Hydrogen-bonded rotaxanes: structure and dynamics of mechanically interlocked molecular shuttles
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CHAPTER 1

Towards Fluorescent Molecular Shuttles

Abstract

Artificial molecular machines capable of converting chemical, photochemical or electrochemical energy into mechanical motion constitute a fascinating challenge in the field of nanoscience. These molecular scale architectures exploit a bottom-up approach, which depends on the design and construction of molecular assemblies that are able to transport efficient actuation on length scales much smaller than traditional microscale actuators.

Mechanically interlocked molecules such as rotaxanes are suitable candidates for molecular machines due to their ability to switch between two or more states as a result of induced relative movement of their noncovalently interacting components on application of external stimuli. The alteration of relative positions of the interlocked components constitutes a mechanical switch, possibly with varying physical properties such as conductivity, circular dichroism and fluorescence. The use of changes in fluorescence as an output signal attracts considerable attention since it allows detection of the state of the system with high time resolution and high sensitivity.
1.1 Mechanically interlocked molecules

The miniaturization race of the components of mechanical devices leads scientists to design and construct new molecular machines and motors at the nanometer scale. The fabrication of mechanical machines of molecular size is highly important not only for the growth of nanoscience, but also for the development of a bottom-up approach to nanotechnology. The inspiration for the design of topologically non-trivial molecules comes from the objects and artistry around us, the chemical literature, and abstract thought. Especially, the supramolecular architectures of the biological world are prime examples of the possibility and utility of nanotechnology towards artificial molecular machines.

In principle, molecular machines can be designed starting from several kinds of molecular and supramolecular systems, including DNA. Most of the systems constructed so far are based on mechanically interlocked molecules – those in which the molecules consist of two or more components that are connected together. Interlocked molecules such as catenanes, rotaxanes, knots and Borromean rings have captured the imagination of the scientific world for more than two decades. Rapid advances in the synthesis of mechanically-interlocked molecules in recent times have enabled precise control of the architectures and topologies of the molecules; hence, it has enabled chemists to develop specifically desired functions based on these unique structures. For instance, the application of switchable, mechanically-interlocked, small molecules has been demonstrated in solid-state electronic devices, mechanised nanoparticles, and nano-electromechanical systems.

Catenanes (from the Latin catena meaning chain) are composed of mutually interlocked rings, whereas rotaxanes (from the Latin rota and axis, meaning wheel and axle, respectively) contain a linear dumbbell-shaped component – comprised of a central rod section terminated by bulky end groups or stoppers – around which one or more rings are trapped in an abacus-like manner. In the absence of sterically bulky end groups (stoppers) at both ends of the thread/axle component, rotaxanes become “pseudorotaxanes” which can equilibrate with the separated axle-type and ring components (Figure 1).

A) B) C)

Figure 1. Schematic representation of A) a catenane, B) a rotaxane and C) the threading-dethreading equilibrium involving the axle and ring components of a pseudorotaxane.
There are various kinds of rotaxanes such as [2]rotaxane, [1]rotaxane, Janus rotaxane and polyrotaxane (Figure 2).

![Figure 2. Cartoon representation of different types of rotaxane species.](image)

The importance and attraction of employing rotaxanes in the construction of molecular machines originates from the fact that (i) the mechanical bond facilitates a large variety of mutual arrangements of the molecular components whilst conferring stability to the system, (ii) the interlocked design limits the amplitude of the inter-component motion in the three dimensions, (iii) the stability of a specific arrangement (co-conformation) is determined by the strength of the intercomponent interactions, and (iv) such interactions can be modulated by external stimulation. Rotaxanes are good prototypes for the fabrication of both linear and rotary molecular machines due to the two interesting molecular motions, namely, reversible displacement of the ring along the axle, and rotation of the ring around the axle (Figure 3A and Figure 3B, respectively). The systems of the first type constitute one of the most common implementations of the molecular machine concept and are termed molecular shuttles.

