Backstory

An interdisciplinary effort to understand chemical organizations at the origin of life

C. Jeancolas,[1,2] A.Y. Singh,[3] S. Jain,[3,*] S. Krishna,[4,*] and P. Nghe[4,*]

This backstory features the perspectives of three group leaders of a Franco-Indian collaboration on the origin of life, involving efforts to engineer evolvable chemical systems. The researchers explain how they overcame the difficulties to bring empiricist and theorist cultures together and the importance of such synergy for the future of origin of life research.

How life originated on earth is perhaps one of the most fundamental questions that humankind has contemplated. Until the last century, trying to answer such question had mainly engaged philosophers since very little was known about the workings of living objects. Only in the last century, we gained enough knowledge with the help of appropriate technology about these workings to scientifically approach the question of their origin.

Even the simplest living cells are exceedingly complex and explaining their origin requires one to propose a process of self-organization of chemicals into a complex network of interactions. To this aim, a collaboration between Dr. Philippe Nghe, (biophysicist at ESPCI, Paris), Dr. Sandeep Krishna (theoretical physicist at NCBS, Bengaluru), and Dr. Sanjay Jain (theoretical physicist at University of Delhi, Delhi) under the grant from the Indo-French Center for the Promotion of Advanced Research (IFCPAR) studies the chemical organization and evolution of RNA autocatalytic networks.

The Paris and Delhi group visits NCBS. From left to right: Philippe Nghe, Angad Yuvraj Singh, and Shashi Thuttupalli

On the one hand, RNA is often regarded as one of the primordial biomolecules because of its ability to store genetic information like DNA and to catalyze chemical reactions like proteins can do. On the other hand, autocatalysis, that is the ability of a molecule to catalyze its own synthesis, must have been central so that chemicals could have sustained themselves and given birth to complex chemistries. In an autocatalytic network of chemical species, each member catalyzes the production of at least one other member of the network. Thus, an autocatalytic network collectively reproduces all of the members of the network giving rise to self-replication, an important property of living systems. This feature is a prerequisite to achieve Darwinian evolution thought to be an essential behavior of life, as the famous NASA definition of life states it as "a self-sustaining chemical system capable of Darwinian evolution". This evolutive dynamics is defined by the combination between reproduction with heredity, variation, and selection. Bringing together these features in a chemical system by implementing Darwinian dynamics in autocatalytic networks composed of catalytic RNA molecules (also called ribozymes) is the aim of the interdisciplinary grant from the IFCPAR.

Experimentally, the team used Azoarcus group I intron ribozymes to develop a technology for screening a large diversity of autocatalytic networks with Darwinian properties using microfluidic droplets and next-generation sequencing (Ameta et al., 2021a). This has allowed the characterization of network dynamics and study of variation in the network composition through the addition of other RNA ribozymes. The same was further validated using kinetic modeling. The rules discovered implied trade-offs between composition persistence (concentrations of different ribozymes in the solution remain unchanged) that is a prerequisite for heredity and variation, which is necessary for innovation. Thereafter, to expand the property of variation to generate novel RNA sequences, the team used the catalytic properties of Azoarcus group I intron to diversify a homogeneous population of small RNA fragments into elongated, diversely folded, and even circular RNA species along with RNA self-reproduction, all predicted by a kinetic model (Jeancolas et al., 2021). To go beyond RNA, the team also reviewed the recent literature on the efforts to implement Darwinian evolution in autocatalytic networks in various chemical reaction systems (Ameta et al. 2021b, 2022) and it has set up a unified theoretical framework to characterize all the possible autocatalytic motifs in any chemical system (Blokhuis et al., 2020). As a result, these studies pave the way to better understand the origin of life by engineering evolvable chemical systems bringing together empirical and theoretical approaches.

This backstory features the perspectives from the three leaders of this collaboration.

What are your general background and interests?

Krishna: I remember when I was in high school someone came and talked to our class about Chaos theory, Lorenz discovery, and the so-called Butterfly effect. Around then or a little later, I also remember reading some popular science books about the fascinating patterns formed in non-equilibrium systems. Ever since then, I have been interested in the nonlinear dynamics of systems very far from equilibrium. I think it was therefore almost inevitable that my research interests lie at the interface of physics and biology. After all, living organisms are the quintessential examples of such non-equilibrium systems, and they exhibit fascinatingly complex dynamical phenomena. Moreover, they also evolve over longer timescales, which itself is a nonlinear and far-from-equilibrium process. I have studied a range of biological systems ranging from gene networks within cells, to cell populations to multi-species ecosystems, with the broad aims of: (i) extracting fundamental physical principles behind the nonlinear, far-from-equilibrium phenomena seen in living systems, and also (ii) understanding the functioning and evolution of specific biological systems.

