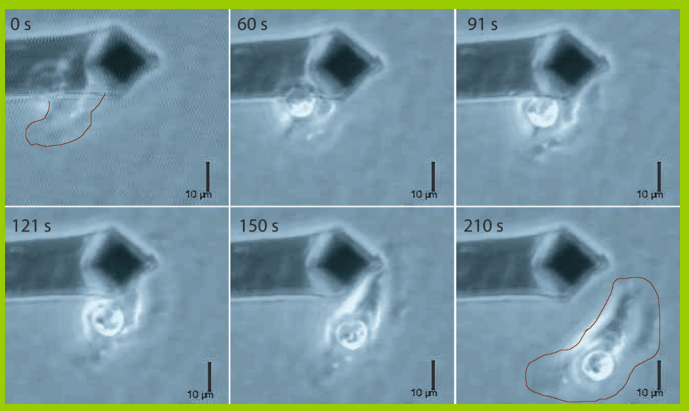
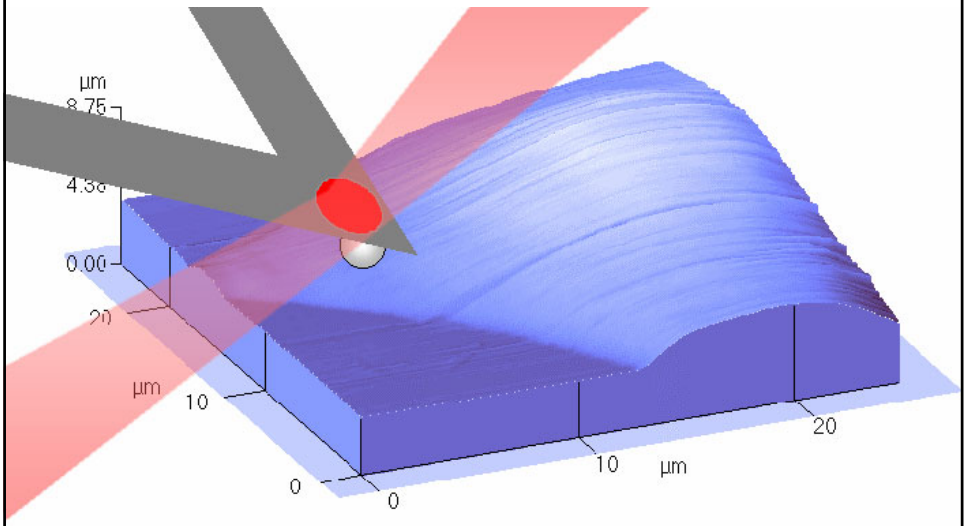
Scanning Force Microscopy

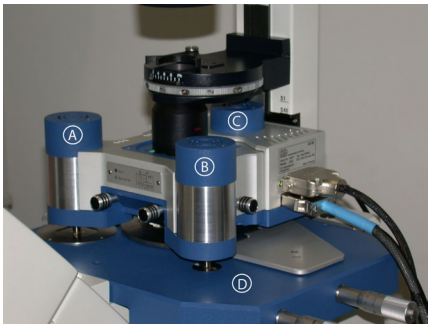
i.e. Atomic Force Microscopy

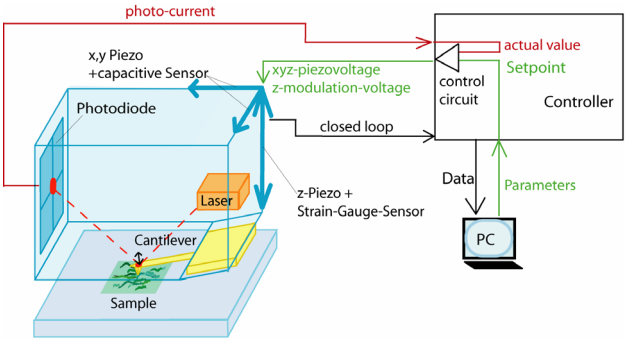


Josef A. Käs
Institute for Soft Matter Physics
Physics Department

Atomic Force Microscopy







Single Molecule Force Spectroscopy

Zanjan 04

CeNS Center for NanoScience

Hermann E. Gaub
Applied Physics
University Munich

Ludwig Maximilians Universität

Molecular-Resolution Images of Langmuir–Blodgett Films and DNA by Atomic Force Microscopy

A. L. Weisenhorn,* M. Egger,† F. Ohnesorge,† S. A. C. Gould, S.-P. Heyn,† H. G. Hansma, R. L. Sinsheimer,‡ H. E. Gaub,† and P. K. Hansma

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Received March 20, 1990. In Final Form: June 13, 1990

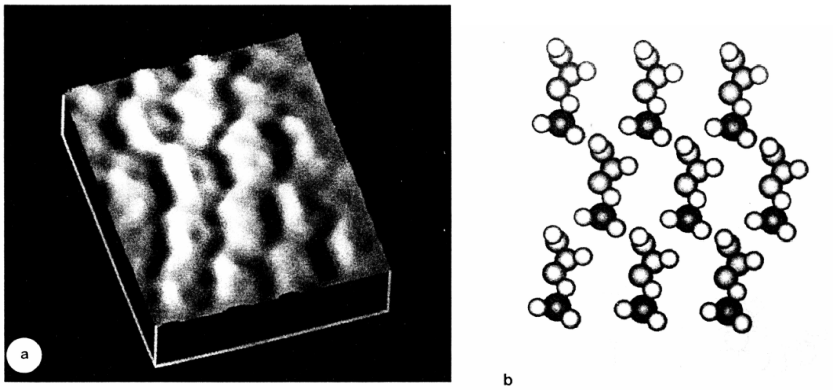
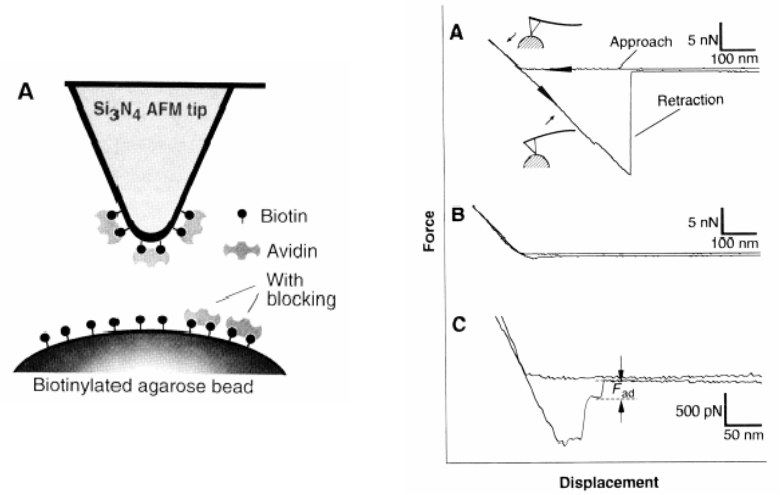


