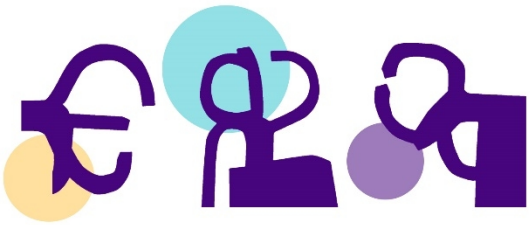
LDM'2023 DONOSTIA
ORIGINS OF LIFE



BOOK OF ABSTRACTS

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ProtoMet

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SCIENTIFIC PROGRAM (first complete version) -- OLDM2023

Palacio Miramar, Donostia-San Sebastian, Basque Country, Spain --- Oct 2-4 (2023)

TIME / DAY	Monday, Oct 2	Tuesday, Oct 3	Wednesday, Oct 4
8:00 – 9:00	Registration & welcome (krm)		
9:00 – 10:00	- Jack Szostak	- Karin Oberg	- Ram Krishnamurthy
10:00 – 10:30 10:30 – 11:00	- Edoardo Gianni (OC1) - Christof Mast (OC2)	- Grégoire Danger (OC6) - Martina Preiner (OC7)	- Andrés dl Escosura (OC11) - Yannick Geiger (OC12)
11:00 – 11:30	*Coffee break*	*Coffee break*	*Coffee break*
11:30 – 12:15 12:15 – 12:45 12:45 – 13:00	- Matthew Powner - Klara Hlouchova (OC3) - Short (2min) poster comm.	- Dora Tang - Anju Tomar (OC8) - Short (2min) poster comm.	- Peter Walde - Nathalie Katsonis (OC13) - Short (2min) poster comm.
13:00 – 14:30	*Lunch break*	*Lunch break*	*Lunch break*
14:30 – 15:30	- Irene Chen	- Daniel Segré	- Claudia Bonfio
15:30 – 16:00 16:00 – 16:15	- Antonio Lazcano (OC4) - Short (2min) poster comm.	- Emilie Werner (OC9) - Short (2min) poster comm.	- Nemanja Cvjetan (OC14) - Short (2min) poster comm.
16:15 – 16:45	*Coffee break*	*Coffee break*	*Coffee break*
16:45 – 17:30 17:30 – 18:00	- Joana Xavier - Quoc Phuong Tran (OC5)	- Joseph Moran - Robert Pascal (OC10)	- Sheref Mansy - Alvaro Moreno (OC15)
18:00 – 18:30			Recap & conclusion (krm)

KEYNOTES: 45 min + 15 min (questions/debate) // Invited: 35 min + 10 min (q/d) // OC: 25 min + 5 min (q/d)

Keynote Speakers

Why did Biology Begin with RNA and not some other Genetic Material?

Jack W. Szostak [a]

[a] *Howard Hughes Medical Institute,*
Department of Chemistry
University of Chicago
Chicago, IL, USA
jwszostak@uchicago.edu

Keywords: prebiotic chemistry • origin of life • nonenzymatic replication • RNA World

Abstract: The prebiotic synthesis of the canonical ribonucleotides is likely to have been accompanied by the synthesis of related nucleotides such as arabino-, threo-, and deoxy-nucleotides. How might modern RNA might have emerged from this primordial heterogeneity? We have found that nonenzymatic template-directed primer extension with activated ribonucleotides is generally more efficient than with other classes of nucleotides. On the other hand, noncanonical nucleotides in template strands can be copied over to yield an RNA product. Our observations suggest that nonenzymatic copying served as a chemical selection mechanism that allowed relatively homogeneous RNA to emerge from a complex mixture of prebiotically synthesized nucleotides and oligonucleotides.

References

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Keeping it together: from protocells to phages

Irene A. Chen

Department of Chemical and Biomolecular Engineering, University of California, Los Angeles, 5531 Boelter Hall, CA 90024, USA.
ireneachen@ucla.edu

Keywords: protocell • in vitro evolution • ribozyme • RNA world • phage

Abstract: Life may have begun from self-replicating RNA molecules. The encapsulation of RNA inside vesicles creates the potential for emergent properties[1]. We have found that encapsulation leads to greater ribozyme activity, improved folding, and even faster evolution inside vesicles, indicating advantages to cell-based early life[2,3]. In addition, our studies on phage-based nanomaterials demonstrates important advantages of delivering cargo at the nano- rather than molecular scale. Our in vitro and in vivo studies show the importance of considering spatial organization for both understanding the origin of life and developing applications in biotechnology[4,5].

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How to make a habitable planet: an astrochemical perspective

Karin I. Öberg[a]

[a] *Department of Astronomy, Harvard University, 60 Garden St, Cambridge, 02138 MA, USA*
koberg@cfa.harvard.edu

Keywords: astrochemistry • astrobiology • habitability • planet formation • protoplanetary disks

How did the Earth become a planet hospitable to origins of life? And how often should we expect planets around other stars to be habitable? The answers to both these questions lie in the formation and distribution of molecules in planet-forming disks around young stars. By studying the chemistry of these disks, we can begin to map out under which conditions young planets have access to the elements most associated with life (carbon, oxygen, nitrogen, sulfur and phosphorous), to water, and even to organic molecules. I will review our current understanding of this chemistry, as well as how this understanding has been achieved through a combination of astronomical observations, including chemical imaging of disks with the powerful microwave telescope ALMA and infrared spectroscopy with JWST, theory, and laboratory experiments aimed at recreating some of the exotic chemistry characteristic of planet-forming environments. Still, many questions regarding our planet's and our own astrochemical origins remain, and I will also discuss how ongoing observational and laboratory efforts, as well as current and future Solar System missions are setting us up to more deeply address the question of how to make a habitable planet.

Metabolism as memory in the origin of life

Daniel Segrè [a]

[a] *Department of Biology, Faculty of Computing and Data Science, Bioinformatics Program, Biological Design Center, Boston University, Boston, MA 02215, USA*
dsegre@bu.edu

Keywords: collective self-reproduction • compositional inheritance • flux balance analysis • metabolic networks • network expansion algorithm • artificial chemistries

Metabolism constitutes the engine of all living cells. Its structure, encoded in an abstract network of stoichiometric relationships, defines the space of possible metabolic states reachable by an organism. Even before the emergence of biopolymers as carriers of information, and before the rise of transcription/translation, it is possible that metabolism itself could act as a rudimentary inheritance system, in which molecular compositions would be transferred across generations of self-reproducing protocells and be subject to early adaptive processes[1]. In addition to playing a role as a short-term memory system for the reproduction of protocellular aggregates, metabolism also carries in its current structure a long-term memory of its ancient history[2]. Systems biology approaches can help understand the rise of self-sustaining protometabolic networks, explore alternative scenarios for metabolic evolution[3], and reconstruct plausible paths for the emergence of life.

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Prebiotic Systems Chemistry - Reinventing the Chemistries Leading to Origins of Life

Ramanarayanan Krishnamurthy [a]

[a] *Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.*

rkrishna@scripps.edu

Keywords: Prebiotic Systems Chemistry • Glyoxylic Acid • Protometabolic Pathways

Understanding the chemical origins of life has been interpreted, as a first step, demonstrating the formation of the chemical building blocks of life such as amino acids, nucleic acids, metabolites and lipids.[1] How these are formed in a prebiotic context on early Earth is primarily guided by considerations of (a) geochemical constraints (b) prebiotic chemical inventory and/or (c) the (absolute) necessity to mimic their biological pathways right from the beginning.[2] In this context, formaldehyde has played a central role in the formation of sugars, amino acids and nucleosides.[3] We present an alternative source molecule, glyoxylic acid (carboxylated formaldehyde), whose reactions not only lead to these biological building blocks but harbor the potential for (a) systems chemistry with feedback and (b) naturally transitioning to the types of chemistries and chemical pathways observed in biology.[4]

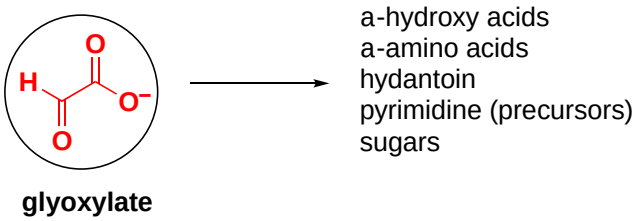

Fig 1. The prebiotic chemistry of glyoxylic acid.

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Shaping early life: primitive cells, primitive membranes

Claudia Bonfio[a]

[a] *Institut de Science et d'Ingenierie Supramoleculaires, 8 All. Gaspard Monge, 67000 Strasbourg, FR*
bonfio@unistra.fr

Keywords: protocell • lipid • selection

The complexity of modern biochemistry suggests that a systems biochemistry approach is required to understand and potentially recapitulate the network of prebiotic reactions that led to the emergence of life.

Early cells probably relied upon interconnected chemistries to link nucleic acids, peptide-based catalysts and membranes. In this context, I will discuss our recent advancements about:

- what, how and when membrane-based compartments appeared on early Earth;
- whether primitive membranes could be compatible with prebiotic chemistries and metal-driven catalytic processes;
- what biophysical or biochemical mechanisms could enable primitive cell cycles to retain continuity of function.

Addressing all these points can help us to elucidate the prebiotic pathways that led to the emergence of populations of functional primitive cells and, from there, the rise of life as we know it.

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Invited Speakers

On the cyanosulfidic origins of peptides, nucleotides, and metabolism

Matthew W. Powner[a]

[a] *Chemistry Department, UCL, 20 Gordon Street, London, WC1H 0AJ, UK*
matthew.powner@ucl.ac.uk

Keywords: prebiotic • systems chemistry • peptides • nucleotides • metabolism

Living organisms are highly complex chemical systems that exploit a small constellation of universally conserved metabolites. The chemical unity of these metabolites provides compelling evidence that a simple set of predisposed reactions predicated the appearance of life on Earth. The complexity of prebiotic chemistry until recently had suggested that elucidating life's origins was an insurmountable task, but prebiotic systems chemistry is now providing unprecedented scope to explore the origins of life and an exciting new perspective on a 4-billion-year-old problem. At the heart of this systems approach is an understanding that individual classes of metabolites cannot be considered in isolation if the chemical origin of life on Earth is to be successfully elucidated. In this talk several recent advances that suggest that nucleotides and proteinogenic peptides are predisposed chemical structures that can be facilely synthesised in water will be presented.[1-8]

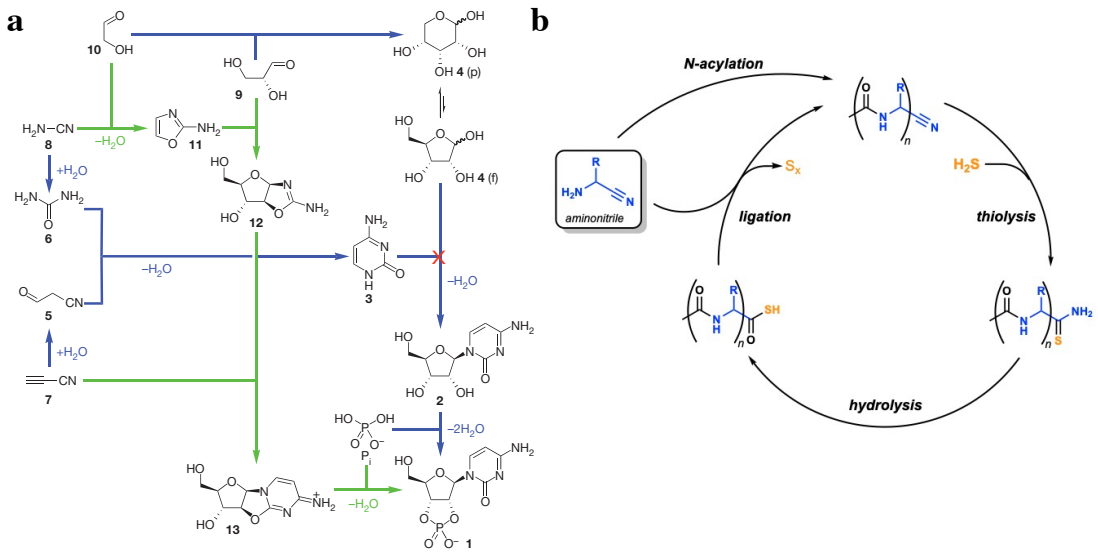

Fig 1. Chemoselective prebiotic synthesis of **a)** nucleotides; **b)** peptides.

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The Future of Autocatalysis in Origins Research

Joana C. Xavier [a,b]

[a] *Dayhoff Labs (London, United Kingdom)*
[b] *Imperial College London (Department of Chemistry, London, United Kingdom)*
xavier@dayhofflabs.com

Keywords: autocatalysis • origins of life • cofactors • metabolism • networks

Autocatalysis has for a while been considered a particularly relevant concept in studies of life's origins, for providing a mechanism of self-reproduction independent of genes. But the road between known autocatalytic systems and the first cells is vast and mostly uncharted. Is classic autocatalysis relevant for the origin of life as we know it? What is different between a) a complex network with autocatalytic motifs in a tight relation with a dynamic geochemical environment and b) an autocatalytic reaction in stable laboratory conditions? Recent results with biochemical networks show that a flexibilization of the concept of autocatalysis is required for it to apply in the origins of metabolism, in particular with regards to catalytic constraints imposed by the environment[1,2]. Metal catalysts are essential for all life known, and they seem to be even more essential at the emergence of complex biochemical networks before the origin of the genetic code[2,3]. Thermodynamics seems to favor the core of biochemistry[4], so hitherto unknown kinetically favorable pathways must have played a role in the establishment of universal organic (auto)catalysts in prebiotic networks. Micro-compartments can provide mechanisms for complexification and the emergence of new autocatalytic motifs[5] and their role in prebiotic geochemistry must be further explored. More, large-scale, standardized, quantitative experiments are direly needed for a foundational model of network (auto)catalysis where Artificial Intelligence will be immensely impactful. Future advances should be grounded on data and healthy dialogue towards stronger and testable theoretical frameworks of life's origins[6].

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Possible role of coacervates in the origin of life

T-Y Dora Tang[a,b]

[a] Max Planck Institute for Cellular Molecular Biology and Genetics
Pfotenhauer Strasse 108, 01307 Dresden, Deutschland
[b] *Saarland University, Department of Biology, 66123 Saarbrucken*
tang@mpi-cbg.de

Keywords: coacervates, protocells

In the 1920's Oparin hypothesized that membrane free compartments formed by coacervation would have provided a viable route to compartmentalize prebiotic reactions as a precursor to the modern cell. Studies which support this hypothesis are limited in that the precise chemical composition and conditions on prebiotic earth remain a mystery. Despite this, using bottom-up approaches allows us to generate physically relevant protocell models in the lab. This provides a means to unravel the effect of compartmentalization by coacervation during the origin of life.

Here, I will present strategies for the design and synthesis of protocell models based on liquid-liquid phase separation of oppositely charged components (coacervates) and describe how these compartments could be viable protocells models to bring molecules in close proximity and to tune reaction outcomes.

Nonenzymatic Metabolic Reactions and Life's Origins

Joseph Moran[a,b]

[a]*Université de Strasbourg, CNRS, ISIS UMR 7006, F-67000 Strasbourg, France*
[b]*University of Ottawa, Department of Chemistry and Biomolecular Sciences, Ottawa, ON, K1N 6N5, Canada*
moran@unistra.fr

Keywords: protometabolism • prebiotic chemistry • nonenzymatic • catalysis • self-organization

All dynamic self-organized systems found in nature are driven into existence when an energetic stress is relaxed under specific constraints. Whatever self-organized chemistry formed the basis for life should be no different. Due to the difficulty of making fundamental changes to a complex system that must operate continuously to support itself, the major chemical features of "protometabolism" may still be found in metabolism.[1] To look for clues to the initial energetic stress (i.e., redox gradients, etc.) and constraints (i.e., natural catalysts, proton gradients, scale, temperature, etc.) that would have enabled self-organization, our team is experimentally evaluating the conditions under which nonenzymatic versions of highly conserved metabolic processes might occur.[2-8] We are also evaluating whether metabolites, especially coenzymes, can act as catalysts to reinforce existing reactions within the network or to enable new ones – a necessary condition for the initial network to grow and to become more complex.[9] This talk will summarize our progress towards these goals.

References

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Iron porphyrins as potentially prebiotic catalysts?

Peter Walde[a] and Nemanja Cvjetan[a,b]

[a] *Department of Materials, ETH Zürich, Vladimir-Prelog-Weg 5, 8093 Zürich*
[b] *Current address: Department of Chemistry, University of Alberta, Edmonton, Canada*
peter.walde@mat.ethz.ch

Keywords: hemin • micelles • vesicles • catalysis • peroxidase

Assuming that vesicular protocells with a network of internal chemical transformations (**Fig. 1**) leading to vesicle growth and division once existed as prebiological compartment systems, it is likely that iron played a role as a catalyst.[1] Whether abiotically formed iron porphyrins played such a role is controversial.[2] Using ferric protoporphyrin IX (ferric heme *b* = hemin) as model iron porphyrin, we investigated its peroxidase-like activity in aqueous solution in the presence of micelle-forming amphiphiles and in the presence of vesicles as hosts for hemin.[3] Key findings from this fundamental study are presented and future challenges are discussed.

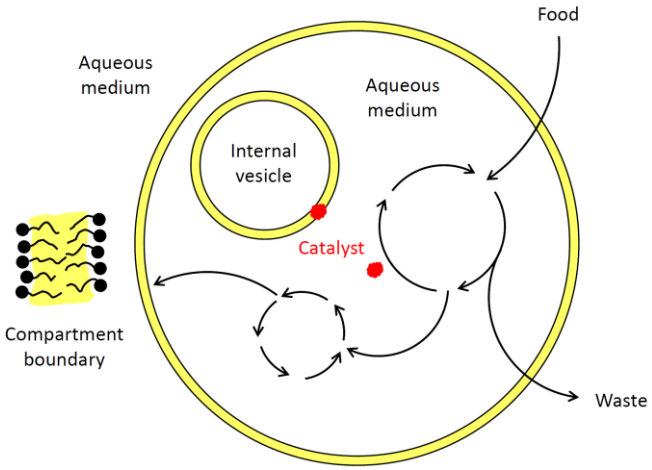

Fig 1. Highly schematic representation of a spherical vesicle as protocellular compartment system.

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Growing and Dividing Protocells

Sheref S. Mansy[a,b]

[a] *DiCIBIO, University of Trento, Trento, Italy*
[b] *Department of Chemistry, University of Alberta, Edmonton, Canada*
sheref@ualberta.ca

Keywords: protocell • protometabolism • Darwinian evolution

There is currently no plausible path for the emergence of a self-replicating protocell because prevalent formulations of model protocells are built with fatty acid vesicles that cannot withstand the concentrations of Mg^{2+} needed for the function and replication of nucleic acids. Although prebiotic chelates increase the survivability of fatty acid vesicles, the resulting model protocells are incapable of growth and division. Here, we build on our past findings to show that protocells made of mixtures of cyclophospholipids and fatty acids can grow and divide in the presence of Mg^{2+}-citrate. Importantly, these protocells retain encapsulated nucleic acids during growth and division, can acquire nucleotides from their surroundings, and are compatible with the non-enzymatic extension of an RNA oligonucleotide. Our work shows that prebiotically plausible mixtures of lipids form protocells compatible with the conditions necessary for the emergence of Darwinian evolution. Potential supporting metabolic systems to sustain the protocells will also be discussed.

