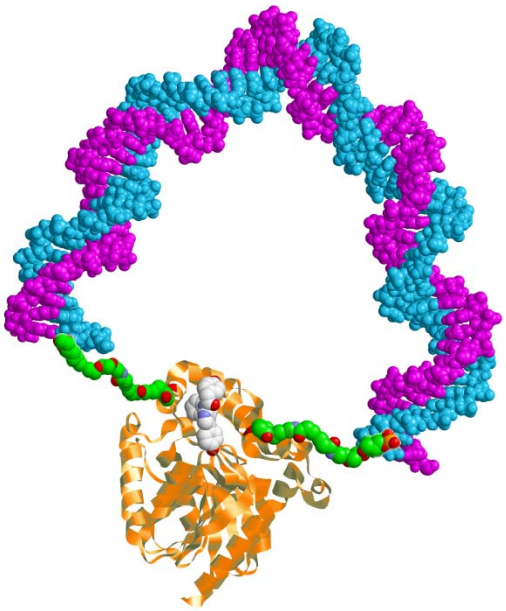
PAST PROJECTS

Mechanical control of Renilla Luciferase

Chiao-Yu Tseng, Yilin Zhan

Enzymes are *deformable molecules*: the molecular structure can exist in essentially a continuum of different conformations. These are accessible by applying mechanical stress to the molecule. Mechanics then provides a general molecular mechanism to modulate enzymatic *reactions*, by applying mechanical stresses which deform the enzyme. There are approximately 10^4 known different enzymatic reactions; each one can in principle be thus subjected to molecular control !

Here we control the activity of the enzyme luciferase from Renilla reniformis through a DNA spring attached to the enzyme. On the one hand, this study establishes that mechanical stress applied through the DNA springs is indeed a general method for the artificial control of enzymes. On the other hand, this system turns out to be especially convenient for the experimental study of the nonlinear mechanics of both the protein and the DNA spring. The reason is that the enzymatic reaction in question produces (albeit with small quantum yield) a blue photon, so measurements of the reaction speed are immediately transformed to measurements of light intensity.



In the construction of Fig. 1, a single-stranded DNA oligomer (typically 40 – 60 bases long) is covalently attached by the ends to two specific surface sites on the enzyme (labeled by Cys residues introduced by mutagenesis). Hybridization to the complementary strand rigidifies the DNA spring, which exerts a mechanical stress on the enzyme.

Fig. 1 *Cartoon of a protein-DNA chimera; the protein (Renilla Luciferase) and DNA spring are drawn approximately to scale.*

Mechanical control of the enzyme is shown in Fig. 2, where the light intensity produced by the chemical reaction is modulated by the stress exerted by the DNA spring on the enzyme.

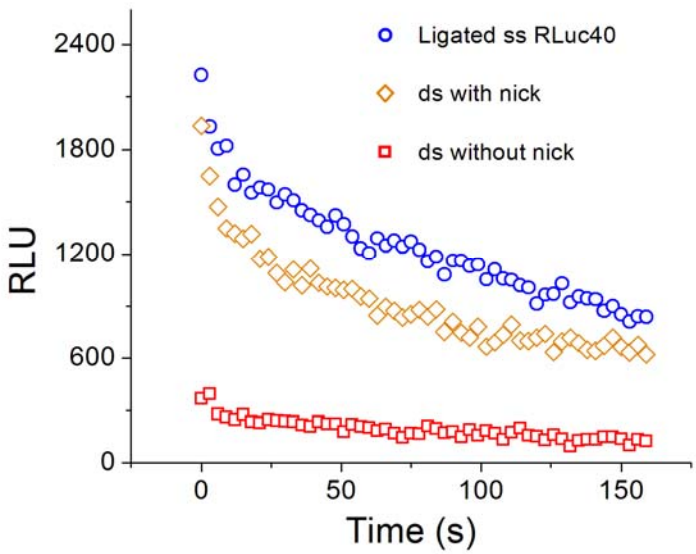


Fig. 2 *Time course of luminescence intensity (arbitary units) of the RLuc40 chimera (the supra-molecular construction of Fig. 1, with a 40mer DNA spring) for different states of mechanical stress. Blue circles: ss DNA (zero stress); orange diamonds: ds DNA with nick (small stress); red squares: ds DNA with no nick (larger stress). Luminescence intensity is proportional to reaction speed. The latter decreases in the course of time due to a combination of effects: substrate consumption, product inhibition, and inactivation of the enzyme after about 100 enzymatic turnovers.*

The molecule of Fig. 1 can be seen as a system of two coupled *nonlinear* springs: a DNA "leaf spring", which is compressed, and a protein spring, which is extended. Both springs operate across a softening transition into the nonlinear elasticity regime. Fig. 3 shows a model of the free energy of the system (energy of the DNA + energy of the enzyme) deduced from the measurements. The scenario is that of a phase transition: as a parameter is varied (here the restoring force of the enzyme spring in the nonlinear regime, F_0), the absolute minimum of the free energy switches from a state where mostly the enzyme is deformed to a state where mostly the DNA spring is deformed.

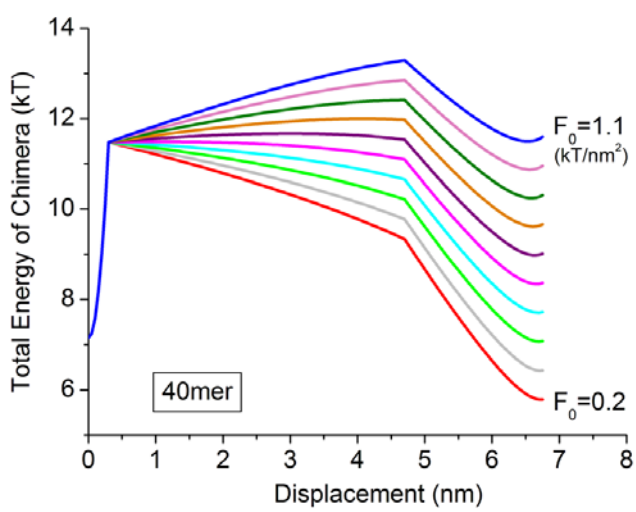


Fig. 3 *Elastic free energy (model) for the chimera construction of Fig. 1, as a function of the deformation of the enzyme. The different curves correspond to different values of the restoring force of the enzyme in the nonlinear regime. This is a model of two coupled nonlinear springs at finite temperature (the enzyme and the DNA), each undergoing a reversible softening transition beyond a critical "yield" deformation.*

In summary, the supra-molecular construction of Fig. 1 gives access to the nonlinear mechanics of these molecules.

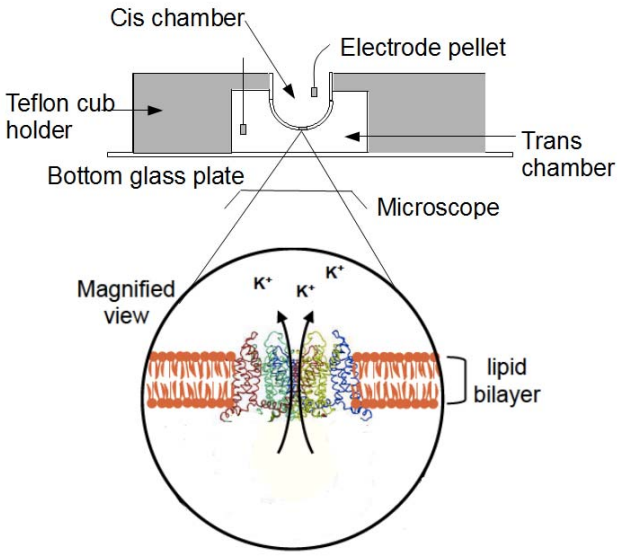
- C-Y. Tseng and G. Zocchi, "Mechanical control of Renilla Luciferase", J. Am. Chem. Soc. **135**, 11879 (2013).
- C-Y. Tseng and G. Zocchi, "Equilibrium softening of an enzyme explored with the DNA spring", Appl. Phys. Lett. **104**, 153702 (2014).
- Yilin Zhan and Giovanni Zocchi, "Flexibility of DNA/PNA, DNA/LNA, DNA/RNA hybrids measured with a nanoscale transducer", EPL **119**, 48005 (2017).

