# Protein Patterning on Si by AFM Anodic Oxidation

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## Introduction

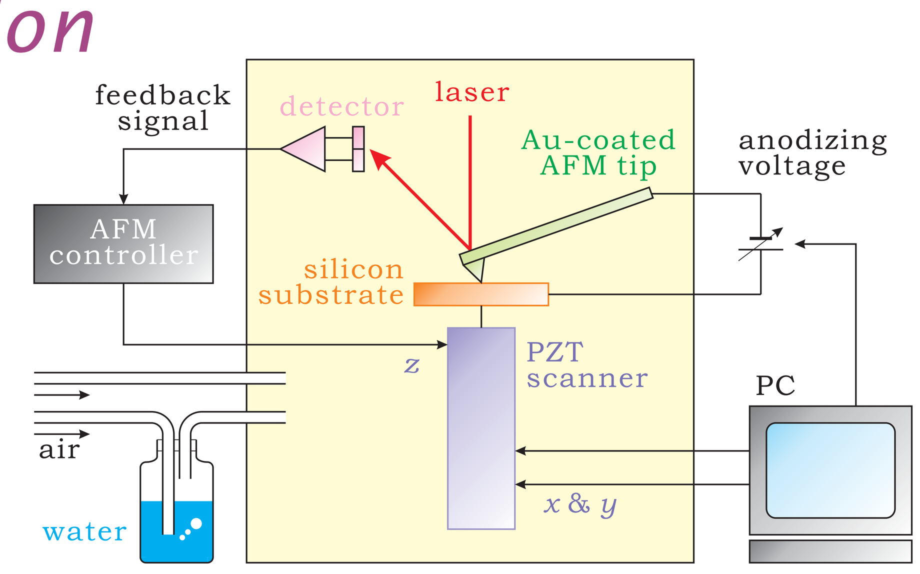
Various methods of protein patterning on Si has been reported in relation to realization of bioelectronic devices [1].

In recent years, there is also an emerging interest in so-called "protein chips". For miniaturization and integration of such devices, patterning of protein molecules at a high spatial resolution is required.

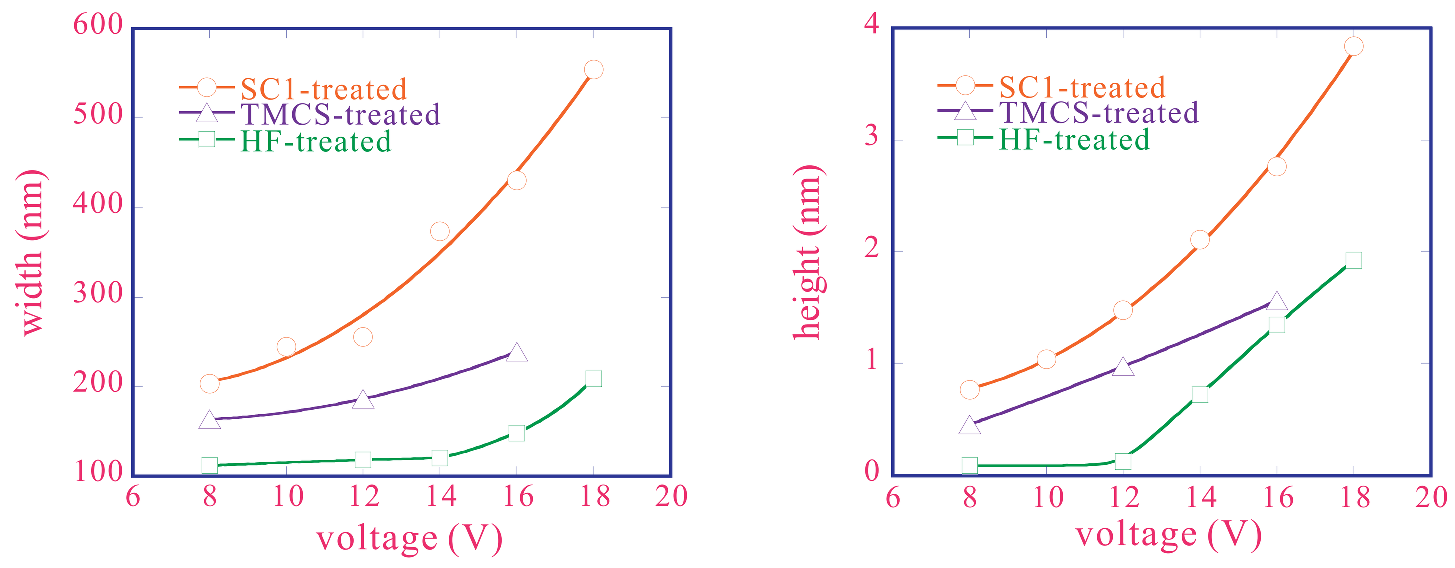
In this study, protein patterning based on the AFM (atomic force microscope) anodic oxidation process [2] was developed.

