**The active template approach to interlocked molecules**

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**The active template approach to interlocked molecules takes advantage of the ability of metal ions to both organise precursor fragments for mechanical bond formation and to mediate the final covalent bond-forming reaction that captures the interlocked structure. Since its inception just a decade ago, this new methodology has expanded rapidly from a single reaction for rotaxane synthesis to a range of metal-mediated bond formations for the synthesis of complex interlocked molecules. Here we introduce the active template concept, its key advantages for the synthesis of interlocked molecules and outline recent advances that have been made using this technology. We will conclude with comments about future directions and challenges.**

Interlocked molecules, the archetypal examples of which are catenanes, and rotaxanes, , have progressed over the last half-century from a significant synthetic challenge to readily accessible chemical species[[1]](#endnote-2). The first synthetic breakthroughs in the field were provided by Wasserman, who demonstrated in 1960 that catenane formation could be achieved simply by taking advantage of the random threading of macrocycle precursors through pre-formed rings prior to cyclisation[[2]](#endnote-3), and soon after by Schill and Lüttringhaus, who showed in 1964 that the low yield of the Wasserman’s approach could be significantly improved by employing a covalent template[[3]](#endnote-4). Although successful, both of these approaches foundered on low synthetic efficiencies: Wasserman’s statistical approach employed simple building blocks but led to inherently low yields of the interlocked target; Schill and Lüttringhaus’ covalent template directed approach led to significantly higher yields in the mechanical bond forming step but required the laborious synthesis of complex precursors.

The synthetic “big bang” moment for the field came in 1983 when Sauvage and co-workers disclosed the first metal ion templated synthesis of a catenane[[4]](#endnote-5). Sauvage’s approach was revolutionary because it demonstrated for the first time that interlocked molecules could be obtained in an efficient manner from relatively simple building blocks by employing non-covalent interactions to orient the covalent components prior to mechanical bond formation. This “passive template” synthetic philosophy has driven research on interlocked molecules for over three decades, with a small number of privileged non-covalent templates that deliver the interlocked structure in high yield dominating the field, including Stoddart’s electron-deficient/electron-rich - template, Leigh’s hydrogen bonding tetralactam macrocycles, Beer’s anion-directed approach, and various systems that take advantage of hydrophobic binding1. This has allowed the study of the mechanical bond for a range of applications, perhaps most famously as components of molecular machines for which Sauvage and Stoddart received the 2016 Nobel Prize for Chemistry along with Feringa[[5]](#endnote-6),[[6]](#endnote-7).

The metal ion-directed methods developed by Sauvage employed metal-ligand interactions as a passive “glue” to organise the covalent components prior to mechanical bond formation by “stoppering” of the axle component (in the case of rotaxane synthesis; **Figure 1a**) or “clipping” of the macrocycle (applicable in both rotaxane and catenane synthesis). Arguably however, this fails to take full advantage of the chemistry of metal ions, the dominant application of which in modern chemistry is as catalytically active species for the synthesis of complex molecules. In 2006, Leigh and co-workers introduced the active template (AT) concept[[7]](#endnote-8) in which the full potential of the templating moiety is realised; a metal ion bound in the cavity of a macrocycle mediates the formation of a new covalent bond between two suitably functionalised half axles *through* the cavity of the ring, leading to efficient mechanical bond formation (**Figure 1b**).

In this review, we will introduce the active template concept and chart its development from the original report to a powerful synthetic tool for the production of a range of complex interlocked molecules. Along the way, we will discuss the key differences and advantages of this novel methodology from those previously developed, the chemical requirements of a successful active template reaction, recent applications of the methodology and future directions for this relatively new synthetic approach.

**[H1] The active template CuAAC reaction**

The Cu-mediated alkyne-azide cycloaddition reaction[[8]](#endnote-9),[[9]](#endnote-10) has rapidly become a tool of choice in the synthesis of functional non-natural products due its broad substrate scope and typically high synthetic efficiency. The active template (AT-CuAAC) modification of this ubiquitous “click”[[10]](#endnote-11) reaction was the first example to be reported by Leigh and co-workers in 2006 and is the best-developed of current active template methodologies[[11]](#endnote-12). In the original report, a CuI ion bound to a pyridine ligand disposed endotopically in the cavity of the macrocycle mediated the formation of a triazole linkage. The reaction was proposed to proceed by coordinating the alkyne and azide moieties of the half-axle components to the Cu ion on opposite faces of the macrocycle, which activates them to the 1,3 dipolar cycloaddition reaction (**Figure 1c**). A respectable 57% isolated yield of rotaxane **4** was achieved when one equivalent of all components was used, the balance of the material being the non-interlocked axle and recovered macrocycle **3**. The yield based on macrocycle **3** could be raised to 94% when 5 equivalents of alkyne **1** and azide **2** were employed. Even when a sub-stoichiometric quantity of the CuI ion was added, yields as high as 82% were obtained demonstrating that the metal ion can turn over in an active template process.

Soon after[[12]](#endnote-13), Leigh and co-workers extended the AT-CuAAC reaction to a range of pyridine-containing macrocycles. The key finding of this study was that, although macrocycle rigidity, number of Lewis basic donor atoms and steric hindrance near the binding site all affected the yield of the interlocked product, as long as the endotopic ligand was reasonably unencumbered and mono- or bi-dentate the desired rotaxane was indeed formed. This suggested that the active template approach could be relatively tolerant to modification of the macrocycle component. The one variable that was not explored in this report was the effect of macrocycle size on the reaction outcome, an important factor in rotaxane synthesis as the diameter the macrocyclic cavity determines the size of the stoppers required to hold it on the axle. An educated first “guess” of the effect of macrocycle size in an active template reaction might be that reaction efficiency would fall as the space within the macrocycle available for the bond formation to take place is reduced. In 2011 Goldup and co-workers published the first study of macrocycle size in the AT-CuAAC reaction and reached the surprising conclusion that instead, smaller bipyridine macrocycles led to enhanced yields of [2]rotaxane products; the smallest macrocycle investigated produced [2]rotaxanes with up to 100% selectivity[[13]](#endnote-14). This finding allowed the synthesis of a selection of functionalised rotaxanes in excellent yield based on a range of simple, small stopper units (**Figure 1d**). Subsequently, the same authors demonstrated that by modifying the reaction conditions essentially quantitative yields could be obtained with all macrocycle sizes, increasing the generality of the AT-CuAAC reaction still further[[14]](#endnote-15).

