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ERDEC-TR-425

**PREDICTIVE BINDING PARAMETERS
FOR DNA-DNA ASSOCIATION
WITHIN A FLUID STREAM**

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RESEARCH AND TECHNOLOGY DIRECTORATE

July 1997

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PREFACE

The work described in this report was authorized under Project No. 10161102BH67, Environmental, Pathogen Detection. This work was started in November 1993 and completed in November 1994.

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Predictive Binding Parameters for DNA-DNA Association Within a Fluid Stream

1. Introduction

DNA hybridization studies have shown that predictions of association times and of hybridization efficiencies are possible. Given the starting and ending concentrations of single stranded (ssDNA), the G,C,A,T content of the DNA, the temperature, and the ionic conditions, hybridization times can be predicted as the reactions proceeds in a test tube. These parameters become important when predicting the importance of DNA hybridization as a tool for capturing and distinguishing microbes responsible for pathogenesis in humans. This study projects the use of DNA oligonucleotides to look at hybridization under very different conditions and uses conventional mathematical tools to predict binding rates.

Historically, hybridization rates were determined using a variety of analytical techniques. Radioligand techniques detect at picogram levels¹, and avidin-biotin couplings as signal molecules can detect at the nanogram level.^{2,3} If the polymerase chain reaction (PCR) is used prior to signal generation, femtogram quantities equivalent to one genome can be detected.^{4,5,6} Our approach measures hybridization as it occurs in real time at the nanogram level without the need for attachment of a signal generating molecule. Also, our approach circumvents the need to measure beginning and ending concentrations of ssDNA analytically.

In this study of hybridization rates, we used the unconventional environment of a real-time monitoring device, BIAcore™ manufactured by Pharmacia Biosensor, Piscataway, New Jersey. The instrument measures molecular interactions as they occur. Biotinylated oligonucleotides were placed on a sensor chip and mass changes on the surface of the chip were monitored. Mass changes were a result of ligand binding at specific binding sites. The sensor surface was continually monitored for changes in the minimal angle of reflected monochromatic light. Mass changes sensitive and specific enough to calculate affinities of binding were measured. Changes in the minimal angle of reflectance were recorded as resonance units (RU) and converted to molar equivalents.

Although the ability to achieve DNA-DNA hybridization at room temperature, in five minutes under low stringency conditions (in BIAcore) has been shown previously⁷, this study analyses the hybridization reaction using second order rate kinetic substitutions. Reactions followed to completion correlated with mathematical predictions of association rates. Minimal parametric changes had little to no effect on hybridization rates.

2. Materials and Methods

2.1 Source and description of materials

Oligonucleotides of 20 basepairs (bp) and 40 bp were obtained from Dr. Kim Rogers, USA Environmental Protection Agency, Las Vegas, Nevada. Avidin was obtained from Serva Biochemicals, Westbury, N.Y. Methods in BIAcore followed the original work as described by Wood. (7) A biotinylated 20 bp oligonucleotide (BUNI), a 40 bp (with 20 bp complementary to BUNI) oligonucleotide (BETA), and a 40 bp non-complementary oligonucleotide (FETA) were used.

2.2 Equilibrium experiments

Avidin was covalently immobilized to the sensor matrix using carboxyl amine coupling. The biotinylated oligonucleotide (BUNI) was injected followed by the 40 bp complementary strand (BETA). As BUNI and BETA hybridized, the reaction was followed for 10 minutes. The completed reaction was evaluated using second order rate kinetics.⁸ Non-complementary FETA did not bind to BUNI in a separate experiment.

2.3 Kinetics experiments

Increasing concentrations of BETA were inoculated over a base of avidin/BUNI. Baseline was reestablished by washing with 100 mM HCl. Association rates for each concentration were obtained and the %BETA associated with BUNI was obtained using second order rate kinetics.

2.4 Parametric changes

Three differing concentrations of BETA at two differing flow rates were tested. The objective of this experiment was to observe any effects of minor parametric changes on hybridization.

3. Results

3.1 Equilibrium experiments

As shown in Figure 1, BUNI and BETA reached equilibrium at 300 seconds. Evaluation of the mathematical predictive value of second order rate kinetics suggests that single stranded DNA consumption can be predicted in BIAcore using this approach. (See Appendixes A, B, C, and D).

