The Biophysics of DNA Hybridization with Immobilized Oligonucleotide Probes

Vincent Chan,* David J. Graves,* and Steven E. McKenzie*‡
*Department of Chemical Engineering, University of Pennsylvania, Philadelphia, and ‡Department of Pediatrics, University of Pennsylvania School of Medicine and the Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104 USA

ABSTRACT A mathematical model based on receptor-ligand interactions at a cell surface has been modified and further developed to represent heterogeneous DNA-DNA hybridization on a solid surface. The immobilized DNA molecules with known sequences are called probes, and the DNA molecules in solution with unknown sequences are called targets in this model. Capture of the perfectly complementary target is modeled as a combined reaction-diffusion limited irreversible reaction. In the model, there are two different mechanisms by which targets can hybridize with the complementary probes: direct hybridization from the solution and hybridization by molecules that adsorb nonspecifically and then surface diffuse to the probe. The results indicate that nonspecific adsorption of single-stranded DNA on the surface and subsequent two-dimensional diffusion can significantly enhance the overall reaction rate. Heterogeneous hybridization depends strongly on the rate constants for DNA adsorption/desorption in the non-probe-covered regions of the surface, the two-dimensional (2D) diffusion coefficient, and the size of probes and targets. The model shows that the overall kinetics of DNA hybridization to DNA on a solid support may be an extremely efficient process for physically realistic 2D diffusion coefficients, target concentrations, and surface probe densities. The implication for design and operation of a DNA hybridization surface is that there is an optimal surface probe density when 2D diffusion occurs; values above that optimum do not increase the capture rate. Our model predicts capture rates in agreement with those from recent experimental literature. The results of our analysis predict that several things can be done to improve heterogeneous hybridization: 1) the solution phase target molecules should be about 100 bases or less in size to speed solution-phase and surface diffusion; 2) conditions should be created such that reversible adsorption and two-dimensional diffusion occur in the surface regions between DNA probe molecules; 3) provided that 2) is satisfied, one can achieve results with a sparse probe coverage that are equal to or better than those obtained with a surface totally covered with DNA probes.

INTRODUCTION

Hybridization, or pairing, of complementary biomolecules is a basic principle of molecular biology used in methods such as Southern and Northern blotting and in nucleic acid amplifications such as the polymerase chain reaction (PCR). Such hybridization can take place in solution or with one molecule of the pair attached to a solid phase. Traditionally, solid-phase hybridizations are conducted on nitrocellulose or nylon membranes and are time consuming because of the time needed to achieve detectable hybridization and the need to extensively wash off nonspecifically bound molecules. Hybridization of DNA from free solution to an array of many different immobilized DNA probes on a surface in microscopic spots is very new but is potentially one of the most promising analytical techniques in molecular biology. From such an array, in theory, the identity or sequence of an unknown DNA target can be distinguished by the patterns of hybridized molecules. In keeping with the emerging convention for immobilized arrays, the known immobilized DNA molecules are called probes and the unknown DNA molecules in the solution are called targets. Targets are usually labeled either directly with fluorescent or radioactive molecules or indirectly with conjugates that then bind fluorescent, chemiluminescent, or radioactive molecules. During the past few years, considerable work has been done on the development of practical devices based on this principle for disease diagnosis and large-scale genome sequencing (Chetverin and Kramer, 1994; Eggers et al., 1994; Jacobs and Fodor, 1994; Lamture et al., 1994; Maskos and Southern, 1992, 1993; Mirzabekov, 1994; Pease et al., 1994; Southern et al., 1994). This new technology has the potential to replace traditional techniques because it is less labor intensive and uses less of the expensive DNA probe material.

Advances in solid-phase synthesis have allowed researchers to attach biomolecules like DNA oligonucleotides or peptides to solid surfaces and create dense patterns of specific diverse molecules on a single surface (Chrisey et al., 1994; Jacobs and Fodor, 1994; Maskos and Southern, 1992; Pease et al., 1994; Southern et al., 1994). Several groups have constructed oligonucleotide arrays with as many as 10^5 different types of DNA molecules on a single silicon or glass surface (Pease et al., 1994; Southern et al., 1994). They demonstrated that these arrays of DNA were capable of detecting a single base mismatch of fluorescently tagged or radioisotopically labeled complementary DNA targets. All of these published studies utilized short DNA molecules both as solution targets and as immobilized probes, and

Received for publication 24 February 1995 and in final form 2 September 1995.

Address reprint requests to Dr. David J. Graves, Department of Chemical Engineering, 311A Towne Building, 220 S. 33rd St., Philadelphia, PA 19104-6393. Tel.: 215-898-7951. Fax: 215-573-2093; E-mail: graves@cheme.seas.upenn.edu.

0006-3495/95/12/2243/13 $2.00

focused mainly on experimental aspects of DNA-DNA hybridization such as hybrid detection and surface chemistry. There is, however, a lack of knowledge about fundamental aspects of heterogeneous hybridization, such as the kinetics of diffusion and adsorption of DNA molecules on solid supports. As a result, a more quantitative understanding of this process would give molecular biologists and engineers more information for designing a better system in terms of efficiency and selectivity. For the desired genetic analysis to be done accurately and efficiently, the detailed physical and chemical parameters governing the combined transport and reaction processes must be understood more completely. Practical questions in the design and use of immobilized probe arrays include: What probe density is optimal? How big should the probes and targets be? What degree of nonspecific adsorption is permissible? and, What concentration of target is best? The critical question is how these parameters will influence the capture (or hybridization) rate.

Many other important biological processes involve the interaction of solutes and surfaces. These include biological events such as cell signaling after ligand-receptor interactions and biotechnology applications such as substrate cleavage by immobilized enzymes. Recent mathematical models of ligand-receptor binding are summarized by Lauffenburger and Linderman (1993). We turned to the body of theoretical knowledge in these fields as an initial point for models of DNA hybridization. For the last 26 years, theoretical and experimental researchers have been interested in how the reduction of dimensionality (RD) enhances the rate of capture on a surface as originally proposed by Adam and Delbruck (1968) in their seminal paper. They hypothesized that nonspecific adsorption of molecules on a surface and the subsequent two-dimensional (2D) diffusion to the capture site can enhance the overall capture rate. When one partner in a bimolecular reaction is immobilized on a surface, the rate of capture will in general depend on events in the bulk (three dimensions) as well as on the surface (two dimensions), and on the relative ratio of solute diffusion to the intrinsic reaction rate. The predicted capture rate can be approached by consideration of several published theories (Berg and Purcell, 1977; DeLisi, 1980; Wang et al., 1992): 1) the maximum capture rate, that to a surface that is uniformly reactive and a perfect sink (Berg and Purcell, 1977); 2) capture by a surface with dispersed small capture sites which act as perfect sinks (Berg and Purcell, 1977; Wang et al., 1992); 3) capture by a surface with dispersed small capture sites with intrinsic reaction limitation (Axelrod and Wang, 1994). In those physical situations with dispersed small capture sites, the presence or absence of surface diffusion mediated by nonspecific adsorption to non-capture site regions of the surface can be explicitly included in the predicted reaction rate.

Axelrod and Wang (1994) recently developed a combined reaction-diffusion rate-limited model for ligand-receptor interactions that occur on a cell surface. Their theory was based on the Brownian nature of a ligand's motion and the nonspecific adsorption/desorption of ligands on the cell surfaces. Although the authors (Axelrod and Wang, 1994) identified the model as reaction limited, it is actually a hybrid between a diffusion- and reaction-controlled model because the overall kinetics of the binding are sensitive to both the reaction and surface diffusion rates. Their model is similar in nature to the modified Smoluchowski theory reviewed by Torney and McConnell (1983) and applied by Gaspers et al. (1995) in their data analysis, which described (2D) reaction between two reactants as a combination of the surface diffusion rate and the intrinsic reaction rate.

We have modeled the biophysical situation in heterogeneous DNA-DNA hybridization to predict capture rates based on the literature of receptor-ligand interactions. In particular, because of the evidence that there is an intrinsic reaction limitation, we apply and extend the Axelrod and Wang model (subsequently referred to as AW) to DNA hybridization. We then compare capture rate predictions with those from other models. In contrast to AW, our theoretical model explicitly varies the size of the interacting molecules (DNA in our case) both in solution and on the surface and correlates the experimental hybridization rate with measurable parameters such as the surface diffusion coefficient, equilibrium adsorption constant, and the nonspecific adsorption/desorption rates, all of which depend on the DNA size. We define an optimal surface probe density. Finally, we compare our model results with recent data from the literature (Maskos and Southern, 1992) and show agreement between the two.

THEORY AND DEFINITIONS

Model and assumptions

An overview in the reaction-diffusion process in heterogeneous DNA hybridization is shown in Fig. 1. The diagram indicates that hybridization can occur either directly through three-dimensional (3D) diffusion from free solution to the probe or through nonspecific reversible adsorption from free solution to regions not covered with immobilized DNA, followed by 2D diffusion of the target to the probe. The nonspecific adsorption rate (k_a, cm/s) and desorption rate (k_d, s^{-1}) together uniquely define an adsorption equilibrium constant (K_e, cm).

