Inferring Parameters for an Elementary Step Model of DNA Structure Kinetics with Locally Context-Dependent Arrhenius Rates
Appendix

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A Local Context

Here we describe how to calculate the local context of a transition, i.e., formation or breakage of a base pair. We use 0-based numbering for numbering bases. That is, in a multi-strand complex, for each strand of length l, the first nucleotide at the 5' end of the strand is numbered 0 and the last nucleotide at the 3' end of the strand is numbered $l-1$.

The local context of a base pair forming or breaking is a pair (l, r), where l and r are the half-contexts on the left and right sides of the base pair forming or breaking, respectively. Each half context is one of seven possibilities: stack, loop, end, stack+loop, stack+end, loop+end, stack+stack. Right half contexts are illustrated in Algorithm 1. Algorithm 1 finds the half contexts, and Algorithm 2 uses Algorithm 1 to find the local context. These algorithms use *dot-parens-plus-mult* notation to represent a secondary structure (state), which is obtained from the *dot-parens-plus* notation. The dot-parens-plus notation uses the symbols '(', ')', '.', '+', and ' '. Matching parentheses represent bases which have formed a base pair, a dot represents a free base pair, and a plus represents a break between strands. For example, '((((((+))))))' means that bases 0, 1, 2, 3, 4, and 5 of the first strand are paired with bases 5, 4, 3, 2, 1, and 0 of the second strand, respectively. When all base pairs between strands break, we replace the plus sign by a space. For example, '..... .....' means that no base pair is formed. The dot-parens-plus-mult notation, inserts '*' at the start and end of a dot-parent-plus notation and before and after all '+' signs and spaces in the dot-parent-plus notation. Thus, '((((((+))))))' and '..... .....' change to '*((((((*+*))))))*' and '*.....* *.....*', respectively. If two states s_1 and s_2 differ by a single base pair, their dot-parens-plus-mult notation differs at exactly two positions, say e_1 and e_2. The left half context is determined by positions $l_1 = e_1 - 1$ and $l_2 = e_2 + 1$, while the right half context is determined by positions $r_1 = e_1 + 1$ and $r_2 = e_2 - 1$.

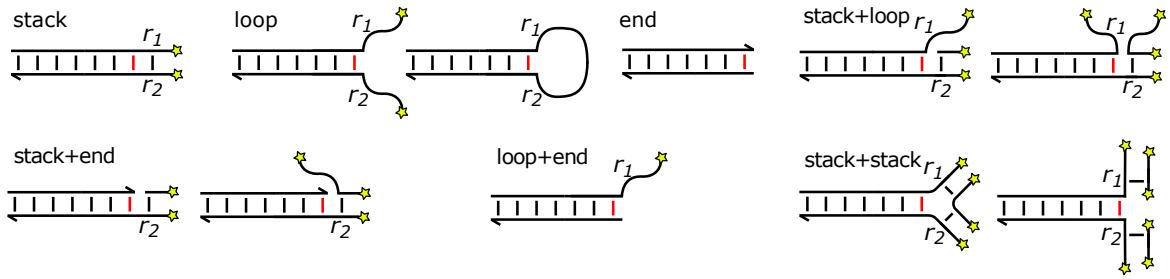
B Interacting DNA Strands State Space

To generate our reduced state space for a reaction, we use Algorithm 3 in combination with a reaction-specific set of initial states $\mathcal{S}_{\text{init}}$ and final states $\mathcal{S}_{\text{final}}$ and a reaction-specific function that returns `NeighborStates`(s), the neighboring states for a state s. Algorithm 3 uses a breadth-first search approach: initially, the queue Q and candidate state space $\mathcal{S}_{\text{init}}$ are composed of just the initial states. For every state in the queue, unexplored successor states are added to the candidate state space and then queued for exploration. In this work, we use only one initial state and one final state per reaction.

Next we explain how we calculate the neighboring states of a state s in our reduced state space, for each of the five different reaction types that arise in our dataset. As noted in our paper, for a given reaction, a structure is in in our reduced state space if and only if its bases occur in either the initial or final state of the reaction. Moreover, base pairs may only form or break at the edge of a hybridized domain. For toehold-mediated 3-way strand displacement and toehold-mediated 4-way strand exchange, to efficiently obtain mean first passage times with sparse matrix computations, we further heuristically prune the state space of each reaction.

Algorithm 1: Find the half context on one side of a base pair forming or breaking

The seven possible right half contexts are illustrated in the graphic below.



Function `HalfContext`(d, f_1, f_2)

Input: d is a dot-parens-plus-mult notation, f_1 and f_2 are either the indices l_1, l_2 that determine the left half context or the indices r_1, r_2 that determine the right half context.

Output: The half context appearing in positions f_1 and f_2.

$c_1 \leftarrow d[f_1]$
$c_2 \leftarrow d[f_2]$
if $c_1 =$ '(' and $c_2 =$ ')' **then**
  counter $\leftarrow 0$
  for k in $[f_1, f_2]$ **do**
    if $d[k] =$ '(' **then** counter $\leftarrow$ counter + 1
    else if $d[k] =$ ')' **then** counter $\leftarrow$ counter - 1
    if counter $= 0$ **then**
      if $k = f_2$ **then** return stack
      else return stack+stack
else if ($c_1 =$ '(' and $c_2 =$ '(') or ($c_1 =$ ')' and $c_2 =$ ')') or ($c_1 =$ ')' and $c_2 =$ '(') **then** return stack+stack
else if ($c_1 =$ '(' and $c_2 =$ '.') or ($c_1 =$ ')' and $c_2 =$ '.') or ($c_1 =$ '.' and $c_2 =$ ')') or ($c_1 =$ '.' and $c_2 =$ '(') **then** return stack+loop
else if $c_1 =$ '(' and $c_2 =$ '*') or ($c_1 =$ ')' and $c_2 =$ '*') or ($c_1 =$ '*' and $c_2 =$ ')') or ($c_1 =$ '*' and $c_2 =$ '(') **then** return stack+end
else if ($c_1 =$ '.' and $c_2 =$ '*') or ($c_1 =$ '*' and $c_2 =$ '.') **then** return loop+end
else if $c_1 =$ '*' and $c_2 =$ '*' **then** return end
else if $c_1 =$ '.' and $c_2 =$ '.' **then** return loop

Algorithm 2: Find the local context of a base pair forming or breaking

Function `LocalContext`(s_i, s_j)

Input: States s_i and state s_j, which differ by exactly one base pair. (Either of the states can have an extra base pair compared to the other.)

Output: $\langle l, r \rangle$, which is the local context of the base pair breaking or forming in transition from state s_i to state s_j.

$d_i \leftarrow$ dot-parens-plus-mult notation of s_i
$d_j \leftarrow$ dot-parens-plus-mult notation of s_j
$e_1 \leftarrow$ the first position where d_i and d_j differ
$e_2 \leftarrow$ the second position where d_i and d_j differ
$l \leftarrow$ `HalfContext` $(d_i, e_1 - 1, e_2 + 1)$ `// Algorithm 1`
$r \leftarrow$ `HalfContext` $(d_i, e_1 + 1, e_2 - 1)$
return $\langle l, r \rangle$

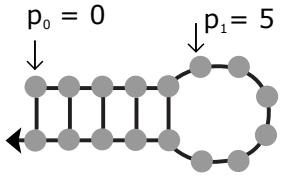
Algorithm 3: Generate state space

Function `GenerateStateSpace`
- $\mathcal{S} \leftarrow \mathcal{S}_{\text{init}}$, $Q \leftarrow \mathcal{S}_{\text{init}}$
- **while** $Q \neq \emptyset$ **do**
  - $\mathcal{N} \leftarrow \emptyset$
  - **foreach** $s \in Q$ **do**
    - **foreach** $s_p \in$ `NeighborStates(`s`)` **do**
      - **if** $s_p \notin \mathcal{S}$ **and** $s_p \notin \mathcal{S}_{\text{final}}$ **then**
        - $\mathcal{S} \leftarrow \mathcal{S} \cup s_p$
        - $\mathcal{N} \leftarrow \mathcal{N} \cup s_p$
  - $Q \leftarrow \mathcal{N}$
- $\mathcal{S} \leftarrow \mathcal{S} \cup \mathcal{S}_{\text{final}}$
- return $\mathcal{S}$

B.1 Hairpin Closing and Opening

Let l be the length of a strand that has a hairpin structure and let $m < l/2$ be the length of its stem in the fully closed position. Each state corresponds to a partial opening of the hairpin from the left and/or right ends, and is represented by a tuple $\langle p_o, p_1 \rangle$, where $0 \leq p_0 \leq p_1 \leq m$. The tuple indicates that the bases p_0 to $p_1 - 1$ are paired with bases $l - p_1$ to $l - p_0 - 1$ respectively, and no other base pairs are formed. Algorithm 4 calculates and returns the set of neighbors of a hairpin state s, and includes an example of a hairpin state. The state spaces of hairpin opening and closing are equal, except that the initial and final states are swapped. In hairpin closing, the initial state ($\mathcal{S}_{\text{init}} = \{\langle 0, 0 \rangle\}$) has no base pairs. The final state ($\mathcal{S}_{\text{final}} = \{\langle 0, m \rangle\}$) has m base pairs.

Algorithm 4: Calculate the neighbor states of a hairpin state $s = \langle p_0, p_1 \rangle$



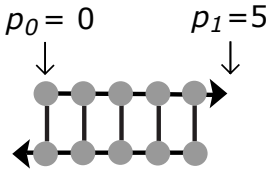
Function `NeighborStates(`$s = \langle p_0, p_1 \rangle$`)`
- `// This function returns all possible neighbors of state` s
- $\mathcal{N} \leftarrow \emptyset$
- `// Consider possibly valid new states, then remove the invalid ones`
- **if** $\langle p_0, p_1 \rangle = \langle 0, 0 \rangle$ **then**
  - **for** $p \in [0, m-1]$ **do**
    - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p, p+1 \rangle$
- **else**
  - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 1, p_1 \rangle \cup \langle p_0 + 1, p_1 \rangle \cup \langle p_0, p_1 - 1 \rangle \cup \langle p_0, p_1 + 1 \rangle$
- **foreach** $s' = \langle p'_0, p'_1 \rangle \in \mathcal{N}$ **do**
  - `// The state in which no base pair has formed is shown by` $\langle 0, 0 \rangle$
  - **if** $p'_0 = p'_1$ and $p'_0 \neq 0$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle 0, 0 \rangle$
- **foreach** $s' \in \mathcal{N}$ **do**
  - **if** `!AllowedState(`s'`)` **then** $\mathcal{N} \leftarrow \mathcal{N} \setminus s'$ `// Remove invalid states`
- return $\mathcal{N}$

Function `AllowedState(`$s' = \langle p_0, p_1 \rangle$`)`
- **if** $!(0 \leq p_0 \leq p_1 \leq m)$ **then** return False
- return True

B.2 Helix Association and Dissociation

Let l be the length of a strand in the helix. Each state corresponds to a partial opening of the helix from the left and/or right ends, and is represented by a tuple $\langle p_0, p_1 \rangle$, where $0 \leq p_0 \leq p_1 \leq l$. The tuple indicates that all bases numbered p_0 to $p_1 - 1$ in one strand are paired with bases numbered $l - p_1$ to $l - p_0 - 1$ in the other strand, respectively, and there are no other base pairs in the state. Algorithm 5 calculates and returns the set of neighbors of a helix state s, and includes an example of a helix state. The state spaces of helix association and dissociation are equal, except that the initial and final states are swapped. In helix association, the initial state ($\mathcal{S}_{\text{init}} = \{\langle 0, 0 \rangle\}$) has no base pairs. The final state ($\mathcal{S}_{\text{final}} = \{\langle 0, l \rangle\}$) has l base pairs.

