Modeling of Competitive Kinetics of DNA Hybridization Reactions

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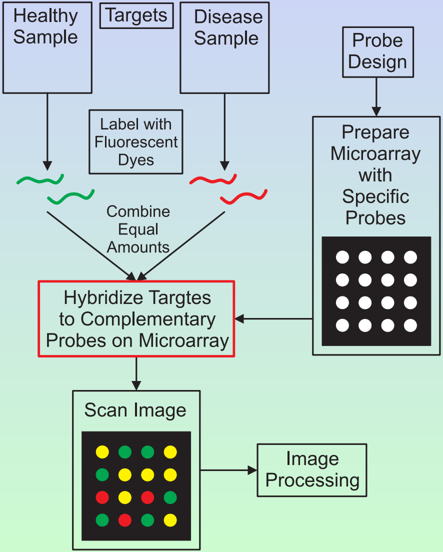
Outline

Microarray

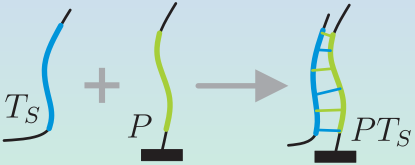

Healthy Sample
Targets
Disease Sample
Probe Design
Label with Fluorescent Dyes
Prepare Microarray with Specific Probes
Combine Equal Amounts
Hybridize Targtes to Complementary Probes on Microarray
Scan Image
Image Processing

Microarray Hybridization

The ultimate goal:

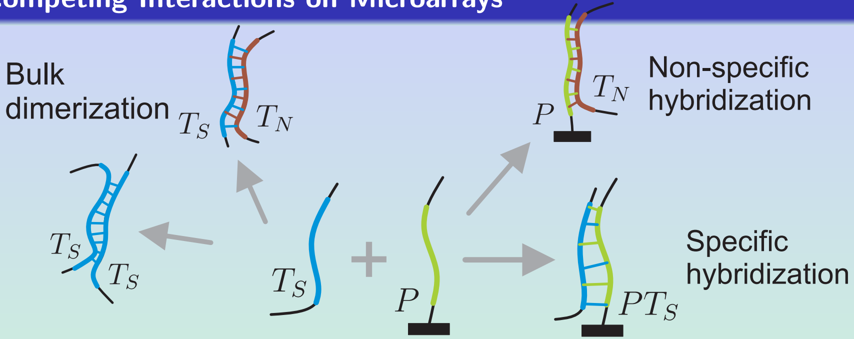
Accurate modeling of hybridization reactions on microarray taking into account competitive probe-target interactions and unimolecular folding.

Competing Interactions on Microarrays



Specific hybridization

Competing Interactions on Microarrays



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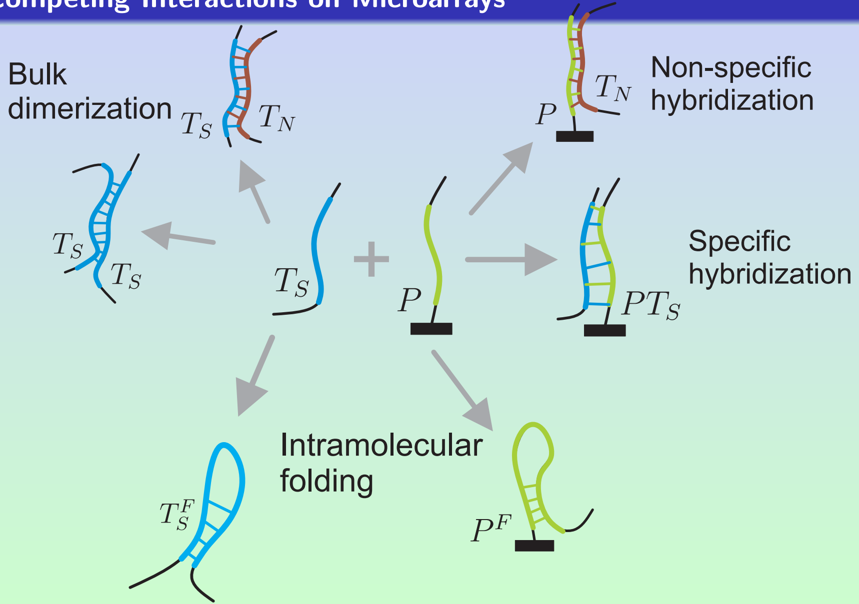

Hybridization Models

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   - (University of Utah) Bishop, Blair, Chagovetz. 2006.
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4. Multiplex-multi-state models: Competitive reactions of duplex formations and unimolecular folding of probes and targets.

