Programming an in vitro DNA oscillator using a molecular networking strategy

运用分子网络策略构建体外DNA振荡子

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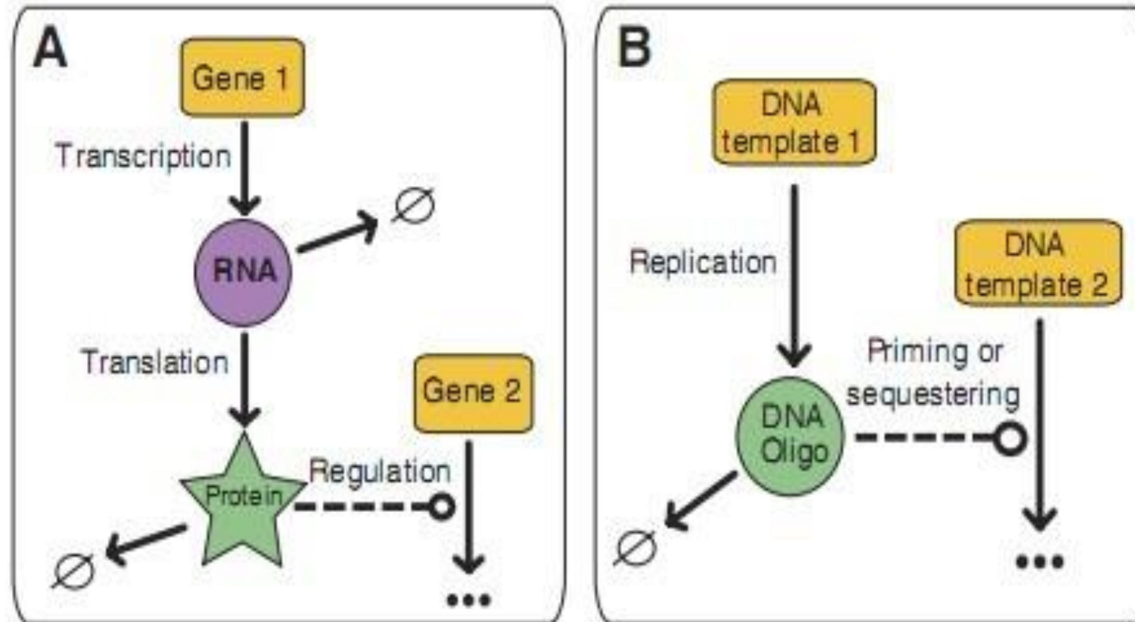
Background

- Living organisms perform and control complex behaviours by using webs of chemical reactions organized in precise networks however, it is extremely difficult to rationally create such network architectures in artificial, non-living and well-controlled settings

- In vitro systems serious challenges remain, the cascadability of the various modules, the precise control of the sequence of events and the correct balance between production and destruction of the dynamic species

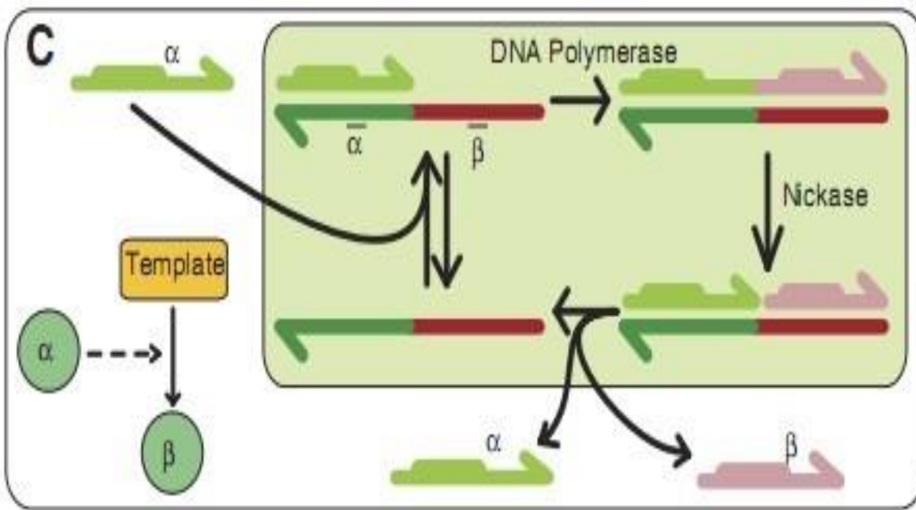
- gene regulatory networks we noticed that their architecture relies mostly on a limited set of basic events: activation inhibition and destruction.

