Learning about Conformational changes in ion channels with Fluorescence

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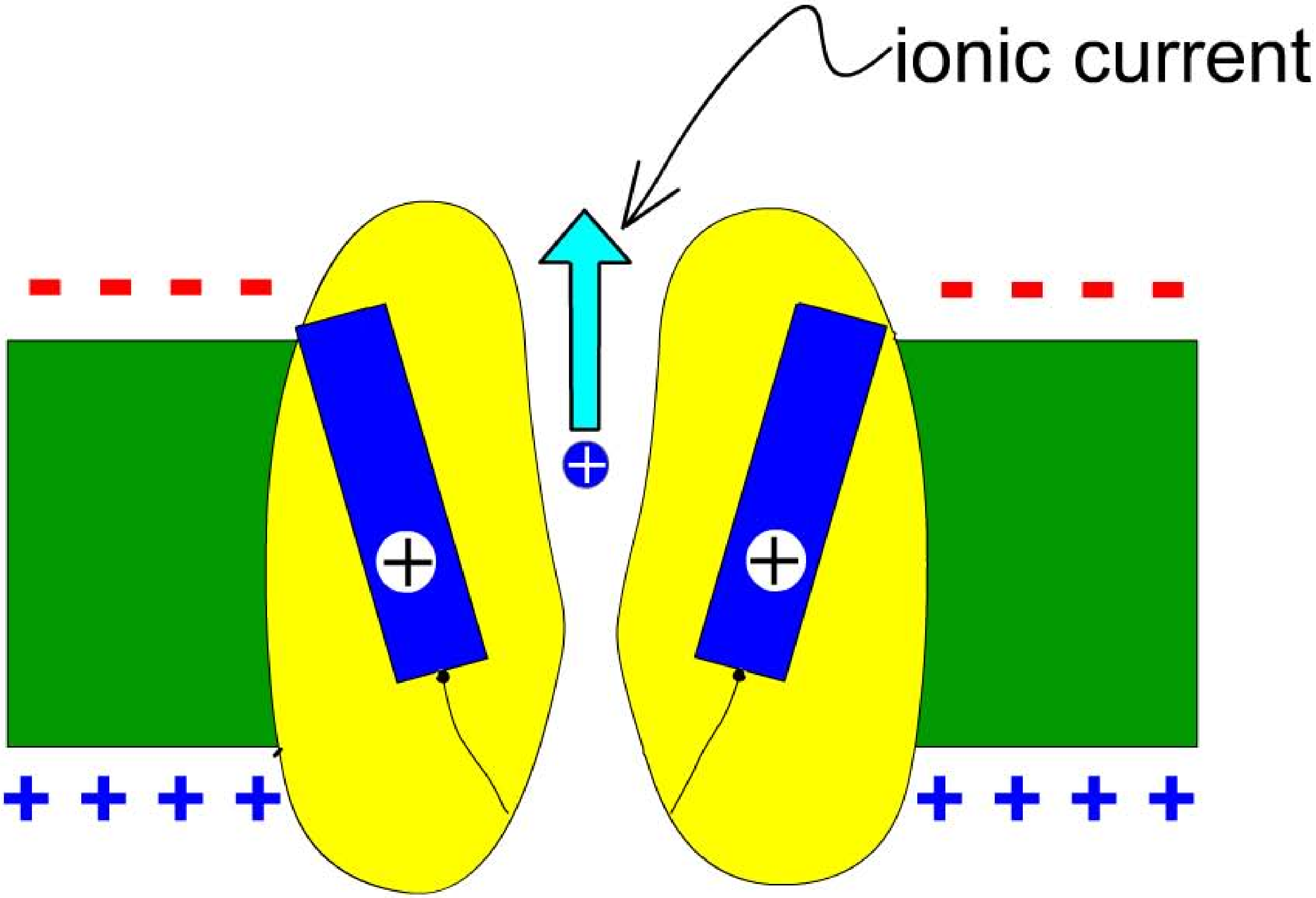
A voltage-gated Ion channel

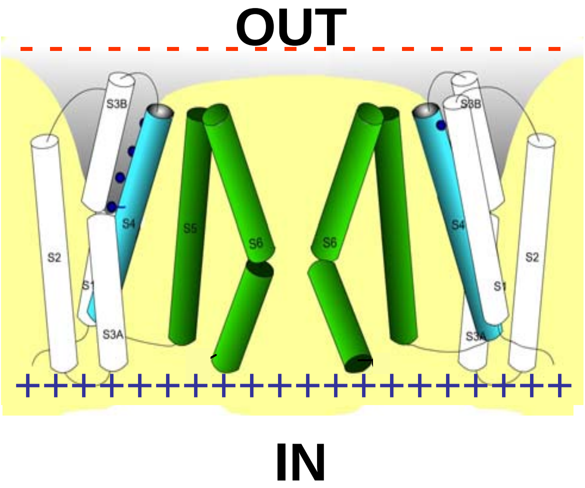


OPEN

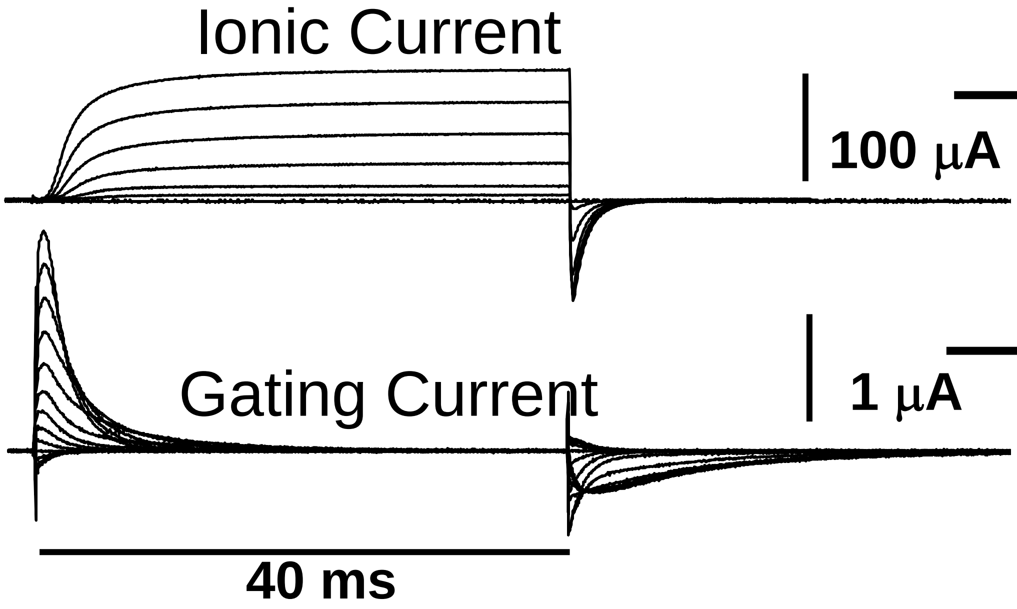
Conformational changes detected at the macroscopic level

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?



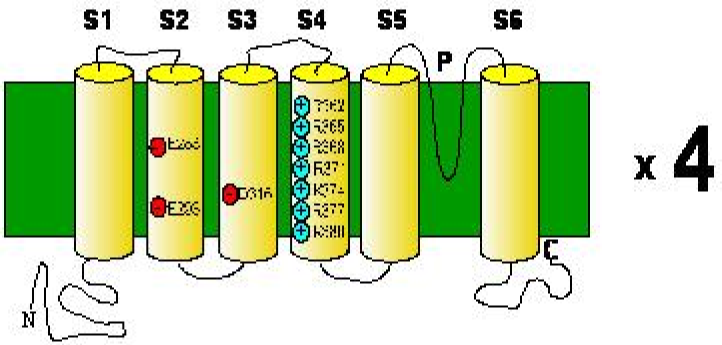
The conduction of ions through the protein:

-----the actual function----

The rearrangement of intrinsic charges in the protein:

----voltage dependent Conformational changes-----

*Shaker*B



362 365 368 371 374 377 380

***Shaker*B S4** AIL**R**VI**R**LV**R**VF**R**IF**K**LS**R**HS**K**

Rat μ1 DI ISAL**R**TF**R**VL**R**AL**K**TITVIPGLKT

216 219

Rat μ1 DII SVL**R**SF**R**LL**R**VF**K**LA**K**SWPTL

660 663

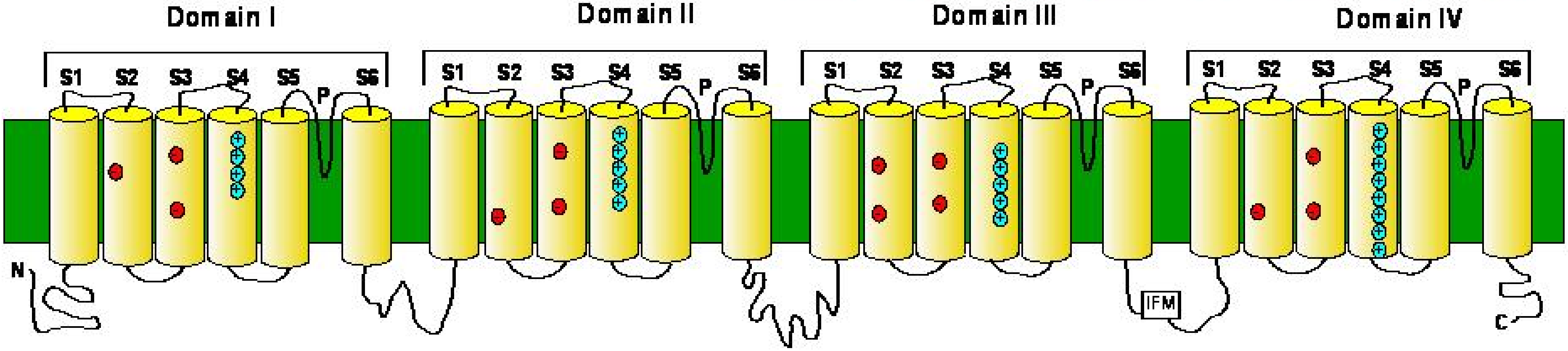
Rat μ1 DIII LGPI**K**SL**R**TL**R**AL**R**PL**R**ALSR

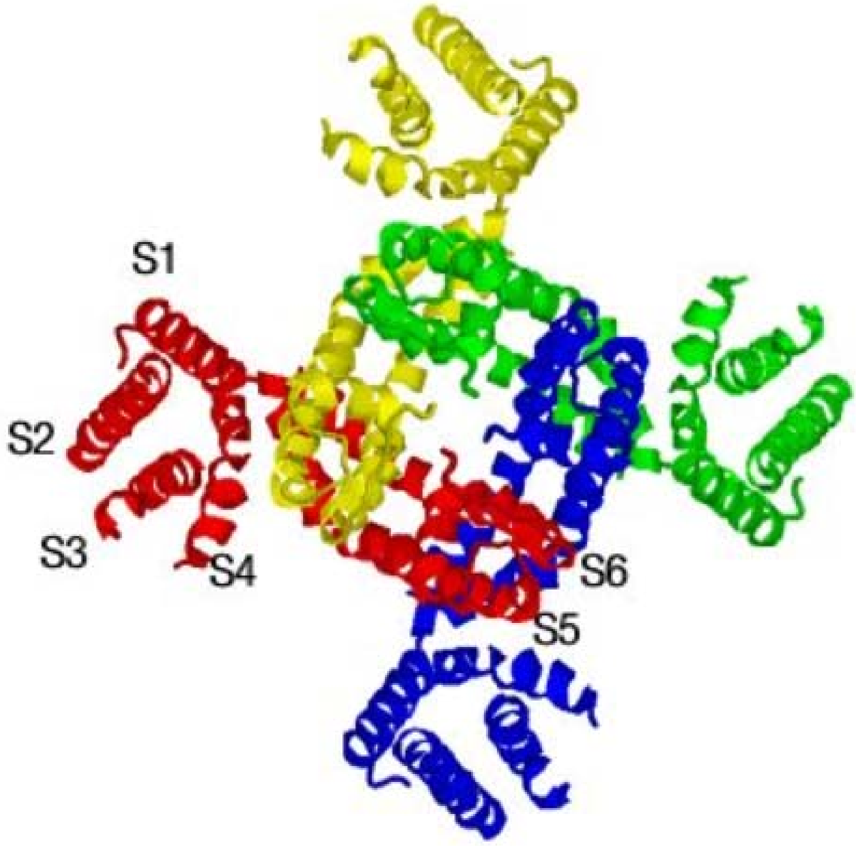
1115 1119

Rat μ1 DIV SPTLF**R**VI**R**LA**R**IG**R**VI**R**LI**R**GA**K**GI**R**

1436 1441

Rat μ1

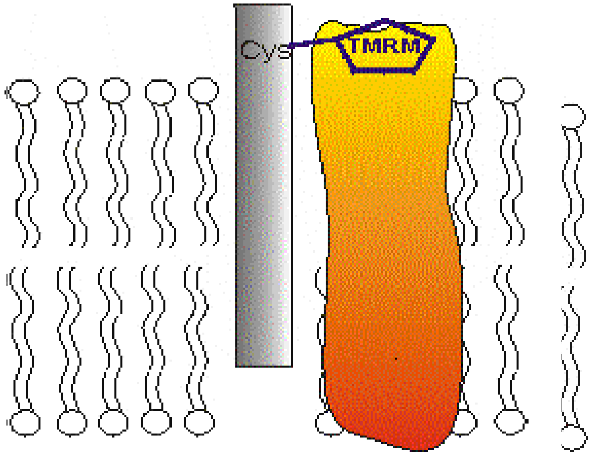




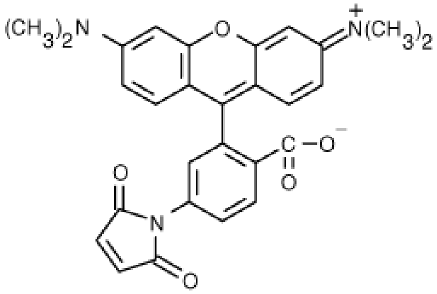
X-ray structure of Kv1.2 (pdb id 2A79)
Long et al (Science, 2005)

