The Game of Order and Complexity: Spontaneous Formation of Protocells?

Christian Mayer [1], Ulrich Schreiber [2], María J. Dávila [1], Oliver J. Schmitz [3], Yildiz Großmann [3]

[1] Institute of Physical Chemistry, CENIDE, University of Duisburg-Essen, 45141 Essen, Germany
[2] Department of Geology, University of Duisburg-Essen, 45141 Essen, Germany; [3] Institute of Applied Analytical Chemistry, University of Duisburg-Essen, 45141 Essen, Germany;

Life is characterized by significant complexity combined with a high degree of order. Therefore, early steps towards the origin of life must have necessarily led into this direction. The most powerful process leading to states of increased complexity and order is Darwinian evolution. However, this requires self-reproducing systems. So which processes, instead of Darwinian evolution, could have developed complex prebiotic structures in the early beginnings?

First, a necessary precondition for development is a permanent non-equilibrium state. This is most easily achieved by a periodic variation of physical conditions, such as switching between states of wetness and dryness, or by fluctuating temperature or pressure conditions. This combined with the formation and selection of complex molecular structures has the power to lead from simple chaotic mixtures toward larger, more defined entities. Perfect conditions for such a development are found in a planetary crust.

Recent experiments in our lab rely on pressure cycling, simulating the conditions in the Earth's crust in a depth of 1 km. We can show that, in presence of simple prebiotic molecules, these conditions lead to the periodic formation of vesicles. These vesicles in turn select peptides generated randomly in a pool of amino acids. The resulting vesicle-peptide structures are constantly selected for their stability, leading to an ongoing optimization process. In total, a process is formed which leads to continuous increase in order (selected peptide sequences) and complexity (growing size of the peptides).

Fig. 1 shows the experimental result of such an autonomous selection process, a functional peptide structure formed inside a membrane of the "surviving" vesicles. It has the capability to stabilize the vesicle, alter its size and allow for an increased water permeability in order to reduce osmotic pressure load. Having spontaneously developed, the system represents a surprisingly high degree of order and complexity and may lead the way to actual protocells.

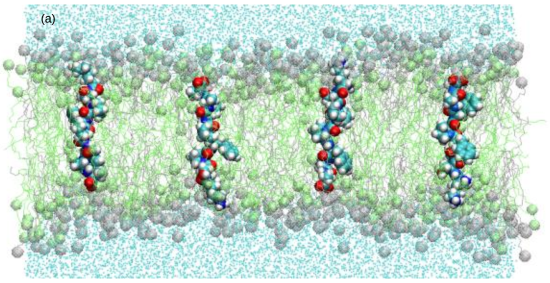


Fig. 1:

Initial stage of a membrane structure formed from small molecules by a spontaneous structural development during pressure cycling. In a subsequent step, the integrated peptide molecules form hexamers that act as pores in the membrane.

References

Mayer, C., Schreiber, U., Dávila M. (2015) Periodic vesicle formation in tectonic fault zones – an ideal scenario for molecular evolution, *Origins of Life and Evolution of Biospheres* 45: 139-148.
Mayer, C., Schreiber, U., Dávila, M. (2017) Selection of prebiotic molecules in amphiphilic environments, *Life* 7: 3.
Mayer, C., Schreiber, U., Dávila, M., Schmitz, O.J. et al. (2018) Molecular evolution in a peptide-vesicle system, *Life* 8: 16.
Mayer, C. (2020) Life in the context of order and complexity, *Life* 10, 5.
Davila, M., Mayer C. (2022) Membrane structure obtained in an experimental evolution process, *Life* 12: 145.
Davila, M., Mayer C. (2023) Structural phenomena in a vesicle membrane obtained through an evolution experiment, *Life* 13: 1735.

An AI-based holistic view of protein space

Joint work with Guy Yanai, Nir Ben-Tal, Liam Longo and Rachel Kolodhy.

We trained a protein language model to create meaningful maps of protein space. The training goal of our model is to co-embed the protein sequences and protein structures, so that the two embeddings of a protein domain are close to each other, and further away from those of other domains. We will show how the resulting model generates meaningful maps of the protein universe based on sets of sequences. In particular, it captures well known evolutionary relationships documented in the hierarchical domain classification ECOD, and even more complicated relationships between families of CATH domains that have different topologies. Furthermore, it captures meaningful relationships between domains that are classified differently in these hierarchies.

