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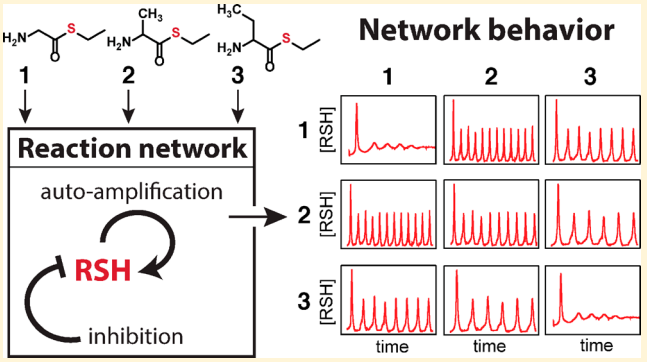
Robustness, Entrainment, and Hybridization in Dissipative Molecular Networks, and the Origin of Life

Brian J. Cafferty,[†] Albert S. Y. Wong,[†] Sergey N. Semenov,[†,⊥] Lee Belding,[†] Samira Gmür,[†] Wilhelm T. S. Huck,[∥] and George M. Whitesides*[,†,‡,§]

[†]Department of Chemistry and Chemical Biology, Harvard University 12 Oxford Street, Cambridge, Massachusetts 02138, United States
[∥]Institute for Molecules and Materials, Radboud University Nijmegen, Heyendaalseweg 1 35, 6525 AJ Nijmegen, The Netherlands
[‡]Wyss Institute for Biologically Inspired Engineering, 60 Oxford Street, Cambridge, Massachusetts 02138, United States
[§]Kalvi Institute for Bionano Science and Technology, Harvard University, 29 Oxford Street, Cambridge, Massachusetts 02138, United States

Supporting Information

ABSTRACT: How simple chemical reactions self-assembled into complex, robust networks at the origin of life is unknown. This general problem—self-assembly of dissipative molecular networks—is also important in understanding the growth of complexity from simplicity in molecular and biomolecular systems. Here, we describe how heterogeneity in the composition of a small network of oscillatory organic reactions can sustain (rather than stop) these oscillations, when homogeneity in their composition does not. Specifically, multiple reactants in an amide-forming network sustain oscillation when the environment (here, the space velocity) changes, while homogeneous networks—those with fewer reactants—do not. Remarkably, a mixture of two reactants of different structure—neither of which produces oscillations individually—oscillates when combined. These results demonstrate that molecular heterogeneity present in mixtures of reactants can promote rather than suppress complex behaviors.



INTRODUCTION

The ability to design, and thus understand, networks of molecular reactions is one goal of research on the origins of life.[1−3] How stable networks formed from simpler, individual reactions and processes, and how they persisted within changing environments and molecular compositions in what was plausibly (although not necessarily) a chaotic environment, are central problems in this field.[4−6] Characteristics of networks of reactions showing complex, cooperative behavior that would have enabled them to persist through large and irregular changes in both their environment and chemical composition have not, however, been defined.

Using a well-characterized network of organic reactions that oscillates in time in the concentration of products and intermediates, we have examined how increasing heterogeneity in the composition of reactants influenced the ability of this network to sustain its oscillations in the face of changing conditions. Introducing *mixtures* of reactants into this network demonstrated three characteristics: (i) *Entrainment*. Mixing reactants that do *not* oscillate in the network, with a reactant that does, enables the mixture to oscillate. (ii) *Increased robustness*. Networks that are heterogeneous in their composition can be more stable to changes in the environment (here, space velocity) than more homogeneous ones. (iii) *Hybridization*. Two reactants, which do not oscillate separately, can oscillate when combined. These results show that heterogeneity in molecular composition of reactants, and cooperativity between separate reactions in a network, can lead to *greater* stability in network behavior than that of simpler, compositionally homogeneous systems. Our findings thus suggest—remarkably, and counterintuitively—that the inherent lack of selectivity in chemical reactions, and the ensuing mixtures present in plausible prebiotic environments, could in fact have *aided* in the formation and development of reaction networks, rather than shifting them to greater instability and fragility.

