JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

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Photoclickable Dendritic Molecular Glue: Noncovalent-to-Covalent Photochemical Transformation of Protein Hybrids

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Supporting Information

ABSTRACT: A water-soluble dendron with a fluorescein isothiocyanate (FITC) fluorescent label and bearing nine pendant guanidinium ion (Gu^+)/benzophenone (BP) pairs at its periphery ($Glue^{BP}$-FITC) serves as a "photoclickable molecular glue". By multivalent salt-bridge formation between Gu^+ ions and oxyanions, $Glue^{BP}$-FITC temporarily adheres to a kinesin/microtubule hybrid. Upon subsequent exposure to UV light, this noncovalent binding is made permanent via a cross-linking reaction mediated by carbon radicals derived from the photoexcited BP units. This temporal-to-permanent transformation by light occurs quickly and efficiently in this preorganized state, allowing the movements of microtubules on a kinesin-coated glass plate to be photochemically controlled. A fundamental difference between such temporal and permanent bindings was visualized by the use of "optical tweezers".

In living systems, fusion of certain biological motifs is known to occur in a stepwise fashion through intermediates upon temporal adhesion.[1−7] Such a dynamic nature is essential to prevent unfavorable kinetic traps from interfering with the cascade sequence of assembly events. An example of stepwise fixation in biology is immobilization of leukocytes at vascular endothelial cells,[1−3] where leukocytes are immobilized temporarily via selectin at the initial stage and then permanently via integrin. A similar mechanism operates for blood coagulation,[4,5] where fibrinogen utilizes its cell-adhesive peptide sequence to adhere onto cell surfaces before being polymerized for permanent fixation.

Here we report $Glue^{BP}$-FITC (Figure 1) as a "photoclickable molecular glue" that allows for efficient transformation of temporal (noncovalent) adhesion into permanent (covalent) fixation by UV light (Figure 2). This molecular glue is composed of a water-soluble dendron that accommodates guanidinium ion (Gu^+) pendants at its periphery. For optical visualization, the core part of this dendron is fluorescently labeled with fluorescein isothiocyanate (FITC). We recently reported that a prototype of this dendritic molecular glue adheres to proteins,[8,9] phospholipid membranes,[10] and clay nanosheets[11] via formation of a multivalent salt bridge between the Gu^+ pendants and oxyanions.[12] In proximity to each Gu^+ pendant, the newly designed $Glue^{BP}$-FITC bears a benzophenone (BP) unit, whose photoexcited triplet state can generate a reactive carbon radical.[13] When $Glue^{BP}$-FITC adheres to a

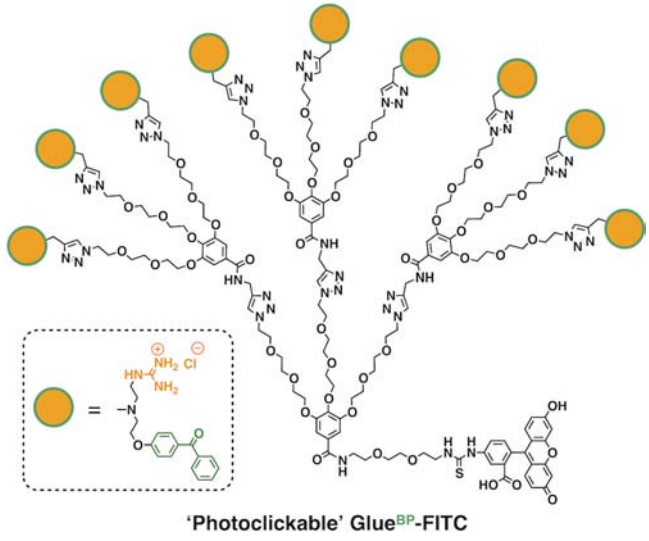

Figure 1. Schematic structure of the "photoclickable molecular glue" $Glue^{BP}$-FITC. Orange-and-green circles represent pendant adhesive guanidinium ion (Gu^+)/photoreactive benzophenone (BP) pairs.

