Chemical Evolution Reimagined

Moran Frenkel-Pinter*[1,2,3,4], Kavita Matange[1,3], Vahab Rajaei[1,3], John T Costner[1,3], Adelaide Robertson[1,3], Jennifer Seoyoung Kim[1,3], Anton S Petrov[1,2,3], Jessica C. Bowman[1,3], and Loren Dean Williams*[1,2,3]

[1]NASA Center for the Origins of Life, [2]NSF-NASA Center of Chemical Evolution, [3]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400 USA, [4]Institute of Chemistry, The Hebrew University of Jerusalem, Israel 91904

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*Corresponding authors:

Prof. Loren Dean Williams
School of Chemistry and Biochemistry
Georgia Institute of Technology
315 Ferst Drive NW
Atlanta, GA 30332-0400
Ph: (+1) (404) 385-6258
Fax: (+1) (404) 894-2295
loren.williams@chemistry.gatech.edu

Dr. Moran Frenkel-Pinter
The Center for Nanoscience and Nanotechnology
Institute of Chemistry
The Hebrew University of Jerusalem
Givat Ram Campus
Jerusalem 91904, Israel
Ph: (+972)-2-6585313
Moran.fp@mail.huji.ac.il

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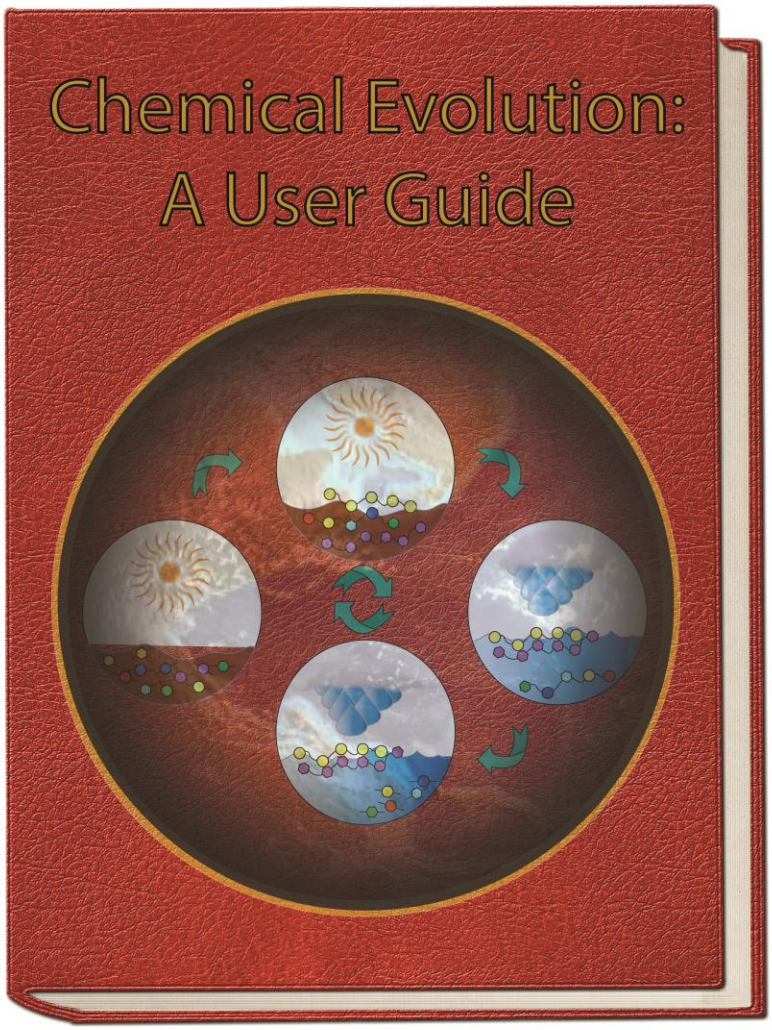

Abstract

Some of the most interesting open questions about the origins of life and molecular sciences center on chemical evolution and the spontaneous generation of new complex and functional chemical species. The spectacular polymers that underlay biology demonstrate an untapped, by modern science, creative potential. We hypothesized that prebiotic chemical evolutionary processes leading to biopolymers were not idiosyncratic one-off events. We have developed an experimental platform that accomplishes chemical evolution in the laboratory. In this paper we describe this platform and report empirical outcomes, some of which were not foreseen. We have constructed experimental platform to study evolution of chemical systems that: (i) undergo continuous recursive change with transitions to new chemical spaces while not converging, (ii) demonstrate stringent chemical selection, during which combinatorial explosion is avoided, (iii) maintain synchronicity of molecular sub-populations, and (iv) harvest environmental energy that is invested in chemical reactions. We have established general guidelines for conducting chemical evolution. Our results suggest that chemical evolution can be adapted to produce a broad array of molecules with novel structures and functions.

