Electronic Supplementary Material (ESI) for Chemical Science.

Supporting information

Selection of Diverse Polymorphic Structures from a Small Dynamic Molecular Network Controlled by the Environment

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1. Materials and methods

All chemicals, unless otherwise stated, were purchased from commercial suppliers (*Sigma-Aldrich, TCI Europe, Acros Organics, Merck Chemicals or Alfa Aesar*) and used as received. Acetonitrile (ULC-MS grade), water (UPLC-MS grade) and trifluoroacetic acid (HPLC grade) were purchased from *Biosolve BV*. Water was doubly distilled prior to use.

Library preparation and sampling

Building block **1** was dissolved in 2.0 mM concentration in borate buffer (50 mM, pH 8.5) and diluted to 1.0 mM concentration by addition of borate buffer and/or cosolvent to the desired percentage (up to 50%). For fast oxidation of the libraries a 100 mM solution of sodium perborate in double distilled water was used. The solutions were kept in a UPLC vial (12 x 32 mm) with a Teflon-lined cap. Stirred samples contained a cylindrical micro-stirrer bar (2 x 5 mm, Teflon-coated, purchased from VWR) and were stirred at 1200 rpm using an IKA RCT basic hotplate stirrer, unless otherwise specified. Shaken samples were shaken at 1200 rpm and 25 °C using Eppendorf thermomixer *comfort*.

UPLC and LC-MS analysis

An aliquot of 5.0 µL was taken into a UPLC vial insert and diluted with 20 µL of an iPrOH/H_2O (1:1 V/V) mixture 1 minute before the injection. A volume of 3.0 µL of the mixture was injected. UPLC analyses were performed on a Waters Acquity H-class instrument equipped with diode array UV/Vis detector. LC-MS analyses were performed on a Xevo G2 UPLC/TOF with ESI ionization, manufactured by Waters. All analyses were performed at 35 °C, with a flow rate of 0.3 mL/min, using a reversed-phase UPLC column (Acquity UPLC HSS T3, 100 Å, 1.8 µm, 2.1 x 150 mm). UV absorbance was monitored at 254 nm. Positive-ion mass spectra were acquired using electro-spray ionization and the same UPLC method. Injection volume was 5.0 µL with dilution as previously described.

UPLC method

Solutions containing building block **1** and its oxidation products were analyzed using the following method (linear gradient):

Solvent A: UPLC/MS grade water purchased from Biosolve (0.1% trifluoroacetic acid added).

Solvent B: UPLC/MS grade acetonitrile purchased from Biosolve (0.1% trifluoroacetic acid added).

Time (min)	A%	B%
0	60	40
1	60	40
2	40	60
12	20	80
13	5	95
14	5	95
15	60	40
17	60	40

Negative staining transmission electron microscopy

A small drop (5 µL) of sample was deposited on a 400 mesh copper grid covered with a thin carbon film (Agar Scientific). After 30 seconds, the droplet was blotted on filter paper. The sample was then stained twice (4 µL each time) with a solution of 2% uranyl acetate deposited on the grid and blotted on the filter paper after 30 seconds each time. The grids were observed in a Philips CM120 cryo-electron microscope operating at 120 keV. Images were recorded on a slow scan CCD camera.

Cryo transmission electron microscopy

A 2.8 µL aliquot of sample was deposited on freshly glow discharged holey carbon-coated grid (Quantifoil 3.5/1, Quantifoil Micro Tools, Jena, Germany). After blotting the excess liquid, the grid was vitrified in liquid ethane on a vitrobot (FEI, Eindhoven, the Netherlands) and transferred to a Philips CM 120 electron microscope equipped with a Gatan model 626 cryo-stage, operating at 120 keV. Micrographs were recorded under low-dose conditions with a slow-scan CCD camera.

