RAPID DNA HYBRIDIZATION REACTIONS USING ISOTACHOPHORESIS

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ABSTRACT

We use ITP to control and increase the rate of homogenous DNA hybridization reactions. We present a novel physical model, new validation experiments, and new demonstrations of this assay. We studied the coupled physics and chemistry of the dynamics of preconcentration, mixing, and chemical reaction kinetics under ITP. Our experimentally-validated model enables closed form solution for two-species hybridization reaction under ITP, and predicts 10,000 fold speed-up of chemical reaction rate at concentrations of order 10 pM, with greater enhancement of reaction rate at lower concentrations. We experimentally demonstrate 800 fold speed-up using ITP compared to the standard case of reaction in a simple, well mixed reaction chamber.

KEYWORDS: Isotachophoresis, DNA Hybridization, Reaction Kinetics, Molecular Beacons

INTRODUCTION

Isotachophoresis (ITP) is an electrophoresis technique which uses two buffers consisting of a high mobility leading electrolyte (LE) and a low mobility trailing electrolyte (TE). [1] It focuses sample species whose mobility is bracketed by those of the LE and TE. We use so-called peak mode ITP to simultaneously preconcentrate, rapidly mix, and expedite reactions between target RNA or DNA species extracted from biological samples and molecular beacon probes.

Molecular beacons are partially self-hybridizing synthetic oligonucleiotides which use a fluorophore and a quencher to produce a sequence specific fluorescence signal. [2] We use ITP to focus target and molecular beacons into a sharp, order 10 micron, ITP interface which we use as a customizable, 10 pL reaction volume to achieve unprecedented speed up of reaction and sensitivity .

THEORY

We denote the concentrations of free molecular beacons, free targets, and hybridized beacons-target complexes as B, T, and BT respectively. Following Tsourkas *et al.*, the hybridization of DNA and molecular beacons can be expressed as $B+T \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} BT$, where k_{on} and k_{off} are the on- and off-rate of the reaction, respectively. [3]

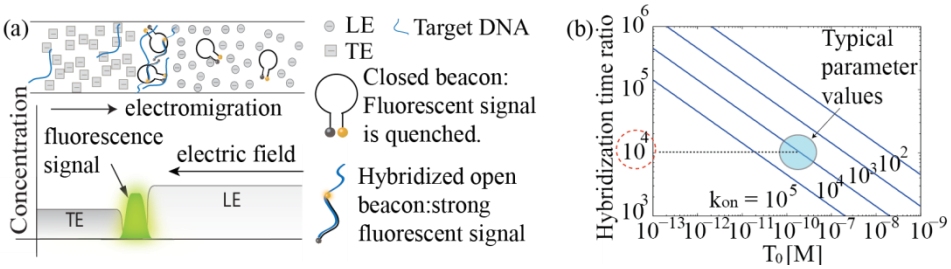

Figure 1:(a) *Schematic showing DNA hybridization at an 10 micron (10 picoliter) ITP interface, speeding up hybrid product rate by over 10,000 fold and increasing the absolute fluorescence signal intensity by 10^6 times at O(10 pM).* ***(b)*** *Ratio of characteristic hybridization time predicted by our model for various initial target concentration and on-rate constant values.*

By considering the fluxes into the control volume surrounding the ITP interface, as well the reaction kinetics within the control volume, we derived a set of non-linear ODEs describing the average concentration of species at the interface over time. These governing equations assume a constant interface width, δ, in which all species have an overlapping Gaussian distributions in space.

$$\begin{cases} \dfrac{d\overline{c_B}}{dt} = \dfrac{\eta_{LE} V_{ITP}}{\delta} B_0 - k_{on} \dfrac{3}{\sqrt{\pi}} \overline{c_B c_T} + k_{off} \overline{c_{BT}} \\ \dfrac{d\overline{c_T}}{dt} = \dfrac{\eta_{TE} V_{ITP}}{\delta} T_0 - k_{on} \dfrac{3}{\sqrt{\pi}} \overline{c_B c_T} + k_{off} \overline{c_{BT}} \\ \dfrac{d\overline{c_{BT}}}{dt} = k_{on} \dfrac{3}{\sqrt{\pi}} \overline{c_B c_T} - k_{off} \overline{c_{BT}} \end{cases} \quad (1)$$

where $\bar{c}_i$ with $i = B,\ T,$ and BT are defined as average concentration over the control volume, η_{LE} and η_{TE} denote ITP focusing rate coefficients in LE and TE zones respectively, B_0 and T_0 are initial beacons and target concentrations.

