APPLICATIONS OF ROTAXANES IN DYNAMIC MEMORY, BIOELECTRONICS, AND MEMBRANE TRANSPORT

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INTRODUCTION

Interlocking molecules such as rotaxanes initially gained interest due to their interesting topology and synthetic challenge, but recent efforts have proven that they can be used in many important applications. A rotaxane is a molecule that contains a ring surrounding a rod portion in which the ends are large enough to prevent the ring from slipping off (Figure 1). One of the first syntheses of a rotaxane in 1967 was achieved in only 6% yield.[1] Since then, a methodology has been devised enabling their creation in near quantitative yield.[2] In general, the three main methods of preparing rotaxanes are clipping, threading, and slipping (Figure 1).[3] Clipping involves forming the ring around the preformed dumbbell component. During threading, the bulky blocking groups are attached to the ends of the axle after the ring has been threaded on it. Lastly in the slipping method, the preformed ring is forced over one of the blocking groups and onto a thermodynamically stable area on the axle.

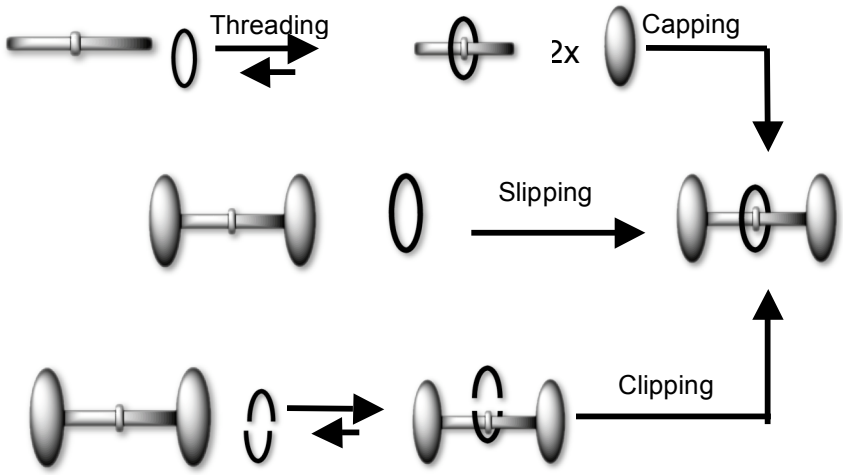

Figure 1. Assembly of a rotaxane

Because the ring is not covalently bound to the rod, it can move freely between the stoppers. The functionality and polarity of the end groups, ring, and rod can be altered allowing control over this movement. This unique feature has enabled chemists to devise numerous applications involving rotaxanes. Recent work has also been done on sensory polymers[4] and catalysis,[5] but these applications are in the early stages of development and will not be discussed here. Reported herein are the roles they can play in molecular electronics, bioelectronics, and cellular transport. These examples show very different ways in which the interactions between the two components of the molecule can be controlled to achieve a specific function.

REDOX ACTIVATED SWITCHES FOR DYNAMIC MEMORY STORAGE

The most frequently studied application of rotaxanes has been in the area of molecular switches.[6] The ring of a rotaxane is free to move along the rod component, but when functionality with an affinity for the ring component was installed on the rod, the ring's position could be controlled. Stoddart and coworkers showed that when two identical sites were installed on the rod, the ring shuttled back and forth between them.[6] When two different recognition sites with two different affinities were installed on the rod, a rotaxane was created where the ring spent more time on one site than the other. Stimuli have been applied that changed the ring's preference of recognition sites and thus the molecule behaved as a switch. Rotaxane switches have been activated through a variety of stimuli such as heat,[7] pH change,[8] and light.[9] Since none of these types of switches have been incorporated into devices, as have the redox-activated switches mentioned below have, they will not be discussed in this report. Also, because the field is rapidly progressing, a new rotaxane switch not yet used in a device will be discussed.