Introduction

Around four billion years ago, prebiotic chemistry established the molecular keystones of biology, paving the path to life (1). Chemical and geological processes on the ancient Earth caused increases in the complexity of organic molecules, leading ultimately to RNA, DNA, protein, polysaccharides, membrane-forming amphipaths and to the roots of biology.

Prebiotic chemistry presents some of the most fascinating and difficult questions in the field of chemical sciences (2-10). Here we monitor systems that change over time in behaviors that can be described as chemical evolution. We investigate the ability of environmental cycling to continuously drive chemical change. Our overall goal here is to determine if chemical evolution is a general class of processes that can be understood, directed, and repurposed (11).

Advances in our understanding of principles underlying chemical evolution have come from the field of systems chemistry (12-21). Oscillatory networks of organic reactions are sustained by compositional heterogeneity, but not by homogeneity (19). In addition, dynamic combinatorial chemistry (DCC) teaches us about chemical evolution (22, 23). In DCC, monomers (building-blocks) link and shuffle, exchanging between oligomers. DCC has achieved host–guest functionality (24, 25), elaborate folds (26) and self-replication (27). DCC is directed toward specific targets and is generally driven by equilibrium processes.

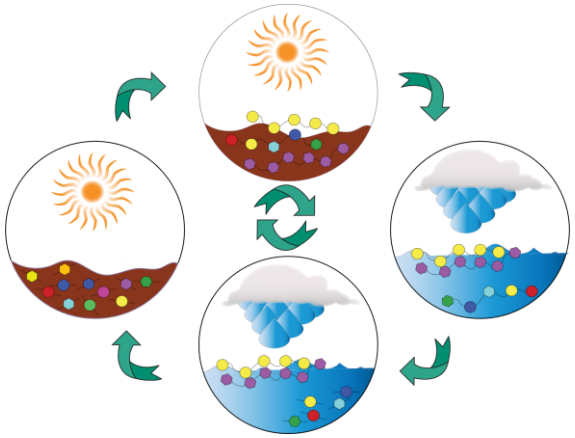

Fig. 1. **Wet-dry cycling.** During dry conditions, molecules condense to form more complex molecules. During wet conditions, complex molecules are partially hydrolyzed back to simpler molecules. Experimental wet-dry cycling mimics conditions on land surfaces of the earth.

Additional advances in chemical evolution have come from wet-dry cycling (28). Wet-dry cycling causes oscillations between linkage of building-blocks to form oligomers, and depolymerization of oligomers into smaller fragments (Fig. 1). For instance, glucose oligomerizes during wet-dry cycling to form oligosaccharides (29). Similarly, hydroxy acids oligomerize during wet-dry cycling to form polyesters (28). Mixtures of amino acids and hydroxy acids oligomerize under wet-dry cycling to form depsipeptides, which contain both ester and amide bonds (Fig. 2) (7, 30-35). Based in part on an understanding of wet-dry cycling, Hud and coworkers suggested a model of chemical progression of proto-RNA to RNA (2).

Finally, mutually catalytic systems (36) and reproducing catalytic micelles (37) reveal mechanistic information of chemical evolution. Auto-catalytic production of macrocycles has been used for selecting functional molecules (20, 38).

Chemical Evolution of Complex Mixtures in Practice. A primary goal in this work is to empirically study complex chemical systems, with a goal of establishing rules that will enable development of a robust, facile and applicable platform for performing chemical evolution in the lab. Chemical evolution is defined here as a system with molecules that exhibits continuous chemical change, during exploration of chemical landscapes. Here, during wet-dry cycling of

various complex mixtures, chemical evolution links building blocks via ester and thiol ester bonds, then converts those to amides and other linkages. The system transitions from one chemical space to another.

