Workshop OQOL'09:
OPEN QUESTIONS ON THE ORIGINS OF LIFE 2009

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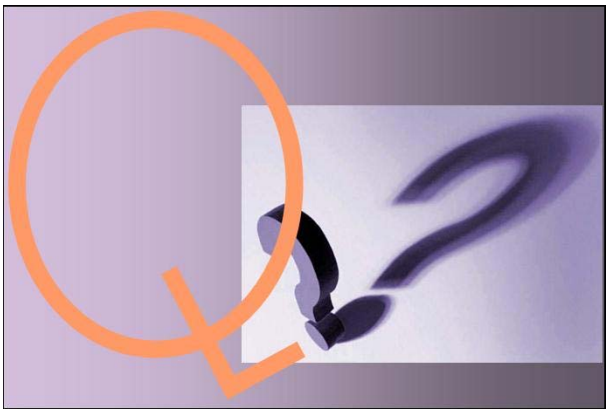
Book of Abstracts

[FIRST DRAFT]



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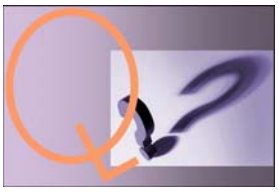
Workshop OQOL'09
Abstracts for the following selected question

- **Contingency versus determinism in the origin of life/origin of proteins.**

Premise. The origin of life is often seen in terms of two basic, opposite schemes, determinism and contingency. Generally, the two principles work hand in the hand, as each "choice" made by contingency must then comply to the natural laws and, in turn, contingency arises from a given thermodynamic asset. However, when we ask the basic question of whether the origin of life follows an obligatory deterministic pathway (absolute determinism), or whether it is due to the vagaries of contingency, the two views become again drastically opposite to each other. More precisely, according to the deterministic view (as represented most notably by Christian de Duve), the origin of life is seen as an event of very high probability: actually, it had to come out inevitably from the starting and boundary conditions (the so called "gospel of inevitability"). The opposite view (advocated, for example, by Jacques Monod), implies that the origin of life was due to the occurrence of several independent factors, each of them perhaps not un-deterministic, whose simultaneous and unpredictable interaction led to successive events, up to the origin of life.

> **The question**. Do you agree that the choice between these two extreme points of view cannot be done on a rational, scientific basis, and is instead for each scientist a matter of philosophical or religious belief? And, if you do not agree, which *scientific* arguments would you offer in favour of one or the other lines of thought?



Pier Luigi Luisi,
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Abstract: The largest majority of scientists on the origin of life accept the view that that life originates from the inanimate matter via a series of steps of increasing complexity governed by the natural laws. Within this camp, there are several authors who consider the origin of life on Earth as an obligatory event. In particular they see the emergence of life as a series of steps linked to each other by a causal link, each step being the consequence of the previous one and determining causally the next one. We can ascribe those to the side of the "absolute determinism", and one of the foremost Authors of this kind is Christian de Duve. One finds however this idea in several other authors, although with various degrees of emphasis and awareness. I would like to make the point that there is an intrinsic fallacy in this argument. One cannot invoke the natural laws, and then pretend that they regulate themselves so as to go towards a preferential, thermodynamically unlikely, direction. The absolute determinism would have its validity only if the emergence of cellular life would correspond to a spontaneous process under (more or less) thermodynamic control, leading namely to a state of energy minimization. It is instead rather obvious that it is not so- not only the cellular structure, but also its basic constituents –the ordered sequences of proteins and nucleic acids, or the genetic code- are not the products of spontaneous reactions, but rather the products of a series of evolutionary happenstances. I make further the point that this absolute determinism hides a form of creationism, and I dubbed these authors as "crypto-creationists": they refute to accept, unconsciously, the bitter pill of contingency (chance) as the basic determinant of the origin of life- and adhere instead to an anti-thermodynamic notion of an intelligent purpose.

Are there any proof that life has been shaped by contingency? For life at large this is rather clear: think of the asteroid that eliminated the dinosaurs 60 millions years ago. Or think to the accidental "invention" of oxygen by cyano-bacteria, which destroyed all but a few forms of life on Earth at that time- certainly life would have developed very differently without these events of contingency- actually mankind might have not arisen at all without them.

Of course contingency and determinism come always hand in hand, (asteroids follow the gravitation laws, and release of oxygen obeys thermodynamics, etc) but when we say that the origin of life is primarily the product of contingency, we mean that the basic critical steps for the emergence of life- production of protein catalysts, the arising of ATP or the genetic code, etc., etc.- are not under thermodynamic control, but rather they are shaped by the simultaneous occurrence of *per se* independent parameters (concentration, pH, salinity, presence of matrices, etc). I believe that it is impossible to envisage the origin of life and its evolution to human consciousness as a deterministically obligatory process. It is equally true that the scenario of contingency is very unpleasant to mankind, as it is understood as if life would be the blind product of chance; and this fear is one of the main origin of the anti-Darwinist movement and the insurgence of creationism or intelligent design. Emotional and archetypical factors are very important in what scientists believe or not. To this regard, let me cite the famous sentence of one of the pioneers of contingency, Jacques Monod, who wrote in his 1971 Chance and Necessity:
"*we would like to consider ourselves as necessary, inevitable, ordained for all eternity. All religions, all philosophies, and even part of science, testify to the unwearyingly, heroic effort of mankind, desperately denying its own contingency*".

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Abstract (draft): what is the relationship between the underlying physical properties of matter and the emergent and evolving living systems? I suggest that there is a bottom up relationship of constraint on possible form and behavior, but that constraint does not determine the particular paths of actualization that life takes. My view is that there is a nested hierarchy of constraint on possibility (physics constrains chemical arrangements, actual chemical arrangements constrain biological arrangements, etc.) but that while the possibility space is determined from below, the actual domains of what has arisen and evolved in the history of life is not fully determined by the underlying physical and chemical properties.

The other topic I would be happy to speak in would be "Is life an emergent property" I would argue that if emergence identifies a type of novelty and causal power then life qualifies as emergent from the chemical and physical properties of its constitutive parts. Novelty refers both to properties of the whole (living being) that the parts do not possess, and underdetermination or underspecificity of the actualized forms by the range of possibilities warranted by the behavior of the component parts. Causal powers come in two types: causal interactions that become realized when the whole emergences (that are not realized by the parts in isolation) and so-called "downward causation" which means that there is causal significance, usually in the constraint on behavior of the parts in virtue of being in an emergent whole.

Andrew Pohorille
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The debate about deterministic *vs*. indeterministic origins of life is quite old. One view holds that the origin of life is an event governed by chance, and the result of so many random events is unpredictable. This view was eloquently expressed by Monod. In his book “Chance or Necessity” he argued that life was a product of “nature’s roulette.” In an alternative view, expressed in particular by deDuve and Morowitz, the origin of life is considered a deterministic event. Its details need not be deterministic in every respect, but the overall behavior is predictable.

From the methodological point of view we need to consider the emergence of life as, at least partially, deterministic phenomenon. As it is hopeless to predict numbers in roulette, it would be fool’s errand to try to understand “nature’s roulette”. This, however, does not mean that if stochastic events played a role in the transitions from inanimate to animate matter, even an important one, it would be impossible to predict their outcomes. A similar reasoning is common in other fields of science and forms the basis of one of the most important areas of physics – statistical mechanics. The central requirement is that there are some underlying principles or constraints governing the behavior of the system. Unraveling these principles and constraints coming from physics and chemistry and their effects on the origins of life is the key to the problem here. In addition, since the laws of physics and principles of chemistry are the same everywhere in the Universe, this reasoning implies some level of universality of life, no matter where it originated.

One set of constraints comes from prebiotic organic chemistry. Contrary to a "random chemistry" assumption adapted in a number of models of protobiological evolution, primordial chemistry was quite constrained. In particular, Arthur Weber has demonstrated that synthetic potential of carbon chemistry under mild aqueous conditions in the absence of catalysts is quite limited as a result of thermodynamic and kinetic constraints. Even if the synthetic limitations of early metabolism are relaxed by introducing high-energy compounds that can capture some of the free energy released in downhill reactions and subsequently use it to drive uphill reactions, the diversity of chemical transformations remains restricted by the requirements that only –10 kcal/mol of carbon is available for biosynthesis, and that energy-rich molecules can be synthesized only by irreversible reactions with large, favorable free energies. The repertoire of possible prebiotic reactions can be further expanded to transformations that are kinetically forbidden without assistance, once enzymatic or non-enzymatic catalysis is included. Even then, many synthetic constraints remain if one makes a biochemically plausible assumption that chemical reactions in which the substrates and the products are separated by high energy barriers is less likely than reactions involving low energy barriers. A somewhat related reasoning about synthetic constraints led Morowitz *et al.* to a conclusion that the reversed citric acid cycle was at the origin of metabolism.

Equally important are principles of and constraints on physical interactions at the molecular level. To self-reproduce and evolve, organic matter must self-organize into functional structures capable of responding to environmental changes. This process is based on physical rather than chemical interactions, *i.e.* interactions that do not involve the formation of chemical bonds. Folded proteins, membranes forming cell walls and the DNA double helix are examples of structures stabilized by such interactions. In every biological process these interactions are often formed and broken in response to internal and external stimuli. This requires that their strength must be properly tuned. If they were too weak, the system would exhibit uncontrolled response to natural fluctuations of physical and chemical parameters. If they were too strong biological processes would be too slow and energetically costly. Furthermore, strength of physical interactions depends critically on the solvent. Polar molecules can be dissolved in polar solvents, such as water, but not in non-polar ones. Electrostatic interactions between these molecules are reduced in polar liquids, compared to those in the gas phase, such that they become compatible with other physical interactions. In addition, water exhibits a remarkable trait that it also promotes *hydrophobic interactions* between non-polar molecules. The hydrophobic effect is responsible for self-organization of nanoscopic structures such as micelles, membranes and globular proteins. Thus, water is an excellent solvent for life mainly because it promotes self-organization of matter into structures that are sufficiently versatile, robust yet flexible to support functions of a living system. Only very few other solvents might have similar properties. This illustrates how physical interactions greatly limit environments that are suitable for life.

The constraints of physics and chemistry act at different levels from reducing the number of possible chemical reactions and imposing rules on their self-organization into networks to forming a molecular basis for the self-assembly of macromolecular structures into functional units. Are they sufficient to imply a considerable level of determinism in the origins of life? We recently addressed this question through modeling the emergence of metabolism and enzymatic catalysis using chemically and biochemically plausible assumptions. This work was supported by a grant from the Templeton Foundation. Our computer simulations indicate that although most "protocells" exhibit little catalytic activity, some encapsulate metabolisms composed of series of consecutive chemical reactions, which occasionally organize into autocatalytic cycles, even without genome. Even though the underlying processes are highly stochastic and the mathematical formulation of the model is fully probabilistic, these features persist in the

populations. This demonstrates a considerable level of determinism. In addition, several concepts inherent to Darwinian evolution, such as the “species” (defined as similar metabolic networks), fitness to the environment and inheritance appear to hold for the population, but not for individual protocells. A somewhat similar conclusion was reached by Lancet *et al.*, They showed that compositions of protocells without genomes could persist over generations, thus forming “compositional genomes”. Further insight into the role of contingency and determinism can be obtained from high throughput experiments that have been initiated in several groups.

In summary, even though we are still far from definitive answers to the question that was posed here, a number of experimental and theoretical tools are now available that allows us to shift the problem from the realm of religion and speculations to the realm of rational, scientific inquiry.

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A scientifically rational choice between contingency and determinism can and should be made in favour of determinism. This is because the immense combinatorial space apparently offered by molecular diversity for exploration is actually highly constrained by the solutions that cells – and populations of cells – have to find. Life must always solve the universal problems of prospering in heaven yet surviving in hell, of diversifying yet specialising, and of reconciling competition and collaboration. In the scenario of a pre-biotic ecology [1, 2], Life originated as an ecosystem of multi-molecule assemblies or *composomes* [3] exchanging constituents in a flow of creation and degradation to solve the universal problems. In this scenario, the limiting constraints were (1) molecular complementarity [4] acting on many, interacting ingredients rather than a few independent ones, (2) non-specific interactions involving large classes of molecules rather than specific interactions involving only a few molecules [5], (3) non-specific interactions between many molecules leading to simple consequences rather than specific interactions between a few molecules leading to complicated or chaotic consequences. The scenario of a prebiotic ecology is particularly attractive because it offers explanations: the composome has its descendant in the hyperstructure which is a large assembly of many, interacting, often unstable molecules and macromolecules that performs a function. The importance of the composome in Life's early solutions is reflected in the importance of the hyperstructure in Life's recent solutions [6]. The solutions found by modern cells are in terms of hyperstructure dynamics and include: the dual nature of the most stable constituent of the cell [7], replication of this constituent plus fission to increase diversity and robustness [3, 8, 9], and altruistic lysis. Hence, we would argue that choosing between contingency and determinism ought to depend on answering the question 'what is a cell' [10]? And what we see now is that a modern bacterial cell is a set of hyperstructures and that this cell is itself part of a population .

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[Additional abstracts]

(Contingency vs determinism)

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Chance and the observer: epistemological remarks on the issue of contingency and determinism

I disagree with the assertion according to which the choice between contingency and determinism cannot be done on a scientific basis. The two alternatives can be made understandable and the choice itself can be made possible, in principle, on a scientific basis if we deconstruct the objectivistic view of scientific knowledge through an epistemological analysis of the modalities of construction of our descriptions of the natural world. In other words an attempt to propose a solution to this question without appealing to metaphysical issues – and at the same time without necessarily contradicting them - can be made if we consider contingency and determinism as two concepts which do not refer to the properties of reality itself but rather to the properties of the descriptions we formulate as scientific observers.

According to the epistemological assumptions characteristic of a constructivist point of view on knowledge (Maturana and Varela, 1980, 1984; Maturana, 1988, Ceruti, 1989; Bich, 2008; Damiano, 2009), these two concepts (contingency and determinism) need to be considered as possible ways of interpreting the capability or not of our models to allow us to successfully interact with natural systems: in this specific domain to deal with the problem of the origin of life and, also, of the transitions in evolution. What from an objectivist point of view looks like a hypothesis about Nature itself, according to this perspective becomes instead a hypothesis on the limits of our attempts to describe it, and as such it can be dealt with on a scientific basis.

A deterministic solution to this issue can be conceived, at least in principle, in specific cases when contingency depends on the “quantitative” insufficiency of our models to describe the behavior of the system under study, that is, when we need to hypothesize the intervention of some external factors. In these cases there is no theoretical limit to our possibility to build a more comprehensive description by increasing the resolution power of the starting model (Rosen, 1991).

With regard to contingency, the solution - an example of which could be Monod's thesis about "gratuity" (Monod, 1970) - is strictly connected to the problem of diachronic emergence, as it would imply a limit in principle to our capability to describe natural processes without the possibility to resort to more comprehensive descriptions. The inexplicable phenomena, in this case the origin of life, would appear to the observer as contingent. A result of this kind would be of paramount importance from both the theoretical and epistemological points of view as it would provide biology of a "negative theorem" comparable to the fundamental ones formulated in physics and mathematics (Bailly and Longo, 2006), thus opening the way to the elaboration of new modalities of description specific for the biological domain (Bich, 2008, Bich and Damiano, 2008).

