DNA-Based Oscillator Reactions

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DNA as a universal substrate for chemical kinetics

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(Used DNA Strand Displacement Reactions to Create Oscillators)
(Simulations only)
Lotka–Volterra Chemical Oscillator:

\[ X_1 = \text{prey (reproduces)} \]
\[ X_2 = \text{predator (consumes prey)} \]

(A) The formal chemical reaction system to be implemented with original (unscaled) and scaled rate constants.

Initial concentrations of \( X_1 \) and \( X_2 \) are 2 and 1 unscaled and 20 and 10 nM scaled.

(C) Plot of the concentrations of:
- \( X_1 \) (Red curve) and
- \( X_2 \) (Green curve)

Plot for the ideal system (Dashed line)
Corresponding DNA species: (Solid line).

**Ideal chemical reactions:**

1: \[ X_1 + X_2 \xrightarrow{k_1} 2X_2 \]
2: \[ X_1 \xrightarrow{k_2} 2X_1 \]
3: \[ X_2 \xrightarrow{k_3} 0 \]

<table>
<thead>
<tr>
<th>Unscaled</th>
<th>Scaled</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 )</td>
<td>1.5</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>1</td>
</tr>
<tr>
<td>( k_3 )</td>
<td>1</td>
</tr>
</tbody>
</table>

Lotka–Volterra Chemical Oscillator:

(B) Reaction modeling of DNA implementation:
- Each CRN reaction corresponds to a set of DNA reactions.
- Concentration oscillations are in the range of about 0–2.
- Under typical nucleic-acid experimental conditions, maximal second-order rate constants for strand displacement reactions are about 106/M/s, and maximum concentrations are on the order of 10–5 M.

Scaling:
- We scale the original system by a time-scaling factor of $\alpha = 300$, and
- A concentration-scaling factor $\beta = 10^{-8}$ employs units of seconds and molar, and
- use $C_{\text{max}} = 10^{-5}$ M and $q_{\text{max}} = 106/M/s$.
- Maximum strand displacement rate constant $q_{\text{max}} = 106\ M^{-1}\ s^{-1}$.

Initial Concentrations:
- initial concentration of auxiliary species $G_i$, $T_i$, $L_i$, $B_i$, $L_S$, and $B_S_i$ is $C_{\text{max}} = 10\ \mu$M.
- The initial concentrations of strands $X_1$ and $X_2$ is $\gamma^{-1}20\ nM = 40\ nM\ and\ \gamma^{-1}10\ nM = 20\ nM$.

Buffering:- Species $X_2$ requires a buffering module because $\sigma_2 < \sigma$ ($\sigma = \sigma_1 = k_1$ and $\sigma_2 = 0$).
- Buffering-scaling factor $\gamma^{-1} = q_{\text{max}}(q_{\text{max}} - \sigma)^{-1} = 2$.

**Figure B**

DNA reaction modules

\[
\begin{align*}
1 & \quad X_1 + L_1 \xrightarrow{q_1} H_1 + B_1 \\
2 & \quad X_2 + H_1 \xrightarrow{q_{\text{max}}} O_1 \\
3 & \quad O_1 + T_1 \xrightarrow{q_{\text{max}}} 2X_2 \\
4 & \quad X_1 + G_2 \xrightarrow{q_2} O_2 \\
5 & \quad O_2 + T_2 \xrightarrow{q_{\text{max}}} 2X_1 \\
6 & \quad X_2 + G_3 \xrightarrow{q_3} O_3 \\
7 & \quad X_2 + L_S \xrightarrow{q_{\text{max}}} H_{S2} + B_{S2}
\end{align*}
\]

**Figure C**

Simulation of ideal and DNA reactions

\[\text{X}_1\ \text{X}_2\ \text{ideal}\ \text{DNA}\]

(C) Plot of the concentrations of $X_1$ (Red curve) and $X_2$ (Green curve) for the ideal system (Dashed line) and the corresponding DNA species (Solid line).