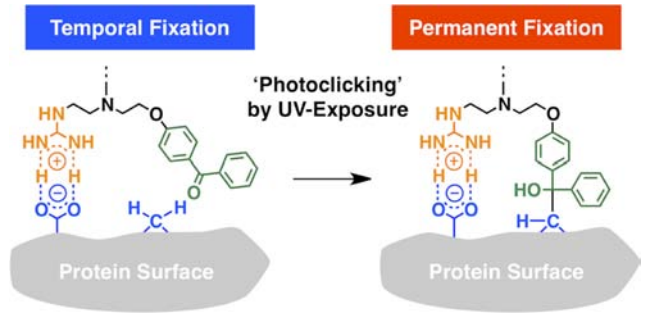

Figure 2. Schematic illustration of the "photoclickable molecular glue" concept: $Glue^{BP}$-FITC covalently binds to a protein via photoexcitation of benzophenone (BP) units after noncovalent adhesion mediated by formation of salt bridges between guanidinium (Gu^+) ions and protein oxyanions.

Received: January 30, 2013
Published: March 12, 2013

   dx.doi.org/10.1021/ja401059w | J. Am. Chem. Soc. 2013, 135, 4684−4687

protein, this carbon radical efficiently gives rise to the formation of a covalent bond between Glue^{BP}-FITC and the target protein (permanent fixation; Figure 2).[14] In a proof-of-concept study, we utilized Glue^{BP}-FITC for photocontrol of the translational movements of microtubules on a kinesin-coated glass surface[15] driven by ATP-fueled hand-over-hand motions of kinesin.[16] We also highlight results of an investigation using "optical tweezers" that shows how the affinity of microtubules with kinesin changes upon photoclicking of adhering Glue^{BP}-FITC.

Photoclickable Glue^{BP}-FITC (Figure 1)[17] was synthesized by incorporation of FITC into the focal core of a dendron carrying azide groups, a "click" reaction between the resultant FITC-labeled, azide-bearing dendron and dendrons carrying both an alkyne-substituted focal core and pendant Boc-protected Gu^{+}/BP pairs, and removal of the Boc groups. To investigate the photoinduced reactivity of Glue^{BP}-FITC, a mixture of Glue^{BP}-FITC (3 μM) and bovine serum albumin (BSA) (10 μM) was irradiated with a Xe arc lamp (305−315 nm bandpass filter) in Tris-HCl buffer ([Tris] = 20 mM, pH 7.0) over a period of 10 min. In electronic absorption spectroscopy, we observed a decrease in the absorption intensity at 270−320 nm (Figure S3 in the Supporting Information),[17] analogous to the case reported for photochemical covalent binding of BP with actin.[18] The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profiles developed by staining with Coomassie brilliant blue (CBB) (Figure 3a) did not change with the exposure time (iii−iv) but were substantially identical to those of (i) BSA and (ii) its non-irradiated mixture with Glue^{BP}-FITC. However, by reference to the SDS-PAGE profiles developed by FITC emission (λ_{ex} = 488 nm) without CBB (Figure 3b), we found that the protein fractions corresponding to the bands in Figure 3a(iii−vi) were fluorescent. Since the bands were not fluorescent unless the mixture of BSA and Glue^{BP}-FITC was UV-irradiated [Figure 3a and b(ii)], we conclude that Glue^{BP}-FITC was covalently attached to BSA upon UV irradiation. This photochemical reaction is rather quick, considering that the band was highly emissive even after UV irradiation for 1 min [Figure 3b(iii)]. It is also noteworthy that the secondary structure of BSA, as observed by its circular dichroism (CD) spectral profile at 200−250 nm (Figure S4), was virtually unchanged by this covalent immobilization process.[17]

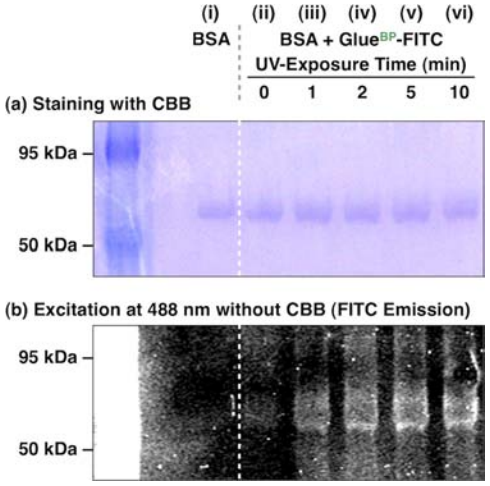

Figure 3. SDS-PAGE profiles of BSA (5.0 μM) in (i) the absence and (ii−vi) the presence of Glue^{BP}-FITC (3.0 μM) (ii) before and (iii−vi) after UV irradiation at 310 nm (Xe arc lamp, 305−315 nm bandpass filter) for (iii) 1, (iv) 2, (v) 5, and (vi) 10 min, developed by (a) staining with CBB or (b) fluorescence emission of FITC excited at 488 nm.