## AFM Anodic Oxidation

By applying a dc voltage between the substrate and the conductive AFM tip, the surface of the substrate is locally oxidized by anodic oxidation mediated by the adsorbed water.




The block diagram of the AFM anodic oxidation system.




The width and height of the oxide structures drawn at a constant tip velocity of 2.3 μm/s with different voltages on various Si surfaces; (a) Si surface with a thin (ca. 0.6 nm) oxide layer prepared by the SC-1 process, (b) hydrophobic surface prepared by treating the SC1 oxide with trimethylchlorosilane (TMCS) and (c) bare Si surface obtained by removing the SC1 oxide with HF.

## Experiment

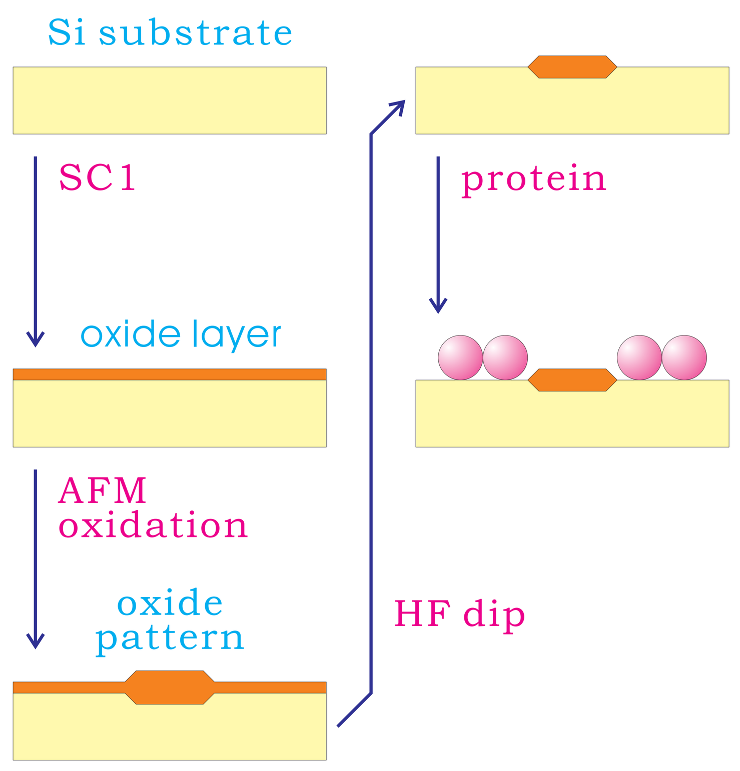
Processes for negative and positive patterning of protein molecules on Si were developed.

substrate: 8-12 Ωcm, n-type Si(111), 250 μm
protein: ferritin ($\phi$=12nm), 21 μM solution

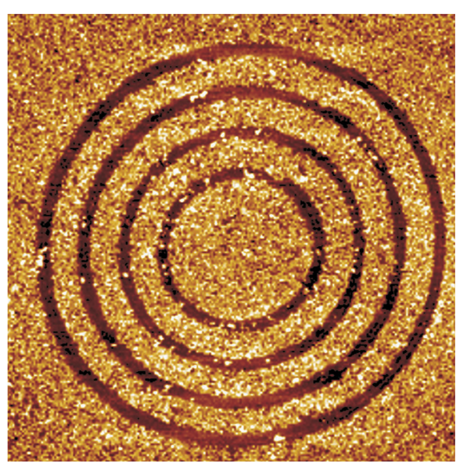
## Negative Patterning (I)

Firstly, a simple process for negative patterning of ferritin molecules on Si was developed.

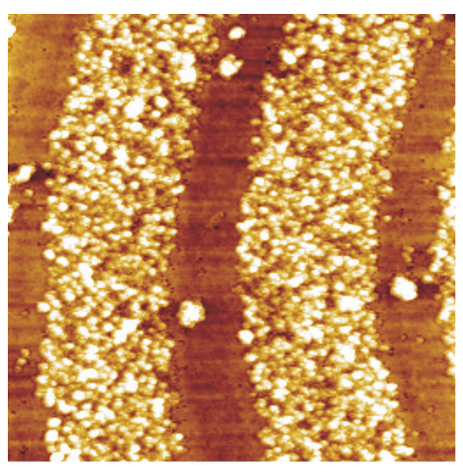
In this process, the ferritin molecules stick to the bare Si surface, whereas they are washed away from the oxide surface.




A process for negative patterning of ferritin molecules on Si.



