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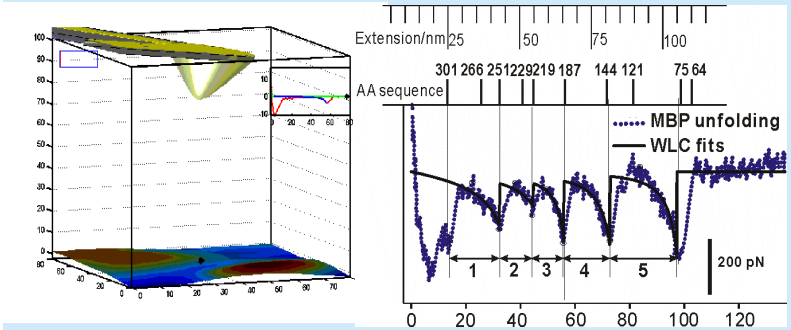
Mechanical Unfolding of Single Proteins and AFM-based Force Mapping



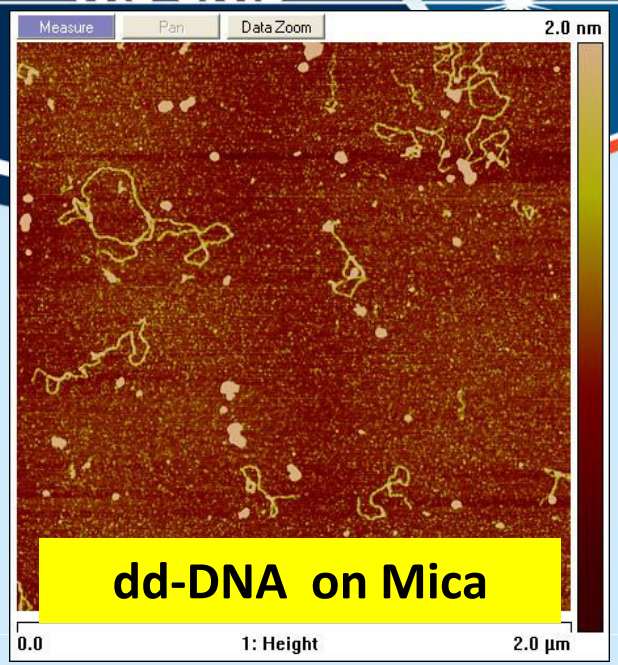
Shan Zou

Biomolecular Sensing and Imaging
100 Sussex Drive, Ottawa, ON, K1A 0R6
shan.zou@nrc.ca

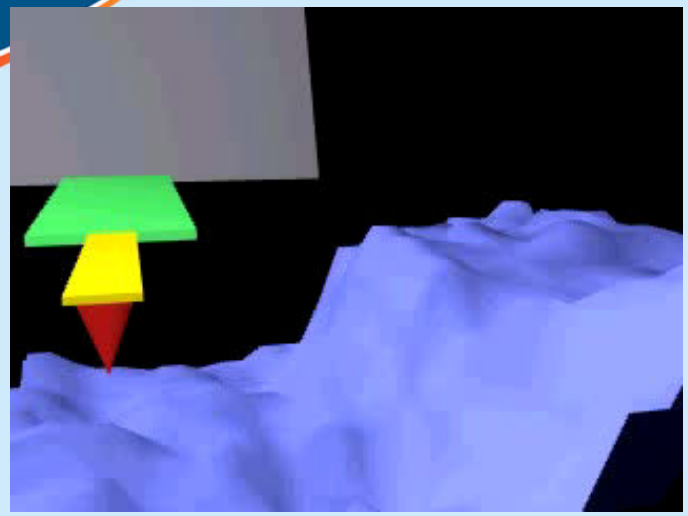
National Research Council Canada | Conseil national de recherches Canada

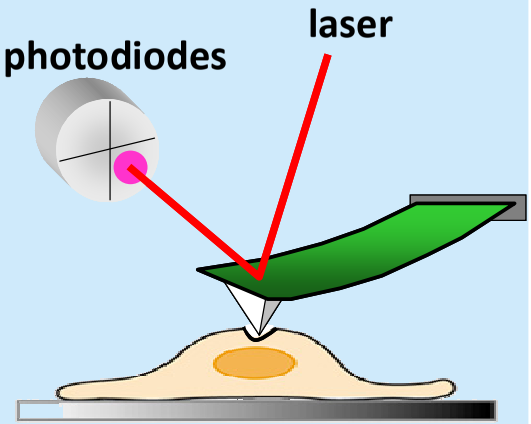
Canada

Atomic Force Microscopy (AFM)



dd-DNA on Mica

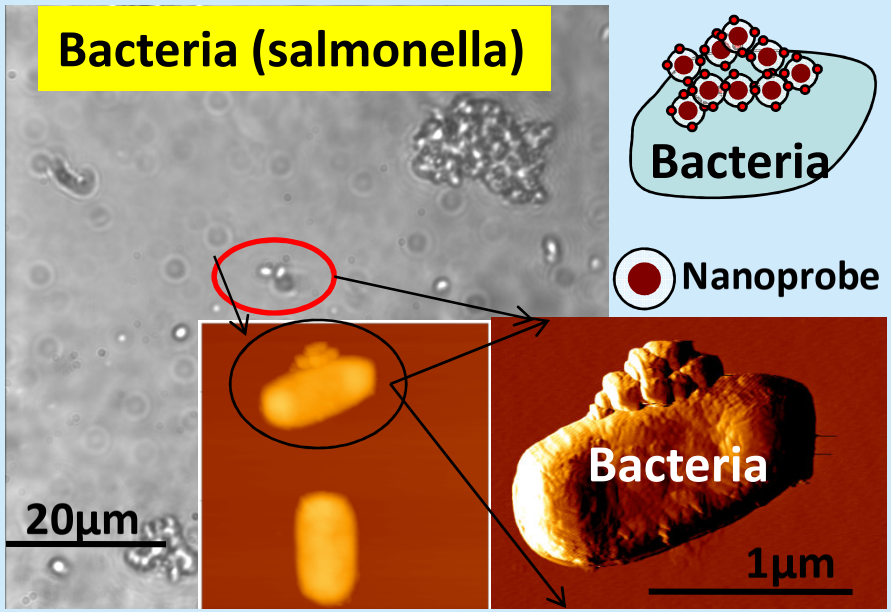




Piezo, feedback control

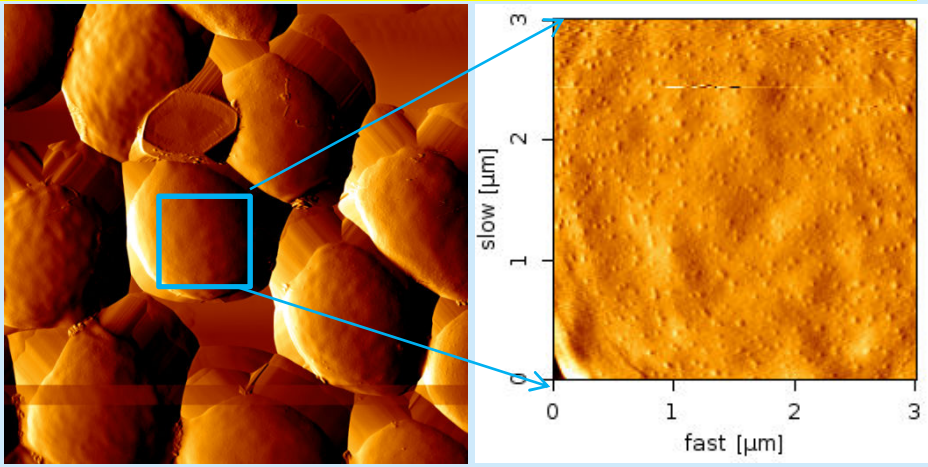
High spatial resolution!

Bacteria (salmonella)



With C. Paquet(SIMS) , and S. Ryan, J. Tanha (IBS)

Yeast cells exposed to Se nanoparticles



~60 nm Nanoparticles were observed on the cell surfaces. (With Zoltan Mester, INMS)

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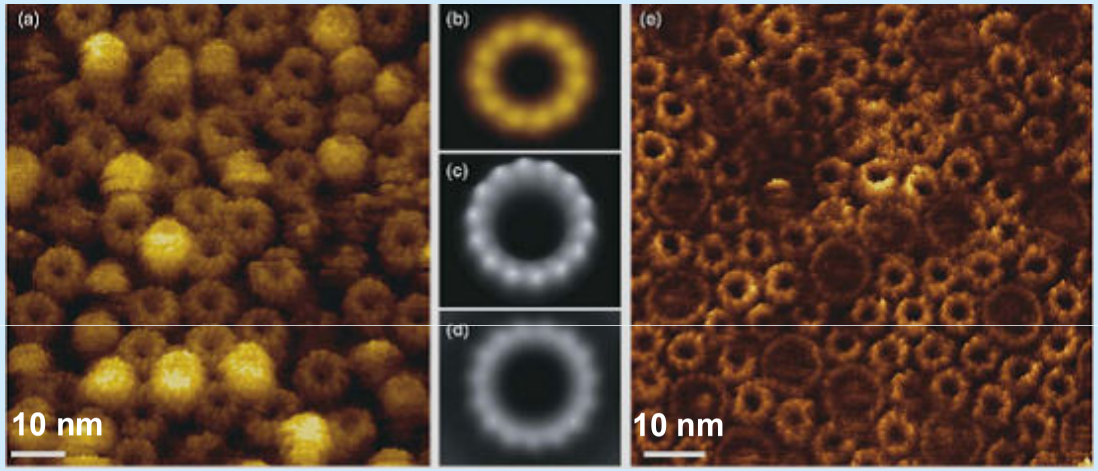
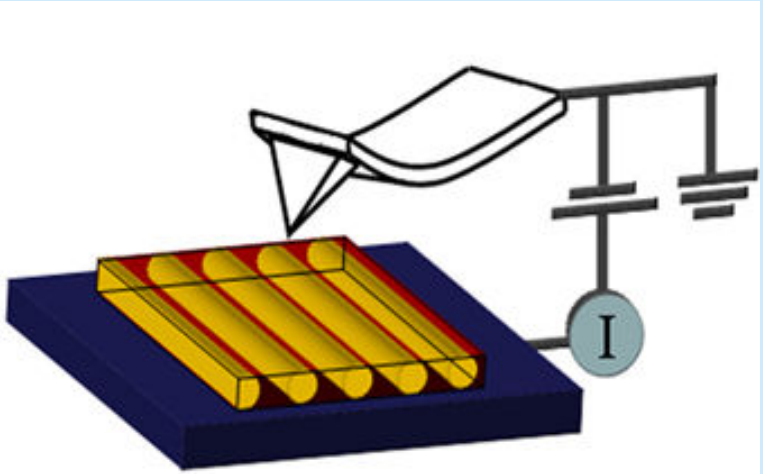
High Resolution AFM

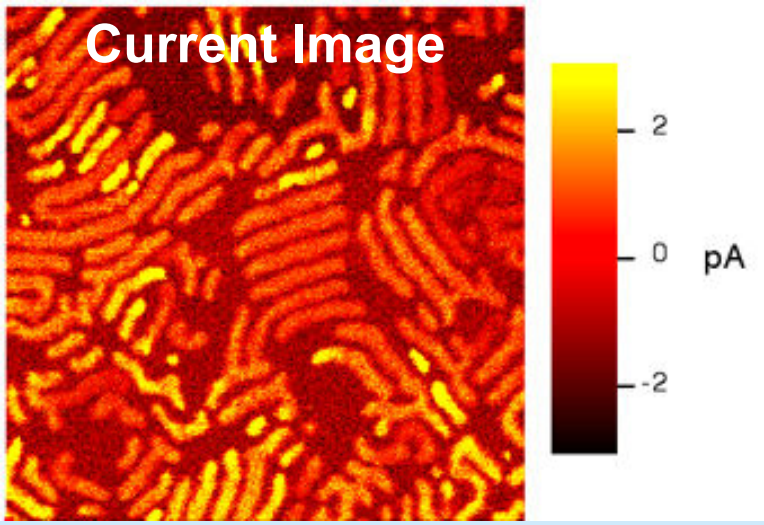


Oligomeric assemblies of native membrane proteins by AFM. (a) Topography (raw data) of sodium-driven rotors from FoF1-ATP synthases of Ilyobacter tartaricus. Averaged topographs of ion-driven rotors from FoF1-ATP synthases of (b) I. tartaricus, (c) spinach chloroplasts and (d) Spirulina platensis; (e) The raw data AFM image of a core complex surrounded by seven peripheral antenna (LH2) complexes to the structural model derived from it.

Scheuring S. and Sturgis J. (2005) Science 309, 484-487

Conductive Probe AFM





NRC Canada, University of Toronto, University of Bristol

Li JK; Zou S; Rider D; Manners I; Walker GC *Adv. Mater.* 2008, *20*, 1989-1993.

Wang YS, Zou S, Winnik MA, Manners I *Chemistry Eur. J.* 2008, in press.

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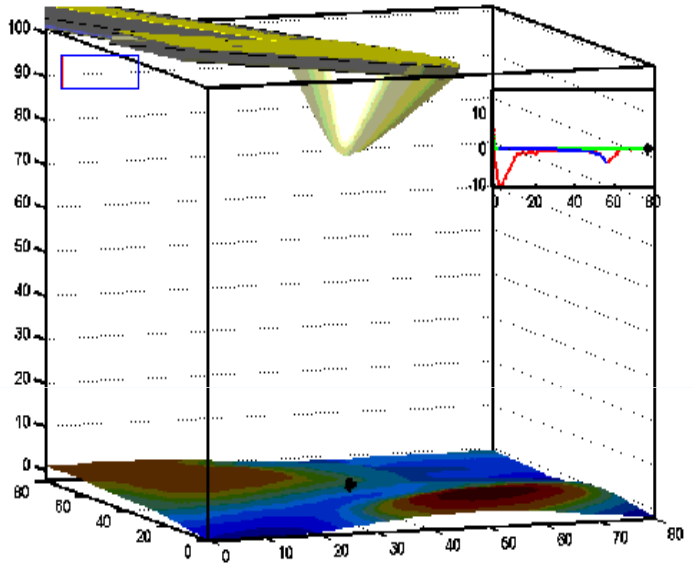
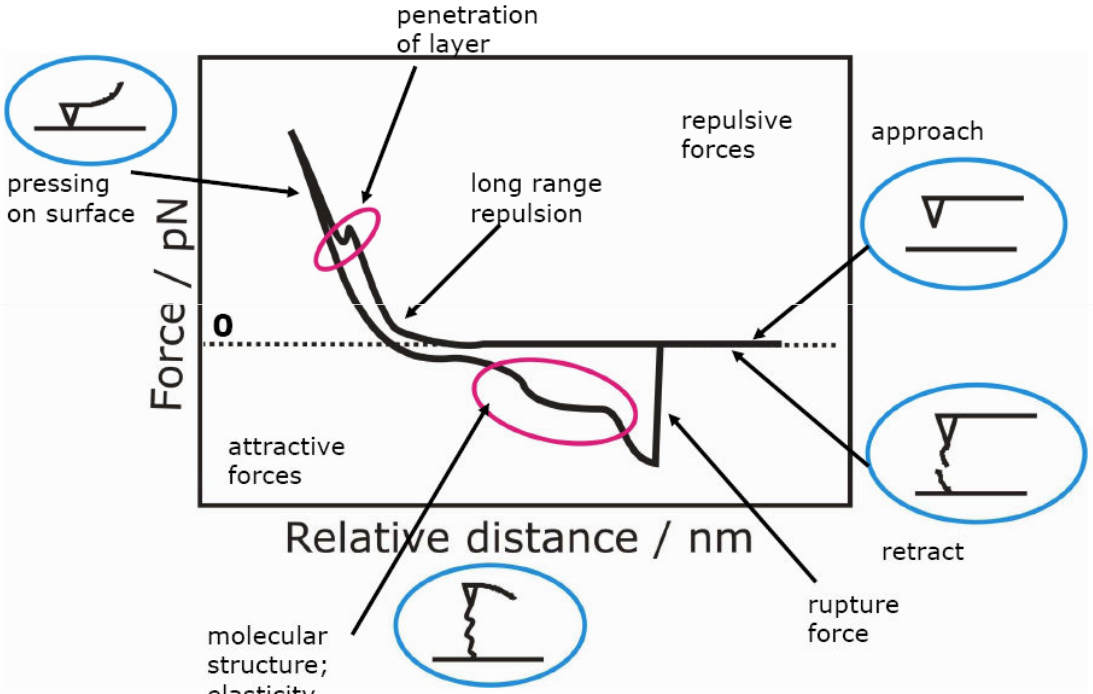
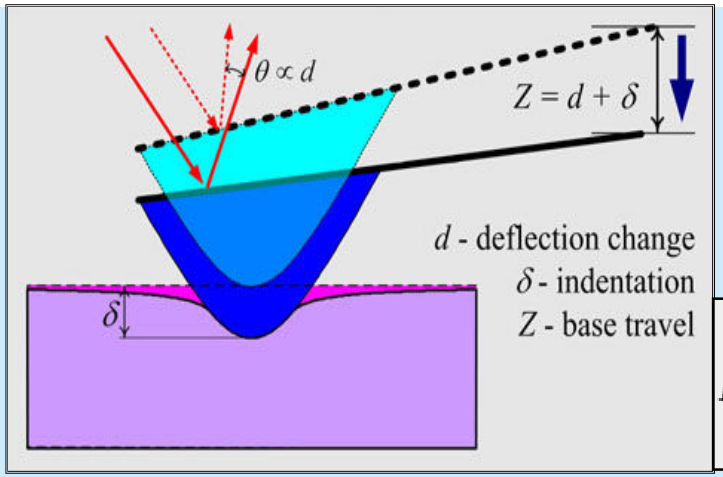
AFM-based Single Molecule Force Spectroscopy (SMFS)



