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Making Biochemistry-Free (Generalized) Life in a Test Tube

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7.1. Summary

As we are discovering a large number and variety of extrasolar planets, many of which could harbor some form of life, it is time to ask the question: Is biochemistry-based life the only chemical support for life? On Earth, all living systems (i) process information, (ii) metabolize, (iii) self-reproduce and (iv) evolve. These are traditionally associated with the presence of a boundary, metabolism and information-carrying polymers. But, can processes (i)–(iv) take place in a non-biochemical chemical system? We present progress in this area resulting from experiments on system boot-up generated in a one-pot batch reactor during the autonomous chemically controlled non-equilibrium synthesis and self-assembly of functional polymer vesicles from a homogeneous blend of small, non-biochemical molecules. We follow their dynamical evolution that integrates metabolism, growth, reproduction and descent with modification under autonomous chemical control, achieved by implementing a polymerization induced self-assembly (PISA) scenario, which solves the concentration problem and generates an enabling and versatile free-energy gradient. As chemicals ("fuels") are consumed in the polymerization

The First Steps of Life,
coordinated by Ernesto DI MAURO.

reaction, energy is dissipated and entropy changes result in morphological changes, the expulsion of "spore-like molecules" and joint physicochemical evolution. We monitor the consequences of the copolymer synthesis and the resulting evolution of their molecular self-assembly in "vivo". We find that this transient (or dissipative) self-assembly process leads to vesicles with diameters between 0.5 μm and tens of micrometers. They exhibit several autonomous, emergent, life-like properties, including periodic growth and partial collapse, system self-reproduction, together with homeostasis, competition, chemo-phototaxis and adaptation. We also briefly discuss the extension of the above by combining PISA with click or oscillatory chemistry, which yield higher complexity entities. Together, these results offer insights into completely chemistry-based artificial life, as well as into the origin of proto-cells en route to proto-life and the simplest pre-LUCA living systems. This provides the first material realization of Bernal's "generalized life".

7.2. Introduction and background

Life on planet Earth provides us with the most complex manifestations of matter that we know. It uses chemistry, that is, processes "in which atoms and ions change partners" (Feynman et al. 2005) with their attendant "consequences, both in the structure and composition of matter and in the accompanying energy changes" (Adesnik et al. 2002). Using the subset of carbon chemistry we call biochemistry, living systems on Earth are thermodynamically open chemical systems operating in out of equilibrium conditions. We do not know why life exists on Earth or its origin, and we do not know if it exists in other celestial bodies beyond our 4.567 Ga planet (Morris et al. 2019). The fossil record indicates that it was already present on the Earth about 3.7 Ga ago (Fortey 1999). Phylogenetic analysis demonstrates that the three major domains in which life manifests today, namely, Bacteria, Archaea and Eukaryotes, share a Last Universal Common Ancestor (LUCA). We believe that this ancestor must have been far less complex than what we see today. Living systems range in sizes between micrometers (bacteria) and tens of meters (for aspen). Yet, in spite of their extraordinary diversity and complexity, all living systems display four basic properties or functions (Eigen 1995; Morris et al. 2019; Nurse 2021; Munuzuri and Pérez-Mercader 2022). These functions are as follows: (i) the handling and processing of information (Feynman 1982; Cheetham 2011), (ii) metabolism (Cheetham 2011; Smith and Morowitz 2016), (iii) self-reproduction (Volkenstein 1983) and (iv) evolution, both (a) adaptive and (b) non-adaptive (Lynch 2007). In the environments where they exist, by themselves or in interaction with other living species, all extant living systems universally implement these functions using biochemistry.

In extant life, the "processing of information" consists, on the input, of information (contained in the living system's genome and its molecular machinery; Morris et al. 2019), which is then transformed and output in useful forms with which the system is capable of executing the above functions enabling its existence in its environment. With that information, the living system can harness the energy in its environment, and using simpler molecules found in this environment, it synthesizes the chemical molecules that realize its existence. Eventually, some of these parts are used by the living system to self-reproduce, that is, to make fresh "copies" of itself (its "children"). For many reasons, which include the limited fidelity of molecular copying, self-replication (especially in a complex environment) and molecular and systemic degradation, these may not be perfect "copies". They are viable copies, in the sense that all that is required is that the copies themselves can carry out their own independent lifecycle (these "children", an embodiment of von Virchow's "omnia vita ex vita" dictum (Virchow 1859), can coexist for some time with their parents and join their parents' already existing population as they are born, age, and execute their own lifecycle). Finally, the chemistry of extant living systems can change sufficiently and make populations whose individual living systems evolve following adaptive and/or non-adaptive strategies (Lynch 2007).

How did all this come about? We do not know, and given the historical nature of this question, we may never know with certainty. But most of the current scientific thinking on this revolves around the notion that what we see today is the result of co-evolutionary processes (Knoll 2003). That is, processes involving the coupled evolution of life, its chemistry and environment, that is, the co-evolution of life and its planetary environment. Perhaps there was an evolution from the simple to the complex (and from LUCA to today).

However, because of its specification and definition, LUCA already used extant biochemical molecules and was therefore considerably complex. Hence, the notion that perhaps there were simpler predecessor chemical systems constituting simpler lineages that eventually evolved into more complex systems, and finally closer to LUCA and its epoch. These hypothetical systems are called "protocells" and have become an area of experimental research in recent years (Rasmussen 2008). Presently, and because of the lack of historical evidence, we can only make guesses as to how protocells operated or how they appeared, or if so, how they evolved into lineages that eventually led to LUCA. But we do know that if they existed, they must have been constrained by the laws of physics and chemistry, just like the rest of the planet and the Universe.

But, did protocells exist or could they have existed? And, if they existed, what were they like? What can we learn in the chemistry laboratory today about their de novo synthesis? Is biochemistry necessary or just sufficient to implement the four properties common to all life? Can we put together chemical systems that using small molecules (without biochemistry) display the above properties using chemistry and the processes in which atoms change partners and generate changes in structure and energy to implement them? What would we learn?

If such chemically operated protocells could be built, they would fall into the category and be an example of what Bernal conceptualized (Bernal 1965, 1966) as "generalized, or cosmic, biology". This "generalized life" refers to all chemistry based and operated systems that using simple molecules and physical sources of free-energy such as light, execute (unify, display, express) the four properties or shared functions of biochemistry-based (extant natural) life. That is, generalized life displays/expresses the *same functions* as natural life, but with a *different mechanism*. In this context, protocells, together with chemical evolution, are an important and integral part in the evolutionary journey from non-living to living matter.

