

# Immobilizing DNA on gold via thiol modification for atomic force microscopy imaging in buffer solutions

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Received 18 November 1993

Thiols, dialkylsulfides, and dialkyldisulfides are known to be chemisorbed with high affinity on gold. We have prepared DNAs of specific length and sequence carrying thiol groups at each end. For this purpose, primers with an HS-(CH<sub>2</sub>)<sub>6</sub>-arm at the 5'-end were used to amplify segments of plasmid DNA via the polymerase chain reaction. These thiolated DNAs bind strongly to the large, ultraflat Au surfaces which we have recently described [Hegner, M. et al. (1993) *Surface Sci.* 291, 39–46], and can be imaged by AFM in liquids (aqueous solutions or propanol). The lengths obtained in the AFM images are consistent with the DNA being in a native B-conformation.

Atomic force microscope; DNA; Gold; Chemisorption; Immobilization; PCR

## 1. INTRODUCTION

Scanning probe microscopy (SPM), which includes scanning tunneling microscopy (STM) [1], atomic force microscopy (AFM) [2], and related techniques, allow in principle ultrastructural investigations of biological macromolecules under native conditions, i.e. in 'physiological' buffers [3] and in the presence of biologically relevant ligands. AFM does not depend on the conductivity of the biological material and it is therefore the favored technique. In addition to mimicking to some extent 'physiological conditions' and to allowing biological macromolecules to be examined in their native state, imaging by AFM in liquids allows the force between the tip and sample to be reduced by a factor 10 to 100 as compared to the force required in air or vacuum. This is clearly important if one wants to avoid deformation of the soft biological material by the tip-sample interaction in contact mode measurements [4].

Air-dried DNA has been imaged by STM or AFM on various substrates (e.g. mica, gold) ([5]; see also [6] and references therein) or by embedding in carbon films [7]. Others have electrochemically adsorbed DNA onto gold surfaces [8–10] or fixed it onto chemically modified mica [11–13]. Highly oriented pyrolytic graphite (HOPG) can produce artefacts mimicking DNA [14–16], and therefore it is not used anymore as a substrate for DNA imaging.

In water solution DNA has been imaged by AFM on aminosilylated mica [13], and on bare mica [12].

Gold is in principle a very convenient substrate for a number of reasons. It is chemically rather inert, but

accessible to chemisorption via thiolate bonding. Au thiolates are known to be quite stable (heat of adsorption, tens of kcal/mol [17–19]). Thiol groups can be introduced in a (biological) macromolecule by appropriate modification; alternatively, self-assembled monolayers can be formed onto gold surfaces using heterobifunctional compounds, anchored to gold via thiolate bonds and then exposing the appropriate chemical functions to the (water) medium [20].

We have recently described the facile preparation of large, flat polycrystalline gold surfaces (mean roughness on 25  $\mu\text{m}^2$ , 2–3 Å) [21]. Macromolecules and organelles anchored to such homogeneous surfaces should be easy to recognize.

In the present paper we describe the immobilisation of well-defined DNA fragments onto these flat gold surfaces. In essence, defined DNAs were produced by the polymerase chain reaction using oligonucleotide primers with a thiol group at the 5'-end. The resulting DNA fragments were anchored to flat gold surfaces via thiolate bonds at the two ends, thereby leaving the DNA backbone relatively free to interact, e.g. with enzymes or DNA binding proteins. Others have studied such interactions in samples which were adsorbed on mica and dried in air [22]. The DNA images reported below were obtained by AFM, although other scanning probe microscopies could be used, at least in principle.

## 2. MATERIALS AND METHODS

### 2.1. The DNA-fragments

Thiol-substituted deoxy-oligonucleotides (Microsynth, Windisch, Switzerland) to be used as primers in PCR were obtained by esterification of a 6-mercapto-hexanol-group to the 5'-phosphate of oligonucle-

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otides (20-mers) using a  $\beta$ -cyanoethyl-phosphoramidite C6-Thiol-Modifier (Clontech Laboratories, Inc. Palo Alto, CA).