For over 60 years, chemists interested in the chemical origins of life have synthesized molecules under model prebiotic conditions—especially reactions occurring with defined reactants, using laboratory conditions chosen largely for synthetic familiarity and convenience—and demonstrated beautiful and instructive examples of molecular possibilities plausibly relevant to the prebiotic world.[1,7−10] These results do

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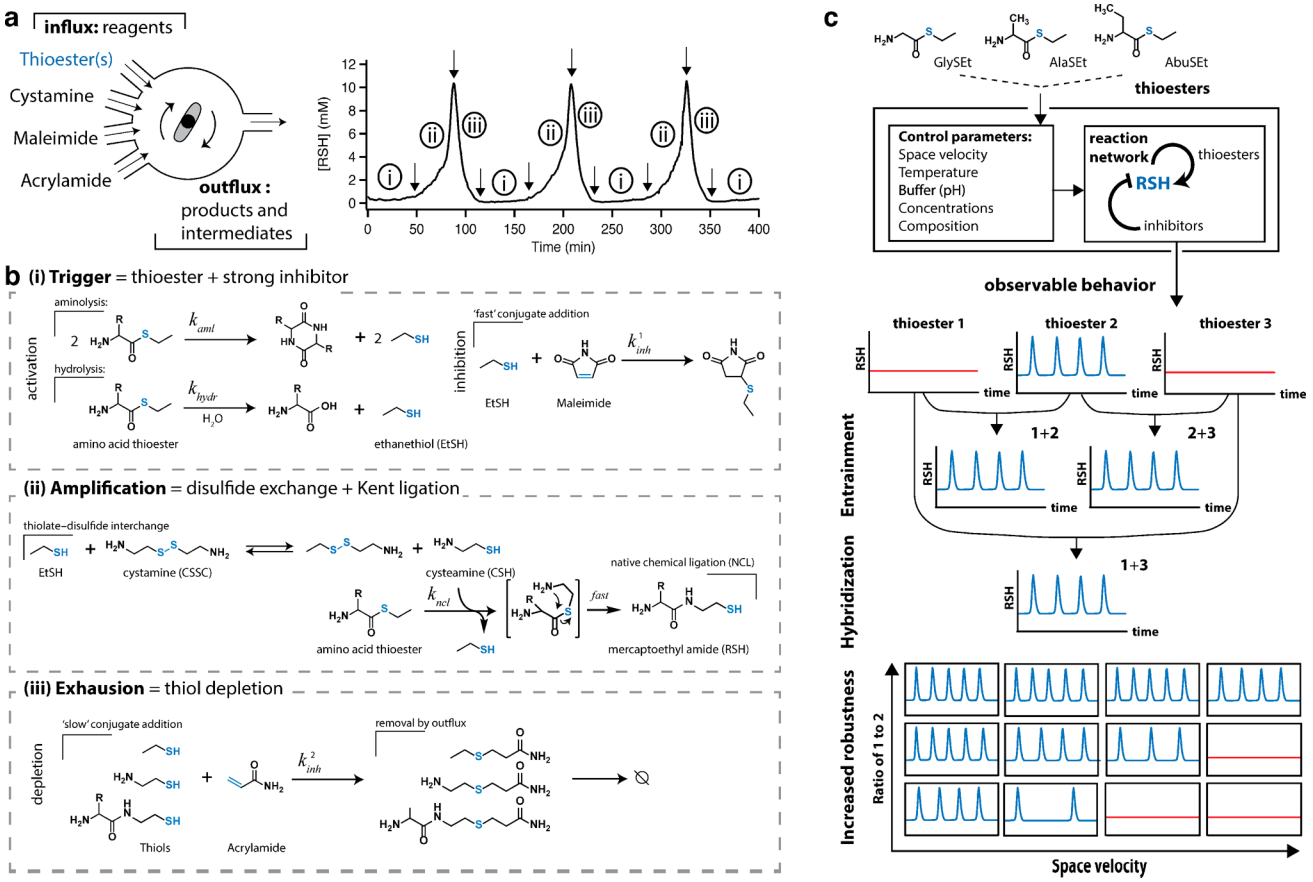

Figure 1. Overview of the network of organic reactions, and its observable behavior. (a) Schematic showing the influx of reagents (thioester(s), cystamine, maleimide, and acrylamide) and outflux of products in the continuously stirred-tank reactor, and a pictorial representation of an oscillatory network. The three chemical steps of the reaction network, (i) triggering, (ii) amplification, and (iii) exhaustion, are shown with arrows indicating transition points between steps. (b) Detailed reaction scheme of the oscillating network based on three chemical steps. The formation of ethanethiol in the triggering step occurs either through hydrolysis (k_{hyd}), or through aminolysis (k_{aml}). The formation of mercaptoethyl amide in the amplification step occurs through native chemical ligation (k_{ncl}). Thiol is consumed through reaction with maleimide (k^1_{inh}) during the triggering step, and acrylamide (k^2_{inh}) during the exhaustion step. (c) Schematic representation of glycine ethyl thioester (GlySEt), L-alanine ethyl thioester (AlaSEt), and L-α-amino butyric acid ethyl thioester (AbuSEt). The diagram shows control parameters that can be manipulated to influence the system. Observable behaviors include stable oscillations (blue line) and steady states (red line). Examples of entrainment, hybridization, and increased robustness of oscillatory behavior are shown with a set of three thioesters, two that do not oscillate (1 and 3) and one that does oscillate (thioester 2). Increased robustness is shown as the ability of heterogeneous networks (as compared to more homogeneous ones) to oscillate over larger ranges of space velocities and to maintain frequencies of oscillation that are less sensitive to change in space velocity. (The oscillations are pictorial representations and do not perfectly reflect the behavior of oscillation in thiol concentration that was observed experimentally.)