![Figure 3. Schematic representation of the intercomponent motions that can occur in a rotaxane: A) ring shuttling and B) rotation.](image)

### 1.2 Template-Directed Synthesis

The most important advance in the science of mechanically interlocked compounds has been the advent of template-directed synthesis. It is almost impossible to give a concise definition for the term “template” which accounts for all chemistry to which it has been related. Templates cover the whole range from biochemistry with its complex apparatus for DNA replication to biomineralization and the formation of structured inorganic materials to the covalently templated synthesis of macrocycles to the preparation of supramolecular catalyst or host-guest complexes and to metal organic reactions for the synthesis of small ring systems. However, all these have in common that a template...
must serve different purposes. (i) It organizes reaction partners in such a way that a desired product is formed that would not form easily in the absence of the template. Therefore, a template controls reactivity through organizing the reaction partners in a favorable arrangement. (ii) In order to achieve this, the template needs to bind to the reaction partners. Molecular recognition is thus a necessary requirement for template synthesis and the binding sites of the components must be complementary to each other. (iii) The control of reactivity and the recognition of the reaction partners imply that information is stored in the template and transferred to the product of the reaction. Hence, the third important aspect is information transfer. In fulfilling these three tasks, templates facilitate to tame molecular complexity and provide access to otherwise inaccessible products. Donor-acceptor,\textsuperscript{22, 23} metal-ligand,\textsuperscript{24} electrostatic\textsuperscript{25} or π-π interactions\textsuperscript{26} play a fundamental part in the template driven processes of molecular recognition\textsuperscript{27, 28} and self-assembly.\textsuperscript{29-31}

Several different methods have been devised for the synthesis of rotaxanes, which are categorized mainly as kinetic and thermodynamic approaches (Figure 4).\textsuperscript{32} The kinetic method requires an irreversible process for the construction of the rotaxane in the final step (Figure 4, 1-3), whereas the thermodynamic one is based on an equilibrium to form the rotaxane in the final step (Figure 4, 4-6). Among them, the most straightforward process is the clipping approach,\textsuperscript{33} in which the interlocked structure is obtained by macrocyclization of an acyclic ligand around the template site of an already stoppered thread.

\textbf{Figure 4.} Cartoon representation for typical synthetic methods of rotaxanes.\textsuperscript{34}
1.3 Design of Molecular Shuttles

In classical template syntheses of rotaxanes the inter-component recognition motif included in the template process ‘lives on’ in the product, affording a preferred location or binding site for the ring on the thread.14 When two identical sites (‘stations’) are included in the dumbbell component of the rotaxane, the macrocyclic ring can shuttle back and forth between the two stations.20, 35 Such a system constitutes a degenerate molecular shuttle (Figure 5).35, 36

Figure 5. A) The two co-conformations associated with a degenerate rotaxane, B) Representation of the potential energy of the system as a function of position of the ring along the axis. The number of dots in each potential well corresponds to the relative population of the respective co-conformation in a statistically distribution ensemble.

If two recognition sites with very different binding affinities can be grafted into the linear portion of the dumbbell, however, a rotaxane can exist as two different equilibrating co-conformations, whose populations reflect their relative free energies as determined by the strengths of noncovalent binding interactions in the two different forms (Figure 6).

According to the classical stereochemical definition, the term “conformation” describes “the spatial arrangement of the atoms affording distinction between stereoisomers which can be interconverted by rotations about formally single bonds”. Some authorities extend the term to include inversion at trigonal pyramidal centres and other polytopal rearrangements.37 However, in the field of supramolecular chemistry the competitive noncovalent interactions between the components lead to molecular motions that are not restricted to the atoms. Instead, the molecular motions affect parts of an interlocked molecule, which can move from one recognition site to another, depending on the binding affinities of the recognition units. Therefore, in order to describe the translational isomers
of mechanically interlocked molecules the term “co-conformation” was introduced. In a wider sense, it describes the spatial position of one mechanically interlocked component, which can move along or around the other “stationary” component.

