Conditional Dicer Substrate Formation via Shape and Sequence Transduction with scRNAs

The reaction pathway is depicted in Figure 1. Target test tubes are depicted in Figure 2 based on the specification of (Wolfe et al., *J Am Chem Soc*, 2017; Supplementary Section S2.2) with the following definitions. The total number of target test tubes is $|\Omega| = \sum_{n=1,\ldots,N} \{\text{Step 0, Step 1, Step 2}\}_n + \text{Crosstalk} = 3N+1$; the target test tubes in the multistate test tube ensemble, Ω, are indexed by $h = 1, \ldots, 3N+1$. $L_{\max} = 2$ for all tubes.

Reactants for system n

- Target: X_n
- scRNAs: $\{\mathrm{A{\cdot}B},\ \mathrm{C}\}_n$

Elementary step tubes for system n

- Step 0_n: $\Psi_{0_n}^{\text{products}} \equiv \{\mathrm{X},\ \mathrm{A{\cdot}B},\ \mathrm{C}\}_n$; $\Psi_{0_n}^{\text{reactants}} \equiv \{\mathrm{A},\ \mathrm{B{\cdot}C}\}_n$; $\Psi_{0_n}^{\text{exclude}} \equiv \{\mathrm{X{\cdot}A}\}$
- Step 1_n: $\Psi_{1_n}^{\text{products}} \equiv \{\mathrm{X{\cdot}A},\ \mathrm{B}\}_n$; $\Psi_{1_n}^{\text{reactants}} \equiv \{\mathrm{X},\ \mathrm{A{\cdot}B}\}_n$; $\Psi_{1_n}^{\text{exclude}} \equiv \emptyset$
- Step 2_n: $\Psi_{2_n}^{\text{products}} \equiv \{\mathrm{B{\cdot}C}\}_n$; $\Psi_{2_n}^{\text{reactants}} \equiv \{\mathrm{B},\ \mathrm{C}\}_n$; $\Psi_{2_n}^{\text{exclude}} \equiv \emptyset$

Crosstalk tube

- Crosstalk tube: $\Psi_{\text{global}}^{\text{reactive}} \equiv \cup_{n=1,\ldots,N}\{\lambda_n^{\text{reactive}}\}$; $\Psi_{\text{global}}^{\text{crosstalk}} \equiv \Psi_{\text{global}}^{L \le L_{\max}} - \cup_{n=1,\ldots,N}\{\lambda_n^{\text{cognate}}\}$

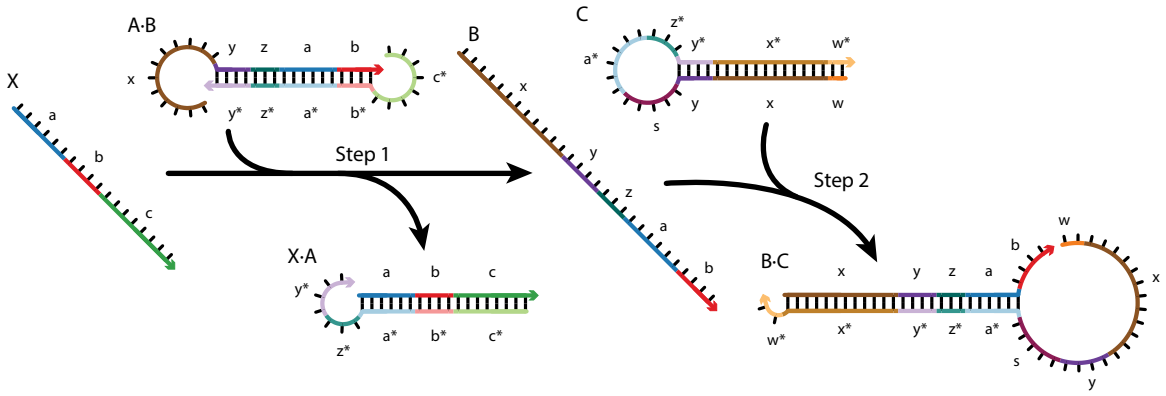
The reactive species and cognate products for system n are:

- $\lambda_n^{\text{simple}} = \{\mathrm{A{\cdot}B},\ \mathrm{C}\}_n$
- $\lambda_n^{\text{ss-out}} = \{\mathrm{X},\ \mathrm{B},\ \mathrm{C}^{\text{out}}\}_n$
- $\lambda_n^{\text{ss-in}} = \{\mathrm{A}^{\text{toe}},\ \mathrm{C}^{\text{loop}}\}_n$
- $\lambda_n^{\text{reactive}} = \{\mathrm{A{\cdot}B},\ \mathrm{C},\ \mathrm{X},\ \mathrm{B},\ \mathrm{C}^{\text{out}},\ \mathrm{A}^{\text{toe}},\ \mathrm{C}^{\text{loop}}\}_n$
- $\lambda_n^{\text{cognate}} = \{\mathrm{X{\cdot}A},\ \mathrm{B{\cdot}C},\ \mathrm{X{\cdot}A}^{\text{toe}},\ \mathrm{B{\cdot}C}^{\text{loop}}\}_n$

based on the definitions (listed $5'$ to $3'$ using the sequence domain notation of Figure 1):

