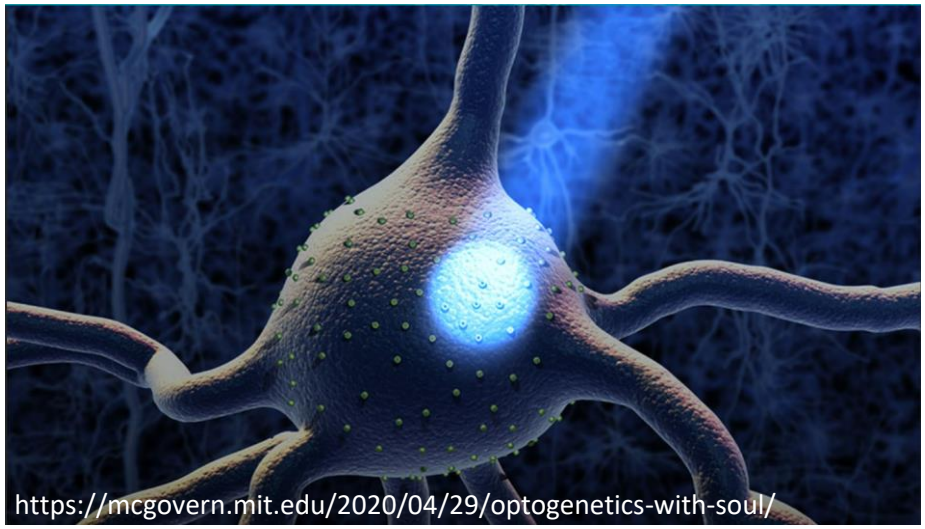
Molecular mechanism and functionalization of optogenetic tool from the protein perspective

2020/12/10

Literature Seminar

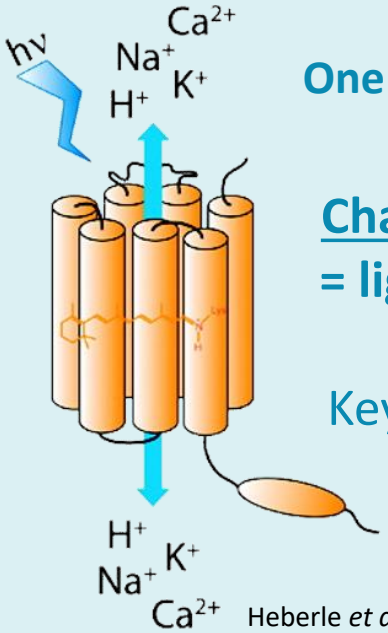
B4 Habazaki

What is Optogenetics?



https://mcgovern.mit.edu/2020/04/29/optogenetics-with-soul/

Optogenetics is the technique that allows precise and fast control of the activity of targeted brain cells!



One protein in Rhodopsin family

Channelrhodopsin-2 (ChR2)
= light-gated cation channel

Key protein of optogenetics

Heberle *et al. J. Am. Chem. Soc.* **2009**, *131 (21)*, 7313-7319.

A new ChR2 achieved minimally invasive optogenetic stimulation

Brain damage…

Buchen, *Nature* **2010**, *465*, 26-28.

Non-invasive!

https://www.clea-japan.com/products/animal/inbred

A significant disadvantage of optogenetics is **permanent damage to the brain**…

Now it is possible to activate any mouse brain region, non-invasively using ultra light-sensitive Channelrhodopsin mutant, SOUL!

Feng *et al. Neuron* **2020**, *107 (1)*, 38-51.

Contents

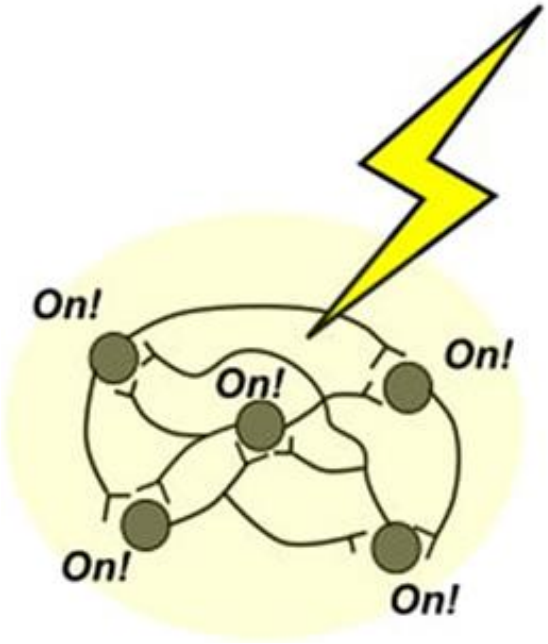
Contents

Artificial control of neuronal activity

Question of neuroscientists :

How does the **activity of certain neurons** in living animals relate to the function of the brain?

- **Observing** the relationship between neural activity and brain function
- **Manipulating** neural activity and examining what happens in the brain



Electrical stimulation

- The previous major method of artificial induction of neuronal activity

⭕ Fast control of neuronal activity

❌ Permanent damage to the brain

❌ Unspecific excitation of a wide spread neuronal network

https://kids.frontiersin.org/article/10.3389/frym.2017.00051

Artificial control of neuronal activity

Question of neuroscientists :

How does the **activity of certain neurons** in living animals relate to the function of the brain?

- **Observing** the relationship between neural activity and brain function
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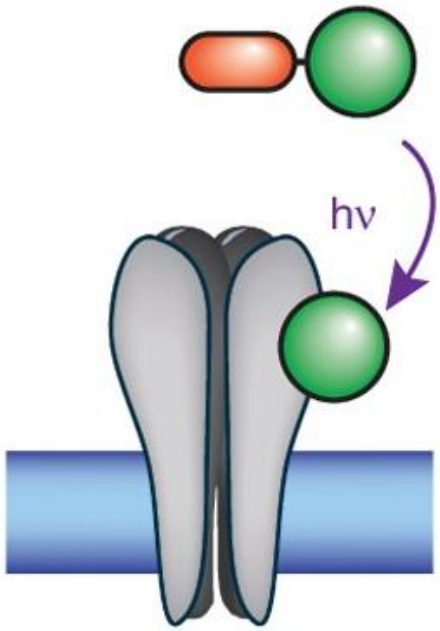


Caged reagent

- Neurotransmitters whose activity is controlled by light

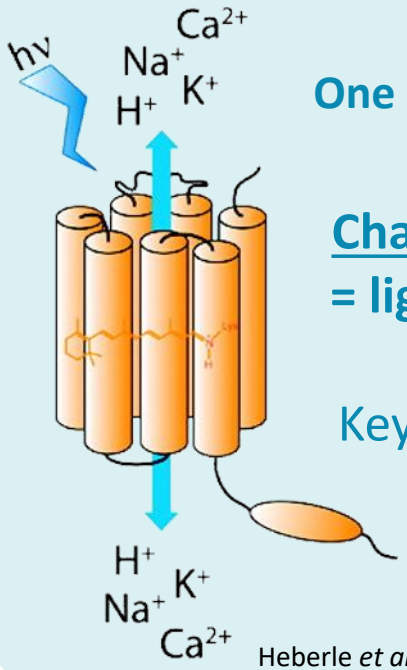
✓ Less invasive than electrical stimulation

✓ Excitation of specific location

✗ Unspecific excitation of a wide spread neuronal network

Kramer *et al. Nat Neurosci* **2013**, *16*, 816-823.

New technique to control neuronal activity using light; Optogenetics



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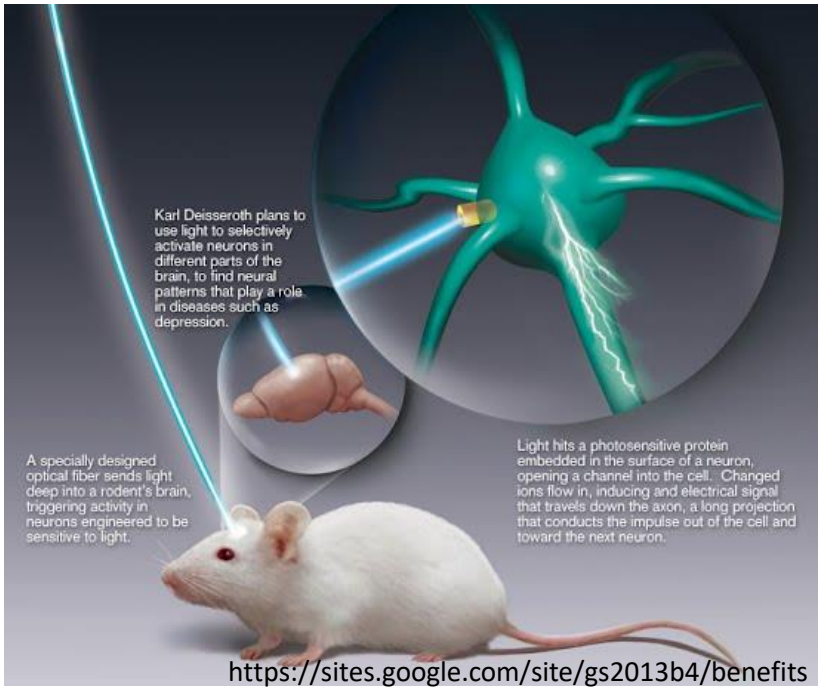


https://sites.google.com/site/gs2013b4/benefits

Needs : Cell type-specific neural activity manipulation technique was desired.

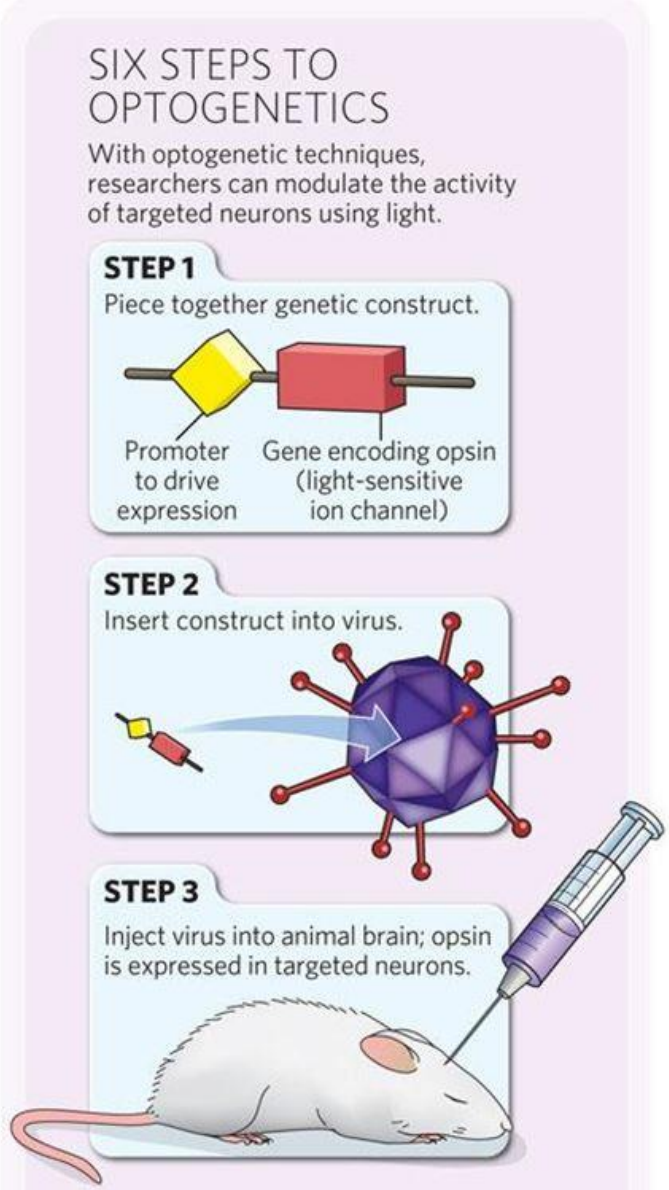
Discovery : ChR2 was discovered as a **light-sensitive protein** which has **channel** function.

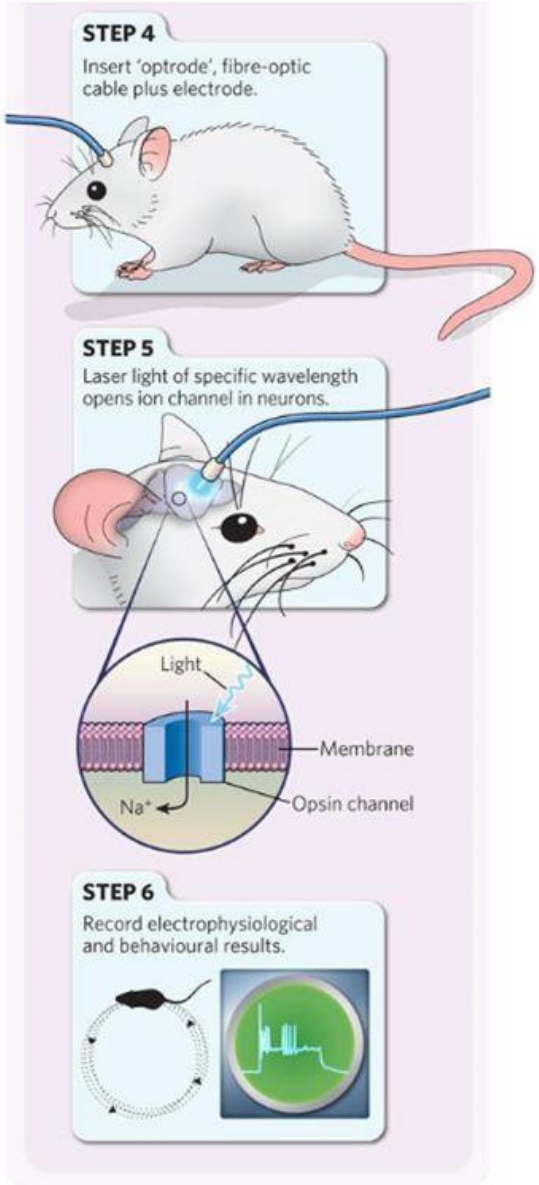
Optogenetics

Idea : Expressing ChR2 in neurons induces light-induced depolarization

Deisseroth *et al. Nat Neurosci* **2005**, *8*, 1263-1268.

New technique to control neuronal activity using light; Optogenetics



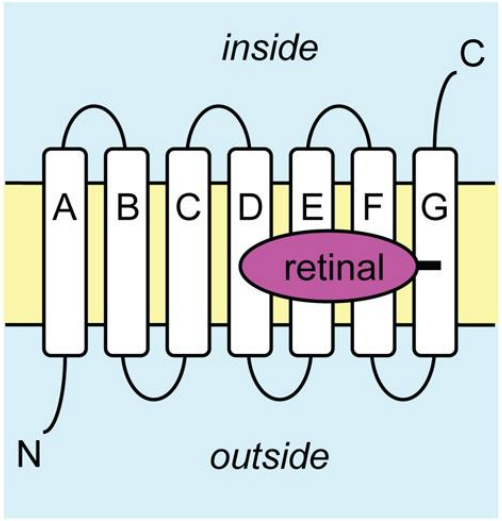


- **Fast** control (**millisecond-scale**)!
- Excitation of **specific location**!
- Excitation of **specific cell type**!
- **Reversible** control!

Buchen, *Nature* **2010**, *465*, 26-28.

