Learning about Conformational changes in ion channels with Fluorescence

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A voltage-gated ion channel
Conformational changes detected at the macroscopic level

Electrophysiological Methods

1. Oocyte Cut-open Voltage clamp
2. Whole cell patch clamp
3. Bilayers voltage clamp
4. Liposomes “Nernst clamp”

What do we record?

- Ionic Current
- Gating Current

Out

The conduction of ions through the protein:
-----the actual function-----

The rearrangement of intrinsic charges in the protein:
-----voltage dependent
Conformational changes-----
X-ray structure of Kv1.2 (pdb id 2A79)
Long et al (Science, 2005)

OPEN-INACTIVATED state
Detection of conformational change

Tetramethylrhodamine maleimide

\[
\text{CH}_3\text{N} - \text{O} - \text{N} \text{(CH}_3\text{)}_2
\]

\[
\text{C}=\text{N} - \text{O} - \text{N} \text{(CH}_3\text{)}_2
\]

\[
\text{C}=\text{O}
\]

-90 mV

\[\Delta F/F \text{ 12.5%}\]

decrease in F

0 mV

quenched

unquenched

256 ms
The fluorescence change in M356C is due to quenching

A

M356C

Counts

570 585 600 615 630 645

Wavelength (nm)

B

A359C

Counts

570 585 600 615 630 645

Wavelength (nm)

C

counts

540 555 570 585 600 615 630

Wavelength (nm)

D

Holding Potential (mV)

560 565 570 575

Wavelength (nm)

Phenol

M356C

A359C

NMG-MES

KI

Methanol

Ethanol

3-Methyl-1-Butanol

Isopropanol

H2O

Kl

Phenol

MethButanol

Isopropanol
Depolarization Induces a Conformational Change in the m2 Muscarinic Receptor

Gating currents

Fluorescence signal

Voltage dependent binding to Xenopus oocytes
Na Channel
Sodium Channel

The diagram illustrates the sodium channel at different voltage potentials. At 0 mV, the channel is open, allowing sodium ions to flow. As the voltage increases to -130 mV and further, the channel closes, preventing ion flow. The graph below the channel shows the current $I_g$ as a function of time, with $Q$, $\frac{2}{3}Q$, and $\frac{Q}{3}$ indicating the points of channel opening and closing.
Fast component immobilized by inactivation in Domain IV

Domain IV (R1448C)

18 μA

50 mV

-130 mV

1 μA

0.5% ΔF/F

20 ms

8 μA

0 mV

-130 mV

10.24 ms

10.24 ms
Comparison of **fluorescence**, gating and **ionic** traces of labeled mutants

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Gating charge</th>
<th>Ionic current</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-30 mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-70 mV</td>
<td></td>
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<td></td>
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</tbody>
</table>

*DOMAIN I (S216C)*  *DOMAIN II (S660C)*  *DOMAIN III (L1115C)*  *DOMAIN IV (S1436C)*

1 ms
The S4s of the first three domains do not activate in a specific sequence. The activation of S4 in domain IV requires a prior activation of one of the three S4 segments.
Electrochromic Effect

Ground State

Photoexcitation

Excited State

Electrochromic Shift

\[ \Delta \frac{1}{\lambda} = \Delta \frac{1}{\lambda}^{\text{solv}} - (\mu_e - \mu_g) E \cos \alpha \]
Mapping the electric Field with electrochromic probes
FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)

Donor

\[ h\nu_d \]

\[ K_{et} \propto R^{-6} \]

Acceptor

\[ h\nu_a \]
\[ E = \frac{k_{ET}}{(k_{ET} + k_n)} \]

Forster theory:
\[ k_T(R) = \frac{1}{\tau_D \left[ \frac{R_0}{R} \right]^6} \]

Where
\[ R_0 = 8.79 \times 10^{-5} \left( J_D q_D n^{-4} \kappa^2 \right)^{1/6} \]

Then,
\[ R = R_0 \left[ \frac{(1 - E)}{E} \right]^{1/6} \]
\[ R = R_0 \left[ \frac{\tau_{DA}}{(\tau_D - \tau_{DA})} \right]^{1/6} \]
Figure 8-20

Efficiency of energy transfer as a function of distance in dansyl-(L-prolyl)ₙ-α-naphthyl semicarbazide oligomers with \( n = 1 \) to 12. The curve was fit to the data with Equation 8-57. [From L. Stryer and R. P. Haugland, Proc. Natl. Acad. Sci. USA 98:719 (1967).]
Another method: Using hydrophobic ions as FRET acceptors (reference) in the bilayer

Gating current trace of Dipicrylamine (dpA)