A practical framework to tackle these questions is to apply an "outside-in" design strategy for a system (Brooks 1995), in which we start with the general properties of the system and then try to reproduce those properties with a lower level and more detailed structure in the logical or mechanistic hierarchy. Think of the separate functions to be implemented and then find a way to couple them so as to generate a cooperative (emergent) system where "the whole is more that the [simple] sum of its parts" (Bridgman 1936). In theory, a potentially successful strategy to couple and integrate these functions is to identify features shared by the general functions and assemble the system in such a way that the shared attributes are necessary, complementary and accessible to the properties. These common physical (or chemical) features provide a framework over which to link the functions sought after (Lin et al. 2021; Munuzuri and Pérez-Mercader 2022).

Although the above is very conceptual and more easily said than done, an example from the history of technology can illustrate this "outside in" approach. Let us consider artificial powered flight. In nature, we have birds, insects and mammals that fly. They use wings which they flap (Tennekes 1998). This is not what Langley, and seven years later, the Wright brothers did to achieve artificial powered flight. They understood the principles of flight and had a profound understanding of the "border of attack" of a wing, which combined with the effects of a moving air mass generated by a rotating propeller of the right pitch could provide a means of using Bernouilli's principle to lift a plane into flight. They did not use feathers or wing flapping. They used the combination of physical principles that enables flight in

natural life, as in birds, bats or insects, but implemented in a totally different (and artificial) fashion with wood, metal, linen, and using the internal combustion engine.

A shared attribute for enabling the handling (processing) of information (property (i) shared by all living systems) and metabolism (property (ii) above) for an open (i.e. out of thermodynamic equilibrium) system to autonomously boot-up is provided by the presence of a free-energy gradient. This requires the information to already be present or generated in the system and enabled, as well as communicated. Given the correct boundary conditions (Munuzuri and Pérez-Mercader 2022), this free-energy gradient implies the existence of an entropy gradient, which, if it has the correct sign, can be used as a means to enable the production of localized order (lowering the entropy), as long as the gradient is maintained (Prigogine 1967) and the available information can be transported for its processing. Assuming a constant temperature, in such a situation the system's entropy could be adjusted by an energy flow from the environment and, depending on the Gibbs free-energy consumption and entropy production rates, eventually the system's disorder would dominate and lead to its self-replication (Murray and Hunt 1993; Volkenstein 2009). In practice, the above translate into the need for the system to be spatially extended within some medium from which it is phase separated for the presence of gradients in chemical potential.

In order to metabolize, the system needs to process molecules it finds in its environment and use them as materials for the synthesis of presumably more complex molecules with which it makes, at least, its own parts and also those that will implement its self-reproduction. As Morowitz (1968) pronounced, and as quoted by Harold (1986, p. 22), "The necessary condition for this is that the system be connected with a source and a sink and the work be associated with a flow of energy from source to sink". For this, the system requires the existence of a free-energy gradient with respect to its external environment in order to perform the synthesis of its own more complex parts (molecules). But the system is an open system in some environment (Prigogine 1967), and with passage of time its entropy will increase and eventually the system will degrade and, possibly, disintegrate and "die".

Of course, extant living systems avoid this fate by generating appropriate copies of themselves through the process of self-reproduction, and the new systems can be called their "children" (Volkenstein 2009). Furthermore, due to imperfections in reproduction or changes occurring within the parents during their lifetimes, these populations will contain individuals which are differently (for the better or the worse) suitable to perform their functions in their environment and can therefore lead to a form of selection. They could, in principle, even compete among

themselves or acquire traits from their environment (e.g. some available catalyst) and enter into competitive exclusion (Hardin 1960; Mayr 2001; Martin and Hine 2008; Katla et al. 2023) and a "struggle for life" (Gause 2003).

The required entropy and concentration gradients for information handling and the internal autonomous synthesis by a living system of the substances necessary for its life can, in principle, be provided if the system exists in a volume that partitions (separates) it from the environment in which it exists, and furthermore, if it is capable of entrapping (at least partially) the molecules necessary to sustain the chemical reactions to power it while maintaining its thermodynamically open character.

Can we make/construct/create/design a material system that is somehow capable of autonomously implementing the above at molecular levels? It would need to be a dissipative system in which self-assembly takes place (Halley and Winkler 2008; Riess et al. 2020). Can such material system be implemented with chemistry? Is biochemistry needed?

In the following, we discuss experimental work that, not using biochemistry, demonstrates notable progress and promise in the answer to these questions.

7.3. Laboratory implementation of an artificial autonomous, and self-organized functional system

(I) Booting up a polymer system

Synthetic chemistry's "arithmetic demon", vesicles and "exorcising" the "demon"

The synthesis of chemicals is usually an uphill battle, and even more so in carbon chemistry, as it often requires multistep reactions followed by separation, purification and many other steps. Typically, any of these conversion rates are smaller than 1. Thus, put together, they give rise to an overall conversion rate, which can be exponentially much less than 1. In other words, after several of those steps, the amounts of chemicals that can be produced are very small. This situation is known as the "arithmetic demon" (Ireland 1969; Serratosa 1990; Gilbert and Martin 2016) and ways to combat it include a reduction in the number of synthetic steps, catalysis, "clever" use of the affinity between molecules (as in click chemistry, for example), "stocking up" of the ingredients in very large quantities, or concentrating the reactants into a small volume where the reactions can then take place by avoiding dilution effects.

In life, to deal with the arithmetic demon, nature seems to have opted for a combination of the above, including the use of "small containers" within which the complex reactions required by life take place, making extensive use of liquid–liquid phase separation as it occurs in "colloidal" matter. Of course extremely evolved, extant living systems such as bacteria, archaea or the individual and its cells in the complex system which constitute eukaryotes, have a permeable wall which separates them from their environment and therefore solves the concentration problem by providing a (global) free-energy gradient, while also keeping the system thermodynamically open. These natural membranes are made of phospholipid molecules. These are amphiphilic molecules, that is, block co-polymer molecules (Jones 2013), which, given a solvent (H_2O for life on Earth), combine a solvophilic block back to back with a solvophobic block (Jones 2013; Morris et al. 2019). If they are made of non-lipidic polymer blocks, they are known as "amphiphilic block copolymers" or ABCs. Given a high enough concentration of them in a solution (the critical micelle concentration (CMC)), they will self-organize and adopt equilibrium (Halley and Winkler 2008) self-assembled cooperative configurations into space-partitioning morphologies, such as micelles, tubes, vesicles and a number of ordered structures (Israelachvili 2006). The vesicles in natural life are somewhat permeable compartments (see below) made by membranes formed with phospholipid, lipid and fatty acid related amphiphiles, and containing a liquid "lumen" (when made of non-lipid polymeric amphiphiles, they are also called "polymersomes").

Can we make these molecules from artificial components simpler and easier to synthesize and from simpler components (Rosen 2000) than phospholipids? In extant natural life using simpler parts found in the environment, amphiphiles are synthesized by the living system. This synthesis has to be robust and precise, as the lengths of the solvophilic and solvophobic blocks have to be fairly uniform throughout the membrane. Otherwise, their self-assembly will not lead to uniform membranes, which will contain excessive defects and not be able to confine essential chemical materials at a sufficient level and for long enough times so as to allow for necessary functional activities (Jones 2013).