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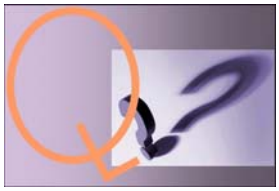
Workshop OQOL'09
Abstracts for the following selected question

- **Is life an emergent property?**

Premise. Although emergence is a notion with many complex sides, the general view is that emergent properties are those novel properties that arise when parts or components assemble together into a higher hierarchic order – novel in the sense that they are not present in the parts or components. Most of modern scientists would consider cellular life an emergent property, as the single components are 'per se' not living. Then…

> **The question**. Do you think there are sufficient data now to say that life is indeed an emergent property, arising from the interactions and self-organization of non living parts? – Or do you still see a kind of "vitalistic" flavour in the statements that define life as an emergent quality?



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Spain.

Abstract: **Gödel, Biology and emergent properties**

The Gödel theorem of undecidability applied to any biological system states that "within a cell, properties exist that are neither provable nor disprovable on the basis of the rules that define the system". It means that, on the basis of the rules that governs cell behaviour, there might exist or appear properties from which we cannot say if they can or cannot be derived from the rules of the cell system. On the other hand, the incompleteness theorem states that "in a sufficiently well known cell in which decidability of all properties are required, there will be contradictory properties". The biological translation of that theorem is extremely important because it asserts that, no matter how well we know a particular cell system, we can find properties and/or behaviours that seem contradictory between them. The interpretation of Turing about Gödel's results applied to biological systems may be as follows: "there may appear functions, structures and properties in general of biological systems that cannot be computed by any logical machine". If the cell was a Turing machine, there should exist a finite procedure (i.e., an algorithm) telling how to compute its behaviour. A computable function is exactly the function that can be calculated using a mechanical calculation device given unlimited amounts of time and storage space. Gödel theorems tell us that we cannot anticipate the appearance or the non appearance of new properties in the cell and some times properties will appear that will be contradictory between them no matter how deeply the cell is known.

A key question in Biology is emergence. Biological systems are plenty of emergent properties and, particularly Evolutionary Biology shows us how frequent and abundant they are through the history of living beings on Earth. The appearance of evolutionary novelties, at least some of them, gives support to Gödel's statements. Biological features, particularly evolutionary ones, are not predictable most of the times, and they can be considered as emergent novelties/properties within that particular system formed by living entities on Earth. In summary, emergent biological phenomena may appear within a particular system that follows Gödel's theorems. We can define a new biological system adding new rules that may integrate, to avoid inconsistencies, the emergent feature. However, the new one, although more sophisticated and reach, will be exposed, following Gödel's statements, to new unpredictable phenomena.

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In as much as there is exceedingly little knowledge about the actual origin(s) of life, it seems reasonable to extend to its beginnings the same fundamental evolutionary nature that has been recorded throughout life's cellular history. Therefore, the concept that this evolution could have reached back into prebiotic chemistry is, at least for now, legitimate and one worth exploring. It would also mean that life could be reasonably categorized as an emergent system.

Eventually, however, the proofs that "interactions and self-organization of non living parts" could be progressive, systemic and prebiotically relevant must be analytical. Analytical approaches will be very useful in recognizing and quantifying the "novel properties that arise when parts or components assemble together into a higher hierarchic order"; they can also explore systematically the effects of selective molecular traits in reaching that higher hierarchical order.

One such molecular trait is that of chiral asymmetry, which is fundamental to terrestrial life and may have had a-biotic roots. Chirality is an inherently associative property in that the enantiomers of a chiral molecule do not have exact physical symmetry, will react differently with other chiral molecules and, by the different rates of their diasteromeric transition states, may lead to kinetic resolution of enantiomers in polymerization reactions.

We will discuss this possible aspect of molecular evolution on the basis of analytical and computational findings.

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I believe it is useful to distinguish at least three senses of emergence: (i). Are there properties of the "whole" system which are not deducible from their parts plus the local interactions of the parts? There are numerous examples of this type of emergence. One consists in mapping a universal Turing machine onto Conway's Game of Life. The Game of Life is a 2 dimensional cellular automaton with the same "physics" or Boolean rule, realized by each square on the lattice, which may be infinite dimensional. Patterns such as gliders and glider guns, describable at a NEW LEVEL of DESCRIPTION that needs no further reference to the Boolean "physics", are then used to construct the universal Turing machine. On an infinite 2 dimensional lattice, the halting problem arises, so many behaviors of the lattice cannot be deduced from the local Boolean rules. This is a form of emergence. The same property has recently been discovered mapping universal Turing machines onto infinite 2 dimensional Ising models. (ii) The emergence of collectively autocatalytic sets, as in the theory I have developed and described in the abstract for the question on the origin of catalytic cycles, depends upon primitives called molecules, reactions, and catalysis. At a sufficient diversity the ratio of reactions to molecules is sufficiently high, given a probability that a molecule catalyzes a randomly chosen reaction, collectively autocatalytic sets emerge. This is emergence because the whole collectively autocatalytic set achieves catalytic closure and can reproduce or maintain itself given "food". None of its parts have this property. Catalytic closure is an emergent property. Furthermore, the emergence of the collectively autocatalytic set is an example of Robert Laughlin's "laws of organization", and is notable in that it is not reducible to any specific underlying physics. Of course, entities that instantiate "molecules", "reactions", and "catalysis" are needed. But the theory is mathematical, and the law of organization is mathematical, and can be realized by multiple physical platforms, perhaps even were the constants of nature changed. Thus it is independent of physical laws, and not reducible to them. (iii) The EVOLUTION of autocatalytic sets occurs in a non-ergodic universe which will never make all possible proteins length, eg 200. We are on a unique diachronic trajectory. History enters when the space of the possible vastly exceeds what can happen. Autocatalytic sets can evolve and co-evolve by natural selection and make novel niches with one another that depend upon their chemical and physical properties. Thus they are capable of Darwinian pre-adaptations which we cannot pre-state. It follows that we cannot make probability statements about their evolution - indeed the biosphere's evolution of collectively autocatalytic cells - nor are their sufficient natural laws to describe that evolution, as I discuss in detail in "Investigations" and "Reinventing the Sacred". But the lack of sufficient natural laws means that the "becoming" of a biosphere of living systems is both partially non-lawful, hence emergent with respect to physics, and also non-random. What is selected cannot always be pre-stated, but succeeds in the ever changing contexts of opportunities for adaptations which in turn change the opportunities for adaptations. In this sense, evolutionary theory cannot be epistemologically closed, hence the evolution of life again is ever emergent.

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We have developed a simple chemical system capable of self-movement in order to study the chemical-molecular origins of movement, perception and cognition. The system consists simply of an oil droplet in an aqueous environment. The aqueous phase contains a surfactant that modulates the interfacial tension between the drop of oil and its environment. We embed a chemical reaction in the oil phase that reacts with water when an oily precursor comes in contact with the water phase at the liquid-liquid interface. This reaction not only powers the droplet to move in the aqueous phase but also allows for sustained movement. The direction of the movement is governed by a self-generated pH gradient that surrounds the droplet. In addition this self-generated gradient can be overridden by an externally imposed pH gradient, and therefore the direction of droplet motion may be controlled. Also we noticed that convection flow is generated inside the oil droplet to cause the movement, which was also confirmed by simulating the fluid dynamics integrated with chemical reactions (Matsuno et al., 2007, ACAL, Springer, p. 179.). We can observe that the droplet senses the gradient in the environment (either internally generated or externally imposed) and moves predictably within the gradient as a form of primitive chemotaxis (Hanczyc, M., et al., 2007, J. Am. Chem. Soc., 129, p. 9386).

By creating a pH gradient and concomitant convection flow, the droplet behaves as if it can perceive the environment. We believe that the geometry of the interface shape can control sensitivity to the environment (Ikegami et al., 2008, BioSys., 91, p.388). This geometry-induced fluctuation is the source of fluctuation of motion, which we think is tightly linked with the idea of biological autonomy. There is empirical evidence to support the above ideas.
Some form of internal bias is necessary for breaking symmetry to cause self-movement and the bias may be the result of perception of the environment.

Such simple oil droplet systems show autonomy in the sense that the droplets move in response to the self-generated pH and the environmental gradient. In our modeling, we demonstrated that an computational autopoietic cell could move by continuously self-repairing the membrane, but in this case failed to show any gradient-climbing behavior (Suzuki, K. and Ikegami, T, 2008, Artificial Life, in press). This may be due to the fact that the autopoietic cell can only survive in the narrow range of environments that support a certain substrate density. Compared with that autopoietic cell model, our oil droplets are more stable and they strive to maintain their boundary structures. We hypothesize that the pH gradient around the droplet results in an unbalanced interfacial tension at the interface. The droplet then responds by motion in order to maintain a balanced interfacial tension. Once the tension forces around the droplet are balanced the droplet would stop moving. In this way, we contend that a kind of homeostasis is a basis for self-movement. Homeostasis is not simply preserving cell identity but also promotes self-movement with minimal cognitive behaviors as discussed in [Ikegami,T. and Suzuki, K., 2008, BioSys., 91, p.388].

[Additional abstracts]

(Is life an emergent property?)

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Emergence in complex systems theory is understood as the origin of new properties within the bottom up approach in hierarchical systems. For example, the chemical bond is an emergent phenomenon in the progression from atoms to molecules. It does not exist in the world of atoms but it is essential for understanding molecules. Similarly, genetic information is an emergent property of the transition from chemistry to biology. In the context of origin of life questions digital information is an emergent property of polynucleotide replication, the genetic code is an emergent property of the dynamical interactions of replicating ensembles of polynucleotides. Although the emergent property appears on the higher hierarchical level, its prerequisites are laid down already at the lower level: Conventional molecules can be formed only by atoms without closed electronic shells, or in other words atoms with closed shells do not form ordinary chemical bonds. It seems to be worthwhile to consider other emergent properties in an analogous way.

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It would be surprising to find respectable scientists who still hold a “vitalistic” view of life. This does not, however, mean that the point is moot. Instead it can be rephrased – what are the essential emergent properties of life and what processes bring them to existence? There are three types of emergent traits that are particularly relevant to the origins of cellular life:

1. macromolecular, sub-cellular and cellular structures,
2. novel cellular functions
3. organization of functions into cooperative, regulated, reproducing networks.

Two types of phenomena are essential for shaping these emergent quantities. One are subtly regulated physical (non-covalent) interactions. The others are evolutionary processes.

The formation of macromolecular, sub-cellular and cellular structures exhibits typical characteristics of emergent phenomena. In particular, it would be difficult to anticipate the existence of vesicles from observing single amphiphilic, membrane-forming molecules. Similarly, it would be difficult to predict the structure and function of ribosomes or energy transduction systems only from the knowledge of each individual component. However, equipped with our understanding of non-covalent interactions between individual parts and how they are affected by the environment we can explain, at least qualitatively, how many of these structures, from folded proteins to membrane-bound protocells, form and why they are stable. In many instances, our knowledge is even deeper – we can predict formation and properties of some of these structures. Much of this knowledge comes from fields different than the origin of life,

primarily from physical and biophysical chemistry. It is being extensively used in biotechnology, and medical and pharmaceutical sciences.

The emergence of novel cellular functions is a fascinating aspect of the origins of life. How presumably very limited inventory of initial structures and functions improved, increased and diversified to allow for increases in complexity of protocells? This issue has been recently addressed by a number of researchers. In a research project supported by the NASA Astrobiology Institute, we have been investigating this problem using techniques of *in vitro* protein evolution and protein design. We have shown that several novel functions can emerge through a relatively small number of mutations to a common protein scaffold. They are often (but not always) associated with refolding of the original structure. Simple analysis of mutations does not reveal the nature of a novel function – a characteristic feature of emergent traits. This simple example illustrates the pivotal role of evolutionary processes in generating emergent quantities.

Organization of functions into cooperative, regulated, reproducing networks is probably the most complex and the least understood emergent property at the origins of life. Although a few examples of successful coupling of a small number of functions and a few realistic theories, mostly in the genetic domain, exist, efforts along these lines have not been yet successful for a number of reasons. This gap in our knowledge greatly limits our ability to construct laboratory models of protocells. Closing this gap is likely to be at the frontier of research on the origin of life.

Leonardo Bich
CE.R.CO. – University of Bergamo, Italy

Life: emergence, not self-organization

I do not think there are sufficient data to assert that life is an emergent phenomenon so far. Nevertheless emergence is still a crucial concept in order to understand what makes an organism an integrated system of a specific kind, something more complex than a simple aggregation of non-living parts. I will sustain this last assertion from three points of view: theoretical, epistemological and heuristic.

First of all, an emergentist approach to life can allow us to distinguish some specific properties of living systems - as their autonomy and their endosystemic self-dependence of the metabolic organization (Varela, 1979; Maturana and Varela, 1980; Rosen, 1991) - from the properties of those processes of self-assembly and pattern generation which are also characteristic of other classes of systems like chemical dissipative structures (Prigogine and Stengers, 1979) and which can be classified under the traditional notion of self-organization (Crutchfield, 1994; Bich, 2008b).

This theoretical distinction has also important epistemological implications as these two classes of phenomena – emergence and self-organization – can be classified according to two different descriptive relations they depend on, respectively non-deducibility and unpredictability. These descriptive relations underlie two different kinds of modellisations. The first relation, in fact, focuses on intrinsic limits in the possibility of modellisation of natural systems and appeals to a dynamical use of alternative but nonetheless complementary models; the second one, instead, focuses on the limits in the accuracy of observables and makes use of bottom-up models. Therefore, if we assume this framework, the emergentist approach to the investigation of life can have relevant implications for the development of a suitable heuristic for biology. In fact it opens the way to the elaboration of a specific modelistic for the biological domain. Also, it makes necessary to identify which are the pertinent components at any different descriptive level, as they

do not necessary need to coincide with those observed at the basic physical or chemical one (Bich, 2008a). A top-down or a bottom-up approach can be chosen according to the purposes of the scientific observer and the characteristics of the specific case under study.

With regard to the second part of the question, if we do not assume a strong ontological approach on emergence there is no reason to point out any risk of "vitalism". An emergentist framework which focuses on the role of organization - coherently also with the thought of the first British emergentists (Morgan, 1923; Broad, 1925) - and on the relation between different descriptive levels or domains, is in fact coherent with physicalist assumptions.

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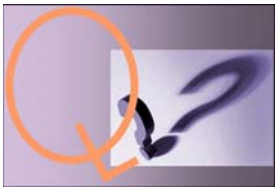
Workshop OQOL'09
Abstracts for the following selected question

- **Heterotrophic versus autotrophic scenarios.**

Premise. One of the important questions relating to the origin of life problem today is the *heterotrophy/autotrophy* dichotomy. In an (extreme) heterotrophic scenario, the organic material supposed to have accumulated in a prebiotic world by high-energy processes (such as those of the Miller type in a primordial atmosphere, or by impact delivery to the Earth from extraterrestrial sources) is assumed to generate the critical self-organization processes culminating in life's origin. In sharp contrast, in an (extreme) autotrophic scenario, this kind of organic material is considered irrelevant and it is, instead, postulated that the substrates and intermediates of the chemical processes that organized themselves toward life were generated through synthetic processes within self-organized structures (e.g. from free-energy reach C-1- or C-2-organics, combined with strong inorganic reductants).