Nghe: I did my PhD on complex fluids and microfluidics. I always had an attraction for topics where there is a tight connection between theory and experiments. I then switched during my postdoc to studying evolutionary constraints in gene networks. Synthetic approaches to the origin of life lie at the frontier between soft matter and evolutionary biology, thus it was a good fit. I was lucky to engage with this field when I started my own group, as I was looking for a widely open scientific question.

Sanjay: I did my Ph.D. and postdoctoral work in the areas of string theory, quantum gravity, and statistical mechanics. Subsequently, as a faculty member at the Indian Institute of Science, I started working on the origin of life problem, specifically on models of self-organization in prebiotic chemical networks. At the University of Delhi and the Santa Fe Institute, I have been interested in the emergence of complexity in general, and also in the nature of organization of bacterial cells.

[1]Laboratoire Biophysique et Évolution, UMR Chimie Biologie Innovation 8231, ESPCI Paris, Université PSL, CNRS, Paris, France

[2]Department of Philosophy, Durham University, Durham, UK

[3]Department of Physics and Astrophysics, University of Delhi, Delhi, India

[4]Simons Centre for the Study of Living Machines, National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India

*Correspondence: jain_physics@yahoo.co.in (S.J.), sandeep@ncbs.res.in (S.K.), philippe.nghe@espci.psl.eu (P.N.)

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The Paris and Delhi group visits NCBS. From left to right: Sandeep Ameta, Yoshiya Matsubara, and Sandeep Krishna

What is the story behind this collaboration?

Krishna: During my PhD, with Sanjay as my supervisor, we had used theoretical models to explore some ways in which autocatalytic sets could spontaneously emerge and grow in complexity. However, at that time, the early 2000s, there did not exist experimental systems which were suited to test the ideas and assumptions behind these models. And this is why I had started to explore other areas at the interface of biology and physics after my PhD. This interest had been lying dormant for many years before I met Philippe.

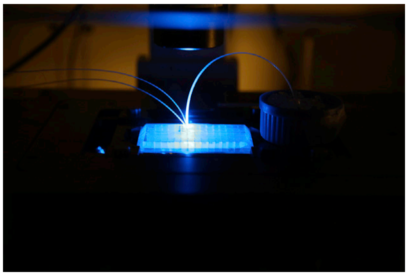
Nghe: Sandeep and I started to discuss projects of quantitative evolution of bacteria after a conference. It occurred that all of us (Sandeep, Sanjay, and I) were or had been working on the fascinating topic of origin of life, notably the idea that early evolution may have started from autocatalytic networks without template-based replication. As our expertise is complementary (theory on the one hand and experiments on the other hand), it was natural for us to start a collaboration and apply for funds.

Jain: After working with Sandeep on the origin of life question in the late 1990s and early 2000s, I was attracted to biology. It is noteworthy that Sandeep, Philippe, and I all turned to biology after working in the origin of life field, and within biology, worked, independently, on the simplest life-forms, bacteria. Studying biochemical networks in bacteria as well as bacterial physiology has undoubtedly informed our interests in the origin of life field. In particular, thinking of bacteria as complex autocatalytic sets enclosed in growing containers and describing their dynamics through coarse grained models has helped us in this project to formulate models of autocatalytic set evolution in protocells.

How difficult was putting together a team for this project? Was it difficult to get funding for this project?

Krishna: Philippe was already thinking very deeply about autocatalytic networks and Sandeep Ameta, who was already part of his team, was an expert on RNA biochemistry and the ideal person for us to learn about the Azoarcus system and its potential. What was hardest perhaps was to reorient our theoretical approaches to consider the new experimental possibilities. Were the questions we were asking many years ago the right ones? Were there other questions we should be asking? Once we received the IFCPAR grant and were able to use the Simons Foundation grant, we had at my institute to give Sandeep Ameta new opportunities to continue his work on the Azoarcus system, the collaboration was well settled.

Nghe: After a first attempt, we benefited from a French-Indian collaboration scheme supported by ministries of foreign affairs of the respective countries, called IFCPAR. We indeed felt lucky that such a fundamental project could be funded. This may be due in part to the fact that the funding scheme is firstly focused on creating bridges between teams of our two countries and the build-up of knowledge.