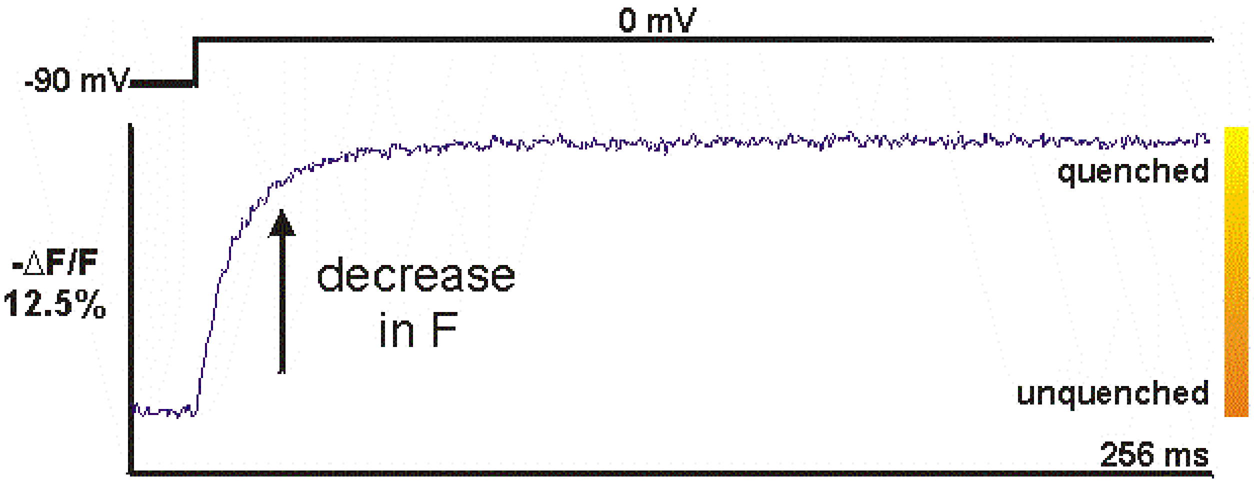
OPEN-INACTIVATED state

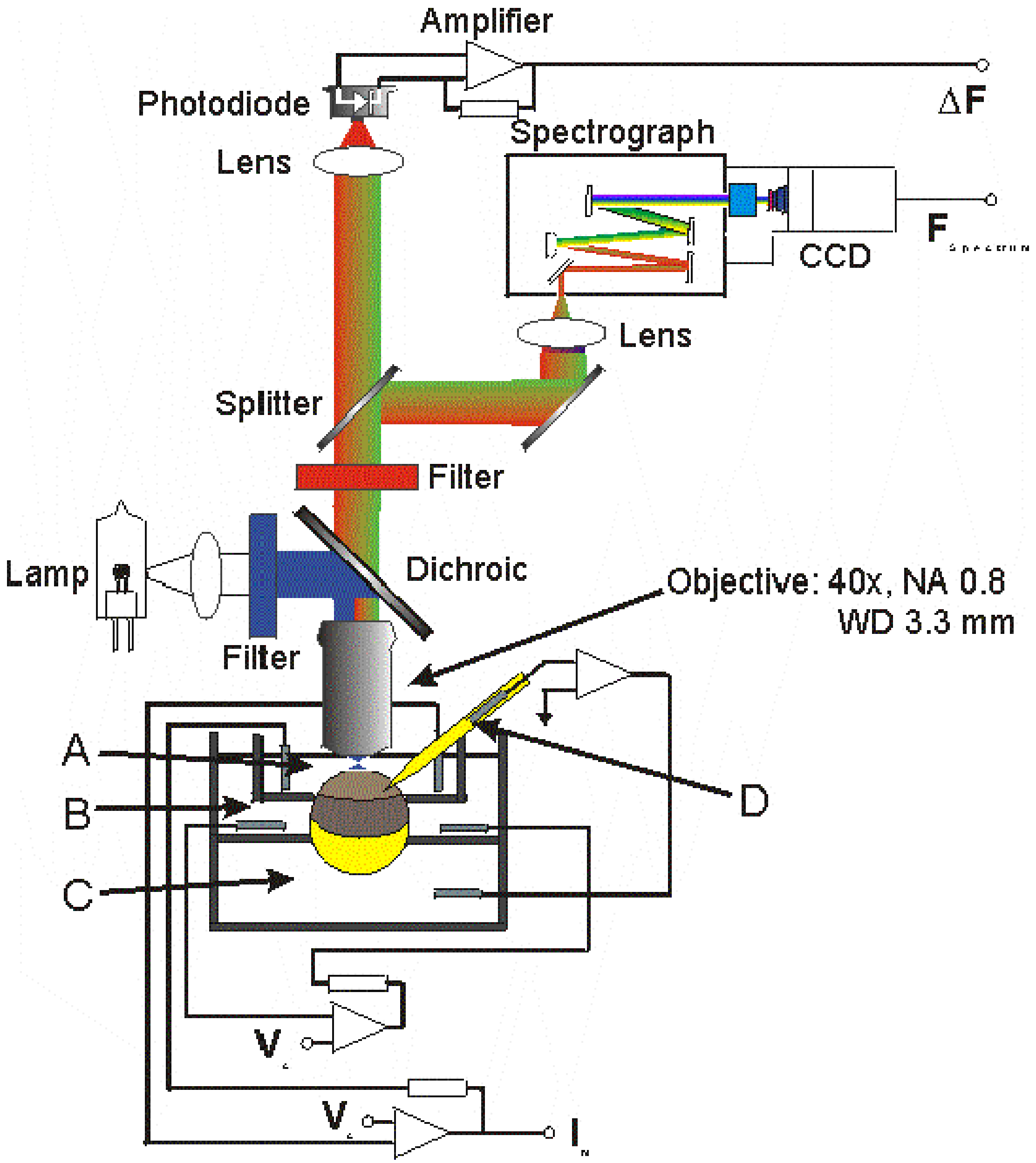
Detection of conformational change



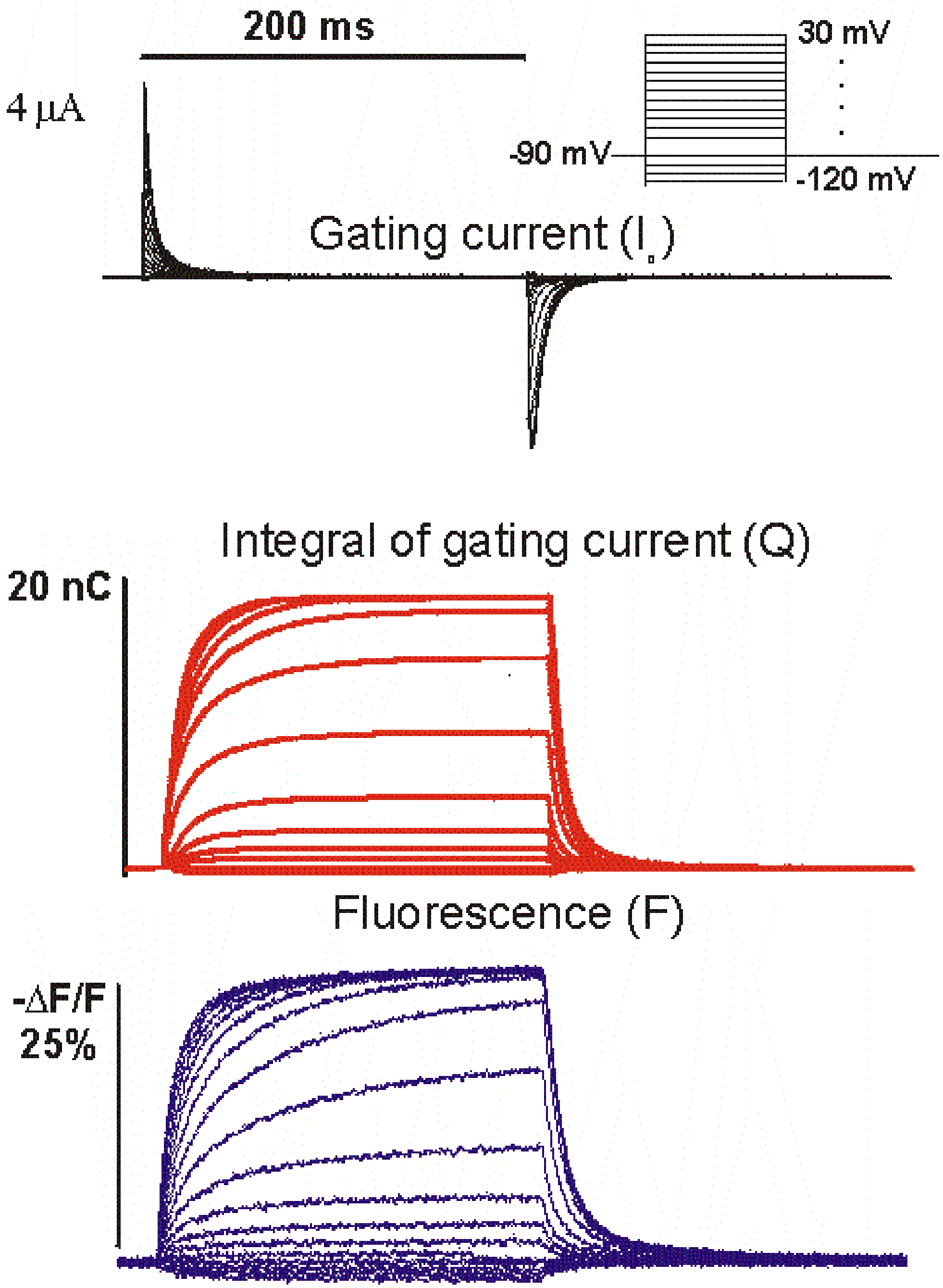
Tetramethylrhodamine maleimide



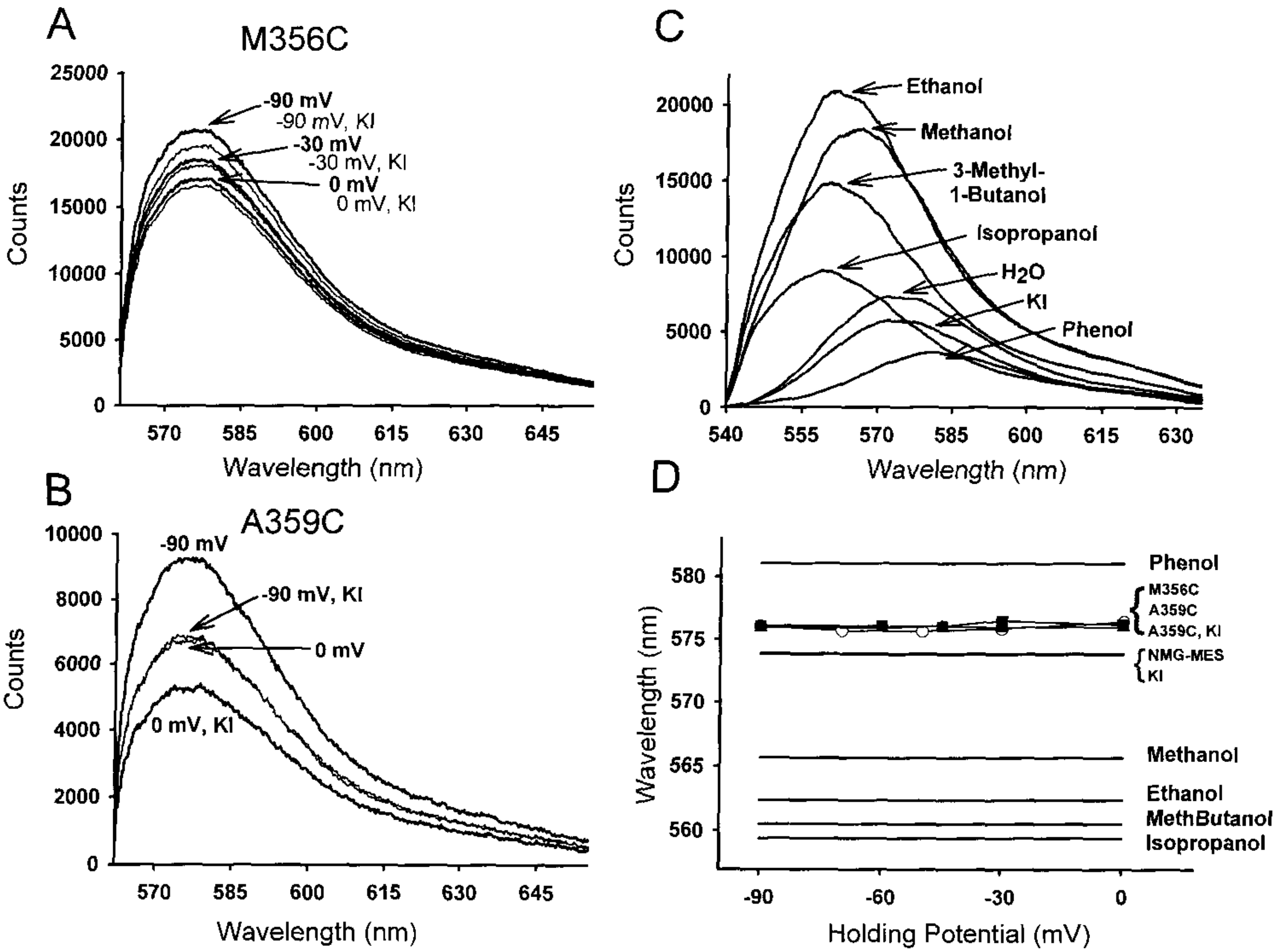



Amplifier
Photodiode
ΔF
Lens
Spectrograph
CCD
$F_{spectrum}$
Lens
Splitter
Filter
Lamp
Dichroic
Filter
Objective: 40x, NA 0.8
WD 3.3 mm
A
B
C
D
V_c
V_c
I_m

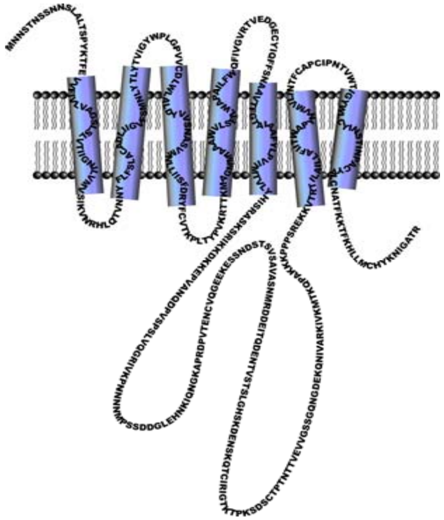
Experimental data: M356C W434F



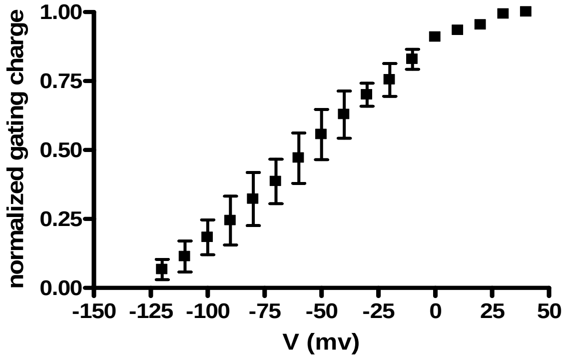
The fluorescence change in M356C is due to quenching

