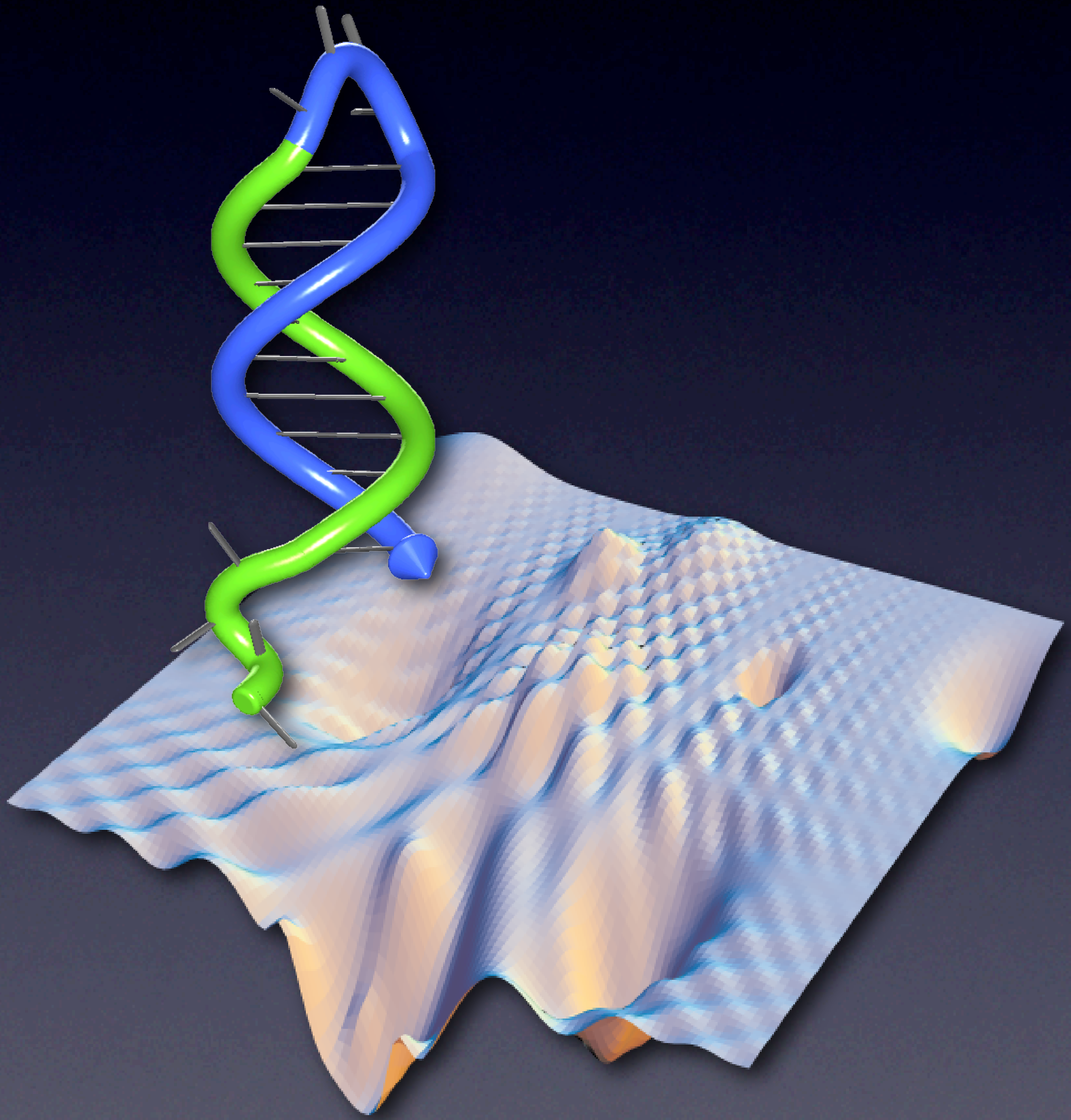
Coarse-Graining DNA Free Energy Landscapes



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Bernard Yurke
Lucent Bell Labs

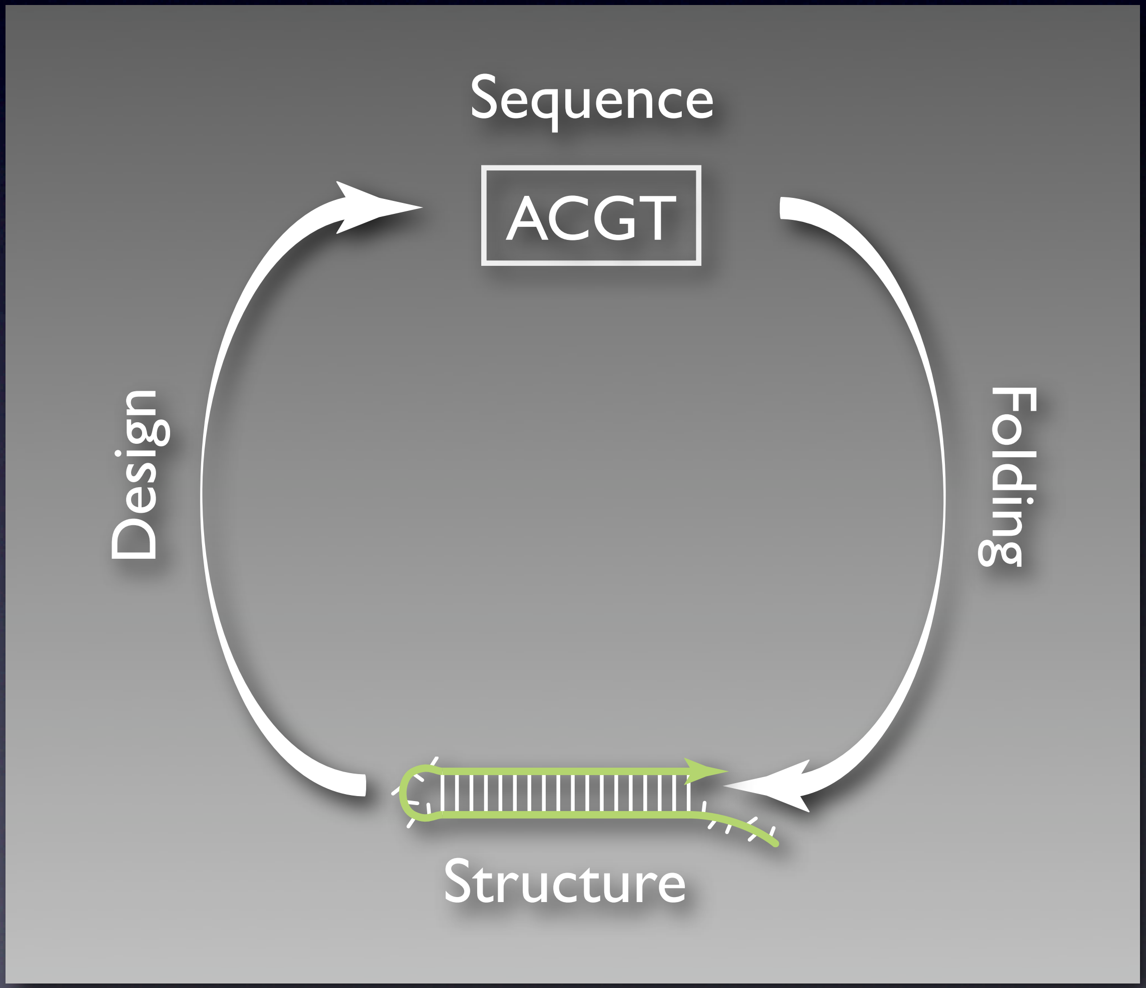
April 12, 2007

Computational Nucleic Acid Engineering

Why nucleic acids?