ESCRTing Eukaryogenesis: membrane remodeling at the prokatotic-eukaryotic junction

Natalie Elia

The emergence of eukaryotic cells ~1.5–2 billion years ago was a transformative event in life's history. Unlike prokaryotes, eukaryotes are compartmentalized, with membrane-bound organelles that communicate via vesicular transport. These features likely existed in the earliest eukaryotes were key to the evolution of complex life. Recent phylogenomic studies place the origin of eukaryotes within the archaeal domain, specifically among Asgard archaea— a newly discovered archaeal superphylum that contain genes which encode for eukaryotic signature proteins (ESPs). Among these proteins are components of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery—a conserved membrane-remodeling system present in all domains of life. In eukaryotes, ESCRTs drive membrane constriction and fission in diverse processes, including cytokinesis, endosomal sorting, viral budding, and membrane repair. The ability of ESCRT proteins to remodel membranes together with their identification in Asgard archaea raises the possibility that these ancestral ESCRTs contributed to early steps in the evolution of cellular complexity. To test this notion, we perform an in depth biochemical and cellular characterization of ESCRT complexes encoded in Asgard archaea. Our cryo-EM structures and functional studies shows that Asgard ESCRTs can remodel eukaryotic-like membranes and provide supporting evidence for the presence of these proteins at the junction between prokaryotes and eukaryotes.

From binding to catalysis: emergence of a rudimentary enzyme conferring intrinsic antibiotic resistance

Claudèle Lemay-St-Denis, Stella Cellier-Goetghebeur, Maxime St-Aubin, Keigo Ide, Janine N. Copp, Soichiro Tsuda, Nir Ben-Tal, Rachel Kolodny, Joelle N. Pelletier

Type B dihydrofolate reductases (DfrB) were discovered for their insurmountable antibiotic resistance phenotype shortly after the clinical introduction in the 1960's of the synthetic antibiotic trimethoprim to target the ubiquitous bacterial dihydrofolate reductase FolA. DfrB are evolutionarily unrelated to FolA, and the rapid emergence of antibiotic resistance based on a protein that has no similarity (sequential or structural) to the target enzyme yet catalyzes the same reaction, is unprecedented. Even more surprising is that DfrB is the only enzyme built of the Src Homology 3 (SH3) fold, that otherwise mediates protein-protein interactions. The emergence of catalysis starting from a non-catalytic protein is exceedingly rare. We show that DfrB enzymes followed an entirely different evolutionary model for the emergence of catalysis from a non-catalytic domain, based on their unique mechanism of homotetramerization to form a highly symmetrical active site. Combining computations and experiments we suggest that the evolution of catalytic activity in the DfrB domain is intrinsically linked to its structural evolution to form the characteristic homotetrameric complex. Indeed, while no active-site residues are conserved across all characterized catalytically active homologues, prediction of the complex proves to be a powerful predictor of catalytic activity. We propose that the catalytic activity of the DfrB homotetramer is a promiscuous property of its primary binding function, a hallmark of the SH3 fold, highlighting a unique evolutionary pathway to functional diversification driven by evolution of quaternary structure.

Cerium Phosphate and the origin of life: from utilizing formamide to form nucleosides and nucleotides to enantioselective adsorption of amino acids

Shoval Gilboa[1], Nitai Arbell[1] and Yaron Paz[1]

