Catalysis as a Robust Feature and Catalytic Promiscuity as a Recurrent Trait in Peptide Based Self-Replicators

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ABSTRACT: Recently, the first self-replicating molecules have been described that are capable of catalyzing different reactions in addition to their own formation. These findings raise the question whether such catalytic promiscuity is a widespread characteristic, or whether it is feature restricted to a special subset of replicators. Another related issue is whether catalytic activity of these systems is sensitive to alterations in the peptide structure. Both of these questions are relevant in the context of evolution. Widespread catalytic promiscuity would be beneficial if replicators are to acquire a metabolism that involves catalysis of different chemical reactions. Furthermore, in order to enable adaptation and acquisition of new traits through mutation and selection, it is desirable that self-replicating molecules can mutate and explore structure space while retaining their catalytic activity. Here we demonstrate that catalytic promiscuity of a class of peptide based self-replicators is indeed a recurrent trait in a significant fraction of the probed structure space. Specifically, we investigated eighteen self-assembly driven self-replicators, each made from a different single building block, and six replicators that emerged from a binary building block mixture. Most of these were found to catalyze both the retro-aldol reaction of methodol, as well as the cleavage of fluorenylmethoxycarbonyl (FMOC) groups. No obvious correlation exists between the efficiencies with which replicators catalyze these two reactions, indicating that the reactions have different requirements with respect to catalyst structure. The degree of catalytic activity varied with replicator structure spanning up to three orders of magnitude. Of the binary mixtures, most gave replicators with activities in between those of the replicators made of the corresponding individual building blocks. However, in one instance, where specific interactions promote the formation of a specific two-building-block replicator mutant, this species had an activity that exceeded that of the corresponding single-building-block replicators. These observations imply that evolutionary enhancement of a specific catalytic activity of self-replicating molecules should be possible also in a regime where mutation rates are relatively high.

INTRODUCTION

How chemistry can be turned into biology is one of the biggest unanswered questions in contemporary science.[1-4] So far, experimental approaches to address this question revolve around developing chemical systems that harbor the key characteristics of life: self-replication, metabolism and compartmentalization.[5-8] Building on efforts directed at addressing each of these aspects in isolation, research recently has started to attempt their pairwise integration. Systems that combine nucleic-acid replication and compartmentalization[9-13]have been reported and, more recently, also the integration of replication and metabolism is starting to be explored.[14-16] The latter features replicators that are able to catalyze, besides their own self-replication, also other chemical reactions. Pioneering work by Rebek et. al. has demonstrated a synthetic self-replicator capable of exhibiting catalytic activity, but the solvents required for self-replication and catalysis were mutually incompatible.[17] Another approach used RNA replicases derived from the naturally occurring Azoarcus bacterium to implement a rudimentary catabolism.[16,18] More recently, we succeeded in integrating self-replication and proto-metabolic activity, where catalytic activity emerges spontaneously alongside self-replication.[14-15] In this system a specific pentapeptide (GLKFK) was attached to a benzene-1,3-dithiol core, that, upon oxidation in buffered aqueous solution, generated a dynamic combinatorial library (DCL) of disulfide macrocycles with various sizes (Figure 1a). These macrocycles interconvert through disulfide exchange. After an initial lag phase, hexamer macrocycles nucleate by forming a small stack. The stacking of macrocycles is stabilized by β-sheet interactions between the pentapeptides and π-π stacking of the aromatic core. The supramolecular fibers elongate by incorporating more hexamer macrocycles at their ends, depleting them from the macrocycle exchange pool and leading to a re-equilibration of the DCL and the generation of more hexamer macrocycles. The transfer of material from solution to the growing fiber end is mediated by the fiber sides.[19] When applying mechanical agitation, fibers can fragment and hence generate more fiber ends to grow from, allowing for exponential replication.[20]

The stacking of macrocycles into fibers inadvertently brings the lysine residues in the pentapeptide backbone into close proximity, thereby lowering the apparent pK_a of the ε-amino group by approximately three units.[14] This results in the partial

deprotonation of ammonium groups in the lysine side chains at close to neutral pH. At the same time the β-sheets also provide hydrophobic microenvironments along the fibers, that can act as substrate or cofactor binding sites.[15]