Biology demonstrates diverse functionality

Structure prediction and design both amenable to computational treatment



Programmable, biocompatible, nanoscale construction material

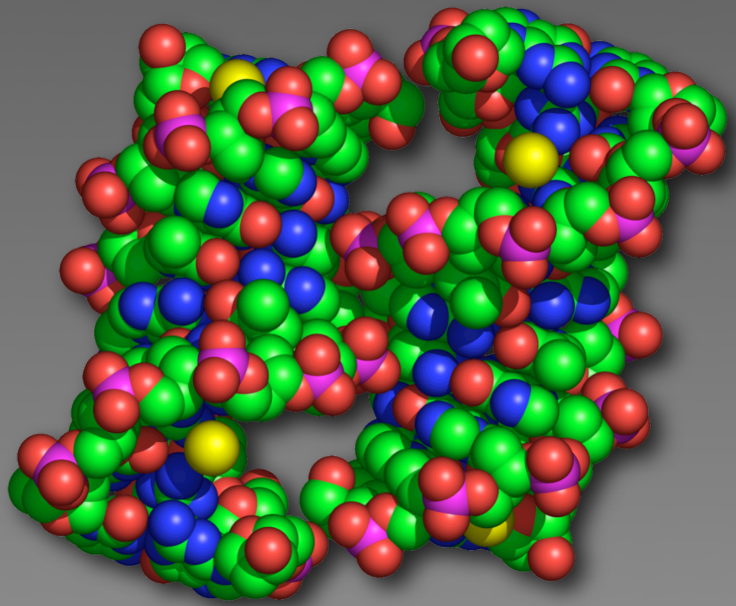
Goals

Develop algorithms to map coarse-grained nucleic acid free energy landscapes – Identify the kinetic and equilibrium properties that dominate experimental observables

Characterize the function of specific synthetic nucleic acid devices using a combination of computational and experimental approaches

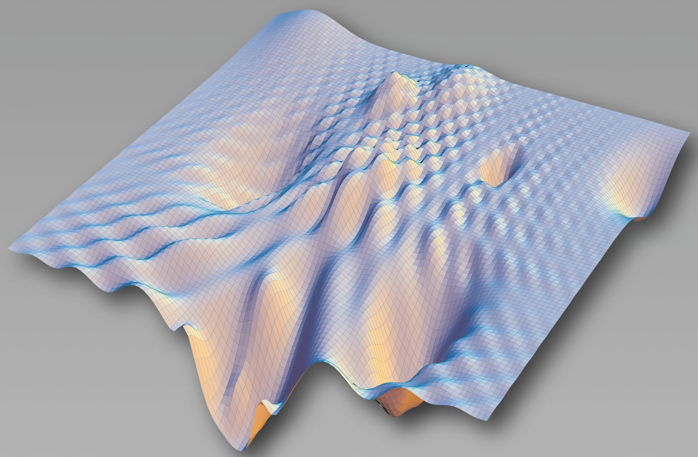
Free Energy Landscape

Tertiary Structure



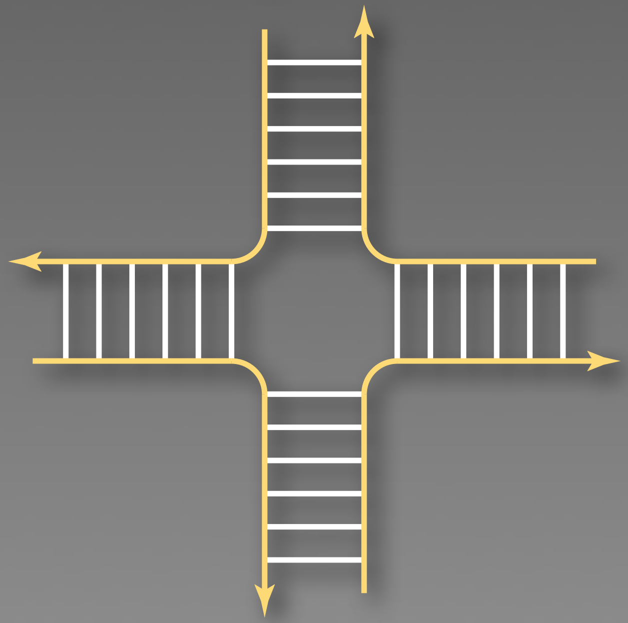
List of atomic coordinates and bonds

Free Energy Landscape



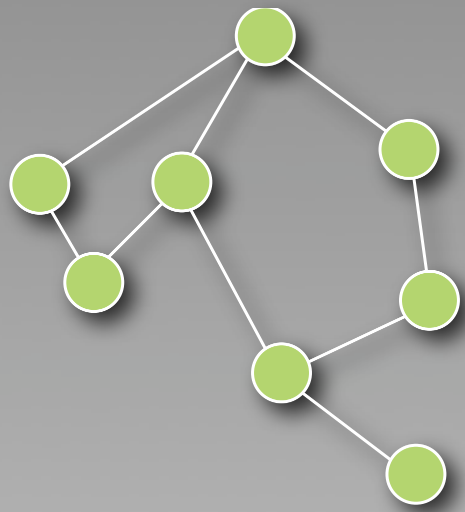
Continuous representation

Secondary Structure



List of base pairs

Free Energy Landscape



Graph representation

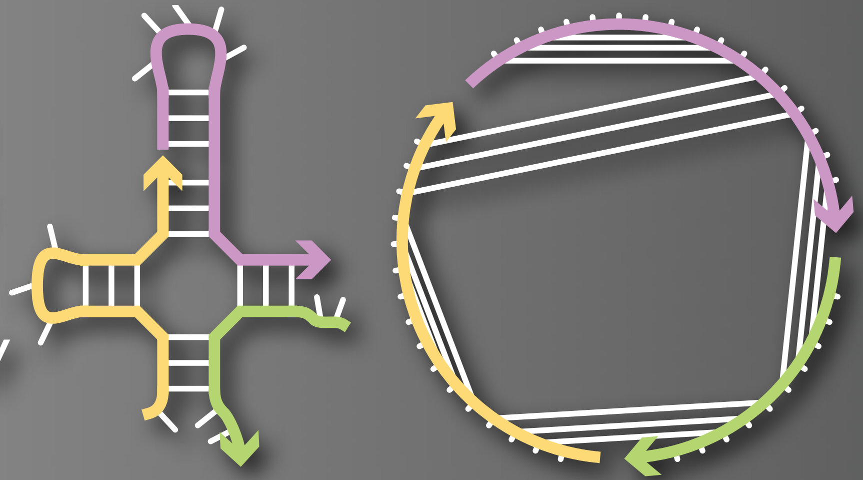
Each node is a secondary structure
Each edge is an elementary kinetic step
Size of graph scales exponentially with strand length – efficient algorithms needed

Project 1: Thermodynamic Analysis of Interacting Strands

Partition function for a complex

New algorithm for calculating the partition function of an unpseudoknotted complex of multiple interacting strands

