The Study of Barnacle Cyprid Adhesion with Atomic Force Microscopy

In Yee Phang[1,2], Nick Aldred[3], Anthony S. Clare[3], G. Julius Vancso[1,2]

[1] Materials Science and Technology of Polymers, MESA+ Institute for Nanotechnology, University of Twente, P. O. Box 217, 7500 AE, Enschede, The Netherlands

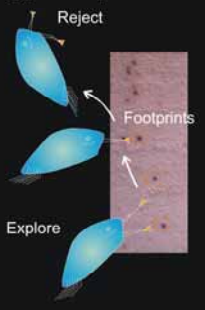
[2] Dutch Polymer Institute, P. O. Box 902, 5600 AX, Eindhoven, The Netherlands

[3] School of Marine Science & Technology, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom.

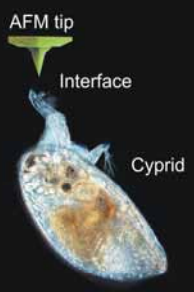
Introduction

The understanding of biointerfaces in contact with seawater is crucially important in tackling the problems of marine biofouling. The aim of our research is to address a particular problem, i.e. barnacle adhesion, to the biointerface and the corresponding fouling process.[1-5] Therefore, Atomic Force Microscopy is used to investigate this specific interface.

Cyprid exploration



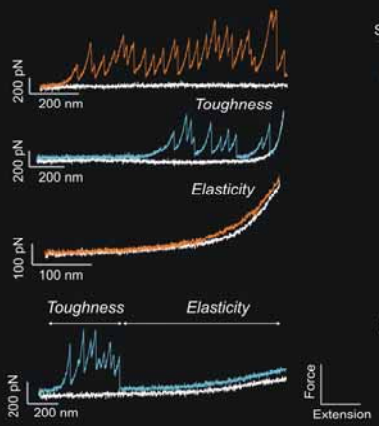
Biointerface



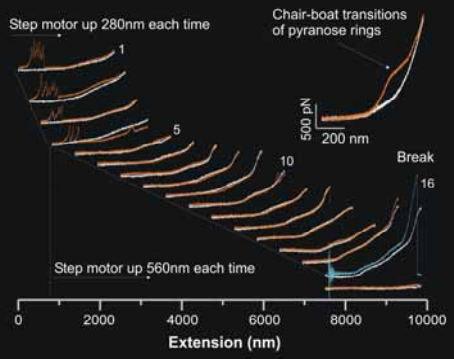
Atomic force microscopy (AFM)
Dimension D3100, Veeco

AFM force spectroscopy of footprint proteins

Toughness and elasticity



Stepwise extension and transition plateau



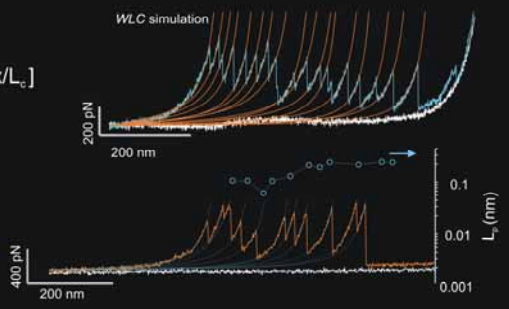
Footprint proteins
- can dissipate large amount of energy
- behave like elastic materials
- can be stretched to high extension.

Worm-Like Chain (WLC) polymer elasticity model

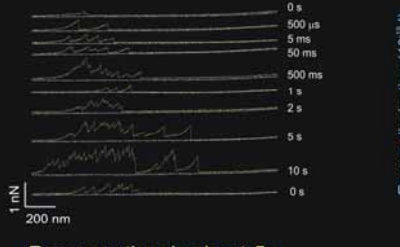
$$F(x)=(kT/L_p)[\ 0.25(1-x/L_c)^{-2} - 0.25 + x/L_c\ ]$$

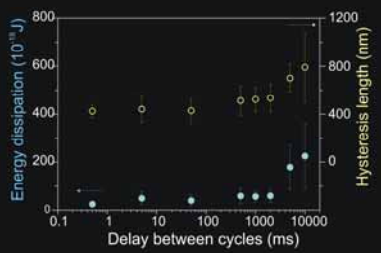
Where
k is Boltzmann's constant
T is temperature
L_p is the persistence length
L_c is the contour length