Algorithm 5: Calculate the neighbor states of a helix state $s = \langle p_0, p_1 \rangle$



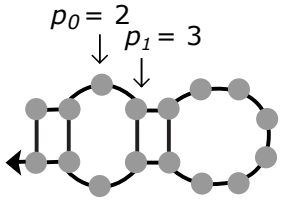
Function `NeighborStates` ($s = \langle p_0, p_1 \rangle$)
- `// This function returns all possible neighbors of state` s
- $\mathcal{N} \leftarrow \emptyset$
- `// Consider possibly valid new states, then remove the invalid ones`
- **if** $\langle p_0, p_1 \rangle = \langle 0, 0 \rangle$ **then**
  - **for** p in $[0, l-1]$ **do**
    - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p, p+1 \rangle$
- **else**
  - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 1, p_1 \rangle \cup \langle p_0 + 1, p_1 \rangle \cup \langle p_0, p_1 - 1 \rangle \cup \langle p_0, p_1 + 1 \rangle$
- **foreach** $s' = \langle p_0', p_1' \rangle \in \mathcal{N}$ **do**
  - `// The state in which no base pair has formed is shown by` $\langle 0, 0 \rangle$
  - **if** $p_0' = p_1'$ and $p_0' \neq 0$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle 0, 0 \rangle$
- **foreach** $s' \in \mathcal{N}$ **do**
  - **if** `!AllowedState(`s'`)` **then** $\mathcal{N} \leftarrow \mathcal{N} \setminus s'$ `// Remove invalid states`
- return $\mathcal{N}$

Function `AllowedState(`$s' = \langle p_0, p_1 \rangle$`)`
- **if** $!(0 \leq p_0 \leq p_1 \leq l)$ **then** return False
- return True

B.3 Bubble Closing

Let l be the length of the hairpin strand, $m < l/2$ be the length of the stem in the fully closed position, and f be the position where a bubble is formed. Each state corresponds to a partial opening of the bubble from the middle, and is represented by a tuple $\langle p_0, p_1 \rangle$, where $0 < p_0 \leq f \leq p_1 < m$. The tuple indicates that all bases numbered 0 to $p_0 - 1$ are paired with bases numbered $l - p_0$ to $l - 1$, respectively, and all bases numbered p_1 to $m - 1$ are paired with bases numbered $l - m$ to $l - p_1 - 1$, respectively, and there are no other base pairs in the state. Algorithm 6 calculates and returns the set of neighbors of a bubble closing state s, and includes an example of a bubble state. In the initial state ($\mathcal{S}_{\text{init}} = \{\langle f, f+1 \rangle\}$), all base pairs in the hairpin stem have formed except for a bubble of size 1 in the stem at position f. In the final state ($\mathcal{S}_{\text{final}} = \{\langle f, f \rangle\}$), all base pairs have formed.

Algorithm 6: Calculate the neighbor states of a bubble state $s = \langle p_0, p_1 \rangle$



Function `NeighborStates` ($s = \langle p_0, p_1 \rangle$)
  `// This function returns all possible neighbors of state` s
  $\mathcal{N} \leftarrow \emptyset$
  `// Consider possibly valid new states, then remove the invalid ones`
  $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 1, p_1 \rangle \cup \langle p_0 + 1, p_1 \rangle \cup \langle p_0, p_1 - 1 \rangle \cup \langle p_0, p_1 + 1 \rangle$
  foreach $s' = \langle p_0', p_1' \rangle \in \mathcal{N}$ **do**
    `// The state in which all base pairs have formed is shown by` $\langle f, f \rangle$
    if $p_0' = p_1'$ and $p_0' \neq f$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle f, f \rangle$
  foreach $s' \in \mathcal{N}$ **do**
    if `!AllowedState(`s'`)` **then** $\mathcal{N} \leftarrow \mathcal{N} \setminus s'$ `// Remove invalid states`
  return $\mathcal{N}$
Function `AllowedState`($s' = \langle p_0, p_1 \rangle$)
  if $!(0 < p_0 \leq f \leq p_1 < m)$ **then** return False
  return True

B.4 Toehold-mediated 3-way Strand Displacement

Let l be the length of the substrate. For simplicity, let l also be the length of the invader, m be the toehold length, and $l - m$ be the length of the incumbent. Each state is represented by a tuple $\langle p_0, p_1, p_2, p_3 \rangle$, where $0 \leq p_0 \leq p_1 \leq p_2 \leq p_3 \leq l$ and $p_2 \geq m$. The tuple indicates that all bases numbered p_0 to $p_1 - 1$ in the substrate are paired with bases numbered $l - p_1$ to $l - p_0 - 1$ in the invader, respectively, all bases numbered p_2 to $p_3 - 1$ in the substrate are paired with bases numbered $l - p_3$ to $l - p_2 - 1$ in the incumbent, respectively, and there are no other base pairs in the state. Algorithm 7 calculates and returns the set of neighbors of a toehold-mediated 3-way strand displacement state s, and includes an example of a toehold-mediated 3-way strand displacement state. In the initial state ($\mathcal{S}_{\text{init}} = \{\langle 0, 0, m, l \rangle\}$), the substrate is completely attached to the incumbent, but completely detached from the invader. In the final state ($\mathcal{S}_{\text{final}} = \{\langle 0, l, l, l \rangle\}$), the substrate is completely detached from the incumbent, but completely attached to the invader. Algorithm 8 adapts algorithm 7 for toehold-mediated 3-way strand displacement with mismatches between the invader and the substrate. In the algorithm, *mp* is a pointer to the mismatch position in the displacement domain.

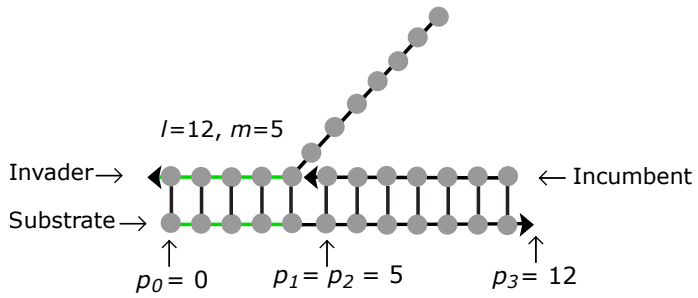
Note that in algorithms 7 and 8, to efficiently obtain mean first passage times with sparse matrix computations, we further heuristically prune the state space of each reaction (described in the algorithms).

B.5 Toehold-mediated 4-way Strand Exchange

Let *complex* be the first helix and *complex1* and *complex2* be the two strands in this helix. Let *reporter* be the second helix and *reporter1* be the strand in this helix that is complementary to *complex1* and *reporter2* be the strand in this helix that is complementary to *complex2*. Let l be the length of the helices excluding their toehold. For simplicity, let m be the toehold length of *complex1* and *reporter1* and let n be the toehold length of *complex2* and *reporter2*. Each state is represented by a tuple $\langle p_0, p_1, p_{0'}, p_{1'}, p_2, p_3 \rangle$. The tuple indicates that all bases numbered p_0 to $p_{0'} - 1$ in *complex1* have paired with bases numbered $l + m - p_{0'}$ to $l + m - p_0 - 1$ in *reporter1*, respectively, all bases numbered 0 to $p_2 - 1$ in *complex1* are paired with bases numbered $l + n - p_2$ to $l + n - 1$ in *complex2*, respectively, all bases numbered p_1 to $p_{1'} - 1$ in *reporter2* are paired with bases numbered $l + n - p_{1'}$ to $l + n - p_1 - 1$ in *complex2*, respectively, all bases numbered 0 to $p_3 - 1$ in *reporter2* are paired with bases numbered $l + m - p_3$ to $l + m - 1$ in *reporter1*, respectively, and there are no other base pairs in the state. Algorithm 9 calculates and returns the set of neighbors of a toehold-mediated 4-way strand exchange state s, and includes an example of a toehold-mediated 4-way strand exchange state. In the initial state ($\mathcal{S}_{\text{init}} = \{\langle l + m, l + n, l + m, l + n, l, l \rangle\}$), *complex1* and *complex2* are completely bound except in their toeholds (have formed the *complex* helix), *reporter1* and *reporter2* are completely bound except in their toeholds (have formed the *reporter* helix), and no base pairs have formed between the *complex* helix and the *reporter* helix. Hence, each helix has two complementary strands except for their toeholds. In the final state ($\mathcal{S}_{\text{final}} = \{\langle 0, 0, l + m, l + n, 0, 0 \rangle\}$), the *reporter* and *complex* helices have completely exchanged strands and two new helices, which have complementary strands, are formed.

Note that in algorithm 9, to efficiently obtain mean first passage times with sparse matrix computations, we further heuristically prune the state space of each reaction (described in the algorithm).

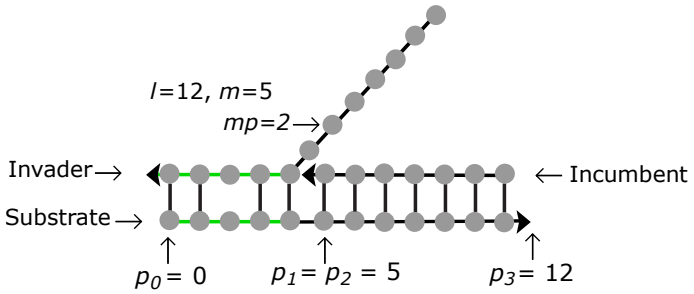
Algorithm 7: Calculate the neighbor states of a toehold-mediated 3-way strand displacement state $s = \langle p_0, p_1, p_2, p_3 \rangle$



Function `NeighborStates` ($s = \langle p_0, p_1, p_2, p_3 \rangle$)
- `// This function returns all possible neighbors of state` s
- $\mathcal{N} \leftarrow \emptyset$
- `// Consider possibly valid new states, then remove the invalid ones`
- **if** $p_0 = p_1$ **then**
  - `// If the invader and the substrate are detached, they can form a base pair`
  - **for** p in $[0, p_2 - 1]$ **do**
    - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p, p+1, p_2, p_3 \rangle$
- **else**
  - `// The invader and substrate can form or break a base pair`
  - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 1, p_1, p_2, p_3 \rangle \cup \langle p_0 + 1, p_1, p_2, p_3 \rangle \cup \langle p_0, p_1 - 1, p_2, p_3 \rangle \cup \langle p_0, p_1 + 1, p_2, p_3 \rangle$
  - `// The incumbent and substrate can form or break a base pair`
  - $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1, p_2 - 1, p_3 \rangle \cup \langle p_0, p_1, p_2 + 1, p_3 \rangle \cup \langle p_0, p_1, p_2, p_3 - 1 \rangle \cup \langle p_0, p_1, p_2, p_3 + 1 \rangle$
- **foreach** $s' = \langle p'_0, p'_1, p'_2, p'_3 \rangle \in \mathcal{N}$ **do**
  - `// States in which the substrate and invader are detached are shown by` $\langle 0, 0, p'_2, p'_3 \rangle$
  - **if** $p'_0 = p'_1$ and $p'_0 \neq 0$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle 0, 0, p'_2, p'_3 \rangle$
  - `// States in which the substrate and incumbent are detached are shown by` $\langle p'_0, p'_1, l, l \rangle$
  - **if** $p'_2 = p'_3$ and $p'_2 \neq l$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle p'_0, p'_1, l, l \rangle$
- **foreach** $s' \in \mathcal{N}$ **do**
  - **if** `!AllowedState(`s'`)` **then** $\mathcal{N} \leftarrow \mathcal{N} \setminus s'$ `// Remove invalid states`
- return $\mathcal{N}$

