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Sugar synthesis in a protocellular model leads to a cell signalling response in bacteria

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The design of systems with life-like properties from simple chemical components may offer insights into biological processes, with the ultimate goal of creating an artificial chemical cell that would be considered to be alive. Most efforts to create artificial cells have concentrated on systems based on complex natural molecules such as DNA and RNA. Here we have constructed a lipid-bound protometabolism that synthesizes complex carbohydrates from simple feedstocks, which are capable of engaging the natural quorum sensing mechanism of the marine bacterium *Vibrio harveyi* and stimulating a proportional bioluminescent response. This encapsulated system may represent the first step towards the realization of a cellular 'mimic' and a starting point for 'bottom-up' designs of other chemical cells, which could perhaps display complex behaviours such as communication with natural cells.

Although they are the third major class of biomolecules, sugars have been somewhat neglected in prebiotic chemistry, save as necessary building blocks[1,2] in an RNA-world[3]. This is despite their unrivalled potential to convey information due to their density of functionality and large number of configurational permutations[4]. Perhaps this neglect stems from the absence of any direct genetic templated role for most carbohydrates in current biotic chemistry. D-ribose, it could be argued, is simply a contingent scaffold to allow nucleobase display in nucleic acid polymers; other carbohydrates have been shown to be capable of providing a backbone structure[1,2,5]. Yet, this current absence in models does not preclude an early role for sugars in key developmental steps in prebiotic chemistry. Indeed, one of the strong supporting elements of RNA-world theory is a self-replicative ability based on autocatalysis and information transfer in the same molecule (that is, RNA). As Luisi[6] has highlighted, these properties in other molecules, perhaps in sugars, would 'greatly simplify any scenario concerning the origin of life'.

Considerable research effort in recent years has focused on creating a so-called 'minimal' artificial cell: one with all the components and characteristics to be described as 'alive'[7–9]. The construction of an artificial cell has traditionally followed one of two approaches: a so-called top-down approach, in which genes are sequentially knocked out from organisms until only the minimal genome required for sustaining life is left[10,11] and a bottom-up approach, which aims to create a cell from scratch[12–14]; such approaches, although theoretically not limited to biological materials or concepts, still typically use lipid membranes as a material for delineation of the cell[15]. This compartmentalization (cellular-ization) is important if the commonly cited[6,16] requirements for a living system are to be met: those of metabolism, a boundary or container and information transfer.

Here we describe a possible first step toward the realization of key goals: the development of a lipid vesicle container capable of initiating a quorum sensing response in *Vibrio harveyi* by means of the autocatalytic sugar-synthesizing formose reaction. By demonstrating here that an autocatalytic cycle[17] that synthesizes diverse sugars may be (1) compartmentalized (placed in a semi-permeable protocell), and (2) driven by a primitive form of metabolism (fuelled by small molecule precursors), we show a novel possibility of systems based on non-biological reactions that may be considered to have some characteristics of a protocell. A result of this reaction is the coupling to natural quorum sensing/language mechanisms of living bacterial cells, which provides a step towards the fulfilment of the criteria for information transfer, and therefore a step towards a construct that might be assessed by a successful 'Turing' test[18] for life.

Results

Design of a compartmentalized metabolism. The formose reaction[19] is complex and dynamic, producing a large variety of linear and branched carbohydrates via an autocatalytic mechanism[17] from concentrated (>1.0 M) formaldehyde feedstock at high pH and in the presence of metal cations (typically calcium; few others work well[20], see Supplementary Information). We considered the transfer of this reaction to a small bounded vessel, to maintain high local concentrations and suitable conditions, to be a vital aspect of establishing the potential of this complex and emergent reaction at the heart of a possible protometabolism (Fig. 1).

Several factors and potential problems of the vesicular formose reaction were addressed. First, the stringent reaction conditions required a robust membrane for encapsulation. Second, formaldehyde was required at high concentration within the vesicle for successful reaction; this necessitated minimal leaking or passage of formaldehyde across any possible membrane barrier. Third, in addition to formaldehyde, the reaction required calcium hydroxide as a catalyst (a source of both metal ions and alkali). This salt usually exists as a suspension under the reaction conditions, incompatible with the direct synthesis of vesicles; this suggested that *in situ* formation of this catalyst would be required.

In an effort to find an appropriate membrane system capable of fulfilling all of these criteria, a range of lipid (and other) building blocks capable of self assembly into potential cell-like reaction compartments were screened (using both dynamic light scattering analysis and the permeability of a fluorescent probe, see Supplementary Information) to determine whether they would form cohesive compartments and withstand conditions (high pH, feedstock, concentration and ionic strength/salt concentration).

