THERMALLY INDUCED PHASE TRANSITIONS OF BIOMOLECULES OBSERVED VIA NANOMECHANICAL MOTION FROM MICROCANTILEVERS

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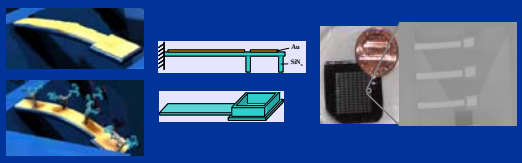
INTRODUCTION

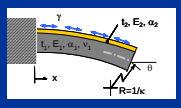
Configuration and conformational changes in biomolecules, including phase transitions, play a critical role in many bio processes. Given that they are driven by free energy reductions that usually involve enthalpy changes, they are often detected by calorimetric measurements. We report the use of the microcantilever array to study the thermal stability of macromolecules. The sensitivity of these cantilevers combined with their fast response time has allowed us to use these sensors for the thermal analysis of phase transitions in adsorbed molecular films. Their extremely high surface-to-volume ratio permits detection of surface stresses which are too small to observe on a macroscale to become an important sensing mechanism. The array format allows us to multiplex our experiments

THE MICROCANTILEVER

Microcantilevers have become important micromachined structures for many sensing applications. Microcantilever-based sensors directly translate changes in Gibbs free energy due to molecular interactions into mechanical responses. When biomolecules are immobilized onto one surface of the cantilever, changes in the surface stress on that side of the cantilever induce bending. The cantilever transduces a biochemical signal into a mechanical one. One can follow surface processes by measuring the deflection of the cantilever tip.



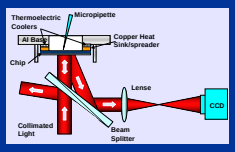
- *Stoney's formula:*

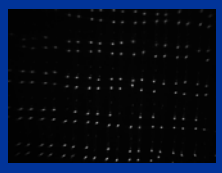


$$\Delta\gamma = \frac{\Delta\theta \cdot E_1 t_1^2}{6\cdot(1-\nu_1)L} = \frac{\Delta h \cdot E_1 t_1^2}{2\cdot(1-\nu_1)L^2}$$

a surface stress of 1 mJ/m² will result in a deflection of 1 nm at the end

- *Optical Detection Method:*

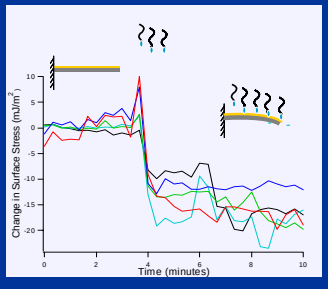


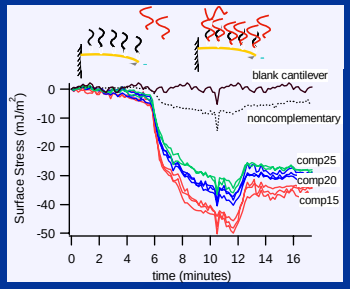


DNA IMMOBILIZATION AND HYBRIDIZATION

When thiolated ssDNA **immobilize** onto a cantilever, there is a decrease in the surface stress as the cantilever bends away from the gold surface.

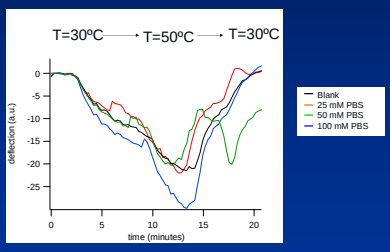
When complimentary DNA molecules in 50mM PBS **hybridize** onto a cantilever, there is a further decrease in the surface stress.