The assumptions in this combined diffusion-reaction limited model of heterogeneous DNA-DNA hybridization include the following: 1) The surface is covalently linked to a fixed number of probe DNA molecules that are equally spaced and exposed to the same chemical environment. 2) Each of the immobilized DNA molecules reacts irreversibly with only one complementary DNA target from free solution. 3) The number of available probes is constant throughout the hybridization and is independent of the reaction rate. Assumption 3 appears valid if one is interested in the initial (maximal) rate of hybridization. 4) The surface is an infinite flat plate that is H cm away from a DNA source of a fixed and finite concentration (i.e., there are no end effects). 5) Surface coverage of the nonspecifically

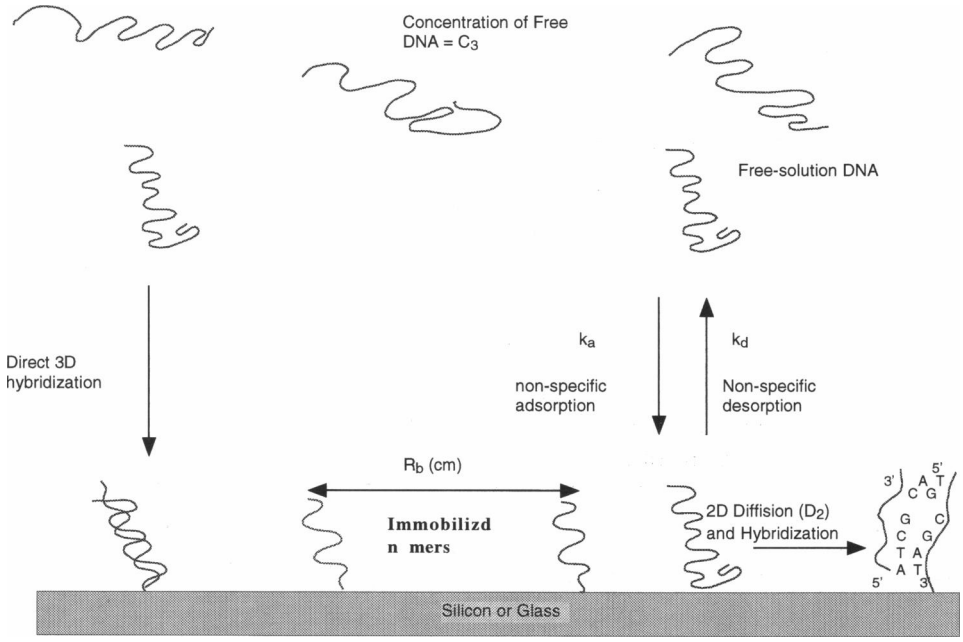

FIGURE 1 Physical model of heterogeneous DNA hybridization to an array of immobilized probe molecules on a surface.

adsorbed DNA targets is well below a monolayer, so that the lateral interactions between the adsorbed molecules can be neglected. It was reported by Tilton et al. (1990a) that the 2D diffusion coefficient of proteins strongly depends on their concentration at the surface.

The detailed mathematical and physical arguments of the reaction-limited model follow those of Axelrod and Wang (1994) and are summarized in Appendix A. The use of the initial rate to model heterogeneous hybridization is strongly supported by the experimental methods used by several experts in heterogeneous hybridization on glass and silicon surfaces. Pease et al. (1994) detected fluorescence signals from heterogeneous hybridization by applying a hybridization mixture with labeled DNA targets to immobilized oligonucleotide surfaces at 15°C for 15 min. Lamture et al. (1994) as well as Maskos and Southern (1992, 1993) detected a radioactive signal from hybridization at a lower temperature of 4°C for 0.5–2 h (with duplex density of 1.5×10^9-4.3×10^{10} molecules/cm^2). From their experimental measurements, they showed that the final concentration of hybridized molecules on the surface ranged from 0.017% to 4.4%. These results showed that a low fractional degree of hybridization is sufficient for detecting hybridized molecules in clinical applications. Furthermore, rapid results would greatly extend the range of applications relative to the classical method of Southern blotting, which often takes 24 h or more.

In heterogeneous DNA-DNA hybridization, each DNA molecule immobilized on the surface as a probe can react to completion with only one complementary DNA molecule from free solution. Experimental evidence indicates that DNA-DNA hybridization in free solution and on surfaces is often a reaction rate-limited process (Bloomfield et al., 1974; Meinkoth and Wahl, 1984; Wetmur, 1991; Wolf et al., 1987). One group measured a rate constant for oligonucleotide association in solution to be about 4×10^6 M^{-1} s^{-1}, which was two to three orders of magnitude smaller than the diffusion-limited rate (Bloomfield et al., 1974). Another group (Parkhurst and Parkhurst, 1995) applied fluorescence resonance energy transfer to study the hybridization rate between a 16 mer and its complement in solution and determined a rate constant of 5.7×10^5 M^{-1} s^{-1}, which is four orders of magnitude smaller than the estimated diffusion-limited rate of 8.9×10^9 M^{-1} s^{-1}. When one of the two complementary oligonucleotides is immobilized, it is likely that the steric limitations will cause the process to be even more reaction rate limited than in solution. Very few data on heterogeneous hybridization exist. From a study of heterogeneous hybridization on nitrocellulose membranes at high solution concentrations of DNA, the kinetics were found to be first order in the immobilized DNA concentration by one group (Meinkoth and Wahl, 1984). This implies a reaction-limited situation.

The following specifications are particular to DNA hybridization. First, the surface is assumed to possess two types of sites, namely a region where DNA can adsorb on the target DNA and a nonspecific adsorption/desorption region. Each immobilized DNA molecule is assumed to occupy a semispherical region of radius R_a and to be separated from neighboring molecules by a distance R_b. R_b can be calculated directly from the surface density of immobilized DNA assuming a regular square array of DNA on the surface:

$$R_b = \sqrt{\frac{1}{C_s \times 4}} \tag{1}$$

where C_s is the concentration of immobilized DNA probes on the surface (molecules/unit area), which can be measured, for example, by radioactivity, using a gas phase array

detector (Lamture et al., 1994). In our calculations, R_b was varied from a minimum value of $2R_a$, which indicates the closest packing of DNA on a surface, to a maximum of 0.01 cm, which indicates a very low surface concentration of immobilized DNA. The definition of completed hybridization in this model is similar to that of successful collision rate defined by Axelrod and Wang (1994) in ligand-receptor binding on cell surfaces. It is the collision between the target DNA molecules and the immobilized probe DNA molecules that leads to an irreversible binding (under the given experimental conditions) between them through Watson-Crick base interactions. The process of two-dimensional diffusion is well recognized in biological systems such as cell membranes as well as on man-made materials (Burghardt and Axelrod, 1981). Several research groups successfully measured the lateral mobility of synthetic lipids on cell membranes (Kubitscheck et al., 1994) and proteins on various substrates (Burghardt and Axelrod, 1981; Gaspers et al., 1994; Tilton et al., 1990a). Nevertheless, there is still controversy about the effect of 2D diffusion on the actual rate of reactions in immobilized enzyme systems and its role in physiological functions at the cellular level. The existence of nonspecific DNA adsorption has been shown experimentally by several researchers (Boyle and Lew, 1994; Chetverin and Kramer, 1994; Eggers et al., 1994; Lamture et al., 1994; Maskos and Southern, 1992, 1993; Pease et al., 1994; Southern et al., 1994; Vogelstein and Gillespie, 1979).

Model parameters

Three parameters were defined and studied by AW: the two-dimensional (2D) fraction f, the reduction of dimensionality (RD) enhancement η, and the global efficiency γ. We specifically modified these for the case of heterogeneous DNA hybridization and have extended the model to define another equation, a scaling correlation for the design of an optimal system.

The first important parameter is the 2D fraction f_2^{irr}, which is the ratio of the hybridization rate on a surface due to lateral diffusion F_{2D}^{irr} (moles/probe-s) to the total hybridization rate from both surface and solution phase processes F_t^{irr} (moles/probe-s):

$$f_2^{irr} = \frac{F_{2D}^{irr}}{F_t^{irr}} \tag{2}$$

$$f_2^{irr} = \left[\left(\frac{3\pi}{16}\right)\left(\frac{\sigma_2}{\sigma_3}\right)\left(\frac{\chi_3}{\chi_2}\right)\left(\frac{D_3}{D_2}\right)\left(\frac{R_a}{K_e}\right) \cdot \left(1 + \frac{8\chi_2 D_2 R_a}{\pi\sigma_2 k_d (R_b^2 - R_a^2)}\right) + 1\right]^{-1} \tag{3}$$

A second important parameter in heterogeneous hybridization is the reduction of dimensionality (RD) enhancement factor η^{irr}, which is the ratio of the total reaction rate with 2D diffusion to the rate when D_2 is set equal to zero:

$$\eta^{irr} = \frac{1 + \frac{2}{3}\left(\frac{R_b^2\sigma_3}{\chi_3 R_a^2 H}\right)}{1 + \frac{2}{3}\left(\frac{R_b^2\sigma_3}{\chi_3 R_a^2 H}\right)(1 - f_2^{irr})} \tag{4}$$

where H is the distance from the surface at which the target concentration is at its bulk value.