The simplicity of the AT-CuAAC, its generality with respect to substrates and macrocycle size, and the ready availability of the reaction components has led to this being the most studied and applied of all active template procedures published to date.

**[H1] Passive vs. Active Template Approaches**

Although there are superficial similarities between metal ion-based passive template syntheses and the AT-CuAAC reaction (both require the metal ion to bind in the cavity of the macrocycle and coordinate the axle precursors), the passive and active template approaches differ fundamentally in the underlying driving force for mechanical bond formation. The passive template approach relies on the formation of a thermodynamically stable, suitably pre-organised complex between the covalent components (**Figure 1a**, intermediate **III**). The interlocked architecture is then captured by a final covalent bond forming reaction (“stoppering) that is, to a first approximation, independent of the threading state of the molecule[[15]](#endnote-16). Thus, in the passive template process shown in **Figure 1a**, the yield of the product can be superficially linked to the position of the equilibrium between threaded complex **III** and non-productive species **I** and the yield optimised by designing conditions that bias the equilibrium to favour the former.

In contrast, the active template approach (**Figure 1b**) relies on the metal ion that mediates the final covalent bond formation being sequestered within the macrocycle, leading to bond formation taking place through the cavity of the macrocycle faster than it takes place in bulk solution. In this analysis, the active template phenomenon is fundamentally kinetically driven with the selectivity for the threaded product depending on the same factors that determine selectivity in catalytic processes; the position of the pre-equilibrium between the starting materials and activated complexes (e.g. **VIII** and **X**), the relative rates with which these complexes progress towards products, and any background reactions that compete with the desired bond formation (e.g. the direct reaction of substrates **VI** and **VII** without the involvement of the metal ion or off-ligand reactions mediated by metal ions not ligated by a macrocycle). In the case of the process shown in **Figure 1b** the yield of rotaxane will depend on the relative values of *K***VIII**, *k*AT, *K***X** and *k*axle all of which are expected to vary with the conditions of the reaction.

The distinction between the thermodynamic nature of the passive template phenomenon and the kinetic nature of the active template approach may seem somewhat philosophical but it has direct practical consequences, including the different considerations required when optimising passive and active template syntheses (**Box 2**).

**[H3] Role of Mechanism in the Active Template Approach.** Based on the above discussion it should be clear that the detailed mechanism of the bond formation employed in an active template reaction is extremely important as it determines the availability and relative rate of pathways that lead to mechanical bond formation and competing pathways that lead to non-interlocked products. These different pathways can be superficially grouped as those that involve bond formation from opposite faces of the macrocycle (productive intermediate **VI**, **Figure 1b**), bond formation from the same face of the macrocycle (unproductive intermediate **VIII**) and background reactions not involving the macrocycle which necessarily lead to non-interlocked axle. In the case of the original AT-CuAAC reaction, the latter was determined to be the origin of the incomplete conversion of macrocycle **3**12; kinetic studies demonstrated that “free” CuI is both catalytically competent and indeed mediates the CuAAC reaction that leads to non-interlocked products faster than the corresponding AT-CuAAC reaction leading to rotaxane **4**. Thus, even when the quantity of unbound CuI is extremely low, the CuAAC pathway is competitive with the AT-CuAAC pathway. Having identified this trend, Leigh and co-workers were able to optimise the reaction further by employing 10 equivalents of macrocycle **3** to significantly reduce the concentration of unbound CuI. Under these conditions the yield of rotaxane **4** based on half-axle components **1** and **2** rose to >95%.

The situation becomes more complicated when more than one viable active template pathway is present leading to different interlocked structures as Leigh and co-workers observed in the case of rigid pyridine macrocycle **9**. When **9** was employed in excess over CuI in the AT-CuAAC reaction of alkyne **1** and azide **3**, [3]rotaxane **10** was also isolated in addition to the expected [2]rotaxane (**Figure 2a**). This provides strong evidence for a di-Cu pathway in the triazole forming step12. Later work by Goldup and co-workers with bipyridine macrocycles **11** of varying size and substituents allowed a detailed model of the key intermediate in the bipyridine-mediated AT-CuAAC reaction to be derived (**Figure 2b**)14. The results suggest that, at least in the case of the smallest macrocycles, a mono-metallic pathway via intermediate **I** is favoured leading to [2]rotaxanes. However, when larger macrocycles bearing a benzylic ether linkage were employed at high temperature, a bimetallic pathway via intermediate **II** becomes important. By optimising the conditions of the reaction, the yield of the [3]rotaxane can be raised to ~50% based on the macrocyclic component or the [2]rotaxane can be formed in >95% yield in all cases.

These examples demonstrate that the active template approach can be used as a relatively unusual mechanistic probe in which the mechanical state of the product provides insight into the composition of key intermediates en route to the mechanical bond formation. Conversely this insight can be used to allow the synthesis of new interlocked products. Goldup and co-workers used the detailed mechanistic model to design a kinetic self-sorting approach to hetero[3]rotaxanes in which the order of macrocycles on the axle is determined by the mechanism of the reaction[[16]](#endnote-17).

**[H3] Turnover of reaction components.** One consequence of the kinetic nature of AT methodologies is that there is no requirement that the axle and macrocycle combine to form a stable complex with the metal ion in either the interlocked or non-interlocked arrangements. This is in contrast to passive template syntheses in which both components bind the metal ion to form a stable complex both before and, importantly, after the final covalent bond forming reaction. As a consequence, regardless of the inherent selectivity for the interlocked product, an active template reaction can in theory be driven to complete conversion with respect to the macrocycle (typically the more synthetically expensive component) by addition of excess half-axle components; any macrocycle complex resulting in “failed” bond formations will not necessarily remain associated with the products and can thus be recycled back into the reaction and ultimately go on to produce the interlocked product. Similarly, it is theoretically possible to employ substoichiometric quantities of the metal ion in an active template reaction as it can be recycled from interlocked product to unreacted macrocycle. Both of these key synthetic advantages were demonstrated in the Leigh group’s first report of the AT-CuAAC reaction11 (**Figure 1c**) and have been exploited in many subsequent reports of active template processes.

