Cantilever deflection associated with hybridization of monomolecular DNA film

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Abstract
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Keywords
DNA, electrostatics, spatial dimensions, nucleotides, biosensors, gold, atomic force microscopes, biomolecules, chemical sensors, surface charge

Disciplines
Biomechanical Engineering | Biomechanics and Biotransport | Biotechnology | Nanoscience and Nanotechnology

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Cantilever deflection associated with hybridization of monomolecular DNA film

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Recent experiments show that specific binding between a ligand and surface immobilized receptor, such as hybridization of single stranded DNA immobilized on a microcantilever surface, leads to cantilever deflection. The binding-induced deflection may be used as a method for detection of biomolecules, such as pathogens and biohazards. Mechanical deformation induced due to hybridization of surface-immobilized DNA strands is a commonly used system to demonstrate the efficacy of microcantilever sensors. To understand the mechanism underlying the cantilever deflections, a theoretical model that incorporates the influence of ligand/receptor complex surface distribution and empirical interchain potential is developed to predict the binding-induced deflections. The cantilever bending induced due to hybridization of DNA strands is predicted for different receptor immobilization densities, hybridization efficiencies, and spatial arrangements. Predicted deflections are compared with experimental reports to validate the modeling assumptions and identify the influence of various components on mechanical deformation. Comparison of numerical predictions and experimental results suggest that, at high immobilization densities, hybridization-induced mechanical deformation is determined, primarily by immobilization density and hybridization efficiency, whereas, at lower immobilization densities, spatial arrangement of hybridized chains need to be considered in determining the cantilever deflection. © 2012 American Institute of Physics. [http://dx.doi.org/10.1063/1.3698204]

I. INTRODUCTION

Microcantilever-based sensors are an intriguing new alternative for conventional chemical and biological sensors, because of their extremely high sensitivity and miniature sensing elements. The sensing strategy involves coating one surface of a micromachined cantilever with a receptor species that has high affinity for the analyte molecule. Binding of the ligand on the sensitized surface induces a mechanical deformation of the microcantilevers, thus transducing the surface chemical reaction into a measurable quantitative signal. Thundat and his colleagues\(^1\) made the seminal observation that atomic force microscope (AFM) cantilevers deflect, due to changes in relative humidity, and thus opened a myriad of possibilities for the use of AFM cantilevers for chemical and biological sensing. They predicted possibilities of adsorbate detection of the order of picograms and immediately followed up with another study, in which they detected mercury adsorption on cantilever from mercury vapor in air with picogram resolution.\(^1,2\) Since these initial reports, microcantilever-based sensors have been investigated for sensing of chemicals,\(^3,4\) biomolecules,\(^5-12\) explosives,\(^13-15\) and markers for cancer.\(^16-22\)

DNA hybridization is a simple and prominent example of biomolecular recognition and detection, since it is fundamental to most biological processes. Fritz et al.\(^6\) monitored hybridization of surface-immobilized single stranded DNA (ssDNA) with oligonucleotide length of 12 nucleotides (nt) and with 3 different concentration values of the target ssDNA molecules. Cantilever deflections of about 3 nm, 15 nm, and 21 nm were reported for hybridization with complimentary strand concentrations of 80 nM, 400 nM, and 2000 nM, respectively. Cantilever deflection was also found to be different for hybridization of ssDNA with strands that had a single base-pair mismatch, indicating that microcantilever-based sensors have intrinsic sensitivity to discriminate single nucleotide polymorphisms. Since this work, cantilever deflection due to ssDNA hybridization has been utilized as a validation experiment for new techniques, and cantilever deflection signals up to \(~100 \text{ nm}\) have been reported for those experiments.\(^9-12,23\)

Hansen et al.\(^7\) also demonstrated that hybridization-induced cantilever deflection can be used to discriminate base-pair mismatches with 20-nt and 25-nt probe DNA molecules. They used 10 nt DNA oligonucleotides as complementary target molecules, which contain either one or two internal mismatches. The results showed that the number and position of mismatch pairs affects the deflection of the cantilever.