Rigorous treatment of representation and distinguishability issues



R.M. Dirks, J.S. Bois, J.M. Schaeffer, E. Winfree, N.A. Pierce, *SIAM Rev*, 2007

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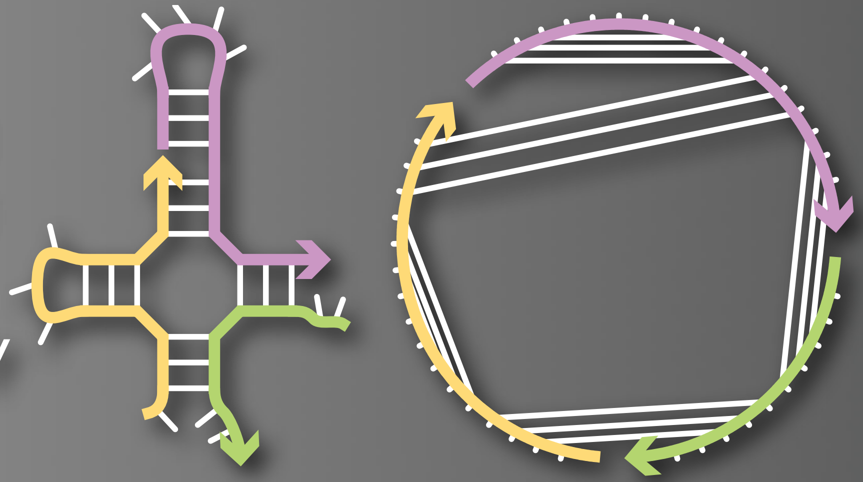


Concentration determination in the thermodynamic limit

For large systems (e.g. a test tube), the equilibrium concentration of each complex species can be obtained by solving a convex programming problem

Efficient solution of the (high-dimensional) primal problem is achieved via solution of the (low-dimensional) dual problem

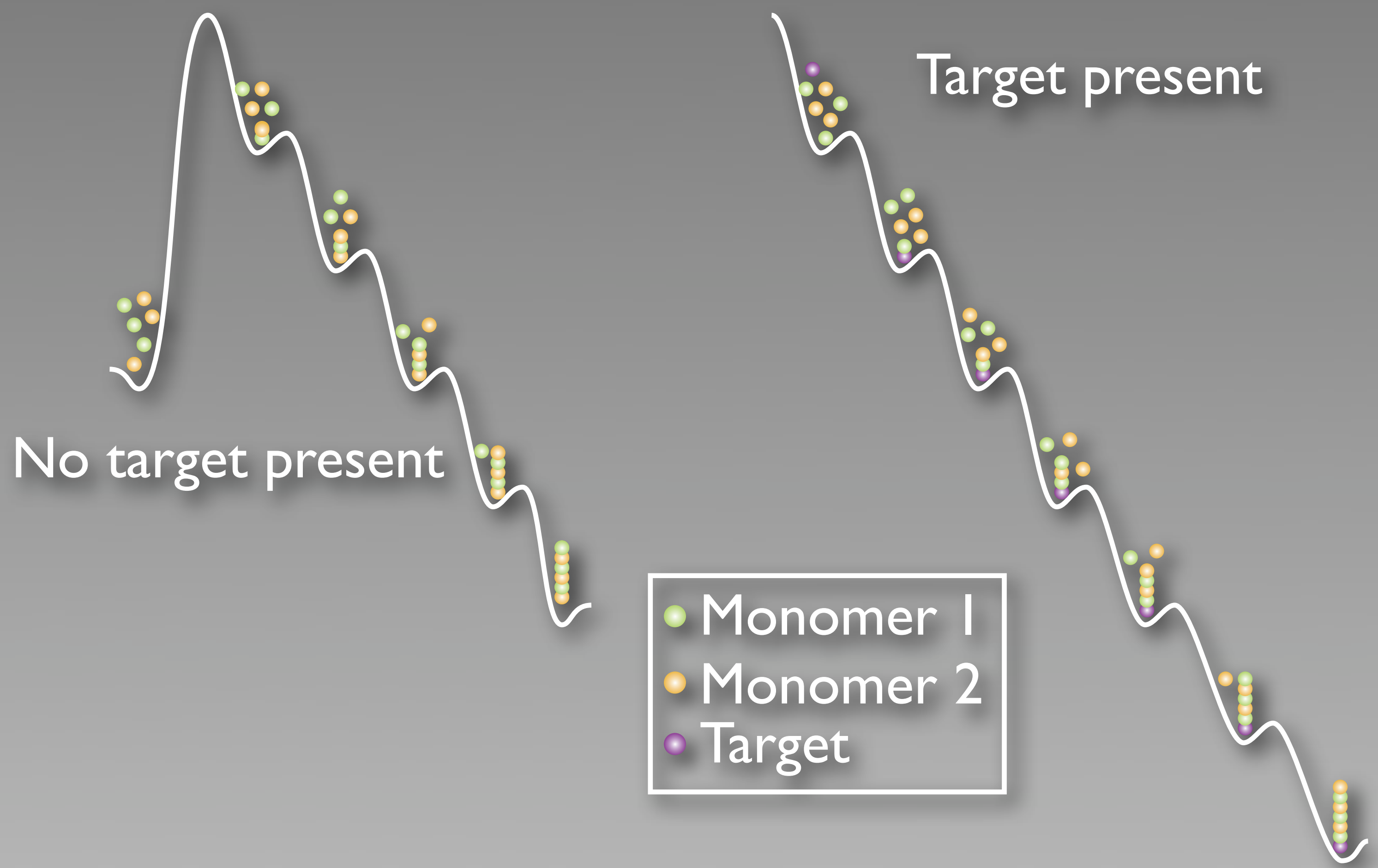
R.M. Dirks, J.S. Bois, J.M. Schaeffer, E. Winfree, N.A. Pierce, *SIAM Rev*, 2007

Project 2: Analysis of HCR free energy landscapes

Hybridization Chain Reactions (HCR)

