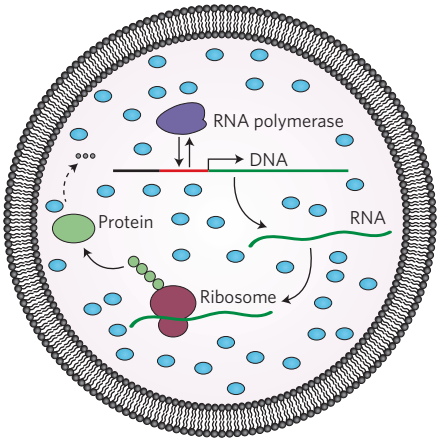

Figure 1 | Genetic processes in artificial cells. The transcription of RNA from DNA by RNA polymerase and the production of protein by ribosomes from RNA can be reconstituted *in vitro* using a synthetic gene expression system. To better imitate the crowded and compartmentalized environment of a cell, the reaction can be encapsulated in lipid bilayer membranes (shown in black) and macromolecular crowding agents (light blue circles) can be added. With such a system, LeDuc and colleagues[5] find that macromolecular crowding has a strong influence on transcription efficiency and the performance of simple genetic systems.

indicated through the increased cell-free production of a green fluorescent protein. The researchers also found that crowding apparently made gene expression more robust with respect to perturbations by ions, which tend to influence enzyme function. Furthermore, they found that crowding alters the behaviour of a simple gene regulatory module that contained a negative feedback loop. Finally, LeDuc and colleagues constructed an artificial cell (Fig. 1) from lipid membrane vesicles that encapsulated a synthetic expression system and a genetic construct. The cells could express green fluorescent proteins using the genetic construct, and enhanced gene expression was observed when macromolecular crowding agents were added to the cells.

In the past few years, impressive progress has been made in the construction of cell-free gene circuits. Several groups have developed purified gene expression 'kits' that can be used to construct artificial gene circuits that would be difficult to obtain if crude cell extracts were used as *in vitro* transcription–translation systems[9,10]. These kits have been used, for example, to produce cytoskeleton-like filaments within artificial lipid bilayer vesicles[11], and to create synthetic gene circuits[10]. However, crowding has not explicitly been taken into account as a design parameter for artificial cell-like systems until now.

The continued integration of different aspects of cellular biochemistry into cell-free reaction systems will in the short term lead to the development of chemical systems at an intermediate level of complexity — somewhere between the complexity of well-stirred reaction beakers of traditional chemistry and that of living cells. The investigation of crowding, confinement and spatial organization in such systems could help to elucidate important factors that make cells appear different from conventional chemical systems. This in turn should aid the development of a cell-free biotechnology and could eventually lead to the emergence of a bio-analogue cell-scale nanotechnology. Artificial cell-like systems could then be developed that offer complex chemical processes and products that are not accessible with conventional chemistry. Such systems could ultimately provide an alternative to the genetic and metabolic engineering of existing organisms, and would potentially be more economic and cause less ethical concern. ❐

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MOLECULAR COMPUTING

In situ computation of cell identity

Cascade reactions can be used to carry out logic operations on the surface of cells and identify the presence of particular collections of cell surface markers.

Thomas E. Schaus and Peng Yin

A cell membrane contains a collection of proteins, known as surface antigens, that is unique to each cell type and can be used to identify both the cell and its functional state. Identification is typically carried out using a set of molecular probes that target and label individual antigens. These labels are then imaged with a microscope or other instrumentation, and the researcher or the instrumentation itself analyses the data to compute the identity of the cell. In certain applications, however, it would be useful if this analysis could be carried out without any human intervention and the final result of the computation — that is, the identity of the cell — could be displayed directly on the cell itself.

Writing in *Nature Nanotechnology*, Sergei Rudchenko, Milan Stojanovic and colleagues at Columbia University have now shown that targeted DNA sequences can be used to carry out antigen-dependent logic-gate computations on the surface of a cell[1].

The researchers use a collection of probe molecules that first target specific antigens

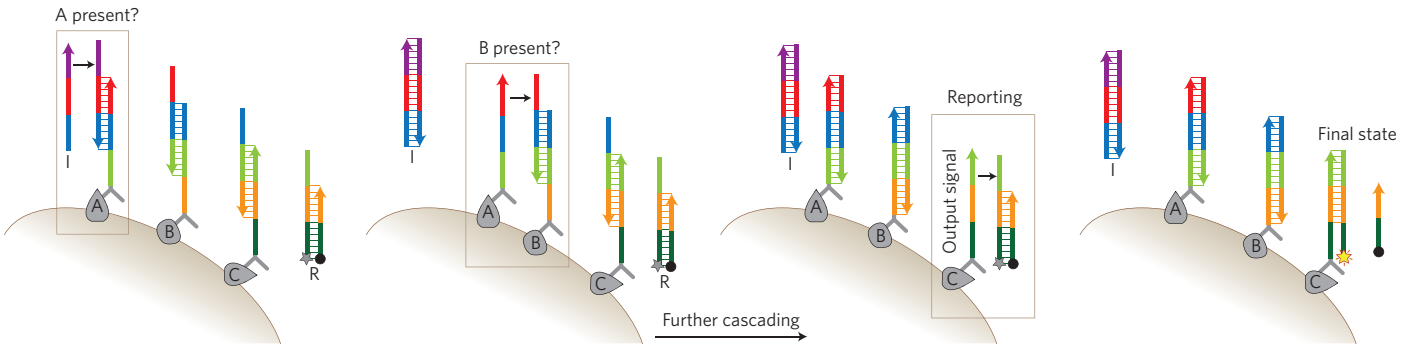

Figure 1 | A three-input AND gate (A AND B AND C) using a programmed cascade of reactions on a cell surface. Three probe molecules (to antigens A, B and C), each of which are composed of an antibody (grey) conjugated to one strand of a synthetic DNA duplex (shown as a series of coloured domains), are added to a solution and bind to their target antigens on the surface of a cell (brown). An initiator molecule (Ini) is then added (left panel), which binds to the complementary strand of probe A by means of the toehold sequence (purple), and removes it, exposing a new signal sequence (red). The newly active probe A then interacts with probe B in a similar fashion, and probe B subsequently activates probe C (middle panels). The exposed strand of activated probe C represents the unique output signal of the cascade, which is possible only when antigens A, B and C are all present. In the example shown, probe C binds, separates and de-quenches a fluorophore strand from a soluble reporter duplex (R), leaving the cascade in its fluorescent (yellow star), completed state (right panel).