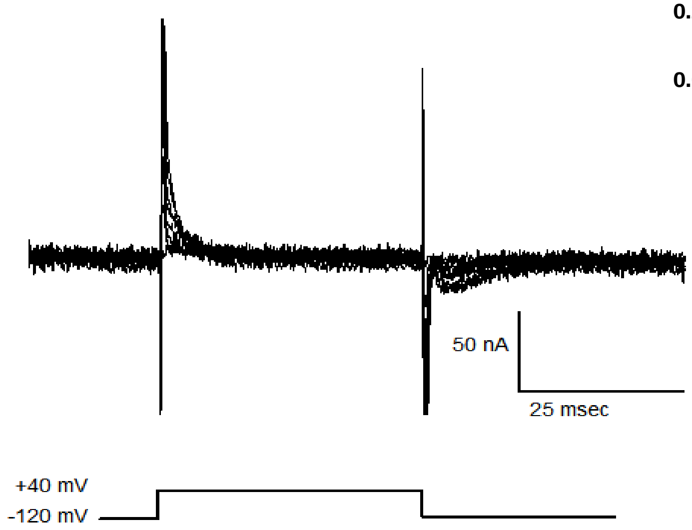


Depolarization Induces a Conformational Change in the m2 Muscarinic Receptor

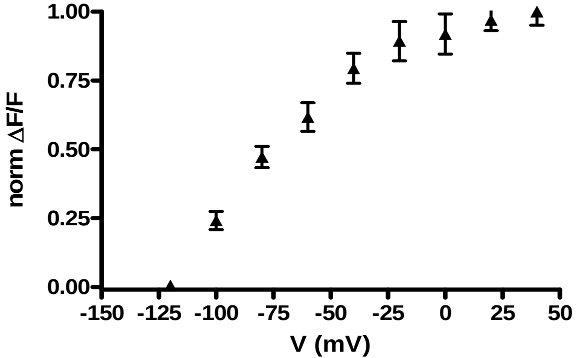


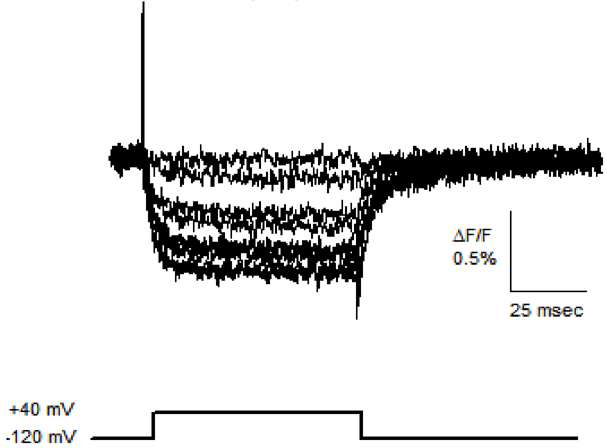
Gating currents



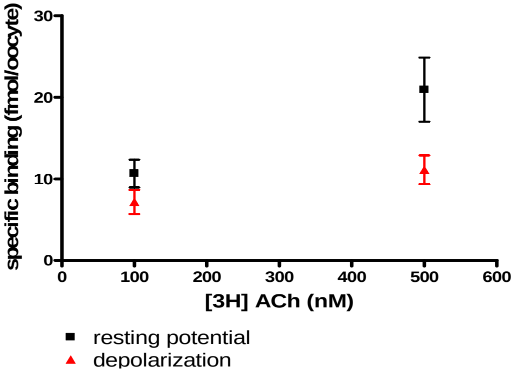


Fluorescence signal

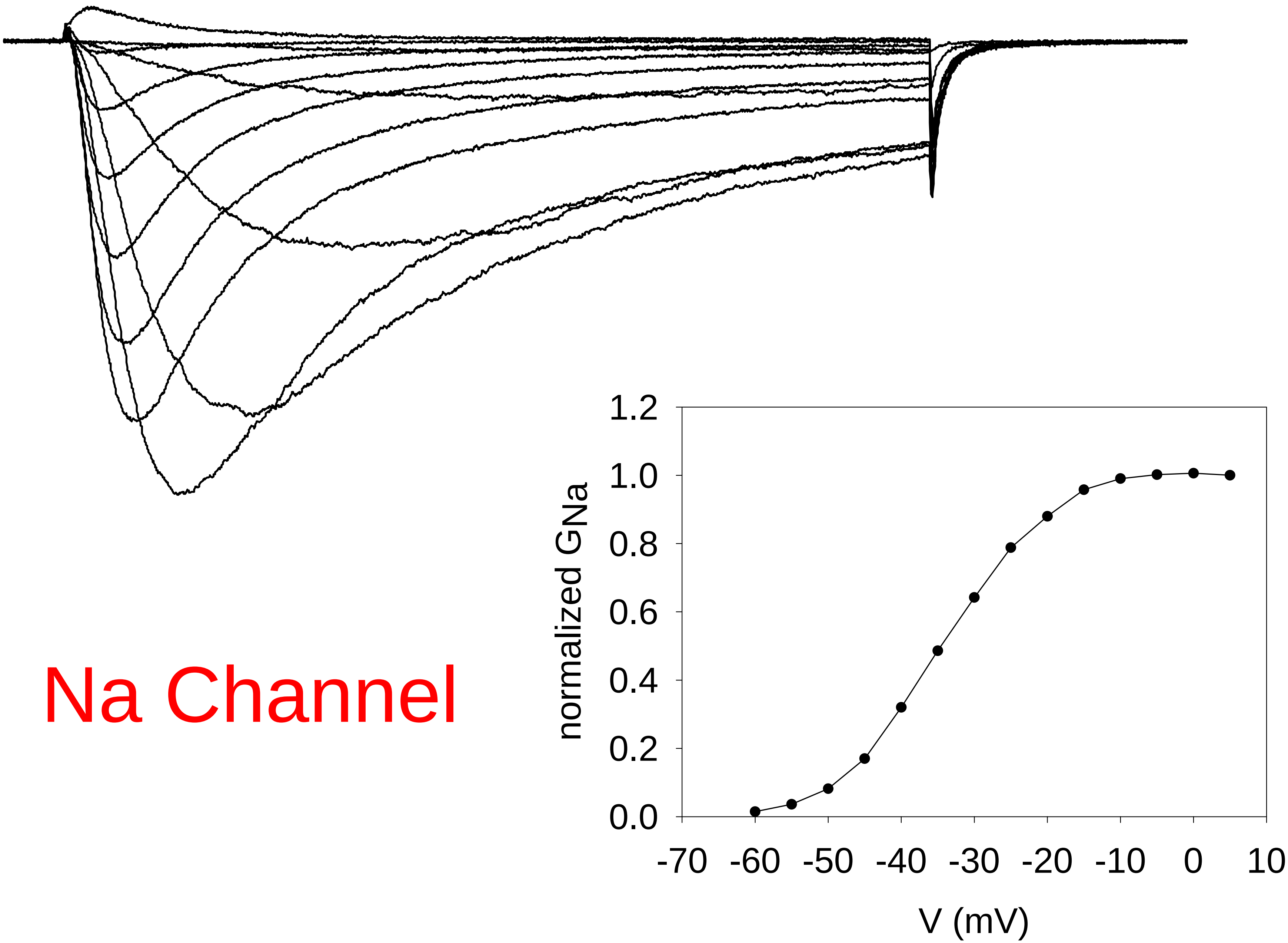




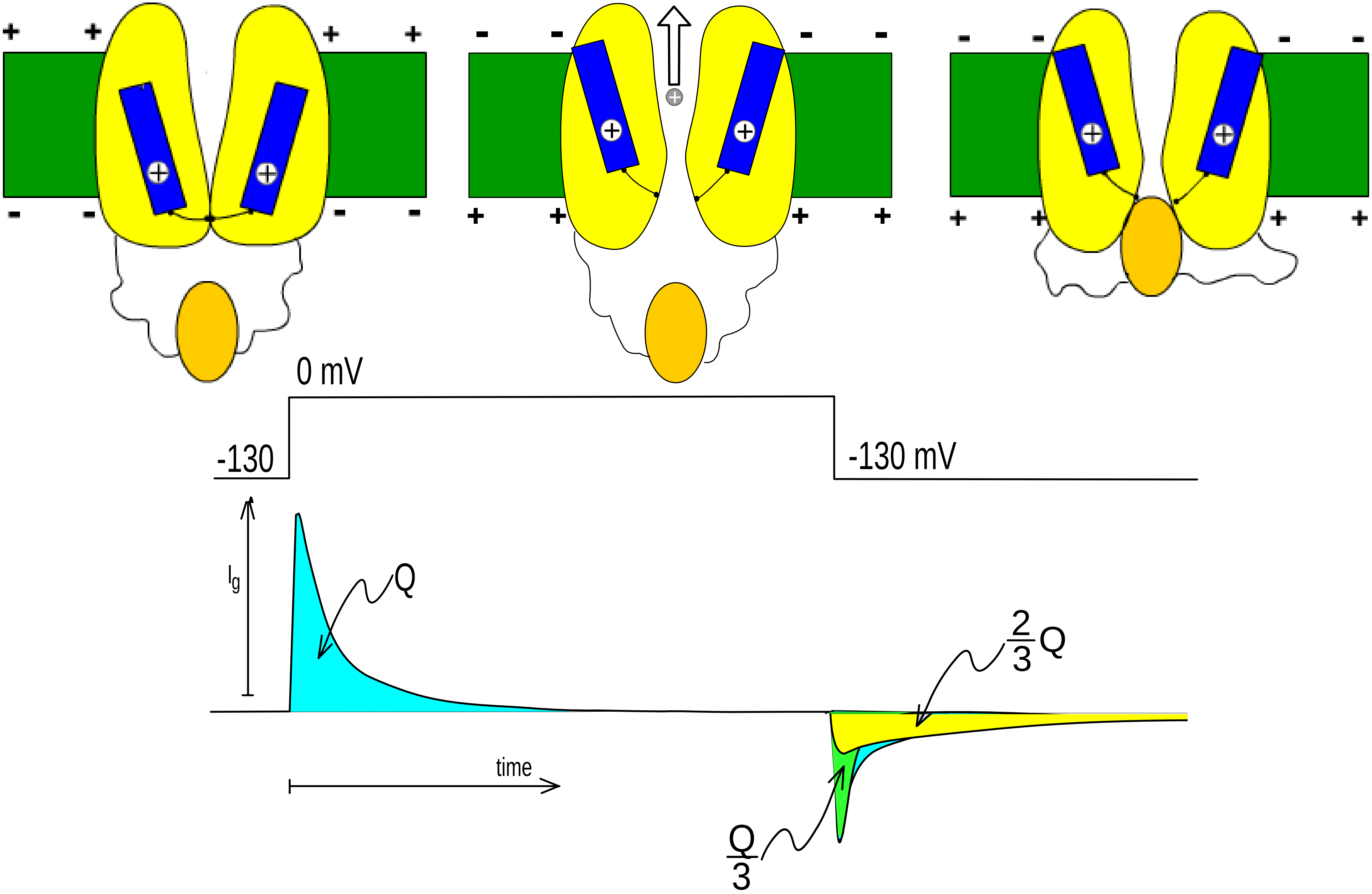
Voltage dependent binding to Xenopus oocytes



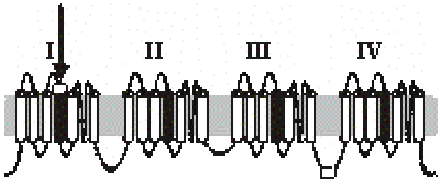
Na Channel



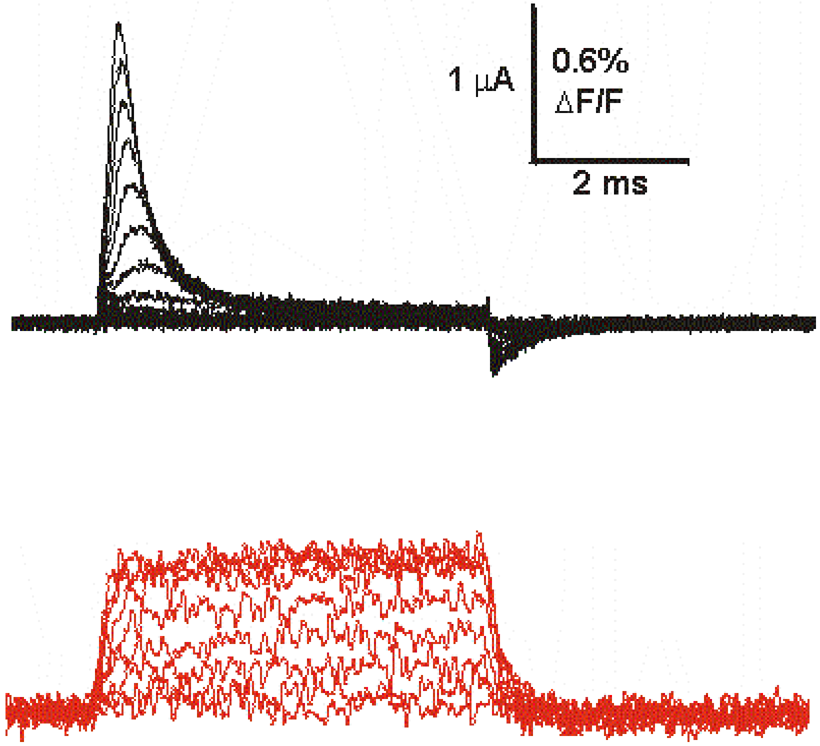
Sodium Channel

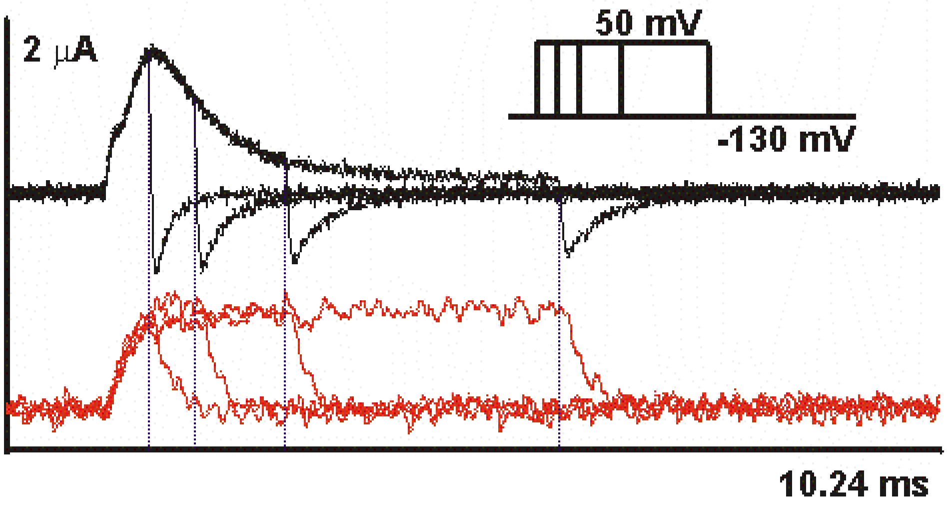


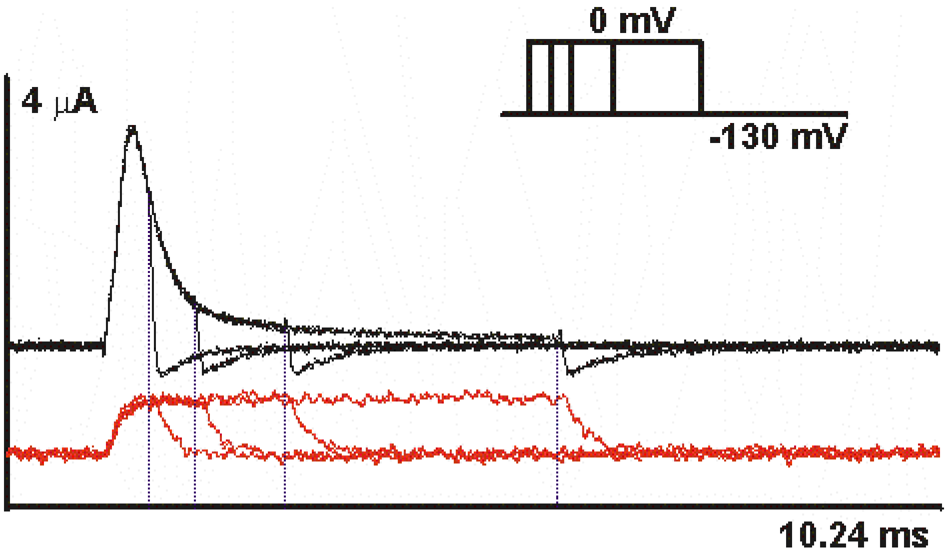
Lack of immobilization in domain I



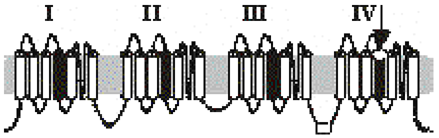
Domain I (S216C)



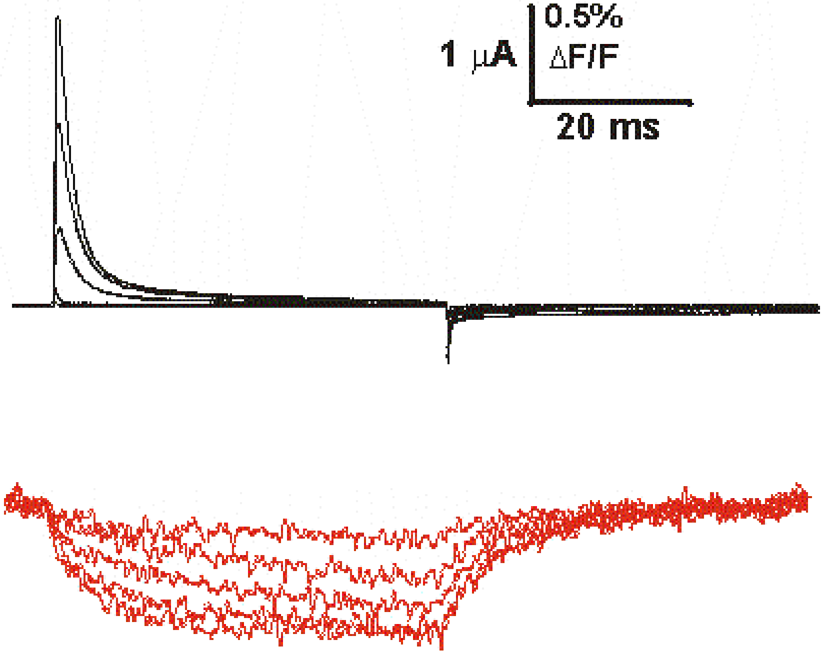


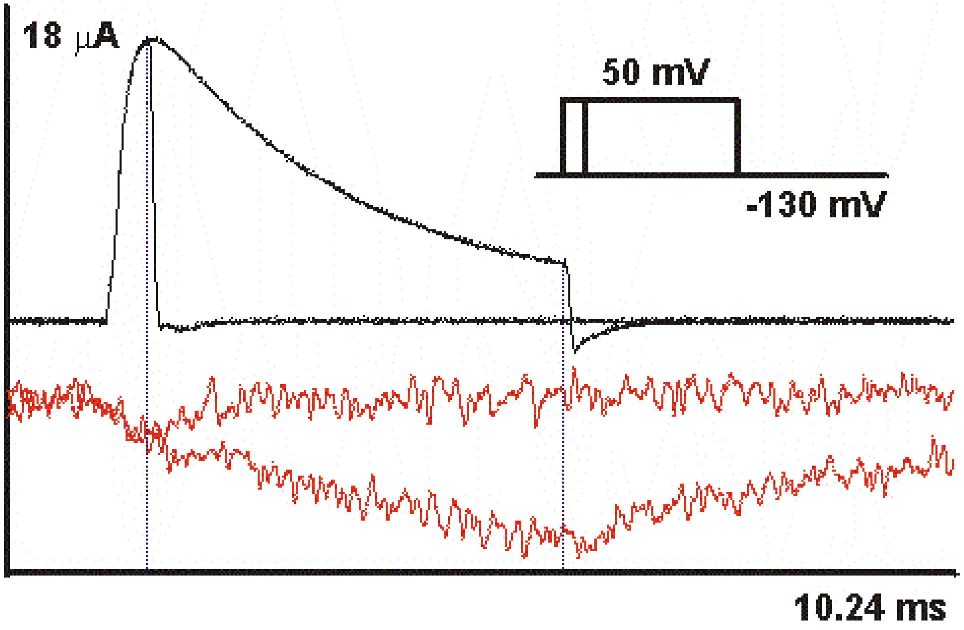


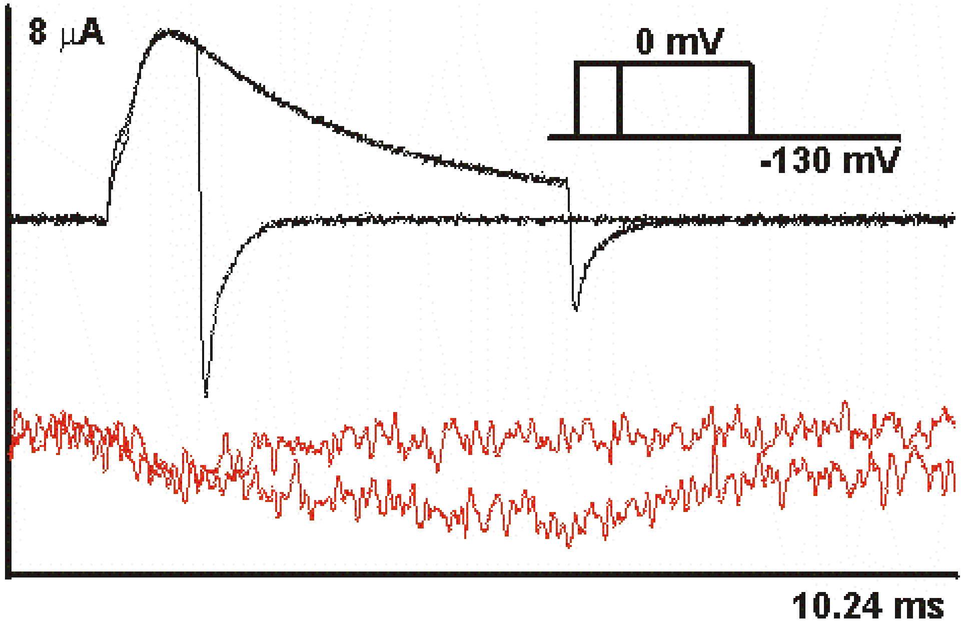
Fast component immobilized by inactivation in Domain IV

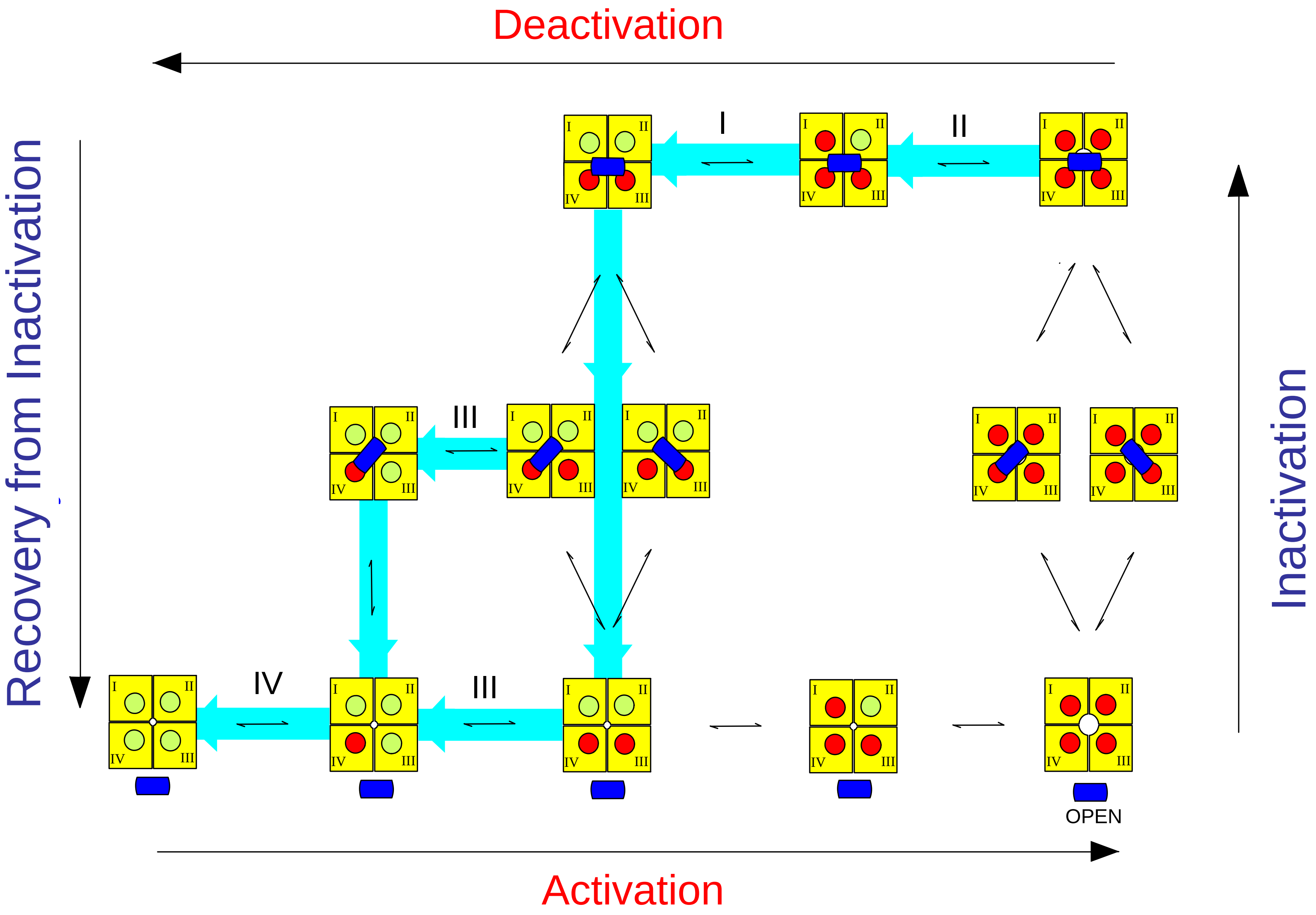


Domain IV (R1448C)