Stachowiak and co-workers\(^11,12\) conducted experiments to investigate the influence of ssDNA strand length, immobilization density, and hybridization efficiency on the hybridization-induced microcantilever deformation. The salt concentrations in immobilization and hybridization buffer were varied to achieve different immobilization densities and hybridization efficiencies. Changing the salt concentrations from 0 to 1000 mM resulted in an increase in immobilization density from 0.06 to 0.12 nm\(^2\), and similar change of salt concentration in a hybridization buffer resulted in an increase of hybridization efficiency from 30% to 80%. Three different molecular lengths of 10 nt, 20 nt, and 30 nt were used, and both immobilization density and hybridization efficiency were found to be lower for longer DNA chains. Hybridization-induced cantilever deflections corresponding...
to different chain lengths, immobilization densities, and hybridization efficiencies collapsed on to a single curve when expressed as a function of the coverage of hybridized chains. These results indicated that the effects of the immobilization density, hybridization efficiencies, and chain length are coupled, and the cantilever deflection may primarily depend on the surface coverage of hybridized chains.

In order to explain the underlying mechanism for hybridization-induced deflection, Fritz\textsuperscript{24} hypothesized that the cantilever deflection is the result of two competing mechanisms: electrostatic repulsion between negative charges on the DNA strands and relaxation of steric hindrance as disordered ssDNA transition to ordered DNA strands. The increase in negative charges during hybridization results in an expansion of the cantilever surface due to the electrostatic repulsion and, consequently, bending of the cantilever. Alternatively, when surface-bound, single-stranded oligonucleotide undergo hybridization, conformational changes from a disordered strand to rod-like double helix result in relaxation of the steric hindrance and contraction of the surface. The competing mechanisms were proposed to explain cantilever bending observed during hybridization experiments. During the initial phase of DNA hybridization, the relaxation of steric hindrance leads to relaxation of cantilever bending, but as the hybridization proceeds, the surface starts expanding, due to buildup of charge interactions among neighboring molecules. However, it is important to note that the DNA hybridization experiments are performed in buffers with high salt concentrations. The positive ions in the solutions may shield the electrostatic repulsion between the strands, and the magnitude of inter-chain repulsion may depend on the ionic composition of the hybridization buffer.

Besides the electrostatic effects, hydration forces between the chains may also lead to hybridization-induced cantilever deflection.\textsuperscript{25–27} To study the effect of the hydration forces, Mertens \textit{et al.}\textsuperscript{28} conducted experiments to investigate the influence of relative humidity on deflection of micro-cantilevers immobilized with ssDNA and double-stranded DNA (dsDNA). Deflection of cantilevers with ssDNA and dsDNA strands increased to about 150 nm and 200 nm, respectively, as the relative humidity was changed from 0 to 100\%. These results indicate that hydration forces play an important part in determining cantilever deflection.

Hagan \textit{et al.}\textsuperscript{29} modeled the hybridization-induced cantilever deflections based on both electrostatic repulsions and hydration forces between DNA strands. The microcantilever was modeled as a membrane, and the DNA strands were modeled as straight rods immobilized on the membrane surface. Repulsive interactions between the DNA strands led to increases in their spacing and rotation of rods, resulting in cantilever bending. Cantilever deflections due to a high immobilization density of 0.17 chains/nm\textsuperscript{2} and hybridization efficiency of 100\% were investigated. Based on the numerical results, it was concluded that the cantilever deflection induced by uniformly distributed DNA strands is much smaller in magnitude compared to experimental observations. However, numerical prediction based on disordered arrangement of DNA strands and 100\% hybridization efficiency was found to match the experimental observations.

In this paper, we report a model for hybridization-induced bending of micro-cantilever based on the minimization of the energy functional that accounts for cantilever bending energy and DNA inter-chain interactions. Influence of different immobilization densities, hybridization efficiencies, and chain distribution on the cantilever bending is considered. Cantilever is idealized as an elastic beam, while the energy of DNA is estimated using interaction potentials that account for both electrostatic and hydration forces. Predicted results are compared to experimentally reported deflections to identify the influence of immobilization density, hybridization efficiency, and chain distribution on cantilever deflection.

