Effective affinities in microarray data

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Abstract

In the past couple of years several studies have shown that hybridization in Affymetrix DNA microarrays can be rather well understood on the basis of simple models of physical chemistry. In the majority of the cases a Langmuir isotherm was used to fit experimental data. Although there is a general consensus about this approach, some discrepancies between different studies are evident. For instance, some authors have fitted the hybridization affinities from the microarray fluorescent intensities, while others used affinities obtained from melting experiments in solution. The former approach yields fitted affinities that at first sight are only partially consistent with solution values. In this paper we show that this discrepancy exists only superficially: a sufficiently complete model provides effective affinities which are fully consistent with those fitted to experimental data. This link provides new insight on the relevant processes underlying the functioning of DNA microarrays.

1. Introduction

In all living cells the genes are transcribed, i.e., copied into messenger RNA (mRNA), at different rates [1]. These rates depend on the type of cell, on the stage of the cell life cycle and on other external stimuli, like changes of pH, temperature or on the presence of chemicals. The abundance of a specific mRNA defines the so-called gene expression level. It is of central importance to understand when, in which tissue and in which amount a given gene is expressed. This knowledge is for instance crucial in understanding several diseases that originate from deregulations in the gene transcription process, i.e., those pathologies triggered by genes which are overexpressed or underexpressed.

DNA microarrays have become pivotal devices in molecular biology as they allow a genome-wide screening of gene expression levels in a single experiment. Both commercial and home-made microarrays are nowadays available. One of the leading companies in the DNA-microarray market is Affymetrix, which produces high-density oligonucleotide microarrays [2]. In Affymetrix arrays, photolithographic techniques are used to grow on a solid substrate
Table 1. The stacking free energy parameters $\Delta G$ for RNA/DNA hybrids measured in solution at a salt concentration of 1 M NaCl and at 45°C [5]. The upper strand is RNA (with orientation 5′–3′) and lower strand DNA (orientation 3′–5′). The helix initiation energy is $\Delta G_{\text{init}} = 3.14$ kcal mol$^{-1}$.

| Sequence       | $-\Delta G$ (kcal mol$^{-1}$) | Sequence       | $-\Delta G$ (kcal mol$^{-1}$) |
|----------------|-------------------------------|----------------|-------------------------------|
| rAA dTT        | 0.83                          | rAC dTG        | 1.99                          |
| rAG dTC        | 1.62                          | rAU dTA        | 0.70                          |
| rCA dGT        | 0.70                          | rCC dGA        | 1.92                          |
| rCG dGC        | 1.32                          | rCU dGA        | 0.73                          |
| rGA dCT        | 1.21                          | rGC dCG        | 2.56                          |
| rGG dCC        | 2.65                          | rGU dCA        | 0.93                          |
| rUA dAT        | 0.42                          | rUC dAG        | 1.31                          |
| rUG dAC        | 1.37                          | rUU dAA        | −0.08                         |

single-stranded DNA sequences which are 25 nucleotides long; these are normally referred to as probes. The array is placed in contact with a solution containing RNA molecules, i.e., the targets, extracted from biological samples. Those targets that are complementary to probe sequences tend to bind to these, a process known as hybridization. Biotin molecules are attached to a fraction of the nucleotides in the target sequences. Once hybridization has occurred and the unbound targets are washed away, streptavidin molecules, which carry fluorescent labels, are added to the solution. The latter bind with high affinity to the biotin so that the amount of hybridized probe–target duplexes can be determined experimentally by optical measurements.

Two specific aspects of Affymetrix arrays are:

1. several probes are complementary to the same target molecule (these probes form the so-called probe set), and
2. each perfect matching (PM) probe has a partner probe which differs by a single nucleotide in the middle position, the so-called mismatch (MM) probe.

The use of multiple probes for the same target RNA increases the reliability of the determination of gene expression levels in Affymetrix arrays, which are obtained from simultaneous measurements of several fluorescent signals. The signals measured from MM probes can be used as a test for the quality of the hybridization experiment. Usually, one expects that PM probes give a stronger signal than the corresponding MM probes. However, ‘bright mismatches’, i.e., higher signals from MM than PM probes, are observed quite frequently [3].