Synthetic control of ion channels

Andrew Wang, Amila Ariyaratne

Ion channels are an important group of proteins whose function entails large conformational motion. These molecules are pores embedded in the cell membrane which open and close on millisecond timescales, selectively allowing ionic currents to flow in and out of the cell. Specifically, voltage gated channels open and close in response to the polarization of the cell membrane; they are the molecular switches which sustain the action potential in nerve cells. The basic process is that these molecules undergo deformations in response to the large electric field across the membrane (of order 100 mV / 4 nm).

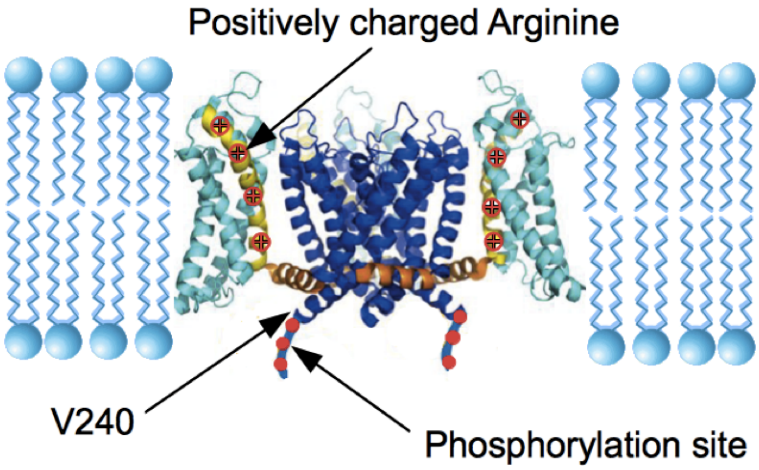
It is a matter of considerable interest to design new modes of control for ion channels: these can then be used in neuroscience research. Our approach is that such deformable molecules can always be controlled mechanically. Thus we design artificial control mechanisms based ultimately on exerting stresses on the molecule.



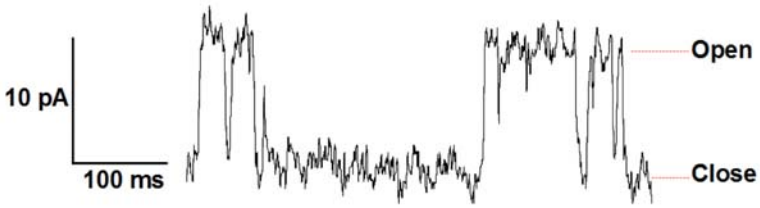
Cell free electrophysiology setup for testing ion channel response.

One example is to add phosphorylation sites to a voltage gated channel. The electric field due to the additional charges may bias the opening probability of the channel. With this construction we put the response of the channel under the control of a specific Kinase, adding a new layer of chemical control. The construction is genetically encoded and can, in principle, be transferred into the living neuron. We explore a number of different designs for the artificial control of channels. The response of the modified channels is tested in a supported bilayer electrophysiology setup; the channels are expressed in bacteria and reconstituted in vesicles; the latter are fused to the artificial bilayer.

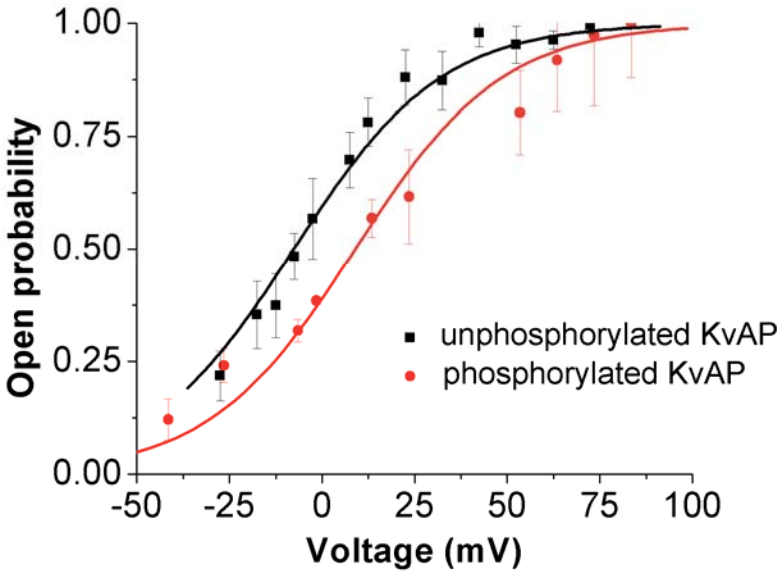
This work is in collaboration with the group of Elisha Moses at the Weizmann Institute in Israel: http://www.weizmann.ac.il/complex/EMoses//home.html .



Ion channel geometry with artificial phosphorylation sites. Opening and closing of the channel is controlled by the position of the α-helix (yellow) containing the positively charged Arginines; phosphorylation (addition of two negative charges) at the position shown may bias the position of this α-helix through electrostatic interactions, thus biasing the opening probability of the channel. The structure shown (Mammalian Shaker Kv 1.2, PDB 2A79) is that of a potassium channel with a structure similar to the KvAP used in our studies.



Single-channel current trace of the modified KvAP ion channels after phosphorylation



Dependence of the opening probability (the fraction of time the channel is open) on the membrane voltage for the modified KvAP ion channels before and after phosphorylation. The red dots indicate the phosphorylated ion channels, while the black squares are from the unphosphorylated ion channels. The effect of phosphorylation is to inhibit the channels by translating the opening probability curve towards more positive voltages.

This appears to be the first demonstration of an artificial phosphorylation site which modulates channel activity. The study exemplifies a general strategy to add chemical control to voltage gated channels.

- A. Wang and G. Zocchi, "Artificial modulation of the gating behavior of a K+ channel in a KvAP-DNA chimera", PLoS ONE 6(4), e18598 (2011).
- A. Ariyaratne and G. Zocchi, "Artificial phosphorylation sites modulate the activity of a voltage gated potassium channel", Phys. Rev. E **91**, 032701 (2015).

The elastic energy of sharply bent DNA

Hao Qu, Chiao-Yu Tseng, Yong Wang, Daniel Sanchez, Juan Wang

Double stranded DNA has been recently used as a "molecular spring" to mechanically perturb the conformation of proteins, ribozymes and peptides [1, 2], and therefore mechanically control a variety of chemical reactions. We are thus interested in characterizing quantitatively the mechanics of the DNA springs. The relevant regime is one of sharp bending of the DNA ($x << L << \ell_d$ where x is the end-to-end distance (EED) of the DNA, L the contour length, $\ell_d \approx 50$ nm the persistence length); this is also a regime of interest in many problems of DNA packaging (e.g. inside viruses). But here we look at the polymer physics problem: is it possible to "replace" the complex chemical structure of the molecule with a small number of effective parameters as far as the mechanical properties are concerned ?

We address this question with the stressed molecule shown in Figure 1, where the ds DNA part (red & blue) is bent and the ss part (blue) is stretched. We measure the elastic energy $E_{tot} = E_d + E_s$ of this molecule by thermodynamics methods, and extract the bending energy E_d of ds DNA (since E_s , the stretching energy of ss DNA, is well known).

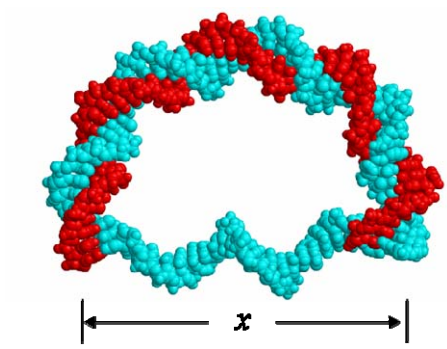


Fig. 1 *This DNA molecule stores a large elastic energy, resulting from the bending of the ds (upper) part and stretching of the ss (lower) part).*

Increasing the number of bases N_s in the ss part of the molecule has the effect of softening the ss spring, thus increasing x (the EED). Measuring the elastic energy for varying N_s is then equivalent to measuring for varying x.