Contents

Structure of ChR2



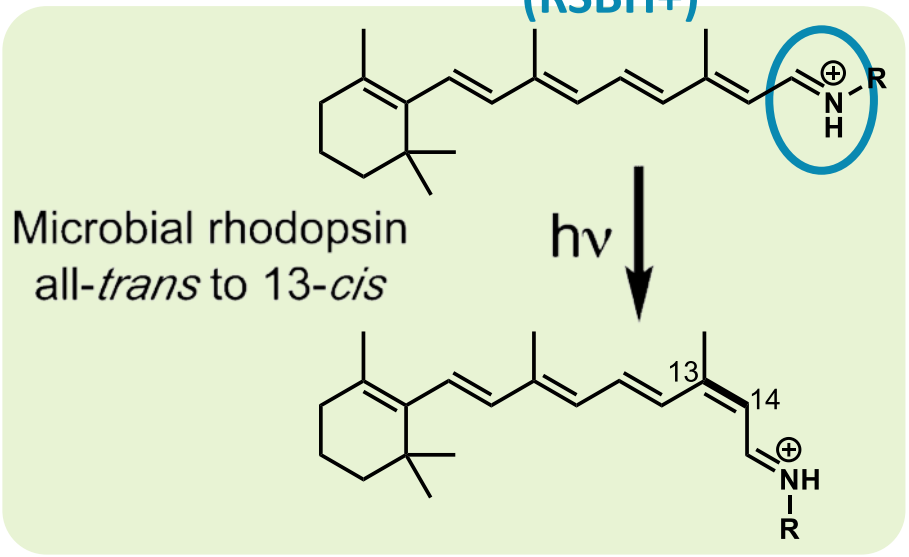
Photoreceptor protein; Rhodopsin

- Apoprotein; Opsin
- Chromophore; retinal

Opsin protein

- Seven transmembrane α-helices (TM)

Retinal Schiff Base (RSBH+)

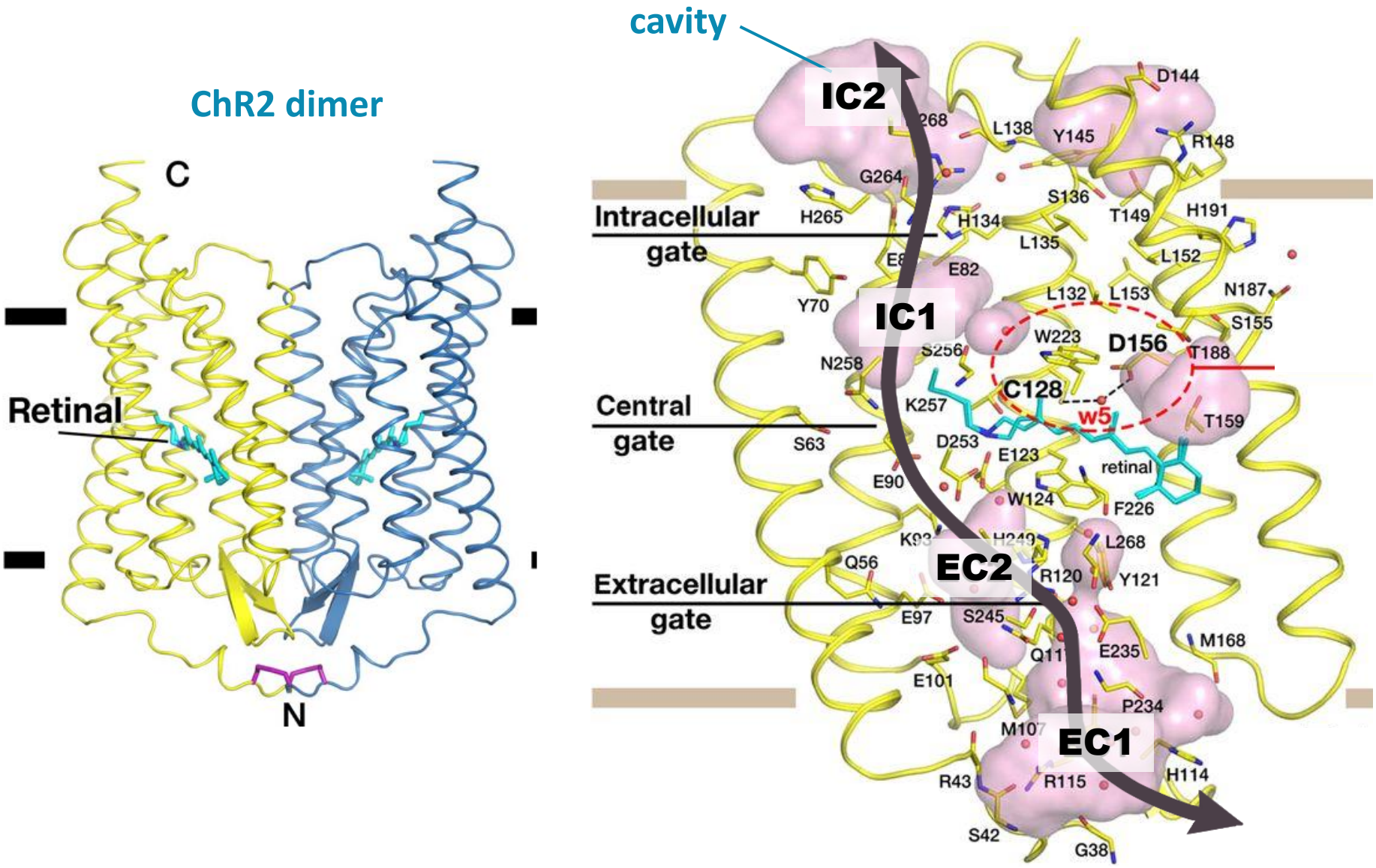


Retinal chromophore

- Forming a Schiff base linkage with a lysine residue in the middle of TM7
- Isomerization from all-*trans*-retinal to 13-*cis*-retinal by the photoactivation

Ernst *et al. Chem. Rev.* **2014**, *114 (1),* 126-163.

Structure of ChR2



Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Methods used to investigate the molecular mechanisms of ChR2

Analysis of molecular mechanism

Structural Analysis

X-ray crystallography
Cryo-EM, XFEL
Computer simulation

Spectroscopy

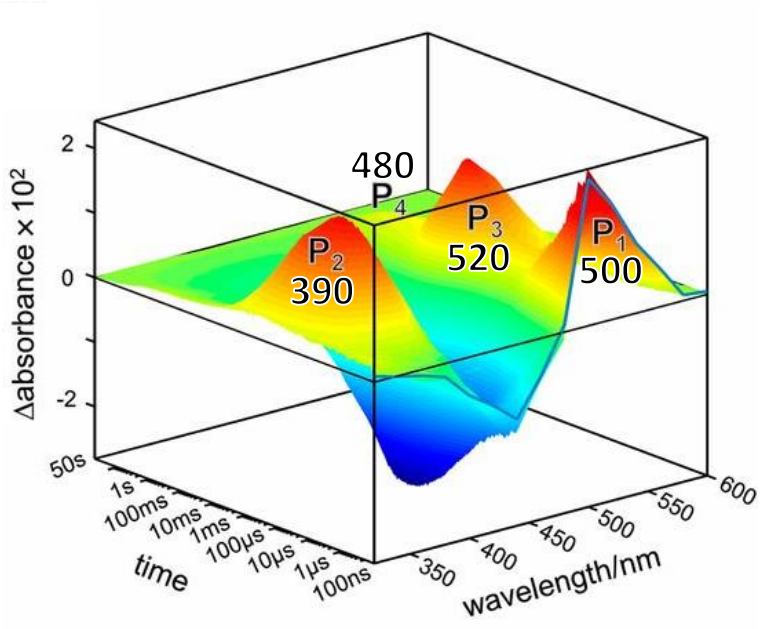
Time-resolved spectroscopy
Raman spectroscopy
EPR, NMR

Electrophysiological Studies

Patch-Clamp Recordings

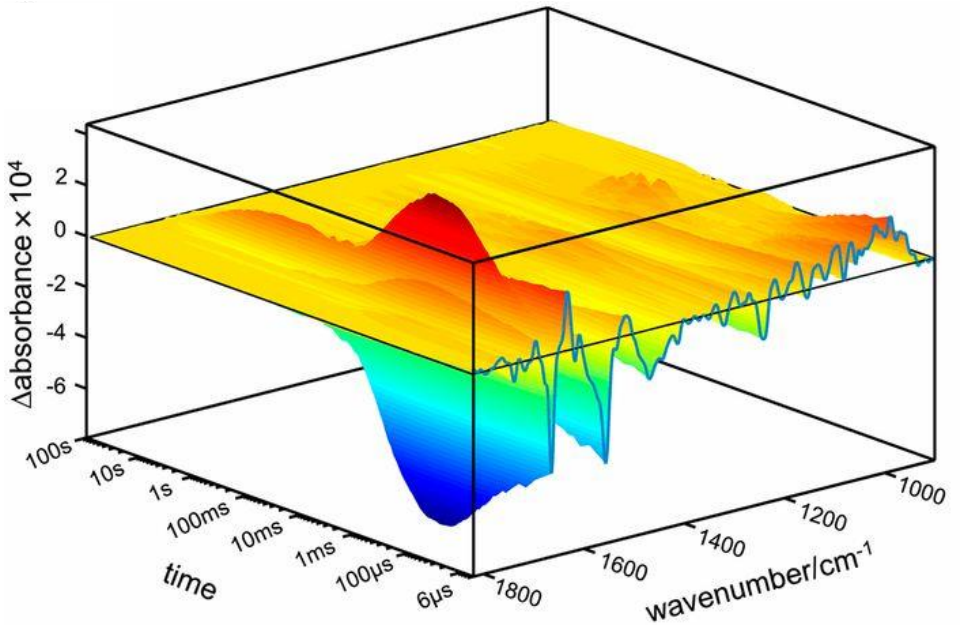
Methods used to investigate the molecular mechanisms of ChR2

Time-resolved spectroscopy



UV/Vis spectroscopy

- Isomeric state of **retinal**
- Environment of **retinal Schiff base**
  (e.g. Protonation state (RSB or RSBH+))

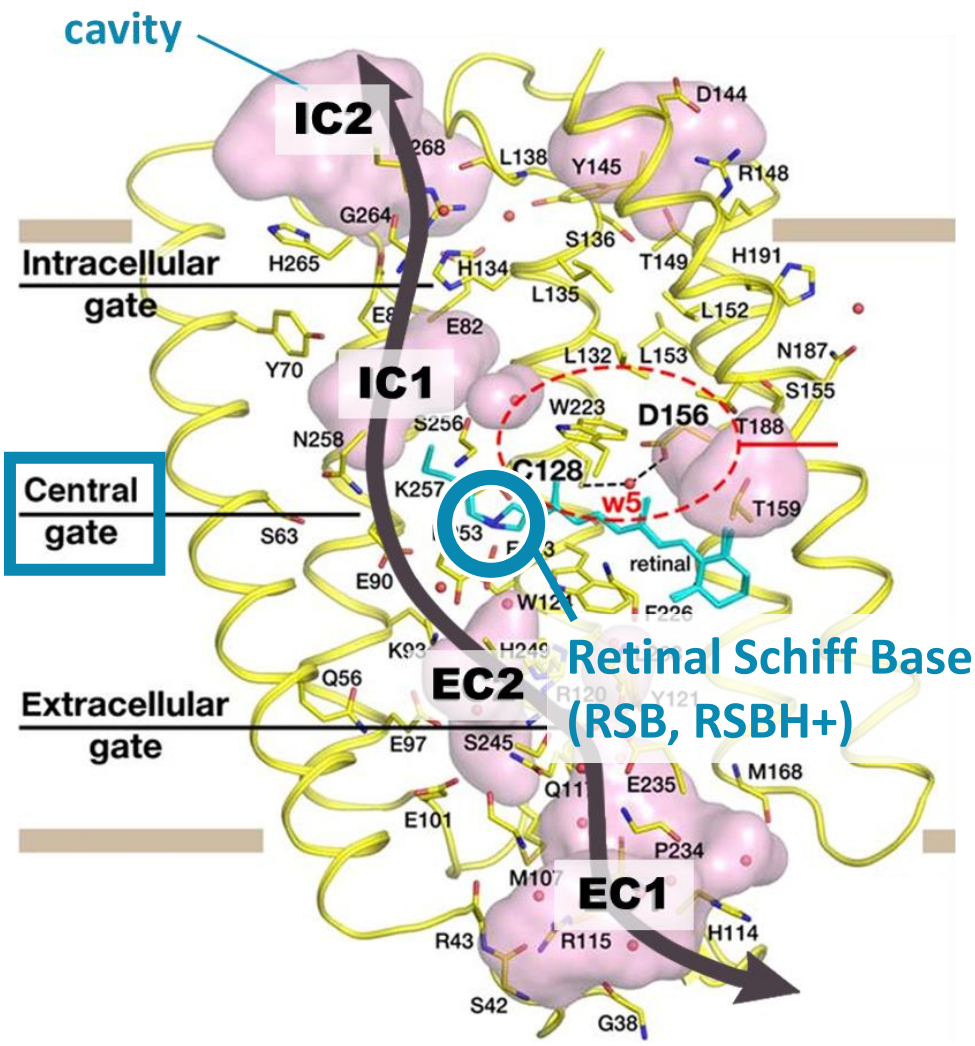


FTIR spectroscopy

- Changes in **interactions**
  (e.g. Hydrogen bonding)
- Large **conformational changes** in the protein backbone

Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281.

Photocycle of ChR2



Important points in the optical response of ChR2

① **Photoisomerization of retinal**
= Trigger of photoactivation

② **Change in the protonation state of RSB**
= Important switch of channel gating

③ **Changes in the H-bonding networks**
= Key factors of conformational change

☆Channel gating does not rely on the steric effect of retinal isomerization!

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Photocycle of ChR2

Channel closing phase

③Conformational change

②D156 reprotonation

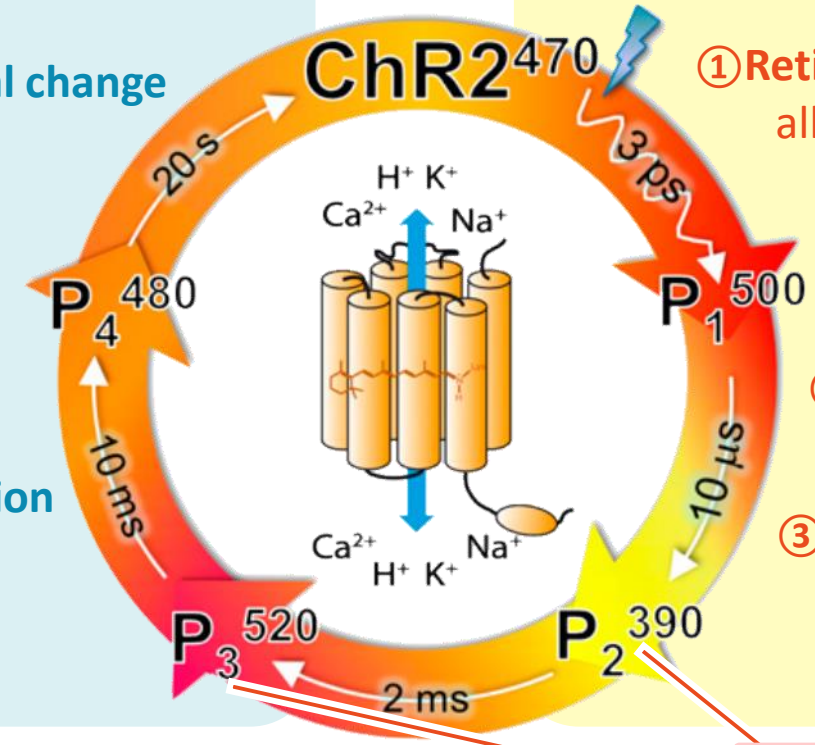
①Retinal reisomerization
Channel closing

Channel opening phase

①Retinal isomerization
all-*trans*→13-*cis*

②RSBH+ deprotonation
RSBH+→D256

③Conformational change
Channel opening



②RSB reprotonation
D156→RSB

Conducting state

Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281.

