MAX PLANCK INSTITUTE
FOR MEDICAL RESEARCH

Azobenzene Photoswitches for Optical Control of Neuronal Excitability

MtL Class Chemical Biology

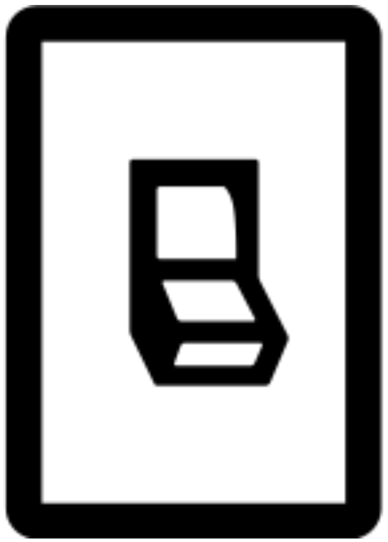
Department of Chemical Biology
Max Planck Institute for Medical Research, Heidelberg

Institute of Chemical Sciences and Engineering ISIC
EPFL Lausanne, Switzerland

Photopharmacology

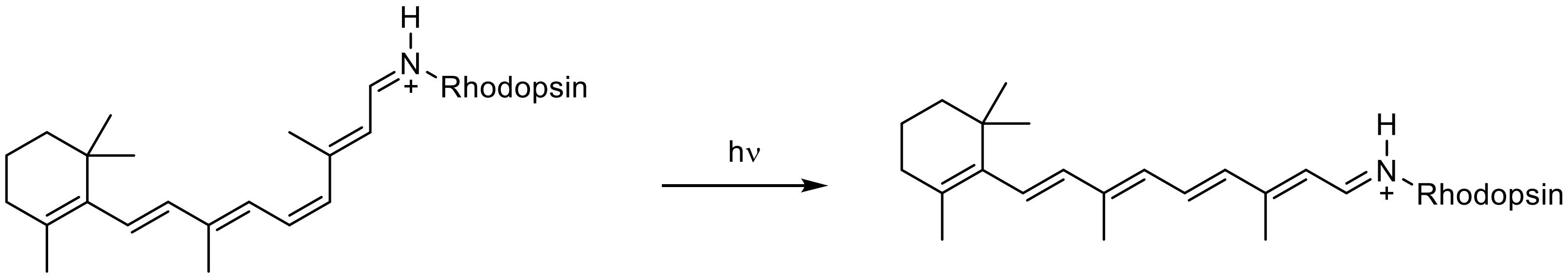
Rewiring biology to respond to light

OFF



ON

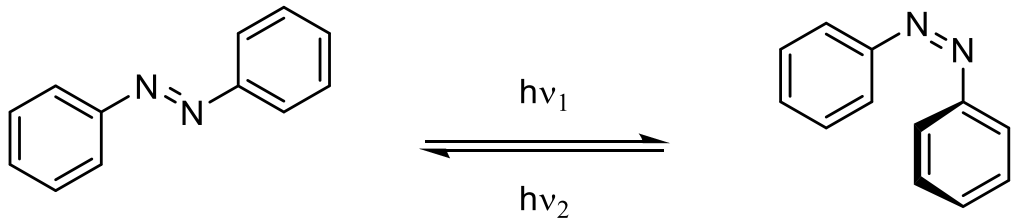
Retinal – Nature‘s photoswitch



11-*cis*-retinilidene

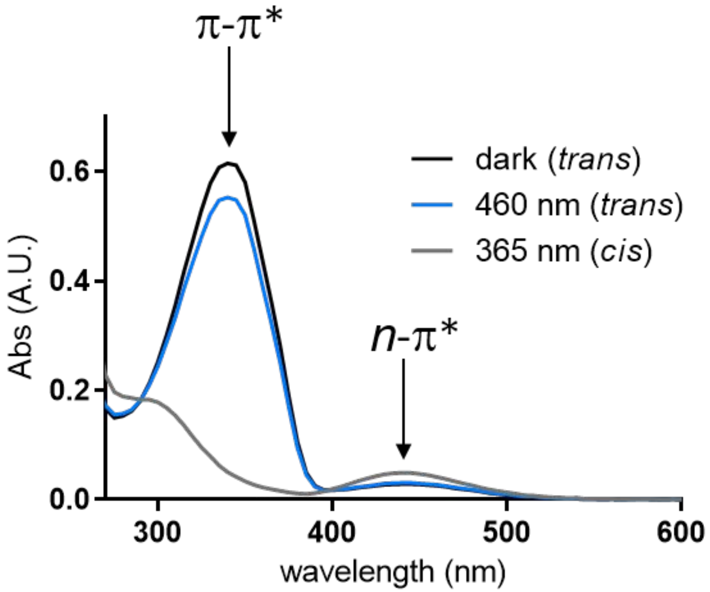
all-*trans*-retinilidene

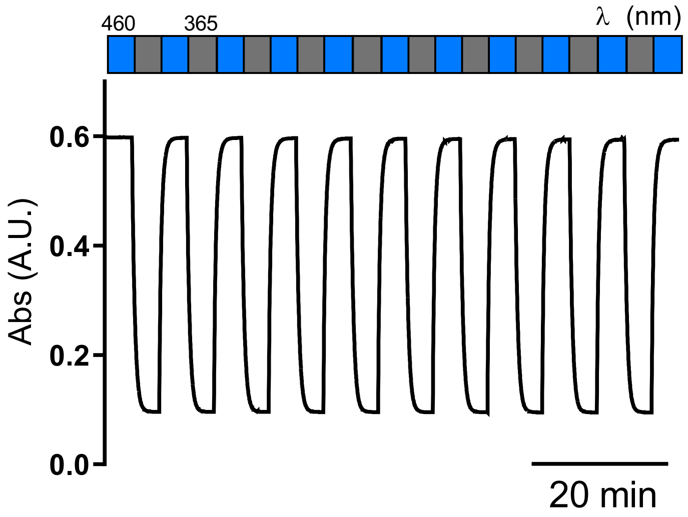
Azobenzene – A synthetic photoswitch

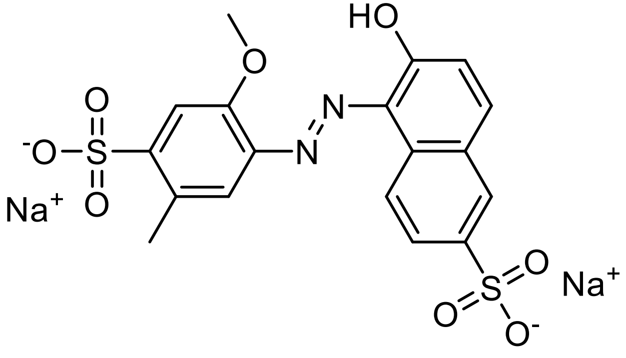


trans-azobenzene *cis*-azobenzene

$\lambda_{opt.\ cis}$ = 365 nm (MeCN)
$t_{½}$ ≈ 2 days (MeCN)
$\Delta d_{4,4'}$ ≈ 3 Å

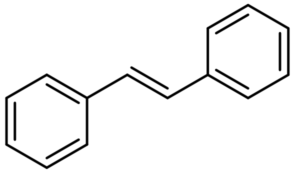




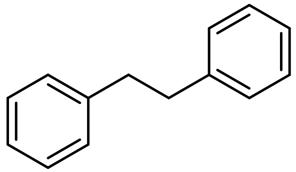


Allura Red AC

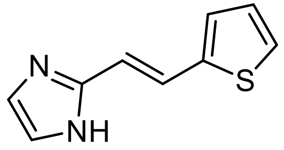
Azologization of common pharmacophores (“Azosters“)