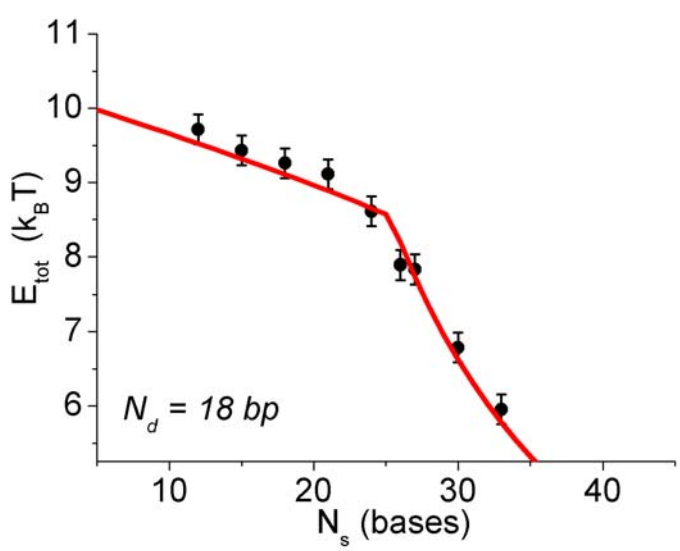


Fig. 2 *The elastic energy E_{tot} measured for the stressed molecul of Fig. 1, for varying N_s (number of bases in the ss part), at fixed N_d = 18 (number of bp in the ds part). The "knee" in the curve corresponds to the appearance of a kink in the ds DNA. The line is a fit using the formula below for the bending energy of ds DNA.*

We find that bent ds DNA develops a kink where the local torque exceeds the critical value $\tau_c \approx 30\ pN \times nm$. In this regime the energy is linear in the kink angle (i.e. the torque is constant $= \tau_c$ at the kink). The bending energy E_d in the linear and nonlinear regimes depends on only two effective parameters, the bending modulus $B = kT\ \ell_d \approx 200\ pN \times nm^2$ and the critical torque τ_c, and is given by the following approximate expression (plotted in Fig. 3):

$$E_d(x) = \begin{cases} \tau_c \arccos\left(\dfrac{x}{2R}\right) & \text{for} \quad 0 < x < x_c \\ \dfrac{5B}{L}\ \dfrac{x_0 - x}{2L} - T \ln\left(\dfrac{2L - x}{2L - x_0}\right) & \text{for} \quad x_c < x < x_0 \end{cases}$$

where $R = L\left(1 - \dfrac{2}{45}\gamma^2\right)$, $x_0 = \langle x \rangle_{f=0} = 2L\left(1 - \dfrac{LT}{5B}\right)$ is the EED at zero force, and $\gamma = L\tau_c/(2B)$. The upper form corresponds to the DNA being kinked, the lower to the DNA being smoothly bent. The critical EED x_c is found by equating the upper and lower expressions. The contour length of the DNA is $2L = 0.33\ \text{nm} \times N_d$. This expression refers to a situation of zero torque boundary conditions at the ends of the molecule (as if the two ends of the DNA were pulled together by a string).

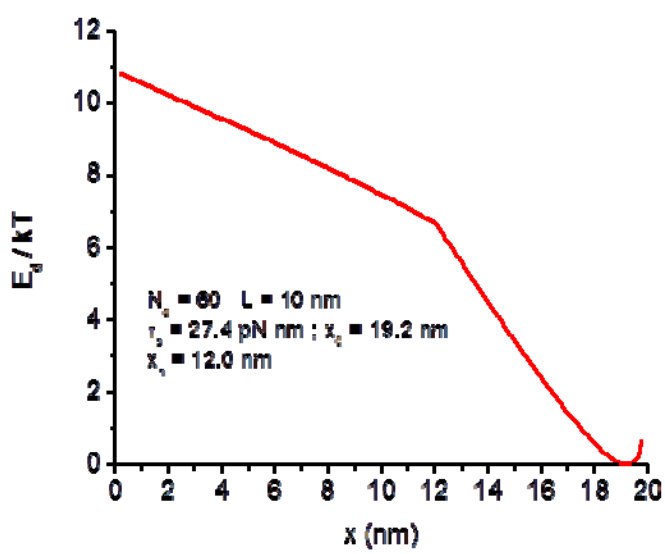


Fig. 3 *The bending energy function (in units of kT) for a 60 bases long ds DNA molecule (contour length 2L = 20 nm) plotted vs EED x (in nm) according to the formula above, for*

$B = 200\ pN \times nm^2$

$\tau_c \approx 27.4\ pN \times nm$

The part of the curve for $x > x_c$ (to the right of the break) is the worm-like-chain energy; the part for $x < x_c$ corresponds to the presence of a kink in the molecule.

It is the beauty of polymer physics that some physical properties of large molecules can be represented through a small number of effective parameters which "summarize" the complex chemical structure: for DNA elasticity in the linear (worm-like-chain) regime, such is the role of the persistence length; to include the nonlinear regime of large bending, one additional parameter suffices, namely the critical torque τ_c.

- D. Sanchez, H. Qu, D. Bulla and G. Zocchi, "DNA kinks and bubbles: Temperature dependence of the elastic energy of sharply bent 10-nm-size DNA molecules", Phys. Rev. E **87**, 022710 (2013).
- J. Wang, H. Qu, and G. Zocchi, "Critical bending torque of DNA is a materials parameter independent of local base sequence", Phys. Rev. E **88**, 032712 (2013).
- H. Qu, Y. Wang, C-Y. Tseng, and G. Zocchi, "Critical torque for kink formation in double stranded DNA", Phys. Rev. X **1**, 021008 (2011).
- Hao Qu and Giovanni Zocchi, "The complete bending energy function for nicked DNA", Europhys. Lett. **94**, 18003 (2011).
- H. Qu, C-Y. Tseng, Y. Wang, A. J. Levine, and G. Zocchi, "The elastic energy of sharply bent nicked DNA", Europhys. Lett. **90**, 18003 (2010).
- A. Wang and G. Zocchi, "Elastic energy driven polymerization", Biophys. J. **96**, 2344-52 (2009).

[1] G. Zocchi, "Controlling proteins through molecular springs", Ann. Rev. Biophys. **38**, 75-88 (2009).

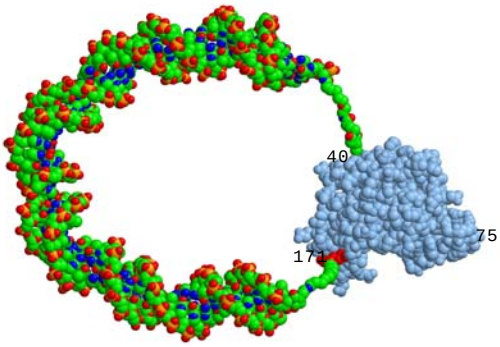
[2] Röglin L.; Altenbrunn F.; Seitz O., "DNA and RNA-controlled switching of protein kinase activity**",** Chembiochem **10**, 758-65 (2009).

Mechano-chemistry with the enzyme Guanylate Kinase

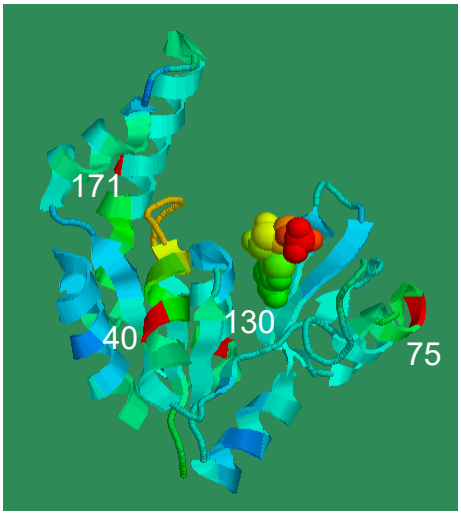
Chiao-Yu Tseng, Andrew Wang

The aminoacid sequence determines the folded structure of a protein, but this structure is "soft". Indeed, ligand binding events often elicit large conformational changes of the folded structure – the phenomena of allostery and induced fit.