Two well-characterized double-stranded DNAs were used as templates: a standard plasmid (pBluescript KS<sup>+</sup>) and the plasmid pro-SL4, which includes a segment (668 bp, length ~225 nm) of wild-type pro-sucrase-isomaltase cDNA in pBluescript KS<sup>+</sup> [23]. The plasmid pBluescript KS<sup>+</sup> was linearized with *ScaI* and the plasmid pro-SL4 with *XmnI*. The fragments were amplified by PCR (Perkin Elmer Cetus; Ampli Taq) using either thiol-modified oligonucleotides or, as a control, unmodified oligonucleotides. The resulting length was specified by the location of the oligonucleotides in the plasmid sequence (upstream position 29 oligonucleotide 5'-d(GCGGCGGGTGTGGT-GGTTAC)-3'; downstream position 1609 oligonucleotide 5'-d(GCACCGCCTACATACCTCGC)-3'). In order to avoid oligomerization of the oligonucleotides during the PCR reaction, the polymerization mixture contained dithiothreitol (DTT) (final concentration 2 mM). The two thiolated DNA fragments obtained were a 1581-bp pBluescript fragment and a 2249-bp pro-SL4 fragment, respectively. After PCR, the DNA fragments were extracted with phenol, precipitated with ethanol, and purified by agarose gel electrophoresis. Since residual agarose, even in minute amounts, might be mistakenly interpreted as DNA threads, the thiolated DNA fragments were further purified by chromatography on Elu Tip d (Schleicher and Schuell, Feldbach, Switzerland) according to a standard protocol [24]. Purified DNA was dissolved in either 10 mM Tris-HCl, pH 7.5, or 10 mM ammonium acetate.

The DNA fragments can be stored for several months at +4°C, but should not be frozen (after thawing, they appear as bundles). Prior to use, a 200-fold excess of DTT is added, i.e. to a concentration of approx. 0.1  $\mu$ M and incubated for 30–60 min. The DTT which is chemisorbed to gold at this concentration does not seriously interfere with our measurements.

## 2.2. The gold-substrate

The template-stripped gold surfaces were prepared as described earlier [21]. Following the stripping with tetrahydrofuran the mica sheet was lifted gently using a slight vacuum suction. The gold-platelets were dried in a desiccator for 15 min prior to use.

## 2.3. Anchoring the DNA

Routinely, a droplet of 1  $\mu$ l (0.3–0.5 fmol) of DNA in DTT and either 10 mM ammonium acetate or Tris-HCl buffer was transferred onto the template-stripped gold surface, and left for 45–60 min at room temperature at 100% relative humidity; then dried for 10–30 min in a desiccator, and rehydrated in either ammonium acetate or Tris-buffer. This cycle of drying and rehydration — which should not irreversibly denature the DNAs [25] — was found to lead to a fair amount of DNA being anchored to the gold surface. After mounting in the AFM instrument, the fluid cell was flushed with either ammonium acetate or Tris buffer, which removed non-thiolate-anchored DNA. In principle, thiolated DNA fragments can be anchored to gold directly from the droplet solution without drying; however, the amount bound was then found to be quite small, which required long scanning times.

In control experiments DNA fragments were radioactively labelled with [<sup>32</sup>P]dCTP and their degree of adsorption was assessed with a Phosphorimager (Molecular Dynamics, Krefeld, Germany).

## 2.4. AFM measurements

All AFM measurements were carried out with a Nanoscope III atomic force microscope (Digital Instruments Inc., Santa Barbara, CA). Image acquisition in the constant-force imaging mode was done in liquids (water, propanol, ethanol, buffer (10 mM Tris-HCl, pH 7.5/1 mM EDTA) or in 10 mM ammonium acetate with or without 0.1  $\mu$ M DTT) using the fluid cell from Digital Inc. We used various monocrystalline silicon cantilevers with integrated silicon tips with spring constants ranging from 0.08 to 0.17 N/m.

