Observing invisible machines with invisible light: The mechanics of molecular machines

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Rotaxanes comprise macrocycles that can shuttle between docking stations along an axle. Here we explore the nanosecond shuttling mechanism by reversing the relative binding affinities of two stations through ultraviolet-induced transient reduction. We monitor ensuing changes in the CO-stretching bands of the two stations and the shuttling macrocycle using an infrared probing pulse. Because hydrogen-bond scission and formation at the initial and final stations lead to well-resolved changes in the respective CO-stretch frequencies, the departure and arrival of the macrocycle can be observed separately. We find that the shuttling involves two steps: thermally driven escape from the initial station, followed by rapid motion along the track ending either at the initial or final station. By varying the track’s length, we find that the rapid motion approximates a biased one-dimensional random walk. However, surprisingly, the direction of the overall motion is opposite that of the bias.
4.1 Introduction

Recent developments in the generation and control of motion on a molecular scale have enabled the construction of prototypical molecular machines.\textsuperscript{9,10,30,40,89,132–140} The mechanics of such molecular machines are fundamentally different from those of macroscopic machines. As a machine’s size and speed approach those of the solvent molecules surrounding it, macroscopic concepts such as viscous friction lose meaning, and thermal fluctuations lead to intrinsic randomness in the machine’s behavior.\textsuperscript{62,64} A detailed understanding of the motion of molecular machines requires experiments with both structural sensitivity at the atomic level and sufficient time resolution. Time-resolved vibrational spectroscopy meets both these requirements.\textsuperscript{141,142} Probing the stretch modes of specific, localized, chemical bonds affords a direct view of the conformational changes underlying operation of individual components of the molecular machine. The time resolution of the probing is determined by the pulse

![Chemical structures of the [2]rotaxane shuttle in the neutral, radical anion and shuttled radical anion states.](image)
duration, which can be less than 100 fs. Here, we use time-resolved vibrational pump-probe experiments on a light-triggered rotaxane shuttle to unravel its operating mechanism.

Rotaxanes consist of mechanically interlocked wheel- and axle-like components. Fig. 4.1 shows the chemical structure of this type of molecular machine, together with its operation cycle, which we characterized previously using spectroelectrochemistry and transient UV-Vis spectroscopy. In the neutral molecule, the macrocycle is hydrogen bonded predominantly (>99%) to the succinamide station (succ). After excitation of the naphthalimide station (ni) with a 355 nm pulse, the rotaxane undergoes rapid (∼1.6 ns) intersystem crossing to the triplet state. In this state, the ni station is reduced by an external electron donor to form a radical anion (ni•−). The ni•− station has a much greater affinity for the macrocycle than the succ station (equilibrium constant >1000). Consequently, the macrocycle shuttles over the thread and forms hydrogen bonds to the ni•− station. After slow (∼100 µs) charge recombination between ni•− and the radical cation of the electron donor, the macrocycle travels back over the thread, binds to the succ station and the system is ready to shuttle again. In the following, rotaxanes with track length n will be referred to as C_n.

4.2 Results and discussion

4.2.1 The different stages of operation viewed through vibrations

The vibrational absorption spectrum of the C_{12} rotaxane in its initial state is shown in Figure 4.2a. All peaks in the rotaxane spectrum can be assigned by comparison with spectra of the constituent components. The symmetric and antisymmetric CO-stretch modes of the ni station are observed as peaks 1 and 7, respectively (the latter also contains a contribution from the CO-stretch vibration of the macrocycle), and the CO-stretch mode of the succ station as peak 5. In the time-resolved experiments, we trigger the rotaxane with a 4 ns duration 355 nm pulse, and observe the subsequent vibrational absorption change using a 100 fs mid-IR probe pulse * (see chapter 2). Figs. 4.2b and 4.2c show normalized UV-IR transient spectra of the C_{12} rotaxane at different delay times. The spectra at early delays (<120 ns) show how the triplet state evolves to the radical anion state (ni•−). Subsequently, the spectrum evolves further as the macrocycle shuttles from the succ to the ni•− station (Fig. 4.2c). For the assignment of the peaks in Fig. 4.2c we use steady-state spectra of the initial, charged, and final states. Peaks 2 and 3 (labeled ARRIVAL) are the CO-stretch modes of the ni•− station when it is free, and when it is hydrogen bonded to the macrocycle, respectively. The temporal evolution of these peaks therefore directly mirrors the arrival of the macrocycle at the ni•− station. Similarly, peaks 4 and 5 (labeled DEPARTURE) are the CO-stretch modes of the succ station when free, and when hydrogen bonded to the macrocycle. The behavior of these two peaks therefore mirrors the departure of the macrocycle from the succ station. Peak 6 is due to the CO-stretch mode of the macrocycle when its NH groups are hydrogen bonded to the

