Supramolecular Polymers

Stretching and Rupturing Individual Supramolecular Polymer Chains by AFM**

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Dedicated to Professor Helmut Ringsdorf
on the occasion of his 75th birthday

Inspired by nature, supramolecular chemistry utilizes multiple, reversible cooperative intermolecular interactions, such as hydrogen bonding, to fabricate self-organized structures with a very rich structural hierarchy.[1] The corresponding intermolecular interactions (forces)[2] govern the interplay of order and mobility,[3] as well as the properties and functionality (recognition, reaction, transport, regulation etc.) in such systems.[3,4]

In recent years, supramolecular polymers[5,6] (also called reversible polymers) comprised of bifunctional monomeric units that are reversibly aggregated through relatively strong noncovalent interactions were described.[6–9] A prime example are the supramolecular polymers formed through self-complementary recognition of the quadruple hydrogen-bonded 2-ureido-4[1H]-pyrimidinone (UPy) motif, introduced by Sijbesma, Meijer et al.[6,10,11] Owing to the highly dynamic character of the aggregation, these materials show a truly unique combination of tunable properties, such as viscosity, composition, or effective chain length on one hand, and short equilibration times on the other hand.[6] Even though these properties are a direct consequence of the presence of multiple weak interactions, typically the equilibrium complexation constants and complex lifetimes serve as measures for the strength of the interactions.[12] Recently, theoretical work by van der Gucht et al.[13] shed some light on the forces between surfaces in the presence of reversible polymers. From an experimental point of view, however, little is known about the actual underlying inter- and intramolecular forces.[14]

AFM-based single-molecule force spectroscopy (SMFS)[15–18] appears to be suitable to address interaction forces and to directly probe individual supramolecular polymer chains. As illustrated in Scheme 1, such experiments involve the probing of a dynamic "macromolecule" that comprises reversibly aggregated self-complementary building blocks. As we will show herein, the interactions between individual surface-immobilized UPys[19] and the stretching of reversible supramolecular polymer chains were probed in AFM force-displacement (fd) measurements in hexadecane (HD) and DMSO/propan-2-ol mixtures. Our results open the pathway to elucidate the properties of these dynamic, reversibly aggregated polymers as a function of various external parameters on the molecular scale.

The systematic investigation of the single-chain mechanical properties of supramolecular UPy polymers was based on the measurement of the individual molecular interactions present in these supramolecular polymers. For this purpose, we first prepared the monolayer architecture **3** on Au(111), which comprised surface-immobilized ω-hydroxy PEG chains, by coupling UPy isocyanate **2** to the terminal alcohol functionalities of a layer of **1** (Scheme 1).[20] Similar to **5**,[19] this architecture ensures that the UPy units are available for complexation in a solution-like environment, as indicated by differential pulse voltammetry experiments with the ferrocenyl-labeled UPy derivative **6** (see Supporting Information). Layers of **3** thus expose chemically accessible UPy units tethered by PEG spacers to gold surfaces. As the mechanical properties of these spacers are known,[21] we can identify and verify the stretching of single macromolecules in SMFS measurements.

Force displacement curves were measured in HD between **3**, which was immobilized on Au(111), and AFM tips that were functionalized with **5**. The data were converted into force–extension curves and fitted with the m-FJC model (Figure 1; see also the Experimental Section, Equation (1)). The observed stretching is attributed to the stretching of the PEG segment of **3**. The Kuhn length of PEG was found to be 0.65 ± 0.08 nm and the segment elasticity is 6.2 ± 0.6 nN nm^{-1}. These fit parameters agree well with reported data[21] and thus confirm the successful stretching of single PEG chains. The observed extensions agree favorably with the contour length of the linker ($\approx 12 \pm 3$ nm; PEG chain is 9.5 ± 1.0 nm). As single PEG chains are stretched, the rupture forces of a single UPy–UPy complex can be directly determined as 180 ± 21 pN in HD ($T = 301$ K, loading rate: 35 nN s^{-1}, see also Supporting Information).

