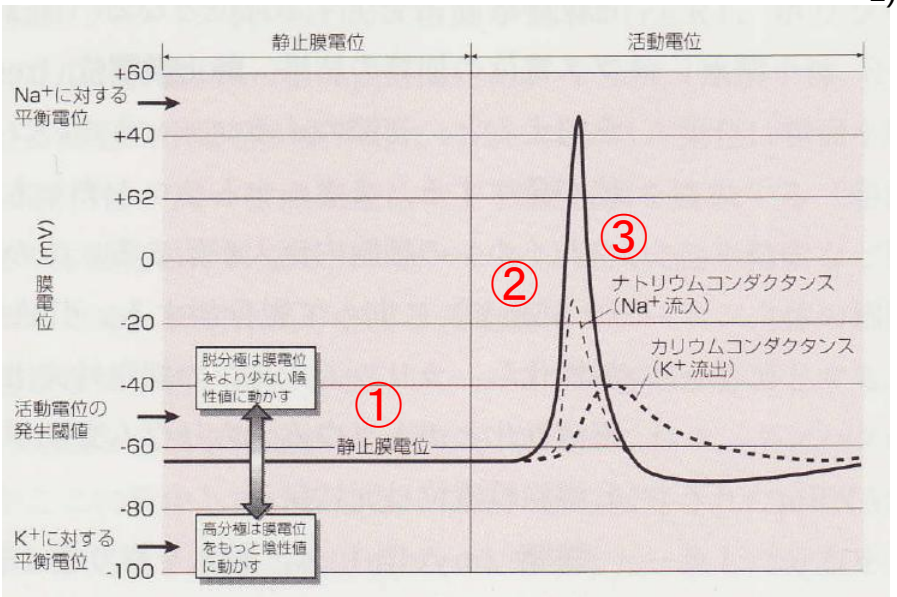
Voltage-sensitive Probes

20160114 Atsushi Hayata

Contents

Transmembrane potential -active potential-

1)



① Polarization (different ion concentrations among inside and outside the membrane)

↓ Stimulation

② Depolarization (Na^+ influx)

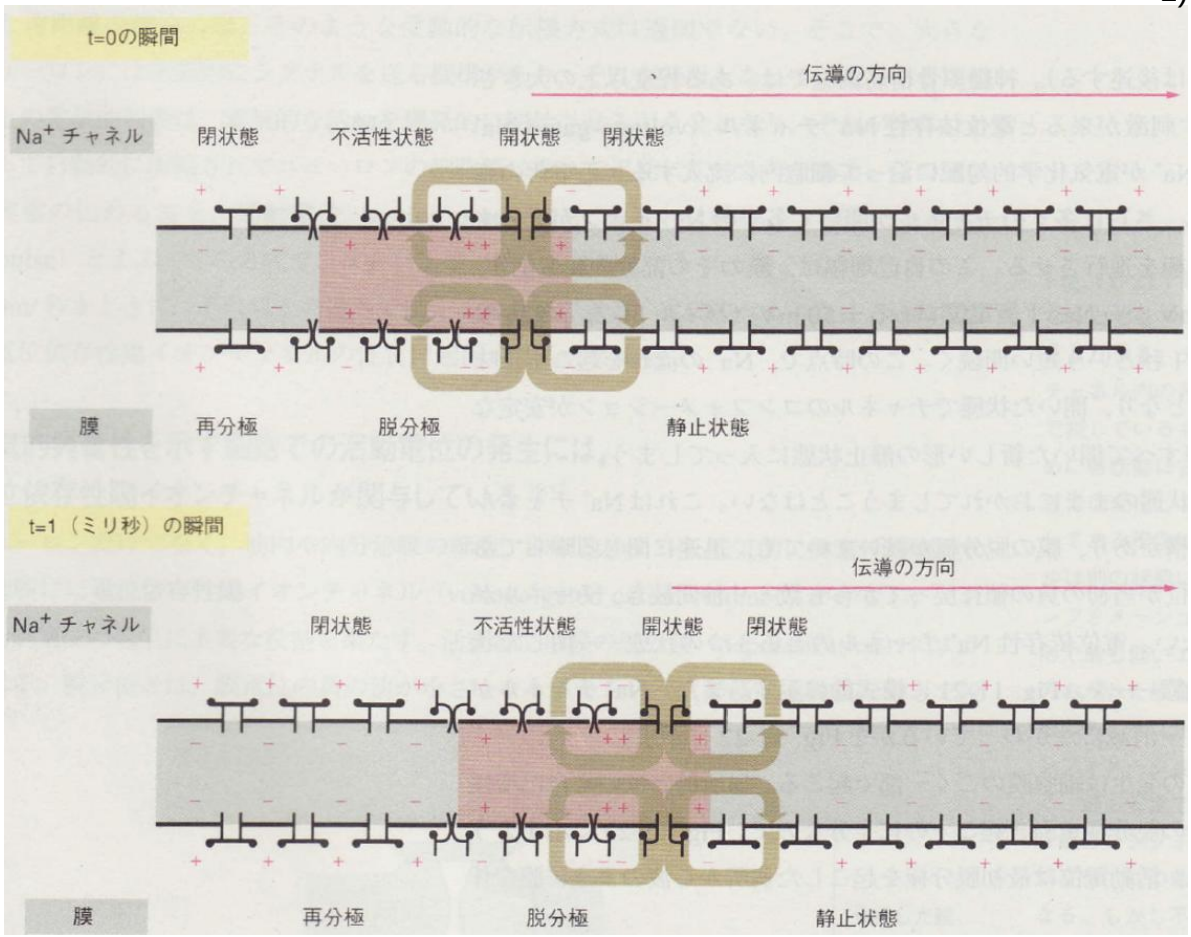
↓

③ Polarization (K^+ efflux)

(1) 脳・神経科学入門講座　上　羊土社

Transmembrane potential -signal transmission-

1)

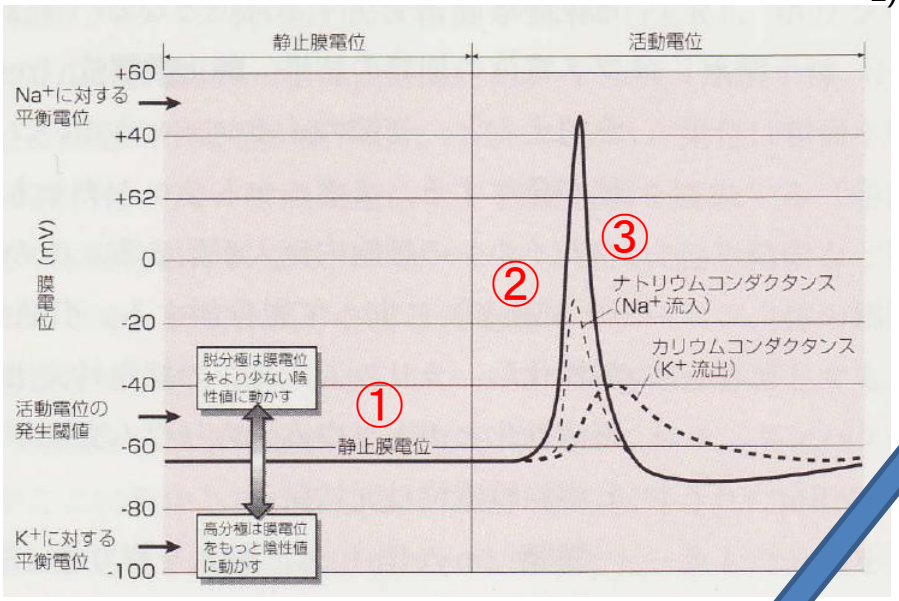


Signal transmission with action potential

(1) 細胞の分子生物学　第三版　教育社

Transmembrane potential -detection-

1)



1. Electrophysiology
2. Ca^{2+}-sensitive probes
3. Voltage-sensitive probes

① Polarization (different ion concentrations among inside and outside the membrane)

↓ Stimulation

② Depolarization (Na^+ influx) → Voltage sensitive Ca^{2+} channel (Ca^{2+} influx)

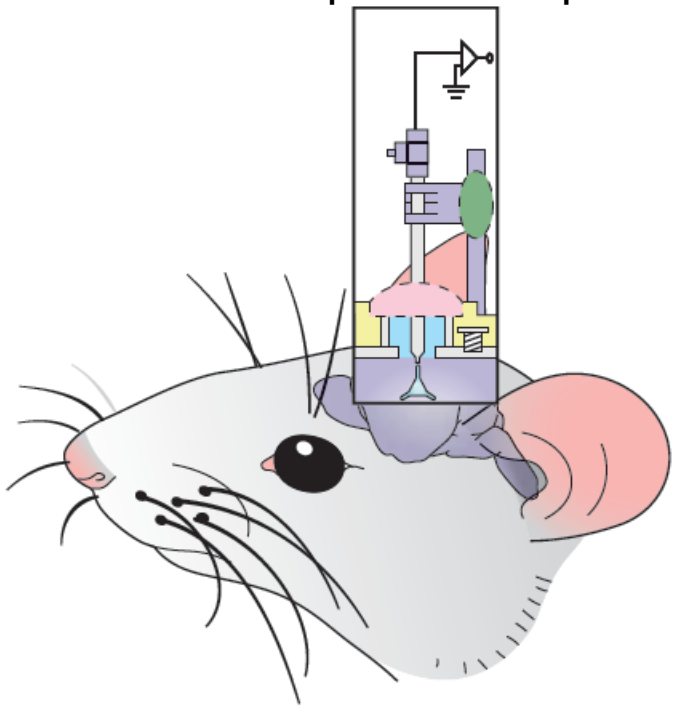
↓

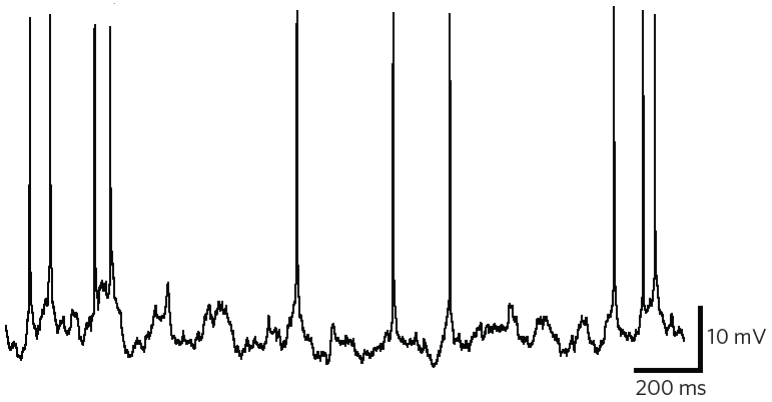
③ Polarization (K^+ efflux)

