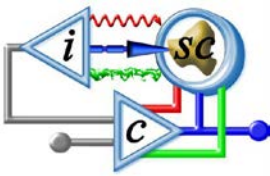
Vanderbilt Institute for Integrative Biosystems Research and Education



Instrumenting and Control the Single Cell

Challenges of Single Ion-Channel Biosensors

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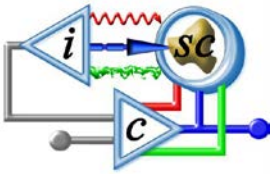
John P. Wikswo (Vanderbilt)

APS March Meeting
March 3, 2003

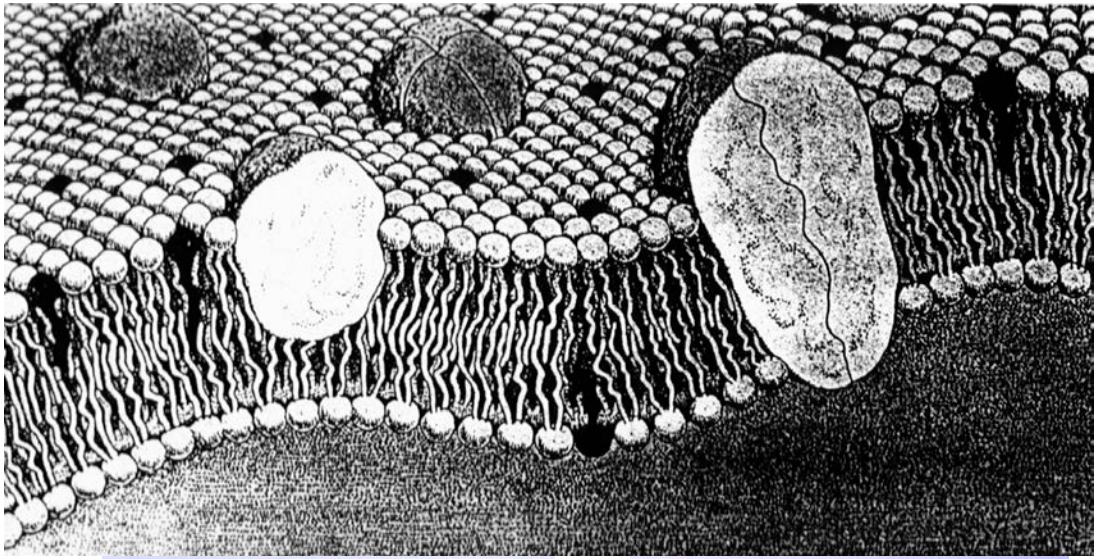
Receptors as Biosensors

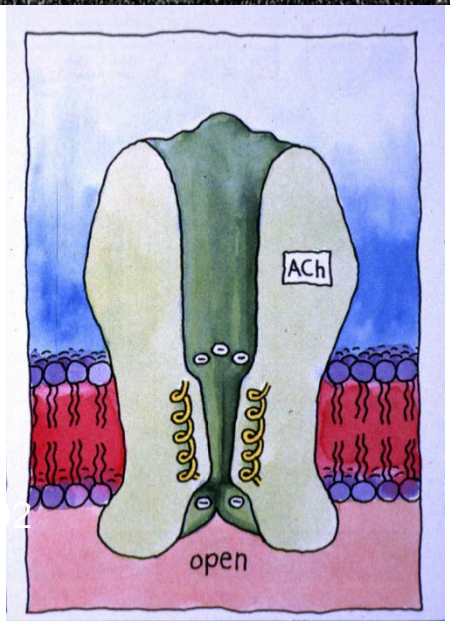


- Receptor binding can be used to detect chemical and biological agents, drugs, and environmental toxins. This can be accomplished in several ways
  - Fluorophores
  - Field effect transistors
  - Surface plasmon resonance
  - Labeled nanoparticles
  - Nanocantilevers
  - Ion Channels