independently. The cell-bound probes then undergo a cascade of reactions that carry out a prescribed logic calculation, terminating in the display of a unique output DNA sequence if the calculation is evaluated as 'true'. Each probe molecule is composed of an antibody and a synthetic DNA duplex. One strand of the duplex is conjugated directly to the antibody, whereas the complementary strand is the cascading signal, passed between probe molecules through a mechanism known as toehold-mediated strand displacement[2,3]. Therefore, the targeting antibody plays an identification role, and the DNA executes the computation.

With the approach, Stojanovic and colleagues are able to demonstrate three fundamental logic gates: AND, OR and NOT. For example, (A AND B) is computed in the following way. Two probe molecules, A and B, are added to a solution and attempt to find their targets on the surface of a cell. A single-stranded initiator sequence is then added to the solution, binds to an exposed toehold sequence of the complementary strand of probe A, and removes it, thereby exposing the entire length of the conjugated strand of probe A. This conjugated strand is now active and can, in turn, bind to and remove the complementary strand of probe B. Finally, activated probe B binds to a soluble, fluorescent reporter strand marking the presence of A and B. This computation can be extended to more than two antigen inputs by simply inserting another probe layer into the cascade, and Stojanovic and colleagues demonstrate a three-input AND gate (simplified in Fig.1) in which the signal propagates at about one step every ten minutes.

The OR gate was developed by making two probes using the same DNA sequences, but different antibodies; the presence of either antigen input will pass the same signal. Demonstration of (A NOT B) was slightly trickier: a triggered probe A displays the final conjugated output signal strand outright, but in the presence of probe B, probe A displaces the complementary strand of probe B and once again becomes double stranded and inactive. The caveat here is that probe A must interact with any probe B before interaction with the target strand, and so a delayed, sequential target strand addition may be required to prevent a race condition.

Over 200 types of cell are histologically identifiable in the human body, and further classification into stem cell or other types are increasingly of interest[4,5]. Accurate identification depends on having sufficient information available. Although a single, highly specific antigen is sometimes known, it is frequently necessary to evaluate multiple surface antigens. Under the simple assumption that each cell type has a random collection of 500 surface antigen types from a human-wide pool of 5,000 types, there is a 10% chance (P) of finding a given antigen on a cell, and a P^N chance of finding N different antigen types on the same cell. Typically, therefore, positive identification of three random antigens would be enough information to accurately differentiate a cell type, even without a negative selection for probes that bind other cells. More realistically, the distribution of probabilities of a given antigen type on a cell is not uniform, and will contain some very common 'housekeeping' proteins and some quite unusual ones[6]. Even then, simple probability calculations suggest that only four or five antigens would suffice to specifically identify a cell, which is within the range of the reported method and would require only the simple AND gates to operate.

The approach of Stojanovic and colleagues has a number of attractive features. In particular, the method is accessible and reliable because it uses standard antibodies and easily acquired synthetic DNA. The system is also modular and, in principle, scalable. Because the probe–probe interaction is only transient, potential signalling effects of antigen co-localization are minimized. Antigens must, however, be mobile on the cell surface, and the antibodies may still act as agonists or antagonists to individual antigens.

Applications that could take advantage of this *in situ* computation include *in vitro* light microscopy with a spectrally limited number of labels, *in vitro* capture of particular cell types from a mixed population, and perhaps even *in vivo* drug targeting, which has the potential to dramatically reduce dose-limiting side effects and toxicity. Stojanovic and colleagues demonstrate the operation of their system in whole blood, illustrating the robustness of their method against enzymatic, nucleic acid and buffering challenges. However, the major barrier to *in vivo* use may be the adequate distribution of probes in the body, as

DNA by itself is ill-suited to the tasks of rapid diffusion and membrane crossing. Medicinal chemists traditionally use small molecules with masses of less than 500 Da and relatively low polarity for these tasks[7], and the probes developed by Stojanovic and colleagues far exceed these rules of thumb. These probes may still find viable targets within or directly accessible from the bloodstream, such as liver cells or some tumours, but for other targets alternative delivery methods are required.

Several natural extensions of this system are possible. In particular, although the approach has been demonstrated with plentiful target antigens, signal cascading will be limited by the least abundant probe present. Therefore, the system could be improved by developing a catalytic scheme in which an individual probe propagates its signal to multiple downstream partners. This could lead to faster and higher signal generation, as well as a binary output typical of logic circuits, although challenges related to signal thresholding and leakage must be overcome. Other extensions, enabled by the modular nature of these DNA schemes, include scaling to relatively complex computations[8], the triggering of other self-assembling systems[9] and interaction with other DNA machinery[10]. Significant challenges remain before any of these developments or applications can be realized, but Stojanovic and colleagues have, nevertheless, developed a new approach to *in situ* computation. Although the evaluation of live cells previously relied on *post hoc* computation, the team have now harnessed the power of DNA nanotechnology to choreograph the computations directly on the cell surface. ❐

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