Glue^{BP}-FITC can covalently unite different proteins. For a demonstration, we chose a microtubule/kinesin biomachinery system. Kinesin is a motor protein that walks hand-over-hand along a microtubule strand,[16,19] fueled by repeated cycles of binding of ATP followed by its hydrolysis to ADP.[20] This machinery system plays a vital role in biological events such as cell division[21,22] and substance transportation,[23−25] for which kinesin and the microtubule are programmed to undergo noncovalent heterotropic hybridization. For the purpose of exploring possible effects of Glue^{BP}-FITC on the machinery motion, we prepared a flow cell with an interior volume of ∼20 μL in which Cy5-labeled fluorescent microtubules (8.8 ng/mL) were placed on a kinesin-coated glass plate.[17] The flow cell was then treated with Glue^{BP}-FITC (10 μM) and UV-irradiated for 2 min centered at 300 nm (handy UV lamp).[17] As shown in Figure 4a(iii) and b(iii), even after the admission of ATP buffer

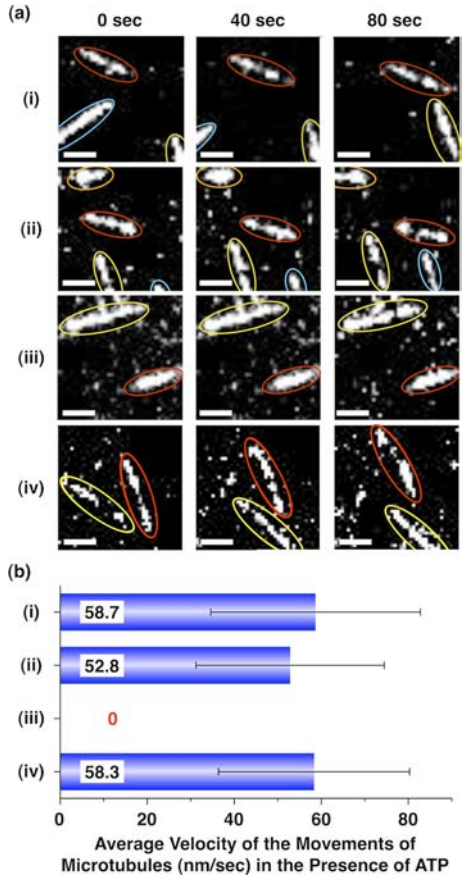

Figure 4. Effects of photoclickable Glue^{BP}-FITC (10 μM) on the translational movements of Cy5-labeled microtubules (8.8 ng/mL) on a kinesin-coated glass plate at 26 °C in ATP buffer.[26] Conditions: (i) +microtubules; (ii) +microtubules → +Glue^{BP}-FITC; (iii) +microtubules → +Glue^{BP}-FITC → +UV exposure for 2 min; (iv) +Glue^{BP}-FITC → +UV exposure for 2 min → +microtubules. For the UV exposure, samples were irradiated with UV light centered at 300 nm using a handy UV lamp. Before fluorescence microscopy, all of the samples were rinsed with ATP buffer. (a) Fluorescence microscopy traces (λ_{ex} = 635 nm) of Cy5-labeled microtubules in 0−80 s. Scale bars = 3 μm. (b) Average velocities of the microtubules.

dx.doi.org/10.1021/ja401059w | J. Am. Chem. Soc. 2013, 135, 4684−4687

([ATP] = 2 mM) to induce the hand-over-hand motions of kinesin,[26] the microtubules became completely immobile on the kinesin-coated glass plate, as observed by fluorescence microscopy (λ_{ex} = 635 nm). In contrast, without irradiation [Figure 4a(ii)], the microtubules moved at an average velocity of 52.8 nm/s [Figure 4b(ii)], which is comparable to the average velocity of 58.7 nm/s observed in a control experiment without Glue^{BP}-FITC [Figure 4a and b(i)]. On the other hand, when the kinesin-immobilized glass plate was first treated with Glue^{BP}-FITC (10 μM) and then irradiated for 2 min at 300 nm prior to hybridization with microtubules,[17] admission of ATP buffer as a fuel resulted in movement of the microtubules [Figure 4a and b(iv)]. Therefore, the Glue^{BP}-FITC-mediated photoreaction did not deteriorate the motor function of kinesin. BP in Glue^{BP}-FITC was essential for the covalent immobilization, as the use of a dendritic glue without BP[10] under identical conditions resulted in no photoinduced arrest of the microtubules (Figures S6 and S7).[17]