Membrane Ion Channel
~ 1 nm pore

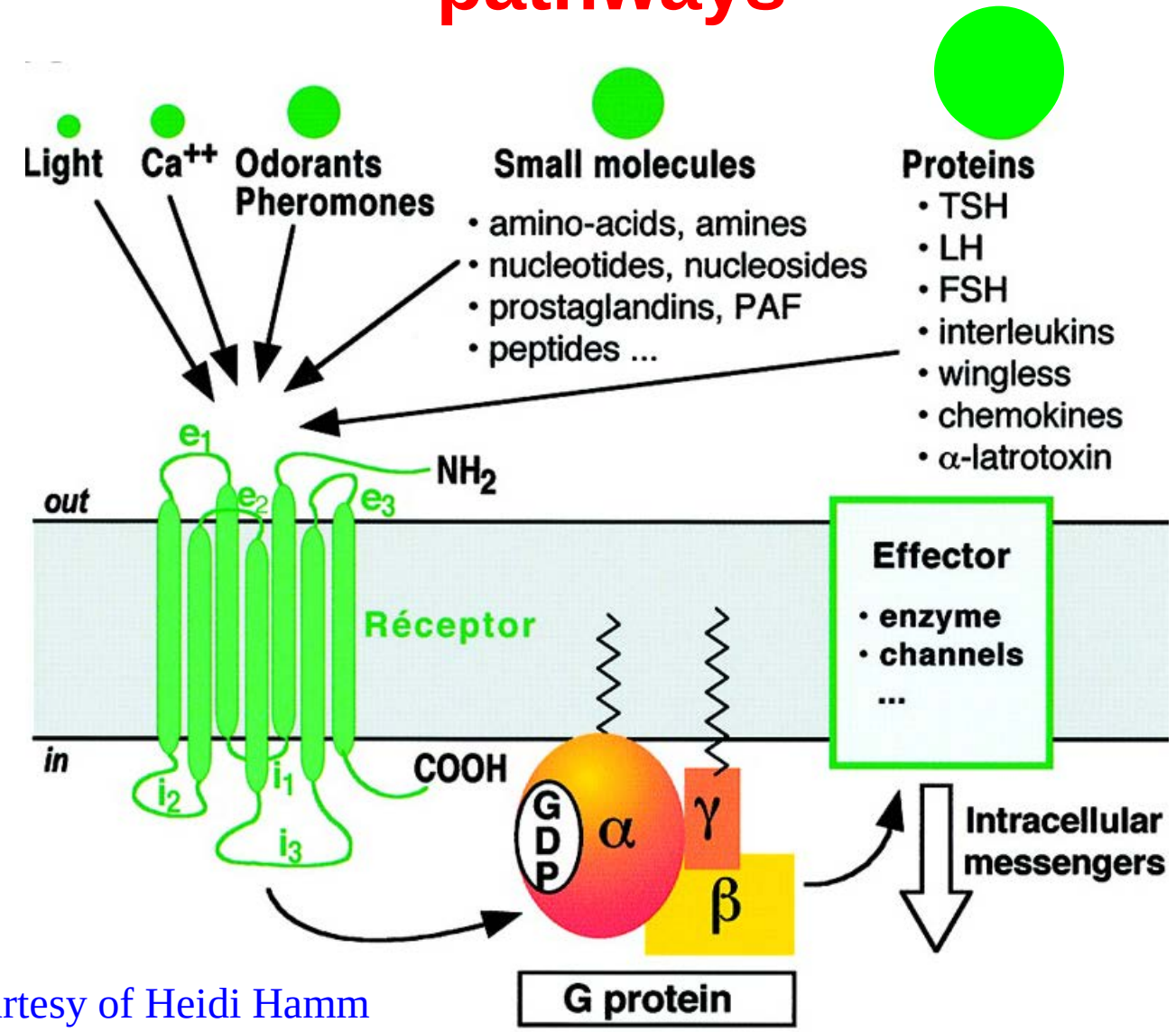
- Membrane-spanning protein
- MscL, αHL, and connexins: large pores with no ionic selectivity.
- K^+ channels: ion selective and often rectification even in symmetric solutions
- Gating controlled by electric or mechanical forces and ligands
- Gated ion channels can assume discrete conducting states