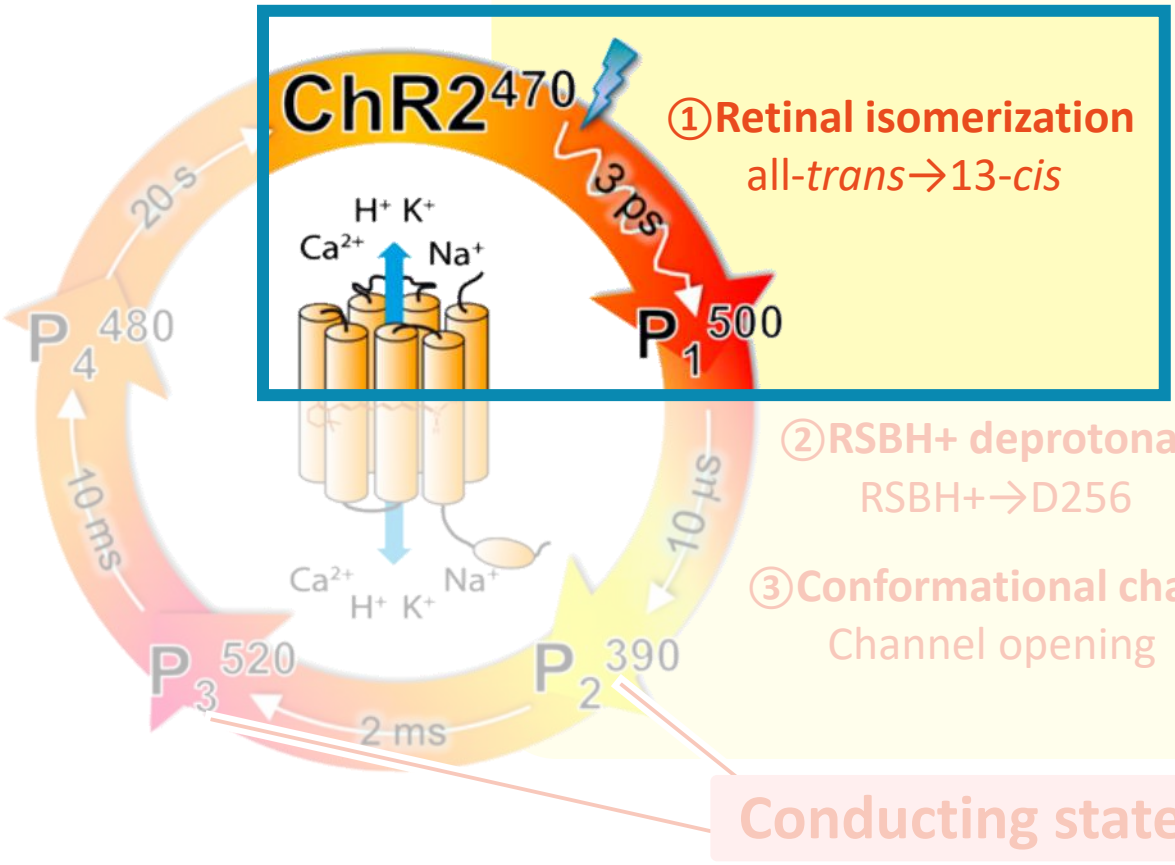
Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

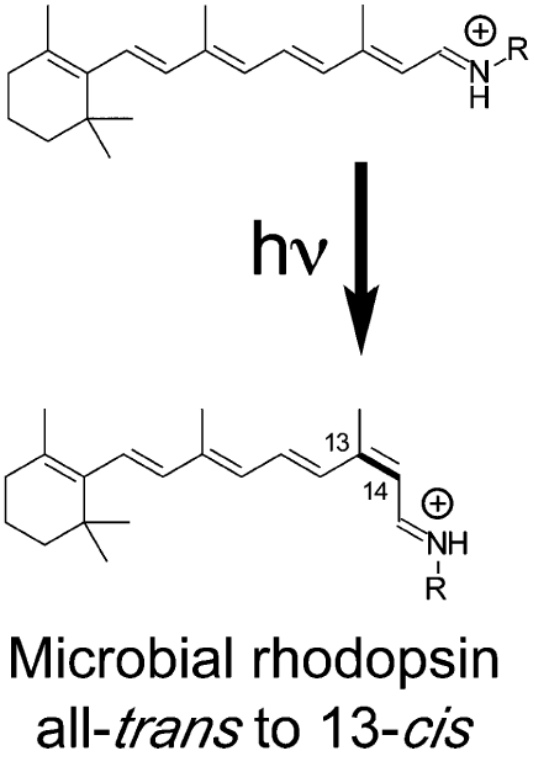
Heberle *et al. J. Am. Chem. Soc.* **2009**, *131 (21),* 7313-7319.

Ernst *et al. Chem. Rev.* **2014**, *114 (1),* 126-163.

Light absorption and photoisomerization of retinal

Channel opening phase





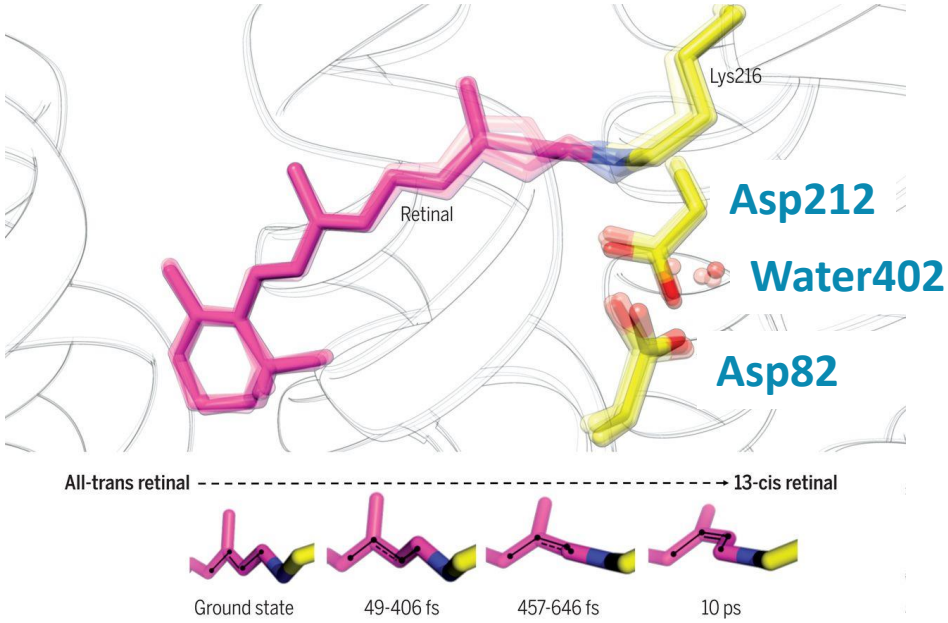
Microbial rhodopsin
all-*trans* to 13-*cis*

- The initial photochemical reaction in rhodopsins is known to be **one of the fastest and most efficient chemical events** in biology!

Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281. Ernst *et al. Chem. Rev.* **2014**, *114 (1),* 126-163.

Light absorption and photoisomerization of retinal

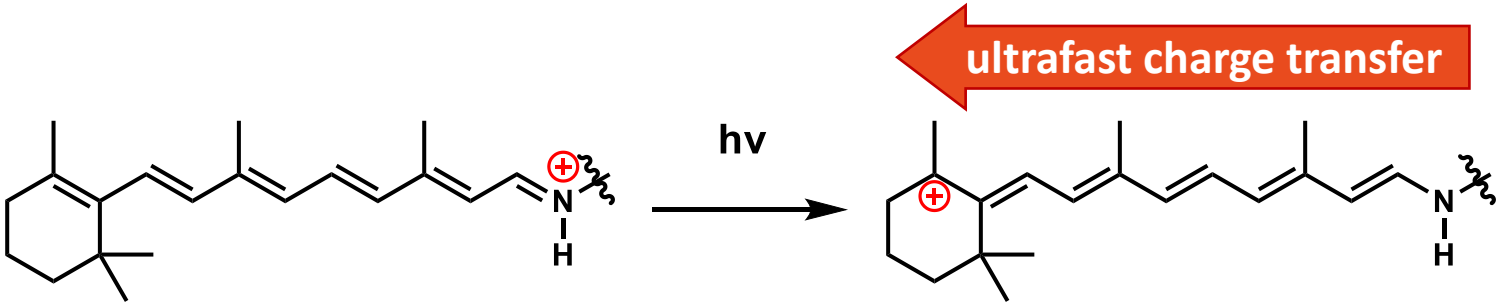
Observation using X-ray free-electron lasers (XFELs) of Bacteriorhodopsin



The ultrafast structural changes to the retinal was probed!

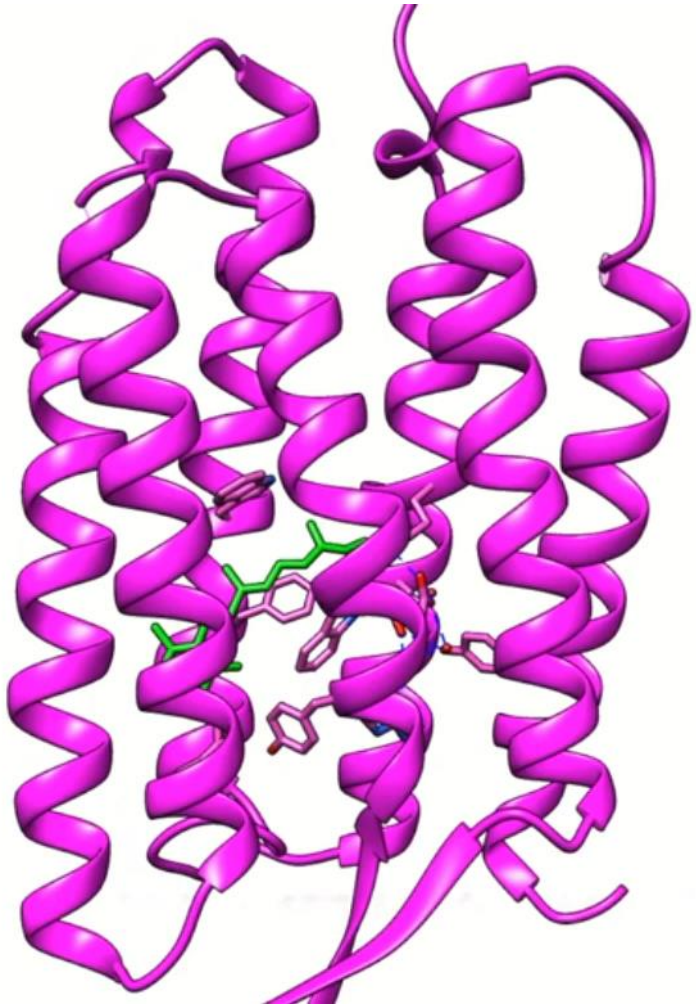
Ultrafast charge transfer along retinal is a driving force for **ultrafast response of the counterion cluster**.

→Possibly contributing to the stereoselectivity and efficiency.



Standfuss *et al. Science* **2018**, *361*, 6398.

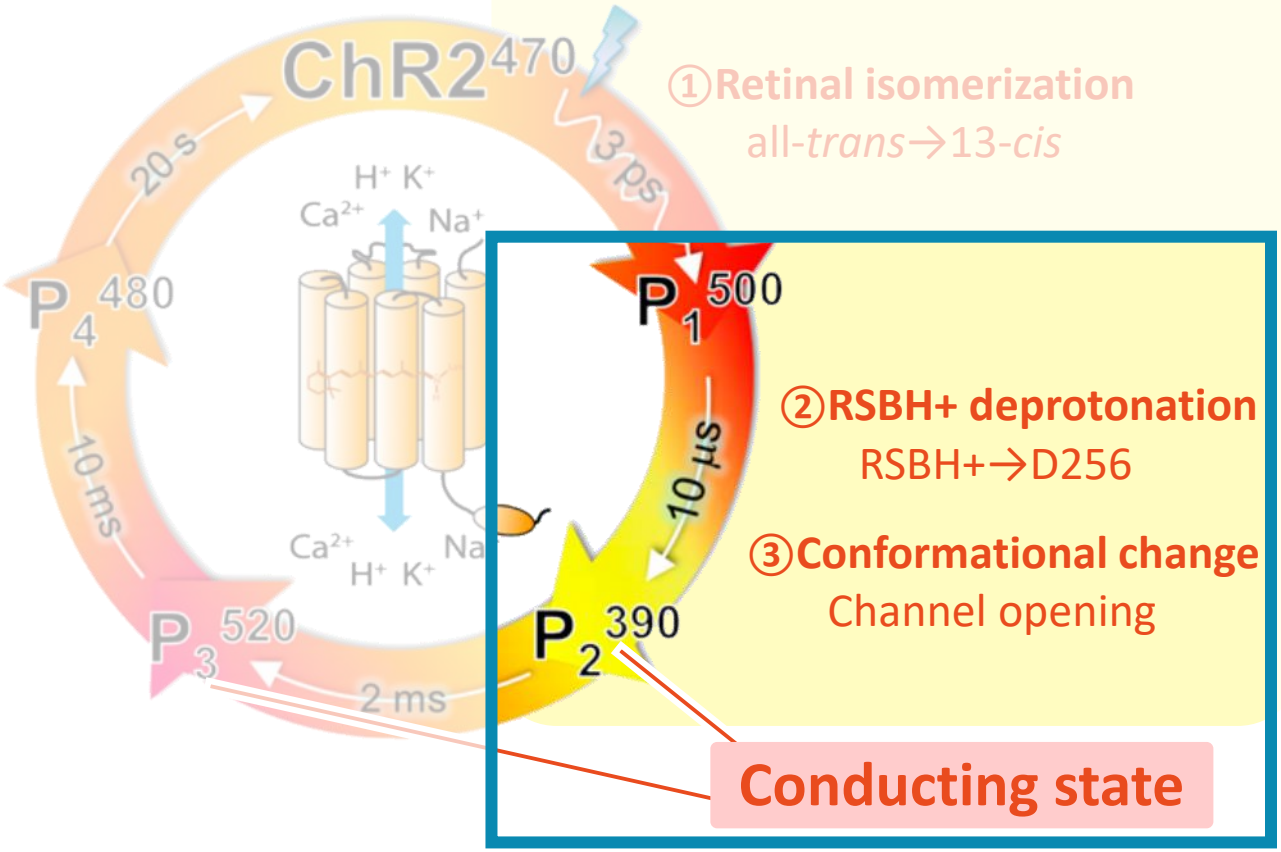
Light absorption and photoisomerization of retinal



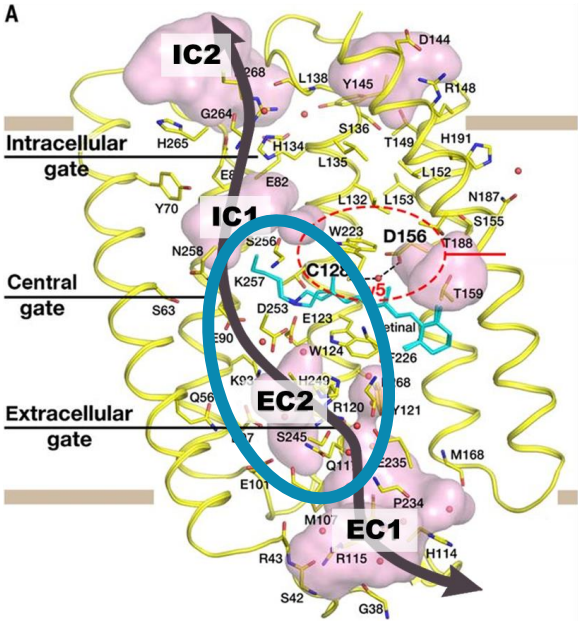
Standfuss *et al. Science* **2018**, *361,* 6398.

Rearrangement of hydrogen-bond network and channel opening

Channel opening phase



Opening of Central gate & Extracellular gate



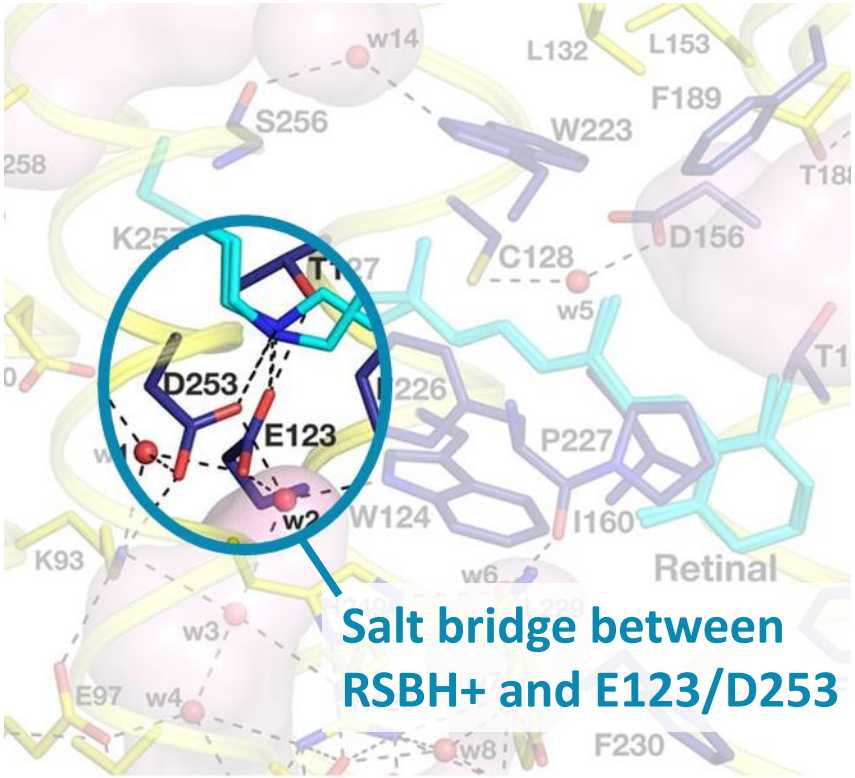
Ultrafast isomerization of retinal can not directly cause major structural changes.

→**The high-energy H-bonding network causes structural changes with energetic relaxation!**

Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281. Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Rearrangement of hydrogen-bond network and channel opening

Proton transfer from RSBH+ to D253 and rearrangement of D253



Spectroscopic data :

- **D253** serves as **the proton acceptor** of RSBH+.
- **The strong interaction of the protonated D253 with the E123** carboxylate is observed after isomerization of retinal.