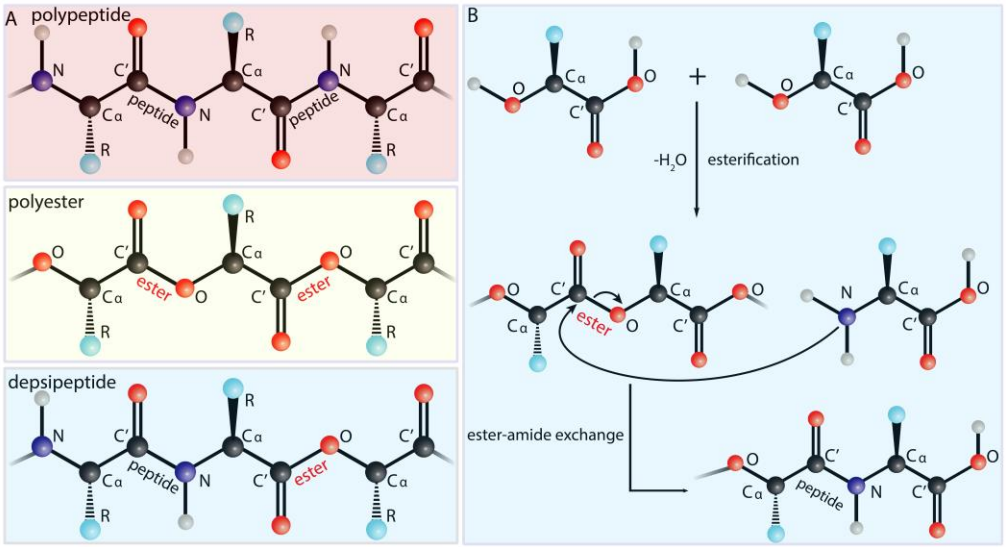

Fig. 2. A) Polypeptide, polyester and depsipeptide. B) Esters, formed by condensation dehydration reactions between hydroxy acids, are converted to depsipeptides by ester-amide exchange.

Our experimental platform suggests that chemical evolution is robust and does not require highly specific combinations of purified reagents or interventions such as purifications by chromatography. Complex mixtures, during gentle environmental oscillations, are appear to governed by general rules, some of where not foreseen. We have observed that environmental energy is harvested, molecular subpopulations are synchronized, and chemical selection is stringent such that combinatorial explosion is constrained. Exploration of new chemical species is spontaneous and gradual. By performing and characterizing chemical evolution of complex mixtures, we are broadly poised to understand and manipulate it.

Results

The experimental platform. Experiments were performed with a variety of initial mixtures containing either 2 building-blocks, 8 building-blocks or 22 building-blocks. Both one-step dry-downs and prolonged wet-dry cycling were performed. These dry-down systems are combinations of thermodynamically or kinetically controlled reactions that all share water as a reactant or product. The mixtures are nested, in that each 2 building-block mixture is a subset of the 8 building-block mixture, which is a subset of the 22 building-block mixture. Building-blocks were not constrained by prebiotic plausibly; we are investigating general chemical evolution rather than prebiotic chemical evolution. Wet-dry cycling was performed for 15 cycles where each cycle spanned two days. All reactions occurred at 45°C and were kept under anoxia to maintain reduced thiols. Reactions were monitored after each cycle by C18 HPLC-UV-Vis, LC-MS and NMR (Supplementary Figures S5-S37 and Table S1). Experimental details are described in Fig. 3. Sample HPLC chromatograms are shown in Fig. 4. Chemical characterization is described in the Supplementary Materials.

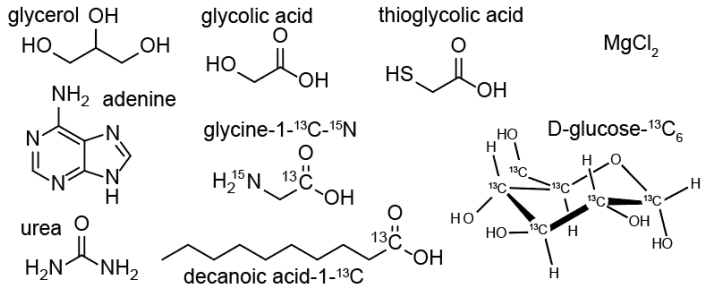

Fig. 3. **The 8 building-block mixture. Nine chemical components (called MFP Set 3) were used to initiate chemical evolution.** The building-blocks include glycolic acid, thioglycolic acid, glycine, glycerol, urea, glucose, decanoic acid, and adenine. $MgCl_2$ is not considered a building-block.

The primary focus of this report is the 8 building-block mixture (Fig. 3, MFP Set 3). This mixture is moderately complex, containing an alpha-hydroxy acid, an alpha mercaptoacid, an amino acid, urea, an aldohexose, a triol, an amphipathic long chain carboxylic acid, a purine and a hydrated divalent cation.

