Time Delay in Structural Shifts: Modeling Multiple States

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BBSI at Pitt

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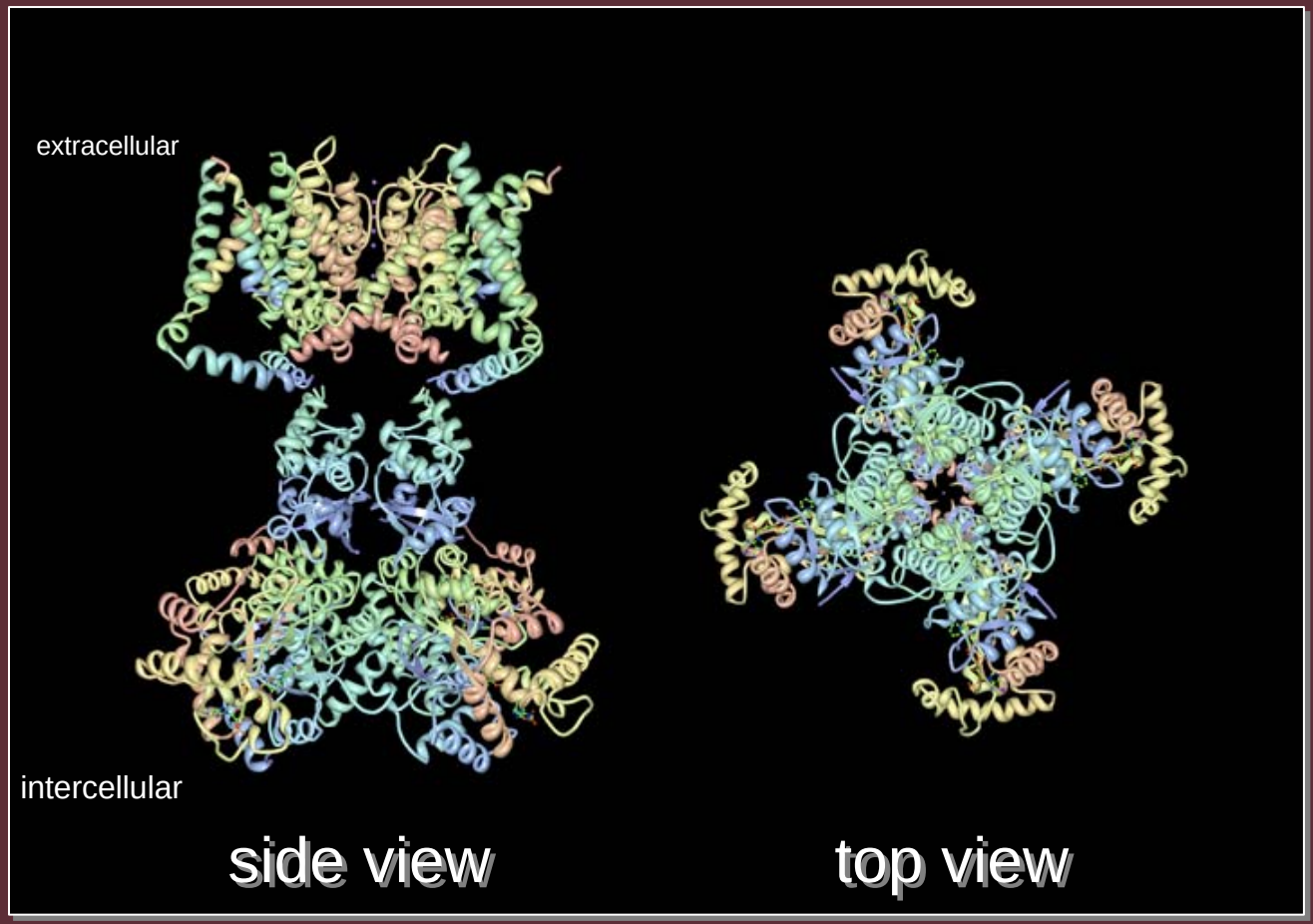
N-type Shaker K+ channels are composed of four subunits



