

Complex Molecules That Fold Like Proteins Can Emerge Spontaneously

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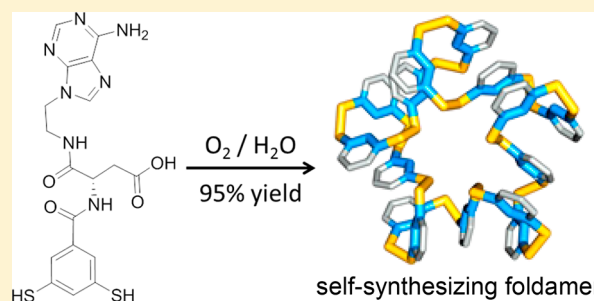
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Supporting Information

ABSTRACT: Folding can bestow macromolecules with various properties, as evident from nature's proteins. Until now complex folded molecules are the product either of evolution or of an elaborate process of design and synthesis. We now show that molecules that fold in a well-defined architecture of substantial complexity can emerge autonomously and selectively from a simple precursor. Specifically, we have identified a self-synthesizing macrocyclic foldamer with a complex and unprecedented secondary and tertiary structure that constructs itself highly selectively from 15 identical peptide-nucleobase subunits, using a dynamic combinatorial chemistry approach. Folding of the structure drives its synthesis in 95% yield from a mixture of interconverting molecules of different ring sizes in a one-step process. Single-crystal X-ray crystallography and NMR reveal a folding pattern based on an intricate network of noncovalent interactions involving residues spaced apart widely in the linear sequence. These results establish dynamic combinatorial chemistry as a powerful approach to developing synthetic molecules with folding motifs of a complexity that goes well beyond that accessible with current design approaches. The fact that such molecules can form autonomously implies that they may have played a role in the origin of life at earlier stages than previously thought possible.



INTRODUCTION

The chemistry of life relies on biopolymers (proteins, nucleic acids) folding into specific conformations that dictate their properties. It is generally believed that the complex folded structures encountered in biology are the result of millions of years of evolution. Much of the research on synthetic foldamers^{1–13} is driven by the desire to bypass evolution, go beyond the constraints of using only nature's building blocks, and directly access structures that fold like proteins, but are based on completely synthetic subunits. The ultimate goal is achieving new and sophisticated functions that require the molecular complexity of extended folded structures. This goal is still largely out of reach and foldamers able to exhibit specific function have remained rare,^{14–16} due to the huge challenge of obtaining new modes of folding in designed proteins,^{17,18} molecules that mimic peptides or nucleic acids,^{5,8,9} and in completely abiotic molecules.^{1–4,6,7,10–13}

The approach taken to accessing new synthetic foldamers has until now relied almost exclusively on design, followed by multistep synthesis. An impressive new range of backbones have been developed that fold into a variety of well-defined architectures, including secondary structures such as helices and sheets.¹⁹ Yet, the design approach tends to be based on

relatively simple and small-range assembly motifs, primarily driven by interactions between residues close to each other in the oligomeric chain of monomer units. Foldamers that rely on long-range interactions (between residues further apart in the oligomeric chain, as observed in the folding of proteins and nucleic acids) have remained difficult to access due to a lack of reliable design rules. Hence, alternative approaches are needed for accessing fundamentally new classes of foldamers that rely on long-range interactions.

Dynamic combinatorial chemistry^{20,21} has been suggested as a useful selection tool for accessing such new folded structures. In brief, in a dynamic combinatorial library (DCL) building blocks react with each other to give rise to a mixture of oligomeric compounds that continuously exchange these building blocks between them. When a specific library member is able to form efficient intramolecular noncovalent interactions, inducing it to fold, this compound should be more stable than other library members that are unable to engage in such interactions. Hence, the library composition should shift in favor of the foldamer. Indeed, several groups have reported