Oral Communications

In vitro evolution of ribozymes for self-synthesis

Edoardo Gianni[a], Christopher J.K. Wan[b], James Attwater[c] and Philipp Holliger[a]

[a]UKRI MRC Laboratory of Molecular Biology, Cambridge, UK
[b]Francis Crick Institute, London, UK
[c]UCL Department of Chemistry, London, UK
egianni@mrc-lmb.cam.ac.uk

Keywords: RNA, directed evolution, self-replication, ribozyme

Self-replication and open-ended evolution are key hallmarks of living organisms, but their emergence from chemical systems at the origins of life remains unclear. RNA has the potential to enable these processes, but current limitations in speed and accuracy of artificial polymerase ribozymes preclude full ribozyme self-replication. In order to overcome these challenges, we have developed a novel *in vitro* self-synthesis based selection method. Using this, we observe - for the first time - a polymerase ribozyme[1,2] catalysing its own evolution, recapitulating selective pressures likely present on the early earth. Studying the results of this evolutionary process, we identified mutations that improve ribozyme self-synthesis, and revealed general principles on how a replicator can overcome primordial challenges to sustain itself.

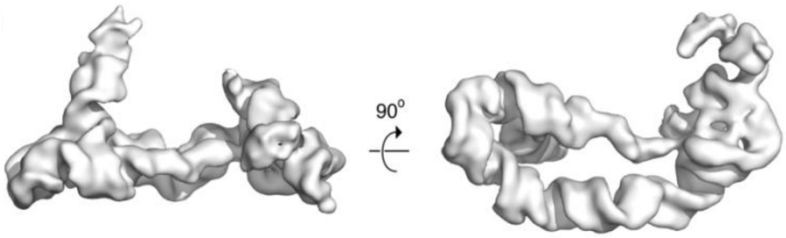

Fig 1. CryoEM density of triplet polymerase ribozyme heterodimer (from McRae et al[2])

References

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Physical non-equilibrium environments as prebiotic selector

Thomas Matreux [a] , Paula Aikkila [a] , Corinna Kufner [b], Dominik B. Bucher [c], Wolfgang Zinth [d], Dieter Braun [a] and Christof B. Mast [a]

[a] *Systems Biophysics, Department of Physics, Ludwig Maximilians University Munich, Amalienstr. 54, 80799 Munich, Germany*
[b] *Harvard-Smithsonian Center for Astrophysics, Department of Astronomy, Harvard University, 60 Garden Street, Cambridge, MA 02138, USA*
[c] *Lehrstuhl für Physikalische Chemie, Technische Universität München, Lichtenbergstr. 4, 85748 Garching b. München, Germany*
[d] *Biomolecular Optics and Center for Integrated Protein Science, Ludwig Maximilians University Munich, Oettingenstrasse 67, 80538 Munich, Germany*
christof.mast@physik.uni-muenchen.de

Keywords: non-equilibrium • biophysics • thermophoresis • phase transitions

Life is an out-of-equilibrium process, so its origin must also have been decisively shaped and driven by the non-equilibrium systems present 4 billion years ago. We have studied how simple heat flows through geological networks of interconnected chambers created chemical niches with complex mixtures of prebiotically relevant substances, each with different concentration ratios. These "micro-labs" could thus enable a variety of prebiotic reactions and massively increase their yield and selectivity compared to bulk systems. We exemplify this with the trimetaphosphate (TMP) driven phosphorylation of cytidines and the dimerization of glycine. Due to its strong thermophoresis, the otherwise rare TMP is concentrated by heat fluxes significantly more than its reactants, leading to orders of magnitude higher product yields. As another non-equilibrium physical system, we investigated the sequence selectivity of UV radiation on modeled genome pools created from subsets of codon sequences. Comparison with existing chronologies for codon and amino acid evolution suggests a possible role for UV light as a selection pressure during the evolution of early life.

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Why these amino acids and not others?

Mikhail Makarov[a], Vyacheslav Tretyachenko[a], Valerio G. Giacobelli[a], Stephen D. Fried[b] and Klara Hlouchova[a]

[a] *Department of Cell Biology, Faculty of Science, Charles University, Prague 128 43, Czech Republic*
[b] *Department of Chemistry and T. C. Jenkins Department of Biophysics, Johns Hopkins University, Baltimore, Maryland 21218, United States*
klara.hlouchova@natur.cuni.cz

Keywords: protein evolution • amino acid alphabet • origins of life • chemical evolution

All extant cells known to humankind build proteins from the same 20 coded amino acids. However, the study of origins of life implies that earlier cells functioned with a smaller alphabet and that other non-canonical amino acids were abundant and available to use while some of the canonical amino acids evolved only at later stages of life's evolution. That is intriguing within today's biology where each of the 20 canonical amino acids occupies a unique and seemingly indispensable role. This profound evolutionary transition in our cells' history therefore raises urgent questions: Could early proteins support a functional proto-biosphere (and how?) and what factors accompanied the selection of today's protein alphabet? Why weren't some of the most prebiotically abundant amino acids included in the Central Dogma?

To uncover the biophysical properties of proteins available at distinct evolutionary periods, we characterized highly combinatorial libraries composed of different amino acid repertoires [1,2]. I will uncover the properties of proteins from early vs. canonical amino acids and describe the consequences of inclusion of the most prebiotically abundant non-canonical amino acids [1-3]. I will further elaborate on functions that could be available to proteins composed of the early amino acids and on functional aspects that could drive the later expansion of the protein alphabet [3, unpublished data].

Our work indicates that structured conformations were readily available already to early protein alphabets (that were most probably highly acidic), capitalizing on interactions that are less frequent or in some cases rare in today's biology.

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On the nature of cellular genomes before DNA: insights from the RNA virosphere

Antonio Lazcano[a,b]
[a]*Miembro de El Colegio Nacional*
[b]*Universidad Nacional Autónoma de México*

Keywords: RNA viruses, RNA ancestral genomes, RNA polymerase, gene duplication

Although the chemical nature and the origin of the primordial replicative genetic polymers is largely unknown, the catalytic, regulatory and structural properties of RNA molecules and ribonucleotides, combined with their ubiquity in cellular processes suggest that it is an early, perhaps primordial, stage during which RNA molecules played a much more conspicuous role in heredity and metabolism. Although it is unlikely that RNA viruses are direct descendants of primitive RNA-based life forms, their study may provide insights into the replication strategies, genomes sizes and organization, mutation and evolutionary rates and gene generation processes of early cellular genomes prior to the emergence of DNA.

The only common features shared by all known RNA viruses are, of course, an RNA genome, and a monophyletic right-hand monomeric RNA-dependent polymerase (RdRp). Reconstruction of the evolutionary history of RNA viruses is hindered by the high level of mutation, which rapidly erodes the information contained in their sequences. Because of the absence of editing and repair mechanisms, RNA viral genomes are limited by Eigen's limit and tend to have small sizes (~2 kbp to ~33 kbp), and the high level of mutation rapidly erodes evolutionary information contained in sequences. However, the same is not true of their tertiary structures. Analysis of diverse RNA viruses suggests that viral RDRps, as well as their cellular homologs (*E. coli* DNA pol I, II & IV) may be vestiges of the ancestral proteinic polymerase from a hypothetical RNA/protein world stage. A key event in the evolution of genomes must have been the emergence of an array of editing and repair mechanisms, and we posit that one of the very first to evolve was 3',5' exonuclease activity, represented today by the coronaviral exonuclease, which has been hijacked from cellular host. Three-dimensional analysis of RNA viral encoded proteins shows that gene duplications have played a key role in the size increase of RNA viruses, and suggest that the same mechanism may have operated, together with recombination, in putative ancient cells with RNA genomes.

Nonlinear Dynamics meet Biomolecule Synthesis– How Feedstocks Modulate Kinetics and Product Distributions in a Prebiotic Autocatalytic Reaction

Quoc Phuong Tran[a,b], Ruiqin Yi[d] and Albert C. Fahrenbach [a,b,c]

[a]School of Chemistry and [b]Australian Centre for Astrobiology, [c]UNSW RNA Institute, University of New South Wales, Sydney, NSW 2052, Australia; [d]Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo 152-8550
q.tran@student.unsw.edu.au

Keywords: cyanamide • formose reaction • kinetics • reaction network • ribonucleotide precursors

How protometabolism emerged from prebiotic chemistry has remained a topic of intense debate in the scientific community. It is hypothesised that biomolecule synthesis intertwined with nonlinear dynamics may provide a potential clue. Using the formose reaction as a model for prebiotic autocatalysis, we added other feedstocks proposed to be present on early Earth to investigate their effects on the kinetics and product distributions of the resulting reaction network. In one case study, cyanamide was shown to inhibit the formose reaction by reacting with formose sugars to form aminooxazole derivatives while also forming tetrose and pentose aminooxazolines, precursors for TNA and RNA synthesis. Implications regarding chemical evolution and future work involving microfluidics will also be discussed.

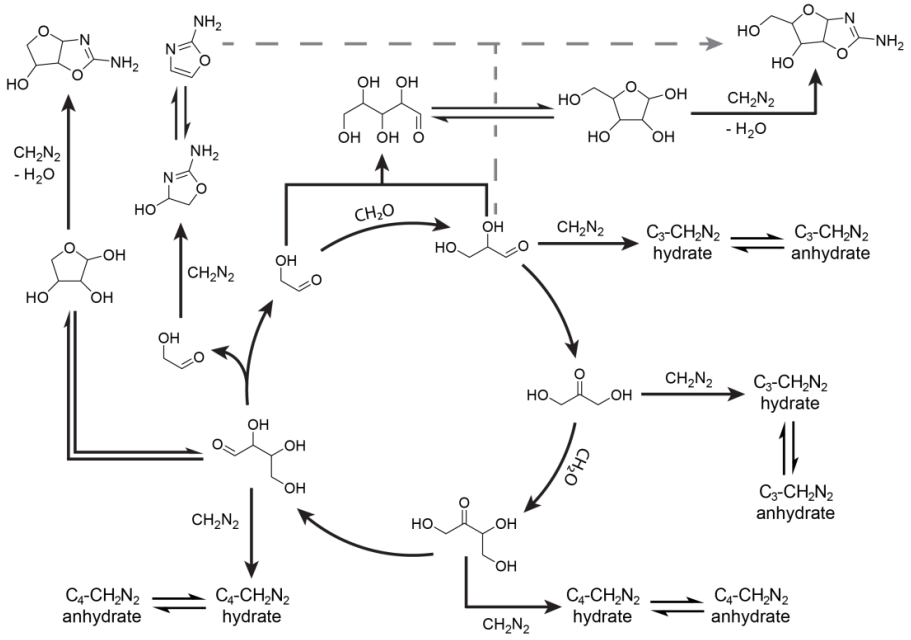

Figure 1. Some pathways for the proposed reaction network of formose intermediates and cyanamide based on the autocatalytic cycle proposed by Breslow and experiments herein.

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Origin and composition of the early Earth's reservoir of exogenous organic matter and its impact on the emergence of prebiotic chemical systems

Danger G.[a,b], Vinogradoff V. [a], L. Le Sergeant[a] d'Hendecourt and Pascal R[a].

[a] *Aix–Marseille Université, CNRS, PIIM, Institut Origines, Marseille, France.*
[b] *Institut Universitaire de France, IUF*
gregoire.danger@univ-amu.fr

Keywords: astrochemistry • molecular diversity • prebiotic systems • organic matter

Some of the organic matter in our solar system may have originated in dense molecular clouds. These clouds are made up of silicate grains surrounded by ices including H_2O, CO_2, CO, CH_3OH and NH_3. In some areas, the cloud will collapse on itself to form a solar nebula that will potentially evolve into a planetary system such as our solar system. During this evolution, these ices will undergo numerous modifications leading to a complexification of the organic matrix. The agglomeration of these grains carrying more or less evolved organic matter have formed the small bodies of our solar system, from the asteroids to the comets far away. These small objects could have then served as a reservoir of organic matter for the development of prebiotic chemistry on the surface of a telluric planet such as the primitive Earth.

Using experimental approaches developed in our laboratory [1], we try to reproduce the conditions of formation of interstellar grains and to study their molecular composition. Following this strategy, we aim to determine the role of solid phases in the origin and evolution of organic matter (volatile and non-volatile) in these astrophysical environments [2]. Linked to the question of the origin of biochemical systems on Earth, our research could help determine how such organic matter may have serve in the emergence of prebiotic chemical processes, representing the first stages towards the appearance of life [3].

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Mineral surfaces as protoenzymes: how to connect cofactors, rock pores and heterogeneous catalysis

Delfina P. Henriques Pereira[a,b], Tuğçe Beyazay[c], Kendra S. Belthle[c], Harun Tüysüz[c], Martina Preiner[a,b]

[a] Max Planck Institute for Terrestrial Microbiology, Marburg, Germany
[b] Microcosm Earth Center, Marburg, Germany
[c] Max Planck Institute for Coal Research, Mülheim/Ruhr, Germany
martina.preiner@mpi-marburg.mpg.de

Keywords: hydrogen • cofactors • minerals • catalysis • serpentinization

The last universal common ancestor (LUCA) arose in an environment of rocks and water on the early Earth about 4 billion years ago. We can connect LUCA's metabolism to its geochemical roots through top-down comparative bioinformatics [1] and through bottom up geochemical laboratory studies, using minerals and inorganic redox partners (H_2, metal ions) instead of catalysts as enzymes [2]. We aim to connect central metabolic cofactors and enzymatic reactions that were present in LUCA to early Earth geochemical reaction partners in order to better understand the transition from environmental reactions to genetically encoded metabolic functions. The hypothesis: cofactors are the missing link between abiotic and biotic (enzymatic) catalysis. Here, we show a connection between abiotic and biotic hydrogen (electron) transfer. Hydrogen gas, H_2, is generated in various geochemical settings, among them serpentinization, a water-rock interaction process during which iron-containing minerals transfer electrons to the protons of water. H_2 has been a source of electrons and energy since there was liquid water on the early Earth, and it fuelled early anaerobic ecosystems in the Earth's crust. It is also the electron donor for the most ancient route of biological CO_2 fixation, the acetyl-CoA pathway and abiotic, geochemical organic syntheses resembles segments of the pathway occur in hydrothermal vents today [3].

In metabolism itself, H_2 is being transformed into biochemical electron donors, cofactors such as the dinucleotide NADH which can be seen – simply put – as hydride (H^-) donors. We successfully activated hydrogen on minerals found in serpentinizing systems (awaruite Ni_3Fe, magnetite Fe_3O_4) reduce NAD^+ to NADH under aqueous conditions at temperatures found at the cooler end of serpentinizing systems [4]. We furthermore were able to conduct these principles onto other biochemical electron donors and acceptors (flavins such as F420, FAD and Riboflavin). These results underline the connection between central molecular transitions in metabolism and abiotic, geochemical catalysis with hydrogen as a common denominator.

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UV-driven reduction of cysteinyl peptides with cyclic disulfide molecules.

Anju Tomar,[a] Nita Sahai [b] and Sheref Mansy [c]

[a] *Department CIBIO, University of Trento, Via Sommarive 9, 38123 Povo (TN), Italy*
[b] *School of Polymer Science and Polymer Engineering, University of Akron*
[c] *Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton AB T6G 2G2, Canada.*
anju.tomar@unitn.it

Keywords: Lipoic acid ● Glutathione ● UV light ● Disulfide ● Membrane

The primordial soup must have contained metal ions. In biological system, protein bound metal ions are important in mediating catalysis and redox chemistry. The interaction of peptides and metal ions involves donor moieties, such as carboxylates, amino groups, and thiolates. Cysteinyl peptides must be reduced in order to coordinate iron-sulfur clusters.[1] However, mixtures of ferric ions and cysteinyl peptides leads to the reduction of ferric to ferrous ions and the concomitant formation of disulfide bridged, oxidized cysteinyl peptides. So, we were interested in determining whether there could be a photochemically driven alternative amenable to surface conditions. As lipoic acid is structurally similar to fatty acids and has been previously shown to be photo-reduced with UV light,[2] we characterized the influence of lipoic acid on oxidized cysteinyl peptides. Due to the hydrophobicity of lipoic acid, it can flip across the membrane and reduce the oxidized peptides present in it on irradiation with UV light. The hetero complex of lipoic acid and reduced peptide was one of the products of reaction which indicated the protection of lipoic acid from UV light.

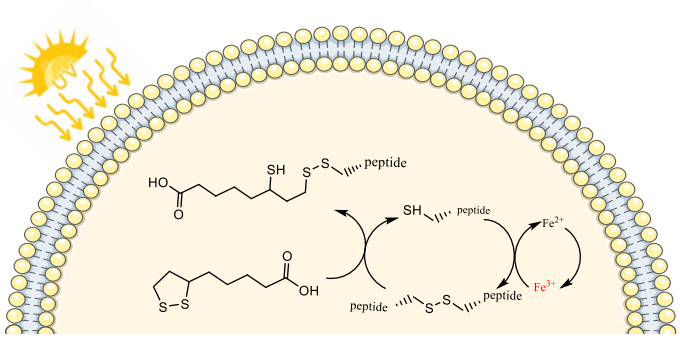

Fig. 1 Schematic representation of reduction of oxidized peptides and thiol-disulfide exchange between peptides and lipoic acid in the membrane on irradiation with UV light of 300 nm.

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Metal/ADP complexes promote phosphorylation of ribonucleotides and ribonucleosides[1]

Emilie Werner,[a] Silvana Pinna,[a] and Joseph Moran[a]

[a] *Institut de Science et d'Ingénierie Supramoléculaires, Université de Strasbourg & CNRS (UMR 7006), 8 Allée Gaspard Monge 67000 Strasbourg, France*

emilie.werner@etu.unistra.fr

Keywords: phosphorylation • adenine derivatives • catalysis • feedback • metabolism

Adenine derivatives are precursors to essential biomolecules such as cofactors and coenzymes. Famously, adenosine 5'-triphosphate (ATP), also known as life's universal energy currency, plays a central role in biochemistry by promoting polymerisation and phosphorylation reactions. However, it is unclear why and how adenine derivatives became central to metabolism. One hypothesis is that adenine derivatives were chemically best suited to promoting phosphoryl transfer. In this communication, we show that adenosine-5'-diphosphate (ADP) enables the non-enzymatic phosphoryl transfer from acetyl phosphate to other ribonucleotides and ribonucleosides in the presence of Fe^{III} or Al^{III} at room temperature in water. No other nucleoside diphosphates were found to promote the reaction. This work demonstrates how biomolecules can feed back into metabolic pathways, thereby strengthening or enlarging the chemical network. This system may represent an intermediary evolutionary stage between substrate-level[2-5] and enzymatic phosphorylation[6] (**Fig. 1**).

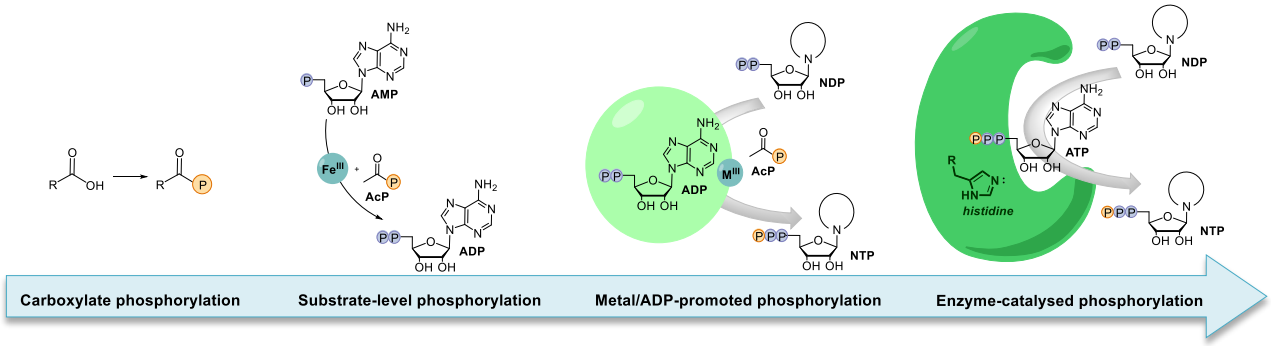

Fig 1. A plausible evolution of phosphorylation in protometabolism.