(1) 脳･神経科学入門講座　上　羊土社

1. Electrophysiology

Illustration of patch-clamp[2)]





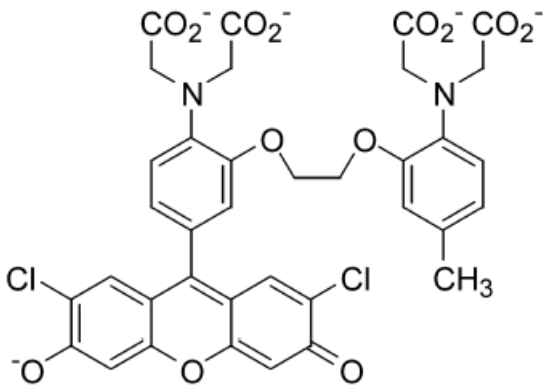
Galvani first reported the direct stimulation of nerves over 200 years ago.[1)]

<u>advantage</u>
Electrical activity is recorded directly.
Time resolution.
High sensitivity.

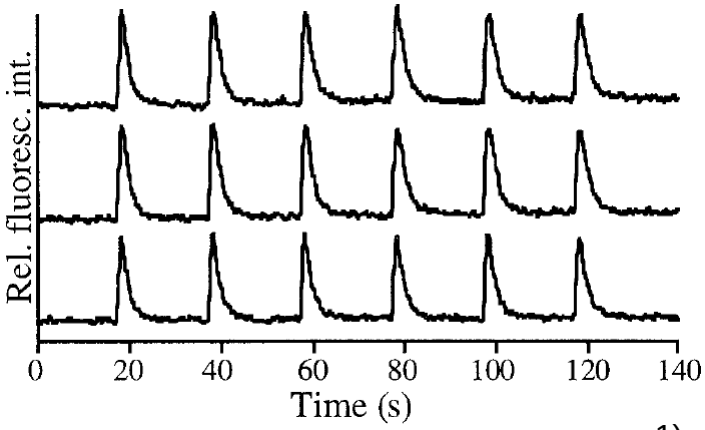
<u>disadvantage</u>
Physical contact with the tissue is necessary.
Spatial resolution.

(1) Galvani, L. *Bonon. Sci. Art. Inst. Acad.* **1791**, *7*, 364-415.
(2) Scanziani, M.; Häusser Michael *Nature* **2009**, *461*, 930-939.

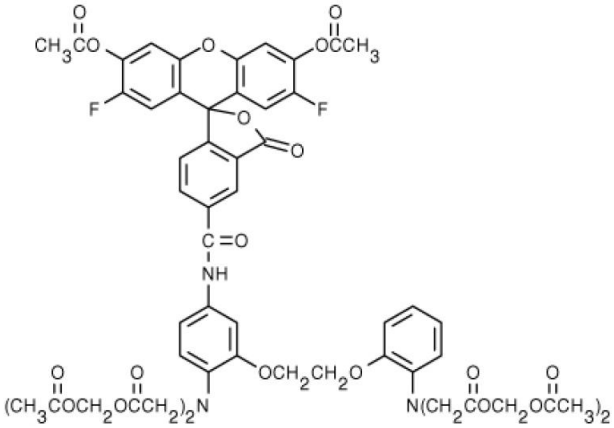
2. Ca^{2+}-sensitive probes



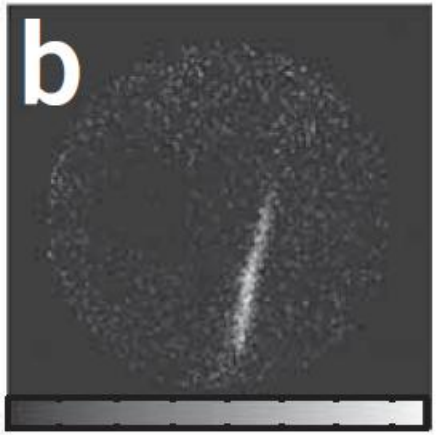
fluo-3



Fluorescence change of fluo-3.[1)]



Oregon Green®488 BAPTA-1, AM

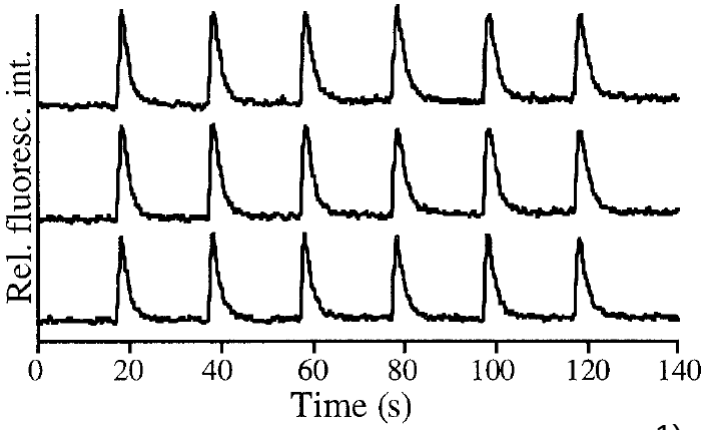


Imaging of Ca^{2+} spiking.[2)]

(1) Jacobs, J. M.; Meyer, T. *J. Neurosci.* **1997**, *17*, 4129-4135.
(2) Flusberg, B. A.; Nimmerjahn, A.; Cocker, E. D.; Mukamel, E. A.; Barretto, R. P. J.; Ko, T. H.; Burns, L. D.; Jung, J. C.; Schnitzer, M. J. *Nature Methods* **2008**, *5*, 935-938.

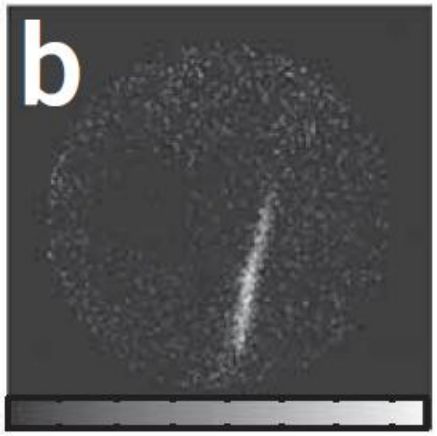
2. Ca^{2+}-sensitive probes

advantage
Spatial resolution.
High sensitivity.



Fluorescence change of fluo-3.[1)]

disadvantage
Electrical activity is not recorded directly.
Slow time course of the intracellular calcium signal (time resolution).



Imaging of Ca^{2+} spiking.[2)]

(1) Jacobs, J. M.; Meyer, T. *J. Neurosci.* **1997**, *17*, 4129-4135.
(2) Flusberg, B. A.; Nimmerjahn, A.; Cocker, E. D.; Mukamel, E. A.; Barretto, R. P. J.; Ko, T. H.; Burns, L. D.; Jung, J. C.; Schnitzer, M. J. *Nature Methods* **2008**, *5*, 935-938.

Short summary

1. Electrophysiology

advantage
Electrical activity is recorded directly.
Time resolution.
High sensitivity.

disadvantage
Physical contact with the tissue is necessary.
Spatial resolution.

2. Ca^{2+}-sensitive probes

advantage
Spatial resolution.
High sensitivity.

disadvantage
Electrical activity is not recorded directly.
Slow time course of the intracellular calcium signal (time resolution).

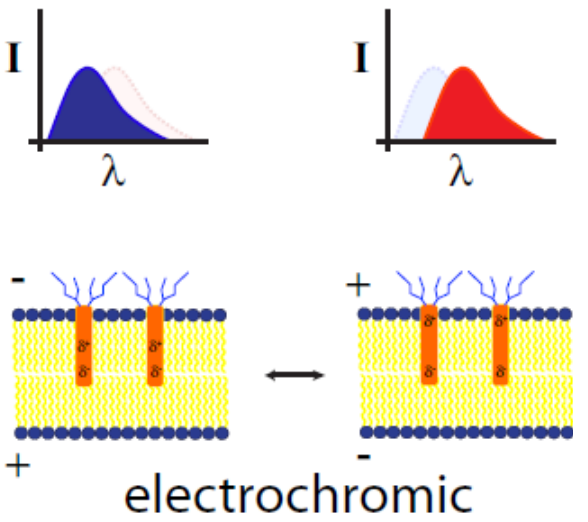
3. Voltage-sensitive probes

advantage
Electrical activity is recorded directly.