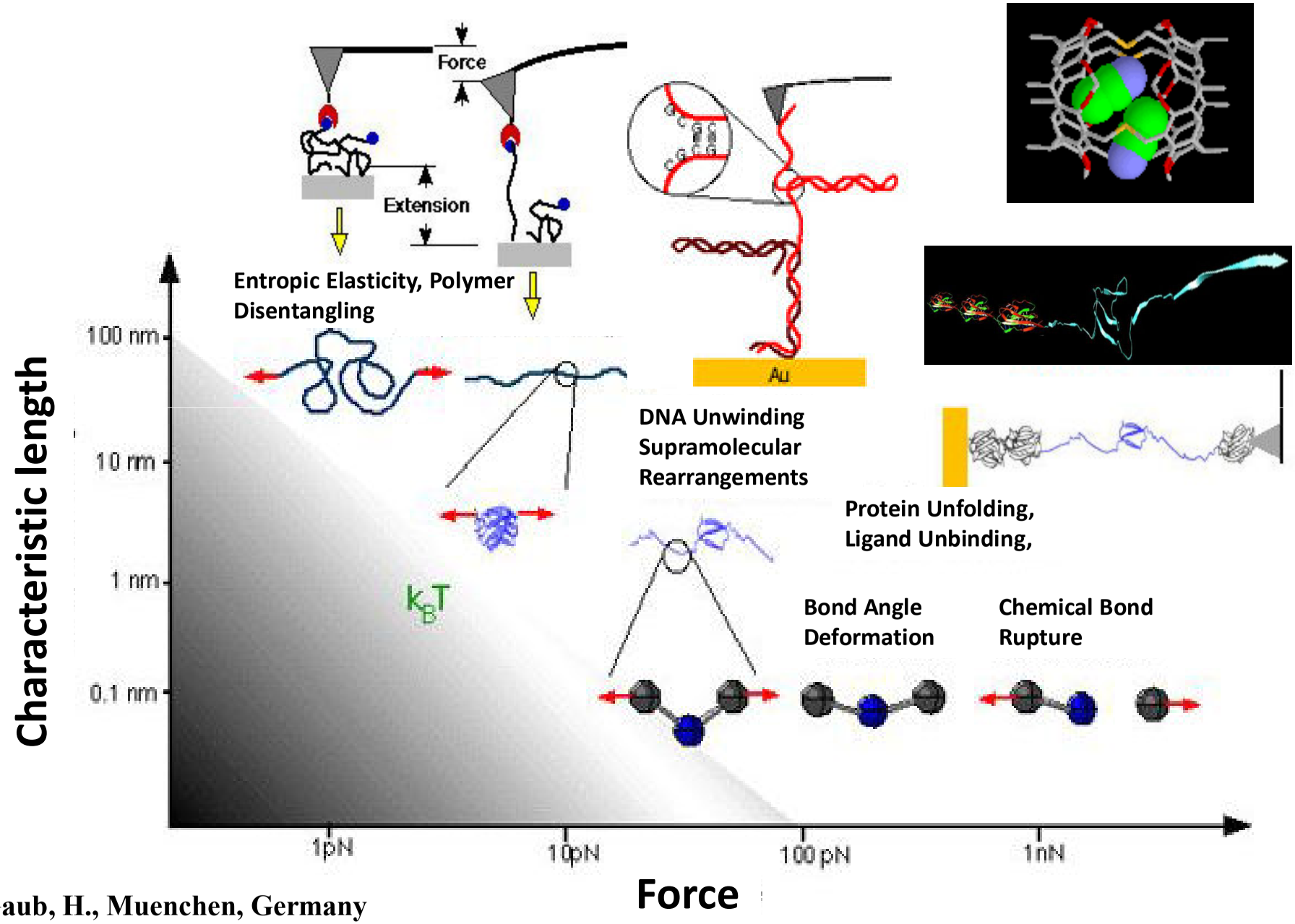
Interpretation of force-distance curves





$$F = \frac{4E\sqrt{R}}{3(1-\sigma^2)}\delta^{3/2}$$

Stretching & Interaction Forces by SMFS



Gaub, H., Muenchen, Germany

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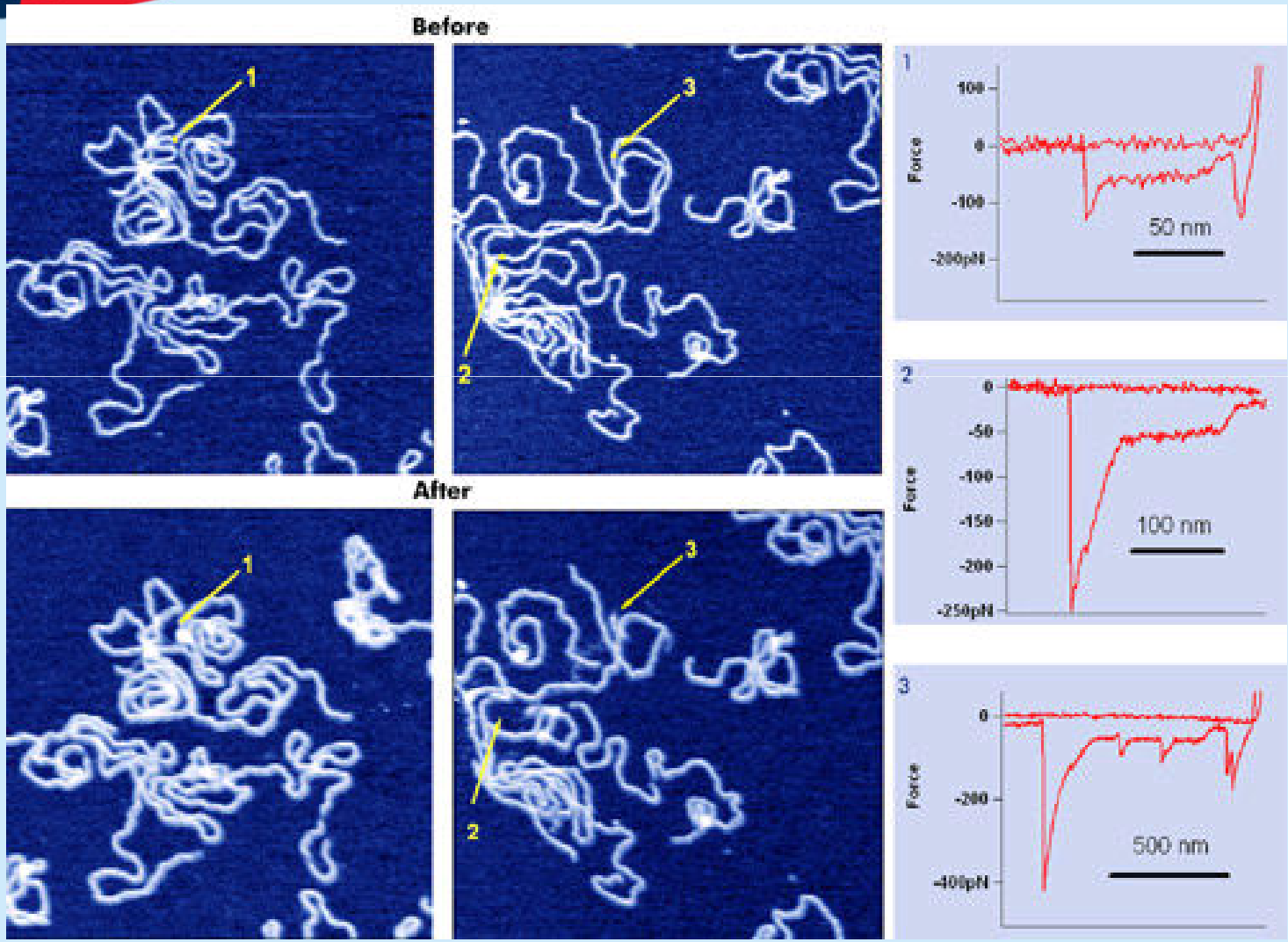
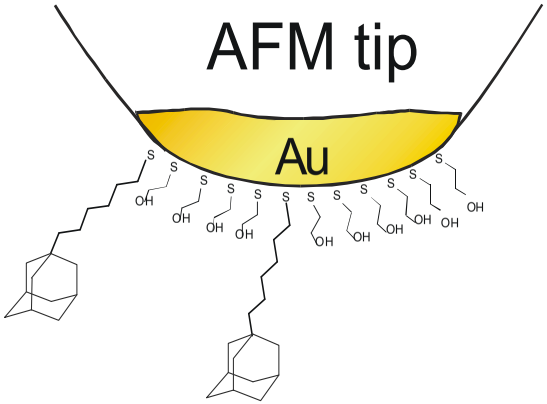
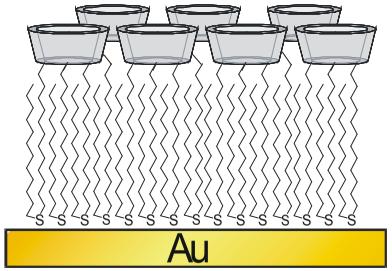
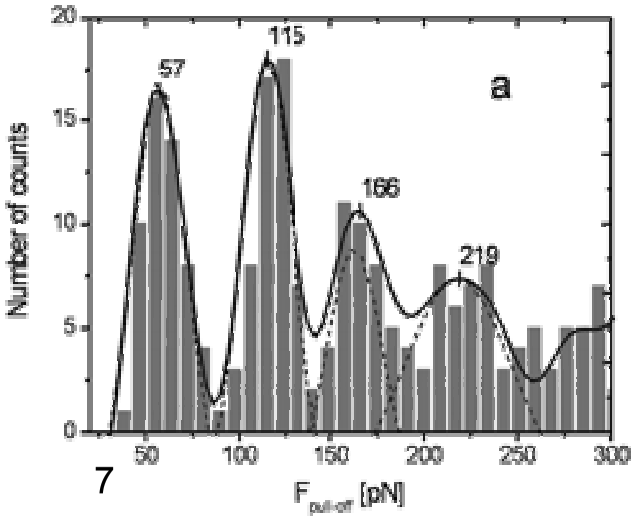
Unzipping of single double stranded DNA molecules by SMFS



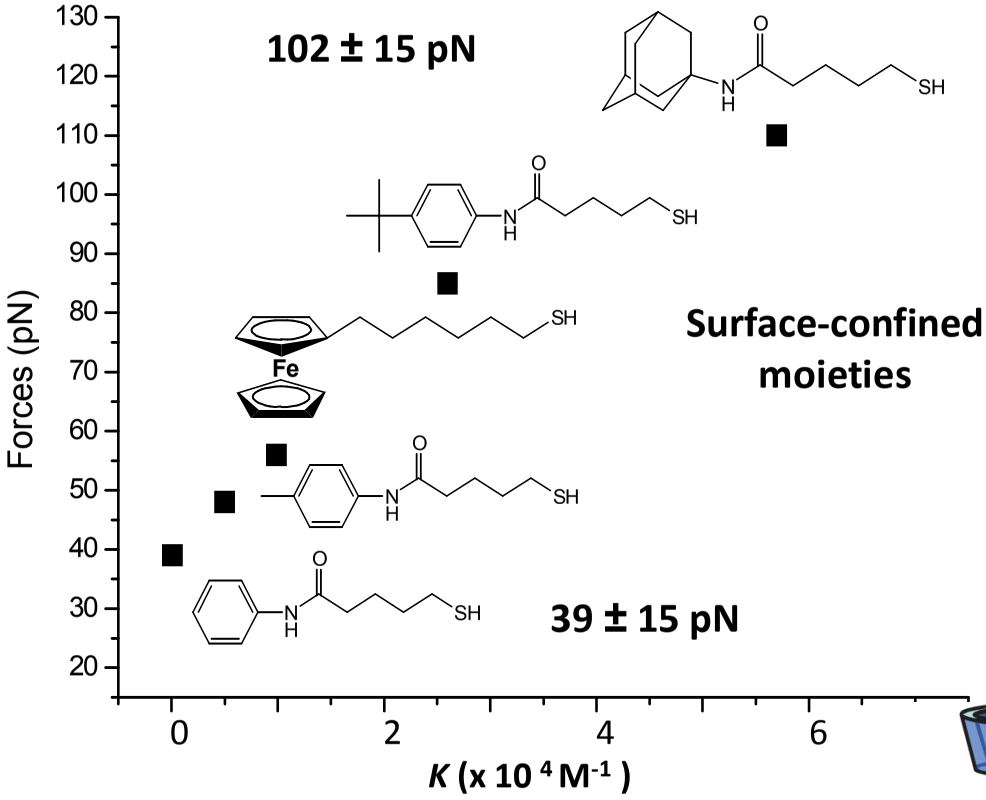
Host-Guest (ligand-receptor) Interactions



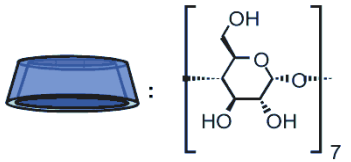


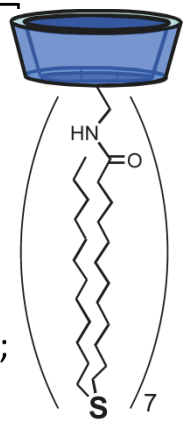


7



Host: β-cyclodextrin (β-CD)





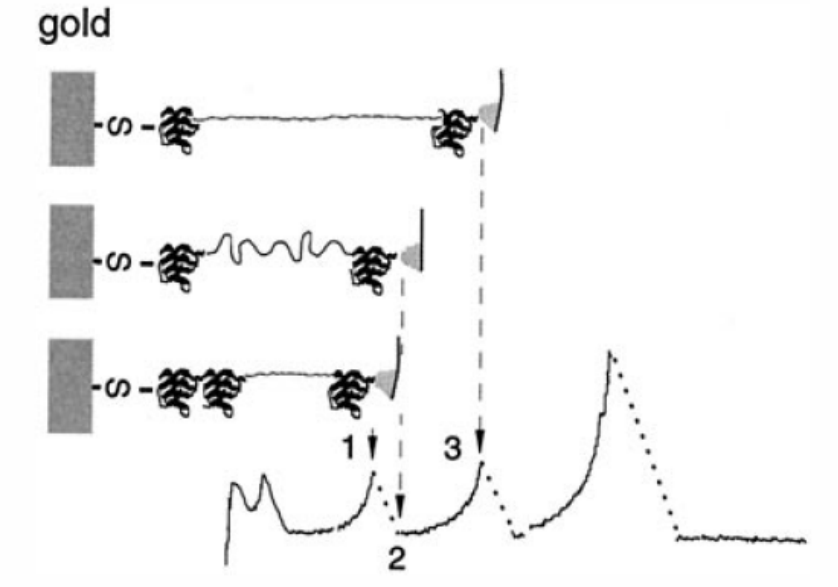
Auletta T; de Jong MR; Mulder A; van Veggel, FCJM; Huskens J; Reinhoudt DN; **Zou S**; Zapotoczny S; Schönherr H; Vancso GJ; Kuipers L ***J. Am. Chem. Soc.,*** 2004, 126, 1577.

Zou, S.; et.al. ***Langmuir,*** 2003, 19, 8618.

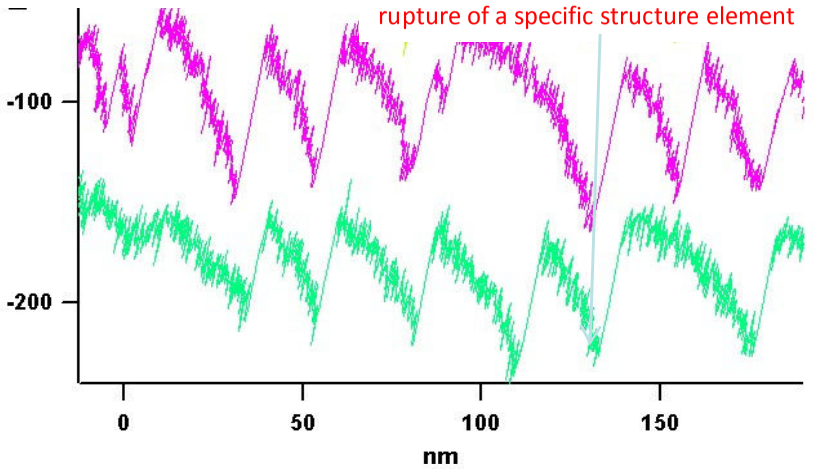
Zou, S.; et.al. ***Angew. Chem. Int. Ed.*** 2005, *44*, 956-959.

Zou, S.; et.al. ***J. Am. Chem. Soc.,*** 2005, *127*, 11230.

Mechanical Unfolding of Adhesion Proteins



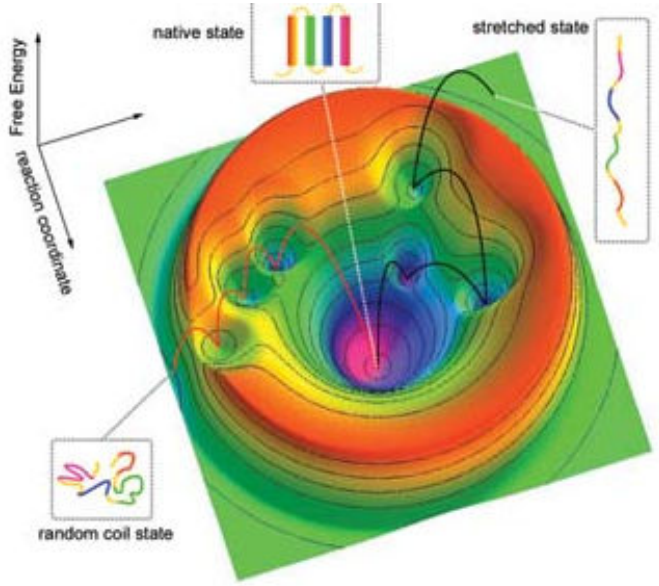
Bovine Fibronectin III, Titin



Single modules of multi-domain adhesion proteins unfold in a largely two-state manner. The forced unfolding of each domain is initiated by the rupture of a specific structure element (most commonly secondary structure), which leads to rapid unfolding of the whole domain into a random coil chain.

Exposing the protein to heat or chemical denaturants is the classical way of driving a protein out of its folded conformation through this energy landscape to an unfolded conformation (red pathway). However, the exact pathway of mechanical unfolding or reaction coordinate is not necessary the same (black pathway).

A funnel-shaped energy landscape



MECHNICAL denaturation of non-mechanical protein???

➔

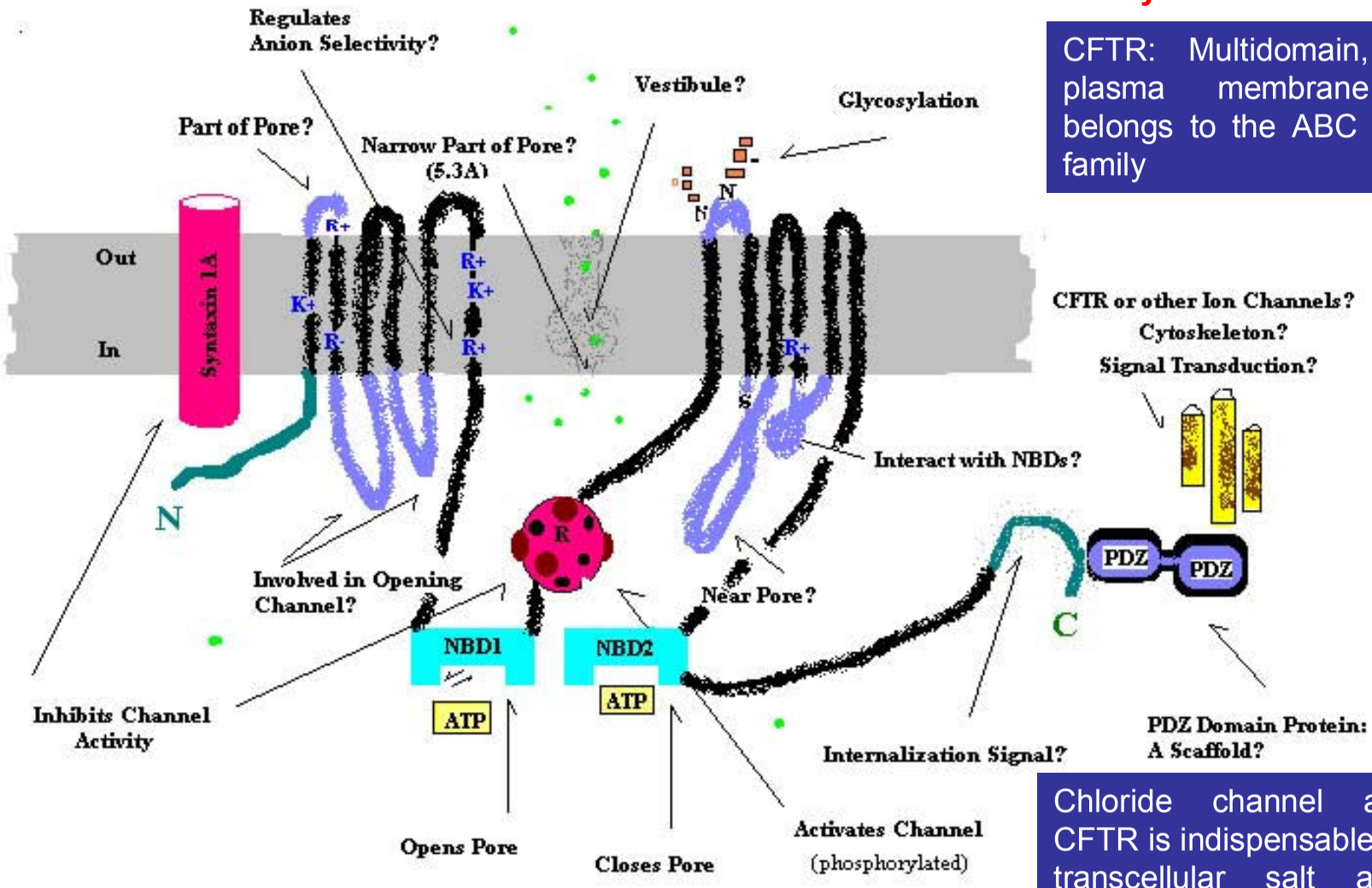
Single Molecule Study on STRUCTRAL TRANSITIONS

Specifically, how much the typical two-state behavior in thermal/salt induced denaturation of MBP would be preserved in mechanical denaturation?