The hybridization of complementary strands in solution, or the reverse process of DNA/RNA melting, has been widely investigated in the past years [4]. Measurements of melting temperatures of short oligonucleotides have yielded estimates of the enthalpy and entropy differences $\Delta H$ and $\Delta S$ between a double helix and the two separate strands. It turns out that $\Delta H$ and $\Delta S$ can be well approximated by a sum over local terms depending on pairs of neighbouring nucleotides, plus eventual boundary terms. This defines the so-called nearest-neighbour model [4]. Table 1 gives an example of nearest-neighbour free energy parameters obtained from measurements of melting temperatures of DNA/RNA duplexes in solution. The free energy differences are obtained from $\Delta G = \Delta H - T \Delta S$, assuming that the experimentally measured $\Delta H$ and $\Delta S$ are temperature independent.
The hybridization process in microarrays is not identical to that in solution, as one of the two strands is surface-bound. A review of recent work on the hybridization on surface-immobilized DNA [6] shows that the rate constants for hybridization are lower than those predicted by the nearest-neighbour model in solution. The comparison was done with experiments with a single species target and probes of equal length [7–9].

Several studies [3, 10–15] recently discussed the role of the Langmuir isotherm and variants thereof in connection with DNA microarrays. Research toward a physics-based modelling of hybridization in Affymetrix arrays can roughly be divided into two approaches. The first approach is to identify empirical functions with many degrees of freedom, that are fitted to experimental data [3, 16]. The other approach is molecular based, and employs the hybridization energies in solution; it then requires a rescaling of parameters like the effective temperature [12, 17]. The aim of this paper is to link these two apparently different viewpoints. We shall show indeed that, when the appropriate quantities are compared, i.e. the effective affinities, the two models yield fully consistent results.

This paper is organized as follows. Section 2 reanalyses the binding affinities as introduced by Naef and Magnasco [3] and Binder and Preibisch [16]. We carry out a sensitivity analysis and show which features are robust and which are sensitive. In section 3, effective affinities are calculated using a molecular model based on the binding free energies of Sugimoto et al [5] and the extension by Carlone and Heim [17]. From this model, the influence of different additions to the molecular model on the effective affinities is calculated and analysed. Section 4 concludes the paper and summarizes the main results.

2. Effective affinities for Affymetrix arrays

We turn now to the determination of the effective affinities from the analysis of Affymetrix data. We follow here and further the procedure originally introduced by Naef and Magnasco [3] and extended more recently by Binder and Preibisch [16].

Naef and Magnasco fit the brightness $B$ of perfect-matching probes as a function of their sequence composition:

$$\ln \left( \frac{B}{[RNA]} \right) = \sum_{li} S_{li} A_{li},$$

where $l = A, C, G, T$ is the letter index and $i = 1, \ldots, 25$ the position along the probe. $S_{li}$ is a Boolean variable equal to 1 if the probe sequence has letter $l$ at position $i$ and 0 otherwise, and thus $A_{li}$ are per-site, per-letter affinities. The median of the PM brightnesses $[RNA]$ is used in this expression as a surrogate for the RNA concentration, which is not known for most Affymetrix data.

In Affymetrix experiments, the brightness $B$ will saturate, once the majority of the probes are bound to targets. Capturing such saturation requires the use of Langmuir isotherms; the approach above (equation (1)) neglects saturation effects, and hence is only expected to work in the so-called Henry regime [18] signified by brightnesses much lower than the maximal value. Since only few probes reach saturation, neglecting saturation is justifiable.