A closed form analytical model can be obtained by further assuming slow off-rate ($k_{off} \ll k_{on}T_0$) and one dominant reactant concentration at the ITP interface ($T_0 \gg B_0$). The solution of our analytical model is presented in Table 1, and is compared with the standard hybridization case of a simple reaction (*sans* ITP) in a well mixed reaction chamber.

Table 1. *Analytical solution comparison of ITP hybridization and standard (reaction chamber) hybridization*

	ITP-enhanced hybridization	Standard hybridization
Hybridization product concentration	$c_{BT} = \frac{\eta V_{ITP}}{\delta} B_0 t \left(1 - e^{-\frac{1}{2}\frac{3}{\sqrt{\pi}}\frac{\eta V_{ITP}}{\delta} k_{on} T_0 t^2}\right)$	$c_{BT} = B_0\left(1 - e^{-k_{on}T_0 t}\right)$
Characteristic time scale	$t_{ITP} \sim \sqrt{\frac{1}{\frac{\eta_{TE} V_{ITP}}{\delta} k_{on} T_0}}$	$t_{st} \sim \frac{1}{k_{on}T_0}$

Importantly, we find that the speed-up in reaction time is inversely proportional to the square root of the initial concentration of the abundant reactant. **This leads, for example, to 10,000 fold speed-up of chemical reaction rate at order 10 pM concentrations**. In Figure 1b, speed-up in rate of reaction via ITP is predicted from the model for various initial target concentrations and relevant kinetic rate constants of molecular beacons. Furthermore, while the maximum concentration of the hybrid in the standard case is limited by the initial concentration of molecular beacons, ITP focusing results in a continuosly increasing hybridization product concentration. This results in a 1,000,000-fold increase in the absolute concentration of the hybridized product. The assay yields a 1000-fold increase in signal enhancement over the control case (ITP of beacons without target).

EXPERIMENTAL

We validated the model using a set of controlled experiments in which we hybridize DNA-based molecular beacons with synthetic complementary oligonucleotides. We mix target DNA with TE, and mix molecular beacons with LE. This ensures that the reactants remain separated until they simultaneously and progressively focus into the same 10 pL ITP reaction volume, at which point they begin to react and hybridize (c.f. Figure 1a). In order to monitor reaction rate, we recorded the fluorescence signal at seven locations along the length of the channel. We normalize the signal by the signal of beacons alone, to obtain the fraction of hybridized beacons.

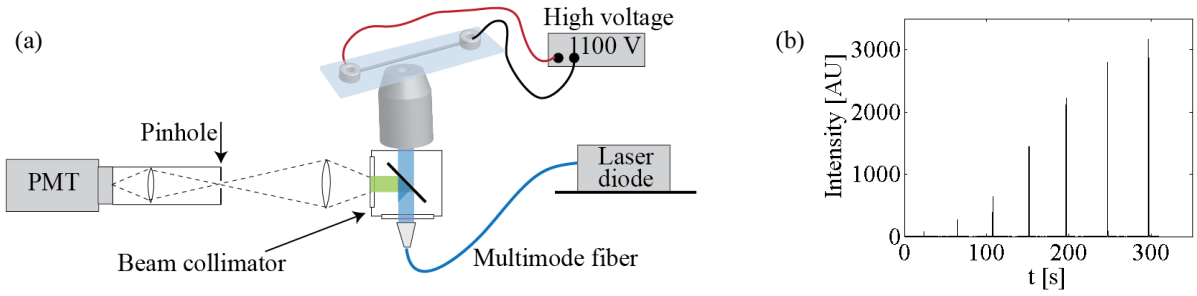

Figure 2: (a) *Schematic of the experimental setup used to study DNA hybridization under ITP. Our set-up consists of a straight 4 cm long microfluidic channel, laser excitation, high-voltage supply, and a custom point confocal fluorescence system. We use laser diode illumination and photomultiplier tube detection for rapid, sensitive detection of reactions.* ***(b)*** *An example raw data from the setup. These temporal fluorescence signals (isotachopherograms) are collected from our custom point detector and integrated in time. We use these signal integrals to quantify concentration of hybridization product.*

RESULTS AND DISCUSSION

In Figure 3a we summarize our model validation study by comparing between a direct numerical solution of the governing equations, our analytical model, and experimental results at various target concentrations. We see good agreement between the model and experimental data (inset of Figure 3a). We use our analytical model to propose a new time-scale normalization which completely collapses data across beacon concentrations, target concentrations, and ITP conditions. This collapse of data is shown in the main plot of Figure 3a.