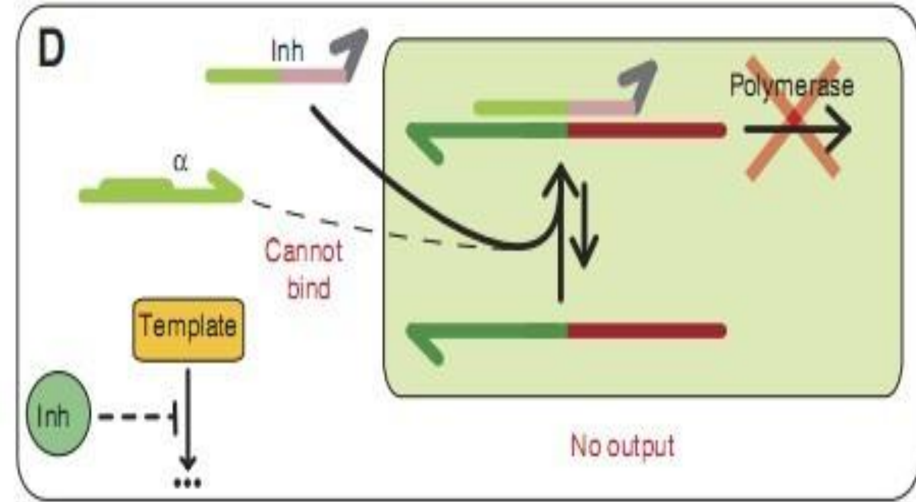
Designing reaction networks



(A) Schematic description of the canonical gene regulation pathway.

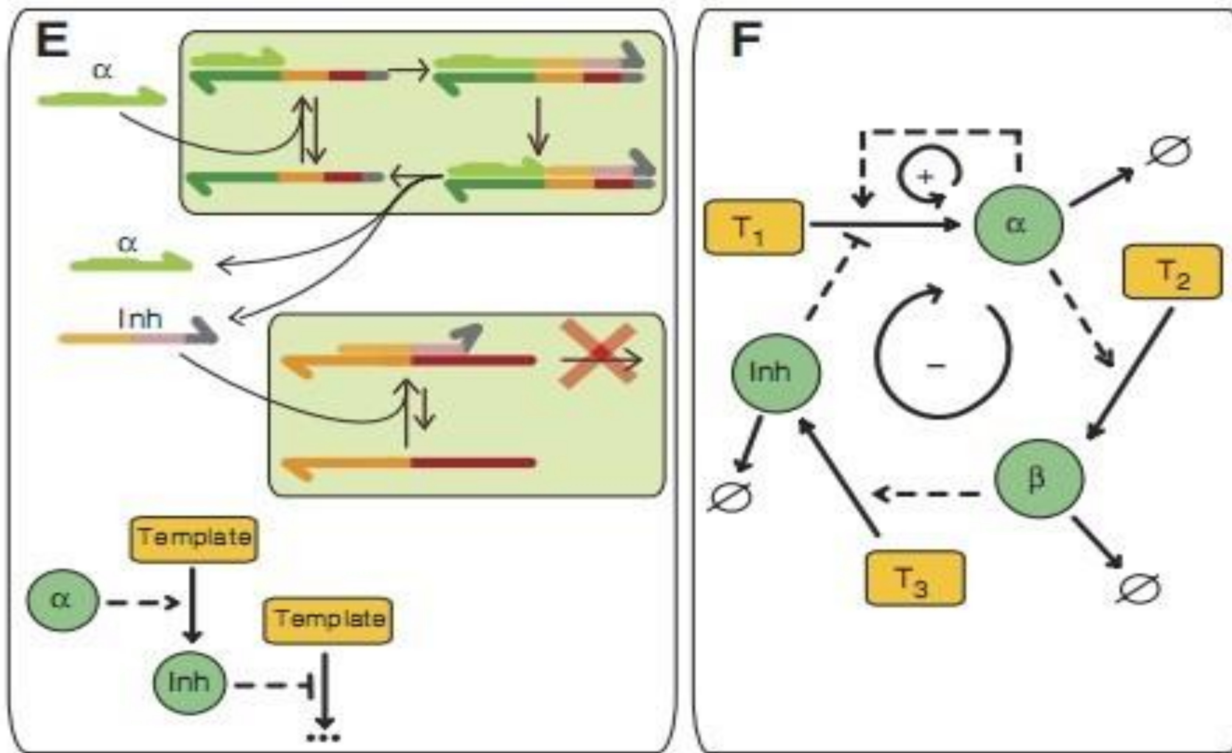
(B) A similar architecture is implemented, but genes are replaced by single-stranded DNA templates,while dynamic species (RNA and proteins) are replaced by small oligomers obtained from replication of the templates.