2. Supplementary figures

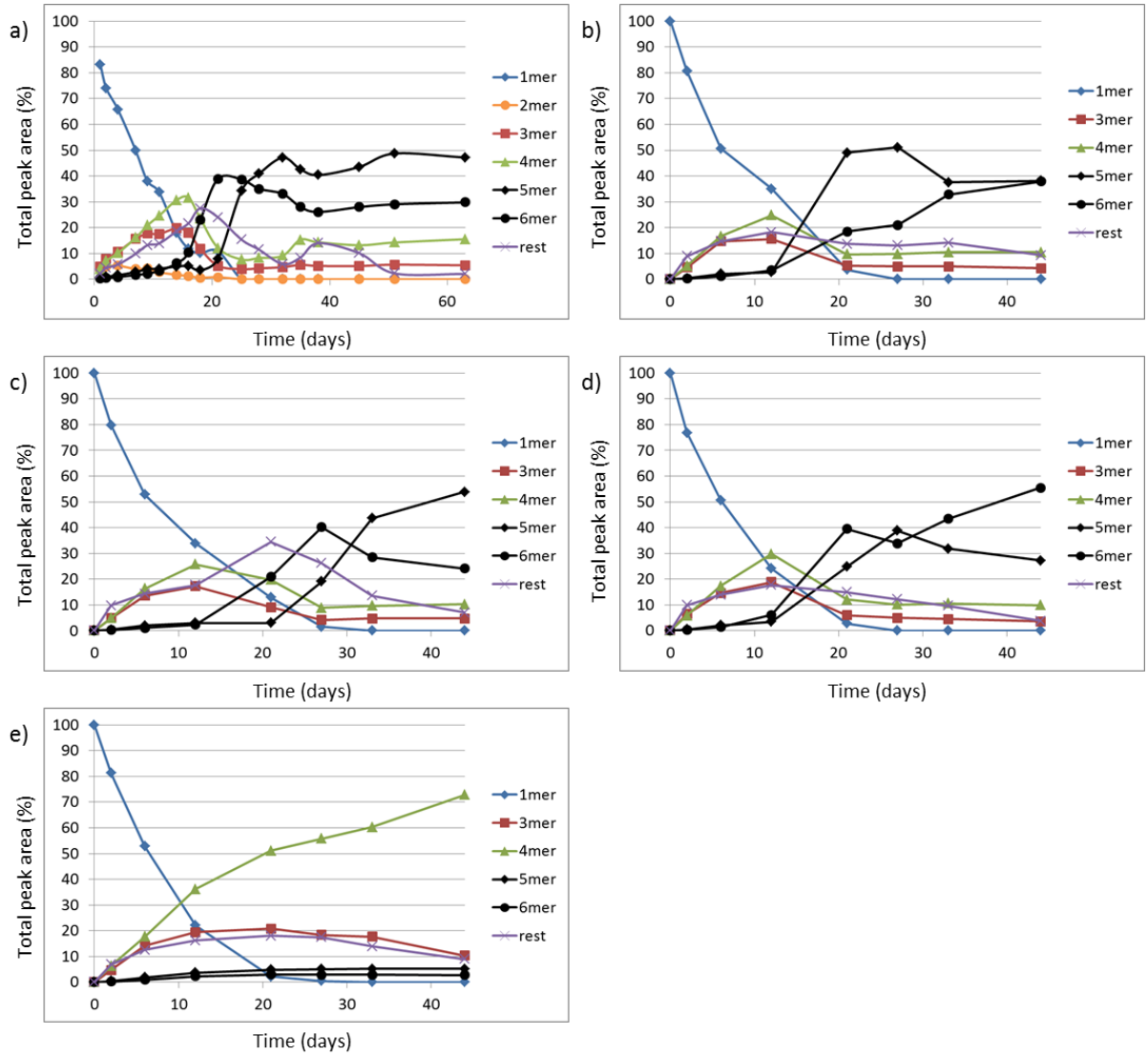

Figure S1. Five exemplary DCLs made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 10% of 1-propanol as a cosolvent show different outcomes: $\mathbf{1}_5$ and $\mathbf{1}_6$ co-exist in replicates (a) to (d) while in (e) $\mathbf{1}_4$ is the dominant product.