The experimentally measured fluorescence intensity $I_s$ of a probe with sequence $s$ does not approach zero at zero concentration of the matching target: there is a background signal, probably due to non-specific binding. To take this into consideration, we distinguish two contributions to the fluorescence intensity: a constant background intensity $I_0$ and the brightness $B$ due to specific binding:

$$I_s = I_0 + B,$$
Figure 1. Position-dependent effective affinities fitted from Affymetrix data of the HGU95A chip set. Three different background values are subtracted: $I_0 = 0, 50$ and 100. The three topmost curves are the affinities for nucleotides C and the three lowest curves for the nucleotides A. The affinities for T and G are almost degenerate.

in which $B$ is the brightness as in equation (1). We tried different background subtractions schemes in order to test the robustness of the data. Figure 1 shows the position-dependent affinities $A_{ij}$ obtained from fitting the experimental data to equations (1) and (2) for background intensities of $I_0 = 0, 50$ and 100 (constant background level). In the fit, the distance of the data to the model was minimized in the logarithmic scale. We note that although the shape of the fitted position-dependent affinities remain the same in the three cases, the amplitudes vary by a factor of 4. In all cases the shape is consistent with what was found in [3, 16]: the position-dependent affinities are approximately symmetrical with respect to the central position of the probe ($i = 13$) and the highest affinity is for nucleotides C and the lowest for A in the probe sequence. The affinities for the G and T bases are almost degenerate and show less position dependence than the affinities for the C and A bases.

In the case of $I_0 = 0$ we have a rather low signal. This is somehow expected as in that case the non-specific part of the signal may dominate, which induces a loss of specificity. When higher values of $I_0$ are taken, a non-trivial signal starts to emerge. As $I_0$ increases, the amplitude of the strongest effective affinity increases to 0.2 and 0.4 for respectively $I_0 = 50$ and 100.

In figure 2 we plot the fitted affinities $A_{ij}$ for probe sets with an average intensity above 500. This case corresponds to signals well above the background level and thus the results should be weakly dependent on the value of $I_0$ chosen, as is indeed the case.

As mentioned above, using the median of the PM brightnesses [RNA] as an estimate for the RNA concentration is the only thing one can do in the absence of knowledge of its true value. Affymetrix, however, performed a set of experiments in which some target sequences are added in solution (spiked-in) at a known concentration. The results, known as the Latin square data set, are publicly available from the Affymetrix web site [19]. We used these data to refit the effective affinities from equation (1), using the true target concentration $c_s$ of sequence $s$, rather than the median of the intensities. Due to the large number of parameters, this procedure yields typically values of $A_{ij}$ that are too noisy. To limit the number of fitting parameters we therefore have fitted $A_{ij}$ only at some fixed positions $i = 1, 4, 7, 10 \ldots 25$ and taken for the other values of $i$ a linear interpolation between the two fitted numbers. Note that the Latin square set also contains a series of reference intensities measured in the absence of the transcripts in solution.
As in figure 1, but disregarding all the data for probe sets with an average intensity below \( I_0 = 500 \). The effective affinities are less sensitive to the choice of \( I_0 \), compared with the fits of figure 1.

Figure 3. Fit of spike-in data of the HGU95A microarray using equation (1). Here, we subtract from the intensity the known background intensity at zero concentration. (i.e., \( c_s = 0 \)), a procedure that yields a direct estimate of the background signal \( I_0 \). The position-dependent affinities obtained from the fitting of the Latin square set are shown in figure 3. The results, although still somewhat noisy, follow the general trend already shown in figures 1 and 2.

The fact that the position-dependent affinities are lower for G than for C and for A than for T is consistent with the hybridization data in solution, as pointed out in [20]. This apparent ‘asymmetry’ is due to the asymmetry between DNA strands of the surface-bound probes and the RNA strands of the target molecules in solution.

The fact that the effective affinities for G and T are close is quite surprising, given the clear differences in binding free energies in solution; we will argue below that this is due to hybridization between RNA target molecules in solution.
3. Effective affinities resulting from molecular-based models

To obtain more insight into the relation between the hybridization free energies of table 1 and the effective affinities of references [3, 16] and which we analysed in the previous section, we extract effective affinities from a model which was recently proposed by two of us [17].

