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

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Tricks of the trade
Small gems of know-how are often acquired at the expense of a lot of failure. This appendix is written in the hope that it prevents others from wasting time reinventing the wheel when performing the experiments outlined in this thesis. A lot of the information in this appendix is borne from experience and some of the reasoning behind the problems and solutions are, to my regret, very speculative. Lack of time usually prevented the systematic investigation of the problems encountered. The first two sections describes more thoroughly the experimental method in chapter 2 by providing the motivation behind the procedures as well as pointing out some pitfalls. The last section discusses the nature and methods employed to avoid the various artifacts which have plagued our experiments. Before we get into details, a general rule concerning the ni based rotaxanes and their transient spectra:

If the ni$\text{-}^*$ species does not decay hyperbolically, something is wrong.

With that said, the remainder of the appendix will outline how to prevent the above.

A.1 Sample preparation of naphthalimide samples

The foundation of the sample preparation used for the experiments discussed in chapters 3, 4, 5, 6, and 8 is presented in chapter 6 of the PhD thesis of G.W.H. Wurpel. The methods described therein are designed for UV-visible pump probe experiments. Some alterations had to be made to better suit IR-probe experiments.

A.1.1 Standard sample preparation

In the following we describe the method employed for a rotaxane sample preparation of a standard UV-pump IR-probe experiment. The motivation behind the steps will be given in separate subsections. The following is a list of the essential requirements of the sample:

- The rotaxane concentration used is 1 $\times$ 10$^{-4}$ M (see section A.1.3).
- The external electron donor concentration is approximately in 100 fold excess to that of the rotaxane, 1 $\times$ 10$^{-2}$ M.
- The solvent does not absorb any IR radiation at the required wavelengths and cause any photochemical reactions (see section A.1.2 and A.1.3).
- The sample is prepared under dry (see chapter 5) and inert atmosphere (N$_2$) which we achieve by using Schlenk flasks with teflon screw caps (these have the essential quality that they can pass through a glovebox airlock, see section A.1.6).
- The sample is kept in a sealed sample cell, with a path length ranging from 5–10 mm (see sections A.1.3, A.1.6, and A.2.1).

The following is a step-by-step procedure for the preparation of the sample and assumes a basic knowledge of working under inert atmosphere:
A.1 Sample preparation of naphthalimide samples

- The rotaxane powder is first dissolved in a minimal amount of chloroform (see section A.1.3).

- The solution is transferred to a oven- or flame-dried Schlenk flask with a teflon screw cap (see sections A.1.6 and A.1.7).

- The chloroform is evaporated under reduced pressure or by blowing N\textsubscript{2} over the sample solution.

- The electron donor (1,4-diazabicyclo[2,2,2]octane, DABCO) is weighed under dry and inert atmosphere and placed in a schlenk flask.

- The electron donor is dissolved in the required solvent (CD\textsubscript{3}CN, see section A.1.2) and added to the rotaxane.

- The rotaxane is left to dissolve, the solution can be sonicated to speed this up.

- The solution is degassed by bubbling argon through the sample for at least 20 min (see section A.1.6).

- The sample is transferred to a sealed CaF\textsubscript{2} sample cell (see sections A.1.3, A.1.6).

To obtain the driest solution of electron donor and CD\textsubscript{3}CN, the solution should be dried over molecular sieves (3 Å pores, this specifically catches water) in two separate stages.

A.1.2 Solvent

We use 99.8 % D purity CD\textsubscript{3}CN from Eurisotop. The HDO content of the solvent is <0.01 %. We dry the solvent twice over 3 Å pore size molecular sieves (20 % (v/v) of the sample), dried at 300°C before use. The typical concentration of H\textsubscript{2}O is 21 ppm as determined from Karl-Fischer titration (see chapter 5 section 5.4.1). All the HDO undergoes isotopic exchange with the molecular sieves (a zeolite material rich in OH groups) to form H\textsubscript{2}O.

We use CD\textsubscript{3}CN for two very important reasons:

- CD\textsubscript{3}CN absorbs minimally in the spectral range around 1600 cm\textsuperscript{-1}.

- CD\textsubscript{3}CN does not absorb the UV radiation of the pump laser (355 nm), nor does it react with the ni species in the excited or radical anion state.

