Prebiotic Chemistry

International Edition: DOI: 10.1002/anie.201905427
German Edition: DOI: 10.1002/ange.201905427

How Small Heterocycles Make a Reaction Network of Amino Acids and Nucleotides Efficient in Water

*Peter Tremmel, Helmut Griesser, Ulrich E. Steiner, and Clemens Richert**

Abstract: *Organisms use enzymes to ensure a flow of substrates through biosynthetic pathways. How the earliest form of life established biosynthetic networks and prevented hydrolysis of intermediates without enzymes is unclear. Organocatalysts may have played the role of enzymes. Quantitative analysis of reactions of adenosine 5'-monophosphate and glycine that produce peptides, pyrophosphates, and RNA chains reveals that organocapture by heterocycles gives hydrolytically stabilized intermediates with balanced reactivity. We determined rate constants for 20 reactions in aqueous solutions containing a carbodiimide and measured product formation with cyanamide as a condensing agent. Organocapture favors reactions that are kinetically slow but productive, and networks, over single transformations. Heterocycles can increase the metabolic efficiency more than two-fold, with up to 0.6 useful bonds per fuel molecule spent, boosting the efficiency of life-like reaction systems in the absence of enzymes.*

Life relies on a network of reactions that occur in aqueous medium.[1] Creating such a network from small molecules alone, to reenact what may have happened when life arose from inanimate material in prebiotic evolution, has proven difficult.[2] A number of steps of primary metabolism have been observed to occur without enzymes,[3–5] but reaction networks producing peptides, nucleic acids, and high-energy pyrophosphates are hard to generate experimentally. Without enzymes that lower activation barriers, regulate chemical flux, and provide reaction sites that shield reactive intermediates from water, these pivotal biomolecules usually do not form in significant quantities.

One reason for this may be the properties of water. Water is a nucleophile, and many reactive species react with water. Synthetic chemists usually exclude water from their reaction mixtures,[6] and only introduce water to quench remaining reagents at the end of reactions. Many reactions in the cell are related to synthetic transformations in laboratory syntheses and can suffer from hydrolysis of intermediates. This includes peptide synthesis and RNA synthesis.

Nature performs translation and replication in water but shields the reactive species in the active site of enzymes and the ribosome. These biocatalysts are encoded in genes, and it is difficult to see how polypeptides and RNA chains were produced before the ribosome had evolved.[7] There must have been fairly evolved processes that turned out either type of biomolecules without falling prey to hydrolysis. How this was achieved on a molecular level is unclear. A molecular principle that minimized hydrolytic loss and steered molecules to achieve a flux of compounds through biosynthetic pathways must have existed.

Perhaps, the molecules found in biology have special properties that made them survive in molecular evolution.[8] Amino acids and biologically relevant carboxylic acids survive under harsh reaction conditions,[9,10] suggesting that they constitute minima in structure space. Likewise, there are several potentially prebiotic processes that lead to peptides.[11–13] Similarly, nucleobases,[14] nucleotides,[15–17] and oligonucleotides[18] have been found to form in the absence of enzymes. Synergistic reactions may favor the formation of peptides in the presence of ribonucleotides and vice versa.

Nucleoside phosphates play key roles in metabolism, genetics and protein biosynthesis, and are believed to have acted in similar roles in the earliest organisms.[19] Building on classical assays involving amino acids, ribonucleotides, and carbodiimides,[20] we recently found a condensation buffer that leads to the simultaneous formation of oligoribonucleotides, peptides, and cofactors, starting from ribonucleotides, amino acids, and cofactor precursors.[21] The reactions occur in homogeneous aqueous solution containing a carbodiimide or other activators as chemical fuel. The condensation buffer drives genetic copying, de novo formation of RNA strands,[22] and the formation of peptido-RNAs with peptides that are N-terminally linked to (oligo)ribonucleotides.[23] Peptide growth is faster than the background oligomerization of amino acids,[23] and the reaction system is robust, producing biochemically relevant molecules with all 20 proteinogenic amino acids.[24]

Among the enzyme-free experimental systems known to us, the condensation-buffer system produces the largest number of classes of biomolecules. A typical assay will run continuously for days or weeks without human intervention, making it a prime candidate for studying the principles of an enzyme-free reaction network. Understanding how it functions without falling prey to a single dominant reaction or premature hydrolysis requires a quantitative understanding.

[*] Dr. P. Tremmel, H. Griesser, Prof. C. Richert
Institut für Organische Chemie, Universität Stuttgart
70569 Stuttgart (Germany)
E-mail: lehrstuhl-2@oc.uni-stuttgart.de
Prof. Dr. U. E. Steiner
Department of Chemistry, University of Konstanz
78457 Konstanz (Germany)

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under: https://doi.org/10.1002/anie.201905427.