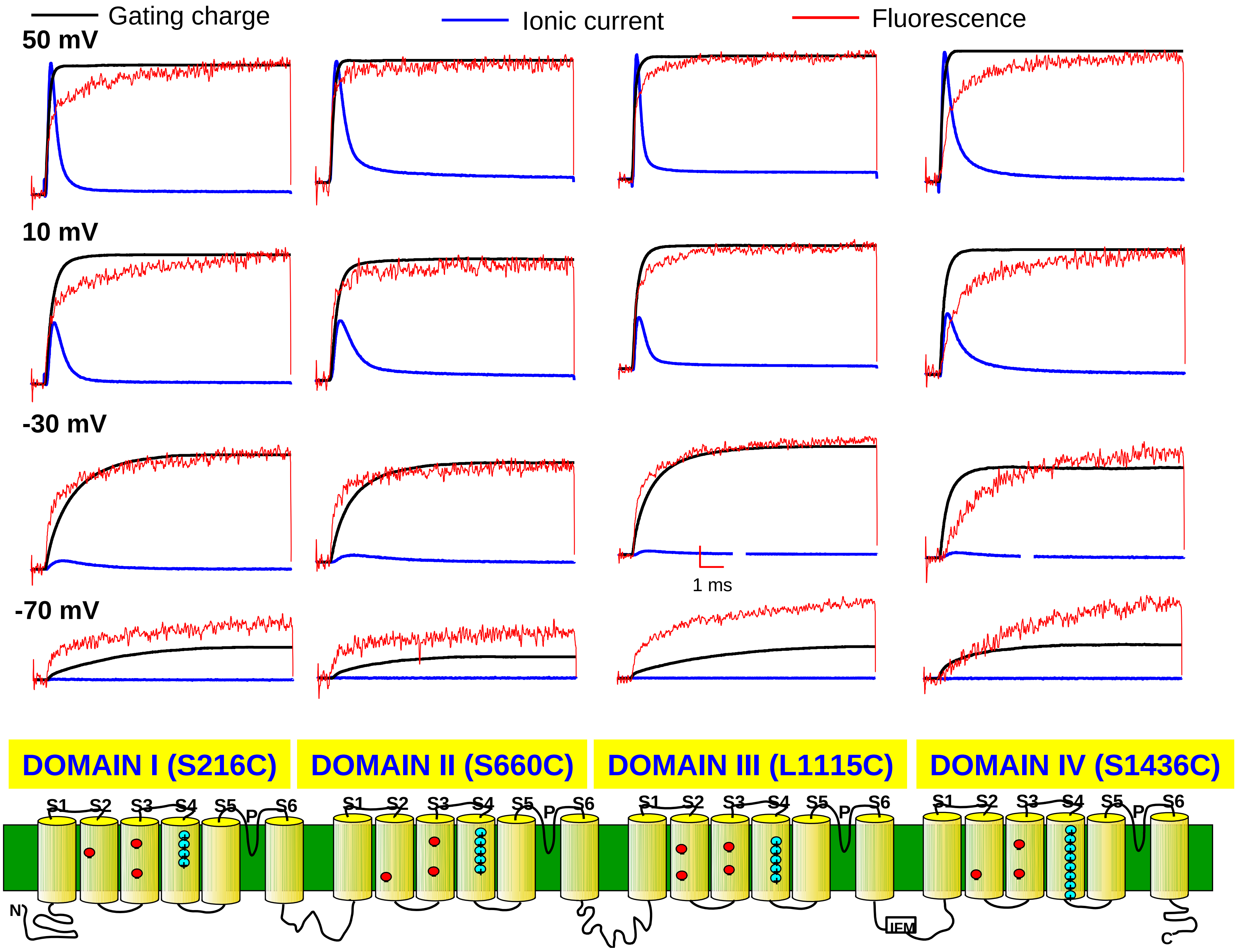




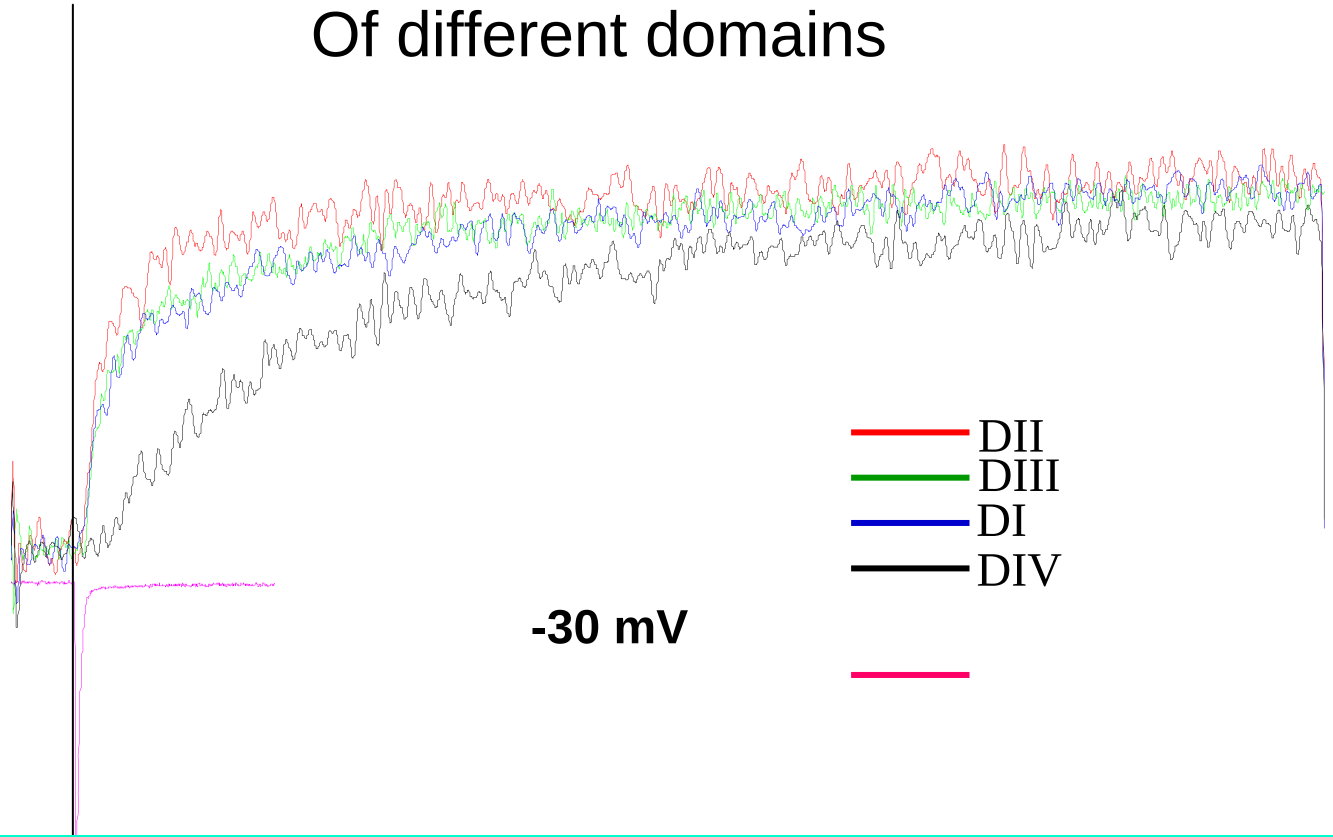



Deactivation
I
II
III
IV
III
OPEN
Activation
Recovery from Inactivation
Inactivation

Comparison of fluorescence, gating and ionic traces of labeled mutants

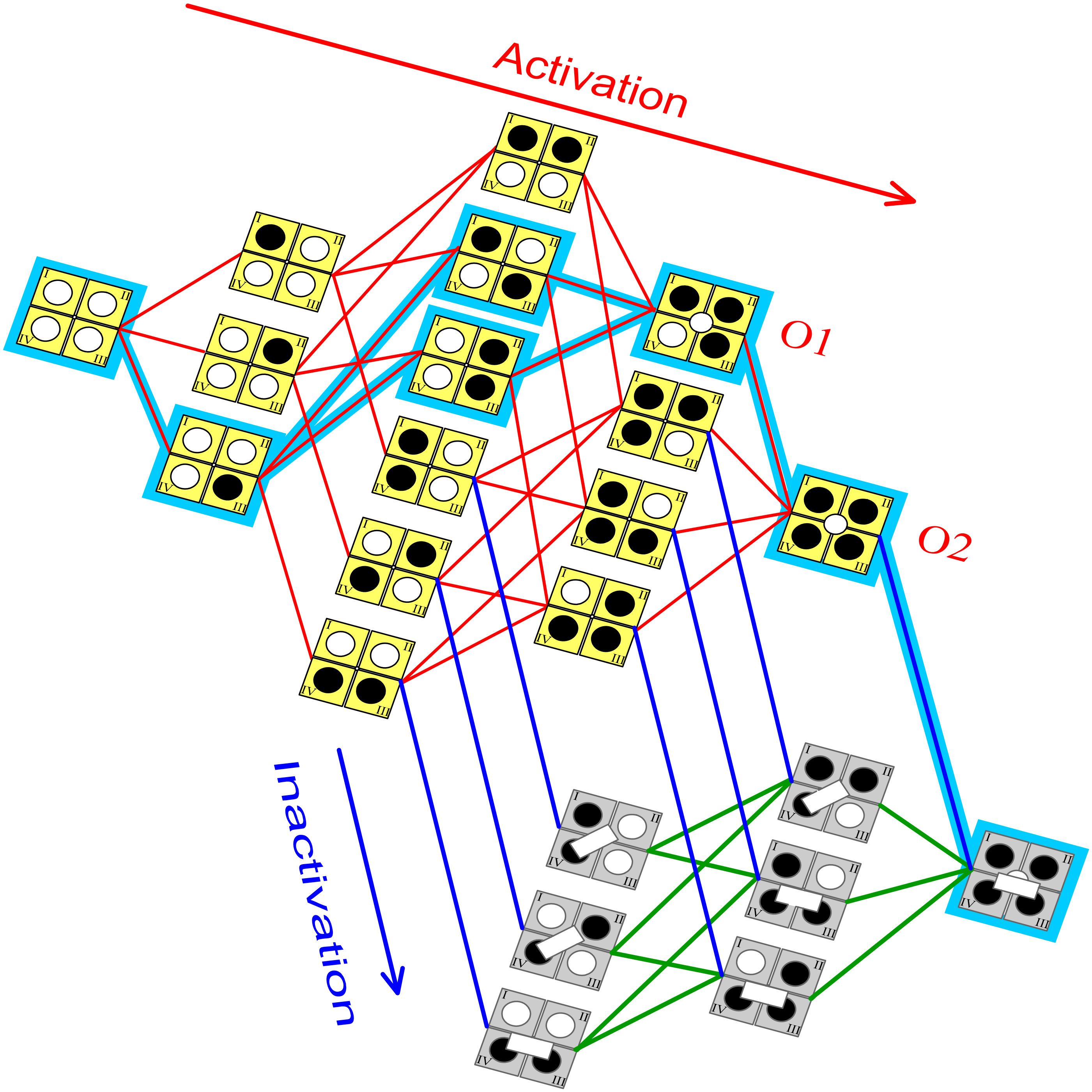


Time course of F signal
Of different domains

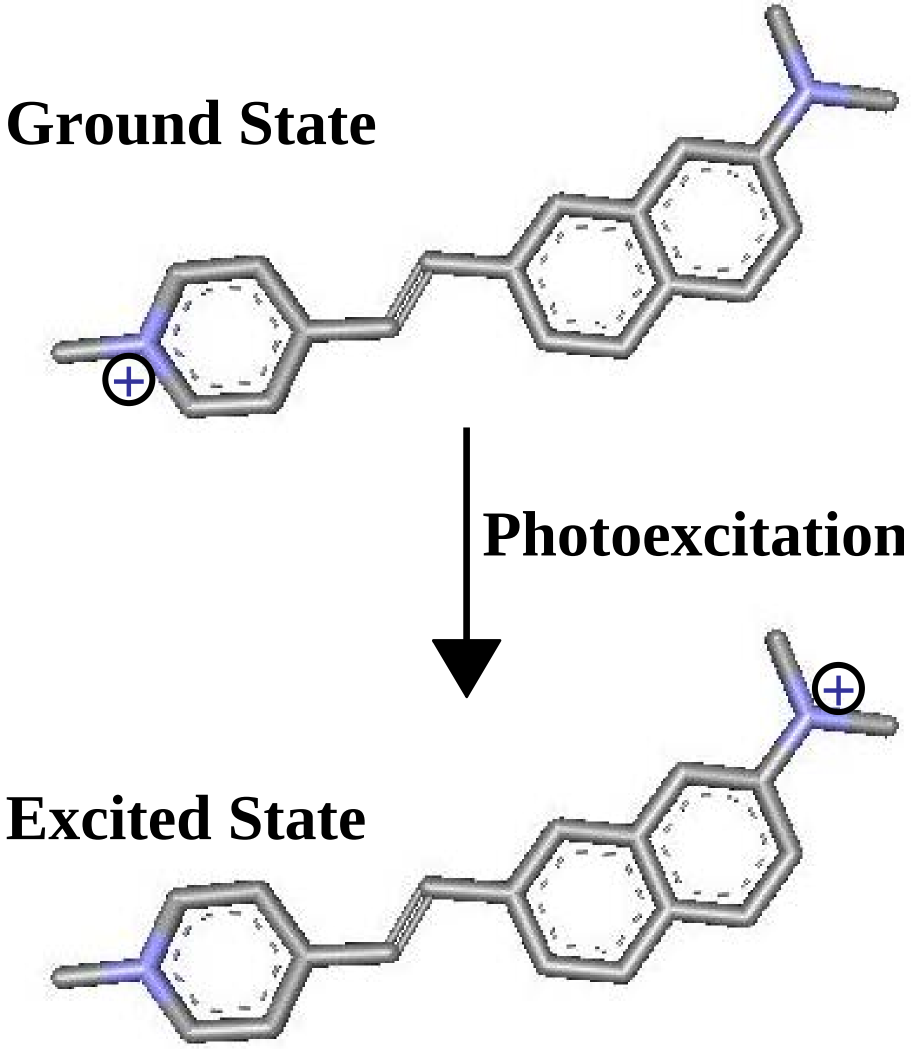


The S4s of the first three domains do not activate in a specific sequence.

The activation of S4 in domain IV requires a prior activation of one of the three S4 segments.


Activation
O1
O2
Inactivation
I
II
III
IV

Electrochromic Effect



Ground State

Photoexcitation

Excited State

Electrochromic Shift

$$\Delta 1/\lambda = \Delta 1/\lambda^{\mathrm{solv}} - (\mu_e - \mu_g)\mathbf{E}\ \mathrm{Cos}\,\alpha$$