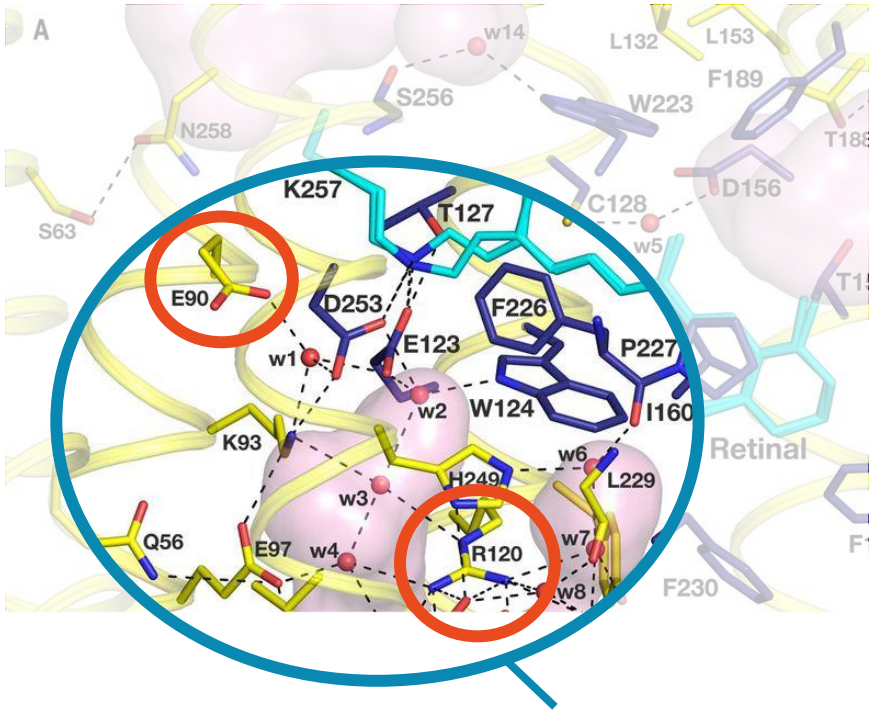
Isomerization causes the deprotonation of RSBH+ and the rearrangement of D253.

Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281.

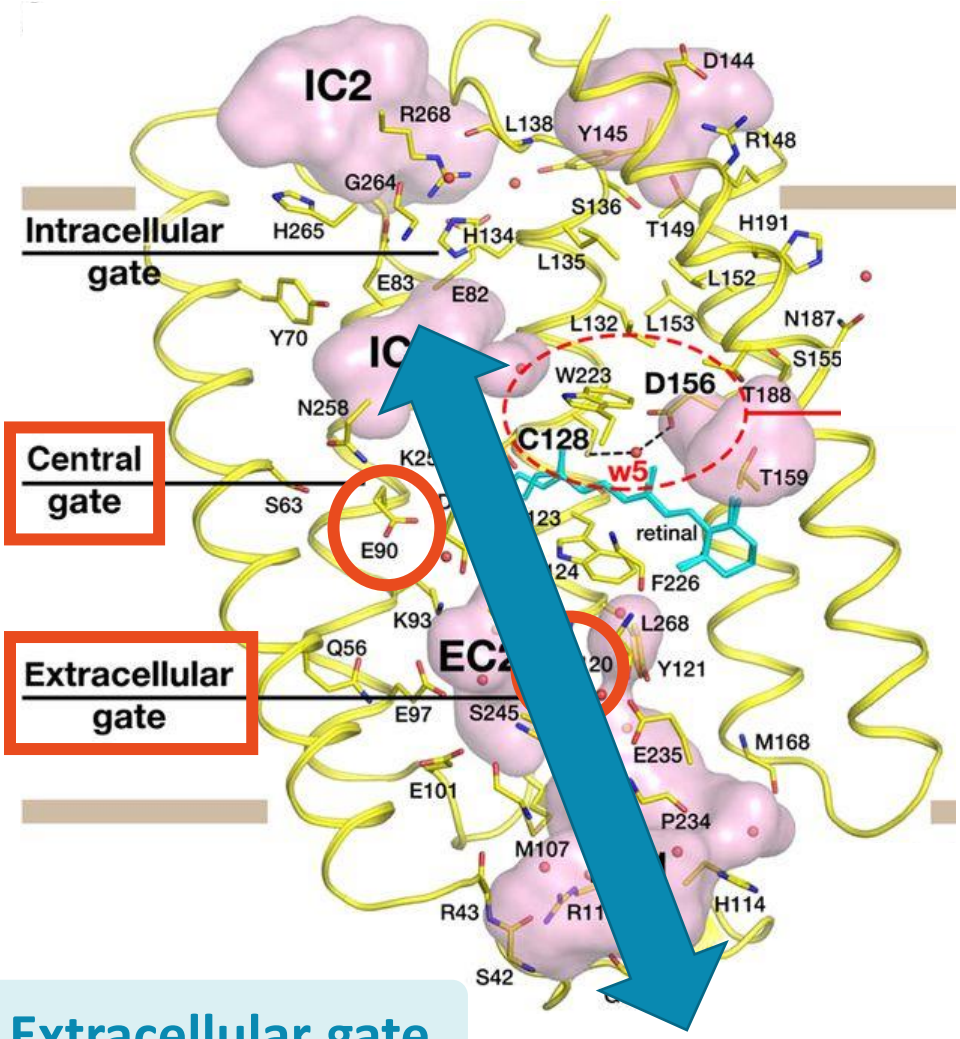
Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Rearrangement of hydrogen-bond network and channel opening

Rearrangement of H-bonding network and opening of two gates



H-bonding network containing D253

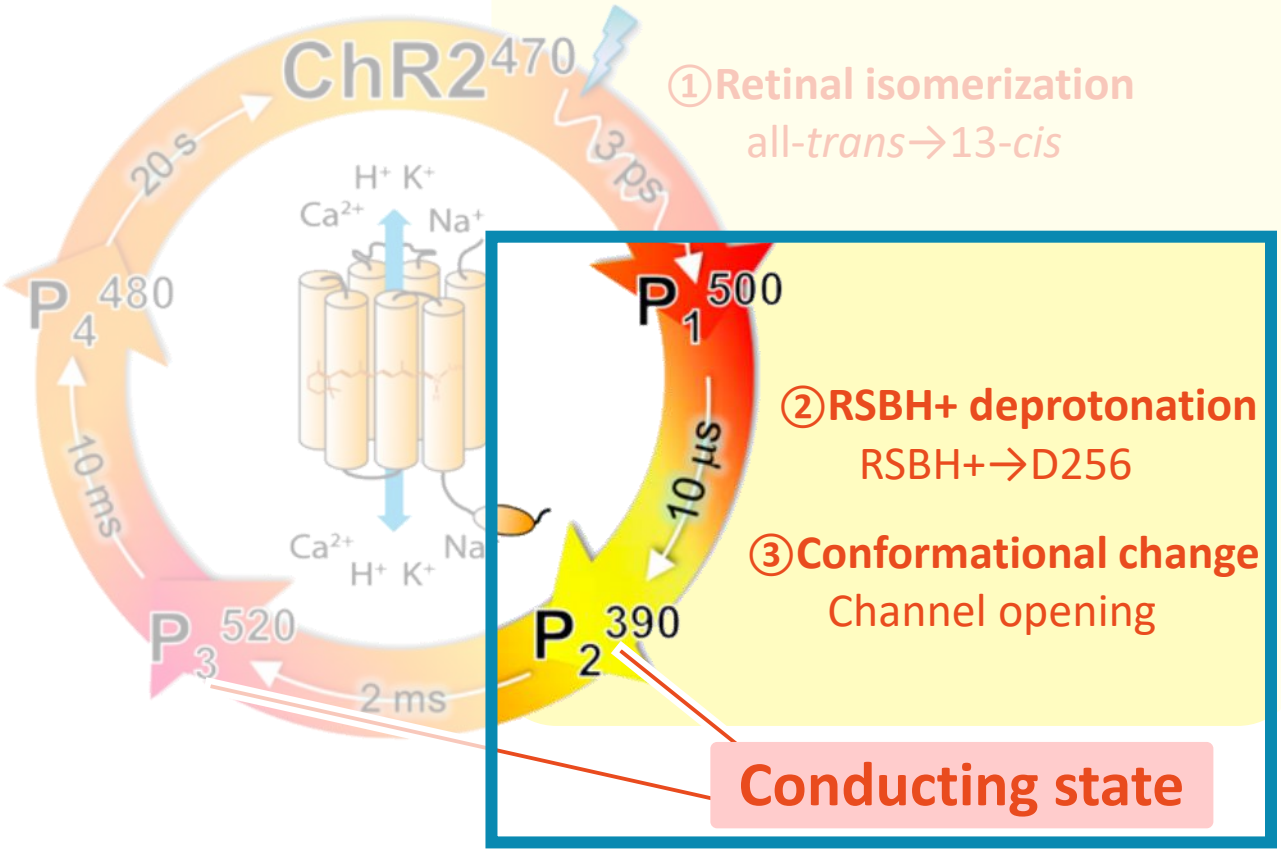


Opening of Central and Extracellular gate

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Rearrangement of hydrogen-bond network and channel opening

Channel opening phase



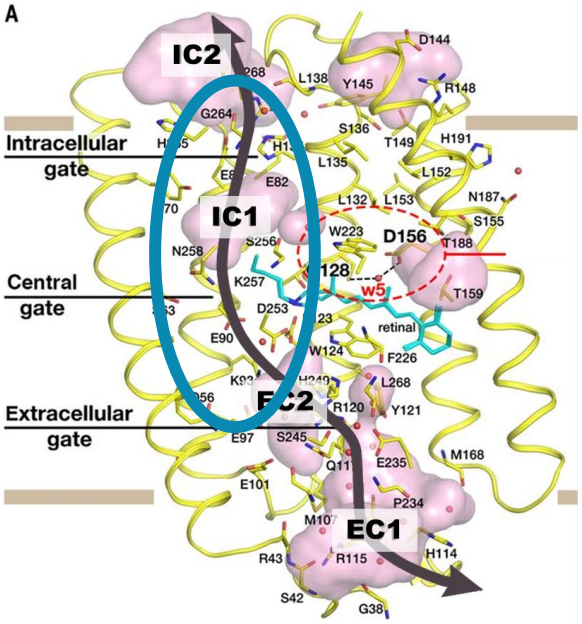
①Retinal isomerization
all-*trans*→13-*cis*

②RSBH+ deprotonation
RSBH+→D256

③Conformational change
Channel opening

Conducting state

Opening of Intracellular gate



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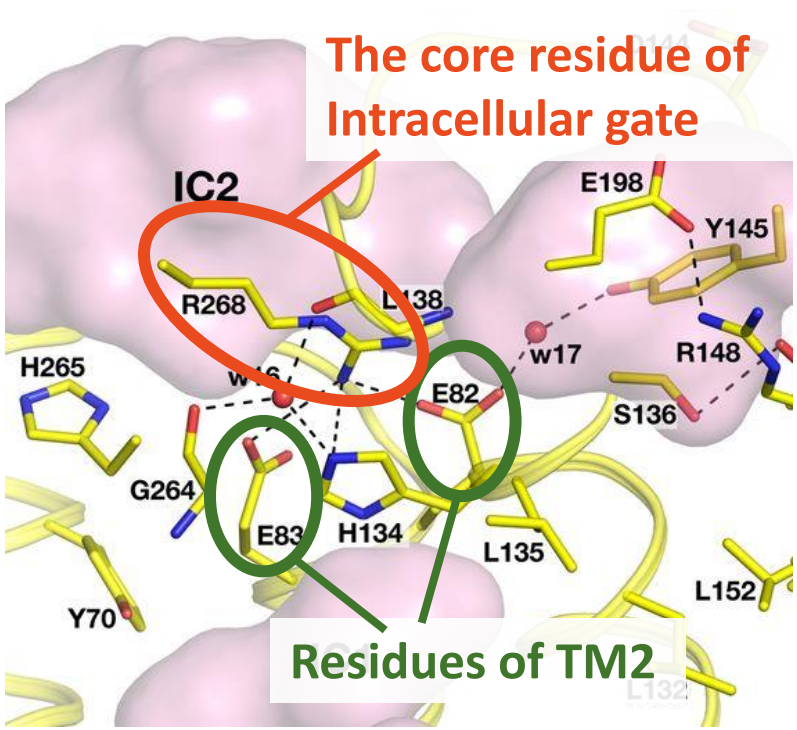
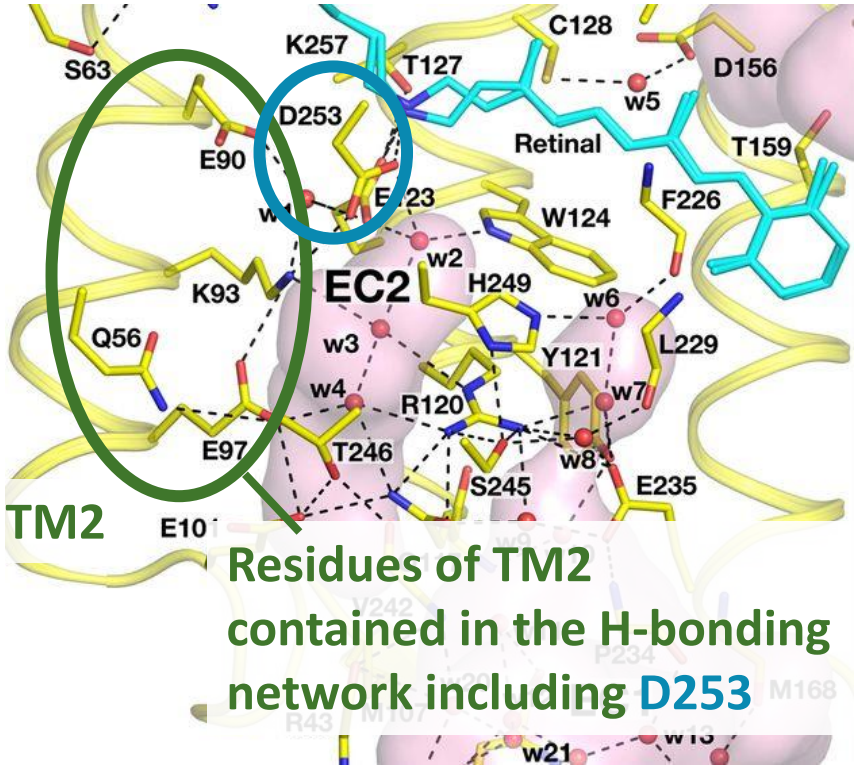
Rearrangement of hydrogen-bond network and channel opening

Rearrangement of H-bonding network and opening of the remaining gate

Structural & Spectroscopic data :

- The global reorientation of TM 2 occurs during channel opening.





Hegemann, Gerwert *et al. Angew. Chem. Int. Ed.* **2015**, *54,* 4953-4957.