This model is based on ideas from Held et al [12]. As it uses as input the binding free energies between DNA and RNA strands in solution reported in table 1, we will refer to it as the molecular-based model. Additionally, it incorporates the effect of binding in solution of RNA to RNA in an approximate way, fitted to the intensities measured on an Affymetrix chip. The intensity $I_s$ of sequence $s$ is assumed to be proportional to the fraction of hybridized probes at the surface, described by a Langmuir model. In detail, it is given by [17]

$$I_s = I_0 + \frac{\alpha_s c_s Z_s}{1 + \alpha_s c_s Z_s} I_{\text{max}},$$

where $c_s$ is the total concentration of targets with sequence $s$ in solution, $Z_s$ is the partition sum over states in which target $s$ is bound to the probe, and $\alpha_s$ is the fraction of targets in solution which are free, and not hybridized in solution.

In the model of reference [17]

$$Z_s = \exp(-\beta \Delta G_s),$$

where $\beta = 1/(RT)$ is the inverse temperature, and $\Delta G_s$ is the total binding free energy for a perfectly formed helix of 25 base pairs between the RNA target and DNA probe. This binding free energy is described by

$$\Delta G_s = \sum_{i\neq i'} S_{i,i'} S_{i+1,i'} \Delta G(l,l') + \Delta G_{\text{init}}.$$  

As before, $S_{i,i'}$ is a Boolean variable equal to 1 if the probe sequence has letter $l$ at position $i$ and 0 otherwise. Thus, the sum in equation (5) runs over all 24 stacking parameters $\Delta G(l,l')$, which depend on the identity of two neighbouring nucleotides $l$ and $l'$ in the surface-bound DNA strand. $\Delta G_{\text{init}}$ represents a helix initiation cost [4]. For the stacking parameters the model uses RNA/DNA free energies given in table 1, as obtained from experiments in solution [5]. Note that, differently from the approach of [3] and [16], the free energies used here are position independent. In [17], the inverse temperature $\beta$ in equation (4) is taken as a fitting parameter.

We stress that in [17] the hybridization free energy $\Delta G = \Delta H - T \Delta S$ was taken at $T = 37 \, ^\circ C$, while an Affymetrix hybridization experiment is performed at $T = 45 \, ^\circ C$, which is the value we consider here (see table 1). Although the temperature differs by only 8 $^\circ C$, the $\Delta G$ on average differ by about 20%, since $\Delta H$ and $T \Delta S$ are rather close. We took the sequences of the Latin square set (25 nucleotides of length) and generated the $\Delta G$ of each sequence at both temperatures. A plot of $\Delta G_{37}$ versus $\Delta G_{45}$ shows that the values are narrowly distributed along a straight line. This implies that a difference between the two choices of parameters can be reabsorbed in a rescaling of $\beta$ in equation (4).

Of practical interest is the total concentration $c_s$ of targets with sequence $s$. Due to hybridization of single-stranded RNA in the solution, the concentration of free targets, which can bind to the probes, is lower than the total concentration of targets in solution. In the model of [17], this is taken into account by reducing the total concentration $c_s$ in solution by a factor of $\alpha_s$ given by

$$\alpha_s = \frac{1}{1 + c_0 \exp (\beta' \Delta G_R)},$$

where $\beta'$ and $c_0$ are fitting parameters and $\Delta G_R$ is the (sequence-dependent) RNA/RNA binding free energy for duplex formation in solution, taken from [4]. Note that also $\alpha_s$ is
highly sequence dependent: CG-rich targets will have high affinity to the complementary surface bound probes, but will also have a strong tendency to hybridize in solution. It has been shown that a unique choice (i.e., probe independent) of the parameters $I_{\text{max}}, \beta, \beta'$ and $c_0$ fits the experimental data well [17].

There are many similarities, and also some discrepancies, between the intensities $I_i$ in the Naef and Magnasco (NM) approach equation (2) and in the molecular-based model equation (3). The binding free energy in the NM approach is captured in the summation on the right-hand side of equation (1), which is very similar to the summation in equation (5) in the molecular-based model. NM uses a summation over single base pairs with position-dependent affinities, while the molecular-based model uses (in equation (5)) a summation over pairs of base pairs (allowing for stacking energies), with a position-independent strength. As we already mentioned, NM does not feature saturation, while the molecular-based model does through the denominator in equation (3). Finally, the clear position dependence in the effective affinities obtained with the NM approach is not included in the molecular-based model of [17].