11 x 11 $\mu m^2$



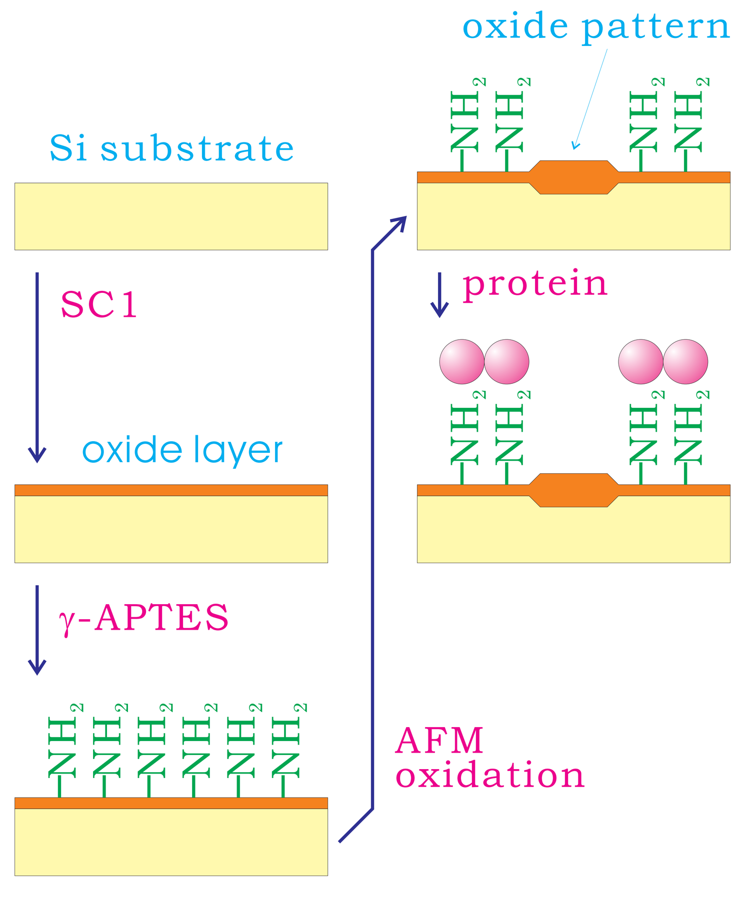
2 x 2 $\mu m^2$

AFM images of the surface after immobilization of ferritin molecules, which cover the Si surface except for the concentric circles of oxide formed by AFM anodic oxidation.

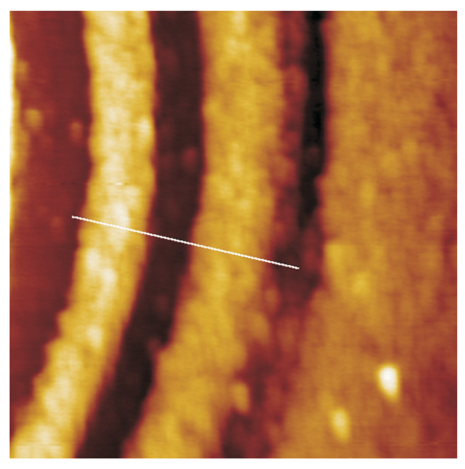
## Negative Patterning (II)

Secondly, a modified process for negative patterning was developed.

In this process, the oxide surface is treated with $\gamma$-aminopropyltriethoxysilane ($\gamma$-APTES) to introduce amino groups, to which the ferritin molecules are attached.

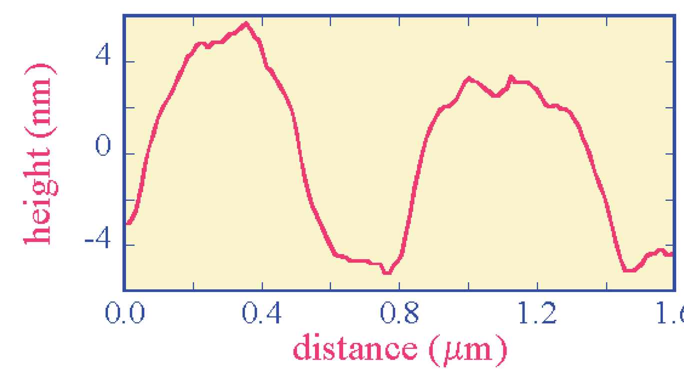



Another process for negative patterning of ferritin molecules on Si.



3 x 3 $\mu m^2$

AFM image of the surface after immobilization of ferritin molecules.