One of the most basic and interesting questions about heterogeneous hybridization is how well it compares to the hybridization rate of targets to a uniformly reactive surface. A comparable question was posed by Berg and Purcell (1977) in receptor-ligand interactions. This can be answered by the third parameter, global efficiency. Global efficiency compares the total hybridization rate per probe with the capture rate F_m that would occur if the entire glass or silicon surface were a perfect sink:

$$\gamma^{irr} = \frac{F_t^{irr}}{F_m} = \left(1 + \frac{2(1 - f_2^{irr})R_b^2\sigma_3}{3R_a^2\chi_3 H}\right)^{-1} \tag{5}$$

The closer the global efficiency is to unity, the closer the hybridization rate is to the maximum attained with a uniformly reactive surface. In other words, it describes how closely a dispersed array of a small number of targets comes to the maximal capture rate.

Principal values for DNA-DNA hybridization

To calculate f_2, η, and γ, we need to consider σ_n, χ_n, D_n, K_e, R_b, R_a, and k_d. These are several parameters in the 2D fraction that find direct application in hybridization and are worth examination. It must be noted that there are at present no data on diffusion or Brownian motion of DNA molecules in two dimensions, so we have assumed the persistence length $\sigma_2 = \sigma_3$ in all of the calculations of this paper. χ_2 and χ_3, the reaction success probabilities, are chosen as less than or equal to 0.001, according to arguments of AW. This is a diffusion-limited reaction theory in which reaction control was brought about by a low reaction probability. This results in low but equal probabilities for 2D and 3D systems. Potential changes of the reaction probability that may be caused by a change of probe or target size are not addressed in this model.

The value for the Brownian persistence distance of a spherical particle, σ, is equivalent to the Brownian mean square displacement and can be derived from the Langevin approach (Russel, 1981):

$$\langle\sigma_{2,3}{}^2\rangle^{1/2} = \frac{\sqrt{kTm}}{3\pi\eta R_g} \tag{6}$$

where k is Boltzman's constant, T is the absolute temperature (K), m is the molecular weight of DNA, R_g is the radius of gyration of DNA, and η is the viscosity of water. It appears legitimate to use the Langevin approach to calculate the Brownian persistence distance of the single-strand DNA

particles, which have the structure of a random coil or wormlike chain and for which σ has the same physical meaning as for any Brownian particle (the distance traveled by a particle between collisions). First of all, Brownian motion can be applied to small particles ranging from 1 nm to 10 μm (Russel, 1981). All of the DNA molecules used in our calculations fall within this range. Furthermore, it is well known that DNA molecules behave as Brownian particles in solution, from the results of computer simulations (Dwyer and Bloomfield, 1993) and experimental studies (Ferrari and Bloomfield, 1992; Gosnell and Zimm, 1993; Liu and Giddings, 1993). For example, Bloomfield et al. applied a Brownian dynamics algorithm to simulate the diffusion coefficient of DNA molecules by modeling DNA as a wormlike chain of hydrodynamically equivalent spherical frictional elements. They correctly calculated the 3D diffusion coefficient of 160 base-pair DNA. Although the multi-point binding between DNA target and its complementary probe is very complex, a simple collision mechanism between the two complementary molecules still applies in heterogeneous hybridization as long as DNA behaves as a Brownian particle. The exact dynamics of these flexible polyelectrolytes could be solved for by application of the mean field theory (Granfeldt et al., 1992), but our enhanced AW theory based on the Brownian properties of DNA should give a reasonable estimate for this heterogeneous reaction-diffusion process.

R_g is either estimated from molecular simulations or calculated from experimental data by other groups (Bloomfield et al., 1974; Dwyer and Bloomfield, 1993; Ferrari and Bloomfield, 1992; Garcia de la Torre et al., 1994; Gosnell and Zimm, 1993; Liu and Giddings, 1993; Strasburger and Reinert, 1973). For a solution containing a random coil polymer of DNA with more than 1000 bases, the radius of gyration can be estimated as follows (Bloomfield et al., 1974; Gosnell and Zimm, 1993):

$$R_g = \left[\frac{a}{3}(L - 3a)\left(\frac{L}{2a}\right)^{\epsilon}\right]^{1/2} \tag{7}$$

where L is the contour length of the coil and is assumed to be 0.34 nm per base pair (Bloomfield et al., 1974), a is the persistence length of the coil, which is equal to 50 nm (Gosnell and Zimm, 1993), and ϵ can be estimated as 0.05 at very low Na^+ concentrations (Gosnell and Zimm, 1993). For other DNA molecules, the experimental values of the hydrodynamic radius ($d/2$) of DNA were measured and converted to a root mean square radius of gyration R_g by applying the Flory correlation (Flory, 1953; Liu and Giddings, 1993):

$$0.665R_g = \frac{d}{2} \tag{8}$$

The value of the equilibrium constant K_e for nonspecific adsorption was calculated from adsorption isotherms of 36 mers on glass surfaces as determined by Kosicki et al. (Eggers et al., 1994; Lamture et al., 1994), which supports a Langmuir model. We have also seen Langmuirian adsorption in initial adsorption studies of single-stranded DNA on bare glass (unpublished result). K_e was found by applying a simple Langmuir equation that describes many cases of polymer adsorption very well (Adamson, 1990):

$$\theta_{DNA} = \frac{K_e[DNA]}{1 + K_e[DNA]} \tag{9}$$

where [DNA] is the concentration of DNA in solution and θ_{DNA} is the surface coverage (moles/cm^2) of adsorbed DNA. At very low [DNA] in most hybridization situations (Pease et al., 1994; Southern et al., 1992) this reduces to

$$\theta_{DNA} = K_e[DNA] \tag{10}$$

Thus K_e is just the experimental slope of DNA surface concentration versus solution concentration plot as [DNA] $\rightarrow$ 0. The maximum K_e for other DNAs can be roughly estimated by assuming that only one monolayer of DNA is nonspecifically adsorbed on the surface and is in equilibrium with the DNA in solution from Eqs. 1 and 9:

$$K_e = \frac{1}{4R_g^2[DNA]_{equilibrium}} \tag{11}$$

where $[DNA]_{equilibrium}$ is the concentration of DNA in solution that is in equilibrium with adsorbed DNA on the surface. These equations appear valid because nonspecific adsorption of DNA molecules on glass or silica is reversible (as opposed to irreversible specific adsorption). This physical property is widely used in a commercially available DNA purification technique using so-called glass milk (Boyle and Lew, 1994; Vogelstein and Gillespie, 1979). This is a method of purifying DNA from an agarose matrix after electrophoresis. The agarose-DNA mixture is dissolved in a buffer with high NaI concentration (3 M) and mixed with a suspension of small glass particles. The adsorbed DNA is then eluted with good efficiency (90%) by suspending the mixture in a buffer with a lower ionic strength. It has been calculated from the experimental data of Vogelstein et al. (1979) and Boyle and Lew (1994) that as much as 0.2 μg/cm^2 of DNA can be adsorbed on glass or silica surfaces. Both large DNA (48,000 bp) as well as small DNA (100 bp) can be recovered by this method.

The experimental values of the diffusion coefficient for various sizes of DNA molecules in solution were obtained from published experimental and simulation data (Bloomfield et al., 1974; Dwyer and Bloomfield, 1993; Ferrari and Bloomfield, 1992; Garcia de la Torre et al., 1994; Gosnell and Zimm, 1993; Liu and Giddings, 1993). They range from 1.88×10^{-6} cm^2/s for 6 base pairs to 0.9×10^{-8} cm^2/s for 40,461 base pairs. We should caution the reader that these do not appear to be totally in agreement (especially the 3D diffusion value for 160 bp DNA), but we have used them in the absence of better information. Diffusion coefficients for double-strand DNA were applied in the calculations because there are nearly no diffusion data on single-strand DNA in the literature. Although real hybridization experi-

ments utilize single-strand DNA, our results include a wide range of diffusion parameters and thus should apply to single-strand as well as double-strand DNA.

Capture rate prediction

It is important to know how the rate of the hybridization or capture per unit area (mole/cm^2-s) changes with the density of the DNA probe array. In general, the maximum capture rate per unit area of a molecule by a spherical perfect adsorber with a as the radius was defined by Berg and Purcell (1977).

$$J = \frac{D_3 C_0}{a} \tag{12}$$

When there are dispersed uniform sinks with s as the radius embedded in the full reflectance surface of a sphere, the total capture rate per unit area is equal to

$$J = \frac{D_3 C_0}{a}\left(\frac{Ns}{Ns + \pi a}\right) \tag{13}$$

Thus the actual rate term is a product of the maximum rate and a term that varies from 0 ($Ns \ll \pi a$) to 1 ($Ns \gg \pi a$). It must be noted that the effects of nonspecific adsorption and surface diffusion are not considered in Eqs. 12 and 13.