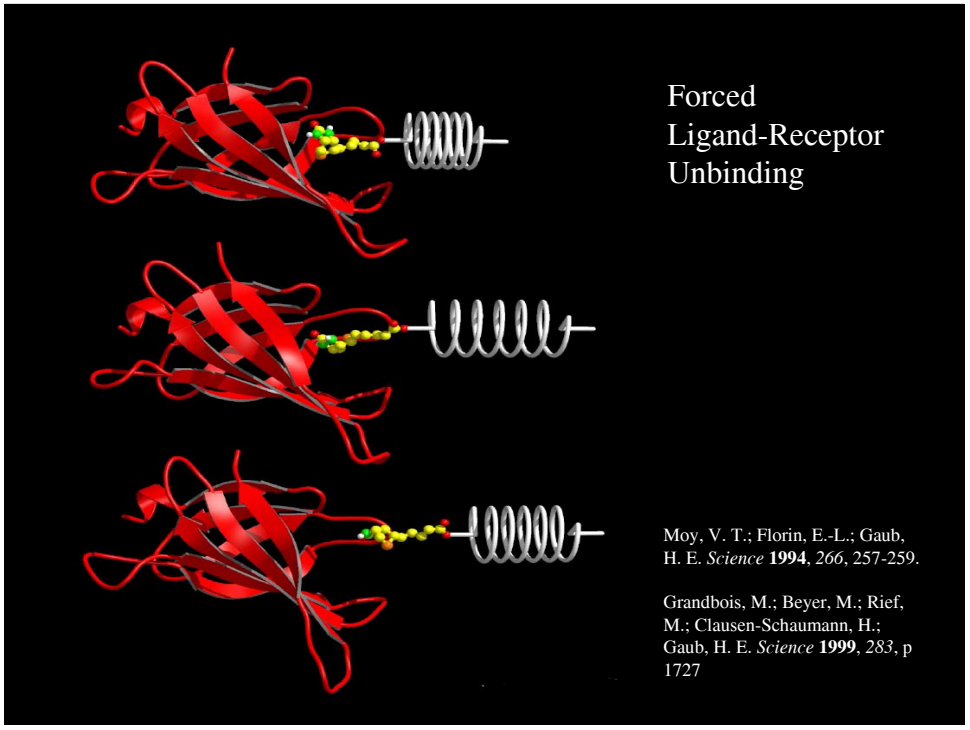
FIG. 2. (a) High resolution AFM image of the surface of a 1-α-DMPG membrane taken in buffer (10 m*M* Hepes, 150 m*M* NaCl, pH 7.4) at room temperature. The image size is 24 × 30 Å^2, the height is about 0.5 Å. (b) Model arranged from adapted 3-D crystal data representing the surface of the membrane.

Adhesion Forces Between Individual Ligand-Receptor Pairs

Ernst-Ludwig Florin, Vincent T. Moy, Hermann E. Gaub*

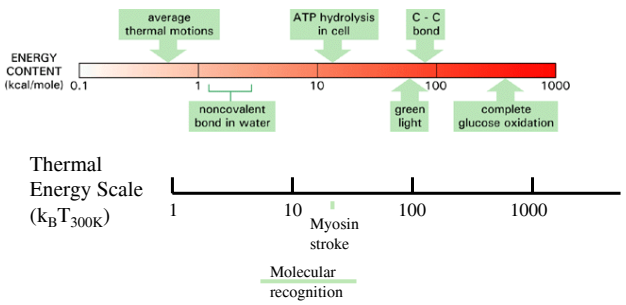
SCIENCE • VOL. 264 • 15 APRIL 1994 **415**





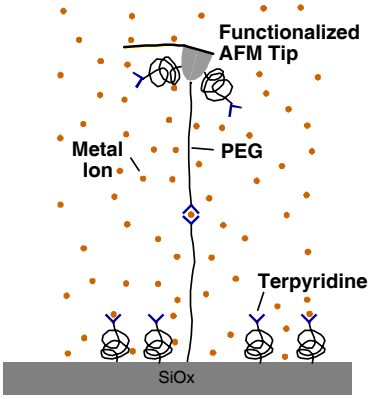
Keep in mind:

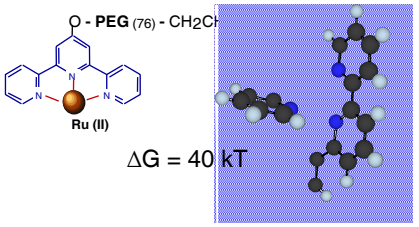
$1k_BT_{300K}$ = 25 meV = 4 pNnm = 0.6 kcal/Mol = 2.5 kJ/Mol

