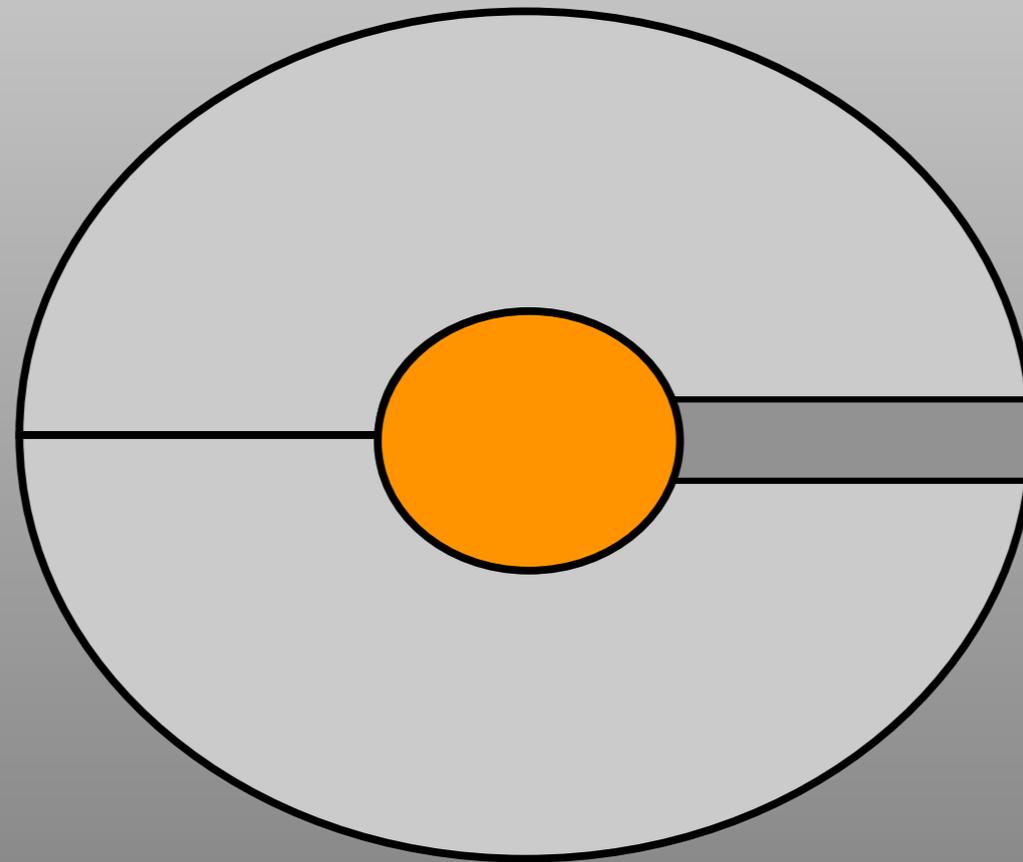


Photopharmakologie



Lichtabhängige Kontrolle zellulärer Rezeptoren

Dr. Martin Sumser (Trauner Lab)

Überblick

- Einführung in die Neurophysiologie und Methodik derselben
- Pharmakologie / Photopharmakologie
- Strategien zur Lichtschaltbarkeitmachung von Rezeptoren
- Design eines artifiziellen Photoschalters
- Das Prinzip der Photopharmakologie
- Anwendung von photochromen Liganden in der Neurobiologie
- Anwendung von konjugierten Photoschaltern in der Neurobiologie

Das Membran- und das Ruhepotential

Cell types	Resting potential
Skeletal muscle cells	-95 mV ^[3]
Smooth muscle cells	-60 mV
Astroglia	-80 to -90 mV
Neurons	-60 to -70 mV
Erythrocytes	-9 mV
Photoreceptor cells	-40 mV

extracellular (outside)

http://en.wikipedia.org/wiki/Resting_potential

lipid bilayer

$V_m = -70 \text{ mV}$ (typical resting potential of a neuron)

intracellular (inside)

inside negative

Das Membran- und das Ruhepotential

Cell types	Resting potential
Skeletal muscle cells	-95 mV ^[3]
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Erythrocytes	-9 mV
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extracellular (outside)

http://en.wikipedia.org/wiki/Resting_potential



intracellular (inside)

inside negative

Das Ruhepotential ist kein thermodynamisches Gleichgewicht, sondern resultiert aus der Aktivität von Ionenpumpen (primär der Na^+/K^+ -ATPase) und speziellen Ionenkanälen, die am Ruhepotential offen und daher leitfähig sind. Normalerweise sind diese Kanäle selektiv für Kalium.

Die unterschiedliche intra- und extrazelluläre Verteilung von Ionen definiert das Ruhepotential

TABLE 2.1

Extracellular and Intracellular Ion Concentrations

<i>Ion</i>	<i>Concentration (mM)</i>	
	<i>Intracellular</i>	<i>Extracellular</i>
Squid neuron		
Potassium (K ⁺)	400	20
Sodium (Na ⁺)	50	440
Chloride (Cl ⁻)	40–150	560
Calcium (Ca ²⁺)	0.0001	10
Mammalian neuron		
Potassium (K ⁺)	140	5
Sodium (Na ⁺)	5–15	145
Chloride (Cl ⁻)	4–30	110
Calcium (Ca ²⁺)	0.0001	1–2

Nernst Gleichung

$$E = \frac{R \cdot T}{z \cdot F} \cdot \ln \frac{[X]_{out}}{[X]_{in}}$$

Berechnung des Nernstpotentials

Nernst calculator

Temperature	T [K]	298		gas constant	R	8.314
charge	z	1		Faraday constant	F	96485
concentration	[x] out [mM]	140				
concentration	[x] in [mM]	10				
reversal potential	V rev [mV]	67,77				

Die unterschiedliche intra- und extrazelluläre Verteilung von Ionen definiert das Ruhepotential

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Berechnung des Nernstpotentials

Nernst calculator

Temperature	T [K]	298		gas constant	R	8.314
charge	z	1		Faraday constant	F	96485
concentration	[x] out [mM]	5				
concentration	[x] in [mM]	140				
reversal potential	V rev [mV]	-85,57				

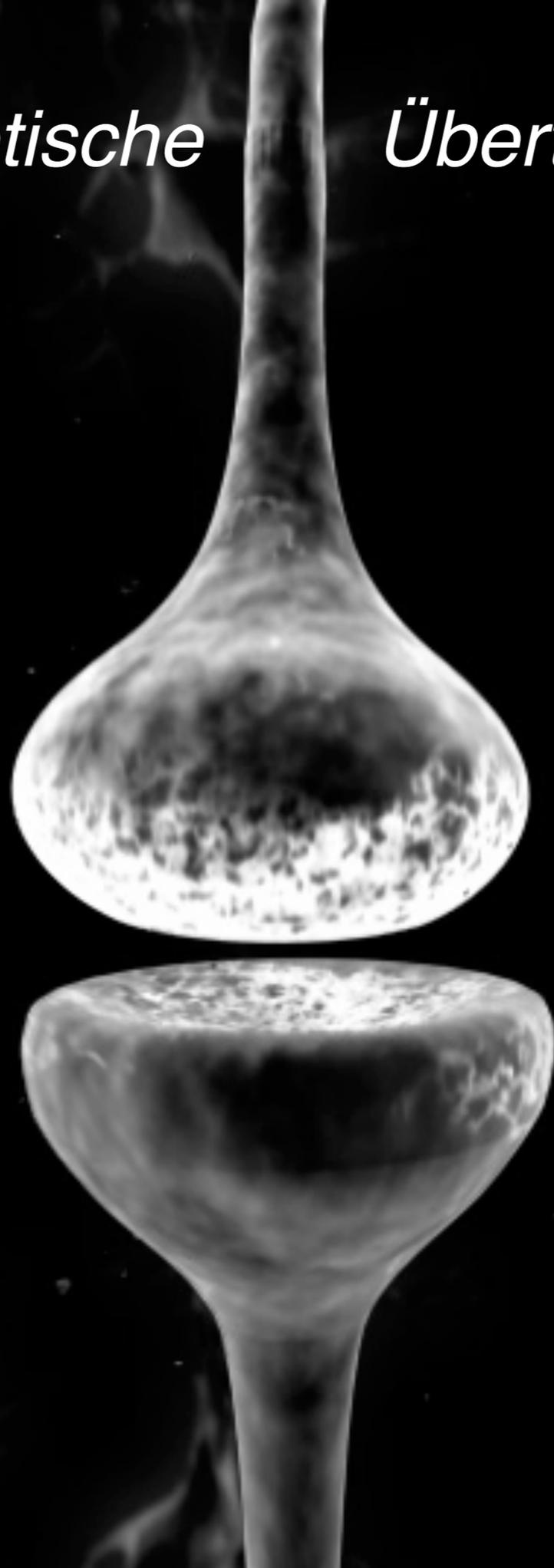
Umkehr- oder Gleichgewichtspotentiale

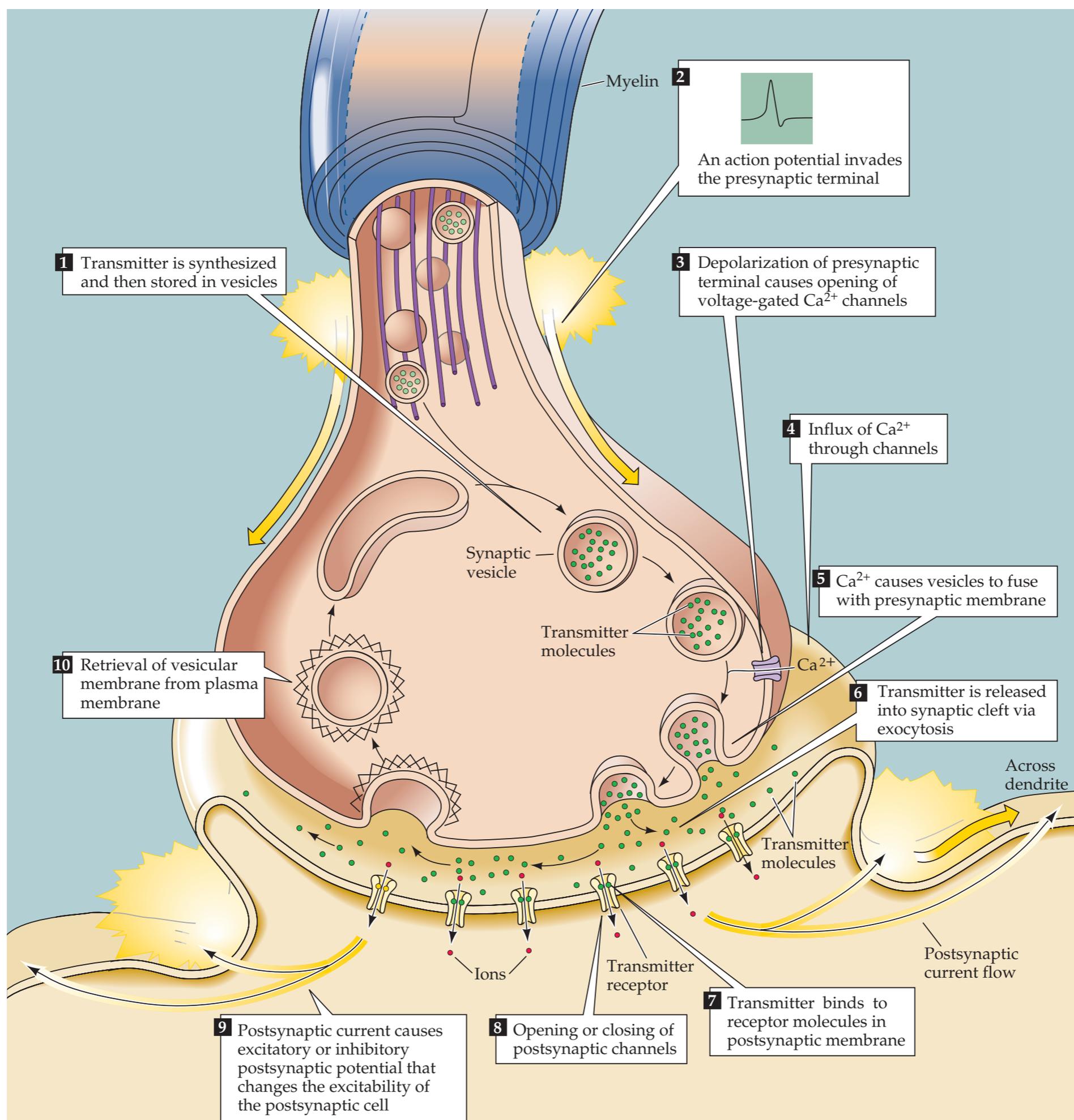
<u>ion</u>	<u>equilibrium potential (neurons)</u>
Na ⁺	+ 55 mV
K ⁺	- 90 mV
Cl ⁻	- 86 mV
Ca ²⁺	+ 123 mV

Wenn sich in einer Membran bei -70mV spannungsgesteuerte Kanäle öffnen, wird Na⁺, Ca²⁺ und Cl⁻ in die Zelle strömen und K⁺ hinaus.

Synaptische

Übertragung



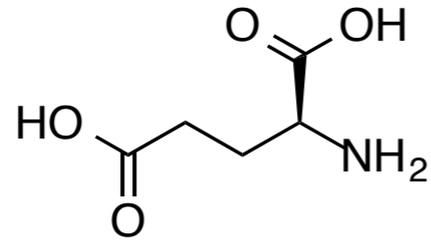


Neurotransmitter

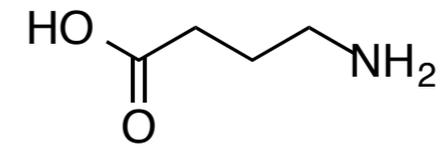
excitatory

inhibitory

more in
the CNS
(humans)



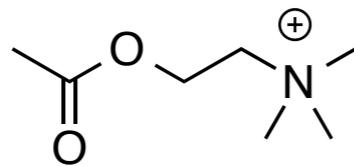
L-glutamate



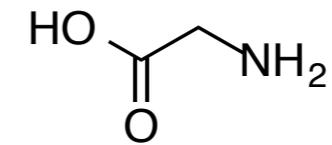
GABA

(γ-aminobutyric acid)

more in
periphery
(humans)



acetylcholine



glycine

Neurotransmitter

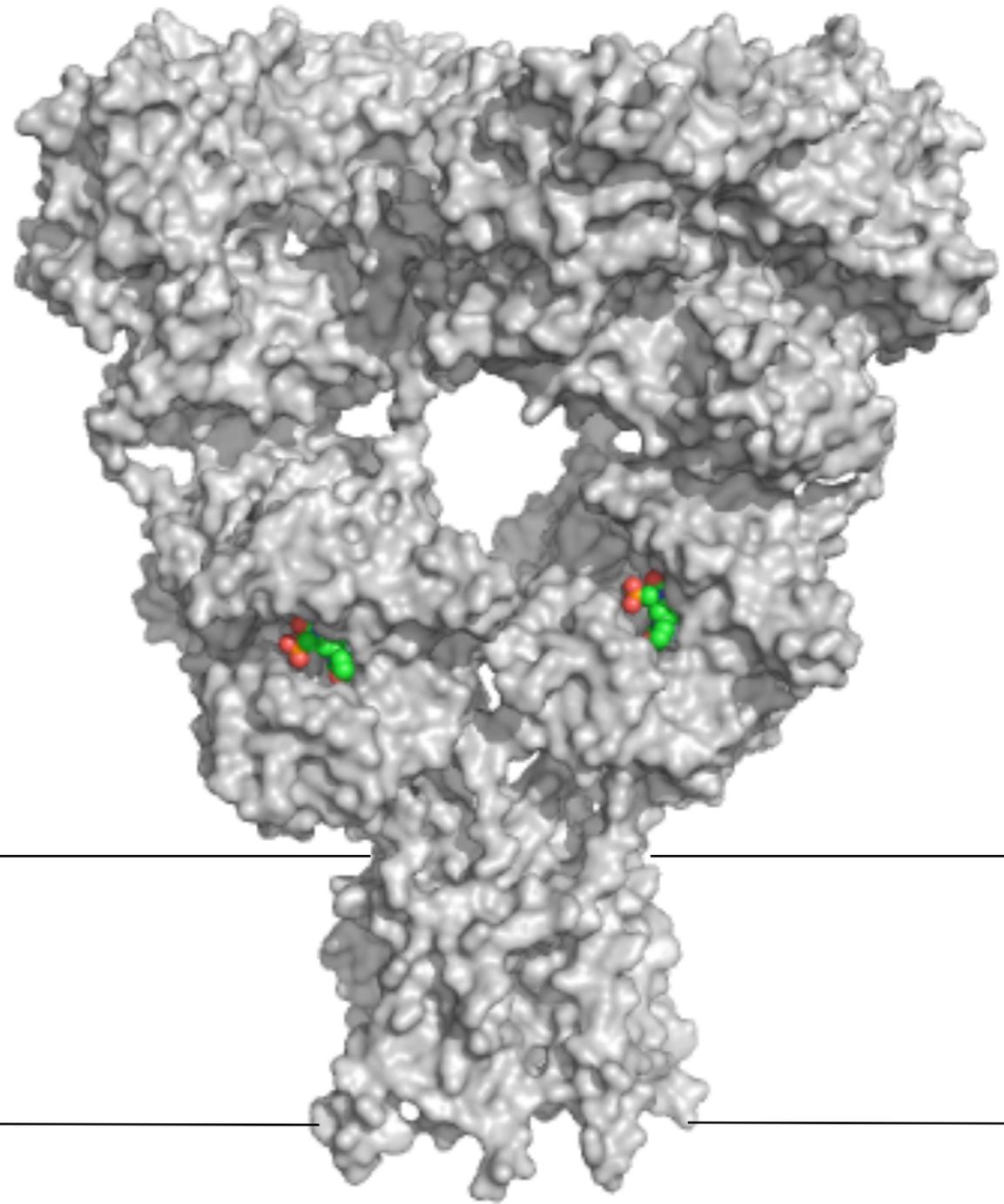
TABLE 6.1
Functional Features of the Major Neurotransmitters

<i>Neurotransmitter</i>	<i>Postsynaptic effect^a</i>	<i>Precursor(s)</i>	<i>Rate-limiting step in synthesis</i>	<i>Removal mechanism</i>	<i>Type of vesicle</i>
ACh	Excitatory	Choline + acetyl CoA	CAT	AChE	Small, clear
Glutamate	Excitatory	Glutamine	Glutaminase	Transporters	Small, clear
GABA	Inhibitory	Glutamate	GAD	Transporters	Small, clear
Glycine	Inhibitory	Serine	Phosphoserine	Transporters	Small, clear
Catecholamines (epinephrine, norepinephrine, dopamine)	Excitatory	Tyrosine	Tyrosine hydroxylase	Transporters, MAO, COMT	Small dense-core, or large irregular dense-core
Serotonin (5-HT)	Excitatory	Tryptophan	Tryptophan hydroxylase	Transporters, MAO	Large, dense-core
Histamine	Excitatory	Histidine	Histidine decarboxylase	Transporters	Large, dense-core
ATP	Excitatory	ADP	Mitochondrial oxidative phosphorylation; glycolysis	Hydrolysis to AMP and adenosine	Small, clear
Neuropeptides	Excitatory and inhibitory	Amino acids (protein synthesis)	Synthesis and transport	Proteases	Large, dense-core
Endocannabinoids	Inhibits inhibition	Membrane lipids	Enzymatic modification of lipids	Hydrolysis by FAAH	None
Nitric oxide	Excitatory and inhibitory	Arginine	Nitric oxide synthase	Spontaneous oxidation	None

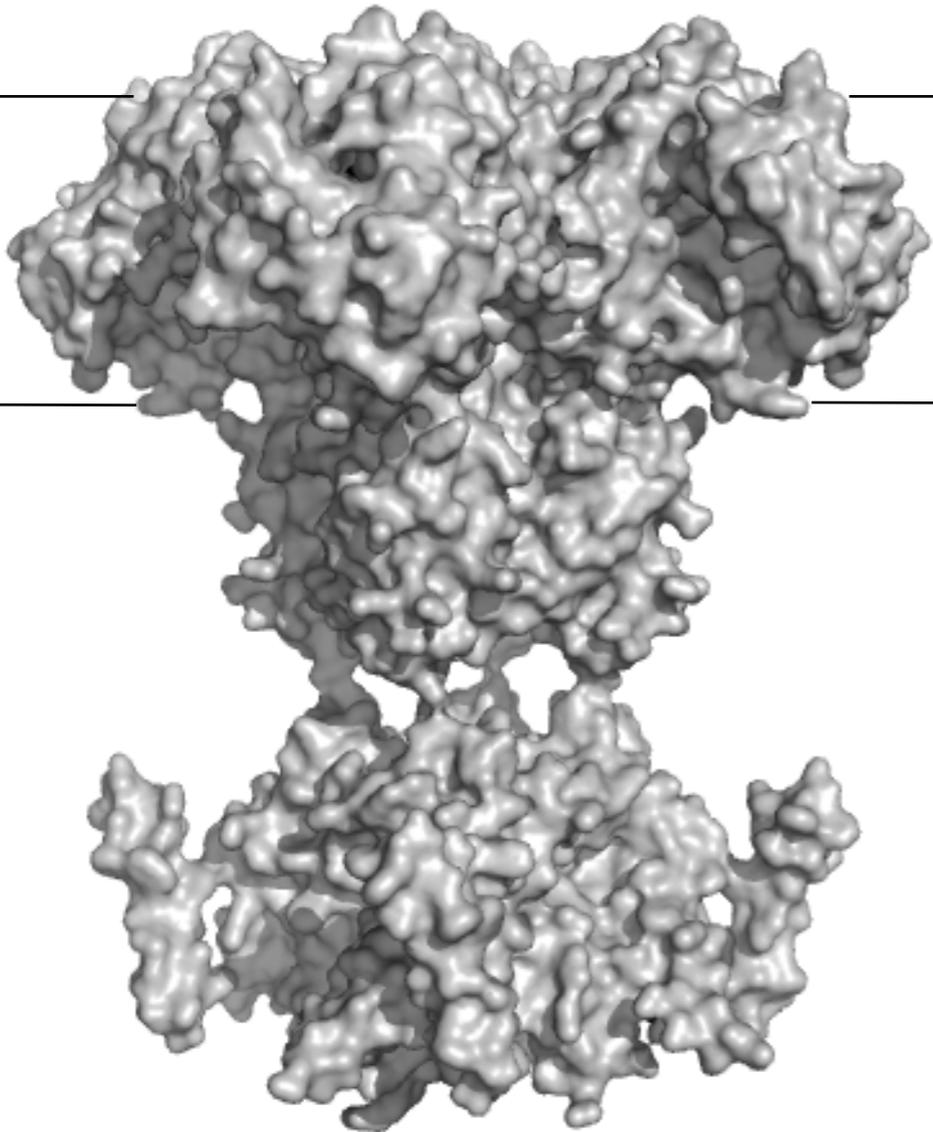
^aThe most common postsynaptic effect is indicated; the same transmitter can elicit postsynaptic excitation or inhibition depending on the nature of the ion channels affected by transmitter binding (see Chapter 7).

10 nm

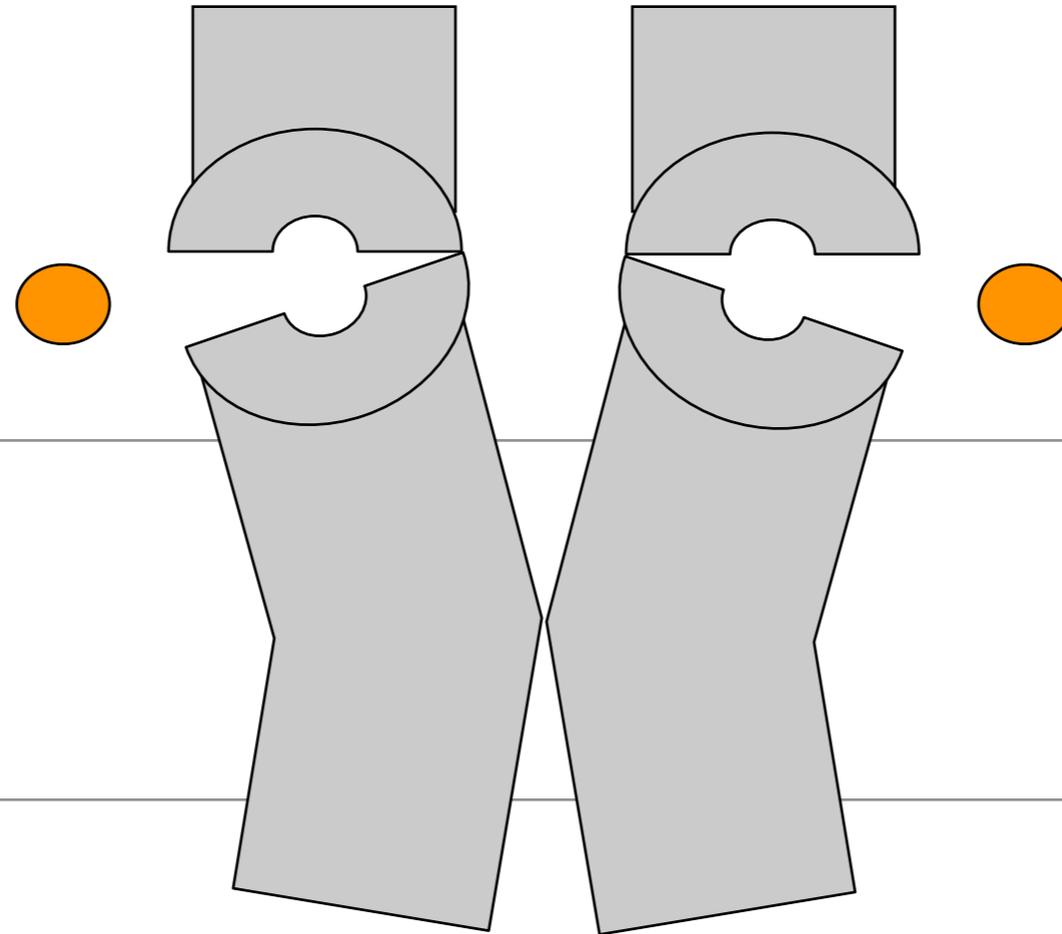
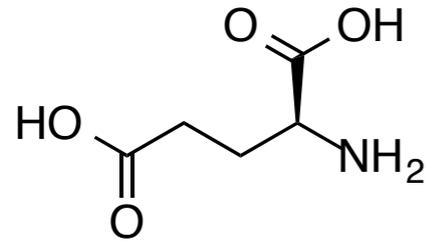
spannungsgesteuerter Kaliumkanal



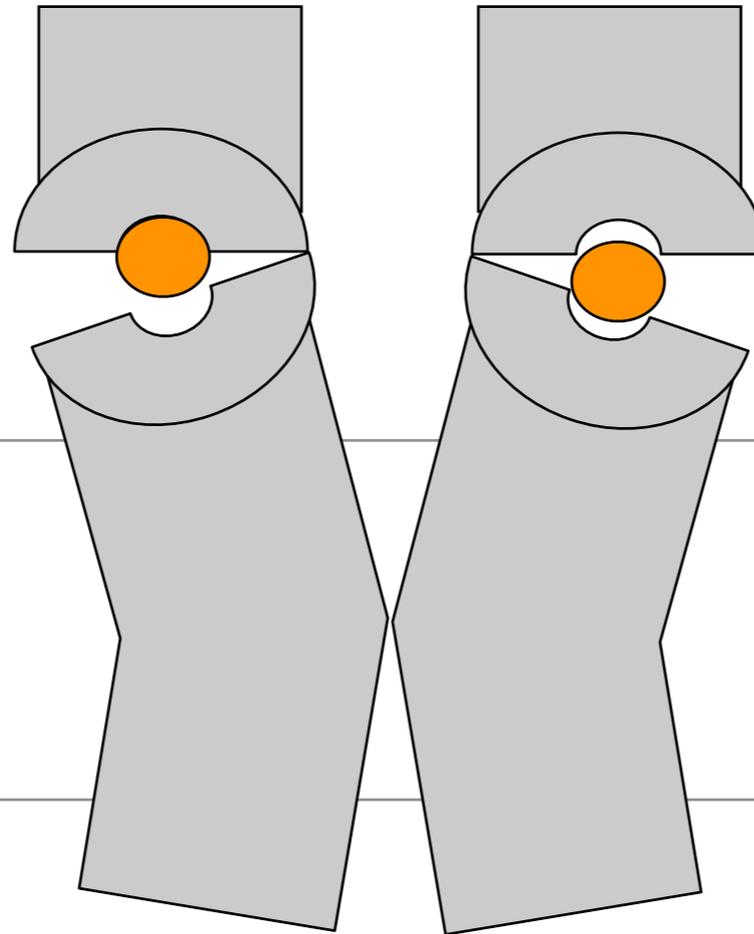
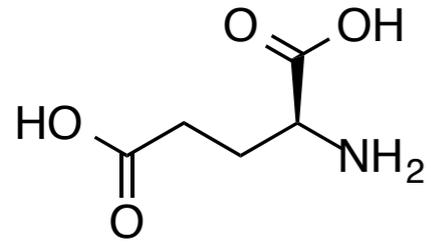
Ionotroper Glutmatrezeptor



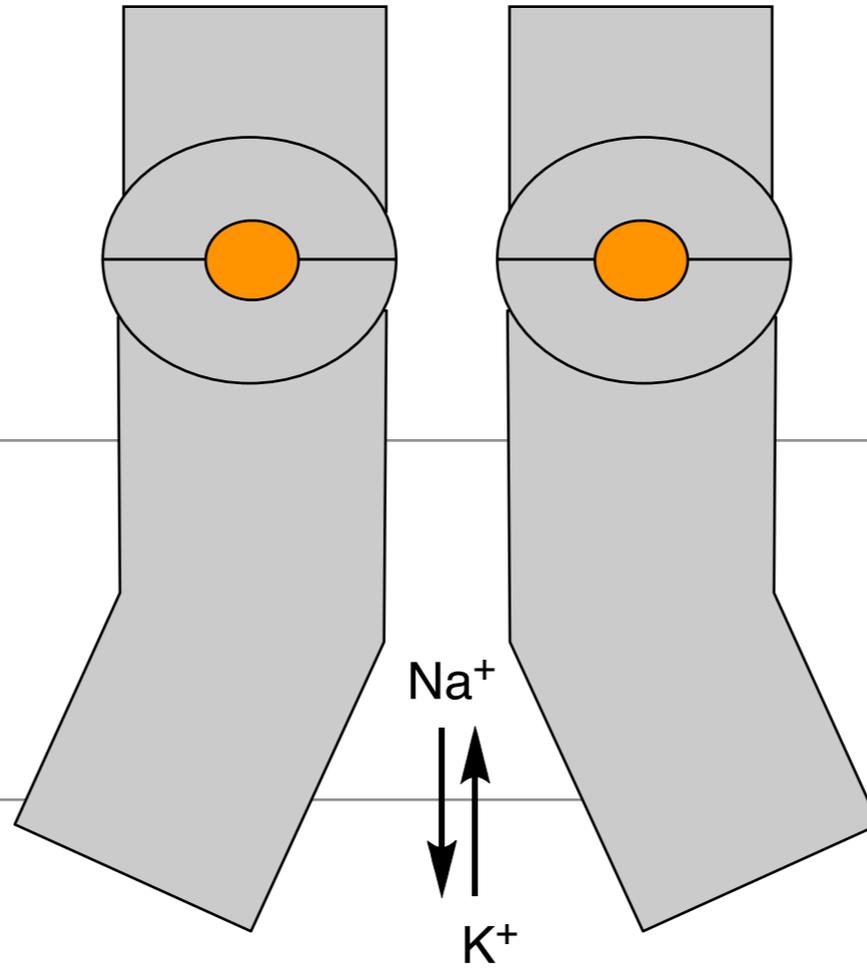
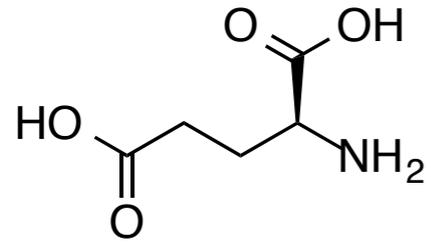
Ionotrope Glutamatrezeptoren (iGluRs)



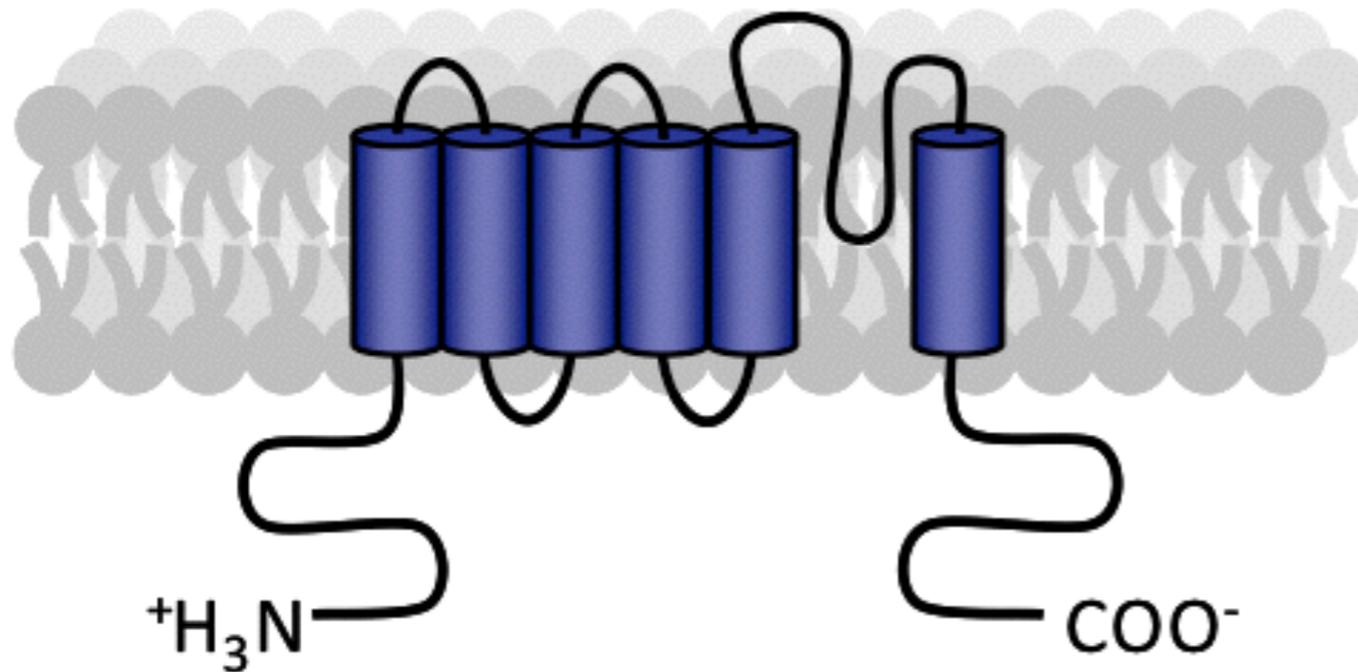
Ionotrope Glutamatrezeptoren (iGluRs)