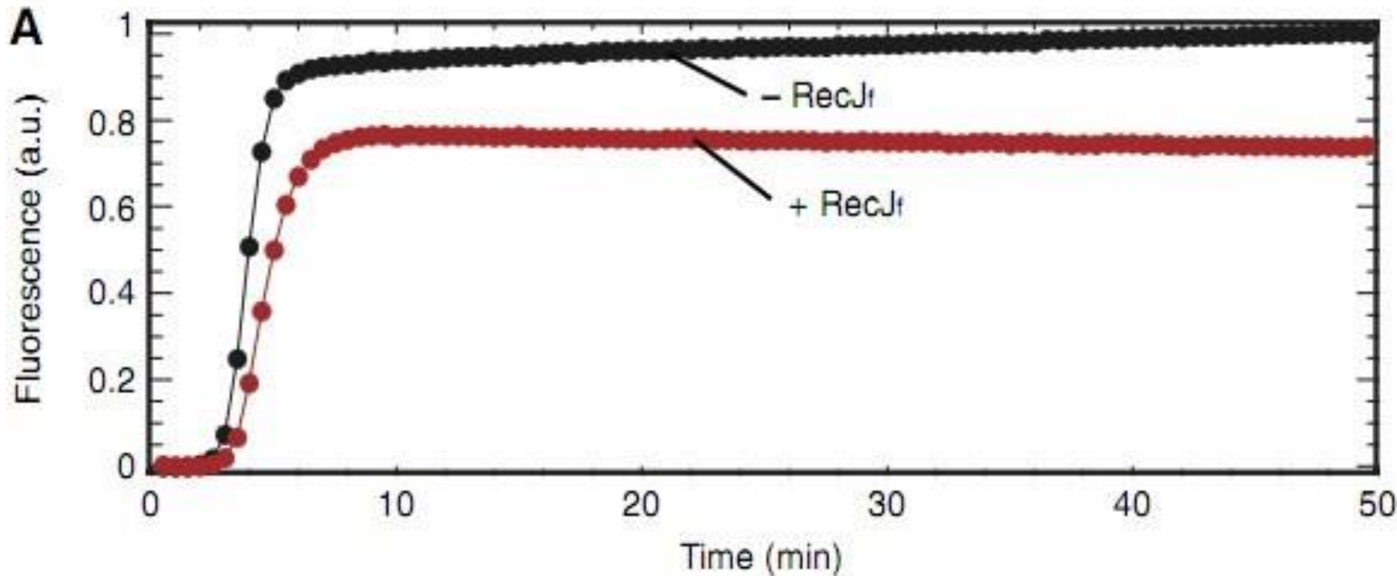
(C) Molecular description of the activation mechanism. The input oligomer a binds to the template and is elongated by a polymerase. a displays a recognition sequence, which allows a nicking endonuclease to nick the newly extended strand. This step releases the input a, the output strand b and the template, ready for a new turnover.

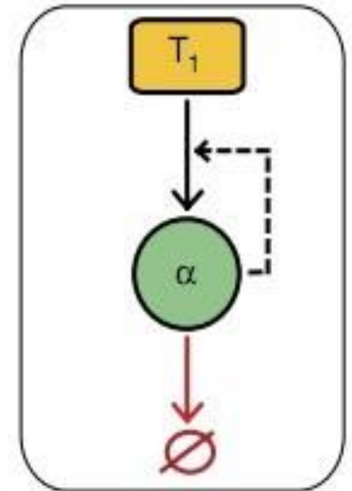
(D)Inhibition mechanism. The inhibitor Inh is designed to bind strongly to the template but, due to a pair of mismatches at its 3’ end, it is not recognized as a polymerization primer. Therefore, the template is reversibly sequestered as an unproductive partial duplex