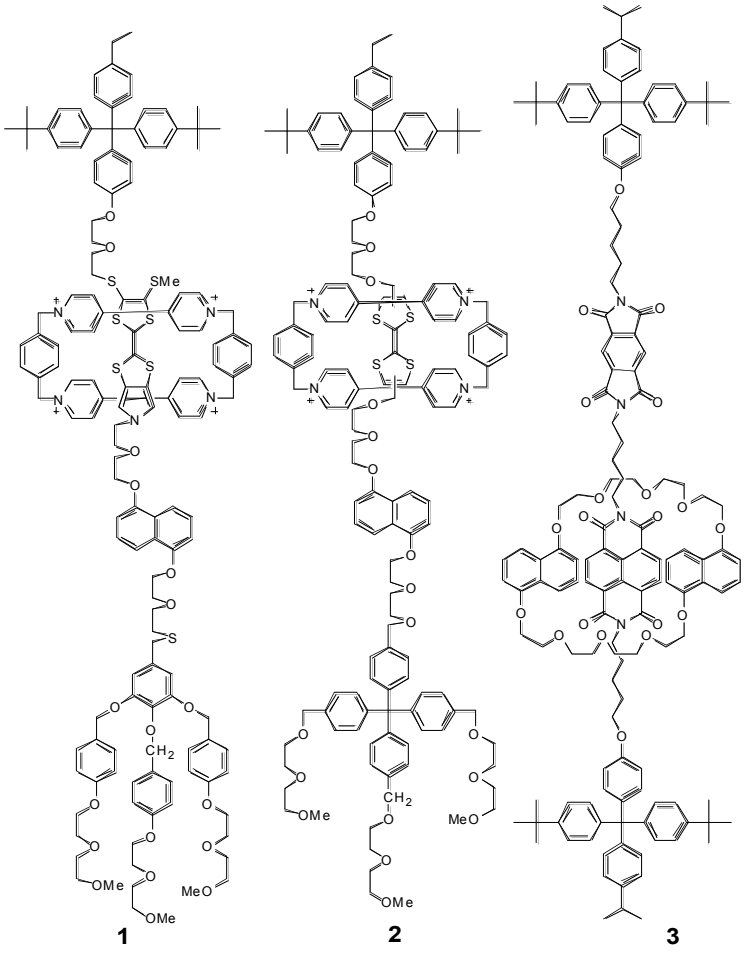

Figure 2. Rotaxanes synthesized for the use in molecular electronics

Some of the most recent switches have incorporated a tetrathiafulvalene derivative. This motif has been extensively studied and is commonly used in supramolecular chemistry.[10] Amphiphilic rotaxane (**1**) comprised of one monopyrrolotetrathiafulvalene (MPTFF) unit and one 1,5-dioxynapthalene (DNP) site on the rod along with a cyclobis(paraquat-*p*-phenylene) ($CBPQT^{4+}$) ring was constructed (Figure 2) using the clipping method by Stoddart and co-workers.[11] As observed by cyclic voltammetry, UV-vis spectroscopy, and ^1H NMR spectroscopy, switching occurred when MPTFF was oxidized while the ring was encircling it, which is designated as the ground state. Upon oxidation, a MPTFF radical cation was formed, and the ring, repelled by Coulombic forces, moved over to the DNP

site. Once the MPTFF unit was reduced back to its neutral starting state, the molecule existed as a metastable isomer isoelectronic to its ground state, the only difference being the location of the ring (Figure 3). This isomer presumably has a different conductivity than the ground state and thus makes these molecules useful in molecular electronics. The molecule can equilibrate back to the ground state by either thermal equilibration or by reduction and oxidation of the $CBPQT^{4+}$ ring. Unfortunately it was discovered that in acetonitrile at room temperature only half of the ground state material existed in the conformation having the ring encircling the MPTFF site. Therefore, only half of the rotaxanes are working as switches. Attempting to improve the ground state co-conformation ratio, Stoddart and co-workers synthesized rotaxane **2** (Figure 2) having a tetrathiafulvalene (TFF) unit instead of the MPTFF unit.[12] In addition to finding that the mechanism for switching was the same, they also observed that **2** existed solely as the co-conformations in which the ring encircled the TFF unit because it formed a tighter donor-acceptor complex than with MPTFF. Both **1** and **2** have been incorporated into devices as discussed below.

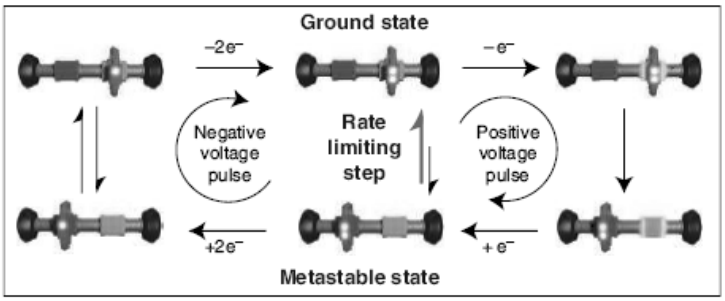

Figure 3. Switching mechanism[6]

