Supporting Information

Photoisomerization of an individual azobenzene molecule in water: an on-off switch triggered by light at a fixed wavelength

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ABBREVIATIONS

αHL: α-hemolysin

DMSO: dimethylsulfoxide

DTT: dithiothreitol

EDTA: ethylenediaminetetraacetic acid

HPLC: high performance liquid chromatography

IASD: 4-acetamido-4'-((iodoacetyl)amino)-stilbene-2,2'-disulfonic acid

IVTT: in vitro transcription and translation

ESMS: electrospray ionization mass spectrometry

PEG-5000-maleimide: *o*-(2-maleimidoethyl)-*o*'-methyl-polyethyleneglycol 5000

Rf: ratio of the migration position of a compound to the distance to the solvent front on TLC

TCEP: tris(2-carboxyethyl)phosphine hydrochloride

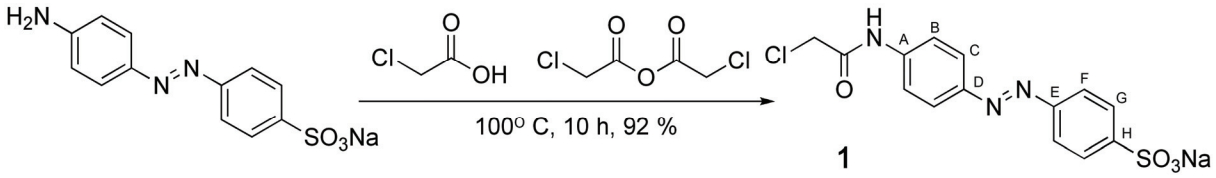
TLC: thin layer chromatography

Tris: tris(hydroxymethyl)aminomethane

WT: wild type

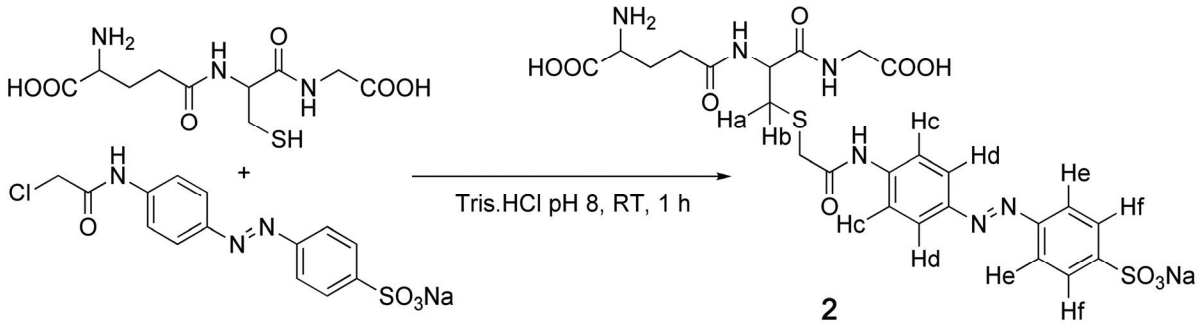
MATERIALS AND METHODS

Synthesis of sodium 4-((4-(2-chloroethanamido)phenyl)diazenyl)benzenesulfonate, 1.



Sodium 4-((4-(2-chloroethanamido)phenyl)diazenyl)benzenesulfonate was prepared according to a procedure adapted from that of Woolley and coworkers for 3,3'-bis(sulfo)-4,4'-bis(chloroacetamido)azobenzene[1]. Solid sodium 4'-sulfato-4-aminoazobenzene (1.17 g, 3.9 mmol, 1 equivalent; Lancaster) was mixed with chloroacetic acid (3.36 g, 36 mmol, 9 equivalents; Aldrich) and chloroacetic anhydride (5.7 g, 33 mmol, 9 equivalents; Aldrich). The mixture of solids was heated to 100°C (above the melting temperature) and stirred for 10 h. The mixture was then cooled to room temperature and washed with dichloromethane, in which the product is insoluble, to extract the remaining chloroacetic acid and chloroacetic anhydride. The product was further washed with dichloromethane with vacuum filtration and dried under vacuum to yield 1.26 g (3.6 mmol, 92 %) of an orange solid. The product appeared pure as determined by NMR, MS and TLC. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 4.50 (s, 2H, CH_2), 7.80-7.97 (m, 6H, arom), 8.02 (d, 2H, J = 8.5 Hz, H_F), 10.87 (s, 1H, NH). ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 44.49 (CH_2), 120.46 (C_B), 122.80 (C_C), 124.67 (C_F), 127.58 (C_G), 142.47 (C_A), 148.76 (C_H), 151.19 (C_D), 152.58 (C_E), 165.98 (C=O). ESMS: m/z 352.28 [M- Na^+]$^-$, calculated 352.02.

Reaction of 1 with gluthathione to form sodium 4-((4-(2-(2-(4-amino-4-carboxybutanamido)-3-(carboxymethylamino)-3-oxopropylthio)ethanamido)phenyl)diazenyl)benzenesulfonate, 2.