not (in our view) mimic either the probable molecular or environmental heterogeneity of that world, or its dynamic (as opposed to steady-state) aspects. What has emerged from this body of work is a number of theories of the origin of life, which propose that life emerged spontaneously from the self-assembly, or spontaneous organization, of the organic products of reactions, occurring in complex mixtures of molecules formed abiotically from simple precursors and sequences of reactions.[11−16] How these reactions form networks depends on the principles (reactivity, concentration, mass and heat transport, association with or dissociation from surfaces, irradiation, spatial and temporal gradients in free energy, and others) that govern the self-assembly of dissipative processes[17] and how their interactions might have led to the stable (or persistent) networks of processes characteristic of metabolism.[6,18]

"Robustness" of reaction networks—which we define for this work as stability of a property or function to changes in molecular composition and in reaction conditions—is centrally important to understanding the emergence of complex and time-dependent systems,[19,20] including those—we presume—present prebiotically. Robustness will thus constrain the range of compositions and reaction conditions that allow the spontaneous emergence of dynamic complexity from quasi-equilibrium simplicity or steady states.[21] We ask: Under what conditions do chemical reactions in complex mixtures organize spontaneously into robust networks capable of persistence and adaptation under changing conditions, and ultimately of chemical evolution?

We are investigating the behavior of model networks that oscillate in the concentration of products and intermediates (Figure 1a). The ability of a reaction network to generate a collective behavior (here, oscillation) provides an experimentally tractable method of characterizing the collective behavior of the network (rather than of its individual components). "Oscillation" is not, by itself, a necessary condition for the emergence of the networks of metabolism, but is—in our view—arguably sufficiently similar in complexity and dynamic

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behavior to small sub-components of metabolism that it provides a useful model for a peri-biotically relevant dissipative network of reactions. The network examined in this work (and characterized previously[22]) generates amides by the reaction of amines with thioesters—a type of reaction specifically relevant to non-ribosomal peptide synthesis (for example, of gramicidin, bacitracin, and vancomycin)[23] and chemically related to the synthesis of fatty acids and polyketides.[24] We have designed this system so that it incorporates an auto-amplifying step, and—when carried out in a continuous stirred-tank reactor (CSTR) intended to model the flux of reactants, intermediates, and products in metabolic networks across the boundaries of a protocell—oscillates.

The chemical oscillator can be separated into three sequential chemical steps (Figures 1b). (i) "Triggering", which includes formation of ethanethiol from thioesters by hydrolysis and aminolysis and destruction by subsequent conjugate addition to maleimide. Initiation of the amide-forming reaction occurs upon depletion of maleimide in the reactor. (ii) "Auto-amplification", which involves the autocatalytic formation of cysteamine and an amino acid amide produced from native chemical ligation (Kent ligation[25]). (iii) "Exhaustion", which depletes thiols by their conjugate addition to acrylamide. After the exhaustion step, products and reactants are removed from the reactor by mass transport (e.g., flow through the CSTR), the reactor refills with fresh reactants, and a subsequent triggering step reinitiates the cycle of reactions. The behavior of the network is influenced by space velocity (s^{-1}, defined as the ratio of flow-rate to reactor volume, and is the reciprocal of the mean residence time), concentration of reactants, chemical composition of reactants, pH of the solution, and temperature. To monitor the network, we follow the concentration of thiols as they leave the CSTR by allowing them to react with Ellman's reagent, and quantify the labeled thiols by UV−vis spectrophotometry (Figure S1).

We have examined the behavior of this network to changes in the structure of the thioester, and to changes in space velocity (Figure 1b). We examined L-alanine ethyl thioester (AlaSEt), glycine ethyl thioester (GlySEt), and L-α-amino-butyric acid ethyl thioester (AbuSEt)—a series that enable us to use physical-organic approaches[26,27] to extend our studies across a series of related molecular structures. We focused on the effect of changes in the composition of the thioester, because this component is central to the formation of thiols in both the triggering (determined by the first-order rate of hydrolysis k_{hyd} and the second-order rate of aminolysis k_{aml}) and auto-amplification (determined by rate of native chemical ligation k_{ncl}) steps (Table 1). The values of k_{hyd} and k_{ncl} for AbuSEt and GlySEt respectively are greater than and less than those for AlaSEt, and only GlySEt was found to form ethanethiol by aminolysis (Figure S2). Surprisingly, studies of these three thioesters demonstrate that *heterogeneity* in the components of this reaction network can stabilize network behavior by entrainment, increased robustness, and hybridization.

