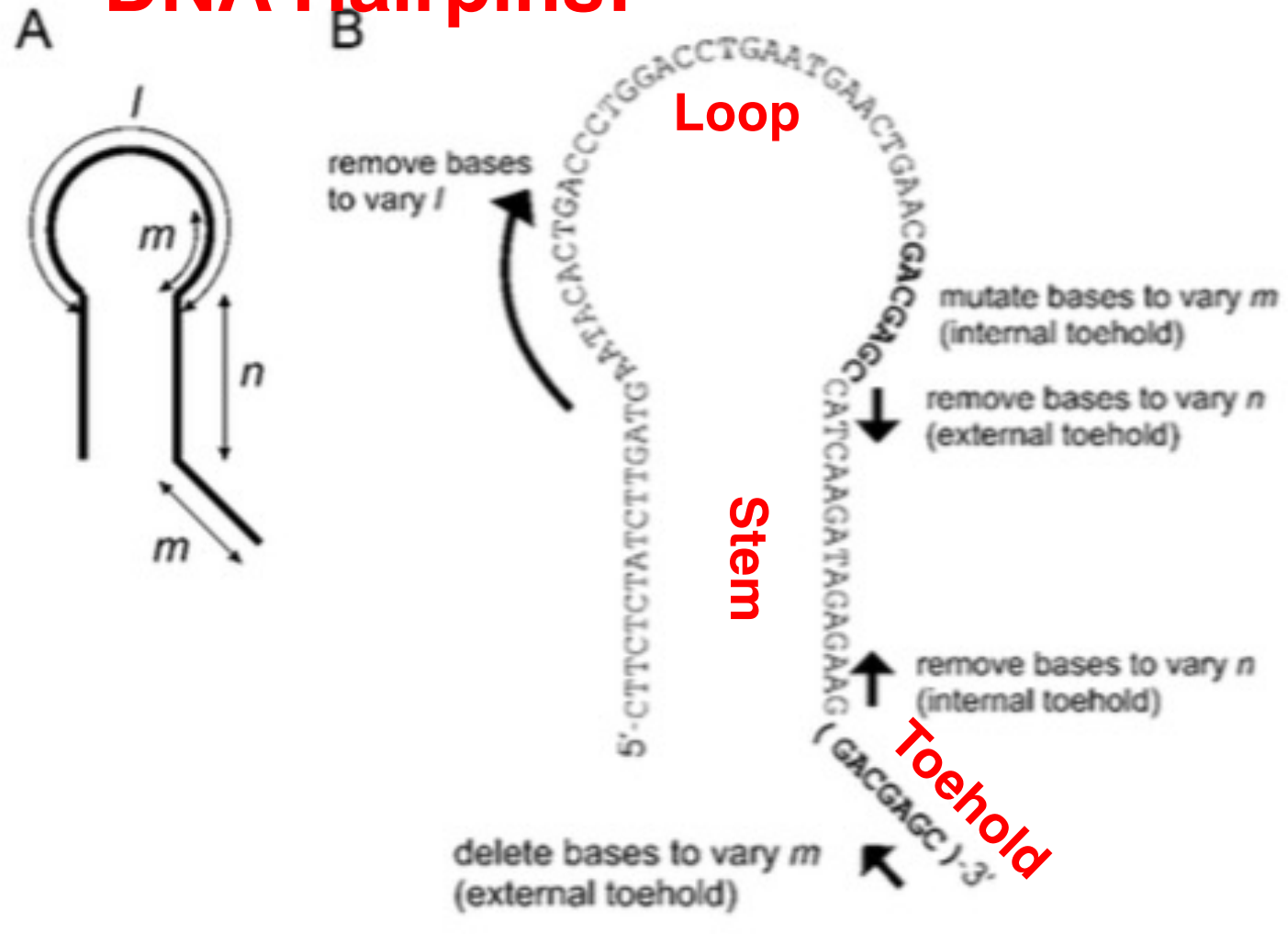
DNA Hybridization Reactions Using DNA Hairpins

John Reif

DNA Hairpins:



Hairpin description and design. (*A*) Definition of hairpin dimensions: loop domain length, *l*; neck length *n*; and toehold length, *m* (the toehold may be internal or external; see Fig. 1, *C* and *D*). (*B*) The sequence of hairpin *H*. The ways in which this sequence was adapted to generate hairpins with different values of *l*, *m*, and *n* are indicated.

Green, S. J., Lubrich, D. & Turberfield, A. J. DNA hairpins: fuel for autonomous DNA devices. Biophysical journal 91, 2966-2975, doi:10.1529/biophysj.106.084681 (2006).

Types of DNA Hybridization Reactions Using DNA Hairpins

This Lecture: Solution-based DNA Hybridization Reactions

Next 2 Lectures:

- DNA Hairpins Localized on a nanotrack or 2D DNA origami
- DNA Hairpins Localized on a Cell Surface

References:
Solution-based DNA Hybridization Reactions

- ***Dirks, R. M. & Pierce, N. A. Triggered amplification by hybridization chain reaction. Proceedings of the National Academy of Sciences of the United States of America 101, 15275-15278, doi:10.1073/pnas.0407024101 (2004).***

- ***Green, S. J., Lubrich, D. & Turberfield, A. J. DNA hairpins: fuel for autonomous DNA devices. Biophysical journal 91, 2966-2975, doi:10.1529/biophysj.106.084681 (2006).***

- ***Seelig, G., Yurke, B. & Winfree, E. Catalyzed relaxation of a metastable DNA fuel. Journal of the American Chemical Society 128, 12211-12220, doi:10.1021/ja0635635 (2006).***

Hairpin Chain Reaction (HCR)

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Triggered amplification by hybridization chain reaction (HCR)

Robert M. Dirks[†] and Niles A. Pierce[‡§]

Table 1. HCR systems

System	Strand	Sequence*
Basic	H1	5′-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGCGTG-3′
	H2	5′-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACTTTG-3′
	I	5′-AGTCTAGGATTCGGCGTGGGTTAA-3′
Aptamer[†]	H1	5′-CATCTCGGTTTGGCTTTCTTGTTACCCAGGTAACAAGAAAGCCAAACC-3′
	H2	5′-TAACAAGAAAGCCAAACCGAGATGGGTTTGGCTTTCTTGTTACCTGGG-3′
	I^{ATP}	5′-CCCAGGTAACAAGAAAGCCAAACCTCTTGTT*ACCTGGGGGAGTATTGCGGAGGAAGGT*-3′
	I	5′-CCCAGGTAACAAGAAAGCCAAACC-3′

*In the hairpin sequences, loops are underlined and sticky ends are overlined.
[†]Aptamer nucleotides (8) are italicized.

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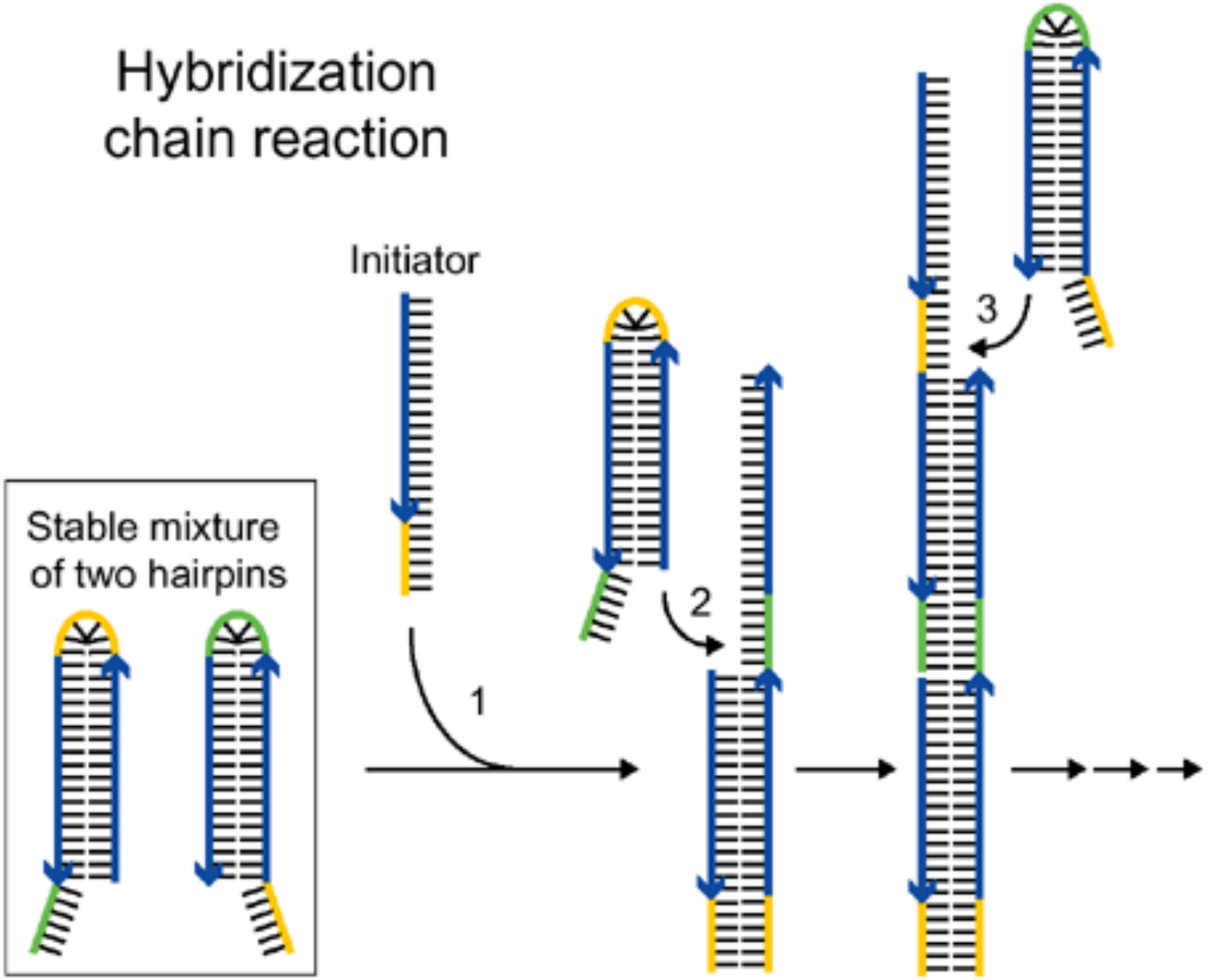
HCR Reaction in Solution:



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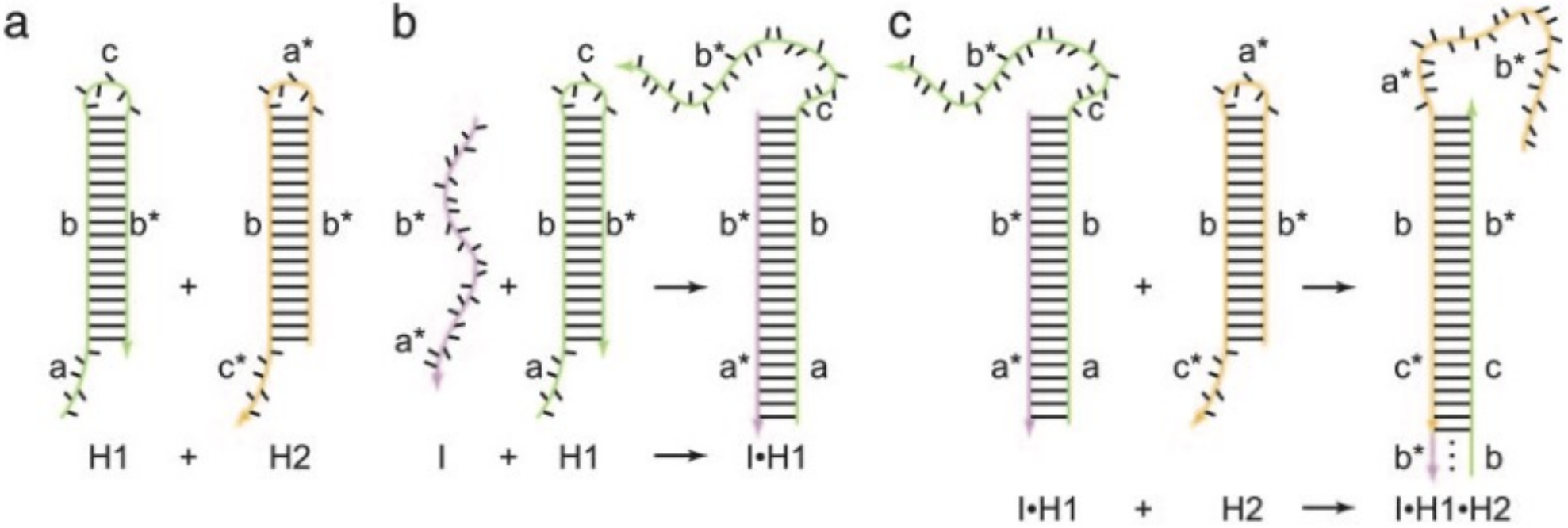

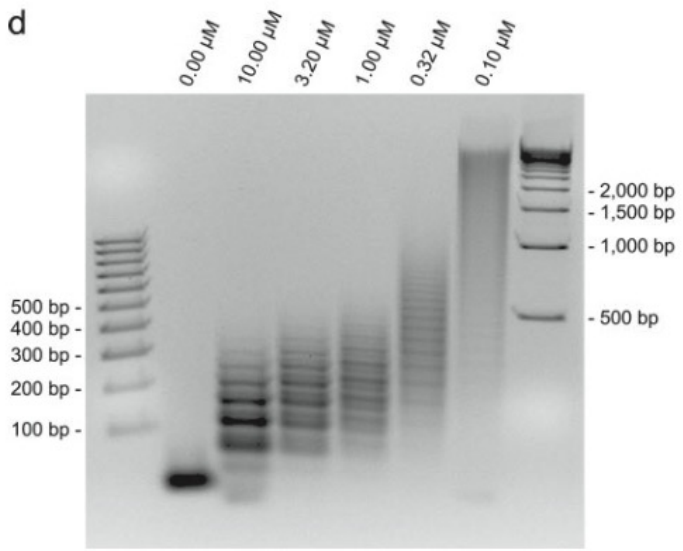

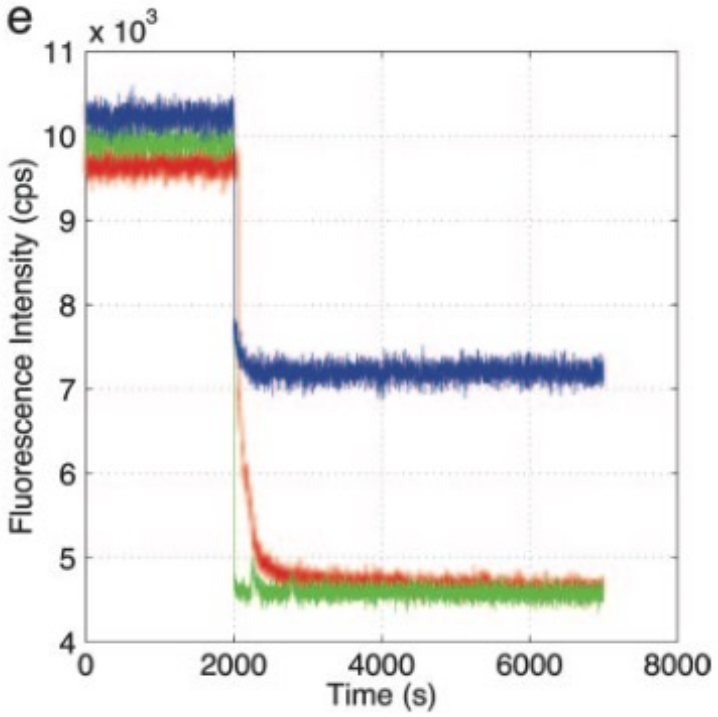

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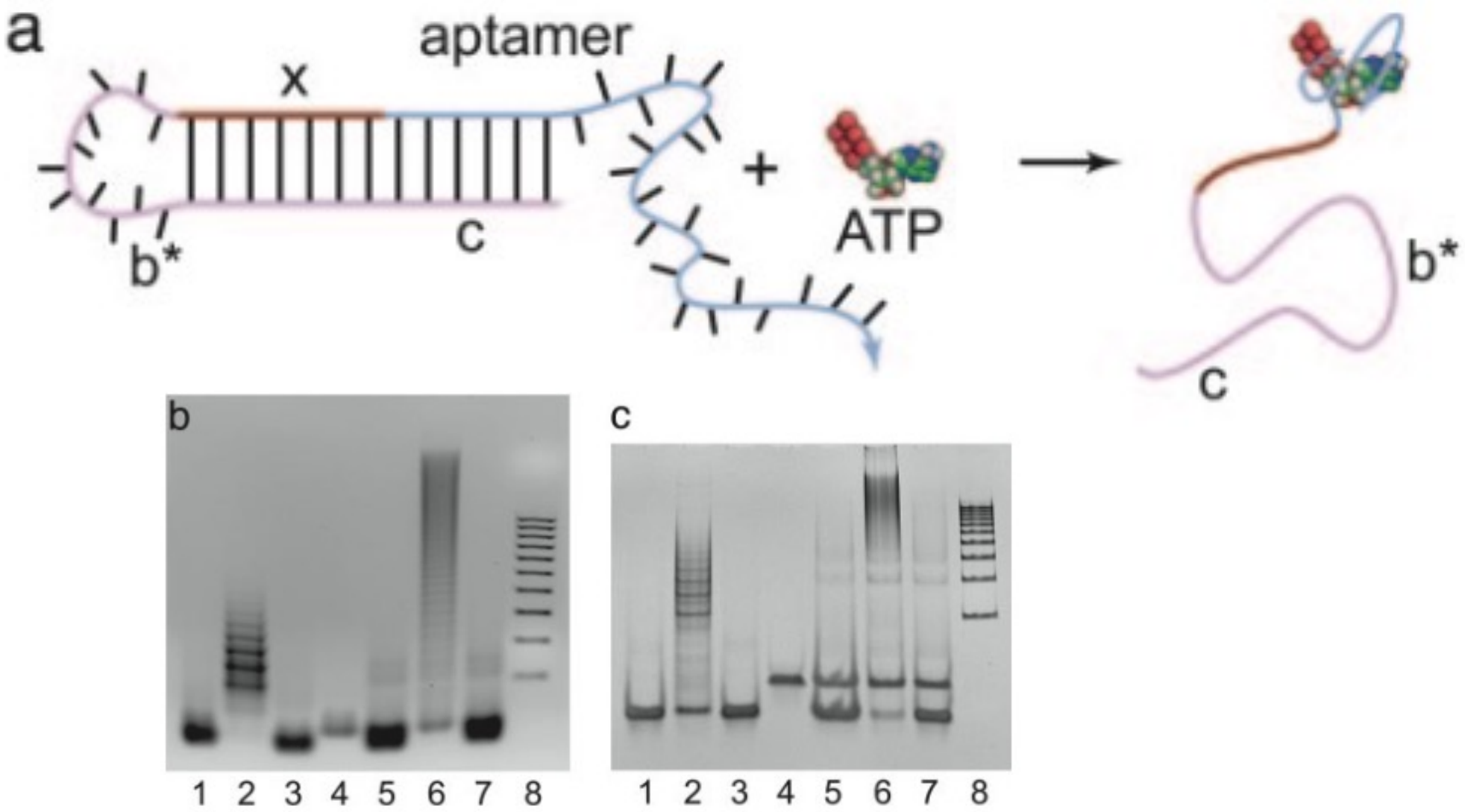
Using an Aptamer to Trigger HCR Reaction in Solution:



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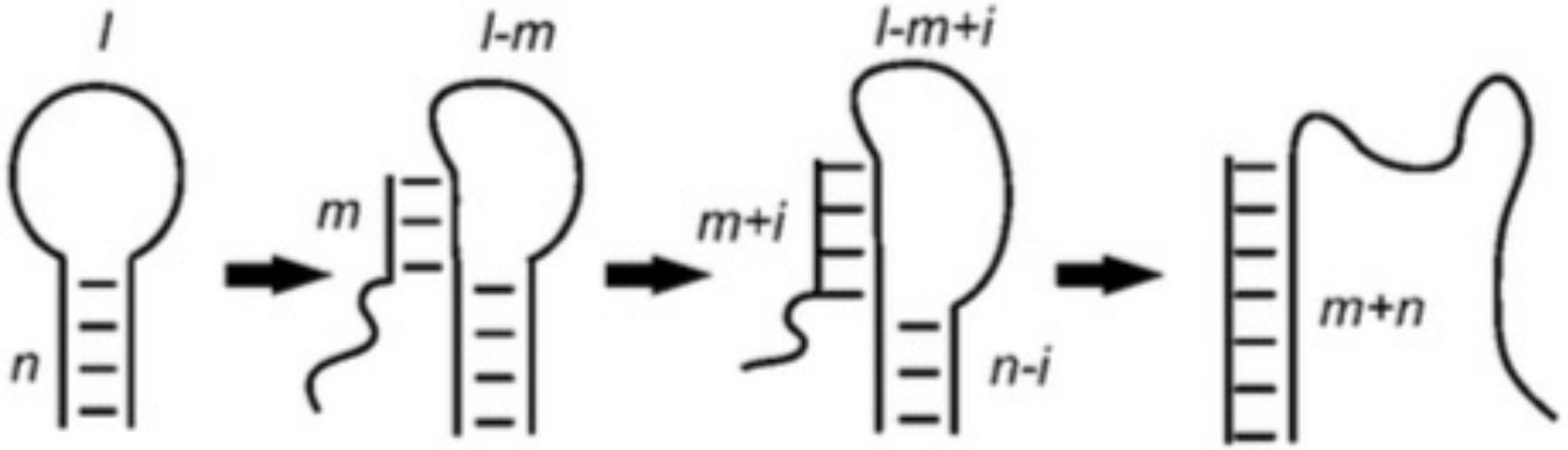
Reaction of a Catalytic Opening Strand with an Internal Toehold

Green, S. J., Lubrich, D. & Turberfield, A. J. DNA hairpins: fuel for autonomous DNA devices. Biophysical journal 91, 2966-2975, doi:10.1529/biophysj.106.084681 (2006).

Reaction of a Catalytic Opening Strand with an Internal Toehold



Reaction of a catalytic opening strand with an internal toehold of length m with a hairpin of loop length l and neck length n.

