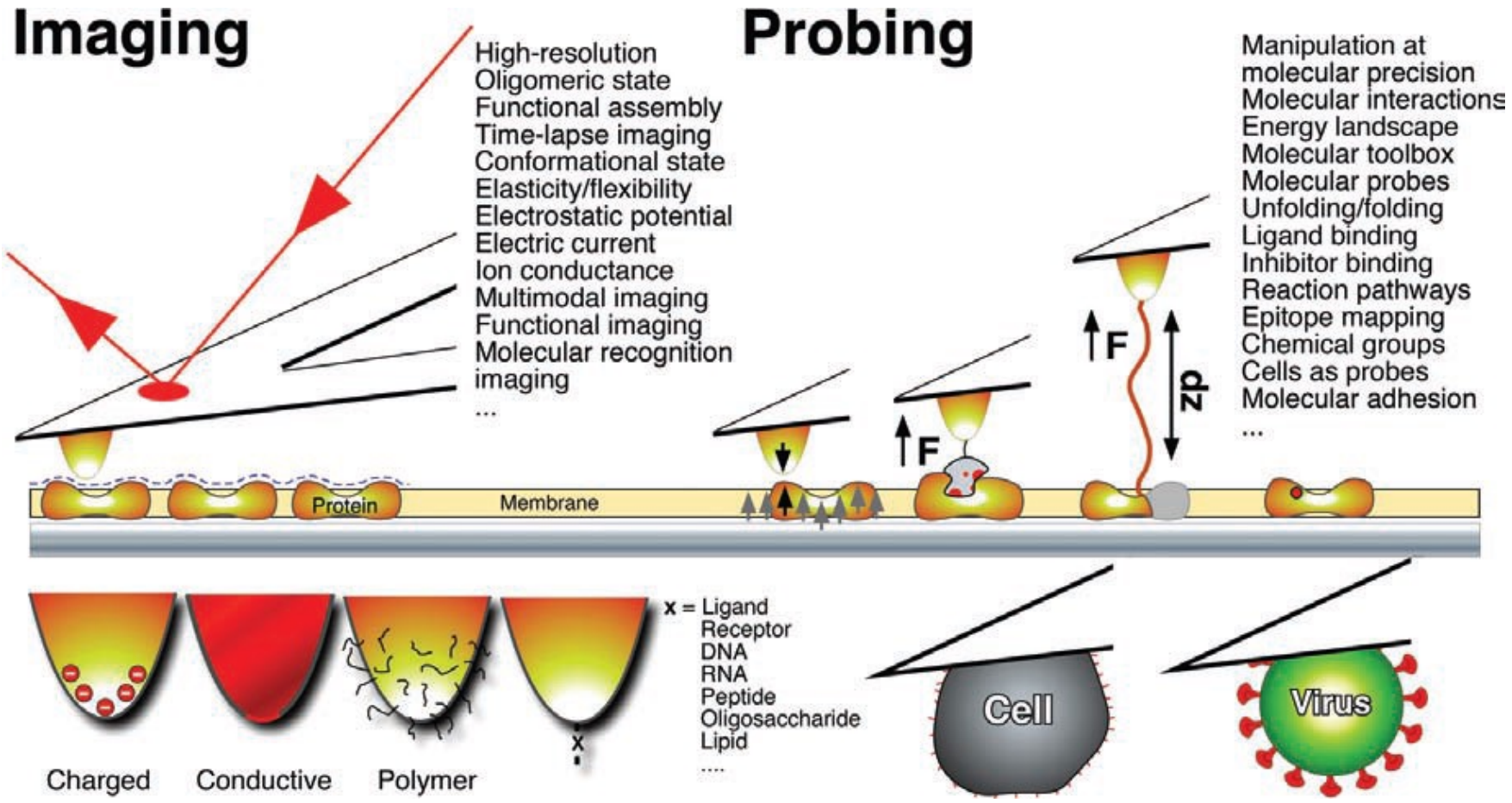
Single molecule force spectroscopy

AFM: lab on a tip



S. Scheuring, D. Muller, H. Stalhberg, H.-A. Engel, A. Engel, *Eur. Biophys. J.* **31**, 172 (2002)

Single molecule manipulation

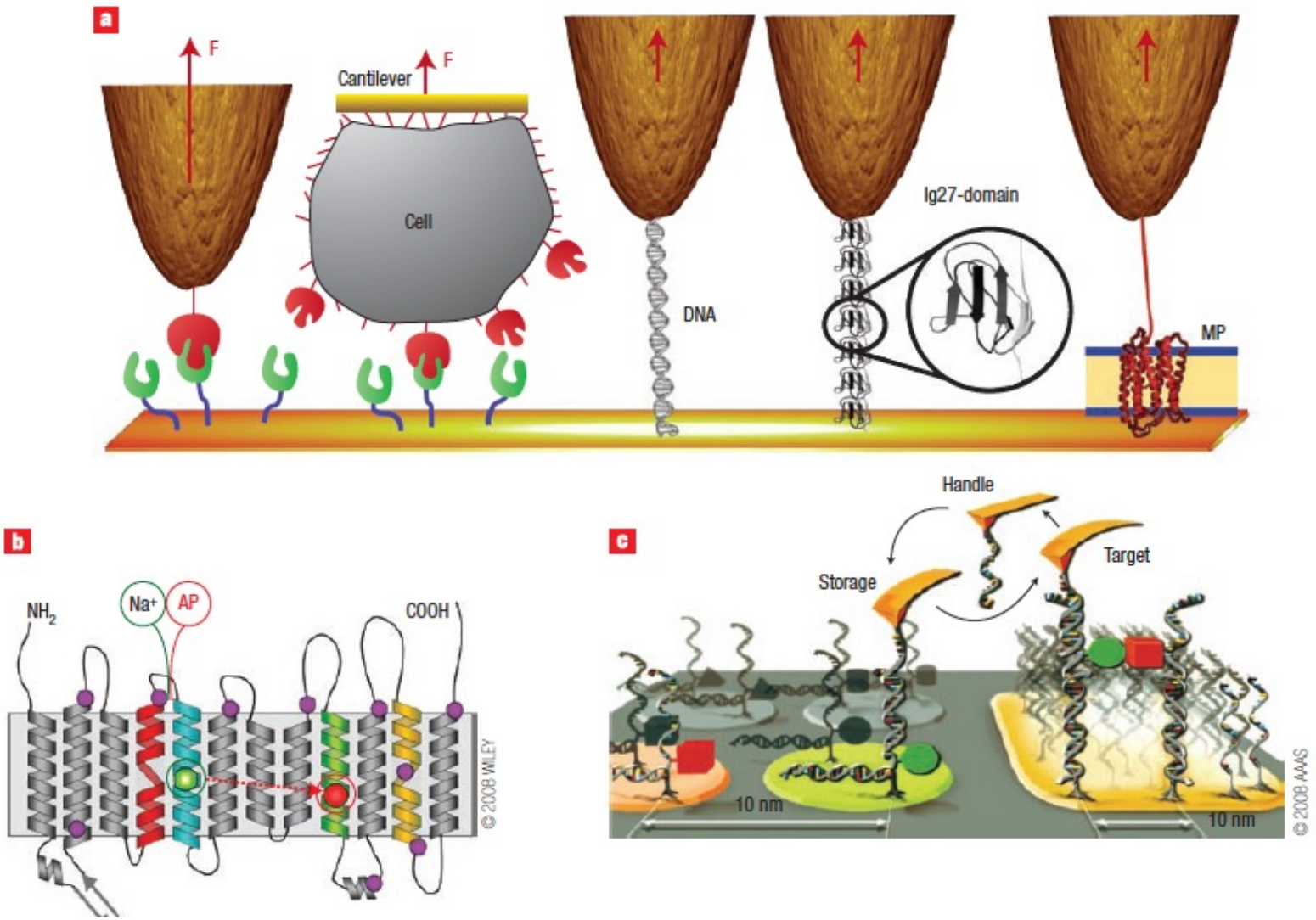
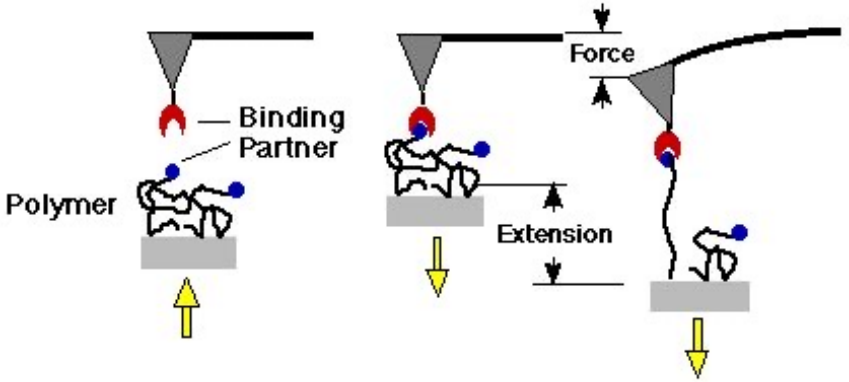
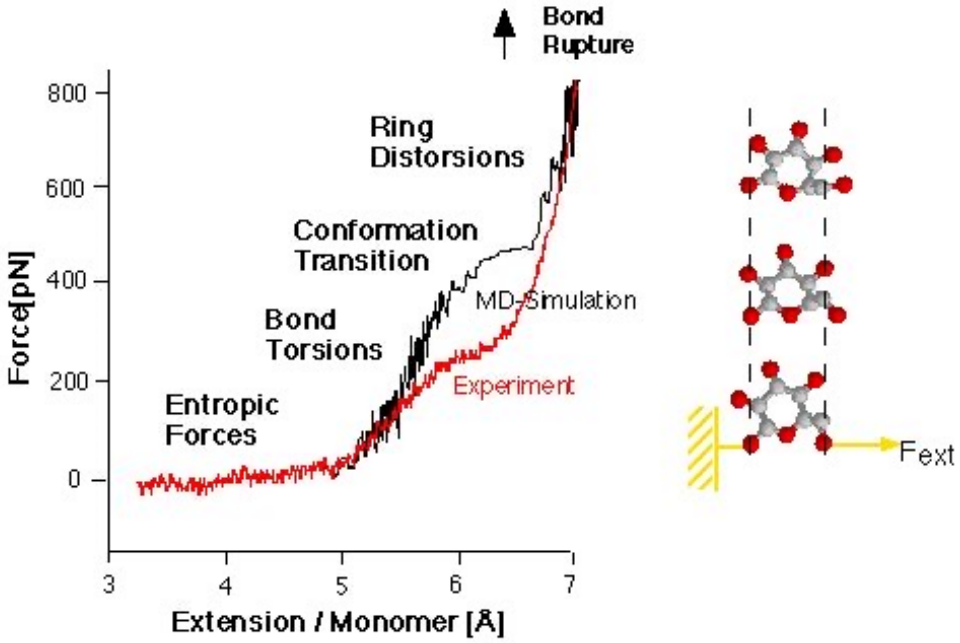
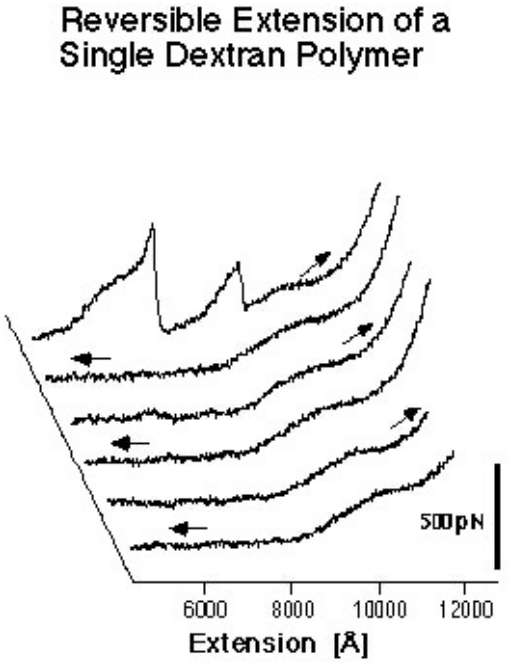


