



HAL
open science

Rotaxanating Metallo-supramolecular Nano-cylinder Helicates to Switch DNA Junction Binding

Catherine a J Hooper, Lucia Cardo, James S Craig, Lazaros Melidis, Viktoriia Sadovnikova, Ross Egan, Aditya Garai, Florian Burkert, Louise Male, Nikolas J Hodges, et al.

► **To cite this version:**

Catherine a J Hooper, Lucia Cardo, James S Craig, Lazaros Melidis, Viktoriia Sadovnikova, et al.. Rotaxanating Metallo-supramolecular Nano-cylinder Helicates to Switch DNA Junction Binding. Journal of the American Chemical Society, 2020, 142 (49), pp.20651-20660. 10.1021/jacs.0c07750 . hal-03054411

HAL Id: hal-03054411

<https://hal.science/hal-03054411v1>

Submitted on 11 Dec 2020

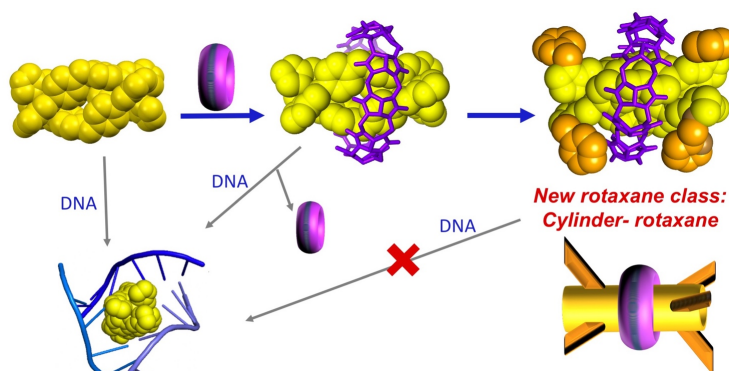
HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Rotaxanating metallo-supramolecular nano-cylinder helicates to switch DNA junction binding.

Catherine A.J. Hooper, Lucia Cardo, James S. Craig, Lazaros Melidis, Viktoriia Sadovnikova, Ross Egan, Aditya Garai, Florian Burkert, Louise Male, Nikolas J. Hodges, Douglas F. Browning, Roselyne Rosas, Fengbo Liu, Fillipe V. Rocha, Mauro A. Lima, Simin Liu,* David Bardelang,* and Michael J. Hannon.*

ABSTRACT: A new class of rotaxane is created, not by encapsulating a conventional linear thread, but rather by wrapping a large cucurbit[10]uril macrocycle about a three-dimensional, cylindrical, nanosized, self-assembled supramolecular helicate as the axle. The resulting *pseudo*-rotaxane is readily converted into a proper interlocked rotaxane by adding branch points to the helicate strands that form the surface of the cylinder (like branches and roots on a tree trunk). The supramolecular cylinder that forms the axle is itself a member of a unique and remarkable class of helicate metallo-drugs that bind Y-shaped DNA junction structures and induce cell death. While *pseudo*-rotaxanation does not modify the DNA-binding properties, proper, mechanically-interlocked rotaxanation transforms the DNA-binding and biological activity of the cylinder.



■ INTRODUCTION

Drawing on nature for inspiration, supramolecular chemistry has created a variety of complex, often exotic, nanoscale structures. Mechanically linked molecules have been of particular interest, and rotaxanes have been formed by threading molecular strands through a variety of different classes of macrocycle.¹⁻³ Applications of rotaxanes have ranged from protecting peptides from rapid enzymatic degradation, to creating materials with switchable surface properties.¹⁻⁸ Yet the threads used, to date, have been principally linear, 1-dimensional or 2-dimensional, organic, covalent molecules.

An alternative, but equally exciting, aspect of supramolecular chemistry, has been to take the designs and lessons of supramolecular chemistry and to apply them back to the biology that provided the initial inspiration for the field. Indeed self-assembled metallo-supramolecular helicates^{9,10} are enjoying a resurgence of interest for their application in biology.¹¹⁻²⁰ They offer quite large nanoscale surfaces of defined topography, of comparable scale to key structures found in biomolecules. We have used these external surfaces to access unique DNA- and RNA-recognition properties,²¹⁻²³ demonstrating with X-ray crystal structures the remarkable binding of cylindrical metallo-helicates in the heart of DNA and RNA 3-way junction structures,²⁴⁻²⁸ where the nucleic acid bases form perfectly stacked

aromatic interactions²⁹ with the external aromatic surfaces of the metallo-helicates. Nucleic acid junction structures are exciting targets found both in biology and nucleic acid nanoscience.³⁰⁻³⁵ Our junction-binding cylinders arrest the proliferation of cancer cells and the HIV virus.³⁶⁻³⁹

Given that the supramolecular nano-cylinder helicates thread so beautifully *into and through* junctions and bulges in nucleic acids we were intrigued to see whether we could push the boundaries of supramolecular assembly to similarly thread such self-assembled nanoscale metallo-supramolecular helicates through *synthetic* macrocycles to create new types of rotaxanes and *pseudo*-rotaxanes in which the thread was not a simple chain but a three dimensional, self-assembled, **metallo-cylinder**. Since the cylinders recognise DNA-junction structures through their external surfaces, we anticipated that wrapping another molecule around this region of the cylinder might also interfere with (and ultimately control) the DNA-binding. Rotaxanation in DNA-binding agents has not been explored previously.

We report now, for the first time, the wrapping of a synthetic macrocycle around the external surface of a self-assembled metallo-supramolecular nanostructure to create a *pseudo*-rotaxane, subsequent conversion to a proper (mechanically -interlocked) [2]-rotaxane, and the effect of rotaxanation on the biomolecular recognition and biological properties of the metallo-supramolecular cylinders. **Examples of larger threads in**

rotaxanes include *pseudo*-rotaxanes created by clipping macrocycles around carbon nanotubes,^{40,41} a rotaxane with a spherical fullerene in the centre,⁴² and DNA rotaxanes⁴³ but this work is distinct in representing (to the best of our knowledge) the first example of a proper interlocked rotaxane assembled around a three-dimensional metal-assembled supramolecular helicate as axle. An interesting report of rotaxanation of a helical peptide has also appeared, but in that case the small rotaxane ring wraps just around its linear chain (primary structure) rather than encapsulating a helical secondary structure.⁴⁴ The large three-dimensional cylindrical surface that our metallo-supramolecular helicate axle affords also brings another opportunity: rather than attaching a branched organic unit at each end of the thread as stopper we now clip unbranched units onto each thread of the helicate axle exploiting the metal-coordination and supramolecular structure to achieve the branching effect which mechanically constrains the ring. We show that the rotaxanation allows us to switch the DNA-binding properties and thus the biological activity of the cylinder.

■ RESULTS AND DISCUSSION

The first challenge was to identify a suitable ‘ring’ that might be wrapped about these metallo-supramolecular helicates. They are tetracations formed by wrapping three organic ligand strands about two metal centres (Fig. 1).¹¹ They are cylindrical in shape, and around 2 nm in length and 1 nm in diameter - much greater than the dimensions normally associated with rotaxanation or encapsulation - but we identified that cucurbit[10]uril (Fig. 1) might offer a cavity of suitable dimensions.^{45,46}

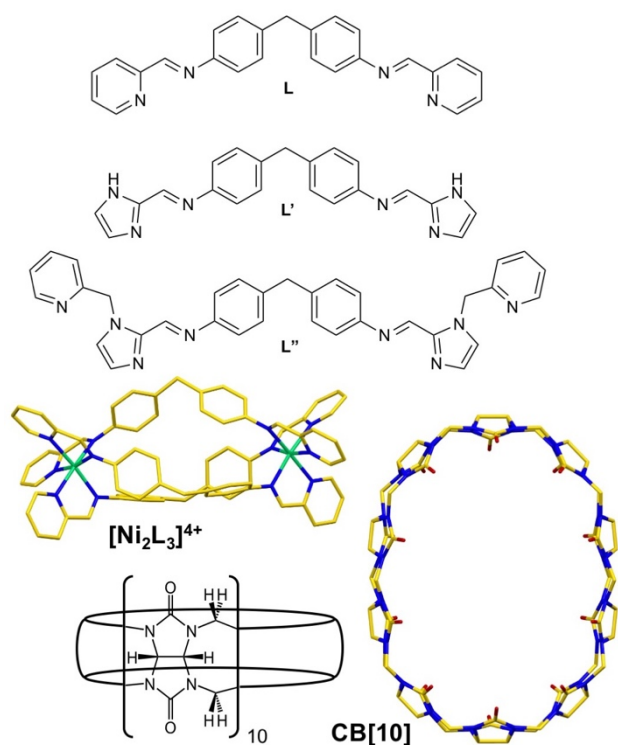


Figure 1: The ligands used in this study together with the structure of the [Ni₂L₃]⁴⁺ cylinder (CSD-NITBIB) and cucurbit[10]uril (from CSD-LAZPIM) and illustrating the flexibility and potential ellipsoidal distortions CB[10] can accommodate in response to guests).