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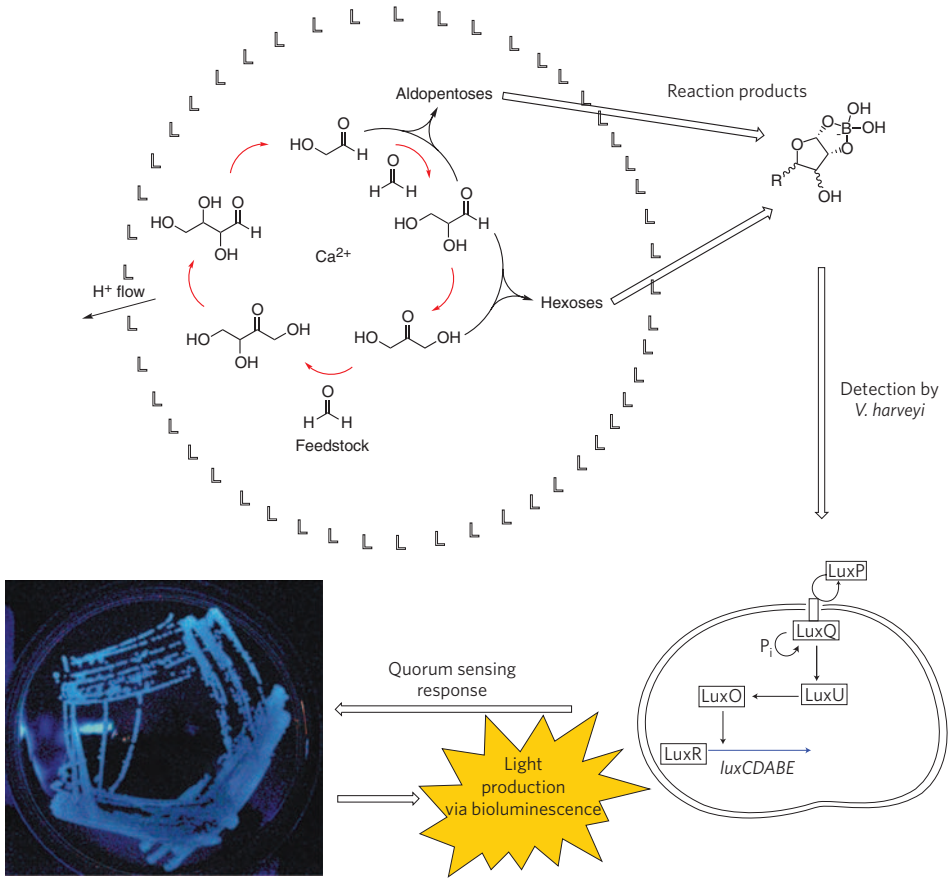

Figure 1 | The chemical cell concept. The components of the autocatalytic formose reaction are encapsulated within a lipid (L) vesicle (top). The increased pH outside the vesicle initiates the production of carbohydrates. Carbohydrate–borate complexes are formed (top right), and these diffuse through the medium to interact with the bacterium *Vibrio harveyi* (bottom right). Successful binding to the LuxP/LuxQ signal transduction proteins of the *V. harveyi* bacterium results in a protein phosphorylation response, and the subsequent expression of the genes *luxCDABE* (P_i, inorganic phosphate). The proteins produced give rise to a detectable bioluminescent output (bottom left, a photograph of an agar plate of *V. harveyi* stimulated with formose). In this way, the products of the protocellular metabolism of a chemical cell allow signalling to the cells of a natural organism.

Of those tested, the synthetic phospholipid 1,2-di-*O*-phytanoyl-*sn*-glycero-3-*O*-phosphocholine (DPhPC) was found to be optimally robust and capable. Dynamic light scattering showed that discrete vesicles of DPhPC remained present over many hours at pH 12, with good stability and consistency (no significant change in vesicle size, diameter 160–200 nm). Permeability was tested using the fluorescent dye 5-carboxyfluorescein, which was encapsulated within the vesicles at high concentrations, sufficient to quench fluorescence[21]. With this dye marker, leakage from the vesicle compartment into the bulk solution owing to any possible membrane degradation or diffusion is indicated by greatly increased fluorescence; no leakage was observed over several hours from DPhPC vesicles under the testing conditions required with base and/or calcium present (see Supplementary Information). We also examined the vesicles using fluorescence microscopy, with the incorporation of a fluorescent dye into the vesicle membrane for visualization[22] (see Supplementary Information). Vesicles were observed at high pH for several hours, and no disruption events were observed.

