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Participant	Title	Abstract
Vikram Alva Kullanja	On the Origin of Proteins from Peptides	Contemporary proteins arose by combinatorial shuffling and differentiation from a basic set of domain prototypes, most of which were already established at the time of the Last Universal Common Ancestor, 3.5 billion years ago. The origin of domains themselves, however, is poorly understood. We are pursuing the hypothesis that they arose by fusion and accretion from an ancestral set of peptides active as co-factors of RNA-dependent replication and catalysis (the 'RNA world'). We reasoned that if this hypothesis is true, comparative studies should allow a description of this peptide set. To this end, we compared domains of known structure using a sequence- and structure-based approach and identified 50 fragments that occur in domains of different folds, yet show significant similarities in sequence and structure. Based (1) on their widespread occurrence, (2) on their involvement in the most ancient functions (e.g. nucleic acid-binding and metal-binding), (3) on their occurrence in the most ancient folds (e.g. the P-loop NTPases and ribosomal proteins), and (4) on their enrichment in nucleic acid-binding folds, we propose that these fragments possibly represent the observable remnants of the RNA-peptide world from which the first folded domains arose.
Moritz Ammelburg	Evolution of an Enzyme	Proteins constitute the most important class of functional molecules in living matter. The domain is their main higher-order module featuring the emergent property of autonomously folding into intricate but well-defined structures. The evolution of these basic units themselves, however, is poorly understood. We identified a rather simple supers-secondary structure element, whose duplication, fusion and subsequent divergence gave rise to three different topologies of folded proteins. This homologous group is not only structurally diverse, but also functionally versatile. Through the first biochemical and structural characterization of a sequence intermediate within this group, an archaeal riboflavin kinase, we elucidate the nature of the link between DNA-binding proteins of this family and bacterial/eukaryotic riboflavin kinase enzymes. The latter differ from their archaeal counterparts in being specific for ATP instead of CTP as the phosphoryl group donor. Analyses of the amino acid sequences and the molecular architectures of both types of kinases establish the highly unusual CTP-dependence as the ancestral property. Further comparison to related transcription factors illustrates how a gain of enzymatic activity from basal DNA binding activity was accompanied by a decrease in internal symmetry. Interestingly, both groups of kinases convergently evolved toward a higher specificity for riboflavin as the phosphoryl group acceptor. Taken together our study emphasizes the importance of evolutionary intermediates in tracing the origins of structural and functional diversity of proteins.
Siddharth Hegde	Colors of Extreme Exo-Earths	The search for extrasolar planets has already detected rocky planets and several planetary candidates with minimum masses that are consistent with rocky planets in the habitable zone of their host stars. A low-resolution spectrum in the form of a color-color diagram of an exoplanet is likely to be one of the first post-detection quantities to be measured for the case of direct detection. In this poster, we explore potentially detectable surface features on rocky exoplanets and their connection to, and importance as, a habitat for extremophiles, as known on Earth. Extremophiles provide us with the minimum known envelope of environmental limits for life on our planet. The color of a planet reveals information on its properties, especially for surface features of rocky planets with clear atmospheres. We use filter photometry in the visible as a first step in the characterization of rocky exoplanets to prioritize targets for follow-up spectroscopy. Many surface environments on Earth have characteristic albedos and occupy a different color space in the visible waveband (0.4–0.9 microns) that can be distinguished remotely. These detectable surface features can be linked to the extreme niches that support extremophiles on Earth and provide a link between geomicrobiology and observational astronomy. This poster explores how filter photometry can serve as a first step in characterizing Earth-like exoplanets through its geological history, thereby prioritizing targets to search for atmospheric biosignatures.
