

'Making is Understanding': Non-equilibrium structure and dynamics—giving chemistry direction.

Over the last quarter-century the Leigh group have led a transformation in the understanding of molecular-level dynamics, ushering in a new era of non-equilibrium (supra)molecular chemistry. The Leigh group have pioneered the control of molecular-level dynamics using ratchet mechanisms [2003]. Ratchet mechanisms give chemical processes directional bias (kinetic asymmetry) from random thermal motion. The Leigh group have introduced original concepts for the synthesis of entangled molecular structures (e.g. interlocking auxiliaries [2004], catalytic 'active template' synthesis [2006], 2D molecular weaving [2020]), developed synthetic strategies to complex knots and links [2012], created 'molecular assemblers' [2013] and other functional dynamic nanosystems [2003], initiated the field of programmable small-molecule robotics [2016], demonstrated conformational selection in small-molecule catalysis [2016], and established molecular nanotechnology as a research frontier for energy transduction and metabolism [2016]. The Leigh group's designs reverse engineer biomolecular machinery, delivering insights into how molecules 'walk' [2010] and track-based synthesizers operate [2013], how the acceleration of chemical reactions enables energy to be transduced by catalysts (such as motor proteins) [2016, 2021, 2022], and how dynamic (supra)molecular systems are driven away from equilibrium through catalysis [2021]. **The Leigh group has applied theories from physics, to the design of molecules that operate directionally through chemistry, revealing fundamental new insights into biology, and how the inanimate becomes animate.**

The introduction of Brownian ratchet mechanisms into synthetic molecules in the early 2000s is widely regarded as one of the most significant developments in the control of molecular-level dynamics to date [*...perhaps the most important result so far accruing from the synthesis of molecular machines is the insight provided into the fundamental mechanisms by which molecular motors function*], R. D. Astumian, *Chem. Sci.* **2017**, *8*, 840]. Seminal contributions from the Leigh group in this regard include the invention of the first molecular Brownian ratchets [2003 & 2004], the first molecular energy ratchet [2003], the first molecular information ratchet [2007], and the first linear molecular motor (a non-adiabatic molecular incarnation of the Maxwell Demon thought experiment [2007]). Leigh's group have developed increasingly sophisticated molecular machines, including examples that synthesize peptides in a programmable sequence reminiscent of the ribosome [2013] (extended to continuous-carbon-backbone polymers [2020]). They developed the first synthetic small-molecules that 'walk' along tracks [2010], the first autonomous chemically fueled molecular motors and pumps [2016 & 2021], motors driven by pulses of chemical fuel [2017], small-molecule robotics [2016] and a programmable 'molecular assembler' [2017] described in an accompanying N&Vs article as '*Science fiction becomes fact*'.

Over the last decade the Leigh group have introduced synthetic strategies that have allowed access to increasingly complex molecular knots and links. They have used molecular knots to induce both chiral [2016] and allosteric [2017] catalysis, studied the effects of entanglement across length scales [2018], synthesized the most complex knots and links to date [2012-2022], extrapolated these concepts to the two-dimensional weaving of polymer chains [2020], and forged a burgeoning new field of 'molecular nanotopology'.

1. Brownian ratchets: Out-of-equilibrium molecular-level dynamics

Engineering approaches that focus on Newtonian mechanics (momentum, inertia etc) are not useful design concepts for the nanoscale, where statistical mechanisms dominate. The idea of using random thermal fluctuations to drive directed motion has its origins in the visionary works of von Smoluchowski [*Phys. Z.* **13**, 1069 (1912)] and Feynman [*The Feynman Lectures on Physics* (1963)], but breakthrough papers from Astumian (theory) and the Leigh group

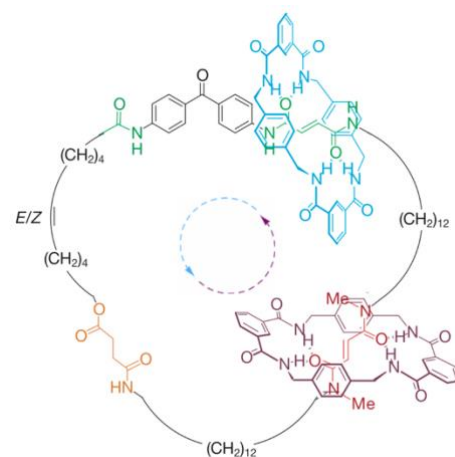


Fig. 1. Molecular Brownian ratchets (2003).

