Synthesis with Single Macromolecules: Covalent Connection between a Neutral Dendronized Polymer and Polyelectrolyte Chains as well as Graphene Edges[a]

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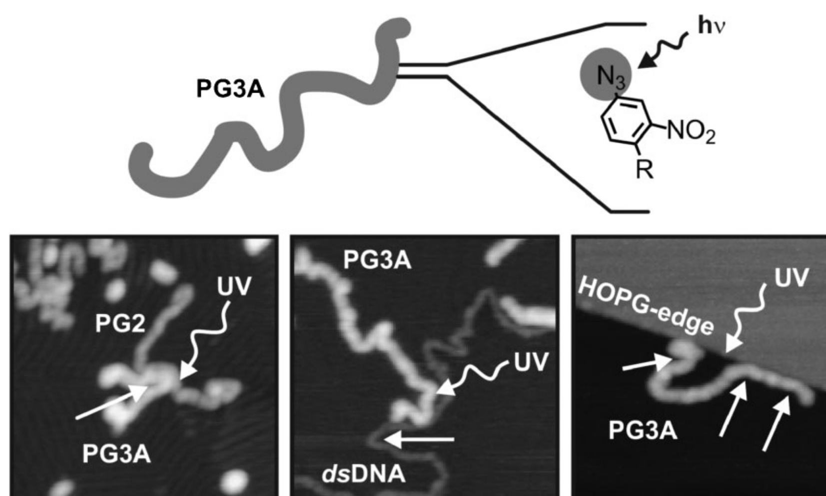
Single chains of a neutral, dendronized polymer with peripheral azide groups (**PG3A**) are co-deposited onto molecularly modified graphite substrates with a positively charged dendronized polymer (**PG2**) as well as with negatively charged plasmid *ds*DNA. **PG3A** is also prepared near graphite step-edges. Single **PG3A** chains are moved with a scanning force microscope tip, into close contact with either of the two polyelectrolytes, as well as the step-edge at predetermined positions. Treating these structures in situ with UV light leads to photochemically induced cross-linking between the **PG3A** chains carrying azide groups and **PG2**, *ds*DNA, and graphite step-edges, respectively, which is proven by mechanically challenging the "welding" points by pulling on the molecules with the SFM-tip.



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[a] Supporting information for this article is available at the bottom of the article's abstract page, which can be accessed from the journal's homepage at http://www.mrc-journal.de, or from the author.

Introduction

Covalent chemistry between single molecules on solid substrates was pioneered by Hla et al.,[1] who combined two iodo benzenes on a Cu surface, with biphenyl, by using the interactions between the scanning tunneling microscope tip and the molecules. Other bimolecular reactions[2] and even polymerizations[3] initiated by tip–molecule interactions followed. Related are experiments in which chemical reactions on solid substrates were initiated by other means (e.g., electrochemical or photochemical ones) but the

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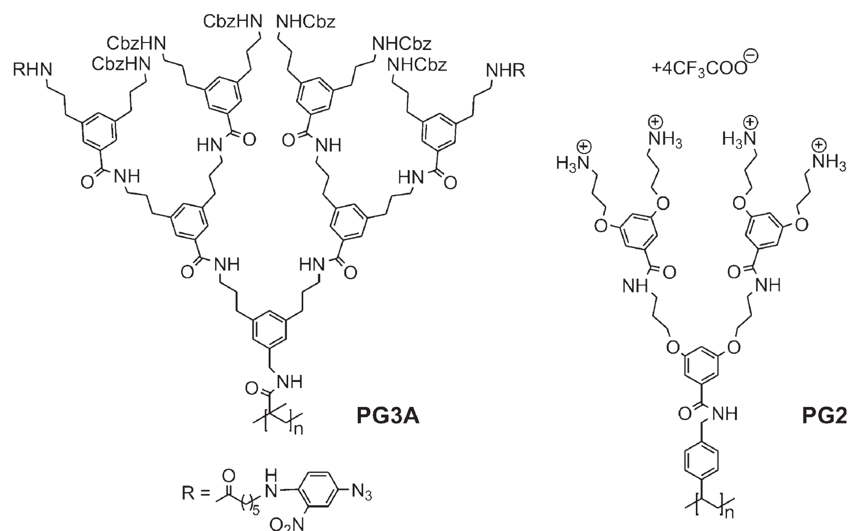

