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Electronic Supporting Information

Compact and stable SNAP ligand-conjugated quantum dots as a fluorescent probe for single-molecule imaging of dynein motor protein

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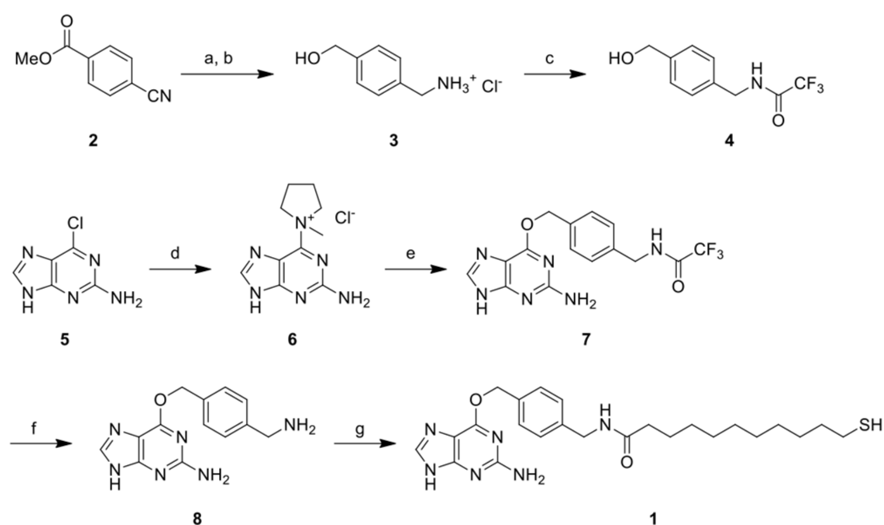
Experimental details

1. Materials

Cadmium 2,4-pentanedionate (98%) and 11-mercaptoundecanoic acid (MUA) were purchased from Alfa Aesar. Selenium powder 100 mesh (99.99%) and tellurium 40 mesh (99.997%) were purchased from Aldrich. Tri-*n*-octylphosphine oxide (TOPO, > 95%), tri-*n*-octylphosphine (TOP), hexadecylamine (HDA), bis(trimethylsilyl) sulfide (TMS_2S), tributylphosphine (TBP), and diethylzinc (1.0 M in hexane) were purchased from Tokyo Chemical Industry. Dimethylcadmium (10 wt% in hexane) was purchased from Stream chemicals. *n*-Octadecylphosphonic acid (ODPA) was purchased from PCI Synthesis. Expect where otherwise noted, all solvents and reagents for the synthesis of BG-SH ligands were purchased from Wako Pure Chemical Industries Ltd. (Japan), Tokyo Chemical Industry (Japan), and Aldrich Chemical Co. (USA) and were used without further purification.

2. Synthesis

1) Synthesis of BG-SH ligand (**1**)



Scheme 1. a) $LiAlH_4$, THF, 50 °C; b) HCl, 67% (2steps); c) $CF_3COOC_2H_5$, TEA, MeOH, r.t., 17h, 50%; d) 1-methylpyrrolidin, DMF, r.t., 24h, 50%; e) **4**, NaH, DMF, r.t., 24h, 50%; f) K_2CO_3,MeOH, r.t., 11h, 80%; g) 11-mercaptoundecanoic acid *N*-succinimidyl ester, DMAP, CH_2Cl_2, r.t., 5h, 40%.

