Supporting Information for

Complete ON/OFF Photoswitching of the Motility of a Nano Biomolecular Machine

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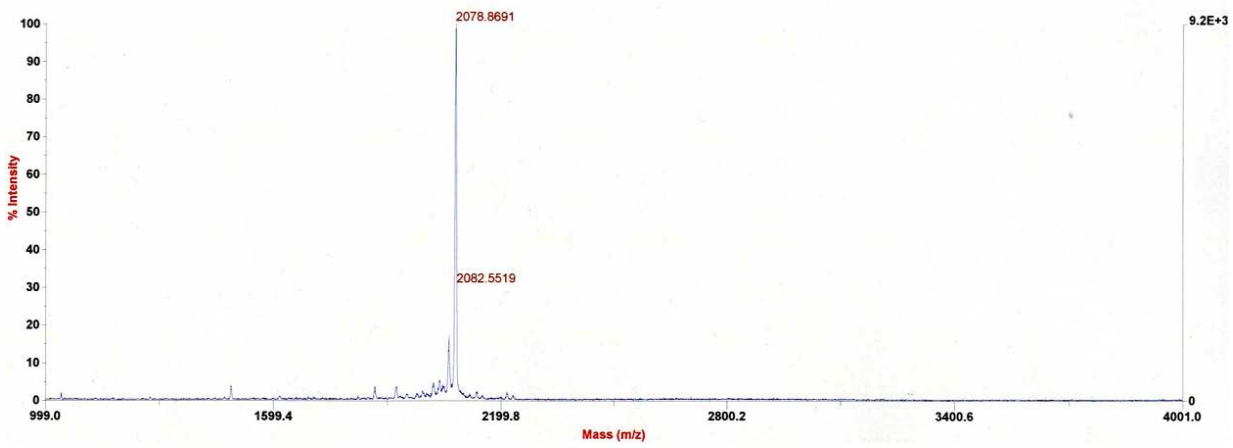
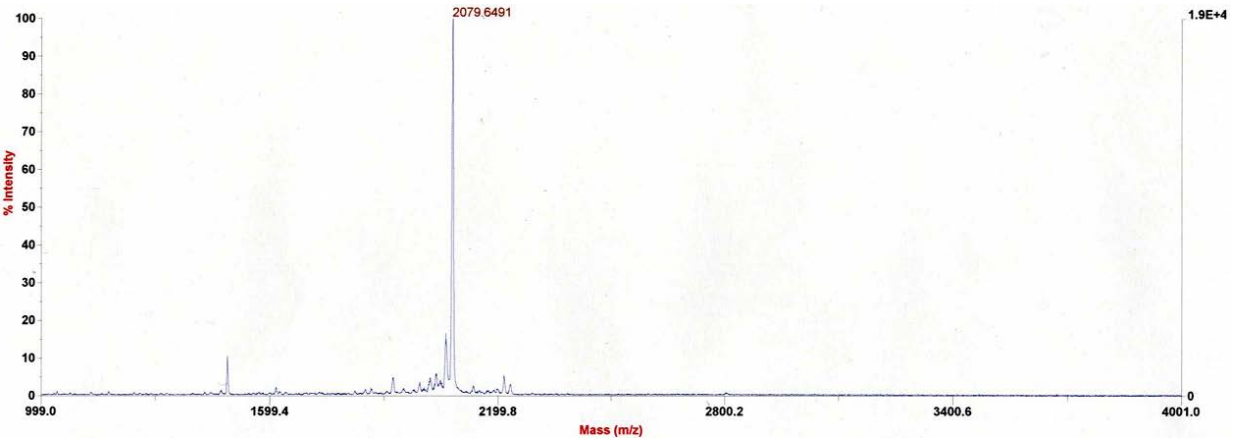
(1) MALDI-TOF Mass spectra of compounds 1-9.

(a)



(b)



(c)

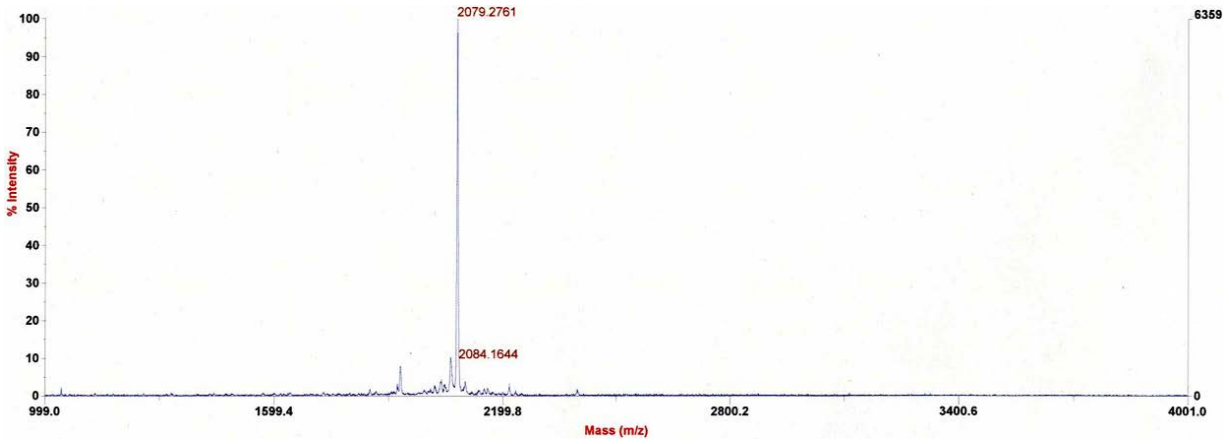


Figure S1. MALDI-TOF-MS spectrum of (a) compound **1**: *m/z*=2078.86 $[M+H]^+$ (calcd. 2078.14), (b) compound **2**: *m/z*=2079.64 $[M+H]^+$ (calcd. 2079.12), and (c) compound **3**: *m/z*=2079.27 $[M+H]^+$ (calcd. 2079.12).