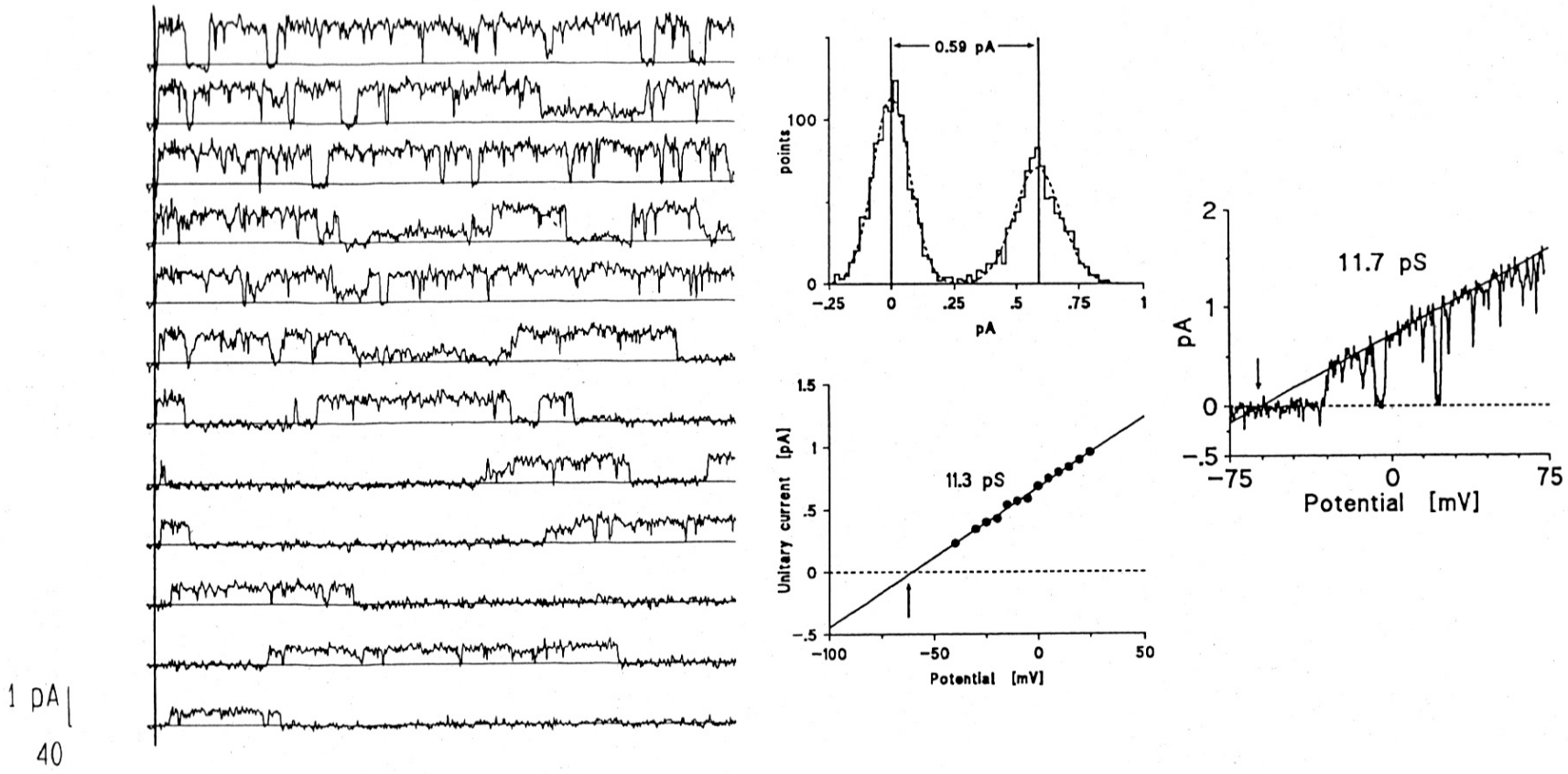
citable Membranes, B. Hille

G-protein-coupled receptors exhibit complex signalling pathways



Courtesy of Heidi Hamm

Transmembrane ion channels have a time- and voltage- or ligand-dependent conductance that can serve as the basis for a biosensor