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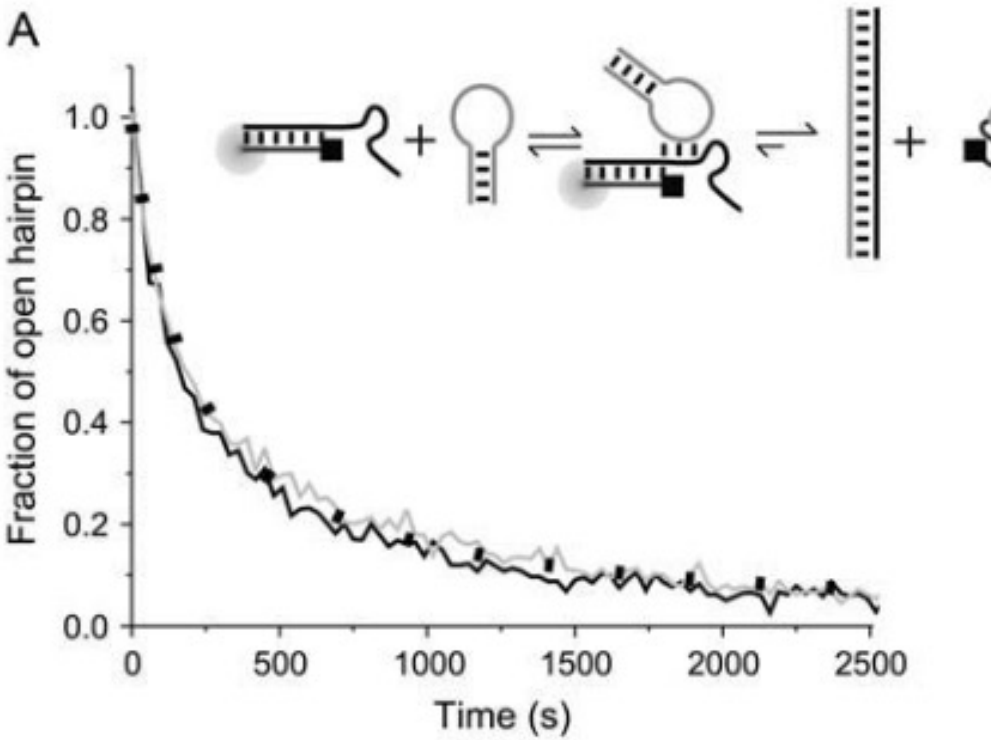
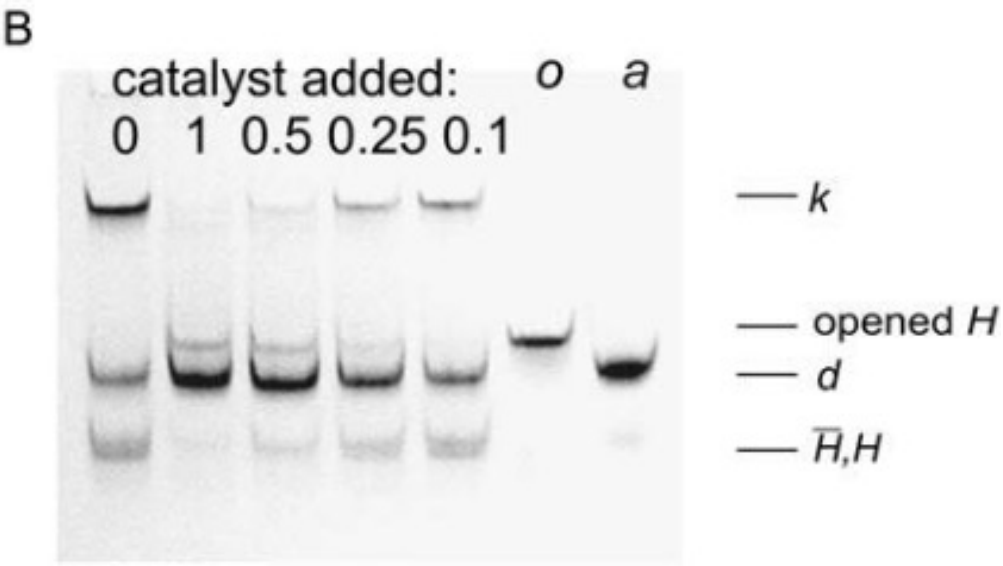
Catalysis of hairpin hybridization by an opening strand:





Displacement of an opening strand bound to hairpin H (l = 40, n = 16) by the complementary hairpin H.

- The schematic diagram shows the FRET labels on the opening strand used to observe the progress of the reaction.
- Black and gray lines show the time dependence of the reaction for opening strands with seven-base internal and external toeholds, respectively.

Catalysis of the reaction between complementary hairpins (l = 40, n = 16) by an opening strand with a seven-base external toehold.

Bands in the native polyacrylamide gel correspond to the reactants (individual hairpins H, H and kissed complex k), the duplex product d, and the intermediate formed by the opening strand bound to H.

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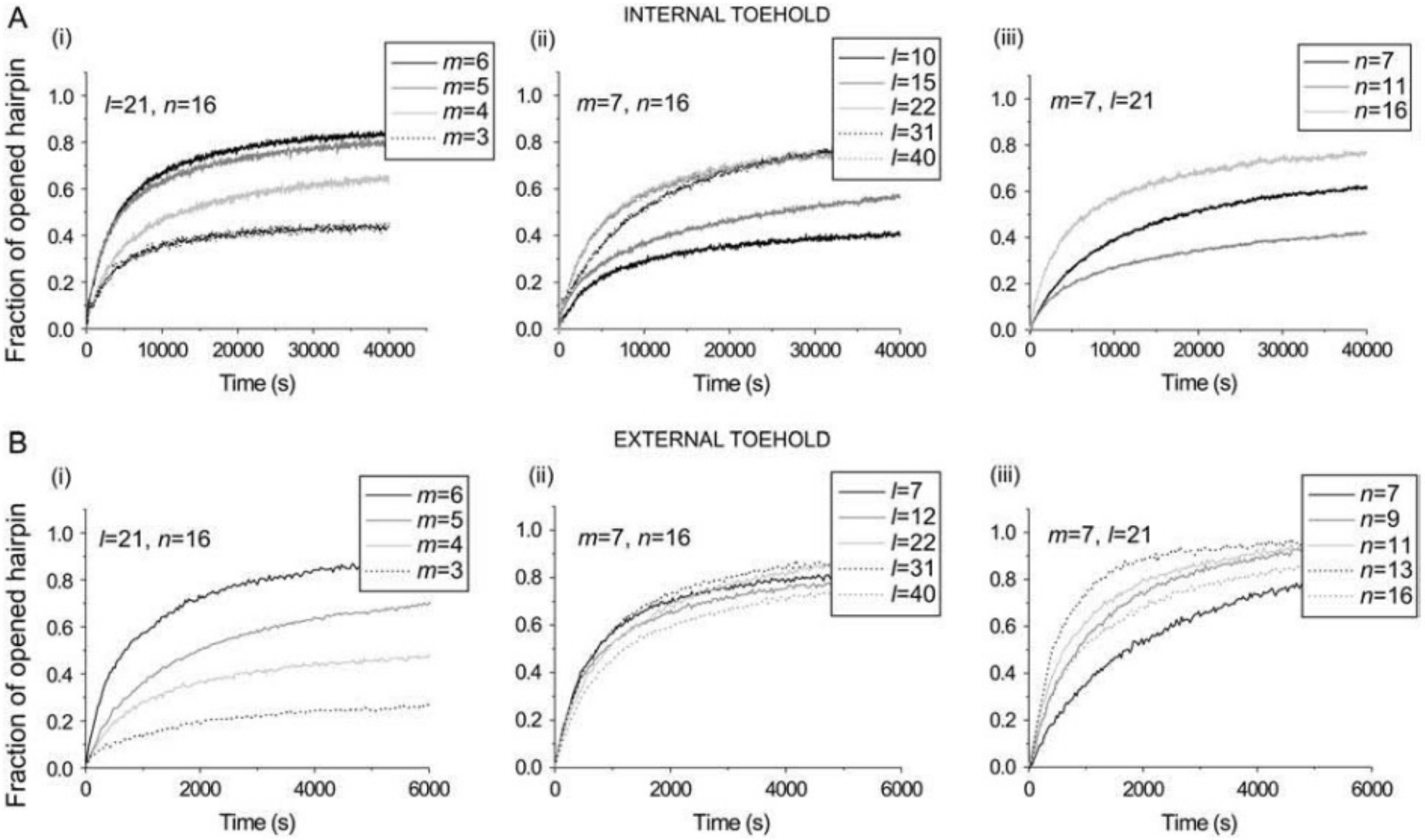
Opening Hairpins using Internal vs External Toeholds:



(i) Toehold length m varied

loop length, l = 21 and neck length, n = 16, were fixed.

(ii) Loop length l varied

m = 7 and n = 16 were fixed.

(iii) Neck length n varied

m = 7 and l = 21 were fixed.

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Reaction of a Catalytic Opening Strand with an Internal Toehold

Energetics of Hybridizations between Hairpins and Opening Strands

Free energy changes for hybridization between a hairpin ($l = 21$, $n = 16$) and opening strands with different toeholds

Toehold length, position	Fraction hybridized*	ΔG^0_{total} (kcal mol^{-1})†	$\Delta G^0_{toehold}$ (kcal mol^{-1})‡	$\Delta G^0_{loop} = \Delta G^0_{toehold} - \Delta G^0_{total}$ (kcal mol^{-1})
5, e	0.88	−13.9	−8.2	5.7
4, e	0.67	−12.4	−6.8	5.6
3, e	0.38	−11.3	−4.6	6.7
5, i	0.86	−13.5	−8.9	4.6
4, i	0.73	−12.7	−6.7	6.0
3, i	0.47	−11.7	−5.3	6.4

*The fraction hybridized is deduced from steady-state donor fluorescence from a dual-labeled opening strand.
†Corresponds to the measured equilibrium constant.
‡Calculated using nearest-neighbor model (11).

