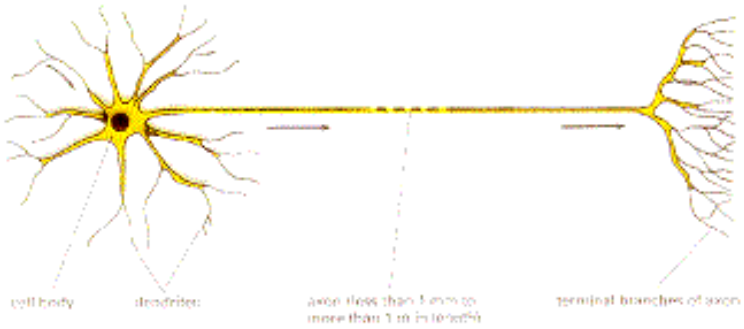
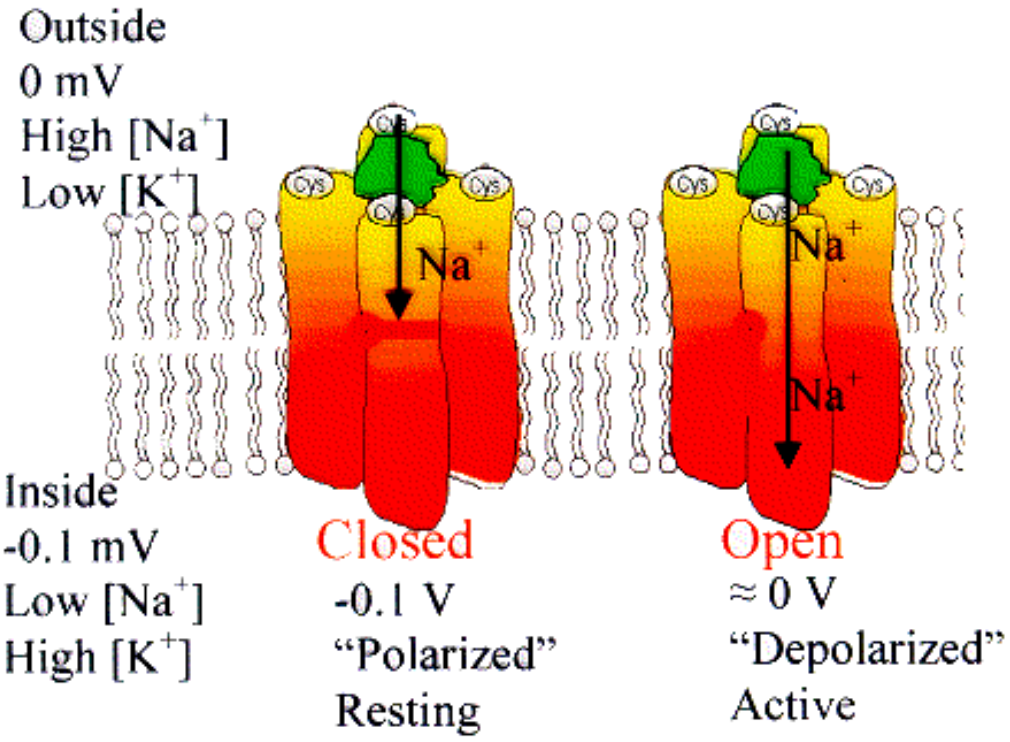
How Ion Channels Move to Create Action Potentials

Nerve cells contain ion channels

Opening/closing of (Na, K) ion channels lead to Action Potential – electrical wave.

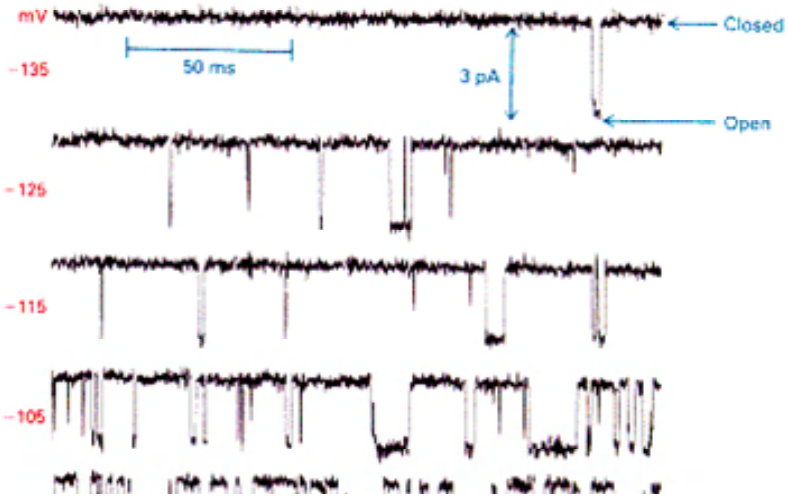




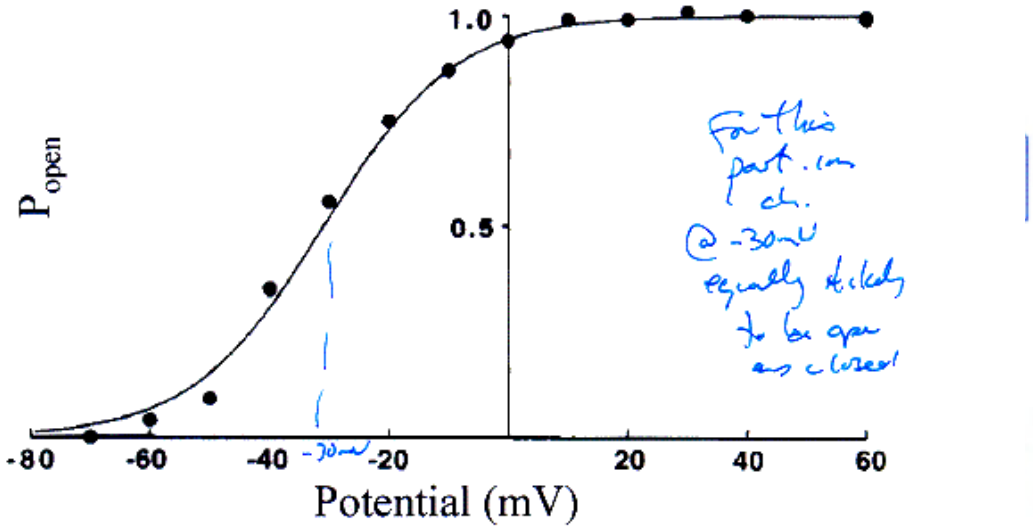
How does action potential occur?

Voltage dependence of on/off transitions.