The above observations prompted us to investigate how the binding strength between kinesin and the microtubules changed upon adhesion with Glue^{BP}-FITC and subsequent photoclicking. For this purpose, we made use of "optical tweezers", which utilize a focused laser beam to generate a trapping force in the piconewton range[27,28] for holding a polystyrene (PS) bead. Figure 5a shows a schematic illustration of the principle of optical tweezers for our experiment. Through a strong His_6 tag (kinesin)/anti-His_6 antibody (PS bead) interaction, PS beads (4.7 pM) were attached to adenosine 5′-(β,γ-imido)triphosphate (AMP-PNP)−kinesin (no ATPase activity; 5.7 nM) that had been hybridized beforehand with microtubules (0.4 mg/mL) immobilized on a glass plate.[17] The above setup was constructed within a flow cell having an interior volume of ∼10 μL (Figure 5a). With a 1064 nm laser beam, 20 individual PS beads were held one-by-one and pulled to check whether they could be peeled off at a given trapping force, as determined by bright-field optical microscopy. The trapping force for holding a PS bead was varied over the range 30−200 pN,[17] where junction points other than that formed by the kinesin/microtubule hybridization are supposed not to break up.[29−31] Thus, when the trapping force is insufficient for the kinesin/microtubule hybrid to break up, the PS bead, which may have initially moved along a pulled direction, is eventually released from the optical tweezers and returned to the origin spontaneously (Figure 5c). On the other hand, when the kinesin/microtubule hybrid break up, the bead loses positional control after the laser trapping is turned off (Figure 5d). It should be noted that 1:1 kinesin/microtubule hybrids have been reported to break up when the kinesin part is pulled by an applied force of ∼17 pN.[32,33] Nevertheless, the stoichiometry of the kinesin/microtubule hybridization was unspecified and differed from bead to bead. Thus, we conducted three sets of the above experiments, each using a separately prepared flow-cell setup, and the data at each individual trapping force were averaged (Figure 5b).

Before the treatment with Glue^{BP}-FITC, the average number of residual PS beads that were not peeled off, among 20 beads tested, decreased monotonically from 15.3 to 5.7 as the applied trapping force was increased from 33 to 131 pN (Figure 5b, green circles). However, after the treatment with Glue^{BP}-FITC (4 μM),[17] the number of residual PS beads obviously increased (Figure 5b, blue circles), and the peel-off profile as a function of the applied trapping force showed an inflection point at 100 pN. In the range where the applied trapping force was larger