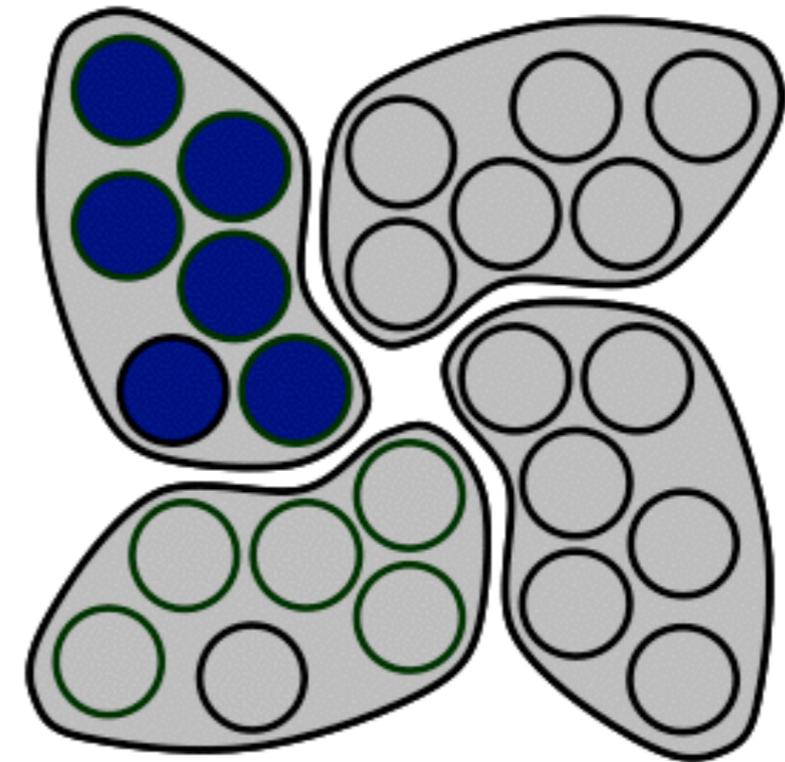
Ionotrope Glutamatrezeptoren (iGluRs)



Spannungsgesteuerte Kaliumkanäle (Kv)

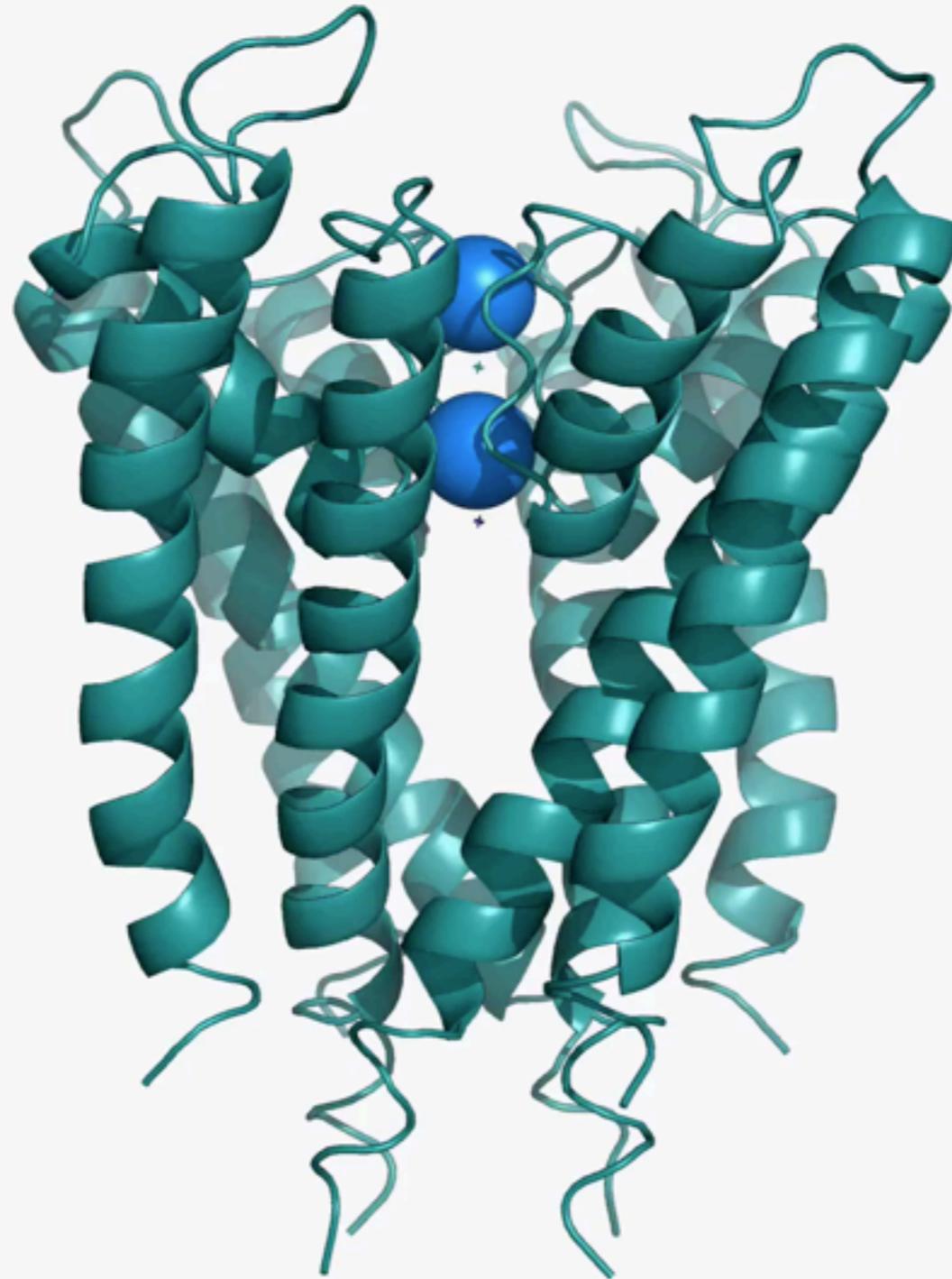


Potassium channel topology



Channel tetramer

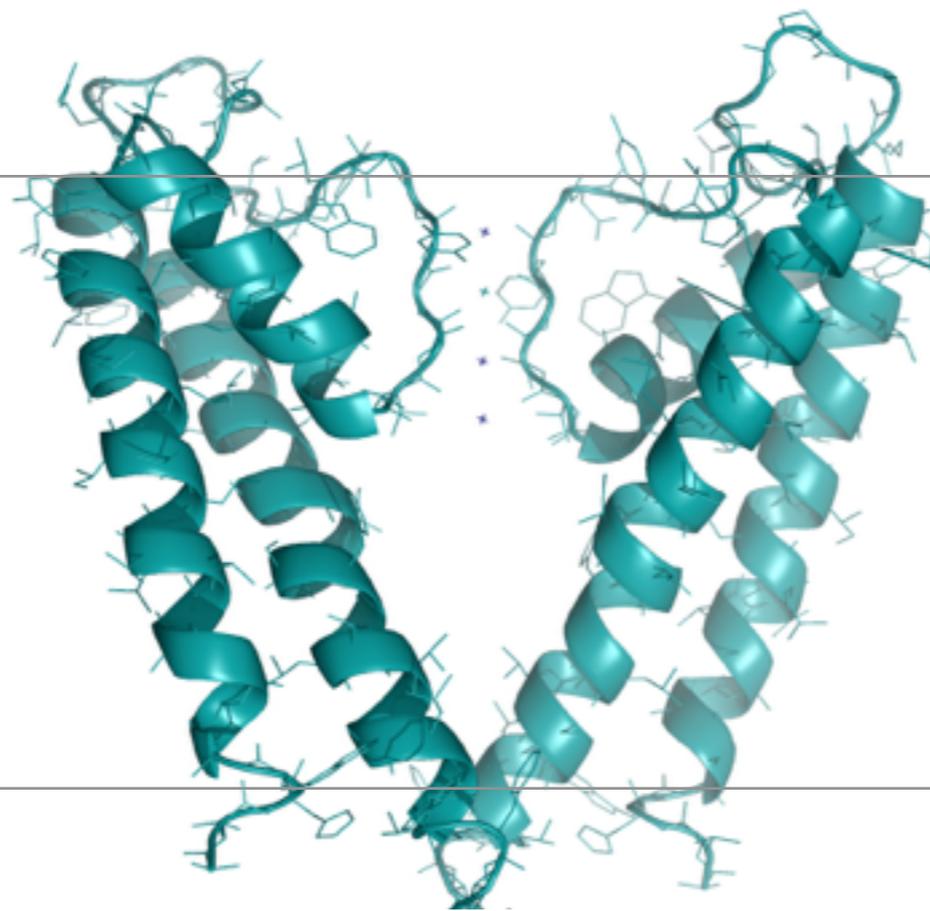
Die Röntgenstruktur eines K_v -Kanals



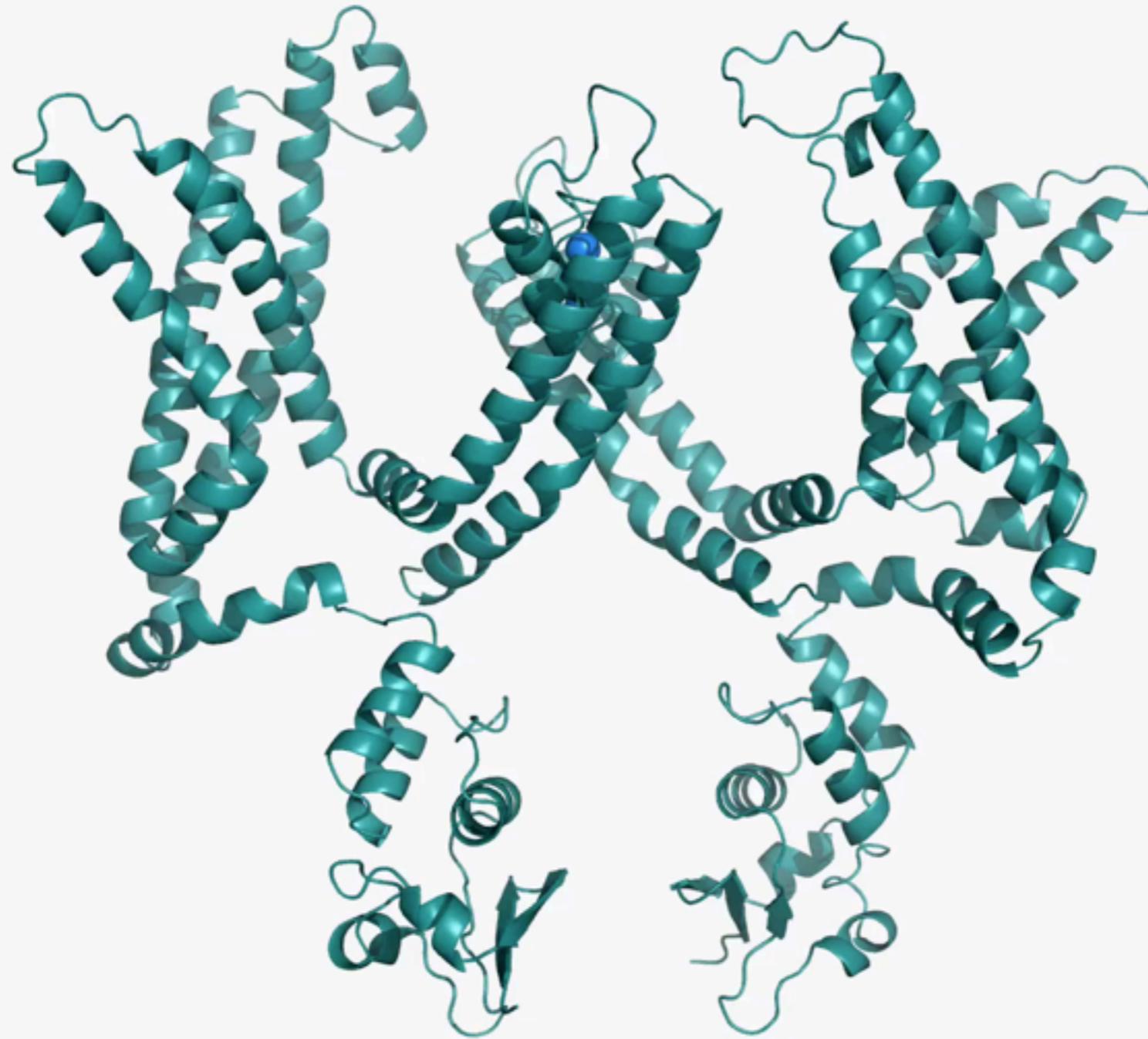
Rod MacKinnon (Nobel Prize 2005)

KcsA

Der Selektivitätsfilter

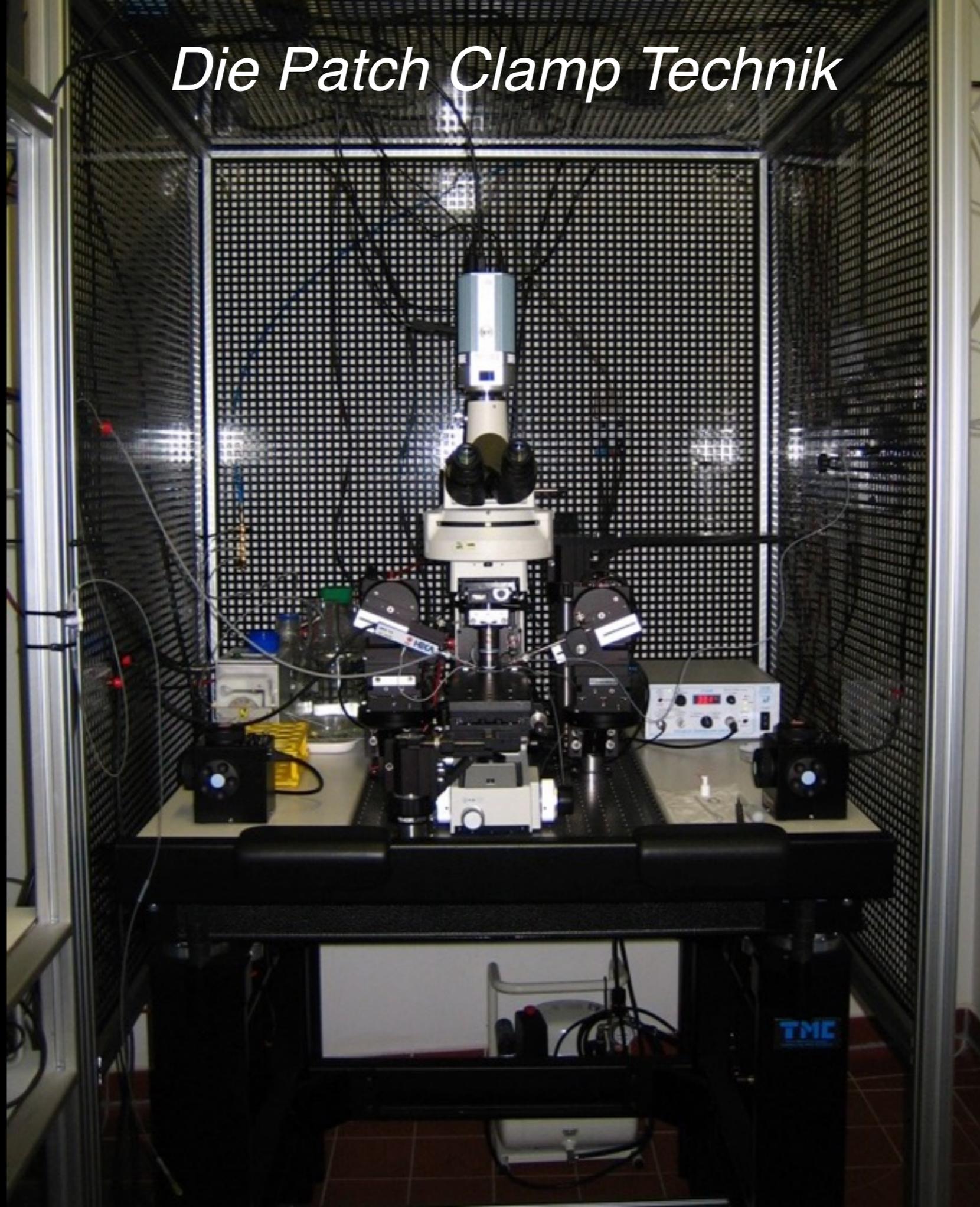


Der Spannungssensor

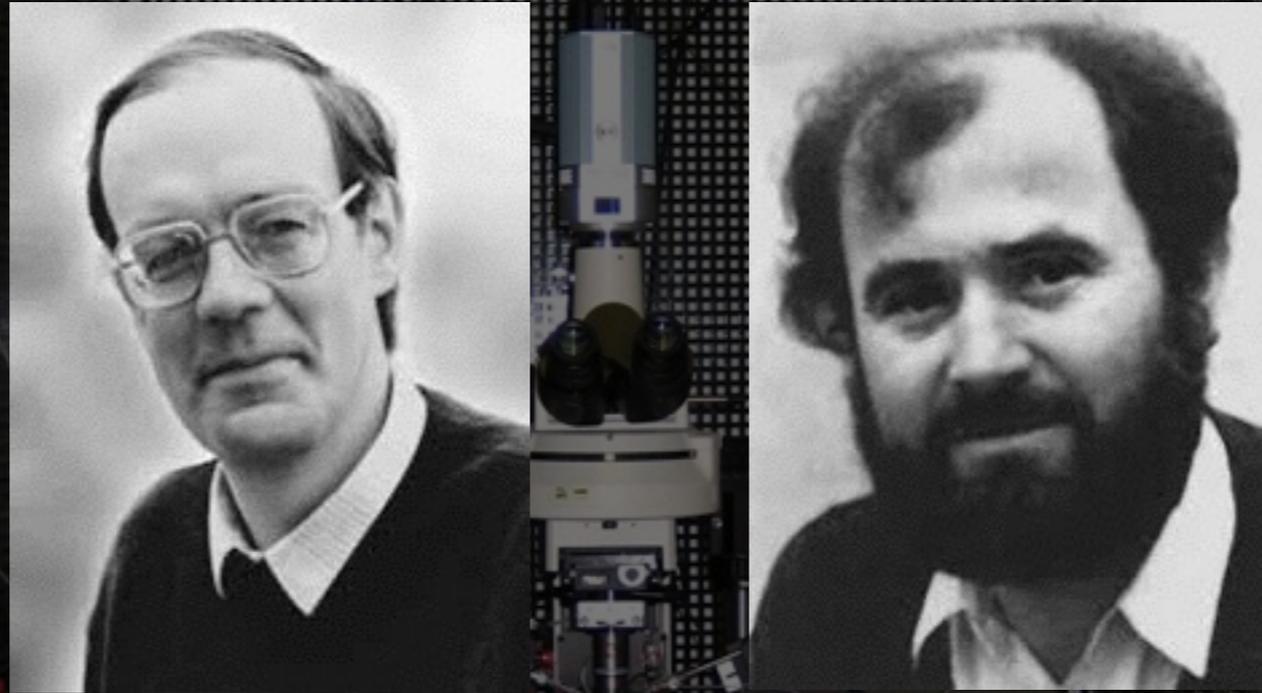


K_{v1.2}

Die Patch Clamp Technik

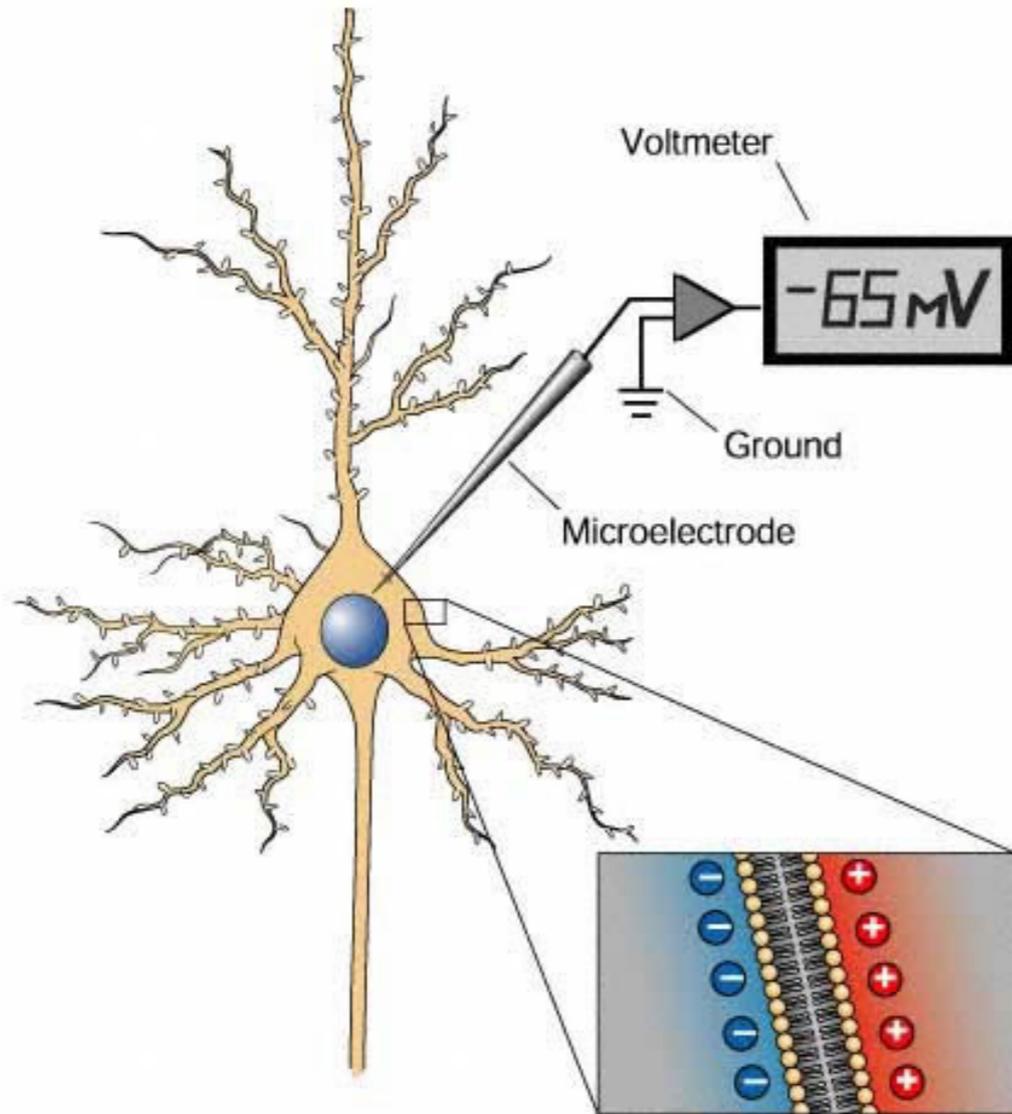


Die Patch Clamp Technik

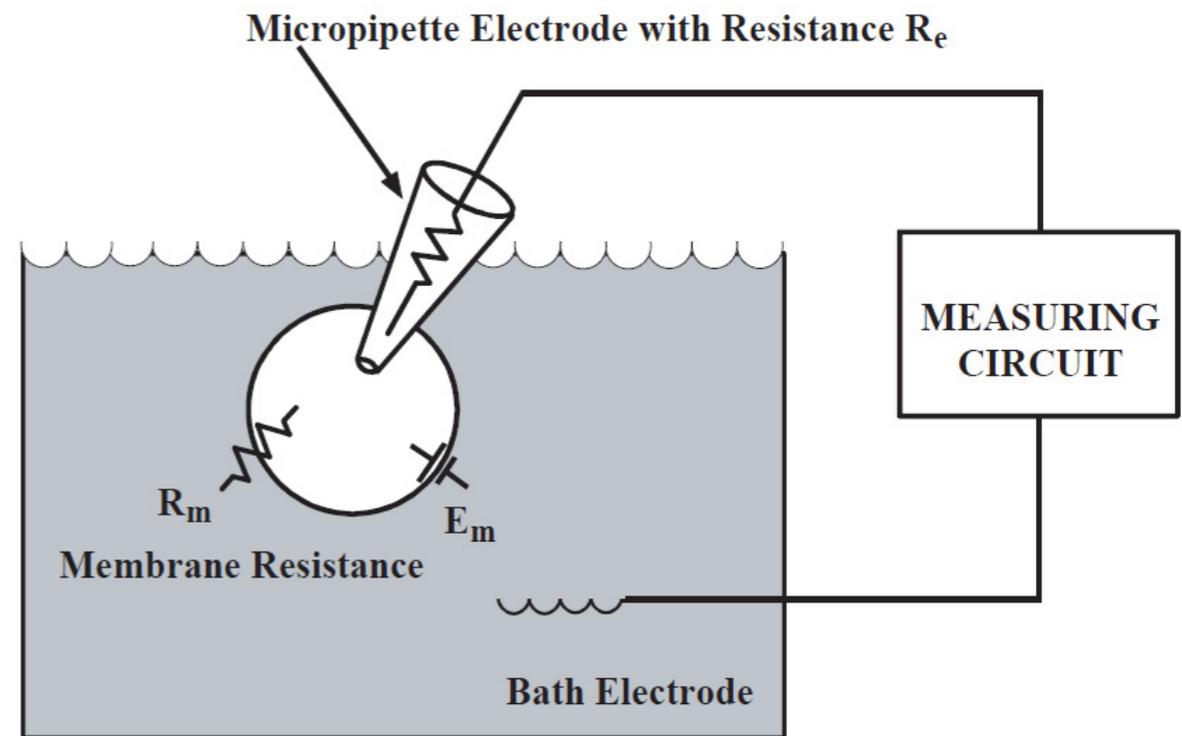


Bert Sakmann und Erwin Neher
Nobelpreis 1991

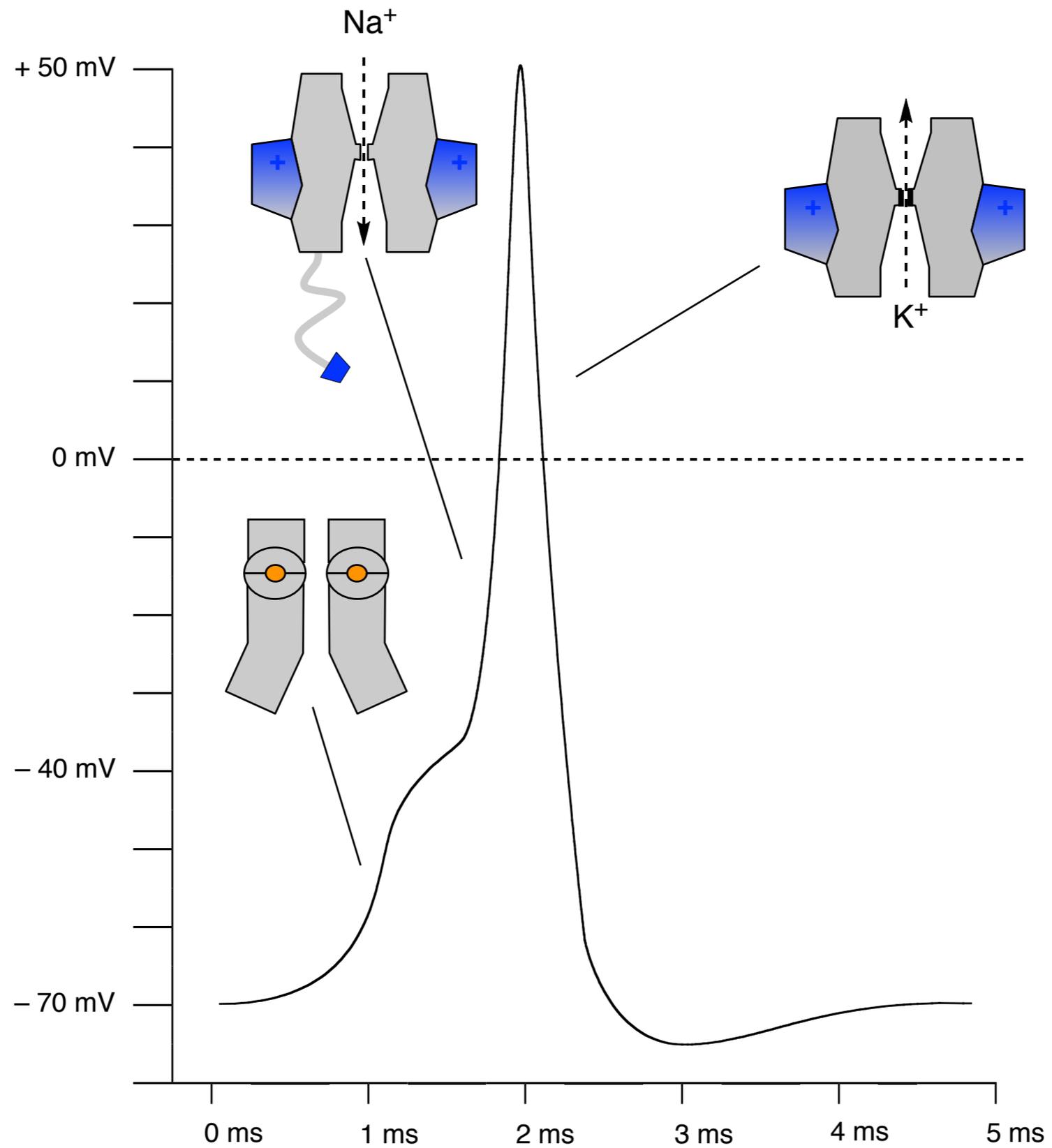
Die Patch Clamp Technik



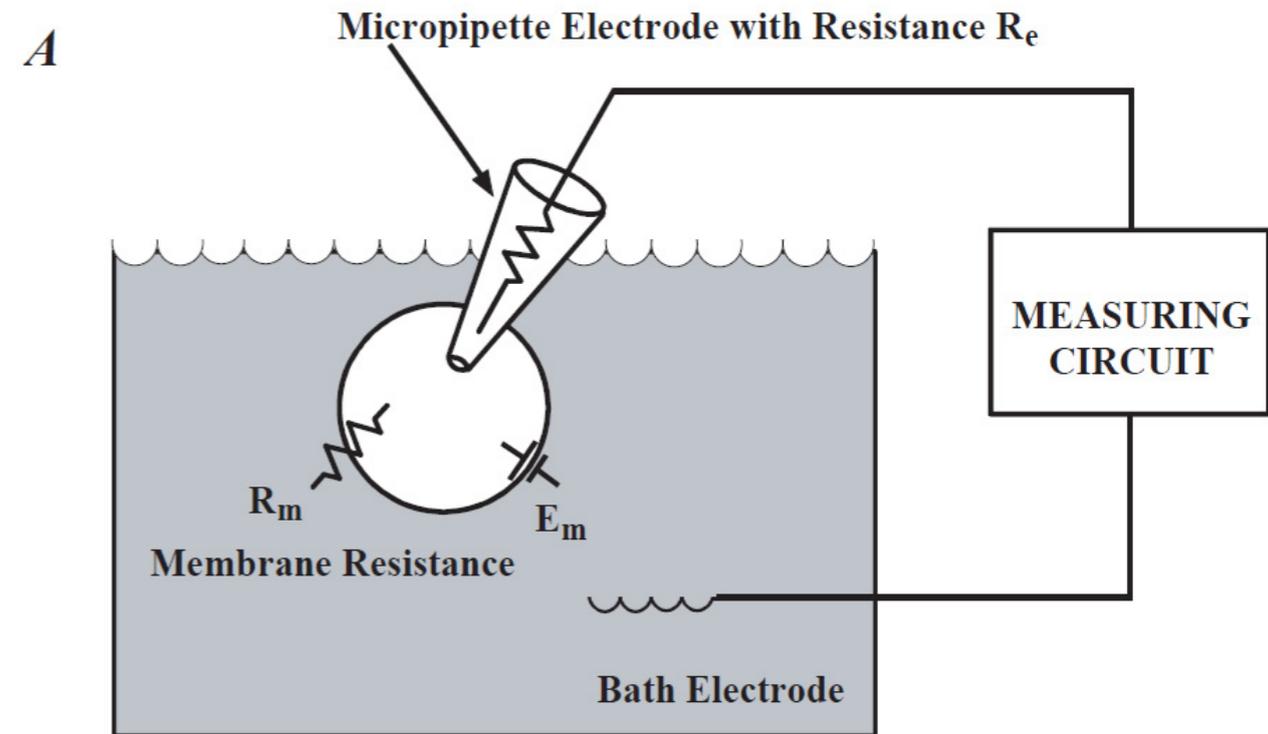
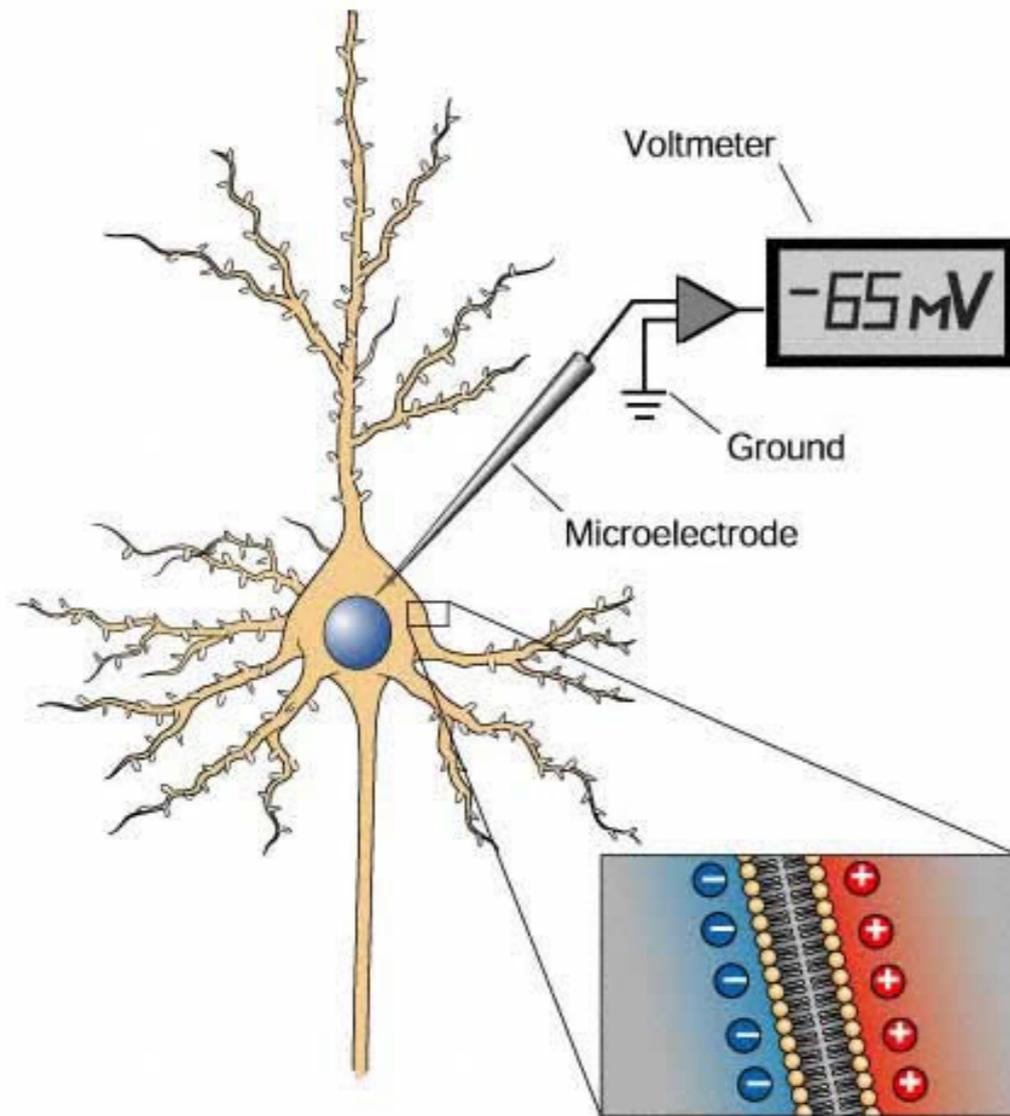
A