Reduced *L*-gluthathione (50 μL of a fresh 0.1 M stock solution in water) and compound **1** (50 μL at 0.2 M in DMSO) were mixed in 200 mM Tris.HCl buffer at pH 8.0 (900 μL). The reaction was complete within 1 h, as assessed by the disappearance of *L*-gluthathione by silica gel TLC on plates containing a fluorescent indicator (eluant: EtOH:H_2O:NH_4OH, 7.5:1.5:1, visualized by 1) UV then 2) ninhydrin: Rf(*L*-gluthathione) = 0.37, Rf(**1**) = 0.92, Rf(**2**) = 0.67). The product was purified by preparative silica gel TLC, and extracted from the gel with the eluant. The silica gel was filtered off and the filtrate was evaporated to dryness. ^{1}H NMR (400 MHz, D_2O); Protons were assigned by COSY: CH-$\underline{CH_2}$-CH_2 (1.99 ppm, m, 2H), CH-CH_2-$\underline{CH_2}$ (2.38 ppm, m, 2H), CH-$\underline{CH_aH_b}$-S (H_a, 2.83 ppm, dd, 1H, J = 14 Hz; H_b, 3.01 ppm, dd, 1H, J = 4.8, 14 Hz), S-$\underline{CH_2}$-CO (3.28 ppm, s, 2H), $\underline{CH}$-CH_2-CH_2 (3.64 ppm, t, 1H, J = 5.6, 6 Hz), NH-$\underline{CH_2}$-COOH (3.76, s, 2H), $\underline{CH}$-CH_2-S (4.47 ppm, m, 1H, J = 4.8 Hz), H_c (7.31 ppm, d, 2H, J = 8.4 Hz), H_d (7.46 ppm, d, 2H, J = 8.4 Hz), H_f (7.55 ppm, d, 2H, J = 8.0 Hz), H_e (7.75 ppm, d, 2H, J = 8.0 Hz).

UV/VIS analysis: thermal relaxation and cis state spectrum.

Spectra were recorded by using a Cary 3 UV-visible spectrophotometer, thermoregulated at 25°C with a circulating bath. Irradiation with a 300 W Xe lamp and a 380 nm interference filter of 2.5 x 10^{-5} M azobenzene derivative **1** in the electrophysiology buffer (1 mL; 10 mM Tris.HCl pH 8.5, 100 µM EDTA, 2 M KCl; dilution from a stock of 0.1 M **1** in DMSO) was performed in a quartz cuvette of 1 cm pathlength equipped with a small magnetic stirrer. No further changes in the UV spectrum occurred after 20 min. Thermal relaxation rates were determined by following the increase of the absorbance at 346 nm, the maximum of absorption by the trans state (ε_{346} = 30000 $M^{-1}cm^{-1}$; see derivation of equations for details).

A spectrum of the pure cis form of **1** was obtained by using an HLPC fitted with a photodiode array detector (Waters). The spectrum was normalized by using the absorbance at the isobestic points, where the extinction coefficients had been determined for the pure trans state (ε_{238} = 13900 $M^{-1}cm^{-1}$, ε_{292} = 8500 $M^{-1}cm^{-1}$, ε_{416} = 2800 $M^{-1}cm^{-1}$). Selected extinction coefficients for the cis form of **1** are: ε_{252} = 19100 $M^{-1}cm^{-1}$, ε_{309} = 8300 $M^{-1}cm^{-1}$, ε_{380} = 740 $M^{-1}cm^{-1}$, ε_{432} = 3500 $M^{-1}cm^{-1}$. The trans and the cis isomers were separated on a C2/C18 column (# µRPC C2/C18 ST 4.6/100 from Amersham Biosciences) eluted with a linear gradient from 0:100 to 30:70 of acetonitrile:water over 30 min. The retention times of the cis and the trans forms were respectively 14 and 20 min.

Protein labelling.

The α-HL T117C-D8 mutant and α-HL WT labeled with [^{35}S]-*L*-methionine (# 51001 H from MP Biomedicals, Inc. at 10 mCi/ml = 370 MBq/ml; final activity in IVTT mix: 0.4 mCi/ml = 14.8 MBq/ml) were prepared by in vitro transcription and translation (IVTT) by using an E. coli T7-S30 extract system for circular DNA (# L1130 from Promega). T117C-D8 has an octa-aspartate tail to facilitate the separation of $(WT)_6(T117C\text{-}\mathbf{1}\text{-}D8)_1$ from the other heteroheptamers upon SDS-polyacrylamide gel electrophoresis[2, 3]. The IVTT mix (75 μL) was incubated for 90 min at 37ºC and then placed on ice for 5 min to stop the reaction. 200 mM Tris.HCl buffer, pH 8.0, (25 μL) containing 1 mM fresh DTT (# D-5545 from Sigma) was then added and the mixture was passed through a P6 Micro Bio-Spin chromatography column (# 732-6221 from BioRad) at 1000 g for 5 min to remove molecules of low molecular weight. 200 mM Tris.HCl buffer, pH 8.0, (91 μL) containing 1 mM fresh DTT was added to the filtrate (50 μL, equivalent to 37.5 μL of undiluted IVTT mix), and the mixture was left for 5 min at room temperature. Compound **1** was dissolved at 0.5 M in DMSO. The reduced filtrate was reacted with three aliquots (each of 3 μL) of this stock solution, added at 20 min intervals, over a total period of 80 min. The protein solution was again passed through a BioRad P6 Micro Bio-Spin chromatography column at 1000 g for 5 min to remove the excess reagent. The filtrate (80 μL, equivalent to 20 μL of undiluted IVTT mix) was mixed with WT αHL (50 μL of undiluted IVTT mix) and the mixture was used to gently resuspend washed rabbit red blood cell membranes[4]. The protein was allowed to oligomerize on the membranes for 1 h at 37ºC. The membranes were then centrifuged at 21000 g for 5 min at 4ºC, the supernatant was removed and the pellet was gently resuspended in 1x sample loading buffer[5] (50 μL) and loaded onto a 5 % SDS-PAGE gel[5]. The running buffer[5] contained 0.1 mM of