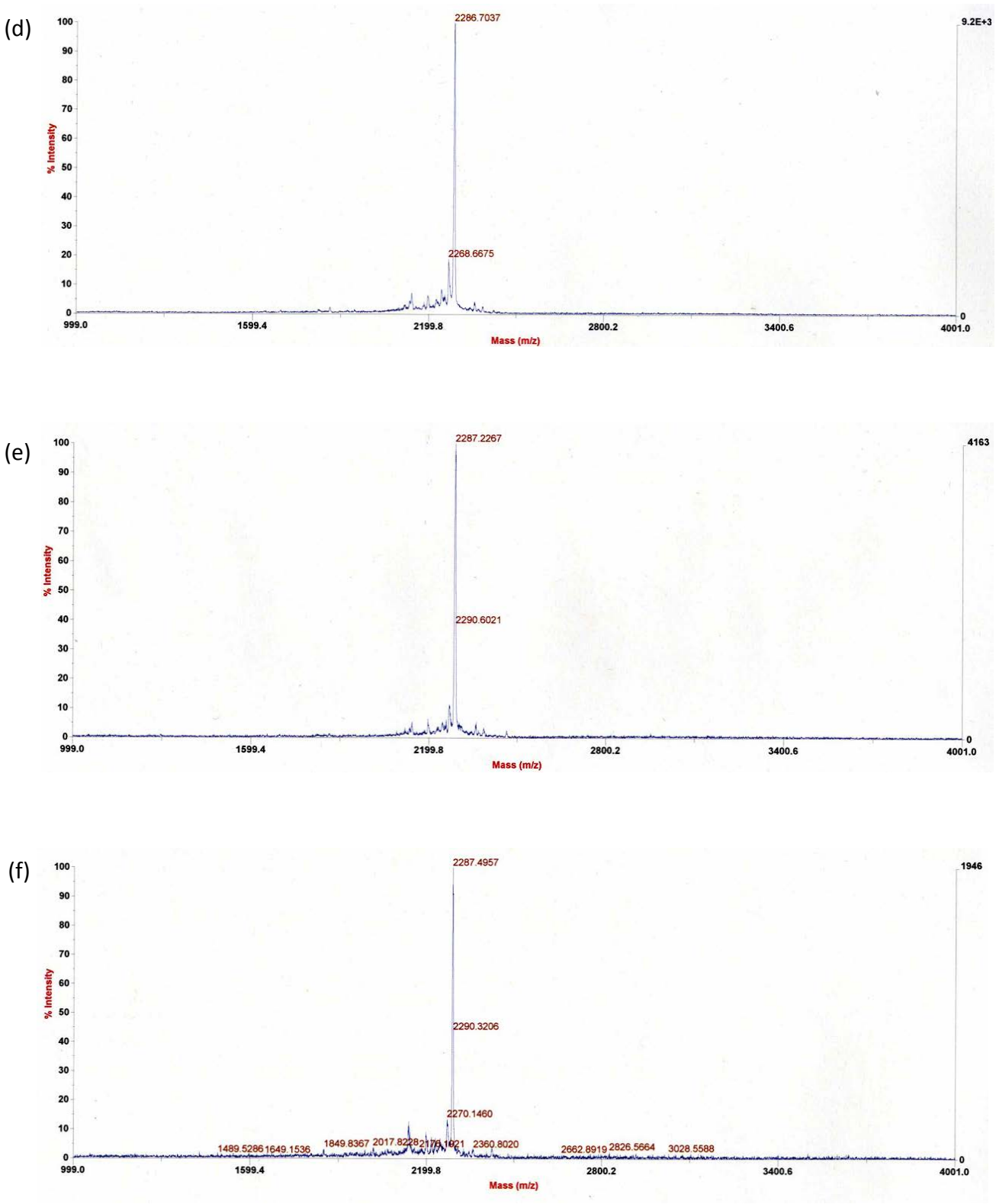
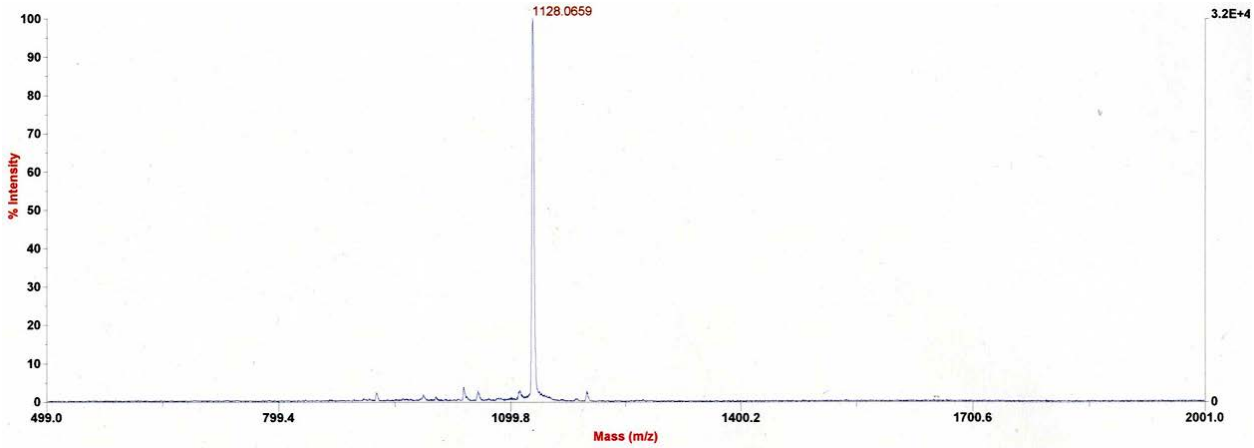
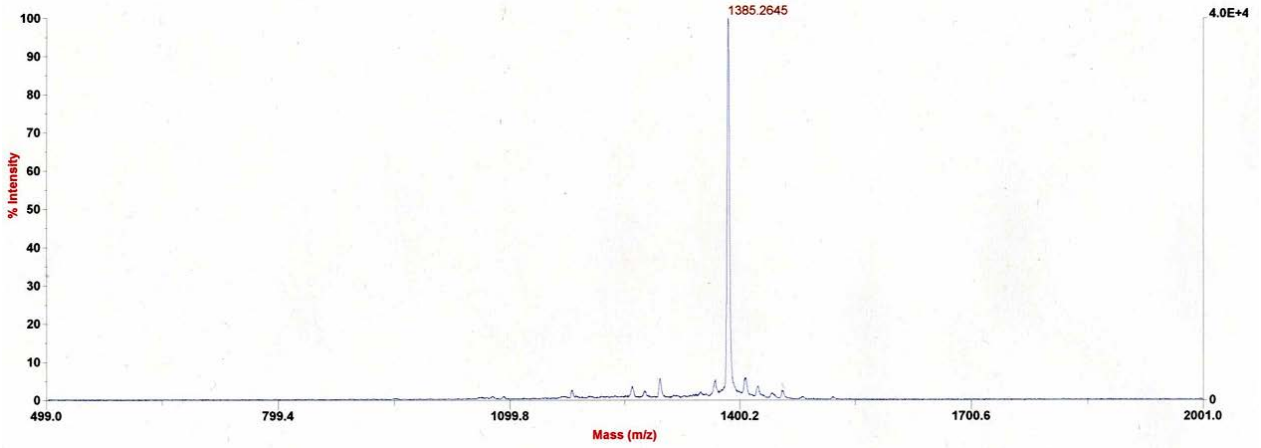


