**Previews**

# Selection of assembly complexity in a space of tetrapeptides

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In this issue of *Chem*, Ulijn and co-workers show how peptide designs can be selected in a vast sequence space by reversible enzymic exchange, which produces arrays of tetrapeptides, and this is modulated by the nature of the environment.

Synthetic chemists spend a large amount of time on controlling every aspect of the environment of their reactions in order to gain maximum control over the outcomes of those reactions. As the number of components of any system increases, the complexity of the interactions between those components explodes, and it rapidly becomes impossible to precisely elucidate all the different interactions that are occurring. This is the situation in most systems that are not confined to laboratory settings, and the explication of these kinds of complex systems is a major challenge for chemists studying systems that approach the complexity of biological systems. Because of its significance in biology, and the potential role it might have played in the origins of life, the behavior of peptide bond formation under such complex environmental conditions has been a major strand of research into the behavior of complex and dynamic chemical systems. The generation of complex systems based on peptide chemistry has inspired many studies exploring the importance of such environmental conditions, and there are also questions about how selection at the sequence level can occur in complex chemical and biochemical systems.

Biological catalysts not only help the formation of the peptide bond but also can recognize and differentiate between different amino acid substrates. A key question is how biology was able to build the catalysts, made from peptides, before the machinery to effi-ciently build peptides became accessible. However, recent work shows that it is also possible to form peptides without activation or heterogeneous supports. This means that it is possible that selection in the environment could help the sequence and functional selection of peptides.[1] Indeed, the generation of complex mixtures of oligopeptide species was established in environmentally controlled methods of generating complex systems of peptide mixtures capable of showing the characteristic dynamic behaviors. Thus, the generation of complexity from relatively simple precursors in this way is a vital step in understanding the transition from chemically "simple" systems, where all the relevant parameters can be controlled, to stochastic systems that exhibit more complex behaviors as a result of the large populations involved.

The generation of complexity in such systems does not, however, simply entail the endless generation of more and more components, and an important aspect of the study of these systems is the study of the emergence of higher-order behaviors through selection processes acting on the whole system. In simple undirected systems, such as the one-pot formose reaction (which is the base-mediated condensation of formamide), this reaction can continuously lead to combinatorial explosions. Here, simple building blocks capable of function exist, but they are in insuffi-cient concentration to self-organize, adapt, and thus generate complexity. One can explore the effect of recursion on such complex mixtures by "seeding" the product mixture into a fresh version of the reaction, with the inclusion of different mineral environments, over a number of reaction cycles. This reaction can self-limit[2] the overall number of products assembled, which decreases as the number of cycles increases. The number of products decreases because the reactions occur recursively, or in cycles, enhanced by the mineral environment. This process leads to selectivity, thus limiting the combinatorial explosion. In this case, the involvement of mineral surfaces with simple reactions can help direct the emergence of some of the building blocks found in RNA—ribose and uracil—and these can be detected under very simple conditions. This again highlights the importance of the environmental conditions not just for the generation of complex mixtures but also for the selection process that can amplify or suppress certain behaviors, allowing for stochastic systems to begin to demonstrate more coordinated behaviors.[3]

Living systems are characterized by an ability to sustain chemical reaction networks far from equilibrium. It is likely that life first arose through a process of continual disruption of equilibrium states in recursive, complex reaction networks driven by periodic, potentially recursive, environmental changes.[4] For example, systems that show the emergence of proto-enzymatic functions can be achieved via recursive polymerization reactions using amino acids and glycolic acid. Again, a critical driver is the environment—in this case, a range of different mineral environments—and in just four

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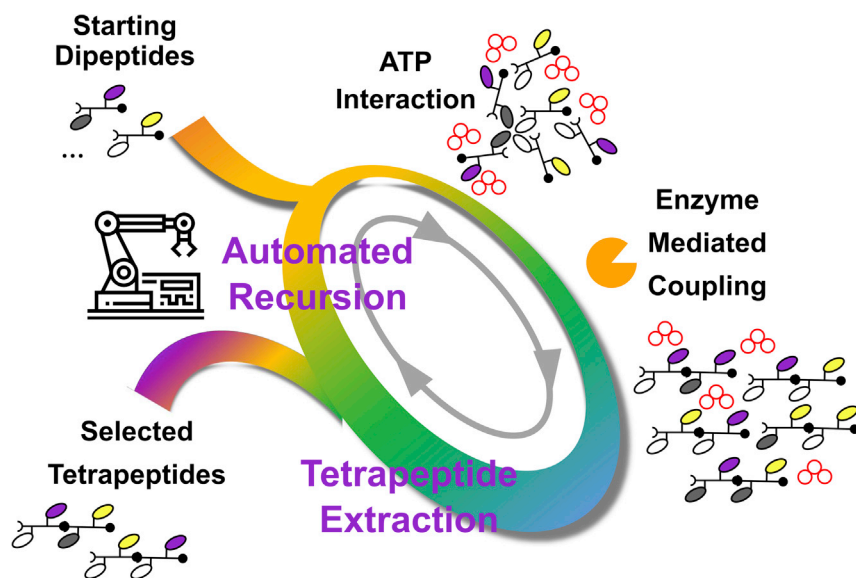
**Figure 1. Potential workflow for an automated recursive platform for applying evolution-like selective pressures to enzyme-mediated dipeptide coupling systems**
Tetrapeptides amplified by environmental conditions can be extracted and used as a new feedstock for subsequent iterations, allowing the system to evolve beyond the equilibrium point of the system.