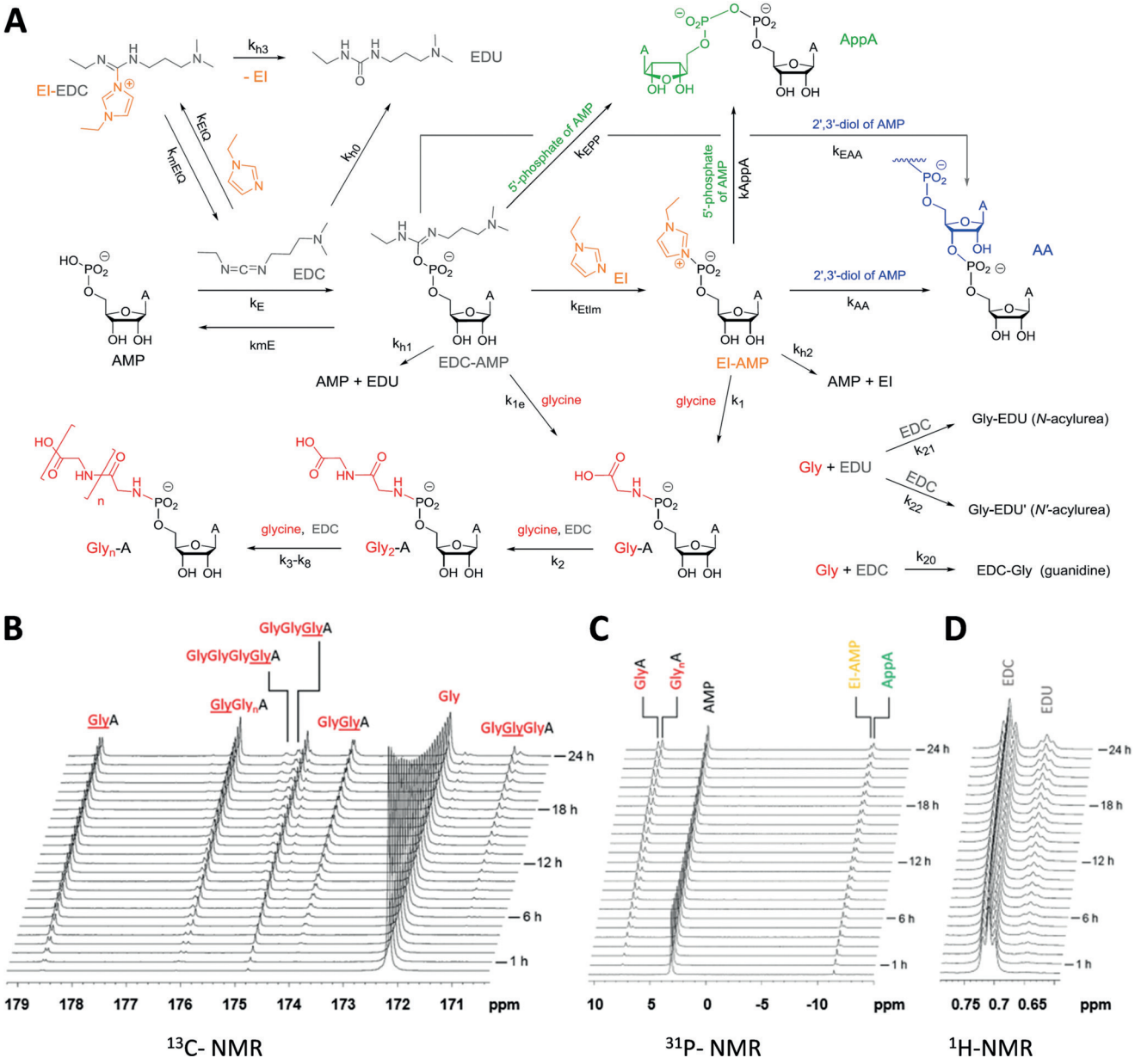


Figure 1. Reaction network for AMP, glycine, EDC, and 1-ethylimidiazole in aqueous buffer. A) Reactions with rate constants shown below arrows. B) ^{13}C-NMR spectra showing peptide chain growth, C) ^{31}P-NMR spectra with signals for nucleotidic species, and D) ^{1}H-NMR spectra used to monitor conversion of EDC to EDU. Conditions: 0.2 M AMP, 0.2 M glycine, 0.08 M EDC, 0.15 M ethylimidazole, 0.08 M $MgCl_2$, 0.5 M HEPES, pH 7.5, 1 °C.

Herein, we report rate constants for 20 core processes of the network, together with a chemical-flux analysis over 24 h. The results show a principle that we call organocapture: a small heterocycle captures reactive intermediates by reacting with them, suppressing hydrolysis, and modulating reactivity. Organocatalysts could have played enzyme-like roles in early stages of molecular evolution.