Received: October 30, 2018

Published: December 18, 2018

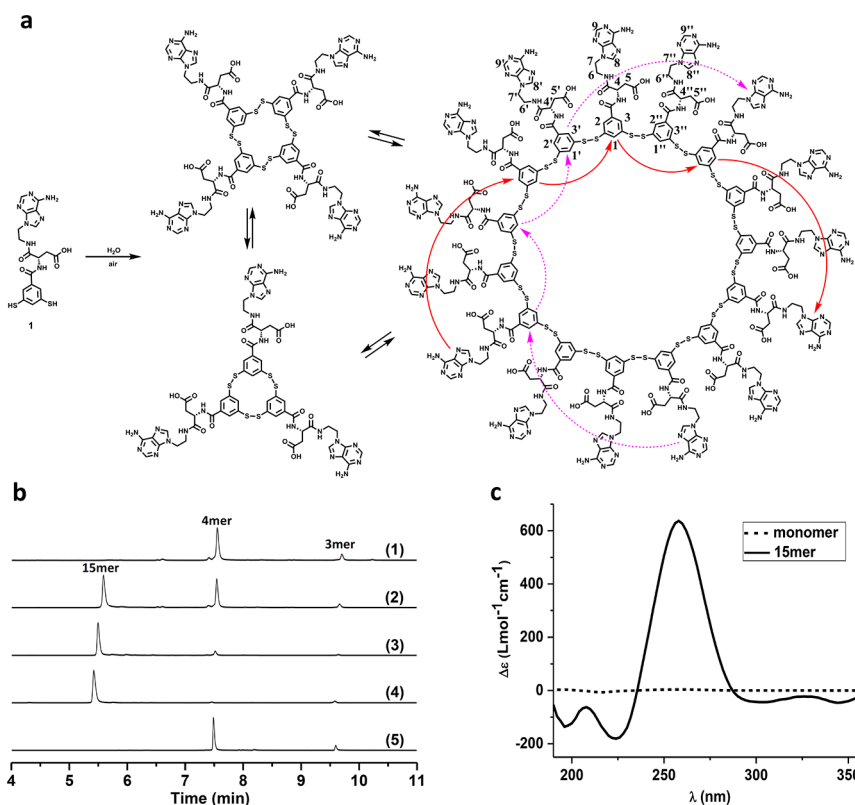


Figure 1. Selective formation of the 15mer. (a) Dynamic combinatorial library of amino-acid nucleobase building block **1**. Arrows indicate two sets of π -stacks observed between phenyls and adenines. The remaining three stacks are not shown for clarity. (b) UPLC chromatograms (absorption at 254 nm) showing library compositions after 14 days of stirring (in 50 mM borate buffer, pH = 8.2) at a building block concentration of (1) 0.050 mM; (2) 0.50 mM; (3) 2.0 mM; (4) 0.50 mM in the presence of 1.0 M NaCl; and (5) 0.50 mM in the presence of 50% acetone. (c) CD spectrum of monomer **1** (dotted line) and 15mer (solid line) in water at 298 K.

folding-driven changes in library compositions.^{22–29} However, until now this approach has failed to deliver fundamentally new folding motifs. This lack of success is surprising, given that dynamic combinatorial methods have proven successful in the discovery of new and often unexpected host–guest systems,^{30,31} interlocked structures,^{31,32} and self-replicating molecules.³³ All of these systems rely, like foldamers, on noncovalent interactions as the prime selection criterion.

We now report results that should prompt a revival of the combinatorial approach to foldamers. We discovered a new macrocycle that folds into a complex but precisely defined secondary and tertiary structure, constructing itself out of 15 identical peptide-nucleobase subunits. Folding of the structure drives its synthesis in 95% yield from a mixture of interconverting molecules of different ring size in a one-step process. Single-crystal X-ray crystallography and NMR analyses reveal a new folding pattern based on an intricate network of hydrogen bonds and π -stacking interactions featuring long-range interactions.