Scheme 1. Chemical structures of the neutral and azide-carrying dendronized polymer **PG3A** and its counterpart, the charged dendronized polymer **PG2**.

successful course of which was monitored by the STM.[4] A couple of years ago, the first experiment was reported, in which two macromolecules were prepared as single entities on a solid substrate, moved together by the SFM and connected by photochemical treatment. The connection was then proven again with the help of the SFM, by dragging the combined macromolecules across the surface.[5] These experiments, for which a third generation dendronized polymer[6] similar to **PG3A** (Scheme 1) was used, have the beauty that they can be carried out under ambient conditions. The actual "gluing" process came about by this polymer's peripheral azide groups, which decompose into the highly reactive nitrenes upon irradiation. At the site where the two chains were in tight contact, these nitrenes caused a structurally ill-defined intermolecular cross-linking. Because of the potential importance of such protocols for the bottom-up approach to the nanosciences, in the following years, this experiment was further developed to maturity.[7] The key-steps were (i) the introduction of a dense monolayer ("carpet") of long chain fatty acids between the solid substrate (highly oriented pyrolytic graphite, HOPG) and the deposited macromolecules to be subjected to such a "move-connect-prove" sequence and (ii) the use of the structurally precisely defined dendronized polymer **PG3A**. This way, any irrationalities stemming from unknown adsorbents on HOPG and the not fully known chemical structure of the polymer were prevented. The carpet technology also improved the dragging and imaging of the deposited dendronized polymers. On this basis, it was an obvious next step to not only try to connect two neutral macromolecules with one another, but rather to try to expand the range of employable components to charged ones. This would open access for this technology to the repertoire of polyelectrolytes the bio world offers. An issue that had to be considered in this regard is the need for co-preparation of neutral and charged species on the same substrate. In light of the present interest in graphemes,[8] it was an additional challenge to explore whether the edges of these unique sheet-like entities could be attacked and thus modified at predetermined sites by the gluing protocol. We here disclose our findings regarding gluing of the neutral **PG3A** with the positively charged **PG2** when co-prepared on a carpet of the long chain fatty acid $C_{29}H_{59}CO_2H$ on HOPG and with *ds*DNA, which is negatively charged, when co-prepared on a carpet of the long chain alkyl amine $C_{12}H_{25}NH_2$. Finally, attempts will be made to glue **PG3A** on HOPG/$C_{12}H_{25}NH_2$ to the edges of the topmost graphene planes.

Results and Discussion

The dendronized polymers **PG3A**[7] and **PG2**[9] were deposited as single molecules from dilute solutions onto the basal plane of HOPG, modified with a spin-coated molecular monolayer.[10] The latter defines the substrates' surface properties including its surface charge, its wettability, and the friction during the manipulation of macromolecular adsorbates across the surface. Single molecules of the neutral **PG3A** were immobilized on both HOPG pre-coated with a long chain fatty acid ($C_{29}H_{59}CO_2H$) or a long chain alkyl amine ($C_{12}H_{25}NH_2$), while the positively charged **PG2** was deposited on the fatty acid monolayer only.

Figure 1(a) displays an SFM image of a monolayer of $C_{29}H_{59}CO_2H$ on HOPG, prior adsorption of the dendronized polymers. The flat lying molecules form straight lamellae with the head groups micro-phase-separated. The lamella spacing is 7.5 nm as determined by the fast Fourier-transform (FFT) of the height image [inset Figure 1(a)], and their orientation reflects the three-fold symmetry of the underlying HOPG. The spacing is approximately twice the length of $C_{29}H_{59}CO_2H$ (7.8 nm). Since the head-group of the fatty acid can be negatively charged, this well-defined monolayer serves as an excellent substrate for the adsorption of the positively charged dendronized polymer **PG2** but also for its neutral counterpart **PG3A**. The co-adsorption of neutral and azide-carrying **PG3A** and the charged **PG2** on HOPG, pre-coated with a monolayer of $C_{29}H_{59}CO_2H$ is shown in Figure 1(b). The third generation dendronized polymer **PG3A** can be distinguished from the