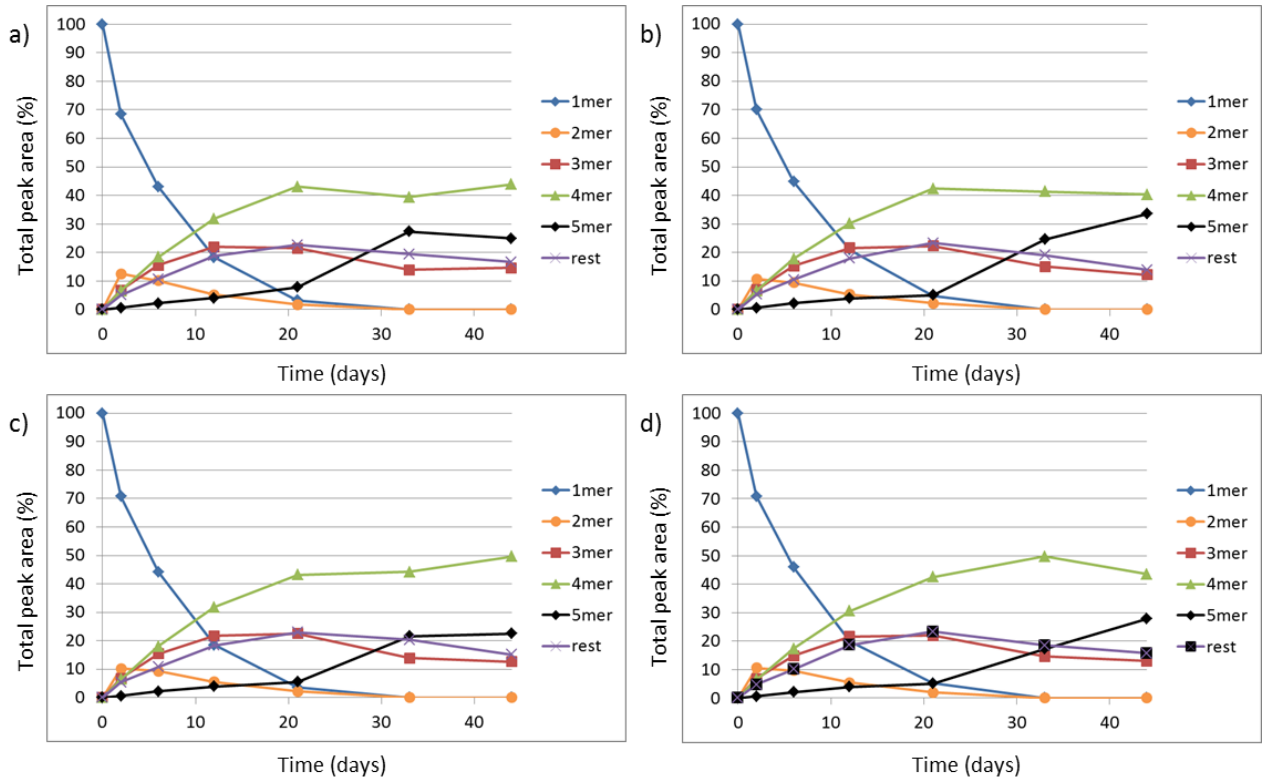

Figure S2. Four repeats of DCLs made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 15% of 1-propanol as a cosolvent show similar outcomes: emergence of $\mathbf{1}_4$ as the dominant compound.

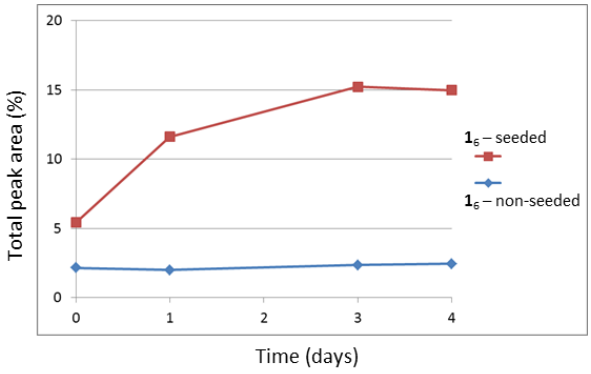

Figure S3. A library seeded with $\mathbf{1}_6$ (70%) was added to a solution of building block **1** (80% oxidized, red) and showed significant growth of $\mathbf{1}_6$ in comparison to a non-seeded sample (blue). This indicates $\mathbf{1}_6$ is a self-replicator. Only the hexamer concentration is shown for clarity.