RESULTS AND DISCUSSION

We designed chimeric building block **1**, which contains an amino-acid and a nucleobase subunit, both key structural elements involved in the folding of proteins and nucleic acids, respectively. We reasoned that the presence of these two types of subunits, which are central in nature's folded macromolecules, should maximize the chances of accessing foldamers in our dynamic libraries. At the same time, and unlike nature, having both subunits present within a single building should

give rise to fundamentally new foldamers. Building block **1** was synthesized in six steps as outlined in the [Supporting Information](#). The building block is equipped with two thiol groups, which, upon exposure to atmospheric oxygen, oxidize to give rise to an equilibrium mixture of different macrocyclic disulfides,³⁰ which interconvert through thiol–disulfide exchange ([Figure 1a](#)). Ordinarily, in the absence of any noncovalent interactions, DCLs of building blocks with the benzenedithiol core of **1** are dominated by small trimeric and tetrameric macrocycles (the dimer is too strained to form in significant quantities). Indeed, a small DCL made from a 50 μ M solution of building block **1** in borate buffer (50 mM, pH 8.2) consisted mostly of cyclic tetramer (top trace in [Figure 1b](#); composition monitored by UPLC). However, repeating the same experiment at higher building block concentration (500 μ M) led to the emergence of an unusually large macrocycle, consisting of 15 subunits of **1**, as evident from mass spectrometric analysis of the relevant peak in the UPLC chromatogram ([Figure S20](#)). Increasing the concentration of the building block or addition of NaCl (1.0 M) to the mixture enhanced the formation of the 15mer, accounting for up to 95% of the library material ([Figure 1b](#), trace 4). Experiments with other salts revealed similar effects ([Figure S4](#)), indicating that the exact nature of the salt is not important and that the formation of the 15mer benefits from a high ionic strength, suggesting that the salt acts to reduce charge repulsion within the 15mer. The 15mer is most likely the thermodynamic product, as partial reduction followed by reoxidation led to the almost quantitative re-formation of the 15mer ([Figure S6](#)).

