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Article

Two Sides of the Same Coin: Emergence of Foldamers and Self-Replicators from Dynamic Combinatorial Libraries

Charalampos G. Pappas,*,# Bin Liu,# Ivana Marić, Jim Ottelé, Armin Kiani, Marcus L. van der Klok, Patrick R. Onck, and Sijbren Otto*

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ABSTRACT: The ability of molecules and systems to make copies of themselves and the ability of molecules to fold into stable, well-defined three-dimensional conformations are of considerable importance in the formation and persistence of life. The question of how, during the emergence of life, oligomerization reactions become selective and channel these reactions toward a small number of specific products remains largely unanswered. Herein, we demonstrate a fully synthetic chemical system where structurally complex foldamers and self-replicating assemblies emerge spontaneously and with high selectivity from pools of oligomers as a result of forming noncovalent interactions. Whether foldamers or replicators form depends on remarkably small differences in building block structures and composition and experimental conditions. We also observed the dynamic transformation of a foldamer into a replicator. These results show that the structural requirements/design criteria for building blocks that lead to foldamers are similar to those that lead to replicators. What determines whether folding or replication takes place is not necessarily the type of noncovalent interaction, but only whether they occur intra- or intermolecularly. This work brings together, for the first time, the fields of replicator and foldamer chemistry.

INTRODUCTION

The inner workings of current life feature a division of labor: genetic information is stored in nucleic acids, while most catalytic processes are mediated by proteins. The latter require folding for proper function, while for nucleic acids, and in particular for RNA, folding hampers their replication. While the tasks of catalysis and information storage and propagation may be divided, the molecules that perform these functions are connected in a complex manner: The functional interplay between proteins and nucleic acids form the central dogma of biochemistry and the machinery associated with the synthesis of proteins based on the information contained in nucleic acids is an astounding product of evolution. The separation of genotype and phenotype that is achieved through the division of labor between proteins and nucleic acids is itself a powerful enabler of further evolution. Given their central role in biology, uncovering how molecules capable of storing and copying information and molecules capable of folding into well-defined conformations may emerge from simple precursors is relevant to the grand challenges of the origin and synthesis of life. These topics have been addressed separately in the fields of self-replicator chemistry and the field of foldamers.

Molecules that can spontaneously self-replicate remain less than a handful. Following pioneer work by von Kiedrowski,[1] synthetic self-replicating systems containing RNA[2−4] and peptides[5−7] have been implemented. Despite the fact that the majority of these studies involve elaborate design, we have recently discovered that self-replicating structures can emerge autonomously from a mixture of interconverting molecules.[8] In the latter case, self-replication is driven through assembly into β-sheets, leading to the formation of supramolecular polymers.[9]

Synthetic foldamers[10−15] have also been demonstrated featuring various non-natural backbones, capable of molecular recognition[16] and catalysis.[17] These structures rely heavily on design, followed by multiple-step synthesis. An alternative pathway for accessing dynamic tertiary folded architectures has been recently proposed by us. Following a systems chemistry approach,[18] we showed the spontaneous emergence of foldamers of unrivaled structural complexity, where efficient intramolecular interactions drive their synthesis in high yield and in a one-step process.[19]

Despite its importance in biology, the relationship between the processes of self-replication and foldamer formation has received comparatively little attention, largely because, for the

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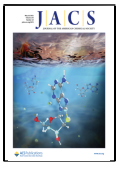

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vast majority of cases, the building blocks from which replicators are made bear little to no resemblance to those that make up foldamers. Yet it is well established in biology that RNA can be replicated and can also be folded into structures that may exhibit catalytic activity. Thus, the fields of foldamers and replicators may be more closely connected than is currently evident.

We now show that folding and replication are two sides of the same coin. Small changes in conditions or in the structure of a building block or in the composition of a two-building-block system can swing the balance from the formation of foldamers to the formation of self-replicators. We also show that folded structures of considerable structural complexity can transform into self-replicating supramolecular assemblies through reversible covalent chemistry. Whether foldamers or self-replicators form is governed primarily by whether noncovalent interactions occur within or between molecules, respectively. We speculate that this duality could constitute a first step toward the emergence of a primitive genotype–phenotype separation.

