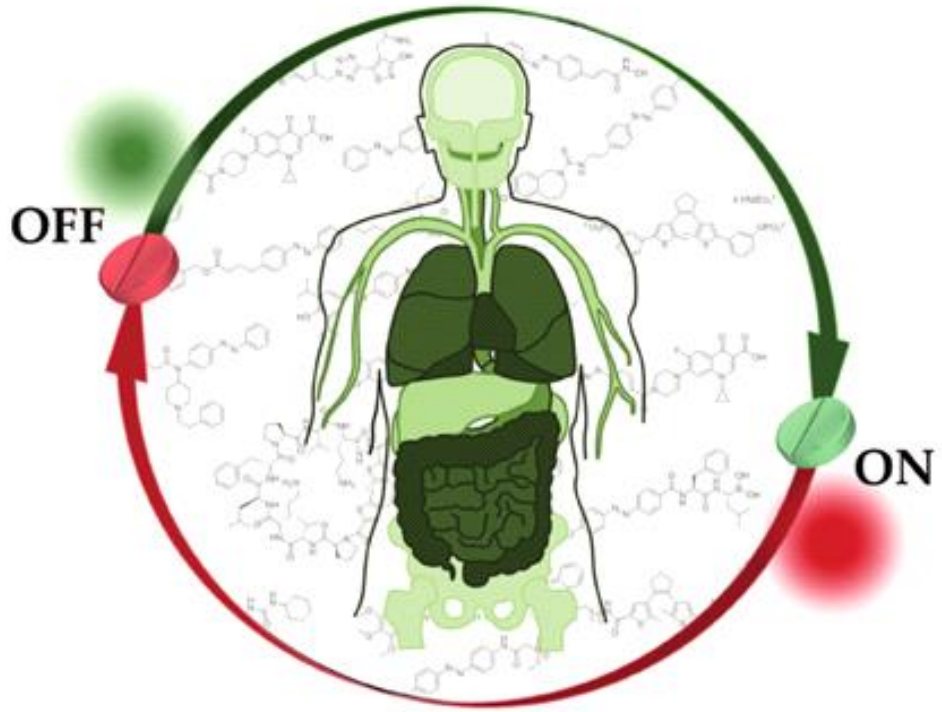
Photopharmacology



Group Meeting: 07/13/2017

Dominik Kölmel

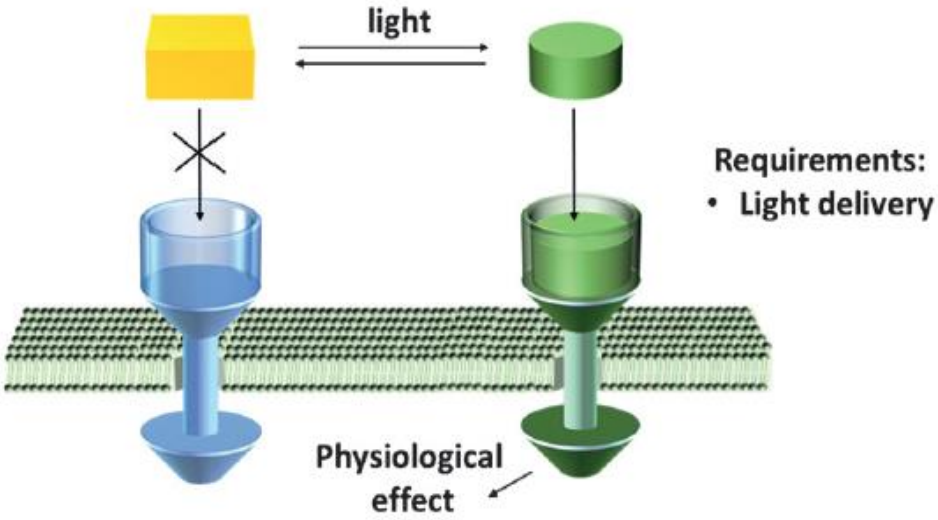
MacMillan Group

Outline

- Advantages and Challenges of Photopharmacology
- Photoswitches:
  - Azobenzenes
  - Diarylethenes
- Requirements and Design Strategies
- Selected Examples

Photopharmacology

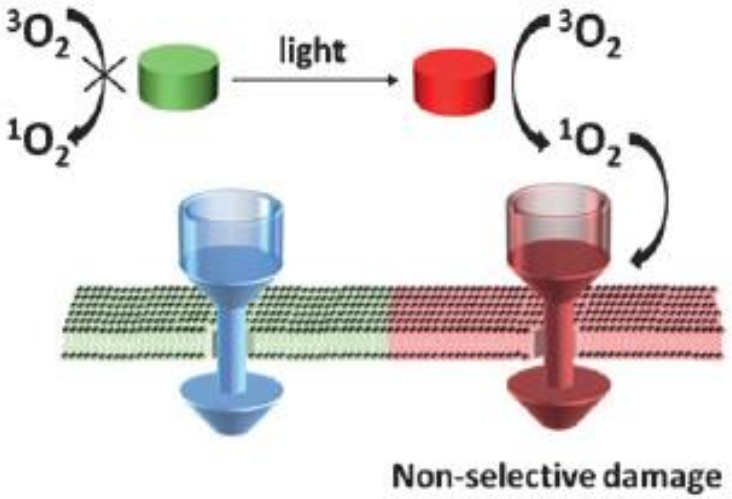
- definition:
  control of biological activity with synthetic photopharmaceuticals
- photopharmaceuticals:
  pharmacophore with incorporated photoswitch
- reversible switching between on and off state
- on state: high biological activity
- off state: no/low biological activity



M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed*. **2016**, *55*, 10978.

Photodynamic Therapy

- definition:
  light-triggered generation of cytotoxic $^1O_2$
- photosensitizer:
  transfers energy from light to $^3O_2$ to form $^1O_2$
- non-selective destruction of cells/tissue
- $^1O_2$ has short lifetime: local tissue ablation



Requirements:
- **Light delivery**
- **Presence of oxygen**

M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed*. **2016**, *55*, 10978.

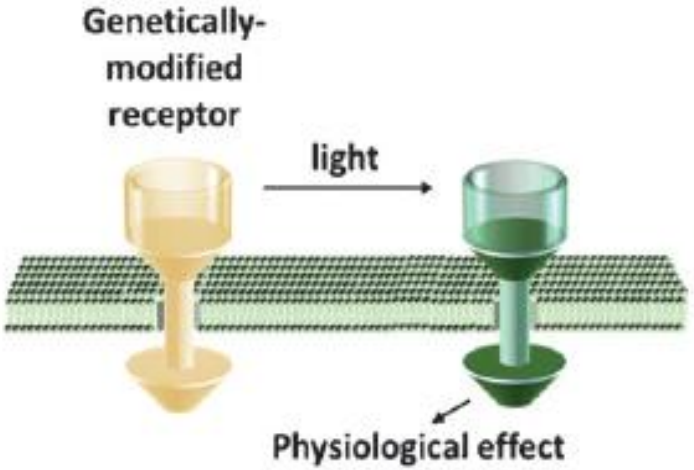
Optogenetics

- definition:
  control of biological activity with light-sensitive, genetically engineered biomolecules

- photoswitches are typically derived from photoresponsive rhodopins

- mainly used to manipulate ion channels in neurons

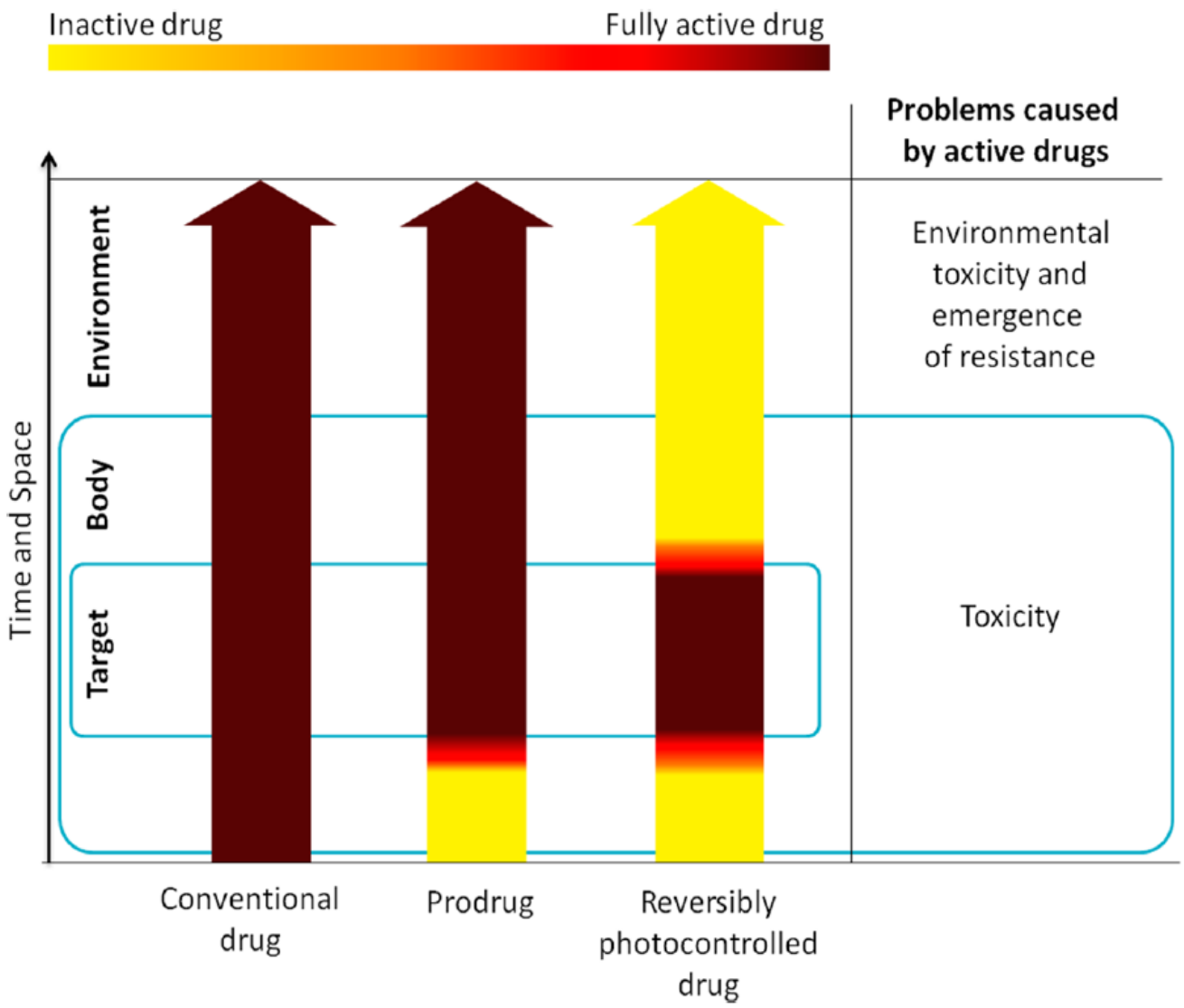
- requirement for genetic manipulation limits potential therapeutic applications



Requirements:
- **Light delivery**
- **Genetic manipulation**

M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed*. **2016**, *55*, 10978.

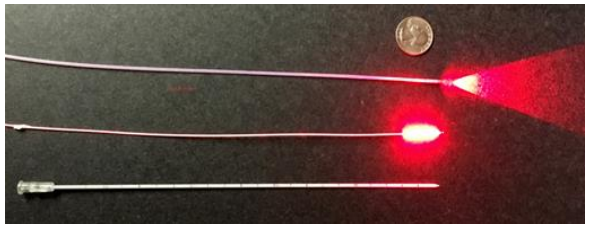
Advantages of Photodynamic Therapy



W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc*. **2014**, *136*, 2178.