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*The time resolution of the experiment is determined by the pump pulse, which is 4 ns. The 100 fs probe pulse provides us with the spectral bandwidth necessary to probe the entire rotaxane absorption spectrum in one laser pulse.
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Figure 4.2: a: FTIR Spectrum of $10^{-4}$ M C$_{12}$ rotaxane and $10^{-2}$ M DABCO in CD$_3$CN, 11 mm pathlength (solvent subtracted). b: Transient UV-IR spectra of the C$_{12}$ rotaxane at delays ranging from 10 ns to 120 ns after UV excitation. c: Transient UV-IR spectra of the C$_{12}$ rotaxane at delays ranging from 120 ns to 2000 ns after UV excitation. The labeled vibrations in the transient spectrum correspond with the labeled CO groups of Fig. 4.1.
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Figure 4.3: Observed time-dependence of the absorption peaks 2, 3, 4, 5, and 6 normalized to the maximum intensity of peak 1, each of which corresponds to a specific CO bond in a specific state of the operation cycle, see Fig. 4.1. The curves represent least-squares fits of bi-exponential decays.

ni*– station. The corresponding absorption decrease of the CO-stretch mode of succ-bound macrocycles as they leave the succ station is superimposed on (temporally static) peak 7.

4.2.2 Dynamics of the operation

We have shown that vibrational spectroscopy enables us to observe the departure and arrival of the shuttles separately. The shuttling rate of the macrocycle can be obtained from the dynamics of these bands, each corresponding to a specific component of the rotaxane. The time evolution of the intensity of peaks 2, 3, 4, 5, and 6 is shown in Fig. 4.3. We find that the departure and arrival of the macrocycle can be well described with the same time constant. We performed a global least-squares fit to the data 2.8, in which the charging and shuttling are each described by a single rate constant. From the fit (see curves in Fig. 4.3) we obtain $k_{\text{charge}} = 2.8 \pm 0.4 \times 10^7 \text{ s}^{-1}$, which corresponds to the charging lifetime of $35\pm5$ ns. We find that for the C_{12} rotaxane $k_s^{C_{12}} = 1.30 \pm 0.03 \times 10^6 \text{ s}^{-1}$, which corresponds to a shuttling time of $0.77 \pm 0.02 \mu s$, in agreement with previous UV-Vis experiments.30

The fact that the departure and arrival exhibit the same dynamics implies that during the shuttling process, an individual macrocycle spends only a short time on the thread compared to the average shuttling time of the ensemble. The observed average shuttling time is therefore not determined by the time the shuttle spends on the CH$_2$ chain, but rather by the time it takes for the shuttle to escape from the initial station. The sequence of events in the shuttling of this rotaxane is thus as follows: occasional escape from the succ station, followed by a rapid
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motion over the thread, ending at either the \textbf{ni}^*− (where it can no longer escape because of the stronger hydrogen-bonding) or the initial \textbf{succ} station (where it will wait until the next escape). Below we investigate the two steps of this mechanism in more detail.