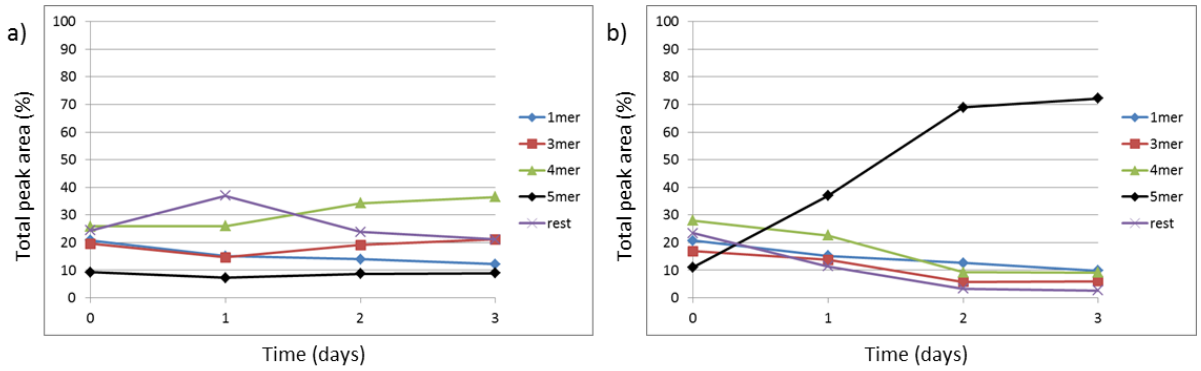

Figure S4. Libraries of building block **1** seeded with $\mathbf{1}_5$ (65%) produced different outcomes depending on the conditions. (a) There was no preference for any macrocycles to emerge from the library containing only buffer, however (b) $\mathbf{1}_5$ emerged as the dominant species in a library containing 10% 1-propanol. This indicates $\mathbf{1}_5$ is self-replicator in the presence of 10% 1-propanol.