Having discovered a suitable robust encapsulating material, we probed the permeability of the resulting vesicle cells towards formaldehyde—the feedstock for our intended protometabolism, which for successful sugar synthesis would need to be retained at high concentration (>1.0 M). Formaldehyde was encapsulated within vesicles at concentrations greater than those required for the reaction, and the concentration of lost or leaked formaldehyde outside the vesicles was quantified at various time points using a coupled spectrophotometric redox-enzyme assay allowing precise and sensitive detection (see Supplementary Information). The results showed that the rate of formaldehyde crossing the membrane was so low that the vesicles maintained an interior formaldehyde concentration of >1.0 M even after 4 hours (see Supplementary Information); they also confirmed a very low external concentration of formaldehyde significantly lower than the amount necessary for a productive formose reaction to occur.

Next, a method for the *in situ* formation of the formose catalyst, calcium hydroxide, within the vesicles was devised. Calcium hydroxide itself is insoluble, and although many soluble calcium salts exist, calcium ions themselves cross phospholipid membranes relatively slowly[23]. In contrast, protons much more readily cross membranes, via a different mechanism from that of metal ions[24]. Together, these suggested that calcium hydroxide formation could be achieved *in situ* by exogenous addition of alkali to vesicles containing soluble but internally trapped calcium. Such a system would thus be driven/switched by the existence of a pH difference, created simply by addition of a source of OH^- to the solution external to the vesicle. To determine that basic conditions could be created inside a vesicle in this way, the fluorescent pH indicator pyranine was encapsulated in a vesicle at neutral pH. Addition of hydroxide ions to the external solution around the vesicle suspension rapidly resulted in increased fluorescence of internal pyranine (see Supplementary Information), indicative of a marked rise in internal pH. In this way, external pH control could be used to drive an internal formose reaction.