Because of the number of starting materials, intermediates, and products, a multicomponent model was called for. Figure 1 A shows the reaction Scheme for our assays with adenosine 5′-monophosphate (AMP), glycine (Gly), 1-ethylimidazole (EI), and *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC) as a condensing agent. The first step of a given pathway is activation by addition to the condensing agent. For AMP, this produces the isourea EDC-AMP, which can either dissociate back to the starting materials, react with the organocatalyst to form EI-AMP, or suffer hydrolysis, liberating AMP and ethyl dimethylaminopropyl urea (EDU). Either EDC-AMP or EI-AMP can react with nucleophiles, including the amino group of Gly, the 5′-phosphate of another nucleotide, or a ribose diol, producing a phosphoramidate (Gly-A), a pyrophosphate (AppA), or a phosphodiester (AA), respectively. Activation of the carboxylate of free Gly or ribonucleotide-bound glycine (Gly-A), followed by nucleophilic attack of another amino acid, then leads to chain growth. Background reactions producing guanidines or *N*-acylureas are also considered, as well as an organocatalytic pathway to EDC hydrolysis via EI-EDC. Amino-acid oligo-

merization in the absence of ribonucleotides was previously shown to be minimal,[23] and was therefore not included in the quantitative model. Likewise, further growth in the phosphodiester channel, back reactions, and other low-level reactions were not included in the quantitative treatment to keep the number of variables manageable.

Assays were run at 1 °C in a slight modification of general condensation buffer (1 M HEPES, pH 7.5, 0.8 M EDC hydrochloride, 150 mM EI, 80 mM $MgCl_2$).[21] Initially, the concentrations of AMP and glycine were set to 0.2 M, as in our earlier studies.[24] The formation and consumption of the species was monitored by NMR. Assignment of peaks relied on two-dimensional spectra, and quantitative data was extracted from 1D experiments. The formation of peptido-chains was followed by ^{13}C-NMR spectroscopy, nucleotidic species (AMP, EDC-AMP, EI-AMP, phosphodiester AA, and pyrophosphate AppA) were detected via ^{31}P-NMR spectroscopy, and the conversion of EDC to EDU was monitored by ^{1}H NMR spectroscopy (see Figure 1 for typical spectra).

Based on this data, the kinetic model was evolved, using a set of coupled differential equations and the software *Mathematica*. To avoid ambiguous results, caused by multi-parameter fits with too many floating parameters, we expanded the complexity of the system step by step, starting with just EDC in buffer, and ending with the full set of components of Figure 1 A. A rate constant determined in a low complexity mixture was fixed, and new rate constants were determined in the next, more complex mixture. A detailed description of each assay is given in the Supporting Information. Figure 2 shows representative kinetic plots, and Table 1 provides the list of rate constants.

After determining the rate constant for the hydrolysis of EDC[25] under our reaction conditions and its reactivity toward ethylimidazole, the oligomerization of AMP was studied with and without EI. These assays were initially performed at 0.2 M AMP to ensure sufficiently strong phosphodiester signals for fitting (Figure 2 A). Quantitative analysis confirmed that EI accelerates phosphodiester formation, but, surprisingly, slows down pyrophosphate formation. Further, the ribonucleotide induces much faster consumption of EDC than the reaction with water alone, by forming the isourea EDC-AMP. This highly reactive species is mostly hydrolyzed in the absence of EI, but it is captured as EI-AMP in the presence of the heterocycle.

The subsequent assays with Gly as an additional reaction partner showed how the amino acid competes for the activation agent, but they gave unsatisfactory fits at very high concentrations of reactants (Figure 2 B), due to pH shifts over the course of the assays. More focused assays with lower AMP concentration (0.04 M) and 2.5 equiv Gly avoided such effects and provided reliable rate constants for the peptide-forming channel. Thus, the full reaction network was quantitatively described (Supporting Information, Figure S19).

Figure 2 C shows the evolution of peptide chains of increasing length in the presence of EI, and Figure 2 D gives the formation of the same species in the absence of the organocatalyst. The data shows that the organocatalyst boosts product formation, even though the absolute value of the rate constant for the formation of Gly-A is smaller than without