In the schematic representation illustrated in Figure 6, it has been assumed that the ring preferentially locates over the station A (state 0) until a stimulus is applied which switches off the stronger of the two recognition sites. As a result, the system is brought into a non-equilibrium state that subsequently relaxes in line with the new potential energy landscape. This implies that the macrocycle moves to the second weaker recognition site (station B) until a new equilibrium is reached (state 1). Upon application of another stimulus, the original potential energy landscape is restored, and hence another conformational equilibration occurs through the shuttling of the ring back to station A. State 0 and state 1 are often referred to as ground state and metastable state co-conformations of rotaxanes. By switching the recognition properties of one of the two recognition sites off and on again, the relative proportions of the two species can be controlled. In appropriately designed systems, the switching process can be controlled reversibly using a wide variety of different stimuli including pH changes, competitive binding, redox control, and light.

Light is one of the most attractive means of supplying energy to nanodevices, including molecular machines. Photochemically powered molecular switches can be driven in a waste-free manner, and their introduction into molecular electronic and nanoelectronic materials can have a remarkable effect due to their properties related to photo-to-mechanical energy transduction. Just like electrons, photons can induce (write) and detect (read) molecular actuations. Besides, luminescent output can respond very quickly, can be of low cost for the detection of photons and can be highly sensitive and easily carried out in a small place. Light-driven translational motion has been accomplished in rotaxanes with use of photo-induced electron transfer (ET), excited-state changes in hydrogen-bonding and photoisomerization (cis-trans) of double bonds. The alternation of relative positions of the interlocked components constitutes a molecular switch, which may be capable of read out using various physical properties of the rotaxane such as conductivity, circular dichroism and fluorescence. Utilizing fluorescence change as an output signal is preferable since it allows detecting the state of the system with high time resolution and high sensitivity.
Figure 6. Schematic operation of a two-station rotaxane as a controllable molecular shuttle, and representation of the potential energy of the system as a function of the position of the ring relative to the axle upon switching off and on station A. The number of dots in each position reflects the relative population of the corresponding co-conformation in a statistically distributed ensemble. Structures A) and C) correspond to equilibrium states, whilst B) and D) are metastable states.

1.4 Fluorescent Rotaxanes

For switchable rotaxanes containing chromophores, the displacement of the ring upon application of an external energy input alters the relative orientations of the two components of the interlocked molecule, which subsequently leads to changes in processes such as electron transfer or energy transfer. Since the fluorescent signal provides many advantages as mentioned above, light-driven molecular machines employing fluorescent outputs have received great attention in recent years. However, reports based on rotaxanes that can be induced to switch between two different fluorescent states are rare because of the lengthy synthesis and the difficulty of predicting the photophysical properties accurately. Photo-excitation of the luminescent units
included either in the dumbbell or in the macrocycle of a rotaxane engenders luminescent signals, which can be utilized for conformational identification. Co-conformational equilibria of rotaxanes can be investigated by utilizing either the enhancement or quenching of the fluorescence signal. In the following paragraphs, bistable rotaxanes will be discussed based on the types of the fluorescence outputs used to monitor the shuttling processes. For example, fluorescent [2]- and [3]-rotaxanes, 1 and 2, containing a strongly fluorescent perylene diimide group were reported by Baggerman et al. in which the hydrogen-bonding interactions between the amide groups of the macrocycle and the carbonyls of the imide change the optical spectroscopic properties of the perylene chromophore (Figure 7). The absorption and emission spectra are red shifted and the fluorescence lifetimes of the rotaxanes are shortened compared to those of the corresponding threads. Furthermore, the extents of shifts in the rotaxane are dependent on polarity and hydrogen bond accepting ability of the solvents due to the competing interactions of the two components of the rotaxane with the solvent molecules.