reaction cycles, product mixtures from recursive reactions exhibited greater catalytic activity and truncation of product space toward higher-molecular-weight species than did non-recursive controls. Many approaches to the exploration of chemical complexity, and the origin of life, focus on how the molecules found in biology might be made—from the simplest plausible starting materials—in the absence of biological processes. Another approach could be to view the emergence of the chemistry of biology as a process whereby the environment effectively directs complex mixtures toward structure, function, and genetic systems over time.[5] This does not require the molecules found in biology today to be made initially and leads to the hypothesis that environment can direct chemical soups toward order and eventually living systems. As such, unconstrained condensation reactions can be steered by changes in the reaction environment, such as the order of reactant addition and the addition of salts or minerals. The use of -omics techniques to survey the resulting chemical ensembles has been used to show that the environ-

mental programming of distinct, significant, and reproducibly different product mixtures leads to different chemical products. Further, these differences in composition have consequences manifested in clearly different structural and functional properties. By simple variation of the environmental parameters, differentiation of distinct chemical ensembles is possible. Hence, the environment can program complexity emerging from such unconstrained reactions.

In this issue of *Chem*, Ulijn and co-workers use a system of dipeptides to generate complex systems by their enzyme-facilitated condensation in a dynamic library of tetrapeptides.[6] The authors explore the behavior of these dynamic systems by introducing environmental conditions that provide selective pressures and result in self-organized system behaviors that generate interpretable patterns within the combinatorial complexity of the generated system. The complexity of the generated tetrapeptides is intrinsically related to the complexity of the chosen dipeptide precursors and is directed by

the selection pressures applied. This work provides a fascinating glimpse into the way systems can generate (by assembling relatively simple precursors, along with finely tuned selective pressures, in a defined fashion) a complexity that can exhibit well-defined, interpretable, and potentially even predictable behaviors. This work represents an exciting step toward exploring the importance of this assembly-pathway to the generation of systems that use stochastic processes to generate system-level behaviors.

A fascinating idea would be to leverage this approach to introduce the kind of recursion described above to develop these systems into closed loops that can then evolve on a whole-system level. Systems that are capable of such exploration by optimizing the efficacy of antimicrobial peptides via direct evolution have been previously shown.[7] In particular, a closed-loop approach that combines a genetic algorithm, machine learning, and *in vitro* evaluation to improve the antimicrobial activity of peptides against *Escherichia coli* was built. Using such automated, closed-loop robotic systems in conjunction with evolutionary algorithms allows selection to explore beyond biological control systems. A possible question is whether the process of Ulijn and co-workers could be built into a closed-loop system to build on the complexity from the environment. This approach opens the prospect of using these hybrid hardware and software systems to explore the vast sequence space available to oligomeric and polymeric materials as they increase in complexity (Figure 1).[8]

The development of chemo-biological systems embodied in a hybrid robot undergoing selection might allow the field to leap from directed evolution to a more open-ended multiple-fitness-based search. In this regard, evolution (once the preserve of biology) has been widely emulated in software, whereas physically embodied systems that can evolve have

been limited to electronic and robotic devices and have never been artificially implemented in populations of physically interacting chemical entities. We wonder whether combining these systems into a liquid-handling robot built with the aim of investigating populations of interacting experiments, perhaps in artificial cells, could allow the exploration of an automated evolutionary process.[9]

A problem in systems chemistry and biochemical systems is how the process of selection and evolution should be quantified and explained. Recently, a new theory called assembly theory has shown that both selection and evolution can be explained via the notion of molecular complexity.[10] By investigating both the complexity of the molecule and its abundance in the system, one can quantify the amount of evolutionary processing. By following the emergence of complexity and selection in physical systems, such as with the work of Ulijn and co-workers, it will be possible to design biochemical and chemical systems capable of autonomously evolving from the bottom up. This approach

might open a new window to the origin of life, the understanding of evolutionary biochemistry, and the design of new life forms.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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# Selective palladium recovery by a highly porous polyisothiocyanurate

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**Precious metals, particularly palladium (Pd), are in short supply, and their effective recovery from waste depends on metal-specific adsorbents that provide energy-efficient and environmentally friendly solutions. In this issue of *Chem*, Coskun and co-workers introduce a new porous organic polymer with exceptional porosity and stability and record-high capacity and selectivity toward Pd.**

Palladium (Pd) has an important role in modern technological applications, especially in catalytic converters.[1] In

the world of chemistry, the power of Pd-based catalysts has enabled numerous important organic reactions

forging challenging bonds.[2,3] However, the biggest constraint currently is the supply. Pd mine production has maintained a stable status over the past several years (~215 tons/year), whereas global demand keeps rising as a result of the increasing number of technologies that require this element (Figure 1).[1] This is reflected by the

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