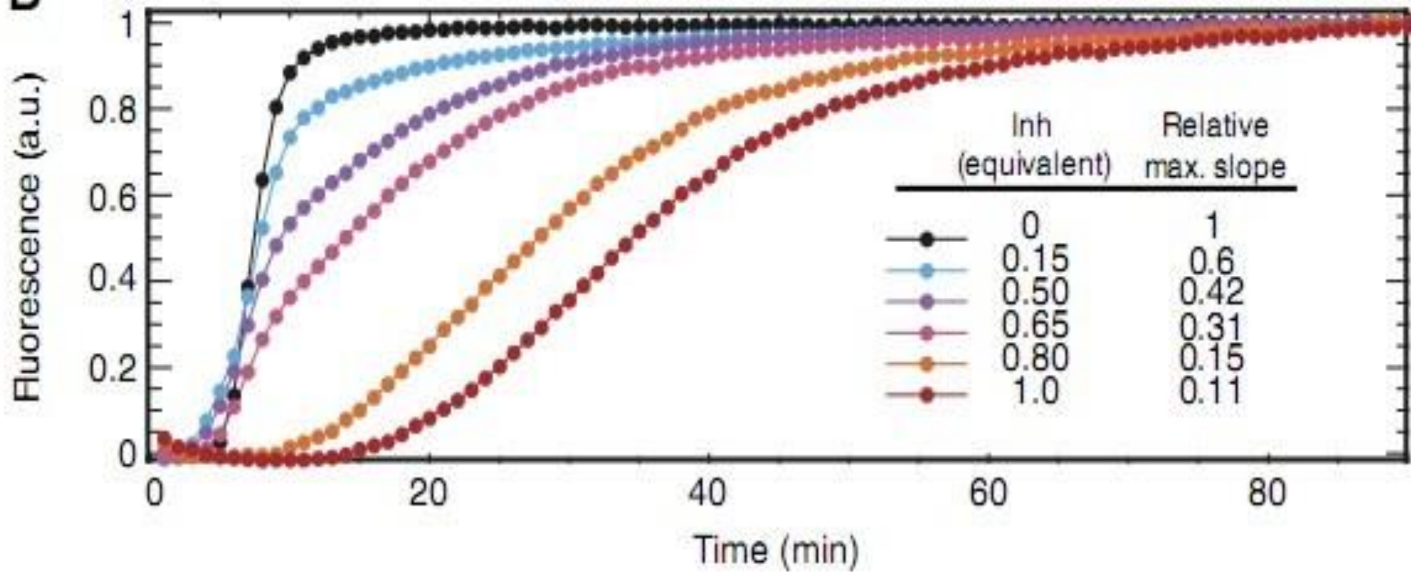
(E) Cascading. Previous activation or inhibition blocks can be connected to each other by simply matching their sequences

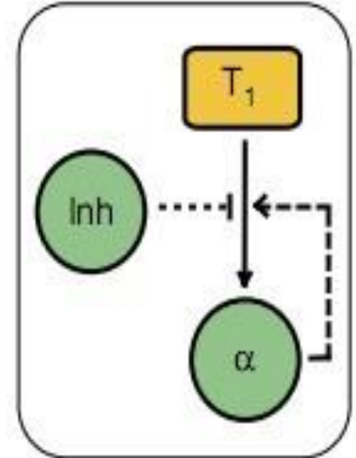
(F) Implementation, within this framework, of an oscillator comprising a positive-feedback loop (+) and a delayed negative-feedback loop (-)



