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Stretching Single-Stranded DNA on a Surface

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ABSTRACT

A sufficiently long strand of DNA bends and balls up, like any other polymer longer than its persistence length. The ability to stretch a DNA molecule lengthwise on a substrate has garnered a great deal of attention in the past several years. There has been notable success in stretching double-stranded DNA (dsDNA), but it is more difficult to stretch single-stranded DNA (ssDNA), both because of the very short persistence length and because it is very "sticky," and attaches nonspecifically to surfaces. In this issue of *Nano Letters*, Woolley and Kelly present a new method for stretching single-stranded DNA strands on surfaces.

There are many reasons for wanting to stretch both double- and single-stranded DNA. Stretched DNA could one day be sequenced using scanning probe techniques, such as AFM or STM. cDNA chip makers may benefit from such an approach in order to optimize the accessibility of the attached sequences, and hence their density. Optical microscopy now has a breadth of resolution-increasing techniques that could possibly take advantage of fluorescently labeled stretched DNA, allowing for parallel detection of sequences.[1] Another possible use of a stretched single-stranded DNA (ssDNA) template could be enzyme activity studies. It is often argued that surface-based assays are impaired by uncontrolled interactions, but, on the other hand, DNA is tightly packed in cells. In particular, it would be interesting to study the interaction of DNA binding proteins with such an elongated ssDNA, possibly shedding light on whether the phosphate backbone or the base pairs are involved in the binding process! Moreover, a controlled approach whereby ssDNA would be stretched above a channel[2] would circumvent this limitation and certainly be welcome by single-molecule biophysicists. Finally, the ability to encode information in DNA sequences may also be used for patterning materials and nanocrystals, and stretched strands of ssDNA may be most useful for this.[3]

Extension of single DNA molecules, either double-stranded DNA (dsDNA) or ssDNA has been studied extensively in the past decade.[4−10] One of the keys to success in this type of experiment is to prevent the DNA molecules from sticking to the neighboring surfaces. It is thus no surprise that stretching DNA on surfaces has turned out to be a rather tricky task. There are different flavors of protocols to achieve this goal, but all of them rely first on the specific attachment of DNA molecules by their extremities to the surface, and second on a means to extend the attached strand over the surface to which it is bound. The first step has long been the least controlled one, the main problem being the

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nonspecific attachment of DNA at random positions along its length. This problem was studied in detail a few years ago, and the parameters required for specific attachment of the strands by their extremities only are now pretty well known.[11] The second step is open to more freedom: some researchers rely on a liquid flow,[12,13] some others on a receding meniscus,[14] some on a more or less controlled combination of both, or for instance electrophoresis,[8,15] among many other possibilities. The basic mechanism taking place during binding seems to be local denaturation of the dsDNA ends and hydrophobic or electrostatic attachment of the base pairs or the backbone to the surface. Obviously, ssDNA would, in similar conditions, snap onto the surface and bind nonspecifically all over the place, because it is already in its denaturated state all along its length. That is what actually happens usually, as the authors of the present work point out.

The clever way they have designed to prevent this from happening is to cover a mica surface with poly-L-lysine. The experimental observation is that, upon sliding a drop of ssDNA solution onto such a surface, one ends up with mostly elongated ssDNA molecules, as demonstrated by AFM measurements. The experimental result is quite clear and opens up a new avenue for investigation. The mechanism of ssDNA stretching is also of considerable interest, and the present preliminary report still leaves open many questions regarding this.