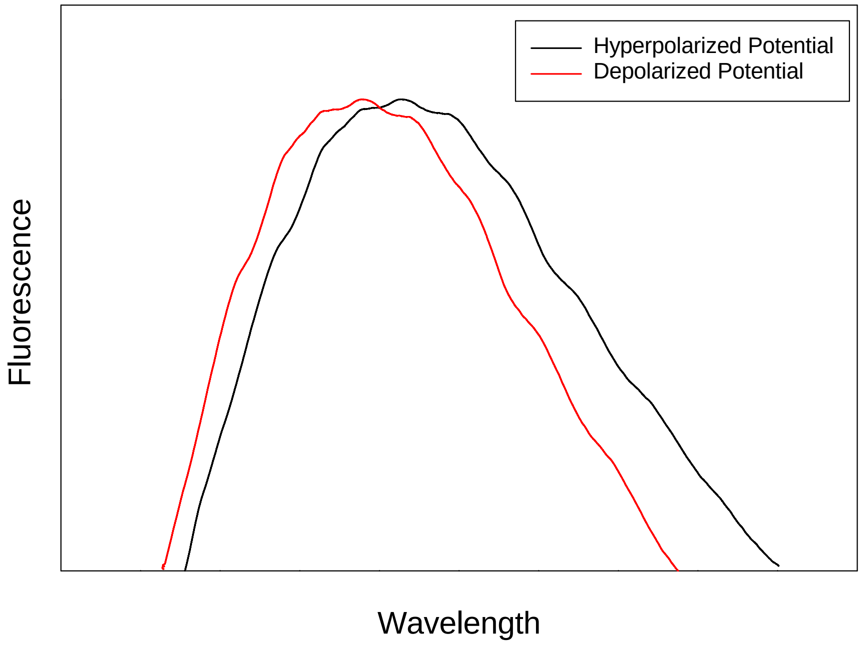
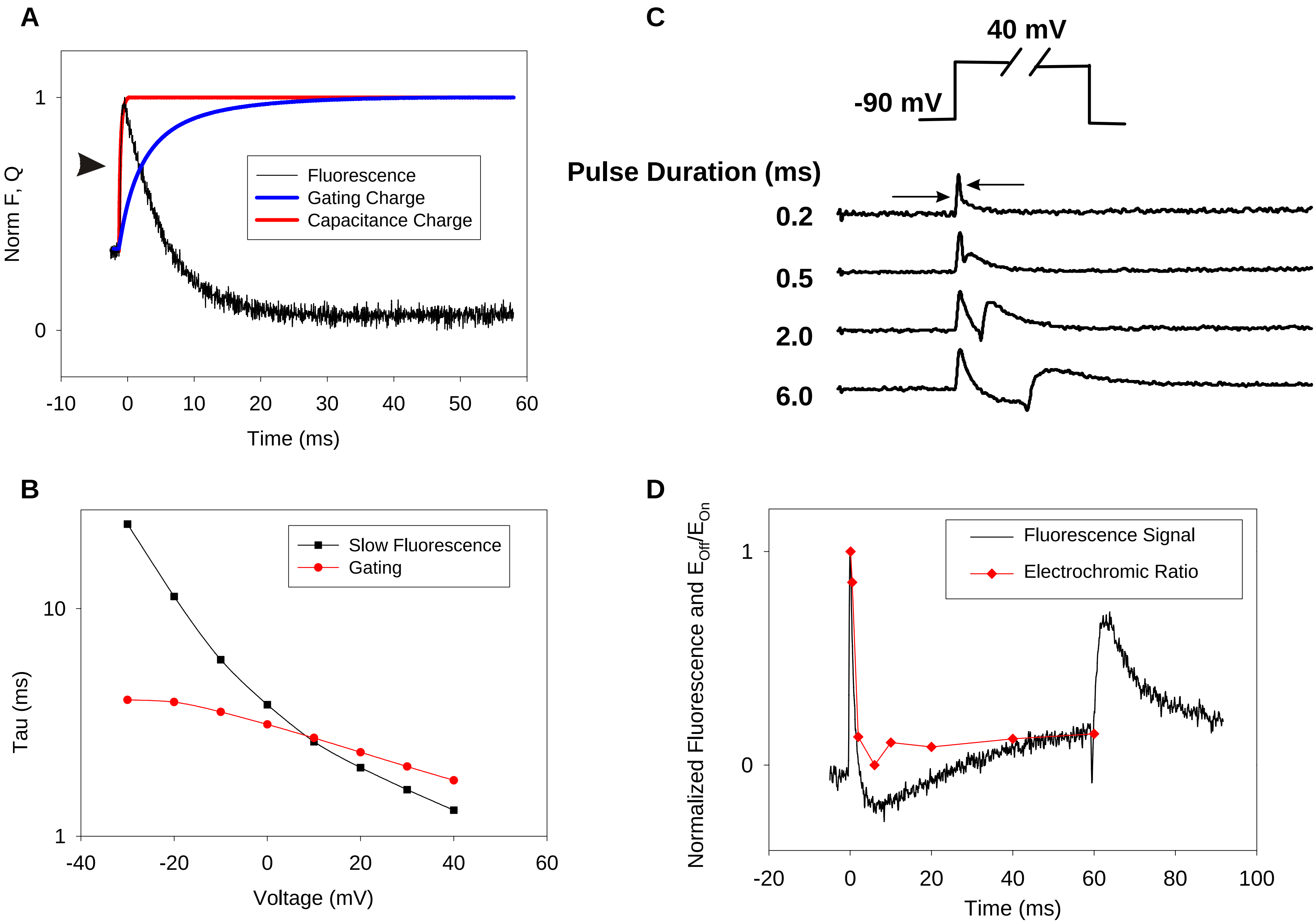
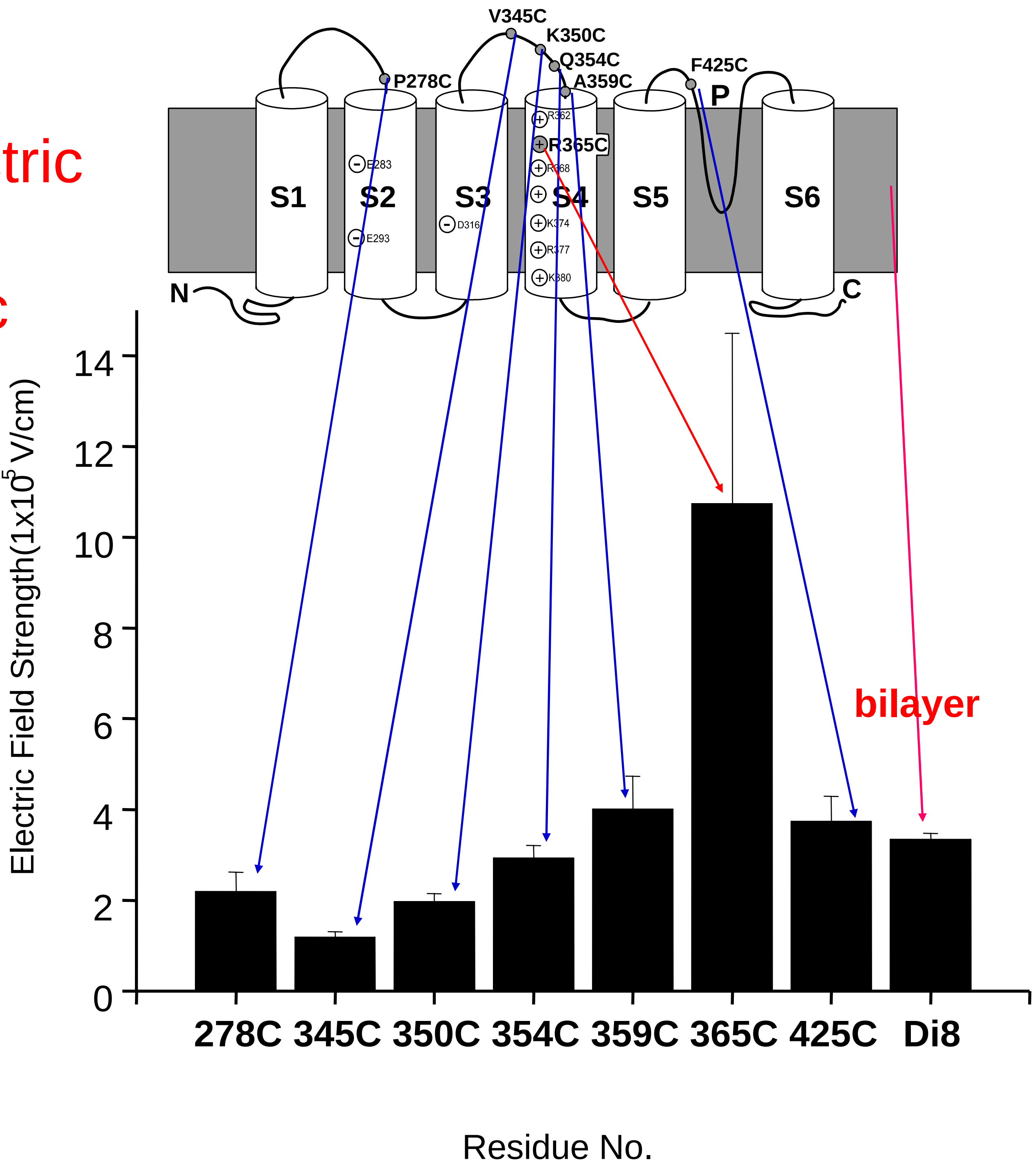


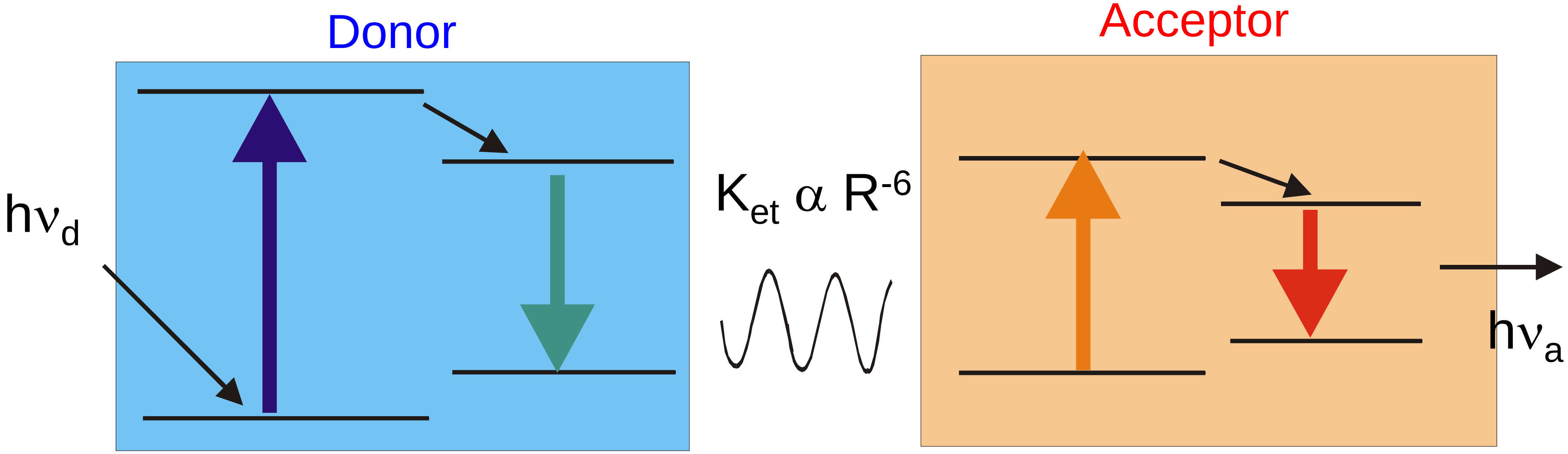
Figure 3

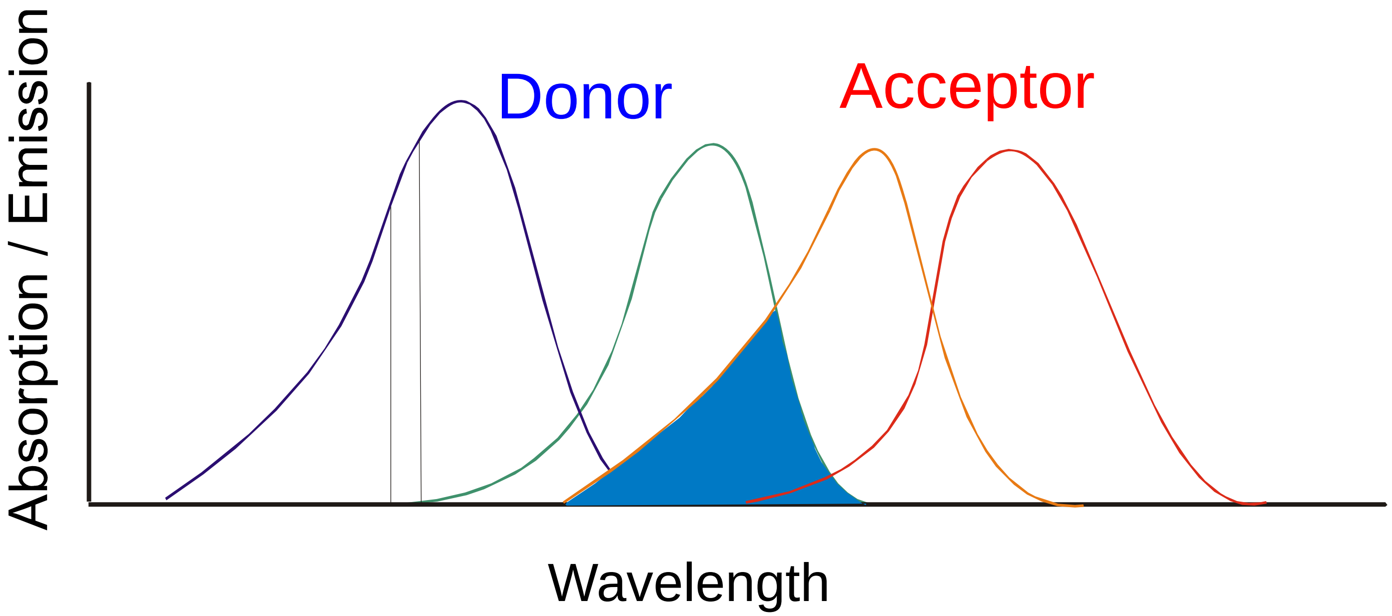

A
Norm F, Q
1
0
-10
0
10
20
30
40
50
60
Time (ms)
Fluorescence
Gating Charge
Capacitance Charge
C
40 mV
-90 mV
Pulse Duration (ms)
0.2
0.5
2.0
6.0
B
Tau (ms)
10
1
-40
-20
0
20
40
60
Voltage (mV)
Slow Fluorescence
Gating
D
Normalized Fluorescence and E_{Off}/E_{On}
1
0
-20
0
20
40
60
80
100
Time (ms)
Fluorescence Signal
Electrochromic Ratio

Mapping the electric Field with electrochromic probes



FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET)





$$E=k_{ET}/(k_{ET}+k_n)$$

Forster theory:

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

Where

$$R_0=8.79 \times 10^{-5} (J_D\ q_D\ n^{-4}\ \kappa^2)^{1/6}$$

Then,

$$R=R_0[(1-E)/E]^{1/6}$$

$$R=R_o[\tau_{DA}/(\tau_D-\tau_{DA})]^{1/6}$$

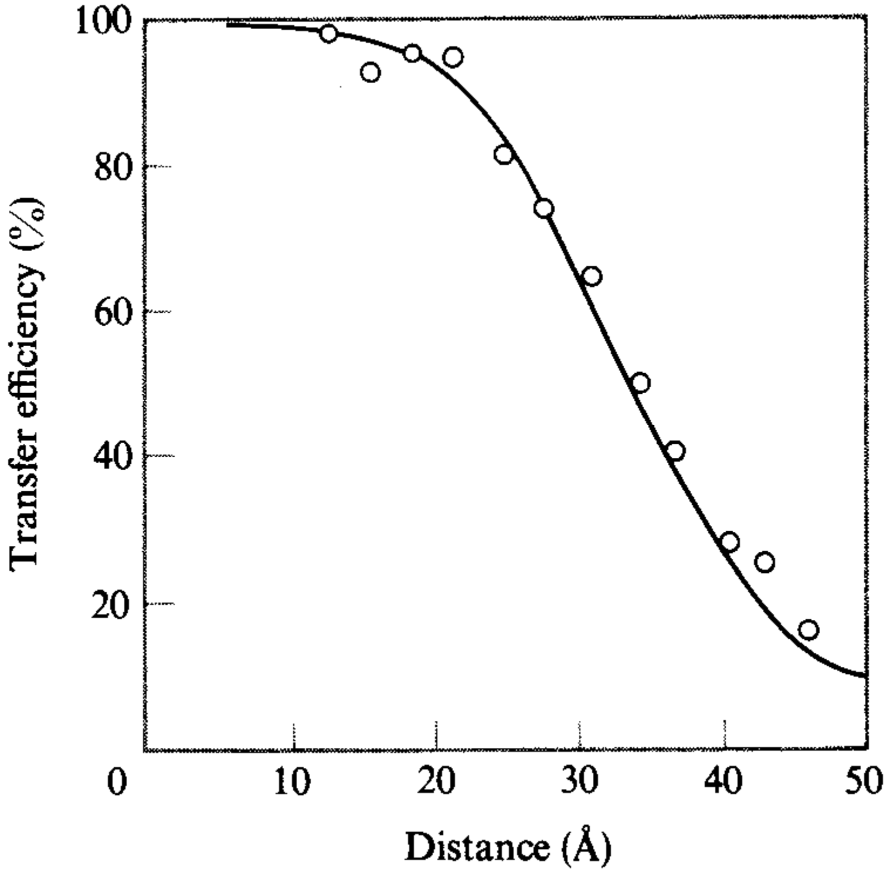


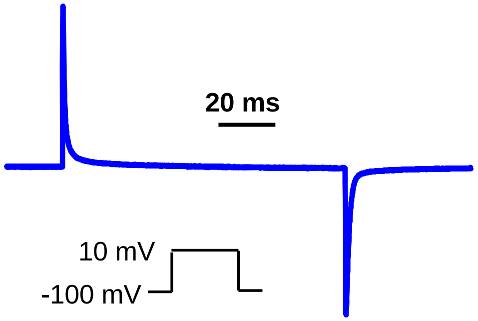
Figure 8-20

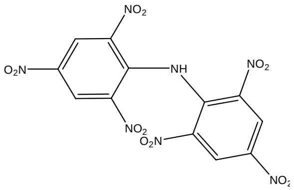
Efficiency of energy transfer as a function of distance in dansyl-(L-prolyl)$_n$-α-naphthyl semicarbazide oligomers with $n = 1$ to 12. The curve was fit to the data with Equation 8-57. [From L. Stryer and R. P. Haugland, *Proc. Natl. Acad. Sci. USA* 98:719 (1967).]

Another method: Using hydrophobic ions as FRET acceptors (reference) in the bilayer

Gating current trace of

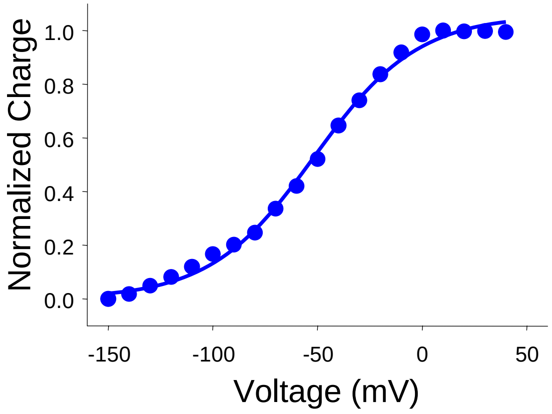
Dipicrylamine (dpA)