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Pairs of DNA Hairpins: Fuel for Autonomous DNA Devices

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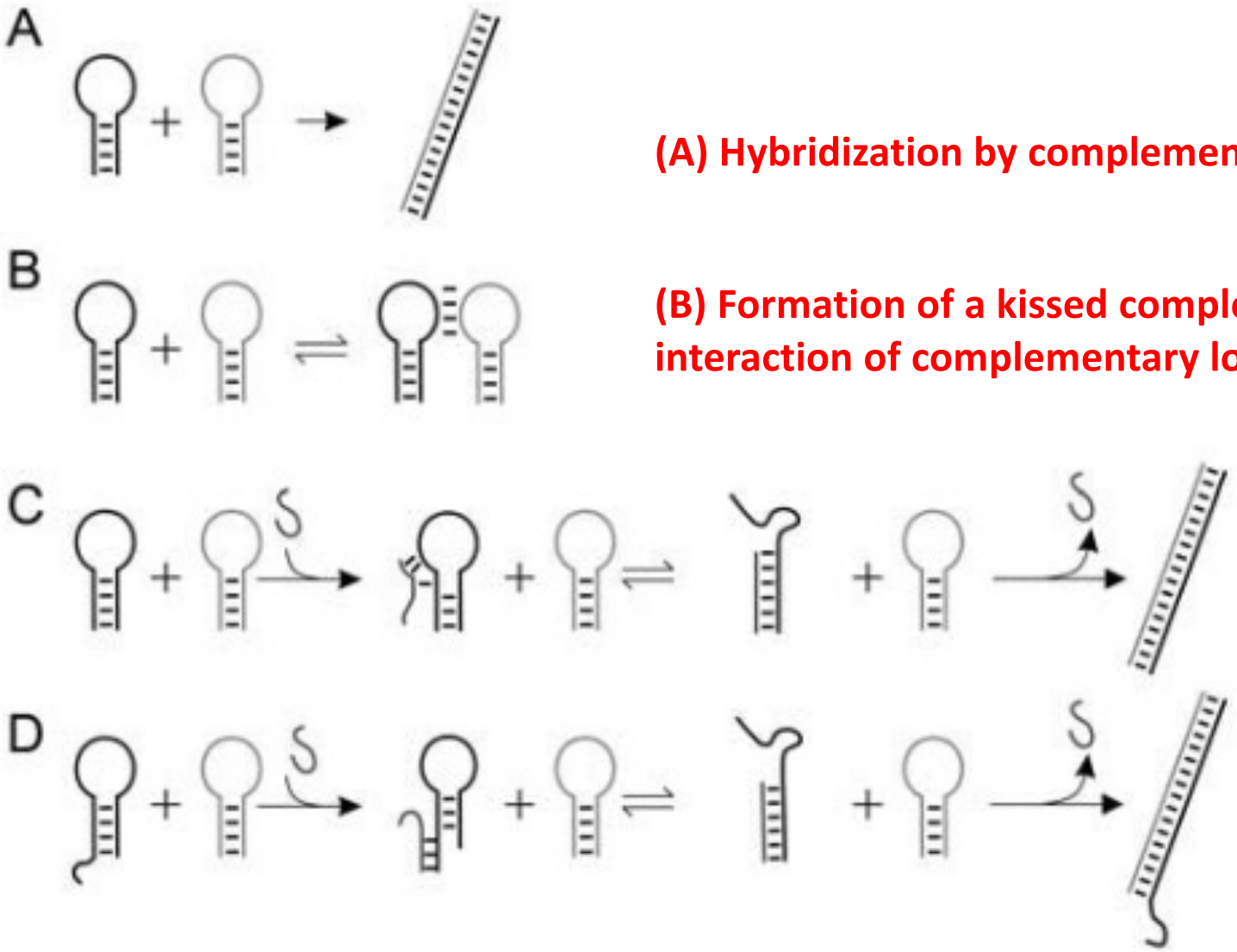

(A) Hybridization by complementary hairpins.

(B) Formation of a kissed complex by interaction of complementary loop domains.

Catalysis of hybridization by an opening strand capable of opening a hairpin by strand invasion initiated at an internal toehold (C) or an external toehold (D).

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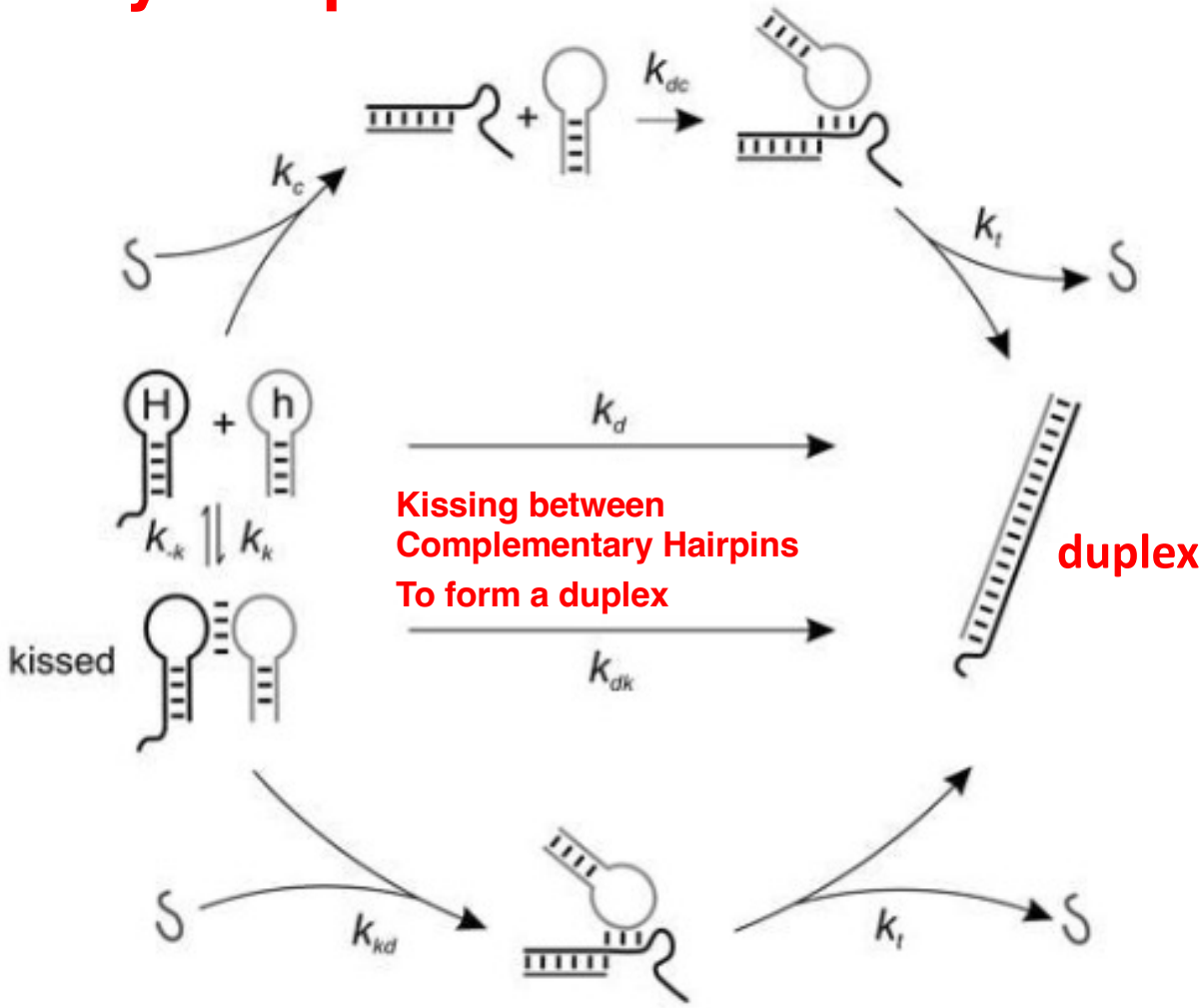
Using Complementary Hairpins:

Complementary Hairpins:

H and h are complementary (separately assembled) hairpins which can kiss



Interactions between complementary hairpins, including catalysis of hybridization by an opening strand. (*Middle*) Spontaneous interactions between complementary hairpins: kissing, and complete hybridization to produce a fully basepaired duplex. (*Upper*) Hybridization between monomeric hairpins catalyzed by an opening strand. (*Lower*) Hybridization between kissed hairpins catalyzed by an opening strand. The catalyst shown initiates strand displacement by binding at an external toehold.

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Using Complementary Hairpins:

Estimated Rate Constants:

Rate constants used to calculate reaction time course

Rate constant	Process	Value	Figure or assumption
k_k	Kissing (association)	3×10^4 M^{-1} s^{-1}	Fit to data shown in Fig. 4 *B ii*
k_{-k}	Kissing (dissociation)	4×10^{-4} s^{-1}	Fit to data shown in Fig. 4 *B ii*
k_{dk}	Duplex from kissed	0 s^{-1}	Measured to be slow*
k_d	Direct duplex formation	5×10^3 M^{-1} s^{-1}	Inferred from Fig. 4 *A ii*†
k_c	Opening of hairpin loop by Opening strand	10^5 M^{-1} s^{-1}	Fit to data shown in Fig. 5 *B iii* (Supplementary Material)
k_{dc}	Reaction of opened hairpin with complementary hairpin, displacing opening strand	5×10^4 M^{-1} s^{-1}	Fit to data shown in Fig. 6 *A*
k_{kd}	Opening of hairpin loop in kissed complex by opening strand	10^5 M^{-1} s^{-1}	Same as k_c‡
k_t	Resolution of three-strand complex (opening strand and hairpins) to form a duplex and free opening strand	(10 s^{-1})	Fast§

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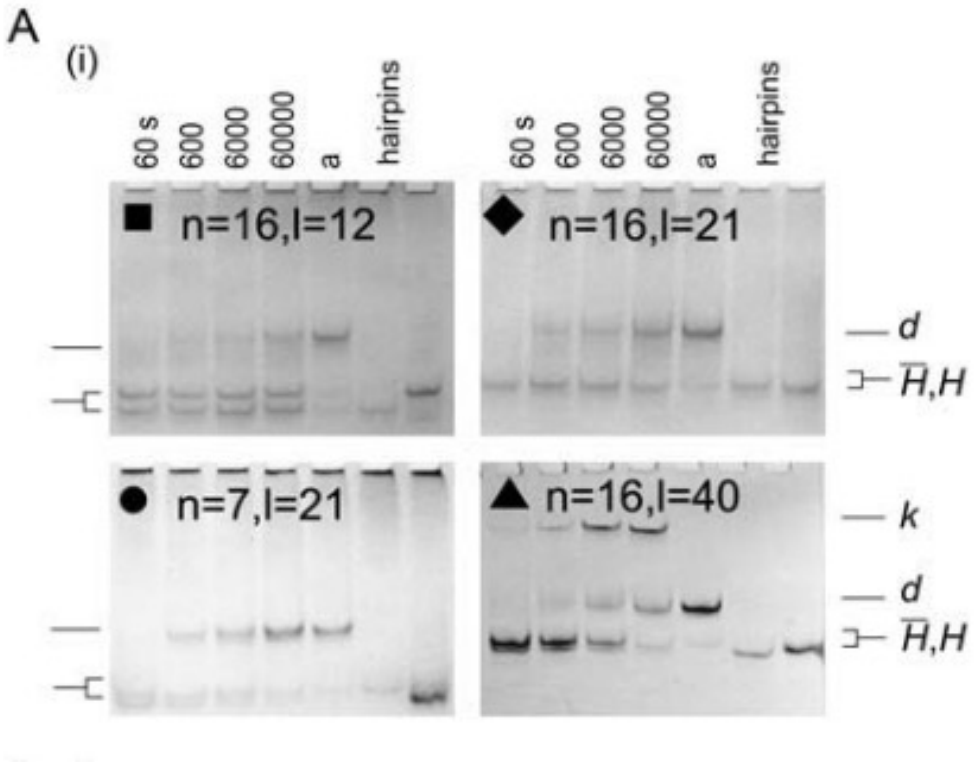
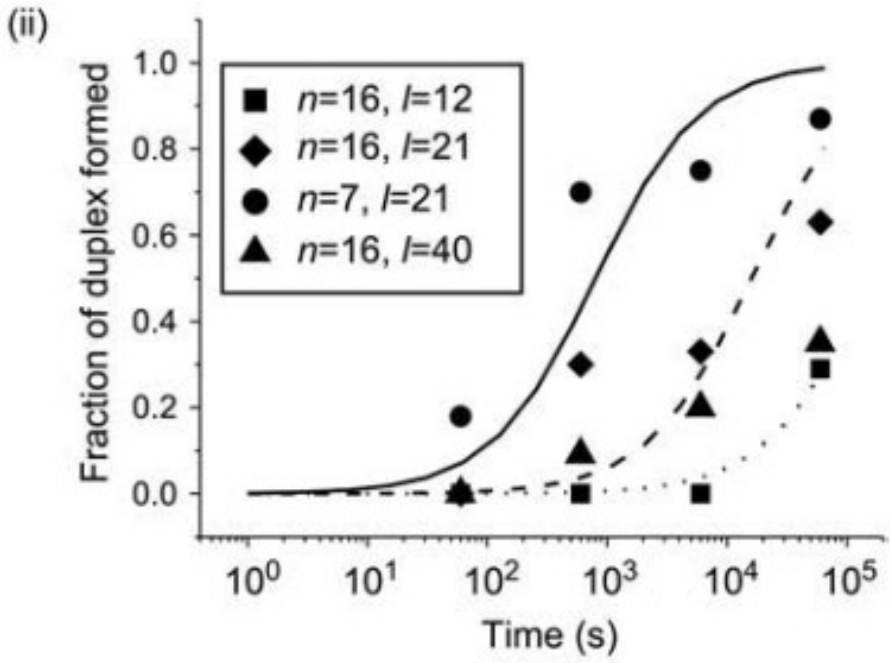
Spontaneous interaction of complementary hairpins: The kissing interaction.





