7. SFM investigations of some of dendronized polymers

7.1. Introduction of SFM investigations

Atomic Force Microscopy (AFM), known also as Scanning Force Microscopy (SFM), has been invented in 1986 by Binning and co-workers. The physical quality measured with this apparatus is the force between a sharp conical tip and a sample surface while scanning the tip across the sample.
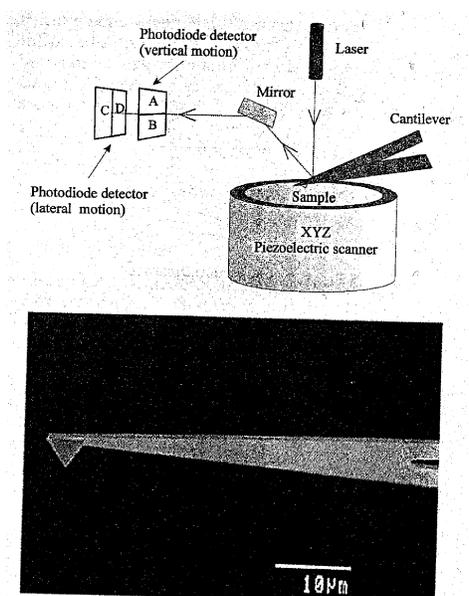

Fig. 22. Principle of SFM measurement.

This allows investigations to be performed on a wide range of materials including polymers. SFM probes the surface of a sample using a sharp tip, with a terminal radius often less than 10 nm. The tip is located at the free end of a few 100 μm long cantilever that has got an elastic modulus that can be as low as a few 0.1 N/m. Forces of a few pN between the tip and the sample surface cause vertical deflections of the cantilever on the Angstrom scale. A laser beam bounces off the back of the cantilever onto a position-sensitive detector (PSD). As the cantilever bends, the position of the laser beam on the detector shifts. The PSD itself can measure displacements of light beams as small as 1 nm. The ratio of the path length between the cantilever and the detector to the length of

the cantilever itself produces a mechanical amplification. As a result, the system can detect sub-Angstrom vertical movements of the cantilever tip. The measured cantilever deflections enable the computer to generate a map of the surface topography.

Since the shape or 'conformation' of a single molecule plays a key role for its physical properties, a great deal of effort in polymer physics have been devoted towards the development of models, which allow the prediction of molecular conformations. These models permit the calculation of thermodynamic of molecular ensembles in various environments, as well as mechanical properties of the single molecule. For large molecule, such as DNA, it is possible to gain insight into their conformations simply by imaging them deposited on a flat solid surface using the Electron or scanning force microscopy. Examples are globular and cylindrical or wormlike molecules such as DNA and RNA,[50-53] nucleosomes,[54] chromosomes,[55,56] viruses,[57] dendrimers,[58-60] arborescent graft polymers,[61] polymacromonomers,[62,63] monodendron jacketed linear flexible polymers and relatively stiff poly(p-phenylene)s.[33] Figure 23 presents the
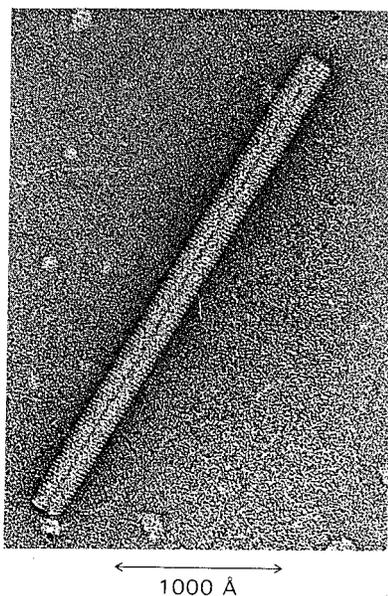

Fig. 23. Electron micrograph of a tobacco mosaic virus (TMV) particle.

electron micrograph of a tobacco mosaic virus, which is the simplest and best understood TMV, with 3000 Å long and 180 Å in diameter. Shape control of the synthetic molecules is mainly achieved by dense grafting and well defined multiple branching. The examples listed above have in common that they all represent rather thick, one might say 'fat', molecules which do not interpenetrate each other but exist as

segregated species. Furthermore, the thermal motion of these molecules is slow compared to the time scale of observation determined by tapping and scanning frequencies. Thus, the shape of the molecules is sufficiently persistent to allow direct probing of each single molecule by a scanning tip. Molecules, which are individually different in size and conformation, can be resolved even if they are neither arranged in an ordered array nor strongly bound to the substrate by chemi-or physisorption, the latter being typical conditions for ultrahigh resolution scanning tunnelling and scanning force microscopy on molecular adsorbate layers.

  Due to the large size of dendronized polymers and the surface forces acting between individual molecules and the substrate, monomolecular films form spontaneously on various substrate by simple casting or dipping procedures with dilute solutions. Even deposition of individual molecules can be achieved in this way.

  The deprotected dendronized polymers are water soluble, which consist of repeat units that are carrying charged groups. These polymers belong to the polyelectralytes. As we know, polyelectralytes are classified into strong and weakly charged ones. In strong polyelectralytes, every (or almost every) charged group is dissociated carrying a net charge. Therefore, the electrostatic Coulomb interactions are dominating over the non-electrostatic interactions, such as van-der-Waals interactions. Weak polyelectralytes are characterized by a small degree of dissociation of the charge groups or a small fraction of charge groups. In this case, the non-Coulomb interactions may be of the same order of magnitude. In short terms, the electrostatic energy will contribute significantly to the conformation of the polyelectralytes.

  In the previous sections, the deprotected (or charged) dendronized polymers bear charged groups on the periphery and belong to the strong polyelectrolyte. In contrast to the traditional polyelectralytes, the charged dendronized polymers (or in other words, dendronized polyelectrolytes) have more charges and more densely jacketed branches. These two factors favour the extension of polymer backbones. In the other hand, longer distance between the charges and backbones of dendronized polyelectrolytes will decrease the effect of charged groups on the conformation of backbone. Besides these, the behaviours of the bulky inner parts of oil-soluble dendrons in water may also play a role in this complicated systems. Nevertheless, SFM has provided a direct means to visualise the conformation of 'fat' polymers.