Das Aktionspotential



Die Patch Clamp Technik



Stromklemme



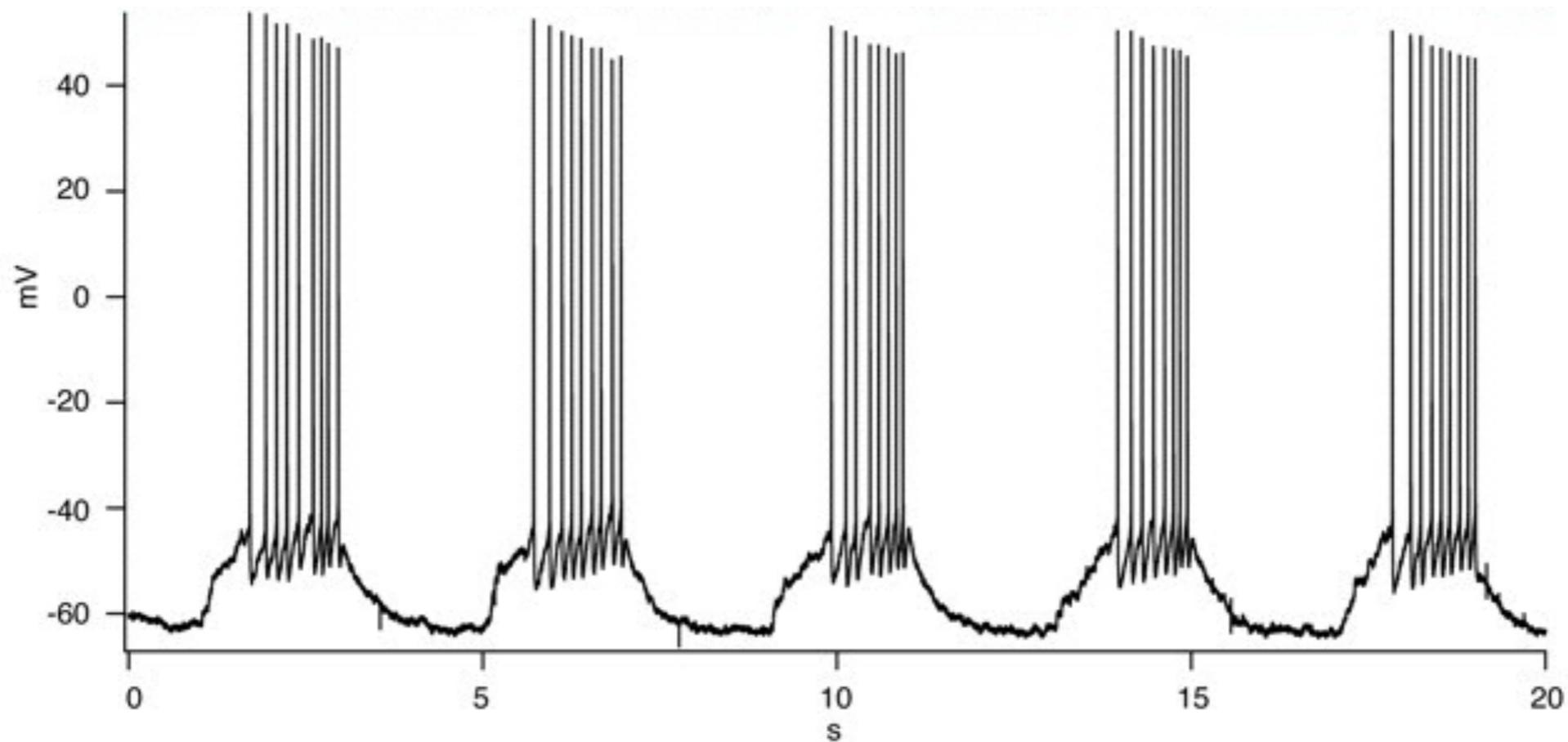
Änderung der Spannung (Aktionspotentiale)

Spannungsklemme

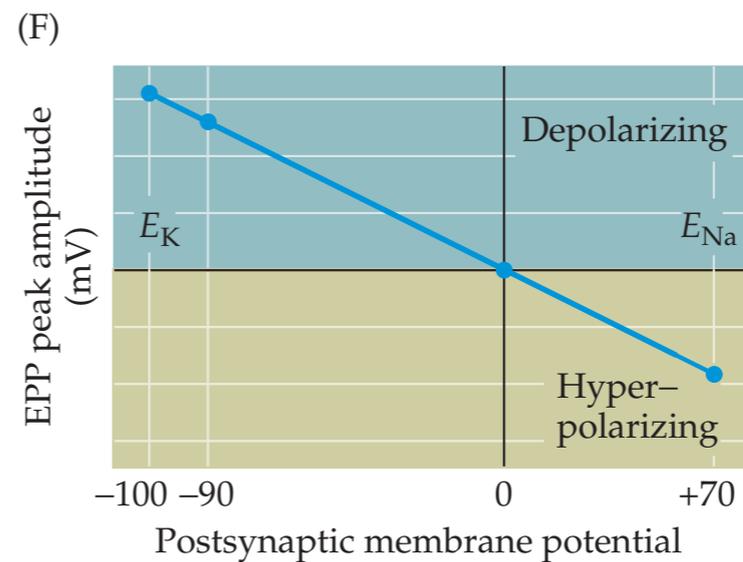
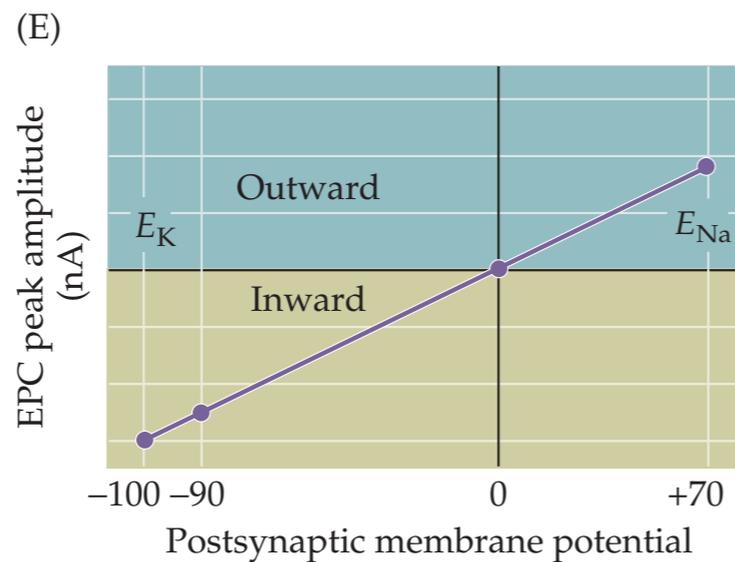
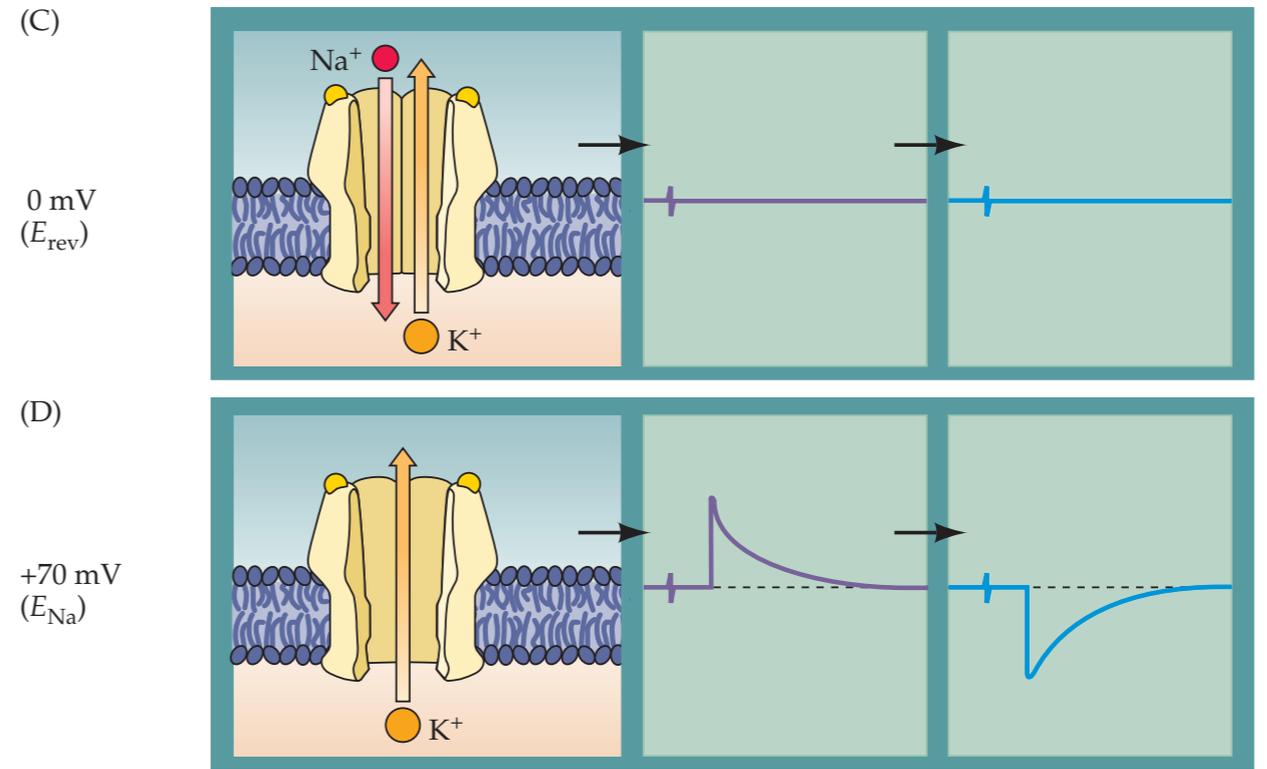
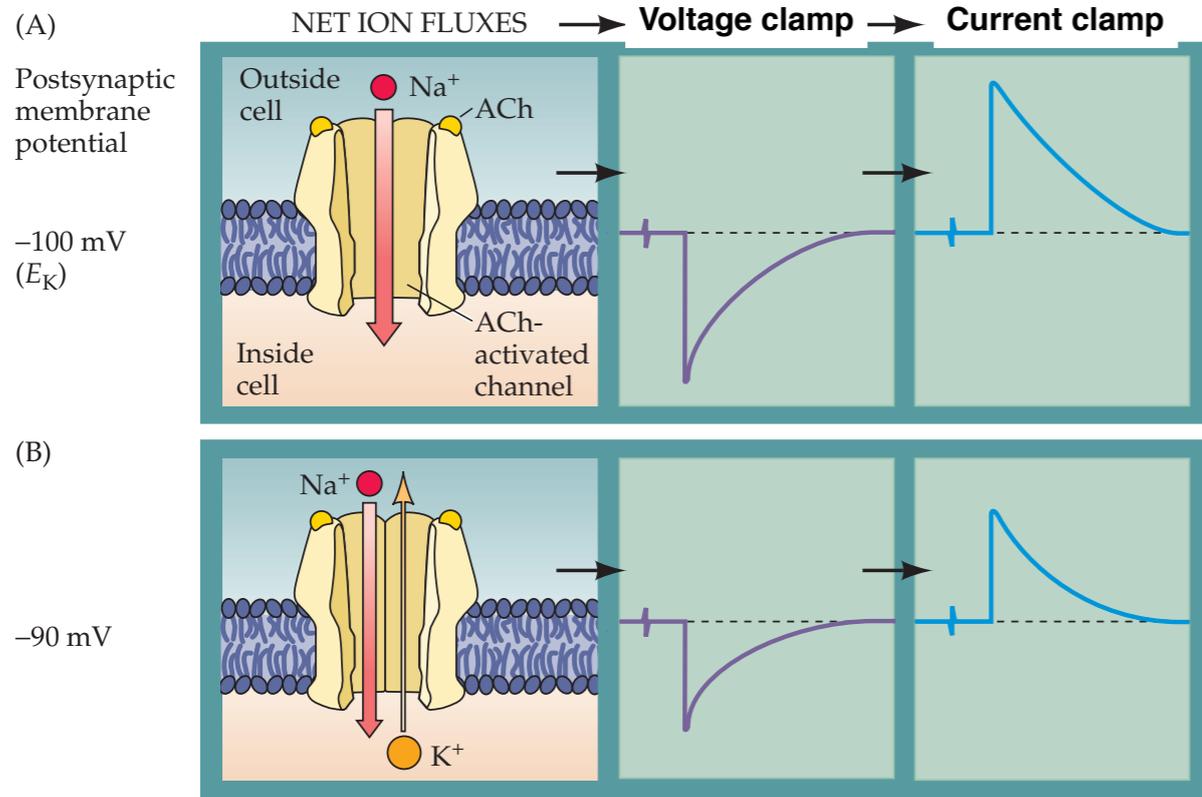


Änderung des Stroms.
Die Zelle wird auf einem vorgegebenen Haltepotential „geklemmt“.

Excitable cells (e.g. neurons) change their transmembrane potential quickly



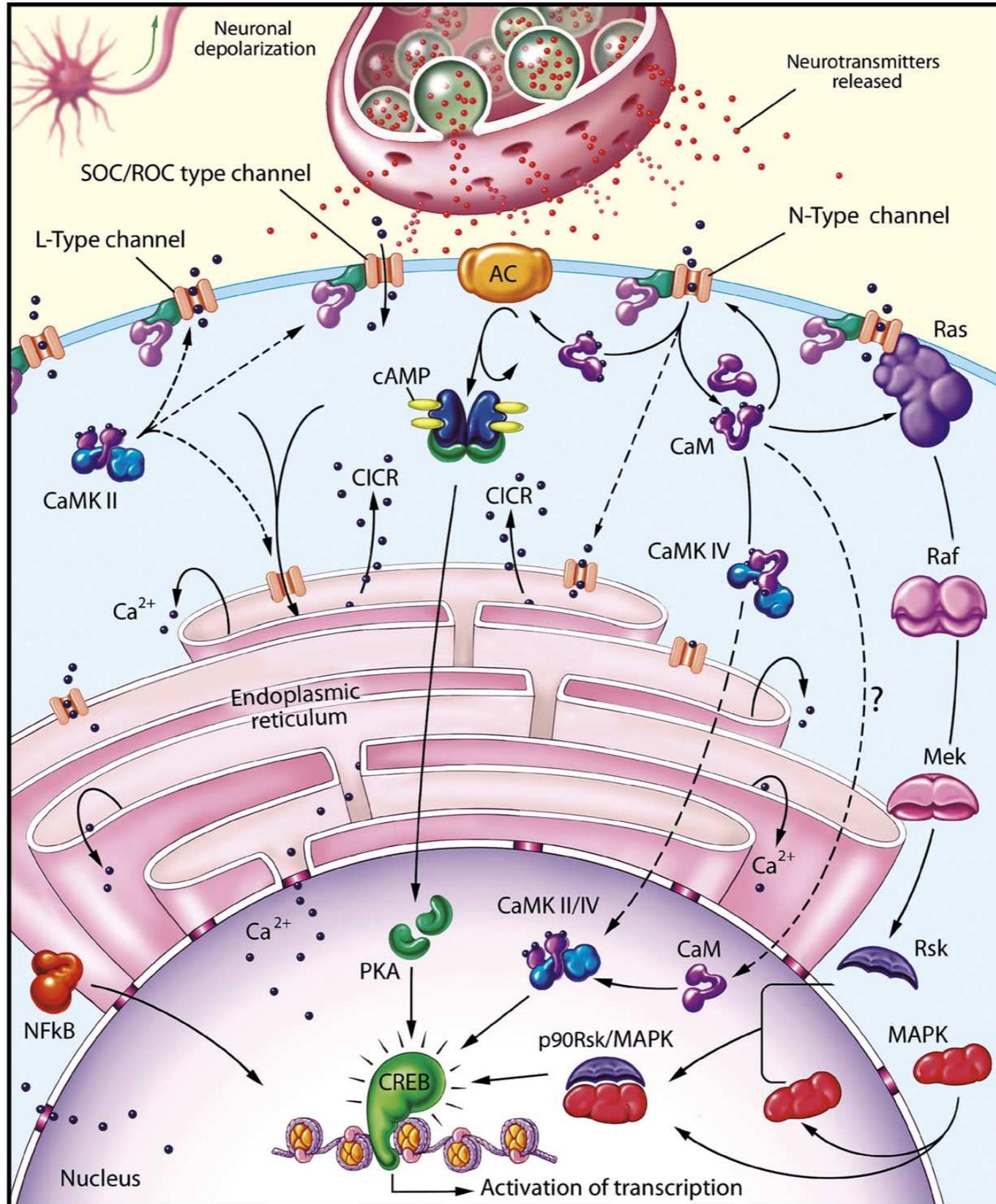
Vergleich: Current - Voltage clamp



Kalziummessungen

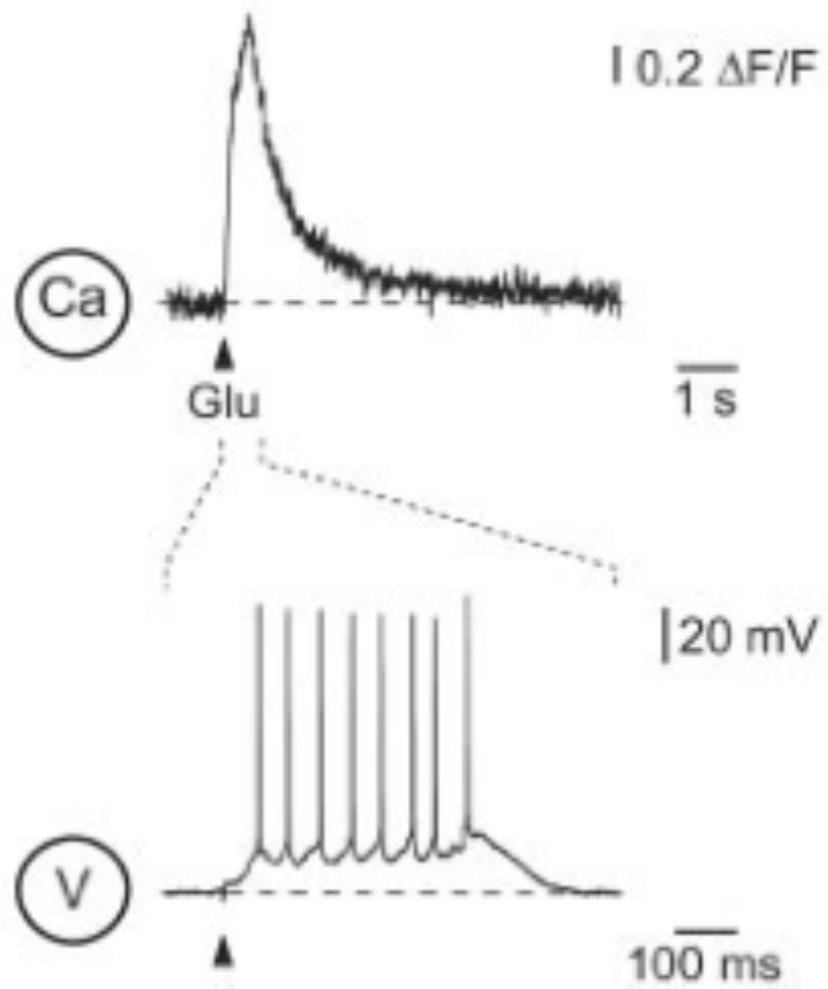
Calcium Imaging

Wozu?

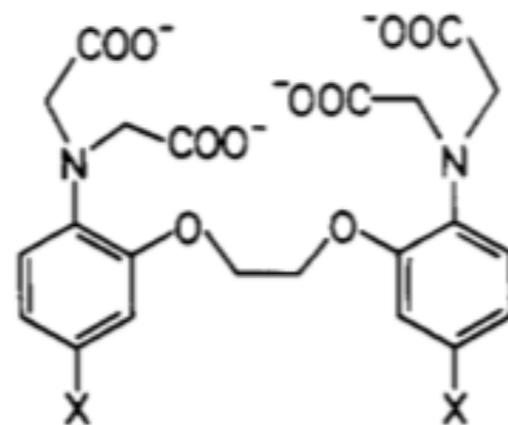
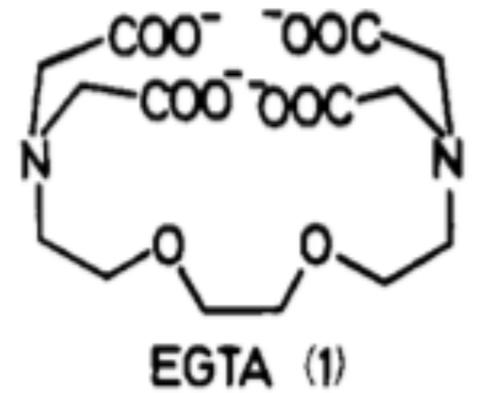


Und deshalb!

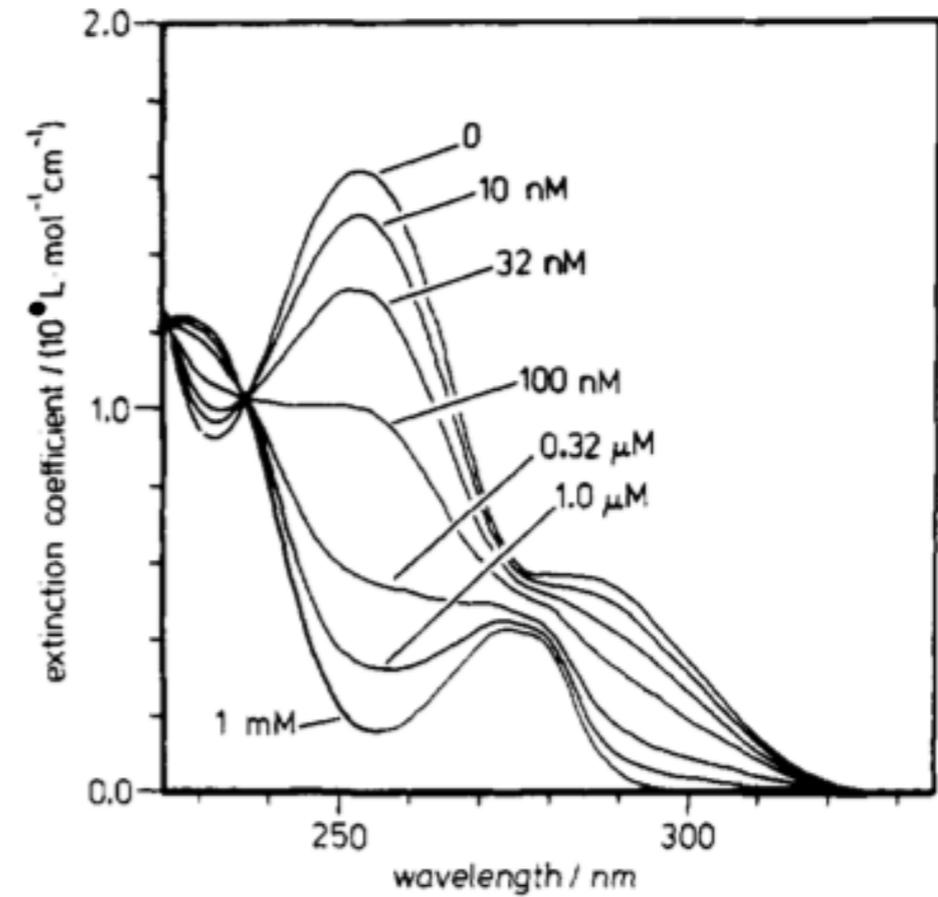
C



Und wie macht man das?

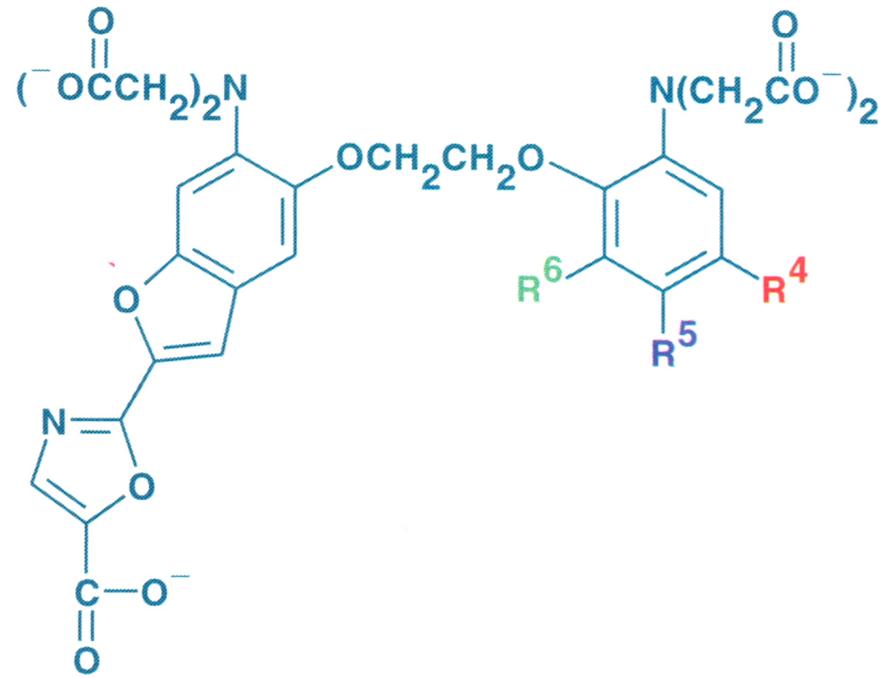


BAPTA



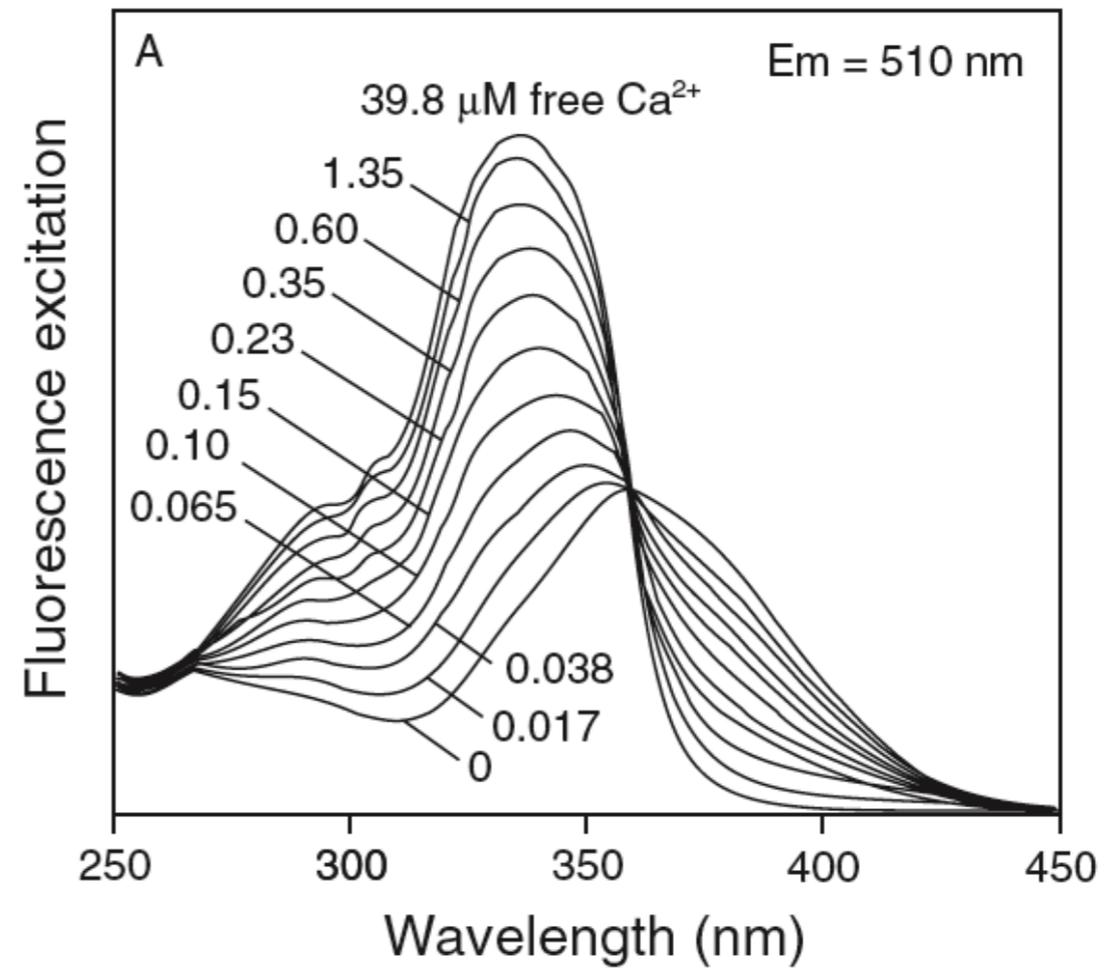
Roger Tsien, Nobelpreis 2008

Und so!

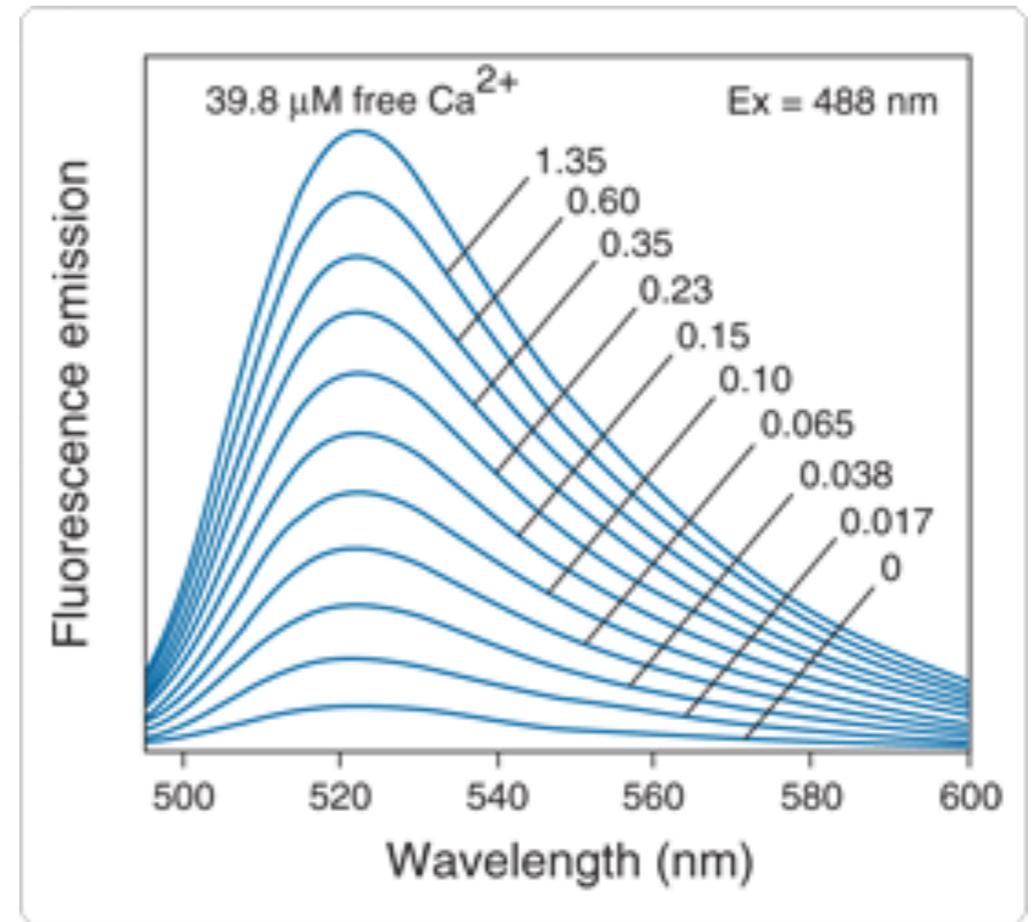
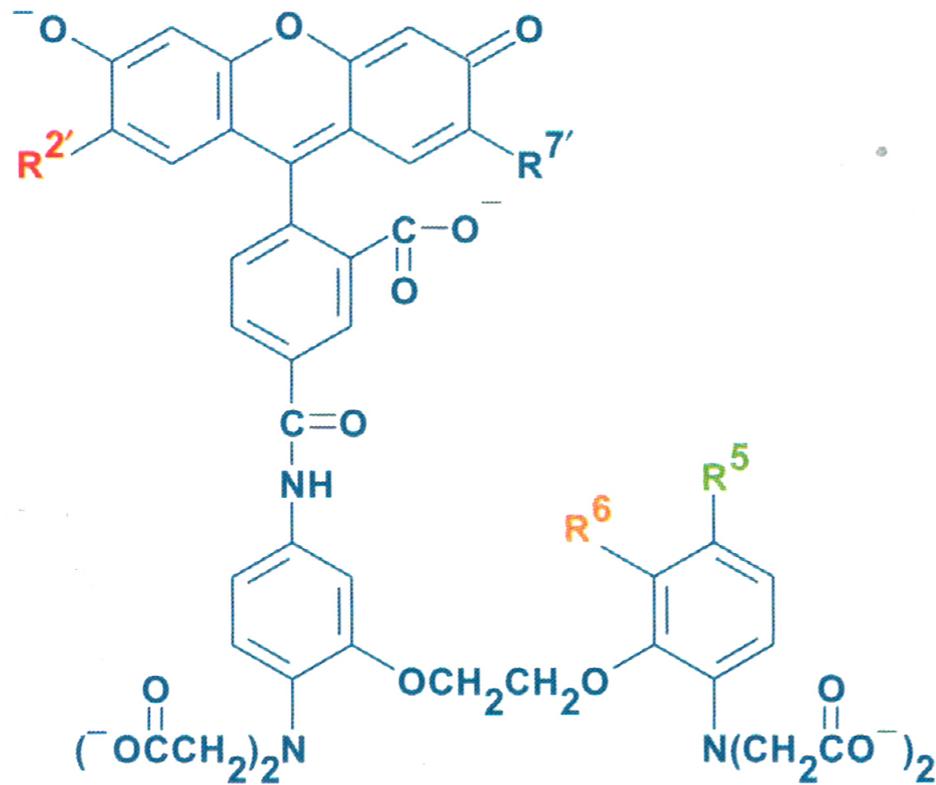


Indicator	$K_d(\text{Ca}^{2+})$	R^4	R^5	R^6
Fura-2	0.14 μM	H	CH_3	H
Fura-5F	0.40 μM	H	F	H
Fura-4F	0.77 μM	F	H	H
Fura-6F	5.30 μM	H	H	F
Fura-FF	5.50 μM	H	F	F

Figure 19.5 Fura indicators with varying Ca^{2+} affinities.