Function `AllowedState`($s' = \langle p_0, p_1, p_2, p_3 \rangle$)
- **if** $!(0 \leq p_0 \leq p_1 \leq p_2 \leq p_3 \leq l$ and $p_2 \geq m)$ **then** return False
- `// Heuristically, further prune the state space to enable sparse matrix computations`
- **if** $p_0 = p_1$ and $p_2 = p_3$ **then** return False `// Disallow the complex to dissociate into three strands`
- **if** $(p_2 - p_1 > 1$ or $p_0 \neq 0)$ and $(0 < m < p_2)$ **then** return False `// When there is a gap of greater than 1 base // pair between the invader and the incumbent, or the invader is not bound to the substrate from the // left end of the strands, disallow the first base pair of the incumbent and the substrate to break // in the existence of a toehold`
- **if** $p_2 - p_1 > m + 2$ **then** return False `// Disallow states which have a gap of size greater than` $m + 2$ `// between the incumbent and the invader`
- return True

Algorithm 8: Calculate the neighbor states of a toehold-mediated 3-way strand displacement state $s = \langle p_0, p_1, p_2, p_3 \rangle$ that has a mismatch between the invader and the substrate



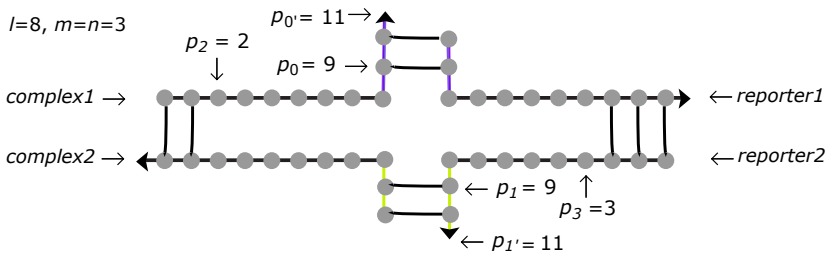
Function `NeighborStates` ($s = \langle p_0, p_1, p_2, p_3 \rangle$)

```
// This function returns all possible neighbors of state s
```
$\mathcal{N} \leftarrow \emptyset$
```
// Consider possibly valid new states, then remove the invalid ones
```
if $p_0 = p_1$ **then**
```
    // If the invader and the substrate are detached, they can form a base pair that is not located
     at the mismatch position
```
    for p in $[0, p_2 - 1]$ **do**
        if $p \neq m + mp - 1$ **then**
            $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p, p + 1, p_2, p_3 \rangle$
else
```
    // The incumbent and substrate can form or break a base pair
```
    $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1, p_2 - 1, p_3 \rangle \cup \langle p_0, p_1, p_2 + 1, p_3 \rangle \cup \langle p_0, p_1, p_2, p_3 - 1 \rangle \cup \langle p_0, p_1, p_2, p_3 + 1 \rangle$
```
    // The invader and substrate can form or break a base pair that is not located at the mismatch
     position
    // For the Machinek et al. [6] study of toehold-mediated 3-way strand displacement with
     mismatches, mp is either 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, or 14.
```
    if $p_0 - 1 \neq m + mp - 1$ **then** $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 1, p_1, p_2, p_3 \rangle$
    else $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 2, p_1, p_2, p_3 \rangle$
    if $p_0 + 1 \neq m + mp - 1$ **then** $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 + 1, p_1, p_2, p_3 \rangle$
    else $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 + 2, p_1, p_2, p_3 \rangle$
    if $p_1 - 1 \neq m + mp - 1$ **then** $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1 - 1, p_2, p_3 \rangle$
    else $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1 - 2, p_2, p_3 \rangle$
    if $p_1 + 1 \neq m + mp - 1$ **then** $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1 + 1, p_2, p_3 \rangle$
    else $\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1 + 2, p_2, p_3 \rangle$
foreach $s' = \langle p_0', p_1', p_2', p_3' \rangle \in \mathcal{N}$ **do**
```
    // States in which the substrate and invader are detached are shown by ⟨0, 0, p2', p3'⟩
```
    if $p_0' = p_1'$ and $p_0' \neq 0$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle 0, 0, p_2', p_3' \rangle$
```
    // States in which the substrate and incumbent are detached are shown by ⟨p0', p1', l, l⟩
```
    if $p_2' = p_3'$ and $p_2' \neq l$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle p_0', p_1', l, l \rangle$
foreach $s' \in \mathcal{N}$ **do**
    if `!AllowedState(`s'`)` **then** $\mathcal{N} \leftarrow \mathcal{N} \setminus s'$ `// Remove invalid states`
return $\mathcal{N}$

Function `AllowedState(`$s' = \langle p_0, p_1, p_2, p_3 \rangle$`)`

if $!(0 \leq p_0 \leq p_1 \leq p_2 \leq p_3 \leq l$ and $p_2 \geq m)$ **then** return False
```
// Heuristically, further prune the state space to enable sparse matrix computations
```
if $p_0 = p_1$ and $p_2 = p_3$ **then** return False `// Disallow the complex to dissociate into three strands`
if $(p_2 - p_1 > 5$ or $p_0 \neq 0)$ and $(0 < m < p_2)$ **then** return False`// When there is a gap of greater than 5 base`
```
// pairs between the invader and the incumbent, or the invader is not bound to the substrate from
// the left end of the strands, disallow the first base pair of the incumbent and the substrate to
// break in the existence of a toehold
```
if $p_2 - p_1 > m + 4$ **then** return False `// Disallow states which have a gap of size greater than` $m + 4$
```
// between the incumbent and the invader
```
return True

Algorithm 9: Calculate the neighbor states of a toehold-mediated 4-way strand exchange state $s = \langle p_0, p_1, p_{0'}, p_{1'}, p_2, p_3 \rangle$



Function `NeighborStates` ($s = \langle p_0, p_1, p_{0'}, p_{1'}, p_2, p_3 \rangle$)

`// This function returns all possible neighbors of state` s

$\mathcal{N} \leftarrow \emptyset$

`// Consider possibly valid new states, then remove the invalid ones`

if $p_0 = p_{0'}$ **then**

`// If` *complex1* `and` *reporter1* `are detached, they can form a base pair`

for p in $[\max\{p_2, p_3\}, l + m - 1]$ **do**

$\mathcal{N} \leftarrow \mathcal{N} \cup \langle p, p_1, p+1, p_{1'}, p_2, p_3 \rangle$

else

`// complex1 can form or break a base pair with reporter1`

$\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0 - 1, p_1, p_{0'}, p_{1'}, p_2, p_3 \rangle \cup \langle p_0 + 1, p_1, p_{0'}, p_{1'}, p_2, p_3 \rangle \cup \langle p_0, p_1, p_{0'} - 1, p_{1'}, p_2, p_3 \rangle \cup \langle p_0, p_1, p_{0'} + 1, p_{1'}, p_2, p_3 \rangle$

if $p_1 = p_{1'}$ **then**

`// If reporter2 and complex2 are detached, they can form a base pair`

for p in $[\max\{p_2, p_3\}, l + n - 1]$ **do**

$\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p, p_1, p+1, p_2, p_3 \rangle$

else

`// reporter2 can form or break a base pair with complex2`

$\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1 - 1, p_{0'}, p_{1'}, p_2, p_3 \rangle \cup \langle p_0, p_1 + 1, p_{0'}, p_{1'}, p_2, p_3 \rangle \cup \langle p_0, p_1, p_{0'}, p_{1'} - 1, p_2, p_3 \rangle \cup \langle p_0, p_1, p_{0'}, p_{1'} + 1, p_2, p_3 \rangle$

if $(p_0 \neq p_{0'}$ or $p_1 \neq p_{1'})$ or $(m = 0$ or $n = 0)$ **then**

`// If complex1 and reporter1 are attached or complex2 and reporter2 are attached or a toehold does not exist, then complex1 and complex2 can form or break a base pair`

$\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1, p_{0'}, p_{1'}, p_2 - 1, p_3 \rangle \cup \langle p_0, p_1, p_{0'}, p_{1'}, p_2 + 1, p_3 \rangle$

`// If complex1 and reporter1 are attached or complex2 and reporter2 are attached or a toehold does not exist, then reporter1 and reporter2 can form or break a base pair`

$\mathcal{N} \leftarrow \mathcal{N} \cup \langle p_0, p_1, p_{0'}, p_{1'}, p_2, p_3 - 1 \rangle \cup \langle p_0, p_1, p_{0'}, p_{1'}, p_2, p_3 + 1 \rangle$

foreach $s' = \langle p'_0, p'_1, p'_{0'}, p'_{1'}, p'_2, p'_3 \rangle \in \mathcal{N}$ **do**

`// States in which complex1 and reporter1 are detached are shown by` $\langle l + m, p'_1, l + m, p'_{1'}, p'_2, p'_3 \rangle$

if $p'_0 = p'_{0'}$ and $0 \leq p'_0 < l + m$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle l + m, p'_1, l + m, p'_{1'}, p'_2, p'_3 \rangle$

`// States in which reporter2 and complex2 are detached are shown by` $\langle p'_0, l + n, p'_{0'}, l + n, p'_2, p'_3 \rangle$

if $p'_1 = p'_{1'}$ and $0 \leq p'_1 < l + n$ **then** $\mathcal{N} \leftarrow (\mathcal{N} \setminus s') \cup \langle p'_0, l + n, p'_{0'}, l + n, p'_2, p'_3 \rangle$

foreach $s' \in \mathcal{N}$ **do**

if `!AllowedState(`s'`)` **then** $\mathcal{N} \leftarrow \mathcal{N} \setminus s'$ `// Remove invalid states`

return $\mathcal{N}$

Function `AllowedState(`$s' = \langle p_0, p_1, p_{0'}, p_{1'}, p_2, p_3 \rangle$`)`

if $!(p_3 \leq p_0$ and $p_3 \leq p_1$ and $p_2 \leq p_0$ and $p_2 \leq p_1$ and $0 \leq p_2 \leq l$ and $0 \leq p_3 \leq l$ and $0 \leq p_0 \leq p_{0'} \leq l + m$ and $0 \leq p_1 \leq p_{1'} \leq l + n)$ **then** return False

`// Heuristically, further prune the state space to enable sparse matrix computations`

if $(p_0 = p_{0'}$ or $p_1 = p_{1'})$ and $(p_2 = 0$ or $p_3 = 0)$ **then** return False `// Disallow the complex to dissociate into three or four complexes`

if $(m = 0$ or $n = 0)$ and $(p_0 = p_{0'}$ or $p_1 = p_{1'})$ and $(l - p_2 > 3 - m/3$ or $l - p_3 > 3 - n/3)$ **then** return False

`// When one of the toeholds does not exist and the reporter and complex have not attached from both sides, disallow the complex and the reporter to break more than` $3 - m/3$ `and` $3 - n/3$ `base pairs, // respectively`

if $(m \neq 0$ and $n \neq 0)$ and $(p_0 = p_{0'}$ or $p_1 = p_{1'})$ and $(p_2 < l - 1$ or $p_3 < l - 1)$ **then** return False `// When both the toeholds exist and the reporter and the complex haven not attached from both sides, disallow the complex and the reporter to break more than one base pair`

if $p_{0'} < l$ or $p_{1'} < l$ **then** return False

if $(p_0 \neq p_{0'}$ and $p_1 \neq p_{1'})$ and $(|p_0 - p_3| + |p_0 - p_2| + |p_1 - p_3| + |p_1 - p_2| > 8 - n/3 - m/3)$ **then** return False

`// When the complex and reporter are attached from both sides, disallow large loops`

return True

C Half Context Frequency

Fig. 1 shows the fraction of all unimolecular elementary steps in the training dataset that involve a given half context, i.e., the fraction $\frac{\#l}{\sum_{l \in \mathcal{C}} \#l}$, where $\mathcal{C} = \{\text{stack}, \text{loop}, \text{end}, \text{stack+loop}, \text{stack+end}, \text{loop+end}, \text{stack+stack}\}$ is the set of half contexts and $\#l$ is the number of unimolecular transitions that involve the half context l in the continuous-time Markov chain (CTMC) of the reactions. Analogously, Fig. 2 shows the fraction of all bimolecular elementary steps in the training dataset that involve a given half context.