Light Delivery

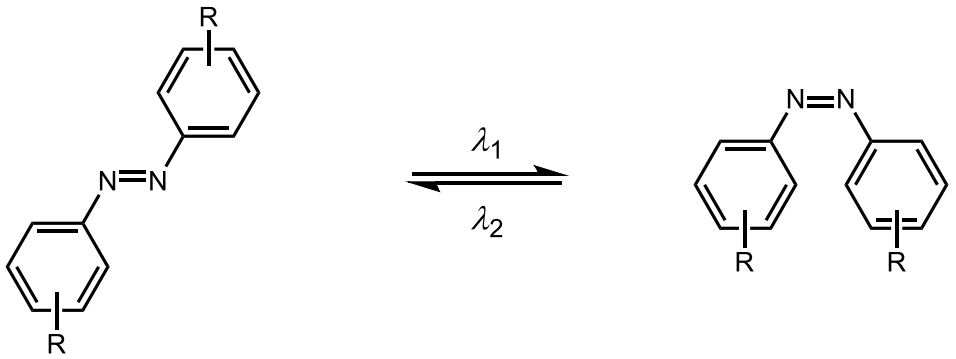
- red light (λ > 600 nm) can greatly reduce phototoxic effects
- phototherapeutic window: 650-900 nm
- penetration depth: 1 cm (@630 nm)
  2 cm (@800 nm)
- limiting factors: light scattering and absorption by endogenous chromophores (λ < 650 nm: hemoglobin, λ > 900 nm: water)
- light sources: lasers and LEDs
- light delivery systems: fiberoptic devices and light diffusers



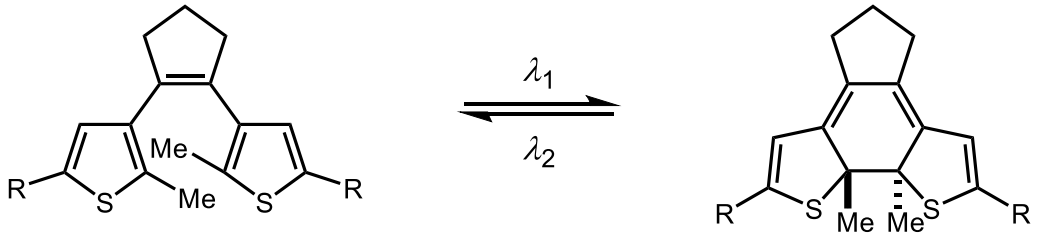
M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed*. **2016**, *55*, 10978.

Photoswitches

- Azobenzenes

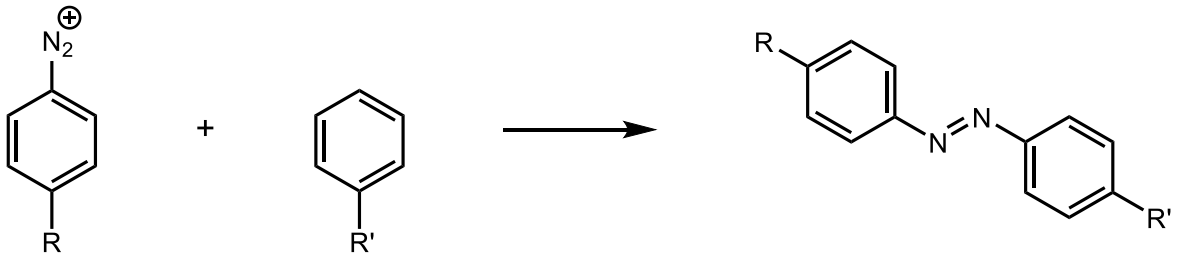


- Diarylethenes

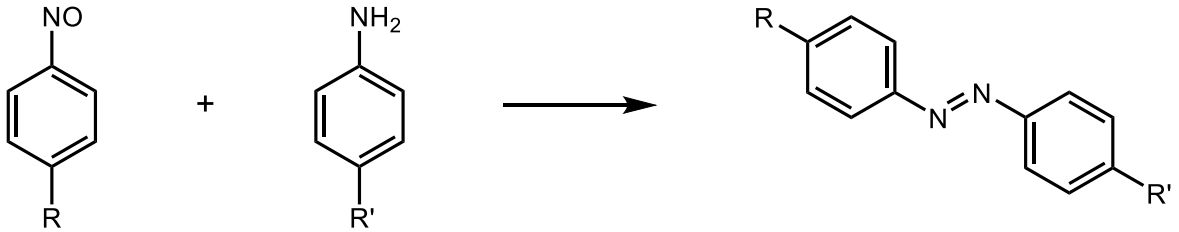


Synthesis of Azobenzenes

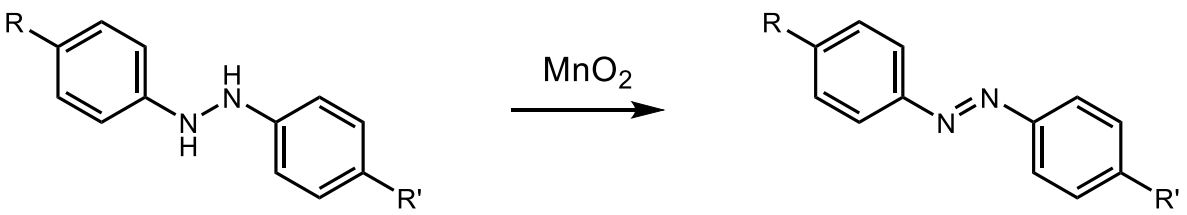
- azo coupling



- Mills reaction

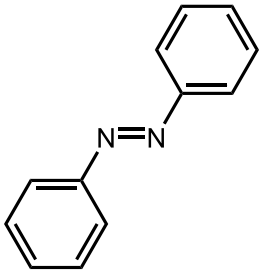


- dehydrogenation of hydrazines

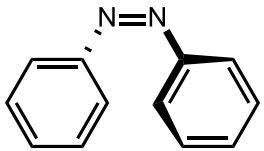


E. Merino, *Chem. Soc. Rev*. **2011**, *40*, 3835.

Azobenzene: Properties



$h\nu$



λ_{max} ($\pi \rightarrow \pi^*$) ≈ 320 nm

λ_{max} (n $\rightarrow \pi^*$) ≈ 450 nm

$\Phi_{trans \rightarrow cis}$ = 0.21 (H_2O/EtOH)

λ_{max} ($\pi \rightarrow \pi^*$) ≈ 270 nm

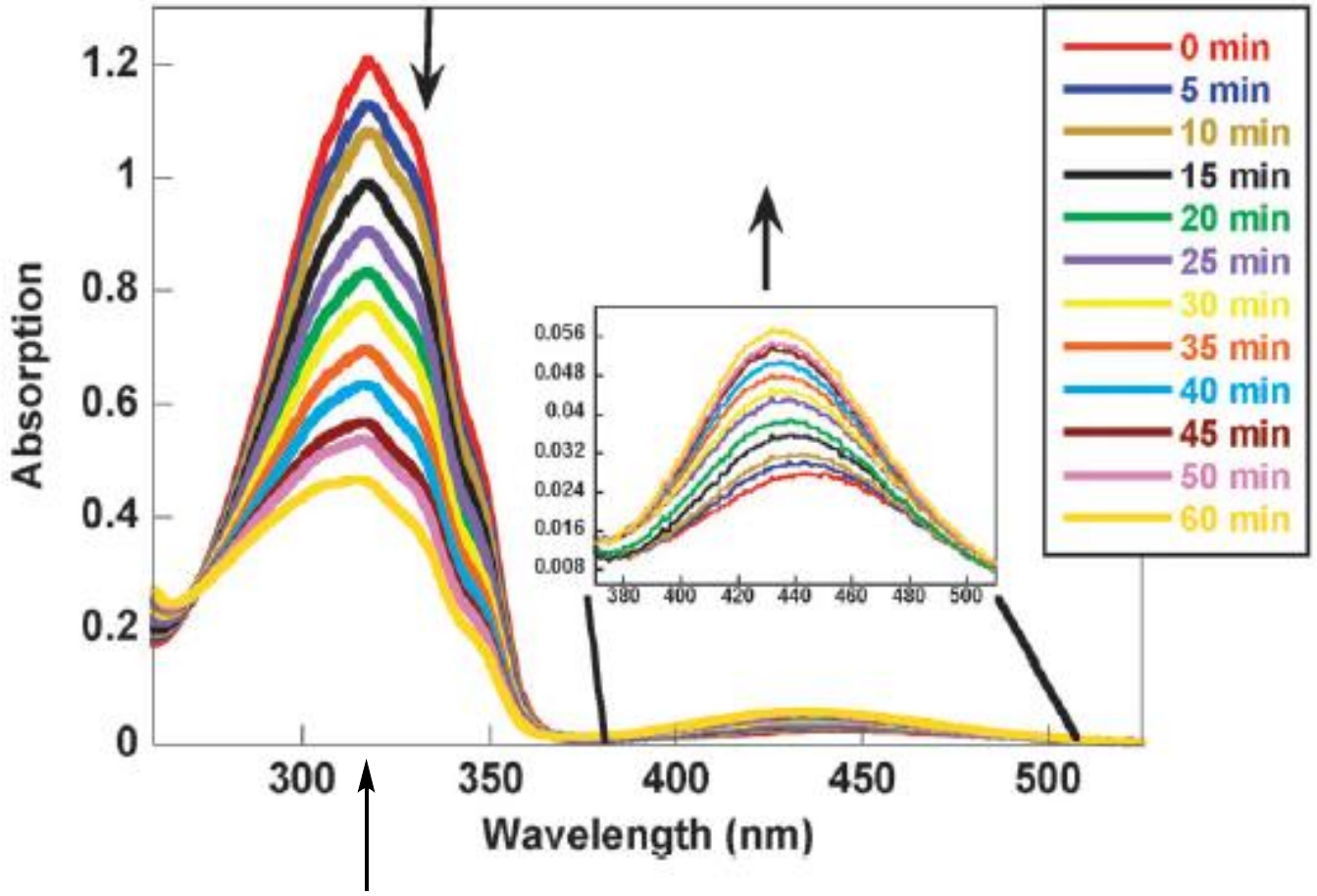
λ_{max} (n $\rightarrow \pi^*$) ≈ 450 nm

$\Phi_{cis \rightarrow trans}$ = 0.40 (H_2O/EtOH)

- excited state lifetime: 1-10 ps
- $t_{1/2}$ (*cis*-isomer) ≈ 2 d (MeCN)
- energy difference: 22 kcal
- distance change: 3 Å
- dipole moment change: 3 D
- photostationary state:
  20:80 (excitation @ 313 nm)
  90:10 (excitation @ 436 nm)