Characterization of NBD1 and NBD2-MBP of CFTR by Integrated AFM, Confocal FL and Single-Molecule Force Spectroscopy

Collaboration with G. C. Walker, G. Lukacs,
University of Toronto, Sick Children Hospital, McGill University
Cystic Fibrosis Foundation

CFTR: Multidomain, polytopic plasma membrane protein; belongs to the ABC transporter family

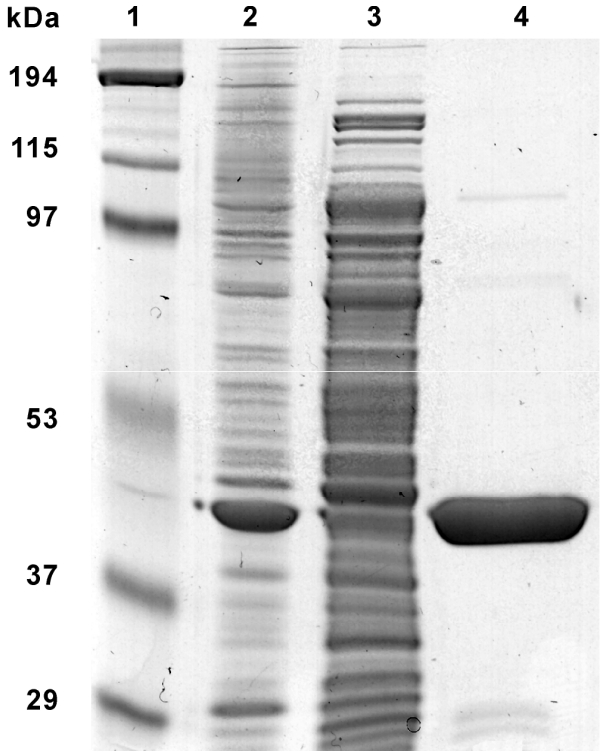


Chloride channel activity of CFTR is indispensable for normal transcellular salt and water transport in numerous organs.

http://www2.montana.edu/cftr/PointandClick/putting_it_all_together.htm

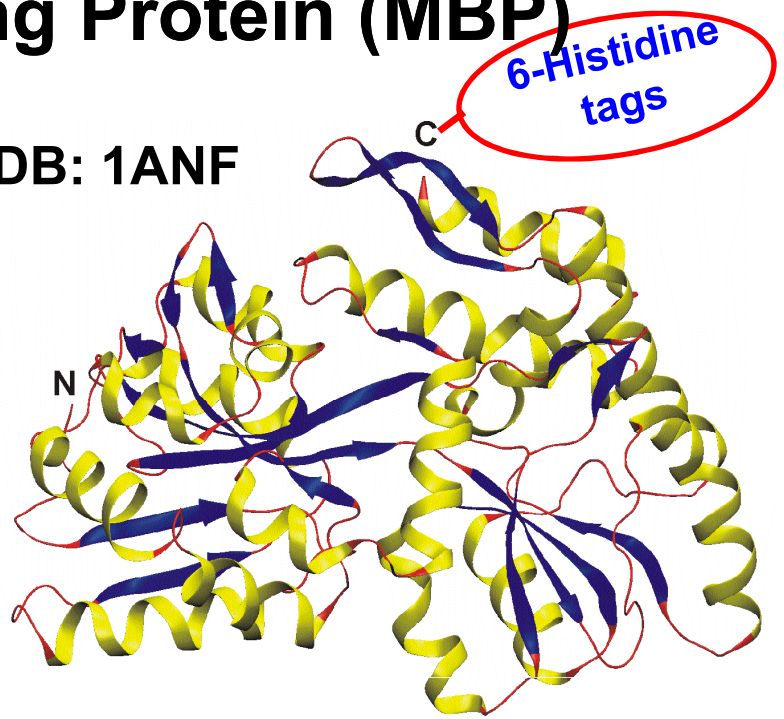
Maltose Binding Protein (MBP)

14% SDS PAGE stained with Coomassie Brilliant Blue R250: Lane 1 –Marker, Lane 2 – Supernatant, Lane 3 – Pellet, Lane 4 – Eluted MBP protein.



Well expressed in the bacteria cytoplasm and was found to be a single two-state unfolding transition upon ***heating.***

PDB: 1ANF



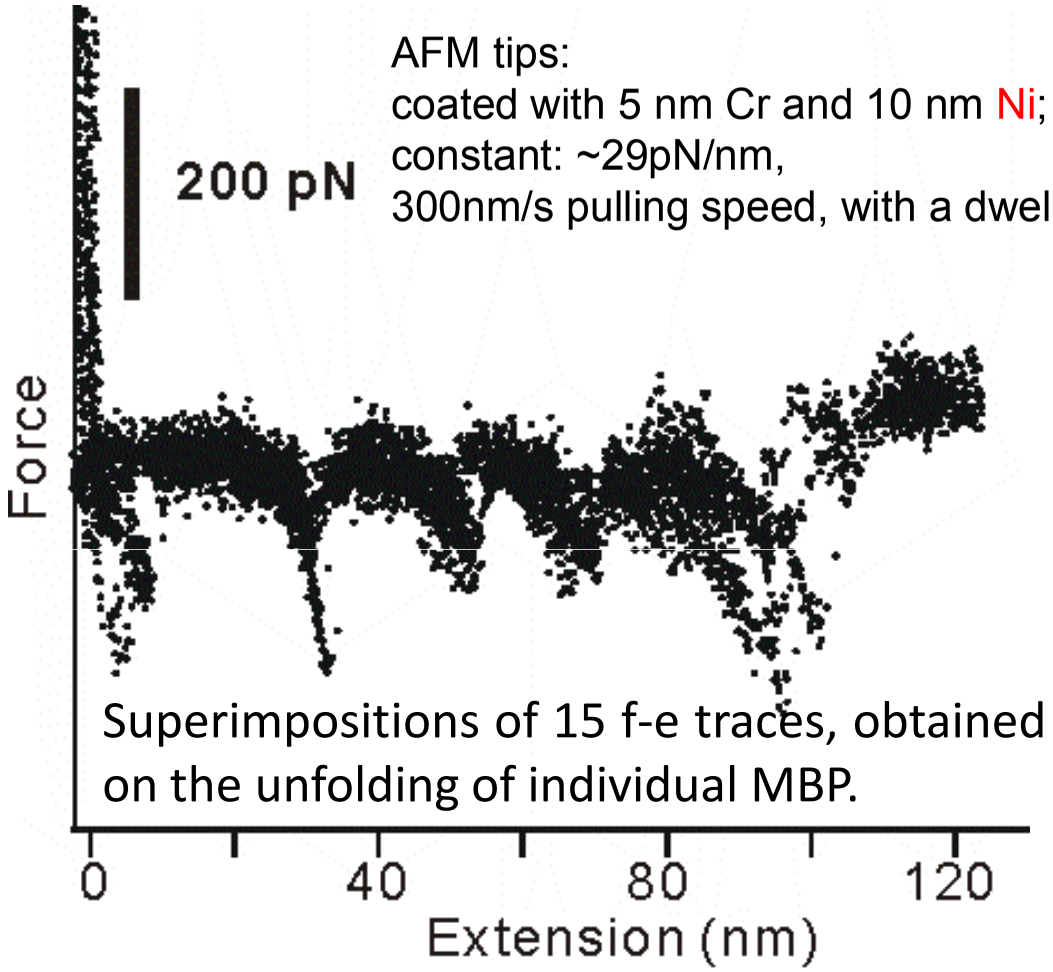
MBP source: in the periplasm of Escherichia coli. – responsible for catabolism of maltodextrin

Widely used as solubilizing agent – generate affinity handles for purification of recombinant proteins

number of Residues: 366
beta-strands: 23; alpha helices: 16

in our study: C terminus modified with 6 His-tags

Unfolding of Single MBP Molecules



Superimpositions of 15 f-e traces, obtained on the unfolding of individual MBP.

AFM tips:
coated with 5 nm Cr and 10 nm Ni; spring constant: ~29pN/nm,
300nm/s pulling speed, with a dwell time of 2 s .

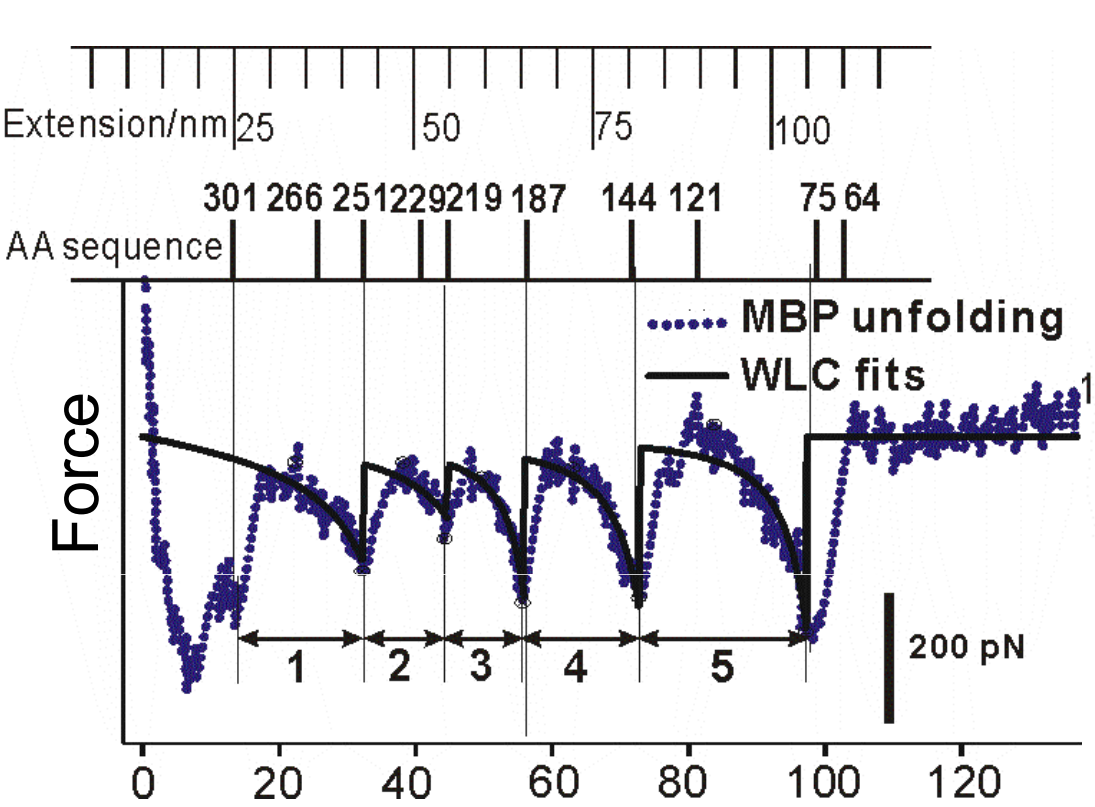
- Six His-tags at C-terminus improve the attachment probability of MBP to Ni-AFM tips by 30 %.

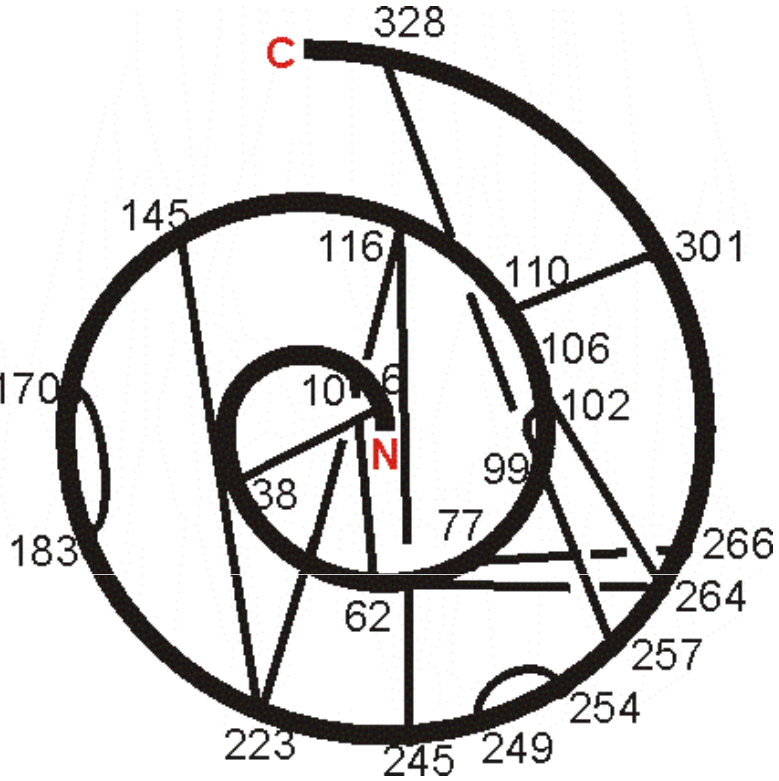
- Common unfolding patterns were highlighted in the denser regions by alignment of the f-e curves.

- **MULTIPLE force transitions → mechanical unfolding of MBP is not two-state, but multiple states.**

Demonstrate that force is not projected along the "thermal" denaturation coordinate.

Unfolding of Single MBP Molecules

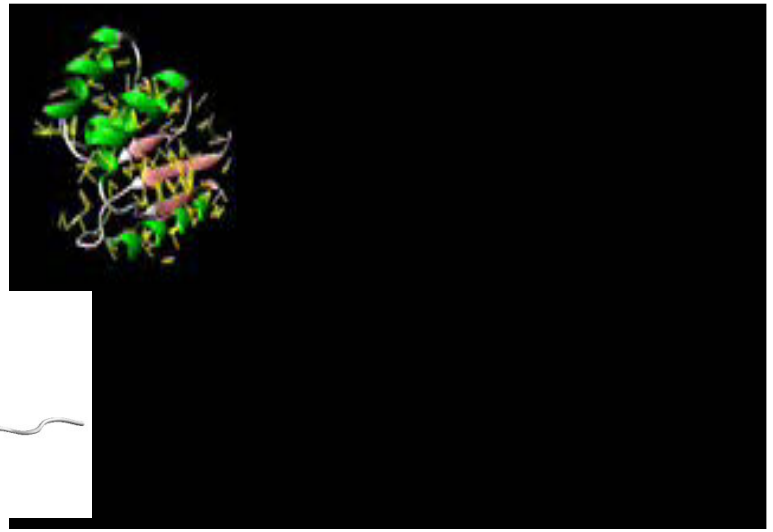


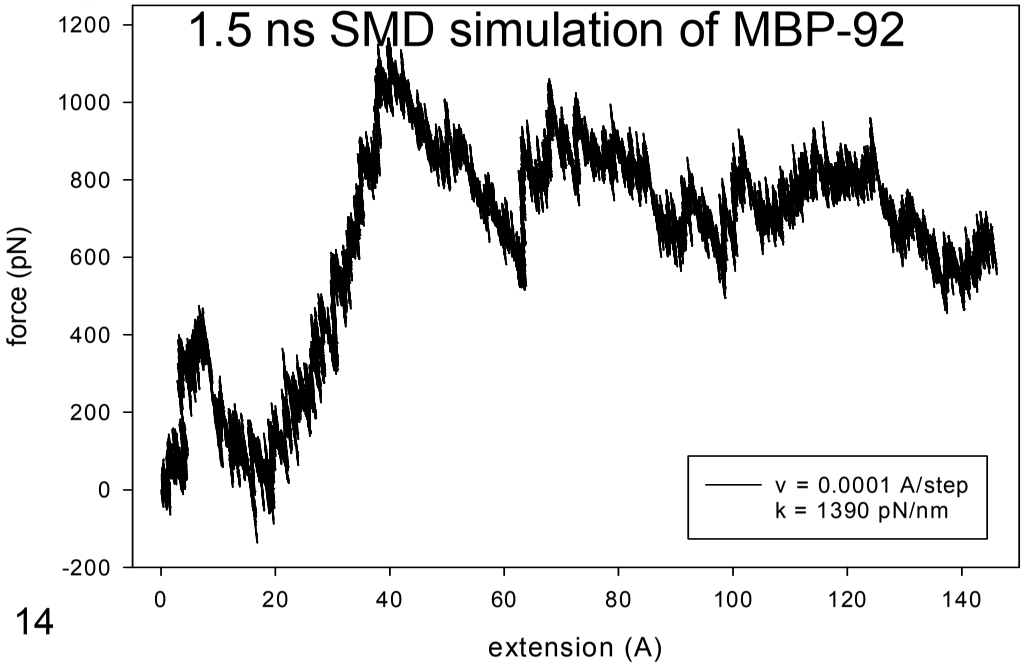


The sequential unfolding events of the MBP only occur at the ends of beta-sheet regions, suggesting that cooperative interactions exist between two neighbouring secondary beta strands within a single protein molecule.

MD Simulation Data

First 92 residues fragment :





The force peak occurs at 40 Å corresponding to the middle beta strand breaking away from the top strand, but still connected to the bottom one. The next one at 75 Å is attributed to the separating of this beta strand from the lower one.

For MBP-92 -- seen unfold sequentially rather than in a two-state process;
-- seen that a "core" of stable structure remained until the full unfolding occurred.

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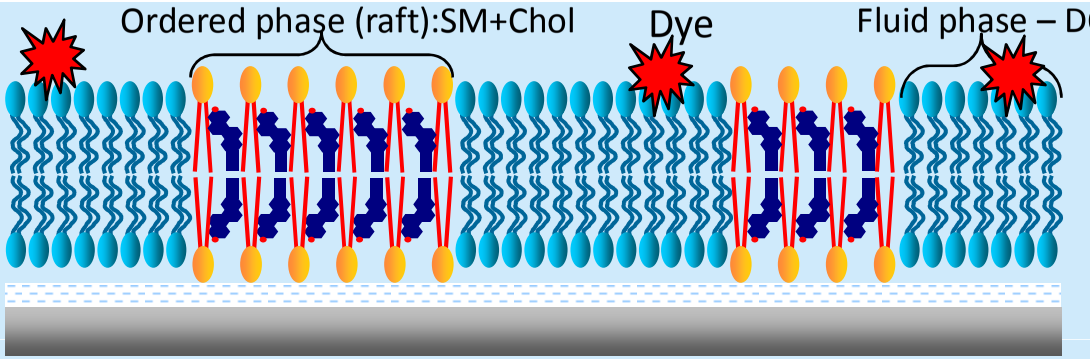
Membrane Restructuring

Collaboration with Dr. Linda J. Johnston

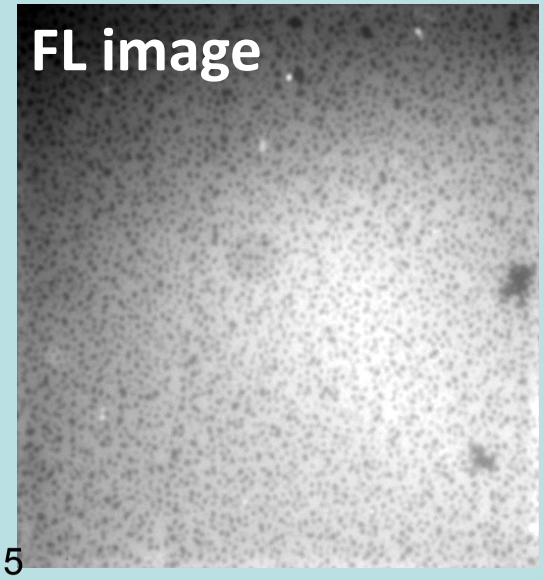
Induced by Enzymatic Generation of Ceramide using Integrated AFM and Fluorescent Microscopy

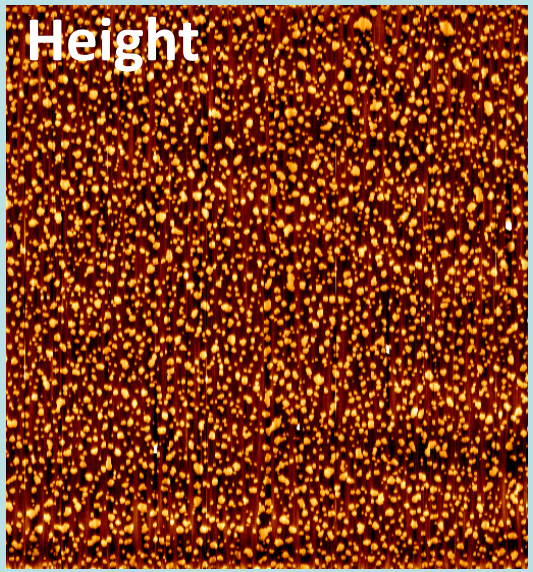
DOPC(TR0.3%): Egg Sphingomyelin: Cholesterol (DEC 2:2:1)

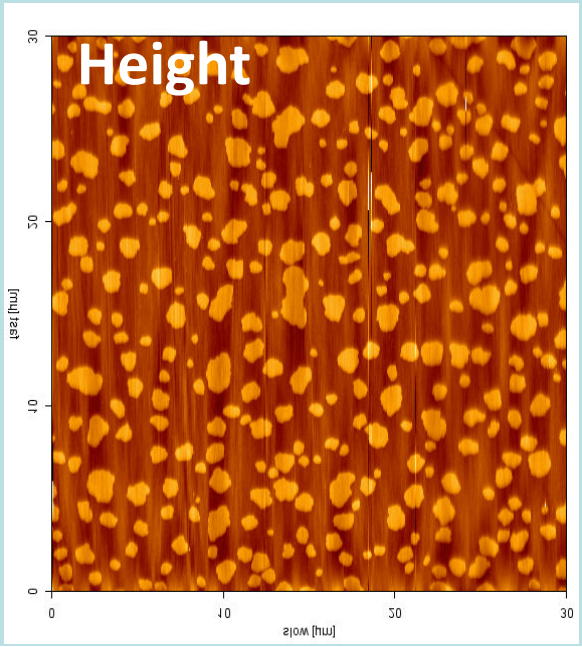


Ceramide involved as a messenger in signaling during Apoptosis cell differentiation and growth suppression.