(Can measure by ensemble or single-channels)



$$P_{open} = \frac{1}{e^{q_a (V_{50} - V)/kT} + 1}$$



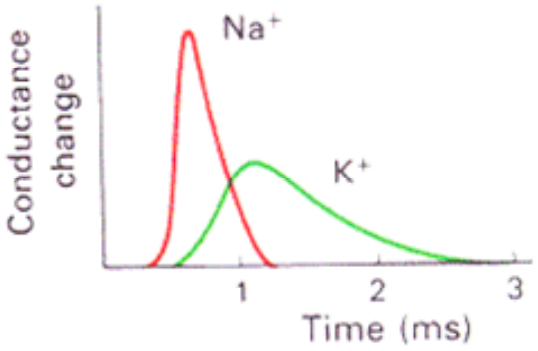
Suggests model where 2 states that differ in energy by qV

Where q is about 13e, or 13e/4 per S1-S4 sub-unit; V= -80mV.

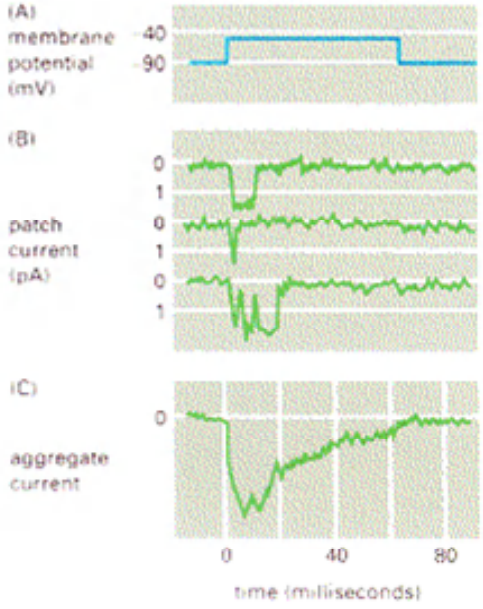
q is part of channel—gating current, not ionic current!

Do ion channels open gradually or all or nothing?

Ensemble



Single Ion Channel



Patlack & Horn, 1982

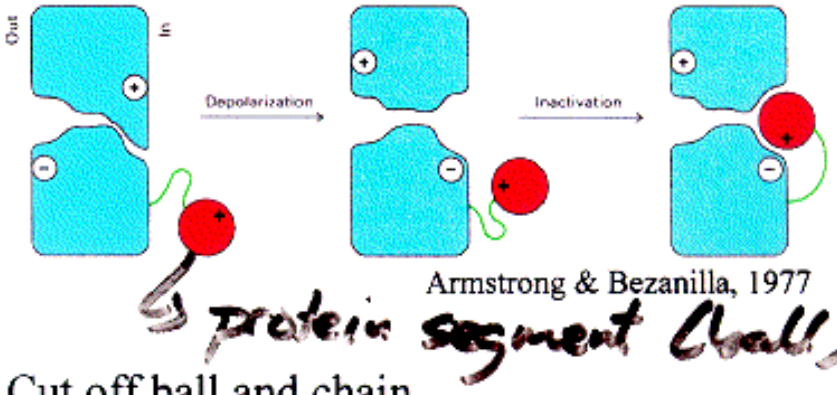
How does gate spontaneously shut-off?
How fast?

Nerve Impulse propagate, not spread, because Na^+ spontaneously shut-off.

What shuts off channel?

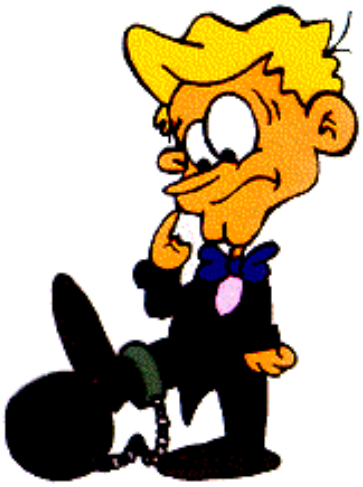
Na channels shut off in a msec
i.e. why you don't have spasms
i.e. why action potential travels rather than just spreads.
Why you can have repetitive firings of nerve.

The Ball and Chain model

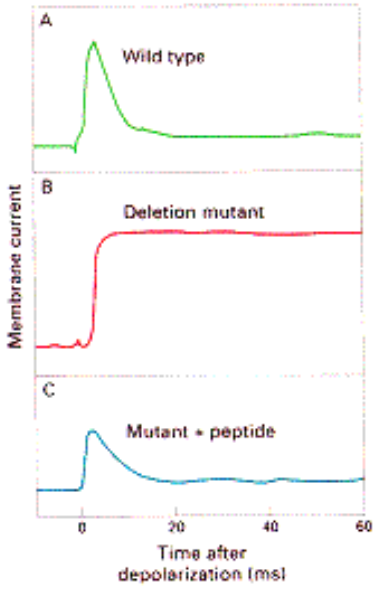


Armstrong & Bezanilla, 1977

Protein segment (ball)



Cut off ball and chain,
and no (fast) inactivation.

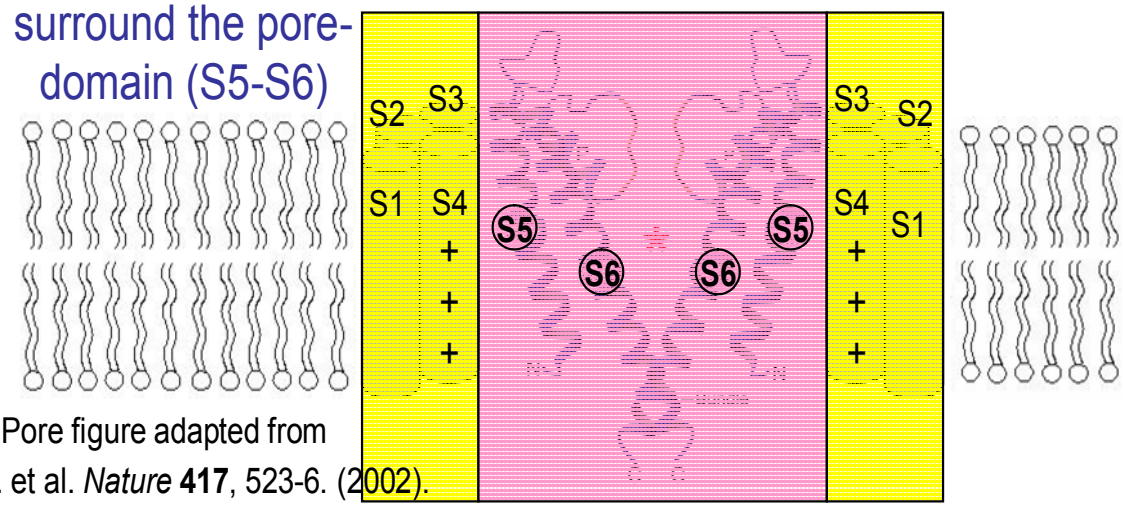


Zagotta, Hoshi, Aldrich, 1990

Structure of Pore-Domain (S5-S6) is known

(KvAP, Kv1.2… all yield the same structure)

b Voltage-sensing domains (S1-S4) surround the pore-domain (S5-S6)



Pore figure adapted from Jiang, Y. et al. *Nature* **417**, 523-6. (2002).