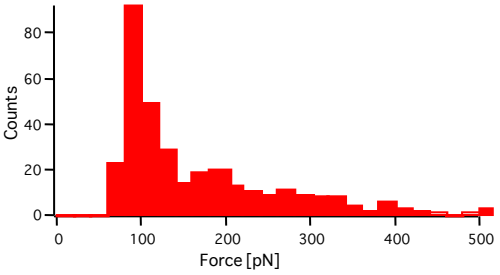


Probing a Single Metallo Organic Bond

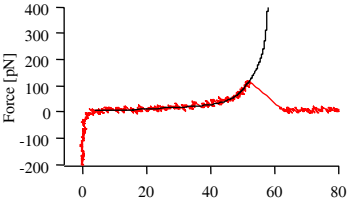
the Ruthenium(II)-Terpyridine Complex

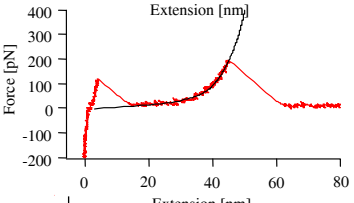


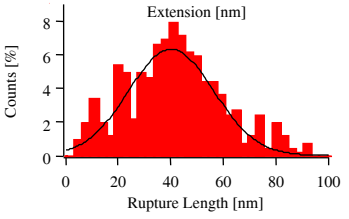


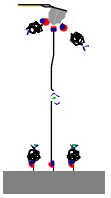


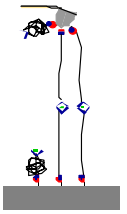
Probing Metalloorganic Bonds



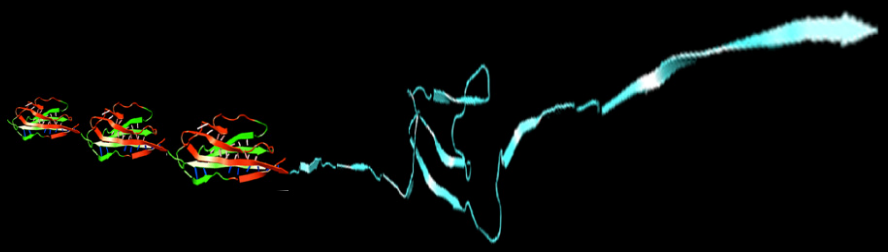






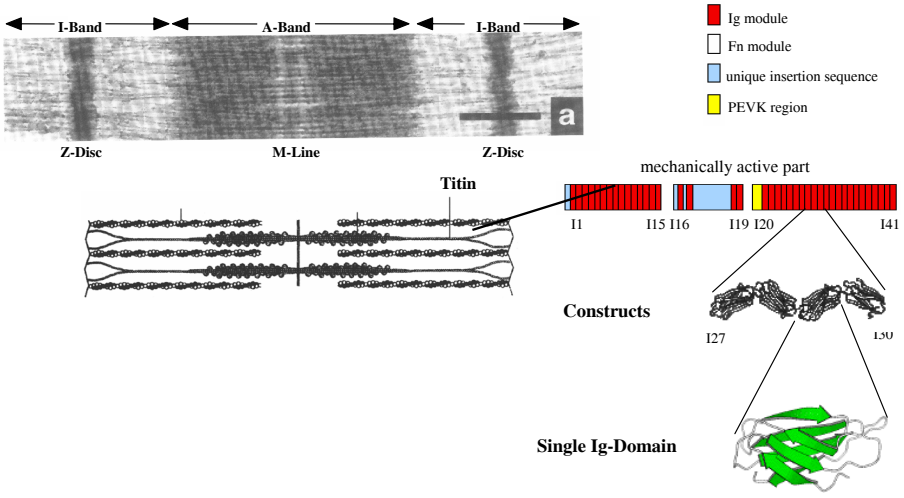


Unfolding Proteins

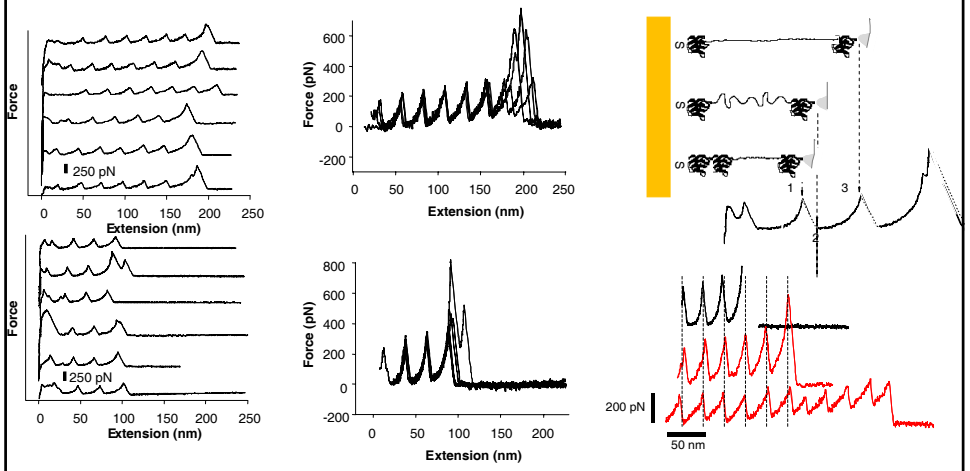


Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. *Science* **1997**, *276*, 1109-1112.
Oesterhelt, F.; Oesterhelt, D.; Pfeiffer, M.; Engel, A.; Gaub, H. E.; Müller, D. J. *Science* **2000**, *288*, 143-146.

The Giant Muscle Protein Titin



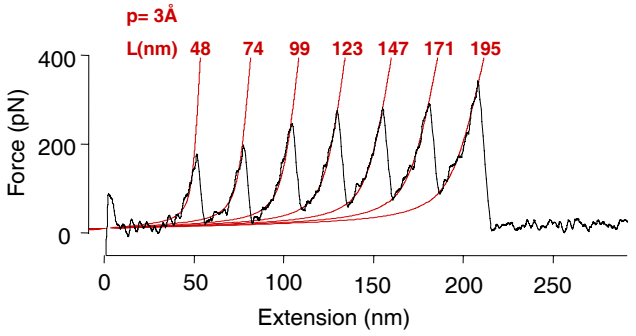
Unfolding 4 and 8 Segment Long Recombinant Titin IgFragments



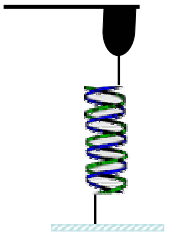
M. Rief, M. Gautel, F. Oesterhelt, J. M. Fernandez and H. E. Gaub, *Science* (1997),Vol 276 , p 1109-