Any solvent with the same properties in which the ni species dissolves can of course be used, provided it’s suitable for photochemical experiment.

A.1.3 Solubility of naphthalimide samples

The solubility of ni rotaxanes in acetonitrile is poor. The bare thread and ni stopper molecules dissolve much more readily in this solvent. Directly dissolving the appropriate amount of rotaxane for a 10\textsuperscript{-4} M solution in acetonitrile results in a suspension, even after extensive
sonication. However, sufficient rotaxane will dissolve for a UV-pump mid-IR probe measurement. When prepared in this fashion, the suspension has to settle before any experiment can be performed; it causes a frequency dependent baseline in FTIR measurements and a large amount of noise (and scatter when using an IR pump) in time-resolved measurements.

We found out, by accident, that the dissolution of rotaxane in chloroform followed by the evaporation of the chloroform results in solid rotaxane which dissolves readily in acetonitrile. A possible explanation is that re-crystallization of the rotaxane from acetonitrile (standard procedure in rotaxane synthesis) results in a kinetic barrier to dissolving in acetonitrile. The rotaxane apparently does not get a chance to form such a solid structure in chloroform.

A.1.4 Preparing N-deuterated rotaxane samples

In the case of N-deuterated ni rotaxanes, the difficulty does not lie in the isotopic substitution step, this process happens rather readily. The trick is to keep the sample deuterated. Even with a concentration of 21 ppm H₂O (~1×10⁻³ M) in the solvent (the lowest attainable amount with the method described in section A.1.2) the sample undergoes complete re-protonation. To prevent re-protonation, we add 0.026% (v/v) C₂D₅OD to the sample as a deuterium “buffer”. The amount of alcohol is sufficient to absorb stray protons in the sample but low enough not to interfere with the shuttling dynamics (see Fig. 5.1, chapter 5). Furthermore, from all the additives we investigated in chapter 5, ethanol interferes the least with the operational speed of the rotaxane.

A.1.5 Concentration of naphthalimide samples

A concentration of 5×10⁻⁴ M rotaxane is possible if the method in section A.1.3 is followed. However, at such a high concentration, the rotaxane crystallizes rather quickly after having been dissolved (~1 hr, which is not enough time to get it in the laser setup for a measurement). We found that if a suspension of rotaxane (C₉) dissolves when heated above 65 °C in a thermostatted sample cell. The solution can then be cooled and kept at 40 °C for the remainder of the experiment (do this only if necessary because the re-crystallization will still occur, but more slowly).

When performing the transient 2DIR experiments of chapter 8, we tried several tricks to further increase rotaxane concentration of the sample. One of them involved using up to 25%(v/v) dichloromethane (DCM) as a co-solvent which improved the solubility of the rotaxane (this only worked for the C₁₂ rotaxane, the C₅ crystallizes very well in DCM). We quickly stopped using this method because the added DCM resulted in poor heat dissipation from the laser excitation. The excitation of a ni species results in a large amount of heat dumped in the sample which causes a change in the local density of the sample. This results in a long lasting (multiple milliseconds) change in the refractive index of the solvent, increasing the noise of the measurement. Additionally, the heat accumulation is such that we charred the CaF₂ window at the point where the pump laser enters the sample.

Aside from signal magnitude, the concentration of the sample has consequences for the dynamics of the ni⁻⁻ species. A too high rotaxane concentration leads to other problems such as triplet-triplet annihilation and faster radical anion recombination rates. Both cases
A.1 Sample preparation of naphthalimide samples

are detrimental to signal magnitude. Triplet-triplet annihilation occurs when two \( n^* \) species encounter one another (which is more probable at a high rotaxane concentration or high laser pump power). Energy transfer occurs resulting in one excited-state singlet and one ground-state molecule.\(^{234}\) The recombination of the \( n^* \) species with the DABCO\(^{**} \) will have a faster rate at higher concentrations on account of it being a bimolecular reaction.

