Elastic Properties of Photoswitchable Azobenzene Polymers from Molecular Dynamics Simulations**

Lars V. Schäfer, E. Matthias Müller, Hermann E. Gaub, and Helmut Grubmüller*

Nanomechanical devices or molecular machines will, for a broad range of applications, most likely be powered by light or other kinds of electromagnetic radiation.\[1–4\] The major reasons are ease of addressability, picosecond reaction times to external stimuli, and compatibility with a broad range of ambient substances, such as solvents, electrolytes, or gases. Azobenzene is a well-studied photoactive system, which can be photoswitched selectively from an extended \textit{trans} to a more compact \textit{cis} conformation by using light of wavelength 365 nm, whereas the reverse \textit{cis}-to-\textit{trans} isomerization is induced by light of wavelength 420 nm (Figure 1a).\[5,6\] Many processes, such as light-driven ion transport through biological membranes,\[7–9\] can be steered by conformational switching of azobenzene chromophores. Azobenzene has been used frequently in synthetic photoresponsive systems for the regulation of the geometry and function of biomolecules.\[10–19\] The isomerization of individual azobenzene molecules has also been studied by scanning tunneling microscopy.\[20,21\]

Recently, a bistable polyazobenzene peptide was synthesized as a model system for a light-powered molecular machine, and its mechanical properties were characterized by means of single-molecule atomic force microscopy (AFM) experiments (Figure 1b, c).\[22,23\] Optical switching of the azobenzene polymers between their extended \textit{trans} and compact \textit{cis} conformations was demonstrated, and the corresponding change in the contour length of the polymer was detected. Thereby, in analogy to an Otto cycle, Gaub and co-workers established an optomechanical operating cycle, in which “optical” contraction against an external force delivered net mechanical work.\[22,23\] Thus, they demonstrated that azobenzene polymers indeed hold great promise for future applications in nanotechnology, for example, as light-triggered molecular switches or cargo lifters. In a related experiment, Vancso and co-workers characterized a redox–mechanical cycle by using electrochemical AFM-based single-molecule force spectroscopy.\[24\]

However, the measured overall length change upon switching between the \textit{cis} and \textit{trans} polymers—and thus the work output—was considerably smaller than could be expected on the basis of the length change of a single
azobenzene monomer and the number of individual monomers. To resolve this discrepancy, and, more generally, to obtain a detailed understanding of the underlying polymer mechanics at the atomistic scale, we carried out explicit-solvent force-probe molecular dynamics (MD) simulations\[^{25,26}\] of polyazobenzene model peptides under mechanical stress, the results of which resemble those of the AFM experiments described in Figure 1c. The simulations provided detailed mechanistic insight that is prerequisite for the efficient and targeted optimization of photoswitchable polymers for future applications in nanotechnology. Because we were only interested in the elastic properties of these model polymers, and not in the photosomerization kinetics, we kept the azobenzenes fixed in the cis or trans conformation during the simulations. We did not include the photosomerization itself into our simulations on the assumption that this process occurs on a much faster time scale than the subsequent structural rearrangement of the polymer. Indeed, sub-picosecond kinetics have been observed for azobenzene photosomerization.\[^{27,28}\] a complex process that involves several energy surfaces.\[^{27-33}\]

By comparing our simulations to the AFM experiments, we gleaned insight into the overall elastic properties of azobenzene polymers and could relate our results directly to the measured elasticity characteristics. We were able to elucidate the crucial role of the peptides that interlink the azobenzene units and to predict the elastic behavior beyond the experimentally accessible force regime. Finally, we used the detailed mechanistic understanding thus obtained to design a photoswitchable polyazobenzene peptide with improved optomechanical properties.

The model azobenzene polymers used in our simulations were constructed from four azotripeptide units (\(n = 4\) in Figure 1b). These (Lys-Azo-Gly), dodecamers have the same stoichiometry as the polymers used in the AFM studies. As in the experiments, DMSO was used as a solvent. To study the effect of the azobenzene conformation (cis or trans) on the elastic properties, we considered three stereochemically different polymers, an all-cis, an all-trans, and a mixed cis–trans–cis–trans system. The mixed polymer was considered because complete photosomerization upon optical pumping was not achieved in the experiments as a result of the overlapping absorption bands of the cis and trans states.\[^{22,23}\] A study of this polymer would also reveal possible cooperative effects between adjacent monomers.

The force-versus-extension traces obtained from the AFM experiments reflect the elastic properties expected for a polymer subjected to a mechanical load. Figure 2a shows the overall force-extension traces of the all-cis, all-trans, and mixed azobenzene model polymers obtained from our force-probe MD simulations (over 50–70 ns each). These traces closely resemble the curves from the AFM experiments (Figure 2a, inset).\[^{22,23}\] At higher forces above 600 pN, deviations from the wormlike-chain (WLC) behavior are seen. These deviations are discussed below. As expected, the force trace of the mixed cis–trans–cis–trans polymer lies midway between those of the all-cis and the all-trans polymers. This result already suggests that cooperative effects between monomers are unlikely. Therefore, such effects cannot explain the unexpectedly small length changes observed in the AFM experiments.

