



Universiteit  
Leiden  
The Netherlands

## Feeling sugar-protein interactions using carbon nanotubes : a molecular reognition force microscopy study

Klein, D.C.G.

### Citation

Klein, D. C. G. (2004, November 11). *Feeling sugar-protein interactions using carbon nanotubes : a molecular reognition force microscopy study*. Retrieved from <https://hdl.handle.net/1887/106077>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/106077>

**Note:** To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/106077> holds various files of this Leiden University dissertation.

**Author:** Klein, D.C.G.

**Title:** Feeling sugar-protein interactions using carbon nanotubes

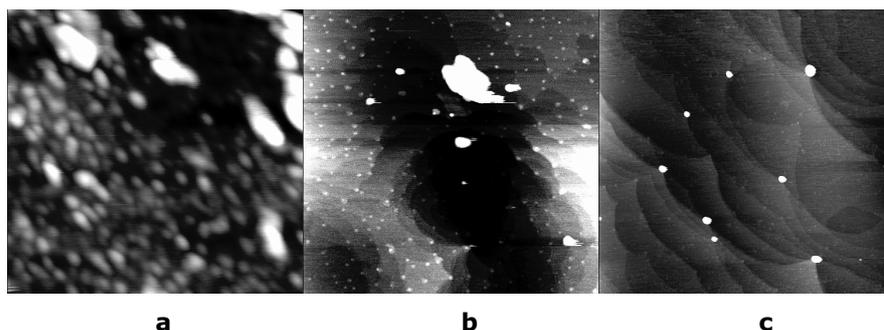
**Issue Date:** 2004-11-11

## Appendix gold crystal preparation

### Preparation of a gold crystal containing a hydroxyl-terminated SAM

The gold single crystal, with a mechanically polished (111) surface, was sputtered and annealed in ultra-high vacuum in order to clean the surface and to allow atomically flat terraces to be formed. This first cleaning step had to be performed only once. The steps that will be described below have to be repeated each time that the gold surface is regenerated.

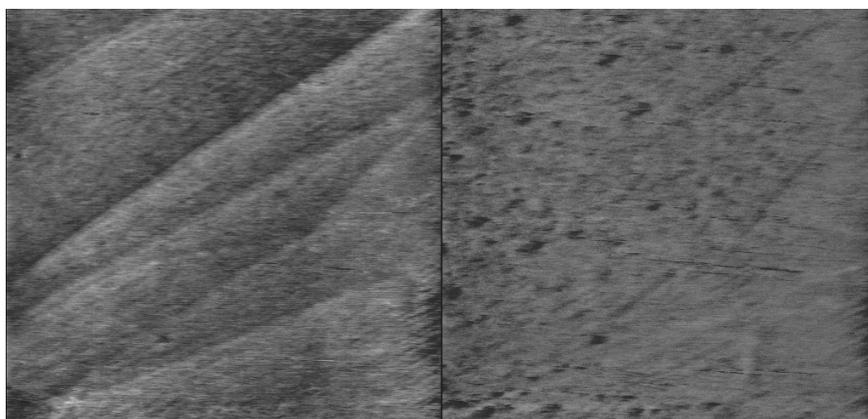
Before allowing a SAM to form on the gold crystal, possible protein and lipid remainders of the previous experiment had to be removed. First, the crystal was incubated overnight with detergent (Hellmanex 0.5%), rinsed very well with deionized water and dried in a nitrogen flow. Globular features with a diameter of around 20 nm are found on the gold crystal, as can be seen in Figure 1 a. Probably



**Figure 1** AFM images of three stages in the preparation of a clean Au (111) substrate. All three images were recorded in tapping mode in air, scales are different. (a) Gold crystal after overnight incubation with detergent, rinsed and dried. The AFM image dimensions are 500 nm x 500 nm x 10 nm. (b) Gold crystal after heating in air to 615 °C for 20 minutes. The AFM image dimensions are 1  $\mu\text{m}$  x 1  $\mu\text{m}$  x 4 nm (c) After rinsing with ethanol, and drying with nitrogen. Image dimensions are 2  $\mu\text{m}$  x 2  $\mu\text{m}$  x 10 nm. Most of the contaminant particles originally present on the gold surface are rinsed away, but the low density of residual impurities that act as pinning sites cannot be removed by this method.