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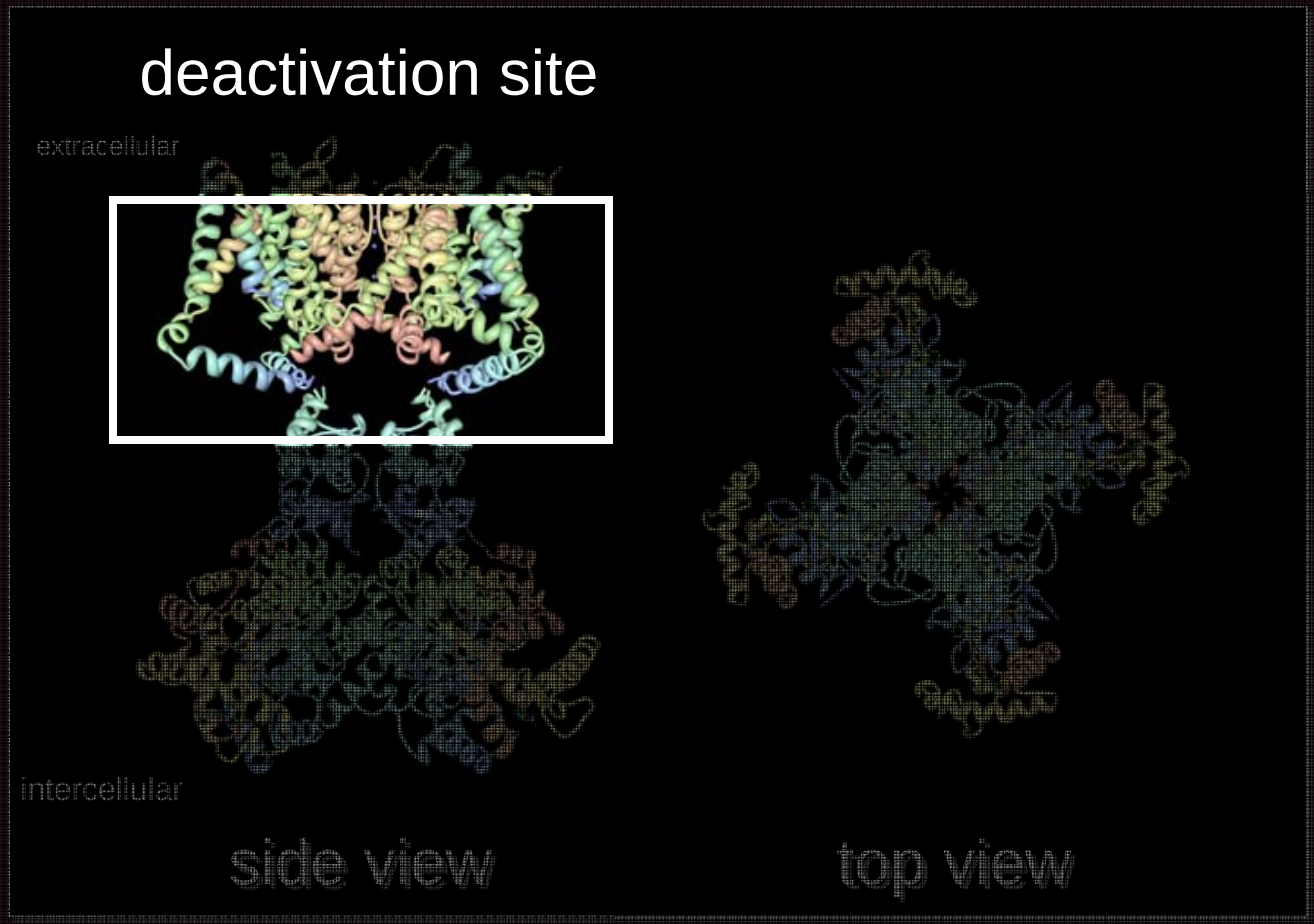
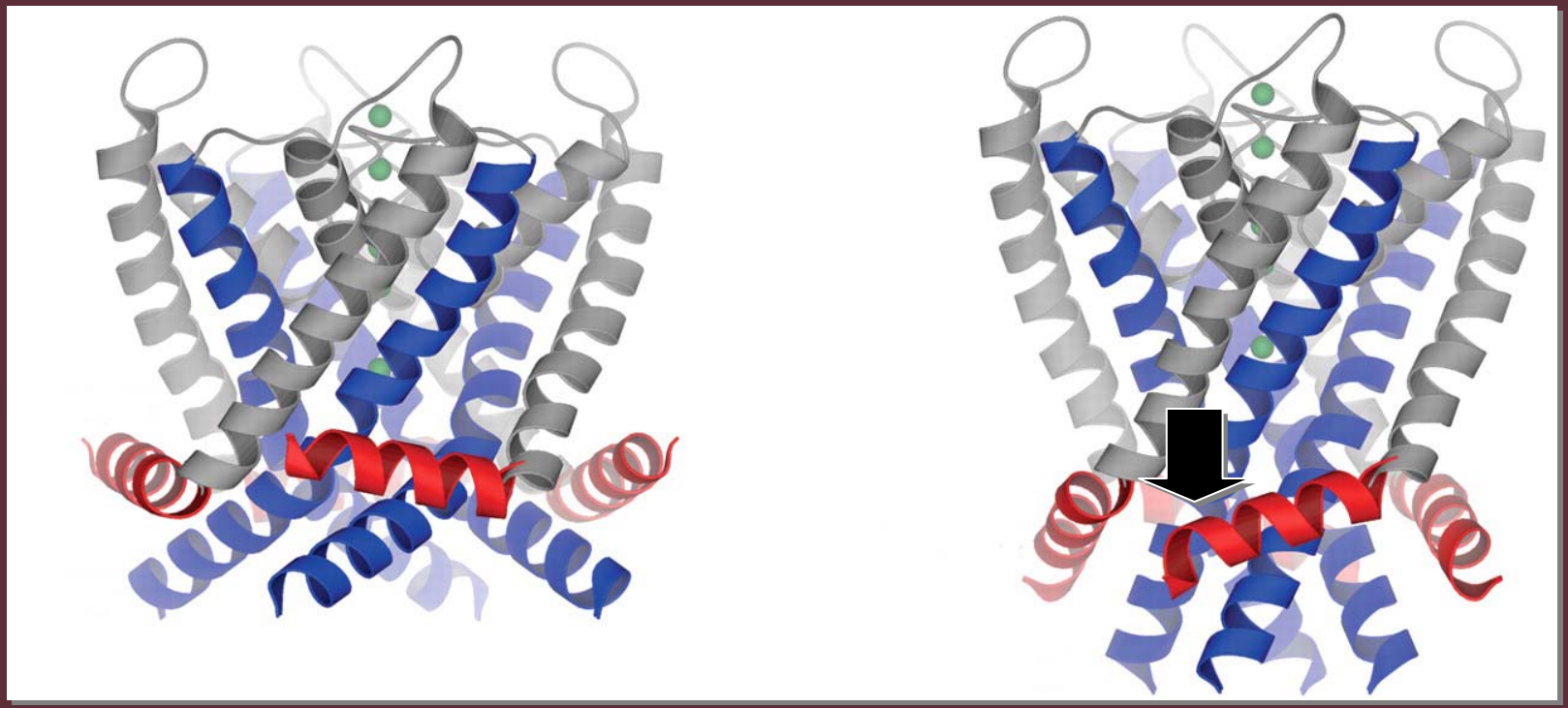


