Azobenzene as a Photoregulator Covalently Attached to RNA: A Quantum Mechanics/Molecular Mechanics-Surface Hopping Dynamics Study

Padmabati Mondal,1,a) Giovanni Granucci,2 Dominique Rastädter,1 Maurizio Persico,2, b) and Irene Burghardt1, c)  
1) Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany  
2) Dipartimento di Chimica e Chimico Industriale, Università di Pisa, v. Moruzzi 13, I-56124 Pisa, Italy

The photoregulation of nucleic acids by azobenzene photoswitches has recently attracted considerable interest in the context of emerging biotechnological applications. To understand the mechanism of photoinduced isomerisation and conformational control in these complex biological environments, we employ a Quantum Mechanics/Molecular Mechanics (QM/MM) approach in conjunction with nonadiabatic Surface Hopping (SH) dynamics. Two representative RNA-azobenzene complexes are investigated, both of which contain the azobenzene chromophore covalently attached to an RNA double strand via a β-deoxyribose linker. Due to the pronounced constraints of the local RNA environment, it is found that trans-to-cis isomerization is slowed down to a time scale of ~15 picoseconds, in contrast to 500 femtoseconds in vacuo, with a quantum yield reduced by a factor of two. By contrast, cis-to-trans isomerization remains in a sub-picolisecond regime. A volume-conserving isomerization mechanism is found, similarly to the pedal-like mechanism previously identified for azobenzene in solution phase. Strikingly, the chiral RNA environment induces opposite right-handed and left-handed helicities of the ground-state cis-azobenzene chromophore in the two RNA-azobenzene complexes, along with an almost completely chirality conserving photochemical pathway for these helical enantiomers.

1. INTRODUCTION

The light-induced control of conformational changes in nucleic acids by introducing covalently or non-covalently bound photoswitches has proven a promising route towards DNA and RNA functionalization1–6. Applications range from photoregulation of gene expression7 and functional aptamers8, to the tailored design of photoresponsive oligonucleotide walkers9, tweezers10, and diverse nanoarchitectures.

In this context, azobenzene photoswitches play a prominent role1–6,11,12, besides other photo-switchable chromophores like spiropyrans which have also been employed in DNA control13. The azobenzene chromophore switches reversibly between the planar trans form and the non-planar cis form, upon absorption of UV light inducing trans-to-cis conversion, or visible light inducing cis-to-trans conversion14–16. While the planar trans azobenzene isomer intercalates readily between aromatic nucleobases, the non-planar cis form, which is shorter by ~3 Å, tends to unstack from a DNA or RNA helix17–20. As a result, preferential destabilization of nucleic acid double helices has been observed after trans-to-cis conversion5,6,17,18,21. In a paradigm system involving azobenzene covalently linked to DNA or RNA oligomers via a D-threono linker5,17, differences in melting temperatures ($T_m$) between trans vs. cis azobenzene substituted oligomers of the order of $\Delta T_m = T_m^{trans} - T_m^{cis} \sim 10^\circ C - 20^\circ C$ were observed, which can be augmented by inserting multiple azobenzenes into the duplex structure22.

Due to the widely different time scales of azobenzene isomerization (femtoseconds to picoseconds) as compared with the much slower DNA or RNA conformational response (nanoseconds to milliseconds), complementary techniques are required to simulate the relevant steps. In particular, Molecular Dynamics (MD) simulations including an effective switching potential19,23 were employed to model the fast isomerization step, while enhanced sampling techniques like Replica Exchange Molecular Dynamics (REMD) were used to explore longer time scales where the effects of DNA and RNA unfolding become manifest20. Markov State Models (MSM) are the method of choice to provide an embedding of MD and REMD type information into a kinetic description adapted to long-time conformational changes24.

Here, we are concerned with the shortest, ultrafast time scale, in order to investigate whether azobenzene works as an efficient photoswitch in the highly specific nucleic acid environment, and whether azobenzene isomerization proceeds similarly to the gas phase or solution phase. A priori, the DNA or RNA local environment could entail significant modifications of the mechanism and time scales. To this end, we use hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) simulations25–30 in conjunction with Surface Hopping (SH) dynamics26,31,32 in order to obtain a detailed picture of the isomerization process in azobenzene-RNA complexes on a picosecond time scale. In a related approach, QM/MM-SH simulations were recently used to...
study peptide folding and unfolding driven by azobenzene photoisomerisation\textsuperscript{32}. The simulations reported in the present work represent an important benchmark for more approximate Molecular Mechanics (MM) based treatments\textsuperscript{19,20,33,34}.

Connecting to our recent work\textsuperscript{20,33,35} in the context of RNA regulation, we focus on azobenzene-linker-RNA complexes – rather than complexes with DNA – in the present study. To reduce the bias associated with the study of a single system, we carried out parallel investigations of two azobenzene-RNA complexes as depicted in Figure 1, both of which contain the azobenzene chromophore anchored to one of the RNA strands \textit{via} a $\beta$-deoxyribose linker\textsuperscript{33,35}. This linker-azobenzene combination has recently been shown to induce a destabilization effect comparable to the widely applied D-threominitol-linked azobenzene\textsuperscript{35,36}, with excellent efficiencies and high thermal stability. In addition, the $\beta$-deoxyribose linker has been shown to impose minimal disturbance on the duplex stability\textsuperscript{33,35,36}. The two RNAs that were selected correspond to (i) a double-helix structure representing a fragment of a longer double helix that was synthesized and studied both experimentally and theoretically\textsuperscript{33,35}, and (ii) a 14-mer hairpin structure capped by a tetraloop motif – a frequently occurring RNA motif – which was studied in Ref. 20 using REMD simulations.