Footprint protein extension can be simulated by *WLC* mode



Energy dissipation and refolding dynamic

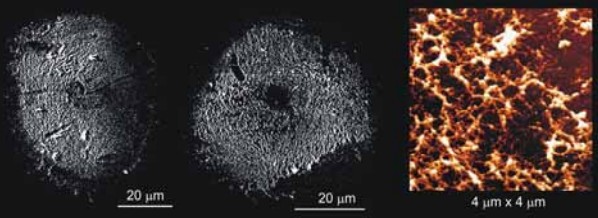




Recovery time is about ***5*** s

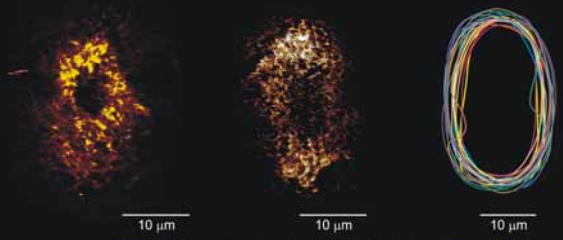
AFM footprint morphology (NH_2-Functionalized glass)

Semibalanus balainoide



Balanus amphitrite

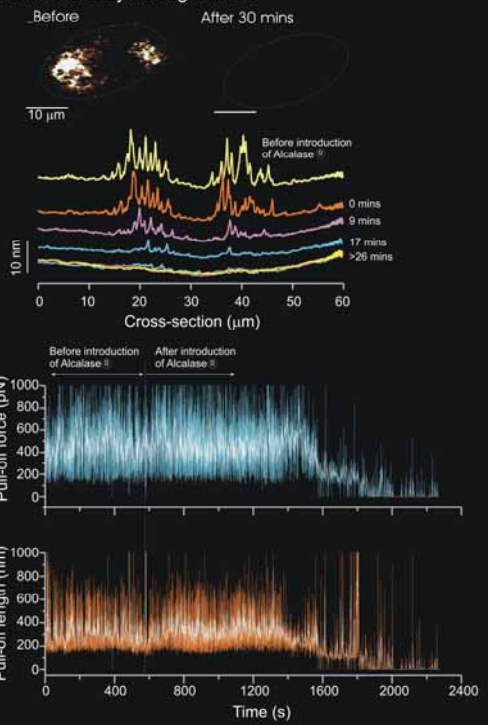
Outline of 19 footprints



The size of footprints depostied on NH_2-functionalized glass surface is similar to the attachment pad of cyprid (*B. Amphitrite*). The volume of footprint deposited is estimated to be 2×10^{-18} m^3.

Effect of serine protease, Alcalase® on footprint

AFM *in situ* enzyme degradation



Footprints can be removed from the surface by serine protease, Alcalase® .The in situ degradation process can be followed by AFM imaging mode and force spectroscopy, respectively.

Conclusions

AFM is a powerful nanoscale characterization technique. Morphology of barnacle cyprid footprints of S. Balanoide and B. Amphitrite were visualized by AFM. Nanomechanical properties of the footprint proteins were studed by AFM based force spectroscopy. in situ enzymatic degradation of footprints adhesives during exposured to Alcalase was monitored by AFM.

References
1. I. Y. Phang, N. Aldred, A. S. Clare, G. J. Vancso. Biofouling, **2006**, 22, 245.
2. I. Y. Phang, N. Aldred, A. S. Clare, G. J. Vancso. NanoS, **2007**,*01*,34
3. I. Y. Phang, N. Aldred, A. S. Clare, G. J. Vancso. J. R. Soc. Interface, **2008**,*5*,397.
4. Aldred, N., I.Y. Phang, S. L. Conlan, A. S. Clare, G. J. Vancso. Biofouling, **2008**,*24*,97.
5. I. Y. Phang, N. Aldred, A. S. Clare, G. J. Vancso. **2008**,*Submitted*.

Financial support by the Dutch Polymer Institute (DPI) is gratefully acknowledged. I. Y. Phang thanks SYNTHESYS for funding the work with Prof. Jens T. HØeg at University of Copenhagen.