4-(Aminomethyl)-benzyl alcohol (3)

To a solution of Methyl 4-cyanobenzonate (**2**) (5 g, 31 mmol) in anhydrous THF (50 mL) were added $LiAlH_4$ in anhydrous THF (200 mL) slowly at 0 ^oC. The reaction mixture was stirred for additional 1 h at room temperature. After 15 h of stirring at 50 ^oC, the reaction was cooled to room temperature and quenched with H_2O (5.9 mL), 15% NaOH (5.9 mL) and H_2O (17.7 mL). The resulting precipitate was filtered by celite, washed with THF, and filtrate was concentrated in vacuo. The residue was added in 1 M HCl (50 mL) and extracted with EtOAc (50 mL). The aqueous layer was concentrated *in vacuo*. The clude residue was added to acetone (50 mL) and the suspension was filtered to give **3** (3.60 g, 67%) as a white solid. **^{1}H-NMR** (400 MHz, D_2O, 298 K) δ= 4.26 (s, 2H, -C*H$_2$*-NH$_2$-), 4.64 (s, 2H, -C*H$_2$*-OH), 7.45 (s, 4H, Ar). **MS (ESI)** (m/z) calcd for $C_8H_{12}NO$, $[M+H]^+$: 137.08; found: 138.09.

2,2,2-Trifluoro-N-(4-hydroxymethyl-benzyl)-acetamide (4)

To a solution of **3** (3.47 g, 20 mmol) and triethylamine (1.76 mL, 20 mmol) in MeOH (20 mL) were added trifluoroacetic acid ethyl ester (3.1 mL, 26 mmol) at room temperature.
After 17 h of stirring, the reaction mixture was concentrated *in vacuo*. The residue was extracted with EtOAc and washed with brine, and was dried over Na_2SO_4, and concentrated in vacuo. The crude residue was dissolved in $CHCl_3$ and MeOH, adsorbed on SiO_2 (10 g), and purified by column chromatography with $CHCl_3$: MeOH (20 : 1) to give **4** (3.48 g, 74%) as a white solid. **^{1}H-NMR** (400 MHz, $CDCl_3$, 298 K, TMS) δ= 1.74 (br, 1H, -O*H*), 4.52 (d, J= 5.5 Hz, 2H, -C*H$_2$*-NH$_2$-), 4.69 (s, 2H, -C*H$_2$*-OH), 7.25-7.38 (s, 4H, Ar). **MS (ESI)** (m/z) calcd for $C_{10}H_{10}F_3NO_2Na$, $[M+Na]^+$: 256.06; found: 256.06.

1-(2-Amino-7H-purin-6-yl)-1-methyl-pyrrolidium chloride (6)

1-Methylpyrrolidin (18.0 g, 210 mmol) was added to a suspension of 2-amino-6-chloropurine (**5**) (16.9 g, 100 mmol) in anhydrous DMF (300 mL) and the reaction mixture was stirred for 24 h at room temperature. Acetone (50 mL) was added and the precipitate was filtered affording of pure **6** (16.1 g, 63%) as a white solid.
^{1}H-NMR (400 MHz, DMSO-d6, 298 K, TMS) δ= 2.10 (m, 2H, -C*H$_2$*-), 2.35 (m, 2H, -C*H$_2$*-), 3.65 (S, 3H, -C*H$_3$*), 3.98 (m, 2H, -C*H$_2$*-N$^+$-), 4.61 (m, 2H, -C*H$_2$*-N$^+$-), 7.07 (s, 2H, -N*H$_2$*), 8.33 (S, 1H, H8), 13.4 (S, 1H, H9). **MS (ESI)** (m/z) calcd for $C_{10}H_{15}N_6^+$, $[M]^+$: 219.14; found: 219.14.

N-[4-(2-Amino-9H-purin-6-yloxymethyl)-benzyl]-2,2,2-trifluoro-acetamide (7)

To a solution of **4** (2.34 g, 10 mmol) in anhydrous DMF (100 mL) were added 60% NaH (1.2 g, 30 mmol) under N_2 atmosphere at room temperature. After 30 min, **6** (2.54 g, 10 mmol) and DMAP (122 mg, 1 mmol) were added and the reaction mixture was stirred for 19 h at room

temperature. The solution was concentrated *in vacuo*. The crude residue was dissolved in MeOH, adsorbed on SiO_2 (20 g), and purified by column chromatography with $CHCl_3$: MeOH (9 : 1) to give **7** (1.24 g, 34%) as a white solid. **^{1}H-NMR** (400 MHz, DMSO-d6, 298 K, TMS) δ = 4.40 (d, J=5.8 Hz, 2H, -C*H₂*-CO-), 5.46 (S, 2H, -CH_2-O-), 6.30 (S, 2H, -NH_2), 7.21-7.50 (m, 4H, Ar), 7.81 (S, 1H, H8), 10.01 (S, 1H, -N*H*-CO-), 12.42 (1H, S, H9). **MS (ESI)** (m/z) calcd for $C_{15}H_{13}F_3N_6O_2Na$, $[M+Na]^+$: 389.10; found: 389.10.