2. METHODS

Given that the electronic excitation remains localized on the azobenzene chromophore, a QM/MM treatment is a natural approach to the electronic structure of the azobenzene-linker-RNA complexes. As illustrated in Figure 2, the QM part corresponds to the azobenzene chromophore, while the MM part combines the $\beta$-deoxyribose linker, the RNA duplex, and the surrounding water.

For the QM part, we employ a semi-empirical Configuration Interaction (CI) method based upon Floating Occupation Molecular Orbitals (FOMO-CI)\textsuperscript{37}, which has proven successful in the past in the study of azobenzene derivatives\textsuperscript{31}. Calculations were performed with a development version of the MOPAC program, using a reparametrized AM1 Hamiltonian and additional features as detailed in Refs. 37–39.

The linker and the RNA duplex are described by the Amber ff99SB\textsuperscript{40} force field as implemented in the TINKER 6.1 package\textsuperscript{41}, and the water molecules are of TIP3P type\textsuperscript{42}. The connection atom scheme\textsuperscript{43} is employed to describe the boundary between the covalently linked QM and MM parts. According to this scheme, the C1’ atom of the $\beta$-deoxyribose linker is the connection atom which behaves as a hydrogen atom in the QM part and as a normal carbon atom in the MM part (see Figure 2). The total energy of the system is of additive type and the electrostatic embedding scheme is considered for the calculation of the effective QM/MM Hamiltonian\textsuperscript{43}.

The QM/MM scheme is combined with an approximate representation of the nonadiabatic dynamics using Tully’s Fewest Switches Surface Hopping algorithm\textsuperscript{44} including overlap based quantum decoherence corrections (SH-ODC)\textsuperscript{31,45}. Energy gradients and nonadiabatic couplings are calculated using the FOMO-CI method as a function of the time-evolving trajectories. Trajectories

FIG. 1. Structure of the azobenzene-linker-RNA systems under study. The azobenzene chromophore is covalently bound to the RNA backbone \textit{via} a $\beta$-deoxyribose linker. (a) Duplex structure denoted Azo-RNA1. (b) Hairpin structure, capped with a tetraloop, denoted Azo-RNA2. (c) Detailed structure of linker-azobenzene unit covalently bound to the RNA backbone.

FIG. 2. Representation of the QM/MM set-up for the azobenzene-linker-RNA system in water (l.h.s.), with a detailed representation of the linker-azobenzene moiety, where the QM/MM connection atom is shown in magenta (r.h.s.).
are initiated in the $S_2(\pi - \pi^*)$ state, whose squared transition dipole moment averaged over the ensemble is dominant (see Supporting Information Sec. S1). An ensemble of 100 initial conditions is considered, which are sampled from equilibrated ground-state MD simulations for the relevant complexes. Following MD pre-equilibration, a QM/MM equilibration was carried out using Brownian dynamics in the electronic ground state, with an equilibration time of 50 ps at 300 K. To avoid nonequilibrium effects due to heating at the beginning of the QM/MM equilibration, the first 10 ps are excluded from the 50 ps equilibration data for generating initial conditions. The ground state distribution is chosen from snapshots of 40 ps taken from the QM/MM equilibration trajectory. During the simulation, the populations of the first six singlet states are monitored.

The Supporting Information provides further information regarding the simulation set-up, construction of initial conditions, and absorption spectra obtained within the QM/MM set-up (see Supporting Information Sec. S1 and Sec. S2).

3. RESULTS AND DISCUSSION

In the following, we first analyze the time-dependent electronic state populations and estimated isomerization quantum yields resulting from the QM/MM-SH simulations. Then, we turn to the isomerization mechanism, with emphasis on the role of the RNA environment, the subtle role of chirality, and the interplay of different dihedral angles whose combined dynamics results in a largely volume-preserving photoreactive path.

3.1. Photodynamical time scales and quantum yield

Figure 3 illustrates one of the key results of this study: Trans-to-cis isomerization of azobenzene is slowed down remarkably in the RNA environment, from 500 femtoseconds in the gas phase to $\sim$15 picoseconds in RNA. By contrast, the time scale of cis-to-trans isomerization is almost unaffected and remains less than one picosecond. These findings are very similar for the two RNAs that we studied very similar for the two RNAs that we considered in the RNA environment – pointing towards an important role of stacking interactions that stabilize the excited $S_1$ state for $\sim$10 picoseconds. Apparently, the $S_1$ surface is very flat, or exhibits a shallow local barrier, such that trajectories are substantially delayed before reaching the $S_1/S_0$ crossing region. From the build-up of $S_0$ population, we can infer that most trajectories return to the $S_0/\text{trans}$ conformation, while a lesser portion isomerizes and yields the $S_0/\text{cis}$ product.