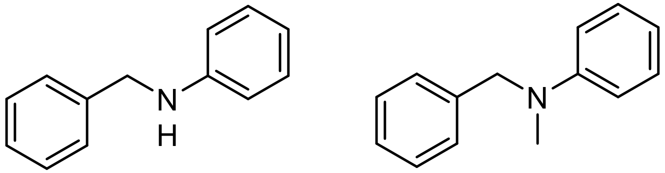
stilbene



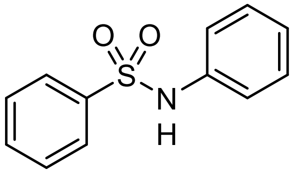
1,2-diarylethane



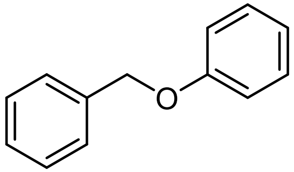
heterocyclic stilbene



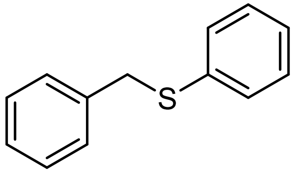
N-benzyl aniline



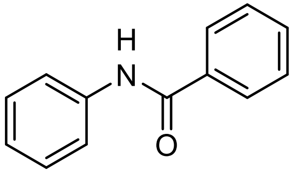
biaryl sulfonamide



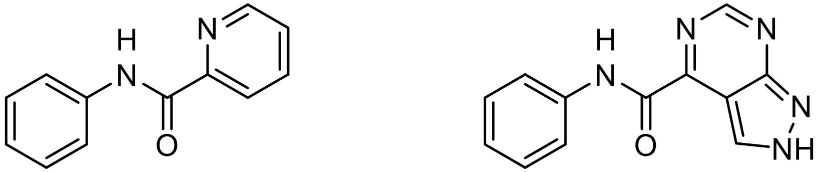
benzyl phenyl ether



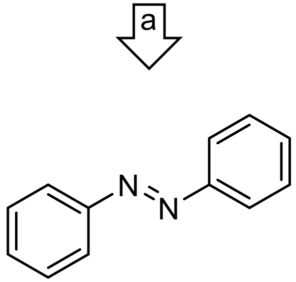
benzyl phen thioether



N-aryl benzamide

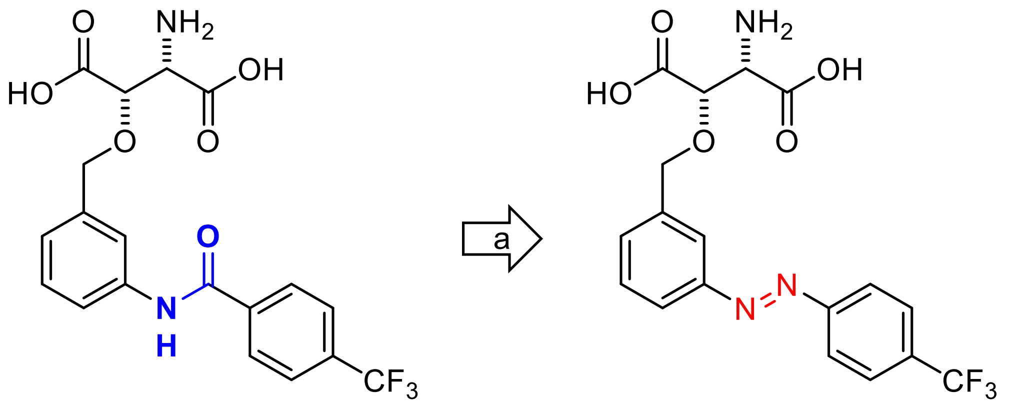


heterocyclic *N*-aryl benzamide

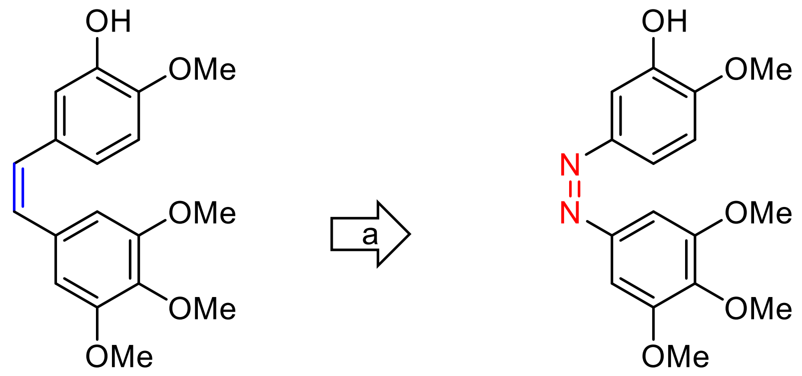


trans-azobenzene

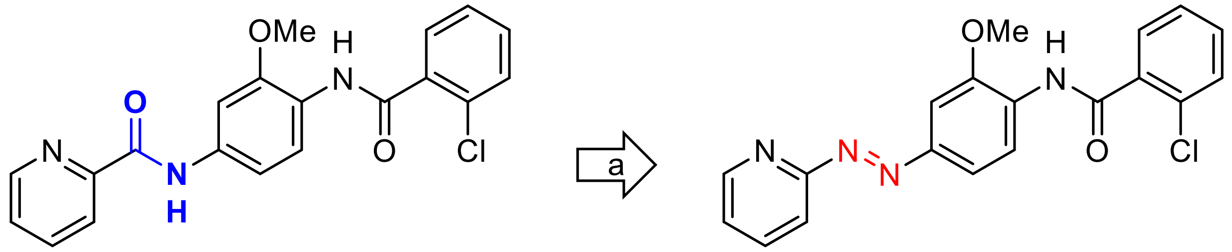
Azosters - Examples



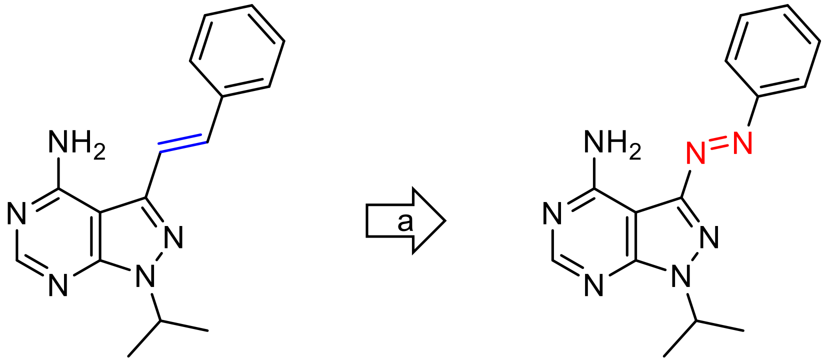
TFB-TBOA **ATT** (EAAT)



Combretastatin A-4 **Photostatin-1** (Microtubules)



VU-415374 **Alloswitch-1** ($mGlu_5$)



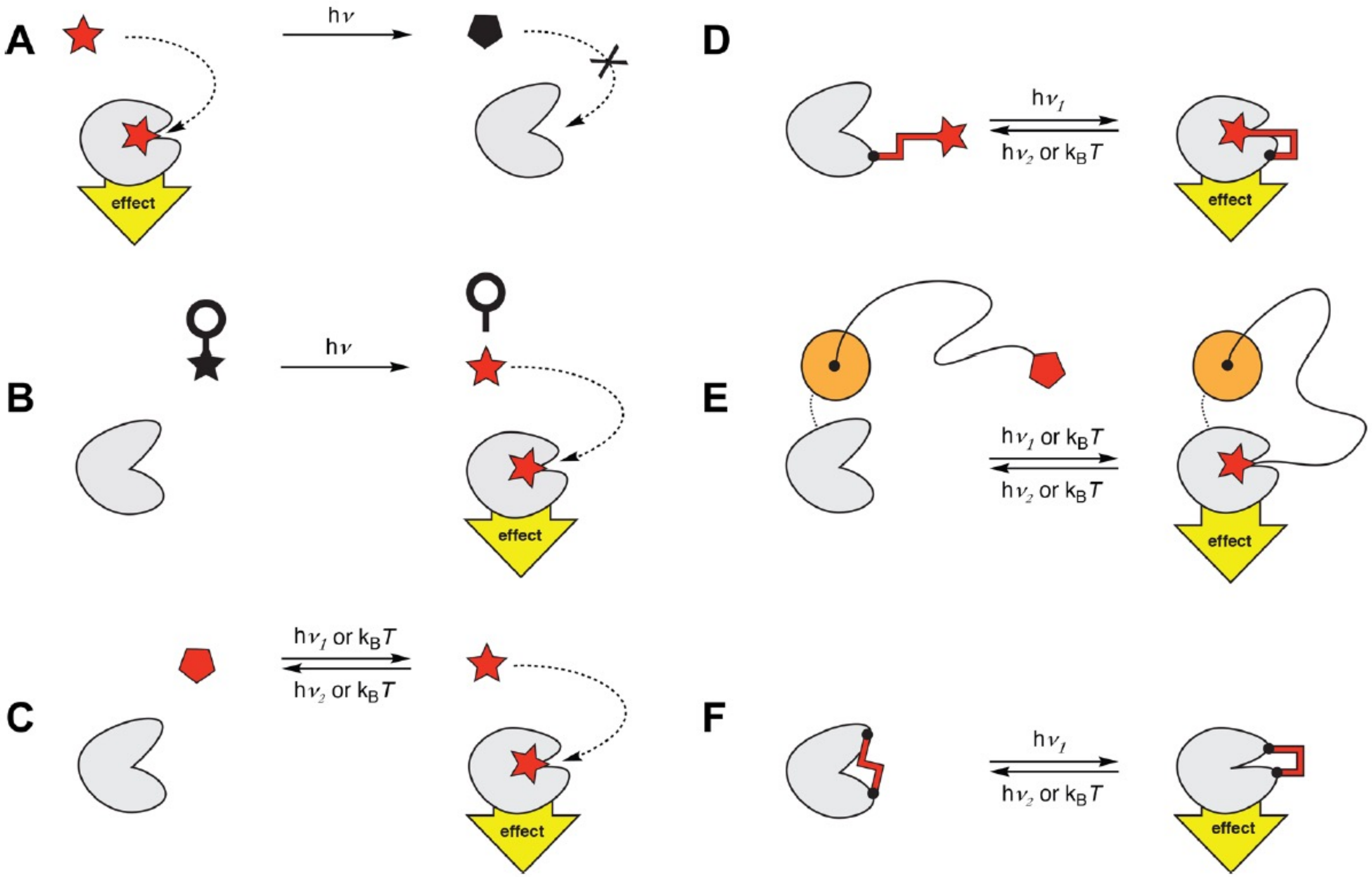
cmpd. **9** cmpd. **4** (RET kinase)

Modalities of Photopharmacology

Irreversible photoinactivation

Irreversible photoactivation (photo-uncaging)

Photochromic Ligand (PCL)



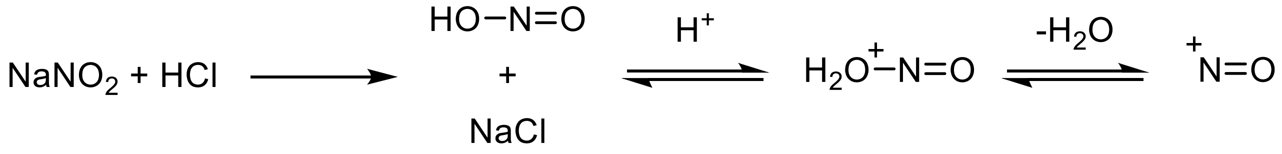
Photoswitchable Tethered Ligand (PTL)

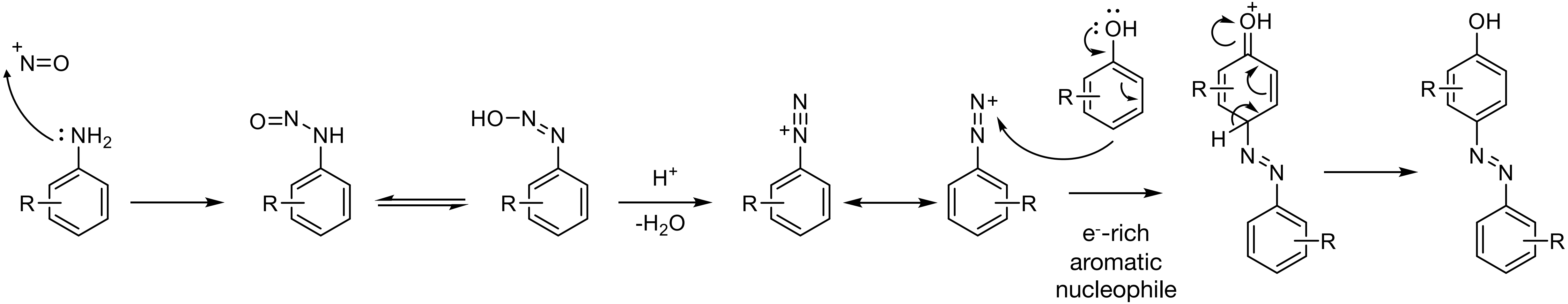
Photoswitchable Orthogonal Remotely Tethered Ligand (PORTL)

Photoswitchable Crosslinker

Chem. Rev. 2018, 118, 21, 10710-10747

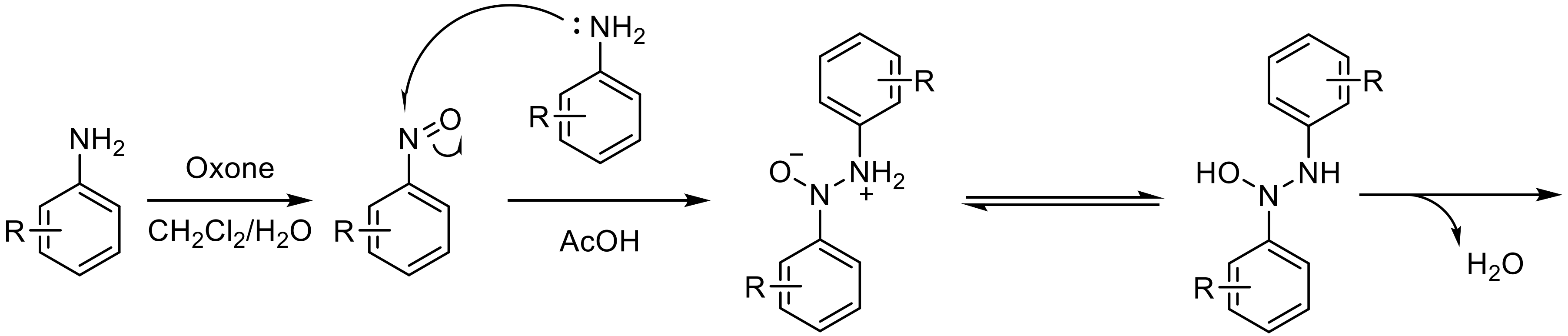
Synthesis of azobenzenes – Azo coupling





Chem. Soc. Rev., 2011,40, 3835–3853

Synthesis of azobenzenes – Baeyer-Mills reaciton



Optical control of ion channels

nature neuroscience

Nat. Neurosci. 7, 1381–1386 (2004).