Figure S2. MALDI-TOF-MS spectrum of (d) compound **4**: *m/z*=2286.70 $[M+H]^+$ (calcd. 2286.20), (e) compound **5**: *m/z*=2287.22 $[M+H]^+$ (calcd. 2287.19), and (f) compound **6**: *m/z*=2287.49 $[M+H]^+$ (calcd. 2287.19).

(g)



(h)



(i)

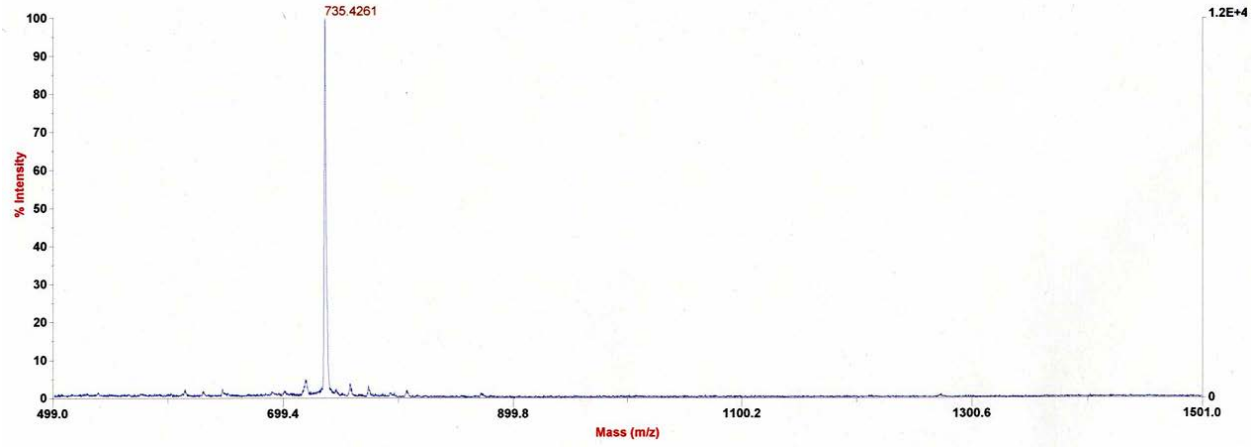


Figure S3. MALDI-TOF-MS spectrum of (g) compound **7**: *m/z*=1128.06 $[M+H]^+$ (calcd. 1128.53), (h) compound **8**: *m/z*=1385.26 $[M+H]^+$ (calcd. 1385.74), and (i) compound **9**: *m/z*=735.42 $[M+H]^+$ (calcd. 735.32).

(2) UV-Visible absorption spectra of compounds 5-9.