For the trajectories starting in cis conformation (Figure 3c/d), a very different scenario is observed: While the $S_2$ state again decays very rapidly, within $\sim$1 picosecond, the $S_1$ state is only transiently populated – or barely populated at all – while the ground state ($S_0$) population rises within $\sim$1 picosecond, concomitantly with the $S_2$ decay. This indicates that $S_1$ is at most a transient intermediate rather than a long-lived intermediate as in the trans-to-cis dynamics. As is known from previous work\cite{31,46}, the potential energy surfaces (PESs) are indeed very different in the cis region as compared with the trans region. Our analysis confirms that the $S_2/S_1$ and $S_1/S_0$ conical intersection\cite{47} seams are very close in the case of the cis-to-trans photochemical pathway, permitting ultrafast $S_2 \rightarrow S_1 \rightarrow S_0$ conversion.

As also shown in Figure 3, the population decay derived from the trajectory ensemble can be fitted to an approximate set of coupled first-order kinetic equations for the state populations $P_n$, $n = 0, \ldots, 2$, for a three-state system comprising the $S_n$, $n = 0, \ldots, 2$, electronic states (see also Ref. 31),

\[
P_2 = \exp(-t/\tau_2) \\
P_1 = \frac{\tau_1}{\tau_1 - \tau_2} \left( \exp(-t/\tau_1) - \exp(-t/\tau_2) \right) \\
P_0 = 1 - P_1 - P_2 \simeq 1 - \exp(-t/\tau_0)
\]

where $\tau_2$ and $\tau_1$ are the lifetimes of the $S_2$ and $S_1$ states,
and \( \tau_0 \) describes the concomitant rise of the \( S_0 \) state (see Table 1). Here, \( \tau_0 \) is treated as an independent decay parameter, and the single-exponential form is found to give good agreement with the relation \( P_0 = 1 - P_1 - P_2 \) for the data at hand, given that \( \tau_1 \) and \( \tau_2 \) typically differ by a factor of ten. In the case of \( cis\text{-}to\text{-}trans \) isomerisation, the fitting procedure for \( S_1 \) did not work well due to the small state population, such that only \( \tau_2 \) and \( \tau_0 \) were determined.

The nature of the population dynamics is very similar for the Azo-RNA1 and Azo-RNA2 complexes, even though the \( S_1 \) and \( S_2 \) lifetimes depend slightly on the environment. Since an analysis with the same FOMO-CI-SH set-up was previously carried out for the azobenzene chromophore in gas phase and in various solvents\(^{31}\), we compare in Table 1 the results for the relevant lifetimes \( \tau_n, n = 0, \ldots, 2 \). This comparative analysis underscores that (i) \( S_1 \) lifetimes in the \( trans\text{-}to\text{-}cis \) isomerisation dynamics in an RNA environment differ by a factor of about 20 from previously observed lifetimes in the gas phase and solution phase, and (ii) in the case of \( cis\text{-}to\text{-}trans \) dynamics, \( S_2 \) lifetimes are slightly augmented, by a factor of two.

Table 1. Comparison of \( S_2 \) and \( S_1 \) lifetimes for \( trans\text{-}to\text{-}cis \) and \( cis\text{-}to\text{-}trans \) isomerisation in vacuo as compared with various solvents and the RNA environment. The values in vacuo and in solution are taken from Ref. 31. In addition, the time scale of the growth of the \( S_0 \) population (\( \tau_0 \)) is given.

| isomerisation | environment | \( \tau_1 \) (ps) | \( \tau_2 \) (ps) | \( \tau_0 \) (ps) |
|---------------|-------------|-----------------|-----------------|-----------------|
| \( trans \to cis \) | in vacuo | 0.525 | 0.191 |
| | methanol | 0.643 | 0.145 |
| | ethylene glycol | 0.534 | 0.091 |
| | Azo-RNA1 | 14.590 | 1.120 | 16.10 |
| | Azo-RNA2 | 10.233 | 0.952 | 11.30 |
| \( cis \to trans \) | in vacuo | 0.085 | 0.446 |
| | methanol | 0.071 | 0.381 |
| | ethylene glycol | 0.082 | 0.352 |
| | Azo-RNA1 | - | 0.702 | 0.97 |
| | Azo-RNA2 | - | 0.527 | 0.84 |

FIG. 4. Schematic representation of the photochemistry of the azobenzene-linker-RNA complexes as deduced from the present study, (a) for the \( trans\text{-}to\text{-}cis \) pathway (shown in red) which exhibits a remarkable slowing-down as compared to the chromophore in vacuo and (b) for the \( cis\text{-}to\text{-}trans \) pathway (shown in green) whose kinetics remains comparable to the situation in vacuo. The \( S_0 \) ground state, \( S_1 (n - \pi^* \text{ state, and } S_2 (\pi - \pi^*) \text{ state are shown explicitly (with calculated Franck-Condon energies indicated as solid blue circles), while the higher-lying singlet states } (S_n, n = 3, \ldots, 5) \text{ are indicated schematically at the Franck-Condon point. The effective reaction coordinate is a complex combination of internal modes, especially involving a concerted motion of the dihedrals. Conical intersections which interconnect all relevant states are also indicated schematically.}

TABLE 2. Comparison of quantum yields for \( trans\text{-}to\text{-}cis \) \((t \to c)\) and \( cis\text{-}to\text{-}trans \) \((c \to t)\) isomerisation in vacuo, in solution, and in an RNA environment. The values in vacuo and in solution are taken from Ref. 31.

| environment | \( \Phi_{t\to c} \) | \( \Phi_{c\to t} \) |
|-------------|-----------------|-----------------|
| in vacuo | 0.21 | 0.54 |
| methanol | 0.24 | 0.57 |
| ethylene glycol | 0.24 | 0.67 |
| Azo-RNA1 | 0.10 | 0.50 |
| Azo-RNA2 | 0.14 | 0.52 |
| experiment (methanol) | 0.12-0.15 | 0.3-0.37 |