The Loeb group has recently reported the synthesis of two bistable molecular shuttles combining 1,2-bis(pyridinium)ethane and benzylanilinium as recognition sites and a dibenzo-24-crown-8 ether (DB24C8) wheel in which the acid-base controlled shuttling of DB24C8 was accompanied by a change in color and/or fluorescence intensity. This motif combines acid/base control of the anilinium template and the strong N–H…O hydrogen-bonding with the enhanced π-stacking properties found for the recognition of 1,2-bis(pyridinium)ethane dications. The addition of acid (CF₃SO₃H) to 3⁺ leads to the displacement of the DB24C8 unit from 1,2-bis(pyridinium)ethane to the benzylanilinium recognition site (Figure 8). The shuttle is returned to its original state upon addition of base (NEt₃) and the shuttling process can be cycled by repeated addition of acid and base.
without any degradation of the compound. This implies that the molecular shuttle 3 behaves like a bistable ON/OFF switch. As designed, the molecular shuttle $\text{H-3}^{3+}$ includes a 4-pyridinium aniline ICT chromophore. Therefore, the switching between $3^{2+}$ and $\text{H-3}^{3+}$ is accompanied by a vivid color change from bright orange to colorless. Exchanging the 3,5-bis-trifluoromethyl)benzyl group for an anthracenyl group introduces the ability to monitor the molecular shuttling by fluorescence spectroscopy. The fluorescence spectrum of the compound shows stunted emission presumably due to the quenching of fluorescence by lone pair electrons of the aniline nitrogen. Protonation partially restores normal anthracene fluorescence.

![Figure 8. Acid/base controlled shuttling in compound 3.](image)

