CHEMISTRY

Can Droplets and Bubbles Think?

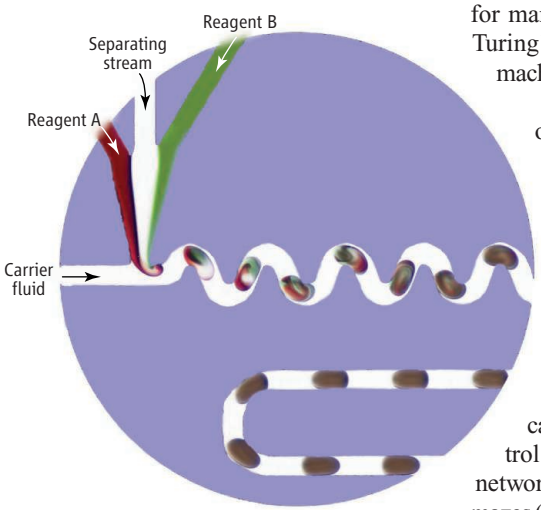
Bubbles flowing through narrow channels can be encoded with information and made to perform logic operations like those in a computer.

Irving R. Epstein

How many of us, at the close of a well-spent evening, have stared into that last glass of beer or champagne and wondered what eternal truths lie in the rising bubbles before us? In this issue, Fuerstman *et al.* on page 828 *(1)* and Prakash and Gershenfeld on page 832 (*2*) report their use of microfluidic technology to construct streams of droplets (liquid-in-liquid) and bubbles (gas-in-liquid) that can encode and decode information or perform logical operations.

Since its emergence in the 1990s, microfluidics has become a powerful technique for a wide variety of applications in biotechnology, engineering, physics, and chemistry. By studying processes in channels with typical dimensions of tens to hundreds of micrometers, researchers can conduct controlled reactions while economizing on the consumption of possibly scarce materials. Flow in such channels can be characterized by two dimensionless numbers, the Reynolds number (*Re*), and the capillary number (*Ca*). When *Re* is low, inertial effects are negligible, and fluid flow is laminar and simple. In the narrow spaces of a typical microfluidic device, this is almost always the case.

The capillary number (*Ca*) characterizes the balance between surface tension and viscous forces. *Ca* determines the behavior of the bubbles or droplets when they reach a point at which a channel splits into two branches (*3*). For *Ca* less than a critical value that depends on the length of the bubble or droplet and the geometry of the channels, surface tension dominates, so the bubble holds together. Like the traveler in Robert Frost's poem *The Road Not Taken* (*4*), the bubble must choose a path. Although Frost's protagonist selects "the one less traveled by," a solitary bubble or droplet forced to settle on one of two paths will pick the channel that offers the lowest resistance to flow. Because the presence of a droplet or bubble increases the resistance of a channel, a second bubble arriving at a junction between two channels of similar but unequal length will take path B after the first takes the shorter path A. As the interval between droplets decreases, more complex sequences of path choices (e.g., AABAAB) can be generated. This simple principle provides the basis for the work reported by both of these groups.



Microfluidics plus chemistry. A microfluidic channel carries water droplets of volume 250 pl in a continuous stream of oil. [Adapted from (*18*)] The droplets act as microreactors in which the reagents are rapidly mixed (upper undulating region) and are then transported with no dispersion (lower smooth region). Such an arrangement can be used to control chemical reaction networks on a millisecond time scale.

Fuerstman *et al.* generate a series of droplets at periodically or aperiodically varying times and pass the resulting stream through a loop that offers the choice between two slightly different channels, thereby modifying the initial interdroplet time intervals and encoding an input signal. Remarkably, unlike Frost's traveler, who "doubted if I should ever come back," Fuerstman *et al.* are able to restore the initial series of intervals, thus decoding the signal, either by reversing the direction of flow or by passing the stream through a second loop.

Prakash and Gershenfeld take a more explicitly computational point of view, representing bits of information as bubbles in a channel, and construct circuits that not only carry out the functions of Boolean AND, OR, and NOT gates but also can be linked together to produce more elaborate arrangements capable of acting as counters, oscillators, or memory arrays. It is perhaps not totally farfetched to imagine a stream of bubbles and a set of rules for manipulating them as the equivalent of Turing's vision of a universal computing machine (*5*).

These demonstrations of the power of relatively simple two-component microfluidic flows make one wonder how far it is possible to go in constructing microfluidic "thinking devices." Can *in fluido* compete with *in silico* or *in cerebro*, at least for certain specialized applications? It is now possible to build microfluidic chips containing hundreds of addressable elements (*6*), and these elements can be made to perform important control and memory tasks (*7*). Microfluidic networks have already been used to solve mazes (*8*) and computationally difficult mathematics problems (*9*). Paradoxically, one obstacle to the practical use of microfluidic devices has been their lack of portability, but recent work (*10*, *11*) suggests ways in which this hurdle may be overcome.

Neurons and computer chips are certainly more complex than chemically inert droplets or bubbles. One way to enhance the capability of microfluidic devices is to combine them (see the figure) with the bistability, oscillations, and waves found in nonlinear chemical dynamics (*12*). On a macroscopic scale, the prototype Belousov-Zhabotinsky reaction has been used in devices that count (*13*), store images (*14*), solve mazes (*15*), and perform logical operations (*16*). Carried out in a microfluidic network, an appropriately chosen set of nonlinear reactions not only mimics but also provides insight into the mechanism of blood clotting (*17*). Although it is difficult to envision microfluidic systems in which the number of connections to a single element begins to approach the number of synapses to an individual neuron, adding the repertoire of chemistry to the flexibility and capability of rapid switching provided by microfluidics should make it possible to perform tasks of considerable complexity.