(experiment) in the late 1990s and early 2000s explained how these concepts apply to, and can be used in the design of, molecular nanotechnology and other out-of-equilibrium chemical systems.

Astumian described theoretical energy and information Brownian ratchets in 1998 [*Eur. Polym. J.* **27**, 474 (1998)]; the Leigh group showed how these physical principles could be applied to the design of synthetic molecular systems in landmark papers in 2003 and 2004 [*Nature* **424**, 174 (2003), Fig. 1; *Science* **306**, 1532 (2004)]. In particular, Leigh's team recognized that molecular components (e.g. the rings in catenanes or rotaxanes) could be considered as Brownian particles on a potential energy surface (the track) and their dynamics thus controlled by incorporating Brownian ratchet mechanisms into synthetic molecular designs: "The way in which the principles of an energy ratchet can be applied to a catenane architecture is not to consider the whole structure as a molecular machine, but rather to view one macrocycle as a motor that transports a substrate—the other ring—directionally around itself" and demonstrating for the first time that "Mechanisms formulated from non-equilibrium statistical mechanics can be successfully used to design synthetic molecular motors" [*Science* **306**, 1532 (2004)].

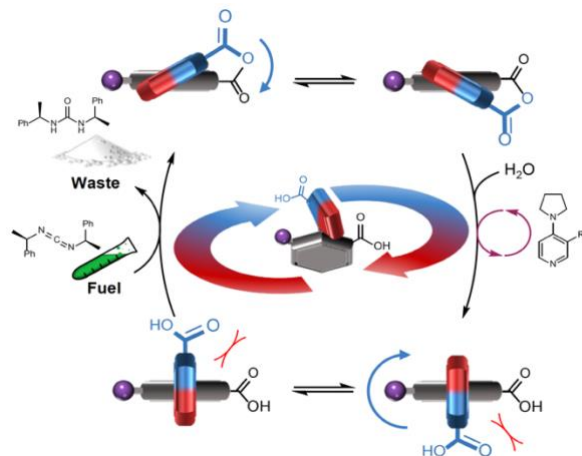


Fig. 2. Single bond rotary motor catalyst (2022).

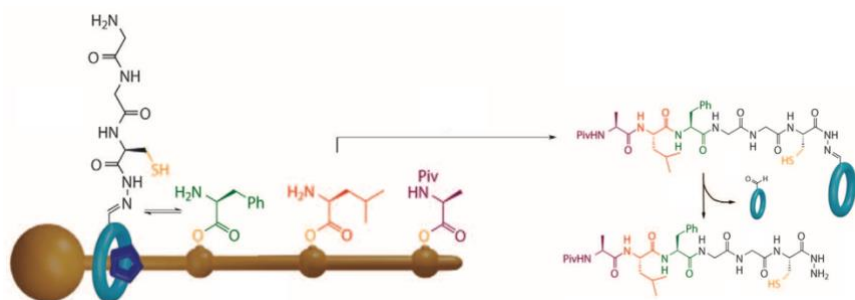


Fig. 3. Molecular peptide synthesizer (2013).

This realization marked a conceptual breakthrough that enabled the design and synthesis of genuine chemical engines, modules that power molecular motors and other complex molecular nanotechnology. The Leigh group's contributions include the first molecular energy ratchets [*Nature* **424**, 174 (2003); *Science* **306**, 1532 (2004)], the first molecular

information ratchets [*Nature* **445**, 523 (2007)], the first linear molecular motors [*J. Am. Chem. Soc.* **128**, 4058 (2006)], the first pulse-fueled [*Science* **358**, 340 (2017)] and the first autonomous chemically fueled molecular motors [*Nature* **534**, 235 (2016)] and pumps [*Nature* **594**, 529 (2021)]. In 2022 the Leigh group reported a 26-atom motor-molecule catalyst that continuously directionally rotates the components 360° about a covalent single bond [*Nature* **604**, 80 (2022); Fig. 2]. This minimalist doubly kinetically gated information ratchet demonstrates conformational selection in catalysis, a significant contributor to enzyme mechanism. 'Making is understanding' reveals how catalysis transduces energy to power molecular nanotechnology in biology.