[1]*Department of Chemical Engineering, Technion, Haifa, Israel*

The abiotic formation of nucleotides from small, simple molecules is of large interest in the context of elucidating the origin of life scenario. Since the pioneering direct synthesis of cytidine-monophosphate from glycolaldehyde [1], few examples of the formation of complete nucleotides from primitive precursors have been reported. This final step, grafting a phosphate group onto a nucleoside, requires an available source of sufficiently labile phosphate groups [2]. Accordingly, this step may be reliant on the presence of phosphate minerals, which may serve as a reservoir of these functional groups [3]. An appropriate candidate as a phosphate-donating mineral for prebiotic synthesis is cerium phosphate ($CePO_4$). Cerium is the most abundant element among the rare-earth elements, making it more abundant than copper and five times more abundant that lead. Many of its compounds are active as catalysts and photocatalysts. Igneous phosphate rocks, which seem to be a key player in the formation of biomolecules, typically contain 1000–32,000 ppm of cerium. Cerium phosphate appears in the Earth's crust as the mineral monazite, which, under some conditions, adopts the rhabdophane ($CePO_4.nH_2O$) structure. This common mineral not only contains a phosphate group, which presumably could be donated during the reaction to form nucleotides but also acts as a low-activity photocatalyst that promotes the formation of electron–hole pairs under UV irradiation, which can be further involved in redox reactions.

Here we show that nucleosides and nucleotides can be formed from formamide in a one-pot reaction utilizing the mineral cerium phosphate ($CePO_4$) as a photocatalyst, a catalyst and a reactant that supplies the necessary phosphate groups.[4] While the most abundant RNA/DNA building blocks were found to be thymidine and thymidine monophosphate, considerable yields of other building blocks such as cytidine, cytidine monophosphate, and adenosine cyclic monophosphate were found. Comparing the yield of nucleosides and nucleotides under light conditions to that in the dark suggests that in the presence of cerium phosphate, light promotes the formation of nucleobases, whereas the formation of nucleotides from nucleosides take place even in the absence of light. The scenario described herein is considerably simpler than other scenarios involving several steps and several reactants. Therefore, by virtue of the principle of Occam's razor, it should be of large interest for the community. Moreover, it will be shown that cerium phosphate tends to adsorb amino-acids (for example: phenylalanine, citrulline) in an enantio-selective manner. This could be related to the chiral symmetry of both forms of cerium phosphate. More studies on this property are under way.

1. M.W. Powner, B. Gerland, J.D. Sutherland, *Nature,* **459**, 239 (2009)
2. F.H. Westheimer, *Science,* **235,** 1173 (1987)
3. B.M. Bizzarri, J.E. Šponer, J. Šponer, G. Cassone, M. Kapralov, G.N. Timoshenko, E. Krasavin, G. Fanelli, A.M. Timperio, E. Di Mauro, R. Saladino*, ChemSystemsChem,* **2***,* e1900039 (2020)
4. S. Gilboa, L. Panz, N. Arbell, Y. Paz, *Life*, **14**, 846 (2024)

Advancing the Understanding of the Butlerov Reaction through First-Principles Chemical Reaction Network Generation

Dhanalakshmi Vadivel*, and Daniele Dondi
Department of Chemistry, University of Pavia, Viale Taramelli 12, Pavia 27100, Italy.

The Butlerov reaction, also known as the formose reaction, involves the transformation of formaldehyde and calcium hydroxide into a complex mixture of sugars. Despite its long history, the reaction mechanism remains a subject of scientific debate, with significant implications for the prebiotic synthesis of carbohydrates from simple compounds. Over time, the reaction progresses toward aldose disproportionation via the Cannizzaro reaction, further complicating mechanistic interpretations. The reaction is considered autocatalytic, with glycolaldehyde formation via retro-aldol fragmentation of tetroses, a concept first proposed by Breslow in 1950. Our study employs automatic reaction network generation and density functional theory (DFT) free energy calculations to analyze compounds formed through aldol condensation and isomerization processes. Our approach enhances the fundamental understanding of the formose reaction mechanism, providing insights that could inspire new research directions in organic chemistry and origins-of-life studies.

Extending the Concepts of Habitability and the Habitable Zone beyond Surface Liquid Water

Dirk Schulze-Makuch[1-3] and Amri Wandel[4]

[1] Astrobiology Group, ZAA, Technische Universität Berlin, Hardenbergstr. 36A, 10623 Berlin, Germany, email: schulze-makuch@tu-berlin.de
[2] German Research Centre for Geosciences (GFZ), Section Geomicrobiology, 14473 Potsdam, Germany
[3] Department of Plankton and Microbial Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries, 16775 Stechlin, Germany
[4] Racah Inst. of Physics, The Hebrew University of Jerusalem, Israel, email: amri@huji.ac.il