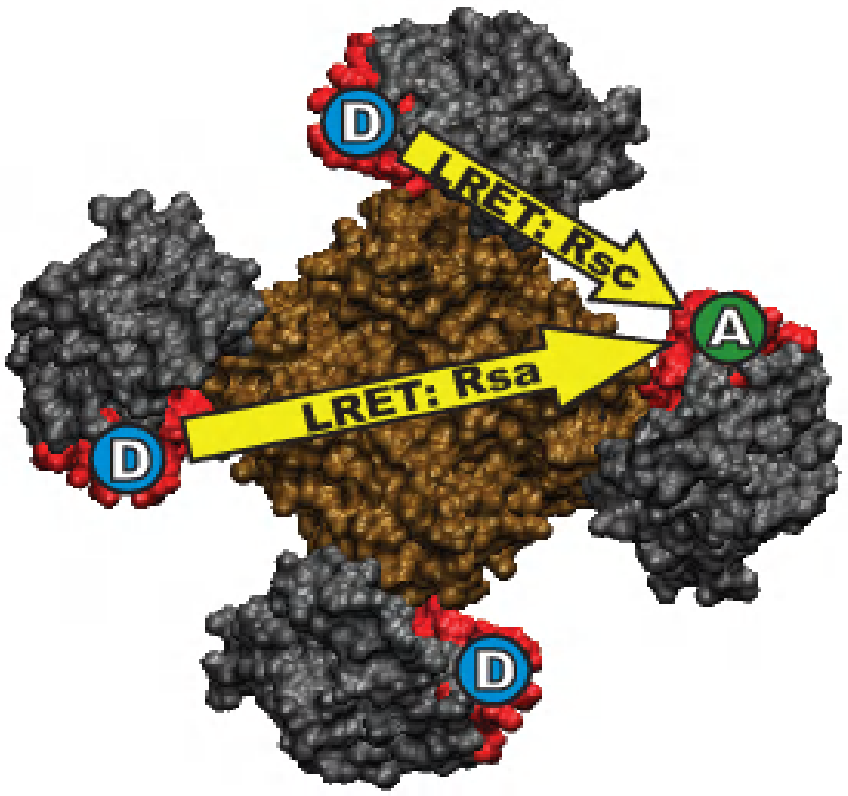
Explains ion selectivity ($K^+ > Na^+$) and rapid ion flux.

Excellent agreement between LRET and Crystallography

But how S4 (and S1-S3) move, remain controversial.

Crystal Structure of S1-S6 Ion Channel
Nobel Prize for Rod MacKinnon, 2006

S1-S4 Voltage Sensor Lies on the Outside of S5/S6

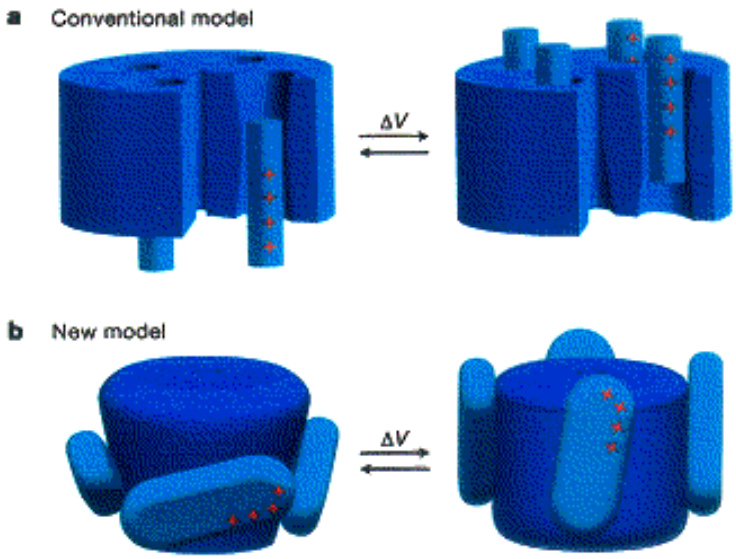


But…

Channel has been crystallized in only one state. There is no crysta structure of a channel in the open and closed state. Also, there wer some serious problems with some (all?) states.

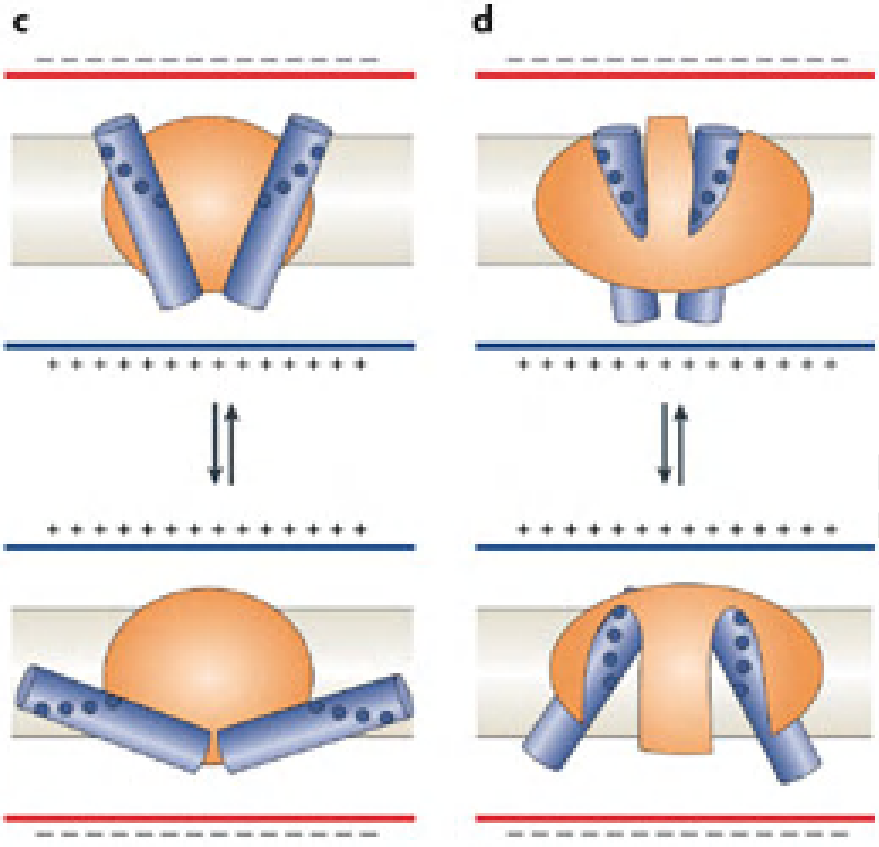
Need alternative techniques…lower resolution but can tell about channel in a more realistic setting.