Time resolution?
Spatial resolution?
Sensitivity? (electron -> photon)

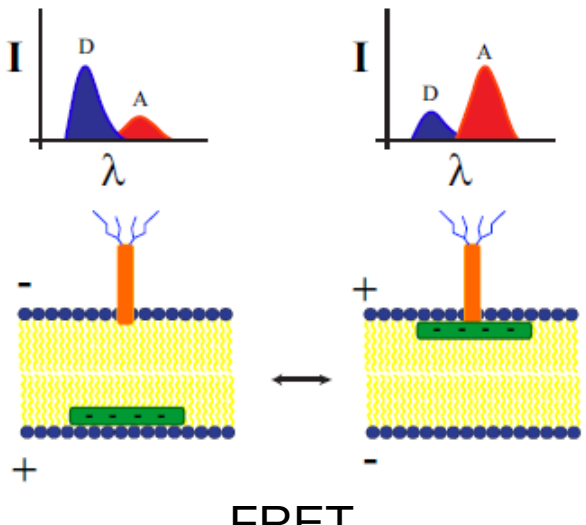
3. Voltage-sensitive probes

3-1.[1)]



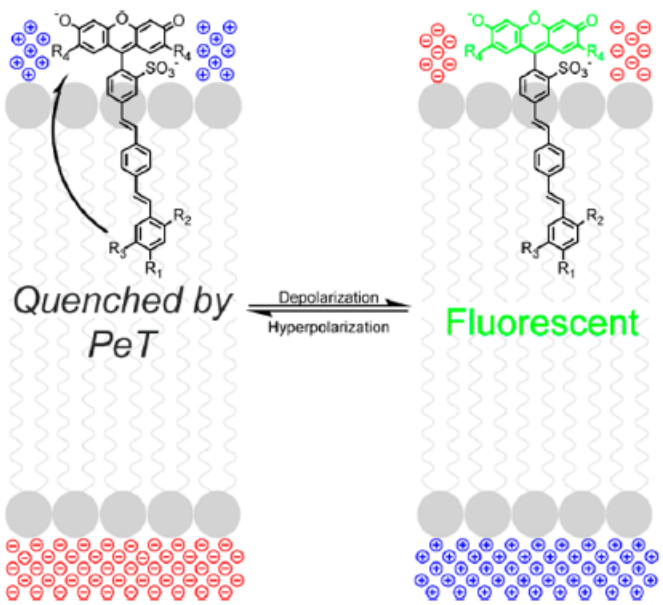
electrochromic

3-2.[1)]



FRET

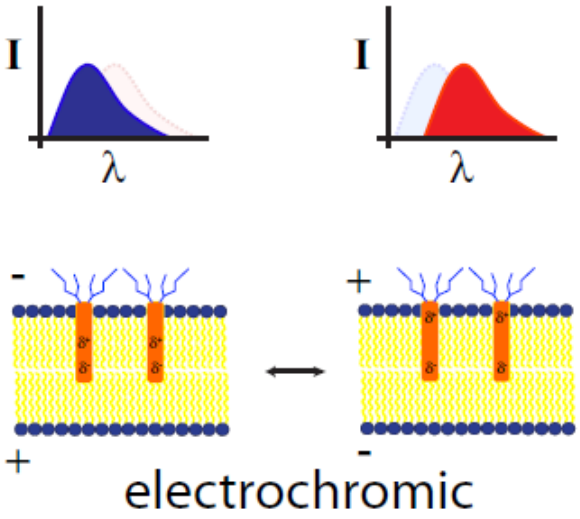
3-3.[2)]

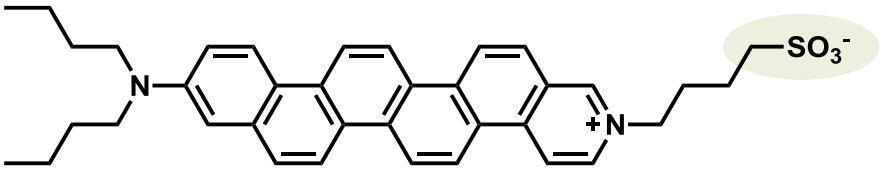


PeT

(1) Peterka, D. S.; Takahashi, H.; Yuste, R. *Neuron* **2011**, *69*, 9-21.
(2) Woodford, C. R.; Frady, E. P.; Smith, R. S.; Morey, B.; Canzi, G.; Palida, S. F.; Araneda, R. C.; Kristan, Jr., W. B.; Kubiak, C. P.; Miller, E. W.; Tsien, R. Y. *J. Am. Chem. Soc.* **2015**, *137*, 1817-1824.

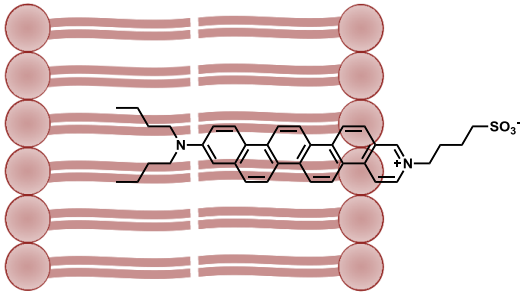
3-1. Electrochromic -ANNINE-6-





ANNINE-6

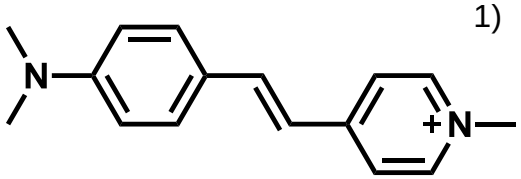
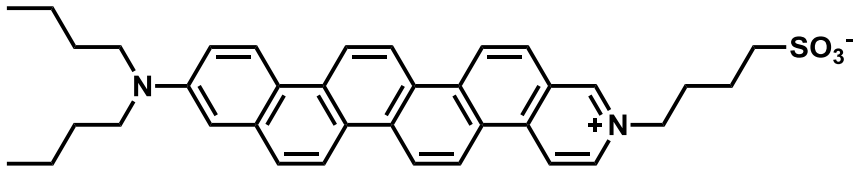
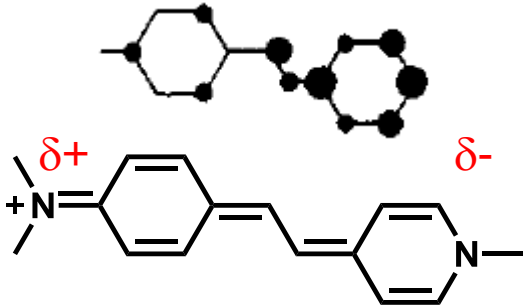
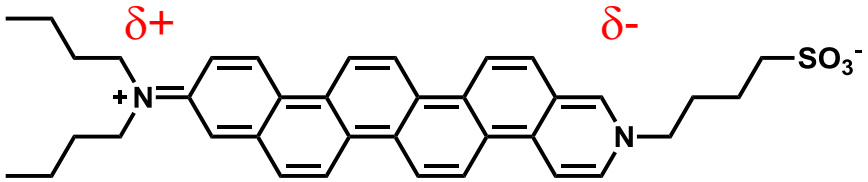
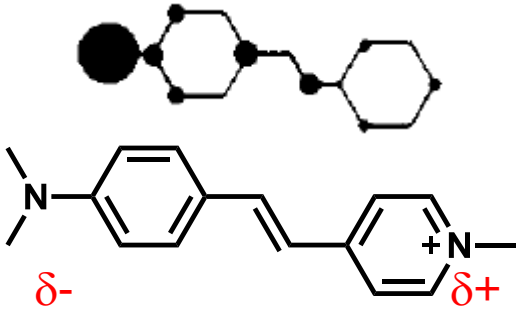
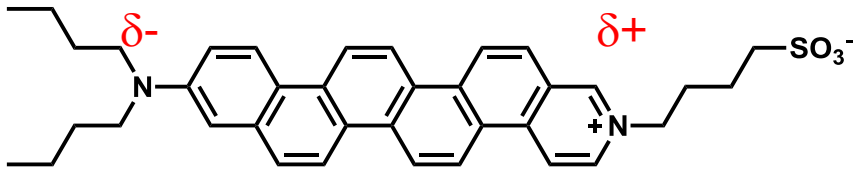
Prevent internalization.
Determine direction of the molecule.