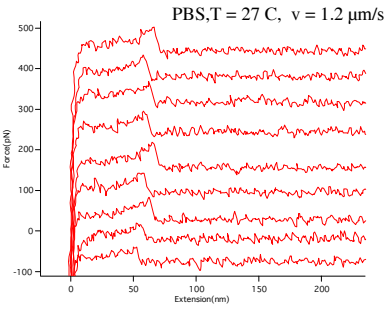
Unfolded Ig 8mer as a Worm Like Chain

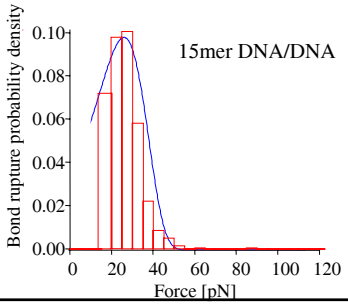
$$F(x)=\frac{kT}{p}\left(\frac{1}{4(1-x/L)^2}-\frac{1}{4}+\frac{x}{L}\right)$$

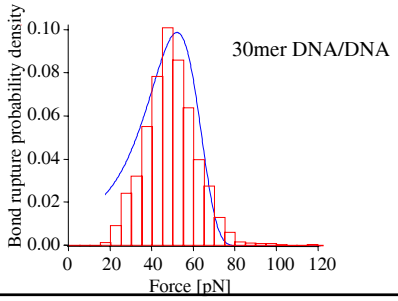


Forced Unbinding of DNA Oligomer Duplexes

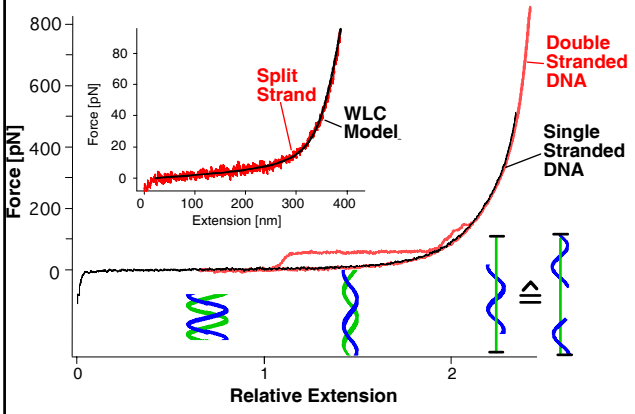


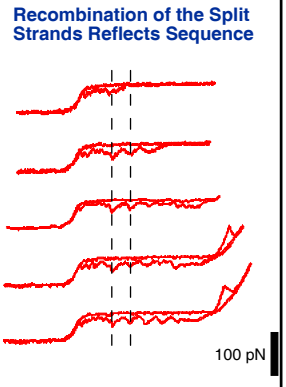






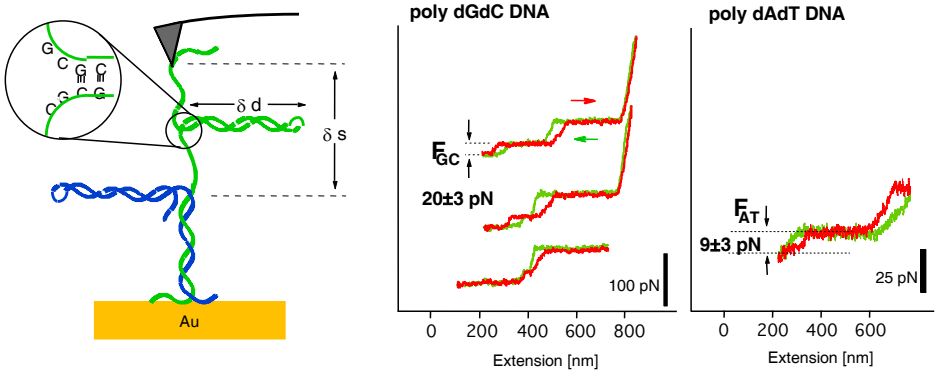
B-S and Melting Transition in l DNA





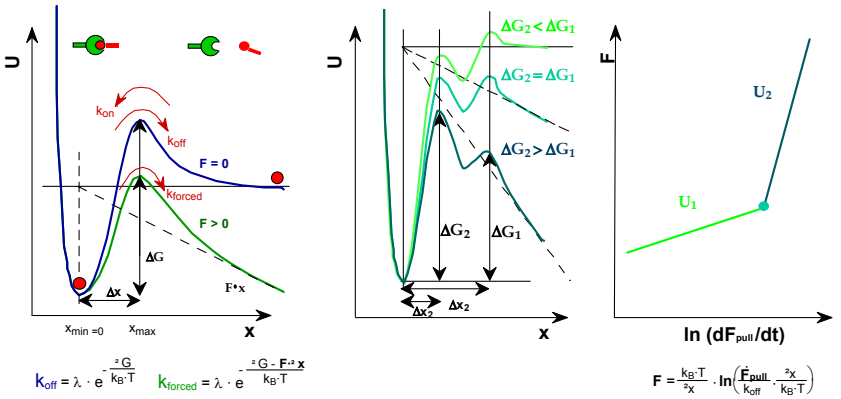
Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Nature Struct. Biol.* **1999**, *6*, 346-348.

Unzipping DNA Hairpins



Rief, M.; Clausen-Schaumann, H.; Gaub, H. E. *Nat. Struct. Biol.* **1999**, *6*, 346-

Energy Landscape Changes under Tension



R.Merkel and E. Evans., Nature, 397, 50-53

Opportunities for Single Molecule Assays in Bio-Analytics

-Mechanics provides orthogonal information
-Extremely low amounts of analyte needed

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.

=> Growing demand in Genomics & Proteomics

Current bottle necks:

Sensitivity e.g. Quantitative SNP detection

Selectivity e.g. False positives

High Throughput 10^6 Assays in parallel

Improved Sensitivity by Smaller Sensors

Molecules as Sensors?