Cucurbit[n]urils (CB[n]s) are a class of macrocycles that are attracting much attention for both host-guest chemistry and rotaxanation studies.⁴⁵⁻⁵¹ They are pumpkin-shaped molecules with a rigid hydrophobic cavity flanked by two identical openings (portals) whose rims are each lined with n carbonyl groups. CB[5,6,7] are ideal sizes for traditional small organics and CB[6,7,8] have been used with organic, linear threads and chains to create rotaxanes.^{48,52} Other applications have included using the host-guest chemistry to attach proteins to surfaces or fluorophores, and for drug delivery.^{47,53} The largest of the class, with an untwisted cavity, is CB[10] (Fig. 1) which can accommodate multiple or larger molecules^{45,46} as demonstrated by its use to capture both a Stoddart ‘blue-box’ (bis-bipyridinium) macrocycle and its methoxybenzene guest to create a Russian-doll style assembly.⁵⁴ Binding interactions include hydrophobic effects in the heart of the cavity and ion-dipole interactions at the rims.⁴⁵⁻⁵⁴

While lower order cucurbiturils have relatively rigid cavities, CB[10] is more flexible. It is observed to be ellipsoidal in its crystal structure (cavity diameter 1.13-1.24 nm; portal diameter 0.95-1.06 nm) in complex with 4,6-bis(4-(ammoniomethyl)phenylamino)-1,3,5-triazene-2(1H)-one,^{45,46} and it is observed to also complex platinum terpyridyls⁵⁵ and porphyrins⁵⁶ which implies further distortion is possible. The previous use of CB[10] for rotaxanation is restricted to a report of a kinetic rotaxane – a *pseudo*-rotaxane in which the thread is encapsulated at room temperature but can enter and leave at elevated temperatures.⁵⁷

To initiate our studies we first explored whether the supramolecular cylinders would bind to CB[10] to give a tube threaded through a ring. CB[10] itself is an insoluble compound, but rapidly dissolves on addition of an aqueous solution of [M₂L₃]⁴⁺ cylinders (M= Fe, Ru, Ni). Electrospray mass spectrometry shows peaks corresponding to [M₂L₃•CB10]⁴⁺ (Fig. S1) and proton NMR spectroscopy of [Fe₂L₃]⁴⁺ with CB[10] confirms formation of a 1:1 complex (Fig. 2). DOSY NMR reveals that a single species is formed, of greater hydrodynamic radius than the free cylinder, and involving both cylinder and CB[10]. The cylinder retains its two-fold and three-fold symmetry in the complex. The proton NMR resonances corresponding to the central CH₂ of the cylinder and the adjacent phenylene protons experience upfield shifts, consistent with a shielded location in the CB[10] cavity, while the imine proton and the pyridine H₃ are shifted downfield, consistent with encountering the carbonyls at the rim of the macrocycle. NOEs are observed from these two cylinder protons (H_{im} and H₃) to the CH₂ proton on the rim of the CB[10] macrocycle (Fig. S2). The NMR is unambiguous confirmation that the cylinder sits symmetrically (on the NMR timescale) in the centre of the macrocycle threading through from one side to the other as a *pseudo*-rotaxane, with the macrocycle sitting over the central diphenylmethanes of the cylinder as desired. To retain its three-fold symmetry within the ten-fold symmetric CB[10], the cylinder must be rapidly spinning or rocking about the metal-metal axis while within the macrocycle. DFT calculations and MD simulations reproduce this solution structure, with the DFT structure showing axial deviation between the host and guest main symmetry axes at the energy minimum (Fig. 2d) and the MD showing the rotation (Fig 6). Use of a small excess of cylinder (Fig. S3) in the NMR allows free and bound cylinder peaks to be observed indicating that exchange is slow on the NMR timescale, however on mixing [Fe₂L₃]⁴⁺ with

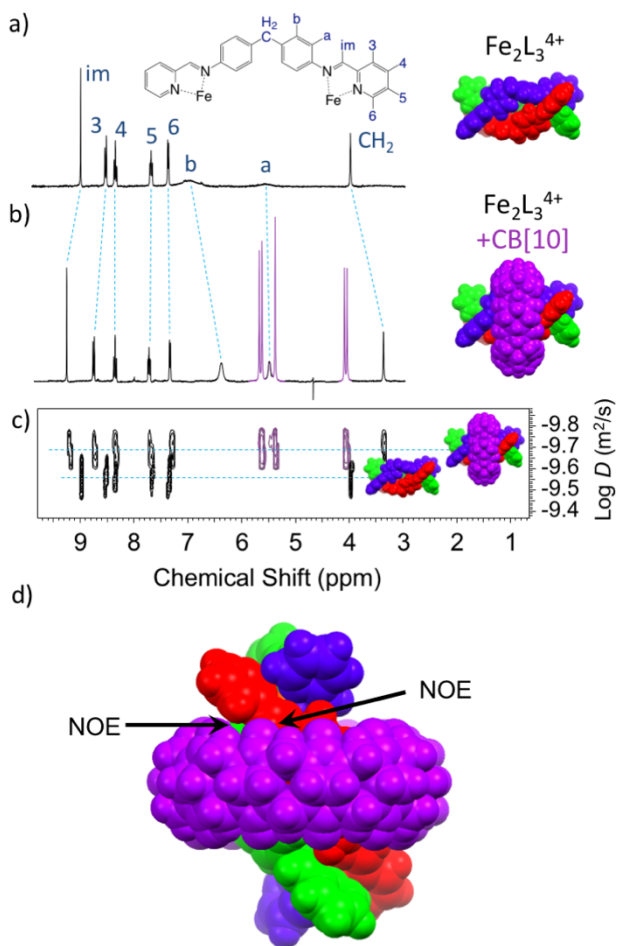


Figure 2: 500 MHz ^1H NMR spectra of the $[\text{Fe}_2\text{L}_3]^{4+}$ cylinder (0.5mM) (a) alone and (b) with 1 equivalent of CB[10] in D_2O illustrating the shifts caused by binding. (c) DOSY NMR spectra (stacked) showing the change in diffusion coefficient on binding consistent with formation of a single species of increased hydrodynamic radius. (d) DFT model of the cylinder + CB[10] also showing the experimentally observed NOEs from cylinder H-im & H-3 to a CH_2 proton on the rim of the CB[10].

$[\text{Ni}_2\text{L}_3\cdot\text{CB}10]^{4+}$ and immediately injecting into an electrospray mass spectrometer a mixture of $[\text{Fe}_2\text{L}_3\cdot\text{CB}10]^{4+}$ and $[\text{Ni}_2\text{L}_3\cdot\text{CB}10]^{4+}$ was observed, the ratio of which remained unchanged over a further hour of sampling (Fig. S4). Together these observations indicate that the exchange takes place over a timescale of milliseconds to seconds, which is consistent with measurements on other cucurbiturils with different guests.⁵⁸ The proton NMR spectrum of $[\text{Ru}_2\text{L}_3]^{4+}$ with CB[10] shows analogous upfield and downfield shifts in the resonances and the paramagnetically shifted proton NMR of $[\text{Ni}_2\text{L}_3]^{4+}$ with CB[10] also shows the binding (Figs. S5 and S6).

While the ruthenium(II) and nickel(II) $[\text{M}_2\text{L}_3]^{4+}$ cylinders have excellent stability, the iron(II) cylinders can experience some hydrolytic degradation over long periods,^{11,37} though they are stabilised by binding to DNA.^{21,22} Encapsulation of the cylinder within the CB[10] macrocycle similarly enhances the cylinder stability; an experiment with 0.9 equivalents of CB[10] revealed no degradation of the bound cylinder in aqueous solution over a period of 194 days (Fig. S7). The insolubility of

unbound CB[10] prevents accurate measurements of binding affinity with guests, however an NMR dilution experiment showed no unbound cylinder (Fig. S8) implying the binding constant is greater than 10^7 M^{-1} , and NMR competition studies show that the cylinder binds more strongly than both methyl viologen and memantine hydrochloride.