3 Models for how S4 moves



New model of how voltage sensor moves to turn channel on/off (gated by voltage)

c) Helix: Twist and Rotation

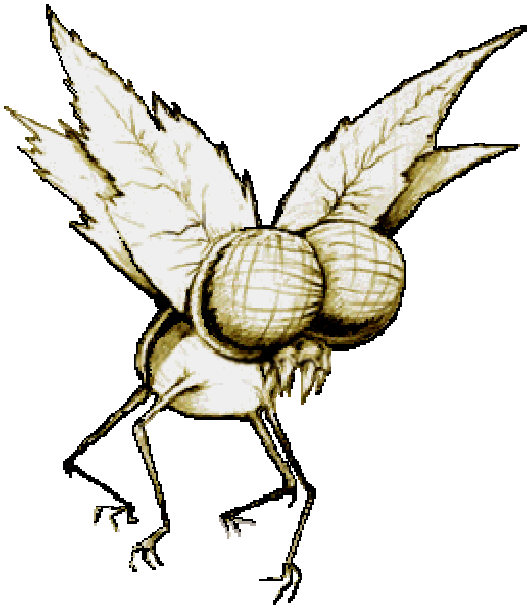


Translation

Rotation plus small rotation

Nature Reviews | Molecular Cell Biology

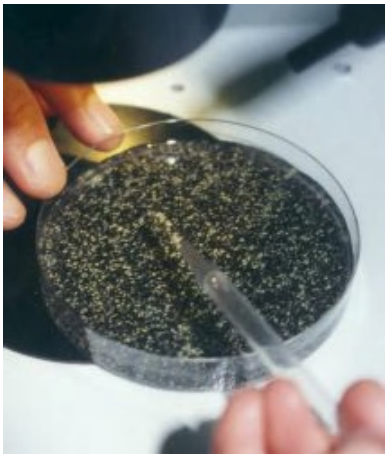
What can fluorescence tell us about Shaker K^+ channel opening/closing?



Shaker Channel– Can measure both Open & Closed States.

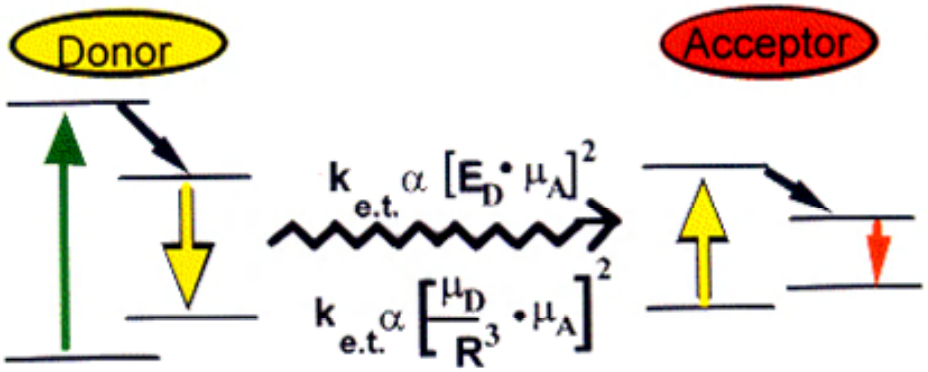
Inject mRNA in oocytes; wait 2-5 days; protein in membrane.

(Use mRNA where ionic current is blocked, if necessary).



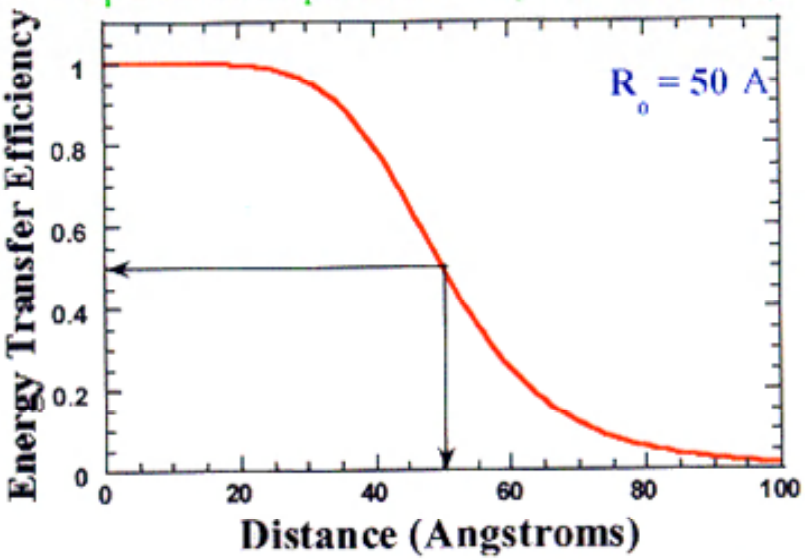
www.mpibp-frankfurt.mpg.de/schwarz/oocytes.html

Energy transfer: What is it? (review)

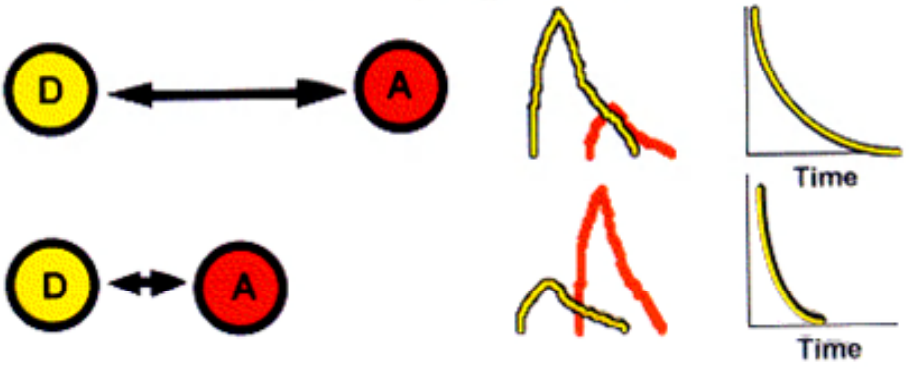


E : Energy transfer efficiency for FRET

Spectroscopic ruler! (δD ≈ few Å)