outside of cell



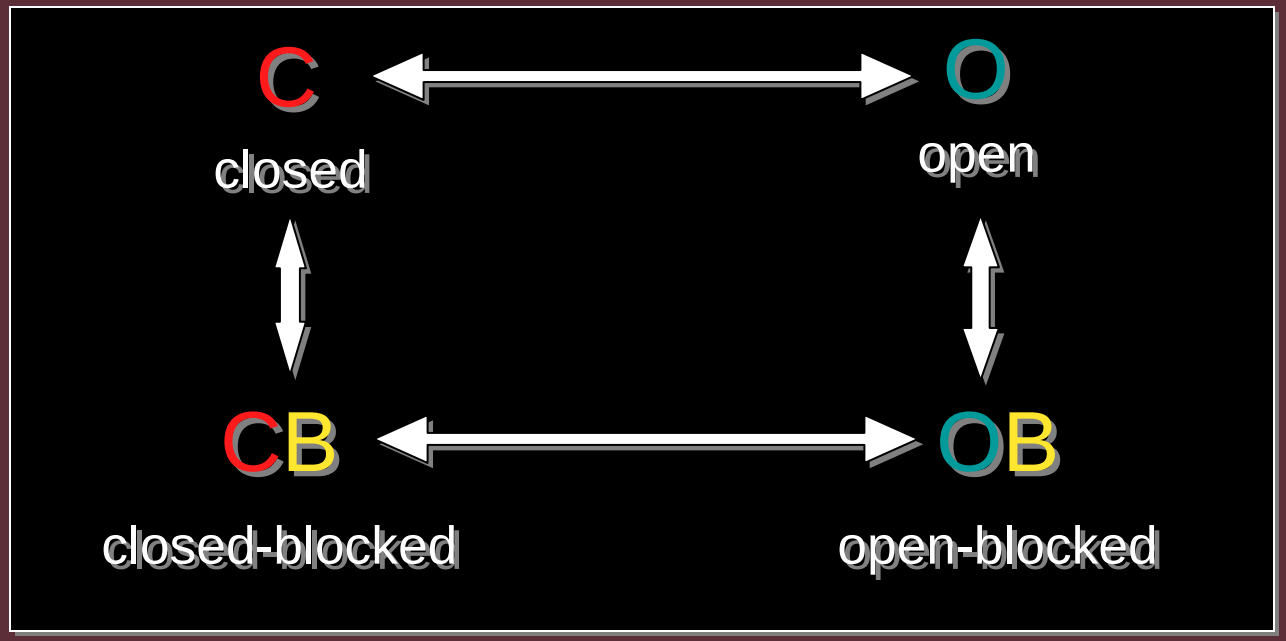
open

inside of cell

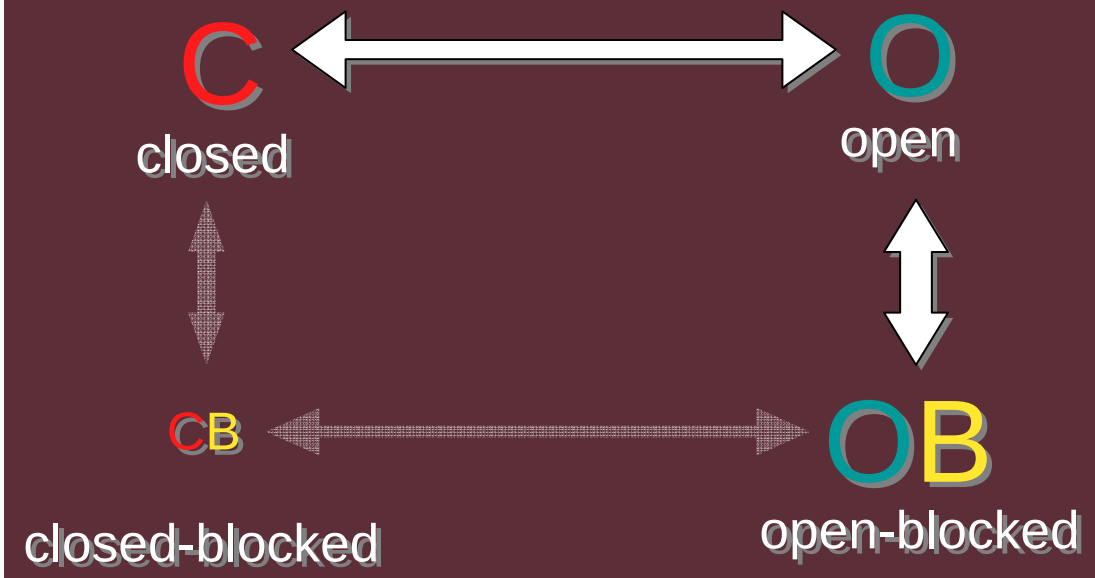
closed

N-type Shaker K+ channel functional states

- open (activated) ⟺ closed (deactivated)
- unblocked ⟺ blocked (inactivated)

