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Direct force measurement between cucurbit[6]uril and spermine using atomic force microscopy

Jeonghan Kim, Youngkook Kim, Kangkyun Baek, Young Ho Ko, Dongwoo Kim, Kimoon Kim *

National Creative Research Initiative Center for Smart Supramolecules and Department of Chemistry, Pohang University of Science and Technology, San 31 Hyojadong, Pohang 790-784, Republic of Korea

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ABSTRACT

The rupture forces of individual host–guest complexes between surface-confined cucurbit[6]uril (CB[6]) and spermine derivatives were measured directly by atomic force microscopy (AFM). While 1,2-dithiolane-attached spermine was immobilized on a gold-coated AFM tip, perallyloxyCB[6] was attached to an allyl-terminated self-assembled monolayer on a gold substrate by olefin metathesis reaction. A histogram and autocorrelation function analysis yielded a rupture force of approximately 120 pN, which is the highest value ever reported for a synthetic host–guest system.

1. Introduction

Supramolecular chemistry enables us to better understand phenomena frequently occurring in nature. Such phenomena utilize reversible short-range interactions including molecular interactions and molecular recognition events in spontaneous self-assembly processes.[1] Although the reversible interactions have been investigated extensively through measuring their thermodynamics using isothermal titration calorimetry (ITC), analytical ultracentrifugation (AUC), and other techniques,[2] the recent development of nanotechnology requires fundamental information about such interactions at a molecular level. Atomic force microscopy (AFM) has been utilized to visualize the surface structures of individual biological molecules and of molecular assemblies.[3] In addition, AFM allows one to directly measure forces between two interacting objects in nanonewton resolution.[4–9] Taking advantage of the unique feature of AFM, many research groups have applied this technique to study molecular interactions of biological molecules including complementary interactions of DNA molecules[4] and biotin–avidin system.[5] Recently, the AFM technique was extended to synthetic molecule pairs such as β-cyclodextrin/ferrocene,[6] ammonium/crown ether,[7] and TMPD/TCNQ charge transfer complex.[8]

Cucurbit[6]uril (CB[6]), a member of the host family cucurbit[*n*]uril (CB[*n*]) comprising six glycoluril units, has a cavity accessible through two identical carbonyl laced portals.[10] The polar carbonyl groups at the portals and the hydrophobic cavity allow the cavitand to form stable host–guest complexes with small molecules such as protonated aminoalkanes.[10] In particular, tetracationic spermine, $NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$, forms an extraordinarily stable complex with CB[6],[11] the stability constant of which was recently measured to be $5.4\times10^{10}\ M^{-1}$ in 0.2 M LiCl.[12] In this complex, the central tetramethylene moiety of spermine threads through the CB[6] cavity, while the two dicationic side arms coordinate to the both portals. Furthermore, the binding constant of spermine to a water soluble CB[6] derivative[14a] in pure water was measured to be as high as $10^{12}\ M^{-1}$, which is the highest affinity ever observed for a CB[6] complex.

Despite rich supramolecular chemistry, practical applications of CB[*n*] were limited until recently mainly because of the difficulty in introducing functional groups on their surfaces. A few years ago, however, we developed a direct functionalization method of CB[*n*],[15a] which allowed us to synthesize a wide variety of tailor made CB[*n*] derivatives and to study their applications such as mimicking ion channels, synthesis of polymers and vesicles, and immobilization of CB[*n*] on solid surfaces.[15,16] The strong interaction between CB[6] and spermine has been exploited in noncovalent modification of the surfaces of vesicles, polymer nanocapsules made of CB[6] derivatives,[16a,c] and in visualization of CB[6]-immobilized surfaces.[15a] The functionalization of CB[*n*] and subsequent immobilization of CB[*n*] derivatives on solid surfaces also led us to study the rupture force of individual complexes between CB[*n*] and guest molecules by AFM. Herein we report, for the first time, the direct force measurement between surface-confined CB[6] and spermine derivatives using AFM.

* Corresponding author. Tel.: +82 54 279 2113; fax: +82 54 279 8129.
E-mail address: kkim@postech.ac.kr (K. Kim).