O^6-(4-Amino-methylbenzyl)guanine (8)

To a solution of **7** (915 mg, 2.5 mmol) in MeOH (90 mL) and H_2O (10 mL) were added K_2CO_3 (1.7 g, 12.5 mmol) under reflux for 3 h at 60 ^oC. The reaction mixture was concentrated in vacuo. The crude residue and dissolved in MeOH and H_2O, adsorbed on SiO_2 (10 g), and purified by column chromatography with $CHCl_3$: MeOH : Triethylamine (5 : 1 : 0.05) to give **8** (588 mg, 87%) as a white solid. **^{1}H-NMR** (400 MHz, DMSO-d6, 298 K, TMS) δ = 3.97 (S, 2H, -CH_2-N_2-), 5.47 (S, 2H, -CH_2-O-), 6.22 (S, 2H, -NH_2, guanine), 7.36-7.47 (m, 4H, Ar), 7.81 (S, 1H, H8). **MS (ESI)** (m/z) calcd for $C_{13}H_{14}N_6ONa$, $[M+Na]^+$: 293.28; found: 293.28.

BG-MUA (1)

8 (135 mg, 0.5 mmol), 11-mercaptoundecanoic acid N-succinimidyl ester (162 mg, 0.5 mmol), and DIEA (0.4ml, 2.5 mmol) were added in anhydrous DMF (5 mL). The mixture was stirred 5 hr at 50°C and then evaporated. The residue was purified by silica chromatography ($CHCl_3$: MeOH = 5 : 1) and concentrated and dried in vacuo to get white powder of **1** (125 mg, 53%). **^{1}H-NMR** (400 MHz, DMSO-d_6, 298 K) δ = 1.24-1.32 (m, 12H, -CH_2-), 1.50-1.53 (m, 4H, HS-CH_2-CH_2-, CO-CH_2-CH_2-), 2.12-2.44 (m, 2H, CO-CH_2-), 2.44-2.50 (m, 2H, HS-CH_2-), 5.46 (S, 2H, -CH_2-O-), 6.25 (S, 2H, -NH_2, guanine), 7.25-7.45 (m, 4H, Ar), 7.83 (S, 1H, H8), 10.05 (S, 1H, -N*H*-CO-). **^{13}C-NMR** (100 MHz, DMSO-d_6, 298 K, TMS) δ = 23.9, 25.1, 28.5, 28.8, 33.0, 35.0, 66.6, 95.3, 127.1, 128.4, 134.9, 138.4, 139.6, 155.8, 159.4, 172.6. **MS (ESI)** (m/z) calcd for $C_{24}H_{35}N_6O_2S^+$, $[M+H^+]$: 471.2537; found: 471.2537.

Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck silica gel glass plates, 60F254. Silica gel chromatography was performed on Kanto Chemical silica gel N60 (40-50 μm). NMR measurements were recorded at 27°C on JEOL ECP-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), using residual undeuterated solvent as an internal reference. ESI-mass spectra were obtained with a Thermo Scientific Exactive.

2) PC-QDs

PC-QDs were prepared form TOPO-coated CdSe/ZnS QDs[1)] (600 nm emission) by the ligand-exchange reaction as a previously reported method [2)]

3) BG-QDs

BG-QDs was prepared from the PC-QDs by the ligand-exchange reaction using BG-SH ligands. A typical procedure is as follows: 20 μL of a DMSO solution of BG-SH (1 □M) was added to 1 mL of an aqueous solution of PC-QDs (1μM, PBS) under vigorously stirring. After 2 hours, BG-QDs were purified by using a desalting column (PD-10, GE Healthcare) with PBS.