Chemical System

Table: Chemical reactions of microarray hybridization

Reaction	Equilibrium Constant	Rate Constants
$p_i + t_j \rightleftarrows p_i t_j$	$K_{p_i t_j}$	$k^a_{p_i t_j}, k^d_{p_i t_j}$
$p_i + p_j \rightleftarrows p_i p_j$	$K_{p_i p_j}$	$k^a_{p_i p_j}, k^d_{p_i p_j}$
$t_i + t_j \rightleftarrows t_i t_j$	$K_{t_i t_j}$	$k^a_{t_i t_j}, k^d_{t_i t_j}$
$p_i \rightleftarrows p_i^f$	$K_{p_i^f}$	$k^a_{p_i^f}, k^d_{p_i^f}$
$t_i \rightleftarrows t_i^f$	$K_{t_i^f}$	$k^a_{t_i^f}, k^d_{t_i^f}$

Kinetic Model

Assumptions

- System consists of two species types: probes p_i and targets t_j.
- Each p_i can react with every t_j forming $p_i t_j$ duplex.
- Each p_i and t_j can react with each of its counterparts of the same species, forming $p_i p_j$ and $t_i t_j$ complexes.
- The forward rate constants are assumed to be same for all species: $k^a_{p_i t_j} = k^a_{p_i p_j} = k^a_{t_i t_j} = k^a$.
- There are no diffusion barriers to the reaction process.

Initial conditions:

At $\tau = 0$

$$(C_{p_i}, C_{t_j}, C_{p_i^f}, C_{t_j^f}, C_{p_i p_j}, C_{p_i t_j}, C_{t_i t_j}) = (C^0_{p_i}, C^0_{t_j}, 0, 0, 0, 0, 0)$$

Kinetic Model

$$\frac{dC_{p_i}}{d\tau} = \sum_{j=1}^{N_t}\left(k^d_{p_i t_j} C_{p_i t_j} - k^a C_{p_i} C_{t_j}\right) + \sum_{j=1}^{N_p}\left(k^d_{p_i p_j} C_{p_i p_j} - k^a C_{p_i} C_{p_j}\right) + \\ + \left(k^d_{p_i^f} C_{p_i^f} - k^a_{p_i^f} C_{p_i}\right), \qquad i = 1, \ldots, N_p$$

$$\frac{dC_{t_j}}{d\tau} = \sum_{i=1}^{N_p}\left(k^d_{p_i t_j} C_{p_i t_j} - k^a C_{p_i} C_{t_j}\right) + \sum_{i=1}^{N_t}\left(k^d_{t_i t_j} C_{t_i t_j} - k^a C_{t_i} C_{t_j}\right) + \\ + \left(k^d_{t_j^f} C_{t_j^f} - k^a_{t_j^f} C_{t_j}\right), \qquad j = 1, \ldots, N_t$$

$$\frac{dC_{p_i p_j}}{d\tau} = k^d_{p_i p_j} C_{p_i p_j} - k^a C_{p_i} C_{p_j} \qquad \frac{dC_{p_i t_j}}{d\tau} = k^d_{p_i t_j} C_{p_i t_j} - k^a C_{p_i} C_{t_j}$$

$$\frac{dC_{t_i t_j}}{d\tau} = k^d_{t_i t_j} C_{t_i t_j} - k^a C_{t_i} C_{t_j}$$

$$\frac{dC_{p_i^f}}{d\tau} = k^d_{p_i^f} C_{p_i^f} - k^a_{p_i^f} C_{p_i} \qquad \frac{dC_{t_j^f}}{d\tau} = k^d_{t_j^f} C_{t_j^f} - k^a_{t_j^f} C_{t_j}$$