Although TFF-containing switches have worked reasonably well, they still have problems associated with them. Firstly, any rotaxane with a $CBPQT^{4+}$ ring contains counter ions. Since it is unknown how counter ions affect the switching mechanism, their elimination would remove a level of uncertainty. Secondly, electrochemical and photophysical investigation gave evidence that in solution, and on monolayers at the air-water interface, there can be folded conformations of the rotaxanes where the hydrophilic chains on the stopper group, or the other recognition site, fold over to interact with the positively charged macrocyle (Figure 4). A neutral switch would presumably lessen these conformations. Stoddart, Sanders, and coworkers[13] have recently synthesized via slippage a neutral rotaxane (**3**) containing one pyromellitic diimide (PmI) unit and one 1,4,5,8-napthalene-tetracarboxylate diimide (NpI) site along with a 1,5-dinaphtho[38]crown-10 ring (Figure2). ^{1}H NMR analysis of rotaxane **3** showed that the crown ether resides primarily over the NpI unit. The co-conformation having the crown ether ring surrounding the

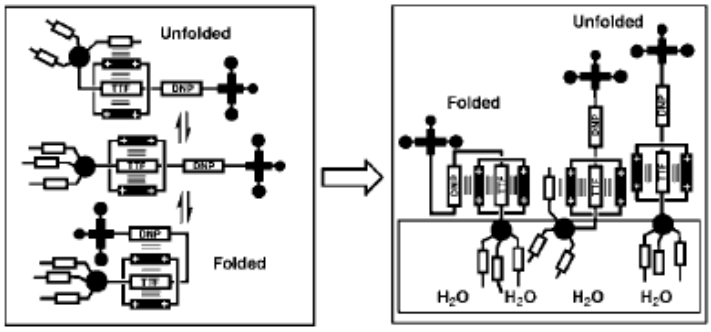

Figure 4. Folded conformations[13]

PmI unit could not be detected using variable-temperature ^{1}H NMR spectroscopy, and free energy differences data acquired from studying degenerate versions of rotaxane **3** indicated that less than 1% of the population of molecules reside in the conformation containing the PmI unit surrounded by the ring. This is what was expected because NpI is known to be a much better electron acceptor unit than PmI. After one-electron reduction, the NpI unit is deactivated and the ring moves to the PmI site. Further reduction of the NpI and PmI site results in a situation where there are no donor-acceptor interactions with the macrocycle. The ability to destabilize the metastable state is favorable because it provides a mechanism to 'reset' the switch without reduction or thermal relaxation. This switch has only been studied in solution, and efforts are currently underway to make the molecule amphiphilic and incorporate it into half devices and full devices.

After studies were done on self-assembled monolayers on the air/water interface[14] and on gold,[15] rotaxanes **2** and **3** were incorporated into a crossbar device (Figure 5).[16] A layer of rotaxanes was sandwiched between an electrode consisting of silicon and another consisting of titanium and aluminum. When tested against controls, such as eicosanoic acid and a version of **1** missing the ring component, the devices with rotaxanes showed switching behavior while the controls did not. The device stored and read out words with ASCII characters when voltages were applied to the electrodes by assigning the low conductance ground state to 0 and the high conductance metastable state to 1. One obstacle in the creation of such solid stable devices is the lack of any direct characterization techniques available to study the molecules themselves. This was demonstrated when the crossbar was constructed using two metal electrodes instead of one metal one and one silicon one. This device showed switching that was independent of the molecule that was contained between the wires. Williams and coworkers[17] showed that this behavior is the result of metal filaments forming between the electrodes.

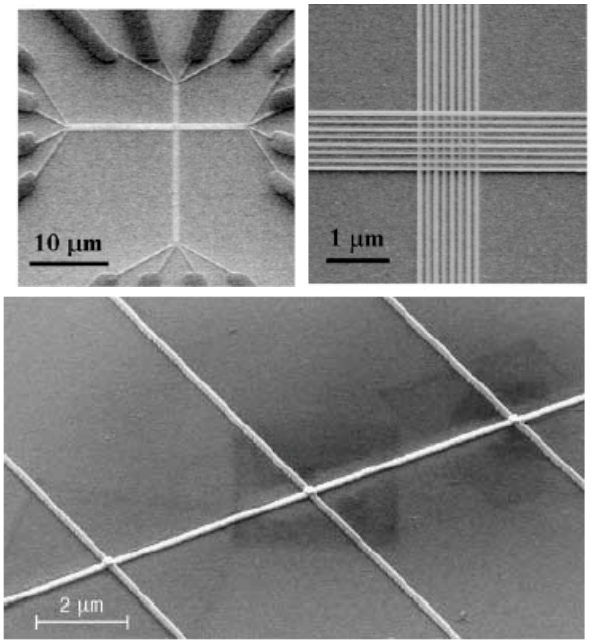

Figure 5. SEM images of crossbar device[16]