Here we ask: can we control proteins mechanically ? (The answer is yes). We have learned how to establish a suitable force field, using DNA "molecular springs" (see Figure). We are establishing a map of the equilibrium response of one model system: the enzyme Guanylate Kinase, which catalyzes phosphoryl transfer from ATP to GMP. We measure how the mechanical stress modulates the binding affinities for the two substrates and the catalytic rate k_{cat}. We characterize the perturbation applied to the protein by the elastic energy of the molecule; we deduce that the deformation of the protein under stress is relatively small in this case. The reason is that the enzyme is mechanically stiffer than the DNA spring. There are many questions: does it make a difference where we apply the mechanical stress ? Is it possible to affect the kinetic parameters separately ? We chose 3 different attachment points for the DNA spring (residues 75/171, 40/171, and 40/130, see Figure) and obtained different specific responses. This means that many different conformational states are accessible (and can be isolated at room temperature) by mechanically stressing the molecule.
In general, these protein-DNA chimeras are a wonderful tool to study mechano-chemical coupling in enzymes.



Cartoon of a protein-DNA chimera; the protein (GK) and DNA spring are drawn approximately to scale. The distance between residues 40 and 171 is ~ 4 nm.



GK structure (1ZNX) with bound GMP; the different spring attachment sites are colored red.

- C-Y. Tseng, A. Wang, and G. Zocchi, "Mechano-chemistry of the enzyme Guanylate Kinase", Europhys. Lett. **91**, 18005 (2010).

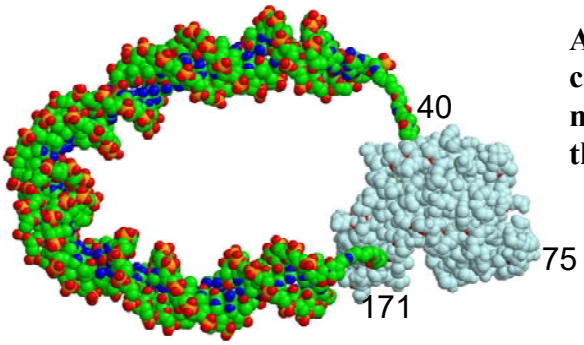
- B. Choi and G. Zocchi, “Guanylate Kinase, Induced Fit, and the Allosteric Spring Probe”, Biophys. J. **92**, 1651-58 (2007).
- B. Choi, G. Zocchi, Y. Wu, S. Chan, L. Jeanne Perry, “Allosteric control through mechanical tension”, Phys. Rev. Lett. **95**, 078102 (2005).

Partitioning of the elastic energy in protein-DNA chimeras

Chiao-Yu Tseng, Andrew Wang

in collaboration with: *Biljana Rolih, Alex Levine*

Just like in a macroscopic solid, in a stressed molecule the elastic energy is not necessarily distributed uniformly across the molecule. In the protein-DNA chimera of the Figure, the DNA spring is 60 bp long. We can measure the elastic energy E_{el} of the whole molecule by the thermodynamic method above, so the question arises how is this energy partitioned between protein and DNA. We attach the DNA spring at different places on the protein, and find relatively small differences in E_{el} . Combining such measurements with some modeling (in collaboration with the group of Alex Levine – UCLA Chemistry) we conclude that for the molecule in the Figure most of the elastic energy is in the DNA spring (so the perturbation on the protein is small). The reason is kink formation in the DNA (see Project "*Elastic energy of sharply bent DNA*"). This is a system of two coupled non-linear springs (the DNA and the protein), so expect qualitative changes in behavior (bifurcations) as control parameters (e.g. the length of the DNA spring) are changed.
This is a specific example of a more general question of partitioning (or "focusing") of elastic energy in macromolecular structures.



A mechanically stressed protein-DNA chimera. The elastic energy of this molecule is of order ~ 15 kT, and most of the energy is in the DNA spring.

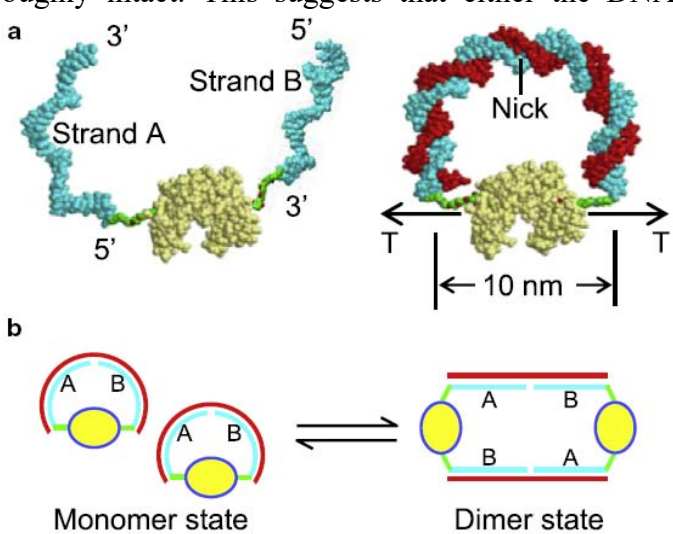
- Tseng, C-Y., A. Wang, G. Zocchi, B. Rolih, A. J. Levine, "The elastic energy of protein-DNA chimeras", *Phys. Rev. E* **80**, 061912 (2009).

Elastic Energy Driven Self-Assembly

Andrew Wang

Protein-DNA chimeras are designer stressed molecules where a DNA "molecular spring" perturbs the conformation of the protein. One purpose of this construction is to mechanically control the enzymatic activity of the protein (see Project *Mechano-chemistry with the enzyme Guanylate Kinase*); another purpose is to study the mechanical response of the protein structure. To characterize the mechanical perturbation the simplest measure is the elastic energy injected into the protein (a stress is an elastic energy per unit volume), but how does one measure the elastic energy of a molecule ?
In the system below, this elastic energy drives a dimerization process: the mechanical stress, provided by the DNA molecular spring, destabilizes the monomer state, but is relaxed in the dimer (Fig. 1). The monomer-dimer equilibrium provides a thermodynamic measurement of the elastic energy F_{el} of the monomer:

$F_{el} = \frac{1}{2} kT \ln \frac{X_D}{X_M^2}$ where X_M (X_D) is the mole fraction of monomers (dimers). For the molecule below, we measure the elastic energy of the monomer $F_{el} \approx 9.2\ kT$, which is too small if the DNA bends according to linear elasticity and the protein structure is roughly intact. This suggests that either the DNA is kinked or the protein partially unfolds. This brings us to the question of how the elastic energy is partitioned between the DNA spring and the protein.



This protein-DNA chimera is mechanically stressed (upper panel), but, because of the nick, it can relax by dimerizing (lower panel).

- Wang, A., and G. Zocchi, "Elastic energy driven polymerization", *Biophys. J.* **96**, 2344 (2009).

Dynamics of conformational changes in DNA

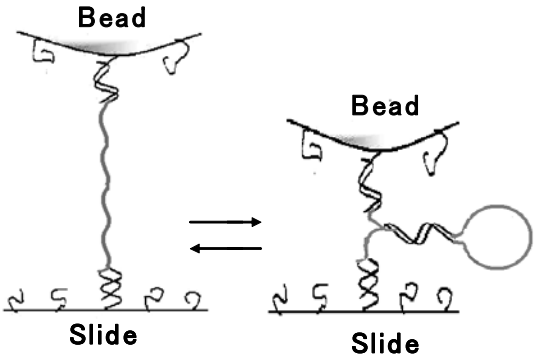
Jeungphill Hanne

in collaboration with: *Nikos Voulgarakis, Alan Bishop, Kim Rasmussen*

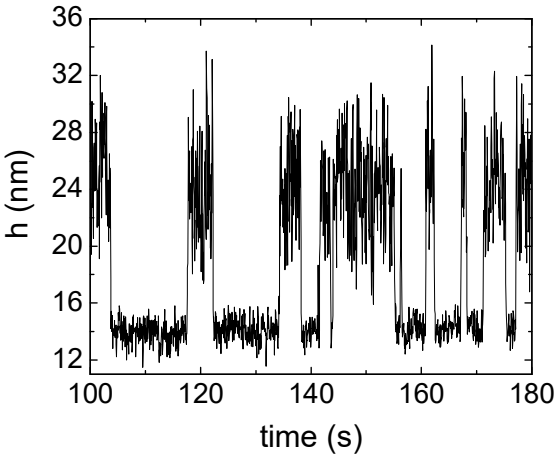
What we know about conformational changes of proteins and DNA comes mostly from structural studies. The dynamics of these processes is much less explored, because of a lack of experimental techniques. Single molecule methods are in principle the most straightforward way to study dynamics.