Time-course PAGE analysis of the interactions between complementary pairs of hairpins with l-base loop domains and n-basepair necks.

- PAGE analysis of the strength of kissing interactions between hairpins with nonhomologous necks as a function of the degree of complementarity for experimental details.
- Using individual hairpins; a complementary hairpins annealed.
- Bands indicated are individual hairpins (H, H), duplex (d), and kissed complex (k).
- Duplex formation as a function of time deduced from gel band intensities. The solid lines are calculated according to second-order reaction kinetics with rate constants 50 M^{-1} s^{-1} (dotted line), 500 M^{-1} s^{-1} (dashed line), and 10,000 M^{-1} s^{-1} (solid line).
- Slower bands labeled k correspond to kissed complexes. The contents of lanes (hairpins K(x,y) and H) are indicated above. x and y are the dimensions of loop subdomains of K(x,y) that are complementary to H.

(B) Time Course of the kissing interaction.

Slower bands labeled k correspond to kissed complexes.
The contents of lanes (hairpins K(x,y) and H) are indicated above. x and y are the dimensions of loop subdomains of K(x,y) that are complementary to H
Regions complementary to hairpin H are indicated by a solid black line, noncomplementary regions by a dashed line.
Time dependence of the formation of a kissed complex, by hairpins with complementary 40-base loops and nonhomologous necks, derived from FRET data. The fitted line assumes reversible second-order reaction kinetics.
The inset shows the positions of the dye labels.

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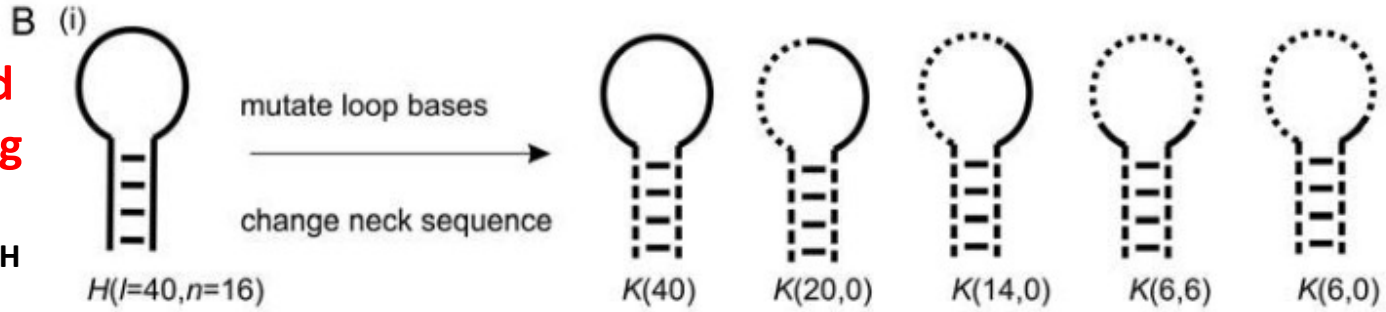
Mutated Designs of Complementary Hairpins: The Kissing Interaction between their 40-base loops.

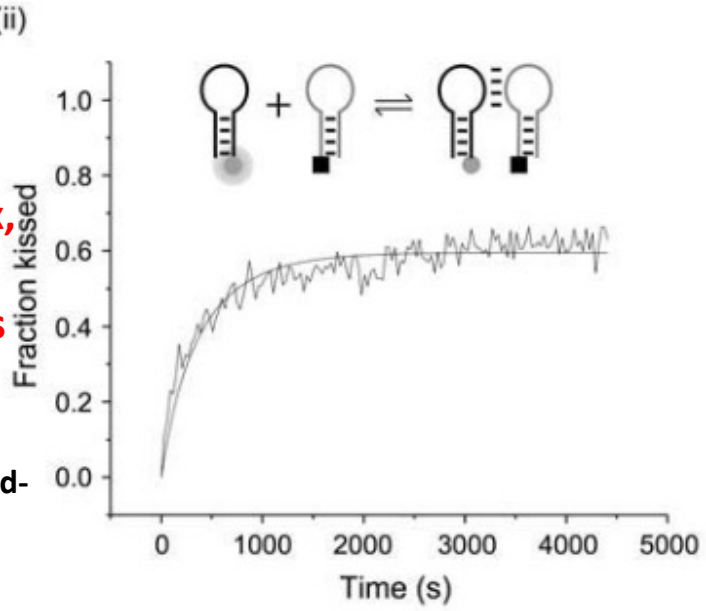
Designs of hairpins used to investigate the kissing interaction:

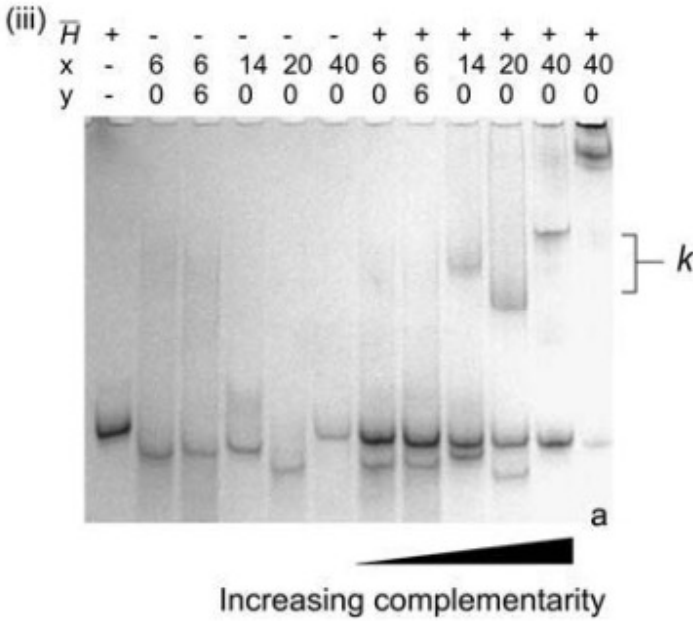
Regions complementary to hairpin H are indicated by a solid black line, noncomplementary regions by a dashed line.



(ii) Time dependence of the formation of a kissed complex, by hairpins with complementary 40-base loops and nonhomologous necks, derived from FRET data.

The fitted line assumes reversible second-order reaction kinetics. The inset shows the positions of the dye labels.





(iii) PAGE analysis of the strength of kissing interactions between hairpins with nonhomologous necks as a function of the degree of complementarity

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Autonomous DNA Walker using no enzymes

[Tuberfield2008] *S. J. Green, J. Bath, and A. J. Turberfield, Coordinated Chemomechanical Cycles: A Mechanism for Autonomous Molecular Motion, Physical Review Letters, 101, 238101 (2008).*

Two-part fuel: complementary (separately assembled) hairpins H1 and H2 (which can kiss)

Walker Operation:

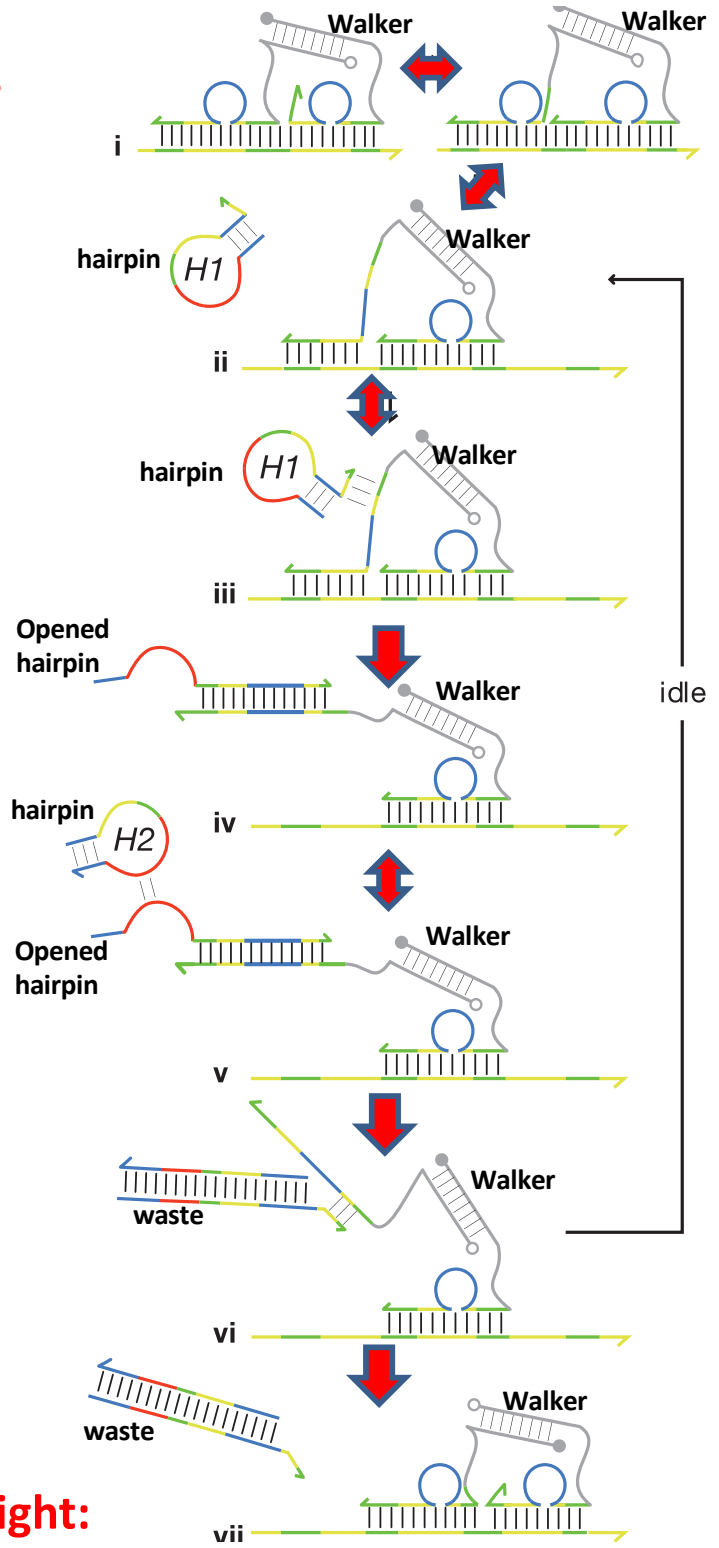
(i) Competition between feet for binding to the track can lift part of the left foot from the track, and

(ii) The lifting of the left foot reveals a toehold domain.