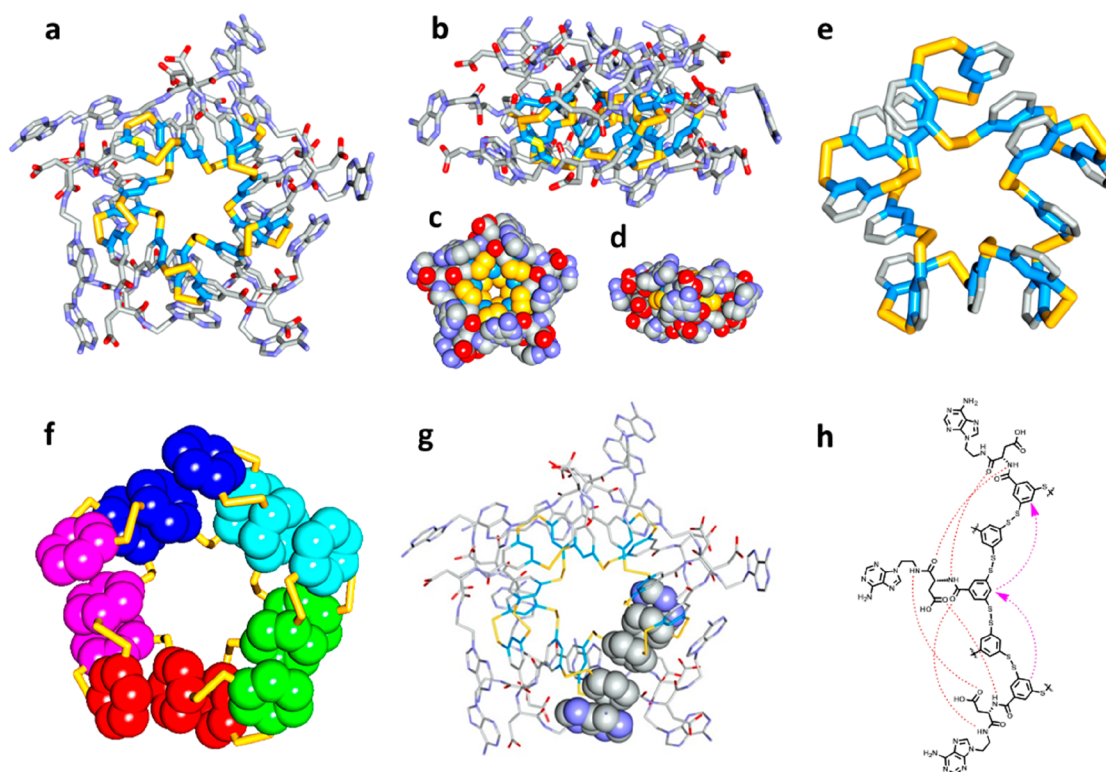


Figure 2. X-ray crystal structure of the 15mer. (a) Top view of the central cavity of the macrocycle. (b) Side view. (c) Top and (d) side view of the 15mer in space-filling representation. (e) The ring of disulfide bonds connecting the phenyl rings. (f) Core part of the foldamer, showing five stacks of three phenyl rings connected by disulfide bonds (two of the five sets of stacked phenyls are indicated by arrows in the extended structure shown in Figure 1a). (g) Top view of the 15mer highlighting one stack of three core phenyl rings and two adenines on the top and bottom of the stack. (h) Set of intermolecular hydrogen bonds formed between the three building blocks that constitute the stack of three phenyl rings. Solvent molecules, hydrogen atoms, and disorders are omitted for clarity. C atoms are shown in gray, N in purple, O in red, and S in yellow; except in panel f the C atoms of the macrocycle core are shown in light blue.

Experiments in the absence of salt but in the presence of 50% acetone (bottom trace in Figure 1b) or other cosolvents (Figure S5) gave only negligible amounts of 15mer, suggesting that hydrophobic interactions are important in stabilizing this compound. These observations, together with the fact that the 15mer is only poorly retained on the UPLC column, suggest that the compound adopts a folded structure. This hypothesis was confirmed when we characterized its structure using tandem mass spectrometry, circular dichroism (CD) spectroscopy, X-ray crystallography, and NMR spectroscopy.

The 15mer was isolated by preparative HPLC (isolated yield: 90%) and then analyzed by means of MALDI TOF/TOF. The results (Figure S7) show that the 15mer fragments into smaller components, ranging from tetramer up to 12mer, indicating that the 15mer is a single macrocycle and not a system of interlocked rings (a catenane).

The CD spectrum of the 15mer showed an intense positive band at 260 nm, which can be attributed to the absorption of the aromatic dithiol³⁴ and the terminal adenine. Importantly, the CD signals of the 15mer are dramatically enhanced compared to those of monomer **1** (Figure 1c), which suggests that the aromatic rings reside in a well-defined chiral environment, even though these rings are relatively remote from the chiral center in building block **1**, located on the amino-acid residue.

Detailed insights into the structure of the 15mer were obtained from single-crystal X-ray diffraction data. Crystals of the 15mer were prepared by slow diffusion of acetone into an aqueous solution of the 15mer. The crystal structure (Figure

2) confirms that the 15mer is a single giant macrocycle³⁵ linked together by 15 disulfide bonds to give a 75-atom ring. Figure 1a shows the extended conformation of this ring for clarity, which, in reality, is collapsed into a compact but intricately folded structure. Figure 2e shows the 75-atom ring in its highly twisted conformation. Overall, the structure is characterized by a hydrophobic core and presents its hydrophilic groups on its surface, similar to what is observed in folded proteins. The most notable structural motif is the stacking of aromatic rings. Five stacks of three phenyl rings can be identified (shown in Figure 2f), capped with an adenine ring at the top and bottom (except where these adenines are recruited for crystal packing), as shown for one of these stacks in Figure 2g. The distances between the three phenyl rings in these stacks are in the range of 3.43–3.45 Å, while the distances between the phenyl and adenine rings are somewhat larger (3.46–3.49 Å), but all in the range typical for π -stacking.³⁶ Interestingly, the rings that end up in the same stack are spaced far apart in the extended structure; as indicated in Figure 1a, a stack is composed of the phenyl rings from the *i*, *i*+2, and *i*+4 residues, while the capping adenines belong to the *i*–3 and the *i*+7 residues. Except for the adenines recruited for crystal packing (disrupting the otherwise 5-fold symmetry of the structure), this arrangement gets repeated five times in an interdigitated fashion (the second stack is indicated by a dotted line in Figure 1a; the other stacks are not shown for clarity). The five stacks of rings are arranged in a tiled fashion, as shown in Figure 2f. Thus, not only can secondary structure elements be identified (corresponding to the stacks of

