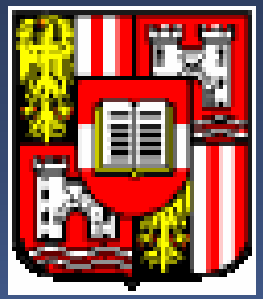


University of Linz
Austria

Institute for Biophysics

Single Molecule Recognition Atomic Force Microscopy

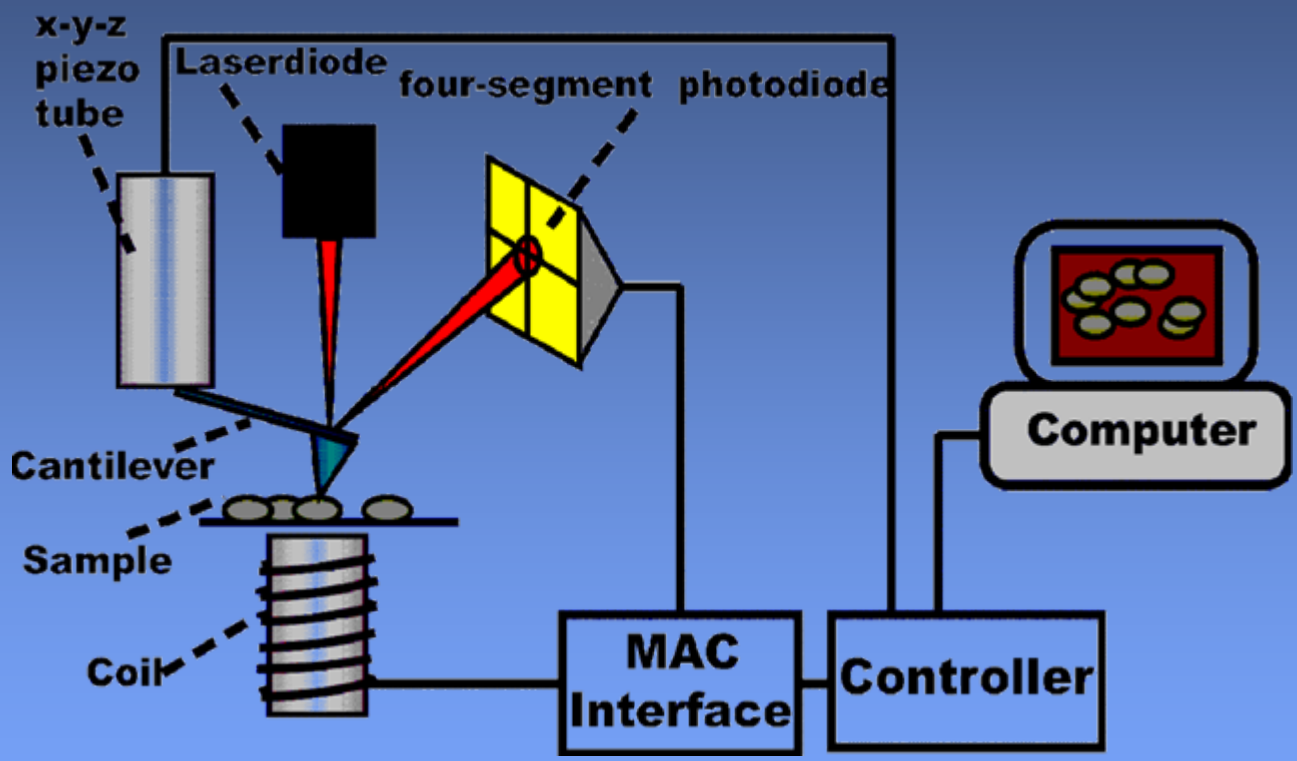
Peter Hinterdorfer

Purdue
10-05-2006

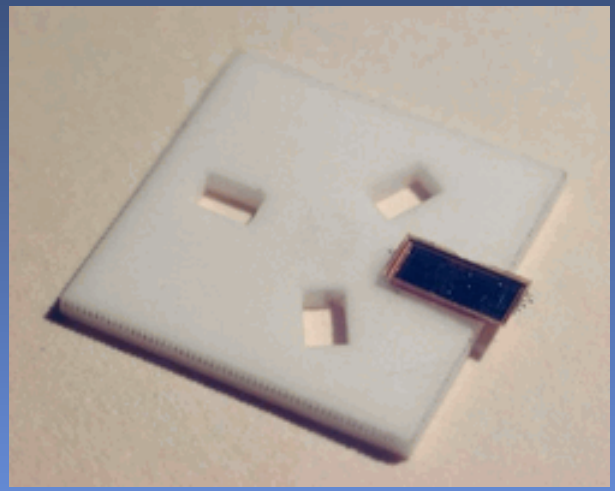
Overview

- MACmode AFM Imaging
- Recognition Force Spectroscopy
- Combining Force Spectroscopy with Imaging
- Simultaneously recorded *T*opography and *Rec*ognition images (*TREC*)
- Summary and Outlook

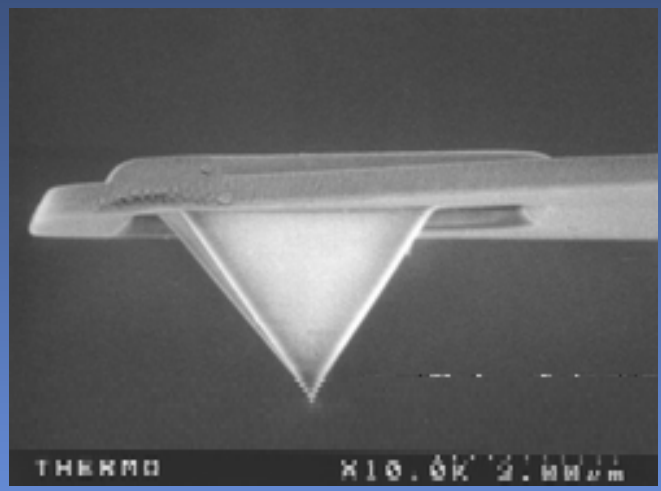
MACmode AFM


x-y-z
piezo
tube
Laserdiode
four-segment photodiode
Cantilever
Sample
Coil
MAC
Interface
Controller
Computer

AFM Tips and Cantilevers



http://www.park.com



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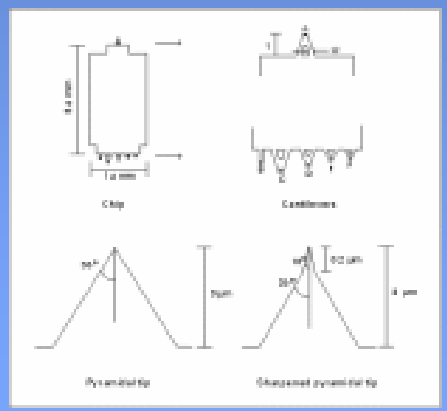
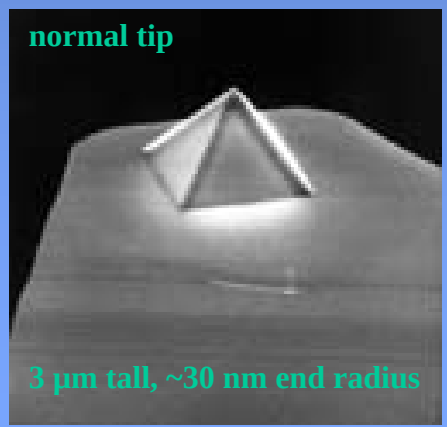
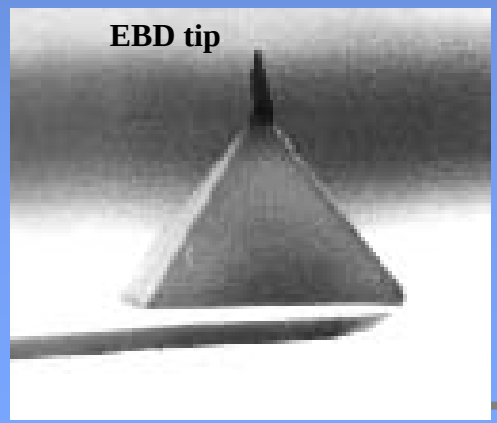


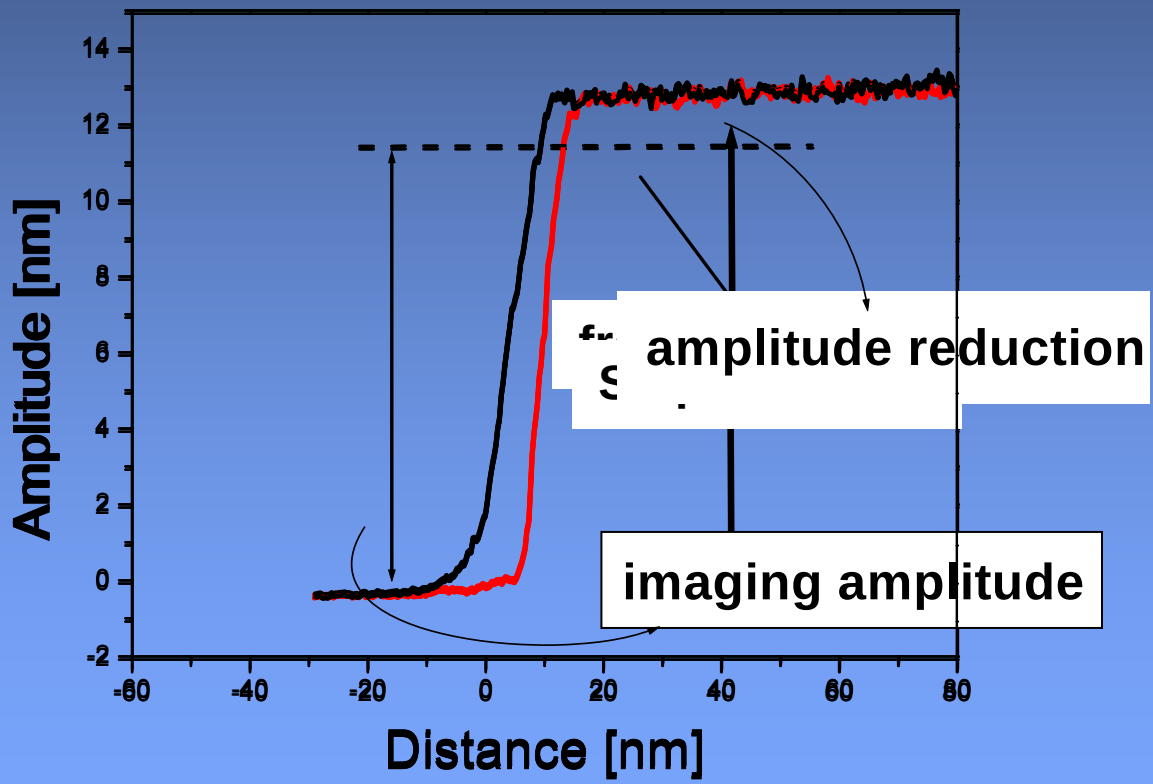




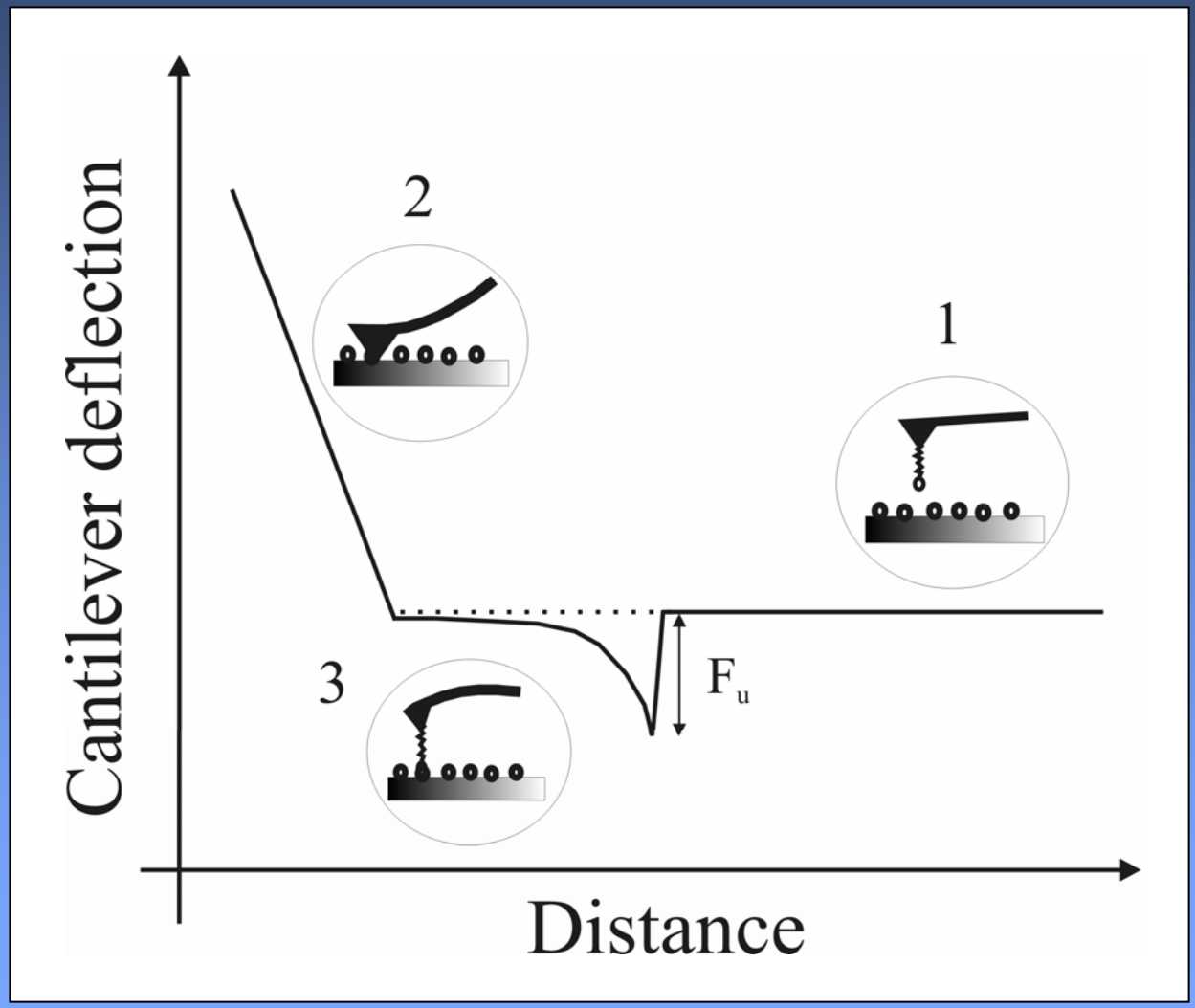
http://stm2.nrl.navy.mil/how-afm/how-afm.html#tips

MACmode Imaging

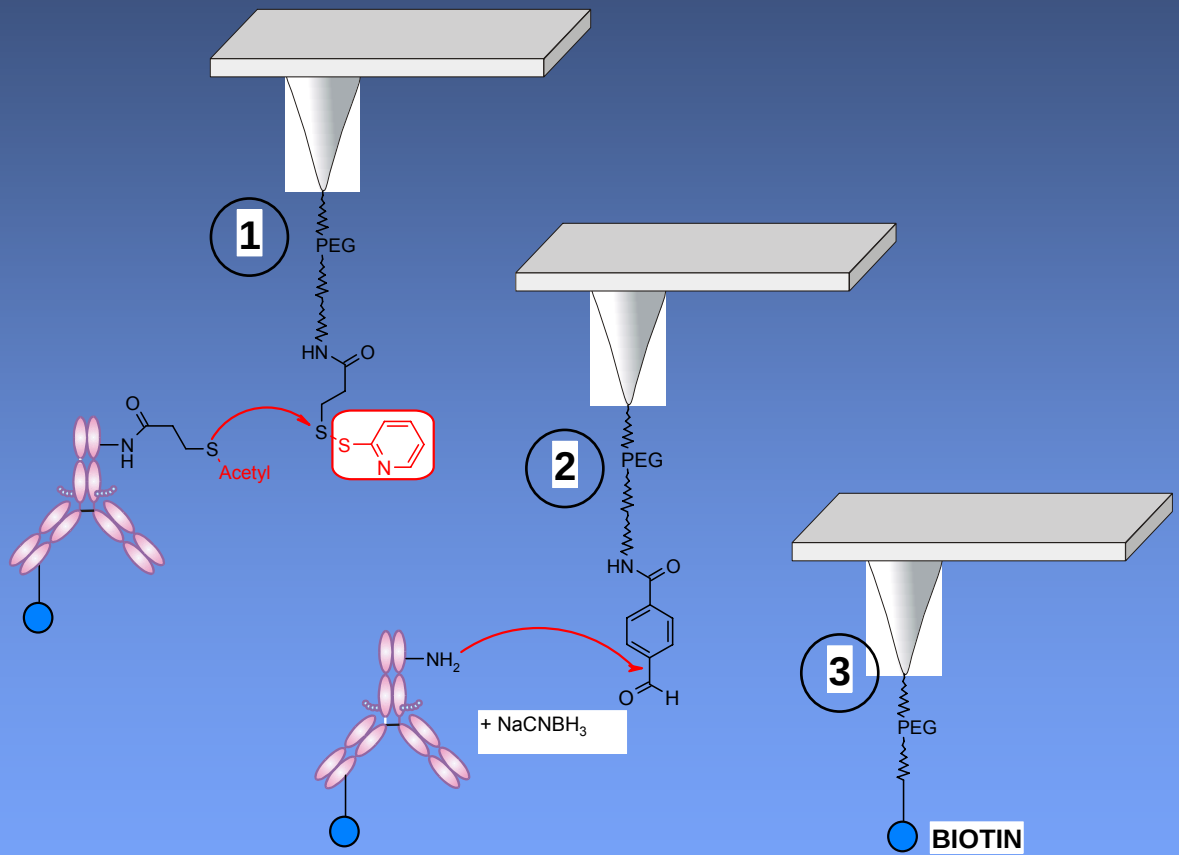
Amplitude-distance cycle using a bare AFM-tip



Force Detection



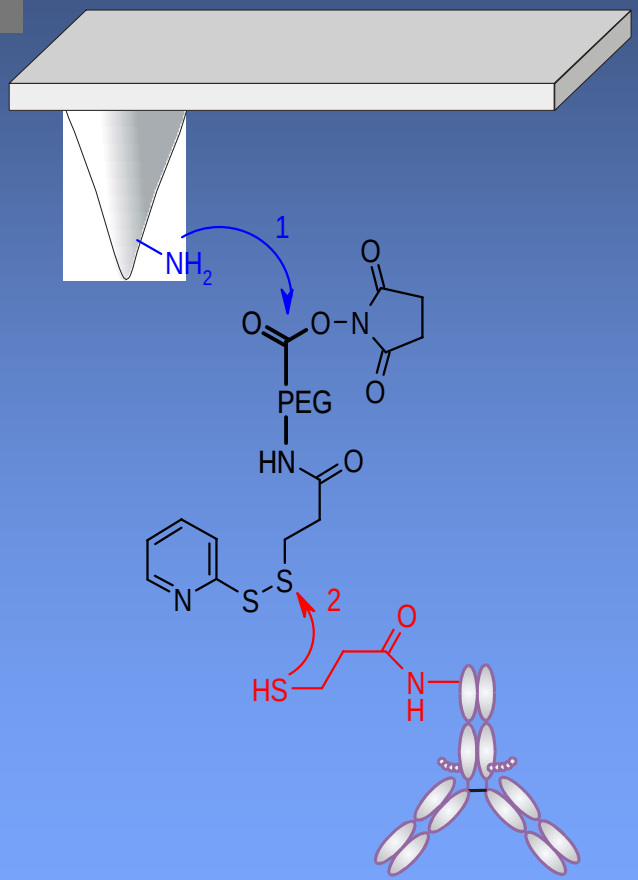
Tip Chemistry (via flexible linker)