Here we use the single-molecule method based on evanescent wave scattering developed in the lab to study the dynamics of opening and closing of DNA hairpins.

This experiment differs from previous studies of hairpins in that the force field is non-homogeneous in space on the scale of the hairpin, and the ends of the DNA hairpin are coupled to the solid surfaces through very short (~ 5 nm) arms. This work is in collaboration with the Bishop-Rasmussen group at Los Alamos National Lab, where they study hairpin opening dynamics using a reduced-degrees-of-freedom statistical mechanics model (the Peyrard-Bishop-Dauxois model of DNA melting). This allows informative comparisons between model and experiments.



The 1 micron diameter bead is tethered to the slide by a single 74 bases long ssDNA molecule containing the 40 bases DNA hairpin. The average position of the bead moves up and down as the hairpin opens and closes.



Part of a time series of the bead's position with respect to the slide, showing the DNA hairpin tether repeatedly switching from the open (larger h) to the closed (smaller h) state.

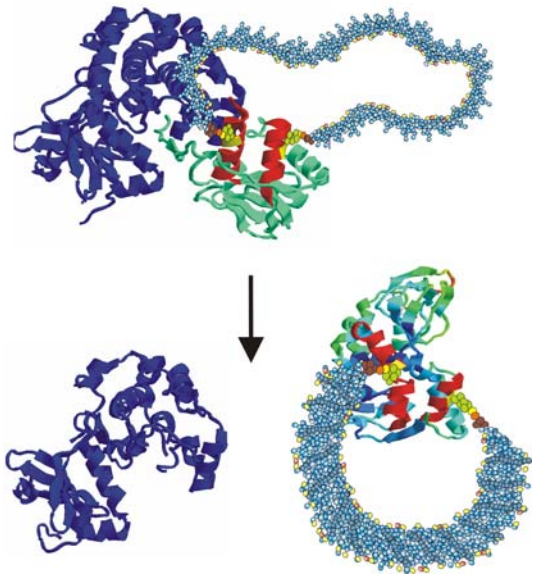
- J. Hanne, G. Zocchi, N. K. Voulgarakis, A. R. Bishop and K. Ø. Rasmussen, "Opening rates of DNA hairpins: experiment and model", Phys. Rev. E **76**, 011909-916 (2007).

Mimicking cAMP dependent Allostery of PKA

Brian Choi

Protein Kinases are enzymes which phosphorylate protein substrates, and are typically involved in signal transduction. Protein Kinase A (PKA) in particular plays a crucial role in numerous signaling pathways and metabolic processes. PKA is allosterically regulated by cAMP; it is in fact the primary receptor for cAMP in eukaryotic cells. The tetrameric enzyme, composed of two regulatory (RS) and two catalytic (CS) subunits, is inactive, because the RS binds to the CS through a surface of contact which includes the catalytic site, which is thus not accessible in this state. Upon cAMP binding the RS undergoes a conformational change which causes the CS to dissociate from the complex, activating catalysis. Part of this conformational change consists of the relative displacement of the red α-helices displayed in the Figure below.

The aim of this project is to study this allosteric mechanism by mechanically forcing conformational changes of the RS which elicit dissociation of the CS. We have shown that directly applying the mechanical stress on two elements of the protein's secondary structure which are known to move with respect to each other in the cAMP-induced conformational change indeed activates the complex, roughly as effectively as the presence of cAMP. The mechanical stress is exerted by a "molecular spring" made of a short piece of DNA, which we chemically couple to the regulatory subunit by attaching the ends of the DNA to Cysteine residues introduced at specific locations by site directed mutagenesis. The stiffness of the DNA spring can be varied externally by hybridization with complementary DNA of varying lengths, providing external control over the mechanical stress.



The cAMP-induced conformational change of the regulatory subunit (RS: green) consists in part of a relative displacement of the two red α-helices. We directly apply a mechanical stress to the RS which favors this conformational motion, by attaching a molecular spring to the two helices. Under tension (lower part of the Figure), the spring pulls the helices apart. We obtain dissociation of the catalytic subunit (CS: blue) and consequently activation of the enzyme.

- B. Choi and G. Zocchi, "Mimicking cAMP Dependent Allosteric Control of Protein Kinase A through Mechanical Tension", JACS **128**, 8541-48 (2006).

Artificial Allosteric Control of Maltose Binding Protein

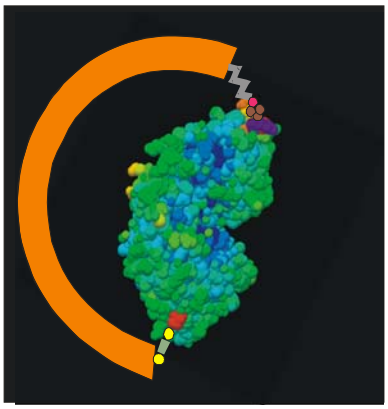
Brian Choi

in collaboration with: *Stephen Canale, Yim Wu, Sum Chan, Jeanne Perry*

Allosteric control is the mechanism whereby a control molecule binds to a site on a protein, inducing a conformational change at a distant site, which affects the function of the protein. It is the fundamental molecular control mechanism in the cell, the basis of many steps in the signaling pathways, including regulation of gene expression.

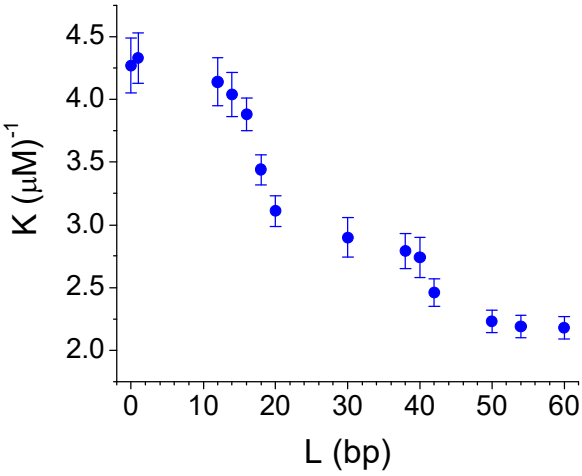
We are building artificial allosteric control modules on different proteins, based on a new molecular engineering approach, which allows to exert a mechanical tension on the protein, externally controlled. The fundamental question we address is the physical nature of allosteric control (what does it take to make the protein change conformation ?). Biotechnology applications we foresee for the new molecules include a new generation of amplified molecular probes, and "smart drugs".

In the case of the Maltose Binding Protein (MBP), we show that a mechanical stress which favors the "open" conformation of the molecule, applied by means of a molecular spring attached as shown below, lowers the binding affinity for maltose. In fact, the binding affinity can be modulated by varying the mechanical stress.



Tension in the DNA "molecular spring" favors the open conformation of the protein, lowering the binding affinity for the substrate.

Mechanical modulation of the binding affinity of MBP for maltose. As the stiffness of the molecular spring is increased (increasing L = length of the complementary strand hybridized to the ss DNA of the chimera above), the binding affinity K decreases.



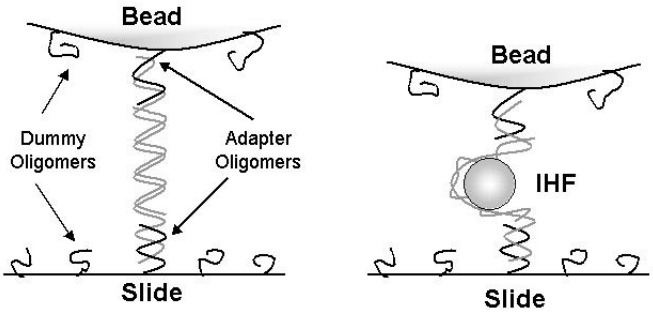
- B. Choi, G. Zocchi, S. Canale, Y. Wu, S. Chan, L. Jeanne Perry, "Artificial allosteric control of Maltose Binding Protein", Phys. Rev. Lett. **94**, 038103 (2005).