**[H3] Access to unstable interlocked molecules.** Whereas the initial product of a successful passive template synthesis is typically more thermodynamically stable than the non-interlocked components, the product in an active template reaction need not be and can even be significantly *less* thermodynamically stable than its non-interlocked components. All that is required is that the active template pathway proceed with an appreciable rate. The ability to form [2]rotaxanes in high yield using the bipyridine macrocycles in the AT-CuAAC reaction provides a striking example of this. Although the products of reactions with the smallest macrocycle are extremely sterically hindered (e.g. Fig 1d13) and expected to be unstable with respect to de-threading, they are still formed in excellent yield. This is in part due to the favourable free energy of triazole formation, which compensates for the build-up of repulsive steric interactions, and in part because the steric interactions in the rotaxane are not fully developed in the transition state leading to the interlocked product.[[17]](#endnote-18) In contrast, when macrocycles of a similar size were employed in Sauvage’s passive template approach the yield of the interlocked product fell dramatically[[18]](#endnote-19), presumably because steric interactions disfavour formation of the threaded complex.

**[H1] Generality of the AT Concept**

As discussed above, the active template approach requires the final covalent bond formation to take place through the cavity of the macrocycle with the functionalised half-axle components disposed on opposite faces of the ring. If these requirements are met, almost any bond formation can, with some caveats, be harnessed to generate a new active template reaction. This principle has been amply demonstrated over the last decade: since the AT-CuAAC reaction11 was first disclosed, active template couplings based on bond formations mediated by Cu[[19]](#endnote-20),[[20]](#endnote-21),[[21]](#endnote-22),[[22]](#endnote-23),[[23]](#endnote-24),[[24]](#endnote-25),[[25]](#endnote-26), Pd[[26]](#endnote-27),[[27]](#endnote-28),[[28]](#endnote-29), Ni[[29]](#endnote-30),[[30]](#endnote-31),[[31]](#endnote-32) and Zn[[32]](#endnote-33) ions have been developed with a large variety of functional groups produced in the coupling step (**Table 1**). The relatively rapid development of a range of active template couplings might suggest that such developments are “easy” to achieve and that simply substituting a similar macrocyclic ligand in an established metal-mediated bond formation will result in a successful active template coupling. This view is unfortunately naïve; as suggested above, the detailed mechanism of the reaction is extremely important.

Firstly, it is a necessary pre-requisite that at least one of the axle components, the metal ion and the macrocycle are in some way associated during the step that leads to mechanical bond formation. Secondly, it is typically desirable that the final covalent bond forming reaction is irreversible as, all other factors being equal, the non-interlocked product is entropically favoured. Thirdly, the intermediate leading to the proposed mechanical bond formation must be stereoelectronically biased to lead to reaction through the macrocycle cavity. Not all reactions meet these three basic criteria (e.g. **Box 3**) and thus it is wise to consider the detailed mechanism of a proposed new active template process.

The points above notwithstanding, the potential to design active template reactions based on existing catalytic processes is an extremely powerful and attractive. Indeed, it is impressive that active template couplings leading to 10 different axle functional groups have been disclosed in the last decade alone. This increases the synthetic diversity of interlocked molecules available as the only restriction on the axle moiety is that it contains the functional group produced in the coupling step (c.f. the passive template approach in which both axle *and* macrocycle must contain the components required for efficient templation).

Looking to the future, given the diversity of reactions presented, it is striking that almost all active template reactions rely on aromatic-N ligands. This may in part be due to the relative ease with which endotopic aromatic-N ligands can be designed, in contrast, for instance, to the tetrahedral P ligands commonly used in catalysis. Furthermore, it is noteworthy that to date no PdII/0 cross coupling active template reactions have been disclosed; the Pd-mediated sp-sp and oxidative Heck active template couplings published rely on PdII to gather the reaction components with oxidation of the Pd0 by-product taking place off-cycle. It seems likely that diversification of the ligand motifs in the active template approach will lead to further developments in the types of bond formations that can be employed.

**[H1] Applications of the AT Approach**

**[H3] Synthesis of complex targets.** Although many of the early studies focussed on rotaxane synthesis, the active template concept is clearly applicable to other interlocked and entangled targets; embedding a metal ion in any looped portion of a molecule can permit the formation of a molecular crossing point and thus a mechanical bond. Active template catenane formation was reported simultaneously in 2009 by the groups of Saito[[33]](#endnote-34) and Leigh using AT-Glaser and AT-CuAAC reactions respectively(**Figure 3a**)[[34]](#endnote-35),[[35]](#endnote-36). In both cases the yields reported are relatively low compared with the corresponding rotaxane forming reaction (50-64%) even with five equivalents of the pre-macrocycle component and relatively extended reaction times (~5 days) due to the need to work under high-dilution conditions. These results highlight the dual challenge in catenane synthesis; the need to form a new macrocycle while also templating mechanical bond formation. Thus, although catenane synthesis is possible using the active template approach, it is yet to be turned into a genuinely useful and general tool.

In 2011, the AT-CuAAC reaction was extended to knotted structures by Leigh and co-workers in an approach that combines passive and active templating methodologies. Looped intermediate **14** was formed by coordination of the bipyridine moieties to CuI and the second CuI ion bound to the pyridine unit mediated the AT-CuAAC reaction to capture knotted structure **15**[[36]](#endnote-37) (**Figure 3b**). In addition to the novelty of the approach, it is worth noting that trefoil knot **15** (**Figure 3c**) was the smallest reported to date. That such a small knot can be produced in reasonable yield is once again thanks to the fact that the intermediate leading to the knot need not be thermodynamically favoured in the equilibrium, simply kinetically viable in the AT-CuAAC bond formation.