rings), but since these stacks adopt well-defined orientations with respect to each other, also tertiary structure is present, which has only very recently been achieved in designed foldamers.³⁷ Apart from π -stacking interactions, also hydrogen bonds play an important role in stabilizing this folded structure. Between the building blocks that constitute the π -stack (residues i , $i+2$, and $i+4$), five hydrogen bonds are observed between the NH and CO groups (Figure 2h).

The solution-phase ^1H NMR spectrum (Figure 3a) of the isolated 15mer (D_2O , 298 K) is consistent with the X-ray

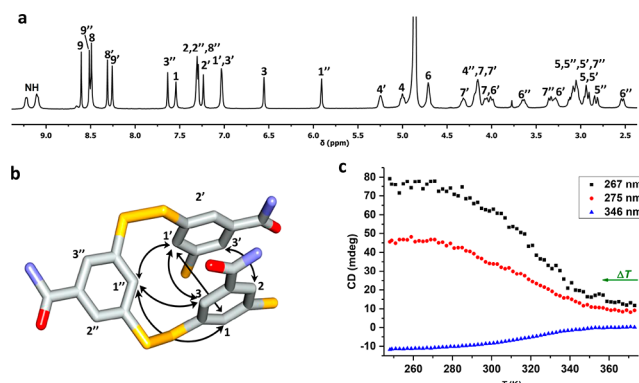


Figure 3. NMR and CD characterization of the 15mer. (a) ^1H NMR spectrum of the 15mer (500 MHz, D_2O , 298 K), with signal assignments corresponding to the labeling shown in Figure 1a. (b) Repeating unit of the 15mer with observed NOEs. (c) Changes in the Cotton effect intensities at specified wavelengths in the CD spectra of a DMF solution of the 15mer observed upon cooling from 373 K to 248 K.

crystal structure. The presence of sharp signals is in agreement with a highly ordered compact structure. In the spectrum the monomeric unit **1** appears in three separate sets of signals, indicative of a C_3 symmetry for the 15mer in solution. The signals of the protons of the phenyl rings are distributed over a wide range of chemical shifts (from 5.8 to 7.6 ppm). The large upfield shift of two of these (to 6.6 and 5.8 ppm, respectively, versus 7.2 and 7.3 ppm in monomer **1**) indicates that some of them are adjacent to the face of an aromatic ring. 2D-NMR studies enabled the complete assignment of the spectrum of the 15mer as well as the assignment of several through-space interactions that are consistent with the X-ray crystal structure (Figure 3b). These data indicate that, also in solution, the 15mer adopts a folded structure that is very similar to that observed in the X-ray crystal structure.

We then attempted to unfold the 15mer. Temperature-dependent ^1H NMR experiments on aqueous solutions of the 15mer were performed, but no significant spectral changes were observed, even at temperatures up to 353 K (Figure S12). Acetone was added (which disrupts the process of formation of the 15mer; vide supra), but the NMR spectra remained essentially unchanged (Figure S13), indicating that the 15mer, once formed, does not readily unfold. Unfolding was eventually achieved by dissolving the 15mer in $\text{DMF-}d_7$. At room temperature sharp signals corresponding to the folded 15mer were still observed (Figure S14), but these broadened significantly when the temperature was increased to 363 K, suggesting (at least partial) unfolding. At 373 K one set of relatively sharp signals was observed indicating a 15-fold symmetry of the system and, thus, complete unfolding of the macrocycle. The original spectrum was retained upon cooling

the sample to room temperature. The unfolding–refolding process was further analyzed by variable-temperature CD spectroscopy in dimethylformamide (DMF) (Figure S17). Reversible attenuation and enhancement of the CD signals were observed by heating and cooling the DMF solution (Figure 3c).