We previously reported that the GLKFK-based replicators (made from building block **8** in Table 1) can catalyze the retro-aldol reaction of methodol **1** (Figure 1b) with an activity that is comparable to that of computationally designed enzymes for this reaction.[21] Catalysis most likely proceeds via iminium formation through the ε-amines of the lysine residues. Iminium formation activates the C-C bond towards cleavage and the resulting enamine undergoes tautomerization and subsequent hydrolysis to regenerate the catalyst.[22] In fibers of $\mathbf{8}_6$ less than 10% of lysines were found to be catalytically active. This may be attributed to the relatively poorly ordered stacks resulting from $\mathbf{8}_6$, that provides a rather disorderly spectrum of microenvironments.

The same replicator also catalyzes the cleavage of the FMOC group from FMOC-glycine **4** (Figure 1c).[23] This reaction proceeds through the deprotonation of **4** by the ε- amine on lysine, followed by fragmentation into dibenzofulvene **5**, glycine **6** and CO_2. In this case the dibenzofulvene enhances the rate of thiol oxidation, providing a positive feedback on the production of replicator precursor, amounting to a proto-metabolism. In both retro-aldol and FMOC cleavage reactions the unprotonated lysine residues play a key role: for the retro-aldol reaction they likely transiently form imines with the substrate, while for FMOC cleavage they act as general base. The superior catalytic activity of the replicators compared to their precursors (building blocks or small oligomers thereof) is attributed to the fact that the amine groups in these precursors are further apart and therefore almost fully protonated at neutral pH.

Now that catalytically active self-replicators have become available, a number of questions can be addressed that relate to the potential evolvability of such systems. Firstly, how robust is catalytic activity to changes in the chemical structure of the replicator? In early evolution, replication fidelity is expected to be low and therefore catalytic activity is only likely to persist if it is robust to mutations of the self-replicator.[24] Secondly, it is known from current biochemistry that the invention of new catalytic activity relies strongly on promiscuity in catalysis.[25] Especially in early evolution, enzymatic promiscuity is crucial for organisms to adapt to a changing environment and allow for the development of novel catalytic functions. If a change in selection pressure confers an evolutionary benefit to a slow, promiscuous catalytic transformation, evolution is likely to select mutants with enhanced activity of this transformation.[26-28] The question is now whether catalytic promiscuity is also a widespread feature in synthetic self-replicators.

We addressed these two questions by investigating the catalytic activity of structurally related replicators for the retro-aldol and FMOC cleavage reactions described above and shown in Figure 1b,c. To conduct this study, we prepared a range of new replicators. The best of these are, respectively, nine- or two-fold more active in the catalysis of these two reactions than the previously reported replicator $\mathbf{8}_6$. The results also show that catalytic activity is remarkably robust to changes in the structure of the replicators; much more so than most evolved contemporary enzymes.[29] Finally, catalytic promiscuity is recurrent: as default, the tested replicators catalyze both reactions. The extent to which they catalyze the retro-aldol reaction does not appear to correlate with the rate enhancement of the FMOC cleavage reaction, suggesting that catalytic optimization of each of these reactions would have to occur through separate evolutionary processes, that could start from the same replicator. These are encouraging results for future work on enabling the evolution of catalytic activity in systems of non-biological replicators.