The question. Do you see strong chemical arguments in favor of the one or the other scenario? And which experiments would you do/suggest, in order to possibly clarify this dichotomy?



Robert Pascal
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Abstract Q3: The combination of chemical, biochemical, and geological arguments favours heterotrophic scenarios

The existence of a redox gradient between strong inorganic reducing agents and an atmosphere having a neutral composition (mainly made of CO_2 and N_2) has been considered for decades as being able both to drive the synthesis of organic compounds in specific locations and to constitute a source of free energy for early living organisms. For this reason, autotrophy requires geological processes capable of segregating reagents in different redox reservoirs and then bringing them into contact in place favourable to the development of life. This question cannot be dissociated from that of the composition and evolution of the early atmosphere since the existence of redox gradient between the mantle and the atmosphere is dependent on processes that oxidize the atmosphere. The widespread belief in the past decades was that the atmosphere of the Earth evolved rapidly towards a mixture of CO_2 and N_2 as major constituents because of a fast escape of hydrogen atoms to the outer space.[1] But, it has been considered recently that the photolysis of hydrogen-containing molecules (H_2O, CH_4…) may be less efficient than presently in leading to the escape of hydrogen atoms because the temperature of a CO_2–rich and O_2–free upper atmosphere was much lower.[2] Then, hydrogen escape was probably inefficient to continuously drive the atmosphere towards a neutral composition. Although organic molecules could have been formed by heating dissolved mixture of inorganic precursors in hydrothermal systems, a reduced atmosphere strongly limits the availability of redox gradients as reducing free energy source for early living organisms. On the other hand, activation of a reducing atmosphere by uv-irradiation, lightning, or impacts is likely to have delivered activated organic molecules to the surface, which may have had many geochemical consequences on the evolution of the Earth and give some grounds to a heterotrophic scenario. These activation processes are likely to deliver energy-rich small molecular weight organics prone to undergo complexification processes provided that they are concentrated in specific locations on the surface through a segregation process rather than diluted in the ocean. In this view the question of autotrophy or heterotrophy can only be handled by considering both the chemistry derived from potential energy sources and the geological processes capable of segregating inorganic or organic chemicals in favourable locations. Activated low-molecular weight organics formed by activation in the atmosphere are associated with free energy contents sufficient to drive the formation of essential biochemical intermediates through coupled reactions, which is not the case of many inorganic redox potential proposed to have played a role in an autotrophic origin of life process. Moreover, free energy conversion from redox reactions requires both electron and chemical group transfers, which has been achieved by living organisms after the invention of chemiosmosis.[3]

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Sheref S. Mansy
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Heterotrophic versus autotrophic scenarios

Autotrophic and heterotrophic theories of the origin of life often motivate research with different emphases. While autotrophic origins supporters often carry-out experimental research focused on prebiotic metabolic cycles, heterotrophic assumptions usually result in research focused on the creation of replicating systems. As both approaches make unsubstantiated assumptions and focus on specific molecular reactions in isolation from their larger evolutionary context, a rationale is presented for seeking experimental model autotrophic and heterotrophic mechanisms within protocellular structures.

Eric Smith
Santa Fe Institute, USA.

The complexity and diversity of structures in the living systems we are trying to account for, and the difficulties of experimental control, will probably necessitate that most research on origin of life focus on the interaction of components and processes pre-arranged by the experimenter. Yet our experiments must be chosen to include the question, at each level of structure, "why is there something instead of nothing at all?" From my perspective, admittedly an opinion, many ways of thinking about origin of life -- in particular those addressed in questions: (3) Autotrophy versus heterotrophy, (10) Unity versus confederacy, and (11) Ecology and individuality -- have been framed too much in terms of object distinctions found in modern life, which may not provide the clearest framing for questions about origins.

The talk will focus on two projects:
1) Study of the chemical/energetic modularity of a small-molecule metabolic network that may be a model for "minimal modern life", emphasizing ties to geochemical context and classes of ecologically universal function, and
2) The identification of separate theoretical frameworks to account for ecological metabolic universals, as distinct from processes that contribute to the emergence of individuality in its many forms.

I will argue that metabolic modularity suggests a confederacy of processes linked by synthetic or network-feedback similarity and in many cases associated with primary support from particular energy systems. I will further argue that the emergence of individuality is, in its distinctive principles, a separate class of problems from the stabilization of metabolic universals, even though the two obviously support one another at the level of structures. I will advocate a view of ecology that draws on Venter's metagenomic approach, and also on a "meta-metabolomics" involving ecological flux balance. I will then argue that the emergence of individuality requires a theory for the emergence and stabilization of memory and control, physiological and species-level partitioning of metabolic competences, and the innovation of trophic interfaces in the sense of Borenstein et al. I will suggest that the problems of becoming individual and becoming speciated

are in many cases frustrated constraint satisfaction problems, and suggest consequences for signatures in trophic re-arrangement, punctuated equilibrium, and coordinated extinction events, as well as for re-framing the autotrophy/heterotrophy opposition to separate questions of chemistry from questions of individuation.

Wolfgang Weigand
Friedrich-Schiller-Universität Jena, Germany.

I would like to start with a statement of S. L. Miller: *"It must be admitted from the beginning that we do not know how life began"*. Indeed, we do not know how first bioorganic molecules, catalytical cycles and living cells were formed, but current views on the beginnings of life are divided in two camps: the organic soup "cultists" and the chemoautotrophic surface metabolism "rooters". Proponents of the primordial soup theory suggest that life originated through the organization of organic molecules that were produced in the atmosphere by a Miller–Urey type of reaction or were delivered to Earth from space. Supporter of the surface metabolism theory assume that metabolism arose chemoautotrophically starting from CO_2, CO, N_2, H_2O on the surface of the iron sulfide FeS. As the source of free energy, the oxidative formation of pyrite from iron monosulphide (FeS) and hydrogen sulphide (H_2S) has been suggested. In my opinion, an important argument against the primordial soup hypothesis is that the concentration of dissolved simple organic precursors in a prebiotic broth would be too low for further reactions and to polymerise for self-replication.
In contrast, there are some important experiments which are supporting the chemoautotrophic pathway: Thioacetic S acid forms from CH_3SH as methyl source and CO. That reaction was confirmed experimentally at 100°C in the presence of (Ni,Fe)S minerals. That reaction shows some similarity with the acetyl-CoA biosynthetic pathway catalysed by Fe-S and (Fe,Ni)-S centres in the active part of the enzyme. α-Amino acid activation and peptide formation were discovered when α-amino acids were reacted with CO and H_2S or CH_3SH under the same conditions (CO/(Fe,Ni)S/100°C). It has been also argued that the biochemical process of ammonia formation, from dinitrogen, by iron–sulfur enzymes may be traced back to a prebiotic "pyrite-pulled" nitrogen fixation. The synthesis of ammonia may serve as a model for a primordial nitrogen fixing system and it conforms well with theories of chemoautotrophic origin of life. Consequently, an important key experiment supporting the chemoautotrophic theory would be the successful reaction of CO_2 with H_2S in the presence of FeS and NiS yielding thioacetic S ester as well as a joint activation of CO_2 and N_2 forming amino acids.
Due to these experimental results, I see some chemical arguments in favour of the autotrophic scenario. Nevertheless, the question remains: Can a chemist make a living cell? Of course, he could make life, because he is working in an analogous way than nature and he is carrying out the same reactions according the laws of nature, but the circumstances are too sophisticated and the factor of time has to be taken into account, too. Thus it would be rather unlikely that a chemist will make life and therefore it will be impossible – at least at the moment – to clarify the dichotomy *Heterotrophic versus autotrophic scenarios*.

[Additional abstracts]

(Autotrophy vs. heterotrophy?)

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Which way to life: autotrophy or heterotrophy?

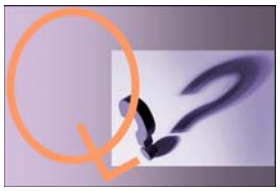
Our current understanding of the basic molecular processes of living organisms has challenged many original assumptions of the heterotrophic theory. However, laboratory experiments have shown how easy it is to produce a variety of organic compounds, including biochemically important monomers, under plausible cosmic and geochemical conditions. The robust nature of these reactions has been demonstrated by the finding that organic compounds are highly ubiquitous, as shown by their presence in carbon-rich meteorites, cometary spectra, and interstellar clouds where star and planetary formation is taking place. Experimental results achieved so far with the FeS/H_2S combination in reducing N_2 and CO are consistent with a heterotrophic origin of life that acknowledges the role of sulfur-rich minerals and other catalysts in the synthesis and accumulation of organic compounds. It will be argued that an updated heterotrophic hypothesis assumes that the raw material for assembling the first self-maintaining, replicative chemical systems was the outcome of abiotic synthesis, while the energy required to drive the chemical reactions involved in growth and reproduction may have been provided by cyanamide, thioesters, glycine nitrile, or other high-energy compounds.

Workshop OQOL'09
Abstracts for the following selected question

- **On the origin of catalytic cycles**

Premise. In a prebiotic scenario, like that assumed by Stanley Miller in his famous experiments, once given the initial conditions, prebiotic reactions flow towards the most stable compounds, being ruled by thermodynamic control. With the 'free ticket' of thermodynamic control, however, chemical prebiotic evolution would not have gone very far. In fact, the question of the origin of life can be abstracted as the question of the origin of enzyme-like controlled catalysis (eventually leading to genetically controlled catalysis), giving rise to sequential metabolic cycles, as opposite to chemically equilibrated reaction pathways. One line of thought considers that films of organic materials, found bound to the hot internal surfaces of inorganic tubes in contemporary hydrothermal systems, may have initiated networks of interaction between different layers that led the way towards metabolic cellular life.

> **The question**. How do you envisage the origin of sequentially catalytized reactions in a prebiotic scenario? And can you provide facts or scientific arguments, not simply beliefs, about this critical point?



Stuart Kauffman
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I begin by noting that catalytic cycles are an accomplished experimental fact. Guenter von Kiedrowski has made collectively autocatalytic sets of single stranded DNA molecules. Reza Ghadiri has made collectively autocatalytic sets of peptides. In these collectively autocatalytic sets, a molecule, A, catalyses the formation of B from B fragments, and B catalyzes the formation of A from A fragments. Ghadirii's results establish firmly that molecular reproduction need not be based on template replication of DNA, RNA or similar molecules although such replication without enzymes is not ruled out in the origin of life. The next issue is the range of molecules that might play the roles of catalysts in catalytic cycles. These include RNA, peptides, and organometalic molecules such as peptides with metalic moieties. The probability that a "randomly chosen" molecule in these diverse classes can function as a catalysis may differ dramatically. Experiments with in vitro evolution of RNA ribozymes suggests a probability that a random RNA sequence catalyzes a specific reaction at about one in a thousand trillion. I know of no data for random peptides, but due to the higher chemical diversity, and data showing that random peptides bind to arbitrary epitopes with a probability of about one in a million, would hazard the guess that the probability a random peptide catalyzes a randomly chosen reaction at about one in a billion. Julies Rebek guesses that the chances an organometalic compound catalyzes a random reaction may be about one in a thousand.

My own work has uncovered what may be a law of organization. Consider a set of molecules that can serve as substrates and products of reactions, and are themselves candidates to catalyze those very reactions. The ratio of molecular diversity to reactions among a set of N different species depends upon the order of the reaction. For cleavage and ligation reactions among linear polymers the ratio of reactions to molecules scales as the length of the longest polymer, say P. For two substrate two product reactions, the ratio of reactions to molecules scales as the diversity of molecular species, say N. N rises much faster than P as linear polymer length and diversity of possible polymers increase. The ratio is unknown for non-linear polymers. Numerical and analytic work show that if the probability of catalysis of a given reaction by a given molecule is above a threshold, then as the diversity of molecular species increases, more and more reactions are catalyzed and a "giant connected catalyzed reaction system" arises. With probability approaching 1.0, this giant component will contain a collectively autocatalytic set. Obviously the size of this set depends critically on the probability that molecules catalyze reactions, so is far smaller and simpler to obtain if the probability of catalysis is high. This suggests organometalic peptides as candidates of choice.

Once catalysis is achieved, it can also be inhibited, either competitively or non-competitively. This unstudied problem will yield autocatalytic sets with complex dynamics. Work on random causal (Boolean) systems shows that these can behave in three regimes, ordered, critical, and chaotic. It may be deeply important that critical networks store information, propagate information, and correlate the most complex and diverse behavior among variables whose activities or concentrations can vary. If dynamically critical autocatalytic sets can exist - and there is evidence cells are dynamically critical - they may be able to coordinate the most complex behaviors within and between such co-evolving sets.

A major point that I close with is that the emergence of collectively autocatalytic sets, now testable using "never before born proteins" or organometalic peptides, is what Robert Laughlin calls a "Law of Organization", not reducible to fundamental physics, but a law in its own right. As such, it is emergent with respect to fundamental physics, as I will describe in my answer to question 2.

Peter Schuster
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Closed cycles of processes show emergent properties. For example, a cycle of chemical reactions acts in total like a catalyst, a cycle of catalytic reactions has the properties of an autocatalyst, a cycle of autocatalytic reactions shows features that are characteristic for higher order autocatalysis – as observed with various symbiotic systems. In the conventional view catalytic cycles may originate from intrinsic catalytic functions or from (more or less) arbitrary or random assignment of catalysis and improvement through variation and selection. Examples of the first case are various metabolic cycles that are thought to have originated from extensive chemical networks through optimization according to thermodynamic criteria and optimal network flow. Networks of catalysts as found in genetic regulation are examples for cycles improved by variation and selection.

Systematic optimization by means of variation and selection becomes possible after the advent of 'digital' replicators like present day nucleic acid molecules. Digital replicators operate by means of a digit-to-digit copying mechanism and every molecule belonging to this class – no matter whether it is a corract copy or a mutation – is a potential replicator as well. Catalytic cycles containing two or more replicators suffer from competition between elements that may lead to loss of essential parts. In symbiotic networks the solution out of the dilemma, favorable catalysis versus competition, is mutual dependence: Otherwise inevitable selection of one component is suppressed by a positive feedback loop, the simplest example being the 'hypercycle' proposed in the nineteen seventies. Positive feedback loops are inherently unstable but can be stabilized by inclusion of negative feedback elements as we see in all systems in nature.

Another path towards catalytic networks of replicators is opened by the fact that neutrality is a ubiquitous phenomenon in polynucleotide or polypeptide space. Many biopolymer molecules are indistinguishable for selection. Neutral replicators that are separated by one or two point mutations form neutral networks in genotype space and are units of selection in the sense that their components are present at constant ratios and no member of the unit can disappear. Competition is suppressed by neutrality and random drift is counteracted by frequent mutation. The proposal is that – existence of replicators given – neutral networks are the basis from which the genetic apparatus, genetic regulation networks, and feedback cycles can originate.

