Optimizing rotary processes in synthetic molecular motors

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We deal with the issue of quantifying and optimizing the rotation dynamics of synthetic molecular motors. For this purpose, the continuous four-stage rotation behavior of a typical light-activated molecular motor was measured in detail. All reaction constants were determined empirically. Next, we developed a Markov model that describes the full motor dynamics mathematically. We derived expressions for a set of characteristic quantities, i.e., the average rate of quarter rotations or “velocity,” V, the spread in the average number of quarter rotations, D, and the dimensionless Péclet number, Pe = V/D. Furthermore, we determined the rate of full, four-step rotations (Ωtot), from which we derived another dimensionless quantity, the “rotational excess,” r.e. This quantity, defined as the relative difference between total forward (Ω+) and backward (Ω−) full rotations, is a good measure of the unidirectionality of the rotation process. Our model provides a pragmatic tool to optimize motor performance. We demonstrate this by calculating V, D, Pe, Ωtot, and r.e. for different rates of thermal versus photochemical energy input. We find that for a given light intensity, an optimal temperature range exists in which the motor exhibits excellent efficiency and unidirectional behavior, above or below which motor performance decreases.

Markov model | unidirectional rotation

The development of nanoscale molecular devices that exhibit controlled movement upon energy input advances progressively (1–11). In the past decade, a spectrum of ingeniously designed molecular structures, ranging from artificial muscles (12) to molecular cars (13), was introduced. In the B.L.F. group, chiroptical switches have evolved into unidirectionally rotating molecular motors (14–17). In fact, an internal, 360° rotation was induced in one specific direction (i.e., clockwise or counterclockwise) upon input of photons and thermal energy. On the road toward applications, it was shown that the phase of liquid crystals can be switched have evolved into unidirectionally rotating molecular motors. In the B.L.F. group, chiroptical switches have evolved into unidirectionally rotating molecular motors (14–17). In fact, an internal, 360° rotation was induced in one specific direction (i.e., clockwise or counterclockwise) upon input of photons and thermal energy. On the road toward applications, it was shown that the phase of liquid crystals can be controlled when doped with small portions of optically enriched molecular motor, and micro objects can be moved (18–20). In addition, molecular motors were recently grafted on gold (21) and quartz surfaces (22).

The general concept of these molecular motors is visualized in Scheme 1 (16, 17). The motor consists of a lower part ("stator," red) and an upper part ("rotor," blue). The rotation takes place around a central double bond (“axle,” black) in four stages, i.e., through transitions between four isomers.

All four conversions in Scheme 1 are in principle reversible (i.e., equilibria are involved). For example, motor molecules with R = OCH3 have photochemical equilibria of 14/86 and 11/89 for the concentration ratios A/B and C/D, respectively. Importantly, the thermal conversions from unstable to stable forms are quantitative (i.e., 100%). Previous research focused on attempts to optimize molecular rotation kinetics through chemical design (24–27). However, it focused on single, thermal reaction steps only. Full and ongoing rotation of individual motor molecules can be realized when both light of appropriate energy (hν) and thermal energy (denoted as Δ) are supplied simultaneously. We therefore envisioned that quantification and optimization of continuous four-stage rotation should be the next challenge to take on. Remarkably, a proper approach to quantify the rotational dynamics and efficiency of synthetic rotational motors is lacking. This is in contrast to the situation for biological molecular motors, generally defined as Brownian motors (28–34) or steppers (35–38), which have been the inspiration of numerous studies.

Here, we report characterization of the rotation dynamics of a synthetic molecular motor (Scheme 1) upon simultaneous input of photons and heat. Next, all empirically determined reaction constants were inserted in a mathematical model based on the theory of Markov processes. Finally, we show how the model can be used to optimize rotational behavior (i.e., efficiency) of existing molecular motors.

Results and Discussion

Empirical Results. Several experiments were necessary to fully characterize the rotation dynamics of the molecular motor system as depicted in Scheme 1 (R = OCH3). In a first experiment, 100% of the stable isomer A (17) was simultaneously illuminated (λ = 365

Scheme 1. General concept of molecular motors. R denotes the stereocchemistry of the stereogenic center at the 2'-position of the rotor. P and M denote helicity of the entire molecule (23). cis and trans denote whether the naphthalene part of rotor is at the identical (cis) or opposite side (trans) as the R-substituent of the stator.