7.2. SFM measurement of deprotected dendronized polymers

Samples for the SFM experiments were prepared by spin casting of dendrimer solution (33 μg/μL) in distilled water, and followed by washing the surface with distilled water and dried with nitrogen.

The measurements were performed with a Nanoscope Multimode SFM (Digital Instruments). It is provided with a scanner (E scanner) that allows investigation to be carried out on a XY scale that goes from ca. 12 μm down to the nanometer scale. This apparatus can perform investigations either in contact Mode SFM, Tapping Mode SFM or Phase Imaging. The standard Si cantilever/tip which posses a spring constant K = 42 N/m. The holder is placed in the microscope and the laser beam is reflected off the free end of the rear side of the red tangular cantilever which is coated with aluminium.
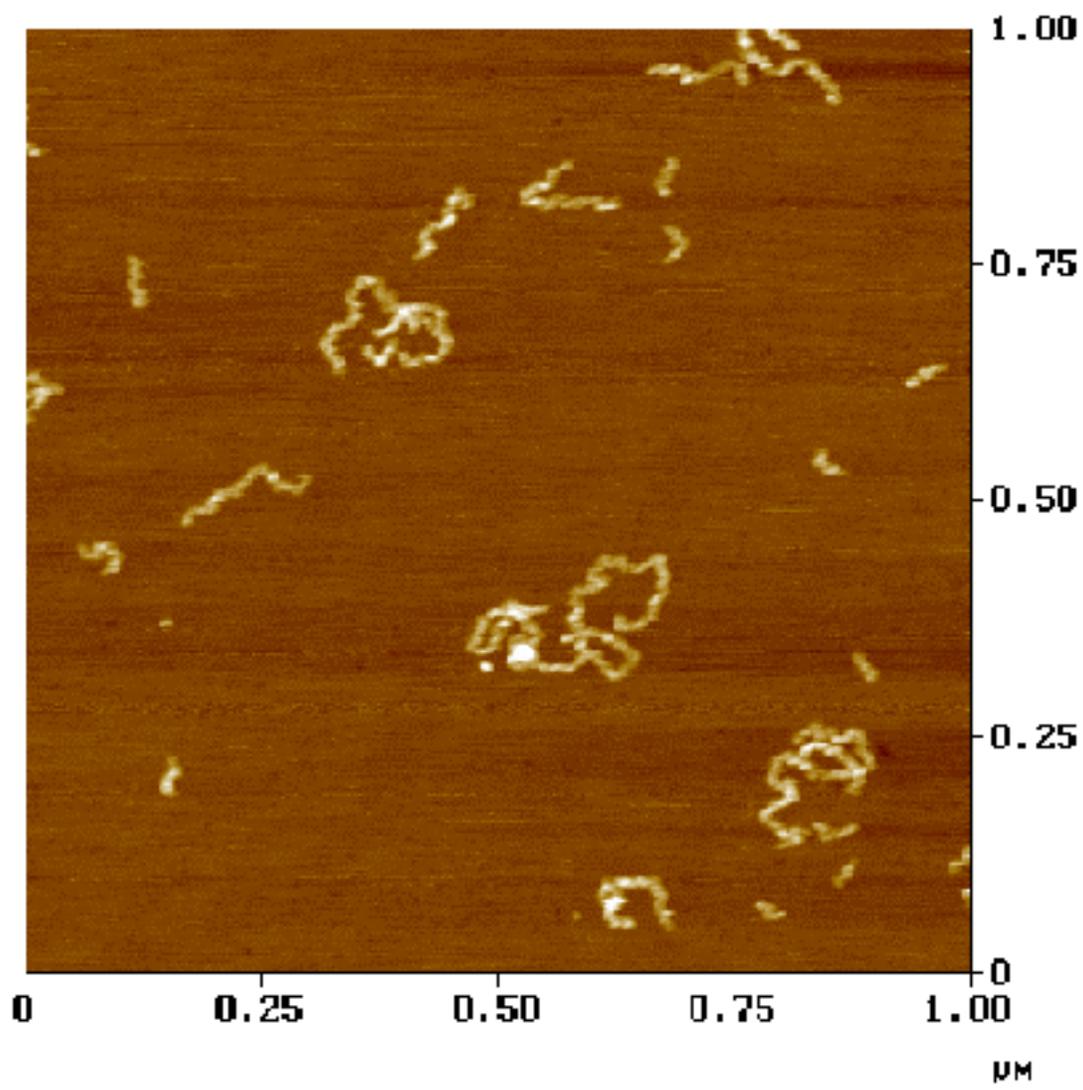
Fig. 24. A height contrast SFM image of G2 dendronized polymer **33b** on mica. (the average height profile is 1.8 ±0.4 nm).
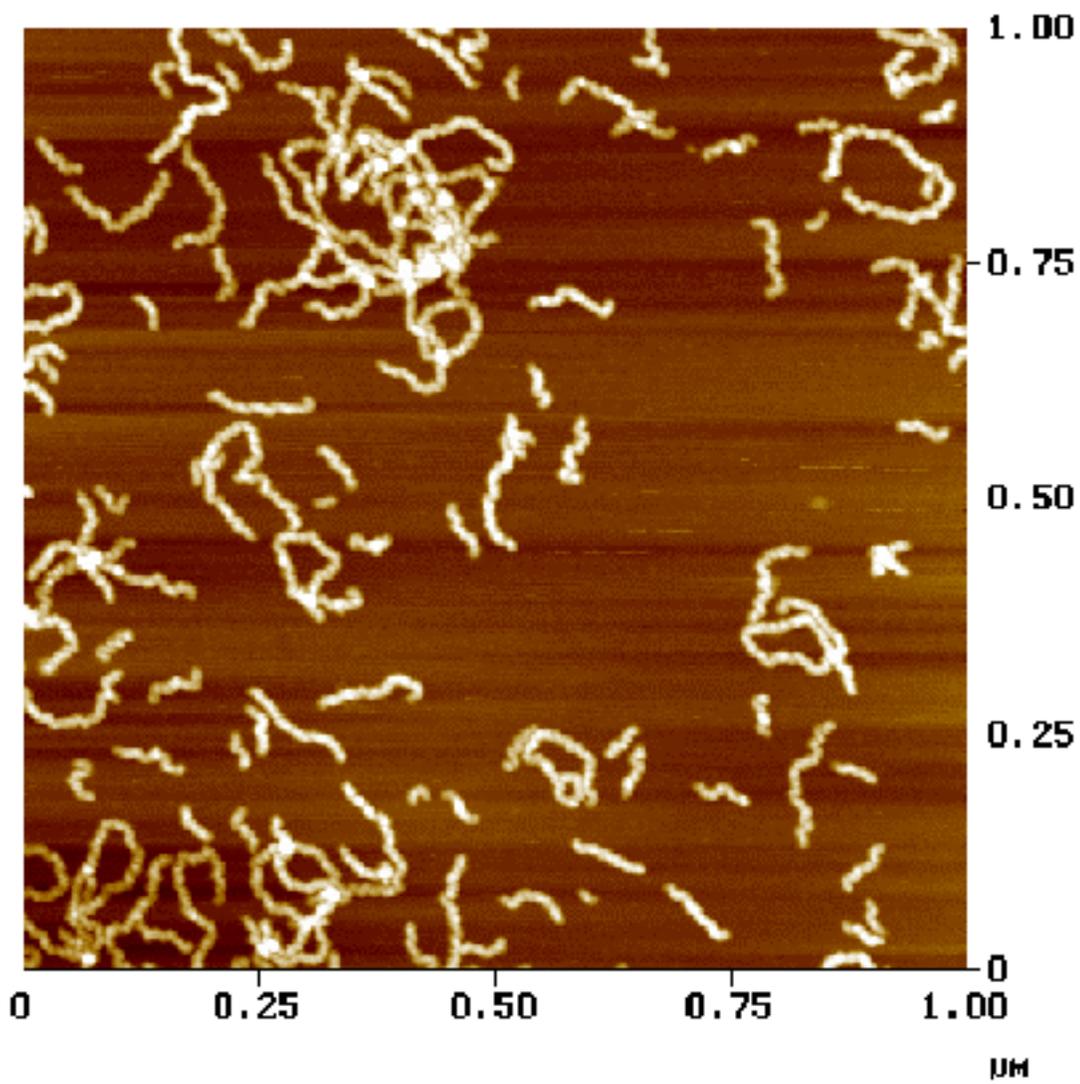
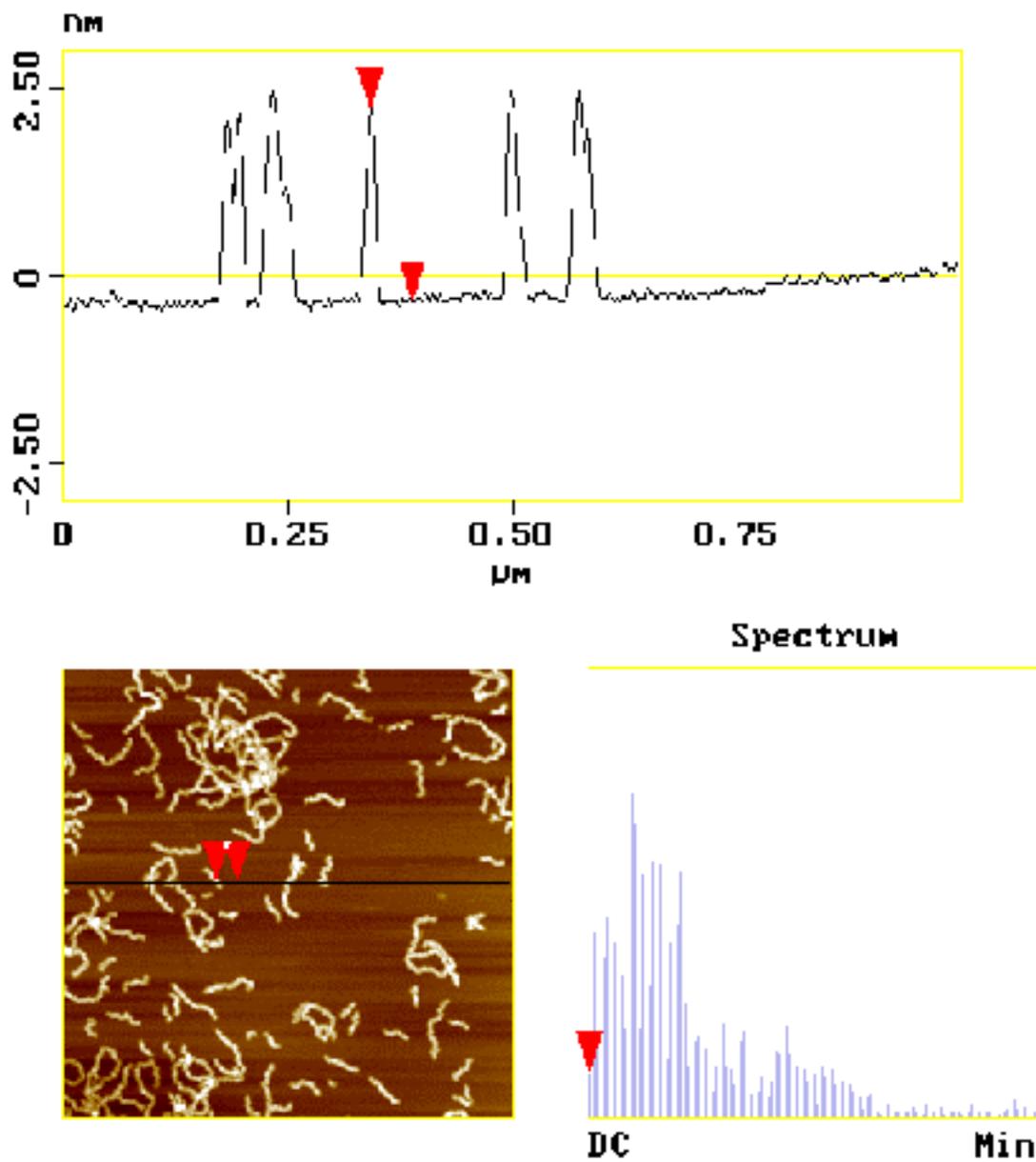**Fig. 25**. A height contrast SFM image of G3 dendronized polymer **36b** on mica.