Table 1. Rate Constants for the Formation of Ethanethiol by Hydrolysis (k_{hyd}) and Aminolysis (k_{aml}), and Formation of Thiol by Native Chemical Ligation (k_{ncl})

thioester	k_{hyd} (s^{-1}, $\times10^{-5}$)	k_{aml} (M^{-1} s^{-1}, $\times10^{-2}$)	k_{ncl} (M^{-1} s^{-1})
GlySEt	9.0	1.7	1.50
AlaSEt	1.1	n.d.[a]	0.47
AbuSEt	0.37	n.d.	0.23

[a]n.d. stands for "not determined" because aminolysis was not observed.

RESULTS AND DISCUSSION

A reaction network containing AlaSEt as the only amino acid thioester, cystamine, maleimide, and acrylamide (see caption of Figure 2 for concentration of each compound) oscillated in the concentration of thiol compounds.[22] Sustaining these oscillations depended delicately on the reaction conditions, and oscillation occurred over a limited range of space velocities (between 1×10^{-2} and 2×10^{-2} min^{-1}). The system did not oscillate using either GlySEt or AbuSEt. This inability to oscillate is not unexpected, because reaction conditions (concentration of reactants, temperature, pH, and space velocity) were originally selected to form an oscillatory network specifically with AlaSEt, and these conditions are not compatible with substitution by AbuSEt or GlySEt.

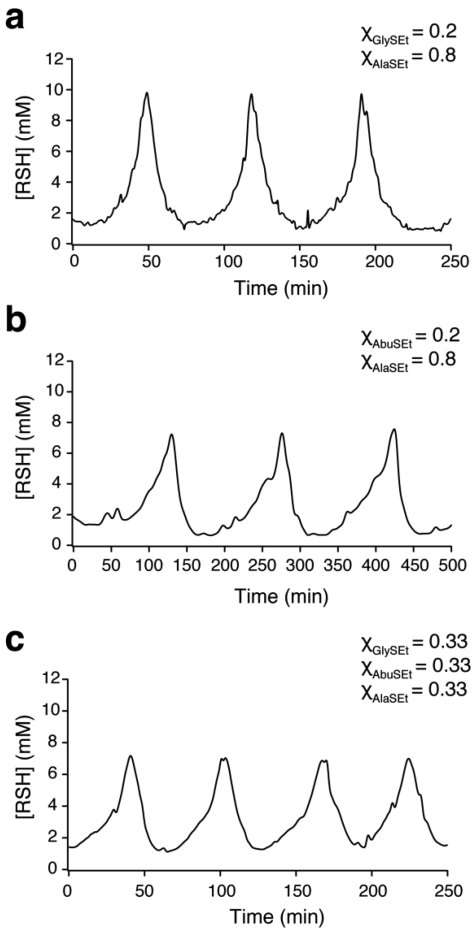

Figure 2. Examples of oscillation in concentration of thiol formed from mixtures of (a) AlaSEt (χ_{AlaSEt} = 0.8) and GlySEt (χ_{GlySEt} = 0.2), (b) AlaSEt (χ_{AlaSEt} = 0.8) and AbuSEt (χ_{AbuSEt} = 0.2), and (c) AlaSEt (χ_{AlaSEt} = 0.33), GlySEt (χ_{GlySEt} = 0.33), and AbuSEt (χ_{AbuSEt} = 0.33). The space velocities were 1.9×10^{-2} min^{-1} for the experiment with AlaSEt and GlySEt, 1.4×10^{-2} min^{-1} for the experiment with AlaSEt and AbuSEt, and 3.6×10^{-2} min^{-1} for the experiment with AlaSEt, GlySEt, and AbuSEt. Initial reaction conditions were $[thioester]_{total}$ = 46 mM, [cystamine] = 92 mM, [maleimide] = 10 mM, [acrylamide] = 320 mM, 1 M potassium phosphate buffer pH 8.0, 28 °C.