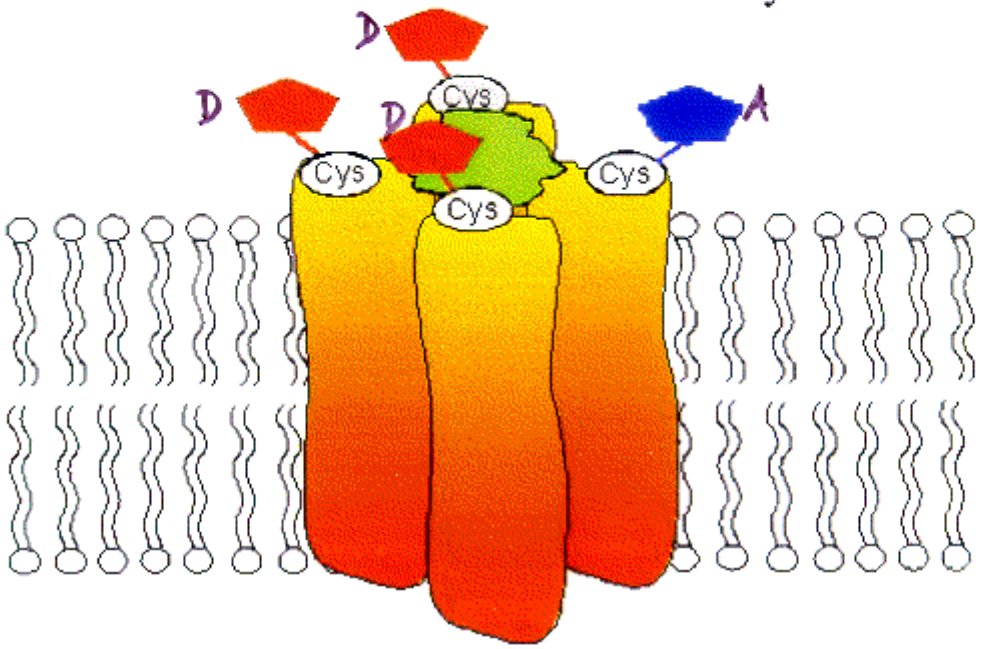


$E = \frac{1}{1+(R/R_0)^6}$



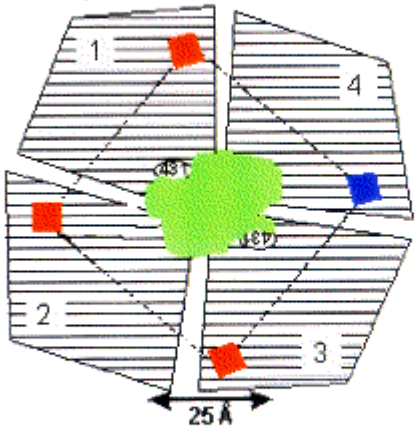
How to measure?
Where to put probes?

Label Channel with different dyes



Depending on how close dyes are,

get different colors, lifetimes



As shape changes,

get different colors

" lifetimes

Problems with oocytes

1. Tremendous amount of autofluorescence

2. Donor only and acceptor only plus desired donor-acceptor only

Answer: Luminescence Resoance Energy Transfer (LRET).

1. Donor has long lifetime– gets away from autofluorescence

2. Can isolate donor-acceptor complex.

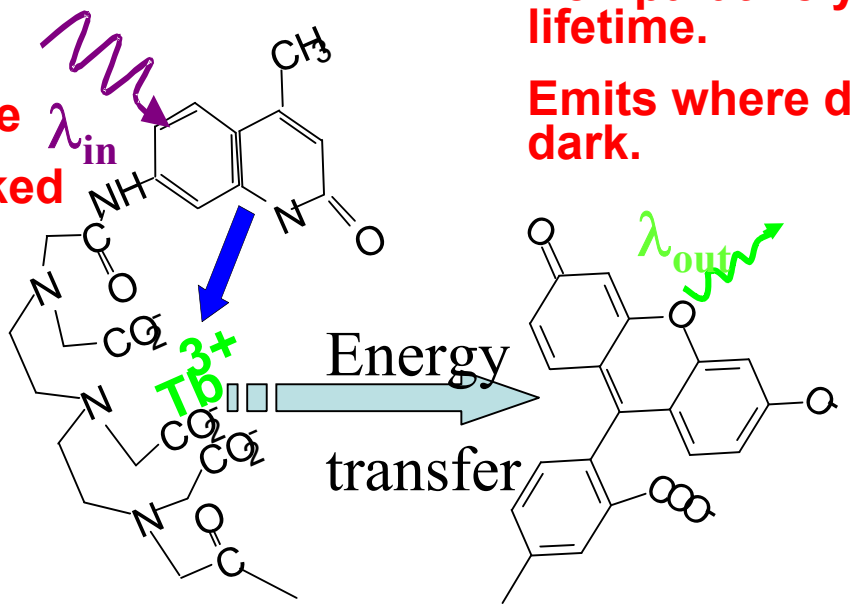
Luminescent Chelates

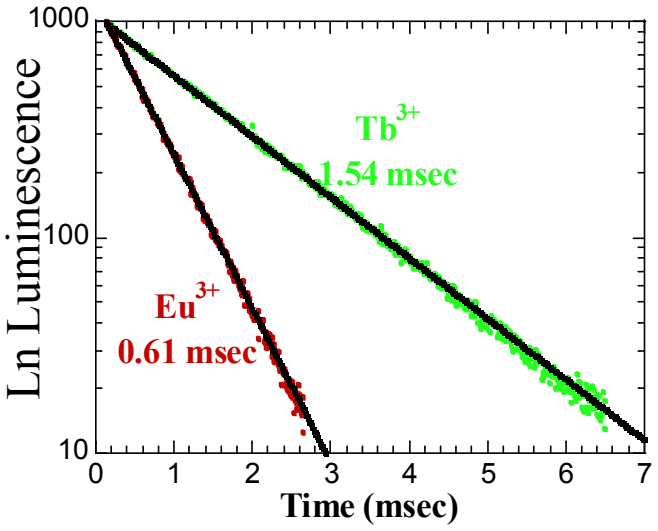
Long lifetime

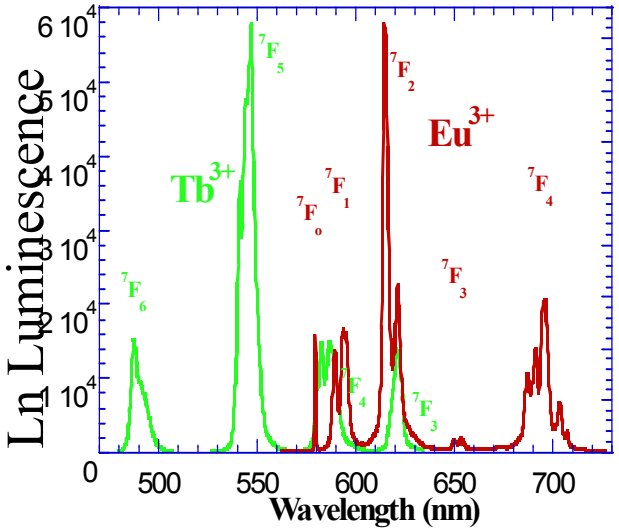
Sharply spiked in λ.