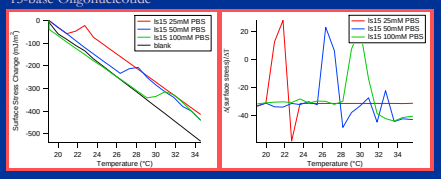
DNA MELTING

As we ramp the temperature of the cantilevers we see deviations from the linear response seen on the blank cantilever. We associate these deviations as the melting transitions as the DNA unravels. Depending on the salt concentration, the stability of the DNA can be seen by shifts at higher temperatures.30

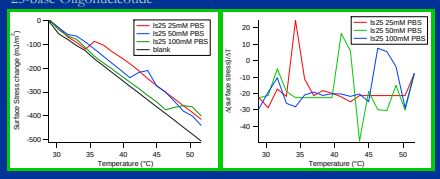


Name	Sequence	Tm,theory at 25mM Na+	Tm,theory at 50mM Na+	Tm,theory at 100mM Na+
ls15 comp15	5'-thiol-GTGGTAGATGAAGGTGAGAG-3' 5'- ATT TGT ATT TAG TAG -3'	24.7	30.6	35.8
ls20 comp20	5'-thiol-TTTTTTTTATTCAATTTATT-3' 5'- AGTAAAATGAATAAAAAAAA-3'	32.2	36.7	42.1
ls25 comp25	5'-thiol-TTTTTTTTATTCATTTTACTT TTTT-3' 5'- AAAAAAGTAAAATGAATAAAAAAAA -3'	37.1	43.8	49.9
ds20	5'-thiol-GTGGTAGATGAAGGTGAG AG-3	46.1	52.9	

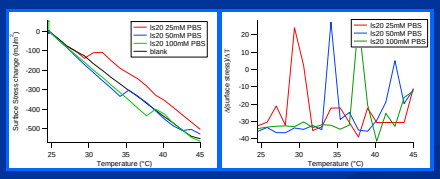
15-base Oligonucleotide



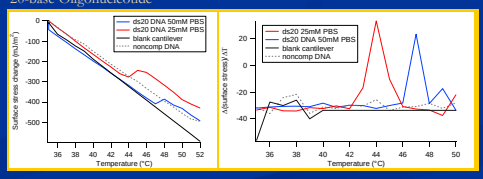
25-base Oligonucleotide



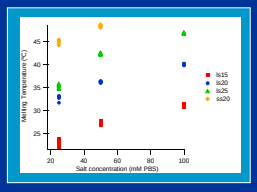
20-base Oligonucleotide

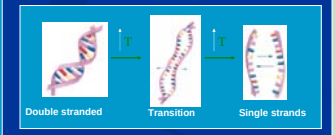


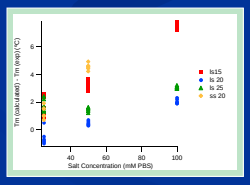
20-base Oligonucleotide



Melting Results







$$T_{m,calculated} = \frac{\Delta H^\circ}{\Delta S^\circ + R\ln C_t}$$

Factors impacting melting temperature:

- Length – Base stacking is the main contribution to the stability of DNA. This favorable interaction increases with the number of bases.
- Solution ionic strength – repulsion between charged phosphate backbones is neutralized in the presence of salt to produce a stable structure
- DNA base composition – G and C bases have 3 hydrogen bonds, A and T have 2 hydrogen bonds

The melting temperature of DNA is suppressed on a surface compared to in bulk because …

- Chain mobility decreased due to one end being tethered
- Electrostatic interactions with the surface different than with the bulk

Factors impacting surface stress:

- Electrostatic repulsions
- Osmotic pressure of counterions
- Hydration forces
- DNA conformational entropy

- Cantilever strain energy

SUMMARY

With the microcantilevers, we are able to explore the stability of DNA under a variety of solution conditions. Differences in the lengths and intermolecular interactions between single and double stranded DNA are highlighted by variations in cantilever deflection. This technique has allowed us to probe of DNA melting dynamics, which allows us to better understand the stability of DNA complexes on surfaces.

ACKNOWLEDGEMENTS

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