Buffering module: DNA implementation of the buffering module used to cancel out the buffering effect.
- A buffering module is needed for each formal species $X_j$ for which $\sigma_j < \sigma$.
- The buffering module for species $X_2$ is shown.
- $X_2$ reversibly displaces $BS_2$ from complex $LS_2$ to produce complex $HS_2$ similarly to the first reaction of the bimolecular module.
- The black domain (13) is unique to this buffering module for species $X_2$.
- Set $qs_j = \gamma^{-1}(\sigma - \sigma_j)$.

\[
X_j + LS_j \xrightarrow{qs_j \over q_{\text{max}}} HS_j + BS_j \quad [12]
\quad \Rightarrow \quad X_j \xrightarrow{qs_j C_{\text{max}} \over q_{\text{max}} C_{\text{max}}} HS_j \quad [13]
\]

Reaction equations 12 are used in simulations; Simplified reaction equations 13 are useful for analysis.

Programming chemical kinetics: engineering dynamic reaction networks with DNA strand displacement


(Used DNA Strand Displacement Reactions to Create Oscillators)
Examples of formal CRNs exhibiting different dynamical behaviors in the mass action setting (based on numerical solutions to mass action ODEs).

(From PhD Thesis, Niranjan Srinivas)

**a** stabilization

\[
\begin{align*}
A & \xrightarrow{1} B \\
A + B & \xrightarrow{0.5} B \\
A & \xrightarrow{2} 2A \\
([A], [B])_0 &= (2, 0) \text{ M}
\end{align*}
\]

**b** oscillation

\[
\begin{align*}
B + A & \xrightarrow{1} 2B \\
C + B & \xrightarrow{1} 2C \\
A + C & \xrightarrow{1} 2A \\
([A], [B], [C])_0 &= (2, 1, 1) \text{ M}
\end{align*}
\]

**c** chaos

\[
\begin{align*}
A & \xrightarrow{30} 2A \\
2A & \xrightarrow{0.5} A \\
B + A & \xrightarrow{1} 2B \\
B & \xrightarrow{10} \text{ (no reaction)} \\
A + C & \xrightarrow{1} \text{ (no reaction)} \\
C & \xrightarrow{16.5} 2C \\
2C & \xrightarrow{0.5} C \\
([A], [B], [C])_0 &= (10, 10, 10) \text{ M}
\end{align*}
\]
Rock-Paper-Scissors
Oscillator:
- A is rock,
- B is paper, and
- C is scissors.
It has 3 autocatalytic reactions:
  B+A → 2B (paper covers rock),
  C+B → 2C (scissors cuts paper), and
  A+C → 2A (rock breaks scissors),
The sum of the species A + B + C is conserved.

- If the rate constants for the three autocatalytic reactions are identical, the product A × B × C is also conserved.
- So the dynamics is constrained to be:
  • on the intersection of the plane A + B + C = constant and
  • the curve A × B × C = constant
- But there is no equilibrium on that intersection, resulting in characteristic triangle-like orbits.

(From PhD Thesis, Niranjan Srinivas)
Overview of CRN-to-DNA implementation:
- Start with a desired dynamical behavior (oscillation, in this case) and a CRN program that captures the desired dynamics.
- We then use the CRN-to-DNA scheme described in this chapter to translate the formal CRN into a DNA strand displacement implementation, where the formal species are represented by single strands of DNA called “signal” species.
- Desired reactions between signal species are mediated by “fuel” species which provide both logic and free-energy for the reaction.
- Some of the fuel species are multistranded complexes which are pre-prepared and purified. In the regime where the fuel species are at high concentration, the signal species approximate the dynamics of the formal species in the original CRN.
- Reactions are performed in “batch reactor” mode, which means that fuel species are not replenished.
(Therefore, the test tube dynamics is expected to deviate from idealized formal CRN dynamics.)