Takuya Ueda
Department of Medical Genome Sciences, University of Tokyo, Japan.

On the origin of specific macromolecular sequences, catalytic cycles and the RNA world: The 'ligand-imprinting hypothesis'

In life, nucleic acid storing he information inseparably coordinate with protein responsible for biotical function. Thus, the origin of life is deemed to be the emergence of the linkage of these two molecules. Nucleic acid and protein are, of course, polymers of nucleotides and amino aids, respectively. If the condensation process of these monomers occurred simultaneously in the vicinity, coupling of genetic and functional molecules might be generated. How protein achieved molecular recognition capability during amino acid condensation process remains as an enigma.

Here I propose "Ligand-imprinting hypothesis" answering these two problems. First amino acids and nucleotides were activated to higher energy level by attaching amino acid to 5' phosphate of nucleotide. We can find this fossil molecule in the first sep of aminoacylation reaction of tRNA. These activated amino acids disposed around a particular ligand molecule prior to polymerization reaction. By condensation reaction of activated amino acids ligand-embedded polypeptides from which modern enzyme has evolved, were generated as a molecule with binding properties. Condensation polymerized of nucleotides took place coupling with amino acid condensation. If the base of nucleotide corresponded to an amino acid, resulted nucleic acids (RNA) could store primitive genetic information.

Robert Shapiro
New York University, USA.

"Metabolism-first" theories of the origin of life propose that a self-reproducing collection of small molecules preceeded replicating polymers in the development of life. This concept has often been described in terms of an autocatalytic reaction cycle, in which sufficient quantities of carbon dioxide or of other simple organic molecules are absorbed in each turn of the cycle to double the amount of material within it. The participating members of the cycle also serve as catalysts for the reactions of the cycle. Variants of the reductive citric acid cycle have often been cited as possible examples of such a system.

The plausibility of such a system has been challenged on a number of grounds. Many alternative possibilities for chemical reaction undoubtedly existed for organic molecules on the early Earth. Many of them would serve to drain material from the cycle, rather than sustain it. Any catalysts that were present would be as likely to facilitate these side reactions as they would the core reactions of the cycle. In the absence of specific enzymes, the organic material present would be likely to form a host of different substances and polymeric tars, as has been found in meteorites.

I will argue that these objections can be remedied if an external energy source can be coupled specifically to a reaction of the central cycle. Thermodynamic factors would then favor the central cycle and draw organic material from competing reactions into it; no specific catalysis would be required. Environmental changes could lead to the evolution of the central cycle into a

more complex self-sustaining reaction network. At some stage, the segregation of the reactive components within a suitable compartment would provide the first primitive cell.

While these ideas may be plausible, a "proof of principle" experiment will be needed to validate them. Some advocates of autocatalytic cycles have attempted to specify the participating components in advance, but this approach has not as yet proved fruitful. I feel that a more empirical approach should be used. An initial mixture of simple reactive organic molecules should be combined with an energy source (such as a redox couple), and the changes in composition of the mixture over time should be followed. A serial transfer protocol could be used to allow the system to be followed for an extended period of time. Failure would be marked by the dispersal of the material into a large group of unreactive end – products. If, however, a limited number of interacting chemicals dominated the mixture and maintained or increased their concencentration with time, then we may have encountered a system capable of self-organization and chemical evolution, propelled by an available source of free energy.

[Additional abstracts]

(Origin of catalytic cycles?)

Robert Pascal
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Abstract Q4: Catalytic cycles as a result of the imperative of kinetic amplification

In chemistry, possibilities of self-organization are ultimately founded on the non-homogeneity of matter (made of atoms or molecules). Starting from this atom-level discontinuity to get macroscopic structures observable at the scale of cells or organisms is the main problem to be solved to understand the origin of life. Crystal formation and surfactant aggregation are examples of thermodynamically controlled processes of structure formation originating in the intrinsic properties of atoms and molecules. However, dynamic self-organizing processes as those required for the origin of life derive more directly from kinetic[1,2,3] than from thermodynamic principles and need the availability of exceptionally efficient amplification processes (replication, autocatalysis). Enzyme-like catalytic polymers require a defined sequence ensuring a three-dimensional structure with binding sites able to accommodate for substrates and transition states. Then a significant catalytic activity is only possible for degrees of polymerization allowing a close contact of folded secondary structures, but the spontaneous emergence of well defined sequences of significant length is highly unlikely without replicating machinery. Moreover, limited amounts of catalysts displaying high kinetic proficiencies would hardly have consequences at a macroscopic scale. On the other hand, inorganic or organic molecular catalysis (small molecule interactions[4]) is able to bring about substantial rate increases by chemical participation or by converting bimolecular reactions into intramolecular ones.[5] But the essential point is that even moderate rate enhancements can be sufficient to influence the dynamics of multitude of molecules provided that they are involved in autocatalytic cycles. Chemical dynamics can then be responsible for a certain level of self-organization and of selection so that autocatalytic cycles are in principle able to invade a favourable environment when they compete for sources of matter or energy.[6] However, the further specificity of life – the ability of accumulating information in the course of evolution and to store it for next generations – requires replicating genetic polymers or alternative information carriers. In this view, autocatalytic cycles or networks capable of amplifying new chemical signals delivered by these replicating genetic polymers and of supplying small chemical

substances needed for their growth may be considered as favourable environments (ecological niches) for their growth and their evolution.

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John Sutherland
School of Chemistry, University of Manchester, UK.

A response to the question concerning the origin of catalytic cycles.

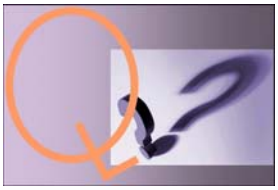
Prebiotic chemistry need not be limited to reactions under thermodynamic control, nor need it give extremely complex mixtures. So, when thinking about the origins of catalysis, one can certainly imagine simple mixtures of compounds still having chemical potential for further reaction. To an organic chemist, the problem of getting catalytic cycles going is the need for catalysis of more than one step of a multistep cycle. The paradox is how to get to the position of having catalysts for several steps when acquisition of a catalyst for just one step does not allow advantage to accrue to the system. One possible way round this is to have 'Jack of all trades' catalysts - single species, or preferably families of related species which catalyse several reactions, albeit none of them particularly well. Such catalysts are known in organic chemistry, think of 'synzymes'. It is possible that molecules like synzymes could be produced prebiotically - we think the chances are good enough to work on this now. Prebiotic 'synzymes' could catalyse further reactions of prebiotic reaction products and start to define cycles. There is no reason why this sort of chemistry could not take place at the same time as RNA assembly, and encapsulation so lets break down some conceptual barriers!

Workshop OQOL'09
Abstracts for the following selected question

- **Plausibility of the RNA world.**

Premise. The origin of life on the basis of a prebiotic family of RNAs is still a preferred scenario. This assumes, however, that RNA is formed prebiotically, while the question 'what made RNA?' is still unanswered. In fact, until now there is not even an accepted view of a robust prebiotic synthesis of mononucleotides, despite the considerable amount of work in the field by exquisite chemists. And, even if that would be discovered, still we would need to find a prebiotic way to couple the units in a 3'-5' configuration to one another. And, finally, even if this also would be known, we would have to find out how a specific macromolecular sequence could be synthesized in many identical copies (see also the question above), to give a concentration of, say, 10^{-12} M in solution (which implies, in turn, more than 10^{13} (quasi) identical copies in one liter, or ca. 10^{7} identical copies in one microliter). One might conclude that the prebiotic synthesis of RNA is still a chimera from the scientific point of view.

> **The question**. Do you share these arguments and rather bleak view? Which experiments or arguments can you suggest to counteract these objections against the "prebiotic" RNA world?



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The RNA world is a hypothetical scenario that has been inspired by the unexpected richness of structural and kinetic properties of RNA molecules. The plausibility of an RNA world is primarily a historical question. There are many problems arising in the design of plausible pathways leading to the first RNA molecules. Looking backwards from present day molecular biology to the origin of the cells and organisms has nothing to very little to offer that can replace an intermediate RNA world provided the result of prebiotic evolution is a DNA + protein world as we see it nowadays.

John Sutherland
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A short, robust synthesis of pyrimidine nucleoside-2',3'-cyclic phosphates from prebiotically plausible starting materials has recently been discovered (manuscript in submission). Furthermore, an intermediate by-product of this synthesis has the potential to be converted to purine nucleoside-2',3'-cyclic phosphates. Thus the prebiotic synthesis of activated ribonucleotides should not be considered an insuperable problem. The oligomerisation of these monomers to give RNA is still a challenge, but there is cause for optimism in the literature, and renewed experimental work in this area is now taking place. The issue of 3',5'- vs. 2',5'-linkage formation is still relevant, but there are chemical possibilities for solving this which might additionally result in oligonucleotide aminoacylation. This leaves open the possibility of the co-evolution of RNA and peptides, and suggests that the 'RNA World' concept in its purest interpretation might be too chemically austere. If lipids can also be synthesised in similar chemical scenarios (and we think they can), then highly complex macromolecular systems might be produced from which life could emerge as a consequence of the rules, and subtleties of organic chemistry.

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On the Likelihood of the Abiotic Formation of RNA molecules. (Response to Question 6)

The publication by Walter Gilbert which coined the phrase "RNA world" called for the assembly of the first RNAs from "a nucleotide pool." The nucleotide components of RNA are substances of considerable chemical complexity, bearing four chiral centers, and the regiospecific connection of the furanose form of the sugar ribose to a particular place on each of four heterocycle entities. If the nucleotides of the pool are to be capable of extensive polymerization, each must bear its phosphate on the same hydroxyl group of the ribose ring.

No driving force of nature, or set of chemical circumstances, is known which would produce the four RNA nucleotides without producing a host of similar substances, with alternative sugars bound to an extensive group of nitrogenous substances, and phosphate (or some substitute) connected at a variety of positions. This array of N-glycosides should be accompanied by an even larger concentration of simpler substances, such as alkyl phosphates, which would serve to terminate chains in any process that favored polymerization. The "progress" that has been made by skilled chemists in this area more reflects an achievement in the total laboratory synthesis of RNA rather than any recapitulation of events on the early Earth.

These circumstances have caused Gerald Joyce and Leslie Orgel to declare that the abiotic formation of RNA would constitute a "near miracle". Many other chemists, myself included, agree with this assessment. If we reject the idea that RNA, and other information-rich biopolymers were present at the start of life, then we arrive at an alternative, satisfactory solution for their origin. RNA first appeared through natural selection in living organisms, as the result of an extensive series of events, each of which had its own justification.

If we accept this argument, then we must conclude that the earliest forms of life functioned through the activities of sets of smaller, abiotically available molecules. To understand the origin of life, we must understand and if possible model these processes.

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Identification the ancient RNA machine for protein biosynthesis

Ribosomes are the universal machines producing proteins. We have identified* an internal architectural element that represents the ancient version of these machines within the concurrent ribosome. The overall fold of the RNA backbone of this region resembles motifs identified in "ancient" as well as "modern" RNA molecules of comparable size. Consistently, the extremely high conservation of this region throughout all known kingdoms of life implies its existence irrespective of environmental conditions. Comprised of three semy-symmetrical substructures of around 60 nucleotides each, this architectural element confines a void that provides the stereochemistry required for peptide bond formation and for the succession of this reaction, hence capable of the production of nascent proteins. The universality of the structure of this region, its central location within the modern ribosome, and the inherent tendency of RNA segment of comparable size to dimerize, indicate that this region may represent the ancient ribosome.

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* Using structural methods, supported by comprehensive mutagenesis experiments and quantum mechanical calculations.

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Evolutionary aspects of solid support replication processes

In general the origin and evolution of chemical replicators are considered in the context of vesicular containment within the protocell concept, and most often a co-evolution of vesicles and replicators is at least implicitly assumed for an effective Darwinian evolution in which a strong geno- phenotype coupling is required. It is, however, well established that mineral surfaces function as excellent catalyst for oligomerization of (activated) nucleotide monomers, and for this as well as for other reasons minerals have been proposed to have played an important role in the origin of life on Earth. Nucleic acid oligomers adsorp to a variety of minerals and other solid supports and such adsorption may provide spacial localization and confinement of chemical replication processes. The implications of such processes will be discussed in terms of protocellular evolution.

[Additional abstracts]
(Plausibility of an 'RNA world'?)

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A modular evolution-based model for the origin of the RNA world

A main unsolved problem in the RNA world scenario for the origin of life is how a template-dependent RNA polymerase ribozyme emerged from short RNA oligomers obtained by random polymerization on mineral surfaces: current estimates establish a minimum size about 165 nt long for such a ribozyme, a length three to four times that of the longest RNA oligomers obtained by random polymerization on clay mineral surfaces.

We have performed computational studies to investigate the structural repertoire yielded by random RNA polymerization. The analysis of very large pools (10^8 molecules) of ssRNA sequences of length 12 to 40 nt showed that topologically simple RNA modules turn out to be the most abundant ones, especially hairpin structures and stem-loops (Stich et al., *J. Theor. Biol.* 2008). A fraction of the ubiquitous hairpin modules could have displayed RNA ligase activity, as they do in current ribozymes, and catalyze the assembly of larger, eventually functional RNA molecules. We demonstrated that such ligation processes allow a fraction of the population to retain their previous modular structure. Therefore, structural and functional complexity can progressively increase even in the absence of template replication (Manrubia and Briones, *RNA* 2007).

This allows us to build up a stepwise model of ligation-based, modular evolution that could pave the way to the emergence of a ribozyme with RNA replicase activity, step at which information-driven evolution would be triggered (Briones et al., *RNA* 2009). Our evolutionary model shows two main advantages with respect to previous hypotheses put forward for the origin of the RNA world: i) short RNA modules resulting from template-independent polymerization on different surfaces or microenvironments might suffice to produce the first functional RNAs, being template replication not needed at this stage; ii) modular evolution shortens adaptation times and allows attaining complex structures that could not be otherwise directly selected. Therefore, ligation-based modular evolution might have bridged the gap between the first random RNA oligomers and a template-dependent RNA polymerase ribozyme at the dawn of the RNA world.

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The Prebiotic Synthesis of RNA and pre-RNA

The idea has been put forth that life originated from self-replicating, catalytic RNA molecules that were formed spontaneously on the surface of the Earth via prebiotic chemistry. The idea is particularly attractive due to the preponderance of catalytic RNA in modern cells and the central role of RNA in translation. After almost 50 years of effort however, unfortunately, the prebiotic synthesis of RNA has proven rather difficult. One solution to this seeming dilemma is that RNA was preceded by a simpler genetic molecule whose prebiotic synthesis was considerably more facile. In fact, this is probably one of the most experimentally testable ideas in the field of the study of the origins of life. We have systematically studied the prebiotic synthesis of a number of nucleic acid analogues over the years, with a special emphasis on robustness of synthesis and stability. The findings accumulated over the last 10 years will be reviewed and prospects for future directions suggested.