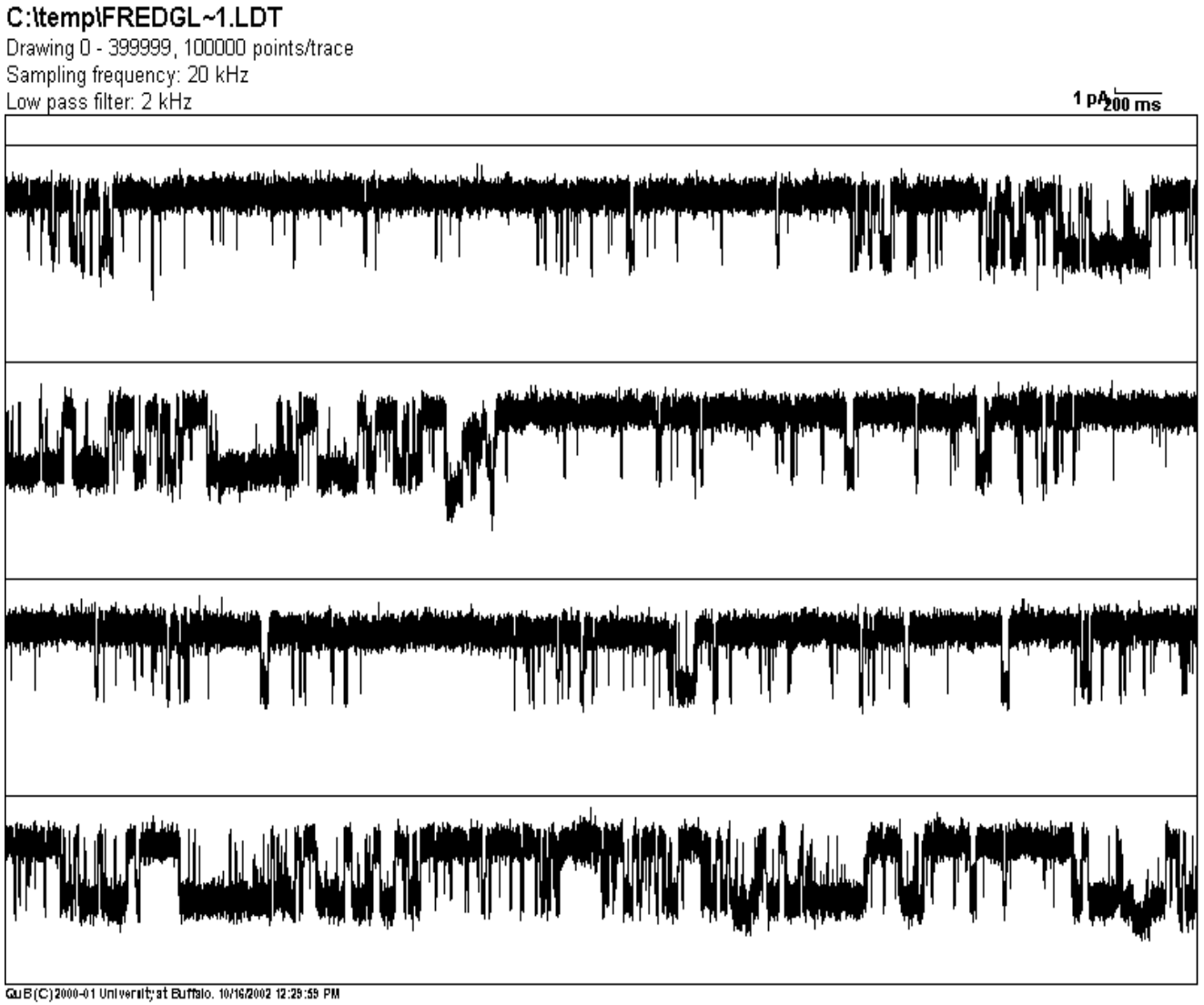


R Wilders and HJ Jongsma, Biophysical J, 65: 2601-2613 (1993)

A particular ligand can control channel conductance

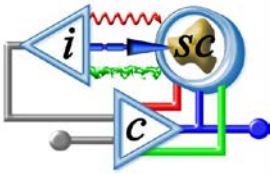
- Typical single-channel recordings of conductance fluctuations of a single gluR0 channel

- GluR0 in outside-out patch from HEK cells, zero Ca^{2+}, symmetric K^+ saline, -60mV.