(From PhD Thesis, Niranjan Srinivas)
Domain notation: A “domain” comprises contiguously located bases whose binding and unbinding occurs as one logical unit. * indicates Watson-Crick complementarity. Arrows indicate 3’ ends.

Toehold exchange Reaction: “Short” (5-7 nucleotide) domains which bind fleetingly to their components at room temperature and reversibly co-localize distinct molecules are called “toeholds”.
- Here toehold t reversibly co-localizes the molecules to form a three stranded intermediate, where the two b domains can exchange base pairs by a process called three-way branch migration.
- Eventually, either toehold u dissociates (leading to the products) or toehold t dissociates (leading to the reactants).
- Notice that the entire process is reversible and toehold u can also carry out toehold exchange.

(From PhD Thesis, Niranjan Srinivas)
The formal species are represented by single-stranded DNA molecules (signal strands):
- With a history domain in black (versioning unit, e.g. $h_{Br}$) and a logical unit.
- The logical unit with three domains:
  - the first toehold (e.g. $f_B$),
  - a branch migration region (e.g. $m_B$), and
  - the second toehold (e.g. $s_B$).
- Signal strands are designed to not interact with each other.
- Signal strands with the same logical unit (e.g. $B_r$ and $B_s$) represent the same formal species (B) and are designed to behave identically in solution.

(From PhD Thesis, Niranjan Srinivas)
DNA Strand-Displacement Implementation of Cyclic Lotka-Volterra Oscillator Module 1: Reaction B + A → 2B.

- The same mechanism can occur with different versions of B and A.
- Names of fuel species are enclosed in a dashed box.  
  (From PhD Thesis. Niranjan Srinivas)
Kinetic Model for Simulation of DNA Strand-Displacement Implementation of Cyclic Lotka-Volterra Oscillator

Module 1: Reaction B + A → 2B:

\[ B + \text{React}_{\text{BABr}} \xrightleftharpoons[k_{sd}]{k_{sd}} \text{ReactInt}_{\text{BABr}} + \text{Back}_{\text{BA}} \]
\[ \text{ReactInt}_{\text{BABr}} + A \xrightarrow{k_{sd}} \text{Flux}_{\text{ABr}} + \text{Waste}_{\text{BA}} \]
\[ \text{Flux}_{\text{ABr}} + \text{Produce}_{\text{ABrBs}} \xrightleftharpoons[k_{sd}]{k_{sd}} \text{B} + \text{ProduceInt}_{\text{ABrBs}} \]
\[ \text{ProduceInt}_{\text{ABrBs}} + \text{Helper}_{\text{BBs}} \xrightarrow{k_{sd}} \text{B} + \text{Waste}_{\text{ABrBs}} \]

- Equations above specify the chemical reaction equations in the strand-displacement level model for the autocatalytic module B + A → 2B.

- Each strand displacement and toehold exchange reaction has been modeled as an effective bimolecular reaction with a rate constant of \( k_{sd} = 2 \times 10^5 \text{ /M /s} \).

- This model assumes that unimolecular reactions (including dissociation of toeholds and branch migration) are effectively instantaneous.

- All fuel molecules have an initial concentration of 300 nM and are not replenished.

(From PhD Thesis, Niranjan Srinivas)
The same mechanism can occur with different versions of C and B. Names of fuel species are enclosed in a dashed box. (From PhD Thesis, Niranjan Srinivas)

The same mechanism can occur with different versions of $A$ and $C$. Names of fuel species are enclosed in a dashed box. *(From PhD Thesis, Niranjan Srinivas)*
Modeling of DNA Strand-Displacement Implementation of Cyclic Lotka-Volterra Oscillator: Modeling the DNA implementation of the oscillator at the level of individual strand displacement and toehold exchange reactions. The fuel species are present at an initial concentration of 300 nM and are not replenished.