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The question whether life originated through the emergence of some autocatalytic metabolic reaction cycle - the metabolism view (Kauffman 2000, Shapiro 2006, Segre et al. 2000, Morowitz et al. 2000, Wachtershauser 1988), or a self-replicating oligomer of variable sequence - the RNA-World view (Gesteland et al. 1999, Joyce 2002, Orgel 1998), remains controversial and a source of continuing debate, though it should be noted that more recent considerations have suggested that a sharp demarcation between the two approaches may not be warranted (Eschenmoser 2007).

In a recent paper (Pross submitted) we have argued that by building on the concept of dynamic kinetic stability (Pross 2004, 2005), the two stages in life's establishment on earth - emergence and evolution, may be conceptually unified and considered as one continuous process governed by a single driving force principle - the drive toward greater dynamic kinetic stability. That view is strengthened by the realization that Darwinian theory has its roots in chemical kinetic theory, adding weight to the idea that biological and (replicative) chemical phenomena share a common underlying physicochemical basis. Indeed, the observation of Darwinian natural selection at the molecular level (Mills et al. 1967, Eigen 1992) is a striking illustration of the intimate chemistry-biology interrelation. Accordingly, we believe insights into the relatively poorly understood and uncertain prebiotic emergence phase can be obtained by relating it to the later, relatively well understood evolutionary phase. Simply put, ***evolutionary patterns gleaned from evolutionary biology may provide insights into the chemistry of emergence.*** Let us now consider possible applications of this way of reasoning to further probe the nature of the primal replicator – metabolic or genomic?

Modern Darwinian thinking endorses the following two central ideas. First, all living systems, whether cyanobacteria, believed to have existed on earth for some 3.5 billion years, or more recent life forms such as we humans, utilize the same basic nucleic acid genomic system. Second, evolution is considered to have taken place by small incremental steps, which in molecular terms is attributed to genome sequence mutation. If we now build on our proposal of

utilizing the pattern observed in evolution as a likely model for describing the process of emergence, we are led to several conclusions. First, both the ***universality*** and the ***stability*** of the genomic system of information storage over the billions of years during which life on earth evolved, suggest that the mode of information storage in the prebiotic phase would have been similar in kind, namely, one based on an oligomeric genomic system, rather than one based on a non-genomic metabolic system. That way of thinking in itself lends support to the RNA-world view, in which the emergence of some genomic RNA-like replicator was the primordial event that led to the emergence of life. However this argument in support of a genomic origin may be taken a step further.

Let us begin by assuming that some unidentified non-genomic autocatalytic metabolic system (for example, one based on the reverse citric acid cycle), rather than a genomic oligomeric system, did emerge prebiotically, and let us also assume (despite the lack of theoretical or experimental evidence) that such a system would be capable of undergoing Darwinian-type evolution. Even accepting those far-reaching assumptions however, it is difficult to see how a prebiotic ***non-genomic*** system would have been able to undergo a series of incremental changes that would have lead to a structurally distinct ***genomic*** system, ***while able to maintain its replicative capability during the transformation.*** Here again evolutionary theory offers useful insights. Consider Maynard Smith's classic model of protein evolution (Maynard Smith 1970). In his model Maynard Smith made clear that the unitary mutation steps in the amino acid sequence during the evolutionary transformation of protein structure ***cannot pass through non-functional intermediates***. Using similar reasoning we conclude that the transformation of a non-genomic replicating system into a genomic one would have required that each and every step in that transformation also pass through functional intermediates – functional here signifying the possession of a replicative capability - and it is far from clear that such a condition can be satisfied. Just how would an evolutionary process, step-wise and incremental in nature, transform a replicating system based on an autocatalytic metabolic cycle such as the reverse citric acid cycle, into a structurally quite distinct oligomeric sequence-based replicator, while maintaining a replicative capability at each step? After all, the replicative modalities are mechanistically distinct - in one version of the metabolic description, for example, it is based on autocatalytic cycle closure, while in the genomic oligomer description it is based on template-directed replication. It is therefore difficult to see the physicochemical basis for the smooth interconversion of one replicative type into the other, though it should be noted that recent work is increasingly emphasizing the importance of reaction networks, not just in metabolic cycles, but in genomic systems as well (Lincoln and Joyce 2009, Dadon et al. 2008). Accordingly, our proposal for a continuous emergence-evolution process, when considered together with a Darwinian model based on the centrality of a genomic oligomer system, reaffirms, we believe, the pre-eminence of a genomic system in the prebiotic phase as well. The suggestion of a peptide-nucleic acid (PNA) oligomer as a possible carrier of earlier genetic information exemplifies this way of thinking (Nielsen 1993), though definitive evidence for any particular pre-RNA entity has yet to be established.

In the context of the Origin of Life debate, Eschenmoser (1994) has expressed the view that chemical theories are significant "if, and only if, they lead to experiments which extend chemical knowledge". In that regard it is important to note that the metabolic vs. genetic replicator dichotomy has an immediate consequence with regard the minimal proto-cell project (Szostak et al. 2001). Both the process of life's emergence and the proto-cell project involve the transformation of inanimate matter into a simple living system. Accordingly, understanding the principles by which Nature chose to undertake this transformation will likely have direct implications on any synthetic attempts to attain that same goal. Biomimetic chemistry par excellence! Thus we conclude by saying that the origin of life field is more than just an intriguing

but speculative, academic exercise, as some might suggest. Its deliberations and conclusions, once firmly based, will necessarily impact on the many challenges that still await us at the problematic biology-chemistry interface.

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Liquid crystal self-assembly of nucleic acids:
a new pathway for the prebiotic synthesis of RNA

We have recently reported that aqueous solutions of complementary DNA and RNA oligomers, as short as 6 bp, exhibit chiral nematic and columnar lyotropic liquid crystalline (LC) phases, even though such duplexes lack the shape anisotropy required for LC ordering. Structural characterization shows that LC phases are produced by the end-to-end stacking of the duplex oligomers into polydisperse linear aggregates, which are then able to orientationally order [1,2]. Furthermore, when only a small fraction of the sequences is complementary, the duplex-forming oligomers segregate from the unpaired strands condensing into LC droplets, thereby maximally concentrating their terminals and holding them in the 3'-5' configuration favorable to ligation [3]. Finally, spontaneous phase separation and liquid crystallization are also found to select complementary and partially complementary strands in concentrated pools of random sequences.

We argue that this set of observations (sketched in the figure) has enlightening implications on the self-assembly of nucleic acids and on the prebiotic emergence of RNA strands long enough to sustain the enzymatic activity required by the "RNA world" scenario. Indeed, in the described LC condensation, complementarity promotes concentration via the intermediary of LC order, which provides a natural template for elongation.

Under periodic variation of temperature and/or concentration and in an appropriate chemical environment – conditions possibly met in tidal pools and currently investigated in our labs -, a self-catalytic process is established for the selective growth of extended complementary strands, since at every ligation longer filaments are formed, that more easily fit within the LC phases and can act as templates for further elongation.

Provided that oligomeric building blocks are available, the described process may act as a proper self-replication mechanism, "selecting" complementary strands but also accommodating some pairing mismatches and thus allowing for copy errors and sequence variation, a prerequisite for the emergence of ribozymes.

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The idea of an RNA world, when initially proposed, represented a major breakthrough in thinking about the origin of life. It can be argued that it is still the only convincing starting point for understanding how biology (in the Orgel sense of residue-by-residue replication of information) might have begun. We should remember that the hypothesis has been supported by much subsequent work in biochemistry, and that it is biological evidence – rather than largely structural inference - that now provides the strongest argument for the validity of the model. The Premise (6) is erroneous in that it assumes that RNA must have been formed prebiotically. In fact, the model tells us little if anything about prebiological chemistry.

Chemists have rather convincingly demonstrated that more than one chemical system of information replication would have been capable of chemically driven replication. A number of structural analogs of RNA or DNA have shown interesting properties in this regard, some of which are quite far removed form RNA itself in structure. Even the base-pairing units (the purines and pyrmidines) can be replaced by other molecules which are capable of forming specific complexes between chains in a manner similar to RNA. A question which is not quite so easy to answer, however, is what conditions of concentration and homogeneity would be required to make this idea work on a prebiological earth. Experiments need to be performed to test if the kind of selectivity which has been demonstrated by Eschenmoser for p-RNA in laboratory experiments, would also apply to reactions carried out with mixtures of sequences.

It is probable that some type of protocell was also necessary to permit selection to occur under prebiological conditions.

Workshop OQOL'09
Abstracts for the following selected question

• Minimal (proto-)cellular world?

• Minimal (proto-)cellular world? (a)

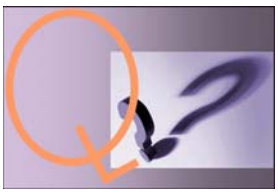
Premise. The simplest cells on Earth contain at least 500-600 genes, and more generally a few thousand. This elicits the question, whether this high complexity is really necessary for cellular life, also in view of the fact that early cells, conceivably, could not have been so complex. Until now, however, the construction of chemical synthetic cells has not been successful, and the attempts to make DNA/Protein "minimal cells" with extant genes and enzymes are still based on systems with approximately a hundred genes. In other words, we are still missing the view of the early protocells—the primitive structures from which modern cells may have arisen.

> **The question**. Do you see a way around the conundrum, that a living cell has to contain several dozens of independent specific macromolecular species and that, nevertheless, this complexity is not reasonably possible in prebiotic times? And/or: how do you envisage the structure of the simplest, early cells?

• Minimal (proto-)cellular world? (b)

Premise. The main building blocks of membranes in present-day prokaryotes are rather different from one another: in bacteria (like in eukaryotes) phospholipids are made of fatty acids, linked to the glycerol group (G3P) by ester bonds, whereas the phospholipids of archeabacteria are isoprenoid derivatives linked to glycerol (the stereoisomer, G1P) through ether bonds. And consider the extremely important role of hopanoids and steroids in modern bacterial and eukaryotic membranes.

> **The question**. Do you think that these radical molecular differences show that the issue of compartments was not relevant until late stages in the origin of life? Or do you consider that compartmentalization was still an early landmark, phospholipid diversity being easily explained as a later evolutionary adaptation to extreme environments, for instance?



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The primary characteristic of early cells is very likely to be the cell cycle

The primary characteristic of early cells could be the genetic regulation. Alternatively, the primary cellular occurrence was cell division, in which case the genetic control of cell behavior must have come about later as the result of the evolutionary selection aimed to make this process more reliable. Such a view implies the selection mechanism that acts without genes. Within the so called “vesicle world” a kind of primitive life process that was able to evolve into more complex cell-like one could have existed on the basis of properties own to vesicles. Vesicles can multiply by forming membrane buds which can be released by breaking the connecting necks. Membranes of daughter vesicles retain the composition and structure of the mother membrane which means that this system exhibits the property of compositional inheritance. Here we shall shortly reveal some experimental evidence about the process of vesicle splitting. Then we shall deal with the theoretical modeling of vesicle self-reproduction. It will be shown that this process only occurs under a condition which relates parameters depending on membrane composition and the parameters determining vesicle doubling time, i.e., vesicle intrinsic properties and properties of the external medium. The obtained criterion can act as the selectivity criterion in the sense that vesicle populations with shorter average doubling times have better survival advantage than other vesicle populations. For further support of vesicular origin of early cells we looked whether in addition to the process of division there are any other generic features of the cell cycle behavior that reflect the properties of simple vesicular systems. One such feature could be the variability of cell generation times, in view that one of the intrinsic properties of the process of vesicle self-reproduction is the variability of vesicle doubling times. Moreover, vesicle self-reproduction process can be divided into phases of spherical and subsequent non-spherical vesicle growth, of which the variable phase is mainly the first one. Incidentally, eukaryotic cells behave in an analogous fashion, as most of the variability of their generation times is due to the variability of the length of their first, i.e., the G1 phase of the cell cycle. In conclusion, by studying properties of vesicles we may get an insight into the structure of early cells but also learn more about functioning of cells as they are now.

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Constructive approach to proto-cellular life

We have been working on the experimental synthesis of the lipid vesicle in which the genetic molecule (RNA) is self-replicated by its encoding protein (RNA-dependent RNA polymerase). As the experimental setup for the *in liposome* RNA-protein self-replication network is not close enough to the pre-biotic soup, we can not answer to the question as to how the primordial life appeared at the beginning. However, carefully avoiding conclusions that strongly depend on which kinds of molecules were used in the experiments, we will discuss basic questions on the requirement of small compartments and the upper limits of the composition complexity for the origin of life.

Not in small compartment but in a bulk solution, the simplest self-replication system composing a genetic molecule an encoded protein, each of which catalyzes the synthesis of each other, the replication will accelerate if the concentration of either of the components increases due to the nature of the mutually catalytic network. Resultantly, the concentration of all the components could have evolved to turn higher and higher. But when it comes to living compartments, e.g., present cells, DNA molecule is a few in copy number while proteins are many. If there were many copies of DNA in a cell, the genetic information would lose the evolvability, because the effect of many mutations independently occurred on many DNA molecules is averaged-out in single cell so that the phenotypic diversity among the cells would turn out to be too small for Darwinian selection. Such hypothesis was demonstrated here by the selection experiment on the lipid vesicle encapsulating the transcription and translation processes. DNA must be as few as possible in number, in addition to other requirements to be genetic molecule; the heritability and combinatorial power of encoding. This small-number rule for genetic molecule per cell limits the compartment size by the diameter of 10 um. In compartments larger than 10 um, a limited number of protein catalysts produced from single genetic molecules scatter and fail to bind the genetic molecule for the self-replication. Small compartments are required for Darwinian evolution.

How many components are needed to run the self-replication in lipid vesicles? The self-replicating system was assembled using one template RNA encoding an RNA-dependent RNA polymerase which is translated by *in vitro* translation system reconstructed from purified translation factors and replicates the original template RNA. To run this *in liposome* RNA-protein self-replicating system required only 144 gene products. Although this number may vary slightly depending on the experimental setup, the order was close to the estimated minimal number of essential genes. The experimentally demonstrated composition complexity is thought to show a complexity at a certain time point in the developing process from pre-biotic soup to present cells.

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Conceptual bases for the emergence of early protocells

Life as we know it today exhibits both a *self-maintaining* and a *self-reproducing* organization. A self-sustaining organization is, in a chemical sense, an organizationally closed (recursive) set of reactions, and it is referred to as "Self-Maintaining System" (SMS). A SMS is conceptually an implementation of an autopoietic system under the physical-chemical laws, since processes manage the regeneration of their own raw materials recursively (self-construction) while maintaining its own organization and structure (Varela *et al.*, 1974). A SMS could increase its complexity. This could be achieved incorporating new elements and/or new functions.

Two main features should have characterized the early protocells: (1) be simple enough to appear easily in the chemical conditions of the early Earth; (2) capacity to allow further prebiotic evolution toward more complex systems, acquiring new properties. The system must provide a framework that gives certain independence from the environment at the same time as it is able to preserve itself and to generate new components of increasing structural complexity.

