CHEMICAL BIOLOGY

How to sweet-talk bacteria

Small sugar molecules produced by an autocatalytic reaction cycle confined inside vesicle-based 'artificial cells' can trigger a response in living bacterial cells.

Geoffrey J. T. Cooper and Leroy Cronin

Recognizing that a living cell is an assembly of cooperating chemical sub-units, which together make up an organism with the required functions for life, raises a number of interesting questions. Among these are: how many components would it take to construct an ensemble that could be considered alive? And, related to this: how can we recognize and define when an artificial system is alive[1–3]? Living systems exhibit many characteristics that distinguish them from inorganic or non-living matter. Life is a complex phenomenon requiring not only individual self-replicating and self-sustaining systems, but also a mechanism that allows organization of information within these systems, which brings about characteristic evolutionary and metabolic dynamics[1].

There are two generally accepted approaches in the attempted construction of a so-called minimal cell — a top-down approach, in which the non-essential parts of the genome are systematically removed from a natural cell (leaving only the minimum required for sustaining life), and a bottom-up approach in which the protocell is constructed from scratch using simple building units[2–5]. From the chemist's point of view, the bottom-up approach is more appealing, and by careful choice of molecular building blocks, it should be possible to design a system fulfilling the requirements

for a living system. These are typically cited as metabolism, compartmentalization (for example, in a membrane vesicle or pre-fabricated microreactor) and information transfer through replication[2,6,7]. In the absence of a rigorous definition of an artificial living system, however, it becomes difficult to determine which features are most important.

One possible approach to establishing which features are relevant to life could be achieved by constructing an artificial protocellular system that is designed to mimic a 'real' living system. The assessment of its life-like properties could then be made by real living systems through chemical interrogation in a Turing-like test. To 'pass' this test, the protocell must first cause a response from the living interrogator and then, in turn, respond to that response, such that the interrogator is unable to distinguish the protocell from a living cell[1].

With their high degree of possible functionalization and conformational permutations, sugars and complex carbohydrates would be capable of providing both structural and information-storage roles in prebiotic chemistry. Despite this, they are largely neglected — perhaps simply because they have no direct genetic role in modern biology. On page 377 of this issue, Davis and co-workers[8] present the bottom-up construction of an elegant system in

which a proto-metabolism — running on simple feedstocks and producing complex carbohydrates — based on the formose reaction is encapsulated within vesicles that can stimulate a bioluminescent response from a living bacterial cell.

The well-known formose reaction involves the calcium-hydroxide-catalysed conversion of formaldehyde into a variety of linear and branched carbohydrates under the correct conditions[9]. By encapsulating formaldehyde and a calcium source inside phospholipid vesicles, a compartmentalized protocell system was constructed whereby the internal 'metabolism' (the formose reaction) of the vesicle could be initiated by a simple change in the pH. The borate complex of a key product from the encapsulated formose reaction could be used to stimulate the natural quorum sensing mechanisms of the marine bacterium *Vibrio harveyi*, giving rise to a proportional bioluminescent response that could be measured. This works because the borate complexes of certain products from the formose reaction are structurally very similar to the natural autoinducer molecule that stimulates quorum sensing, the means by which many bacteria communicate by release and detection of small-molecule 'messengers'.

By careful adjustment and optimization, the conditions necessary for protocell–cell
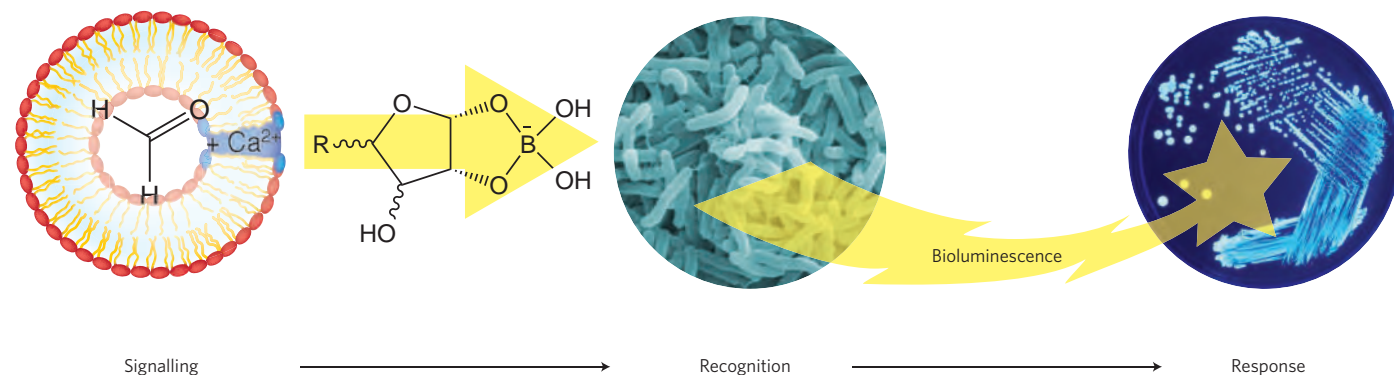

Signalling → Recognition → Response

Figure 1 | A schematic representation of protocell-to-cell communication. A vesicle in which the formose reaction — which converts formaldehyde into more complex sugar molecules — has been initiated releases a 'signalling' molecule that combines with borate in solution. This borate-derivatized sugar is then recognized by the bacterium *V. harveyi* and this stimulates a bioluminescent response. Images from the Dartmouth Electron Microscope Facility, USA, and the Centers for Disease Control and Prevention, USA.

coexistence were established. Addition of the protocells — in which the formose reaction had already been initiated — to active *V. harveyi* culture, containing borate and a pore-forming protein, resulted in significant light production by the bacteria, demonstrating that direct protocell-to-cell communication was occurring (Fig. 1).