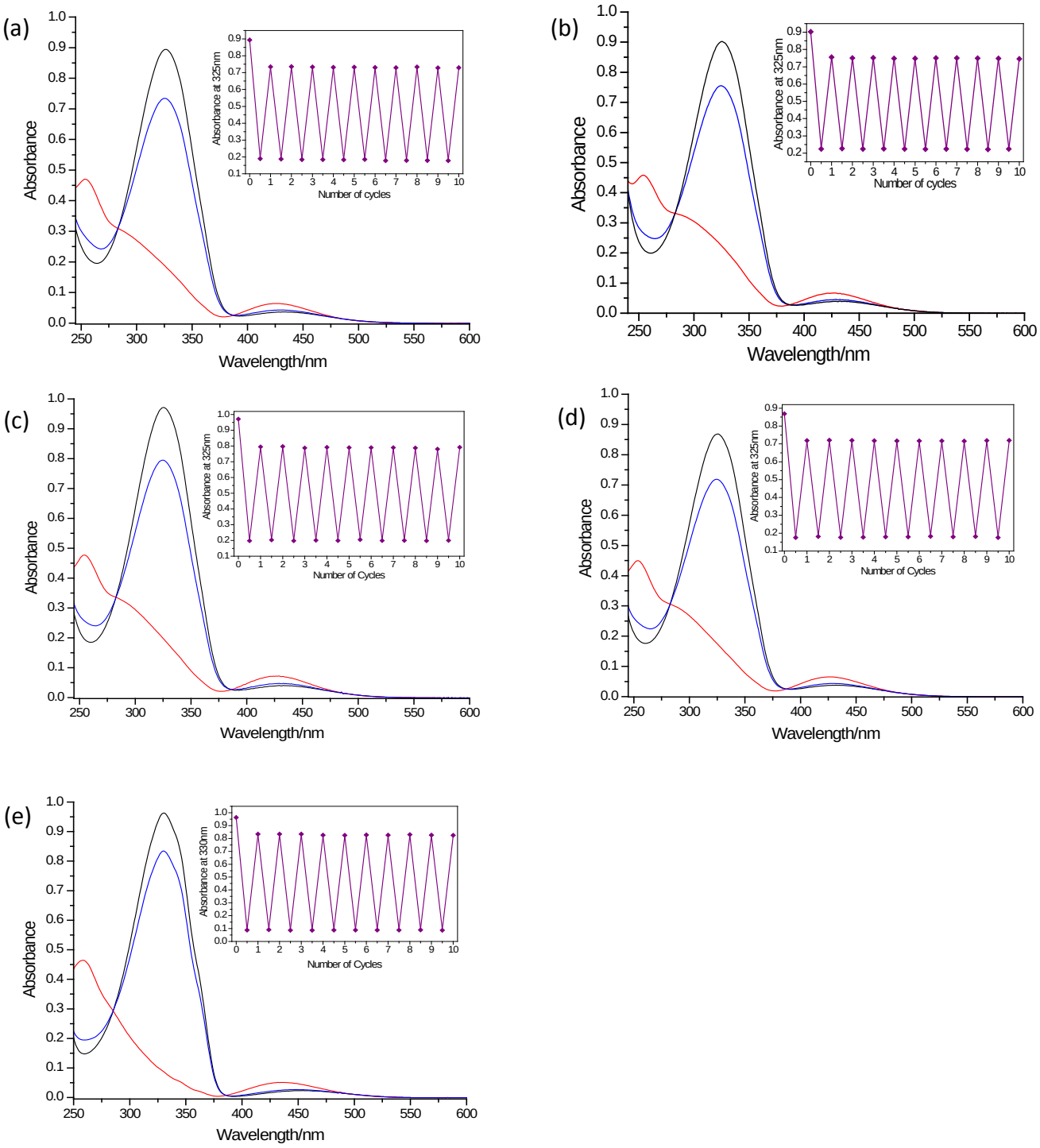


Figure S4. UV-Visible absorption spectra of compounds **5-8** (a-d) in BRB-40 buffer solution and **9** (e) in DMSO at 25 ^{0}C; before photo-irradiation (Black line), PSS under 365 nm light irradiation (Red line), PSS under 436 nm light irradiation (Blue line). The inset shows the absorbance changes at 325 nm after alternating irradiation with 365 nm and 436 nm light for 10 cycles.

(3) Thermal Stability of *cis* isomer of compounds 4-9.

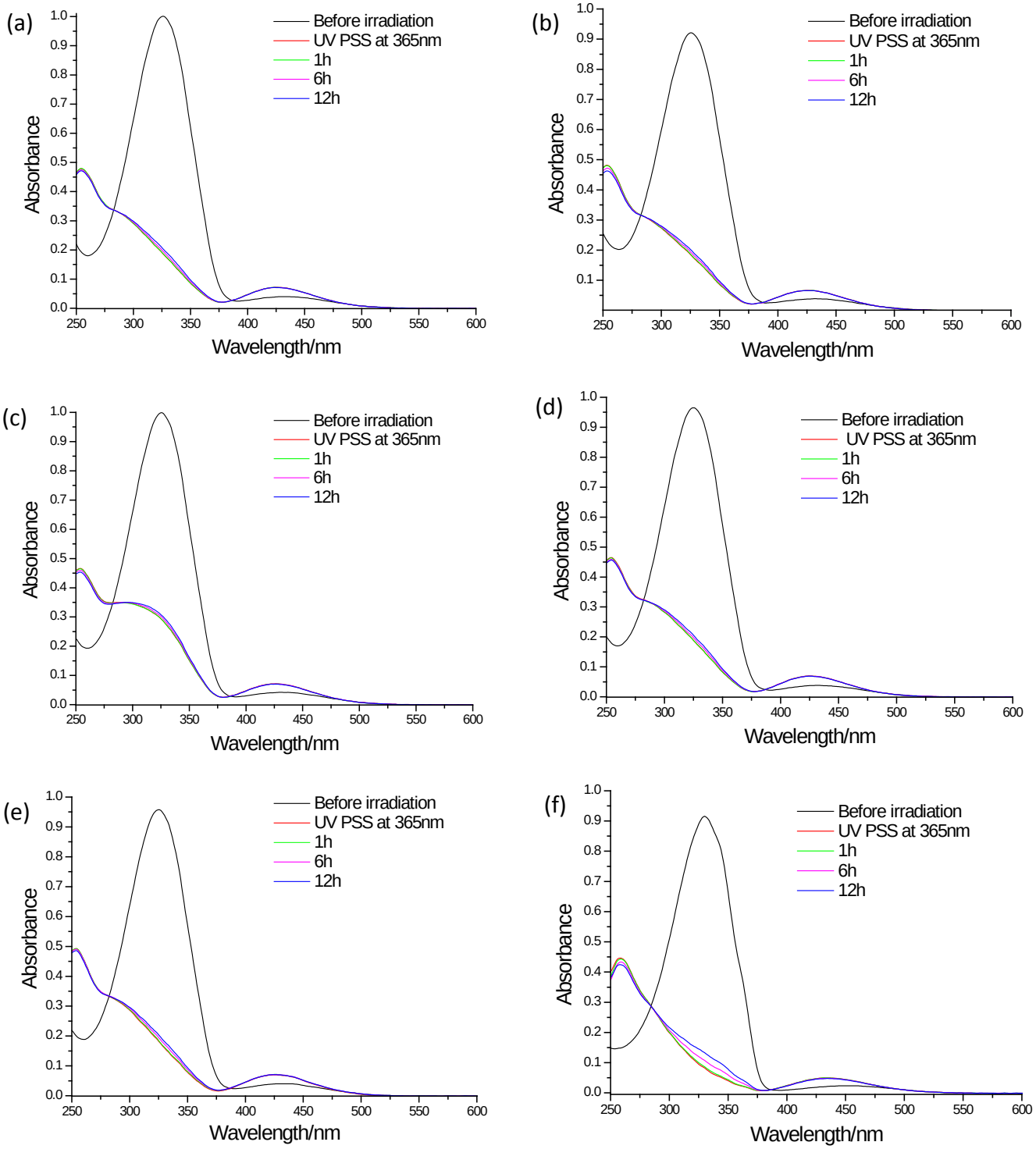


Figure S5. UV-Visible absorption spectra of compounds **4-8** (a-e) in BRB-40 buffer solution and **9** (f) in DMSO after irradiation for 40 s at 365 nm and then incubation in the dark at 25 ^{0}C.