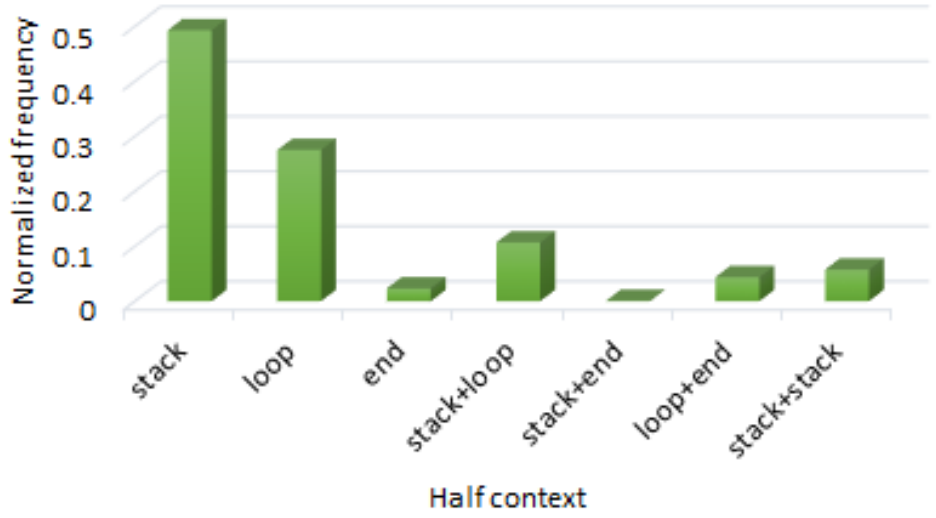


Fig. 1: Normalized frequency of the half contexts in unimolecular transitions.

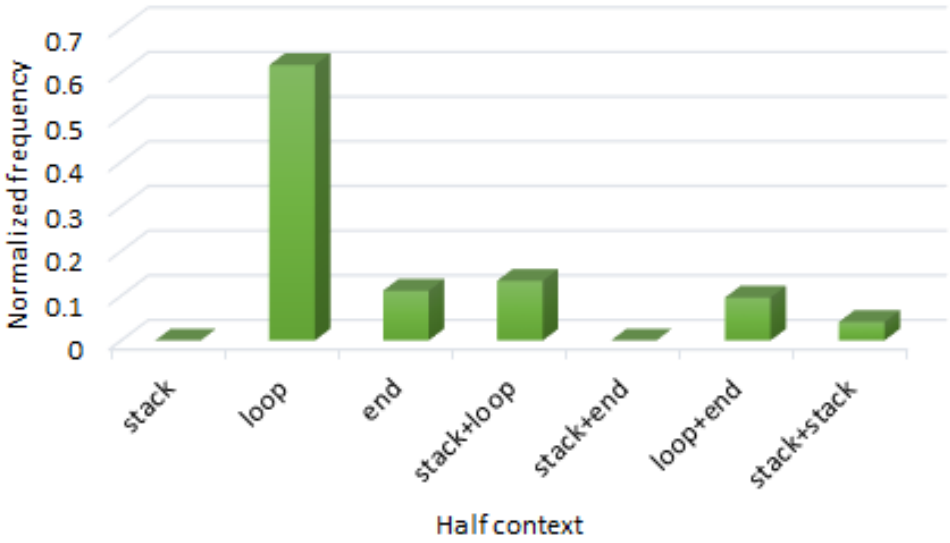


Fig. 2: Normalized frequency of the half contexts in bimolecular transitions.

D Experimental Plot Reproduction

The following plots show the performance of the Metropolis and the Arrhenius models on the training and testing datasets. Dashed lines indicate model fits and predictions and solid lines indicate experimentally determined values. For the MCMC ensemble method, error bars indicate the range (minimum to maximum) of predictions.

D.1 Training Set ($\mathcal{D}_{\text{train}}$)

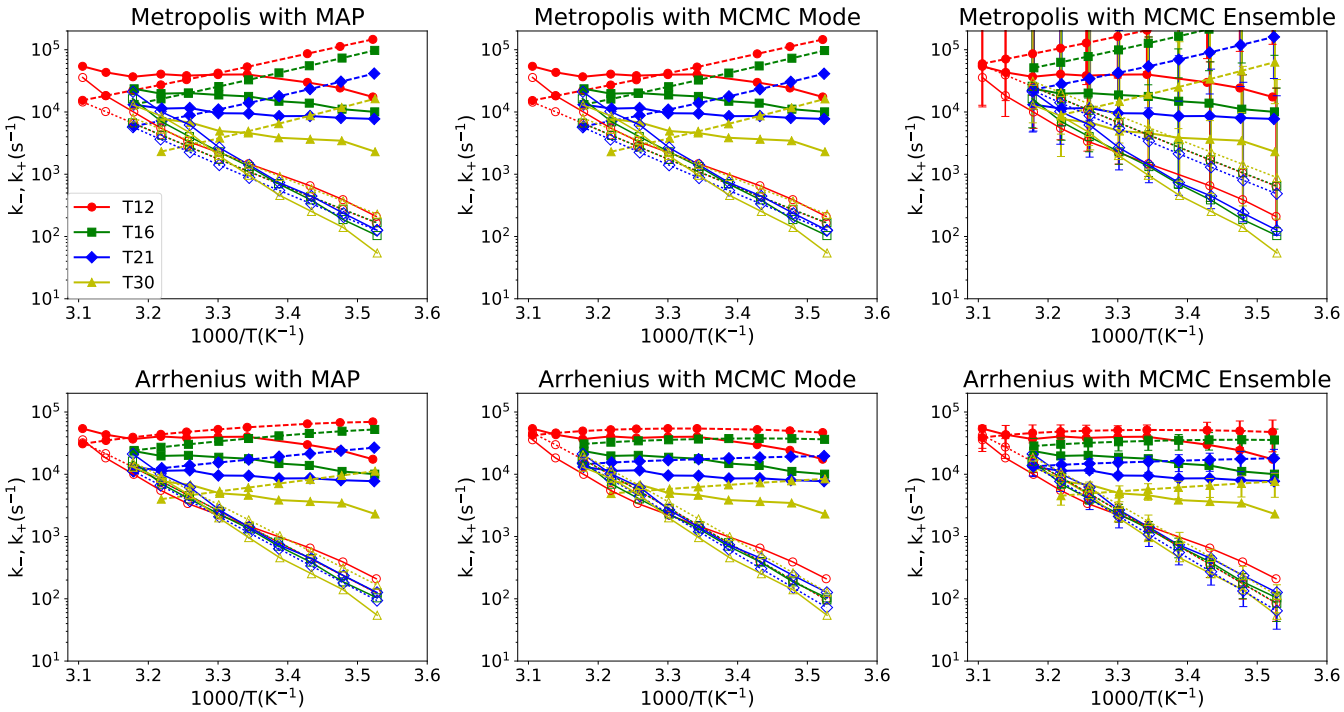


Fig. 3: Model fitting (dashed lines) of reaction rate constants (y axis) for hairpin closing (solid) and opening (open) with sequence $5'$-$CCCAA$-$(T)_n$-$TTGGG$-$3'$ where n is 12,16, 21, or 30, experimental data (solid lines) from Fig. 4 of Bonnet et al. [3].

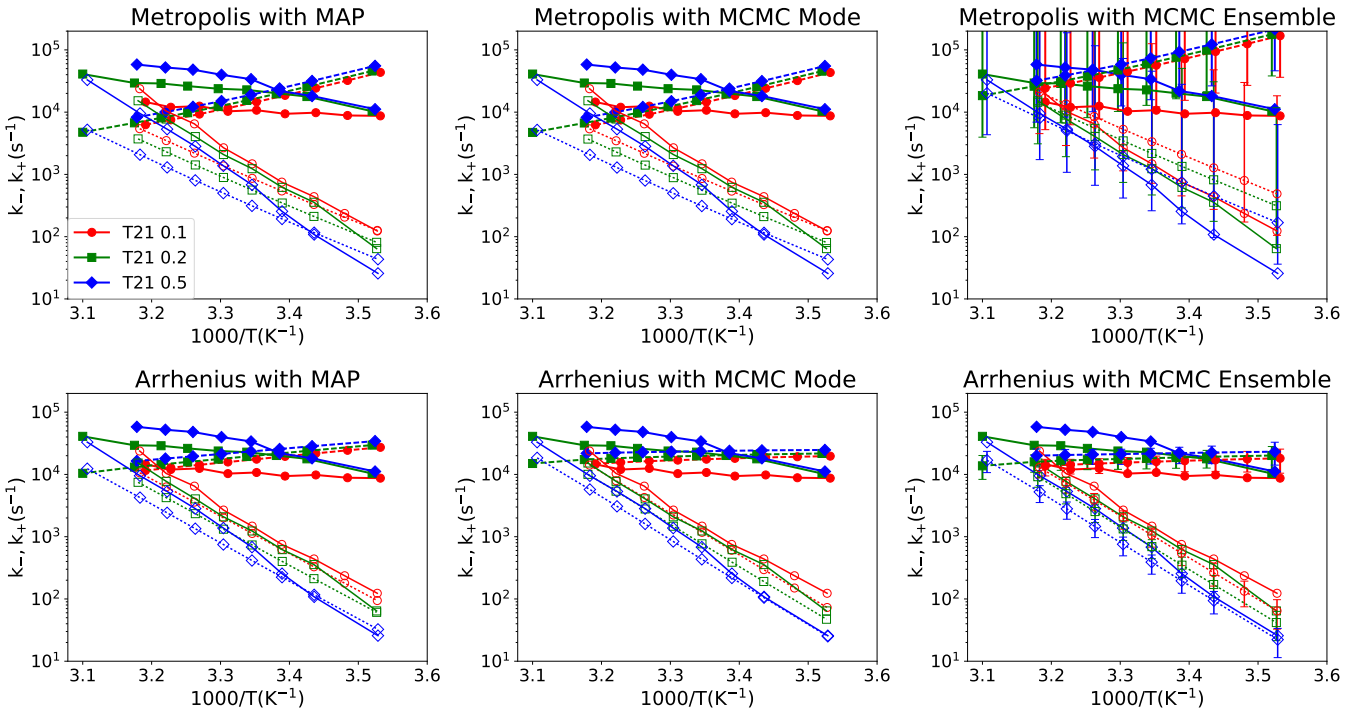


Fig. 4: Model fitting (dashed lines) of reaction rate constants (y axis) for hairpin closing (solid) and opening (open) with sequence 5′-$CCCAA$-$(T)_{21}$-$TTGGG$-3′ at different salt concentrations, experimental data (solid lines) from Fig.6 of Bonnet et al. [3]. Fig. 6 from Bonnet et al. [3] wrongfully notes the use of a poly-A instead of a poly-T hairpin loop, which becomes evident in comparison to Fig. 5 of the same work (private communication with the authors).