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Oscillations were not observed when networks were examined with either AbuSEt or GlySEt at 46 mM (the same concentration of thioester used for the network with AlaSEt). Using simulations, we screened a range of conditions (in initial concentration of thioester, and in space velocity) and evaluated the ability of AlaSEt, AbuSEt, and GlySEt to oscillate on their own. The phase plots in Figure S3 shows that only AlaSEt can sustain oscillations at the concentration of thioester used in this study (46 mM and below).

Entrainment of "Non-oscillatory" Components by an "Oscillatory" Component. Although neither AbuSEt nor GlySEt oscillated on its own, we tested the ability of networks containing *mixtures* of AlaSEt and AbuSEt, or AlaSEt and GlySEt, to oscillate, a process that we call entrainment (see Supporting Information for discussion). The total concentration of thioester entering the reactor was the same as for the previous experiments ([thioester] = 46 mM). Networks with mole fractions of GlySEt (χ_{GlySEt} = $[GlySEt]_0/([GlySEt]_0$ + $[AlaSEt]_0)$) from 0.1 to 0.4 did, in fact, oscillate, as did networks with mole fractions of AbuSEt (χ_{AbuSEt} = $[AbuSEt]_0/([AbuSEt]_0$ + $[AlaSEt]_0)$) from 0.1 to 0.4 (Figures 2, S4, and S5). The entrainment observed in these systems is also limited in concentration: networks with mole fraction of GlySEt or AbuSEt at, or above, 0.5 did not oscillate. Mixtures containing equal concentrations of AlaSEt, GlySEt, and AbuSEt also oscillated (Figure 2c). The network did not oscillate when AlaSEt at a mole fraction of 0.7 was examined alone over a range of space velocity ($1. \times 10^{-2}$ min^{-1} to 2.0×10^{-2} min^{-1}) that supported oscillation for the heterogeneous networks (Figure S6). Numerical modeling also shows that networks containing AlaSEt alone at mole fractions below 0.7 will not oscillate (Figure S3). These findings demonstrate that two or more thioesters are required to oscillate under these conditions.

Increased Robustness of Heterogeneous Networks. Using networks with only AlaSEt and mixtures of AlaSEt and GlySEt, we examined the robustness of the oscillatory state against change in one important parameter—the space velocity—by incrementally increasing space velocity and evaluating its effect on the behavior of the network (Figures 3 and S5). Surprisingly, the range of space velocities that support oscillation increased by more than a factor of 3 as χ_{GlySEt} increased from 0 to 0.4. The change in the period of oscillation with change in space velocity for the network with mixtures of AlaSEt and GlySEt is also smaller than the network with AlaSEt alone: that is, the frequency of oscillation of the network stabilizes with multiple reactants. As χ_{GlySEt} increased from 0 to 0.4, the sensitivity of the period to the change in space velocity decreased by a factor of 8 (Figures 3c and S7). The dynamic behavior of these mixed networks is thus, up to a point, better preserved against an environmental change (here, space velocity) than networks with a single thioester, a characteristic that is similar to that of the homeostasis present in metabolic networks. Networks containing combinations of AlaSEt and GlySEt are more robust than those with only AlaSEt in two ways: (i) they oscillate over a larger range of space velocity, and (ii) their frequency of oscillation is less sensitive to changes in space velocity.

Simulations. To clarify the origin of robustness in the heterogeneous network, we examined the influence of the thioester component on the triggering step by simulating networks using a set of differential equations (see Supporting Information for details on how simulations were performed).