these are micelles of detergent. In order to get rid of these, the crystal was heated in air to 615°C for 20 min. This results in a relatively clean surface as is shown in Figure 1 b, which contains much less contamination, and already shows the (111) terraces of the Au crystal. To remove this residual contamination as much as possible, the gold surface was rinsed with ethanol and dried in a nitrogen flow. Most of the particles were removed, although a low density of impurities was still present that acted as pinning sites, Figure 1 c. To remove these, another sputtering and annealing cycle would be necessary. In addition, when forming a SAM, the alkane thiols will compete with the impurities on the surface, and because of the strong gold-thiol bond, the alkane thiols are likely to “win” this competition and form a clean SAM on the gold crystal.

In order to form a SAM, the cleaned gold crystal was incubated overnight in a 2 mM 11-mercaptoundecanol solution, rinsed with ethanol and dried in a nitrogen flow. The gold crystal containing the SAM, imaged with the AFM in tapping mode in liquid is shown in Figure 2.



**Figure 2** Tapping mode height (a) and phase (b) AFM images of a gold crystal with a SAM of 11-mercapto-undecanol, recorded in 0.01M phosphate buffer at pH 3.4 with a regular tip. Image sizes are 500nm x 500nm x 5nm and 500nm x 500nm x 20°.

## Acknowledgements

The research described in this thesis has been made possible by the help of many people. I would like to thank all the people who designed and fabricated equipment, who purified and provided biological samples, who allowed me to use their custom-made apparatus, and who shared their knowledge with me.

Ik wil Dian van der Zalm van de Fijnmechanische Dienst van de Universiteit Leiden bedanken voor het vervaardigen van een fijnmechanisch besturingssysteem voor de scanning elektronen-microscop. Ook heeft Dian samen met Lau van As en Gerard van Amsterdam van de Leidse Instrumentenmakersschool een heel kleine functionalisatiecel voor koolstof-nanobuis tips ontworpen en gemaakt, een waar kunststukje. Ik ben Raymond Koehler dank verschuldigd voor het ontwikkelen van de "peakdetector".

I am grateful to all the people involved in the Bio AFM Lab for a very nice collaboration, especially Maarten van Es and Allard Katan. I would like to thank Karin Sliedregt-Bol and Gijs van der Marel from the Leiden Institute of Chemistry for synthesizing mannose with a three carbon-linker. We thank Jens Christian Jensenius from the Department of Medical Microbiology and Immunology, University of Aarhus for providing rMBL and for discussions and advice, Henriette Jensenius from Leiden University for initiating the work on MBL and for her contribution to the experiments described in this thesis, Amalia Stamouli from Leiden University for her leading role in the work on LH2, Marc Laus from the Institute of Biology Leiden University for discussion and for supplying mannose, and Margot Beukers from the LACDR at Leiden University for providing CHO cells and advice.

We thank Clara L. Díaz from the Institute of Biology, Leiden University, for rat IgE, Trudy J.J. Logman from the Institute of Biology Leiden for purified PSL, and Werner Baumgartner from the Institute of Anatomy of the University of Würzburg for the cadherin construct and the anti-cadherin antibody. I am grateful to Peter Hinterdorfer from the University of Linz for introducing me to the field of molecular recognition force microscopy and to Rachel McKendry and Christian Riemer from University College London for helping me with the chemical functionalization of AFM tips. I am grateful to Daniel J. Müller from the BioTechnological Center, University of Technology in Dresden for providing purple membrane. I would also like to thank Peter Bøggild from the Microelectronic Centre of the Technical University of Denmark for discussions and for inviting me.

## Acknowledgements

---

I would like to thank Suzi Jarvis of Trinity College Dublin for providing the opportunity to fabricate MWNT tips and for her guidance. I am grateful to Frans Tichelaar from the National Centre for HREM in Delft for making TEM images, Martin Clausen of LION at Leiden University for production of SWNT tips and Geiske de Groot of LION at Leiden University for fabrication of MWNT tips.

I am grateful to all the people involved in the development of Camera scanning probe operation system, especially Els van Tol-Homan, Marcel Rost and Maarten van Es of LION, Leiden University. I would also like to thank Ellie van Rijsewijk, Barry Cats, Riet Nieuwenhuizen for helping me with administrative matters. At last, I would like to thank Tjerk Oosterkamp, Joost Frenken and Jan Kijne for inspiring discussions.