\section{II. THEORETICAL MODEL}

The microcantilever is modeled as a slender multilayer beam, as schematically represented in Fig. 1 and consists of three layers: the SiN\textsubscript{3} base layer, the gold (Au) layer for biomolecule immobilization, and the immobilized DNA strands. The total energy of the DNA-cantilever system consists of two major parts: the bending energy of the cantilever and the inter-chain energy between DNA molecules.

\begin{equation}
E_{\text{total}} = E_{\text{bend}} + \sum_{\text{all molecule pairs}} F^i.
\end{equation}

The gold film thickness is much smaller than overall thickness, and in addition, an elastic modulus of gold is of the same order of magnitude as that of the base silicon nitride; therefore, the cantilever is modeled as a monolithic linear elastic material in order to simplify the bending energy expression. The bending energy of the cantilever, denoted by $E_{\text{bend}}$, can be expressed as a function of the equilibrium radius ($R$) with the cantilever plane stain elastic modulus and thickness ($t$),\textsuperscript{29}

\begin{equation}
E_{\text{bend}} = \frac{Et^3}{12R^2(1-\nu^2)},
\end{equation}

where $R = \frac{L^2}{2\delta}$, $E$ is the Young’s modulus for the cantilever, $L$ is the length of the cantilever, $\nu$ is the Poisson Ratio, and $\delta$ is the cantilever deflection.

The total energy of DNA molecules is modeled as the sum of the pair interaction energies and thus is a function of three groups: the interactions between hybridized dsDNA...
molecules (D-D), between hybridized dsDNA and ssDNA (D-S), and between ssDNAs (S-S).

\[ \sum F^i = \sum F^i_{D-D} + \sum F^i_{D-S} + \sum F^i_{S-S}. \]  

(3)

Strey et al.\textsuperscript{26,27} showed that the energy of D-D interaction is far higher than that of D-S and S-S interactions. As a result, the energy from D-S and S-S interactions is neglected and the total energy of DNA molecules is simplified to be a function only of D-D interactions.

\[ \sum F^i = \sum F^i_{D-D}. \]  

(4)

Strey et al.\textsuperscript{26,27} proposed the functional form for the interaction energy based on the analysis of nematically ordered polymers. The function form was derived, considering the direct interactions \( F_0 \) between molecules and the harmonic entropic fluctuations.

\[ F^i = F^i_0(d^i) + c_k T k_c^{-1} = \sqrt{\frac{\partial^2 F^i_0}{\partial d^i} - \frac{1}{d^i}} \]  

(5)

where \( k_B \) is the Boltzmann constant, \( T \) is the temperature, \( d^i \) is the inter-molecular separation, \( l_p \) is the persistence length of the DNA molecules, \( k_c = k_B T l_p \) denotes the intrinsic bending stiffness of the DNA molecules, and the parameter \( c \) is an empirical determined dimensionless constant of order 1. The free energy \( F^i_0 \) is the summation of all molecular interaction between DNA molecules as a result of the solvent-mediated interactions (hydration forces) and electrostatic repulsions.

A systematic study of the electrostatic energy between two rod-like molecules with surface charges has been reported by Brenner and Parsegia.\textsuperscript{30} They performed theoretical calculations for two molecules with different configurations. The DNA molecules used in the cantilever experiments are usually less than 50 nucleotide long (<17 nm) and are considerably short compared to the persistence length of double-strand DNA.\textsuperscript{31} As a result, the hybridized DNA molecules can be treated as rods or cylinders standing on the surface of the micro-cantilever. With the parallel rods assumption, the energy of electrostatic repulsion per unit length can be written as

\[ F_{EL}(d^i) = a \sqrt{\frac{\pi}{2}} \frac{\exp(-d^i/\lambda_D)}{\sqrt{d^i/\lambda_D}}. \]  

(6)

where \( \lambda_D \) and \( d^i \) are the decay length and the axial separation between molecules, respectively, and \( a \) is determined by the salt concentration in the solution, experimentally.