(II) Polymerization-induced self-assembly

Artificially, polymeric amphiphiles can be synthesized in the laboratory with excellent precision under very mild, oxygen tolerant conditions using the technique of RAFT polymerization (Perrier 2021) applied to a previously available "simple" block. This simpler block is called the macro-Chain Transfer Agent (mCTA). Macro

(the "m" in the acronym mCTA) refers to a solvophilic (or solvophobic) tail. The CTA is a small molecule that under the right conditions can transfer monomer molecules from the reaction solution, switching its place with them at the end of the solvophilic (solvophobic) block (the macro part of the mCTA molecule), where they are bound and start a solvophobic block, eventually making it by repetition of the transfer process into a solvophobic (or solvophilic) block with the CTA at the end. Thus, using RAFT polymerization, we could begin with a solution containing an mCTA, one of the ends of which is hydrophilic, and polymerize a monomer on it, also contained in the solution, that leads to a solvophobic block when it is in a polymer chain. As the polymerization reaction continues, this resulting ABC will become more solvophobic and therefore change its collective self-assembly configuration. For example, as the polymerization goes on and molecules of an ABC are produced, their resulting collective morphology can be expected to change from free ABC molecules in solution, giving rise to emergent cooperative associations of the still polymerizing ABCs into micelles, worms and on to vesicles. This (extraordinary) process is implemented in a polymerization-induced self-assembly (PISA) reaction, where starting from a stirred homogeneous and isotropic mixture of chemicals under mild conditions, the chemical reactions do their molecular assembly and construction job "directed" by the information contained in the reacting molecules and their environment, and use chemistry's power to simultaneously generate energy and structural changes in some medium. Furthermore, these changes can be controlled by a number of (not necessarily constant or deterministic) external factors such as light, temperature or reactant flow, including their diffusion, convection and migration (Gong and Pérez-Mercader 2018; Penfold et al. 2019; Pearce and Pérez-Mercader 2020a). An illustration of the chemical and physical pathway is briefly provided in Figure 7.1.

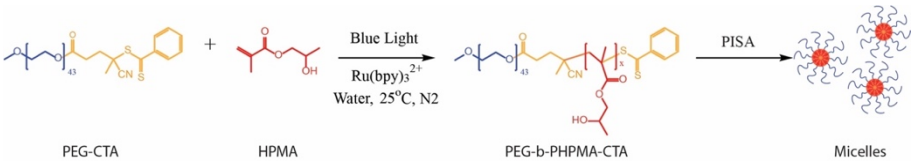

Figure 7.1. *Light mediated polymerization-induced self-assembly. For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

COMMENT ON FIGURE 7.1.– *Synthesis route for preparation of micelles via PET-RAFT PISA reaction using PEG-CTA and HPMA catalyzed by* $Ru(bpy)_3^{2+}$ *under blue light irradiation in an oxygen-poor environment. Under the correct environmental conditions, these micelles will evolve into vesicles with a complex behavior that provide an example of non-biochemical, small molecule "generalized" life (Lin et al. 2021).*

Autopoietic synthesis of a functional container (reactor)

As a development of the work in Szymanski and Pérez-Mercader (2016), one such system was presented in Albertsen et al. (2017), where the mCTA is a polyethylene glycol (PEG) polymer with 43 units of EG and has a molecule of CTA containing three sulfur atoms at its end, which can generate the radicals needed for the polymerization. Put in an aqueous solution containing a suitable monomer (Lansalot et al. 2016) such as HPMA (2-hydroxypropyl methacrylate), plus a ruthenium salt to help produce radicals for the polymerization when bathed with blue light, the chemistry in the system generates amphiphiles using RAFT polymerization (Perrier 2021). In the appropriate conditions, these amphiphiles, controlled by the chemistry going on, self-assemble into vesicular structures large enough to be seen in the optical microscope (Szymanski and Pérez-Mercader 2014, 2016). The latter enables the possibility of following the time and morphological evolution of the self-assembled structures under an optical microscope by capturing movies and then quantitatively analyzing and characterizing some of their collective properties (for details, see Albertsen et al. (2017)).

The initial conditions for the reaction are those of a stirred and physically homogeneous blend of chemicals contained in a transparent but otherwise closed vial (batch operation). The first 8 h of the PISA reaction took place in a 10-mL vial at a temperature of 25^{o}C (Figure 7.2(a)). The 5 mL solution went from a stirred transparent mix to a "cloudy" mixture as the various synthesized ABC self-organized and began to form self-assembled structures as a consequence of the ongoing RAFT polymerization process.

This autonomous process generated vesicular structures out of the originally homogeneous and stirred mixture of far simpler unassembled molecules.

After approximately 8 h, a small aliquot of the reacted solution was transferred to a microscope slide where it was illuminated by pulses of blue light lasting 4 s, followed by a 20 ms burst of green light to take the fluorescence image. The resulting image was recorded by a digital camera and a movie was assembled. These images were then analyzed using a combination of standard software techniques and python algorithms.

We clearly see in Figure 7.2(c) that the PISA reaction is generating vesicles with <10 um diameter and smaller objects.

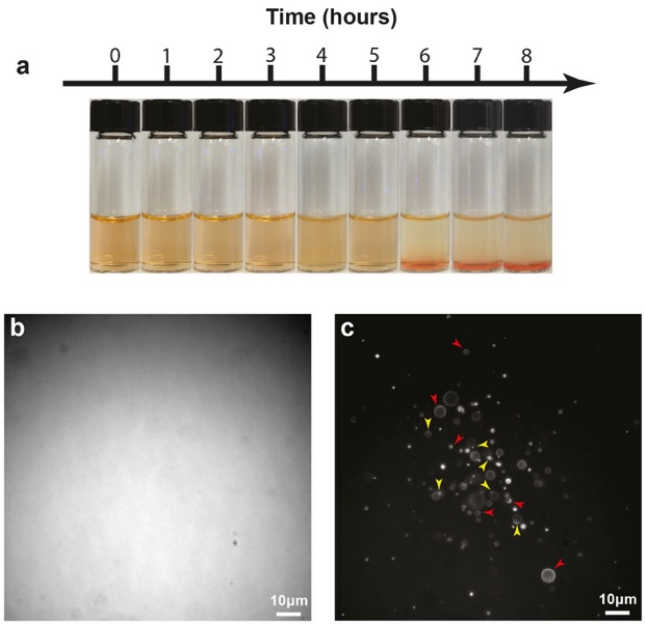

Figure 7.2. *Synthetic autopoiesis: going autonomously from a homogeneous chemical blend to self-reproducing chemical systems. For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