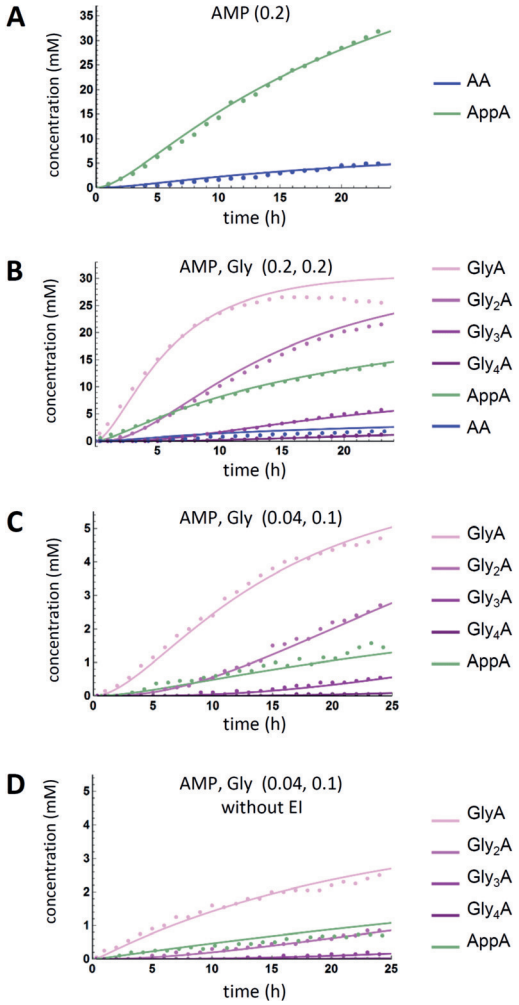


Figure 2. Kinetics of product formation in assays with A) AMP alone, B) AMP and glycine, C) AMP and glycine at concentrations favoring peptide formation, and D) same as (C), but in the absence of EI. Starting materials are given above each plot with molar concentrations in parentheses. Only product-forming reactions are shown. Symbols are experimental data points and lines are calculated with the theoretical model. Conditions: 0.15 M EI, 0.08 M $MgCl_2$, pH 7.5, 1 °C; for (A/B): 0.2 M AMP, 0.2 M glycine, 0.5 M HEPES, and for (C/D): 0.04 M AMP, 0.1 M glycine, 0.4 M EDC, 1 M HEPES. See Figure 1 for shorthands and the Supporting Information for more details.

EI (compare k_{1e} and k_1 in Table 1). This is similar to what was found for pyrophosphate formation (k_{EPP} vs. k_{AppA}) and shows that the organocatalyst does not act as a conventional catalyst, accelerating the rate of these reactions, but rather slows down unproductive reactions more than the desired ones, and modulates the rate of competing reaction channels.

Next, we determined what may be called the metabolic efficiency of the reaction network. For this, we plotted how many useful bonds are formed per EDC molecule consumed. Useful bonds were defined as amide, pyrophosphate, phosphodiester, or phosphoramidate bonds, that is, bonds found in or leading to a biologically relevant species. Figure 3 shows that after the initial build-up of activated species, the efficiency increased toward a steady-state level for all five cases analyzed. In the absence of an organocatalyst, almost 90 % of all EDC molecules were hydrolyzed when AMP was

Table 1: Rate constants of reactions of the network involving AMP, glycine, EDC, and ethylimidazole in condensation buffer.

Assay number	Starting material[a]	Rate constant[b]	Order[c]	Rate constant value
1	EDC	k_{h0}	1st	$4.2\times10^{-4}\ h^{-1}$
2	EI, EDC	k_{EtQ}	2nd	$2.2\times10^{-5}\ mM^{-1}h^{-1}$
		k_{mEtQ}	1st	$1.3\times10^{-2}\ h^{-1}$
		k_{h3}	1st	$<10^{-3}\ h^{-1}$
3	AMP, EDC	k_{EAA}	2nd	$2.0\times10^{-5}\ mM^{-1}h^{-1}$
		k_{EPP}	2nd	$5.2\times10^{-3}\ mM^{-1}h^{-1}$
		k_{E}	2nd	$4.5\times10^{-5}\ mM^{-1}h^{-1}$
		k_{h1}	1st	$1.8\ h^{-1}$
		k_{mE}	1st	$0.1\ h^{-1}$
4	AMP, EDC, EI	k_{AA}	2nd	$7.5\times10^{-5}\ mM^{-1}h^{-1}$
		k_{AppA}	2nd	$8.3\times10^{-4}\ mM^{-1}h^{-1}$
		k_{EtIm}	2nd	$0.5\ mM^{-1}h^{-1}$
		k_{h2}	1st	$0.03\ h^{-1}$
5	AMP, EDC, Gly	k_{1e}	2nd	$7.3\times10^{-3}\ mM^{-1}h^{-1}$
6	AMP, EDC, Gly, EI	k_{1}	2nd	$2.0\times10^{-3}\ mM^{-1}h^{-1}$
		k_{2}	3rd	$1.6\times10^{-6}\ mM^{-2}h^{-1}$
		k_{3}–k_{8}[d]	3rd	$8.5\times10^{-7}\ mM^{-2}h^{-1}$
		k_{20}	2nd	$1.1\times10^{-5}\ mM^{-1}h^{-1}$
		k_{21}	3rd	$1.3\times10^{-7}\ mM^{-2}h^{-1}$
		k_{22}[e]	3rd	$3.0\times10^{-8}\ mM^{-2}h^{-1}$