We propose to extend the term Habitable Zone (HZ) and habitability in their conservative interpretation. While the conservative HZ is defined as the circumstellar region where a planet can sustain liquid water on its surface given an adequate atmosphere, this definition may be extended to include planets inwards of the inner edge and outside of the outer edge of the HZ. Examples are potential habitats with subglacial water on the night side of a tidally locked planet, icy moons orbiting giant planets on which geothermal heat is produced by tidal forces or radioactivity, and hydrogen-rich Hycean worlds. Another type of extension may be Dune-like arid worlds, with an Earth-analog in places like the Atacama Desert (Schulze-Makuch 2024). The objective is to determine an EcoZone (EZ), in which environmental conditions consistent with water-based life may exist.

Schulze-Makuch, D. (2024) We may be looking for Martian life in the wrong place. *Nature Astronomy* 8: 1208-1210, https://doi.org/10.1038/s41550-024-02381-x

Prebiotic chemistry in the Solar System suggested by the OSIRIS-REx Sample Return from Asteroid Bennu and the Mars rovers

Amri Wandel

NASA's OSIRIS-REx mission has returned pristine samples from asteroid Bennu, providing a transformative look into the chemical makeup of the early solar system. Preliminary analyses of the sample—retrieved in September 2023—have revealed:
All five nucleobases of DNA and RNA (adenine, cytosine, guanine, thymine, uracil)
Fourteen amino acids essential for life on Earth **Phosphate-bearing sodium minerals** and **hydrated phyllosilicates Ammonia and other nitrogen-rich compounds**, pointing to complex organic chemistry. These materials suggest formation in **briny, alkaline aqueous environments** on Bennu's parent body, consistent with geochemical conditions conducive to prebiotic synthesis. The findings strongly support theories of organic molecule delivery to early Earth via carbonaceous asteroids. The preservation of these compounds over 4.5 billion years provides vital insight into prebiotic chemistry, planetary evolution, and astrobiological potential in other parts of our solar system—especially in subsurface ocean worlds like Europa and Enceladus.

In addition, NASA's Curiosity and Perseverance rovers have recently made significant strides in the search for organic molecules on Mars, offering new insights into the planet's past habitability. In a groundbreaking analysis, Curiosity's Sample Analysis at Mars (SAM) instrument detected the largest organic molecules ever found on Mars within a 3.7-billion-year-old mudstone —long-chain hydrocarbons that may be remnants of fatty acids, which are vital components of cell membranes in terrestrial life. The preservation of such complex organics suggests that prebiotic chemistry on Mars may have been more advanced than previously thought. **Also the more recent rover,** Perseverance, which has been exploring Jezero Crater, has identified rocks altered by water and containing organic molecules. Notably, a rock dubbed "Cheyava Falls" exhibited organic compounds alongside minerals like calcium sulfate and hematite. While these discoveries do not confirm the existence of past life, they significantly enhance our understanding of the planet's geochemical history and its capacity to preserve organic matter.

Super-flares of the young M-dwarf G 227-22 and prebiotic chemistry

Shira Fraenkel

Super-flares emitted by young M-dwarf stars are usually assumed to originate from thermal emission. G227-22 is a young M dwarf star observed from the second year through the sixth year of the Transiting Exoplanet Survey Satellite (TESS) mission. The star's location near the ecliptic north pole provides a wealth of data from TESS and CARMENES, spanning around five years of observations. We detected a total number of 1962 flares from TESS sector 14 to sector 76. To study the flares' spectral energy distribution, simultaneous follow-up observations were performed with the Ariel University 0.4-m telescope and the Wise Observatory 0.7-m telescope in two different filters (B and Rc), in which one strong flare was detected (E~10^32 erg). Assuming black-body radiation, the joint analysis of the data enables estimating the evolution of the flare temperature and area. However, this assumption seems to be violated at the flare peak, possibly indicating short-lived non-thermal emission. Such emission, mainly if accompanied by UV emission, may have implications for prebiotic chemistry that possibly take place on potentially habitable planets around the star.