The Leigh group have pioneered the design of systems that operate through the integrated interaction of multiple functional components, include synthetic molecules that 'walk' along

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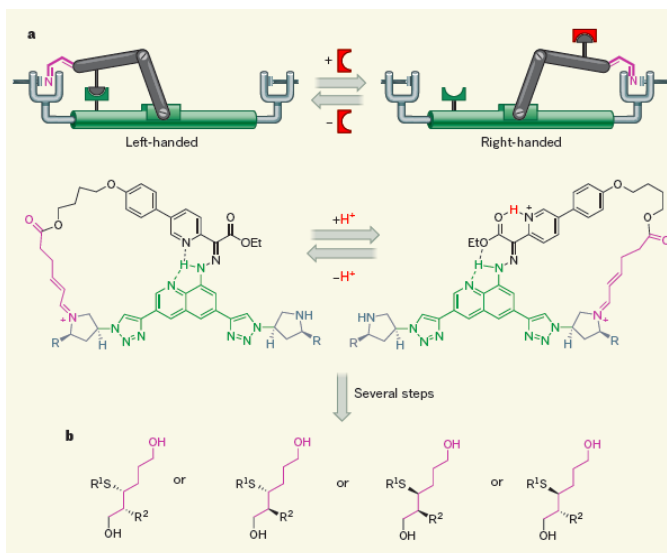


Fig. 4. Molecular robotics (2017).

tracks [*Nat. Chem.* **2**, 96 (2010); *Angew. Chem. Int. Ed.* **50**, 285 (2011) & *Angew. Chem. Int. Ed.* **51**, 5480 (2012)], programmable small-molecule robotics [*Nat. Chem.* **8**, 138 (2016) & *Nature* **549**, 374 (2017), Fig. 4], and ribosome-like molecular synthesizers [*Science* **339**, 189 (2013), Fig. 3; *Nat. Nanotech.* **13**, 381 (2018) & *Chem* **6**, 2964 (2020)]. A read-only molecular analog of Turing's 'automatic machine for computable numbers' incorporates a ratchet mechanism to move a macrocyclic 'reading head' in one direction along a molecular tape through a string-encoded state sequence, generating readable outputs [*Nature* **612**, 78 (2022)]. The introduction of ratchet mechanisms has transformed the understanding and design of molecular systems—biological, chemical and physical—from the macroscopic analogies of the 1990s to the scale-relevant concepts, insights and designs that underpin out-of-equilibrium research in the molecular sciences today.

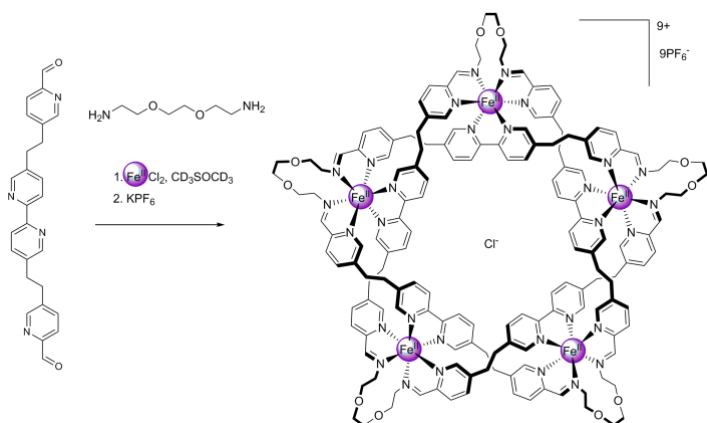


Fig. 5. A molecular pentafoil knot (2012).

The Leigh group have developed both general and specific methods for the synthesis of increasingly complex molecular knots and links, and demonstrated unique characteristics and effects that topology can impart. In 2012 the Leigh group reported the first higher order knot (five crossings) using a circular helicate strategy to generate the necessary number of crossings and the correct connectivity [*Nat. Chem.* **4**, 15 (2012), Fig. 5]. Circular double and triple helicates have subsequently been used to make 3- [*J. Am. Chem. Soc.* **136**, 13142 (2014); *J. Am. Chem. Soc.* **140**, 4982 (2018)], 5- [*Science* **352**, 1555 (2016)], 8- [*Science* **355**, 159 (2017), Fig. 6] and 9- [*Nat. Chem.* **10**, 1083 (2018)] crossing knots, and links with up to 9 crossings, sometimes with intrinsic writhe [*Angew. Chem. Int. Ed.* **57**, 13833 (2018)], including a Star of David [2]catenane, a triply-interlocked link [*Nat. Chem.* **6**, 978 (2014)].