3.1. Extending the molecular-based model

In this work, we introduce several extensions to the latter model. These extensions will cause position dependence in the effective affinities, without ad hoc modifications to the stacking free energy parameters. Most of these extensions are related to the fact that both target and probe are polydisperse in length, and that the duplex can fluctuate and partially unzip. We will first explain these extensions, and then discuss their effect later.

- **Unzipping.** Besides the configuration in which the target is bound to the probe over its full length, other configurations occur in which the target covers only part of the probe. This is taken into account by a ‘zipper’-model. As a result, the partition sum $Z_i$ not only contains a single term exp$(-\beta \Delta G_i)$, but is a summation over many terms, each of which given by equation (5), but in which the index $i$ runs from the first bound pair $m$ to the last bound pair $r \geq m$. This idea is visualized in figure 4.
• **Probe length dispersity.** During the production process of the Affymetrix chips, the probability $p_g$ that the probe grows by an extra nucleotide is only around $p_g \approx 90\%$ [21]. This means that the fraction of probes which reach the final full length of 25 nucleotides is $P(25) = (p_g)^{25}$. The fraction of incomplete probes reaching a length $l < 25$ equals $P(l) = (p_g)^l (1 - p_g)$. We have included the effect of probe length dispersity by including these probabilities in the calculation. The intensity is therefore equal to $I = \sum_{l=1}^{25} P(l) I_l$, where $I_l$ is the Langmuir isotherm corresponding to a probe of length $l$.

• **Non-specific binding.** Even in Affymetrix experiments where no perfect matching targets are present, the intensity does not fall well below 0.5% of the maximal intensity. We attribute this to non-specific binding to the probes. To account for the non-specific binding, we include in our model a constant sequence-independent background intensity $I_0$.

• **Tail repulsion.** The RNA-target molecules often extend beyond the 25 base pairs of the probe; the average target length is 50 base pairs. The tail of the target which sticks out from the base of the probe is hindered significantly by the surface (see figure 4). This causes an entropic repulsion between the target and the surface, lowering the intensity. The mathematics of this effect is presented in the appendix. This effect is not sequence dependent and the parameters $Z_s$ in equation (3) can therefore be multiplied by a constant factor $Z_{S_{\text{tail}}}$, given in equation (A.4).

• **Fluorescent labels.** Due to the fact that in the experiments only the U and C nucleotides can have a label, the fluorescence intensity will scale linearly with the number of U and C nucleotides, which obviously depends on the sequence. We therefore multiplied each Langmuir isotherm by a factor $2^{X_{\text{UC}}}$, in which $X_{\text{UC}}$ is the fraction of U and C in the target sequence. We assumed that the target is simply composed of 25-mers.

### 3.2. Results of the model calculations

We generated 100,000 different random sequences of 25 nucleotides each. For each sequence $s = 1 \ldots 10^5$, we also selected a concentration $c_s$, with a minimal value of $c_{\text{min}} = 1 \text{pM}$ and $c_{\text{max}} = 1 \text{nM}$ (the typical range of target concentrations in Affymetrix arrays); the logarithm of these concentrations $\log(c_s)$ is drawn from a uniform distribution $[\log(c_{\text{min}}), \log(c_{\text{max}})]$. For each sequence $s$, the intensity $I_s$ is calculated using the molecular-based model, equation (3), with the extensions just described. The parameters entering this equation are the stacking free energies given in table 1, as well as the parameter $\alpha_s$ reflecting the reduction of the total concentration of free targets in solution; this latter (sequence-dependent) parameter uses the RNA/RNA binding free energies for duplex formation in solution, taken from [4]. The modifications in the molecular-based model as compared to the model in [17], as well as the different choice of free energy parameters ($\Delta G_{45}$ versus $\Delta G_{37}$), require a refitting of the effective inverse temperature $\beta'$ and a concentration $c_0$, which yielded $\beta' = 0.6$ (kcal/mol)$^{-1}$ and $c_0 = e^{\epsilon\beta'}$, with $\epsilon = 42$ kcal mol$^{-1}$. The fitting procedure for these two parameters follows the procedure of [17].