➔ Restructuring of the cell membrane lipids.







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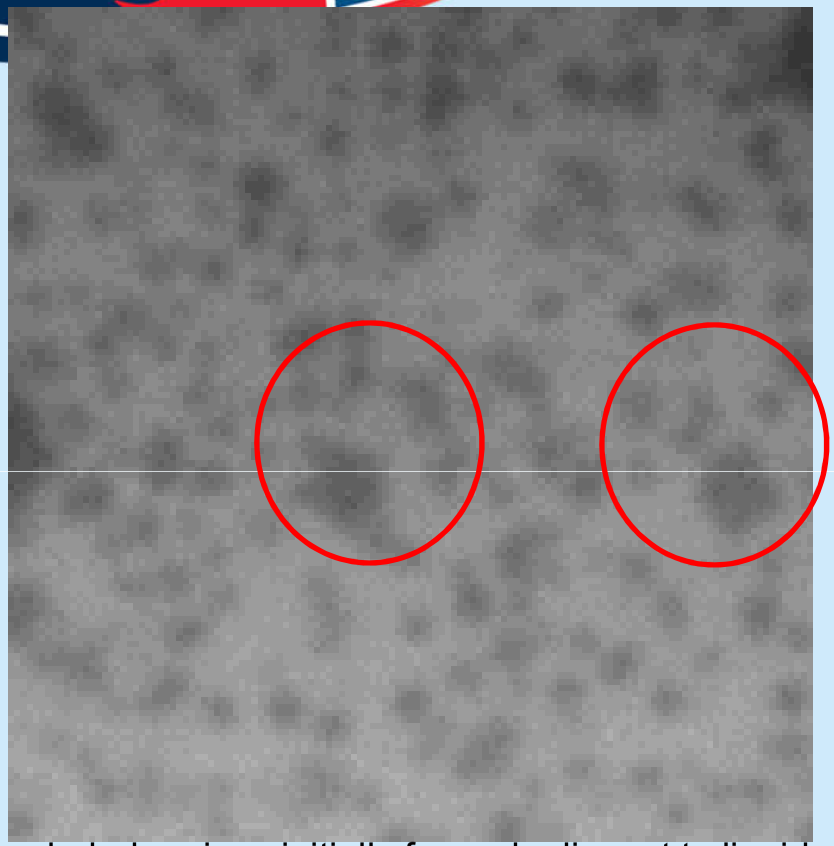
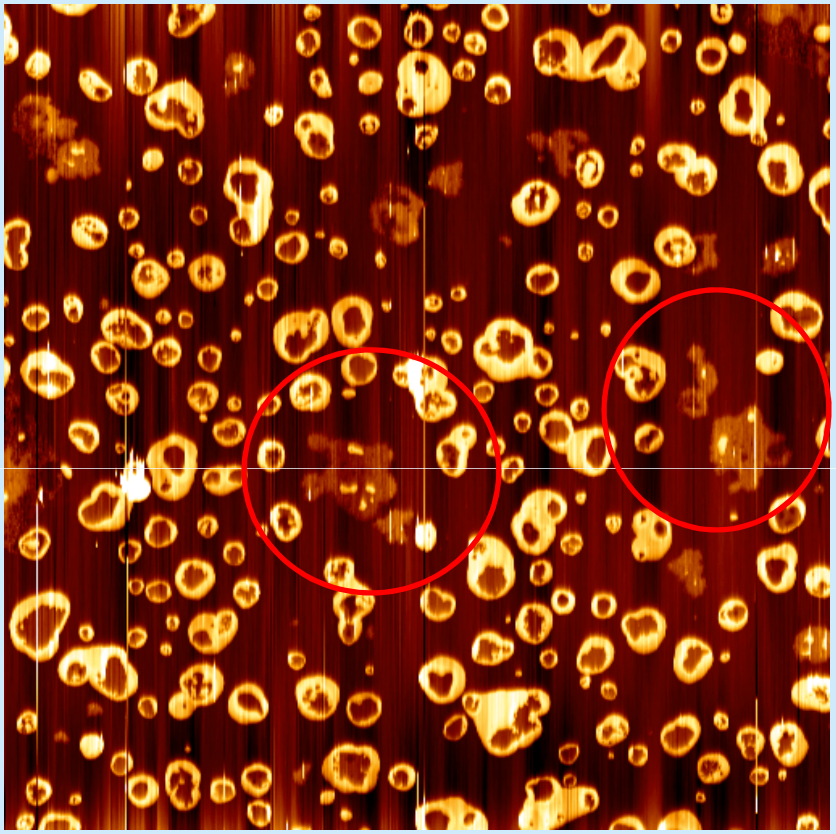
Relevant to SMase-mediated changes to raft domains in cellular membranes





- Dye-excluded regions initially formed adjacent to liquid-ordered domains and grew to large patches that included a number of initial domains.
- AFM showed that ceramide generation led to heterogeneous domains with many small higher regions of a ceramide-enriched phase.
- Ceramide generation leads to cholesterol expulsion from the domains, generating cholesterol enriched regions in the surrounding DOPC phase that excludes dye and has decreased fluidity.

16 Direct visualization for cholesterol expulsion from liquid-ordered domains in response to ceramide production.

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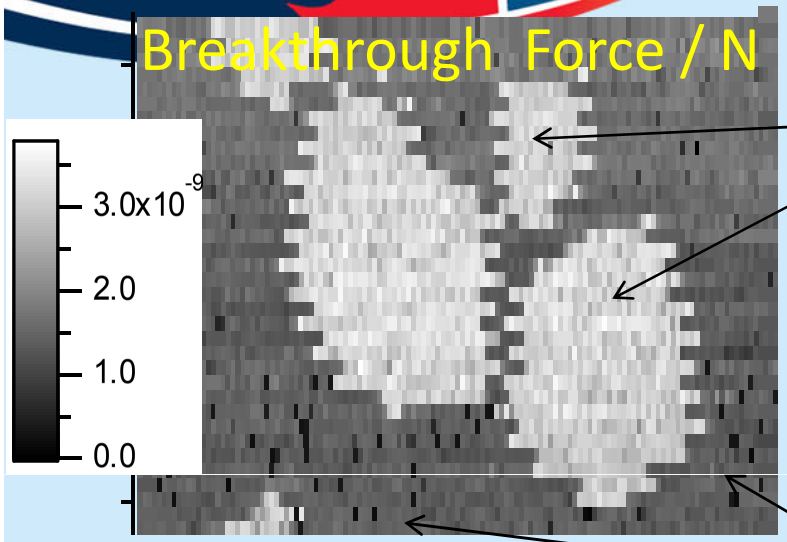
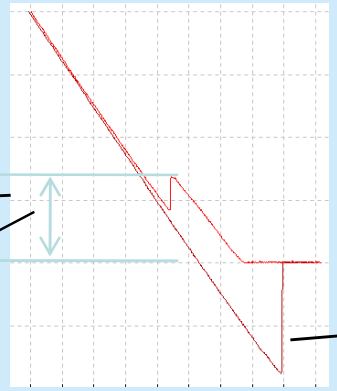
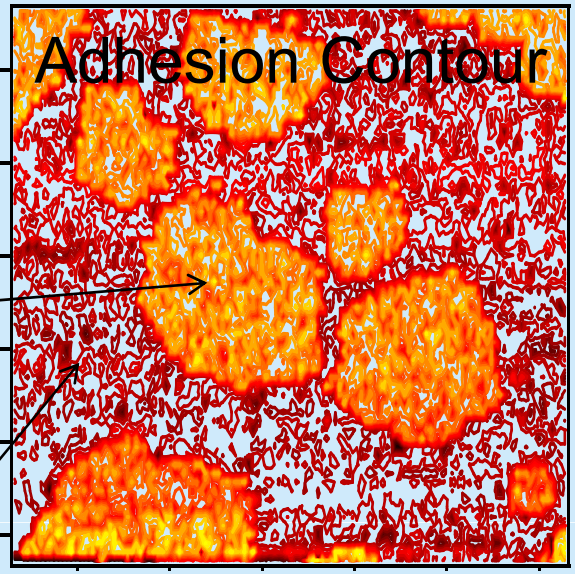
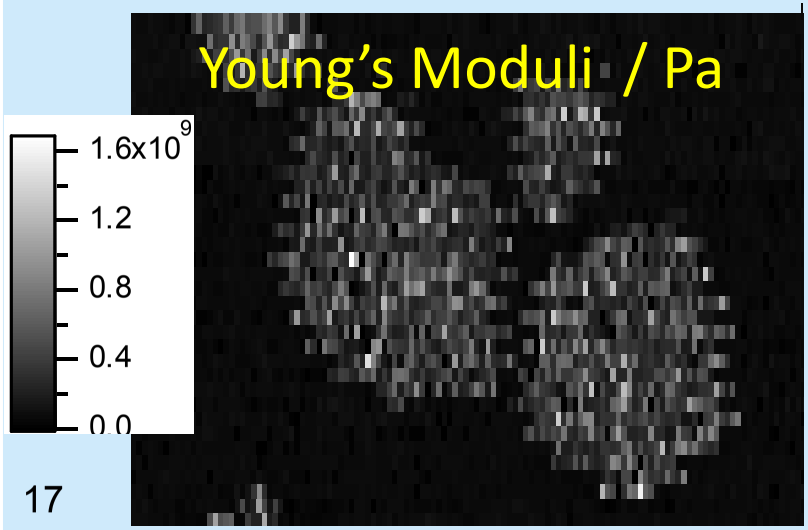
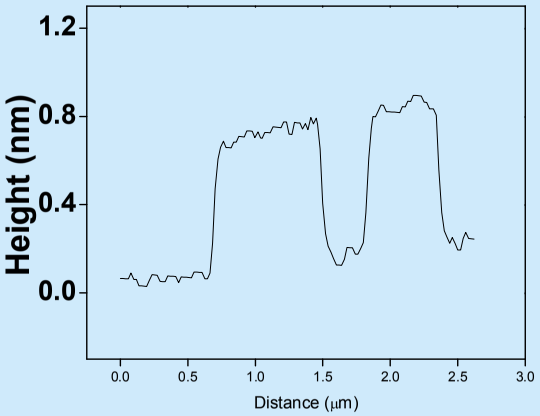
Force mapping

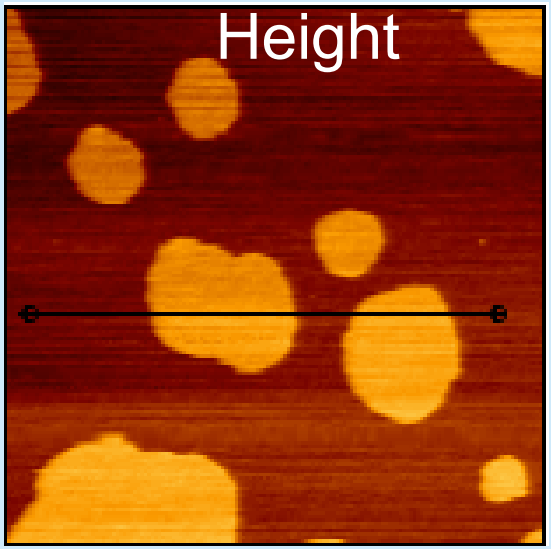












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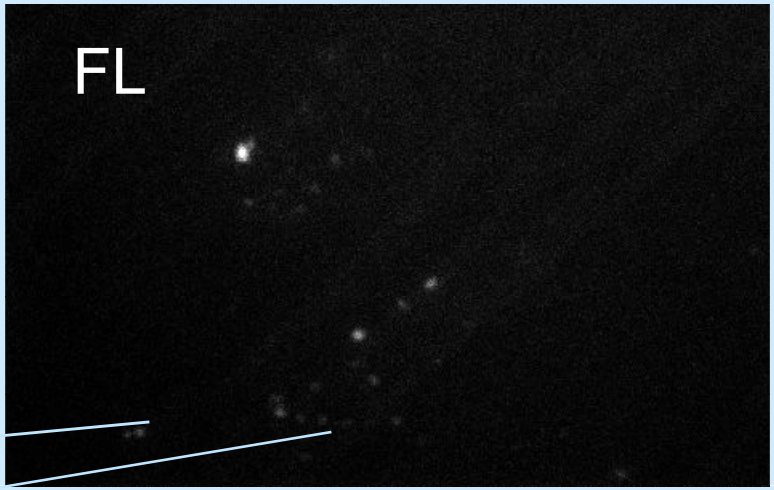
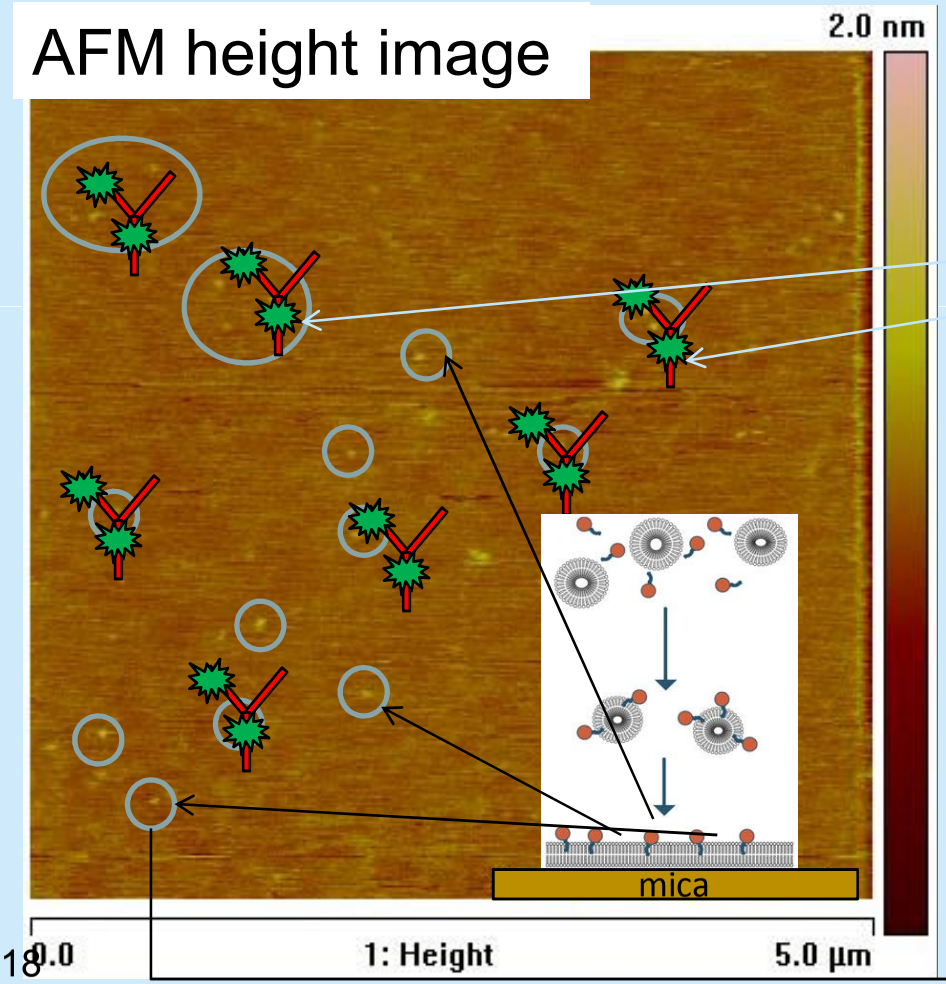
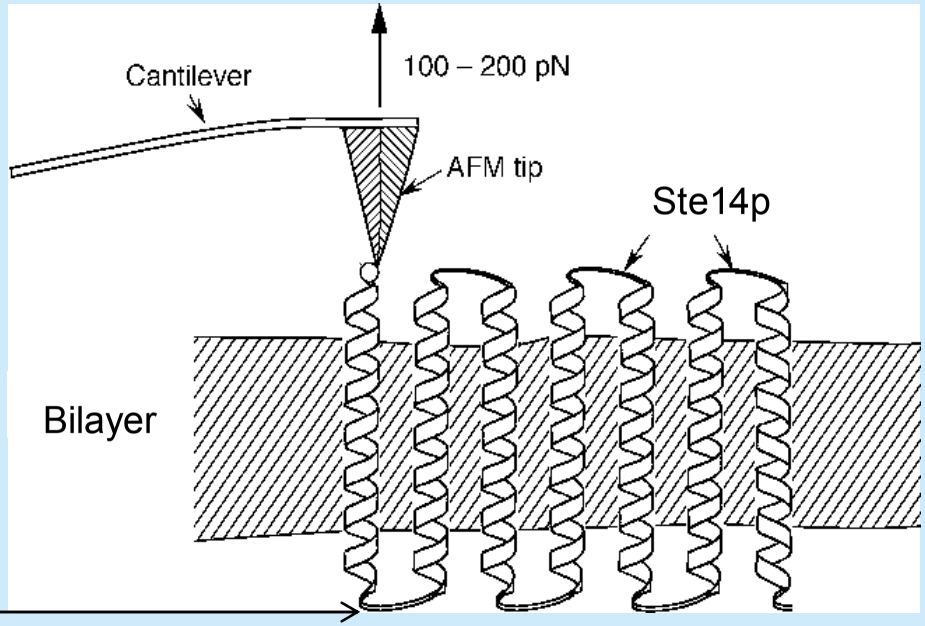
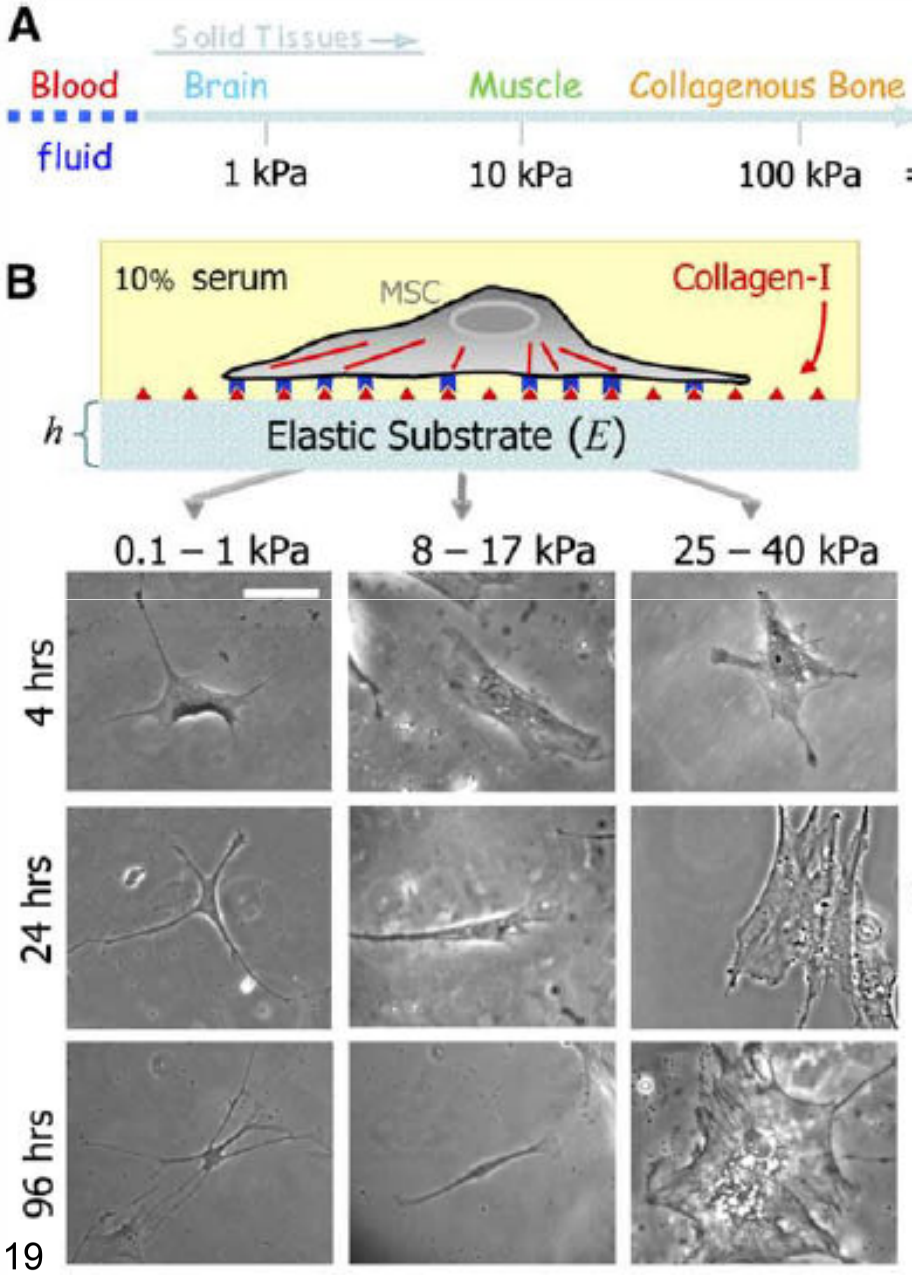
Membrane Protein Interaction by Multimodal Techniques







Matrix Elasticity Directs Stem Cell Lineage



Microenvironments appear important in stem cell lineage specification.

