In June 2004, a group of about 300 curious scientists gathered at MIT to hold the international ‘Synthetic Biology 1.0’ meeting. Whether it is fair or not to mark this as the inaugural event of a nascent discipline, it certainly brought together a group of people who were enthusiastic about exploring what it would take to advance the deliberate and rational engineering of living systems. Now classic achievements in the field that were discussed included the toggle switch in *E. coli* (Gardner et al., Nature 403:339), the repressilator (Elowitz and Leibler, Nature 403:335), and pattern formation (Basu et al., Nature 434:1130), combining molecular biology and engineering-driven modeling. Another project demonstrated the engineering of bacteria to produce amorphadiene (an antimalarial drug precursor) by integrating genes from three different organisms (Martin et al., Nat. Biotechnol. 21:796). These, and other projects, provide a foundation for new ideas of how system-level genetic engineering might develop.

Since then, the field has gained considerable momentum and began to branch into new avenues. The design of synthetic gene circuits in bacteria is still a pervasive activity in the field, and it is also pervasive throughout the various articles collected in this issue of *Current Opinion in Biotechnology*. However, circuit design has also successfully entered the domain of mammalian cells, as illustrated in the article by Tigges and Fussenegger. Familiar circuit motifs such as toggle switches, time-delay circuits, and oscillators have found their way into mammalian cells. The mammalian versions are coupled to specific regulatory systems and are carefully adapted to potential therapeutic applications, for example by using regulatory systems that function via side-effect free physiological triggers.

Another important development focuses on implementing circuits across cellular boundaries to rebuild for example bacterial cell–cell communication or spatial development processes typical of higher organisms, as reviewed by Pai, Tanouchi, Collins, and You in the second contribution. This enables synthetic biology to address novel degrees of functionality in designing biological systems. The improved understanding of the signaling pathways used in the synthetic systems, which are often based on bacterial quorum sensing, allows careful fine-tuning of overall system performance. The integration of diffusible molecules into synthetic networks can provide reliable synchronization over decision making among individuals and can attenuate the effects of noise. Ultimately, the ability to engineer multicellular systems reliably will aid in a variety of applications from therapeutics to simulating and investigating social behavior.

In the next contribution, Benenson analyzes recent progress in using RNA across biological kingdoms for the design of biological computing and
cellular control elements. In bacteria, riboswitches that control intrinsic terminators and small RNAs have been demonstrated to perform at least simple logic functions. In yeast, engineered ribozymes and aptamers provide additional promising and perhaps scalable mechanisms for implementing sophisticated computational functions. The largest diversity of possibilities for exploiting RNA for biological computation and control exists in higher level organisms such as mammalian cells. For example, RNAi based logic statements based on short RNA sequences that can be integrated into large scale circuits appears to be an attractive technology, but needs to be made sensitive to cellular inputs.

A central feature of synthetic biology, exemplified in the three contributions above, is the desire to design relatively large systems, consisting of multiple genes and regulatory elements. However, designing such systems is a major challenge that requires substantial experience, expertise, and detailed knowledge regarding all elements to be incorporated into the synthetic system. To be successful, one must master a wide range of topics including the ability to select the most appropriate circuit design for a given task, familiarity with protein/promoter/ribosome binding site variants with the most suitable rate constants (including the required mathematical modeling effort), cloning expertise (for example developing restriction site strategies for constructing large systems), selecting proper mRNA (de)stabilizing functions, preventing excessive homologous sequences, and so on. It is clear that computational tools must eventually provide significant support for automation of circuit design, circuit optimization, and DNA-sequence writing. While computational tools for synthetic biology initially focused on DNA assembly strategies, they increasingly also support mathematical modeling of the systems being designed and address other fundamental questions in circuit selection, as reviewed in the contribution from Marchisio and Stelling. The authors are careful to point out that computational design for synthetic biology is still an area that is in its infancy, and an important problem that must be addressed now is how to integrate existing tools into an intuitively usable workflow.

One of the crucial challenges in circuit design remains the absence of suites of parts with quasi-continuous coverage of the parameter space to allow thorough and efficient testing of alternative circuit designs to achieve desired function. One way to obtain such a collection of parts is described by Dougherty and Arnold, who summarize progress in exploiting directed evolution for adapting parts to the functional requirements of synthetic circuits. Their review also focuses on the adaptation of enzymes as important elements of engineered biological systems and argues that this will spurt some very important applications for synthetic biology. Dougherty and Arnold conclude by reflecting on certain limits that evolution might impose on the design ambitions of synthetic biology, a theme that is also explored in the last contribution of this issue.

An indispensable part of synthetic biology is the question of orthogonality—the question of whether we can design functions that have minimal interaction with existing system elements, just as thermal and electrical insulators are used in many man-made devices to prevent parts from influencing each other in an undesired or at least uncontrolled fashion. One route that synthetic biology might pursue is to provide alternative cellular biochemistries, ranging from different (chemical) forms of genetic material to ‘never born proteins’ with potentially entirely novel functions. Progress in this field of chemical synthetic biology is summarized in a contribution from Chiarabelli, Stano and Luisi.

As an example of how synthetic biology can influence an existing field, Carothers, Goler, and Keasling provide an overview on recent advances in controlling biological functions required for the production of chemical compounds ‘from cradle to grave’. The advances range from pathway regulation to the significance of RNA tools for high-throughput assembly of novel systems.

As an important complement to the summaries highlighting the potential and progress in the field of synthetic biology, the final contribution strikes a more cautionary tone. Danchin puts forward his view on the fundamental limitations of design in synthetic biology, and highlights the apparent contradiction between purposeful design and required stability and the biological principle of evolution. He advances the argument that designed cells will either be able to reproduce, and therefore retain the capacity to evolve, or will be designed devoid of those genes that are involved in energy-dependent degradation and therefore will age and require periodic reconstruction.

While not an exhaustive account of recent years in synthetic biology, we argue that the collection of articles presented here provides an excellent overview of the current status of the field and its main lines of development. This includes fundamental questions on how to build the infrastructure required for a novel engineering discipline, what might be and what might not be possible in a cellular framework, and a focus on the core lines of current research and around-the-corner applications. We sincerely hope that it will be as pleasurable to read through this issue of Current Opinion in Biotechnology as it was putting it together.