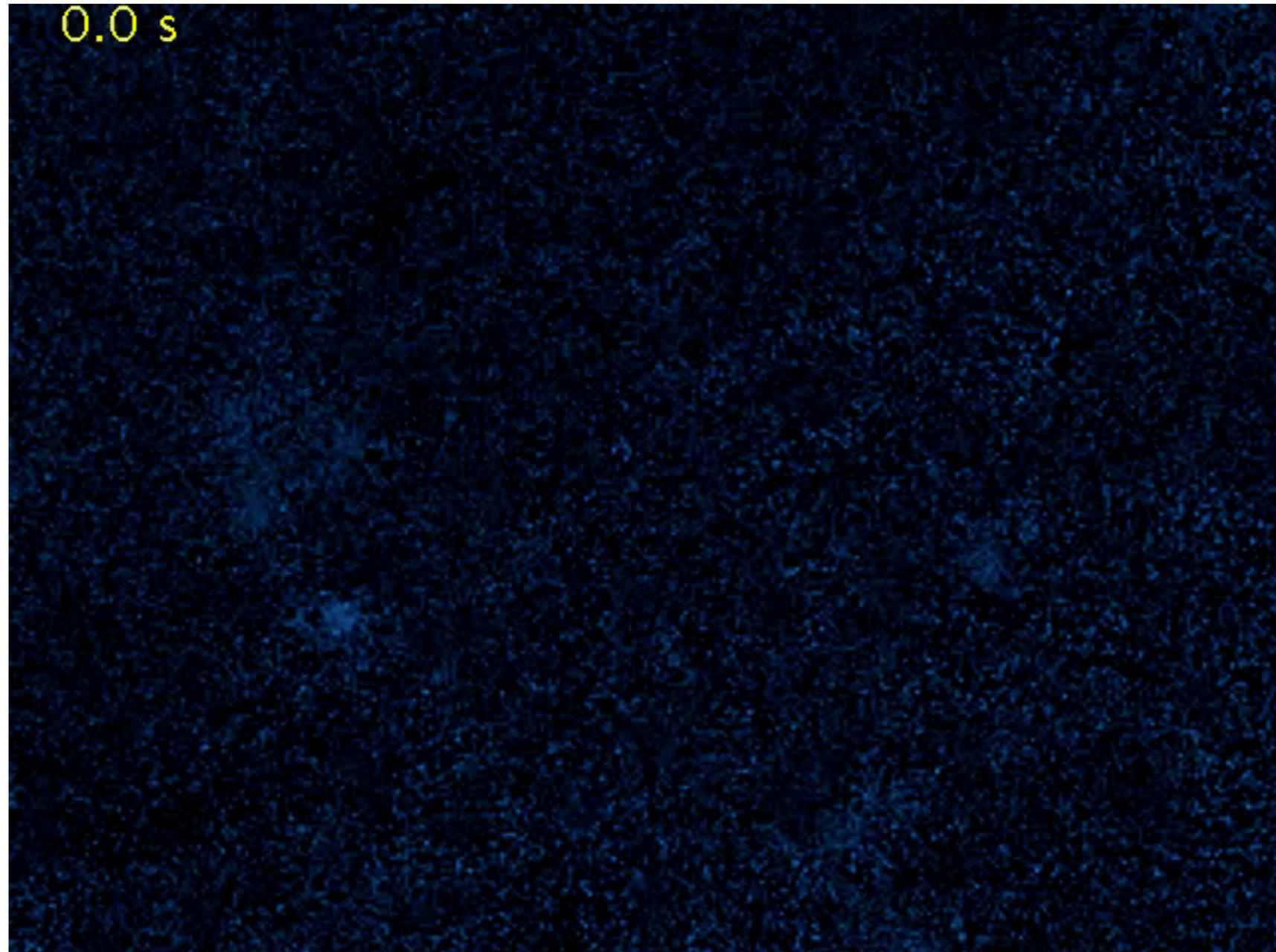


Oder so!



Indicator	K _d (Ca ²⁺)	R ^{2'}	R ^{7'}	R ⁵	R ⁶
Calcium Green-1	0.19 μM	Cl	Cl	H	H
Calcium Green-5N	14 μM	Cl	Cl	NO ₂	H
Oregon Green 488 BAPTA-1	0.17 μM	F	F	H	H
Oregon Green 488 BAPTA-6F	3 μM	F	F	H	F
Oregon Green 488 BAPTA-5N	20 μM	F	F	NO ₂	H

Und wie sieht das dann aus?



Applikation eines Glutamat Analog
auf Zellen des Hippocampus

Zusammenfassung Neurophysiologie

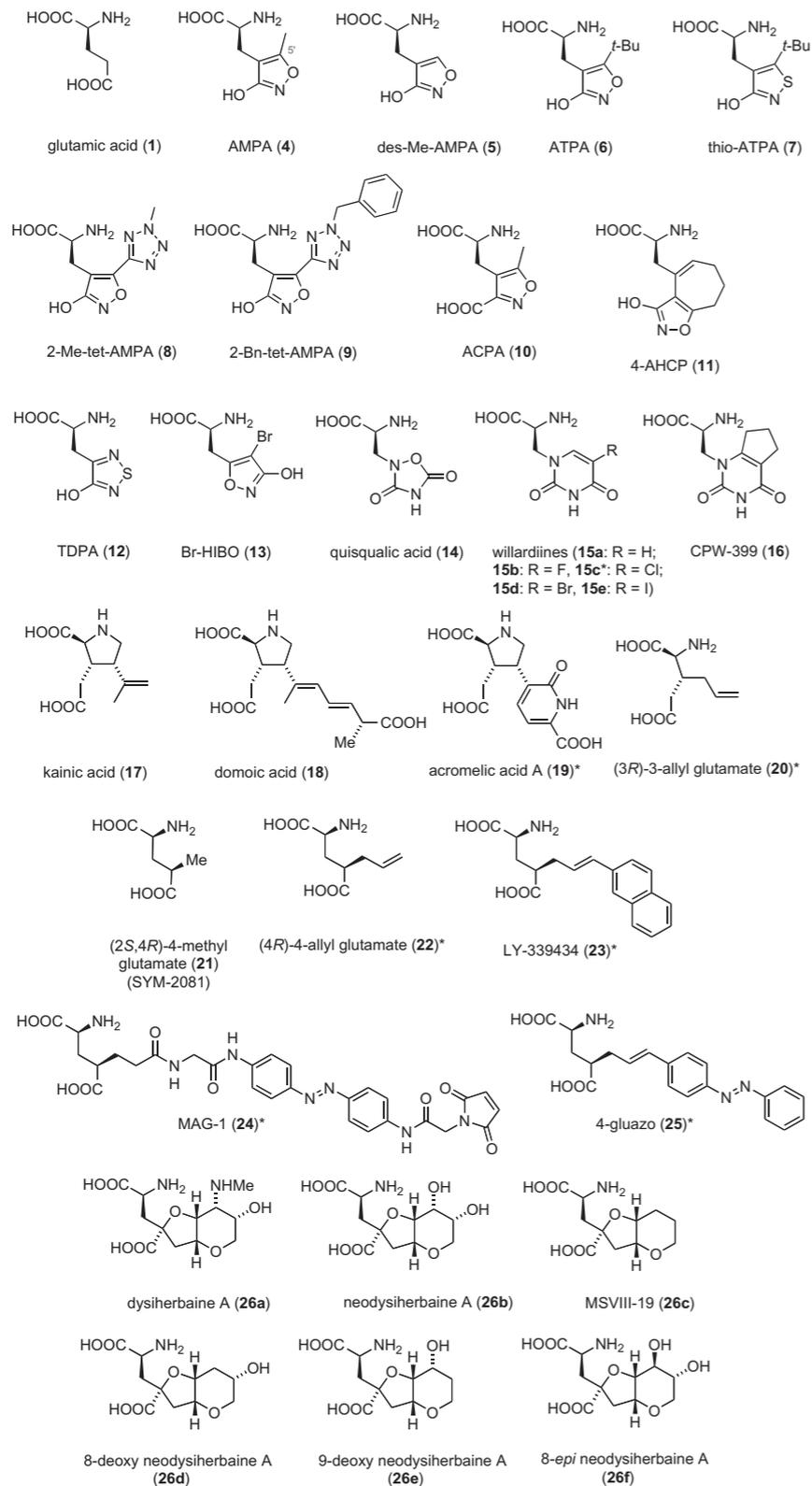
- Neuronen haben ein negatives Ruhepotential von etwa -70mV
- Neuronen kommunizieren durch synaptische Übertragung
- Die Freisetzung von Neurotransmitter Vesikeln, erfolgt durch Kalziumeinstrom in das präsynaptische Terminal (spannungsgesteuerte Ca^{2+} - Kanäle)
- Aktionspotentiale werden durch spannungsgesteuerte Na^+ und K^+ - Kanäle geformt
- Elektrische Signale in Zellen können durch die patch clamp Technik gemessen werden
- Kalzium als wichtigstes intrazelluläres Signalmolekül kann durch fluoreszierende Kalziumsensoren zur Bildgebung von zellulärer Aktivität (Calcium Imaging) benutzt werden

Pharmakologie

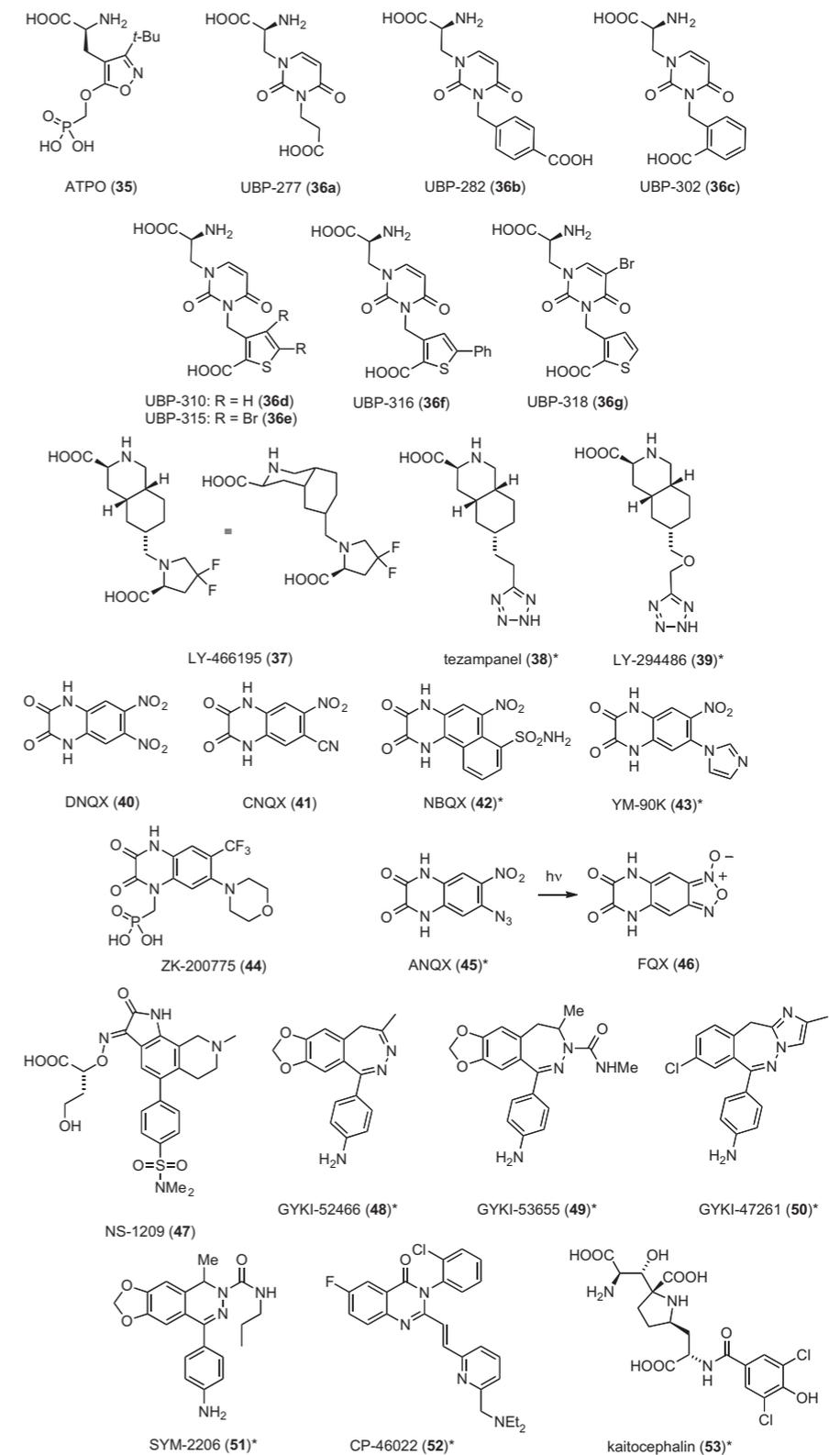
Agonisten

und

Antagonisten

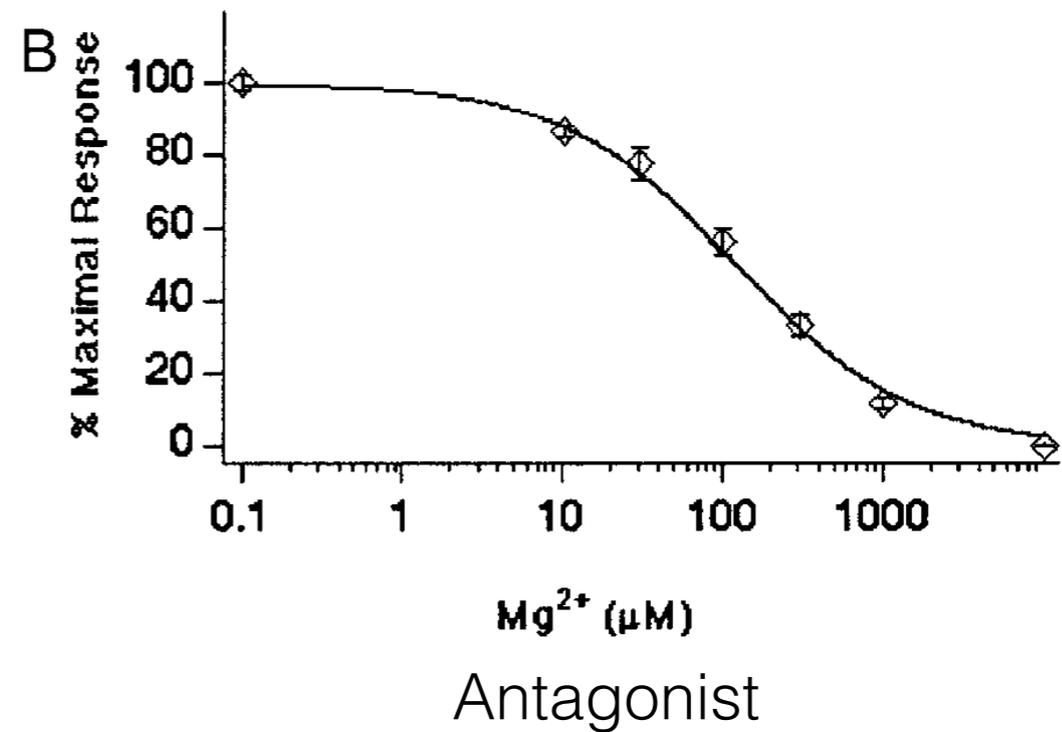
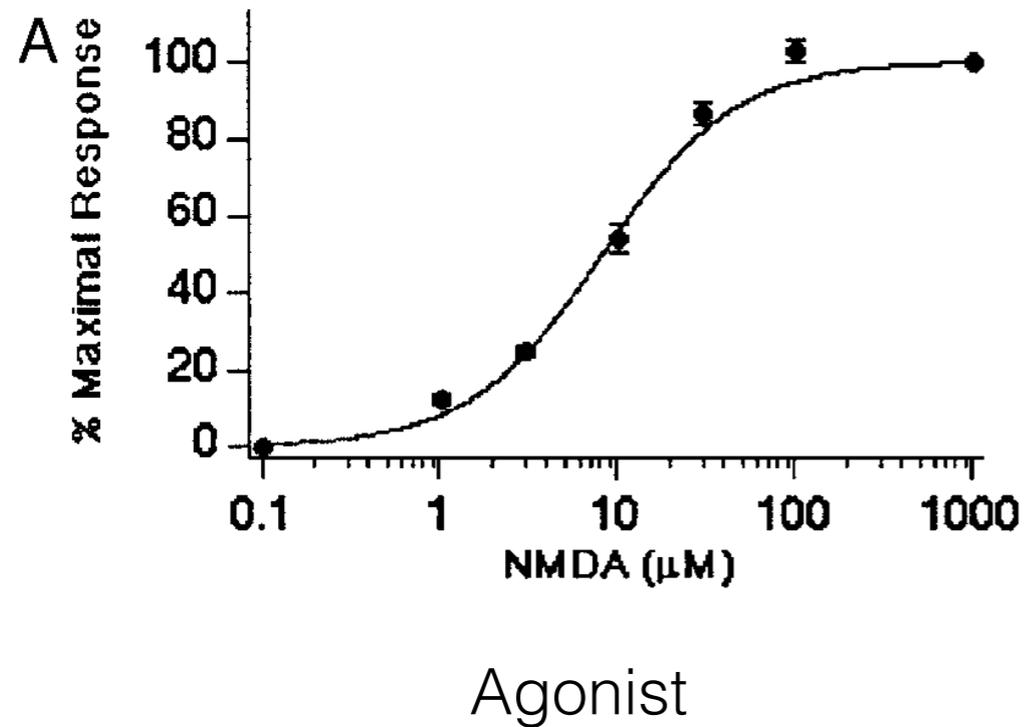


AMPA Rezeptor ionotroper Glutamatrezeptor

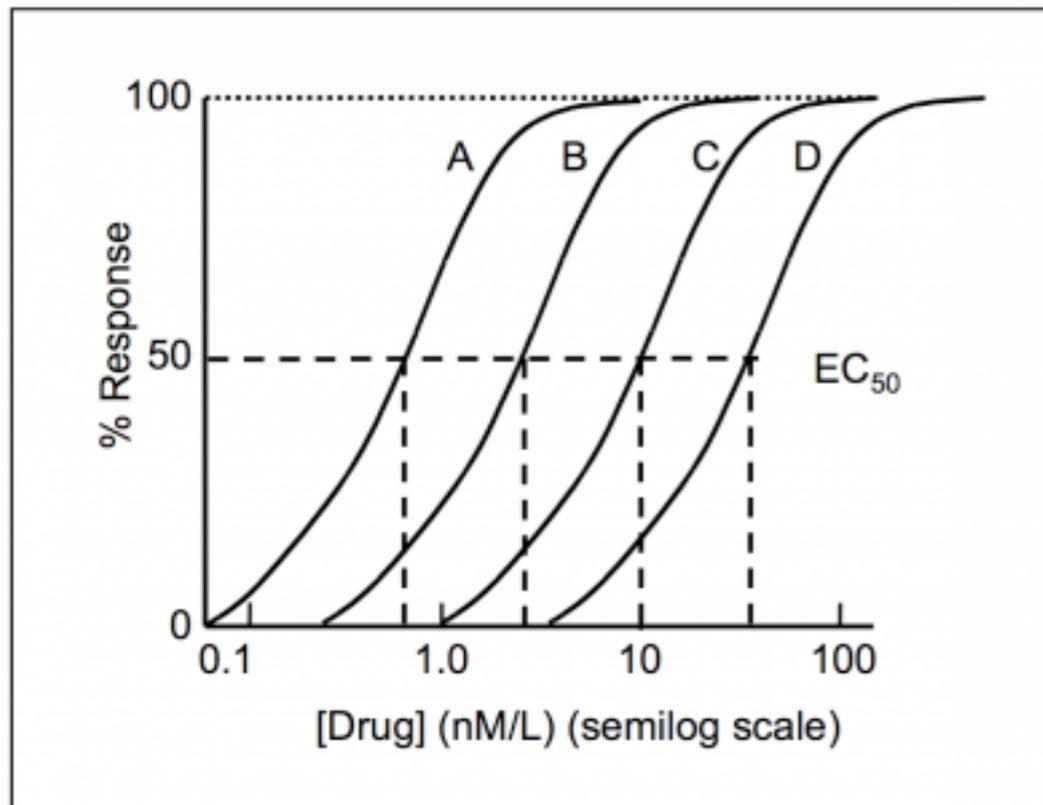


Dosis-Wirkungs-Kurven

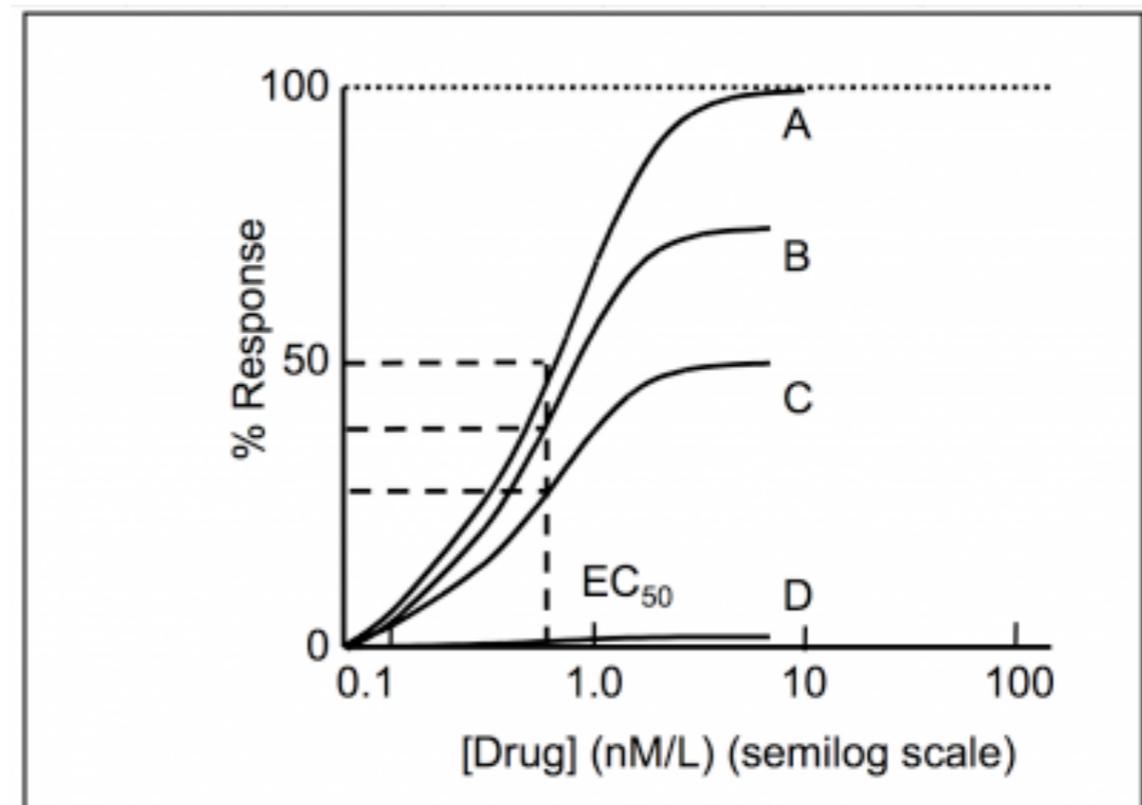
NMDA Glutamatrezeptor



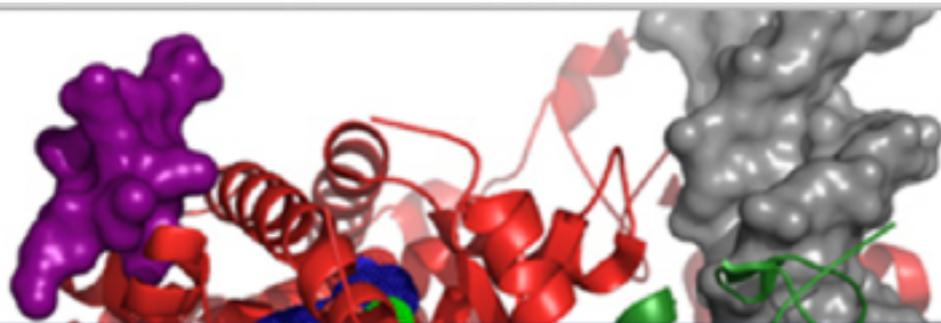
Wirksamkeit und Affinität



Gleiche Wirksamkeit aber unterschiedliche Affinität



Gleicher EC₅₀ aber unterschiedliche Wirksamkeit
partieller Agonismus



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IUPHAR/BPS Guide to PHARMACOLOGY

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An expert-driven guide to pharmacological targets and the substances that act on them.

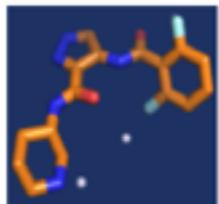
Targets



- ▶ G protein-coupled receptors
- ▶ Ion channels
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- ▶ Kinases
- ▶ Catalytic receptors
- ▶ Transporters
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- ▶ Other protein targets

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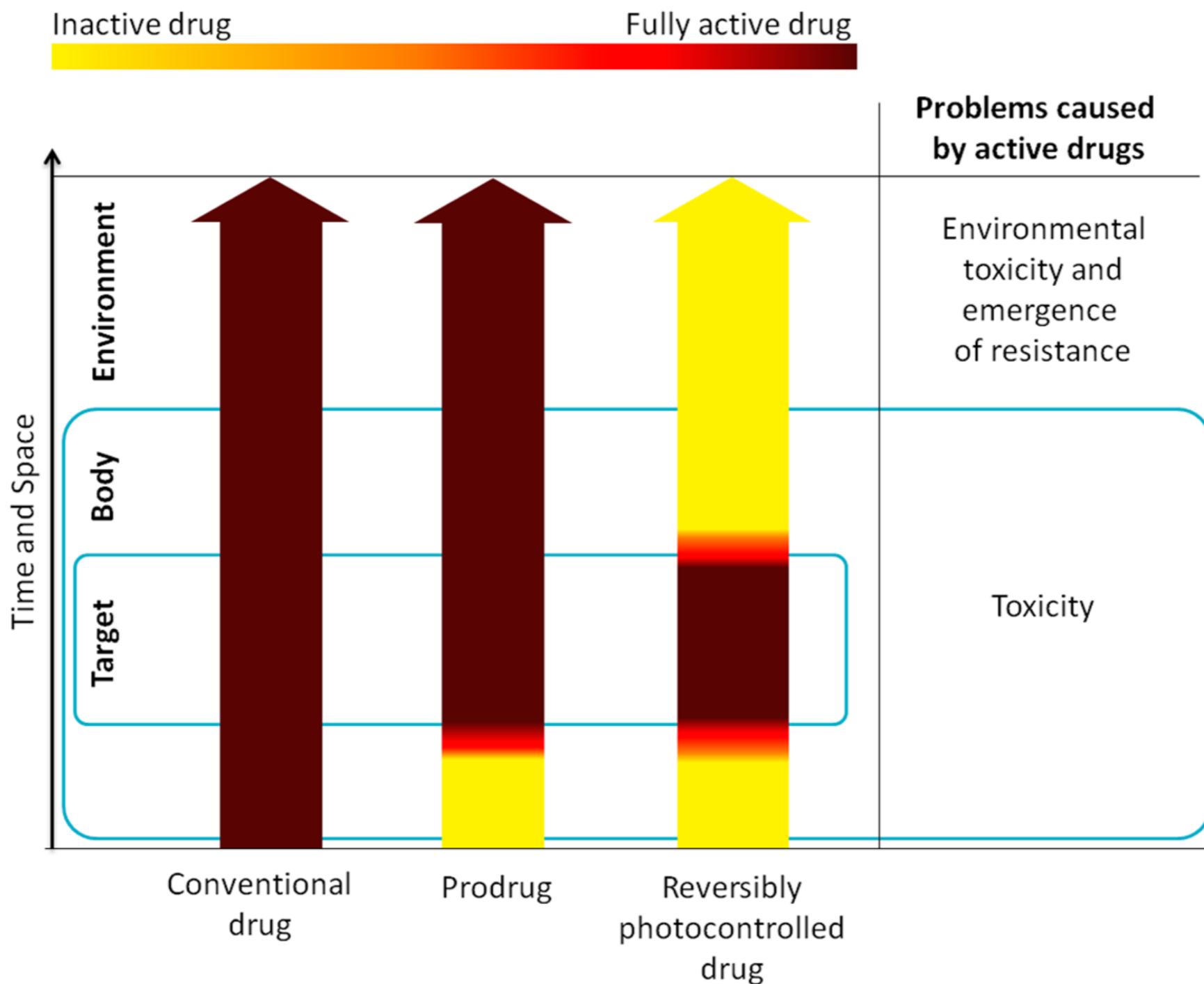


<http://www.guidetopharmacology.org/>

Pharmakologie vs. Photopharmakologie

- Problem Nebenwirkung: geringe Selektivität des Medikaments
- das therapeutische Fenster ist sehr schmal (z.B. zytotoxische Antikrebsmedikamente)
- mit Licht könnten Medikamente lokal am Wirkungsort aktiviert werden

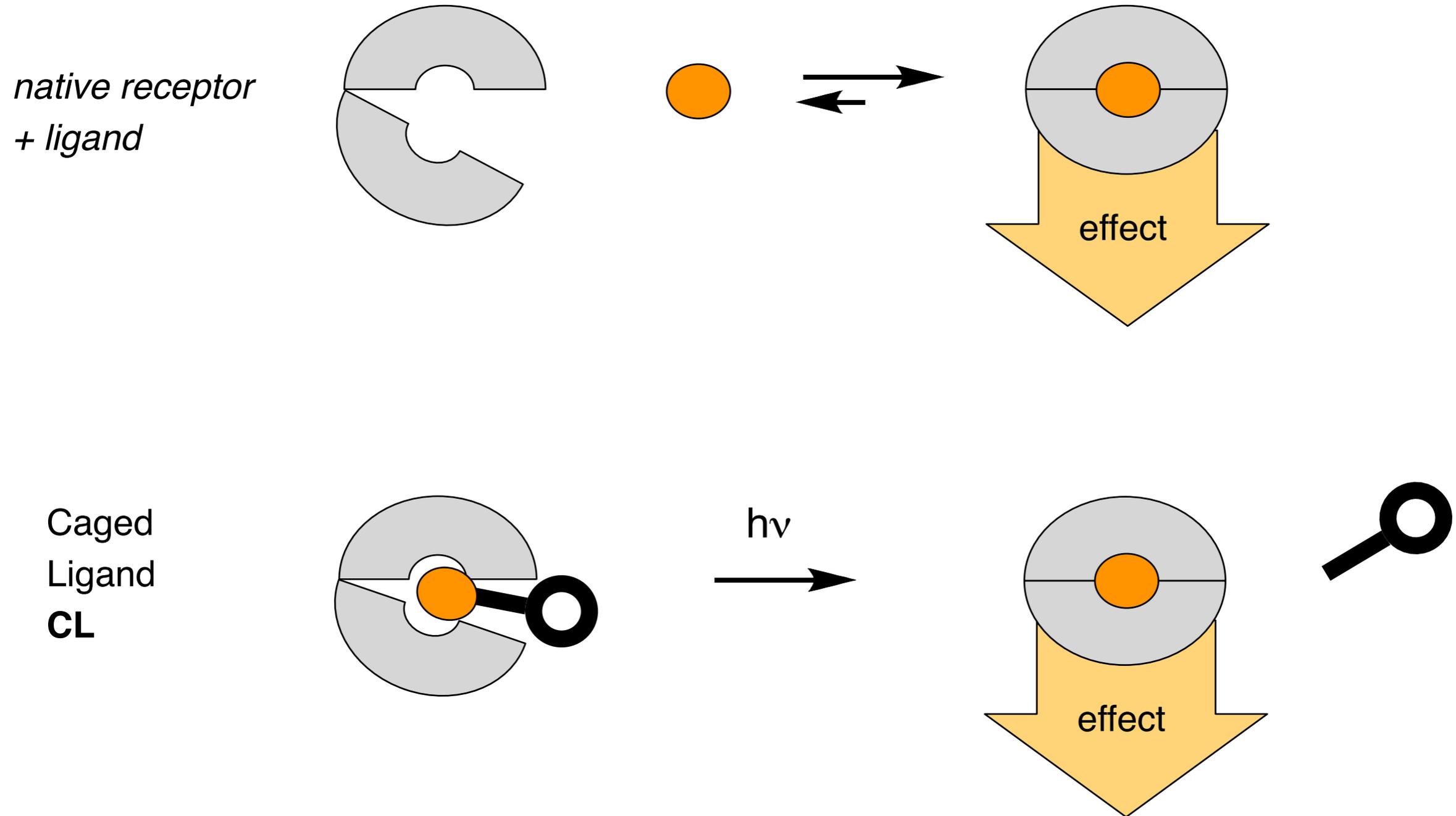
Vorteil der Photopharmakologie



Pharmakologie vs. Photopharmakologie

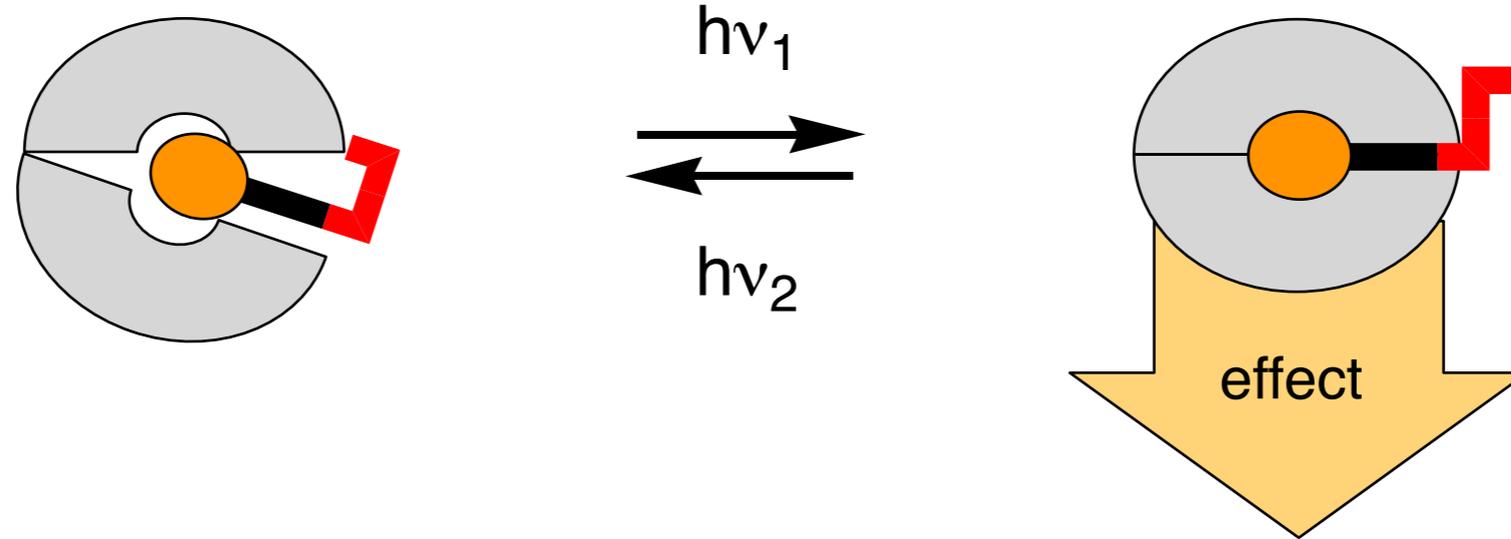
- Problem Nebenwirkung, geringe Selektivität des Medikaments
- das therapeutische Fenster ist sehr schmal (z.B. zytotoxische Antikrebsmedikamente)
- mit Licht könnten Medikamente lokal am Wirkungsort aktiviert werden
- hohe zeitliche und räumliche Genauigkeit
- Licht ist im allgemeinen nicht schädlich
- Problem: Penetration in Gewebe (beste Wellenlänge ca. 650-800 nm)
- Licht kann sehr leicht qualitativ (Wellenlänge) und quantitativ (Intensität) dosiert werden (Photodosierung)

