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Effects of mismatches in three-way strand displacement: destabilizing branch migration or introducing hairpin traps?

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DNA30

Motivation

- Kinetic control of toehold-mediated strand displacement is essential in autonomous molecular machinery and molecular computation.
- Introducing mismatched base pairs can strongly affect kinetic rates. However, the mechanisms of such effects are not fully understood.

Contributions

- In this work, we revisit three-way strand displacement reactions by Machinek et al. [1], using *Multistrand* [2] to simulate reaction trajectories and employing *ViDa* [3] to visualize their energy landscapes.
- Our computational analyses suggest that in three-way strand displacement reactions, mismatches can have a strong **hairpin-mediated** influence on kinetic rates.

Experiments

Kinetic hypotheses by Machinek et al. [1]

- The mismatch near the toehold destabilizes the system once the toehold is bound, forcing the system to spend considerably more time in the toehold-only state.
- Higher probability for spontaneous detachment of the invader and thus a reduced overall reaction rate.

Simulated sequence designs

- Machinek-Perfect: no mismatch in the invader [1]
- Machinek-Proximal: a mismatch (C) close to the toehold [1]
- Proximal-C2T: mismatch T instead of C

Multistrand simulation settings

- Simulation mode: trajectory mode
- Reactant concentration: 100 μM | Temperature: 25 ℃
- Stop condition: the incumbent dissociates from the substrate
- Maximum simulation time: 2000 s

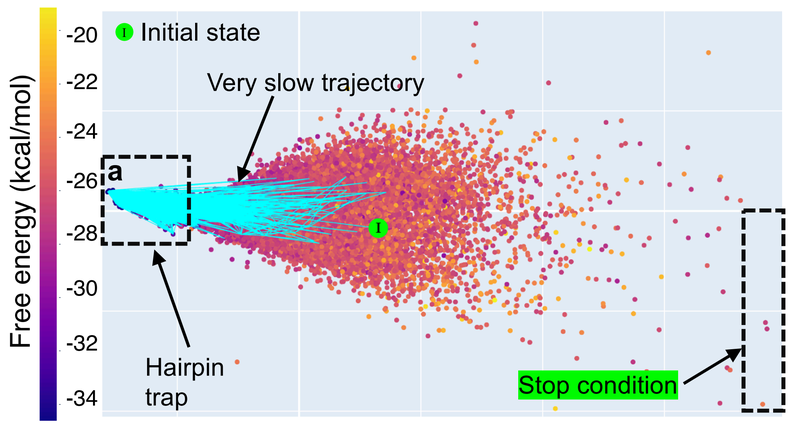
ViDa visualization tool [3]

- Creates low-dimensional embeddings of secondary structure state spaces using the output of kinetics simulators, e.g., *Multistrand* [2].

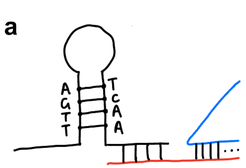
References

[1] Machinek, R.R., *et al.*, Nat. Commun., 5 (1): 5324, 2014.
[2] Schaeffer, J.M., *et al.*, DNA 21, pp. 194-211, 2015.
[3] Zhang C., *et al.,* MLCB, pp. 148-162, PMLR, 2024.

Reaction: Machinek-Proximal



Reaction	Median simulated time (s)	Experimental rate ($M^{-1} s^{-1}$)
Machinek-Perfect [1]	0.14	1.45×10^{6}
Machinek-Proximal [1]	1631.23	1.51×10^{3}
Proximal-C2T	83.96	-



