured on a Bruker DPX-400 (400 MHz). GPC was analyzed using Toso TSKgel GMH_{HR}-H(S) columns and a Shimadzu SPD-10AVP UV detector (254 nm). IR spectra were measured on a Shimadzu FTIR-4000 spectrophotometer. UV-vis. spectra were measured on a Shimadzu UV-260 spectrophotometer. Emission spectra were obtained with a Hitachi F-2500 spectrofluorophotometer.

Materials.

Dichloromethane (Kanto Chemical) was distilled over phosphorus pentachloride (Wako Chemicals). Chloroform (Kishida Chemicals) was distilled over calcium chloride (Wako Chemicals). Triethylamine (Kanto Chemicals) was distilled over calcium hydride. Tetrahydrofuran (THF, Kishida Chemicals) was distilled over sodium. 4-Bromobenzoic acid (Tokyo Kasei), 1-decanol (Kanto Chemicals), 2-methyl-3-butyne-2-ol (Lancaster), and the catalyst system were commercially obtained.

Synthesis [Scheme 1].

All the following reaction procedures were conducted under dry nitrogen.

4-Decyloxycarbonyl-1-bromobenzene (1).

To a solution of 4-bromobenzoic acid (4.27 g, 21.2 mmol) in dry dichloromethane (90 ml) was added 1-decanol (3.51 g, 22.0 mmol), followed by 4-(dimethylamino)pyridine (DMAP) (248 mg, 2.03 mmol). Dicyclohexylcarbodiimide (DCC) (4.78 g, 21.7 mmol) was added and stirred at room temperature under nitrogen until the reaction was complete. The reaction mixture was filtered to remove the precipitated dicyclohexylurea and was evaporated to dryness under reduced pressure. The crude product was purified by silica gel chromatography eluted with hexane/ethyl acetate (80/20 v/v) to give **1** (TLC $R_f = 0.78$). The product was pale-yellow liquid. Yield: 63.2%; $^1\text{H NMR}$ (CDCl_3 , TMS) δ 7.90 (d, 2H, 2CH in phenyl), 7.58 (d, 2H, 2CH in phenyl), 4.30 (t, 2H, OCH₂), 1.76 (m, 2H, -OCH₂CH₂-), 1.43 (m, 2H, CH₂CH₃), 1.27 (m, 12H, -(CH₂)₆-CH₂CH₃), 0.88 (t, 3H, -CH₃); IR (cm^{-1} , NaCl) 2930 (C-H), 1720 (C=O), 1275 (C-O).

Decyl 4-(3-hydroxy-3-methylbut-1-ynyl)benzoate (2).

In triethylamine (100 ml) were dissolved **(1)** (11.0 g, 32.3 mmol), 2-methyl-3-butyn-2-ol (5.09 g, 60.5 mmol),

dichlorobis(triphenylphosphine)palladium (25.3 mg, 3.60×10^{-2} mmol), cuprous acetate (29.1 mg, 1.60×10^{-1} mmol), and triphenylphosphine (53.6 mg, 2.04×10^{-1} mmol). The solution was stirred for 24 h at the reflux temperature. The resulting salt was removed by filtration and the solvent of the filtrate was evaporated to yield a crude product, which was purified by silica-gel chromatography using hexane/ethylacetate (80/20 v/v) as an eluent (TLC R_f = 0.49). The product was yellow viscous liquid. Yield: 98.9%; $^1\text{H NMR}$ (CDCl_3 , TMS) δ 7.97 (d, 2H, 2CH in phenyl), 7.46 (d, 2H, 2CH in phenyl), 4.31 (t, 2H, -OCH₂-), 2.04 (s, 1H, -OH), 1.77 (m, 2H, -OCH₂CH₂-), 1.63 (s, 6H, (CH₃)₂C-), 1.44 (m, 2H, -CH₂CH₃), 1.27 (m, 12H, -(CH₂)₆-CH₂CH₃), 0.89 (t, 3H, -CH₃ in decyl); IR (cm^{-1} , NaCl) 3400 (O-H), 2850 (C-H), 1720 (C=O), 1260 (C-O).

4-(Decyloxycarbonyl)phenylacetylene [DecCPA].