To probe the single-chain mechanical properties of UPy-based supramolecular polymers, AFM measurements were carried out with tips and substrates that were both modified with the short-chain UPy disulfide **5** in the presence of bis(UPy) **4** in HD (10^{-3} mM). Owing to the high complexation constant of $\geq 10^9$ M^{-1} and a complex lifetime greater than 1 s,[11b,12] bis(UPy) forms supramolecular polymers with an effective degree of polymerization of approximately 40 in solution.[22] Figure 2 shows a typical force–extension curve measured under these experimental conditions.

A significant restoring force is observed only for extensions greater than 50 nm. Fits to Equation (1) yielded values for the Kuhn length of 0.68 ± 0.08 nm and a segment elasticity of 7.6 ± 2.5 nN nm^{-1}. The elasticity parameters are within the error margins for the data measured for a single PEG chain

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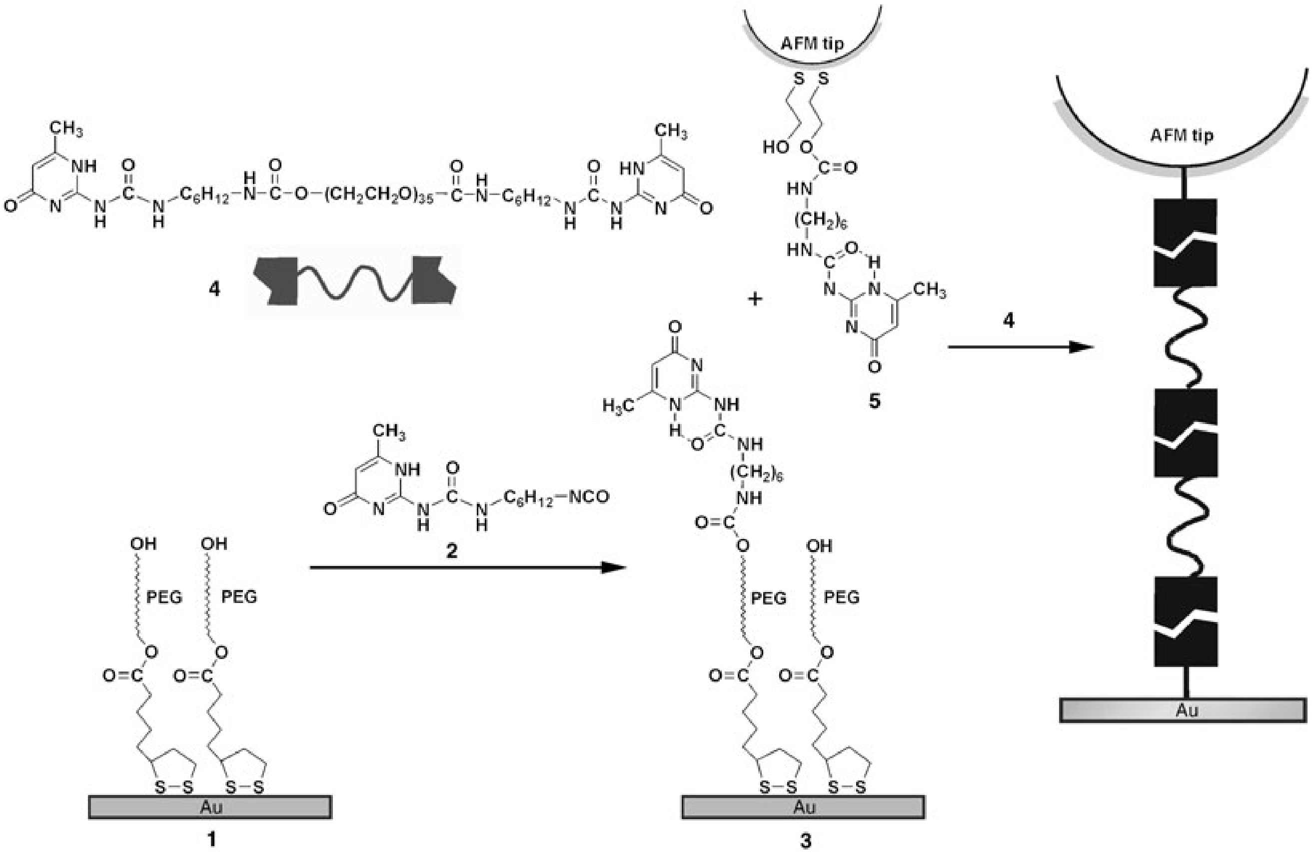