Wang et al. (1992) solved for the capture rate per unit area of dispersed uniform sinks that are perfect adsorbers embedded in the center at the bottom of a cylinder that allows nonspecific adsorption and surface diffusion.

$$J = \frac{\pi D_3 C_0}{4H} \tag{14}$$

Because there is likely to be some reaction limitation in heterogeneous hybridization as discussed previously, we will concentrate here on dispersed sinks (immobilized probes) with a combined reaction-diffusion limitation. The hybridization or capture rate per unit area can be directly determined from the other defined parameters (Appendix C) and is an extension of the original AW theory.

$$J_{\text{total}} = \gamma^{\text{irr}} F_{\text{m}} C_{\text{s}} = \frac{\pi \gamma^{\text{irr}} D_3 C_0}{4H} \tag{15}$$

where J_{total} is the total hybridization rate per unit area (moles/s-cm^2). J_{total} is not directly a function of the concentration of the surface probes, but depends on the global efficiency, which is a highly nonlinear function of R_{b}. J_{total} is an experimentally measurable parameter. The intrinsic reaction limitation enters the calculation through its effects on the global efficiency. Global efficiency has practical importance in designing an efficient DNA chip because it directly dictates the time scale required to obtain a detectable signal in the hybridized device. This correlation is valid when there is an excess amount of target in solution and only a small fraction of the immobilized probes have hybridized with the targets.

Experimental surface diffusion values for DNA molecules and rate constants for nonspecific adsorption/desorption on the surface such as those that are now being measured in our laboratory will be required for accurately predicting the effect of the reduction of dimensionality phenomenon on the overall rate of hybridization. Until such data are available, we have expressed these theoretical results over a range of parameters expected to cover all physically realizable values.

RESULTS

We calculated f_2, η, and γ for various sizes of target and probe DNA. In our calculations, σ_{n}, χ_{n}, D_{n}, K_{e}, R_{b}, R_{a}, and k_{d} explicitly depend on size. We then define a scaling correlation for optimal probe density and compare our capture rate prediction with published experimental values.

Effect of target DNA molecular weight

It is interesting to investigate the effect of the target size on the hybridization process. Transport parameters for several lengths of DNA were obtained from the literature (Bloomfield et al., 1974; Dwyer and Bloomfield, 1993; Ferrari and Bloomfield, 1992; Garcia de la Torre et al., 1994; Gosnell and Zimm, 1993; Liu and Giddings, 1993; Strasburger and Reinert, 1973), and others were calculated as discussed in the Theory section. These parameters are shown in Table 1. It must be noted that these diffusion coefficients are not perfectly correlated with their corresponding molecular weight because they are derived from different computer simulation and experimental methods.

Fig. 2 *A* shows the 2D fraction f_2 plotted as a function of k_{d} for DNA molecules ranging from 6 to 40,461 bases in length. The immobilized probe was fixed in length at a 14 mer in all calculations; this ensures one unique perfectly complementary sequence on average in targets up to 40,461 bp in length, i.e., in 4^{14}. It was assumed that the experimentally achievable maximum surface coverage of immobilized 14 mers is 8.3×10^{-13} moles/cm^2 ($R_{\text{b}} = 7.07 \times 10^{-7}$ cm) for the case in Fig. 2 *A*. In Fig. 2 *A*, f_2 versus k_{d}, the 2D fraction f_2 increases nearly 100-fold in a range of nonspecific desorption rates between 1 and 10,000 s^{-1} for the smallest DNA targets when the equilibrium adsorption/desorption constant is kept constant. The data indicate that the 2D fraction decreases with increasing target size when k_{d} is between 10 and 10^8 s^{-1}. There is a fractional change in f_2^{irr} of 0.9 as the DNA size changes from 40,461 to 6 nt. When the 2D diffusion coefficient is of an order of magnitude similar to that of the 3D diffusion coefficient (for example, 1.88×10^{-6} cm^2/s in the case of 6 mers), molecules that arrive at the probe because of surface diffusion dominate the overall process. DNAs of any larger size approach a maximal 2D fraction at higher k_{d}, but the actual maximum value of the 2D fraction of any given DNA molecule is directly related to its size. This occurs because

TABLE 1 Transport parameters of DNAs in free solution

Base pairs (#)	3D diffusion coefficient* (10^{-8} cm^2/s)	Radius of gyration (R_g)† (10^{-8} cm)	Persistence length† (10^{-10} cm)	Reference
6	188	9.5	15	Garcia de la Torre et al., 1994
30	90	39.3	6.7	Garcia de la Torre et al., 1994
160	6.91	254.4	2.4	Ferrari and Bloomfield, 1992
2686	3.8	1190	2.1	Gosnell and Zimm, 1993
4373	2.7	1600	1.99	Gosnell and Zimm, 1993
5996	2.2	1910	1.96	Gosnell and Zimm, 1993
10,600	1.69	2620	1.89	Liu and Giddings, 1993
21,692	1.1	3864	1.84	Liu and Giddings, 1993
40,461	0.9	5386	1.8	Strasburger and Reinert, 1973

*3D diffusion coefficients were directly taken from the references.
†Radius of gyration and persistence length were calculated from Eqs. 5–7.

both the speed of 2D diffusion and the dynamics of adsorption and desorption are directly related to the molecular weight of the polymer. Direct hybridization from 3D processes is slower in comparison to hybridization from 2D diffusion for small DNA molecules.

Fig. 2 *B* shows the effect of the surface density of immobilized DNA probes on the 2D fraction at a constant value of k_d (10^4 s^{-1}). The 2D fraction is very sensitive to decreases in probe density (increased R_b) because the size of the nonspecific adsorption region increases. An increase of the molecular weight greatly decreases the 2D fraction of the DNA because the adsorption/desorption equilibrium constant decreases according to the size exclusion principle. The 2D fractions for the largest DNA (40,461 bases) can be 1/10 those for the smallest (6 bases) when the immobilized surface density becomes relatively small (coverage $< 4.1 \times 10^{-13}$ moles/cm^2, $R_b > 10^{-6}$ cm). This indicates that transport and adsorption properties can greatly affect the kinetics of heterogeneous hybridization.

Fig. 2 *C* shows the hybridization enhancement resulting from the reduction in dimensionality RD enhancement for solution-phase DNA of different sizes at a k_d of 10^4 s^{-1}. The results reveal that the enhancement is very small at very high concentrations of immobilized probes in all cases, although at low surface coverage an increase in target molecular weight can greatly reduce the RD enhancement. The RD enhancement is about 7,000-fold higher for the 6 base case compared to the 40,461 base case. This change can be attributed to the decrease of K_e and diffusion coefficient as the molecular weight of DNA increases. It may at first seem incongruous that the 2D fraction in Fig. 2 *B* can be high while the RD enhancement in 2 *C* is low (for small DNAs and low R_b values). However, in such cases, the surface packing of DNA probes has become so high that the process is limited simply by the speed of target diffusion from bulk solution (high global efficiency; see below).

The dependence of global efficiency on R_b for DNAs of different molecular weight is seen in Fig. 2 *D*. Note that here the size of the diffusing DNA has little effect, except for the smaller sizes. The global efficiency decreases with increasing molecular weight and decreasing diffusive rate when R_b is larger than 5×10^{-5} cm (surface coverage $< 1.66 \times 10^{-16}$ moles/cm^2) because of the strong dependence of RD enhancement on molecular weight in smaller DNA. At very low surface concentrations, the global efficiency and the hybridization rate per unit area of a 6-base single-stranded DNA can be 10 times and 2,088 times higher, respectively, than those of 40,461 bases, according to Eq. 15.

Effect of the size of immobilized DNA

In a further set of calculations, the DNA in free solution was held at 160 bases, and the size of immobilized DNA was changed from an R_g of 9.5×10^{-8} cm (6 bases with maximum density of 2.76×10^{12} molecules/cm^2) to 5.39×10^{-5} cm (21,692 bases with maximum density of 8.6×10^7 molecules/cm^2). The value of k_d was set at 10^4 s^{-1}, a 2D diffusion coefficient 6.9×10^{-8} cm^2/s was assumed for 160 mers, and the reaction probability in both dimensions was set at 0.001. $\sigma_{2,3}$ is equal to 2.4×10^{-10} cm, and H is equal to 10^{-3} cm. Fig. 3 *A* shows the plot of the 2D fraction plotted against R_b for different sizes of immobilized DNA molecules. The 2D fraction approaches a minimum of 0.05 with 21,692 base immobilized DNA because in this case the fractional area for nonspecific adsorption/desorption is greatly reduced and direct hybridization through 3D diffusion from the solution becomes the dominant mechanism. It must be noted that each curve starts at a different value of R_b because its starting point is directly related to the size of the immobilized target.

Fig. 3 *B* shows the RD enhancement versus R_b for the same cases. The results show that the size of immobilized DNA affects the mechanism in heterogeneous hybridization. RD enhancement ranges from 30 when 6 mers are the probes to 1 when 21,692 mers are probes because larger immobilized DNA molecules allow much less space for nonspecific adsorption/desorption. RD enhancement would be even more significantly reduced if the effect of steric hindrance on the surface diffusion process is taken into account.

