

Cascading transformations within a dynamic self-assembled system

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Molecular subcomponents such as phosphate groups are often passed between biomolecules during complex signalling cascades, the flow of which define the motion of the machinery of life. Here, we show how an abiological system consisting of organic subcomponents knitted together by metal-ion coordination can respond to simple signals in complex ways. A Cu₃ helicate transformed into its Zn₂Cu^I analogue following the addition of zinc(II), and the ejected copper(I) went on to induce the self-assembly of a Cu₂ helicate from other free subcomponents present in solution. The addition of an additional subcomponent, 8-aminoquinoline, resulted in the formation of a third, more stable Cu₃ helicate, requiring the destruction of both the Zn₂Cu^I and Cu₂ helicates to scavenge sufficient Cu^I for the new structure. This system thus demonstrates two examples in which the application of one signal provokes two distinct responses involving the creation or destruction of complex assemblies as the system seeks thermodynamic equilibrium following perturbation.

The complex functions associated with life emerge from systems of molecules interacting in intricate ways^{1–8}. The information that mediates these biomolecular interactions is often conveyed by means of a molecular subcomponent (such as a phosphate group⁹) being passed from molecule to molecule following a thermodynamic gradient. Information received by one molecular component of a system often prompts that component to release a signal of its own, leading to the elegant signalling cascades that allow living organisms to process information^{10–12} in the complex ways that are necessary to adapt to stimuli⁹. Many synthetic molecular machines^{13–19} also depend on the passage of similar chemical signals²⁰ to function. The development of molecular machines with more complex functions will require more complex, multistage signalling mechanisms.

Here, we demonstrate a minimal system in which the application of different signals—through the addition of a metal ion or a small molecule—induces the system to reconstitute in complex ways. This system is shown schematically in Fig. 1. The addition of a limited amount of copper(I) to a mixture of three subcomponents A, B and C induces two of them to come together preferentially. The addition of zinc(II) to this intermediate product mixture displaces part of the copper(I); this displaced copper(I) then leads to self-assembly elsewhere in the system. Addition of molecular subcomponent D then induces complete rearrangement of the system, as all copper(I) is scavenged to generate a final product of greater thermodynamic stability, resulting in the disassembly of the system's other structures.

The dynamic reassembly behaviour of a novel heterometallic Zn₂Cu^I helicate **2** (Fig. 2) is central to the complex behaviour of the system described herein. This work thus builds upon examples presented by other groups in which the coordination geometries of different metal ions and well-defined multitopic ligands have been used to create structural complexity. Such an approach has enabled the construction of grids²¹, triangles^{22,23}, catenanes²⁴ and helicates^{25–29}, which have been prepared from well-designed multitopic ligands. Complex macrocycles³⁰ and a molecular Solomon link, incorporating both Cu^{II} and Zn^{II} (ref. 31), have also been constructed from subcomponents and multiple metal ions.

The reaction between 8-amino-2-quinolinecarboxamide **A** (4 equiv.), 2,9-diformyl-1,10-phenanthroline **B** (2 equiv.) and Cu(CH₃CN)₄BF₄ (3 equiv.) in acetonitrile produces tricopper(I) helicate **1** as the uniquely observed product (Fig. 2), in similar fashion to what has been observed in systems containing other 8-aminoquinolines, **B** and Cu^I (refs 32,33). We reasoned that the different environments of the peripheral metal binding sites compared to the central one might allow the formation of a heterometallic helicate. Indeed, Zn₂Cu^I double helicate **2** could be prepared either from free constituent subcomponents (Fig. 2) or through the reaction of Zn(OAc)₂ (2 equiv.) with **1** in acetonitrile (Fig. 2). This transmetallation did not proceed in DMSO, which suggests that solvent stabilization of the ejected Cu^I provides a crucial part of the thermodynamic driving force for this reaction. Indeed, the solvent stabilization of Cu^I in acetonitrile is reflected in the observation that the equilibrium constant K_{disp} for the disproportionation $2 \text{Cu}^{\text{I}}(\text{solv}) \rightleftharpoons \text{Cu}^{\text{II}}(\text{solv}) + \text{Cu}^0(\text{s})$ becomes more than 20 orders of