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On the evolutionary role of metabolism

Robert Pascal,[a] and Addy Pross[b]

[a] *PIIM and Institut Origines, Aix-Marseille Université, Marseille, France*
[b] *Department of chemistry, Ben-Gurion University of the Negev, Be'er-Sheva, Israel*
robert.pascal@univ-amu.fr

Keywords: Origin of life • Metabolism • Evolution • Irreversibility • Dynamic Kinetic Stability

The dissipation of the energy of a source into a sink must be used by self-organizing entities to compensate for the entropic cost of their growth in order to avoid a violation of the Second Law. Consistently with the views that the role of metabolism must not be limited to anabolism in an origin of life context[1] and that the origin of life should include at least a minimal version of it,[2] new analyses will be presented on the contribution of the metabolism in coupling dissipation with development. Autocatalytic systems constituted of a cyclic arrangement involving unstable chemical intermediates are capable of coupling growth with dissipation. In spite of their lack of variability these systems can mimic essential features of life: they can grow in a far-from-equilibrium state, be transmitted and, importantly, become extinct (in biological terms, they can die). Their peculiarity lies in the fact that the whole system is in a state of Dynamic Kinetic Stability (DKS) rather than selected intermediates. As soon as these systems are established in a DKS state, the direction of evolution is determined towards an increase in DKS.[3] By making a whole range of thermodynamically unstable species available for the process, they substantially widen the chemical space for the self-organization of life. Constraints about kinetics and irreversibility will be analyzed. The mere presence of such autonomous systems constitutes a transmissible factor implying that the whole system is in a replicative DKS state and that its evolution is ruled by kinetic selection. This property could be shared by covalent conjugates or supramolecular aggregates (coacervates or vesicles) to which such systems may become associated, therefore introducing the possibility of genetic variability and open-ended evolution.[4] This approach supports the hypothesis that the introduction of a process enabling kinetic selection took place before the emergence of template-replicated polymers brought about variability, therefore making the latter much less unlikely.

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Programming catalytic and replication functions with minimal nucleobase sequences

Andrés de la Escosura,[a, b] Sonia Vela-Gallego,[a] Marcos Sanz [a] and Alonso Puente [a]

[a] *Department of Organic Chemistry, Universidad Autónoma de Madrid, 28049 Madrid, Spain*
[b] *Institute for Advanced Research in Chemistry (IAdChem), Cantoblanco, 28049 Madrid, Spain*
andres.delaescosura@uam.es

Key words: systems chemistry • supramolecular catalysis • replication networks • nucleobases

The study of complex molecular networks and supramolecular assemblies is a clear objective in the field of systems chemistry, which is expected to have a great impact in the area of origins-of-life research and as biohybrid functional systems in materials science [2,3]. With regards to the origins of life, a pertinent question is whether protocells cells could be constructed from non-natural components. To answer this question, we have research lines towards synthetic minimal nucleobase sequences [3], replication networks [4] and nucleolipid compartments [5]. Merging these components is an interesting approach because it allows exploring some properties of life without the restrictions of the historical pathway that Darwinian evolution took. In this respect, in the talk I will present results on how self-assembly processes and replication networks can be programmed with minimal nucleobase sequences in hybrid synthetic molecules built from simple biological building blocks (Figure 1). Particular attention will be paid to the implementation of catalytic function in those assemblies [6], in an effort to merge in the same type of systems catalysis, replication and compartmentalization.

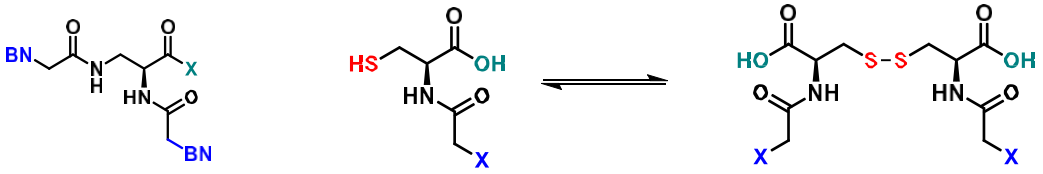

Fig 1. Types of structures developed to explore catalytic and replication functions in chemical networks and supramolecular assemblies. **X** can be a nucleobase or a lipid chain.

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Chiral selective self-replicators

Shuo Yang, Yannick Geiger, Marc Geerts, Marcel J. Eleveld, Armin Kiani and Sijbren Otto

Univ. of Groningen, Stratingh Institute for Chemistry, Nijenborgh 4, 9747 AG Groningen, Netherlands
y.geiger@rug.nl

Keywords: origins of biological homochirality • self-replication • supramolecular self-assemblies • systems chemistry

One of the great mysteries of nature is the origin of biological homochirality, i. e. how life came to be made of chiral building blocks (sugars and amino acids) that exist only as one of the two possible handednesses.[1] Much effort has been devoted in finding ways to amplify an initial chiral imbalance or even to break chiral symmetry.[1] However, only few studies[2] deal with chiral selectivity in self-replicating molecules, which are a key part of early chemical evolution.[3]

In this study, we report several self-replicating systems that show chiral selectivity using dynamic combinatorial libraries.[4] The systems are made of simple peptide building blocks (Fig. 1) that spontaneously oxidize to form macrocycles. These further assemble into fibres that exhibit exponential growth. We found specific macrocycle sizes, such as pentamers, to give chiral selective fibres: they don't emerge spontaneously from a racemic mixture and grow, upon seeding, only from same-chirality material – with an accumulated error of only 10% when seeded in racemic material (Fig. 1). The reason for pentamer replicators being different from e. g. hexamers was investigated via molecular dynamics calculations and comparison with reference systems: the pentamers probably can adopt only a chirality-sensitive conformation ("Cartwheel"), while hexamers can adopt a more forgiving structure ("Pairwise"). This shows how conformational subtleties can have great influence on supramolecular systems.

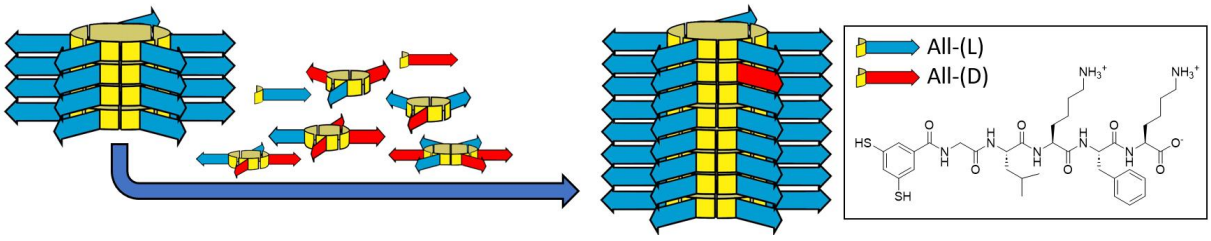

Fig 1. Enantiopure pentamer fibres grow with high chiral selectivity from racemic material.

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Chemical origin of lipids and protocells in the primeval ocean

Nathalie Katsonis

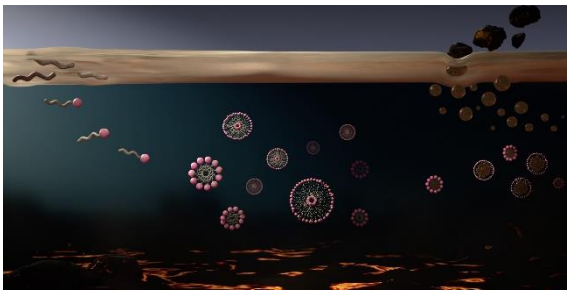
Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, Groningen, The Netherlands,
www.katsonis.eu
n.h.katsonis@rug.nl

Keywords: protocells ● systems chemistry ● light ● prebiotic chemistry ● motility

The early Earth was covered in water. For life to emerge, simple molecules must have concentrated in primitive compartments, commonly referred to as protocells.[1] It is commonly accepted that protocells self-assembled from simple lipids.[2] However, where these lipids came from, and how they self-assembled spontaneously into primitive compartments, remains a blind spot in our understanding of chemical evolution and the origin of life.

Building on geological evidence that an oil slick of hydrocarbons covered the primordial ocean, I propose that the irradiation of the primeval oil slick by the sun produced simple lipids and compartments, with overarching consequences on the emerging chemical complexity of system Earth.[3] Here, I will focus on presenting our recent progress in researching formation and self-assembly of lipids by photo-oxidation of a prebiotically plausible oil slick.

We hope that this work will make an original contribution to prebiotic chemistry, with the results also delivering new insights into the degradation of oil spills in the environment.



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Investigation of peroxidase-like catalytic activity of ferric heme *b* in aqueous micellar systems

Nemanja Cvjetan[a,b] and Peter Walde[a]

[a] *Department of Materials, ETH Zürich, Vladimir-Prelog-Weg 5, 8093 Zürich, Switzerland*
[b] *Current address: Department of Chemistry, University of Alberta, Edmonton, Canada*

cvjetan@ualberta.ca

Keywords: micelles • hemin • peroxidase • catalysis

It is hypothesized that prior to the emergence of life, enzymes, due to their complexity, did not exist. Molecularly "simpler" species probably catalyzed reactions instead of enzymes. Due to its presence in almost all living species, where it serves as a prosthetic group in contemporary enzymes, iron(III)protoporphyrin IX (ferric heme *b* = hemin), is proposed to be one of the catalytic species that was operating at the early stages of life formation.[1] In this work, the focus was on the evaluation of the peroxidase-like catalytic activity of ferric heme *b* in aqueous micellar systems.[2] Micelles provide a local environment different from the bulk, and in that way serve as a cavity for ferric heme *b*, conceptually similar to how apoproteins of heme peroxidases provide an environment for ferric heme *b* in contemporary enzymes.[1] The ability of ferric heme *b* to catalyze one-electron oxidations in the presence of micelle-forming surfactants with hydrogen peroxide as the oxidant was evaluated. Conditions were found under which selected reactions with model substrates can proceed efficiently.[2]

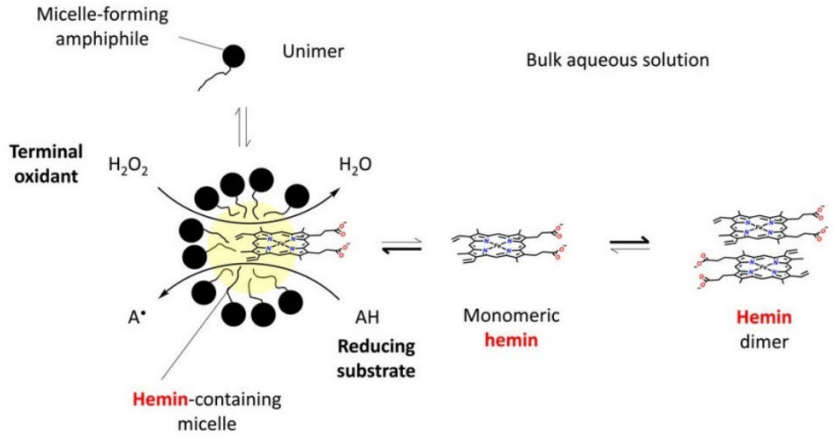

Fig 1. Schematic representation of a micellar system containing ferric heme *b* for catalyzing the oxidation of the substrate AH with hydrogen peroxide as oxidant.[1]

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On the prebiotic origins of regulation: the problem of minimal metabolic robustness

Nino Lauber,[a,b,c] Leonardo Bich,[c] Alvaro Moreno [b] and Kepa Ruiz-Mirazo [a,c]

[a] *Biofisika Institute (CSIC, UPV/EHU), Leioa, Bizkaia, Spain.*
[b] *Donostia International Physics Centre (DIPC), San Sebastián-Donostia, Gipuzkoa, Spain*
[c] *D. Philosophy, University of the Basque Country, San Sebastián-Donostia, Gipuzkoa, Spain*
alvaro.moreno@ehu.eus

Keywords: Biological regulation ● minimal metabolism ● prebiotic evolution ● protocell agency ● dynamic decoupling

In this contribution we will argue that regulatory mechanisms play a fundamental role in the problem of origins of life because they are an organizational requirement for the evolutionary development of increasingly complex protocells. We will discuss the minimal forms of regulation that could appear in compartmentalized proto-metabolic systems, contributing to their dynamic stability, robustness and functional integration, beyond the effects that simpler feedback mechanisms could have also on those aspects. Our line of argument will be based on the characterization of regulation provided by [1], but now applied in the context of protocell systems and, more specifically, to different types of (transport/transduction) mechanisms that might be implemented in the actual boundary --i.e., the lipid membrane-- of those systems. A key question to address will be whether regulation is necessary to bring about *minimal metabolisms* or not. According to [2], a minimal metabolism already involves a non-reducible hierarchical organization of material components and transformation processes in which a functional bootstrapping between synthesis and control relationships is established. The issue under analysis will be whether this is an enabling condition to articulate the first regulatory mechanisms or, instead, the latter are a pre-requisite for the transition from proto-metabolisms to minimal metabolisms. The theoretical implications of the results of our analysis will be discussed -- in particular, with regard to other topics of interest in this field, like the emergence of minimal forms of agency.

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Posters

Successive templated non-enzymatic ligation of short RNAs with high sequence specificity

Adriana Calaça Serrão[a*], Sreekar Wunnava[a*], Avinash Dass[b], Philipp Schwintek[a], Lennard Ufer[a], Lara Anubis[a], Éléonore Moittié[a], Zsófia Meggyesi and Dieter Braun [a]

[a] *Department of Physics, NanoSystems Initiative Munich and Center for Nanoscience, Ludwig-Maximilians-Universität (LMU) München, Amalienstraße 54, 80799 Munich, Germany*
[b] *McMaster University, Department of Physics and Astronomy, 1280 Main Street West Hamilton, Ontario, Canada*
** Equal contribution*
a.serrao@physik.uni-muenchen.de

Keywords: non-enzymatic replication • prebiotic chemistry • cyclic phosphate • RNA ligation

Replication of genetic information is a necessary step for the emergence of life. The 2',3' cyclic phosphate (2',3'>P) is a product of prebiotic phosphorylation pathways and a simple and available activation group for RNA ligation. In our work, we have found that the ligation with 2',3'>P activated oligonucleotides proceeds in alkaline pH 9-11 aqueous solutions with 1mM $MgCl_2$ at temperatures ranging from -20°–25 °C. Under the optimum conditions the reaction yields ~ 25% (7 days, pH 10 and 5°C). Additionally, the formed product is an equimolar mixture of both 2',5' and 3',5' linked oligomers. We expand on previous work by showing both high sequence specificity and successive elongation. One single mutation at the ligation site yields significantly less product (<30%). When using splinted oligomers, we observe up to five successive ligations of both 8 mer and 16 mer, yielding 48 and 96 mer strands, respectively.

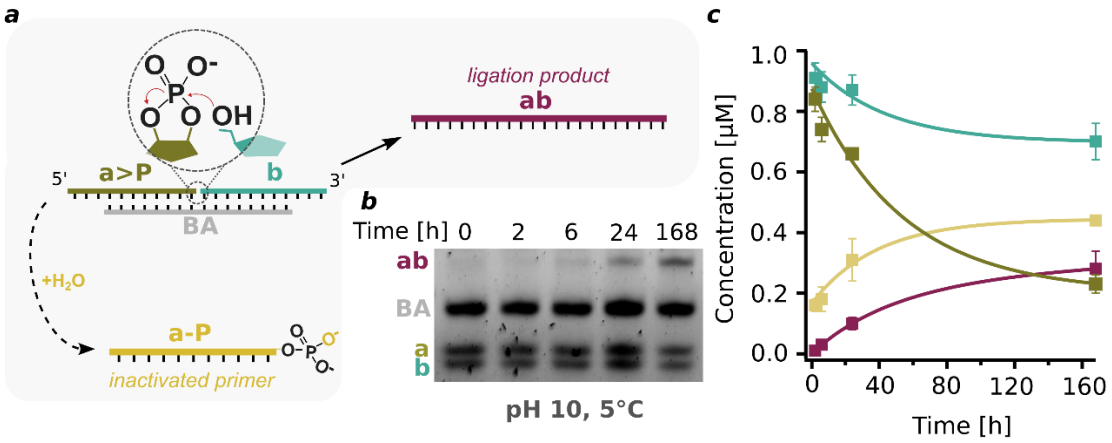

Fig 1. *a*, Schematics of the ligation reaction between the 5'OH and the 2'3'-cyclic phosphate. *b,* PAGE of the reaction at different time points. *c,* Concentration over time of all intervening species.

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In vitro evolution reveals non-cationic protein-RNA interaction mediated by metal ions.

Valerio G. Giacobelli[a], Kosuke Fujishima[b,c], Martin Lepšík[d], Vyacheslav Tretyachenko[a], Tereza Kadavá[e], Mikhail Makarov[a], Lucie Bednárová[d], Petr Novák[f], Klára Hlouchová[a]

[a] *Department of Cell Biology, Faculty of Science, Charles University, BIOCEV, Prague, Czech Republic, 12800.*

[b] *Earth-Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan, 1528550*

[c] *Graduate School of Media and Governance, Keio University, Fujisawa, Japan, 2520882.*

[d] *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic, 16610.*

[e] *Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic, 12800.*

[f] *Institute of Microbiology, The Czech Academy of Sciences, Vestec, Czech Republic, 25250.*

giacobev@natur.cuni.cz

Keywords: RNA-protein interaction • genetic code evolution • protein evolution • metal ions interaction • mRNA-display

RNA-protein interactions have been of utmost importance to life since its earliest forms, reaching even before the last universal common ancestor (LUCA). However, the ancient molecular mechanisms behind this key biological interaction remain enigmatic because extant RNA-protein interactions rely heavily on positively charged and aromatic amino acids that were absent (or heavily under-represented) in the early pre-LUCA evolutionary period. Here, an RNA-binding variant of the ribosomal uL11 C-terminal domain was selected from a ~10^{10} library of partially randomized sequences, all composed of 10 prebiotically plausible canonical amino acids. The selected variant binds to the cognate RNA with a similar overall affinity although it is less structured in the unbound form than the wild-type protein domain. The variant complex association and dissociation are both slower than for the wild-type, implying different mechanistic processes involved. The profile of the wildtype and mutant complex stabilities along with MD simulations uncover qualitative differences in the interaction modes. In the absence of positively charged and aromatic residues, the mutant uL11 domain uses ion bridging (K^+/Mg^{2+}) interactions between the RNA sugarphosphate backbone and glutamic acid residues as an alternative source of stabilization. This study presents experimental support to provide a new perspective on how early protein-RNA interactions evolved, where the lack of aromatic/basic residues may have been compensated by acidic residues plus metal ions.

“Synthesis of RNA building-blocks from prebiotic substances via heterogeneous catalysis and photocatalysis”

Shoval Gilboa,[a] and Yaron Paz [a]

[a] *Department of Chemical Engineering, Technion-Israel Institute for Technology, Haifa, Israel*
shovalgilboa@campus.technion.ac.il

Keywords: RNA Building-blocks • Heterogeneous catalysis • Non-enzymatic • One-pot reaction • Nucleotides

Throughout the years, many theories have been suggested on how life first emerged. The RNA world hypothesis suggests that in the primordial world simple molecules were formed under appropriate conditions, and then increased their complexity to form nucleotides, and eventually chains of RNA. The RNA molecule has an essential role in life; on the one hand, it can be self-replicated, allowing for genetic propagation. On the other, it functions as a catalyst in more complex reactions, such as protein synthesis.