Lorenz Keil	In situ probing of a Polymerization Trap using an Interferometer	In situ probing of a Polymerization Trap using an Interferometer Lorenz Keil, Moritz Kreysing, Christof Mast and Dieter Braun Approaching possible scenarios for the origin of life (1), the formation of polymers is crucial. It is is exceedingly difficult to create sufficient long polymers from concentrations which could have been realistic on the early earth. Since life has to be nourished from an out of equilibrium setting, suitable geological boundary conditions were explored to drive early evolution. We have suggested and demonstrated (2, 3) that thermal gradients allow the strong accumulation of molecules in bulk fluids (3). A spatial confined thermal gradient drives the combination of thermophoresis and thermal convection, resulting in the accumulating of even single nucleotides. Longer polymers are retained exponentially better (3). The combined replication and accumulation of DNA in a laser-driven thermal trap was demonstrated (4). Very recently, we could show in theory and demonstrate with sticky oligomers, that the thermal trap hyper-exponentially enforces the polymerization of long polymers. The reason is that the trap enhances the concentration of monomers, which are trapped much better after polymerization and further enhances the polymerization (5). We would like to demonstrate this effect with realistic RNA monomers and confirm the expectations provided for RNA based on measurements of thermophoresis. But the detection of small molecules without changing their physical and chemical properties pose major problems. Also, the trapping volumes used in previous studies are too small for mass spectrometry, gel electrophoresis or other detection methods. Therefore, we approach the molecule detection with a simple imaging of the refractive index inside the molecular trap using an optical interferometer. Estimations predict that RNA monomers with 5mM concentration modulate the refractive index by several wavelengths across the 200µm of optical path length. The trap is large enough to provide enough material for subsequent analysis. We will show the state of the art of this approach. The fast heating time constant on the order of some seconds allows to compare the accumulated against non-accumulated trap. Future fluorescence detection is possible. For example we approach the polymerization of cGMP (6) once we have confirmed the mechanism and can control the initial conditions for low monomer concentration without prior polymerization. Based on the theoretical treatment (5), we expect very strong enhancement of polymerization in the thermal trap. 1. Crick F (1970) Central dogma of molecular biology. Nature 227:561–563. 2. Weinert FM, Braun D (2009) An Optical Conveyor for Molecules. Nano Letters 9:4264–4267. 3. Baaske P et al. (2007) Extreme accumulation of nucleotides in simulated hydrothermal pore systems. Proc Natl Acad Sci U S A 104:9346–9351. 4. Mast CB, Braun D (2010) Thermal Trap for DNA Replication. Physical Review Letters 104:188102. 5. Mast CB, Schink S, Gerland U, Braun D (2013) Escalation of polymerization in a thermal gradient. Proceedings of the National Academy of Sciences 110:8030–8035. 6. Costanzo G, Pino S, Ciciriello F, Di Mauro E (2009) Generation of Long RNA Chains in Water. Journal of Biological Chemistry 284:33206–33216.
Moritz Kreysing	Thermal accumulation of replicating oligo-nucleotides triggers natural selection	Moritz Kreysing, Lorenz Keil, Simon Lanzmich, Christof Mast, Stephan Krampf, and Dieter Braun Systems Biophysics, Ludwig Maximilian University, Munich Central to most Origin-of-Life scenarios is the possibility for pre-biotic organic molecules to interact in order to form increasingly complex, catalytic molecular machinery ultimately capable of autonomous replication. While strong evidence for the spontaneous synthesis of single nucleotides [1] recently arose, concentrations required to allow these building blocks to polymerize [2] and gain functionality, still seem improbable for early earth conditions. Previously we demonstrated experimentally as well as in simulation [3,4] that the interplay of temperature gradients and micro-fluidics is capable of accumulating DNA nucleotides against entropic costs, while simultaneously exposing these molecules to cyclic changes in temperature that can be exploited for polymerase chain reactions. While these proof-of-principle experiments still required laser technology for the generation of thermal and convective fields, we now succeeded to show that simple temperature gradients across vertical pores, as found in rocks near submarine hydrothermal vent [4], can be sufficient to accumulate nucleotides efficiently from dilute solutions. In particular we show that depending on the pores' dimensions, it can act as a length-selective molecular filter. Therefore we suggest that equivalent out-of-equilibrium environments could have served as meeting points for long and complex molecules, too rare to find each other in a dilute primordial ocean.