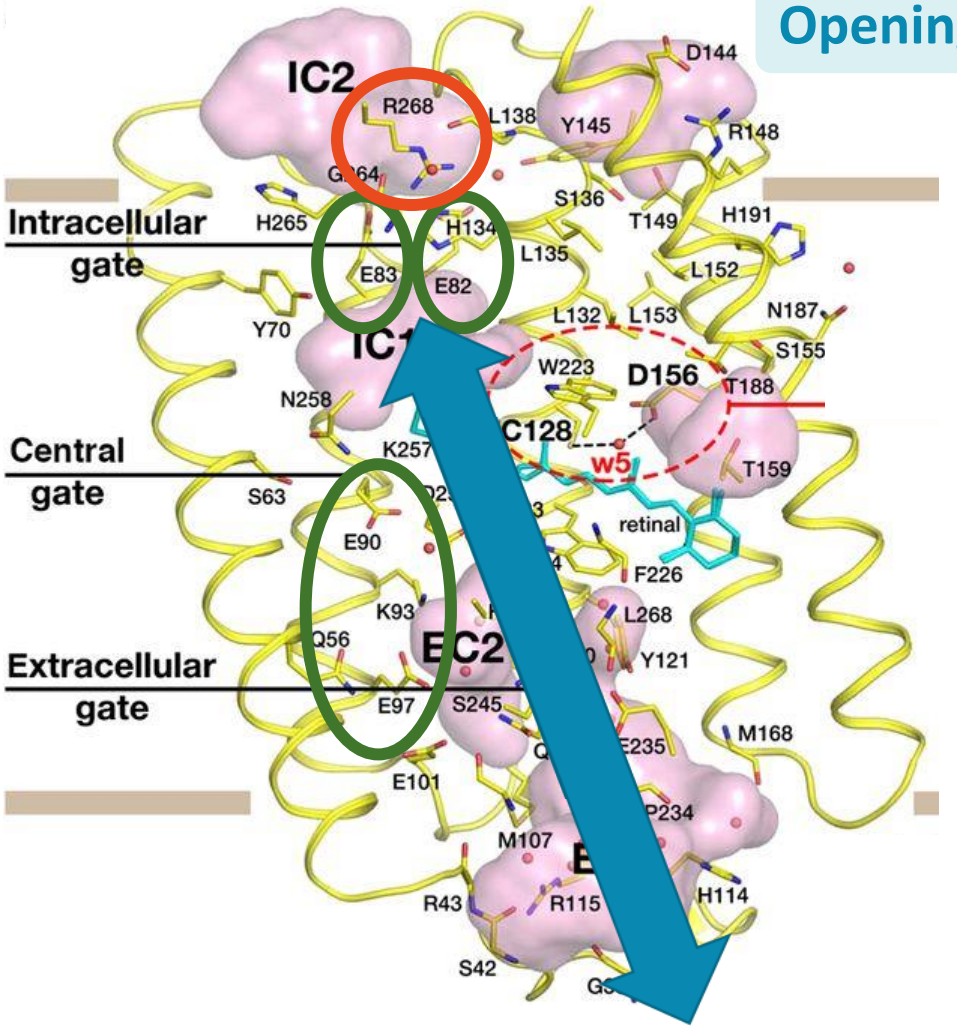
Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

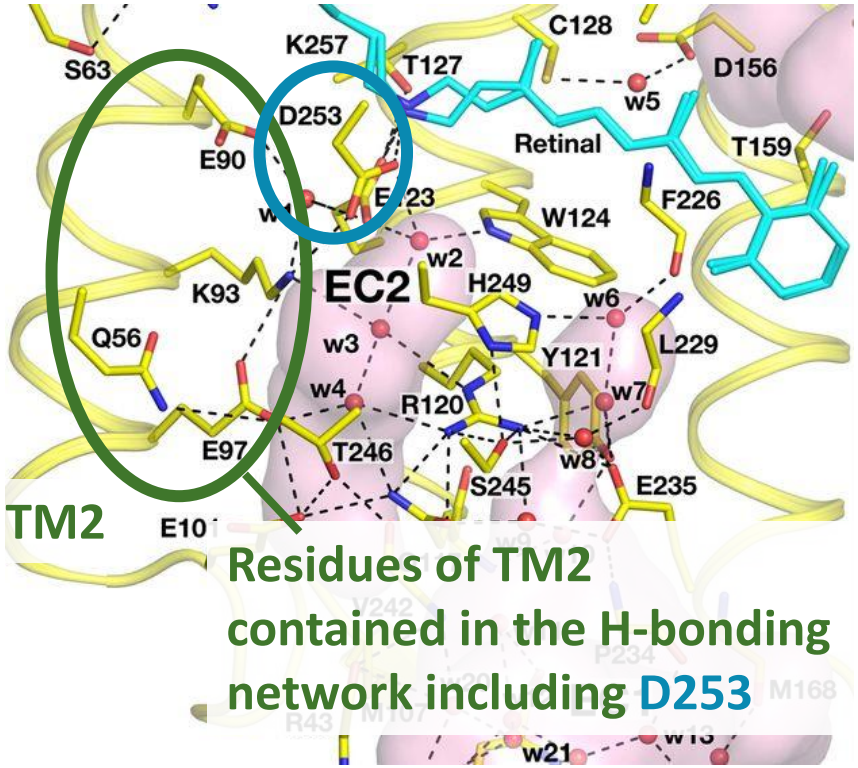
Bartl *et al. J. Biol. Chem.* **2008**, *283,* 35033-35041.

Rearrangement of hydrogen-bond network and channel opening

Rearrangement of H-bonding network and opening of the remaining gate

Opening of Intracellular gate

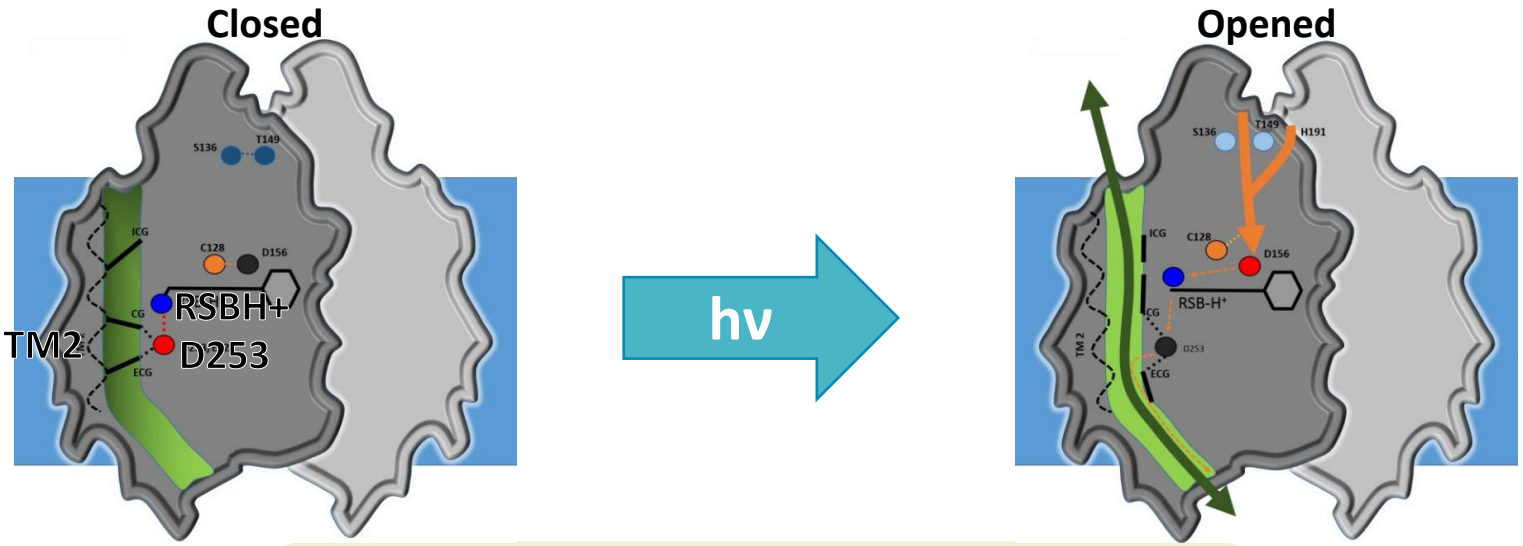




Residues of TM2 contained in the H-bonding network including D253

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Short Summary



Photoisomerization of retinal

↓

Deprotonation of RSB

↓

Changes in the H-bonding networks

↓

Changes in the protein conformation

↓

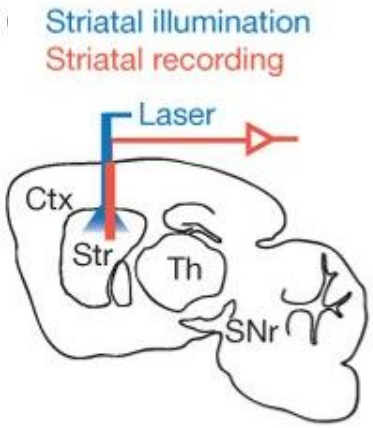
Opening of three gates

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

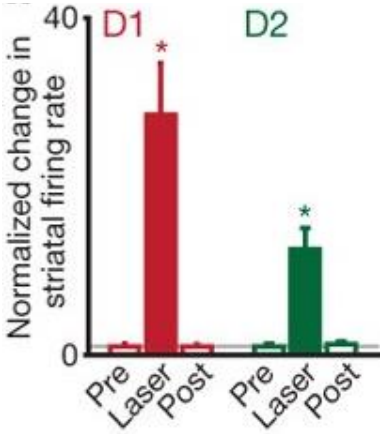
Contents

Application of optogenetics

Regulation of parkinsonian motor behaviours by optogenetic control



D1→direct-pathway
D2→indirect-pathway



- Direct-pathway activation completely restored motor behaviour to pre-lesion levels in in Parkinson's disease-model mice!

Kreitzer *et al. Nature* **2010**, *466,* 622-626.

Contents

Development of less invasive optogenetic tools

Brain damage…

Buchen, *Nature* **2010**, *465*, 26-28.

Optogenetics

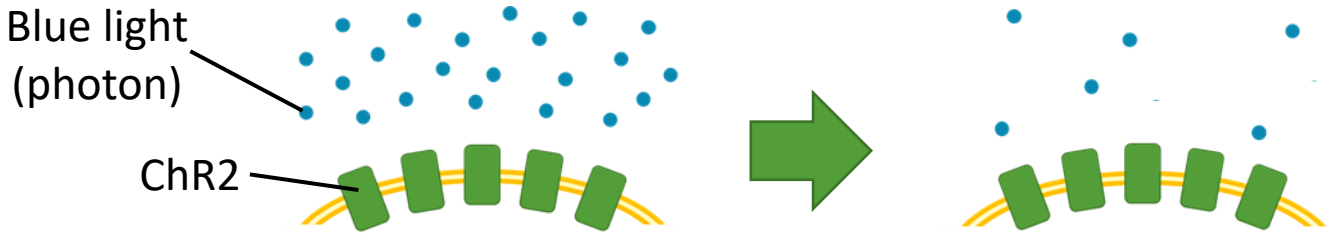
✗ **Permanent damage to the brain…**

Development of **less invasive optogenetic tools** is a very important challenge!

One of the strategies of less invasive optogenetic tools :

Engineering **high light-sensitive** ChR2 mutants!

… ChR2 mutants that can drive a certain amount of photocurrent even by very weak light (only a little photon).

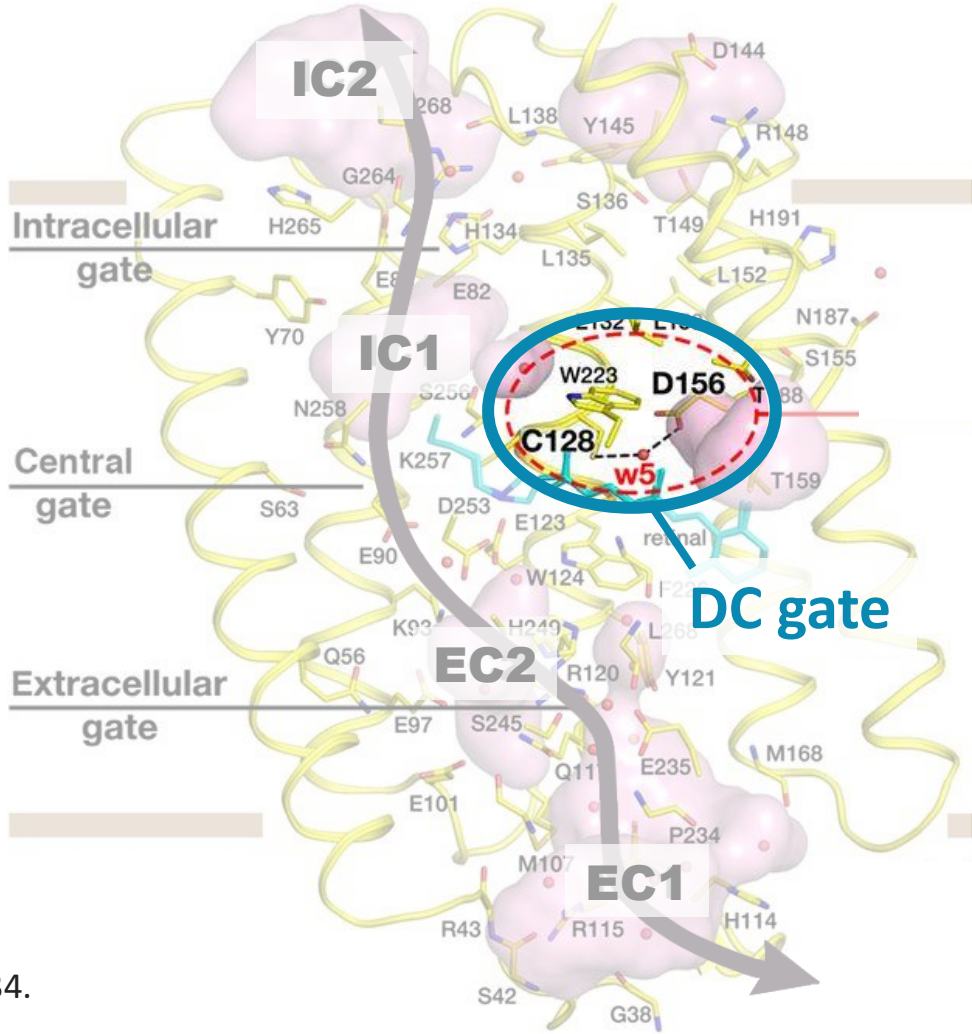


Highly light-sensitive ChR2 mutant ①; **SFO family**

Step-Function Opsin (SFO) family

- **Mutation : C128X or D156A**
  (X = S, T, A)
- **Phenotype :**
  Significantly stable open channel state

  →Accumulation of the conducting state



Deisseroth, Hegemann *et al. Nat Neurosci* **2009**, *12, 229*-234.

Bamann *et al. Biochemistry* **2010**, *49 (2), 267*-278.

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

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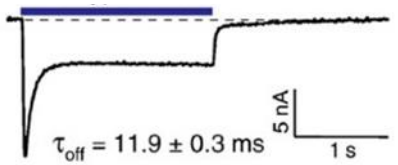
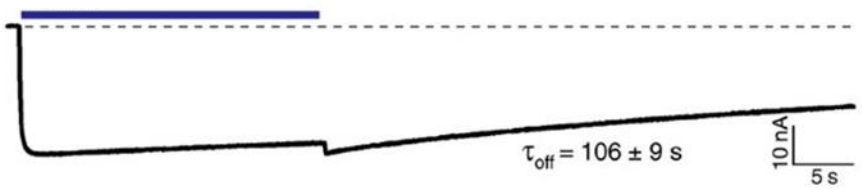
Wild-type ChR2



SFO (C128S)

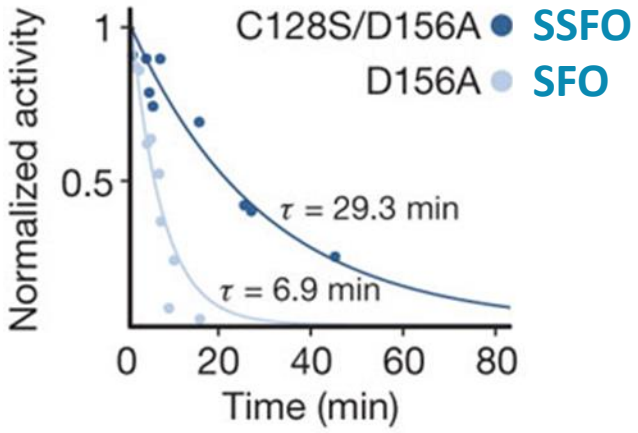


Deisseroth, Hegemann *et al. Nat Neurosci* **2009**, *12, 229*-234.

Stable Step-Function Opsin (SSFO)

- **Mutation : C128X and D156A**
- **Phenotype :**

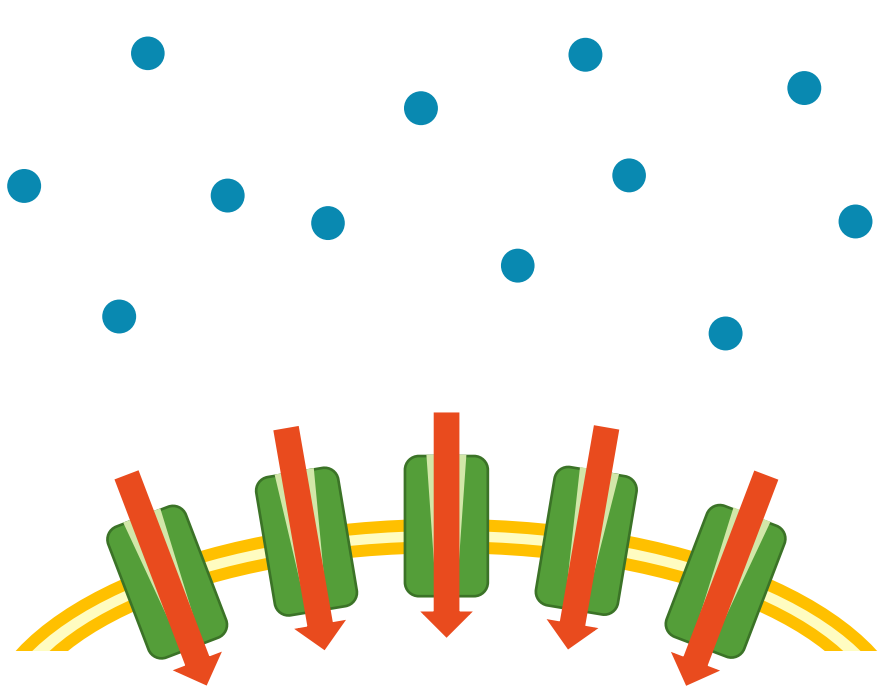
  More stable open channel state!