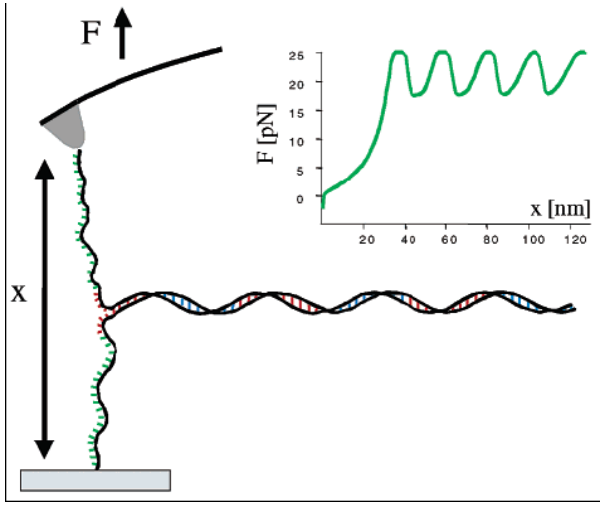
Figure 3 Single molecule manipulation, control and design. **a**, Applying AFM to probe interaction forces (F) of single biomolecules. These examples measure ligand–receptor interactions in their isolated form (left) and embedded in their cellular environment (probe replaced by a biological cell); stretching of a DNA molecule; unfolding of Ig27-titin; and unfolding of a membrane protein (MP). **b**, SMFS can detect and locate interactions (circles) on the structure of membrane proteins (here proton/sodium antiporter NhaA from *E. coli*). A ligand (Na^+ ion) or inhibitor (AP, 2-aminoperimidine) binding to the ligand-binding site (green circle) establishes different interactions activating (green circle) or deactivating (green and red circles) the antiporter (composed of 12 transmembrane helices). **c**, Single molecules can be mechanically assembled by picking up from discrete storage sites with a DNA oligomer at the AFM probe and depositing them at a target site with nanoscopic precision. Reproduced with permission from refs 52, 53 and 63.

Single molecule force spectroscopy



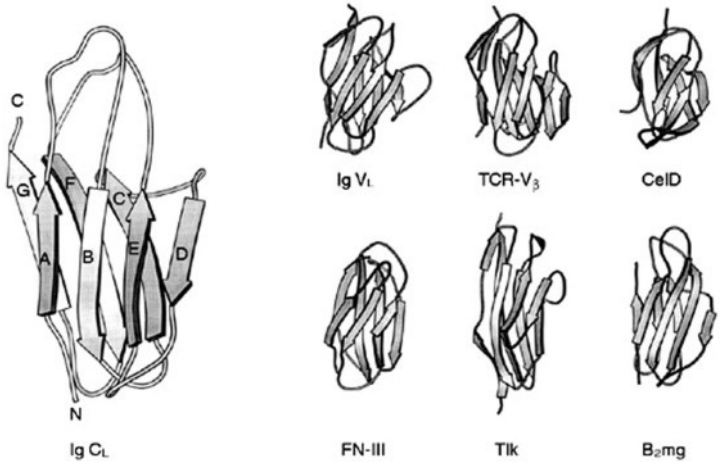


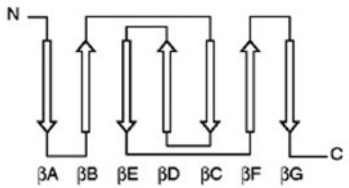




Single molecule manipulation

One common feature of many mechanical proteins is that they contain multiple, individually folded protein domains.
Two important examples are the **immunoglobulin (Ig)-type fold** and the **fibronectin-type fold** (the most common of which is fibronectin type 3 or FN-III).
Both are so-called **b-sandwich structures:** these domains might unfold and refold as proteins execute mechanical functions.





Force-induced extension of the **protein titin**, for example, which is responsible for the passive elasticity of muscle, can cause its constituent Ig and FN-III domains to unravel.

$40 \times 25 \times 25$ Å (1 Å $= 10^{-10}$ m)

Structural diversity of Ig-type domains. The constant domain of antibodies (Ig C_L) represents the common core present in all Ig-like structures, composed of seven antiparallel β strands labeled A–F. The two-dimensional topological diagram (bottom, left) shows the connected Greek key motif of this structure.

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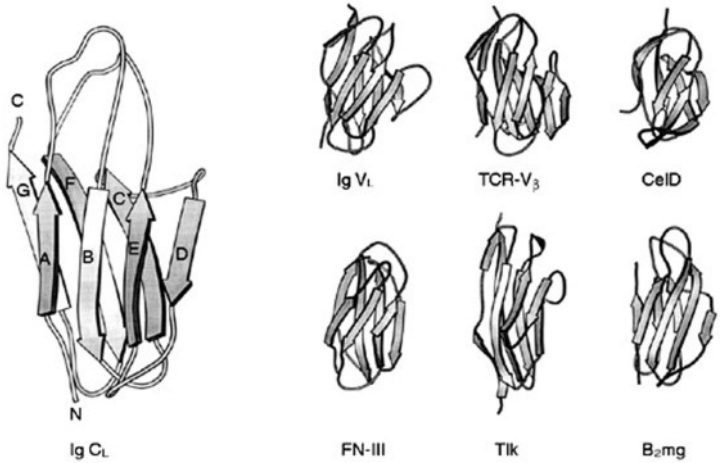
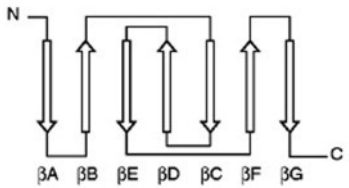


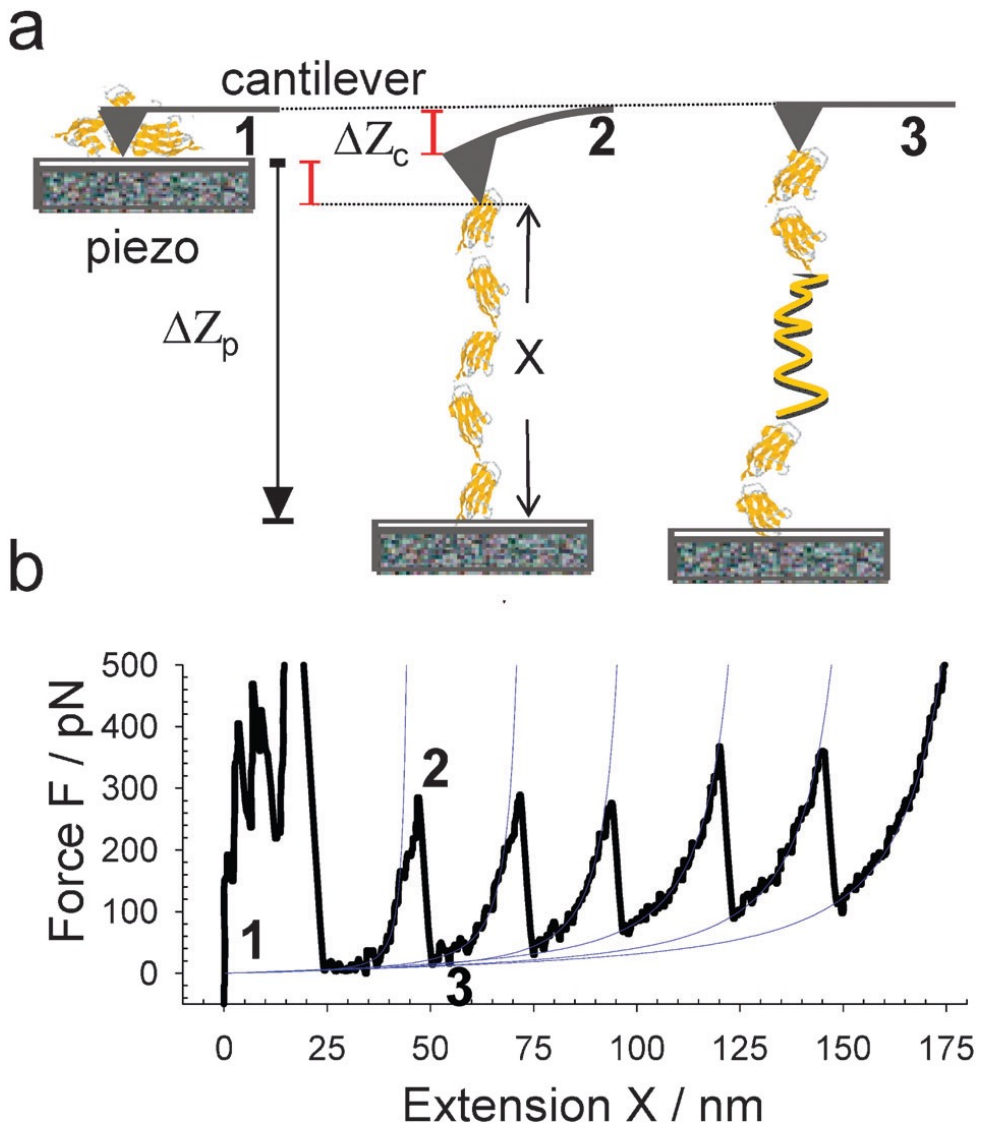


Unfolding and refolding of domains, as part of a particular mechanical function, could be **a mechanism by which tension is maintained as a protein is extended or relaxed**. Unfolding might also contribute to the function of fibronectin by exposing cryptic protein interaction sites that are important in ECM assembly.