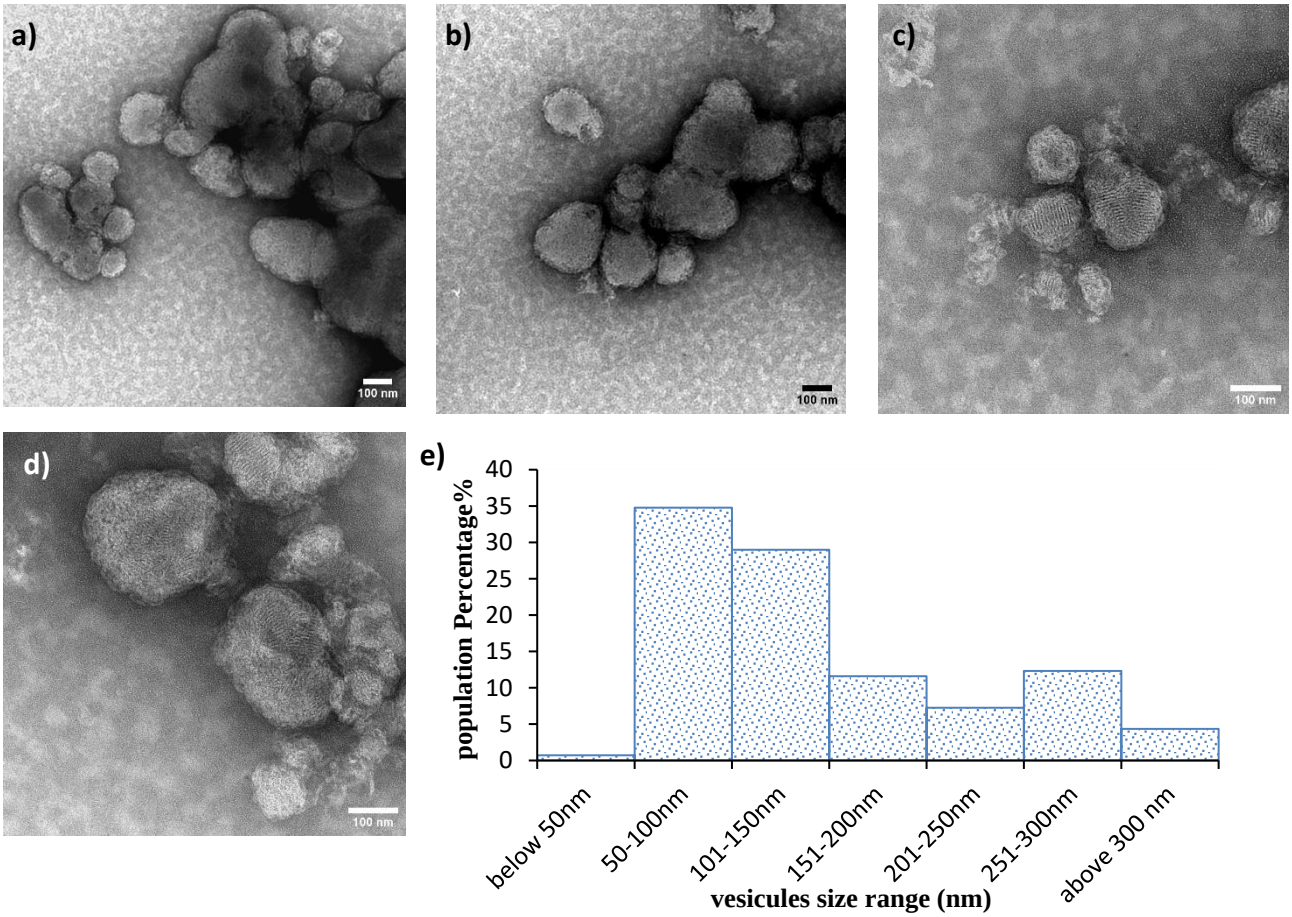

Figure S5. The morphology and size distribution of a fully oxidized DCL made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 50% of 1-propanol in B_2O_3 buffer (50 mM, pH 8.5) after 40 days. a-d) TEM micrograph (negatively stained) of the library showing vesicular aggregates with distinct periodic deformities in their structures. e) the size distribution of vesicular aggregates based on the measurement of 138 round vesicles.

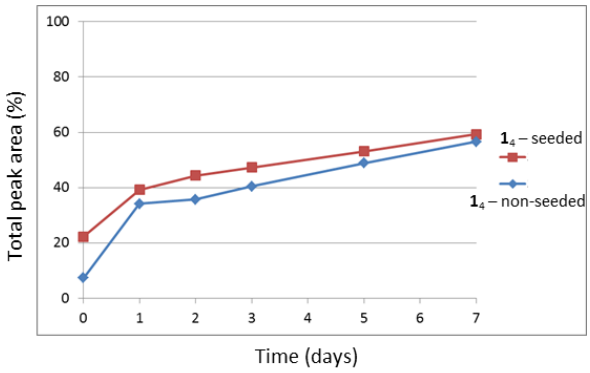

Figure S6. A library of building block **1** (80% oxidized) seeded with $\mathbf{1}_4$ (90%) in the presence of 50% 1-propanol did not show significant growth of $\mathbf{1}_4$ in comparison to a library without seed. Only tetramer concentration is shown for clarity.

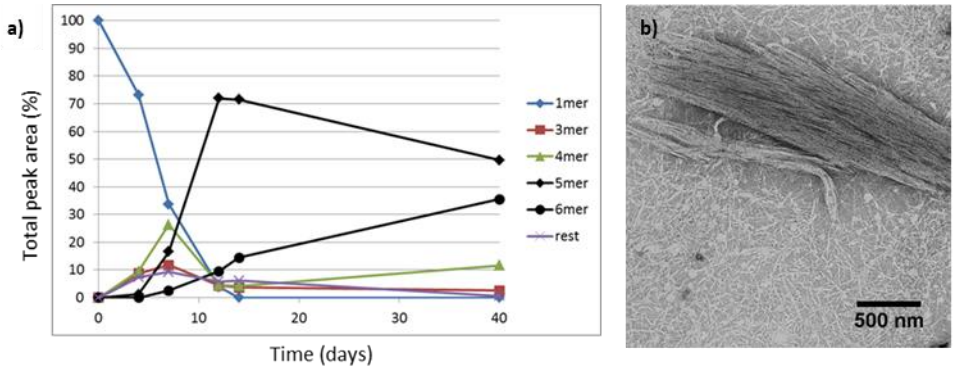

Figure S7. (a) Evolution of the product distribution of the DCLs made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) show that both $\mathbf{1}_5$ and $\mathbf{1}_6$ macrocycles co-exist when in the presence of 10% of 1-butanol and (b) a TEM micrograph (negatively stained) of the same library on day 40 shows fibrous structures.

