ABSTRACT

In the era of genomics and proteomics, our knowledge about biological systems has become vast and impossible to fully comprehend. Inspired by the physical sciences, many researchers have thus invoked minimal systems approaches, in the context of synthetic biology, to reduce the complexity of biological systems and focus on the minimal number of components to reproduce and reconstitute fundamental biological phenomena in a controlled cell-free environment. Our group is particularly interested in arriving at a minimal system to divide a vesicular compartment, or in other words, a potential minimal protocell. This contribution briefly discusses the impact that microfluidics may have in such an effort.

KEYWORDS: Formatting, Layout, Manuscript, PDF-file

INTRODUCTION: SYNTHETIC BIOLOGY OF MINIMAL SYSTEMS

In the light of ever growing information that we have on biological systems, the challenge for quantitative biology and biophysics is to identify appropriate procedures and protocols that allow the researcher to strip down the complexity of a biological system to a level that can still be reliably modelled, but retaining the essential features of its “real” counterpart. The virtue of physics has always been the reductionist approach, which allowed scientists to identify the underlying basic principles of seemingly complex phenomena, and subject them to rigorous mathematical treatment. Biological systems are obviously among the most complex phenomena we can think of, and it is fair to state that our rapidly increasing knowledge from genomics and proteomics does not make it easier to identify a small set of fundamental principles of the big concept of “life” that can be defined and quantitatively understood.

In this context, the emerging field of Synthetic Biology, in its biophysical understanding, represents the striving for a better quantification of biological, particularly cellular systems, by a so-called “bottom-up biology”. Physicist Richard Feynman once phrased the famous quote “What I cannot create, I do not understand”. In strict sense, following this quote, we would only fully understand a biological system if we were able to make it from scratch. Of course, it appears to be a rather hopeless enterprise to make a “modern” cell, leave alone a whole organism, in all its complexity. On the other side, life has arisen from a presumably much simpler subsystem, containing unknown and probably no longer existing key molecules. The success of several functional in vitro assays for biological subsystems, functioning in environments of dramatically reduced complexity, suggests that it is indeed possible to reconstitute essential features and distinct modules of the cell from small and physically controllable sets of molecules, and by doing this learn more about the fundamental physical and chemical laws which nature builds the phenomenon of life on.

THE CONCEPT OF A MINIMAL CELL

There are many motivations for the development of minimal cellular systems, or - to call them more provocatively – artificial cells. One obvious motivation is to find possible models of how primordial cells could have developed to become the first major organizational units of life, compartmenting biological information, and thus forming the first true individuals set apart from their environment - from then on being subject to the mechanisms of Darwinian evolution. Another motivation is the more technical one, with biomedical implications: if we manage to create functional models of cells, we may in the future be able to replace the real ones that fail or somehow misbehave in our organisms. This would be a completely novel approach to what is presently aimed at and partly achieved by stem cell technology – but with fewer, or at least different, ethical implications. The third, and certainly in short terms most relevant, motivation is the striving for better quantitative analysis and modeling of biological systems - as the quantitative researcher is often frustrated when working with native cells under physiological conditions.

Minimal systems of cellular modules have in the past decades tremendously helped to elucidate underlying physical and chemical laws that govern complex phenomena of living matter. Dissecting the cellular interaction network module by module will, although it does not give us a complete view of the full system, at least help us in understanding the principles that might have been assembled and combined in ancient forms of living systems. In fact, by admiring the
immense entanglement of cellular networks, with their stunning complexity that raises very little hope for understanding the full system, we have to admit that cells as we know them today do not tell us much about the first physical principles and (bio-)chemical modules that governed their evolution.

Among the most remarkable features in modern cells and organisms is not only the general processing and inheriting of genetic information, but also the possibility to adapt to environmental conditions, and the robustness of the biochemical machinery against external and internal disturbances. It is thus well conceivable that nature’s solution to a specific biological problem, e.g., cell division or generally the budding or fusion of vesicles from and to membranes, is not the most straightforward in terms of underlying physical mechanisms, and could be realized in simpler systems with fewer molecular players. This is particularly important to keep in mind when considering the possibility of generating an artificial cell, or, more modestly, engineering a specific functionality by employing a set of biological devices, i.e., proteins.

**HOW CAN MICROFLUIDICS HELP IN REALIZING A MINIMAL CELL?**

One of the most critical tasks in the design of a minimal cell is efficient compartmentation of its components. As a minimum requirement, the compartments have to be selectively permeable and biologically benign, more elaborate ones should be able to divide and replicate. Vesicles based on amphiphilic, self-assembling molecules, such as phospholipids, have in the past been considered the most promising candidates for cell-mimicking compartments. A simpler, but for the compartmentation of solution-based systems equally suitable system could be water droplets surrounded by non-polar solutions such as oil. Here, encapsulation of a protein expression machinery together with a gene of choice already represents the basis for Darwinian evolution. The first landmark study elegantly realizing compartmentation of a cell-free transcription/translation was published in 1998 by Tawfik and Griffiths, who utilized water-in-oil-emulsions to encapsulate different genes into small water droplets separated by mineral oil, achieving true genotype-phenotype coupling. The technical limitation of this approach, i.e. the heterodispersity of these droplets when generated by simple emulsification, could several years later be overcome, when the technical developments of microfluidics in general fluid handling became overly apparent. To our knowledge, our own group first published the concept of creating large amounts of homodisperse water-in-oil droplets for cell-free protein expression in soft polymer microstructures (Dittrich et al. 2005), which was later followed up on and technically perfected by other groups. Today, droplet based microfluidics has many exciting applications in chemistry and biology, with a huge promise for minimal systems design compatible with large number screening.

However, in spite of the many premises and potentials of droplet-based microfluidics, the more attractive perspective for a development of minimal protocells is the optimized generation of large quantities of homodispersely sized phospholipid vesicles filled with biological molecules such as proteins and nucleic acids. Although much work has been done in recent years employing minimal biological systems based on vesicles, the possibility of functional insertion of proteins, manipulation and detection has so far been limited, mainly due to the low-tech generation methods, requiring, e.g., the low salt conditions, at which vesicles are usually grown to large (i.e., easily manipulated and detected) sizes. Thus, it is a very attractive goal to employ microfluidic strategies to efficiently create and load vesicles with proteins or generally, biomolecules of choice. One very promising approach has recently been introduced by Stachowiak et al. (2008), utilizing microfluidic jetting for the generation and simultaneous filling of giant membrane vesicles.

In my talk, I will specifically discuss about our adaptation of this technology to generate minimal model systems of cell division. Here, we utilize an assay to generate dynamic protein self-organization of bacterial cell-division proteins (Loose et al., 2008) which may eventually lead to the protein-induced division of the vesicle.
REFERENCES