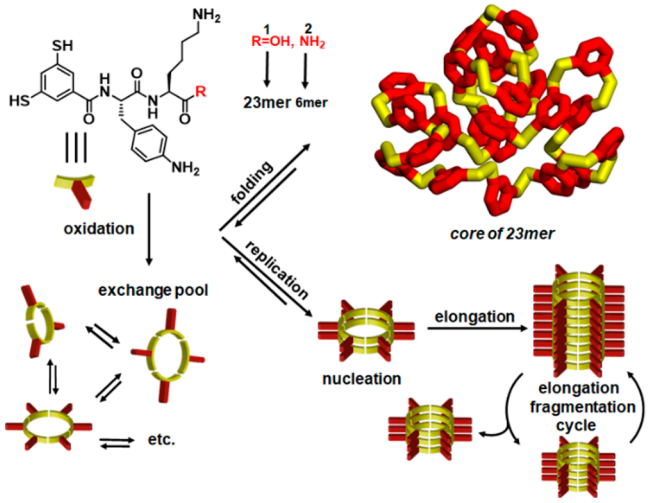
RESULTS AND DISCUSSION

We have previously described the spontaneous emergence of a complex discrete folded structure composed of 23 identical subunits, starting from a dynamic combinatorial library (DCL) made from dipeptide-appended dithiol building block **1** (Scheme 1). The dipeptide is composed of a modified phenylalanine (p-NH_2 substituted) and a lysine amino-acid residue and is zwitterionic at neutral pH.[20] We have since discovered that relatively small changes in environmental conditions can affect the formation of the folded structure. Libraries made from 2.0 mM building block **1** in the presence of 1.0 M sodium bromide led to the emergence of the hexamer macrocycle (Figure 1a), as evident from ultraperformance liquid chromatography/mass spectrometry (UPLC/MS) analysis. Hexamer formation was also observed in the presence of a variety of different sodium salts (1.0 M sulfate, perchlorate, citrate; Figures S4 and S5). However, in the presence of 1.0 M KCl or 1.0 M $MgCl_2$ foldamer $\mathbf{1}_{23}$ was produced and no $\mathbf{1}_6$ was detected (Figure S6). Given that the effects of these salts do not follow typical salting out trends (i.e., the Hoffmeister series), we speculate that specific interactions involving the bromide, sulfate, perchlorate, or citrate anions occur, most likely with ammonium groups of **1**.

Seeding experiments were performed in order to investigate whether this dynamic reconfiguration occurs autocatalytically. Thus, 10 mol % of the preformed $\mathbf{1}_6$ was added into a fresh library, and UPLC analysis showed that the time scale of the emergence of hexamer was shortened dramatically, which confirms the autocatalytic nature of the formation of $\mathbf{1}_6$ (Figure 1b).[21]

To further demonstrate the close association between the two different modes of organization leading to folding or self-replication, we formed a DCL through oxidizing building block **1** by oxygen from the air in a solution containing 100 mM sodium citrate (instead of the 1.0 M concentration used above). UPLC analysis of the library after 8 days revealed the formation of $\mathbf{1}_{23}$ in high yield (85%). Upon seeding this sample with $\mathbf{1}_6$, the giant macrocycle fully converted into the self-replicating species (Figure S7). This interconversion was accompanied by the formation of laterally associated fibrillar

Scheme 1. Schematic Representation Showing the Emergence of Foldamers and Self-Replicators from Dynamic Combinatorial Libraries Made by Oxidizing Dipeptide-Functionalized Dithiol Building Blocks[a]



[a]The selective formation of specific oligomers is driven by noncovalent interactions occurring either intramolecularly (for folding) or intermolecularly (for replication).