(4) HPLC purity of compounds 1-3.

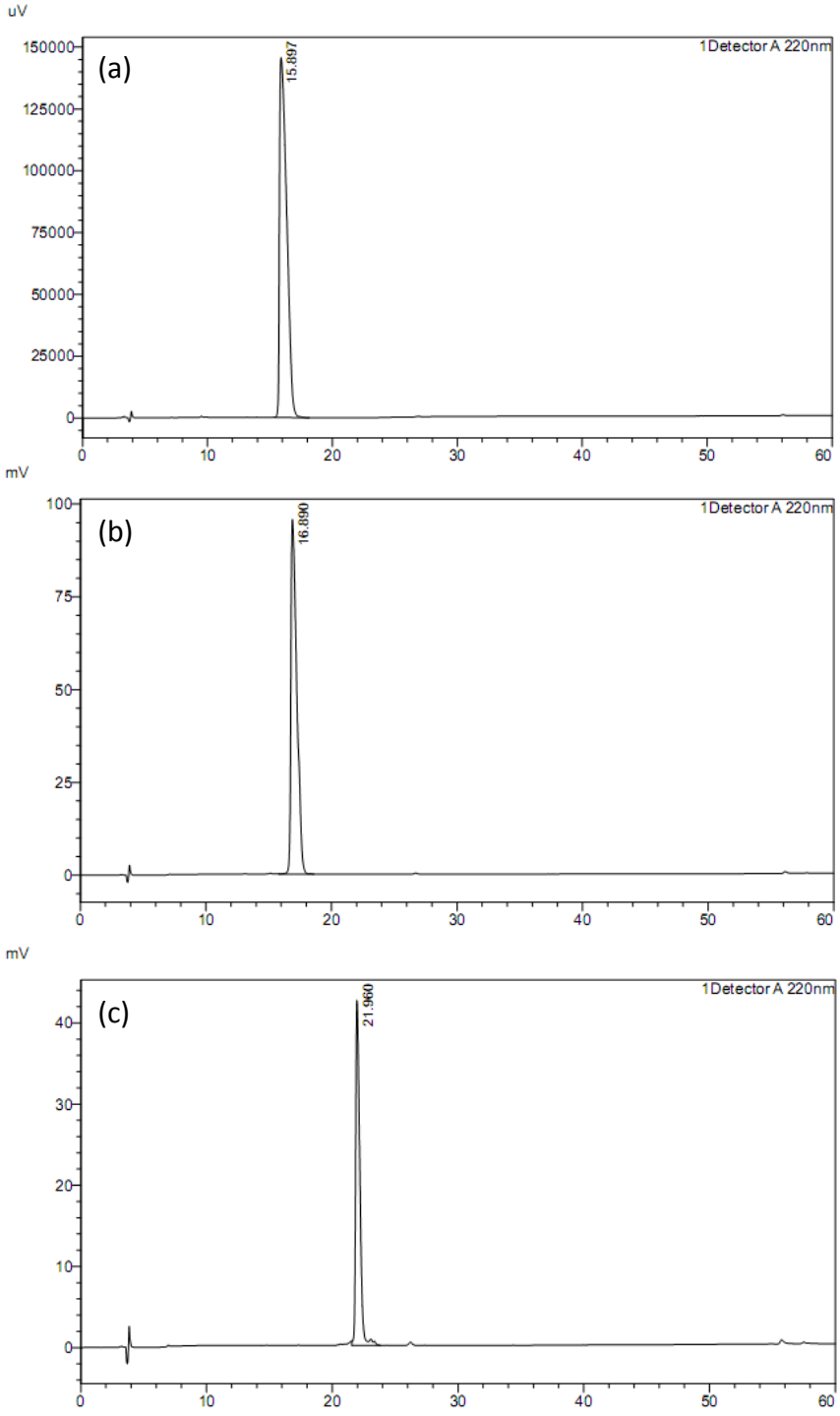
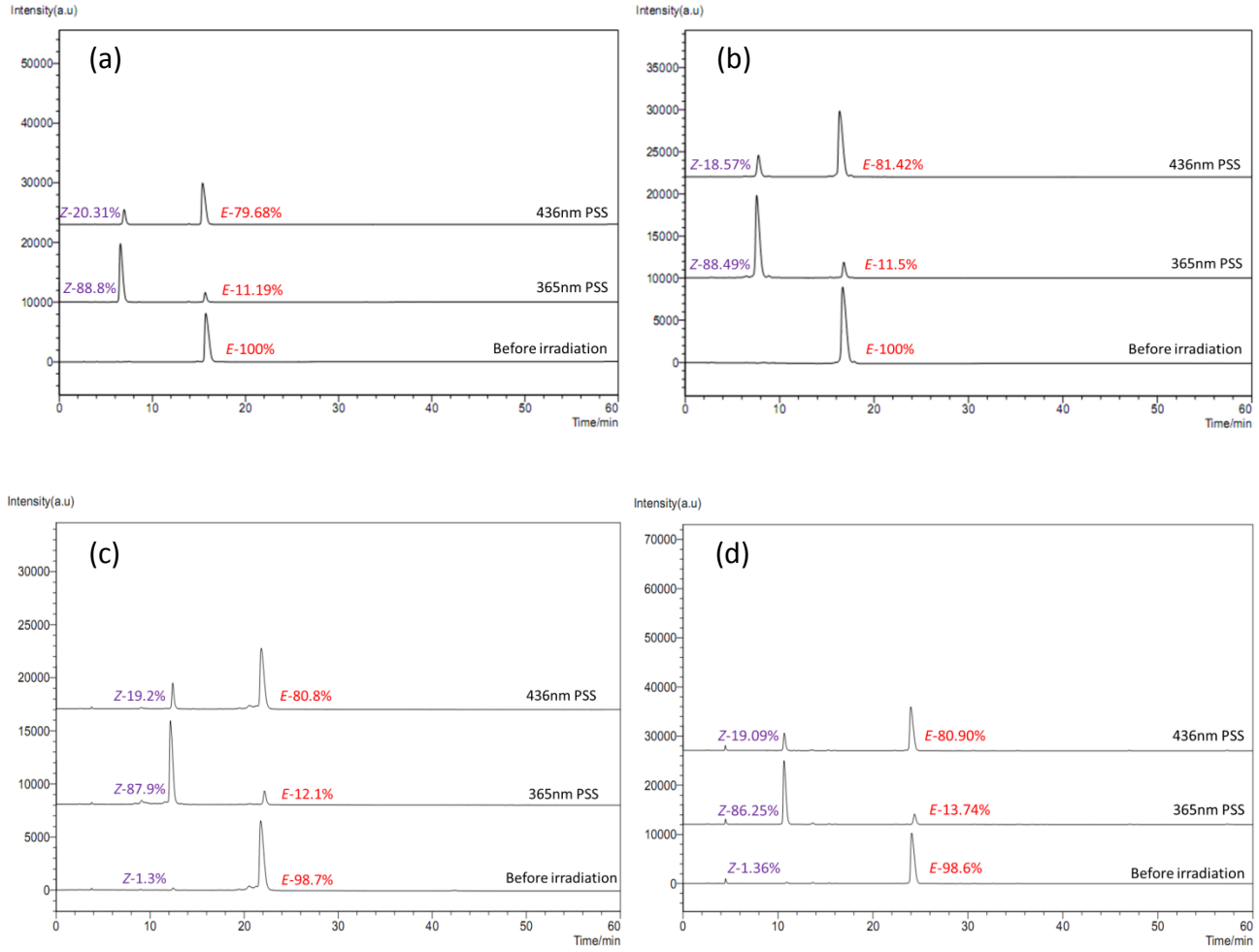


