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Parasitic Behavior of Self-Replicating Molecules

Meniz Altay, Yigit Altay, and Sijbren Otto*

Abstract: Self-replication plays a central role in the origin of life and in strategies to synthesize life de-novo. Studies on self-replication have focused mostly on isolated systems, while the dynamics of systems containing multiple replicators has received comparatively little attention. Yet most evolutionary scenarios involve the interplay between different replicators. Here we report the emergence of parasitic behavior in a system containing self-replicators derived from two subtly different building blocks **1** and **2**. Replicators from **2** form readily through cross-catalysis by pre-existing replicators made from **1**. Once formed, the new replicators consume the original replicators to which they owe their existence. These results resemble parasitic and predatory behavior that is normally associated with living systems and show how such life-like behavior has its roots in relatively simple systems of self-replicating molecules.

Understanding the origins of life^[1] and the de-novo synthesis of life are among the grand challenges in contemporary science and an important focus in systems chemistry.^[2] Self-replicating systems^[3] play a key role in scenarios of the origin of life and are a promising possible starting point for the de-novo synthesis of life. Self-replicating molecules have been developed based on DNA,^[4] RNA,^[5] peptides^[6] or synthetic molecules.^[7] The majority of these systems feature only a single self-replicating molecule. Yet approaches to the origin of life and its de-novo synthesis will inevitably involve systems in which multiple replicators co-exist and interact. Evolution involves the selection from among competing replicators and, most likely, also cooperation between replicators. Thus the dynamics that can occur in systems of co-existing replicators are an important new focus in the development of systems of self-replicators towards life. Early work in this field involves systems of replicators based on RNA,^[5d,8] α -helical peptides^[6d,9] and synthetic replicators.^[7d-f,10]

Using a dynamic combinatorial approach to self-replication,^[7e-g,10] based on pseudopeptide building blocks^[11] we recently developed replicating cyclic disulfide oligomers made from dithiol building blocks.^[12] In a typical dynamic combinatorial library (DCL) made from an individual building block such as dithiol **1** (Scheme 1A), as oxidation takes place, a mixture of differently sized macrocycles forms, that continuously interconvert through thiol-disulfide exchange (Scheme 1B).^[13] When one of the library members is able to bind to copies of itself, this compound is stabilized and the equilibrium shifts towards more of that macrocycle, resulting in self-replication and the formation of stacks of the replicator. Mechanical energy can break the stacks, thereby increasing the number of ends from which the stacks grow and enabling exponential replication.^[14]

Unlike most other replicators, in these combinatorial systems, the structure of the building blocks does not predetermine the

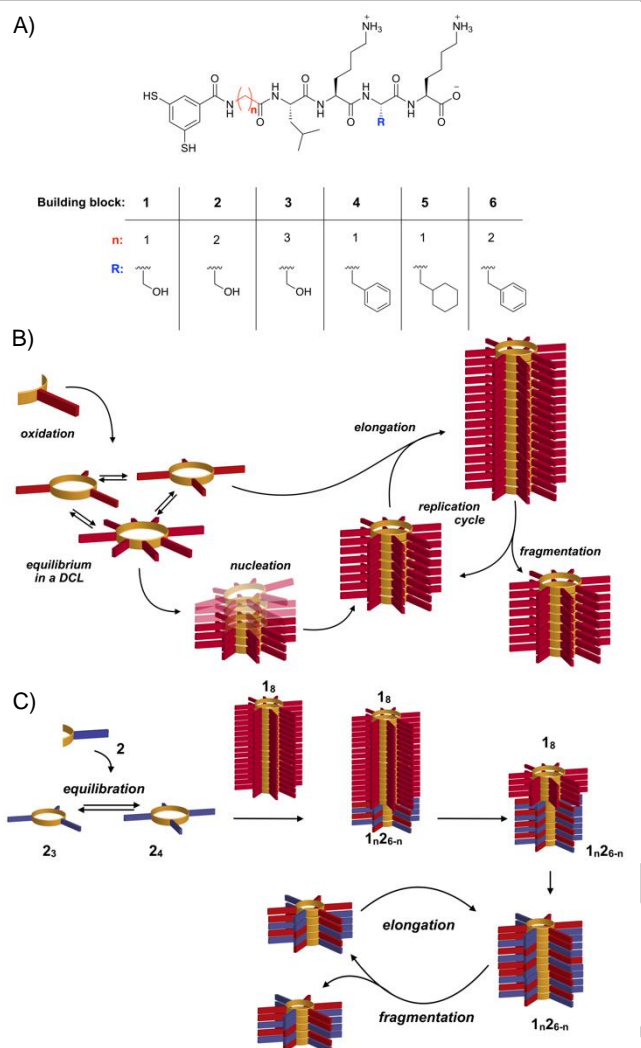
nature of the replicator that emerges. The ring size and building block composition of newly formed replicators are also influenced by mechanical agitation,^[12a] the solvent environment^[15] and pre-existing replicators.^[16] The latter studies revealed mechanisms of co-operation and co-existence by which replicators diversify and assist in each other's formation. We now report an example where a set of newly formed replicators exhibits exactly the opposite: parasitic behavior. Emergence of the parasitic replicator relies on cross-catalysis by a structurally closely related pre-existing replicator, which is subsequently consumed by the very replicators that it brought into existence. While several reports describe the emergence of parasites in systems where enzymes mediate replication of nucleic acids,^[17] this is the first report of the emergence of a parasite in a system of autonomous self-replicators.