Light-activated ion channels for remote control of neuronal firing

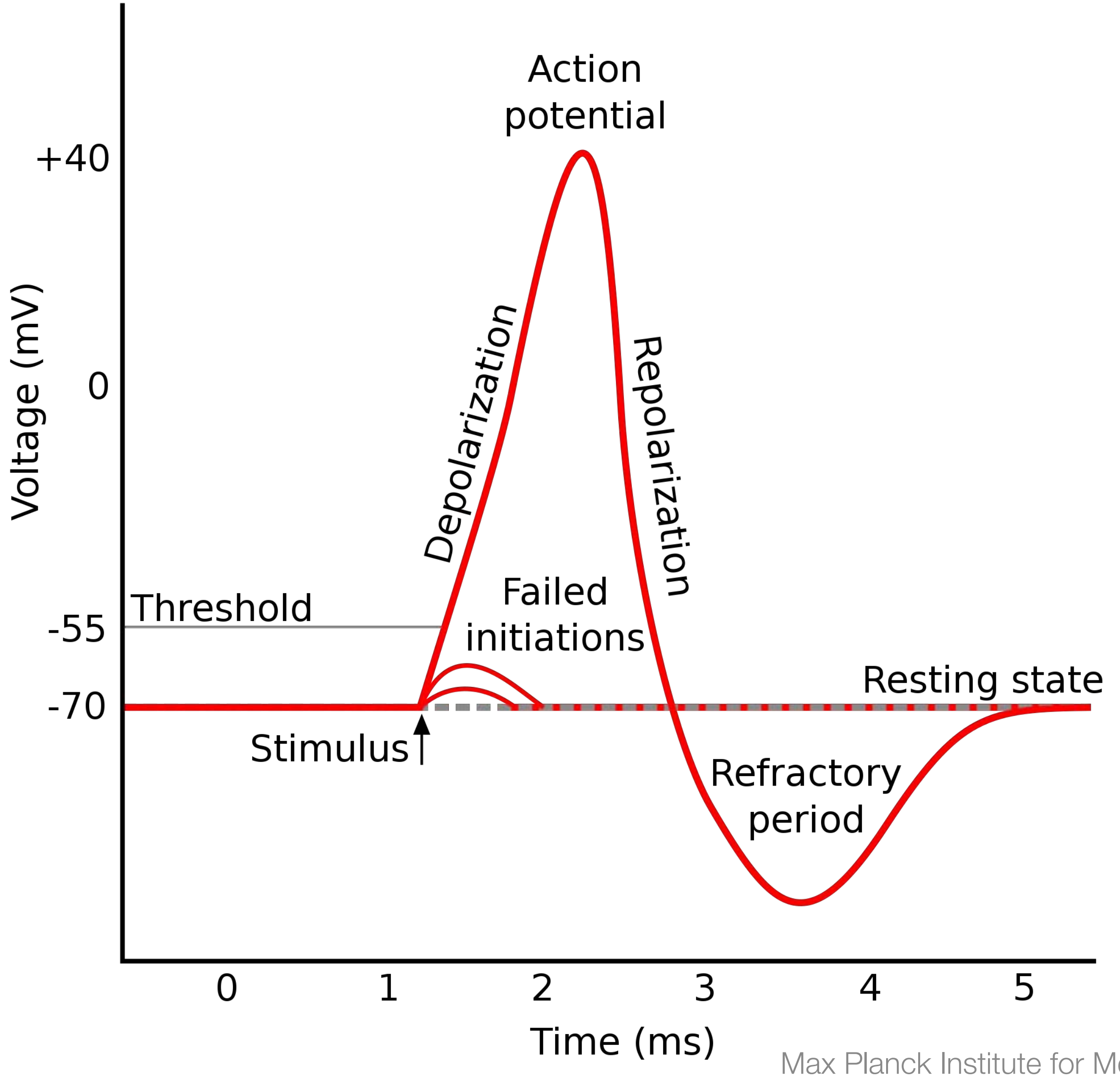
Matthew Banghart[1,3], Katharine Borges[2,3], Ehud Isacoff[2], Dirk Trauner[1] & Richard H Kramer[2]

Neurons have ion channels that are directly gated by voltage, ligands and temperature but not by light. Using structure-based design, we have developed a new chemical gate that confers light sensitivity to an ion channel. The gate includes a functional group for selective conjugation to an engineered K^+ channel, a pore blocker and a photoisomerizable azobenzene. Long-wavelength light drives the azobenzene

Many techniques exist for controlling neural activity, but they all have considerable limitations. Traditional electrical and chemical methods require invasive electrodes or chemical delivery systems that cannot control patterns of activity in densely packed neural tissue. Optical techniques utilizing caged neurotransmitters[5,6] are less invasive and can be more precise, but reversal of the effects of the uncaged transmitter is limited by its diffusion kinetics. Recently,

Voltage-gated K^+ ion channel → Light-gated K^+ ion channel

Background: Action potential (AP) firing in neurons



- → Depolarization driven by activation of voltage-gated sodium channels (influx of Na^{+} ions)
- → Activation of voltage-gated potassium channels at peak
- → Efflux of K^{+} ions driving force for repolarization and hyperpolarization

Optical control of ion channels

nature neuroscience

Nat. Neurosci. 7, 1381–1386 (2004).

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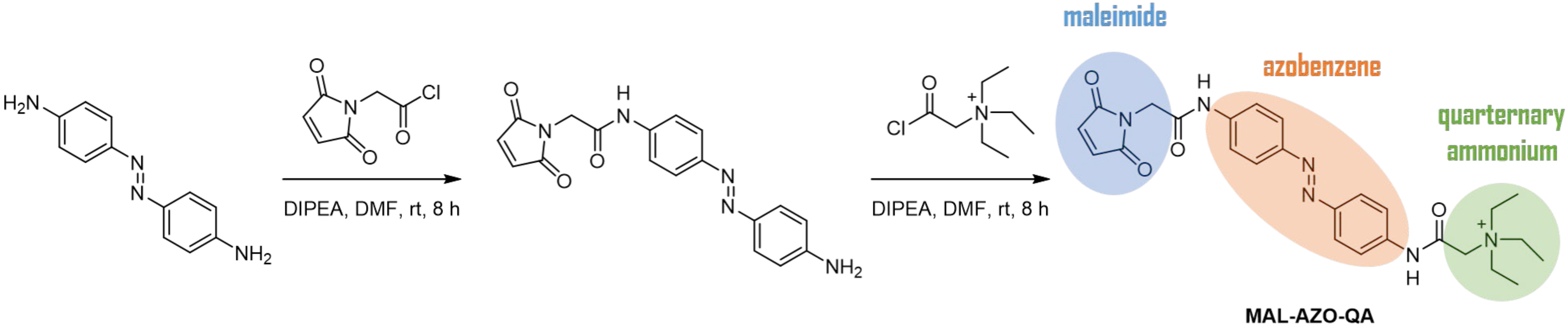
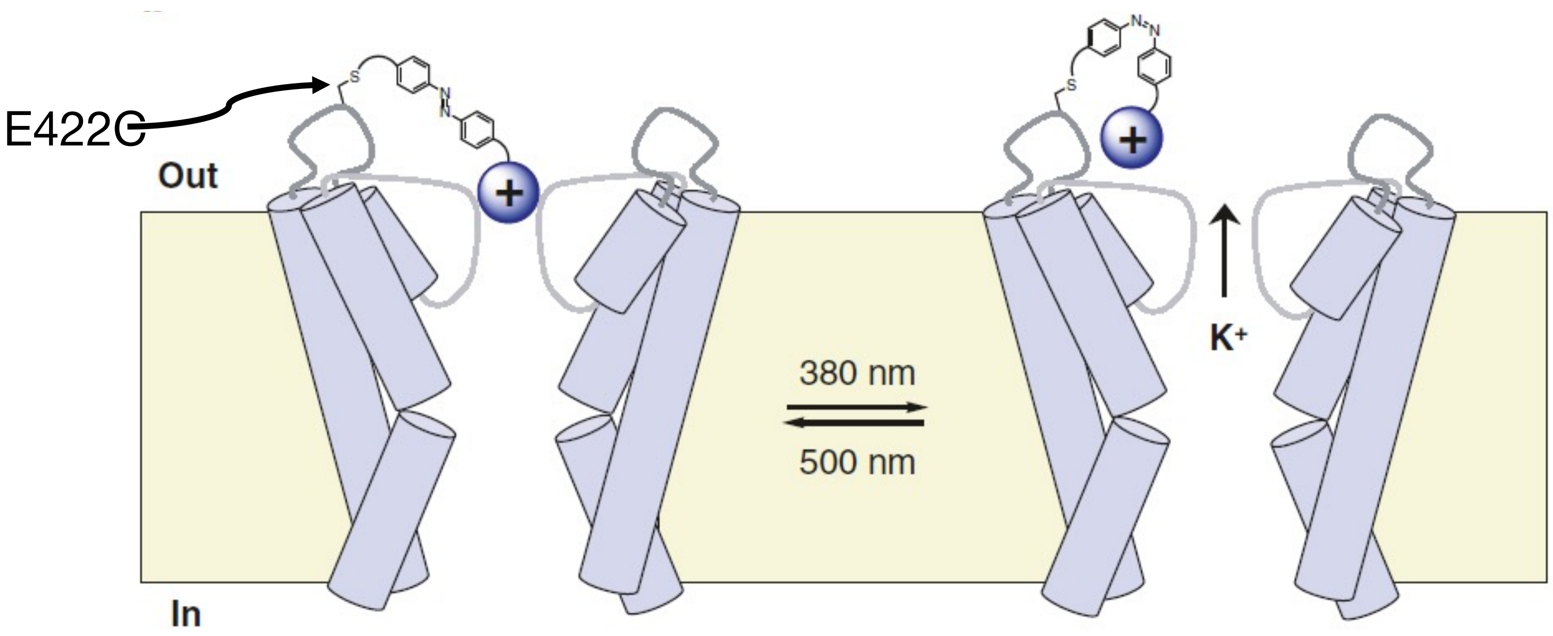
<u>Voltage</u>-gated K^+ ion channel → <u>Light</u>-gated K^+ ion channel

Synthesis of MAL-AZO-QA

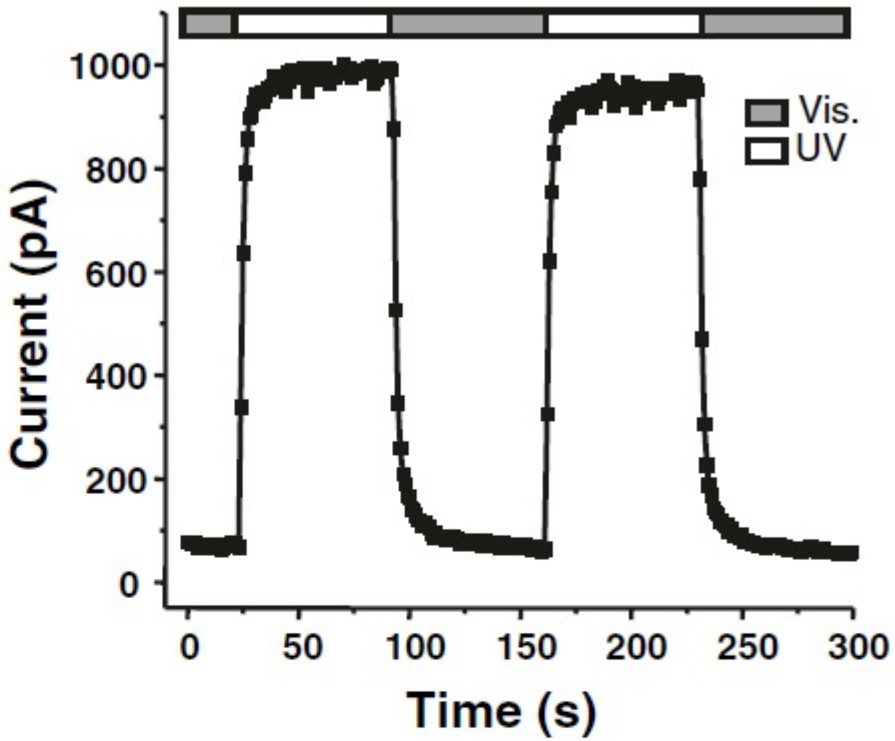


Design of SPARK (synth. photoisomerizable azobenzene-regulated K^+ ion channel)