[a] Components used to determine rate constant. [b] See Figure 1 for reactions and the Supporting Information for further details. [c] Pseudo-first-order reaction (hydrolysis), second-order reaction, or multi-step reaction treated as third-order reaction. [d] Rate constants for peptide-chain growth beyond the first dipeptide were assumed to be equal. [e] Determined at 0.2 M AMP concentration.

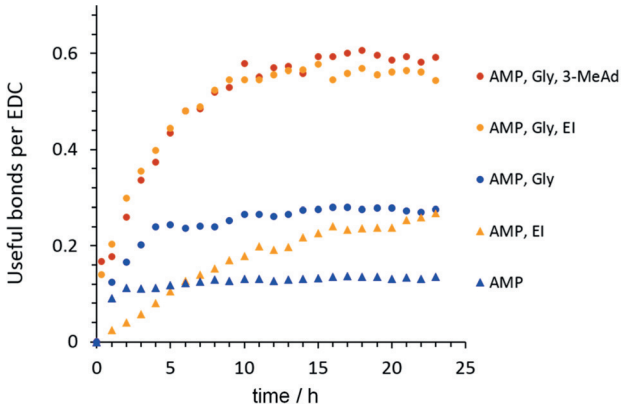


Figure 3. Efficiency of product formation in the absence and presence of organocatalysts 1-ethylimidazole (EI) or 3-methyladenine (3-MeAd) in condensation buffer with AMP/glycine.

the only biological reactant. When Gly was added, the yield per EDC consumed rose slowly, reaching approximately 25% within 5 h.

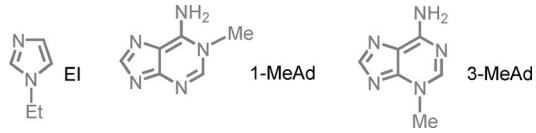
EI markedly increased the efficiency of the reaction network. The kinetics were slower, particularly when the ribonucleotide was the only biomolecule, but the steady-state level reached was more than twice that found in the absence of an organocatalyst for either reaction system (AMP alone or AMP and Gly). With EI, up to 58% of all carbodiimide molecules consumed led to a useful bond in the reaction mixture with AMP and Gly. This value was even higher (greater than 75%) when calculated per EDU molecule formed because a significant portion of EDC reacts to form EI-EDC, a latent form of EDC, from which the carbodiimide is slowly liberated ($k_{mEtQ} = 1.3\times10^{-2}\ h^{-1}$, Table 1 and Supporting Information, Figures S29 and S33).

Importantly, the boost in productivity is not limited to EI. We studied 1-methyladenine and 3-methyladenine, two methylated nucleobases that have been employed as leaving groups of activated ribonucleotides in oligomerizations.[26] Their purine ring systems may provide interactions in the transition state that are not accessible with smaller heterocycles like imidazole. Table 2 shows that either methylated adenine has a beneficial effect, producing activated species that are more long-lived than EDC-AMP. Each organocatalyst has a different effect on the relative extent of product formation, though. Assays with 3-methyladenine gave up to 60% metabolic yield per EDC molecule consumed (red data points in Figure 3), indicating how powerful an organocap this methylated base is.

A boost in yield was also found for a different activating agent. Chapter 7.2 of the Supporting Information shows results with cyanamide, a condensing agent more likely to have been found on prebiotic earth than EDC.[27,28] Reactions are much slower in cyanamide, but the reactivity pattern is similar to that with EDC (Supporting Information, Figure S43–47). Without EI, even 2 M cyanamide did not lead to detectable peptide-chain formation after 18 d, whereas half an equivalent of EI gave well detectable dipeptido-nucleotide and approximately one order of magnitude more intense peaks for Gly-A. After 60 d at 22 °C and low Mg^{2+} concentration, Gly-A and $(Gly)_2$-A had reached similar levels, and the first phosphodiester bonds were detectable (Supporting Information, Figure S44).