Results and Discussion

Building block design. In order to probe how robust catalytic activity by our self-replicators is toward mutation in the peptide sequence and to probe how widespread promiscuity is, we required a large range of replicators with different peptide sequences. We developed and screened eighteen fiber-forming, pentapeptide-based self-replicators, made from different single building blocks **8**-**24** (Table 1). Of these **8**, **12**, **13**, **14**, **17** and **18** were reported previously.[14,19-20,30-35] The investigated building blocks can be loosely grouped, depending on their peptide sequence. The first group (**8-14**) features two lysine residues (important for catalysis) at positions 3 and 5 in the peptide chain. Within this group we modified the fourth amino acid positioned between these two lysine residues. This position tolerates considerable structural changes, without losing the ability to form self-replicators.[30-31] We modified this position from apolar and bulky (phenyl alanine, isoleucine) to small (alanine) and polar (serine). Whereas most replicators are hexameric macrocycles, in some instances these structural modifications are accompanied by a change in the macrocycle size of the self-replicators. Building block **12** with a tyrosine in position 4 yielded pentamer or hexamers, depending on the conditions during fiber emergence (Figure S14-17). Building blocks **13** and **14** gave octamers. These differences follow previously described selection criteria, where less strongly interacting (i.e. less hydrophobic) peptides give rise to larger rings.[30] In the second group (**15-19**) we replaced one of the lysine residues on the third or fifth position with other positively charged residues, such as ornithine or arginine or by a negatively charged glutamic acid (**19**). These modifications affect the residues that are directly involved in catalysis. In the third group, we reversed the order of hydrophilic and hydrophobic amino acids (**20-22**) to ensure preservation of β-sheet forming tendencies, but with lysine residues positioned closer to the fiber core and thereby most likely in a more hydrophobic environment. The fourth group contained building blocks in which we altered the second amino acid in the sequence from leucine to phenylalanine (**23-24**).

In the fifth group, we also explored six replicators that emerged from binary mixtures of some of these building blocks. Notably, the combination XGLKFK/XGKLKL (**8**+**22**) was recently found to form a non-statistical distribution of mixed hexamers, in which the alternating **8-22-8-22-8-22** hexamer is favored as a result of steric zipper formation between neighboring building blocks within the macrocycle.[36]

Replicator preparation and characterization. All replicators were prepared at 1.0 mM building block concentration in conditions that favored the emergence of the specific replicator (50-200 mM of borate- or phosphate buffer at 40 °C and stirred at 1200 rpm; SI section S1.3). The molecular composition of the samples was determined with ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis (SI section S2.3). The resulting DCLs formed fibers as evident from negative-stain transmission electron microscopy (TEM) analysis (SI Section S2.4).