Cytoplasm

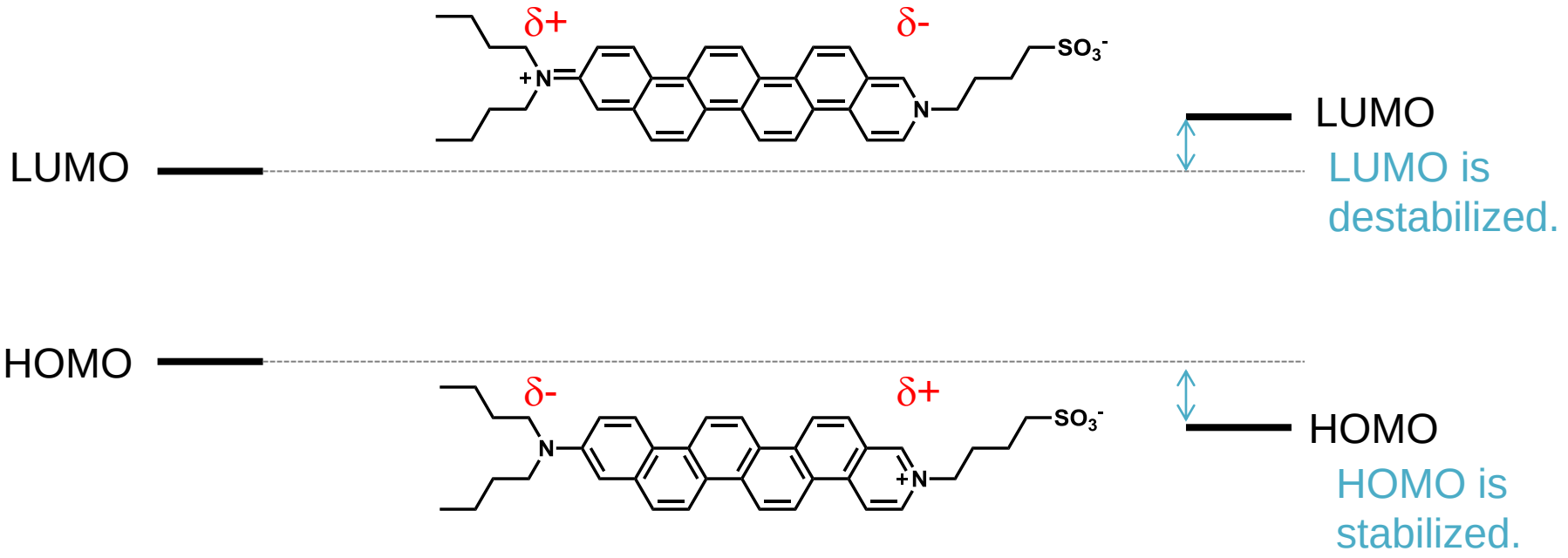
Extracellular medium

3-1. Electrochromic -HOMO, LUMO-

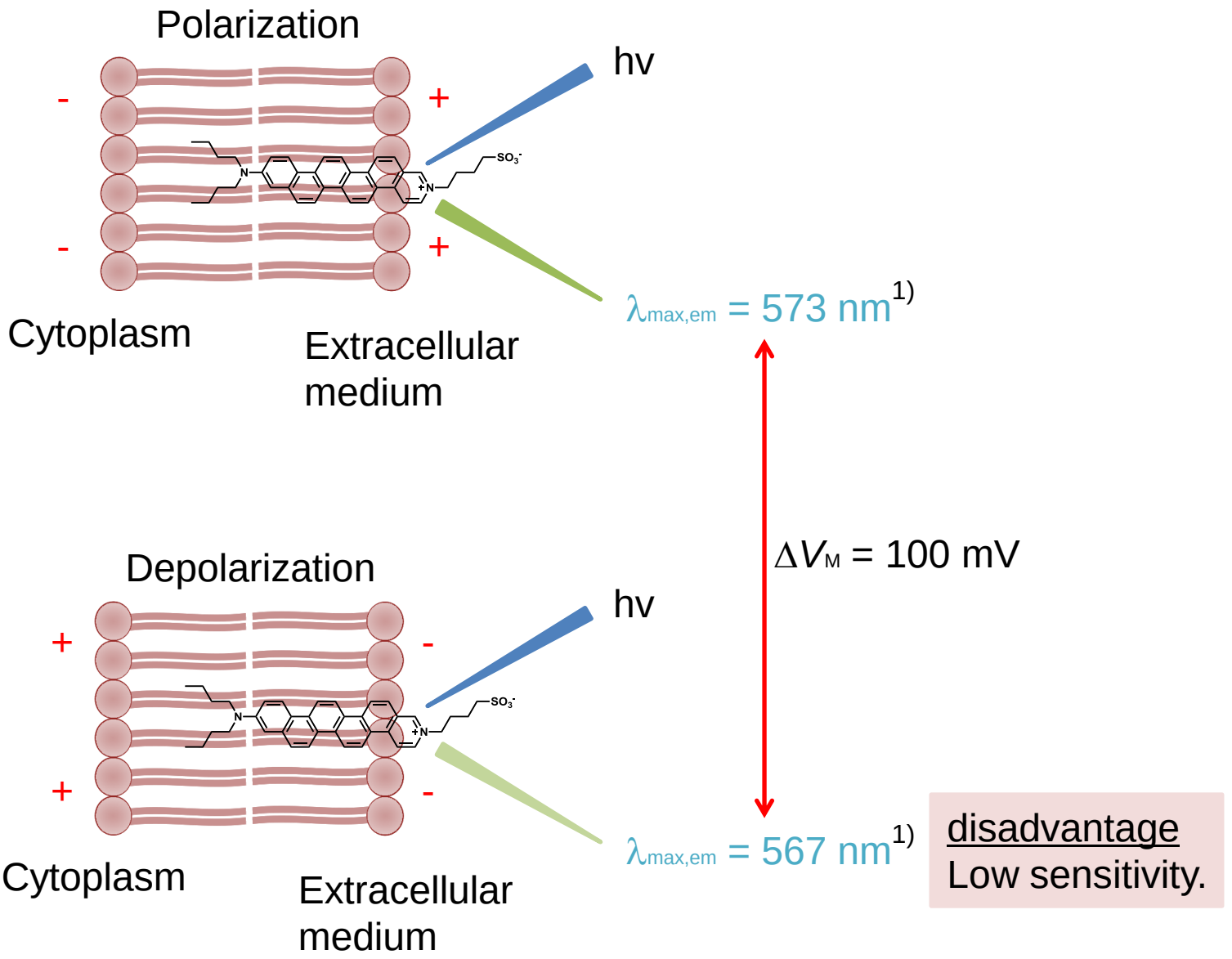
	 1)	 ANNINE-6
LUMO		
HOMO		

(1) Loew, L. M.; Bonneville, G. W.; Surow, J. *Biochemistry* **1978**, *17*, 4065-4071.

3-1. Electrochromic -energy shift-

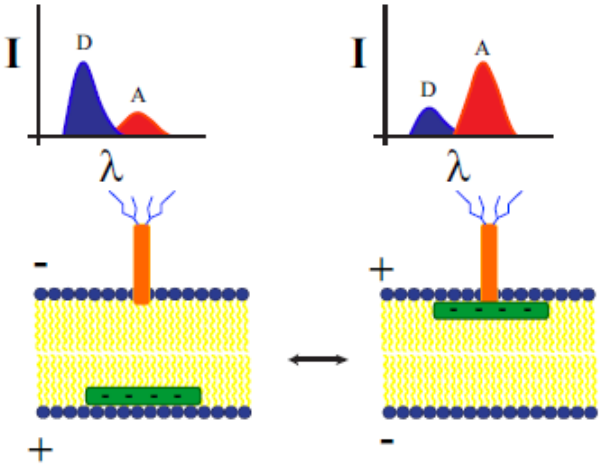


3-1. Electrochromic -disadvantage-



(1) Kuhn, B.; Fromherz, P. *J. Phys. Chem. B* **2003**, *107*, 7903-7913.

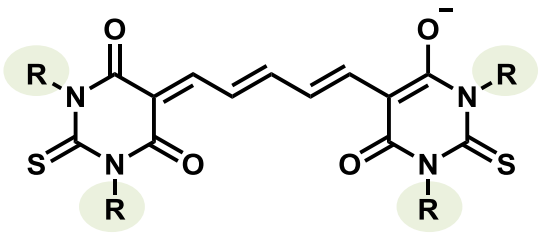
3-2. FRET -DisBAC$_6$ and CC1-DMPE-



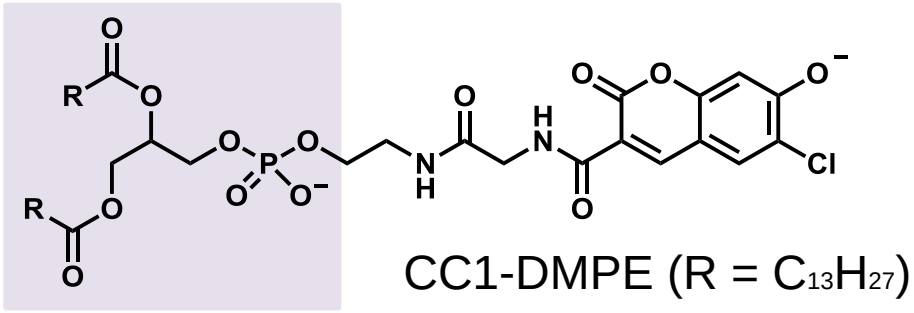
Extracellular medium

Cytoplasm

Depolarization Polarization



DiSBAC$_6$ (R = C_6H_{13})



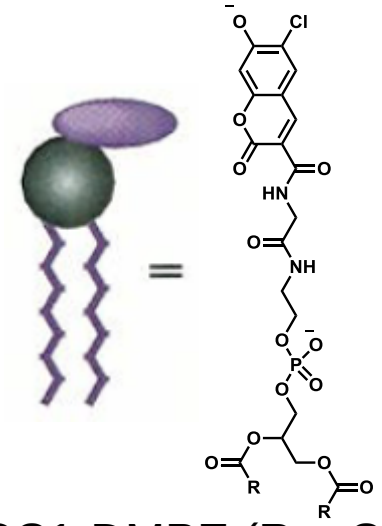
CC1-DMPE (R = $C_{13}H_{27}$)

Increasing lipophilicity.
Localization to the membrane.