Clearly, the striking lengthening of the \( S_1 \) lifetime in the \( trans\text{-}to\text{-}cis \) dynamics must be due to steric constraints of the RNA environment that mainly affect the \( trans \) form of the chromophore. A slowing down of the azobenzene isomerization in solvents and constrained environments has been previously reported\(^{31,48,49}\), even though the effect was by far not as pronounced and typically limited to an increase of the isomerization time by a factor of two to three. Noticeably, though, a full suppression of isomerisation\(^{50}\), mainly due to steric effects\(^{51}\), has been found for regularly packed self-assembled monolayers of trans-azobiphenyls, with \( S_1 \) lifetimes of the same order of magnitude as in the present work.

The quantum yield for \( trans\text{-}to\text{-}cis \) \((\Phi_{t\to c})\) and \( cis\text{-}to\text{-}trans \) \((\Phi_{c\to t})\) isomerisation is 10% and 50%, respectively, which is smaller than in the gas phase and in solution. This clearly indicates that steric effects are very pronounced and tend to hinder isomerisation. The quantum yields for azobenzene isomerisation in the gas phase, in solution, and in the RNA environment are given in Table 2 for comparison. As in Table 1, all results are obtained by the same simulation method. These results are in line with recent experimental\(^{52}\) and MD-based computational\(^{34}\) studies on the photoisomerisation quantum yield of azobenzene in DNA where the quantum yield for the \( trans\text{-}to\text{-}cis \) conversion was found to depend largely on the local DNA sequence\(^{53}\) as well as on temperature\(^{53}\), and strongly decreases with decreasing
local volume in the nucleic acid environment. We confirm this trend using the first QM-based computational study of azobenzene photoisomerisation in an RNA environment.

### 3.2. Photochemical pathways

To summarize our observations, Figure 4 shows a schematic picture of the photodynamics of trans and cis azobenzene in RNA, as obtained from the ensemble of propagated QM/MM-SH trajectories. The effective reaction coordinate is a complex combination of local modes, especially involving a concerted motion of neighboring dihedrals, as further discussed below. In the following, we briefly recapitulate the photochemical pathways delineated in Figure 4. Detailed information, e.g., regarding the energetic distribution of the trajectory ensemble at the hopping geometries, is provided in the Supporting Information (Sec. S3).

After the initial excitation from the trans ground state to the S2 state, the close-lying S3, S1 and S0 states are temporarily populated to some extent by nonadiabatic transitions. Within less than a picosecond (i.e., 0.7-1 ps), the population is transferred from S2 and the partially populated upper states to the S1 state, at geometries that remain close to the Franck-Condon region. A majority of trajectories are then found to stay on the S1 surface for an extended time interval, presumably due to existence of a local minimum and shallow barrier preceding the S1/S0 conical intersection at CNNC=90°. This PES topology, which is modified as compared with the gas phase, is most likely created by the stacking of trans azobenzene with the upper and lower bases, hindering trans-to-cis photoisomerisation (see the discussion below). At this point, a subset of reactive trajectories have high enough energy to proceed to the S1/S0 conical intersection. A trajectory is considered to reach the conical interaction if it meets a threshold criterion for the energy difference between two states, i.e., $\Delta E < 0.1$ eV. The remaining trajectories are unreactive and return to $S_0$, mainly by hopping from the transoid region, where the S1/S0 energy difference is still large. In both reactive and unreactive cases, the dynamics tends to be slow, of the order of 10 picoseconds. Overall, almost 80% of the population is transferred back to the trans ground state via unreactive trajectories. The complementary subset of trajectories which reach the conical intersection are eventually equidistributed between the trans and cis ground-state geometries, rendering the overall quantum yield small, with $\Phi_{t\rightarrow c} \approx 10\%$.

By contrast, starting in the S2 state of the cis isomer, trajectories directly reach the three-state conical intersection region between S2, S1, and S0, within <1 ps, promoted by the steep S2 surface. No participation of the S3, S1 and S0 states is noticed when starting from the cis isomer. At the conical intersection, population is transferred either directly to the S0 state or via S1 to S0 almost immediately, such that the S1 lifetime of the cis isomer could not be determined. Since the total population decays through the conical intersection which provides access to the trans and cis channels in a symmetric fashion, the final ground-state populations are found to be equally distributed, such that the cis-to-trans photoisomerisation quantum yield is 50%.

### 3.3. Isomerisation mechanism

To gain some insight into the effect of the local RNA environment on the isomerisation process, Figure 5 shows selected snapshots from a reactive trans-to-cis trajectory in the Azo-RNA2 system. This trajectory is among the few realizations that isomerize rapidly, but similar scenarios have been observed for other reactive and unreactive trajectories. The figure illustrates the time-evolving center-of-mass distance between the azobenzene rings and the upper and lower base pairs. Despite the isomerisation event (at ~1.5 ps), the relative distances evolve smoothly and stacking interactions are preserved to a large extent. This underscores the important role of stacking, and also the flexibility of the chromophore’s non-covalent binding interactions within its local RNA environment. As further discussed in the Supporting Information, the isomerization event indeed does not exert a major disrupting effect on the interaction with the near-
est neighbouring RNA bases (see Supporting Information Sec. S4).