The force field and simulation protocol used were validated by comparison with the AFM results. To this end, the force-probe MD force-extension data were modeled using an extended WLC model,\[^{34}\] WLC model \[Eq. (1)]\[^{34}\]  

\[
F(r) = \frac{k_B T}{L_p} \left( \frac{1}{4} \left( 1 - \frac{r}{L_c} \right)^2 - \frac{1}{4} \left( 1 + \frac{r}{L_c} \right)^2 \right),
\]

in which \(F\) is the force, \(T = 300\) K the temperature, \(r\) the polymer extension, \(L_c\) the contour length, and \(L_p\) the persistence length. Table 1 lists the WLC parameters obtained as well as the extension difference per switched azobenzene monomer, \(\Delta l\), for all simulated systems, together with the values obtained from experiment.

Because experimental WLC data are only accessible for the all-trans polymer,\[^{23}\] we compare in Table 1 only the parameters of the all-trans polymers. The contour length \(L_c\) per azotripeptide unit given in reference \[22\] was not extracted from the measured force traces, but determined by molecular modeling. Therefore, we only compare the
The WLC fits were also used to extract the extension differences $\Delta l$ between the trans and cis polymers from the simulations. As illustrated in Figure 2b, $\Delta l$ determines the work output upon contraction against an external load. Therefore, an increase in $\Delta l$ improves the efficiency of the optomechanical energy conversion caused by the polymers.

The WLC parameters of all-trans polymers obtained from experiment and from our force-probe MD simulations are listed in Table 1.

### Table 1: WLC parameters of all-trans polymers obtained from experiment and from our force-probe MD simulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment</th>
<th>Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_0$ [nm]</td>
<td>1.90</td>
<td>2.05</td>
</tr>
<tr>
<td>$R_L$ [nm]</td>
<td>0.50</td>
<td>0.97</td>
</tr>
<tr>
<td>$R_{WLC}$ [nm]</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>$\Delta l$ [nm]</td>
<td>0.60</td>
<td>1.45</td>
</tr>
<tr>
<td>$\Delta l_{\text{free}}$ [nm]</td>
<td>0.50</td>
<td>0.97</td>
</tr>
</tbody>
</table>

[a] WLC parameters were obtained by modeling the force-extension data with the extended WLC model [Eq. (1)]. The extension difference $\Delta l$ per switched azobenzene monomer is given at a force of 200 pN to enable direct comparison with the experimental data. Two different values are given for $R_L$; these values reflect the quality of the fits. $R_{WLC}$ was obtained from a WLC fit with both $L_0$ and $R_L$ as adjustable parameters, whereas $R_{WLC}$ was obtained from a fit with $L_0 = 0.5$ nm, which is the value obtained from the experimental data.

Indeed, the extension difference due solely to the azobenzene units is 1.70 Å per monomer (dashed green line in Figure 2c), which is the value expected from polymer tilt and differential stiffness. This contribution to $\Delta l$ is larger than the total extension difference of 1.45 Å for the polymer (Figure 2a). Figure 2d shows that the difference of about 0.25 Å can be attributed to the lysine residues, which therefore partly compensate the “optical” lengthening and contraction of the azo groups. The force-extension curves of the glycine residues are rather independent of the azobenzene conformation (Figure 2d, inset).

As shown schematically on the right-hand side of Figure 3d, the compensation by lysine residues is due to the isomerization of the lysine backbone between an extended anti and a more compact syn conformation around the backbone dihedral angle $\psi$. These two conformers are populated to different extents, depending on whether the
adjacent azobenzene moiety is cis or trans (Figure 3). Figure 3b,c shows exemplarily the backbone dihedral angles $\Psi$ of Lys7 in the all-cis and in the all-trans polymers, respectively, as a function of the applied force (black), together with the average $\Psi$ angle and the statistical error (blue and red for cis and trans, respectively). In Figure 3d, the average backbone dihedral angle $\Psi$ of all lysine residues is shown as a function of the applied force. For the all-cis polymer, the lysine residues already adopted the extended anti conformation at very low forces of between 0 and 300 pN (blue lines). These transitions also reveal themselves as steps in the force-extension trace of the all-cis model polymer (blue arrows in Figure 2).

In the mixed polymer, Lys7, which is bonded directly to a cis azobenzene moiety (Figure 3a), also adopted the anti conformation very early (dash-dotted black line in Figure 3d), in a similar manner to Lys4 in the all-cis polymer. In contrast, Lys4 and Lys10 with adjacent trans azobenzene units underwent syn-to-anti transitions only at rather large forces of between 600 and 800 pN (solid and dashed black lines, respectively). The same behavior was observed for all lysine amino acids in the all-trans polymer (red lines).