(iii) This can bind the complementary toehold domain of H1, initiating a strand- displacement reaction that opens the neck of H1 and displaces the left foot from the track (iv).

(v) Part of the opened loop H1 can act as a second toehold to initiate hybridization with H2 to form a stable waste product (the H1 H2 duplex),

(vi) displacing H1 from all but the initial toehold domain of the lifted foot and allowing the foot to rebind the track to the left or right with equal probability.



Walker moves left to right:

Catalyzed Relaxation of a Metastable DNA Fuel

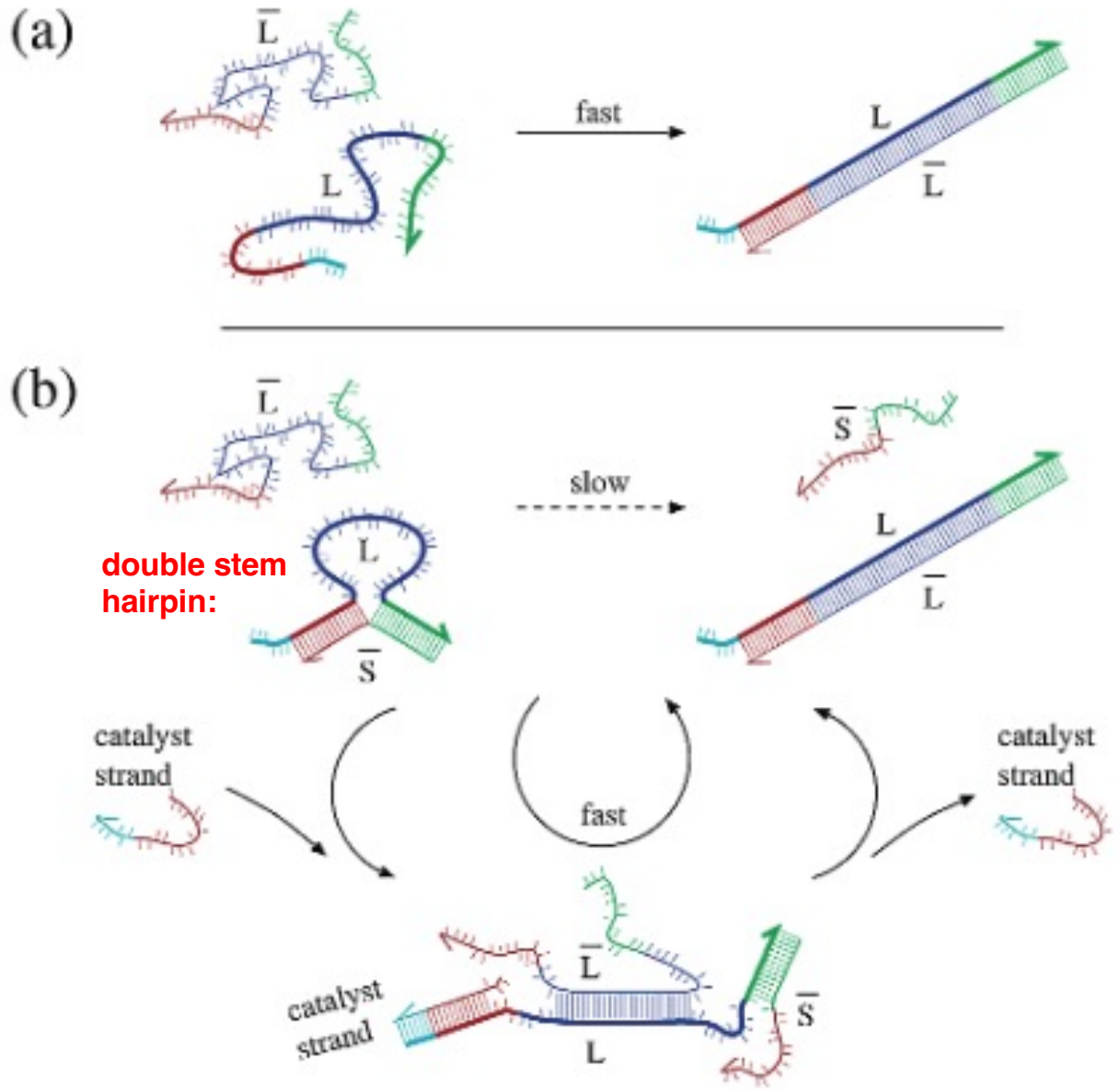
Seelig, G., Yurke, B. & Winfree, E. Catalyzed relaxation of a metastable DNA fuel. Journal of the American Chemical Society 128, 12211-12220, doi:10.1021/ja0635635 (2006).

Uses DNA fuel complexes each consisting of two kissing metastable hairpins with double stems

Catalyzed Relaxation of a Metastable DNA Fuel

Seelig, Yurke, Winfree

Reactions:



Hybridization reaction between two complementary strands L and its complement.

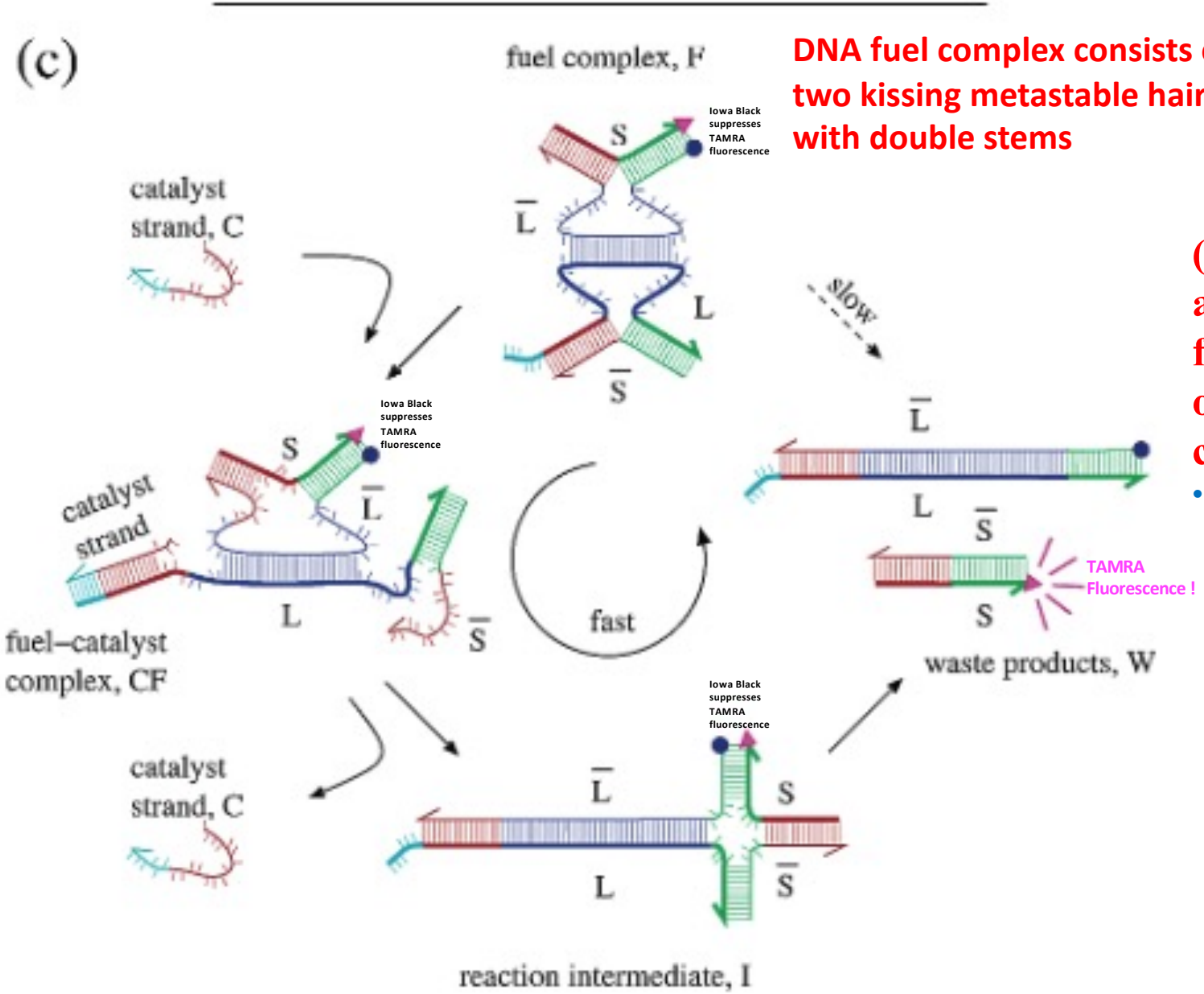
Kinetic control over a DNA hybridization reaction, as previously proposed.

- **The 6 nt toe-hold (cyan) greatly enhances the rate at which the catalyst strand binds and partially displaces *S*h by three-strand branch migration.**

Seelig, G., Yurke, B. & Winfree, E. Catalyzed relaxation of a metastable DNA fuel. Journal of the American Chemical Society 128, 12211-12220, doi:10.1021/ja0635635 (2006).

Thin and thick line segments of the same color are complementary to each other (red, 15 bp; blue, 40 bp; green, 15 bp; cyan, 6 bp).

Catalyzed Relaxation of a Metastable DNA Fuel, Cont



DNA fuel complex consists of two kissing metastable hairpins with double stems

(c) DNA nanostructures and reaction pathway for the catalyzed decay of a metastable fuel complex.

- Note that all base-paired domains must be double-helical, but by construction, the kissing loops of the fuel complex cannot be topologically linked. Fluorophore and quencher positions are indicated.