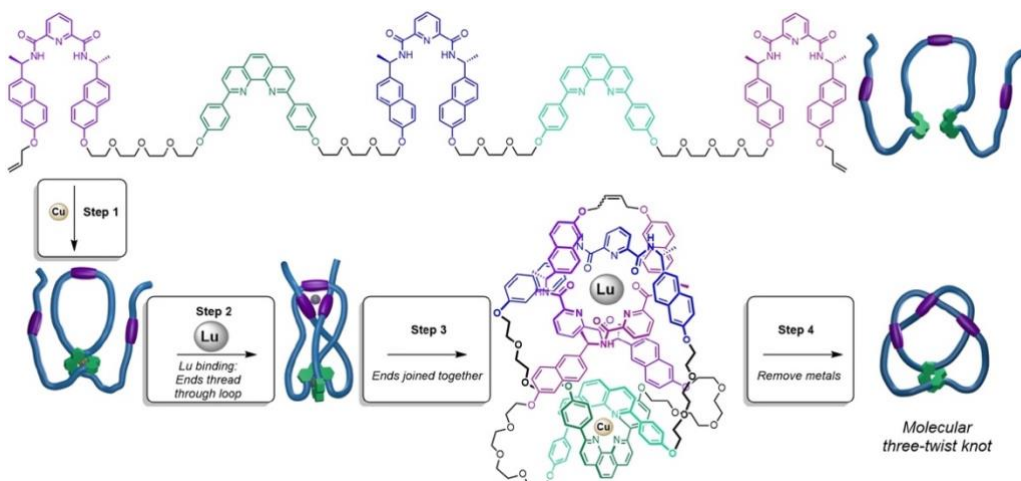


Fig. 7. Knot synthesis by metal ion 'chaperones' [2020].

2. Molecular Nanotopology

The physical significance of topological complexity (knots and links) is apparent in areas as diverse as colloids, liquid crystals, optical beams, soap films, super fluids and the origins of the early universe. In molecular terms, knots are found in DNA and proteins and form spontaneously in any polymer of sufficient length and flexibility. Over the last decade the Leigh group have led advances in the field of 'molecular nanotopology' (the topological counterpart to 'molecular nanotechnology'; see Stoddart, J. F. *Dawning of the age of molecular*

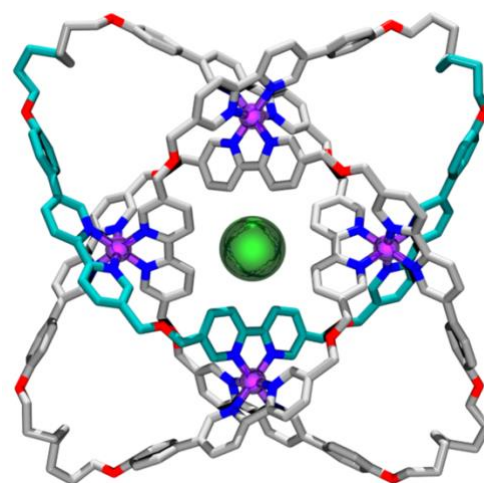


Fig. 6. Molecular braiding (2017).

Biology uses chaperones to direct the folding of peptide chains into precise 3D protein structures. The Leigh group have introduced a similar concept for folding and entangling single synthetic molecular strands into different knots by interspacing

different metal ion binding sites along the thread [*Nature* **584**, 562 (2020), Fig. 7]. The resulting strand can be programmed to fold into different knot topologies according to the sequence of metal ions it is exposed to.

