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Knotted Fishing Line, Covalent Bonds, and Breaking Points

Every angler knows that a fishing line breaks easily at the place of a tight knot. In their report (1) "How strong is a covalent bond?" Michel Grandbois *et al.* present a "fly fishing method" for measuring the force needed to break long polysaccharide chains. In these experiments, a single molecule was attached with covalent bonds (Si-C, Si-O) to the tip of an atomic force microscope (AFM) at one end and to a substrate at the other. The polymers they used were very long (thousands of sugar rings) and were relaxed in a solution before attachment to the surfaces. Persistence length of such polymers roughly corresponds to the dimension of the composing monomers, as demonstrated in a report by S. B. Smith *et al.* (2), in which single-stranded DNA was used. Chains of this size (a thousandfold longer than their persistence length) are usually knotted and frequently will have more than one knot (3). This knotty property of long polymers was not discussed by Grandbois *et al.* (1), and it casts doubt on the interpretations of the data and the conclusions in the report.

Grandbois *et al.* observed a breaking force that was much smaller than one would expect if the C-C or C-O bonds within the polysaccharide chains breaking. They then infer that the polymer itself did not break, but that the multiple bonds at each end (attaching the polymer to the AFM and to the substrate) were breaking, one at a time (1). This interpretation is rather unlikely, because such one-by-one breaking of identical covalent bonds should produce a succession of peaks of roughly the same height (4). Grandbois *et al.*, however, observed a continuous steep increase between the consecutive peaks. Also, Grandbois *et al.* state that the observed breaking force was smaller than the theoretical strength of Si-C bonds. Therefore, it is likely that the actual attaching bonds did not break. What, then, were the small peaks of increasing height that they observed and analyzed?

We hypothesize a third possibility: that the observed peaks preceding the final breakage could be the "signatures" of progressive tightening of complex knots in the polysaccharide chain, whereby this tightening could be opposed by entanglement with other chains attached in the vicinity. Why, then, might the change of the attaching chemistry change the strength of the polymer, which would remain chemically unchanged? Small differences in a strength of a solvent (caused, for example, by the presence of mercaptoethylamine) could affect the tightness of the formed knots: A good solvent would loosen

the knot, and thus change the breaking force.

If Grandbois *et al.* were in fact measuring the breakpoints of knotted polysaccharide chains, why would the breaking force of a knotted polymer then be measured at about half the expected breaking force of an unknotted polymer? In an attempt to answer this question, we performed an experiment with a fishing line that had a nominal resistance of 10 kg. After tying a trefoil knot in the line, we tested the subsequent resistance. The line broke, exactly at the knot, under a weight of about 6 kg.

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Response: Science is based on the falsification of hypotheses, and we are grateful for this comment by Stasiak *et al.*, which challenges the hypothesis we proposed in our report by launching a competitor.

In contrast to the assumption made by Stasiak *et al.*, it was not the case that the rupture peaks that we observed were always in increasing order. In fact, we frequently found peaks in descending order (Fig. 1). The insert in figure 2 in our report (1) may have mislead readers; however, it is only one example among many. Also, because the insert shows a close-up view of the trace at the position marked by the arrow, the micro-ruptures shown at about 2 nN and not, as Stasiak *et al.* assume, at half the force.

We agree with the assumption made by Stasiak *et al.* that knotted polymers will break at lower forces than unentangled ones. This topic was addressed recently by Saita *et al.* (2). Polymers, however, break only once, and we do not see how one could use this property of knotted lines to explain the multiple

breaking of molecular bonds that we observed in our investigations.

The tightening of a molecular knot will manifest itself in force curves in several ways. Let us, for ease of discussion, distinguish between equilibrium and nonequilibrium processes. In cases where the knotted states are separated by energy barriers on the order of the thermal energy, kT , one would expect a fully reversible behavior: the knots would tighten or disentangle during the experiment. With changing external force, the average time the polymer spends in either of the two states would change. The apparent stiffness of a segment would thus be decreased slightly. But, as a result of the ratio of knotted length to contour length of the polymer, this effect would be marginal and, most likely, not detectable.

In cases where the energy barriers between the knotted states were so high that a disentanglement would not occur spontaneously during the experiment (for example, as a result of steric restrictions caused by side groups, as suggested by Stasiak *et al.*), the tightening of the knots could indeed result in discontinuous force scans. The discontinuities under discussion correspond to energy barriers on the order of covalent bonds, so the restrictions would have to be severe. Following the reasoning of Stasiak *et al.*, the restriction imposed by bulky side groups would, on pulling of the polymer, greatly increase with decreasing size of the loop of the knot. This would be a percolation-type problem. Thus, only when the diameter of the mesh became comparable to the size of the side group would an energy much higher than kT be built up. These conditions would result in a very narrow window of forces and polymer elongation (after the discontinuity) within which this effect could contribute to the force curves.

We can estimate the range of this effect as follows: Let us assume that a loop is tightened and is stuck at a side group. This situation would be possible only if the loop had

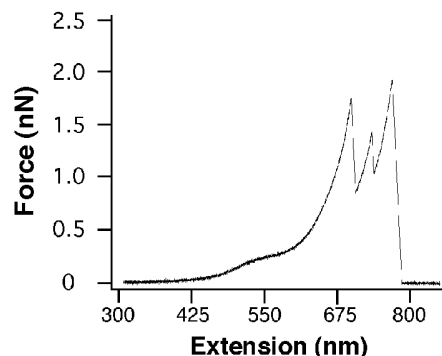


Fig. 1. Multiple bond rupture events of a covalently attached polysaccharide showing rupture forces in random order. Experimental conditions were the same as those in figure 2 of our report (1).

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reached a diameter that was comparable to the size of the side group; otherwise, as a result of thermal excitations, the side group would easily pass through the loop. On pulling, the barrier would be overcome and the loop would be tightened further. A second side group could be pulled through the loop (resulting in a subsequent discontinuity in the force scan) only if the loop did not close smaller than the size of the side group. Because the diameter of a loop would decrease linearly with the length of the polymer, this second event could not occur "later" than a few angstroms. Triple or higher discontinuities, which we predominantly observed in the experiment, cannot be explained by the tightening of knots.

We do find, in our histograms of polymer elongation after a discontinuity [figure 3 in (1)], a fraction of about 5% of events for which this criterion would hold, and so we cannot exclude the possibility that these events might reflect the tightening of knots. The remaining 95% of events, however, which show polymer elongation of more than 10 Å, cannot be explained in this way.

A knot can have a complex topology, like that which results from trying to pull a tight

knot in a rope through an entangled mess made by the rest of the rope (a situation that all of us fishermen are familiar with). However, the analogy to molecular events is not valid because molecular knots have no friction, only activation barriers of the kind we discuss above. The conformation of the disentangled mess of a hemp rope does not change spontaneously as one tries to pull the knot through. In the case of a molecular mess, however, thermal excitations would create a multitude of conformations during pulling that would allow a molecular knot to move through the mess. In analogy to DeGennes's description of polymer diffusion (3), this might be called "forced reptation."

We are still convinced that our explanation of the data in our report (1) is correct. Nevertheless, this discussion has yielded an interesting catch, and it may inspire further thought and experimentation.

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