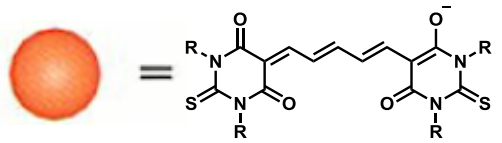
Anchor to the cell membrane.
Determine direction of the molecule.

(1) Gonzalez, J. E.; Tsien, R. Y. *Chem. Biol.* **1997**, *4*, 269-277.

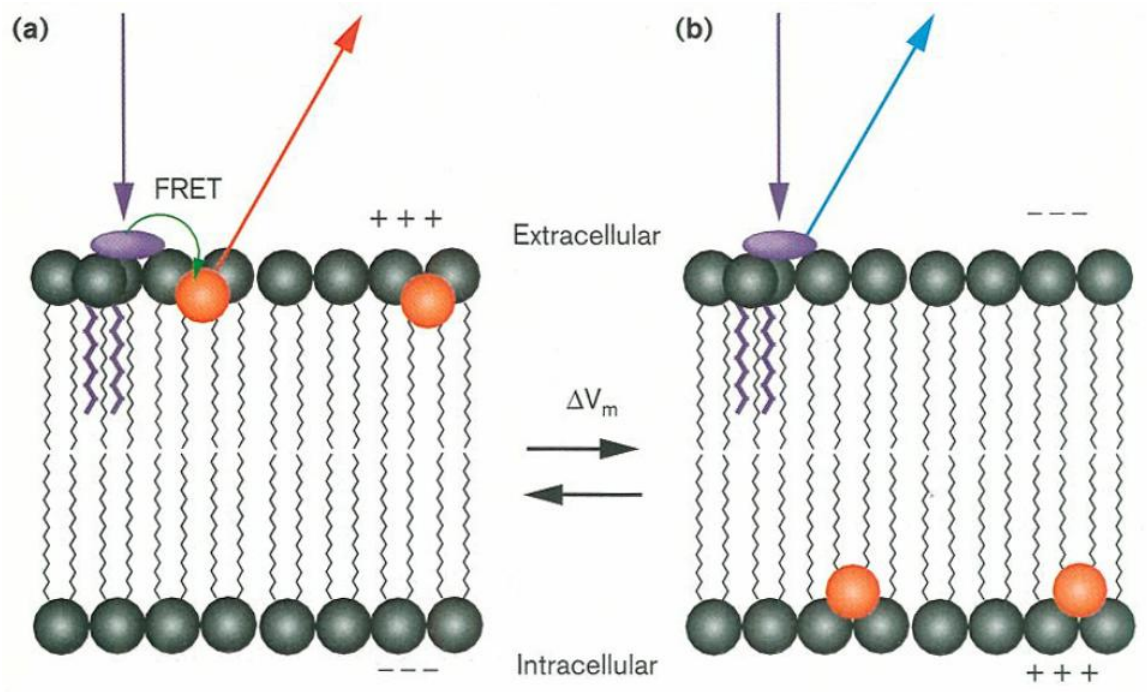
3-2. FRET -explanation-



CC1-DMPE (R = $C_{13}H_{27}$)

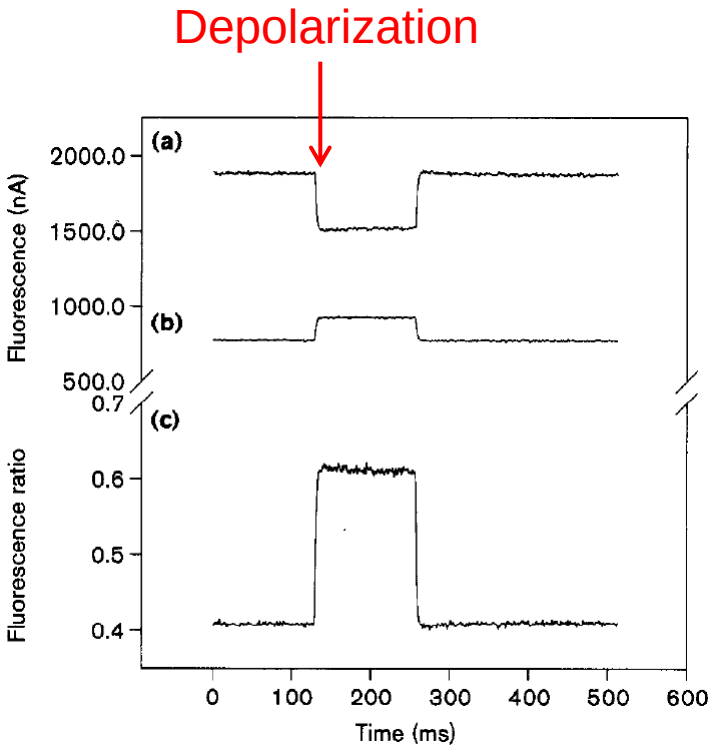


DiSBAC$_6$ (R = C_6H_{13})



Polarization

Depolarization



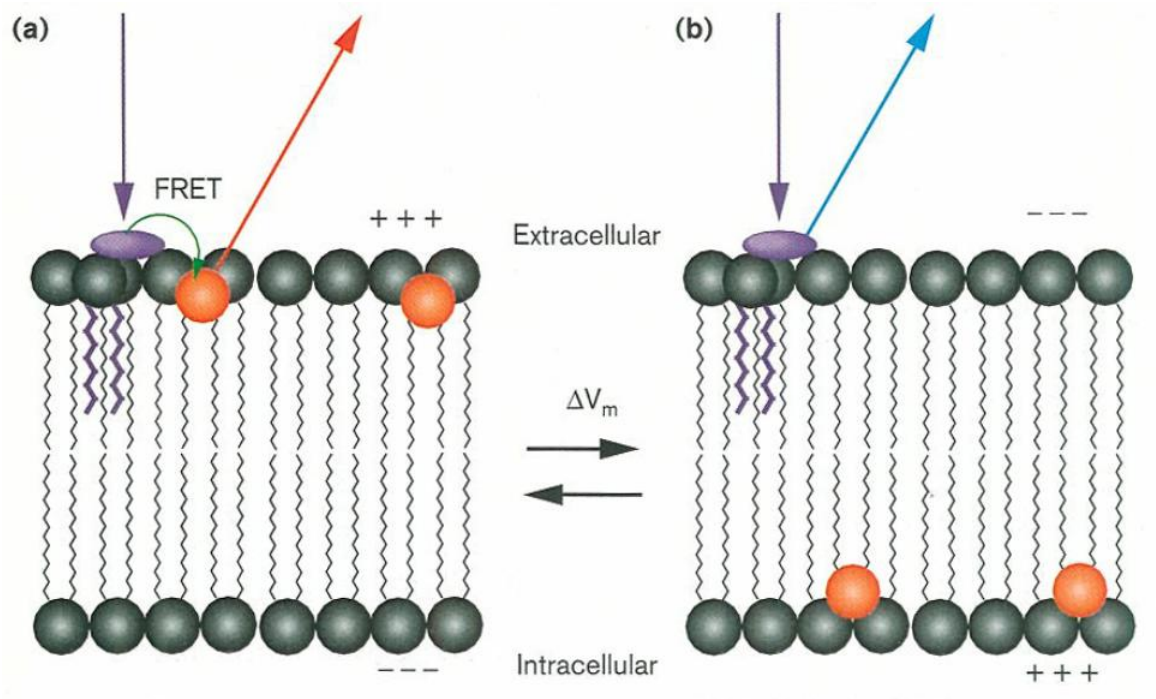
Fluorescence change of (a) DiSBAC$_6$, (b) CC1-DMPE. (c) The ratio of (a) and (b).

(1) Gonzalez, J. E.; Tsien, R. Y. *Chem. Biol.* **1997**, *4*, 269-277.

3-2. FRET -advantage and disadvantage-

advantage
High sensitivity.
Spatial resolution.

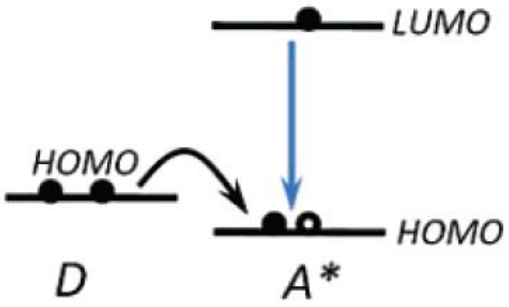
disadvantage
Slow translocation of mobile charges (time resolution).
Changing the membrane capacitance.



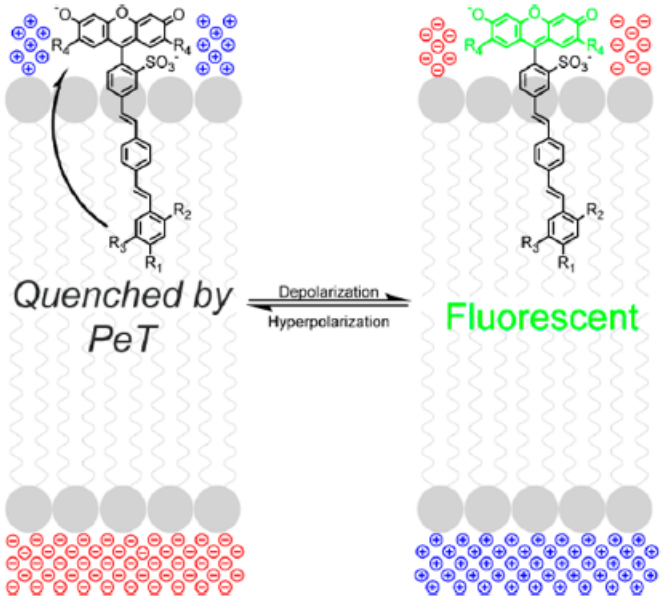
(1) Gonzalez, J. E.; Tsien, R. Y. *Chem. Biol.* **1997**, *4*, 269-277.

