NOVEL SIMULATION TOOL COUPLING NON-LINEAR ELECTROPHORESIS AND REACTION KINETICS

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ABSTRACT

We developed a new, fast and accurate simulation tool which enables the prediction of multi-species reaction kinetics coupled to nonlinear electrophoretic transport. We present here the capabilities of the code, its validation against published experiments, and demonstrate its use in simulation and prediction of complete biosensing assays.

KEYWORDS

Simulation, electrophoresis, isotachophoresis, hybridization, reaction kinetics.

INTRODUCTION

Electrophoretic separation and concentration techniques are extensively used in a wide range of chemical and biochemical applications, including drug discovery, genetics, and food analysis. Recently, there is growing interest in the use of on-chip electrophoretic techniques for rapid biosensing and point-of-care diagnostics.[1] Fast computational tools are an essential part of any such assay development, as they enable insight into physical and chemical processes and significantly reduce experimental time. Several simulation tools for prediction of non-linear electrophoretic transport now exist.[2] However, to the best of our knowledge, none of the existing codes accounts for important non-equilibrium reactions, such as those which govern nucleic acid hybridization, or antibody-antigen binding.

We here present, for the first time, a new, fast and open source simulation tool, which couples non-linear electrophoretic transport (elecrtromigration-advection-diffusion) with chemical reaction kinetics. Our code, named *SpressoRXN* is constructed as a module to Stanford's open source electrophoresis simulation tool, Spresso.[3] Our code enables simulation of non-linear electrophoretic transport combined with multi-species reaction kinetics. This new capability, together with existing features of Spresso (Fig 1), can be used for the design and optimization of novel biosensing assays.

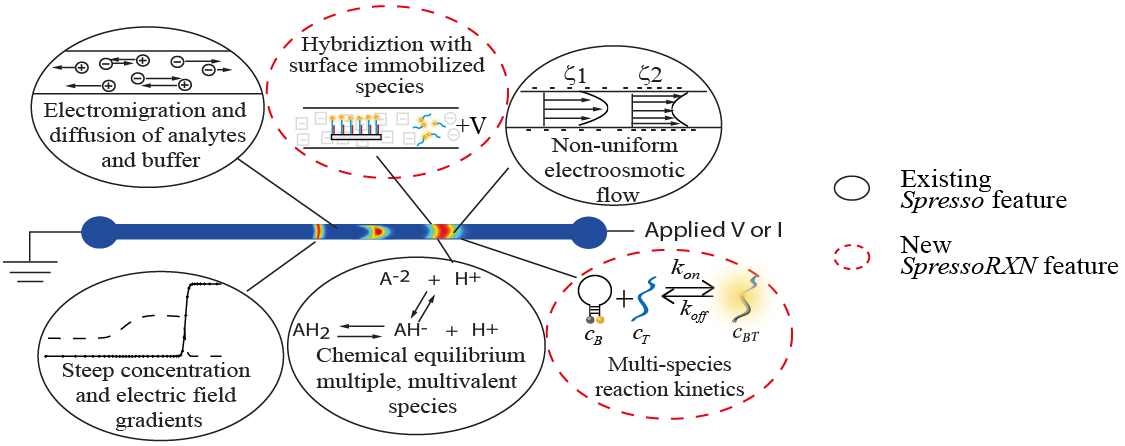

Figure 1: Schematic showing SpressoRXN's features and capabilities. The new (dashed red circles) and inherited (solid black circles) capabilities of the simulation tool are presented. When put together, these features enable fast simulation of complex non-linear electrophoretic transport coupled with multi-species reaction kinetics.

GOVERNING EQUATIONS

A key assumption in our simulation is that the concentration of any reactants is significantly lower than those of the background electrolytes. This assumption holds well for the majority of biochemical processes of interest. We are thus able to decouple the chemical reaction and electric field solvers, resulting in fast simulation times. We first use Spresso to solve for the electric field in space and time. Then, for each pair of reactants, c_i^R, c_j^R, producing a product, c_{ij}^P, we solve a set of transport-reaction equations of the type:

$$\frac{\partial c_{ij}^P}{\partial t} = \frac{\partial}{\partial x}\left[\mu_{ij} E c_{ij}^P + D_{ij}\frac{\partial c_{ij}^P}{\partial x}\right] + k_{on}^{ij} c_i^R c_j^R - k_{off}^{ij} c_{ij}^P \qquad i,j = 1 \ldots N$$

where μ_{ij} is the effective electrophoretic mobility, D_{ij} is the molecular diffusivity, E is the electric field (obtained from Spresso) and k_{on}^{ij}, k_{off}^{ij} are respectively the on- and off-rates of the reaction. Currently, we address only second order reactions which are useful in describing hybridization and binding events.