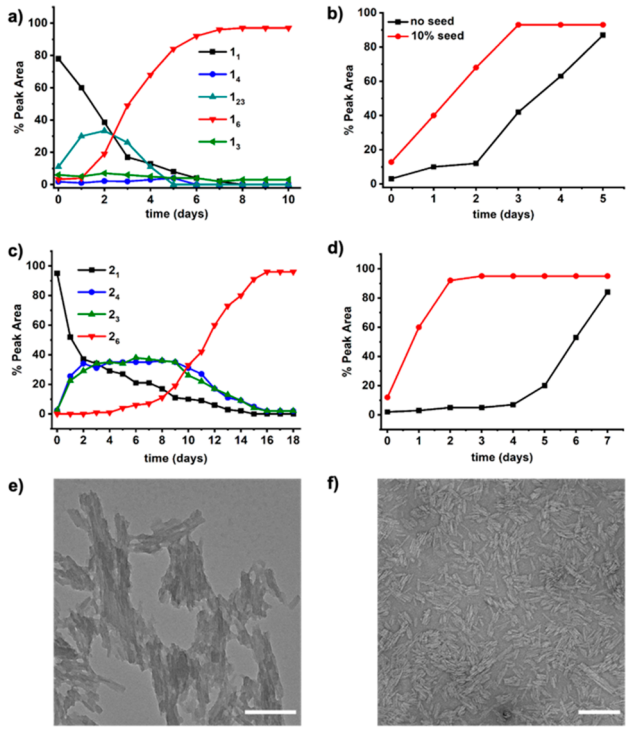


Figure 1. Kinetic profile of a dynamic combinatorial library made from **1** (2.0 mM in 12.5 mM borate buffer, pH = 8.0, in the presence of 1.0 M sodium bromide) stirred at 1200 rpm, (a) nonseeded and (b) after the addition of 10 mol % of the preformed hexamer at day 0. Kinetic profile of a dynamic combinatorial library made from **2** (2.0 mM in 12.5 mM borate buffer, pH = 8.0) (c) nonseeded and (d) after the addition of 10 mol % of the preformed hexamer in 80% oxidized library using sodium perborate (80 mM), compared to a nonseeded sample that was also treated with sodium perborate. Negative stain TEM micrographs of samples dominated by (e) $\mathbf{1}_6$ and (f) $\mathbf{2}_6$; the scale bar corresponds to 200 nm.

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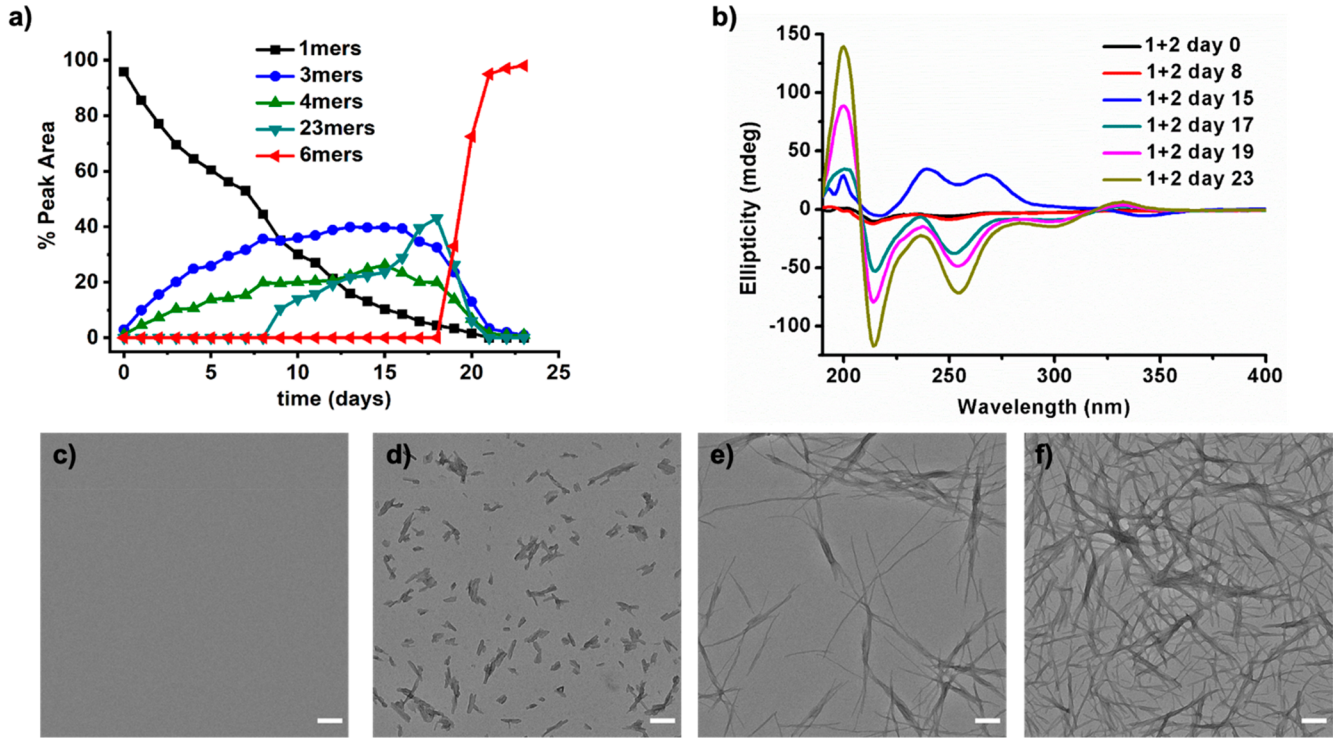