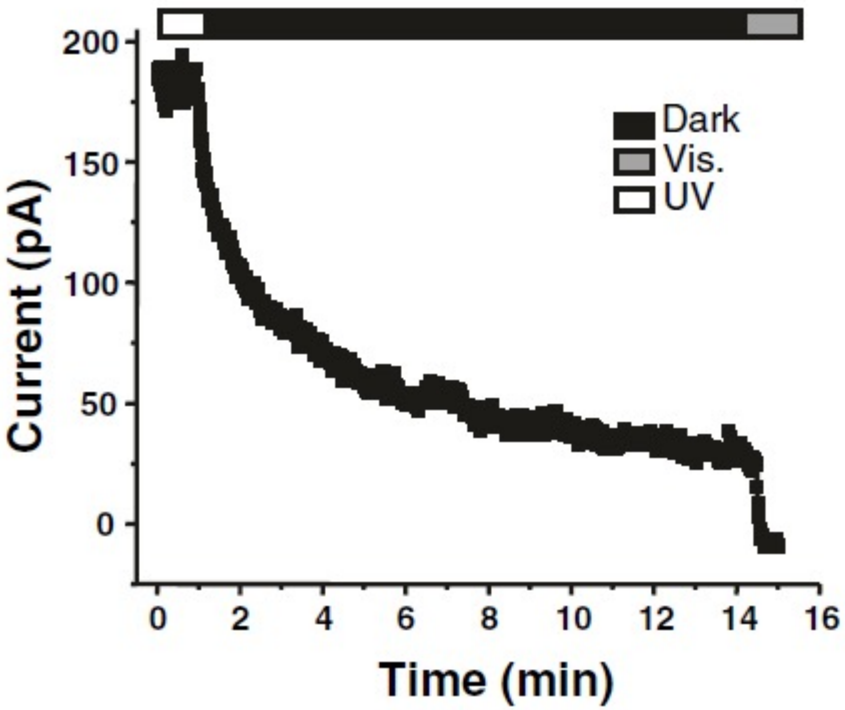
- Site-selective conjugation to Cys via 1,4-Thiol-Michael-Addition (**MAL**)
- **QA** pore blocker for “SHAKER“ K^+ ion channels
- blocks pore only in stretched *trans*-**AZO** configuration ($\Delta d_{\text{MAL-QA trans}} \approx 17$ Å; $\Delta d_{\text{MAL-QA cis}} \approx 10$ Å)



Reversible optical control of SPARK channels in oocytes

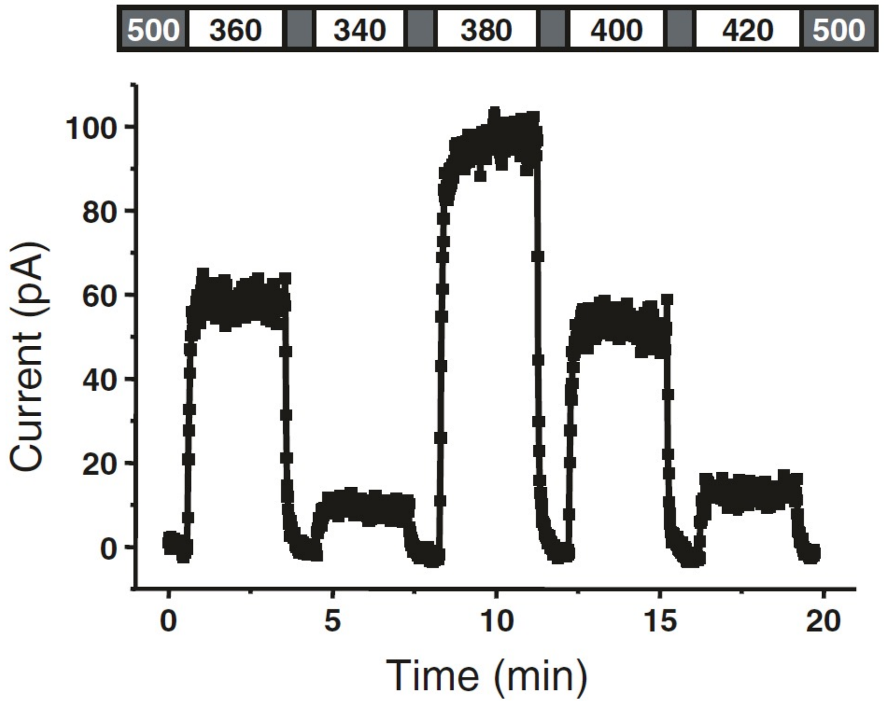


100 µM MAL-AZO-QA for 30 min; alternating irradiation with Vis. (500 nm) and UV (380 nm) light

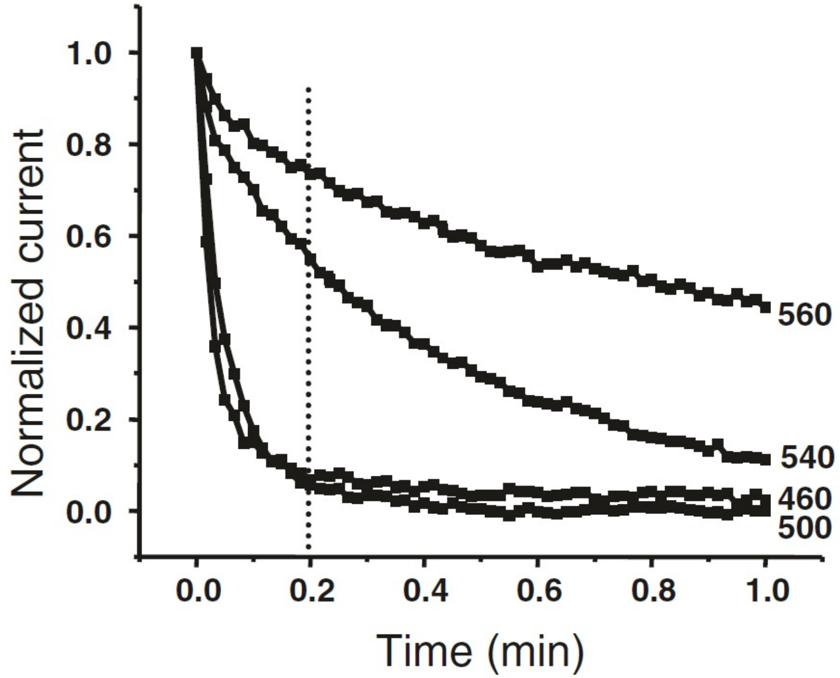


100 µM MAL-AZO-QA for 30 min; biexponential decrease in current corresponds to thermal relaxation of azobenzene

Spectral sensitivity and photoconversion rates

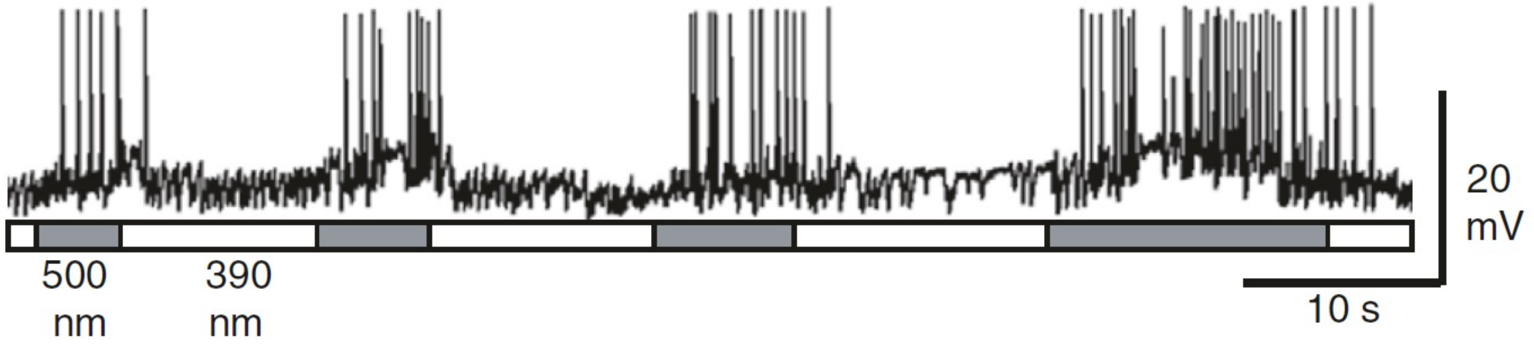


Wavelength-dependent blocking (500 nm) and unblocking (340-420 nm) of SPARK channels



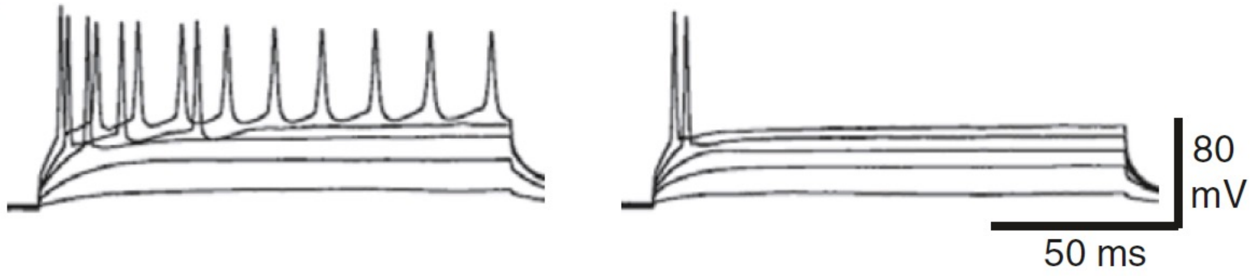
Rates of SPARK channel blocking during irradiation with indicated wavelenghts