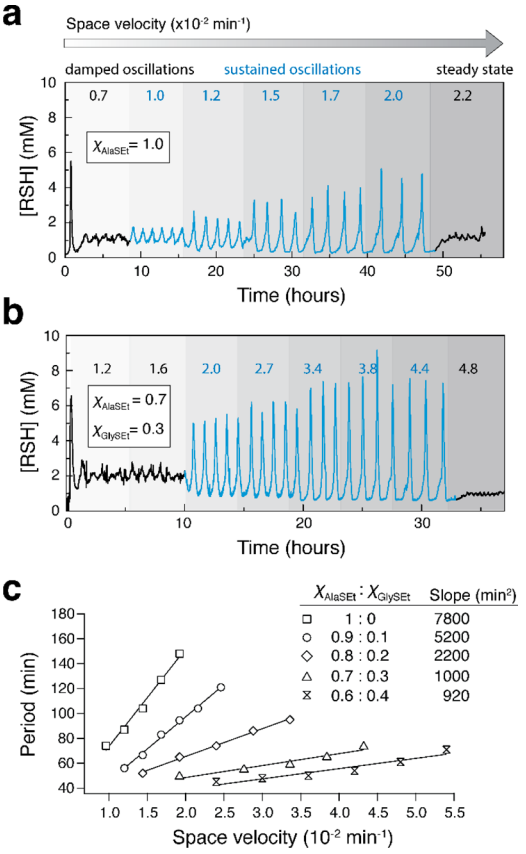

Figure 3. Increased robustness against change in space velocity. Examples of experimental data obtained for experiments for which the space velocity (listed above the trace) was changed incrementally for (a) a network containing only AlaSEt and (b) a network containing χ_{GlySEt} = 0.3 and χ_{AlaSEt} = 0.7. For each mixture, three states of the network were observed as space velocity was increased over time: dampened oscillation, stable oscillations, and a non-oscillatory steady state. Stable oscillations are shown in blue. The space velocity was increased incrementally for each experiment over time with values (in units of 10^{-2} min^{-1}) listed in the plot. (c) Influence of space velocity on the stability and period of oscillation for networks with different mole fractions of GlySEt and AlaSEt (labeled in plot). Lines are linear fits of the data and emphasize trends (their slopes are proportional to the sensitivity of the network to change in space velocity). Values of the slopes of these fits are shown in the plot. Initial reaction conditions are given in Figure 2. Experiments were performed in triplicate.

Specifically, we compared the rate of formation of ethanethiol through hydrolysis in networks with a single thioester (with different values of k_{hyd}) to networks with mixtures of AlaSEt and GlySEt (Figure 4). Figure 4a shows the influence of the rate of formation of ethanethiol on the stability of networks that have a single thioester (gray areas represent combinations of k_{hyd} and space velocity that oscillate). This plot shows that larger rates of formation of ethanethiol are required to support oscillation at larger space velocities. Larger rates of formation of ethanethiol do *not*, however, significantly increase the range of space velocities that support oscillation in networks with a single thioester.

In contrast, numerical simulations of networks with AlaSEt and GlySEt show that the range of space velocities that support oscillation increases, up to a point, with an increase in χ_{GlySEt} (Figures 4b and S8). These simulations suggest that the increased robustness observed with heterogeneity reflects two

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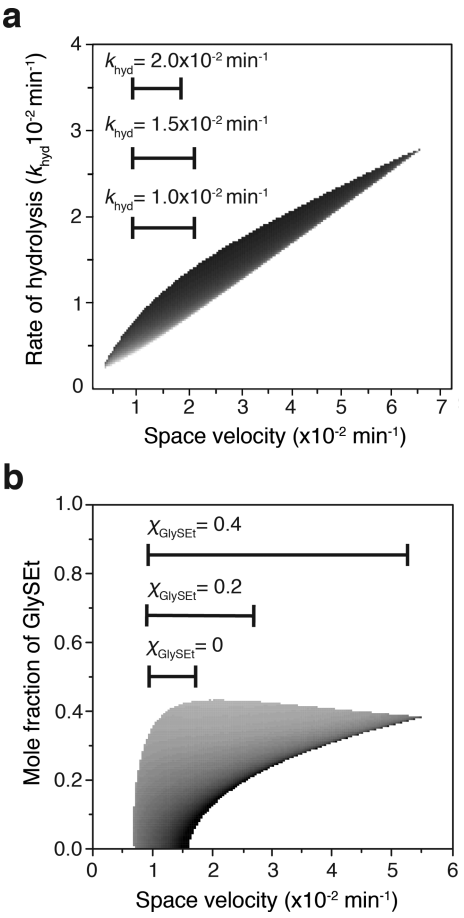

Figure 4. Influence of rate of formation of ethanethiol and space velocity on the oscillatory behavior of the network. (a) Numerical simulations showing the dependence of the rate of formation of ethanethiol by hydrolysis (k_{hyd}) during the triggering step on the range of space velocities that support stable oscillations. The space velocities that form stable oscillations are shown in gray, and conditions that do not show oscillation are shown in white. Grayscale shows how the period changes with space velocity (darker color indicates longer period). Bars show the length of the range of space velocities that form oscillations for three values of k_{hyd}. (b) Plot of simulations showing the influence of χ_{GlySEt} on the stability of the network with GlySEt and AlaSEt at different space velocities. Bars show the length of the range of space velocities that support oscillation for three values of χ_{GlySEt}. See Supporting Information for the list of ordinary differential equations used in simulations.

complementary factors that govern formation of ethanethiol during the triggering step: (i) At larger space velocities, the concentration of both thioesters in the reactor increases more rapidly during the triggering step than at smaller space velocities (Figure S9). (ii) Formation of ethanethiol from GlySEt is more sensitive to changes in concentration than from AlaSEt, as it is second-order in the concentration of GlySEt (k_{aml}[Gly]^2). Thus, increasing space velocity increases the rate of formation of ethanethiol from GlySEt more than it does from AlaSEt (Figure S10), and this increase enables these heterogeneous networks to compensate for changes in space velocity better than networks with a single thioester. These factors are also responsible for a decrease in the sensitivity of the period of oscillation to changes in space velocity for heterogeneous networks, because increasing the rate of formation of ethanethiol decreases the period of oscillation (Figure S11).