Courtesy of A. Auerbach, SUNY-Buffalo

Single Ion Channel Biosensors



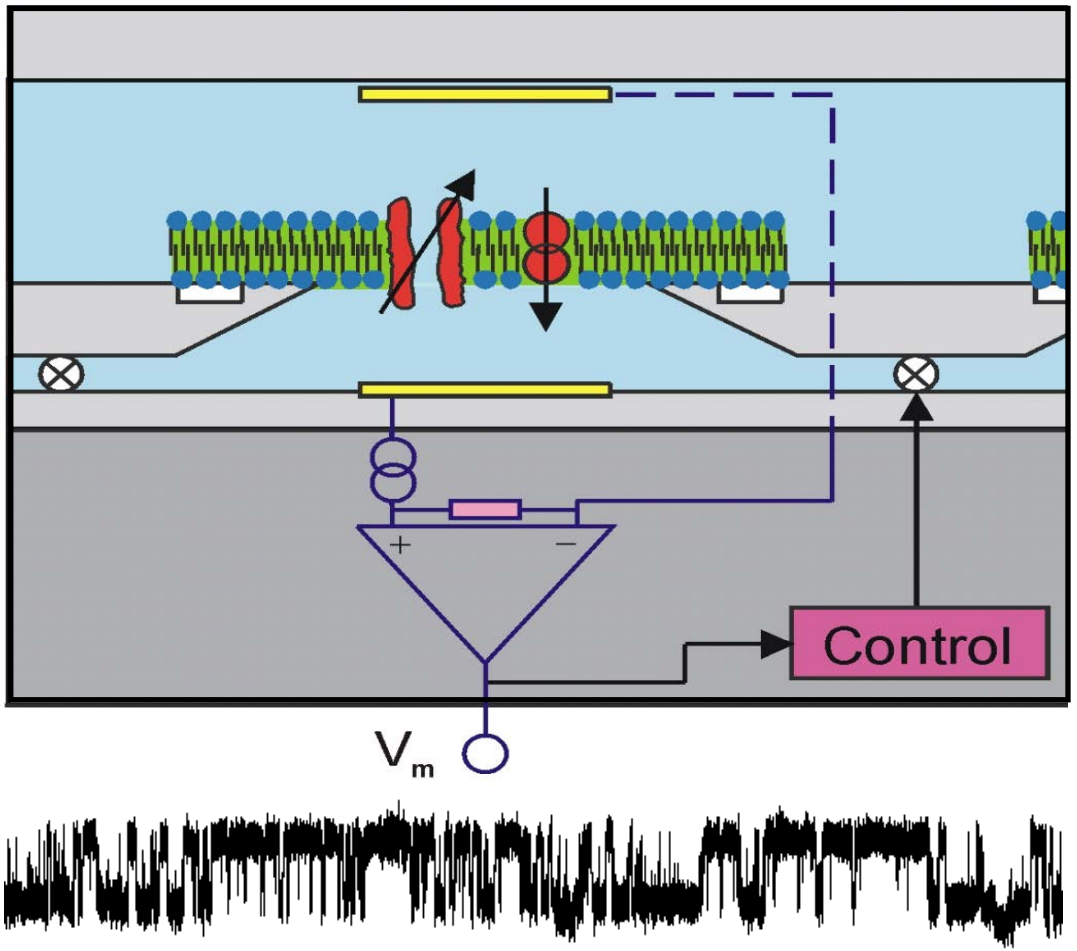
- Single ion channels might be useful as biosensors
  - Can be specific to a particular ligand
  - Can be natural or genetically engineered
  - Can be controlled by voltage or chemistry
  - Might allow DNA sequencing of bioagents
  - Best used in a high resistance lipid membrane
    - Can simulate expected local environment
    - Can allow normal function
    - Demonstrated single-channel sensitivity
    - Gigaohm seal reduces noise

The Appeal

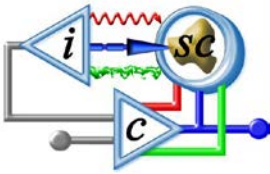
- In many cases, voltage- and ligand-gated channels and their signaling cascades exhibit a sensitivity, specificity, and efficiency of optimal physical devices such as photomultipliers
- Rhodopsin molecules and cyclic GMP-gated channels of retinal rods form single-photon detectors.
- Ion channels are high-gain amplifiers that transduce a small electrical, mechanical, or chemical signal into a large response
- A single ion channel can detect a single molecule.
- What are the limitations?

How do you make an ion-channel biosensor?

- Make two small silicon bottles
- Connect with a small hole
- Cover the hole with a lipid membrane
- Put channel in the membrane
- Put different test solutions in one chamber
- Measure the current through the channel



What is the gain of a ligand-gated ion channel?