H. M. D. Bandara, S. C. Burdette, *Chem. Soc. Rev*. **2012**, *41*, 1809.

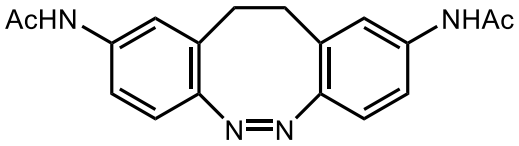
Absorption Spectrum of Azobenzene



irradiation @ 316 nm

H. M. D. Bandara, S. C. Burdette, *Chem. Soc. Rev*. **2012**, *41*, 1809.

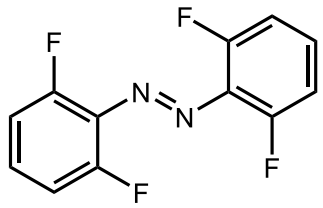
Visible-Light-Switchable Azobenzene



$\lambda_{trans \rightarrow cis}$ = 500-550 nm

$\lambda_{cis \rightarrow trans}$ = 407 nm

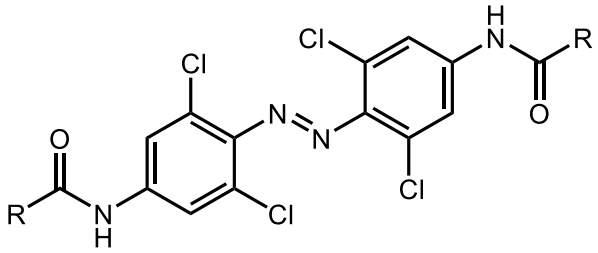
$t_{1/2}$ (*trans*-isomer) ≈ 4.8 h



$\lambda_{trans \rightarrow cis}$ > 450 nm

$\lambda_{cis \rightarrow trans}$ = 410 nm

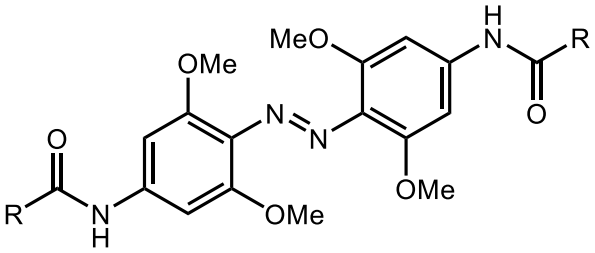
$t_{1/2}$ (*cis*-isomer) ≈ 700 d



$\lambda_{trans \rightarrow cis}$ = 635 nm

$\lambda_{cis \rightarrow trans}$ = 450 nm

$t_{1/2}$ (*cis*-isomer) ≈ 3.5 h



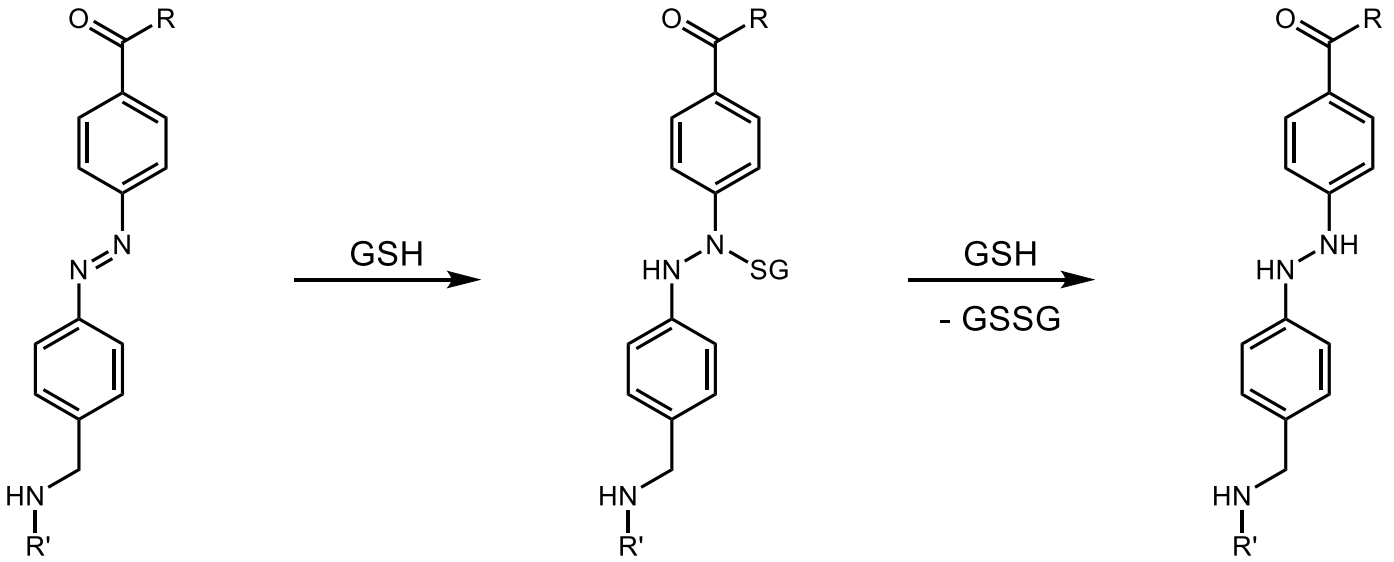
$\lambda_{trans \rightarrow cis}$ = 635 nm

$\lambda_{cis \rightarrow trans}$ = 460 nm

$t_{1/2}$ (*cis*-isomer) ≈ 2.4 d

W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc.* **2014**, *136*, 2178.

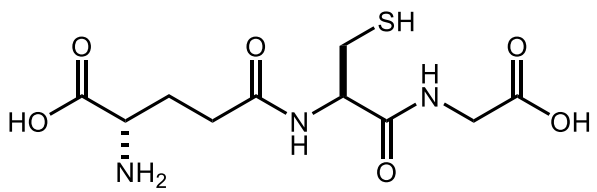
Biological Stability of Azobenzenes I



k_{red} (*trans*) = 6 h^{-1} M^{-2}

k_{red} (*cis*) = 600 h^{-1} M^{-2}

GSH =

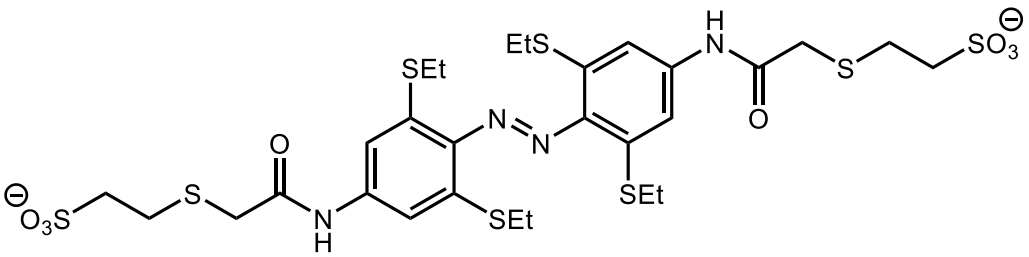


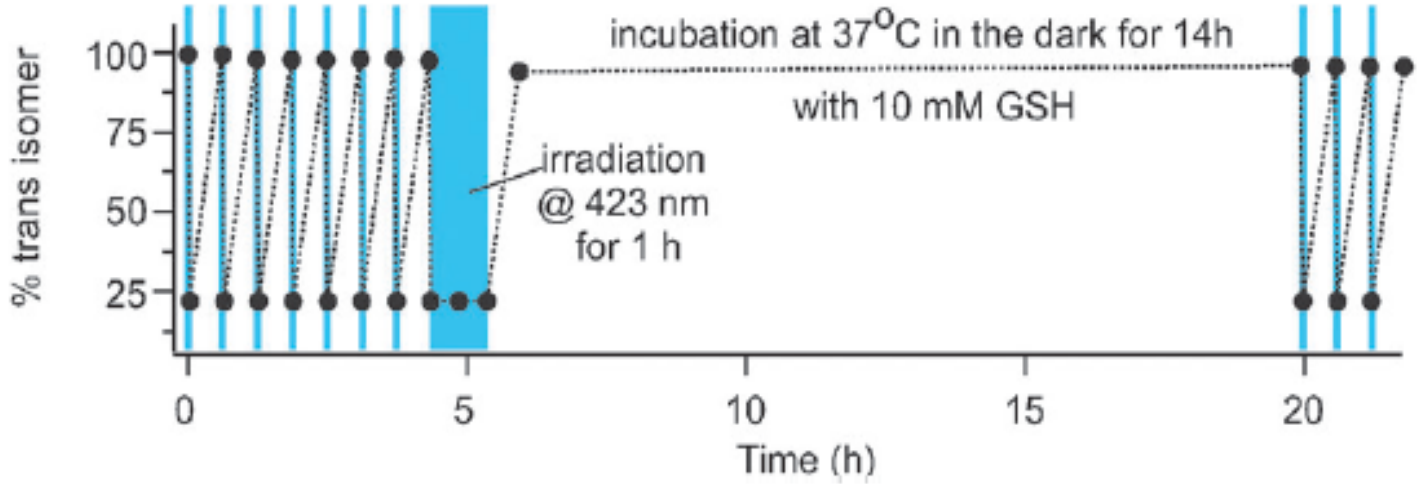
glutathione

- azobenzenes can be prone to reduction by endogenous thiols
- intracellular glutathione concentration: up to 10 mM
- mildly electron donating substituents increase stability

C. Boulègue, M. Löweneck, C. Renner, L. Moroder, *ChemBioChem* **2007**, *8*, 591.

Biological Stability of Azobenzenes II

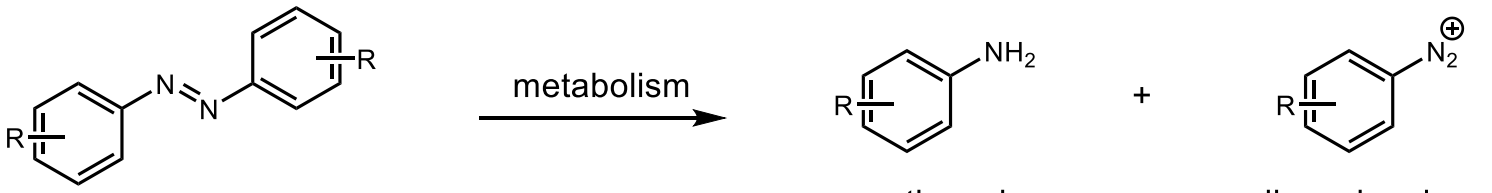




S. Samanta, T. M. McCormick, S. K. Schmidt, D. S. Seferos, G. A. Woolley, *Chem. Commun*. **2013**, *49*, 10314.

Metabolization of Azobenzenes

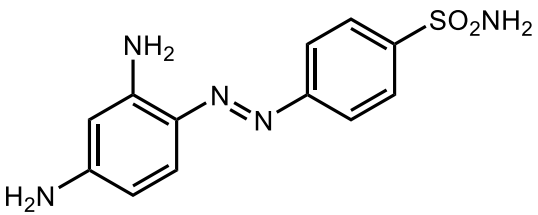
- potentially toxic/carcinogenic metabolites



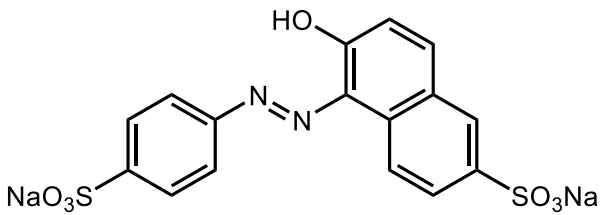
aromatic amines diazonium ions

- however: metabolization depends on structure

- biologically safe azobenzenes are used as approved drugs and food colorants



prontosil
antibacterial drug

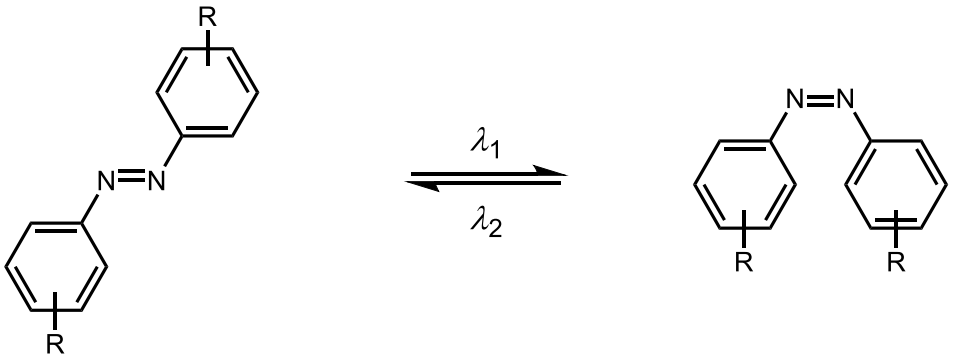


sunset yellow FCF (E110)
used in food, cosmetics, and drugs

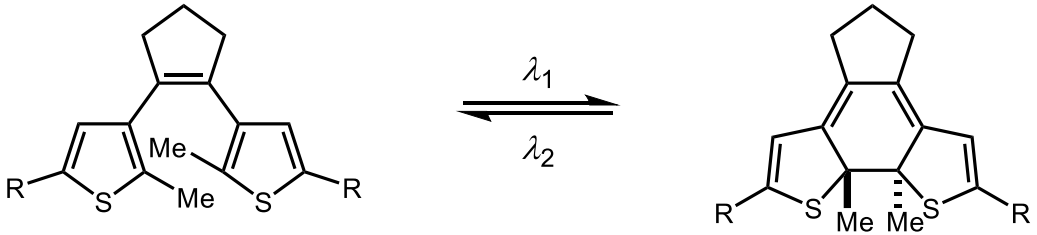
M. A. Brown, S. C. de Vito, *Crit. Rev. Environ. Sci. Technol*. **1993**, *23*, 249.