RESULTS

Code validation: Isotachophoresis (ITP) in an electrophoretic technique which uses a discontinuous buffer to focus species of interest at a high electric field gradient formed at the interface between high (LE) and low (TE) mobility ions. In recent work, Bercovici *et al.* [4] provided an analytical model and validation experiments for hybridization of nucleic

acids under ITP. Briefly, two complementary DNA strands, *A* and *B*, were mixed in the TE and LE respectively. Both *A* and *B* concentrate at the ITP interface, resulting in an accelerated reaction rate. We reproduced the experimental conditions reported in [4] in our simulation, and used this as a validation case for our code. Such an ITP assay is particularly interesting as a validation case, as it involves sharp electric field gradients, discontinuous buffers, discontinuous electroosmotic flow, and reaction kinetic rates which vary with space and time. Fig 2 presents the fraction of hybrid concentration, f_{ITP}, *versus* time for the three different concentrations of species *A* $(1, 10, 100\ nM)$ and a fixed $(1\ nM)$ concentration of *B*. We obtain good agreement with experimental results, with slight under prediction at short times, and over prediction at long times. We attribute these differences to sample dispersion due to non-uniform electroosmosis, which is not yet modeled in our code.

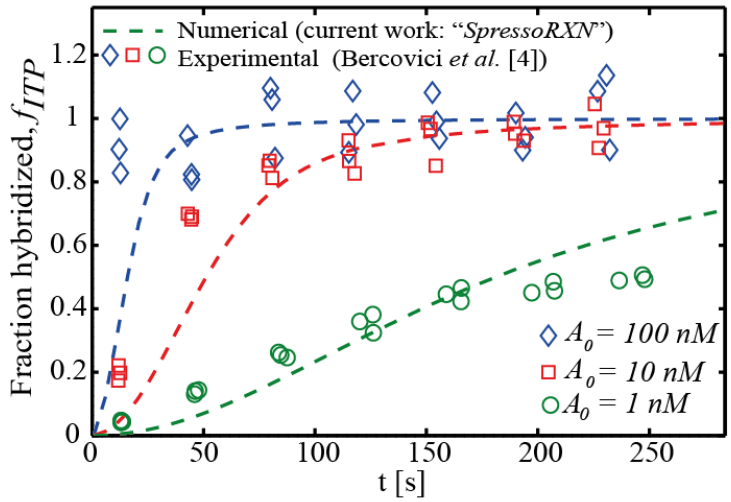

Figure 2: Comparison of SpressoRXN simulation results against experimental ITP-aided hybridization data from [4]. Species A is mixed in the TE, whereas species B is mixed with the LE. The two species concentrate and focus at the ITP interface. Shown is the fraction of B hybridized versus time. The LE buffer consists of 250 mM HCl, 500 mM Bistris and 2 mM $MgCl_2$ whereas the TE buffer consists of 100 mM Tricine and 100 mM Bistris. We set the mobility of DNA as $3.8 \cdot 10^{-8}\ m^2/Vs$, and the reaction on-rate as $k_{on} = 4750\ M^{-1}s^{-1}$, based on independent measurements in [4]. We set the off-rate to $k_{off} = 2 \cdot 10^{-6}\ M^{-1}$.

Reactions in the bulk: While an analytical solution for predicting hybridization under ITP conditions exists,[4] it is based on several restrictive assumptions including the existence of one species in excess concentration, constant interface width, overlapping Gaussian shape concentration distribution, and pH-independent analyte mobility. Using our code we are able to provide prediction and optimization for more realistic and general assays in which reacting species have different mobilities (and are pH dependent), reactant concentration are on the same order of magnitude, or where dissociation rates play an important role. Fig 3 demonstrates the use of the code for such a case, showing that the analytical model significantly overpredicts the hybridization rate at long times.