We used two closely related building blocks **1** and **2** (Scheme 1A) featuring two thiol units to promote covalent thiol-disulfide exchange and a short peptide chain composed of alternating hydrophilic and hydrophobic amino acids to promote self-assembly through β -sheet formation. As we reported previously, building block **1** spontaneously forms a self-replicating cyclic octamer (**1₈**).^[12b] Building block **2** contains an additional methylene unit in the amino acid that connects the peptide to the aromatic dithiol core. We reasoned that this modification would make nucleation of any replicators formed from **2** more difficult by increasing the degrees of freedom in the peptide chain. Indeed, in contrast to building block **1** and most previously studied peptide-based building blocks in this family,^[12,14-16] the spontaneous emergence of replicators from DCLs made from building block **2** was sluggish. When a DCL (1.0 mM in **2** in 50 mM borate buffer, pH=8.2) was exposed to air under constant mechanical agitation, cyclic trimers (**2₃**) and tetramers (**2₄**) emerged as the main products (Figure 1A). Repeating this experiment at a constant oxidation level (65%, ensuring sufficient free thiol to mediate disulfide exchange) yielded <9% cyclic hexamer replicator (**2₆**) after two months (see Figure S10; for evidence that **2₆** is a self-replicator, vide infra).

Given that replicators derived from building block **1** assemble readily into fibers,^[12b] we investigated whether these fibers could act as templates and cross-catalyze the formation of replicators from building block **2**. Thus, we first prepared a DCL by dissolving **2** in aqueous borate buffer (50 mM, pH 8.2) to a concentration of 1.0 mM. After 24h of stirring in the presence of air the library had oxidized to approximately 75%. We then added 0.2 mol eq. (with respect to building block) of replicator **1₈** and monitored the library composition over 9 days by UPLC.^[18] A set of cyclic hexamer replicators **1_n2_{6-n}** emerged rapidly and grew to dominate the mixture after 4 days (Figure 1B). Repeating this experiment using 0.5 mol eq. of replicator **1₈** led to the somewhat faster emergence of **1_n2_{6-n}** (Figure 1C), suggesting a cross-catalytic role of **1₈**. To confirm that the emergence of the hexameric replicators was indeed promoted by **1₈** we set up a negative control experiment from an equimolar mixture of **1** and **2** (**[1]=[2]=0.5 mM**) to which we did not add any **1₈**. We did not observe any cyclic hexamers in this sample even after 7 days (see SI Figure S23).