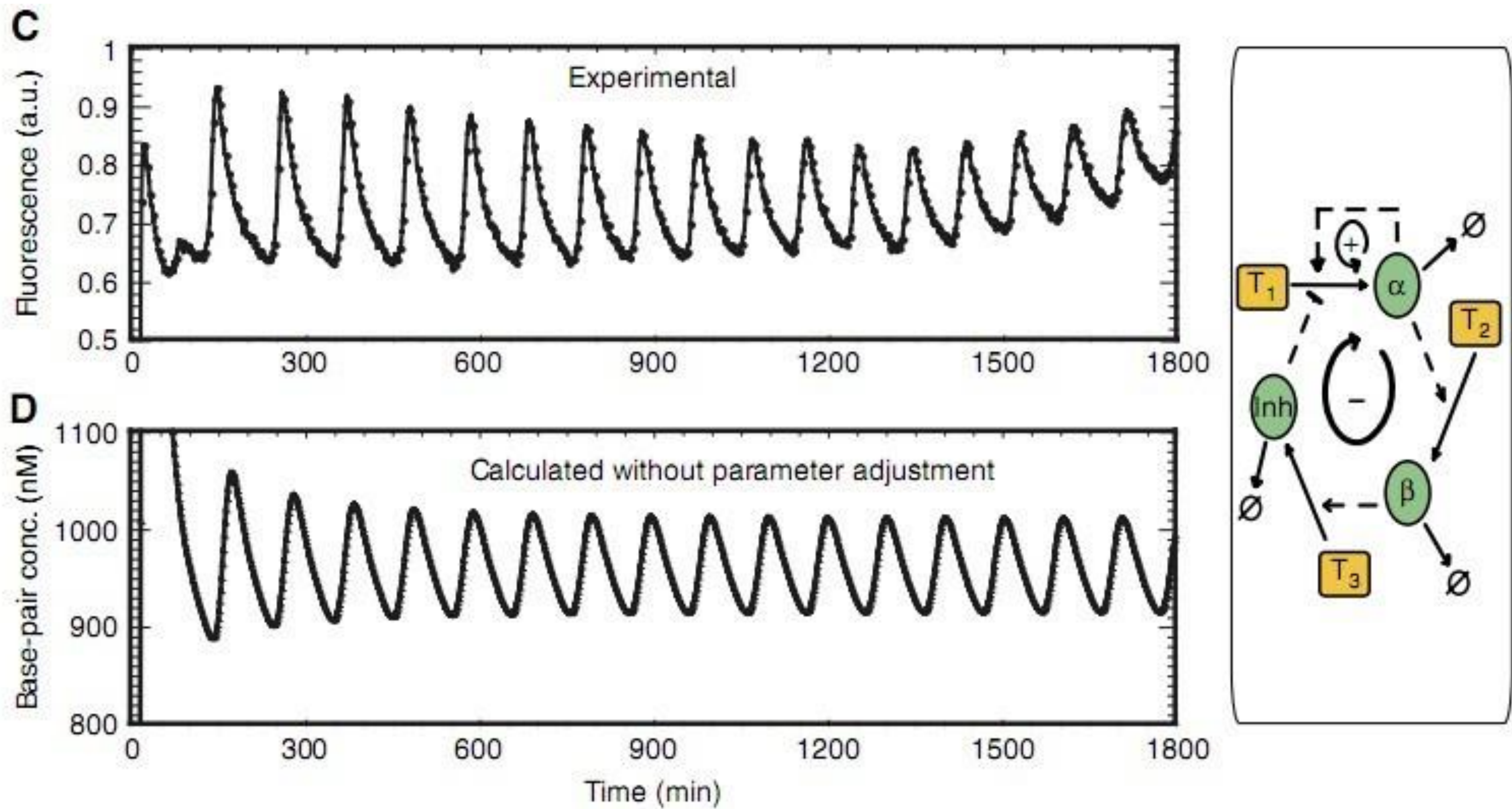


(A) One-node positive-feedback loop (autocatalytic module). In the presence of Bst Polymerase (80 Uml^{-1}) and nicking enzyme Nt.bstNBI (200 U ml^{-1}), template T1 (60 nM) performs an exponential amplification of its input a. The fluorescence reaches a plateau when the template gets saturated with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



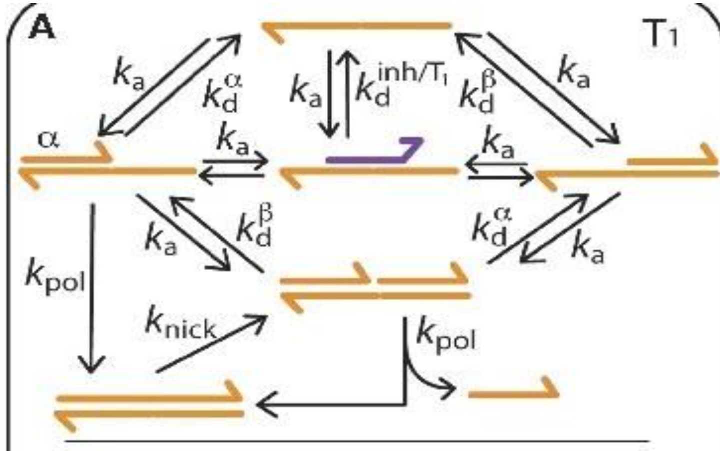


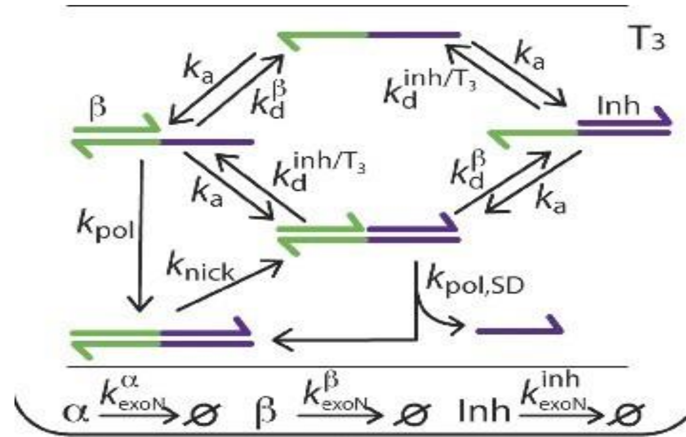
(B) Inhibited amplification. Increasing amounts of inhibitor (from 0 to 1 eq. of T1) decrease the amplification rate of the previous system (-RecJf).