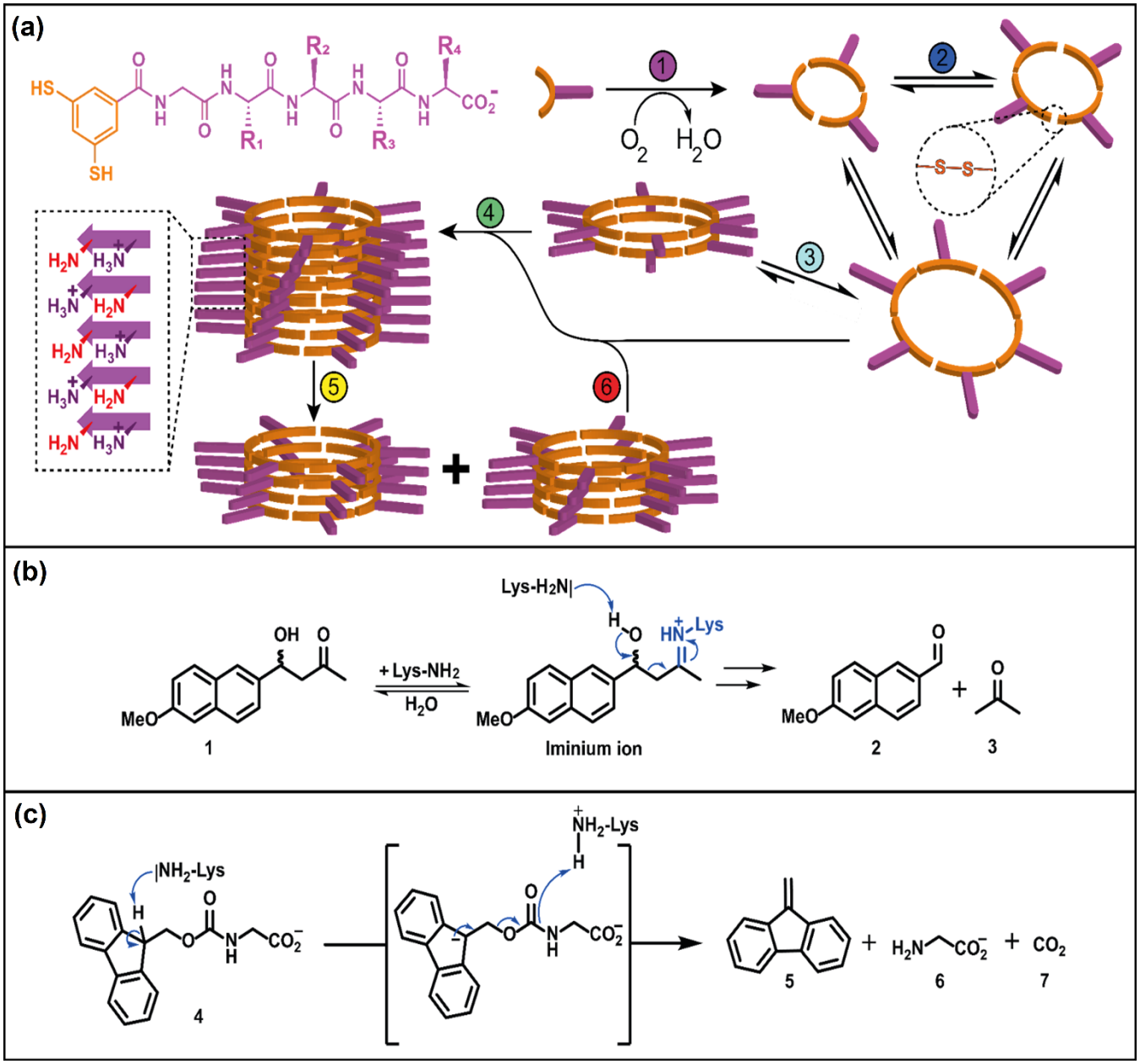

Figure 1. (a) Schematic structure and proposed mechanism of (hexamer) replicator fiber emergence, starting from a building block with a benzene-1,3-dithiol core appended to a pentapeptide that starts with a glycine, followed by alternating hydrophobic and hydrophilic (L-)amino acids. Upon oxidation of thiols to disulfides (1), these building blocks form DCLs of interconverting macrocycles (2). After spontaneous nucleation of a distinct macrocycle size (here a hexamer; (3)), the product distribution shifts towards the production of more of the macrocycle that assembles (4). The fibers keep growing from the fiber ends and when mechanical energy is supplied, the elongating fibers break, creating more fiber ends (5) from which the fibers can grow, enabling exponential replication (6). The dense packing of peptide side-chains in the fiber diminishes the apparent p*K*a of the ammonium groups, compared to that of un-assembled macrocycles, as a result of Coulombic repulsion, enabling catalysis through the resulting amines.[11] (b) Amine-mediated catalysis of the retro-aldol reaction of methodol **1** through an iminium intermediate yields 6-methoxy-2-naphthaldehyde **2** and acetone **3**. (c) Brønsted-base-mediated cleavage of FMOC-glycine **4**, yielding dibenzofulvene **5**, glycine **6** and CO_2.

All replicator assemblies were further characterized with circular dichroism (CD) (SI section S2.2) and thioflavin T fluorescence (SI section S2.1) to probe the presence of β-sheet like structures.[37] Considerable differences in CD spectra and thioflavin T fluorescence were observed for the different replicators, suggesting that the replicator fibers have different degrees of internal structure.

To confirm whether a DCL produced self-replicators, seeding experiments were conducted (SI section S2.5). A small aliquot of pre-formed replicator seed was added to a DCL, before significant amounts of replicator had spontaneously formed and the subsequent growth of the replicator was monitored by UPLC or UV/VIS and compared to growth in an unseeded control. All systems showed accelerated replication kinetics compared to the unseeded control experiments.