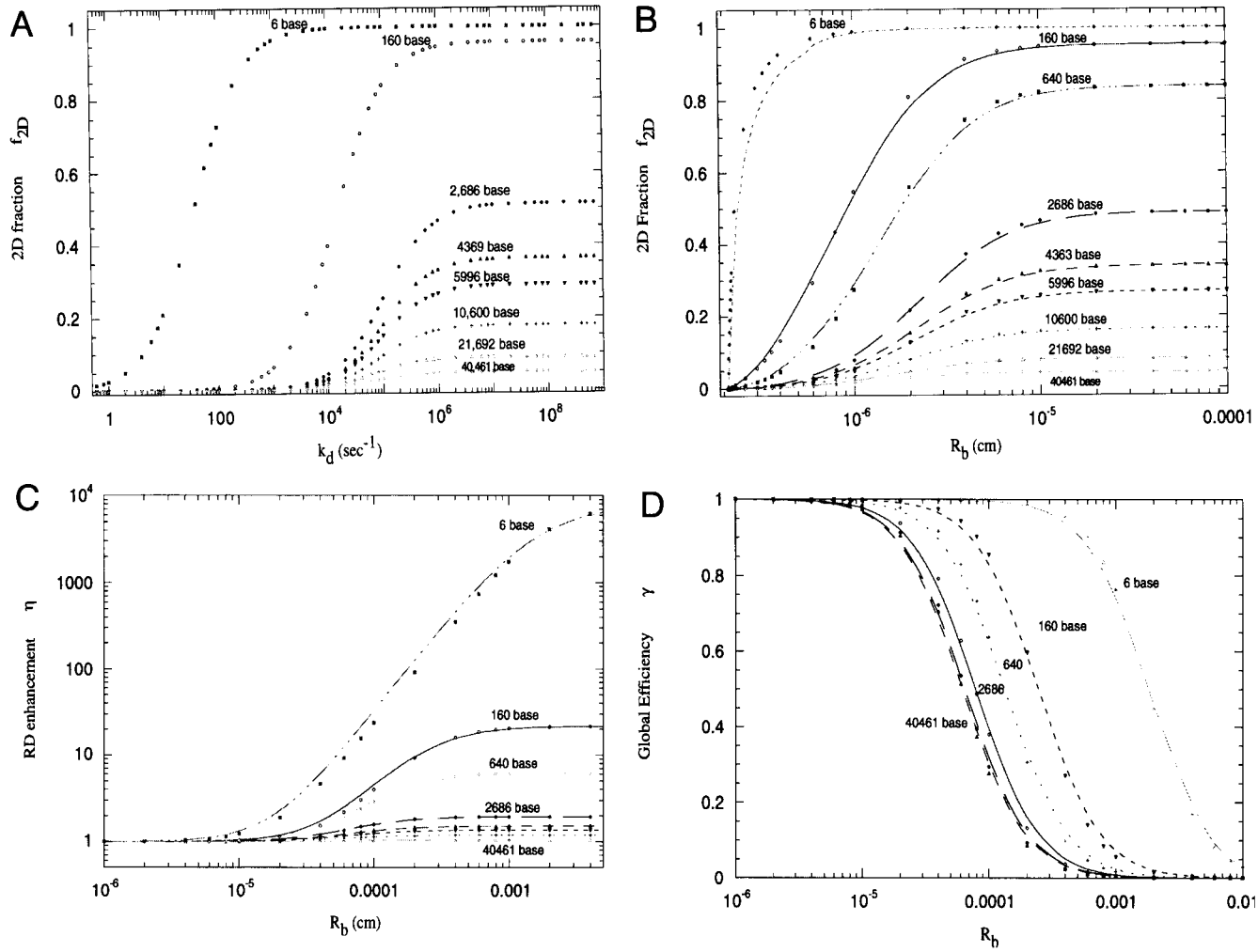

FIGURE 2 Effect of the molecular weight of the target DNA. (*A*) 2D fraction f_2 as a function of nonspecific desorption rate k_d for various target sizes ($R_b = 7.07 \times 10^{-7}$ cm; $R_a = 1.9 \times 10^{-7}$ cm; $\chi = 0.001$; $H = 10^{-3}$ cm. D_3, D_2, and $\sigma_{2,3}$ for probes of 160 to 40,461 bases are listed in Table 1. Values of K_e are calculated from Eq. 16. Sizes of free DNA in decreasing order are 40,461; 21,692; 10,600; 5,996; 4,363; 2,686; and 160 bases). Increasing the DNA molecular weight decreases f_2. (*B*) f_2 as a function of inter-probe spacing R_b for various target sizes ($k_d = 10^4$ s^{-1} and other constants are the same as those used in *A*). Increasing the DNA molecular weight decreases f_2. (*C*) RD enhancement η as a function of R_b for various target sizes. All constants are the same as those used in *A*. Increasing the size of DNA target decreases η. (*D*) Global efficiency γ as a function of R_b for various target sizes. Other constants are the same as those used in *A*. Increasing the size of DNA target decreases γ when R_b is larger than 10^{-5} cm.

Fig. 3 *C* shows the global efficiency versus R_b. The graph indicates that a global efficiency reaches 1.0 as the amount of immobilized DNA on the surface increases, but the values of R_b at which this occurs vary with the probe size. The values of R_b and the probe density for optimum hybridization as well as the maximum packing density of different probes are listed in Table 2.

A scaling correlation for probe density

In Fig. 3 *C* we define a optimal probe density for efficient capture, θ', as that value when the global efficiency first deviates from unity by 1%. Table 2 shows the value of θ' needed to achieve efficient capture. This density is compared with that of the closest packing. When this minimum target density is plotted against probe size on log-log axes, Fig. 4 shows that a linear relationship is found. These data suggest a direct scaling correlation for designing an optimum system of heterogeneous hybridization:

$$\frac{C_1}{C_2} = \left(\frac{W_1}{W_2}\right)^{-1.37} \tag{16}$$

where C_1 is the minimum surface coverage and W_1 is the molecular weight or number of bases needed to attain efficient capture, and C_2 and W_2 are a pair of unknown values for these variables. Using the values in Table 2, the optimal surface coverage for any probe size can be predicted with Eq. 13. Actual data for C_1 and W_1, when available, should prove more useful than the theoretical values in Table 2.

We can apply this correlation to find the maximum size of probes that can be used for sequencing by hybridization,

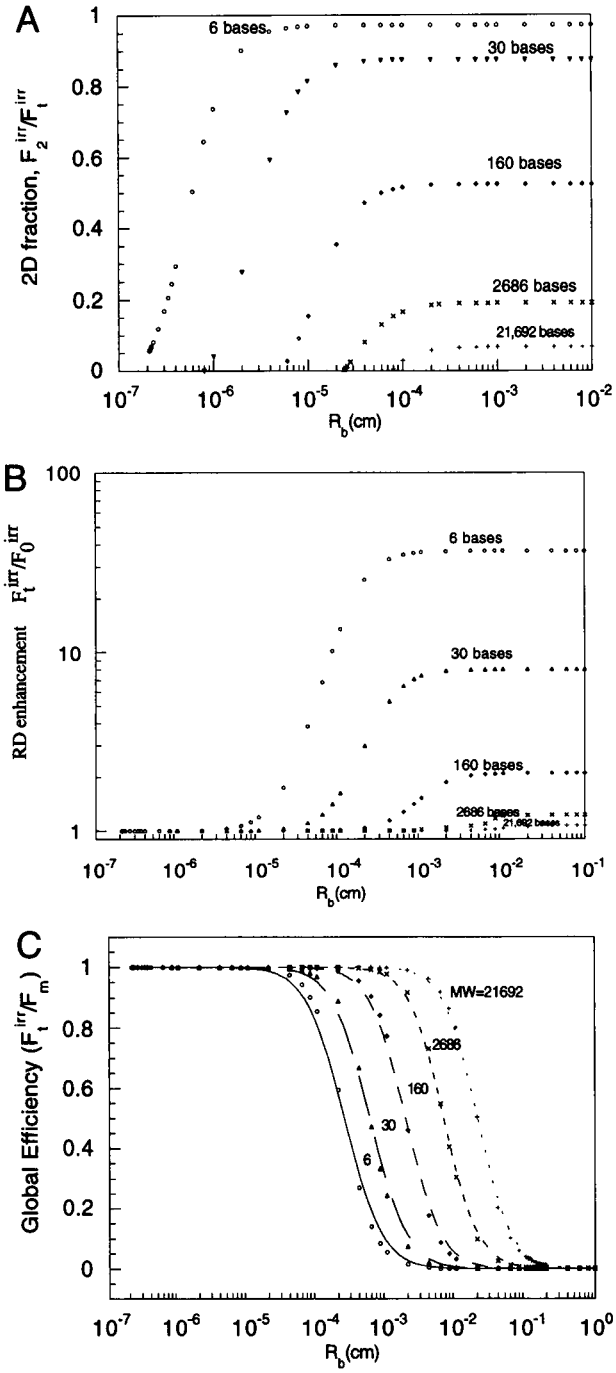

FIGURE 3 Effect of molecular weight of probe DNA. (*A*) 2D fraction f_2 as a function of inter-probe spacing R_b for various probe sizes (a DNA size of 160 bases in solution is maintained for all calculations. Values of R_a from Table 1: $k_d = 10^4\ s^{-1}$; $\chi = 0.001$; $D_3 = D_2 = 6.91 \times 10^{-8}\ cm^2/s$; $H = 10^{-3}$ cm; σ_2, 3of 160 bases $= 2.4 \times 10^{-10}$ cm). Increasing the size of the DNA probe greatly reduces f_2. (*B*) RD enhancement η as a function of R_b for various probe sizes. Increasing the size of DNA probe reduces η. All constants are the same as those used in *A*. (*C*) Global efficiency γ as a function of R_b for various probe sizes. All constants are the same as those used in *A*.

