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Mechanism of Building Block Exchange in Stacks of Self-Replicating Macrocycles

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Abstract Self-replicating molecules containing labile reversible covalent bonds are becoming increasingly topical. Yet their robustness to component exchange and the threat of loss of information has received very little attention. We show here that supramolecular interactions can efficiently protect self-replicating molecules from otherwise rapid component exchange.

Key words self-replication, self-assembly, exchange, supramolecular polymers, dynamic combinatorial chemistry, systems chemistry

Self-replication¹ is of key importance to the question of the origin of life² and in the quest for the first de novo synthesis of life. In the process of replication molecular information is copied and transferred to the next generation of replicating molecules. Adequate propagation of such information requires not only sufficiently accurate copying, but also sufficiently long persistence of this information. The latter issue is particularly relevant for the rapidly increasing number of self-replicating systems that feature relatively labile reversible covalent bonds,^{3,4} but received comparatively little attention. We now report a detailed mechanistic investigation into the pathways that lead to information loss through building-block exchange in systems of stacked self-assembled self-replicating macrocycles. We conclude that exchange is effectively limited to the stack ends, while the replicator compositions inside the stacks remain stable for months, even though exchange kinetics of nonassembled macrocycles occurs on a timescale of minutes.

We based our study on a previously reported system of self-assembly-driven self-replicators, shown in Figure 1.⁴ A thiol-functionalized peptide building block **1** forms a dynamic combinatorial library of disulfide macrocycles, among which hexamers **1₆** nucleate and elongate into su-

Figure 1 Thiol functionalized peptide building blocks **1** and isotopically labeled **1*** form a mixture of interconverting macrocycles upon formation of disulfide bridges (the structure of the cyclic trimer is shown as an example). Among the macrocycles, hexamers selectively self-assemble by nucleating and elongating into supramolecular nanofibers that undergo mechanically induced fragmentation.

pramolecular nanofibers. This assembly process drives the synthesis of the very macrocycles that assemble, causing these molecules to self-replicate. The replicator fibers undergo fragmentation by mechanical forces in solution, increasing the number of ends from which they grow, leading to sigmoidal growth of hexamer concentration, which is typical of autocatalytic processes. Notably, autocatalysis in this system enables exponential replication.^{4c}

The mechanisms of the fiber assembly, equilibration and deconstruction processes were analyzed via UPLC-MS. Under the chromatographic conditions (acidic aqueous acetonitrile mixtures) the fibers disassemble and hexamers are eluted individually. We used building blocks **1** and its isotopically labeled analogue **1**^{*} into which ¹⁵N-labeled glycine was incorporated (Figure 1). Macrocycles of the same size formed by any combination of **1** and **1**^{*} co-elute during chromatography, whereby ratios between the two building blocks are easily assessed by MS analysis of the corresponding chromatographic peak. The use of **1** and **1**^{*} in the same mixture results in the potential formation of many combinations of macrocycles with different isotopic labeling patterns. These macrocycles are in constant exchange in solution (Figure 2).

Figure 2 Exchange between building blocks in solution leading to formation of mixed macrocycles (of which four are shown)

In order to study the exchange pathways of building blocks between different macrocycles, we prepared supramolecular block copolymers by growing labeled **1**^{*}₆ on both fiber ends of previously prepared unlabeled **1**₆ fibers, in 1:1 ratio (Figure 3, a).⁵ Hexameric macrocycles are represented as colored rectangles as shown in Figure 3 (a; blue for **1**₆; red for **1**^{*}₆, and different shades of purple for the mixed macrocycles). Using peptide building blocks **1** and **1**^{*} introduces enough diversity to be able to study the pathways of formation, approach to equilibrium and deconstruction, unencumbered by enthalpic factors which would be present upon mixing building blocks with different peptide sequences.^{4e}

Figure 3 shows schematically how mixing can occur over time in a system prepared as a 1:1 block copolymer of unlabeled **1**₆ and labeled **1**^{*}₆ macrocycles, resulting in two parallel trends, both driven by the entropy of mixing of labeled and unlabeled material: (1) At the level of the fiber, the distribution of labeled material over the length of an average fiber becomes uniform. (2) At the level of individual

macrocycles: From initially pure **1**₆ and **1**^{*}₆ macrocycles, mixed hexamers **1**₁**1**^{*}₅, **1**₂**1**^{*}₄, **1**₃**1**^{*}₃, **1**₄**1**^{*}₂, **1**₅**1**^{*}₁ (in various isomeric forms) will form in statistical (binomially distributed) amounts. In Figure 3 and in the following, purple rectangles indicate both similar probabilities of finding a labeled or an unlabeled macrocycle and also an increased probability to find a mixed macrocycle, rather than a homogeneous one, in a particular position of the average fiber.