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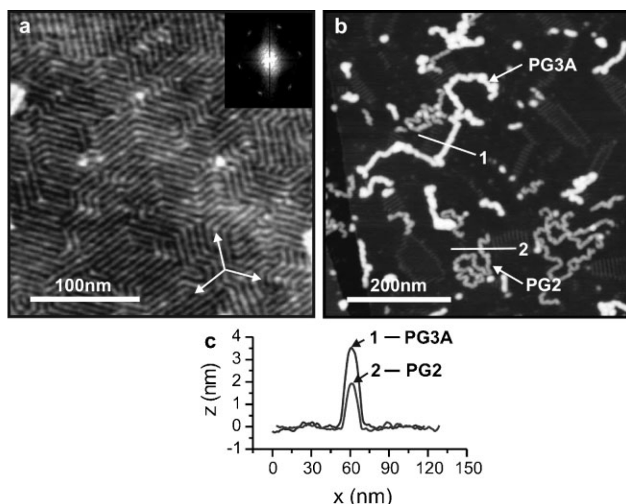

Figure 1. HOPG coated with a monolayer of $C_{29}H_{59}CO_2H$, prior to adsorption of the dendronized polymers (a), **PG3A** and its counterpart, the charged dendronized polymer **PG2** on such a monolayer (b), and cross-sections (c) along white lines **1** and **2** in (b). The two macromolecules **PG3A** and **PG2** are clearly distinguishable in their height.

second generation **PG2** by its larger contour-height. Cross-sections, exemplarily shown in Figure 1(c), give an average height for **PG2** and **PG3A** of 2.06 nm ($N = 123$) and 3.56 nm ($N = 210$), respectively. In the background, one can recognize the preserved lamellae of flat lying fatty acid molecules.[11]

The cross-linking of individual strands of **PG3A** and its counterparts **PG2**, *ds*DNA or step-edges of HOPG utilizing a "move-connect-prove" sequence,[5,7] was carried out in situ, while the SFM-tip was approached to the surface. The illumination time needed for a successful cross-linking experiment in this particular geometry, was determined in model experiments monitoring the kinetics of the photolysis of the azide carrying **PG3A** by Fourier-transform IR (FT-IR) absorption spectroscopy. The absorption bands of the peripheral azide groups of the **PG3A** were observed at wavelengths of 270 and 471 nm, besides the absorption of the aromatic moieties of the polymer core at 240 nm. They were compared to the chemically similar, but not azide functionalized polymer **PG3**.[9] The absorption of the azide overlaps sufficiently with the wavelength of the UV light (254 nm) used in the cross-linking experiment (see Supporting Information).