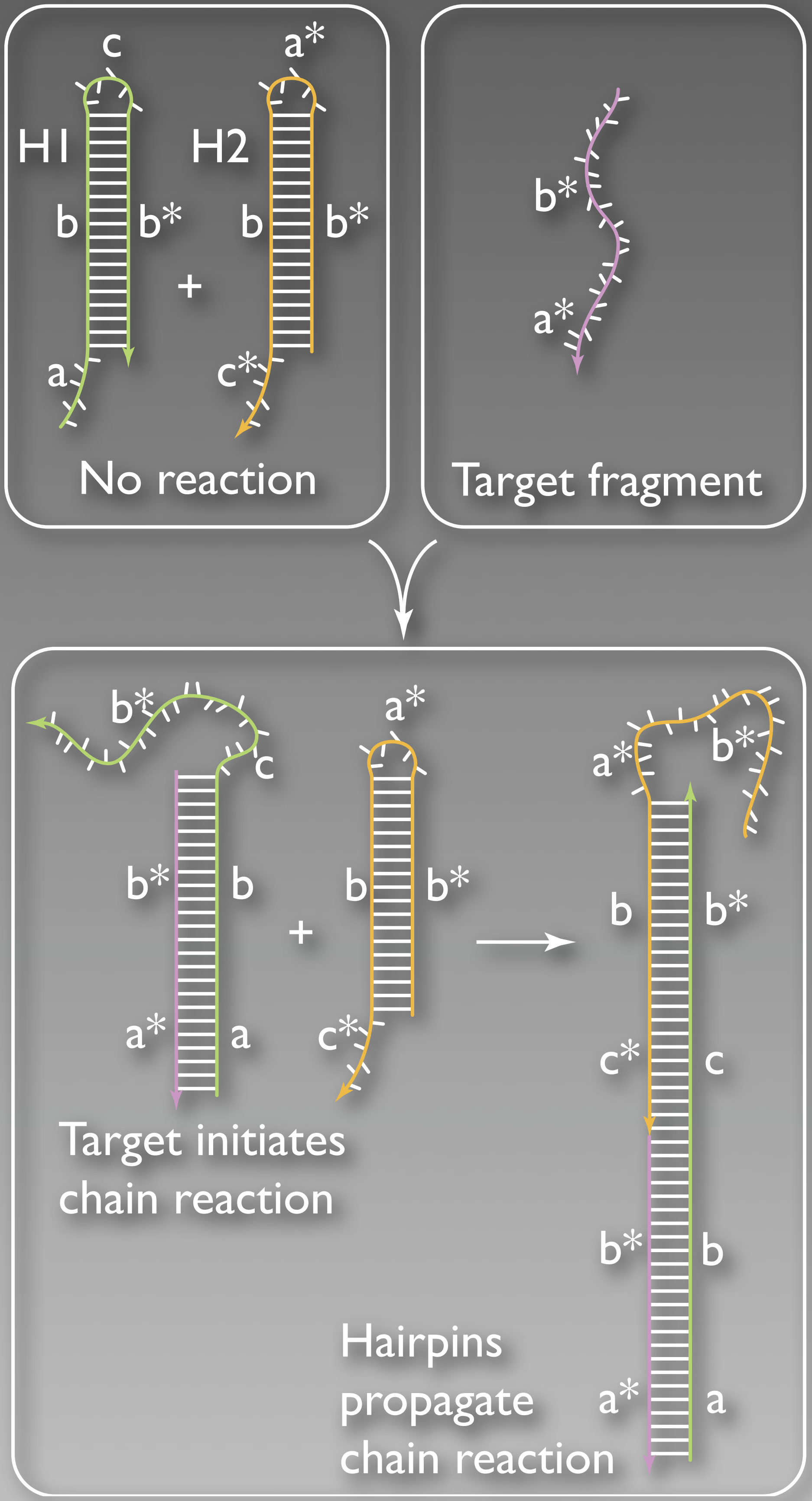
Amplifying transducers for biosensing

Triggered self-assembly of metastable DNA or RNA monomers



Conceptual free energy landscapes

Basic HCR Mechanism



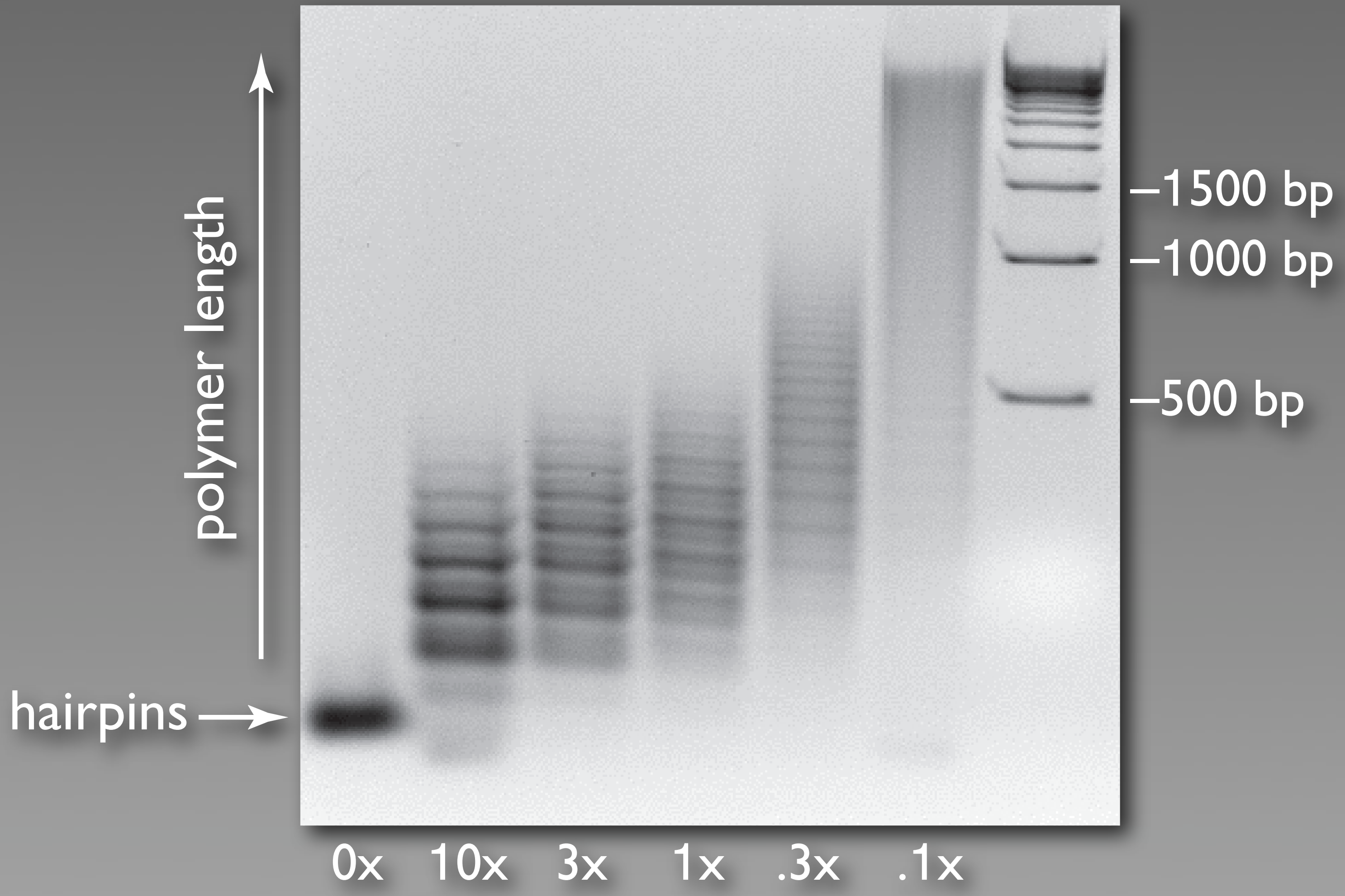
No reaction

Target fragment

Target initiates chain reaction

Hairpins propagate chain reaction

Detecting a target DNA fragment



Target Concentration

1 μM H1 and H2 Hairpins

Average molecular weight varies inversely with initiator concentration

R.M. Dirks, N.A. Pierce, Proc Natl Acad Sci USA, 2004

Probing the metastability of HCR

Are the hairpins metastable in the absence of target?

Anneal H1 and H2 without target to see if hairpins polymerize

R.M. Dirks, J.S. Bois, N.A. Pierce, unpublished

Probing the metastability of HCR

Are the hairpins metastable in the absence of target?