3. QD characterization

Fluorescence spectra of QDs were measured with a spectrofluorometer FP-8000 (JASCO Corporation) at the excitation wavelength of 488 nm. Dynamic light scattering measurements were carried out with Nano-ZS (Malvern) at pH = 7.4 (10 mM PBS). Fluorescence autocorrelation curves of QDs in aqueous solution were measured by using a compact FCS (C9413-01MOD, Hamamatsu Photonics) with a 473 nm semiconductor laser as an excitation light source. Fluorescence lifetime of QDs was measured using a lifetime fluorescence spectrofluorometer (Horiba) equipped with a LED laser (484 nm).

4. Preparation of SNAP-GFP

EGFP cDNA was amplified by TTCTCGAGATGGTGAGCAAGGGCGAG and ACGCGGCCGCTAGTGGTGATGGTGATGATGCTTGTACAGCTCGTCCATGCC including his-tag sequence and inserted to XhoI-NotI site of pSNAP-tag(T7)-2 (NEB). pSNAP-EGFP was transformed into *E. coli* KRX competent cells (Promega) grown in LB media with ampicilin at 37°C on shaking table. To induce production of the targeted protein, isopropyl beta-*D*-1-thiogalactopyranoside (0.1 mM) and *L*-Rhamnose (1 %) were added to the LB media, and then incubated with shaking gently for 12 hr at 18°C. The cells were lysed and suspended with binding buffer (50 mM Tris-HCl, pH=8.0). The purification was performed using Ni-NTA column (GE healthcare) and the expression was checked by CBB staining method.

5. Agarose gel electrophoresis

BG-QDs, PC-QDs, BG-QDs + SNAP-GFP (1:5 molar ratio), and PC-QDs + SNAP-GFP (1:5 molar ratio) were run on a 1% agarose gel (25 mM TAE buffer) that was applied on 100 V for 30 min. Fluorescence spots (> 600 nm) of the QDs were observed under irradiation of light at 460 nm using a luminescent image analyzer (GE Healthcare, Image Quant 4010).

6. Preparation of SNAP-tagged dynein

As SNAP-tagged dynein, we expressed and purified GST dimer of *Dictyostelium* cytoplasmic dynein motor domain (GST380) as previously described.[3] The equivalent construct of human cytoplasmic dynein 1 (GST-D382)[4] was provided courtesy of Dr. Ken'ya

Furuta, National Institute of Information and Communications Technology, Japan. To conjugate the SNAP-tagged dynein on BG-QDs, the dynein and the quantum dots were mixed with the mixing ratio of 10:1 (dynein: QD) in assay buffer (20 mM PIPES-KOH, 10 mM potassium acetate, 4 mM $MgSO_4$, 1 mM EGTA, pH 7.0) and incubated for 30 min at room temperature. Tubulin was purified from porcin brain through four cycles of polymerization and depolymerization using a high-molarity PIPES buffer to remove microtubule-associated proteins effectively.[5)] Then, purified tubulin was labeled with Alexa Fluor 488 (A20000, Life technologies) or biotin (21336, Life technologies) as described previously.[6)] Fluorescently labeled microtubules were prepared by copolymerization of 103 μM non-labeled tubulin, 2 μM Alexa488-labeled tubulin and 3 μM biotin-labeled tubulin for 30 min at 37 °C and stabilized with paclitaxel (169-18616, Wako).