Photoswitches

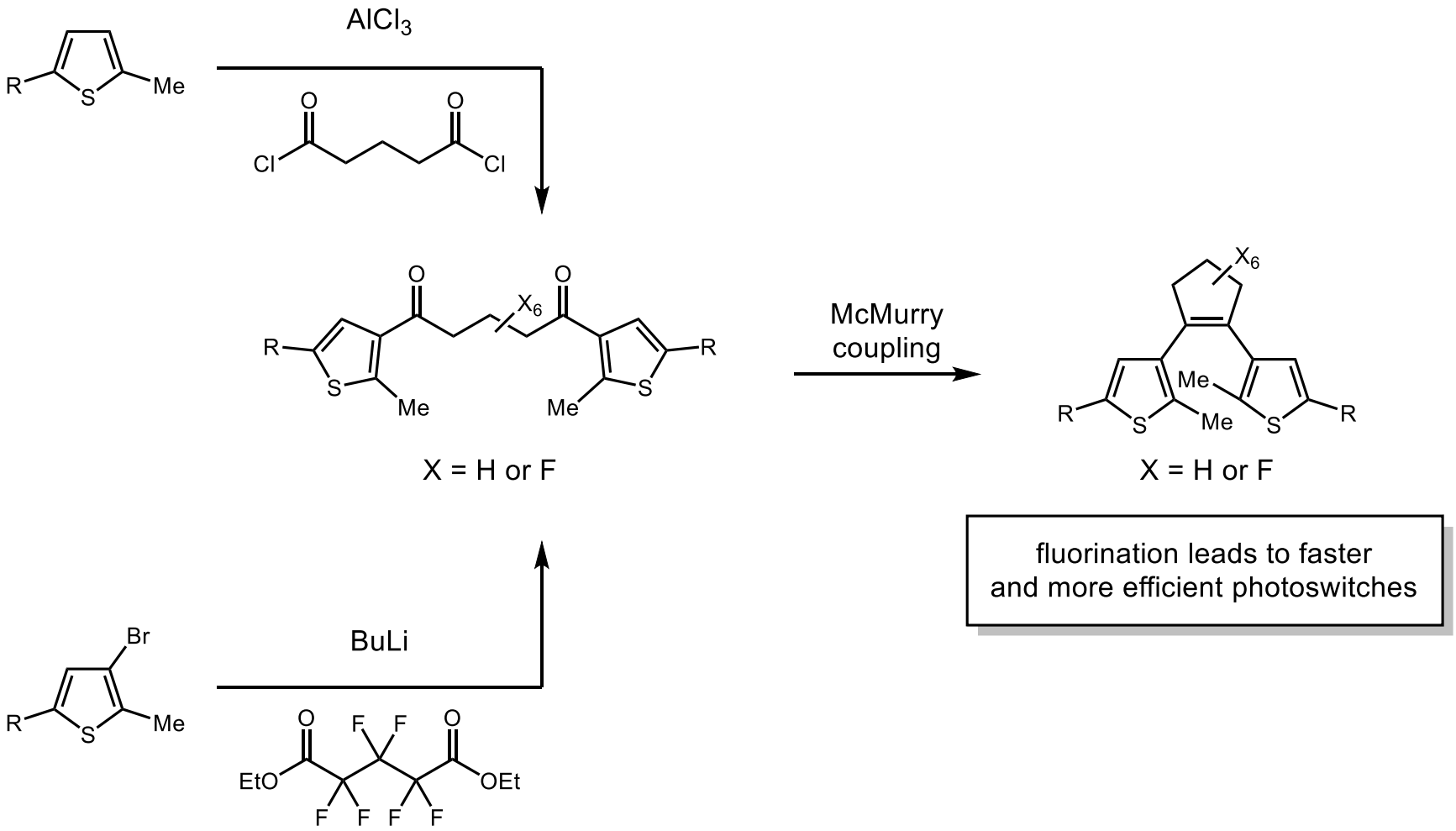
- Azobenzenes



- Diarylethenes



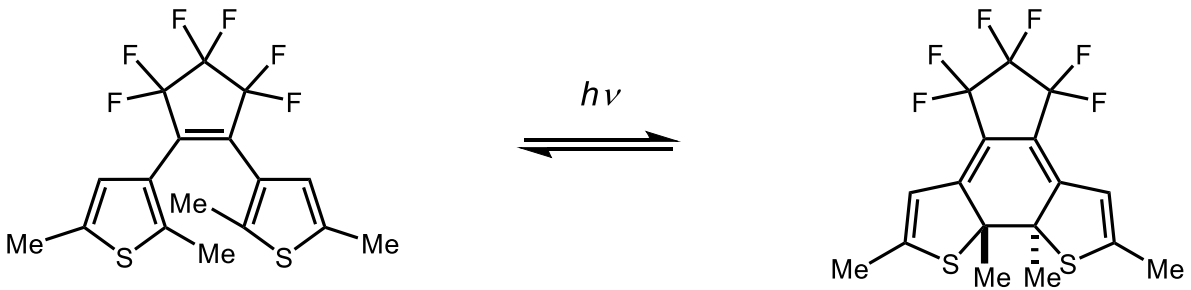
Synthesis of Diarylethenes



fluorination leads to faster
and more efficient photoswitches

L. N. Lucas, J. J. D. de Jong, J. H. van Esch, R. M. Kellogg, B. L. Feringa, *Eur. J. Org. Chem*. **2003**, 155.

Diarylethenes: Properties



λ_{max} = 303 nm

$\Phi_{o \rightarrow c}$ = 0.40 (hexanes)

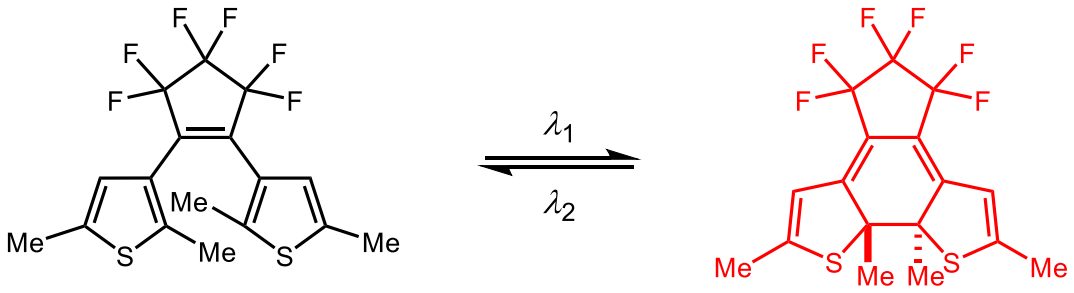
λ_{max} = 505 nm

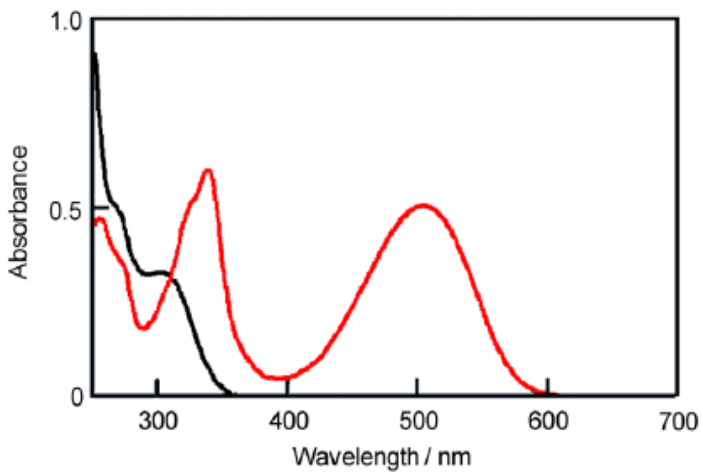
$\Phi_{c \rightarrow o}$ = 0.12 (hexanes)

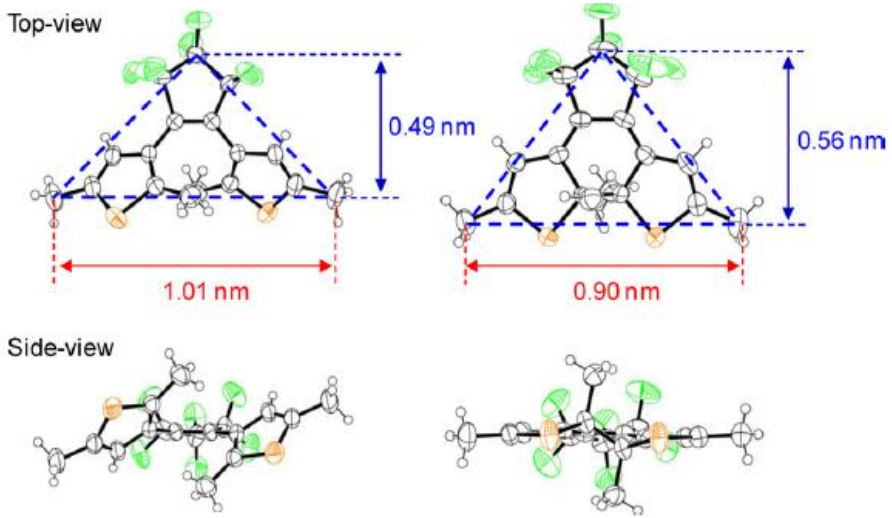
- excited state lifetime: ca. 20 ps
- $t_{1/2}$ (closed state) ≈ 5 × 10^5 a
- bistable photoswitch
- distance change: 1 Å
- dipole moment change: small
- high fatigue resistance (>10^4 switching cycles)

M. Irie, T. Fukaminato, K. Matsuda, S. Kobatake, *Chem. Rev*. **2014**, *114*, 12174.

Photoswitching of Diarylethenes

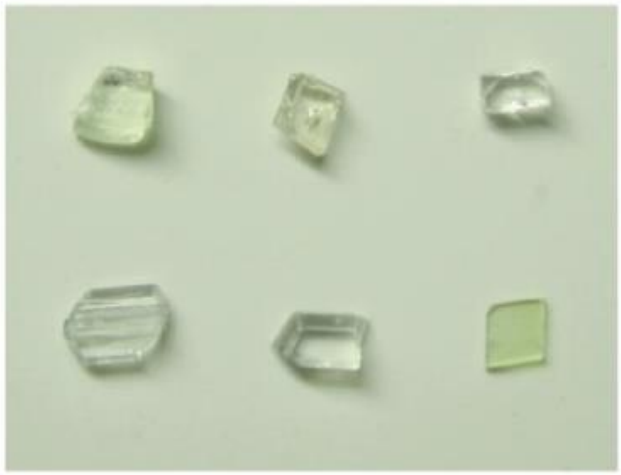






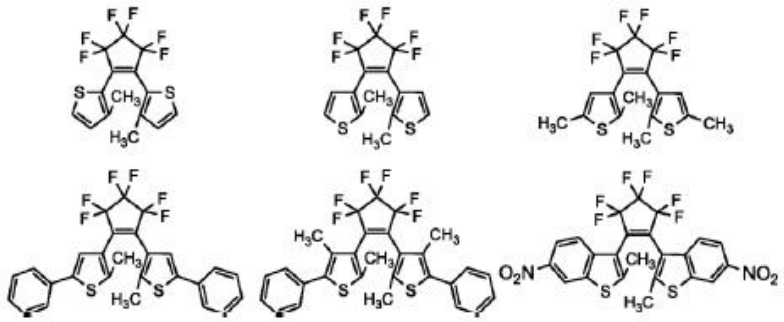
M. Irie, T. Fukaminato, K. Matsuda, S. Kobatake, *Chem. Rev.* **2014**, *114*, 12174.

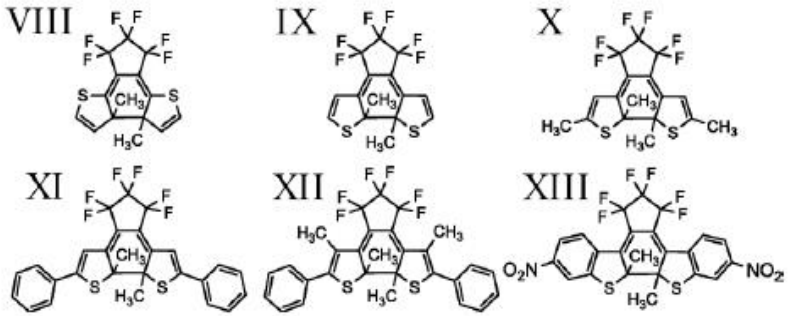
Color-Change of Diarylethene Switches



UV

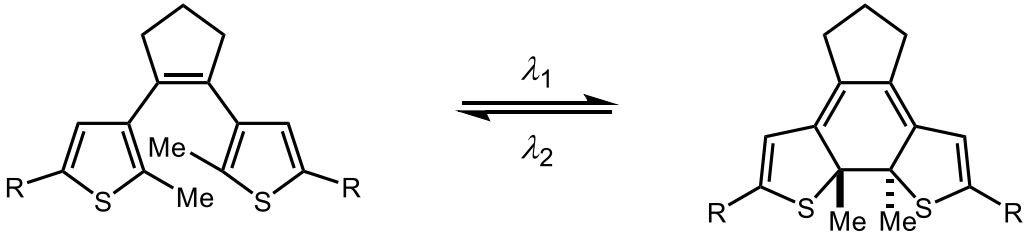
Vis.