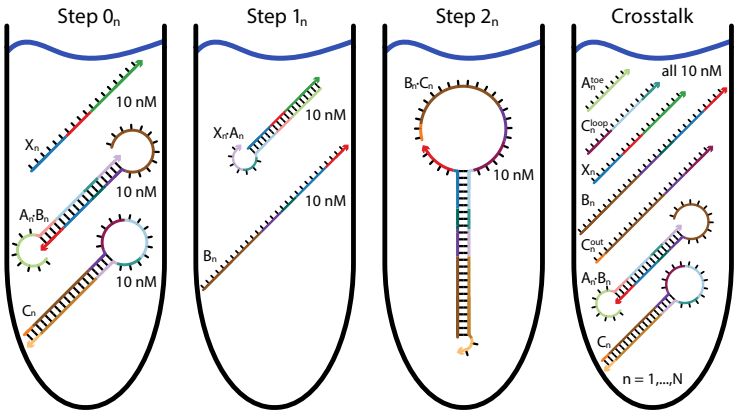
- $\mathrm{A} \equiv$ c*-b*-a*-z*-y*
- $\mathrm{A}^{\text{toe}} \equiv$ c*
- $\mathrm{B} \equiv$ x-y-z-a-b
- $\mathrm{C} \equiv \mathrm{C}^{\text{out}}\text{-}\mathrm{C}^{\text{in}}$
- $\mathrm{C}^{\text{loop}} \equiv$ s-a*-z*
- $\mathrm{C}^{\text{in}} \equiv$ a*-z*-y*-x*-w*
- $\mathrm{C}^{\text{out}} \equiv$ w-x-y-s
- $\mathrm{X} \equiv$ a-b-c

Note: $\mathrm{C}_n^{\text{loop}}$ includes portions of both C_n^{in} and $\mathrm{C}_n^{\text{out}}$. Including $\mathrm{C}_n^{\text{loop}}$ in $\lambda_n^{\text{reactive}}$ is not redundant with inclusion of C_n because pairing to the loop would cause a pseudoknot and hence will not be checked by the ensemble except if the interaction opens the hairpin. We want to be able to check nucleation with the loop even when the hairpin remains closed, so we include $\mathrm{C}_n^{\text{loop}}$ in $\lambda_n^{\text{reactive}}$.



Step	Reaction	Function	Mechanism
1	X + A·B → X·A + B	detect target X (sequence 'a-b-c')	toehold/toehold nucleation, 3-way branch migration, spontaneous dissociation
2	B + C → B·C	form Dicer substrate targeting independent target Y (sequence 'w-x-y-z')	toehold/loop nucleation, 3-way branch migration

Figure 1: Reaction pathway for conditional Dicer substrate formation via shape and sequence transduction with small conditional RNAs (scRNAs; Hochrein et al., *J Am Chem Soc*, 2013). scRNA A·B detects target X (comprising sequence 'a-b-c'), generating intermediate B that assembles with scRNA C to generate Dicer substrate B·C (targeting independent sequence 'w-x-y-z' for silencing). Top: Reaction pathway schematic. Bottom: Elementary step details.



Tube	On-targets (Ψ_h^{on})	Off-targets (Ψ_h^{off})
Step 0_n	$\{\text{X, A·B, C}\}_n$	$\{\text{A, B·C}\}_n \cup \Psi_{0_n}^{L\leq L_{\max}} - \{\text{X·A}\}$
Step 1_n	$\{\text{X·A, B}\}_n$	$\{\text{X, A·B}\}_n \cup \Psi_{1_n}^{L\leq L_{\max}}$
Step 2_n	$\{\text{B·C}\}_n$	$\{\text{B, C}\}_n \cup \Psi_{2_n}^{L\leq L_{\max}}$
Crosstalk	$\cup_{n=1,\ldots,N}\{\lambda_n^{\text{reactive}}\}$	$\Psi_{\text{global}}^{L\leq L_{\max}} - \cup_{n=1,\ldots,N}\{\lambda_n^{\text{cognate}}\}$

Figure 2: Target test tubes for conditional Dicer substrate formation via shape and sequence transduction with scRNAs. Top: Target test tube schematics. Bottom: Target test tube details. Each target test tube contains the depicted on-target complexes (each with the depicted target structure and a target concentration of 10 nM) and the off-target complexes listed in the table (each with vanishing target concentration). To simultaneously design N orthogonal systems, the total number of target test tubes is $|\Omega| = 3N + 1$. $L_{\max} = 2$ for all tubes. Design conditions: RNA in 1 M Na^+ at 37 °C.