Recurrent organization could appear starting from just relatively simple sets of chemical aggregates. Focusing on the plausibility of such organized systems through the prebiotic evolution, some conditions should be addressed:

a) Molecularity. Prebiotic systems were built by common surrounding materials in the early Earth. Extant cells are a form of physical-chemical organization of matter. Metabolic reactions represent a particular subset of the whole of suitable chemical reactions in which catalysers act as constraints modifying the dynamics of conversions between "blocks". Only most sophisticated modulators could explain massive endergonic or polymerization processes.
b) Recursive self-maintained. The condition of recursivity in terms of organizational closure is also needed for self-maintenance (Cornish-Bowden & Cárdenas, 2007).
c) Autopoiesis. The system must be autopoietic and, as a consequence, a physical border should be included as a necessary condition for the realization of its self-maintenance (Varela *et al.*, 1974).
d) Thermodynamic coherence. Since the system must be open, i.e., capable to exchange energy and/or matter with the environment, the processes within in must be energetically and mechanistically coupled. There must be a form of energy "currency" as it happens in present cells to guarantee the kinetic coupling among different processes (Skulachev, 1992). Flow of matter and energy across a membrane are necessarily coupled with a self-

maintained recurrent chemical reaction network capable of making its own boundary (Ruiz-Mirazo *et al.*, 2004).

e) Stoichiometric coherence. Stoichiometric balance is needed, as a direct consequence of the mass conservation law acting on the chemical reaction network with extension to open systems (Montero *et al.*, 2008).

f) Self-reproduction. The system must have a specific way to reproduce itself. This property would be a consequence of a particular way of realisation of autopoiesis in which the growth processes are larger than the decay processes. Eventually this may include the possibility of generate differences on network progeny efficiency and competition, allowing the increase of complexity in some sort of pre-Darwinian evolution.

g) Increase of structural and functional complexity. Structural complexity could be achieved by system growth throw the incorporation of new elements in the network. Functional complexity is related to new closed constraints ("constraints closure") by means of the combined action of a membrane and a set of catalysts giving kinetic confinement. Finally complexity must be kept within the system (redundance, feed-back, buffering…).

In this contribution, we present a discussion on some of these aspects. The paper is illustrated with a recently proposed model (Olasagasti *et al.*, 2007; Montero *et al.*, 2007) that represents a theoretical metabolic SMS that satisfies some of the aforementioned questions. The model provides a better understanding of how early cells could have developed before achieving highly complex tasks such as the synthesis of informational genetic molecules.

Future work will consider a system that includes a kind of informational inheritable molecule or structure linked to differences on network efficiency. This will open the door to a differentiation of "genotype" and "phenotype".

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Early protein-synthesis machinery

The smallest bacterial genome contains 182 genes (*Carsonella ruddii*; Nakabachi et al., 2006), about 45% code for the translational apparatus (rRNAs, ribosomal proteins, tRNAs, synthetases, factors). It follows that a minimal translational apparatus with all principal functions of recent systems might need about 80 genes, which is about half the number required in the bacteria *E. coli*. We can imagine a further simplification, when we consider translation of leaderless mRNA (lmRNA), the simplest mRNA lacking initiation and termination regions and probably prevailing in early translation. This is suggested by the fact that lmRNAs are still present in organisms of all three domains. Accordingly, lmRNAs are considered as molecular fossils from the days of the last universal common ancestor (LUCA). Interestingly, a new type of ribosomes was detected recently, which is specialized for translation of exclusive lmRNAs rather than normal mRNAs; the corresponding ribosomes contain a highly reduced content of ribosomal proteins in the small subunit (Kaberdina et al., 2009).

We can go a further step back in evolution of the translational apparatus, when we consider the strong evidence indicating a co-evolution of tRNAs, synthetases and ribosomes. Indeed, all three classes of molecules/complexes can be dissected in evolutionary old and young parts. The old parts of tRNAs are related to old synthetases domains and to the large ribosomal subunit, which probably is older than the small one.

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Question 9 (b):
Early origin of phospholipid membranes but late specialisation of membrane components

There are two basic kinds of membrane phospholipids in extant organisms. Bacterial and eukaryal membrane phospholipids are fatty acid esters linked to *sn*-glycerol-3-P (G3P). Archaea possess isoprenoid ethers built on *sn*-glycerol-1-P (G1P). While there are some exceptions to the nature of the lateral chain, fatty acid or isoprenoid, and that of the linkage (ether versus ester), exceptions to the opposite chirality of the two types of phospholipids have never been observed. Since the two key enzymes leading to G1P and G3P, G1P- and G3P- dehydrogenase (G1PDH and G3PDH), are not homologous, they might have originated during the speciation of the two prokaryotic domains, opening the possibility that the last universal common ancestor (cenancestor) lacked a membrane (was acellular) or possessed no phospholipid membranes at all. We have previously shown that G1PDH and G3PDH belong to two separate superfamilies universally distributed, suggesting that members of both superfamilies existed already in the cenancestor. We suggested that the cenancestor was capable of synthesizing phospholipids enzymatically but leading probably to mixtures of lipids based on both glycerolphosphate stereoisomers. We also showed that many archaea possess homologues to nearly all known bacterial genes involved in fatty acid metabolism, showing the potential to synthesise fatty acid phospholipids. This, together with the presence of universally conserved proteins intimately linked to membranes, such as those of a respiratory chain and membrane ATPases used to generate free energy to the expense of a chemiosmotic gradient, argues in favour of a cenancestor already endowed with membrane phospholipids and an earlier origin of the lipidic nature of the cellular compartment.

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[Additional abstracts]

(Proto-cell world?)

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Routes to minimal living cells: How experiments may suggest plausible paths

Comparative genomics indicates that a minimal number of ca. 200 genes represents the core of a hypothetical minimal genome for a living microorganism in a very permissive environment [1]. Such minimal genome, being about 40% of the genome of Mycoplasma genitalium (an obligate parasite), still constitutes a very complex system, cannot be considered prebiotic.

On the other hand, the concept of minimal autopoietic systems [2] suggest that minimal living cells may in principle involve a very small number of *functions*. The above mentioned minimal genome, actually, essentially codifies for such minimal number of functions; however, since it derives from the analysis of modern organisms, its size takes into account the complexity, the evolution, and the rules of modern cellular metabolism. Can a simpler biochemical logic be exploited for the construction of living primitive cells? The elimination of DNA, for example, whould lead to a RNA-based cell. The elimination of catalytic proteins, on the other hand, would lead to a ribozyme-based cells [3]. How plausible are these models?

To date, however, only few experimental attempts have been devoted to this question, whereas there have been reported several experimental studies on the physical mechanisms underlying protocell dynamics – from its assembly to self-reproduction – based on modern protein biosynthesis, which is taken as a paradigm of cellular metabolism.

In this contribution, I will first discuss the point of minimal cellular functions, illustrating and criticizing some minimization principles, and later show how experiments on minimal cells [4] can also be informative on the nature of primitive cells. In particular, it will be shown how recent studies on unexpected catalytic properties of some simple peptides (peptide synthetase activity) may be significant for further speculations and investigations on primitive cells, that also include possible ribosome-free pathways to macromolecules, by a combinatorial fragment condensation approach [5]. Finally, even if we would know the chemical nature of minimal primitive cells, we would still miss the knowledge on the paths of their formation/assembly. This is a relevant open question on the origin of cells, that may be experimentally investigated by using semi-synthetic approaches as model systems [6].

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I envisage that the early cells have the simplest cell forms with a minimal number of molecules and the smallest genome. Such cells are called as Minimal Cells (*1*), and may be constructed in the immediate future. To image the early cells in the proto-cellular world is very alike to image synthetic cells that might be a simplest form. Therefore, to construct the Minimal Cells as in living state is a promised way to understanding of the early cells.

According to a current standard, a prototype of the Minimal Cells can be constructed by encapsulating an *in vitro* protein expression system inside liposome to achieve the internal protein synthesis. The liposome has important role as an interface between environment and the Minimal Cell. Moreover, the interface is responsible for the material transportations via membrane, an energy generation, and liposome growing and self-division for the self-reproduction of the minimal cell.

In this session I focus on the liposome membrane of the Minimal Cells, and introduce some experimental progresses toward the developments of F_oF_1-ATP synthase, permeable machinery, and a lipid synthesizing system (*2*) on the liposome of the Minimal Cells.

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Spontaneous Assembly of Cell-Like Structures From Likely Prebiotic Materials: Problems and Prospects

It has been suggested by a number of theoreticians, including Gantí, that cellularity is a precondition for a living system. Indeed over the years many researchers have sought to synthesize structures morphologically resembling cells under prebiotic conditions, for example the protenoid microspheres of Sydney Fox or the Jeewanu particles of Bahadur and coworkers. These structures clearly contain no lipid and are thus perhaps not “protocells”. Conversely, many likely prebiotic organic amphiphiles such as fatty acids only produce micelles or vesicles under rather narrow conditions, for example high ionic strength or abundant divalent cations seem to inhibit the self-assembly of cell-like structures such as vesicles. We have recently during an investigation the aqueous chemistry of HCN discovered a number of cell like-structures of extremely homogeneous size distribution which are produced robustly from these simple reactions. We have investigated the chemical and morphological structure of these and their interactions with amphiphilic species. Their potential importance as intermediates in the process of proto-cellular development on the primitive Earth, and possible implications for life-detection on other planets and in the geological record will be discussed.

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Abstract Q9(b). Phospholipids May Have Co-evolved with Chemiosmosis

An answer to the question can be expected by considering the utility of compartments and their components in relation with the development of the cell machinery, which may provide a scenario ruled by driving forces for the evolution of the membrane. Its main role in early living beings was probably that of avoiding the dilution of components, and especially macromolecular ones, in the environment. For this reason, it required rudimentary surfactants to have appeared early. A thermodynamic analysis shows that in addition to its role in building a membrane, the formation fatty acid– or that of other functional derivatives of hydrocarbons – from low molecular weight organic molecules is in principle capable of sustaining an early metabolism in a heterotrophic scenario. This observation supports an early emergence of compartments since fatty acids have been shown to spontaneously form vesicles by themselves or in mixture with other organics.[1] Vesicles made of this kind of surfactants may have harboured genetic polymers while their permeability was favourable to a spontaneous exchange of metabolites[2] and alkali cations.[3] As the metabolism became more complex new kinds of hydrophobic substances became probably available to build the membrane. However, as long as fatty acids remained present in the cell, their weak acidity – allowing them to be present under a non-ionized form capable of translocating across the membrane at neutral or weakly acidic pH – induced proton permeability and precluded the emergence of a further role of the membrane i.e. the possibility of development of proton concentration gradients between the internal and the external side. This function may then have appeared later than compartments since stable gradients of concentration of ions, especially those of protons, required impermeable membranes.[3] The completion of this task required both the selection of surfactants such as phospholipids remaining in a fully ionized state due to the low pK_A of phosphate esters[4] and the presence of transporters for substrates and metabolites to compensate for its low permeability. It is then proposed that chemiosmosis co-evolved with the development of efficient biochemical pathways to get rid of fatty acids in the membrane. The development of chemiosmosis machinery thus required (i) phospholipids-based membranes (ii) the availability of a biochemical machinery capable of reducing the fatty acid concentration below the threshold needed for a stable enough proton concentration gradient. This kind of evolutionary process can also account for a two stage evolution in which free energy became available from Na^+-translocating ATP synthases[5] before the invention of H^+-translocating ATP synthase since a translocation of alkali metal cations is possible only for membranes containing high levels of hydrophobic weak acids.

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Could be the Ribocell a realistic proto-cell model?

The Ribocell is an hypothetical cellular model that has been proposed some years ago as a minimal cell model (Szostak et al 2001). It consists in a self-replicating minimum genoma coupled with the self-reproduction of the lipid vesicle where it is contained. This model assumes the existence of two hypothetical ribozymes one (R_{Lip}) able to catalyze the conversion of molecular precursors (P) into lipids (L) and the second one (R_{Pol}) able to translate and duplicate RNA strands by pair base (NTP) linking. Therefore, in an environment rich of both lipid precursors (P) and activated nucleotides (NTPs) the ribocell can self-reproduce if the two mechanism: the genoma self-replication and the membrane reproduction (growth and splitting) are somehow synchronized. The aim of this contribution is to explore the feasibility of this models starting from the assumptions that all the needed substrates are available in the external environment: *the blue lagoon approximation*, and that the two ribozymes are already present and encapsulated in the lipid vesicle: *the first ancestor approximation*. Therefore, the main questions we try to answer is if the synchronization can emerge spontaneously by coupling metabolic reactions, i.e. the lipid production and the genoma duplication, along with chemical-physical processes like solute transport and water diffusion across the membrane, osmotic pressure, elongation and bending of the lipid bilayer. In particular: is the first ancestor in the blue lagoon able to reaches a stationary condition where it oscillates continuously between two states after an before the division? Is there a concentration threshold for the genetic material to avoid that the daughters cell remain without the minimal genetic kit to be alive ? Or, in other worlds, how much is this model robust to random fluctuations ?

In order to get insights in the Ribocell time behaviour we present and discuss an *in silico* model of this hypothetical proto-cell setting the kinetic parameters from experimental data available in literature (see for instance Mansy et al. 2008) and exploring the range of stability for the non-assigned ones. The Ribocell time course will be described with a deterministic approach by solving the kinetic differential equation set associated with the proposed metabolic network, and also with a stochastic approach by using the software ENVIRONMENT recently introduced (Mavelli & Ruiz-Mirazo 2006, 2007a) and already used to study the time behaviour of the minimal lipid peptide cell (Ruiz-Mirazo & Mavelli 2007, 2008). The deterministic approach shows that with a reasonable set of kinetic parameters our *in silico* Ribocell can reach a stationary oscillating regime of growth and division between two steady values of the internal aqueous volume. The generation time reaches a steady values of 500 days that can be enormously reduced increasing the membrane permeability to the substrates P and NTP. As a consequence of this, the Ribocell seems to be not a very plausible prebiotic model without the help of a mediated transport by proteins. Finally, we deal with the Ribocell in the more general perspective of building up a minimal cell (Luisi et al. 2006a, b) by coupling an internal metabolic network that produce lipids (Mavelli & Ruiz-Mirazo 2006) with the dynamics of the vesicle membrane (Mavelli & Ruiz-Mirazo 2007a,b, Mavelli et al. in press) and looking for some general constraints that should be satisfied in order to observe the instauration of an stationary division regime.