An extension of the observation that the metal ion can turn over in the active template approach is that the same metal ion-ligand motif can mediate more than one covalent bond formation to generate more than one mechanical bond. This was first demonstrated in the formation of “handcuff” [3]rotaxanes in which the ligand employed in active template CuAAC or Cadiot-Chodkiewicz couplings bridged the two macrocyclic units[[37]](#endnote-38). More recently, this result has been extended to the synthesis of true “molecular sheaf” systems in which multiple axles are threaded through a single macrocycle37,[[38]](#endnote-39) (**Figure 3c**). The first successful approach reported by Leigh and co-workers made use of an active template Ni-mediated sp3-sp3 homo-coupling to thread up to three axles through a terpyridine macrocycle to give [4]rotaxane **16**. Similarly, Anderson and co-workers achieved the synthesis of doubly threaded [3]rotaxane **17** using an AT-Glaser coupling. Rotaxanes **16** and **17** are notable because the threading of multiple axles through a single macrocycle is a longstanding challenge that has been hard to address using passive template methodologies; passive template syntheses require that a stable complex is formed with all axles present, necessitating extremely large macrocycles and thus extremely large stoppering units[[39]](#endnote-40). The active template approach to some extent alleviates this problem and thus makes access to such multiply threaded species possible. In contrast, when Saito and co-workers took a mixed active/passive template approach to the synthesis of doubly threaded [3]rotaxane **18**[[40]](#endnote-41)**,**[[41]](#endnote-42) (**Figure 3d**) and related rotacatenanes[[42]](#endnote-43), the use of a passive template step necessitated the use of larger macrocycles and thus extremely large stoppering units.

The ability to synthesise small, crowded rotaxanes using the active template approach was exploited by Goldup and co-workers in the synthesis of mechanically planar chiral rotaxanes using a chiral auxiliary approach[[43]](#endnote-44). Their synthesis relies on the crowded nature of diastereoisomers (d-*R*mp)-**19** and (d-*S*mp)-**19** (**Figure 3e**) in which the elements of mechanical and covalent chirality are forced into close proximity and so interact strongly. Although mechanically epimeric rotaxanes have previously been reported[[44]](#endnote-45) this was the first example of their separation using standard chromatographic techniques. Once separated, the diastereoisomers were converted to the corresponding enantiomers by a grafting reaction[[45]](#endnote-46) that replaces the chiral sugar-derived stopper with an achiral amine nucleophile.

 One of the most complex molecules synthesised so far using the AT-CuAAC reaction is a rotaxane pro-drug **20** disclosed by Papot and co-workers (**Figure 3e**)[[46]](#endnote-47). Rotaxane **20** contains a hydrophilic sugar-derived stopper group (green), a sugar-derived enzyme-cleavable group (orange), a self-imolative linker (grey) and a stopper derived from the complex anti-cancer natural product paclitaxel (purple). Rotaxane **20** was synthesised in a respectable 27% yield for such a complex molecule after preparative HPLC. The mechanical diastereoisomers (see above) of **20** were not separated. The authors demonstrated that the mechanical bond of **20** prevents enzymatic cleavage of the ester bond between the paclitaxel unit and the stopper, which in turn suppresses the antimitotic activity of the drug. However, in the presence of -galactosidase, the enzyme cleavable group (orange) is removed which results in cleavage of the self-imolative linker (grey) and ultimately cleavage of the macrocycle (blue) and disassembly of the rotaxane. The axle, freed from the rotaxane, is subsequently saponified to produce free, biologically active paclitaxel. Thus, rotaxane **20** is a -galactosidase-dependent pro-drug form of paclitaxel that relies on the mechanical bond for its function.

The examples presented demonstrate the potential of the active template approach in the synthesis of complex interlocked structures including systems with multiple mechanical bonds, complex mechanical stereochemistry and dense, reactive functionality. This in turn opens up new structures for study and will hopefully lead to new applications of interlocked molecules in various areas.

**[H3] The Active Template Approach in the Synthesis of Interlocked Materials.** Materials based on interlocked molecules have been the source of much attention for the past three decades. In recent years, the active template approach has been bought to bear on the synthesis of molecules with potential materials applications. In 2012, the groups of Anderson[[47]](#endnote-48) and Gladysz[[48]](#endnote-49),[[49]](#endnote-50),[[50]](#endnote-51) independently reported the synthesis of conjugated oligoyne rotaxanes using the AT-Glaser reaction. This approach has since been extended by Anderson and co-workers to active template Cadiot-Chodkiewiz sp-sp heterocouplings and increasingly complicated examples including conjugated porphyrin nano-rings (**21**)[[51]](#endnote-52), longer polynes up with up to 12 conjugated triple bonds[[52]](#endnote-53) (**22**), doubly threaded oligoyne rotaxanes52 (**17**), and [9]cumulenes (**23**) by isomerisation of the oligoyne product of the Glaser coupling[[53]](#endnote-54) (**Figure 4a**). Studies of the properties of these insulated conjugated molecules revealed that encapsulation by the macrocycles stabilised the conjugated structures; dodecayne rotaxane **22** was stable to temperatures >220 °C52, [9]cumulene rotaxane **23** was sufficiently stable to allow the detailed study of its electronic properties53, and a modified hexa-yne rotaxane was stable enough to be used in single molecule conductance experiments[[54]](#endnote-55). Encapsulation was also shown to lead to efficient energy transfer between the threaded components**Error! Bookmark not defined.**,[[55]](#endnote-56),[[56]](#endnote-57).

Poly[n]rotaxanes have previously been synthesised by threading of macrocycles onto pre-formed polymers or polymerisation of threaded monomers. However, the precision synthesis of oligomeric rotaxanes, including those with distinguishable and precisely placed macrocycles remains an outstanding challenge. In 2016 Goldup and co-workers demonstrated an iterative AT-CuAAC approach to rotaxane oligomers (**Figure 4b**) that takes advantage of the use of small macrocycles, removing the need for bulky groups between the reactions sites. By employing a monomer unit containing an azide and a protected alkyne function, [7]rotaxane **24** containing six identical macrocycles was produced with >90% synthetic efficiency over each round of coupling. Furthermore, varying the macrocycle in each AT-CuAAC coupling step allowed the synthesis of [4]rotaxane **25** containing three distinct macrocycles precisely placed on the axle in 75% yield over 9 chemical steps. Aside from demonstrating the power of the active template approach for the precision engineering of interlocked structures, this study suggests that formation of the mechanical bond alters the conformation of the axle significantly by favouring an extended all *syn* arrangement that minimises steric repulsion between the macrocycles. More recently, by exploiting the crowded nature of these small rotaxanes Goldup and co-workers extended this approach to the stepwise protecting group free synthesis of [3] and [4]rotaxanes[[57]](#endnote-58).