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nm) and heated (55.5 °C) to induce rotation of the motor molecules. See supporting information (SI) for details. The development of concentrations of all four isomers in course of time was monitored by $^1$H NMR spectroscopy (Fig. 1). Care was taken to ensure that light intensity and temperature were identical and constant throughout both experiments.

When pure A was irradiated and heated, a relatively fast cis-trans isomerization into B was observed. The fraction of A decreased from 1.00 to 0.10 in 1.3 $\times$ 10$^4$ s, passing through a shallow minimum. The fraction of B reached a maximum of 0.59 at $t = 6.6 \times 10^3$ s and then slowly decreased to a final value of 0.43 at equilibrium. The third isomer in the cycle (C) was formed next, and its fraction reached a final value of 0.17. The fourth isomer (D) was not observed during the first 1.2 $\times$ 10$^3$ s of the experiment and from that moment, fraction D slowly increased to a final magnitude of 0.28. The order of formation of isomers is in line with the unidirectional rotation, once the motor is excited, happens on the scale of tens of picoseconds (27, 39–42), whereas the interval between two jumps (i.e., two quarter rotations) typically ranges from seconds to hours. It demonstrates that we were able to keep the illumination intensity, or fraction, of the four isomers of the motor molecule of type B does not react at all during $\Delta t$.

To characterize the system, we note that motor rotation is a stochastic process. The dynamics of a given molecule will be a random walk through Scheme 2, which is an example of a Markov process. The basic property of a Markov process is that every next place in either direction. The scheme allows for so-called “self-transitions,” such as $p_{BA}$, which represents the probability that a molecule of type B does not react at all during $\Delta t$.

To model reaction dynamics, we first defined a time-dependent row vector $X(t) = [A(t), B(t), C(t), D(t)]$. Its elements equal the normalized concentration, or fraction, of the four isomers of the molecular motor, at a time $t$ (see Fig. 1). Next, a $4 \times 4$ matrix $P_0$ was determined from Scheme 2. This matrix governs how $X$ changes in a small time interval $\Delta t$ by the collection of all reactions, i.e.:

$$X(t + \Delta t) = X(t) P_0 \tag{1}$$

The elements of $P_0$ are related to the probabilities $p_{KL}$ in Scheme 2 (with $K, L \in \{A, B, C, D\}$). They are given by

$$p_{KL} = k_{KL} \cdot \Delta t \quad \text{(for } K \neq L \text{)} \tag{2a}$$

$$p_{KK} = 1 - \sum_{L \neq K} p_{KL} \tag{2b}$$

\textbf{Modeling the Evolution of the Molecular Fractions.} We proceed by deriving a Markov model for molecular rotation dynamics. In Scheme 2, a schematic overview of all mathematically possible transitions (denoted by arrows) in our motor system is depicted.

![Scheme 2](image_url)

**Scheme 2.** Schematic representation of four-stage rotational behavior of a molecular motor. For example, $p_{AB}$ describes the conversion probability from isomer A to B, during a time interval $\Delta t$. The scheme allows for so-called “self-transitions,” such as $p_{BA}$, which represents the probability that a molecule of type B does not react at all during $\Delta t$. Cross-conversions of A into C and B into D and vice versa are split in positive (+, clockwise) and negative (−, counterclockwise) rotational movements because these conversions can take place in either direction.

**Fig. 1.** Development of four motor isomers ([M]-trans-A, (P)-cis-B, (M)-cis-C, and (P)-trans-D starting from ([M]-trans-A)$_0$ = 1.00. Symbols: empirical data. Lines: modeling with matrix $P_0$ (see Eqs. 1 and 2).
The matrix $P_0$ was determined for our specific molecular system, using Eqs. 2 a and b and the 12 empirical reaction constants. Making use of standard linear algebra after letting $\Delta t \to 0$, we solved Eq. 1 for the initial conditions in experiments 1 and 2 (see SI). The calculated isomeric fractions for experiment 1 are visualized as solid lines in Fig. 1. The motor system finally reached a dynamic equilibrium. This implies that $X$ converged to a vector $W$, according to: $X(t \to \infty) \to W = [w_A, w_B, w_C, w_D]$. The elements $w_K$ of $W$ form the equilibrium fractions of the four isomers. From Eq. 1, it follows directly that

$$W \cdot P_0 = W \quad \text{[3]}$$

Thus, $W$ is a left-side eigenvector of $P_0$ with eigenvalue 1. We calculated $W$ from Eq. 3 and the reaction constants; $W = [w_A = 0.13, w_B = 0.43, w_C = 0.17, w_D = 0.27]$.