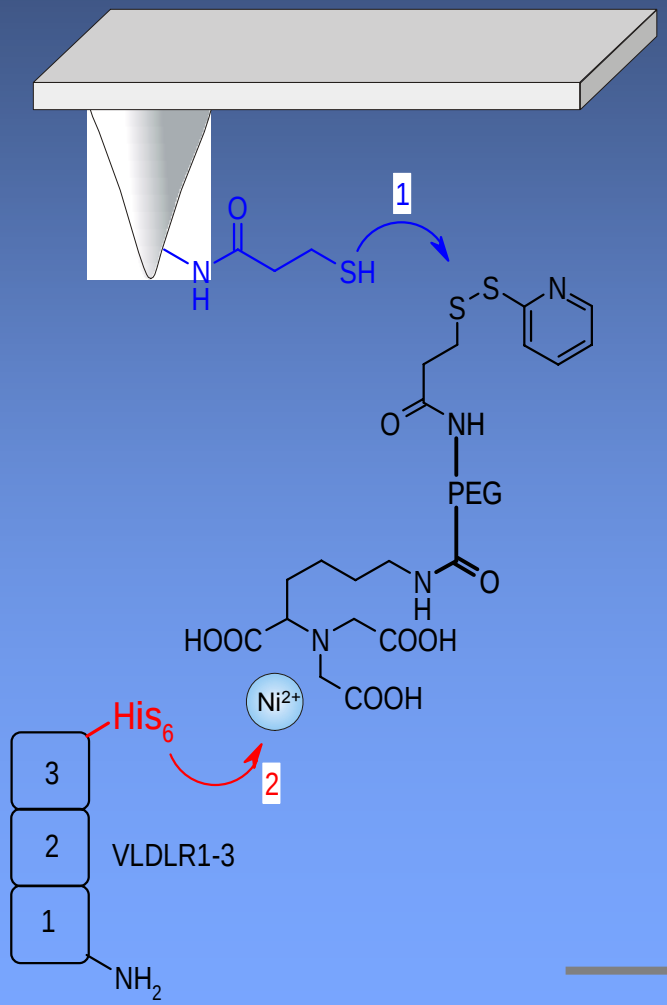
Riener et al., Anal. Chim. Act. 497 (2003) 1367

SH- or His6-coupling

I. tip-PEG-PDP + HS-biomolecule



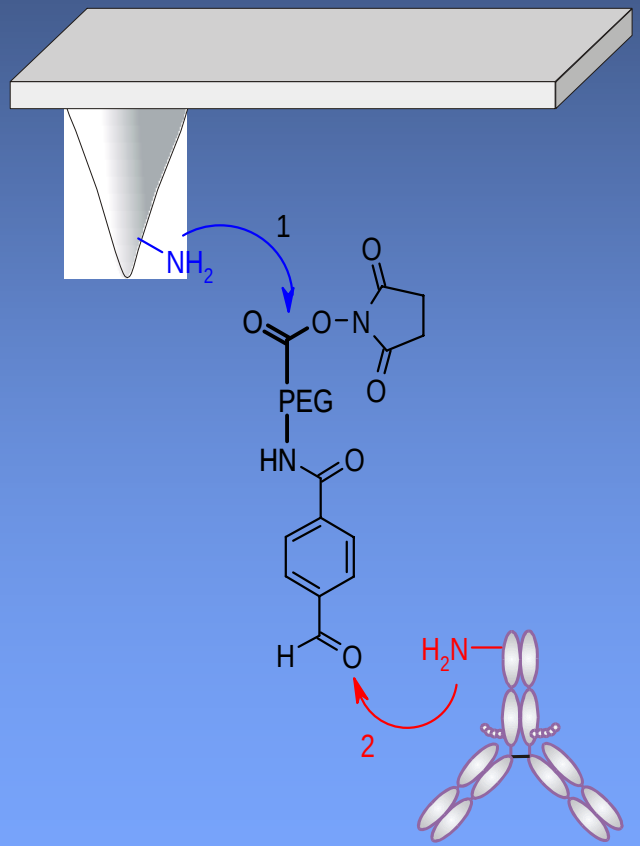
II. tip-SH + PDP-PEG-NTA + His_6-protein



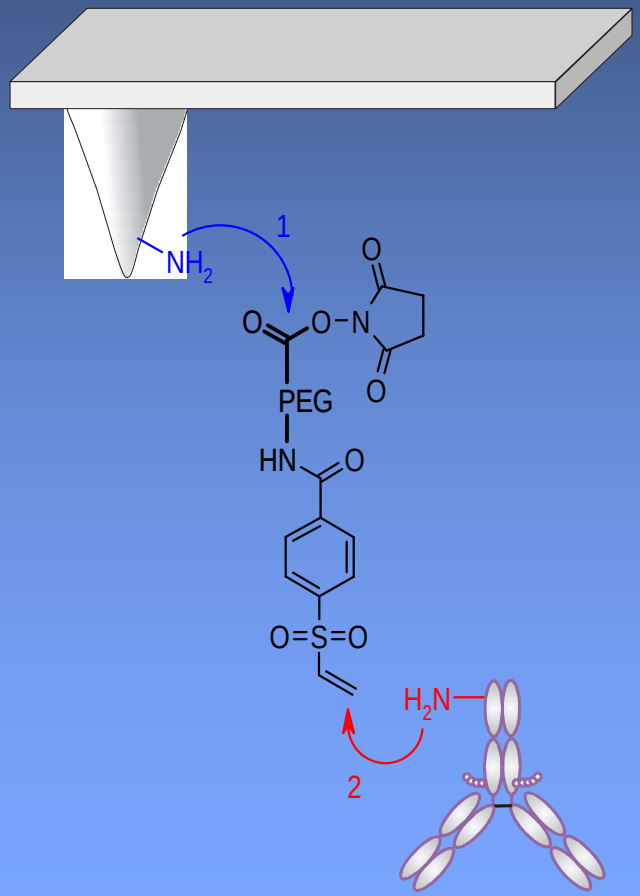
Riener et al., Anal. Chim. Act. 497 (2003) 1367

Direct NH2-Coupling

III. tip-PEG-aldehyde + NH2-protein

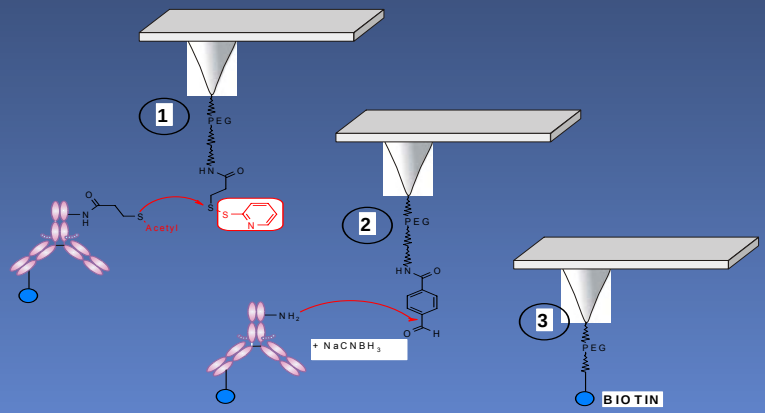


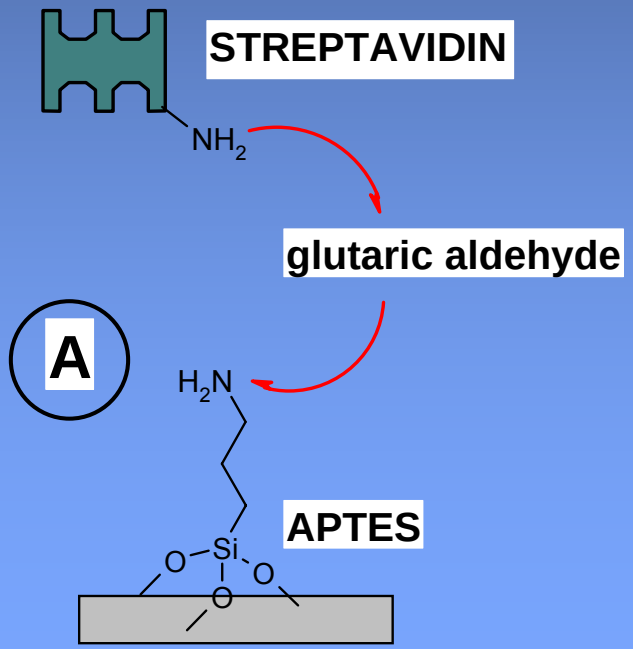
IV. tip-PEG-vinylsulfon + NH_2-protein

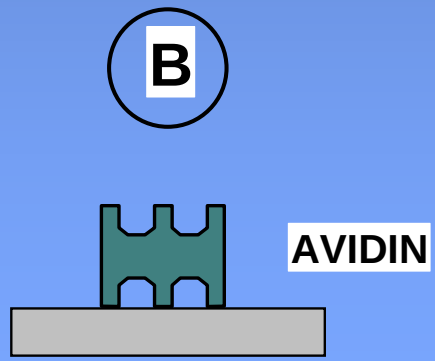


Riener et al., Anal. Chim. Act. 497 (2003) 1367

Surface Chemistry

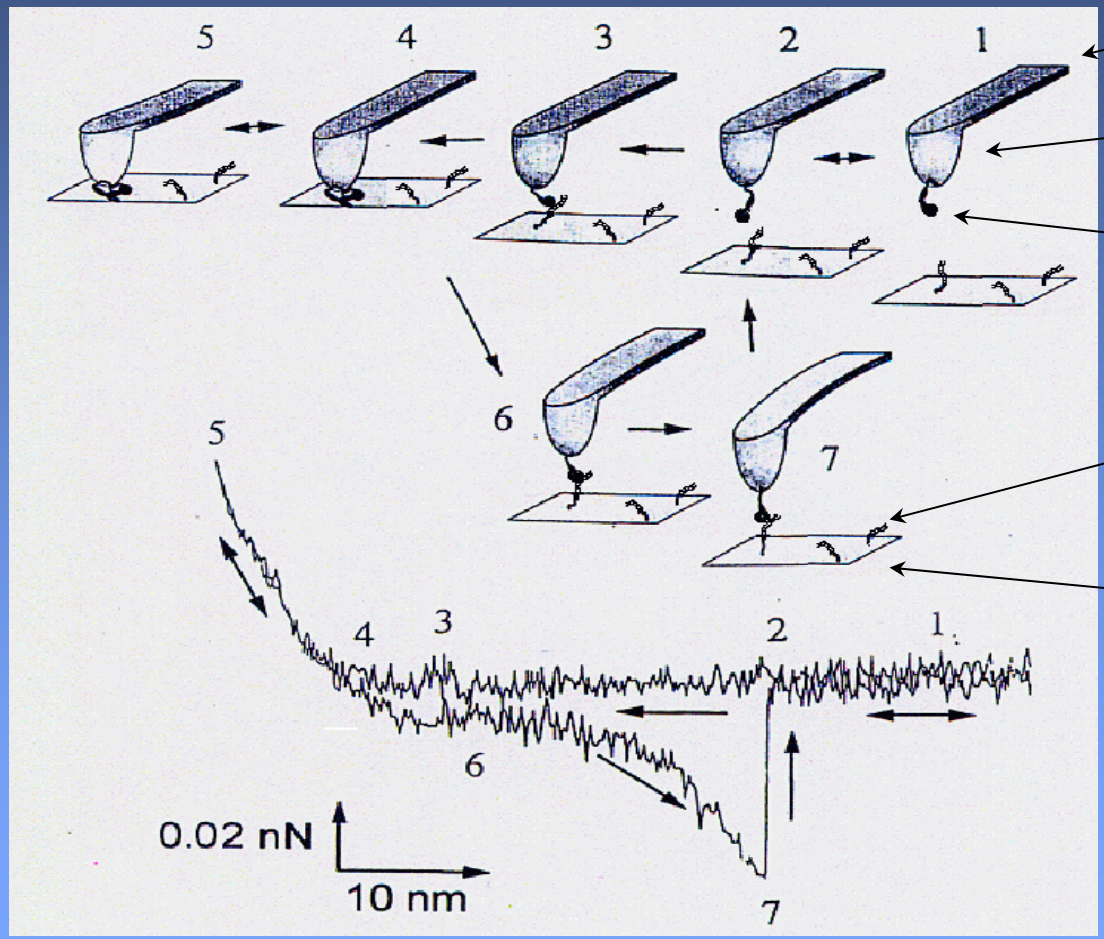






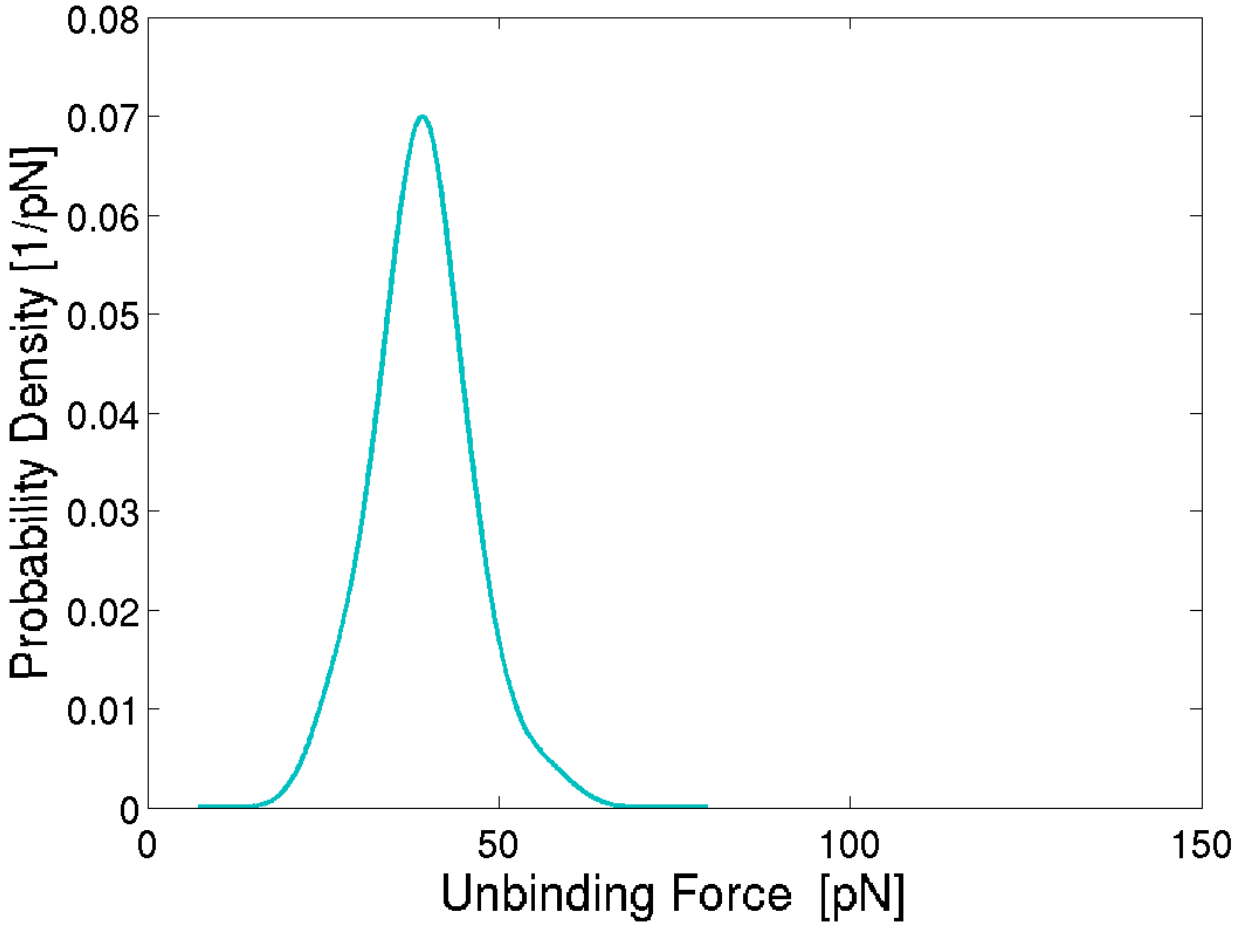
Riener et al., Anal. Chim. Act. 497 (2003) 59