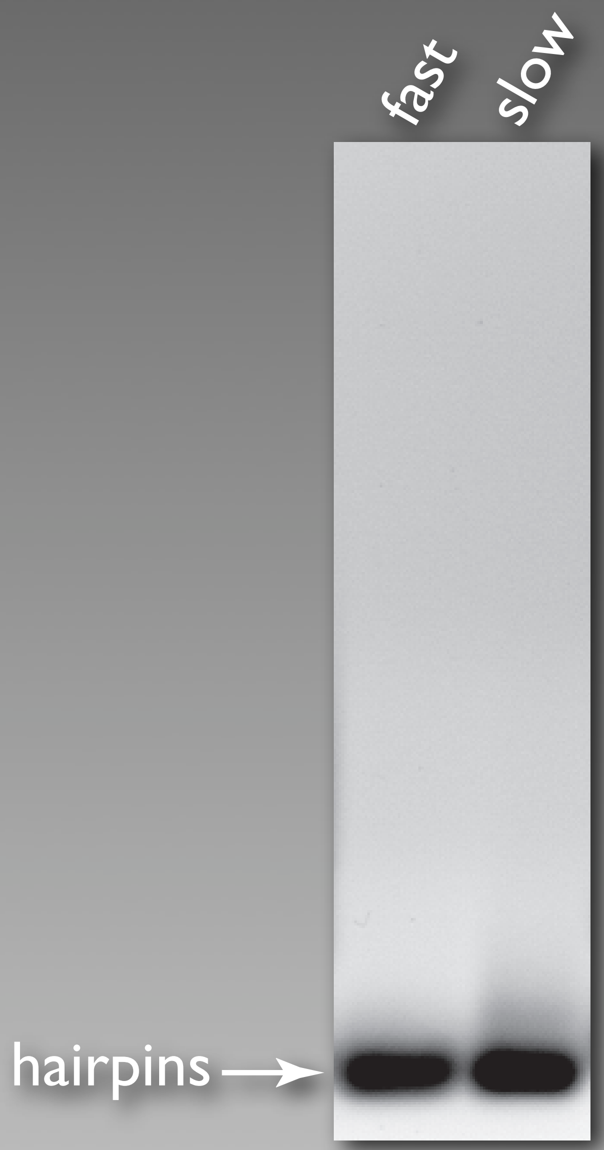
Anneal H1 and H2 without target to see if hairpins polymerize

No polymerization observed with 36-hour anneal

Competing hypotheses:

- Hairpins are metastable and kinetic trap is difficult to escape via annealing
- Hairpins are stable

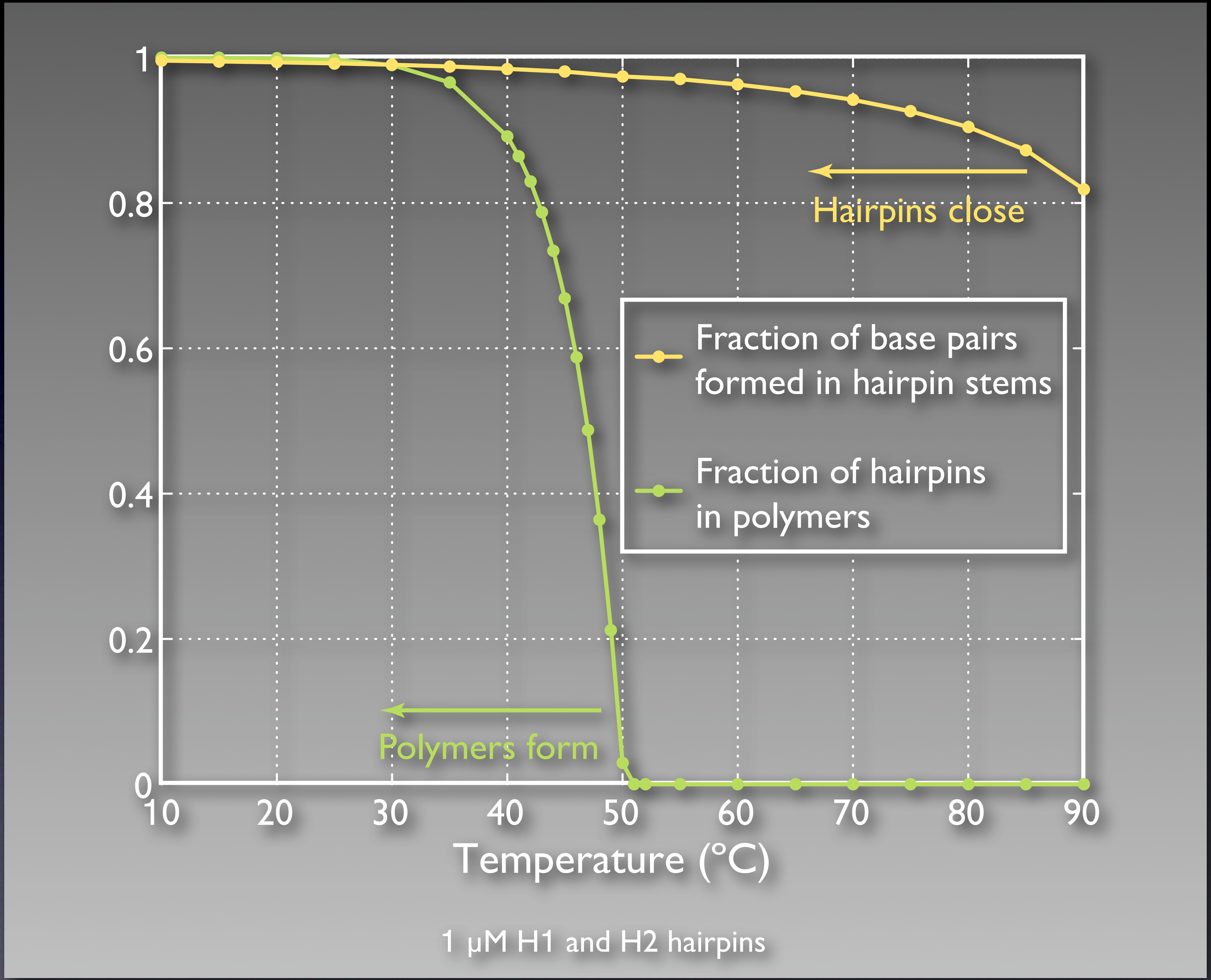
A surprising result



Fast anneal: 90s at 90 °C, cool on bench top
Slow anneal: 95 °C - 20 °C, cool 30 min/°C

R.M. Dirks, J.S. Bois, N.A. Pierce, unpublished

Predicted annealing behavior



As cooling proceeds, hairpins are predicted to close before polymerization becomes energetically favorable

Probing annealing behavior

Trigger polymerization using an RNA target and then digest to obtain HCR products with no bound target

Do they break up into hairpins?