Figure S6. HPLC chromatograms showing >95% purity of (a) compound **1**, (b) compound **2** and (c) compound **3**. Conditions of the RP-HPLC analysis; Column-5C_{18}-MS-II, 4.6×250mm(Waters); Eluent-CH_3CN/H_2O containing 0.1% TFA; Solvent gradient-10 to 35% for 1 h; Flow rate-1ml/min at 25 ^{0}C. Injection volume-20 µL was used to analyze the purity of each compound and 220 nm was used as a monitoring wavelength.

(5) HPLC analysis on the conversion ratio from *trans* to *cis* and *cis* to *trans* forms of the compounds 4-9.

The photo conversion ratio from *trans* to *cis* and *cis* to *trans* of the azo unit in compounds **4-9** upon irradiation with 365 nm light and 436nm light was measured with HPLC analyses. HPLC was performed on SHIMADZU HPLC system. Conditions of the RP-HPLC analysis; Column-5C_{18}-MS-II, 4.6×250mm(Waters); Eluent-CH_3CN/H_2O containing 0.1% TFA; Solvent gradient-20 to 45% for 1 h; Flow rate-1ml/min at room temperature (25 ^{0}C). Injection volume-20 μL was used to analyze the ratio of each isomer and the isobestic point in this eluent condition (283 nm) was used as the monitoring wavelength.



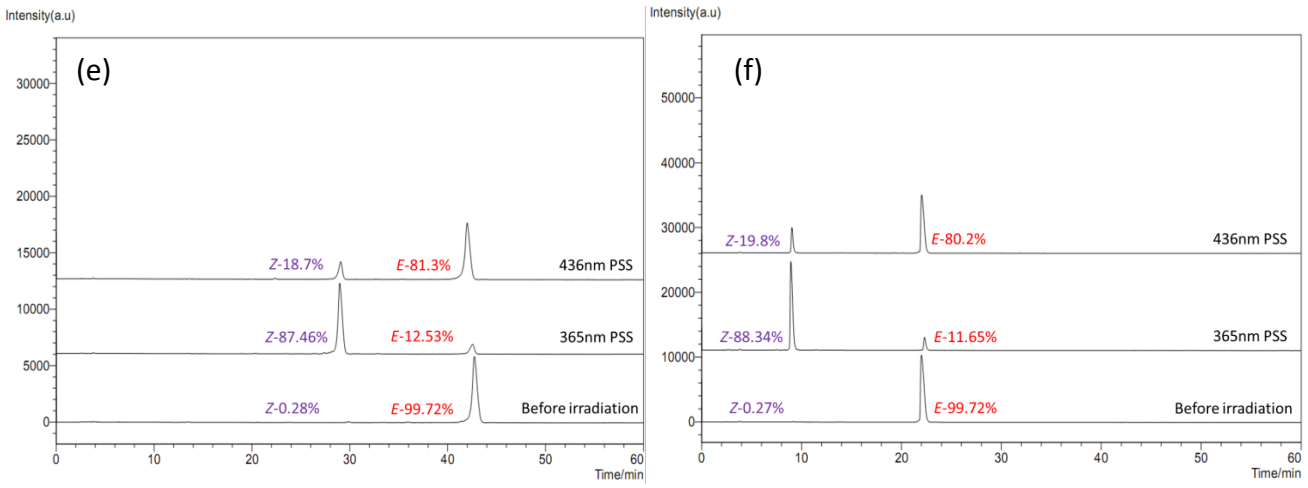


Figure S7. HPLC chromatograms showing the *cis* (*Z*) and *trans* (*E*) isomer ratio of (a) compound **4,** (b) compound **5**, (c) compound **6**, (d) compound **7**, (e) compound **8** and (f) compound **9** at before irradiation, after 365 nm light irradiation under PSS and after 436 nm light irradiation under PSS respectively.