Force-Distance Cycle

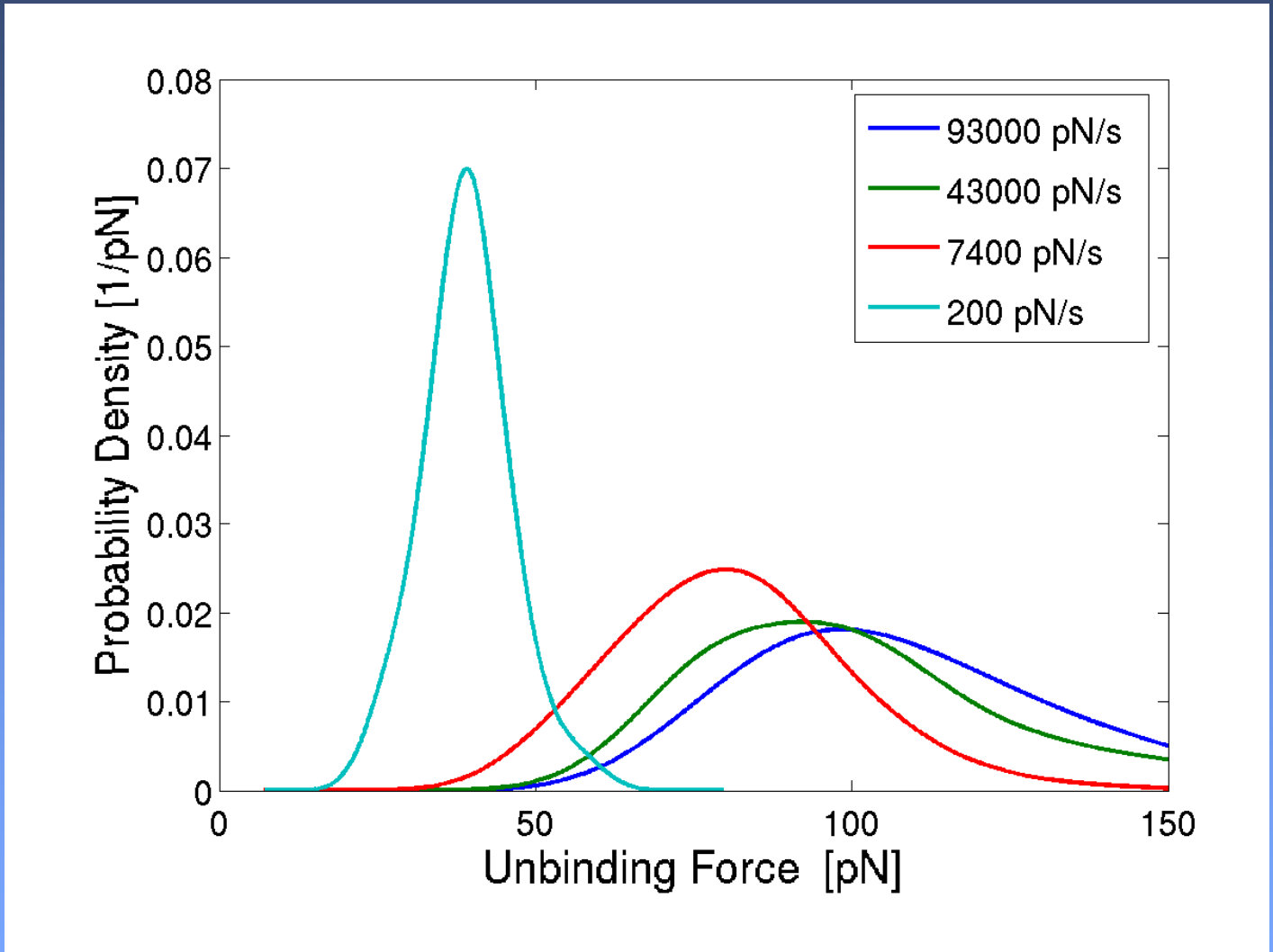


Hinterdorfer et al., PNAS 93 (1996) 3477, Nanobiol. 4 (1998) 177

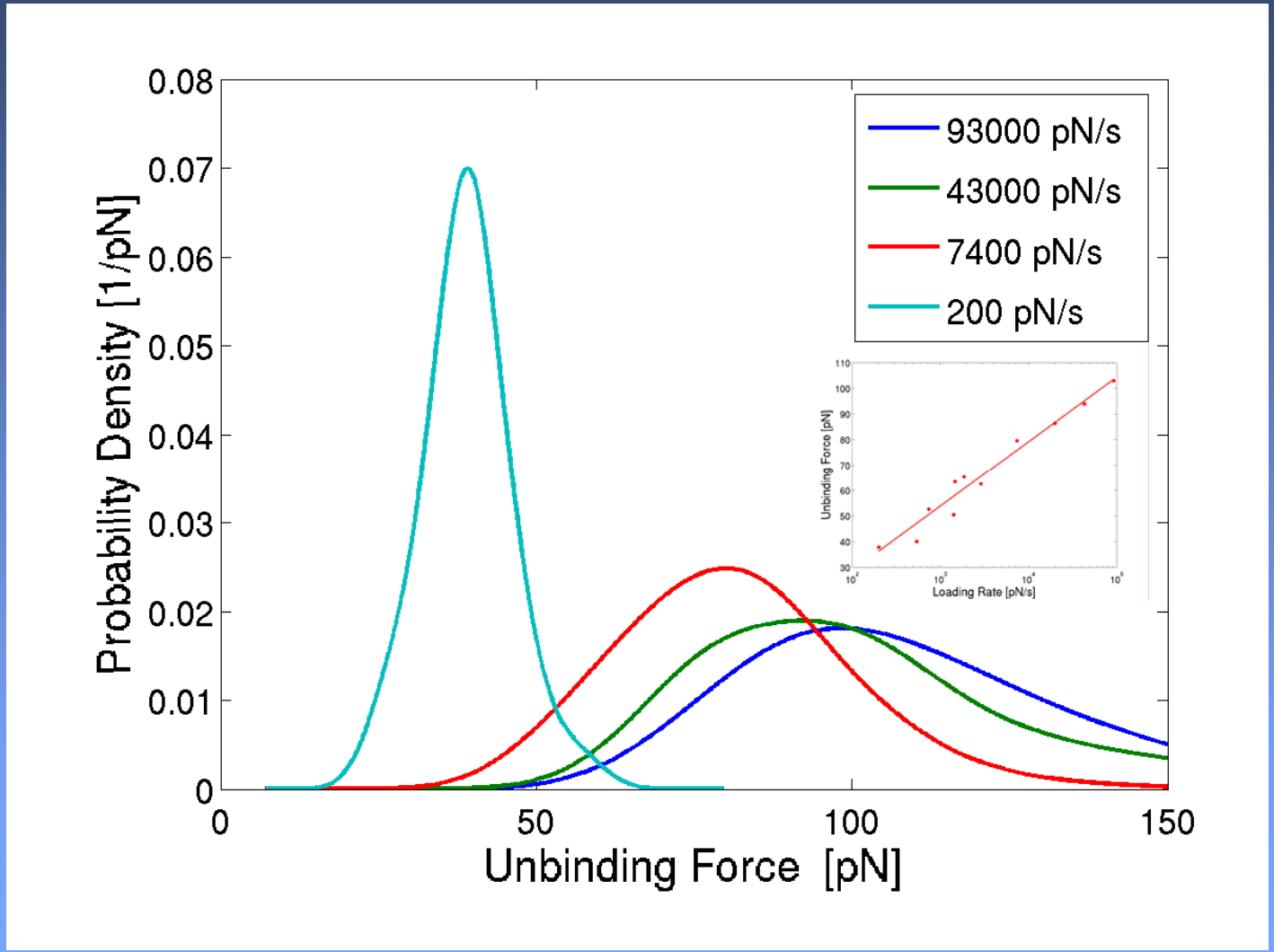
Probability Density



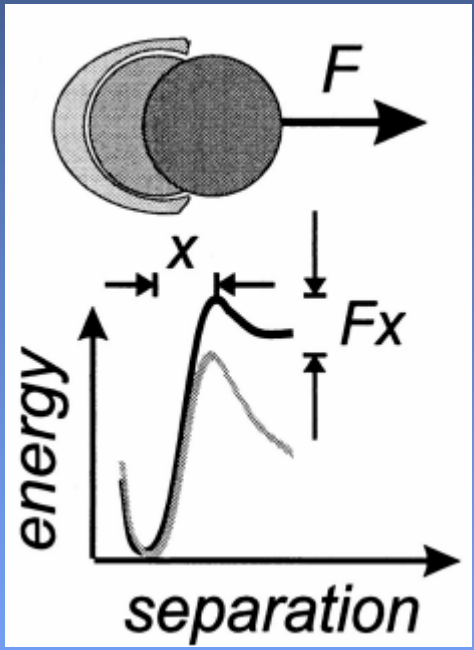
Probability Density



Probability Density



Theory of Force-Spectroscopy



Master Equation

$$dN(t)/dt = -k_d(rt)N(t)$$

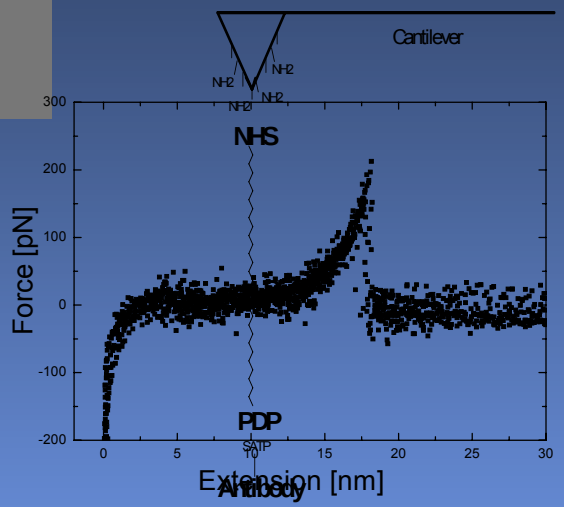
Boltzmann Ansatz

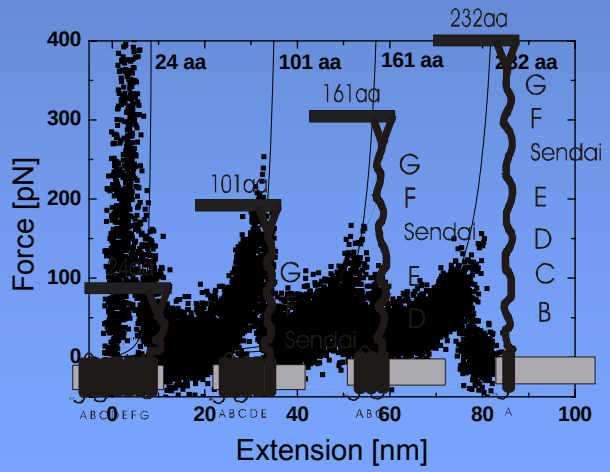
$$k_d(F) = k_d(0)\, e^{Fx/kT}$$

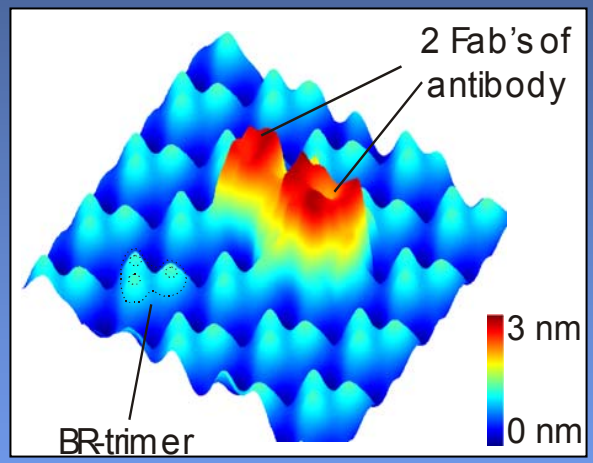
$$F^*(r) = k_BT/x_ß \cdot \ln(r \cdot x_ß / k_{off} \cdot k_BT)$$

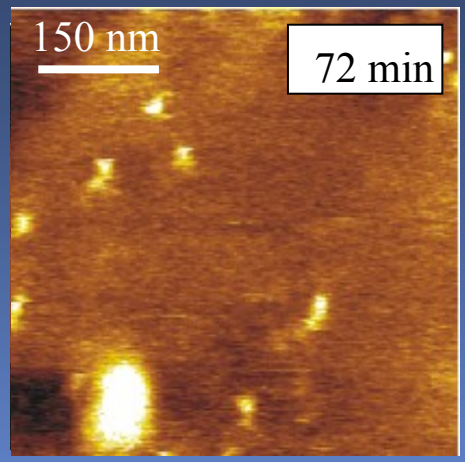
E. Evans, T. Strunz

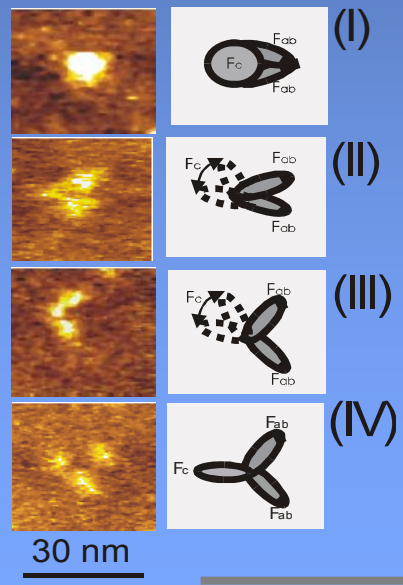
Molecular Complexes & Forces







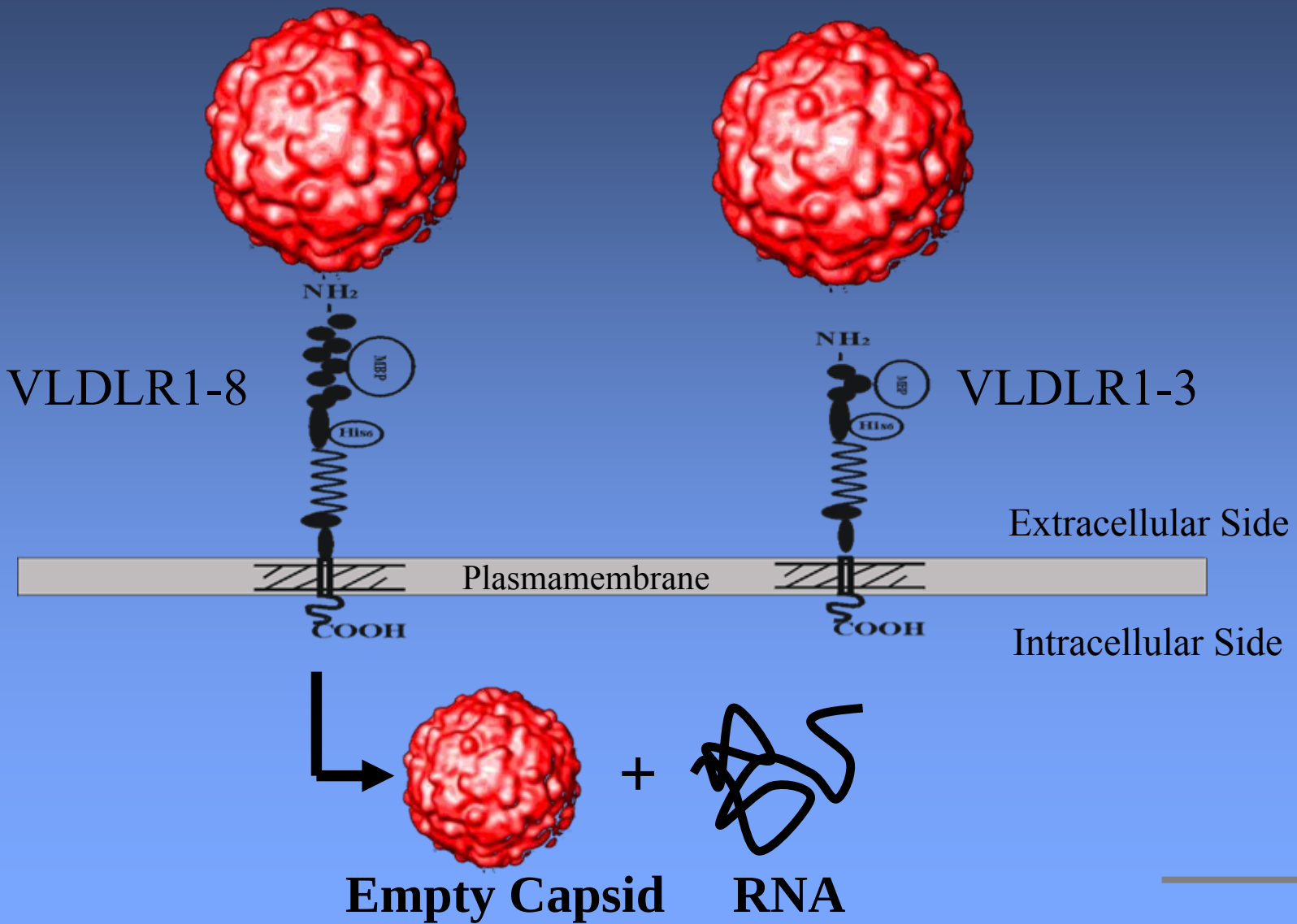




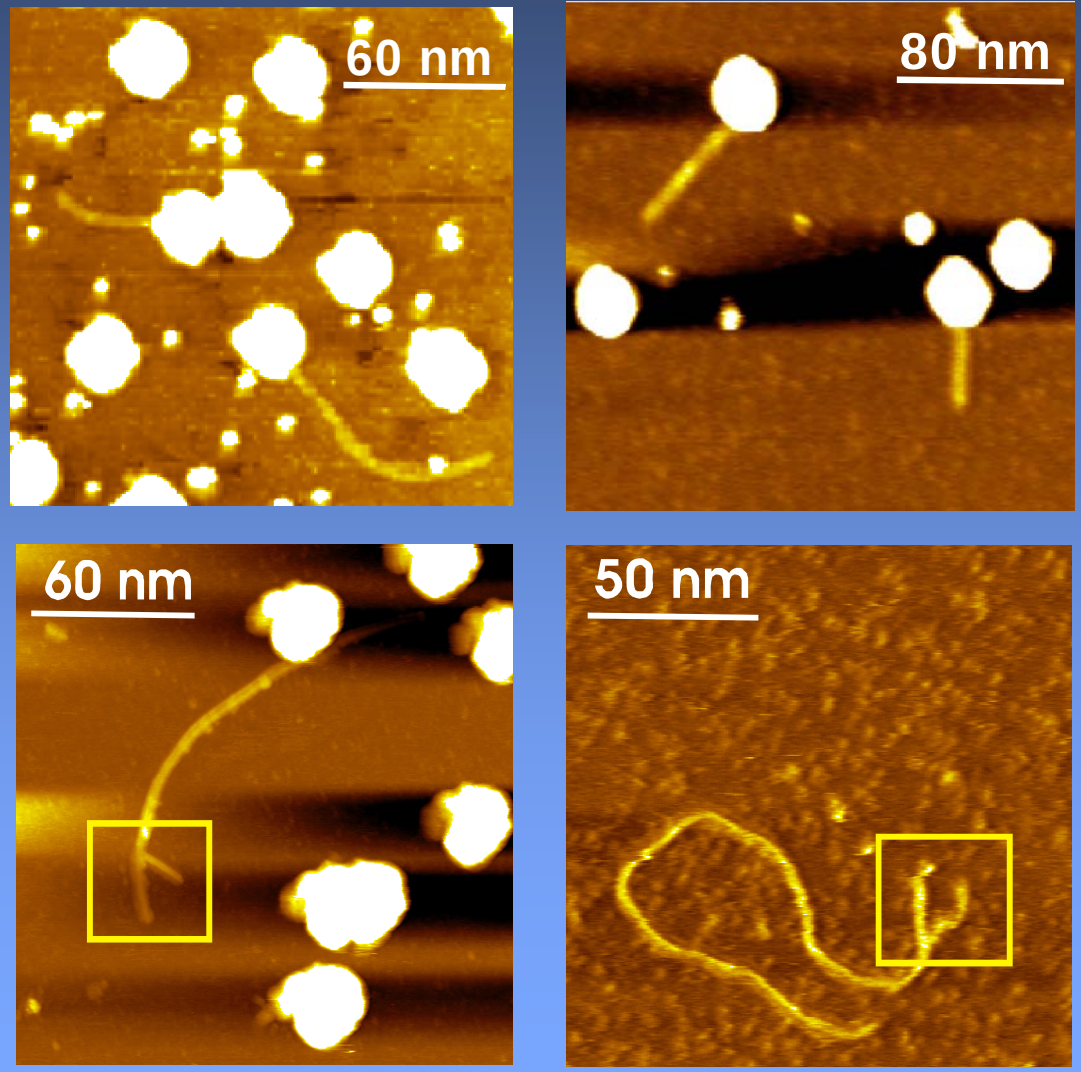
Kienberger et al., J. Mol. Biol. 347 (2005) 597-606