Figure 2. (a) Kinetic profile of a dynamic combinatorial library made from a mixed system composed of equimolar amounts **1** and **2** (at a total concentration of 4.0 mM in 12.5 mM borate buffer, pH = 8.0) stirred at 1200 rpm. (b) Time-dependent CD spectra of the library. Negative stain TEM micrographs showing the formation of fibrillar assemblies containing (c) 0%, (d) 20%, (e) 60%, and (f) 90% of mixed hexamers. Scale bar: 200 nm.

assemblies (Figure 1e, Figure S8) visualized using transmission electron microscopy (TEM).

Having demonstrated that a foldamer can be transformed into a self-replicator upon changing the environment, we explored whether mutations of the chemical structure might result in similar effects. We reasoned that charge repulsion is more severe in the compact foldamer $\mathbf{1}_{23}$ than in the more "open" fibrous assemblies of $\mathbf{1}_6$, consistent with the salt-induced shift toward replicator formation described above. Thus, increasing charge repulsion between the building blocks by providing these with a net charge was expected to favor replicator formation. Hence, we designed cationic building block **2**, which differs from zwitterionic building block **1** in having the carboxylate terminus amidated. The synthesis of **2** was performed on a solid phase using Rink Amide resin. UPLC/MS analysis of DCLs made at a concentration of 2.0 mM **2** in 12.5 mM borate buffer, pH = 8.0, revealed the formation of small rings (trimers and tetramers) at the early stages of the process. After a lag phase of 8 days, the product distribution suddenly changed with the emergence of the cyclic hexamer macrocycle (Figure 1c, Figures S9 and S10).

In order to investigate whether the formation of $\mathbf{2}_6$ is autocatalytic, we oxidized a solution of **2** to 80% (conversion of thiols into disulfides) using sodium perborate (80 mM), followed by slower further oxidation mediated by oxygen present in the air. Subsequently, 10 mol % of $\mathbf{2}_6$ was added, and UPLC analysis showed that the initial lag phase was dramatically diminished; hexamer grew rapidly, accounting for up to 90% of the library material after 2 days of continuous stirring (Figure 1d). The formation of fibrous supramolecular assemblies was evident from TEM analysis (Figure 1f).