In the experimental Affymetrix data set, the average intensity is around 3% of the maximal intensity. In all our simulations, we adjusted the temperature to reproduce this average intensity. The resulting temperatures range from 494 to 550 K. There is still a gap between the experimental temperature of 318 K, but including the effects mentioned above has significantly decreased this gap in the original molecular-based model, where the effective temperature was 700 K [17]; in turn the latter model had already a much more realistic effective temperature than the Held model where the effective temperature exceeded 2000 K [12]. To obtain the effective affinities $A_i$, associated to the molecular-based model, we minimize the difference between the
Figure 5. Effective affinities, obtained with the molecular-based model, versus position in the probe, for the four different nucleotides. In panel (a) only the binding energy is taken into account; the effective temperature $T$ is $800 \text{ K}$. In (b), the hybridization in solution is also taken into account, as in the molecular-based model of [17]; the resulting effective temperature becomes $T = 570 \text{ K}$. The effect of using the ‘zipper’ and the probe length distribution is shown in (c), resulting in an effective temperature of $T = 525 \text{ K}$. In (d) all effects mentioned in the text are taken into account and the effective temperature is reduced to $T = 494 \text{ K}$.

(This figure is in colour only in the electronic version)

The intensity $I_s$ as predicted by the molecular model in equation (3) and the intensity $I_e$ resulting from the effective affinities and given by

$$\ln(I_e) = \sum_{ni} S_{ni} A_{ni} - \ln(c_0),$$

in analogy to equation (1). More precisely, the effective affinities $A_{ni}$ result from a minimization of the sum over all 100,000 sequences of the squared difference between the logarithm of the intensity $I_s$ and the logarithm of the intensity $I_e$ resulting from the effective affinities.

The first data set comprises a simple two-state model, in which a target is either free in solution, or fully bound to a probe. Hybridization in solution is not taken into account, i.e., $\alpha_s = 1$. The results are shown in figure 5(a). The effective affinities do not depend on the position, apart from the two edge nucleotides which enter in only one pair of neighbouring base pairs (see equation (5)). Note that the affinities increase with the ordering $A < T < G < C$, as expected from the values of the free energies of table 1.

Next, the hybridization in solution is taken into account by using two extra parameters $\beta'$ and $c_0$ which have the values of $\beta' = 0.6 \text{ (kcal/mol)}^{-1}$ and $c_0 = e^{\epsilon \beta'}$ with $\epsilon = 42 \text{ kcal mol}^{-1}$, respectively. Still the effective affinities are not position dependent; see figure 5(b). However, the order of the curves has changed: $A < T < G < C$.

Figure 5(c) shows the effective affinities when polydispersity of the probe length distribution and the effect that a duplex can zip open has been taken into account. These two effects lead to position-dependent effective affinities. The effect on the side of the microarray surface is larger than that on the solution side. Furthermore, the effective affinities of $G$ and $T$ have become more alike.
The last panel of figure 5 shows the effective affinities when also the effect of noise, entropy of the tails, and the fact that only U and C carry fluorescent labels are taken into account. The biggest effect is that the effective temperature is lowered. Furthermore, the sequence has become A < G ≈ T < C, in agreement with the order of effective affinities observed in experiment (see figure 2). Note also that the scale of amplitudes of the effective affinities ranges from about −0.2 to 0.2 (see figures 5(c) and (d)). This is fully consistent with the values obtained in section 2.

4. Conclusions

In this paper we have analysed the relation between the effective affinities as originally introduced by Naef and Magnasco [3] and those obtained from a molecular-based model [17], which uses hybridization free energies in solution. We show that these two models yield very similar effective affinities. This implies that free energies in solution are adequate parameters to describe hybridization in Affymetrix microarrays, at least if an effective temperature is used.