Taken together, this data shows how heterocycles can turn an inefficient enzyme-free reaction system into one with surprising metabolic efficiency. Because the small molecules do not accelerate most of the useful reactions but increase

Table 2: Product distribution in assays with different organocatalysts after 24 h.[a]



Organocap	OC-AMP[b] [mM]	AppA [mM]	AA [mM]	Gly-A [mM]	Gly_n-A [mM]
–	0.5[c]	11	<0.1	17	14
EI	8	14	1–2	26	28
3-MeAd	5	6	1	32	16
1-MeAd	41	11	1	20	13

[a] Conditions, for all four assays 0.2 M AMP, 0.2 mM [1-^{13}C]-glycine, 0.5 M HEPES, 0.08 M $MgCl_2$, pH 7.5, 1 °C. Concentrations of the organocatalysts shown: 0.15 M 1-ethylimidazole (EI), 0.075 M 1-methyladenine (1-MeAd), and 0.075 M 3-methyladenine (3-MeAd). [b] OC denotes organocap moiety. See Figures S34–S42 in the Supporting Information for data on regioisomers and Table S4 in the Supporting Information for pK_a values. [c] Concentration of EDC-AMP.

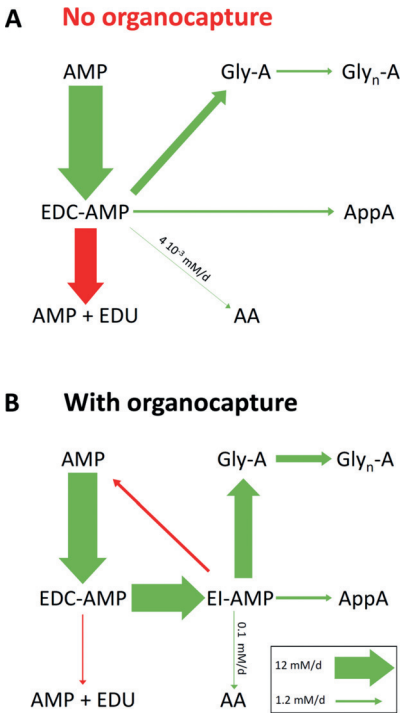


Figure 4. Chemical flux of AMP molecules through different reaction channels within 24 h, A) in the absence, and B) in the presence of ethylimidazole. The box at the bottom shows how the width of arrows scales with the chemical fluxes, except where stated numerically on arrows. Green arrows are for reactions producing useful bonds, and red arrows are for hydrolysis processes consuming chemical fuel without producing useful bonds. Conditions: 0.04 M AMP, 0.1 M glycine, 0.4 M EDC, 0.08 M $MgCl_2$, 1 M HEPES, pH 7.5, 1 °C, with or without 0.15 M 1-ethylimidazole.

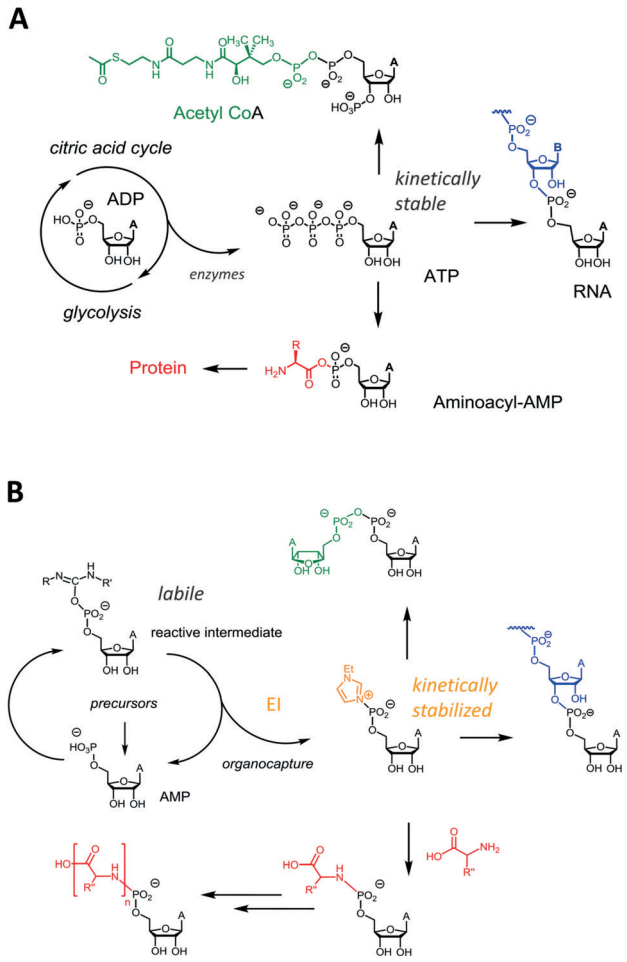


Figure 5. The organocatalyst-activated form of AMP is reminiscent of ATP as intermediate for reactions critical for a living system. A) Processes in the cell, B) reactions in the system studied here.

yields by modulating reactivity and suppressing loss channels, we propose the term organocapture for this.