Notably, these relatively short peptides are capable of giving rise to self-replication, a property that was previously reported mostly for systems containing longer pentapeptide sequences that were biased toward β-sheet formation through alternating hydrophobic and charged amino acid residues.[22]

The fact that very specific ring sizes form is in line with previous observations for self-replicating systems (which tend to form the ring size for which multivalency is just sufficient to allow self-assembly)[22] and foldamers (for which different folds often have substantially different energies, so a particular fold and accompanying ring size tend to form with high selectivity).[20]

Having in hand two very similar building blocks that, at low ionic strength, give either foldamers (for **1**) or replicators (for **2**), we set out to investigate the behavior of DCLs made by mixing these two building blocks. We initially prepared libraries containing an equimolar amount of **1** and **2** (at a total concentration of 4.0 mM in 12.5 mM sodium borate buffer, pH = 8.0) and stirred the solution in the presence of air. At the early stages of the process, mixed trimers ($\mathbf{2}_3$, $\mathbf{2}_2\mathbf{1}_1$, $\mathbf{2}_1\mathbf{1}_2$, $\mathbf{1}_3$) and tetramers ($\mathbf{2}_4$, $\mathbf{2}_3\mathbf{1}_1$, $\mathbf{2}_2\mathbf{1}_2$, $\mathbf{2}_1\mathbf{1}_3$, $\mathbf{1}_4$) dominated the libraries (Figure 2a). After 8 days, we observed the emergence of additional library members, consisting of 23mers, which eventually accounted for up to 40% of the library material.

Separation of the set of giant macrocycles was challenging with the available chromatographic techniques. However, mass analysis confirms that the family of large macrocycles is composed of a series of mixed 23mers, even though we cannot identify their exact composition. Finally, after 19 days, the emergence of a new set of species was observed composed of a family of mixed hexamers ($\mathbf{2}_6$, $\mathbf{2}_5\mathbf{1}_1$, $\mathbf{2}_4\mathbf{1}_2$, $\mathbf{2}_3\mathbf{1}_3$, $\mathbf{2}_2\mathbf{1}_4$, $\mathbf{2}_1\mathbf{1}_5$, $\mathbf{1}_6$) reaching complete conversion after 23 days (Figures 2a and

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S11−S32). Seeding experiments confirmed the autocatalytic nature of the growth of the hexamer family (Figure S33). Circular dichroism (CD) experiments were used to probe the changes in the chiral ordering of the building blocks in this multiphase dynamic transition (Figure 2b). CD-silent spectra were found for monomers and small rings. After 2 weeks, with the emergence of the folded structures, two characteristic peaks were observed at 240 and 266 nm, respectively, which are attributed to a chiral arrangement of the aromatic dithiol cores, even though the aromatic rings are relatively remote from the chiral centers of the amino acids. A completely different chiral environment was formed after the emergence of the replicators, accompanied by the appearance of positive helicity at 197 nm and negative helicity at 216 nm, indicative of a β-sheet type assembly. Significant enhancement of the CD signal was noticed upon further conversion into hexamers (Figure 2b). The CD spectra observed upon formation of the mixed 23mers and mixed hexamers are very similar to the CD spectra of $\mathbf{1}_{23}$ and $\mathbf{2}_6$, respectively, which are shown in Figure S34, suggesting that the mixed building block assemblies have structures that are very similar to that of the pure foldamer and replicator. The formation of a β-sheet type assembly in the case of $\mathbf{2}_6$ was further confirmed using thioflavin T experiments, showing the enhancement in fluorescence that is indicative of the presence of β-sheets (Figure S35). Molecular dynamics simulations on the $\mathbf{1}_6$ and $\mathbf{2}_6$ fibers show that in both systems the aromatic dimercaptobenzene cores of the macrocycles assemble through π-stacking (Figure 3a,b and d,e, showing two