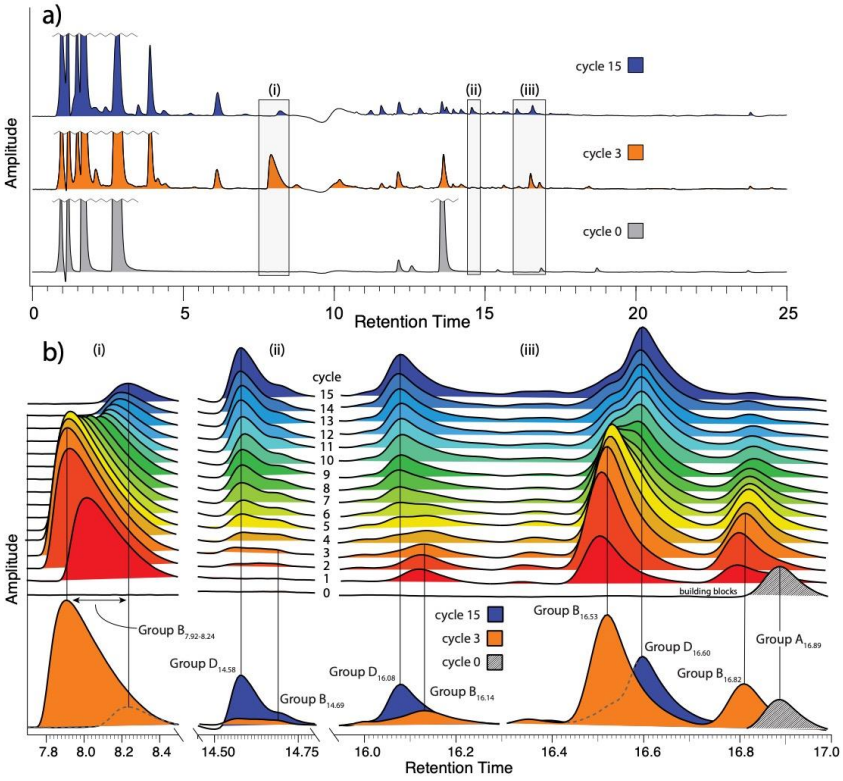

Fig. 4. a) Chromatograms of 15 and 3 wet-dry cycles, initiating with a mixture of eight species that undergoes water chemistry (starting mixture is shown as cycle 0). (b) An enlargement of select regions of the chromatogram showing all 15 cycles (top). A superimposition of the same regions of the chromatograms of cycles 0, 3, and 15 is also shown.

Observing Chemical Evolution. The realization of chemical evolution is indicated by chromatograms of 15 wet-dry cycles of MFP Set 3 (Fig. 4), and by analysis of populations and chemical structures of the molecules that compose those peaks. The chemical composition of the system continuously changes during each of 15 cycles. Every chromatogram is different from the flanking chromatographs. The system does not converge and does not approach convergence after 15 cycles. Differences between cycles 14 and 15 are at least as great as between any earlier pair of contiguous cycles. The system progresses from one chemical space to another.

Combinatorial Compression. Combinatorial explosion is an impediment to theory and practice of chemical evolution. Complex or even relatively simple mixtures undergoing chemical transformations tend to combinatorically explode (17, 39-41). The formose reaction, for example, leads to a very large number of carbohydrate species (41, 42).

We were initially surprised to observe that under the reaction conditions used here, the number of products remains low, and relationships between reactants and products are not exponential or even additive. The predicted number of products radically over-estimates observed number (Fig. 5f). In fact, increasing reactant number and diversity causes subtraction of products. We use the phrase 'combinatorial compression' to describe the low number of products and the systemic subtraction of some products when the number of reactants is increased.

Combinatorial compression was observed by comparing reactions of complex mixtures to those of simple mixtures. The 8 building-block mixture (Fig. 3) and various 2 building-block subsets of the 8 building-block mixture were dried down under the same conditions and characterized by HPLC (Fig. 5a-c). For example, a total of 15 primary product peaks were observed in two independent 2 building-block mixtures (glucose with thioglycolic acid or glycolic acid with glycerol). The 8 building-block mixture, which contains all four of these building-blocks plus an additional four, in addition to magnesium, gives only 11 primary product peaks. Ten of 15 product

peaks observed in the two 2 building-block systems have been subtracted and are absent from the 8 building-block mixture. This product subtraction is observed when comparing single-step dry-downs of the 8 building-block set with any 2 building-block subsets.