Comparatively short lifetime.

Emits where donor is dark.

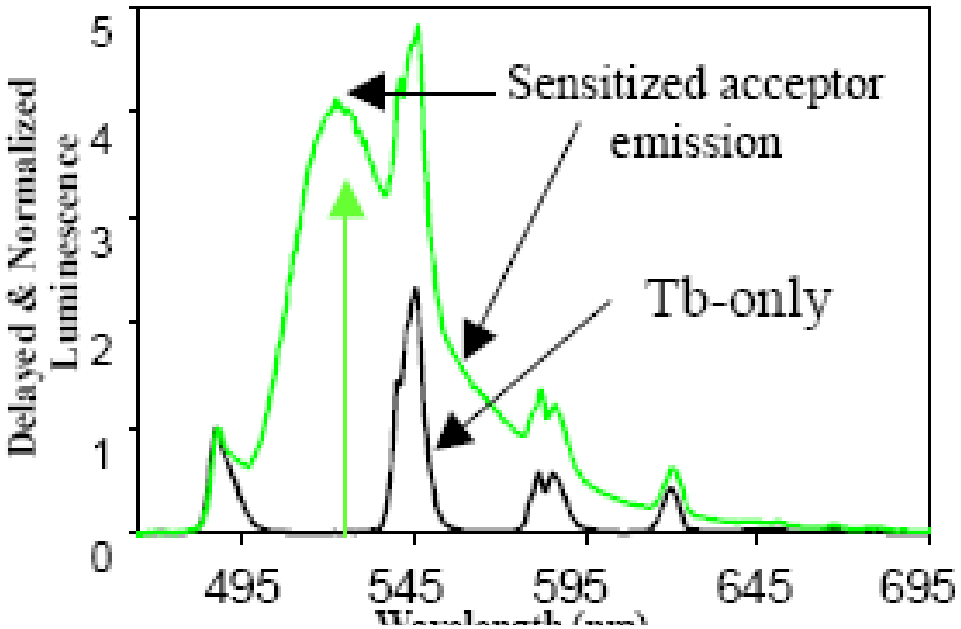


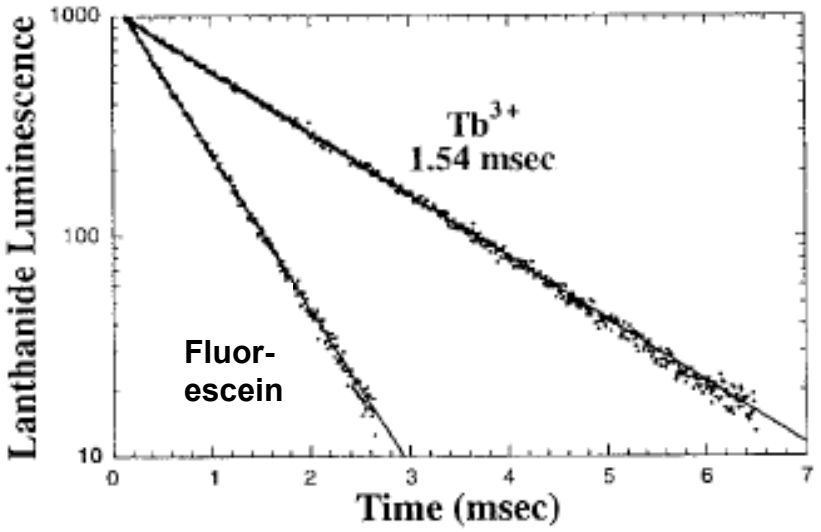




Can see D-A even if incomplete labeling: D-only, A-only.