3-3. PeT -VF 2.4. Cl-

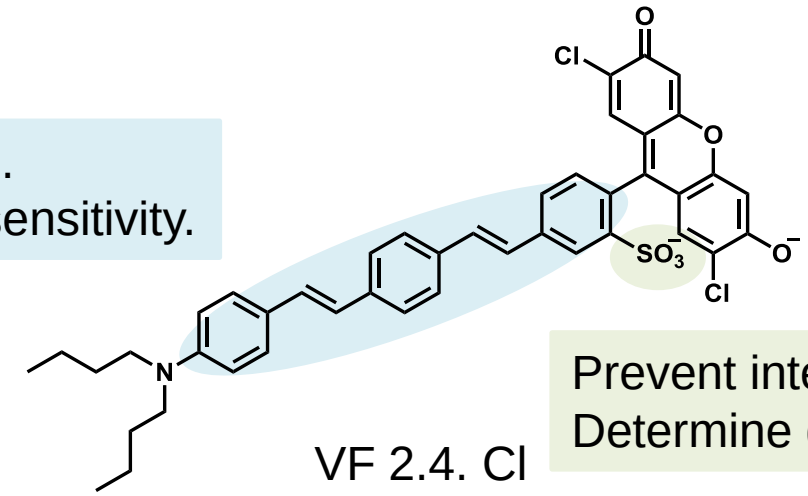
c 2)



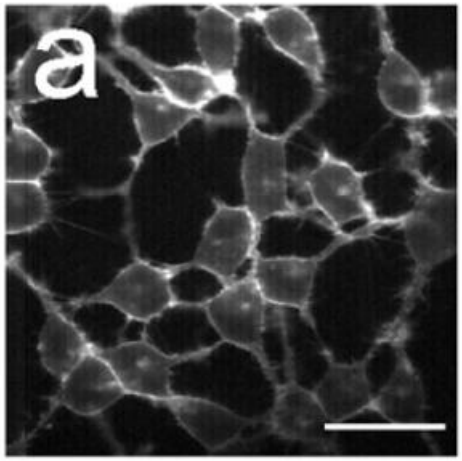
Mechanism of PeT.



Long molecular wire.
Increasing voltage-sensitivity.



VF 2.4. Cl



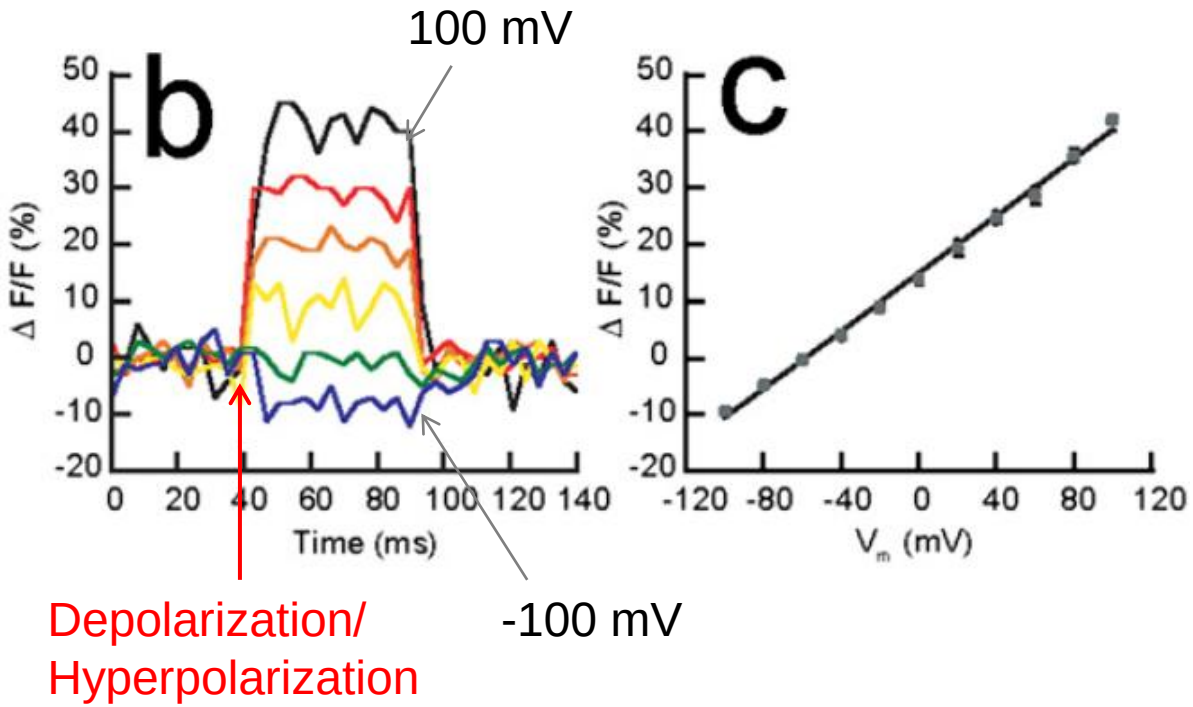
HEK 293 cells stained with 2 μM VF 2.4. Cl

Prevent internalization.
Determine direction of the molecule.

(1) Miller, E. W.; Lin, J. Y.; Frady, E. P.; Steinbach, P. A.; Kristan, Jr. W. B.; Tsien, R. Y. *P Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2114-2119.
(2) Li, L-s. *Nano Lett.* **2007**, *7*, 2981-2986.

3-3. PeT -voltage sensitivity-

Voltage-sensitivity



(b) Fractional changes in VF 2.4. Cl fluorescence during a series of voltage steps to +100 or -100 mV from a holding potential of -60 mV.
(c) Fractional changes in VF 2.4. Cl fluorescence from (b) plotted against membrane potential .

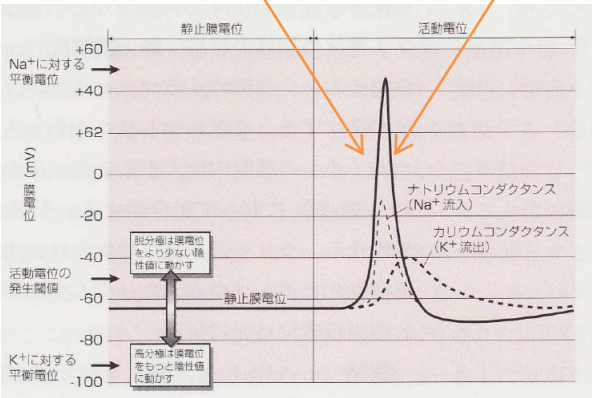
(1) Miller, E. W.; Lin, J. Y.; Frady, E. P.; Steinbach, P. A.; Kristan, Jr. W. B.; Tsien, R. Y. *P Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2114-2119.

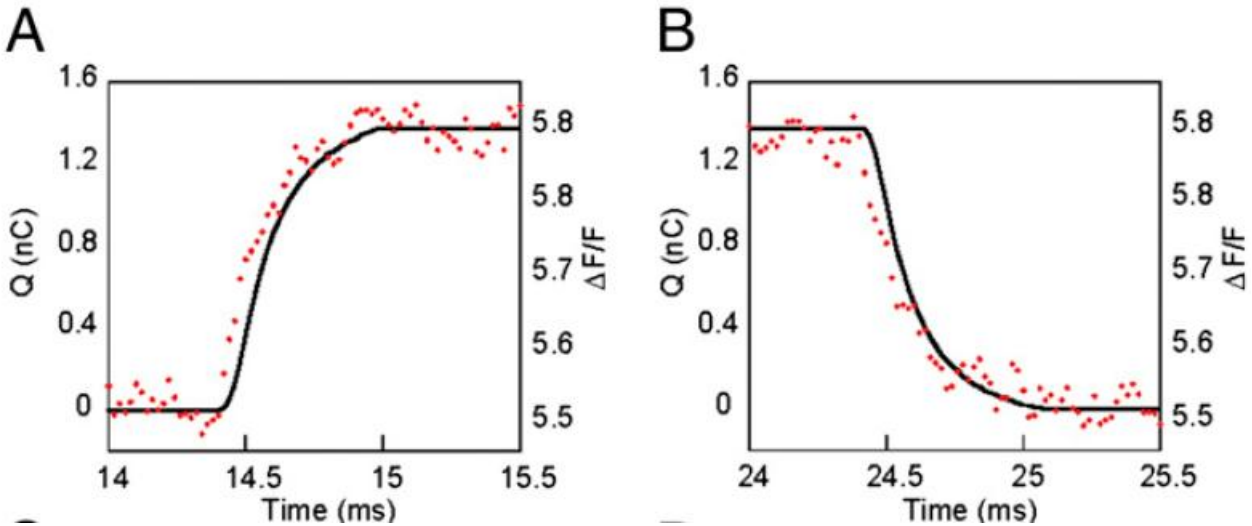
3-3. PeT -time resolution-

Kinetics of signals
(time resolution)