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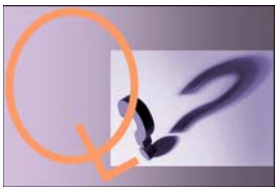
The vision of cells that underpins much of origins-of-life thinking is based on notions of simple, unstructured 'cells' that no longer exist – and arguably never have existed. An aesthetically attractive origin-of-life model would afford better insights into modern cells and populations and, reciprocally, would assimilate recent discoveries about what bacteria really are. There is now ample evidence that firstly bacteria are stuffed with extensive assemblies of molecules and macromolecules termed hyperstructures and secondly that bacteria have their meaning only as members of populations. In the 'ecosystems first' hypothesis, modern cells never arose from simple, primitive structures and the quest for the minimal cell is a search for an imaginary Holy Grail. In our hypothesis, the ancestors of hyperstructures were composomes and selection was for populations of interacting hyperstructures. These populations were, we contend, at least as complicated as their descendants. The real conundrum faced by the earliest cells and their descendants is how to negotiate the vastness of phenotype space created by molecular diversity so as to produce the reproducible, coherent behaviours on which selection can act. For this, new concepts are needed. One of these concepts is competitive coherence which describes the way biological systems achieve coherence with both their history and their environment.

Workshop OQOL'09
Abstracts for the following selected question

- **Life as unity or confederacy ?**

Premise. Many sciences have conventionally (if implicitly) referred to "life" as a unitary concept, and all too often, we speak of "the origin of life" as if it were essentially one kind of unified event: a transition from "no life" to "life" on Earth. An alternative premise would be that life is a collection of coupled but still distinguishable subsystems, each with its own recognizable dynamics and requirements for stability. In that case the origin of life could involve a sequence of transitions understandable in somewhat independent terms. For instance, one could take separately the appearance of self-reproducing systems and the formation of vesicles, biogenesis of proteins different from setting up metabolic cycles, origin of reductive power different from prebiotic chemistry, etc The degree of both contingency and of what some have called "irreducible complexity" in life will depend strongly on how tightly or loosely its subsystems are coupled.

The question. Do you agree with this possible alternative view of life origin? And if yes, what is the proper way to apply the notions of interdependency versus subsystem independence, in the understanding of both the modern function of life and of its origin? Can a different understanding of the organization and stability of life today lead to better sequences of investigations of life's origins? If "life" is not a totally unitary notion, but rather a confederacy of coupled processes, can the recognition of this decomposition help us define the nature and process of origins of life in ways that do not lead to contradiction and confusion?



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The complexity and diversity of structures in the living systems we are trying to account for, and the difficulties of experimental control, will probably necessitate that most research on origin of life focus on the interaction of components and processes pre-arranged by the experimenter. Yet our experiments must be chosen to include the question, at each level of structure, "why is there something instead of nothing at all?" From my perspective, admittedly an opinion, many ways of thinking about origin of life -- in particular those addressed in questions: (3) Autotrophy versus heterotrophy, (10) Unity versus confederacy, and (11) Ecology and individuality -- have been framed too much in terms of object distinctions found in modern life, which may not provide the clearest framing for questions about origins.

The talk will focus on two projects:
1) Study of the chemical/energetic modularity of a small-molecule metabolic network that may be a model for "minimal modern life", emphasizing ties to geochemical context and classes of ecologically universal function, and
2) The identification of separate theoretical frameworks to account for ecological metabolic universals, as distinct from processes that contribute to the emergence of individuality in its many forms.

I will argue that metabolic modularity suggests a confederacy of processes linked by synthetic or network-feedback similarity and in many cases associated with primary support from particular energy systems. I will further argue that the emergence of individuality is, in its distinctive principles, a separate class of problems from the stabilization of metabolic universals, even though the two obviously support one another at the level of structures. I will advocate a view of ecology that draws on Venter's metagenomic approach, and also on a "meta-metabolomics" involving ecological flux balance. I will then argue that the emergence of individuality requires a theory for the emergence and stabilization of memory and control, physiological and species-level partitioning of metabolic competences, and the innovation of trophic interfaces in the sense of Borenstein et al. I will suggest that the problems of becoming individual and becoming speciated are in many cases frustrated constraint satisfaction problems, and suggest consequences for signatures in trophic re-arrangement, punctuated equilibrium, and coordinated extinction events, as well as for re-framing the autotrophy/heterotrophy opposition to separate questions of chemistry from questions of individuation.

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This question poses several conceptual problems.

COMMENT NUMBER 1: It is widely accepted that something with the degree of complexity of the simplest present-day cells cannot suddenly appear. Thus, it is assumed that a more or less long *cumulative process* has been necessary. Accordingly, during this process different, progressively complex forms of organization would have appeared. However, it is hard to explain this cumulative process, because neither the one-shot, order for free “self-organization” associated with

the kinds of uniform non-linear dynamical systems that mathematicians usually study, nor Darwinian evolution (DE) seem to provide a satisfactory explanatory mechanism. The latter because DE requires a kind of system almost as complex as present-day simplest organisms, and the former because SO cannot ensure an iterative accumulation of organizational complexity. So, what kind of mechanism would explain this primitive cumulative process, which, starting from chemical evolution, led to the earlier systems capable to evolve by natural selection?

Recently, Fox Keller (2007) has suggested a form of evolution that is alternative both to natural selection and to emergent self-organization: evolution by composition. Recovering a half-century old idea proposed by H. Simon (1962), she argues that an iterative process may lead to increasingly complex systems, based on hierarchical and modular structures: If stable heterogeneous systems, initially quite simple, merge into composite systems that are themselves -- mechanically, thermodynamically (TD), chemically-- stable, such composite systems in turn would provide the building blocks for further construction, generating a process in which novelty arises through composition (or combination) and finally becomes integrated into the changing population by selection for increased relative stability.

This idea of evolution by composition supposes a modular-based type of organization. But, on the other hand, in many aspects, living systems show a highly holistic and distributed organization, where decomposition in simpler organizational modules does not work. A possible explanation of this apparently contradictory feature is that what initially were independent systems tend to become, first, inter-dependent modules and later, after an evolutionary process, components of highly integrated networks. In other words, organizational novelties have taken over old ones, making inter-dependent formerly in-dependent structures or organizations because this way the global, more integrated system gained stability and functional efficiency. As C. Woese (2002) has argued, early cellular organization was necessarily *modular and malleable*. According to this view, many of the structures responsible for more recent innovations have been formed by cutting and pasting earlier structures.

The problem, however, is how this mechanism can be applied to *early* prebiotic evolution, since the "primitive cells" alluded by Woese are relatively complex forms of organization (namely, they suppose a genetically driven metabolic organization). Accumulation of "modules" is relatively easy to conceive when we consider processes of assembling of TD conservative structures. But modularity is much harder to see when, instead, we are considering dissipative self-maintaining chemical organizations, because they are highly distributed. For, what ensure the cyclic reproduction of certain local interactions and therefore, the organizational stability of the system are the dynamical properties of the global organization. To speak of "modules" in this organizational context requires a certain form of dynamical decoupling within the system, but this in turn requires a relatively complex level of organization.

An intermediate solution may consist in the association between processes of assembling of TD conservative structures and distributed, holistic dissipative organizations. Actually, the importance of processes of self-assembling, and, more generally, of processes leading to the construction of novel (usually bigger or more complex) structures capable of playing functional roles in prebiotic organizations (like membranes or complex molecular aggregates) is nowadays widely acknowledged. For example, self-assembling of lipid molecules in a closed membrane/vesicle can be associated with a self-maintaining reaction network because the functional advantage of ensuring the adequate concentrations of certain key components (thus improving the self-maintenance of the network); Another example may be template assembling nucleotide molecules in a polymer if this polymer becomes functionally relevant for the system (say, by allowing active transport through a phospholipid membrane).

COMMENT NUMBER 2: The temporal unfolding of primitive forms of already genetically instructed organizations seems to support the claim that *historicity* has to be introduced as a key feature. In a sense, this is obviously true. Here it is important to clarify what we mean by "historicity". However, what is usually meant by "historicity" is a lineage-heritable process, where contingent events leave decisive consequences. Thus, historicity is understood as a process different that what has happen in the history of the universe, where history is rather understood as the unfolding of laws or necessary organizational constraints. In this sense, historicity would be rather a *consequence* of the type of organization we have already described, not a requirement to define it.

COMMENT NUMBER 3: Does the essence of life lie on some form of individuality or (proto) organism or, rather, it lies "in the intersection of lineages and metabolism", as Dupré & O Malley (2009) have recently defended. If so, there would not be any sharp distinction between life and non-life, but a continuum of variably complex cooperative systems.

My personal view is that, however embedded in evolutionary and ecological webs may living systems appear, their metabolic functioning still points to a basic organizational core that should be properly characterized. If the essence of biological organization is conceived as a web of diverse interactions *among* rather than *within* current living systems (extending the idea of living system to molecular replicators, viruses, organelles, parasites, symbionts, etc.), it will not be possible to determine whether organisms should be taken as a basic starting point (i.e., as a highly integrated and cohesive type of organization that gets progressively complex) or just as some occasional result of an on-going dynamics of loose cooperative relations among different kinds of biological entities. This is an important issue, because it could turn out that without such a highly integrated and cohesive individual organization, living systems would not be able to build a wider and more complex historic-collective meta-network (which, in turn, provides the necessary conditions for their long-term maintenance and evolution). Furthermore, without a strong idea of individual (metabolic) organization, it would be very difficult to account of concepts like functionality, agency, unit of selection,… or to make a clear-cut distinction between organisms and other forms of cooperative or "ecological" networks. And, after all, life seems to demonstrate, in the course of evolution, that increasingly complex forms of individual agents have emerged and developed, bringing forth progressively sophisticated interactive and cognitive capacities.

This is not to deny that a strong entanglement between the "individualistic" and the collective and historical dimensions. Therefore, although, from very early phases, one should think the phenomenon of life in terms of a collection of individual (proto-cellular) organizations, which generate (and, at the same time, are being generated by) a larger populational and trans-generational web of relations (even if such a global/populational level is still very far from what we understand nowadays as an evolving ecosystem/biosphere) I think we should keep as a central connecting theme in the OL research program the construction of individual organisms.

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The question whether life is a unity or confederacy goes to the very heart of the Origin of Life debate. In essence it asks whether life's emergence began with some unique physicochemical event followed by an extended process of complexification (whose details are yet to be clarified), or life began with the emergence of two or more identifiable subsystems which then merged into the holistic entity of some early life form. The unitary hypothesis is exemplified by the RNA-world view (Gesteland et al. 1999, Joyce 2002, Orgel 1998), which postulates the fortuitous formation of some replicating entity, RNA or RNA-like, which then underwent a process of complexification leading to the emergence of some minimal living system with its associated subsystems. The confederacy view is exemplified by Dyson's "double origin hypothesis" (Dyson 1985), where two subsystems – an independently formed replicating entity and an independently formed metabolic entity - combined to create a complex system, exhibiting both replicative and metabolic capabilities. The question is of practical importance since current attempts to synthesize a minimal living system (reviewed in Luisi 2006) will clearly benefit from an understanding of the path originally taken by nature to achieve that goal. In this commentary we wish to provide arguments favoring the unity view over the confederacy view and note that the above question connects directly with the replication-first – metabolism-first dichotomy (Shapiro 2006, Kauffman 2000, Segre et al. 2000, Morowitz et al. 2000, Orgel 2008, 2000, 1992, Pross 2004, Lazcano and Miller 1999, Lifson 1997, Maynard Smith and Szathmary 1995).

1. Application of Occam's Razor - "Entities should not be multiplied unnecessarily".

If, according to the confederacy view, we consider metabolic and replicative capabilities as exemplifying two key subsystems that would have needed to emerge and combine to generate a living system, application of Occam's Razor to that view suggests it to be less likely. Consider, both replication-first and metabolism-first schools of thought remain controversial and subject to on-going polemic debate due to the fact that persuasive chemical arguments against both approaches have been raised. Accordingly we would argue that the requirement for the ***independent*** emergence of each of these two quite special capabilities – replication and metabolism - necessarily weakens the confederacy argument. A theory which is based on the validity of ***one*** uncertain premise seems more desirable (or at least less undesirable) than one that is based on the validity of ***two*** uncertain premises. So, at least with regard the specific merging of metabolic and replicative subsystems, the confederacy viewpoint takes on the combined deficiencies of the two competing schools. However a more general problem with the confederacy approach needs to be considered, one associated with the thermodynamics of aggregation processes.

2. Kinetic and Thermodynamic Considerations

If we build an origin of life hypothesis on a confederacy viewpoint, then a critical issue that needs to be explained is the conversion of simpler equilibrium (or strictly-speaking, pseudo-equilibrium) subsystems into the far-from-equilibrium holistic systems that constitute the simplest living beings, e.g., a bacterial cell. Thus, even if the independent emergence of the particular subsystems is accepted (despite the difficulties mentioned above), it remains far from clear how the amalgamation of two or more equilibrium (or pseudo-equilibrium) systems can result in the formation of a far-from-equilibrium composite. Thermodynamic considerations suggest that a physical merging of the three key life subsystems (i.e., metabolic, replicative and

compartmental) may succeed in creating a cell, morphologically speaking, but that cell would likely find itself in a pseudo-equilibrium state, that is it would be ***dead.*** In chemical terms that system, by the very conditions by which it was created, would not constitute the dynamic far-from-equilibrium state associated with a living cell.

The physicochemical (as opposed to the historical) dilemma in understanding the origin of life requires us to come up with a mechanism by which established physicochemical principles would explain the ***in-principle*** conversion of a ***simple equilibrium (or pseudo-equilibrium)*** system into a ***complex far-from-equilibrium*** system. The unity approach appears to us to address this question more satisfactorily in that we have recently argued that a simple non-metabolic replicating entity could be expected to be transformed by kinetic selection into a metabolic (in the energy-gathering sense) replicating system, and this key transition could be viewed as a first step toward the generation of a far-from-equilibrium replicating system (Pross 2008, 2004). Thus we would argue that a unity view, through a kinetic analysis of replicating systems, may be better able to provide a causal explanation for the spontaneous emergence of (kinetically stable but thermodynamically unstable) metabolic far-from-equilibrium dynamic replicating systems.

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Biological autonomy and systemic integration

Considering how strictly subsystems are integrated in a living organism can be a fruitful way in order to understand more in depth what makes an organism a system of a certain class. In fact it allows us to shift our point of view from the properties of specific molecular components, from which to reconstruct life, to the properties of the system they realize, that is, to the conditions the constituents must satisfy in order to be part of it. The different ways this problem is dealt with are at the basis of the theoretical frameworks which characterize different lines of thought in biology.
I think that a preliminary step in order to answer this question should consist in avoiding the radical dichotomy between the two extreme positions on this issue: the decomposition of organisms due to a machine-like modularity (Simon, 1973) and the holistic view of living systems as not analyzable wholes. This step brings us to a "middle-way" approach to the problem, which faces two sub-poles of the dichotomy. The first one consists on a thesis on the partial decomposability of living systems into coupled but semi-independent subsystems, an example of which is the one proposed by Tibor Ganti through his Chemoton theory (Ganti, 2003). The second one is based on a more strict interdependency of sub-processes which can only be characterized in the light of the higher level system they integrate. This line of thought has been brought forth by the tradition of studies on biological autonomy (Maturana and Varela, 1980; Varela, 1979; Rosen, 1991, Cornish-Bowden and Càrdenas, 2008).