In summary, our simulations showed that lysine residues bonded to trans azobenzene moieties tend to adopt the compact syn conformation (at up to 600–800 pN), whereas lysine residues adjacent to cis azo groups already undergo syn-to-anti conformational transitions at low to moderate forces below 300 pN. This preference for the extended anti conformation of lysine residues adjacent to cis azobenzene units explains the observed compensation effect. The difference in the length of the polymer chain because of cis–trans isomerization of the azobenzene units is reduced from 1.7 Å to 1.3–1.5 Å. However, in the AFM experiments, a value of only 0.6 Å was measured. We conclude that in the AFM experiments only about 40% of the azobenzene moieties were effectively photosomerized upon optical pumping. The major reason for the failure to attain the maximum value, which is about 70–80% as a result of the overlapping absorption bands of the trans and cis isomers,[35] in the AFM experiments is probably that only about half of the polymer was excited by the evanescent light field, in agreement with the estimate of the authors.[22,23,36]

The force-probe MD simulations also allow the investigation of the mechanics of the polymers at high forces and thus enable prediction of their elastic behavior even well beyond the force regime accessible to AFM. Only at forces larger than 1000 pN were mechanically induced cis-to-trans transitions of the azobenzene units observed in our simulations (data not shown). These isomerizations occurred by rotation about the central N–N bond, in agreement with recent ab initio calculations.[36] The high forces observed are consistent with the large thermal barrier to isomerization of 108 kJ mol$^{-1}$,[37] which, as a result of the orthogonality of the rotation coordinate with respect to the pulling coordinate, is largely unaffected by the applied force.[36] Also in the AFM experiments no mechanical transitions were observed up to 500 pN.[22,23] At large forces of about 600–800 pN, the simulations also predict syn-to-anti transitions of the lysine residues in the all-trans polymer. For the short polymer used in this study, these transitions are visible as steps in the force-extension curve (red arrows in Figure 2a). For the much larger polymers used in the AFM experiments, these individual steps would not be resolved, but a tendency towards larger extensions would be observed in the force-extension traces of the trans polymers at forces larger than about 600 pN.

The dependence of the degree of extension of the lysine amino acids on the conformation of the azobenzene moiety they are bonded to demonstrates that the peptides interlinking the photoactive azobenzene units are not simply “passive glue”, but rather, through intermonomeric correlations, actively affect the elastic properties of the polymer. In the case at hand, the compensating effect of the lysine residues reduces the overall “optical” length contraction and thereby diminishes the work output of the polymer. We next investigated the possibility of using the structural and dynamical insight obtained from our studies to tailor and optimize photoswitchable polymers. For example, with the aim to increase the rigidity of the polymer backbone, we replaced the lysine and glycine residues with proline residues, so that less compensation and, accordingly, a larger work output could be expected.

Figure 4 shows the elastic behavior of the all-cis, all-trans, and mixed (Pro-Azo-Pro)$_4$ dodecamers as determined from a set of force-probe MD simulations similar to those described above. The stiffer proline linkers indeed lead to an increased extension difference between the cis and trans polymers of 1.70 Å per azo unit (dashed green line), as compared to 1.45 Å for the lysine-containing polymer (see Figure 2). Figure 4b shows the individual force-extension curves of the azobenzene and proline moieties in (Pro-Azo-Pro)$_4$. Apparently, and in contrast to (Lys-Azo-Gly)$_4$, the extension difference between the cis and trans azobenzene units is no longer compensated by the interlinking residues, which explains the increased $\Delta l$ value of 1.70 Å. Accordingly, the work output is expected to be enhanced by about 15–20% relative to that of the lysine-containing polymer.

Efficient and targeted optimization of photoswitchable polymers is a major challenge and requires detailed microscopic interpretation of the polymer mechanics. Herein, we
provided insight on the basis of force-probe molecular dynamics simulations into the mechanics of photoswitchable polyazobenzene peptides under mechanical stress. Comparison of the WLC parameters revealed that the overall elastic properties of the simulated azobenzene model polymers agree very well with the results of AFM experiments.[22,23] Our simulation of the WLC parameters revealed that the overall elastic behavior of the model polymers was applied by moving the spring (Eqs. (2))

\[ V_{spring}(z) = k_s [z(z(t) - z_{spring}(t))^2] \]

in which \( k_s = 500 \text{ kJ mol}^{-1} \text{ nm}^{-2} \) is the force constant of the spring, \( z(0) \) is the position of the pulled N terminal atom, and \( z_{spring}(t) \) is the position of the spring. The C terminal, \( C_t \), was fixed by a stationary harmonic spring with a constant velocity \( z(0) = z_{spring}(0) \). Mechanical stress to probe the elastic behavior of the model polymers was applied by moving the spring with a constant velocity \( z = 0.1 \text{ nm ns}^{-1} \) to yield simulation times of 50–70 and 10–14 ns, respectively, per force-probe MD trajectory. The overall simulation time was about 0.5 ns. Simulation details are given in the Supporting Information.

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