M. Irie, T. Fukaminato, K. Matsuda, S. Kobatake, *Chem. Rev*. **2014**, *114*, 12174.

Switching Diarylethenes in both Directions with Visible Light

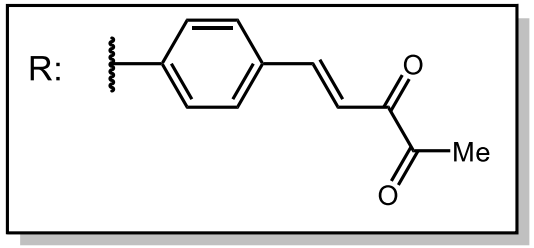


λ_{max} = 405 nm

λ_{max} = 579 nm

$\Phi_{o \rightarrow c}$ = 0.90 (MeCN)

$\Phi_{c \rightarrow o}$ = 0.028 (MeCN)



- biacetyl moiety acts as intramolecular triplet sensitizer
- triplet sensitizer prevents by-product formation from excited singlet state
- just one isomer in photostationary state upon excitation at either absorption maximum

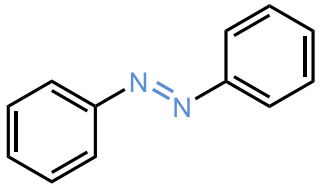
S. Fredrich, R. Göstl, M. Herder, L. Grubert, S. Hecht, *Angew. Chem. Int. Ed*. **2016**, *55*, 1208.

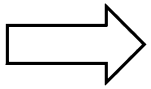
Requirements for Photoswitchable Drugs

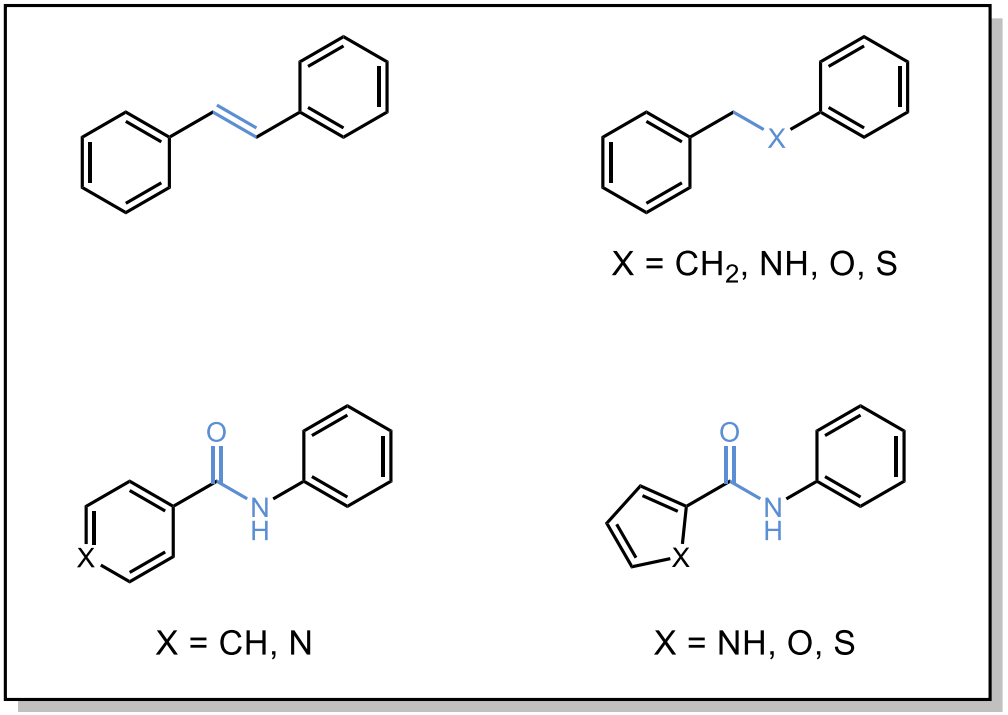
- preferably large change in activity upon photoisomerization
- sufficient concentration change upon irradiation
- photoswitching with red light (high penetration depth, low phototoxicity)
- thermal re-isomerization on suitable time scale
- sufficient metabolic stability
- no general toxicity (drug and its metabolites)
- water soluble in both photoisomeric states

W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc*. **2014**, *136*, 2178.

Design Strategies: Azologization



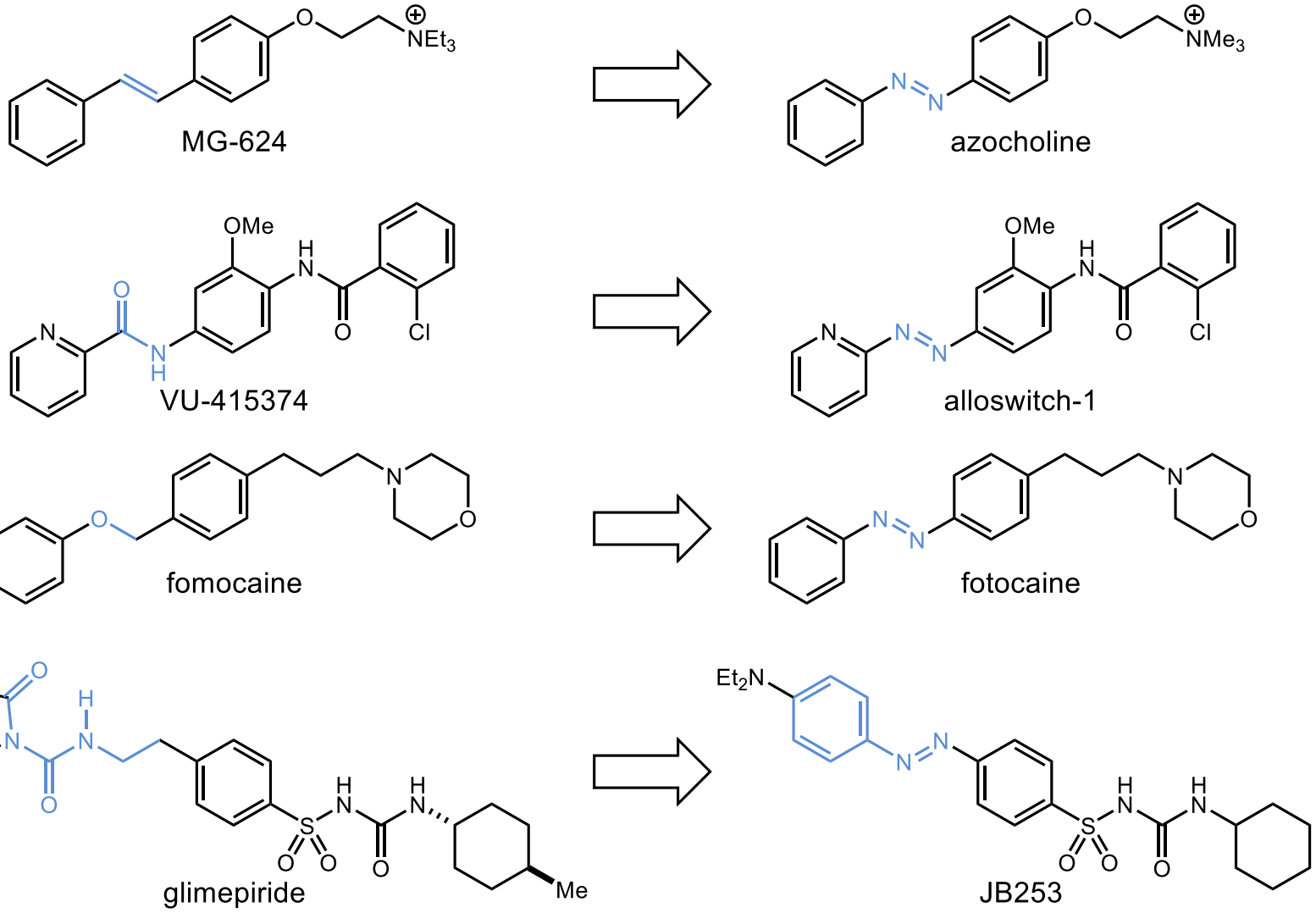




- some functional groups can be considered "azosters"

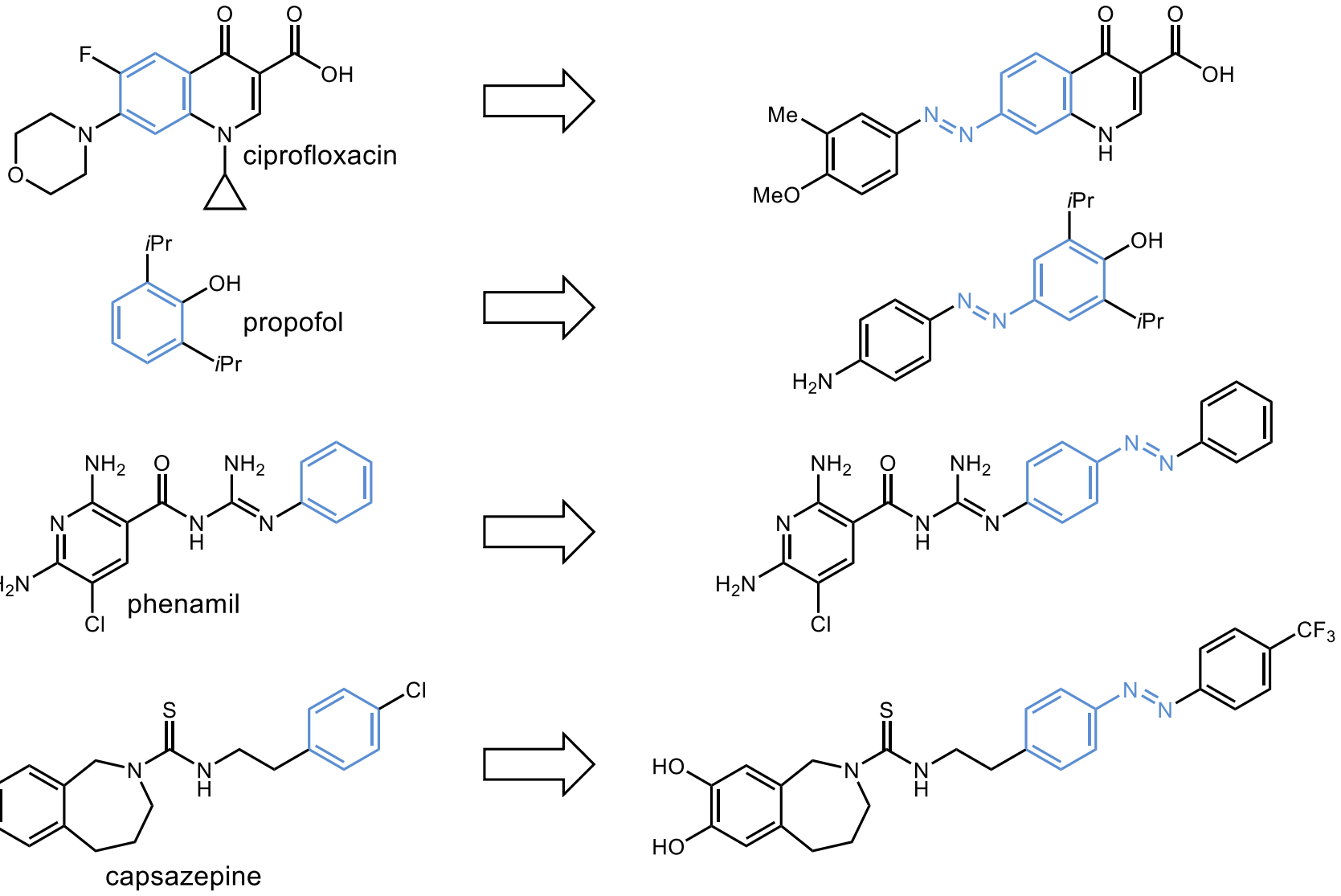
J. Broichhagen, J. A. Frank, D. Trauner, *Acc. Chem. Res*. **2015**, *48*, 1947.