Catalysis by single-building-block replicators. We determined the catalytic efficiencies of replicators made from building blocks **8**-**24** for the retro-aldol reaction of methodol **1** (Figure 1b) and the cleavage of FMOC-glycine **4** (Figure 1c) by

measuring the initial rates of these reactions following previously established protocols.[11] We monitored all reactions in triplicate over a time span of 162 min, with direct injections from the reaction vials. To guarantee representative sampling, we utilized a custom-built sample tray, that allows for stirring of the reaction vials inside the UPLC sample chamber during measurements.[38] We monitored the formation of retro-aldol product **2** (which absorbs at 313 nm), upon mixing replicator fibers (25 μM in building block) with methodol **1** (200 μM) in 50 mM borate buffer with 1% acetonitrile at pH 8.2 and 25 °C, while stirring at 1200 rpm. FMOC cleavage was monitored using a similar protocol: After mixing a solution of replicator fibers (25 μM in building block) with **4** (200 μM) in 50 mM borate buffer with 1% acetonitrile at pH 8.2 and 25 °C, stirred at 1200 rpm, we followed the disappearance of **4** (monitored at 254 nm). Monitoring the resulting alkene **5** was less suitable as it gave broad peaks in the UPLC chromatograms (Figure S2). Peak areas were converted to concentrations utilizing previously established calibration curves.[11] The changes in peak areas were subsequently fitted by linear regression to obtain the initial rates. Measuring initial rates avoids complications due to product inhibition or two-phase kinetics (where a burst phase is followed by a slower subsequent reaction), previously observed in designer enzymes.[39-41]

The initial rates of the set of 18 different replicators for both catalytic transformations are shown in Figure 2. The observed initial rates stretch over two orders of magnitude for the retro-aldol reaction and span three orders of magnitude for FMOC cleavage. All replicators had at least some catalytic activity for both reactions, except for $\mathbf{19}_6$. This replicator has three ionizable groups in its sequence (GLKFE) in the form of the amine side chain of lysine, the carboxylic acid side chain of glutamic acid, and the carboxy terminus. At neutral pH this building block can be expected to have a net negative charge, which precludes the presence of non-protonated amine groups, that are postulated to mediate catalysis (vide supra).

As expected, alterations of the lysine residues affected catalytic activity. Replicators made from building blocks XGLRFK **17** and XGLKFR **18**, in which one of the two lysines of parent building block **8** was replaced by an arginine, showed reduced activity towards the retro-aldol reaction of **1** and the cleavage of **4**. When one of the lysines was replaced by ornithine (the side chain of which is one CH_2 shorter than that of lysine) to obtain XGLKFO **15** or XGLOFK **16**, catalysis was reduced for FMOC cleavage, but enhanced for the retro-aldol reaction.

Across the board catalytic activity of the probed pentapeptide-based replicators appears remarkably robust to changes in the amino-acid sequence. As long as amine functional groups were present, the amino-acid sequence could be changed substantially and catalytic activity and catalytic promiscuity of the replicator fibers persisted. This is an encouraging observation in the context of early evolution. Only if the catalytic activity is maintained in a substantial part of structure space, changes in the peptide sequence become possible without rapidly losing catalytic activity, allowing further evolutionary exploration. Furthermore, early evolution is most likely accompanied by low replication fidelity (high mutation rates), so in order for catalytic activity the persist, it must be tolerant towards mutations.

The replicator structure space that we surveyed is dominated by hexameric replicators. While only few replicators with different ring sizes were studied, the resulting data suggest that ring size does affect catalytic efficiency: Hexamer $\mathbf{12}_5$ outperformed pentamer $\mathbf{12}_6$ by a factor 2 in retro-aldol catalysis and by a factor 3 in FMOC cleavage. Remarkably, octamer fibers of XGLKAK **13** proved to be the most efficient catalyst of the retro-aldol reaction (while only being average in activity towards the cleavage of **4**). In contrast, the other octamer replicator that was investigated, obtained from XGLKSK **14**, showed only average retro-aldol activity and hardly any activity towards FMOC cleavage.

The replicators made from the building blocks in group 3, in which the amine residues are placed closer to the core of the fibers, were showing relatively high activity for both the retro-aldol reaction and FMOC cleavage reactions, suggesting that bringing the catalytically active amines in a more hydrophobic environment benefits activity.