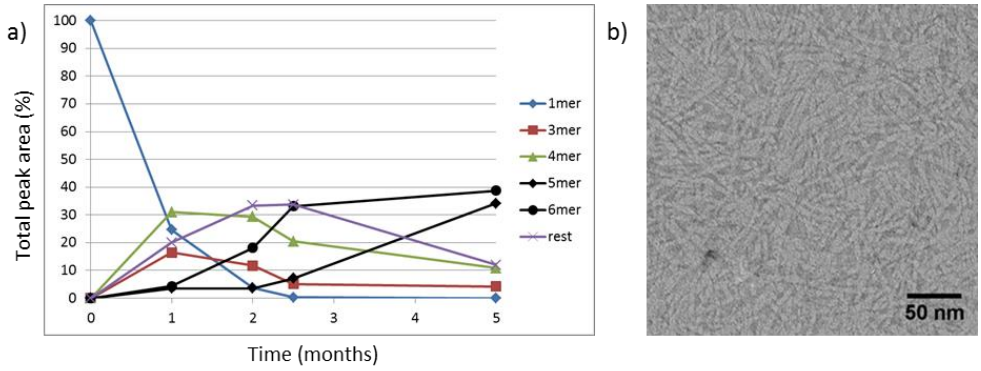

Figure S8. (a) Evolution of the product distribution for a DCL made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 10% of ethanol as a cosolvent shows $\mathbf{1}_5$ and $\mathbf{1}_6$ co-existing but emerging much later than with experiments containing other co-solvents. (b) TEM micrograph (negatively stained) of the library containing 10% ethanol (4 months) showing a fibrous structure.

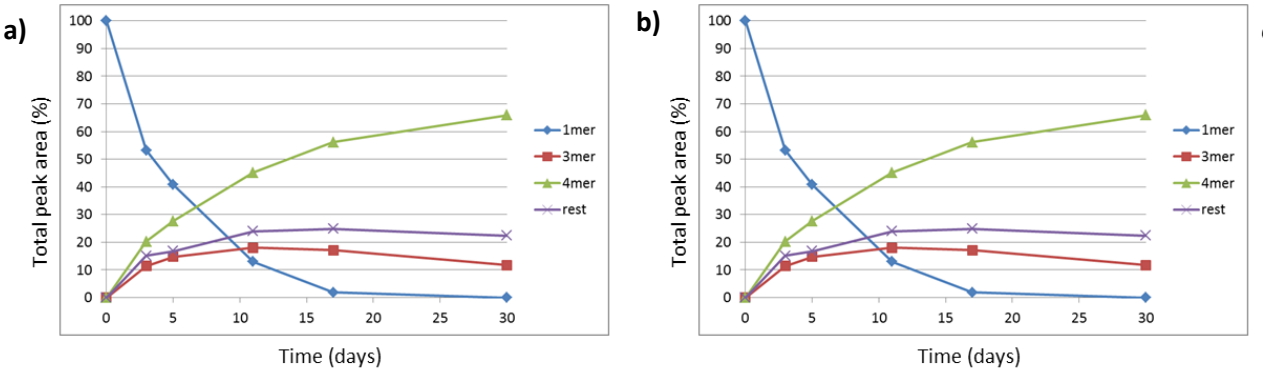

Figure S9. Evolution of the product distribution for the DCLs made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with (a) 30% and (b) 50% tetrahydrofuran as a cosolvent showing $\mathbf{1}_4$ as the main species.

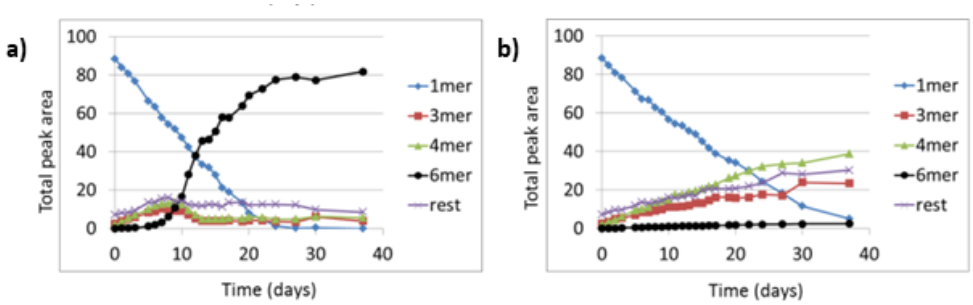

Figure S10. Libraries made from building block **1** (1.0 mM) produce different outcomes depending on agitation: (a) shaking which yields $\mathbf{1}_6$ as the dominant species, while (b) no agitation which yields no preference for specific macrocycles. These observations indicate that the nanoribbons composed of $\mathbf{1}_6$ grow by a breakage-elongation mechanism.