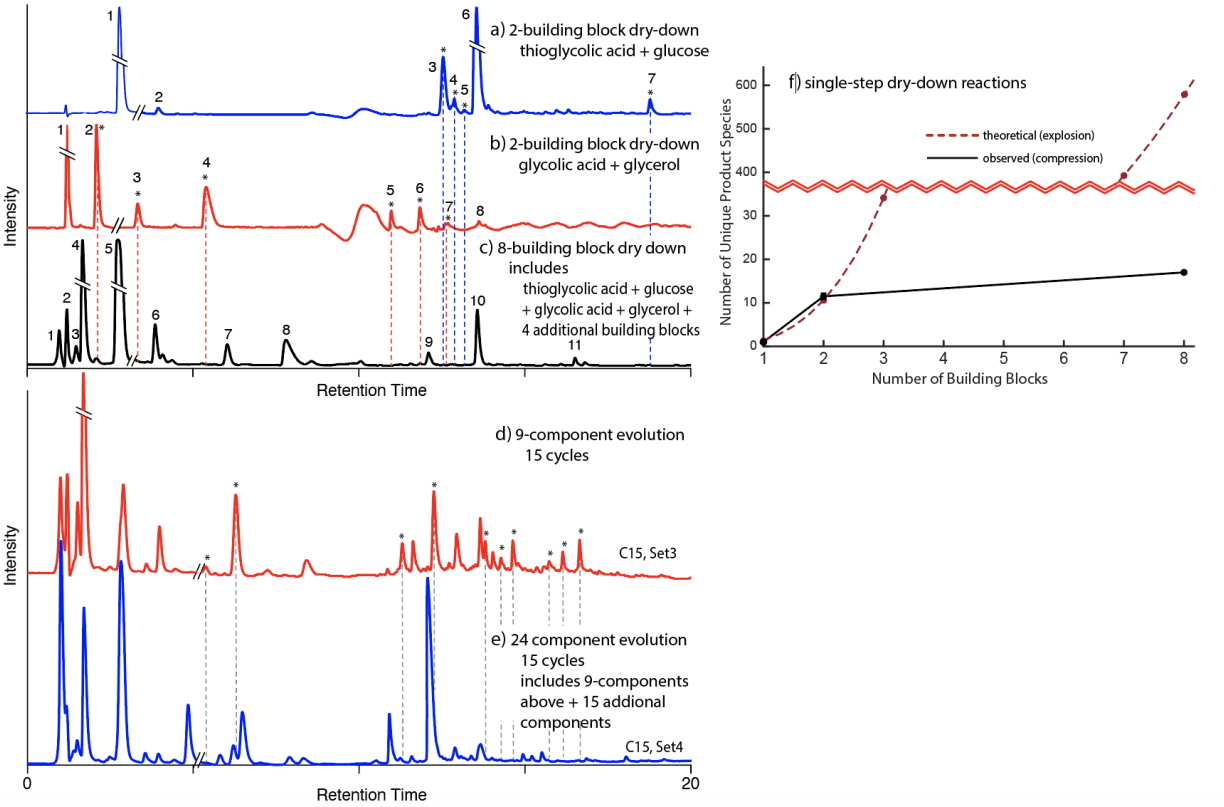

Fig. 5. **Combinatorial compression**. a) A dry-down reaction of a binary mixture of thioglycolic acid and glucose reveals 7 primary product peaks. b) A dry-down of a binary mixture of glycolic acid and glycerol reveals 8 primary product peaks. c) A dry-down reaction of a complex 8 building-block mixture that includes thioglycolic acid, glucose, glycolic acid and glycerol reveals only 11 primary product peaks. Product peaks in the binary mixtures that are absent from the complex mixture are marked with asterisks. These dry-down reactions were conducted for 72 hours at 45°C and product mixtures were separated using C18-HPLC. d) A chromatograph of the 9 component system after 15 wet-dry cycles at 45°C. Product mixtures were separated using C18-HPLC. e) A chromatograph of the 24 component system after 15 wet-dry cycles at 45°C. Product mixtures were separated using C18-HPLC. Peaks observed in the 9-component system that are absent from the 24-component system are marked by asterisks. f) Observed and theoretical number of products from single-step dry-down reactions at 45°C. Thus far, we have data for 1 building-block, 2 building-block, and 8 building-block experiments. We have measurements for multiple 2 building-block systems (error bars are SD). The theoretical number of products was calculated for length < 4 assuming no branching with each permutation being distinct.

A 2 building-block single-step dry-down reaction of glucose with thioglycolic acid gives seven product peaks. Four of seven product peaks of this 2 building-block dry-down were subtracted from the 8 building-block dry-down. Peaks 3-5 and 7 in Fig. 5a are absent from Fig. 5c. The 2 building-block dry-down of glycolic acid and glycerol gives eight primary product peaks. In the 8 building-block dry-down, six of eight 2 building-block peaks were subtracted. Peaks 2-7 in Fig. 5b are absent from Fig. 5c.