Hydration forces are attributed to a hydration bonding network between neighboring DNA strands in water. Leikin et al.\textsuperscript{32} reported that dsDNA is surrounded by at least two hydration shells, which contain about 20 water molecules per base pair. This leads to a strong repulsion between DNA molecules when the separation is within several decay length. They also suggested that the free energy per unit length due to hydration forces between rod-like molecules should be of the same form as the electrostatic repulsions. Similarly, the expression for the hydration force–induced interactions is expressed as

\[ F_H^i(d^i) = b \sqrt{\frac{\pi}{2}} \frac{\exp(-d^i/\lambda_H)}{\sqrt{d^i/\lambda_H}}, \]  

(7)

where \( \lambda_H \) (≈0.29 nm) is the correlation length (decay length) of water and \( b \) is also determined empirically. So the final free energy for a pair of DNA molecules (\( F_0^i \) in Eq. (5)) is written as

\[ F_0^i(d^i) = a \sqrt{\frac{\pi}{2}} \frac{\exp(-d^i/\lambda_D)}{\sqrt{d^i/\lambda_D}} + b \sqrt{\frac{\pi}{2}} \frac{\exp(-d^i/\lambda_H)}{\sqrt{d^i/\lambda_H}}. \]  

(8)

The DNA molecules are initially considered to be standing parallel on the surface of the cantilever. As the separation between DNA chains is increased, \( F_0 \) decays exponentially. Since the DNA strands are only immobilized on the top surface of the cantilever, increase in DNA separation will lead to cantilever bending. Since the length of DNA strands \( (h=\text{several nm}) \) is two order of magnitude smaller than the cantilever thickness \( (t=500-1000 \text{ nm}) \), cantilever deflections of the order of \( 10-100 \text{ nm} \) will only result in small rotations of the DNA strands. Therefore, the DNA strands are assumed to stay nearly parallel throughout the cantilever deflections. The relation between inter-chain separation and radius of curvature of the bent cantilever is expressed as

\[ d^i(d_0, R) = d_0 \left(1 + \frac{r}{2R}\right), \]  

(9)

where \( d_0 \) is the initial separation before cantilever bending between the \( i \)th molecule pair. As a result, the total energy of the system, which is the summation of the cantilever bending and total free interaction energy, is a function of the initial ensemble of hybridized DNA molecules and equilibrium radius of the cantilever.

\[ E_{\text{total}} = E_{\text{bend}}(R) + \sum_{\text{DNApairs}} F(d^i(d_0, R)) = E_{\text{total}}(d_0, R). \]  

(10)

The total energy is minimized to determine the equilibrium radius of curvature for different ensembles to investigate the effect of different immobilization densities and hybridization efficiencies.

III. INITIAL DNA ENSEMBLES

In the cantilever bending experiments, the ssDNA molecules are first immobilized on the cantilever with a certain immobilization density. Subsequently, a certain percentage of ssDNA chains (quantified by the hybridization efficiency) bind with the complementary targets, forming hybridized dsDNA chains, leading to cantilever bending. The number of the hybridized dsDNA chains on the surface is determined by the immobilization density and hybridization efficiency, while their arrangement will depend both on the spatial distribution of DNA chains during immobilization and hybridization steps. The immobilization density and hybridization
efficiency may be experimentally determined, but it is hard to directly measure the chain arrangement on the cantilever surface. For a given hybridization and immobilization efficiency, four different ensembles of hybridized DNA chains are constructed in order to determine the influence of spatial arrangements on the hybridization-induced cantilever bending. Each ensemble consisted of 1600 DNA chains.

In the first ensemble (average spacing), the hybridized chains were assumed to be arranged in a hexagonal close-packed arrangement with uniform spacing. Hexagonal closed pack arrangements were created for the given immobilization density and hybridization efficiency ($\phi$). The interchain spacing, $d_i$, was computed to match the predicted coverage of hybridized chains, calculated according to the following equation:

$$d_i = d_0/\sqrt{\phi}.$$  \hspace{1cm} (11)

Close-packed hybridized dsDNA ensembles were generated for the desired coverage of hybridized chains, and the cantilever deflections were computed through minimization of bending and hybridized dsDNA interaction energy.