Sodium hydride (60 wt %, 690 mg, 17.3 mmol) was added to a toluene solution (120 ml) of **(2)** (11.0 g, 32.0 mmol), and the mixture was heated to 105 °C with stirring for 2 h. After the usual work up, the product was purified with silica-gel chromatography using toluene/hexane (50/50 v/v) as an eluent (TLC R_f = 0.50). Yellow liquid was obtained in 53.4% yield. $^1\text{H NMR}$ (CDCl_3 , TMS) δ 7.99 (d, 2H, 2CH in phenyl), 7.55 (d, 2H, CH in phenyl),

4.31 (t, 2H, OCH₂), 3.22 (s, 1H, C≡CH) 1.76 (m, 2H, -OCH₂CH₂-), 1.43 (m, 2H, -CH₂CH₃), 1.27 (m, 12H, -(CH₂)₆-CH₂CH₃), 0.88 (t, 3H, -CH₃); IR (cm⁻¹, NaCl) 3250 (H-C), 2960 (C-H), 2100 (C≡C), 1700 (C=O), 1275 (C-O); Anal. Calcd for -(C₁₉H₂₆O₂)_n: C, 79.68; H, 9.15. Found: C, 79.95; H, 9.61.

Poly[4-(decyloxycarbonyl)phenylacetylene] [Poly(DecCPA)] (4).

To the monomer (3) (172 mg, 6.01 x 10⁻¹ mmol) in chloroform (2.50 ml) was added the (bicyclo[2.2.1]hepta-2,5-diene)chlororhodium (I) dimer {[Rh(norbornadiene)Cl]₂} (0.80 mg, 1.7 x 10⁻³ mmol) in dry triethylamine (0.50 ml), and the solution was stirred for 4 h at room temperature. The polymerization mixture was poured into methanol, and the polymer was purified by reprecipitation from chloroform solution into methanol and then dried *in vacuo* for 24 h. Yellow solid was obtained in 88.4% yield. ¹H NMR (CDCl₃, TMS) δ 7.62 (br, 2H, CH in phenyl), 6.66 (br, 2H, CH in phenyl), 5.75 (br, 1H, HC=C- in main chain), 4.19 (br, 2H, -OCH₂-), 1.69 (br, 2H, -OCH₂CH₂-), 1.35-1.25 (br, 14H, -(CH₂)₇-), 0.85 (br, 3H, -CH₃); IR (cm⁻¹, KBr) 3000 (C-H), 1720 (C=O), 1277 (C-O); GPC (based on polystyrene standard): M_w = 4.23 x 10⁵, M_w/M_n = 2.80; Cis%: > 98 (from ¹H NMR measurement; see the following section); UV-vis. (7.05 x 10⁻⁵ mol/l

THF solution) $\lambda_{\max} = 395 \text{ nm}$ ($\epsilon = 3790$), $\lambda_{\epsilon>50} = 520 \text{ nm}$; Anal. Calcd for $-(\text{C}_{19}\text{H}_{26}\text{O}_2)-$: C, 79.68; H, 9.15. Found: C, 78.92; H, 9.33.

Determination of stereo-regularity in the main chain (Cis%).¹⁰⁻¹²⁾

The cis% of the configuration of the double bonds in the main chain was determined by the ratio of the integral (i.e. area) of the peak at about δ 5.7, 6.6 and 7.6 ppm in ^1H NMR spectra.¹⁰⁾ In addition, polyacetylenes involves 4 types of backbone configurations: cis-transoidal, cis-cisoidal, trans-transoidal, and trans-cisoidal.¹¹⁾ It has been shown that the poly(phenylacetylene)s prepared with $[\text{Rh}(\text{norbornadiene})\text{Cl}]_2$ possess about 100% cis-transoidal main chain structure.¹²⁾ It was found that a cis-content of this poly(DecCPA) had also the over 98% stereoregular main chain by ^1H NMR measurement.

Instrumentation of a TIRFM.

A spectroscopic device was incorporated into a TIRFM [Figure 1 *a*] based on an optical microscope (IX-70-22FL/PH, OLYMPUS, Japan).⁸⁾ The linearly polarized 441.6-nm-line 70 mW output of a helium-cadmium continuous wave (CW) laser (M.4.70, Liconix, USA) was used. After passing through a neutral density filter, a focusing lens, and a cubic prism, the

laser beam was totally reflected. The incident angle at the quartz slide-to-solution was 78 degrees to the normal, and the critical angle was 74.5 degrees. The fluorescence emission from the specimen was collected with an oil-immersion microscope objective (1.40 NA, 60 x, Plan Apo, Nikon, Japan). For imaging, the fluorescence was filtered by a barrier filter (BA475, OLYMPUS, Japan) and then focused by a relay lens onto an ICCD camera (ICCD 325, Photek, USA). The images were recorded on a video cassette recorder through an image processor (ARUGUS C5510, Hamamatsu Photonics, Japan).