Controlling neuronal excitability with MAL-AZO-QA

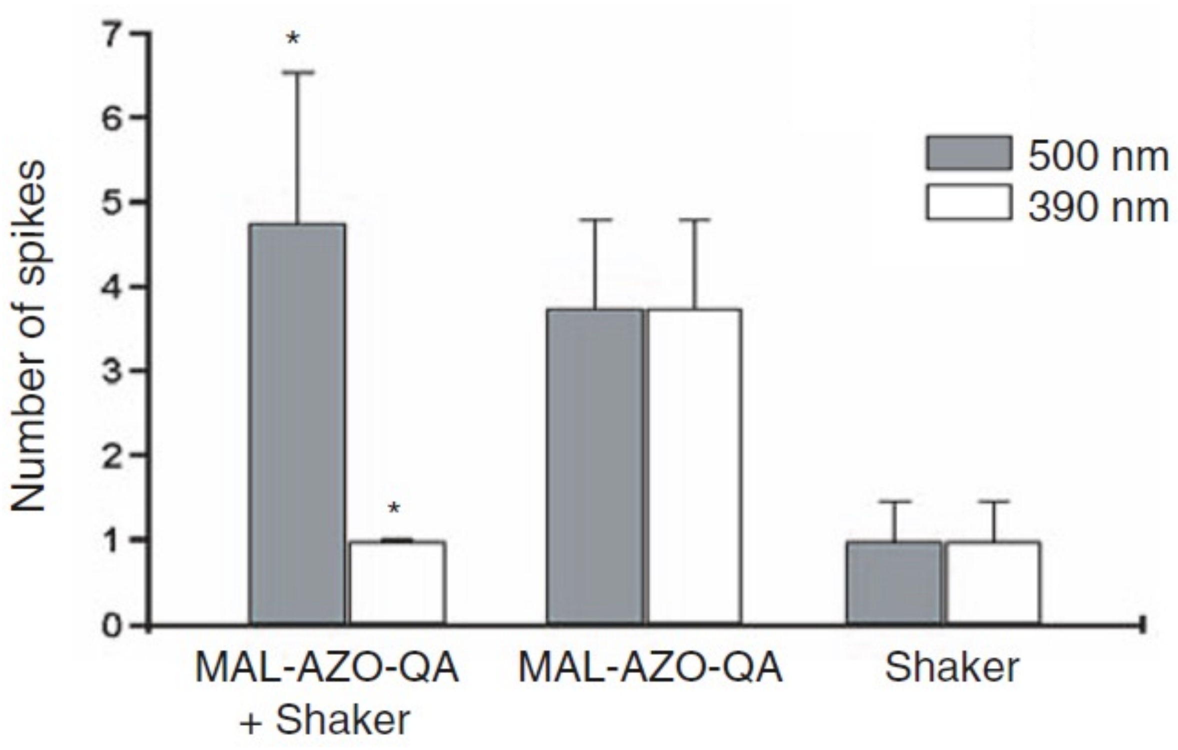


Silencing of AP firing in neurons upon illumination with 390 nm light and reviving by exposure to 500 nm light

Repetitive AP firing in 500 nm light (left), but only a single AP in 390 nm light
(depicted are single-cell traces of 5 neurons)



Controlling neuronal excitability with MAL-AZO-QA



- 390 nm light decreased number of APs in cultured neurons by 79 %
- No effect on cells treated with MAL-AZO-QA only
- No effect on cells expressing Shaker channels only

→ Non-invasive and reversible control of AP firing in living neurons with spatial and temporal control

Potential application & Outlook

- Spatiotempral precision allows dissection of neuronal networks *in vitro* and *in vivo*

- Strategy may be extended to ionotropic (ligand-gated ion channels) or metabrotoic receptors by replacement of QA with corresponding ligand

- Vision Restoration: conferring light-sensitivity on endogenous neuronal ion channels to restore retinal light response in mice (e.g. Sci Rep 7, 45487 (2017). https://doi.org/10.1038/srep45487)

Further Reading

- https://pubs.acs.org/doi/abs/10.1021/acs.accounts.5b00129
- https://pubs.acs.org/doi/10.1021/cr300179f
- https://pubs.acs.org/doi/abs/10.1021/acs.chemrev.8b00037

Chemogenetic control of nanobodies

Collaborators

Miroslaw Tarnawski

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Jan Ellenberg

Shotaro Otsuka

Thorsten Müller

Hans-Georg Kräusslich

Helen Farrants
Birgit Koch, Julien Hiblot

BioRxiv, **2019,** 683557
Nature Methods 2020

GFP fusion proteins are abundant

GFP

PDBID: 3K1K

addgene

The nonprofit plasmid repository

5696 Results for: GFP

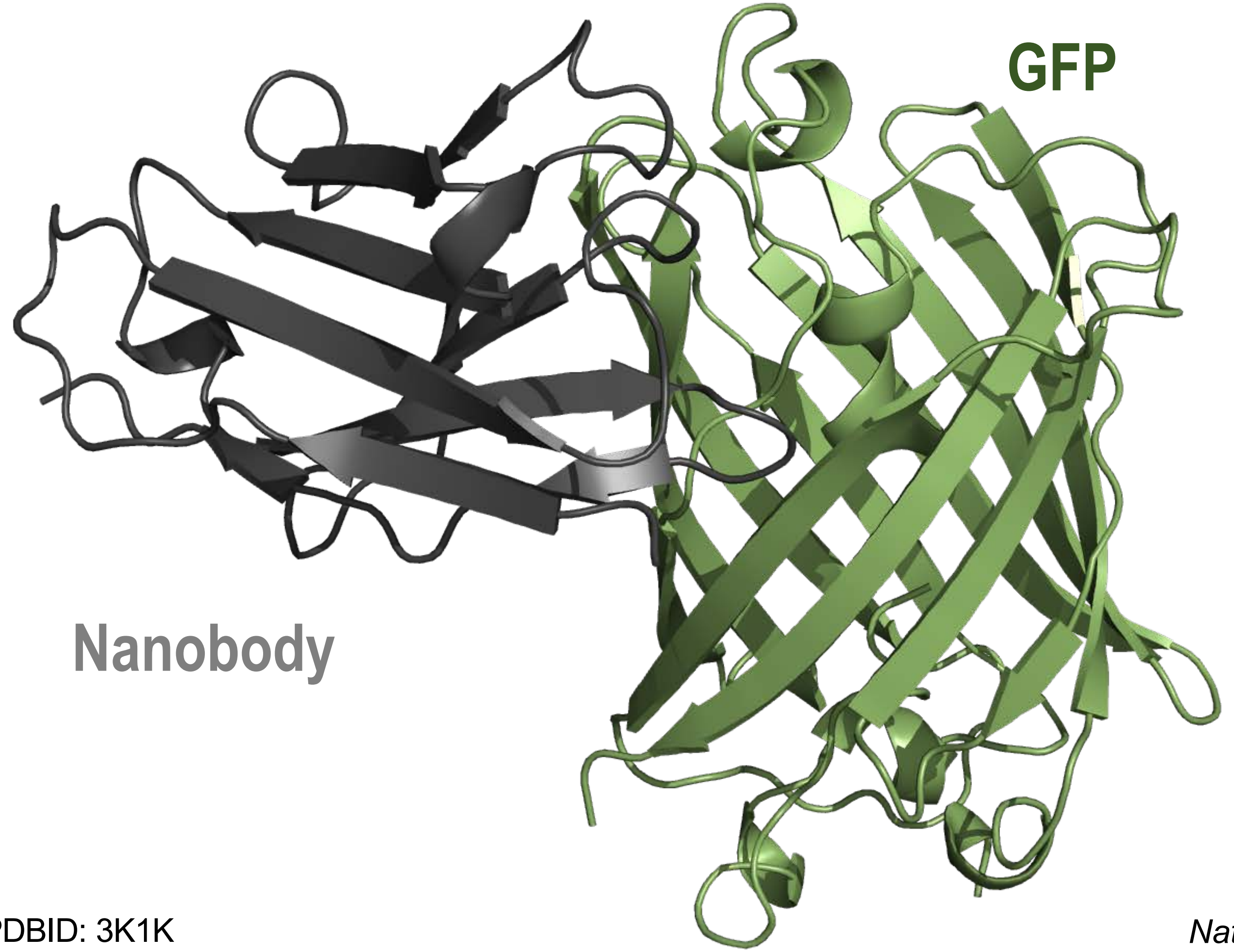
938 Results for: YFP

Yeast GFP Clone Collection

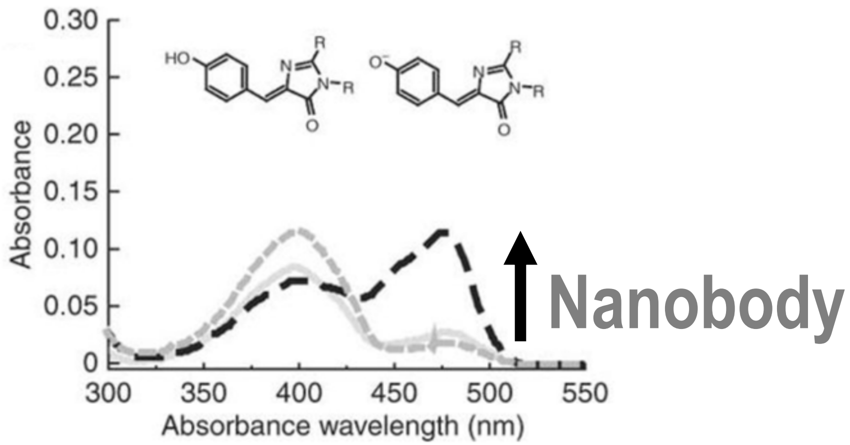
ThermoFisher SCIENTIFIC

4159 EGFP-tagged open reading frames

The “enhancer” nanobody binds GFP



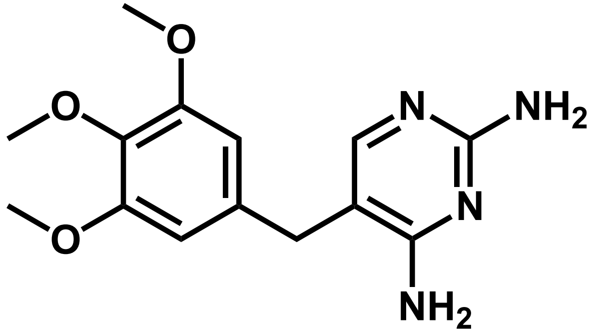
wtGFP absorption spectra



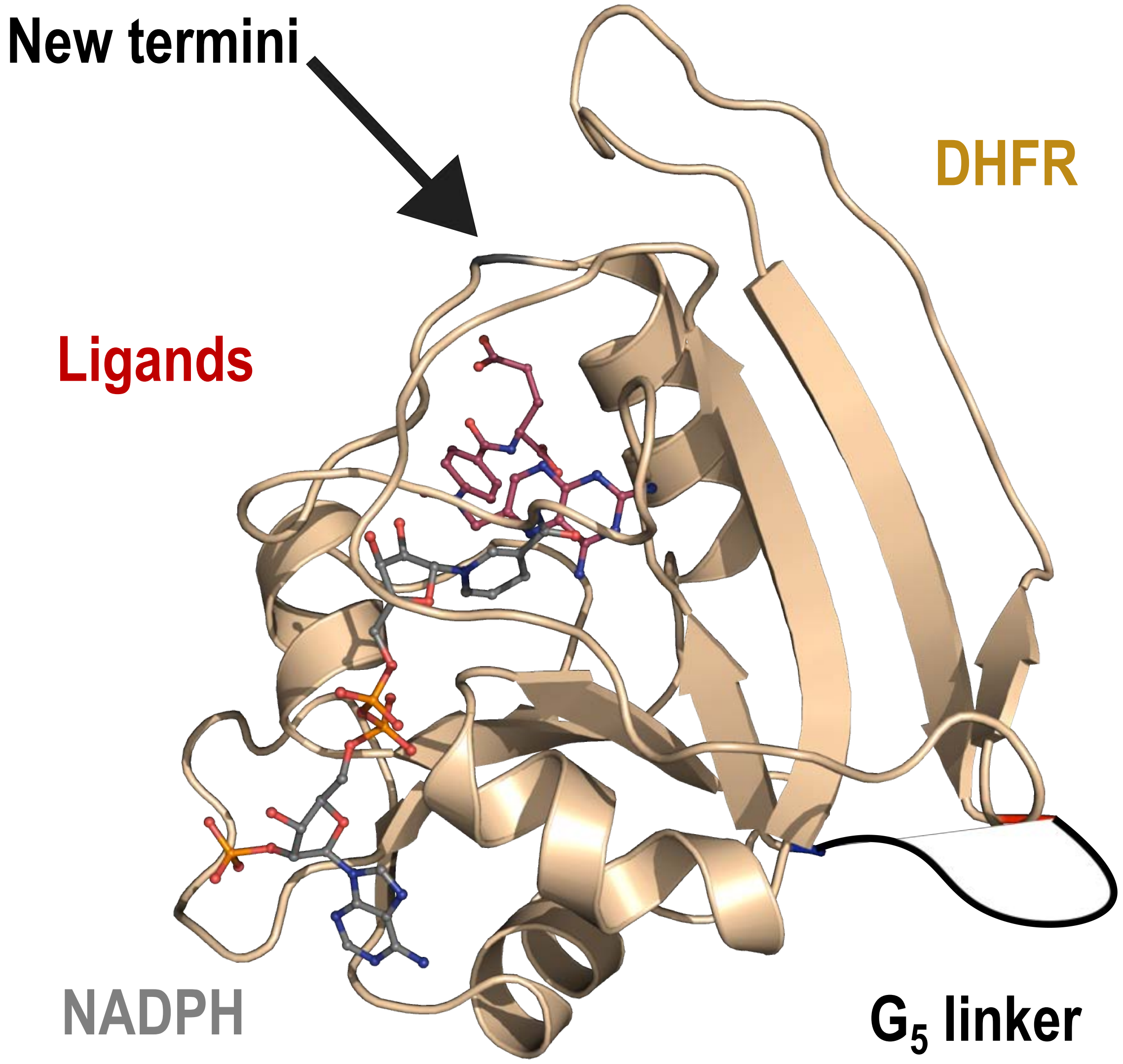
PDBID: 3K1K

Nat. Struct. Mol. Biol. 2010, **17**, 133-38.