Compression is also observed during wet-dry cycling. After fifteen wet-dry cycles of the 8 building-block (MFP set 3 at 45°C, Fig. 5d) 30 primary peaks are observed in the HPLC chromatogram. By comparison, the same treatment of our 22 building-block system (MFP set 4, Fig. 5e) produces just 34 peaks in the HPLC chromatogram. Many products of the 8 building-block reaction are subtracted from the 22 building-block reaction. The general observation is that the number of product species does not increase significantly with the number of input species in single-step dry-downs or in multi-step wet-dry cycling.

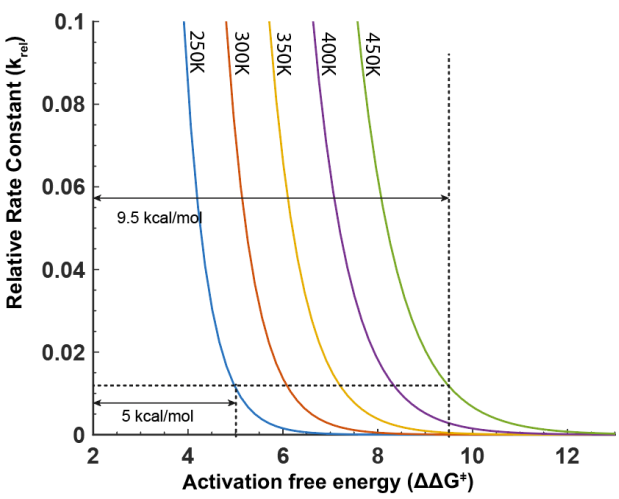

Fig. 6. Low temperature provides selectivity in $\Delta G^{\ddagger}$ constraining the number of products Shown here are activation free energy ($\Delta G^{\ddagger}$) versus relative rate constant (k_{rel}) isotherms at various temperatures computed with transition state theory [$k_{(rel)} = T(\exp(-(\Delta G^{\ddagger}/RT)))$]. Assuming that products are observed only if $k_{(rel)}$ is greater than some cutoff (say $K_{(rel)} > 0.01$), products are observed at high temperature for a broad range of $\Delta G^{\ddagger}$ from 0 to 9.5 kcal, whereas products are observed at low temperature for a narrower range of $\Delta G^{\ddagger}$ from 0 to 5 kcal.

The Basis of Combinatorial Compression. The driving forces of thermodynamically controlled reactions and the rates of kinetically controlled reactions

depend upon temperature. For thermodynamically-controlled reactions at 45°C, a small difference of around 1.5 kcal/mol in reaction free energies ($\Delta\Delta G_{rxn}$) gives 10-fold selectivity (42). Similarly, for kinetically-controlled reactions, a difference of around 1.5 kcal/mol in the activation free energies ($\Delta\Delta G^{\ddagger}$) gives 10-fold selectivity (Fig. 6). As temperature decreases, thermodynamic and kinetic selectivities rise rapidly. The temperature of our wet-dry reactions appears to account in part for differences in our results (compression) from those of previous approaches (explosion). We have observed that the compression/explosion balance depends on temperature. As temperature rises, the systems tip toward explosion. As temperature decreases, the systems tip towards compression. A second contribution to combinatorial compression would be competition between reactions for the common product or reagent. For example, Le Chatelier's principle dictates that forward progression of one reaction can cause reversal of another reaction if there are differential stoichiometries in water consumption.

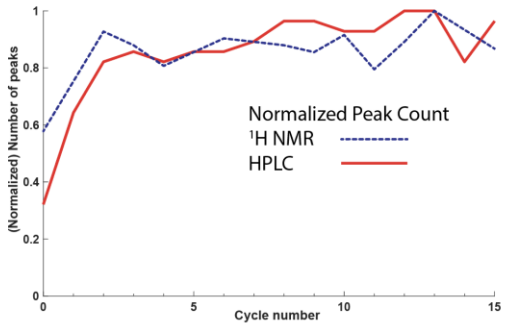

Fig. 7. Normalized peak count in HPLC and ^{1}H-NMR analyses during chemical evolution of the 8 building-block system. These bulk measurement data demonstrate that the system does not combinatorically explode during chemical evolution and that the change in number of species determined by HPLC-UV is consistent with that determined by ^{1}H-NMR.