The correspondence between model and empirical data are very accurate, given the considerable standard error of NMR ($\approx 3\%$) and the experimental challenge of keeping the input of photons and heat constant during the experiments. This result establishes that the matrix $P_0$ and the empirically determined reaction constants adequately describe the fraction development of experiments 1 and 2, once again justifying our approach. We note that matrix $P_0$ is easily modified to be applied to rotational motors with any number of cyclic stages as well as nonrotational (i.e., linear) motors (45).

Rotation Dynamics. The Markov process described by $P_0$ is appropriate for studying the development of the fractions of isomers A, B, C, and D in time. However, it does not allow one to determine all dynamic parameters. It is essential to realize that in dynamic equilibrium, the molecules keep on rotating (like in experiments 1 and 2), although the fraction values of isomers A, B, C, and D do not change anymore. To calculate the full rotation dynamics in equilibrium, a more extended Markov process and matrix, $P$, closely related to $P_0$, is introduced. For this, we consider all possible conversion pairs or jump types (the arrows in Scheme 2) as the unknowns in our problem, e.g., AA or BC. The proper state space $S$, thus reads: $S = \{AA, AB, AC+, AC-, AD, BB, BC, BD+, BD-, BA, CC, CD, CA+, CA-, CB, DD, DA, DB+, DB-, DC\}$. Because $S$ has jump types as elements, it allows us to find the dynamic properties in equilibrium. Within $S$, the signs $+$ and $-$ for cross-conversions ($A \leftrightarrow B$ and $C \leftrightarrow D$) refer to a clockwise and counterclockwise jump, respectively. We assume equal probabilities for both paths: $p_{AC+} = p_{AC-} = \frac{1}{2}k_{AC}\Delta t$, with equivalent expressions for the other cross-probabilities, BD, DB, and CA. Thus, four extra conversion probabilities are introduced into matrix $P$ compared with the initial matrix $P_0$. The elements of the resulting $20 \times 20$ matrix $P$, incorporating all conversion probabilities, are formally given by (with $KL, MN \in S$):

$$P_{KL,LMN} = p_{MN} \quad \text{if} \quad L = M \quad \text{[4a]}$$

$$P_{KL,LMN} = 0 \quad \text{if} \quad L \neq M \quad \text{[4b]}$$

During an interval $\Delta t$, a fixed fraction of molecules will be making a certain conversion. The equilibrium fraction making a jump $KL \in S$ in a time interval $\Delta t$ is formally denoted by $W_{KL}$. Because only molecules in state K can make the transition $KL$, $W_{KL}$ is proportional to $w_K$ (the equilibrium isomeric fraction calculated from $P_0$ see Eq. 3) and to the transition probability $p_{KL}$:

$$W_{KL} = w_K \cdot p_{KL} \quad \text{[5]}$$

The next ingredient to determine rotation dynamics is the jump observable ($\phi$). It is a function that assigns how manyquarter rotations a molecule is making during a jump. For example: $\phi$ (AA) = 0, $\phi$ (AB) = 1, $\phi$ (AC+) = 2, $\phi$ (AC-) = -2, $\phi$ (AD) = -1. A motor that performed a random walk through Scheme 2 of $n$ steps, i.e., for a period $t = n\Delta t$, will have reached $\Phi_n$ quarter rotations at time $t$. Thus, $\Phi_n$ the so-called net jump counter, is the sum of all jumps:

$$\Phi_n = \sum_{i=1}^{n} \phi_i \quad \text{[6]}$$

Because each molecule performs its own, specific random walk, each molecule has its own net jump count ($\Phi_n$), after a time $t$. In equilibrium, the collection of net jump counts ($\Phi_n$) of all molecules is distributed according to a normal distribution, due to the central limit theorem (43, 44). Hence, for each isomer $K$, the distribution of net jump counts is given by:

$$\mu_{K}(\Phi) = \frac{c_K(\Phi)}{2\pi DT} e^{-\frac{(\Phi - \nu)^2}{2DT}} \quad \text{[7]}$$