The mechanism by which the approach to equilibrium proceeds may involve three different mixing pathways A, B, and C, as shown in Figure 4. Mechanism A only involves exchange at the fiber ends: macrocycles transfer from fiber ends, where exchange can potentially happen (Figure 2) to the solution. Mechanism B involves fiber ends exchange as in mechanism A, but also features fiber breakage, exposing fiber cores to the exchanging solution. Mechanism C implies exchange between macrocycles in solution and macrocycles in the fiber cores and is observed for many supramolecular polymers.⁶ We will show below that exchange at sites other than the fiber ends does not occur to a significant degree in our system, which excludes mechanism C.

Figure 5 shows a comparison between the MS spectra of hexamer macrocycles immediately after fiber growth (Figure 5, a) and after 45 days of stirring at 200 rpm (Figure 5, b). A merged envelope of isotope peaks in the final MS spectrum, rather than the bimodal distribution of the two original envelopes of the peaks, shows that mixed macrocycles are formed (Figure 2). This indicates that the stability of the initial fibers is indeed only transient.

Figure 3 Supramolecular block copolymers (a) of **1**₆ (blue rectangles) and **1**^{*}₆ (red rectangles) undergo mixing over time, along the equilibration pathway. Colors show the distribution of the isotope label (blue = unlabeled; red = fully labeled; purple = partially labeled) along the fiber length of an average hexamer in an average fiber during the mixing process. An intermediate gradient state (b) is reached, eventually yielding fibers where hexamers have a uniform probability of containing **1** and **1**^{*} (c).

Figure 4 Mixing pathways. (a) Mechanism A: fiber end exchange; slow mixing expected. (b) Mechanism B: breakage combined with fiber end exchange; mixing sped up by breakage. (c) Mechanism C: exchange with building blocks in the fiber cores.

Figure 5 MS spectra of hexamer ions from $1+1^*$ fibers containing isotopically labeled macrocycles grown on previously prepared isotopically unlabeled macrocycle fibers: (a) immediately after preparation; (b) after 45 days stirring at 200 rpm. $[1]+[1^*] = 3.8$ mM.

To confirm the block-copolymer nature of the supramolecular aggregates at the start of the experiment, progressive reduction of the fibers was performed by using dithiothreitol (DTT) as a reducing agent.⁷ As shown in the Supporting Information (Figure S1), DTT irreversibly destroys the disulfide bonds of the hexamers predominantly at the fiber ends, reducing the latter back to building blocks (**1** and

1*). This experiment showed high selectivity in the first stages of the reduction process (Figure S1, c–g): the peaks due to the isotopically labeled macrocycles **1***₆ at the fiber ends are disappearing from the MS spectra first, followed by the unlabeled macrocycles **1**₆. In the first half of the reduction experiment, the selectivity towards **1*** hexamers is 80–90%, depending on the time interval between preparation and execution of the first experiment, indicating that the outer blocks are almost exclusively targeted. The small amount (10–20%) unlabeled **1**₆ that is also reduced may be a result of fiber breakage. These data confirm that growth and deconstruction pathways predominantly involve reactions at the fiber ends.

To gather further insights into the equilibration pathways, we performed identical reduction experiments at different times during equilibration of a stirred fiber sample. The expectation is that a freshly prepared block copolymer loses all of the labeled macrocycles in the initial stages of reduction (Figure 6, a), leaving only unlabeled macrocycles to be detected in the second half of the reduction process. In contrast, the composition of a perfectly mixed fiber does not vary as the fibers are progressively reduced (Figure 6, c), as the constituent macrocycles are fully mixed. Intermediate gradient distributions (Figure 6, b) yield a more gradual change in the isotopic distribution during the reduction experiment.

Figure 6 Reduction experiments are performed on a sample by gradually deconstructing macrocycles from the fiber ends by reduction. The remaining mixture is allowed time to mix, after which another sample is subjected to gradual reduction. The same experiment performed at various stages of the equilibration pathway provides insights into the isotopic distribution within the fibers.

For every measurement, we calculated the fraction of isotopically labeled building blocks in the fibers F , defined in Equation 1, and plotted it against the equivalents of reducing agent used. In this equation $[1^*]_{\text{fibers}}$ is the concentration of 1^* as supramolecular fibers in the system, $[1]_{\text{fibers}}$ is the concentration of 1 as supramolecular fibers in the system, $m/z(1_6^{4+} + 1_6^{*,4+})$ is the average mass to charge ratio of the isotopic peak distribution of the mixture of 1 and 1^* in the supramolecular fibers (calculated from integration of the MS spectra), $m/z(1_6^{4+})$ is the average mass to charge ratio of the isotopic peak distribution of pure 1 in the supramolecular fibers (also calculated from integration of the MS spectra), and $2/3$ is a correction factor that accounts for the observation by MS of hexamers of 1 with a quadruple charge.