Charge voltage relationship of dpA in oocyte membranes

Calculated $R_o$ between TMR and dpA is 20 Å
Large movement

Small movement

depolarization

repolarization

Large movement

Small movement

depolarization

repolarization
Individual experiments average

363C - ABD

average

WT

425C

349C

354C

363C

367C
Lanthanide-based Resonance Energy Transfer (LRET)

LRET utilizes luminescent lanthanides (i.e. Tb$^{3+}$, Eu$^{3+}$) with sensitizer and reaction group as fluorescent donors

- Lifetimes are easily measurable ($\tau \sim 0.2 - 1.5$ ms)

- Lanthanides fluoresce isotropically, reducing errors from orientation factor $\kappa^2$

- Sharply spiked emission spectrum and temporal elimination of direct acceptor excitation enables specific measurements of acceptor sensitized emission
Donor only
\[ \tau_d = 1.6 \text{ ms} \]

Sensitized emission
\[ \tau_{ad} = 92 \mu s, 566 \mu s \]

\[ R_{SC} = 28 \text{ Å} \]
\[ R_{SA} = 41 \text{ Å} \]

\( R_{SA} \) predicted from \( R_{SC} \) using Pithagorean Theorem \( = 40 \text{ Å} \)
INVERTED CUT-OPEN FLUORESCENCE SETUP
Acquisition

-90 mV

50 mV: \( \tau_{ad} = 173 \ \mu s \)

-120 mV: \( \tau_{ad} = 65 \ \mu s \)

Pulse potential

\( R_{SC} \)

\( Q \)

Fit, \( R_{SC} \)

Fit, \( Q \)
The results show that upon depolarization distances from toxin to:
   S4 decrease by 0.8 Å  
       (361 and 365 in S4)  
   S3-S4 linker decrease by 2 Å  
       (351, 352, 353)  
   S3 Increase by 1 Å  
       (333, 335)  

Conclusion:
transmembrane displacement is about 2 Å  
With S4 and S3 moving in opposite directions
Genetically encoded LRET

LBT peptide

YIDTNNDGWYEGDELLA

Time, ms

Normalized Intensity

<table>
<thead>
<tr>
<th>Distance Å</th>
<th>Transfer Efficiency</th>
</tr>
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<tbody>
<tr>
<td>6-His</td>
<td>Cu²⁺Ni²⁺</td>
</tr>
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</table>

Tb³⁺ only

Tb³⁺ + Cu²⁺
Lanthanide based Transfer-LRET using a genetically encoded tag

**Lanthanide Binding Tag**

**Fluorescein maleimide**

Expression of modified channels in XL-1 Blue

Solubilization of membranes proteins in detergent

Purification of His-tagged channels on cobalt resin

Reconstitution of channels in proteoliposomes

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**Expression of modified channels in XL-1 Blue**

**Solubilization of membranes proteins in detergent**

**Purification of His-tagged channels on cobalt resin**

**Reconstitution of channels in proteoliposomes**

**10mM KCl Crown Ether Valinomycin Liposomes**

Normalized fluorescence intensity

0 200 400 600 800 1000
Example:

**Demonstration of rotational movement of the voltage sensor of KvAP**

These experiments are also done in oocytes under voltage clamp allowing better potential control and time resolution.
Conformational changes detected at the single molecule level

Optical Methods: fluorescence
SPR & SPCE

- Surface plasmon resonance (SPR) is used to excite fluorophores near a specially prepared thin metal surface on glass substrate.
- Surface plasmon-coupled emission (SPCE) is used in the configuration below to capture the emission of the fluorophores excited by SPR as described by Lakowicz et al.

Figure 1. Schematic of the application of surface plasmon resonance to fluorescence in aqueous solution. The evanescent field strength decreases away from the metal surface. Molecules within the evanescent field are excited (stars). However, molecules within approximately 10 nm of the metal surface will be quenched and those beyond the evanescent field are not excited.

Figure 2. Simulation of reflectance of 647 nm laser excitation off the surface of the plasmon chip. The “dip” is the angle at which photons are most efficiently absorbed and maximally excite surface plasmon. The critical angle for SF11glass at an H₂O interface and the maximum acceptance angle for the Olympus APO 100x NA 1.65 objective are shown as dotted lines.
Surface-coupled plasmon emission

aqueous solution with fluorophores (n~1.33)

Ag or Au

high index glass hemispherical lens

Start of

End of
CONCLUSION
The position of S4 at the level of R362 is constrained with respect to S1 and S2 in CLOSED state.

Translation ~6.5 A
Rotation ~180°
Tilt ~30°