Strand displacement gate

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 $|\Omega| = \sum_{n=1,\ldots,N}$ {Step 0, Step 1}$_n$ + Crosstalk = $2N+1$; the target test tubes in the multistate test tube ensemble, Ω, are indexed by $h = 1, \ldots, 2N+1$. $L_{\max} = 2$ for all tubes.

Reactants for system n

- Target: X_n
- Gate: $\{A{\cdot}B\}_n$

Elementary step tubes for system n

- Step 0_n: $\Psi^{\text{products}}_{0_n} \equiv \{\text{X, A}{\cdot}\text{B}\}_n$; $\Psi^{\text{reactants}}_{0_n} \equiv \{\text{A, B}\}_n$; $\Psi^{\text{exclude}}_{0_n} \equiv \{\text{X}{\cdot}\text{A}\}$
- Step 1_n: $\Psi^{\text{products}}_{1_n} \equiv \{\text{X}{\cdot}\text{A, B}\}_n$; $\Psi^{\text{reactants}}_{1_n} \equiv \{\text{X, A}{\cdot}\text{B}\}_n$; $\Psi^{\text{exclude}}_{1_n} \equiv \emptyset$

Crosstalk tube

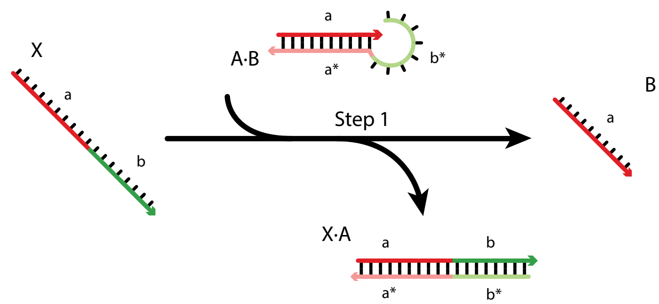
- Crosstalk tube: $\Psi^{\text{reactive}}_{\text{global}} \equiv \cup_{n=1,\ldots,N}\{\lambda^{\text{reactive}}_n\}$; $\Psi^{\text{crosstalk}}_{\text{global}} \equiv \Psi^{L \le L_{\max}}_{\text{global}} - \cup_{n=1,\ldots,N}\{\lambda^{\text{cognate}}_n\}$

The reactive species and cognate products for system n are:

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

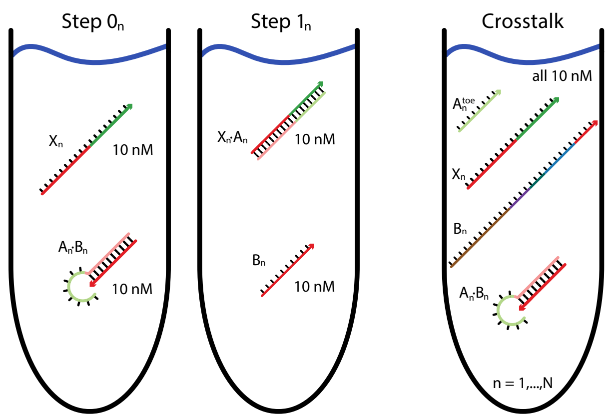
based on the definitions (listed $5'$ to $3'$ using the sequence domain notation of Figure 1):

- A ≡ b*-a*
- A^{toe} ≡ b*
- B ≡ a
- X ≡ a-b



Step	Reaction	Function	Mechanism
1	X + A·B → X·A + B	detect target X (sequence 'a-b')	toehold/toehold nucleation, 3-way branch migration

Figure 1: Reaction pathway for strand displacement gate. A·B detects target X (comprising sequence 'a-b-c'), generating unstructured output B. Top: Reaction pathway schematic. Bottom: Elementary step details.



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

Figure 2: Target test tubes for strand displacement gate. 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 $|\Omega| = 2N + 1$. $L_{\max} = 2$ for all tubes. Design conditions: RNA in 1 M Na^+ at 23 °C.