In 1992, less than a decade after the first template synthesis of a catenane, Daryle H. Busch (the ‘Father’ of template synthesis) suggested that “*The ultimate aspiration of chemists working on interlocked structures might be to weave molecules as if they were macroscopic threads*” [D. H. Busch. *Structural definition of chemical templates and the prediction of new and unusual materials. J. Inclusion Phenom. Mol. Recognit. Chem.* **12**, 389 (1992)]. In 2020 the Leigh group reported the first weaving of ligands to form an interwoven 3x3 grid. This was used to synthesize both a molecular endless (7_4) knot [*Nat. Chem.* **13**, 117-122 (2021), Fig. 8] and to weave polymer chains in two-dimensions [*Nature* **588**, 429 (2020), Fig. 9]. The layered 2D

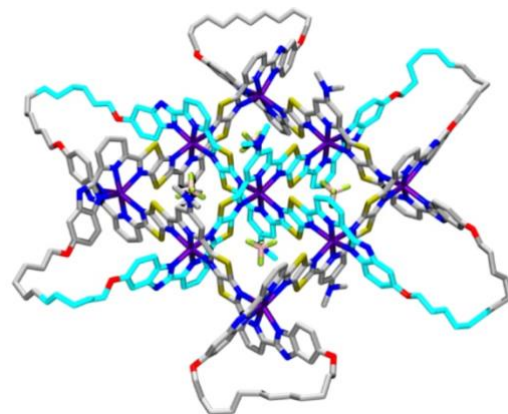


Fig. 8. Molecular endless (7_4) knot (2020).

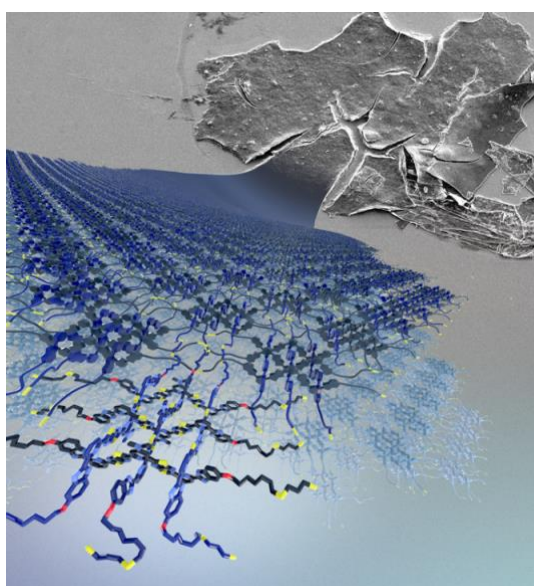


Fig. 9. 2D molecular weaving (2020).

molecularly woven material has long-range order, is twice as stiff as the constituent linear polymer, and delaminates and tears in the manner of a macroscopic textile. When incorporated into a polymer-supported membrane it acts as a net, slowing the passage of large ions while letting smaller ions through. In 2022 the Leigh group developed the rapid assembly of large 2D arrays of precise entanglement sequences through Vernier templating [*Science* **375**, 1035 (2022)].

The ability to weave molecular strands in regular arrays in two-dimensions—forming molecularly woven fabrics—is the realization of Busch’s ‘ultimate aspiration’ for interlocked structures. It opens up new possibilities and research directions at the intersection of three major fields: polymer science, two-dimensional materials and molecular nanotopology.

3. Catenanes, rotaxanes and hydrogen bonded molecular shuttles

The Leigh group’s pioneering of methods to control non-equilibrium structure and dynamics arose from simple rotaxane and catenane chemistry. In 1995 the Leigh group reported the one-step synthesis of benzylic amide catenanes [*Angew. Chem. Int. Ed.* **34**, 1209 (1995)]. Carrying out similar processes under thermodynamic control (organic ‘magic’ rings) gives virtually quantitative yields [*J. Am. Chem. Soc.* **121**, 1599 (1999)]. Structural and mechanistic studies enabled related rotaxanes to be designed [*J. Am. Chem. Soc.* **118**, 10662 (1996)]. Divergent hydrogen bonding sites occur in adjacent amino acid residues in peptide chains, leading to the synthesis of the first peptide rotaxanes [*Angew. Chem. Int. Ed.* **36**, 728 (1997)] and shuttles [*J. Am. Chem. Soc.* **119**, 11092 (1997)].