Rising edge

Falling edge

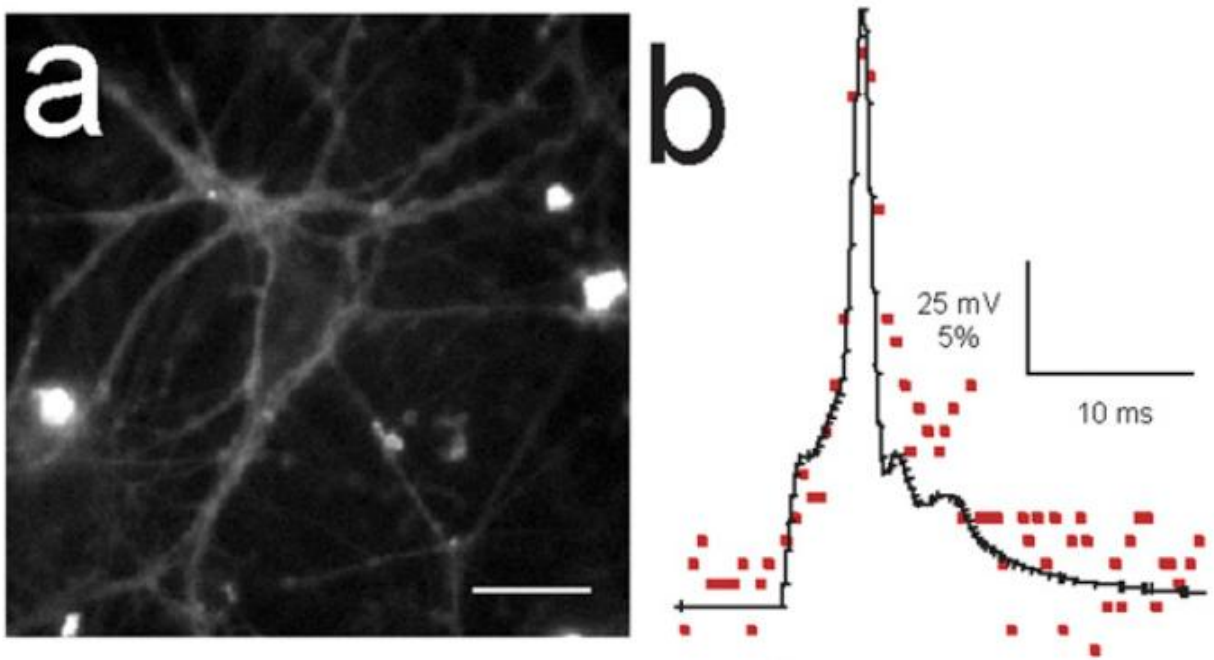




(A) Rising edge and (B) Falling edge of a 100-mV depolarizing step from -60 mV in HEK cells stained with VF 2.4. Cl.
Black, solid trace is the integrated current measured electrophsiologically.
Red points are the optical recording.

(1) Miller, E. W.; Lin, J. Y.; Frady, E. P.; Steinbach, P. A.; Kristan, Jr. W. B.; Tsien, R. Y. *P Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2114-2119.

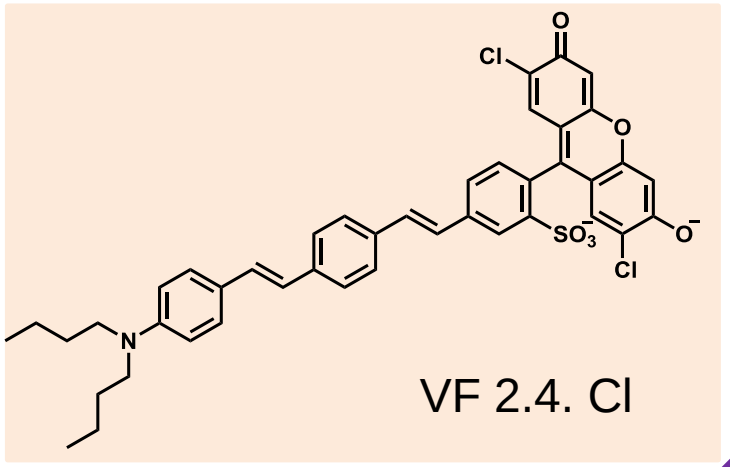
3-3. PeT -rat neurons-



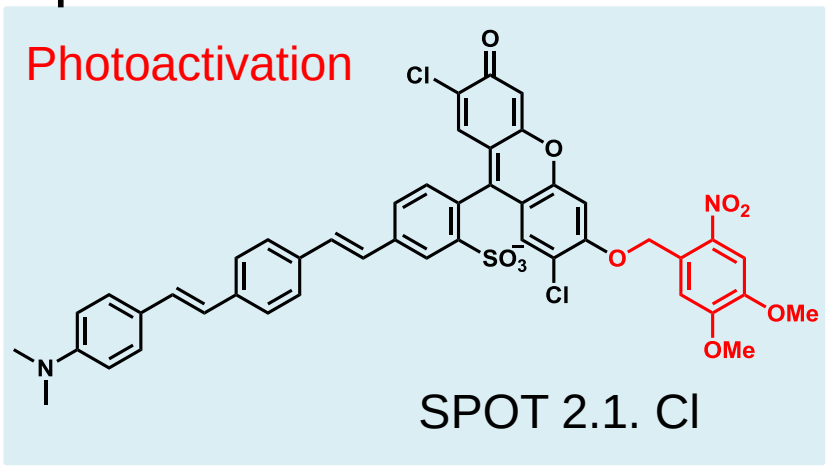
(A) Rat hippocampal neurons stained with 2 μM VF 2.4. Cl.
(B) VF 2.4. Cl can detected evoked action potentials in rat hippocampal neurons in single trials.
The black trace is the recorded electrophysiology signal.
Individual points represent the optical signal from VF 2.4. Cl.

(1) Miller, E. W.; Lin, J. Y.; Frady, E. P.; Steinbach, P. A.; Kristan, Jr. W. B.; Tsien, R. Y. *P Natl. Acad. Sci. U.S.A.* **2012**, *109*, 2114-2119.

PeT-based probes

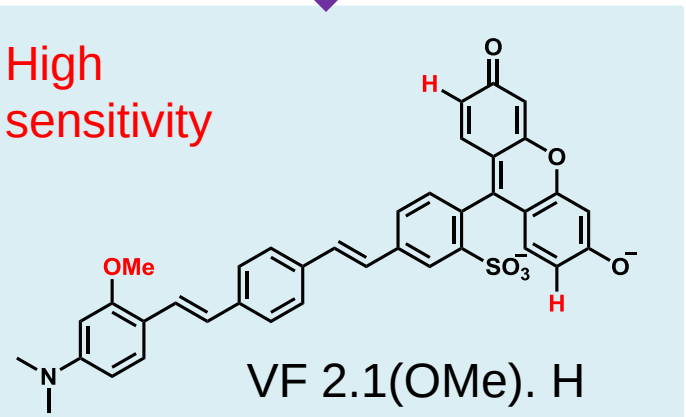


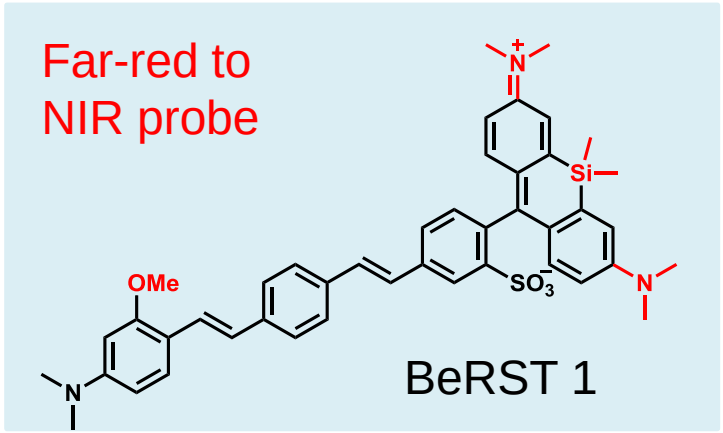
3)



1)

2)





(1) Woodford, C. R.; Frady, E. P.; Smith, R. S.; Morey, B.; Canzi, G.; Palida, S. F.; Araneda, R. C.; Kristan, Jr. W. B.; Kubiak, C. P.; Miller, E. W. Tsien, R. Y. *J. Am. Chem. Soc.* **2015**, *137*, 1817-1824.

(2) Huang, Y-L.; Walker, A. S.; Miller, E. W. *J. Am. Chem. Soc.* **2015**, *137*, 10767-10776.

(3) Grenier, V.; Walker, A. S.; Miller, E. W. *J. Am. Chem. Soc.* **2015**, *137*, 10894-10897.

Summary

3. Voltage-sensitive probes

advantage
Electrical activity is recorded directly.

Time resolution?
Spatial resolution?
Sensitivity? (electron -> photon)

3-1. Electrochromic

disadvantage
Low sensitivity.

3-2. FRET

advantage
High sensitivity.
Spatial resolution.

disadvantage
Slow translocation of mobile charges (time resolution).
Changing the membrane capacitance.