To explore the effect of the encapsulation on the cylinder's DNA-recognition properties we investigated the binding of the nickel cylinder•CB[10] complex (as representative of the three complexes) to DNA 3-way junctions (3WJ)^{24-28, 30-31} using gel electrophoresis. The assay (Fig. 3a) uses a 3WJ formed from three different complementary DNA strands. The 3WJ structure is (entropically) unstable at room temperature in absence of the cylinder and the DNA exists as three single strands. When cylinder binds, the 3WJ is stabilised and observed in the gel. Radiolabelling one strand allows a simple on/off gel-shift binding assay that is readily visualised. Figure 3a shows that the cylinder•CB[10] complex stabilises the 3WJ and the effect of the cylinder•CB[10] complex is the same as the cylinder alone, though higher loadings are required. This indicates that

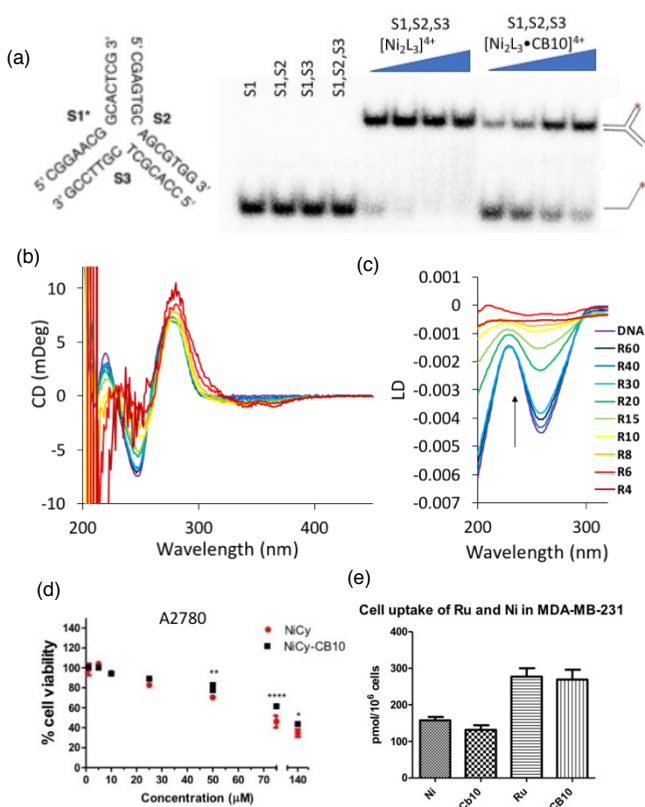


Figure 3: (a) PAGE gel showing that the $[\text{Ni}_2\text{L}_3\cdot\text{CB}10]^{4+}$ complex stabilises DNA 3WJ in the same way as $[\text{M}_2\text{L}_3]^{4+}$ cylinders alone. (* = radiolabel) (b) CD and (c) LD spectra of ct-DNA (100 μM DNA concentration; 10 mM sodium chloride; 1 mM sodium cacodylate) with $[\text{Ni}_2\text{L}_3\cdot\text{CB}10]^{4+}$. R values represent DNA base pairs/complex. The spectra show binding and DNA coiling and importantly are analogous to those obtained with free $[\text{Ni}_2\text{L}_3]^{4+}$ (Fig. S8) (d) MTT cell survival assay (72 hours treatment of A2780 cells), demonstrating that $[\text{Ni}_2\text{L}_3\cdot\text{CB}10]^{4+}$ and $[\text{Ni}_2\text{L}_3]^{4+}$ have similar effects on cell survival (triplicate independent repeats; two way ANOVA analyses). (e) Cell uptake studies (triplicate) demonstrating that CB[10] does not affect the cell uptake. The greater uptake of the Ru (over Ni) cylinder is consistent with previous observations in 1G5 (Jurkat T-) cells.³⁷

the cylinder can leave the CB[10] cavity and bind to the anionic DNA 3WJ cavity; while binding of an intact cylinder•CB[10] complex to the 3WJ cannot be excluded this would be expected to give a different gel shift. **The cylinder binding to the 3WJ is in competition with the binding to the CB[10]; Adding small amounts of solid CB[10] or of the soluble methylviologen CB[10] complex to the pseudorotaxane leads to small reductions in 3WJ formation (Fig. S9).** In these experiments, the binding energy to the 3WJ also incorporates the energetic cost of assembling the 3WJ from the DNA single strands. Given that the 3WJ is not observed in absence of cylinder at room temperature, the binding constant of cylinder to the 3WJ structure itself must be (significantly) greater than that to CB[10].

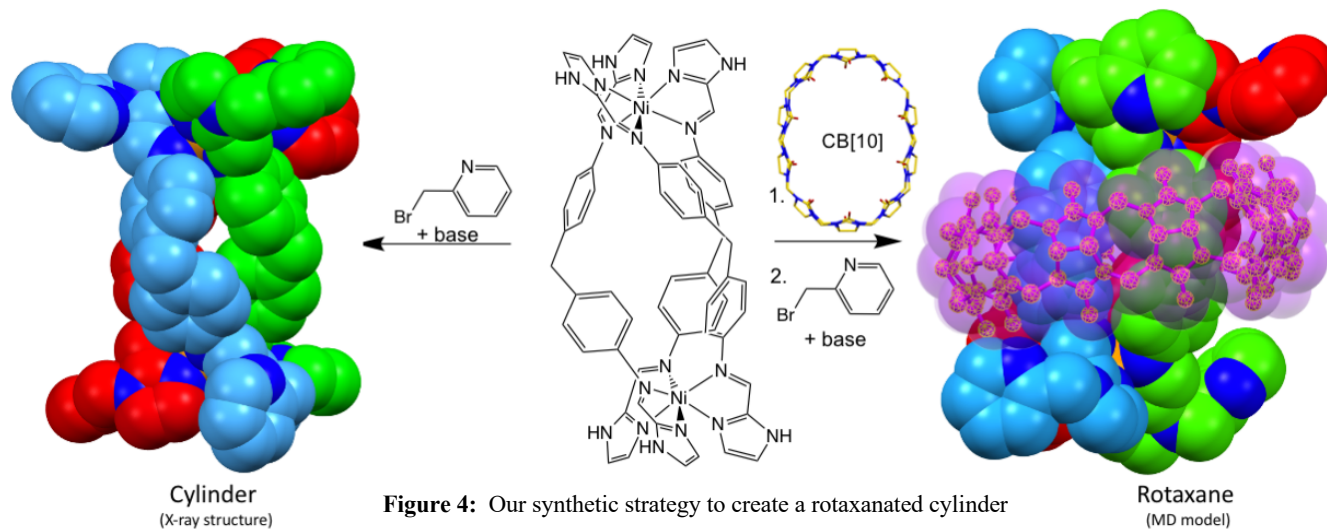
We also studied the binding to calf-thymus DNA (a polymeric genomic DNA), using circular and flow linear dichroism spectroscopies (Fig. 3b,c). The effects are again the same as the free cylinder binding: The circular dichroism confirms binding of cylinder (an induced CD signal is observed in the cylinder spectroscopy between 300-400 nm) and retention of a B-DNA structure (typical signature between 200-300 nm). The CD spectra are the same as those observed with cylinder alone (Fig S10), although at high loading some scattering is observed consistent with release of CB[10] which (uncomplexed) will be insoluble. The flow linear dichroism experiments probe the orientation of ct-DNA in a Couette flow cell, with the magnitude of the peak at 260 nm reflecting the orientation.^{21,22} Cylinders are known to coil ct-DNA (leading to a loss of orientation);^{21,22} the spectra on addition of the cylinder•CB[10] complex confirm the same DNA-coiling effects and are analogous to those observed with free cylinder (Fig S10). We conclude that while the CB[10] does bind the cylinder in solution to form the pseudo-rotaxane, the cylinder would prefer to bind to anionic DNA. For the 3WJ, which is not pre-formed in solution, the extra energy of forming the structure means that there is competition between binding CB[10] or 3WJ. When presented with a polymeric DNA anion the cylinder leaves the CB[10] cavity and binds its preferred partner. Preferential binding to the anionic DNA over the neutral CB[10] cavity is unsurprising, given the anticipated electrostatic contribution to that binding energy, though it contrasts with spermine that preferentially binds CB[7] over DNA.⁵⁹

Consistent with the *in vitro* DNA binding, when we treat cells (A2780 ovarian and MDA-MB-231 breast cancer lines) with the cylinder•CB[10] complex, we observe very similar inhibitions of cell proliferation (as assessed by an MTT assay) to

those with the corresponding free cylinder (Figs. 3d and S11). There is a very slight enhancement in activity for the ruthenium cylinder but a slight drop in activity for the iron and nickel cylinders. Uptake studies (assessed by ICP-MS) indicate that the encapsulation inside CB[10] has little effect on the extent of overall uptake (Fig. 3e). Cucurbiturils are frequently used as drug delivery agents and platinum metallo-drugs (e.g. oxaliplatin) encapsulated inside cucurbit[7]uril show some enhanced effects⁶⁰⁻⁶⁵ due to better uptake and/or pharmacology (less degradation and side reactions with thiols). The lack of dramatic enhancement for the cylinder perhaps reflects the already excellent cell uptake of cylinders^{36,37} and the lack of competing side reactions. The DNA-binding studies indicate that whether the cylinders have entered alone or in complex with CB[10], their effects when they reach the target DNA are expected to be the same. Consistent with this, a dinuclear ruthenium complex (from Keene, Collins *et al.*)^{66,67} that also binds non-covalently to DNA, and that can partially insert (but **is too large to thread**) into CB[10], similarly showed only small effects of complexation on its *in cellulo* behaviour (though the DNA-binding of that agent is different and the complex only mildly cytotoxic).