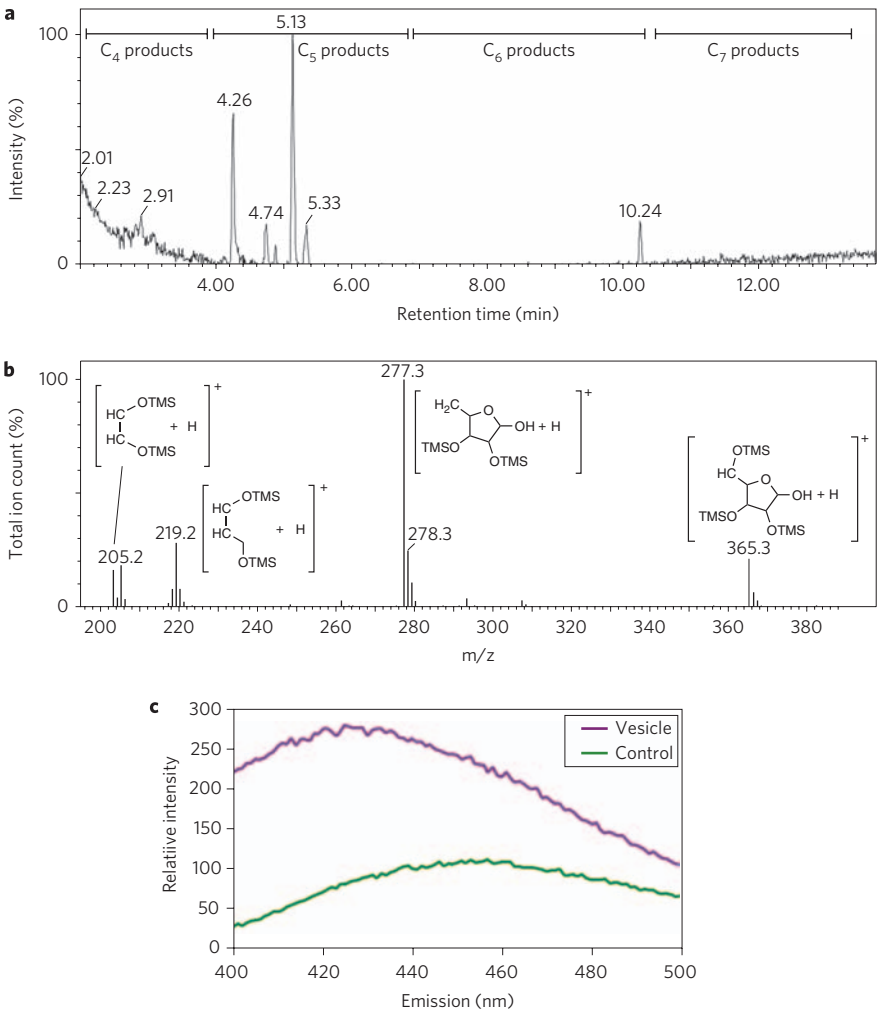

Figure 2 | Evidence for protometabolic formation of carbohydrates inside a vesicle. a, Gas chromatography–mass spectroscopy trace of *per*-trimethylsilyl-derivatized contents of the reaction inside vesicles. The observed peaks correspond to carbohydrate products with a distribution that can be referenced to that of the bulk-phase formose reaction (see Supplementary Information). **b**, An illustrative mass spectrum of the gas chromotography peak eluting at ~5.1 minutes in **a**. Assignment of the fragments corresponding to each peak (shown) allowed identification of the compound as an aldopentose. The spectrum was comparable to that of synthesized *per*-trimethylsilyl pentose samples (see Supplementary Information); other gas chromotography peaks were characterized similarly (see Supplementary Information). **c**, The emission spectra of 4-(dimethylamino)naphthalene-1-boronic acid, in the presence of the products of the reaction and a control sample. 4-(dimethylamino)naphthalene-1-boronic acid is a fluorescent dye which increases in fluorescence in the presence of carbohydrates[53].

Initiation and analysis of the compartmentalized formose reaction. With the key requirements now established, the compartmentalized formose protometabolism was initiated. First the chemical components were assembled; formaldehyde and calcium chloride were encapsulated in a DPhPC vesicle. Next, the external solution was exchanged by gel filtration for sodium chloride solution at sufficient concentration to act as an osmotic buffer. Finally, sodium hydroxide solution was added to the surrounding external aqueous medium, and the internal reaction process was initiated by heating to 37 °C.

Parallel analytical techniques were used to ensure successful detection of any possible carbohydrate products indicative of successful metabolism, even at the low concentrations associated with the low reaction volume (and therefore low absolute amounts) available within vesicles. First, an extensive characterization of a bulk-phase formose reaction was carried out (see Supplementary Information), principally using gas chromatography–mass spectrometry (GC–MS), to ensure a suitable basis for comparison and analysis of formose products produced inside the vesicle. GC–MS of *per*-trimethylsilyl-derivatized products of the vesicle reaction gave peaks (Fig. 2a,b, additional reference material in Supplementary Information), which retention time and mass spectrometry confirmed as carbohydrates. Consistent with our goal of non-bulk reaction evolution, the product peak distributions in vesicles differed both in type and temporally from those of a bulk reaction. Formose product distribution is known to be very sensitive to reaction conditions, and the kinetics may be modulated by alteration of the size and/or volume of the microscopic vesicular reaction compartment[25,26]; analysis revealed a carbohydrate composition of 65% pentose and 15% hexose, with the remaining carbohydrates split between tetroses, heptoses and components of the formose autocatalytic cycle. This represents a significant change from the bulk-phase reaction, for which the product composition was estimated as 38% hexose, 15% pentose, 18% tetrose and 19% heptose. Successful carbohydrate product formation was also confirmed by other methods including ^{1}H NMR spectroscopy (see Supplementary Information), which revealed the emergence of resonances in the characteristic carbohydrate region ($\delta = 3.5$–5.0 ppm). The fluorescent carbohydrate indicator 4-(dimethylamino)naphthalene-1-boronic acid (DMNBA)[27] was also used. This water-soluble

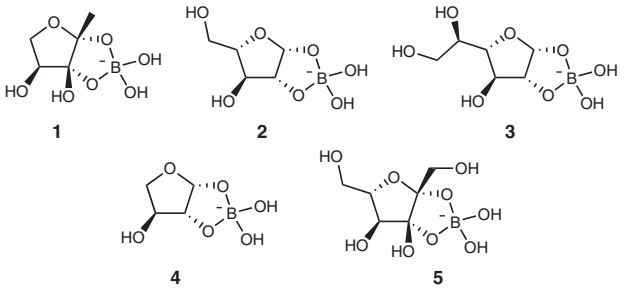

Figure 3 | Illustration of the structural analogy between signalling molecule autoinducer-2 (AI-2) and products of the formose reaction. The structures shown are the natural autoinducer-2 (**1**), and four of the formose reaction products identified as borates: D-xylofuran-1,2-*O*-borate (**2**), D-galactofuran-1,2-*O*-borate (**3**), L-threofuran-1,2-*O*-borate (**4**) and D-sorbofuran-1,2-*O*-borate (**5**).

fluorescent dye gives a characteristic fluorescence increase upon binding to carbohydrates. DMNBA gave a strong fluorescent response to vesicle metabolism products characteristic of the presence of carbohydrates (Fig. 2c). In all cases, negative control reactions—omitting one of the key metabolic components (formaldehyde, calcium, pH gradient)—gave no observable carbohydrate formation. Together these experiments demonstrated successful sugar synthesis using a protocellular encapsulated reaction.

Control experiments were also performed that provided additional evidence that the reaction was taking place only within the internal vesicle volume. When the lipid was omitted from reaction setup, no carbohydrate products were observed, indicating that the reactants required concentration/localization within vesicles for successful reaction. Together with the negligible formaldehyde concentration observed external to the protocells (as described above) these results further confirmed that the observed reaction was taking place within vesicles.