Scheme 1. Molecular thin layers of ω-hydroxy-terminated PEG are obtained by self-assembly of disulfide **1** from solution in chloroform onto Au(111) surfaces, followed by reaction with isocyanate **2** to form a layer **3** that exposes UPy moieties. Supramolecular polymers, which are formed by self-complementary association of the telechelic PEG derivative **4** in hexadecane solution, can be probed by AFM (right) by utilizing gold-coated AFM tips that are functionalized with a self-assembled monolayer of the asymmetrically substituted UPy disulfide **5** in hexadecane.

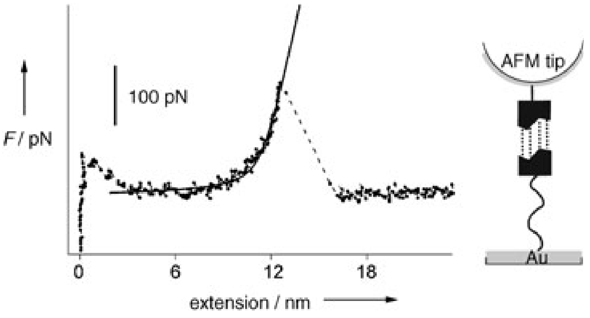

Figure 1. Force–extension curve measured between a gold-coated AFM tip, which is functionalized with a SAM of UPy disulfide **5**, and a Au(111) sample that is functionalized with a layer of **3** in hexadecane. The fit of the data to the m-FJC model is shown as a solid line.

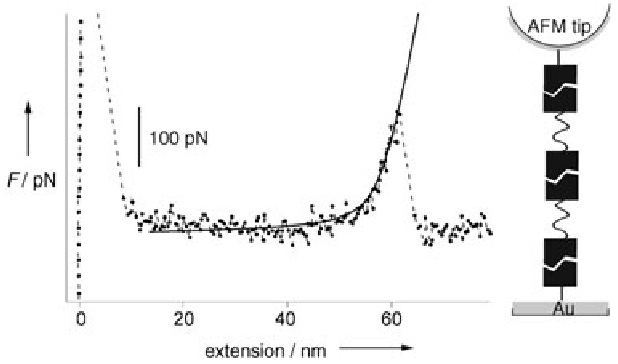

Figure 2. Force–extension curve measured between a gold-coated AFM tip and a Au(111) sample that are both functionalized with layers of **5** in the presence of the bifunctional bis(UPy) derivative **4** in hexadecane. The fit of the data to the m-FJC model is shown as a solid line.

which thus indicates that also in the current experiment a single PEG "chain" is being stretched. The significantly longer stretching length (⩾ contour length of a single PEG segment) indicates that indeed supramolecular polymers were stretched, which appears to be reasonable if the lifetimes of UPy complexes in HD are considered. By contrast, fd curves recorded in 15% DMSO in propan-2-ol showed a markedly different stretching length distribution. In this case, only very short chains were stretched, in a similar fashion to the data observed for the dimer experiment in HD. A very similar reduction in stretching length was observed in blocking experiments with a trifluoromethyl-functionalized UPy derivative (see Supporting Information).

The mean value of the rupture forces observed for the supramolecular polymers in HD of 172 ± 23 pN lies within the error margins for the rupture force of the dimeric complex. Our data suggests, however, that the rupture forces decrease slightly with increasing lengths of the stretched supramolecular polymer chains (see Supporting Information). This observation is in agreement with the theory developed by Evans et al.,[23,24] who predict a decrease in the magnitude of the single-complex rupture force with increasing spacer length and an increasing number of consecutive bonds along one molecule.[25]

Stretching lengths of more than 150 nm were detected in HD (Figure 3a) which corresponds to a "degree of polymerization" of up to 15, assuming a "monomer length" of 12 nm. The probability of stretching a supramolecular polymer chain that bridges the gap between the substrate and the tip appears to be an exponential function of the chain length (Figure 3a, inset). In the presence of DMSO, the hydrogen bonds are disrupted[6,10] and consequently the stretching and rupture of long supramolecular polymers is not observed (Figure 3b).