Bacterial dihydrofolate reductase (DHFR) can be circularly permutated

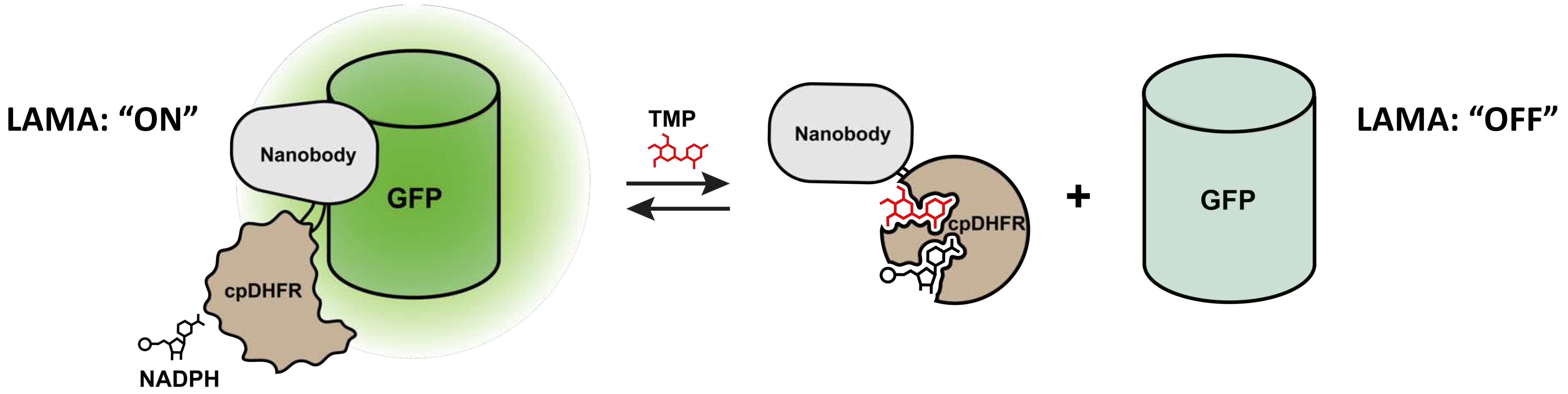


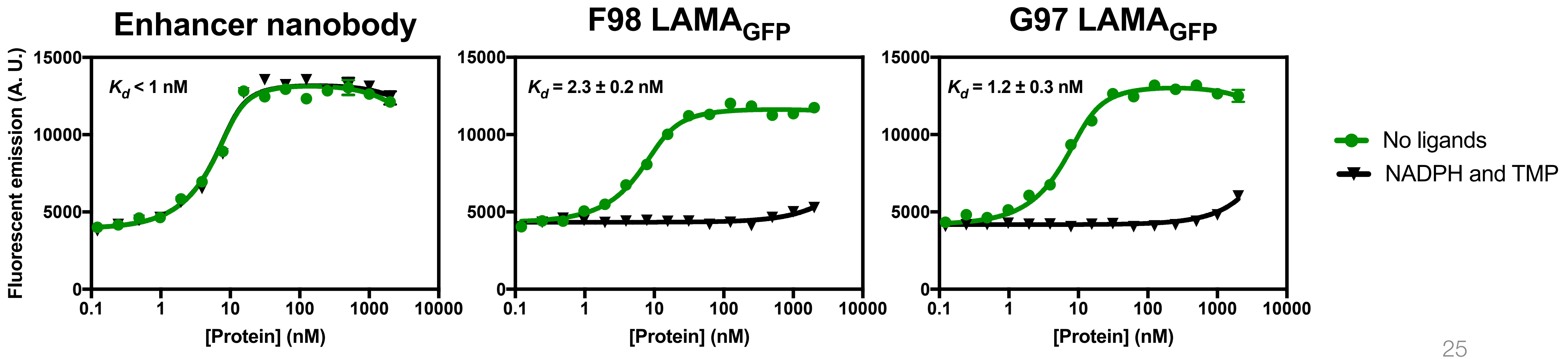
Trimethoprim (TMP)



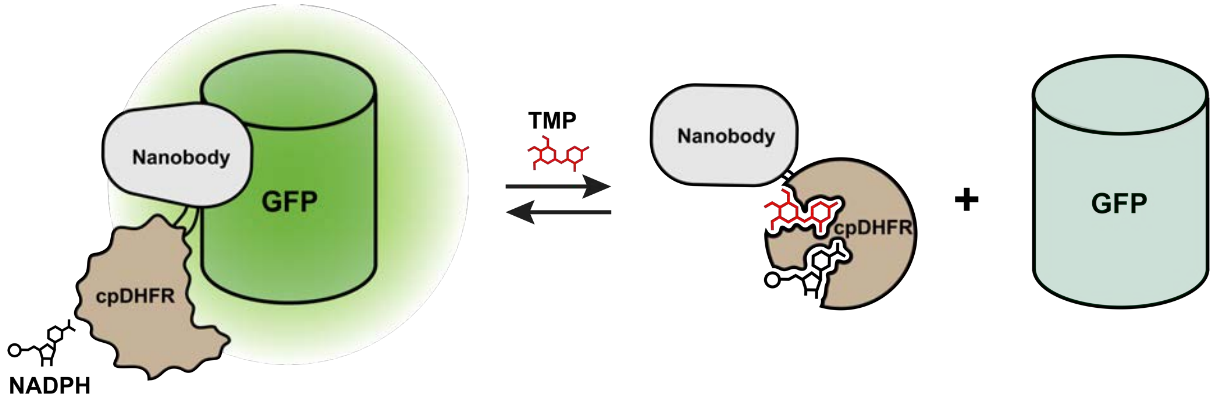
PDBID: 1RH3

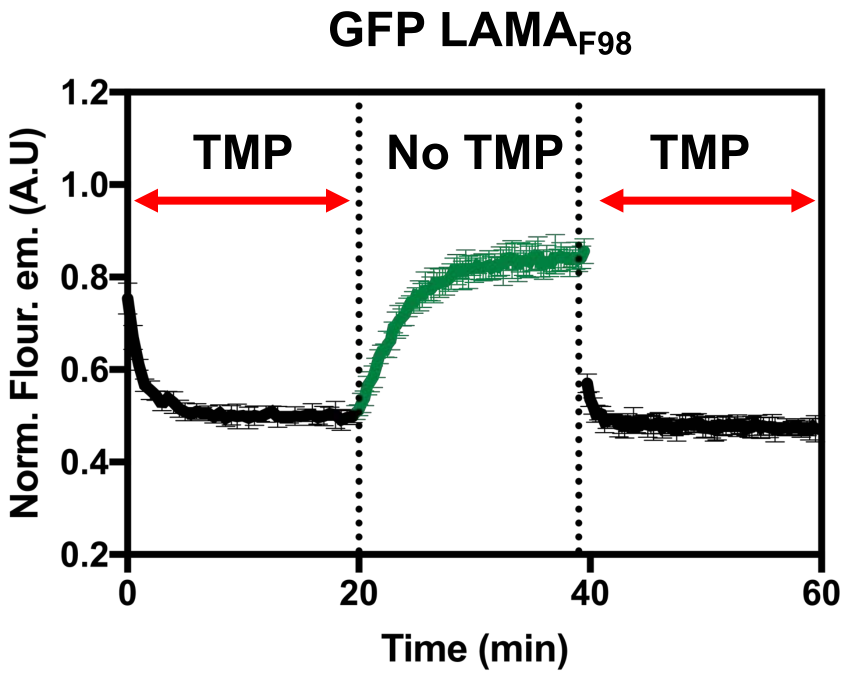
LAMAs: Ligand-based Affinity Modulator of Antibody-fragments