An alternative approach to extended interlocked materials is the use of metalo-supramolecular chemistry to assemble suitably functionalised building blocks using metal ligand interactions[[58]](#endnote-59). Crowley and co-workers demonstrated this in the context of rotaxanes synthesised using the AT-CuAAC reaction by post-synthetically modifying a rotaxane to include a terpyridine unit in both the macrocycle and axle. Addition of an FeII salt to **26** led to the assembly of oligomeric species of between 11 and 13 monomer units[[59]](#endnote-60).

The application of active template reactions in the synthesis of interlocked materials is still in its infancy but important lead results have already been reported. Hopefully these will inspire further developments that exploit the synthetic flexibility of the active template approach.

**[H3] Interlocked Molecules as Catalysts and Hosts.** Although a great many interlocked ligands have been synthesised since Sauvage’s first passive template synthesis, the application of these systems as ligands to support catalytically competent metal ions has not been reported. This is perhaps unsurprising because interlocked ligands synthesised using the passive template approach typically lead to coordinatively saturated complexes that are poorly suited to catalysis. In 2015, the groups of Goldup and Leigh independently reported the first examples of rotaxane ligands in catalysts synthesised using active template methodologies. Leigh and co-workers reported an active template Goldberg reaction to synthesise rotaxane **27** that contains a chiral macrocycle with an endotopic metal-binding site[[60]](#endnote-61). The Michael addition reaction between diethyl malonate and nitrostyrene proceeded with higher enantioselectivity in the presence of the Ni complex of chiral rotaxane **27** than that of chiral acyclic ligand **28 (Figure 5a)**. However, this study also highlighted one of the key challenges in the development of rotaxane ligands for catalysis; the reaction with rotaxane **27**, although more selective was over an order of magnitude slower than acyclic ligand **28** (27 days vs 2 days). Thus, although complexes of **27** are coordinatively unsaturated and the crowded nature of the cavity formed by the mechanical bond bears attractive similarities with an enzyme active site (flexible, well-defined 3D space), it appears this same crowding can lead to low catalytic activity.

Goldup and co-workers took a different approach to the development of rotaxane catalysts by placing the catalytic function near to but not directly within the macrocycle[[61]](#endnote-62) (Figure 5b). Rotaxane gold complex [Au(**29**)Cl] was synthesised in excellent yield using the AT-CuAAC reaction and its activity in an Au-mediated cyclopropanation reaction studied. Surprisingly, precatalyst [Au(**29**)Cl] was found to be inactive but non-interlocked axle complex [Au(**30**)Cl] was catalytically competent. Subsequent studies revealed this to be due to inhibition by the Lewis basic N-donors in the macrocycle. This inhibition could be reversed by competitive binding of catalytically innocent cations into the cavity of the macrocycle which resulted in a catalyst species that was both highly active *and* more diastereoselecitve than non-interlocked complex. The increased selectivity was attributed to the sterically hindered environment provided by the threaded macrocycle. Importantly, the nature of the cationic guest controlled the degree of diastereoselectivity, presumably by altering the steric environment around the metal centre, demonstrating that [Au(**29**)Cl] produces an allosterically regulated catalyst in which the guest controls both activity and selectivity.

Leigh and Goldup’s interlocked catalysts use the mechanical bond to control the action of a catalyst on exogenous substrates. There is also significant interest in interlocked systems which control functionalisation of the axle component, for instance as a potential method for post synthetic modification of polymers[[62]](#endnote-63). Saito and co-workers demonstrated that a CuI ion bound within the cavity of a phenanthroline macrocycle was able to mediate a double hydro-amination reaction of the threaded diyne functionality generated in an AT-Glaser reaction to produce a pyrrole moiety (**Figure 5c**)[[63]](#endnote-64). The conversion of diyne rotaxane **31** to pyrrole rotaxane **32** was found to be significantly more efficient than the corresponding reaction of the non-interlocked macrocycle and axle suggesting that the mechanical bond plays a role in the transformation. Key to the success of this approach is that the axle does not contain ligating functionality that would inhibit the activity of the metal ion, an important benefit of the active template approach.

 Beer and co-workers have pioneered the use of active template couplings in the synthesis of hosts for anions by developing a modification of the AT-CuAAC reaction in which the terminal acetylene coupling partner is replaced with an iodoacetylene, leading to formation of an iodotriazole product25. The first examples reported employed bis-iodotriazole macrocycles that coordinate to CuI through the triazole ligand. These complexes then mediate active template formation of the iodotriazole to produce the interlocked product in reasonable yield (18-41%). The triazole donors were subsequently coordinated to ReI to orient the C-I bonds into the cavity of the rotaxane **33** and generate a cavity containing three polarised C-I bonds that readily participated in halogen bonding interactions with a range of anions (**Figure 6a**). Rotaxane **33** selectively binds halide anions over harder oxy-anions, in line with other halogen bonding hosts[[64]](#endnote-65). Later work extended this approach to systems with up to four polarised C-I bonds and these hosts were found to bind anions even in the presence of water24. In competitive solvents, the relative order of binding was reversed with iodide binding more strongly than chloride.

Recently, Beer and co-workers extended this approach to chalcogenido-triazole macrocycles **34b** and **34c**, which bind Cu in a bidentate manner through the Se and Te atoms respectively (**Figure 6b**) 23. These macrocycles were then employed in the AT-CuAAC reaction to produce rotaxanes **34b** and **34c** and their ability to bind anions through chalcogen bonding interactions was investigated. Rotaxane **34c** was found to bind anions more strongly than the corresponding protonated system **34a** through a X•••Te chalcogen bond whereas the less polarised C-Se bond of **34b** was found to be ineffective. Not only does this work introduce a new interaction to the field of rotaxane anion hosts, it is also the first and only example of a non-N ligand based macrocycle for use in an active template process.

Tucker and McClenaghan applied the active template approach to develop a host for small molecules. They synthesised rotaxanes based on the Hamilton receptor for barbital using the AT-Glaser (**36**) and AT-CuAAC (**37**) reactions (**Figure 6c**)[[65]](#endnote-66). While diyne rotaxane **36** bound barbital (**35**), albeit with a reduced binding constant compared with the non-interlocked macrocycle, triazole rotaxane **37** did not bind the guest at all. The reduced binding of **36** for barbital compared with the macrocyclic host was attributed to the steric hindrance provided by the mechanical bond. However, the failure of **37** to bind is harder to rationalise using steric arguments alone and is likely to be due to competing interactions between the Lewis basic triazole moiety and the hydrogen bond donor sites in the macrocycle.