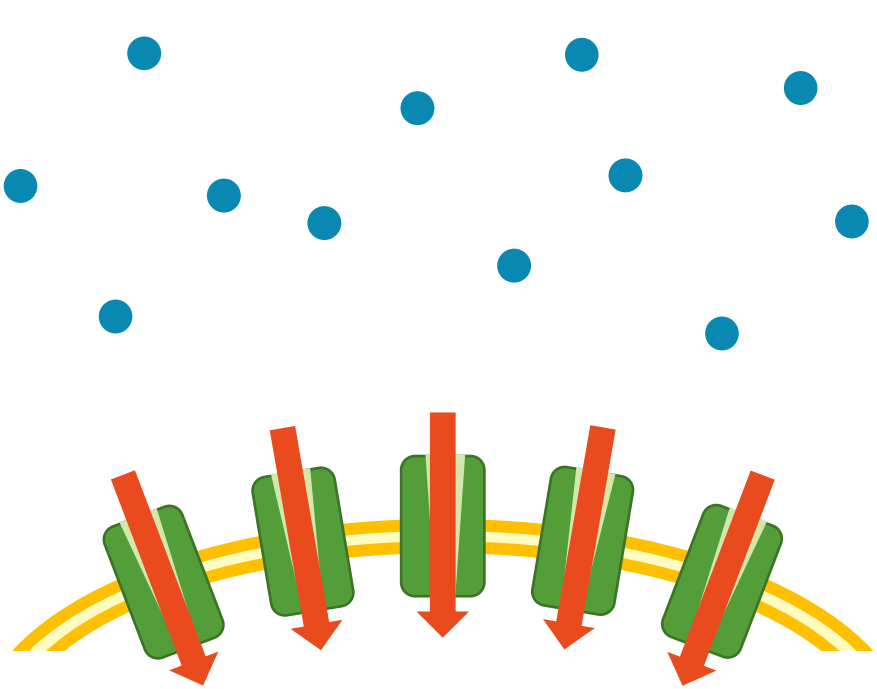
Deisseroth, Hegemann, Yizhar *et al. Nature* **2011**, *477, 171-178*.

Highly light-sensitive ChR2 mutant ①; **SFO family**

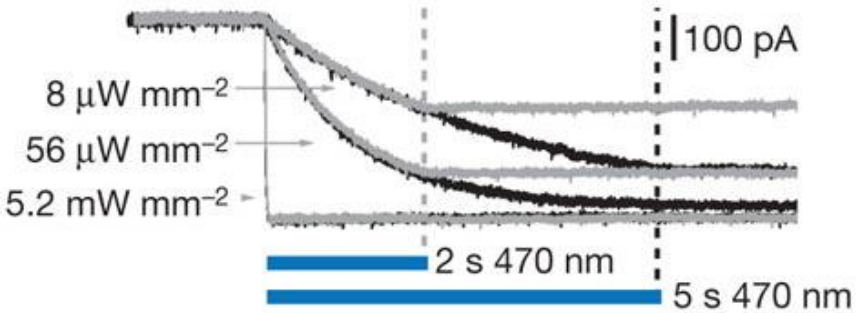
SFO/SSFO act as photon integrators across time!



WT



SFO/SSFO family

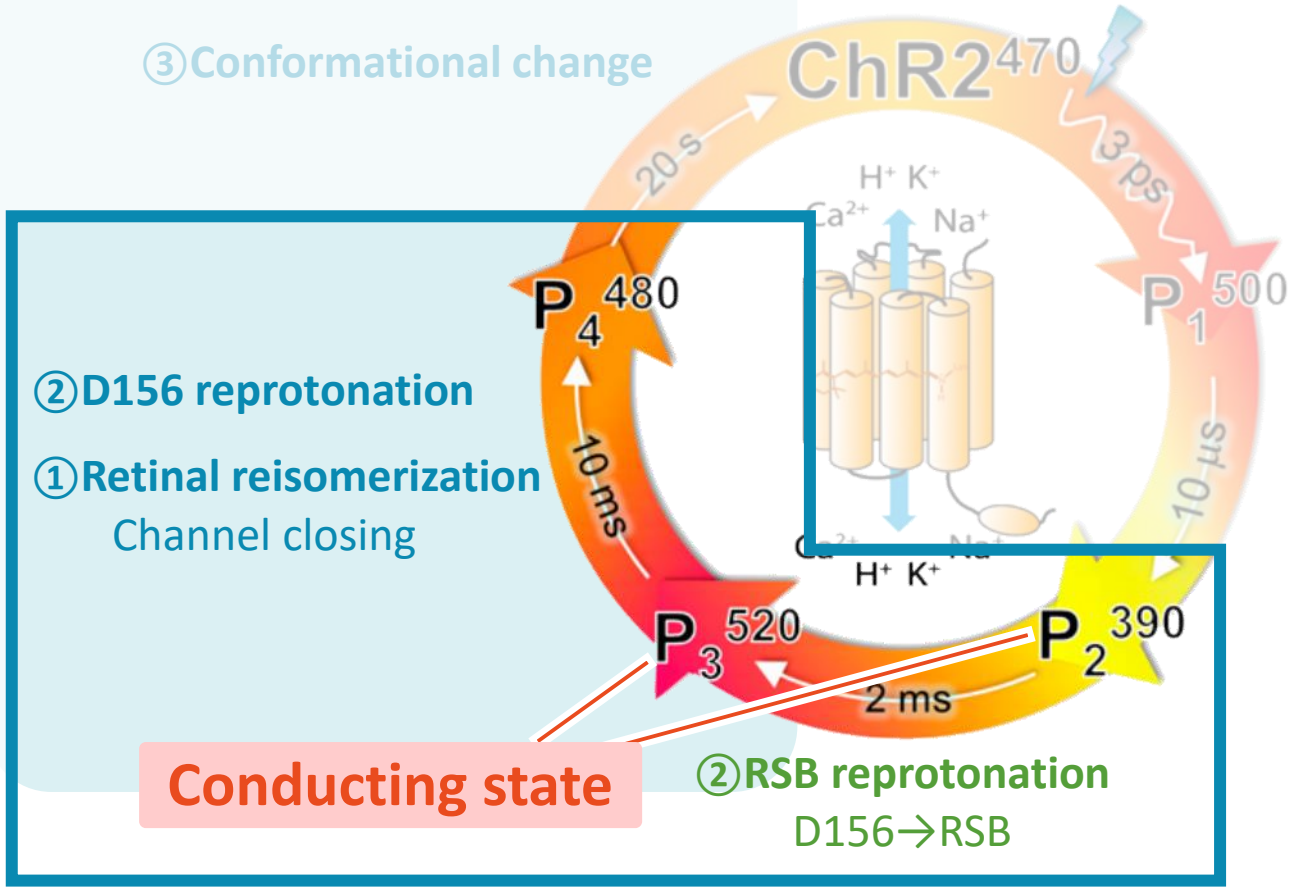


Deisseroth, Hegemann, Yizhar *et al. Nature* **2011**, *477, 171-178*.

Highly light-sensitive ChR2 mutant ①; **SFO family**

Channel closing phase

③Conformational change



②D156 reprotonation

①Retinal reisomerization

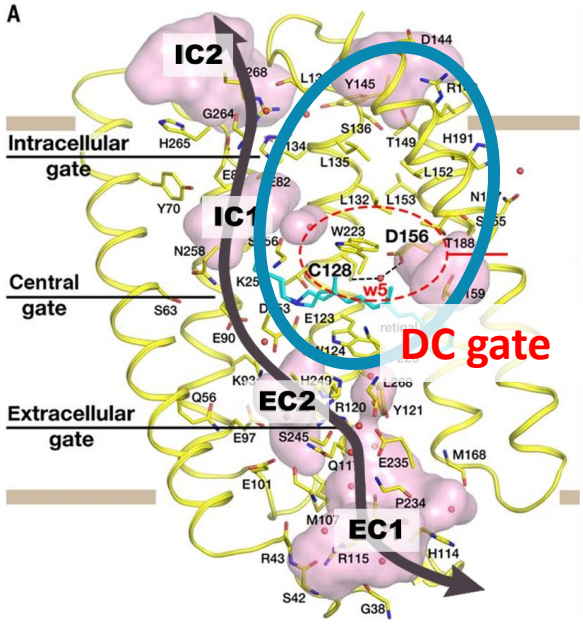
Channel closing

Conducting state

②RSB reprotonation

D156→RSB

Reprotonation and channel closing

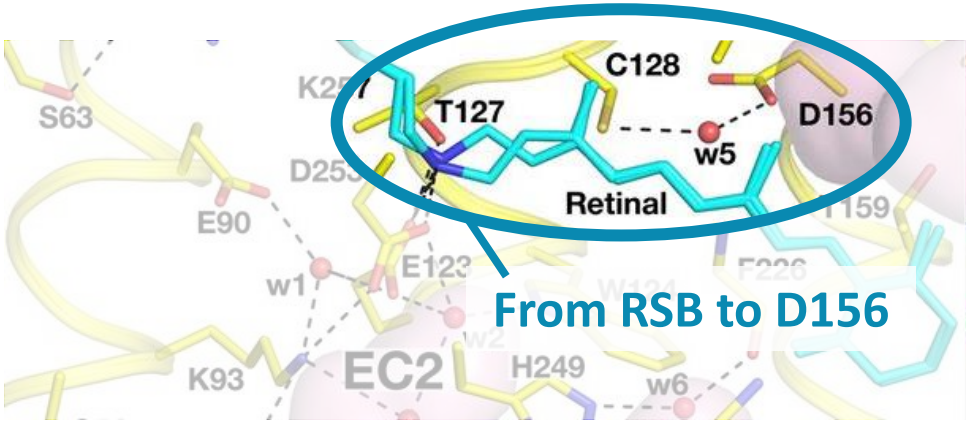


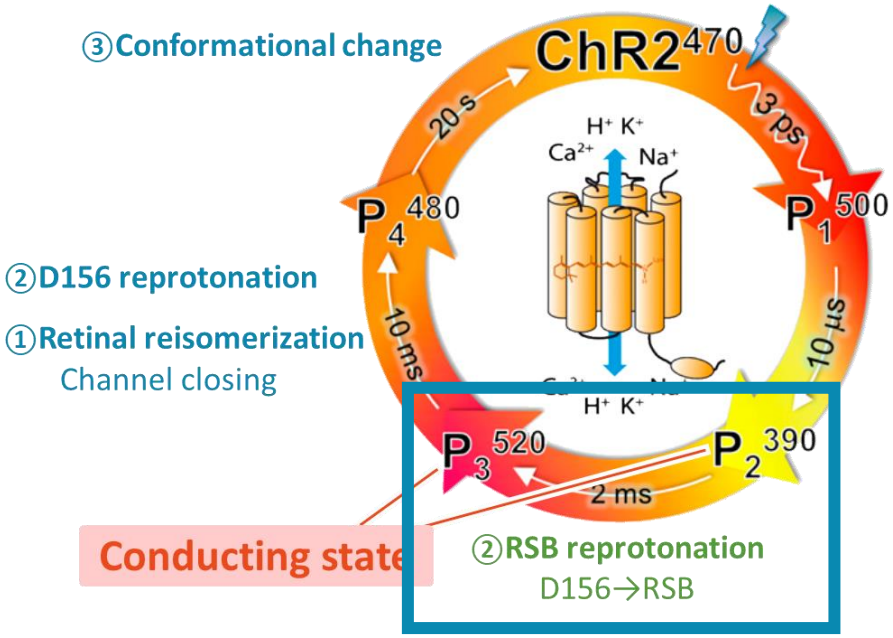
The mutations of SFO family **slow down the steps related to channel closing**.

→**Accumulation of conducting states!**

Heberle *et al. PNAS* **2013**, *110 (14),* E1273–E1281. Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Highly light-sensitive ChR2 mutant ①; **SFO family**





Spectroscopic data :

- **D156 and C128 forms strong H-bond** in the DC gate.
- **D156** serves as **the proton donor** of RSB.

In the conducting states, the H-bonding network from RSB to D156 is formed through T127 & C128.

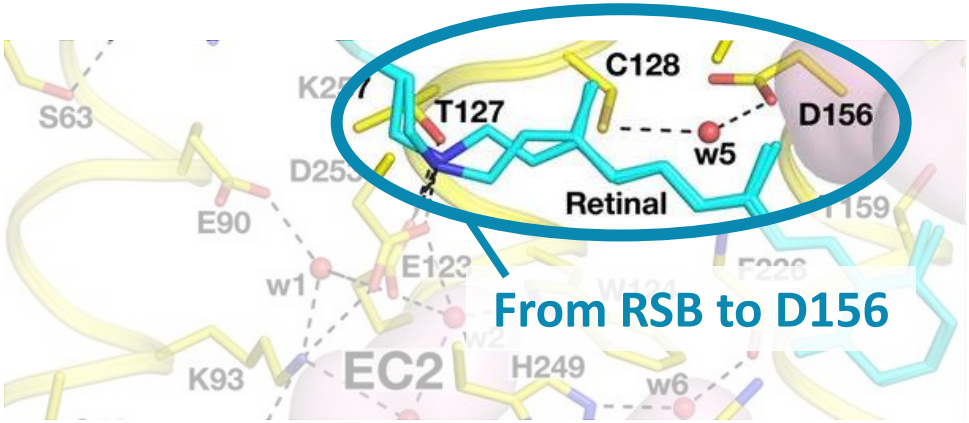
① **In the transition from P_2^{390} (RSB) to P_3^{520} (RSBH+), the proton is transferred from D156 to RSB.**

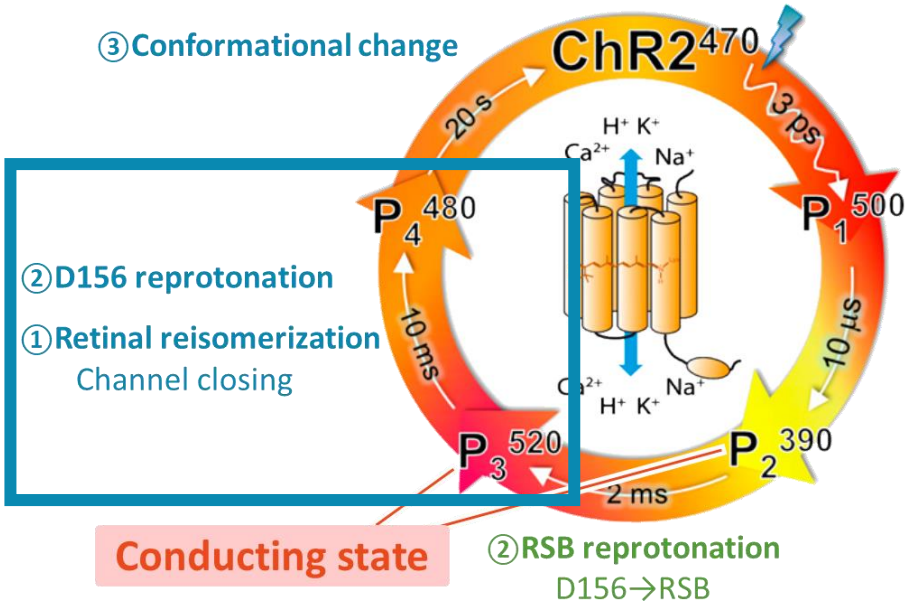
Heberle *et al. PNAS* **2013**, *110 (14),* E1273–E1281.

Heberle *et al. Photochem. Photobiol. Sci.* **2010**, *9,* 194-198.

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Highly light-sensitive ChR2 mutant ①; **SFO family**





Spectroscopic data :

- **D156 is reprotonated** when the channel is closed.

Structural & Electrophysiological data :

- **A proton is taken up** from inside the cell.

② In P_3^{520}, RSBH+ points toward the cytoplasmic side **due to** stabilization by the deprotonated D156.

→**Reprotonation of D156 is the trigger of channel closing!**

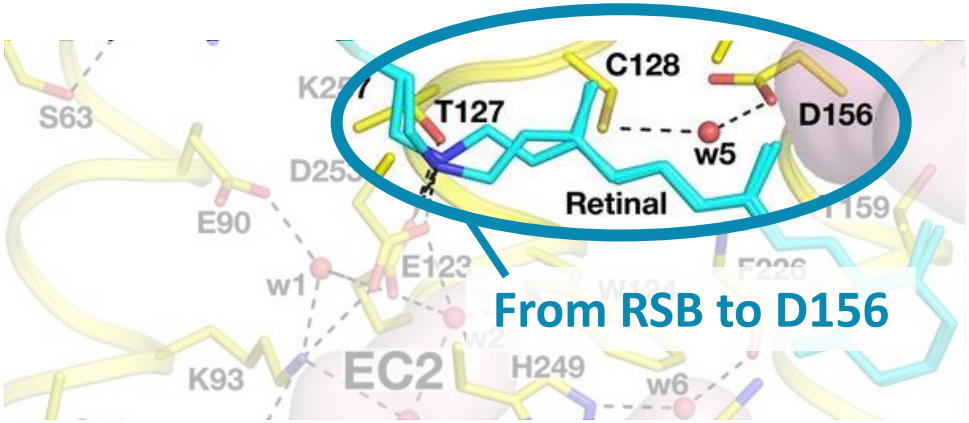
Bamberg *et al. PNAS* **2009** , *106 (30),* 12317-12322.