fresh sodium thioglycolate. The gel was run at 50 V overnight. It was then dried at 50ºC under vacuum and placed against a Kodak Bio Max MR autoradiography film (# Z350370-50EA from Sigma-Aldrich) for 3 h. (Figure S4). The band corresponding to $(WT)_6(T117C\text{-}\mathbf{1}\text{-}D8)_1$ was excised and the protein was extracted in 10 mM Tris.HCl buffer, pH 7.5, (200 µL), containing 100 µM EDTA and 2 mM fresh DTT. The gel was rehydrated overnight with gentle rotation at 4ºC and crushed with a pestle. The protein was recovered by filtering off the polyacrylamide gel by using microfilterfuge tubes with 0.2 µm cellulose acetate membranes (# 7016-024 from Rainin), by centrifugation for 30 min at 16000 g at 25ºC.

To assess the extent of modification, T117C-**1**-D8 and the unmodified T117C-D8 monomers were treated with IASD [4-acetamido-4'-((iodoacetyl)amino)-stilbene-2,2'-disulfonic acid; # A484 from Molecular Probes-Invitrogen Detection Technologies] or PEG-5000-maleimide [*o*-(2-maleimidoethyl)-*o*'-methyl-polyethyleneglycol 5000; # 63187 from Fluka].

Modification with PEG-5000-maleimide: 200 mM Tris.HCl buffer, pH 8.0, (10 µL) containing 1 mM fresh DTT was added to the monomer (30 µL, equivalent to 7.5 µL of undiluted IVTT mix). The protein was reduced for 5 min at 25ºC and PEG-5000-maleimide (10 µL, 0.05 M in water) was added (10 mM final). The mixture was allowed to react for 15 min at 25ºC and then quenched with 2x Laemmli sample loading buffer (50 µL, contains 1.43 M β-mercaptoethanol)[5].

Modification with IASD: 200 mM Tris.HCl buffer, pH 8.5, (10 µL) containing 1 mM fresh DTT was added to the monomer (30 µL, equivalent to 7.5 µL of undiluted IVTT mix). The protein was reduced for 5 min at 25ºC and two aliquots of IASD (each 5 µL; 0.1 M in water)

were added (20 mM final) at 20 min intervals over a total of 1 h. The reaction was quenched with 2x sample loading buffer (50 μL, contains 1.43 M β-mercaptoethanol).

The samples were loaded onto a 12% SDS-PAGE gel. The running buffer contained 0.1 mM of fresh sodium thioglycolate. The gel was run at 50 V overnight. For improved band separation, the gel was run until the tracking dye had moved into the bottom reservoir and a 25 kDa colored protein marker (# 161-0374 from BioRad) had reached the bottom of the gel. It was then dried at 80ºC and placed against a Kodak Bio Max MR autoradiography film (# Z350370-50EA from Sigma-Aldrich) for 18 h. (Figure S5). A clear difference in electrophoretic mobility could be observed between T117C-**1**-D8 and the previously unmodified T117C-D8 monomers after treatment with PEG-5000-maleimide. T117C-D8 was modified by PEG-5000-maleimide, causing a large gel shift. T117C-**1**-D8 was not modified by PEG-5000-maleimide, because the cysteine had already been modified by **1**.

Electrical recording with UV irradiation

A folded bilayer was made over a 100-μm aperture in a 25 μm-thick Teflon sheet, glued between two Teflon chambers. The aperture had been pretreated with a solution of 10% hexadecane in pentane (10 μL) on each side. Initially, the buffer in the chambers (10 mM Tris.HCl, pH 8.5, 100 μM EDTA, 2 M KCl) was below the level of the aperture. A drop of 1,2-diphytanoyl-sn-glycero-3-phosphocholine in pentane (20 μL; 10 mg/ml, # 101506 Avanti Polar Lipids Inc) was added to each side and the pentane allowed to evaporate. A bilayer was then formed by raising the level of buffer in both chambers above the aperture. αHL $(WT)_6(T117C\text{-}\mathbf{1}\text{-}D8)_1$ (0.05 μL) was added with magnetic stirring to the cis chamber. The insertion of a single pore was judged by the appearance of a current step (-89 pA at an applied voltage of -50 mV). The trans chamber was fitted with a sapphire window (# 43300, Oriel) for irradiation of the bilayer. This chamber, rather than the cis chamber (as is normal in our laboratory), was grounded because of a small current leak at the irradiation window.

The irradiation set-up consisted of a 300 W Xenon lamp (# 6258, Oriel), a F/1 type condenser (# 66984, Oriel), an IR water filter (# 6117, Oriel) refrigerated by a circulating bath, a multiple filter holder (# 62020, Oriel), and a fiber optic focusing assembly (# 77800, Oriel) with a fiber optic bundle (# 77577, Oriel) to direct the light through the sapphire window of the chamber. A heat filter (# 716 GK 106, Comar) was used in addition to the water filter for the removal of IR radiation. Metallic neutral density filters with optical densities of 0.2 (# 59660), 0.5 (# 59690), 0.7 (# 59694) and 1.5 (# 59705) were from Oriel. 380 nm (# 380 IL 50) and 500 nm (# 500 IL 50) interference filters were from Comar. The 330 nm (# 59154) band-pass filter was from Oriel and the 465 nm (# 465 BP 50) band-pass filter from Comar.