In 2014 Goldup and co-workers serendipitously observed stabilisation of a reactive organometallic species during in the AT-CuAAC synthesis of a simple rotaxane[[66]](#endnote-67); when base was added to accelerate the reaction between azide **38**, alkyne **39** and macrocycle **40** the isolated product was triazolide **41** *even after* aqueous work up with EDTA and column chromatography. Triazolide **41** could even be produced in high yield under aqueous conditions (**Figure 6d**)! The dramatic stabilisation of the CuI-C bond in **41** is attributed to the steric hindrance provided by the crowded mechanical bond between the short axle and small macrocycle. Indeed, when a larger macrocycle or more a flexible axle were employed the corresponding triazolide was not isolated. In addition to providing mechanistic evidence for a CuI triazolide intermediate in the AT-CuAAC reaction (**41** appears to be a “trapped” intermediate of the reaction rather than the product of CuI insertion as readdition of CuI to the corresponding rotaxane fails to lead to formation of **41**), the unexpected stability of **41** suggests that sterically hindered rotaxanes accessible using active template approaches may allow the design of kinetically stabilised ligands for various applications (*cf* Sauvage’s “catenand” effect[[67]](#endnote-68)).

The above examples demonstrate that, in addition to high synthetic efficiency, the active template approach offers opportunities to generate interlocked molecules with diverse functionality and function. The reduced synthetic restrictions on structure compared with passive template approaches will hopefully lead to new applications of mechanically bonded structures and the production of optimised interlocked catalysts, hosts, ligands and sensors.

**[H3] Molecular Machines.** Soon after the development of the AT-CuACC reaction Leigh and co-workers disclosed a degenerate molecular shuttle in which two triazole ligands in the axle act as stations for a metal ion bound in the cavity of the macrocycle11b. This first report set the scene for much of the later work on bistable stimuli responsive molecular shuttles in which the functional group formed in an active template reaction typically acts as one of the stations31,[[68]](#endnote-69),[[69]](#endnote-70),[[70]](#endnote-71). One of the most recently disclosed of these, but perhaps most obvious given the history of the field, is shuttle **42** which is a triazole-pyridine analogue of Sauvage’s seminal phenanthroline-terpyridine shuttle[[71]](#endnote-72); the bidentate pyridine-triazole station is formed in the final AT-CuAAC coupling that forms the mechanical bond (**Figure 7a**). When bound to CuI the macrocycle predominantly occupies the bidentate station but is translocated to the tridentate station when CuI is replaced by ZnII.

Historically, molecular shuttles have typically taken advantage of the templating motif used in the passive template synthesis of the interlocked molecule as one of the macrocycle binding sites. Although this alignment between the method of synthesis and the function of the target is elegant it comes with a disadvantage; typically, the templating interaction is extremely favourable and so the activation barrier to shuttling is often relatively high. One of the principle advantages of the active template approach to shuttles is to allow the synthesis of molecular shuttles with significantly reduced intermolecular interactions and thus lower barriers to molecular motion while still maintaining good positional integrity; a preference of just 7.3 kJmol-1 is sufficient for >95% of the molecules to exist in the preferred co-conformation. Leigh and co-workers disclosed the first example of such a shuttle by employing single H-bond contacts as the primary non-covalent interaction between the macrocycle and station (**Figure 7b**)20,[[72]](#endnote-73). Shuttle **43** was synthesised using an AT-Cadiot Chodkiewicz reaction that produces a diyne functional group in the axle that does not have significant attractive interactions with the macrocycle. The stations in the shuttle are the amide unit and the dimethylaminopyridine (DMAP) unit. In the neutral state the macrocycle predominantly occupies the amide station due to the hydrogen bond between the amide N-H and the bipyridine macrocycle. Protonation of the DMAP station results in a new preferred co-conformation in which the macrocycle is hydrogen bonded to pyridinium unit. Calculations suggest that the preferred co-conformations in the neutral and protonated states are favoured by 16.3 kcalmol-1 and 3.8 kcalmol-1 respectively suggesting reasonable co-conformational integrity in both states.

The most complex molecular machine synthesised using the active template approach to date is undoubtedly Leigh and co-workers’ “peptide synthesiser” rotaxane **47** (**Figure 7c**)[[73]](#endnote-74),[[74]](#endnote-75), in which a catalyst bound to macrocycle sequentially transforms functional groups in the axle component to produce a sequence-specific peptide product. The role of the AT-CuAAC in the synthesis is to allow the assembly of the interlocked structure in high yield from macrocycle **44**, alkyne **45** and azide **46**, loading the macrocyclic on to the axle information strand, without introducing significant inter-component interactions that would hinder or slow the operation of the machine. The catalyst unit was then attached to the macrocycle by hydrazone formation. The application of the active template approach in the synthesis of such a complex structure demonstrates the synthetic power of this methodology.

**[H1] Conclusions and future challenges**

To conclude, the active template approach has come a long way in the decade since the principle was established by Leigh and co-workers; 10 new active template bond forming reactions have been developed; the approach has been extended to a range of other structures including catenanes and knots; and active template reactions have been applied to the synthesis of functional architectures and interlocked materials. For comparison, it took a decade from Sauvage’s first report of a catenane for his Cu-phenanthroline template to be extended to a rotaxane[[75]](#endnote-76)! While the high rate of progress in the active template approach surely reflects the larger and more developed community of modern synthetic supramolecular chemistry, not to mention the improved analytical techniques available to characterise the products, it is still an impressive start for a new field.

However, this progress has been largely driven by a relatively small number of groups, and the majority of publications concerning the active template approach focus on the development of new AT methodologies, rather than their application to new targets and functions. That such a useful methodology has not been applied more widely may simply reflect the conservative nature of synthetic chemistry but may also relate to the availability of the key starting material, the macrocycle. Indeed, in many cases macrocycles required for the active template approach, particularly small bipyridine macrocycles, were typically only available in relatively low yield (10% or less in the macrocyclisation step). Recent efforts have improved this significantly however and this will hopefully open the field up to new entrants70.