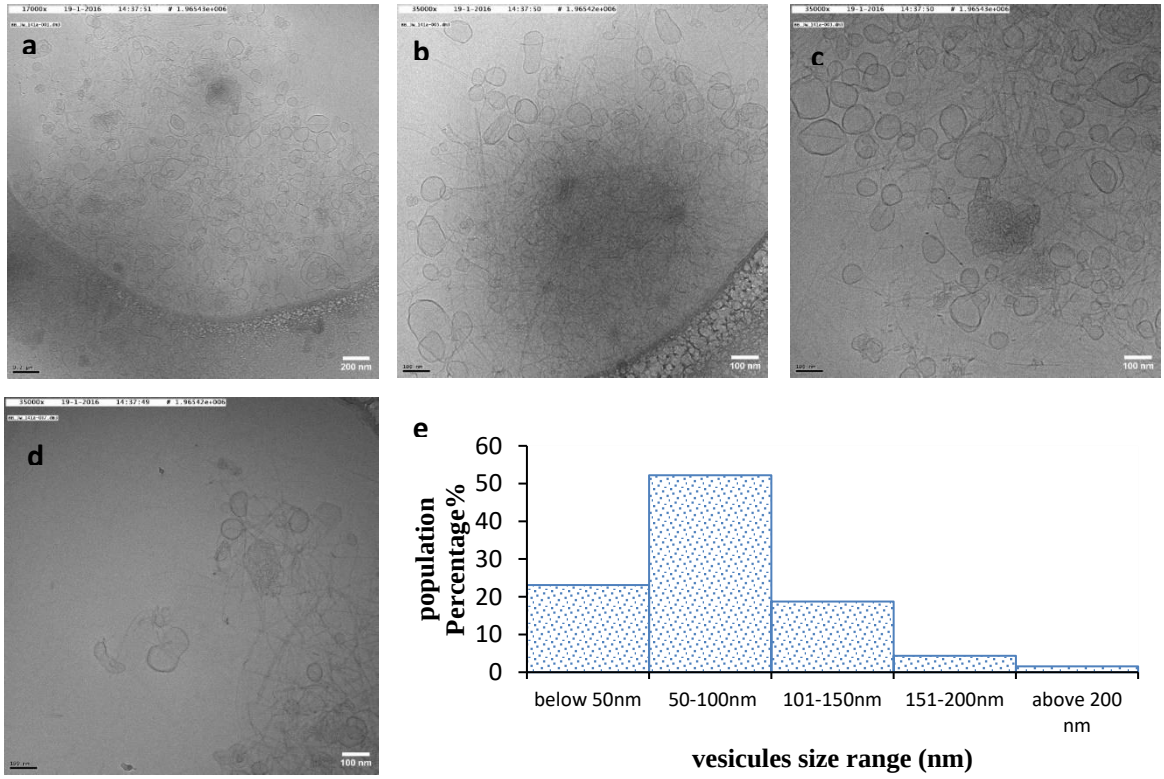

Figure S11. The morphology and size distribution for a DCL made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 10% of methanol in B_2O_3 buffer (50 mM, pH 8.5) as a cosolvent after 2.5 months. a-d) Representative set of cryo-TEM micrographs of the library showing vesicular structures. e) The size distribution of the structures based on the measurement of 335 round vesicles.