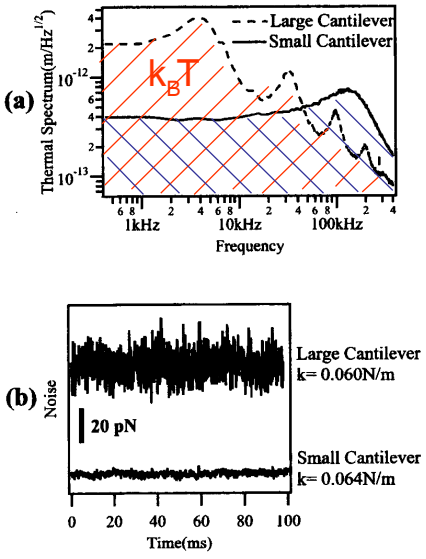
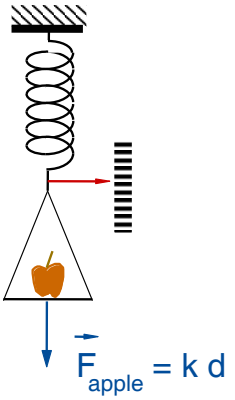


FIG. 1. (a) Thermal spectra of a large (200-μm-long) and small (10-μm-long) cantilever immersed in water far from the sample surface. (b) Measured force noise for the same two cantilevers in a 3 kHz bandwidth.

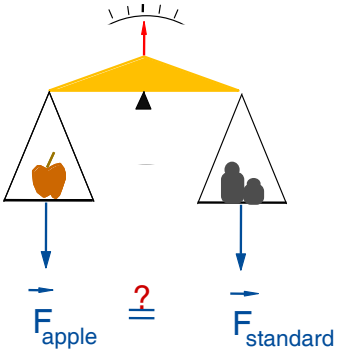
Viani, Schäffer, Chand, Rief, Gaub, Hansma *J. Appl. Phys.* **(1999)**, 4, p2258-

Absolute Versus Differential Force Measurements

Scales measure absolute forces

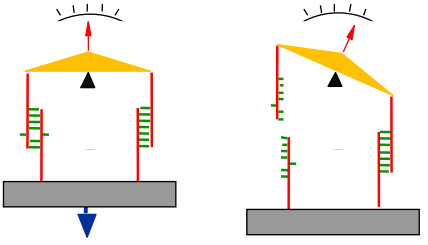


Balances compare
unknown forces with a standard

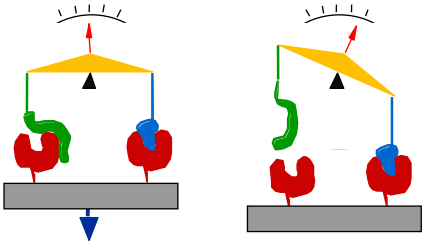


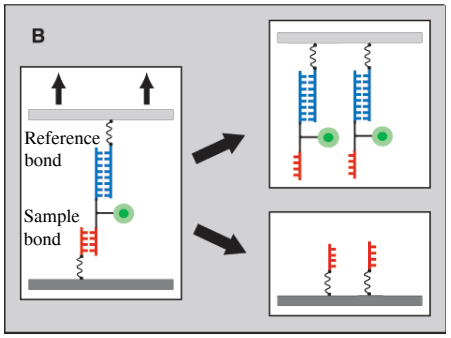
Singe Molecule Differential Force Assay

Detecting
nucleic acid mismatches



Discriminating energetically
equivalent binding modes

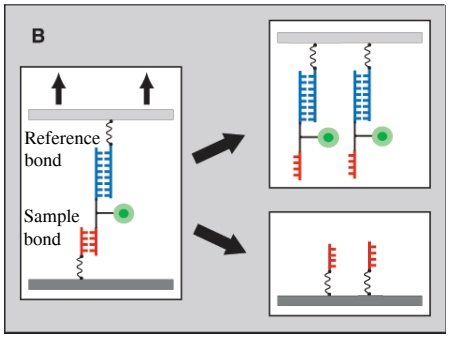




Single Molecule
1bit AD-Conversion

Parallel Format

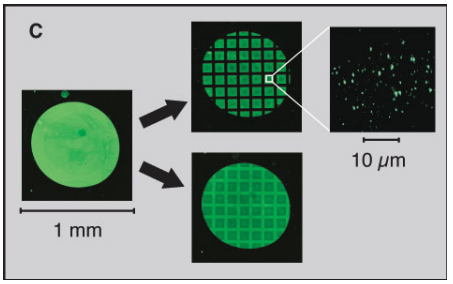
$$\Delta n \approx \Delta I \approx \Delta P_{bond}$$