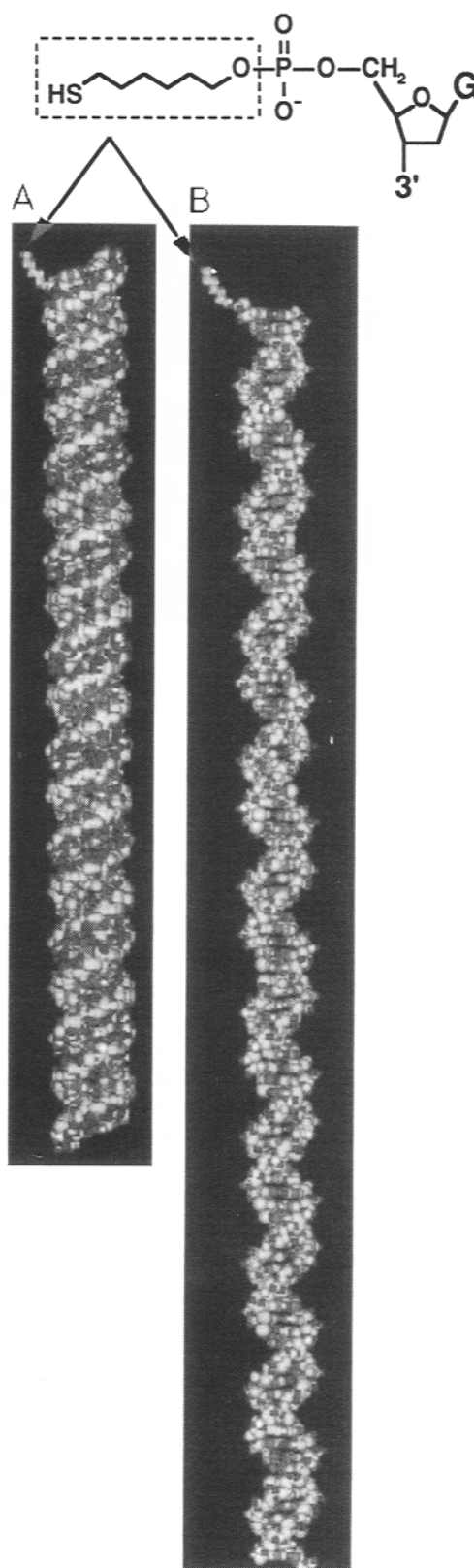


Fig. 1. Van der Waals models of DNA fragments with the HS(CH<sub>2</sub>)<sub>6</sub>-arm at the 5'-end (100 bp). (A) In the A-conformation; (B) in the B-conformation.

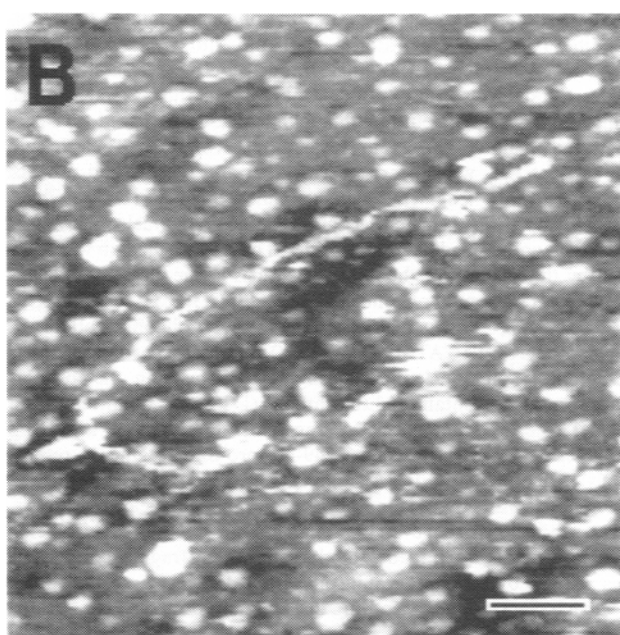
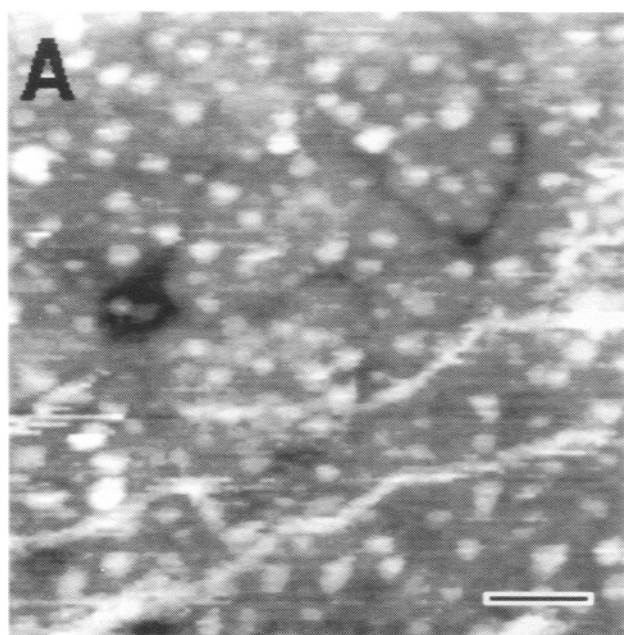