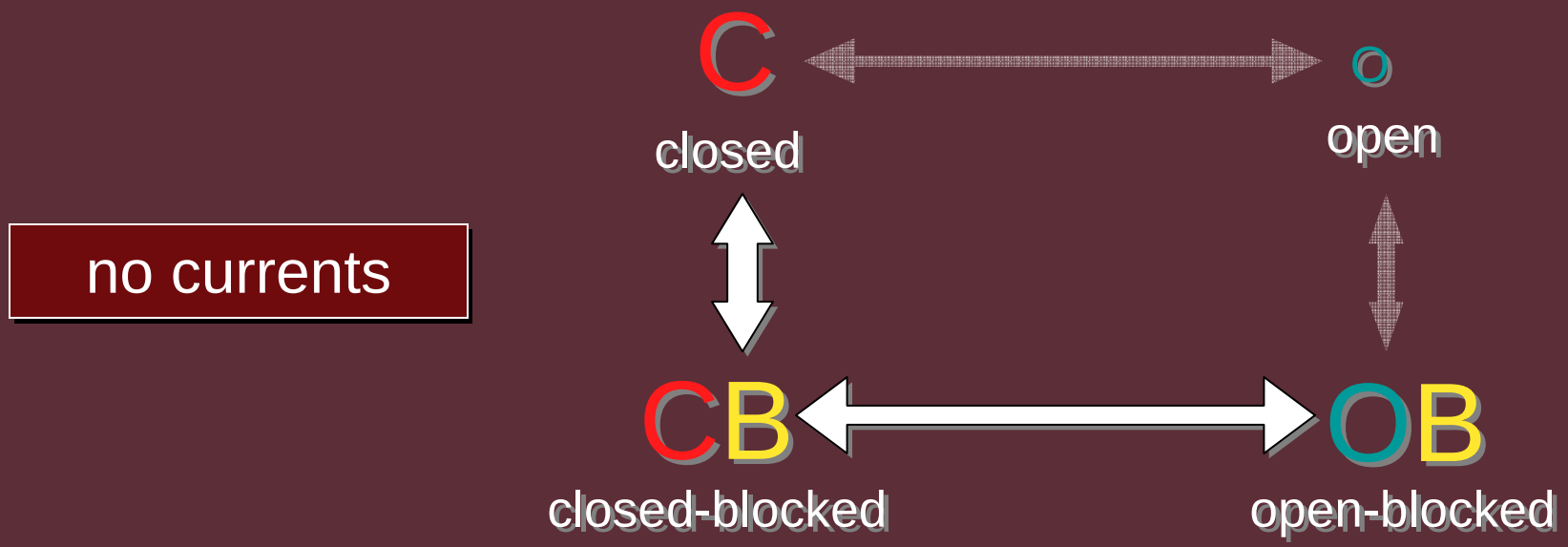


“recovery”: two routes to C



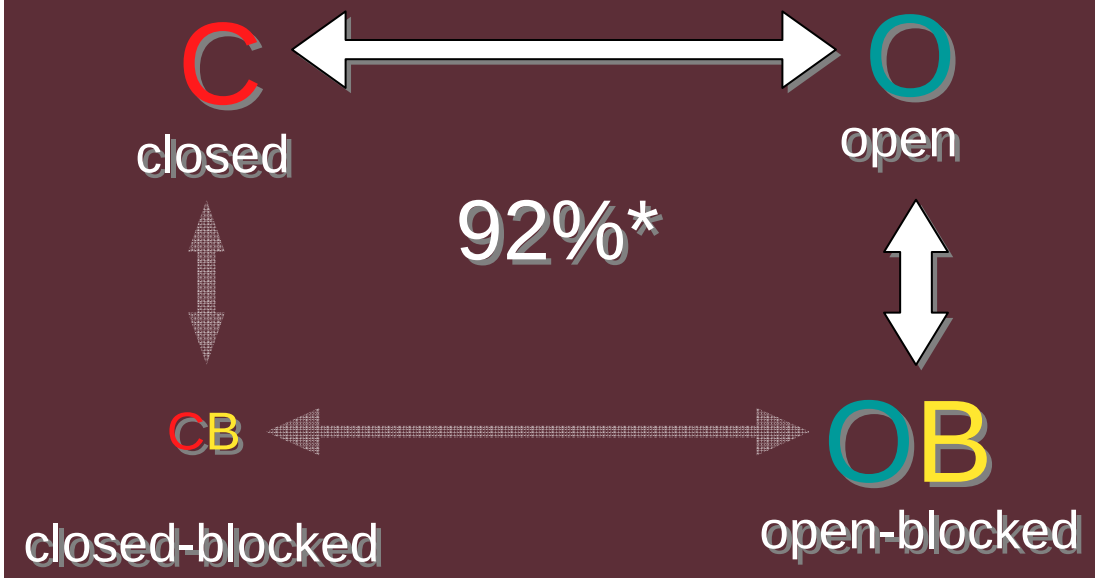
substantial

ionic (“tail”) currents



no currents