Fig. 7 confirms combinatorial compression during wet-dry cycling using two different types of bulk measurements, consistent with proposals for analysis by Mamajanov (17). The peak counts of HPLC chromatograms and ^{1}H NMR spectra show similar behavior and remain constant after cycle 3.

Population Synchronicity. Analysis of the peak integrals of HPLC chromatograms (Fig. 4) demonstrated coordinated behavior within groups of molecules during chemical evolution (Fig. 8). The populations of ensembles of molecules were synchronized. Peaks were grouped by shared behaviors. Group A consists of building-blocks plus their immediate condensation products, which form before the first dry-down cycle (43). Group B is an ensemble of molecules that increases sharply in population during cycles 1-3 and falls in population during cycles 5-15. Group C increases in population during cycles 1-5 and remains nearly constant (or falls slightly) during cycles 6-15. Group D begins to form in cycle 3, after which it increases in population until cycle 15. Cycle 3 marks a system-side inflection point, indicated by directional changes in populations of synchronized Groups B, C, and D. At or near cycle 3, populations within Group B begin to fall, populations within Group C reach a plateau, and populations within Group D begin to increase. The increase in the populations with Group D was ongoing as the experiment was terminated at cycle 15.

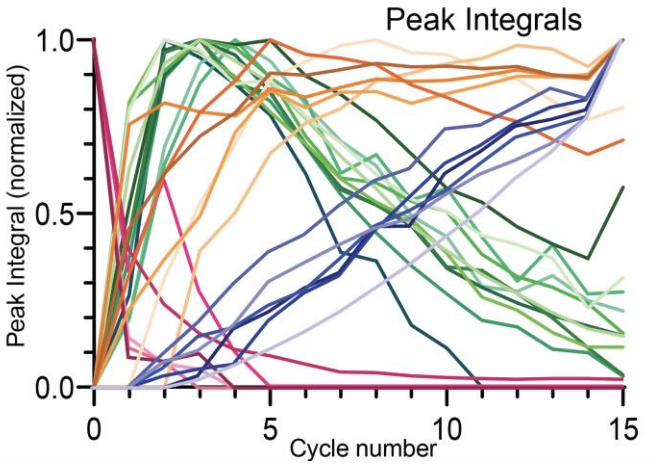

Fig. 8. Normalized changes in populations of molecules during chemical evolution. Group A molecules (red/pink) fall at various rates throughout the process. Group B molecules (green) reach a maximum population near cycle 3 and fall off over the next 12 cycles. Group C (yellow/orange) molecules increase in population curing cycles 1 to 5 and thereafter fall very slowly. Group D (blue) molecules begin increasing in population at cycle 2 or 3 and increase until cycle 15. Data were obtained by fitting of modified Gaussians to HPLC chromatograms. Peak assignments are given in the supplementary materials (Supplementary Information).

Chemical Evolution is not an idiosyncratic property of MFP set 3. Other sets of input building-blocks with lesser or greater levels of complexity also demonstrated chemical evolution. Chemical evolution does not require feeding, but as far as we know, does not exclude it. In this report we have not fed the chemically evolving systems. Five replicas of 15 cycle chemical evolution of MFP Set 3 were conducted. The process is reproducible and is deterministic.

***Chemical Spaces and Bulk Measurements*.** Even though the complex chemical nature of our platform imposes analytical challenges, extensive information regarding the nature of the products can be gathered by combining various analytical techniques, including LCMS, HPLC, NMR, and UV absorbance. We observe that some building blocks are much more reactive than others; various products were observed that contained linkages to one or more glycolic acid, glycerol and thioglycolic acid. UV absorbance spectra of various product peaks match spectra of known esters and thioesters. On the other hand, it appeared that decanoic acid and adenine were not reactive under our conditions. Notably, our chromatography technique focused on hydrophobicity-based separation, in which resolution is poor for hydrophilic species. However, due to the expected predominant chemical reactions in the system (e.g. formation of thioester, ester, and amide bonds), we have expected that most of these product peaks will be well resolved in our analytical separation, an expectation that has been met based on peak shape and deconvolution.

Discussion

We describe an experimental platform and demonstrate proof of concept of general chemical evolution. In sum, the chemical systems here can; (i) undergo continuous and recursive change with transitions to new chemical spaces, and do not converge, (ii) demonstrate stringent chemical selection, and maintain combinatorial compression, (iii) exhibit population synchronicity, and (iv) harvest environmental energy to drive chemical reactions. Some of the basic characteristics of our platform are:

General Chemical Evolution. We tested the hypothesis that certain types of chemical systems can undergo continuous molecular change, with unending progression form one chemical space to the next and the next. The progression transforms bond types, functional groups and molecular masses. During chemical evolution one ensemble of molecules is fodder for the next ensemble of molecules, which is fodder for the next, *ad infinitum*.