With the huge variety of well-known organisms and potential responses available, it may be argued that the construction of a system that can give rise to a one-time response from a bacterium is not particularly difficult to achieve. This is a significant result, however, because it demonstrates the potential for truly bottom-up construction of protocellular systems that can co-exist and interact with living cells. If such a system could be scaled up to cope with many different reaction networks that could interlink with one another, then the prospects of developing emergent life-like chemistries are rather good. Future directions aside, the elegance of the current work lies in the simplicity of the core components — namely formaldehyde and calcium hydroxide — and the fact that they give rise to far more complex products and that they only do so when the system is triggered into activity by an external stimulus (here a pH change). This means that rather than looking for a simple

response from the bacteria to an added reagent, what we see here is a sequence of events where the protocell first manufactures the signalling molecule before releasing it. Only then can a response be observed from the living cells.

Clearly, to approach the requirements for a 'living' protocell, further layers of complexity need to be added. Early 'next step' implementations could take the form of a counter-response from the protocell on successful induction of bioluminescence in the bacteria. This could include events such as vesicle budding or other morphogenesis, cessation of carbohydrate production, closing of membrane pores or an entirely different chemical response that can be measured externally. Also, the recyclability of the protocell metabolism would need to be addressed to allow carbohydrate production and/or release to be actively moderated.

Finally, to allow the evolution of the system, protocells would need to have some means of self-replication whereby successive generations retain the functionality of the parent species, but with the inbuilt ability to adapt to a local changing environment. Meanwhile, the local chemical conditions would also have to allow growth of bacterial cells, which is not possible with the current system. However, Davis and co-workers suggest

that this might be overcome by the use of extremophiles[8] — bacteria that can better tolerate the fluctuating conditions required by the protocells.

In terms of addressing the 'Turing test' for life, this research paves the way for the construction of increasingly more intricate protocellular systems that are able to communicate chemically with different living 'interrogator' cells and distinguish them by different responses or behaviours. As this cross-talk between protocells and cells becomes ever more complex, the real question becomes: is the proof of life actually found by listening to the language rather than in the recreating the hardware? ❏

Geoffrey J. T. Cooper and Leroy Cronin are part of WestCHEM, Department of Chemistry, The University of Glasgow, Glasgow G12 8QQ, UK.
e-mail: L.Cronin@chem.gla.ac.uk

References
1. Cronin, L. *et al. Nature Biotechnol.* **24,** 1203–1206 (2006).
2. Szostak, J. W., Bartel, D. P. & Luisi, P. L. *Nature* **409,** 387–390 (2001).
3. Glass, J. I. *et al. Proc. Natl Acad. Sci. USA* **103,** 425–430 (2006).
4. Deamer, D. *Trends Biotechnol.* **23,** 336–338 (2005).
5. Rasmussen, S. *et al. Science* **303,** 963–965 (2004).
6. Noireaux, V. & Libchaber, A. *Proc. Natl Acad. Sci. USA* **101,** 17669–17674 (2004).
7. Tangen, U. *et al. Complexus* **3,** 48–57 (2006).
8. Gardner, P. M., Winzer, K. & Davis, B. G. *Nature Chem.* **1,** 377–383 (2009).
9. Butlerow, A. *Annalen* **120,** 295–298 (1861).

HETEROCYCLIC CHEMISTRY

New reactivity realized

At arguably one of the prettiest locations in England, the Nineteenth Lakeland Symposium brought together an international group of delegates from academia and industry to discuss a breadth of topics at the cutting edge of synthetic and heterocyclic organic chemistry.

Timothy C. Gallagher

Owing to the ubiquity of C–H bonds within organic molecules, the ability to selectively react and manipulate just one of them amongst many would have profound implications for the field of synthetic organic chemistry. 'C–H activation' provides a method of accessing a bond that is normally regarded as relatively unreactive, and frequently poses a significant issue in terms of control — there are often many C–H bonds available, and selectivity for specific bonds is intimately tied to effectiveness and efficiency. This topic has progressed rapidly in recent years and was a major theme at the latest Lakeland Symposium, held at Grasmere in England's Lake District.

A variety of mechanisms may be exploited to achieve net C–H activation, some of which are classical and others more recently recognized[1]: certain C–H bonds within arenes and heteroarenes, alkenes and alkanes can be accessed — with alkanes presenting a particularly difficult challenge. Transition metals, particularly rhodium, palladium and iridium, frequently have an important role in activation and subsequent functionalization of C–H bonds, with the added bonus that their effective use depends on their ability to be used in catalytic quantities.

The flexibility of rhodium-based catalysis to allow conversion of C–H bonds to C–C bonds within a variety of environments

was discussed by Jon Ellman (University of California, Berkeley). Activation of a β-C–H bond within unsaturated imines allows direct coupling to alkenes and alkynes and, in turn, provides a new and direct entry to substituted pyridines[2] (Fig. 1a). More broadly, the ability to selectively manipulate C–H bonds within a pre-existing and functionalized heterocyclic scaffold offers a highly versatile approach to the complexity and diversity that is sought within the pharmaceutical and fine chemicals sectors.

Conventional wisdom states that cross-coupling reactions require both C–halogen and C–metal components. However, the elegant and fundamentally important