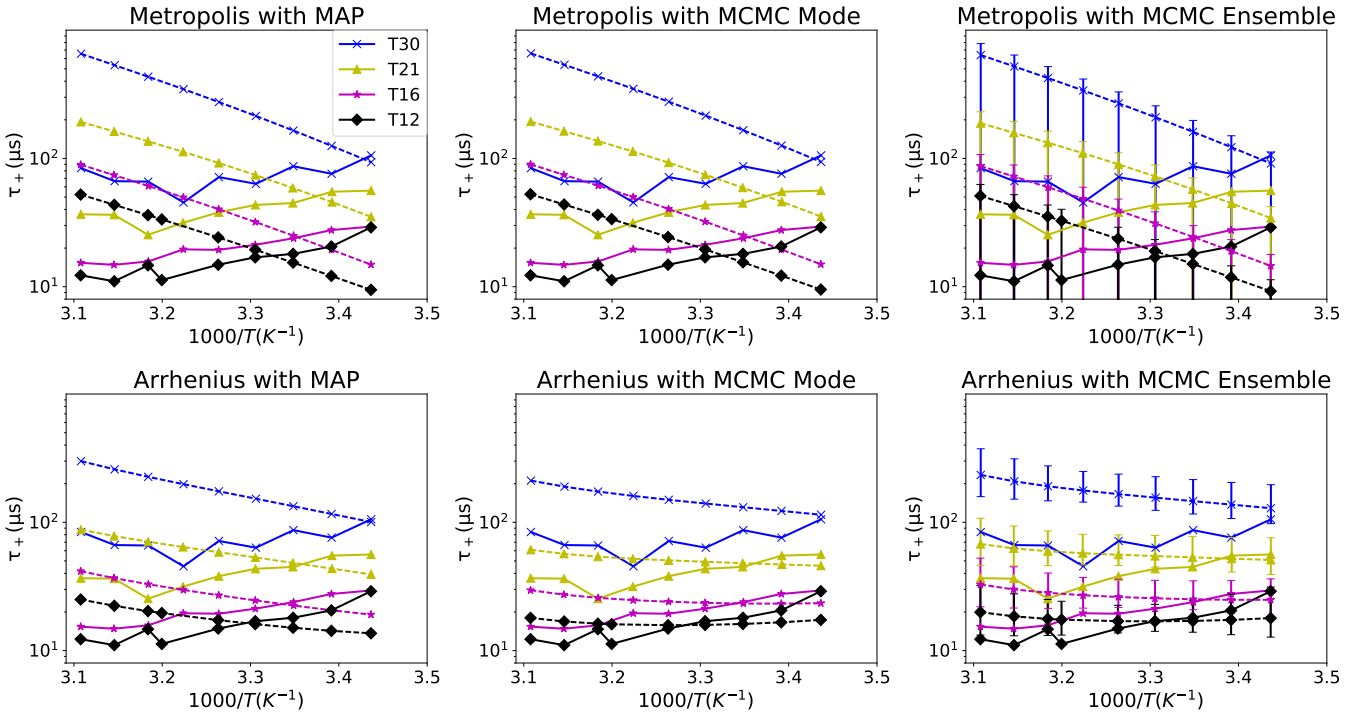


Fig. 5: Model fitting (dashed lines) of reaction timescales (y axis) for hairpin closing with sequence 5′-$CCCAA$-$(T)_n$-$TTGGG$-3′ where n is 12,16, 21, or 30, experimental data (solid lines) from Fig. 3.28 of Bonnet [2].

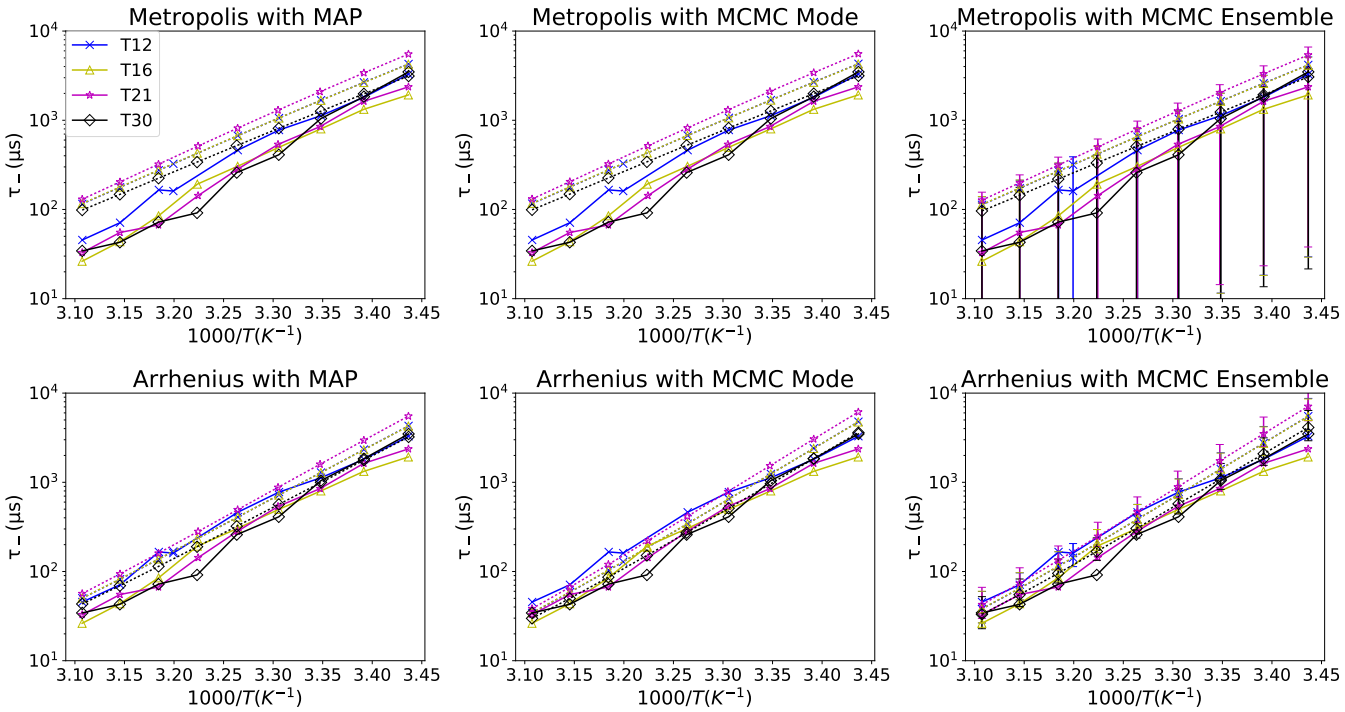


Fig. 6: Model fitting (dashed lines) of reaction timescales (y axis) for hairpin opening with sequence 5′-$CCCAA$-$(T)_n$-$TTGGG$-3′ where n is 12,16, 21, or 30, experimental data (solid lines) from Fig. 3.28 of Bonnet [2].

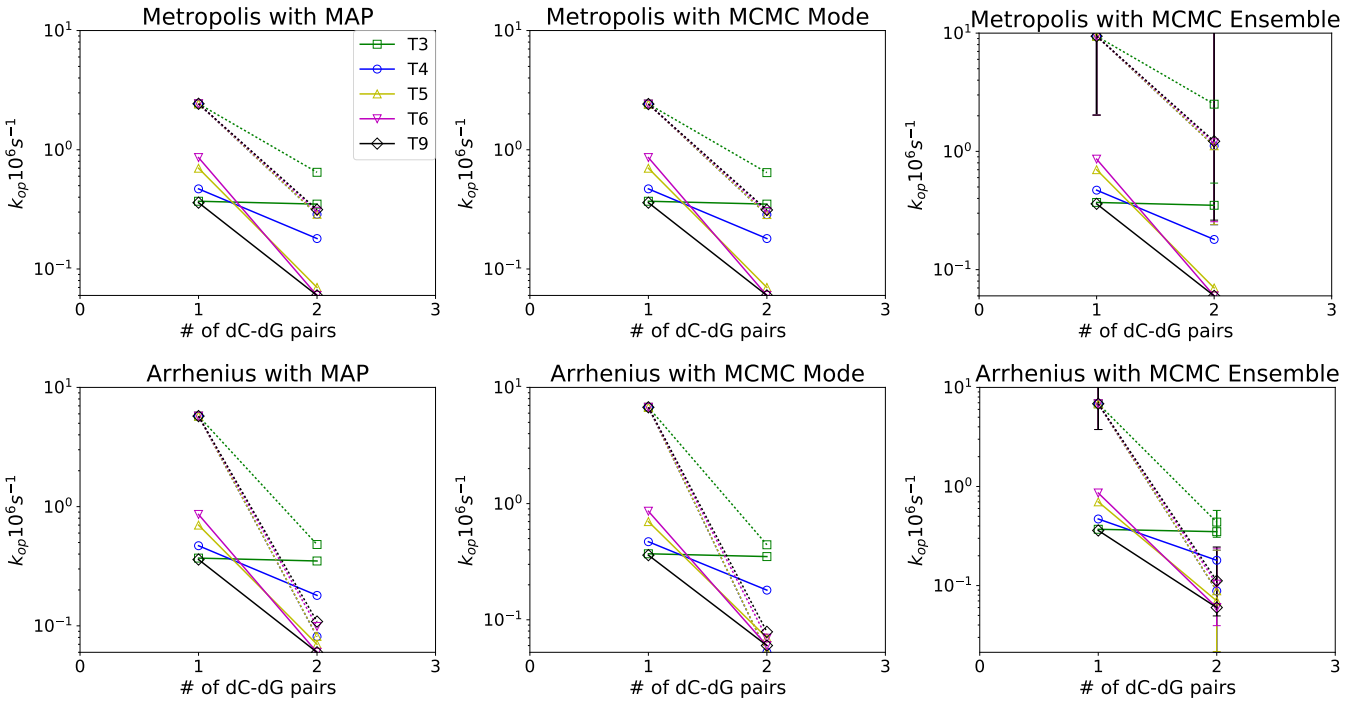


Fig. 7: Model fitting (dashed lines) of reaction rate constants (y axis) for hairpin opening with sequence F-$(dC)_y$-$(dT)_x$-$(dG)_y$ (x ranging from 3 to 9) as a function of dC-dG pairs (y ranging from 1 to 2), experimental data (solid lines) from Table 1 (Fig. 3b) of Kim et al. [5].