“recovery”: two routes to C



substantial

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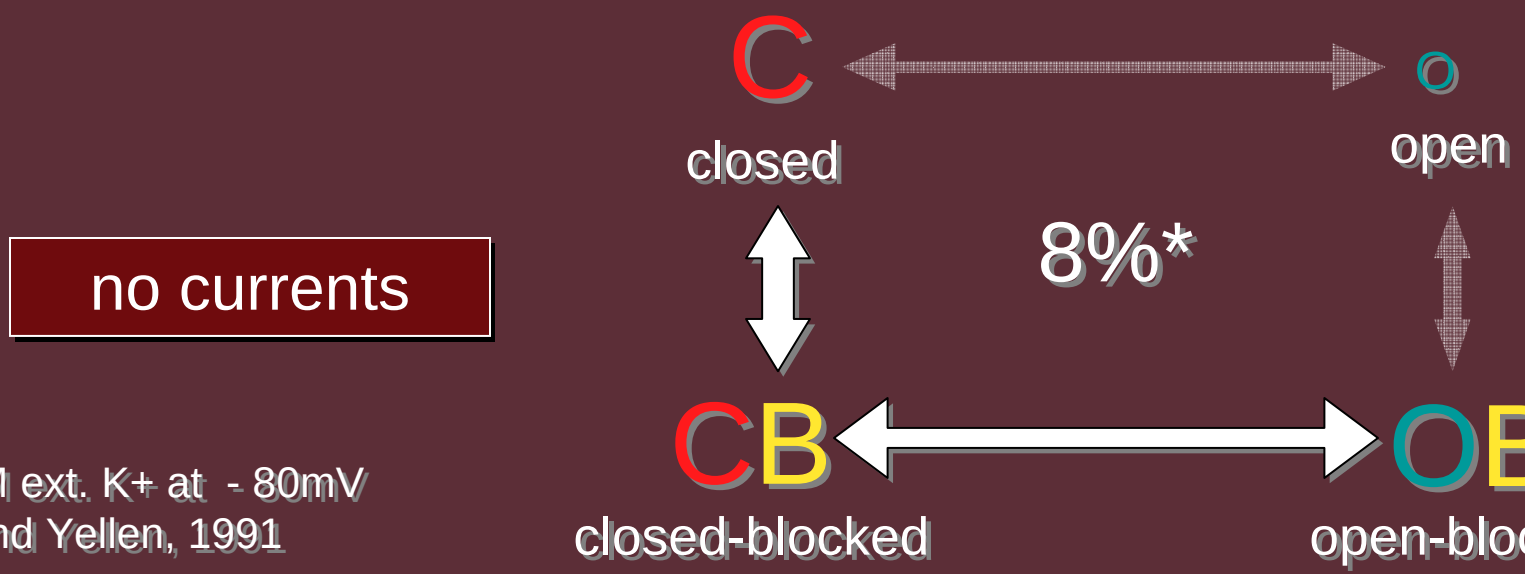
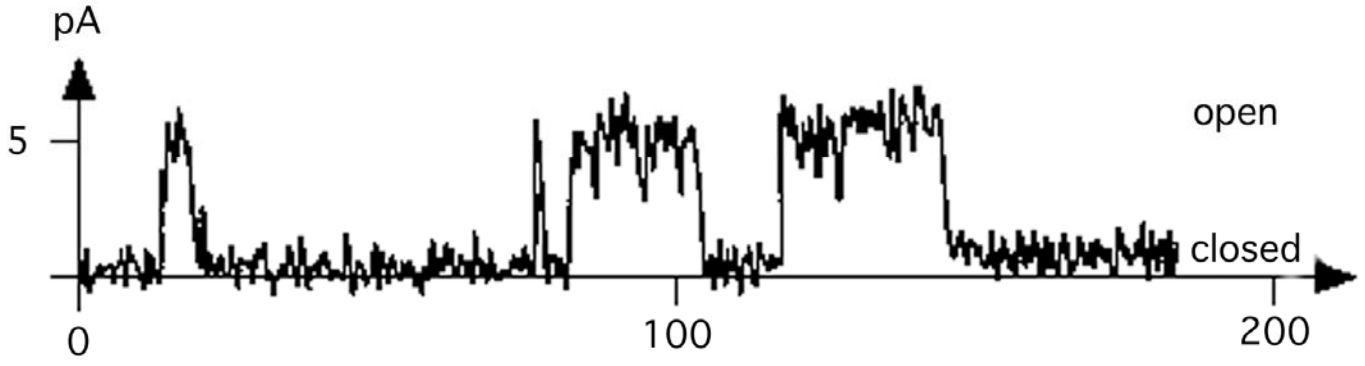