Firstly, the fact that the effective affinity for G is lower than that for C and that the affinity for A is lower than that for T is consistent with hybridization data in solution, as pointed out in [16, 20]. Here, we have shown the role of target–target hybridization in solution, which in the molecular-based approach [17] is described by a parameter α (see equation (6)). The effect of α is of diminishing the differences in the effective affinities between different nucleotides so to make the effective affinities for G and T almost ‘degenerate’ (see figure 5). This is consistent with the data of Naef and Magnasco [3], Binder and Preibisch [16] and our results of section 2. The basic physics behind this effect is quite clear. The small difference between the effective affinities for G and T, in spite of the large difference in binding free energies in solution between these two nucleotides, is caused by the fact that G-rich sequences tend to hybridize strongly in solution, thereby diminishing their concentration available for binding to the probes.

We note that the calculation of the previous section yields effective affinities which are position dependent, mostly caused by the ability of the probe–target complex to partly open up at the ends. To a lesser extent, the target–surface repulsion and the polydispersity of the probes also play a role. The profiles of the effective affinities calculated in section 2 are somewhat smoother than those deduced from the molecular-based model. This difference is, however, small. The most important aspect of our analysis is, however, that the molecular-based model (1) reproduces the degeneracy between the affinities of G and T, and (2) yields amplitudes for the affinities quantitatively close to those calculated in section 2.

We finally comment on other possible ways of linking effective affinities to hybridization free energies obtained from melting experiments in solution. A recent study [22] attributed the differences between the two quantities to the effect of biotin molecules on the binding. This is an alternative point of view compared to our approach which emphasizes instead the effect of hybridization in solution between partially complementary single-stranded RNA molecules. In this respect it would be interesting if measurements of melting temperatures experiments of biotinilated RNA and DNA duplexes in solution similar to that of [5] could be performed. These experiments would allow one to quantify the effect of biotin on binding. To our knowledge such experiments have not yet been performed.

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Appendix. Entropic repulsion between substrate and target tail

We model the single-stranded DNA segment as a freely jointed chain with Kuhn length \( b \). The probability distribution that a segment of \( N \) Kuhn steps extends to a distance \( \vec{r} \) from its origin is given by a Gaussian distribution:

\[
\Gamma(\vec{r}, N) = \left( \frac{3}{2\pi Nb^2} \right)^{3/2} e^{-3r^2/2N b^2}.
\]  (A.1)

To determine the number of polymers starting from a height \( z \) above the surface and not crossing the wall, we use the method of mirror images. Using the same configuration as in figure A.1: the fraction of walks of length \( N \) originating from \( \vec{r} = (0, 0, z) \) and terminating at \( \vec{r}' = (0, 0, z') \) is equal to \( \Gamma(\vec{r}' - \vec{r}, N) \). A part of these cross the wall. This fraction is equal to \( \Gamma(\vec{r}' + \vec{r}, N) \), i.e., the number of walks originating in \( -\vec{r} \) and terminating in \( \vec{r}' \). Therefore the fraction of walks of total length \( N \) starting in \( \vec{r} \) and terminating in \( \vec{r}' \) and which do not cross the wall is given by the difference:

\[
e^{\Delta S_{r_1}}/R = \int_{z > 0} d\vec{r}' \left[ \Gamma(\vec{r}' - \vec{r}, N) - \Gamma(\vec{r}' + \vec{r}, N) \right]
= \text{Erf} \left( \frac{z}{b} \sqrt{\frac{3}{2N}} \right),
\]  (A.2)

where \( \text{Erf}(x) \) denotes the error function defined as

\[
\text{Erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} dt.
\]  (A.3)

We recall that the Kuhn length is related to the persistence length as \( b = 2l_p \) and that for single-stranded DNA \( l_p \approx 5 \text{ bp} \).

We sum next over all possible tail lengths. Before hybridization the target molecules are fragmented at random locations, with an average fragment length of about 50 bp. We find thus:

\[
e^{\Delta S_{r_1}/R} = (1 - \gamma) \sum_{N=0}^{\infty} \gamma^N \text{Erf} \left[ \frac{m + m_0}{10} \sqrt{\frac{3}{2N}} \right],
\]  (A.4)

in which \( \gamma = 49/50 \) is the probability for chain continuation, and \( m_0 \) is the ratio of the spacer distance and the length of a single base pair.
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