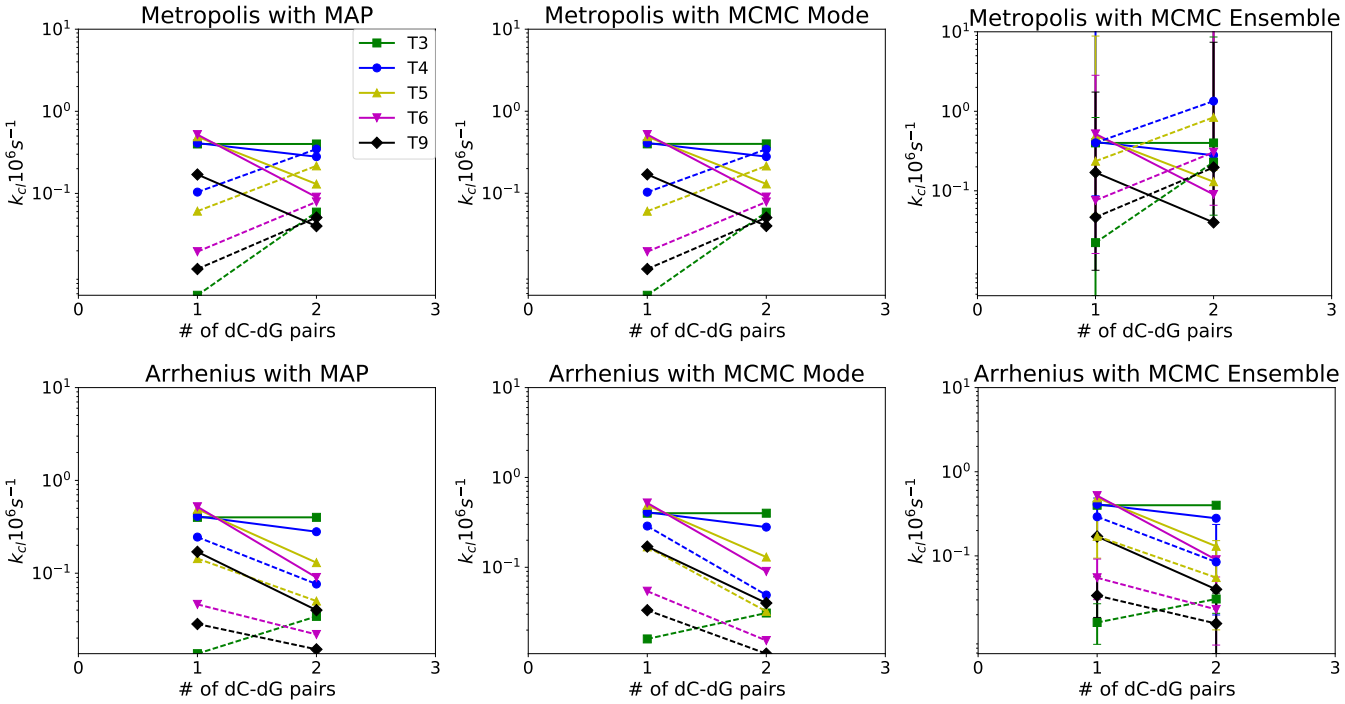


Fig. 8: Model fitting (dashed lines) of reaction rate constants (y axis) for hairpin closing with sequence F-$(dC)_y$-$(dT)_x$-$(dG)_y$ (x ranging from 3 to 9) as a function of dC-dG pairs (y ranging from 1 to 2), experimental data (solid lines) from Table 1 (Fig. 3b) of Kim et al. [5].

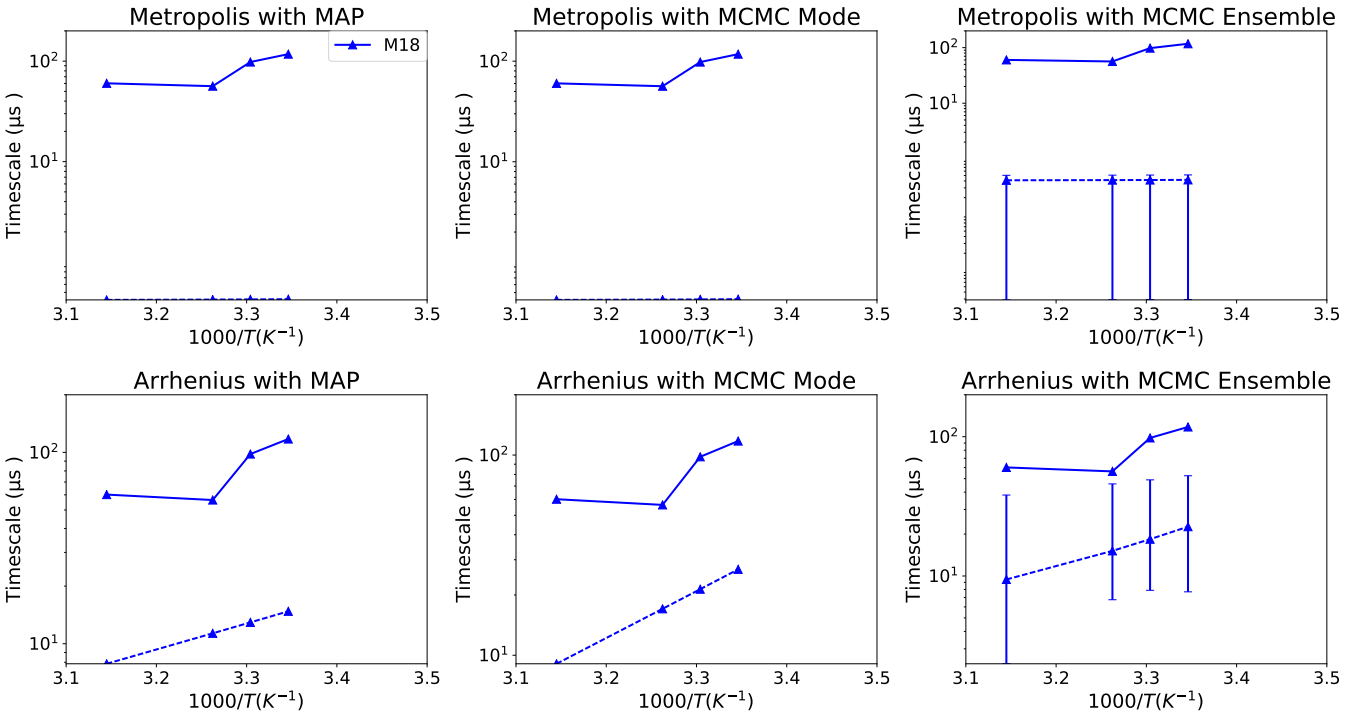


Fig. 9: Model fitting (dashed lines) of reaction timescales (y axis) for bubble closing with sequence M_{18}, experimental data (solid lines) from Fig. 4 of Altan-Bonnet et al. [1].

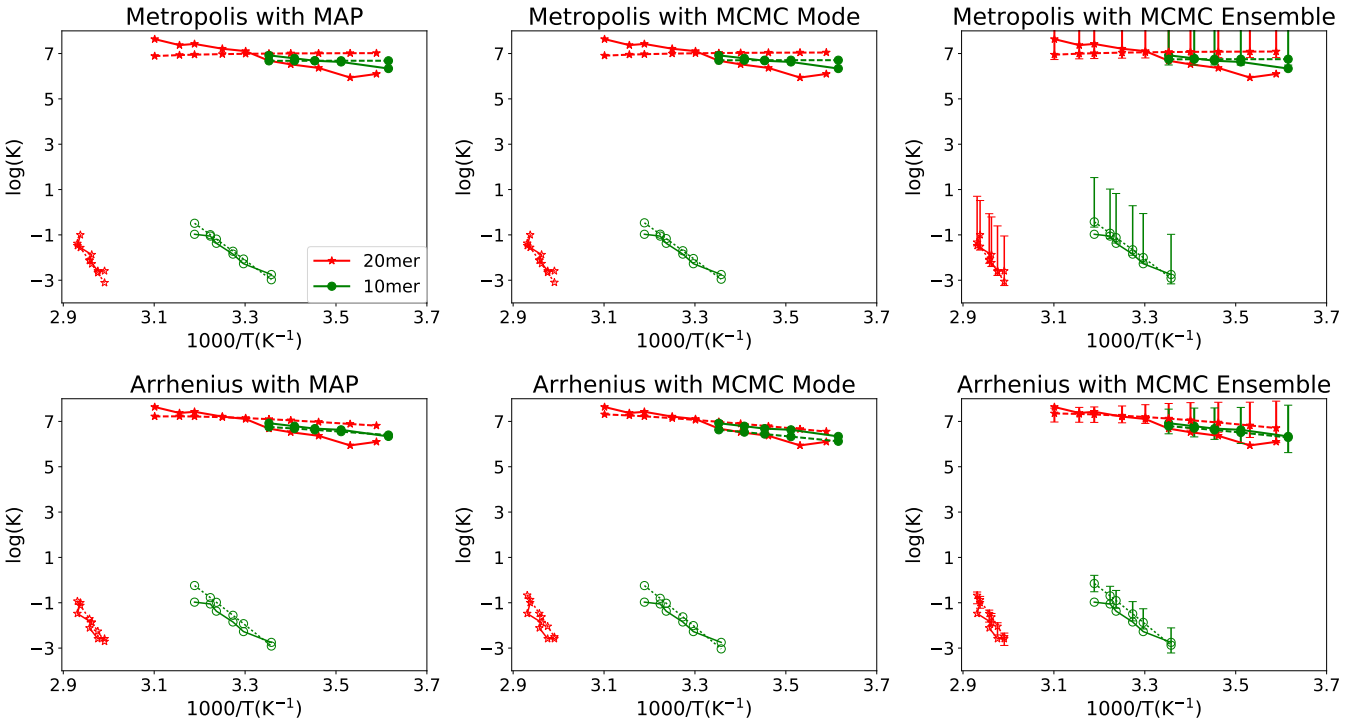


Fig. 10: Model fitting (dashed lines) of reaction rate constants (y axis) for helix association (solid) and disassociation (solid), experimental data (solid lines) from Fig. 6 of Morrison and Stols [7]. 10mer and 20mer are variation in the length of the strand.