Future directions will surely include the application of the approach to new and challenging targets and ever more complex structures, including those with applications in catalysis, sensing and materials. The extension of the AT approach to non-N ligands, as demonstrated by Beer and co-workers is surely overdue, in particular, ligands capable of supporting cross coupling reactions such as Pd and Ni-mediated processes, given the power and ubiquity of these reactions in synthetic chemistry more widely. Another opportunity that has so far remained overlooked is the possibility of developing organocatalytic active template reactions by embedding suitable organocatalytic functionality within the cavity of a macrocycle. The extension of the approach to a general and high-yielding catenane forming reaction is also yet to be achieved. Finally, a clear understanding of the role of macrocycle size in reactions other than the bipyridine-mediated AT-CuAAC reaction is also desirable**Error! Bookmark not defined.**.

To achieve this, one aim of this review is to highlight the potential for researchers in the field of catalysis to contribute the development of new methods for the synthesis of interlocked molecules; the detailed understanding they would bring to the area would surely significantly accelerate progress. Conversely, the application of active template reactions to the analysis of reactions mechanisms could contribute significantly to the field of catalysis.

Finally, the greatest challenge in organic synthesis is to be able to make any molecule efficiently, regardless of the structure. Arguably, the active template approach takes us one step closer to this lofty aim in the context of mechanically bonded molecules by allowing, in principle, almost any axle to be included in the cavity of a macrocycle. Although this has in the past been termed a “traceless” synthesis, because it is not obvious where the bond formation has taken place or how it was templated, the macrocycle still bears the ligand unit used to direct the reaction and this still serves to reduce the structural diversity of the available products. Perhaps in future, a truly traceless active template reaction in which no component of the interlocked structure retains the tell-tale signature of the synthesis will become available and the ultimate aim of being able to achieve any target with little or no limitations on the covalent structure of the components will be within our grasp.

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**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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**Figure captions**

**Figure 1**: **Passive and active template synthesis of rotaxanes. a**, Schematic representation of the metal ion-directed passive template approach to rotaxanes by stoppering showing the key steps that determine selectivity. **b**, Schematic representation of the active template approach to rotaxanes showing the key steps that determine selectivity. **c**, Leigh’s original active template CuAAC reaction. Alkyne **1** and azide **2** each bearing bulky stopper units are reacted in the presence of a macrocycle **3** and a Cu (I) complex. The copper ion is coordinated by the pyridine nitrogen in the macrocycle and mediates formation of the triazole through the cavity. **d**, Examples of functionalised rotaxanes (**5** - **8**)synthesised using the AT-CuAAC reaction with small macrocycles.

**Figure 2**: **The formation of [3]rotaxanes in the AT-CuAAC reaction**. **a**, Formation [3]rotaxane **10** in the AT-CuAAC reaction with pyridine macrocycle **9**. **b**, A detailed mechanistic model for the formation of [3]rotaxanes in the bipyridine-mediated AT-CuAAC reaction in which the pathway followed depends on ring size, temperature, Cu loading and solvent. A monometallic pathway via intermediate **I** in which a single metal ion is coordinated by macrocycle, alkyne and azide favours formation of the [2]rotaxane. The reaction conditions and substrates can be tuned to favour a bimetallic pathway — leading to the [3]rotaxane — via intermediate **II** where one copper ion is coordinated by macrocyle and -coordinated to the acetylide while a second is coordinated by a second macrocycle, azide and -coordinated to the acetylide. R = C(4-*t*Bu-C6H4)3

**Table 1**: **Active template reactions described to date** Ligand structures are shown in the table headed with donor atoms shown in red. Red and green balls indicated sterically bulky stopper units.

**Figure 3**: Complex interlocked molecules synthesised using the active template approach: **a**, Catenanes **12** and **13** produced by AT-Glaser and AT-CuAAC couplings respectively in moderate yield. **b**, Molecular knot **15** synthesised by a mixed active/passive template approach via intermediate **14**. **c**, Multiply-threaded [4]rotaxane **16** (R1 = 4-*t*Bu-C6H4) and [3]rotaxane **17** (R2 = 3,5-di-*t*Bu-C6H3) synthesis by AT-sp3-sp3 homocoupling of alky bromides and AT-Glaser coupling respectively. **d**, Doubly threaded [3]rotaxane **18** synthesised by AT-Glaser coupling and subsequent passive template-directed threading. **e**, Separable crowded mechanical epimers (d-*R*mp)-**19** and (d-*S*mp)-**19** used in the auxiliary synthesis of enantiopure mechanically planar chiral rotaxanes. **f**, Papot’s rotaxane prodrug **20** that demonstrates the level of molecular complexity that can be accessed using the AT-CuAAC reaction.

**Figure 4**: Interlocked electronic and oligomeric materials can be synthesised using the active template approach: **a**, insulated conjugate structures synthesised using AT-Glaser and AT-Cadiot-Chodkiewicz couplings (conjugated porphyrin nanoring **21**, oligoyne [2]rotaxanes **22**, cumulene rotaxane **23**). **b**, homo[6]rotaxane **24** and hetero[4]rotaxane **25** synthesised using an iterative AT-CuAAC coupling strategy. **c**, Crowley’s interlocked metallo-supramolecular polymer ([Fe(**26**)]2+)n (n = 11 - 13) using an AT-CuAAC strategy.

**Figure 5**: Interlocked molecules as catalysts. **a**, Leigh’s chiral interlocked ligand **27** and its performance in a Ni-mediated Michael addition. **b**, Goldup’s rotaxane-AuCl [Au(**29**)Cl] complex and its behaviour as a stimuli responsive cyclopropanation pre-catalyst. **c**, The diyne-containing axle of rotaxane **31** is converted to a pyrrole axle by a reaction with aniline catalysed by a Cu(I) ion that is coordinated by the macrocycle. R = 4-C6H4-C(4-*t*Bu-C6H4)3

**Figure 6**: Interlocked host molecules synthesised using the active template approach: **a**, Beer’s halogen bonding anion host **33** and the binding constant with selected anions. **b**, Beer’s chalcogen-bonding host **34c** and a comparison between the binding constants with various anions and the hydrogen bonding-only host **34a**. **c**, Tucker and McClenaghan’s Hamilton receptor rotaxanes synthesised through AT-Glaser (**36**) and AT-CuAAC (**37**) reacions of which, only **36** is a host for barbital (**35**). **d**, Goldup’s rotaxane CuI-triazolide **41** that can be synthesised under aqueous conditions due to stabilisation provided by the mechanical bond. R = C(4-*t*Bu-C6H4)3