Having established that *pseudo*-rotaxanation with CB[10] was readily achieved and that it placed the macrocycle in the correct position over the central section of the cylinder but that it had little effect on the biological properties due to competitive de-threading, we next explored if we could lock the macrocycle onto the cylinder as a proper rotaxane. **Traditional rotaxanes are formed by attaching two bulky stoppers, one at each end of the thread.** By using a 3-dimensional helicate as the axle we open up an alternative possibility to create the branching needed to lock the ring onto the structure, by attaching multiple (smaller) groups that protrude out of the cylindrical surface at the ends of the helicate axle. To explore this we modified the design of the cylinder, replacing the pyridines with 2-imidazole units. The 2-imidazole offers a position to which we can add further functionalisation, through alkylation of the non-coordinated nitrogen in the imidazole ring, and we selected 2-picoyl as the alkylating group. Thus we introduce three branch points onto the cylinder surface at each end. This new design approach is shown in Fig. 4.

We first prepared two non-rotaxanated cylinders, $[\text{Ni}_2\text{L}'_3]^{4+}$ and $[\text{Ni}_2\text{L}''_3]^{4+}$, analogous to the parent cylinders but bearing the 2-imidazole and 2-imidazole-N-picoyl groups in place of the pyridines. They are readily prepared from the condensation of the 2-imidazole and 2-imidazole-N-picoyl carboxaldehydes



with bis-1,4-diaminophenylmethane and subsequent coordination to nickel(II). While the corresponding iron(II) complexes were also prepared, $[\text{Fe}_2\text{L}'_3]^{4+}$ had a lower solution stability than the corresponding nickel(II) complex and so we focused our studies on the nickel complexes. The X-ray crystal structures of the $[\text{M}_2\text{L}'_3]^{4+}$ cations ($\text{M} = \text{Ni}, \text{Fe}$) were obtained (as the hexafluorophosphate salts) and confirmed the expected structure (Figs. 4 and S12), with the retention of the triple-helical cylindrical structure, the retention of the central core of the cylinder structure which is crucial for its binding to both DNA 3-way junctions and CB[10], and now the introduction of the picolyl units sticking out of the helix and in such a position that they should not interfere with the CB[10] binding site but would potentially sterically prevent it from threading/dethreading.

We then explored whether we could post-synthetically transform $[\text{Ni}_2\text{L}'_3]^{4+}$ into the alkylated $[\text{Ni}_2\text{L}'_3]^{4+}$ by reaction with alkylating agent 2-(bromomethyl)pyridine and Hunig's base in acetonitrile; heating at reflux overnight afforded the desired hexa-alkylated product, $[\text{Ni}_2\text{L}'_3]^{4+}$, as confirmed by mass spectrometry and (paramagnetic) proton NMR spectroscopy. Small amounts of the penta-functionalised cylinder were also observed by mass spectrometry. To access the rotaxane we then repeated the same reaction in the presence of CB[10]. While acetonitrile did not prove to be a suitable solvent, reaction in aqueous methanol at reflux overnight, afforded the rotaxane as a cream precipitate in ~60% isolated yield. The mass spectrum (Fig. 5 and S14) shows a dominant peak corresponding to $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ (correct mass and isotope distribution). Smaller peaks corresponding to penta-alkylated rotaxanes are also observed but only a tiny trace peak corresponding to unrotaxanated $[\text{Ni}_2\text{L}'_3]^{4+}$ indicating that the rotaxane (as opposed to a *pseudo*-rotaxane) is formed in which the ring is now constrained by the picolyl groups to remain on the cylinder. The (paramagnetically shifted) proton NMR spectrum shows the

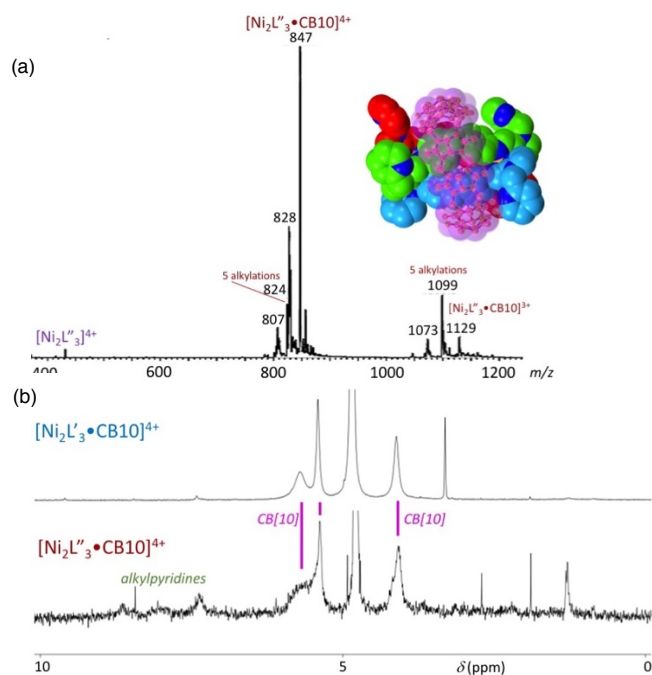


Figure 5: (a) Mass Spectrum of the $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ rotaxane (b) ^1H NMR Spectrum (0-10ppm shown only) of the $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ rotaxane and comparison with imidazole pseudo-rotaxane $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ showing presence of CB[10] and the alkylpyridine groups on the modified cylinder.

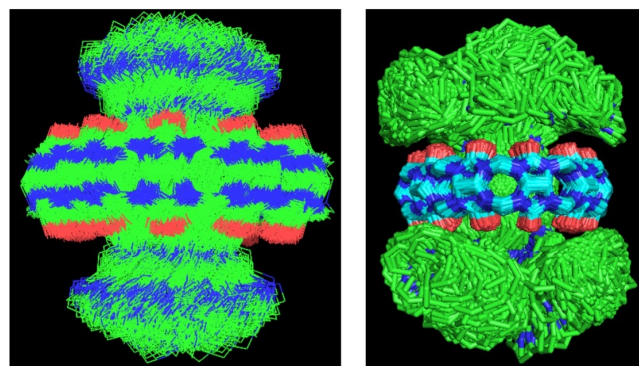


Figure 6: Combined and superimposed views of Molecular Dynamics simulations of the *pseudo*-rotaxanated $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ (left) and proper rotaxanated $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ (right) complexes showing the free rotation of the $[\text{Ni}_2\text{L}'_3]^{4+}$ cation in the CB[10] and the more restricted motion caused by the picolyl groups for $[\text{Ni}_2\text{L}'_3]^{4+}$; in the $1\mu\text{s}$ timescale the rotaxanated cylinder does not rotate. Hydrogens are omitted for clarity. Supplementary videos of the simulations are also available. There is a tendency for the CB[10] ring to undergo fluxional distortions during the simulation, including a buckling of the ring at the CH_2 groups that create a heart-shaped ring and allowing it to close up around the cylinder. This is also seen at very low frequency (<0.1%) in simulations of the free CB[10] but is more prevalent in the rotaxane. The paramagnetic broadening in the ^1H NMR does not allow this feature to be confirmed experimentally but reflects the greater flexibility of CB[10] compared to lower order cucubit[n]urils. See also Figs S18 and S22.

presence of peaks corresponding to the cylinder and the CB[10] (Figs. 5 and S11). The cylinder peaks in the rotaxane are further broadened than those observed in the free $[\text{Ni}_2\text{L}'_3]^{4+}$ cylinder; consistent with this, MD simulations (Figs. 4 and 6) indicate that the picolyl groups restrict the spinning motion of the cylinder about the metal-metal axis while rotaxanated inside the CB[10]. The rotaxanated complex $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ was mixed with an equimolar amount of $[\text{Fe}_2\text{L}_3]^{4+}$ and heated under reflux for 24 hours in 10% methanol: water. The rotaxane was unchanged and no Fe_2L_3 pseudo-rotaxanes were observed by mass spectrometry; this contrasts with the analogous mixing experiments with pseudo-rotaxanes and is strong evidence of proper rotaxane formation.

Rotaxanation would also be expected to enhance the inherent stability of the cylinder and to explore this we treated the picolyl functionalised cylinder $[\text{Ni}_2\text{L}'_3]^{4+}$ and the rotaxane $[\text{Ni}_2\text{L}'_3\cdot\text{CB10}]^{4+}$ with edta. Edta binds strongly to Ni^{2+} in water ($\log k > 18$)⁶⁸ and is able to remove nickel from $[\text{Ni}_2\text{L}'_3]^{4+}$ over a period of hours at room temperature. The rotaxanation does not provide additional stabilisation, and the rotaxanated cylinder is competitive with edta for nickel binding (Fig. S21) with significant amounts of rotaxane still present even after treatment with 10 equivalents of $\text{Na}_2(\text{edta})$.