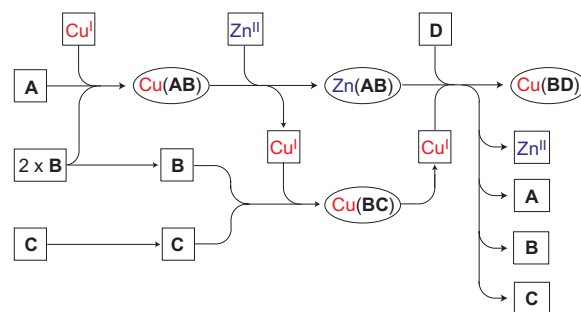


Figure 1 | Schematic of the transformations in a dynamic self-assembled system. The addition of a limited amount of copper(I) to a mixture of subcomponents **A**, **B** and **C** induced **A** and **B** to come together preferentially. The addition of zinc(II) caused a cascade reaction, through which part of the copper(I) was displaced. This ejected copper(I) induced **B** and **C** to come together. The addition of another molecular subcomponent **D** induced complete rearrangement of the system.

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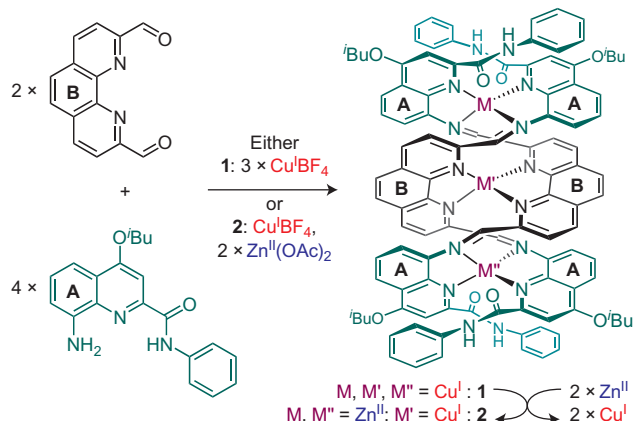


Figure 2 | Syntheses of homometallic double helicate 1 and heterometallic double helicate 2. Reaction of **A** (4 equiv.) and **B** (2 equiv.) with copper(I) (3 equiv.) led to the formation of homometallic double helicate **1**. Reaction of **A** (4 equiv.) and **B** (2 equiv.) with copper(I) (1 equiv.) and zinc(II) (2 equiv.) led to heterometallic double helicate **2**. Addition of zinc(II) (2 equiv.) to **1** led to ejection of copper(I) and formation of **2**.

magnitude smaller in changing solvent from DMSO to acetonitrile³⁴. The precipitation of unseen, finely divided CuOAc was unlikely to have contributed to the driving force for transmetalation, because its solubility in acetonitrile (40 mM, measured by nuclear magnetic resonance (NMR) integration against *t*-BuOH as the internal standard) is greater than the concentrations of Cu^I (29.6 mM) and acetate (39.4 mM) with which the reaction was run.

Crystals of **1** and **2** were grown from vapour diffusion of diethyl ether into a dimethylformamide solution of **1** and nitromethane into an acetonitrile solution of **2**, respectively. The structures of both were solved by single-crystal X-ray diffraction. Severe disorder limited structure quality in both cases. The connectivities of both **1** and **2** are clear, as shown in Fig. 3, but the structures' quality precludes comparison and discussion of bond lengths and angles; indeed, we cannot conclude with certainty that the positions of Zn^{II} and Cu^I are not disordered within **2**. In both **1** and **2**, each ligand orients one amide carbonyl group towards the centre of the helicate, where it may accept a hydrogen bond from the other ligand's amide N–H group and participate in a stabilizing dipole–cation interaction with the Zn^{II} or Cu^I centre. Solution NMR spectra are consistent with the presence of additional amide-group conformations (for **1** and **2**) and possibly the presence of disorder between Zn^{II} and Cu^I (for **2**); these spectra are presented and discussed in the Supplementary Information.