3.2 Kinetic evaluations

Figures 2 and 3 illustrate concentration dependent hybridization at a flow rate of 1 $\mu\text{l}/\text{min}$. Figures 4 and 5 illustrate the same concentration dependent hybridization using a flow rate of 5 $\mu\text{l}/\text{min}$. Conversion of RU to molar amounts at selected time points during the curve, allowed Cot curve substitution estimates of the relationship of bound to unbound single stranded DNA as the reaction progressed.

Concentration dependent reactions showed a 5-fold difference in RU response over a range of 1 μM to 9 μM BETA. Binding was linear at flow rates of 1 $\mu\text{l}/\text{min}$ up to seven minutes for concentrations of 5 μM BETA or less. Non-linearity became apparent at concentrations of 7.5 and 9 μM BETA after nine minutes. At 5 $\mu\text{l}/\text{min}$, linearity was observed at concentrations of from 1 to 5 μM BETA. Reactions slowed at concentrations of BETA above 5 μM . Association kinetics were measurable within a 5 minute time frame.

3.3 Parametric changes

As shown in Tables 1 and 2, changes in flow rate and concentrations did not affect the hybridization reaction between 5 and 9 minutes.

4. Conclusion

Our use of oligonucleotides in BIAcore resulted in the ability to predict rates of reaction based on mathematical assessment. Observations of concentration dependent binding and linearity of reactions provide the ability to decipher useful monitoring windows for a given set of reactions.

Figure 1. Resonance units increase as BUNI hybridizes to BETA, equilibrium at 300 seconds