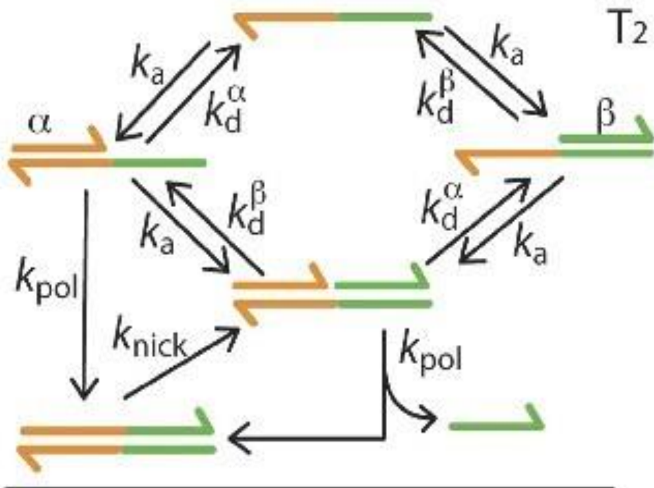


(C) Oscillator. Production of Inh is connected to the presence of a as in Figure 1F. This three-templates (T1 and T3: 30 nM; T2: 5 nM) three-enzymes (Bst, Nt.BstNBI, RecJf) system produces sustained fluorescent oscillations with a period of 100 min, in good agreement with the predicted evolution of the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

raw model







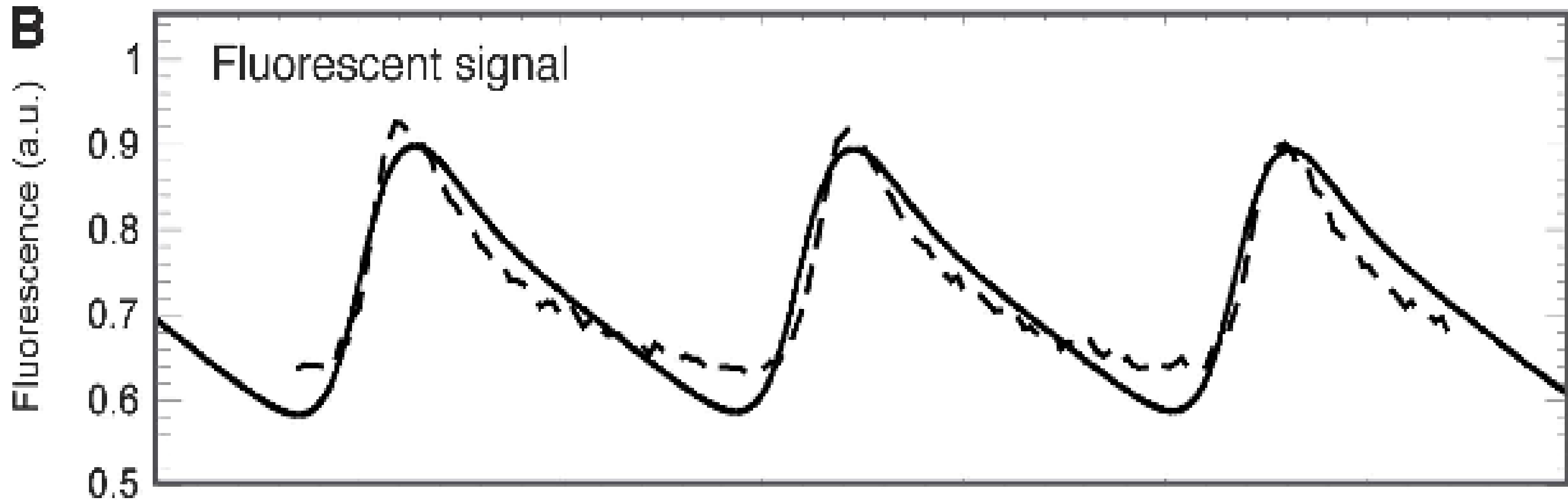
Schematic representation of the reactions included in the kinetic model.