- Gain ≡ the number of ions that pass through the channel for one bound ligand

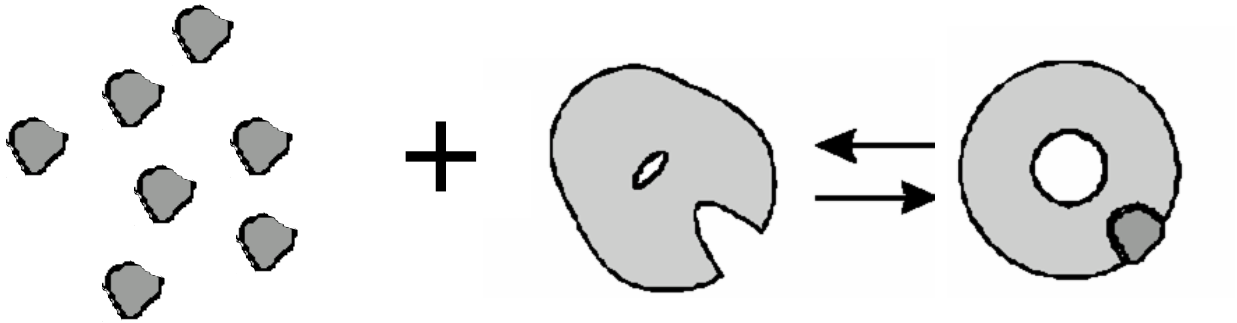
$$I = g \times V \qquad Q = I \times t \qquad N = \frac{Q}{z \times e} \qquad N = \frac{g \times V \times t_{bound}}{z \times e}$$

- 1 ms < t_{bound} < 10 ms
- 10^4 < flux < 10^7 ions per second
- 10 < Gain < 10^5.
- Large channels like gluR0 in normal [K^+] pass about 10^7 ions/s at a 100 mV driving force. In higher [K^+] or *Vm* they will pass more ions. The open time occurs in bursts that typically last for one second. **For these channels, the "gain", *i.e.*, the integrated ion flux/ligand binding, is >10^7**

Does it matter that the binding reaction is bimolecular?

$$L + R_0 \xrightarrow{k} R_1$$

Free Ligand Unbound Bound



Tense (Closed) Relaxed (Open)

"Allosteric Mechanisms in the Activation of Ligand-Gated Channels,"
Meyer B. Jackson, University of Wisconsin - Madison
http://www.biophysics.org/btol/img/Jackson.M.pdf

Ligand binding is a bimolecular reaction

- Unbound Receptor plus Ligand gives Bound Receptor

$$R_0 + L \xrightleftharpoons{k} R_1$$

- The product of [Ligand] and [Unbound Receptor] are important.

$$\frac{[R_1]}{[L][R_0]} = k$$

- There is an energy associated with binding

$$\Delta G = -RT\ell n(k)$$

What you need

- You want the ligand to stick well

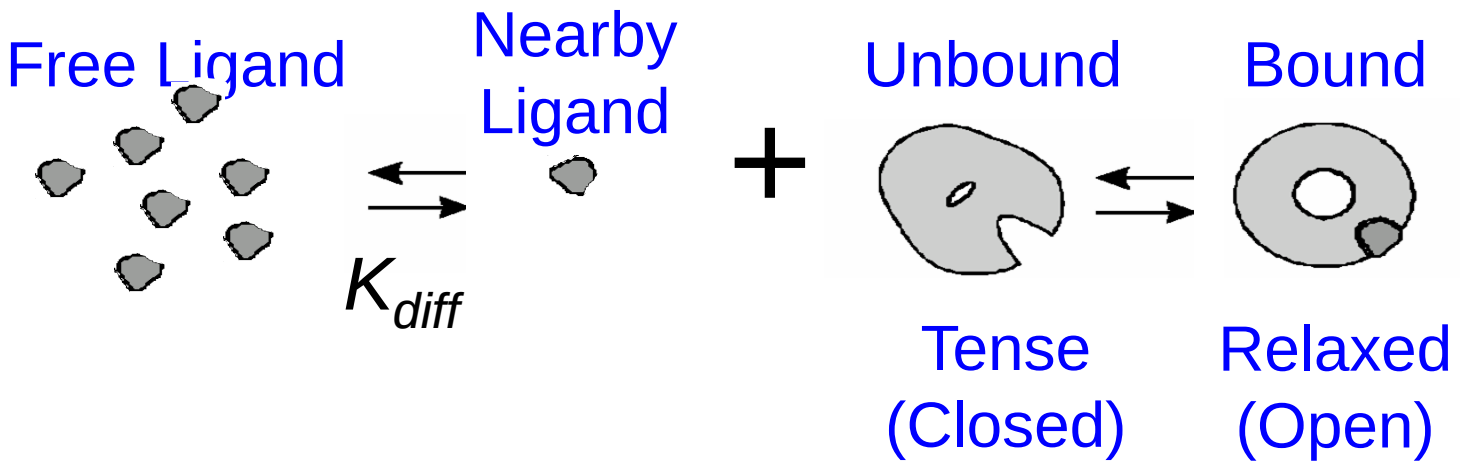
What determines the reaction rate in a bimolecular reaction?