Finally, inspection of group 4 showed that relatively subtle variations in peptide structure can have a large impact on activity. Hexamer replicators made from XGFKFK **23** and XGFKLK **24** proved to be the most efficient catalysts for FMOC cleavage, even though they differ only little in building block structure from XGLKFK **8** and XGLKIK **11**. In contrast, the activity of the corresponding four replicators for the retro-aldol reaction is quite comparable.

Catalysis by mixed-building-block replicators. In principle replicator fibers made from mixtures of building blocks can provide microenvironments that are not accessible by using only a single building block. This notion encouraged us to probe to what extent such systems could show catalytic activity that differs from that of the corresponding single-building-block replicators. We set out to obtain self-replicators from equimolar mixtures of two building blocks for six binary combinations, combining our most studied building block **8**, with building blocks **13**, **14**, **16**, **17** and **22**, spanning a wide range of activities of the corresponding single-building-block replicators. We also included the **17**+**18** mixture, to include modifications of the lysine residues. Replicators that emerged from these mixtures were characterized by CD spectroscopy (SI section S2.2), thioflavin T analysis assay (SI section S2.1),

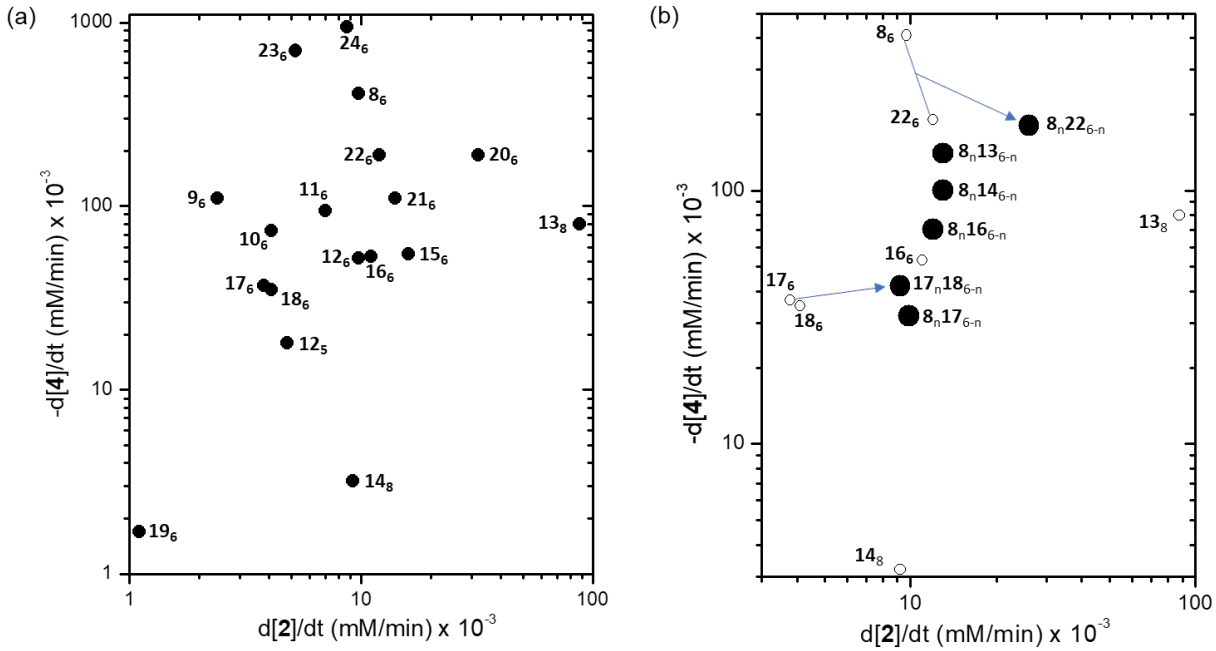

Figure 2. Correlation of the initial rates of the retro-aldol reaction of methodol **1** and the cleavage of FMOC-glycine **4** catalyzed by different replicators made from (a) a single building block, or (b) an equimolar mixture of two different building blocks. Arrows indicate the instances where combining building blocks yielded catalytic activities superior to those of the corresponding single-building-block systems. Error bars are omitted for clarity (errors are provided in Table 1). The numbers correspond to the building blocks listed in Table 1.