Probing annealing behavior

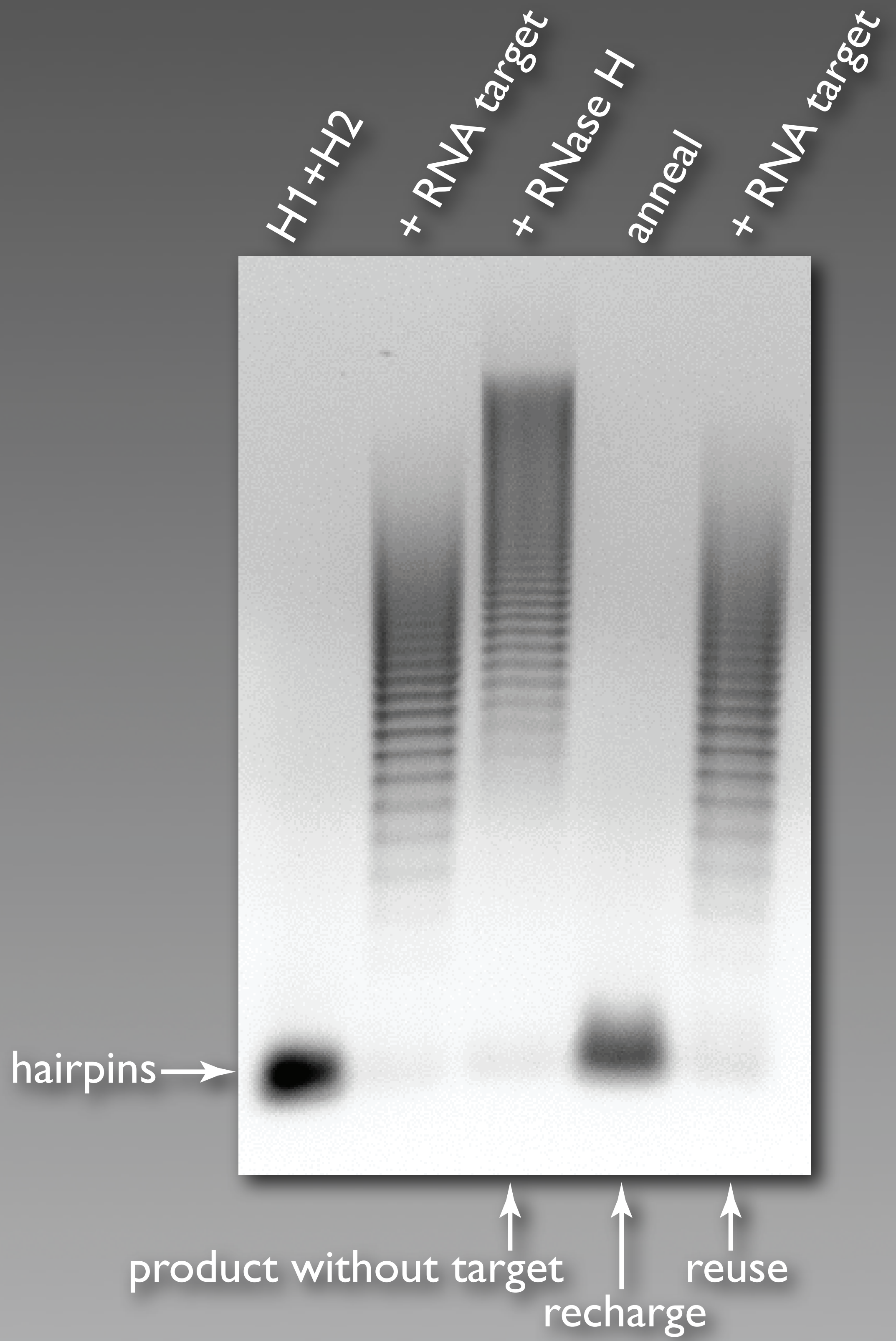
Trigger polymerization using an RNA target and then digest to obtain HCR products with no bound target

Do they break up into hairpins?

No – the polymers become less mobile

Supports the hypothesis that hairpins are metastable

Annealing recharges the sensor

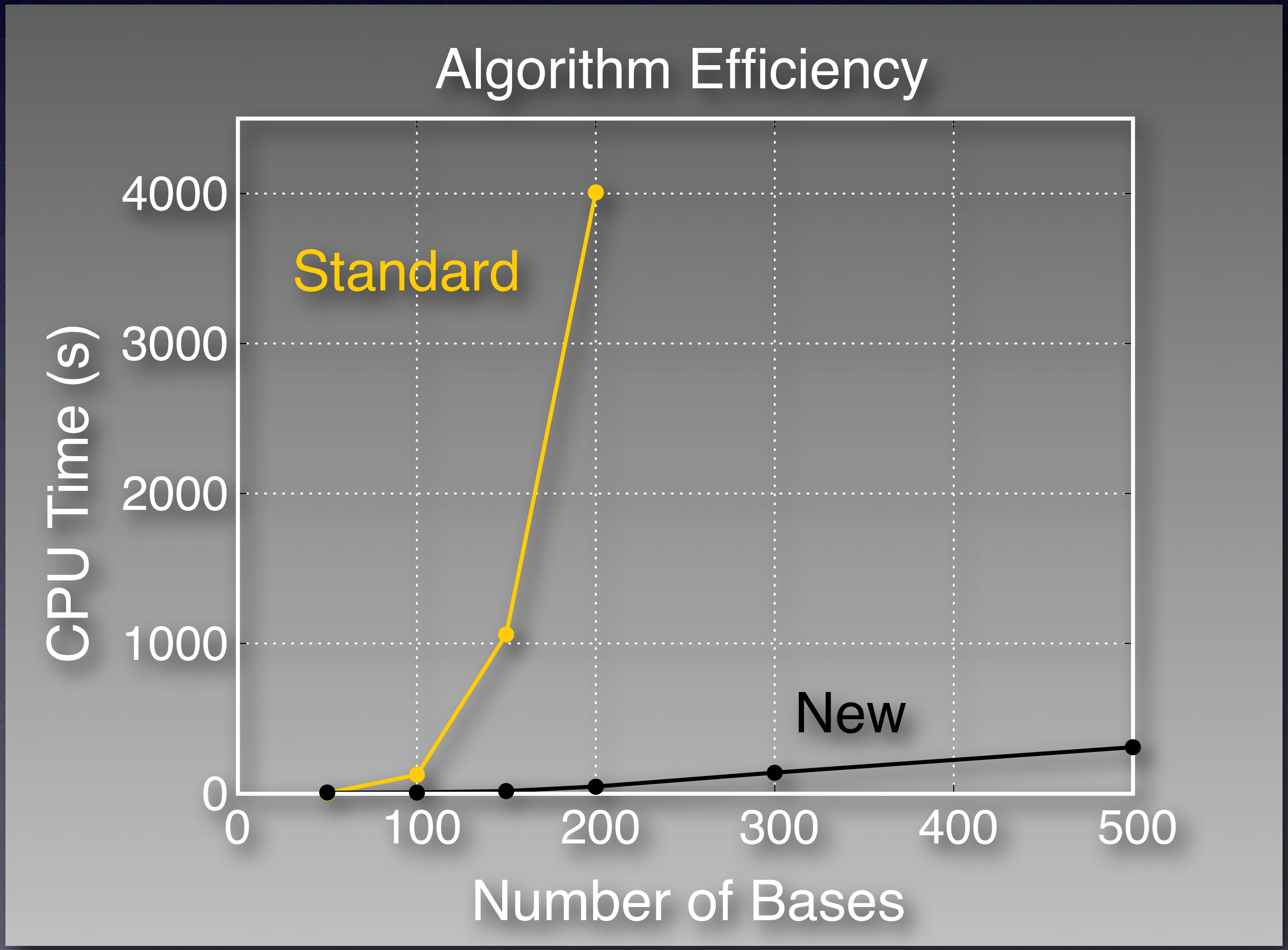


Project 3: Fast Stochastic Kinetics for Interacting Strands

Explore vast landscapes by stochastically generating trajectories from the exact probability distributions (Gillespie, 1976; Flamm et al., 2000)

New algorithm for kinetic simulations of unpseudoknotted complexes of interacting strands

Lower complexity (energy calculations, move generation and selection)