Fig. 26. A section analysis of a height contrast SFM image of G3 dendronized polymer **33b** on mica. (the thicken of the one absorbed individual polymer chain is 3.2 nm , The average thickness is 2.4 ± o.4 nm)
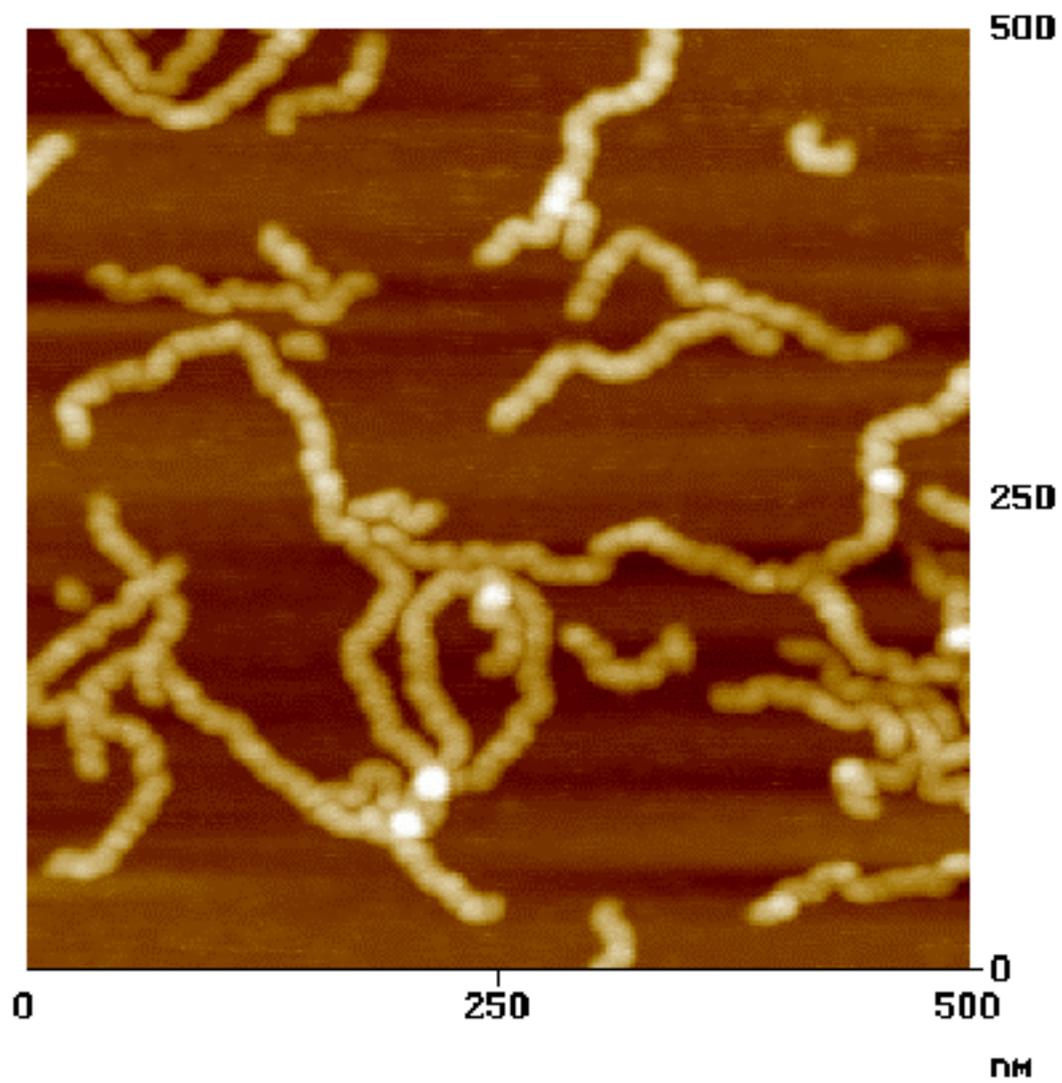
Fig. 27. A height contrast SFM image of G4 dendronized polymer **37b** on mica.
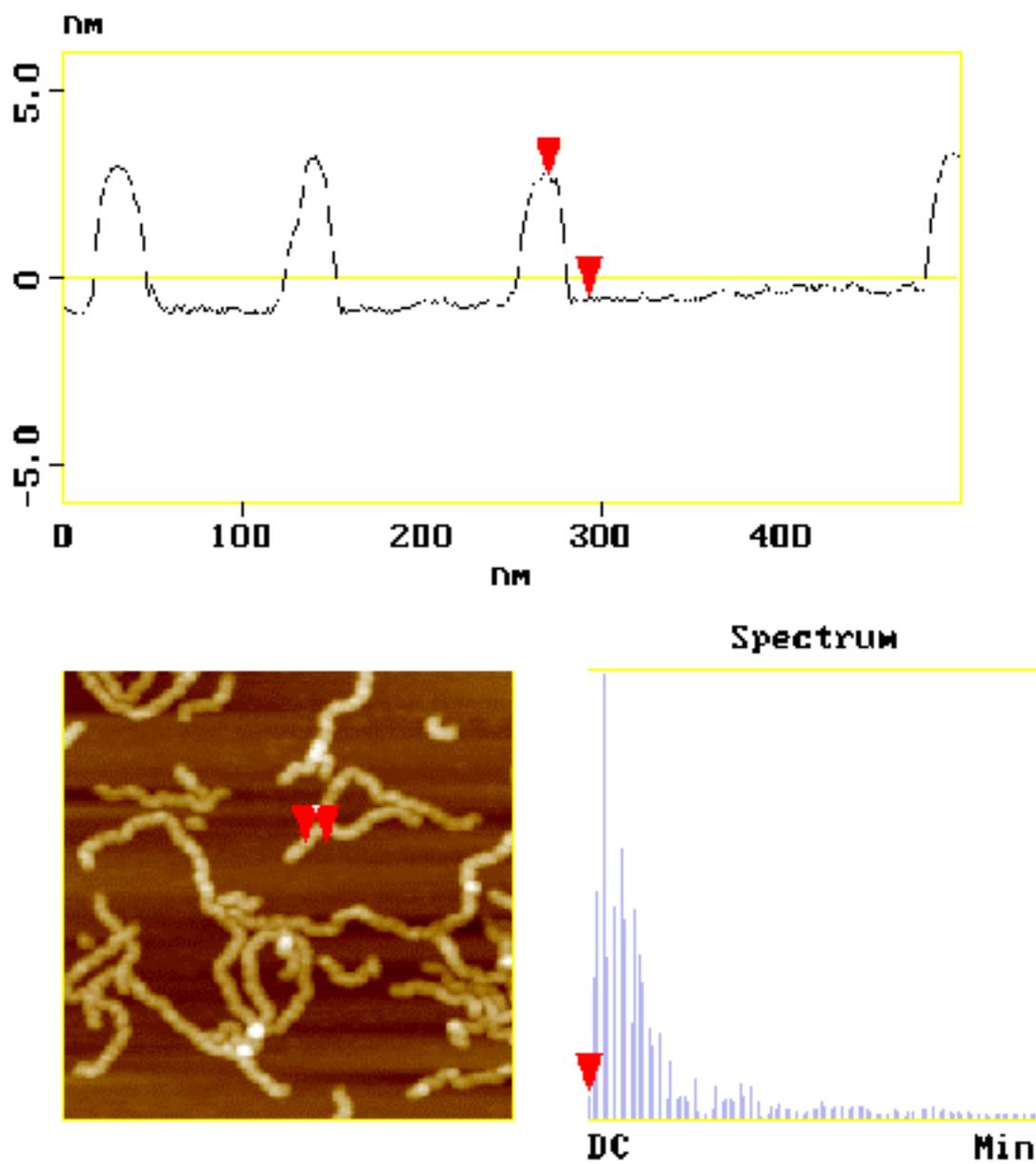
Fig. 28. A section analysis of a height contrast SFM image of G4 dendronized polymer **37b** on mica. (the height of the one individual polymer chain is 4.3 nm, the average height is 3.2 ± 0.6 nm)