mutation detection, etc., within the limit of detection by various techniques. For example, a conventional gas phase array detector of radioactivity can achieve a reasonable

TABLE 2 The minimum value of surface density of immobilized DNA needed to reach maximal efficiency in the reaction limited regime

Base pair (#)	Distance between probes (10^{-5} cm)	Minimum efficient coverage (10^{-16} moles/cm^2)	Closed packed surface density (10^{-16} moles/cm^2)
6	1	41.5	46,000
30	4	2.59	29,600
160	10	0.416	642
2,686	100	0.00416	29.3
21,962	250	0.00066	1.43

signal-to-noise ratio (SNR) of 50 when a duplex density of 3.24×10^8 molecules/cm^2 is reached on the surface. This result is calculated from the experimental data of Eggers et al. (1994) in calibrating the SNR of various detection techniques against the signal. By using the optimum probe density of 6 mers (2.5×10^9 molecules/cm^2) from our calculation as C_1 and the minimum detectable duplex density as the lower limit of C_2, we determine from Eq. 16 that the maximum probe size for optimal hybridization within the detection limit of the gas phase array detector is 26 mers. When a more sensitive technique such as a direct CCD chip detector (Eggers et al., 1994; Lamture et al., 1994) with much higher SNR is applied in the detection of the duplex formed, the maximum size of probe could be as large as 1400 nt for a SNR of 50.

Comparison with experimental data

This theoretical study of DNA hybridization on a glass or silicon surface led to a flux expression (J_{total}) that suggests a direct correlation between the global efficiency and the hybridization rate per unit area. The expression for the hybridization rate per unit area correctly predicts within a

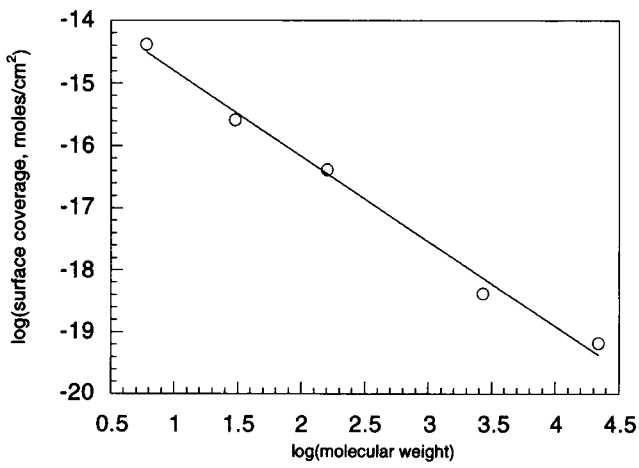

FIGURE 4 Scaling correlation for probe density to obtain maximum efficiency with minimal coverage. The optimal probe density for efficient capture is the value when the global efficiency deviates from unity by 1% and was obtained from Fig. 3 *C*. The least-squares line is given by $y = -1.3661x - 13.442$ with a regression coefficient $R = 0.996$.

factor of 2 the magnitude of the quantity of hybrid formed on a glass surface between a 12 mer target and a 12 mer probe after 5 min at 15°C (Maskos and Southern, 1992). The amount of hybridized DNA target was measured by radioactivity and was found to be 7.3×10^{-14} mol/cm^2, whereas the predicted quantity, calculated from Eq. 15, was 1.84×10^{-13} mol/cm^2 ($D_{2,3} = 94 \times 10^{-8}$ cm^2/s; $H = 10^{-3}$ cm; $\gamma = 1$ and $C_0 = 10^{-8}$ M).

DISCUSSION

We have developed a model of combined diffusion-reaction-limited heterogeneous DNA hybridization. It was shown that hybridization of DNA molecules from the solution phase to the immobilized complementary DNA probes is an efficient process when the DNA surface density and surface diffusion coefficient are sufficiently high. Reduction of dimensionality processes enhance the overall hybridization rates, except in the case of very high surface density or extremely slow surface diffusion processes. One of the experimental proofs on RD enhancement was provided by Gaspers et al. (1995) for the hydrolysis of surface immobilized peptides by adsorbed collagenase. They found that the intrinsic reaction rate for the enzymes on the substrate-coated surface was higher than the value measured in solution.

There are published data comparing 2D and 3D diffusion values for some biomolecules on artificial surfaces such as glass and polymers. The ratio of 2D to 3D diffusion coefficients for proteins is on the order of 0.0003 to 0.01, as determined from the experimental data of various groups using total internal reflection microscopy and fluorescence recovery after pattern photobleaching in different systems (Burghardt and Axelrod, 1981; Gaspers et al., 1994; Huang et al., 1994; Tilton et al., 1990a,b). These included the diffusion of adsorbed bovine serum albumin at a quartz surface (Burghardt and Axelrod, 1981); collagenase on 2-furanacryloyl-L-leucylglycyl-L-prolyl-L-alanine (FAL-GPA) peptides attached to a glass surface (Gaspers et al., 1994); bovine serum albumin at polymer surfaces (Tilton et al., 1990b); and bovine prothrombin fragment 1 on a supported lipid membrane (Huang et al., 1994). When Fig. 2 *C* is compared with Fig. 2 *B*, it is clear that most DNA hybridization is the result of the nonspecifically adsorbed molecules, even in regimes with very low RD enhancement when the 2D diffusion coefficients of target DNA molecules were between 9×10^{-9} and 1.88×10^{-6} cm^2/s. In other words, the magnitude of the surface diffusion coefficient dictates the type of hybridization kinetics, namely direct 3D hybridization or 2D hybridization.

One of the most important results in this model is the global efficiency, because it directly indicates the rate of hybridization per unit area. From Fig. 2 *D*, one can find that γ (and thus the rate of hybridization per unit area) is an increasing function of the surface diffusion coefficient of target DNA and of the density of probes until it reaches a maximum value at a certain R_b value. Furthermore, γ is a decreasing function of the target size. Capture is a diffusion-limited process for a wide range of probe densities or R_b values due to the RD enhancement. This can be explained by the large decrease of RD enhancement through slower 2D diffusion and resulting hybridization when the surface has a very low number of immobilized probes. Thus when there are sufficient dispersed probes, the surface behaves biophysically almost as efficiently as a uniformly adsorbing surface because of the combined benefits of 2D and 3D diffusion. The practical upper limit on R_b (minimum surface concentration) is set by two considerations: 1) the value at which the global efficiency begins to decrease from unity; 2) the value at which an insufficient signal is generated for reliable detection.

One may not need a high density of immobilized DNA to achieve an efficient hybridization process provided that K_e and D_2 have appropriate values. It is known from experimentation and computer simulations that 2D diffusion of proteins on surfaces becomes much slower when the surface concentration of diffusing enzymes increases (Gaspers et al., 1994; Saxton, 1982; Tilton et al., 1990a). Raising the target concentration in the hybridization solution to force more target DNA to be adsorbed on the surfaces may not be advantageous because the actual hybridization rate might be controlled by 2D diffusion. Our calculations show that reduction of dimensionality remains significant in practical heterogeneous hybridization even when the 2D diffusion coefficient is 100-fold smaller than the 3D diffusion coefficient (data not shown), as has been suggested by the experimental values of biomolecular diffusion on solid surfaces (Burghardt and Axelrod, 1981; Gaspers et al., 1994; Huang et al., 1994; Tilton et al., 1990a,b).

To optimize the utility of various biotechniques (e.g., sequencing by hybridization, mutation detection, etc.) that are based on the principle of heterogeneous hybridization, it is important to determine the most appropriate size of DNA to be used as targets as well as the most appropriate hybridization conditions. From Fig. 2, *A–D*, it was shown that RD kinetics becomes more important when the molecular weight of the solution-phase DNA decreases. This result follows both from a change in the nonspecific adsorption/desorption equilibrium constant and from the surface diffusion coefficient, which is assumed to decrease in the same manner as the 3D diffusion coefficient with an increase in size. The actual process would be close to this calculation because it is well known that adsorption dynamics is a size-dependent process (Gaspers et al., 1994; Tilton et al., 1990a,b) and the assumption of maximum DNA packing is appropriate, as shown by experimental data for the nonspecific adsorption of DNA (Eggers et al., 1994, and unpublished results recently obtained in our laboratory) on bare glass, silanized glass, and silicon surfaces. However, one might not be able experimentally to vary k_d and the 2D diffusion coefficient independently. If the actual molecular mechanism of surface diffusion involves "site hopping" or some similar process, the adsorption/desorption rate con-

stants may be inextricably linked to the surface diffusion constant.