Table 1. Initial rates of the retro-aldol reactions of **1** and the FMOC cleavage of **4** catalyzed by self-replicators with indicated ring sizes made from building blocks **8**-**24** or mixtures of these. Building blocks in group 1 differ from each other in the fourth amino-acid residue. Those in group 2 feature mutations in the lysine residues implicated in catalysis. Group 3 has the lysine residues closer to the core, while group 4 has mutations in the second amino acid residue. Group 5 consists of binary combinations of building blocks. Errors represent the standard deviation of three repeats.

	Sequence[a]	Macrocycle size	d[2]/d[t] (mM min^{-1}) x 10^{-3}	-d[4]/d[t] (mM min^{-1}) x 10^{-3}
Group 1 (4th amino acid altered)				
8	XGLKFK	hexamer	9.7 ± 0.12	410 ± 9.2
9	XGLKVK	hexamer	2.4 ± 0.16	110 ± 2.9
10	XGLKMK	hexamer	4.1 ± 0.29	74 ± 4.8
11	XGLKIK	hexamer	7.0 ± 0.25	94 ± 5.1
12	XGLKYK	hexamer	9.7 ± 0.75	52 ± 17
		pentamer	4.8 ± 0.41	18 ± 5.7
13	XGLKAK	octamer	88 ± 2.1	80 ± 12
14	XGLKSK	octamer	9.2 ± 0.38	3.2 ± 2.3
Group 2 (catalytic lysine residues altered)				
15	XGLKFO	hexamer	16 ± 0.99	55 ± 2.6
16	XGLOFK	hexamer	11 ± 0.87	53 ± 4.2
17	XGLRFK	hexamer	3.8 ± 1.9	37 ± 1.2
18	XGLKFR	hexamer	4.1 ± 0.21	35 ± 4.2
19	XGLKFE	hexamer	1.10 ± 0.01	1.7 ± 2.3
Group 3 (inverse sequence)				
20	XGKFKL	hexamer	32 ± 1.1	190 ± 8.8
21	XGKFKF	hexamer	14 ± 0.95	110 ± 11

22	XGKLKL	hexamer	12 ± 0.57	190 ± 4.5
Group 4 (second and fourth amino acids varies)				
23	XGFKFK	hexamer	5.2 ± 0.81	700 ± 27
24	XGFKLK	hexamer	8.7 ± 2.2	950 ± 310
Group 5 (1:1 mixtures of building blocks)				
8+13	XGLKFK/XGLKAK	hexamer	13 ± 0.27	140 ± 13
8+14	XGLKFK/XGLKSK	hexamer	13 ± 0.68	100 ± 7.11
8+16	XGLKFK/XGLOFK	hexamer	12 ± 0.40	70 ± 11
8+17	XGLKFK/XGLRFK	hexamer	9.9 ± 0.15	32 ± 3.2
8+22	XGLKFK/XGKLKL	hexamer	26 ± 1.2	180 ± 7.7
17+18	XGLRFK/XGLKFR	hexamer	9.2 ± 0.18	42 ± 11

X = 3,5-dimercaptobenzene-CO-

TEM analysis (SI section S2.4) and seeding experiments (SI section S2.5). UPLC-MS analysis (SI section S2.3) showed that the replicators incorporated the corresponding building blocks in a statistical manner (close to Gaussian distribution for **8**+**16**, **8**+**17**) or with a bias towards incorporation of building block **8** (for **8**+**13** and **8**+**14**). In the latter case, the remaining block **13** or **14** resided in trimers and tetramers. We also observed a strongly non-statistical hexamer mixture in the **8**+**22** system in which the formation of $\mathbf{8}_3\mathbf{22}_3$ was favored as a result of steric zipper formation.[36] For the **17**+**18** system no information regarding the building block incorporation preferences was obtained due to the fact that both building blocks have identical mass. In all cases we obtained hexamer replicators, even when including blocks **13** or **14**, which, by themselves, prefer to make octamer replicators.

Catalysis results obtained with replicators that emerged from the **8**+**14** and **8**+**16** mixtures yielded activities for FMOC cleavage that were comparable to that of the corresponding single-building-block replicators. Catalysis of the retro-aldol reaction by these mixed systems was somewhere between the activities of the single-building-block systems. Also replicators made from the **8**+**13** mixture showed activities in-between that of the corresponding single-building block systems.

