CONTROL OF COMPLEX BIOMOLECULAR SYSTEMS

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***Abstract*—Nature has evolved extremely intelligent and complex adaptive systems. For instance, a cell fuses genetic processes with nanoscale sensors and actuators to result in one of the most efficient and autonomous molecular factories. These systems operate by integrating multiple levels of system architecture. Sensing, information processing, as well as cellular action are all fused at the local level. At each level of the system architecture, higher-order functionalities or emergent properties are often derived. These complexities cannot be simply extrapolated from its individual components and are far beyond our full understanding. This leads us towards cell mimetic approaches requiring fusion of biotechnology, nanotechnology, and informatics, for controlling and interrogating these complex biological systems. We have demonstrated that properly designed time-varying stimulations can self-organize and adjust the functionalities across multiple length scales to efficiently reach the desired control state. This may yield new insight into unlocking and acquiring novel control modalities of the underlying mechanisms that drive the natural processes of life.**

I. INTRODUCTION

CELLS are extremely intelligent systems with multiple levels of complexity. The DNA/RNA and protein molecules, which drive its natural processes, possess dimensions on the nanometer range. Exploring the governing mechanisms across a wide span of length scales is best stated by P. W. Anderson in his 1972 Science paper [1] as "at each level of complexity entirely new properties appear, and the understanding of the new behaviors requires research which I think is as fundamental in its nature as any other." While understanding of the cellular process is important in life science and medical applications, it is very challenging to discover the underlying mechanisms. These processes are active, dynamic, stochastic, nonlinear, and multi-parametric. The degree of complexity is often beyond intuition alone. Novel tools and approaches for accelerating our study in these aspects are highly desirable.

Fusion of bio-, nano-, and informatics (BNI Fusion technology) enables us to take a different approach to explore the fundamental design rules in a cell. Micro and nanotechnologies facilitate production, manipulation, and characterization of different biological/artificial objects down to the single molecule level. Biotechnological advancements have allowed scientists to physically manipulate genetic pathways to generate nutrients and fuels from waste products or engineer strains of proteins to possess novel functionalities. Information technology provides the framework for organizing and developing complex bio-nano systems. The BNI fusion will culminate in systemic architectures that will rival those that have taken millions of years to come to fruition in nature. With this will come the hopes of achieving a fundamental comprehension of how the interplay of these three areas can be manipulated on the molecular level to produce enhanced emergent properties.

To implement the BNI fusion technology, some conceptual differences between natural and artificial systems should be taken into consideration. In man-made machines, a system is usually assembled from simple components. A biological system builds system architecture upon multiple levels of complex machinery. Several basic characteristics are commonly observed in natural biological designs. These characteristics should be extensively studied in order to understand cellular systems. They include i) specific molecular recognition, ii) amplification of biological signals, iii) stochasticity, and iv) combinatorial control. These aspects are conceptually important for the design of functional molecular systems in the future. In this paper, we present some of our work to understand and mimic these concepts. Designs of artificial and biological molecules are first reviewed. We then discuss single cell manipulations for revealing the operation processes in the cellular systems. Finally, we describe multi-parametric control of cells by combining bio-, nano-, and information technologies.

II. MOLECULAR DESIGN

Advances in bio-nanotechnology have produced molecular-scale devices incorporating neo-functional materials with the generation of biocompatible energies, or a novel framework by which nanosystems can be assembled. For instance, artificially synthesized molecular motors (rotaxane) allow mechanical switching upon chemical or electrical stimulations [2]. In our group, we have developed a rotaxane-powered microcantilever actuator utilizing an integrated approach that combines "bottom-up" assembly of molecular functionality with "top-down" micro and nano fabrication [3][4]. By

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harnessing the nanoscale mechanical motion from artificial molecular machines and eliciting a mechanical response in a microscale device, this system mimics natural skeletal muscle and provides a key component for the development of functional nanosystems.