Another very interesting approach to obtain switchable rotaxanes based on fluorescent units takes advantage of the translocation of a ring along a peptide-based thread, induced by changes in the nature of the local environment. On the basis of the experience gained by the solvent driven molecular shuttles, bistable rotaxanes 4 and 5 were specifically constructed in which translational isomerism of the components can be controlled to either permit or preclude fluorescence quenching by intercomponent electron transfer (Figure 9). Rotaxanes 4 and 5 consists of an anthracene fluorophore (which also acts as a stopper) connected to a glycylglycine hydrogen-bonding station, which, in turn, is attached to a C11 alkyl chain terminated by a second stopper. Double protonation of 5 with excess of trifluoroacetic acid results in the formation of $5\cdot2\text{H}^+\cdot2\text{CF}_3\text{CO}_2^-$. The nitrophenyl and pyridinium moieties in the macrocycles in 4 and 5·2H⁺, respectively, are known to quench the fluorescence of the anthracene unit via distance-dependent electron transfer. In nonpolar solvents, in which the intercomponent hydrogen-bonding interactions are the strongest, the anthracene fluorescence is virtually completely quenched, whereas in solvents with a strong solvophobic profile for alkyl chains such as DMSO, UV-stimulated fluorescence is visible at submicromolar concentrations.
Recently, it has been demonstrated that FRET labeling can also be used to detect small motions in mechanically interlocked molecules. Li et al. reported a fluorescence switch in which the light induced shuttling of the macrocycle along the thread produced changes of the interaction between the two chromophores in the thread. The molecular shuttle \( E_6/Z_6 \) consists of a benzylic amide macrocycle mechanically interlocked onto a thread molecule \( E_7/Z_7 \), featuring two potential hydrogen-bonding stations- a fumaramide group and a succinic amide ester unit separated by \( C_{12} \) aliphatic spacer (Figure 10). Highly fluorescent perylene bisimide and pyrene chromophores are attached to fumaramide and succinic amide ester groups, respectively. The macrocycle contains pyridine units, which are good fluorescence quenchers of the pyrene after protonation. The relative orientation of the pyrene and perylene bisimide transition dipole moments have a great influence on energy transfer and electron transfer processes. Therefore, a molecular shuttle with a macrocycle to control the energy and/or electron transfer between the perylene and pyrene moieties can be designed with proper spacers to control the distance and orientation of the fluorescent units. For rotaxanes \( E_6 \) and \( Z_6 \), the macrocycle precludes intramolecular folding of the thread, subsequently the photoinduced electron transfer from perylene to pyrene is slowed down and the emission of the perylene moiety is enhanced compared with that of the thread \( E_7 \) and \( Z_7 \) (Figure 11). On the other hand, in rotaxane \( Z_6 \), the protonation of the pyridine units in the macrocycle leads to quenching of the emission of the pyrene chromophore through electron transfer from pyrene to the pyridinium ions. As a result, the energy transfer from pyrene to perylene moiety is suppressed, and the fluorescence of the perylene unit becomes weaker.
As shown by the examples described above, a modular construction of molecular machines pursued by integrating fluorescent probes and mechanically switchable systems in a molecular assembly is a quite demanding task. In fact, the majority of artificial molecular machines controlled by molecular shuttles reported so far are operated only as on/off switches and not in an adjustable multilevel mode. The availability of robust artificial switches which exhibit a single signal at desired levels in response to fixed doses of different inducers rather than minute concentration changes of a single inducer would be more desirable in the field of biotechnology. Recently, Li and co-workers have...
designed and constructed a multistable system to explore the properties and operational mechanisms of this kind of artificial shuttles as a prologue to optimizing their performance (Figure 12).\textsuperscript{59} Rotaxane 8 is a multilevel fluorescence switch in which acid/base- and metal-ion complexation/decomplexation induced shuttling of the macrocycle along the thread impels changes in the interaction between the macrocycle and the fluorescent chromophore in the thread. The molecular shuttle comprises a thread containing an anthracene group as a fluorescent chromophore and an aniline-containing oligo(ethylene glycol) macrocycle mechanically interlocked on the thread. A secondary dialkylammonium (-NH$_2$\textsuperscript{+}) and an amide center are located on the thread as hydrogen-bonding stations. At low pH, the macrocycle is assembled around an ammonium cation center through a combination of strong N’-H…O hydrogen bonds and weak C-H…O interactions between the macrocycle and the thread. Addition of base results in the deprotonation of the ammonium recognition site, which causes the macrocycle to move to the amide recognition site. On the other hand, upon addition of Li\textsuperscript{+} ions, the conformation of [2]rotaxane can be altered by a combination of Li-oligo(ethylene glycol) interactions between the amide and pyridine groups. However, addition of Zn$^{2+}$ ions leads to a complexation of pyridine/aniline moiety in the macrocycle and the amine in the thread, and, as a result the macrocycle moves back to the amide side. These diverse processes are all accompanied by different fluorescent responses. As illustrated in Figure 13, rotaxane 8 enables multi-level expression of fluorescence in response to different triggers: (1) complete repression in the absence of any stimulus; (2) slight expression in response to base; (3) low-level expression following addition of Li$^+$; (4) high-level expression in the presence of Zn$^{2+}$.

\textbf{Figure 12.} Graphical representation of the movement process in the multistable molecular shuttle 8.\textsuperscript{59}
Another example for a [2]rotaxane with dual fluorescence address is rotaxane 9 containing an α-cyclodextrin (α-CD) macrocycle, an azobenzene unit, and two different fluorescent naphthalimide fluorophores, as shown in Figure 14.68 CDs have been attractive wheel components in the fabrication of rotaxanes because of their challenging constructions and potential applications in fields such as biomedical materials, nanostructured functional materials and molecular devices. The cis-trans photoisomerization of the azobenzene unit resulted in the motion of α-CD on the thread. On account of the rigidity of the α-CD ring shifting close to the chromophore, which hinders the vibration and rotation of the bonds in the methylene linker, the fluorescence intensities of the chromophores nearer the CD rings are enhanced. Easy regulation and full reversibility of the fluorescence change intensity of the two fluorescent stoppers makes it possible for the rotaxane to be used as a molecular storage medium or switch with all optical inputs and outputs. However, it is of interest to note that this kind of switches is limited in contrast by the photostationary states that are formed: it is usually not possible to convert quantitatively to either E or Z isomers.
Figure 14. Light-driven [2]rotaxane 9 molecular shuttle with dual fluorescence (F_520 and F_395) addresses.