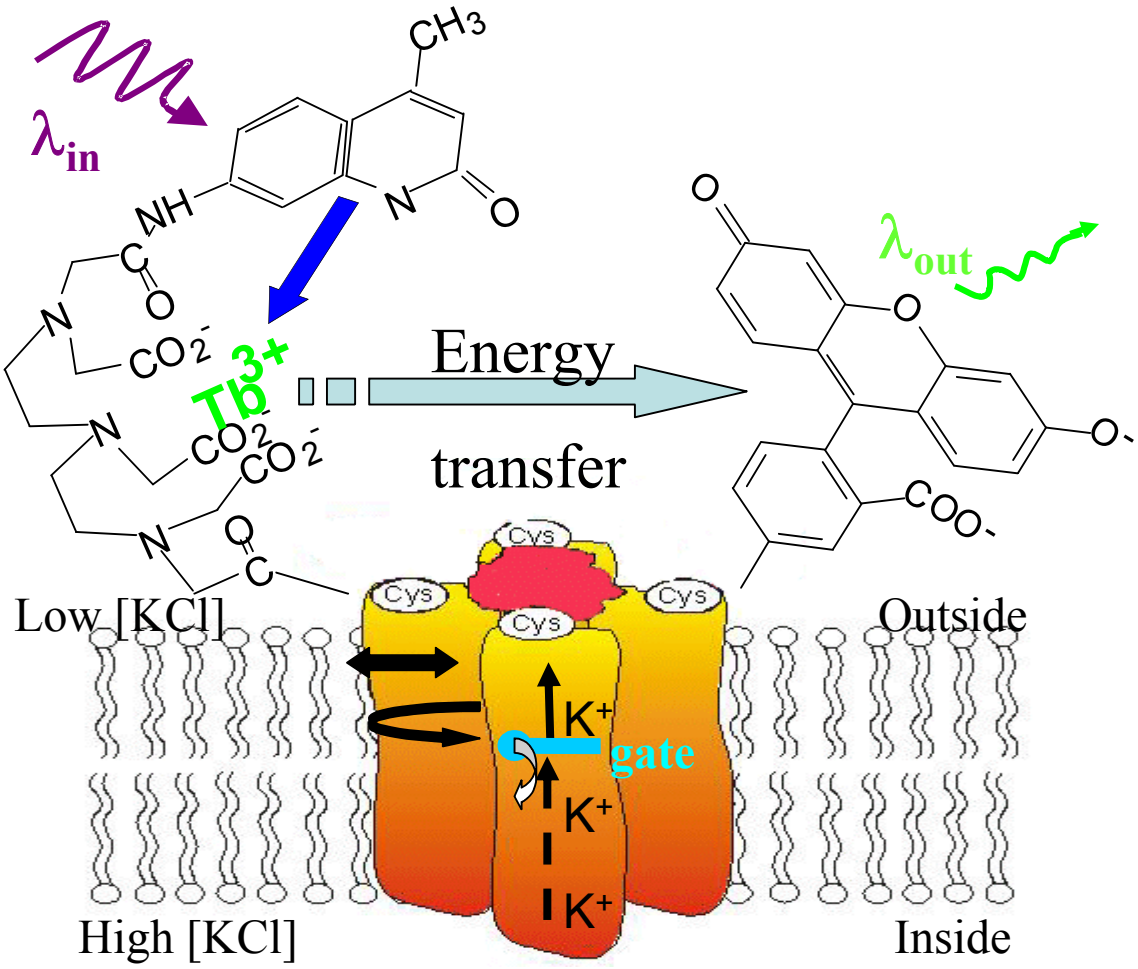
Example of LRET





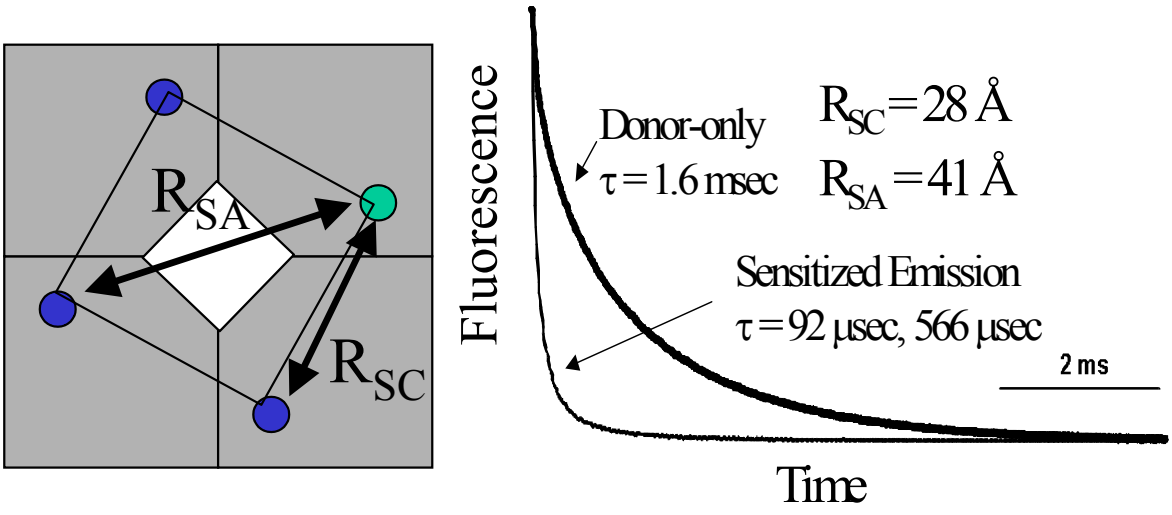
$$E = \frac{1}{1+(\frac{R}{R_o})^6} = 1 - \frac{\tau_{DA}}{\tau_D} = \frac{I_A}{I_{DA}+I_A}$$

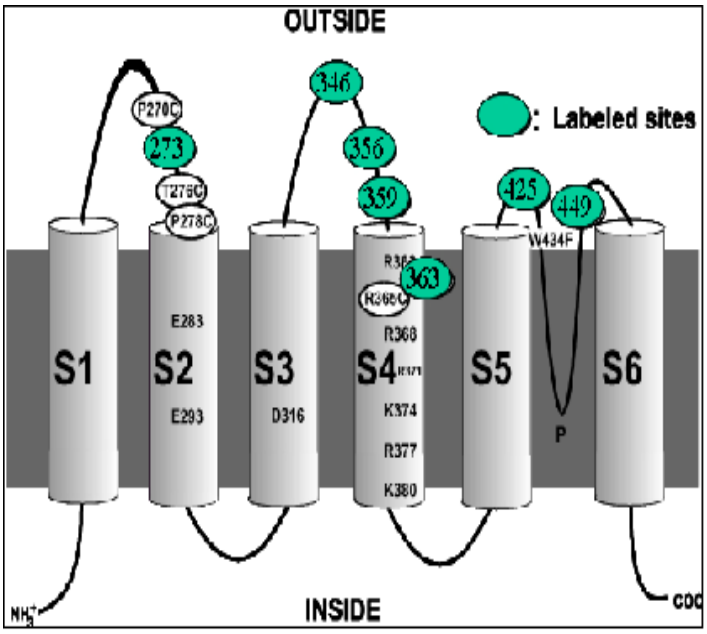
Shaker Potassium Channel



-60 mV (resting, closed)
0 mV (active, open)