Strategien zur Lichtschaltbarkeitmachung von Rezeptoren

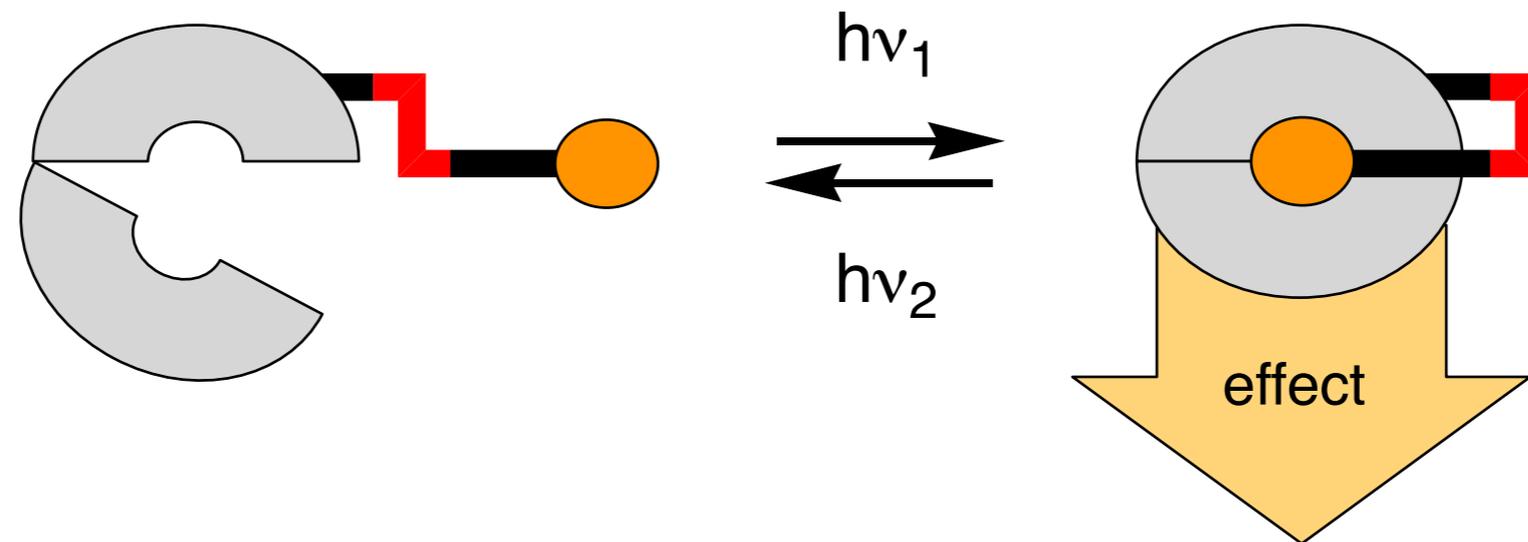


Strategien zur Lichtschaltbarkeitmachung von Rezeptoren

Photochromic
Ligand
PCL



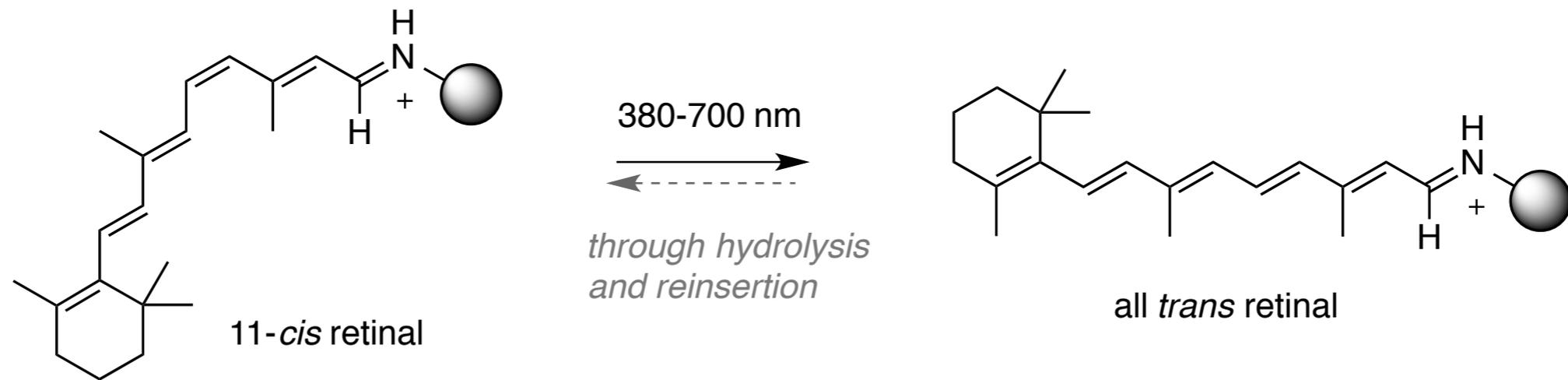
Photoswitched
Tethered
Ligand
PTL



Design von photochromen Liganden

Photopharmakologie

Natürlich vorkommender Lichtschalter

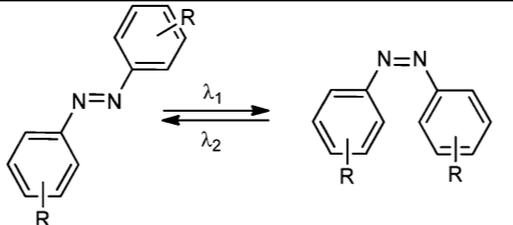
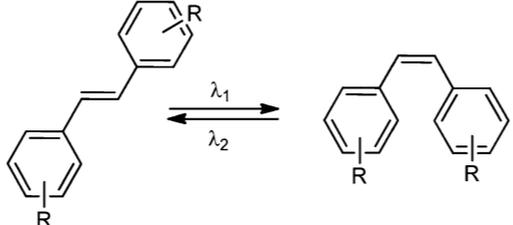
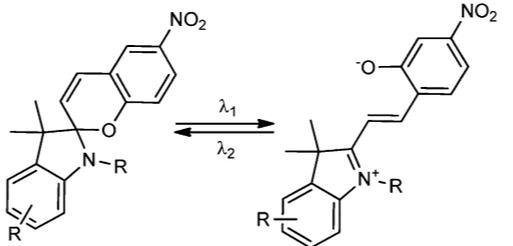
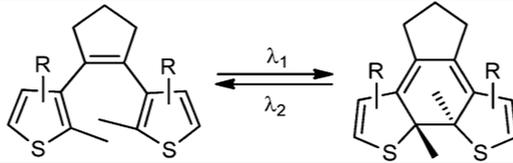
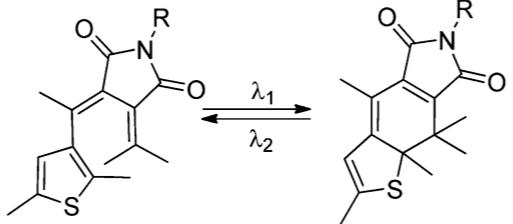
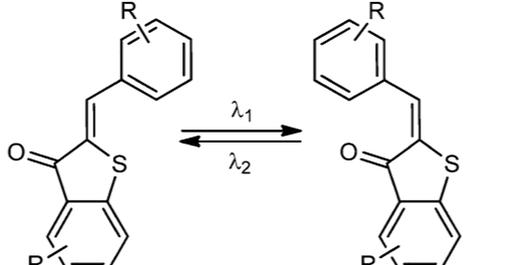


Rhodopsin

Der ideale Photoschalter

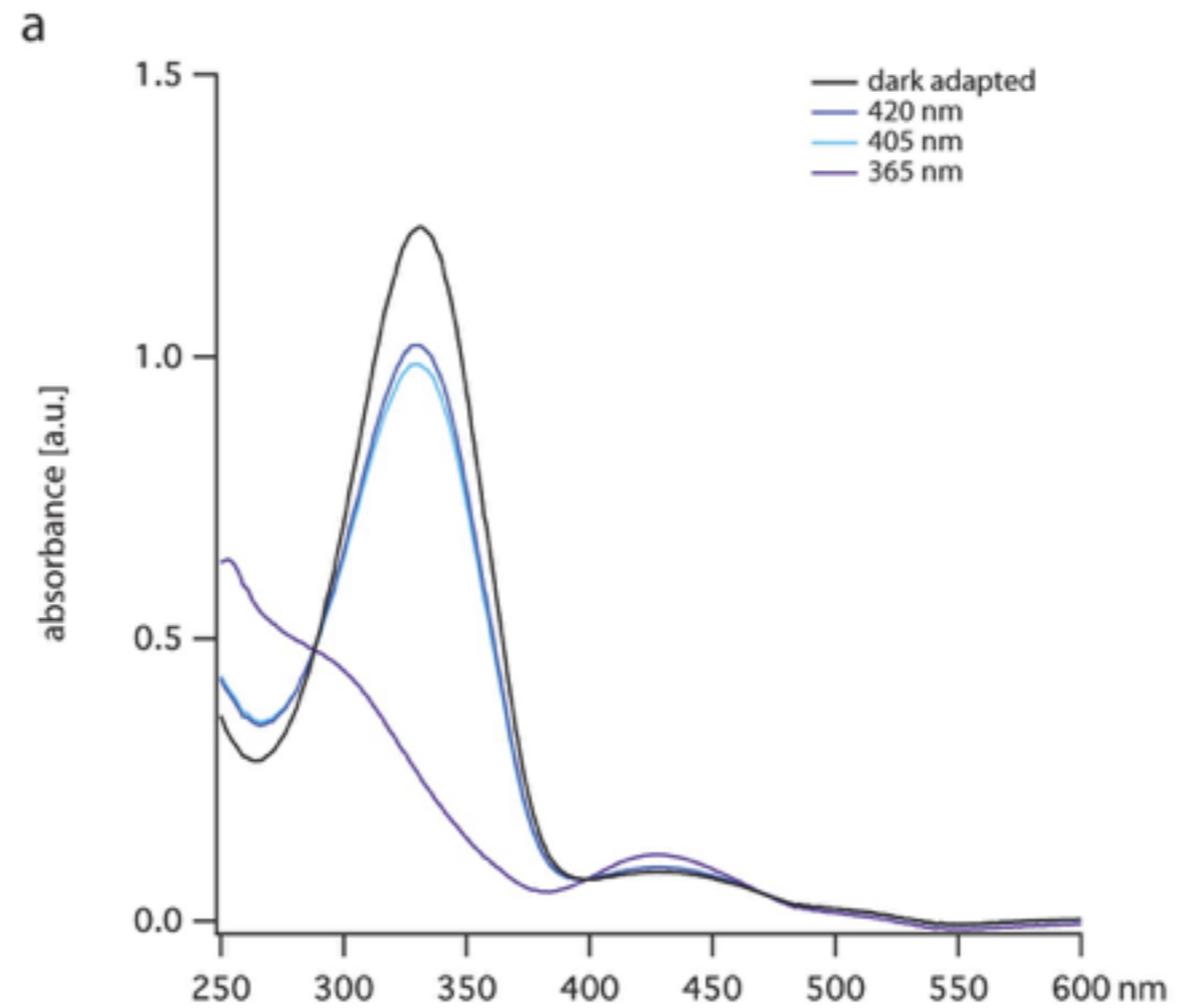
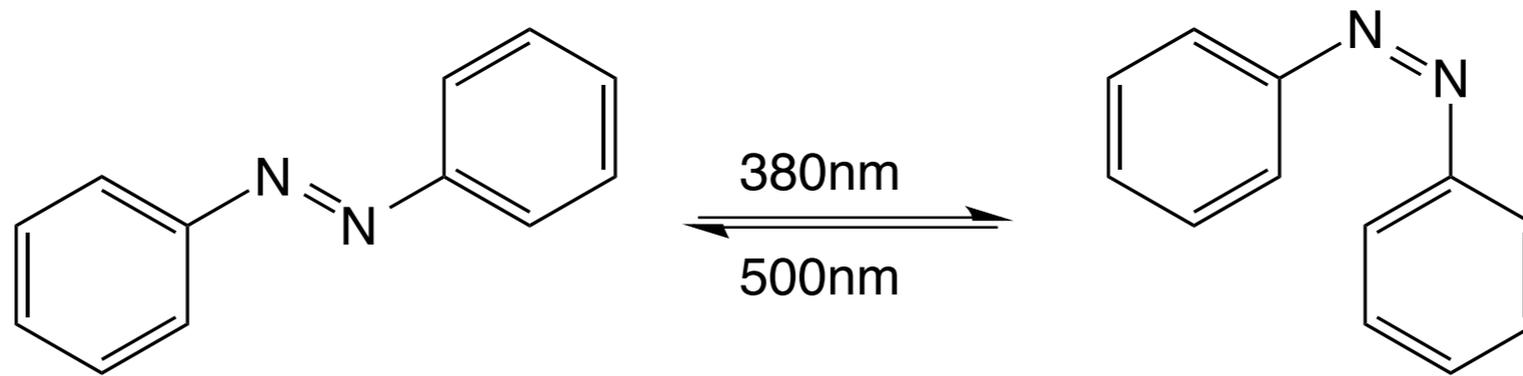
- großer Unterschied in der Aktivität der beiden Isomere
- Licht sollte die Konzentration des aktiven Isomer deutlich erhöhen
- die thermische Relaxierung sollte möglichst in einem für die Anwendung geeigneten Zeitraum erfolgen (Bistabilität)
- Löslichkeit in physiologischem Puffer
- metabolisch stabil
- geringe Toxizität

Artifizielle Lichtschalter

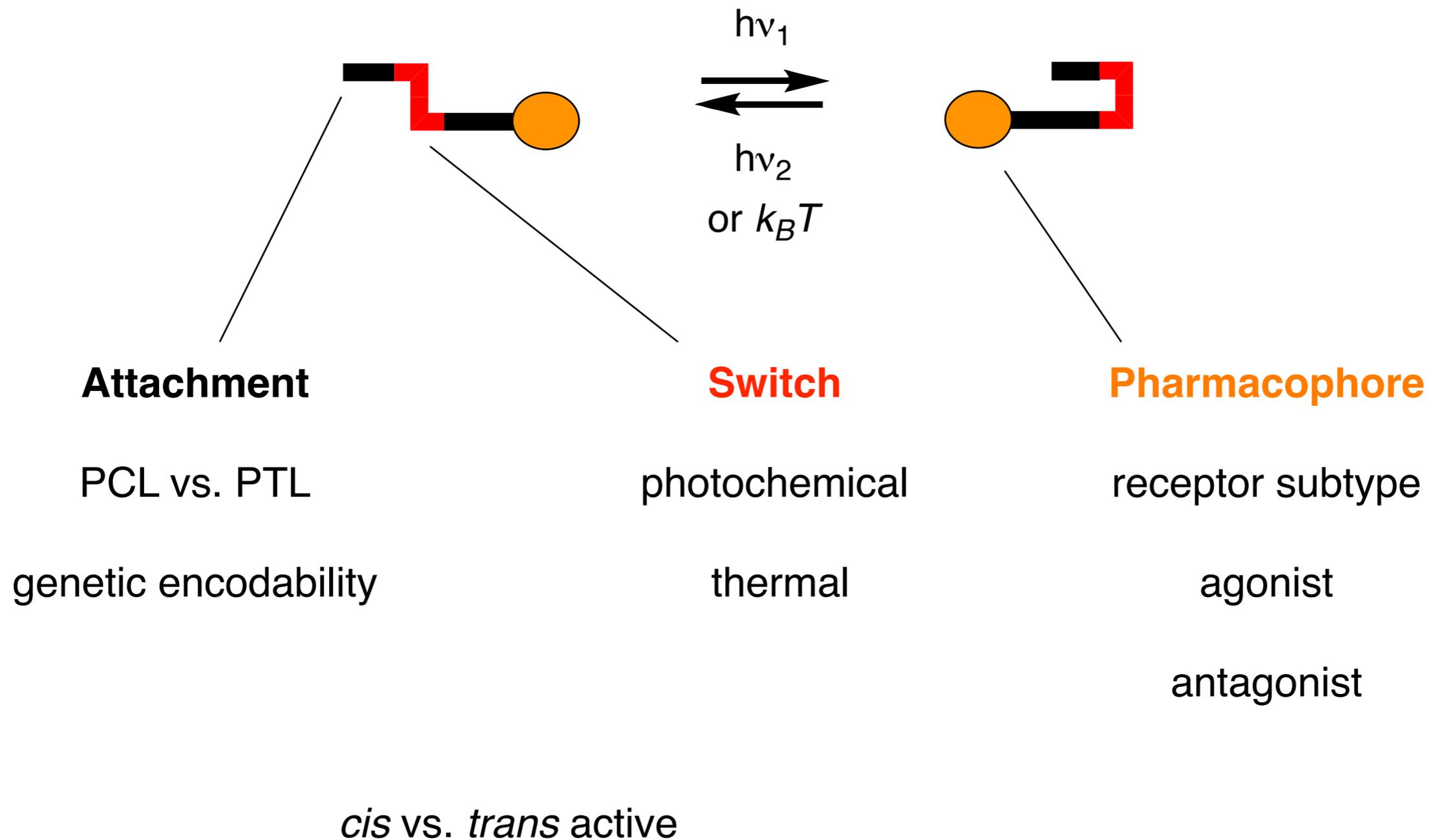
	Photoswitches	Isomerization	λ_1/λ_2	polarity change
A	Azobenzenes		UV/VIS (ΔT)	medium ($\Delta\mu = \sim 3$ D)
B	Stilbenes		UV/UV	small
C	Spiroyrans		UV/VIS (ΔT) or VIS/UV	large ($\Delta\mu = 8-15$ D)
D	Diarylethenes		UV/VIS	small
E	Thiophenefulgides		UV/VIS	small
F	Hemithioindigos		VIS/VIS (ΔT)	medium ($\Delta\mu = \sim 1.6$ D)

Artifizielle Lichtschalter

Das Azobenzol

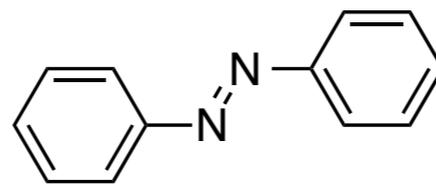


Die chemische Variabilität eröffnet viele Möglichkeiten zur Adaption

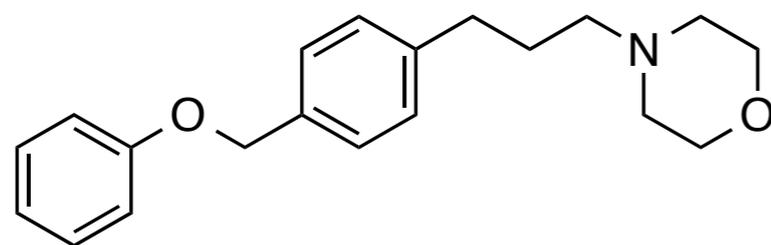
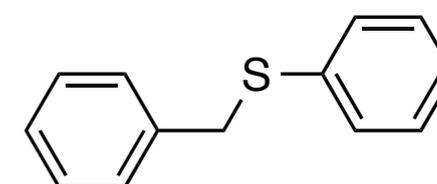
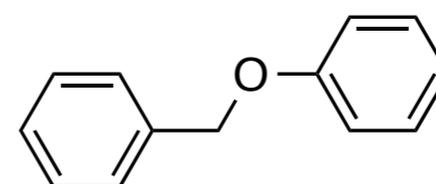
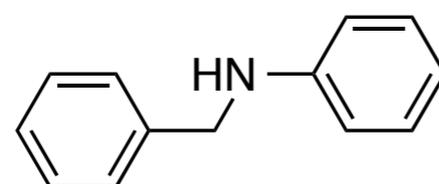
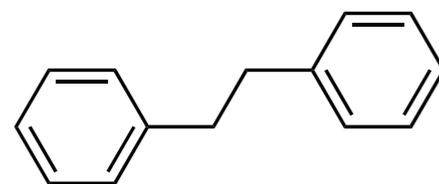
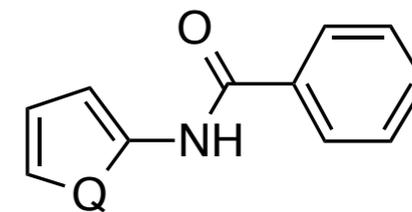
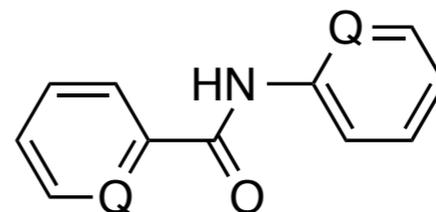
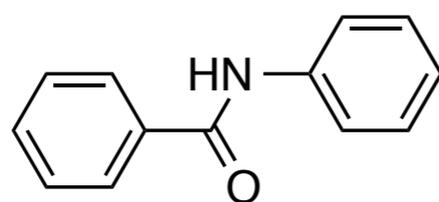
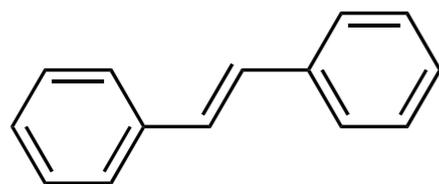


*Das Prinzip der Photopharmakologie
ist der Einbau von Photoschaltern in die
molekulare Struktur der bioaktiven Verbindung*

Azologisierung

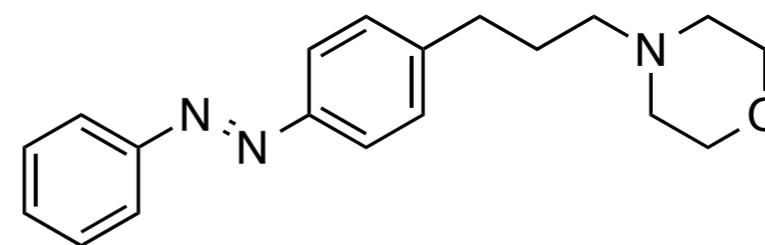


$\hat{=}$



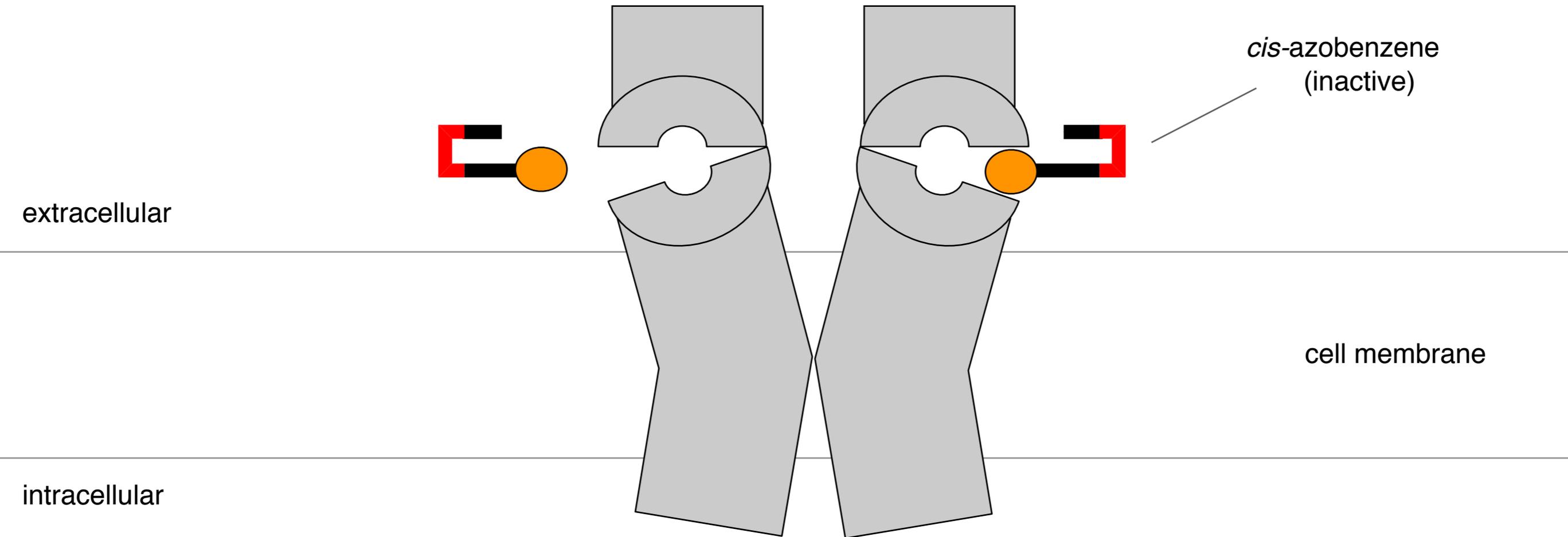
fomocaine

azologization
→

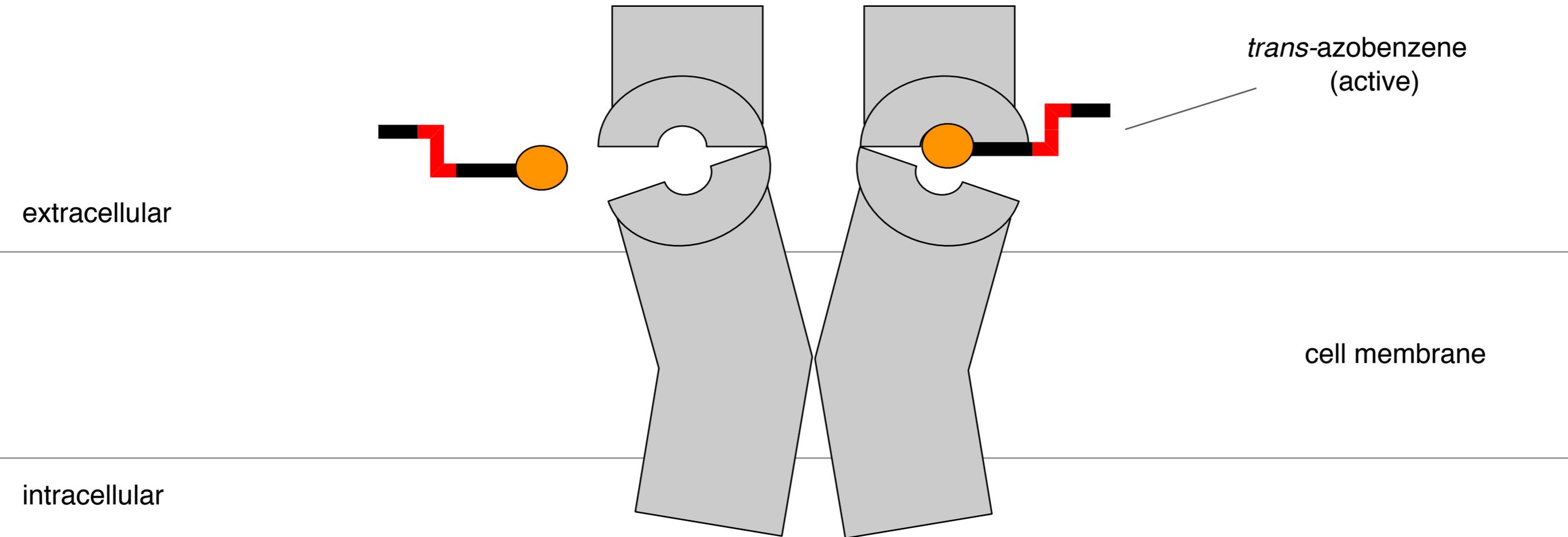


fotocaine

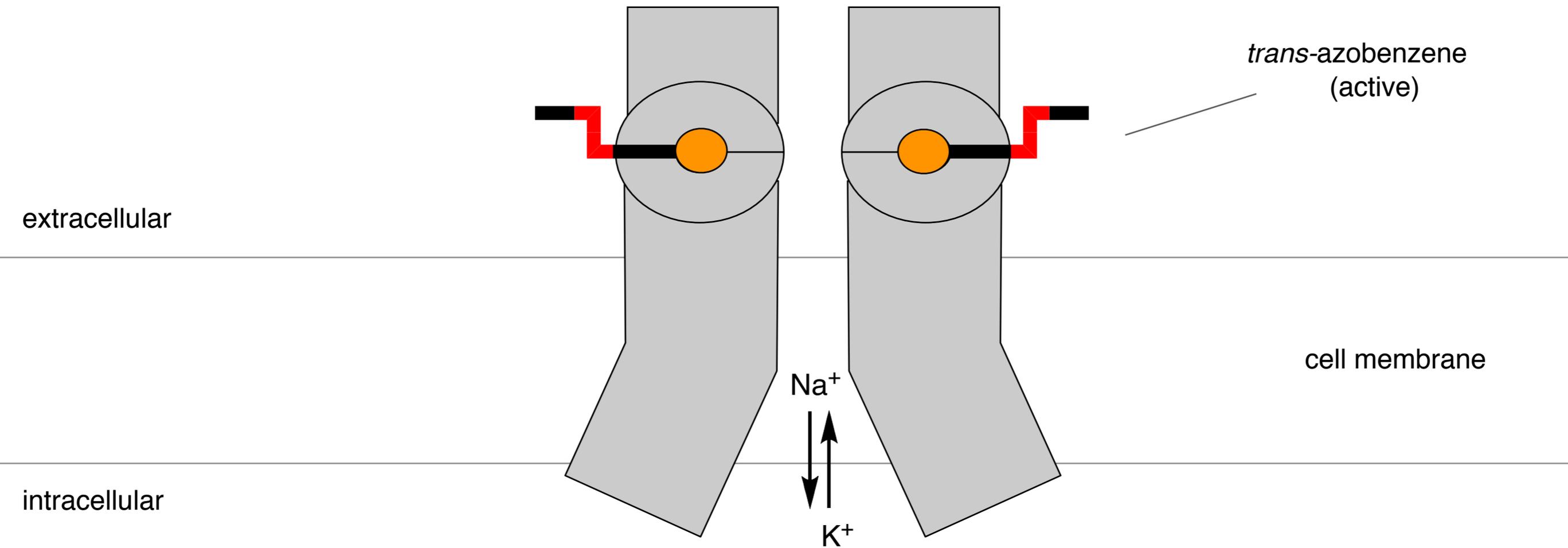
Funktionsweise von PCLs an ionotropen Glutamatrezeptoren



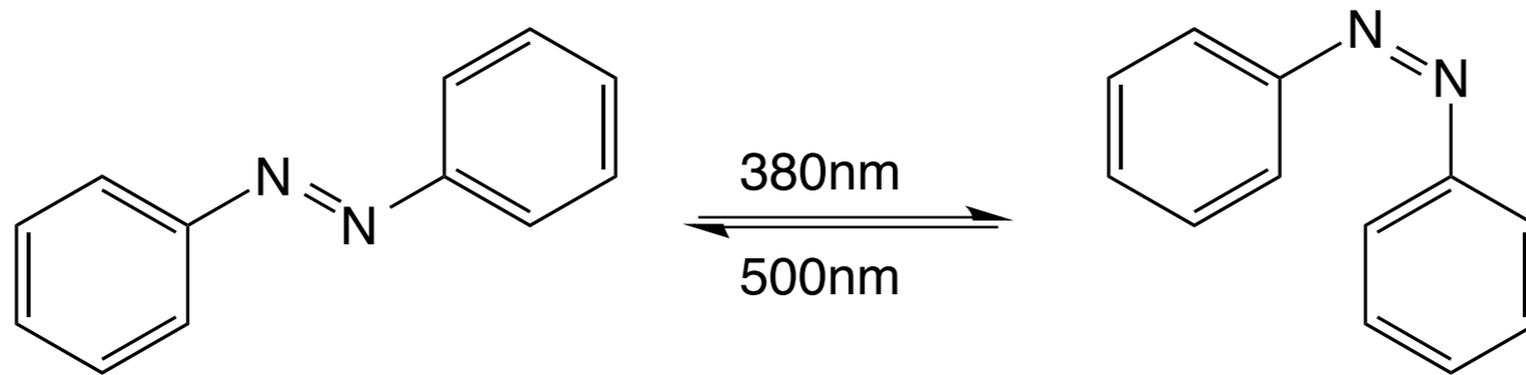
Funktionsweise von PCLs an ionotropen Glutamatrezeptoren



Funktionsweise von PCLs an ionotropen Glutamatrezeptoren



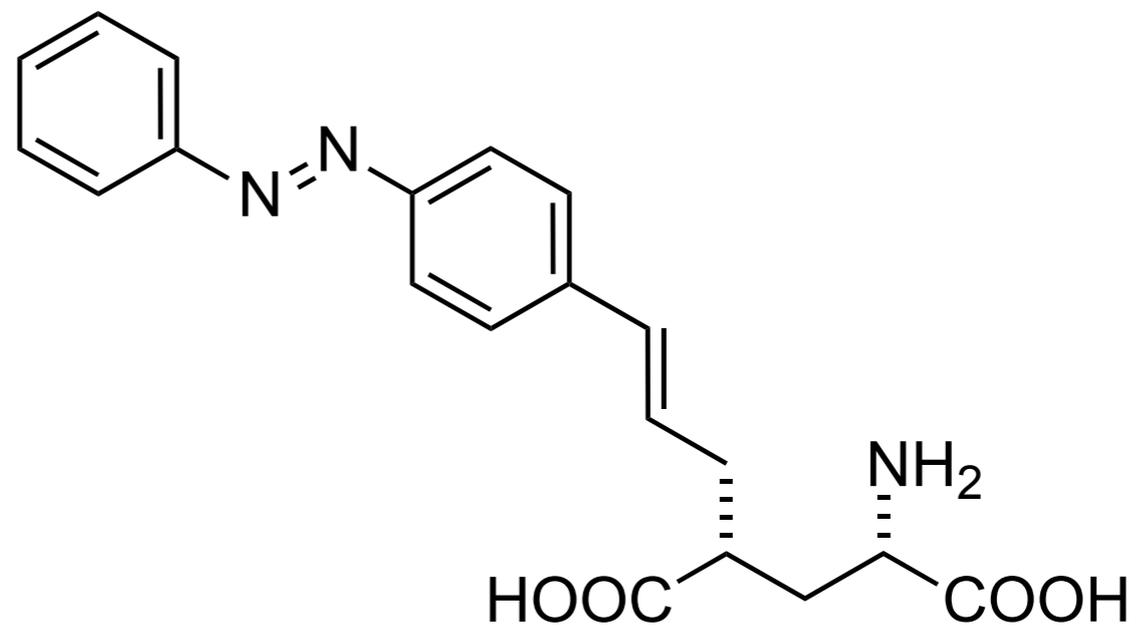
Design von PCLs für einen Glutamatrezeptor



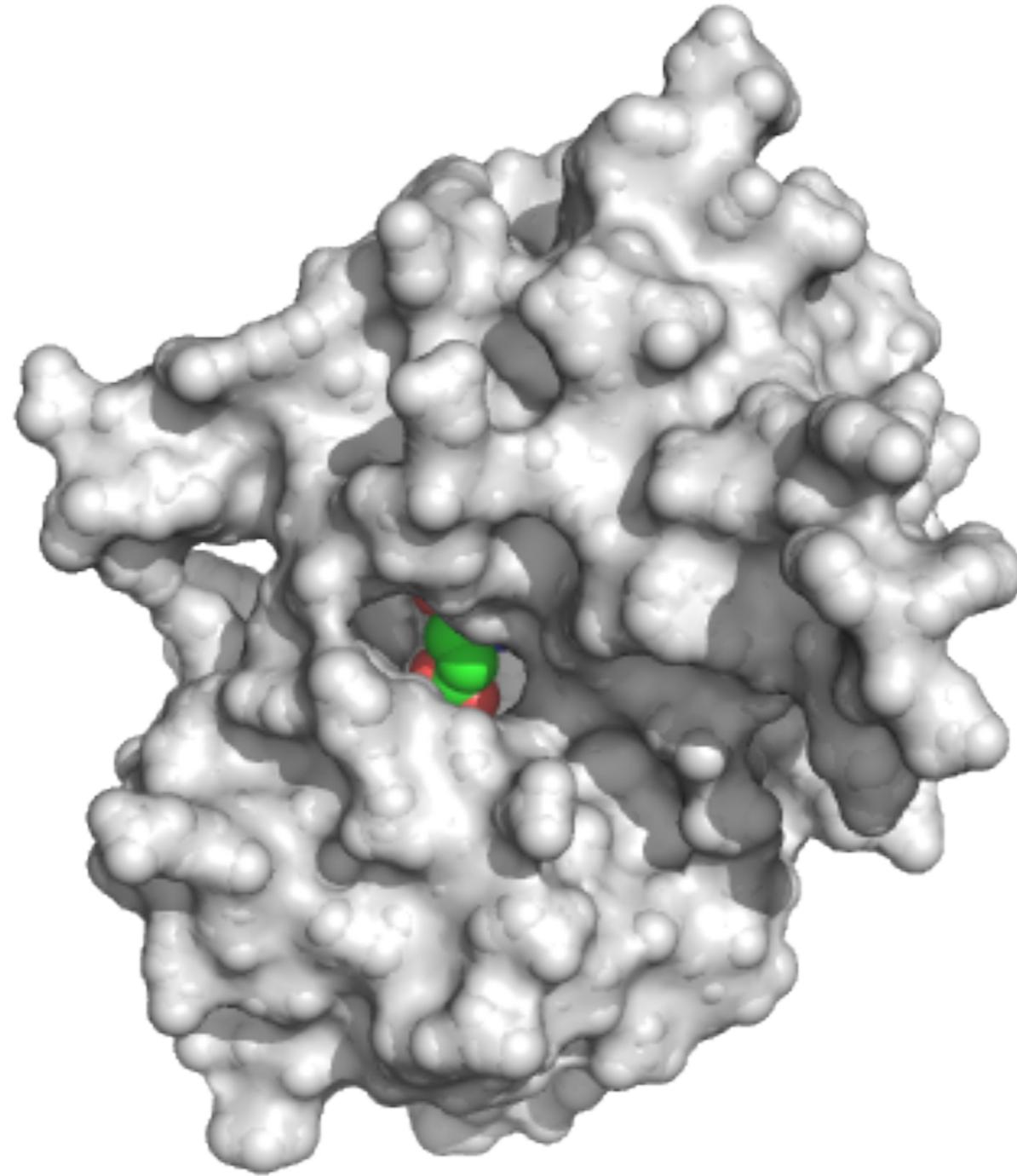
Azoswitch

Linker (vinyl)