Kienberger et al., EMBO Rep. 5 (2004) 579-583

Human Rhinovirus

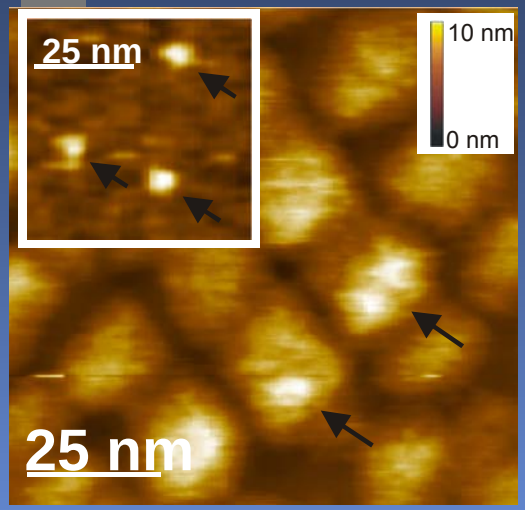


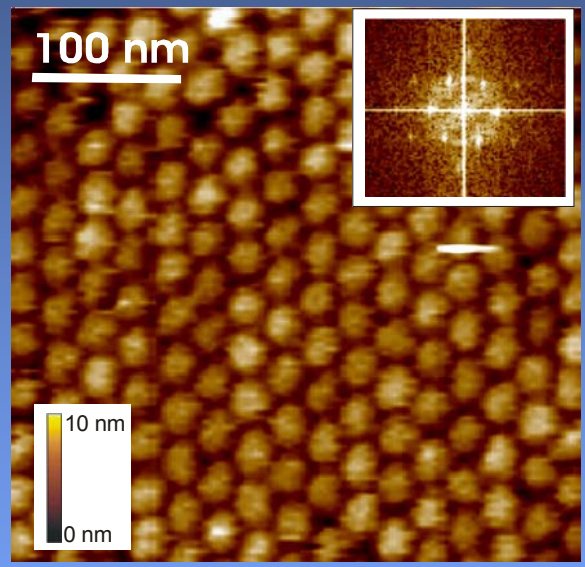
Substructure of RNA

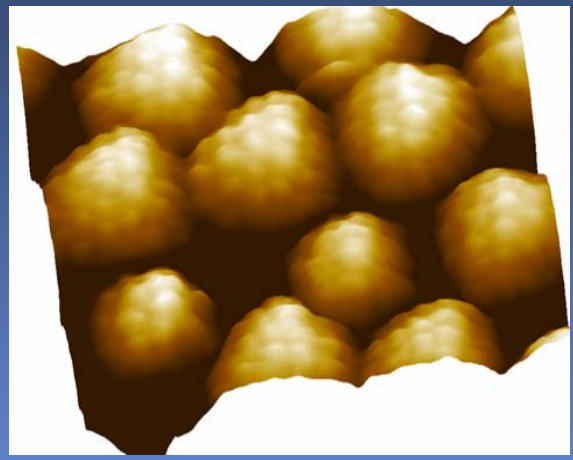


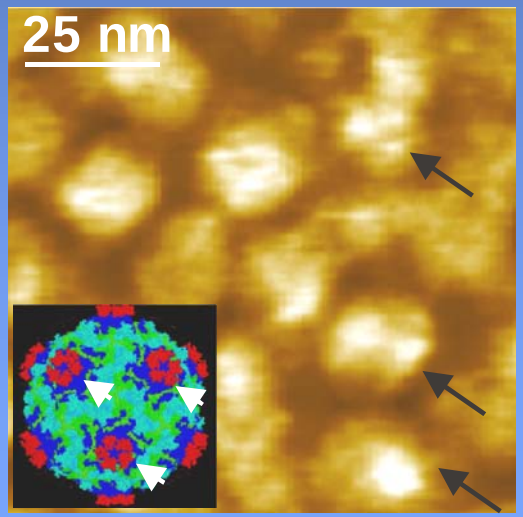
Kienberger et al., J. Virol. 78 (2004) 3203-9

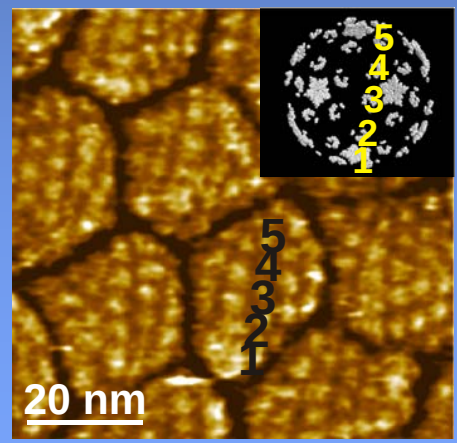
Virus-Receptor Complex







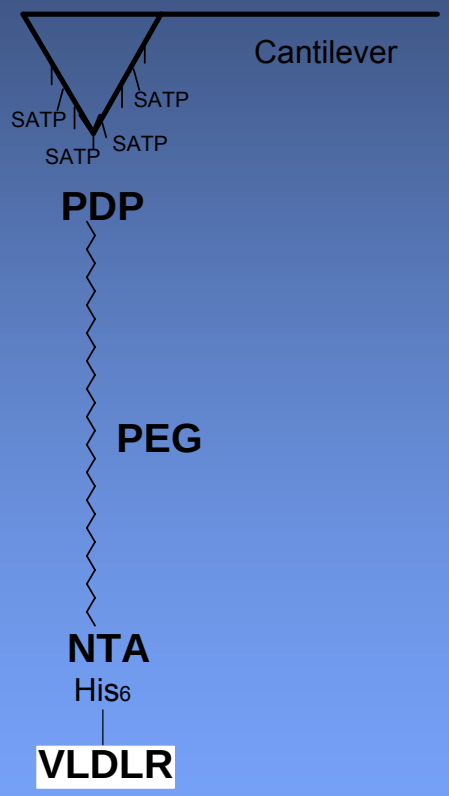


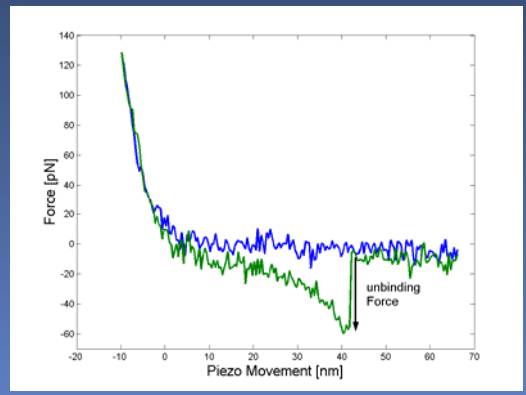


Kienberger et al., Structure, in press

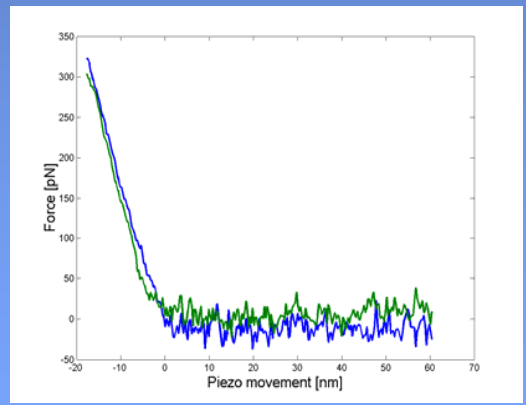
Kienberger et al., J. Virology 78 (2004) 3203-9

Virus-Receptor Interaction



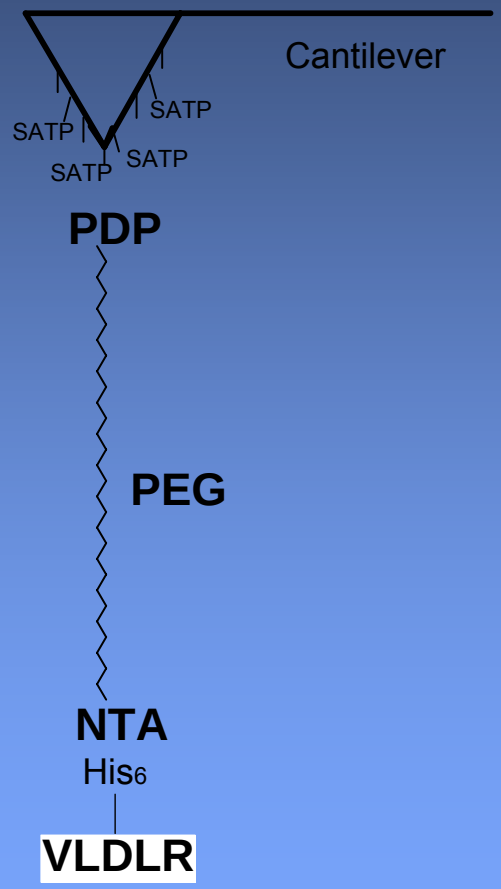


+ Ca^{2+} ↑ ↓ - Ca^{2+}



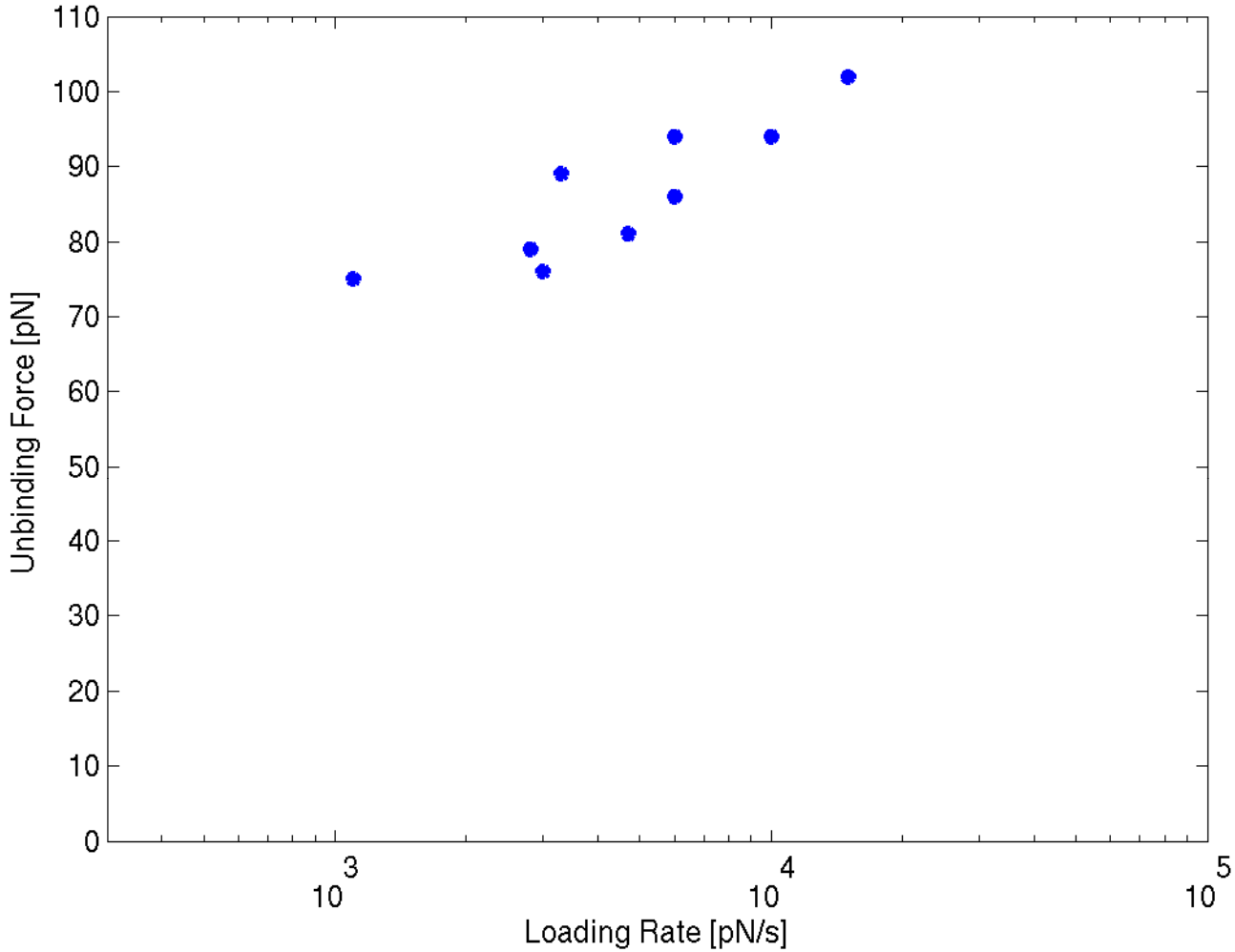
Rankl et al., manuscript in preparation

Receptor Constructs against HRV2



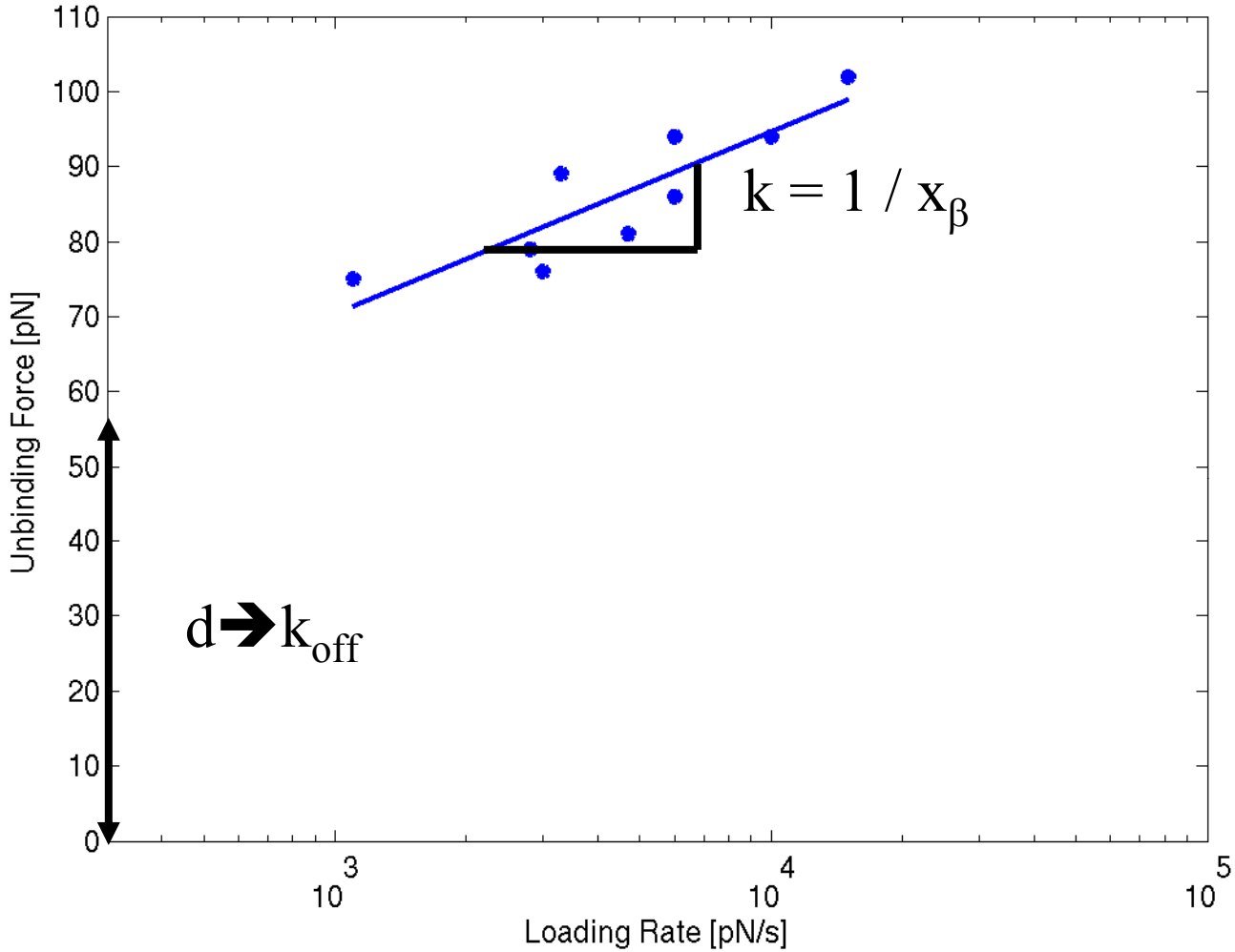
Native	V1-8	V1-3	V333	V33
1	MBP	MBP	MBP	MBP
2	1	1	3	3
3	2	2	3	3
4	3	3	3	His_6
5	4	His_6	His_6	
6	5			
7	6			
8	7			
EGF	8			
Sugar	His_6			
TM				
CP				