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Scheme 1. A) Chemical structures of the building blocks utilized in cross-seeding experiments. B) Cartoon representation for the general replication mechanism for a particular building block (1_8 in this case). C) Proposed mechanism for the emergence of the parasitic replicator (1_n2_{6-n}) in a DCL made from building block 2 upon cross-seeding with 1_8 . First, a small dynamic combinatorial library of cyclic disulfides is made by oxidation of building block 2 . While the cross-seed dissociates from one end, stacking of rings of one particular size (1_n2_{6-n}) shifts the equilibrium in the direction of these library members. Agitation breaks the stacks producing more ends from which the stacks can grow, giving rise to exponential replication.

Remarkably, the emergence of the set of hexameric replicators is accompanied by a decrease in the amount of 1_8 , to the point that this replicator was no longer detectable after 3 days in the experiments shown in Figure 1B and C. Repeating the experiment with 1.0 mol eq. 1_8 confirmed this behavior, although a small amount of 1_8 was still left at the point that the disulfide exchange ceased due to complete oxidation (Figure 1D). These results suggest that the newly formed hexameric replicators act as parasites: they grow at the expense of the original octameric replicators to which they owe their existence. This conclusion was supported by MS analysis of the UPLC peak that contains the co-eluting 1_n2_{6-n} macrocycles with different composition (see SI Figures S49, S52, S57) including up to 6 units of 2 . In contrast, no mixed cyclic octamers (1_n2_{8-n}) could be detected in the experiments shown in Figure 1.

In order to prove that 1_n2_{6-n} , including 1_6 , are replicators and to compare their replication efficiencies, we performed a set of serial transfer seeding experiments (Scheme 2). A second generation sample was prepared by transferring an aliquot (0.2

mol eq.) of the sample corresponding to Figure 1B to a DCL made from building block 2 . Finally, a third generation sample was prepared by transferring 0.2 mol eq. from the second-generation sample to a fresh DCL prepared from 2 .

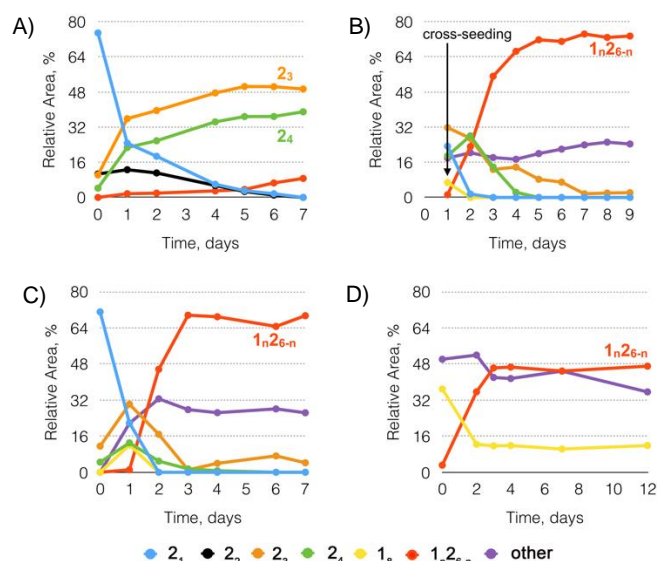
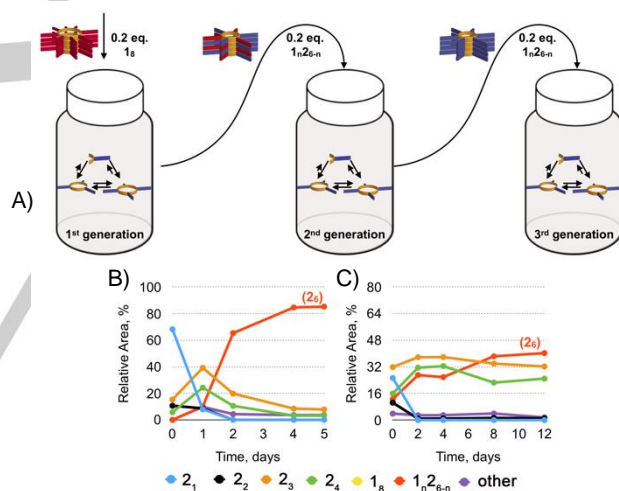


Figure 1. Product distribution over time monitored by UPLC for agitated DCLs that are A) non-seeded; mixed with B) 0.2 mol eq. 1_8 on day 1, C) 0.5 mol eq. 1_8 and D) 1.0 mol eq. 1_8 on day 0.