Furthermore, we present direct experimental evidence that the length sensitivity of this molecular filter is capable of triggering the natural selection of replicating nucleotide chains. Interestingly, we found that the selection pressure towards longer molecules and higher complexity is strongest if the mechanism of replication has only very low efficiency.

References:
1. M. Powner et al., Nature 459 (2009)
2. G. Costanzo et al., ChemBioChem 13 (2012)
3. C. Mast et al., PRL 104 (2010)
3. P. Baaske et al., PNAS (2007)

Franziska Kriegel

Microfluidic Reconstruction of a Prebiotic Nano-Geo-Membrane

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Life is a non-equilibrium process that locally builds up structure and complexity by transforming external energy. In modern eukaryotes, mitochondria use a pH gradient across a membrane to create energy-rich compounds. What is the origin of this intriguingly complex mechanism? Very similar pH gradients are created in geological hydrothermal systems on the seafloor 4 billion years ago when life arose. Strikingly, these micrometer-thin mineral membranes at prebiotic hydrothermal vents consist of catalytic FeS cluster, which are nowadays utilized as electron transfer centers in mitochondrial membrane-proteins – an unbroken evolutionary chain from geochemistry ?

The catalytic and non-equilibrium properties of this inorganic hydrothermal membrane have yet to be tested experimentally. How can a geological non-equilibrium trigger a biochemical non-equilibrium to form the first biochemical molecules? With a high pressure, oxygen-free experiment using standard HPLC equipment, we try to emulate the anoxic conditions at the bottom of the sea on the early Earth, creating FeS membranes under well-defined microfluidic conditions.

We hope that this prebiotic FeS membrane will drive proto-biochemical reactions such as the hydrogenation of carbon dioxide and the oxidation of methane. Our experiment at the microscale under well defined non-equilibrium conditions provide the capability for simulating the environment on the Early earth in a highly defined fashion. We aim to detect under concise C13 labeling various carbon fixation products such as carbon monoxide, formaldehyde, methanol, methane thiol, acetate, amino acids and peptides online. We will show our present progress and first results from this exploratory approach.

We have shown in the past that non-equilibrium yields fascinating combinations of molecule accumulation and replication, which are important for the origin of life as well as for biotechnological applications. This basic research has led to the successful LMU spin-off NanoTemper. We are confident that pH and redox gradients will show equally rich fundamental biophysics and applications. Last but not least, research non-equilibrium carbon-fixation might not only be interesting for Origin of Life research, but could also help to understand novel ways to create renewable fuels.

Simon Alexander Lanzmich

Transfer RNA as an Exponential Replicator – a Bridge towards Translation?

The RNA-world scenario offers interesting perspectives to the question of how life could have developed under early earth conditions. Evolving systems rely on the storage and replication of genetic information. In modern biology, an interlinked machinery of proteins and RNA molecules performs these tasks. But proteins are encoded on RNA and replication of RNA requires the low error rates of proteins, posing a chicken-and-egg problem.

Here, we present an exponential replication mechanism for information stored in RNA complexes. The required non-equilibrium condition is provided by moderate temperature oscillations, which naturally occur within temperature gradients due to thermal convection. Setting could be pores within hydrothermal vents or mounds. Such systems are compatible with enhanced accumulation and enforced polymerization inside thermal traps [1,2]. The sequence of the RNA hairpins is taken from modern transfer RNA (tRNA), a class of molecules that is of general importance in all domains of life, pointing towards its ancient origin [3].

In contrast to other approaches in the field, which implement chemical base-by-base replication by ligation [4], we only rely on rudimentary sequence information and can adapt to various environmental settings. A pool of molecules replicates the information stored in a succession of RNA sequences solely by base-pairing. The energy to drive replication is stored thermally in metastable hairpins. Upon binding to the template, the hybridization of hairpins is catalyzed, creating more stable duplexes and quadruples. The catalytic template is afterward freed from the product by the temperature oscillations, triggering a cross-catalytic chain reaction and hence exponential growth of information-carrying multiplexes. The experiments show that the exponential replication mechanism is highly specific for the correct sequences and exhibits a duplication time of 30s, which equals the thermal oscillation period and is much faster than the generation of false positives in the absence of template [5].

By extending the replication scheme to double-hairpin structures, successions of more than two sequences can be replicated. The resulting complex aligns the amino acid binding sites at the 3' ends of the original tRNAs. Thus, the presented replication suggests a transition towards a first translation system.

[1] C. B. Mast et al., Phys. Rev. Lett. 104, 188102 (2010).
[2] C. B. Mast et al., PNAS 110, 8030–8035 (2013).
[3] H. Kuhn and J. Waser, Nature 298, 585–586 (1982).
[4] C. Deck et al., Nature Chem. 3, 603 (2011).
[5] H. Krammer et al., Phys. Rev. Lett. 108, 238104 (2012).