Rankl et al., manuscript in preparation

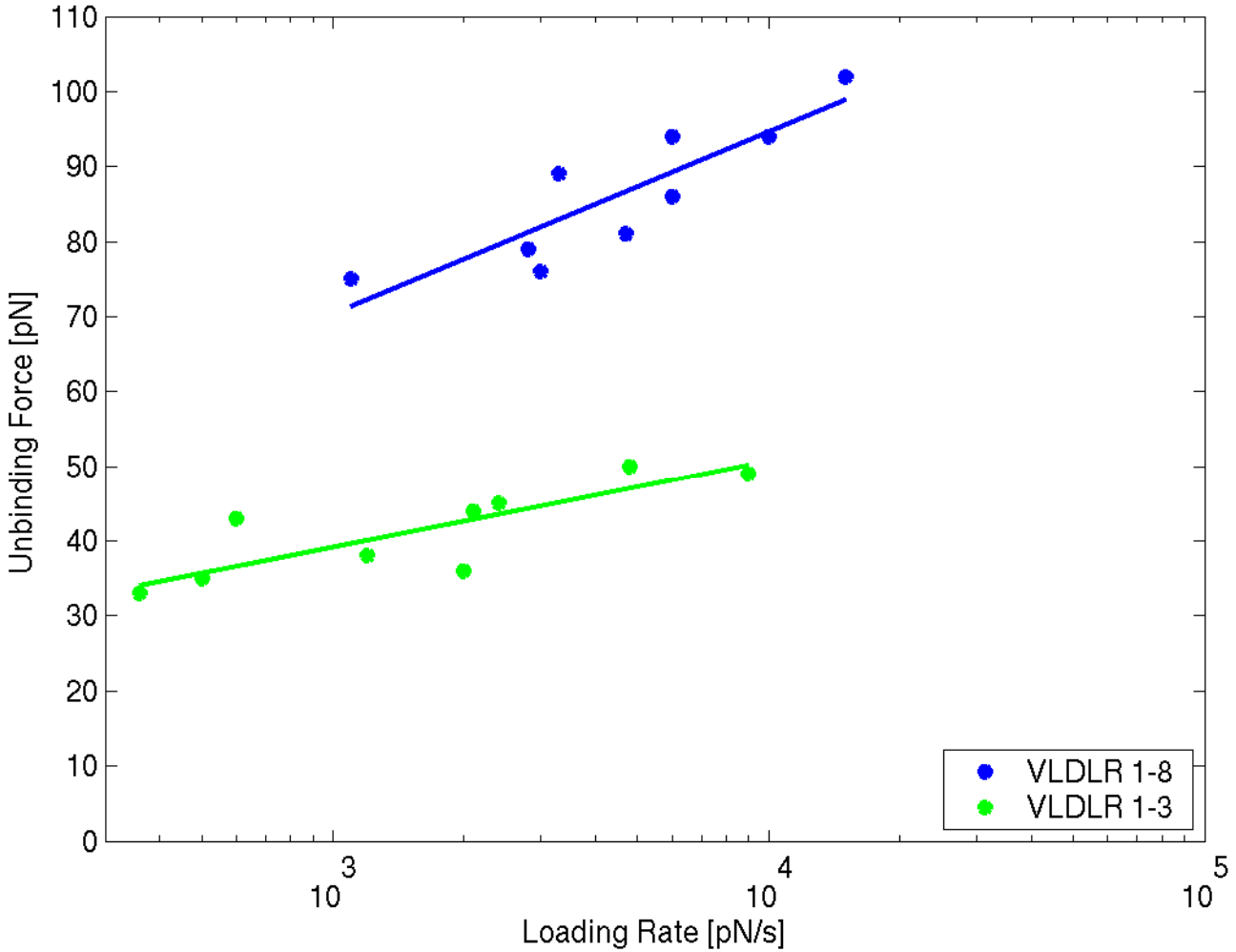


fit linear function f=k*ln r + d

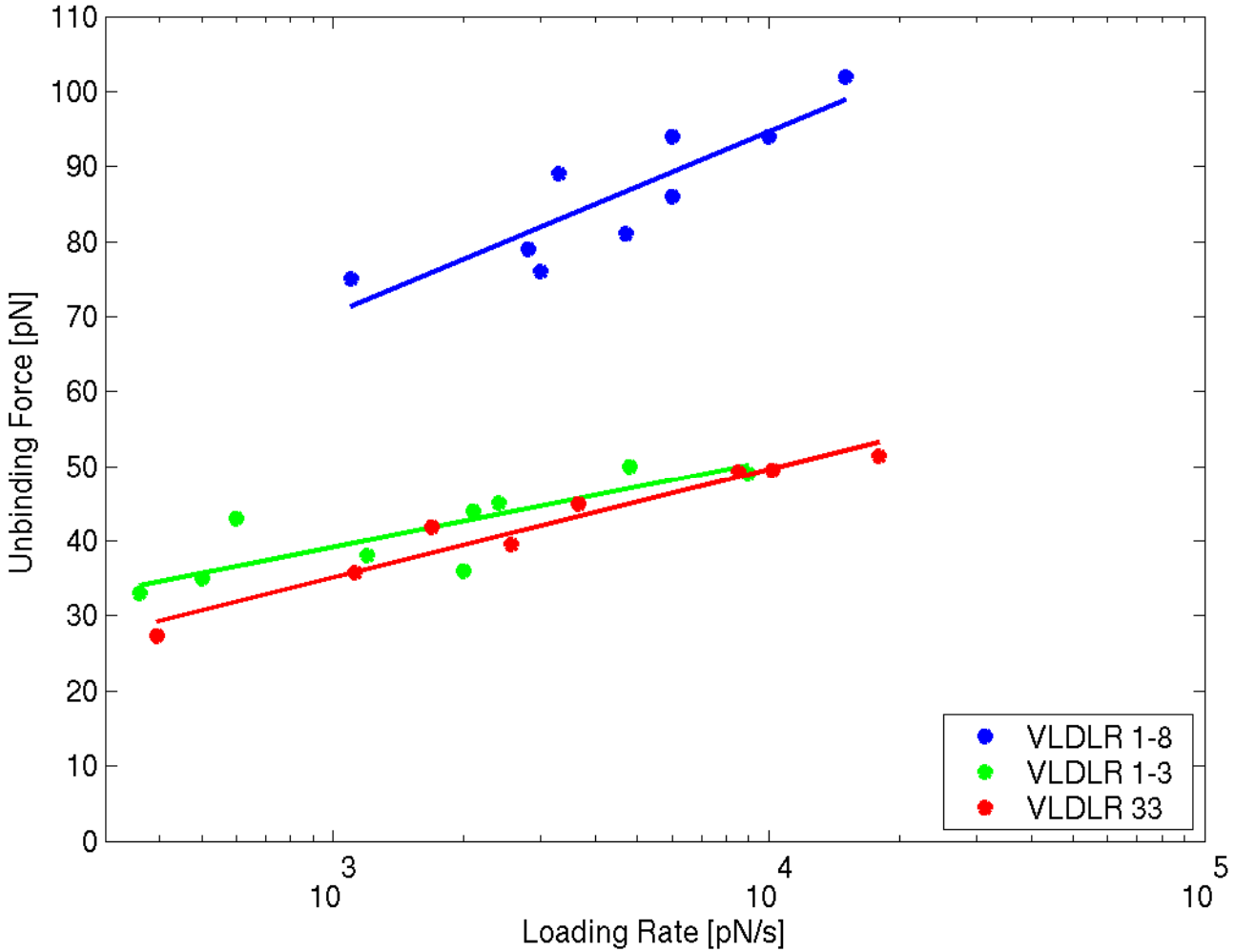
Rankl et al., manuscript in preparation



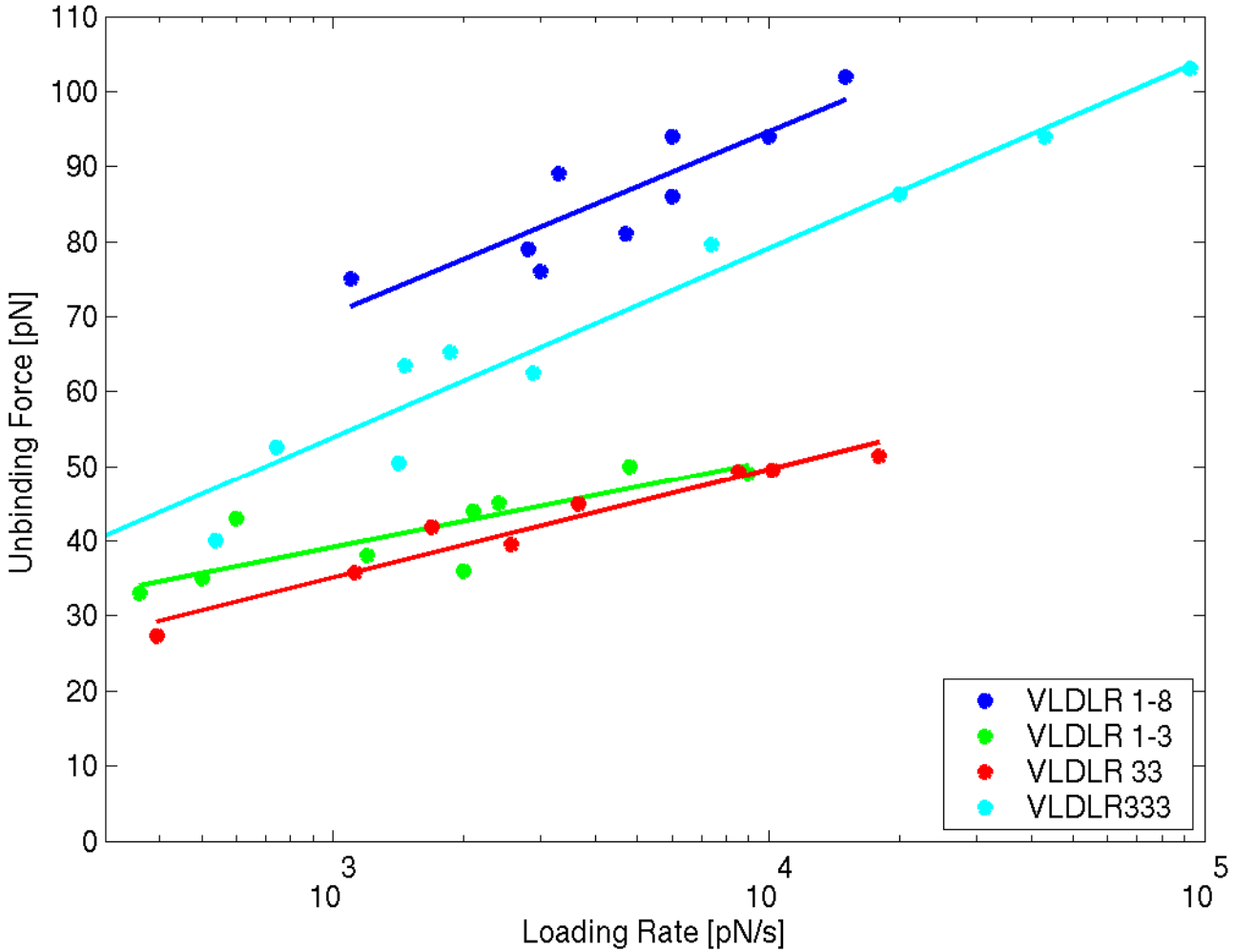
Rankl et al., manuscript in preparation



Rankl et al., manuscript in preparation



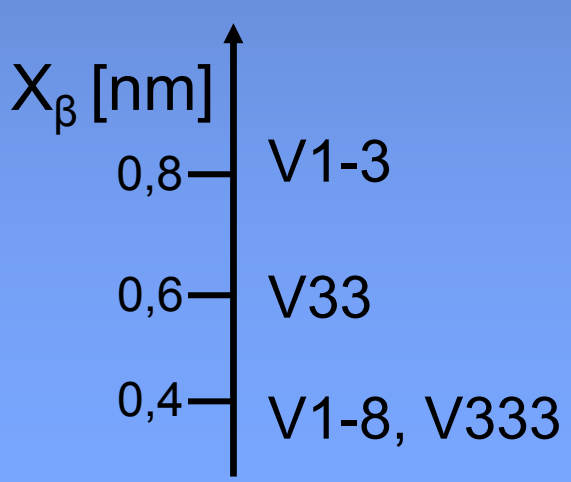
Rankl et al., manuscript in preparation

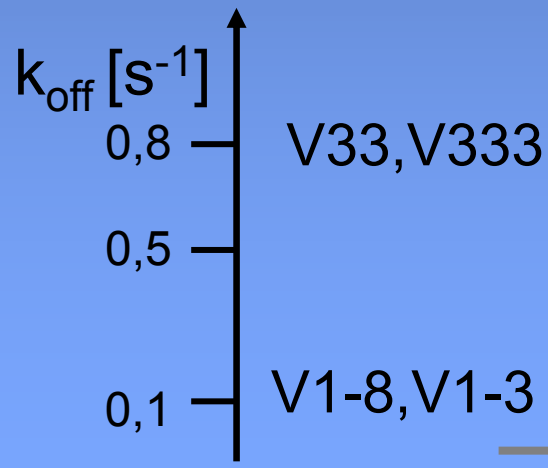


Rankl et al., manuscript in preparation

Results: VLDLR-HRV2 Binding

construct	x_β [nm]	k_{off} [s^{-1}]
V1-8	0,41± 0,18	0,088± 0.067
V1-3	0,79± 0,24	0,11± 0.083
V333	0,37± 0.037	0,75± 0,58
V33	0,62± 0,12	0,83± 0,76