BIOELECTRONICS

Redox enzymes not only have high specificity for their substrates, but also have very high turnover rates, thereby offering an efficient and potent means for energy acquisition and integration into

biofuel cells. However, a major problem arises because in many redox enzymes, the active centers are located deeply inside the protein and are insulated from the electrode. Charge carriers have been used to transfer electrons from the enzyme to the submerged electrode in attempts to solve this problem.[18] Compounds such as ferrocene derivatives have been used that are free in solution, covalently linked to the enzyme, and covalently linked to a cofactor that binds to the enzyme. In all these cases, the goal was to allow the carrier molecule to capture electrons from inside the enzyme[18] and, because the molecule would have freedom to leave the active site, transfer current to the electrode surface. Additionally, conducting polymers have been used to entrap the proteins and mediate the transfer of electrons. Although these techniques did achieve electron transfer, it was to a lesser extent than for the natural enzyme with its natural substrates. Presumably the attachments of the charge carrier groups on the enzyme are nonoptimal and alignment with the electrode was poor. Attaching the enzymes or the substrates to the electrode can alleviate these problems because of the consistency in the attachment. The challenge arises in creating attachments that are capable of transferring electrons to the electrode.

Willner and coworkers have devised several methods to harness the redox capabilities of glucose oxidase (GOx) by creating monolayers of its cofactor, flavin adenine dinucleotide (FAD), on gold surfaces. In solution this enzyme catalyzes the oxidation of glucose in the presence of oxygen when bound to FAD. Gluconic acid and hydrogen peroxide are products of the reaction, which has an overall turnover rate of approximately 700 s^{-1}. When GOx binds to the monolayer assembled FAD, electrons from the reaction can be transferred to the gold surface provided there is a transport mechanism. Several attachments have achieved high levels of electron transfer. When a pyrroloquinoline quinone (PQQ) unit was present in the chain, the electrons would transfer from FAD to PQQ and then to the surface.[19] The turnover rate for this process was found to be equivalent to that of the natural enzyme. FAD has also been attached to gold surfaces through connections to gold nanoparticles[20] and single-walled carbon nanotubes.[21] The turnover rates for these half-cells were 5000 s^{-1} and 4100 s^{-1} respectively. The most recent development in creating electrical communication to GOx has been the use of rotaxanes.[22]

The incorporation of rotaxanes into this strategy increases the intricacy of electron transfer due to the freedom of motion that the ring component has between the enzyme and the surface. A rotaxane containing a $CBPQT^{4+}$ ring encircling a diiminobenzene was synthesized by threading (Figure 6). Synthesized off a gold surface, the other end was capped with FAD. Due to the strength of the donor-acceptor complex, the ring preferentially remained around the diiminobenzene unit. Once FAD was reduced to $FADH_2$, electrons were transferred to the $CBPQT^{4+}$ ring thereby reducing it. The reduced form of the ring no longer had an affinity for the diiminobenzene unit. As a result it was free to move along the rod. The positively charged ring was attracted to the negatively charged gold surface and

shuttled towards it leading to fast electron transfer. It was found though that the electron transfer turnover rate of this reaction was only approximately 400 s^{-1}, which is lower than the rate of the natural process. However, of crucial importance in creating efficient half cells for biofuel devices is the potential at which reduction occurs. The thermodynamic redox potential of FAD at pH 8.0 is -0.51 V. Oxidation occurred here at -4.0 V, a value close to the thermodynamic potential. Maximum power extraction occurs when these values are as close as possible. The control electrode that was missing the ring component exhibited no electron transfer to the electrode. As a result, rotaxanes act as efficient electron transport agents in gold assembled monolayers of GOx creating an anode well suited for incorporation into glucose-based biofuel cells.

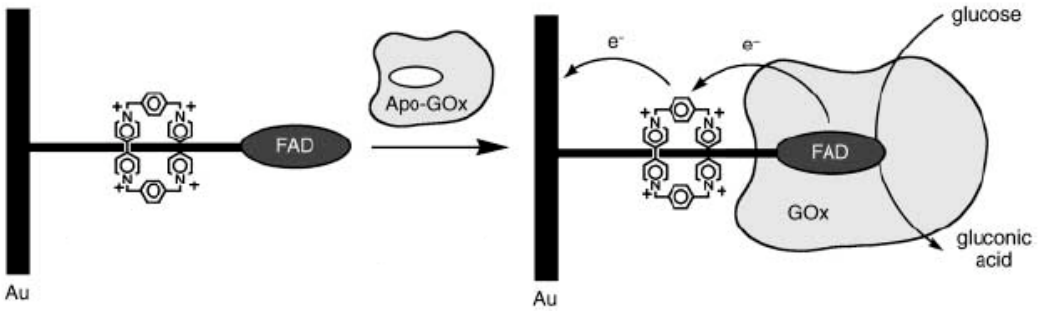

Figure 6. Mechanism of conduction between GOx and gold electrode via rotaxane ring[22]