Yahai Lu

Adaptation to low H2 and syntrophic growth of methanogenic archaea

Methanogenic archaea may have been among the earth's most earliest organisms and today play the vital role in regulating the earth's climate change. Methanocella spp. are a new type of methanogens originally discovered in paddy field soil. As a group of hydrogenotrophic methanogens, a unique feature of these organisms is their adaptation to low H2 and syntrophic growth at thermodynamic limits. The adaptive mechanisms, however, remain unknown. In the present study, we determined the transcripts of 21 genes involved in the key steps of methanogenesis and acetate assimilation of Methanocella conradii HZ254. The methanogen was grown in monoculture or syntrophically with Pelotomaculum thermopropionicum (propionate syntroph) or Syntrophothermus lipocalidus (butyrate syntroph). We found that the genes coding for formylmethanofuran dehydrogenase (Fwd), heterodisulfide reductase (Hdr) and the membrane-bound energy converting hydrogenase (Ech) were markedly up-regulated under syntrophic conditions. The genes coding for Fwd, Hdr and the D subunit of F420-nonreducing hydrogenase (Mvh) form a large transcription unit and therefore the Mvh/Hdr/Fwd complex capable of mediating the electron bifurcation and connecting the first and last steps of methanogenesis was predicted to form in M. conradii. We propose that the adaptation to low H2 and syntrophic growth of Methanocella methanogens is due to the reinforcement of Mvh/Hdr/Fwd complex interaction, thus securing the coupling for the flavin-based electron bifurcation, which is perhaps the earth's earliest mode of energy conservation.

Christof Mast

Molecular Evolution Driven by Thermal Traps

Christof B. Mast, Severin Schink, Ulrich Gerland and Dieter Braun

Biopolymers like RNA, DNA and proteins are the fundamental actors in all life on earth. It is however unclear, how the first RNA polymers with enzymatic activity could arise in a prebiotic scenario: Even in millimolar concentrations, ribonucleic acids only build short polymers with a length of 20 bases. We demonstrate how a reversible polymerization process can be enhanced with the help of a simple thermal gradient [1]. Situated in an elongated compartment comparable to a hydrothermal pore it will create a convective fluid flow and also push biomolecules along the thermal gradient due to thermophoresis. The physical non-equilibrium setting of this so-called thermal trap is able to selectively accumulate longer polymers exponentially better than shorter polymers. Since the formation of longer polymers is coupled to higher local monomer concentrations, polymerization and thermal trapping are mutually self-enhancing. This process is described by a theory of trapped polymerization which we experimentally validated with the reversible polymerization of sticky-ended dsDNA blocks (monomers) in a laser-driven thermal trap. The extrapolation of the theory toward the RNA-world scenario shows that a pore height of 5 cm and a temperature difference of 10 K are sufficient to form RNA polymers longer than the shortest RNA based replicator.

In the experimental setting, the superposition of two perpendicular convection flows and thermophoresis also supported the formation of large (~100µm) and specific DNA aggregates made of polymerizing DNA monomers: The melting temperature of the aggregates and the sticky ends of the DNA monomers match. No aggregates were found using non-polymerizing monomers with randomized sticky-ends. Such specific aggregation of genetic material could have lead to the selection of sequences by their structural stabilization.

The replication of genetic molecules is central to Darwinian evolution. We demonstrate, how a laser-driven thermal trap is able to drive an exponential replication reaction via thermal cycling and at the same time protects the replication products against outward diffusion into the diluted reservoir [2]. In a proxy replication reaction, DNA replicating polymerase is able to double the amount of a 143mer product each 50 s, while the time constant for accumulation is 92 s. Thermal traps could therefore represent a possible non-equilibrium environment for the formation and replication of the first