(a) \(([[A], [B], [C]])_0 = (30, 10, 10)\) nM

(b) \(([[A], [B], [C]])_0 = (60, 10, 10)\) nM

a. Concentrations of Product molecules (dashed lines; - - - ProduceCApAq in red, ProduceBCjCk in blue, ProduceABrBs in orange) and signal strands as a function of time starting with an initial concentration of \((([[A], [B], [C]])_0 = (30, 10, 10)\) nM.

b. The plot in (a), zoomed in so that the oscillatory dynamics of the signal strands are visible.

c. The plot in (b) with an initial concentration of \((([[A], [B], [C]]) = (60, 10, 10)\) nM, all other parameters being the same.

(From PhD Thesis, Niranjan Srinivas)
Examples of spurious “leak” pathways that arise due to blunt-end (zero base toehold) strand displacement.
- These pathways are illustrated in the case of the autocatalytic module $C + B \rightarrow 2C$ but can occur with the other modules as well.
- Locations of invasion are indicated by numbered dashed arrows.

a. The second input (here, $Br$) can invade at locations 1 (the junction) and 2 (the end of the helix) in the React species. Once strand displacement finishes, the Flux molecule may be released and a spurious species can be formed.

b. A similar reaction can happen between the Helper species and the Produce species, releasing the second output of the Produce molecule (here, $C_k$) and resulting in a spurious species.

c. Spontaneous fraying due to thermal fluctuations at the end of the helix in the React molecule may enable the Produce molecule to invade at location 5. Strand displacement can then result in the release of the first output of the produce gate (here, $C_j$) and the formation of a spurious species. Notice that all of these spurious species shown here are capable of participating in some reactions that are also a legitimate part of desired reaction pathways.

(From PhD Thesis, Niranjan Srinivas)
Programming an in vitro DNA oscillator using a molecular networking strategy

Kevin Montagne, Raphael Plasson, Yasuyuki Sakai, Teruo Fujii and Yannick Rondelez

Molecular Systems Biology 7; Article number 466, 2010
doi:10.1038/msb.2010.120

(Used DNA Polymerase and Nickase Enzymic Reactions to Create Oscillators)
(A) Schematic description of the canonical gene regulation pathway.

‘--->’ Indicates an activation interaction

‘---|’ Indicates inhibition

‘--o’ represents either activation or inhibition

‘-+’ indicates decay.

(B) A similar architecture is implemented, but genes are replaced by single-stranded DNA templates, while dynamic species (RNA and proteins) are replaced by small oligomers obtained from replication of the templates.

Kevin Montagne, Raphael Plasson, Yasuyuki Sakai, Teruo Fujii and Yannick Rondelez, Programming an in vitro DNA oscillator using a molecular networking strategy, Molecular Systems Biology 7; Article number 466; doi:10.1038/msb.2010.120
(C) Molecular description of the activation mechanism. The input oligomer $\alpha$ binds to the template and is elongated by a polymerase. $\alpha$ displays a recognition sequence (in bold), which allows a nicking endonuclease to nick the newly extended strand. This step releases the input $\alpha$, the output strand $\beta$ and the template, ready for a new turnover.

(D) Inhibition mechanism. The inhibitor Inh is designed to bind strongly to the template but, due to a pair of mismatches at its 30 end, it is not recognized as a polymerization primer. Therefore, the template is reversibly sequestered as an unproductive partial duplex.
(E) Cascading: Previous activation or inhibition blocks can be connected to each other by simply matching their sequences (shown here using a color code).

(F) Implementation: an oscillator comprising a positive-feedback loop (+) and a delayed negative-feedback loop (-).

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Experiments: Reactions shown were performed at 38.51°C, initiated with 0.1 nM a and monitored (ex. 490 nM; em. 510 nM) using EvaGreen- induced fluorescence.

(A) One-node positive-feedback loop (autocatalytic module):
- In the presence of Bst Polymerase (80 U ml⁻¹) and nicking enzyme Nt.bstNBI (200 U ml⁻¹), template T₁ (60 nM) performs an exponential amplification of its input a.
- The fluorescence reaches a plateau when the template gets saturated with a.
- The low subsequent increase is due to the accumulation of single-stranded a, weakly fluorescent in these conditions.
- In the presence of exonuclease RecJ₁ (30 U ml⁻¹), the reaction reaches a flat steady state instead.