Results and Discussion.

Synthesis and Characterization of Poly(DecCPA).

By using the combined catalyst system, i.e., $[\text{Rh}(\text{norbornadiene})\text{Cl}]_2$ and triethylamine, the polymerization of DecCPA monomer proceeded to give a yellow solid poly(DecCPA) having the high molecular weight and the cis-transoidal main chain controlled. Poly(DecCPA) had also an expanded conjugated high stereo-regular main chain that absorbed the wavelength of 441.6 nm as wavelength of the laser using this system, and emitted fluorescence that peak wavelength of 510 nm (Figure 2). It pointed out the

ability of application with a TIRFM as a following section.

Fluorescence Images for Individual Single Poly(DecCPA) Molecules.

After putting poly(DecCPA) THF solution (1×10^{-9} mol/l, 2.0 μ l) onto the surface of a quartz slide glass, the fluorescence images were measured with the TIRFM [Figure 1 *a*]. Fluorescence of a single molecule was very weak, in order to decrease the background luminescence, we reduced the optical excitation volume, using evanescent-field illumination of TIRFM.⁸⁾ When a laser is incident on a quartz slide-to-medium interface at above the critical angle, the light is totally and internally reflected and an evanescent field is produced just beyond the interface [Figure 1 *b*]. This evanescent field is localized near the interface with the $1/e$ penetration depth of 144 nm, depending on the incident angle of the laser. Combining the low background optics with the local excitation, the background was reduced to 1-3 photons s^{-1} per diffraction limit area, i.e. > 2000-fold lower than that for a conventional epifluorescence microscope.⁸⁾ Thus, single fluorophores, that is, single poly(DecCPA) molecules could be clearly visualized at a full-video rate (1/30 sec frame⁻¹) [Figure 3 *a*]. A typical time course of the fluorescence intensity of an individual bright spot, which is indicated the white arrow, is shown in

Figure 3 *b*. Fluorescence intensity of a single poly(DecCPA) greatly fluctuated during the first 113 frames (0 ~ 3.77 sec). After the irradiation of the laser beam for a certain period of time, that is at the 113th video frames, the fluorescence intensity dropped to baseline in a single-step manner due to desorption, confirming that each single spot is arising from a single fluorophore. The observed process was desorption of a single poly(DecCPA) molecule. Evidence that a single fluorescent spot was produced from a single poly(DecCPA) molecule with TIRFM in THF solution was obtained as follows. The poly(DecCPA) was easily dissolved in THF, and this solution was a dilute one (1×10^{-9} mol/l). Therefore poly(DecCPA) molecules were very difficult to associate under the conditions of the TIRFM measurement. Besides, the fluorescent spot was desorbed in a single step (at 113th frame in Figure 3 *b*). If the single fluorescent spot was associated with the multiple molecules, the desorption must occur the multi-steps. Furthermore, as the concentration of poly(DecCPA) increased, the number of the fluorescent spots increased whereas the fluorescence intensities distribution of the individual spots remained constant, consistent with the fluorescence spots arising from single poly(DecCPA) molecules.

In the period of the first 113 frames (0 ~ 3.77 sec in Figure 3 *b*), this twinkling is the dynamic photon emission phenomenon of single

poly(DecCPA) molecule that we mention specifically as resultant from the time scale –changing very slowly in the time scale of milliseconds and seconds– with changed fluorescent intensity associated with single polymer molecule conformational changing. This molecular dynamic photonic phenomenon, that is, changing of π -conjugated system by the conformational change in the single polymer chain was caused by the micro-Brownian motion in THF solution at room temperature having the thermal energy of $k_B T$, where k_B is the Boltzmann constant and T is the temperature. Although a molecular dynamics was at the picosecond time scale generally, this poly(DecCPA) molecule had the mode of the molecular motion of π -conjugated main chain did this very slowly in a second time scale was shown. In addition, this behavior would be unobservable in bulk studies using the technique that makes the average of large number of molecules to be a measuring sample.

This is the first report that twinkling of a single molecule of a conjugated polymer by slow conformational changing of the conjugated polymer chain was directly detected in solution at room temperature. Novel single-molecule device having dynamic photonic function such as this paper reported using the Brownian motion will be developed in the near future.