Synthetic molecules can be directly applied for interfacing with natural biological systems. In the past decade, there have been great advancements in the synthesis of biomolecules such as DNA and RNA. Various modifications on the oligonucleotide are available to alter the nuclease resistance, specificity, melting temperature, and binding activity to other molecules. By proper design, the synthetic biomolecules can work as molecular sensors or actuators. For example, a molecular beacon is an oligonucleotide probe that has a stem-and-loop structure. It undergoes spontaneous fluorogenic conformational changes upon hybridization to the complementary nucleic acid target [5]. By using a molecular beacon design, we achieved single molecule detection and discrimination of single nucleotide polymorphisms by combining microfluidic components, laser induced fluorescence (LIF) techniques, and confocal measurements [6]. We have also designed specific molecular beacon probes for measuring the intracellular mRNA inside individual cells (Fig. 1) [7]. Furthermore, other novel molecular designs can be used for interfacing, detecting, and manipulating a large variety of molecules. For example, it is possible to take advantage of the oligonucleotide-protein interaction for protein detection [8][9].

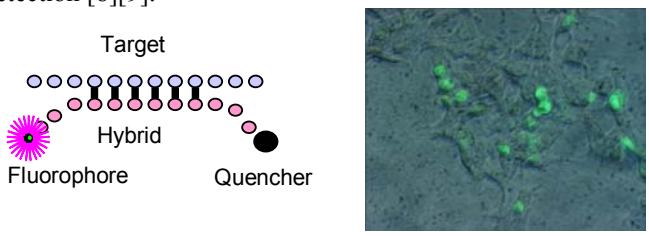

Fig. 1. Transfection of molecular beacon for single cell measurement.

III. Single Cell Study

Biological systems exhibit substantial phenotypic variation. Stochastic events can be observed within the drug responses of different individuals and even in the self-organization of embryonic cells during development. The molecular machinery that controls genetic regulation may account for the large amounts of cell-cell variation observed. While natural biological systems use redundancy and extensive feedback in regulatory pathways to achieve reliable regulation, some cellular systems can exploit this noisiness to randomize outcomes in cases where variability is advantageous. In addition, many biological systems adapt quickly to environmental changes and exhibit significant system stability despite the fact that the individual components are quite sensitive to the environment. The basic knowledge of these stochastic/robust events is fundamental to the understanding of the complex biological systems.

BNI fusion technology provides powerful tools to probe these issues. For example, an image actuated optoelectronic tweezers for the concentration, manipulation, and the sorting of cells has been developed [10]. We have demonstrated single cell manipulation by various means including electrokinetic forces, glass manipulators, and hydrodynamic forces [11]-[15]. We have integrated these capabilities and developed a generic microfluidic platform for performing different cellular studies. *E. coli*, C2C12 myoblasts, and human embryonic kidney (HEK) 293T cells have been successfully cultured in the micro cell culture system (Fig. 2). The micro cell culture system with integrated actuators allows study of the cellular responses under different external stimulations.

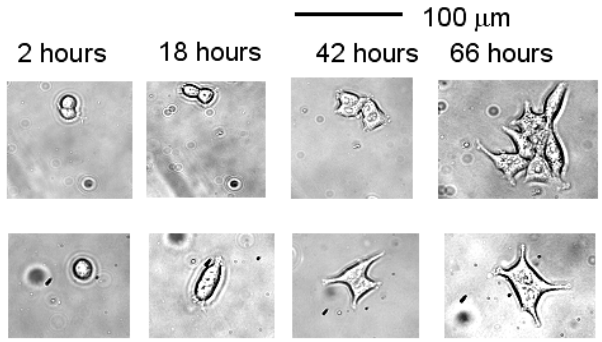

Fig. 2. HEK 293T cell cultured in the microfluidic system. The doubling time of the cells is roughly 1 day.