For networks with AlaSEt alone, the transition point between oscillatory and steady-state behavior at low and high limiting space velocities are represented by Hopf and fold bifurcations, respectively.[22] To understand the nature of these bifurcations for the mixed network, we performed linear stability analysis on a kinetic model of this network with a reduced set of differential equations (see Supporting Information for a description of this analysis). Stability analysis of the mixed network shows that an increase in the value of χ_{GlySEt} from 0 to 0.4 shifts the fold bifurcation to larger space velocity, as a result of the increased rate of formation of ethanethiol. Unexpectedly, the Hopf bifurcation was found *not* to represent the lower limit of the oscillatory state of the mixed network. Instead, sustained oscillations coexist with a stable steady state at space velocities below the Hopf bifurcation (that is, the observed oscillations are likely to result from a subcritical Hopf bifurcation);[28] at these space velocities, the initial conditions of the network (e.g., concentration of thioester and thiol) determine network behavior (Figures S12 and S13, see Supporting Information for discussion).

These results demonstrate that heterogeneous networks can dynamically compensate for changes in their environment through two processes: (i) through a cooperative process in which the contribution of each thioester to the rate of formation of ethanethiol changes non-linearly with changes in space velocity, and (ii) through the appearance of a qualitatively new phenomenon—coexistence of both a locally stable steady state and an oscillatory state.

Hybridization: Oscillation in a Mixture of Individually "Non-oscillatory" Components. Examination of networks with AlaSEt and GlySEt suggested that the individual thioester components could make complementary and compensatory contributions to robustness. The thioester with the larger rate of formation of ethanethiol contributes to the production of thiol most significantly during the triggering step, and the thioester with the smaller rate of formation of ethanethiol contributes most during the auto-amplification step. This complementarity suggests a new mechanism to form oscillatory networks: combining two "non-oscillatory" components contributing complementary behaviors (a network behavior we call *hybridization*, see Supporting Information for discussion).

Because the rates of formation of thiol during the triggering and auto-amplification steps for AbuSEt and GlySEt are respectively greater than and less than those for AlaSEt (Table 1), networks that *combine* the two thioesters could—in principle—provide compensatory contributions to the processes of these reaction steps. To define a molar ratio of GlySEt and AbuSEt that would be most likely to oscillate, we chose a combination of these reactants with a rate of formation of ethanethiol that is similar to AlaSEt. To do so, we used kinetic experiments to match the reaction profiles—specifically, the length of time before auto-amplification—for the formation of amide products by AlaSEt and cystamine to reactions that substitute AlaSEt for mixtures of AbuSEt and GlySEt. Kinetics experiments with cystamine and equal concentrations of GlySEt and AbuSEt show that auto-amplification is initiated at a time similar to that observed with cystamine and AlaSEt (Figure 5a). This result suggested that networks with a 1:1 mixture of AbuSEt and GlySEt would have rates of formation of ethanethiol during the triggering

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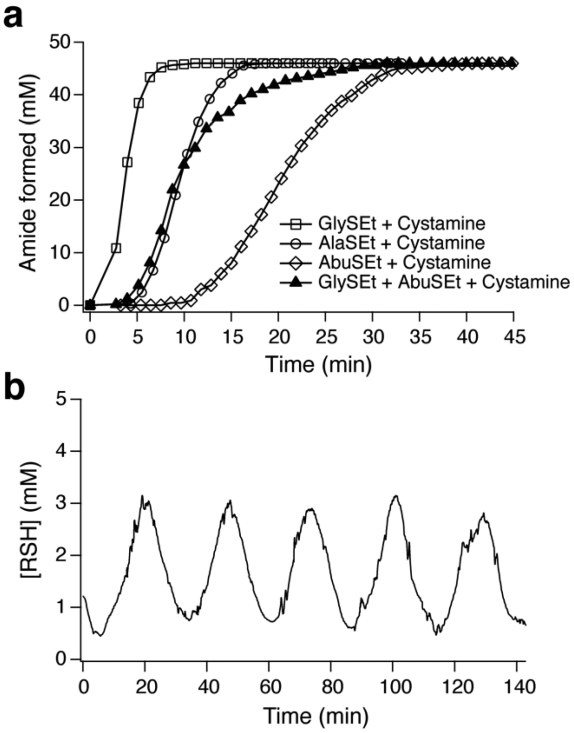