The differing coordination preferences of different metal ions may impose selectivity and discrimination within a self-assembling system, allowing the creation of a more complex structure than is possible with a single metal ion, as several studies have shown^{24,28,31,35–37}. Building upon this earlier work, we were able to take advantage of the preference of **A** and **B** to generate Zn^{II}Cu^I helicate **2** as opposed to Cu^I₂ helicate **1** to allow the realization of a more complex system of transformations than has yet been achieved^{33,38,39} (Fig. 4).

As shown in Fig. 4, helicate **1** is thermodynamically stable in the presence of **B** (2 equiv.) and **C** (4 equiv.): this system expressed **1** in preference to dicopper(I) double helicate **3** when limited in Cu^I. The addition of Zn(OAc)₂ (2 equiv.) to this system, however, gives rise to a cascade of two distinct transformations³⁸: helicate **1** was converted into **2**, and the ejected Cu^I (2 equiv.) reacted with the free **B** (2 equiv.) and **C** (4 equiv.) to generate dicopper double helicate **3**, which co-exists with **2** in solution.

The addition of unsubstituted 8-aminoquinoline **D** (4 equiv.) to this stable mixture of **2** and **3** resulted in the formation of tricopper

helicate **4**. NMR spectra taken at 353 K, 24 h after the addition of **D** to **2** and **3**, showed resonances corresponding to **4**, **A**, **B** and **C**, but no resonances corresponding to **1**, **2** or **3** (Fig. 5). The electrospray ionization (ESI) mass spectrum (Supplementary Fig. S7) revealed the formation of small amounts of the imine condensation product of **A** and **B** around a single zinc template, to which we attribute a set of minor ¹H resonances (marked with * in Fig. 5).

We attribute the greater thermodynamic stability of **4** when compared to **2** and **3** to the more electron-rich character³² of **D** than either **A** or **C**; in other systems we have observed more electron-rich subcomponents to displace less electron-rich ones^{33,39,40}. The preference of helicate **4** to incorporate Cu^I rather than Zn^{II} is attributed to the lack of carbonyl groups in **D** that are well positioned to stabilize a Zn^{II} centre. Because each equivalent of **4** requires three Cu^I ions, the assembly of **4** within the system of Fig. 4 requires the disassembly of both **2** and **3**.

The central Cu^I ion of **2** appears to serve as a linchpin; the lack of any observed Zn^{II} helicate analogous to **2** suggests that such a structure might be destabilized by its greater concentration of charge with respect to the entropically favoured free subcomponents and such mononuclear complexes as **AB**(Zn^{II}), which was observed in the ESI mass spectrum (Supplementary Fig. S7).

To draw an analogy with signal transduction in biological systems, the addition of **D** to the mixture of **2** and **3** may be likened to an initial, thermodynamically favoured phosphorylation event that triggers a cascade, resulting in the release of myriad molecular signals (**A**, **B**, **C** and Zn^{II}) into the local environment. Such signals might then interact with other receptors—or with parts of a more complex self-assembling system—to produce a complex response to a simple stimulus.

In summary, we have shown how the application of a single signal to a complex self-assembling system may trigger multiple disassembly–reassembly events, as the consequences of one reassembly cascade through a system. Structures may be either created or destroyed, as the liberation or consumption of building blocks provokes second-order changes, chemically 'far' from the initial substitution event. The cascade reactions of Fig. 4 demonstrate an advance in the state-of-the-art³⁸ in terms of both the structural complexity of the complexes undergoing rearrangement and, more importantly, the overall systemic complexity: the seven building blocks of Fig. 4 are arranged differently in each of four discrete states, each of which may be accessed from its precursor state through the addition of a single component to the system. The addition of **D** to the system's penultimate state results in the breakage or reconfiguration of 24 discrete N → M and C = N linkages. The degree of complexity