The electrical current was detected by using two Ag/AgCl electrodes, each embedded in a gel of 1.5% agarose in 3 M KCl, amplified with a patch-clamp amplifier (Axopatch 200B, Axon), filtered with a low-pass Bessel filter (80 dB/decade) with a corner frequency of 2 kHz and digitized at a sampling frequency of 5 kHz (Digidata 1200 A/D converter, Axon).

DERIVATION OF EQUATIONS

Half-time of thermal relaxation of azobenzene.

For the thermal relaxation of the cis state to the trans state, we have C $\rightarrow$ T with a rate constant k, where C is the cis state and T the trans state.

Thus $\frac{d[C]}{dt} = -k[C]$ (1)

and $\frac{d[C]}{[C]} = -kdt$

By integrating between time zero and time t, we find:

$[C]_t = [C]_0 e^{-kt}$ (2)

where $[C]_0$ is the initial concentration of the cis state

and $[C]_t$ is the concentration of the cis state at time t

or $\ln\left(\frac{[C]_t}{[C]_0}\right) = -kt$ and $\ln\left(\frac{[C]_0}{[C]_t}\right) = kt$ (3)

When the conversion is complete, we have $[C]_0 = [T]_\infty$ (4)

And at time t, $[C]_t = [C]_0 - [T]_t$ (5)

(4) and (5) give $[C]_t = [T]_\infty - [T]_t$ (6)

(3), (4) and (6) give $\ln\left\{\frac{[T]_\infty}{([T]_\infty - [T]_t)}\right\} = kt$ (7)

According to Beer's law, $A = \varepsilon c l$

Let A_{T-t}, A_{C-t} and A_t be respectively the absorbances of the trans state, the cis state and the total absorbance at 346 nm at time t, when the concentrations of the two states are $[T]_t$ and $[C]_t$.

$A_{T-\infty}$ is the absorbance at 346 nm of the trans state after total conversion of C→T. At this time, the concentration of the trans state is $[T]_\infty = [C]_0$.

Let the extinction coefficients at 346 nm be ε_T and ε_C.

We thus have:

$$A_{T-\infty} = \varepsilon_T [T]_\infty l$$

or $[T]_\infty = \frac{A_{T-\infty}}{\varepsilon_T l}$ (8)

and $A_t = A_{T-t} + A_{C-t} = \varepsilon_T [T]_t l + \varepsilon_C [C]_t l$

since, (6): $[C]_t = [T]_\infty - [T]_t$

Thus $A_t = \varepsilon_T [T]_t l + \varepsilon_C ([T]_\infty - [T]_t) l$

or $A_t = l([T]_t(\varepsilon_T - \varepsilon_C) + [T]_\infty \varepsilon_C)$

and $[T]_t = \frac{1}{\varepsilon_T - \varepsilon_C}\left(\frac{A_t}{l} - \varepsilon_C [T]_\infty\right)$

with (8): $[T]_t = \frac{1}{\varepsilon_T - \varepsilon_C}\left(\frac{A_t}{l} - \frac{\varepsilon_C A_{T-\infty}}{\varepsilon_T l}\right)$ (9)

According to (8) and (9) :

$$[T]_\infty - [T]_t = \frac{1}{l}\left(\frac{A_{T-\infty}}{\varepsilon_T} - \frac{A_t}{\varepsilon_T - \varepsilon_C} + \frac{\varepsilon_C A_{T-\infty}}{\varepsilon_T(\varepsilon_T - \varepsilon_C)}\right)$$

$$\text{or } [T]_\infty - [T]_t = \frac{(\varepsilon_T - \varepsilon_C)A_{T-\infty} - \varepsilon_T(\varepsilon_T - \varepsilon_C)A_t + \varepsilon_C A_{T-\infty}}{\varepsilon_T(\varepsilon_T - \varepsilon_C)l}$$

$$\text{or } [T]_\infty - [T]_t = \frac{1}{l}\left(\frac{A_{T-\infty} - A_t}{\varepsilon_T - \varepsilon_C}\right) \quad (10)$$

(8) and (10) give :

$$\frac{[T]_\infty}{[T]_\infty - [T]_t} = \frac{(\varepsilon_T - \varepsilon_C)A_{T-\infty}}{\varepsilon_T(A_{T-\infty} - A_t)}$$

By replacing in (7), we get:

$$kt = \ln\left(\frac{A_{T-\infty}}{A_{T-\infty} - A_t}\right) + \ln\left(\frac{\varepsilon_T - \varepsilon_C}{\varepsilon_T}\right)$$

Which is the same as:

$$kt = \ln(A_{T-\infty}) - \ln(A_{T-\infty} - A_t) + \ln\left(\frac{\varepsilon_T - \varepsilon_C}{\varepsilon_T}\right)$$

$$\text{and } \ln(A_{T-\infty} - A_t) = -kt + \ln(A_{T-\infty}) + \ln\left(\frac{\varepsilon_T - \varepsilon_C}{\varepsilon_T}\right) \quad (11)$$

where $\ln(A_{T-\infty}) + \ln\left(\frac{\varepsilon_T - \varepsilon_C}{\varepsilon_T}\right)$ is constant

According to (11), we can plot $\ln(A_{T-\infty} - A_t)$ versus t,

where A_t is the total absorbance at 346 nm at time t

and $A_{T-\infty}$ is the absorbance of the trans state at 346 nm when the conversion is complete.

The slope of the line gives k, and from (7): $t_{1/2} = \frac{\ln 2}{k}$.