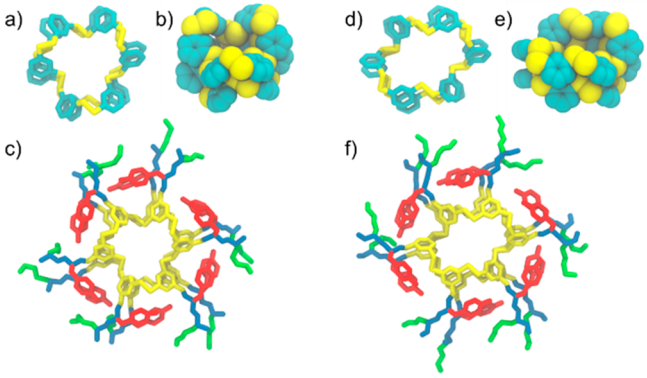


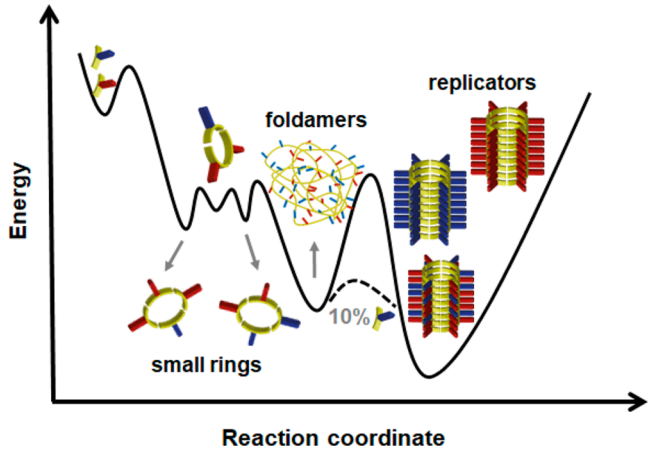
Figure 3. Representative frames taken after 100 ns of a molecular dynamics simulation of stacks of 16 molecules of $\mathbf{1}_6$ (left; panels a−c) and $\mathbf{2}_6$ (right, panels d−f). For clarity only two adjacent macrocycles from the center of the stack (no. 8 and 9) are shown. (a) Top view in wireframe representation; (b) side view in space-filling representation of the dimercaptobenzene core of $\mathbf{1}_6$ (peptide side chains not shown); (c) top view of the complete molecules also showing the interactions between the peptide chains; main chain is shown in blue and side groups in red and green. (d−f) Analogous frames for identical simulations on $\mathbf{2}_6$.

macrocycles taken from the center of the stacks of $\mathbf{1}_6$ and $\mathbf{2}_6$, respectively), while the peptide side chains maintain β-sheet-like arrangements for the duration of the simulations (shown for two selected macrocycles in Figure 3c and f).

The dynamic transition from small rings to large folded rings and finally to replicating structures was also reflected in changes at the nanoscale observed using time-dependent TEM. Ill-defined aggregates were present at the early stages of the process, followed by the appearance of fibrillar structures, which increased in both length and density after the emergence of the self-replicating species (Figure 2c−f).

Next, we investigated how foldamer and replicator formation depends on the ratio of building blocks **1** and **2**. A series of libraries were set up at a total concentration of 4.0 mM in 12.5 mM borate buffer at pH = 8.0, varying the **1**:**2** ratio from 10% to 90% and monitored using UPLC/MS (Figures S36−S44). As previously observed for the equimolar mixture, small rings were the dominant species at the early stages of the process. After 25 days of continuous stirring, libraries containing larger mole fractions of the carboxylate building block **1** (90−60% of **1**) yielded mixed 23mers, accompanied by a small amount of mixed trimers and tetramers. However, after 40 days, mixed hexamers have emerged, with the exception of the library made from a mixture containing 90% **1**. In the latter case, the absence of any self-replicating species might be attributed to the fact that the library reached complete oxidation prior to the emergence of hexamers (disulfide exchange requires a catalytic amount of thiol). Therefore, we reduced the library (by 20%) using dithiothreitol (DTT) and allowed it to oxidize again by oxygen from the air. UPLC/MS analysis revealed the emergence of hexamers. Fibrillar assemblies were found for the mixed libraries regardless of the ratio used (Figure S45). These results raised the question whether $\mathbf{1}_{23}$ may also be converted into $\mathbf{1}_6$ upon prolonging equilibration time through partial reduction. Thus, a library containing only the carboxylate building block (100% of **1**) was subjected to a series of subsequent partial reduction/oxidation steps, which did indeed result in the almost complete conversation of foldamer $\mathbf{1}_{23}$ into replicator $\mathbf{1}_6$ (Figure S46), suggesting that the latter most likely represents the lowest energy state of the system (Scheme 2).