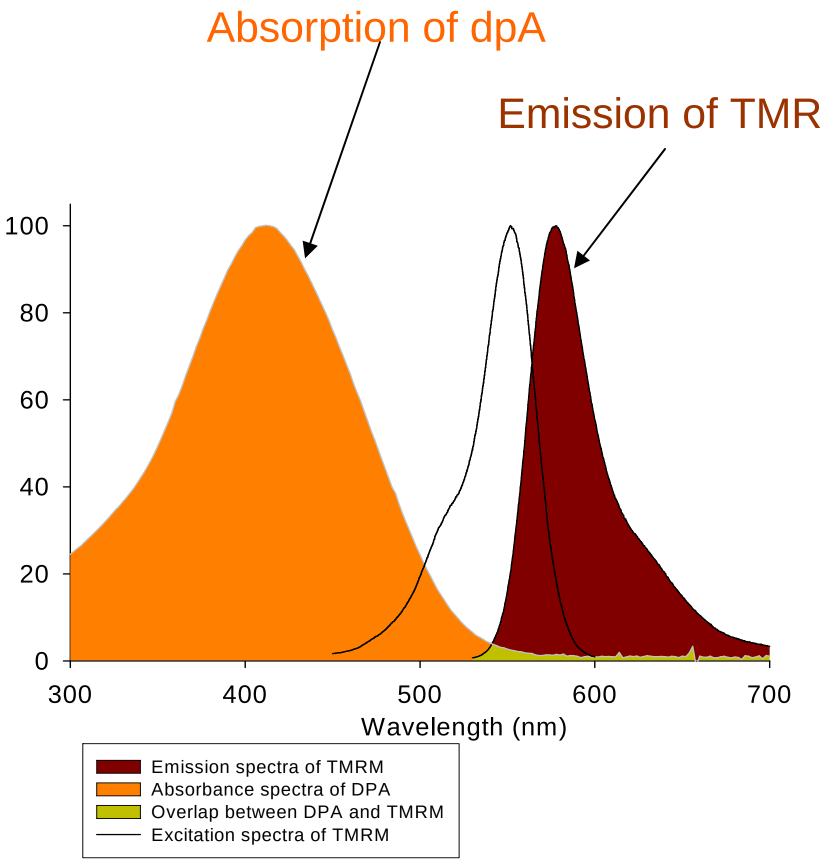




Dipicrylamine

Charge voltage relationship of dpA in oocyte membranes

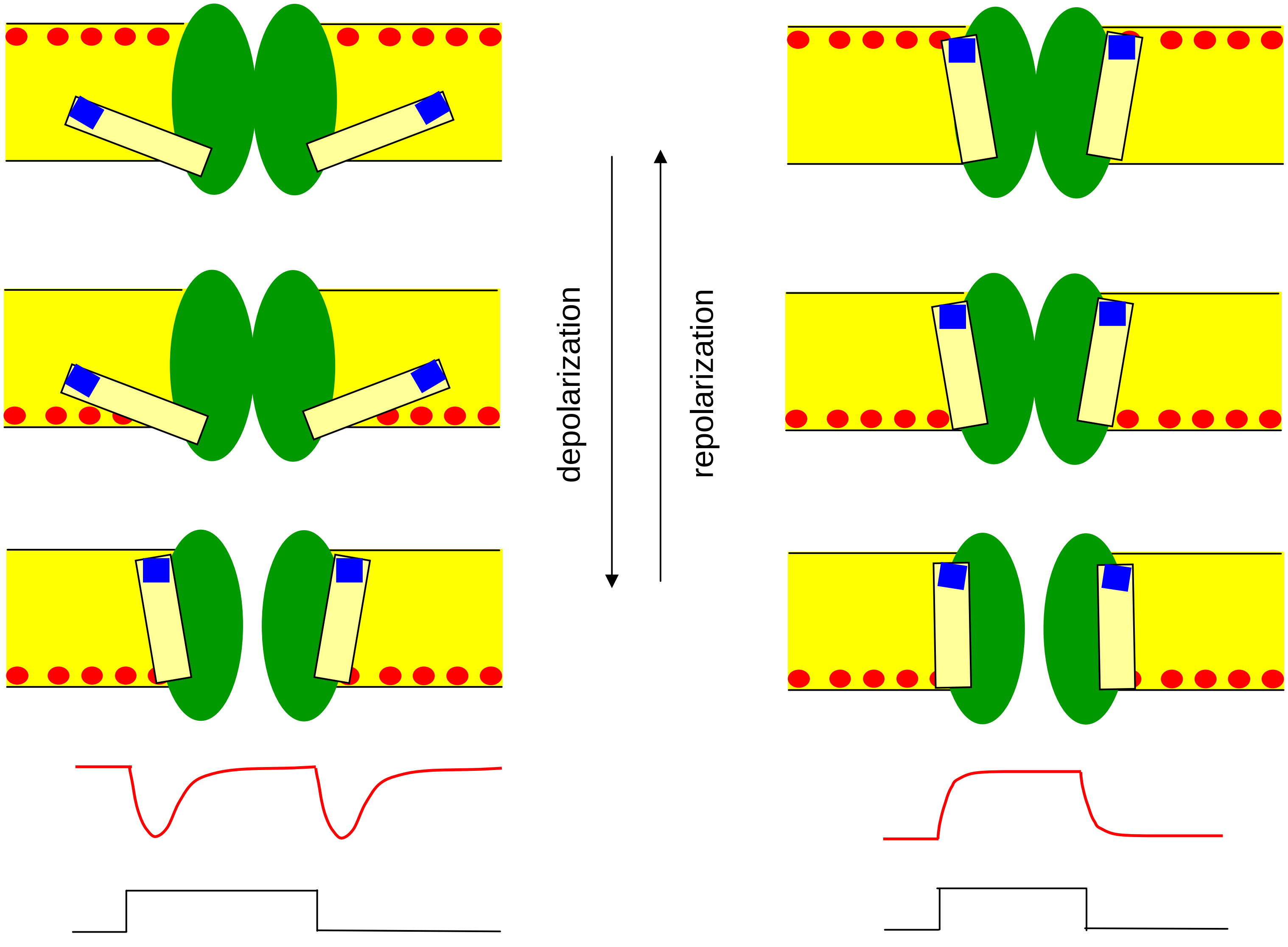


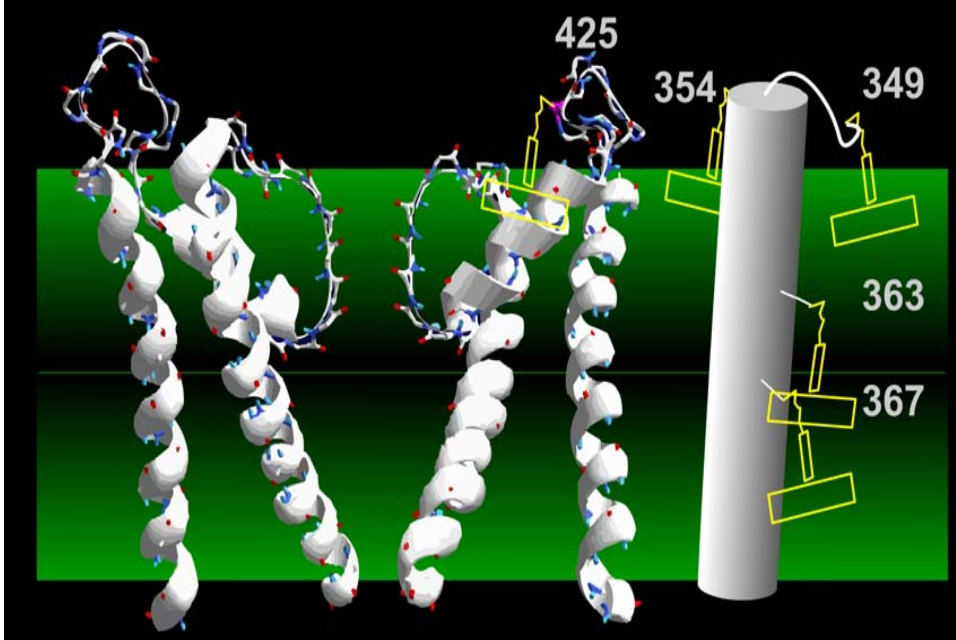


Calculated R_o between TMR and dpA is 20 A

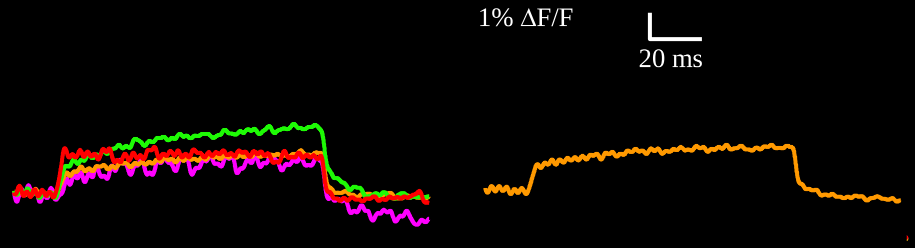
Large movement

Small movement





363C - ABD



average

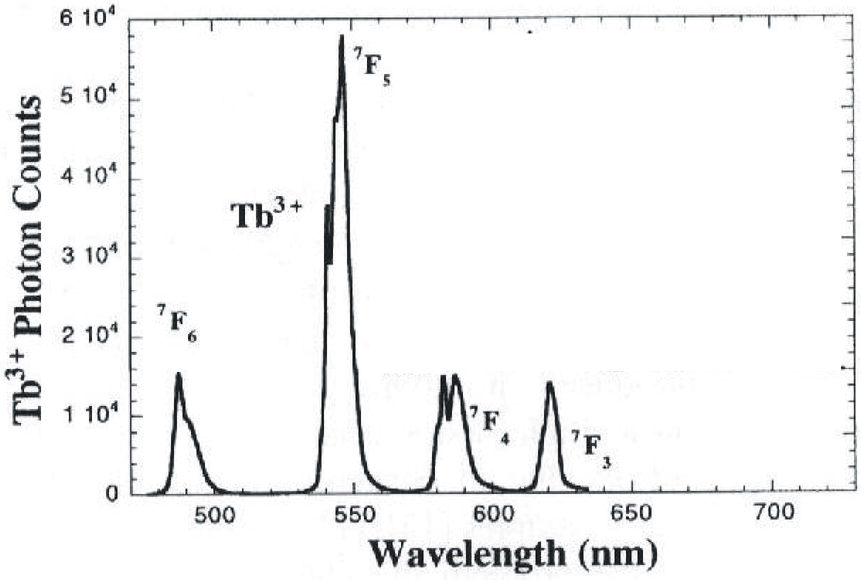
Lanthanide-based Resonance Energy Transfer (LRET)

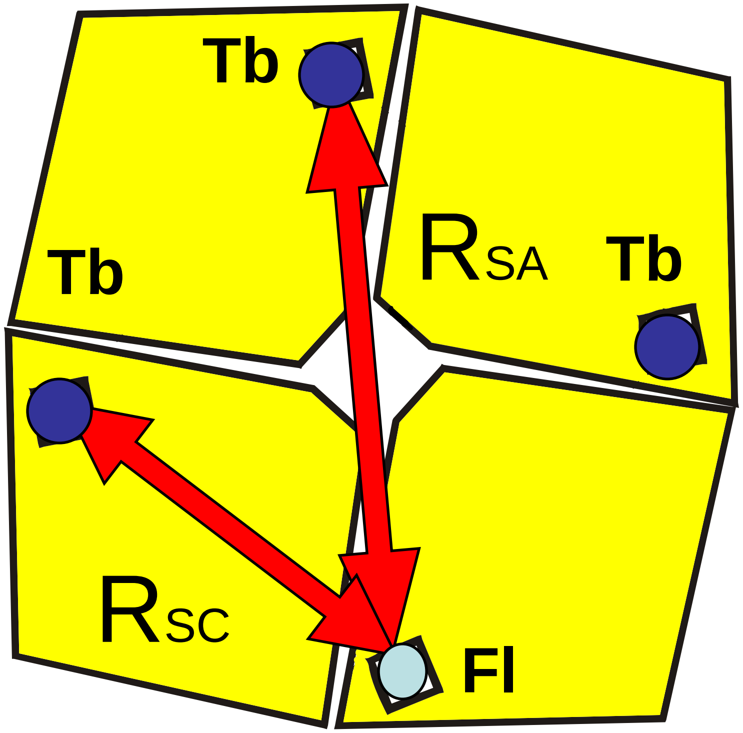
LRET utilizes luminescent lanthanides (i.e. Tb^{3+}, Eu^{3+}) with sensitizer and reaction group as fluorescent donors

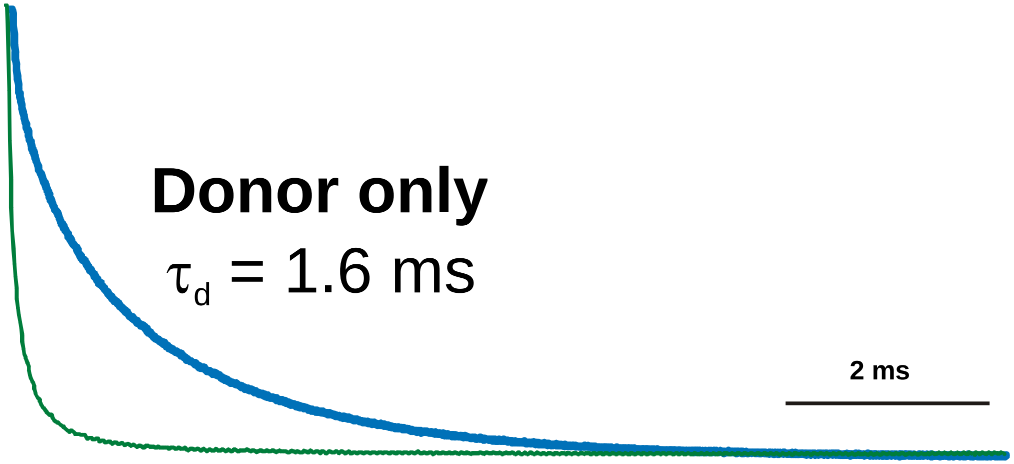
- Lifetimes are easily measurable (τ ~ 0.2 - 1.5 ms)

- Lanthanides fluoresce isotropically, reducing errors from orientation factor κ^2

- Sharply spiked emission spectrum and temporal elimination of direct acceptor excitation enables specific measurements of acceptor sensitized emission