Figure 5 further suggests that the isomerization event follows a volume-conserving mechanism, to exert as little perturbation on the nearest-neighbor environment as possible. This implies that a cooperative dynamics must be involved, similarly to the related pedal-like mechanism that was found in solution phase\textsuperscript{31,49}. Figure S9 shows that substantial changes in the CNN and NNC angles occur very soon after excitation, because in the $S_1(n - \pi^*)$ state, the equilibrium CNN/NNC value is larger than in the ground state\textsuperscript{38,54,55}. Even in the gas phase, an inversion-assisted rotation mechanism of the photoisomerisation is observed\textsuperscript{55}. To better understand the details of the mechanism, we now analyze the time evolution of several dihedrals – i.e., the CNNC (C2-N3-N4-C5), CCNN (C1-C2-N3-N4), NNCC (N3-N4-C5-C6), and CCCC (C1-C2-C5-C6) dihedral angles – as illustrated in Figure 6 for two selected trajectories (see Figure 1c and Supporting Information Sec. S5 for the atom labels and definition of the relevant dihedral angles).

Figure 6a illustrates a reactive trans-to-cis trajectory in the Azo-RNA1 complex, where the sudden change in the CNNC dihedral (red trace) is seen to be accompanied by an almost equal change in the NNCC dihedral (black trace). Indeed, the absolute value of the NNCC dihedral changes more – from $\approx 180^\circ$ to $\approx 0^\circ$ – than the CCNN dihedral (cyan trace), which changes from $\approx 0^\circ$ to $\approx 45^\circ$ via $\approx 80^\circ$ while going from structure 1 to 3 via 2. Note that structure 2 corresponds to a geometry near the conical intersection, preceding the hopping event. From the evolution of the dihedrals, we infer that the N3-N4 twist is immediately followed by a rotation around the N4-C5 single bond. The distant phenyl ring (as seen from the linker position) undergoes partial in-plane rotation around the N4-C5 bond. A very similar scenario is observed for the reactive cis-to-trans trajectory shown in Figure 6b. The CNNC and NNCC evolution is again found to be closely correlated, even though the NNCC dihedral appears to be delayed until the hopping event occurs. In a complementary fashion to Figure 6, Figure S6 of the Supporting Information shows the signed values of the relevant dihedrals. (These exhibit frequent sign changes between $\pm 180^\circ$ in the trans conformation, due to the fluctuating twist of the N3-N4 bond.)

Similar observations were made for the Azo-RNA2 complex, even though some details for the individual trajectories differ (see the Supporting Information Sec. S6.2 for a detailed discussion). In all cases, a concerted mechanism involving several dihedrals is observed, which is consistent with the abovementioned pedal-like mechanism that was found for the azobenzene chromophore in solution\textsuperscript{31,49}. As can be inferred from Fig. 6 and is further explained below, this mechanism is coupled to a collective rearrangement of the local RNA environment.

3.4. Static vs. dynamic effects of RNA environment

In view of the pronounced effect of the RNA environment on the trans-to-cis isomerization kinetics, the question arises whether the local environment mainly exerts a static or dynamic influence. Therefore, we investigated the equilibrium (static) effects of the RNA environment on the shape of the effective torsional potential energy surface by Boltzmann inversion\textsuperscript{56}, based on the CNNC dihedral angle distribution from an ensemble of ground state QM/MM equilibration trajectories for azobenzene in different environments (in vacuo, in ethylene glycol and in RNA). As detailed in the Supporting Information (Sec. S4.2), the analysis reveals that the shape of the ground state potential energy surface around the cis and trans equilibrium geometry in RNA is very similar to the corresponding potentials in solution and in vacuo. Small shifts in the equilibrium values of the CNNC dihedral are observed – about $5^\circ$ for trans-azobenzene, and even less for cis-azobenzene – in opposite directions for azo-RNA1 and azo-RNA2, which can be related to the effect of the chiral environments (see the discussion below). Perhaps contrary to expectation, the effective torsional potentials in RNA are not steeper – but rather slightly broader – than in solution or in vacuo, showing no evidence of a constraining environment. Therefore, we conclude that
the slowing down of the N=N double bond twisting and of the $S_1$ decay are due to dynamic caging, i.e., to the relatively long time needed for the RNA bases to collectively relax geometrically in order to match the twisting of trans-azobenzene. In this sense, steric hindrance in the context of trans-to-cis isomerization should be thought of as a dynamic effect.

3.5. Chiral selectivity

Finally, we address the role of chirality in the photoisomerization event, which is of particular interest in the present context since the RNA environment as such exhibits right-handed helical chirality. With regard to the chromophore, the trans azobenzene isomer is achiral, while the cis isomer exhibits helical chirality and exists in a P (plus, i.e., right-handed) helical form and an M (minus, i.e., left-handed) helical form (see Supporting Information Sec. S5 for details). Likewise, the $S_1/S_0$ conical intersection exhibits symmetric P and M type precursor structures of the two cis isomers.$^{31,57,58}$ For the trans-to-cis photochemical pathway, it is therefore of interest to know whether the P or M type cis form is preferentially generated in the reactive trajectories. Conversely, for the cis-to-trans pathway, we will analyze whether a chirality preserving or a chirality inverting pathway is adopted. Even in an achiral environment, it was found that an asymmetry can exist between these pathways$^{31,57,58}$. This asymmetry could be enhanced in the chiral RNA-linker environment.