From this point of view the issue of unity versus confederacy assumes the form of an opposition between the "material" and the "functional" identification of subsystems (Bich, 2008a). While in the former approach the subsystems are partially independent and can be identified logically and phenomenologically *ex ante* with respect to the realization of the living system they belong to (bottom-up approach), in the latter they can be characterized and identified only *ex-post* and with respect to the unity they integrate, as their condition of existence is the presence of the biological system they realize through their interaction (top down approach). The theoretical framework I sustain here is based on the second approach. It consists in a reinterpretation of the cybernetic notion of circular self-stabilization (Bich and Damiano, 2008) which is not to be applied to single processes or subsystems then coupled together in a linear-like way, but to the whole living system (Bich, 2008b): a second order cybernetic loop of realization and conservation of the unitary organization of the organism. I think that the presence or not of this higher order integrative circularity is what marks the difference between the two middle-way perspectives as it gives the theoretical explanation of the looseness or tightness which the coupling between subsystems can assume.

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[Additional abstracts]

(Plausibility of an 'RNA world'?)

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Life and the single molecule

Based on a series of papers published by Leonard Troland, during the 1920's Hermann J. Muller proposed that life appeared with the abrupt, random formation of a single, mutable gene endowed with catalytic and autoreplicative properties. In sharp contrast with Muller's ideas, Oparin (1938) argued that the essence of life was metabolic flow. For him, life must be seen, in the dialectical sense, as a special form of the motion of matter, always in flow, which included enzymatically based assimilation, growth, and reproduction, but not nucleic acids, whose genetic role was not even suspected during the 1930's. The catalytic versatility of RNA molecules clearly merits a critical reappraisal of Muller's viewpoint, but there are many different definitions of what the RNA world was. The discovery of ribozymes does not imply that wriggling autocatalytic nucleic acid molecules ready to be used as primordial genes were floating in the primitive oceans, or that the RNA world sprung completely assembled from simple precursors present in the prebiotic soup. In other words, the genetic-first approach to life's emergence does not necessarily imply that the first replicating genetic polymers arose spontaneously in isolation from an unorganized prebiotic organic broth due to an extremely improbable accident.

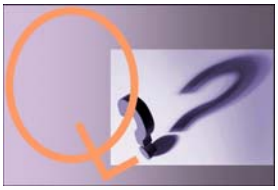
There are many indications of the robustness of the RNA world hypothesis. The genetic-first views of the origin of life would be strongly supported with the synthesis, within the constraint of prebiotic chemistry, of genetic polymers capable of evolving by replication with variation. Although the possibility that membranes were essential from the very beginning is supported by empirical evidence (Mansy et al., 2008), the genetic-first proposal does not requires enclosure within compartments, but such hypothetical model system should be able to evolve and promote catalysis using environmental precursors. There is convincing evidence suggesting that the genetic code and protein synthesis first evolved in such an RNA world, but the question of the ultimate origin of primordial functional protein-encoding sequences in RNA-dominated systems remains open and needs to be addressed.

Workshop OQOL'09
Abstracts for the following selected question

- **Defining the very origin of life**

Premise. Defining life in an universal way is notoriously a difficult or impossible task, but also the notion of "origin of life" appears to be rather confuse. Some authors talk about origin of life at the level of the origin of low molecular weight compounds, obtainable either through hypothermal vents; or the pyrite reaction; or by Miller's type of processes. However, you can have all low molecular weight compounds of this world, and you will never be able to make life, as life only arises at the level of specific macromolecular sequences like enzymes, DNA, RNA.

> **The question**. Do you agree that we should have a critical review of the terminology of "origin of life", and, for example, not use this term at the level of low mol. weight compounds (where we have "prebiotic chemistry", or origin of reductive power…), and restrict it instead to the level of the biogenesis of specific macromolecules and their interactions?



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Replication and Darwinian selection defines life's origin

There are diverse opinions regarding the definition of life's origin, and it is often said that such definition is crucially dependent on how one defines life. I suggest here a stand-alone definition, irrespective of how we define life. Such definition rests upon the most important foundation of biology – Darwin's theory of natural selection and evolution. I claim that life originates upon the spontaneous emergence of the first entity capable of a Darwinian process. It is widely accepted that the most crucial element of such process is self-replication. It should be stressed that many authors argue that the term "replication" only applies to the copying of linearly coded information, e.g. by polynucleotides. Often this is extended to a claim that no life can emerge without the involvement of "digital" polymeric information transfer. In contrast, I use here a chemistry- and pathway-independent terminology whereby "replication" entails the copying of information of any kind, with any measurable level of fidelity, from one generation to another. I propose that life begins at the inception of entity capable of leading to the appearance of its similes. As it is likely that early-on, a replicative process would be highly inefficient, mutations come naturally, hence diversity is an expected outcome. Combined with natural selection, Darwinian evolution would come about. Our own research on the Graded Autocatalysis Replication Domain (GARD) model provides an example of self-replication without nucleic acids. The entities that generate their own (rather imperfect) copies are assemblies of amphiphiles, and the information being copied from one generation to another is compositional, i.e. the ratios of different types of molecules, independent of spatial arrangement. The mechanism by which such replication can emerge, as shown by our computer simulations, is mutually catalytic networks that effect molecular accretion and synthesis. This example can be generalized to help delineate a general definition for life's origin, as well as rigorous criteria for evaluating proposed models for the origin of life.

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Hierarchical Definitions in the Origin of Life

Defining the origin of life must depend on first having a solid definition of life, which can be distinguished from a Theory of Biology. This problem can be further divided into theoretical definitions of origins, as opposed to experimental definitions. In order to be experimentally useful, a set of criteria which would allow the classification of a chemical system as living is required. Life detection in geological samples (extant or extinct, terrestrial or extraterrestrial), is a related problem since "detection" depends on the presence of certain pre-agreed diagnostic characteristics. Leaving aside more exotic possibilities (i.e. silicon-based life), we may build up a hierarchy of necessary but not sufficient characteristics that a living system might need to possess, for example: 1.) be carbon-based, 2.) be composed of a non-equilibrium set of organic compounds (in terms of type and/or chirality with respect to known abiotic mechanisms of synthesis), 3.) be capable of self-reproduction, 4.) be capable of passing on heritable structural and/or functional mutations. This list is open to debate and not exhaustive. The line between extant and extinct life could be drawn between criteria 2 and 3, while that between prebiotic chemistry and life might blur across 1-3. The failure of a system to meet the required criteria can be useful for refining the criteria. Some applications of this idea through time will be discussed.

Sandra Pizzarello
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On defining the origin of life (not really an abstract or fit for printing)

I concur with the premise fully, however, I find it also somewhat quaint. Miller? Hydrothermal vents? Low molecular weight compounds? What about Astrobiology dealing with nucleons and atoms? Yes, the origin of life is so difficult to define that people regularly satisfy themselves by changing the subject.

On the other hand, as much as we agree that life depends on macromolecules and their interactions, macromolecules are assemblages of simpler ones and, in a realistic view of prebiotic evolution, both these smaller molecules and their particular get-together cannot be left out of the discourse regarding the origin of life.

Nor should we forget how ignorant we are of the true extent of a-biotic chemistry or what means (contingencies) that could lead to a pre-biotic stage. For example, we always chuckle when we present the brew of organic material of meteorites as a soup with no way to go. Recently, however, we found meteorites with an organic composition as no other seen before with large amounts of just a few molecular species, all a (possible) key to biochemistry. Not a soup, then, not even a minestrone (certainly not a thermodynamic dead-end).

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Dept. of General Systems Sciences, University of Tokyo, Japan.

Emergence of Self-movement as a precusor of the Darwinian Evolution

A deep understanding of the origin of life requires a careful evaluation of terminology and clarification of objectives, not withstanding the difficulties of defining life itself. A conceptual framework including time, material and process that helps to explain the origin of life will also help us arrive at a definition of life. Our interest lies in how life-like processes can emerge in a collection of molecules. We specifically focus on self-movement as a characteristic of collective matter that is necessary for the emergence of life. Self-movement of collective matter allows for several emergent properties that are necessary for life namely, purposeful behavior, homeodynamics, autopoiesis, primitive cognition, robustness. We envision that self-movement may be a readily accessible process for diverse collections of matter and that primitive self-moving agents able to sense the environment and move with purpose would constitute the first examples of life on earth. Such agents would be capable of competing for resources, escaping dangers, sustaining themselves while at the same time retaining a chemical memory of their past actions. Such a system would foster the development of more complex internal chemical networks which would be responsible both for self-maintenance, self-movement and resource allocation/exploitation. In this sense these primitive agents would be capable of temporal evolution. True Darwinian evolution as an emergent property of the system would come later in the timeline of early earth when the best self-moving agents containing chemical networks acquire the ability to reproduce. We should discuss not only our true ancestor but also other candidates that did not directly contribute to our living systems in order to understand why some material system could not become life. Therefore we believe that it is essential to study the role of self-movement in the origin of life as an early state of matter that precedes replication in order to understand the transition from non-living but self sustaining chemical networks to evolving cellular life. These abstract concepts should be taken seriously to understand the origin of life, while actual materials are useful to realize the abstract concepts. But a careful discussion of abstract concepts around the origin of life is absolutely imperative.

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A systems view on the problem of defining the origin of life boundaries

As this question suggests, we should indeed make a critical review of our ways of conceiving the "origin of life" problem, aiming to establish some common terms in which it could be posed or understood by the majority of researchers in the field. However, I disagree with the concrete proposal of doing so by referring, mainly, to the specific properties of the molecules involved (i.e., whether they are high molecular weight compounds or not). If we share the idea that life is not the property of a single molecule, but of a collection of complexly organized molecules, the boundaries of the problem of the origin of life should not be defined in terms of molecular specificities. Instead, we ought to think in terms of *system* properties, either general (if one considers life as a general property of many different systems) or specific (if one considers life as a special property of a very particular class of systems). In order to mark out the limits of the problem of origins of life, and assuming that such a complex transition cannot take place in a single-step event but it is a process that spans in time (over, let us say, millions of years), one should try to determine a point of beginning and a point of end of that process by characterizing the type organization of the systems involved. In my contribution I will suggest two major properties or concepts to describe the organization of potential candidates for living systems, *autonomy* and *capacity for open-ended evolution*, which respectively mark out the beginning and the end of the process of origin of life. The first one is close to the idea of 'autopoiesis' (Maturana & Varela 1973), since it directly relates to the individual, metabolic (i.e., self-constructive) nature of life. The second is linked to the potential of a self-constructing system to construct other similar systems without an eventual decrease in its level of complexity (von Neumann 1966) and, although it has profound implications for the organization of individuals (phenotype-genotype decoupling), it involves a population of systems in collective evolution. My main claim (Ruiz-Mirazo *et al.* 2004; 2008) will be that the process of the origin of life begins with 'basic autonomous systems', possibly of low molecular complexity (i.e., made of low molecular weight compounds) but already organized as proto-metabolic cells; and it ends with 'genetically instructed metabolisms', or autonomous systems of high molecular complexity (high molecular weight compounds), also organized in cells but in a much more elaborate/intricate way: through a strong 'dynamic decoupling' made possible only with the development of a code of translation between genotype and phenotype. This decoupling is critical to combine the autonomy of individual living beings with their longer-term collective-evolutionary dynamics. So, according to such a scheme, open-ended evolution determines the conclusion of the process of origins of life and the aperture of the process of proper biological expansion and diversification.

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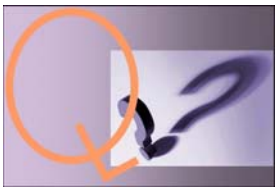
Workshop OQOL'09
Abstracts for the following selected question

- **On the entire field of the origin of life**

FOR A FINAL, COLLECTIVE RECAPITULATION (last discussion session)

Premise. The picture given until now suggests that we have not yet clarified the prebiotic synthesis of RNA, nor the biogenesis of macromolecular sequences, nor the development of the genetic code, nor the structure of the early cells. And probably several other points of ignorance could be added.

> **The question**. would you agree with the statement, that from the conceptual point of view the field has not progressed much since the early experiments of Stanley Miller? And why do you think/not think so?



Robert Shapiro
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The Origin of Life Field has shown little progress since the Miller-Urey experiments of 1953. (Response to Question 14)

In the spring of 1953, widely publicized papers were published by James Watson and Francis Crick on the three-dimensional structure of DNA, and by Stanley Miller on the synthesis of selected amino acids in a spark discharge. The Watson-Crick theory stimulated an enormous explosion of knowledge concerning the molecular basis of life, which continues to the present day. By contrast, the follow-up studies inspired by Miller's experiment have brought no harvest of understanding concerning the mechanism of the origin of life. That question was not even addressed in Miller's experiment. It demonstrated one plausible route, atmospheric synthesis, by which the early Earth may have acquired an inventory of small organic molecules. Other possibilities exist, such as infall from comets and meteorites and synthesis by thermal reactions within the Earth.

The Miller experiment was however an authentic attempt at a prebiotic simulation. Many subsequent studies have confounded such simulations with the very different discipline of total organic synthesis. Proposed "prebiotic" RNA syntheses have required multiple steps, each of which has used a very limited number of purified reagents . Substances that would disrupt the desired reactions have been excluded.The reactions have been carried out under careful supervision in modern laboratories. As such, they represent efforts towards a total synthesis of RNA, rather than a simulation of the chemistry of the early Earth. The probability that such a sequence of steps would take place spontaneously in a natural setting is extremely small.

Such efforts continue and much of chemical interest has emerged from them. However, neither chemists nor laboratories were present on the early Earth. The origin-of –life field remains trapped in a failed paradigm. Much has been learned in related areas, such as the early evolution of life. But until we return to authentic simulations of the chemistry of the early earth, little progress can be expected in our understanding of the principles and circumstances that convert an abiotic mixture into an evolving system.

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Ranking of sites on early Earth as cradles for life

Speculations on the location of proposed prebiotic chemical reactions and sites eventually acting as a scaffold for first living cells, are seldom made. Usually the reaction proposed as a prebiotic one is executed on sometimes very elaborated modern laboratory equipment, difficult to imagine on Earth. The excellent exception is the classic, early experiment by Stanley Miller in the nineteen fifties, who has considered conditions on early Earth and later constructed equipment simulating these conditions of high energy chemistry. The site of reactions is the atmosphere and reservoir of new organic compounds and the sea or lake with the adjacent streams. In spite of no possibility of homochiral formations, Millers approach can get the almost highest ranking of 5 in the 1-6 scale.

The ranking involves supposed physical and chemical characteristics of the particular location and rate of changes on the very wide time scale. On the lowest level of ranking are reactions difficult to be imagined running on early Earth or somewhere in the cosmic space, e.g like Fischer-Tropsch synthesis. In between, there are reactions, like natural chromatography, proceeding, e.g. on raw products of Miller reactions, on chemical products generated by radiolysis, products of catalysis on minerals, on reactions inside layered minerals like montmorillonite, reactions around volcanic vents at the bottom of the seas, products in freezing and thawing water, etc.

Proposed presentation is an extention of non-published ideas of classification of prebiotic phenomena as the “software” (chemical reactions) and conditions in which the reactions were able to run – as the “hardware”.

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