Homochirality is a result of protocellular chemical dynamics

Doron Lancet, Weizmann Institute of science, Rehovot, Israel

An important facet of biology is the homochirality of molecules such as amino acids and sugars. The origin of this symmetry breaking is a long-standing question in molecular evolution. Therefore, many authors assume that an a-priori physical process in the primordial soup is a prerequisite for the origin of life. Over the last two decades, we followed the alternative the autocatalytic concept for enantio-selection [1]. For this, we developed a simulatable, rigorous kinetic formalism [2], based on our Graded Autocatalysis Replication Domain (GARD), as detailed [3,4]. This is a mutually catalytic network model for protocellular reproduction and Darwinian evolution in nanoscopic lipid micelles [5]. The modified model is adapted to the case of chiral molecules by applying an asymmetry constraints expressed among enantiomer molecular recognition events within a nanoscopic assembly. A dynamic attractor [6] shows spontaneous chiral symmetry breaking. This results from stationary compositional states (reproducing composomes), enriched with one relative to the other enantiomer for some of the constituent molecule types, a process that sometimes even culminates to homochirality. It follows that homochirality is very likely an emergent consequence of protocellular chemical behavior, rather than a physical prerequisite necessary for life's origin.

References:

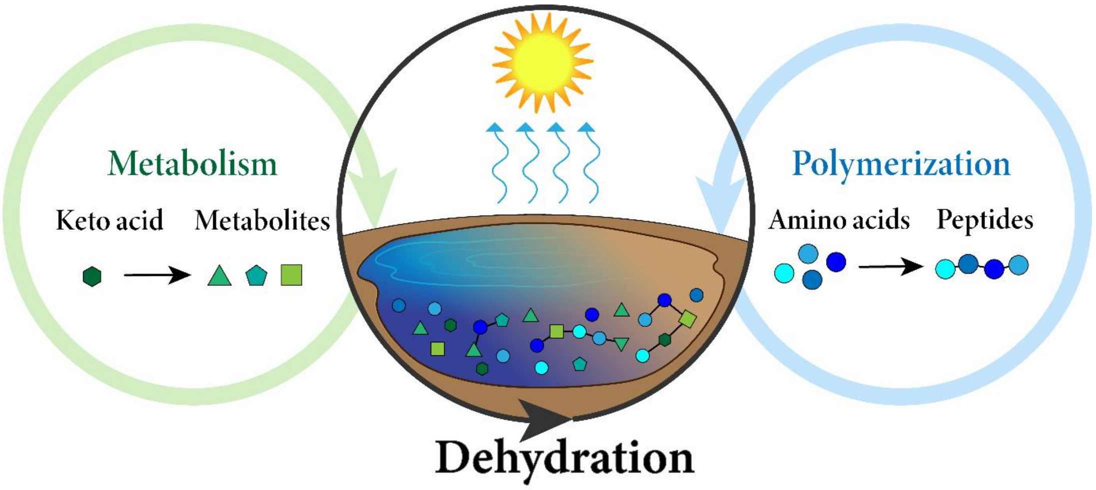
1. Kenso Soai et al., Acc. Chem. Res. (2000) 33, 6, 382–390
2. Kafri et al. Biology Direct 2010, 5:38
3. Segre D. et al. (2000) Proc Natl Acad Sci USA 97(8) 4112
4. Lancet D. et al. (2018) J Royal Soc Interface 15(144): 20180159
5. Kahana A. and Lancet D. (2021) Nat Rev Chem 5, 870–878
6. Kahana et al., (2023) Cell Reports Physical Science 4, 101384 May 17

Robust synthesis of prebiotic precursors in drying reactions of amino acids and keto acids

Eliav Marland[1], Gal Lipson[1], Rotem Edri[1], Or Cohen[1], Aikomari Guzman-Martinez[2], and Moran Frenkel-Pinter[1,3]
[1] *Institute of Chemistry, The Hebrew University of Jerusalem, Israel*
[2] *Department of Chemistry, University of Puerto Rico, Mayagüez, Mayagüez, Puerto Rico*
[3] *The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Israel*

