Synthetic gene circuits with a cell-free toolbox

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Short Abstract — **The recent advances in molecular biology have led to the development of quantitative methods to study biological processes, gene networks in particular. While most of the studies are performed in vivo or in silico, only a few cell-free approaches have been proposed.**
In this work, a cell-free toolbox, prepared specifically to engineer synthetic circuits *in vitro*, is used to study biophysical aspects of gene expression. This constructive approach to biological information can be applied to the synthesis of an artificial cell.

***Keywords* — Cell-free expression, synthetic gene circuits, synthetic cell.**

I. BACKGROUND

Cell-free protein synthesis is increasingly used to produce large amount of proteins *in vitro*. Cell-free systems combine a powerful bacteriophage transcription, in most cases the T7 RNA polymerase, to a cytoplasmic extract from an organism, such as *E. coli*, that provides the translation machinery. These systems have been prepared for many types of applications, mostly in biotechnology. Recently, cell-free protein synthesis was used to reconstitute elementary gene circuits, to emulate pattern formation and to address biopolymer physics questions [1, 2, 3]. These studies were limited, however, by the current available cell-free systems which have not been optimized for quantitative and synthetic biology purposes. In particular, transcription is restricted to a few promoters and only a few synthetic bacteriophage promoters regulated by operators have been characterized. The high protein synthesis rate of cell-free systems needs to be balanced with a high degradation rate of both transcripts and gene products to ensure economical bookkeeping of the information processing. Degradation is also required to set the system at a correct step-wise computation level by preventing accumulation of the synthesized proteins in solution. No mechanisms of messenger RNA inactivation and protein degradation have been described to adjust these parameters. These limitations reduce considerably the potential of cell-free protein synthesis as a system to engineer quantitatively and to run gene circuits *in vitro*.

II. RESULTS

Our laboratory has developed a cell-free expression toolbox specifically adapted for the synthesis of gene circuits *in vitro*. Transcription/translation is carried out in a *E. coli* extract which works with seven *E. coli* sigma factors and two bacteriophage RNA polymerases. The system includes mechanisms to adjust the mRNA inactivation rate and the protein degradation rate. Protein synthesis is controlled by adjusting gene concentrations, promoter strengths, synthesized messengers and proteins lifetime. The toolbox provides unique transcription modularity and a large set of adjustable parameters to engineer synthetic networks.

This cell-free toolbox is used for two purposes: (1) the construction and the study of elementary gene circuits and (2) a constructive approach to synthetic cell. Multiple stage transcription cascades, AND gates and negative feedback loops have been engineered. These circuits can reveal underlying mechanisms such as the competition of sigma factors for the *E. coli* core RNA polymerase that result in transcription auto-regulation. Output signals of these circuits can be tuned in a wide dynamic range depending on mRNA and protein degradation rates. The toolbox is also used to synthesize a synthetic cell using a constructive bottom-up approach. The cell-free extract is encapsulated into cell-sized phospholipids vesicles. Properties of the synthetic vesicles are developed from the internal gene expression. The perspectives and the limitations of this approach will be discussed.

III. CONCLUSION

How far can we go in the synthesis of cell-free gene circuits with a deterministic and quantitative approach? It is the question asked by this work which is also used to address biophysical problems.

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