In contrast the **8**+**17** system yielded replicators which had the same relatively high activity for the retro-aldol reaction as the **8**-only system and the same low activity for FMOC cleavage as the **17**-only system.

Finally, we also observed two cases where mixed-building-block replicators had activities for the retro-aldol reaction that exceeded those of the corresponding single-building-block systems. Such behavior was shown by hexamer replicators that emerged from the **8**+**22** (XGLKFK + XGKLKL) and **17**+**18** (XGLKFR/XGLRFK) mixtures. For the latter system FMOC activity is unaltered compared to that of the single-building block replicators, while for the **8**+**22** system, FMOC activity was equal to that of replicator $\mathbf{22}_6$ and lower than that of $\mathbf{8}_6$.

Taken together, these data show that the incorporation of more than one building block can lead to new microenvironments that can benefit catalysis. However, this appears only the case for a limited number of building block combinations and depends on building block structure in a way that is beyond our current ability to predict or even explain.

Comparing retro-aldol with FMOC cleavage activity. The three replicators with the highest activity for the retro-aldol cleavage of methodol differ from the three replicators that are most active in catalyzing the FMOC cleavage reaction. In fact, the catalytic activity of a given replicator for the retro-aldol reaction shows no significant correlation to its activity for FMOC cleavage. This is perhaps not surprising, given that both reactions are mechanistically quite distinct. However, given these distinct mechanisms, it is surprising to find that catalytic promiscuity is exhibited for the majority of the tested self-replicators. We speculate that this promiscuity is due to the relatively dynamic and heterogeneous nature of the fiber assemblies, which provide a range of different local microenvironments. We previously established for replicator $\mathbf{8}_6$ that only a small fraction of the lysine residues (<10%) is active in catalysis of the retro-aldol reaction.[14]

Conclusions

With this work we substantially extend the number of self-replicating molecules reported to date, for which catalytic activity spontaneously emerges, alongside self-replication. Promiscuous catalytic activity appears a recurrent trait for the investigated class of pentapeptide based self-replicators. Such catalytic promiscuity is a highly desirable feature in early evolution, since it allows for catalysis of different reactions, while only having to replicate a single entity. Analogous to what is observed in the evolution of enzymes,[25,28] we speculate that it should also be possible to evolve catalytically promiscuous self-replicators towards specialist catalysts optimized for one particular transformation.

Catalytic activity proved to be remarkably resilient towards changes in the peptide sequence. This characteristic bodes well for the evolvability of these systems, as it allows the replicator peptide sequence to mutate without immediate loss of function. This robustness is particularly relevant in early evolution where replication most likely occurs with low fidelity and therefore with a high mutation rate.

We also demonstrated that in binary building block mixtures replicators can emerge that may have superior activity, compared to the replicators made from the corresponding individual building blocks. Thus, offering mixtures of building blocks appears a promising strategy for expanding evolutionary space in a combinatorial fashion in search for improved catalytic function.

The investigated class of replicators show some parallels to RNA, in that they can self-replicate and catalyze other reactions. But, unlike RNA, they do not suffer from the folding problem: RNA requires to be unfolded for replication while catalysis only occurs in the folded state.[16,26] For the class of

building blocks investigated here, self-replication and catalytic activity naturally go hand-in-hand as the sites for self-replication (which occurs at the fiber ends) are spatially separated from the sites where catalysis takes place (presumably on the fiber sides) and no conformational rearrangements are needed for replication, nor catalytic activity.

Taken together, these observations raise the intriguing prospect of applying Darwinian evolution, outside the realm of biology and biomolecules, for the discovery and optimization of synthetic catalysts.

ASSOCIATED CONTENT

Supporting Information.
This material is available free of charge via the Internet at http://pubs.acs.org.
Reaction Conditions, UPLC and LC-MS methods and data, CD spectra, ThT assays, TEM images, UV/VIS spectra, MALDI, Seeding experiments.

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Notes

The authors declare no competing financial interest.

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