Rate of photochemical reactions on a surface

The derivation is similar to that for the rate of a photochemical reaction in solution[6].

$$A = \log\left(\frac{I_0}{I_t}\right)$$

where A = absorbance

I_0 = incident light (mmole (photons) s^{-1} cm^{-2})

I_t = transmitted light (mmole (photons) s^{-1} cm^{-2})

Beer's law : $A = \varepsilon c l$

where ε = molar extinction coefficient (M^{-1} cm^{-1})

l = pathlength (cm), c = concentration (M)

Alternatively, $I_t = I_0 e^{(-2.303\varepsilon c l)}$

Consider a 1 cm^2 area of a surface. Let the number of molecules in the area (N') be the same as the number in a very thin layer of solution, where the concentration is c and the thickness of the layer is l cm. N' can be thought of as the number of molecules to a depth of 'l' in a 1 cm^2 window in a $10 \times 10 \times 10$ cm cube (1 L). When N_{AVO} is Avogadro's number:

$$N' = 10^{-2} c \frac{l}{10} N_{AVO}$$

Therefore $cl = 10^3 \frac{N'}{N_{AVO}}$

and $A = 10^3 \varepsilon \frac{N'}{N_{AVO}}$

so that $I_t = I_0 e^{\left(-2.303 \times 10^3 \varepsilon \frac{N'}{N_{AVO}}\right)}$

The rate of a photochemical reaction in the defined area will be the number of molecules per cm^2 that absorb a photon multiplied by the quantum yield (Φ) for the reaction.

Light (number of photons) absorbed per cm^2 = $(I_0 - I_t)N_{AVO}10^{-3}$ molecules cm^{-2}

[the 10^{-3} comes from the fact that the units of I are defined in mmole s^{-1} cm^{-2}]

Therefore $-\frac{dN'}{dt} = \Phi(I_0 - I_t)N_{AVO}10^{-3} = \Phi N_{AVO}10^{-3} I_0 \left(1 - e^{\left(-2.303\times10^3 \varepsilon \frac{N'}{N_{AVO}}\right)}\right)$

Because $e^x = \frac{\sum_{n=0}^{\infty} x^n}{n!}$, $e^{-x} = 1 - x$ for small x

i.e for low total light absorption: $-\frac{dN'}{dt} \approx 2.303\Phi I_0 N'$

Integrating: $\frac{N'_t}{N'_0} = e^{(-2.303\phi I_0 \varepsilon t)}$

where N'_t is the number of molecules at time t

N'_0 is initial number of molecules.

Therefore the fraction photolyzed $\frac{N'_0 - N'_t}{N'_0} = 1 - e^{(-2.303\phi I_0 \varepsilon t)}$

so for example:

at t = $t_{1/2}$, $\frac{N'_0 - N'_t}{N'_0} = 0.5$

so $e^{(-2.303\phi I_0 \varepsilon t)} = 0.5$

Taking ln of both sides:

$$-2.303\phi I_0 \varepsilon t_{1/2} = -0.69$$

$$t_{1/2} \approx \frac{0.3}{\phi I_0 \varepsilon}$$

Therefore for low light absorption the half-life of molecules on a surface is the same as in dilute solution. This is also the mean lifetime, τ, of an individual molecule on a surface (or in a bilayer).

At the single molecule level:

$$\tau \approx \frac{0.3}{\phi I_0 \varepsilon}$$

The ratio between the trans and the cis state lifetimes (at a specified wavelength) is:

$$\frac{\tau_t}{\tau_c} \approx \frac{\phi_c \varepsilon_c}{\phi_t \varepsilon_t}$$

where ϕ_t is the quantum yield of the trans to cis conversion

and ϕ_c is the quantum yield of the cis to trans conversion

Table 1. Effect of the wavelength of the incident light on the ratio of the mean lifetimes, τ_t/τ_c, of the events. In theory (see above): $\tau_t/\tau_c = \phi_c\varepsilon_c / \phi_t\varepsilon_t$. The calculated values were obtained by using both the extinction coefficient of the derivative **1** at the central wavelength of the filter and the integral of the absorbance between the wavelengths for which the transmittance was 50% or higher. The quantum yields were from the literature, determined in H_2O:EtOH, 80:20 [7]. $\Phi_{T\text{-}\pi\pi^*} = 0.21$ and $\Phi_{C\text{-}\pi\pi^*} = 0.15$ were used for the 330 nm band-pass and the 380 ± 10 nm interference filters, and $\Phi_{T\text{-}n\pi^*} = 0.35$ and $\Phi_{C\text{-}n\pi^*} = 0.41$ were used for the 465 nm band-pass and the 500 ± 10 nm interference filters.

Filter	**τ_t/τ_c experimental**	**τ_t/τ_c calculated (ε at central wavelength)**	**τ_t/τ_c calculated (ε from integrated absorbance)**
330 nm band-pass	0.82	0.18	0.21
380 ± 10 nm interference	0.50	0.040	0.033
465 nm band-pass	3.8	1.5	0.66
500 ± 10 nm interference	mainly trans*	1.7	1.4

*When the 500 nm interference filter was used, P_{AZO} was mainly in the trans state and there were too few events to calculate τ_t/τ_c.