A.1.6 Avoiding oxygen

Our early experiments suffered from the presence of oxygen due to experimental reasons (details below, section A.2.1). Oxygen quenches the \( n^* \) species in the triplet state. The reaction between \( O_2 \) and the \( n^* \) species (or excited species in general) is rather complex.\(^{235}\) We found that the presence of oxygen results in an additional loss channel in the dynamics of the \( n^* \) species (see chapter 3). Subsequently, one observes a faster decaying signal which limits the maximum length of shuttling lifetime that can be measured. This is why the length dependence data stops at spacer-length (\( CH_2 \))\(_{16} \) in chapter 4, the \( n^* \) species recombined before shuttling was possible in longer rotaxanes. Furthermore, oxygen quenching also results in the formation of singlet \( O_2 \) which is a very reactive species. The irreversible oxidation of the constituents of the sample with singlet oxygen is a possible source of photo-degradation. In conclusion, dissolved \( O_2 \) must be avoided at all costs.

The most thorough method of removing dissolved oxygen from a sample is to subject it to three freeze-pump-thaw cycles. The UV-vis shuttling experiments by Brouwer et al.\(^{30,128}\) used a T-cell which is essentially a quartz fluorescence cuvette grafted to a round-bottom flask. The advantage of this method is that after degassing, no further handling of the sample is necessary and therefore the chance that \( O_2 \) can contaminate the solution is minimal. Unfortunately, no cuvette exists which transmits IR radiation at our required wavelengths so no T-cell could be constructed for our purposes. The next best method is to degas the sample by bubbling argon through the solution for at least 20 min. The disadvantages of this method are that some solvent evaporates, a glovebox is needed and extra handling is necessary to transfer the sample from the schlenk to the sealed sample cell. The latter increases the chances of oxygen creeping into the sample.

A.1.7 Water

The presence of dissolved water has two effects on a rotaxane sample:

- It acts as a lubricant for the shuttling or pirouetting of the rotaxane (see chapter 5).
- \( H_2O \) absorbs at 1640 cm\(^{-1} \). Too much absorption will obscure the spectrum of the rotaxane around this frequency.

The uncontrolled presence of water will result in a varying shuttling lifetimes between different samples. We therefore started using schlenk flasks with teflon screw-caps in the preparation of the sample which allow for the exclusion of atmospheric water.

We chose to use \( D_2O \) in the lubrication of molecular machines in chapter 5 for the simple reason that it absorbs a lot less in amide I region than \( H_2O \). We were able to measure the shuttling rates of the \( C_{12} \) rotaxane with \( D_2O \) concentrations up to 5.5% (v/v).
A.2 During the experiment

The greatest pump probe signal is obtained when the most overlap is achieved between the UV-pump and IR-probe beams. The IR probe interacts with the largest amount of pumped sample which results in the largest change in absorption. The optimal situation would be when the pump and probe beams are collinear which is, unfortunately, not possible for practical reasons. There is, however, one major drawback to having a small angle between the pump and probe beams which is explained in detail in section A.3.1. The remainder of this section is devoted to other experimental tricks and peculiarities.

A.2.1 Circulating the sample

The early shuttling experiments (chapters 4 and 3) were conducted whilst circulating the sample with a gear pump. The idea behind this was to constantly refresh the sample in order to avoid photo-degradation of the sample due to repeated photo-excitation at the same spot, thus increasing the lifetime of the sample. Whilst the above reasoning is valid, there are three problems which make sample circulation more trouble than it is worth:

- If the system is not air- or water tight, the presence of oxygen and water cause their own set of problems (see section A.1.6 and section A.1.7).
- The circulating introduced a lot of noise in the measurement, probably because of turbulence.
- The volume of liquid necessary to fill the pump is quite large (10 ml), which means a lot of solute is required for an experiment (this problem can in principle be overcome).

In conclusion, unless a hermetically sealed pump-and-cell system is used, circulating the sample does not contribute towards sample longevity. Using a sealed sample cell (volume ranging from 1.8–3.2 ml) is perfectly adequate. When performing standard UV-pump IR-probe experiments, refreshing the sample is not required, provided the pump laser power is not too high (see section A.3.1).