The data from Fig. 3, *A* and *B*, show that an increase in the size of immobilized DNA molecules greatly hinders the 2D diffusion and reaction processes as the 2D fraction and the RD enhancement factor decrease. This results from a large reduction in the fractional area for nonspecific adsorption of DNA targets. From Fig. 3 *C*, it was shown that different sizes of immobilized DNA can achieve the same global efficiency when a 160-base DNA was the target. The global efficiency is the ratio of the actual hybridization rate per DNA probe to the rate of diffusion of a given DNA target to a surface covered by perfect sinks. From the results of Eq. 15, it was shown that the same values of global efficiency on different curves imply the same absolute rates of hybridization because of their proportionality for a given target size. Therefore, heterogeneous hybridization is an equally efficient process when there is a sufficient density of immobilized DNA for a wide range of DNA molecular weights. It becomes a molecular weight-dependent process when there is a lower density of immobilized probes on the surface. Thus, one of the big challenges in the application of this new technology is to improve the rate of the transport of target DNA from the solution to the surface, as shown by this combined reaction-diffusion limited theory.

The log-log plot of minimum surface coverage for efficient hybridization versus molecular weight of immobilized DNA (Fig. 4) was correlated with a straight line by the least-squares method. This yields a scaling correlation for the design of the least expensive and most efficient DNA chip (in terms of moles of DNA required). It must be noted that the quantity of immobilized DNA needed to reach the maximal hybridization efficiency is typically around 10,000-fold smaller than that needed to achieve the close packed surface density of a monolayer. This result implies that one does not require the highest possible density of immobilized probes to achieve an efficient solid-phase hybridization process.

It is not desirable to achieve high hybridization rates by just increasing the surface probe concentration for at least two reasons. First, DNA is expensive and many of the applications envisioned for this technology demand low cost. Second, increased nonspecific hybridization may be seen at high densities. A target that is not complementary to the probe may be counted as a signal when it partially hybridizes simultaneously to two neighboring probes. Such phenomena have been observed (S. P. A. Fodor, personal communication). Furthermore, it is likely that conditions can be found such that DNA can be effectively adsorbed and can diffuse on the non-probe-covered regions of the "DNA chip." This would enhance the hybridization rate at lower concentrations of immobilized probes.

This theory gives some surprising but testable predictions for the effects of probe density, nonspecific adsorption, target size, and concentration on target capture by immobilized oligonucleotide probe arrays. We predict that operation in a regime where global efficiency just reaches 1.0 will maximize capture while minimizing reagent costs and incorrect hybridization (i.e., partial hybridization of a target simultaneously to two closely spaced probes). This prediction presumes that 2D diffusion can take place efficiently. As suggested earlier, this may require a moderate adsorption constant, not so weak as to limit the surface concentration nor so strong as to prevent movement (our experimental measured value of K_e is about 2.5×10^{-5} cm and should be appropriate for effective adsorption and diffusion). Investigators may wish to set up conditions under which 2D diffusion can occur to test this hypothesis. Physical ways to decrease H, the distance from the surface at which the target concentration is at its bulk value, are also predicted to improve the capture rate. Such means might include stirring. Smaller DNA fragments diffuse faster than large ones. Because higher 2D and 3D diffusion constants were found to enhance hybridization in many cases, it may be beneficial to cut the target DNA into small fragments by enzyme digestion or make small fragments of DNA by PCR before carrying out heterogeneous hybridization.

A scaling correlation between the molecular weight of immobilized DNA and its minimum surface coverage to reach the maximal reaction-limited rate in heterogeneous hybridization was presented. This allows the prediction of an appropriate size for an oligonucleotide to be immobilized on the surface for various detection schemes. These results provide a powerful tool for understanding heterogeneous hybridization at a more fundamental level and designing a DNA chip in a more logical manner.

CONCLUSIONS

This analysis has provided considerable insight into the process of heterogeneous hybridization, an important new analytical technique that is almost certain to become extremely important within the next 5 years. We have shown that for rapid and efficient hybridization, the solution-phase (target) DNA that is diffusing to the probe-covered surface should be small (ideally 100 bases or less). It is very desirable to achieve a high level of hybrid pair formation in the minimum time and to form hybrids that do not include mismatch or multiple partial match sequences. Our analysis reveals that sparse surface probe coverage can often be just as fast as a complete monolayer of probe under the appropriate conditions. A large distance between surface probes in such a case will simultaneously decrease the probability of multiple partial matches by a large target (bridging) and the resulting incorrect hybrid formation. The conditions that permit good efficiency with sparse coverage are those that allow surface diffusion of the target from a nonselective but adsorptive region to the sparsely dispersed probes. Therefore, one should try to create conditions conducive to surface diffusion and to ensure that the probes are uniformly distributed rather than being clustered in island regions. Sparse probe coverage may also provide some

cost benefits in DNA chip preparation because of the quantity of reagents required or the decreased time of reaction, although this consideration is expected to be of secondary importance. We provide a scaling correlation that predicts just how far apart the individual probe molecules can be while still providing maximal efficiency. Finally, the equations implicitly predict that good stirring (small H) will be beneficial. Graphical results were not presented for different degrees of stirring, but these can be generated easily for any desired specific H values.

APPENDIX A

The reaction-limited model of heterogeneous hybridization

The total hybridization rate F_t consists of that due to the direct probe-target collision plus that due to nonspecific adsorption followed by surface diffusion:

$$F_t = F_{2D} + F_{3D} \tag{A1}$$

where F_{2D} and F_{3D} are the hybridization rates that occur through 2D diffusion and direct 3D diffusion, respectively. The Brownian dynamics model of ligand-receptor binding is adapted here (Axelrod and Wang, 1994). The collision rate (1/s) of a Brownian solute in free solution, which is the single-strand DNA, with a surface having unit area is

$$R_{3D} = \frac{\langle \nu \rangle_3 C_3}{4} = \frac{F_{3D}}{\chi_3} \tag{A2}$$

where C_3 is the concentration of DNA molecules in solution (number of molecules per unit volume) and $\langle \nu \rangle_3$ is the instantaneous average speed of the Maxwell distributed molecules in three dimensions. As stated earlier, DNA molecules can approach the target laterally after adsorbing on the surface, and the rate of arrival per second per unit length is given by

$$R_{2D} = \frac{\langle \nu \rangle_2 C_2}{\pi} = \frac{F_{2D}}{\chi_2} \tag{A3}$$

where C_2 is the surface concentration (number of molecules per unit area), $\langle \nu \rangle_2$ is the 2D velocity of nonspecifically adsorbed DNA, and χ_n is the successful hybridization probability per collision from either 2D or 3D transport to the target. In general, the subscripts 2 and 3 are used to represent two- and three-dimensional quantities throughout.

For any Brownian molecules, their velocity in any dimension is finite and correlated by

$$\langle \nu \rangle_n = \epsilon_n \sigma_n \tag{A4}$$

where ϵ_n is the frequency of collision experienced by a single particle and σ_n is the Brownian persistence distance. The effective diffusion coefficient of Brownian particles in any dimension can be defined as

$$D_n = \frac{\epsilon_n \sigma_n^2}{2n} \tag{A5}$$

where n is the dimensionality. The 2D and the 3D hybridization rates can be expressed in terms of the diffusion coefficient, the successful hybridization probability per collision from either 2D or 3D transport to the target, and other measurable or calculable parameters:

$$F_{2D} = \frac{8\chi_2 R_a D_2 C_2}{\sigma_2} \tag{A6}$$

$$F_{3D} = \frac{(3\pi/2)\chi_3 R_a^2 D_3 C_3}{\sigma_3} \tag{A7}$$

It is assumed that immobilized DNA irreversibly binds to the complementary molecules when a successful collision occurs. The 2D collision flux and total collision flux are then given by

$$F_{2D}^{irr} = \pi(R_b^2 - R_a^2)(k_a C_3 - k_d C_2) \tag{A8}$$

$$F_t^{irr} = \frac{\pi R_b^2 D_3}{H}(C_0 - C_3) \tag{A9}$$

where the 2D hybridization rate is directly proportional to the amount of nonspecifically adsorbed DNA and the total hybridization rate includes both processes. C_0 is the concentration of free DNA far away from the surface and C_3 is the concentration of free DNA at the surface.

APPENDIX B

Total hybridization or capture rate per unit area

The total hybridization rate per DNA probe can be expressed in terms of the global efficiency and the adsorption rate per probe according to Eq. 12:

$$F_t^{irr} = \gamma^{irr} F_m \tag{12}$$

The adsorption rate per probe is equal to the flow rate per probe (F_m, moles/s) that occurs when the entire glass or silicon surface is a perfect adsorber of DNA targets in solution (Axelrod and Wang, 1994).

$$F_m = \frac{\pi R_b^2 D_3 C_0}{H} \tag{B1}$$

From Eq. 1, the density of the immobilized probes can be expressed in the following form:

$$C_s = \frac{1}{4R_b^2} \tag{B2}$$

The total hybridization rate per unit area is simply the product of the hybridization rate per probe and the density of the immobilized DNA probes on the surface.

$$J_{total} = F_t^{irr} C_s = \frac{\pi \gamma^{irr} D_3 C_0}{4H} \tag{B3}$$

It is shown that the hybridization rate per unit area (moles/cm^2·s) is directly proportional to the global efficiency as H, D_3, and C_0 are constants.