Finally, we investigated the extent to which foldamer formation depends on the structure of the building block. Foldamer formation was critically dependent on the presence of the nucleobase, but not specific for adenine, as replacement of adenine by guanine also gave the 15mer in 95% yield (Figure S18), while without any nucleobase only cyclic trimers and tetramers were detected (Figure S19).

CONCLUSIONS

These results establish dynamic combinatorial chemistry as a promising tool for identifying new foldamers with unprecedented folds of substantial structural complexity that are currently impossible to predict or even rationalize after the event. This method allows selective access to oligomers of precisely defined length in remarkably high yield and with minimal synthetic effort. Having thus established an efficient discovery tool for synthetic structures that fold like proteins, a new frontier in foldamer science is opening up, which should enable the field to become populated with fundamentally new folds. Indeed, in the months following the discovery of the 15mer described herein, we succeeded in identifying a handful of other foldamers of different ring size, based on different building blocks. Their structural characterization is currently underway and will be reported in due course. Accumulation of a sufficiently large number of such structures should, on the long-term, allow rules to be identified that may bring the holy grail in foldamer science a step closer: accessing new folds by design. Our findings are also relevant in the context of the origin of life, as they prove that folded structures of considerable complexity can emerge selectively and spontaneously from a mixture of interconverting molecules, simply as a result of their thermodynamic stability. Such folded structures could have existed and played a role in the origin of life at earlier stages than previously thought possible.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b11698.

Synthetic procedures, NMR spectra, UPLC and LC-MS methods, methods of DCL and sample preparation, mass spectra, X-ray crystallography (PDF)

Crystallographic data (CIF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Hjalmar P. Permentier (Department of Pharmacy, Analytical Biochemistry, University of Groningen, Groningen, The Netherlands) for MALDI-TOF experiments. This research was supported by the ERC (AdG 741774), the EU (MCIF 745805–DSR), NWO (VICI grant), Zernike Dieptestrategie and the Dutch Ministry of Education, Culture and Science (Gravitation program 024.001.035), the Polish National Science Center (UMO-2013/09/B/ST5/00326 and UMO-2018/29/B/ST5/00280), and the University of Trieste (project FRA2016).