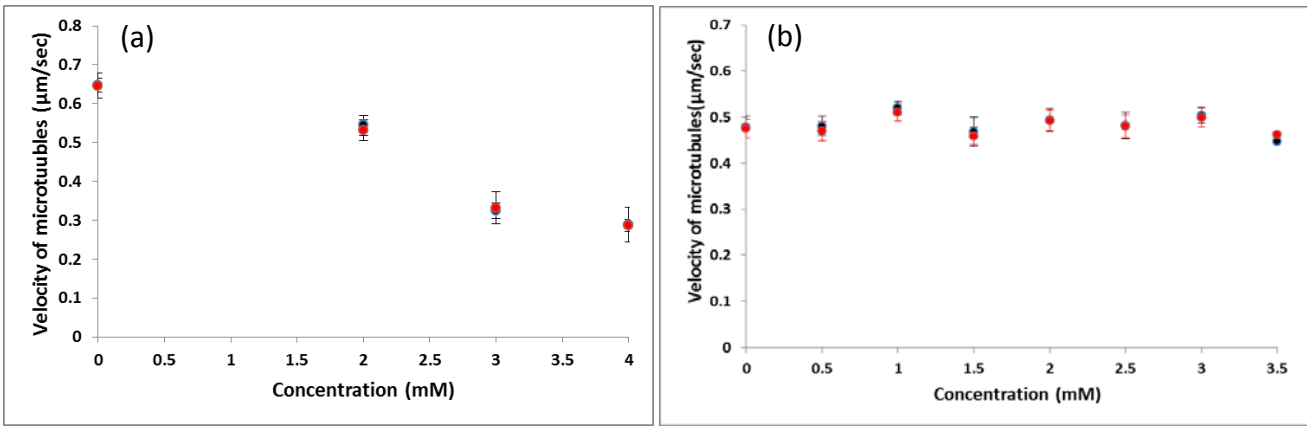


Figure S8. Gliding velocities of microtubules plotted with respect to the concentration of (a) compound **3**, (b) compound **7** at 1.0 mM ATP concentration. Black circles: gliding velocity of microtubules in non-irradiated state; Red circles: gliding velocity after 365 nm light irradiation for 40 s; Error bars represents the standard deviation for 10 microtubules.

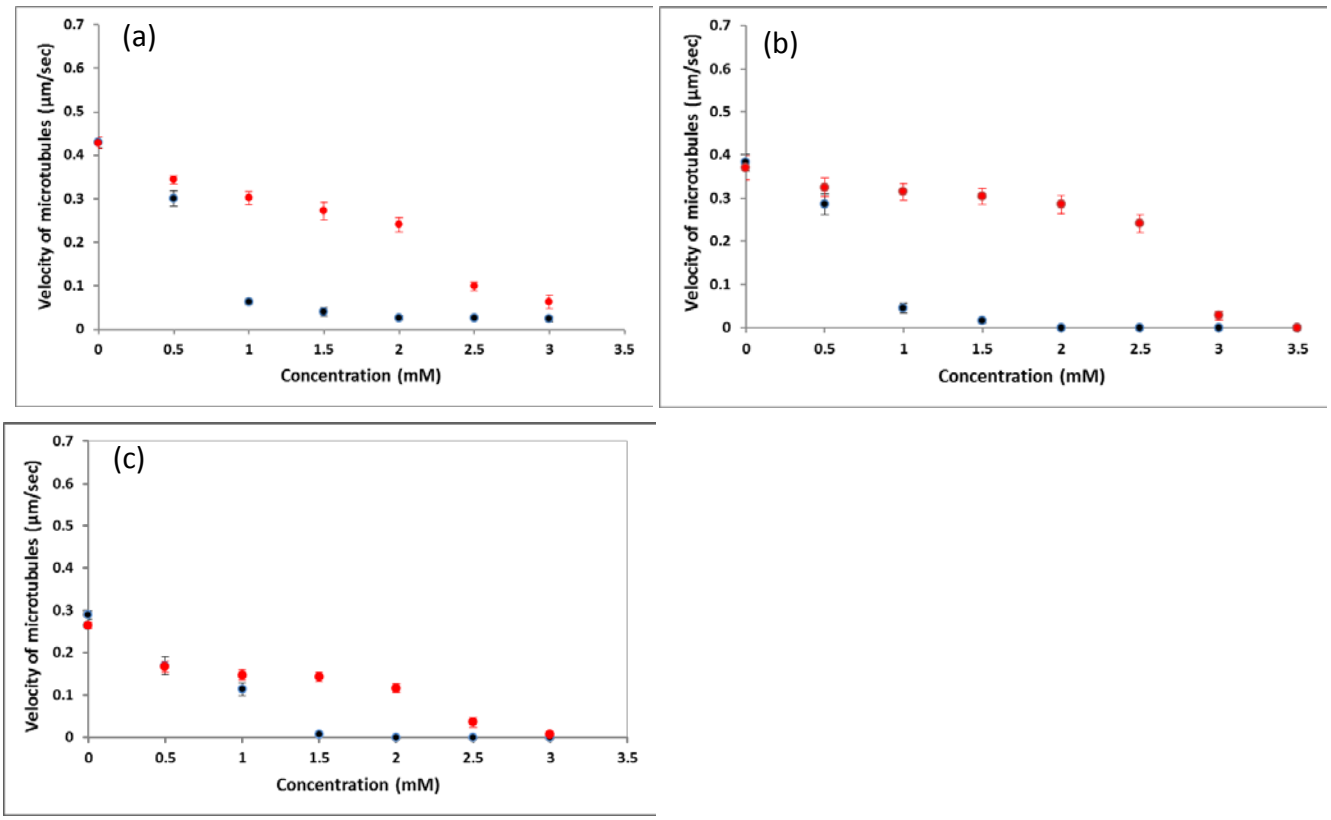


Figure S9. Gliding velocities of microtubules plotted with respect to the concentration of (a) compound **6**, (b) compound **8** at 0.1 mM ATP concentration and of (c) compound **8** at 0.05 mM ATP concentration. Black circles: gliding velocity of microtubules in non-irradiated state; Red circles: gliding velocity after 365 nm light irradiation for 40 s; Error bars represents the standard deviation for 10 microtubules.