Fig. 2. A: AFM image of a 1581 bp DNA fragment (length  $\sim 520$  nm) in 10 mM ammonium acetate. Scan size is  $630 \times 630$  nm; bar = 100 nm. B: AFM image of a 2249 bp DNA fragment (length  $\sim 750$  nm) in 10 mM ammonium acetate. Scan size  $650 \times 650$  nm; bar = 100 nm.

### 3. RESULTS AND DISCUSSION

We have recently described [21] a procedure to prepare very flat and large 'template-stripped' polycrystalline Au (111) surfaces with a mean roughness of 2–3 Å over some  $25 \mu\text{m}^2$  areas. In the present paper we report that DNA can be bound to these flat gold substrates via thiolates and can be imaged by AFM.

An  $\text{HS}(\text{CH}_2)_6$ -arm was linked via an ester bond to the 5'-phosphate of oligonucleotides (see Fig. 1), which were then used as primers in PCR in the presence of DTT. Fig. 1A and B show van der Waals models of one end of such DNA fragments (in the A or B conformation, respectively).

AFM measurements were carried out in the presence of aqueous solutions, which mimic 'physiological' conditions and keep the forces acting on the DNA samples as small as possible (i.e. approx. 1 nN). Larger forces damage and sweep away the DNA fragments (data not shown).

The DNA bound to the gold surface under the conditions detailed in section 2 amounted to approximately 10% of the applied quantity, as determined by analysis of  $^{32}\text{P}$ -labelled samples. However, still less than that, i.e. 1–5%, was eventually imaged by AFM. This may be due to anchoring of the DNA at one end only, perhaps because of steric hindrance by the extra nucleotide that Taq DNA polymerase may introduce at the 3'-end of the DNA [26]. In addition, the relatively short spacer used could also lead to partial steric hindrance. A longer spacer was not used in order to avoid interaction of the polymethylene chain with the DNA bases. Non-an-

chored DNA fragments are likely to be swept away by the AFM tip and thus not be imaged.

Figs. 2 and 3 show representative AFM images of thiolated DNA fragments. The DNAs in Fig. 2A and