Structural diversity of Ig-type domains. The constant domain of antibodies (Ig C_L) represents the common core present in all Ig-like structures, composed of seven antiparallel β strands labeled A–F. The two-dimensional topological diagram (bottom, left) shows the connected Greek key motif of this structure.

Single molecule manipulation

Single-molecule techniques analyze individual members in complex, heterogeneous populations, enabling to reveal the diversity of hidden molecular behaviors and rare events.

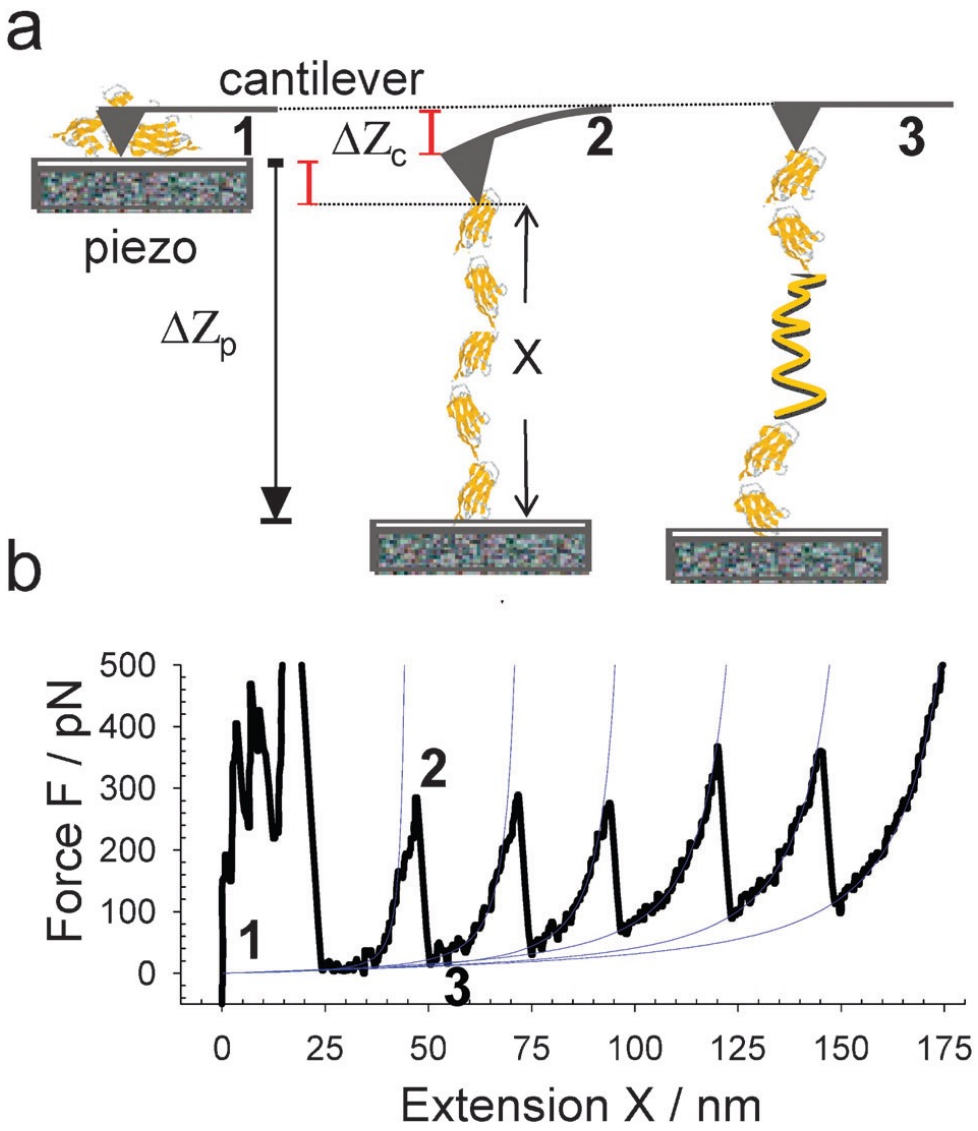


Single-molecule assays permit the direct measurement of the molecular elasticity of polysaccharides, proteins or nucleic acids, otherwise impossible, and the acceleration of certain molecular reactions, such as protein unfolding or ligand–receptor bond rupture.

$$X = \Delta Z_P - \Delta Z_c$$
$$F = k_c \Delta Z_c$$

P.E. Marszalek, Y. F. Dufrene
Chem. Soc. Rev., 2012, 41, 3523–3534

Single molecule manipulation



The resolution of force measurements depends on the dimensions of the AFM cantilever (the smaller the better) and on the range of measured frequencies

using small cantilevers in the measurement bandwidth of 1 kHz, the force noise that limits the resolution can be as small as 3–5 pN (resolution can be improved to below 1 pN)

$$X = \Delta Z_P - \Delta Z_c$$
$$F = k_c \Delta Z_c$$

How much should be the force to “break” a bond?

The energy of a molecular bond is approx 3 eV = 360 KJ / mole = 6 10(-19) J / bond

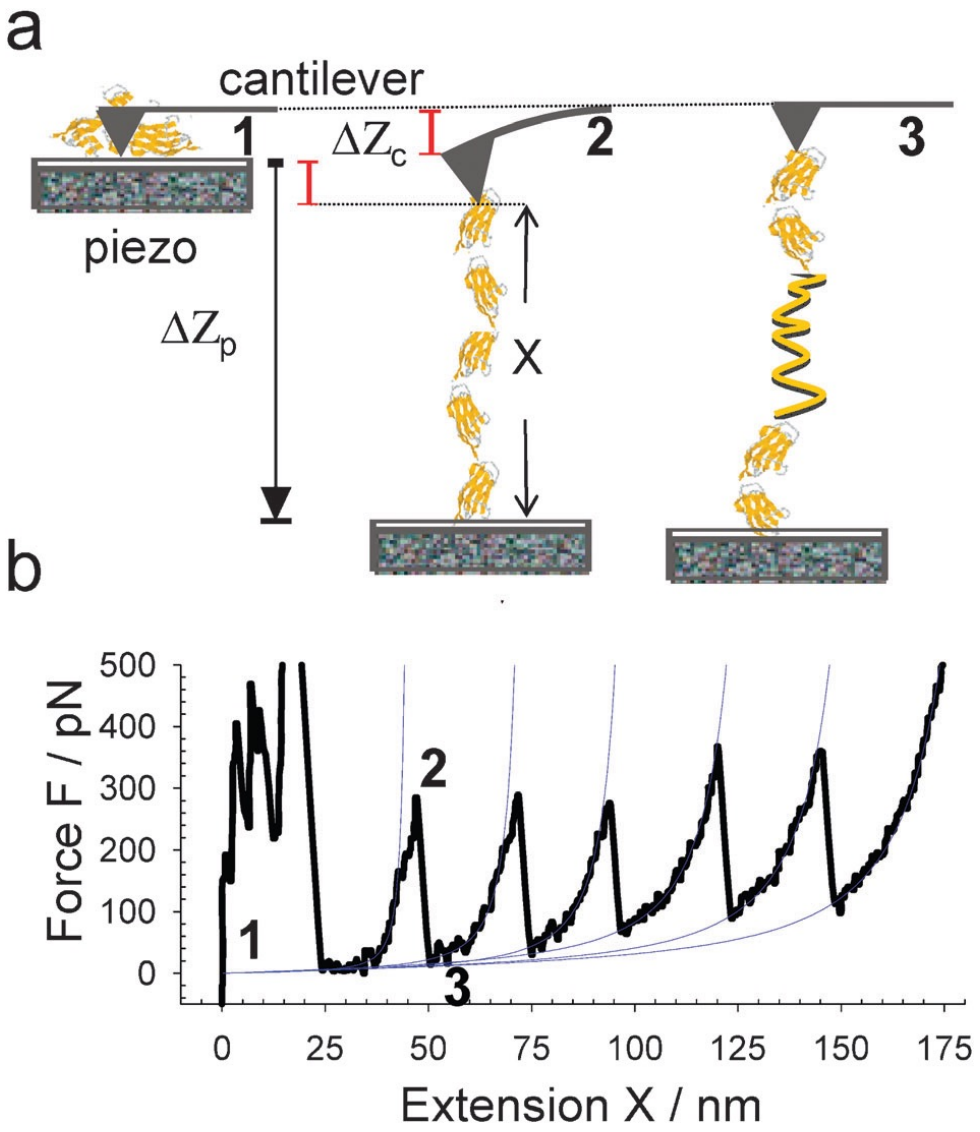
The length of a molecular bond is approx 0.3 nm or 3 Angstroem but to break a bond is sufficient to stretch it by less. Let us say 1 A

ENERGY = FORCE x DISTANCE

FORCE = ENERGY / DISTANCE = 6 10 (-19) J / 0.1 10 (-9) m

= 600 pN or less than 1 nN or 10 (-9) Newton

Single molecule manipulation



Mechanical proteins are frequently composed of either individually folded globular domains that are connected to each other by short flexible linkers (e.g. titin in muscle) or tightly stacked short helical segments that form elongated single-domain structures (e.g. ankyrin in the RBC skeleton),or longer helical domains that form coiled-coils structures (e.g. spectrin in the RBC skeleton, or myosin in muscle).