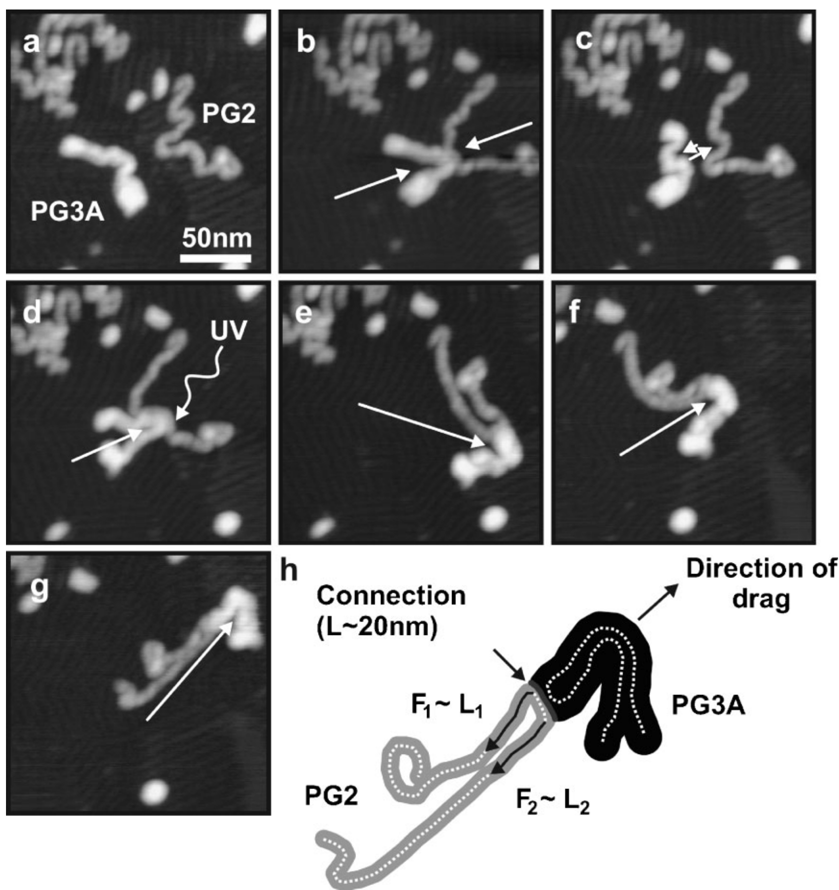

Figure 2. "Move-connect-prove" sequence (a–g) with the neutral and azide-carrying dendronized polymer **PG3A** and its counterpart, the charged dendronized polymer **PG2** on a monolayer of $C_{29}H_{59}CO_2H$ on HOPG. A sketch of the conformation of the two connected strands of **PG3A** and **PG2** in the last sequence step is shown in (h).

Figure 2(a)–(g) provide a "move-connect-prove" sequence of **PG3A** and **PG2** on such a monolayer of $C_{29}H_{59}CO_2H$ on HOPH, which shows that an azide functionalized dendronized polymer can be connected to a non-functionalized counterpart via in situ cross-linking. Starting from the initial conformation shown in Figure 2(a), the two molecules have been moved toward each other, as indicated by the two arrows in Figure 2(b). The same two molecules were moved apart again [Figure 2(c)], which clearly shows that adhesion forces between the two strands can be neglected in this cross-linking experiment. After pushing them back together [Figure 2(e)], the sample was illuminated in situ for 5 min with UV-light at 254 nm in order to cross-link the two molecules at their contact, utilizing the peripheral azide functionalization of **PG3A**. In order to test the junction mechanically, the linked molecules were dragged near the junction across the surface [Figure 2(e)–(g)] to show that the junction stays intact during this mechanical stressing. Hereby, the thicker **PG3A** acts as a handle to drag the thinner **PG2** which is exposed to the attractive force only across the established junction. Assuming a friction force per unit length

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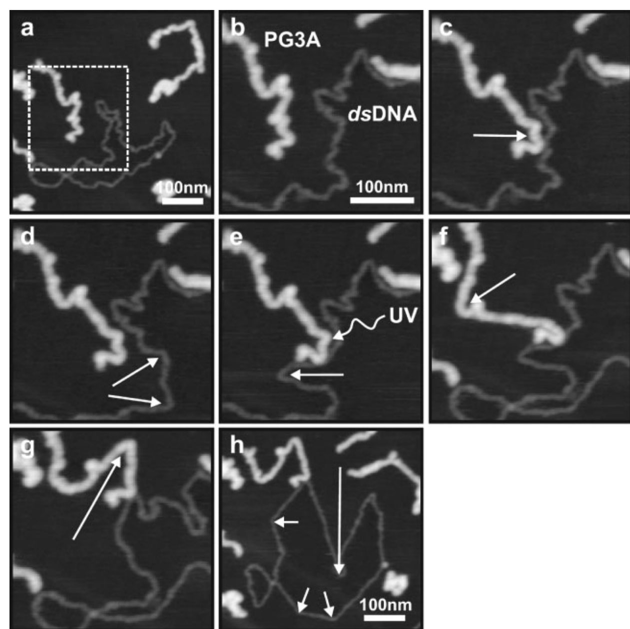

Figure 3. "move-connect-prove" sequence with the neutral and azide-carrying dendronized polymer **PG3A** and plasmid DNA **puC19** on HOPG coated with a monolayer of $C_{12}H_{25}NH_2$.

of the dragged **PG2** strand and the substrate, the junction is charged by a force F_1 and F_2, proportional to the chain length $L_1 = 132$ nm and $L_2 = 117$ nm of the two arms of the **PG2** strand, respectively, as sketched in Figure 2(h). On the other hand, individual chains of first and third generation dendronized polymers (from a homologous series PG1, **PG2**, PG3) break upon dragging, when the length of the dragged arms exceeds a generation independent length of $L_{1,2} \approx (120 \pm 20)$ nm.[12] This indicates that the connection between the two macromolecules is not weaker than the backbone bonds within the individual chains of **PG2**. With a contact length of $L_c \approx 20$ nm between **PG3A** and **PG2**, a fully stretched **PG3A** carries 154 azide groups at its periphery. Thus it has to be assumed that the junction between the two strands is composed of multiple covalent bonds.