Interestingly, a marked preference for P vs. M helicity appears in the cis species of the Azo-RNA1 vs. Azo-RNA2 complexes as a result of the initial equilibration procedure. That is, it turns out that the initial helicity of cis-azobenzene is of M type for Azo-RNA1 and of P type for Azo-RNA2. The reason for this intrinsic difference seems to lie in the preference of cis azobenzene to reside either in the major groove, as is the case for Azo-RNA1, or in the minor groove, as is the case for Azo-RNA2. In earlier MD studies of the Azo-RNA2 complex with a d-threoinol linker,$^{20}$ we also found a minor groove preference of cis azobenzene.

In the following analysis, we will make use of the fact that the algebraic sign of the CNNC dihedral during the isomerization process informs about the P and M helicity, i.e., a positive sign correlates with P helicity while a negative sign correlates with M helicity.$^{37,59}$ A complementary analysis can be given in terms of the change of the NNCC and CCNN dihedrals$^{31}$, which is especially useful when analyzing the initial chirality preserving or chirality inverting property of the isomerisation pathway (see Supporting Information Sec. S7): The pathway is chirality conserving if the CNNC twist is accompanied by a conrotatory torsion of the N-C bonds such that the CCNN and NNCC dihedrals fall into a narrow range around ±90°. Conversely, a pronounced chiral selectivity is observed for the trajectories starting from the trans (upper panels) and cis (lower panels) isomer, both for reactive (red) and unreactive (green) trajectories.

For the trans initial conditions (Figure 7a/b), we focus upon the reactive trajectories (red bars) whose hopping geometries fall into a narrow range around ±90°, where the conical intersection is located. By contrast, for the unreactive trajectories (green bars), the CNNC dihedral at the $S_1/S_0$ hopping point is spread over a wide range, i.e., −100° to −180° and 60° to 180°, consistent with the observation that most unreactive trajectories hop far before reaching the conical intersection, and consequently go back to the trans ground state, resulting in a small photoisomerisation quantum yield. As can be seen from Figure 7a, there is no clear preference for the P-type or M-type cis photoproducts in Azo-RNA1, while a certain preference for the M-type cis photoproduct is found for the Azo-RNA2 complex. However, no definite conclusion can be drawn from the limited set of reactive trajectories. This is confirmed by a complementary analysis based upon the CCNN and NNCC dihedrals, as reported in the Supporting Information (Sec. S7).

Conversely, a pronounced chiral selectivity is observed...
when considering the cis-to-trans photoisomerisation dynamics, as can be inferred from Figure 7c/d. Starting from the equilibrated M vs. P forms of the cis isomer of Azo-RNA1 and Azo-RNA2, it is seen that the M vs. P forms also prevail at the respective hopping geometries. Indeed, an almost perfectly chirality conserving pathway is found in both cases. This conclusion is confirmed by a detailed analysis of the NNCC and CCNN dihedral angles as described in the Supporting Information (Sec. S7): During the initial dynamics (∼200 fs) of the Azo-RNA1 complex, the NNCC dihedral tends to 0° starting from negative values, and its counterpart in the Azo-RNA2 complex also tends to 0° starting from positive values. This confirms that the M-helical form of Azo-RNA1 and the P-helical form of Azo-RNA2 remain conserved on the path towards the S1/S0 conical intersection. Clearly, the chiral RNA environment is the determining factor inducing this pronounced selectivity. For comparison, a ∼75% predominance of the chirality conserving pathway was found for the gas-phase cis-to-trans isomerization in Refs. 57,58, while the study of Ref. 31 did not provide evidence for such a preferential pathway in gas phase or solution. (However, a nearly complete stereospecificity was found for a related arylazo pyrazole photoswitch60.)

Notably, a narrow, almost normal-type distribution of CNNC dihedrals around ±90° is observed both for the reactive and unreactive cis-to-trans trajectories of AzoRNA1 and AzoRNA2 in Figure 7. All trajectories reach the vicinity of the conical intersection and thereby the quantum yield of photoisomerisation is 50%.

4. CONCLUSIONS

To summarize, we have investigated the photoisomerization mechanism of an azobenzene chromophore covalently attached to an RNA double strand using state-of-the-art semiempirical QM/MM-SH methodology that has proven reliable in the treatment of azobenzene in various environments over recent years31,37–39,50,61. Conconcerted nonadiabatic dynamics in six singlet electronic states is followed after photoexcitation to the bright S2 state. Our investigation highlights several key features: First, ultrafast internal conversion from the S2 state to the S1 state initiates the photochemical process (apart from minor participation of the S3 and S4 states, very similarly to the photochemistry of the bare azobenzene chromophore in gas phase55,62 or in solution31,49. Second, the main effect of the extremely constrained RNA environment is felt in the S1 dynamics: The local interactions with the RNA bases lead to an S1 lifetime of ∼15 picoseconds for the trans azobenzene chromophore preceding isomerization, as compared with 500 femtoseconds in vacuo. Further, the trans-to-cis photoisomerization quantum yield is reduced to ∼10%. By contrast, the electronically excited cis isomer remains short-lived, on a time scale of ∼500 femtoseconds, with a 50% cis-to-trans quantum yield. We attribute this pronounced difference to the fact that the upper and lower base pairs exert a highly stabilizing effect on the trans azobenzene isomer – but not on the cis isomer. This picture is confirmed by representative QM/MM trajectories. For the same reasons, the isomerization mechanism is found to be volume-preserving and proceeds by a concerted motion in several dihedral angles, resulting in a pedal-like motion that also prevails in the isomerisation in solution31,49. This motion is accompanied by a collective rearrangement of the local RNA environment that gives rise to steric constraints which are of dynamical rather than static nature.