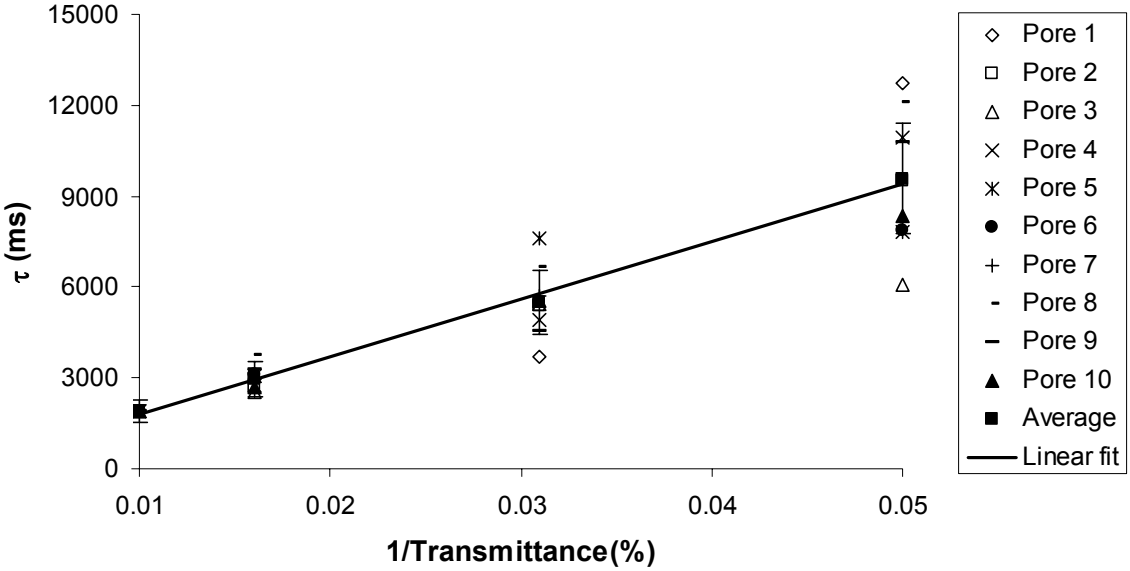
Table 2. Recapitulation of current recordings of P_{AZO} in the cis state in the dark. Each of the sixteen experiments presented in the following table were conducted as described in Figure S2, which shows Pore 1. No events were seen when the cis state was held in the dark for a total of 518 min. The trans state was recovered upon irradiation. Up to five experiments of this type have been conducted on the same pore, Pore 7. The buffer was 10 mM Tris.HCl, pH 8.5, 100 μM EDTA, 2 M KCl on both sides of the bilayer and the applied potential was -50 mV. Irradiation was at 330 nm and 25°C.

Pore	**Duration of the cis state (min)**
Pore 1	125
Pore 2	9
	32
Pore 3	14
	14
Pore 4	10
Pore 5	31
	29
	32
Pore 6	30
Pore 7	36
	31
	32
	31
	31
Pore 8	31
TOTAL	**518**

Figure S1. Mean lifetime, τ, of the events versus 1/transmittance of a neutral density filter. (a) trans events and (b) cis events. The lifetime of the events should be inversely proportional to the amount of incident light (I_0, see above), which was varied experimentally with neutral density filters (ODs of 0.2, 0.5, 0.7 and 1.5) for ten different pores. The irradiation wavelength was 330 nm. The buffer was 10 mM Tris.HCl, pH 8.5, 100 μM EDTA, 2 M KCl on both sides of the bilayer and the applied potential was -50 mV. The temperature was 25°C.

Notes: (1) In each experiment, the value of τ without a neutral density filter (1/transmittance(%) = 0.01) was normalized to the mean value of the event duration for all ten experiments, to compensate for variations in lamp output. The values of τ with the various filters were then adjusted for each experiment based on the normalization of the τ value without a neutral density filter. (2) The greater the optical density of the filter, the longer and fewer are the events, and the larger is the error. That is why the points are more scattered for higher 1/transmittance(%) values.

(a) TRANS


15000
12000
9000
6000
3000
0
τ (ms)
0.01
0.02
0.03
0.04
0.05
1/Transmittance(%)
Pore 1
Pore 2
Pore 3
Pore 4
Pore 5
Pore 6
Pore 7
Pore 8
Pore 9
Pore 10
Average
Linear fit

(b) CIS

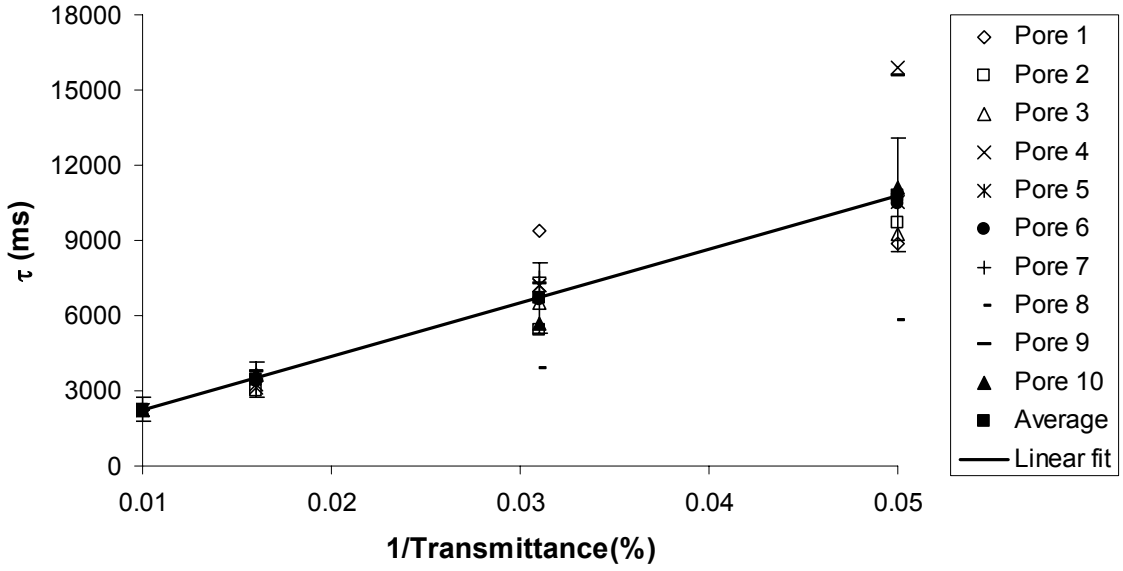

18000
15000
12000
9000
6000
3000
0
τ (ms)
0.01
0.02
0.03
0.04
0.05
1/Transmittance(%)
Pore 1
Pore 2
Pore 3
Pore 4
Pore 5
Pore 6
Pore 7
Pore 8
Pore 9
Pore 10
Average
Linear fit