Examples for Azologization Approach



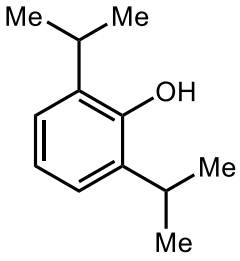
J. Broichhagen, J. A. Frank, D. Trauner, *Acc. Chem. Res.* **2015**, *48*, 1947.

Examples for "Azo-Extension" Approach

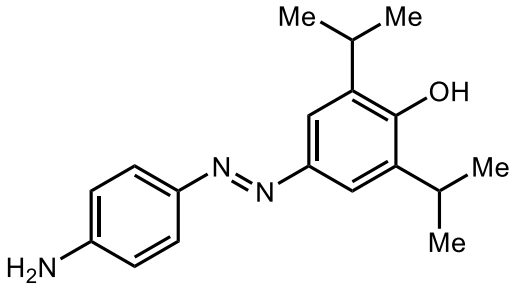


J. Broichhagen, J. A. Frank, D. Trauner, *Acc. Chem. Res.* **2015**, *48*, 1947.

Photoswitchable Anesthetic

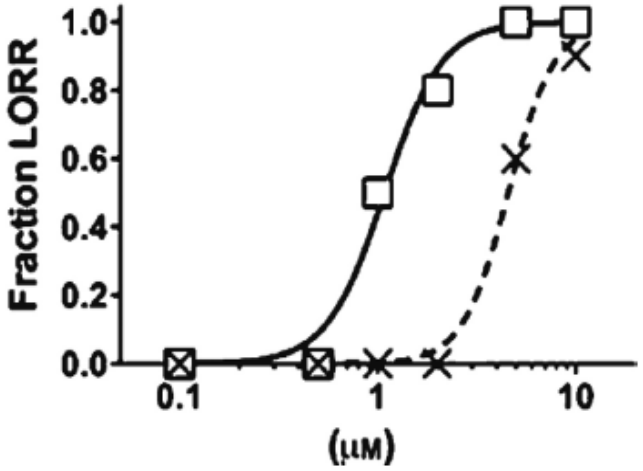


propofol



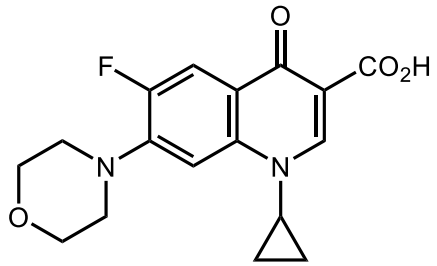
azo-propofol

- propofol: widely used intravenous anesthetic
- mode of action: potentiation of GABA-induced currents
- photoswitching (*trans* → *cis*) at 400 nm followed by rapid thermal isomerisation (*cis* → *trans*)
- EC_{50}(*trans*) = 1.1 μM
- EC_{50}(*cis*) = 4.6 μM
- *Xenopus laevis* showed loss of righting reflex (LORR) when exposed to 3 μM azo-propofol
- spontaneous righting upon UV light exposure

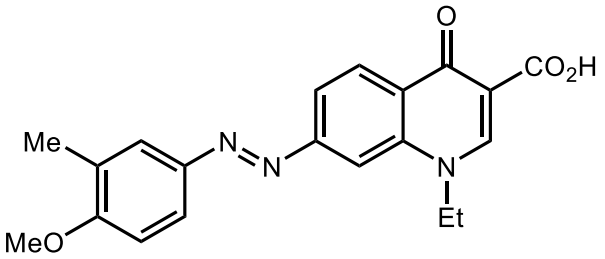


M. Stein, S. J. Middendorp, V. Carta, E. Pejo, D. E. Raines, S. A. Forman, E. Sigel, D. Trauner, *Angew. Chem. Int. Ed.* **2012**, *51*, 10500.

Photoswitchable Antibiotic



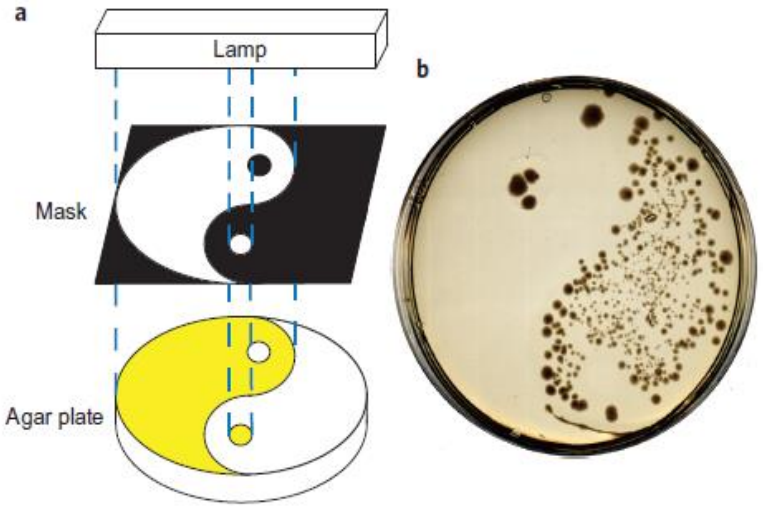
ciprofloxacin



photoswitchable quinolone

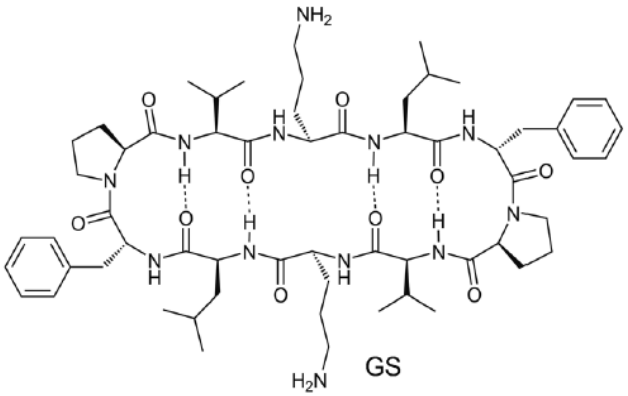
before irradiation: 95% *trans*
after irradiation: 89% *cis*

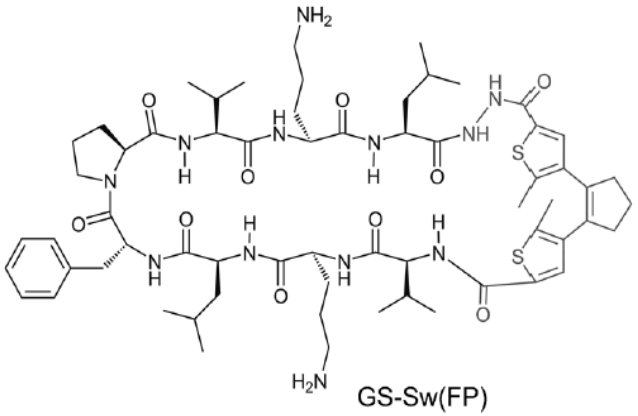
- quinolones: widely used, broad-spectrum antibiotics
- binds to DNA gyrase and blocks DNA replication
- minimal inhibitory concentration (*E. coli*):
  >64 µg/mL (*trans*)
  16 µg/mL (*cis*)
- antibiotic is activated at 365 nm
- loss of activity after 3 h (auto-inactivation)
- activation at 365 nm (50 µg/mL) inhibits growth of *E. coli*



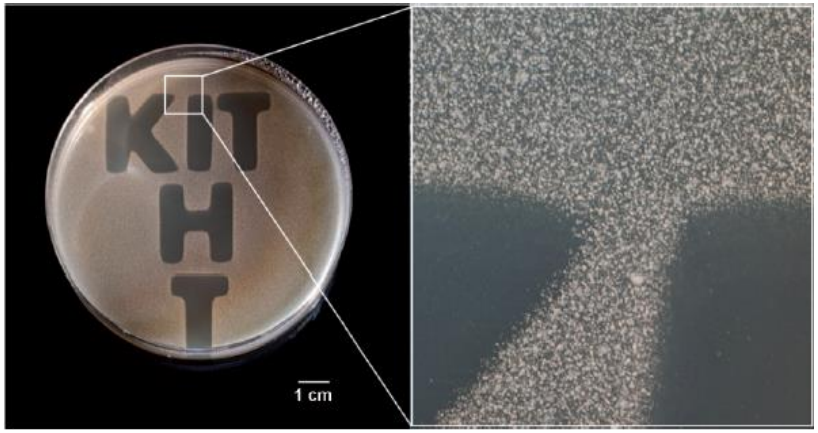
W. A. Velema, J. P. van der Berg, M. J. Hansen, W. Szymanski, A. J. M. Driessen, B. L. Feringa, *Nat. Chem*. **2013**, *5*, 924.

Photoswitchable Gramicidin S





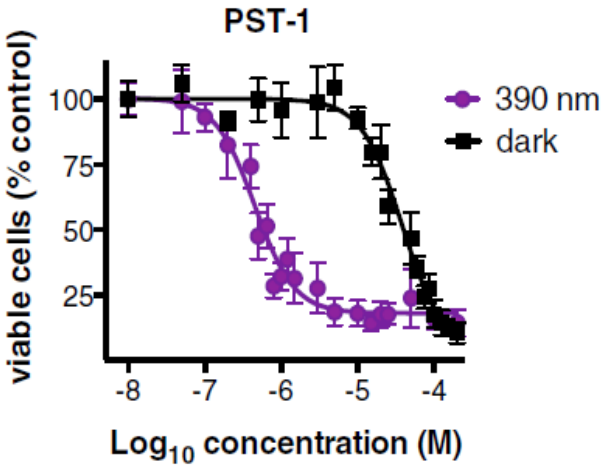
- gramicidin S (GS): antimicrobial cyclic peptide
- amphiphilic structure leads to membrane permeabilization
- open form is almost as active as GS
- closed form is significantly less active
- photoswitching with UV (λ = 256 nm) and visible light (λ = 530 nm)
- closed form (8 μg/mL) can be activated with visible light to inhibit growth of *S. xylosus*

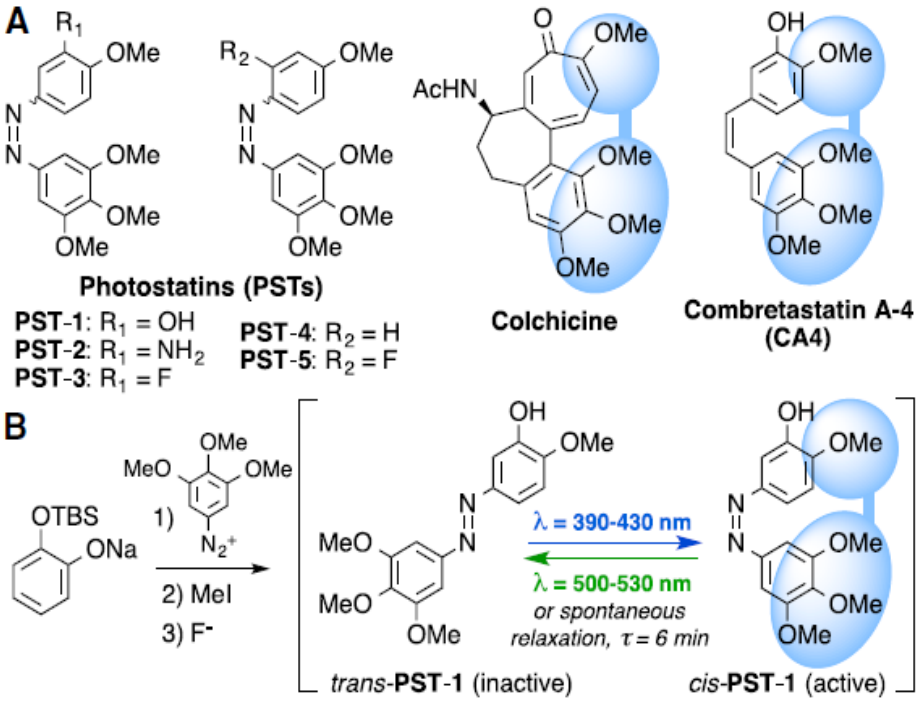


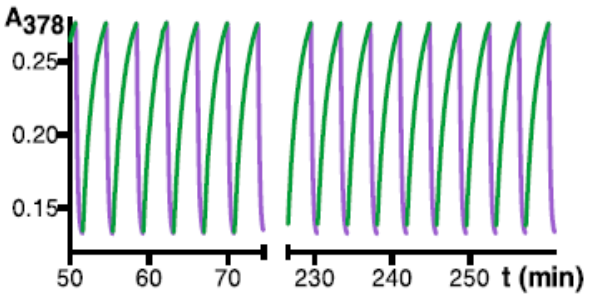
O. Babii, *et al*., *Angew. Chem. Int. Ed*. **2014**, *53*, 3392.