Useful insights of the underlying cellular designs can be obtained by monitoring the dynamics of cellular activities at the single cell level. Measurement of the cell-cell variation in gene expression is crucial to many natural biological processes [16]. The first artificial gene-metabolic oscillator has been recently demonstrated [17]. Understanding single cell dynamics and cell-cell variation are important for the design of these circuits. Our microfluidic approach allows us to measure the gene expression of individual cells and trace them over a long period of time. IPTG-inducible *tac* promoter in *E. coli* was used as the model system for demonstrating the capability of our microfluidic platform. Figure 3 shows traces of individual cell intensity exposed to IPTG concentration from 0.92 to 0.96 mM. In general, we observed considerable variations in the green fluorescence protein intensity. Some traces of typical cell responses were highlighted to illustrate the large variations of the expression dynamics. The large distribution in GFP was expected due to the stochastic nature in gene expression. Despite the large variation in fluorescence intensity, approximately 80% of the intensity curves had a shape similar to the average intensity curves. However, the expression rate and time scale varied significantly between individual cells. We also estimated the normalized expression rates at different time. Typically, the original expression rate (basal transcription) was negligible. The expression rates gradually increased after introduction of the inducer and reached steady state roughly after 150 min. The IPTG

concentration did not significantly affect the time constant for the expression rates to reach steady state indicating that the transcriptional efficiency has minimal effect on the time constant in this particular system model.

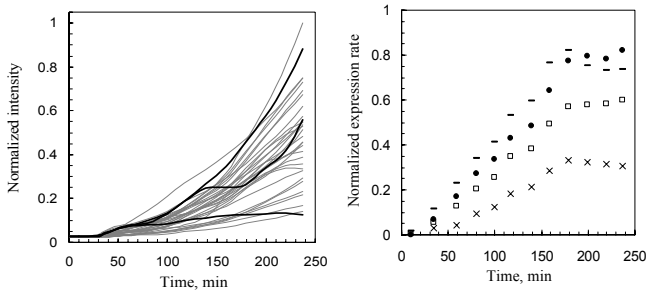

Fig 3. (Left) GFP expressions of individual *E. coli* cells exposed to 0.92-0.96 mM of IPTG. Several individual traces are highlighted to illustrate typical behaviors of the gene expressions dynamics. (Right) Normalized expression rates of the cells at different time. Each symbol represents a different IPTG concentration. • 0.96-0.81 mM; — 0.81-0.77 mM; □ 0.3-0.15 mM; × 0.15-0.04 mM.

IV. Multi-parametric Control

Many cells regulate gene expression by integrating multiple external signals. One of the principle challenges in the life sciences is the understanding of how cells receive, process, and respond to the information form the environment. These processes are controlled by the signal transduction network. A signal transduction pathway is a cascade of biochemical reactions inside the cell that eventually modifies cellular activity, such as the activation of transcription factors. Since most of the cellular phenotypes are the result of gene expression, active control of the transcription factor activity has great importance in cell biology. Unfortunately, our understanding of the signal transduction pathways is far from complete and a rational design of stimuli for regulating the signal transduction networks is extremely difficult.

Combining regents, such as drugs or cytokines, has been known to be effective for manipulation of signal transduction pathways and for disease treatment [18]. In a typical biological experiment, the pathways are isolated and studied individually. Important information in the regulation of the cellular process is lost. However, a rational design of stimuli for regulating the signal transduction pathways is extremely difficult due to the complexity of the networks. Extensive optimization of the dosage is required to establish the potential additive and synergistic effects. We developed a novel approach to cooperate multiple stimuli for controlling complex biological networks. The technology is fundamentally different from the traditional approaches of conducting biological research, in which each component is isolated and studied independently. Our BNI fusion technology, on the other hand, allows us to take a systematic approach permitting the regulation of complex biological networks.

V. Conclusion

We envision emulating nature's strategy of seamless integration between the nano transducer and the distributed multilevel information management. With the promise of highly intelligent emergent functions, the devices and systems based on BNI fusion technology are expected to make significant impacts on the future.

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