Finding the suitable conditions for the formation of RNA building blocks, without enzyme intervention and in a one-pot reaction, is a big challenge in contemporary research. This study investigates the abiotic and non-enzymatic formation of RNA building blocks from the simple molecule Formamide. Different reaction conditions and their effect on the resulting products were analyzed, including various catalysts, reaction temperatures, illumination, and reaction duration, Fig 1. Furthermore, the adsorption behavior of several biological building blocks was studied to better understand and optimize the reaction mechanism.

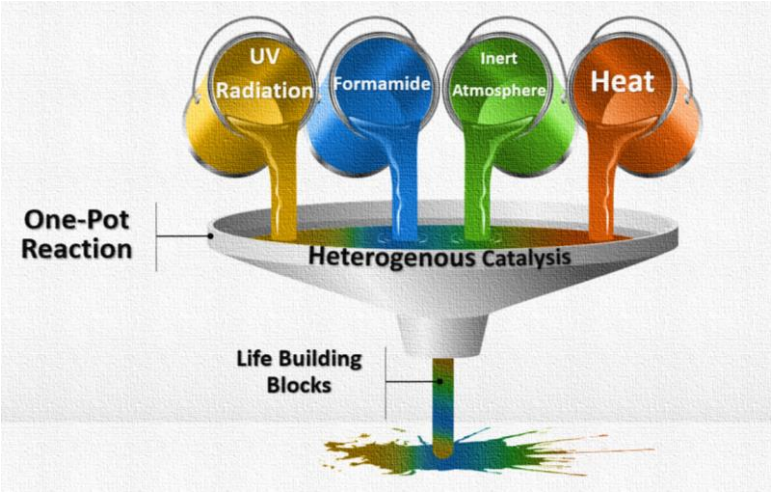

Fig 1. Our approach for the formation of life building blocks in primordial world

Phosphate minerals (PM) have been used as catalysts for the formation of RNA building blocks. It was found that these minerals, under heating, UV-irradiation and an inert atmosphere, promoted the formation of nucleotides and nucleotide derivatives, in a one-pot reaction. This finding may shed light on the formation of primitive RNA chains in the primordial world.

Functional role of oligopeptides under clay mineral influence

Daniel Santamaría [a] and Andrés de la Escosura [b]

[a] *Geology and Geochemistry Department, Universidad Autónoma de Madrid, Cantoblanco 28049 Madrid, Spain*
[b] *Organic Chemistry Department, Universidad Autónoma de Madrid, Cantoblanco 28049 Madrid, Spain*
Presenting: daniel.g.santamaria@uam.es

Keywords: functional oligopeptides • clay minerals • coevolution • catalysis • self-assembly

In this poster, we will discuss the point of transition from prebiotic geochemistry and organic chemistry to biochemistry. This subject is underpinned by the concept that functional biomolecules such as oligopeptides should have interacted and coevolved with rock silicates. Our interest is mainly focused on di- and tripeptides with the potential to self-assemble and/or catalyze reactions of interest. It has been proposed that functional short peptides could have been precursors of larger structures with folded and flexible domains.[1] On the other hand, silicates must have been present in our planet since its initial stages, as they were widely distributed in planetesimals, and so they like played an important role in chemical evolution and the origins of life.[2] In this context, a new research program in our lab stands on the hypothesis that clay mineral surfaces can induce large physical and chemical effects on oligopeptides, and such interactions could give rise to structures with new functionalities relevant to the emergence of life. As a first step to address this hypothesis we propose to make interact nanoparticles of clay minerals with different catalytic and self-assembling di- and tripeptides, to study the adsorption isotherms, their supramolecular behaviour and the emergence of complex catalytic functions (Fig. 1).

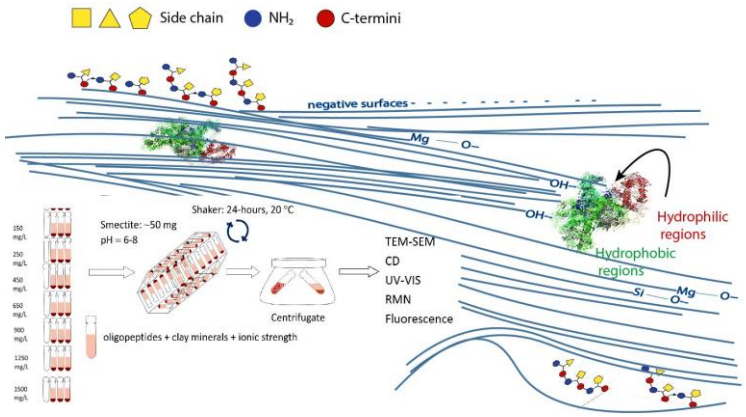

Fig 1. Scheme showing our research approach to study functional peptides interaction with clays.

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Catalytic RNAs and their role in an RNA-peptide-world

Kathrin Halter, Felix Müller, Ewa Węgrzyn, Dr. Ivana Mejdrova, Prof. Dr. Thomas Carell

LMU München, Institute of Chemical Epigenetics, Würmtalstr. 201, D-81377 München
kathrin.halter@lmu.de

Keywords: RNA-peptide world • RNA catalysis • flexizyme • hammerhead ribozyme

Ribonucleic acids (RNAs) play a key role in the theories about the origin of life. They function both as carriers of information and catalysts in reactions which puts them in the spotlight of these theories. Considering a complex mixture of the early prebiotic soup and the presence of amino acid-modified nucleotides found in contemporary transfer-RNAs (tRNAs), considered as molecular fossils, we investigate a putative RNA-peptide world as a complementing concept to the RNA-world[1]. Recently RNA-templated amino acid transfer between two complementary strands containing the modified RNA nucleobases (m)nm⁵U and (m⁶)aa⁶A and peptide chain elongation could be demonstrated in our group[2]. The increased chemical space of RNA modified by amino acids could have been a driving force to evolution. Moreover, catalytically active RNA ribozymes raise special interest in the prebiotic context. It has been shown that there exist small aminoacylating ribozymes recognizing the tRNA 3'- terminal CCA motif and that this concept can be applied to aminoacylate shorter oligonucleotides[3]. To expand the way towards a more complex ribosomal translation machinery, the combination of the templated peptide synthesis with amino acid loading by ribozymes is under investigation. Furthermore, it is of interest what structural role ribozymes played in prebiotic catalysis during evolution in an RNA-peptide world. Therefore, the hammerhead ribozyme is used to screen the effect of non-canonical motifs on catalytic efficiency.

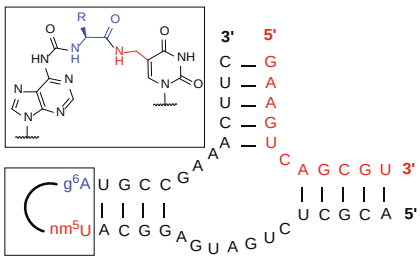

Fig 1. Modified hammerhead ribozyme: The effect on catalytic activity and efficiency by hairpin formation between the two non-canonical nucleobases nm⁵U and g⁶A is investigated.

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White and green rust chimneys accumulate RNA in a ferruginous chemical garden

Vanessa Helmbrecht,[a] Maximilian Weingart,[b] Frieder Klein,[c] Dieter Braun,[b] and William D. Orsi,[a,d]

[a] *Address 1 (Department for Geo- and Environmental Sciences, Ludwig-Maximilians-Universität, 80333 Munich, Germany)*
[b] *Address 2 (Systems Biophysics, Ludwig-Maximilians-Universität, 80799 Munich, Germany)*
[c] *Address 3 (Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, MA 02543, USA)*
[d] *Address 4 (GeoBio-Center^{LMU}, Ludwig-Maximilians-Universität, 80333 Munich, Germany)*
v.helmbrecht@lrz.uni-muenchen.de

Keywords: emergence of life • alkaline vents • RNA accumulation • white rust • green rust

Mechanisms of nucleic acid accumulation were likely critical to life's emergence in the ferruginous oceans of the early Earth. How exactly prebiotic geological settings accumulated nucleic acids from dilute aqueous solutions, is poorly understood. As a possible solution to this concentration problem[1], we simulated the conditions of prebiotic low-temperature alkaline hydrothermal vents in co-precipitation experiments to investigate the potential of ferruginous chemical gardens to accumulate nucleic acids via sorption. The injection of an alkaline solution into an artificial ferruginous solution under anoxic conditions (O_2 <0.01% of present atmospheric levels) and at ambient temperatures, caused the precipitation of amakinite ("white rust"), which quickly converted to chloride-containing fougerite ("green rust"). RNA was only extractable from the ferruginous solution in the presence of a phosphate buffer, suggesting RNA in solution was bound to Fe^{2+} ions. During chimney formation, this iron-bound RNA rapidly accumulated in the white and green rust chimney structure, as it was depleted from the surrounding solution. Our findings reveal that in the oceans of the early Earth, white and green rust chimneys were likely key geochemical features that can rapidly sequester and accumulate RNA[2]. This represents a new mechanism for nucleic acid accumulation, in addition to wet dry cycles, and may have promoted RNA survival in a dilute prebiotic ocean.

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Selective phosphorylation to 2',3'-cyclic phosphate nucleotides and their subsequent polymerization

Juliette Langlais,[a] Nikolas Wetzel [a] and Dieter Braun [a]

[a] *Physics, LMU Munich, Geschwister-Scholl-Platz 1, D-80539 München, Germany*
Juliette.langlais@physik.uni-muenchen.de

Keywords: Phosphorylation • RNA • Polymerisation • Trimetaphosphate • Prebiotic

The phosphorylation of nucleosides into nucleotides and their further polymerization into RNA oligomers are significant steps for the emergence of life. 2',3'-cyclic phosphate mononucleotides have been shown to polymerize in alkaline drying conditions. Among the canonical nucleobases, G has the highest efficiency toward polymerisation. 2',3'-cyclic nucleotides have been reported as products of prebiotic phosphorylation, which makes them interesting reactants for further polymerization.[1]
This study focuses on how to obtain 2',3'-cyclic phosphate nucleotides from phosphorylation reactions, in conditions that also make their polymerisation possible. Under alkaline drying condition and with moderate heating, the reaction of nucleosides with trimetaphosphate produces 2',3'-cyclic nucleotides, as well as short oligonucleotides.

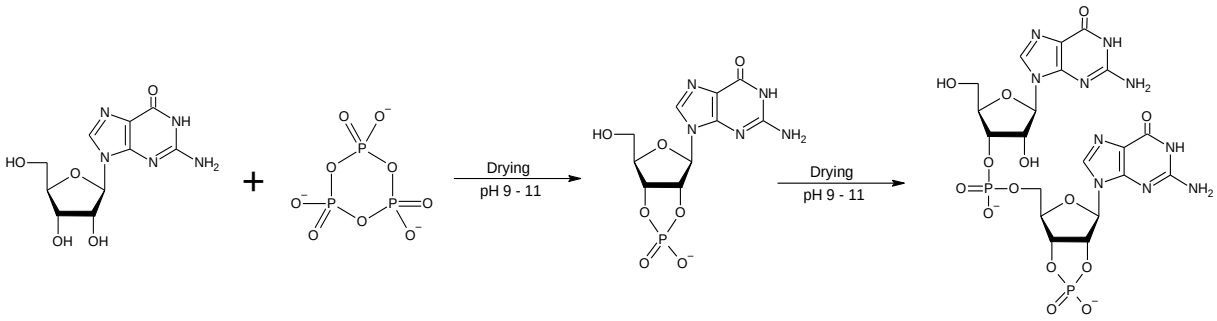

Fig 1. Scheme of the phosphorylation of guanosine nucleosides by trimetaphosphate produces 2',3'-cyclic phosphate nucleotides and their polymerization products in similar conditions.

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Geothermal non-equilibria drive ionic and pH gradients

Thomas Matreux[a], Almuth Schmid[a], Paula Aikkila[a], Kristian Le Vay[b], Bettina Scheu[c], Hannes Mutschler[b], Dieter Braun[a] and Christof B. Mast[a]

[a] *Systems Biophysics, Department of Physics, LMU Munich, Amalienstr. 54, 80799 Munich, Germany*
[b] *Biomimetic systems, TU Dortmund, Germany*
[c] *Earth and Environmental Sciences, LMU Munich, Germany*
th.matreux@physik.lmu.de

Keywords: non-equilibrium • heat flows • phosphate accessibility • ribozyme habitat • thermophoresis

Rocks and their constituent phases likely played an essential role as molecular feedstock during the emergence of life on earth. We aim to combine this geological scenario with physical non-equilibria such as thermal gradients, offering unique opportunities for molecular selection.

Prebiotic reactions often require a defined set of ion concentrations. One example is the activity of some important RNA enzymes that vanishes without divalent magnesium salt, whereas an excess of monovalent sodium salt reduces enzyme function. However, leaching experiments show that relevant geomaterials such as basalts release mainly sodium and only little magnesium. A ubiquitous non-equilibrium solution to this problem are heat flows through thin rock fractures, driving thermogravitational convection and solute thermophoresis. The superposition of both effects actively enriches magnesium ions against sodium and establishes a habitat for ribozyme function from basaltic leachates[1]. The process plausibly occurs within systems of connected rock cracks, which increases the strength and stability of the selective accumulation. Interestingly, thermal gradients also lead to the formation of pH gradients in mixtures of only formic acid and sodium hydroxide, which can be understood and predicted by a separation of timescales[2].

While phosphate is essential to all life, its prebiotic accessibility poses major problems. For instance, one of the most abundant phosphate minerals on the early Earth, Apatite, is insoluble at the neutral and alkaline pH values compatible with nascent life. We show that heat flows can spatially separate the constituents of Apatite, phosphate and calcium, under acidic conditions. The neutralization of the phosphate-enriched and calcium depleted fraction yields considerable amounts of free phosphate. The resulting concentrations are sufficient to form more reactive phosphate species such as trimetaphosphate upon heating. Using a variety of minerals, glasses, and clays, we show which surfaces can promote such reactions.

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Enhanced RNA polymerization by amino acids in a dry state

Saroj K. Rout,[a] Sreekar Wunnava [a] and Dieter Braun[a]

[a] *Systems Biophysics, Department of Physics, Ludwig Maximilian University Munich Geschwister-Scholl-Platz 1, 80539 Munich, Germany*
s.rout@physik.uni-muenchen.de

Keywords: Origin of life • RNA • 2',3' cyclic phosphate ribonucleotide • Amino acid

The prebiotic origin of molecular complexity requires various components to develop synergistic relationships[1]. While RNA needs to be sufficiently long to initiate a function like replicative cycles[2], prebiotic RNA syntheses are essentially random and inefficient polymerizations of nucleotides. Here, we explore how simple amino acids satisfy a coevolutionary scenario to alter the reaction kinetics and increase the yield and selectivity of RNA syntheses under dry conditions. Quantitative LC-MS analyses of products from a polymerization reaction of 2', 3'-cNMP (N= A, U, C, G) in the presence of amino acids in a 5:1 mole ratio at pH 10 reveal that amino acids promote RNA polymerization, with different relative yields for nucleobases; C>A>U>G. The RNA polymerization correlates with the hydrophobicity of the amino acid sidechains; Leu/Ile/Val>Ala>Gly>Lys>Asp. The lesser relative yield for cGMP results from an efficient inherent polymerization without amino acids[3]; cCMP polymerization, in contrast, is strongly increased with amino acids to over 100-fold. Thus, in a reaction of both cGMP and cCMP, the disparity in their polymerization efficiency is suppressed by amino acids to produce a better distribution of mixed sequences with an increased yield. Similar results are also obtained with cAMP+cUMP polymerization, which may be essential for downstream evolution[4].

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Cyclic isothermal strand separation driven by air flux across open pore

Philipp Schwintek[a] and Dieter Braun [a]

[a] *Systems Biophysics, Ludwig Maximilian University Munich,, Amalienstraße 54, 80799 München, Germany*

Philipp.schwintek@physik.uni-muenchen.de

Keywords: isothermal • FRET • oligonucleotides • evaporation • accumulation

The evolution of oligonucleotides faced many challenges on early earth. Chemical reactions require high concentrations and RNA replication requires strand separation. Usually physical non-equilibria, such as steep temperature gradients[1], are required to solve these issues. However, gradients of multiple kelvins across micrometer sized pores are rare. In this work, we show that at isothermal conditions, a gas flux across an open pore can periodically separate oligonucleotide strands, thus enabling replication reactions: Molecules accumulate against a diluting water flux and are continuously cycled through salt gradients. FRET measurements reveal periodic melting of DNA. We deployed finite elements simulations and show that they agree with our fluorescent measurements.

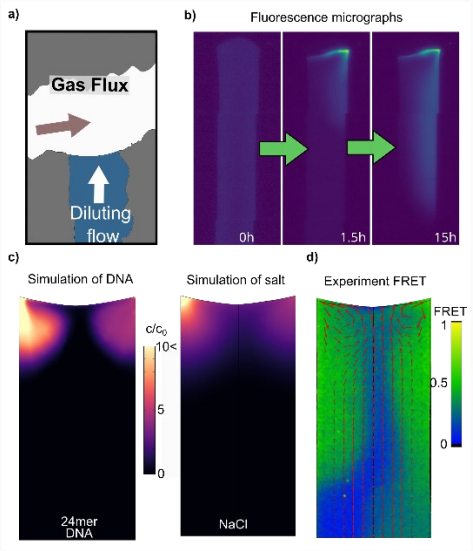

Fig 1. a) Geological setting of a gas flux across an open pore on early earth. **b)** Fluorescent micrographs of 16mer DNA showing accumulation at the interface over time. **c)** Finite element simulation of DNA as well as salt accumulating at the water air interface. **d)** FRET measurement of a 24mer DNA overlayed with the simulated flow pattern (red).

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Interplay between abiotic organic molecules and minerals in aqueous environments: a key for prebiotic chemistry ?

Vinogradoff V. [a], Chevrier V.[b], Meinert C.[c], Grauby O.[d], Rimola A.[e], Mates-Torres E.[e], Danger. G.[a] and Pascal R[a].

[a] *CNRS, Aix-Marseille University, Institut Origines, PIIM UMR 7345, Marseille, France*
[b] *Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR 72701, USA*
[c] *Université Côte d'Azur, ICN, UMR CNRS 7272, Nice, France*
[d] Aix-Marseille Université, CINAM UMR-CNRS 7325, Marseille, France
[e] Universitat Autònoma de Barcelona (UAB), Departament de Química, Bellaterra, Spain
vassilissa.vinogradoff@univ-amu.fr

Keywords: astrochemistry • organic-mineral interactions • geochemistry • sugars

Mineral and organic matter co-exist in many natural systems and are so closely mixed in terrestrial sediments and hydrothermal environments that their mutual interplay is challenging to understand [1]. Together, they exhibit new physicochemical properties due to their interactions. For that reason, organic-mineral interactions are often thought as primordial systems for prebiotic chemistry [2].

Our approach is to investigate such interactions in aqueous conditions applied to Solar System objects, assuming as starting point abiotic common organic matter in the presence of some of the earliest available minerals of the Solar System. Through two examples, we will present the high interest of studying such modest systems in laboratory, which was barely investigated before. In the first system, we highlight the influence of abiotic organic molecules (benzyl compounds), common in many environments, which modify the nature and structure of newly formed phyllosilicates derived from common igneous minerals (olivine and feldspar) [3]. In the second system, we will present for the first time the capability of low-temperature serpentinization to initiate and catalyse the Formose reaction from formaldehyde only, likely one of the most important reactions to produce sugar compounds for prebiotic chemistry [4]. In both cases, the synthetic analogues produced are composed of organic compounds and hydrated minerals thoroughly mixed, a cohesion that could not be reproduced by mechanically mixing components together. Our possible early Earth analogs (and of others planetary environments-Mars) can hence reproduce the first stage of organic matter complexification with minerals for incorporation to prebiotic systems. Our results justify the necessity to address the question of the mineral (omnipresent at the surface of the early Earth) in this chemical organization, not only as a catalyzer, substrate or compartment, but also as a real reactant in the process.