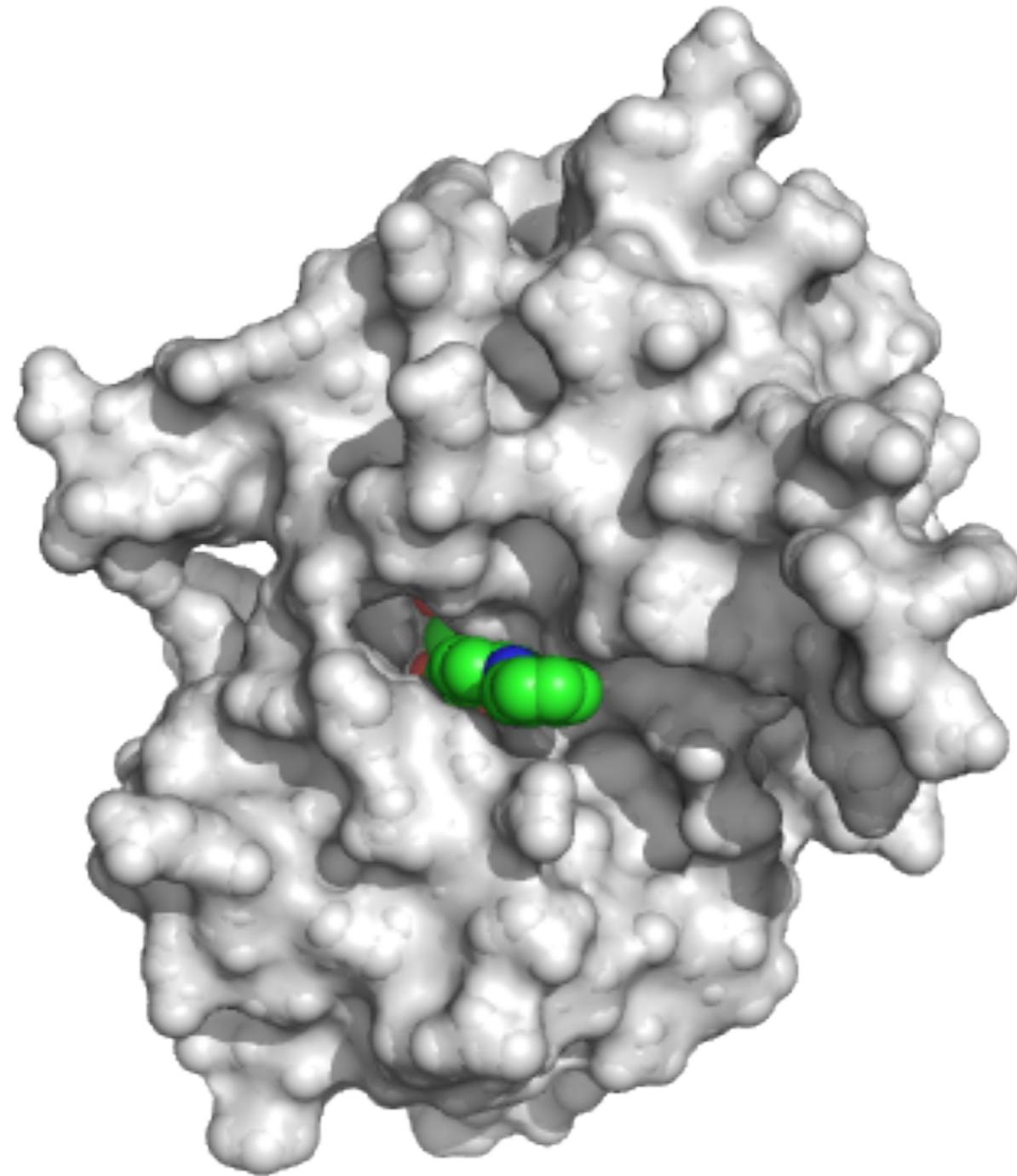
Ligand (glutamate)

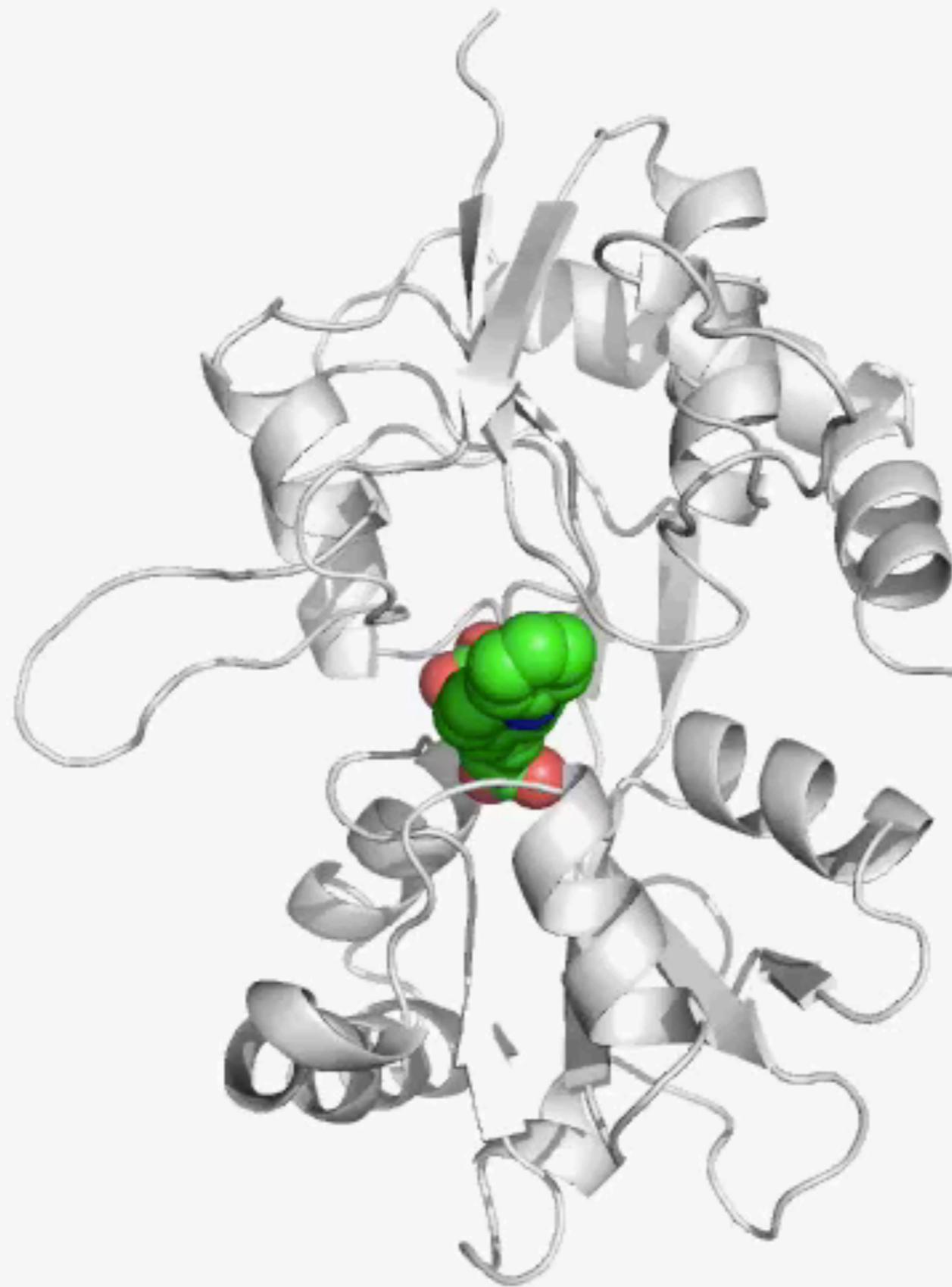


Design von PCLs für einen Glutamatrezeptor

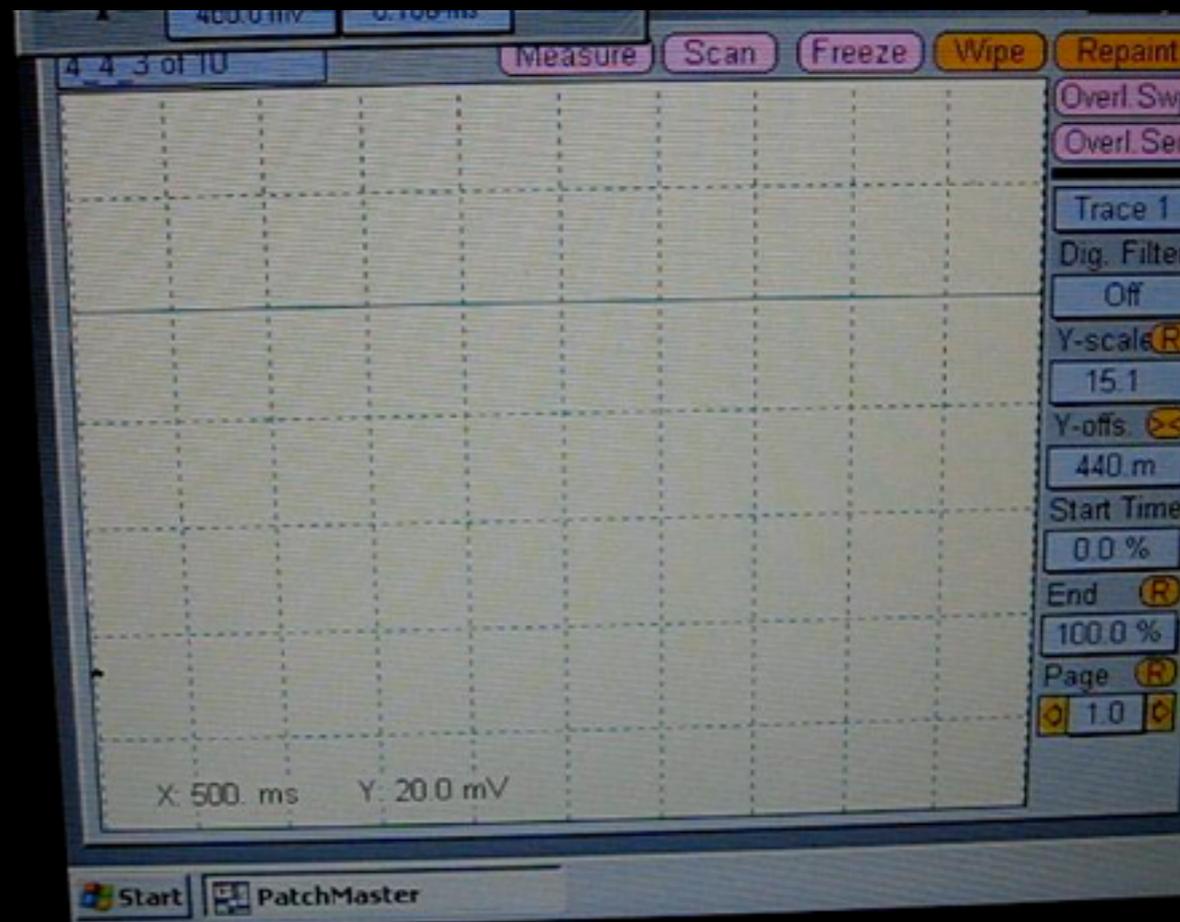
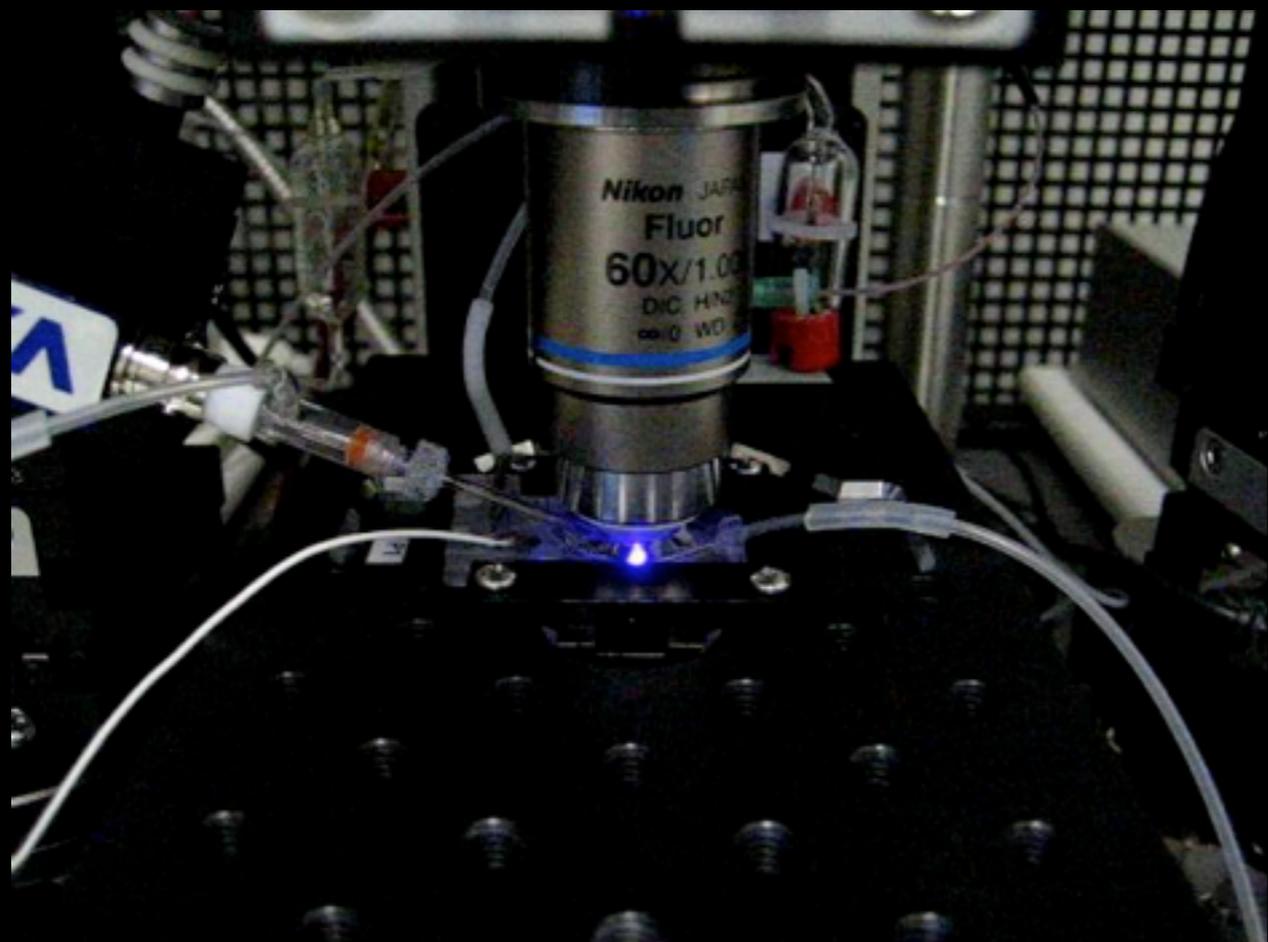


Design von PCLs für einen Glutamatrezeptor

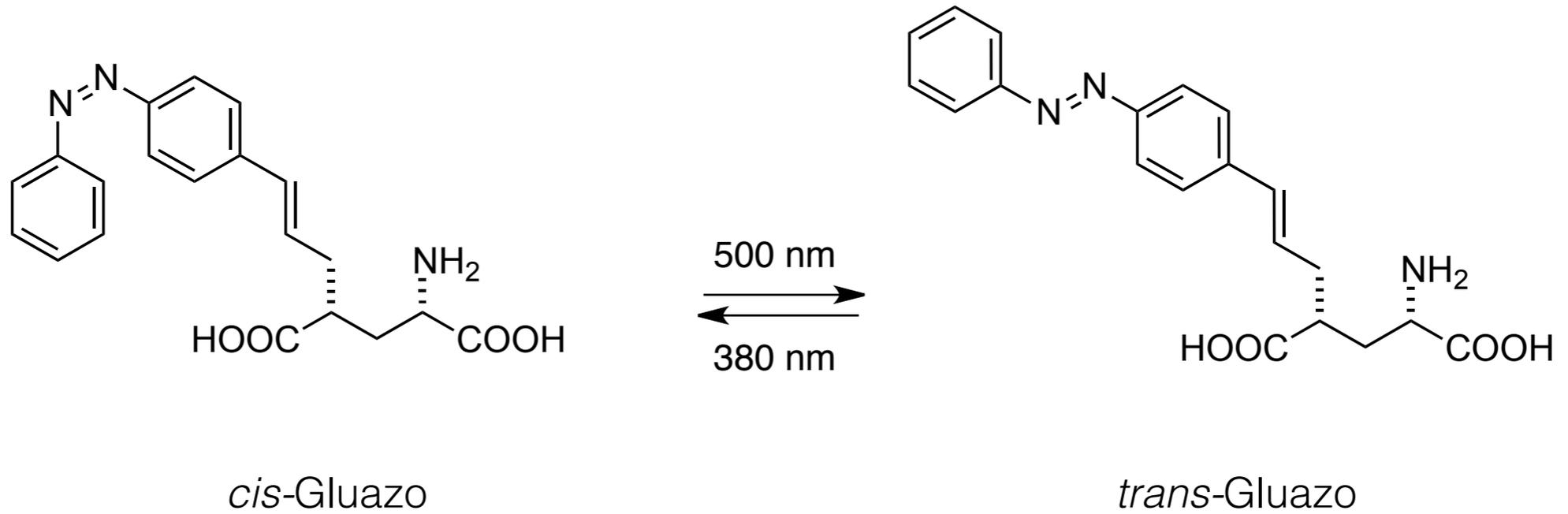




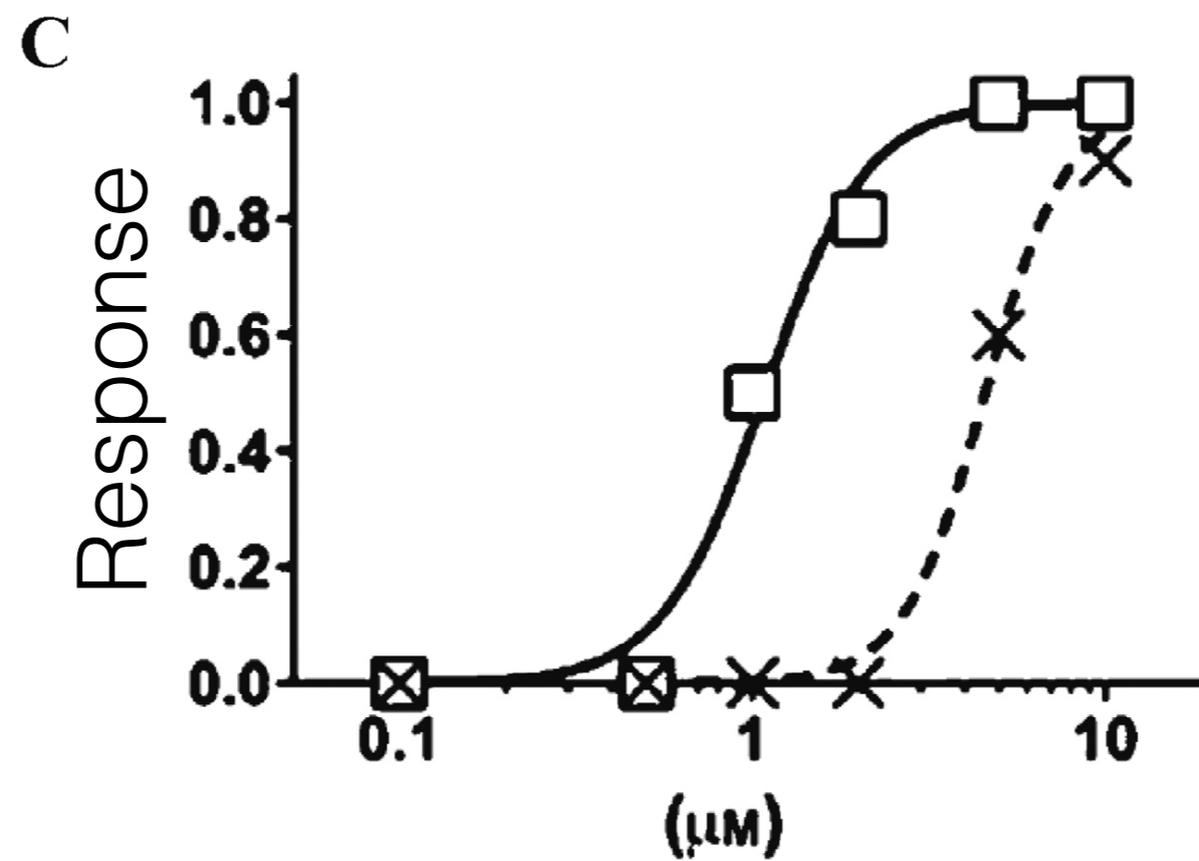
Reiter et al., Biochemistry (2013)



Ein lichtschaltbarer Neurotransmitter



Dosis-Wirkung-Kurve bei PCLs

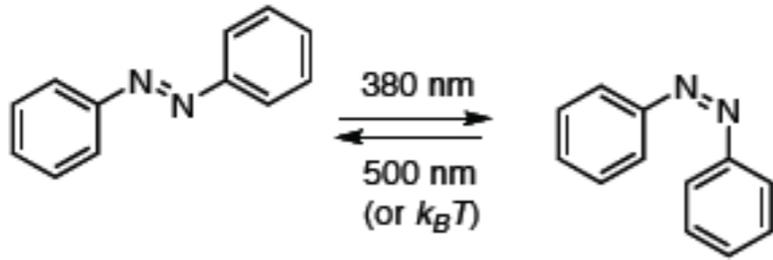


□ *trans*

x *cis*

Spektrales Tuning von Azobenzolen

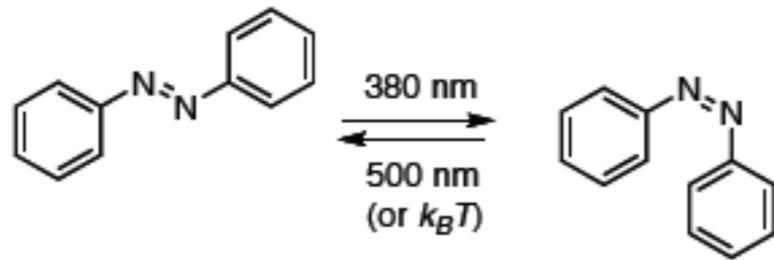
regular azobenzenes:



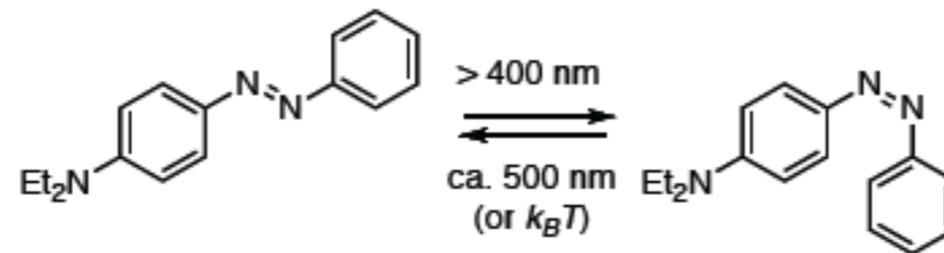
increasingly red-shifted and thermally less stable

Spektrales Tuning von Azobenzolen

regular azobenzenes:

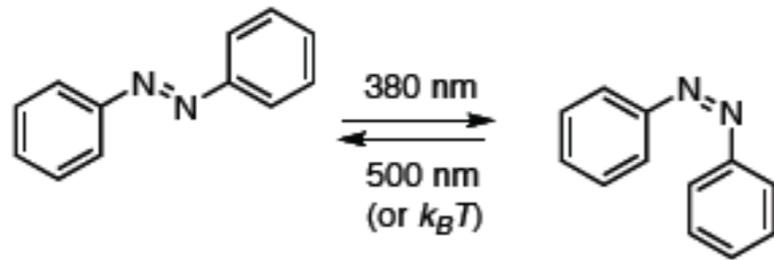


amino azobenzenes:

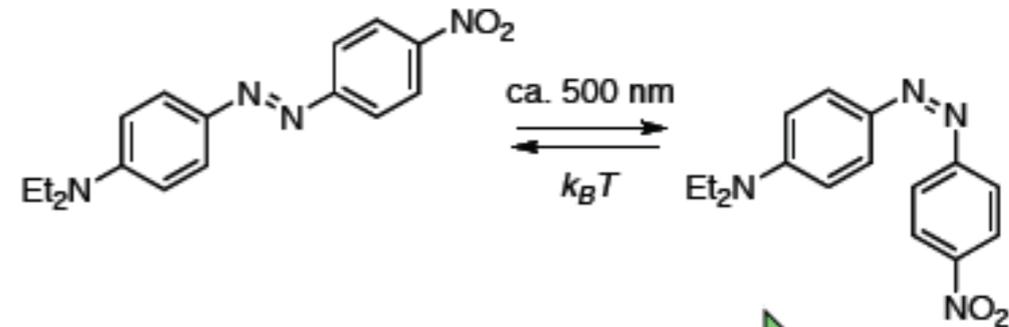


Spektrales Tuning von Azobenzolen

regular azobenzenes:

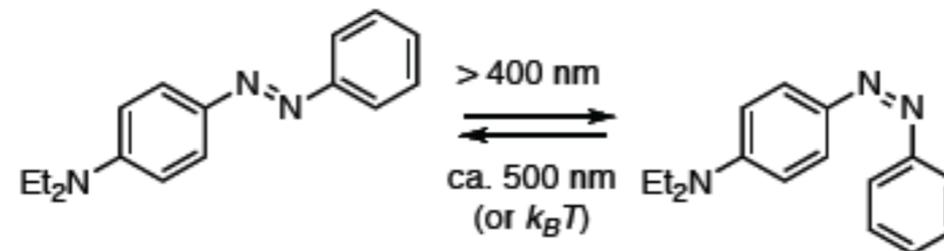


pseudostilbenes (push-pull):



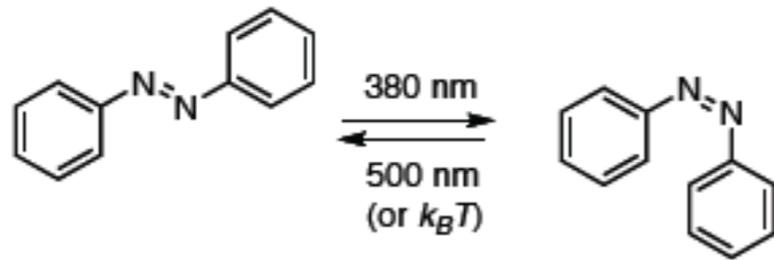
increasingly red-shifted and thermally less stable

amino azobenzenes:

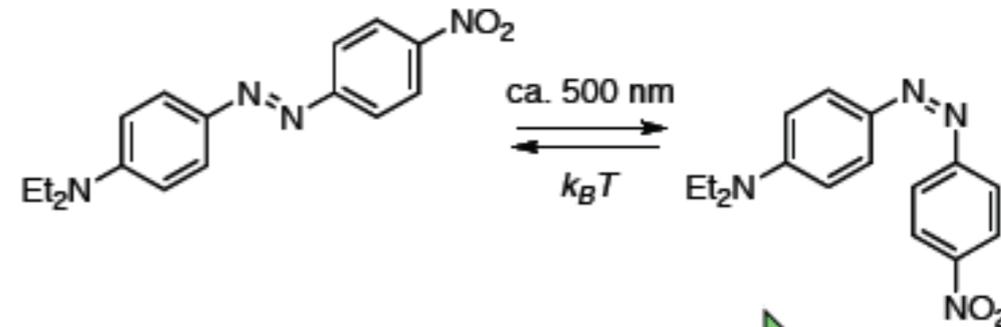


Spektrales Tuning von Azobenzolen

regular azobenzenes:

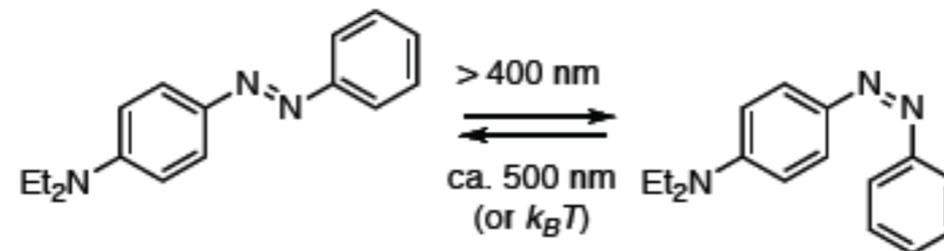


pseudostilbenes (push-pull):

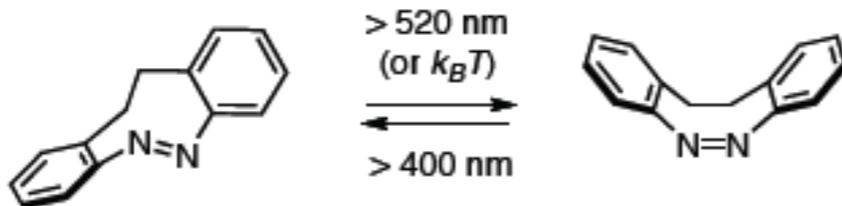


increasingly red-shifted and thermally less stable

amino azobenzenes:

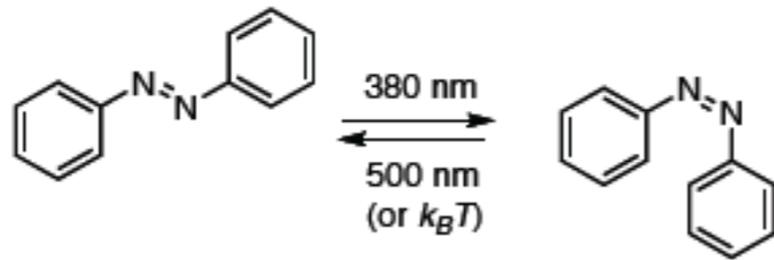


cyclic azobenzenes:

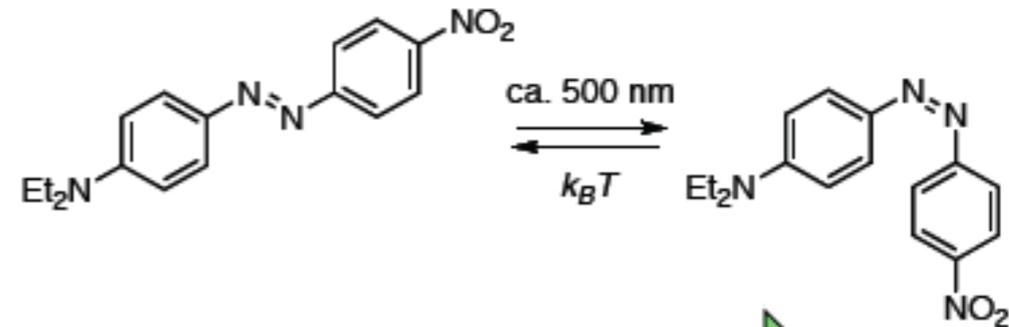


Spektrales Tuning von Azobenzolen

regular azobenzenes:

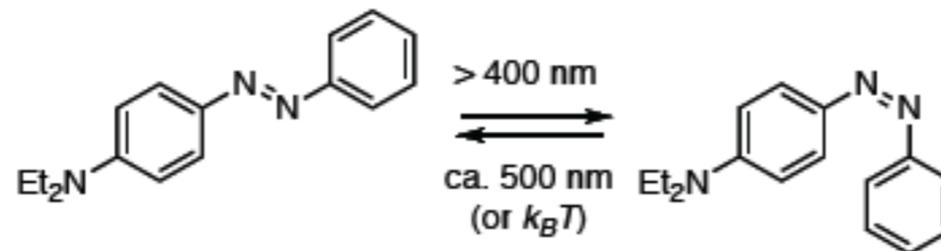


pseudostilbenes (push-pull):

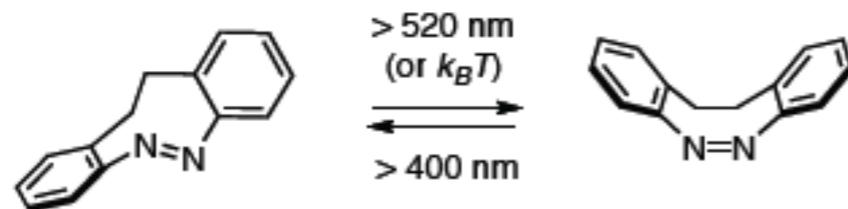


increasingly red-shifted and thermally less stable

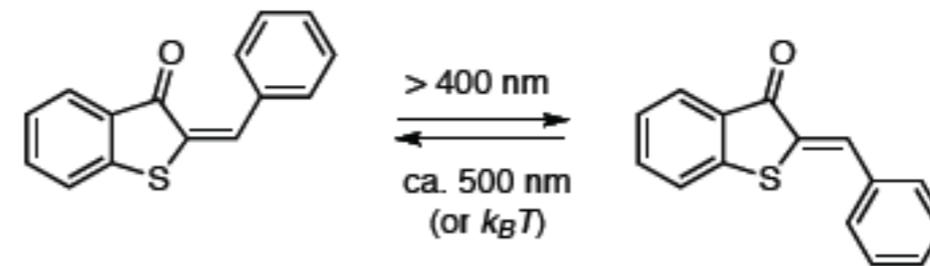
amino azobenzenes:



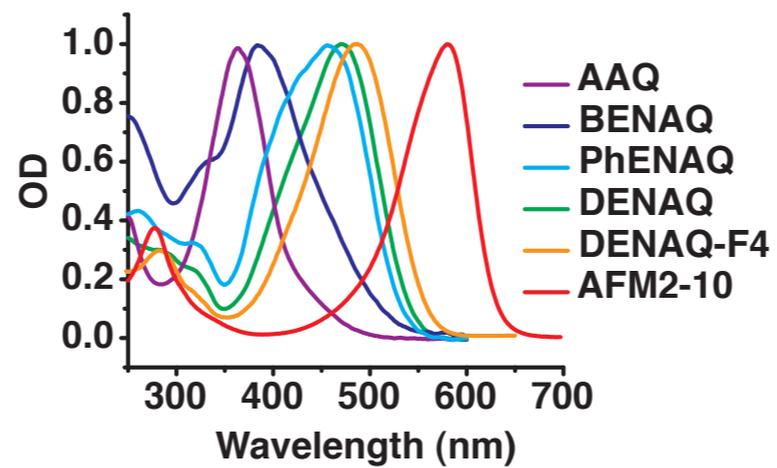
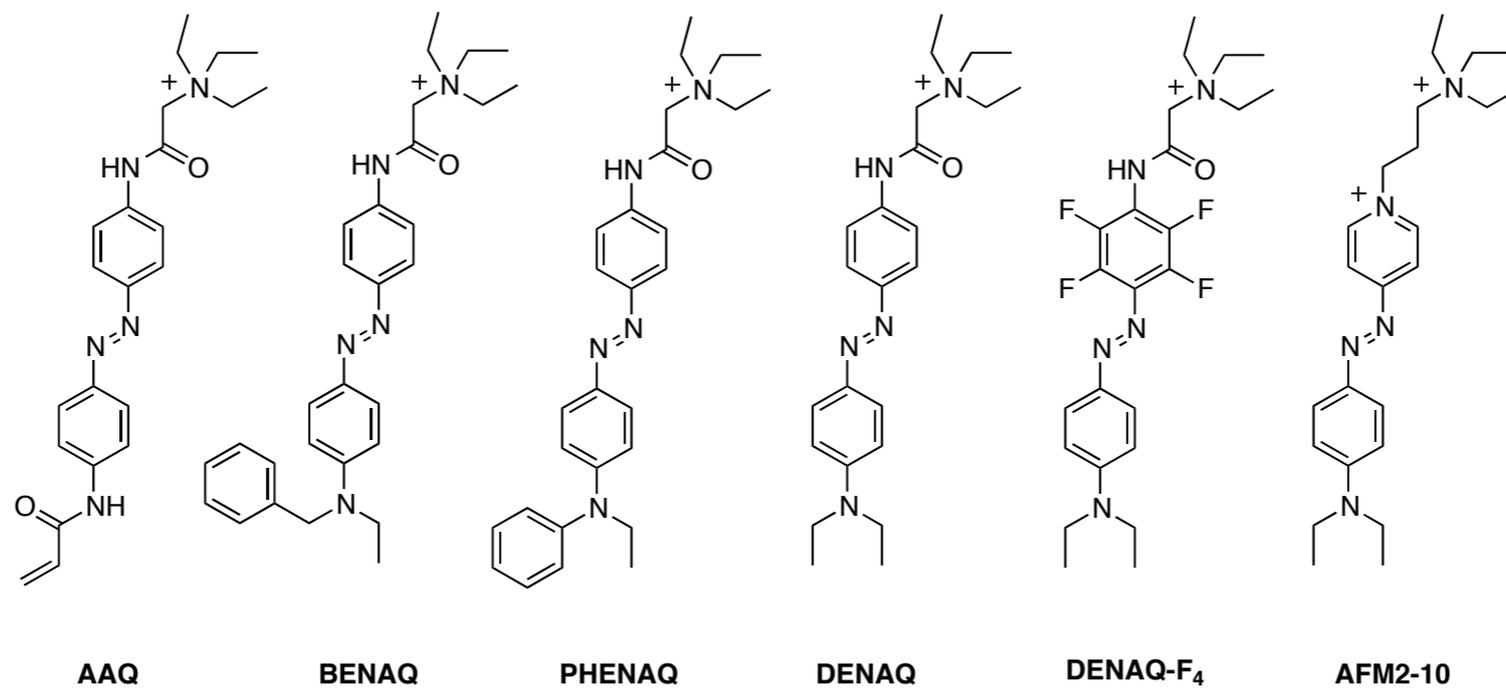
cyclic azobenzenes:



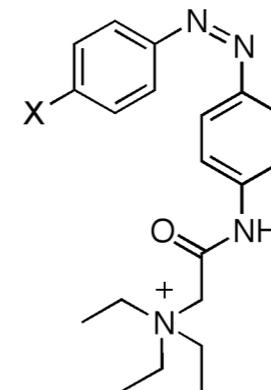
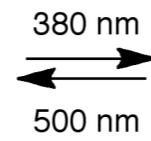
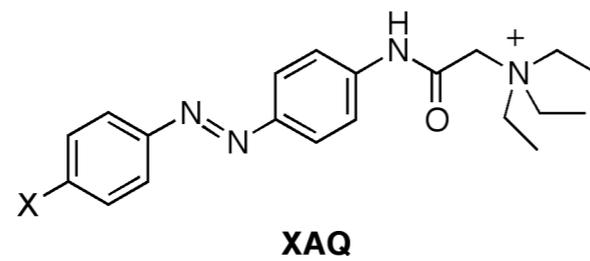
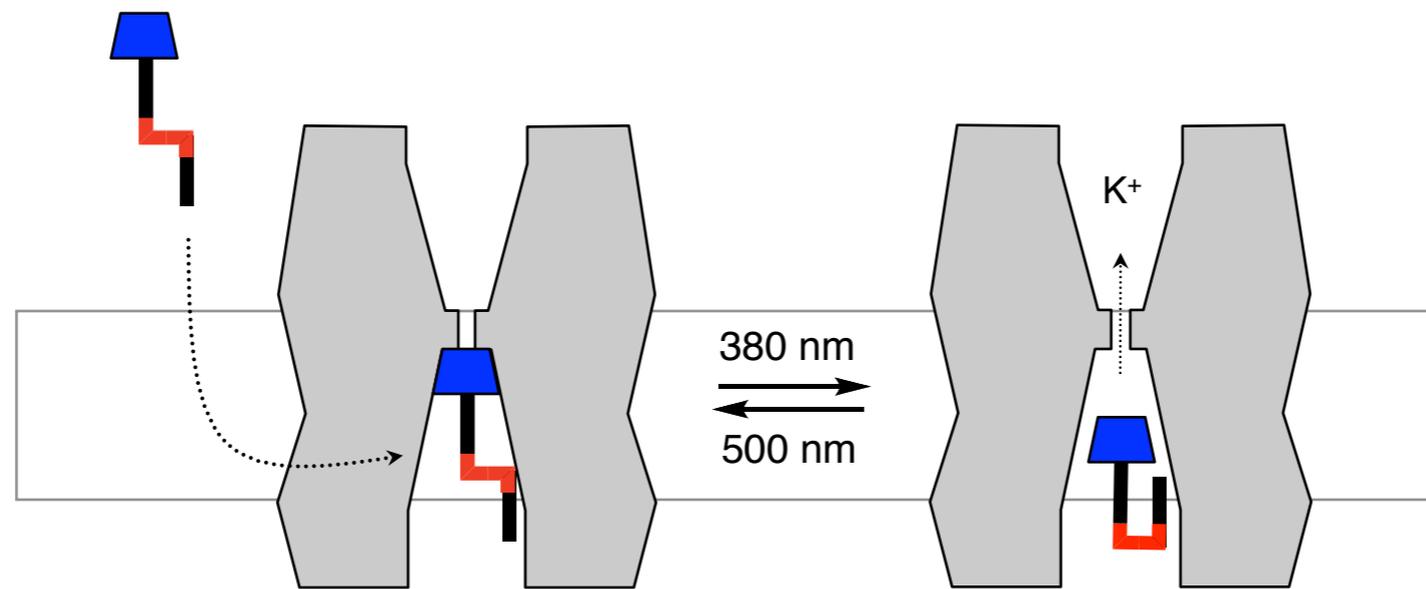
hemithioindigos:



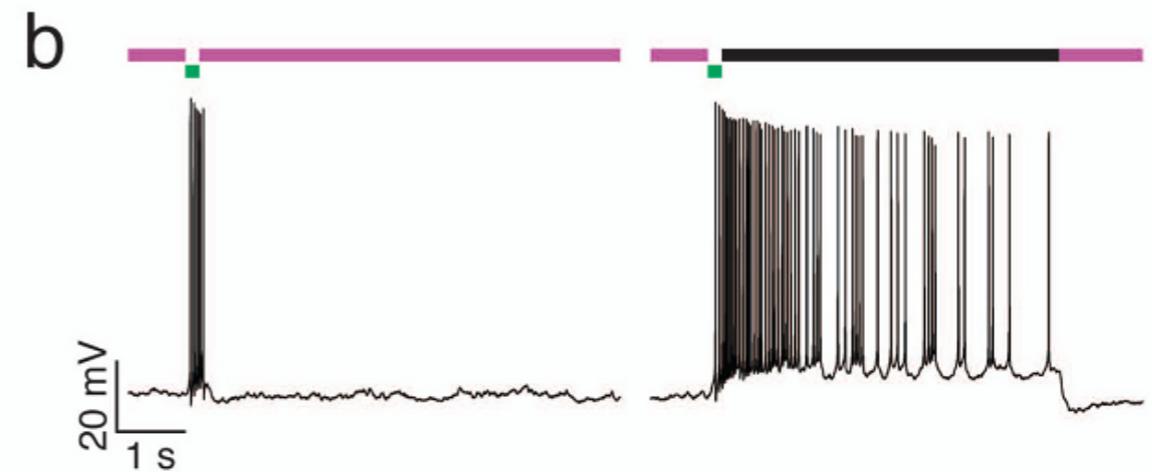
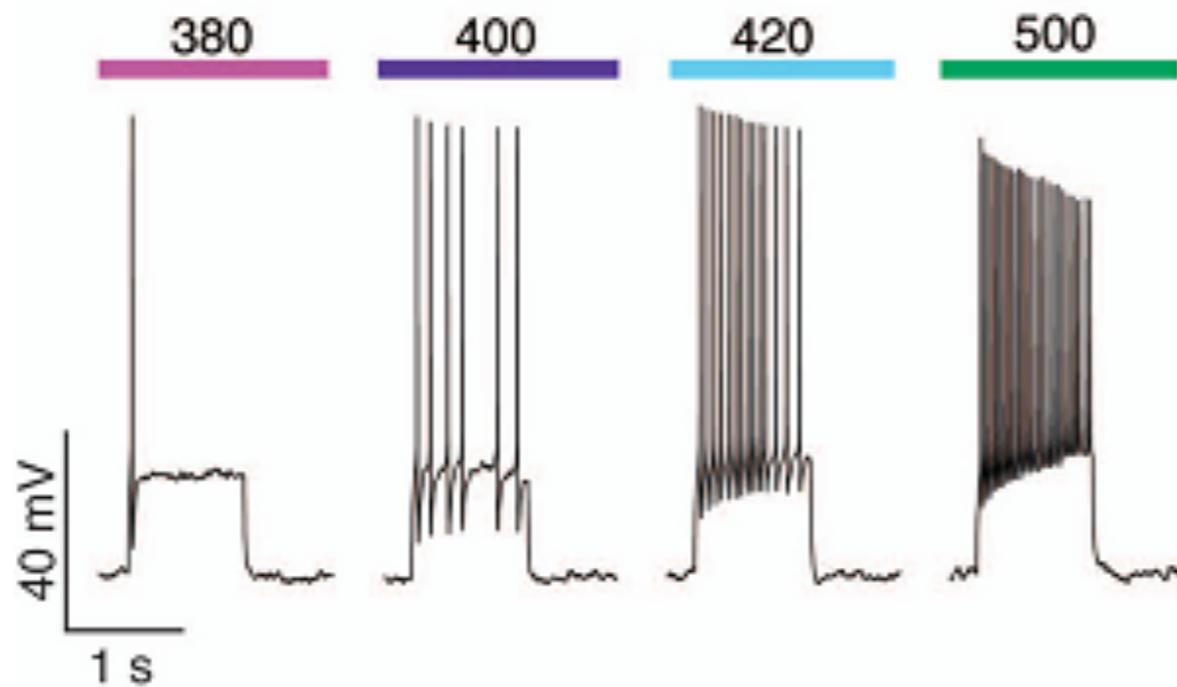
Spektrales Tuning von PCLs



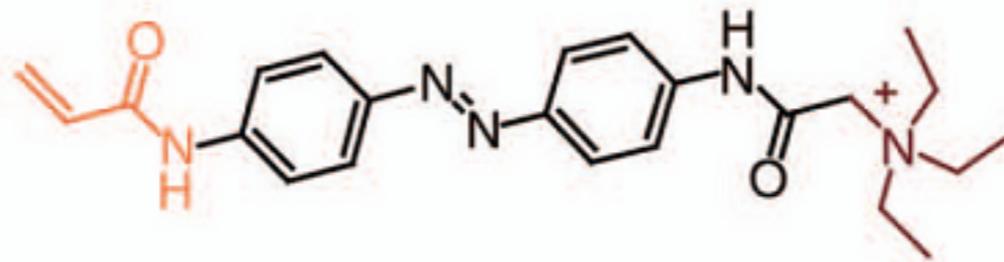
Photochrome K_V -Kanalblocker



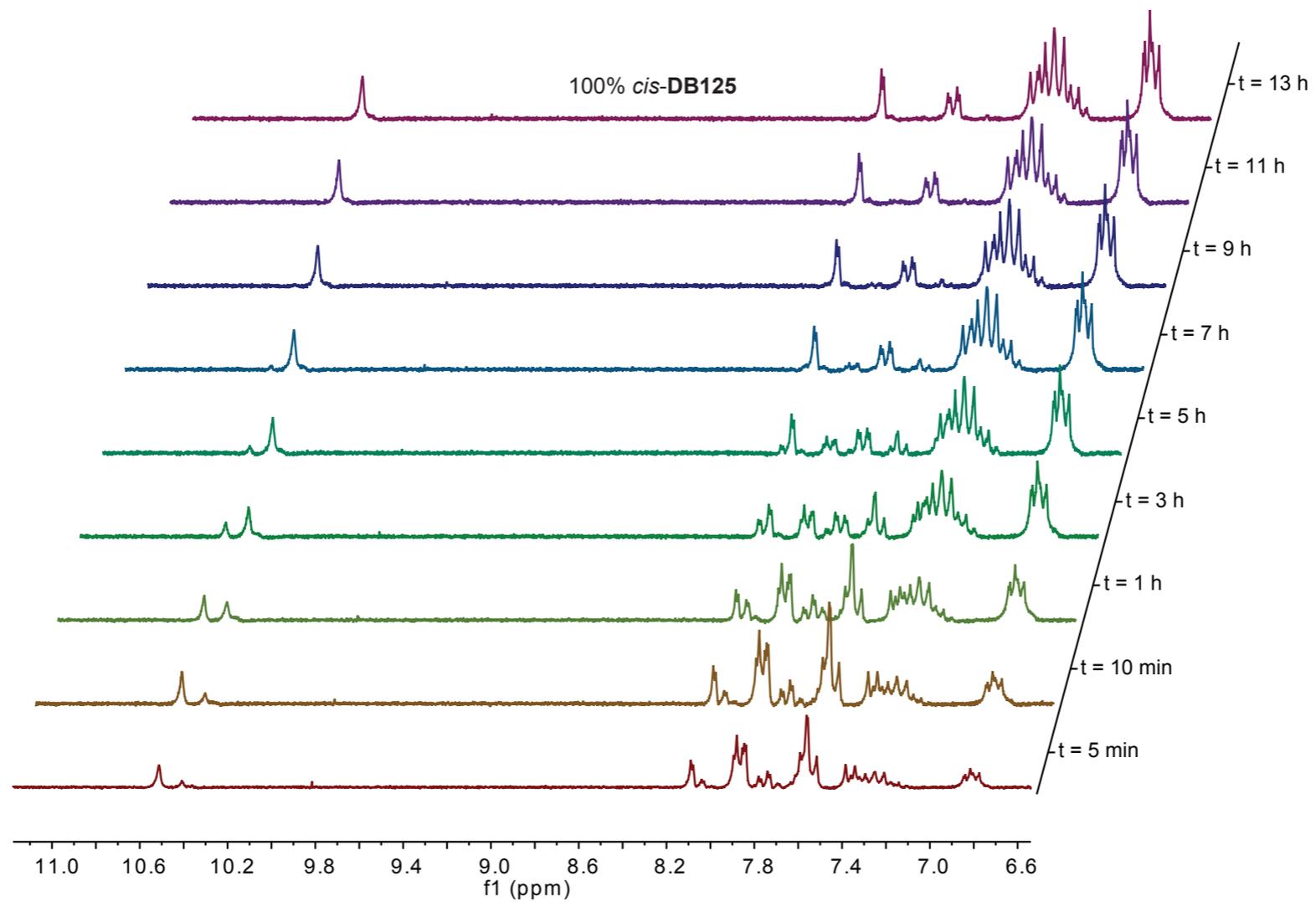
Photodosierung - Kontrolle der aktiven Konzentration durch Licht



AAQ



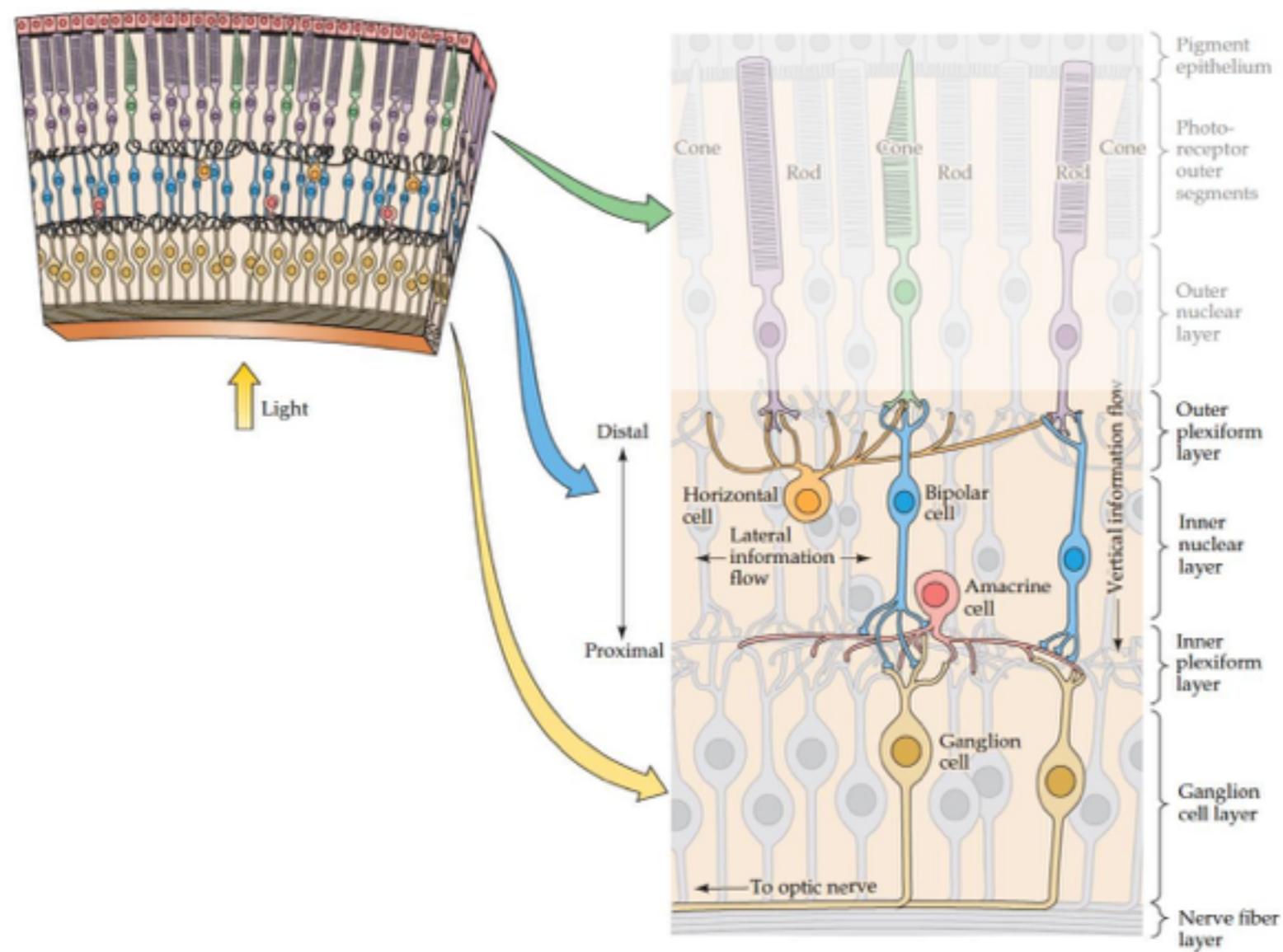
Beobachtung der Isomerisierung im NMR



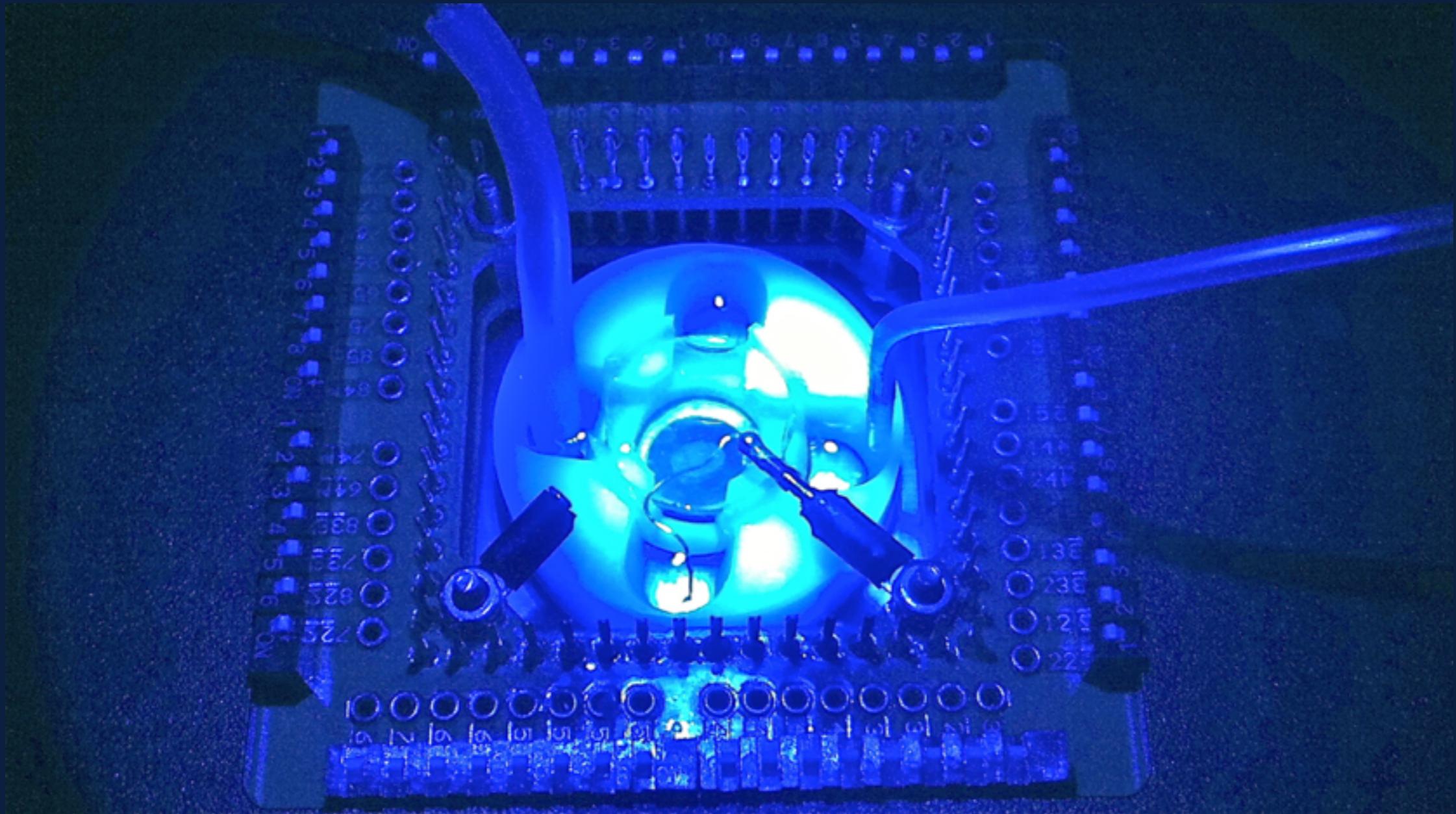
Anwendung von photochromen Liganden in der Neurobiologie

*Photopharmakologische Wiederherstellung der
„Sehfähigkeit“ in blinden Mäusen*

Die Retina

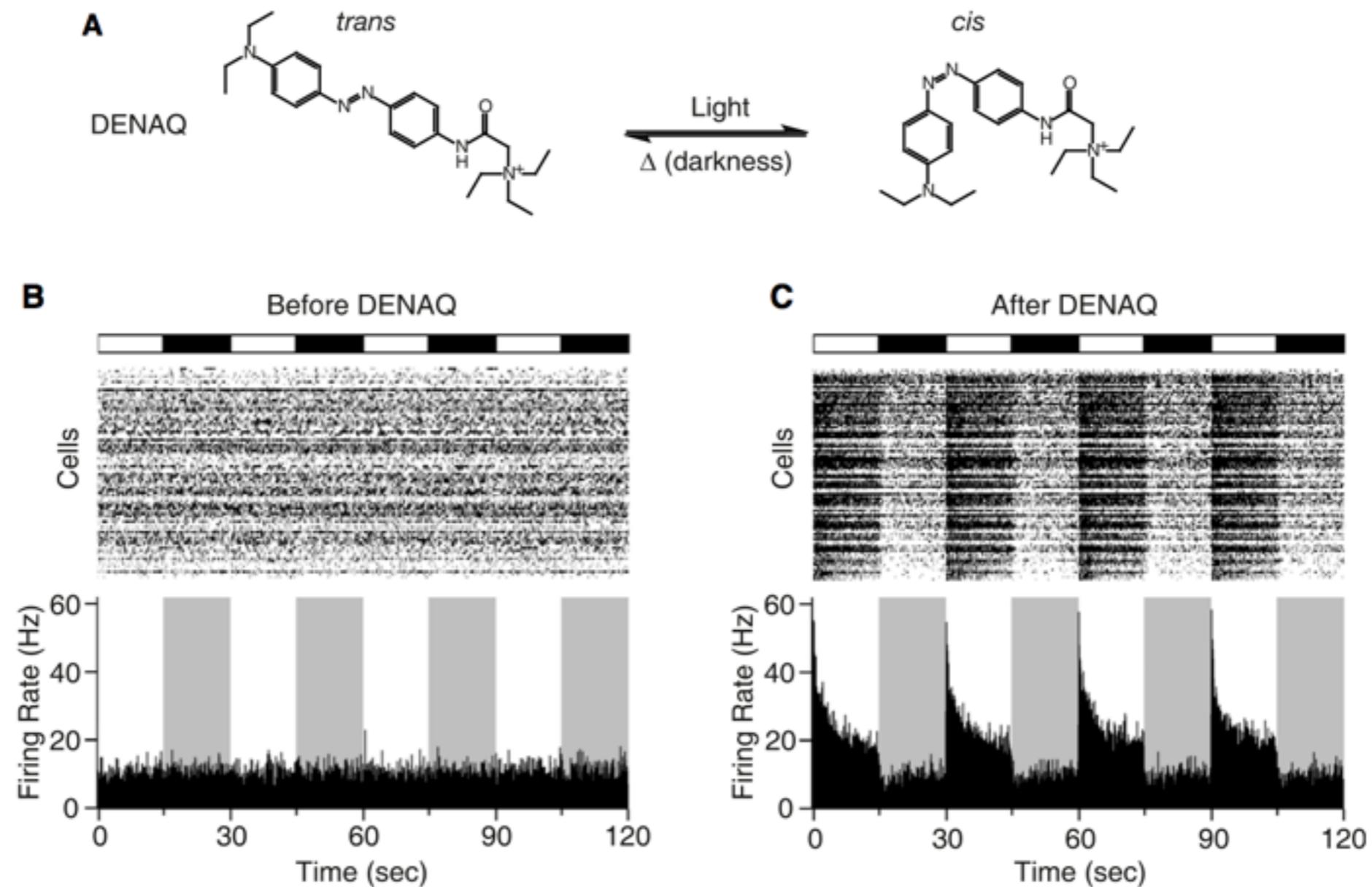


Multielektrodenmessung



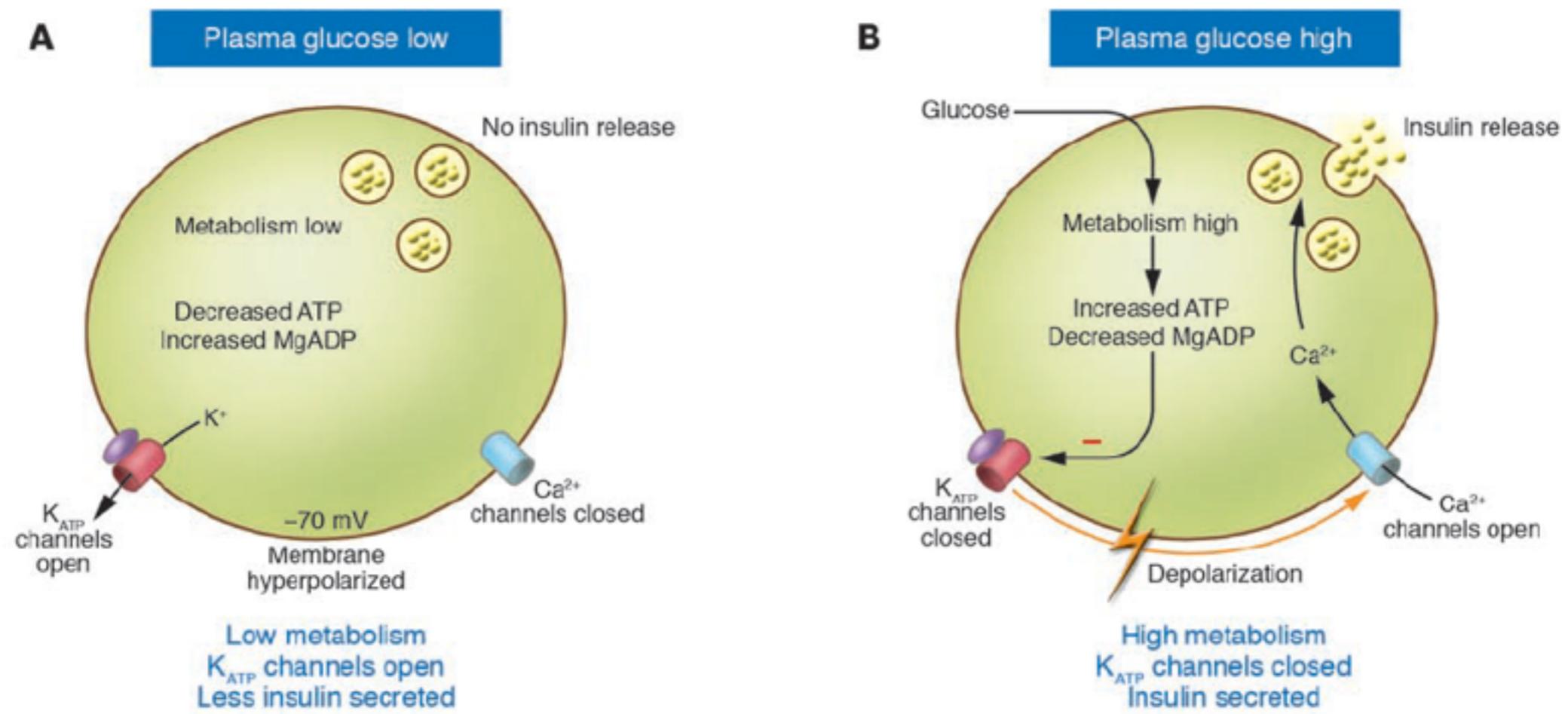
Wiederherstellung der Sehfähigkeit durch Photopharmakologie

2nd generation K-channel blockers

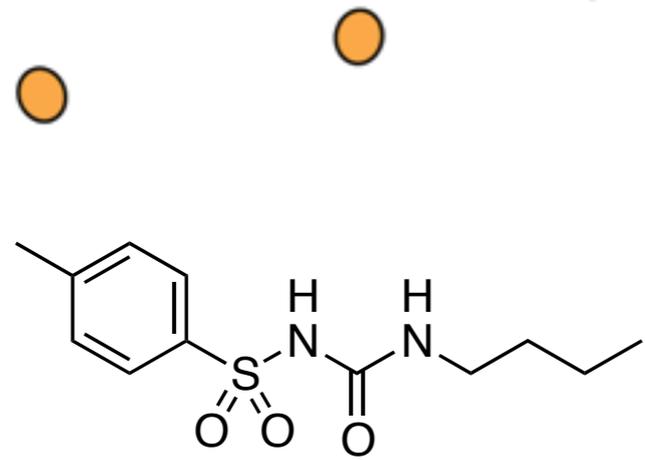
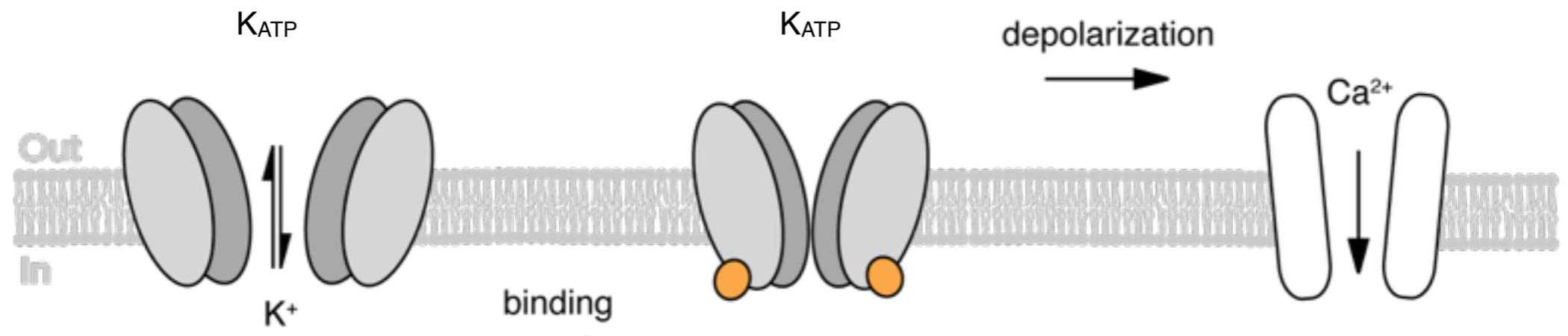


Licht gesteuerte Freisetzung von Insulin

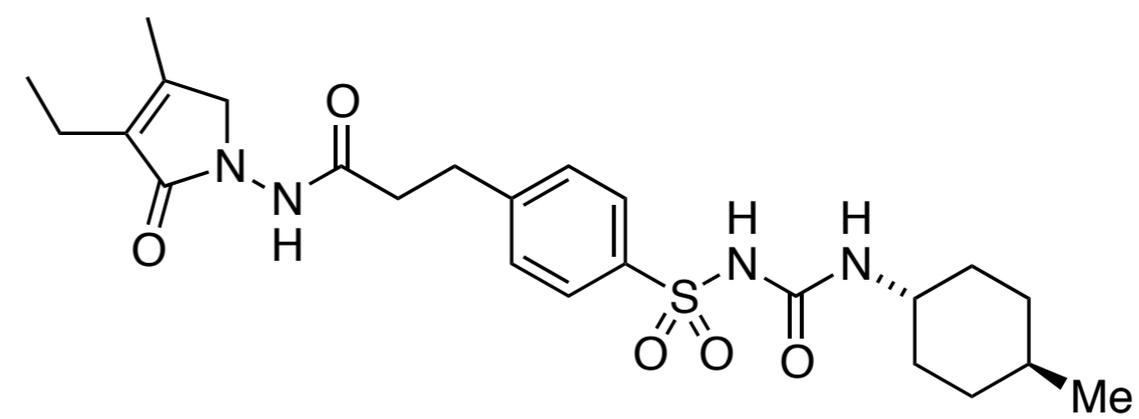
Biochemischer Mechanismus der Insulinfreisetzung