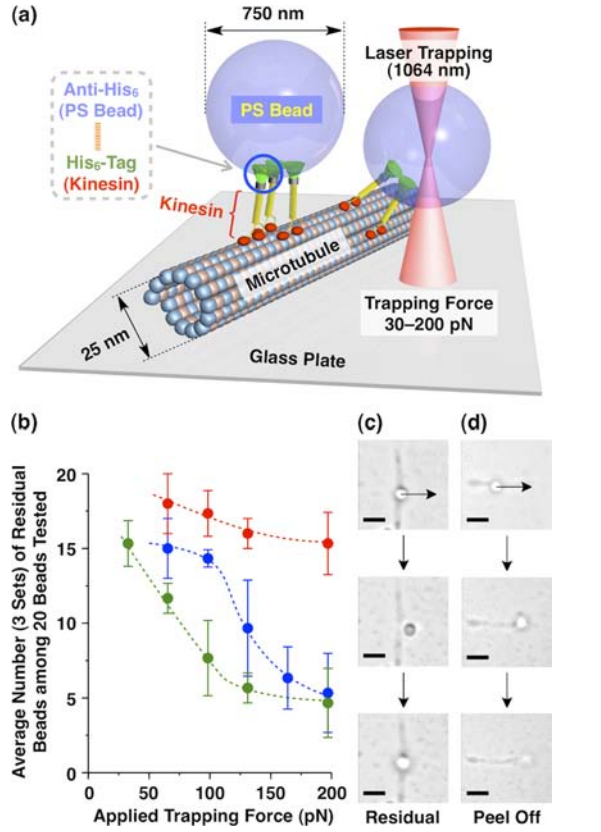

Figure 5. (a) Schematic illustration of the experimental setup using "optical tweezers" for evaluating the peel-off profiles of PS beads at 24 °C in bead buffer.[26] In a flow cell (∼10 μL), PS beads (4.7 pM) having anti-His_6 antibodies were allowed to attach noncovalently to the His_6 tag sequence of (AMP-PNP)−kinesin (no ATPase activity; 5.7 nM) that had been hybridized beforehand with microtubules (0.4 mg/mL) immobilized on the glass substrate. The the cell was then UV-irradiated at 300 nm (handy UV lamp). (b) Average numbers of residual PS beads that were not peeled off at various trapping forces applied. Samples were untreated (green) or treated with Glue^{BP}-FITC (4 μM) without (blue) or with (red) UV exposure for 3 min. (c, d) Bright-field microscopy traces of PS beads (c) returning and (d) peeled off after the laser trapping was turned off. Scale bars = 1 μm.

than 100 pN, the peel-off profile appeared to resemble that observed without Glue^{BP}-FITC (green circles). If it is supposed that the binding stoichiometry at each bead remained unchanged upon treatment with Glue^{BP}-FITC, the observed difference in the peel-off profiles indicates that even non-covalent adhesion of Glue^{BP}-FITC reinforced the kinesin/microtubule hybrid to a certain extent. We then performed UV irradiation centered at 300 nm (handy UV lamp) for 3 min to transform the noncovalent adhesion into a covalent one (Figure 2).[17] Obviously, the number of residual beads greatly increased, as only 4.7 PS beads were peeled off at a maximum trapping force of 200 pN (Figure 5b, red circles). As expected, unless the sample was pretreated with Glue^{BP}-FITC, the UV irradiation hardly changed the peel-off profile (Figure S10).[17]

In conclusion, we have demonstrated that water-soluble Glue^{BP}-FITC (Figure 1) having nine pendant guanidinium ion (Gu^+)/benzophenone (BP) pairs can serve as a "photoclickable molecular glue" that covalently attaches to proteins or unites different proteins through noncovalent preorganization. As studied by "optical tweezers", the temporal-to-permanent photochemical transformation gives rise to a notable change

dx.doi.org/10.1021/ja401059w | J. Am. Chem. Soc. 2013, 135, 4684−4687

in binding strength. This transformation proceeds quickly and efficiently, most likely due to the location of the photoreactive BP units in close proximity to the Gu^+ pendants adhering to the proteins. As exemplified by the use of BSA and kinesin as target proteins, neither noncovalent nor covalent binding appreciably deteriorates the secondary structures of the proteins or their biological functions. In relation to the accelerated proliferation of tumor cells, kinesin, which plays a key role in cell division,[20] has attracted particular attention.[34] In view of the fact that tumor cells express anionically charged heparan sulfate on their surfaces,[35] Gu^+-appended photoclickable $Glue^{BP}$ might be a potent candidate for the development of targeted cancer phototherapy.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis of $Glue^{BP}$-FITC; ^1H NMR, ^{13}C NMR, and MALDI-TOF-MS spectral data; electronic absorption and CD spectra; preparation of microtubule and kinesin samples for microscopy; and related experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the Center for NanoBio Integration at the University of Tokyo. This work was partially supported by Grants-in-Aid for Research Activity Start-up (23850007 to K.O.) and Scientific Research on Innovative Areas "Emergence in Chemistry" (20111010 to T. Aida) from MEXT. N.U. thanks JSPS for financial support through a Young Scientist Fellowship.