Here, $\mu_{K}(\Phi)$ denotes the fraction of molecules of isomer species K, that have $\Phi = \Phi$ at a time $t$. Eq. 7 represents a drifting Gaussian distribution, which is a general solution for biased diffusion processes (46–48). The expression includes a prefactor $c_K(\Phi)$, which depends on the isomer involved. See SI for a more elaborate consideration of the prefactor. Furthermore, it contains a rotation velocity or "drift," $V$, and a broadening term $2DT$, of which the quantity $D$ acts as a diffusion constant. The quantities $V$ and $D$, measured in quarter rotations, are the same for all isomers because they are global properties of the Markov process. It is straightforward to find an expression for $V$ (44, 45). It equals the sum of all jumps per time interval $\Delta t$:

$$V = \frac{1}{\Delta t} \sum_{KL \in S} \phi(KL) \cdot W_{KL} = \sum_{KL \in S} \phi(KL) \cdot w_K \cdot k_{KL} \quad \text{[8]}$$

Here, we used Eqs. 5 and 2 to find that $V$ can be calculated from the equilibrium fractions $w_A, w_B, w_C,$ and $w_D$ (Eq. 3) and the reaction constants. In our case, $V$ has units of quarter rotations per second. The broadening $D = D_0 + D_m$ of the net jump counts is given by a more involved formula. Its first term, $D_0$, exhibits a standard form to determine broadening:

$$D_0 = \frac{1}{\Delta t} \sum_{KL \in S} [\phi(KL) - V\Delta t]^2 \cdot W_{KL} \quad \text{[9]}$$

For the second term we find (43, 44)

$$D_m = \frac{2}{\Delta t} \sum_{KL \in S} (\phi(KL) - V\Delta t) \cdot W_{KL} \left[ \sum_{k=1}^{n} \sum_{MN \in S} (P_k^{(k)}_{KL,LMN}) (\phi(MN) - V\Delta t) \right] \quad \text{[10]}$$

where $S_0$ is a subset of $S$, excluding self-conversions. Because numerical values for all parameters in Eqs. 8-10 can be inferred from our empirical data, one can calculate $V$ and $D = D_0 + D_m$ by using Eqs. 2, 4, and 5.

Having obtained expressions for $V$ and $D$, we calculated the full rotation dynamics (see Eq. 7). We assumed the conditions of experiment 1, i.e., we started with 100% A and made use of the empirical reaction rates. For our specific motor at 55.5 °C, we found: $V = 1.1 \times 10^{-4}$ s$^{-1}$ and $D = 2.0 \times 10^{-4}$ s$^{-1}$. In Fig. 2, we plotted $\mu_{K}(\Phi)$, the fraction of molecules that performed a given number of quarter rotations, $\Phi$, for all isomers together. This graph can equivalently be described as a plot of the probability density of reaching a certain number of quarter rotations at a time $t$. We depicted two curves, one at $t = 5 \times 10^5$ s (red) and another for $t =
with their lower equilibrium concentrations $w_A$ and $w_C$. This is
saturation similar to the one for longer, the two curves approach each other. As for the temperature
decreases dramatically. Because the light input is not dominant any
lower light-induced steps are rate limiting in this temperature region. At

temperatures, dependent rates were determined by using empirical values:
the four isomers. Because, e.g., $w_B > w_A$, more motors will have made $4n + 1$
quarter rotations, than $4n$ quarter rotations ($n \in \mathbb{Z}$). This is illustrated in the
Inset, which zooms in on the curve at $t = 10^6$ s.

$10^6$ s (black). These distributions have the shape of spiked Gaussians, with well-defined, global $V$ and $D$ values. The spiky appearance is due to the prefactor $c_K(\Phi)$ which differs for each isomer as $c_K(\Phi) \propto w_K$. Therefore, the Gaussians in Fig. 2 are modulated periodically, with a period of four quarter rotations. For example, isomer $B$, with $w_B = 0.43$, is responsible for the large spikes. The local minima, on the other hand, are due to the isomers $A$ and $C$, with their lower equilibrium concentrations $w_A$ and $w_C$. This is illustrated in Fig. 2 Inset, which zooms in on the curve at $t = 10^6$ s.

We stress that the full dynamics of the equilibrium motor system is captured in Eqs. 7-10. As an independent check, we also numerically calculated $\mu^{(K)}(\Phi)$ from the four-variable differential equation that describes the evolution of $A$, $B$, $C$ and $D$. For large times, like in Fig. 2, the simulations are indistinguishable from the curves calculated with Eqs. 7-10.