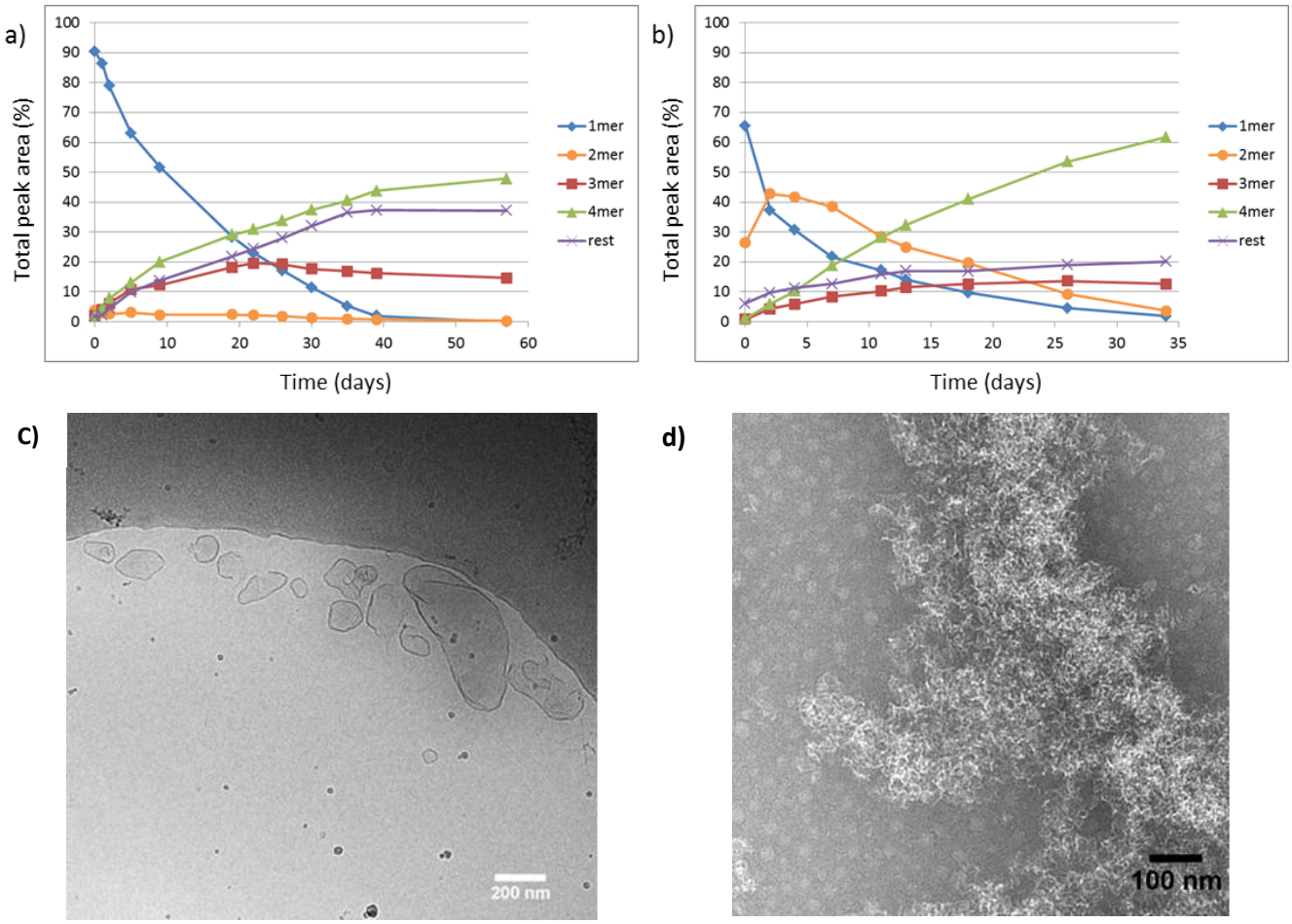

Figure S12. Evolution of the product distribution for the DCLs made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with (a) 10% and (b) 50% of 2-propanol as a cosolvent; (c) Micrographs of a fully oxidized DCL made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 10% of 2-propanol in B_2O_3 buffer (50 mM, pH 8.5) showing vesicle. (d) Micrographs of a fully oxidized DCL made from building block **1** (1.0 mM, stirred at 1200 rpm, room temperature) with 50% of 2-propanol in borate buffer (50 mM, pH 8.5) showing amorphous aggregates.

Table S1. Summary of the conditions and the outcomes of DCLs prepared from building block **1** in the presence of different cosolvents.

Solvent	V/V %	time	dominant species	structure	self-replication	agitation
Methanol	10%	2 months	6-mer & 4-mer	Fiber& vesicle	Y&N	Stirred
Methanol	10%	4 months	only 4mer	Vesicle	N	Stirred
Ethanol	10%	2 months	5-mer & 6-mer	Fiber	Y	Stirred
1-propanol	10%	1 month	5-mer& 6-mer	Fiber	Y	Stirred
1-propanol	15%	1 month	4-mer	Amorphous aggregates	N	Stirred
1-propanol	50%	1 month	4-mer	Vesicular aggregates	N	Stirred
2-propanol	10%	2 months	4-mer	Vesicles	N	Stirred
2-propanol	50%	1 month	4-mer	Amorphous aggregates	N	Stirred
1-butanol	10%	40 days	5-mer & 6-mer	Fiber	Y	Stirred
THF	10%	40 days	6-mer	Nanoribbons	Y	Stirred
THF	10%	40 days	6-mer	Nanoribbons	Y	Shaked
THF	10%	40 days	4-mer, 3-mer	No assembly	N	Non-agitated
THF	30%	1 month	4-mer	Amorphous aggregates	N	Stirred
THF	50%	1 month	4-mer	Amorphous aggregates	N	Stirred