To quantify the energy barrier that the macrocycle must overcome to escape from the \textbf{succ} station we measured the shuttling rate of a C$_9$ rotaxane \footnote{We chose the C$_9$ rotaxane for the temperature studies due to its superior solubility in CD$_3$CN.} over a 45 K temperature range (see Fig. 4.5). The temperature increase due to the partial conversion of the pump energy into heat is negligible (see subsection 4.4.2). The temperature dependence of $k_s^{C_9}$ displays Arrhenius behavior, indicating that the escape from the \textbf{succ} station is effectively a single-barrier event, and that the energy required by the macrocycle to cross it is provided by thermal fluctuations. We find that the data can be well described with the Eyring equation (eq. 4.1),

$$k_s(T) = \frac{k_B T}{h} e^{-\frac{\Delta H^\ddagger}{RT}},$$

(4.1)

where $k_B$ is the Boltzmann factor, $R$ is the gas constant, and $T$ the temperature of the system. From a least-squares fit (Fig. 4.5), we find that the enthalpy of activation $\Delta H^\ddagger$ is 26 ± 1 kJ mol$^{-1}$ and the entropy of activation $\Delta S^\ddagger$ is −32 ± 3 J mol$^{-1}$ K$^{-1}$. The energy barrier $\Delta H^\ddagger$ is consistent with the breaking of approximately 4 hydrogen bonds, assuming a binding energy of $-7.5 \pm 0.8$ kJ mol$^{-1}$K$^{-1}$ per hydrogen bond.\footnote{The negative activation entropy suggests that some ordering of the system is required for escaping from the \textbf{succ} station. Folding of the thread onto itself can be excluded, as this should be evident in the \textbf{ni} and/or \textbf{ni}^*− CO-stretch response in the transient spectra.\footnote{We chose the C$_9$ rotaxane for the temperature studies due to its superior solubility in CD$_3$CN.}} The negative activation entropy suggests that some ordering of the system is required for escaping from the \textbf{succ} station. Folding of the thread onto itself can be excluded, as this should be evident in the \textbf{ni} and/or \textbf{ni}^*− CO-stretch response in the transient spectra.\footnote{We chose the C$_9$ rotaxane for the temperature studies due to its superior solubility in CD$_3$CN.}

The ordering might involve a relative positioning of macrocycle and thread that is favorable for breaking the macrocycle-succ hydrogen bonds.

The next step in the shuttling process is the fast motion of the macrocycle over the thread, ending at either the \textbf{succ} station or the \textbf{ni}^*− station. To investigate the nature of this fast motion, we explored how changing the carbon-chain length affects the probability of the macrocycle ending at the \textbf{ni}^*− station. This probability is proportional to the observed shuttling rate, as the rate at which escape from the \textbf{succ} station occurs is independent of the carbon-chain length. The shuttling rates of rotaxanes with thread lengths $n = 5, 9, 12, 16$ are shown in Fig. 4.4.

The rate of shuttling is observed to decrease dramatically with increasing thread length. We analyze the data by modeling the motion of the macrocycle over the thread as a one-dimensional random walk. In the simplest version of this model, the C$_n$ chain is modeled as a track of $n$ local free-energy minima, between which the macrocycle makes random jumps, to end up at either the \textbf{ni}^*− or \textbf{succ} station. If we assume equal probabilities for the macrocycle to make one step toward either station, the probability of the macrocycle arriving at the \textbf{ni}^*− station is given by (eq. 4.2):

$$P_s(n) = \frac{1}{n+1}.$$  

(4.2)

A least-squares fit of the rate data to this equation (with the proportionality factor between the observed rate constant and $P_s(n)$ as the only fit parameter) is shown as the grey dotted curve in Fig. 4.4. Although the model reproduces the observed trend, the quantitative agreement is
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Figure 4.4: Thread-length \((n)\) dependence on the shuttling rate constant \(k_s\) fitted with an unbiased random walk model, and a biased random walk model (eq. 4.3). The error bars represent \(\pm 1\sigma\) (see section 2.8). The inset shows a schematic representation of the shuttling mechanism.