$$F = \frac{[1^*]_{\text{fibers}}}{[1]_{\text{fibers}} + [1^*]_{\text{fibers}}} = \frac{m/z(1_6^{4+} + 1_6^{*,4+}) - m/z(1_6^{4+})}{m/z(1_6^{*,4+}) - m/z(1_6^{4+})} \cdot \frac{2}{3}$$

Equation 1 Relation between the fraction of isotopically labeled building block in the replicator fibers (F) and the experimentally observable isotope distribution

Starting with a 1:1 mixture of 1_6 and 1_6^* , when fibers have not been reduced yet, $F = 0.5$. As we progressively add more reducing agent, F decreases (Figure 7), indicating that the remaining macrocycles in the fiber cores are richer in unlabeled macrocycles. As shown in Figure 7, the initial slope (which we refer to as M) of the plot of F against the amount of added reducing agent is less negative as the 1_6^* - 1_6 - 1_6^* block-cofiber samples are given more time to equilibrate. The nonlinear shape of the F plot reflects the block-copolymer nature of the fibers. The initial slope M of the plot serves as a measure of the degree of mixing of the labeled building blocks from the cores to the periphery of the fibers: the higher the mixing, the higher (i.e., the less negative) the slope M , eventually converging on $M = 0$ for complete isotope exchange (vide infra). Thus as mixing occurs, the distribution in Figure 3 (a: block copolymer) changes towards the one in Figure 3 (b: gradient distribution). This

Figure 7 Fraction of labeled building block in the fibers (F) plotted against the equivalents of reducing agent DTT (with respect to $[1] + [1^*]$) supplied to a system of supramolecular fibers prepared by growing 1^* hexamers on previously prepared 1 hexamer fibers ($[1] + [1^*] = 3.8$ mM) and stirred at 200 rpm. Blue: 0 days; light blue: 4 days; light green: 12 days; green: 45 days.

observation confirms that equilibration is proceeding at the fiber level, but is not yet complete (i.e., $M \neq 0$), even after 45 days.

Control experiments for a system where we prepared hexamer fibers from a mixture of unlabeled and labeled building blocks 1 and 1^* gave rise to a completely random homogeneous distribution of labels, corresponding to Figure 3 (c). The resulting plot of F as a function of the number of equivalents of reducing agents shows a flat line ($M = 0$) with a value of F close to 0.5, indicating uniform mixing (see Supporting Information Figure S2).

Thus, we have evidence of slow building-block exchange within agitated fiber samples at the level of the replicating macrocycles from the data in Figure 5 and evidence of exchange at the level of the fiber from the data in Figure 7. The timescale of exchange is orders of magnitude slower than building-block exchange between nonassembled macrocycles in solution, which, under comparable conditions, is complete within the time it takes to perform the UPLC analysis (minutes).

In order to investigate the contributions of mechanisms A and B (Figure 4) to the approach of equilibrium, we also monitored exchange within the replicator fibers in the absence of stirring. MS spectra of hexamer macrocycle ions before the exchange experiment and at day 45 are essentially identical (see Supporting Information Figure S3), indicating that exchange is extremely slow if the sample is not agitated. This data at the level of the macrocycles was corroborated at the level of the fibers by comparing the values of M during the exchange process for the agitated and nonagitated fibers. The results are shown in Figure 8 and

Figure 8 Initial slope (M) of the plots of F vs. degree of reduction, against exchange time for block copolymers of 1_6 containing isotopically labeled macrocycles grown on previously prepared isotopically unlabeled macrocycle fibers, comparing nonagitated samples (blue circles) with samples agitated at 200 rpm (black squares).

indicate that M gradually becomes less negative for the stirred sample, while it remains essentially unchanged for the quiescent sample.

Taken together, the data suggest that building-block exchange takes place predominantly at the fiber ends. In quiescent samples fiber exchange of building blocks is prohibitively slow; macrocycles in the fiber cores will only exchange if they become exposed at a fiber end through a stochastic fiber disassembly-assembly process. Given the length of the replicator fibers (several hundred nanometers)^{4c} such exposure through random dynamic variation in fiber length is highly unlikely. Hence, only agitation-mediated fiber breakage will allow macrocycles that were initially hidden within the fiber cores to become exposed at the fiber ends, where they can then undergo building-block exchange. But even with agitation, exchange is still not complete after 45 days, even though nonassembled macrocycles exchange building blocks within minutes. Thus, supramolecular interactions are remarkably efficient in conserving the structural make-up of, and information contained within, self-assembled self-replicating molecules.

Our results imply that information contained in peptide-based replicators can be relatively robust, even where all reactions and interactions through which this information is generated are reversible and can therefore also induce loss of information. An important requirement is that the time required to propagate the information (i.e., the time needed for replication) is shorter than the timescale with which information is lost. Thus, limited stability of information carriers is perhaps less problematic than often believed, as long as systems are sufficiently dynamic; that is, replication dynamics are sufficiently fast.