$$\frac{[R_1]}{[L][R_0]} = k$$

- **The detector is a SINGLE receptor**
- Assume one receptor in a (1 micron)^3 bottle
- **$[R] = 1.3 \times 10^{-24}$ mole/femtoliter = 1.3 nM**
- Binding rate = $1/k$ events per second
- So what is the binding rate?

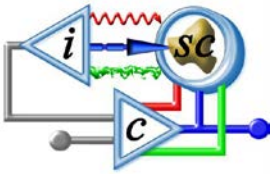
Diffusion limits the binding rate

- For many reactions, the rate of reaction is determined by how long it takes a free ligand to diffuse into the vicinity of the receptor
- $K_{diff} \approx 10^8\ M^{-1}sec^{-1}$ so $K_{on} \approx K_{diff}\,[L]$
- If [L] = 1 nM, $1/K_{on} \approx$ 0.1 binding event/second



1 mM	1 μm	1 nM
10 μs	10ms	10 s

What happens once the ligand is bound?

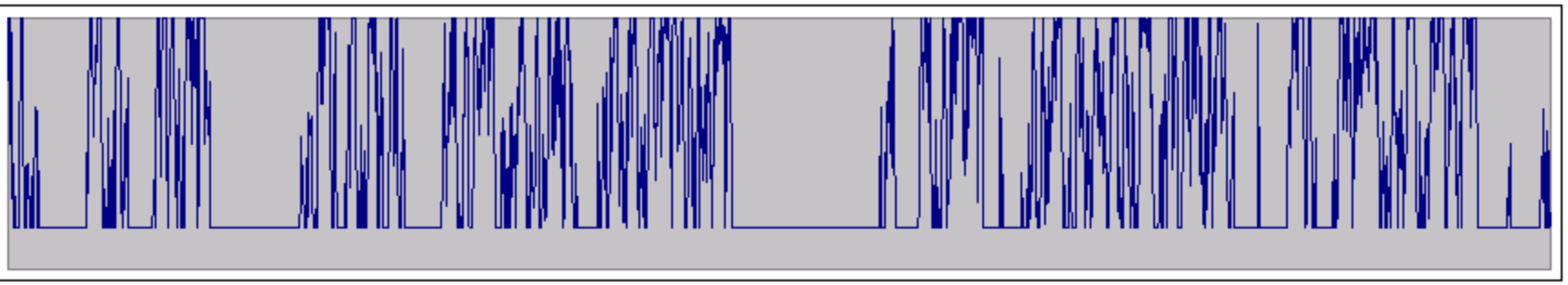


- For a good sensor, once the ligand is bound, it should stay bound long enough to get a good measure of the conductance.
- To measure a concentration, rather than simply the presence of a single molecule, the sensor has to be reused, and hence the ligand must first unbind (dissociate).
- Suppose 10 seconds to measure and 100 seconds to recover. $K_{off} > 0.01$. Can detect [L] ≈ 1 nM
- 10 events will require 1000 sec or 17 minutes

What you need

- You want the ligand to stick well
- You don’t want the ligand to stick so well that you can’t reuse the channel
- You have to measure for a long time to get enough data to get a concentration