■ REFERENCES

- (1) Lokey, R. S.; Iverson, B. L. Synthetic molecules that fold into a pleated secondary structure in solution. *Nature* **1995**, *375*, 303–305.
- (2) Hamuro, Y.; Geib, S. J.; Hamilton, A. D. Oligoanthranilamides. Non-peptide subunits that show formation of specific secondary structure. *J. Am. Chem. Soc.* **1996**, *118*, 7529–7541.
- (3) Nelson, J. C.; Saven, J. G.; Moore, J. S.; Wolynes, P. G. Solvophobic driven folding of nonbiological oligomers. *Science* **1997**, *277*, 1793–1796.
- (4) Bassani, D. M.; Lehn, J. M.; Baum, G.; Fenske, D. Designed self-generation of an extended helical structure from an achiral polyheterocyclic strand. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1845–1847.
- (5) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X. L.; Barchi, J. J.; Gellman, S. H. Residue-based control of helix shape in beta-peptide oligomers. *Nature* **1997**, *387*, 381–384.
- (6) Berl, V.; Huc, I.; Khoury, R. G.; Krische, M. J.; Lehn, J. M. Interconversion of single and double helices formed from synthetic molecular strands. *Nature* **2000**, *407*, 720–723.
- (7) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. A field guide to foldamers. *Chem. Rev.* **2001**, *101*, 3893–4011.
- (8) Goodman, C. M.; Choi, S.; Shandler, S.; DeGrado, W. F. Foldamers as versatile frameworks for the design and evolution of function. *Nat. Chem. Biol.* **2007**, *3*, 252–262.
- (9) Horne, W. S.; Gellman, S. H. Foldamers with heterogeneous backbones. *Acc. Chem. Res.* **2008**, *41*, 1399–1408.
- (10) Saraogi, I.; Hamilton, A. D. Recent advances in the development of aryl-based foldamers. *Chem. Soc. Rev.* **2009**, *38*, 1726–1743.
- (11) Guichard, G.; Huc, I. Synthetic foldamers. *Chem. Commun.* **2011**, *47*, 5933–5941.
- (12) Zhang, D. W.; Zhao, X.; Hou, J. L.; Li, Z. T. Aromatic amide foldamers: structures, properties, and functions. *Chem. Rev.* **2012**, *112*, 5271–5316.
- (13) Le Bailly, B. A. F.; Clayden, J. Dynamic foldamer chemistry. *Chem. Commun.* **2016**, *52*, 4852–4863.
- (14) Muller, M. M.; Windsor, M. A.; Pomerantz, W. C.; Gellman, S. H.; Hilvert, D. A rationally designed aldolase foldamer. *Angew. Chem., Int. Ed.* **2009**, *48*, 922–925.
- (15) Chandramouli, N.; Ferrand, Y.; Lautrette, G.; Kauffmann, B.; Mackereth, C. D.; Laguerre, M.; Dubreuil, D.; Huc, I. Iterative design of a helically folded aromatic oligoamide sequence for the selective encapsulation of fructose. *Nat. Chem.* **2015**, *7*, 334–341.
- (16) Becart, D.; Diemer, V.; Salaun, A.; Oiarbide, M.; Nelli, Y. R.; Kauffmann, B.; Fischer, L.; Palomo, C.; Guichard, G. Helical oligoureia foldamers as powerful hydrogen bonding catalysts for enantioselective C–C bond-forming reactions. *J. Am. Chem. Soc.* **2017**, *139*, 12524–12532.
- (17) Fletcher, J. M.; Harniman, R. L.; Barnes, F. R. H.; Boyle, A. L.; Collins, A.; Mantell, J.; Sharp, T. H.; Antognozzi, M.; Booth, P. J.; Linden, N.; Miles, M. J.; Sessions, R. B.; Verkade, P.; Woolfson, D. N. Self-assembling cages from coiled-coil peptide modules. *Science* **2013**, *340*, 595–599.
- (18) Hosseinzadeh, P.; Bhardwaj, G.; Mulligan, V. K.; Shortridge, M. D.; Craven, T. W.; Pardo-Avila, F.; Retti, S. A.; Kim, D. E.; Silva, D. A.; Ibrahim, Y. M.; Webb, I. K.; Cort, J. R.; Adkins, J. N.; Varani, G.; Baker, D. Comprehensive computational design of ordered peptide macrocycles. *Science* **2017**, *358*, 1461–1466.
- (19) Nair, R. V.; Vijayadas, K. N.; Roy, A.; Sanjayan, G. J. Heterogeneous foldamers from aliphatic-aromatic amino acid building blocks: current trends and future prospects. *Eur. J. Org. Chem.* **2014**, *2014*, 7763–7780.