Naive mesenchymal stem cells (MSCs) are shown here to specify lineage andcommit to phenotypes with extreme sensitivity to tissue level elasticity.

AFM was used for Matrix Elasticity and Cell Mechanics Measurements.

The results have significant implications for understanding physical effects of the in vivo microenvironment and also for therapeutic uses of stem cells.

DE Discher Cell (2006)126, 677.

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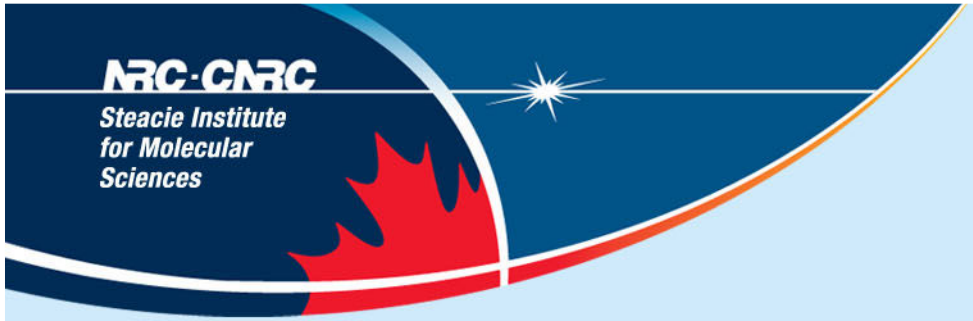
AFM-based SMFS is Capable of:

- nanometer scale features of biological samples in physiological-like environment.
- dynamical events (protein folding-unfolding) at nanometer scale resolution.
- nano-mechanical properties of living cells.

Thank you for your attention!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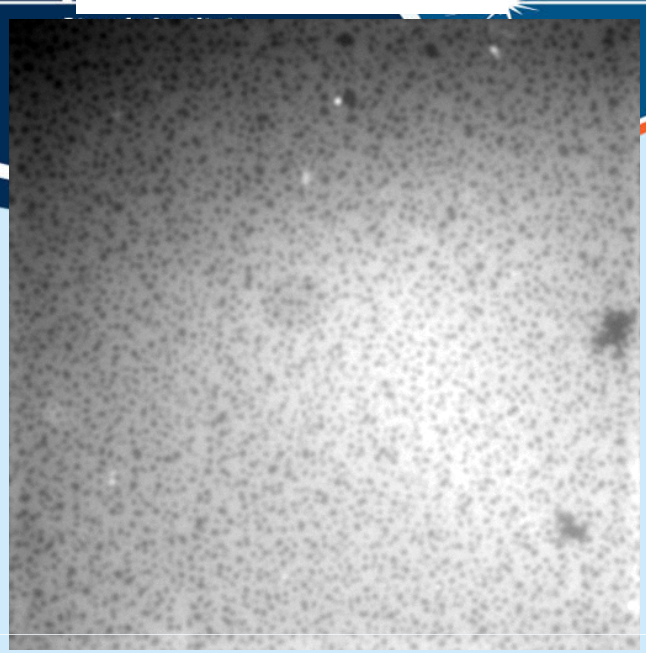
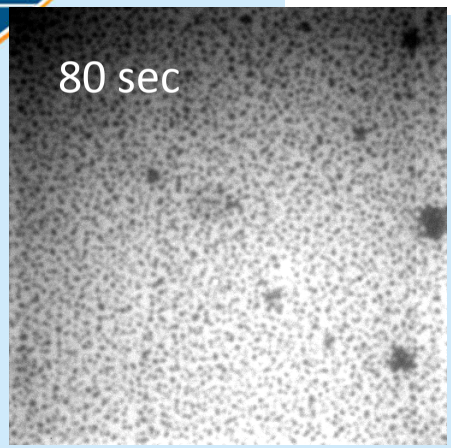
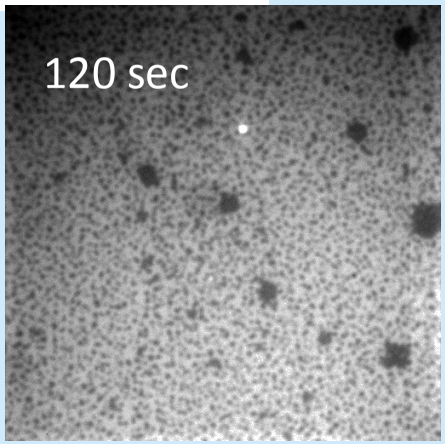
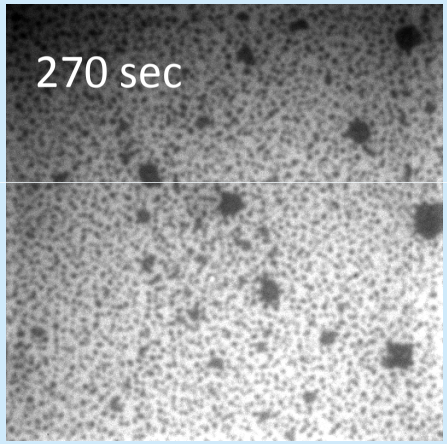
Before SMase

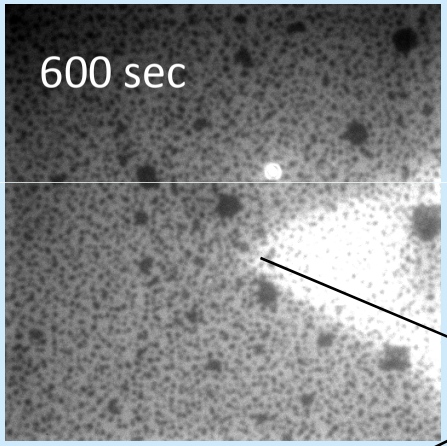


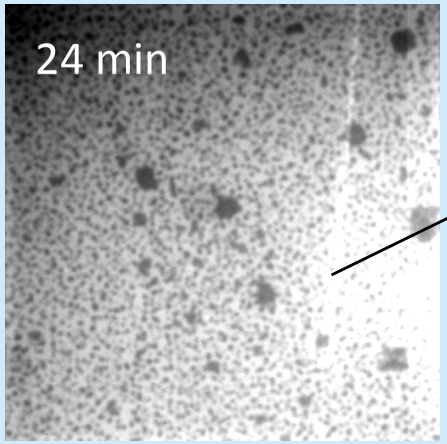
After SMase

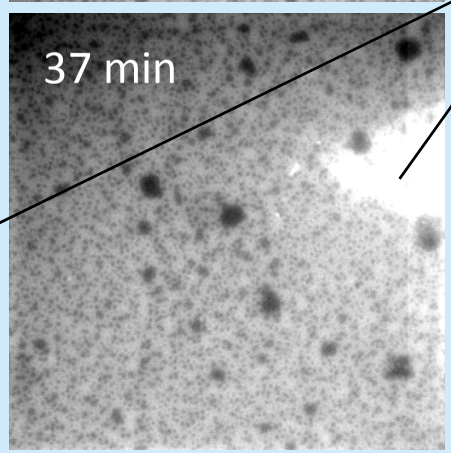






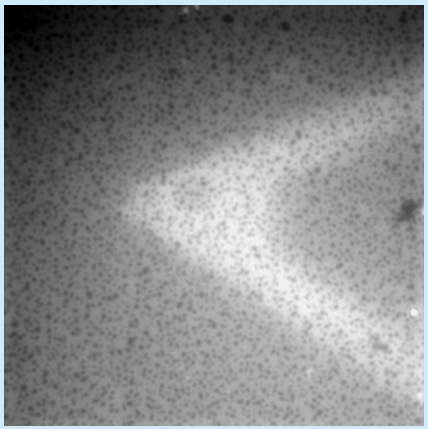


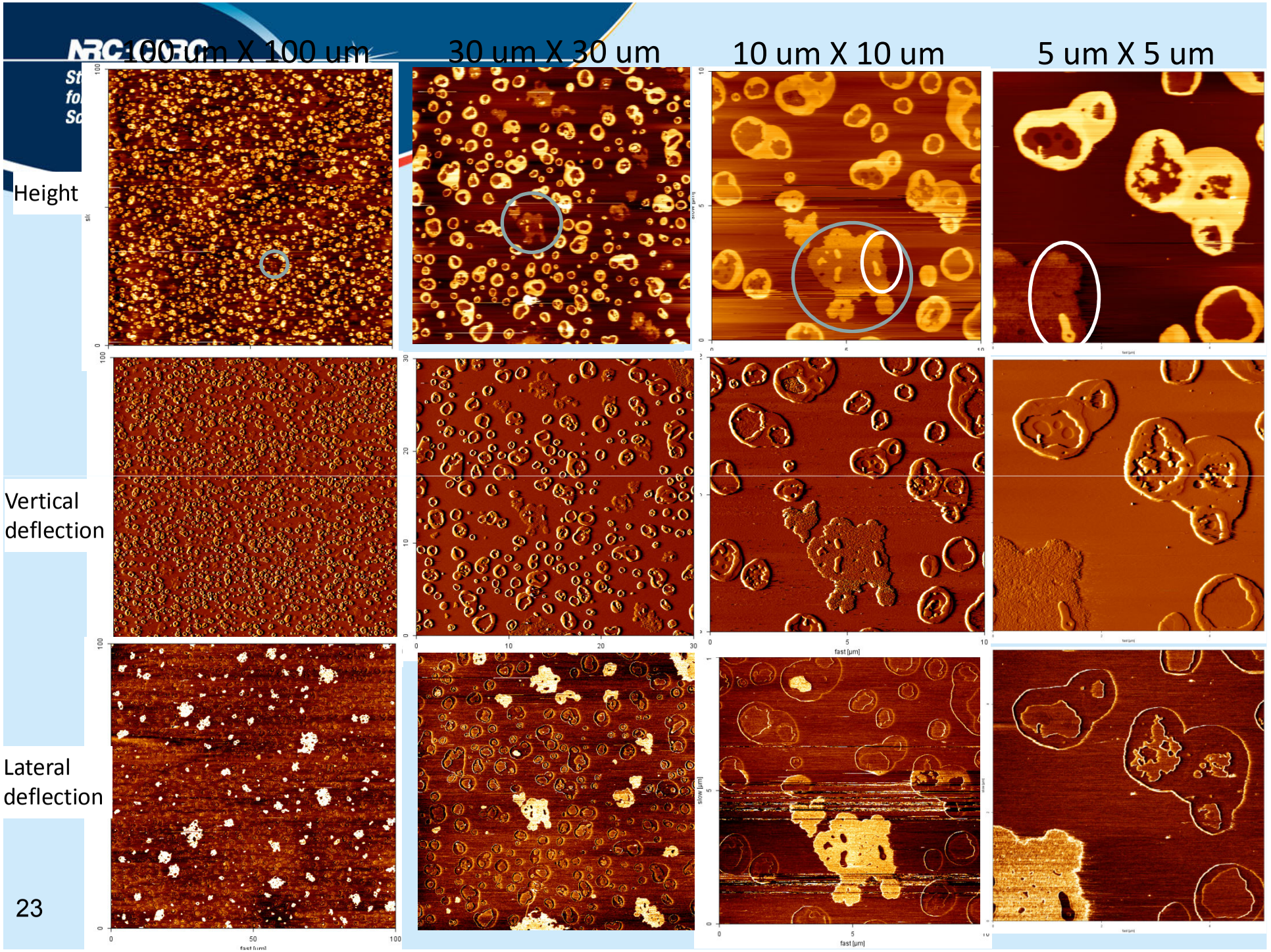




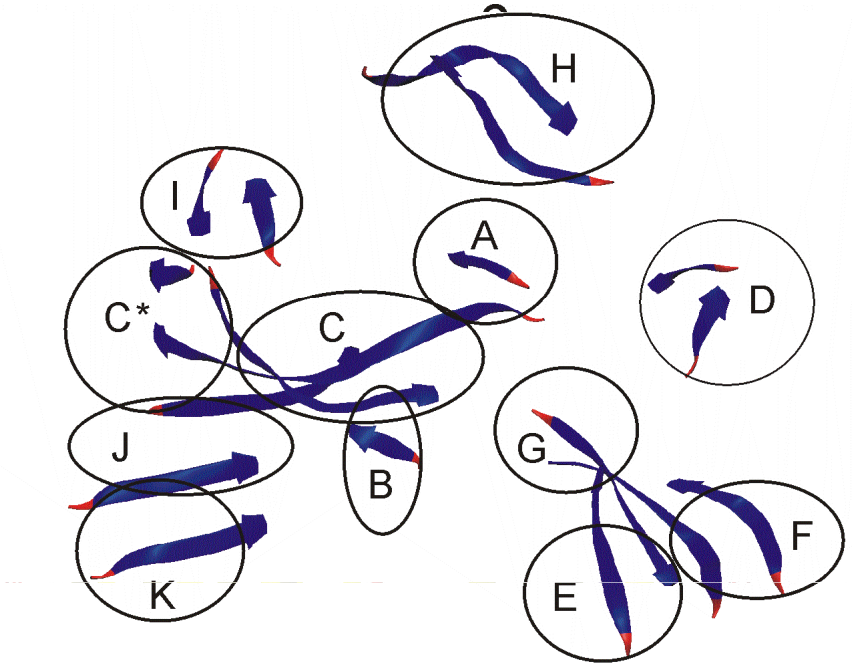
AFM Tip

Dye-excluded regions initially formed adjacent to liquid-ordered domains and grew to large patches that included a number of initial domains.




100 um X 100 um
30 um X 30 um
10 um X 10 um
5 um X 5 um
Height
Vertical deflection
Lateral deflection
fast [µm]
slow [µm]

Sequential Unfolding of MBP



Well match ➔ β sheets unfold as groups of secondary structure.

Note that this does not imply that the β-strand hydrogen bond cleavages necessarily initiate at the ends of β-sheet regions, since the cantilever probes with a minimum time constant of 100 microseconds – all chain dynamics within that time period are integrated into a single response. Transitions associated with groups of alpha helical structure are not observed, which indicates that beta sheet structures of MBP are more resistant to mechanical unfolding those alpha helical structures.

Extension (nm)	25	38		43		54	66		81	90			106	109	
Assigned positions	301	265		251		222	187		144	119			78	64	
β-strand ends	301	264	254	249	245	223	183	170	145	116	110	106	99	77	62	38	10
Division	0aa	1aa		2aa		-1aa	4aa		-1aa	3aa			1aa	2aa	

The probabilities of observing transitions at different strand ends are not equally distributed.

Maltose Binding Protein Unfolding

- Multiple unfolding force transitions observed in the unfolding experiments of single MBP molecules
- C terminus attachments destabilize secondary structure unit nearest to it, thus the unfolding starts from C terminus.

Funding from NSERC, Cystic Fibrosis Foundation, CRC

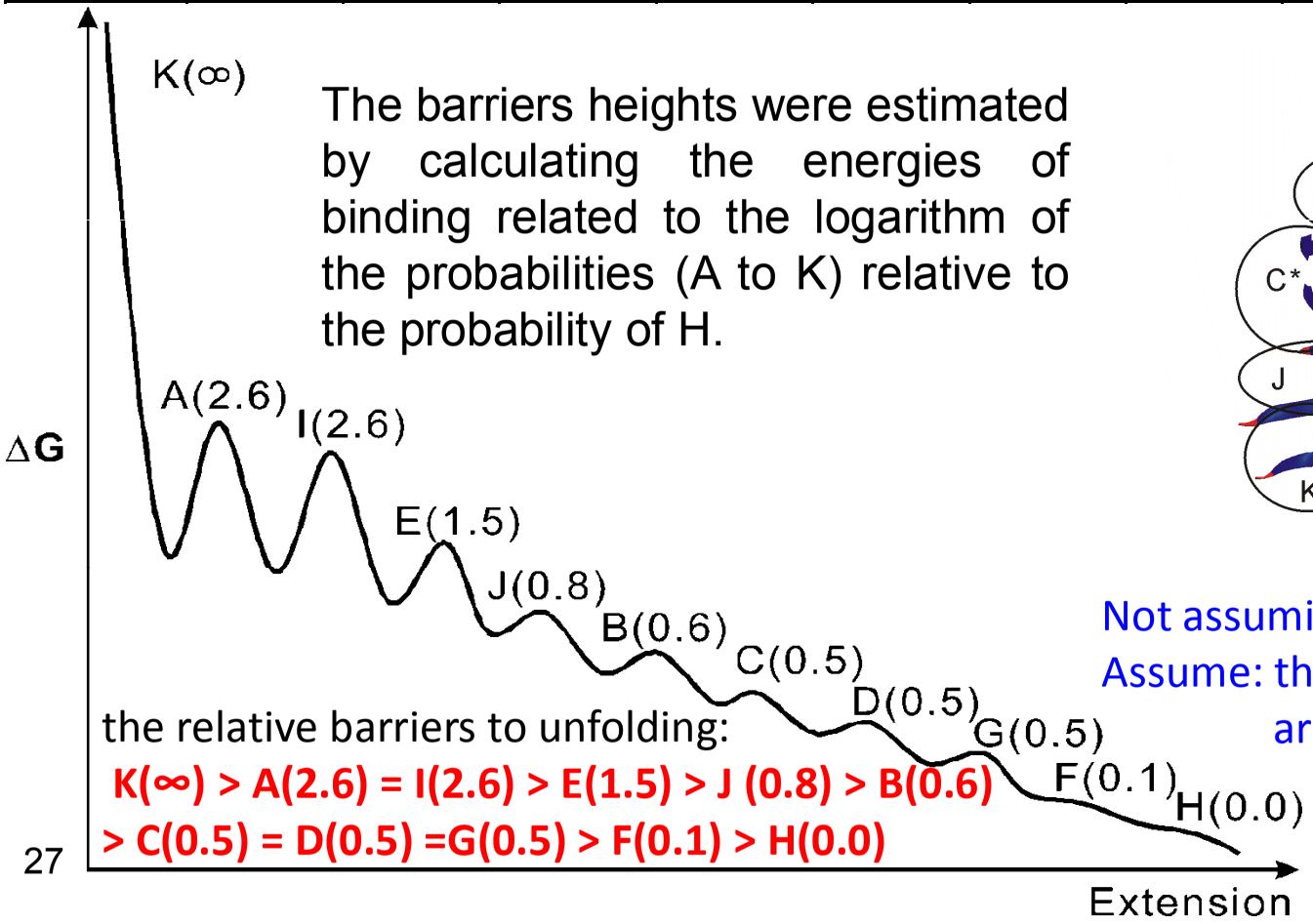
Probabilities of Force Transitions of MBP

Beta sheet / Curve name	A 328→257	B 301→110	C 264→106 266→77* 264→62*	D 254→249	E 245→116	F 223→145	G 223→116	H 183→170	I 102→99	J 62→10	K 38→9
C235		301	265	251	121	145	219	187		63	
1034	(339)					144		185			
78		310						187		65	
624		301				148	228	181		63	
67			78	251	119-116	144		188			
307				253			220	163		63	
618			78	247		145	220		102	60	
50			263-271			144		186			
417			259-269			145	222	174			
M636			266	249		144	222	185			
M1034	(338)	300		251	118	146		188			
M871				249(b)		145		193			(43)
M234	(345)	306	264			144	222	191		65	
M314	325	302	260			144	224				
M998	(340)	301		251				185			
probability	0.067	0.467	0.533	0.533	0.200	0.800	0.533	0.867	0.067	0.400	0

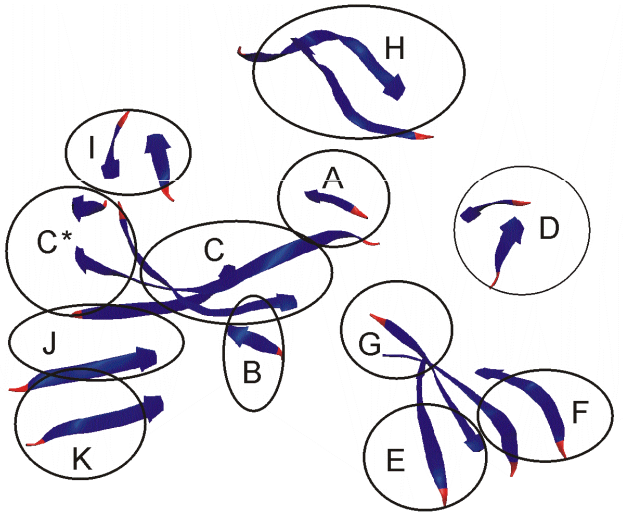
The barriers heights can be estimated by calculating the energies of binding related to the logarithm of the probabilities

Suggested Multiple Barrier-crossing Unfolding Process w/o Applied Forces

Beta sheet	A 328→257	B 301→110	C 264→106 266→77* 264→62*	D 254→249	E 245→116	F 223→145	G 223→116	H 183→170	I 102→99	J 62→10	K 38→9
probability	0.067	0.467	0.533	0.533	0.200	0.800	0.533	0.867	0.067	0.400	0



The barriers heights were estimated by calculating the energies of binding related to the logarithm of the probabilities (A to K) relative to the probability of H.