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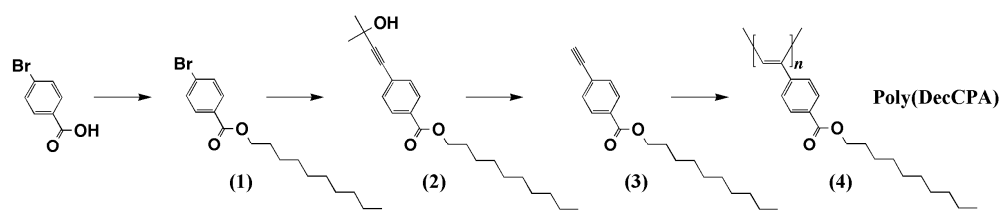
CAPTIONS FOR A SCHEME AND FIGURES

Scheme 1. Synthesis of poly[(4-decyloxy)phenylacetylene] [Poly(DecCPA)].

Figure 1. (a) Schematic drawing of the optical system for single fluorophore imaging (see instrumentation of TIRFM for details). (b) Schematic of the principle of measurement in visualization of individual single conjugated polymer.

Figure 2. Fluorescence spectrum of poly(DecCPA) THF solution at room temperature. Concentration is 1.04×10^{-5} mol/l. Wavelength of excitation (λ_{ex}) is 442 nm.

Figure 3. TIRFM imaging. (a) A micrograph of poly(DecCPA) molecules. Arrow indicates typical fluorescence spot due to a single molecule of poly(DecCPA). Scale bar = 5.0 μm . (b) Dynamic behavior of a fluorescence image intensity of a single molecule observed at the video rate (30 frames = 1.00 sec).



Scheme 1. K. Shinohara *et al.*

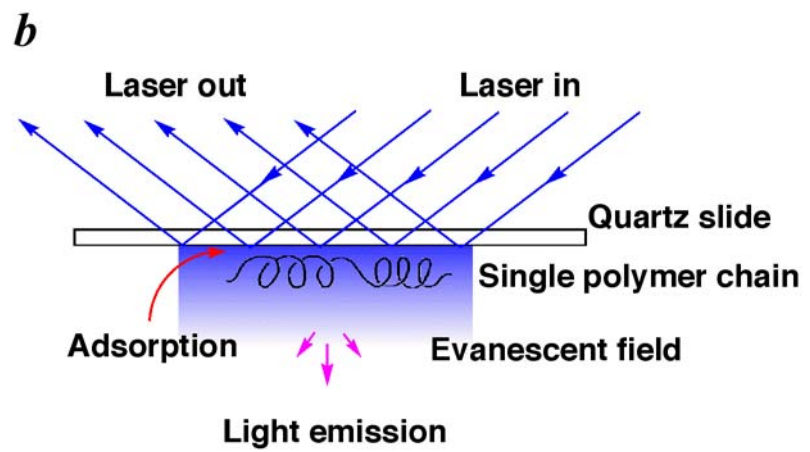
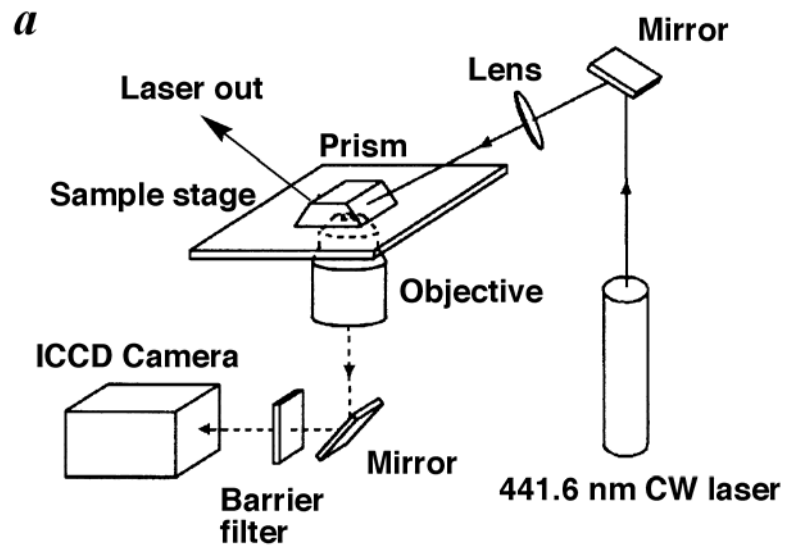