Multiply-threaded rotaxanes are structures of considerable interest;⁶⁹⁻⁷¹ Considering only the ligands of the helicate the rotaxane might be viewed as a [4]rotaxane with two metal bound to the three strands. However, mass spectrometric analysis demonstrates that on demetallation the ligands are no longer sterically constrained in the CB[10] cavity and dethread (Fig. S21). The metallo-supramolecular assembly of the triple-helicate is thus crucial to the design and rotaxane preparation and we consider a [2]rotaxane nomenclature to be more appropriate for this

particular supramolecule, though it might be possible to access [4]rotaxanes by this approach in the future using bulkier groups.

The non-rotaxanated $[\text{Ni}_2\text{L}'_3]^{4+}$ and $[\text{Ni}_2\text{L}''_3]^{4+}$ cylinders bind DNA analogously to the original pyridyl cylinders (Figs. 7 and S15-17). PAGE gel electrophoresis experiments confirms that both are able to bind and stabilise DNA 3-way junctions, just as the original pyridyl cylinders do, with $[\text{Ni}_2\text{L}'_3]^{4+}$ less effective than the parent (perhaps due to imidazole deprotonation) and $[\text{Ni}_2\text{L}''_3]^{4+}$ slightly more so (perhaps because of its larger surfaces which may extend into the 3WJ grooves). Circular dichroism spectra confirm that both cylinders bind calf thymus DNA (inducing a CD signal in the cylinder spectroscopy) which retains a B-DNA structure, and the linear dichroism demonstrates that both cylinders give rise to DNA-coiling as observed with pyridyl-based cylinders. Cell proliferation (MTT) assays confirmed that the functionalised (but non-rotaxanated) cylinder $[\text{Ni}_2\text{L}''_3]^{4+}$ exerts a cytotoxic effect in cells (IC_{50} , 72 h, MDA-MB-231 $55 \pm 10 \mu\text{M}$; SKOV3 $52 \pm 5 \mu\text{M}$). Thus, as anticipated, making changes to the ends of these cylinders does not, of itself, prevent the key DNA-binding and biological activities.

The contrast with the rotaxane $[\text{Ni}_2\text{L}''_3 \cdot \text{CB}10]^{4+}$ is striking (Fig. 7). The rotaxane can no longer stabilise the DNA 3WJ in the gel electrophoresis experiment. It also no longer coils DNA (rather a small increase in DNA orientation is observed). Thus wrapping the CB[10] macrocycle around the central phenylene units has switched off the DNA junction binding and DNA coiling as anticipated. The greater DNA orientation observed in a Couette cell in the linear dichroism experiment indicates some form of DNA binding occurs, while the circular dichroism confirms that the DNA remains in its B form. Molecular dynamics simulations (Fig. S17) suggest that the rotaxane may associate through a partial entry into the grooves, and that this leads to some small elongation of the DNA. Association with the bases at the ends of the DNA strand is also seen in the simulations together with some capacity to bridge across two pieces of DNA that could also lead to enhanced orientation in a Couette cell.

Just as the rotaxanation switches off the key DNA binding features of the cylinders, it also switches off the biological action with the rotaxane showing no cytotoxic effect in cell proliferation (MTT) assays (IC_{50} , 72 h $\gg 140 \mu\text{M}$) in MDA-MB-231 (breast) cell lines (Fig. S11).

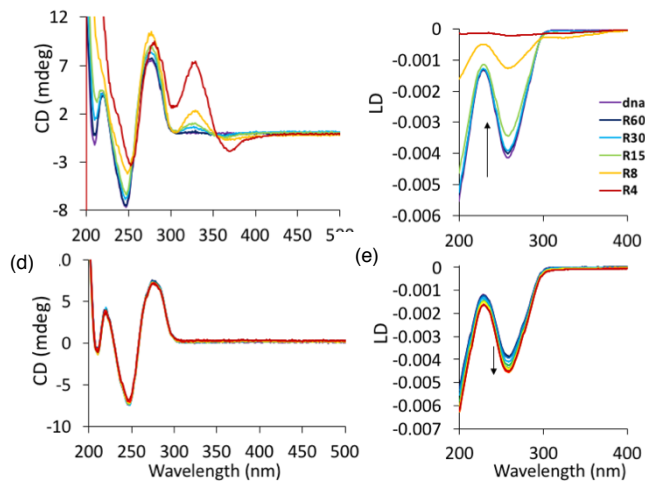
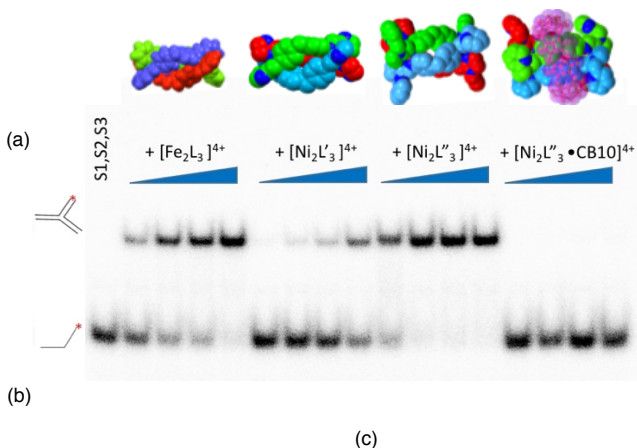


Figure 7: (a) PAGE gel showing that the $[\text{Ni}_2\text{L}'_3]^{4+}$ and $[\text{Ni}_2\text{L}''_3]^{4+}$ complexes stabilise DNA 3WJ but the rotaxanated $[\text{Ni}_2\text{L}''_3 \cdot \text{CB}10]^{4+}$ cylinder does not. (b) CD and (c) LD spectra of ct-DNA (100 μM DNA concentration; 10 mM sodium chloride; 1 mM sodium cacodylate) with $[\text{Ni}_2\text{L}'_3]^{4+}$ and (d) the corresponding CD and (e) LD spectra of ct-DNA with $[\text{Ni}_2\text{L}''_3 \cdot \text{CB}10]^{4+}$. R values represent DNA base pairs/complex.

CONCLUSIONS

We have demonstrated a new approach to design of rotaxanes by employing cylindrical metallo-supramolecular helicates as the axle, with the ring held in place by branch points which emanate from the strands of the helix. These intriguing new agents are assembled by combining two designs on which the field of supramolecular chemistry was founded: the metallo-helicates (pioneered by Lehn)⁹ and rotaxanes (Stoddart and Sauvage).^{1,2} The metallo-cylinders (a class of triple-helicates) used as the rotaxane axle, offer unique and unprecedented DNA binding properties; their activity has stimulated a great interest in designing metallo-supramolecular drugs.^{11,12} Pseudo-rotaxanation is not sufficient to modify the bio-activity of these cylinders *in vitro* or *in cellulo*, but by proper rotaxanation those properties are modified.

Switching the activity of a metallo-drug on rotaxanation represents an unconventional approach: indeed incorporation of metallo-drugs into proper rotaxanes is rare and restricted to mononuclear platinum⁷² and ruthenium⁷³ drugs attached to the terminal stopper of a rotaxane, with the rotaxane part essentially behaving as an attached organic vector. Organic drugs such as paclitaxel have also been attached as a rotaxane stopper⁷⁴ while Papat and Leigh have used a potentially bioactive peptide as a linear thread in a hydrogen-bond assembled rotaxane and combined it with a sophisticated enzyme-cleavage rotaxane degradative release.⁷⁵⁻⁷⁶

Our results raise the tantalising possibility of creating systems in which a triggered rotaxanation or de-rotaxanation (de-threading) inside a cell might be used to switch a metallo-drug action on or off. This might be applicable to a range of different molecular and metallo-supramolecular designs and we are actively exploring the further potential of this approach.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.xxxxxxxx>.

Experimental details & Supplementary Figures S1-S22 (PDF)
cif files for $[\text{Ni}_2\text{L}'_3][\text{PF}_6]_4$ and $[\text{Fe}_2\text{L}'_3][\text{PF}_6]_4$ (.txt)
Movies of the MD simulations in Fig. 6 (.mov)

■ AUTHOR INFORMATION

Corresponding Authors

Michael J. Hannon - School of Chemistry & Physical Sciences for Health Centre, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. Email: m.j.hannon@bham.ac.uk

David Bardelang - Aix-Marseille Univ, CNRS, ICR, Marseille, France. Email: david.bardelang@univ-amu.fr

Simin Liu - The State Key Laboratory of Refractories and Metallurgy, School of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, China. Email: liusimin@wust.edu.cn

Authors

Catherine A.J. Hooper[#] - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Lucia Cardo^{#†} - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

James S. Craig - Physical Sciences for Health Centre, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Lazaros Melidis - Physical Sciences for Health Centre, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Viktoriiia Sadovnikova[§] - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Ross Egan - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Aditya Garai - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Florian Burkert - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Louise Male - School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Nikolas J. Hodges - School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Douglas F. Browning - School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

Roselyne Rosas - Aix Marseille Univ, CNRS, Centrale Marseille, FSCM, Spectropole, Marseille, France

Fengbo Liu - The State Key Laboratory of Refractories and Metallurgy, School of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, China.