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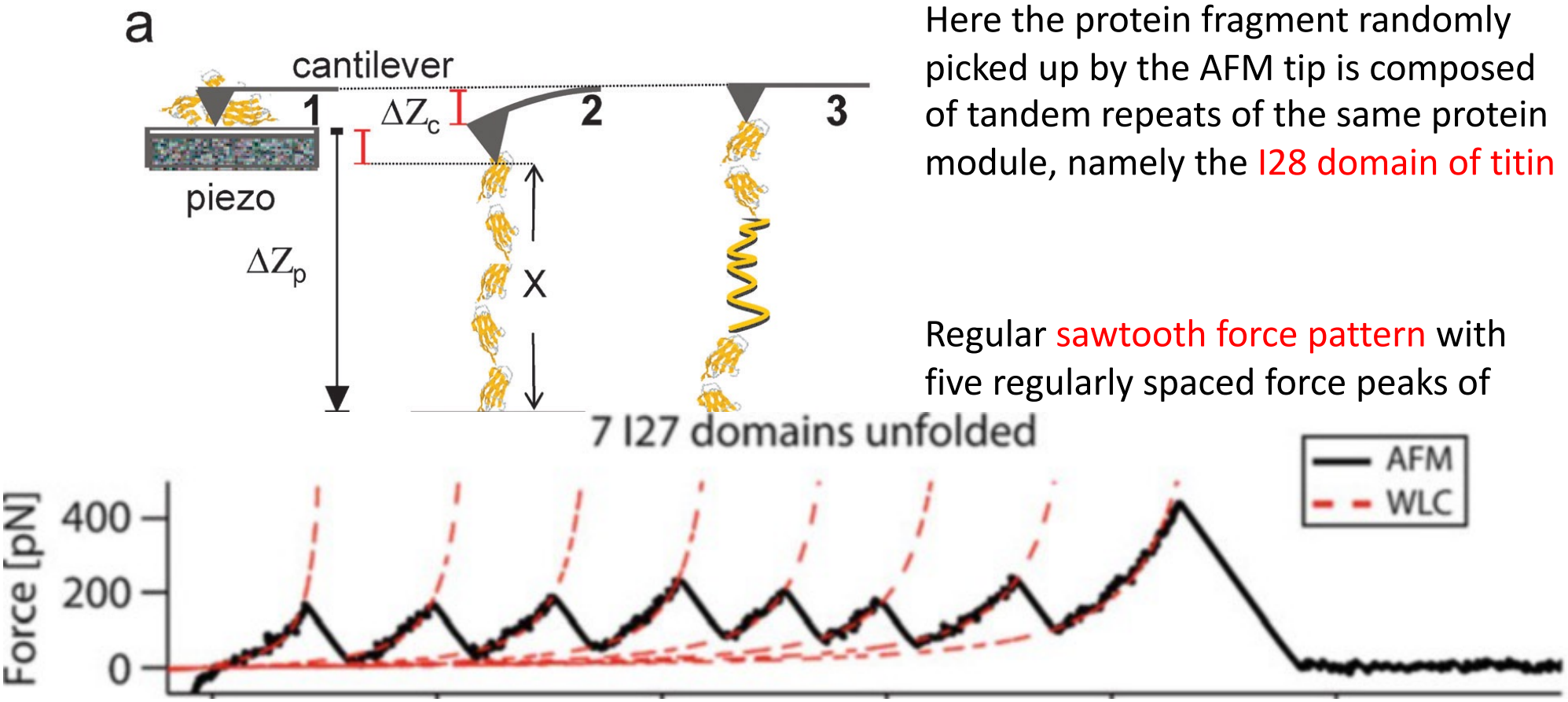
Single molecule manipulation



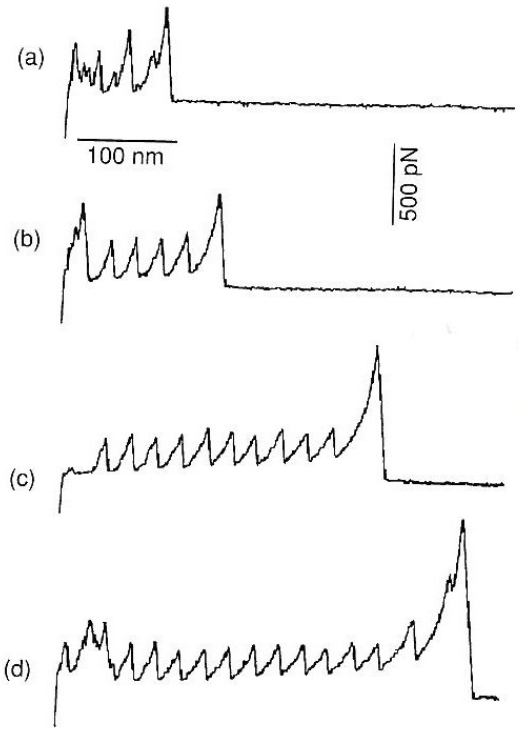
Here the protein fragment randomly picked up by the AFM tip is composed of tandem repeats of the same protein module, namely the I28 domain of titin

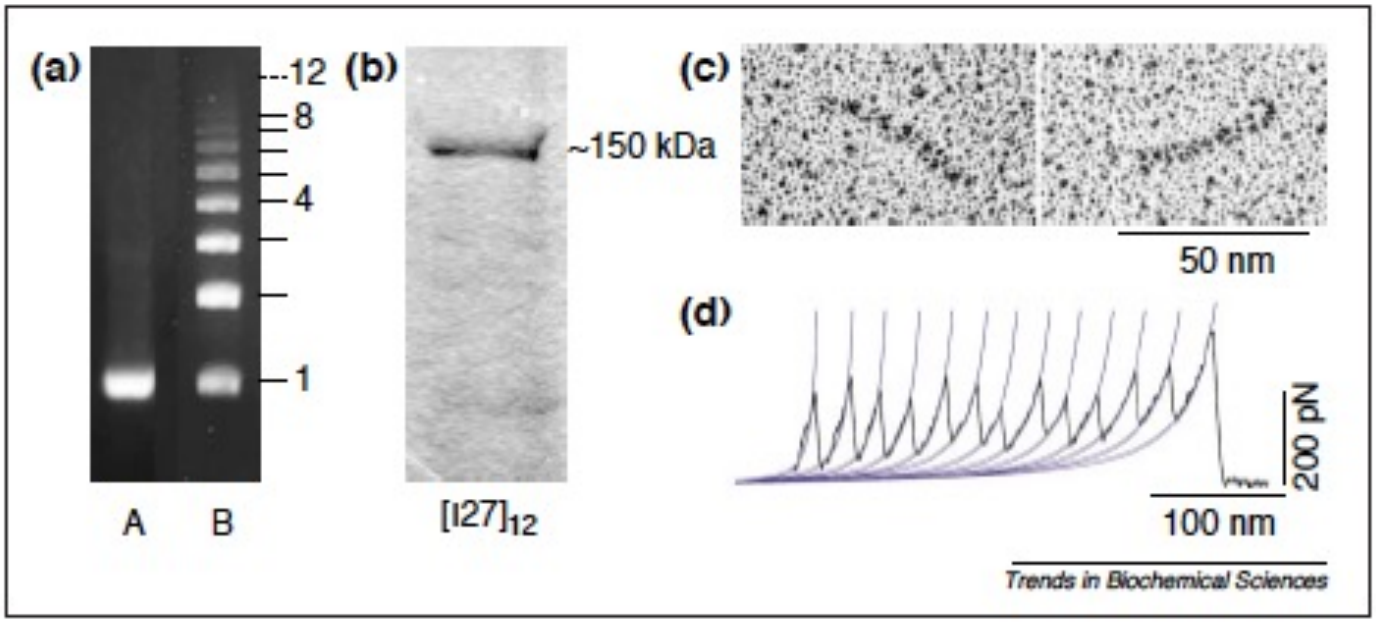
Regular sawtooth force pattern with five regularly spaced force peaks of

Fig. 33.5 Typical force–extension plot of the unfolding of a polyprotein consisting of seven I27 domains from the titin protein (also called I91 domains). Each peak corresponds to an unfolding event of a single domain. The unfolding force for each domain is ~200 pN. The dashed red line indicates a family of worm-like chain fits with a contour length spacing of 28.5 nm between unfolding events