Scheme 2. A) Schematic representation of the serial transfer seeding experiments. Product distribution over time monitored by UPLC for DCLs that are B) second-generation and C) third-generation samples. The product distribution for the first-generation sample is shown in Figure 1B.

Through these serial transfer experiments, we were able to obtain almost pure 2_6 in the second generation as the mass spectrum shows (see SI Figure S57). Therefore, the third generation seeding mainly probes the autocatalytic behavior of 2_6 . After 12 days, 2_6 accounted for 40 % of the overall library composition. Comparing these data with that for the spontaneous emergence of 2_6 (Figure 1A) shows that 2_6 is indeed a replicator. However, comparing the kinetic data for the growth of 1_n2_{6-n} in samples with decreasing content of 1 shows that 2_6 is a less efficient replicator than the set of mixed-building-block 1_n2_{6-n} replicators.

Notable in these seeding experiments is the absence of any octamer replicators (1_n2_{8-n}). So cross-catalysis appears to be strictly unidirectional: octamers promote the formation of hexamers but not the other way around.^[19] This conclusion was

confirmed in experiments in which we added 0.2 mol eq. **2₆** or **1_n2_{6-n}** as seed to an agitated DCL made from building block **1** (1.0 mM). After 5 days the library composition was dominated by trimers and tetramers and no **1₈** was detected (see SI Figures S60-61). These results confirm the parasitic nature of the set of hexamer replicators.

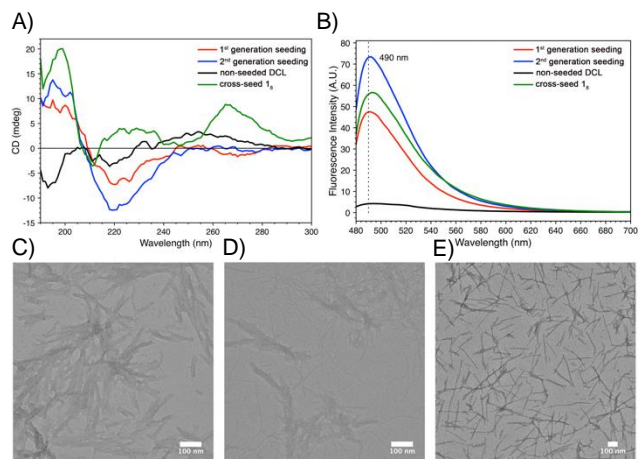


Figure 2. A) CD spectra (recorded at identical concentrations), B) Thioflavin T emission spectra for DCLs made from only cross-seed **1₈**, from peptide **2** without cross-seed and the first and the second generation of seeding. TEM micrographs for A) cross-seed **1₈**, B) first generation **1_n2_{6-n}** and C) almost pure **2₆** obtained in the second generation.

We characterized the newly formed replicators using circular dichroism (CD) spectroscopy, thioflavin T fluorescence assays and transmission electron microscopy (TEM). While the non-seeded DCL made from peptide **2** (mostly **2₃** and **2₄**) initially showed a negative helicity around 196 nm characteristic for random coil conformations, CD spectra for **1₈** and hexamers **1_n2_{6-n}** showed negative helicity around 220 nm and positive helicity at 196 nm, indicative of β -sheet structure (Figure 3A).^[20] Thioflavin T assays were also in agreement with a β -sheet amyloid-fibril-like structure^[21] for all replicator samples, as evident from a more than 40-fold increase in emission intensity at 490 nm compared to non-seeded trimer and tetramer dominated DCLs (Figure 2B). The β -sheet structure is more pronounced in second generation serial transfer samples dominated by **2₆** than in samples of **1₈**. Analysis by TEM showed that **1₈** formed laterally associated short fibers (around 100 nm) (Figure 2C). In the course of the serial transfer experiments the average fiber length increased to around 150 nm for the first-generation replicators and to 350 nm for the second generation. We tentatively attribute the increased fiber length and enhanced β -sheet structure of fibers of **2₆** (as compared to those of **1₈**) to the stronger hydrophobic interactions within the stacks arising from the additional methylene unit in **2** as compared to **1**. Since the rate of replication depends on the number fiber ends,^[14a] and since longer fibers means fewer fiber ends, the increase in length of fibers of **2₆** can (partially) account for the reduced rate of replication of these fibers relative to those of **1₈**.