We find that very good agreement is obtained by introducing a small bias in the probabilities of making a step in the forward or backward direction. Assuming a probability \(p\) of hopping forward (in the direction of the \(\text{ni}^*\) station), and \(1-p\) of hopping backward, the probability of arriving at the \(\text{ni}^*\) station becomes (eq. 4.3)

\[
P_s(n) = \frac{1 - \left(\frac{1-p}{p}\right)^n}{1 - \left(\frac{1-p}{p}\right)^{n+1}}.
\]

From a least-squares fit (with \(p\) and an overall scaling factor as the only fit parameters), we find that the best description of the data is obtained for \(p = 0.442 \pm 0.003\) (red curve in Fig. 4.4). This value of \(p\) implies that the event of the macrocycle moving one step towards the \(\text{ni}^*\) station is slightly less probable than the event of it moving towards the \(\text{succ}\) station \((1-p = 0.56)\), i.e., the random translational motion of the macrocycle along the track has a small bias towards the \(\text{succ}\) station. At present, we can only speculate as to the driving force behind this bias. An enthalpic driving force seems improbable in view of the short-range nature of the hydrogen-bond interactions that give rise to the overall \(\Delta H\) of the shuttling. It is more likely that the bias is mainly of entropic origin. This could be the case if the thread conformation is on average slightly more favorable to macrocycle translation at the \(\text{succ}\)
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side than at the \( \text{ni}^\bullet^- \) side. Interestingly, the overall motion of the macrocycle from \( \text{succ} \) to \( \text{ni}^\bullet^- \) occurs against the bias (which is toward the \( \text{succ} \) station). This is possible only because there is a global free-energy minimum at the \( \text{ni}^\bullet^- \) station.

Our findings can be summarized by combining eqs. (4.1) and (4.3) to obtain an expression for the shuttling rate as a function of temperature and track length (eq. 4.4):

\[
k_s(T, n) \propto k_B T e^{-\Delta G^\dagger \over RT} \times P_s(n).
\]

Each term describes one stage of the shuttling mechanism (see inset of Fig. 4.4). The probability \( P_s(n) \) for the random walk to end at the final station is \( \ll 1 \) (for the shortest shuttle, \( P_s(5) = 0.09 \), for the longest \( P_s(16) = 0.005 \)), so that many escapes (tens to hundreds) of the macrocycle from the initial station are required before a successful shuttling event occurs. This leads to a certain amount of randomness in the timing of the arrival of the shuttle.

A more detailed understanding of the bias against shuttling requires an exploration of the rotaxane’s free-energy landscape using Molecular Dynamics simulations. Since shuttling is such a rare event, this will require state-of-the-art simulation methods, and we hope that our results will stimulate work in this direction. On a more fundamental level, it may be pointed out that the unpredictability of the arrival time of the shuttle does not imply unpredictability of the arrival itself. A consideration of the probability for a macrocycle to make the transition from one station to another is essentially an attempt to describe the shuttling motion—about which our experiments provide ensemble thermodynamic and kinetic information—at the single-molecule level. Fluctuation theorems\(^{146}\) provide a connection between thermodynamic parameters and single-molecule measurements of molecular mechanical events.\(^{147}\) In particular, theoretical work on time-symmetry breaking in such measurements has shown that the free-energy change per operation cycle should be about \( 4\sim8k_BT \) for an individual molecular machine to rectify thermal fluctuations to such an extent as to advance mostly forward in time (i.e., toward the conformation corresponding to the free-energy minimum).\(^{65}\) The free-energy difference which drives the rotaxane’s shuttling motion (the difference between the \( \text{succ} \) and \( \text{ni}^\bullet^- \) bound macrocycle) is \( -18 \text{ kJ/mol} \),\(^{30}\) which corresponds to \( 7.3k_BT \). This is just enough to ensure that the shuttling occurs in the forward time direction.\(^{65}\)