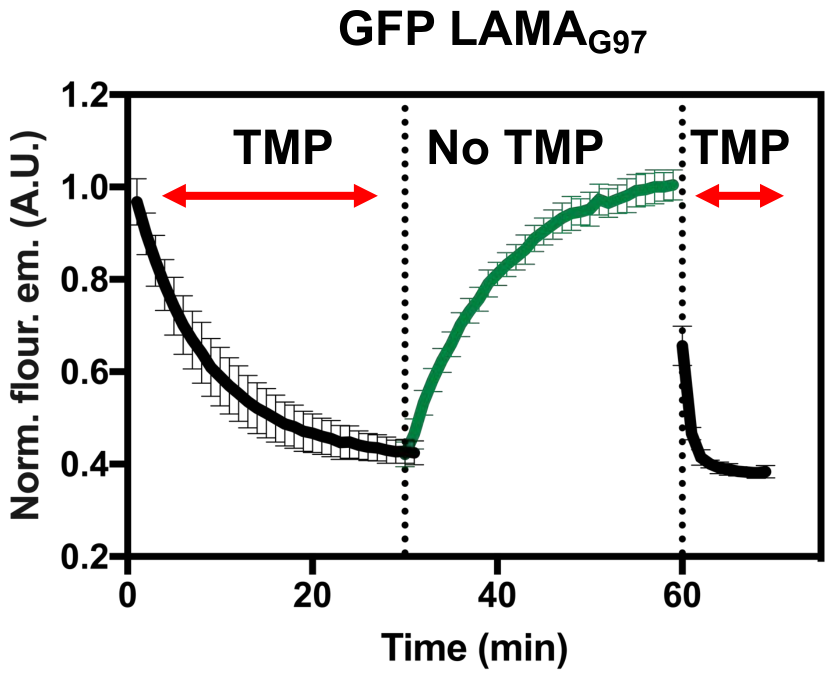




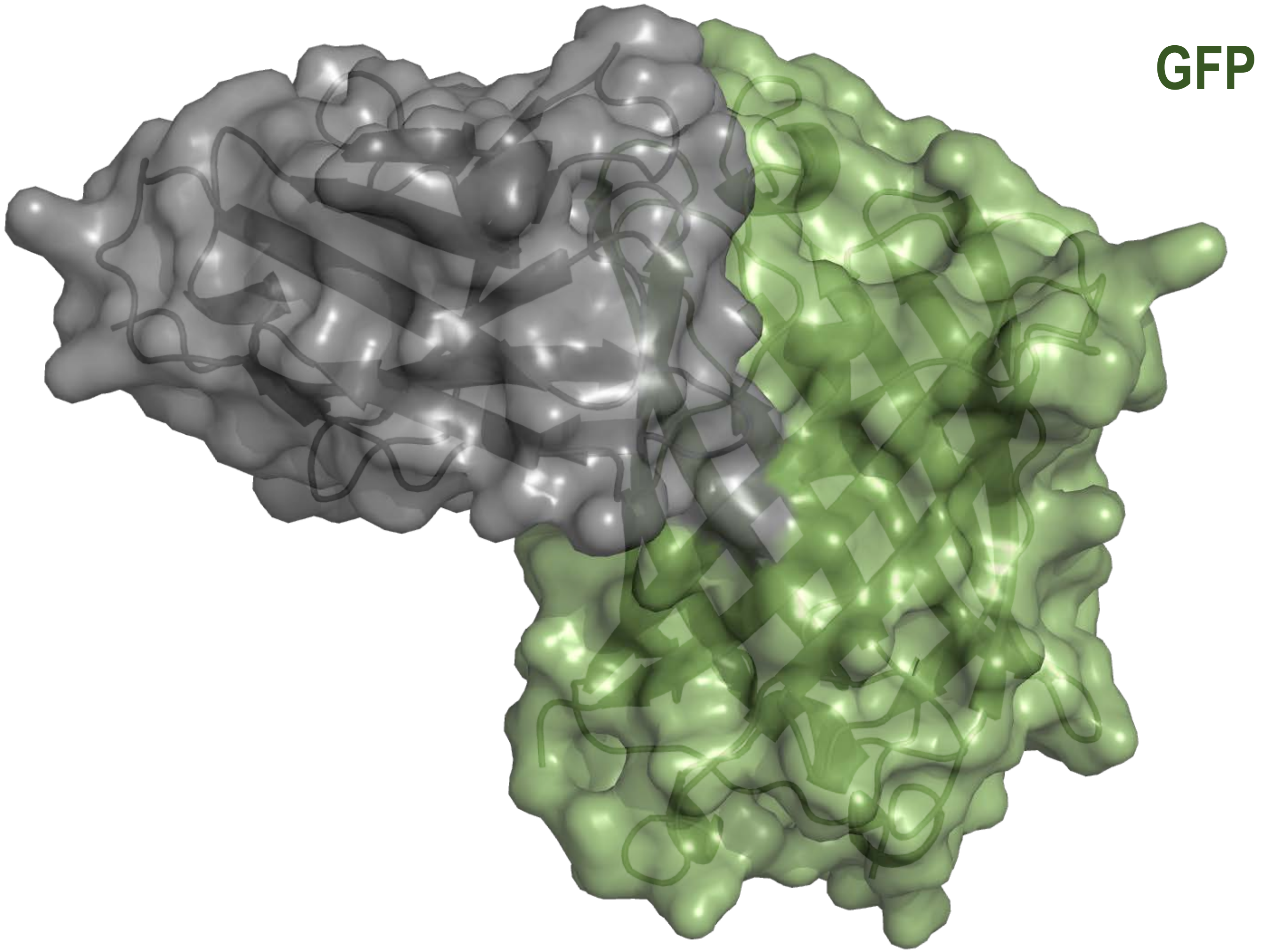
The LAMA is reversibly turned “on” and “off”

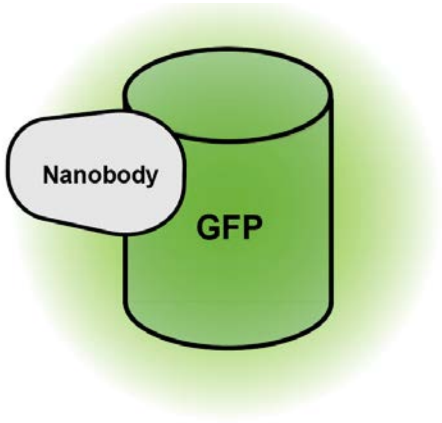






Structure of “enhancer” nanobody bound to GFP

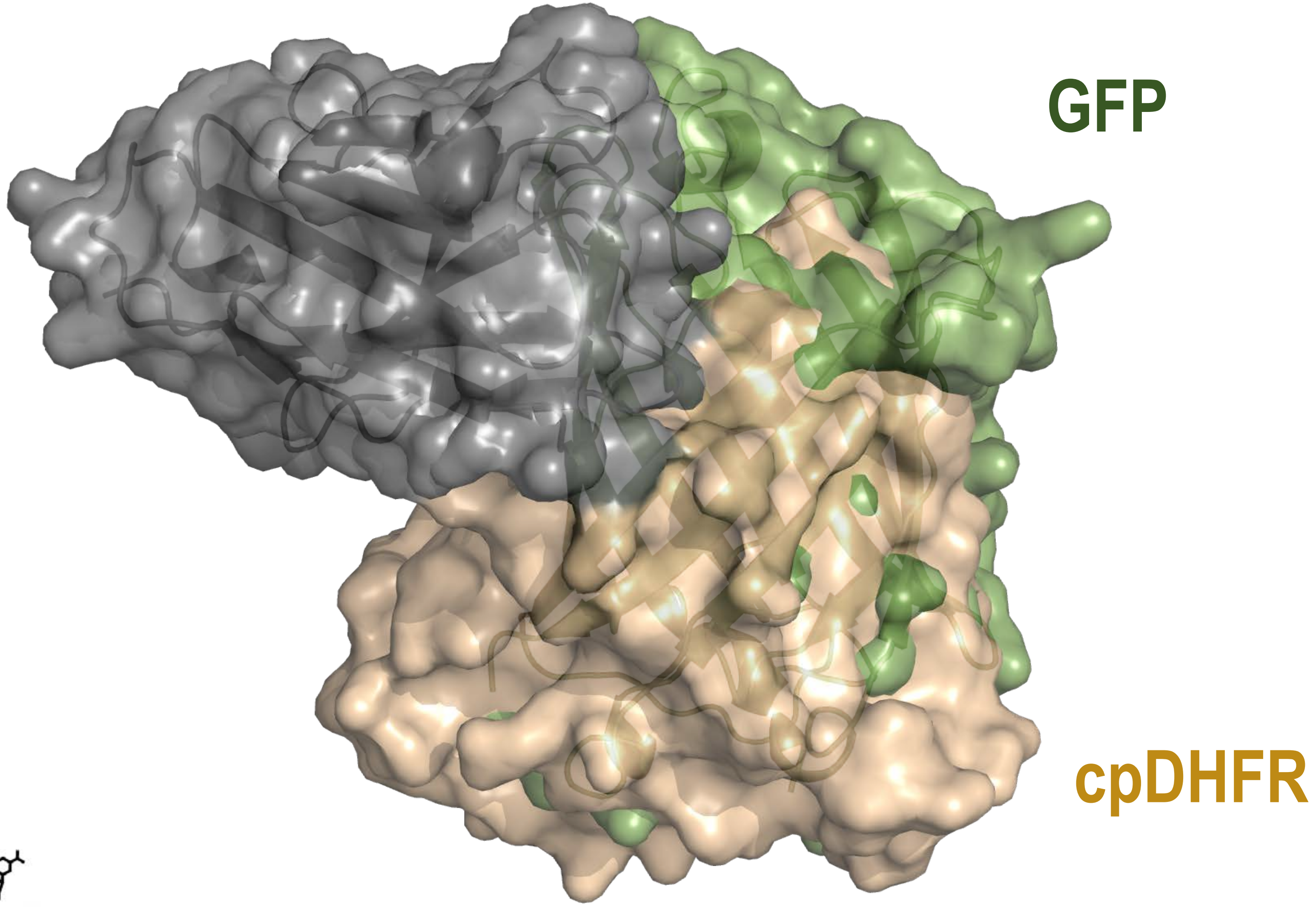


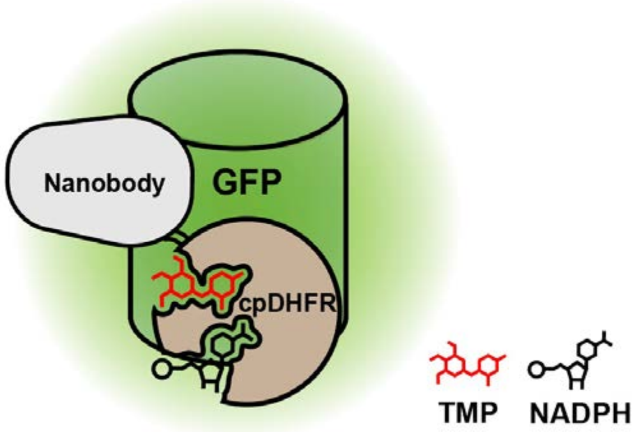


PDBID: 3K1K

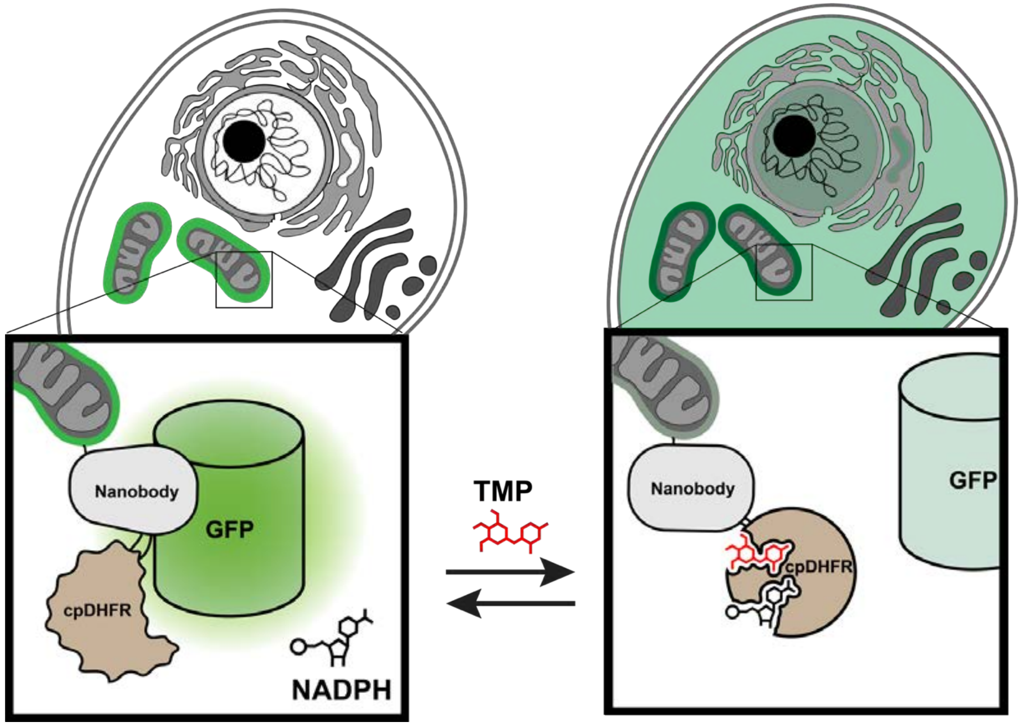
Steric clash between GFP and cpDHFR in LAMA (GFP LAMA$_{F98}$)