Optimizing Motor Dynamics. Interestingly, it is possible to adjust motor performance by tuning the two types of energy supplied to the system: heat and light. Reaction constants $k_{BA}, k_{CB}, k_{AD}$, and $k_{DA}$ describe the thermally activated jumps from the unstable to the stable forms. Hence, they follow the relation $k = (k_B T e^{\Phi} (-\Delta G^T/RT)) / h$. On the other hand, the rates $k_{AB}, k_{AC}, k_{BD}, k_{CA}, k_{CD}, k_{DB}$, and $k_{DC}$ scale with the illumination intensity. Obviously changes in the reaction rates result in different rotation dynamics, via Eqs. 2 and 4. This triggers the question if temperature and light intensity can be tuned in such a manner that the motors rotate optimally.

To substantiate on this interesting issue, we calculated $V$ (see Fig. 3A) and $D$ (see Fig. 3B) in the temperature range $250 < T < 370$ K, for the specific motor used in our experiments. We considered two different illumination intensities. The first (solid curves) equals the value in our experiments whereas for the second (dashed) we assume a light input that is 10 times smaller. The temperature dependent rates were determined by using empirical values: $\Delta G^1$ ($B \rightarrow C$) = 25.6 kcal mol$^{-1}$, $\Delta G^1$ ($C \rightarrow B$) = 30.3 kcal mol$^{-1}$ and $\Delta G^1$ ($D \rightarrow A$) = 25.3 kcal mol$^{-1}$. $\Delta G^1$ ($A \rightarrow D$) = 30.0 kcal mol$^{-1}$.

As can be seen from Fig. 3A, $V$ increases both with increasing $T$ and increasing light input (black vs. red curves). At higher temperatures, $V$ reaches saturation. The saturation value depends on the light input (it differs a factor 10 for the two curves), because the light-induced steps are rate limiting in this temperature region. At lower $T$, however, the thermal steps become rate limiting, so that $V$ decreases dramatically. Because the light input is not dominant any longer, the two curves approach each other. As for the temperature dependence of $D$, we observe two S-shaped curves. At higher $T$, saturation similar to the one for $V$ is seen. At lower $T$, however, $D$ also reaches a plateau value, unlike $V$. The reason for the latter is that $D$ is not limited by the ever slower thermal steps, because it takes all possible jumps into account (see Eqs. 9 and 10). Hence, the light-induced steps also determine $D$ at low $T$, as confirmed by the factor of 10 between the curves with high and low light input.

Péclet Number. A measure giving insight in motor performance is formed by the dimensionless Péclet number $Pe$. It is defined as (taking a quarter rotation as a unit step) $35, 38$:


Thus $Pe$, which originated in fluid dynamics, quantifies the ratio of directed motion and diffusive spreading. For molecular motors, $Pe$ is optimal if all forward rate constants are matched (i.e., equal) and the backward rate constants are small. Indeed, if the forward rates have very dissimilar values, “traffic jams” (or pile up of a certain isomer) will limit the rotation efficiency so that $Pe$ is small. The spikes in Fig. 2 are the signature of such “piling up.” Nevertheless, even for optimized synthetic molecular motors, under continuous irradiation, $Pe$ is limited to unity, i.e., $|Pe| \leq 1$. This is a direct consequence of the stochastic nature of the rotation process. It illustrates the fundamental difference between synthetic molecular engines and daily-life macroscopic engines. The latter have $Pe \gg 1$ because their movement (work) is tightly coupled to distinct quantities of supplied energy.

As shown in Fig. 3C, $Pe$ reaches a maximum value of 0.75 at $\approx 340$ K for the motor and light intensity used in experiments 1 and 2. This fact can be understood as follows. At low $T$, the rates of the thermal steps are much smaller than the light-induced rates, i.e., the forward rates are not matched at all, and $Pe$ is small. Upon increasing $T$, the temperature-dependent rates increase until values close to the light-sensitive rates are reached. At that point, $Pe$ arrives at a maximum. As $T$ increases further, the thermal steps start to dominate the light-induced rates, and $Pe$
in contrast to the case of... necessity that the forward reaction constants (k’s) are “matched,” in contrast to the case of Pe. Hence, a motor that is 100% unidirectional, may have a low Pe due to dissimilar forward rates. This emphasizes that we need to quantify both Pe and an independent quantity for unidirectionality, the rotational excess (r.e.). Note that a motor with Pe = 1 must also be unidirectional, so that r.e. = 1. The definition of r.e. is inspired by similar quantities in chemistry, such as enantiomeric and diastereomeric excess that describe selectivity in asymmetric catalysis (49) and chiral resolution (50). We define:

\[ r.e. = 100\% \frac{\Omega_+ - \Omega_-}{\Omega_+ + \Omega_-} \]  