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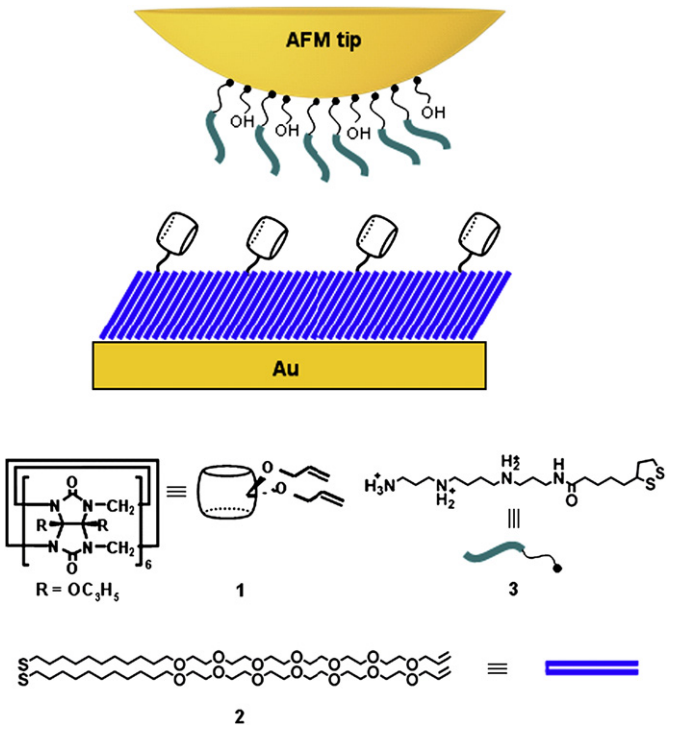
2. Results and discussion

2.1. Modification of AFM tips and solid surfaces

To measure the interaction between CB[6] and spermine by AFM, a spermine derivative was immobilized in self-assembled monolayers on gold-coated AFM tips, while a CB[6] derivative was chemically attached to allyl-terminated self-assembled monolayers on gold substrate by olefin metathesis, as illustrated in Scheme 1. For immobilization of spermine on a gold-coated AFM tip, 1,2-ditholane-derivatized spermine **3** was first synthesized (Scheme 2). A gold-coated tip (spring constant k_c=0.16 Nm^{-1}) was chemically modified with **3** and the remaining gold surface was passivated with 6-mercapto-1-hexanol to prevent nonspecific adsorption. Successful deposition of the spermine derivative **3** on gold was confirmed indirectly by an SPR experiment (see Supplementary data).



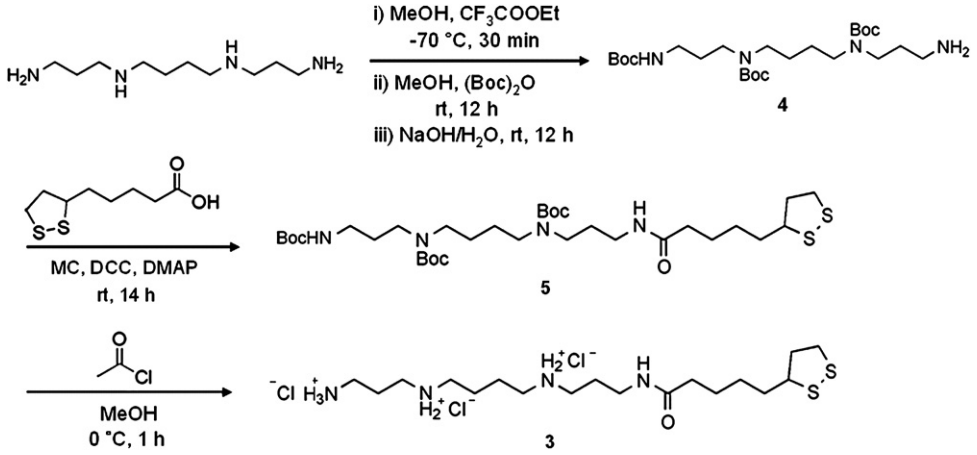
Scheme 1. Schematic representation of direct force measurements between a **1**-immobilized substrate and **3**-immobilized gold-coated AFM tip.

A CB[6]-immobilized substrate was prepared in two steps. First, a self-assembled monolayer (SAM) of **2** on a gold-coated silicon substrate was prepared by dipping the substrate in a solution of **2** in ethanol. The gold substrate with SAM of **2** was immersed into a dichloromethane solution of perallyloxyCB[6] (**1**) and the second generation Grubbs catalyst, $Cl_2P(Cy)_3$(IMes)Ru(=CHPh), IMes=1,3-(2′,4′,6′-trimethylphenyl)imidazol-2-ylidene, for 10 min. The attachment of **1** on the SAM was confirmed by reflectance FTIR spectroscopy (see Supplementary data). The intensity of the characteristic carbonyl peak of CB[6] at 1750 cm^{-1} increased with increasing the reaction time. The intensity reached a plateau within 10 min.

