Review

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# Modularity, context-dependence, and insulation in engineered biological circuits

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**The ability to link systems together such that they behave as predicted once they interact with each other is an essential requirement for the forward-engineering of robust synthetic biological circuits. Unfortunately, because of context-dependencies, parts and functional modules often behave unpredictably once interacting in the cellular environment. This paper reviews recent advances toward establishing a rigorous engineering framework for insulating parts and modules from their context to improve modularity. Overall, a synergy between engineering better parts and higher-level circuit design will be important to resolve the problem of context-dependence.**

## Modularity and context-dependence in engineered biological systems

Synthetic biology, that is, the use of molecular biology techniques to forward-engineer cellular behavior, is a rising branch of biological research [1]. One aim of the field is to gather a better understanding of natural systems by rewiring their subsystems and exporting them to new settings. The ability to redesign living systems has especial potential in the biotechnology industry, promising several breakthroughs in human health and the environment. Designing living systems does not simply rely on the engineering of parts, such as proteins or genetic sequences. By contrast, it essentially requires the ability to link parts together to create sophisticated functionalities. Linking parts together to achieve a predictable behavior is a major challenge in the field. This paper reviews some of the latest advances toward establishing a rigorous engineering framework to overcome this challenge.

As the engineering of biological systems progresses from building simple functional modules to creating large sophisticated systems, a bottom-up approach to design becomes desirable [1–3]. Within a bottom-up design process, basic parts, such as promoters, terminators, ribosome binding sites, and gene coding sequences, are assembled together to create simple functional modules, such as toggle switches [4], oscillators [5–7], and cascades [8]. These modules are then combined with each other to obtain more complicated systems, such as the artificial tissue homeostasis circuit proposed to regulate the concentration of β cells in the pancreas [9] or a synthetic payload delivery device that delivers macromolecules into the cytoplasm of cancer cells *in vitro* [10].

When designing systems bottom-up it is fundamental that the basic parts and the functional modules retain their essential properties unchanged once they are part of a larger system. This modularity assumption allows reliable prediction of the behavior of a system from the behavior of its component building blocks, and has been crucial in many other engineering disciplines, including for the development of large-scale integrated circuits in electronics [11]. In fact, modularity spares the need to co-optimize or even redesign building blocks once they interact with each other, and thus makes the design process scalable, systematic, and substantially faster. In biological engineering, unfortunately, the progress has been hampered by the fact that the salient properties of both basic parts and functional modules depend on their context, which includes the surrounding parts and modules [12]. In the following some of the main reasons for this context-dependence are reviewed, focusing on rigorous engineering solutions that have been recently proposed to insulate parts and functional modules from their context, thus enforcing modularity.

This review mostly considers prokaryotic cells because they provide a reasonably well characterized model system for the implementation of synthetic biological circuits. Furthermore, the basic elements focus on the modularity of promoters and ribosome binding sites because these form the main control knobs for tuning the expression of genes. Topics such as modularity of protein domains are beyond the scope of this review and are covered in separate review articles (see e.g., [13]).

Basic parts and functional modules have key properties that should stay unchanged upon being combined. Promoter activity, the strength of ribosome binding sites, and terminator efficiency should be independent of the genetic context. Similarly, the period and amplitude of an oscillator, the switching-time of a toggle switch, and the sensitivity of a cascade, should not depend on the surrounding systems. If the robustness of the features of a module were implied by the robustness of the basic component parts, we

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could only focus on making robust parts. Unfortunately, as it will be illustrated later, modularity of basic parts does not imply modularity of the circuit they constitute. Hence, a distinction is made between the modularity of basic parts and the modularity of functional modules.

## Modularity of basic parts

The salient features of a basic part are often affected by surrounding genetic sequences as a result of unknown structural interactions between adjacent genetic sequences and factors. For example, the efficiency and position of transcriptional termination markedly depend on the sequences upstream of the terminator [14]. The activity of a promoter depends on the sequences that surround it – in particular, the sequences upstream of the −35 site and downstream of the −10 site affect transcription initiation and promoter escape, respectively [15]. The strength of a ribosome binding site is affected by interactions with the 5′ untranslated region (UTR) sequence and by complicated secondary structures that form across the 5′ UTR sequence and the initial gene coding sequence [16] (Figure 1A). These facts confound system design because, for example, the same combination of promoter and ribosome binding site will result in different and unpredictable protein production rates depending on the specific gene being expressed.

