Switching in Vivo

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Azobenzenes in a New Light—Switching In Vivo**

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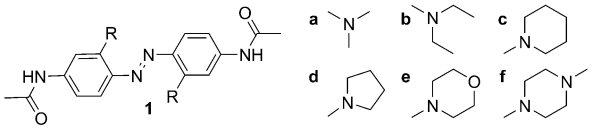
azo compounds · isomerization · molecular switches · photochromism · photocontrol

Controlling the spatial arrangement of structures in a reversible manner on the molecular level opens up vast possibilities in chemistry, as well as in material and biological sciences.[1] Light offers in this context special opportunities, as it is (ideally) nondestructive and can be applied with high spatial resolution. Azobenzenes have been investigated for decades as powerful molecular switches and have been applied in a many very different areas ranging from switchable catalysts[2a] to liquid crystals[2b] and biomolecules.[2c] The parent azobenzene structure can be conveniently changed between the *cis* and the *trans* structure by irradiation at roughly 320 nm and 440 nm, respectively. As the *trans* isomer constitutes the thermodynamically more stable form of the two, the isomerization from *cis* to *trans* can also be achieved thermally. Critical aspects in view of potential appliances include the *cis*/*trans* ratio in the photostationary state, the lifetime of the two isomers, and the long-term stability (potential degradation) over time. Depending on the environment of the switch, other requirements, such as solubility, the effect of pH, temperature, solvent, etc. on the isomerization, and compatibility with the system, have to be satisfied. For instance, the necessity of using UV light to induce the switching processes implies serious limitations in living systems.[3] From a practical point the penetration of UV light into organisms is limited by strong scattering. Furthermore, UV light can severely damage cells and tissues. Therefore, increasing efforts have been made to design azobenzene switches that can be triggered by visible or even IR light.

There have been different approaches to modify the azobenzene switch. One concept is based on the incorporation of electron-donating or electron-withdrawing groups in *ortho* and *para* position.[4] Woolley and co-workers prepared, for instance, various 2,2′-disubstituted amino azobenzenes (Scheme 1).[5] Although promising red shifts are observed, these compounds also display an increased rate of thermal *cis*→*trans* isomerization. Additionally, these compounds suffer from photobleaching effects. The mechanism of this process is still not clear. Photo-oxidation involving the *o*-amino group has been discussed as possible explanations.



Scheme 1. 2,2′-Diamino-substituted azobenzenes.

Trauner and co-workers made use of this fast back-isomerization in their design of photochromic ion channel blockers (Figure 1).[6] They employed push–pull substitution

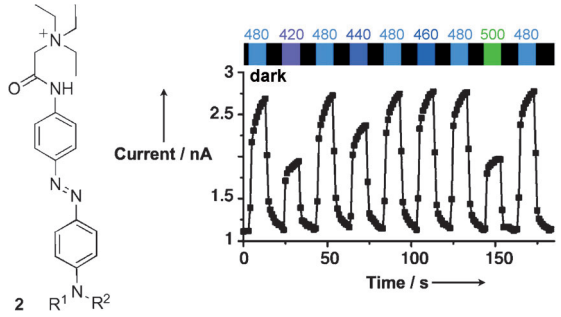


Figure 1. Reversibility of photoswitching and the action spectrum of **2** (R^1, R^2 = Et) on $K_v3.1$ channels expressed in HEK293 (potassium current looped at 1 Hz). Peak current is plotted as a function of time. Cycles of darkness and illumination (420–500 nm) are indicated. Reprinted from Ref. [6] with permission from the American Chemical Society.

pattern to achieve the desired red shift. Depending on its substituents, compound **2** acts as a *trans* (R^1, R^2 = Et) or a *cis* blocker (R^1 = Ph, R^2 = Et). Irradiation of **2** (for R^1, R^2 = Et) at λ = 420–500 nm converts the *trans* blocker to the non-binding *cis* state, which induces a current flow in a potassium channel. When the light source is switched off, the blocker turns itself off. Even more interesting is that **2** (R^1 = Ph, R^2 = Et) acts as a *cis* blocker as it does not interact with the system in its thermodynamically more stable *trans* state, and thus displays lower toxicity. Kamei et al. incorporated a similar switch in single-stranded DNA, and the structure could also be switched with visible light.[7]

Now, Woolley and his group have presented a new set of compounds based on *o*-methoxy-substituted azobenzenes (**4** and **5**; Scheme 2).[8] Although the absorption properties of