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Quasi-2D microfluidic alkaline vent model to study mineral precipitation, gradient formation and particle accumulation

Maximilian Weingart,[a] Siyu Chen[b], Clara Donat[b], Vanessa Helmbrecht[c], William D. Orsi[c], Dieter Braun[a], Karen Alim[b]

[a] *Systems Biophysics, Ludwig-Maximilians University Munich, Amalienstraße 54, 80799 München, Germany*
[b] *CPA and Department of Bioscience, Technical University Munich, Ernst-Otto-Fischer-Straße 8, 85748 Garching b. München, Germany*
[c] *Department of Earth and Environmental Sciences, Ludwig-Maximilians University Munich, Richard-Wagner Straße 10, 80333 München, Germany*
m.weingart@physik.uni-muenchen.de

Keywords: alkaline vents • pH gradients • mineral precipitation • concentration problem • origin of life

Alkaline vents facilitate the precipitation of warm, alkaline fluids exhaled into slightly acidic ocean water, thus providing the necessary gradients to drive molecular reactions at the origins of life. The 3D chimney-like structure of the precipitates, however, prevented any visualisation and testing of potentially reaction fueling gradients to date.
We developed a quasi-2-dimensional microfluidic model of alkaline vents that allows spatio-temporal visualisation of the iron-mineral formation process. To simulate the vent conditions an alkaline fluid is injected into an acidic, Fe(II)-rich solution inside a thin chamber of 500µm thickness. Upon contact of the fluids, we observe a diverse set of precipitatation morphologies, mainly influinced by flow-rate and ion-concentration of the fluids. Using microscope imaging and pH dependent dyes, we show that disordered, finger-like precipitates can facilitate formation and maintenance of pH gradients on the microscale and accumulation of dispersed particles in confined geometries.
Our model is established to investigate the potential of microscale gradients across a semi-permeable boundary for early compartmentalisation, accumulation and chemical reactions at the origins of life.

Understanding the polymerization of 2′,3′-cyclic nucleotides: templation, chirality and the role of minerals.

Sreekar Wunnava[a], Dieter Braun[a]

[a] *Systems Biophysik, Faculty of Physics, LMU Munich, Amalienstrasse 54 80799 Munich, Germany*

s.wunnava@physik.uni-muenchen.de

Keywords: cyclic nucleotides • polymerization• RNA • dry-wet cycles

Generation of RNA from mononucleotides is a crucial step in the emergence of life. Recently we showed that 2′,3′-cyclic nucleotides polymerize under dry conditions starting from an alkaline solution[1]. In the current study, we attempt to explore the role of supra-molecular structure in the polymerization of 2′,3′-cyclic GMP. Apart from improving the reaction yields as compared to our previous work, we took cues from the templated ligation reactions to enhance the polymerization of cCMP on a polyG template. Furthermore, we show that a polymerization mix containing monomers of different chirality, is self-selective i.e. the formation of hetero-chiral oligonucleotides is suppressed. We also explore the role of minerals, such as biotite as prebiotic-buffers on early Earth.

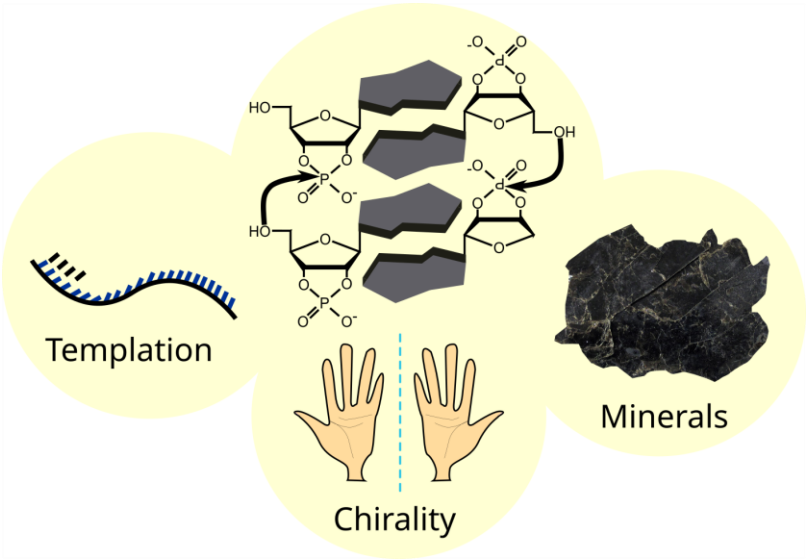

Figure 1. Graphical abstract: Probing the role of supra-molecular arrangement in the polymerization of 2′,3′-cyclic nucleotides.

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Isoxazole Nucleosides as Building Blocks for a Plausible Proto-RNA

Felix Xu [a], Antony Crisp [a],Thea Schinkel [a], Romeo C.A. Dubini [a], Sarah Hübner [a], Sidney Becker [a,b], Florian Schelter [a], Petra Rovó [a,c], and Thomas Carell [a,*]

[a] *Department of Chemistry, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13, 81377 Munich, Germany*
[b] *Current address: Max Planck Institute of Molecular Physiology, Department of Chemical Biology, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany*
[c] *Current address: Institute of Science and Technology Austria (ISTA),*

felix.xu@cup.uni-muenchen.de
Keywords: Prebiotic chemistry • Origin of Life • RNA • Proto-RNA • Isoxazoles

The question of how RNA, as the principal carrier of genetic information evolved is fundamentally important for our understanding of the origin of life. The RNA molecule is far too complex to have formed in one evolutionary step, suggesting that ancestral proto-RNAs (first ancestor of RNA) may have existed,[1] which over time evolved into the RNA of today. Here we show that isoxazole nucleosides, which are quickly formed from hydroxylamine, cyanoacetylene, urea and ribose,[2] are plausible precursors for RNA. The isoxazole nucleoside can rearrange within an RNA-strand to give cytidine, which leads to an increase of pairing stability. If the proto-RNA contains a canonical seed-nucleoside with defined stereochemistry, the seed-nucleoside can control the configuration of the anomeric center that forms during the in-RNA transformation. The results demonstrate that RNA could have emerged from evolutionarily primitive precursor isoxazole ribosides after strand formation.

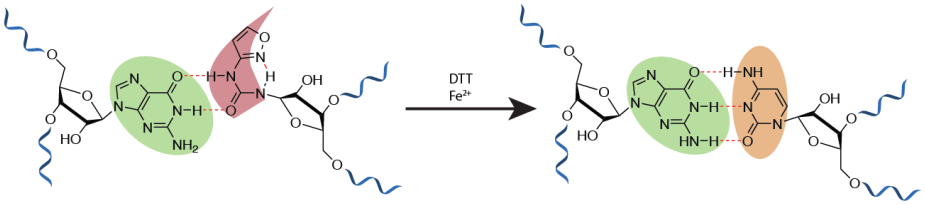

Fig 1. Isoxazole proto-RNA nucleosides, with a stabilizing H-bond, can directly rearrange in RNA to give the C-nucleoside, while amplifying the stereochemical information already fixed in the strand during the reaction.

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Origin of life building blocks under early earth conditions

Nikolai Diukarev [a], Erik Boinowitz [a] and Thomas Carell [a]

[a] *Institute of Chemical Epigenetics, LMU Munich, Würmtalstr. 201, 81377 Munich, Germany*

nikolai.diukarev@cup.uni-muenchen.de
erik.boinowitz@cup.uni-muenchen.de

Keywords: plausible early earth conditions • building blocks of life • oximes • nitriles

Biomolecules such as DNA, RNA and proteins are central to every organism in existence. The first step towards the emergence of life thus required the synthesis of nucleosides and amino acids as the essential building blocks for these biopolymers. To this day, a plausible pathway towards these basic building blocks of life under the conditions which were present on the early earth remains unknown. In the following, we will demonstrate a plausible synthesis of amino acids, pyrimidines and purines from reactive starting material conceivable in an atmosphere containing only N_2, CO_2, SO_2 and H_2O, as was most likely present on the early earth during the time when life first emerged.[1] We report that hydroxylamine and complex aldoximes can serve as important feedstock molecules for the synthesis of the building blocks of life and can thus be considered biological markers for life in the universe.

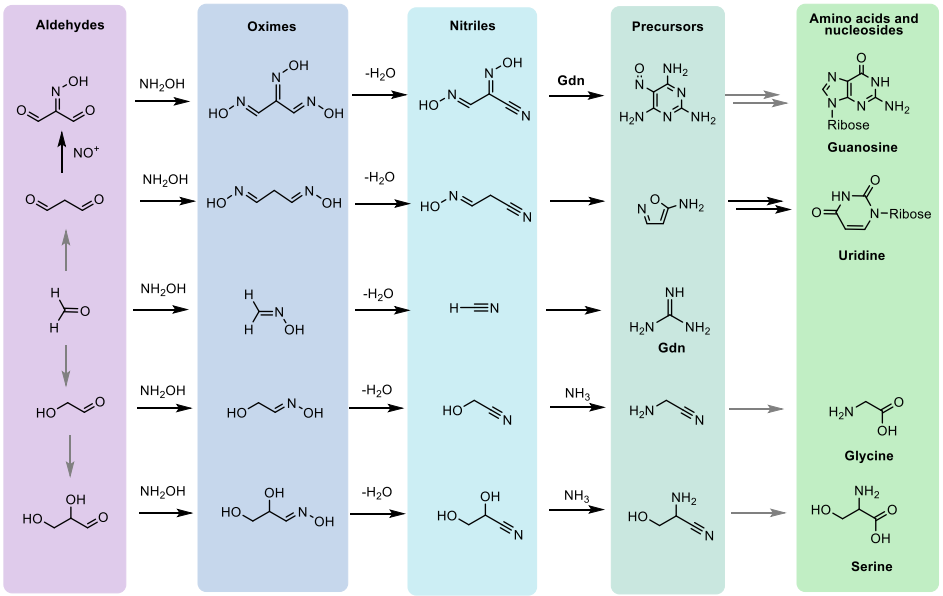

Fig 1. Prebiotically plausible pathway towards amino acids and nucleosides from formaldehyde

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Compression in evolving chemical mixtures

Pau Capera-Aragones[a], Kavita Matange [b,c], Vahab Rajaei [b,c], Loren Dean Williams [b,c] and Moran Frenkel-Pinter [a,b,c]

[a] *Institute of Chemistry, The Hebrew University of Jerusalem, Israel 919004*
[b] *NASA Center for the Origins of Life*
[c] *School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, USA*
Pau.Cap@mail.huji.ac.il

Keywords: Chemical evolution • Dry-wet cycling • Combinatorial compression • Combinatorial explosion • Systems chemistry

Complex or even relatively simple mixtures undergoing chemical transformations tend to combinatorically explode. The rise of chemical selectivity is one of the main challenges in the study of chemical evolution and origins of life. Recent work[1] has shown that at lower temperatures than typically explored, combinatorial compression, i.e., a reduced number of chemicals compared to that expected by combinatorics, is observed. The mechanisms underlying combinatorial compression are yet to be understood. In our work, we used thermodynamic and kinetic theory together with computer simulations to track the evolution of chemical concentrations under a wide range of parameter scenarios. We have now successfully defined a set of rules that are required for combinatorial compression: chemical connectivity, thermodynamic or kinetic dominance, continuous feeding of the compressor, and appropriate temperature or reaction time. Our results shed new light on the way in which chemical evolution operates at the very fundamental level and guide future experiments of chemical evolution towards generation of chemical spaces that can self-maintain high reactivity and endless evolution.

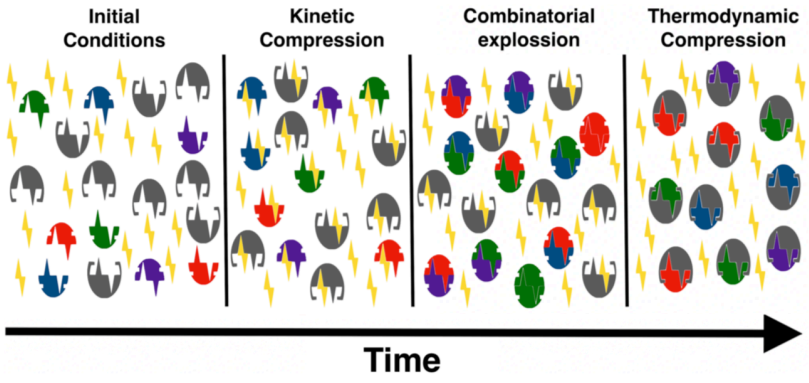

Fig 1. Representation of combinatorial explosion, where nearly all possible combinations of chemicals occur, and combinatorial compression, where only few combinations of chemicals are present. Two types of compression are showed: Kinetic which occur at short times due to the kinetic dominance, and thermodynamic which occur at long times due to thermodynamic dominance of a chemical.

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Emergence of structure in DNA replication from biased short oligomers

Felix Dänekamp*[a], Adriana Calaça Serrão*[a], Zsófia Meggyesi[a] and Dieter Braun[a]

[a] *System Biophysics, Department of Physics, LMU Munich, Amalienstr. 54, 80799 Munich, Germany*
f.daenekamp@physik.uni-muenchen.de

Keywords: template • polymerization • emergence • DNA • sequencing

The replication of short oligonucleotides leading to longer polymers is a central step in the origin of nucleic acids. We studied how the sequence landscape of biased binary DNA oligomer pools evolves upon templated polymerization with *Bst* strand displacing polymerase. By sequencing different time points, we found that after isothermal replication, the initial nucleotide bias of the pool disappears through inversions of bias in the elongated sequences, see fig. 1. At the same time, the average nucleotide present at each position in the elongated sequences remains biased and varies both with position and initial bias. Besides this convergence to overall homogeneous nucleotide composition without loss of dependence on the initial state, we observed the emergence of highly periodic motifs in the sequences that elongated quickly.

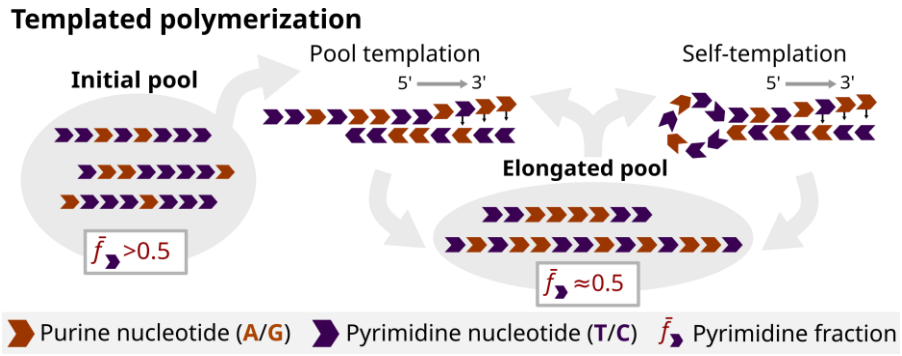

Fig 1. Schematics of templated polymerization mechanisms. Sequences are initially templated by other sequences from the biased initial pool. Once they get long enough, self-templation may occur. Both mechanisms lead to an overall homogeneous nucleotide composition through incorporation of inversely biased nucleotides complementary to the similarly biased template sequences.

Chemical thermodynamics for growing protocells

Atsushi Kamimura,[a] Yuki Sughiyama [a] and Tetsuya J. Kobayashi [a]

[a] *Institute of Industrial Science, The University of Tokyo, Komaba, Meguro-ku, Tokyo 153-8505, Japan*
kamimura@sat.t.u-tokyo.ac.jp

Keywords: Thermodynamics • Protocells • Autocatalytic reactions • Cell growth

Protocellular compartments have commonly been considered as model systems for understanding the origins and evolutions of early cells, as well as designing encapsulated reactors for biotechnology. As a prototypical setup, we generally examine open chemical reaction systems (CRSs), wherein autocatalytic chemical reactions are encapsulated within a volume that can change in size in conjunction with the reactions. The thermodynamics of such CRSs is crucial for elucidating the physical conditions required for their growth.

In this study, we establish a thermodynamic theory for growing CRSs [1] by extending the geometric structure of non-growing CRSs [2]. It provides environmental conditions to determine the fate of the CRSs, whether they will grow, shrink, or reach equilibrium. We also identify thermodynamic constraints: one that restricts the possible states of the CRSs and another that further limits the region where a nonequilibrium steady growing state can exist.

Furthermore, we consider stoichiometric constraints, where the changes in the number of chemicals are constrained according to the stoichiometry of the reactions. Such constraints are typically imposed by the topological features of cellular metabolic networks. As a result, the possible states of the systems are further restricted depending on the initial conditions of the system. Consequently, even in the same environment, the fate of the CRSs can differ based on the initial conditions.

These results are derived from general thermodynamic considerations based solely on the second law of thermodynamics, emphasizing the significant influence of the variable volumes of the system on the reaction dynamics in the growing states.

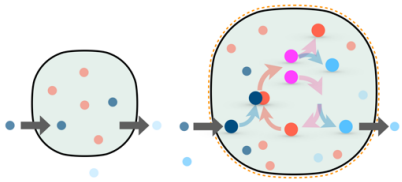

Fig 1. A protocell increases in size in conjunction with autocatalytic chemical reactions.

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A thermodynamic threshold for Darwinian evolution in molecular replicators

Artemy Kolchinsky[a]

[a] *Universitat Pompeu Fabra, Dr Aiguader 88, 08003 Barcelona, Spain*
artemyk@gmail.com

Keywords: nonequilibrium thermodynamics • autocatalysis • evolution

Summary: Understanding the thermodynamics of Darwinian evolution has important implications for biophysics, evolutionary biology, and the study of the origin of life. We show that for autocatalytic replicators in a nonequilibrium steady state, the critical selection coefficient (minimal fitness difference visible to selection) is lower bounded by the Gibbs free energy dissipated per replication event. Our bound applies to a large class of molecular replicators, including many types of autocatalytic sets, polymer-based replicators, and multistep autocatalytic mechanisms. It also applies to various real-world molecular replicators.

Description: Recent work in nonequilibrium thermodynamics has uncovered fundamental bounds on the thermodynamic costs of various biological processes, including chemical sensing, copying of polymer-stored information, and autocatalytic growth and replication. Due to their generality, these bounds shed light on universal thermodynamic properties of life-like systems, including not only modern organisms but also synthetic organisms, protobiological systems, and possible non-terrestrial lifeforms.

One of the most important properties of living systems is that they exhibit *Darwinian evolution*. A population of replicators undergoes Darwinian evolution when replicators with higher fitness outcompete replicators with lower fitness, and thereby come to dominate the population. The ability of higher fitness replicators to outcompete lower fitness ones is not a truism, and generally depends on the fitness difference between replicators as well as various environmental and demographic factors. The strength of Darwinian evolution can be quantified via a bound on the *selection coefficient* s, a measure of relative fitness difference between replicators. In a given population and environment, the minimal selection coefficient which can affect evolutionary outcomes (such as fixation probabilities) represents the "resolution limit" of Darwinian evolution, below which fitness differences are indiscernible. Quantifying critical selection coefficients is a major focus of research in evolutionary biology and origin-of-life studies. Until now, however, there has been no analysis of how the strength of Darwinian evolution depends on the thermodynamic properties of the replicators.