Although the close-packed distribution of hybridized dsDNA is easy to construct, it neglects most details in the real experiments. The first ensemble simply combines the two steps, immobilization and hybridization, together and may not represent the real surface arrangement of hybridized dsDNA chains. The immobilization and hybridization steps were considered separately in generating the next three ensembles of hybridized dsDNA chains.

In the second ensemble (random selection), the ssDNA were assumed to be immobilized on the surface with hexagonal closed pack arrangement. Distribution of hybridized dsDNA was generated, assuming that all the immobilized ssDNA chains have equal probability for hybridization. A certain proportion of the ssDNA chains were randomly selected and converted to hybridized dsDNA in order to match the required hybridization efficiency. Random selection is the simplest way to make an ensemble which has a high degree of disorder due to the hybridization. Five hundred different ensembles were generated for each combination of immobilization density and hybridization efficiency, and the cantilever deflections were computed for each ensemble through minimization of the bending and hybridized dsDNA interaction energy.

In the third ensemble (energy minimization), the ssDNA were again assumed to be immobilized on the surface with hexagonal closed pack spacing; however, the hybridized dsDNA distributions were generated assuming that hybridized sites will be distributed on the surface such that interaction energy between the ssDNA chains is minimized. A Monte-Carlo method–based procedure was used to identify the distribution of hybridized dsDNA sites that have the lowest interaction energy for hybridized dsDNA chains. In each step of the energy minimization procedure, a single hybridized and non-hybridized site were selected for exchange, and this exchange was accepted or rejected depending on the change in interaction energy and acceptance probability. This process was repeated for approximately $10^5$ steps until the total interaction energy did not undergo further reduction. The hybridized dsDNA distributions corresponding to minimum interaction energy were used to compute the cantilever deflections for each immobilization density and hybridization efficiency.

In the fourth ensemble (Gaussian-perturbed), the chain distributions computed through Monte-Carlo–based energy minimization were perturbed by imposing a random displacement at each hybridized dsDNA site. The random displacements followed a Gaussian distribution, with a mean value of zero and a range specified as a fraction of interchain separation of immobilized ssDNA molecules. Ensembles with a different range of perturbations were used for computing the cantilever deflection in order to investigate the influence of disorder magnitude on hybridization-induced bending.

IV. CANTILEVER BENDING COMPUTATION

Cantilever deflections are computed through minimization of the total bending and hybridized dsDNA interaction energy given in Eq. (1). A function minimization program based on the golden section method was utilized to minimize the total energy. The cantilever deflections are computed for hybridized dsDNA strands of three different lengths—10, 20, and 30 nt, immobilization densities varying from 0.046 to 0.171 nm$^{-2}$, and hybridization efficiencies varying from 10% to 100%. The combination of chain lengths, immobilization densities, and hybridization efficiencies are specified in Table I. For each case, cantilever deflections were computed for all the different ensembles of hybridized dsDNA (discussed above). In order to obtain statistically significant trends, deflections were computed for 500 different realizations of random selection and Gaussian-perturbed ensembles.

V. SIMULATION RESULTS AND DISCUSSIONS

Representative distributions of hybridized dsDNA chains, corresponding to an immobilization density of 0.13 nm$^{-2}$ and hybridization efficiency of 50%, for the four different ensembles are presented in Fig. 2. In order to quantify the chain distributions and to verify the underlying assumptions for the

![Table I. Simulation details for the four different ensembles.](image-url)
different ensembles, the average occupation density of nearest neighbor sites by a hybridized dsDNA chain was calculated as the ratio of hybridized dsDNA chains to the total number of possible sites at that neighbor level and is plotted in Fig. 3 for each of the ensembles.

In the representative realization of average spacing ensemble shown in Fig. 2(a), all the hybridized DNA strands are arranged in hexagonal closed packed manner. Since the first ensemble is based on combining the immobilization and hybridization procedures together, all the neighbor sites are filled, as shown in the occupation density plot in Fig. 3, and we refer to this ensemble as “average spacing” ensemble. The nearest neighbor distances decrease with increase in hybridization efficiency, as shown in Eq. (11).