Polyprotein force spectroscopy

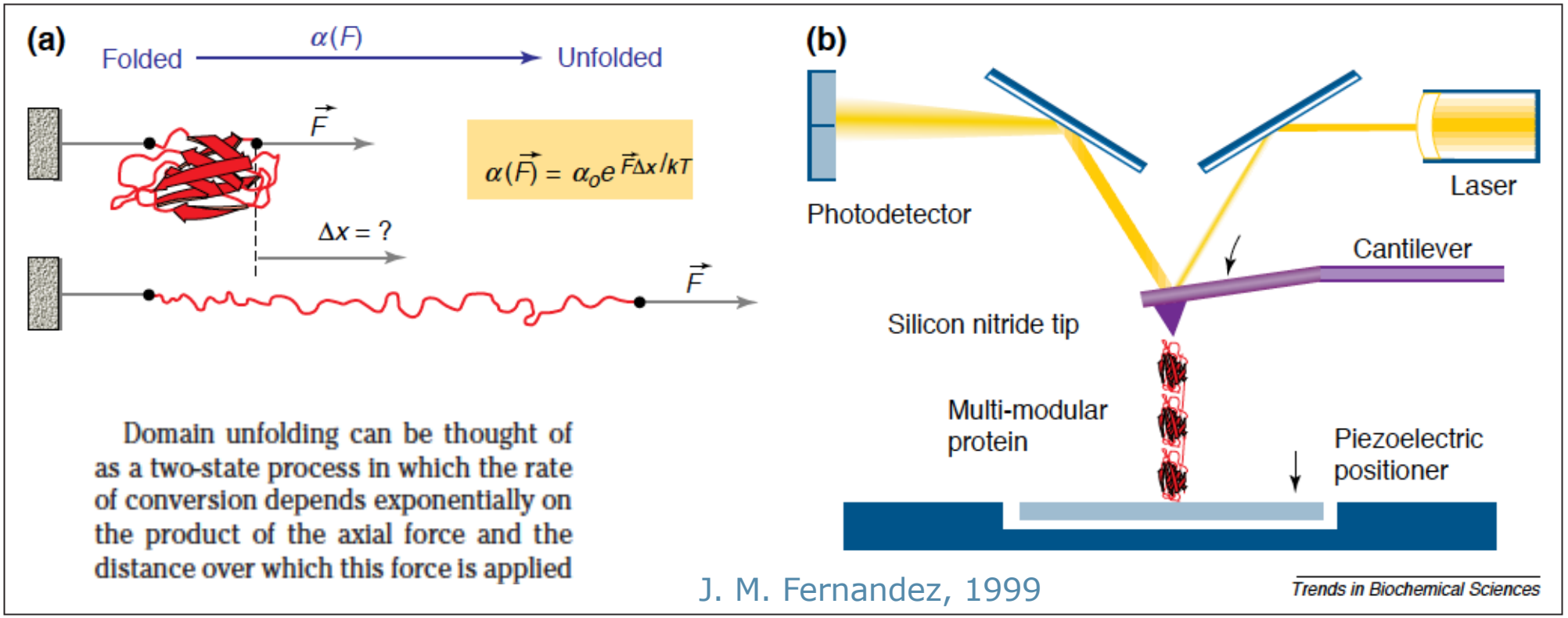
Engineered poly I27 Titin





Protein force spectroscopy

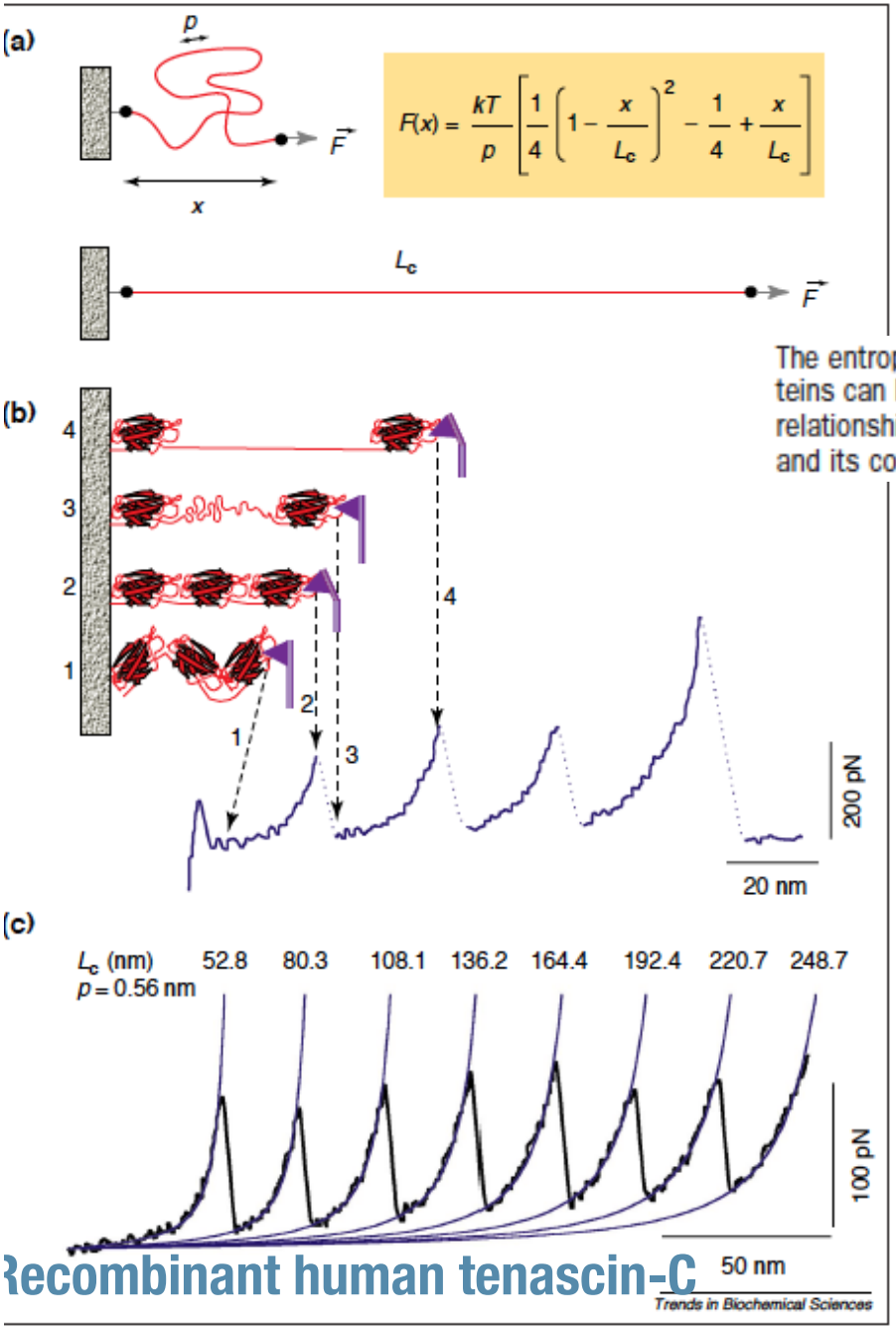
When a polymer is relaxed, it forms a coiled structure (maximizes the entropy of its segments). Extension of the polymer generates an opposing force (reduction in entropy) called ENTROPIC ELASTICITY: small extensions require little force, resistance to extension rises rapidly as the polymer approaches its full length



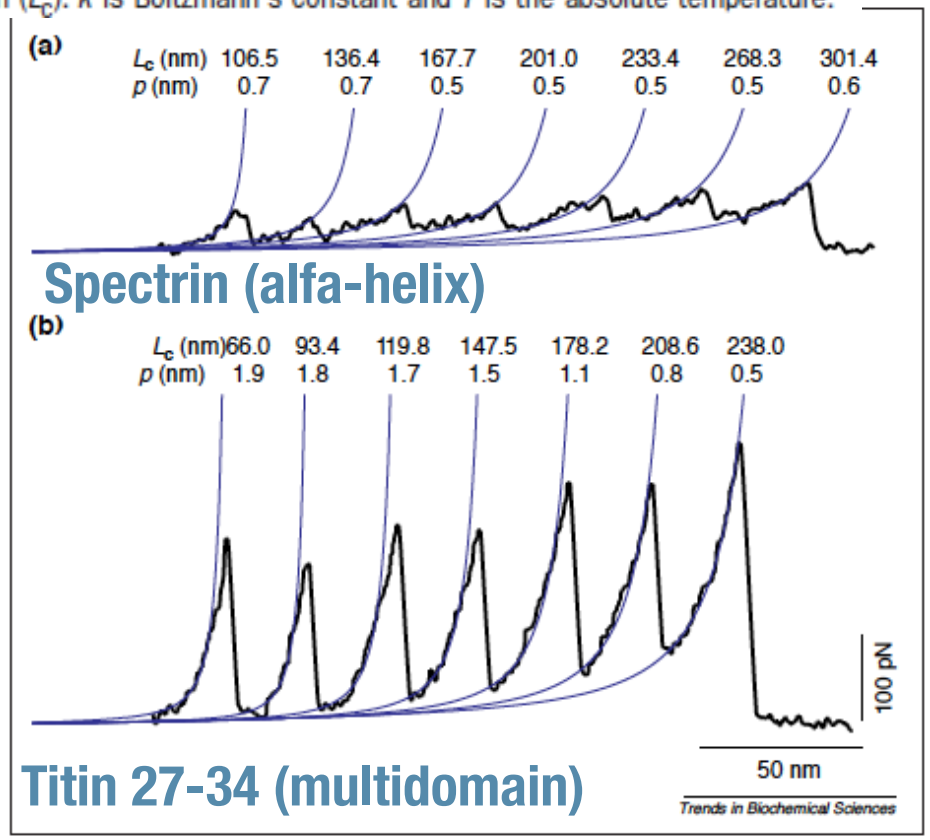
The unfolding of protein domains by an external force. **(a)** When axial stress is applied to a folded domain the protein will unravel. The inset shows an equation describing this transition, where F is the applied force, Δx is the distance over which the unfolding event occurs, α_0 is the rate constant in the absence of an applied force, k is Boltzmann's constant and T is the absolute temperature. Thus, the rate at which protein unfolding occurs increases exponentially with the applied force. This equation is similar to that describing the dissociation of non-covalent bonds placed under an external force[38,39]. **(b)** The force–extension mode of the atomic force microscope (AFM). When pressed against a layer of protein attached to a substrate, the silicon nitride tip can adsorb a single protein molecule. Extension of a molecule by retraction of the piezoelectric positioner results in deflection of the AFM cantilever. This deflection changes the angle of reflection of a laser beam striking the cantilever, which is measured as the change in output from a photodetector.

Polyprotein force spectroscopy

At small displacements, reduction in the number of conformations gives rise to entropic elasticity forces
At large extensions, tension in the molecular backbone may lead to enthalpic elasticity effects (bond deformation, rupture of intramolecular hydrogen bonds and even conformational changes of the entire molecule)



The entropic elasticity of proteins and domain unfolding. **(a)** The entropic elasticity of proteins can be described by the WLC (worm-like chain) equation (inset), which expresses the relationship between force (F) and extension (x) of a protein using its persistence length (p) and its contour length (L_c). k is Boltzmann's constant and T is the absolute temperature.