7. Single-molecule fluorescence imaging

Surface of cover glasses (C022221S, Matsunami Glass) was cleaned by sonication in 1N KOH and by plasma treatment (Diener), and then silanized with N-2-(aminoethyl)-3-aminopropyl-triethoxysilane (KBE-603, Shin-Etsu Chemical). To make PEG-coated and PEG-biotin-coated glasses, the amino-silanized glasses were incubated with PEG solution (200 mg/mL NHS-PEG [ME-050-TS, NOF] in 50 mM MOPS, pH 7.5) or PEG-biotin solution (1 mg/mL NHS-PEG-biotin [BI-050-TS, NOF] and 200 mg/mL NHS-PEG in 50 mM MOPS, pH 7.5) for 3 hours at room temperature, respectively.[7)] Glass chamber for observation was prepared by separating a PEG-coated glass and a PEG-biotin-coated glass with a 30-μm layer of double sticking tape (5603, Nitto-Denko).[8)] The microtubules were immobilized on the PEG-biotin-coated glass surface via Neutravidin (31000, Thermo). Then, glass surface was blocked with 1% (w/v) Pluronic F-127 (P2443, Sigma-Aldrich) and 1 mg/mL casein (218680, Merck Millipore) in assay buffer. Finally, the chamber was filled with imaging solution containing 300 pM QD-dynein, 20 mM PIPES-KOH, 10 mM potassium acetate, 4 mM $MgSO_4$, 1 mM EGTA, 1 mM ATP, 1% (w/v) Pluronic F-127, 1 mg/mL casein, 2 mM dithiothreitol, 0.2 mg/mL glucose oxidase, 40 μg/mL catalase, 1 mM glucose, 10 μM paclitaxel, pH 7.0. The sample was illuminated by a blue laser (Sapphire488-500, Coherent) or a green laser (Sapphire 552-200, Coherent). The images of microtubules and QDs were observed under total internal reflection fluorescence microscopy (IX81, Olympus) with a 100x objective lens (UPlanSApo, NA 1.40, Olympus) and recorded at the frame rate of 10 frames per second using iXon3 EM CCD camera (Andor).

References

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Additional figures

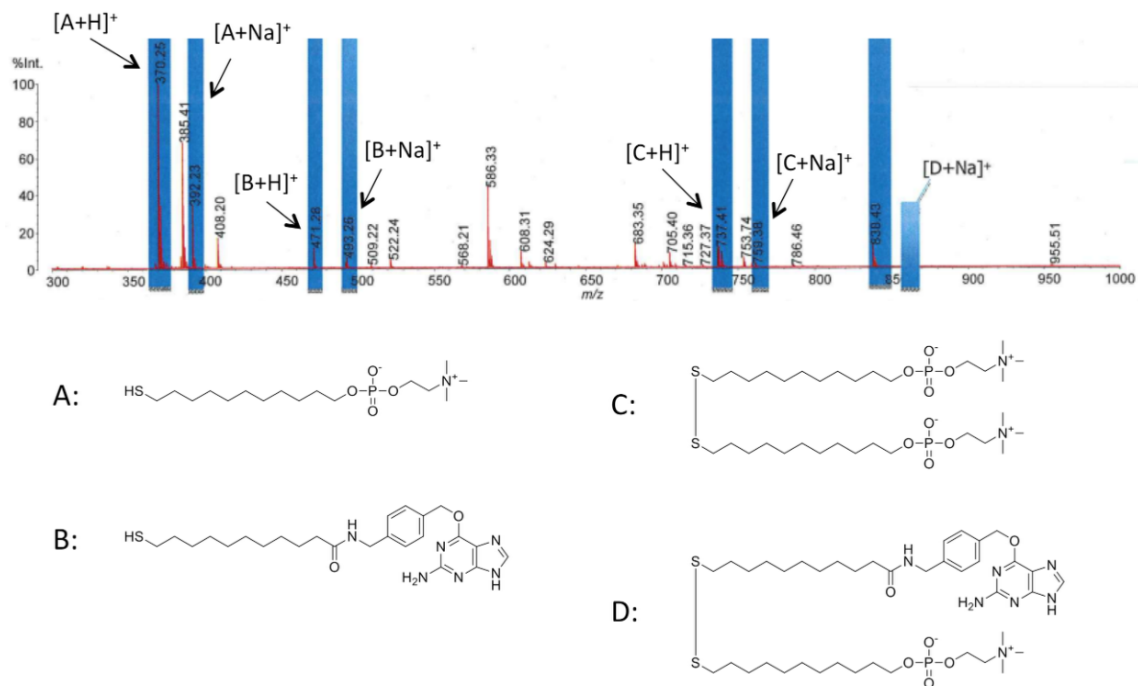

Fig. S1 MALDI-TOF mass spectrum of BG-QD.