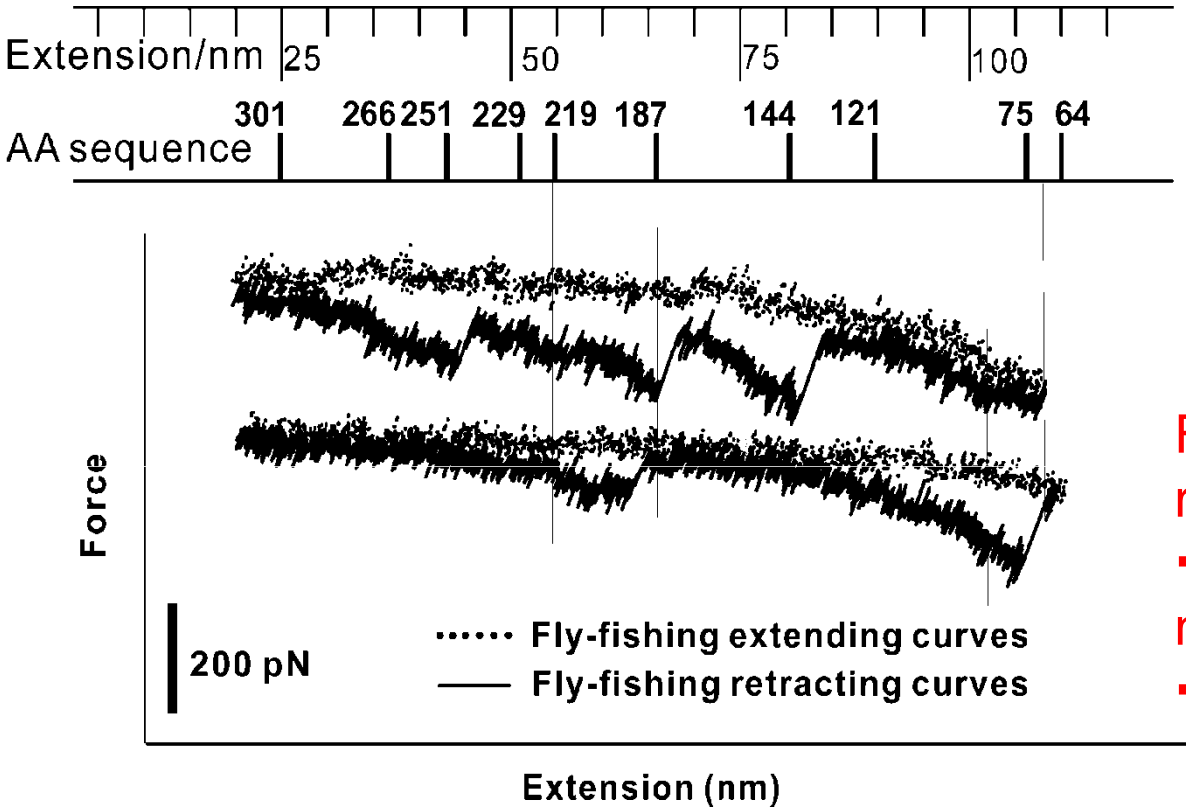


the relative barriers to unfolding:

K(∞) > A(2.6) = I(2.6) > E(1.5) > J (0.8) > B(0.6) > C(0.5) = D(0.5) =G(0.5) > F(0.1) > H(0.0)

Not assuming: the protein is misfolded;
Assume: the barrier heights to unfolding are close to k_bT in energy.

Unfolding and Refolding of MBP – “flying fishing”

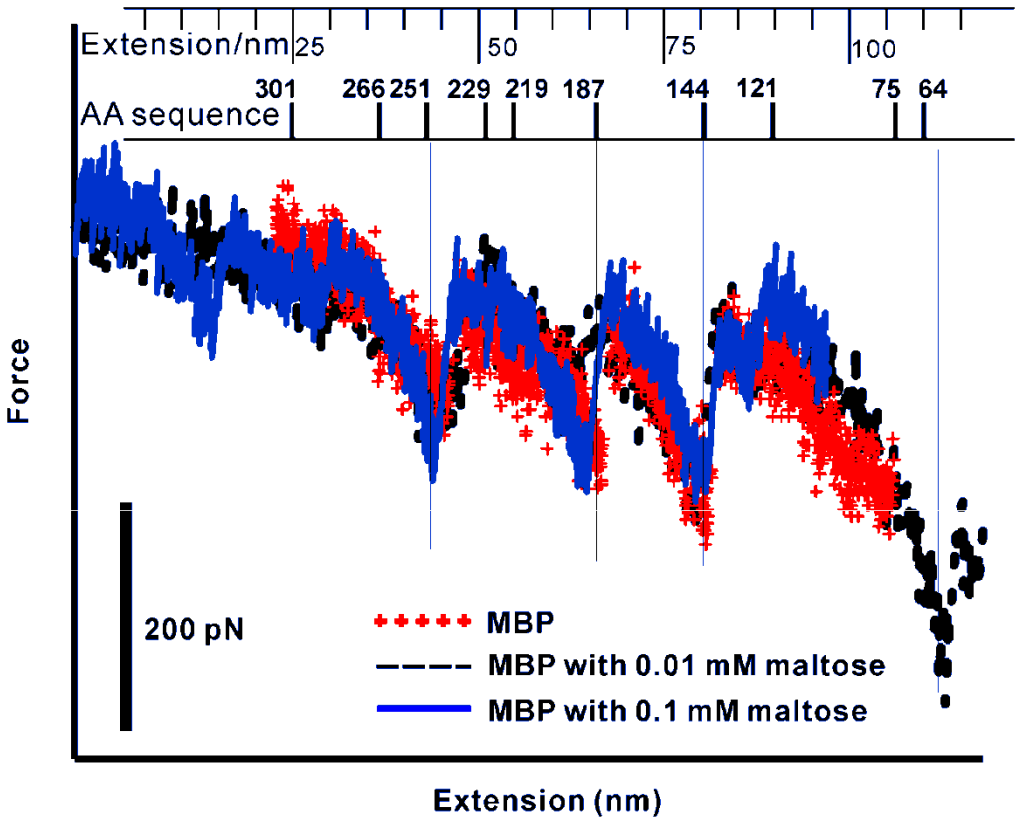


By reducing the pulling force of the cantilever, so-called fly-fishing mode, the protein molecule was allowed to relax and refold.

Fewer force transitions in refolding traces
➔ slow refolding rate (fast mis-fold)
➔ larger refolding barrier

The unfolded protein was relaxed to a small extension (dotted lines), resulting in elastic behavior that is typical of a simple, entropic spring-like, polymer chain. The protein molecule was allowed to relax 2 seconds, during which time it partially refolded back to its folded state. When stretched again, the refolded domains unfold again to produce the characteristic f-e traces, shown as retracting curves (solid lines). Some, but not all, of the transitions observed in pulling the fully relaxed proteins were monitored again (bottom solid lines).

Sequential Unfolding of MBP with Maltose



In the presence of maltose, the probability for the AFM tips to capture MBP at the His-tag end decreased by at least 50% ➔ indicates that upon binding the ligand, MBP undergoes a conformational change which reduces accessibility of the C-terminal region.

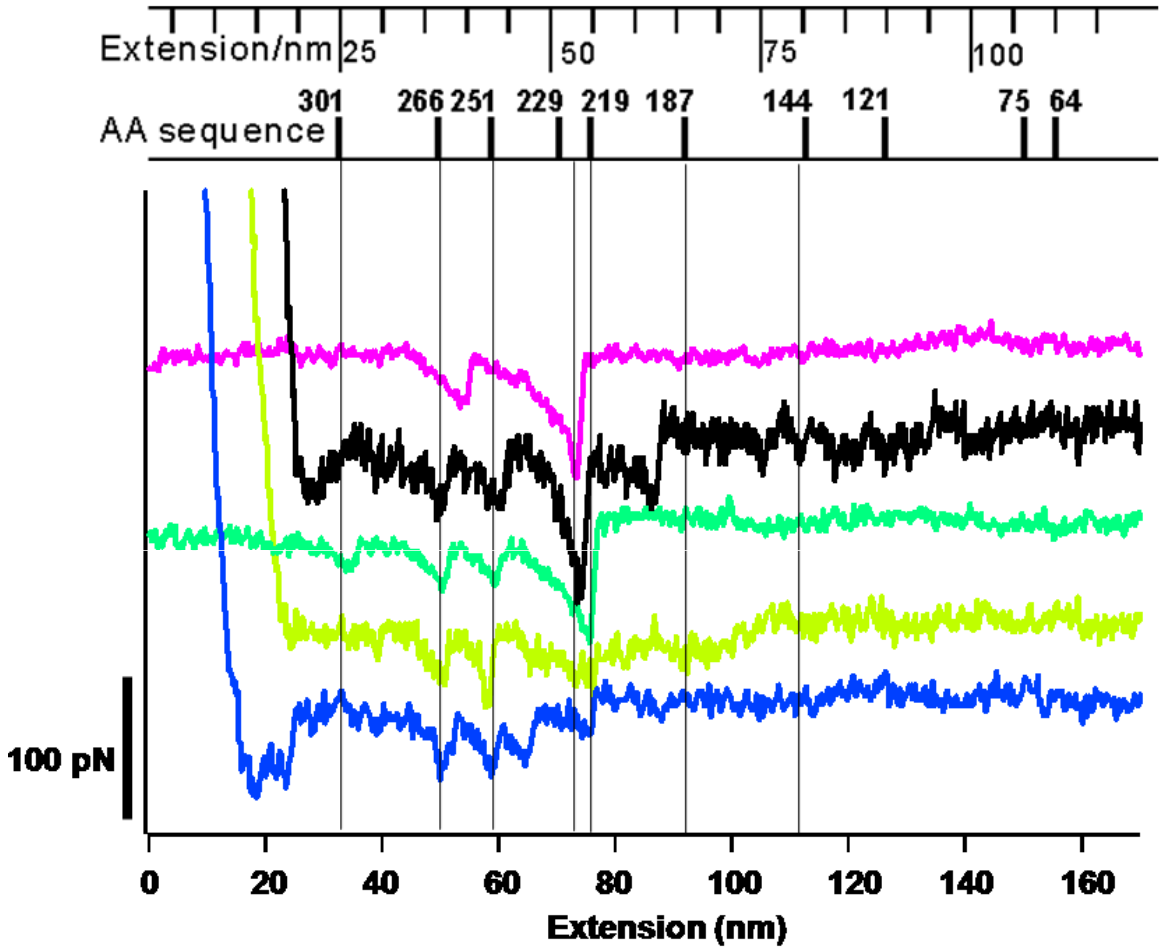
With and without ligands, MOST of the unfolding force transitions of MBP match with each other, indicating that the main unfolding barrier positions are not influenced by the ligand binding.

Noticed that our results CONTRAST those with recently reported optical tweezers data, where two-state behavior was observed.

?? a protein conformation bound to mica surface that disrupted protein folding or caused partial denaturation.

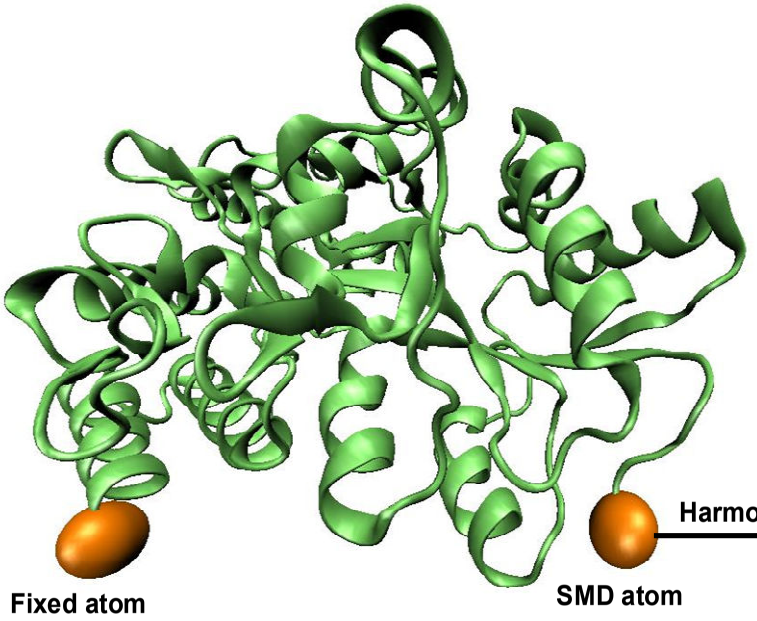
➔ Change sample surface!

Sequential Unfolding of MBP – on Gold Substrates

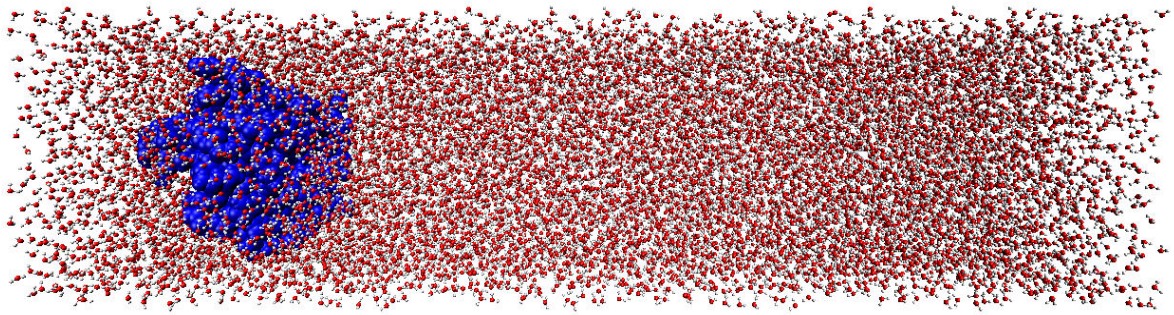


The **SAME** characteristic force transitions -- for negatively charged mica and amylose-gold substrates.

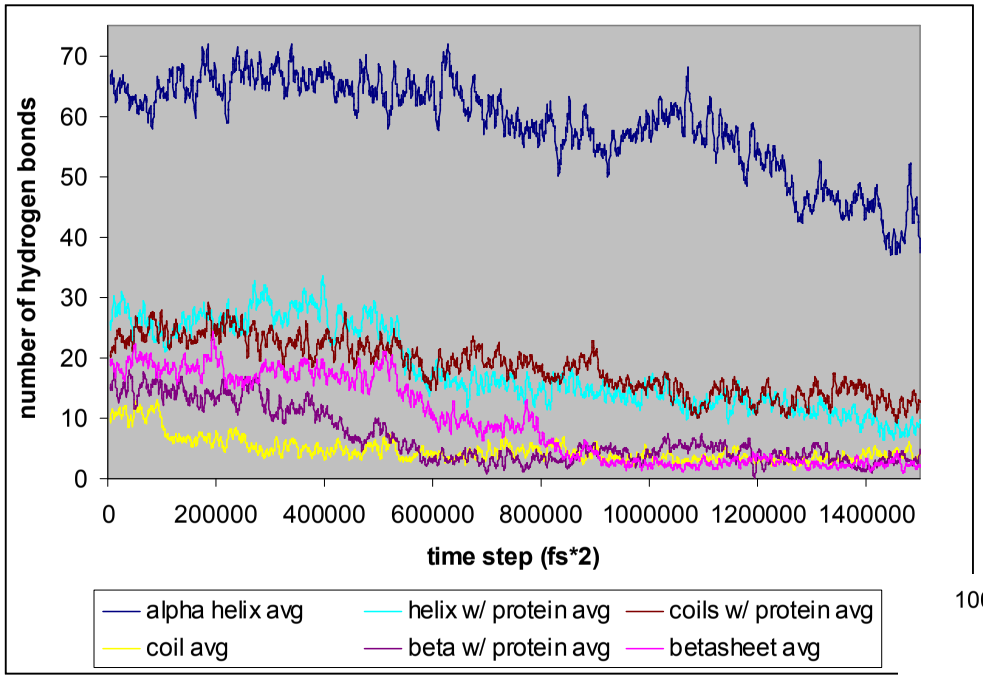
So that if surface binding caused significant unfolding, then an effect should have been observed. It seems likely that surface adsorption slows down unfolding (by reducing the reaction coordinate diffusion coefficient), allowing intermediate steps to be identified.



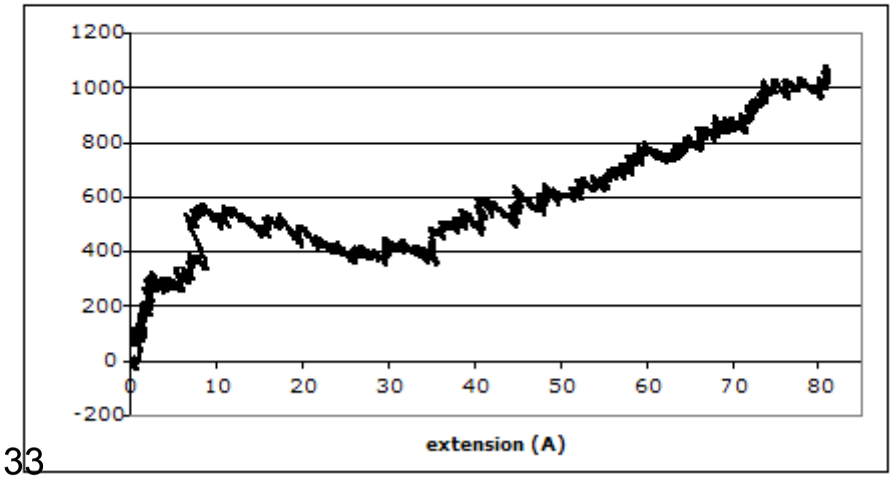
The setup of an SMD simulation. The SMD reference file determines which atoms are fixed in space and which ones are fixed to the moving restraint point (dummy atom) via a harmonic potential.