A representative realization of the random selection ensemble is shown in Fig. 2(b) and was generated assuming a uniform immobilization of ssDNA and equal probability of hybridization for all the sites. The generated ensembles show clusters of hybridized chains, which result in a range of interchain separations. The occupation density of neighbor sites plotted in Fig. 3 is uniform for the different neighbor level, confirming the modeling assumption that all sites have equal probability for hybridization.

A realization of energy minimization ensemble is shown in Fig. 2(c) and was generated based on assumption that a ssDNA chain will hybridize in a manner that minimizes the interaction energy between the hybridized strands. The energy minimization produces a more ordered pattern of hybridized dsDNA distribution in comparison to the second ensemble, with a similar range of interchain separations. The neighborhood occupation density shown in Fig. 3 is lower for the close neighbors and increases for higher order neighbors.

The Gaussian-perturbed ensemble was generated through spatial perturbations of hybridized chain positions calculated in the third ensemble. The representative realization plotted in Fig. 2(d) corresponds to a perturbation of ±20% of initial ssDNA spacing applied to the realization plotted in Fig. 2(c). Similar ensembles with a different range of spatial perturbation were generated to investigate the influence of spatial disorder on the hybridization-induced bending. The perturbed, hybridized dsDNA arrangement does not have a clear definition of neighbor orders and neighbor separation values; therefore, the neighborhood occupation density was not calculated for this arrangement.

VI. BENDING PREDICTIONS

In our computational framework, predicted cantilever bending is linearly dependent on the chain length, because the DNA strands are assumed to stay nearly parallel throughout the cantilever deflections. Predicted cantilever bending is divided with DNA chain length in order to simplify the discussion of computational results.

Cantilever bending per nucleotide calculated for an immobilization density of 0.13 nm$^{-2}$ is plotted as a function of hybridization density in Fig. 4(a) for the four different ensembles.
ensembles. In addition, the bending predictions per nucleotide, corresponding to a hybridization efficiency of 50%, are plotted as a function of immobilization densities in Fig. 4(b) for the four different ensembles. In the case of a random-selected and Gaussian-perturbed ensemble, an average of bending predictions from 500 different ensembles is plotted and error bars correspond to the total range of the spread in the predictions.

As shown in Figs. 4(a) and 4(b), bending predictions are strongly dependent on the range and distribution of hybridized chain spacing in an ensemble. For the same immobilization density and hybridization efficiency, the ensemble with perfect arrangement has the smallest predicted displacements and the predicted displacement increase as the chain distribution becomes more disordered from the third to second and fourth ensemble. The perturbed ensembles predict much higher deflection values than the regularly arranged ensembles due to the large entropy in hybridized chain arrangement. In all cases, cantilever deflection increases with an increase in hybridization efficiency. Initial immobilization density is one of the dominant factors that affect the induced cantilever deflection. When the immobilization density is small, the hybridized chains have large separations, and the predicted deflections are almost negligible for all the four ensembles. As the immobilization density increases, the deflection results increase exponentially with an increase in immobilization density.

Predictions from the first three ensembles are similar in magnitude with the smallest deflection predicted for fully packed hexagonal arrangement of hybridized chains (average spacing ensemble) and largest deflections for a randomly selected ensemble, in which immobilized single stranded chains have equal probability for hybridization. Predictions corresponding to an energy minimization ensemble that corresponds to hybridization of single-stranded chains with minimum interaction energy lie in the middle of the first two ensembles. The interaction potential due to hydration and electrostatic repulsion has an exponential dependence on the hybridized chain spacing, and thus, the bending results are dominated by chains in close proximity to each other. The bending predictions are higher for ensembles that have a large range of intrachain spacing. At low hybridization density, the predictions from the energy minimization ensemble are close to that of the average spacing ensemble as the hybridized chains are spaced apart, but at the high hybridization density, the predictions from the random selection and energy minimization ensemble start converging, as hybridized chains are in closer proximity. Cantilever predictions corresponding to the first three ensembles converge to the same value as the hybridization efficiency approaches 100%, because, at full coverage, all three ensembles are exactly the same.