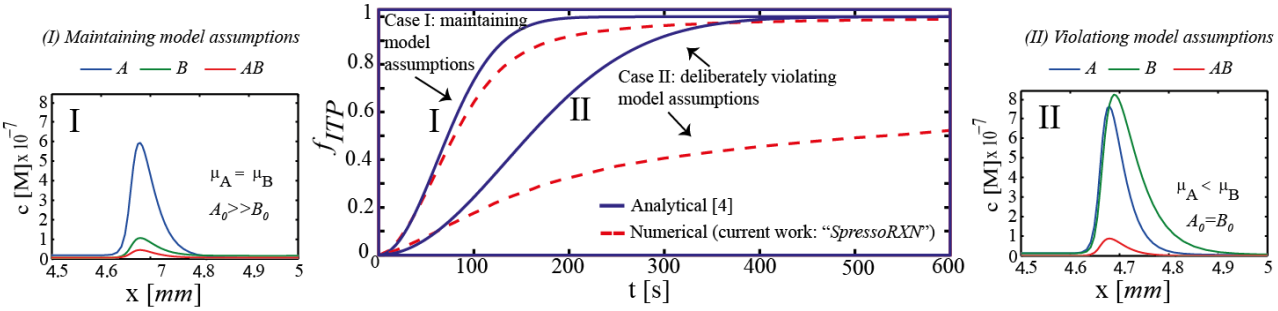

Figure 3: Prediction of rapid DNA hybridization using ITP. Comparison of the analytical model [4] and our numerical simulation for (I) a case in which all analytical model assumptions are maintained. Results are in good agreement, providing additional validation for our numerical simulation. (II) A more general hybridization case in which the analytical model assumptions are deliberately violated. Shown is the fraction of reactant B hybridized vs. time. In case (I) we simulated the hybridization of 100 nM of species A pre-mixed with TE, and 10 nM of species B pre-mixed with LE. Furthermore, to meet model assumptions we used equal mobility $\mu_A = \mu_B = 20 \cdot 10^{-9}\ m^2/Vs$, and a small equilibrium constant $k_{off}/k_{on} = 2 \cdot 10^{-10}$. In case (II) we deliberately deviated from model's assumptions, taking equal initial concentrations of $A_0 = B_0 = 10\ nM$, different mobility values, $\mu_A = 20 \cdot 10^{-9}, \mu_B = 30 \cdot 10^{-9}\ m^2/Vs$ and a relatively large dissociation constant $k_{off}/k_{on} = 10^{-6}$. Our new simulation tool enables to diverge from the restrictive analytical assumptions and investigate the more interesting and realistic cases of ITP-aided hybridization.

Reactions with surfaces: Garcia-Schwarz and Santiago [5] recently presented a novel assay making use of ITP and functionalized hydrogels for high sensitivity detection of nucleic acids. Their method uses two reactions: first, target sequences and fluorescently labeled probes (reporters) preconcentrate under ITP resulting in rapid hybridization. Then, the ITP zone enters a hydrogel where immobilized capture probes bind to excess reporters, thus reducing background fluorescence and improving the signal to noise ratio (SNR).[5] In Fig 4. we present a numerical simulation of the assay's principle, which includes three reacting species, one of which is immobilized in the channel. Simulation results clearly show the improved SNR due to the capturing of excess reporters, and open the door to numerical predictions and optimization of such assay.

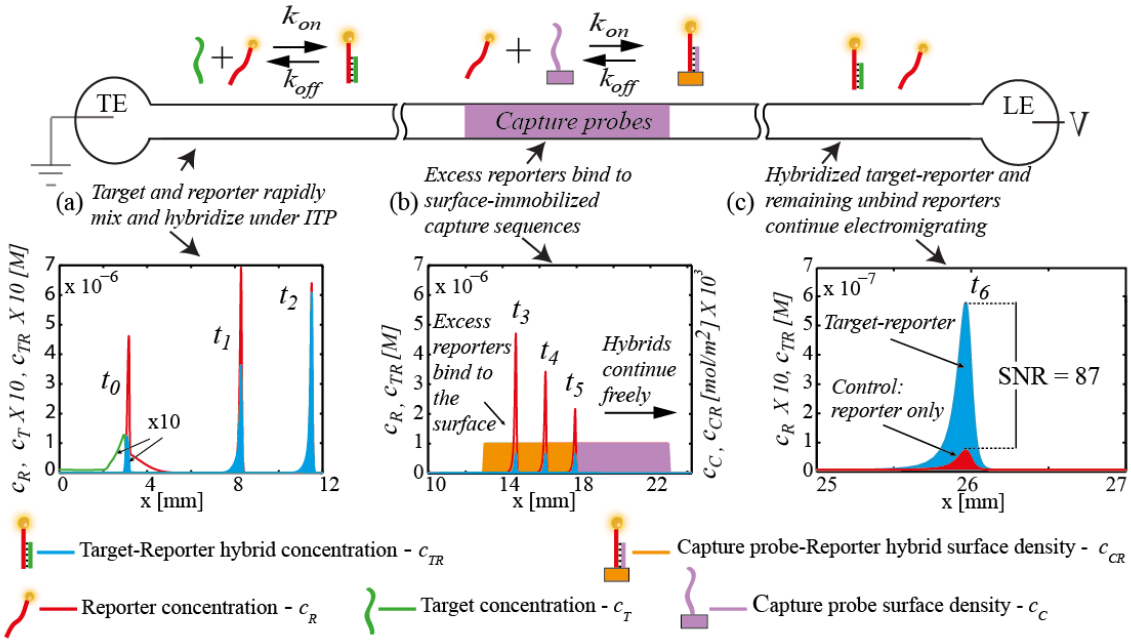

Figure 4: Schematic and simulation results of a complete DNA detection assay [5] involving reactions under ITP in the bulk, and with reacting surfaces. (a) A low concentration (1 nM) target sequence (mixed with TE) and matching labeled probes (1 μM, injected at a finite amount) focus and hybridize via ITP. (b) The high concentration zone electromigrates into a 10 mm long surface functionalized with reporter-specific capture probes. Excess reporters bind to the surface while target-reporter hybrids continue freely. (c) Target-reporter hybrids and any uncaptured reporters continue electromigrating as sharp ITP zone toward the detection site. The total concentration of reporters is compared to their concentration in a control case (with no target) to yield the assay's SNR .

CONCLUSIONS

We developed a new simulation tool which enables, for the first time, predictions of a wide range of assays involving non-linear electrophoresis and chemical reaction kinetics. We validated the code against existing experiments and demonstrated its applicability to predictions of complete assays involving hybridization under ITP in the bulk as well as on surfaces. We believe this code can significantly contribute to the development and optimization of new on-chip biosensing techniques.

ACKNOWLEDGEMENTS

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