Figure 5. Hybridization of two "non-oscillatory" thioesters. (a) A kinetic plot from (^{1}H NMR) showing the formation of the ethanethiol amide product in autocatalytic reactions between the thioesters and cystamine. Data are for AlaSEt, GlySEt, and AbuSEt individually and a mixture containing equal concentrations of GlySEt and AbuSEt. Reaction conditions were $[\text{thioester}]_{\text{total}}$ = 46 mM, [cystamine] = 46 mM, [sodium phosphate] = 500 mM, pH 7.5, 25 °C, D_2O. (b) Oscillations for a network containing equal concentrations of AbuSEt and GlySEt. Reaction conditions were [AbuSEt] = 23 mM, [GlySEt] = 23 mM, [cystamine] = 92 mM, [maleimide] = 10 mM, [acrylamide] = 320 mM, [potassium phosphate] = 200 mM (pH 8.0), 28 °C, and a space velocity of 0.072 min^{-1}.

step similar to networks including only AlaSEt. Networks with a 1:1 mixture of GlySEt and AbuSEt did in fact oscillate (Figure 5b), even though neither GlySEt nor AbuSEt oscillates as a single component. Kinetics experiments show that maleimide is consumed almost entirely by ethanethiol formed from GlySEt at the concentration of maleimide and thioesters examined in the network (Figure S14). These observations suggest a third characteristic of heterogeneity: multiple reactions can act in a complementary way to yield complex behaviors.

CONCLUSIONS

This work demonstrates that heterogeneity can stabilize a complex behavior in a network (that is, it can make the system more robust to changes in reactants and environmental conditions than the same network operating with pure components) rather than destabilizing it. It demonstrates that (i) AlaSEt (an "oscillatory" thioester) can entrain GlySEt and AbuSEt ("non-oscillatory" thioesters) to form oscillatory networks (entrainment); (ii) a combination of GlySEt with AlaSEt can both increase the range of space velocities that support oscillation and increase the stability of the period of oscillation against changes in space velocity (increased robustness); and (iii) a network of GlySEt and AbuSEt—neither of which produces oscillations individually—can oscillate (hybridization). These results establish that heterogeneity present in *mixtures* of structurally similar amino acid thioesters can increase the robustness of the network, because each can contribute to the characteristics of the network in compensatory ways. They suggest, more broadly, that when a network of reactions operates under variable external conditions, different components of the network can compensate for one another in maintaining a complex behavior, and thus increase the robustness of the network.

Interest in robustness extends across many areas of science and technology involving networks.[19,29−31] Mathematical analysis has helped to understand the robustness of biochemical networks,[32−36] but often fails to appreciate the details of the chemistry that defines these, and other, molecular networks. The ability to understand reaction networks fully (especially those that occurred before the emergence of nucleic acids, enzymes, and other organic catalysts), and to create new systems that incorporate only relatively simple molecules, requires a firm understanding of the characteristics of simple networks. The work presented here demonstrates a new approach—one focused on understanding the contribution of individual reactions on network behavior. It shows the counterintuitive behaviors that emerge from examination of the self-organization and adaptation of simple networks to changes in reactants and conditions.

The reactions taking place in this network (amide formation, thiol-thioester exchange, and disulfide exchange) are relevant to reactions during the origin and early development of life for at least two reasons: (i) Thioesters were almost certainly involved in the prebiotic formation of many molecules that are important in contemporary biology.[1,37,38] (ii) Auto-amplification and autocatalysis are strong candidates for processes that would have generated high local concentrations of molecules important for early biological systems.[39,40] "Oscillation" is not a property obviously required for the networks that became "metabolism", but there are many mechanistic similarities between the two (e.g., feedback, delay, switching of network processes). By studying simple networks composed of types of reactions that are relevant to prebiotic chemistry, we provide evidence for an unexpected mechanism——where reaction networks are maintained by compensating rates of reactions between different constituents of a mixture——that could have supported the emergence and adaptation of networks of reactions in the heterogeneous (in molecular composition and in reaction conditions) pre- and peri-biotic Earth.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.9b02554.

Synthesis of compounds, flow reactions, analysis methods, list of differential equations, mathematical modeling, additional discussion, and supporting Figures S1−S14 and Table S1 (PDF)

AUTHOR INFORMATION

Corresponding Author

*gwhitesides@gmwgroup.harvard.edu

ORCID

Wilhelm T. S. Huck: 0000-0003-4222-5411

George M. Whitesides: 0000-0001-9451-2442

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Present Address

[⊥]S.N.S.: Department of Organic Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

Notes

The authors declare no competing financial interest.

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