4.3 Conclusion

We have elucidated the operation mechanism of a molecular shuttle. The CO groups found in both the macrocycle and the thread provide excellent structural probes for monitoring the shuttling motion with time-resolved vibrational spectroscopy. The absorptions of these groups are spectrally well separated, allowing us to monitor the behavior of each of the machine’s components individually. From the time-dependent absorption intensities of the individual components, we have been able to conclude that an individual shuttling event is much faster than the observed ensemble. The temperature dependence of the shuttling rate reveals that the enthalpy of activation equals the enthalpy of formation of four hydrogen bonds. Therefore, the operational speed of the device is limited by the escape rate of the macrocycle from the initial station. The track-length dependence of the shuttling rate can be described
well with a biased one-dimensional random walk. The direction of the bias is, unexpectedly, towards the initial station. At this moment, we can only speculate as to the nature of this bias. Experiments capable of observing the return of the macrocycle from the final station back to the initial station could provide additional information on this matter. Our studies show that the investigated rotaxane-based shuttles are reliable in the sense that the macrocycle will eventually arrive at the $\text{Ni}^{2+}$ station. At the same time, they lead to the conclusion that the time at which this arrival occurs becomes more and more unpredictable with increasing length of the shuttle’s track.

### 4.4 Additional methods and data relevant to this chapter

#### 4.4.1 Temperature dependence of shuttling

Fig. 4.5 shows the temperature dependence on the shuttling rate of the C$_9$ rotaxane. The

![Graph showing temperature dependence of shuttling rate](image)

**Figure 4.5:** C$_9$ shuttling rate ($k_s^{C_9}$) at different temperatures fitted with the Eyring equation (eq. 4.1). The uncertainty of the temperatures above 300 K are determined by the fluctuation in sample temperature caused by the temperature controller when maintaining a desired temperature which is ±2.5°. The uncertainty of the temperatures under 300 K is taken from the accuracy of the thermocouple used to measure sample temperature (±0.1 K). The uncertainty in shuttling rate represent ±1σ.

The rates are obtained from a least-squares global fit of the model described above (section 4.2.2) on the delay dependence of peaks 2, 3, 4, 5, 6 of the C$_9$ rotaxane at 294, 299, 308, 318, 328, and 338 K. The curve is the result of the least-squares global fit of the Eyring equation (equation 4.1).
4.4.2 Dissipation of the heat generated by the excitation of the UV pump

From the energy of the UV photon, the naphthalimide triplet energy, and the reduction and oxidation potentials of the naphthalimide and the electron donor respectively, we estimate that $2.8 \times 10^{-19}$ J of heat is dissipated due to vibrational cooling of the naphthalimide. By solving the equation of heat diffusion (heat diffusivity $D=1.07 \times 10^{-7}$ m$^2$s$^{-1}$)\textsuperscript{148}, it is easily shown that already after 0.5 ns this heat has spread out over a range of $\sim 10$ times the size of the rotaxane, with a maximum temperature increase of less than 10 mK. Hence, in our experiments, thermal activation effects of the pump pulse are negligible.

Random walk models

As discussed above, the unbiased random walk model does not describe the data well. We extended the unbiased random walk model in order to investigate whether this disagreement arises because the macrocycle occupies a finite space on the thread. If $m$ is the number of CH$_2$ units occupied by the macrocycle, the probability of the macrocycle arriving at the ni$^+$ station becomes [144]

$$P_s(n) = \frac{1}{n-m+1}.$$  \hspace{1cm} (4.5)

A least-squares fit using this equation (resulting in $m=4.9$, grey dashed curve in Fig. 4.6) still does not result in a quantitative description of the data (see reduced $\chi^2$ values in table below). As discussed above, the biased random walk model agrees well with the data ($\chi^2 = 5.25$).

<table>
<thead>
<tr>
<th>Model</th>
<th>Reduced $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unbiased ($p = 0.50$)</td>
<td>248.84</td>
</tr>
<tr>
<td>Unbiased, $m = 4.9$</td>
<td>80.87</td>
</tr>
<tr>
<td>Biased, $p = 0.44$</td>
<td>5.25</td>
</tr>
</tbody>
</table>
Figure 4.6: Figure: Thread-length ($n$) dependence on the shuttling rate $k_s$ fitted with an unbiased random walk model (eq. 4.2), an unbiased random walk model with a correction for macrocycle occupation (eq. 4.5), and a biased random walk model (eq. 4.3). Table: Summary of the reduced $\chi^2$ from the fits shown to the left.