The effect became more visible when the chemical flux of molecules through the reaction network over 24 h was determined. For this, we integrated the time-dependent fluxes using our quantitative model. Figure 4 shows the result for the most important reactions of the network involving AMP and Gly. The width of arrows is proportional to the total flux of molecules through the respective channel. In the absence of EI, hydrolysis is the most frequent reaction for the activated form of the ribonucleotide (red arrow in Figure 4 A). In the presence of the organocatalyst (Figure 4 B), this channel dries up almost completely. Here, EI captures the EDC-AMP, converting it to EI-AMP, which is hydrolyzed to a much smaller extent, allowing for productive processes instead via the peptide, pyrophosphate, and phosphodiester channels.

How life-like is the reaction system observed and what can one learn from the results? The system sets up a reaction network that links core biomolecules and has the ability to create genetic information, peptides, and compounds with high-energy pyrophosphate bonds. The network can overcome the dilemma of the need for water and the risk of hydrolytic loss of precious compounds. Like extant biology, it pivots around an activated form of AMP, as shown schematically in Figure 5. Reactivation cycles have been called "a property long sought in non-enzymatic oligomerization".[29]

While biology uses ATP as the kinetically stable activated form, whose pyrophosphate unit can be readily brought to react in enzyme-catalyzed reactions,[30] the model system studied by us produces EI-AMP as the central compound that hydrolyzes much more slowly than its isourea precursor EDC-AMP. The organocapture process thus builds on the existing activation/hydrolysis cycle for AMP and expands it, resulting in a better reactivity profile and minimized hydrolysis.

Both ATP and EI-AMP are precursors of a series of key molecules of life. ATP is the most universal energy currency of the cell. At the same time, ATP is one of the building blocks for RNA synthesis (transcription) and is also consumed when tRNAs are aminoacylated for protein synthesis. Further, ADP is found in important cofactors of the primary metabolism, such as acetyl-CoA, the central hub of metabolism (Figure 5 A).[19] Similarly, using the product of organocapture, the condensation-buffer reaction network supports primitive versions of genetics, peptide synthesis, and metabolism in a homogeneous aqueous solution, bringing together chemical sub-systems.[21,31]

Small heterocycles of the size of EI and methyladenines are much easier to form spontaneously than protein or RNA

enzymes and are thus much more likely to have existed on the early earth. Organocapture makes the reaction network more resistant to hydrolysis, but it also makes it more robust, as different activation agents can lead to the same intermediates. This means that different modes of activation can be utilized, depending on what is available in the environment the reaction system finds itself in. Again, this is a trait typical for living systems and an important prerequisite for survival.

The modulating effect of organocatalyst is significant. With EI, the peptide-forming channel dominates, consuming most of the metabolic energy (Figure 4). The formation of pyrophosphates is the second most populated reaction pathway, and the synthesis of oligonucleotides consumes the smallest fraction of the chemical fuel. This is a reasonable adjustment for a primitive organism. The methyladenines as organocaps change the relative flux through the channels in a significant and subtle way. The concentration of organocatalyst may have been regulated through RNA sequences, such as aptamers.[32] Riboswitches that bind heterocycles, including nucleobases, regulate gene expression to this day.[33] Taken together, this suggests that organocatalysts could have played roles of primitive enzymes in prebiotic systems.

Water interferes with many reactions of organic synthesis. Here we show how the water/hydrolysis paradox can be overcome for reactions producing peptides, oligoribonucleotides, and molecules possessing high-energy pyrophosphate groups in the absence of enzymes. Heterocycles from compound classes that form readily under prebiotic conditions can play roles reminiscent of those of enzymes in fully developed organisms. They react quickly with activated forms of biomolecules via organocapture. In the captured form, the biomolecules are reactive toward other biomolecules, but much less reactive toward water, favoring successful biotransformations. Organocapture breaks down a strongly exothermic hydrolysis reaction of a labile activated species into a series of well-controlled, less exothermic reactions, coupled to or indeed performing biosyntheses. This is typical for life. Different organocaps favor different reaction channels, thus directing what may be called a primitive metabolic network. To our knowledge, the system described here is the most life-like reaction network using small molecules in aqueous solution known.

Acknowledgements

We thank S. Motsch, M. Bechthold, C. Pfeffer, and D. Göhringer for sharing results, Dr. E. Kervio for discussions and Volkswagen Foundation for funding (grant Az 92 768, under the program Life?).

Conflict of interest

The authors declare no conflict of interest.