Sensing of protocellular primary metabolic products by natural cellular partner *V. harveyi*. Having established protometabolic reactions within a lipid vesicle, potential signalling from this protocellular system was probed. A method of detecting metabolites created as chemical outputs by this system was conceived based on a novel biological assay for carbohydrates and formose using the so-called autoinducer-2 quorum sensing system[28] of *V. harveyi*. Quorum sensing describes the process by which low-molecular-weight signal molecules (such as the autoinducers) released by certain bacterial species can reflect the local cell population density and provoke cell-signalling responses. Bioluminescence of the marine bacterium *V. harveyi* is stimulated by at least two different types of signal molecules: acyl homoserine lactones (named autoinducer-1, AI-1, because its detection can induce further release) typical of Gram-negative bacteria[29], and a second based on a single unusual, furanosyl boronate named autoinducer-2 (AI-2; **1** in Fig. 3)[30]. AI-2-type systems are present in a large number of other bacterial species, both Gram positive and negative, including many pathogens[31,32]. Access to diverse autoinducer libraries for *V. harveyi* has, despite considerable interest[33–35], been largely limited to AI-1 like molecules[36–38], with few prescient examinations of the AI-2 system[39–41].

Struck by the apparent similarity of possible primary metabolic reaction products (**2**–**5**) to the natural AI-2 (**1**) (Fig. 3)[39], we identified borate-derivatized formose carbohydrates as potential signalling molecules and investigated their effect on *V. harveyi*. Assays of their activity were performed on the MM32 mutant strain, in which the abilities to detect AI-1 and produce AI-2 are both 'knocked out'[42–44]. Thus, MM32 (unable to produce AI-2 and capable of detecting only AI-2-type molecules) is an ideal reporter for AI-2 and any potential related molecules activating the same signalling pathway. Encouragingly, the products of bulk formose reactions (both Ca(II)- and Pb(II)-catalysed) induced significant light production (Fig. 4a) with EC_{50}s (the concentration at which half the maximum response is observed) of the order of 10^{-5} M, and gave the first indications of the potential for this system to mediate putative communication between protocellular formose and *V. harveyi*. Strikingly, comparison with the bulk product profile showed that the pattern of induction potency over time closely followed the evolution of different product molecule structures; a bioluminescence maximum from products formed at 105 minutes demonstrated not only that potent quorum sensing signalling/communication molecules are evolved by the formose reaction, but that this signal varies with a characteristic temporal periodicity. Metabolite structural features were found to be correlated with light-induction signal strength; standard complexes of individual carbohydrates were prepared and assayed for interaction with the MM32 system (Fig. 4b), and it was observed that of the 4-, 5- and 6-carbon sugar substrates tested, certain substrates resulted in significant induction (see Supplementary Information). Carbohydrates without borate showed little or no induction, but the addition of borate in the formation of complexes gave responses with EC_{50}s in the 10^{-4} to 10^{-5} M range. Notable configurational effects were also observed; the steroechemically related lyxose, threose and sorbose complexes, which bear the closest configurational relationship to the naturally-occurring AI-2 signalling molecule, gave the highest induction (for further discussion see Supplementary Information).

Following this successful interpretation by *V. harveyi* of the chemical output of our protocellular system, we tested artificial cell–cell (formose protocell–*V. harveyi*) interaction. When the protometabolic products of the vesicular formose reaction were introduced into *V. harveyi* culture, the bacteria showed a clear luminescent response (Fig. 4c). Estimations of concentrations of carbohydrate autoinducer analogue suggested the formation of 6–8 μmol of carbohydrate metabolic product per ml of vesicle solution; this value is consistent with an efficient formose protometabolism in the total vesicle-enclosed volume present (see Supplementary Information). Negative controls (performed as described in previous section) including 'empty' vesicles and non-metabolizing chemical cells gave no luminescent response. The response by *V. harveyi* provides further evidence for the presence of low-concentration carbohydrates, produced by a bounded metabolism.

Establishing more direct protocell-to-cell interaction

Stimulation of *V. harveyi* by protocell metabolites suggested that more direct signalling might be possible. The intial ambient conditions of the formose protocell (high pH and ionic strength/salt concentration) were different to those required by *V. harveyi* for growth and signalling. An environment in which the presence of protocells was no longer toxic to *V. harveyi* was generated in two ways: minimization of salt used for osmotic protection and increased buffering of *V. harveyi*. Optimization of vesicle integrity under high osmotic imbalance using dynamic light scattering and redox–enzyme assays (see Supplementary Information) allowed creation of robust protocell vesicles stable even over 4 hours in a minimum sodium chloride concentration of 25 mM. The resulting vesicles were no longer toxic when added to *V. harveyi* (see Supplementary Information). Furthermore, modification of *V. harveyi* medium with 25 mM HEPES at pH 7.8 dramatically improved the buffering capacity and hence the ability of *V. harveyi* to withstand addition of even the pH 12.5 protocell medium; bacteria continued to grow successfully.