Dynamics of Protein-DNA interactions

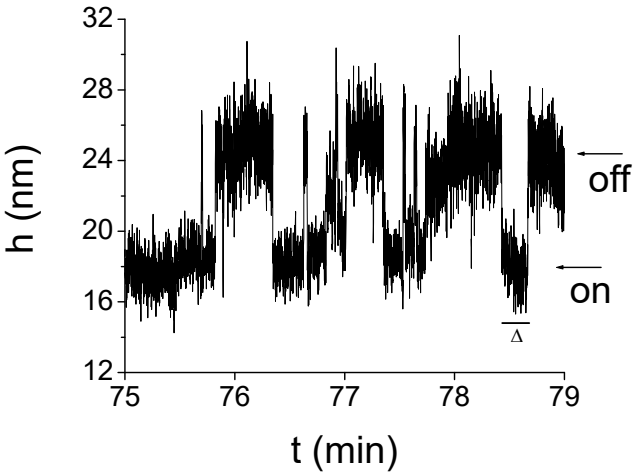
Sanhita Dixit, Mukta Singh-Zocchi, Jeungphill Hanne

DNA binding proteins can induce substantial deformations of the DNA, since typical binding energies are comparable to the work required to locally bend or twist the DNA. Single-molecule methods offer unique opportunities to study the dynamics of such complex bio-molecular interactions. In this experiment we "see" in real time the process of a single protein binding to and falling off from its specific binding site on the DNA. When the protein (Integration Host Factor (IHF) of E. coli) binds, the DNA bends into a half-circle around it. By monitoring the bending of a single 25 nm long DNA probe, we detect single protein binding events and determine the lifetime of the bound state. The conformational change of the DNA probe is detected by optically monitoring the displacement of a μm size bead tethered to a surface by the DNA. Since in the bound state the DNA loops around the IHF, a mechanical tension on the DNA tends to eject the protein. In the experiment, we measure how the rate for the protein to fall off the DNA depends on the mechanical tension in the DNA. This spectrum of off-rates gives insight into the energy landscape of this molecular bond.

In the experiment, we monitor in real time a single protein molecule (IHF) binding and falling off a short (70 bp) piece of DNA.



The graph is a direct measurement of the "molecular dance" sketched in the cartoon above: when the protein binds ("on"), the DNA bends and its end-to-end distance shortens from 26 to 18 nm; when the protein unbinds ("off"), the DNA stretches out again.



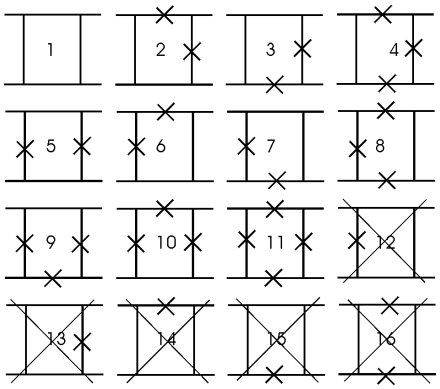
- S. Dixit, M. Singh-Zocchi, J. Hanne, and G. Zocchi, "Mechanics of Binding of a Single Integration-Host-Factor Protein to DNA", Phys. Rev. Lett. **94**, 118101 (2005).

Statistical Mechanics of DNA melting

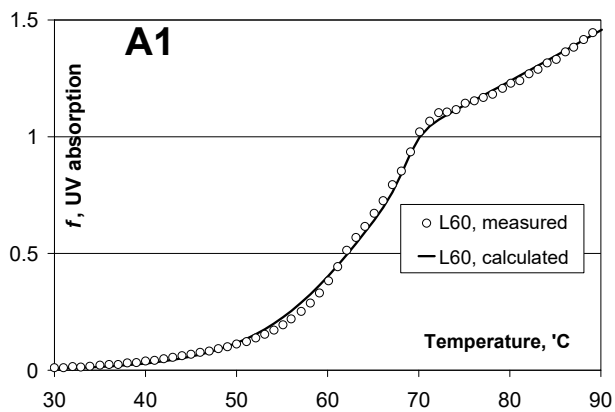
Vassili Ivanov, Yan Zeng, in collaboration with: *Dmitri Piontkovski*

We are interested in developing reduced-degrees-of-freedom models of conformational transitions in biological macromolecules. We have developed a statistical mechanics model ("2×2 model") for DNA melting in which base stacking and pairing are explicitly introduced as distinct degrees of freedom. Unlike previous approaches, this model describes thermal denaturation of DNA in the whole experimentally accessible temperature range (including past the strand separation temperature). Cooperativity arises from simple microscopic rules, as does the temperature dependence of the effective dimer free energies in the corresponding nearest neighbor thermodynamic model.

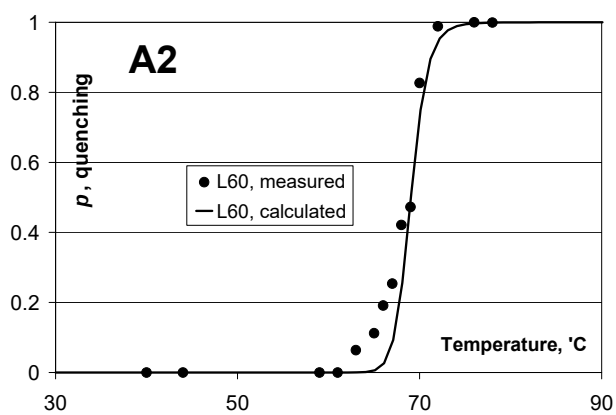
The partition function of the model can be written in a transparent transfer matrix form, and the model is exactly solvable in the homogeneous thermodynamic limit.



The diagram shows the different possible states of the nearest neighbor (NN) dimer in the 2×2 model. Each NN dimer has two pairings (vertical lines) and two stackings (horizontal lines). Crosses indicate broken bonds. The horizontal lines represent the strands. There are sixteen states of the dimer; admissible states of the 2×2 model are the states from 1 to 11; the states from 12 to 16 are prohibited by geometric constraints.



Normalized melting curve *f* (UV absorption measured at 260 nm) for a DNA 60mer (L60). The experimental data are the circles; the 2×2 model is the solid line.



Measured and predicted dissociation curves *p* for L60. The measurements in (A2) (filled circles) were obtained from the quenching method (see Project "Bubbles in DNA"). The 2×2 model is plotted using the same parameter values as in (A1).

- V. Ivanov, Y. Zeng, and G. Zocchi, “Statistical mechanics of base stacking and pairing in DNA melting”, Phys. Rev. E **70**, 051907 (2004).

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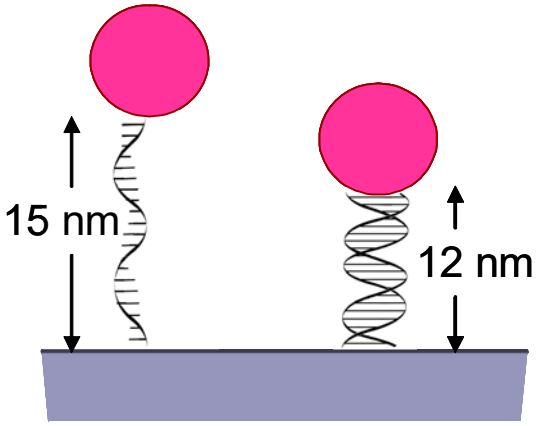
Single Molecule Detection of DNA Hybridization

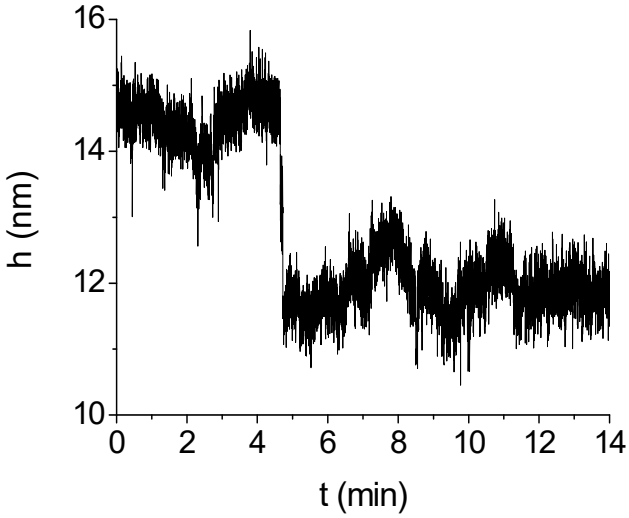
Mukta Singh-Zocchi, Sanhita Dixit, Vassili Ivanov

We detect nanometer scale conformational changes of single DNA oligomers through a micro-mechanical technique. The method can detect single hybridization events of label-free target oligomers. Its extremely high sensitivity make it attractive as a platform for medical diagnostic assays.