no currents

*160 mM ext. K+ at - 80mV
Demo and Yellen, 1991

Experimental Detection of States



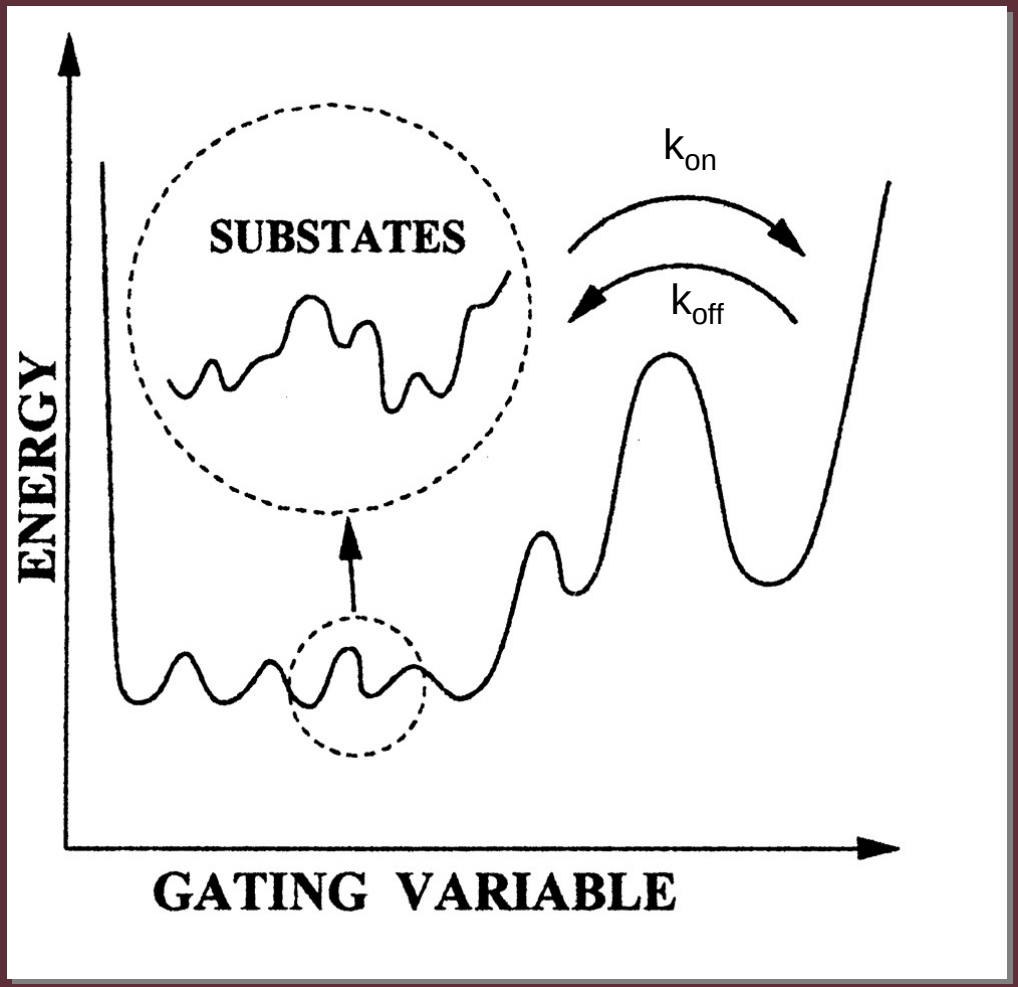
resolution ~ .1 msec

Early (lasting) assumptions:

- thermal (energy) fluctuations and ligation drive state shifts
- shift probability, but not exact time, knowable
- channel unchanged by ion current

Liebovitch, L. S.; Krekora. *Proceedings of the Institute for Mathematics and its Applications*.

Why consider intermediate steps?



Goychuk, Igor and Hanggi, Peter (2002) Proc. Natl. Acad. Sci. USA 99, 3552-3556.

(1) $[A] + [C] \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} [AC] + [B] \underset{k_{-2}}{\overset{k_2}{\leftrightarrow}} [ABC]$

(2) $[A] + [C] \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} [AC_{int}] \underset{k_{-2}}{\overset{k_2}{\leftrightarrow}} [AC] + [B] \underset{k_{-3}}{\overset{k_3}{\leftrightarrow}} [ABC_{int}] \underset{k_{-4}}{\overset{k_4}{\leftrightarrow}} [ABC]$

at equilibrium:

(1) $0 = k_1[A][C] + k_{-2}[ABC] - k_{-1}[AC] - k_2[AC][B]$

(2) $0 = \frac{k_2 k_1}{k_{-1}+k_2}[A][C] + \frac{k_{-3}k_{-4}}{k_{-3}+k_4}[ABC] - \frac{k_{-1}k_{-2}}{k_{-1}+k_2}[AC] - \frac{k_3 k_4}{k_{-3}+k_{-4}}[AC][B]$

at equilibrium:

$$(1)\ 0 = \underline{k_1}[A][C] + \underline{k_{-2}}[ABC] - \underline{k_{-1}}[AC] - \underline{k_2}[AC][B]$$