- (20) Li, J. W.; Nowak, P.; Otto, S. Dynamic combinatorial libraries: From exploring molecular recognition to systems chemistry. *J. Am. Chem. Soc.* **2013**, *135*, 9222–9239.
- (21) Corbett, P. T.; Leclaire, J.; Vial, L.; West, K. R.; Wietor, J. L.; Sanders, J. K. M.; Otto, S. Dynamic combinatorial chemistry. *Chem. Rev.* **2006**, *106*, 3652–3711.
- (22) Case, M. A.; McLendon, G. L. A virtual library approach to investigate protein folding and internal packing. *J. Am. Chem. Soc.* **2000**, *122*, 8089–8090.
- (23) Oh, K.; Jeong, K. S.; Moore, J. S. Folding-driven synthesis of oligomers. *Nature* **2001**, *414*, 889–893.
- (24) Krishnan-Ghosh, Y.; Balasubramanian, S. Dynamic covalent chemistry on self-templating peptides: Formation of a disulfide-linked beta-hairpin mimic. *Angew. Chem., Int. Ed.* **2003**, *42*, 2171–2173.
- (25) Woll, M. G.; Gellman, S. H. Backbone thioester exchange: A new approach to evaluating higher order structural stability in polypeptides. *J. Am. Chem. Soc.* **2004**, *126*, 11172–11174.
- (26) Roy, L.; Case, M. A. Protein core packing by dynamic combinatorial chemistry. *J. Am. Chem. Soc.* **2010**, *132*, 8894–8896.
- (27) Lao, L. L.; Schmitt, J. L.; Lehn, J. M. Evolution of a constitutional dynamic library driven by self-organisation of a helically folded molecular strand. *Chem. - Eur. J.* **2010**, *16*, 4903–4910.
- (28) Lafuente, M.; Atcher, J.; Sola, J.; Alfonso, I. Adaptive correction from virtually complex dynamic libraries: The role of noncovalent interactions in structural selection and folding. *Chem. - Eur. J.* **2015**, *21*, 17002–17009.
- (29) Tsiamantas, C.; de Hatten, X.; Douat, C.; Kauffmann, B.; Maurizot, V.; Ihara, H.; Takafuji, M.; Metzler-Nolte, N.; Huc, I. Selective dynamic assembly of disulfide macrocyclic helical foldamers with remote communication of handedness. *Angew. Chem., Int. Ed.* **2016**, *55*, 6848–6852.
- (30) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. Selection and amplification of hosts from dynamic combinatorial libraries of macrocyclic disulfides. *Science* **2002**, *297*, 590–593.
- (31) Lam, R. T. S.; Belenguer, A.; Roberts, S. L.; Naumann, C.; Jarrosson, T.; Otto, S.; Sanders, J. K. M. Amplification of acetylcholine-binding catenanes from dynamic combinatorial libraries. *Science* **2005**, *308*, 667–669.
- (32) Ponnuswamy, N.; Cougnon, F. B. L.; Clough, J. M.; Pantos, G. D.; Sanders, J. K. M. Discovery of an organic trefoil knot. *Science* **2012**, *338*, 783–785.
- (33) Carnall, J. M. A.; Waudby, C. A.; Belenguer, A. M.; Stuart, M. C. A.; Peyralans, J. J. P.; Otto, S. Mechanosensitive self-replication driven by self-organization. *Science* **2010**, *327*, 1502–1506.
- (34) Frederix, P. W. J. M.; Ide, J.; Altay, Y.; Schaeffer, G.; Suring, M.; Beljonne, D.; Bondarenko, A. S.; Jansen, T. L. C.; Otto, S.; Marrink, S. J. Structural and spectroscopic properties of assemblies of self-replicating peptide macrocycles. *ACS Nano* **2017**, *11*, 7858–7868.
- (35) O'Sullivan, M. C.; Sprafke, J. K.; Kondratuk, D. V.; Rinfrey, C.; Claridge, T. D. W.; Saywell, A.; Blunt, M. O.; O'Shea, J. N.; Beton, P. H.; Malfois, M.; Anderson, H. L. Vernier templating and synthesis of a 12-porphyrin nano-ring. *Nature* **2011**, *469*, 72–75.
- (36) Claessens, C. G.; Stoddart, J. F. pi-pi interactions in self-assembly. *J. Phys. Org. Chem.* **1997**, *10*, 254–272.
- (37) De, S.; Chi, B.; Granier, T.; Qi, T.; Maurizot, V.; Huc, I. Designing cooperatively folded abiotic uni- and multimolecular helix bundles. *Nat. Chem.* **2017**, *10*, 51–57.