A microfluidic chip

How did you deal with the differences between the Indian and the French cultures (social and scientific) at one level and with those between empiricists and theorists at another level?

Nghe: The cultural aspect of the collaboration, in terms of country, has not been an obstacle whatsoever. I must say that French and Indian humor is rather similar! Regarding the experiment—theory interplay, it is always a challenge. An important parameter here is that we all had physics as a main background, where the interplay between experiment and theory is well established. The relationship between theory and experiment is however much less clear in the field of the origin of life. Indeed, most theoreticians have a background in evolution/ecology, whereas experimentalists would mostly be chemists/biochemists. Those two communities typically do not exchange, and their respective practice does not include interaction with each other's approach. In our case, the connection occurs at the level of dynamical systems, viewed from the theory of networks and biochemical reaction networks made of RNA. Understanding the details of the reactions takes time, and one has to find the correct balance between theoretical simplification for the sake of generality, while appropriately addressing the experimental system at hand.

Krishna: Between Indian and French cultures, I did not see much of an issue; I always found Philippe and Sandeep Ameta very easy to talk to and discuss with. I thoroughly enjoyed all my visits to ESPCI and my discussions with them and other students in the group. I think we also shared much in terms of our approach to science, so I never felt any difficulty in communicating or in choosing between different priorities.

Did you face challenges deciding where to publish the research findings coming out of this project and how was the decision made?

Nghe: Deciding where to publish in the origin of life field is never obvious if the project is interdisciplinary or relies on a mixture between theory and experiments. In the best case, it is possible to publish in a high-impact generalist journal, which can happen given the general interest for origin of life. However, there are not many intermediate journals, as the origin of life community is rather small and specialized journals are more confidential. Otherwise, one has to focus on a main central topic (e.g. chemistry and biochemistry) and target a journal accordingly, presenting the other aspects of the study (e.g. theoretical models) as a secondary aspect, for instance by pushing it to supplementary

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The Paris Group visits Delhi University. From left to right: Angad Yuvraj Singh, Shagun Nagpal Sethi, Camille Lambert, Cyrille Jeancolas, Yoshiya Matsubara, Philippe Nghe, and Sanjay Jain information. Intermediate interdisciplinary journals would definitely improve the visibility of the origin of life community.

Krishna: Not really, in most cases at least one of us already had a fairly clear opinion about possible journals where we could publish our results. These opinions, as is usual, are based on finding a sweet spot between the trade-offs of what readership would be most interested in, where we would get most visibility, and which journals might actually accept our manuscript.

What did you learn from this collaboration?

Krishna: I think the main surprise for me, having not really followed the literature on origin of life issues until Philippe contacted me, was just how much advance had been made on the experimental side. In addition to the autocatalytic sets made of RNAs that we use in the project, researchers have found a whole range of interesting systems with which one can explore questions about the emergence of diversity, complexity, and self-reproduction in simple but relevant chemical systems.

Nghe: Pedagogy is key in interactions when both parties have built their own vision of a field that is new to each of them, and one should not hesitate to spend time on very basic aspects. This sounds quite obvious *a posteriori*, but the initial attraction is sometimes toward novelty, at the cost of communicability between collaborators. Collaboration helps to keep contact with tangible ideas.

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What are your perspectives on: The future of the project, the interdisciplinary aspect of such collaborations, and the future of origin of life research?

Nghe: The project has been a stepping stone for further collaborations between our institutes as we manage to secure funding. We are therefore amplifying our effort to build RNA autocatalytic networks capable of evolution, combining theory and experiments. The possibility to more systematically test origin of life scenarios (or rather crucial steps of them) in the laboratory is obviously a game changer. As mentioned above, it will take time for the different disciplines to understand the relationships between their respective findings: How can the biologists relate life-*as*-we-know-it to synthetic model systems? How can the chemists approach evolutionary concepts such as heredity? How can theoreticians propose models that are detailed enough for experimental testing? Those questions

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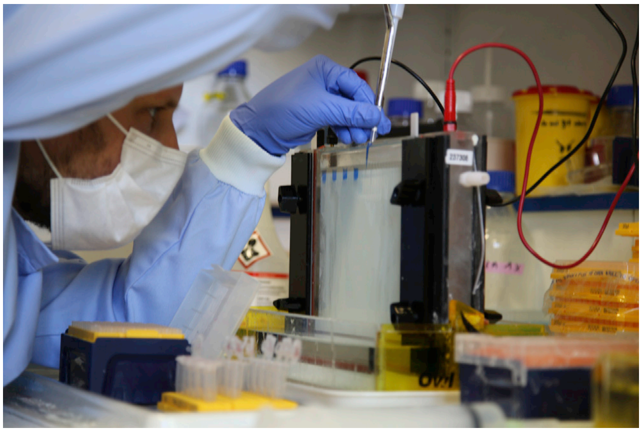