Figure S2. Current recording of P_{AZO} in the cis state for 125 min. The light was on at the beginning of the recording and P_{AZO} flips back and forth between the trans (upper level) and the cis state (lower level). The light was switched off (-hν) with P_{AZO} in the cis state and no transitions to the trans state were seen during the next 125 min. The trans state was recovered upon irradiation (+hν). The buffer was 10 mM Tris.HCl, pH 8.5, 100 μM EDTA, 2 M KCl on both sides of the bilayer and the applied potential was -50 mV. Irradiation was at 330 nm and 25°C.

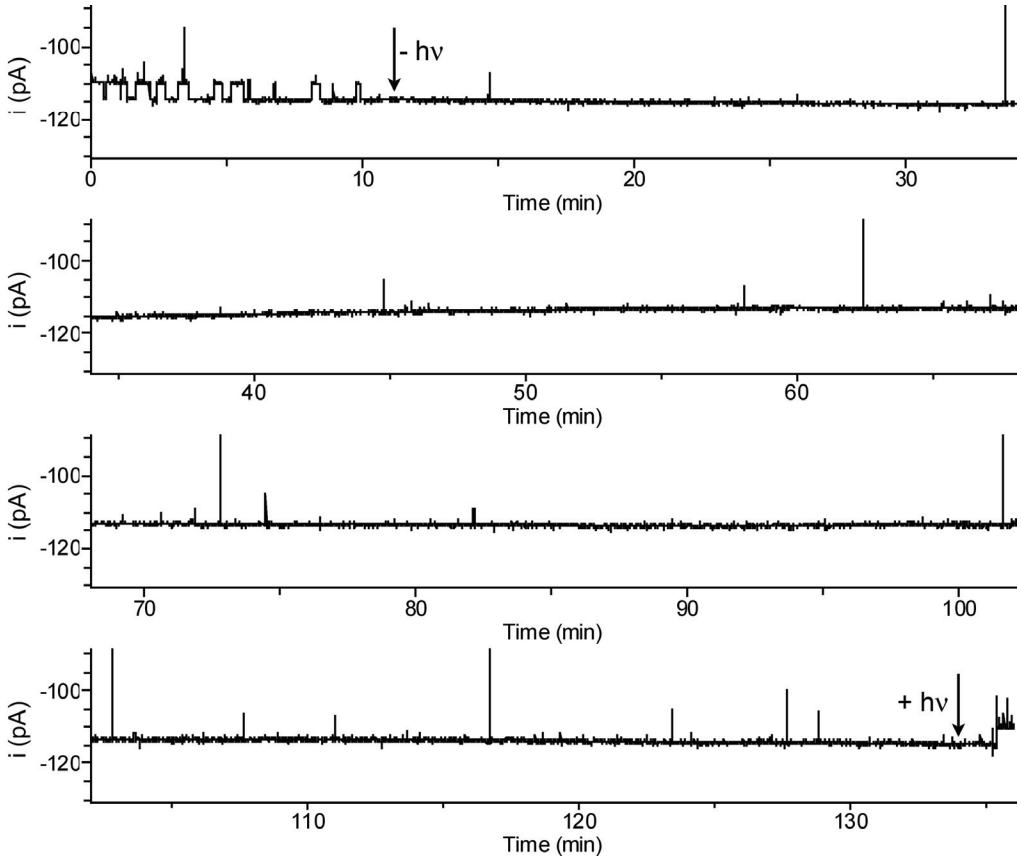
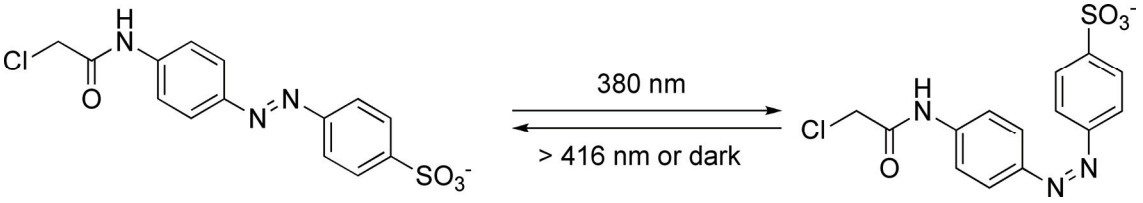


Figure S3. **(**a) Trans and cis isomers of azobenzene **1** and (b) their UV spectra. Unbroken line: trans state. Dotted line: cis state. Dashed line: solution of **1** irradiated at 380 ± 10 nm until no further change in the spectrum was observed. The spectrum of the cis state was obtained by injecting a solution of **1** irradiated at 380 ± 10 nm into an HPLC fitted with a photodiode array detector. The cis spectrum was then normalized based on the extinction coefficients of the trans state at the isobestic points.