Having established a medium for the coexistence of protocells with *V. harveyi*, we next examined incorporation of components into the protocell structure that would allow release of metabolites

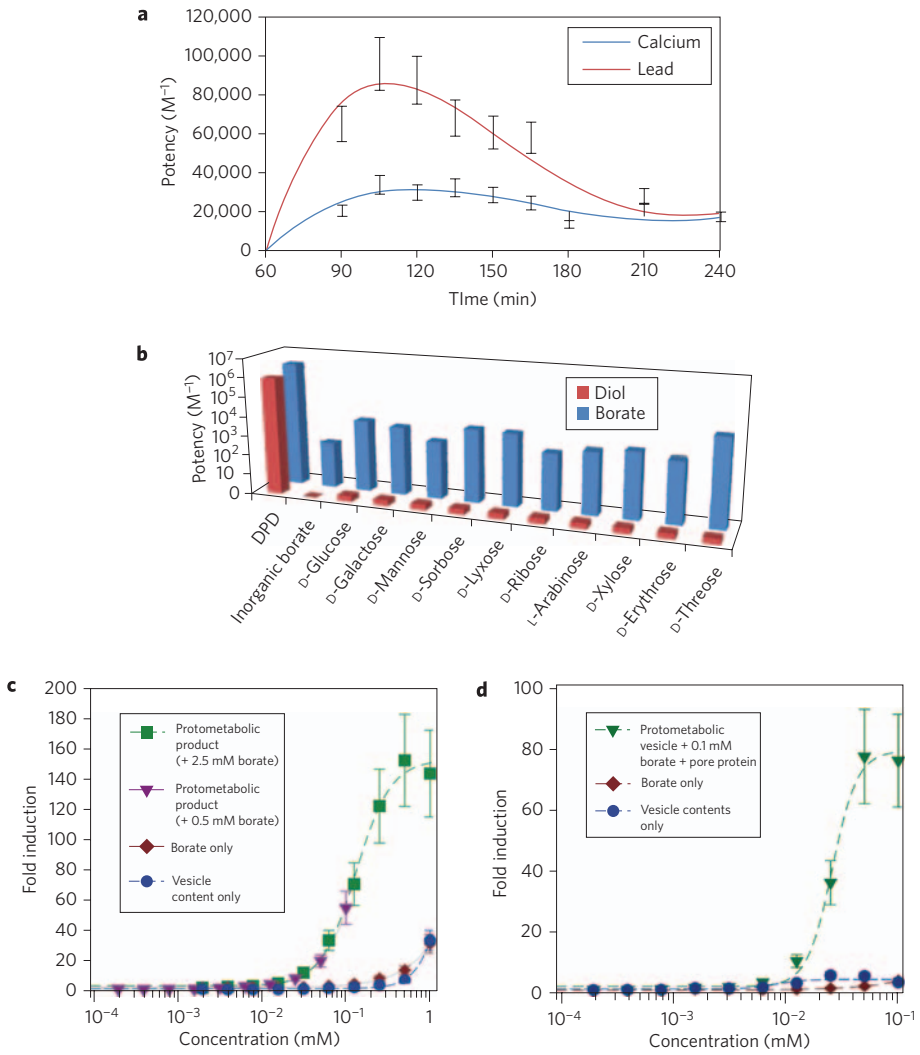

Figure 4 | Induction of bioluminescence in *Vibrio harveyi* by carbohydrate-borate complexes. a, Reaction time course of effect of Ca(II)- and Pb(II)-accelerated formose reaction products on light production by *V. harveyi*. 'Potency' was determined as the reciprocal of the fitted EC_{50} of the formose product mixture at each time point. Error bars indicate the established error over replicates in the biological assay. **b**, Effect of standard carbohydrate borate complexes (blue) on light production by *V. harveyi*. Control compounds (red) are carbohydrates without borate. DPD, (*S*)-4,5-dihydroxy-2,3-pentanedione, is the precursor to the organic borate AI-2 (compound **1**). **c**, Induction of bioluminescence in *V. harveyi* by borate complexes of vesicle contents. Vesicle contents without borate and with boric acid alone were included as negative control samples. Error bars indicate the established error over replicates in the biological assay. **d**, Induction of bioluminescence in *V. harveyi* by directly added vesicles containing formose contents. Vesicle contents without borate and with boric acid alone were included as negative control samples. Error bars indicate the established error over replicates in the biological assay.

to initiate signalling to *V. harveyi*. Although chemical disruption of lipid-assembly in protocells using surfactant (for example, Triton X-100) proved possible (see Supplementary Information), it also proved detrimental to *V. harveyi* viability and response. As an additional protocellular component we therefore used the pore-forming protein α-haemolysin[45,46]. α-Haemolysin is secreted by the bacterium *Staphylococcus aureus* and can insert into phospholipid membranes, forming pores for transport without destroying the vesicles[45,46]. Release assays using encapsulated 5-carboxyfluorescein (see Supplementary Information) demonstrated that α-haemolysin subunits successfully self-assembled and formed pores in protocells without any significant effect on the growth of the *V. harveyi* cells (see Supplementary Information).