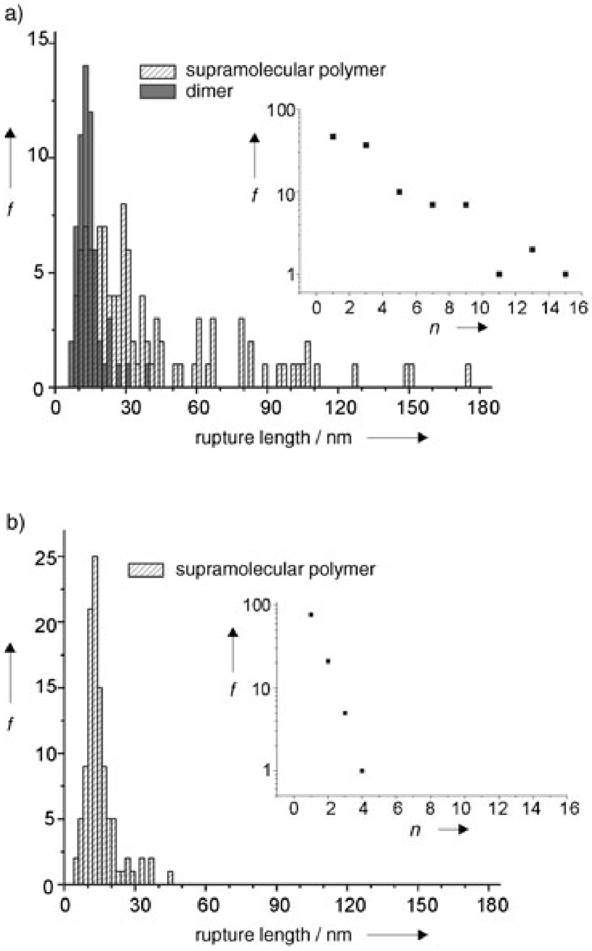

Figure 3. a) Histogram of extension lengths observed for the dimeric interactions and the supramolecular polymers in hexadecane. Inset: Frequency of extension events (*f*) versus the number of repeat units (*n*) in stretched supramolecular polymers calculated on the basis of the length of one repeat unit. b) Histogram of extension lengths observed for the supramolecular polymers in 15% DMSO in propan-2-ol. Inset: Frequency of extension events versus the number of repeat units *n* in stretched supramolecular polymers.

We observed in the AFM experiments that the chain lengths obtained for supramolecular polymers are shorter than predicted. However, independent of the length, an exponential decay of the distribution of effective length versus the degree of polymerization is expected on the basis of the model.[22] The sampling of the rupture length in the AFM experiment may lead to an underestimate because the attachment sites for the UPy telechelic derivatives on the AFM tip are not restricted to the very end of the tip, while the supramolecular polymer "chain" may also be attached to the substrate at a location outside the tip–sample contact area. Furthermore, possible effects of the tip–substrate gap distance (the polymer may form after the tip and the substrate may become detached) and the rate of the loading have not yet been addressed systematically. A systematic investigation of the frequency of successful stretching events probed versus independently adjusted tip–sample separation distances and equilibration times would open the path to elucidate the kinetics of polymerization at a single-molecule level.

In conclusion, the AFM data show that supramolecular polymers can indeed be investigated on the single-molecule level. In PEG-based telechelic bis(UPy) materials, the individual reversible linking sites along a supramolecular polymer chain, that is, the complexes based on self-complementary recognition of 2-ureido-4[1H]-pyrimidinone, act as independent bonds in series. As the material properties including viscosity, composition, or chain length are functions of various external parameters and stimuli, which can be investigated in situ, highly useful information for the design and construction of nanometer scale devices and stimuli-responsive systems will now become directly accessible from SMFS experiments.