Filippe V. Rocha - Department of Chemistry, Federal University of São Carlos, São Carlos, Brazil.

Mauro A. Lima - Department of Chemistry, Federal University of São Carlos, São Carlos, Brazil.

Present Addresses

[†] Lucia Cardo, Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Paseo Miramón 182, 20014 Donostia-San Sebastián, Spain.

[§] Viktoriiia Sadovnikova, *Infirmierie Protestante, 1-3 Chemin du Penthod, 69300 Caluire, France.*

Author contributions

[#] These authors contributed equally: Catherine Hooper and Lucia Cardo

■ ACKNOWLEDGMENTS

This work was funded by the EPSRC Physical Sciences for Health Centre (EP/L016346/1), the EU DNAREC Marie Curie Training Site (MEST-CT-2005-020842), an EU Marie Curie Fellowship (H2020-MSCA-IF-2018-844145), FAPESP (2019/11242-1) and the University of Birmingham. Simulations used the Bluebear and Castles HPC facility (U. Birmingham).⁷⁷ FB was supported by a student exchange from LMU Munich. We thank the Centre for Chemical and Materials Analysis (U. Birmingham), the EPSRC UK National Crystallography Service at the University of Southampton for the collection of the crystallographic data,⁷⁸ Dr. Mirela Pascu (U. Birmingham) for some initial experiments (on L') and Dr. Tim Barendt, Prof. Zoe Pikramenou (both U. Birmingham), Prof Alexandre Martinez (ENS Lyon) and Prof Benoît Colasson (U. Paris Descartes) for helpful discussions.

■ REFERENCES

- (1) Stoddart, J. F. Mechanically Interlocked Molecules (MIMs)—Molecular Shuttles, Switches, and Machines (Nobel Lecture). *Angew. Chem. Int. Ed.* **2017**, *56*, 11094-125.
- (2) Sauvage, J.-P. From Chemical Topology to Molecular Machines (Nobel Lecture). *Angew. Chem. Int. Ed.* **2017**, *56*, 11080-93.
- (3) Zhang, L.; Marcos, V.; Leigh, D. A. Molecular machines with bio-inspired mechanisms. *Proc. Natl. Acad. Sci., USA* **2018**, *115*, 9397-9404.
- (4) Woltering, S.; Gawel, P.; Christensen, K. E.; Thompson, A.L.; Anderson, H.L. Photochemical Unmasking of Polyynic Rotaxanes. *J. Am. Chem. Soc.* **2020**, DOI: 10.1021/jacs.0c05308
- (5) Danon, J.J.; Leigh, D.A.; McGonigal, P.R.; Ward, J.W.; Wu, J. Triply Threaded [4]Rotaxanes. *J. Am. Chem. Soc.* **2016**, *138*, 12643-12647
- (6) Lewis, J.E.M.; Beer, P.D.; Loeb, S.J.; Goldup, S.M. Metal ions in the synthesis of interlocked molecules and materials. *Chem. Soc. Rev.* **2017**, *46*, 2577-2591
- (7) Ambrogio, M.W.; Thomas, C.R.; Zhao, Y.-L.; Zink, J.I.; Stoddart, J.F. Mechanized Silica Nanoparticles: A New Frontier in Theranostic Nanomedicine. *Acc. Chem. Res.* **2011**, *44*, 903-913
- (8) Pairault, N.; Barat, R.; Tranoy-Opalinski, I.; Renoux, B.; Thomas, M.; Papot, S. Rotaxane-based architectures for biological applications. *C. R. Chimie* **2016**, *19*, 103e112.
- (9) Ayme, J. F.; Lehn, J. M. From Coordination Chemistry to Adaptive Chemistry. *Adv. Inorg. Chem.* **2018**, *71*, 3-78.
- (10) Albrecht, M.; Chen, X.; Van Craen, D. From Hierarchical Helicates to Functional Supramolecular Devices. *Chem. Eur. J.* **2019**, *25*, 4265-73.
- (11) Cardo, L.; Hannon, M. J. Non-covalent Metallo-Drugs: Using shape to target DNA and RNA junctions and other nucleic acid structures. *Metals in Life Sciences* **2018**, *18*, 303-324 (Ed. R. Sigel, H. Sigel, A. Sigel), De Gruyter.
- (12) Pöthig, A.; Casini, A. Recent Developments of Supramolecular Metal-based Structures for Applications in Cancer Therapy and Imaging. *Theranostics*, **2019**, *9*, 3150-3169.
- (13) Allison, S.J.; Cooke, D.; Davidson, F.S.; Elliott, P.I.P.; Faulkner, R.A.; Griffiths, H.B. S.; Harper, O.J.; Hussain, O.; Owen-Lynch, P.J.; Phillips, R.M.; Rice, C.R.; Shepherd, S.L.; Wheelhouse, R.T.; Ruthenium-Containing Linear Helicates and Mesocates with Tuneable p53-Selective Cytotoxicity in Colorectal Cancer Cells. *Angew. Chem. Int. Ed.* **2018**, *57*, 9799-9804.