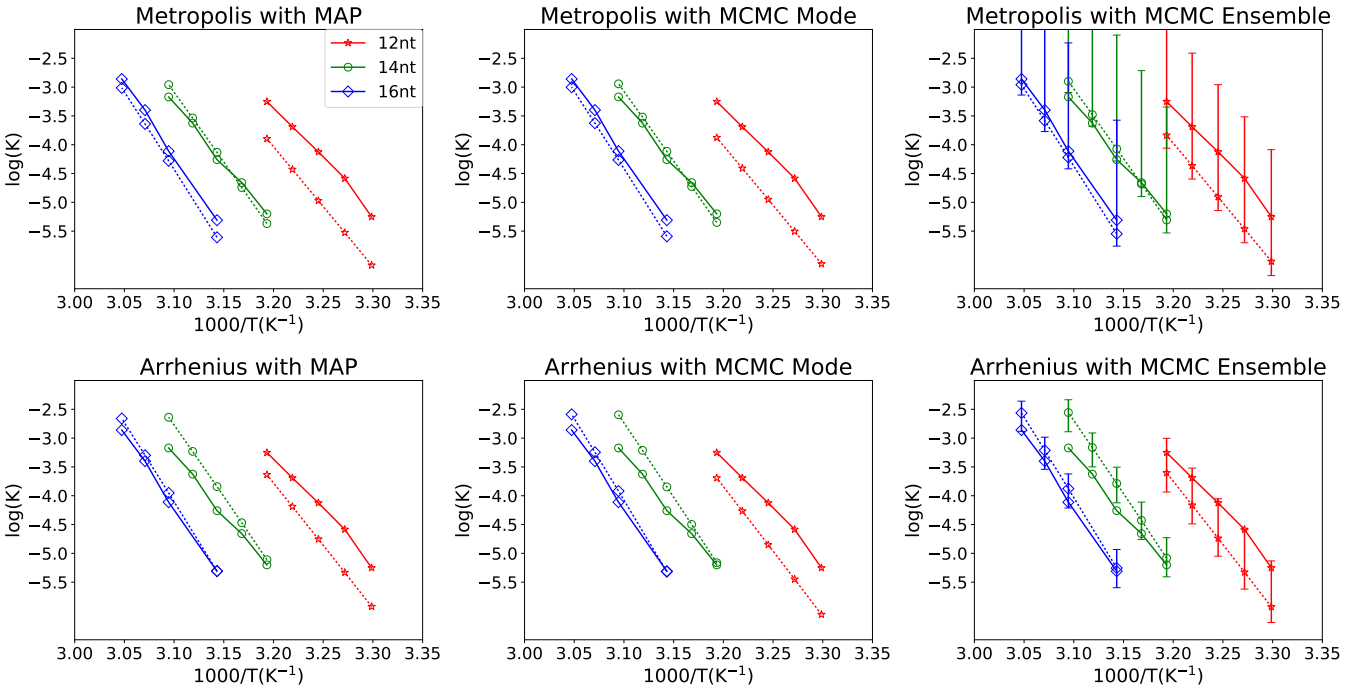


Fig. 11: Model fitting (dashed lines) of reaction rate constants (y axis) for helix disassociation, experimental data (solid lines) from Fig. 6 of Reynaldo et al. [8]. 12nt, 14nt, and 16nt are variations in the length of the strand.

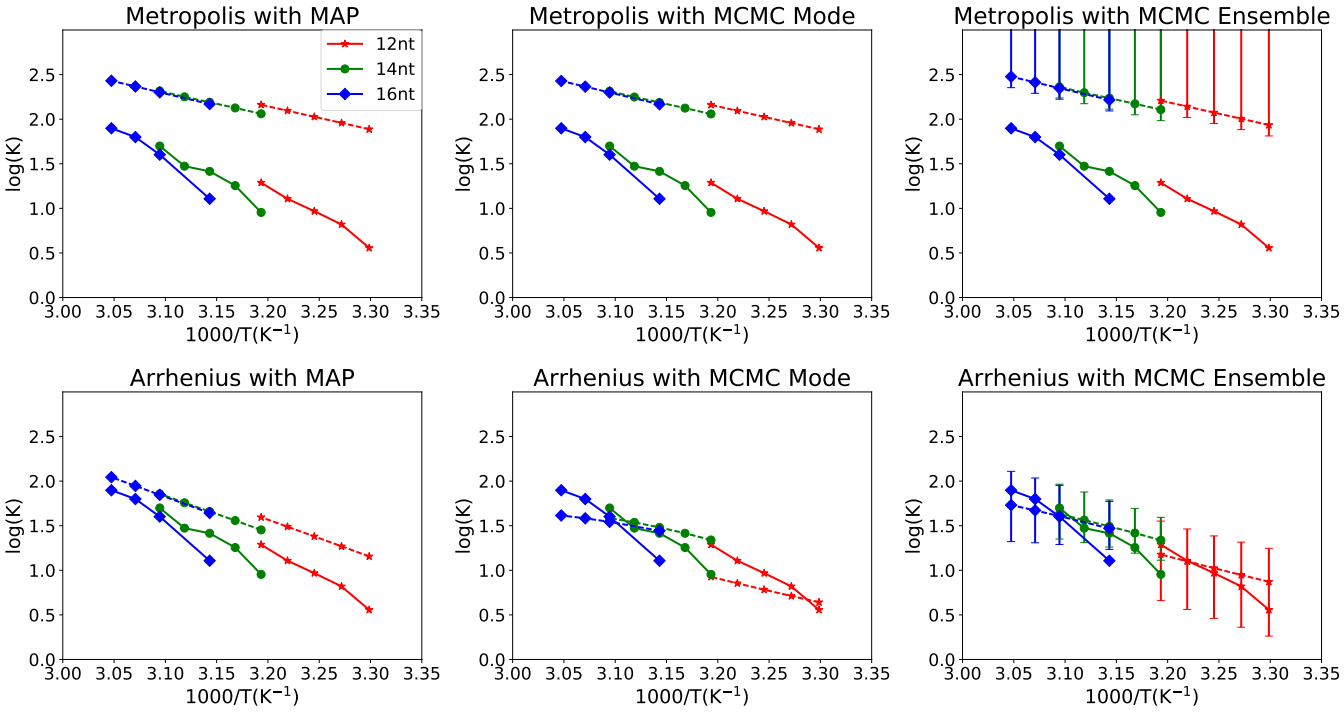


Fig. 12: Model fitting (dashed lines) of reaction rate constants (y axis) for toehold-mediated 3-way strand displacement, experimental data (solid lines) from Fig. 6 of Reynaldo et al. [8]. 12nt, 14nt, and 16nt are variations in the length of the strand.

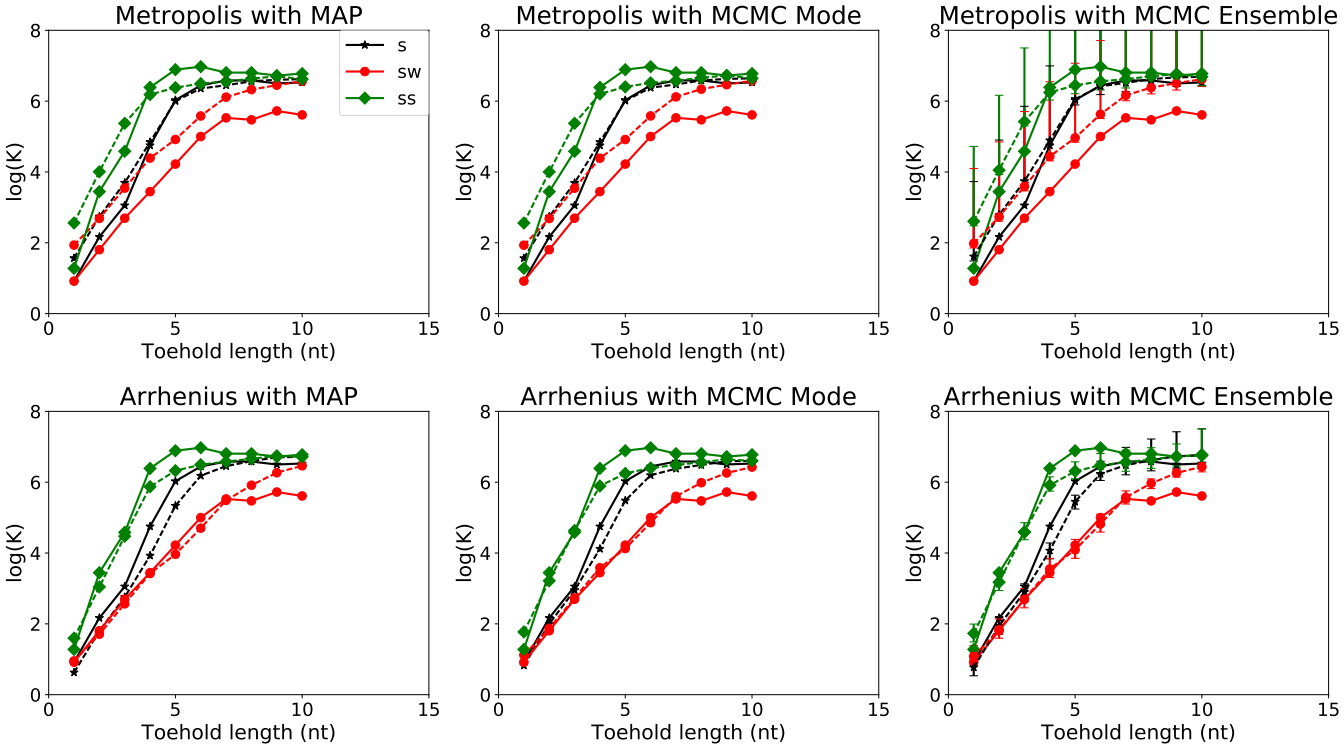


Fig. 13: Model fitting (dashed lines) of reaction rate constants (y axis) for toehold-mediated 3-way strand displacement, experimental data (solid lines) from Fig. 3b of Zhang and Winfree [9]. The toehold is varied between strong (ss), regular (s) and weak (sw) binding strength by varying the G/C content of the toehold sequence.

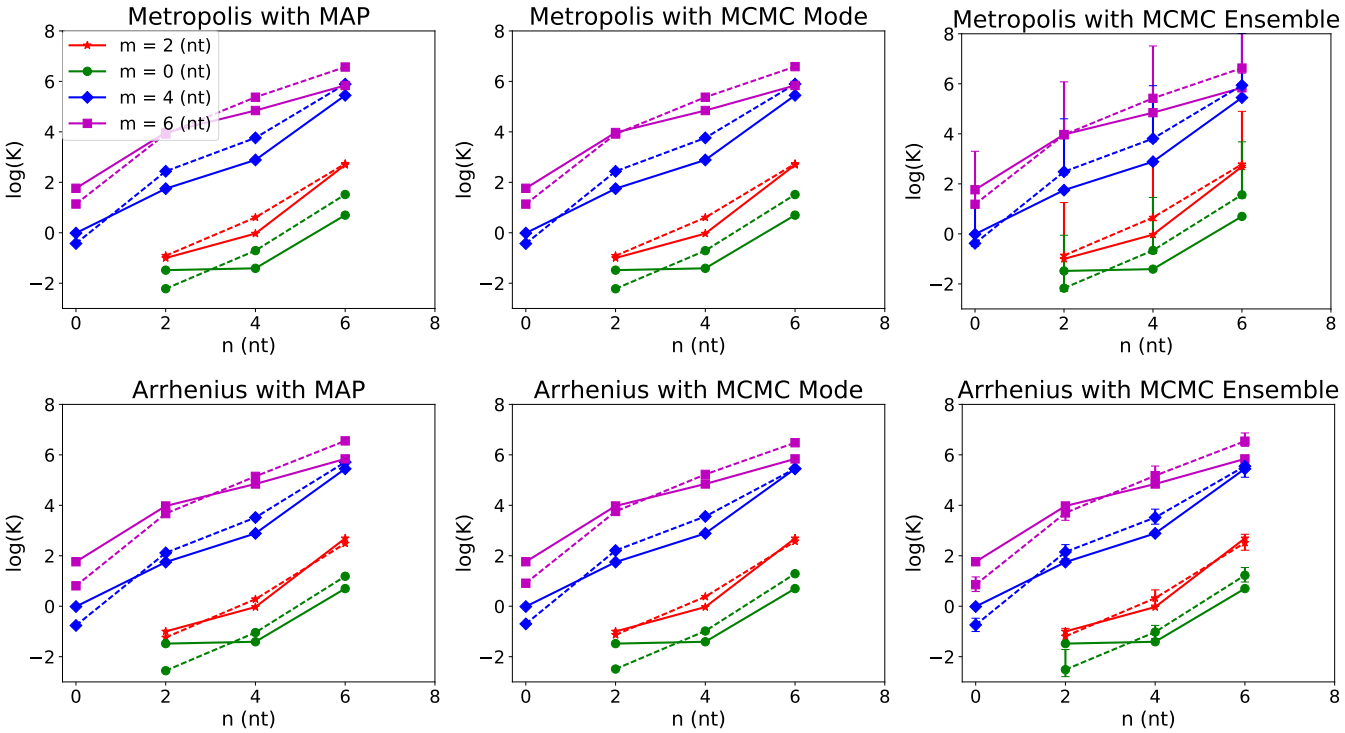


Fig. 14: Model fitting (dashed lines) of reaction rate constants (y axis) for toehold-mediated 4-way strand exchange, experimental data (solid lines) from Table 5.2 of Dabby [4]. m (shown on the legend) and n (shown on the x-axis) are variations in the length of the toehold domains (see Appendix B.5).