Over the next decade (1997-2007) the group introduced numerous novel and influential methods for controlling component dynamics in catenanes and rotaxanes and developed an array of stimuli to induce switching in molecular shuttles, including polarity changes [*J. Am. Chem. Soc.* **119**, 11092

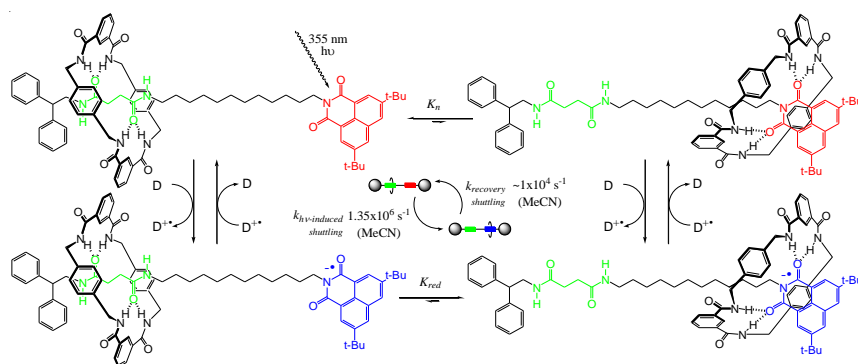


Fig. 10. Light-fueled molecular shuttle (2001).

(1997)], light [*Science* **291**, 2124 (2001). Fig. 10; *J. Am. Chem. Soc.* **123**, 11327 (2001); *Angew. Chem. Int. Ed.* **42**, 2296 (2003); *Science* **328**, 1255 (2010)], anion binding [*Angew. Chem. Int. Ed.* **43**, 1222 (2004); *Angew. Chem. Int. Ed.* **47**, 8036 (2008)], electrochemistry [*J. Am. Chem. Soc.* **125**, 8644 (2003); *J. Am. Chem. Soc.* **130**, 2593 (2008)], entropy-driven changes [*Angew. Chem. Int. Ed.* **42**, 5886 (2003)], reversible covalent chemistry [*Chem. Commun.* 2262 (2004)], competitive binding [*Angew. Chem. Int. Ed.* **45**, 77 (2006)] and allosteric binding [*Angew. Chem. Int. Ed.* **45**, 1385 (2006)].

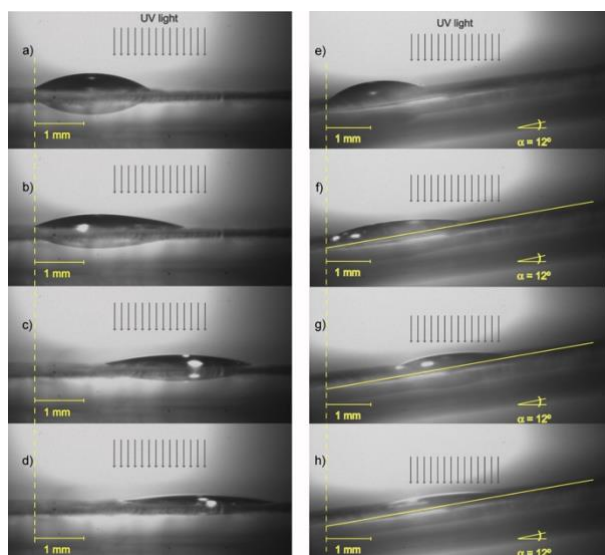


Fig. 11. Macroscopic transport by artificial molecular machines (2005).

These brought about property changes in some of the earliest functional molecular machines, including: photoswitches for fluorescence [*J. Am. Chem. Soc.* **126**, 12210 (2004)], chirality [*J. Am. Chem. Soc.* **125**, 13360 (2003)], dissipative catalysis [*Angew. Chem. Int. Ed.* **58**, 9876 (2019)], early examples that operated in polymer films [*Angew. Chem. Int. Ed.* **44**, 3062 (2005)], on surfaces [*Science* **299**, 531 (2003)], and at the single-molecule level [*Nat. Nanotech.* **6**, 553 (2011)]. Landmark demonstrations included the light-switchable response of a monolayer of fluorinated hydrogen bonded shuttles to change the contact angle of liquid droplets [*Nat. Mater.* **4**, 704 (2005), Fig. 11]. This effect proved sufficiently powerful to transport droplets across a surface, the first example of macroscopic transport by synthetic molecular machinery.