- (14) Li, X.; Wu, J.; Wang, L.; He, C.; Chen, L.; Jiao, Y.; Duan, C. Mitochondrial-DNA-Targeted IrIII-Containing Metallohelices with Tunable Photodynamic Therapy Efficacy in Cancer Cells, *Angew. Chem. Int. Ed.* **2020**, *59*, 6420-6427.
- (15) Kumar, S.V.; Lo, W.K.C.; Brooks, H.J.L.; Crowley, J.D. Synthesis, structure, stability and antimicrobial activity of a ruthenium(II) helicate derived from a bis-bidentate "click" pyridyl-1,2,3-triazole ligand, *Inorg. Chim. Acta*, **2015**, *425*, 1-6.
- (16) Domarco, O.; Lotsch, D.; Schreiber, J.; Dinhof, C.; Van Schoonhoven, S.; Garcia, M. D.; Peinador, C.; Keppler, B. K.; Berger, W.; Terenzi, A. Self-assembled Pt2L2 boxes strongly bind G-quadruplex DNA and influence gene expression in cancer cells. *Dalton Trans.* **2017**, *46*, 329-332.
- (17) Eskandari, A.; Kundu, A.; Ghosh, S.; Suntharalingam, K. A Triangular Platinum(II) Multinuclear Complex with Cytotoxicity Towards Breast Cancer Stem Cells. *Angew. Chem., Int. Ed.* **2019**, *58*, 12059-12064
- (18) Hrabina, O.; Malina, J.; Kostrhunova, H.; Novohradsky, V.; Pracharova, J.; Rogers, N.; Simpson, D.H.; Scott, P.; Brabec, V. Optically Pure Metallohelices That Accumulate in Cell Nuclei, Condense/Aggregate DNA, and Inhibit Activities of DNA Processing Enzymes *Inorg. Chem.* **2020**, *59*, 3304-3311
- (19) Adamski, A.; Fik, M. A.; Kubicki, M.; Hnatejko, Z.; Gurda, D.; Fedoruk-Wyszomirska, A.; Wyszko, E.; Kruszka, D.; Dutkiewicz, Z.; Patroniak, V. Full characterization and cytotoxic activity of new silver(I) and copper(I) helicates with quaterpyridine *New J. Chem.* **2016**, *40*, 7943-7957.
- (20) Qin, H.; Zhao, C.; Sun, Y.; Ren, J.; Qu, X. Metallo-supramolecular Complexes Enantioselectively Eradicate Cancer Stem Cells in Vivo *J. Am. Chem. Soc.* **2017**, *139*, 16201-16209.
- (21) Hannon, M. J.; Moreno, V.; Prieto, M.J.; Molderheim, E.; Sletten, E.; Meistermann, I.; Isaac, C. J.; Sanders K. J.; Rodger, A. Intramolecular DNA coiling mediated by a metallo supramolecular cylinder. *Angew. Chem., Intl. Ed.*, **2001**, *40*, 879-884.
- (22) Meistermann, I.; Moreno, V.; Prieto, M. J.; Moldrheim, E.; Sletten, E.; Khalid, S.; Rodger, P. M.; Peberdy, J. C.; Isaac, C. J.; Rodger, A.; Hannon, M. J. Intramolecular DNA coiling mediated by metallo-supramolecular cylinders: differential binding of P and M helical enantiomers. *Proc. Natl. Acad. Sci., USA* **2002**, *99*, 5069-5074.
- (23) Ducani, C.; Leczkowska, A.; Hodges, N. J.; Hannon, M. J. Noncovalent DNA-Binding Metallo-Supramolecular Cylinders Prevent DNA Transactions in vitro. *Angew. Chem. Intl. Ed.* **2010**, *49*, 8942-8945.
- (24) Oleksi, A.; Blanco, A.G.; Boer, R.; Usón, I.; Aymami, J.; Rodger, A.; Hannon, M.J.; Coll, M. Molecular recognition of a three-way DNA junction by a metallo-supramolecular helicate. *Angew. Chem. Intl. Ed.* **2006**, *45*, 1227-1231.
- (25) Phongtongpasuk, S.; Paulus, S.; Schnabl, J.; Sigel, R. K. O.; Spingler, B.; Hannon, M. J.; Freisinger, E. Binding of a Designed Anti-Cancer Drug to the Central Cavity of an RNA Three-Way Junction. *Angew. Chem. Intl. Ed.* **2013**, *52*, 11513-11516.
- (26) Boer, D. R.; Kerckhoffs, J. M. C. A.; Parajo, Y.; Pascu, M.; Uson, I.; Lincoln, P.; Hannon, M. J.; Coll, M. Towards functionalizable DNA frames: Self assembly of two-component 3D DNA arrays through induction of DNA three-way junction branch points by supramolecular cylinders. *Angew. Chem. Intl. Ed.* **2010**, *49*, 2336-2339.
- (27) Cerasino, L.; Hannon, M. J.; Sletten, E. DNA Three-Way Junction with a Dinuclear Iron(II) Supramolecular Helicate at the Center: A NMR structural study, *Inorg. Chem.* **2007**, *46*, 6245-6251.
- (28) Malina, J.; Hannon, M. J.; Brabec, V. Recognition of DNA Three-Way Junctions by Metallo-supramolecular Cylinders: Gel Electrophoresis Studies *Chem. Eur. J.* **2007**, *14*, 3871-3877.
- (29) Martinez, C.R.; Iverson, B.L. Rethinking the term "pi-stacking". *Chem. Sci.* **2012**, *3*, 2191-2201.
- (30) Duskova, K.; Lamarche, J.; Amor, S.; Caron, C.; Queyriaux, N.; Gaschard, M.; Penouilh, M.-J.; de Robillard, G.; Delmas, D.; Devillers, C.H.; Granzhan, A.; Teulade-Fichou, M.-P.; Chavarot-Kerlidou, M.; Therrien, B.; Britton, S.; Monchaud D. Identification of three-way DNA junction ligands through screening of chemical libraries and validation by complementary in vitro assays. *J. Med. Chem.* **2019**, *62*, 4456-66.
- (31) Duskova, K.; Lejault, P.; Benchimol, É.; Guillot, R.; Britton, S.; Granzhan, A.; Monchaud, D. DNA junction ligands trigger DNA damage and are synthetic lethal with DNA repair inhibitors in cancer cells. *J. Am. Chem. Soc.* **2020**, *142*, 424-35.
- (32) van Rixel, V. H. S.; Busemann, A.; Wissingh, M. F.; Hopkins, S. L.; Siewert, B.; van de Griend, C.; Siegler, M. A.; Marzo, T.; Papi, F.; Ferraroni, M.; Gratteri, P.; Bazzicalupi, C.; Messori, L.; Bonnet, S. Induction of a Four-Way Junction Structure in the DNA Palindromic Hexanucleotide 5'-d(CGTACG)-3' by a Mononuclear Platinum Complex. *Angew. Chem., Int. Ed.* **2019**, *58*, 9378-9382.
- (33) Chien, C.-M.; Wu, P.-C.; Satange, R.; Chang, C.-C.; Lai, Z.-L.; Hagler, L.D.; Zimmerman, S.C.; Hou, M.-H. Structural Basis for Targeting T:T Mismatch with Triaminotriazine- Acridine Conjugate Induces a U-Shaped Head-to-Head Four-Way Junction in CTG Repeat DNA. *J. Am. Chem. Soc.* **2020**, *142*, 11165-11172
- (34) Brogden, A. L.; Hopcroft, N. H.; Searcey, M.; Cardin, C. J. Ligand bridging of the DNA Holliday junction: molecular recognition of a stacked-X four-way junction by a small molecule. *Angew. Chem., Int. Ed.* **2007**, *46*, 3850-3854.
- (35) Zhu, J.; Haynes, C.J. E.; Kieffer, M.; Greenfield, J.L.; Greenhalgh, R.D.; Nitschke, J.R.; Keyser, U.F. Fe^{II}₄L₄ Tetrahedron Binds to Nonpaired DNA Bases. *J. Am. Chem. Soc.* **2019**, *141*, 11358-11362.
- (36) Hotze, A. C. G.; Hodges, N. J.; Hayden, R. E.; Sanchez-Cano, C.; Paines, C.; Male, N.; Tse, M.-K.; Bunce, C. M.; Chipman, J. K.; Hannon, M. J. A supramolecular triple-stranded iron cylinder with unprecedented DNA binding action is a potent cytostatic and apoptotic agent without exhibiting genotoxicity. *Chem. Biol.* **2008**, *15*, 1258-1267.
- (37) Cardo, L.; Nawroth, I.; Cail, P. J.; McKeating, J. A.; Hannon, M. J. Metallo supramolecular cylinders inhibit HIV-1 TAR-TAT complex formation and viral replication in cellulose. *Sci. Rep.* **2018**, *8*, 13342 (2018).
- (38) Pope, A. J.; Bruce, C.; Kysela, B.; Hannon, M. J. Issues surrounding standard cytotoxicity testing for assessing activity of non-covalent DNA-binding metallo-drugs. *Dalton Trans.* **2010**, *39*, 2772-2774.
- (39) Cardo, L.; Sadovnikova, V.; Phongtongpasuk, S.; Hodges, N. J.; Hannon, M. J. Arginine conjugates of metallo-supramolecular cylinders prescribe helicity and enhance DNA junction binding and cellular activity. *Chem. Commun.* **2011**, *47*, 6575 - 6577.
- (40) Pérez, E. M. Putting Rings around Carbon Nanotubes. *Chem. Eur. J.* **2017**, *23*, 12681-12689.
- (41) Barrejón, M.; Mateo-Alonso, A.; Prato, M. Carbon Nanostructures in Rotaxane Architectures. *Eur. J. Org. Chem.* **2019**, 3371-3383.
- (42) Xu, Y.; Kaur, R.; Wang, B.; Minameyer, M.B.; Gsaenger, S.; Meyer, B.; Drewello, T.; Guldi, D.M.; von Delius, M. Concave-Concave π - π Template Approach Enables the Synthesis of [10]Cycloparaphenylene-Fullerene [2]Rotaxanes *J. Am. Chem. Soc.* **2018**, *140*, 13413-13420.
- (43) Ackermann, D.; Schmidt, T.L.; Hannam, J.S.; Purohit, C.S.; Heckel, A.; Famulok, M. A double-stranded DNA rotaxane. *Nat. Nanotechnol.* **2010**, *5*, 436-442.
- (44) Moretto, A.; Menegazzo, I.; Crisma, M.; Shotton, E.J.; Nowell, H.; Mammi, S.; Toniolo, C. A Rigid Helical Peptide Axle for a [2]Rotaxane Molecular Machine. *Angew. Chem. Int. Ed.* **2009**, *48*, 8986-8989
- (45) Yang, X. R.; Liu, F. B.; Zhao, Z. Y.; Liang, F.; Zhang, H. J.; Liu, S. M. Cucurbit[10]uril-based chemistry. *Chinese Chem. Lett.* **2018**, *29*, 1560-1566.
- (46) Liu, S. M.; Zavalij, P. Y.; Isaacs, L. Cucurbit[10]uril. *J. Am. Chem. Soc.* **2005**, *127*, 16798-16799.
- (47) Shetty, D. P.; Khedkar, J. K.; Park, K. M.; Kim, K. Can we beat the biotin-avidin pair?: cucurbit[7]uril-based ultrahigh affinity host-guest complexes and their applications. *Chem. Soc. Revs.* **2015**, *44*, 8747-8761.
- (48) Isaacs, L. Stimuli Responsive Systems Constructed Using Cucurbit[n]uril-Type Molecular Containers. *Acc. Chem. Res.* **2014**, *47*, 2052-2062.