Even though time-resolved spectroscopic observations for these and related systems are not yet available, our predictions are in line with similar trends observed for azobenzene photoisomerization in constrained environments and adsorbed on surfaces31,48,50,54,61,63.

Finally, we found that RNA chirality plays a subtle role in the process and leads to preferential P type (right-handed) or M type (left-handed) cis-to-trans isomerization pathways. Strikingly, the equilibrated Azo-RNA1 vs. Azo-RNA2 cis species are of M type vs. P type, respectively, and conserve their helicity during their short-time dynamics such that the preferred M-type vs. P-type forms are also observed at the hopping geometry. While some degree of chiral selectivity has been previously found for cis-to-trans azobenzene isomerization in the gas phase or in less specific environments, the almost full conservation of M-type vs. P-type helicity during the excited-state dynamics of the Azo-RNA complexes is a unique feature of the chiral RNA environment.

Most of these conclusions are remarkably similar for the two types of chromophore-linker-RNA complexes that were investigated in this work, with the exception of the minor vs. major groove preference of the cis azobenzene chromophore, which is opposite for the two complexes, thus entailing opposite preferences for P vs. M helicity. Apart from this difference, the similarity of the mechanism and excited-state lifetimes is striking. Our results underline the crucial role of local stacking interactions in selectively stabilizing the trans form of the chromophore, which should be accounted for as one of the key guiding principles when designing new, optimally tailored RNA-linker-azobenzene combinations. Specific local modifications, using suitable linkers and substituents, could lead to reduced S1 lifetimes and enhanced quantum yields, but possibly at the expense of reduced differences in melting temperatures associated with the trans vs. cis isomers. In view of connecting to longer times scales, the present benchmark study paves the way for multiscale simulations30 as well as the design of accurate effective switching potentials to model light-responsive functional oligonucleotides and nanoassemblies.

ACKNOWLEDGMENTS

We gratefully acknowledge support by the Research Center SFB 902 ("Molecular Principles of RNA-based
References