Gel loading (Mykhailo Vybornyi)

remain open. Interestingly, a strong motivation for the field arises from the rather recent discovery of exoplanets, which considerably broadens our perspective on understanding origins of life beyond the specific, historical, scenario of the origin of life on Earth. In this sense, astrophysicists have brought back to the field a universal viewpoint, in a literal sense. Hopefully, there are currently several interdisciplinary initiatives for origin of life, at the local, national, or international level. Interdisciplinary workshops will help building stronger integration between communities. Very simply, I would say that the key to progress and collaboration is to develop funding schemes that prioritize highly risky and curiosity-driven projects.

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Jain: An interesting part of this project has been the coming together of experiments on autocatalytic sets, theory tied closely to experiment, and abstract theory. This is reminiscent of disciplines like high energy physics where there exists a continuum between experiment, phenomenology, and theory. This project provided opportunities to theorists to see implementation of their ideas into experiments or help them reorient their theoretical models in line with the requirements of the experiments. Autocatalytic sets are theoretical examples of chemical systems which, in suitable environments, can spontaneously grow in "organizational complexity". The discovery or construction of simple experimental examples of these would be of great value and would guide both theory and experiments in the future. While the origin of life field needs to go a long way to achieve a real fusion of theory and experiment, it seems this is a good time to strengthen this interface. Another aspect of this project has been its interdisciplinarity. It has brought together concepts or methods that have originated in biochemistry, biophysics, dynamical systems, and statistical mechanics. It is essential to break barriers between disciplines to understand the origin of life. Life, after all, is the coming together of atoms and molecules in so many diverse ways.

Krishna: I think, as is often the case with emerging or growing interdisciplinary areas, contact and communication is key. People from different disciplines need to put in the effort to understand what is going on and how people are thinking in other disciplines and to overcome communication barriers. This should not simply be left to individual effort and enterprise; we need to push our communities and institutions to bring in structural changes that enhance such communication and contact across disciplines. By structural changes I mean, for example, changes in reward structures (e.g., recognition of the value of interdisciplinary work, recognition of the time and effort it takes to overcome interdisciplinary barriers), changes in funding policies (e.g., recognition that the direction of truly novel interdisciplinary collaboration may not always be predictable in the way grant proposal reviews often seem to expect), and changes in the way science is taught (e.g., expose students to the nature of scientific knowledge production and historical processes that have led to the emergence of old and new disciplines over time). While the future is undoubtedly interdisciplinary, I think theorists like me need to catch up on all these amazing new experimental possibilities. We have to ask more pointed questions, and look for more testable predictions from our models, while not forgetting the larger questions we are out to answer. It is a very exciting time, and I think a tight two-way interaction between theory and experiments can really lead to many new insights into the nature of the first self-reproducing entities.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Ameta, S., Arsène, S., Foulon, S., Saudemont, B., Clifton, B.E., Griffiths, A.D., and Nghe, P. (2021a). Darwinian properties and their trade-offs in autocatalytic RNA reaction networks. Nat. Commun. *12*, 842.

Ameta, S., Matsubara, Y.J., Chakraborty, N., Krishna, S., and Thutupalli, S. (2021b). Self-reproduction and darwinian evolution in autocatalytic chemical reaction systems. Life *11*, 308.

Ameta, S., Blokhuis, A., Jeancolas, C., and Nghe, P. (2022). 'Chapter 13: toward evolution in chemical reaction networks. Prebiotic Chemistry and Life's Origin. Royal Society of Chemistry, 379–423.

Blokhuis, A., Lacoste, D., and Nghe, P. (2020). Universal motifs and the diversity of autocatalytic systems. Proc. Natl. Acad. Sci. USA *117*, 25230–25236.

Jeancolas, C., Matsubara, Y.J., Vybornyi, M., Lambert, C.N., Blokhuis, A., Alline, T., Griffiths, A.D., Ameta, S., Krishna, S., and Nghe, P. (2021). RNA diversification by a self-reproducing ribozyme revealed by deep sequencing and kinetic modelling. Chem. Commun. *57*, 7517–7520.