Table 1 Kinetic and thermodynamic parameters

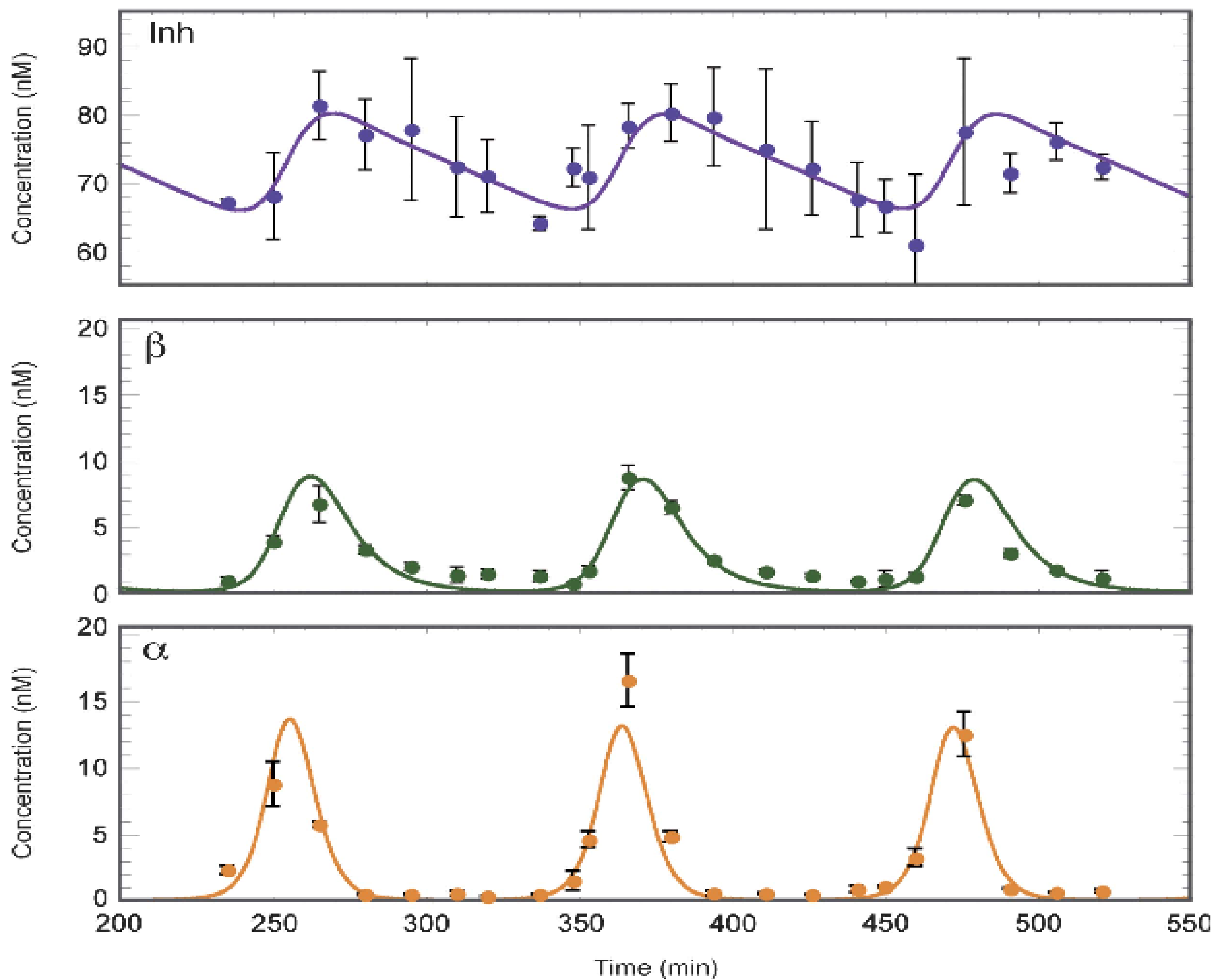
	Parameter	Measured values[a]
Kinetic association constant ($10^6\,M^{-1}\,min^{-1}$)	k_a	26 ± 6
Kinetic dissociation constants (min^{-1})	$k_d^\alpha = k_a/K_a^\alpha$	2.3 ± 0.7
	$k_d^\beta = k_a/K_a^\beta$	0.81 ± 0.4
	$k_d^{Inh/T_1} = k_a/K_a^{Inh/T_1}$	0.0057 ± 0.002
	$k_d^{Inh/T_3} = k_a/K_a^{Inh/T_3}$	0.0021 ± 0.001
Enzymatic pseudo first-order rates (min^{-1})		
Bst polymerase	k_{pol}^α	17 ± 4
	$k_{pol,SD}^{Inh}$	6.9 ± 3
Nt.BstNBI nickase	$k_{nick}^{T_1}$	3.0 ± 1
$RecJ_f$ exonuclease	k_{exo}^α	0.32 ± 0.1
	k_{exo}^β	0.37 ± 0.1
	k_{exo}^{Inh}	1.2 ± 0.2

- The three enzymatic catalyses were analysed one by one: each satisfyingly conformed to the standard Michaelis–Menten model

- Using these independent experimental parameters, it became possible to numerically integrate the ‘raw model’.