Figure 24, 25 and 27 are height contrast SFM images of polymer **33b**, **36b** and **37b** adsorbed on mica by spin-casts technique. The polymers carry 4, 8 and 16 positive charged per repeat unit, respectively. The individual strands are well-resolved and exhibit lengths between a few 10 nm and over 300 nm. They seem to have some

secondary structure, Since there are periodicity along each strand that may be due to helicity. Unfortunately, we are not in a position to prove this challenging proposal.

From the sectional analysis of the polymers (Figure 26, 28), the monolayer thickness of polymers 33b, 36b and 37b were determined to be 1.8 ± 0.4 nm, 2.4 ± 0.4 nm and 3.2 ± 0.6 nm, respectively. These values are in consistent with the trend of increasing generation (from G2 to G4 generation). From the periodicity along each strand, it seems to have second structure. Further experiments are needed to explore the detailed structures of these unusual nanoobjects.

7.3. Complex formations of dendronized polymers with DNA

As we know, isolated DNA molecules would energetically prefer to be in the relaxed state. In fact, virtually all DNA within both procaryotic and eucaryotic cells exists in the negative supercoiled state. As such it seldom, if ever, occurs as free DNA, but is complexed with specific DNA-binding proteins to form compacted molecules called
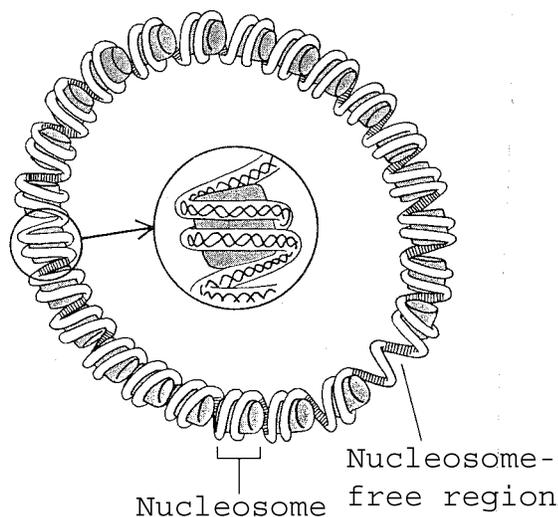

Fig. 29. A schematic depiction of Nucleosomes giving chromatin strands a beadlike appearance.

chromatin. How this compaction occurs is best understood for eucaryotic chromatin, whose most prominent DNA-binding components are the histones. Histones are relatively small, positively charged, arginine- and lysine-rich proteins that aggregate together to form dicrete ellipsoid-shaped packets (histone cores) around which the DNA supercoils. The resulting nucleosomes (see Figure 29) give Chromatin its beaded appearance. In forming the nucleosomes of eucaryotic cells, 200-base-pair-long segments of DNA negatively (left-handedly) supercoil twice around the histone cores. The energy lost by supercoiling may be partially compensated by the energy gained from the ionic and hydrogen bonds formed between the histones and the DNA. The number of supercoils in solution need not be the same as found in chromatin.

Recently, using more genteelly preparative procedures, non-viral gene delivery systems based on DNA complexes with polycations have attracted significantly attention.[64-68] These complexes can form spontaneously as a result of co-operative electrostatic interactions between phosphate groups of the DNA and oppositely charged groups of the polycation. Characterization of the reactions of polyion coupling between DNA and polycation and interactions of formed Polycation / DNA complexes with charged species present in the biological milieu is important for the use of these systems in gene delivery.