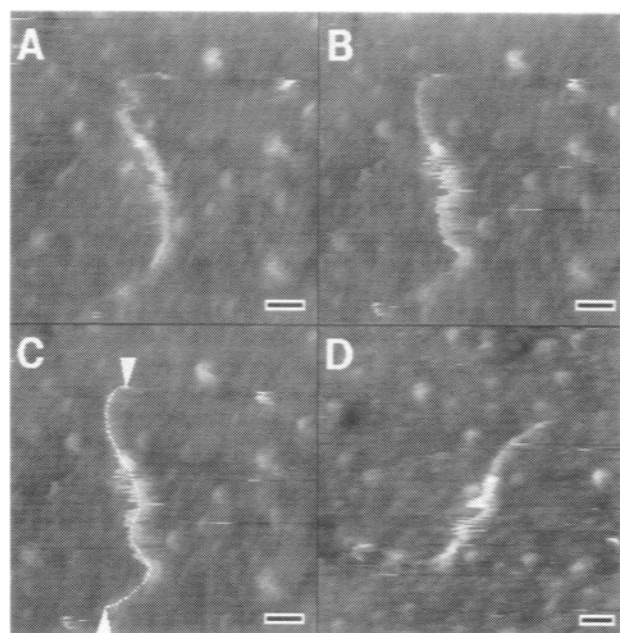


Fig. 3. AFM images of the same 2249 bp DNA fragment (length  $\sim 750$  nm) of Fig. 2B, but measured in propanol. Scan size  $920 \times 920$  nm (A–C), or  $1 \mu\text{m} \times 1 \mu\text{m}$  (D). Bars = 100 nm. (A) and (B) Simultaneously captured data sets of the same fragment, but with different scanning directions: (A) left to right and (B) right to left. (C) the arrow heads indicates the anchoring ends; the white dotted line in between gives the length of the fragment. (D) Fragment oriented  $30^\circ$  to the fast-scan axis.

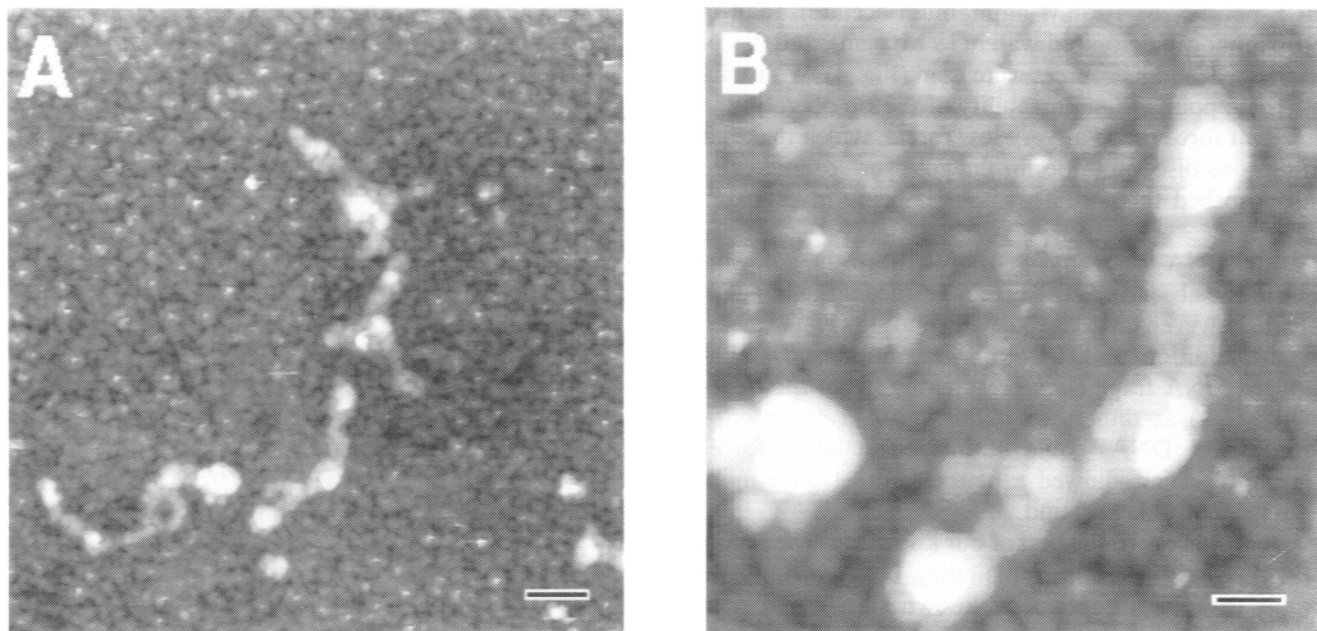


Fig. 4. AFM of non-reduced DNA fragments: the supercoils are (presumably) dimers or oligomers. (A) scan size  $3.3 \times 3.3 \mu\text{m}$ ; bar = 300 nm. (B) Scan size  $10 \times 1.0 \mu\text{m}$ ; bar = 100 nm.

B (in aqueous ammonium acetate) consisted of 1581 bp and 2249 bp, and were, as the figures indicate, approximately 520 nm and 750 nm long, respectively. The measured lengths of these DNA fragments corresponded therefore to those of DNAs in B-conformation ( $3.38 \text{ \AA/bp}$ ), rather than to those in A-conformation ( $2.56 \text{ \AA/bp}$ ) [27–29].