Finally, we investigated the extent to which structurally related peptides are able to show similar cross-catalytic effects. First, we probed whether replicators other than **1₈** can also induce the formation of replicators from building block **2**. We seeded DCLs made from **2** with 0.2 mol eq. of replicators **4₆**, **5₆** and **6₅** but failed to detect any replicators, despite the fact that the ring size of the replicator seeds **4₆** and **5₆** now matches the ring size of the **2₆** replicators, while the spacer length in **6** matches that in **2**. Only mixtures of trimers and tetramers

coexisting with the seeds were obtained in these seeding experiments (see SI Figure S4). Second, we investigated the effect of elongating the spacer in **2** by an additional methylene unit to give building block **3**. We prepared DCLs from building block **3** and seeded these with 0.2 mol eq. **1₈** or **2₆**. Again, we did not observe the emergence of any new replicators (see SI Figure S5). Thus, it appears that cross-catalysis of formation of **1_n2_{6-n}** by **1₈** is specific to these particular peptide sequences.

In conclusion, we observed how a set of 6-ring replicators emerged, aided by a pre-existing 8-ring replicator, only to consume the 8-rings to which the new replicator owed its existence. We speculate that the 6-ring replicator fibers nucleate at some of the ends of the 8-ring replicator fibers (Scheme 1C). The fact that the 8-ring replicators are efficiently broken down (a process that occurs at the fiber ends),^[14b] suggests that the 8-ring fibers remain exposed to the solution at at least one of their fiber ends (i.e. for most 8-ring fibers not more than one fiber end is capped with 6-ring replicators). This behavior is reminiscent of parasitic behavior as it occurs in biology: the set of six-ring replicators benefit from cross-catalysis by the 8-ring replicator in a non-mutualistic way as the 6-ring replicators do not cross-catalyze the formation of 8-ring replicator. Like in biology and in previous RNA-based systems^[17] the parasite is smaller than its host (albeit not much), replicates faster and extracts resources (building blocks) from its host, causing it some harm. Unlike in biology, the host replicator nor its parasite has a metabolism. Notably, the 6-ring replicator even causes the (partial) demise of the 8-ring replicator and utilizes the building block that was previously contained in the 8-rings for its own growth, which starts to resemble predatory behavior. These unique observations illustrate the rich dynamics that multi-replicator systems can exhibit. Appreciating and understanding such dynamics is essential for directing the evolution of multi-replicator systems towards the de-novo synthesis of life.

Acknowledgements

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Keywords: Systems Chemistry • de-novo life • self-replication • parasites • self-assembly