[12]

Here, \( \Omega_+ \) denotes the number of full forward rotations per unit time. The definition of \( \Omega_+ \) is much more stringent than that of \( \Omega_- \). We define a full forward rotation as a rotation from A to A (or B to B, etc.) in the forward direction that contains no backward jumps in between. A similar definition holds for \( \Omega_- \), which describes the rate of full backward rotations. If the motor is fully unidirectional, we have \( \Omega_+ = 0 \), and \( r.e. = 100\% \). If there are as many forward as backward rotations, the motor does not rotate on average and \( r.e. = 0\% \).

A calculation of \( \Omega_+ \) requires knowledge about the exact sequence of partial rotations. As soon as a backward jump takes place, a rotation should be disregarded. To take this into account, we adapt our Markov approach. Intuitively, it works in the following way (a mathematically adequate description can be found in SI). First, we define five mathematical “graphs” similar to Scheme 2. Four of these are used as “counters” for full forward rotations: \( G_A \), \( G_{AB} \), \( G_{BC} \), and \( G_D \). The fifth graph, \( G_- \), acts as a pool of molecules that rotated backwards in their last attempt. To intuitively explain the function of “subgraphs” \( G_A \), \( G_{AB} \), \( G_{BC} \), and \( G_D \) as well as of \( G_- \), let us suppose that a molecule has just jumped backwards from state B to state A. Therefore, it is by definition part of graph \( G_- \). Next, the molecule rotates forward by a quarter turn to become B. At that point, it is transferred to graph \( G_A \), which follows molecules that started as isomer A and are “trying” to make a full forward rotation. Indeed, if the molecule keeps rotating forward only, without going backwards at all, it stays within \( G_A \). Upon completion of a full turn, we add one full rotation (+1) to our counter. If, on the other hand, a backward rotation takes place before the full rotation is completed, the complete rotation attempt is disregarded. This is done by transferring this molecular state to \( G_- \) again. From there, another attempt can be made for a full rotation, etc.

By properly connecting the subgraphs \( G_A \), \( G_{AB} \), \( G_{BC} \), \( G_D \), and \( G_- \), a complete graph is formed that describes the Markov problem fully. From this extended graph, a \( 20 \times 20 \) matrix \( P^{*} \) was derived, which serves as the basis for our calculations of \( \Omega_+ \). The method we use to actually compute \( \Omega_+ \) is related to the way we calculate \( \Omega_- \) above. An analogous procedure is used for \( \Omega_- \).

In Fig. 4A, we show the rate of full forward rotations (\( \Omega_- \), black) and full backward rotations (\( \Omega_- \), blue), as well as their difference (\( \Omega_{eff} = \Omega_- - \Omega_+ \), red), calculated versus temperature. Again, we assumed two light intensities: the photon input of experiments 1 and 2 (solid lines) and 1/10 of that (dashed lines). Remarkably, a clear optimum in \( \Omega_{eff} \) is found in both cases. The maximum value of \( \Omega_- \) scales with the light input: It is 10 times higher for a 10-times-higher intensity (\( 1.1 \times 10^{-4} \) and \( 1.1 \times 10^{-5} \) full rotations per second, respectively). Moreover, the temperature at which the maximum occurs also depends on light input. It is located at \( \approx 370 \) K for the standard light intensity and at \( \approx 350 \) K for 1/10 the intensity. Finally, for high temperatures (\( T > 450 \) K), \( \Omega_{eff} \) is \( \approx 100 \) times smaller for the lower light input than for the higher intensity.