Numerous long-standing questions in research of origins of life center on the history of extant biopolymers. While sequences of proteins have been tuned by eons of evolution, it has been proposed that the polypeptide backbone is the product of prior chemical evolution that occurred about four billion years ago. Both polymerization and metabolism require dehydration. All extant biopolymers are products of condensation-dehydration reactions (e.g. peptide bond formation), and metabolic cycles frequently involve dehydration steps (e.g. dehydration of adenosine diphosphate (ADP) to adenosine triphosphate (ATP)). Former research has demonstrated formation of proto-polymers under drying conditions which favor dehydration reactions, yet there is an absence of research on proto-metabolism under drying conditions, and research on keto acid-amino acid reactions has mainly focused on the effects of catalysts or extreme conditions (e.g. temperature, pressure). Importantly, metabolism and polymerization of keto acids and amino acids are highly intertwined in extant biology. Due to high abundance of both amino acids and keto acids on the prebiotic Earth, we hypothesized that in dry-down reactions under mild conditions, co-polymerization reactions will occur between those molecules to form heterogenous co-polymers.

To test that hypothesis, we performed simple dry-down reactions for one week between α- and γ- keto acids (pyruvic acid and levulinic acid, respectively) and amino acids (Glycine, Alanine and Valine) under a variety of conditions. Our results show that reactions between pyruvic acid and various amino acids produced oligomers up to 6-mers. Control reactions involving either keto acid or amino acid alone did not lead to significant product formation. LC-MS analysis indicates that both pyruvic acid and amino acid monomers are heavily consumed in the reactions to form a multitude of heterogenous products which could have been key players on the path to proto-peptides and proto-metabolism. Our study exemplifies how metabolism-first and polymer-first models can be reconciled via the study of co-polymers composed of keto acids and amino acids under dried conditions.



[1] Frenkel-Pinter, M., Samanta, M., Ashkenasy, G., & Leman, L. *J. Chem. Rev*. **120(11),** 4707–4765 (2020).
[2] K. B. Muchowska, S. J. Varma, J. Moran. *Chem. Rev.* **120**, 7708-7744 (2020).
[3] D. Ross, D. Deamer. *Life* **6**, 28 (2016)

Catalysis by Simple Hydroxybenzenes: Implications for Prebiotic Chemistry and Enzyme Evolution

Meital Reches

Institute of Chemistry and The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel

Catalysis was likely central to the emergence of life, as it remains vital to all living systems today. Yet, the origins and evolution of catalytic function—particularly the transition from simple molecules to complex enzymes—remain unresolved. While enzymes arise from the folding of genetically encoded amino acids, these amino acids themselves may have existed under prebiotic conditions. How such simple building blocks gave rise to complex, functional macromolecules is still a major open question.

Recent studies have shown that both encoded amino acids and their metal complexes exhibit weak catalytic activity. We now report that L-3,4-dihydroxyphenylalanine (DOPA)—a non-coded amino acid found in marine mussels—can catalyze two distinct hydrolysis reactions. Our findings attribute this activity to the hydroxybenzene moiety of DOPA. Expanding on this, we show that other hydroxybenzene-containing molecules, including tannic acid (TA), also catalyze hydrolysis of *p*-nitrophenylacetate (pNPA) and acetylthiocholine (AChI).

Catalytic efficiency correlates with the number and positioning of hydroxyl groups on the aromatic ring. Notably, TA exhibits catalytic activity at the same order of magnitude as acetylcholine esterase, with remarkable thermal stability. These findings support the potential role of simple, naturally occurring hydroxybenzenes in early catalytic systems and suggest their utility as robust, prebiotically relevant catalytic scaffolds.

References

1. Agazani, O., Tulpin, A. & Reches, M. (2021) An Individual Amino Acid as a Possible Prebiotic Catalyst, *ChemSystemsChem*, 3(3) 2100018.

2. Agazani, O., Boas, D., Tiwari, V. S., Cohen, O., Shalev, D. E. & Reches, M. (2025) Simple Hydroxybenzene Molecules as Thermally Stable Catalysts, *ChemSystemsChem*, accepted.

Using β-Sheet Peptides to Model Emergent Properties of Life

Nathaniel Wagner

Self Replicating Amphiphilic β-Sheet Peptides can be used to model various phenomena in Prebiotic Systems Chemistry related to Emergent Properties of Life. In this talk we introduce several prototype models that exhibit Fiber Growth, Replication, Isomer Selection, Chirality, Nucleopeptide Production and Hydrolysis. We briefly discuss each model and their analytical solutions and computer simulations, as well as relevant experimental results.