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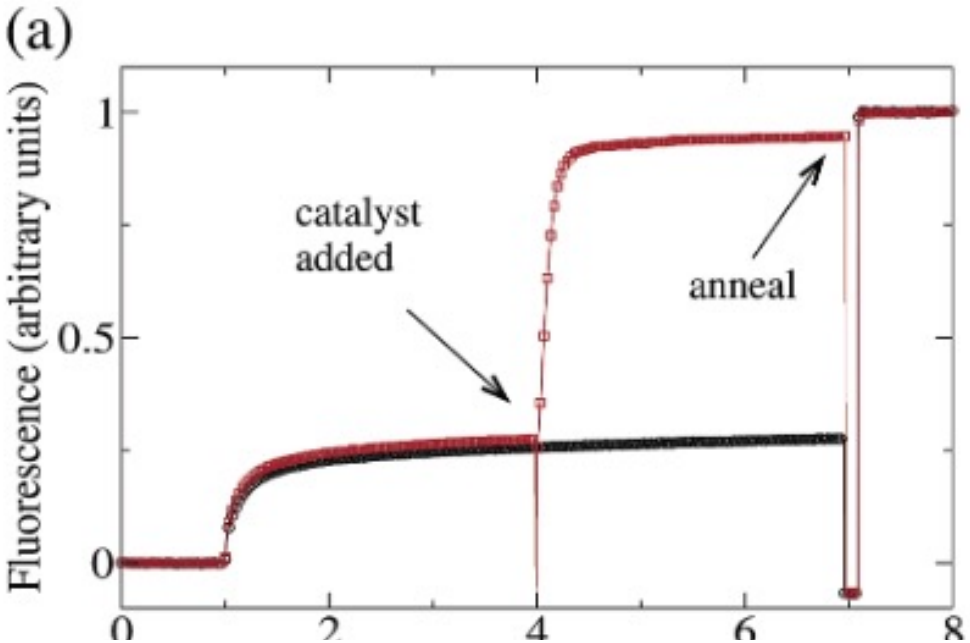
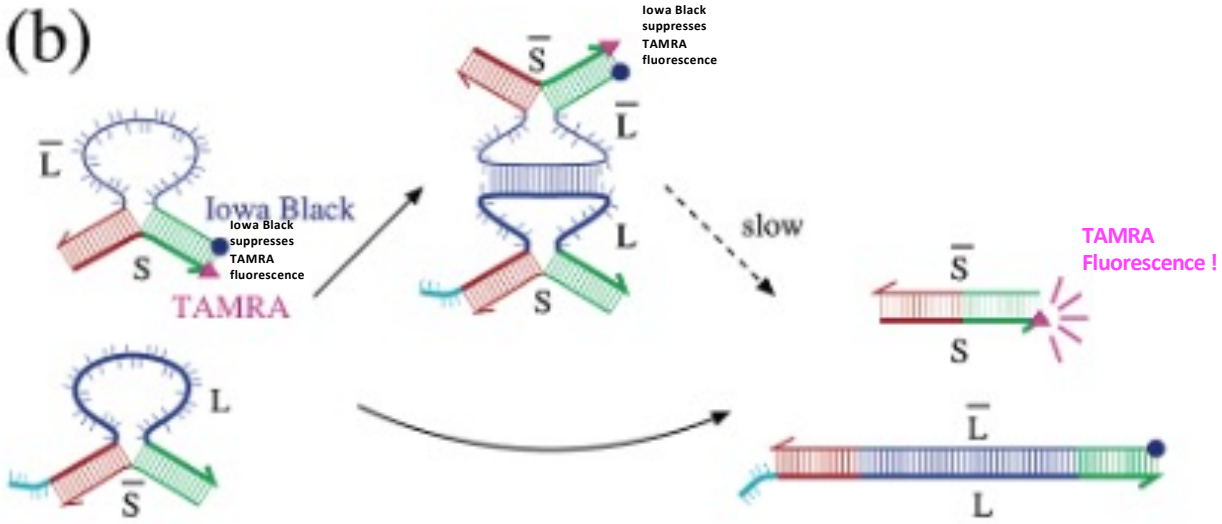
Catalyzed Relaxation of a Metastable DNA Fuel, Cont



Formation of the metastable"fuel"complex:

consists of two kissing metastable hairpins with double stems



Pathways for the loop-loop reaction.

The location of the fluorophore (TAMRA) and quencher (Iowa Black) are indicated.

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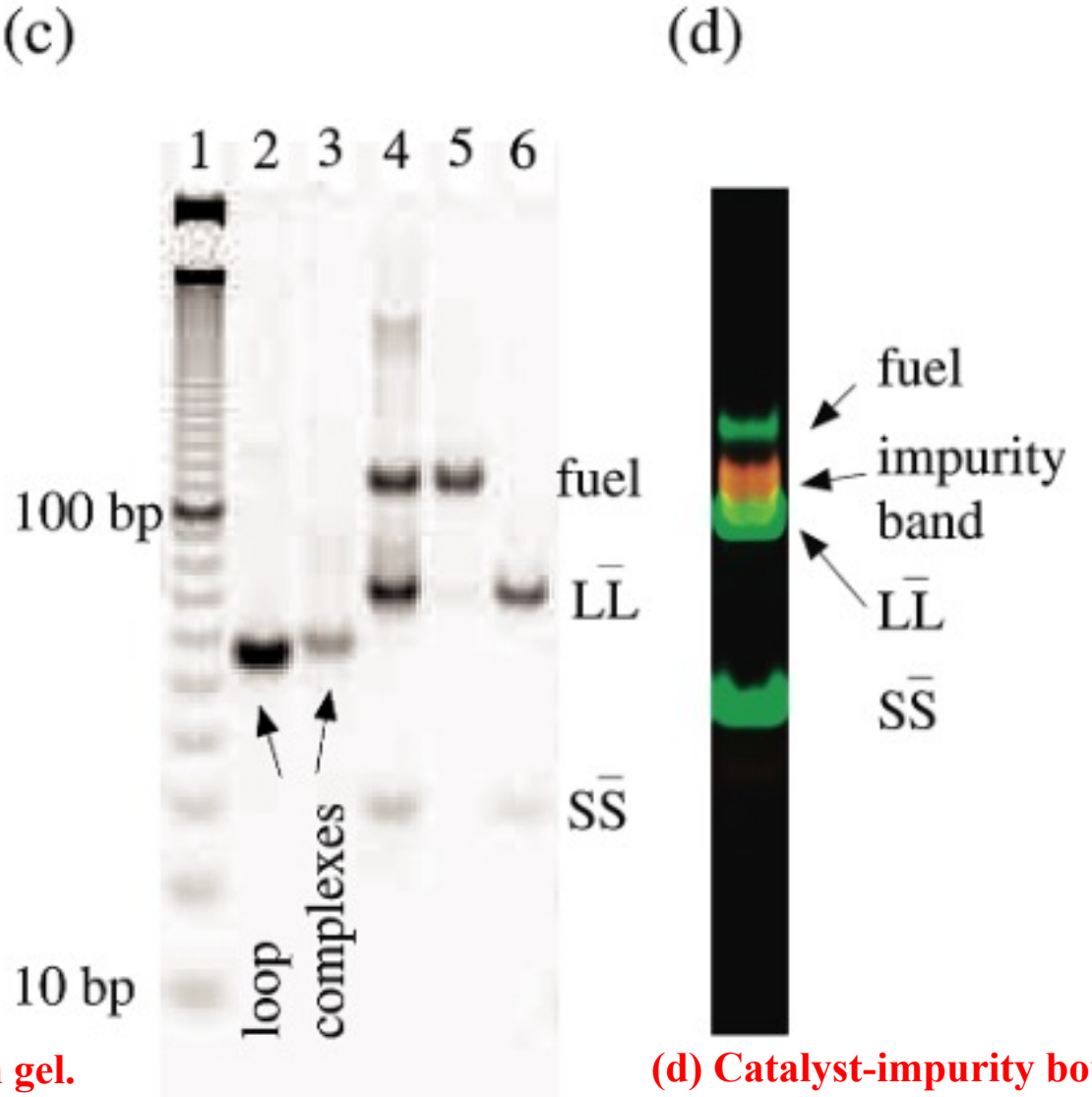
Catalyzed Relaxation of a Metastable DNA Fuel, Cont



(c) Formation gel.

Lane 1, ten bp ladder; lane 2, unlabeled loop; lane 3, dye/quencher-labeled loop; lane 4, mixture of two loops (after ~ 12 h); the three bands correspond to the fuel complex (lowest mobility), the long double-stranded waste product *LL*h (middle), and the short double- stranded waste product *SS*h; lane 5, purified fuel complex; and lane 6, purified fuel, annealed. Dye- and quencher-labeled molecules tend to be less visible in the gel. This effect is more pronounced for the smaller molecules *SS*h and *SL*h.

(d) Catalyst-impurity bound state.

Images taken at two different excitation/ emission wavelengths are overlaid. SybrGold-stained DNA appears green, while the Cy-5-labeled catalyst strand appears red. The catalyst is bound to an impurity state migrating slower than the long waste product *LL*h but faster than the fuel complex.

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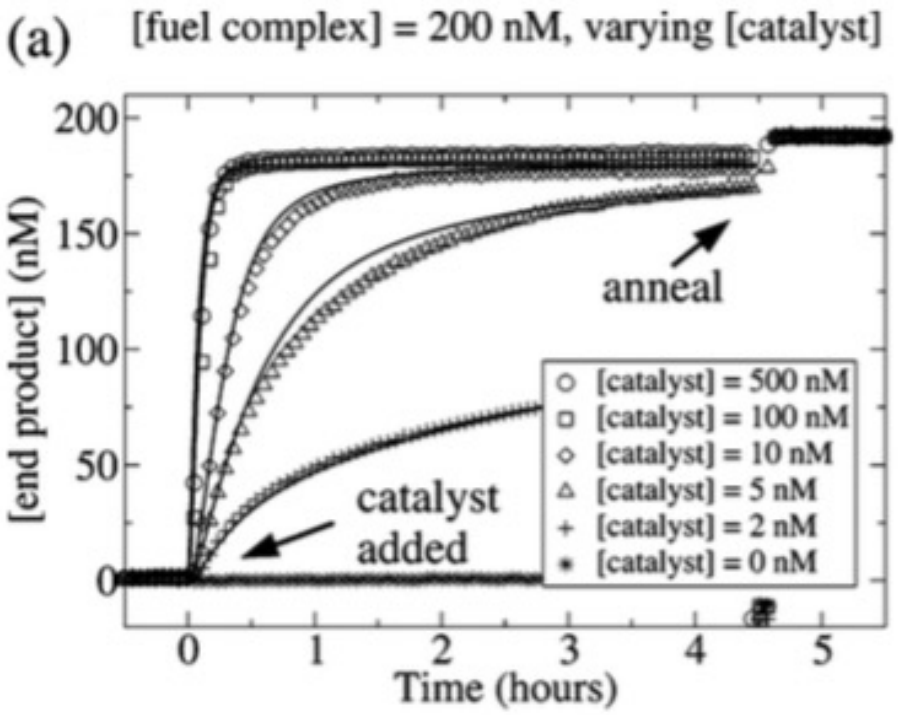
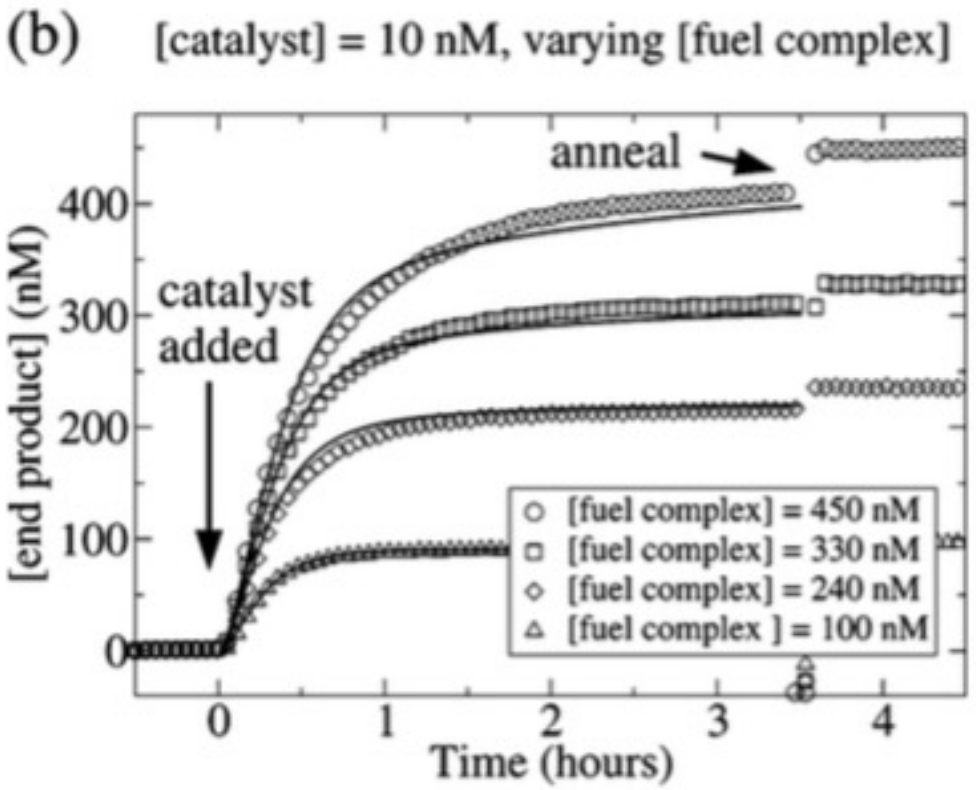
Catalyzed Relaxation of a Metastable DNA Fuel, Cont

Catalysis experiments with purified fuel complex.