We explain these interesting results in the following way. At temperatures below 310 K, the thermal rates are much slower than the photochemical rates (\( k_A \ll k_{hA} \)). The thermal rates are therefore rate determining and inhibit relatively fast full rotations to take place. For this reason, \( \Omega_{eff} \) is basically the same for both light inputs. When the temperature rises, the thermal rates increase and \( \Omega_{eff} \) increases correspondingly. A maximum is reached when the thermal rates and light-induced rates match, such that each forward quarter rotation can be followed by another step forward. However, as the temperature increases further, the thermal rates become dominant. Moreover, the probability of a backward thermal step becomes larger than the probability of a light-induced forward jump. At that point, \( \Omega_{eff} \) will start decreasing due to our stringent demands: only uninterrupted rotations are included. For a lower light input, this point is reached for a lower temperature, explaining the shift of the maximum in Fig. 4A. Once again, this shows the importance of an accurate balance between photon and heat input to achieve the highest rate of total rotations (i.e., motor efficiency).

In Fig. 4B, the r.e. of the motor studied is plotted versus temperature, for the two light intensities considered. At temperatures \( < 275 \) K, r.e. is close to 0%. In the region at \( \approx 300 \) K, a steep rise to \( > 99\% \) is observed, after which, r.e. flattens off to a value \( > 99.8\% \) for higher temperatures. Once more, the curves for different light intensities are shifted over the temperature axis. These observations are rationalized by the fact that the thermal
reaction rates have dropped to practically zero in the region <275 K, i.e., much below the photochemical rates. Therefore, full rotations do not take place through thermal steps from states B to C and D to A, but primarily through photochemical reactions between states A and C and B and D. Because these conversions take place in both directions, backward and forward, in a 50-to-50 ratio, r.e. is 0%. When the temperature is increased, the thermal conversions that take place quantitatively (>99%) in the forward direction, become competitive with the photochemical rates, and r.e. increases consequently to almost 100%. For the lower light intensity, this point is reached at lower temperatures, explaining the shift in Fig. 4B. We conclude that for temperatures that are high enough, these motors are almost perfectly unidirectional.

**Optimal Conditions for the Motor Studied.** Having calculated $V$, $D$, $Pe$, $\Omega_{\text{eff}}$, and r.e., we can finally investigate the question at which conditions the motor studied rotates optimally. We assume the light intensity in experiments 1 and 2. Fig. 4 reveals that the highest motor efficiency is achieved between 345 and 370 K, because at these temperatures, $\Omega_{\text{eff}}$ is near its maximum and r.e. is >99%. Furthermore, Fig. 3 shows that the $Pe$ is near its maximum, whereas $V$ has practically reached its saturation value. Hence, the motors are both very efficient in their reaction dynamics of synthetic molecular motors. A specific unidirectional four-stage rotating motor was completely characterized by $\Omega_{\text{eff}}$, as well as almost perfectly unidirectional (r.e. = 1). Remarkably, it is not useful to raise the temperature further. Motor rotation becomes neither faster nor more efficient at higher temperatures. This, at first sight, counterintuitive result, forms one of the main conclusions of our Markov study.

**Summary**

We applied the theory of Markov processes to quantify the general reaction dynamics of synthetic molecular motors. A specific unidirectional four-stage rotating motor was completely characterized by a temperature and photon-cycled experimental routine based on $^1$H NMR spectroscopy. All its relevant reaction rates were obtained empirically. By using Markov theory, the probability density of observing a certain number of quarter rotations at time $t$ was calculated. Spiked, drifting Gaussians were obtained. Next, expressions were found for the quarter rotation velocity $V$ and rotational spread $D$. This allowed us to determine the $Pe$, $(V/D)$, 0 ≤ $Pe$ ≤ 1, for rotational motors, which is a measure for motor performance. Furthermore, we defined the quantity r.e. ≤ 1 as the relative ratio of the rate of full forward rotations ($\Omega$) versus the rate of full backward rotations ($\Omega$). The effective rate of full rotations is given by $\Omega_{\text{eff}} = \Omega - \Omega$. We demonstrated that $V$, $D$, $Pe$, $\Omega_{\text{eff}}$, and r.e. can be optimized by tuning the temperature and light input, thus achieving maximum efficiency and unidirectionality. Remarkably, there is an optimal temperature range for motor rotation at a given light intensity. Increasing the temperature beyond that range reduces motor performance.

Our work presents a pragmatic and powerful combination of theory and experiment for optimization and control of molecular motor performance. This provides a pivotal step forward toward possible nanoscientific applications.

**Matlab Routines**

Matlab routines to calculate Figs. 3 and 4 are included in SI.

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