		biopolymers - essential ingredients for the start of molecular evolution. [1] Escalation of polymerization in a thermal gradient. Mast CB, Schink S, Gerland U, Braun D (2013) Proceedings of the National Academy of Sciences 110:8030–8035. [2] Thermal Trap for DNA Replication. Mast CB, Braun D (2010) Physical Review Letters 104:188102
Yamila Miguel	Exploring spectra of Mini-Neptunes orbiting different stars: the effect of stellar flares	Ground and space based surveys resulted in the discovery of a growing number of hot mini-Neptunes. Since no such planets exist in our Solar System, their atmospheric composition and structure remains poorly understood. These planets are also interesting targets for future observations, therefore, addressing their atmospheric structure and composition is a major issue and the aim of our work. We address the differences in Mini-Neptunes atmospheres according to the observables semimajor axis and stellar type. Our models explore the detectable atmospheric features for a wide range of stellar types. Many main sequence M stars present strong chromospheric activity that produces high-energy radiation. We particularly study the effect of this radiation in hot mini-Neptunes atmospheres, simulating the effects of a flare and exploring the change in the photochemistry.
Friederike Möller	see Franziska Kriegel	see Franziska Kriegel
Matthias Morasch	Does cGMP polymerize in water?	Matthias Morasch, Johannes Langer, Dieter Braun, Christof Mast The formation of information carriers with the capability of enzymatic function was likely a crucial step towards the evolution of life. Ribonucleic acids (RNA) are a most natural molecular candidate. However, the abiotic polymerization of RNA, even when using certain chemically activated monomers, is inefficient and leads only to very short oligomers. We investigate a previously described, possibly more effective polymerization process from purely cGMP to homo-G RNA polymers in water [1]. This polymerization was argued to be initiated by monomer stacking and leads to the formation of RNA with up to 50 bases. Gel data suggests that the polymerization is favoured at millimolar concentrations of cGMP and temperatures of around 80°C. If true, the molecule would be a very good candidate for the hyperexponential polymerization enforcement of a thermal molecule trap [2]. However, for such an experiment to work, the starting point requires the preparation of non-polymerized cGMP at low concentration. Since arguable the stacking is very strong in cGMP, and putatively the polymerization from dry cGMP is so fast, new strategies need to be developed. Such preparations will equally confirm or reject the suggested reaction pathway [1][3]. If confirmed, the creation of a defined starting condition will also allow the study of the cGMP's polymerization kinetics. At this point, we could validate the experiments done by Costanzo et al. [3] using fluorescence labelling and gel electrophoresis. The next goals are to use more sensitive analytical methods such as capillary gel electrophoresis to detect the longest possible polymers. We will also discuss several new ways to probe the suggested polymerization of cGMP. The new experiments will equally likely probe polymerization from cAMP and cCMP. We hope that they will shed light into the possibly very intriguing polymerization reactions of RNA. References 1. Costanzo G et al. (2012) Generation of RNA Molecules by a Base-Catalysed Click-Like Reaction. ChemBioChem 13:999–1008. 2. Mast CB, Schink S, Gerland U, Braun D (2013) Escalation of polymerization in a thermal gradient. Proceedings of the National Academy of Sciences 110:8030–8035. 3. Costanzo G, Pino S, Ciciriello F, Di Mauro E (2009) Generation of Long RNA Chains in Water. Journal of Biological Chemistry 284:33206–33216.
Sarah Rugheimer	Spectral Fingerprints of Mini and Super Earths	Kepler is detecting a range of potentially rocky planets in the habitable zone, some smaller and some bigger than Earth. The mass and resulting gravity will change the observable spectrum for such planets - was well as our ability to detect those signature, including biosignatures, with future missions. We explore the spectral changes of Mini-Earths and Super-Earths for various stellar types in the Habitable Zone, and the detectability of biosignatures.
James Saenz	Hopanoids and the evolution of membrane ordering.	Understanding the physiology of hopanoids lies at the heart of two long-standing riddles in biology and Earth history: What function do hopanoids serve in bacteria? And what information can be gleaned from their detection in rocks, sediments, and oils? Emerging data demonstrates that hopanoids are functionally similar to sterols in their ability to order membrane lipids and to form liquid ordered domains. Liquid ordered phases are one of two biochemically active membrane states, which until now were thought to be a unique consequence of the interactions between eukaryotic membrane lipids. The formation of a liquid ordered phase was thought to be crucially dependent on the ordering properties of sterols. Since the vast majority of bacteria lack sterols, this phase was assumed to play no role in bacterial membrane physiology. However, we show that diplopterol, the simplest bacterial hopanoid, has similar ordering properties and that hopanoids are indeed bacterial “sterol surrogates”, with the ability to order saturated lipids and to form a liquid ordered phase in model membranes. These observations transform our understanding of membrane evolution, suggesting that the emergence of ordered biochemically active liquid membranes preceded the evolution of sterols. This not only suggests that liquid-ordered membranes may be common outside the Domain Eukarya, but it decouples the evolution of this trait from the requirement for molecular oxygen.