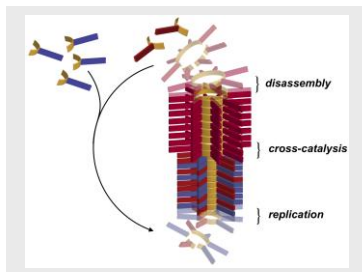
- [1] a) R. Pascal, A. Pross, J. D. Sutherland, *Open Biol.* **2013**, *3*, 130-156; b) P. L. Luisi, *Isr. J. Chem.* **2015**, *55*, 906-918; c) J. D. Sutherland, *Angew. Chem. Int. Ed.* **2016**, *55*, 104-121; d) J. W. Szostak, *Angew. Chem. Int. Ed.* **2017**, *56*, 11037-11043.
- [2] a) R. F. Ludlow, S. Otto, *Chem. Soc. Rev.* **2008**, *37*, 101-108; (b) K. Ruiz Mirazo, C. Briones, A. de la Escosura, *Chem. Rev.* **2014**, *114*, 285-366; c) G. Ashkenasy, T. M. Hermans, S. Otto, A. F. Taylor, *Chem. Soc. Rev.* **2017**, *46*, 2543-2554.
- [3] a) V. Patzke, G. von Kiedrowski, *Arxivoc* **2007**, *v*, 293-310; b) A. J. Bissette, S. P. Fletcher, *Angew. Chem. Int. Ed.* **2013**, *52*, 12800-12826; c) G. Clixby, L. Twyman, *Org. Biomol. Chem.* **2016**, *14*, 4170-4184; d) T. Kosikova, D. Philp, *Chem. Soc. Rev.* **2017**, *46*, 7274-7305; e) H. Duim, S. Otto, *Beilstein J. Org. Chem.* **2017**, *13*, 1189-1203.