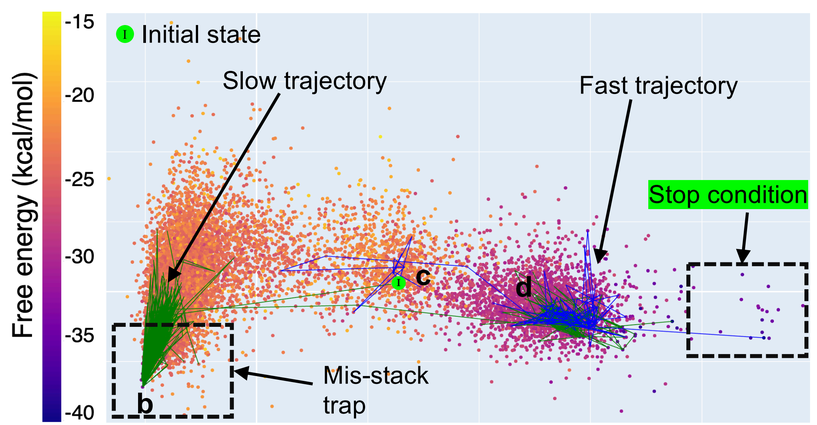
Machinek-Proximal
"*Bad*" hairpins (**a**):
- Impede the branch migration process
- Significantly slow down the overall reaction

Proximal-C2T
- No stable hairpin formation
- Mismatch destabilizes branch migration
- Much faster reaction than Machinek-Proximal

	Invader ━	Substrate ━	Incumbent ━	ΔG (kcal mol^{-1})
	GGTGAGTTTGAGGTTCAATGTGGA	CCCTCCACATTCAACCTCAAACTCACC	TGGTGTTTGTGGGTGTGGTGAGTTTGAGGTTGA	
a	.......((((...)))).(((((	...)))))...((((((((((((((((	..............)))))))))))))))).	-34.623

Reaction: Machinek-Perfect

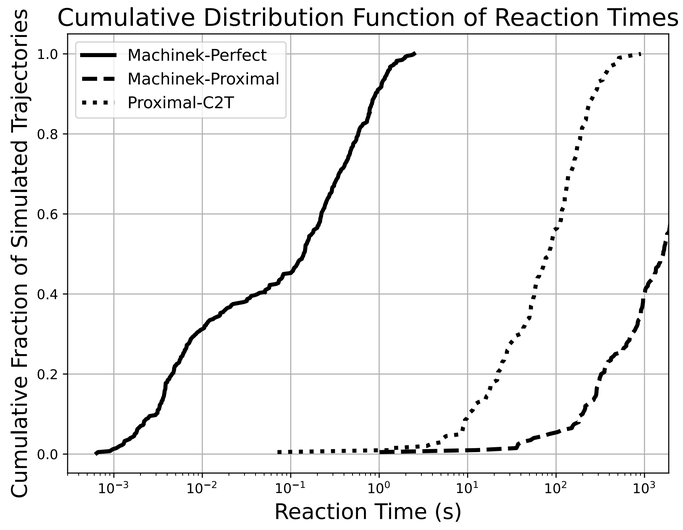
*ATGTGGA & TCCACAT: toeholds **C: mismatch

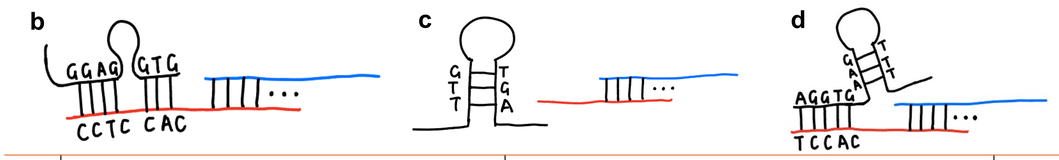


Machinek-Perfect
Two reaction modes:

(1) Slow pathway:
Mis-stack formation
(2) Fast pathway:
No mis-stack formation

"*Good*" hairpins (**c** & **d**):
- Provide "escape route" (**c**)
- Prevent mis-stack formation (**d**)





	Invader ━	Substrate ━	Incumbent ━	ΔG (kcal mol^{-1})
	GGTGAGTTTGAGGTTGAATGTGGA	CCCTCCACATTCAACCTCAAACTCACC	TGGTGTTTGTGGGTGTGGTGAGTTTGAGGTTGA	
b	.(((.....((((..........	.)))))))..((((((((((((((((	..............))))))))))))))))	-32.518
c	.......(((....))).....	..........((((((((((((((((	..............))))))))))))))))	-24.752
d	......(((......))).(((((	...)))))..((((((((((((((((	..............))))))))))))))))	-30.499