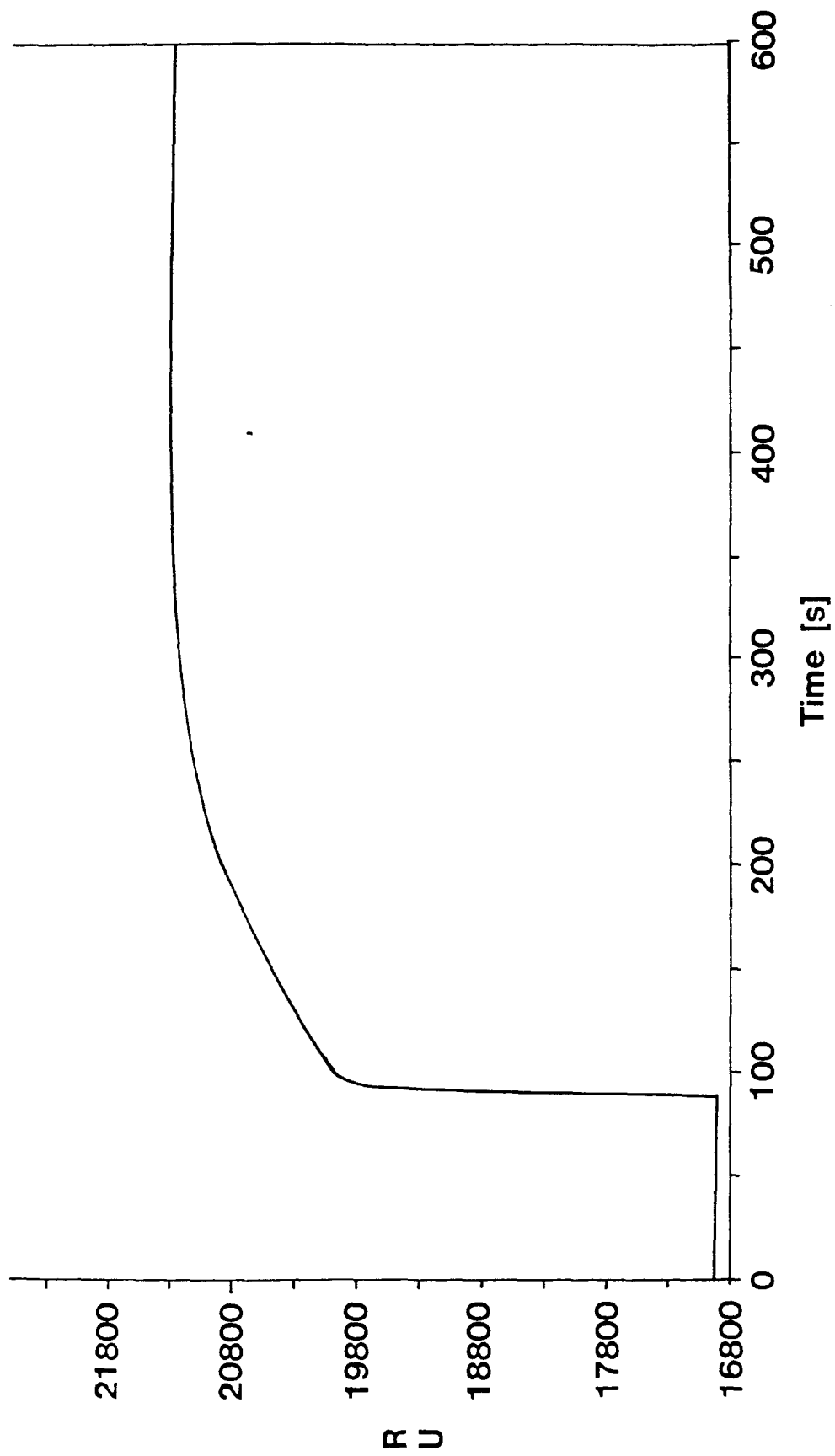


Figure 2. Percent BETA association at a flow rate of 1 $\mu\text{L}/\text{min}$

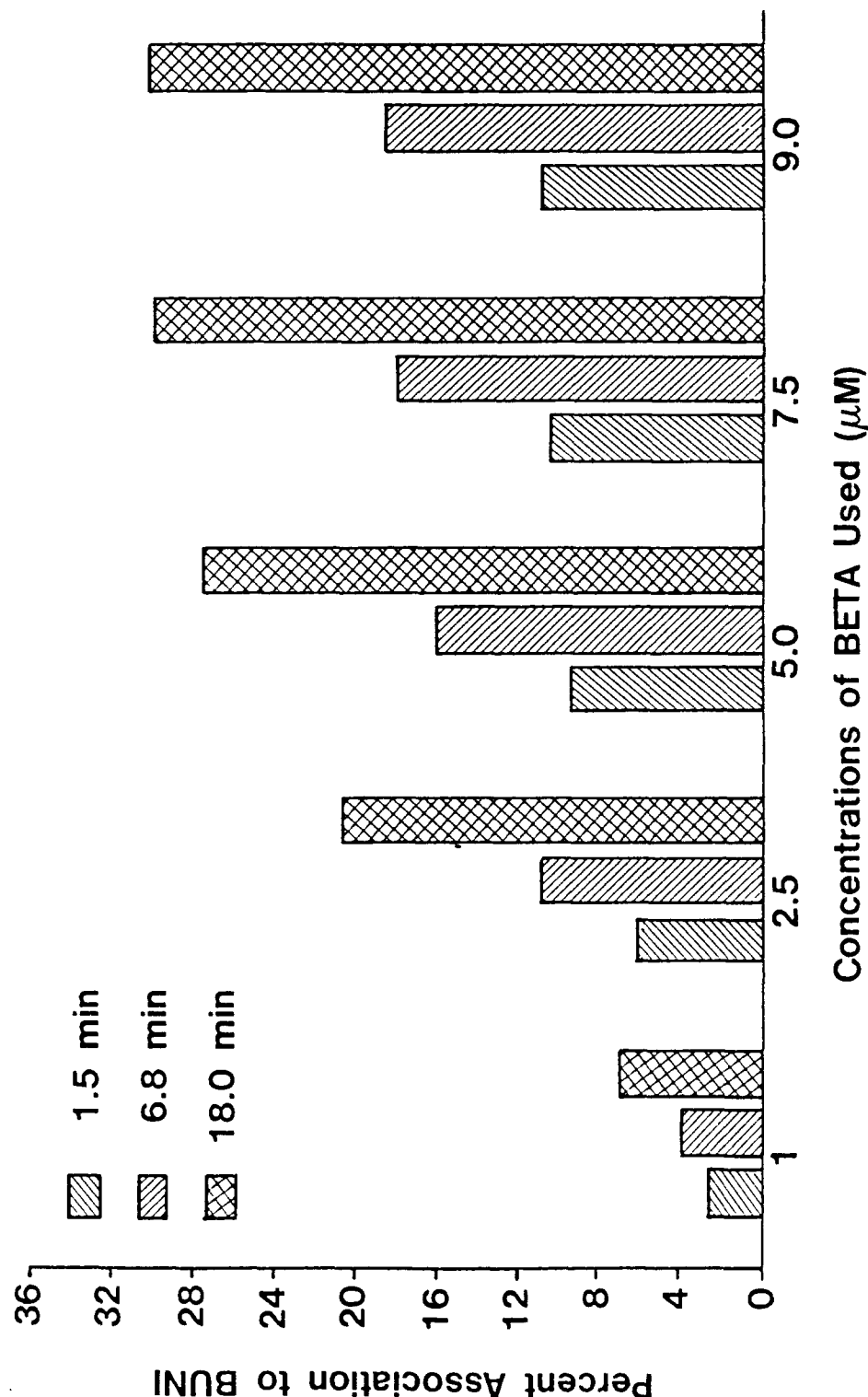


Figure 3. Relationship of concentration of reactants to percent association over time at a flow rate of 1 $\mu\text{L}/\text{min}$