Figure 1. K. Shinohara *et al.*

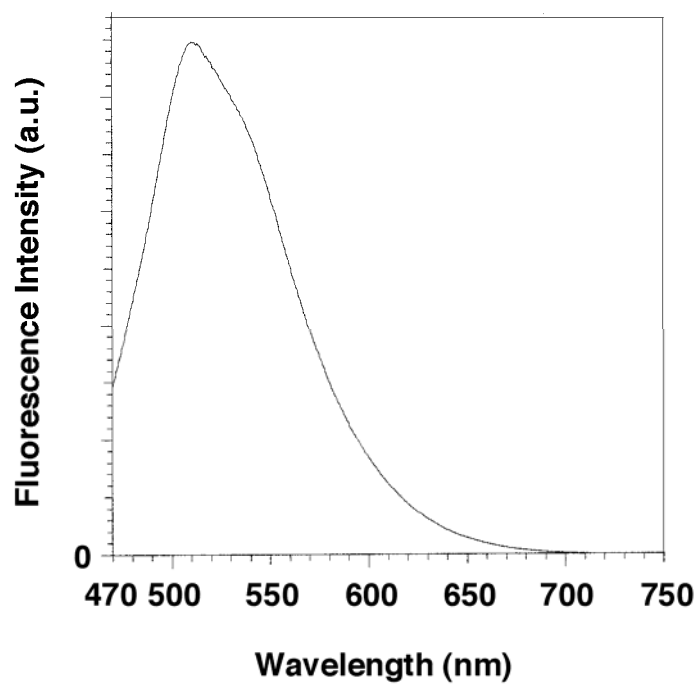


Figure 2. K. Shinohara *et al.*

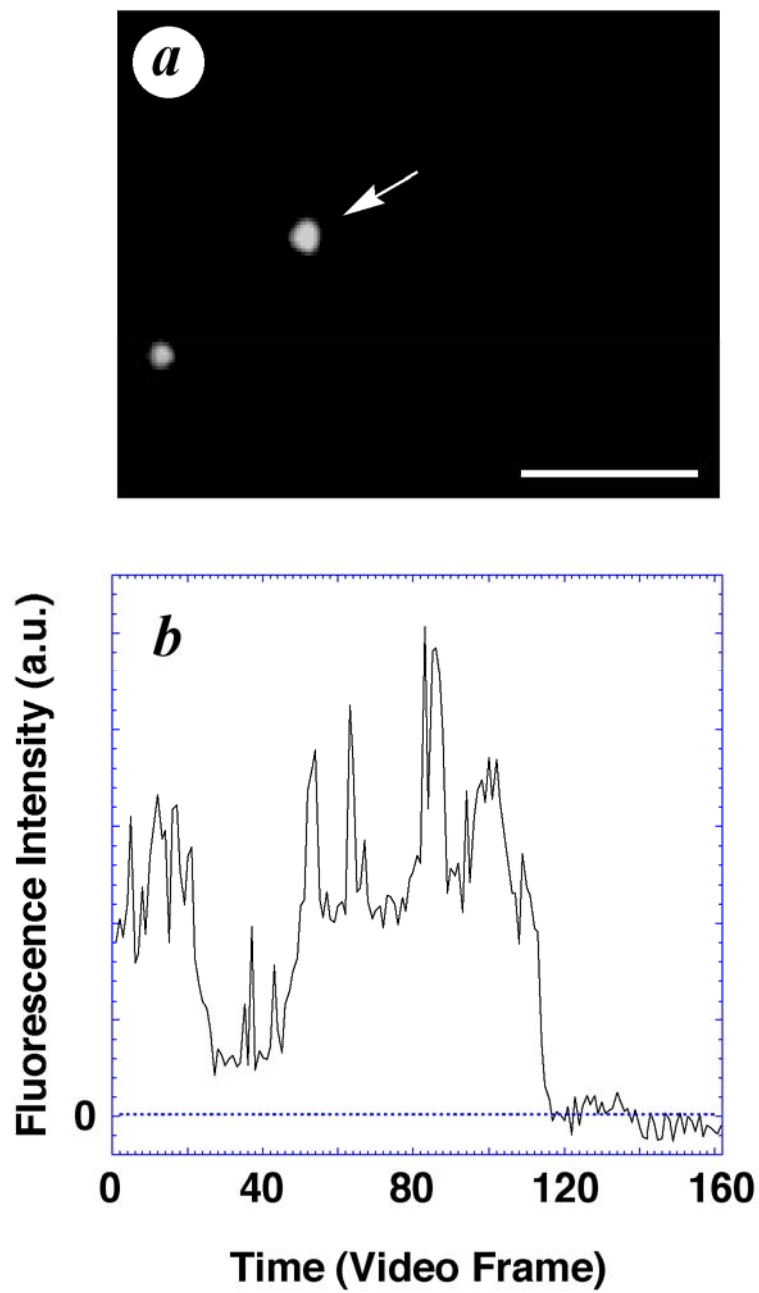


Figure 3. K. Shinohara *et al.*