

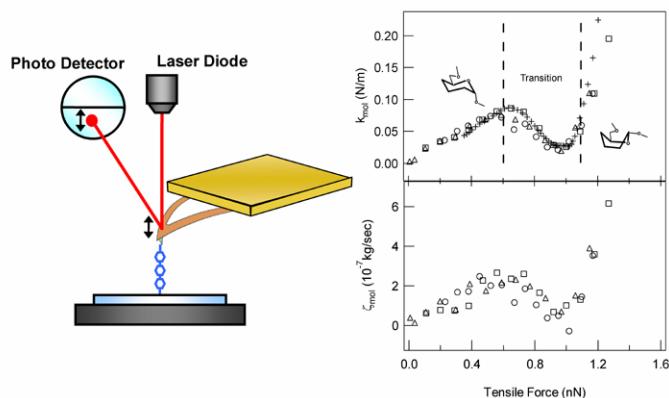
# The dynamic properties of single biomolecules

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## Measuring the thermal noise response of single molecules

The Brownian motion of single polysaccharide molecules under tension has been measured using AFM and used to extract a measure of the energy dissipation of the motion (see Fig. 1). This has contributions from work done against the solvent, and internal work done (against bond bending for example), as well as the molecular stiffness. The internal friction of single molecules is sensitive to conformational change and has hitherto not been accessible by experiment.

When single cellulose molecules are extended, the chain uncoils following the predictions of the freely jointed chain model, and the internal friction of cellulose is found to rise steadily with applied force. However, in the case of dextran, a plateau is observed in the force extension curve, due to a force-induced conformational change in the pyranose ring, which flips from the chair to the more extended boat conformation. This is reflected by a minimum in the internal friction of the molecule. There is also a minimum in chain stiffness due to the extra length released by the conformational change. The minimum in internal friction reflects the increased ease with which pyranose rings can change conformation. It is hoped that internal friction measurements will reveal previously unseen conformational changes in more complex molecules such as proteins during folding.



**Fig. 1:** Measuring the dynamic mechanical properties of single molecules with an AFM cantilever. The variation in single molecule dextran elastic constant and internal friction with applied force.

## Tethering protein concatamers

To apply the method to single protein concatamers, we require a durable means of tethering the molecules. Single protein molecules can be suspended between a cantilever and substrate using non-specific tethering, but the tether breaks before sufficient data can be captured to give an accurate noise spectrum. Tethering molecules through covalent bonds should enable longer duration experiments to be carried out with ease, and has the added advantage that the exact location of the tether point is known. A protocol to tether a modified 5-domain concatamer of I27 between a gold coated cantilever and gold substrate, via distinct covalent links is being developed. The gold-binding functionality of the C-terminal cysteine residue, and the ability of N-terminal histidine residues to bind succinamide will be exploited.

## Publication

Kawakami, M., Byrne, K., Khatri, B., Mcleish, T.C.B., Radford, S.E. and Smith, D.A. (2004) Viscoelastic properties of single polysaccharide molecules determined by analysis of thermally driven oscillations of an atomic force microscope cantilever. *Langmuir*. **20**, 9299-9303

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