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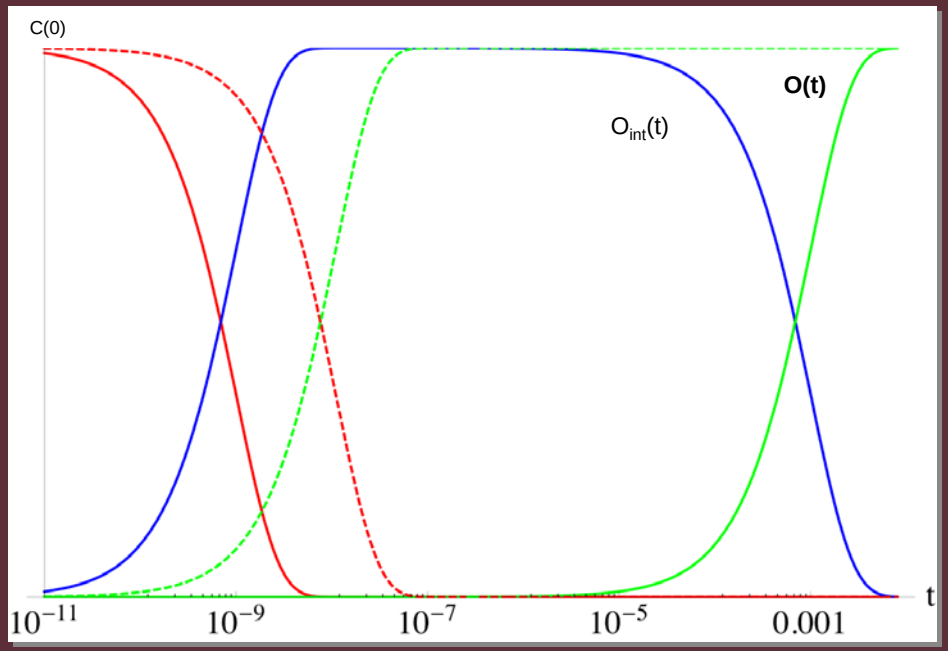
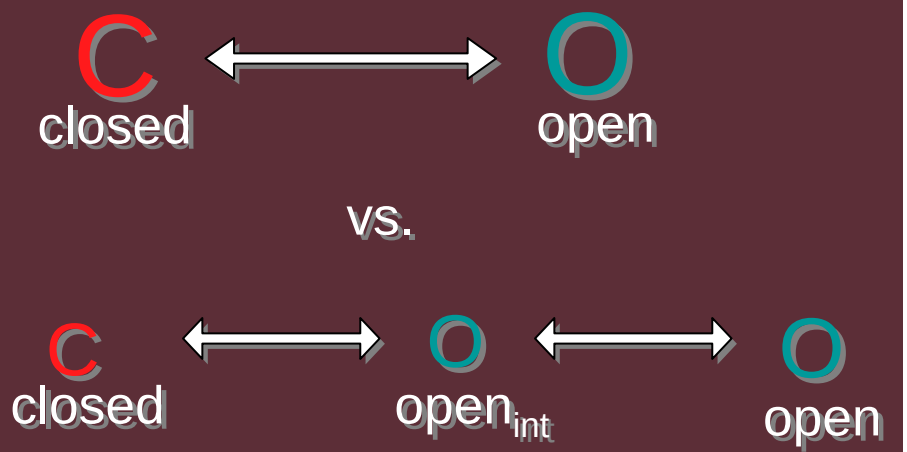
Adjustable Delay, ΔG Preserved



dashed, no delay; solid, delayed

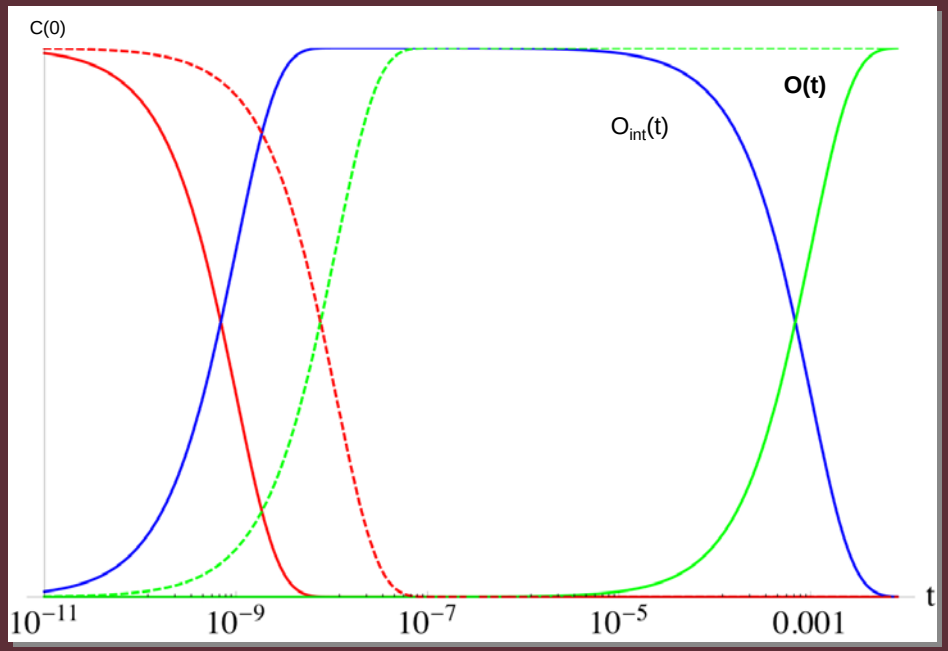


$\log k_1$	$\log k_{-1}$	$\log k_2$	$\log k_{-2}$	O(t) equil.	80%	95%	99%
9	4	3	-4	5.067×10^{-8}	t = .0016	t = .0030	t = .0046
9	3	2	-4	5.067×10^{-8}	t = .016	t = .030	t = .046
8	4	3	-5	5.067×10^{-7}	t = .0016	t = .0030	t = .0046
7	4	3	-6	5.067×10^{-6}	t = .0016	t = .0030	t = .0046

Equilibrium and delay values for varying rate coefficients.