J.M. Schaeffer, J.S. Bois, R.M. Dirks, N.A. Pierce, E. Winfree, in preparation

Project 4: Mapping coarse-grained free energy landscapes

Goal

Identify the macrostates and rates that dominate experimental measurements

Difficulty

The landscape is too large to enumerate the microstates (secondary structures)

Approach

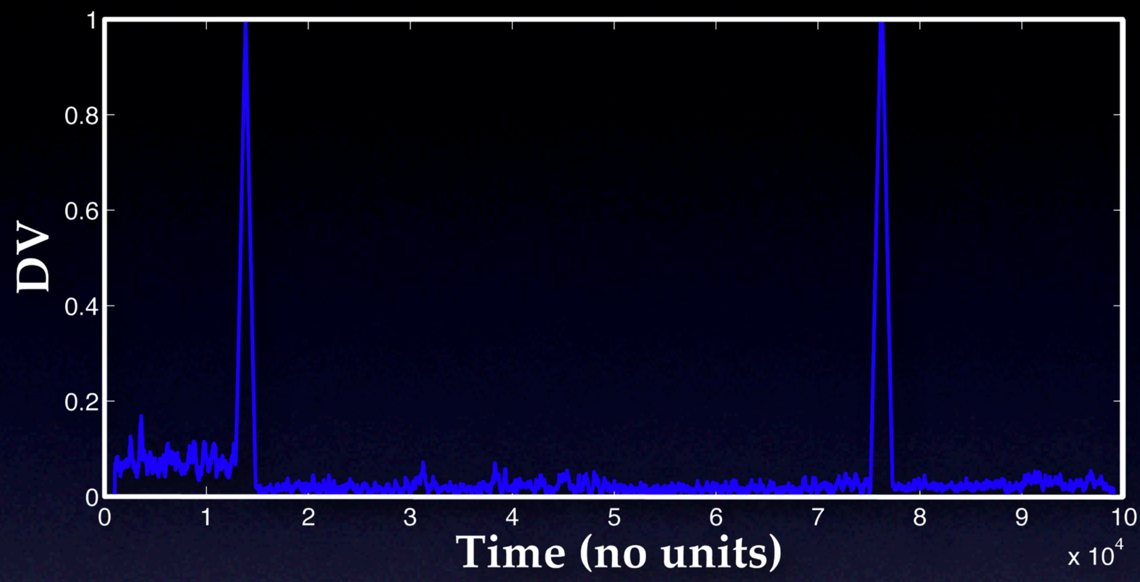
Use stochastic trajectories to explore the landscape

Identify transitions between macrostates by comparing occupancy distributions along trajectories for a specified relaxation time scale τ

Combine repeated distributions into macrostates

Estimate transition rates from first-passage times

Detecting Macrostate Transitions



J. Othmer, N.A. Pierce, unpublished

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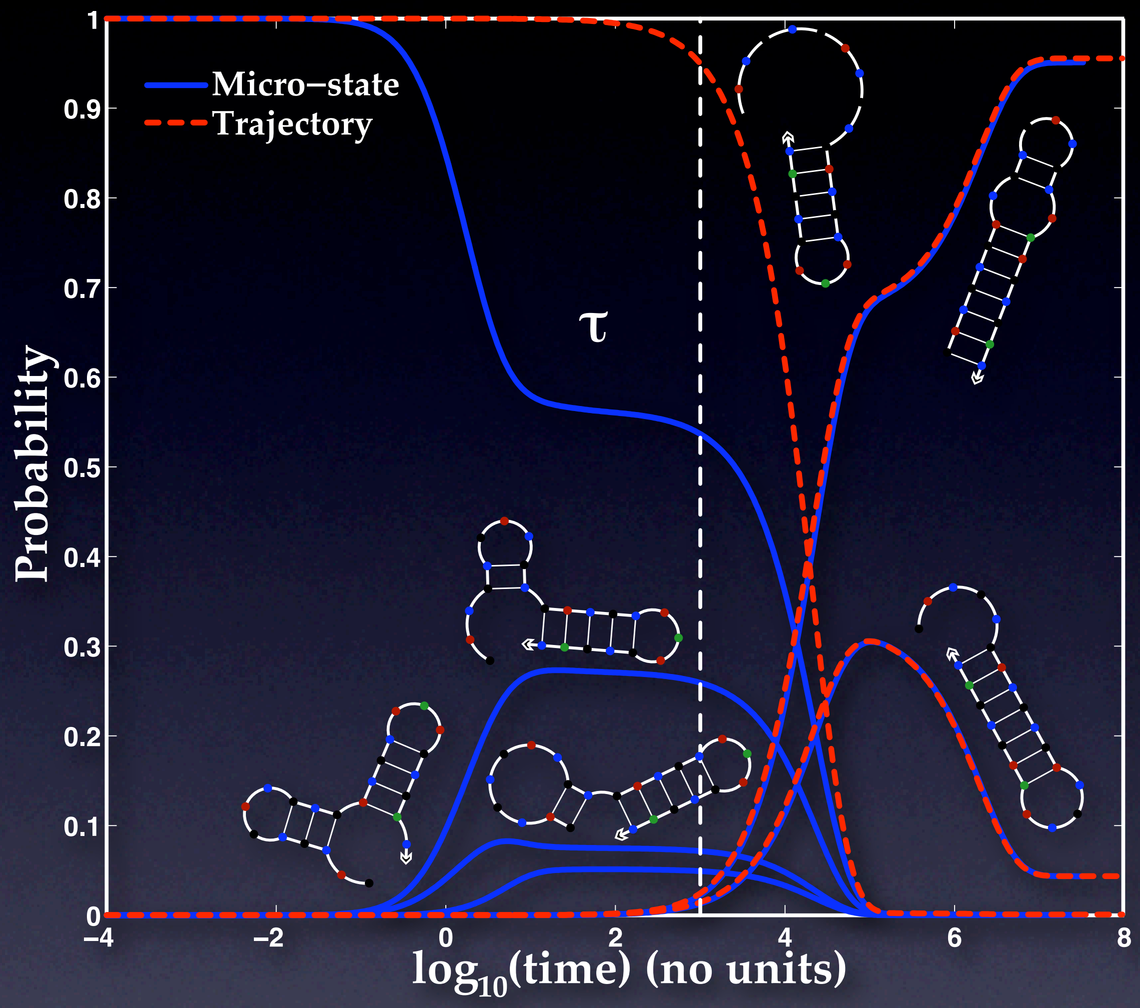
Macrostate Kinetics Example