Ulrich Schreiber	Origin of Life in deep reaching gas-permeable tectonic faults – evidence for pre-biotic organic molecules in hydrothermal quartz veins from the Archaean Yilgarn province	Origin of Life in deep reaching gas-permeable tectonic faults – evidence for pre-biotic organic molecules in hydrothermal quartz veins from the Archaean Yilgarn province Schreiber1, U., Mayer1, C., Dyker2, G., Kirnbauer3, T., Sattler4, T., Schöler4, H.F., Tubbesing4, C., 1)University of Duisburg-Essen, Essen, (2)Ruhr-University Bochum, (3) Technische Fachhochschule „Georg Agricola“, Bochum, (4)University of Heidelberg (all Germany) The discussion on the origin of life encounters difficulties when it comes to estimate the conditions of the early earth and to define plausible environments for the development of the first complex organic molecules. Until now, the role of the earth's crust has been more or less ignored. First continental crustal cores may have been developed some tens to hundreds of million years after formation of earth. Due to tectonic stress the proto continents were sheared by vertical strike-slip faults at an early stage. These deep-reaching open, interconnected tectonic faults may provide possible reaction habitats ranging from nano- to centimetre and even larger dimensions that sum up to several cubic kilometres for the formation of prebiotic molecules. Their fillings consist of supercritical and subcritical waters and supercritical and subcritical gases. Here, all necessary raw materials including phosphate for the development of prebiotic molecules exist in variable concentrations and in sufficient quantities. Furthermore, there are periodically changing pressure and temperature conditions, varying pH-values, metallic surfaces, clay minerals and a large number of catalysts. While cosmic and UV-radiation are excluded, nuclear radiation intervenes the chemical evolution of the molecules inside the crust. Carbon dioxide (CO_2) is of crucial importance. It can be present in an almost pure form as a supercritical fluid ($scCO_2$) in a crustal depth less than 1 km (critical point of pure CO_2: 74 bar; 31°C). $ScCO_2$ is a non-polar solvent. Inside strike-slip faults, a two-phase system formed by supercritical CO_2 in liquid water provides the environment for condensation and polymerisation of hydrogen cyanide, nucleobases, nucleotides and amino acids. Based on these conditions, prebiotic molecules could have been condensed to long-chained molecules, from which first cell structures could have been formed by chemical evolution. According to this model (Schreiber et al. 2012) pre-biotic organic molecules could have been formed in tectonic faults of the first Archaean cratons under conditions which allow reactions similar to the Fischer-Tropsch synthesis. These faults are often documented by quartz- and ore mineralization. During the growth of these quartzes, fluids from the surrounding crust sections are enclosed which conserve the chemical composition of the given fluid medium. Therefor we have extended the study to hydrothermal quartz veins from the Archaean Yilgarn craton, as well as to hydrothermal quartz boulders from a 2.7 to 3 billion years old conglomerate near Murchison (West Australia). In one of the samples from the conglomerate, a wide spectrum of organic compounds such as bromomethane, butane, benzene and toluene have been detected. The time interval between the quartz formation, its erosion and its sedimentation is unknown. Possibly, the analyzed quartz sample was formed in a hydrothermal vein long before any living cells have existed on earth. In this case, the given result would be the first indication for pre-biotic organic chemistry. The analytical results of the oldest fluid inclusions allow for a more detailed understanding of the synthetic processes which have occurred in the rising fluids inside the fault zones and which may have led to the formation of early pre-biotic organic molecules. Based on the findings, laboratory experiments will be designed which are meant to reproduce these processes and to yield further understanding on their mechanism. Further, they should yield a collection of possible products which in the early history of earth may have formed the basis for the first biomolecules. Schreiber U., Locker-Grütjen O., Mayer C. (2012): Hypothesis: Origin of Life in Deep-Reaching Tectonic Faults. Origins of Life and Evolution of Biospheres, February 2012, Volume 42, Issue 1, pp 47-54

Michael Stich

Evolution of RNA replicator populations and simple polymerization reactions