The authors reason that the absence of multivalent cations in their low ionic strength buffer increases intrastrand repulsion. One should probably not be too much concerned by intrastrand attraction, except if the sequence specifically contains stretches of base pairs than could hybridize with one another, something relatively unlikely to happen in circular ssDNA. In the case of ssDNA obtained by denaturation of dsDNA, this risk could easily be prevented by using dilute concentrations. What is certain is that, in low ionic strength buffers, polyelectrolyte theory for ssDNA predicts about 1/2 negative charge per phosphate group, slightly more than for dsDNA.[16,17] We know from dsDNA anchoring on charged surfaces such as poly-L-lysine that phosphate backbone binding to the surface depends on the pH of the buffer (see, for instance, the corresponding experiments with poly-L-lysine in ref 11). The reason for that dependence is probably once again local denaturation of the dsDNA ends, which transforms dsDNA into ssDNA, for which negative charge surface density increases. But pH has also an effect on the protonation of the poly-L-lysine surface, and, for a neutral surface, charge image repulsion prevents binding of the negatively charged DNA onto the surface.[11] There is still room for a pH-dependence study that could complement this report, and that would certainly shed light on the relevant mechanisms.

The authors choose three types of DNA molecules: ϕX174 (circular ssDNA, 5386 bp), M13mp18 (circular ssDNA, 7249 bp), and coliphage λ (linear dsDNA, 48 502 bp). There are two types of DNA here, once λ DNA has been denatured (and hopefully snap-cooled to prevent renaturation): circular and linear. Circular dsDNA has already been stretched previously using molecular combing.[11] The specificity of circular ssDNA over linear ssDNA is that there are a priori no "ends" in it. One can thus imagine that small, circular ssDNA may exhibit kinks and openings that may define more favorable points of attachment on a surface (more readily accessible base pairs for instance). Another possibility would be that the circular topology prevents the DNA strand from staying long enough in close contact to the surface for hydrodynamic reasons. This points to the fact that the choice of circular DNA leaves open some questions concerning the binding mechanism. A future study with linear ssDNA molecules of different sizes may shed more light on whether there really is a well-defined relationship between the optimal poly-L-lysine coverage and ssDNA length.

An alternative explanation for the results observed here is that, at sufficiently low poly-L-lysine coverage, nonspecific binding of ssDNA may occur on poly-L-lysine patches. Of course, the lesser the number of anchoring patches, the fewer binding events will occur. dsDNA combing on poly-L-lysine is convincing evidence that full coverage by poly-L-lysine does not prevent dsDNA stretching, as long as the pH is properly chosen. We have seen that the mechanism for dsDNA binding relies on a subtle equilibrium between surface charges and the existence of small stretches of ssDNA. The pH used in the present experiments (pH 8) falls in the range allowing specific binding on poly-L-lysine quoted in ref 11 for glass surfaces. Even though there is no published value on the optimal pH for combing on mica, because the fluorescent observation used in this type of measurement is not possible (probably due to dye quenching), the same processes are probably taking place. It may explain why the authors have had difficulty seeing elongated DNA on densely covered surfaces. In particular, at basic pH, mica is negatively charged, so that we do not expect any binding of the still negatively charged phosphate backbone of ssDNA. On the other hand, poly-L-lysine provides positively charged sites. One thus expects the shorter ssDNA to bind only at a single site when the poly-L-lysine coverage is low. For large molecules, the probability to have multiple, separated binding sites is significantly increased at larger patch coverage, so that we expect a pattern of loops to be observed[18] or clumped molecules, a trend that should diminish at lower coverage. This fact is precisely what is observed (if we interpret correctly the authors' description that "not many [molecules] are elongated"). Because we have seen that the binding of circular dsDNA is probably reduced compared to linear ssDNA, we can also explain the decrease in observed stretched molecules at low poly-L-lysine coverage for these molecules. A reasonable number of binding events will be obtained only at higher coverage, something that does not lead to multiloop patterns simply because of their small size. In principle, using *linear* ssDNA of small size should give exactly the same results as for the λ ssDNA strands, with the difference that a higher poly-L-lysine coverage could be used, and hence, a higher ssDNA density.

No matter the precise mechanism, the authors have presented a new and interesting advance in stretching ssDNA.

Given the wide range of possible uses, there is thus no doubt that this interesting work will have followers.

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