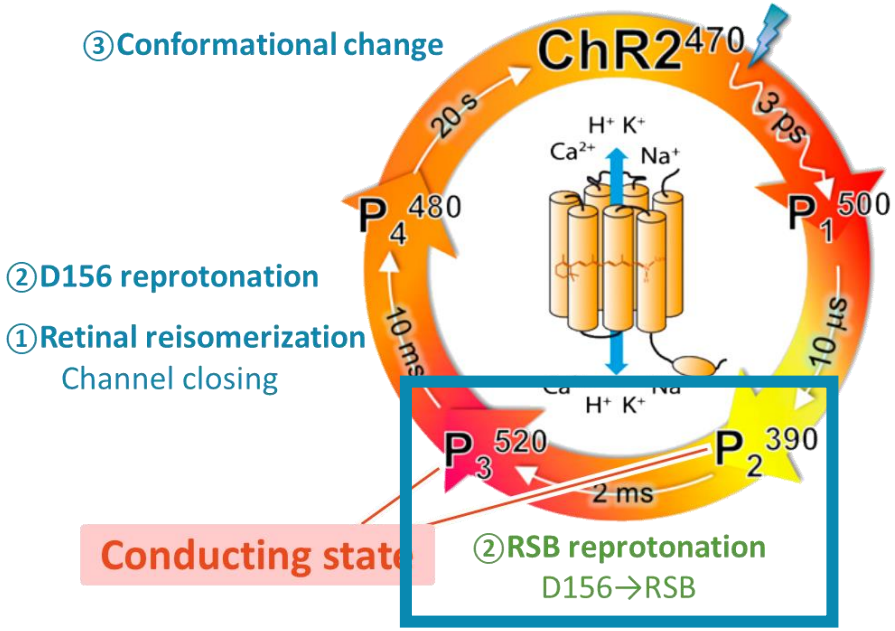
Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281.

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Highly light-sensitive ChR2 mutant ①; **SFO family**

Mechanism of D156A mutation





D156A mutation

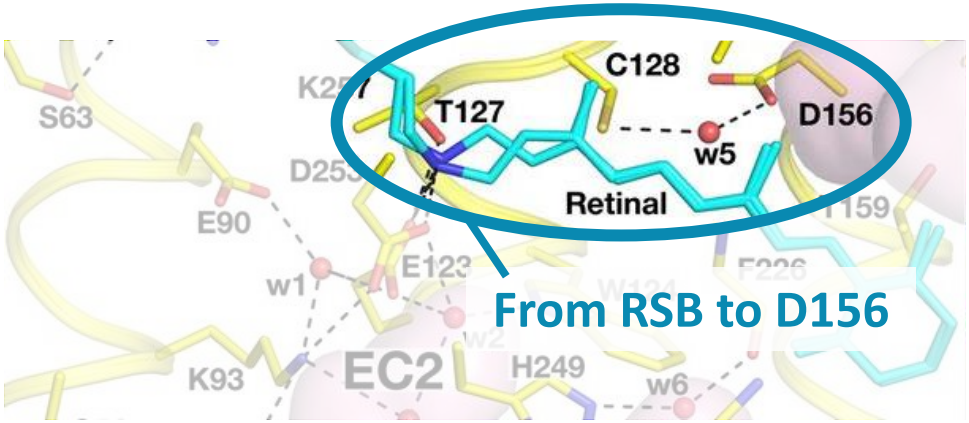
- **Missing of proton donor**

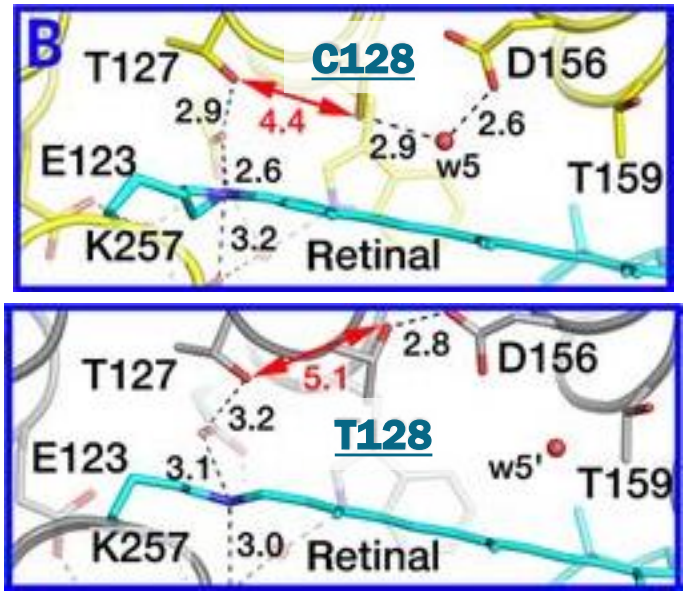
→Accumulation of conducting P_2^{390}

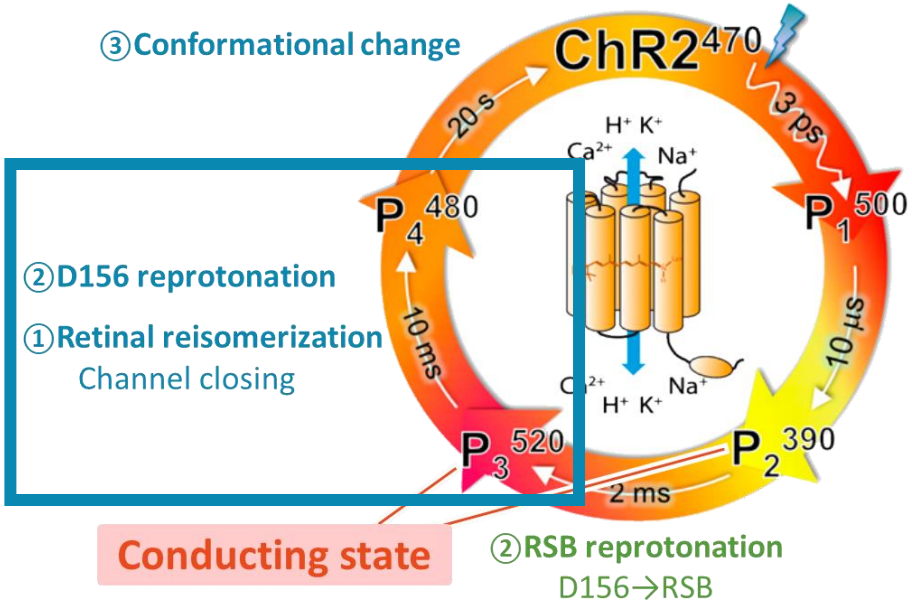
Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281. Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Highly light-sensitive ChR2 mutant ①; **SFO family**

Mechanism of C128X mutation







C128X mutation (X = S, T, A)

- **The mutation disturbs the structure of the retinal pocket.**
  →The deprotonated D156 is stabilized in a different interaction network.
  →Accumulation of conducting P_3^{520}

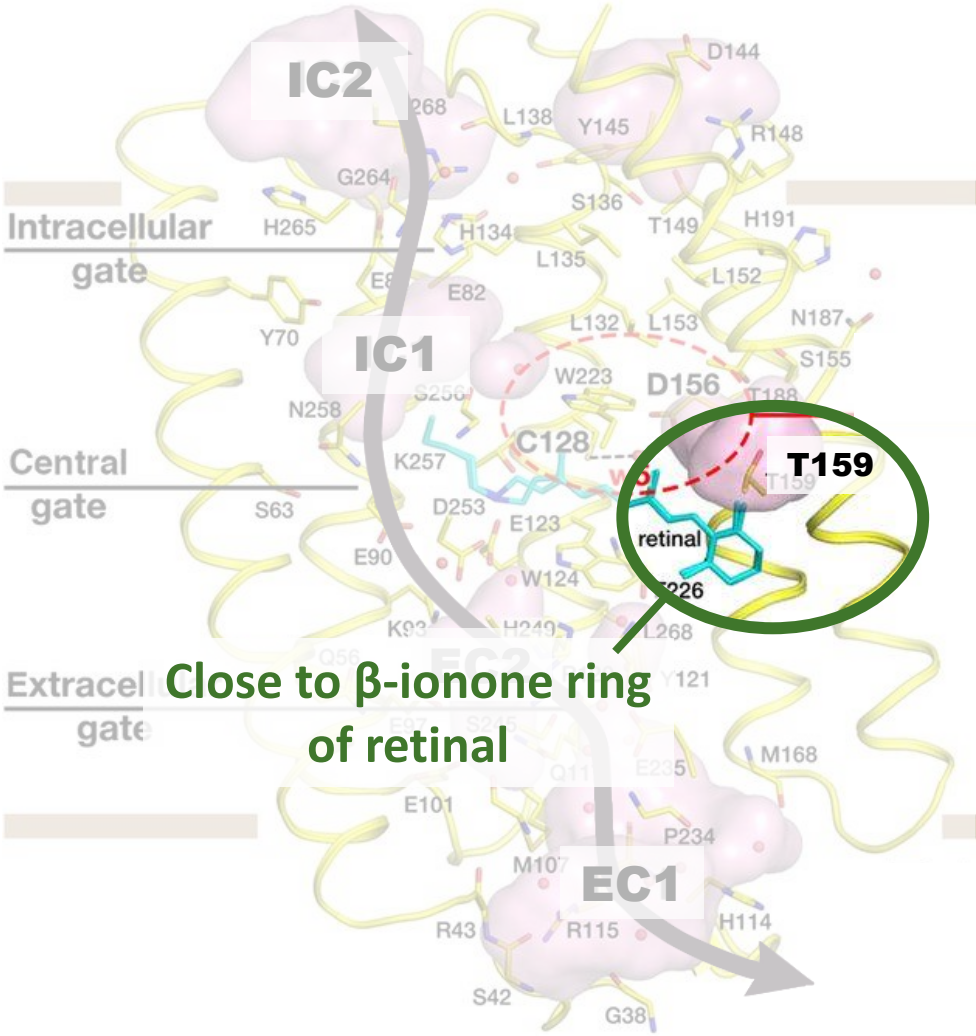
Heberle *et al. PNAS* **2013**, *110 (14),* E1273-E1281. Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

Highly light-sensitive ChR2 mutant ②; **TC mutant**

TC mutant

- **Mutation : T159C**
- **Phenotype :**

  Dramatic increase of photocurrent amplitude



Oertner *et al. PNAS* **2011**, *108 (18),* 7595-7600.

Gordeliy, Bamberg, Büldt *et al. Science* **2017**, *358,* 6366.

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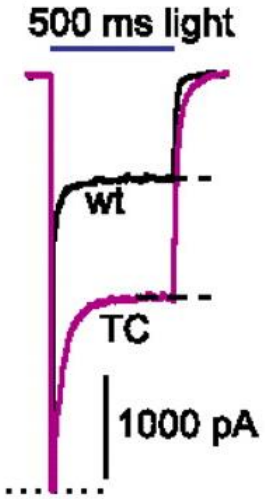
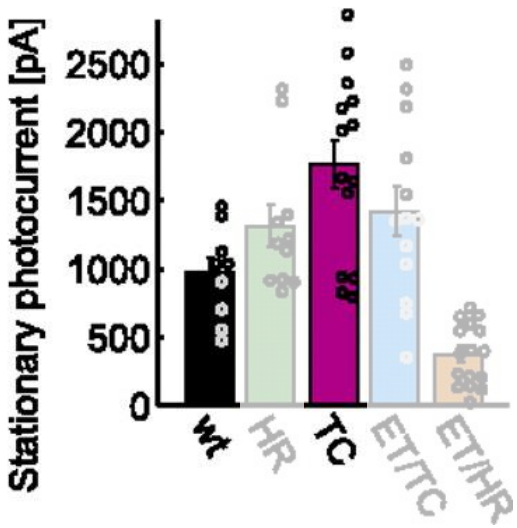
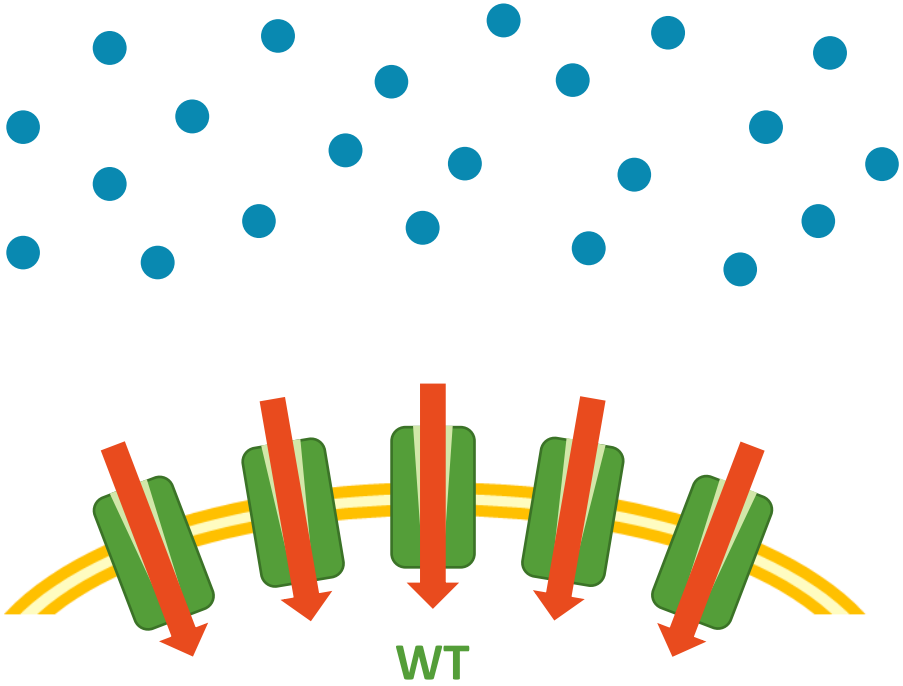
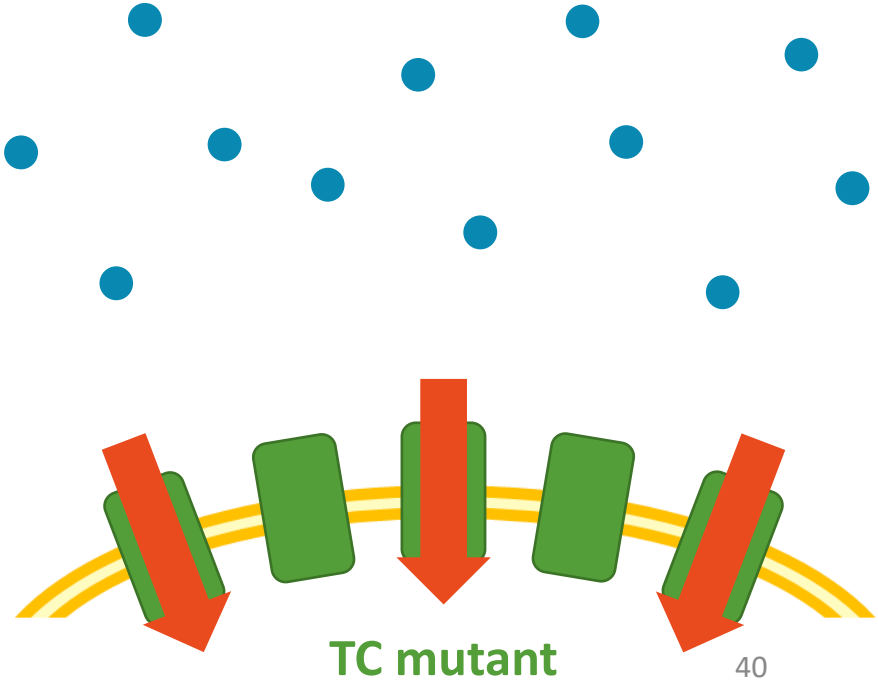




Oertner *et al. PNAS* **2011**, *108 (18),* 7595-7600.





New ultra light-sensitive ChR2; SOUL

Idea

SFO family (C128X, D156A) + TC mutant (T159C) → SOUL (C128S, D156A, T159C)

Highly light sensitive | Highly light sensitive | Ultra light sensitive!

Brain damage…

https://blogs.lt.vt.edu/stems/2014/05/01/optogenetics/

Non-invasive!

https://www.clea-japan.com/products/animal/inbred

Highlight :

Achieving **non-invasive activation** of **any region** of the mouse brain!