COMMENT ON FIGURE 7.2.– *(a) The first 8 h of the PISA reaction mixture, left to right; (b) the solution initially after 8 h of blue light illumination in the vial, as seen on the microscope slide; (c) the solution after 3 h of blue light illumination on the microscope. Note the size of the vesicular polymersomes in (c), the sample was stained with 4 µM rhodamine 6 G to record the fluorescence micrographs. Yellow arrowheads in (c) point to monomer droplets within the membrane of the vesicles, and red arrowheads point to polymersomes where the monomer has spread over a larger fraction of the membrane surface (Albertsen et al. 2017).*

7.4. More physics and chemistry working together: phoenix, self-reproduction via spores, population growth and chemotaxis

(I) New emergent phenomena: vesicle growth and collapse

Further study of the time evolution of the vesicles shows another interesting phenomenon. This can be seen in Figure 7.3, which consists of 12 correlative

fluorescence micrographs. With the help of the arrowheads printed there, we appreciate that there are vesicles that grow with time (green arrowheads) and then collapse, to then grow again and repeat the cycle. We call this phenomenon "phoenix", because it reminds us of the Greek legend, where the Phoenix creature collapsed into its ashes and revived again. Quantitative study of this behavior can be carried out by measuring and plotting the diameter of the vesicle as a function of time. The results are shown in Figure 4.4.

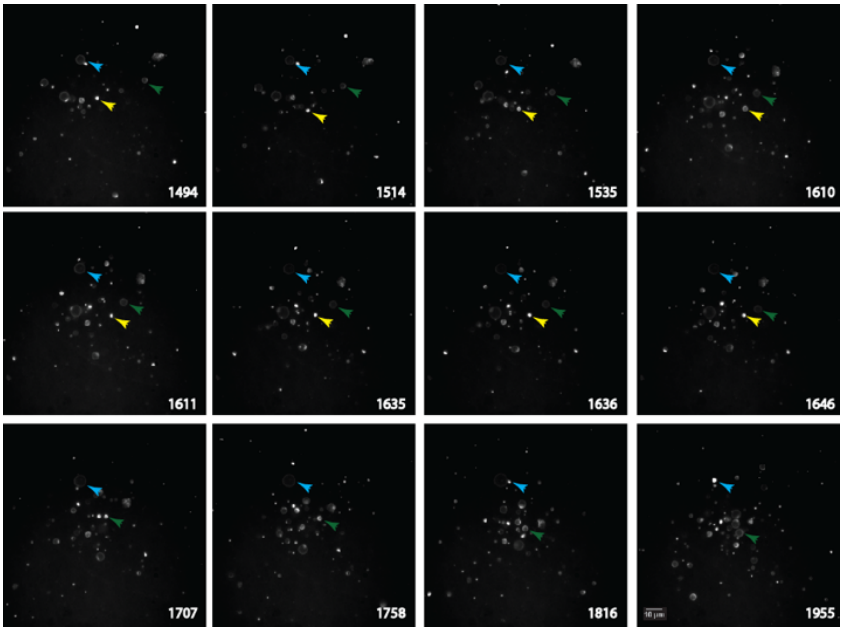

Figure 7.3. *Time lapse of the phoenix behavior of the polymersomes. For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

COMMENT ON FIGURE 7.3.– *The green arrows highlight growing polymersomes and the yellow and blue arrows point to polymersomes just after their collapse. The scale bar applies to all frames and corresponds to 10 μm. The number in the bottom right of the frame denotes the frame number. The data were collected at five second intervals. The sample is stained with rhodamine 6G (Albertsen et al. 2017).*

We see how the vesicle grows in size until a maximum size is reached and it suddenly collapses. The growth can be almost sigmoidal (as seen in some of the cycles shown in Figure 7.4), but it can also be power law, exponential or logarithmic growth, but always reaching a maximum size (which for a given vesicle decreases

its maximum diameter with the number of times the Phoenix cycle is executed), after which the vesicle collapses.

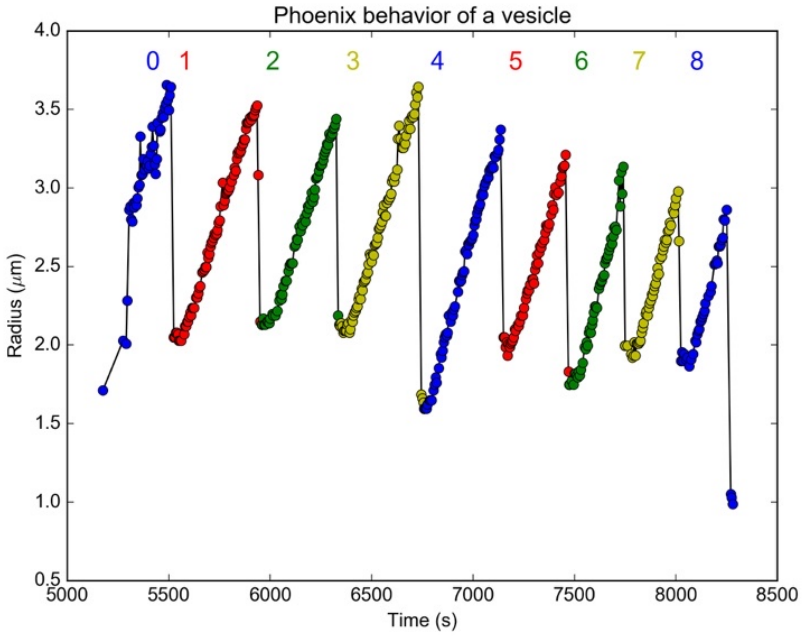

Figure 7.4. *(a) With each successive cycle the maximum size of the polymersome decreases. Different polymersomes were tracked and their area measured using a computer algorithm. The oscillations span thousands of seconds (Albertsen et al. 2017). For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

Although the vesicle collapse may sound counterintuitive, it is actually the kind of behavior expected on the basis of the Rayleigh–Plesset form of the Navier–Stokes equations for the hydrodynamics of a bubble with its own internal pressure (Plesset and Prosperetti 1977) immersed in a pressure field. In our phoenix vesicles, it leads to a highly nonlinear form of "chemo-cavitation" controlled and brought in by chemistry and the nature of the physics of the materials we are synthesizing. Interestingly, although there are other contributions like changes in surface tension and the presence of defects, the hydrodynamics is mostly powered by PISA, its chemistry and by the osmotic pressure differential building up at the membrane due to different chemical concentrations inside and outside of the vesicles (Lin et al. 2021; Figure 7.5). Interestingly, for a variety of conditions, the Rayleigh–Plesset

equation predicts that the "bubble" (vesicle) will undergo successive cycles of growth and collapse.