S4: Geometry & data of Shaker channel

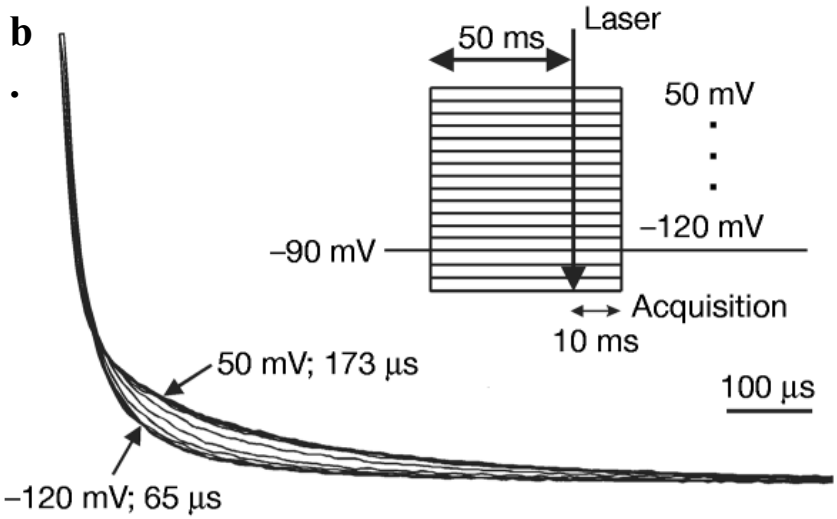


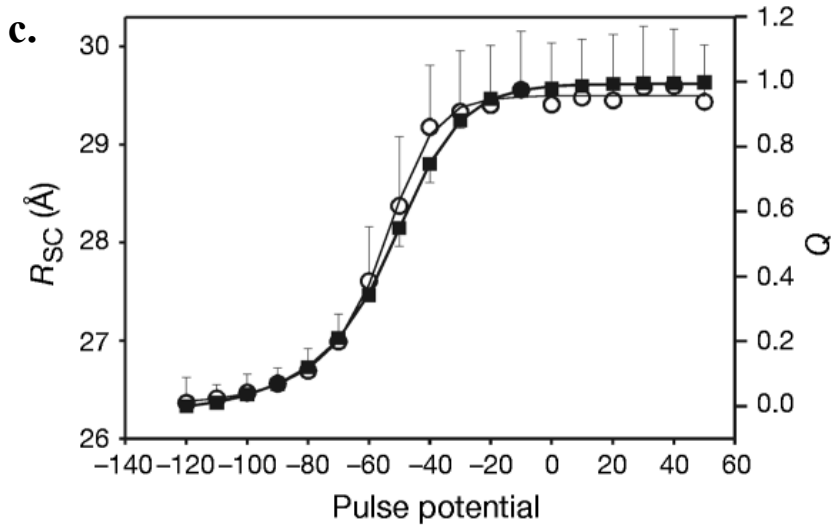


Cha, Nature, 1999

Two exponential= two-distances

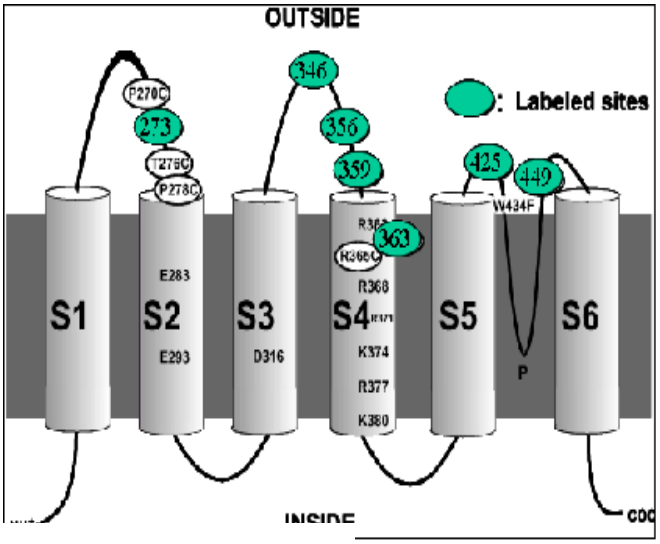
Voltage dependent movement

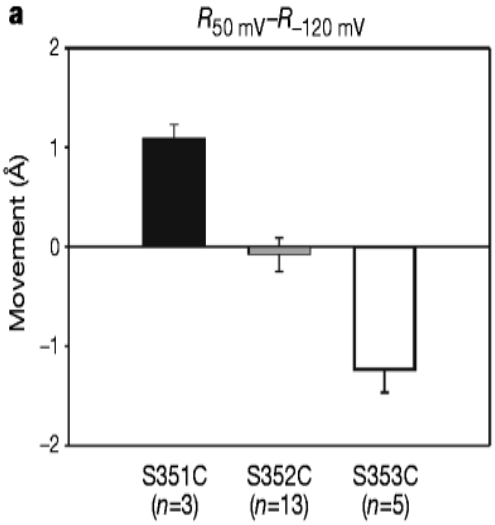




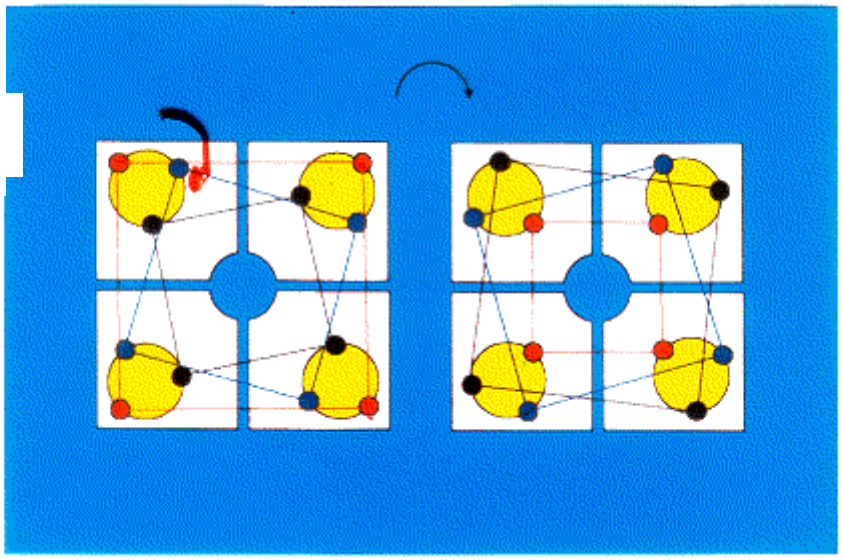
LRET is tracking Gating charge movement

Three neighboring residues, 351, 352, 352 Move in different directions





- S351C gets closer
- S352 C unchanged
- N353C get farther

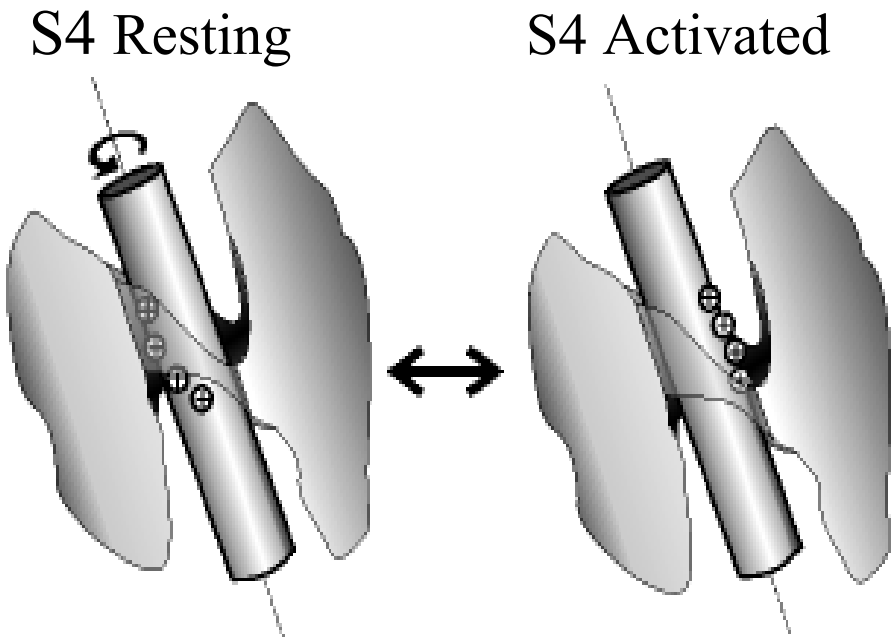


Red residue (S351C) initially far, then close.
Blue residue unchanged.
Black residue initially close, then far.

Shaker voltage sensor twists, does not translate too much.

How it all adds up:

Shaker voltage sensor twists, does not translate too much.



Cha, Nature, 1999

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.