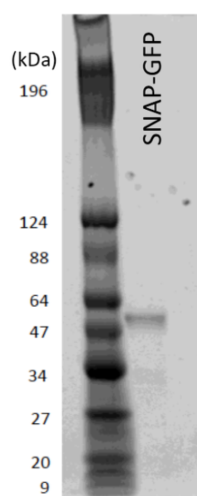

Fig. S2 SDS-PAGE of SNAP-GFP (47.7 kDa).

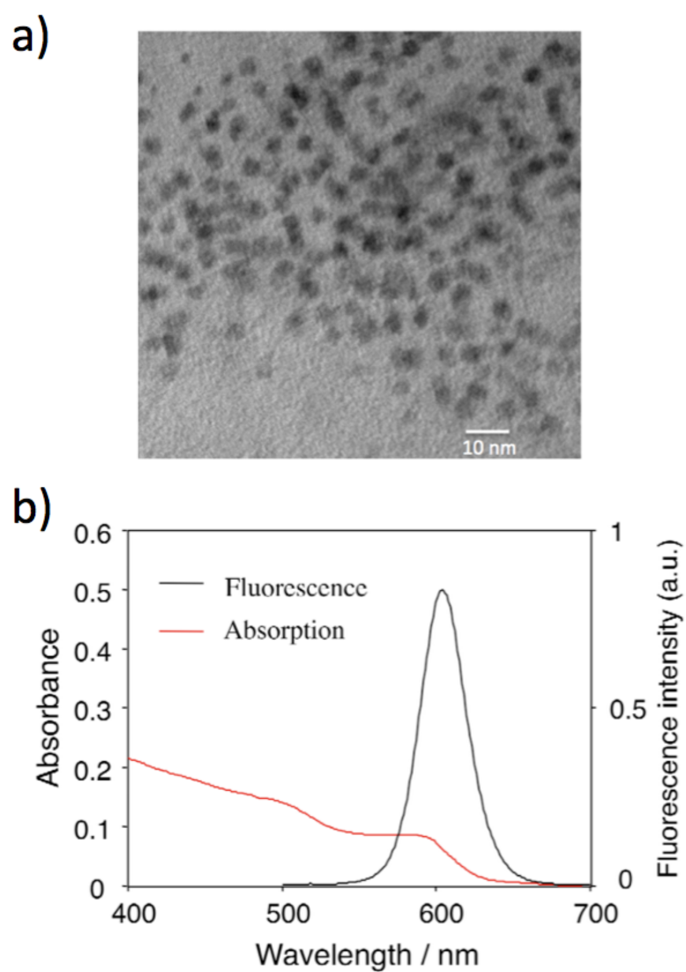

Fig. S3 Transmission electron microscopy (TEM) image (a) and absorption/fluorescence spectra (b) of BG-QD. The TEM image was obtained using a H800 (HITACHI) at 200 kV accelerating voltage.