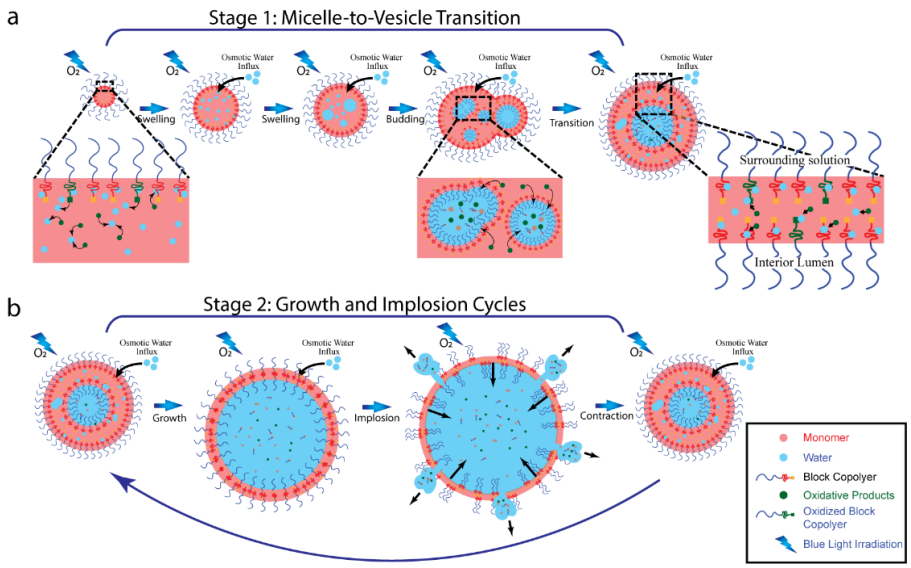

Figure 7.5. *Schematic representation of a "phoenix" cycle. For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

COMMENT ON FIGURE 7.5.– *(a) Stage 1: under exposure to oxygen and blue light irradiation, supramolecular polymer objects transit from micelles to vesicles through a series of intermediate morphologies due to degradation-induced osmotic water influx. (b) Stage 2: the resulting vesicles undergo size growth-implosion cycles which follow the hydrodynamic evolution predicted by the Rayleigh–Plesset equations for cavitation. After these implosion events, the system undergoes a form of reproduction (not represented here), which involves the release of basic conformational information carrying molecules (Albertsen et al. 2017; Lin et al. 2021).*

(II) Reproduction and increase in population size

The birth, growth and death cycles are seen to be accompanied by an increase in population, probably due to the release during collapse of unreacted mCTA or ABC with the CTA appended at the still active ends of some PHPMA chains (Lin et al. 2021) attached to the PEG. In other words, as they collapse, these vesicles replicate,

as indicated by the counting of these structures and shown in Figure 7.6. While at first the structures look like small spherical objects, after some time the number of vesicles that appear in the image (i) suddenly overtakes the droplets, and after approximately 4,000 s into the microscope observation (ii) the number of vesicles grows at a faster rate and with a growth pattern different from the one for the structures in (i). The blue light induces the creation of the phoenix cycles as this vesicle growth rate decreases considerably when the blue light intensity is reduced. For more details, see the Supporting Information in Andersen et al. (2017).

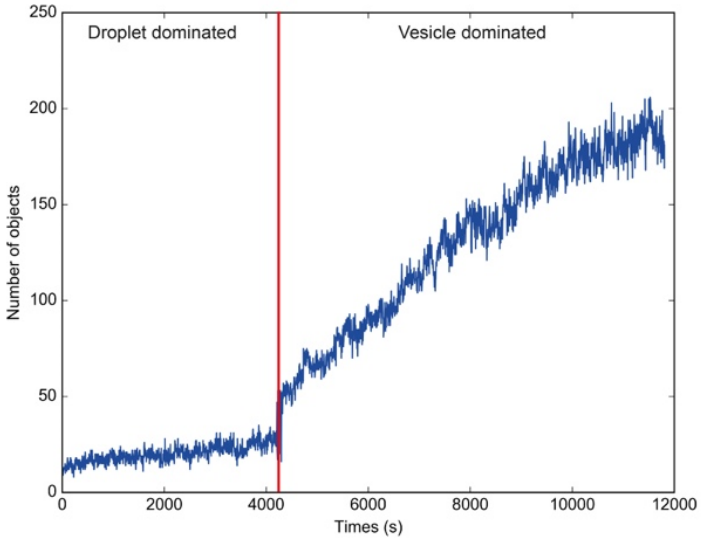

Figure 7.6. *The noisy blue curve shows the change in the type and number of the observed collective structures before ~4,000 s, when the sample was dominated by bright and relatively small filled spherical structures whose number grows at a constant rate, and after ~4,000 s, when the image was dominated by an accelerating and growing number of vesicular structures (Albertsen et al. 2017). For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

During the predicted hydrodynamic collapse phase of the vesicle, a fraction of the contents of the vesicle is spilled into the surrounding medium. Then it begins to grow again. The subsequently lower osmotic pressure inside the newly reconstituted vesicle can allow for the polymerization reaction in the PISA process to continue, which helped by the inertia of the vesicle's membrane, facilitates a fresh vesicle growth. During the growth and internal dynamical physicochemical evolution of the

vesicle, other processes take place inside, which include chemical degradation of the materials in the lumen, as well as polymer sequestration and congestion in the single bilayer membrane, to mention a few important factors. The degradation by reactive oxygen species (ROS) is important because, when it occurs (Lin et al. 2021), it leads to inefficient polymerization due to radical quenching by the ROS, which eventually translates into changes in membrane integrity. The release of inside material into the medium includes incompletely reacted macro-CTA molecules and partially formed amphiphiles. These ("living polymers") will leak out to the vicinity of the imploding vesicle. In the outside medium, they find the materials necessary to complete the amphiphile synthesis and reach a concentration equal or greater than the CMC and thus give rise to new phoenix systems. We can think of the above partially reacted macro-CTA molecules as "seeds" or "spores", since it is from them that the "mother-system" self-reproduces. The cyclic process can continue through a remarkable number of cycles and the parallel with the generic cell division cycle of extant biology represented in Figure 7.7 is clear (see Figure 7.4).