Classical template syntheses involve stoichiometric quantities of an otherwise passive template. In 2006 the Leigh group introduced the conceptually distinct 'active template' approach [*J. Am. Chem. Soc.* **128**, 2186 (2006), Fig. 12; *Chem. Soc. Rev.* **38**, 1530 (2009)] in which a metal ion plays a dual role, acting as both a template for entwining the precursors and catalyzing covalent capture of the threaded reactants. It proceeds under kinetic control, generally requires only a catalytic quantity of the template, and is traceless. The active template strategy has been successfully applied to numerous metal-catalyzed reactions (CuAAC 'click' reaction [*J. Am. Chem. Soc.* **128**, 2186 (2006); *J. Am. Chem. Soc.* **129**, 11950 (2007)], Glaser and Ullmann couplings [*Angew. Chem. Int. Ed.* **46**, 5709 (2007); *Angew. Chem. Int. Ed.* **47**, 4392 (2008)], oxidative Heck [*J. Am. Chem. Soc.* **129**, 12092 (2007)], Pd-catalyzed Michael addition [*Angew. Chem. Int. Ed.* **47**, 3381 (2008)], Ni- and Cu-catalyzed coupling reactions and cycloadditions [*J. Am. Chem. Soc.* **132**, 315 (2010); *J. Am. Chem. Soc.* **132**, 5309 (2010); *J. Am. Chem. Soc.* **132**, 6243 (2010)], Goldberg reaction [*J. Am. Chem. Soc.* **137**, 7656 (2015)] and adopted by many groups around the world [see Goldup, S. M. et al, *The active template approach to interlocked molecules*, *Nat. Rev. Chem.* 61 (2017)].

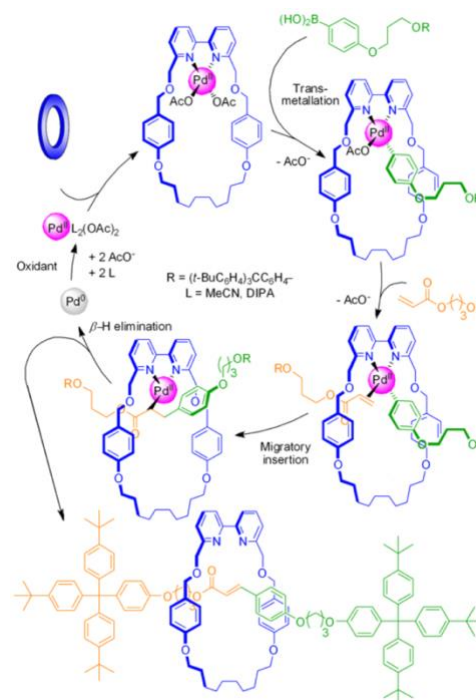


Fig. 12. Active template synthesis (2006).

In 2017 the Leigh group extended the active template concept to metal-free systems [*J. Am. Chem. Soc.* **139**, 8455 (2017); *J. Am. Chem. Soc.* **140**, 6049 (2018)], leading to some of the simplest routes to interlocked molecules developed to date. This catalysis-driven system also enabled the construction of the first artificially chemically fueled molecular pump [*Nature* **594**, 529 (2021); Fig. 13], a

dissipatively assembled polyrotaxane whose design illustrates the fundamental advances in non-equilibrium structure and dynamics made over the last two decades.