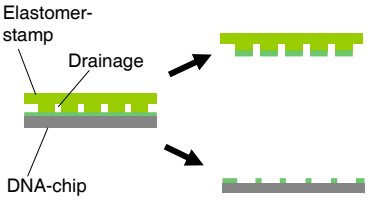


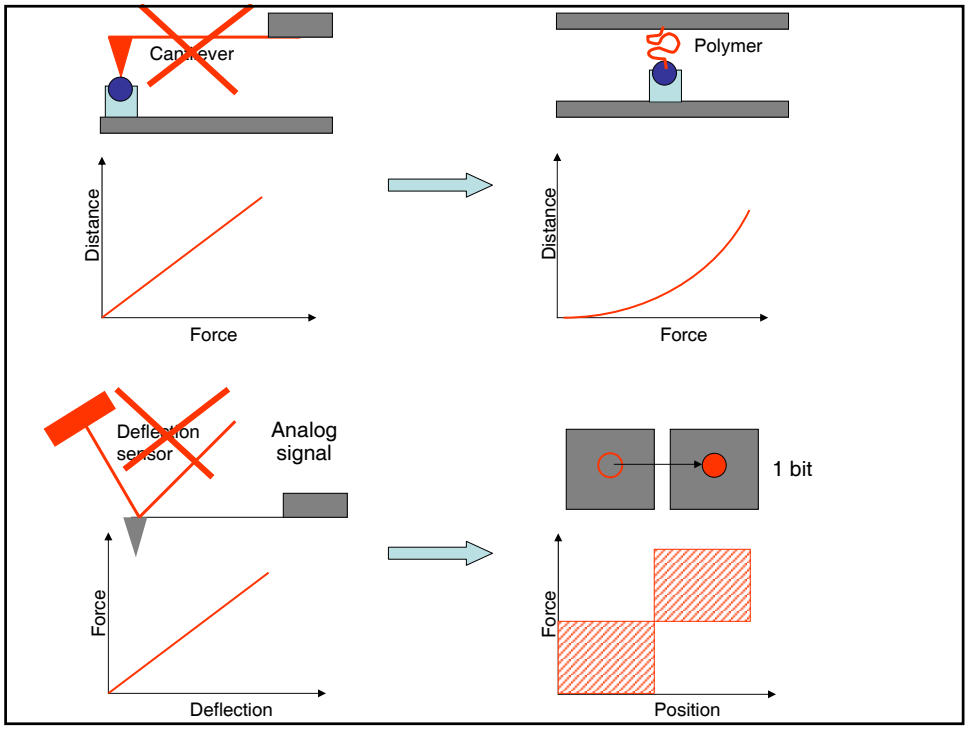
Single Molecule
1bit AD-Conversion

Parallel Format

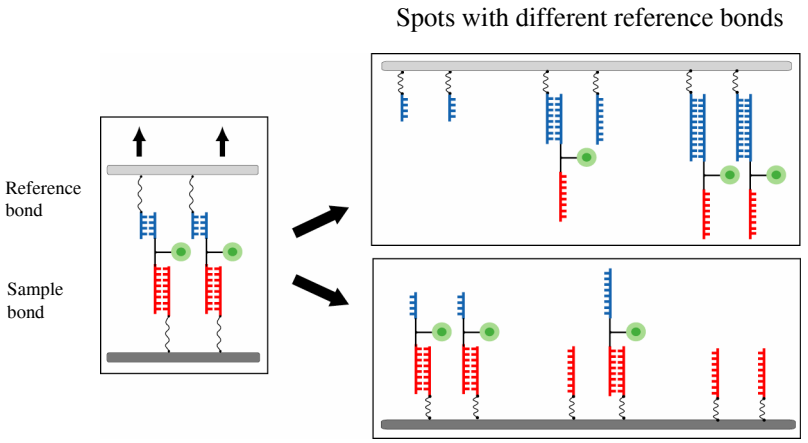
$$\Delta n \approx \Delta I \approx \Delta P_{bond}$$