The single DNA tether, under tension because of the repulsive forces between the bead and the slide, is extended beyond the contour length of the corresponding ds DNA. Upon hybridization of the target, the micron size bead (not to scale in the drawing) is pulled closer to the slide. The bead's motion is detected by evanescent wave scattering.



Detection of a single hybridization event. Tether: 40mer; target: 30mer. Hybridization is detected from the nanometer scale conformational change of the probe oligomer.



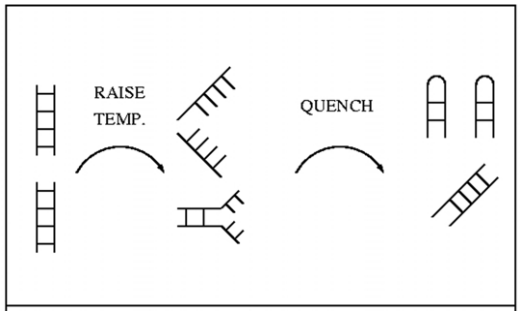
- M. Singh-Zocchi, S. Dixit, V. Ivanov, and G. Zocchi, "Single molecule detection of DNA hybridization", Proc. Natl. Acad. Sci. USA **100**, 7605-10 (2003).

Bubbles in DNA

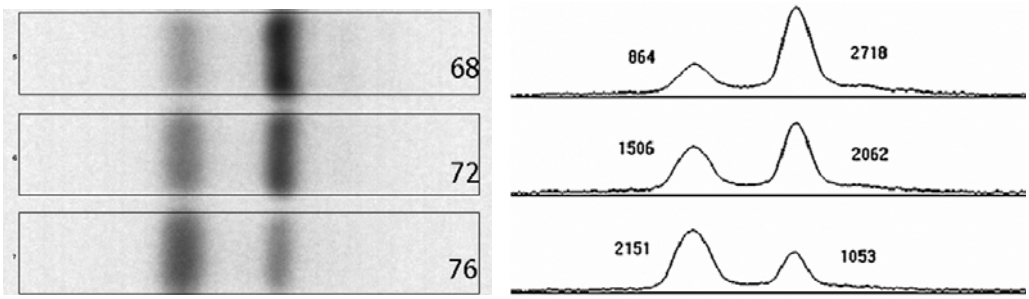
Yan Zeng, Awrasa Montrichok

As the temperature is raised, the DNA double helix melts through the formation of "bubbles" (single-stranded regions), eventually separating into two single strands. As a model system for conformational transitions in polymers, this thermal denaturation has been studied extensively. Nonetheless, the question of what conformations are statistically significant during melting is not clear.

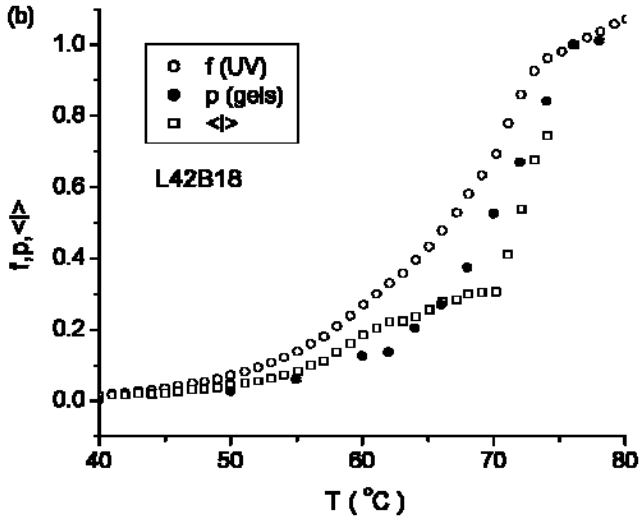
We have developed a new ensemble method to study the melting transition of DNA oligonucleotides, which can quantify the presence of intermediate states [1]. The principle is to trap intermediates in a quenched state. Using this method, we measure the average length of the denaturation bubble, and the statistical weights of the bubble states throughout the transition [2]. For internal bubbles, we find a nucleation size of ~ 20 bases, and a broad distribution of bubble sizes. In contrast, for bubbles opening at the ends of the molecule there is no nucleation threshold [3]. An analysis of the statistical weight of intermediate states versus length of the molecule L shows that the transition becomes strictly two-state only for L ~ 1.



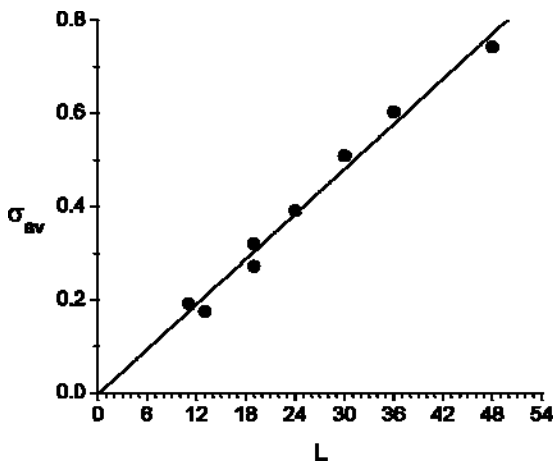
Sketch of the quenching method used to trap intermediate states.



In this gel (running right to left), the slow (fast) band corresponds to duplexes (hairpins). The temperatures (in C) to which the aliquots were heated before quenching are indicated on the lanes. The plot on the right shows the intensity profiles; the numbers are proportional to the areas under the peaks and are used to calculate the fraction of dissociated molecules *p*.



Melting curves for the sequence L42B18 (length L = 42, length of the AT-rich "bubble forming" region B = 18). Clamped at the ends by GC-rich regions and having an AT- rich middle region of length B, this duplex forms a single bubble in the middle when the temperature is increased. The open circles represent the fraction of open base-pairs f (from UV absorption measurements); the filled circles represent the fraction of open (dissociated) molecules p (from the quenching method); the squares represent the average relative length of the bubble <l> calculated from p and f. For increasing temperature the bubble size <l> grows smoothly from zero and reaches a plateau for <l> ≈ 0.3 ~ B/L, the relative size of the AT-rich middle region.



σ_{av} is a measure of the frequency of intermediate states (averaged over the transition region), here plotted versus the length of the molecule L (in bp) for eight sequences which form bubbles at the ends. For a strictly two-state transition, $\sigma_{av} = 0$; extrapolation of the data indicates that this happens only for L = 1.

[1] A. Montrichok, G. Gruner, and G. Zocchi, "Trapping intermediates in the melting transition of DNA oligomers", Europhys. Lett. **62**, 452 (2003).

[2] Y. Zeng, A. Montrichok, and G. Zocchi, "Length and statistical weight of bubbles in DNA melting", Phys. Rev. Lett. **91**, Number 14, 148101 (2003).