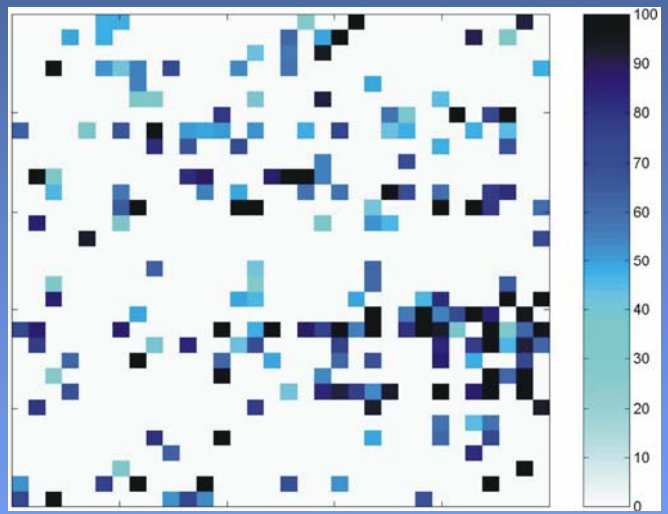




Rankl et al., manuscript in preparation

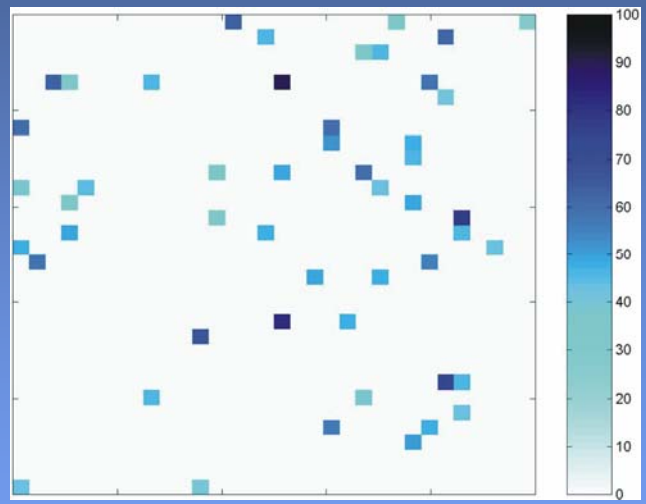
Lateral Force Mapping

Recognition Map



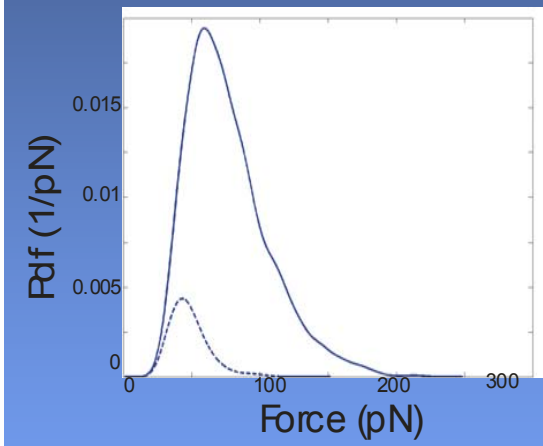
50 nm

Block



50 nm

Binding Probability

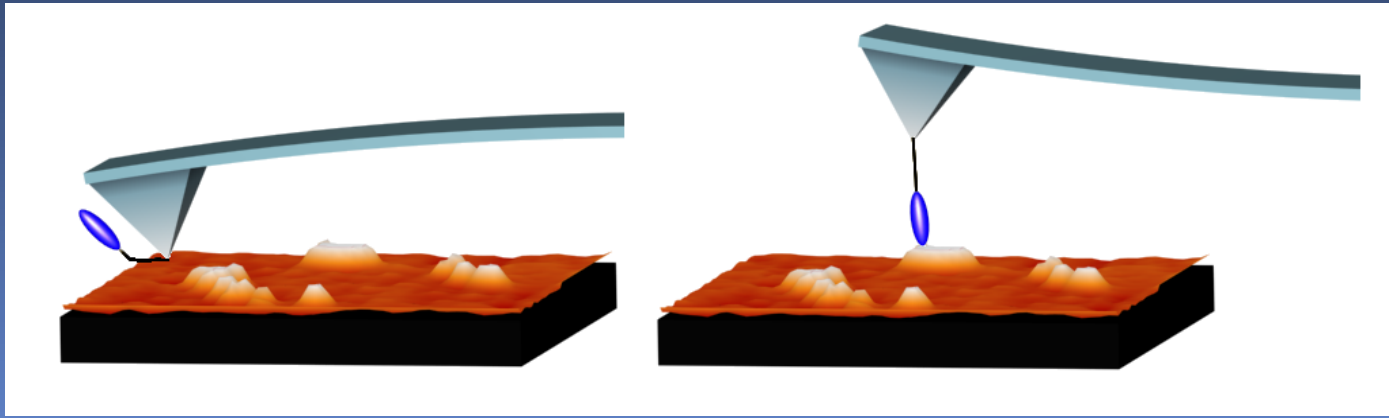


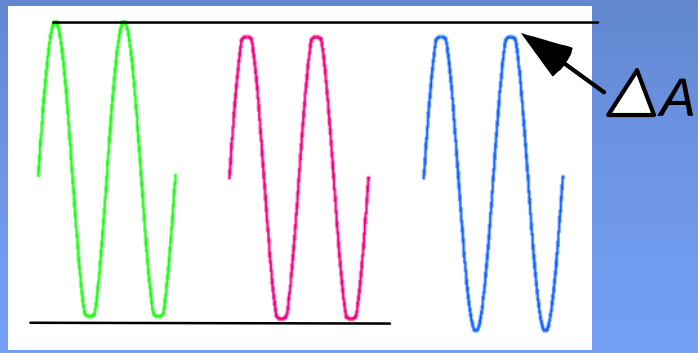
64 X 64 Pixels

T_{Exp} = 14 min

Stroh et al., Biophys. J. 87 (2004) 1981

Principles of TREC





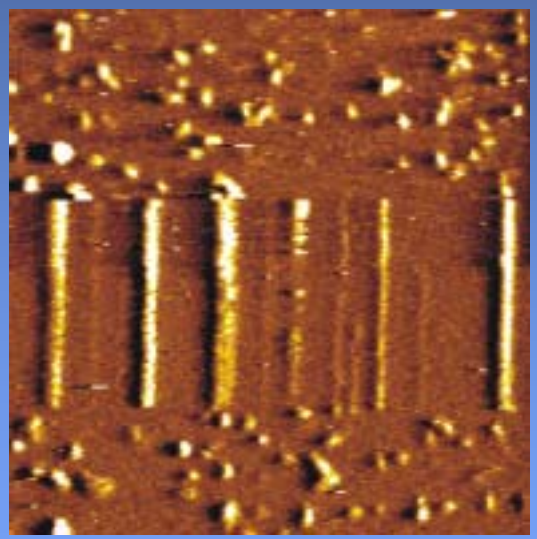
Recognition:
Amplitude Reduction on Top

Topography:
Amplitude Reduction on Bottom

MacMode Force Traces

Envelope of cantilever oscillation

Single molecules on mica

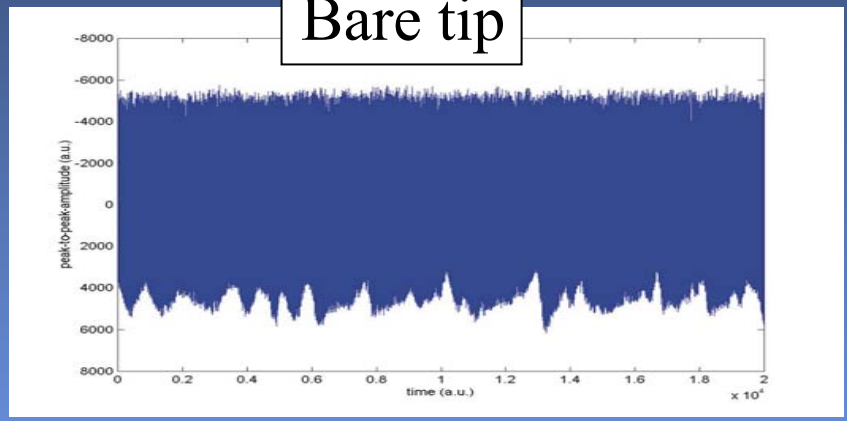


Slow scan axis disabled

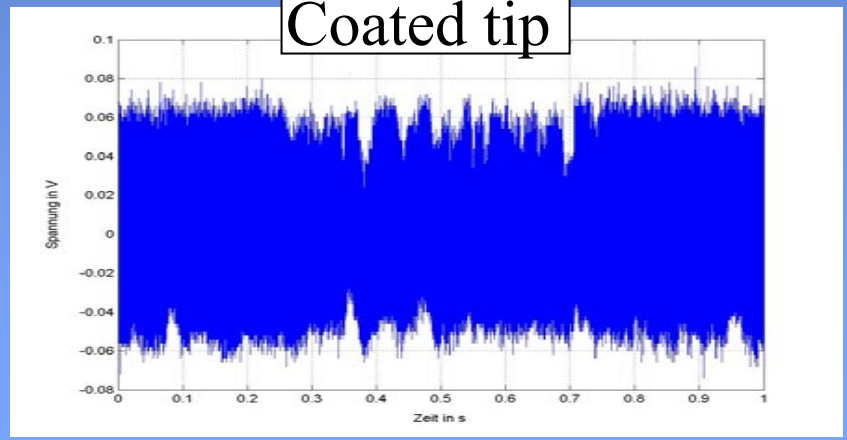
Slow scan axis enabled

150 nm

Bare tip

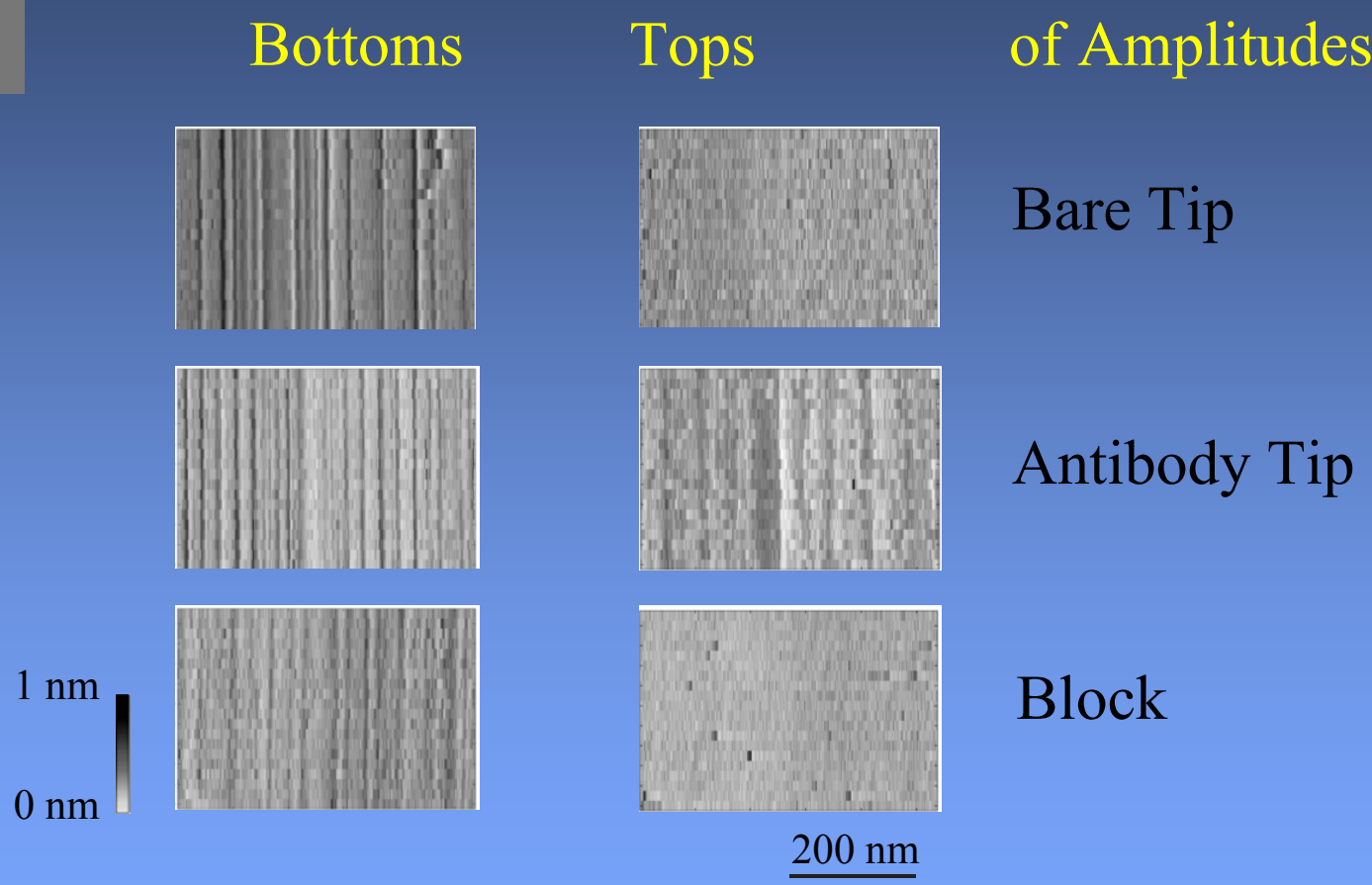


Coated tip



Stroh et al., Biophys. J. 87 (2004) 1981

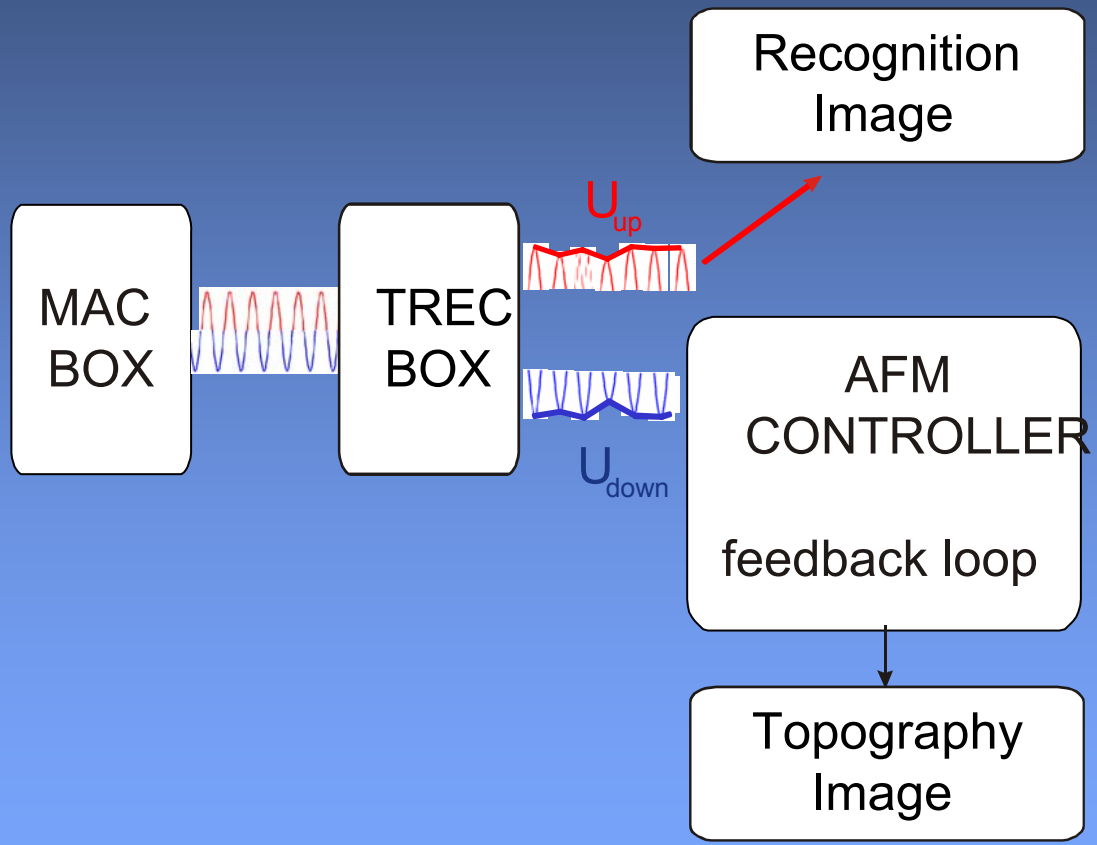
Repeated Linear Scans



Stroh et al., Biophys. J. 87 (2004) 1981

TREC Scheme

TREC = Simultaneous **T**opography and **REC**ognition Imaging

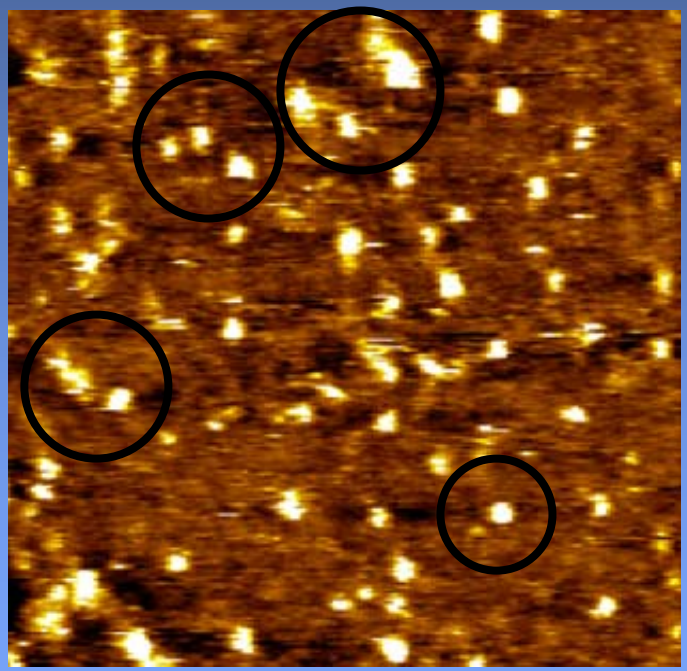


Stroh et al., Biophys. J. 87 (2004) 1981

Topography & Recognition

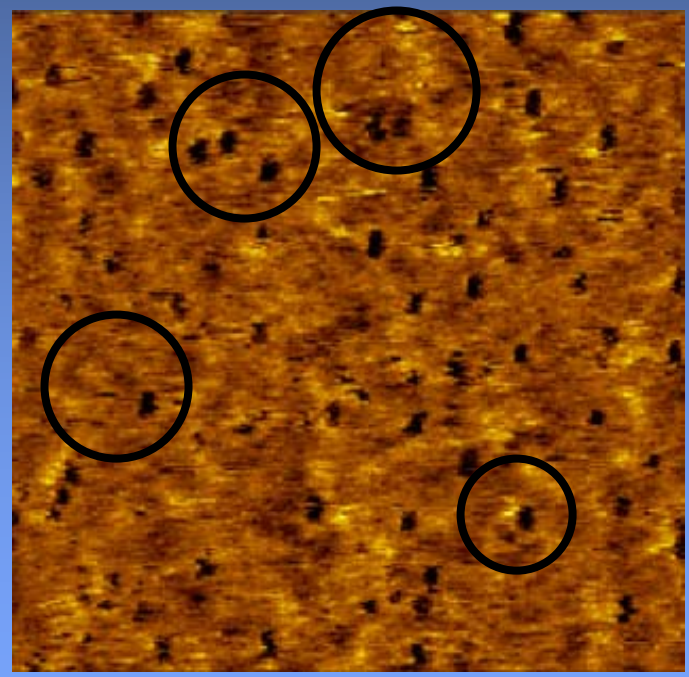
512 x 512 Pixels T_{Exp} = 8 min

Topography image of avidin adsorbed on mica



150 nm

Simultaneously acquired recognition image

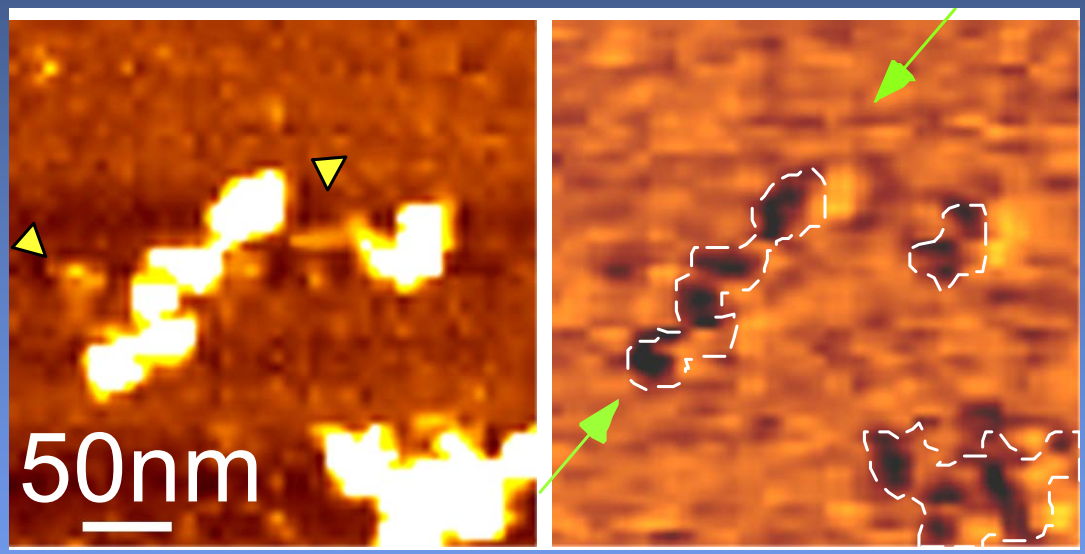


150 nm

Ebner et al., ChemPhysChem, 6 (2005), 897

Application to Chromatin

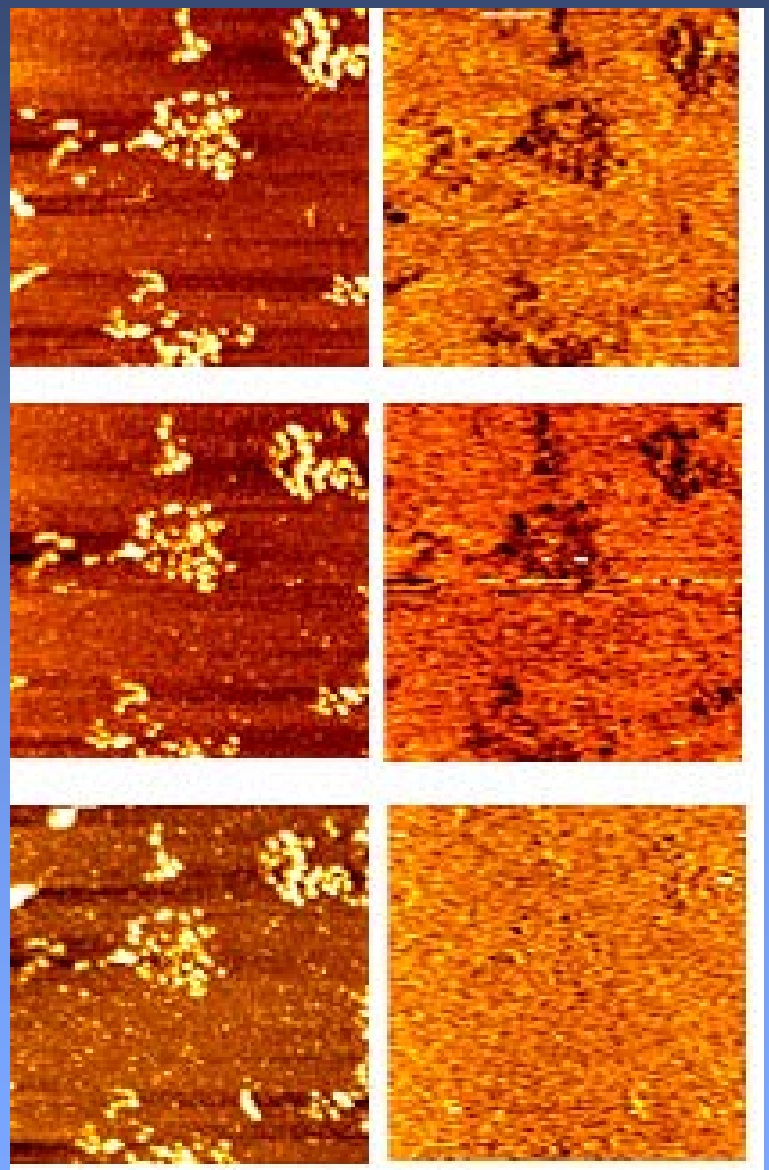
Topography **Recognition**



mmtv chromatin/anti-histone H3 on tip

Stroh, Wang, et al., PNAS 101 (2004) 12503

Recognition is specific



Anti Histone H3 on non-acetylated MMTV

+30μg/ml BSA

- No blocking

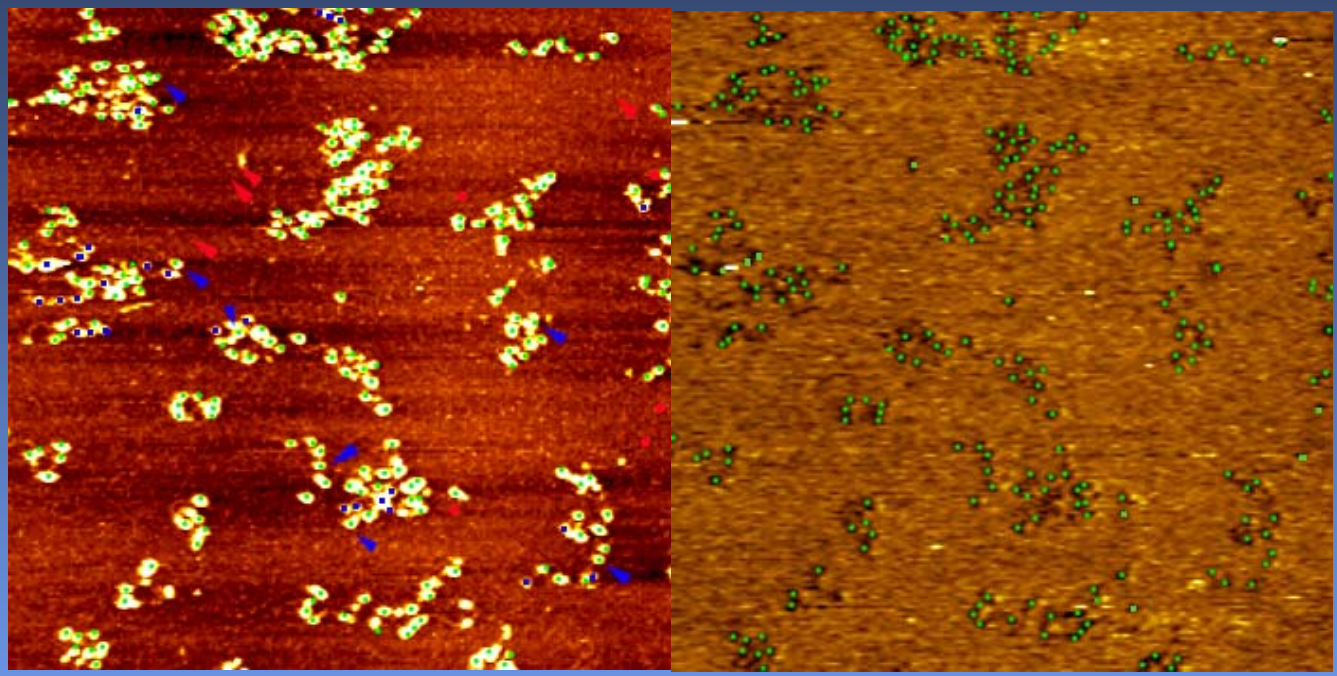
+ 50μg/ml

ARTKQTARKSTGGKAPRKQLC

(aa 1-20 of H3)

Stroh, Wang, et al., PNAS 101 (2004) 12503

Accuracy and Repeatability



Green = 'hit'

Blue = 'miss'

Red = 'false hit'

Arrows = change on rescan

<u>first scan</u>
96±1% (false1.1%±0.1%)
<u>second scan</u>

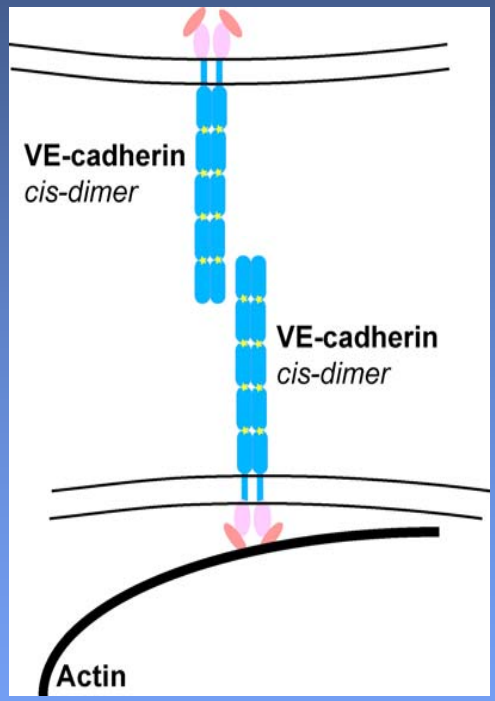
92±2%, (false 2.8%±0.5)

Stroh, Wang, et al., PNAS 101 (2004) 12503

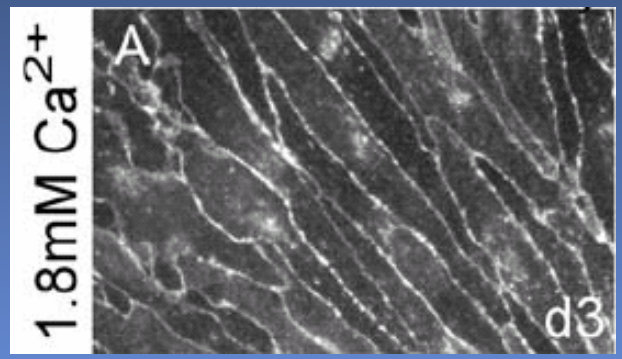
Distribution of trans-interacting VE-cadherins on MyEnd surface