(B) Inhibited amplification: Increasing amounts of inhibitor (from 0 to 1 eq. of T₁) decreases the amplification rate of the previous system (RecJ₁).

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(C) Oscillator.
- Production of Inh is connected to the presence of a as in Figure 1F.
- This three-templates (T₁ and T₂: 30 nM; T₃: 5 nM) three-enzymes (Bst, Nt.BstNBI, RecJ) system produces sustained fluorescent oscillations with a period of 100 min.
- Agrees with predicted evolution of total concentration of base pairs (D).

Kevin Montagne, Raphael Plasson, Yasuyuki Sakai, Teruo Fujii and Yannick Rondelez, Programming an in vitro DNA oscillator using a molecular networking strategy, Molecular Systems Biology 7; Article number 466; doi:10.1038/msb.2010.120
Synthetic in vitro transcriptional oscillators

Jongmin Kim and Erik Winfree

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Supplementary Info:
http://www.dna.caltech.edu/Papers/transcriptional_oscillators2011_SI.pdf

(Used DNA Hybridization and Enzyme Reactions to Create Oscillators)
Schematics for vitro transcriptional oscillators. 

(A) Reaction diagram for the two-switch negative-feedback oscillator.

- On the top left is a block diagram, wherein arrowheads indicate activation or production and circular ends indicate inhibition.
- The block diagram corresponds to the detailed diagram as highlighted by gray shading:
- T21A1 (ON-state switch Sw21) and T21 (OFF-state switch Sw21) are summarized by the Sw21 block;
- RNA inhibitor rl2, together with its threshold, DNA activator A2, and their complex, A2rl2, is summarized by the rl2 block; and
- similarly for the Sw12 and rA1 blocks.

**Coloring:** The sequence domains are color coded to indicate identical or complementary sequences; for the switch templates, the dark blue sequence domain inside the rectangle indicates the T7 RNAP promoter sequence with arrows pointing in the direction of transcription, with transcription domains indicated by light blue dashed circles.
- Transcription by RNAP is shown as black dashed arrows - Degradation of RNA within RNA-DNA hybrids by RNase H is shown as black dotted arrows.
- Hybridization reactions are reversible; the arrowhead corresponds to the thermodynamically favorable direction, and the reverse reactions are expected to be so slow as to be negligible

**For fluorescence monitoring:**
- OFF-state switches are labeled with fluorophores, T21 with Texas Red (red circle) and T12 with TAMRA (green circle), and both activators A1 and A2 are labeled with Iowa Black RQ quenchers (black circle).
- Four types of hybridization reactions are indicated by arrows: activation (magenta), inhibition (orange), annihilation (brown), and release (blue).

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(B) List of hybridization and enzyme reactions.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activation</td>
<td>T21 + A1 → T21A1</td>
</tr>
<tr>
<td>Inhibition</td>
<td>T21A1 + dl1 → T21 + A1dl1</td>
</tr>
<tr>
<td>Annihilation</td>
<td>A1 + dl1 → A1dl1</td>
</tr>
<tr>
<td>Release</td>
<td>A1 + dl1 → A1dl1</td>
</tr>
<tr>
<td>RNAP</td>
<td>A1dl1 + rA1 → A1dl1 + A</td>
</tr>
<tr>
<td>RNaseH</td>
<td>rA1dl1 → dl1</td>
</tr>
<tr>
<td>RNaseH (fast)</td>
<td>T21A1 → T21A1 + rl2</td>
</tr>
<tr>
<td>RNaseH (slow)</td>
<td>T21 → T21 + rl2</td>
</tr>
<tr>
<td>T12A2 + rl2 → T12 + A2rl2</td>
<td></td>
</tr>
<tr>
<td>T2 + A2rl2 → A2rl2</td>
<td></td>
</tr>
</tbody>
</table>