Finally, having established a medium condition for protocell–cell coexistence and selective release of protocell metabolites, all necessary components were assembled. First, formose protocells were created and metabolism initiated. Next, active protocell-containing medium was added directly to active *V. harveyi* culture, containing all the key additional components (α-haemolysin, buffer, low salt, borate). The resulting significant light production by *V. harveyi* indicated successful protocell-to-cell signalling (Fig. 4d). Negative control experiments, in which key components were omitted (as described above) showed no induction of light (see Supplementary Information).

Discussion

The exploration of 'bottom up' approaches to synthetic life through the introduction of reactions into cell-like containers such as vesicles is an area of research that has been elegantly and much explored recently[6]. However, to date, few researchers have examined non-biological systems; emphasis has typically been placed instead on existing biomolecules, for example through enzyme-catalysed processes[12,13,47]. The incorporation of new, atypical reactions requiring harsher conditions represents a significant technical challenge, but is an important step if we are to consider artificial life not necessarily based on known biological systems or strategies.

The encapsulation of complex emergent reactions capable of an autocatalytic cycle, and hence a protometabolism—such as the formose reaction within a vesicular boundary that has been demonstrated here—represents, we believe, an important early step in such research. It has been predicted[6,16] that such a system might form the basis for the construction of a prototypical chemical entity displaying possible characteristics of life, featuring autocatalytic metabolism, within a far-from-equilibrium membrane-based vessel and coupled to information transfer. The indication of a significant change in formose product composition represents an exciting result of such encapsulation, not least because the increased yield of pentoses has potential implications in the origin of nucleic acids.

Despite significant advances in artificial cell technology[7–9], there also remains the problem of determining and/or defining whether or not a given system is indeed 'alive', and thus quantifying the success of the approach taken. It seems possible that metabolism may be a key element[6,16]. However, one suggested approach[18] to establishing relevant features is the testing of mimicry in which cellular systems are assessed by real living systems through chemical interrogation. This may provide a focus for one strand of synthetic biology, with the possible advantage of avoiding the requirement for a definition of life and replacing it with the operational scenario of the test. This 'Turing-like test' could be used to assess progress in the construction of the cellular protometabolism that we have established here as we have demonstrated a primitive form of communication between two cellular entities (one artificial, one natural).

There are still difficulties to overcome before this can be considered true communication, in particular two-way signalling and continued commensal growth of both protocells and bacterial cells. The latter is currently prohibited by pH for the species examined here. However, we are currently examining the possibility of using extremophiles able to withstand the pH of the formose in communication; genetic engineering of the *lux* light-emitting cassette into such bacteria could allow longer and increased interaction between our vesicle protocells and living cells. It should also be noted that here we used two extremes of metabolite release from the protocell—non-selective and forced selection using an evolved protein pore. The development of selective release based on alternative strategies (such as selective lipid permeability[14] or as a result of the reaction mechanism itself) of more direct relevance to understanding protometabolism is an important next step. We hope that more detailed study, refinement and development of the system we have presented here will reveal more about the mechanism of this fascinating protometabolic reaction when held in a small-volume reactor, and might also suggest possible chemical origins of bioactive carbohydrates[48,49] (for example, in communication and in the central structures of other key biomolecules). The protocellular system described here creates D-ribose as one of its many sugar products/outputs. Although selectivity is not yet possible, it has been suggested that the interaction of pentoses with phospholipid membranes may allow a mechanism for selective sequestering of ribose[48]. This would raise the possibility of such or similar systems having a role not only in an alternative prebiotic chemistry based on sugars—'a sugar-world'—but also as a prelude/precursor to an RNA-world and, as such, a potential prebiotic 'missing chemical link'.

Methods

Typical synthesis of DPhPC vesicles. An appropriate amount (typically 1.0 mg) of DPhPC was lyophilized from chloroform to a thin layer inside a glass vial. The lipid was suspended in an aqueous solution by vortexing for 5 minutes, followed by sonication for 20 minutes. The suspension was subjected to 3–5 rapid freeze–thaw cycles, and was passed 11 times through a polycarbonate membrane with 0.1-μm-sized pores using a mini-extruder (Avanti polar lipids). The vesicle solution was diluted to 2.5 ml with sodium chloride solution of an appropriate concentration to act as an osmotic buffer, and passed through a PD10-size exclusion column pre-equilibrated with the same sodium chloride solution (25 ml) using 3.5 ml of the same sodium chloride solution as an eluent.