2.2. AFM measurements

Figure 1a shows a representative pull-off force measured in water between a **1**-immobilized substrate and a **3**-modified AFM tip. A stepwise feature was observed in 90% of the force–distance curves, which is likely due to the discrete bond ruptures of molecular interactions between the substrate and tip. This behavior was repeatedly observed throughout the whole measurements, implying that the binding events are reversible. The separation distance among individual peaks in the force–distance curves is different because of the granular feature of the gold-coated AFM tip and substrate. Only single pull-off events were observed in the remaining 10% of the force–distance curves.

Two control experiments were performed. In the first control experiment, an AFM tip was modified with 6-mercapto-1-hexanol and its interaction with **1**-immobilized substrate was measured. The force–distance curves showed only one pull-off event with much smaller pull-off forces (Fig. 1b). This observation is consistent with the fact that alcohols form no or weak inclusion complexes with CB[6] in aqueous solution.[12,13] The second control experiment was carried out with a **2**-modified substrate and **3**-modified AFM tip (see Supplementary data). This force–distance curves also showed only single smaller pull-off event (Fig. 1c), indicating that there is no significant binding between a terminal allyl group and spermine.[17] The smaller forces observed in the two control experiments presumably arise from nonspecific interactions such as van der Waals interactions between the chemically-modified tip and substrate. These two control experiments confirmed that the multiple binding events shown in Figure 1a are attributed to the formation of host–guest complexes between the surface-confined CB[6] and spermine derivatives.



Scheme 2. Synthetic scheme for **3**.

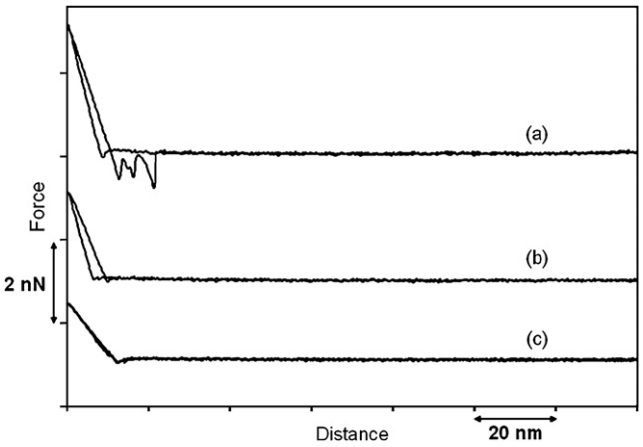


Figure 1. Representative force–distance curves obtained in water between (a) a **1**-immobilized substrate and a **3**-modified AFM tip, (b) a **1**-immobilized substrate and a 6-mercapto-1-hexanol-modified AFM tip, (c) a **2**-modified substrate and a **3**-modified AFM tip.

2.3. Statistical analysis

The statistical analysis of the individual rupture forces revealed quantized maxima. A histogram of the pull-off forces measured between a **1**-immobilized substrate and a **3**-modified AFM tip and its autocorrelation function are shown in Figures 2 and 3, respectively. In the histogram, the quantized maxima with a periodicity of approximately 120 pN were observed, as indicated by arrows in Figure 2, which was confirmed by its autocorrelation function analysis (Fig. 3).[6a] In the AFM force measurement, a multivalent effect is negligible, especially, at a low range of rupture force, since its probability is extremely low from the statistical viewpoint. The 120 pN periodicity, therefore, strongly indicates that the rupture forces observed in the experiment result from the breaking of the host–guest complexes between surface-confined CB[6] and spermine derivatives.