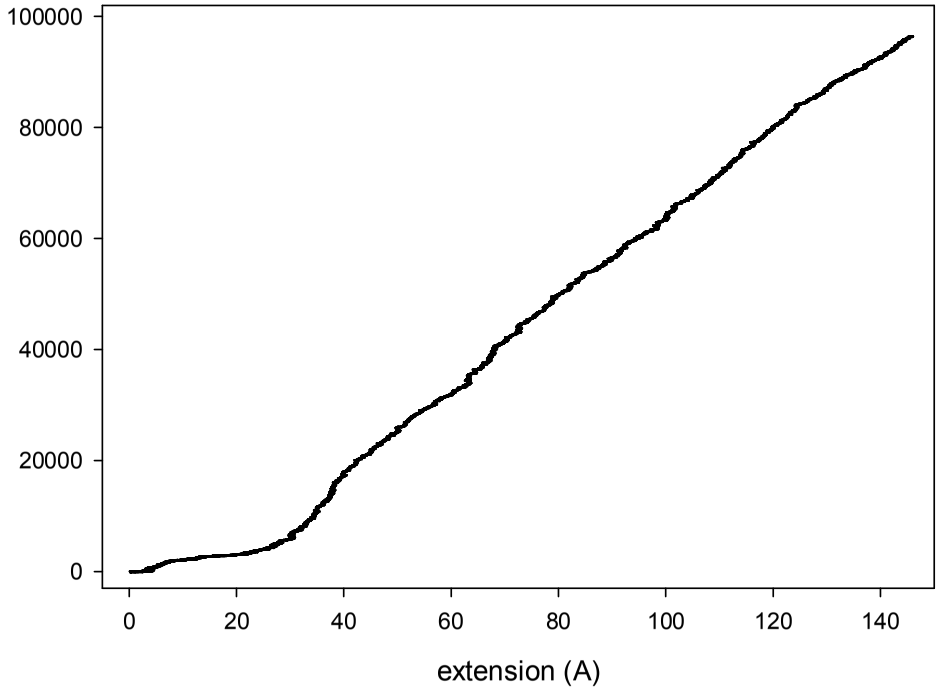


Setup for SMD simulations. Water box in red, MBP-29 in blue. Total system size: 190 x 50 x 50 Å^3 containing 39513 atoms. The pulling direction in this picture is towards the right.



force curve: pulling speed is 0.00015 A/fs with a k of 695 pN/nm. This is about 0.65 ns. The peak around 10 A, corresponds to the separation of the pulled helix from the next one.





Calculation of the total work from the force extension curve.

The Young modulus determination from Force-Distance curves

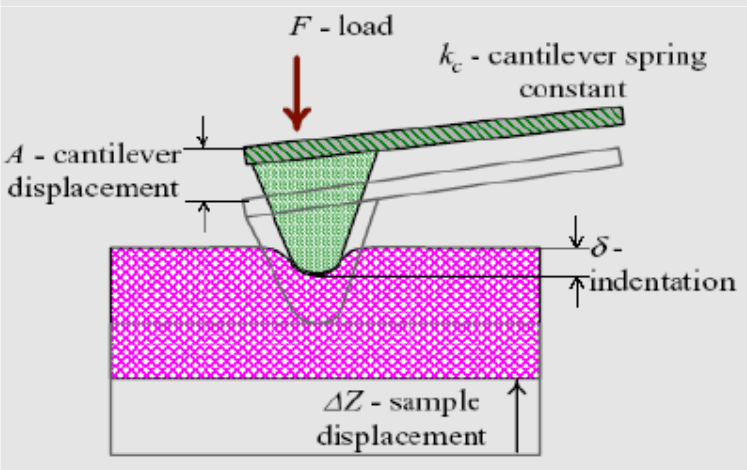
$F = kd$ (Hooke's law for the cantilever)

$F \sim E\,\delta^{\alpha}$ (Hertz law for tip-surface contact)

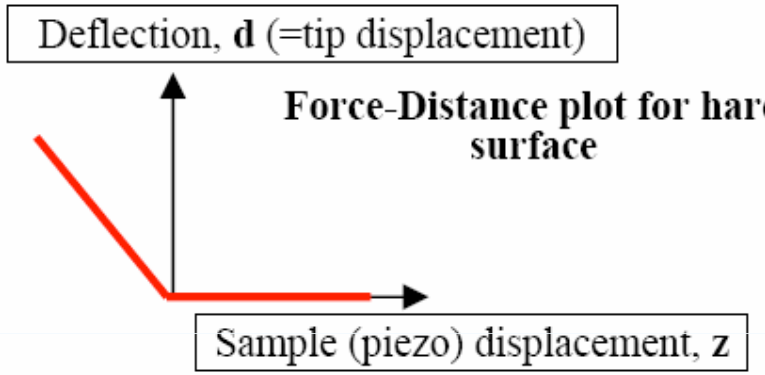
$Z = d + \delta$

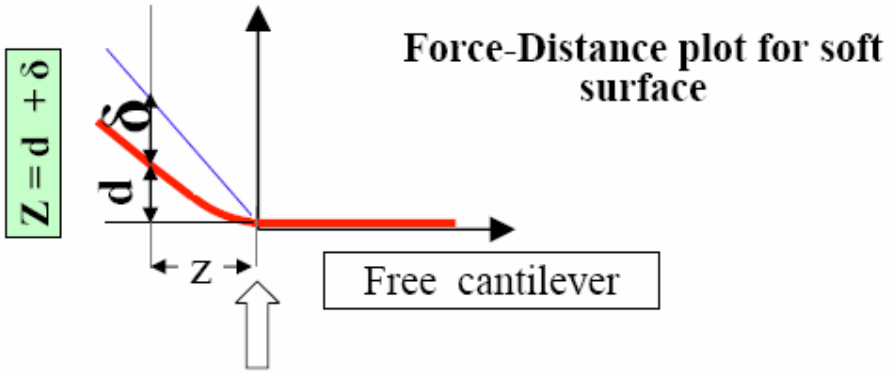
$\alpha = 2$ for cone/plane contact

$\alpha = 3/2$ for sphere/plane contact



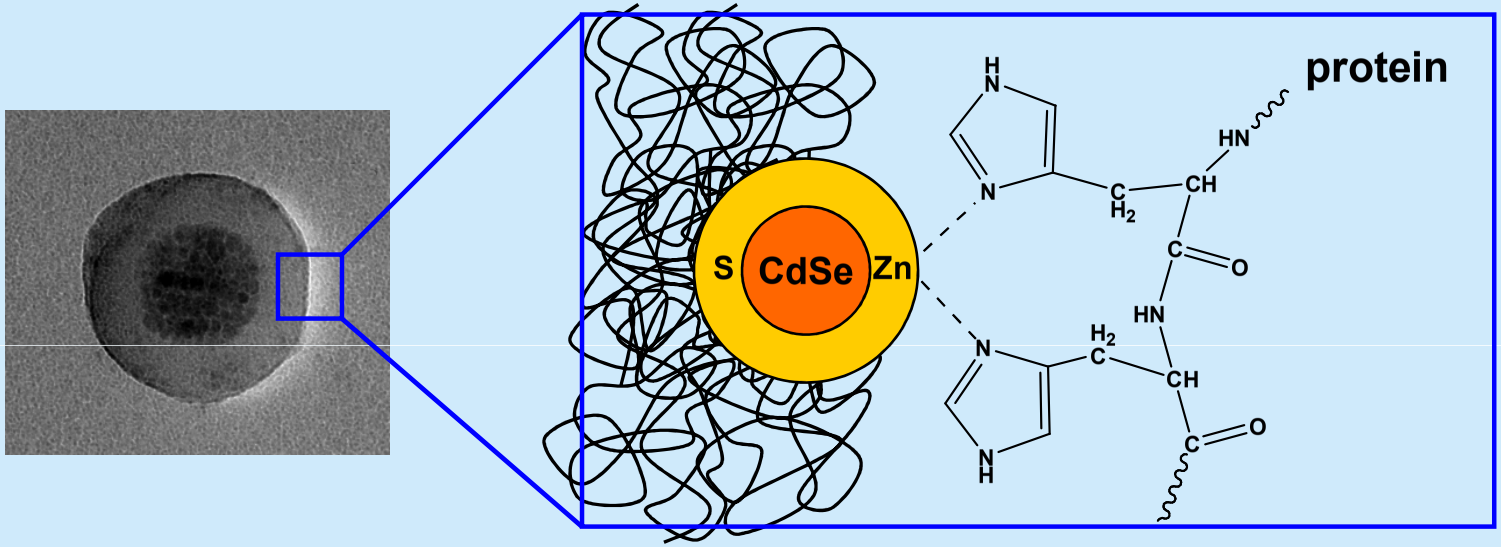
Common problem of such measurements is large error in z-position of the point of the tip-surface contact (dF/dz=0)





Piezo displacement (z) = tip displacement (d) + surface deformation (δ)

These are my probes
-total diameter ~100nm
-QDs conjugated to proteins are deposited on the surface



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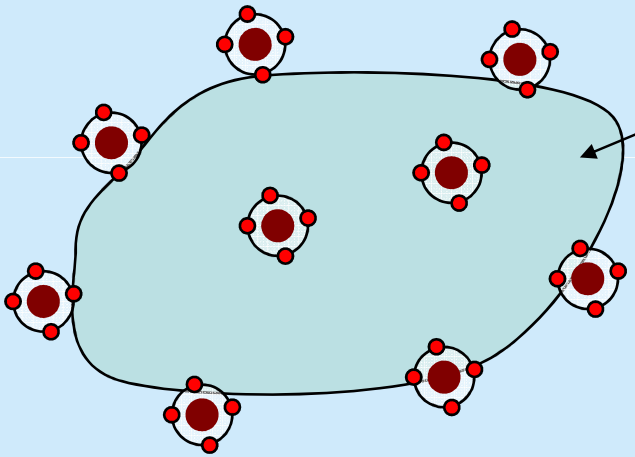
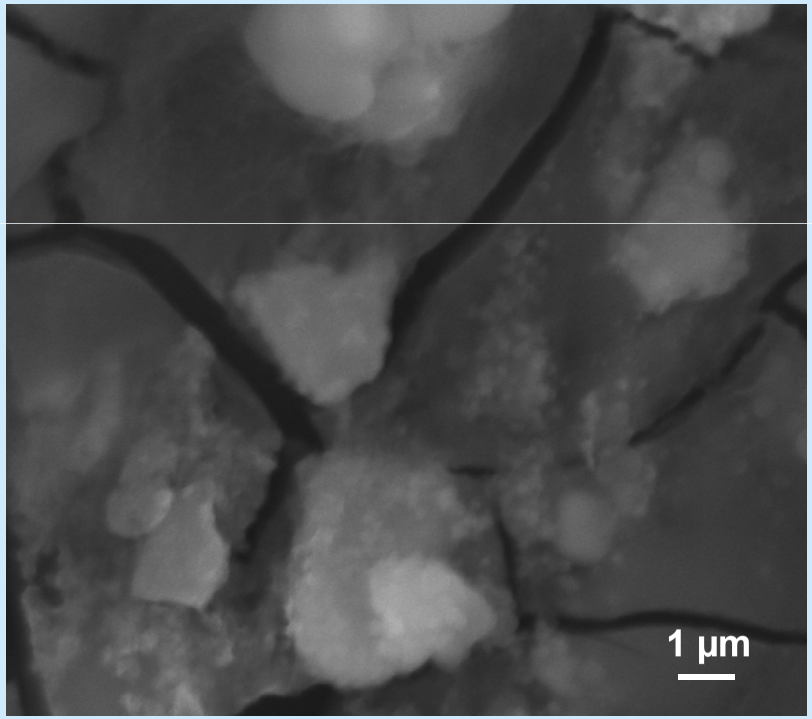
This is a schematic of the bacteria and nanoprobe conjugate

- Nanoprobe (polymer colloid) ~100nm
- QD+protein ~5-10nm

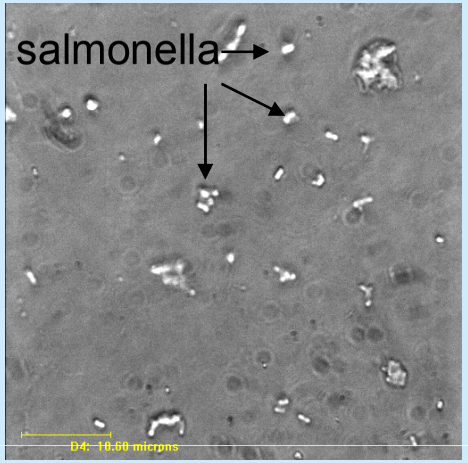


Here are SEM images and optical/fluorescence images

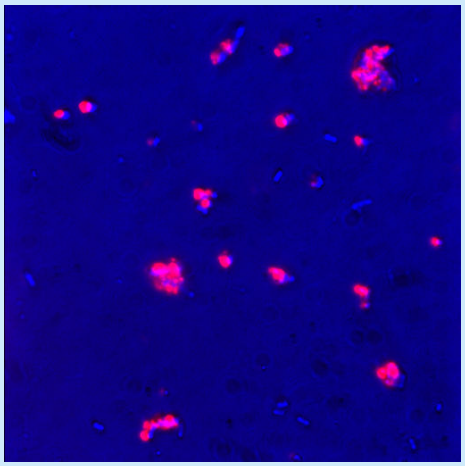
Salmonella-nanoprobe complex



Optical Microscopy



Transmission



Fluorescence-transmission composite

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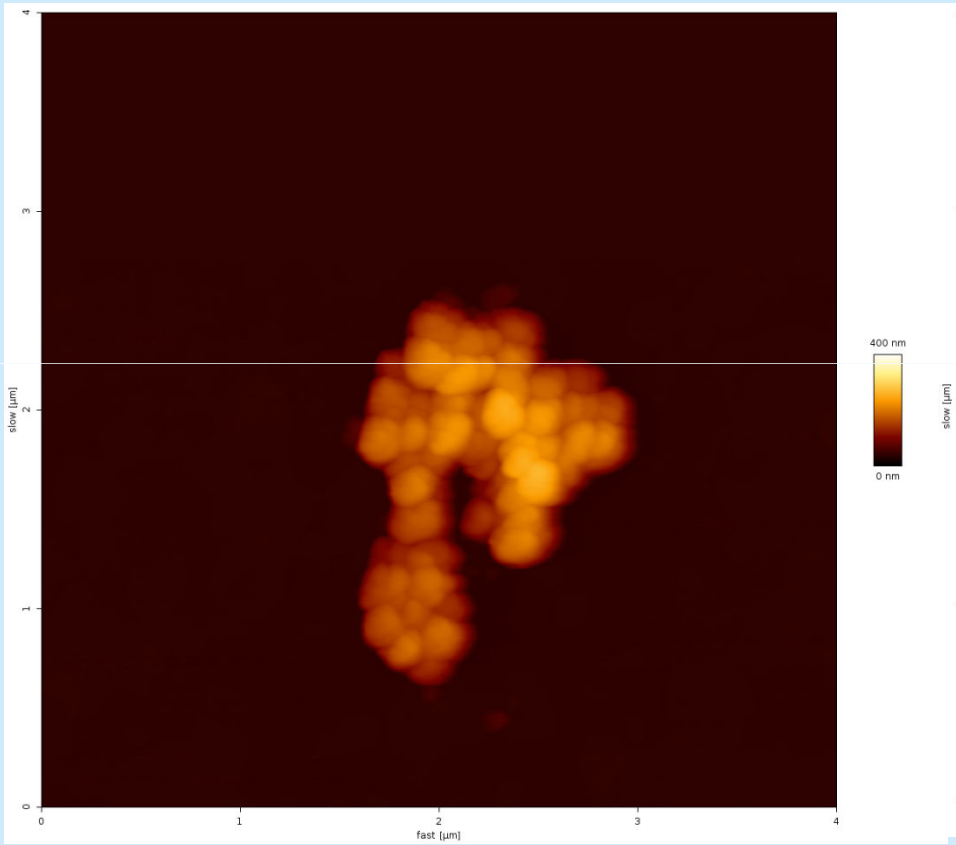

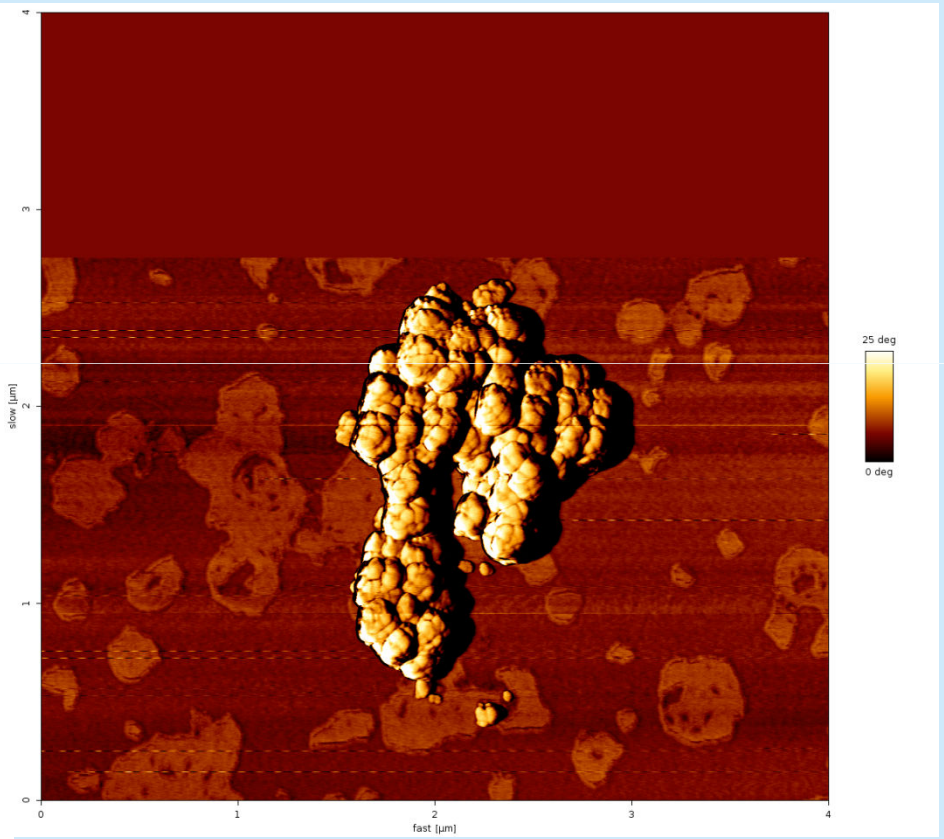

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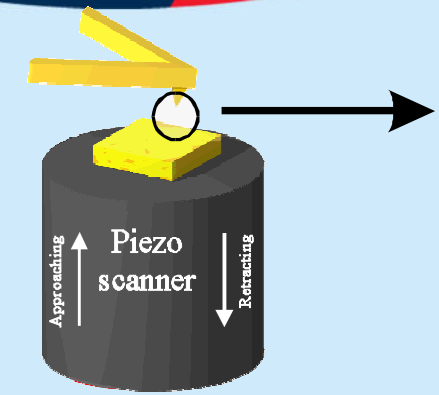
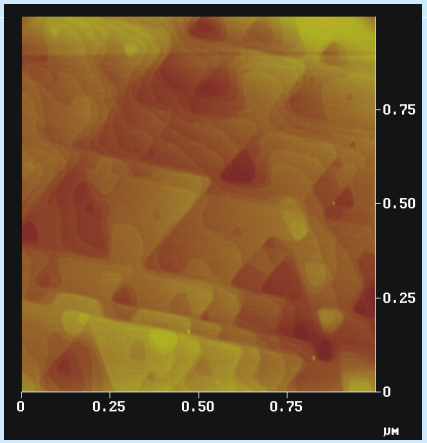
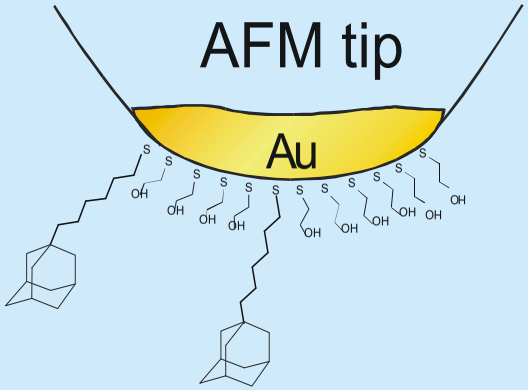
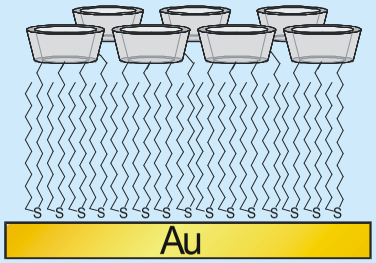
Stretching and Rupturing Individual Supramolecular Dimers and Polymers

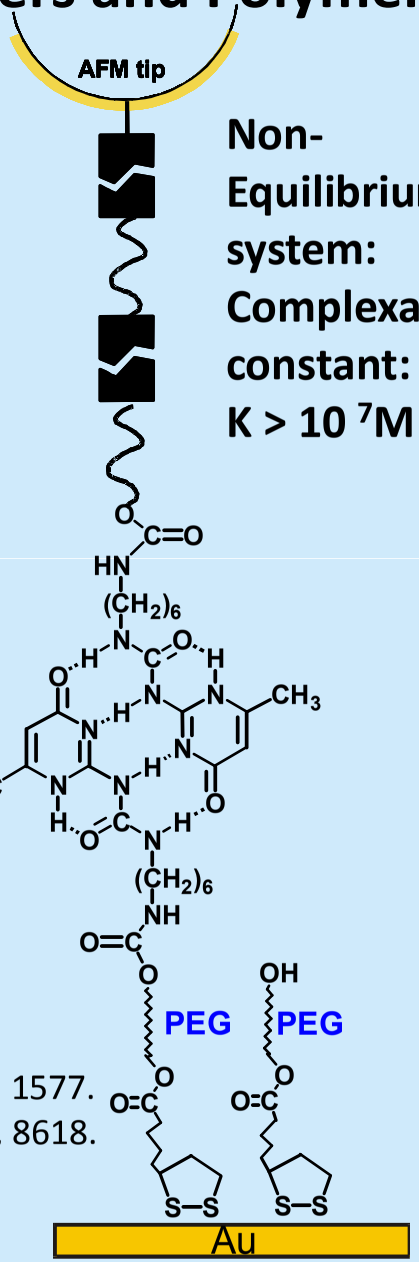








Equilibrium system: complexation constant: $K < 10^{4}\ M^{-1}$



Auletta T; de Jong MR; Mulder A; van Veggel, FCJM; Huskens J; Reinhoudt DN; **Zou S**; Zapotoczny S; Schönherr H; Vancso GJ; Kuipers L ***J. Am. Chem. Soc.,*** 2004, 126, 1577.