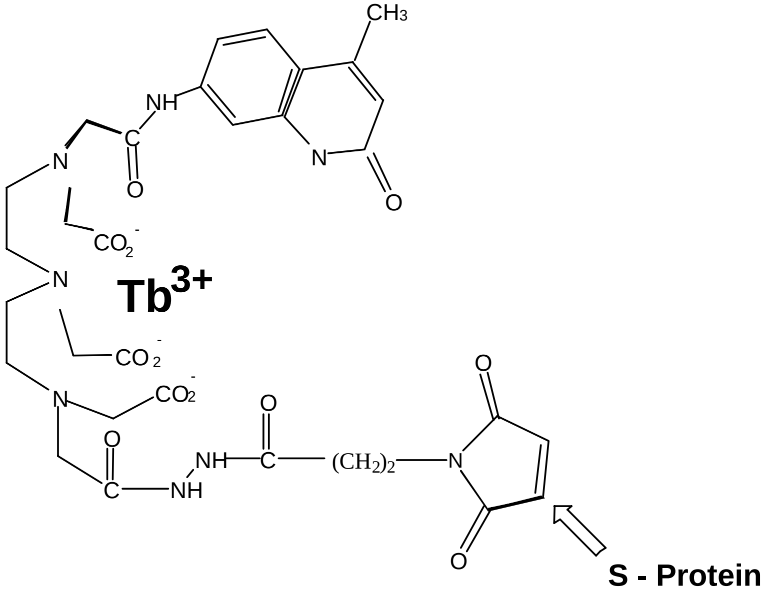






Donor only

τ_d = 1.6 ms



Sensitized emission

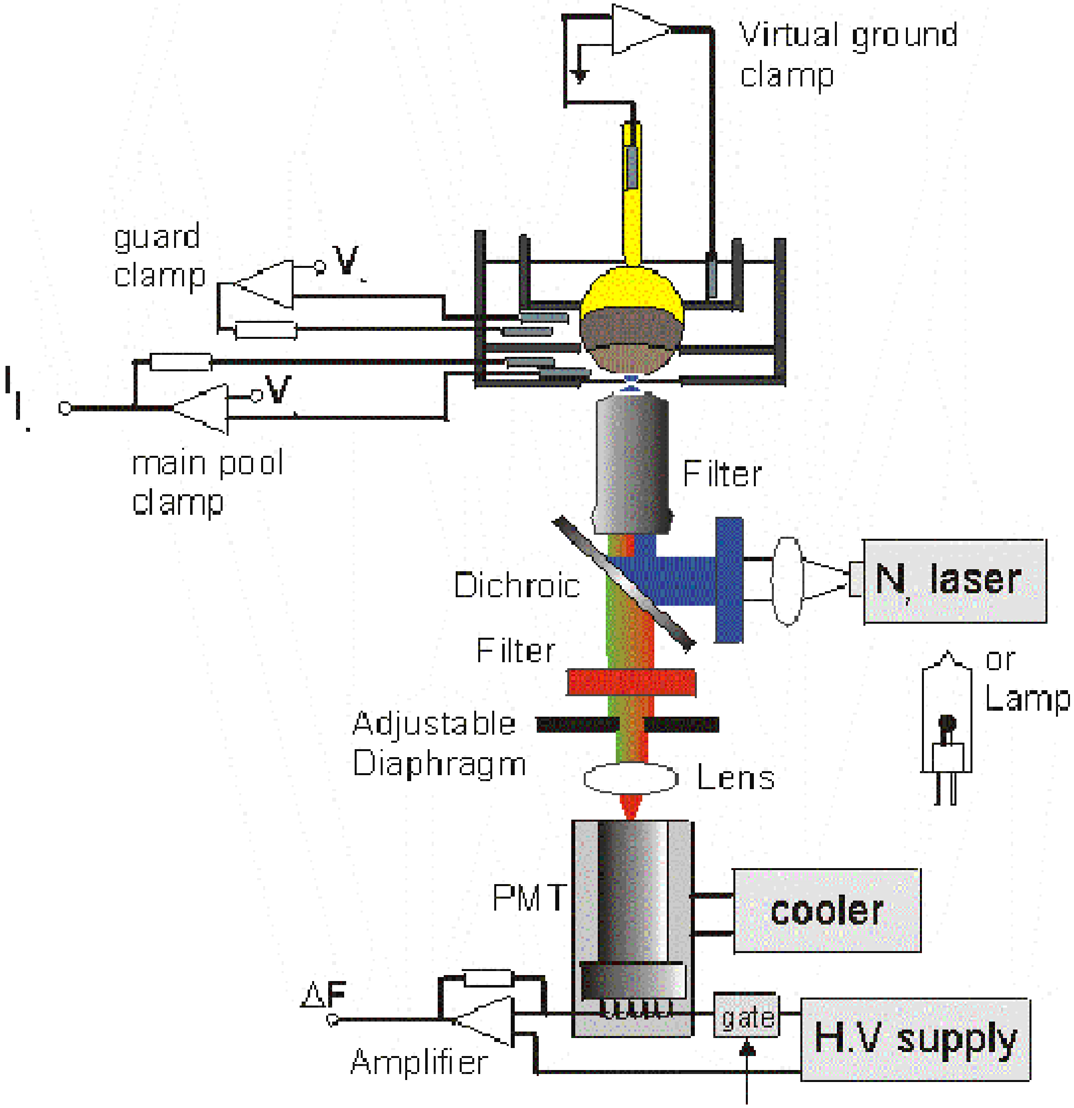
τ_{ad} = 92 µs, 566 µs

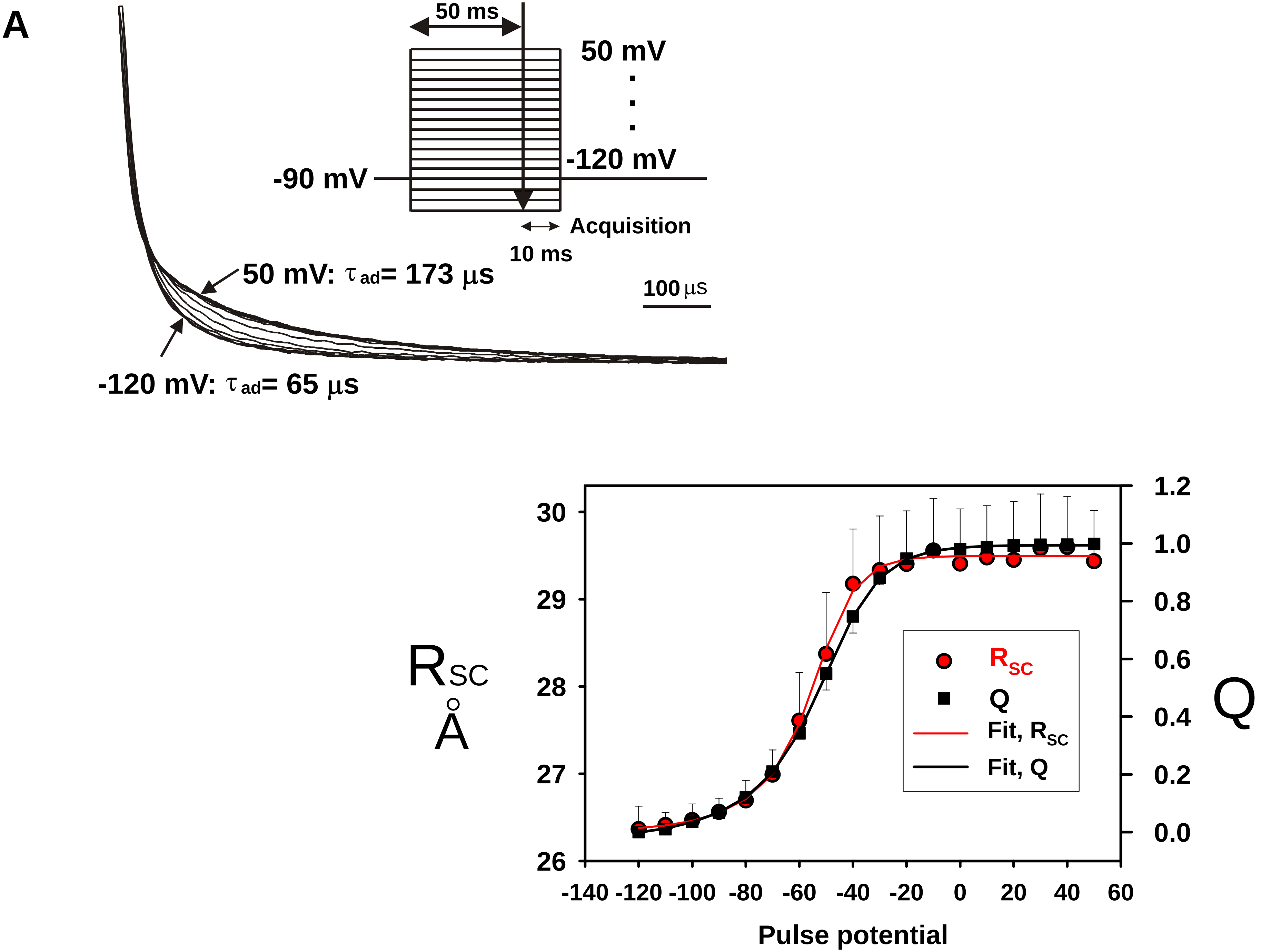
R_{SC}=28 Å

R_{SA}=41 Å

R_{SA} predicted from R_{SC} using Pithagorean Theorem = 40 A

INVERTED CUT-OPEN FLUORESCENCE SETUP




A
50 ms
50 mV
-120 mV
-90 mV
Acquisition
10 ms
100 µs
50 mV: τad= 173 µs
-120 mV: τad= 65 µs
RSC
Å
Q
30
29
28
27
26
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-140
-120
-100
-80
-60
-40
-20
0
20
40
60
Pulse potential
RSC
Q
Fit, RSC
Fit, Q

The results show that upon depolarization distances from toxin to:

- S4 decrease by 0.8 Å
  - (361and 365 in S4)
- S3-S4 linker decrease by 2 Å
  - (351,352,353)
- S3 Increase by 1 Å
  - (333, 335)

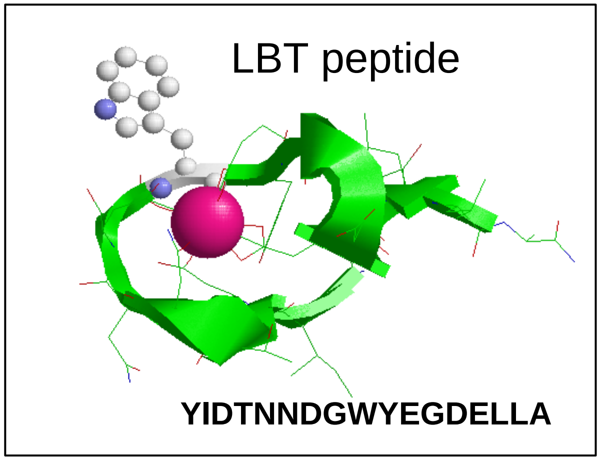
Conclusion:

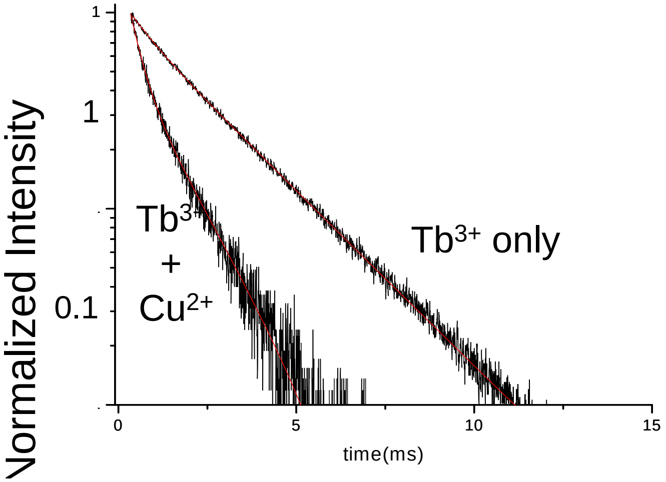
transmembrane displacement is about 2 Å
With S4 and S3 moving in opposite directions

ate

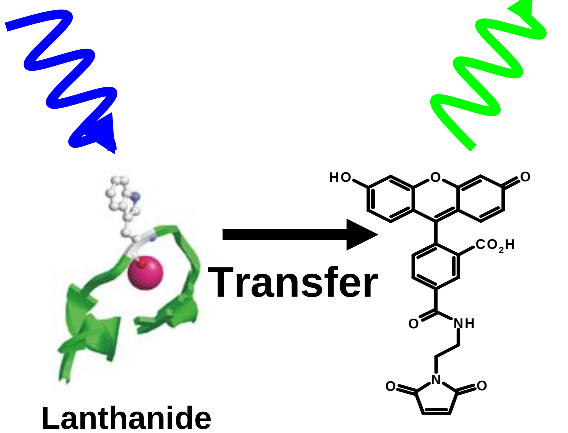
D. Posson, P. Selvin & C. Miller

Genetically encoded LRET



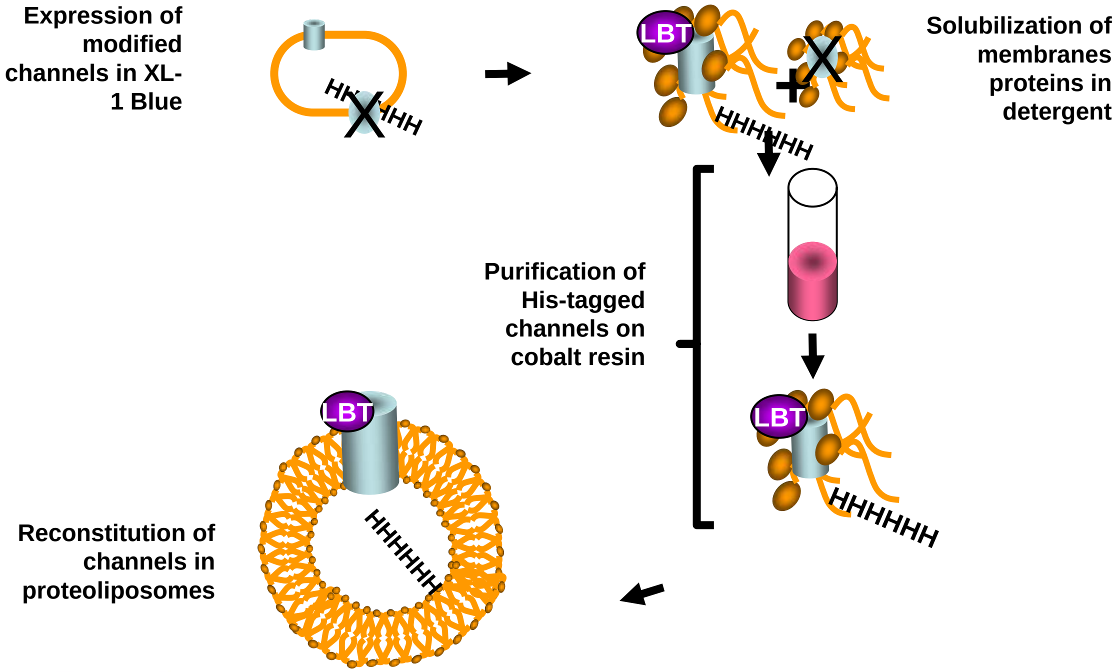


Lanthanide based Transfer-LRET using a genetically encoded tag



Lanthanide Binding Tag

Fluorescein maleimide

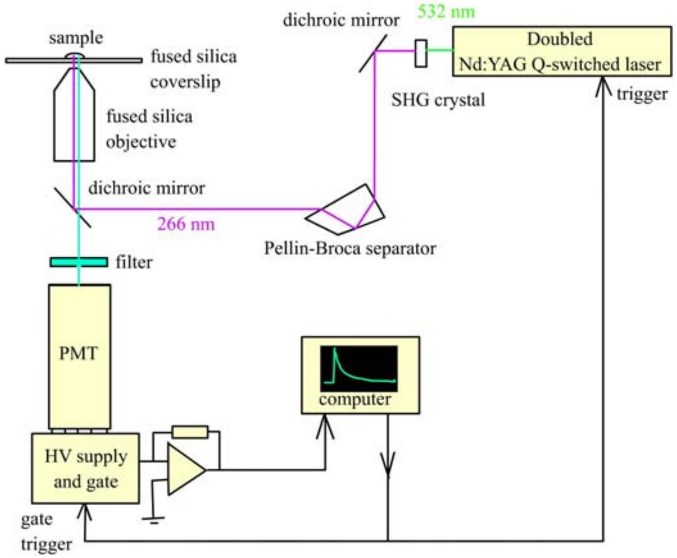


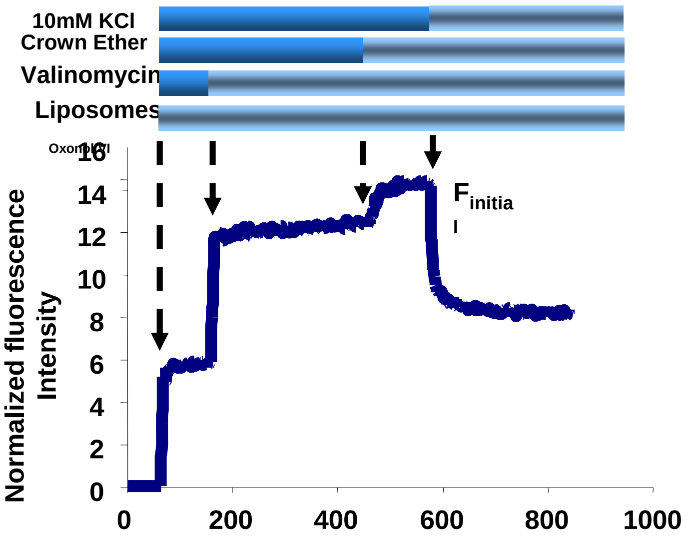
Expression of modified channels in XL-1 Blue

Solubilization of membranes proteins in detergent

Purification of His-tagged channels on cobalt resin

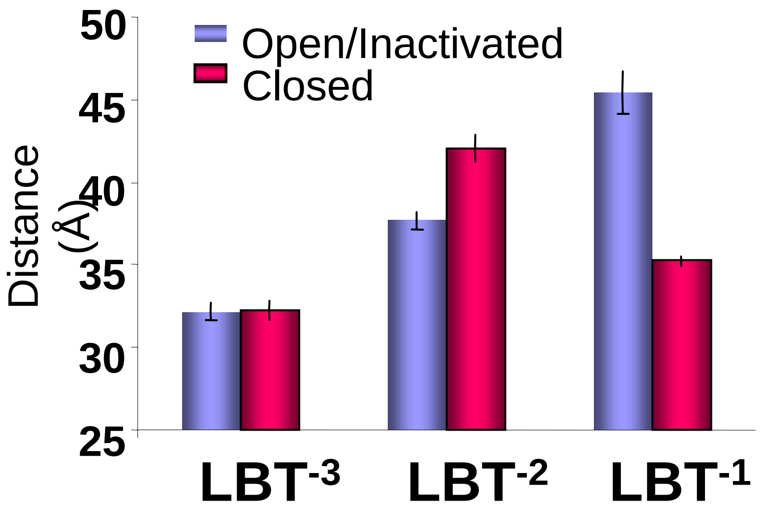
Reconstitution of channels in proteoliposomes



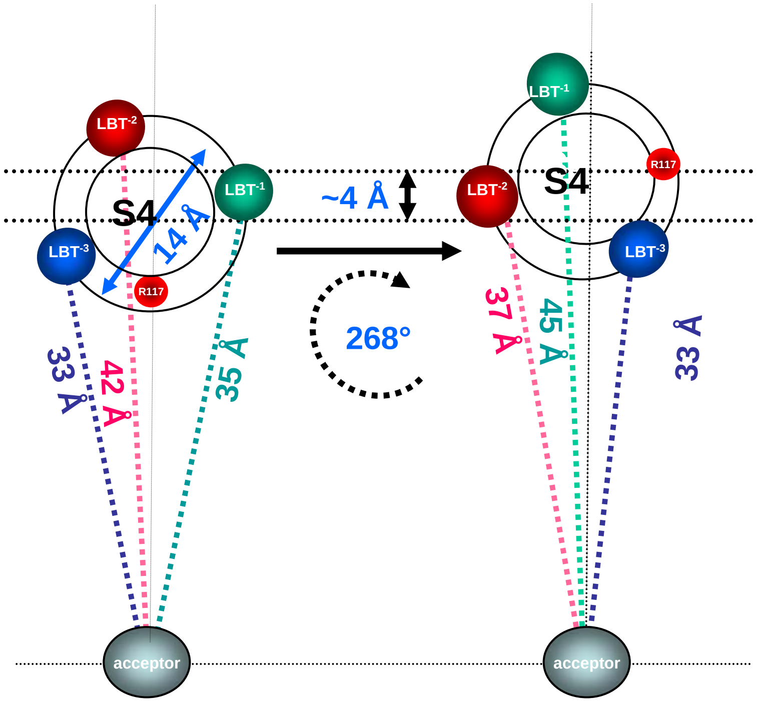


Example:

Demonstration of rotational movement of the voltage sensor of KvAP



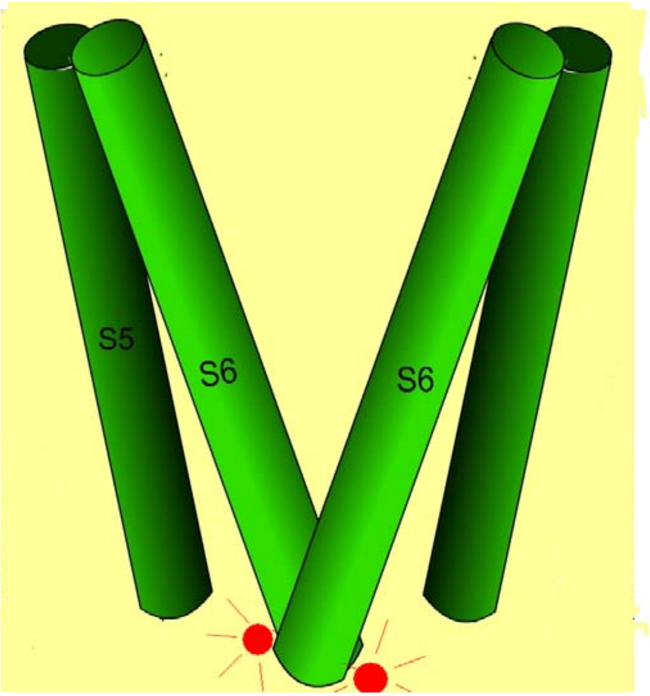
Distance changes in KvAP



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

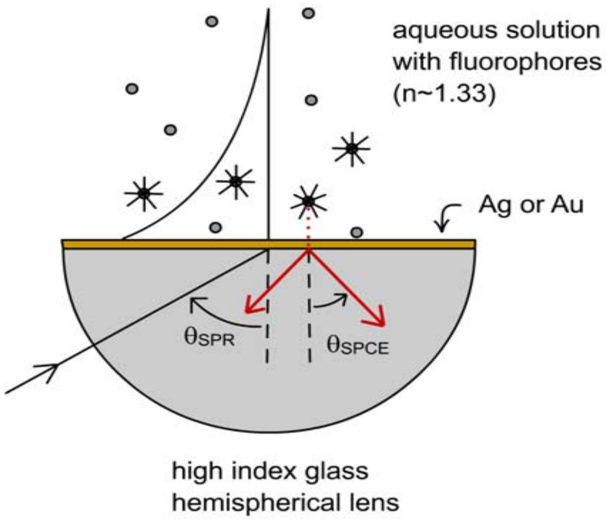


Figure 1. Schematic of the application of surface plasmon resonance to fluorescence in aqueous solution. The evanescent field strength decreases away from the metal surface. Molecules within the evanescent field are excited (stars). However, molecules within approximately 10 nm of the metal surface will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