MEMBRANE TRANSPORT

Membrane permeability is a large problem in drug effectiveness. The pharmaceutical industry invests much time and effort into developing drugs that bind tightly and selectively to their targets, yet if the compound is unable to access its target, it will have little medicinal value. Although several synthetic membrane transporters have been developed, more are needed, especially considering the structural diversity of synthetic drugs. Since polar or charged molecules have trouble crossing lipid bilayers, it is no surprise that many small molecule transporters function by a mechanism that compensates by shielding the functionality from the hydrophobic interior of the membrane. For example, glutathione has been transported when attached to a cholate-spermidine-Ellman's reagent derivative.[23] This molecule folded upon itself to encompass the polar groups and expose the nonpolar substituents. This umbrella mechanism allowed passive passage through the membrane. Smithrud and coworkers[24], noticing the mechanistic freedom of the components of the rotaxane, have devised a new cellular transport agent.

A rotaxane (**4**) consisting of a di-arginine derivatized dibenzo-24-crown-8 ring was synthesized (Figure 7) using a threading mechanism. The stopper groups were comprised of a bulky aromatic group and a cyclophane pocket. Fluorescein and fluorescein-protein kinase C inhibitors were held in place by the diarginine units and bound in the cyclophane pocket. In the absence of the transport agent,

fluorescein did not impermeate the membrane and was not detected in the cell. However, when bound to **4**, the fluorophore was noticeably transported into COS-7 cells. The complex was judged to be nontoxic to the cell line because mitotic behavior was observed in their presence. At this time no current investigation has been done on the mechanism of transport but it was proposed that transport occurs through the ability of the complex to alter conformation based on its environment. In aqueous conditions the aromatic components on the ring would move closer to the encapsulated guest. In the apolar environment of the membrane, the ring can move, allowing optimal salt bridging and hydrogen bonding between the diarginine components on the ring and the bound guest. The rotaxane bound to a variety of other molecules such as small peptides, but no cell transport studies have been done. The effectiveness of this agent will be evaluated once a larger selection of substrates is investigated. For instance, the mentioned peptides are only two or three amino acids in length. It would be interesting to determine the size limit for transport. Also other classes of molecules could be tested such as oligonucleotides.

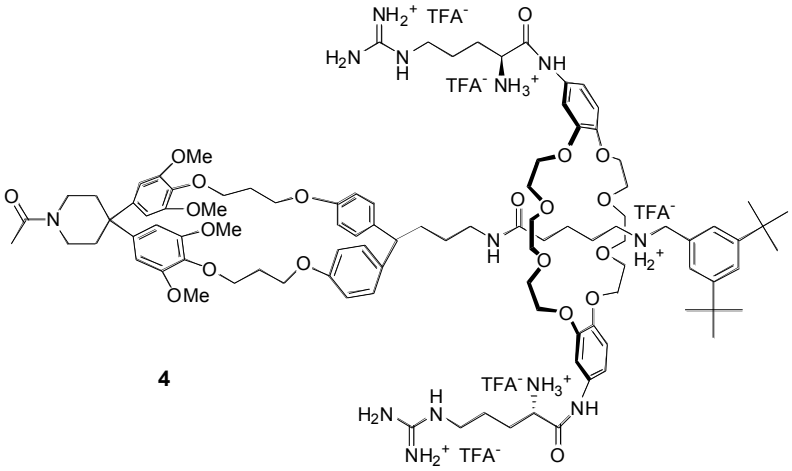

Figure 7. Membrane transport rotaxane

CONCLUSION

A variety of applications of rotaxanes have been presented. Variation of the rod structure has led to a diverse set of functions. Installing different recognition units enabled the creation of switches that have received much notice in the field of molecular electronics and may lead to the development of useful future computing technologies. Enzyme-binding rotaxane monolayers enable electron transfer that wouldn't occur without the motion of the ring. Powerful biofuel cells may be the result of this discovery. Lastly the ring motion provided enough fluidity for the molecule to reestablish favorable binding free energy when in different environments, allowing the transport of molecules through membranes in which they would normally be unable to pass through. Additional utilization of the rotaxane ring's mechanical freedom will undoubtedly lead to the creation of novel and interesting applications in the future.

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