Photostatins: Optical Control of Mitosis and Cell Death I

- microtubules: dynamic polymer of the cytoskeleton
- tubulin polymerization inhibitors can induce cell death (→ cancer chemotherapy)
- current inhibitors are nonspecific
- irradiation of PST-1 at 380-420 nm results in 90% *cis*-isomer
- EC_{50}(dark) = 38 µM
- EC_{50}(390 nm) = 0.5 µM

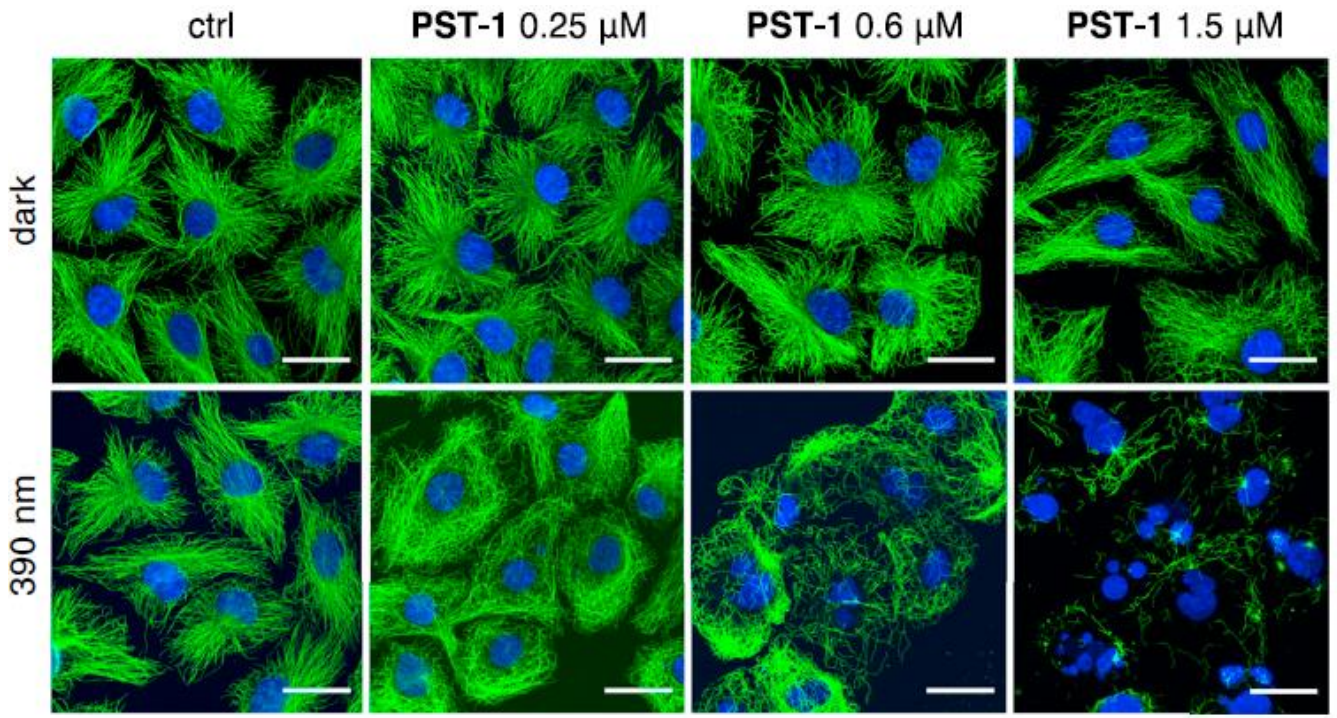






M. Borowiak, et al., *Cell* **2015**, *162*, 403.

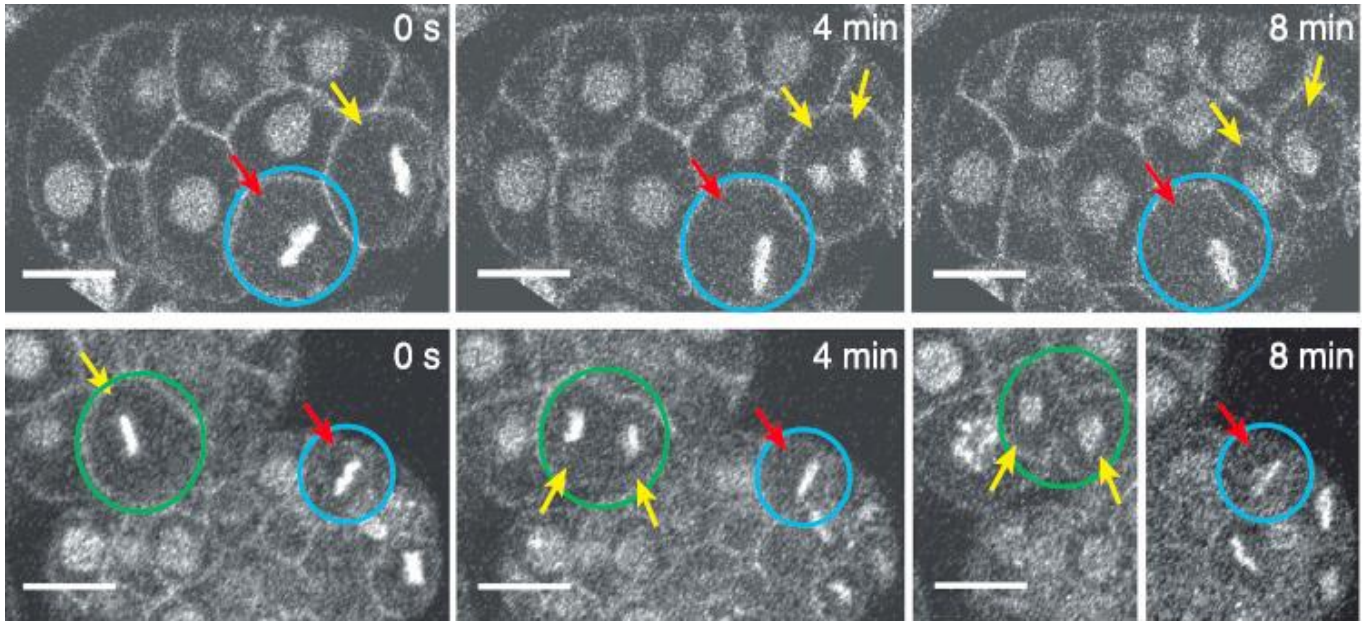
Photostatins: Optical Control of Mitosis and Cell Death II



- human breast cancer cell line was incubated for 20 h
- no toxicity in the dark
- *cis*-PST-1 induces mircrotubule breakdown and nuclear fragmentation above 0.6 µM

M. Borowiak, et al., *Cell* **2015**, *162*, 403.

Photostatins: Optical Control of Mitosis and Cell Death III

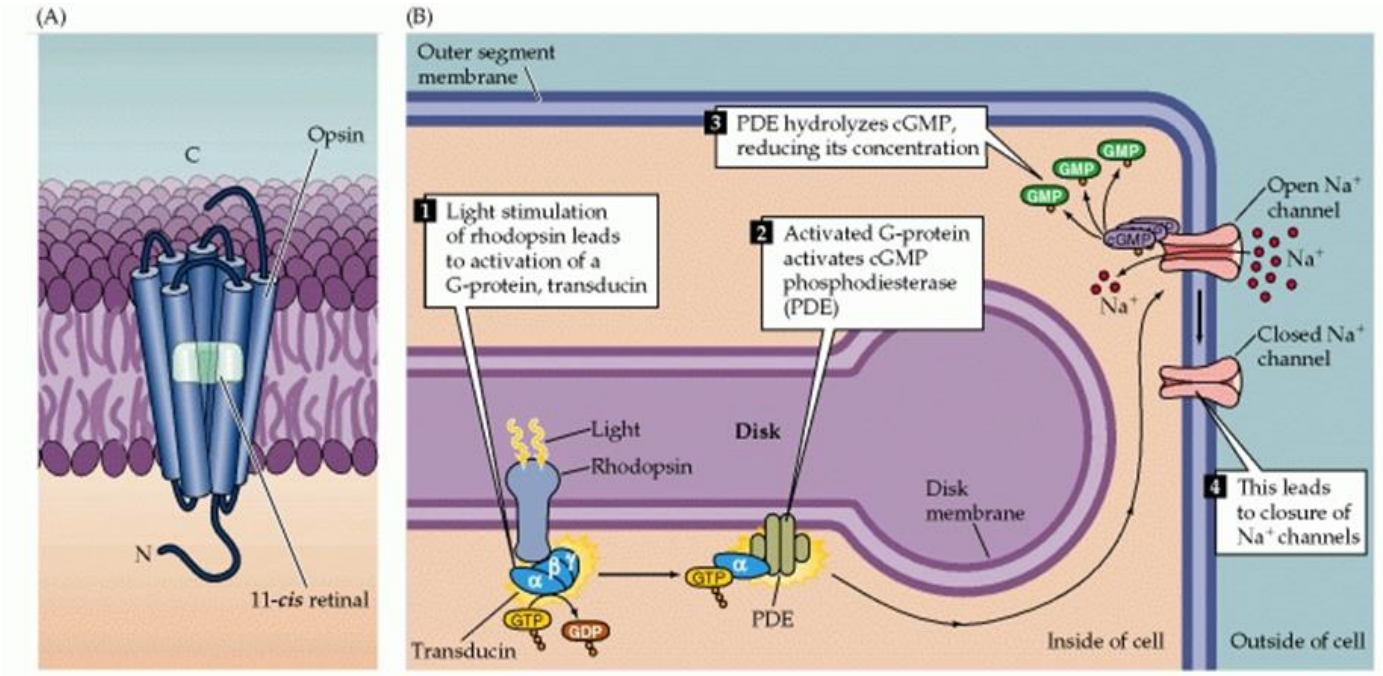


- photoswitch enables fully reversible optical control over mitosis
- *C. elegans* embryos were incubated with 40 µM PST-1
- illumination at 405 nm induced mitotic arrest
  (blue circle, stationary chromosomes)
- illumination at 405 nm followed by 514 nm had no effect
  (rescue protocol, green circle)
- single-cell spatial resolution