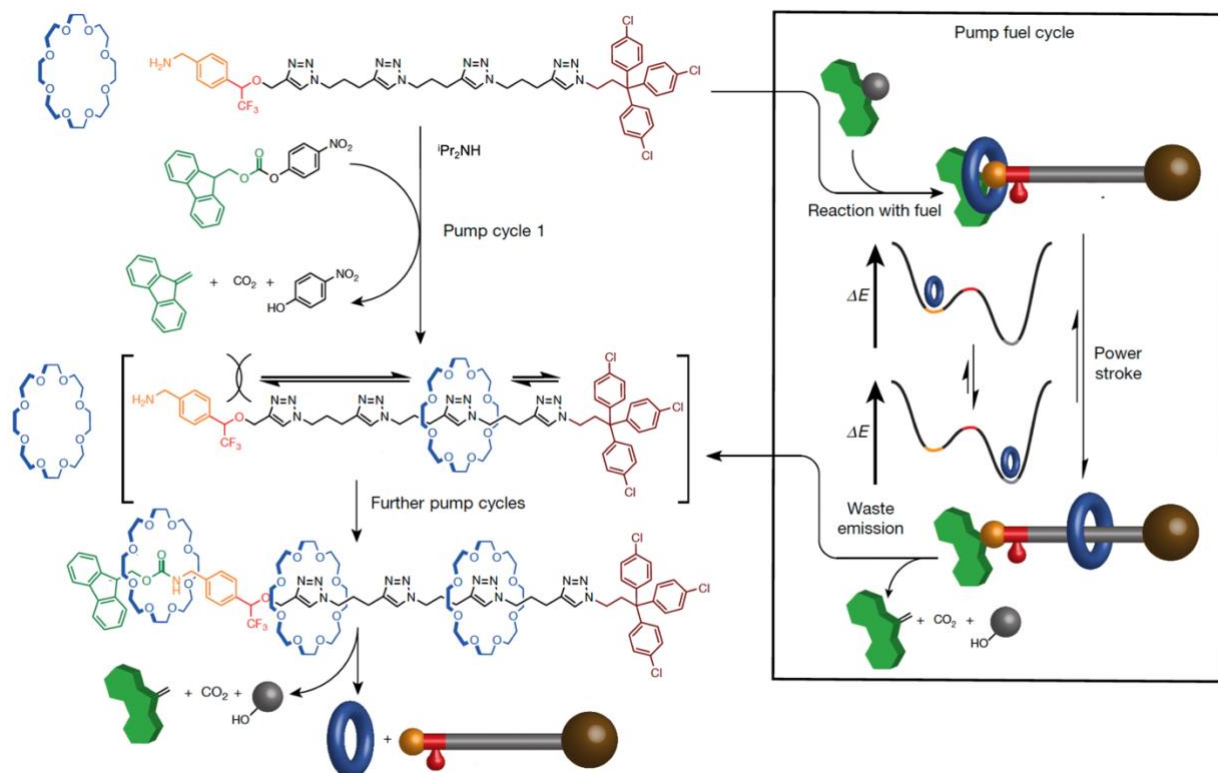


Fig. 13. Catalysis-driven molecular pump (2021).

The significance of non-equilibrium structure and dynamics in the molecular sciences

Ratchet mechanisms have transformed the understanding and design of molecular systems—biological, chemical and physical—in a move away from the macroscopic analogies that dominated thinking regarding molecular dynamics (e.g. pistons, springs, etc) in the 1990s and 2000s, to the properly scale-relevant concepts that underpin out-of-equilibrium research in the molecular sciences today. Ratcheting has established molecular nanotechnology as a research frontier for energy transduction and metabolism, and is enabling the reverse engineering of biomolecular machinery, delivering insights into how molecules ‘walk’ and track-based synthesizers operate, how the acceleration of chemical reactions enables energy to be transduced by catalysts (such as motor proteins, and increasingly by synthetic catalysts), and how dynamic (supra)molecular systems are driven away from equilibrium through catalysis. The recognition of ratchet mechanisms in biological systems, and their invention in synthetic systems, is proving significant in numerous areas, including supramolecular chemistry [Prins: *Angew. Chem. Int. Ed.* **60**, 12648 (2021)], systems chemistry [van Esch, Klajn, Otto: *Chem. Soc. Rev.* **46**, 5474 (2017)], dynamic covalent chemistry [Hartley: *Angew. Chem. Int. Ed.* **60**, 12648 (2021)], DNA nanotechnology [Turberfield: *Phys. Rev. Lett.* **101**, 238101 (2008)], polymer science [Hermans: *Chem. Soc. Rev.* **46**, 5476 (2017)], materials science [Boekhoven: *Chem* **6**, 552 (2020)], molecular biology [Weiss: *Mater. Horiz.* **4**, 310 (2017)], heterogeneous catalysis [Dauenhauer: *ACS Catal.* **9**, 6929 (2019)], endergonic synthesis [Knowles: *J. Am. Chem. Soc.* **141**, 1457 (2019)], and many other disciplines of nanoscience and nanotechnology.

Put simply, ratchet mechanisms give chemistry direction. Kinetic asymmetry, the underlying principle behind their operation, is the dynamic counterpart of structural asymmetry (i.e. chirality). Given the ubiquity of ratchet mechanisms in biology, and its significance for behaviour and function from systems to synthesis in all manner of different contexts, it is surely just as fundamentally important.