■ REFERENCES

(1) Rosen, S. D. *Annu. Rev. Immunol.* **2004**, *22*, 129.
(2) Schmidt, S.; Friedl, P. *Cell Tissue Res.* **2010**, *339*, 83.
(3) Ebert, M. L.; Schaerli, P.; Moser, B. *Mol. Immunol.* **2005**, *42*, 799.
(4) La Corte, A. L. C.; Philippou, H.; Ariës, R. A. S. *Adv. Protein Chem. Struct. Biol.* **2011**, *83*, 75.
(5) Sánchez-Cortés, J.; Mrksich, M. *Chem. Biol.* **2009**, *16*, 900.
(6) Jahn, R.; Scheller, R. H. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 631.
(7) Hutagalung, A. H.; Novick, P. J. *Physiol. Rev.* **2011**, *91*, 119.
(8) Okuro, K.; Kinbara, K.; Tsumoto, K.; Ishii, N.; Aida, T. *J. Am. Chem. Soc.* **2009**, *131*, 1626.
(9) Okuro, K.; Kinbara, K.; Takeda, K.; Inoue, Y.; Ishijima, A.; Aida, T. *Angew. Chem., Int. Ed.* **2010**, *49*, 3030.
(10) Suzuki, Y.; Okuro, K.; Takeuchi, T.; Aida, T. *J. Am. Chem. Soc.* **2012**, *134*, 15273.
(11) Wang, Q.; Mynar, J. L.; Yoshida, M.; Lee, E.; Lee, M.; Okuro, K.; Kinbara, K.; Aida, T. *Nature* **2010**, *463*, 339.
(12) Springs, B.; Haake, P. *Bioorg. Chem.* **1977**, *6*, 181.
(13) Vodovozova, E. L. *Biochemistry (Moscow)* **2007**, *72*, 1.
(14) Dormán, G.; Prestwich, G. D. *Trends Biotechnol.* **2000**, *18*, 64.
(15) Tomishige, M.; Vale, R. D. *J. Cell Biol.* **2000**, *151*, 108.
(16) Yildiz, A.; Tomishige, M.; Vale, R. D.; Selvin, P. R. *Science* **2004**, *303*, 676.
(17) See the Supporting Information.
(18) Tao, T.; Lamkin, M.; Scheiner, C. J. *Arch. Biochem. Biophys.* **1985**, *240*, 627.
(19) Vale, R. D. *Cell* **2003**, *112*, 467.
(20) Hackney, D. D. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6865.
(21) Endow, S. A. *Eur. J. Biochem.* **1999**, *262*, 12.
(22) Drummond, D. R. *Semin. Cell Dev. Biol.* **2011**, *22*, 927.
(23) Caviston, J. P.; Holzbaur, E. L. F. *Trends Cell Biol.* **2006**, *16*, 530.
(24) Goldstein, A. Y.; Wang, X.; Schwarz, T. L. *Curr. Opin. Neurobiol.* **2008**, *18*, 495.
(25) Gagnon, J. A.; Mowry, K. L. *Crit. Rev. Biochem. Mol. Biol.* **2011**, *46*, 229.
(26) ATP buffer (pH 6.8): [PIPES] = 80 mM, [ATP] = 2 mM, [$MgCl_2$] = 1 mM, [EGTA] = 0.5 mM, [glucose] = 4.5 mg/mL, [glucose oxidase] = 2.16 mg/mL, [catalase] = 36 μg/mL, [creatine phosphate] = 2 mM, [creatine kinase] = 10 μg/mL, [2-mercaptoethanol] = 0.5%, [paclitaxel] = 20 μM. Bead buffer (pH 6.8): [PIPES] = 80 mM, [$MgCl_2$] = 1 mM, [EGTA] = 0.5 mM, [paclitaxel] = 20 μM, [casein] = 4 mg/mL, [bead] = 4.7 pM.
(27) Neuman, K. C.; Block, S. M. *Rev. Sci. Instrum.* **2004**, *75*, 2787.
(28) Ashok, P. C.; Dholakia, K. *Curr. Opin. Biotechnol.* **2012**, *23*, 16.
(29) Tsapikouni, T. S.; Missirlis, Y. F. *Colloids Surf., B* **2010**, *75*, 252.
(30) McGurk, S. L.; Green, R. J.; Sanders, G. H. W.; Davies, M. C.; Roberts, C. J.; Tendler, S. J. B.; Williams, P. M. *Langmuir* **1999**, *15*, 5136.
(31) Lee, C.-K.; Wang, Y.-M.; Huang, L.-S.; Lin, S. *Micron* **2007**, *38*, 446.
(32) Kawaguchi, K.; Ishiwata, S. *Science* **2001**, *291*, 667.
(33) Uemura, S.; Kawaguchi, K.; Yajima, J.; Edamatsu, M.; Toyoshima, Y. Y.; Ishiwata, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5977.
(34) Zhang, Y.; Xu, W. *Anticancer Agents Med. Chem.* **2008**, *8*, 698.
(35) Fuster, M. M.; Esko, J. D. *Nat. Rev. Cancer* **2005**, *5*, 526.