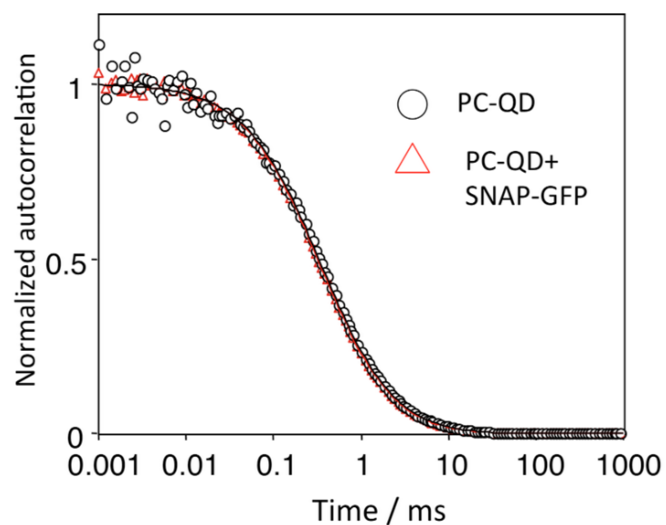

Fig. S4 Fluorescence autocorrelation curves for PC-QD and PC-QD+SNAP-GFP (1:5) in PBS.

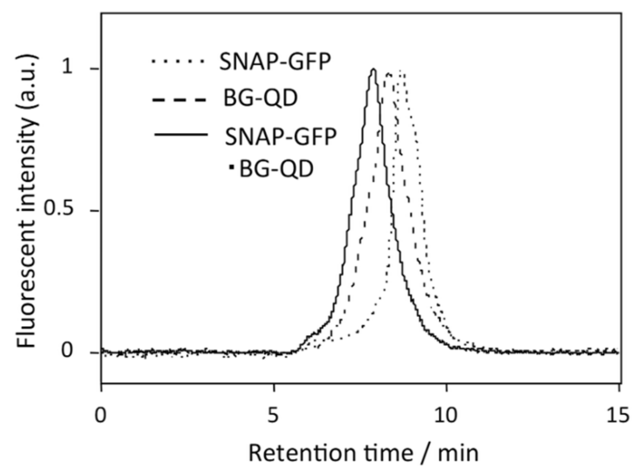

Fig. S5 Size-exclusion gel chromatography for SNAP-GFP, BG-QD and SNAP-GFP・BG-QD. Column: TSK gel G4000 SW_{XL} (TOSOH); Eluent, 0.01 M Na_2CO_3 aqueous solution; Elution rate: 1mL/ min.

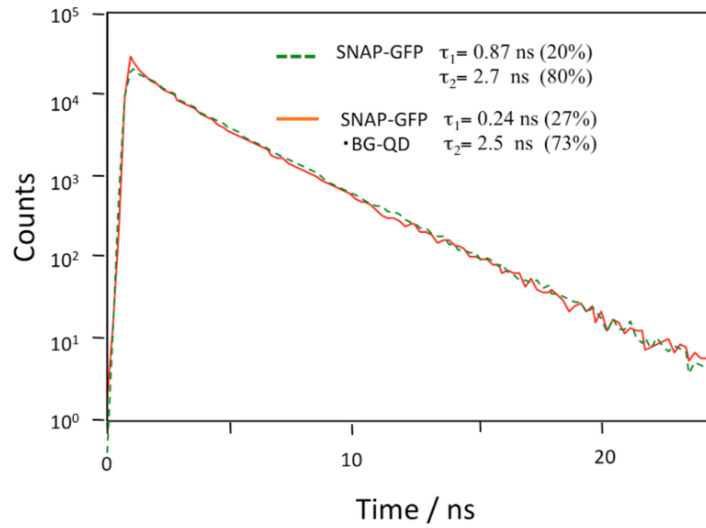

Fig. S6 Fluorescence lifetime of GFP emission in SNAP-GFP and SNAP-GFP ・ BG-QD in PBS .

Movie Information

Movie-1 (mp4)

Total internal reflection fluorescence microscopy shows BG-QD conjugated *Dictyostelium* cytoplasmic dynein (magenta spots) moving along microtubules (cyan lines) in the presence of 1 mM ATP. The both width and height of the field are 10.9 µm.

Movie-2 (mp4)

Same as *Dictyostelium* dynein (Movie-1), BG-QD conjugated human cytoplasmic dynein 1 (magenta spots) moving along microtubules (cyan lines) in the presence of 1 mM ATP. The width and height of the field is 7.5 µm and 5.8 µm, respectively.