**Figure 7**: Examples of molecular machines synthesised using AT reaction. **a**, Sauvage-like shuttle **42** synthesised using the AT-CuAAC reaction. b) “Low interaction” shuttle **43** synthesised using the AT-Cadiot-Chodkiewicz coupling. c) “Peptide synthesiser” **47** molecular machine precursor assembled using the AT-CuAAC reaction. R = C(4-*t*Bu-C6H4)3

**Boxes**

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**[b1] Box 1.** **Key Features of Active Template Methodologies**

|  |
| --- |
| Often extremely high yielding using readily available starting materials. |
| Give rapid access to otherwise difficult/impossible to synthesise architectures. |
| Strategy can be applied using a range of different bond forming reactions. |
| Only one recognition site required in the product, increasing structural diversity. |
| Sub-stoichiometric amounts of template/catalyst can be used |
| Principally a kinetic template effect |
| Product distribution can provide detailed information about reaction mechanisms |

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**[b2] Box 2.** **Selectivity in passive and active template reactions**

**Passive Template**: If we assume the rate of the stoppering reaction is the same for threaded complex **III** and unthreaded pre-axle **I** (typically the case) we can simply write the selectivity of interlocked *vs* non-interlocked products in the system shown in **Figure 1a** as:

$$\frac{Rotaxane IV}{Axle V}=\frac{[III]}{[I]}= \frac{Keq\left[I\right]\left[II\right]\left[M\right]}{[I]}=Keq\left[II\right][M]$$

Similar equations can be derived for more complex systems with multiple productive and non-productive species present. Using this simple model, unsurprisingly the yield in a passive template reaction is expected to be high when *K*eq favours threaded complexes. Thus, the yield of the reaction can be enhanced by employing non-competitive solvents or other logical modifications to conditions (e.g. temperature, concentration etc.) that favour **III**.

**Active Template**: In the active template process shown schematically in **Figure 1b**, the relative yield of rotaxane **IX** compared with non-interlocked axle **XI** depends on the relative rate at which they are produced. The rate of production of the interlocked product depends on the concentration of **VIII** and thus *K***VIII**, [M], [**II**], [**VI**] and [**VII**], and the rate at which it progresses to product, which depends on *k*AT. Similarly, the rate of production of non-interlocked axle **XI** depends on the concentrations of the same components, K**X** and *k*axle. Using this simple model, we can write an equation for the product ratio of rotaxane **IX** and axle **XI** as:

$$\frac{Rotaxane IX}{Axle XI}=\frac{k(AT)[VIII]}{k(axle)[X]}= \frac{k\left(AT\right)\{K\left(VIII\right)\left[II\right]\left[M\right]\left[VI\right]\left[VII\right]\}}{k(axle)\{K(X)\left[II\right]\left[M\right]\left[VI\right]\left[VII\right]\}}=\frac{k\left(AT\right)K\left(VIII\right)}{k(axle)K(X)}$$

From this analysis, the yield of the active template reaction cannot simply be maximised by enhancing the pre-equilibria to favour **VIII** as the same modification may also reduce the rate with which it progresses to product. Similar equations can be derived where multiple productive and unproductive pathways are in operation. In particular, off-ligand reactions that do not involve the macrocycle will significantly reduce the yield of interlocked product and so it is highly desirable that either i) the “free” metal ion is not catalytically competent or ii) the concentration of the metal ion is minimised by strong binding to the macrocycle and by ensuring that less than a stoichiometric quantity is employed. The optimisation of active template reactions is thus much more like the process employed in the development of catalytic reactions where multiple variables must be considered in parallel.

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**[b3] Box 3. Example of a Stereoelectronically Disfavoured AT Reaction**

The attack of nucleophiles on aldehyde electrophiles bearing a proximal chelating unit activated by a Lewis acidic CuII PyBox complex is reported to proceed via complex **A77**. This model suggests that if this process were designed to take place within a macrocycle, as in **B**,then the aldehyde will be disposed such that nucleophilic attack will take place on the same face of the macrocycle as that to which the aldehyde is coordinated, leading to non-interlocked products.

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**Glossary terms**

Catenanes - molecules composed of macrocycles threaded through one another like links in a chain

Rotaxanes - molecules in which macrocycle(s) are threaded on linear “axle” components and locked in place by bulky “stopper” units

Mechanical bond – when two or more independent covalent species are threaded through one another in such a way that they cannot be separated without breaking a covalent bond they are said to be “mechanically bonded”. The mechanical bond arises due to the inability of atoms bonds to pass through one another. The archetypal examples of mechanically bonded structures are catenanes and rotaxanes.

Stoppering – The introduction of stoppers onto the end of a threaded complex to trap the threaded architecture and form the mechanical bond

Clipping – cyclisation of a pre-macrocycle (sometimes termed a “u-shape”) around a preformed axle (to make a rotaxane) or, starting from a threaded complex to form a catenane.

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**ToC blurb:** The active template approach to interlocked molecules uses metal ions to both preorganize reaction components and catalyse the final covalent bond formation that captures the interlocked structure. This Review looks at the history of the method, its application in the synthesis of ever more complex interlocked molecules and future directions.

|  |  |
| --- | --- |
| Entry | Metal + Ligand |
| 1 | Cu(I) + **I**11 or **II**12,13 or **VII**25 or **IX**24 or **X**24,23 |
| 2 | Cu(I) + **III**19Ni(II)/Cu(I) + **II**30Pd(II) + **I** or **II**26 |
| 3 | Cu(I) + **III**19 |
| 4 | Cu(I) + **II**20 |
| 5 | Pd(II) + **I** or **II**27 |
| 6 | Ni(II) + **IV**29 or **V**37 |
| 7 | Pd(II) + **VI**28 |
| 8 | Cu(I) or Zn(II) + **I** or **II**31 |
| 9 | Cu(I) + **III**21 |
| 10 | Ni(II) + **VIII**22 |
| 11 | Cu(I) + **VIII**25 or **IX**24 or **X**24,23 |

 [↑](#endnote-ref-76)