Microvascular endothelial cell line from mouse myocardium (MyEnd)
Vascular endothelial cadherin (calcium-dependent adherent protein) (VE-cadherin)

Selective adhesion between cells



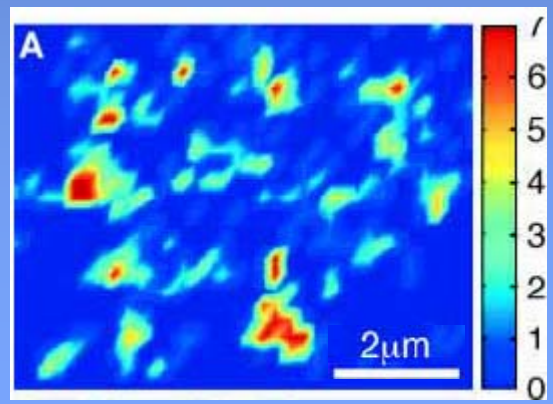
Immunofluorescence labeling of VE-cadherin



(Baumgartner W et al., Histochem Cell Biol, 2004)

Single molecule fluorescence imaging

$$\frac{5\times10^{3}}{\mu m^{2}}$$ *cis* dimers



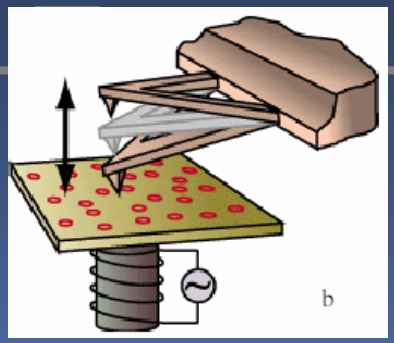
(Baumgartner W et al, J Cell Sci, 2003)

- Lateral resolution is not better than 200 nm

- No information about topography

AFM Simultaneous Topography and RECognition (TREC) TECHNIQUE

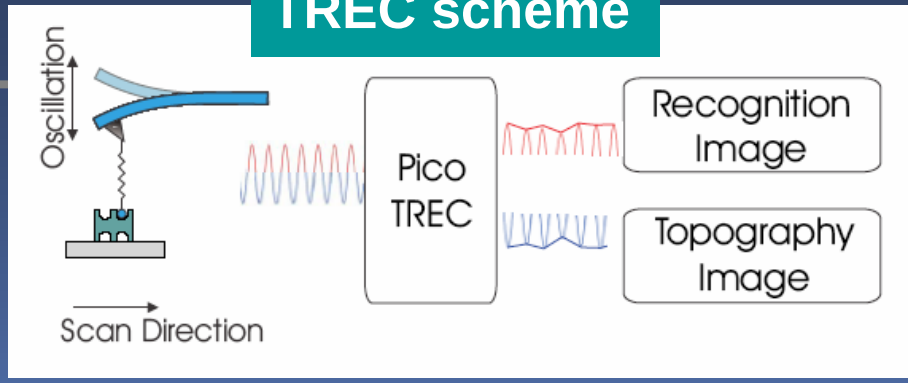
MAC Mode



- Good for "soft" samples (proteins, cells, etc.)
- Gentle imaging technique
- Physiological environment

→ **Requirement for TREC**

TREC scheme

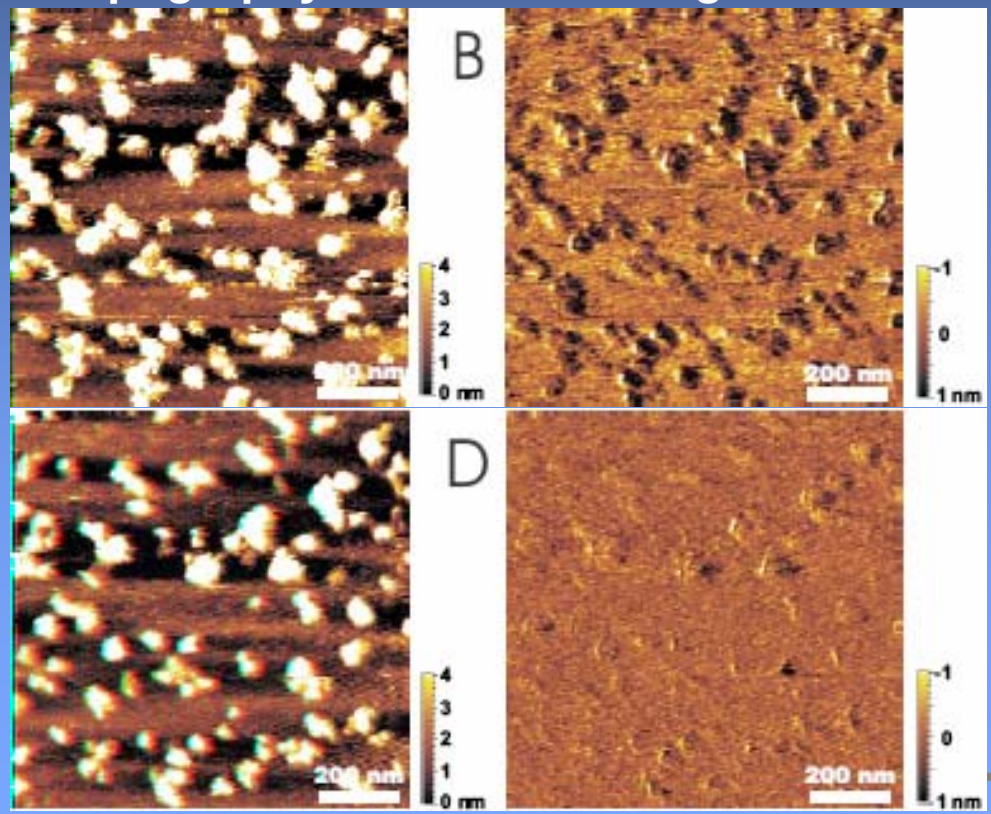


model system: avidin-biotin

AFM tip:
biotin via PEG linker
mica surface:
avidin molecules

blocking with streptavidin

topography **recognition**



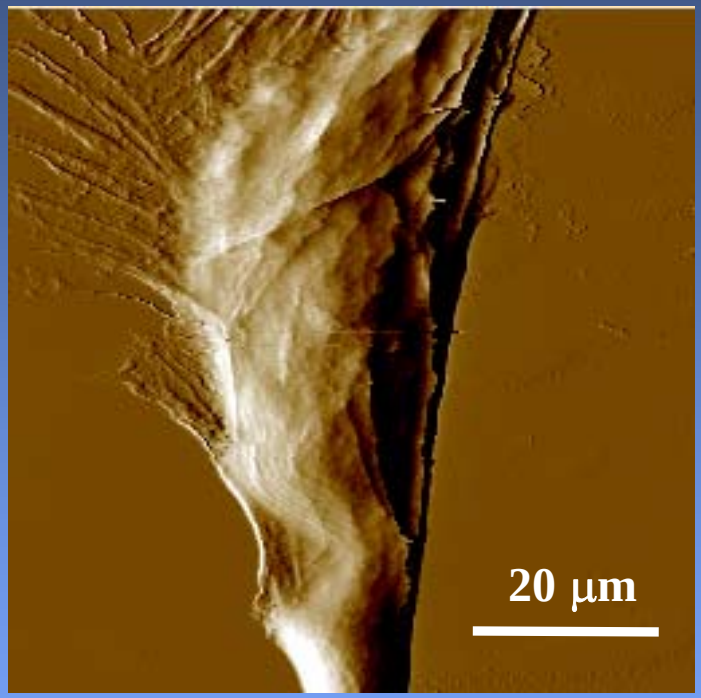
(Stroh C. et al. BiophysJ 2004, PNAS 2004; Ebner A. et al. ChemPhysChem,2005)

Morphology of MyEnd cells

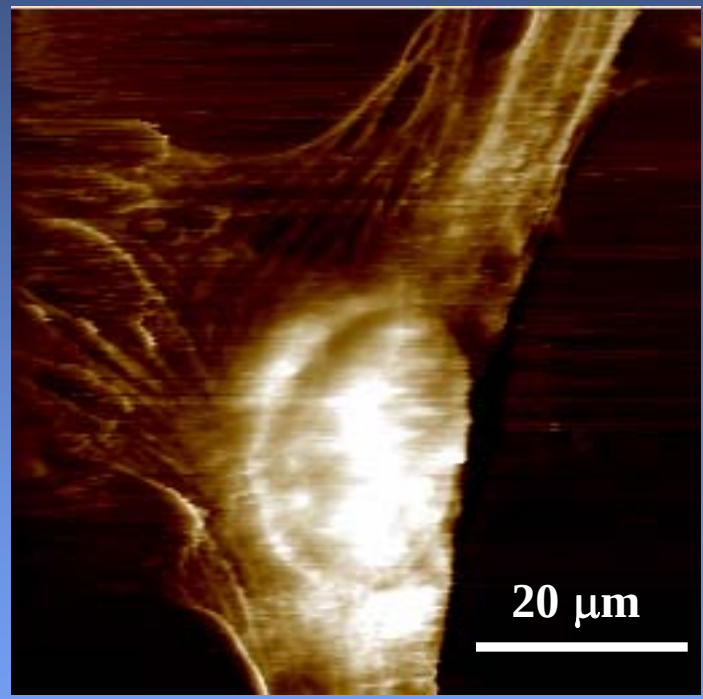
single live cell

AFM PicoPlus; Large scanner; Cantilever: tip E ($\nu \approx 7{,}5$ kHz); HBSS at RT

Contact mode



Mac mode



Lateral mobility of receptors on cell membrane

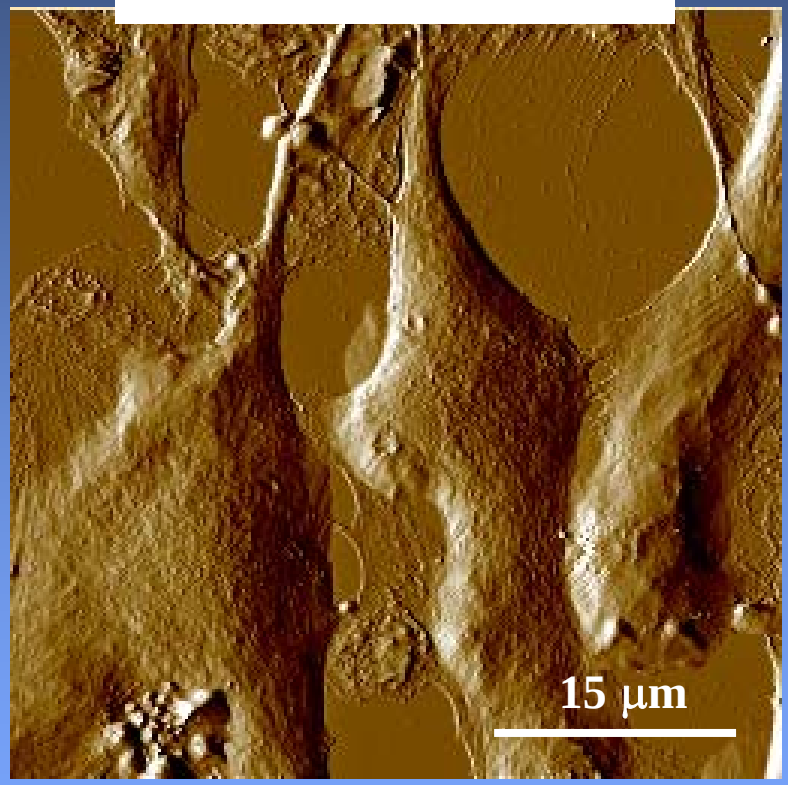
Fixation of cells!

Morphology of MyEnd cells

fixed cells

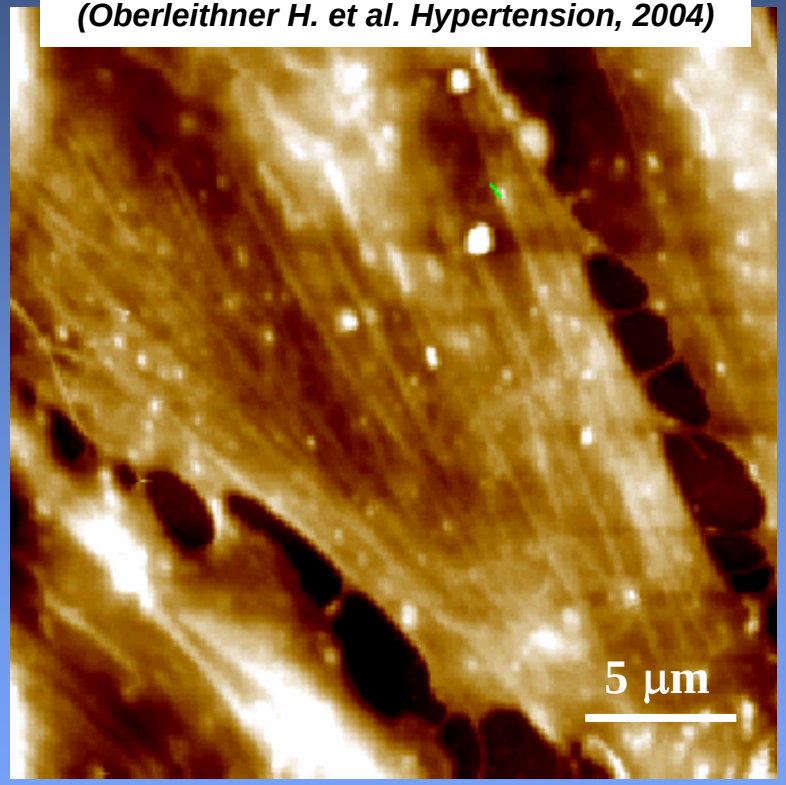
fixation with glutaraldehyde

normal fixation in buffer



gentle fixation in medium

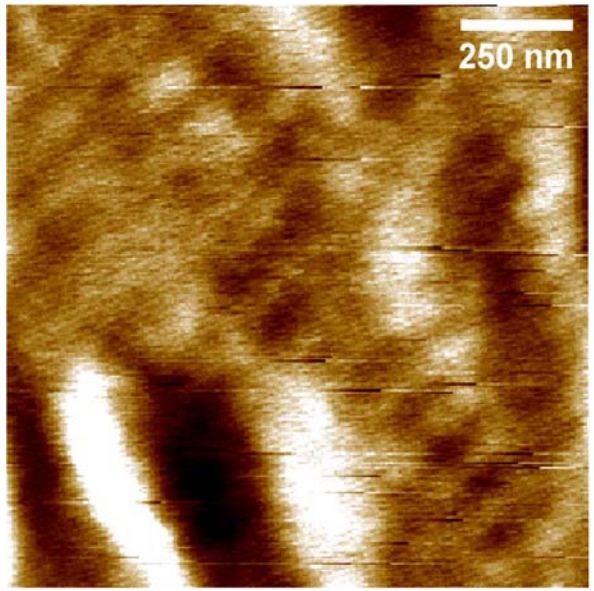
(Oberleithner H. et al. Hypertension, 2004)

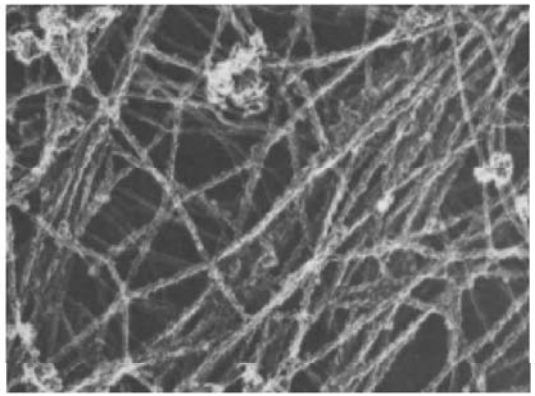


Filamentous network at the cell cortex is mostly conserved!

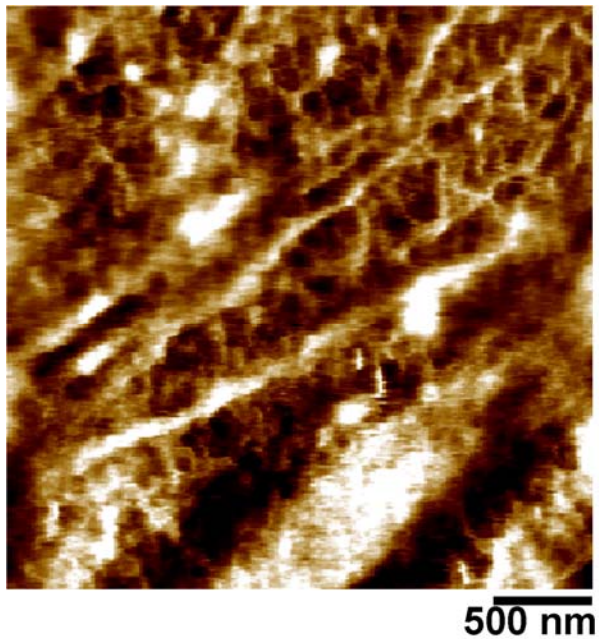
Topography of gently fixed MyEnd cells

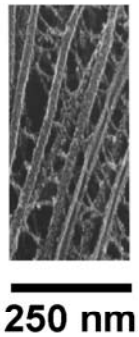
AFM-MAC mode **Transmission EM**





F-actin filaments



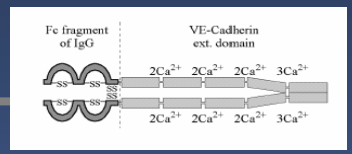


microtubules

(Heuser and Kirschner, JCB, 1980)