- [4] a) G. von Kiedrowski, *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 932-935; b) T. Li, K. C. Nicolaou, *Nature* **1994**, *369*, 218-221; c) A. Luther, R. Brandsch, G. Von Kiedrowski, *Nature* **1998**, *396*, 245-248.
- [5] a) N. Paul, G. F. Joyce, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12733-12740; b) E. J. Hayden, G. von Kiedrowski, N. Lehman, *Angew. Chem., Int. Ed.* **2008**, *47*, 8424-8428; c) T. A. Lincoln, G. F. Joyce, *Science* **2009**, *323*, 1229-1232; d) N. Vaidya, M. L. Manapat, I. A. Chen, R. Xulvi-Brunet, E. J. Hayden, N. Lehman, *Nature* **2012**, *491*, 72-77; e) J. W. Szostak, *J. Syst. Chem.* **2012**, *3*, 2; f) M. P. Robertson, G. F. Joyce, *Chem. Biol.* **2014**, *21*, 238-245; g) T. S. Jayathilaka, N. Lehman, *ChemBioChem* **2018**, *19*, 217-220.
- [6] a) D. H. Lee, J. R. Granja, J. A. Martinez, K. Severin, M. R. Ghadiri, *Nature* **1996**, *382*, 525-528; b) S. Yao, I. Ghosh, R. Zutshi, J. Chmielewski, *J. Am. Chem. Soc.* **1997**, *119*, 10559-10560; c) B. Rubinov, N. Wagner, H. Rapaport, G. Ashkenasy, *Angew. Chem. Int. Ed.* **2009**, *48*, 6683-6686; d) J. Nanda, B. Rubinov, D. Ivniitski, R. Mukherjee, E. Shtelma, Y. Motro, Y. Miller, N. Wagner, R. C. Luria, G. Ashkenasy, *Nature Commun.* **2017**, *8*, 434.
- [7] a) T. Tjivikua, P. Ballester, J. Rebek Jr., *J. Am. Chem. Soc.* **1990**, *112*, 1249-1250; b) A. Dieckmann, S. Beniken, C. D. Lorenz, N. L. Doltsinis, G. von Kiedrowski, *Chem. Eur. J.* **2011**, *17*, 468-480; c) D. Ajami, S. Kamioka, A. C. Sather, R. J. Hooley, J. Rebek, *Heterocycles* **2011**, *82*, 1203; d) I. Bottero, J. Huck, T. Kosikova, D. Philp, *J. Am. Chem. Soc.* **2016**, *138*, 6723-6726. e) T. Kosikova, D. Philp, *J. Am. Chem. Soc.* **2017**, *139*, 12579-12590; f) J. W. Sadownik, T. Kosikova, D. Philp, *J. Am. Chem. Soc.* **2017**, *139*, 17565-17573.
- [8] P. G. Higgs, N. Lehman, *Nat. Rev. Genet.* **2015**, *16*, 7-17.
- [9] a) D. H. Lee, K. Severin, M. R. Ghadiri, *Curr. Opin. Chem. Biol.* **1997**, *1*, 491-496; b) D. H. Lee, K. Severin, Y. Yokobayashi, M. R. Ghadiri, *Nature* **1997**, *390*, 591-594; c) G. Ashkenasy, R. Jagasia, M. Yadav, M. R. Ghadiri, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10872-10877; d) Z. Dadon, N. Wagner, S. Alasibi, M. Samiappan, R. Mukherjee, G. Ashkenasy, *Chem. Eur. J.* **2015**, *21*, 648-654.
- [10] a) V. del Amo, D. Philp, *Chem. Eur. J.* **2010**, *16*, 13304-13318.
- [11] I. Alfonso, *Chem. Commun.* **2016**, *52*, 239-250.
- [12] a) J. M. A. Carnall, C. A. Waudby, A. M. Belenguer, M. C. A. Stuart, J. J.-P. Peyralans, S. Otto, *Science* **2010**, *327*, 1502-1506; b) M. Malakoutikhah, M. Colomb-Delsuc, H. Fanlo-Virgos, J. J. -P. Peyralans, M. C. A. Stuart, S. Otto, *J. Am. Chem. Soc.* **2013**, *135*, 18406-18417; c) P. Nowak, M. Colomb-Delsuc, S. Otto, J. Li, *J. Am. Chem. Soc.* **2015**, *137*, 10965-10969.
- [13] a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2000**, *122*, 12063-12064; b) S. P. Black, J. K. M. Sanders, A. R. Stefankiewicz, *Chem. Soc. Rev.* **2014**, *43*, 1861-1872.
- [14] a) M. Colomb-Delsuc, E. Mattia, J. W. Sadownik, S. Otto, *Nat. Commun.* **2015**, *6*, 7427; b) E. Mattia, A. Pal, G. Leonetti, S. Otto, *Synlett.* **2017**, *28*, 103-107.
- [15] G. Leonetti, S. Otto, *J. Am. Chem. Soc.* **2015**, *137*, 2067-2072.
- [16] a) J. W. Sadownik, E. Mattia, P. Nowak, S. Otto, *Nat. Chem.* **2016**, *3*, 264-269. b) Y. Altay, M. Tezcan, S. Otto, *J. Am. Chem. Soc.* **2017**, *139*, 13612-13615.
- [17] a) D. R. Mills, R. L. Peterson, S. Spiegelman, *Proc. Natl. Acad. Sci. U. S. A.* **1967**, *58*, 217-224; b) Y. Bansho, T. Furubayashi, N. Ichihashi, T. Yomo, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 4045-4050; c) S. Matsumura, A. Kun, M. Ryckelynck, F. Coldren, A. Szilagyi, F. Jossinet, C. Rick, P. Ngnhe, E. Szathmary, A. D. Griffiths, *Science*, **2016**, *354*, 1293 – 1296.
- [18] The total UPLC peak area is comparable for mixtures with substantially different compositions (see supporting information), indicating that the molar absorptivities of the building block is essentially independent of the macrocycle in which it resides. Thus peak areas correlate directly with macrocycle concentrations (expressed in units of building block).
- [19] This behavior differs from that observed for similar systems of self-replicators in which cross-catalysis is reciprocal (see references 15 and 16b). The factors behind this difference are not yet understood.
- [20] N. J. Greenfield, *Nat. Protoc.* **2017**, *1*, 2876-2890. Longer wavelength features in the CD spectrum are due aromatic rings that are placed in a chiral environment in the stacks and not readily interpretable. See for a detailed analysis of the CD spectra of a similar replicator: P. W. J. M. Frederix, J. Idé, Y. Altay, G. Schaeffer, M. Surin, D. Beljonne, A. S. Bondarenko, T. L. C. Jansen, S. Otto, S. J. Marrink, *ACS Nano*, **2017**, *11*, 7858-7868.
- [21] H. Levine, *Protein Sci.* **1993**, *2*, 404-410.

Entry for the Table of Contents

COMMUNICATION

We show how a 6-ring replicator grows off a pre-existing 8-ring replicator, only to consume the 8-rings and utilize its components for its own replication, resembling parasitic and predatory behavior.



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