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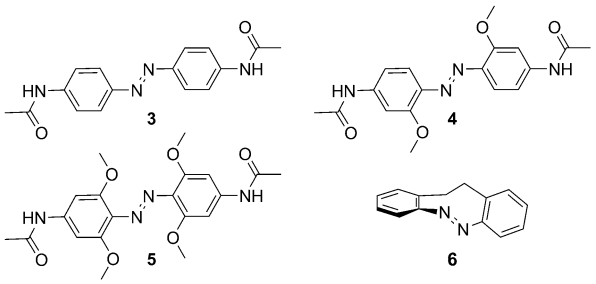

Scheme 2. Parent azobenzene **3** and *o*-methoxy-substituted azobenzenes **4** and **5** presented by Woolley and co-workers, and cyclic azobenzene **6** investigated by Siewertsen et al.

these compounds in the *trans* state are very similar to those of the parent azobenzenes **3** (they even exhibit blue-shifted π–π* bands), they show an increased absorption for the n–π* band at longer wavelengths. More importantly, the n–π* transition of the *cis* isomer of **5** occurs at significantly lower wavelength (36 nm) as than the analogous transition of its *trans* isomer. This behavior now allows the switching of this azobenzene analogue in the visible range in both directions, where usually only the change from *cis* back to the *trans* state is possible. There is one report of an azobenzene switch exhibiting a similar property.[9] The bridged azobenzene **6** (Scheme 2) investigated by Siewertsen et al. also shows two distinct absorption bands for the n–π* transition. In compound **6** the *cis* form, however, represents the thermodynamically more stable isomer.

Remarkably, the isomerization of compound **5** (in DMSO) produces a photostationary state corresponding to roughly 80% *cis* isomer after irradiation with green light (530–560 nm) and can be converted back to a large fraction of *trans* isomer (about 85%) using blue light (450–460 nm) (Figure 2). Additionally, the half-life of **5** (2.4 days) is considerably longer than that of the parent compound **3** and allows its application for most biological processes. Also, the behavior of **5** under biological conditions (25 mM phosphate buffer, pH 7.0) was investigated. Compound **5** display very good solubility, which is not the case for **3** and **4**. Even pH values above pH 5 did not disturb the spectral characteristics. The only restriction in the applicability of this new azo switch

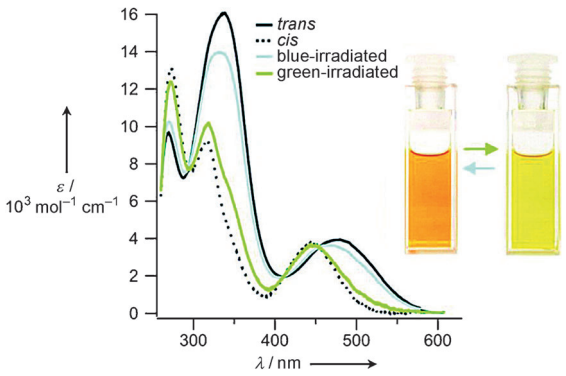

Figure 2. Photoisomerization of **5** measured in DMSO at 25 °C. Reprinted from Ref. [8] with permission from the American Chemical Society.

in biological settings is the sensitivity of **5** towards reductive conditions: The half-life of **5** in a 1 mM solution of reduced glutathione is only 1.5 h, which limits its use to more oxidizing environments.

The reason for the unusual photochemical properties of the azo switch **5** were investigated by computational modeling. The shift in the absorption could be correctly replicated. The mapping of the absolute values of the highest occupied molecular orbital (HOMO) for each of the isomers onto the bond density surface revealed that in both cases the HOMO is centered on the central azo N atoms. In the planar *trans* isomer the HOMO is located in close proximity to the electron-rich O atoms of the methoxy groups, raising its relative energy. The twist of the phenyl rings in the *cis* isomer, however, minimizes this interaction, resulting in absorption behavior similar to that of the parent compound **3**.

With this work Woolley et al. provides a new powerful tool for controlling the spatial arrangement of molecular structures in biological systems in vivo. Their explanation for the performance of **5** should also lead to the design of even better switches. A larger gap between the n–π* bands of the *trans* and the *cis* isomesr should lead to more complete switching, which would further increase the utility. Furthermore, improved stability against glutathione would be highly desirable. In summary, this new azobenzene switch represents a very attractive tool for the control of biological functions in vivo by light. This should pave the way for new endeavors in this exciting research area.

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