The project was supported by a special opportunity award from the Whitaker Foundation and by National Institutes of Health grant R01 DK 16691.

REFERENCES

Adam, G., and M. Delbruck. 1968. Reduction of dimensionality in biological diffusion processes. *In* Structural Chemistry and Molecular Biology. A. Rich and N. Davidision, editors. W. H. Freeman and Company, San Francisco. 198–215.

Adamson, A. W. 1990. Physical Chemistry of Surfaces. John Wiley and Sons, New York.

Axelrod, D., and M. D. Wang. 1994. Reduction of dimensionality kinetics at reaction limited cell surface receptors. *Biophys. J.* 66:588–600.

Berg, H., and E. M. Purcell. 1977. Physics of chemoreception. *Biophys. J.* 20:193–239.

Bloomfield, V. A., D. M. Crothers, and I. Tinoco. 1974. The Physical Chemistry of Nucleic Acids. Harper and Row, New York.

Boyle, J. S., and A. M. Lew. 1994. An inexpensive alternative to glassmilk for DNA purification. *Trends Genet.* 11:8.

Burghardt, T. P., and D. Axelrod. 1981. Total internal reflection/ fluorescence photobleaching recovery study of serum albumin adsorption dynamics. *Biophys. J.* 33:455–468.

Chetverin, A. B., and F. R. Kramer. 1994. Oligonucleotide arrays: new concepts and possibilities. *Biotechnology.* 12:1093–1099.

Chrisey, L. A., P. M. Roberts, V. I. Benezra, W. J. Dressick, C. S. Dulcey, and J. M. Calvert. 1994. Selective attachment of synthetic DNA to self-assembled-monolayer functionalized surfaces. *Mater. Res. Soc. Symp. Proc.* 330:179–185.

DeLisi, C. 1980. The biophysics of ligand-receptor interactions. *Q. Rev. Biophys.* 13:201–230.

Dwyer, J. D., and V. A. Bloomfield. 1993. Brownian dynamics simulations of probe and self-diffusion in concentrated protein and DNA solutions. *Biophys. J.* 65:1810–1816.

Eggers, M., M. Hogan, R. K. Reich, J. Lamture, D. Ehrlich, M. Hollis, B. Kosicki, T. Powdrill, K. Beattie, S. Smith, R. Varma, R. Gangadharan, A. Mallik, B. Burke, and D. Wallace. 1994. A microchip for quantitative detection of molecules utilizing luminescent and radioisotope reporter groups. *Biotechniques.* 17:516–525.

Ferrari, M. E., and V. A. Bloomfield. 1992. Scattering and diffusions of mononucleosomal DNA: effects of counter valence and salt and DNA concentration. *Macromolecules.* 25:5266–5276.

Flory, P. J. 1953. Principles of Polymer Chemistry. Cornell University Press, Ithaca, NY.

Garcia de la Torre, J., S. Navarro, and M. C. Lopez Martinez. 1994. Hydrodynamic properties of a double-helical model for DNA. *Biophys. J.* 66:1573–1579.

Gaspers, P. B., A. P. Gast, and C. R. Robertson. 1995. Enzymes on immobilized substrates: reaction. *J. Colloid Interface Sci.* 172:518–529.

Gaspers, P. B., C. R. Robertson, and A. P. Gast. 1994. Enzymes on immobilized substrate surfaces: diffusions. *Langmuir.* 10:2699–2704.

Gosnell, D. L., and B. H. Zimm. 1993. Measurement of diffusion coefficients of DNA in agarose gel. *Macromolecules.* 26:1304–1308.

Granfeldt, M., B. Jonsson, and C. E. Woodward. 1992. A mean-field Monte Carlo technique for studies of electric double layers and flexible polyelectrolytes. *J. Phys. Chem.* 96:10080–10086.

Huang, Z., K. H. Pearce, and N. L. Thompson. 1994. Translational diffusion of bovine prothrombin fragment 1 weakly bound to supported planar membranes: measurement by total internal reflection with fluorescence pattern photobleaching recovery. *Biophys. J.* 67:1754–1766.

Jacobs, J. W., and S. P. A. Fodor. 1994. Combinatorial chemistry-applications of light-directed chemical synthesis. *Trends Biotechnol.* 12:19–26.

Kubitscheck, U., P. Wedekind, and R. Peters. 1994. Lateral diffusion measurement at high spatial resolution by scanning microphotolysis in a confocal microscope. *Biophys. J.* 67:948–956.

Lamture, J. B., K. L. Beattie, B. E. Burke, M. D. Eggers, D. J. Ehrlich, R. Fowler, M. A. Hollis, B. B. Kosicki, R. K. Reich, S. R. Smith, R. S. Varma, and M. E. Hoagon. 1994. Direct detection of nucleic acid hybridization on the surface of a charged couple device. *Nucleic Acids Res.* 22:2121–2125.

Lauffenburger, D. A., and J. J. Linderman. 1993. Receptors. Oxford University Press, Oxford.

Liu, M.-K., and J. C. Giddings. 1993. Separation and measurement of diffusion coefficients of linear and circular DNAs by flow field-flow fractionation. *Macromolecules.* 26:3576–3588.

Livshits, M. A., V. L. Florentiev, and A. D. Mirzabekov. 1994. Dissociation of duplexes formed by hybridization of DNA with gel-immobilized oligonucleotides. *J. Biomol. Struct. Dyn.* 22:783–795.

Maskos, U., and E. M. Southern. 1992. Oligonucleotide hybridisation on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesized in situ. *Nucleic Acids Res.* 20:1679–1684.

Maskos, U., and E. M. Southern. 1993. Study of oligonucleotide reassociation using large arrays oligonucleotides synthesized on a glass support. *Nucleic Acids Res.* 21:4663–4669.

Meinkoth, J., and G. Wahl. 1984. Hybridization of nucleic acids immobilized on solid supports. *Anal. Biochem.* 138:267–284.

Mirzabekov, A. D.. 1994. DNA sequencing by hybridization—a mega-sequencing method and a diagnostic tool. *Trends Biotechnol.* 12:27–36.

Parkhurst, K. M., and L. J. Parkhurst. 1995. Kinetic studies by fluorescence resonance energy transfer employing a double labeled oligonucleotide: hybridization to the oligonucleotide complement and to the single-stranded DNA. *Biochemistry.* 34:285–292.

Pease, A. C., D. Solas, E. J. Sullivan, M. T. Cornin, C. P. Holmes, and S. P. A. Fodor. 1994. Light-generated oligonucleotide arrays for rapid DNA sequence analysis. *Proc. Natl. Acad. Sci. USA.* 91:5022–5026.

Russel, W. B. 1981. Brownian motion of small particles suspended in liquids. *Annu. Rev. Fluid Mech.* 13:425–455.

Saxton, M. J. 1982. Lateral diffusion in an archipelago: effects of mobile obstacles. *Biophys. J.* 39:165–173.

Southern, E. M., S. C. Case-Green, J. K. Elder, M. Johnson, K. U. Mir, L. Wang, and J. C. Williams. 1994. Arrays of complementary oligonucleotides for analyzing the hybridisation behavior of nucleic acids. *Nucleic Acids Res.* 22:1368–1373.

Strasburger, J., and K. E. Reinert. 1971. Diffusion of DNA at very low concentration. *Bioploymers.* 10:263.

Thompson, N. L., T. P. Burghardt, and D. Axelrod. 1981. Measuring surface dynamics of biomolecules by total internal reflection fluorescence with photobleaching recovery or correlation spectroscopy. *Biophys. J.* 33:435–454.

Thompson, N. L., K. H. Pearce, and H. V. Hsieh. 1993. Total internal fluorescence microscopy: applications to substrate-supported planar membrane. *Eur. Biophys. J.* 22:367–378.

Tilton, R. D., A. P. Gast, and C. R. Robertson. 1990a. Surface diffusion of interacting proteins. Effect of concentration on the lateral mobility of adsorbed bovine serum albumin. *Biophys. J.* 58:1321–1326.

Tilton, R. D., C. R. Robertson, and A. P. Gast. 1990b. Lateral diffusion of bovine serum albumin adsorbed at the solid-liquid interface. *J. Colloid Interface Sci.* 137:192–203.

Torney, D. C., and H. M. McConnell. 1983. Diffusion-limited reaction rate theory for two dimensional system. *Proc. R. Soc. Lond. A.* 387:147–170.

Vogelstein, B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Nat. Acad. Sci. USA.* 76:615–619.

Wang, D., S.-Y. Gou, and D. Axelrod. 1992. Reaction rate enhancement by surface diffusion of adsorbates. *Biophys. Chem.* 43:117–137.

Wetmur, J. G. 1991. DNA probes: applications of the principles of nucleic acid hybridization. *Crit. Rev. Biochem. Mol. Biol.* 26:227–259.

Wolf, S. F., L. Haines, J. Fisch, J. N. Kremsky, J. P. Dougherty, and K. Jacobs. 1987. Rapid hybridization kinetics of DNA attached to submicron latex particles. *Nucleic Acids Res.* 15:2911–2926.