Fluorescence signal (top panel, dashed line) and discrete concentration measurements of total individual oligomers a, b and Inh taken between t=235 and 520 min (Supplementary information S3). The plots show the mean of three replicates ± s.d. The solid curves are the best simultaneous agreement (‘optimized model’) for fluorescence signal and oligomer concentrations, respectively



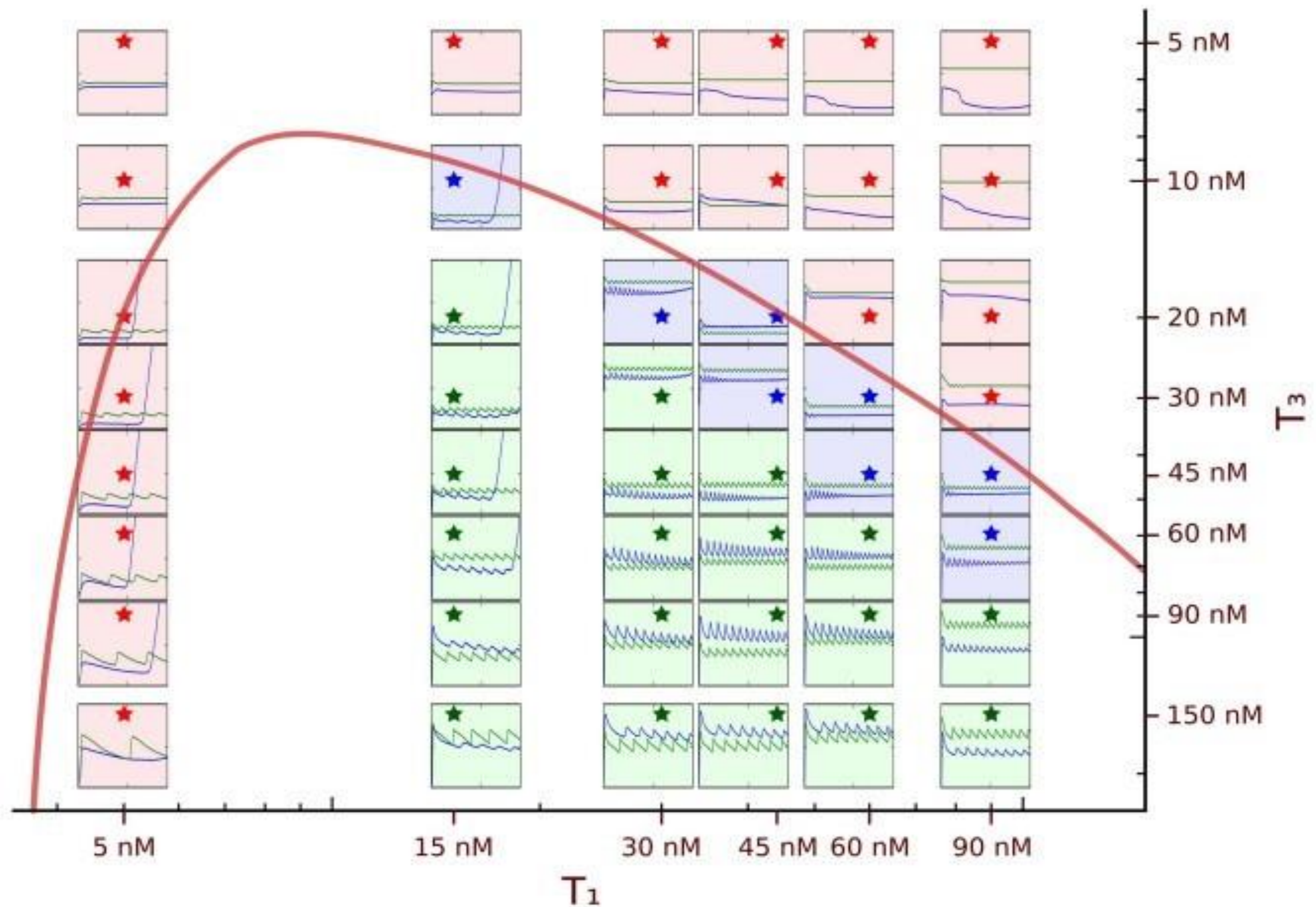
Inh
β
α
Concentration (nM)
90
80
70
60
20
15
10
5
0
200
250
300
350
400
450
500
550
Time (min)

optimized model

- Without any adjustment in the parameters, the agreement between calculated and measured values was only qualitative.

- Built an ‘optimized model’ that includes these uncertainties, by allowing all parameters to drift within a 30% window around their input values.

- The ‘optimized model’ precisely describes the period,amplitude and phase position of the experimental observables



While the structure of the network is encoded at the DNA sequence level, it is possible to tune its behaviour by changing the template concentrations. To test the robustness of our predictions in other locations of its control parameter space,we used the optimized model to calculate the phase diagram

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