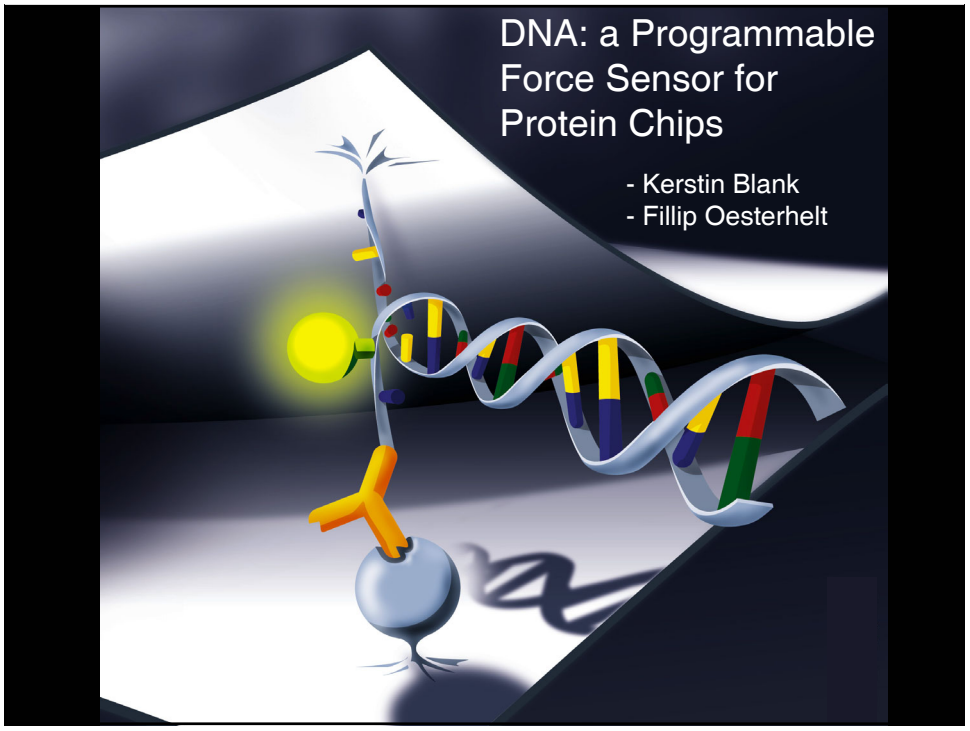




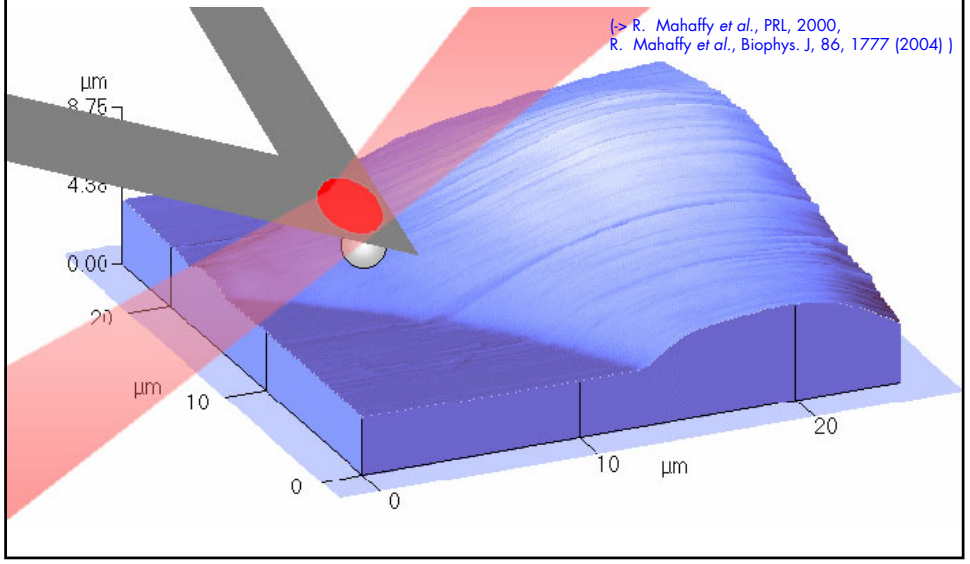


Chemically-Programmable Molecular AD Converter

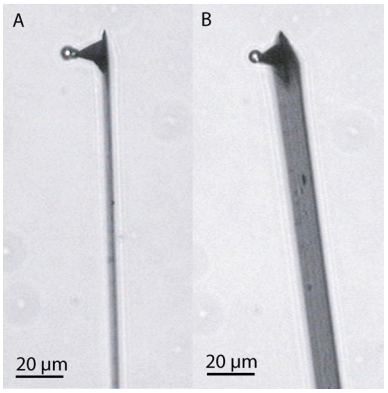


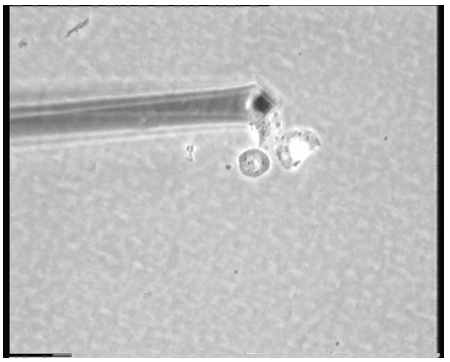


AFM-based Measurements of Cell Elasticity



AFM-based Measurements of Cell Elasticity





Analyzing Viscoelastic AFM-Data

The Hertz-model (sphere indenting an infinite elastic half plane):

$$f_{bead} = \frac{4}{3} K R^{1/2} \delta^{3/2}$$ **with *K =E/(1-ν²)***

viscoelastic extension:

$$\delta = \delta_0 + \tilde{\delta}^* e^{i\omega t} \quad \textbf{with} \quad \tilde{\delta}^* = \delta' + i\delta''$$

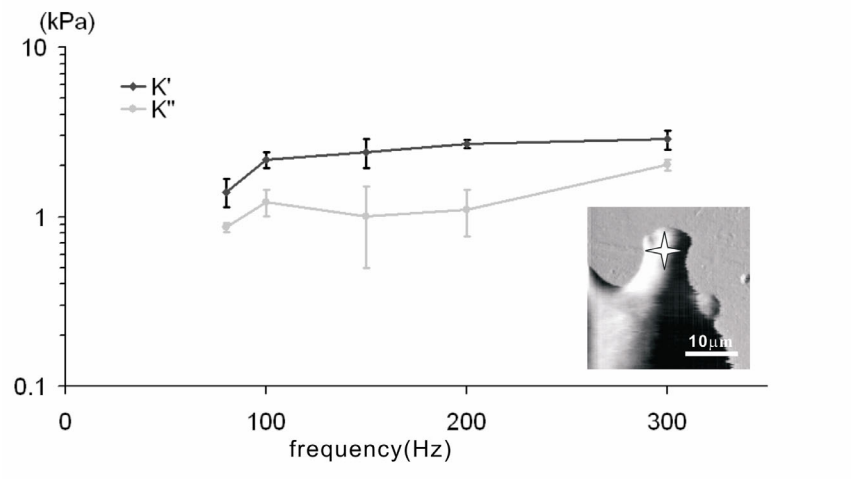
$$\Rightarrow \quad f_{bead} \approx \frac{4}{3} R^{1/2} \left(K_0 \delta_0^{3/2} + \frac{3}{2} K_1^* \delta_0^{1/2} \tilde{\delta}^* \right)$$

$$f_0 \equiv \frac{4}{3} K_0 R^{1/2} \delta_0^{3/2}$$

$$f_{osc}^* \equiv 2 K_1^* R^{1/2} \delta_0^{1/2} \tilde{\delta}^*$$

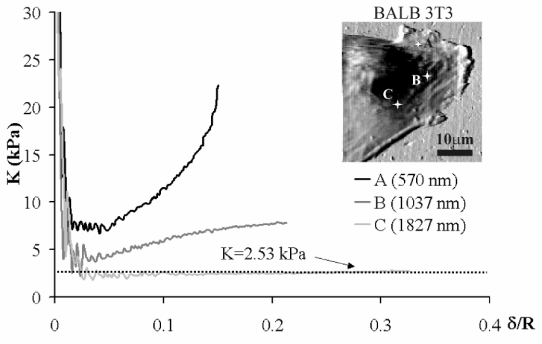
$$\Rightarrow \quad K_1^* = \frac{f_{osc}^*}{2\tilde{\delta}^* (R\delta_0)^{1/2}} = K' + iK''$$

Analyzing Viscoelastic AFM-Data



Correcting for Substrate Effects

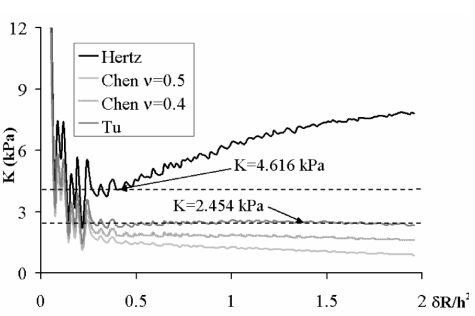
(R. Mahaffy *et al.*, Biophys. J, 86, 1777 (2004))

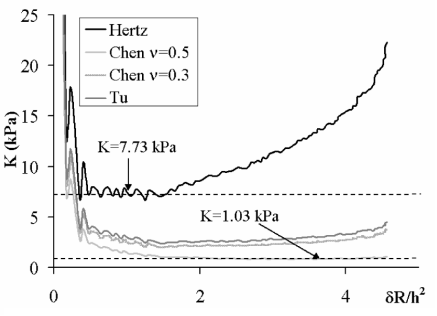


Correcting for Substrate Effects

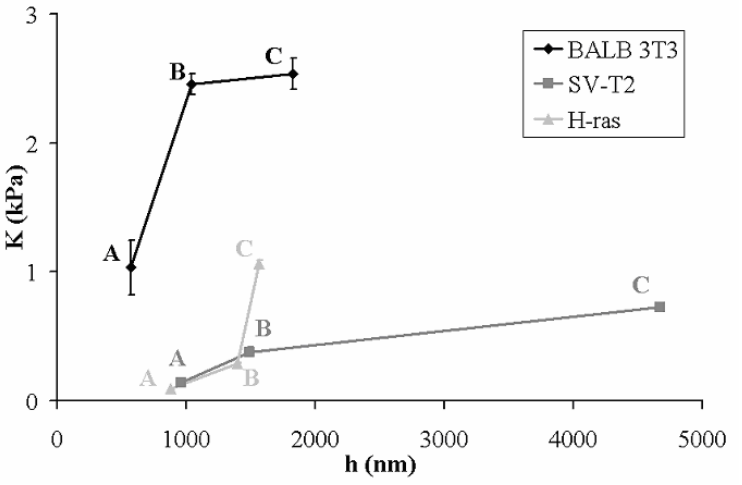
(R. Mahaffy *et al.*, Biophys. J, 86, 1777 (2004))

- Tu (for non adhered parts) and Chen model (for adhered parts) correct for substrate effects and simultaneously determine the Poisson ratio.