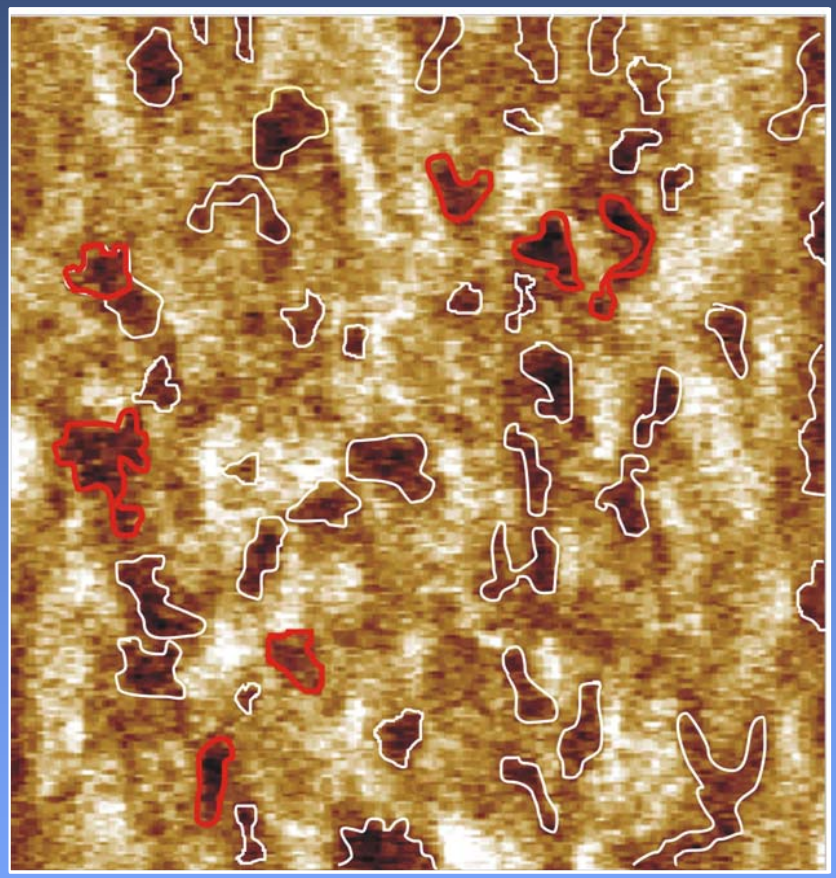
TREC on fixed MyEnd cells / VE-cadherin

AFM tip (ν~7.5 kHz):
VE-cadherin-Fc via PEG-linker



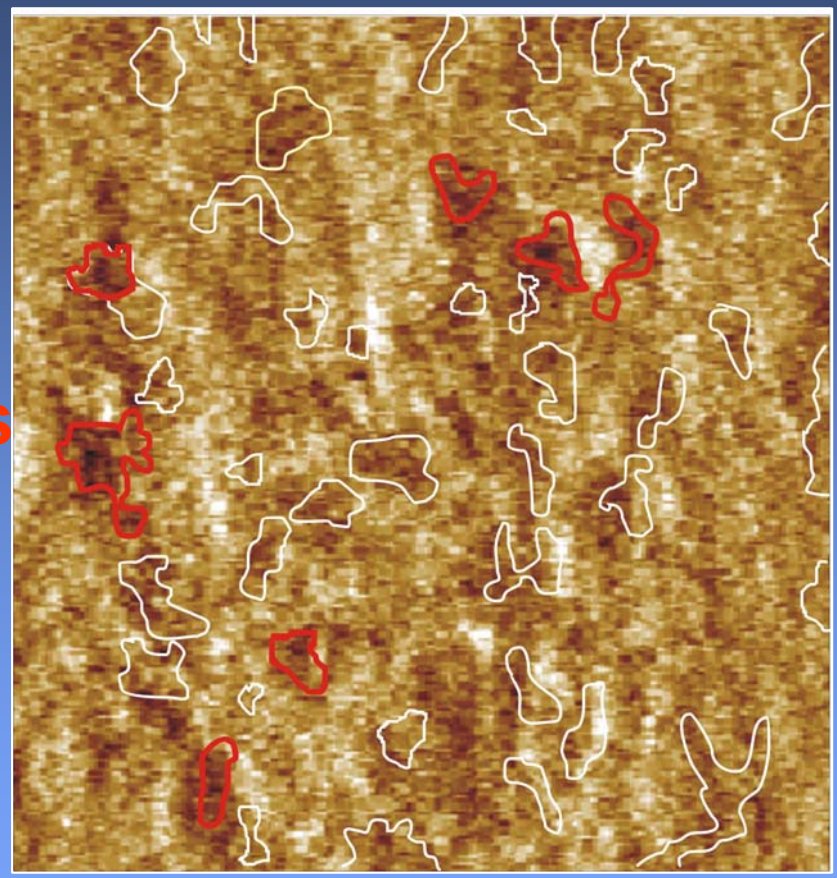
scan size: 1.7μm x 1.7μm
scan speed: ~ 3 μm/s

recognition map

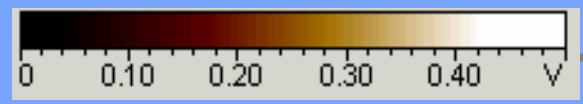


300 nm

blocking with 5mM EDTA

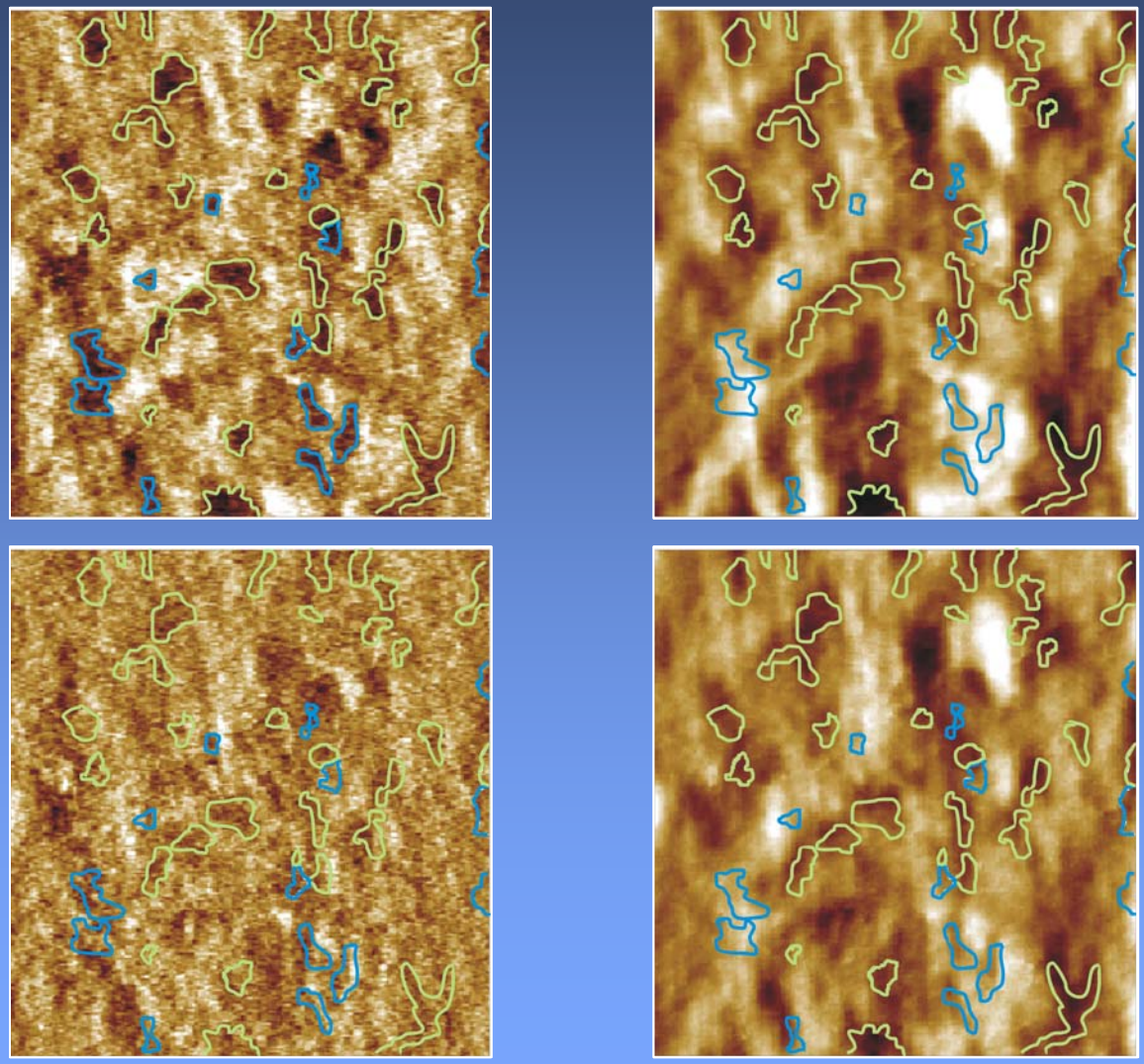


300 nm



TREC on fixed MyEnd cells / VE-cadherin

recognition **topography**



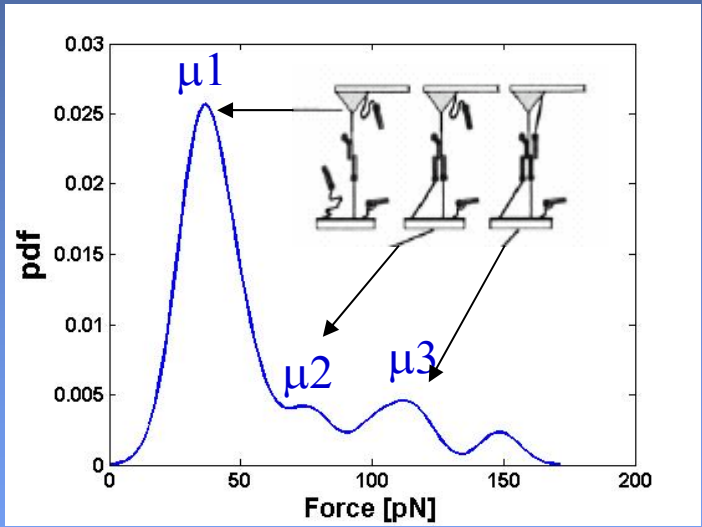
size of VE-cadherin's microdomains from ~ 30 nm to ~ 500 nm

+EDTA

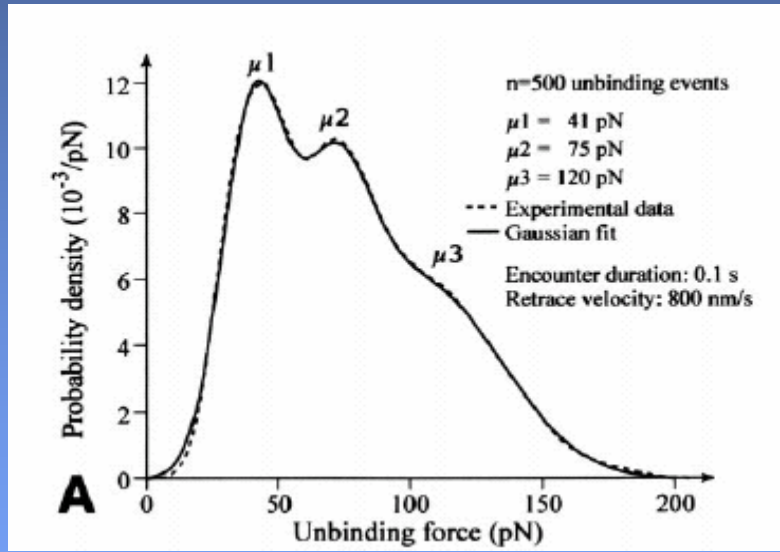
Force measurements: VE-cadherin *cis*-dimers interaction

AFM tip:
VE-cadherin-Fc
via PEG-linker

MyEnd surface



VE-cadherin *cis*-dimers on mica



(Baumgartner W et al, PNAS, 2000)

TREC on fixed MyEnd cells / Fibrinogen

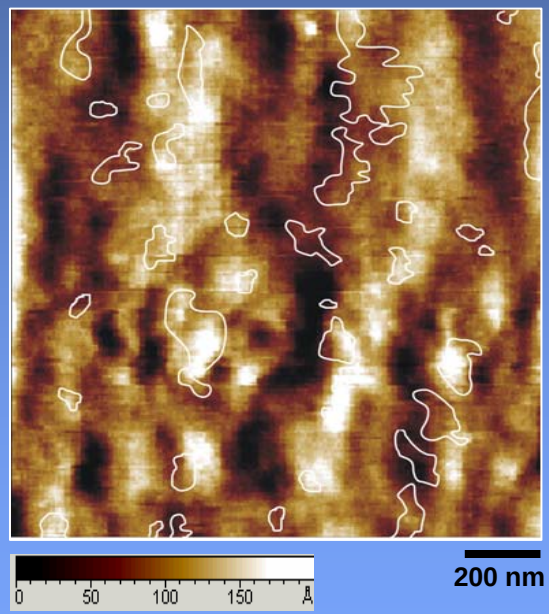
Specificity of fibrin(ogen) to endothelial cells

-VE-cadherin

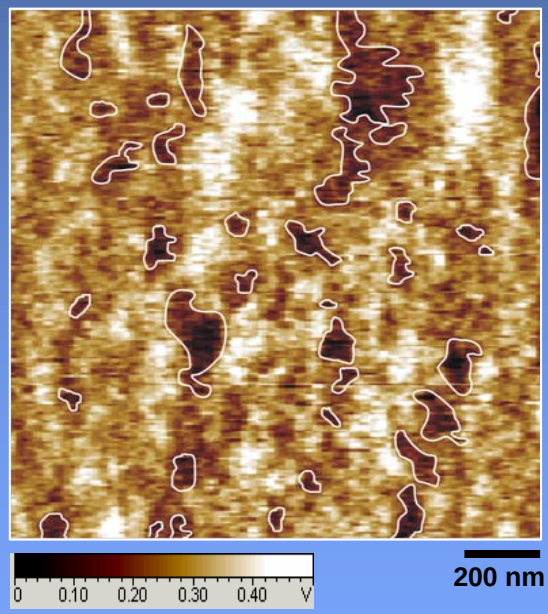
-Integrin $\alpha_v\beta_3$

-Intercellular adhesion molecule (ICAM-1)

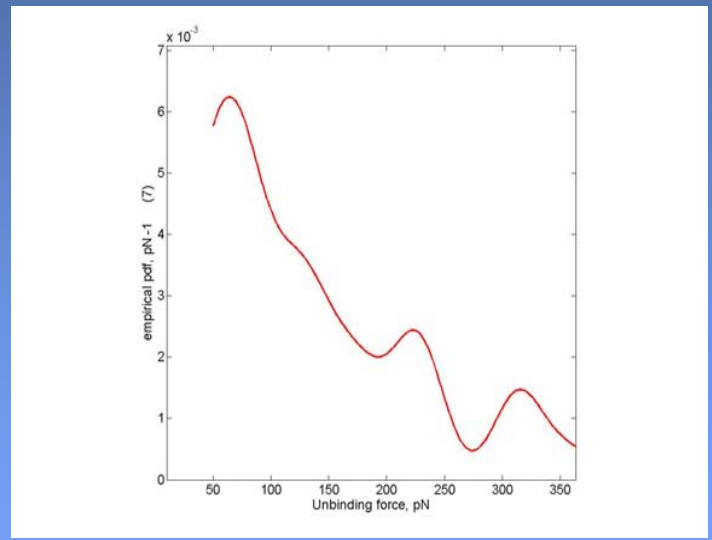
topography



recognition



force distribution



Summary

- MacMode Imaging under Physiological Conditions
- Single Molecule Recognition Force Spectroscopy
- Simultaneous Mapping of Topography and Molecular Recognition
- Nanometer Lateral Resolution at fast Acquisition Rates

People involved

AFM-group **University of Linz** **Technical support**

Ferry Kienberger
Christian Rankl
Cordula Stroh
Lilia Chtcheglova
Andreas Ebner
Theeraporn Puntheeranurak
Rong Zhu

Manfred Geretschläger
Günther Freudenthaler

Surface Chemistry: Hermann Gruber, Linda Wildling, Christoph Hahn, Christian Riener

People involved

Reinat Nevo, Ziv Reich **(Weizmann Institute)**
Rosita Moser, Dieter Blaas **(Vienna Biocenter)**
Werner Baumgartner **(University of Aachen)**
Harald Müller **(University of Kassel)**
Sandra Smith-Gill **(NIH)**
Carina Huber; Margit Sara **(BOKU Vienna)**
Gerald Pfister, Georg Wick **(OEAW, Innsbruck)**

Arizona State University

Hongda Wang, Brian Ashcroft, Stuart Lindsay

Molecular Imaging, Tempe

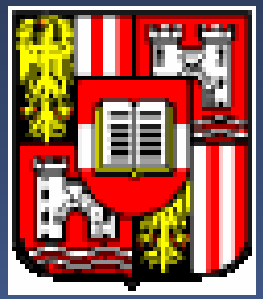
Gerald Kada, Travis Johnson, Jeremy Nelson, Tianwei Jing

http://www.molec.com/linz2007.html

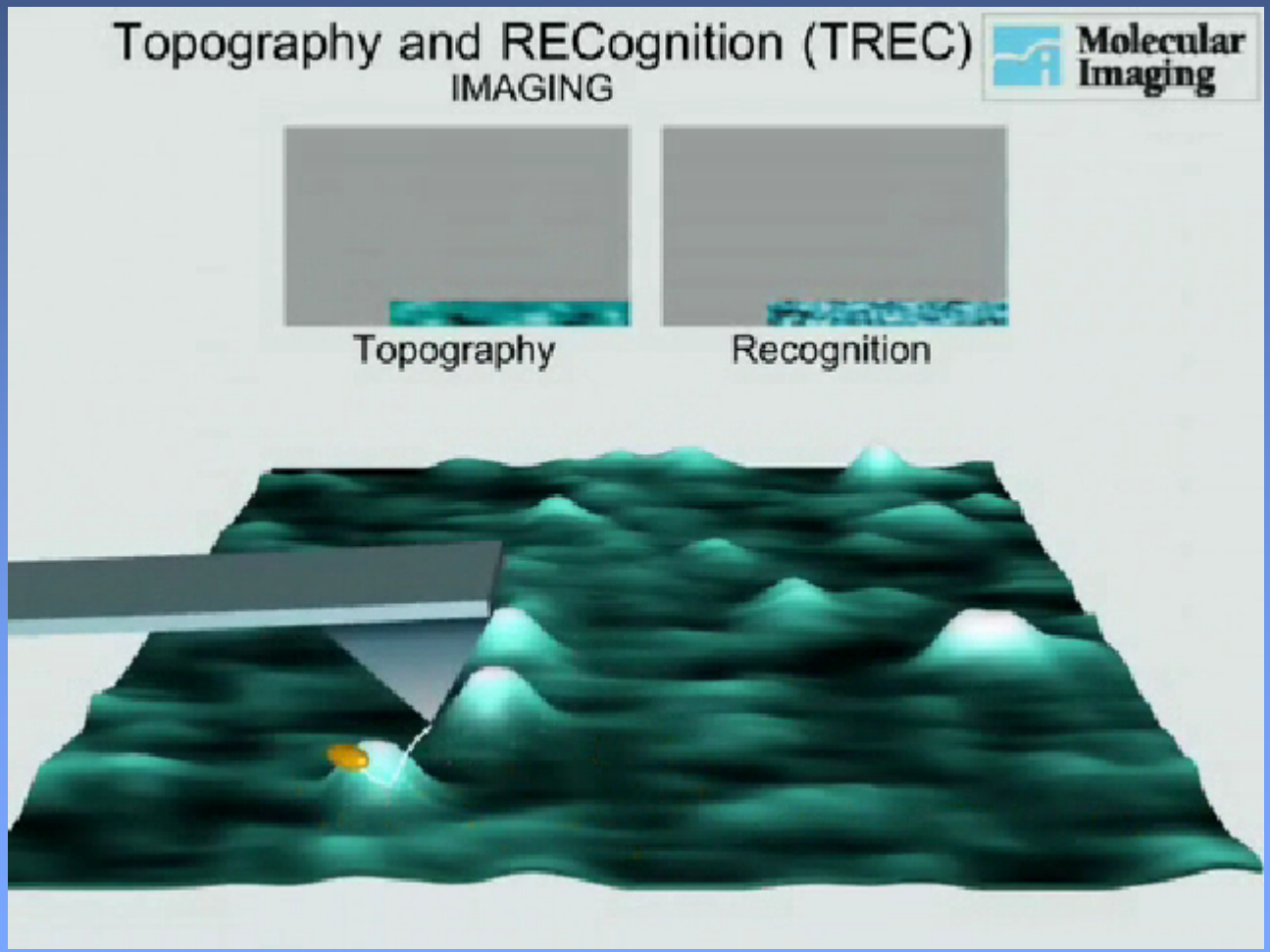
Biological single-molecule research, nano-science, nano-medicine, bio-nanotechnology

Techniques:

- atomic force microscopy
- dynamic force spectroscopy
- optical tweezers
- nanofabrication methods
- self-organization
- fluorescence microscopy
- optical spectroscopy



University of Linz
Austria

Institute for Biophysics

Molecular Imaging