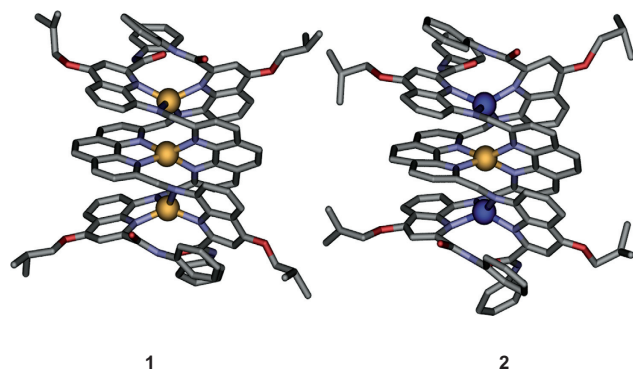


Figure 3 | Crystal structures of 1 and 2: C, grey; N, lilac; O, red; Cu, yellow; Zn, blue. Hydrogen atoms, molecules of solvent of crystallization and counterions are not shown. **1** was crystallized by vapour diffusion of ether into a dimethylformamide solution of **1**. Crystals of **2** were obtained by vapour diffusion of nitromethane into an acetonitrile solution of **2**.

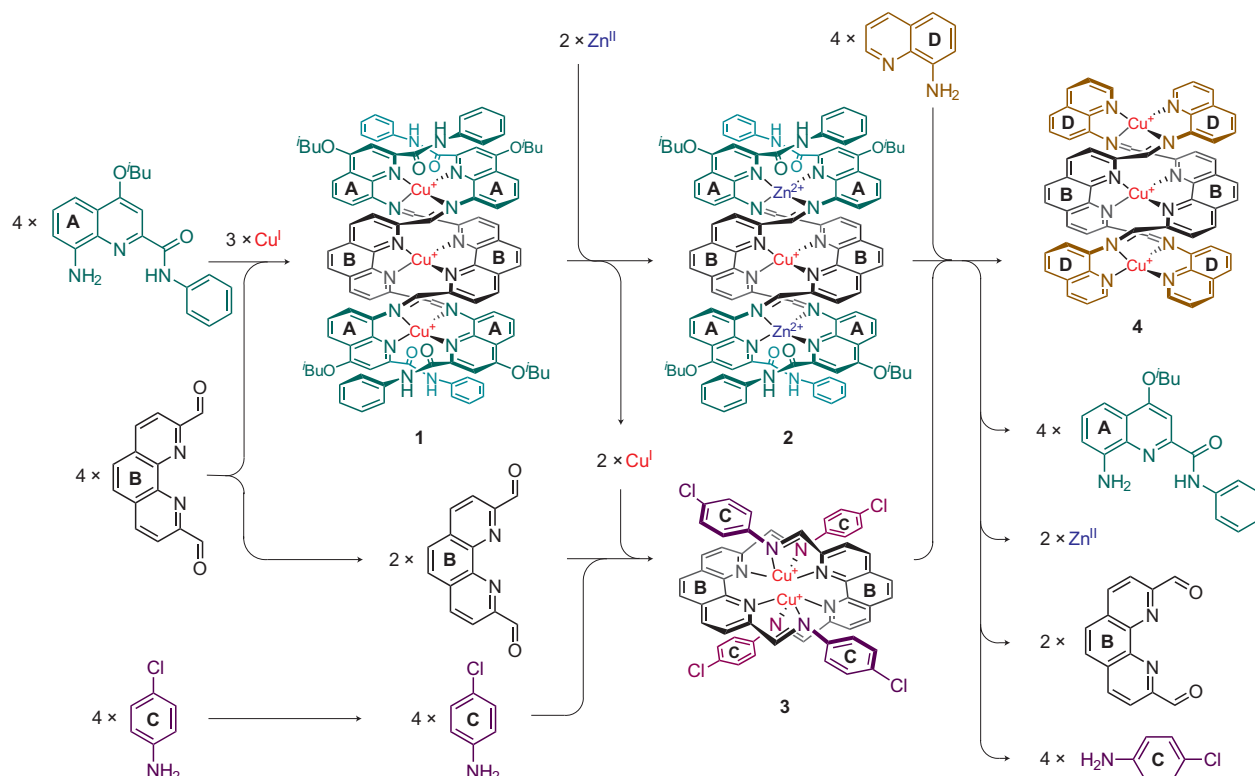


Figure 4 | Self-assembling system in which the addition of one signal (Zn^{II} or 8-aminoquinoline) induced two distinct transformations. On addition of copper(I) to a mixture of **A**, **B** and **C**, homometallic double helicate **1** formed preferentially, leaving excess **B** and **C** in solution. The addition of zinc(II) to this mixture caused a cascade reaction, displacing part of the copper(I) from **1**, resulting in the simultaneous generation of helicates **2** and **3**. The addition of molecular subcomponent **D** brought about the destruction of **2** and **3**, liberating their constituent subcomponents and forming final product **4**.

of this system thus represents a small step towards the intricate and elegant signalling systems found in nature. Similar rearrangement reactions to those studied here may enable the motion of new molecular machines.