Mitigating context-dependence of basic parts is the subject of intense engineering efforts and promising solutions have recently appeared. These solutions usually aim at reducing possible structural interactions through the use of insulators that spatially separate key parts from each other (Figure 1B). This is in analogy to eukaryotic insulators – short pieces of genomic DNA that, when placed between enhancer and promoter and bound to specific factors, prevent interactions between promoter and enhancer, thus reducing promoter activity. Similarly, barrier insulators are able to block the spread of heterochromatin and thus allow a downstream promoter to be active [17,18]. As illustrated in Figure 1B, to render the transcriptional activity of a promoter independent of the genetic context, a promoter cassette can be expanded to include standard adjoining sequences upstream of the −35 box and downstream of the −10 box. A library of promoter cassettes containing these adjoining sequences has been tested across different genetic contexts, demonstrating that promoter activity can be more reliably predicted when the genetic context is changed [19]. To decouple the properties of the promoter from those of the ribosome binding site, the 5′ UTR sequence can be physically separated from the ribosome binding site to avoid unpredictable interference between them. This can be obtained by inserting either a ribozyme or a CRISPR (clustered regularly interspaced short palindromic repeat) target sequence between the 5′ UTR sequence and the ribosome binding site [20–22]. Testing of constructs with different UTR sequences demonstrated that insertion of a CRISPR target sequence substantially reduces variability in protein production rate [20]. Similarly, employment of ribozymes in NOT gates showed robustness to the genetic context [22]. Finally, complicated interference owing to secondary structure across the 5′ UTR and initial gene coding sequence can be further mitigated by the introduction of a standard translation initiation element that contains two Shine–Dalgarno sequences as opposed to containing one only [23].

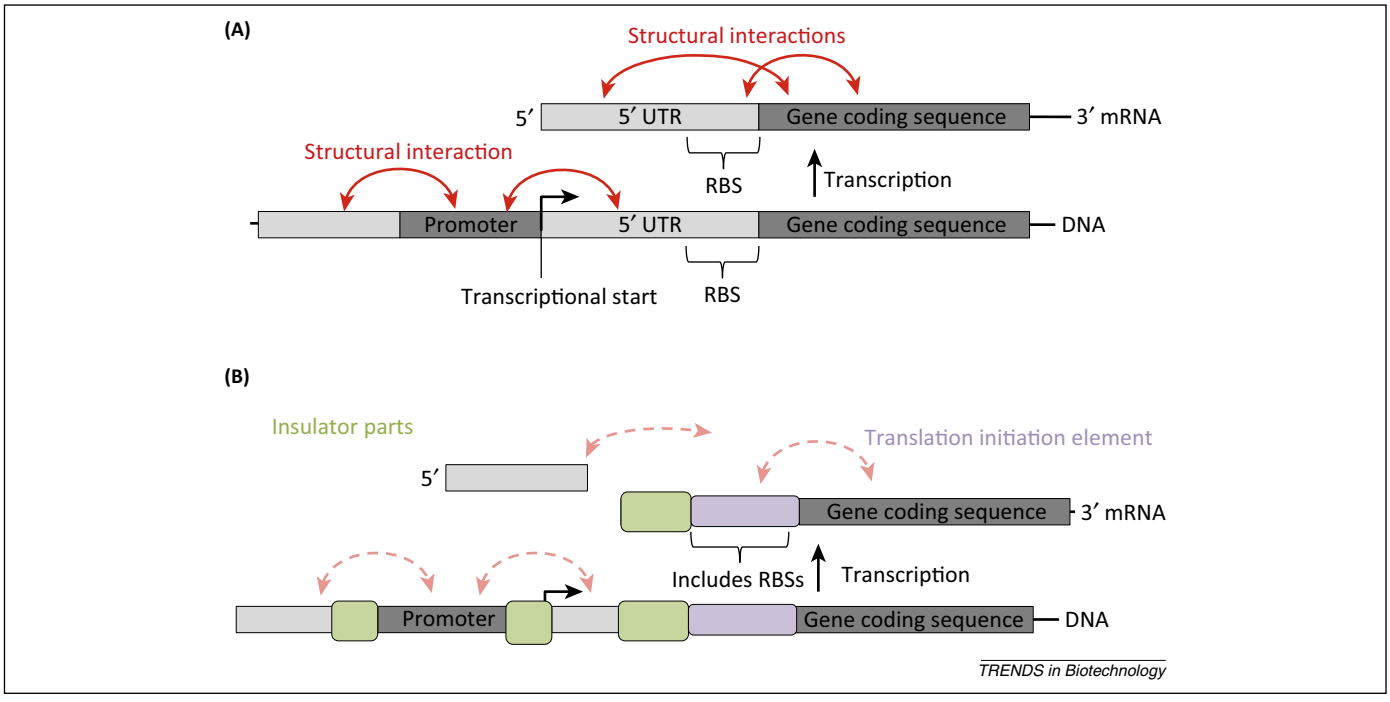


**Figure 1**. Modularity of basic parts. **(A)** Some known structural interactions between genetic sequences affect the activity of promoters and the strength of ribosome binding sites (RBS). **(B)** These structural interferences can be mitigated by physically separating the genetic parts from each other through the insertion of 'insulators'. Upstream and downstream of the promoter, these insulators contain standard sequences that, used for all constructs, guarantee similar promoter activities within different genetic contexts. The 5′ untranslated region (UTR) sequence upstream of the RBS can be cut by using CRISPR targets sequences or ribozymes. Interference from secondary structure formation across the 5′ UTR and initial gene coding sequence can be mitigated by inserting a translation initiation element.