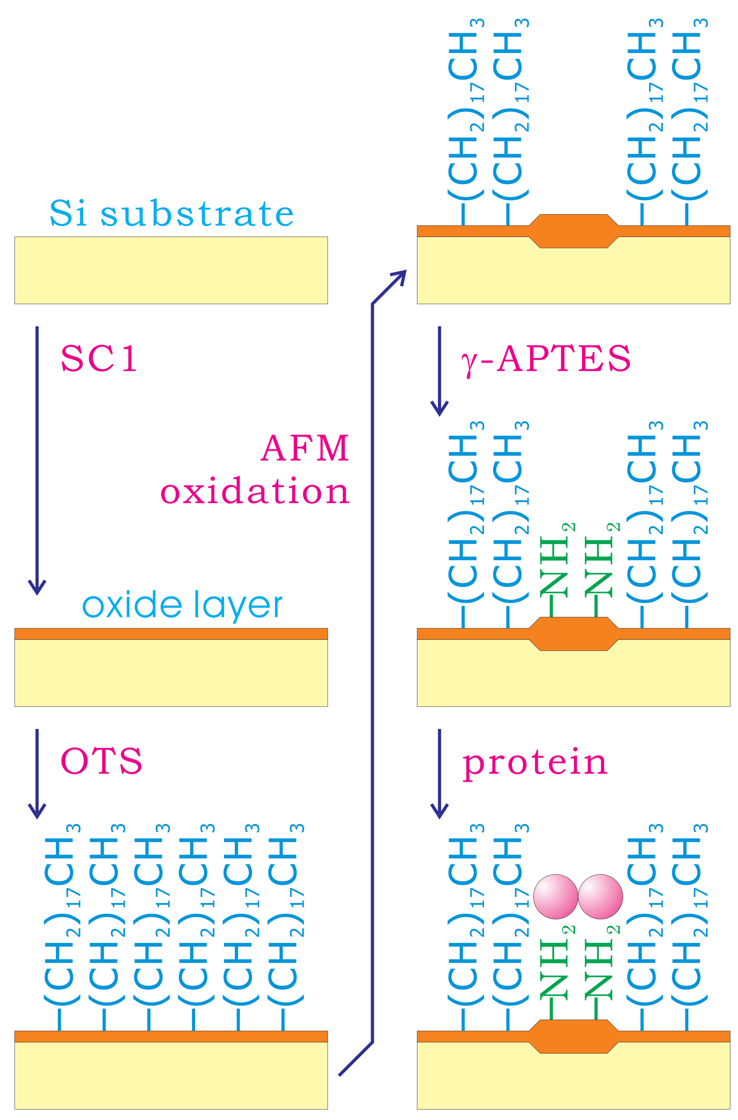

The height profile along the line shown in the AFM image.

## Positive Patterning

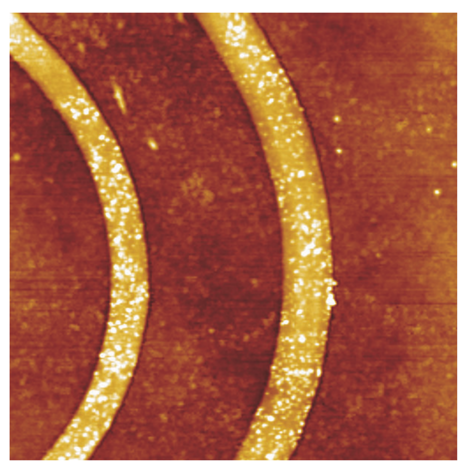
To realize positive patterning, it is necessary to protect the surface from sticking of protein molecules and also from introduction of amino groups during the following processes.

The entire surface is once covered with octadecyltrichlorosilane (OTS).

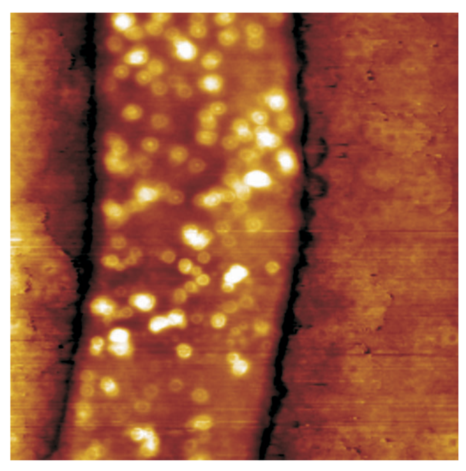
After drawing the oxide pattern, amino groups are introduced with $\gamma$-APTES, to which ferritin molecules are attached.




A process for positive patterning of protein.



5 x 5 $\mu m^2$



1 x 1 $\mu m^2$

AFM images of the surface after positive patterning of ferritin on Si.

## Summary

Based on the AFM anodic oxidation process, submicron patterning of protein molecules on Si was realized. Protocols for both negative and positive patterning were established.

## Acknowledgment

The authors are grateful to Dr. I. Yamashita, Dr. Yu. Mourzina and Dr. K. Suzuki for their advice.

## References

[1] A. S. Blawas, W. M. Reichert, Biomaterials 19 (1998) 595.
[2] W. S. Snow, P. M. Campbell, Science 270 (1995) 1639.