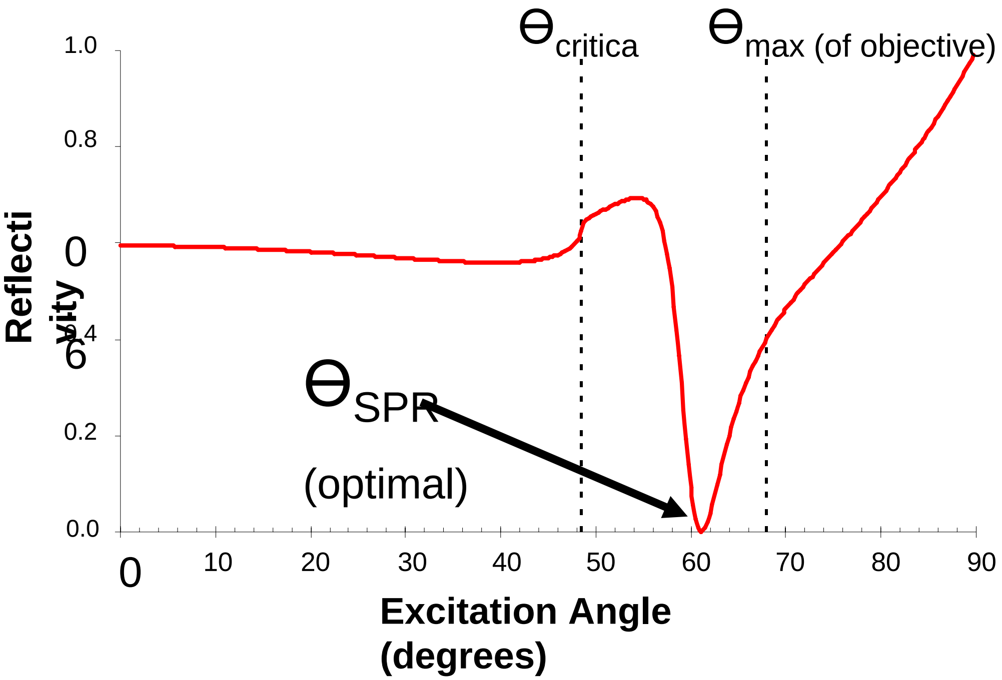
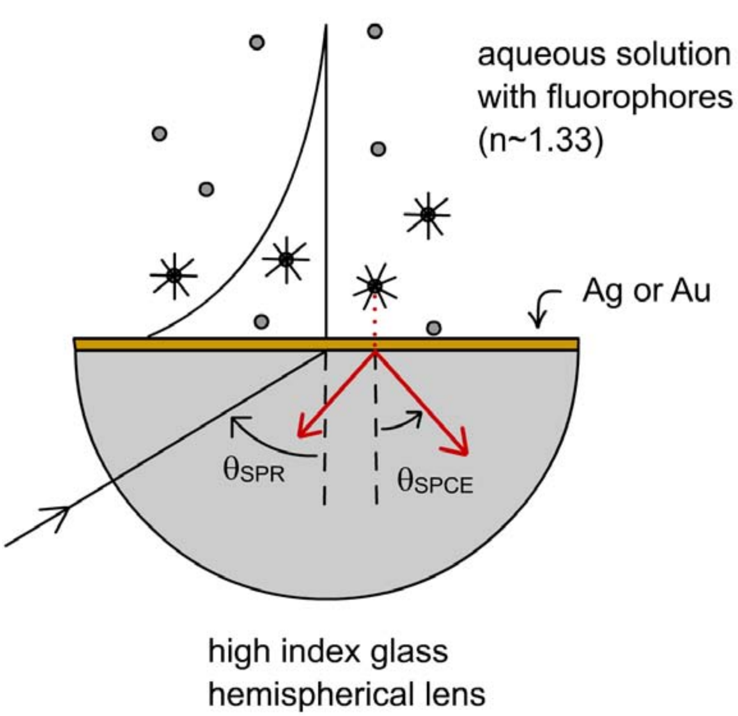
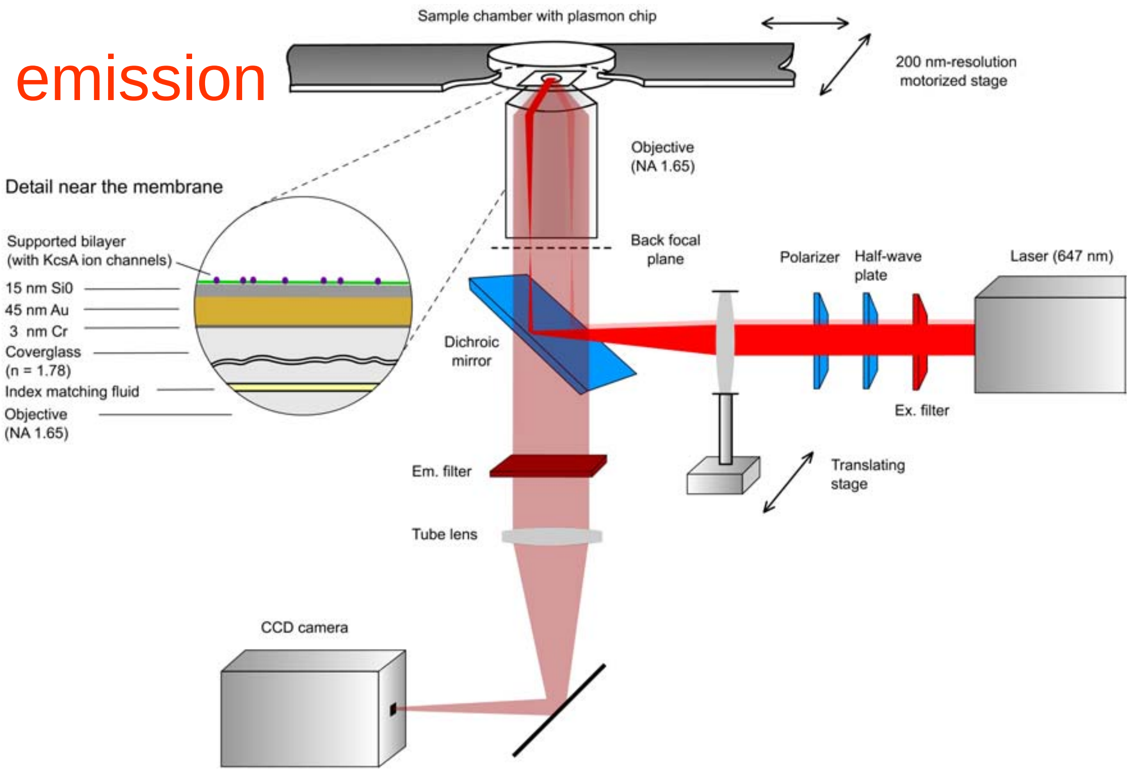


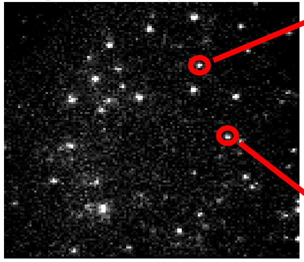
Figure 2. Simulation of reflectance of 647 nm laser excitation off the surface of the plasmon chip. The “dip” is the angle at which photons are most efficiently absorbed and maximally excite surface plasmon. The critical angle for SF11glass at an H_2O interface and the maximum acceptance angle for the Olympus APO 100x NA 1.65 objective are shown as dotted lines.

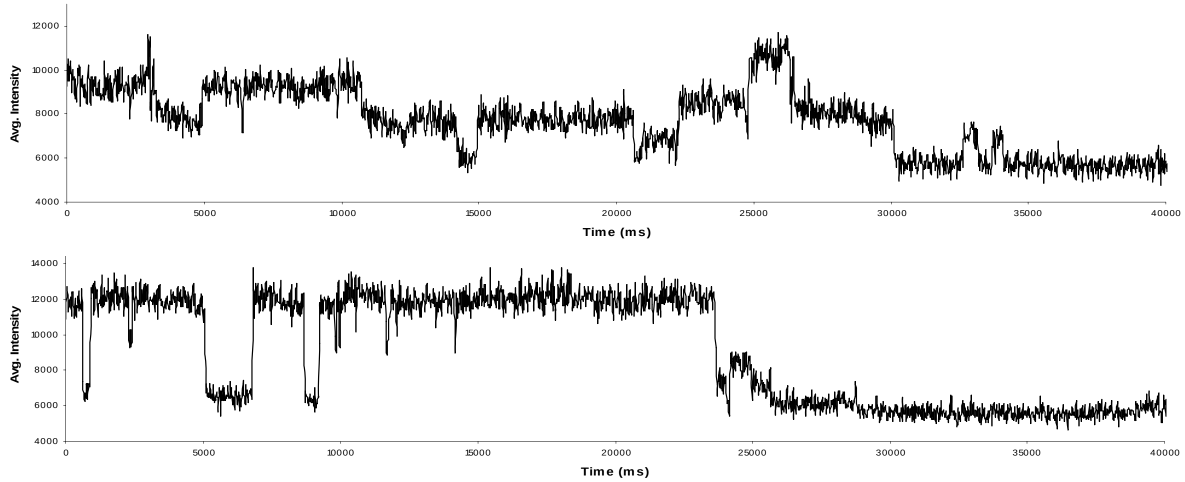
Surface-coupled plasmon emission





Start of





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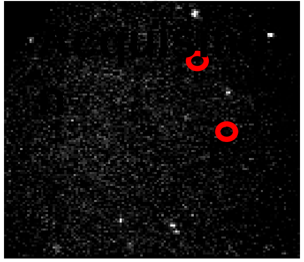
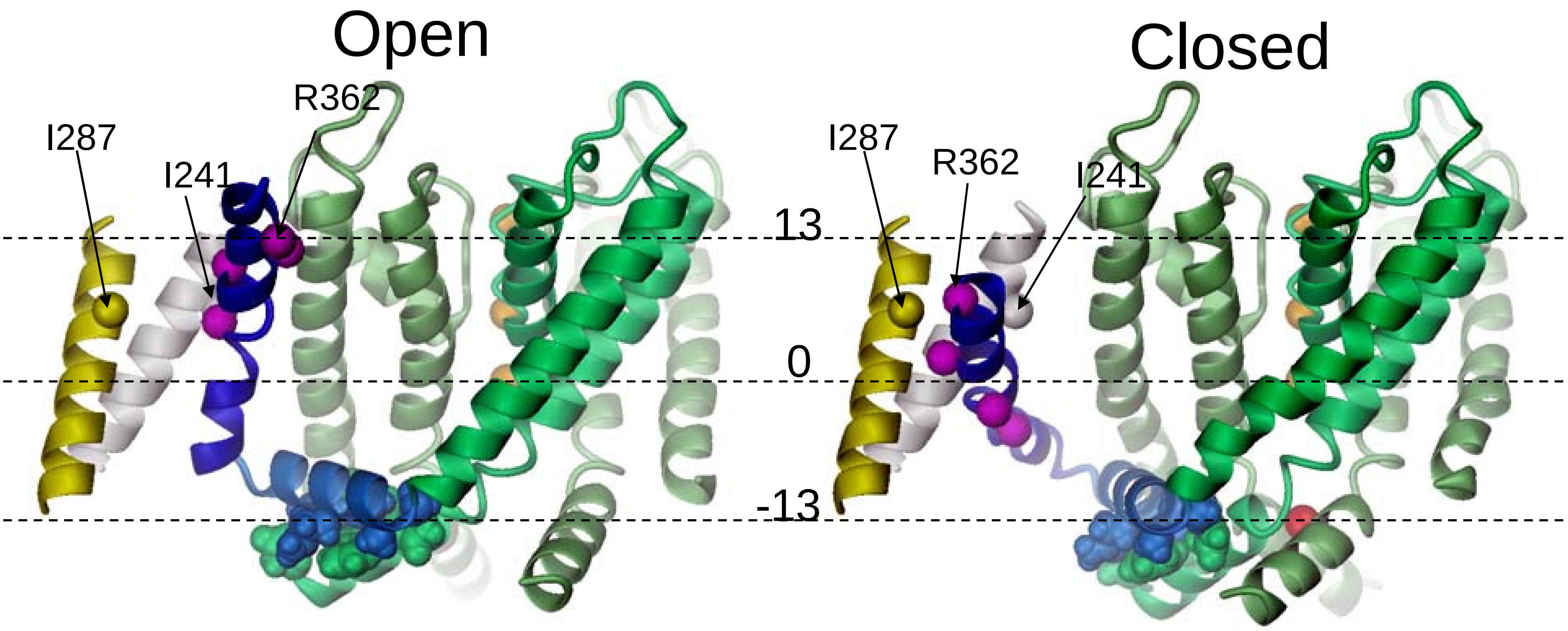
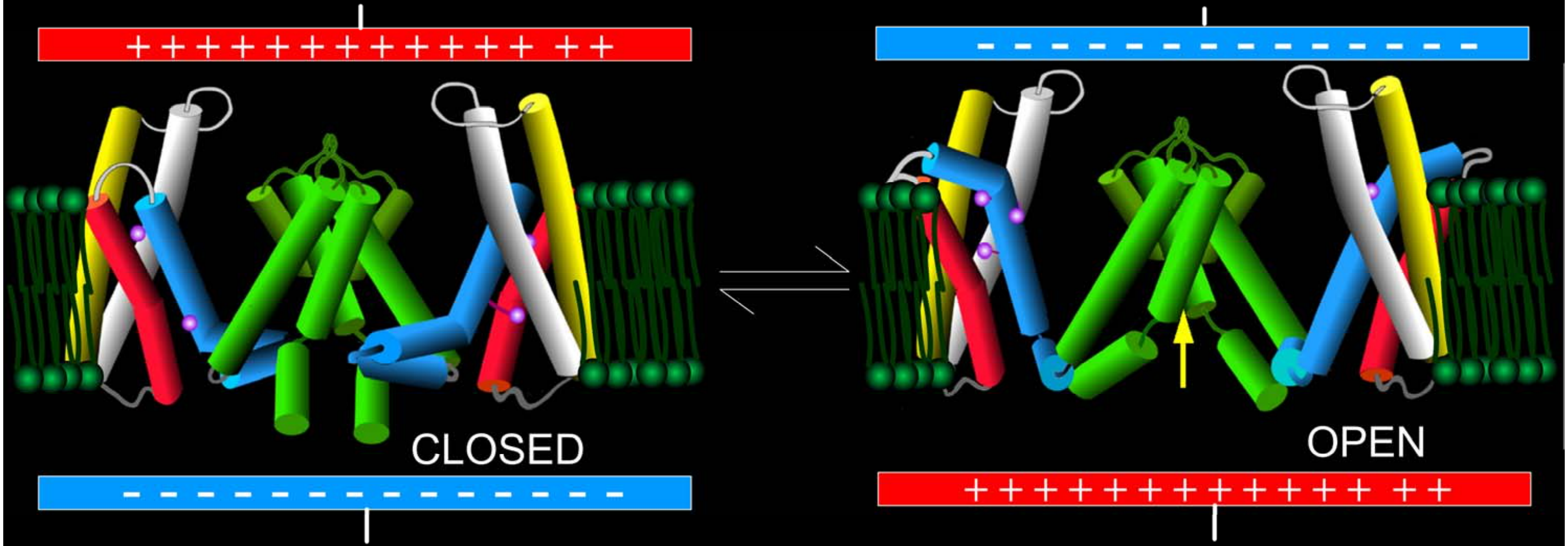


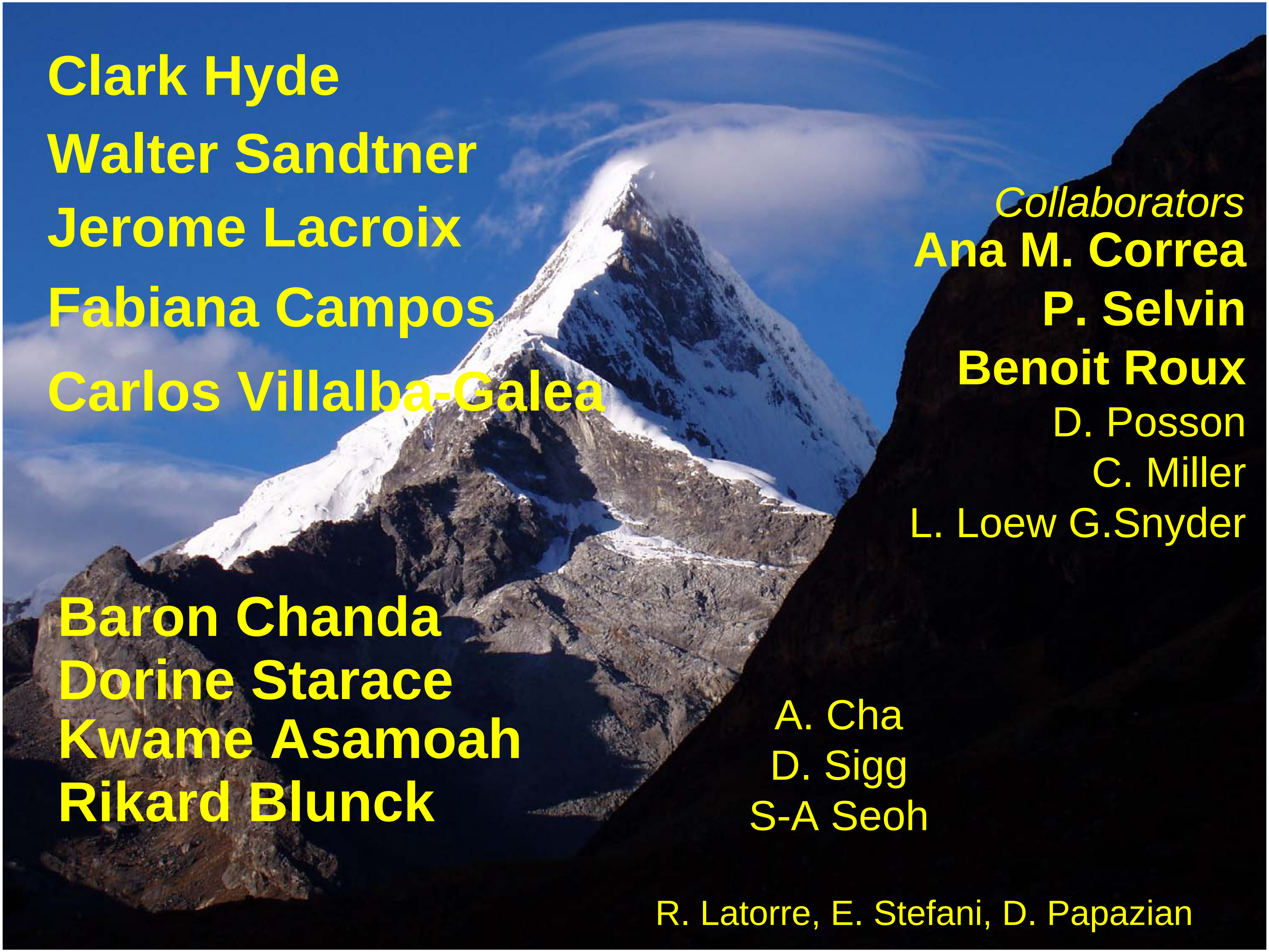
CONCLUSION
The position of S4 at the level of R362 is constrained with respect to S1 and S2 in CLOSED state



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

MINIMAL MODEL


+ + + + + + + + + + + + + + + +
- - - - - - - - - - - - - - -
CLOSED
- - - - - - - - - - - - - -
OPEN
+ + + + + + + + + + + + + + +



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