Scheme 2. Schematic Representation of the Energy Diagram of the Dynamic Transition from Small to Folded and Self-Replicating Structures upon Mixing 1 and 2



In order to investigate whether small rings exhibit any self-assembling propensity prior to the emergence of replicators made from the single building block and from the binary systems, we have oxidized libraries to 90% using sodium perborate. We have observed the formation of aggregates using cryo-TEM, and their assembly was further confirmed using dynamic light scattering (DLS) and Nile-red experiments (Figures S47 and S48). The exact role of the organization of the small rings prior to the formation of autocatalytic and folded structures is currently under investigation.

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CONCLUSIONS

We have demonstrated the use of dynamic combinatorial chemistry as a powerful approach to trigger the autonomous formation of foldamers and self-replicating structures and the conversion of the former to the latter. Small amounts of the cationic building block are sufficient to induce the conversion of a foldamer into a self-replicator in a mixture of zwitterionic and cationic building blocks. Furthermore, salts can induce the formation of replicators from a building block that, in the absence of this salt, forms a foldamer. Given the ease with which systems can be (re)directed from folding to replication suggests that these processes are related. In fact, the design criteria to direct building blocks toward foldamer formation are remarkably similar to those for directing them to replication. In both cases, building blocks need to have the ability to form noncovalent interactions with other identical building blocks. When, after oligomerization (through disulfide bond formation), these interactions take place *within* the same oligomer, these interactions may cause its folding. When the noncovalent interactions take place *between* different (but identical) oligomers, this may lead to self-assembly, which can drive self-replication. Thus, what determines whether folding or replication takes place is not necessarily the type of noncovalent interaction, but only whether they occur intra- or intermolecularly. In our experience, it is very hard to predict which side of the coin (the foldamer side or the replicator side) ends on top. Interestingly, nature, even after billions of years of evolution, still has not fully controlled the flipping of this coin, as evident from diseases like Alzheimer's, in which proteins aggregate into amyloids[23−25] (driven by *inter*molecular noncovalent interactions in a process that is autocatalytic[26]), instead of folding[27] into their functional form (through forming *intra*molecular noncovalent interactions).

It is tempting to speculate about the significance of the foldamer−replicator duality in the context of the early evolution of life. The observation that the same building blocks can give rise to replicators and to foldamers might be a first step in the direction of a primitive genotype−phenotype separation. If replicators and foldamers coexist by virtue of being derived from the same building blocks, the evolutionary selection will take place based on the entire system (replicator + foldamer), with genotype and phenotype being constituted by different molecules (replicator and foldamer, respectively). One would expect that, for systems of this nature in which the foldamer performs a function beneficial to the "fitness" of the system, such functions are selected by evolution. Studies probing such behavior are currently underway in our laboratory.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.1c00788.

UPLC, LC-MS methods and data, library preparation, and additional CD spectra and TEM images (PDF)

AUTHOR INFORMATION

Corresponding Authors

Charalampos G. Pappas − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands;* orcid.org/0000-0003-3019-9607; Email: charalampos.pappas@livmats.uni-freiburg.de

Sijbren Otto − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands;* orcid.org/0000-0003-0259-5637; Email: s.otto@rug.nl

Authors

Bin Liu − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands*

Ivana Marić − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands*

Jim Ottelé − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands;* orcid.org/0000-0001-9875-2320

Armin Kiani − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands*

Marcus L. van der Klok − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands*

Patrick R. Onck − *Zernike Institute for Advanced Materials, University of Groningen, 9747 AG Groningen, The Netherlands*

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.1c00788

Author Contributions

#C.G.P. and B.L. contributed equally

Notes

The authors declare no competing financial interest.

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