The values of rupture force measured by single molecule force spectroscopy can be correlated with thermodynamic parameters of the molecular interaction between host and guest molecules. Previous AFM studies[6–9] suggested that the force quanta and thermodynamic parameters of inclusion complexes follow the same trend, although a direct conversion of rupture force to binding enthalpy is generally not possible. For example, the free binding energy ΔG^0 for the complexes of β-cyclodextrin (β-CD) with ferrocene and adamantanamine derivatives are −22.6 kJ/mol and −27.6 kJ/mol, respectively, for which the rupture force quanta were

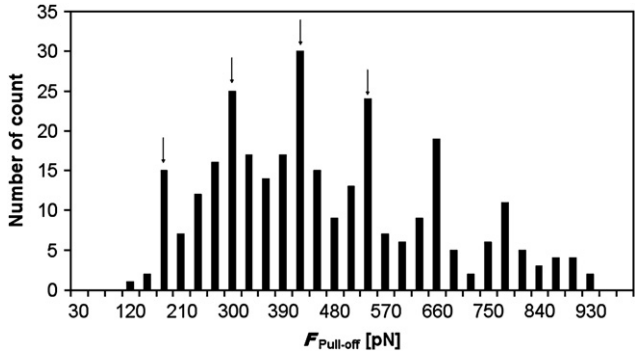


Figure 2. Histogram of rupture forces obtained in water between a **1**-immobilized substrate and a **3**-functionalized AFM tip. The arrows indicate the force maxima with a periodicity of 120 pN.

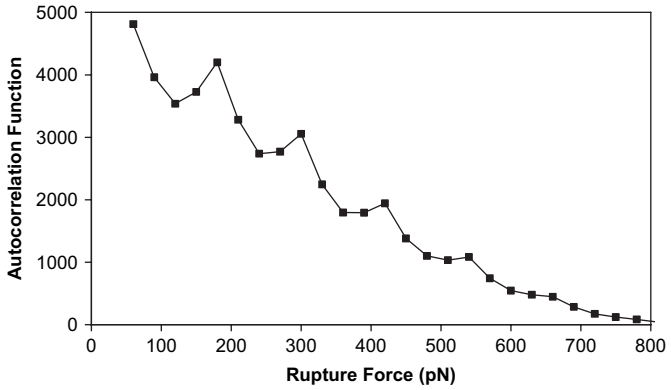


Figure 3. Autocorrelation function of the histogram of the rupture forces shown in Figure 2.

observed to be 55±10 and 102±15 pN, respectively.[6c] In the case of biotin–avidin complex with an extraordinary high affinity of $\sim 10^{15}\ M^{-1}$ (ΔG^0 ~85 kJ/mol),[18] the force was found to be 160±20 pN. Since the binding constant for the complex between a water soluble CB[6] derivative and tetracationic spermine in water is about $\sim 10^{12}\ M^{-1}$ ($\Delta G^0 = -72$ kJ/mol),[14] the rupture force of 120±20 pN for the surface-confined CB[6]-spermine complex looks reasonable, compared with those reported for the previous host–guest complexes.

3. Conclusions

In conclusion, we have shown that the specific interactions between surface-confined CB[6] and spermine derivatives in aqueous media can be measured on a molecular level, using AFM, which may help understand interactions between an individual host–guest pair. The histogram and autocorrelation function analysis showed the quantized rupture force of 120 pN, which is, to the best of our knowledge, the highest value ever reported for synthetic host–guest pairs. This result, together with the thermodynamic parameters such as binding constants, can contribute to better understanding of the host–guest chemistry of CB[6] on a molecular level.

4. Experimental section

4.1. General methods

All the reagents and solvents employed were commercially available and used as supplied without further purification. PerallyloxyCB[6] (**1**) and disulfide compound **2** were synthesized according to the literature.[15,16] NMR data were recorded on a Bruker DRX500 spectrometer. FTIR spectra were recorded on a Perkin–Elmer Spectrum GX FT-IR spectrophotometer.

4.2. Synthesis and characterization

4.2.1. Compound ***4***[19]

Spermine (1.0 g, 4.9 mmol) was dissolved in MeOH under N_2 and the solution was cooled to −70 °C, before ethyl trifluoroacetate (0.65 mL, 5.5 mmol) was added dropwise over 30 min. The temperature was then allowed to rise to 0 °C and the mixture was stirred for an additional 30 min at 0 °C. A solution of di-*tert*-butyl dicarbonate (5 g, 23 mmol) in MeOH was then added over 10 min. The reaction mixture was stirred at rt for 12 h, and water and concentrated aqueous NaOH were added. After the mixture was stirred at rt for 12 h, MeOH was removed under a reduced pressure,

then CH_2Cl_2 and H_2O were added. The aqueous layer was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4. After removal of the solvent, the resulting oil was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (19:1) as an eluent to yield **4** as a pale yellow oil (1.2 g, 48%).