Will future James Bonds be equipped with microfluidic computers or encoders that fit into a shoe and are fueled by a bottle of spring water (or premium vodka)? Probably not, but the possibility of carrying out an impressive variety of tasks usually associated with brains or computers will undoubtedly inspire many more creative uses of microfluidics, both in

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systems like the ones explored in these two reports and in more complex arrangements that exploit chemistry as well.

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CELL BIOLOGY

Cellular Demolition and the Rules of Engagement

Does cell death occur by pulling the plug on Bcl-2 life support or pulling the trigger on Bax activation?

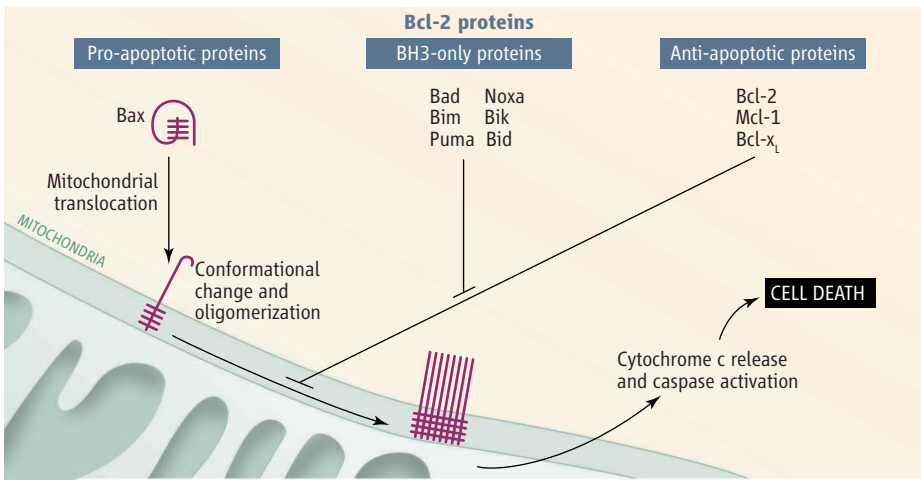
Richard J. Youle

Execution of cellular suicide, or apoptosis, is indisputably under the dominion of one group of proteins, the Bcl-2 family. But within this family, three factions are at odds with each other as they rival to promote or block cell death. Anti-apoptotic members act to restrain pro-apoptotic members. More of an enigma has been the activity of "BH3-only proteins," family members that share a single motif (a BH3 domain) with other Bcl-2 proteins. BH3-only proteins can bind to different subsets of the Bcl-2 family, but whether they assist cell suicide by activating pro-apoptotic members or inhibiting anti-apoptotic family members has been debated. On page 856 of this issue, Willis and colleagues (*1*) conclude that BH3-only proteins work solely by thwarting anti-apoptosis Bcl-2 proteins, thus settling the controversy. We now understand one way to start the cell's death machinery.

Bcl-2 regulates tissue health by inhibiting apoptosis. Bax (and its homolog Bak) has the opposite effect of promoting apoptosis despite a surprisingly similar three-dimensional structure. It is now accepted that anti-apoptotic family members, including Bcl-2, Bcl-x_L, and Mcl-1, restrain Bax and Bak activity. Once activated, Bax and Bak induce permeabilization of the mitochondrial membrane, thereby allowing cytochrome c release and the activation of caspases (which catalyze protein degradation). This cascade of events ultimately leads to breakdown of the cell (see the figure).

Bax was originally identified as a Bcl-2–binding protein and was thought to be inhibited by this interaction (*2*). However, contrary to this "rheostat model," in healthy cells, Bax is not bound to Bcl-2 but exists as a monomer in the cytosol (*3*). How can anti-apoptotic Bcl-2 family members keep pro-apoptotic Bax in check without direct interaction? One solution may center on the activity of BH3-only proteins.

Cell-free models of Bax activation, which do not fully reflect the in vivo situation, show that pro-apoptotic Bax acts synergistically with peptides corresponding to the BH3 domain of only a few BH3-only proteins, notably those from Bid and Bim, to induce cytochrome c release from isolated mitochondria (*4*–*6*). Bid and Bim are thus referred to as "activator proteins." This scenario is consistent with the simple model in which BH3 peptides bind to Bax and cause a conformational change and oligomerization of Bax, thereby activating the mitochondrial cell death pathway. How then do the other BH3-only proteins that do not bind to Bax (so-called "nonactivating" proteins) induce apoptosis? One possibility is that activator proteins could be sequestered by Bcl-2. The activators could be freed from this association if displaced by nonactivating BH3-only proteins. The released activators could then bind to Bax and trigger cell death (*5*). One problem of this model is that activator proteins (Bim and Bid) do not detectably bind to Bax. However, recent work shows that stabilizing the BH3 domain structure (α helices) of Bid and Bim allows the peptides to bind to Bax, keeping the theory plausible (*7*).



Restraining orders. Anti-apoptotic Bcl-2 family members block the translocation of pro-apoptotic Bax perhaps by directly binding and inhibiting Bax on mitochondria following a conformational change. BH3-only proteins promote apoptosis by binding to and inhibiting anti-apoptotic Bcl-2 family protein activity.

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