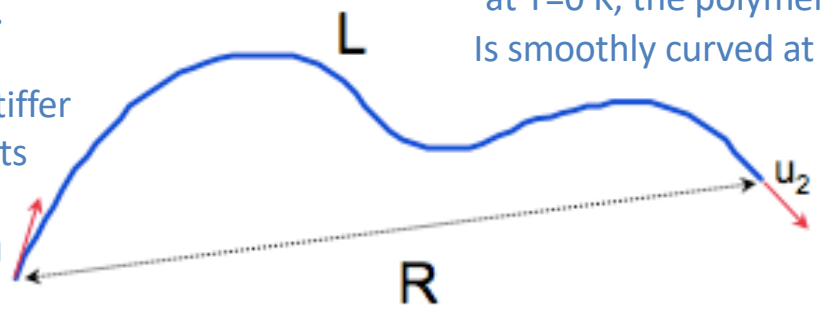


The behaviour of polymers under mechanical stress is described by the **worm-like chain (WLC) model of elasticity**

Equilibrium Statistic of a Worm-like Chain

Isotropic rod continuously flexible.
The worm-like chain model is particularly suited for describing stiffer polymers, with successive segments displaying a sort of cooperativity: all pointing in roughly the same direction

at T=0 K, the polymer adopts a rigid rod conformation
Is smoothly curved at RT



$$\left\langle \vec{u}_1 \cdot \vec{u}_2 \right\rangle = e^{-\frac{L}{P}}$$

The persistence length of the molecule, P, is the decay length through which the initial orientation of the molecule persist. It is a measure of the stiffness of a polymer chain.

This model describes a polymer as a continuous string of a given total (or contour) length. Bending of the polymer at any point influences the angle of the polymer for a distance, referred to as the **persistence length**, that reflects the polymer flexibility. The smaller the persistence length, the greater the entropy of the polymer and the greater the resistance to extension.
The persistence length and the contour length comprise the adjustable parameters of the WLC model.

Courtesy of Prof. C. Bustamante

Single molecule force spectroscopy

The statistical model used for describing the elastic behavior of the polymer is the WORM-LIKE CHAIN (WLC) model (proteins, DNA/RNA) and the FREELY-JOINTED CHAIN (FJC) model (polysaccharides)

WLC model

the polymer is an irregular curved filament, linear on the scale of the persistence length $\mathbf{l_p}$, which represents the stiffness of the molecule.
Molecules with low persistence length have a tendency to form coils.
Extension is limited by the contour length $\mathbf{L_c}$ of the molecule, **i.e.** the length of the linearly extended molecule without stretching the molecular backbone.

In this model, the force **F versus** extension **x** is approximately given by:

$$F(x) = k_b T / l_p [0.25(1 - x/L_c)^{-2} + x/L_c - 0.25]$$

Single molecule force spectroscopy

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FJC model
the polymer is considered as a series of rigid, orientationally independent statistical (Kuhn) segments, connected through flexible joints.
The segment length, or Kuhn length $\mathbf{l}_k$, is a direct measure of the chain stiffness and is related to the contour length by $\mathbf{L}c=\mathbf{n}\mathbf{l}_k$.

In this model, the extension **x** versus force **F** is approximately given by:

$$x(F) = L_c[\coth(Fl_k/k_bT) - k_bT/Fl_k]$$

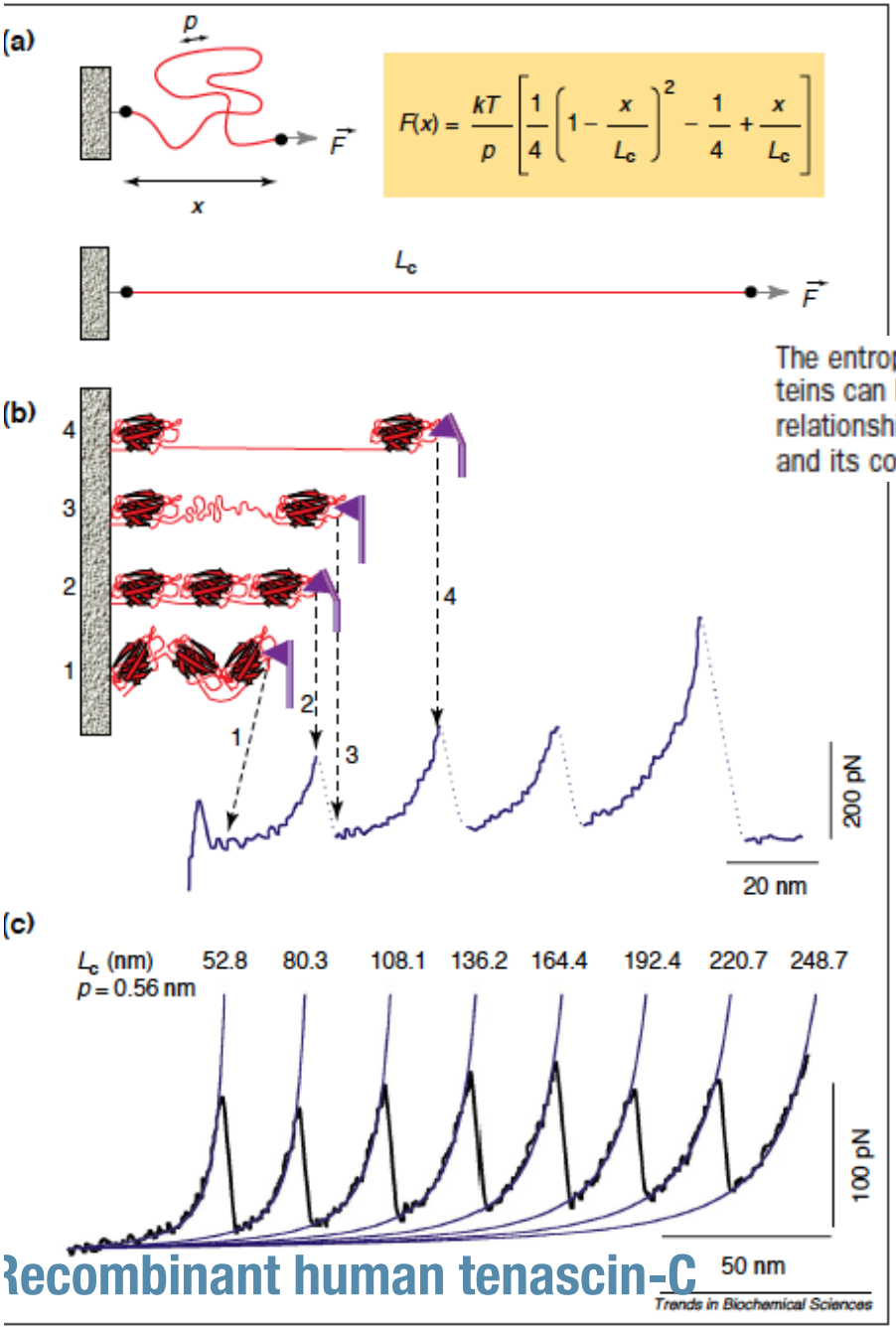
And, allowing Kuhn segments can stretch and align under force with elasticity k_s:

$$x(F) = L_c[\coth(Fl_k/k_bT) - k_bT/Fl_k][1 + nF/k_sL_c]$$

Polyprotein force spectroscopy

Interestingly, the measured unfolding forces generally increase with extension speed. Varying this parameter may provide an estimate of the unfolding off-rate and of the width of the unfolding energy barrier.

Reliable force data on a given molecule require recording several hundred force-curves using many independent tips and samples and proper model to fit data.