Ratcheting and Energy Harvesting. Chemical evolution in our system harvests energy made accessible by environment cycling of water activity. In our system, environmental cycling causes chemical ratcheting, in which a system advances toward an equilibrium state, but without reaching equilibrium, is redirected by the environmental cycle toward a different equilibrium state, then is redirected again, and again, *ad infinitum*.

Wet-Dry Cycling. Liquid water is the medium of biology, and moonlights as the chemical nexus of biochemistry. Water is by far the most frequent and abundant metabolite in biology. Between a third and a half of known biochemical reactions involve consumption or production of water (44), and all universal biopolymers and most metabolites are produced by condensation-

dehydration reactions. The centrality of water as both the medium and primary reactive species in biology is consistent with a model in which water governed chemical evolution during the origins of life. No other substance known to science that can play active roles as both a solvent with unique physical properties and as a hyperactive chemical reagent (44). During general chemical evolution water is a shared reactant, a shared product, and the reaction medium. Oscillating water activity causes near-equilibrium reactions to oscillate in direction and to ratchet in energy and complexity. Chemical evolution does not require feeding; an initial ensemble of building-blocks can undergo unceasing chemical change during environmental cycling.

Condensable Building-Blocks. We chose chemical building-blocks whose reactivity, in both thermodynamic and kinetic senses, might be dependent on water activity. Building-blocks that can covalently link by reactions that absorb water and delink by reactions that release water are candidates for general chemical evolution. Thus, building-block libraries used as input for general chemical evolution contains combinations of hydroxyl groups, carboxylic acids, thiols, amines, esters, thiol esters, amides, acetals, hemiacetals.

Selection and Fitness. General chemical evolution is performed at moderate temperatures. Relatively few products are generated from many substrates. Thus, chemical evolution demonstrates selection based on kinetic and thermodynamic landscapes. At low temperature, lower energy reaction pathways are selected over numerous slightly less favorable possibilities in a phenomenon we call combinatorial compression. We observe that complexity, as defined here by the number of species in an evolving chemical ensemble, is internally limited and does not increase with theoretical complexity.

***Emergence*.** Some systems, biological systems in particular, exhibit emergence, which is properties that cannot be predicted from those of isolated constituents (16, 45). An emergent system appears more organized than anticipated by the behaviors of isolated constituents (46). Emergence opens new chemical niches and can reshape kinetic and thermodynamic possibilities.

Population synchronicity is an example of emergent phenomena of our system. Chemical evolution causes synchronicity of populations of molecules within ensembles; the populations of members of groups of molecules rise and fall together. This synchronicity within ensembles is not predicted from the behaviors of smaller subsystems. The observed dynamics of chemical evolution here appear similar in many respects to allele frequency trajectories in the long term evolution of *E. coli* by Lenski and Desai (47).

Our hypothesis is that mature chemical selection is based on emergent properties of large molecules. These properties include folding, co-assembly and catalysis, which in turn cause additional changes in the governing landscape. If so, chemical evolution selects for molecules with elaborate structures, properties and functions, exemplified by those of biopolymers.

Future Prospects. We do not currently understand the limits of chemical evolution. What happens after 150 cycles or 15,000 cycles? In the next series of experiments, currently in progress, the number of cycles will be dramatically increased. Moreover, we do not yet understand whether chemical evolution can lead to proto-biological structures and functions.

Molecules that are formed easily but are resistant to hydrolysis by kinetic trapping are selected during chemical evolution; their populations selectively increase. For example, wet-dry cycling selects for depsipeptides that are progressively enriched in amide bonds (hydrolyze

slowly) over ester bonds (hydrolyze quickly) (31, 48-50). We hypothesize that prolonged chemical evolution will select for molecules that fold, assemble, and/or co-assemble as these phenomena confer resistance to hydrolysis (3, 51-63) and contribute to kinetic traps. For example, assembled amphipaths hydrolyze more slowly than isolated amphipaths (61). Assembled peptides hydrolyze more slowly than free peptides (55, 57). Folded RNA hydrolyzes more slowly than single-stranded stranded RNA (59). Although it has not been demonstrated here, it seems likely that general chemical evolution will also select for autocatalytic cycles (64).

The chemical species used here are of biological origin. This choice of input molecules (MFP Set 3) reflects cost, availability, and utility for general chemical evolution. The only requirement for participation in chemical evolution is an ability to hydrolyze or condense as water activity oscillates.

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