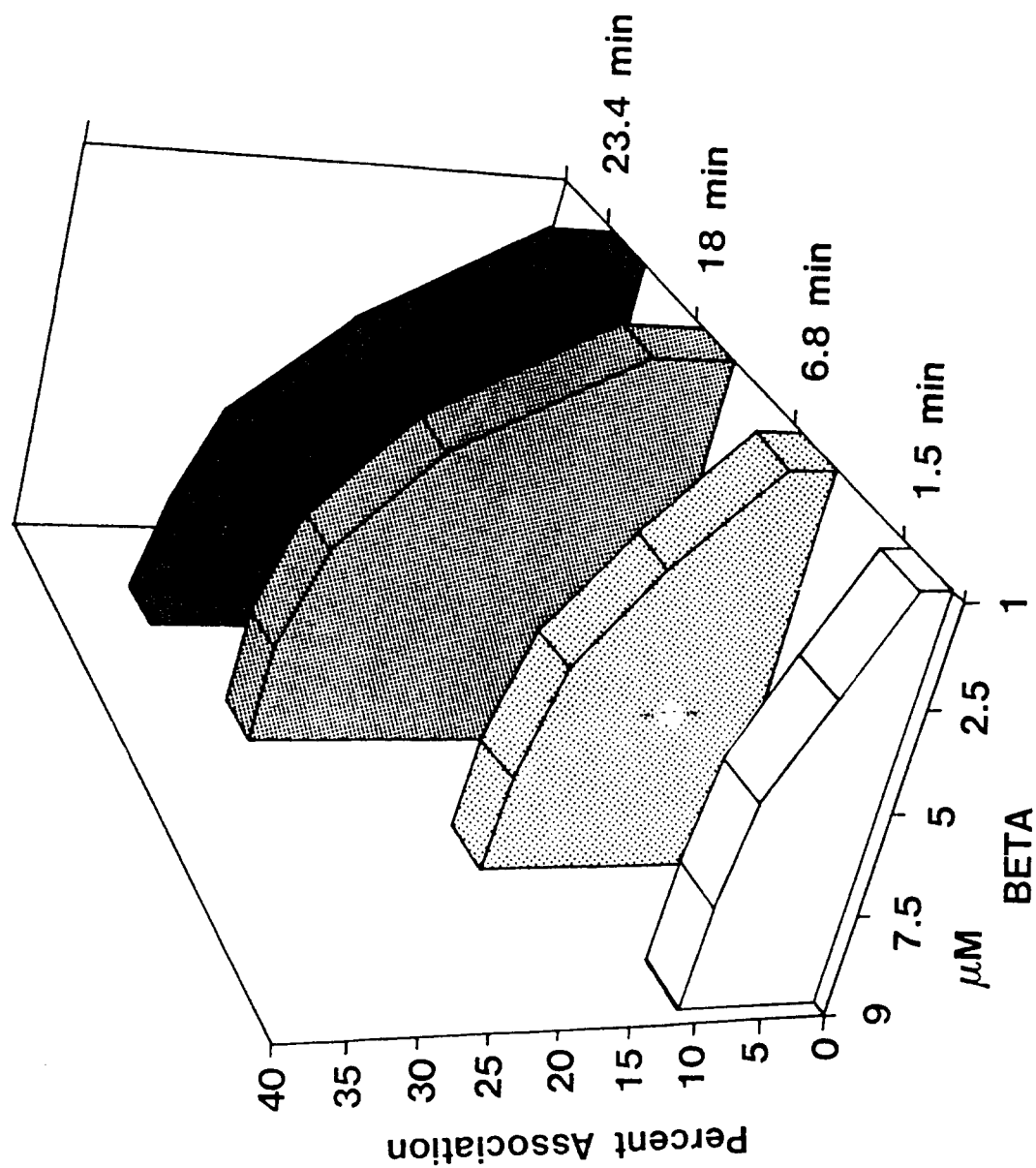


Figure 4. Percent BETA association at a flow rate of 5 $\mu\text{L}/\text{min}$

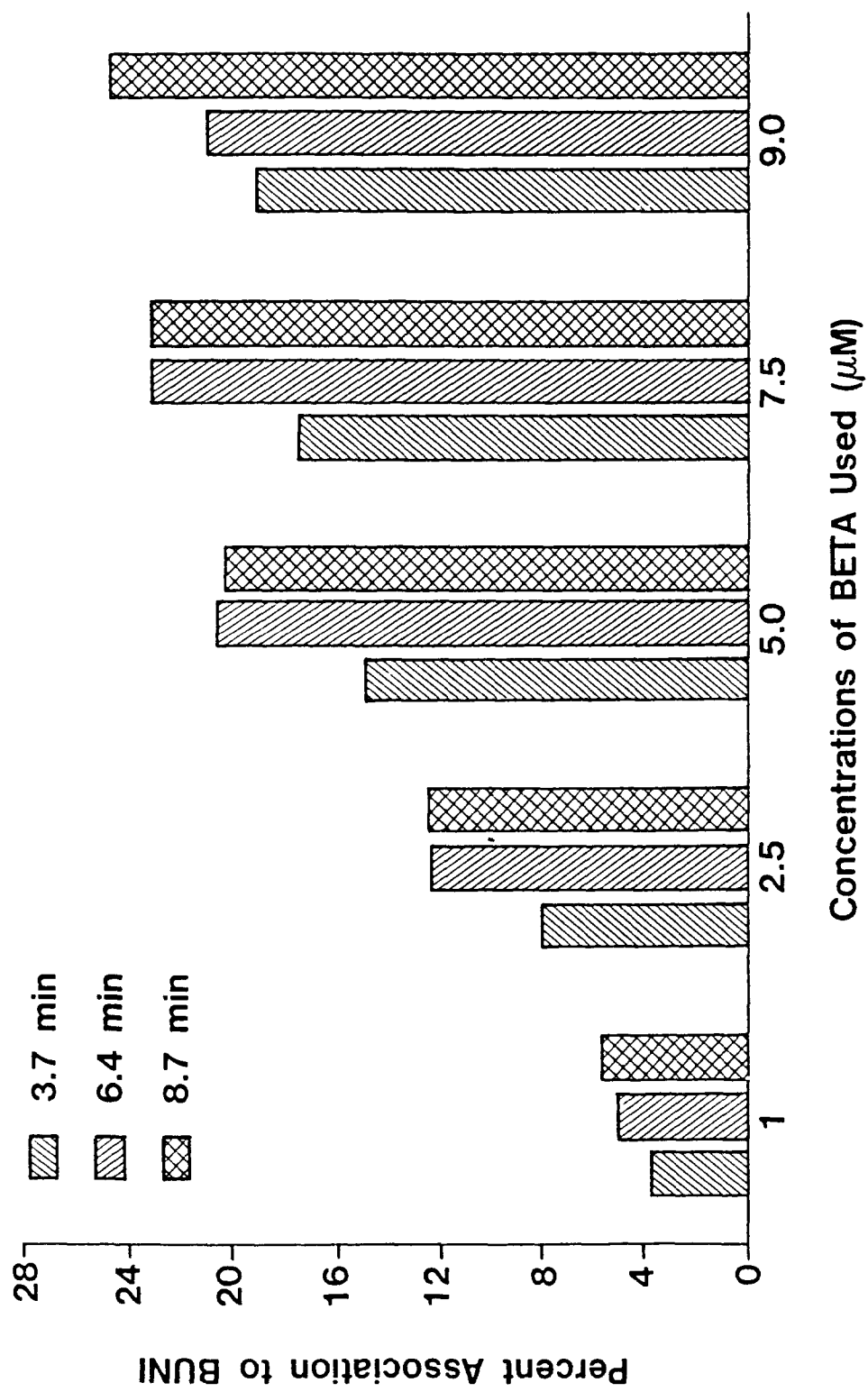


Figure 5. Relationship of reactant concentrations to percent association over time using a flow rate of 5 $\mu\text{L}/\text{min}$