For all hybridization efficiencies and immobilization densities, predictions based on the Gaussian-perturbed ensemble are consistently higher than all other ensembles. Random spatial perturbations used to generate the fourth ensemble increase the range of interchain spacing and ensure that a significant number of hybridized chains is within a few decay lengths of the interaction potential.

In order to further examine the influence of arrangement disorder on the predicted deflection, the ratio of predicted displacements from spatially perturbed ensemble (Gaussian-perturbed) and unperturbed ensemble (energy minimization) are plotted as a function of the reciprocal of immobilization density in Fig. 5 for a different range of spatial perturbations.

![Figure 4](image1.png) **FIG. 4.** Normalized deflection predicted for the four different ensembles. (a) Deflections as a function of hybridization efficiency for immobilization density at 0.13 nm$^{-2}$; (b) deflections as a function of immobilization density for hybridization efficiency at 50%. Insets shows the details of prediction from first three ensembles.

![Figure 5](image2.png) **FIG. 5.** Influence of spatial perturbation on predicted cantilever deflections.
As shown in Fig. 5, increasing the range of spatial perturbations or disorder in the hybridized chain arrangement increases the predicted displacement. The increase in predicted displacement is also strongly dependent on the initial immobilized chain separation. For larger immobilized chain separation (smaller immobilization density), increasing the range of perturbation from ±5% to ±25% of immobilized chain separation increases the predicted displacement from about 5 times to 200 times the deflection predicted for the third ensemble. However, for smaller immobilized chain separation (larger immobilization density), increasing the range of perturbation from ±5% to ±25%, results in predicted displacement increase from about 5 times to 30 times the deflection predicted for the energy minimization ensemble. In addition, the increase in predicted displacements has a linear dependence initial separation of ssDNA chains for a fixed range of spatial perturbations, as indicated by the linear fit plotted in Fig. 5. The slope of linear fits increases with the range of spatial perturbations imposed on the ensembles. The increase in displacements clearly highlights the importance of perturbation in chain arrangement on the cantilever bending.

VII. COMPARISON OF NUMERICAL PREDICTION WITH EXPERIMENTAL REPORTS

Numerical predictions of cantilever bending are compared to the reported experimental measurements for different immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions. Early reports on experiments gave high immobilization densities, hybridization efficiencies, and chain lengths in order to demonstrate the efficacy of our modeling assumptions.}

![Image](https://via.placeholder.com/150)

**TABLE II.** Comparison of reported experimental measurements and numerical predictions.

<table>
<thead>
<tr>
<th>Experimental results</th>
<th>Immobilization density (nm⁻²)</th>
<th>DNA length</th>
<th>δ(h/L)² (10⁻⁵ nm)</th>
<th>Average spacing</th>
<th>Random selection</th>
<th>Energy minimization</th>
<th>Gaussian-perturbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fritz et al.⁶</td>
<td>~0.2</td>
<td>12 nt</td>
<td>~3</td>
<td>~1.5</td>
<td>~5.5</td>
<td>~4.5</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 nt</td>
<td>~6</td>
<td>~2</td>
<td>~7.3</td>
<td>~5.5</td>
<td>...</td>
</tr>
<tr>
<td>McKendry et al.²³</td>
<td>~0.13</td>
<td>12 nt</td>
<td>~2</td>
<td>~1.4</td>
<td>~5</td>
<td>~4</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 nt</td>
<td>~3</td>
<td>~2.4</td>
<td>~9</td>
<td>~6</td>
<td>...</td>
</tr>
<tr>
<td>Wu et al.¹²</td>
<td>~0.15</td>
<td>20 nt</td>
<td>~3.5</td>
<td>~2.4</td>
<td>~9</td>
<td>~6.5</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 nt</td>
<td>~8.5</td>
<td>~3.7</td>
<td>~1.4</td>
<td>~1</td>
<td>...</td>
</tr>
<tr>
<td>High density</td>
<td>Alvarez et al.⁵</td>
<td>~0.13</td>
<td>12 nt</td>
<td>~2.5</td>
<td>~1.4</td>
<td>~5</td>
<td>~4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 nt</td>
<td>17–37</td>
<td>~0.05</td>
<td>~0.1</td>
<td>~0.08</td>
<td>14–34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 nt</td>
<td>8–24</td>
<td>~0.095</td>
<td>~0.2</td>
<td>~0.17</td>
<td>10–24</td>
</tr>
<tr>
<td>Low density</td>
<td>Stachowiak et al.¹¹</td>
<td>0.01–0.1</td>
<td>2–6</td>
<td>~0.15</td>
<td>~0.35</td>
<td>~0.25</td>
<td>5–8</td>
</tr>
</tbody>
</table>
ssDNA, becomes larger. Therefore, for the same immobilization density, the free space between molecules is expected to be smaller for longer DNA chains and, consequently, the chain arrangements are expected to be less disordered for longer sequences in comparison to shorter DNA sequences.