$\frac{k_{-1}k_{-2}}{k_1k_2}$ was maintained to preserve ΔG ~ 6 kcal/mol

Adjustable Delay, ΔG Preserved



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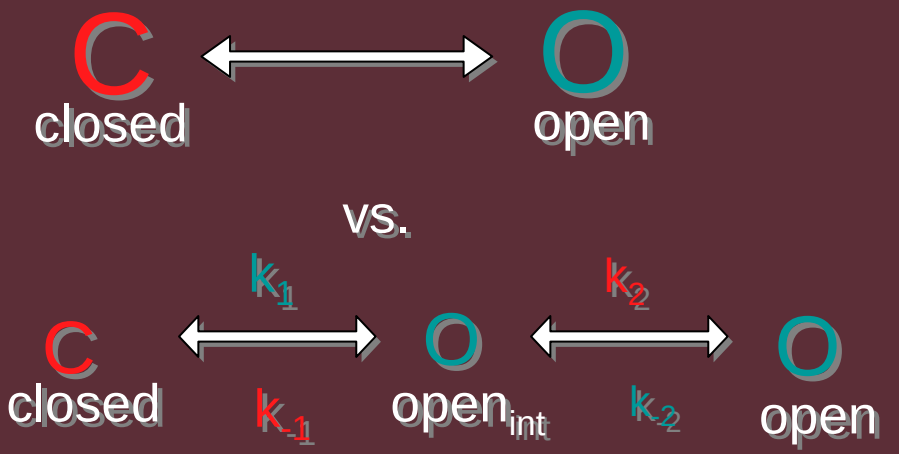

Any intermediate state N must

have sufficiently small

$$\frac{k_{N-1}[N-1]+k_{-N}[N+1]}{k_{-(N-1)}+k_N}$$

to "avoid" detection at equilibrium

log k_1	log k_{-1}	log k_2	log k_{-2}	O(t) equil.	80%	95%	99%
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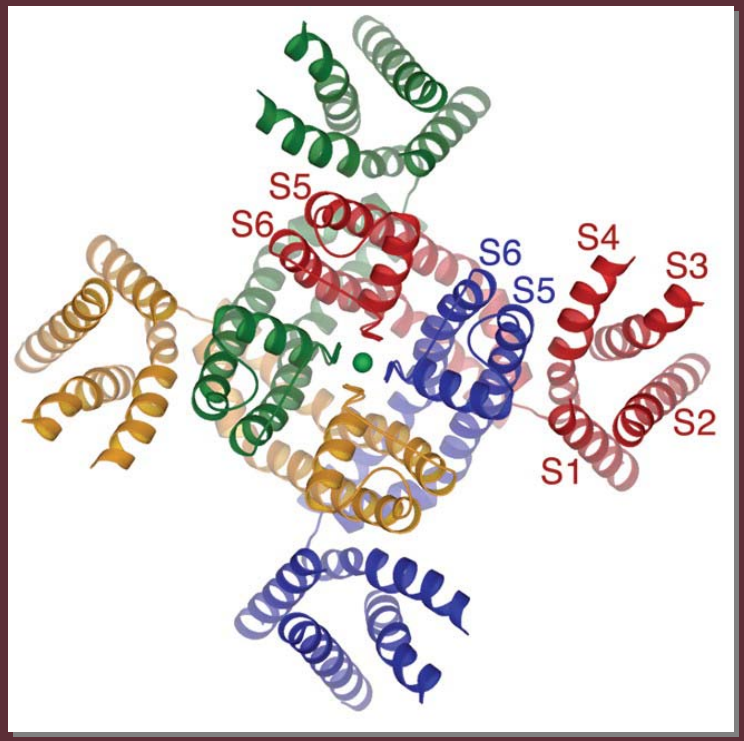
Hyperpolarization shortens delay

Initial delay is likely coupled to movement of voltages sensors (gating charges) on outer helices



top view, looking into cell

MacKinnon, R.; Campbell, E.; Long, S. *Science* (2005). 309, 897-903.

Future Work

Short lifetimes and low k_{off} values explain experimental difficulties of intermediate detection

Intermediate states affect energy landscape but not equilibria or overall ΔG

50% of current drugs target ion channels[6].

Our understanding of channel dynamics and treatment discovery would be improved by:

1. Determining Shaker K+ channel crystal structure in closed conformation[3]

2. Models that describe mechanical coupling of gating charges to pore inactivation

3. Describing selectivity mechanisms in Na+ and K+ channels

References

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