Decay reactions for varying catalyst concentrations



Decay reactions for varying fuel complex concentrations

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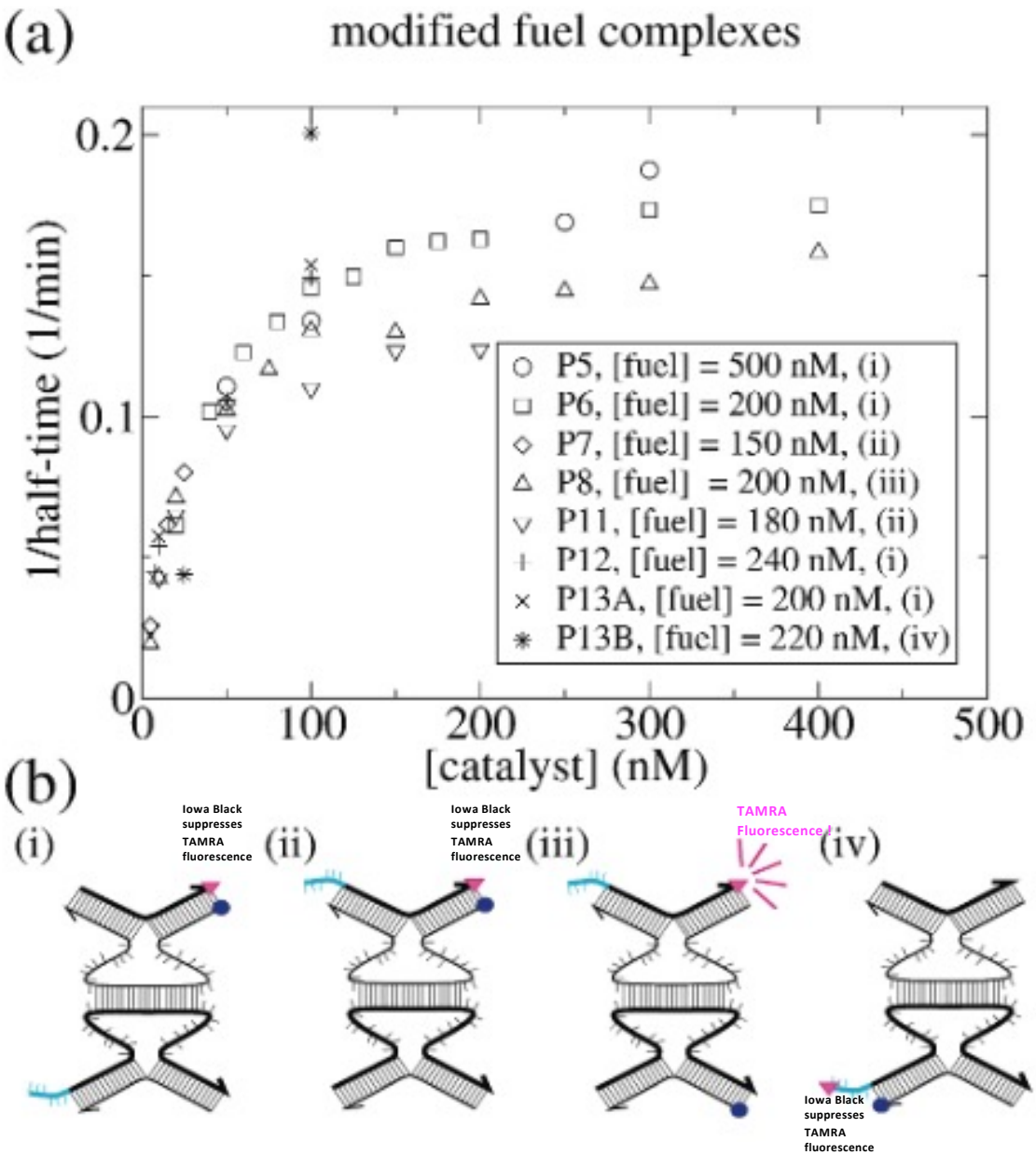
Catalysis for different purifications and modifications.

- Used two different toe-hold positions and three different locations for the dye and quencher in a total of four different complexes.
- As a model-independent measure for the speed of a reaction, we use the inverse of the reaction half-time, which can be interpreted as an overall rate constant.

(a) Inverse of the reaction half-time versus catalyst concentration for the fuel complex variants (i)-(iv) shown in (b).

(b) Toe-hold and dye/quencher positions used when collecting the data shown in (a) for the fuel complex variants (i)-(iv)

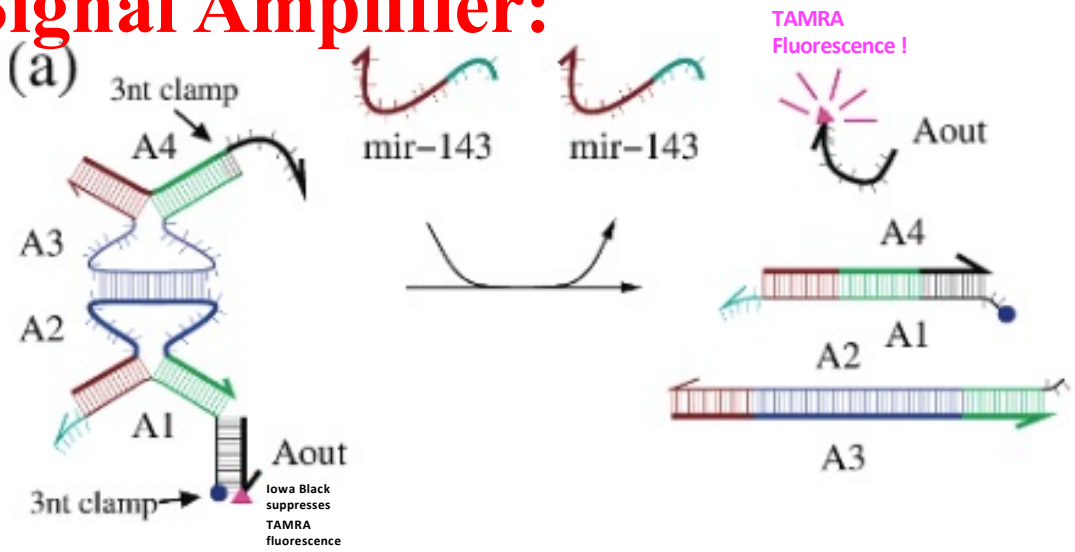


Seelig, G., Yurke, B. & Winfree, E. Catalyzed relaxation of a metastable DNA fuel. Journal of the American Chemical Society 128, 12211-12220, doi:10.1021/ja0635635 (2006).

Application to Catalytic Signal Amplifier:

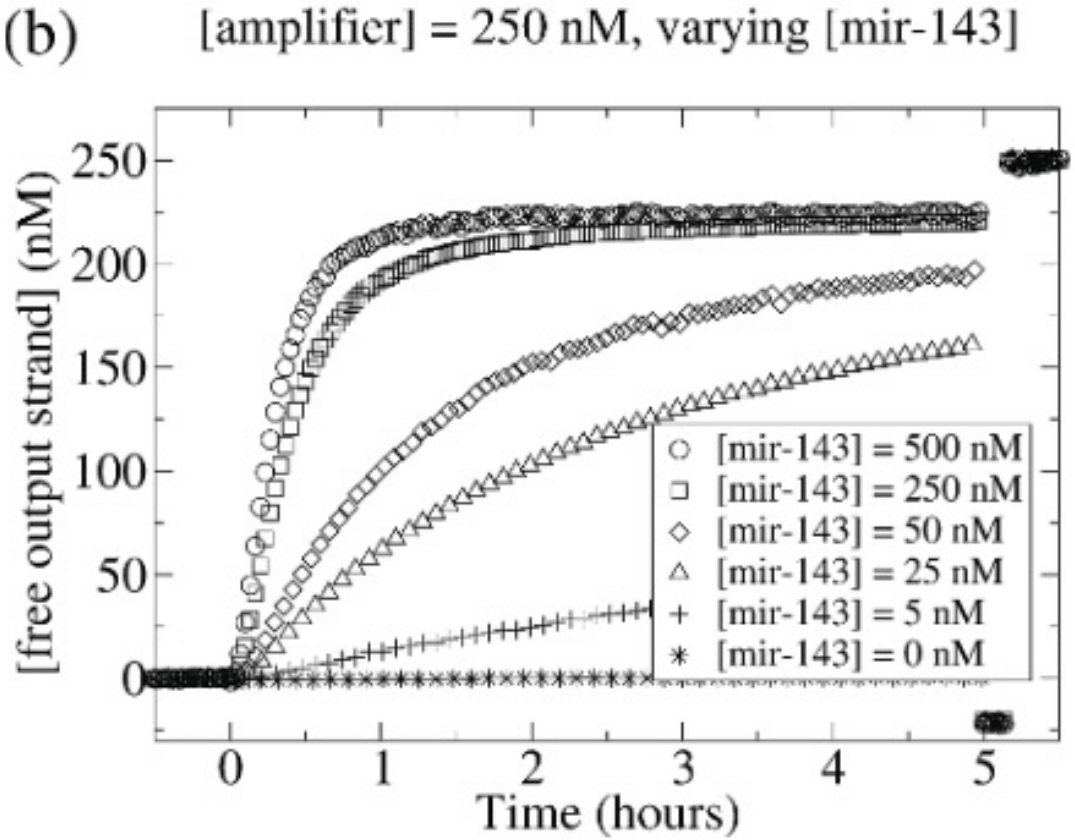
(a) Sketch of the catalytic signal amplifier

- Amplifier strands are labeled *A*out and *A*1-*A*4; the catalyst (dark red with dark cyan toe-hold) has the same sequence as the biological miRNA mir-143.
- The sequences in one arm of the loop (dark red) are adjusted accordingly.
- Blue and green colored regions are the same sequences as in the previous figures.
- The output strand and corresponding extensions of the loop strands are shown in black.
- Three bp clamps introduced for increased stability of the amplifier are indicated.



(b) Fluorescence data for varying concentrations of input strand mir-143.

- Fluorescence increases as the output strand is released.
- Dye and quencher positions are indicated in (a).



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