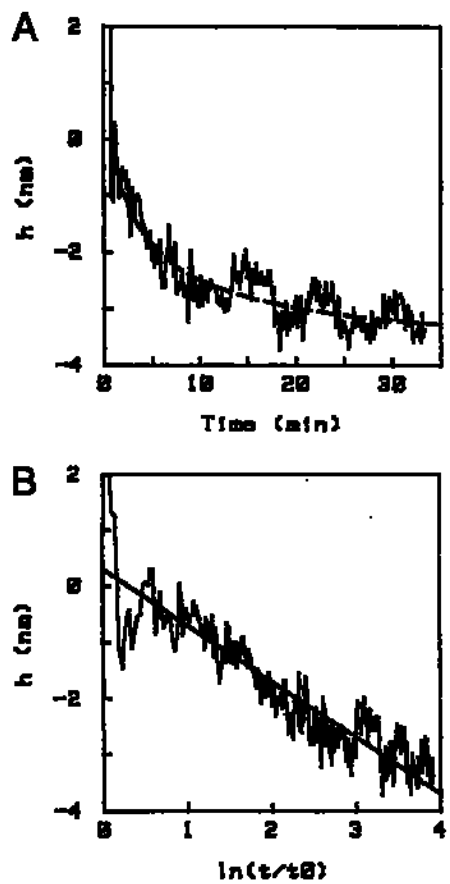
[3] Y. Zeng, A. Montrichok, and G. Zocchi, "Bubble nucleation and cooperativity in DNA melting", J. Mol. Biol. **339**, 67-75 (2004).

[4] Y. Zeng and G. Zocchi, "Mismatches and bubbles in DNA", Biophys. J. **90**, 4522-29 (2006).

Plasticity of proteins

Mukta Singh-Zocchi , Jeungphill Hanne

Globular proteins are peculiar solids that display both local stability of their conformation and the ability to undergo large cooperative changes of shape (conformational changes). If one forces a large deformation of the molecule, such that the structure is necessarily changed, it is not obvious whether the deformed globule can still remain a solid or whether it will melt. Is it possible to plastically deform a protein? We investigate this question with a micro-mechanical experiment on a small region (~ 10 molecules) of a protein monolayer adsorbed on a rigid surface. For the two proteins studied, albumin and myoglobin, we observed that the molecules can be substantially deformed (~ 1–2 nm deformation) by comparatively small stresses applied for sufficiently long times. The deformation is irreversible, and the protein remains in the solid state (i.e., displays a nonzero shear modulus). The dynamics of the deformation is approximately logarithmic in time, similar to creep in solids. These results show that globular proteins adsorbed on a surface can be plastically deformed.



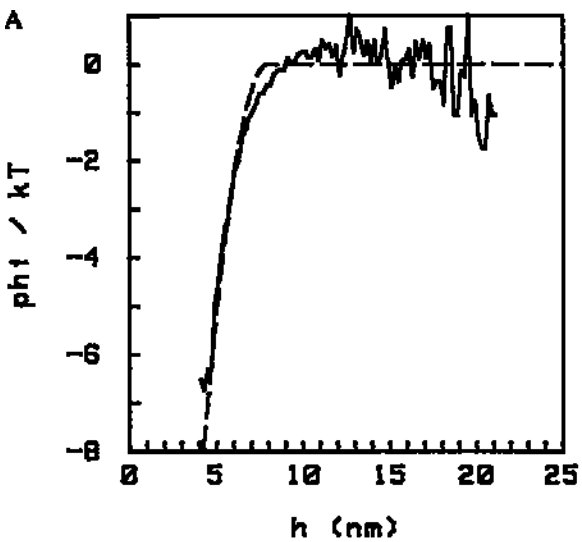
Slow dynamics in the mechanical compression of albumin monolayers. The figure shows the time course of the separation between the bead and the slide. The vertical scale is in nanometers, and $h = 0$ corresponds to the albumin layers coming into contact. The initial condition is with the sphere in the secondary minimum of the DLVO potential, at $h = 10$ nm (not visible in the figure). At $t = 40$ s, solution A (low ionic strength) is exchanged with solution B (high ionic strength), and the sphere falls into the primary minimum (sharp vertical line close to $t = 0$). From then on a slow deformation sets in, with a total amplitude of 3 nm over the 30 min of the experiment. The dashed line is a power law fit.
(*B*) The same data as in *A* plotted versus log (*t*). The solid line is drawn to show that the plot is roughly linear.

- M. Singh-Zocchi, J. Hanne, and G. Zocchi, "Plastic deformation of protein layers", Biophys. J. **83,** 2211-18 (2002).

Depletion Forces with Albumin

Mukta Singh-Zocchi, Anita Andreasen

Two surfaces that come in close contact in a solution with macromolecules present experience an attractive force caused by the osmotic pressure. We measured the distance dependence of this effect by using a micrometer-sized sphere bound to a flat plate through a single molecular attachment in an albumin-containing solution. We obtain the osmotic part of the interaction potential with a resolution of < 1 nm in distance and < 1 kT in energy. This attractive interaction is seen to have a range comparable to the size of the albumin molecule (8 nm). The results are broadly in agreement with a geometric model first proposed by Asakura and Oosawa.



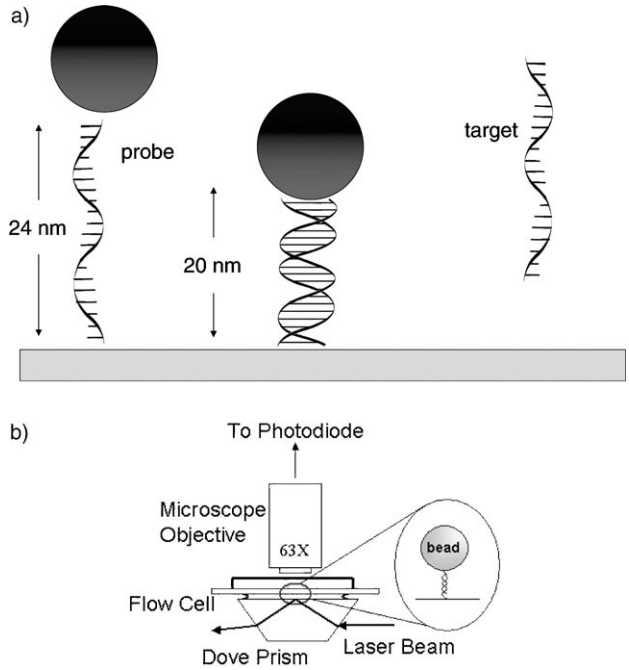
The osmotic part of the interaction potential between the bead and the surface in the presence of albumin in solution. The dashed line is a fit with a cubic form (the result of the Asakura and Oosawa geometric model). The resulting parameters are *a* = 4.0 nm for the ''radius'' of the albumin and P = 2.5 × 10^3 dynes/cm^2 for the osmotic pressure.

- M. Singh-Zocchi, A. Andreasen, and G. Zocchi, "Osmotic pressure contribution of albumin to colloidal interactions", Proc. Natl. Acad. Sci. USA **96**, 6711 (1999).

Single Molecule Experiments by Evanescent Wave Scattering

Giovanni Zocchi

We have developed a new single-molecule method which is a kind of cantilever-less AFM. The method is based on tracking the motion of a micron–size bead attached to a solid surface through a single molecular contact. The bead's motion is monitored with sub-nm resolution by evanescent wave microscopy, while a force is exerted through a flow. The method allows to exert a non-destructive force on a single molecule while simultaneously monitoring nm scale conformational motion of the molecule.



We have demonstrated the method by measuring the entropic elasticity of a long polymer chain [1], by reproducing previously known biotin-avidin bond rupture forces [2], and by detecting nm scale conformational changes of proteins and DNA [3, 4].

a) Principle of the single-molecule sensor. The micron-sized bead is tethered to the glass surface by a single DNA tether, which is kept under tension by the repulsive bead–slide interaction due to other negatively charged polymers attached to the surface. Upon hybridization the tether shortens, and displaces the average position of the bead toward the surface.

b) Schematic of the sensor. The He–Ne laser beam is guided through the prism to create an evanescent wave at the bottom of the flow cell; light scattered by a single bead is collected through the objective.

[1] H. Jensenius and G. Zocchi, "Measuring the spring constant of a single polymer chain", Phys. Rev. Lett. **79**, 5030 (1997).

[2] G. Zocchi, "Force measurements on single molecular contacts through evanescent wave microscopy". Biophys. J. **81**, 2946-53 (2001).

[3] G. Zocchi, "Proteins unfold in steps", Proc. Natl. Acad. Sci. USA **94**, 10647 (1997).

[4] M. Singh-Zocchi, S. Dixit, V. Ivanov, and G. Zocchi, "Single molecule detection of DNA hybridization", Proc. Natl. Acad. Sci. USA **100**, 7605-10 (2003).