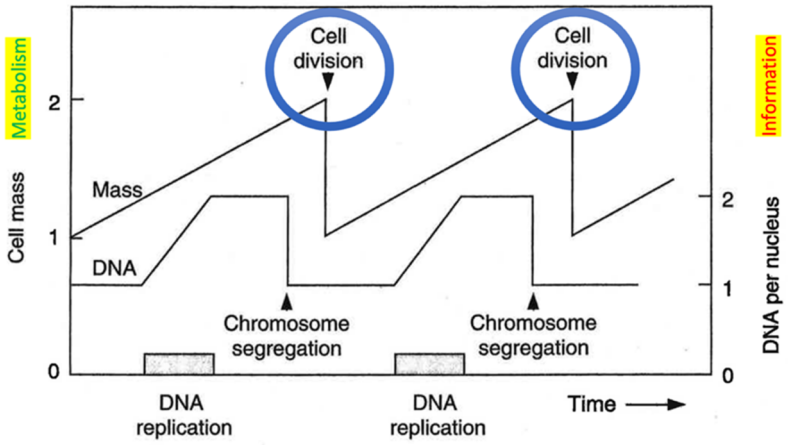

Figure 7.7. *This idealized and abstract depiction of the cell division cycle for living systems shows how in extant life living systems go from one generation to the next in a process that involves the concerted action of metabolism and the change in the information carrying capability of the system. Adapted from Hunt and Murray (1993). For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

Note that reproduction through "spores" seems to be a less costly process than meiosis and mitosis: less energy and complexity than the others, where reproduction requires a far more complex execution (Svetina 2012). This argues for spores as a

more primitive way for reproduction (the hydrodynamics of the spore process are also far simpler than the ones associated with cell division, as discussed above).

Therefore, we see that PISA powered and enabled by chemistry, light and chemical fuels, together with the information contained in the sequence of light pulses and the chemical aliquots in the reacting PISA blend provide a neat, elegant and somewhat simple means to generate, from a completely homogeneous mixture of small molecules, non-biochemical systems with properties (ii) and (iii) at least, common to all natural life. The systems are in addition, self-booting, that is, autopoietic. But, what about properties (i) and (iv)?

(III) Including information and inheritance

In natural life, the control of the chemical system is autopoietic. That is, it is implemented by the internal chemistry of the living system, even though of course it is an open system and therefore is in interaction with its environment, which can affect the system when there are changes in the environment. In extant life, the information is carried in the genome sequence and in the materials that make up the living system: the information transmitted for inheritance uses a polymeric information system. This information is carried in the ordered sequence of the bases in the living system's genome. But this is not the simplest way in which chemical information can be codified, and must have been the result of evolutionary change between the simplest protocells and LUCA. It is highly probable that the simpler forms of protocells did not use a system such as the one we see today.

In the absence of DNA, what could have played the role of information carriers for non-biochemical protocells? What was the reason (and/or vehicle?) why the information had to be passed along from one generation to the next? Presumably the information had to be passed from generation to generation as it would be "very demanding" having to re-invent the wheel in each generation. Or, as Quastler (1964) (implicitly referring to DNA and RNA) put it: "information [in biology] has emerged through the accident of a particular single strand [mutatis mutandis for molecular aliquot combination] becoming the ancestor of the system, that is, through the stability properties of the system descended from that particular single strand". It is a process of "accidental choice remembered". In other words, the necessary information is passed from parent to child, which then inherits it. Both the transmitted information and its vehicle must have been very rudimentary and most probably not in an information storing molecule. Indeed, the information necessary for heredity can be stored in the identity and concentration (aliquots) of the compounds needed for the reaction network to operate and carry out the functions of life (Sagan 1990; Shapiro 2007).

Not surprisingly, our "spores" can play the role of information transmission (heredity) molecules. As previously mentioned, they are incompletely processed ABC molecules whose hydrophobic block is (a) shorter than what it takes to become part of the membrane and (b) carries the CTA attached at its hydrophobic end. These two features make the "spores" in the medium (which still contains enough food) capable of starting new micelles with very similar, but not necessarily identical, properties to the original/mother/parent, which, to begin with, produced the "spore". There is then the potential for variation as, due to their production process, not all "spores" will be identical in length. Therefore, the "spores" can function as carriers of information from one generation to the next.

Furthermore, because the process of "spore" generation is connected with the presence of ROS whose detailed properties are affected by environmental conditions, the "spores" also inherit some non-uniform dependence on the environment, that is, considered as a population of molecules, the "spores" are not all identical due to, among other factors, their polydispersity. The population of "children" generated by the "spores" in one reproductive cycle will then not be uniform, but show some degree of variation. This provides a crude and primitive, albeit practical and effective, form of "descent with modification" (Ridley 2004), already at the level of these small-molecule based "abiotic synthetic protocells".

(IV) Representing life with the methods of theoretical physics

It is interesting to note here that the integration of the four basic properties of life into our non-biochemical system is consistent with the finding that it is possible to represent the properties of livings systems by means of a finite set of equations. As shown in Munuzuri and Pérez-Mercader (2022), it is possible to represent each of the properties (i)–(iv) using a top-level description of the basic mathematical physics and/or chemistry in terms of chemical kinetics. The view in this representation of the living system is that of a system that consists of three theoretical substances representing "food", "cytoplasm" and "waste" configured as a stochastic cubic autocatalytic reaction diffusion system constrained by mass-action kinetics and existing at each of the nodes of an imaginary time-dependent spatial grid. By solving the resulting equations on a computer and analyzing the nature of their numerical solutions we can construct a phase space of their solutions, and the morphologies and phenomenologies they generate. For some ranges of values of the parameters in these equations, we find that there are solutions which incorporate all four properties that characterize life simultaneously. This is important, as it implies that it is not only possible to separately represent the basic properties of life via mathematical functions, which are solutions to some differential equations, but that it is also

possible to unify these four properties into a closed and consistent physico-mathematical representation provided by some equations and their solutions, just as natural living systems do. That is, to represent life it is not necessary to have a material realization, let alone biochemical realizations. Thus, it does not come as a surprise to find that we can realize them materially, and chemically in particular, by using a chemistry which eschews biochemistry.

Put together with the above, we can think of the previous example of the chemical synthesis of simple protocells from a homogeneous blend of chemicals as an example of what Bernal (1967) called "generalized life": it integrates the properties of living systems and does not rely on biochemistry.

7.5. Discussion and conclusions

We have seen how starting with a physically homogeneous blend of a few relatively simple organic, but not biochemical, molecules, we can engineer totally autonomous and self-booting chemical systems, which can integrate properties (i), (ii) and (iii) shared by all natural living systems found on Earth. The system is by design completely "biochemistry free". The key enablers are the use of non-equilibrium chemically fueled PISA and the ensuing self-organization and self-assembly of soft matter systems. These systems work by a surprisingly simple combination of physicochemical processes, where physical effects reinforce the order generation capabilities of chemical phenomena: not only does chemistry shuffle atoms around, but with this it generates both structure and collective behaviors of molecules which couple separate regions of physical (real) space: the outside and inside of the active vesicular structure thus erected by chemistry. The systems self-reproduce by some molecular spores, which can implement a rudimentary form of inheritance and "descent with modification" due to the presence of radicals and molecular degradation.