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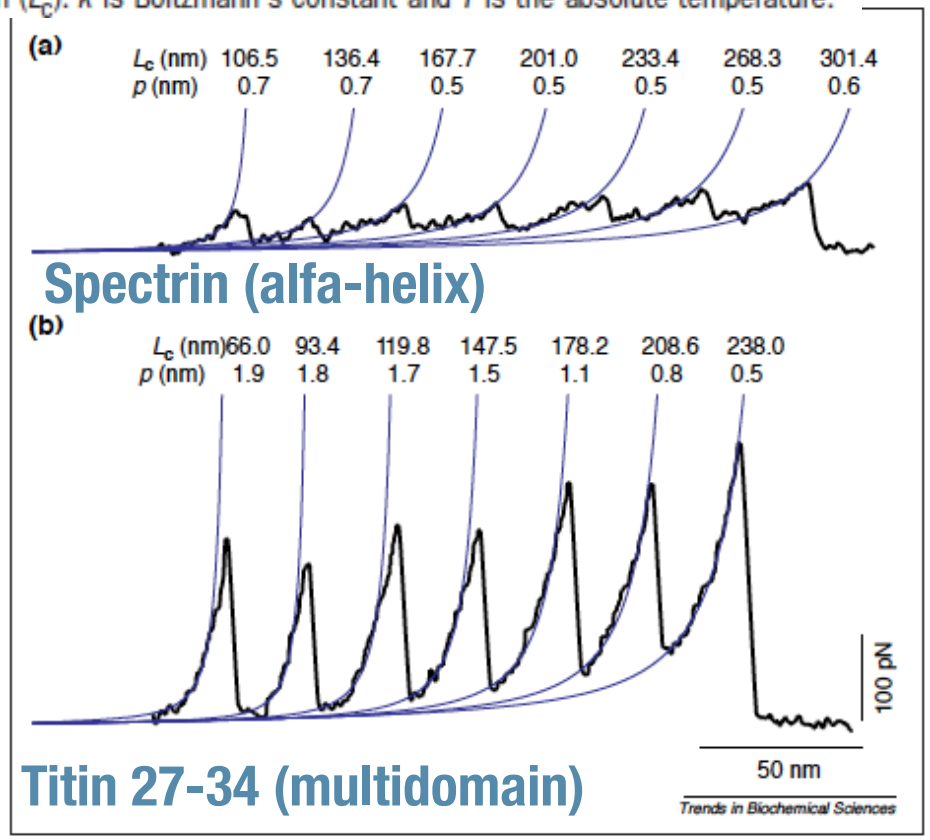
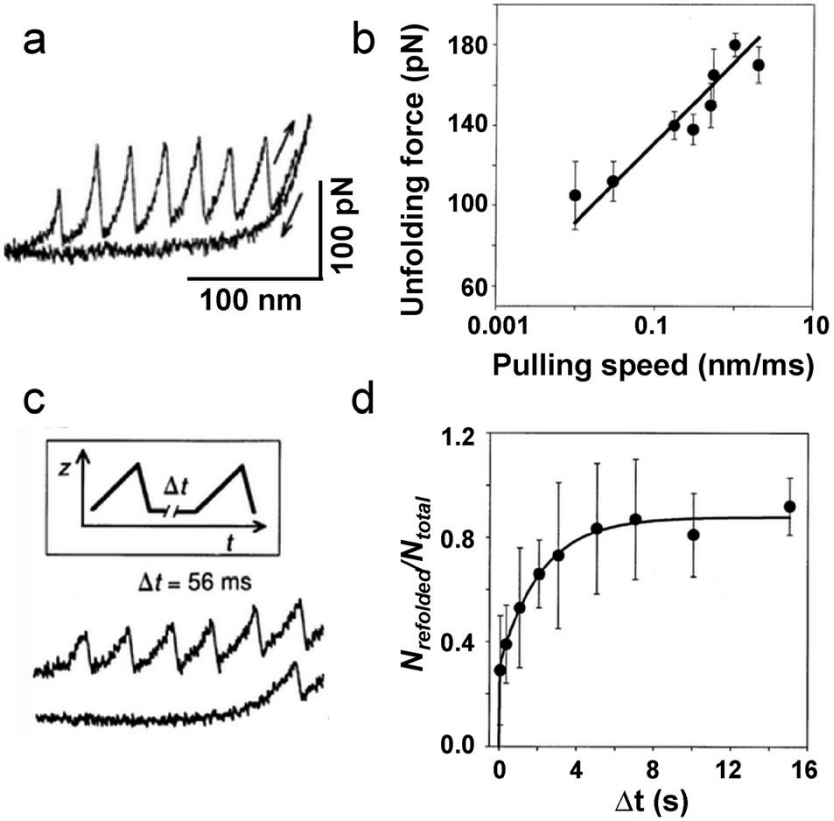


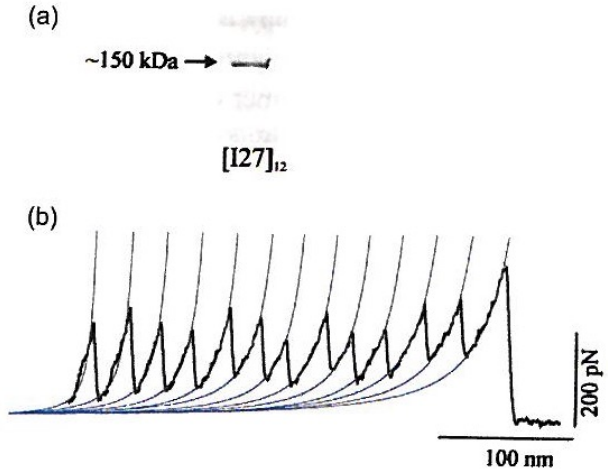
Unfolding-refolding



The data can be fitted using a Monte Carlo approach with a two-state model of the unfolding/refolding process, allowing the extraction of important parameters characterizing the unfolding reaction such as the distance to the unfolding transition state and the unfolding rate constant extrapolated to zero force

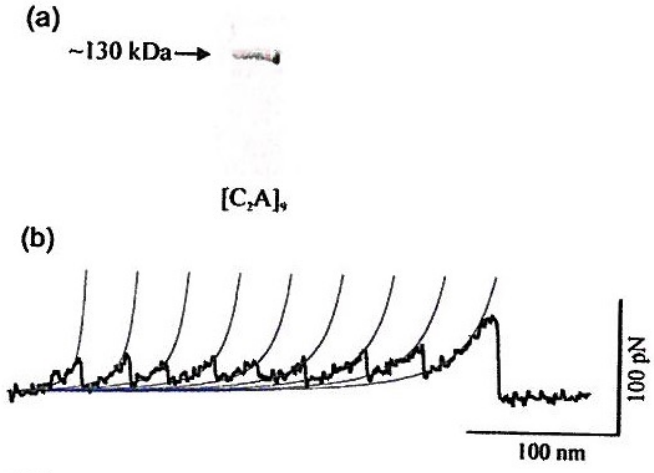
Fig. 3 Repeated unfolding and refolding cycles of tenascin. (a) The same TNfnAll fragment of tenascin was repeatedly stretched at various stretching speeds. (b) The average unfolding force *vs.* stretching speed. (c) A double-pulse experiment where the same molecule is stretched, relaxed for time Δt and stretched again. (d) The fraction of folded modules (counted from the second unfolding pulse) as a function of Δt.

Polyprotein force spectroscopy



F= 204 pN

δLc=28 nm



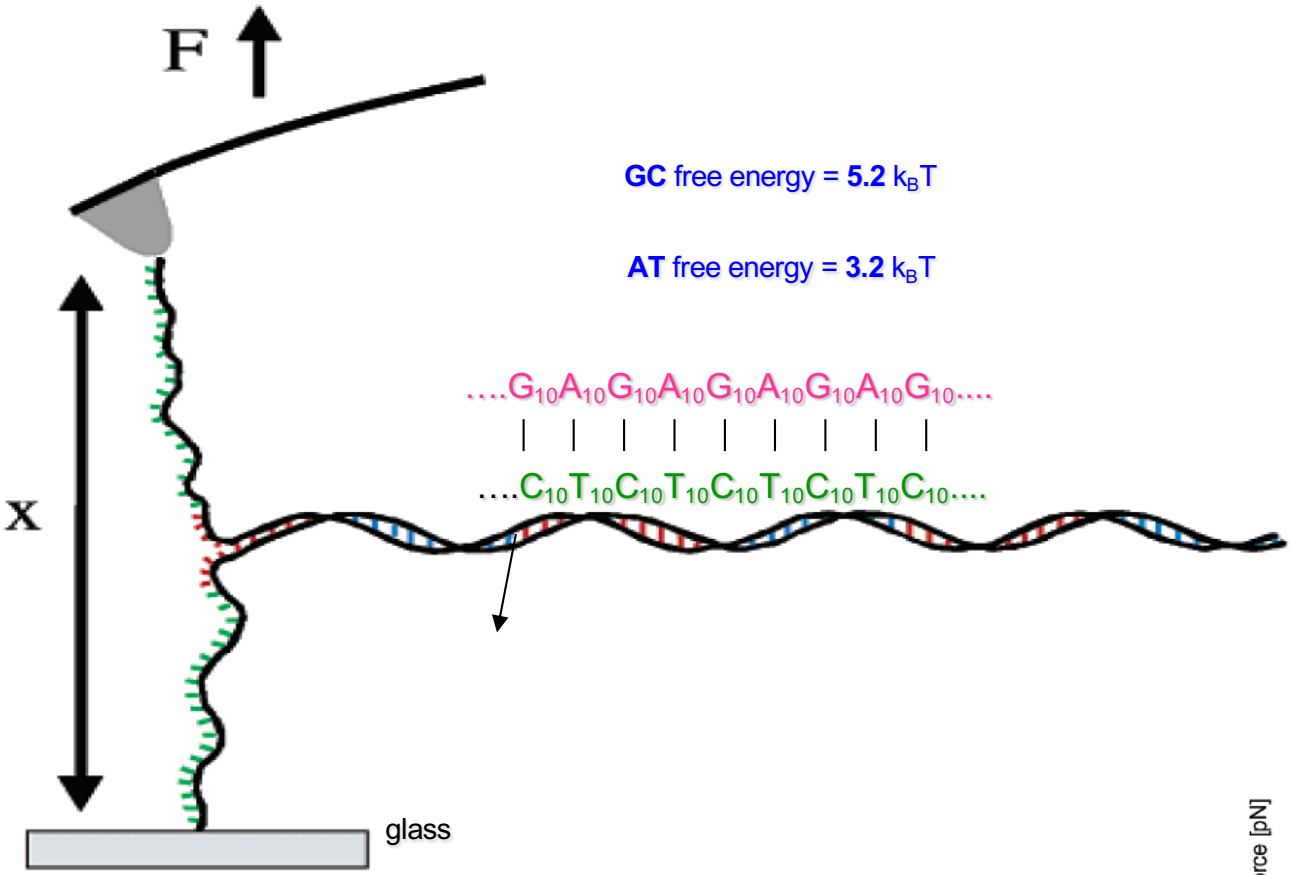
60 pN

38 nm

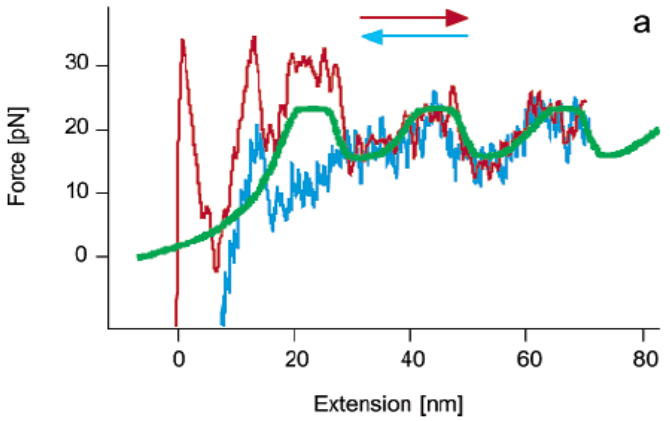
Two proteins with "all beta" structure and β-sandwich topology
-titin has seven β strands which fold face-to-face through backbone H-bonds and hydrophobic core interactions, perpendicular to stretching direction
-C2 β sandwich with 127 aminoacids arranged in 8 antiparallel strands.domains are in a zipper configuration