We demonstrate the existence of a *thermodynamic threshold* for Darwinian evolution. We consider a population of autocatalytic replicators in a nonequilibrium steady state. We suppose that selection is sufficiently strong so that some replicator with fitness f is present in steady state, while another replicator with lower fitness $f' < f$ is driven to extinction. Our main result is the inequality $s \geq e^{-\sigma}$, where $s = 1 - f'/f$ is the selection coefficient and σ is the Gibbs free energy dissipated by the fitter replicator (in units of $k_B T$ per copy). σ is a fundamental measure of the "thermodynamic cost" of replication, and it represents dissipated potential for work: a reaction that dissipates σ of Gibbs free energy can be coupled to a thermodynamically disfavored "uphill" reaction, and thereby perform up to σ of chemical work.

Preprint: Kolchinsky, "A thermodynamic threshold for Darwinian evolution", arXiv: 2112.02809

Exploring ‘minimal metabolism’ as a central concept for understanding the origins of life and developing systems biology

Nino Lauber[a,b,c], Daniele De Martino[a,d], Christoph Flamm[e], Kepa Ruiz-Mirazo[a,c]

[a] *Biofisika Institute(CSIC,UPV/EHU), Leioa, Spain*
[b] *Donostia International Physics Center, Donostia-San Sebastian, Spain*
[c] *Department of Philosophy, University of the Basque Country, Donostia-San Sebastian, Spain*
[d] *Ikerbasque Foundation, Bilbao, Spain*
[e] *Institute for Theoretical Chemistry, University of Vienna, Vienna, Austria*
nino.lauber@ehu.eus

Keywords: metabolism ● origins of life● prebiotic-systems chemistry● functional-bootstrapping

For many years, the origins of life field has been the stage for various, at times contrasting, theories that often relied on a certain level of reductionism, focusing on which type of bio-molecule came first. However, in recent years the emergence of systems biology and systems chemistry has lead to a more non-reductionistic approach to the problem of origins of life, that acknowledges the complexity of living systems and rather focuses how life as whole got organized [1,2]. In this context, ‘minimal metabolism’ is formulated here as a construct that stands at the interface between non-equilibrium, complex chemistries and biological systems, with it’s formation being a major transition from the former to the latter [3]. Thus within this project, a tentative scheme for such a minimal metabolic system is provided, that involves at it’s center a functional bootstrapping between synthesis and control [3]. This concept is then further explored through theoretical, computational models. There two main lines of modeling are pursued. On the one hand, complex chemical reaction networks under simple boundary conditions are explored, using a rule-based modeling approach. On the other hand, simple chemical reaction networks under complex boundary conditions are explored, using an Ising-type lattice-model [4,5].

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The role of cofactors in prebiotic chemistry

Noemí Nogal,[a] Javier Luis-Barrera, [a] Sonia Vela [a] & Andrés de la Escosura [a, b]

[a] *Department of Organic Chemistry, Universidad Autónoma de Madrid, 28049 Madrid, Spain*
[b] *Institute for Advanced Research in Chemistry (IAdChem), Cantoblanco, 28049 Madrid, Spain*
javier.luis@uam.es

Keywords: prebiotic chemistry • nicotinamide cofactors • protometabolisms

Nicotinamide adenine dinucleotide (NAD^+), and its reduced form (NADH), is a very important coenzyme involved in multiple redox reactions within the biochemistry of all living cells. The electron transfer reactions where this cofactor is involved are, in most cases, catalyzed by enzymes. In the context of the origins of life, it is interesting to investigate non-enzymatic routes in which this or other cofactor types, or potential prebiotic derivatives, could participate in reactions that are analogous to their metabolic counterparts.[1] Moreover, it is also desirable to gain deeper knowledge about their action mechanisms and degradation pathways in prebiotic environments and absence of enzymes. Some reactions in which NAD^+ or NADH could assist in the establishment of a protometabolism have actually been researched.[2,3]

In this scenario, our research group, with ample experience on prebiotic systems chemistry,[4,5] has recently initiated a research line focused on the study of different transformations that could interconnect reactive species leading to main prebiotic synthetic routes such as the formose reaction, a possibly primordial synthesis of amino acids, and the cyanosulfidic scenario (Scheme 1). Although this line is in its early stage, in the poster we will discuss some recent advances.

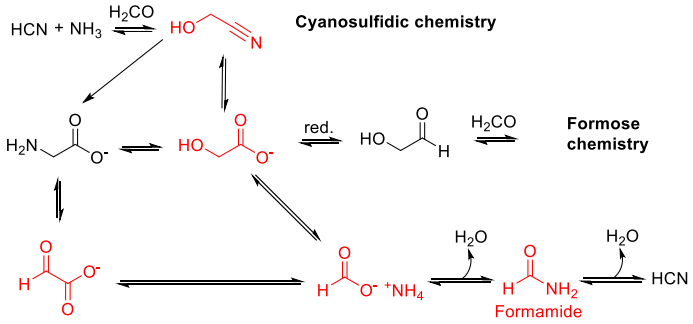

Fig 1. Chemistries that could be affected in one way or another by cofactors.

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Coexistence and non-specific interactions between protocells

Fatma Zohra Mihoubi,[a] and Claudia Bonfio [a]

[a]*University of Strasbourg (Institut de Science et d'Ingénierie Supramoléculaire, 8 allée Gaspard Monge, 67000 Strasbourg France)*

fatma-zohra.mihoubi@etu.unistra.fr

Keywords: protocell • vesicle • coacervate

The emergence of life on Earth likely depended on the formation, and stability, of compartments able to withstand biochemical reactions involving oligonucleotides and peptides. Two models of protocells have appeared as the main contenders for this role: lipid vesicles, composed of prebiotically plausible amphiphiles, such as fatty acids, and coacervates, formed by electrostatic interactions driving liquid-liquid phase separation. Here we discuss the prebiotic conditions required for the coexistence of fatty acid-based vesicles and nucleic acid-peptide coacervates. First, we demonstrate that the key components of coacervates stabilize lipid membranes. Then, we show that electrostatic interactions between primitive biomolecules drive membrane wetting. Our findings pave the way towards tunable transmembrane cargo delivery in mixed populations of coexisting primitive cells.

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Insights into the origins of life: in silico modelling of a ribozyme-constrained metabolism

Joaquin Perez-Grande [a] and Rainer Breitling [a]

[a] *Manchester Institute of Biotechnology, 131 Princess Street, Manchester, M1 7DN, United Kingdom*
joaquin14pg@icloud.com

Keywords: ribozymes • modelling • GEMs • protometabolism • networks

A critical event in the origins of life is thought to have been the emergence of an RNA molecule capable of self-replication and catalyzing reactions, which could have led to the first metabolic networks. Modern ribozymes can be seen as remnants of the molecules that may have participated in self-sustained protometabolic systems, and that were replaced with enzymes in the course of evolution[1]. However, the behavior of ribozymes in metabolic networks and in conjunction with protein-based catalysis it is unclear and hard to test experimentally. In this context, *in silico* metabolic modelling and genome-scale metabolic models (GEMs) offer a systems-level perspective into metabolic processes and networks, and hence could be used to explore the scope, feasibility and limitations of the primordial metabolic systems[2].

In this study, we are taking a computational approach to investigate the role that ribozymes may have played in the origins of metabolism. By comparing and quantifying the metabolic cost of proteins and ribozymes, we find that ribozymes could be seen as less metabolically expensive, which could offset their low catalytic efficiency. This is mainly due to the high costs of protein biosynthesis, which are often overlooked in metabolic modelling and analysis. With this data, we are building a ribozyme-constrained equivalent of the protein-constrained GEMs, in order to gain insights into the metabolic constraints imposed by ribozymes and how these constraints compare to those imposed by protein catalysts[3].

Our work has significant implications for understanding the origins of metabolism. Our novel computational framework describes how ribozymes can be represented in GEMs and demonstrates the conceptual feasibility of a ribozyme-driven metabolic network. Moreover, we show how accounting for biosynthetic costs further constrains the solution space and thus increases the accuracy of metabolic predictions. Overall, this study paves the way for future research on the use of GEMs to explore and characterize metabolic systems in the context of RNA-based catalysis and its role in the origins of life.

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An Open-Source Computational Workflow for the Discovery of Autocatalytic Networks in Abiotic Reactions

Aayush Arya, [a,b] Romulo C. Simbron, [a,c] Siddhant Sharma, [a,d] Alejandro Lozano, [a,e] Jessica Ray, [a] Harrison B. Smith, [f] Jakob L. Andersen, [g] Huan Chen, [h] Markus Meringer, [i] and Henderson J. Cleaves [a,f]

[a] *Blue Marble Space Institute of Science, Seattle, 98104 Washington, United States*
[b] *Johannes Gutenberg-Universität Mainz, 55099 Mainz, Germany*
[c] *Department of Chemistry, University of Colorado Boulder, Boulder, 80309 Colorado, United States*
[d] *Department of Biochemistry, University of Delhi, 110019 New Delhi, India*
[e] *Department of Biomedical Data Science, Stanford University, Stanford, 94305 CA, United States*
[f] *Earth-Life Science Institute, Tokyo Institute of Technology, Ookayama, 152-8550 Tokyo, Japan*
[g] *Department of Mathematics and Computer Science, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark*
[h] *National High MagLab, Florida State University, Tallahassee, 32310 FL, United States*
[i] *German Aerospace Center (DLR), 82234 Oberpfaffenhofen, Wessling, Germany*
siddhaantsharma.ss@gmail.com

Keywords: Molecular Diversity • Prebiotic Chemical Reaction Networks (CRNs) • Autocatalytic Motifs

Complex chemical reaction networks can grow exponentially in terms of the chemical diversity they can generate. It is unknown whether such networks easily discover or shuttle fluxes through autocatalytic sub-networks. Here, we aim to provide a map for experimental chemists studying complex organic reactions by presenting a chemoinformatic workflow to model abiological chemistries by automating the rule-based generation of chemical reaction networks (CRNs) such as the alkaline degradation of glucose, and formose reaction, and their analysis to discover novel compounds and autocatalytic processes. [1] Our pipeline utilizes isomorphism tests to match the output molecular structures to experimentally reported structures as a test of the completeness of our methods. The monoisotopic exact masses of the molecules in the computed CRN product set were calculated, and used to match peaks identified in high-resolution FT-ICR-MS data of the same reaction. We also explored the potential effects of iron ions on generated complex CRNs, and we find that iron ions produce significant changes in the connectivity of various known diversity-generating reaction networks of proposed prebiotic significance. We also looked at the intersection between published libraries of potential prebiotic nucleoside analogues, and the molecular diversity generated in our computed CRNs, and their representations starting from simple prebiotically plausible precursors. This automated, rule-based reaction generation pipeline can enable rapid identification of products of complex prebiotic chemistries, and their underlying synthetic relationships to help identify autocatalysis, and potentially self-organization, in such systems. [2]

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Dimensionality Reduction as a Mechanism for Speeding Up Nucleic Acid Replication in Protocellular Systems

Marco Tuccio[a], Steen Rasmussen[b,c,d]

[a]*University of Turin, Turin, Italy;*
[b]*University of Southern Denmark, Odense, Denmark;*
[c]*Santa Fe Institute, NM USA;*
[d]*European Centre for Living Technology, Venice, Italy*
marco.tuccio@edu.unito.it

Key words: dimensionality-reduction, chemical kinetics, DNA hybridization, DNA replication

Understanding DNA hybridization kinetics is crucial for the development of synthetic biological systems. By investigating the impact of dimensionality reduction on DNA hybridization kinetics, we can gain insights into how to optimize the design of non-enzymatic self-replicating systems. This research has the potential to contribute to the development of more efficient and robust protocellular models, which could also have implications for fields such as biosensing and drug delivery.

We achieve dimensionality reduction in the translational motion of single-stranded DNA by the self-assembly of vesicles decorated with anchored DNA-lipid conjugates on their outer layer[3].

The impact of dimensionality reduction on DNA hybridization kinetics and proto-metabolic efficiency is investigated, with a focus on the Lesion Induced DNA Amplification (LIDA) system[1]. Based on a so-called Zipping Graph Model and due to improved search efficiency of 2D diffusive motion and directionality constraints imposed by the membrane, we demonstrate that dimensionality reduction can significantly speed up nucleic acid replication[2]. We build a mesoscale computational stochastic model of DNA hybridization kinetics that incorporates insights from the polymeric nature of DNA[4] as well as combinatorial properties of hybridization[5].

Accounting for diffusive motion on membranes[6], our model predicts that such dimensionality reduction leads to a faster hybridization rate constant, which in turn accelerates the LIDA replication process. These findings not only contribute to our fundamental understanding of DNA hybridization kinetics in membrane-anchored systems but also provide a foundation for the design and optimization of non-enzymatic self-replicating systems in protocell models. Ultimately, our work suggests that dimensionality reduction may have been a key mechanism through which early living systems increased their metabolic efficiency as the informational DNA molecules are coupled to the metabolic production of building blocks[3].

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Modelling the initial stages of complex coacervate droplet formation

Amber YX. Wu [a] and Styliani Consta [b]

[a] *Department of Medical Biophysics, Western University, London, Ontario, Canada*
[b] *Department of Chemistry, Western University, London, Ontario, Canada*
amberyx.wu@gmail.com

Keywords: complex coacervation • liquid-liquid phase separation • polyelectrolytes • molecular dynamics • computational chemistry

Coacervate droplets are considered a plausible model for protocells due to their spontaneous formation and ability to compartmentalize macromolecules such as nucleic acids and peptides[1]. Although experimental studies have observed and synthesized coacervates under different laboratory conditions[2], little is known about their structure in great details. Here we present atomistic molecular dynamic (MD) simulations of the interactions between water and oppositely charged protein clusters, which would provide much more details than coarse grain modelling, as used in many previous MD studies[3,4]. In addition, since coacervation is known to be a salt-dependent process[5], it is expected that the ions would have a characteristic arrangement of that in droplets compared to the surrounding bulk solution. Observing such partitioning on an atomistic level would serve as a model for the initial stages of complex coacervate formation. We used atomistic MD to compute diagnostics of the structure at different NaCl concentrations, and constructed models of various geometries to analyze the number density.

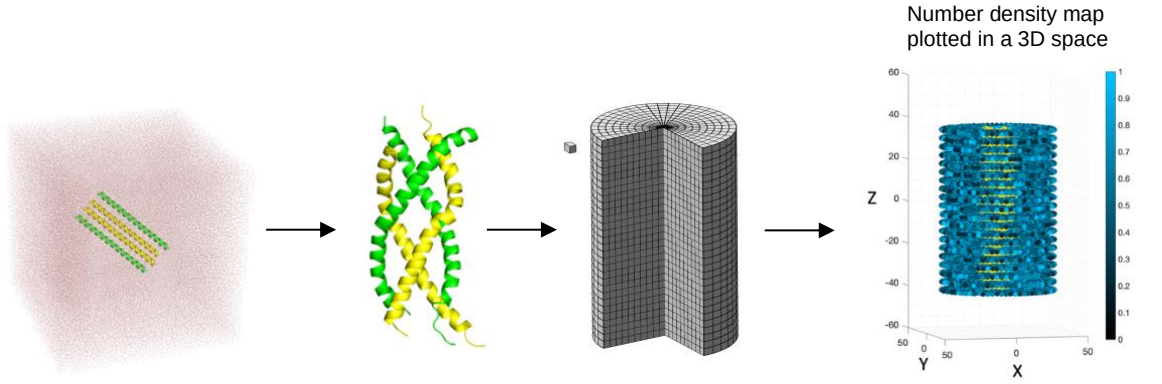

Fig 1. Oppositely charged homopolymers were solvated in explicit solvents and formed coiled coil structures. A cylindrical model was constructed to analyze the number density of the polyelectrolytes and surrounding solvent.

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Replication fidelity of chemical synthetic replicators

Juntian Wu,[a] Omar Markovitch [a] and Sijbren Otto [a]

[a] *Center for Systems Chemistry, Stratingh Institute, University of Groningen, Groningen, The Netherlands*

Presenting author: juntian.wu@rug.nl

Keywords: Self-replication • Replication fidelity • Self-assembly • Systems chemistry

Self-replication is widely considered to be an essential element of the origin of life and Darwinian evolution. Replication fidelity, which is the degree of replication exactness, plays a crucial role in evolution.[1] A vast number of studies on non-enzymatic DNA/RNA polymerization investigated the role fidelity played early on, when the first genetic systems became established.[2, 3] Chemical synthetic replication systems are a promising approach to the de novo synthesis of life and to alternative biochemistries. Exploring how replication fidelity works in a synthetic system can inform on the potential for Darwinian evolution of synthetic replicators.

Our group has constructed a family of synthetic self-replicating systems by using a dynamic combinatorial chemistry (DCC) approach. A typical DCC library starts from a peptide substituted 3,5-dimercaptobenzene building block that can spontaneously form a series of macrocycles. Macrocycles with a specific ring size can stack into fibers and catalyze the formation of macrocycles with the same ring size (self-replication) driven by this stacking. By mixing two structurally closely related building blocks, a series of replicators (mutants) containing different ratios of building blocks can form.[4] Herein, our recent progress on exploring replication fidelity in these synthetic multi-replicators systems will be presented. We studied the fidelity with which a newly formed replicator stacks on a pre-existing replicator. When the newly formed replicator has the same composition as the pre-existing replicator, copying is accurate, otherwise, it represents a mutation step. Seeding experiments were carried out by adding pre-formed replicators to a mixture of replicator precursors. When the samples were seeded with different replicators, different replication kinetics were observed that inform on replication fidelity. Combining model development, data-fitting and parameter estimation, the replication fidelities for different replicators were obtained.

These values inform on the evolvability of the systems and guide the construction and investigation of synthetic systems with different evolvabilities and properties.

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A single phosphorylation mechanism to initiate protometabolism

Joris Zimmermann,[a] Robert J. Mayer,[a] and Joseph Moran [a]

[a] *Institut de Science et d'Ingénierie Supramoléculaires, Université de Strasbourg, CNRS (UMR 7006), 8 Allée Gaspard Monge 67000 Strasbourg, France*
joris.zimmermann@etu.unistra.fr

Keywords: Protometabolism • Carboxy-phosphorylation • PEP • Phosphoryl transfer • Mechanism

The emergence of protometabolism is thought to have arisen from self-organized reaction networks consisting of a small set of simple repeating reaction mechanisms that serve to accumulate key metabolites[1]. Within biological metabolism, phosphorylation helps drive nearly all anabolic pathways as well as biological polymerization. In the core of metabolism, two different types of phosphorylation reactions are found. Primarily, carboxylates are phosphorylated to acyl phosphates. However, phosphoenolpyruvate (PEP) – the metabolite with the most energetic phosphate bond – is synthesized by a different mechanism. Instead of phosphorylation of the carboxylate moiety of pyruvate, the enolate of pyruvate is generated within the enzyme, which subsequently undergoes phosphoryl transfer. Interestingly, the hydrolysis of PEP, which corresponds to the reverse reaction, proceeds through an acylphosphate. We hypothesized that PEP formation via an enolate is the result of biological evolution, and that an ancient nonenzymatic pathway yielded PEP through the direct phosphorylation of the carboxylate of pyruvate followed by intramolecular transfer of the phosphoryl group (**Fig.1**). Here we demonstrate the plausibility of such a nonenzymatic phosphorylation mechanism, which corresponds to the reverse pathway of PEP hydrolysis. A single phosphorylation mechanism may have been sufficient to initiate biochemical networks.