From these morphological considerations we conclude that the DNAs imaged in Fig. 2 were in all likelihood in a *native* B conformation. The agreement between the expected and measured lengths further suggests that they are attached to the gold surface at both ends.

In propanol (which is known to dehydrate DNA) images with better resolution and a width better corresponding to the actual diameter of DNA can be obtained [30]. The DNA in Fig. 3A–D is the same fragment as in Fig. 2B (2249 bp, approx. 750 nm long), now imaged in propanol. The length actually measured (Fig. 3C, white line, between the two arrowheads pointing to the anchoring ends) is  $3.35 \text{ \AA/bp}$ , which is in excellent agreement with that of DNA in B-conformation, i.e.  $3.38 \text{ \AA/bp}$ . The fragment is oriented perpendicularly to the fast-scan axis in Fig. 3A and B. These images were obtained simultaneously, Fig. 3A with the scan direction from right to left, Fig. 3B with the scan direction from left to right; the DNA fragment moves following the cantilever tip with a frequency of 1 Hz and maximum amplitude and speed of approx. 80 nm/s. In spite of the mechanical strain, the DNA fragment was imaged very satisfactorily. In Fig. 3C a white dotted line is drawn on the whole length of the fragment which is

anchored at both ends (arrowheads) to the gold surface. The same DNA fragment is shown again in Fig. 3D, but measured at a scan angle of  $30^\circ$  to the fast scan-axis; this leads to a more parallel movement of the cantilever on the DNA backbone (the bottom end does not show up clearly, because this part was oriented parallel to the fast-scan axis).

The DNA widths of AFM images are known to be larger than the real objects, with the observed width depending on the size of the tip used. Assuming that the radii of our tips are approx. 10 nm (as given by the manufacturer), and that they are circular or parabolic, the appropriate equations [31,32] show that our AFM images (10–18 nm) are indeed compatible with the expected width of DNA. Further improvement of the images will critically depend on the development of finer tips. In addition to this AFM artefact, a broadening of the DNA occurs as one moves away from the anchor points (see Fig. 3A–D). We attribute this to movement of the DNA molecule by the scanning tip, much as the movement of a child's jump rope is much larger in the middle than at the ends.

Scanning probe microscopy is more reliable in the *z*-dimension. In the DNA images of both Figs. 2 and 3, the measured heights were 1.7–2.5 nm, which are close to that of native DNA (i.e.  $\sim 2 \text{ nm}$ ). Thus, the DNA was not detectably compressed by the tip forces which we used (1 nN).

With the low forces used to image DNA the background showed irregular points, with a *z*-dimension in the same range as that of DNA (Fig. 2). These points were far more evident in salt solutions (10 mM Tris-



HCl, pH 7.5, or 10 mM ammonium acetate), than in propanol or in deionized water (18 M $\Omega$ ·cm), and disappeared when higher forces were used (Fig. 4). We attribute these points to an instability of the tip operating in constant force mode at very low forces, which makes it to react to discontinuities in the gold surface and/or to salt adsorbed thereon. This issue is being investigated further.

If the DNA fragments were brought to the gold surface in the absence of DTT, they formed large bundles, which, however, could be partially and slowly unraveled by the addition of DTT. Fig. 4A and B show non-reduced DNA fragments, presumably (from the measured width) as dimers or oligomers in a supercoiled form. These DNAs could be imaged at larger forces (2–4 nN), which allowed a better contrast on a more homogeneous background.

In conclusion, to the best of our knowledge, we report here the first procedure for binding DNA via thiolate bonds to gold surfaces. The known stability of such bonds and the fact that they anchor the DNA threads at their ends only, leaving their backbones lying free on the gold substrate should make our procedure suitable for following dynamic molecular events involving DNA.

**Acknowledgements:** The authors wish to thank Dr. N. Mantei and W. Boll for helpful advice. This work was supported by grants from the ETH Zurich, for which Profs. R. Hütter and R. Ernst are gratefully acknowledged.

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