What about properties (iv-a) and (iv-b)? Experiments (Katla et al. 2023) using PISA's need for radicals to operate combined with non-equilibrium phenomena and PISA's dependence on the choice of catalyst and the environment in which it occurs show, not surprisingly, how independently booted-up and then mixed PISA systems, one of which has an advantage provided by the presence of a catalyst, actually compete for resources in a way that closely follows the competitive exclusion principle, one of the basic features of competition in natural life and at the root of Darwin's "struggle for existence". Additionally, by combining PISA with other facile features of small molecular networks of catalysts, we can generate systems that show adaptation at the molecular and system levels (Pearce and Pérez-Mercader 2021). We can envisage their evolution into more complex systems by

the co-option of more complex molecules and reaction networks to execute more specialized functions.

In conclusion, artificial life based on actual molecular systems, which combine the exchanges of atomic partners and the generation of structures with the information contained in molecules, their bonds and rearrangements, promises a bright future. The use of PISA to tackle the construction of fully artificial, non-biochemical, chemical mimics of life not only solves the arithmetic demon problem, but also brings with it a powerful connection among out-of-equilibrium phenomena and the properties of living systems. This establishes an interaction of the PISA-generated vesicular system with its environment in a way that enables metabolism, self-replication and the integration with information handling.

These systems provide an embodiment with chemistry and by chemistry of Bernal's "generalized life": they can be seen as a material realization of "same functions, different mechanism" for life as we do not know it.

There is much to be done. The results and techniques described here can find applications in artificial life and astrobiology. In the latter case, these help to understand scenarios where simple lineages may have preceded the appearance of more complex life. They can be used to synthesize new advanced materials or construct self-booting and self-programmed soft robots from a homogeneous mixture under mild conditions. Furthermore, they also offer a solid basis for the implementation of more functionalities and can help advance our understanding of how life emerged in our planet from simple beginnings, and how chemical complexity may have developed here or elsewhere in the Universe.

7.6. Acknowledgments

This work would not have been possible without the discussions and collaborations with many of the members of the author's Harvard group, and especially the ones who have co-authored work cited in this chapter. He would also like to thank Stein Jacobsen, Andrew Knoll, Dimitar Sasselov and Jack Szostak in the Harvard Origins of Life Initiative and the students in the Origins of Life Consortium for many discussions. Discussions with Cyrille Boyer, James Cleaves, Esteban Domingo, James Friar, the late Murray Gell-Mann, Juan Manuel Garcia-Ruiz, Dario Gil, Terry Goldman, Jane Kondev, Herbert Levine, Cormack MacCarthy, Stephen Mann, Susanna Manrubia and Geoffrey West are gratefully acknowledged. The author thanks REPSOL S.A. for their support.

7.7. Appendices: Some additional emergent features in PISA "powered" synthetic biochemistry free protocells

We briefly describe some additional features that emerge in these PISA-generated functional systems. These brief comments are intended as a way to illustrate the possibilities for experimentally constructing "generalized life" protocells and the scenarios that they open for further research and applications. We will briefly mention chemotaxis, the presence of competitive exclusion among artificial "species" of our protocells, the effects that result from introducing an additional feedback loop in the PISA synthesis by combining it with click chemistry, and will close with a brief discussion of the representation of natural life at a very high level (i.e. little detail and abstract), using fundamental principles in theoretical physical chemistry.

7.7.1. *Chemotactic behavior*

The functional phoenix vesicles also moved to the center of the image and followed a "tumbling" path that is shown in Figure 7.8.

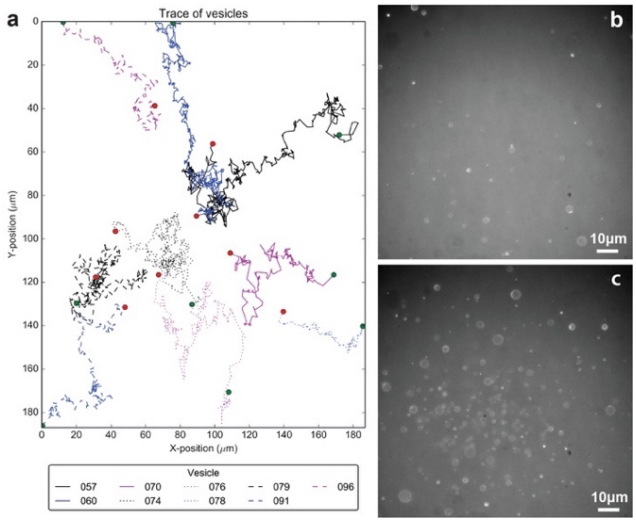

Figure 7.8. *(a) Trace of the movement of a group of vesicles displaying chemotaxis. The collective movement of the vesicle population results in their concentration near the center of the frame where the light was most intense. (b) Micrograph of the solution at the beginning of this experiment and (c) a micrograph at the end of the experiment. The computer trace was generated from a series of micrographs collected during a single experiment (Albertsen et al. 2017). For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

This motion toward the center of the image could be due to thermal fluctuations or the presence of Marangoni effects (Albertsen et al. 2017) in the vesicles due to the formation of defects in their surfaces as the PISA process continued. The former would be most probable when close to the ABC glass temperature. The latter is most probable when the membrane thickness is not uniform, due to the droplet coalescence processes going inside the vesicle and combined with the chemical reaction being maintained by the blue light irradiation (Gong and Pérez-Mercader 2019, 2020). This is a manifestation of motion due to chemical processes affecting the hydrodynamic equilibrium of the vesicles, that is, a form of chemotaxis. It was also observed in more complex autopoietic systems involving the integration of click chemistry together with PISA (Pearce and Pérez-Mercader 2021b), which we will now turn to.

7.7.2. *Adaptive behavior and click-PISA*

An important property of living systems is their ability to adapt. In engineering, there are theorems that show that for a system to display adaptive control, there must be two integrated feedback control loops (see refs. 28 and 34 in Pearce and Pérez-Mercader (2021b)) simultaneously present and properly interconnected in the system. This of course requires a more complex system. The PISA system provides a route to increase chemical system complexity, since self-assembly acts as a positive feedback to the RAFT polymerization because phase separation increases the local monomer concentration in the vicinity of the CTA functional groups (see ref. 33 in Pearce and Pérez-Mercader (2021b)), but it starts with a macro CTA already available in the initial reaction vessel. We can ask if we can increase gap in system complexity between the initial PISA reaction and the final self-assembled active vesicular structures by starting with simpler precursor molecules for the macro CTA. This chemical simplification can be achieved (see ACS Central) by integrating two chemical reactions connected by using the radicals in the RAFT based PISA to reduce a pre-catalyst for an orthogonal copper-catalyzed azide-alkyne click (CuAAC) reaction (see ref. 22 in Pearce and Pérez-Mercader (2021b)). The high affinity associated with click actually combines very well with the rest of the RAFT PISA because its high affinity helps avoid the effects of the arithmetic demon before they can actually start. The reduction Cu(II) PMDETA precatalyst by electron abstraction from the RAFT polymerization to activate the CuAAC reaction drives a negative feedback loop. Remarkably, the resulting two-feedback loops system has the same topological as the canonical systems in control theory (see refs. 28 and 34 in Pearce and Pérez-Mercader (2021b)) for describing adaptation in control

engineering, and it provides a potential mechanism to chemical adaptivity, where the degree of adaptation in the system to external (light) inputs depends on the relative rates of the associated chemical (click and RAFT) and physical (Self-Assembly) processes. For more details, see Pearce and Pérez-Mercader (2021b).