Problem Complexity

Total number of equations

$$\frac{1}{2}\left(5 + N_p + N_t\right)\left(N_p + N_t\right)$$

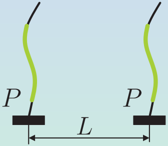
N_p	N_t	Number of equations
1	1	7
5	5	75
10	10	250
100	100	20500
1000	1000	2005000

For larger systems the simulation is computationally expensive.
(Need for parallel ODE solver)

Problem-Size Reduction

1. Equation describing the potential interactions between distinct species of probes could be eliminated from the system

$$\frac{dC_{p_i p_j}}{d\tau} = k^d_{p_i p_j} C_{p_i p_j} - k^a C_{p_i} C_{p_j}$$



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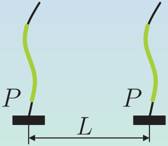


2. Not all probes and targets have equal potential to react and form stable duplexes.
   - Sequence filtering and screening based on different criteria. (>15 consecutive base-pairs, >75% similarity, etc., Kane, 2000).
   - Subsystem of reactions which can occur in a given mixture.

Kinetic Model

Inputs

- Number of probes and targets: N_p and N_t.
- Temperature: T.
- Initial concentrations of probes and targets: $C^0_{p_i}$ and $C^0_{t_i}$.
- Forward rate constants: k^a, $k^a_{p^f_i}$, $k^a_{t^f_j}$.
- Thermodynamic parameters for all DNA complexes.

Outputs

- Concentrations of probes, targets, and duplexes at any given time τ including equilibrium concentrations.

Duplex Thermodynamics

- Thermodynamic transition parameters ΔH, ΔS, and ΔG are determined from published sequence-dependent thermodynamic parameters (SantaLucia's group).

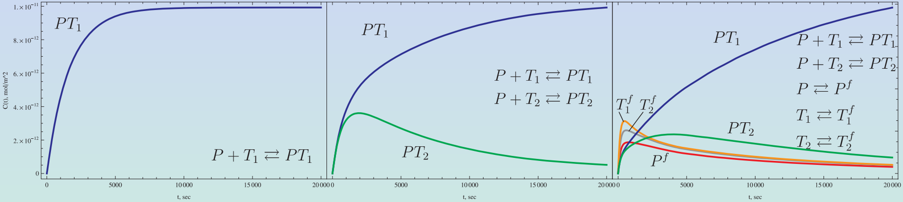
Total free energy

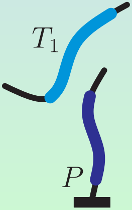
$$\Delta G = \Delta H - T\Delta S$$
$$T_m = \Delta H / \Delta S$$
$$\Delta G = \Delta H \left(1 - T/T_m\right)$$

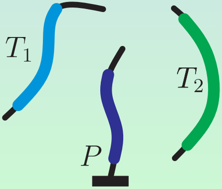
Equilibrium constant and reverse rate constant

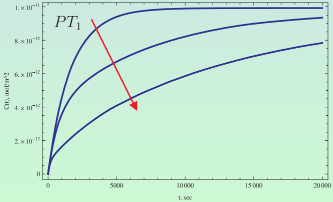
$$K^{eq} = \exp\left(-\frac{\Delta G}{RT}\right) \qquad k^d = \frac{k^a}{K^{eq}}$$

Example: Probe + Specific Target + Nonspecific Target









Conclusion

1. A general analytical description of multiplex-multi-state model is developed.
2. This model describes the kinetic behavior of system with competitive reactions between target mixture, probe set, and corresponding conformations of probes and targets.

Future Work

1. Introduce diffusion of target strands across the probe surface as governed by Second Ficks law:

$$\frac{\partial C_t}{\partial \tau} = D\nabla^2 C_t = D\left[\frac{\partial^2 C_t}{\partial x^2} + \frac{\partial^2 C_t}{\partial y^2} + \frac{\partial^2 C_t}{\partial z^2}\right]$$

2. Implementation of parallel solver for the large ODE systems.

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