Figure 3 displays an analogous "move-connect-prove" sequence for **PG3A** and a negatively charged double stranded plasmid DNA, both immobilized on the long chain alkyl amine $C_{12}H_{25}NH_2$.[13] Figure 3(a) shows that the linear **PG3A** can be readily distinguished from the circular DNA **puC19** by its larger thickness and its topology since **puC19** is a circular plasmid. The mean contour-length of **puC19** double strand adsorbed onto such a substrate, measures $L = (875 \pm 69)$ nm ($N = 83$) which is close to its native B-form length of 815 nm (2 686 bp, 0.34 nm · bp^{-1}). First **PG3A** and the DNA have been moved toward each other [Figure 3(b) and (c)], and it is shown that pure contact does not link the chains such that the junction would hold as one moves one chain apart from the other [Figure 3(d)]. After pushing the chains toward each other into tight contact again [Figure 3(e)], and illuminating the sample for 5 min with UV-light at 254 nm, the induced junction was mechanically tested by pulling first on the **PG3A** [Figure 3(f) and (g)] and then on the DNA [Figure 3(h)], showing that the junction withstands the forces employed upon dragging it across the surface and to straighten the chains coming off the junction. The DNA chain was furthermore over-stretched by a factor of 1.3 during testing the junction to a global contour-length of $L = 1\ 136$ nm ($L_0 = 878$ nm).[13] It shows that the methodology can be quite generally applied to synthesize nanostructures also from very dissimilar macromolecules.

Finally, it has been attempted to connect **PG3A** to step edges on the basal plane of HOPG which are always present after cleaving the substrate, prior sample preparation. Figure 4 displays SFM images of **PG3A** near a step edge (height $\Delta z = 2.2$ nm, see the cross-section in Figure 4(b)) of HOPG pre-coated with $C_{12}H_{25}NH_2$. In order to check that the

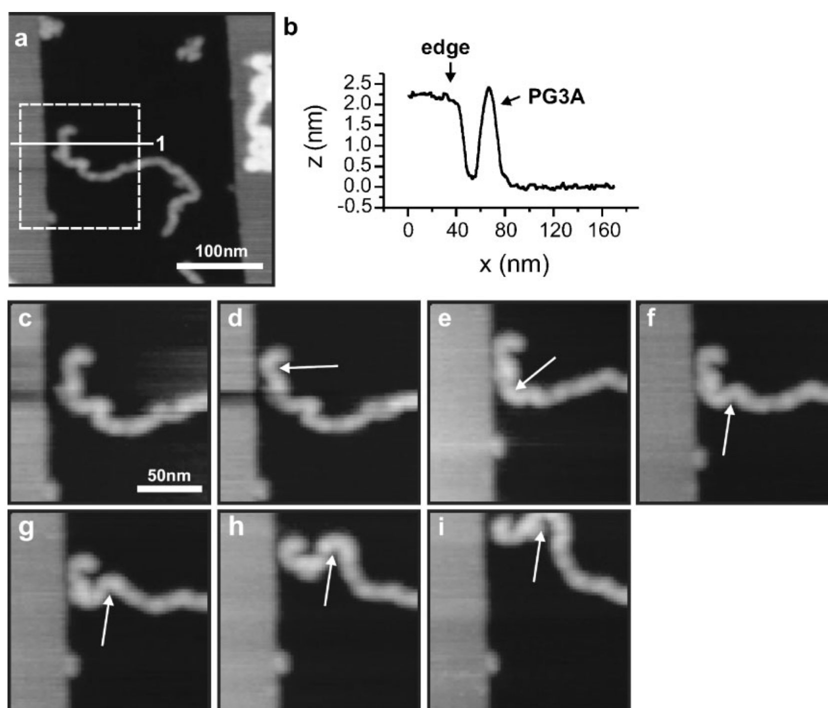

Figure 4. Series of SFM images showing the manipulation of a **PG3A** molecule toward and away from a step edge on HOPG, pre-coated with a monolayer of $C_{12}H_{25}NH_2$. Cross-section (b) is taken from (a) along the white line (1).