The charged higher molecular weight dendronized polymers prepared in this thesis have provided an ideal raw polymers for the direct visualisation of the interaction of polycation / DNA complexes.

For the measurement of polycation / DNA complexes, the surface of mica has been treated with 0.01 % aqueous poly-L-ornithine solution followed by washing the surface of mica with distilled water and drying this with nitrogen. By this treatment, the negative charged surface of mica has been converted into the positive charged ones with which negatively charged DNA can be absorbed. Samples for the SFM experiments were prepared by spin casting of a mixture of 12 µL DNA (10 µg/µL) and 15 µL dendrimer solution (33 µg/µL) in HEPES N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid buffer solution (PH = 7.0), and followed by wash the surface with distilled water and dried with nitrogen. The DNA samples used here are circle plasmid DNA puc19 with 2686 base pairs and linear DNA puc 19 with 2682 base pairs (cutting from the former). It should be pointed out that the resulting mixture of charged dendronized polymer and DNA are soluble in the buffer solution.

The measurements were performed with a Nanoscope Multimode SFM (Digital Instruments), and the operating conditions are the same for the measurement of charged polymers. Figure 30 presents a height contrast image of SFM of the complex of charged G2 polymer **33b** with circle plasmid DNA. From the profile, DNA was wrapped around the surface of charged dendronized polymer without cross-linkage. In some part, the shape of the complex are fatter, indicating more DNA segments were winded.

Figure 31 illustrates a height contrast image of SFM of the complex of charged G4 polymer **37b** with linear plasmid DNA. In the profile, the two chain ends of DNA were wrapped to the surface of G4 polymer, forming a circle of DNA and its complex with polymer.

Another example of the formation of complex of linear DNA with Charged G4 dendronized polymer is given in Figure 32, in which the middle DNA chain segment was wrapped around the surface of the G4 polymer **37b**.