Elasticity of Lamellipodia



(S.Park *et al.*, Biophys. J, submitted)

Elasticity of Lamellipodia

	Net path length (*mm*) l_{30min}	Mean speed (*mm/hr*) *v*	Directionality *D*	Activity (*%/min*) *a*
BALB (n=10)	3.7	7.3	0.7	0.9
SE	2.2	4.5	0.2	0.6
SV-T2 (n=10)	6.0	12.1	0.6	1.7
SE	2.4	4.8	0.2	0.6
t-test (%)	9.7	9.6	53.2	3.0
H-ras (n=10)	12.4	24.7	0.7	2.4
SE	6.0	12.0	0.3	1.1
t-test (%)	5.7	5.7	85.1	5.8

	BALB 3T3 (n=37)	SV-T2 (n=29)	H-ras transformed (n=18)
Average K (kPa)	1.35	0.63	0.57
±	0.32	0.34	0.37

(S.Park *et al.*, Biophys. J, submitted)

Active Force Generation I

- Polymerization:

1. Thermal ratchet vs. polym.-stick-burst
2. Bead motility, nematode sperm motility

(-> Formin)

- Molecular motors:

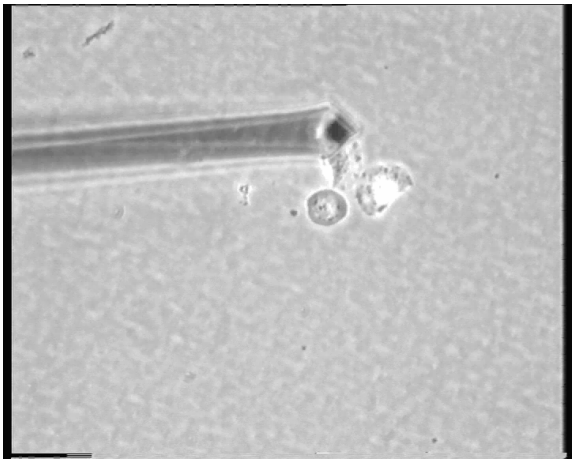
1. Knockouts (Spudich, Gerisch) vs. myosin II in the back of the lamellipodium (Small, Borisy)
2. Traction forces on soft substrates (Sheetz, etc)

(-> myosin I actin-rail model, cell spreading)

- Retrograde flow:

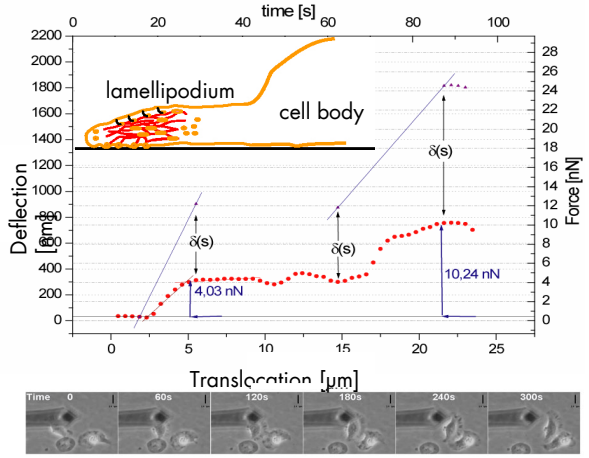
1. Clutch hypothesis

Active Force Generation II



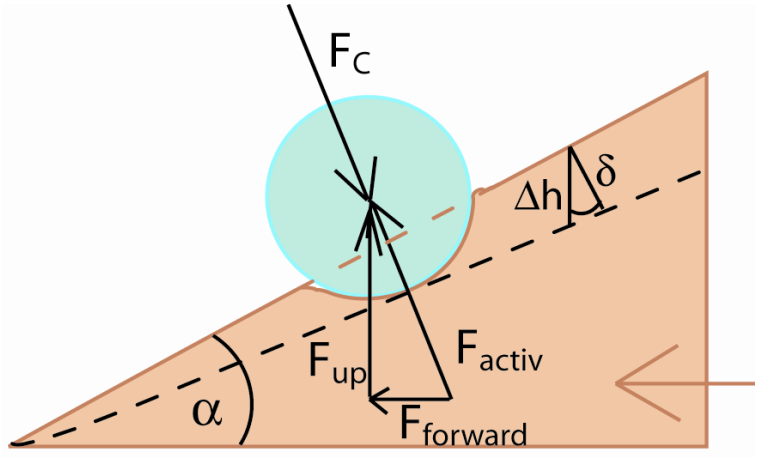
(-> C. Brunner, M. Gögler, A. Ehrlicher, T. Jähnke, B. Kohlstrunk, Propulsive forces of fast moving cells, *Nature*, in preparation (2004))

Active Force Generation II



(-> C. Brunner, M. Gögler, A. Ehrlicher, T. Jähnke, B. Kohlstrunk, Propulsive forces of fast moving cells, *Nature*, in preparation (2004))

Active Force Generation III



Active Force Generation III

Cell (number)	Time (sec)	Slope	F_{up} nN	$F_{forward}$ nN
Lamellipodia (1)	10-20	0,24	4,03	0,97
Soma (1)	65-90	0,14	10,24	1,43
Lamellipodia (2)	15-25	0,25	1,39	0,35
Soma (2)	40-60	0,26	5,55	2,55
Lamellipodia (5)	70-80	0,56	3,3	1,85
Soma (5)	160-230	0,22	11,3	2,43
Soma (6)	30- 150	0,13	8,5	1,12