(a)



(b)

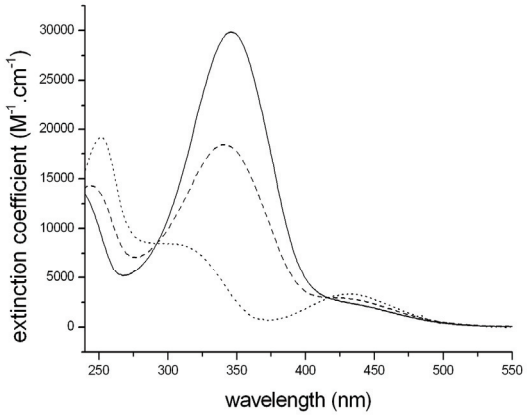


Figure S4. 5% SDS-PAGE gel of αHL heptamers, showing the gel shift caused by the C-terminal octa-aspartate tail (D8) on the single cysteine mutant subunit. Well 1: WT αHL monomer assembled together with T117C-**1**-D8 subunits to give WT_7, WT_6(T117C-**1**-D8)$_1$, WT_5(T117C-**1**-D8)$_2$, etc. Well 2: WT αHL monomer was assembled together with T117C-D8 subunits to give WT_7, WT_6(T117C-D8)$_1$, WT_5(T117C-D8)$_2$, etc. Well 3: WT αHL monomer was assembled on its own to give WT_7. The desired bands were cut from the gel and the protein eluted as described in the text.

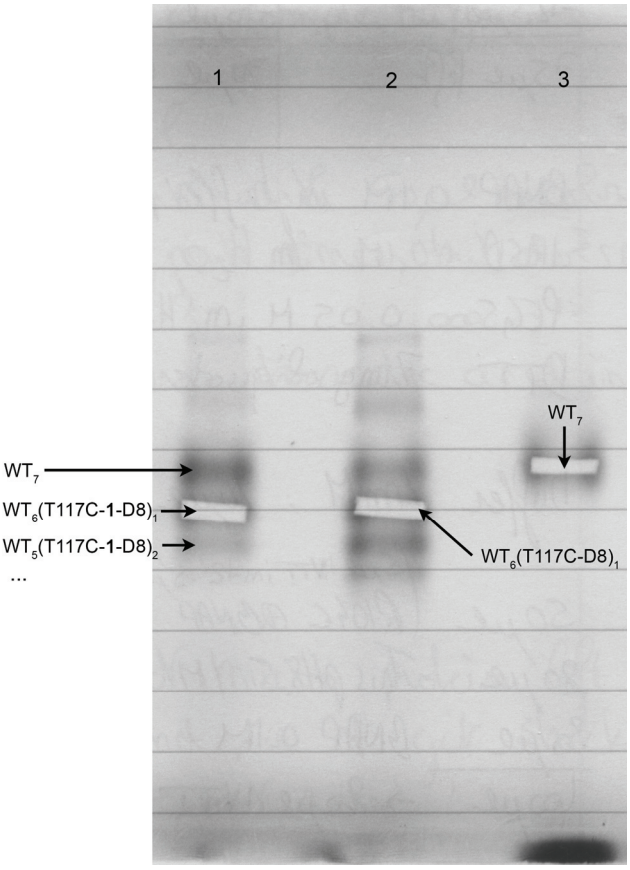
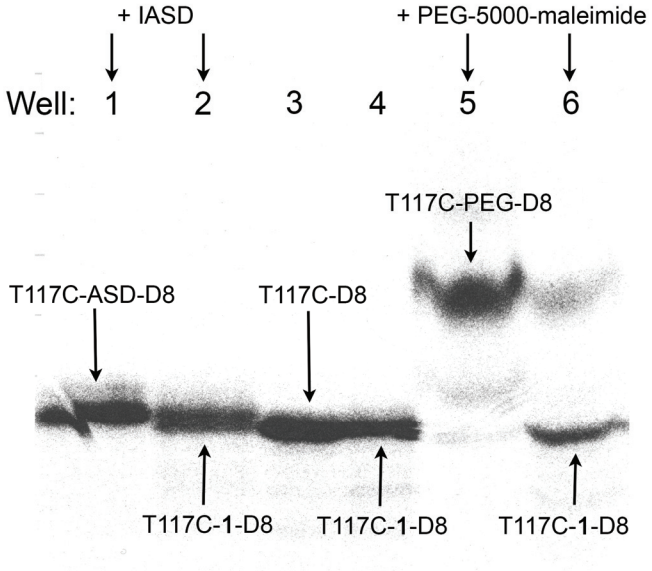


Figure S5. 12% SDS-PAGE gel of the αHL T117C-D8 monomers, showing the gel shifts caused by chemical modification of the single cysteine residue. Well 1: T117C-D8 was reacted IASD. Well 2: T117C-**1**-D8 reacted with IASD. Well 3: T117C-D8. Well 4: T117C-**1**-D8. Well 5: T117C-D8 reacted with PEG-5000-maleimide. Well 6: T117C-**1**-D8 reacted with PEG-5000-maleimide. No significant gel shift was caused by modification of T117C-D8 by **1** (Well 3 v Well 4). Similarly, modification of T117C-D8 by IASD did not produce a large gel shift (Well 1 v Well 2). By contrast, modification of T117C-D8 by PEG-5000-maleimide caused a substantial shift (Well 5). T117C-**1**-D8 was largely unaffected by PEG-5000-maleimide under the same conditions (Well 6), suggesting that the cysteine was mostly modified by **1** and no longer available to react.



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