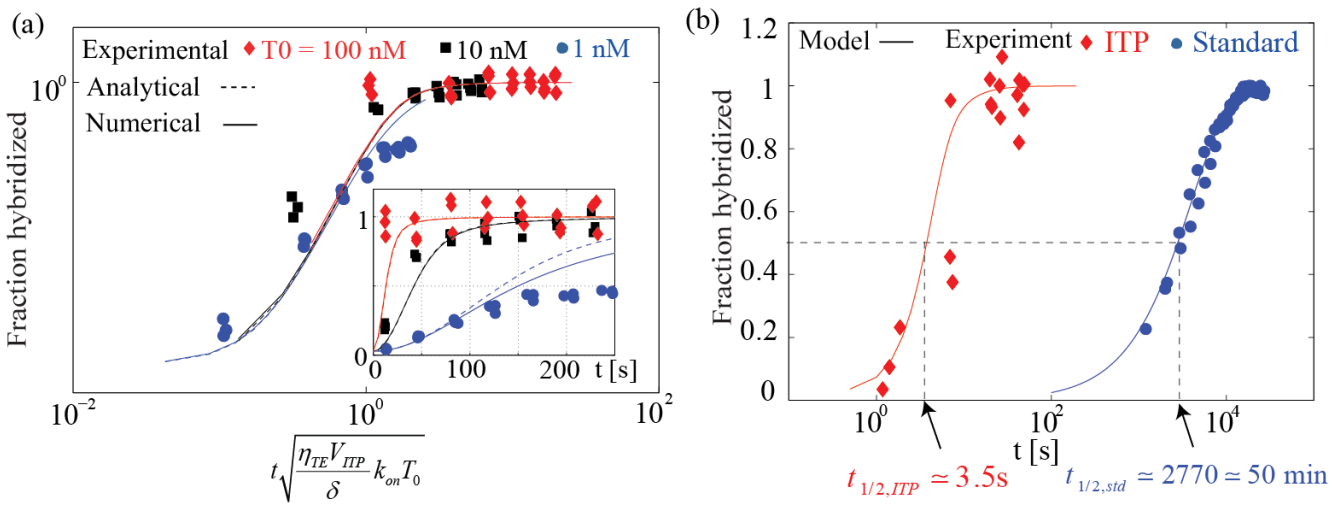

Figure 3: (a) *Analytical and numerical predictions vs. ITP-aided DNA hybridization data with no fitting parameters. (We quantify forward kinetic reaction rate in independent experiments.) Shown are normalized fluorescence signal over time. In the inset we show raw example experimental data at target concentrations of 1, 10, and 100 nM versus 10 nM concentration of molecular beacons (probe length 28-mer; stem length 7-mer). TE was 50 mM Tricine and 100 mM Bistris, and LE was 250 mM HCl, 500 mM Bistris, 2 mM $MgCl_2$, 0.1 % PVP. In the main plot, we show the same data plotted in log-log scale with the time axis normalized by the characteristic time scale predicted by the model.* ***(b)*** *Experimental demonstration of the predicted 800 fold hybridization speed-up for O(10 nM) DNA oligonucleotides. Fraction hybridized plotted in log-log scale for ITP and standard hybridization (without ITP preconcentration) cases as a direct demonstration of rapid hybridization by the assay. The experiment was conducted using 10 nM concentration of molecular beacons and 50 nM target concentration. For ITP, we used TE of 30 mM Tricine and 60 mM Bistris, and the same LE used for Figure 3a. The standard hybridization data were obtained from pressure-driven flow system where reactants were premixed in the LE buffer used in the ITP experiments. The model prediction solid lines are plotted based on independent kinetic rate measurement, interface width, and ITP speed values extracted from the experiment.*

Figure 3b presents an experimental comparison of ITP based DNA hybridization and standard hybridization using 10 nM of molecular beacons and 50 nM of complementary oligonucleotides. Standard (simple mixing-based reaction without ITP) hybridization required 50 min to complete 50 % of the reaction, while ITP-based hybridization required only 3.5 s. This experimental demonstration of 800 fold speed-up is in good agreement with the model prediction.

CONCLUSION

Our work confirms that on-chip ITP can be used to speed up chemical reactions by 10,000 fold by strongly preconcentrating reactant species in simple, straight microchannels. We use peak mode ITP to focus species by up to 50,000 fold into order 10 micron zone lengths; yielding order 10 picoliter volumes which incubate reactions. We have developed and experimentally validated the detailed reaction kinetics model coupled with ITP which can be used to design and optimize a wide range of reaction systems. Our method therefore provides a dramatic increase in sensitivity without sacrificing molecular beacons' specificity. The assay is a unique and highly precise method of studying a wide range of chemical reactions across broad ranges of timescales and reactant concentrations.

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