*4.2.2. Compound **5***

A solution of DCC (210 mg, 1.02 mmol) in CH_2Cl_2 was added to a solution of **4** (500 mg, 1.00 mmol), thioctic acid (210 mg, 1.02 mmol), and DMAP (25 mg, 0.20 mmol) in CH_2Cl_2. The reaction mixture was stirred for 14 h. After removal of the solvent, the resulting oil was purified by column chromatography on silica gel using *n*-hex/EA (1:1) as an eluent to yield **5** as pale yellow oil (500 mg, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ=3.62–3.53 (1H, m), 3.35–3.02 (14H, br), 2.51–2.41 (1H, m), 2.25–2.15 (2H, m), 1.98–1.91 (1H, m), 1.78–1.55 (8H, m), 1.53–1.37 (31H, br); ^{13}C NMR (125 MHz, $CDCl_3$) δ=172.8, 156.5, 156.0, 79.7, 78.9, 56.4, 46.7, 46.2, 44.3, 43.8, 43.4, 40.2, 38.5, 37.4, 36.6, 35.5, 34.7, 28.9, 28.5, 27.7, 26.1, 25.5; HRMS-FAB *m*/*z*: $[M+H]^+$ calcd for $C_{33}H_{63}N_4O_7S_2$: 691.4138, found 691.4130.

*4.2.3. Compound **3***

To a solution of **5** (500 mg, 0.72 mmol) in methanol (5 mL), acetyl chloride (1 mL) was added slowly at 0 °C. After stirring for 1 h, white precipitate was filtered off and washed with methanol and acetonitrile. Removal of the solvent yielded **3** as a white solid (360 mg, 98%). Mp >240 °C; ^{1}H NMR (500 MHz, D_2O) δ=3.72–3.75 (1H, m), 3.31 (2H, t, *J*=6.5 Hz), 3.20–3.22 (2H, m), 3.16 (2H, t, *J*=6.5 Hz), 3.12 (8H, t, *J*=7.5 Hz), 2.45–2.55 (1H, m), 2.29 (2H, t, *J*=7.0 Hz), 2.03–2.16 (H, m), 1.96–2.03 (1H, m), 1.86–1.95 (2H, m), 1.80 (5H, s), 1.55–1.72 (3H, m), 1.38–1.46 (2H, m); ^{13}C NMR (125 MHz, D_2O) δ=177.8, 57.0, 47.4, 47.3, 45.5, 44.9, 40.7, 38.5, 36.9, 36.3, 35.8, 34.0, 28.3, 26.1, 25.3, 24.1, 23.2; HRMS-FAB *m*/*z*: $[M-3HCl+H]^+$ calcd for $C_{18}H_{39}N_4OS_2$: 391.2565, found 391.2570.

4.3. Preparation of 1-anchored self-assembled monolayer (SAM) on gold

*4.3.1. SAM of **2** on gold*

Gold substrates were prepared by evaporating 2 nm of Ti as an adhesion layer, followed by 250 nm Au onto silicon (100) wafer. A gold substrate (10×10 mm) was cleaned by freshly prepared piranha solution for 3 min, then rinsed thoroughly with deionized water and dried in vacuum for 1 h. The cleaned Au substrate was immersed in ethanol (10 mL) solution of **2** (102 mg, 0.1 mmol) for 1 day. The substrate was washed with ethanol several times and dried under a stream of nitrogen to produce SAM of **2** on gold with allyl terminal groups.

*4.3.2. **1**-Anchored SAM on gold*

The SAM of **2** on gold was immersed in CH_2Cl_2 (10 mL) solution containing $[Cl_2P(Cy)_3(IMes)Ru(=CHPh)]$ (IMes=1,3-(2′,4′,6′-trimethylphenyl)imidazol-2-ylidene) (second generation Grubbs catalyst) (8.5 mg, 0.010 mmol) at rt for 5 min. The resulting substrate was thoroughly washed with CH_2Cl_2 and then immersed in a freshly prepared DMF (10 mL) solution containing **1** (15 mg, 0.0090 mmol) at rt for 10 min. The gold substrate was rinsed with DMF and ethanol for several times and dried under a stream of nitrogen to produce **1**-anchored SAM on gold. Its FTIR spectrum is shown in Figure S2.