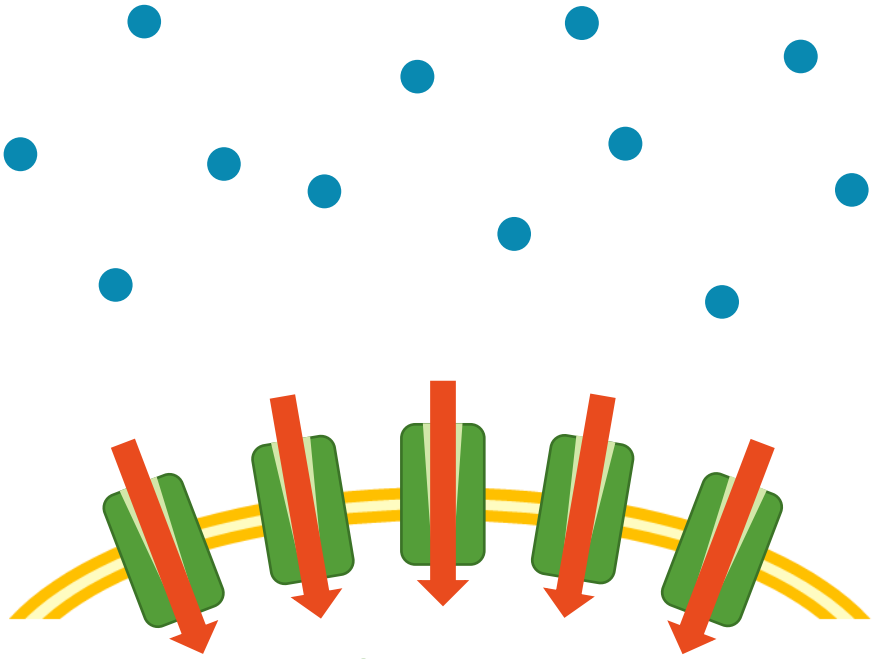
Feng *et al. Neuron* **2020**, *107 (1)*, 38-51.

New ultra light-sensitive ChR2; SOUL

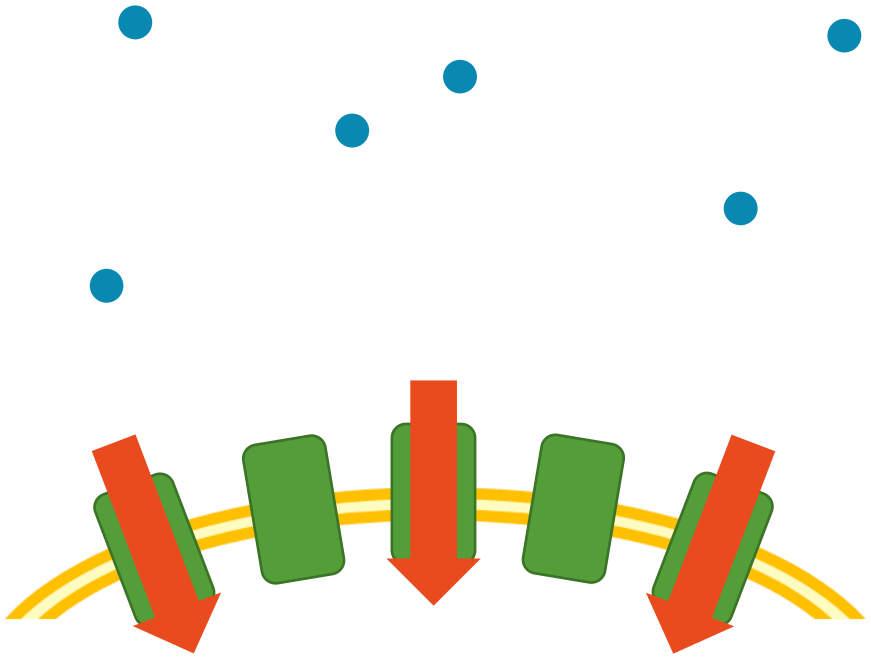
Idea

SSFO family (C128X+D156A) + TC mutant (T159C) → SOUL (C128S+D156A +T159C)

Highly light sensitive | Highly light sensitive | Ultra light sensitive!



SFO/SSFO family

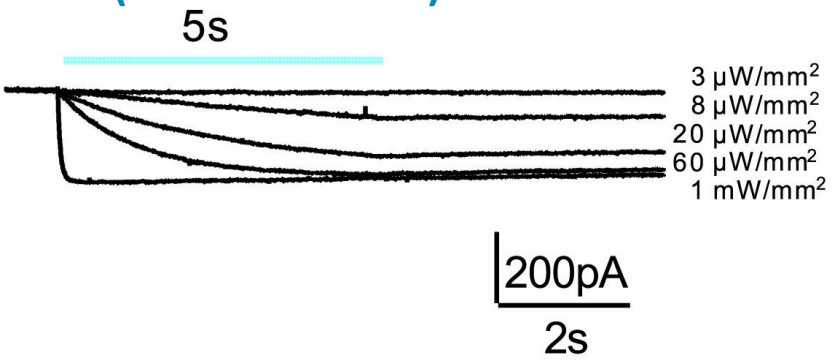


SOUL

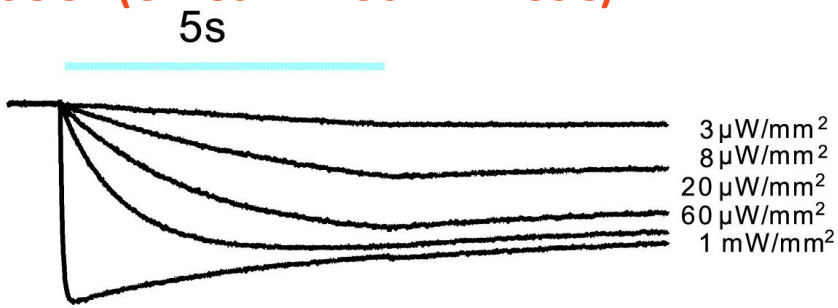
New ultra light-sensitive ChR2; SOUL

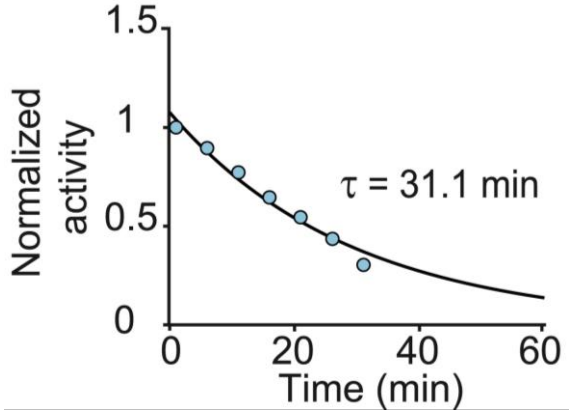
In vitro and *ex vivo* characterization of SOUL

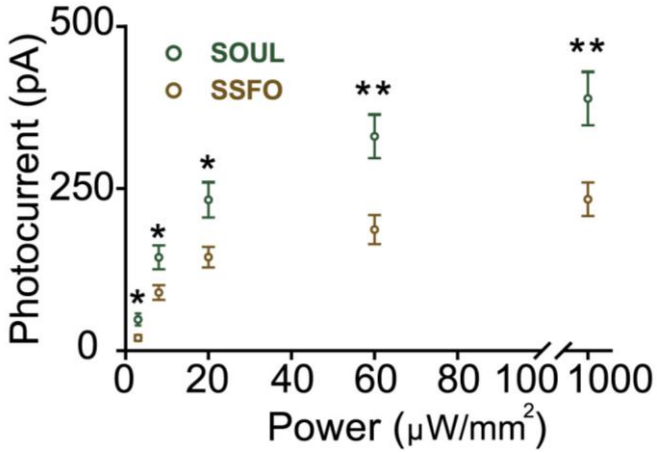
SSFO (C128S + D156A)



SOUL (C128S + D156A + T159C)







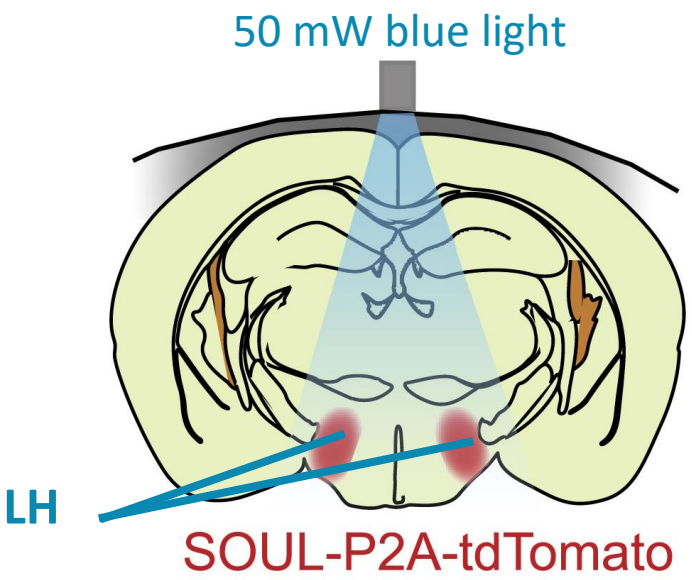
- SOUL retains a **prolonged open state** like SSFO.
- SOUL-expressing neurons had a **significantly higher operational light sensitivity** compared to SSFO.

Feng *et al. Neuron* **2020**, *107 (1)*, 38-51.

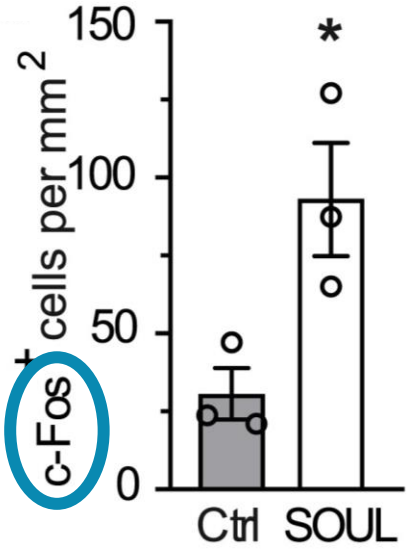
New ultra light-sensitive ChR2; SOUL

In vivo characterization of SOUL

Encoding SOUL into the deepest region in the mouse brain



Marker protein of firing

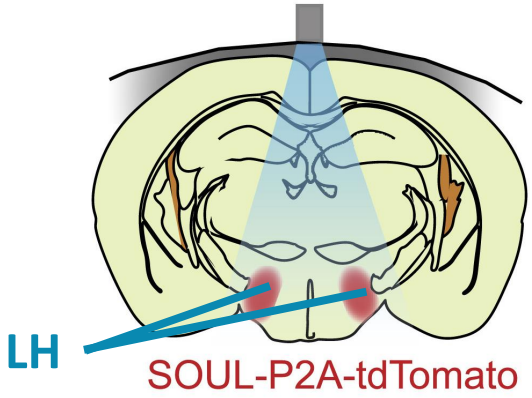


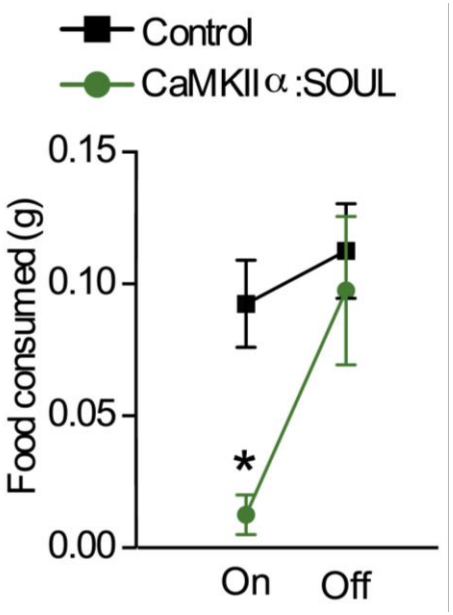
SOUL can be activated via **transcranial optical stimulation** in mice **regardless of the depth**!

Feng *et al. Neuron* **2020**, *107 (1)*, 38-51.

Less invasive optogenetic stimulation using **SOUL**

Transcranial optogenetic control of mouse behavior by SOUL





Previous finding :

Photoactivation of excitatory neurons in LH **suppresses feeding** in food-deprived mice.

The result of this report :

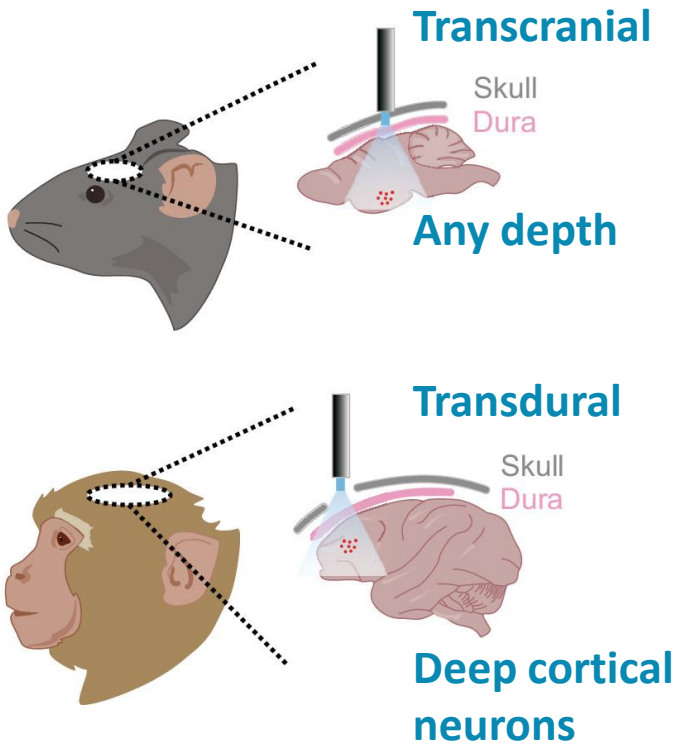
Food-deprived SOUL-expressing mice showed **significantly reduced food consumption** via **transcranial blue light**.

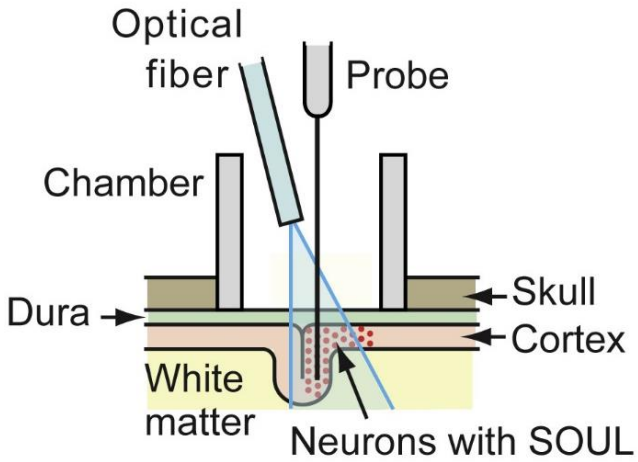
SOUL can manipulate neuronal activity and behavior through **non-invasive transcranial delivery of light** even **in the deepest mouse brain region**!

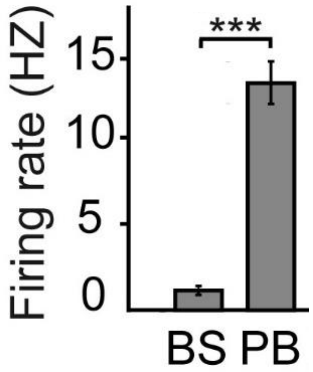
Feng *et al. Neuron* **2020**, *107 (1)*, 38-51.

Less invasive optogenetic stimulation using SOUL

Transdural optogenetic activation of macaque cortex by SOUL







*BS = baseline
PB = post blue light

External optical stimulation of SOUL through native dura achieved **minimally invasive** activation of neurons across **all depths in superficial cortical regions**!

→ Large scale optogenetic activation in primates!

Feng *et al. Neuron* **2020**, *107 (1)*, 38-51. Lawrence & Chang, *J. Neurophysiol.* **2020**, *124 (5)*, 1312-1314.

Contents

Summary & Perspective

- **Optogenetics** is the technique that allows **precise and fast control** of the activity of **targeted brain cells** using **light-gated cation channel**, **channelrhodopsin-2 (ChR2)**.

- The key factors of channel-gating mechanism is…
  ① **Photoisomerization** of retinal
  ② Change in the **protonation state of retinal Schiff base (RSB)**
  ③ **Conformational change** induced by changes in the **H-bonding networks**

- **Non-invasive / minimally invasive** optogenetic activation of **mouse / macaque monkey brain** was achieved by **ultra light-sensitive** ChR2 mutant, **SOUL**.

- SOUL-based optogenetics may be explored for **minimally invasive treatment of neurological and psychiatric disorders**.