Herman E. Gaub (LMU Munich) : Unzipping DNA oligomers

Nano Letters **3**, 4, 493 (2003)



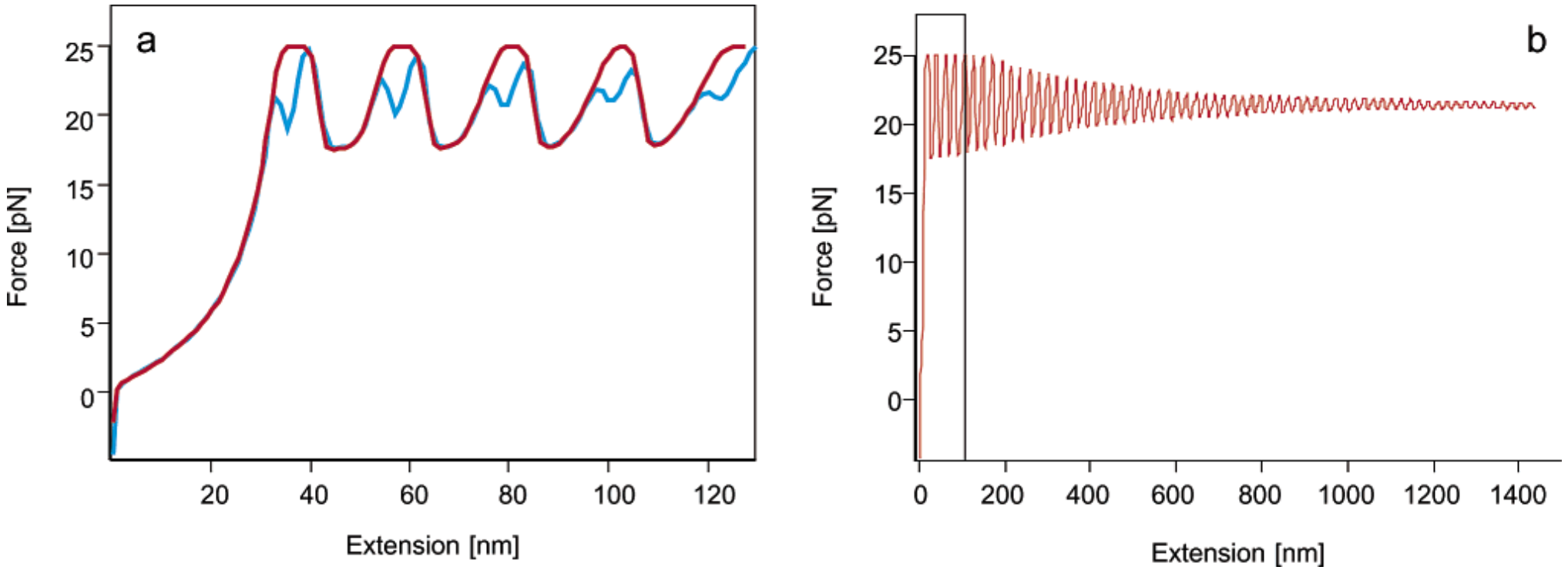
Complementary DNA oligonucleotides are chemically attached to an AFM tip and a glass slide. As the tip is brought in contact with the surface a ds-DNA forms, which can then be unzipped upon retractio the tip.

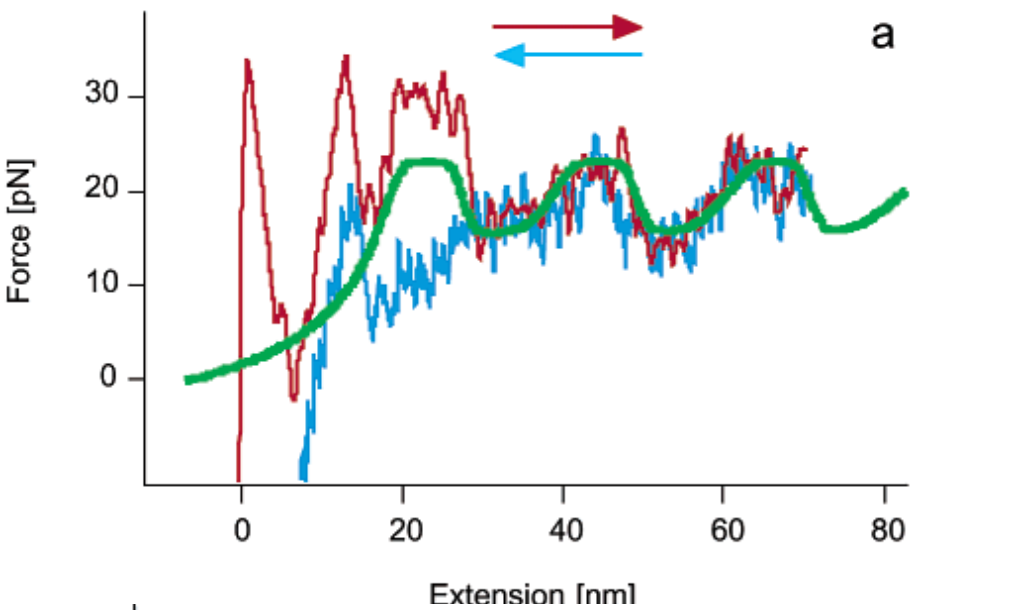


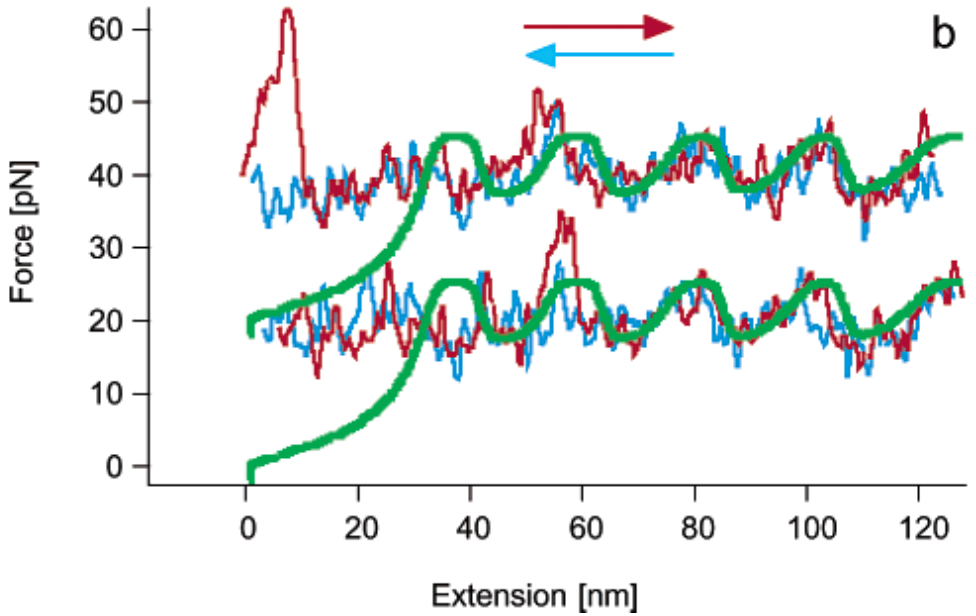
Experimental Results

Herman E. Gaub (LMU Munich) : Unzipping DNA oligomers

Nano Letters **3**, 4, 493 (2003)







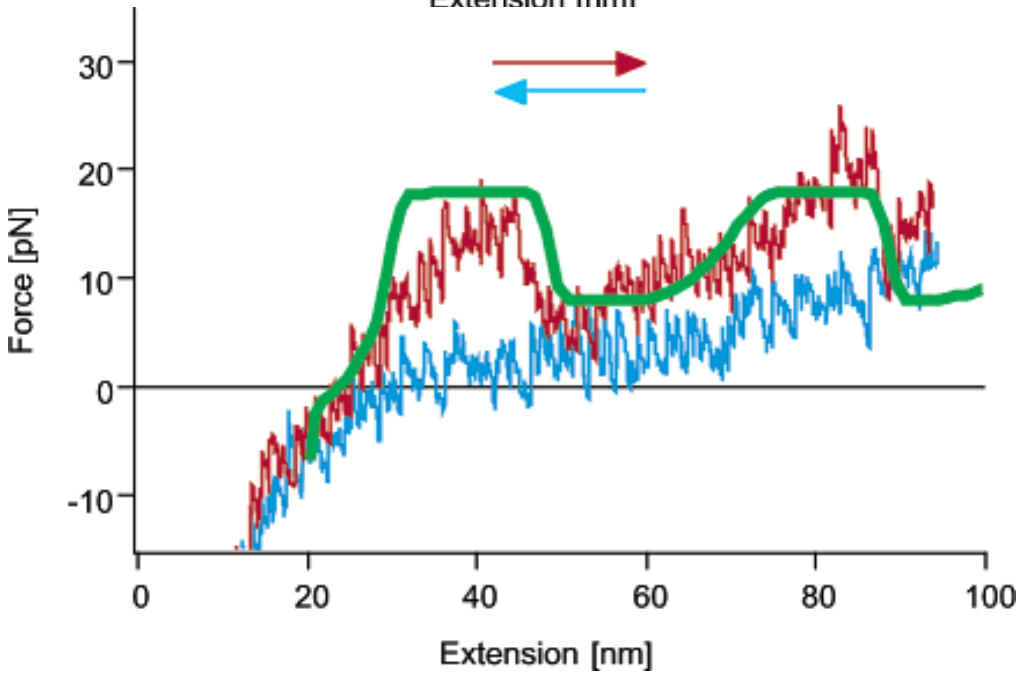


Figure 4. Force vs distance curve upon unzipping a dsDNA molecule with an alternating sequence of 20 pure AT and 20 pure GC base pairs ([dG20dA20dG20dA20dG20]([dC20dT20dC20dT20dC20]) and a simulated curve (green). The force upon unzipping the molecule varies because of the different stability of the AT and GC base pairs. The oscillation in force seen in the data corresponds well to the force vs distance profile calculated from the equilibrium thermodynamic model.