D.2 Testing Set ($\mathcal{D}_{\text{test}}$)

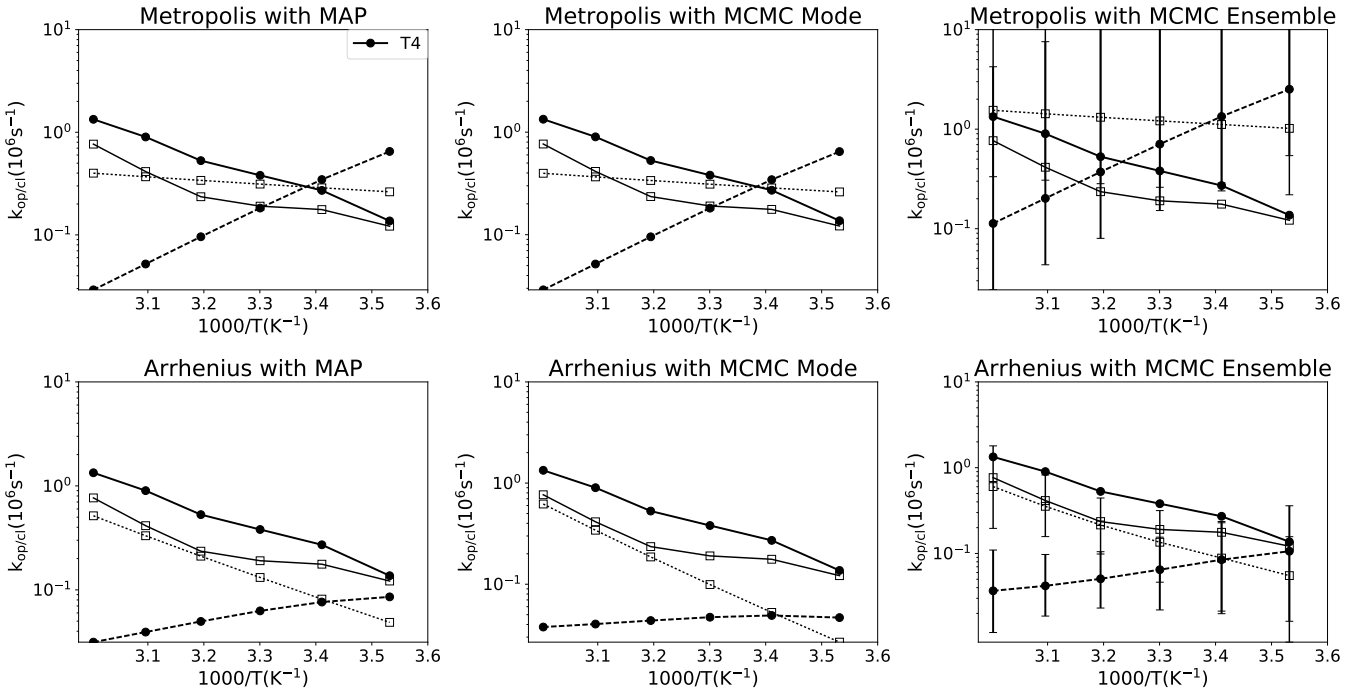


Fig. 15: Model predictions (dashed lines) of reaction rate constants (y axis) for hairpin closing (solid) and opening (open) with sequence F-$(dC)_2$-$(dT)_4$-$(dG)_2$, experimental data (solid lines) from Fig. 5a of Kim et al. [5].

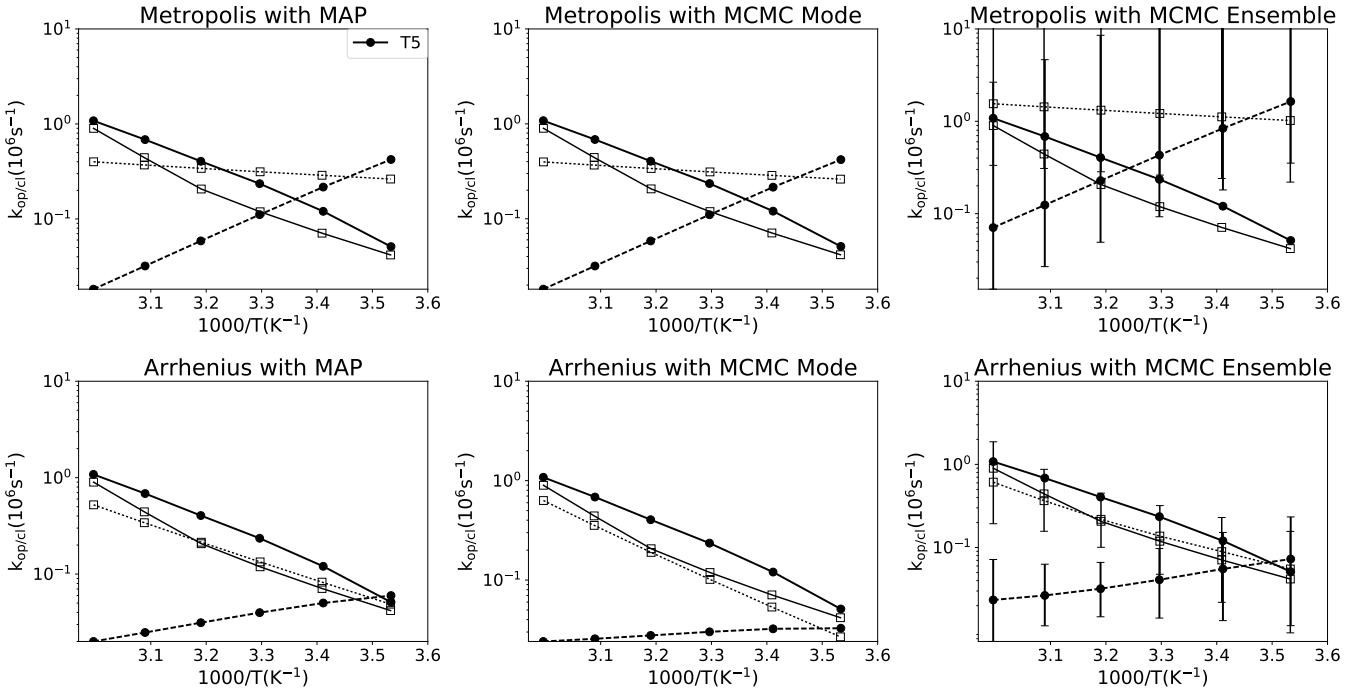


Fig. 16: Model predictions (dashed lines) of reaction rate constants (y axis) for hairpin closing (solid) and opening (open) with sequence F-$(dC)_2$-$(dT)_5$-$(dG)_2$, experimental data (solid lines) from Fig. 5b of Kim et al. [5].

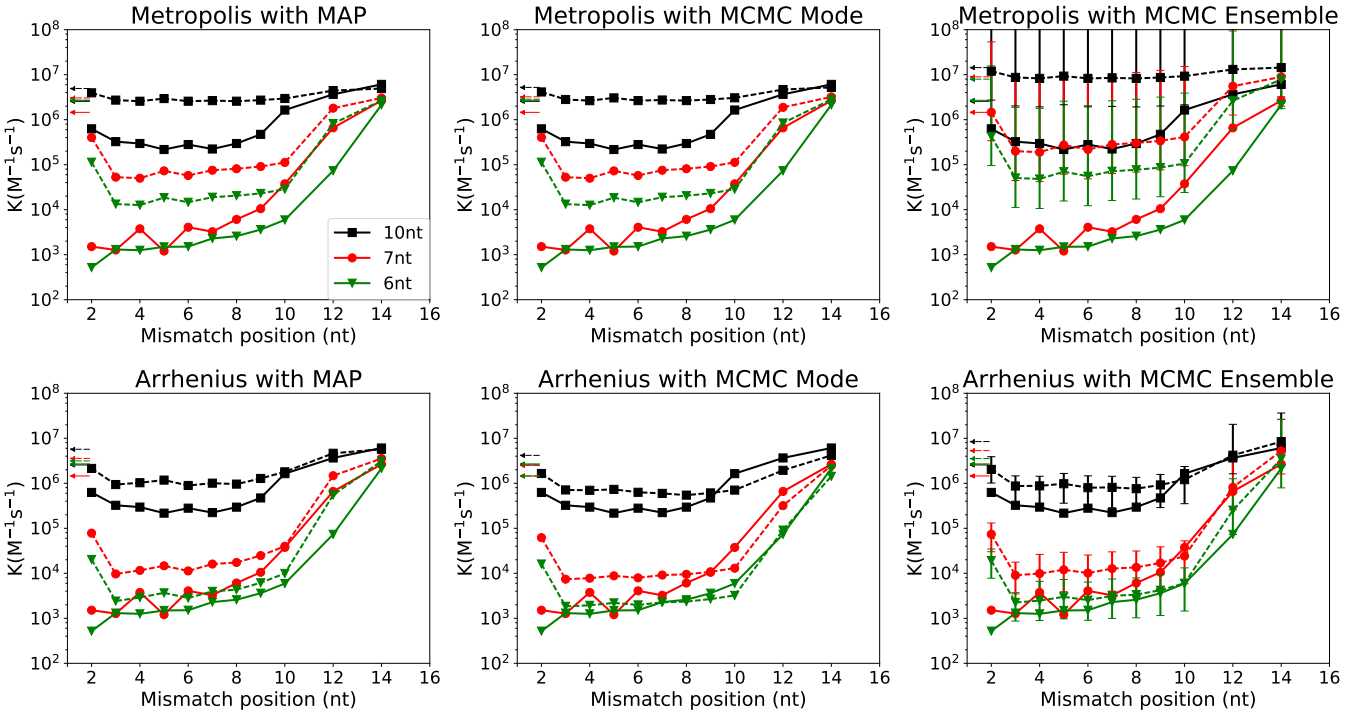


Fig. 17: Model predictions (dashed lines) of reaction rate constants (y axis) for toehold-mediated 3-way strand displacement with mismatches, experimental data (solid lines) from Fig. 2d of Machinek et al. [6]. Arrows indicate no mismatch. The mismatch in the invading strand affects the reaction rate. The length of the toehold domain is ten, seven, and six nucleotides long for ■, ●, and ▼, respectively.

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