Typical procedure for formose reaction in a vesicle. 1.0 mg DPhPC was lyophilized from chloroform to a thin layer inside a glass vial. The lipid was suspended in 1.1 ml 130 mM calcium chloride solution with 1.33 mmol formaldehyde in water by vortexing for 5 minutes, followed by sonication for 20 minutes. The suspension was passed 11 times through a polycarbonate membrane with 0.1-μm-sized pores using a mini-extruder (Avanti polar lipids). The vesicle solution was diluted to 2.5 ml with 1.0 M sodium chloride solution, and passed through a PD10-size exclusion column pre-equilibrated with 1.0 M sodium chloride solution (25 ml) using 3.5 ml of 1.0 M sodium chloride solution as an eluent. The volume of the solution was reduced to 1.0 ml *in vacuo*. The pH of the solution was then increased to 12.5 using 1.0 M sodium hydroxide solution, and the mixture was heated to 37 °C. After 240 minutes, the vesicles were solubilized with 2% Triton X-100 solution, then deionized by passing down a column of DOWEX 50X8 (H^+) and DOWEX 1X400 (^-OH) resins. Negative control samples were prepared by the above procedure, omitting either calcium chloride, formaldehyde, sodium hydroxide, or DPhPC.

Protocol for analysis of vesicular formose reaction mixture by GC–MS. The formose reaction residue was redissolved in water (4.0 ml) and treated with activated Amberlite IR-120 (H^+) resins, and Amberlite IR-410 (HCO_3^-) resins until the solution was neutral. It was then filtered, and the solvent was removed *in vacuo*. The residue was dissolved in dry pyridine (0.25 ml) containing pyridine hydrochloride (5 mg, 0.04 mmol), and trimethylsilylimidazole (0.15 ml). The mixture was stirred at room temperature for 1 hour, then 0.25 μL of each sample was analysed by GC–MS.

Protocol for analysis of vesicular formose reaction mixture by fluorescence. The formose reaction residue was redissolved in HEPES buffer pH 7.8 (1.0 ml). 4-(dimethylamino)naphthalene-1-boronic acid (1 μmol) was added, and the fluorescence spectra ($\lambda_{ex} = 300$ nm; $\lambda_{em} = 400$–500 nm; slit widths: 5 nm excitation, 5 nm emission) were recorded. Negative control spectra using just the dye in aqueous solution were also recorded.

Calcium-accelerated bulk formose reactions. Calcium hydroxide (500 mg, 6.7 mmol) was suspended in water (50 ml). Formaldehyde (2.0 g, 67 mmol) in water (3.0 ml) was added, and the mixture was stirred at 40 °C under Ar for 240 min. At appropriate time intervals after addition of formaldehyde, aliquots (5.0 ml) were taken and added to boric acid (24 mg, 0.39 mmol), and allowed to stir for 10 minutes. The aliquots were subsequently neutralized using activated Amberlite IR120 (H^+) resins, filtered through a 0.2 μm syringe top filter, and the solvent was removed *in vacuo*. The residue was redissolved to an appropriate concentration based on boric acid. See Supplementary Information for lead(II) variant.

Typical procedure for preparation of carbohydrate borate complex standards. A sample of the chosen carbohydrate and an equimolar amount of boric acid were dissolved in water. The mixture was stirred for 2 hours, and the solvent was removed *in vacuo* to afford carbohydrate-borate complexes as a white solid. Characterization by ^{11}B and ^{13}C NMR and mass spectroscopy agreed with previously published data[50,51].

Assay of borate complexes with *V. harveyi*. 20 μl of the appropriate concentration of standard carbohydrate borate complexes or formose reaction aliquots were added to 96-well microtitre plates. The *V. harveyi* reporter strain MM32 was grown for 16 hours at 30 °C with aeration in Luria Bertani medium and diluted 1:5,000 into fresh autoinducer buffer[52] medium. 180 μl of the diluted cells were added to the wells containing samples. Negative control wells contained 20 μl of sterile autoinducer buffer medium. The microtitre plates were incubated at 30 °C. Every 30 minutes, light production and optical density at 490 nm were measured using a Perkin–Elmer Victor^{3}V 1420 multilabel counter. Results are reported as the fold induction of the samples over negative control.

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Author contributions

All authors conceived and designed experiments, analysed the data and discussed the results. P.M.G. performed the experiments and P.M.G. and B.G.D. co-wrote the paper.

Additional information

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