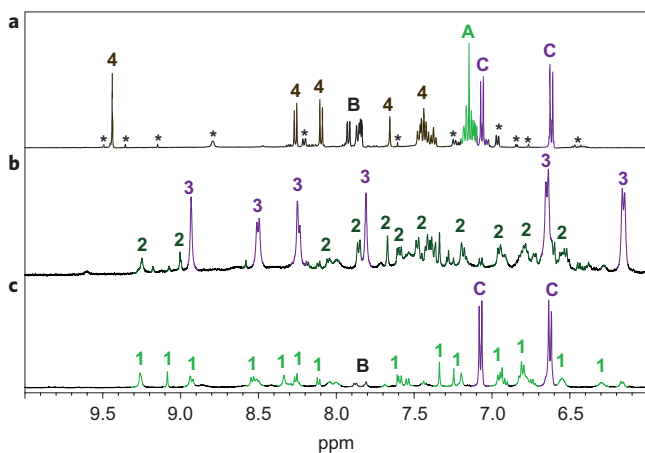


Figure 5 | 1H NMR spectra of the three states of the complex self-assembling system shown in Fig. 4. **a**, The system's terminal state following the addition of 8-aminoquinoline (4 equiv.), yielding **4**, **A**, **B** and **C**; * indicates resonances observed in the 1H spectrum of the mixture of products formed when **B**, **A**, **C** and $Zn(OAc)_2$ are mixed, which are consistent with the condensation product of **A** and **B** about a single Zn^{II} template (see Supplementary Fig. S7). **b**, The system's intermediate state after the addition of $Zn(OAc)_2$ (2 equiv.) to **1**, **B** and **C**, yielding **2** and **3**. **c**, The system following the addition of $Cu(CH_3CN)_4BF_4$ (3 equiv.) to **A** (4 equiv.), **B** (4 equiv.) and **C** (4 equiv.) in CD_3CN , yielding **1**, **B** and **C**.

Methods

Reassembling system of Fig. 4. Into a J-Young NMR tube were added **A** (5.36 mg, 15.9 μ mol), **B** (4.65 mg, 19.7 μ mol), **C** (2.50 mg, 19.7 μ mol), $Cu(CH_3CN)_4BF_4$ (4.66 mg, 14.8 μ mol) and $CDCl_3$ (0.5 ml). The tube was sealed and the solution purged of dioxygen by three vacuum/argon fill cycles then heated for 3 days at 353 K. Volatiles were removed under dynamic vacuum and the remaining brown powder was dissolved in CD_3CN (0.5 ml), giving **1**, **B** and **C** as the sole products observed in solution by 1H NMR (Fig. 5a). To this solution, $Zn(OAc)_2$ (1.00 mg, 9.86 μ mol) was added, the tube was sealed and the solution purged of dioxygen by three vacuum/argon fill cycles. The brown solution was heated at 353 K overnight, yielding a dark green solution with **2** and **3** being the only products observed by 1H NMR (Fig. 5b). To this mixture, 8-aminoquinoline **D** (1.43 mg, 9.86 μ mol) was added; the tube was then sealed and the solution purged of dioxygen by three vacuum/argon fill cycles. The solution was heated to 353 K overnight, after which a dark brown solution was formed in which **4**, **A**, **B** and **C** were the only species observed in solution by 1H NMR (Fig. 5c).