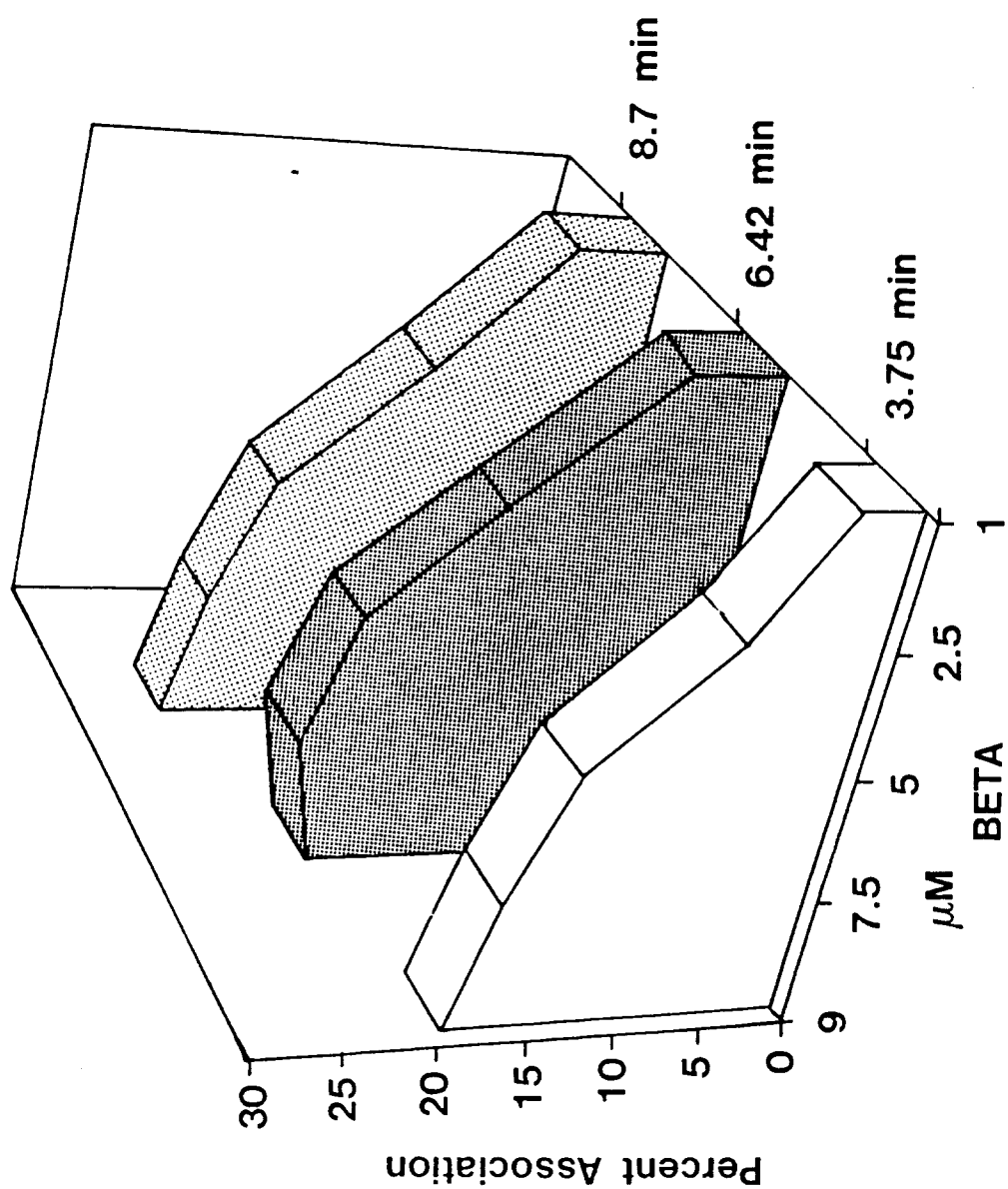


Table 1. Experimental Parameters Varying Flow Rate

Concentrations of Reactants Used in BIAcore at a Flow Rate of 1 μ L/min

Avidin base 1.7 mM

BUNI attached 2.9 mM

BETA, 20 μ l injections of the following concentrations:

9.0 μ M

7.5 μ M

5.0 μ M

2.5 μ M

1.0 μ M

Calculations of the percent associated at the following times into the reaction:

1.5 min

6.8 min

18.0 min

23.4 min

Concentrations of Reactants Used in BIAcore at a Flow Rate of 5 μ L/min

Avidin base 1.84 mM

BUNI attached 3.18 mM

BETA, 35 μ l injections of the following concentrations:

9.0 μ M

7.5 μ M

5.0 μ M

2.5 μ M

1.0 μ M

Calculations of the percent associated at the following times into the reaction:

3.7 min

6.4 min

8.7 min

Table 2. DNA-DNA association in response to minor changes in base probe concentration, measurement time, and flow rate

<u>Ratio of Reacted to Unreacted DNA</u>	<u>Time</u>	<u>Base Conc BUNI</u>	<u>Conc BETA</u>	<u>Flow Rate</u>
1/5.35	6.8 min	2.9 mM	9 μ M	1 μ l/min 20 μ l
1/4	8.7 min	3.2 mM	9 μ M	5 μ l/min 35 μ l
1/3.23	8.6 min	5.4 mM	9 μ M	5 μ l/min 35 μ l

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Appendix A BIAcore conversion formulas for proportionality of RU response to molar equivalents

Conversion formula for protein

$$\frac{\frac{\text{RU response}}{\text{x gm/L}} \times \frac{1000 \text{ RU}}{8.3}}{\text{ml wt of protein}} = \text{molar equivalent on matrix (sensor chip)}$$

Conversion formula for DNA

$$\frac{\frac{\text{RU response}}{\text{x gm/L}} \times \frac{1000 \text{ RU}}{8.3} \times 0.8}{\text{ml wt of oligonucleotide}} = \text{molar equivalent oligo on matrix}$$

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Appendix B Second order rate kinetics calculation for ssDNA

C_0 = ssDNA at time 0

C = ssDNA remaining at time t

k = equilibrium constant

$$C/C_0 = 1/[1 + (kCot)]$$

C/C_0 will equal 1/2 at equilibrium

therefore $kCot = 1$

Cot will equal $1/k$

k will equal $1/Cot$

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Appendix C Experimental parameters for equilibrium experiments

Equilibrium Assessment Parameters; Flow Rate 5 μ L/min

	Injection	Ml Wt	RU Response	Molar Equiv
Avidin	0.22U	66,000	6047	0.76 mM
BUNI	105 ng	7,000	1304	1.24 mM
BETA	150 ng	13,200	1312	0.66 mM

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Appendix D Mathematical derivation of equilibrium conditions of DNA-DNA hybridizations in BIAcore

$$C/Co = 1/(1 + kCot)$$

$$Co = 1240 \text{ nM}$$

$$C = 580 \text{ nM at 300 seconds}$$

$$C/Co = 580/1240 = 0.47$$

$$kCot = 1$$

$$k(1240)(300) = 1$$

$$k = 2.6 \times 10^{-6}$$

$$C/Co = 1/(1 + kCot)$$

$$0.47 = 1/1.9672$$

$$0.47 = 0.51$$