7.7.3. *Competitive exclusion principle and iniferter PISA*

An essential feature of natural life is the presence of competition among species or among individuals of a species. The competition among species follows the "competitive exclusion principle" (CEP), which states that any "two species occupying the same niche will compete with each other to the detriment of one of the species, which will thus be excluded" (Hardin 1960; Mayr 2001; Martin and Hine 2008). The CEP is essential for the functioning of Darwin's "struggle for existence". By using an "iniferter" version of the PISA synthesis that we have been discussing (Katla et al. 2023), it is possible to create two (or more) populations of fully functional protocells. These two populations can be chosen so that they only differ in that one of them is endowed with a photocatalyst which confers advantages in reproduction to the population of the species with a photocatalyst, and not to the other, catalyst-free, species. When put in a common environment, the populations compete for common food, and the population with the photocatalyst eventually displaces and eliminates the other one. These of course are biochemistry free systems, yet their competitive exclusion behavior parallels, even in details, the standard competitive exclusion found for paramecia and other species originally found by Gause (2003). The fact that these PISA-generated non-biochemical protocells show competitive exclusion shows that biochemistry (used by natural extant life) is sufficient but not necessary for establishing competitive exclusion and Darwin's struggle for life.

7.7.4. *PISA and its control by chemical automata*

At some point, we need to integrate the means for strictly chemical control into our system. This requires us to use a manner of control that is chemical at its "root" and capable of carrying out the functions of control associated with computing automata. In other words, we need to implement chemical automata capable of controlling the functioning, and perhaps even the assembly, of a potential "life mimic". Such automata would be "native" chemical automata since once the information is input, the system only has chemistry at its disposal and is fully dependent on chemistry to carry out the required computation.

It turns out that such material native chemical automata at the highest level in the Chomsky hierarchy can be built (Duenas-Diez and Pérez-Mercader 2019a, 2019b, 2020). The use above of the term "material" is important (Minsky 1967; Rich 2008; Linz 2017). It means that by using an actual material chemical realization, we do not have an infinite length tape or an infinite amount of energy at our disposal to operate a potential chemical automaton in the system. The material chemical automata at the highest level in the hierarchy are limited bounded automata (LBAs). They are finite tape Turing machines and are capable of carrying out any computation that can be encoded with context sensitive languages (CSL). For examples of these in bioinformatics of RNA, see Rivas and Eddy (2000). They require two memory stacks (Duenas-Diez and Pérez-Mercader 2019a, 2020; Foulon et al. 2019) and can, for example, be implemented using the chemistry of the Belousov–Zhabotinsky (B-Z) nonlinear oscillatory chemical reaction. The frequency of its redox relaxation oscillations and their amplitudes are effectively stored by the reaction in two different stacks and used to carry out high-level computations (any chemical oscillator can do the same).

7.7.5. *Integrating PISA and information control with the Belousov–Zhabotinsky chemical reaction*

Thus, in principle, we could exert a good level of chemical control using the B-Z example of a redox oscillator. But there is a bonus: oscillations in chemistry involve the presence of "initiator" and "inhibitor" radicals, and radicals are essential in polymerization. The question is, can the B-Z radicals be used to power a PISA system? The answer is yes, and this was reported in Bastakoti and Pérez-Mercader (2017a), from which Figure 7.9 was extracted. In Figure 7.9(a) we see the redox oscillations and in Figure 7.9(b) we see how the chemical fuel is consumed and powers the PISA reaction. Examples of the vesicles that can be produced are shown in Figures 7.9(c) and (d).

Additional work (Bastakoti and Pérez-Mercader 2017b) showed how the B-Z reaction can not only power PISA, but also induce deformation and blebbing of vesicles (Figure 7.10). The latter can be interpreted as the effects of osmotic pressure on a vesicle which is "drinking" water or (not necessarily alternatively) whose membrane has coupled to the internal B-Z reaction in such a way that energy from the reaction is dissipated on the membrane (Seifert et al. 1991).

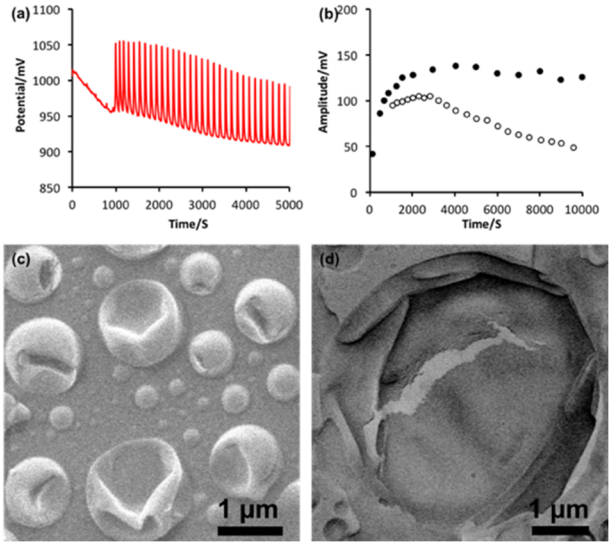

Figure 7.9. *(a) Oscillation profile of the B-Z reaction during amphiphile polymerization between a PEG-CTA and butyl acrylate monomers; (b) amplitude of redox oscillations of the pure B-Z solution (•) and B-Z in polymerization (o). (c and d) SEM image of polymer vesicles after 120 min of polymerization. Adapted from Bastakoti and Pérez-Mercader (2017a). For a color version of this figure, see www.iste.co.uk/dimauro/firststeps.zip*

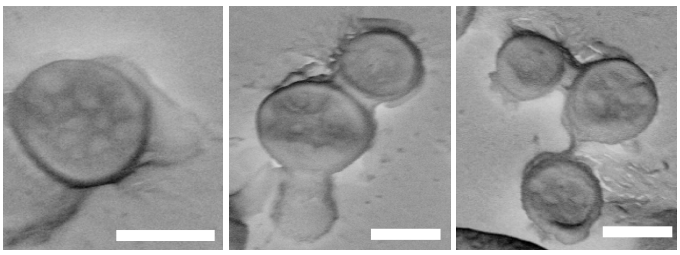

Figure 7.10. *SEM (scanning electron microscopy) images of vesicles showing their appearance and growth of blebs after 80 min of polymerization. The scale bar is 5 µm. These budding and blebbing events result from the reduction of the total interfacial energy due to the B-Z reaction, combined with the gain in interfacial energy due to bud formation (Bastakoti and Pérez-Mercader 2017b)*

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