(C) Theoretical end states of hybridization reactions in the absence of enzymes.
- As the input RNA inhibitor rI2 concentration increases, initially the free DNA activator A2 is consumed without affecting switch state.
- When all free A2 is consumed (i.e., [rI2]1/4[A2tot]−[T12tot]), rI2 displaces A2 from the T12A2 complex in stoichiometric amounts until all A2 is consumed (i.e., [rI2]1/4[A2tot]), resulting in a piecewise linear graph.
- Similarly, the response of switch Sw21 to rA1 input is a piecewise linear graph.
Simple model characterization and experimental results of the two-switch negative-feedback oscillator (Design I).

- The phase diagram of dynamic behaviors shows two domains with respect to changes in a and b (unitless parameters proportional to [T12\textsuperscript{tot}] and [T21\textsuperscript{tot}]) versus n and m (apparent Hill exponents of the activation and inhibition functions).
- (a, b) Phase portraits using unitless dynamic variables proportional to [rA1] and [rI2], x and y. The nullclines for x (violet lines) and y (black lines) are superimposed with temporal trajectories (cyan lines). There are fixed points where x and y nullclines intersect; stable (gray circle) and unstable (red circle) fixed points are marked.
- The system exhibits (a) limit cycle oscillation or (b) damped oscillation to a monostable steady state.
- Parameters are [T12\textsuperscript{tot}]1/4[T21\textsuperscript{tot}]1/4[A1\textsuperscript{tot}]1/4 100 nM, KA1/4KI1/41 mM, n1/4m1/45, t1/4500 s, g1/41, kd1/40.002/s, and respectively kp1/40.04/s or kp1/40.025/s.
- In experiments, normalized fluorescence time courses measured the percent OFF-state switch Sw12 (TAMRA-labeled T12, green) and the percent OFF-state switch Sw21 (Texas Red-labeled T21, red).

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Two examples are shown:
- Reaction #8 with stable oscillations and
- Reaction #13 with strongly damped oscillations.

- Experimental parameters were, respectively, ([T21^{tot}], [A1^{tot}], [dI1^{tot}], [T12^{tot}], [A2^{tot}], [RNAP^{tot}], [RNaseH^{tot}])1/4(250, 250, 700, 120, 350, 92, 8.3) nM and (250, 250, 1000, 80, 500, 125, 15.0) nM.
- Reaction #13 used higher [dI1^{tot}], higher [A2^{tot}], and lower [T12^{tot}]), compared with reaction #8.
- Thus, reaction #13 is mapped to a higher n and a lower a than reaction #8, and lay within the monostable domain rather than the oscillating domain.

- Experimental results were mapped to the phase diagram with respect to a reference oscillation, and shown as ‘stable’ (circles, damping coefficient 00.15/h), ‘damped’ (dots, damping coefficient between 0.15/h and 0.5/h), and ‘strongly damped or too slow to measure’ (crosses, damping coefficient 40.5/h) oscillations.

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Experimental characterization of a two-switch negative feedback oscillator.

(A) The fluorescence time courses report the OFF-state switch Sw12 (TAMRA-labeled T12, green) and the OFF-state switch Sw21 (Texas Red-labeled T21, red); corresponding extended model fits are shown as lines in lighter shades. The non-denaturing gel measurement of the OFF-state Sw21 (black squares, from C below) showed reasonable agreement with fluorescence results.

(B) The total rI2 concentration measurement (blue circles, from C below) showed five complete oscillation cycles; extended model fits are shown as lines (light blue). The concentration of incomplete degradation products was estimated from the band of E35 nucleotides in the denaturing gel (magenta). The rA1 concentration was not measured because most bands were not significantly above background.

(C) The concentrations of RNA signals and incomplete degradation products were measured in a denaturing gel (top and middle) and the OFF-state switch concentrations were measured in a non-denaturing gel (bottom)