1. A. S. Lubbe, W. Szymański, and B. L. Feringa, Chem. Soc. Rev. 46, 1052 (2017).
2. G. Mayer and A. Heckel, Angew. Chem. Int. Ed. 45, 4900 (2006).
3. A. Jäschke, FEBS Lett. 586, 2106 (2012).
4. W. Szymański, J. M. Beierle, H. A. V. Kistemaker, W. A. Velema, and B. L. Feringa, Chem. Rev. 113, 6114 (2013).
5. H. Ito, X. Liang, N. Hishioka, and H. Asanuma, Org. Biomol. Chem. 8, 5519 (2010).
6. J. Li, X. Wang, and X. Liang, Chem. Asian J. 9, 3344 (2014).
7. Y. Kamiya, T. Takagi, H. Ooi, H. Ito, X. Liang, and H. Asanuma, ACS Synth. Biol. 4, 365 (2015).
8. J. A. Phillips, H. Liu, M. B. O’Donoghue, X. Xiong, R. Wang, M. You, K. Sefah, and W. Tan, Bioconjugate Chem. 22, 282 (2011).
9. T. Cha, J. Pan, H. Chen, H. N. Robinson, X. Li, C. Mao, and J. H. Choi, J. Am. Chem. Soc. 137, 9429 (2015).
10. X. Liang, H. Nishioka, N. Takenaka, and H. Asanuma, ChemBioChem 9, 702–705 (2008).
11. H. M. D. Bandara and S. C. Burdette, Chem. Soc. Rev. 41, 1809 (2012).
12. A. A. Beharry and G. A. Woolley, Chem. Soc. Rev. 40, 4422 (2011).
13. J. Andersson, S. Li, P. Lincoln, and J. Andréasson, J. Am. Chem. Soc. 130, 11838 (2008).
14. H. Rau, “in: Photochemistry and Photophysics, ed.; CRC press, Boca Raton, FL, USA,” 1990, 119-141.
15. E. Merino and M. Ribagorda, Beilstein J. Org. Chem. 8, 1071 (2012).
16. K. Morgenstern, Acc. Chem. Res. 42, 213 (2009).
17. H. Kashida, X. Liang, and H. Asanuma, Curr. Org. Chem. 13, 1065 (2009).
18. H. Ito, H. Nishioka, X. Liang, and H. Asanuma, Nuc. Acids Symp. Ser. 51, 171 (2007).
19. M. Biswas and I. Burghardt, Biophys. J. 107, 932 (2014).
20. D. Rastädter, M. Biswas, and I. Burghardt, J. Phys. Chem. B 118, 8478 (2014).
21. H. Asanuma, T. Ito, T. Yoshida, X. G. Liang, and M. Komiyama, Angew. Chem. Int. Ed. 38, 2393 (1999).
22. H. Asanuma, D. Matsunaga, and M. Komiyama, Nuc. Acids Symp. Ser. 49, 35 (2007).
23. M. Böckmann, S. Braun, N. L. Doltsinis, and D. Marx, J. Chem. Phys. 139, 084108 (2013).
24. B. G. Keller, A. Kobitski, G. Jäschke, U. Nienhaus, and F. Noé, J. Am. Chem. Soc. 136, 4534 (2014).
25. H. M. Senn and W. Thiel, Angew. Chem. Int. Ed. 48, 1198 (2009).
26. O. Weingart, Curr. Org. Chem. 21, 586 (2016).
27. H. J. Kulik, J. Zhang, J. P. Kliman, and T. J. Martínez, J. Phys. Chem. B 120, 11381 (2016).
28. A. Strambi, B. Durbeej, N. Ferré, and M. Olivucci, Proc. Natl. Acad. Sci. USA 107, 21322 (2010).
29. V. A. Spata and S. Matsika, J. Phys. Chem. A 118, 12021 (2014).
30. S. Osella and S. Kniippenberg, J. Am. Chem. Soc. 139, 4418 (2017).
31. V. Cantatore, G. Granucci, and M. Persico, Comput. Theor. Chem. 1040–1041, 126 (2014).
32. H. Ito, X. Liang, H. Nishioka, and H. Asanuma, Org. Biomol. Chem. 8, 5519 (2010).
33. M. P. acknowledges the financial support of Pisa University (grant PRA-2016-46). We thank K. Falahati for assistance with the preparation of the figures.
34. J. C. Tully, J. Chem. Phys. 93, 1061 (1990).
35. G. Granucci, M. Persico, and A. Zoccati, J. Chem. Phys. 133, 134111 (2010).
36. M. Pederzoli, J. Pittner, M. Barbatti, and H. Lischka, J. Phys. Chem. 119, 084108 (2015).
37. G. Granucci, M. Persico, and A. Toniolo, J. Chem. Phys. 114, 10608 (2001).
38. T. Cusati, G. Granucci, E. Martínez-Nuñez, F. Martini, M. Persico, and S. Vázquez, J. Phys. Chem. A 116, 98 (2012).
39. C. Ciminielli, G. Granucci, and M. Persico, Chem. Eur. J. 10, 2327 (2004).
40. V. Hornak, R. Abel, A. Okur, B. Stockbich, A. Roitberg, and C. Simmerling, Proteins 65, 712 (2006).
41. J. W. Ponder, (2012), tINKER 6.1; Washington University School of Medicine: St. Louis, MO (http://dashr.wustl.edu/tinker).
42. W. L. Jorgensen and J. D. Madura, J. Am. Chem. Soc. 105, 1407 (1983).
43. A. Kingsland, S. Samai, Y. Yan, D. S. Ginger, and I. Burghardt, J. Phys. Chem. B. 119, 11275 (2015).
44. H. M. Senn and W. Thiel, Angew. Chem. Int. Ed. 55, 2067 (2016).
45. P. Mondal, M. Biswas, T. Goldau, A. Heckel, and I. Burghardt, J. Phys. Chem. B. 119, 11275 (2015).
46. A. Strambi, B. Durbeej, N. Ferré, and M. Olivucci, Proc. Natl. Acad. Sci. USA 107, 21322 (2010).
47. V. A. Spata and S. Matsika, J. Phys. Chem. A 118, 12021 (2014).
48. M. Böckmann, N. L. Doltsinis, and D. Marx, J. Phys. Chem. A 114, 745 (2010).
49. V. Cantatore, G. Granucci, G. Rousseau, G. Padula, and M. Persico, J. Phys. Chem. Lett. 7, 4027 (2016).
51 E. Titov, G. Granucci, J. P. Gotze, M. Persico, and P. Saalfrank, J. Phys. Chem. Lett. 7, 3591 (2016).
52 Y. Yan, X. Wang, J. I. L. Chen, and D. S. Ginger, J. Am. Chem. Soc. 135, 8382 (2013).
53 S. Samal, D. J. Bradley, T. Choi, Y. Yan, and D. S. Ginger, J. Phys. Chem. C 121, 6997 (2017).
54 T. Cusati, G. Granucci, and M. Persico, J. Am. Chem. Soc. 133, 5109 (2011).
55 E. Tan, S. Amirjalayer, S. Smolarek, A. Vdovin, F. Zerbetto, and W. J. Buma, Nature Comm. 6, 5860 (2015).
56 D. Reith, M. Pütz, and F. Müller-Plathe, J. Comput. Chem. 24, 1624 (2003).
57 O. Weingart, Z. Lan, A. Koslowski, and W. Thiel, J. Phys. Chem. Lett. 2, 1506 (2011).
58 Y. Ootani, S. Kiminori, A. Nakayama, T. Noro, and T. Taketsugu, J. Chem. Phys. 131, 194306 (2009).
59 J. A. Gámez, O. Weingart, A. Koslowski, and W. Thiel, J. Chem. Theory Comput. 8, 2352 (2012).
60 Y. Wang, X. Liu, G. Cui, W. Fang, and W. Thiel, Angew. Chem. Int. Ed. 55, 14009 (2016).
61 E. Benassi, G. Granucci, M. Persico, and S. Corni, J. Phys. Chem. C 119, 5962 (2015).
62 T. Schultz, J. Quenneville, B. Levine, A. Toniolo, T. J. Martínez, S. Lochbrunner, M. Schmitt, J. P. Shaffer, M. Z. Zgierski, and A. Stolow, J. Am. Chem. Soc. 125, 8098 (2003).
63 L. Creatini, T. Cusati, G. Granucci, and M. Persico, Chem. Phys. 347, 492 (2008).