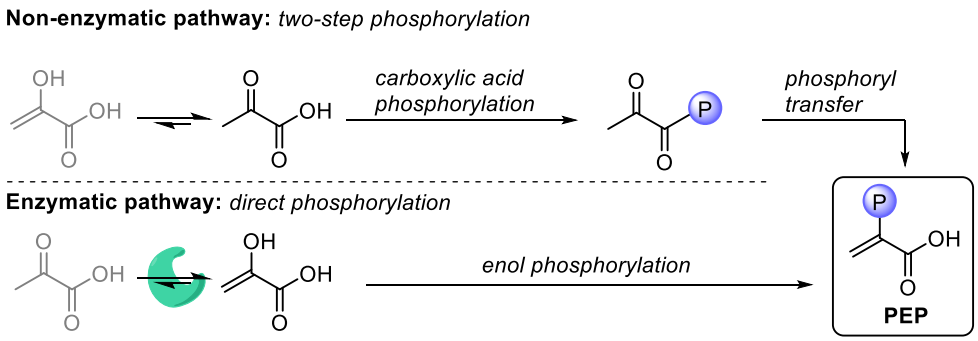

Fig 1. Non-enzymatic vs enzymatic phosphorylation of pyruvate to PEP.

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Physical selection pressures to drive early molecular evolution

Paula Aikkila[a], Thomas Matreux[a], Dieter Braun[a] and Christof Mast[a]

[a] *Systems Biophysics, Department of Physics, LMU Munich, Amalienstr. 54, 80799 Munich, Germany*

p.aikkila@physik.uni-muenchen.de

Keywords: non-equilibrium • biophysics • thermophoresis • UV damage

A crucial step during the origins of life is the emergence of biopolymer building blocks. However, the optimal reaction pathways for their formation usually require feedstocks of pure reactants and defined purification and mixing steps to suppress unwanted side reactions and allow for high product yields. We show that heat flows through thin crack-like compartments purify complex mixtures of prebiotically relevant building blocks with high selectivity by bringing together geomaterials, chemistry and microfluidics in a realistic environment. This non-equilibrium process differentially enriches prebiotically relevant building blocks, distinguishes even mass-identical molecules and boosts their reactivity as seen from the trimetaphosphate (TMP) driven phosphorylation of cytidines and dimerization of glycine. In previous studies we have shown that heat flows through rock cracks can shift the concentration ratio between selectively accumulating magnesium sodium ions, thereby enabling ribozyme function[1]. In the same geological setting, not only heat flows but also UV radiation could have acted as a prebiotic filter. Upon UV radiation, thymine dinucleotides form primarily cyclobutane dimers (CPD) among other UV damages. Above mentioned heat flows through thin rock cracks could have distinguished between damaged and non-damaged short DNA strands and therefore exerted another kind of physical selection pressure to drive molecular evolution.

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Prebiotic synthesis and selection of natural phospholipids

Maiia Aleksandrova,[a] Fidan Rahmatova [a], David Russell [a] and Claudia Bonfio [a]

[a] *Supramolecular Science and Engineering Institute, Strasbourg University, 8 All. Gaspard Monge, 67000 Strasbourg, France*

aleksandrovam@unistra.fr

Keywords: Phospholipids • Protocells • glycerol-1,2-cyclic phosphate

Phospholipids are complex membrane-forming amphiphiles that support the existence of living cells. Unlike other amphiphiles available in the prebiotic world, such as fatty acids, phospholipids tolerate a wide range of environmental conditions and can thus support the chemistry of early life. However, the synthesis of modern phospholipids remains unknown in prebiotically plausible conditions. Here we demonstrate the first prebiotic route to modern biologically relevant lysophospholipids: monodecanoylphosphatidylcholine (lysoDPC) and monodecanoylphosphatidylethanolamine (lysoDPE or MDPE). A library of hydrophilic headgroups was obtained by exploiting the ring-opening of glycerol-1,2-cyclic phosphate under general base catalysis in aqueous solution at 40°C. The ring-opening of cyclic lysophosphatidic acids afforded the formation of complex lysophospholipids. Intriguingly, the intrinsic instability of glycerol-2-phosphoderivatives led to the accumulation of the natural regioisomer, thus suggesting that the selection of modern phospholipids likely arose from the molecular features of primitive amphiphiles available on early Earth.

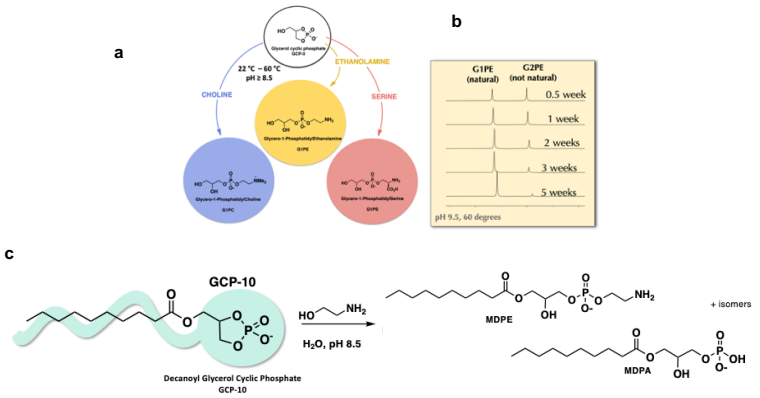

Fig 1. (a) The synthesis of the phospholipid headgroups and the selective degradation **(b)** of the unnatural regioisomer (G2PE), **(c)** Prebiotic synthesis of monodecanoylphosphatidylethanolamine under mild aqueous conditions.

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Coacervation driven by protometabolic NAD^+ reduction enhances NADH production

Rudrarup Bose [a] Anju Tomar [b], Sheref S. Mansy [b,c], and T-Y Dora Tang [a]

[a] *Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany*
[b] *Department CIBIO, University of Trento, Via Sommarive 9, Povo, TN 38123, Italy*
[c] *Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton AB T6G 2G2, Canada*

bose@mpi-cbg.de

Keywords: coacervation • protometabolic reactions • prebiotic metabolites • NADH

Metabolism and compartmentalization are identified as two key features of life. It has been proposed that coacervates, can serve as compartment to host prebiotic reactions during the Origin of Life.[1] There has been continual progress in uncovering new chemical routes to the synthesis of key biological molecules and metabolites and that it is now well established that key biological molecules and metabolites can form coacervates.[2] Despite this, coupling prebiotic chemical reactions to coacervation has been largely unexplored.

To this end, we investigate the effect of the addition of coacervate forming polypeptides on an established protometabolic reaction (see schematic). We show under different salt conditions the material state of polyarginine/NADH coacervates are variable. Furthermore, the presence of coacervates increased the rates of reaction more than two-fold compared to coacervate free conditions. Our data provides a new perspective on the possible synergy between coacervation and protometabolic reactions for Origin of life studies.

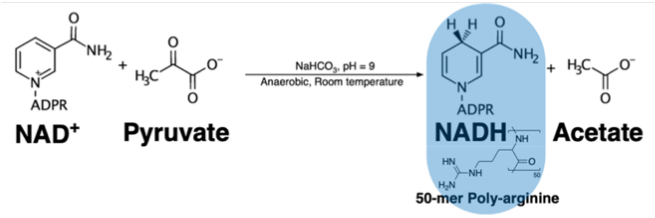

Fig 1. NAD^+ converts to NADH as pyruvate decarboxylates in its presence to acetate.[3] Poly-arginine, present in the reaction mixture, forms coacervate droplets eventually due the production of NADH.

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RNA modifications as molecular fossils of an early protocell world

Chun-Yin, Chan,[a] Johannes, Singer [a] and Thomas Carell [a]

[a] *Faculty for Chemistry and Pharmacy, Ludwig-Maximilians-Universität München, Butenandtstrasse 5-13, 81377 München, Germany*
chach@cup.lmu.de, josich@cup.lmu.de

Keywords: Protocell • Liposome • RNA-Peptide World• RNA World •

tRNAs and the ribosome are considered to be the oldest molecules in cell,[1] modifications found in them could very likely be essential for the survival of an early RNA system and therefore, conserved throughout the evolutionary history. While numerous efforts have been made to support the RNA world theory, the question of how RNA could later evolve and synergise with other biomolecules, like lipids and peptides, and eventually forms a 'protocell', remains elusive. In this study, we show that short RNAs containing *S*-geranyl-2-thiouridine, a highly conserved modification in tRNAs,[2] can anchor on and functionalise zwitterionic liposomes. Our study features a protocell model that also allows the selective binding and accumulation of its complementary canonical strand on the liposome surface by base-pairing. Furthermore, this enables template-directed peptide bond formation on the lipid-RNA surface with the aids of other nucleoside modifications following our recently reported prebiotic pathway.[3] The expanded system shows preference of lipophobic over lipophilic peptides and therefore, enables some selectivity.

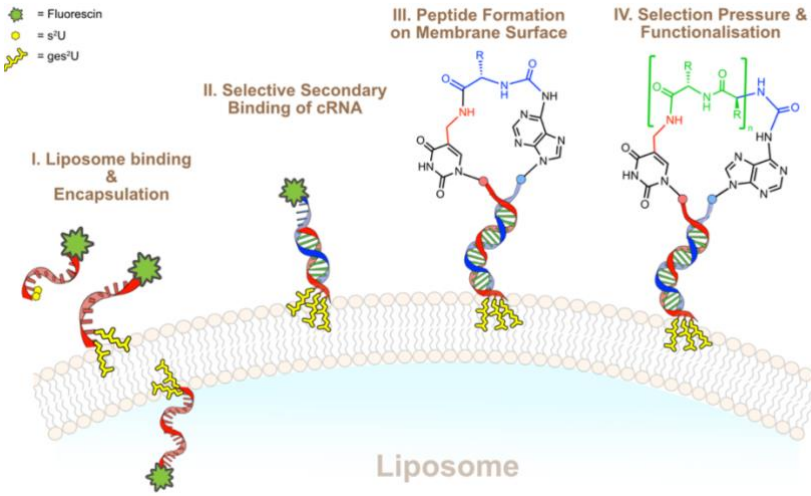

Fig 1. A protocell model that features short RNA oligomers functionalized by natural chemical modifications that allows peptide chemistry to happen in proximity of a liposome surface.

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Prebiotically plausible peptide-lipid interactions

Ivan Cherepashuk,[a] Mikhail Makarov,[a] Robin Krystufek,[c] Sean F. Jordan [b] and Klara Hlouchova [a, c]

[a] *Department of Cell Biology, Faculty of Science, Charles University, BIOCEV, Prague, 12843, Czech Republic*
[b] *Department of Life Sciences, Atlantic Technological University Sligo, Ash Lane, Sligo F91 YW50, Ireland*
[c] *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, 16610, Czech Republic*
cherepai@natur.cuni.cz

Keywords: prebiotic chemistry • random peptides • decanoic acid vesicles • prebiotic evolution

Prebiotic protein chemistry was based on a set of amino acids that is different from a contemporary one.[1] Decanoic acid/decanol vesicles are a good model for prebiotic membranes, and are well-studied.[2] Understanding interaction of such vesicles with prebiotically plausible peptides will give us great insights into the mechanisms that advanced early proto-cell evolution. Yet, interaction between decanoic acid/decanol vesicles and peptides of different amino acid compositions is unknown.

We have conducted a set of preliminary experiments aimed at establishing the presence of random peptide library-vesicles interactions. For this, we synthesized 5-mer peptide libraries of different amino-acid compositions, including some of the most abundant non-canonical, prebiotically plausible amino-acids.[3] Firstly, we established solubility of our peptide libraries in decanol and octanol; our results suggest that only peptides comprised of contemporary amino acid alphabet are soluble in those compounds. There might also be potential binding of peptides with unbranched non-canonical amino acids to the decanoic acid/decanol vesicles, as suggested by the experiment where a vesicle/peptide mixture was filtered through an Amicon Ultra-4 3K filter.[2]

Finally, our experiments with fluorescent imaging of decanoic acid/decanol vesicles suggest that some peptide libraries might interact with the vesicles, and might influence them upon addition of 200 mM NaCl as well.

Our findings suggest that there might be interaction between certain peptide libraries of different amino acid compositions with prebiotically plausible lipids, which might not be based on binding or dissolution.

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Cell-free expression localized and activated at heated air-water interfaces

Alexander Floroni[1], Noël Yeh Martin[1], Christof Mast[1] & Dieter Braun[1]

[1]Systems Biophysics, Ludwig-Maximilian University Munich, Amalienstr. 54, 80799 Munich, Germany

alexander.floroni@physik.uni-muenchen.de

Keywords: Cell-free expression, thermal non-equilibrium, synthetic cell, wet-dry cycles, membrane-free compartmentalization

Heated air-water interfaces create a local water cycle. Molecules in the water phase are concentrated at the warm side by the microscale evaporation. We show that this activates and localizes the RNA transcription and protein translation of a cell-free system. The physically triggered molecular crowding activates the system and enables a localized expression. We find localized expression at air bubbles or extended air-water interfaces, enabling a free design of compartments in simple microfluidic settings. The findings will allow to create interacting multicellular expression systems without the barrier of cell membranes and offer a novel paradigm for long-term feeding of transcription translation systems.

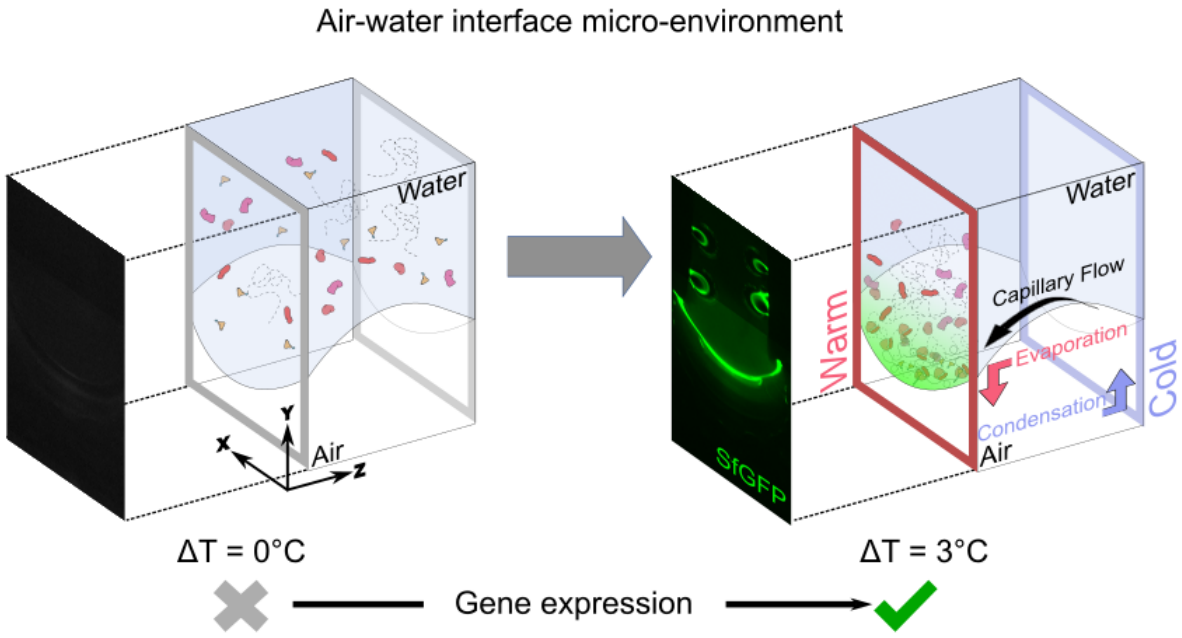

Fig. 1 Schematic representation of an air-water interface: Before (left) and after applying heat to one side of the chamber to produce a temperature difference of 3°C between the front and the back (right). The heat difference creates a non-equilibrium setting where molecules are accumulated at the hot side of the chamber by a capillary flow. When coupled with a Transcription-Translation (TXTL) system that was inactivated through dilution, the accumulation is strong enough to trigger the production of a fluorescent protein (sfGFP) at the interface, effectively reactivating the reaction.

Phase separation in primitive cell membranes

Yuhan Li [a], Claudia Bonfio [a]

[a] *Institut de Science et d'Ingénierie Supramoléculaires (ISIS)*
8 allée Gaspard Monge, BP 70028, 67083 Strasbourg Cedex, France
yuhan.li@unistra.fr

Keywords: phase separation • primitive membrane • cell division

Lipid rafts are key components in modern cells that influence membrane fluidity, trafficking and intercellular communication. Yet, whether lipid rafts also played a role in the emergence and evolution of primitive cells is still unknown. Lipid rafts are membrane domains arising from the phase separation of membrane lipids in coexisting liquid-ordered and liquid-disordered phases. Recent reports suggest that phase-separated membranes, made of modern phospholipids and cholesterol, can undergo division[12] when triggered by an osmotic unbalance. We thus sought to explore whether prebiotically plausible lipid mixtures can self-assemble into phase-separated membranes. Here we show how temperature can trigger phase separation in membranes comprising primitive phospholipids even without cholesterol. Our findings set the stage for studying osmotically-driven division cycles, paving the way towards the emergence of functional, more evolved primitive cells.

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Motility of saltwater droplets in a primeval oil slick

Beatrice Marincioni, Karina Nakashima and Nathalie Katsonis*

University of Groningen (Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, Groningen, The Netherlands)

b.marincioni@rug.nl

Keywords: protocells • systems chemistry • prebiotic chemistry • motility

For life to emerge, simple molecules must have concentrated in primitive compartments, commonly referred to as protocells. It is commonly accepted that protocells self-assembled from simple lipids.[1] However, where these lipids came from, and how they self-assembled spontaneously into primitive compartments, remains a blind spot in our understanding of chemical evolution and the origin of life.

Building on geological evidence that an oil slick of hydrocarbons covered the primordial ocean,[2] we propose that the irradiation of this 'oil slick' by the early sun produced fatty acids as simple lipids.[3] These lipids would stabilize saltwater droplets formed in the oil slick.

Here, I will argue that such water droplets could have co-existed with other forms of protocells. I will also show that saltwater droplets exhibit complex, tactic motility in a slick of oil, based on fundamental physico-chemical mechanisms that involve inhomogeneities in interfacial tension.[4] I will demonstrate that salts classified as chaotropic in the Hofmeister series cause fast droplet movement. I will also show how the presence of phosphates dissolved in water increases droplet speed substantially, because of their effect on the pH.

The evidence that saltwater droplets can travel actively through oil slicks open the door to new plausible scenarios for the formation of fatty acid vesicles as protocells.

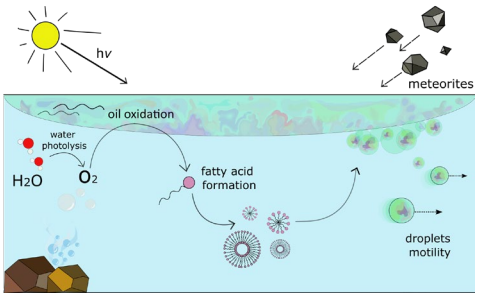

Fig 1. Irradiation of the oil slick produces lipids from which motility of protocells can emerge.

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Prebiotic compartments on the early Earth: Hydrothermal vent coacervate and ocean liposome models

Ella Mullikin,[a] Saehyun Choi,[a,b] Emma Tackman,[a] Christopher H. House,[c] Miriam Freedman,[a] and Chris Keating[a]

[a] *Department of Chemistry, The Pennsylvania State University, University Park, PA 16801 USA*
[b] *Department of Chemistry, University of California, Berkeley, CA 94720 USA*
c Department of Geosciences, The Pennsylvania State University, University Park, PA 16801 USA
emm6558@psu.edu

Keywords: hydrothermal vents • LLPS • protocell • catalysis • proto-metabolism

Compartmentalization is a pivotal step in evolution from purely chemical systems on early Earth to proto-biological systems. One of two prebiotic compartmentalization models considered in this work is complex coacervates, which form from the association of oppositely charged polyelectrolytes in solution. We seek to investigate, for the first time, the interaction of complex coacervates with alkaline hydrothermal vent minerals. The formation of iron sulfide (FeS) minerals in the presence of complex coacervates has been characterized, revealing that minerals can be encapsulated within these compartments. Now, the ability of coacervate-encapsulated FeS and nickel-doped FeS to catalyze proto-metabolic reactions such as fixation of CO_2 using H_2, a product of serpentinization, is being explored. Additionally, a microfluidic setup has been constructed and tested which will enable the study of this phenomenon and related processes in a hydrothermal vent pore-like setting, complete with temperature, pH, and redox gradients that may influence the efficacy of coacervates as prebiotic compartments.