A.2.2 Transient 2DIR

The samples used for the T2DIR experiments deviate from the standard in order to compensate for the small pump-pump probe signal. We opted to use a high rotaxane concentration ($5 \times 10^{-4}$ M, see section A.1.5). In order to beat the faster radical anion recombination (section A.1.5), we heated the sample to 75 °C which significantly increases the shuttling rate (see chapters 4 and 5). We also used the C$_9$ rotaxane, because this intrinsically has a faster shuttling rate than for example the C$_{12}$ (theoretically, the C$_5$ would be the best, but it is less soluble than the C$_9$).

We used the following procedure to prevent scattering of the IR-pump into the probe beam: First the sample should be set such that the IR-pump IR-probe beams are overlapping in the middle of the sample cell. Then, the UV-pump IR-probe signal should be optimized. The windows of the sample cell should be very clean (on the inside as well as on the outside
A.3 Artifacts

We found that high laser power combined with a high-speed sample mover (along with the above alignment procedure) produces the least artifacts.

A.2.3 Photo degradation

During any experiment using the UV-pump laser, the sample repeatedly absorbs UV radiation which eventually leads to photo degradation of the sample. This process is accelerated, obviously, when using higher laser power. In the case of the n-butyl ni stopper molecule and ni threads, photo degradation simply results in a product that no longer participates towards the transient signal. A decrease in signal over time is observed. Unfortunately, the photo degradation of the rotaxane initially leads to the generation of bare thread and a solid deposition (product of the former macrocycle perhaps) on the CaF$_2$ windows. The deposition produces a very prominent artifact (see section A.3.1). There has been some speculation as to what the cause is of the photo degradation: the protons of the methylene groups are acidic (due to the adjacent phenyl and amide groups) and is therefore prone to deprotonation. The latter is more than possible with the excess of DABCO present in the sample (which is a base as well as an electron donor). Further reactions ending up in the destruction of the macrocycle can follow the deprotonation.

A.2.4 Pump laser peculiarities

It is worth the effort to check the lamp and Q-switch timings of the external pump laser, even when they are set at the factory settings. In our case, increasing the delay between the Q-switch trigger pulse and the lamp pump pulse from the factory setting dramatically increased the stability and power of the external pump laser’s output (perhaps this is caused by aging of the laser). With higher repetition rates (>500 Hz) we noticed that the divergence of the output beam changes when tweaking the aforementioned settings (the laser may not be working in the TEM$_{00}$ mode anymore either, judging from the presence of nodes in the beam profile).

A.3 Artifacts

Artifacts are observations caused by the method of the experiment or preparation of the sample rather than the physics or chemistry of the sample. We encountered several artifacts throughout the research and some were the cause of significant delay and frustration. This section discusses these artifacts and how we eliminated or avoided them.

A.3.1 Deposition

The poor solubility and photo degradation of a rotaxane sample results in the deposition of a solid on the window of the sample cell where the pump laser enters the sample. The deposition also absorbs the UV-radiation of subsequent laser pulses. However, in the solid state, the heat dissipation from the solid deposition caused by UV absorption is a lot less efficient than, for example, the rotaxane in solution. The local increase in temperature causes
a change in refractive index of the solvent (CD$_3$CN in our case). In the presence of the pump, the probe beam is refracted differently and hits the detector at a different point than in the absence of the pump. The point where the deviated light hits the detector is less than optimal because the alignment of the probe is optimized before the experiment. The artifact therefore manifests itself as an induced absorption of which the maximum occurs between 500–1000 ns after UV-excitation and can become several factors greater than the signal from the sample. As more laser shots are absorbed, the deposition worsens and the artifact “grows” over time. Additionally, a higher pump-laser power does seem to favor faster deposition on the windows.

The solution to the problem of deposition is simple. Since the artifact occurs at the surface of the window, the point where the pump and probe intersect should be in the center of the sample and the angle between the UV-pump and IR-probe beams should be large enough such that the probe avoids passing through the affected sample region. Unfortunately, increasing the angle between the beams does result in a loss in signal. The deposition has to be removed mechanically; wiping the window sample-side with a lens tissue works perfectly fine. This procedure is however not possible with permanently sealed sample cells. In conclusion, avoid the deposition by ensuring the rotaxane stays dissolved.

A.3.2 Electronic

Electronic artifacts were caused by electrical contact between the laser head and the table. Isolating the laser head from the table with a piece of non-conducting material eliminates this artifact.