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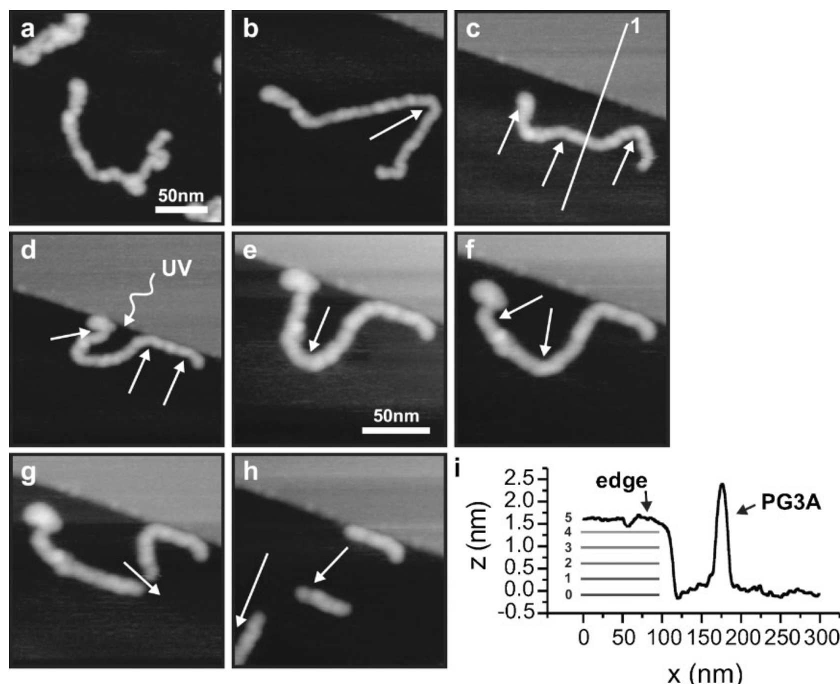

Figure 5. "move-connect-prove" sequence with the neutral and azide-carrying dendronized polymer **PG3A** and a step at the basal plane of HOPG, pre-coated with a monolayer of $C_{12}H_{25}NH_2$. Scale bars in (a) and (b) apply to (b)–(d) and (f)–(h), respectively. Cross-section (i) is taken from image (c) along (1).

chain does not stick by adhesion forces to the step edge, it was first moved toward the step edge [Figure 4(d) and (e)], and then removed from it again [Figure 4(f)–(i)]. Since this was possible without breaking it, the adhesive force is smaller than the force necessary to break a bond in the polymer chain and can be therefore neglected in the next experiment.

Figure 5 displays a "move-connect-prove" sequence with **PG3A** on the basal plane of HOPG, pre-coated with a monolayer of $C_{12}H_{25}NH_2$, being moved toward a step [Figure 5(a)–(d)] and illuminated there at 254 nm. The graphitic step-height measures $\Delta z = 1.6$ nm which is equivalent to approximately 5 graphene layers, assuming a layer spacing of 0.335 nm, as sketched in the cross-section Figure 5(i). Figure 5(e)–(h) show attempts to remove the molecule with its two ends, from the step edge. In order to remove one of them, the chain has to be stretched significantly [Figure 5(e)], before one end detaches [Figure 5(f)]. The other end cannot be removed from the edge without breaking the chain [Figure 5(g) and (h)]. The contact-length between the remaining chain and the step measures $L_C \approx 27$ nm. Also in this case, it has to be assumed that the connection is established by a number of covalent bonds.

These results substantially widen the repertoire in single molecule synthesis where up to now, only connections between two neutral entities were reported. Such connections are now also possible between neutral and positively charged as well as neutral and negatively charged molecular objects. The components' exact chemical nature does not seem to play a role, underlining the considerable potential of azide modified dendronized polymers as molecular glue. Furthermore, the successful covalent connection between **PG3A** and the graphitic step-edge, indicate a remarkable selectivity: Though it is not known whether the **PG3A** chains penetrate the underlying monolayer, it is exclusively the edge of a graphitic layer (graphene) and not its main plane that is attacked under the cross-linking conditions. These findings are considered elementary steps toward molecular constructions, aiming at complex single macromolecules and utilizing their properties on substrates. Such entities could consist of segments like DNA, peptides, and oligosaccharides, glued together by properly equipped dendronized polymers to linear or branched structures, otherwise practically inaccessible. Given the precision with which the gluing sites can be selected, the present work also opens the possibility to construct and study rationally designed model networks comprising of natural and man-made segments. The described experiments with the step-edge should be seen in context of the fascinating option to decorating the edges of single graphene sheets.