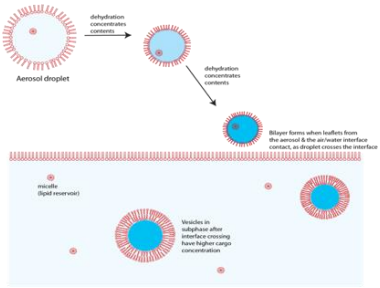

Fig. 1 Proposed mechanism of vesicle generation via aerosolization and re-entry into a surfactant coated ocean.[1]

The other compartmentalization model considered here is membranous vesicles, which form from the assembly of lipids and provide the most direct link to modern cell membranes. We have tested aerosolization and re-entry of prebiotically plausible fatty acid structures in a lipid-coated system mimicking the early ocean surface (Fig. 1). Transport of solutes from an initial solution to a landing solution is observed, indicating cargo-loading of aerosols. Work is in progress to demonstrate the concentration enrichment of cargo via aerosol drying, and future work will explore UV-mediated prebiotic reactions within aerosolized liposomes and the potential role of coacervates to facilitate growth and fission of these compartments.

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Anemochory and the Origins of Life: a study of aerosol to vesicle transformation

Serge Nader,[a] Alexandre Baccouche,[a] Fiona Connolly,[a] John D. Lewis,[b] Desmond Pink,[c] and Sheref S. Mansy [a]

[a] *Department of Chemistry, University of Alberta, Edmonton, AlbertaT6G 2N4, Canada*

[b] *Department of Oncology, University of Alberta, Edmonton, Alberta T6G 2E1, Canada;*

[c] *Nanostics Inc., Edmonton, Alberta T5J 4P6, Canada*

serge.nader@ualberta.ca

Keywords: aerosols • protocells • vesicles • origins of life • prebiotic chemistry

Aerosols are abundant on the Earth and likely played a role in prebiotic chemistry. While much work has centered on the generation of aerosols and their chemistry, little effort has been expended on their fate after settling. Using a laboratory model, we show that aqueous aerosols transform into cell-sized protocellular structures upon entry into aqueous solution containing lipid. Such processes provide for a heretofore unexplored pathway for the assembly of the building blocks of life from disparate geochemical regions within cell-like vesicles with a lipid bilayer in a manner that does not lead to dilution. Our work highlights a new pathway that may have facilitated the emergence of the Earth's first cells.

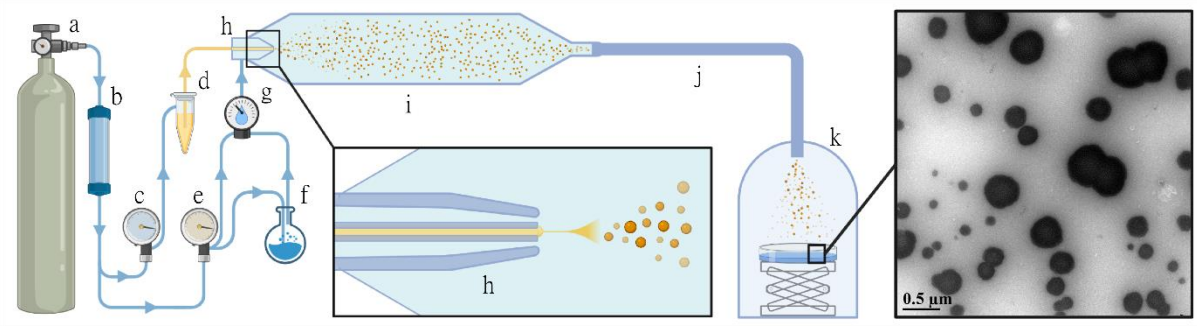

Fig 1. Experimental apparatus used in this study and a transmission electron microscopy photograph showing aerosols transformed into vesicles. [1]

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Vesicle fusion via slow freezing and eutectic melting: Implications for the emergence of a protocell system

Natsumi Noda [a], Tatsuya Shinoda [b], Kazumu Kaneko [c], Yasuhito Sekine [a], Tomoaki Matsuura [a]

[a] *Earth-Life Science Institute (ELSI), Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro, Tokyo*
[b] *School of Life Science and Technology, Tokyo Institute of Technology*
[c] *School of Science, Tokyo Institute of Technology*
natsumi@elsi.jp

Keywords: protocell • LUV • freeze-thaw • eutectic • primitive environment

How did life obtain the system of evolution? The known Earth's life shares cells in common, which can be described as a molecular system that 1) achieve translation of their genetic information 2) inside the compartment isolated from outside. These two features are essential for genetic information to be subjected to natural selection; thus "protocell" system should also have had. In order to construct such complex system, not only forming but also assembling the numerous types of biomolecules would have been required. Since those primitive compartments, when emerged, would have encapsulated only simple contents, the mixing of their contents each other via membrane fusion could have been an important pathway to assemble the encapsulated molecules increasing the complexity of the "protocell" system.
Here we focus on slow freezing and eutectic melting as a plausible mechanism on early Earth that can induce the membrane fusion of lipid vesicles and simultaneous assembling the biomolecules in their functional form. The advantage of eutectic phase in RNA oligomerization and ligation have been recently reported.[1,2] When the solution is cooled slowly (<~1–0.1 K min^{-1}), the dissolved and suspended molecules are expected to be partitioned into the eutectic solution.[3] We prepared large unilamellar vesicles (LUVs, ~100 nm in diameter) with phospholipids, including egg phosphatidylcholine (eggPC), POPC, and PLPC, and subjected the suspension of them to slow freeze-thaw (slow-FT) cycles. After cooling until −28°C at −0.03 K min^{-1} and kept frozen for ~20 hours before thawing, the size of LUV increased to ~1000 nm, suggesting that membrane fusion occurred. The percentages of phospholipid that forms grown vesicles were linearly correlated with the lipid concentration of prepared LUV suspension, supporting that the concentrating LUV through the formation of the eutectic solution has an important role in observed size growth. While the size growth was hardly detected for 100% POPC LUV, adding 12.5% of PLPC resulted in 20% phospholipid forming the grown vesicle after slow-F/T, implying the acyl group of lipids and the related physicochemical property of the membrane possibly affected the efficiency of vesicle fusion. The efficiency of contents mixing during the size growth will be quantified by encapsulating fluorescent molecules as primary simulants followed by functional biomolecules.

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Cooperative pyridoxal-peptide catalysis as precursor of enzymatic transamination

Shunjiro Sodei,[a] Quentin Dherbassy [a] and Joseph Moran [a]

[a] *Institut de Science et d'Ingénierie Supramoléculaires (ISIS), Université de Strasbourg, 8 allée Gaspard Monge, 67000 Strasbourg, France*

sodei@unistra.fr

Keywords: coenzyme • pyridoxal • peptide • transamination • prebiotic chemistry

The coenzyme-protein tandem plays a central role in biology, and likely find its roots in prebiotic chemistry. Together, coenzymes and proteins catalyze many core processes. For instance, transaminase enzymes with the coenzyme pyridoxal-5'-phosphate (PLP) participate in the metabolism of all amino acids through transamination reactions.[1] Building on prior reports of the transamination reactions catalyzed by metal ions[2,3] and PL(P), we recently found that cooperative catalysis by PL(P) and earth abundant metal ions could have constituted an intermediary stage in prebiotic chemistry.[4] In the present work, we hypothesized that the addition of short peptides into the catalytic system could produce further synergistic effect on catalysis and provide a smooth connection to the present enzymatic systems (Fig.1).

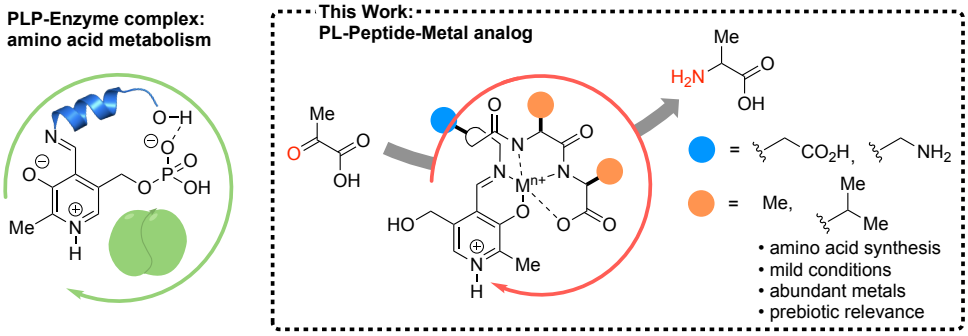

Fig 1. PLP-Transaminase system and its analogous reaction system with PL, peptides, and metals

Here, we report that the addition of peptides has a positive effect on the rate of PLP/metal catalyzed transamination. We are now screening peptide libraries to identify sequences providing more pronounced synergistic effects. However, this preliminary result provides a framework for studying the emergence of the coenzyme-protein pair.

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Liquid crystalline phase separated systems as functional synthetic cells

Ajay Verma[a], Damien Cuvelier[a], Tommaso P. Fraccia[b], Philippe Nghe [a]

[a]*Institut Pierre-Gilles de Gennes, CBI UMR8231, ESPCI Paris, Université PSL, 75005 Paris, France*
[b] *Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Italy*
Presenting author*:ajay.verma@espci.fr*

Keywords: coacervates • liquid crystals • RNA world • molecular evolution • synthetic cells

The realization of functional synthetic compartments, mimicking rudimental cell properties, is a crucial aim of synthetic biology and the origin of life investigation. This principally is a matter of physics, chemistry, and material science; indeed many soft matter systems have been adapted to this purpose. In particular, coacervates microdroplets have recently been proposed as an efficient compartmentalization strategy. Coacervates are liquid-liquid phase separated aqueous systems taking place in solutions of oppositely charged polyelectrolytes, characterized by the formation of membrane-less droplets containing both polymeric species in coexistence with a dilute supernatant phase.
In this frame, we investigated coacervates composed of short nucleic acids (DNA and RNA) oligomers and cationic peptides (Poly-L-Lysine) to obtain synthetic compartments with complex organization patterns and to couple biochemical reactions with compartment properties to probe their molecular evolution potential. To achieve the first goal, we studied the formation of liquid crystalline (LC) phases inside coacervates [1] as a function of DNA oligomers properties (length, flexibility, sequence, structure). We found that in several mixtures of DNA sequences, second phase separation is achieved inside the dense coacervate phase, between isotropic and LC cholesteric phases, leading to the formation of multiphase liquid crystalline coacervates. To achieve the second goal, we are probing the activity of an RNA enzyme, the Azoarcus ribozyme, which is capable of elongating RNA oligomers [2], inside coacervates composed of its own RNA substrate, to seek a relationship between the yield of the Azoarcus reaction and the stability of the whole compartment.

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Motility of droplets in an evolving lipid system

Chanikan Wongkaew, Dhanya Babu, and Nathalie Katsonis *

Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, Groningen, The Netherlands
c.wongkaew@rug.nl

Keywords: Systems chemistry ● Lipid systems ● Motility ● Protocells

For life to emerge, simple molecules must have concentrated in primitive compartments, commonly referred to as protocells.[1] It is commonly accepted that protocells self-assembled from simple lipids.[2] However, how those prebiotically available chemicals spontaneously assembled into the first primitive cells, remains a blind spot in our understanding of chemical evolution and the origin of life. In aqueous solutions, lipids spontaneously form micelles or vesicles. These supramolecular aggregates likely played a role as primitive compartments, possibly transforming into droplets or into one another.[3] Here, I focus on understanding the behaviour of these plausible protocells in complex chemical systems. I show that in a lipid-producing chemical system, both micelles and vesicles can sustain the interfacially-driven motility of droplets, a chemotactic mechanism that had previously only been demonstrated for micelles.[4] In the system where the vesicles are produced after the transient formation of micelles, these aggregates promote the motility of a different oil droplet, which gives rise to competition effects between oil droplets of different compositions. This work paves the way to new pathways for evolutionary exploration as we see how adaptation to a range of physical and chemical environments affects the functional properties of oil droplets as plausible protocells.

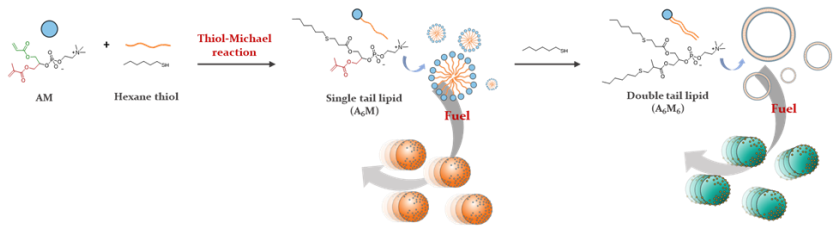

Fig 1. Mechanism of the transient formation of micelles is associated with the emergence of original droplet motility, which is selective towards the chemical composition of the oil droplets.

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e-mails

Name	Surname	e-mail
Paula	Aikkila	p.aikkila@physik.uni-muenchen.de
Maiia	Aleksandrova	aleksandrovam@unistra.fr
Laura	Alonso Saez	lalonso@azti.es
Uxue	Arrizabalaga	uarrizabalaga@azti.es
Rajanya	Banerjee	rajanya.banerjee@unitn.it
Shane	Barrett Kavanagh	shane.kavanagh.22@ucl.ac.uk
Erik Alexander	Boinowitz	erik.boinowitz@cup.uni-muenchen.de
Claudia	Bonfio	bonfio@unistra.fr
Rudrarup	Bose	bose@mpi-cbg.de
Dieter	Braun	mail@dieterb.de
Adriana	Calaça Serrão	a.serrao@physik.uni-muenchen.de
Pau	Capera-Aragones	Pau.Cap@mail.huji.ac.il
Pedro	Caro	pcaro@bcamath.org
Chun Yin	Chan	chhach@cup.uni-muenchen.de
Irene	Chen	ireneachen@ucla.edu
Ivan	Cherepashuk	cherepai@natur.cuni.cz
Nemanja	Cvjetan	cvjetan@ualberta.ca
Felix T.	Dänekamp	f.daenekamp@physik.uni-muenchen.de
Gregoire	Danger	gregoire.danger@univ-amu.fr
Andrés	de la Escosura Navazo	andres.delaescosura@uam.es
Daniele	De Martino	daniele.demartino@ehu.eus
Nikolai	Diukarev	nikolai.diukarev@cup.uni-muenchen.de
Arián	Ferrero Fernández	arian.ferrero@ehu.eus
Christoph	Flamm	xtof@tbi.univie.ac.at
Alexander	Floroni	alexander.floroni@physik.uni-muenchen.de
Carlos	García Ferris	Carlos.Garcia.Ferris@uv.es
Yannick	Geiger	y.geiger@rug.nl
Valerio Guido	Giacobelli	giacobev@natur.cuni.cz
Edoardo	Gianni	egianni@mrc-lmb.cam.ac.uk
Shoval	Gilboa	shovalgilboa@campus.technion.ac.il
Alberto	González Berruezo	alberto.gonzalezb@ehu.eus
Daniel	González Santamaría	daniel.g.santamaria@uam.es
Kathrin	Halter	kathrin.halter@lmu.de
Vanessa	Helmbrecht	v.helmbrecht@lrz.uni-muenchen.de
Klara	Hlouchova	havova1@natur.cuni.cz
Atsushi	Kamimura	kamimura@sat.t.u-tokyo.ac.jp

Nathalie	Katsonis	n.h.katsonis@rug.nl
Artemy	Kolchinsky	artemyk@gmail.com
Ramanarayanan	Krishnamurthy	rkrishna@scripps.edu
Juliette	Langlais	Juliette.langlais@physik.uni-muenchen.de
Nino	Lauber	nino.lauber@ehu.eus
Antonio	Lazcano Becerra	alar@ciencias.unam.mx
Yuhan	Li	yuhan.li@unistra.fr
Raquel	Liébana García	rliebana@azti.es
Javier	Luis-Barrera	javier.luis@uam.es
Sheref	Mansy	sheref.mansy@ualberta.ca
Beatrice	Marincioni	b.marincioni@rug.nl
Christof	Mast	christof.mast@physik.uni-muenchen.de
Thomas	Matreux	th.matreux@physik.lmu.de
Fatma Zohra	Mihoubi	fatma-zohra.mihoubi@etu.unistra.fr
Joseph	Moran	moran@unistra.fr
Alvaro	Moreno Bergareche	alvaro.moreno@ehu.eus
Ella	Mullikin	emm6558@psu.edu
Serge	Nader	serge.nader@ualberta.ca
Karina	Nakashima	k.k.nakashima@rug.nl
Krishnadev	Narayanankutty	krishnadev.narayanankutty@ehu.eus
Natsumi	Noda	natsumi@elsi.jp
Karin	Oberg	koberg@cfa.harvard.edu
Robert	Pascal	robert.PASCAL@univ-amu.fr
Juli	Pereto	Juli.Pereto@uv.es
Carla	Perez Cruz	carlaperez@azti.es
Joaquin	Perez Grande	joaquin.perezgrande@student.manchester.ac.uk
Matthew	Powner	matthew.powner@ucl.ac.uk
Martina	Preiner	martina.Preiner@mpi-marburg.mpg.de
Mirko Alexander	Prokop	mirko.prokop@web.de
Saroj	Rout	s.rout@physik.uni-muenchen.de
Kepa	Ruiz-Mirazo	kepa.ruiz-mirazo@ehu.eus
David	Russell	drussell@unistra.fr
Philipp	Schwintek	Philipp.schwintek@physik.uni-muenchen.de
Daniel	Segrè	dsegre@bu.edu
Siddhant	Sharma	siddhaantsharma.ss@gmail.com
Ben	Shirt-Ediss	ben@shirt-ediss.me
Johannes	Singer	josich@cup.lmu.de

Shunjiro	Sodei	sodei@unistra.fr
Jack W	Szostak	jwszostak@uchicago.edu
T-Y Dora	Tang	tang@mpi-cbg.de
Anju	Tomar	anju.tomar@unitn.it
David	Tourigny	d.tourigny@bham.ac.uk
Quoc Phuong	Tran	q.tran@student.unsw.edu.au
Marco	Tuccio	marco.tuccio@edu.unito.it
Ivan	Ubarrechena Belandia	ivan.ubarrechena@ehu.eus
Gérard	Vauclair	gerardv@ast.obs-mip.fr
Sylvie	Vauclair	sylvie.vauclair@irap.omp.eu
Ajay	Verma	ajay.verma@espci.fr
Vassilissa	Vinogradoff	vassilissa.vinogradoff@univ-amu.fr
Peter	Walde	peter.walde@mat.ethz.ch
Maximilian	Weingart	m.weingart@physik.uni-muenchen.de
Emilie	Werner	emilie.werner@etu.unistra.fr
Chanikan	Wongkaew	c.wongkaew@rug.nl
Amber Yixuan	Wu	amberyx.wu@gmail.com
Juntian	Wu	juntian.wu@rug.nl
Sreekar	Wunnava	s.wunnava@physik.uni-muenchen.de
Joana	Xavier	xavier@dayhofflabs.com
Felix	Xu	felix.xu@cup.uni-muenchen.de
Joris	Zimmermann	joris.zimmermann@etu.unistra.fr