Experimental Section

Materials: Isocyanate **2**[19] was coupled to PEG derivative **1**, which was immobilized through disulfide moieties to Au(111) (5-mM $CHCl_3$ solution, RT),[18,19] to yield surface-immobilized **3** (Scheme 1). Bis(pyrimidinone) **4** was synthesized according to a procedure adapted from Ref. [26] (for details see Supporting Information).
AFM: The AFM measurements were carried out with a NanoScope IIIa multimode AFM (Digital Instruments, Santa Barbara, CA) utilizing a liquid cell and functionalized tips prepared as described previously (see also Supporting Information).[18] The temperature inside the liquid cell was 28 °C. The cantilever spring constants were calibrated by using the thermal noise method.[27] The fd curves were fitted by using IGOR PRO software (version 4.07) to the modified freely jointed chain model (m-FJC), [Eq. (1)], with the extension of the polymer chain x, applied force F, Kuhn length l_K, contour length $L_{contour}$, number of segments n, segment elasticity K_S, Boltzmann constant k_B, and temperature T.[15]

$$x(F) = \left[\coth\left(\frac{F l_K}{k_B T}\right) - \frac{k_B T}{F l_K}\right]\left[L_{contour} + \frac{n}{K_S} F\right] \qquad (1)$$

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[1] a) J.-M. Lehn, *Supramolecular Chemistry: Concepts and Perspectives*, VCH, Weinheim, **1995**; b) *Supramolecular Materials and Technologies* (Ed.: D. N. Reinhoudt), Wiley, New York, **1999**.

[2] J. N. Israelachvili, *Intermolecular and Surface Forces: With Applications to Colloidal and Biological Systems*, Academic Press, New York, **1992**.

[3] H. Ringsdorf, B. Schlarb, J. Venzmer, *Angew. Chem.* **1988**, *100*, 117; *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 113.

[4] a) A. D. Buckingham, A. C. Legon, S. M. Roberts, *Principles of Molecular Recognition*, Blackie, London, **1993**; b) *The Lock and Key Principle: The State of the Art* (Ed.: J.-P. Behr), Wiley, Chichester, **1994**; c) G. Cooke, V. M. Rotello, *Chem. Soc. Rev.* **2002**, *31*, 275; d) J.-M. Lehn, *Science* **2002**, *295*, 2400; e) D. N. Reinhoudt, M. Crego-Calama, *Science* **2002**, *295*, 2403.

[5] J.-M. Lehn, *Macromol. Chem. Macromol. Symp.* **1993**, *69*, 1.

[6] R. P. Sijbesma, F. H. Beijer, L. Brunsveld, B. J. B. Folmer, J. H. K. K. Hirschberg, R. F. M. Lange, J. K. L. Lowe, E. W. Meijer, *Science* **1997**, *278*, 1601.

[7] P. S. Corbin, S. C. Zimmerman, *J. Am. Chem. Soc.* **1998**, *120*, 9710.

[8] B. Gong, Y. Yan, H. Zeng, E. Skrzypczak-Jankunn, Y. W. Kim, J. Zhu, H. Ickes, *J. Am. Chem. Soc.* **1999**, *121*, 5607.

[9] S. Boileau, L. Bouteiller, F. Lauprêtre, F. Lortie, *New J. Chem.* **2000**, *24*, 845.

[10] a) F. H. Beijer, H. Kooijman, A. L. Spek, R. P. Sijbesma, E. W. Meijer, *Angew. Chem.* **1998**, *110*, 79; *Angew. Chem. Int. Ed.* **1998**, *37*, 75; b) F. H. Beijer, R. P. Sijbesma, H. Kooijman, A. L. Spek, E. W. Meijer, *J. Am. Chem. Soc.* **1998**, *120*, 6761.

[11] a) L. Brunsveld, B. J. B. Folmer, E. W. Meijer, R. P. Sijbesma, *Chem. Rev.* **2001**, *101*, 4071; b) A. T. ten Cate, R. P. Sijbesma, *Macromol. Rapid Commun.* **2002**, *23*, 1094.

[12] S. H. M. Söntjens, R. P. Sijbesma, M. H. P. van Genderen, E. W. Meijer, *J. Am. Chem. Soc.* **2000**, *122*, 7487.