3-3. PeT

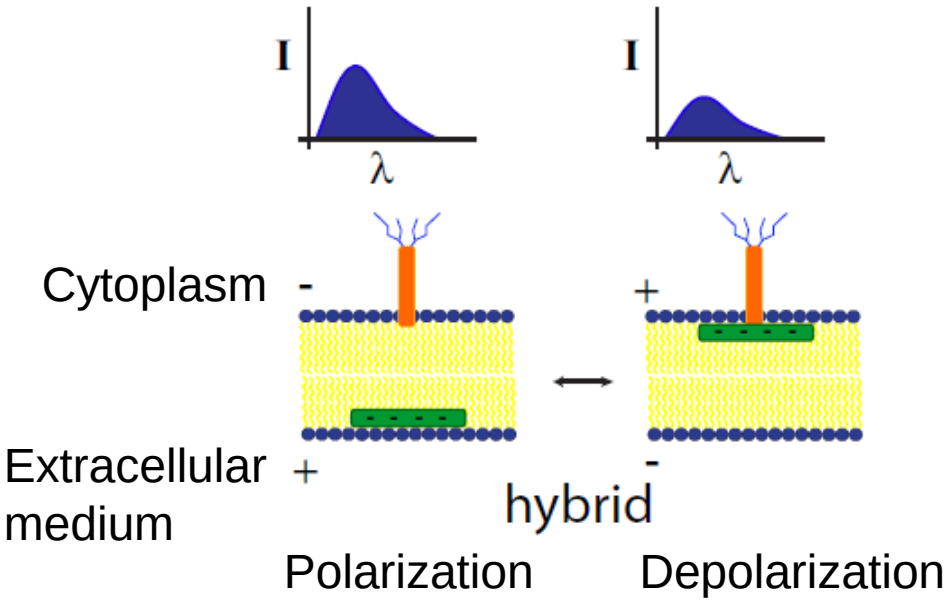
advantage
Electrical activity is recorded directly.
Time resolution.
Spatial resolution.
High sensitivity.

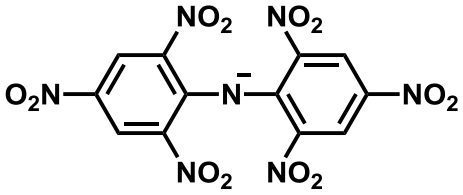
Future perspective
Selective targeting

Genetic expression of PeT-based pH-sensitive protein sensor was reported.[1)]

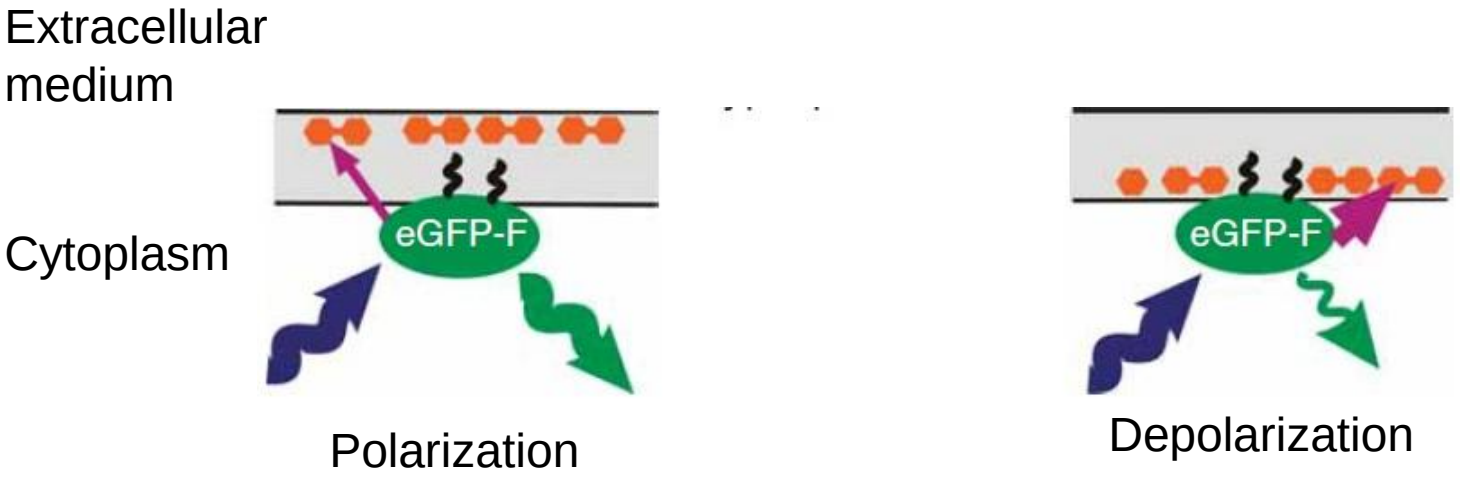
(1) Liu, X.; Jiang, L.; Li, J.; Wang, L.; Yu, Y.; Zhou, Q.; Lv, X.; Gong, W.; Lu, Y.; Wang, J. *J. Am. Chem. Soc.* **2014**, *136*, 13094-13097.

A1. FRET + Genetic Expression



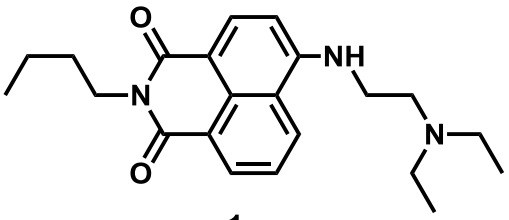


DPA



(1) Chanda, B.; Blunck, R.; Faria, L. C.; Schweizer, F. E.; Mody, I.; Bezanilla, F. *Nat Neurosci* **2005**, *8*, 1619-1626.

A2. PeT based pH-sensitive probe



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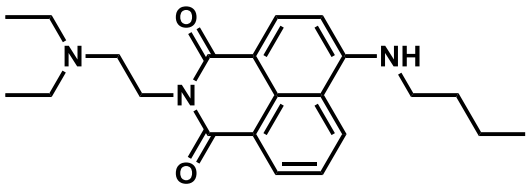

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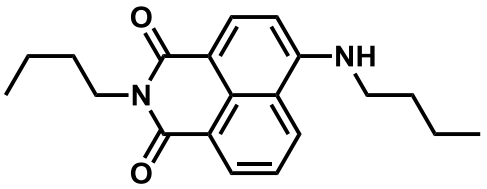

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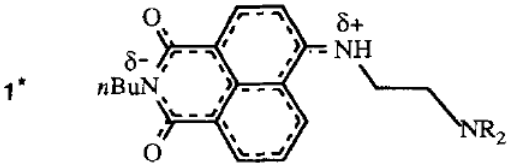

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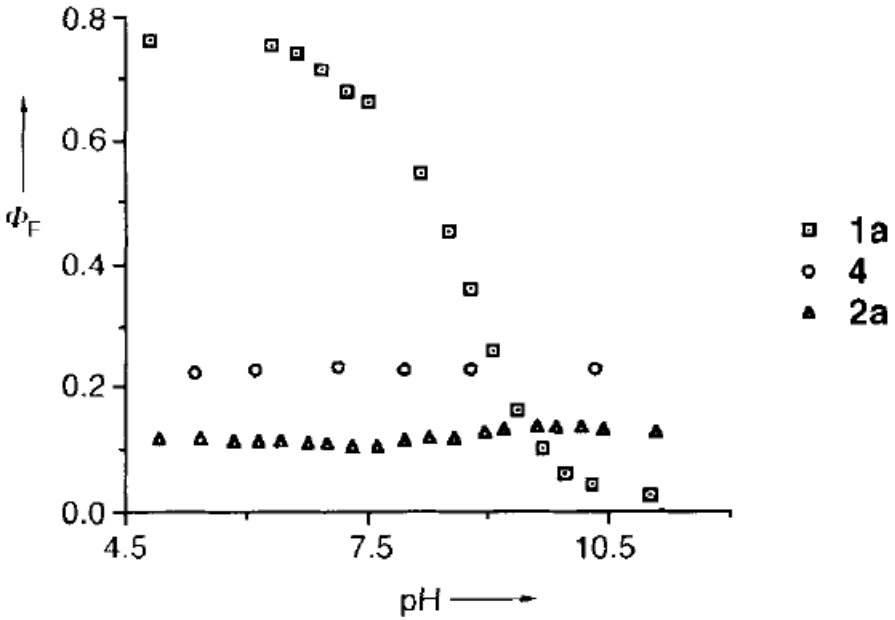

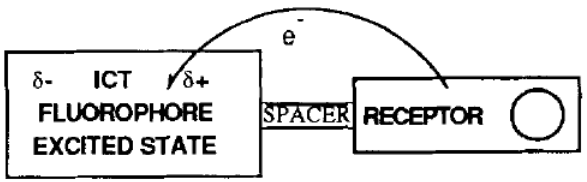

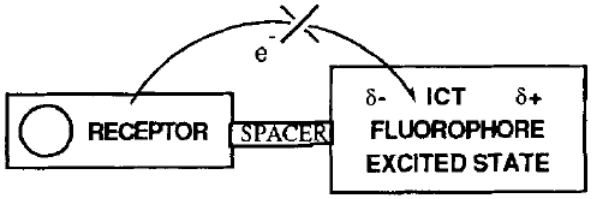

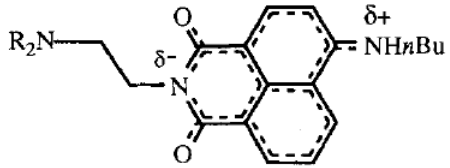

2*

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