4.4. AFM measurement and tip modification

Triangular-shaped, gold-coated, silicon nitride tips (k_c=0.16 Nm^{-1}, Olympus) were immersed in a 1 mM aqueous solution of **3** for 12 h. After thoroughly rinsing with deionized water, the tips were immersed again in a 1 mM aqueous solution of 6-mercapto-1-hexanol for 12 h. The cantilever was then rinsed with deionized water and ethanol. AFM measurements were carried out with a NanoScope IIIa multimode AFM (Digital Instrument) utilizing a liquid cell (Digital Instrument). The uploading rate was 9.6 nN/s. The quantitative analysis of the observed individual pull-off events was performed for 300 force curves at 10 different positions of **1**-anchored SAM. The noise level of the forces measured with the apparatus was estimated to be ±20 pN from the baseline of force curves near its pull-off point. Only the forces larger than 30 pN were included for the quantitative force analysis, considering the noise level originating from the inherent vibration of the AFM cantilever occurring in the pull-off events.

4.5. SPR experiment for measuring surface density of 3 on an Au substrate

Surface plasmon resonance (SPR) experiments were carried out at 25 °C using a BIACore 2000 and Pioneer Sensor Chip J1 (plain gold surface), which has a gold substrate with a size of 7×7 mm. SPR measures the angle of light (θ) reflected from the backside of the gold substrate that is a minimum in intensity. Changes in this angle ($\Delta\theta$) are linearly related to the index of refraction of the solution above the surface and therefore to the density of adsorbed molecules ($\Delta\theta$ of 0.10°=1 ng/mm^2=1000 RU). SPR experiments were performed at the constant flow rate of 10 μL min^{-1} over gold surfaces at 25 °C.

Acknowledgements

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Supplementary data

SPR sensorgram of **3** on an Au substrate, FTIR spectra of SAM of **2** on gold and **1**-anchored SAM, and histograms of rupture forces obtained in control experiments are presented as supplementary materials. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.05.045.

References and notes

1. (a) Lehn, J. M. *Supramolecular Chemistry: Concepts and Perspectives*; VCH: Weinheim, 1995; (b) Reinhoudt, D. N. *Supramolecular Materials*; Wiley: New York, NY, 1999; (c) Buckingham, A. D.; Legon, A. C.; Roberts, S. M. *Principles of Molecular Recognition*; Blackie: London, 1993.
2. Phizieky, E. M.; Fields, S. *Microbiol. Rev.* **1995**, *59*, 94–123.
3. (a) Gaub, H.; Engel, A. *J. Struct. Biol.* **1997**, *119*, 83–237; (b) Baumgartner, W.; Hinterdorfer, P.; Ness, W.; Raab, A.; Vestweber, D.; Schindler, H.; Drenckhahn, D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *9*, 4005–4010.
4. (a) Lee, G. U.; Chrisey, L. A.; Colton, R. J. *Science* **1994**, *266*, 771–773; (b) Moy, V. T.; Florin, E.-L.; Rief, M.; Lehmann, H.; Ludwig, M.; Gaub, H. E.; Dornmair, K. *Proc. SPIE-Int. Soc. Opt. Eng.* **1995**, *2384*, 2–12.
5. (a) Florin, E.-L.; Moy, V. T.; Gaub, H. E. *Science* **1994**, *264*, 415–417; (b) Moy, V. T.; Florin, E.-L.; Gaub, H. E. *Science* **1994**, *266*, 257–259.
6. (a) Schönherr, H.; Beulen, M. W. J.; Bügler, J.; Huskens, J.; van Veggel, F. C. J. M.; Reinhoudt, D. N.; Vansco, G. J. *J. Am. Chem. Soc.* **2000**, *122*, 4963–4967; (b) Zapotoczny, S.; Auletta, T.; de Jong, M. R.; Schönherr, H.; Huskens, J.; van Veggel, F. C. J. M.; Reinhoudt, D. N.; Vansco, G. J. *Langmuir* **2002**, *18*, 6988–6994; (c) Auletta, T.; de Jong, M. R.; Mulder, A.; van Veggel, F. C. J. M.; Huskens, J.; Reinhoudt, D. N.; Zou, S.; Zapotoczny, S.; Schönherr, H.; Vansco, G. J.; Kuipers, L. *J. Am. Chem. Soc.* **2004**, *126*, 1577–1584.
7. Kado, S.; Kimura, K. *J. Am. Chem. Soc.* **2003**, *125*, 4560–4564.
8. Frisbie, C. D.; Skulason, H. *J. Am. Chem. Soc.* **2002**, *124*, 15125–15133.
9. Kim, J.; Liu, Y.; Ahn, S. J.; Zauscher, S.; Karty, J. M.; Yamanaka, Y.; Craig, S. L. *Adv. Mater.* **2005**, *17*, 1749–1753.
10. Reviews: (a) Mock, W. L. *Top. Curr. Chem.* **1995**, *175*, 1–24; (b) Lee, J. W.; Samal, S.; Selvapalam, N.; Kim, H.-J.; Kim, K. *Acc. Chem. Res.* **2003**, *36*, 621–630; (c) Kim, K.; Kim, H.-J. *Encyclopedia of Supramolecular Chemistry*; Atwood, J. L., Steed, J. W.,