[13] J. van der Gucht, N. A. M. Besseling, G. J. Fleer, *J. Chem. Phys.* **2003**, *119*, 8175.

[14] For a very recent report, see: F. R. Kersey, G. Lee, P. Marszalek, S. L. Craig, *J. Am. Chem. Soc.* **2004**, *126*, 3038.

[15] For reviews, see: a) T. Hugel, M. Seitz, *Macromol. Rapid Commun.* **2001**, *22*, 989; b) A. Janshoff, M. Neitzert, Y. Oberdörfer, H. Fuchs, *Angew. Chem.* **2000**, *112*, 3346; *Angew. Chem. Int. Ed.* **2000**, *39*, 3212; c) W. Zhang, X. Zhang, *Prog. Polym. Sci.* **2003**, *28*, 1271.

[16] a) M. Rief, F. Oesterhelt, B. Heymann, H. E. Gaub, *Science* **1997**, *275*, 1295; b) J. E. Bemis, B. B. Akhremitchev, G. C. Walker, *Langmuir* **1999**, *15*, 2799; c) H.-J. Butt, M. Kappl, H. Müller, R. Raiteri, W. Meyer, J. Rühe, *Langmuir* **1999**, *15*, 2559; d) H. Li, W. A. Linke, A. F. Oberhauser, M. Carrion-Vazquez, J. G. Kerkvliet, H. Lu, P. E. Marszalek, J. M. Fernandez, *Nature* **2002**, *418*, 998; e) T. Gutsmann, G. E. Fantner, J. H. Kindt, M. Venturoni, S. Danielsen, P. K. Hansma, *Biophys. J.* **2004**, *86*, 3186.

[17] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, *Nature* **1999**, *397*, 50.

[18] a) H. Schönherr, M. W. J. Beulen, J. Bügler, J. Huskens, F. C. J. M. van Veggel, D. N. Reinhoudt, G. J. Vancso, *J. Am. Chem. Soc.* **2000**, *122*, 4963; b) S. Zapotoczny, T. Auletta, M. R. de Jong, H. Schönherr, J. Huskens, F. C. J. M. van Veggel, D. N. Reinhoudt, G. J. Vancso, *Langmuir* **2002**, *18*, 6988; c) T. Auletta, M. R. de Jong, A. Mulder, F. C. J. M. van Veggel, J. Huskens, D. N. Reinhoudt, S. Zou, S. Zapotoczny, H. Schönherr, G. J. Vancso, L. Kuipers, *J. Am. Chem. Soc.* **2004**, *126*, 1577.

[19] S. Zou, Z. Zhang, R. Förch, W. Knoll, H. Schönherr, G. J. Vancso, *Langmuir* **2003**, *19*, 8618.

[20] The presence of the UPy moieties in the PEG layers and the molecular recognition of labeled self-complementary UPys in solution were assessed by spectroscopic and electrochemical means (see Supporting Information).

[21] a) F. Oesterhelt, M. Rief, H. E. Gaub, *New J. Phys.* **1999**, *1*, 6.1; b) F. Kienberger, V. P. Pastushenko, G. Kada, H. J. Gruber, C. Riener, H. Schindler, P. Hinterdorfer, *Single Mol.* **2000**, *1*, 123.

[22] See the Supporting Information.

[23] a) E. Evans, K. Ritchie, R. Merkel, *Biophys. J.* **1995**, *68*, 2580; b) E. Evans, K. Ritchie, *Biophys. J.* **1997**, *72*, 1541.

[24] E. Evans, P. Williams, in *Physics of Biomolecules and Cells*, Ecoles des Houches d'Eté LXXV, (Eds.: H. Flyvbjerg, F. Julicher, P. Ormos, F. David), EDP Sciences, Springer, Berlin, **2002**, p. 145.

[25] E. Evans, *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 105.

[26] B. J. B. Folmer, R. P. Sijbesma, R. M. Versteegen, J. A. J. van der Rijt, E. W. Meijer, *Adv. Mater.* **2000**, *12*, 874.

[27] J. L. Hutter, J. Bechhoefer, *Rev. Sci. Instrum.* **1993**, *64*, 1868.