M. Borowiak, et al., *Cell* **2015**, *162*, 403.

Restoration of Visual Function

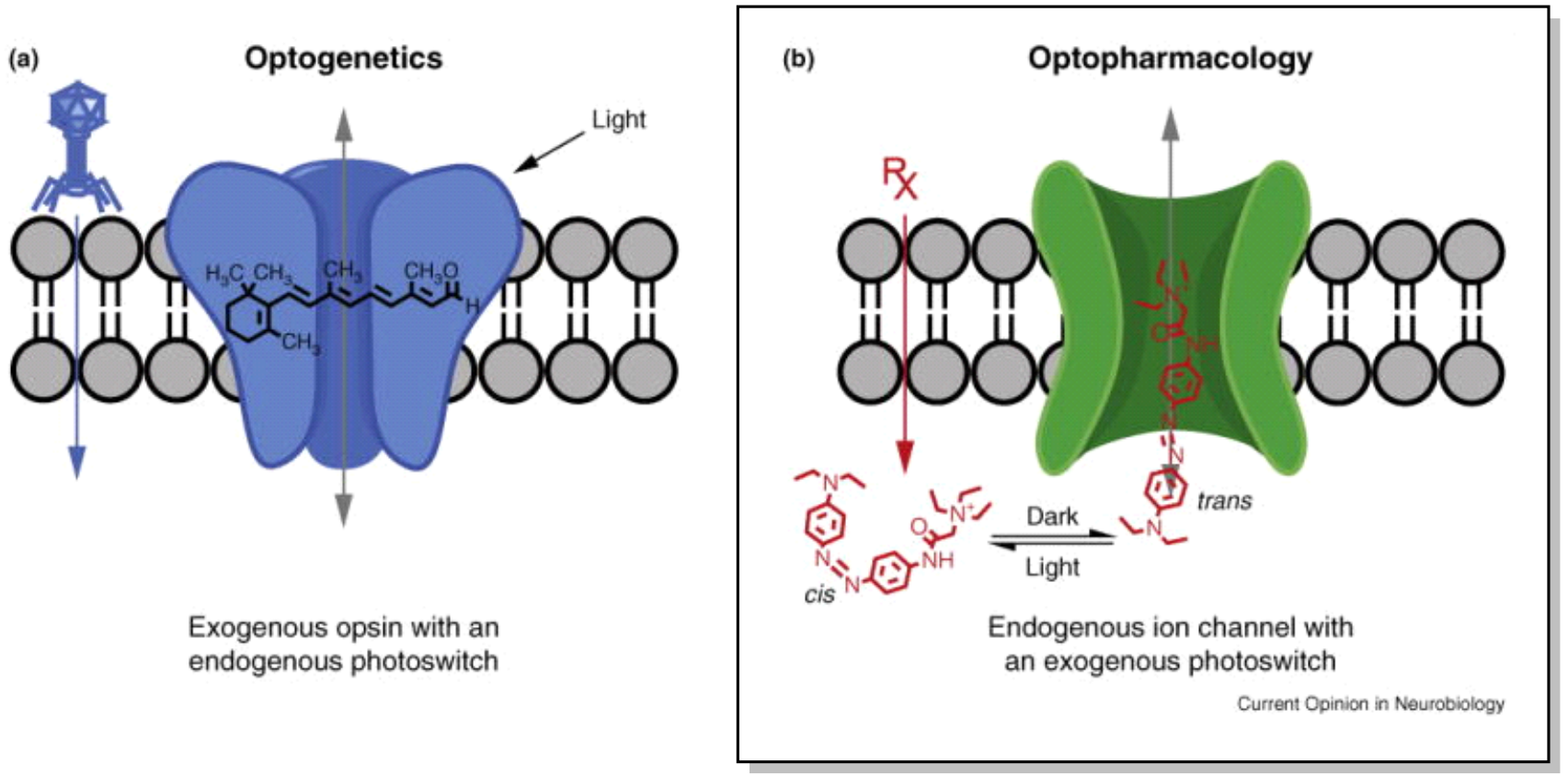
Visual Phototransduction



- visible light induces isomerization of 11-*cis* retinal
- signaling cascade leads to decrease in cGMP concentration
- low cGMP concentration leads to closure of ion channel
  $\rightarrow$ change in electric membrane potential
- disruption of signaling cascade results in blindness
- theorectically, visual function can be restored by direct photoswitching of ion channels

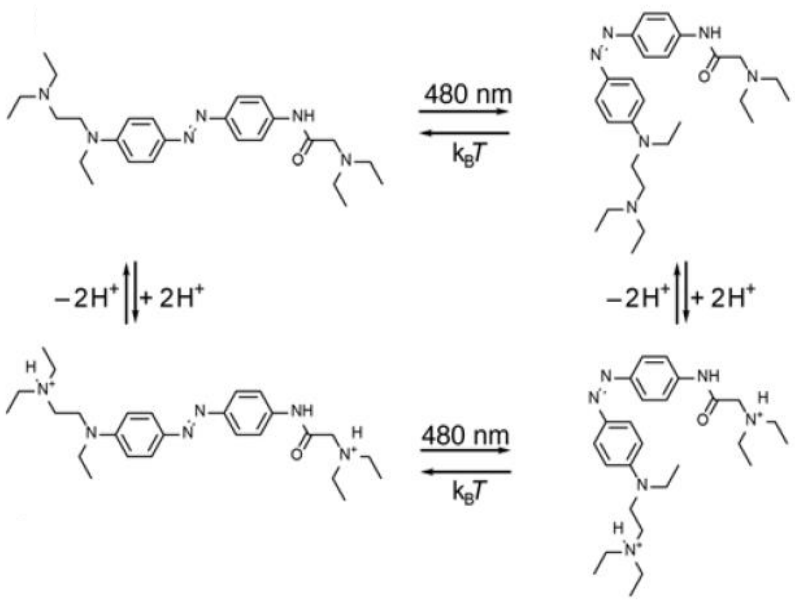
D. Purves, G. J. Augustine, D. Fitzpatrick, *et al.*, *Neuroscience* **2001**, 2nd edition.

Strategies for the Restoration of Visual Function

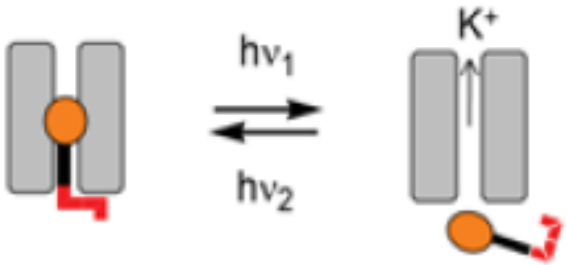


I. Tochitsky, R. H. Kramer, *Curr. Opin. Neurobiol*. **2015**, *34*, 74.

Photopharmacological Control of Bipolar Cells I

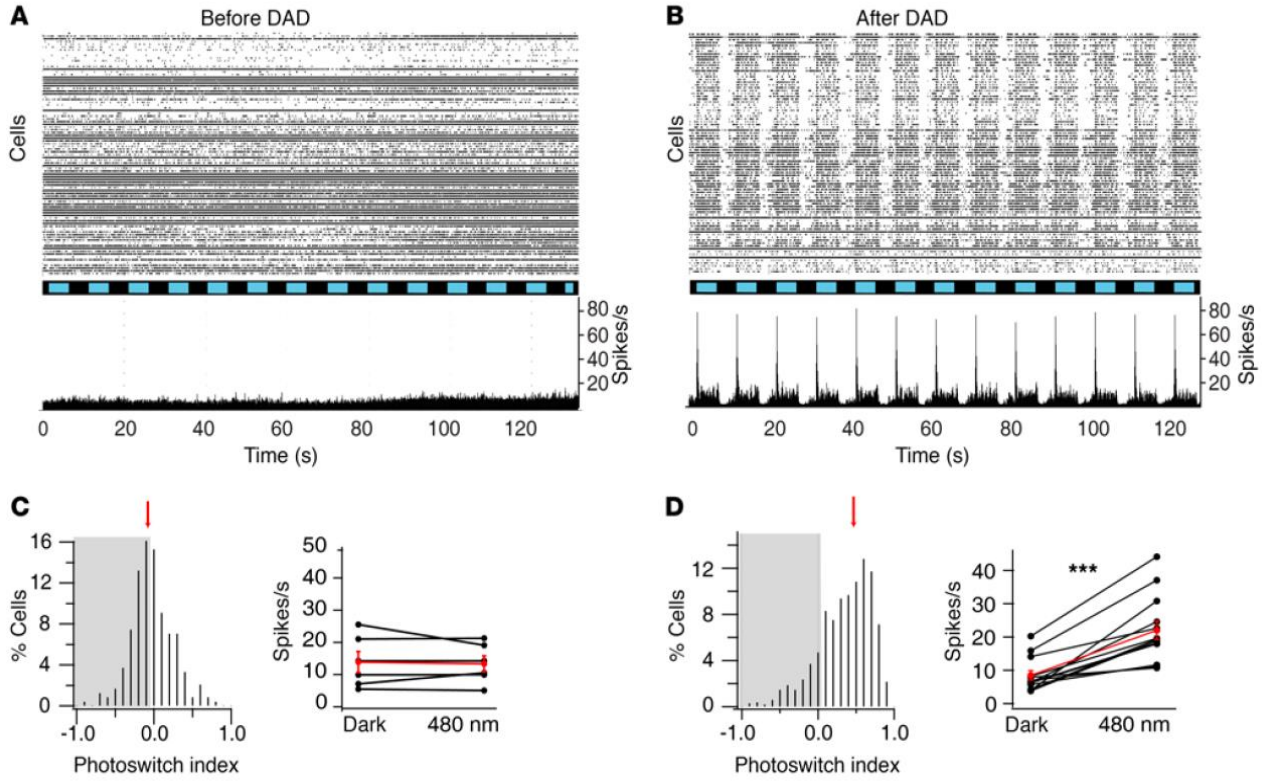


- isomerization to *cis* form with visible light (480 nm)
- thermal relaxation to trans form with τ = 33 ms (DMSO)
- uncharged form can cross membranes
- charged form blocks voltage-gated K^+ channels in *trans* form
- blue light leads to unblocking of ion channels



L. Laprell, *et al*., *J. Clin. Invest*. **2017**, *in press*.

Photopharmacological Control of Bipolar Cells II



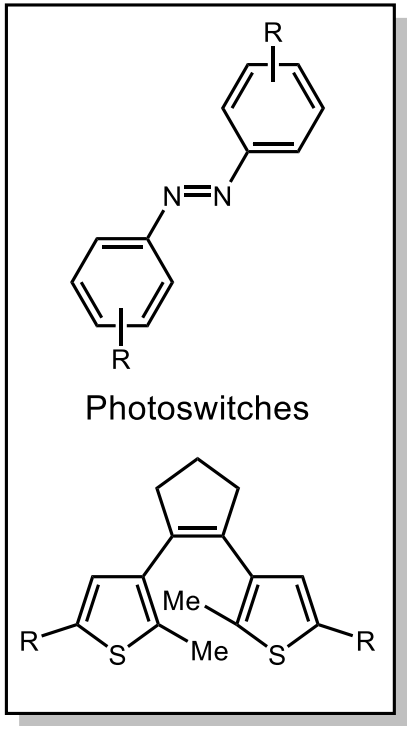
- retinas from blind mice as *ex vivo* model
- genetic knockout leads to loss of photoreceptors
- photoswitch index (PI): normalized change of firing rate upon illumination

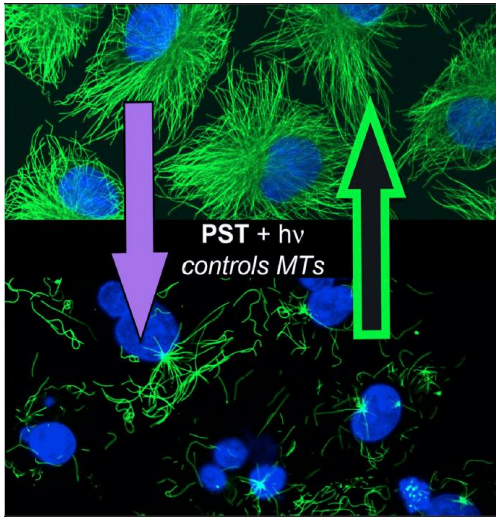
- PI(wild type) = 0.65
- 200 µM photoswitch increased PI from -0.06 to 0.42

L. Laprell, *et al*., *J. Clin. Invest*. **2017**, *in press*.

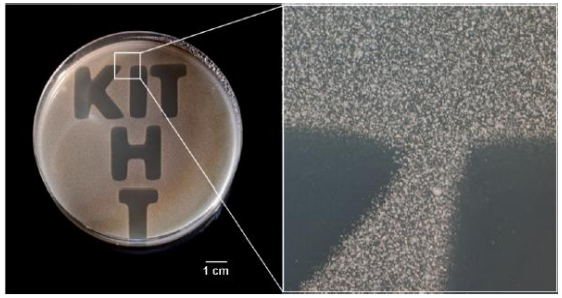
Photopharmacology

Restoration of
Visual Function





Optical Control of
Mitosis and Cell Death



Photoswitchable
Antibiotics