From these preliminary results, it can be concluded that DNA chain can readily form complexes with positive charged dendronized polymers under mild conditions. These resulting complexes can be viusalized individually by SFM. Further investigation is needed to understand the process of how they interact to form complexes.
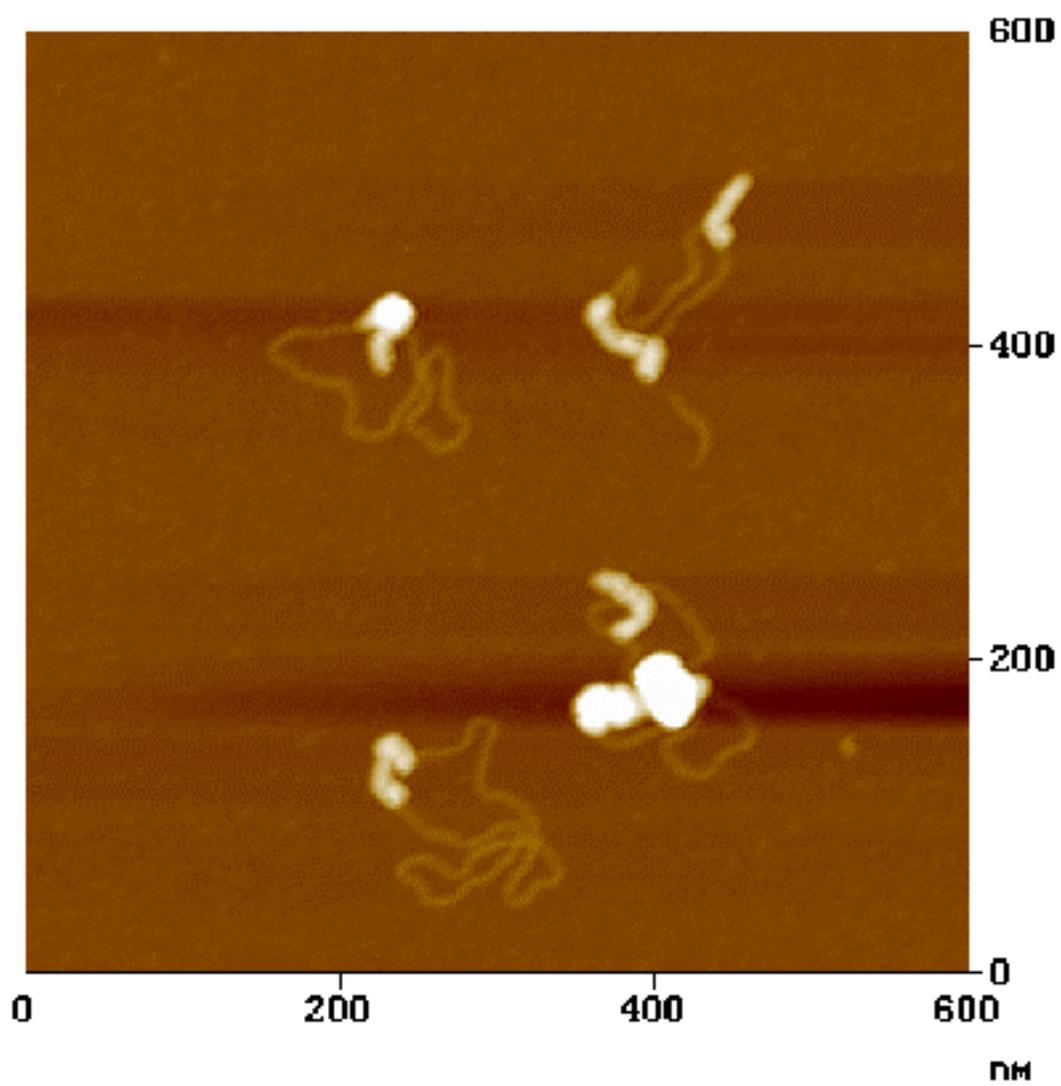
Fig. 30. A height contrast image of SFM of the complex of charged G2 polymer **33b** with circle plasmid DNA.
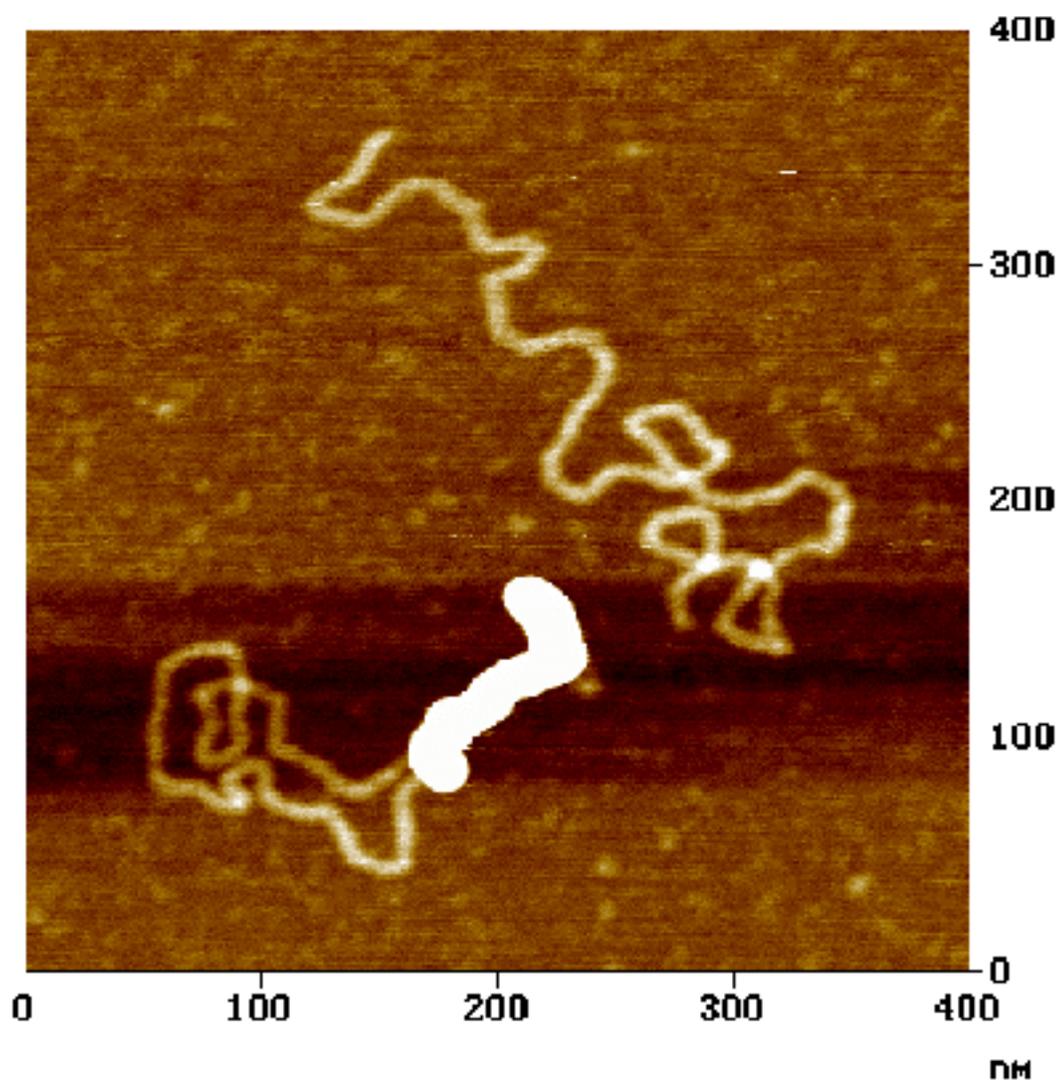
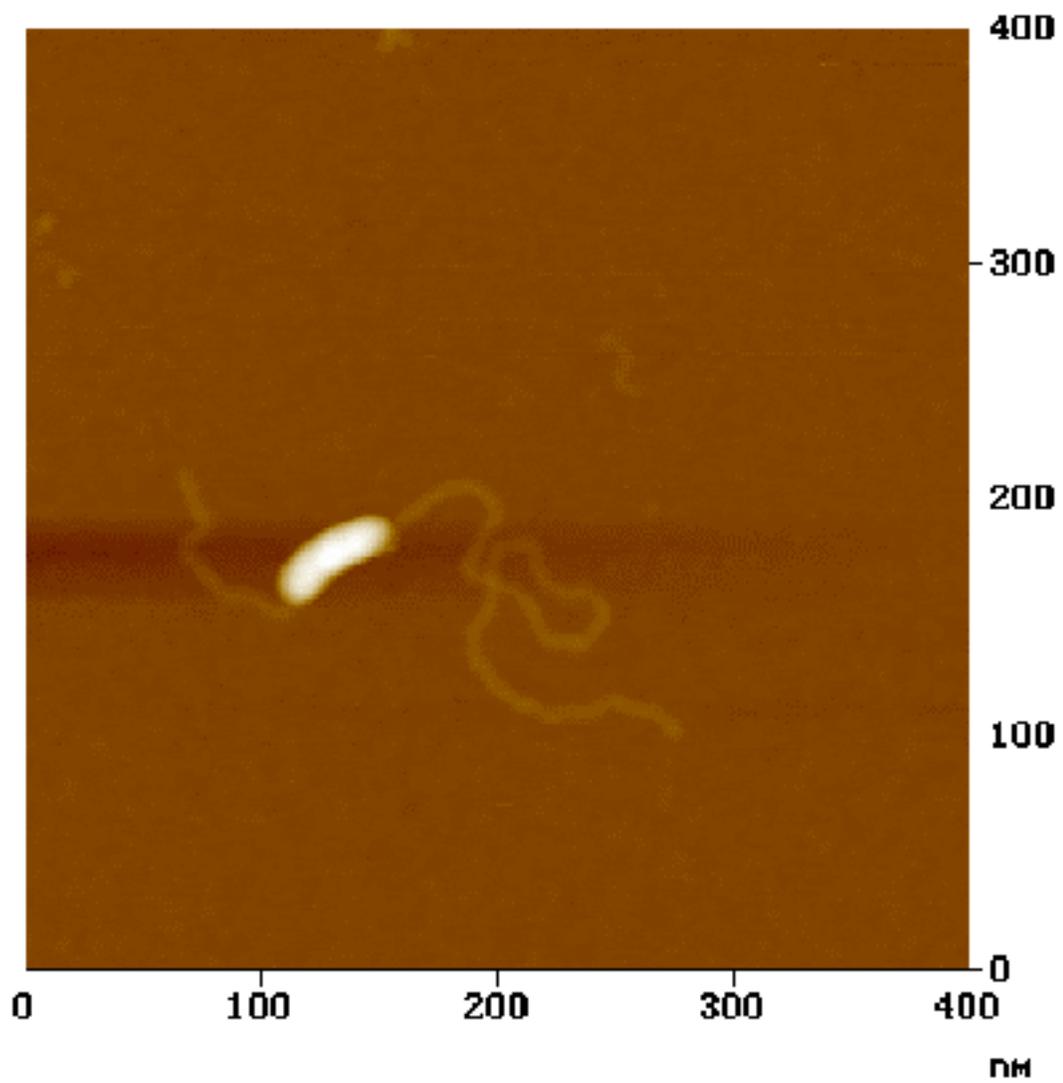**Fig. 31**. A height contrast image of SFM of the complex of charged G4 polymer **37b** with linear plasmid DNA.