Keywords: nucleotides · organocatalysis · peptides · prebiotic chemistry · reaction networks

How to cite: *Angew. Chem. Int. Ed.* **2019**, *58*, 13087–13092
Angew. Chem. **2019**, *131*, 13221–13226

[1] D. Voet, J. G. Voet, *Biochemistry*, 2nd ed., Wiley, New York, **1995**.
[2] L. E. Orgel, *PLoS Biol.* **2008**, *6*, e18.
[3] M. Ralser, *Biochem. J.* **2018**, *475*, 2577.
[4] S. J. Varma, K. B. Muchowska, P. Chatelain, J. Moran, *Nat. Ecol. Evol.* **2018**, *2*, 1019.
[5] G. Springsteen, J. R. Yerabolu, J. Nelson, C. J. Rhea, R. Krishnamurthy, *Nat. Commun.* **2018**, *9*, 91.
[6] A. I. Vogel, A. R. Tatchell, B. S. Furnis, A. J. Hannaford, P. W. G. Smith, *Vogel's textbook of practical organic chemistry*, 5th ed., Wiley, New York, **1991**.
[7] F. H. C. Crick, S. Brenner, A. Klug, G. Pieczenik, *Origins Life* **1976**, *7*, 389.
[8] C. De Duve, *Nature* **2005**, *433*, 581.
[9] S. L. Miller, *Science* **1953**, *117*, 528.
[10] E. T. Parker, M. Zhou, A. S. Burton, D. P. Glavin, J. P. Dworkin, R. Krishnamurthy, F. M. Fernández, J. L. Bada, *Angew. Chem. Int. Ed.* **2014**, *53*, 8132; *Angew. Chem.* **2014**, *126*, 8270.
[11] S. L. Miller, H. C. Urey, *Science* **1959**, *130*, 245.
[12] G. Danger, R. Plasson, R. Pascal, *Chem. Soc. Rev.* **2012**, *41*, 5416.
[13] C. Gibard, S. Bhowmik, M. Karki, E.-K. Kim, R. Krishnamurthy, *Nat. Chem.* **2018**, *10*, 212.
[14] J. Oró, *Biochem. Biophys. Res. Commun.* **1960**, *2*, 407.
[15] M. W. Powner, B. Gerland, J. D. Sutherland, *Nature* **2009**, *459*, 239.
[16] S. Becker, I. Thoma, A. Deutsch, T. Gehrke, P. Mayer, H. Zipse, T. Carell, *Science* **2016**, *352*, 833.
[17] S. J. Roberts, R. Szabla, Z. R. Todd, S. Stairs, D. K. Bučar, J. Šponer, D. D. Sasselov, M. W. Powner, *Nat. Commun.* **2018**, *9*, 4073.
[18] J. P. Ferris, A. R. Hill, Jr., R. Liu, L. E. Orgel, *Nature* **1996**, *381*, 59.
[19] S. A. Benner, A. D. Ellington, A. Tauer, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 7054.
[20] P. Berg, *J. Biol. Chem.* **1958**, *233*, 608.
[21] M. Jauker, H. Griesser, C. Richert, *Angew. Chem. Int. Ed.* **2015**, *54*, 14564; *Angew. Chem.* **2015**, *127*, 14772.
[22] M. Jauker, H. Griesser, C. Richert, *Angew. Chem. Int. Ed.* **2015**, *54*, 14559; *Angew. Chem.* **2015**, *127*, 14767.
[23] H. Griesser, P. Tremmel, E. Kervio, C. Pfeffer, U. E. Steiner, C. Richert, *Angew. Chem. Int. Ed.* **2017**, *56*, 1219; *Angew. Chem.* **2017**, *129*, 1239.
[24] H. Griesser, M. Bechthold, P. Tremmel, E. Kervio, C. Richert, *Angew. Chem. Int. Ed.* **2017**, *56*, 1224; *Angew. Chem.* **2017**, *129*, 1244.
[25] A. Williams, I. T. Ibrahim, *J. Am. Chem. Soc.* **1981**, *103*, 7090.
[26] K. J. Prabahar, J. P. Ferris, *J. Am. Chem. Soc.* **1997**, *119*, 4330.
[27] A. Schimpl, R. M. Lemmon, M. Calvin, *Science* **1965**, *147*, 149.
[28] J. Hulshof, C. Ponnamperuma, *Origins Life* **1976**, *7*, 197.
[29] A. Mariani, D. A. Russell, T. Javelle, J. D. Sutherland, *J. Am. Chem. Soc.* **2018**, *140*, 8657.
[30] F. H. Westheimer, *Science* **1987**, *235*, 1173.
[31] J. D. Sutherland, *Angew. Chem. Int. Ed.* **2016**, *55*, 104; *Angew. Chem.* **2016**, *128*, 108.
[32] M. Egli, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 480; *Angew. Chem.* **1997**, *109*, 494.
[33] R. T. Batey, S. D. Gilbert, R. K. Montange, *Nature* **2004**, *432*, 411.

Manuscript received: May 1, 2019
Revised manuscript received: June 6, 2019
Accepted manuscript online: July 5, 2019
Version of record online: August 9, 2019