Comparisons of the bending predictions and experimental measurements show that, when the immobilized chain separation is larger than a threshold (here, we picked 3.0 nm, 10 times the decay length), a Gaussian-perturbed ensemble has to be considered, due to the large free space between molecules. This implies, at smaller immobilization densities, the disorder in the hybridized dsDNA arrangement is a dominant factor in determining the cantilever bending. For low immobilization density experiments, the spatial disorder may also be influenced by the number of nucleotides in the DNA strands, and thus, more disordered arrangements are required to predict deflections for shorter nucleotides.

For larger immobilization densities, ensembles that account for disorder generated during hybridization of a closed packed ssDNA are sufficient to predict the deflection range reported by Fritz et al.\textsuperscript{6} and Wu et al.\textsuperscript{12} Our calculations show that, with large immobilization densities, the entropy induced by the hybridization method plays an important role.

For all the cases, bending predictions based on the average spacing ensemble (hexagonal closed packing of hybridized chains) do not match reported experimental results. This strong dependence of cantilever deflection on spatial arrangement disorder has important implications for the design of experiments that employ surface-adsorbed receptor molecules. The self-assembly of immobilized molecules must be carefully controlled for reproducibility and reliability of the experiments.

VIII. CONCLUSIONS

We presented a model to examine deflections of a microcantilever resulting from DNA hybridization in this paper. An empirical interaction potential for hybridized DNA chains was used in the simulation to predict hybridization-induced bending. Cantilever bending was predicted based on four different ensembles of hybridized DNA chains’ arrangement. Hexagonal close packing of hybridized DNA is the simplest ensemble to generate, but it neglects the immobilization and hybridization-induced disorder in the chain arrangements. Consequently, the hexagonal close-packed ensemble results in the smallest predictions of cantilever deflections. Hybridized DNA ensembles produced through either random selection or ensuring minimum interaction energy during hybridization of hexagonally closed packed single-stranded DNA resulted in a larger prediction of cantilever bending. Random selection ensemble has more disordered arrangement of chains and higher predicted cantilever deflection in comparison to the minimum interaction energy ensemble. Introducing spatial perturbations in the hybridized dsDNA arrangement leads to larger predictions for cantilever bending. Comparison of numerical predictions with reported experimental results indicates the importance of immobilization density in determining the arrangement of hybridized DNA chains on the surface as well as the hybridization-induced bending. At larger immobilization densities or smaller inter-chain separation, predictions based on ensembles with initial uniform immobilization and partial hybridization of DNA chains are able to predict deflections similar to experimental measurements. At smaller immobilization densities or larger interchain separation, only predictions based on ensembles with spatial perturbation of hybridized DNA strands can match the experimentally measured cantilever deflections. Comparison of numerical predictions and experimental results highlights the importance of immobilization density and spatial disorder imposed during hybridization and hybridization on the hybridization-induced cantilever bending.

ACKNOWLEDGMENTS

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\begin{figure}[h]
  \centering
  \includegraphics[width=\textwidth]{figure.png}
  \caption{Comparison of predicted displacement of Gaussian-perturbed ensemble with experimental measurements reported by Stachowiak et al. (Ref. 11).}
\end{figure}