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Supramolecular Chemistry at the Single Molecule Level**

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Chemical synthesis and sample preparation. The tetrasulfide cavitand was synthesized according to the literature procedure[1]. *N*,*N*,*N*-Triethyl- and *N*,*N*,*N*-trimethyl-(11-mercaptoundecyl)-ammonium bromide were prepared by aminolysis starting from 1-bromo-undec-10-ene, followed by thioester formation and its cleavage to the corresponding thiol.

Gold coated sample substrates (11x11 mm^2, Arrandee, Werther, Germany) were incubated with the 2,8,14,20-tetra-(10-(decylthio)decyl) cavitand and didecylsulfide in a molar ratio of 1:40, solubilized in ethanol/chloroform (1/1) by heating to 60 °C for at least 16 h, which is essential for obtaining highly ordered monolayers[2].

Single molecule force measurements. For AFM force spectroscopy, Si_3N_4 cantilevers (Microlever, Veeco Instruments, Santa Barbara, CA, USA) were dipped in concentrated nitric acid for activation and incubated with a solution of 2 % aminopropyltriethoxysilane (Sigma-Aldrich, Steinheim, Germany) in dry toluene for 2 h at ambient temperature. After silanisation, the cantilevers were washed with toluene and incubated with 1 mM *N*-hydroxysuccinimide-poly(ethylene glycol)-maleimide (Shearwater Polymers, Huntsville, AL, USA) in water for 30 min. The cantilevers were washed with water and functionalized by incubating them overnight at room temperature with a solution of the respective salt, the compounds used being: 6-mercapto-hexyltrimethyl ammonium chloride (111 µM in ethanol), 6-mercaptohexyltriethyl ammonium chloride (128 µM in ethanol), and 2-mercaptoethylamine hydrochloride (1 mM in ethanol; Sigma-Aldrich). Modified tips were stored at 4 °C and remained usable for one week at least. Prior to use in experiment, the modified tips were washed with ethanol.

Force spectroscopy experiments were performed using a commercial AFM head (Multimode IIIa, Veeco Instruments, Santa Barbara, CA, USA) at room temperature. Acquisition of the cantilever deflection force signal and the vertical piezo movement was controlled by a 16 bit AD/DA card (PCI 6052E, National Instruments, Austin, TX, USA) and a high-voltage amplifier (600H, NanoTechTools, Echandens, Switzerland) via a home-built software based on LabView (National Instruments). The deflection signal was low-pass filtered (< 10 kHz) and box averaged by a factor of 5.

The spring constants of all AFM cantilevers were calibrated via the thermal fluctuation method with an absolute uncertainty of approximately 15 %. The values obtained ranged from 13.8 to 17.9 pN nm^{-1}.

All experiments were performed in ethanol. For competition experiments, saturated solutions of the respective free ammonium salts in ethanol were used. In dynamic force spectroscopy experiments, the retract velocity of the piezo element was varied while keeping the approach velocity constant.

Data analysis. Analysis of the force-distance curves measured was performed with a Matlab program (MathWorks, Natick, MA, USA) and corrected to display the actual molecular distances calculated from the z piezo extension, which is especially important for soft cantilevers. The elasticity of the

molecular system was obtained from the slope of the force-distance curves (corrected to molecular extension) on the last 20 data points prior to detachment of the cantilever. The loading rate then was given by the system elasticity times the retract velocity.

Dynamic Force Spectroscopy. For the complexes of the receptor with the ammonium and trimethyl ammonium residues, the loading rate dependence of the most probable bond rupture forces was measured. For each host-guest system, typically 200 to 400 unbinding events were recorded at 8 different retraction velocities ranging from 50 to 8000 nm s^{-1}, yielding loading rates from 150 to 25000 pN s^{-1}. The semilogarithmic plot of the unbinding forces against the loading rates (given by retraction velocity times the molecular elasticity of the system) can be fitted linearly according to the the formula given by Strunz et al.[3]

$$F = \frac{k_B T}{x_\beta} \ln \frac{x_\beta \cdot r}{k_B T \cdot k_{off}}$$

where F denotes the most probable unbinding force, k_BT = 4.11 pN nm (at 298 K) the Boltzmann factor, x_β a molecular length scale, r the loading rate and k_{off} the thermal off-rate under zero load. The slope of the line fit correlates with the last potential barrier along the reaction coordinate of the system, yielding x_β. By extrapolation of the fit to zero external force, the natural thermal off-rate for the system can be obtained[4].

Reference List

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