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References

- Ludlow, R. F. & Otto, S. Systems Chemistry. *Chem. Soc. Rev.* **37**, 101–108 (2007).
- Lehn, J.-M. Programmed chemical systems: multiple subprograms and multiple processing/expression of molecular information. *Chem. Eur. J.* **6**, 2097–2102 (2000).
- Lee, D. H., Severin, K. & Ghadiri, M. R. Autocatalytic networks: the transition from molecular self-replication to molecular ecosystems. *Curr. Opin. Chem. Biol.* **1**, 491–496 (1997).
- Jullien, L., Lemarchand, A., Charier, S., Ruel, O. & Baudin, J. B. Two-site molecules as a road for engineering complexity in chemical systems. *J. Phys. Chem. B* **107**, 9905–9917 (2003).
- Kindermann, M., Stahl, I., Reimold, M., Pankau, W. M. & von Kiedrowski, G. Systems chemistry: kinetic and computational analysis of a nearly exponential organic replicator. *Angew. Chem. Int. Ed.* **44**, 6750–6755 (2005).
- Mukhopadhyay, P., Zavalij, P. Y. & Isaacs, L. High fidelity kinetic self-sorting in multi-component systems based on guests with multiple binding epitopes. *J. Am. Chem. Soc.* **128**, 14093–14102 (2006).