1.5 Scope of this Thesis

Compounds capable of performing large amplitude, non-trivial and controlled mechanical movements upon light stimulation can be obtained by using careful incremental design approaches, the tools of modern synthetic chemistry, and the paradigms of supramolecular chemistry, as well as some inspiration from natural systems. Such achievements allow the development of more sophisticated artificial molecular machines demonstrating complex motions and improved performances in terms of stability, speed, switching, and so forth.

The scope of the work presented in this thesis is to explore different approaches towards unraveling the shuttling dynamics of molecular shuttles based on hydrogen-bonded rotaxanes. Chapter 2 describes the influence of incorporating a new template (ni-gly) in naphthalimide/succinamide-based rotaxanes, which results in a distribution of rotaxane co-conformers in which the macrocycle does not encircle the succinamide station exclusively but also resides near the naphthalimide unit to a significant extent. The thermodynamic parameters show strong enthalpy-entropy compensation and a non-zero heat capacity difference between the two forms.
In Chapter 3, the preparation and dynamic behavior of degenerate rotaxanes are described in which a benzylic amide macrocyclic ring moves back and forth between two naphthalimide glycine (ni-gly) stations by making the passage along a rigid diphenyl ethyne or a flexible aliphatic spacer (C\textsubscript{9}, C\textsubscript{12}, C\textsubscript{26}). The effect of the spacer units on the rate of the shuttling process is evaluated. These kind of molecular shuttles can serve as models to study barriers to ring movement in the non-degenerate bistable [2]rotaxanes employed in molecular electronic devices.

Chapter 4 presents a molecular shuttle in which a benzylic amide macrocycle mechanically is interlocked onto a thread molecule consisting of naphthalimide and perylene imide chromophores as stoppers and fluorescent units, and glycine units positioned next to the chromophores as hydrogen-bonding sites. The macrocycle is predominantly hydrogen-bonded to the perylene imide chromophore in nonpolar solvents, but its position on the thread is strongly dependent on the solvent. \textsuperscript{1}H NMR spectroscopy was used as a tool to probe the position of the macrocycle on the thread as a function of solvent.

Chapter 5 provides details on the electronic spectroscopic properties of the molecular shuttle described in Chapter 4. The relative position of the macrocycle with respect to the solvatochromic perylene imide chromophore gives a strong response in the spectral domain. Hydrogen-bonding interactions between the macrocycle and the imide leads to changes in the optical spectroscopic properties of the perylene imide chromophore: the absorption and emission spectra are red shifted and the fluorescence lifetimes are shortened with respect to those of the corresponding thread. The effects of various solvents on the UV-Vis absorption, steady-state and time-resolved fluorescence spectra are discussed.

The work described in Chapter 6 is a continuation and extension of the work described in Chapter 5. As mentioned previously, the localization of the macrocycle next to the perylene imide chromophore alters the optical properties of the perylene unit. However, the main question remained unanswered: is it possible to use fluorescence spectroscopy to detect the shuttling process in this kind of systems? Here we address this issue by reporting the synthesis of a naphthalimide/ perylene imide-based rotaxane, which enables to monitor the shuttling behavior via fluorescence spectroscopy. When the macrocycle is hydrogen-bonded to the naphthalimide chromophore (ni co-conformer), spectroscopic properties of the rotaxane resemble those of the thread, whilst the localization of the macrocyclic ring in contact with the perylene imide chromophore (pery co-conformer) can be expected to give rise to a significant change. This makes it possible to obtain a complete picture of the shuttling process taking place after selective excitation of ni co-conformer by means of time-resolved fluorescence spectroscopy.
1.6 References

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