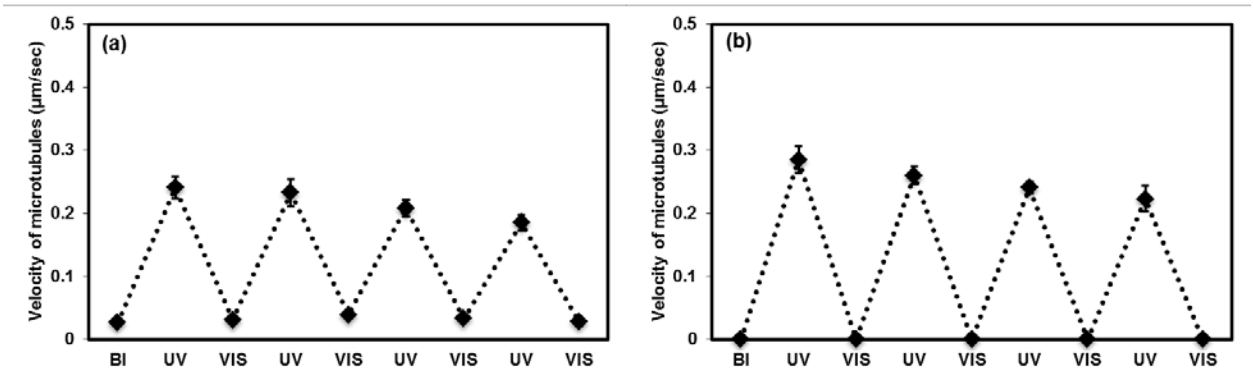


Figure S10. Repeatability of the Photo-controllable change in the gliding velocity of microtubules in presence of (a) compound **6** (2.0 mM), (b) compound **8** (2.0 mM) at 0.1 mM ATP concentration upon alternating irradiation with UV and visible light (BI: Before irradiation, UV: after 365 nm light irradiation for 40 s, VIS: after 436 nm light irradiation for 40 s). Error bars represents the standard deviation for 10 microtubules.

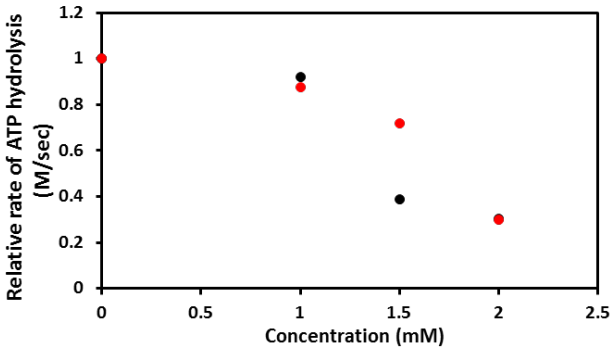


Figure S11. Rate of hydrolysis of ATP (1.0 mM) by kinesin/microtubules at various concentrations of compound **8** in its *trans* and *cis*-rich states. Black and red dots represent the reaction rates in presence of compound **8** in its *trans* and *cis*-rich states, respectively.

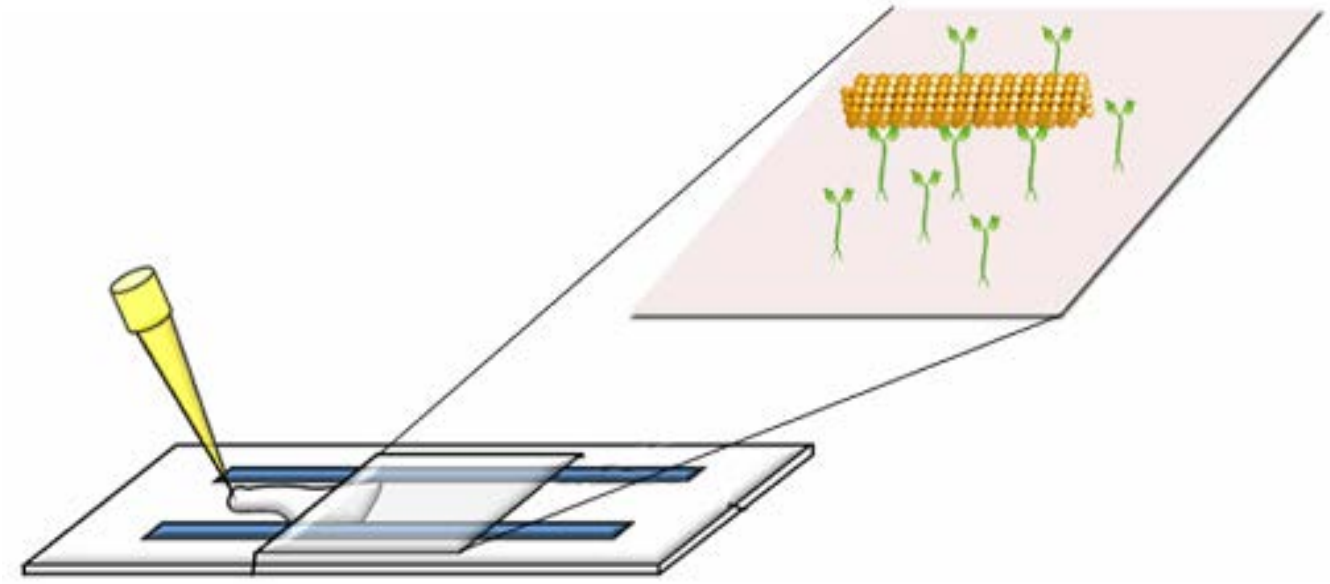


Figure S12. Schematic diagram of the *in vitro* kinesin-microtubule motility assay system.