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Supporting Information

Supporting information for this article is available online at <http://dx.doi.org/10.1055/s-0036-1589709>.

References and Notes

- (1) For reviews, see: (a) Ghosh, I.; Chmielewski, J. *Curr. Opin. Chem. Biol.* **2004**, *8*, 640. (b) Paul, N.; Joyce, G. F. *Curr. Opin. Chem. Biol.* **2004**, *8*, 634. (c) Patzke, V.; von Kiedrowski, G. *ARKIVOC* **2007**, (v), 293. (d) Vidonne, A.; Philp, D. *Eur. J. Org. Chem.* **2009**, 593. (e) Kassianidis, E.; Pearson, R. J.; Wood, E. A.; Philp, D. *Faraday Discuss.* **2010**, *145*, 235. (f) Bissette, A. J.; Fletcher, S. P. *Angew. Chem. Int. Ed.* **2013**, *52*, 12800. (g) Clixby, G.; Twyman, L. *Org. Biomol. Chem.* **2016**, *14*, 4170.
- (2) (a) Ruiz-Mirazo, K.; Briones, C.; de la Escosura, A. *Chem. Rev.* **2014**, *114*, 285. (b) Pross, A. *What Is Life? How Chemistry Becomes Biology*; Oxford University Press: Oxford, **2012**.
- (3) For reviews, see: (a) del Amo, V.; Philp, D. *Chem. Eur. J.* **2010**, *16*, 13304. (b) Moulin, E.; Giuseppone, N. *Top. Curr. Chem.* **2012**, *322*, 87. (c) Otto, S. *Acc. Chem. Res.* **2012**, *45*, 2200. For research papers, see: (d) del Arno, V.; Slawin, A. M. Z.; Philp, D. *Org. Lett.* **2008**, *10*, 4589. (e) Sadownik, J. W.; Philp, D. *Angew. Chem. Int. Ed.* **2008**, *47*, 9965. (f) Turega, S. M.; Lorenz, C.; Sadownik, J. W.; Philp, D. *Chem. Commun.* **2008**, 4076. (g) Xu, S.; Giuseppone, N. *J. Am. Chem. Soc.* **2008**, *130*, 1826. (h) Nguyen, R.; Allouche, L.; Buhler, E.; Giuseppone, N. *Angew. Chem. Int. Ed.* **2009**, *48*, 1093. (i) Mukherjee, R.; Cohen-Luria, R.; Wagner, N.; Ashkenasy, G. A. *Angew. Chem. Int. Ed.* **2015**, *54*, 12452.
- (4) (a) Carnall, J. M. A.; Waudby, C. A.; Belenguer, A. M.; Stuart, M. C. A.; Peyralans, J. J. P.; Otto, S. *Science* **2010**, *327*, 1502. (b) Malakoutikhah, M.; Peyralans, J. J. P.; Colomb-Delsuc, M.; Fanlo-Virgos, H.; Stuart, M. C. A.; Otto, S. *J. Am. Chem. Soc.* **2013**, *135*, 18406. (c) Colomb-Delsuc, M.; Mattia, E.; Sadownik, J. W.; Otto, S. *Nat. Commun.* **2015**, *6*, 7427. (d) Leonetti, G.; Otto, S. *J. Am. Chem. Soc.* **2015**, *137*, 2067. (e) Sadownik, J. W.; Mattia, E.; Nowak, P.; Otto, S. *Nat. Chem.* **2016**, *8*, 264.
- (5) Supramolecular block copolymers were obtained starting with the preparation of 500 μ L of a 3.8 mM solution of unlabeled monomers **1** (in 50 mM pH 8.1 potassium borate buffer) and allowing this solution to oxidize (while stirring in an HPLC vial (12 $\times$ 32 mm) tightly closed with Teflon-lined snap caps using a Teflon-coated magnetic stirrer bar (5 $\times$ 2 mm) at 1200 rpm) resulting in the formation of hexamers **1₆**. These were brought to the appropriate stirring rate and 500 μ L of a 3.8 mM solution of labeled building blocks **1*** were added. Over the course of 2 d, labeled hexamers **1***₆ grow on the previously existing **1₆** fibers.
- (6) da Silva, R. M. P.; van der Zwaag, D.; Albertazzi, L.; Lee, S. S.; Meijer, E. W.; Stupp, S. I. *Nat. Commun.* **2016**, *7*, 11561.
- (7) Pal, A.; Malakoutikhah, M.; Leonetti, G.; Tezcan, M.; Colomb-Delsuc, M.; Nguyen, V. D.; van der Gucht, J.; Otto, S. *Angew. Chem.* **2015**, *54*, 7852.