Zou, S.; Zhang, Z.; Förch, R.; Knoll, W.; Schönherr, H.; Vancso, G. J. ***Langmuir,*** 2003, 19, 8618.

Zou, S.; Schönherr, H.; Vancso, G. J. ***Angew. Chem. Int. Ed.*** 2005, *44*, 956-959.

Zou, S.; Schönherr, H.; Vancso, G. J. ***J. Am. Chem. Soc.,*** 2005, *127*, 11230.

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Single Molecule Manipulation Techniques

Method	Force range (pN)	Dynamical range	Minimum displacement [nm]
Magnetic beads	0.01–100	> 1 s	10
Optical tweezers	0.1–150	> 10 ms	10
Microneedles	> 0.1	> 100 ms	1
BFP	0.5–1000	> 1 ms	10
AFM	> 1	> 1 μs	0.1

Typical application:

Actin stretching,

unzipping and twisting DNA

Membrane anchors, receptor–ligand pairs

Stretching of DNA, protein, polysaccharides, synthetic polymers

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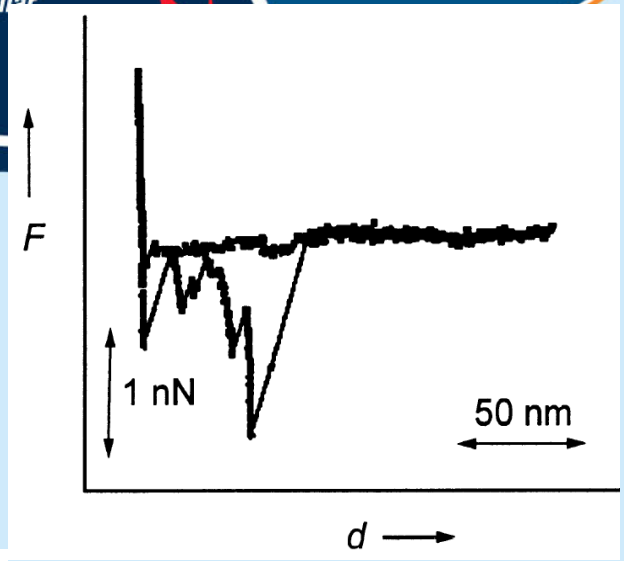
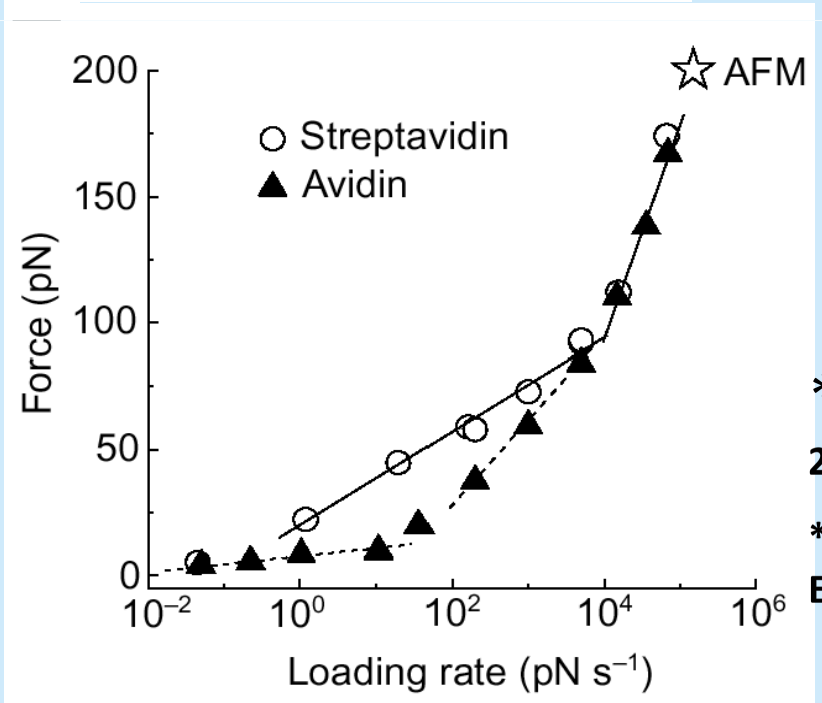
Loading Rate Dependent Single Molecule Force Spectroscopy



Force-induced rupture of double-stranded DNA.

* Lee, G. U.; Chrisey, L. A.; Colton, R. J. *Science* 1994, 266, 771.

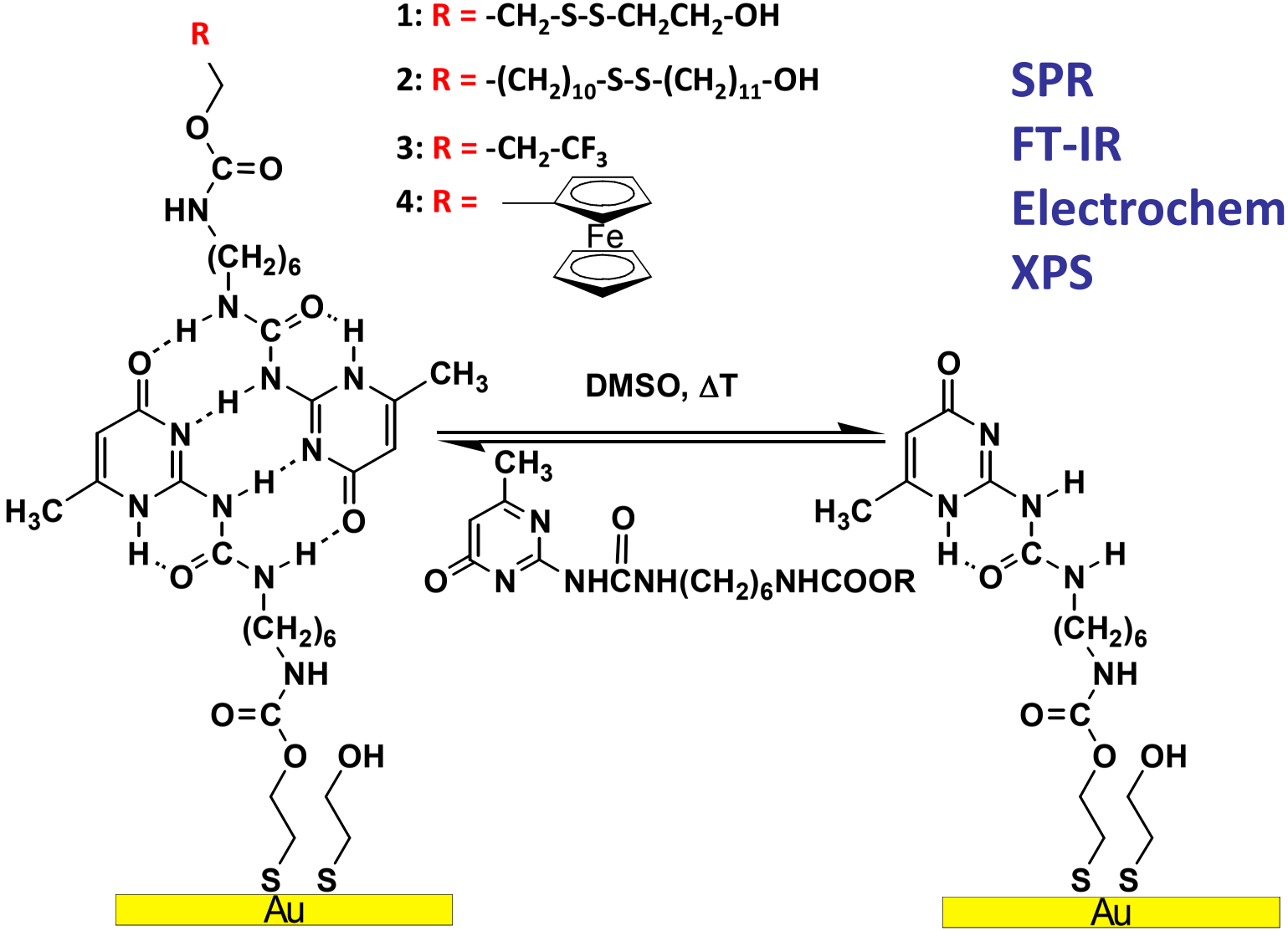


Biotin-avidin bond strengths.

* Florin, E. L.; Moy, V. T.; Gaub, H. E. *Science* 1994, 264, 415.

* Merkel, R.; Nassoy, P.; Leung, A.; Ritchie, K.; Evans, E. *Nature*, 1999, 397, 50-53.

Quadruple Hydrogen Bonding System at Surfaces



SPR

FT-IR

Electrochemistry

XPS

Zou S; Zhang Z; Förch R; Knoll W; Schönherr H; Vancso GJ *Langmuir,* 2003, 19, 8618.

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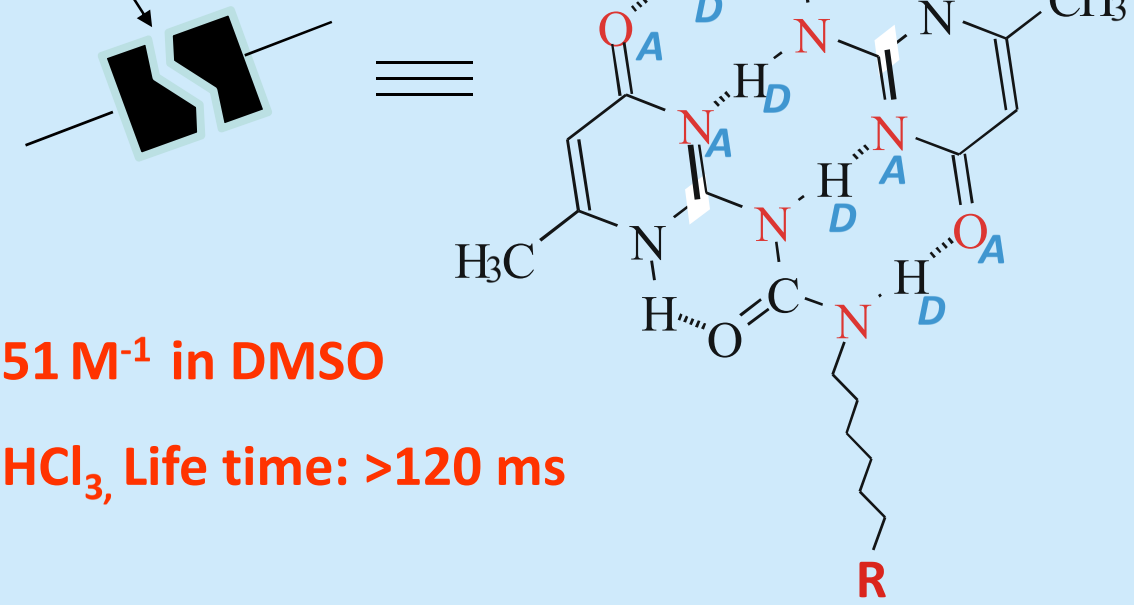
Supramolecular Polymers

Heat or Solvent (DMSO)

Cool or Solvent (Toluene, Hexadecane)

Supramolecular Polymers

Building Blocks



RT: K < 51 M^{-1} in DMSO

K > 10^7 M^{-1} in $CHCl_3$, Life time: >120 ms

Sijbesma RP; Beijer FH; Brunsveld L; Folmer BJB; Hirschberg JHKK; Lange RFM; Lowe JKL; Meijer EW *Science*, 1997, 278, 1601.

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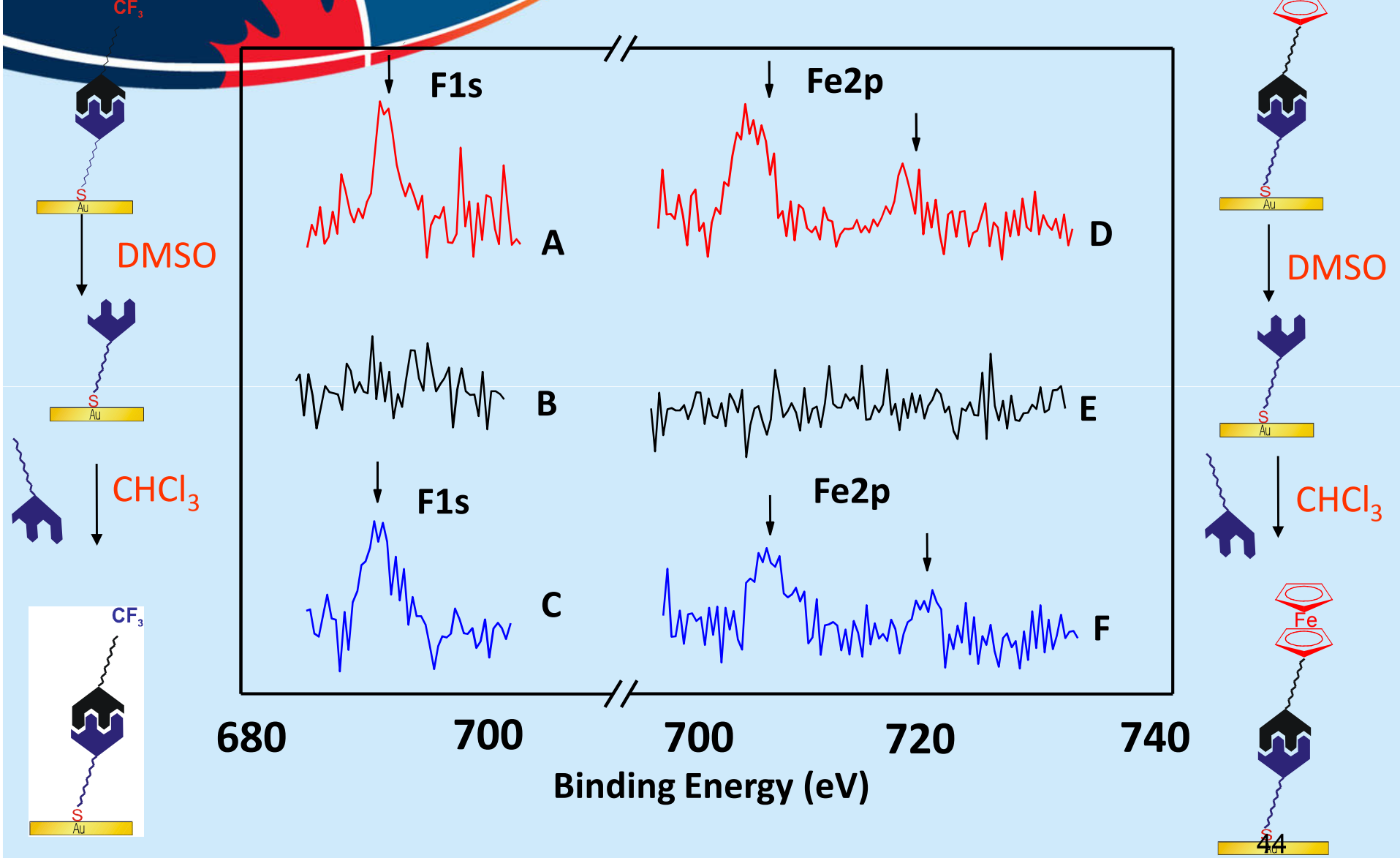
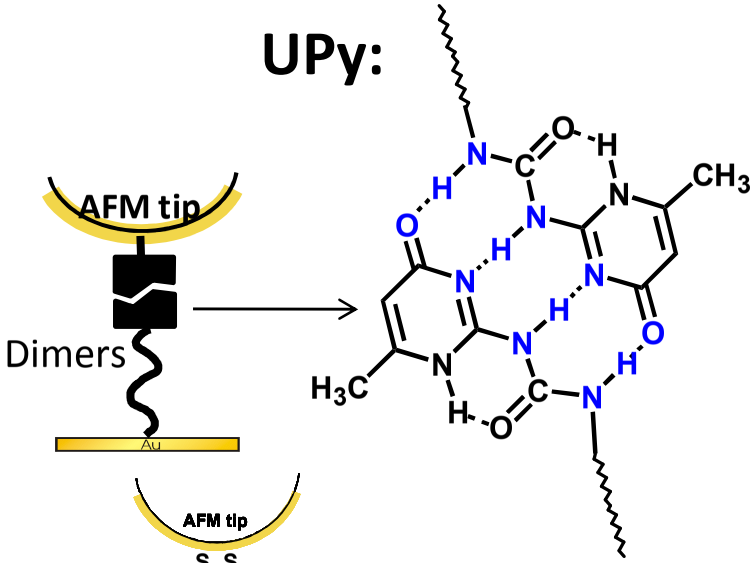
XPS Evidence for Molecular Recognition



H-bonding UPy Complex on Surface

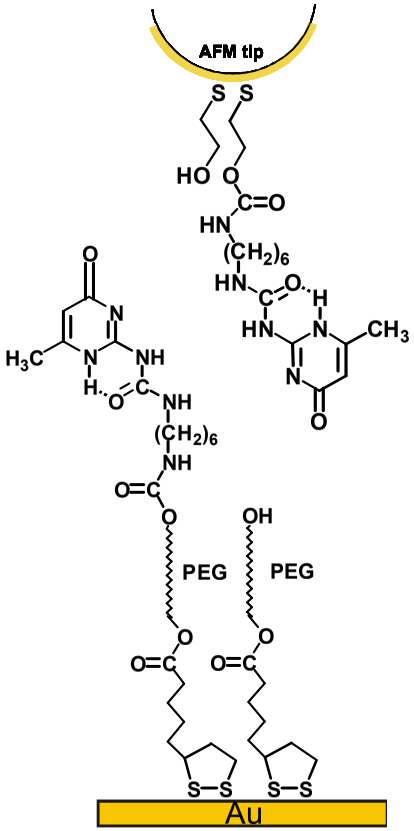


-self-complementary molecular recognition of UPy moieties mediated by quadruple hydrogen bonding sites -- SAMs of UPy disulfide adsorbates on gold-

-dimerization constant can be tuned by solvent and T-

-characterized by XPS, SPR, DPV, AFM, FT-IR –

-*Langmuir,* 2003, 19, 8618.



- H-bonding complexes immobilized on surfaces
- Unbinding forces of UPy complex: ~180 ± 21pN at loading rate: ~35 nN/s
- Stretching individual reversible supramolecular polymers chains
- Rupture force ~ loading rate dependent

Angew. Chem. Int. Ed. 2005, *44*, 956-959.

J. Am. Chem. Soc., 2005, *127*, 11230.