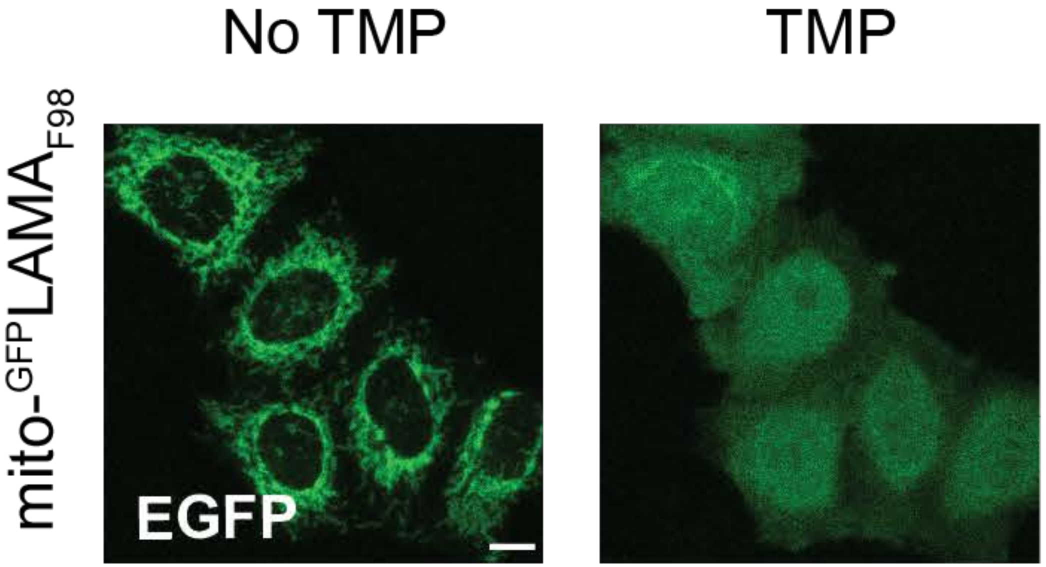
Nanobodies

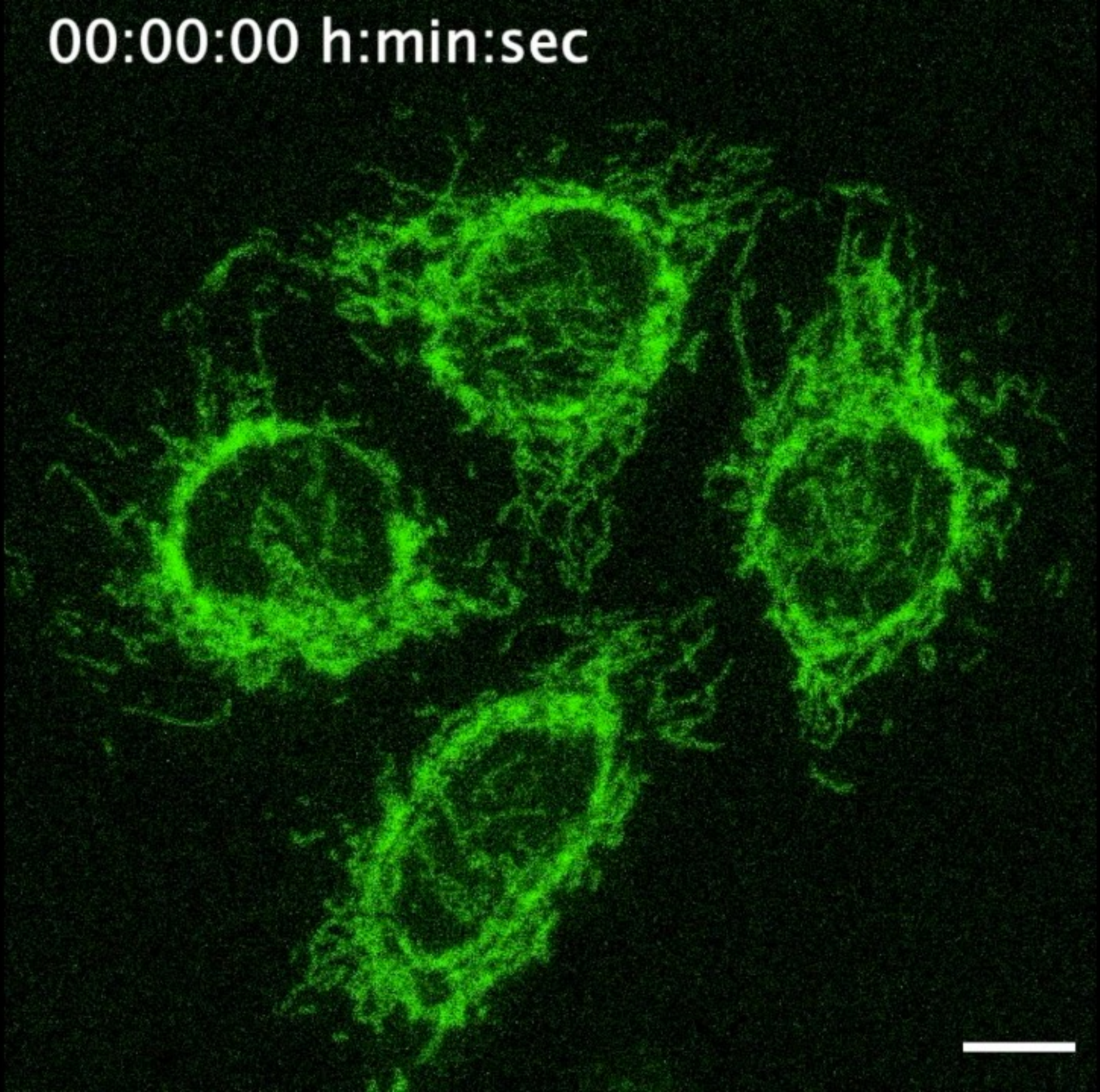




LAMAs can localize GFP fusion proteins in live cells



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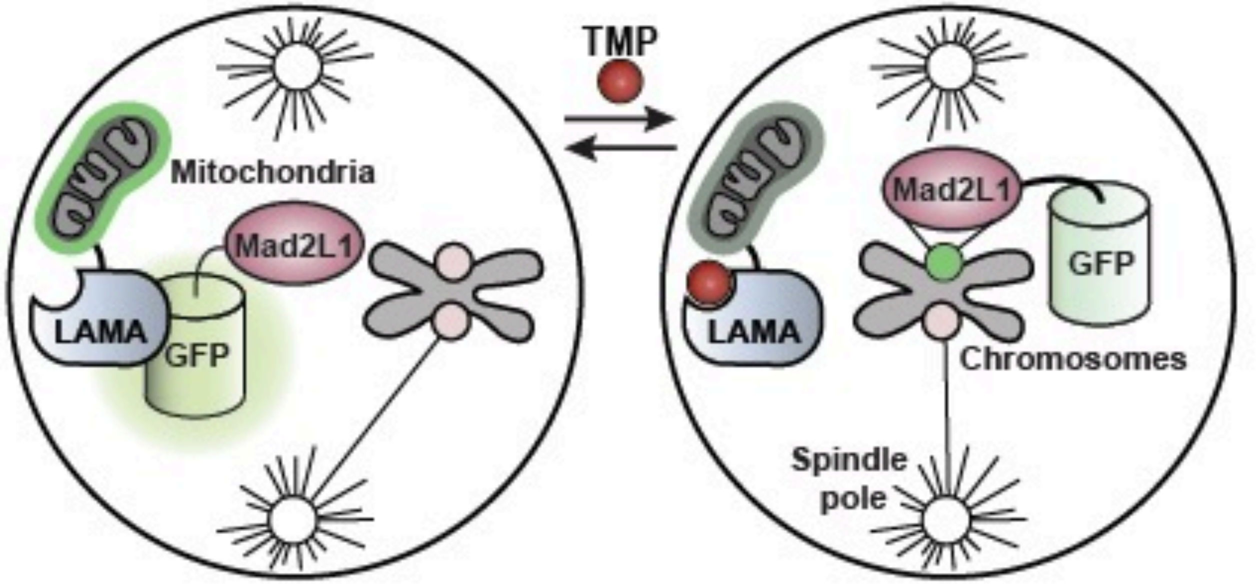
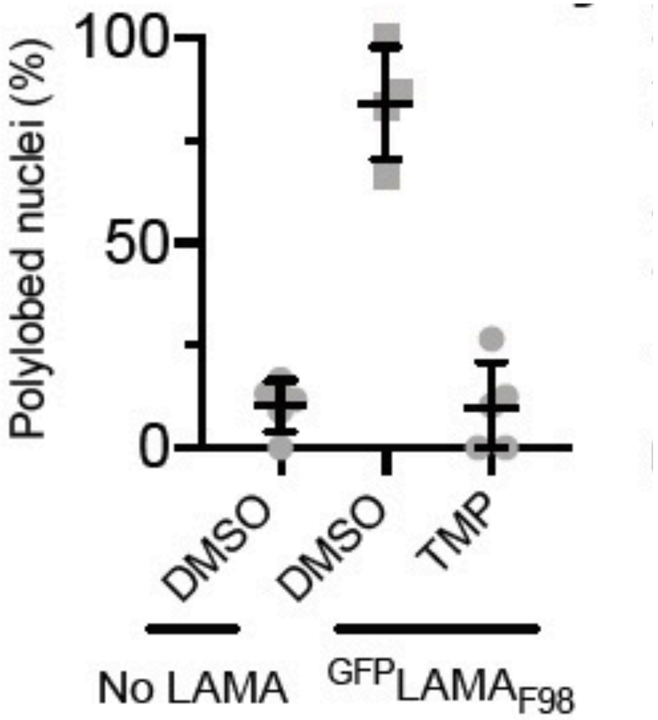
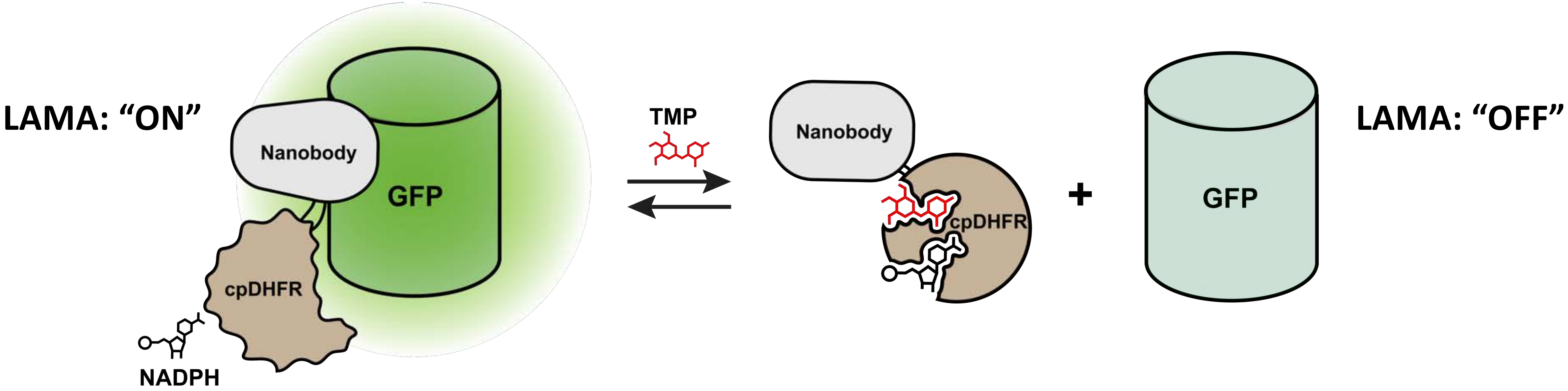
Mislocating Mad2L1 to the mitochondria: Mitotic Checkpoint Override





Chemogenetic control of nanobodies



- GFP LAMAs reversibly localize GFP-fusion proteins in live cells with small molecules
- cpDHFR can be used as an affinity modulator for nanobodies