21 bases
36,567 microstates
4 macrostates

J. Othmer, N.A. Pierce, unpublished

Project 5: NUPACK Nucleic Acid Package

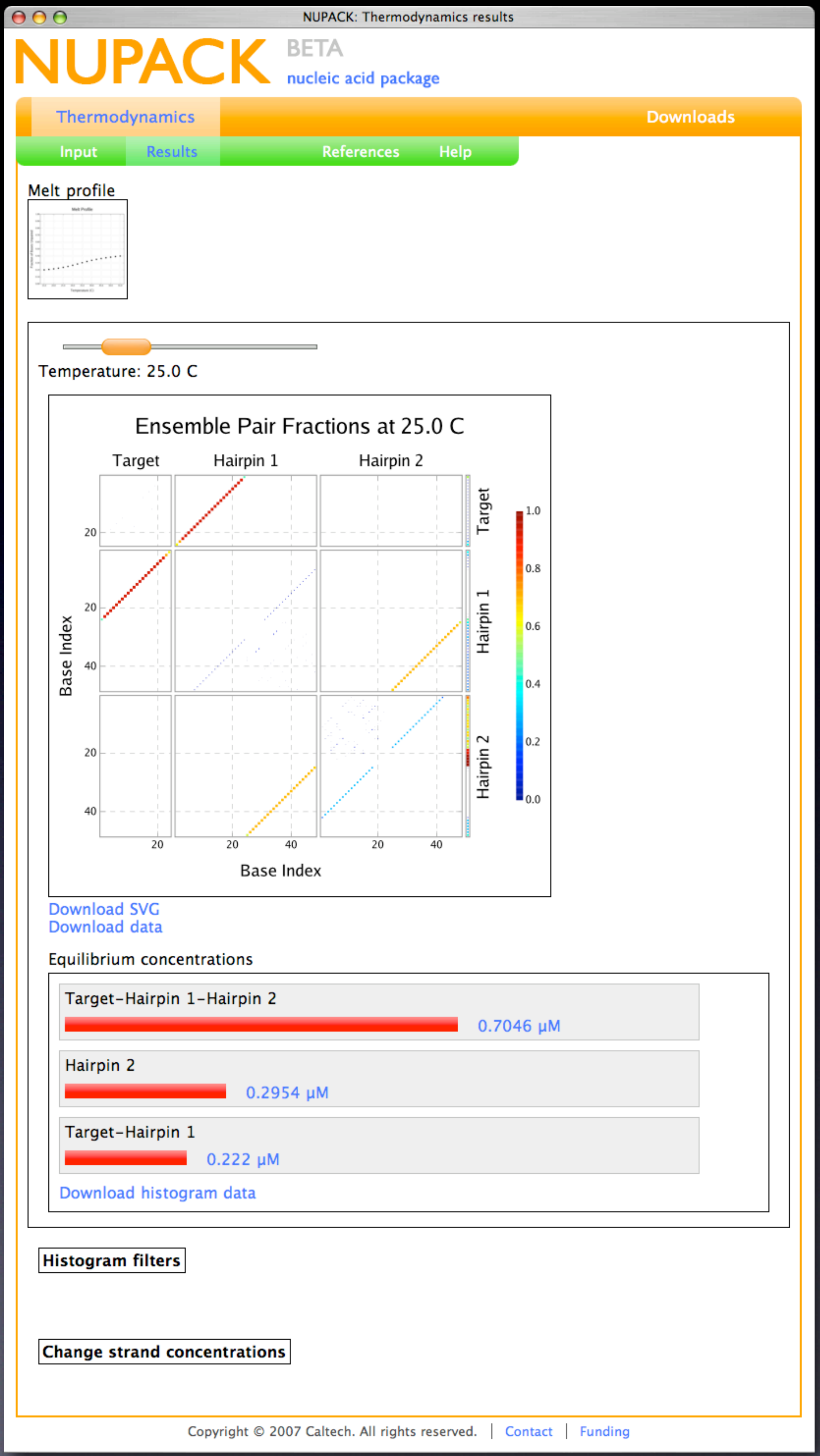
www.nupack.org

A growing software suite for the analysis and design of nucleic acid systems

Web-based interface for efficient hypothesis testing and easy access

Downloadable source code for customization and batch processing

Live demo available



Melt profile

Temperature: 25.0 C

Download SVG
Download data

Equilibrium concentrations

Strand complex	Concentration
Target–Hairpin 1–Hairpin 2	0.7046 µM
Hairpin 2	0.2954 µM
Target–Hairpin 1	0.222 µM

Download histogram data

Histogram filters

Change strand concentrations

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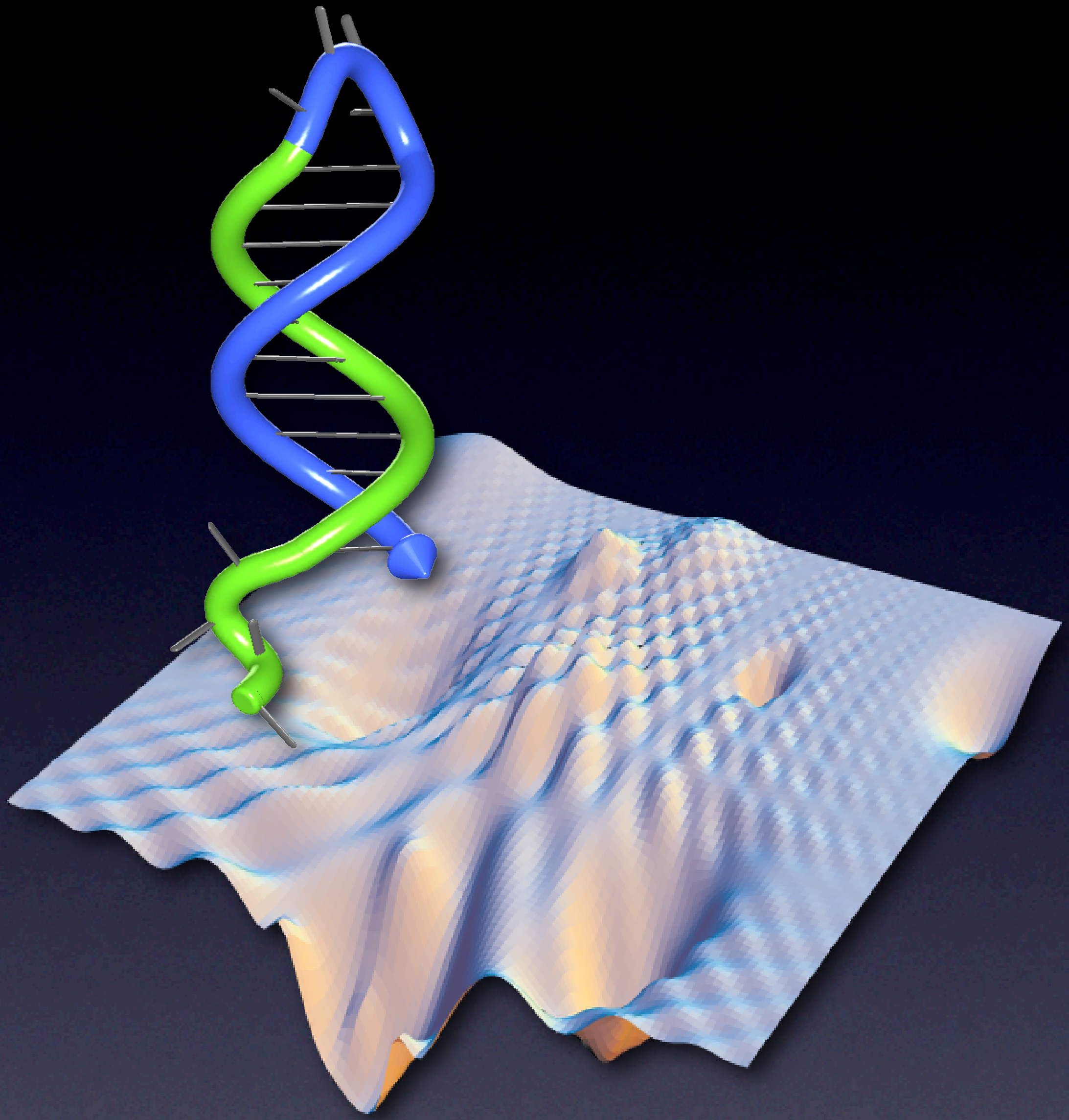

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