A DNA toolbox for engineering *in vitro* life-like behaviors

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Living organisms perform and control complex behaviors by using webs of chemical reactions organized in precise networks. Understanding how life-like behaviors emerge from such complex chemical systems is a challenge for artificial life scientists. An approach is to implement minimal in vitro systems, possessing the characteristic dynamic properties of living systems. In a bottom-up perspective, the ultimate purpose is to lead to the description of minimal functional cells. Taking example on the modularity of biosystemsKitano (2002), complex artificial networks can be obtained by the assembly of elementary building blocksQian and Winfree (2011). In that scope, we developed an experimental framework of dynamic DNA-based modules, that can be assembled to generate large networks with non-trivial dynamic.

This study focuses on the description of a minimal cell as a computing unit. With respect to their environment, simple organisms like bacteria must perform a number of basic computing operations: detection of chemical gradients (chemotaxis), prediction of night and days alternation (circadian rythms) or remembering of past decisions. In molecular terms, these behaviors correspond to various information processing abilities, like adaptation, oscillations, or bistable switching. They are performed within the cell by networks of intercoupled biochemical reactions, one prominent example being the gene regulatory networks.

Our work consisted in building experimental chemical webs that can implement such dynamic functions. We developed a modular DNA toolbox based on a simple biochemical machinery, enabling the construction of arbitrary chemical networks, and their easy in vitro implementation (Montagne et al., 2011). A theoretical work was performed in a continuous feedback loop with the experimental implementation. Simulations of the chemical networks are used for their design, their optimization and their study. Based on the knowledge of the thermodynamic and kinetic parameters of individual reactions, numerical integrations of the corresponding ODE sets enable the assembly of novel networks for predicting their behavior, and to adapt the network topologies for obtaining the target behavior.

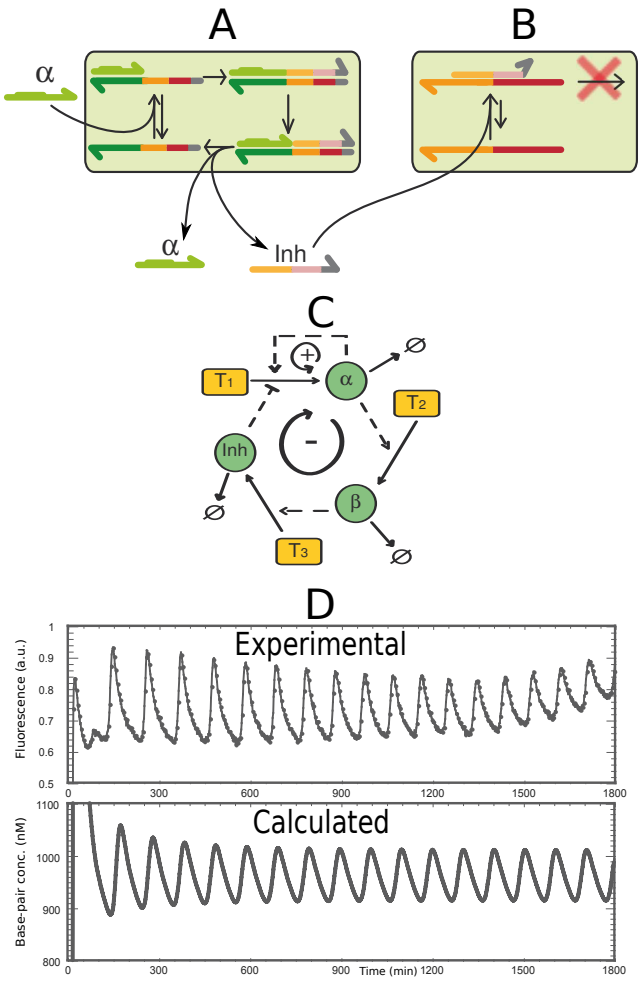

Figure 1: A DNA toolbox. An activation module (A) can be designed for synthesizing a specific oligonucleotide (Inh) when a signal oligonucleotide (α) is present. An inhibition module (B) can be built from the synthesis of an oligonucleotide (Inh) that can specifically interact with a module in order to block its activity. These modules can be assembled in a chemical oscillator (C) that was experimentally designed with a predictable behavior (D).

This system is based on the replication of DNA strands by enzymatic reactions (see Fig. 1). Template DNAs are designed for producing specific message strands, when activated by specific signal strands. Autocatalytic networks are obtained when the signal strand is identical to the message strand. An inhibitor strand can be designed for each template. Full networks can be obtained by assembling these modules, generating positive and negative feedbacks. The dynamics of the system is guaranteed by the presence of an excess of activated nucleotide monomers, and the continuous destruction of the oligomers, for sustaining reaction fluxes.

This toolbox can be used to build non-trivial behaviors. As a proof of concept, we recently reported the de novo construction of a biochemical oscillator, by assembling an autocatalytic unit with a negative feedback loop (Montagne et al., 2011). The dynamic behavior (stability, period, amplitude) of this experimental system can be quantitatively predicted and modulated. We'll discuss how the same toolbox can be used to construct other life-like functions, like bistable or gradient responsive switches, but also logical gates or boolean networks. In the future, compartimentalization of these amorphous systems in vesicles or droplets may provide a good platform for the design of autonomous protocells.

References

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