Experimental Part

Polymers **PG2** and **PG3A** were synthesized according to previously reported procedures.[7,9] Single polymers were deposited onto pre-coated HOPG.[7,10,13] In a first step, 10 μl of a solution of $C_{29}H_{59}COOH$ or $C_{12}H_{25}NH_2$ (Sigma–Aldrich) in chloroform (0.1 mg · ml^{-1}) was spin-coated (all spin coating at 50 rps) onto freshly cleaved HOPG to yield a monolayer. The sample was dried for half an hour under ambient conditions, prior to depositing the various polymers.

For the deposition of pure **PG3A** onto the pre-coated HOPG, 10 μl of **PG3A** dissolved in chloroform (0.04 mg · ml^{-1}) was spin coated onto HOPG pre-coated with $C_{12}H_{25}NH_2$, followed by drying for half an hour under ambient conditions. For co-adsorbing **PG3A** and **PG2**, 10 μl of a solution of the neutral **PG3A** dissolved in chloroform (0.04 mg · ml^{-1}), was spin coated onto HOPG, pre-coated with $C_{29}H_{59}CO_2H$. After drying the sample for 20 min under ambient conditions, 10 μl of **PG2** dissolved in methanol (0.05 mg · ml^{-1}) was additionally spin coated onto the sample, to yield the desired co-adsorption of individualized molecules of **PG2** and **PG3A**. Before SFM-imaging, the sample was dried for additional 30 min under ambient conditions. For the co-adsorption of **PG3A** and DNA onto a monolayer of $C_{12}H_{25}NH_2$ on HOPG in a first step,

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10 μl of an aqueous solution of plasmid DNA (**pUC19**, 2 686 bp, Mobitec GmbH, Göttingen; $1\,ng \cdot \mu l^{-1}$) was applied to the pre-coated HOPG. The standing droplet was spun off after an incubation time of 2 min and the sample was dried for 20 min. In a second step, 10 μl of **PG3A** in chloroform ($0.04\,mg \cdot ml^{-1}$) was spin coated at 50 rps on top of the prior deposited DNA followed by drying the sample for half an hour.

For SFM imaging and lateral manipulation of the macromolecules across the surface, a home-built SFM based on the Multimode head and Nanoscope III controller of Digital Instruments Inc. (Santa Barbara, CA, USA) was used. Besides basic imaging in tapping-mode, the setup allows the movement of the SFM tip in the *x*-*y* plane, along pre-defined traces with additional control of normal force,[6] i.e., the SFM operation can be gently switched from tapping to contact mode. Since every manipulation step requires verification of the resulting position and conformation of the macromolecule, a full image has to be recorded after each step, which slows down the overall manipulation process. To bypass this problem, a fast object-tracking procedure was integrated into the SFM-setup, in which only the small area of interest is scanned with lower pixel-resolution than the full image, but at the same tip velocity. A full SFM-image with 512×512 pixels can be recorded in several minutes, whereas the local fast object tracking, with a resolution of e.g., 64×64 pixels, provides the topographic result on a time scale of a few seconds.

For imaging and lateral manipulation, Olympus etched cantilevers (OMCL-AC240) with a nominal normal spring constant of $2\,N \cdot m^{-1}$ and a tip radius below 10 nm were used. Images were taken by SFM operating in tapping-mode. During manipulation in contact-mode, the SFM-tip was moved with a velocity of $500\,nm \cdot s^{-1}$, whereas the deflection was kept constant at 10 nm, which corresponds to a normal force of about 20 nN.

UV illumination was carried out with a standard spectral Ne-Hg lamp (Pen-Ray 11SC-1, UVP Inc. Upland, CA, USA) with a maximum emission at a wavelength of 254 nm, positioned in front of the Multimode AFM-head at grazing incidence, at a distance of 3.5 cm from the sample. The illumination time was set to $t = 5$ min for all SFM cross-linking experiments.

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