- (49) Barrow, S. J.; Kasera, S.; Rowland, M. J.; del Barrio, J.; Scherman, O. A. Cucurbituril-Based Molecular Recognition. *Chem. Rev.* **2015**, *115*, 12320-12326.
- (50) Assaf, K. I.; Nau, W. M. Cucurbiturils: from synthesis to high-affinity binding and catalysis. *Chem. Soc. Rev.* **2015**, *44*, 394-418.
- (51) Murray, J.; Kim, K.; Ogoshi, T.; Yao, W.; Gibb, B. C. The aqueous supramolecular chemistry of cucurbit[n]urils, pillar[n]arenes and deep-cavity cavitands. *Chem. Soc. Rev.* **2017**, *46*, 2479-2496.
- (52) Cheng, Q.; Yin, H.; Rosas, R.; Gimes, D.; Ouari, O.; Wang, R.; Kermagoret, A.; Bardelang, D. A pH-driven ring translocation switch against cancer cells. *Chem. Commun.* **2018**, *54*, 13825-8.
- (53) See for example Villarroel-Lecourt, G.; Carrasco-Carvajal, J.; Andrade-Villalobos, F.; Solis-Egana, F.; Merino-San Martin, I.; Robinson-Duggon, J.; Fuentealba, D. Encapsulation of Chemotherapeutic Drug Melphalan in Cucurbit[7]uril: Effects on Its Alkylating Activity, Hydrolysis, and Cytotoxicity. *ACS Omega* **2018**, *3*, 8337-8343.
- (54) Gong, W. J.; Yang, X. R.; Zavalij, P. Y.; Isaacs, L.; Zhao, Z. Y.; Liu, S. M. From Packed "Sandwich" to "Russian Doll": Assembly by Charge-Transfer Interactions in Cucurbit[10]uril. *Chem., Eur. J.* **2016**, *22*, 17612-17618.
- (55) Kuang, S.; Hu, Z.; Zhang, H.; Zhang, X.; Liang, F.; Zhao, Z.; Liu, S.M. Enhancement of metal-metal interactions inside a large-cavity synthetic host in water. *Chem. Commun.* **2018**, *54*, 2169-2171.
- (56) Liu, S. M.; Shukla, A. D.; Gadde, S.; Wagner, B. D.; Kaifer, A. E.; Isaacs, L. Ternary complexes comprising cucurbit[10]uril, porphyrins, and guests. *Angew. Chem. Intl. Ed.* **2008**, *47*, 2657-2660.
- (57) Yu, Y.; Li, Y. W.; Wang, X. Q.; Nian, H.; Wang, L.; Li, J.; Zhao, Y. X.; Yang, X. R.; Liu, S. M.; Cao, L. P. Cucurbit[10]uril-Based [2]Rotaxane: Preparation and Supramolecular Assembly-Induced Fluorescence Enhancement. *J. Org. Chem.* **2017**, *82*, 5590-6.
- (58) Masson, E.; Raesi, M.; Kotturi, K. Kinetics Inside, Outside and Through Cucurbiturils. *Isr. J. Chem.* **2018**, *58*, 413 - 434.
- (59) Wang, S.R.; Wang, J.Q.; Xu, G.H.; Wei, L.; Fu, B.S.; Wu, L.Y.; Song, Y.Y.; Yang, X.R.; Li, C.; Liu, S.M.; Zhou, X. The Cucurbit[7]uril-Based Supramolecular Chemistry for Reversible B/Z-DNA Transition. *Adv. Sci.* **2018**, *5*, 1800231.
- (60) Wu, H.; Chen, H.; Tang, B.; Kang, Y.; Xu, J.-F.; Zhang, X. Host-Guest Interactions between Oxaliplatin and Cucurbit[7]uril/ Cucurbit[7]uril Derivatives under Pseudo-Physiological Conditions. *Langmuir* **2020**, *36*, 1235-4.
- (61) Chen, Y.; Huang, Z.; Zhao, H.; Xu, J.F.; Sun, Z.; Zhang, X. Supramolecular Chemotherapy: Cooperative Enhancement of Antitumor Activity by Combining Controlled Release of Oxaliplatin and Consuming of Spermine by Cucurbit[7]uril. *ACS Appl. Mater. Interfaces* **2017**, *9*, 8602-8.
- (62) Cao, L.; Hettiarachchi, G.; Briken, V.; Isaacs, L. Cucurbit[7]uril Containers for Targeted Delivery of Oxaliplatin to Cancer Cells. *Angew. Chem., Intl. Ed.* **2013**, *52*, 12033-12037.
- (63) Jin Jeon, Y.; Kim, S.-Y.; Ho Ko, Y.; Sakamoto, S.; Yamaguchi, K.; Kim, K. Novel Molecular Drug Carrier: Encapsulation of Oxaliplatin in Cucurbit[7]uril and Its Effects on Stability and Reactivity of the Drug. *Org. Biomol. Chem.* **2005**, *3*, 2122.
- (64) Wheate, N. J. Improving Platinum(II)-Based Anticancer Drug Delivery Using Cucurbit[n]urils. *J. Inorg. Biochem.* **2008**, *102*, 2060-6.
- (65) Bali, M.S.; Buck, D.P.; Coe, A.J.; Day, A.I.; Collins, J.G. Cucurbituril binding of trans- $[\{\text{PtCl}(\text{NH}_3)_2\}_2(\mu\text{-NH}_2(\text{CH}_2)_8\text{NH}_2)]^{2+}$ and the effect on the reaction with cysteine. *Dalton Trans.* **2006**, *45*, 5337-5344.
- (66) Li, F.; Gorle, A. K.; Ranson, M.; Vine, K. L.; Kinobe, R.; Feterl, M.; Warner, J. M.; Keene, F. R.; Collins, J. G.; Day, A. I. Probing the Pharmacokinetics of Cucurbit[7, 8 and 10]uril: And a Dinuclear Ruthenium Antimicrobial Complex Encapsulated in Cucurbit[10]uril. *Org. Biomol. Chem.* **2017**, *15*, 4172-9.
- (67) Sun, B.; Musgrave, I. F.; Day, A. I.; Heimann, K.; Keene, F. R.; Collins, J. G. Eukaryotic Cell Toxicity and HSA Binding of $[\text{Ru}(\text{Me}_4\text{phen})(\text{Bb}7)]^{2+}$ and the Effect of Encapsulation in Cucurbit[10]uril. *Front. Chem.* **2018**, *6*, 00595.
- (68) Harris D.H. *Quantitative Chemical Analysis*, 9th Edition, **2015**, WH Freeman & Co., New York.
- (69) McGonigal, P.R. Multiply threaded rotaxanes. *Supramol. Chem.* **2018**, *30*, 782-794.
- (70) Danon, J.J.; Leigh, D.A.; McGonigal, P.R.; Ward, J.W.; Wu, J. Triply Threaded [4]Rotaxanes. *J. Am. Chem. Soc.* **2016**, *138*, 12643-12647.
- (71) Cheng, H.M.; Leigh, D.A.; Maffei, F.; McGonigal, P.R.; Slawin, A.M.Z.; Wu, J. En Route to a Molecular Sheaf: Active Metal Template Synthesis of a [3]Rotaxane with Two Axles Threaded through One Ring. *J. Am. Chem. Soc.* **2011**, *133*, 12298-12303.
- (72) Wang, X.; Smithrud, D.B. Pt-rotaxanes as cytotoxic agents. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6880-3.
- (73) Sojka, M.; Fojtu, M.; Fialova, J.; Masarik, M.; Necas, M.; Marek, R. Locked and Loaded: Ruthenium(II)-Capped Cucurbit[n]uril-Based Rotaxanes with Antimetastatic Properties. *Inorg. Chem.* **2019**, *58*, 10861-70.
- (74) Barat, R.; Legigan, T.; Tranoy-Opalinski, I.; Renoux, B.; Péraudeau, E.; Clarhaut, J.; Poinot, P.; Fernandes, A. E.; Aucagne, V.; Leigh, D. A.; Papot, S. A mechanically interlocked molecular system programmed for the delivery of an anticancer drug. *Chem. Sci.* **2015**, *6*, 2608-2613.
- (75) Fernandes, A.; Viterisi, A.; Coutrot, F.; Potok, S.; Leigh, D.A.; Aucagne, V.; Papot, S. Rotaxane-Based Propeptides: Protection and Enzymatic Release of a Bioactive Pentapeptide. *Angew. Chem. Intl. Ed.* **2009**, *48*, 6443-6447.
- (76) Fernandes, A.; Viterisi, A.; Aucagne, V.; Leigh, D. A.; Papot, S. Second generation specific-enzyme-activated rotaxane propeptides. *Chem. Commun.* **2012**, *48*, 2083-2085.
- (77) Thompson, S.J.; Thompson, S.E.M.; Cazier, J.-B. CaStLeS (Compute and Storage for the Life Sciences): a collection of compute and storage resources for supporting research at the University of Birmingham; DOI 10.5281/zenodo.3250616.
- (78) Coles, S. J.; Gale, P. A. Changing and Challenging Times for Service Crystallography. *Chem. Sci.* **2012**, *3*, 683-9.