Eds.; Marcel Dekker: New York, NY, 2004; pp 390–397; (d) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 4844–4870.
11. (a) Mock, W. L.; Shih, N.-Y. *J. Org. Chem.* **1986**, *51*, 4440–4446; (b) Buschmann, H.-J.; Wego, A.; Zielesny, A.; Schollmeyer, E. *J. Inclusion Phenom.* **2006**, *54*, 241–246.
12. Rekharsky, M. V.; Ko, Y. H.; Selvapalam, N.; Kim, K.; Inoue, Y. *Supramol. Chem.* **2007**, *19*, 39–46.
13. Buschmann, H.-J.; Jansen, K.; Schollmeyer, E. *Thermochim. Acta* **2000**, *346*, 33–36.
14. (a) Zhao, J.; Kim, H.-J.; Oh, J.; Kim, S.-Y.; Lee, J. W.; Sakamoto, S.; Yamaguchi, K.; Kim, K. *Angew. Chem., Int. Ed.* **2001**, *40*, 4233–4235; (b) Kim, Y.; Kim, H.; Ko, Y. H.; Selvapalam, N.; Rekharsky, M. V.; Inoue, Y.; Kim, K., in preparation.
15. (a) Jon, S. Y.; Selvapalam, N.; Oh, D. H.; Kang, J.-K.; Kim, S.-Y.; Jeon, Y. J.; Lee, J. W.; Kim, K. *J. Am. Chem. Soc.* **2003**, *125*, 10186–10187; (b) Kim, K.; Selvapalam, N.; Ko, Y. H.; Park, K. M.; Kim, D.; Kim, J. *Chem. Soc. Rev.* **2007**, *36*, 267–279 and references therein.
16. (a) Lee, H.-K.; Park, K. M.; Jeon, Y. J.; Kim, D.; Oh, D. H.; Kim, H. S.; Park, C. K.; Kim, K. *J. Am. Chem. Soc.* **2005**, *127*, 5006–5007; (b) Jeon, Y. J.; Kim, H.; Jon, S.; Selvapalam, N.; Oh, D. H.; Seo, I.; Park, C.-S.; Jung, S. R.; Koh, D.-S.; Kim, K. *J. Am. Chem. Soc.* **2004**, *126*, 15944–15945; (c) Hwang, I.; Baek, K.; Jung, M.; Kim, Y.; Park, K. M.; Lee, D.-W.; Selvapalam, N.; Kim, K. *J. Am. Chem. Soc.* **2007**, *129*, 4170–4171; (d) Kim, D.; Kim, E.; Kim, J.; Park, K. M.; Baek, K.; Jung, M.; Ko, Y. H.; Sung, W.; Kim, H. S.; Suh, J. H.; Park, C. G.; Na, O. S.; Lee, D.-K.; Lee, K. E.; Han, S. S.; Kim, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 3471–3474.
17. The second control experiment showed somewhat larger pull-off forces compared to the first control experiments (see Supplementary data). The origin of this phenomenon is not clear but may be due to the hydrophobic nature of the 2-modified substrate.
18. (a) Green, N. M. *Biochem. J.* **1963**, *89*, 585–591; (b) Green, N. M. *Adv. Protein Chem.* **1975**, *29*, 85–133; (c) Green, N. M. *Methods Enzymol.* **1990**, *184*, 51–67.
19. (a) Blagbrough, I. S.; Geall, A. J. *Tetrahedron Lett.* **1998**, *39*, 439–442; (b) Wellendorph, P.; Jaroszewski, J. W.; Hansen, S. H.; Franzyk, H. *Eur. J. Med. Chem.* **2003**, *38*, 117–122.