7. Sarma, R. J. & Nitschke, J. R. Self-assembly in systems of subcomponents: simple rules, subtle consequences. *Angew. Chem. Int. Ed.* **47**, 377–380 (2008).
8. Severin, K. The advantage of being virtual-target-induced adaptation and selection in dynamic combinatorial libraries. *Chem. Eur. J.* **10**, 2565–2580 (2004).
9. Gomperts, B., Kramer, I. & Tatham, P. *Signal Transduction* (Academic Press, 2002).
10. Jeong, H., Tombor, B., Albert, R., Oltvai, Z. N. & Barabasi, A. L. The large-scale organization of metabolic networks. *Nature* **407**, 651–654 (2000).
11. de Silva, A. P. & Uchiyama, S. Molecular logic and computing. *Nature Nanotech.* **2**, 399–410 (2007).
12. Gupta, T. & van der Boom, M. E. Redox-active monolayers as a versatile platform for integrating Boolean logic gates. *Angew. Chem. Int. Ed.* **47**, 5322–5326 (2008).
13. von Delius, M., Geertsema, E. M. & Leigh, D. A. A synthetic small molecule that can walk down a track. *Nature Chem.* **2**, 96–101 (2010).
14. Badjic, J. D., Balzani, V., Credi, A., Silvi, S. & Stoddart, J. F. A molecular elevator. *Science* **303**, 1845–1849 (2004).
15. Collin, J.-P., Dietrich-Buchecker, C., Gaviña, P., Jimenez-Molero, M. C. & Sauvage, J.-P. Shuttles and muscles: linear molecular machines based on transition metals. *Acc. Chem. Res.* **34**, 477–487 (2001).
16. Schalley, C. A., Beizai, K. & Vogtle, F. On the way to rotaxane-based molecular motors: studies in molecular mobility and topological chirality. *Acc. Chem. Res.* **34**, 465–476 (2001).
17. Eelkema, R. *et al.* Molecular machines: nanomotor rotates microscale objects. *Nature* **440**, 163 (2006).
18. de Silva, A. P. & McClenaghan, N. D. Molecular-scale logic gates. *Chem. Eur. J.* **10**, 574–586 (2004).
19. Kay, E. R., Leigh, D. A. & Zerbetto, F. Synthetic molecular motors and mechanical machines. *Angew. Chem. Int. Ed.* **46**, 72–191 (2007).
20. Credi, A., Balzani, V., Langford, S. J. & Stoddart, J. F. Logic operations at the molecular level. An XOR gate based on a molecular machine. *J. Am. Chem. Soc.* **119**, 2679–2681 (1997).
21. Petitjean, A., Kyrisakas, N. & Lehn, J.-M. Ion-triggered multistate molecular switching device based on regioselective coordination-controlled ion binding. *Chem. Eur. J.* **11**, 6818–6828 (2005).
22. Schmittl, M. & Mahata, K. Multicomponent assembly of heterometallic isosceles triangles. *Inorg. Chem.* **3**, 822–824 (2009).
23. Champin, B., Sartor, V. & Sauvage, J.-P. A highly rigid ditopic conjugate with orthogonal coordination axes and its zinc(II) and copper(I) complexes. *New J. Chem.* **32**, 1048–1054 (2008).
24. Dietrich-Buchecker, C. O. *et al.* Quantitative formation of [2]catenanes using copper(I) and palladium(II) as templating and assembling centers: then entwining route and the threading approach. *J. Am. Chem. Soc.* **125**, 5717–5725 (2003).
25. Marquis, A. *et al.* Messages in molecules: ligand/cation coding and self-recognition in a constitutionally dynamic system of heterometallic double helicates. *Chem. Eur. J.* **12**, 5623–5641 (2006).
26. Riis-Johannessen, T., Harding, L. P., Jeffery, J. C., Moon, R. & Rice, C. R. Allosteric derpogramming of a trinuclear heterometallic helicate. *Dalton Trans.* 1577–1587 (2007).
27. Canard, G. & Piguet, C. The origin of the surprising stabilities of highly charged self-assembled polymeric complexes in solution. *Inorg. Chem.* **46**, 3511–3522 (2007).
28. Albrecht, M., Liu, Y., Zhu, S. S., Schalley, C. A. & Fröhlich, R. Self-assembly of heterodinuclear triple-stranded helicates: control by coordination number and charge. *Chem. Commun.* 1195–1197 (2009).
29. Hahn, F. E., Offermann, M., Isfort, C. S., Pape, T. & Fröhlich, R. Heterobimetallic triple-stranded helicates with directional benzene-*o*-dithiol catechol ligands. *Angew. Chem. Int. Ed.* **47**, 6794–6797 (2008).
30. Christinat, N., Scopelliti, R. & Severin, K. Multicomponent assembly of boronic acid based macrocycles and cages. *Angew. Chem. Int. Ed.* **47**, 1848–1852 (2008).
31. Pentecost, C. D. *et al.* A molecular Solomon link. *Angew. Chem. Int. Ed.* **46**, 218–222 (2007).
32. Hutin, M., Franz, R. & Nitschke, J. R. A dynamic tricopper helicate. *Chem. Eur. J.* **12**, 4077–4082 (2006).
33. Campbell, V. E. *et al.* Interplay of interactions governing the dynamic conversions of acyclic and macrocyclic helicates. *Chem. Eur. J.* **15**, 6138–6142 (2009).
34. Rosen, B. M. *et al.* The disproportionation of Cu(I)X mediated by ligand and solvent into Cu(0) and Cu(II)X-2 and its implications for SET-LRP. *J. Polym. Sci. Pol. Chem.* **47**, 5606–5628 (2009).
35. Funeriu, D. P., Lehn, J.-M., Fromm, K. M. & Fenske, D. Multiple expression of molecular information: enforced generation of different supramolecular inorganic architectures by processing of the same ligand information through specific coordination algorithms. *Chem. Eur. J.* **6**, 2103–2111 (2000).
36. Piguet, C., Bernardinelli, G., Williams, A. F. & Bocquet, B. Formation of the first isomeric [2]catenates by self-assembly about two different metal ions. *Angew. Chem. Int. Ed.* **34**, 582–584 (1995).
37. Stulz, E., Scott, S. M., Bond, A. D., Teat, S. J. & Sanders, J. K. M. Selection and amplification of mixed-metal porphyrin cages from dynamic combinatorial libraries. *Chem. Eur. J.* **9**, 6039–6048 (2003).
38. Schultz, D. & Nitschke, J. R. Dynamic covalent and supramolecular direction of the synthesis and reassembly of copper(I) complexes. *Proc. Natl Acad. Sci. USA* **102**, 11191–11195 (2005).
39. Schultz, D. & Nitschke, J. R. Designing multistep transformations using the Hammett equation: transimination on a copper(I) template. *J. Am. Chem. Soc.* **128**, 9887–9892 (2006).
40. Schultz, D. & Nitschke, J. R. Kinetic and thermodynamic selectivity in subcomponent substitution. *Chem. Eur. J.* **13**, 3660–3665 (2007).

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Author contributions

J.R.N. conceived the study. V.E.C., J.R.N. and I.H. designed and debugged the experiments. N.D. and X.H. synthesized compound A. V.E.C. synthesized and characterized compounds 1 and 2. V.E.C. and N.D. obtained X-ray quality crystals of 1 and 2. B.K. and I.H. analysed the crystallographic data. V.E.C., J.R.N. and I.H. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

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