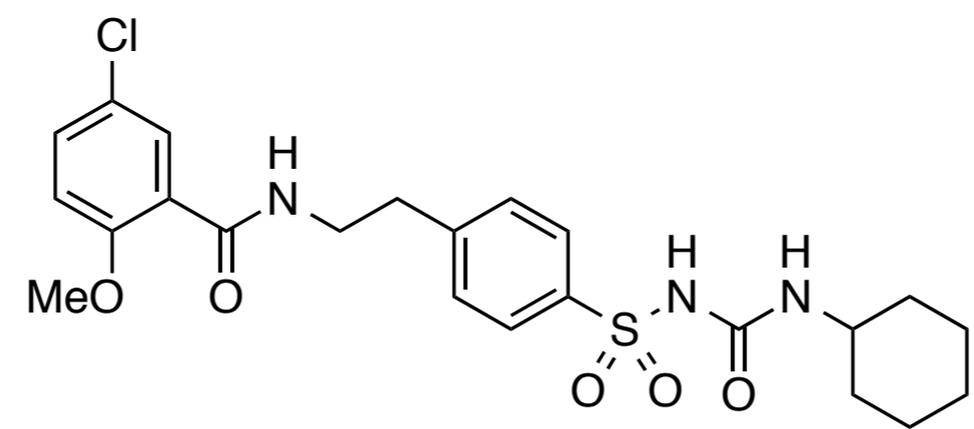
Sulfonylharnstoffe zeigen eine dem ATP ähnliche Wirkung



tolbutamide

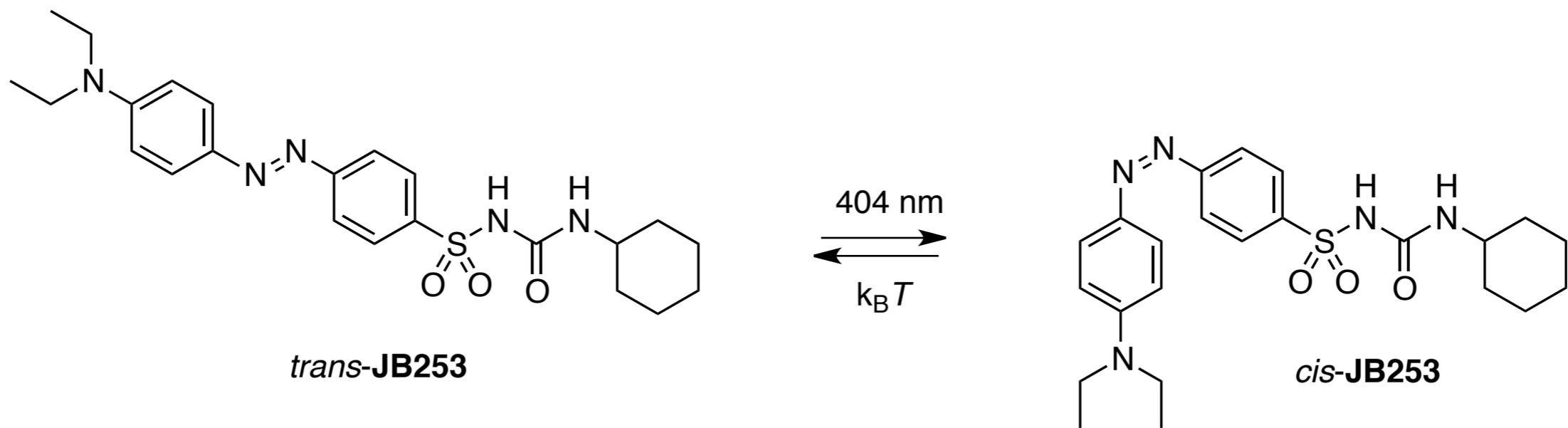
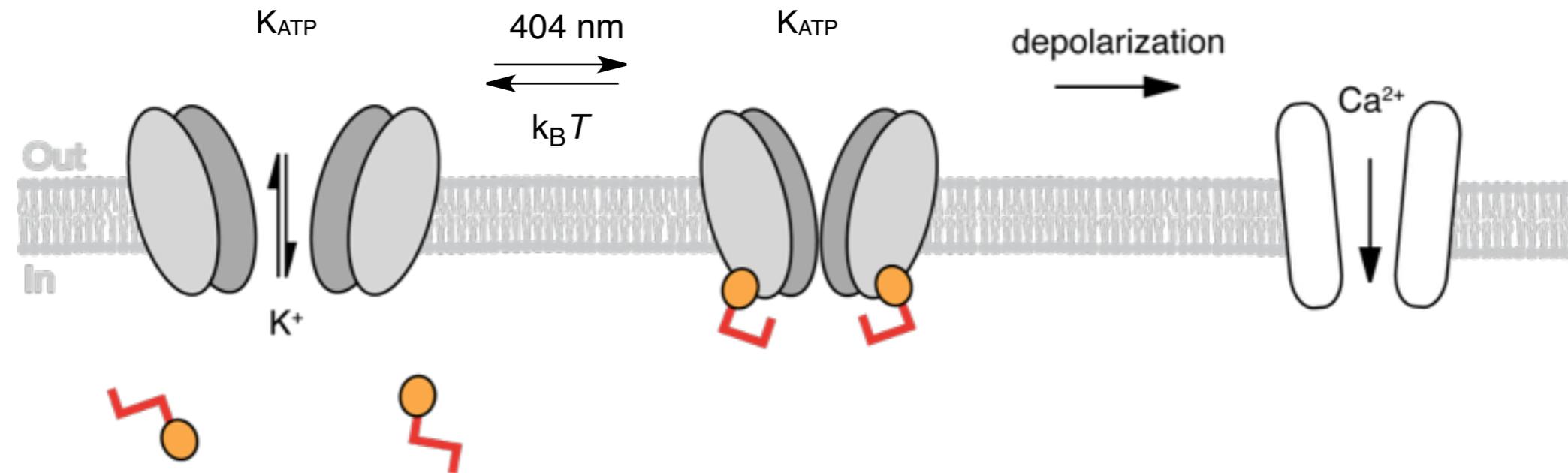


glimepiride

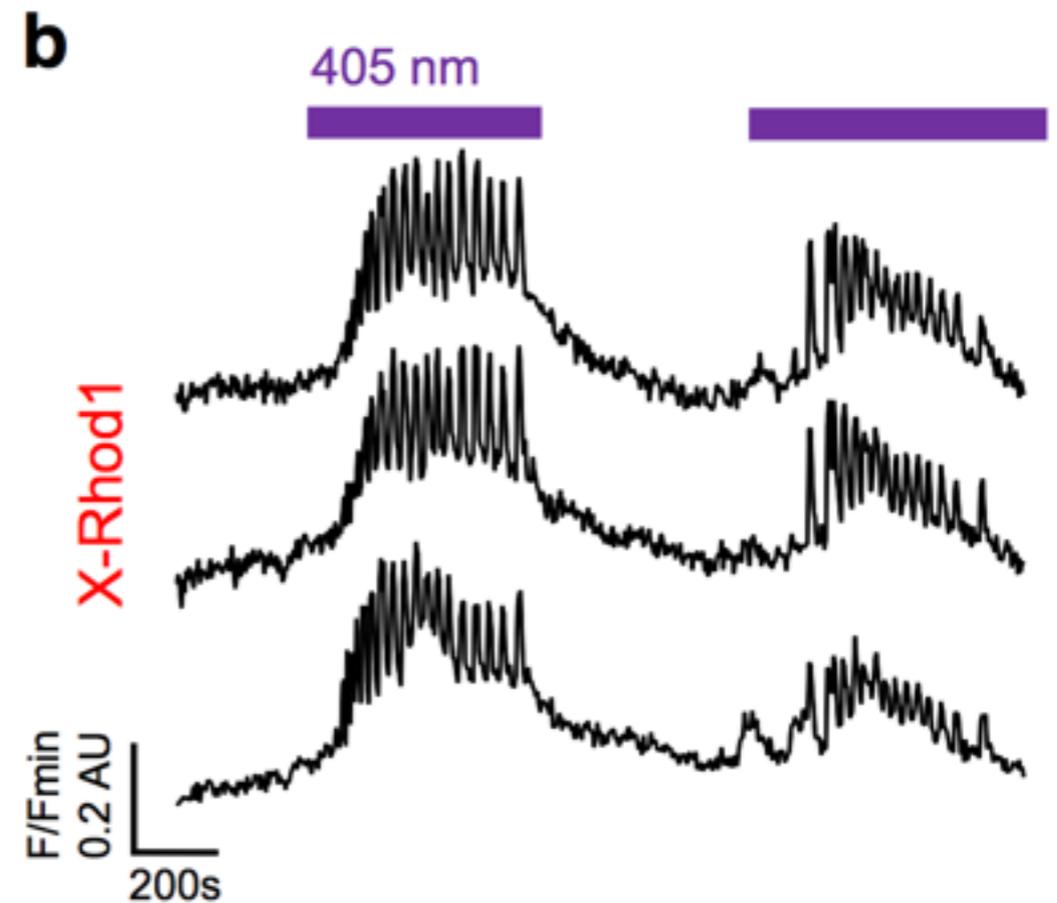
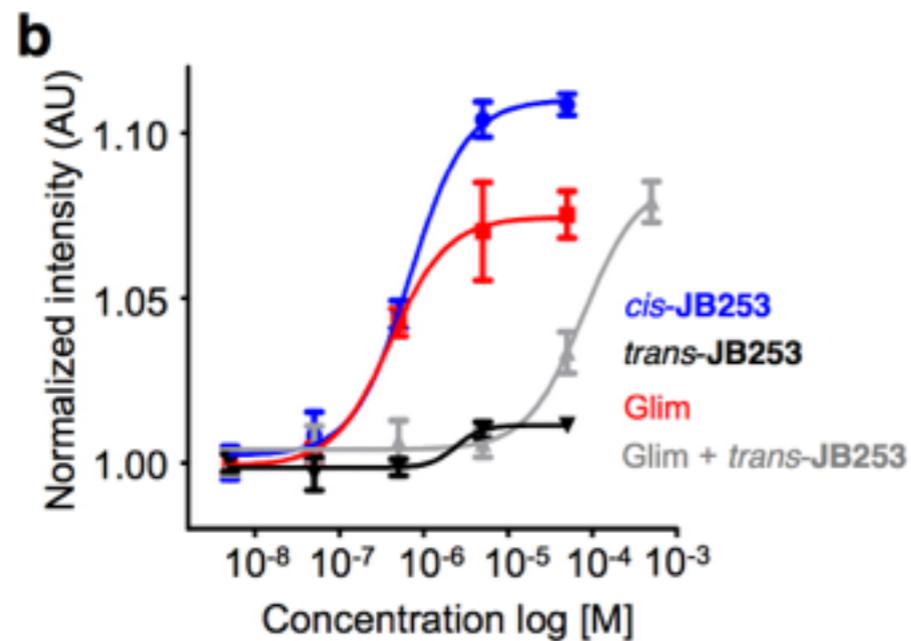
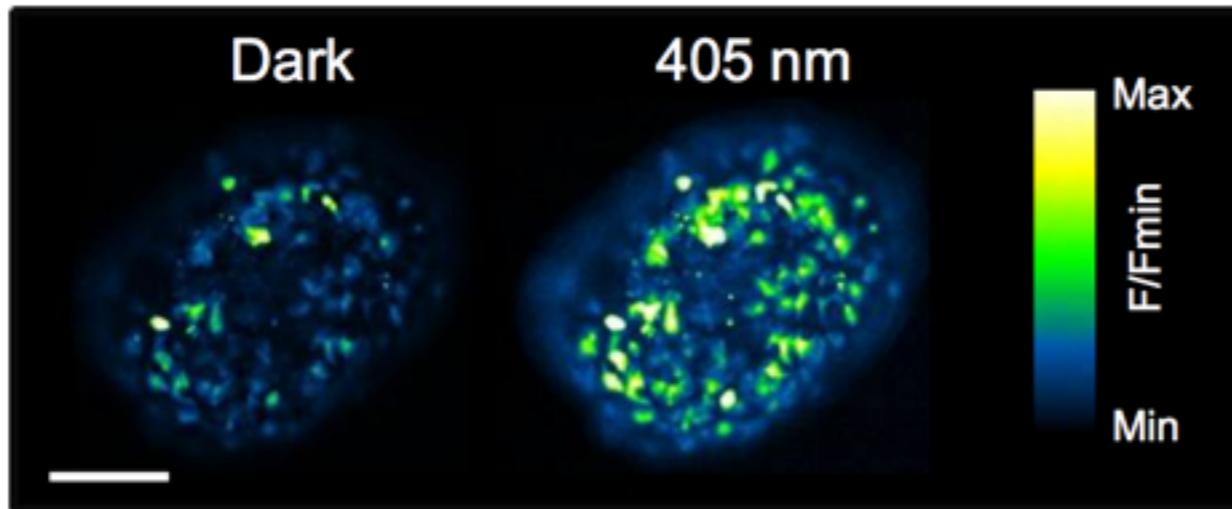


glibenclamide

Ein cis-aktiver lichtschtaltbarer Sulfonylharnstoff

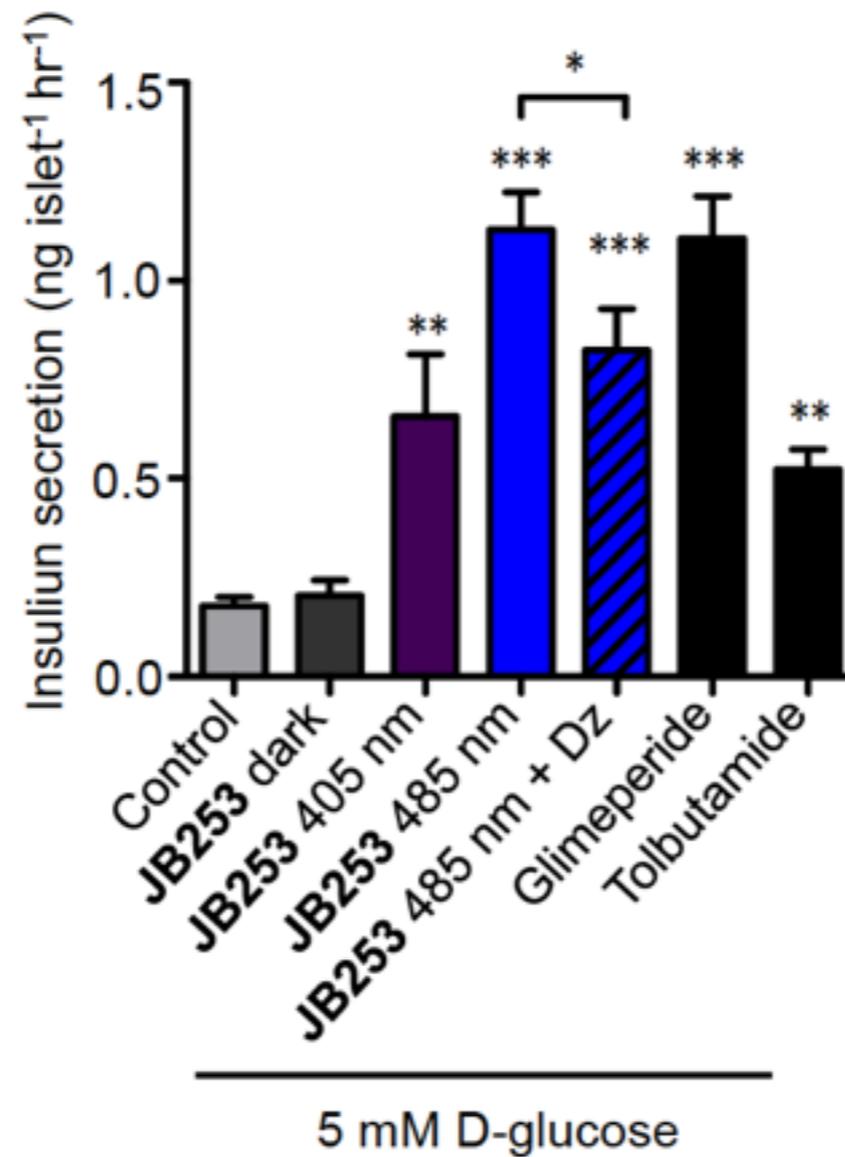


Lichtabhängige Kontrolle von Kalziumoszillationen in beta-Zellen



Ca²⁺ imaging in mouse islet cells

Lichtabhängige Insulinfreisetzung

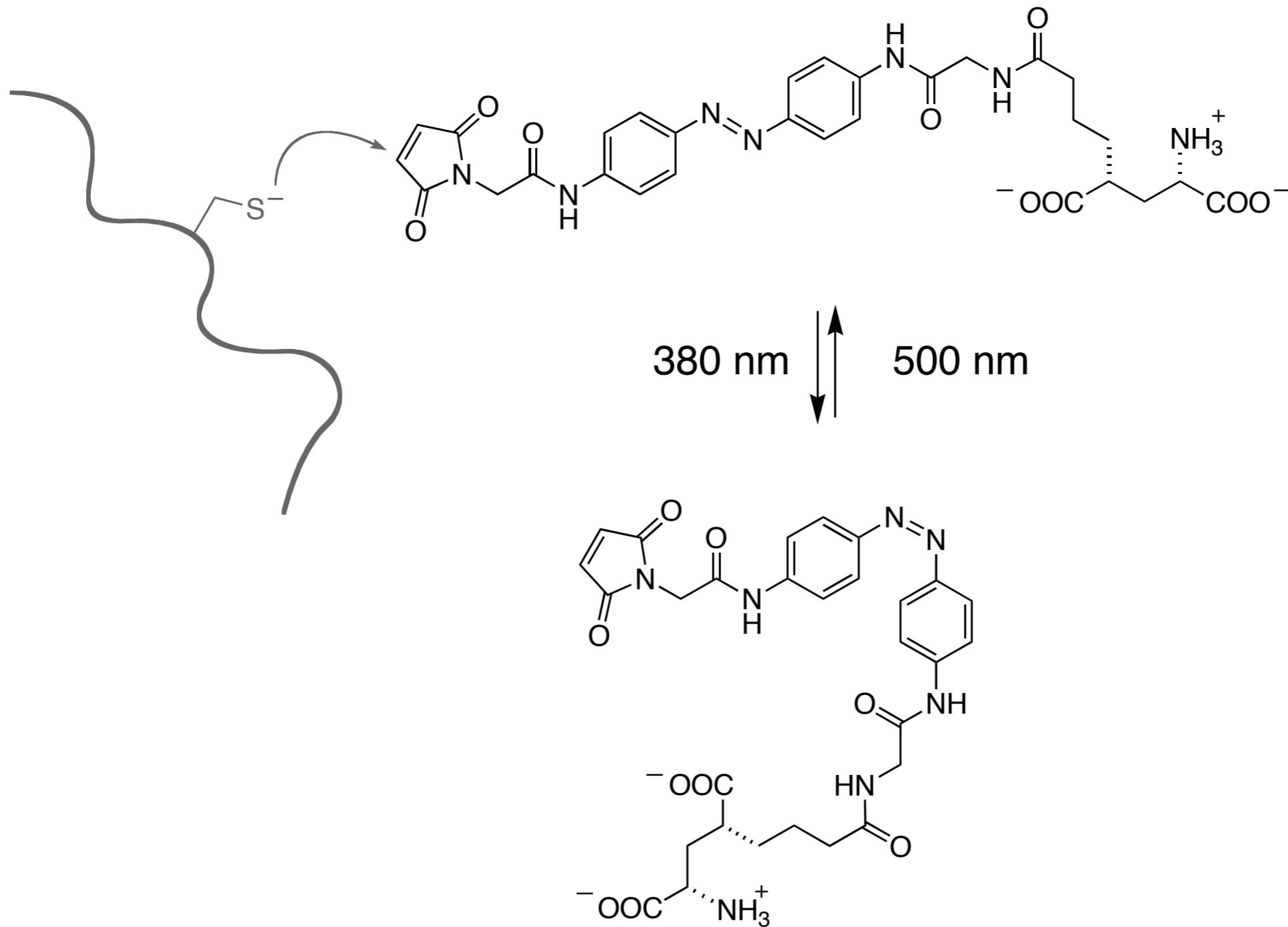


Genetic targeting through tethering

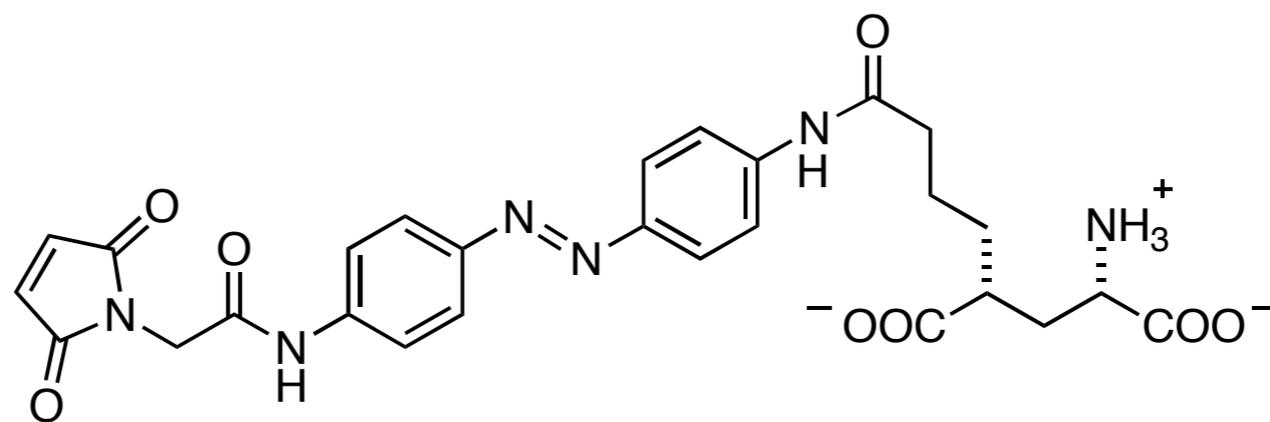
Photoswitched tethered ligands

PTL approach

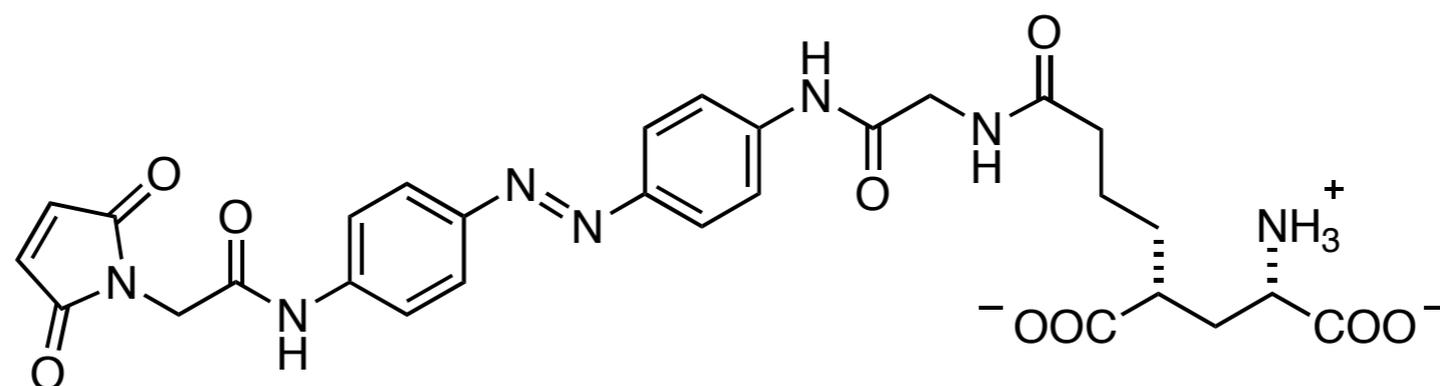
Ein reaktiver molekularer Schalter



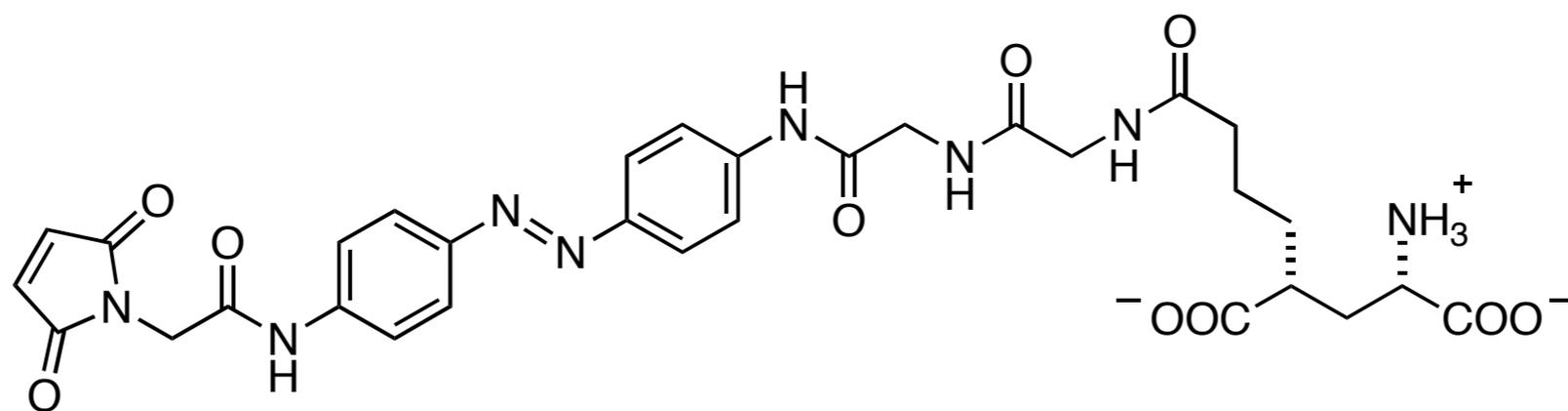
MAGs



MAG-0

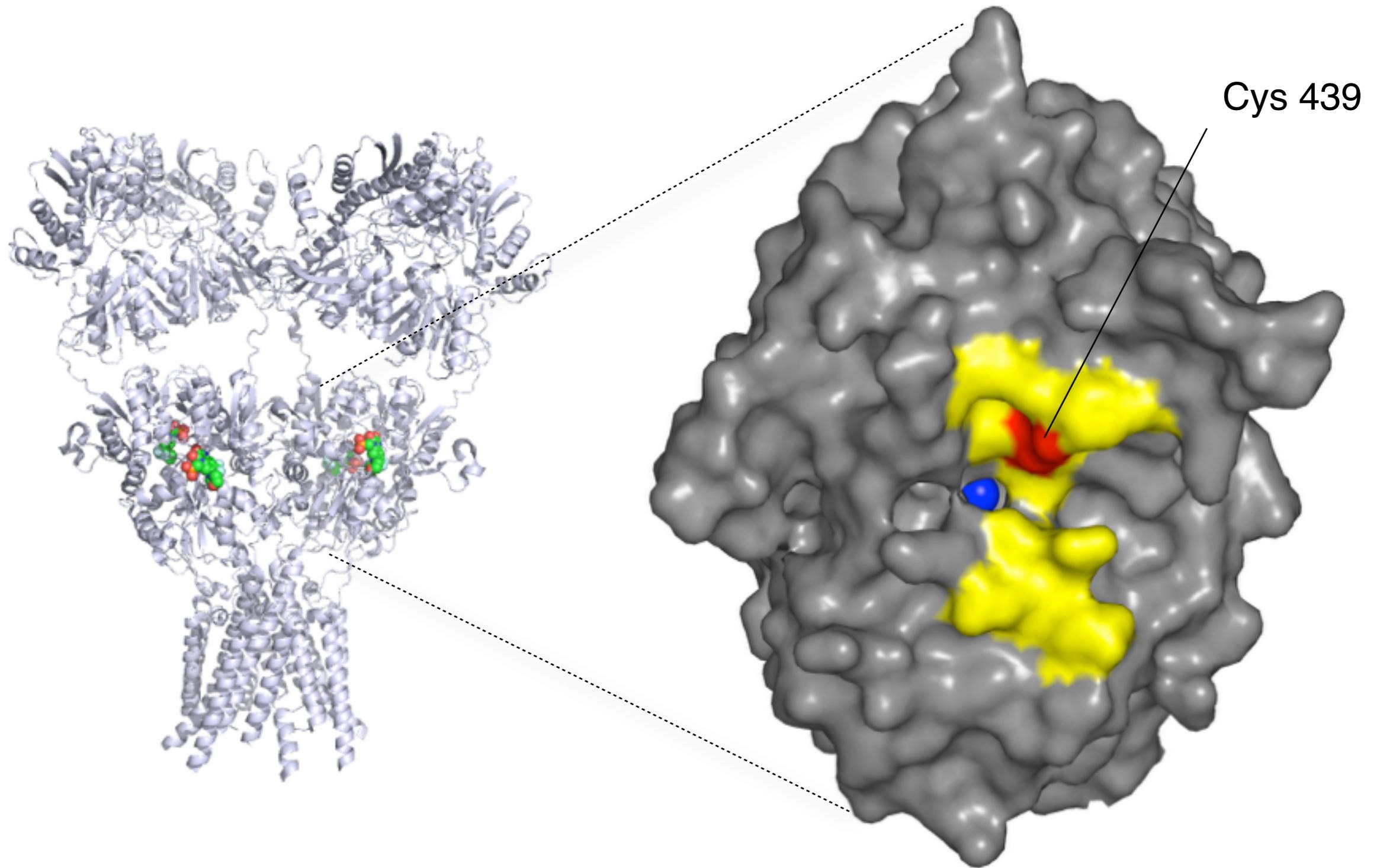


MAG-1

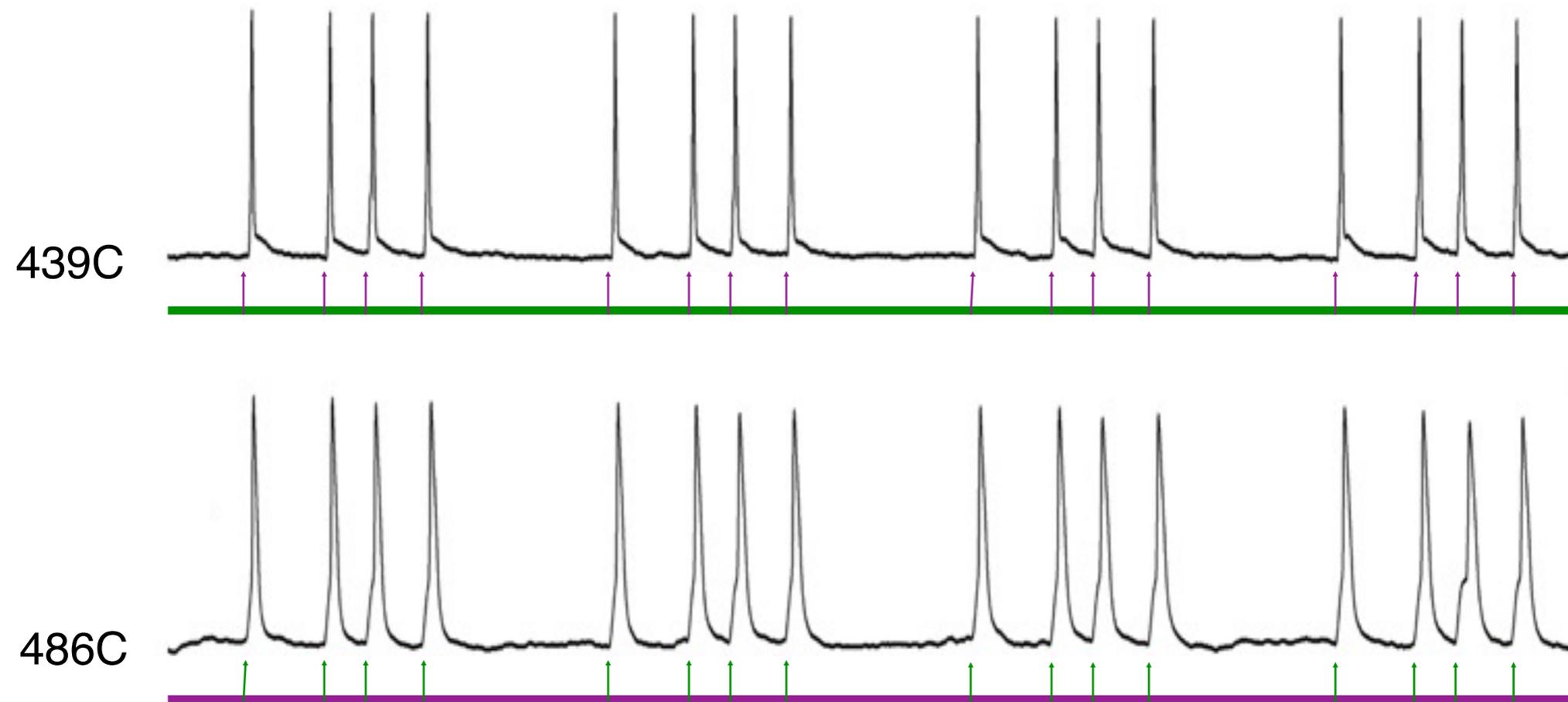
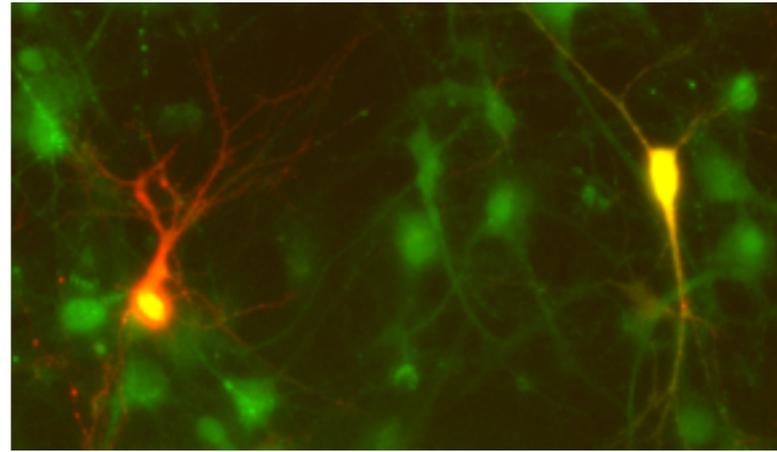


MAG-2

Der Ankerpunkt



Umpolen von LiGluR *trans- vs. cis-active*



Schluss für heute

http://tmedweb.tulane.edu/pharmwiki/doku.php/drug_receptor_theory

Mourot et al., ACS ChemNeurosci (2011)

Schönberger et al., ACS Chem. Biol. **2014**, 5, 514

Szymanski, W., Beierle, J. M., Kistemaker, H. A. V., Velema, W. A. & Feringa, B. L. Reversible Photocontrol of Biological Systems by the Incorporation of Molecular Photoswitches. *Chem. Rev.* **113**, 6114–6178 (2013).

1. Velema, W. A., Szymanski, W. & Feringa, B. L. Photopharmacology: Beyond Proof of Principle. *J. Am. Chem. Soc.* **136**, 2178–2191 (2014).

1. Fehrentz, T., Schönberger, M. & Trauner, D. Optochemical Genetics. *Angew. Chem. Int. Ed.* **50**, 12156–12182 (2011).

The Trauner Lab