Fig. 32. A height contrast image of SFM of the complex of charged G4 polymer **37b** with linear
plasmid DNA.
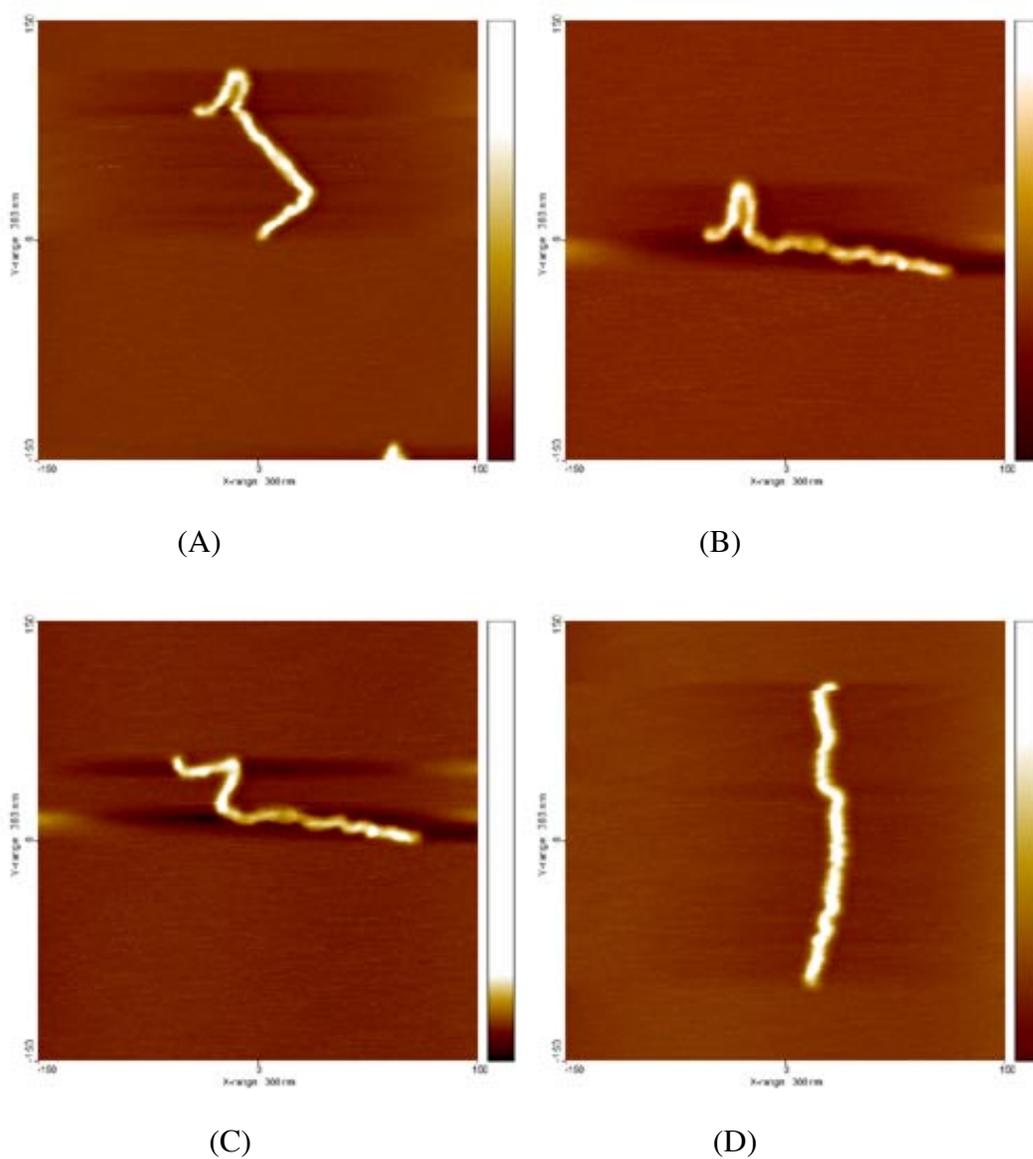
(A)

(B)

(C)

(D)

Fig. 33. Manipulation of a single G2 dendronized polymer **42** absorbed on HOPG by SFM tip, in
which the targeting polymer chain has been manipulated into a linear one by
several consecutive manipulations.

SFM investigation of alkyl chain modified dendronized polymers are in progress. For the G2 polymer **42**, it can be readily absorbed on the HOPG surface. SFM height profile shows that the polymer can align along the symmetric substrate. One of the individual polymer chain has been manipulated to exhibit a linear conformation. The manipulation processes are illustrated in Figure 33. the operation sequence is from A to B and then C and D.