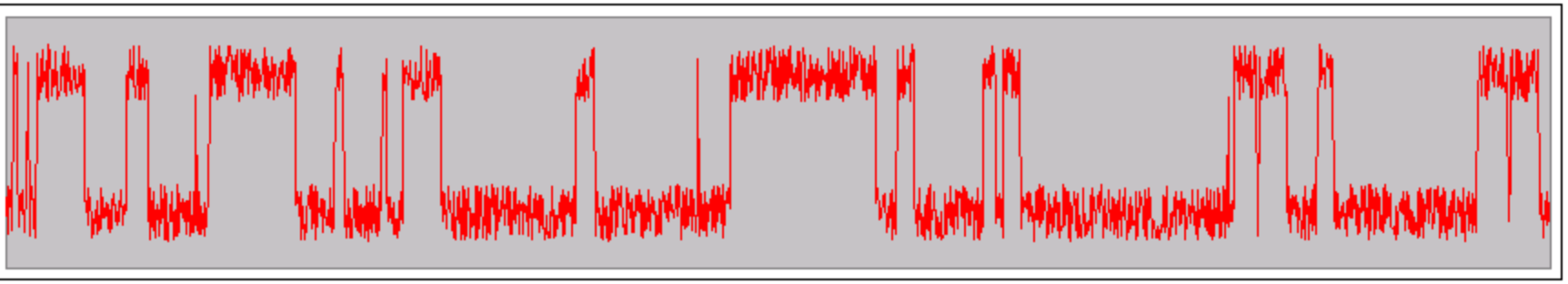
Ligand Position



Diffusing

Bound

Channel Current



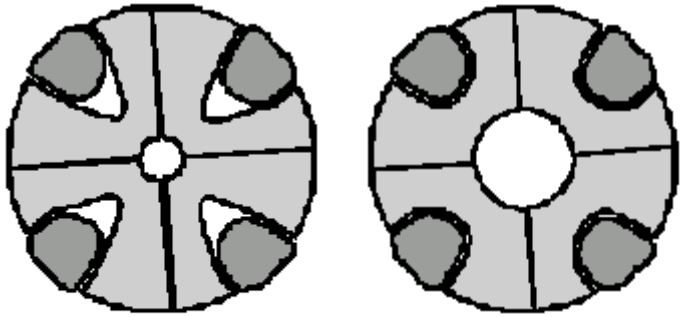
Relaxed

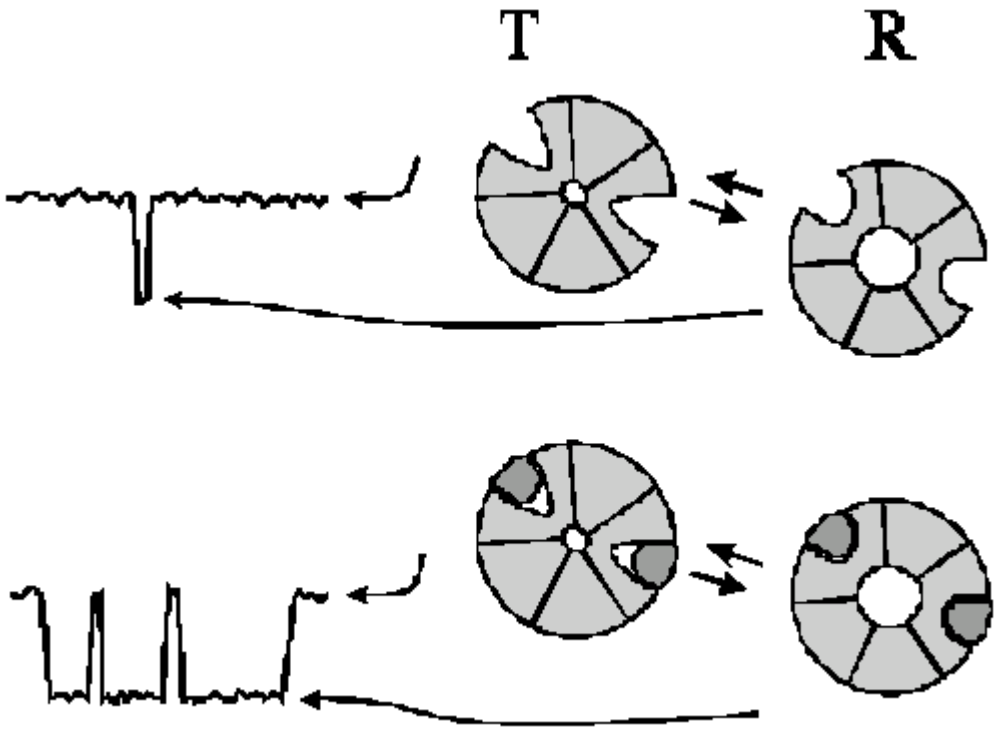
Tense

Average Channel Current	0.462
Binding Probability	0.950
Unbinding Probability	0.020

What are the complications?

- Many ion channels have multiple components that behave cooperatively
- Ion channels blink





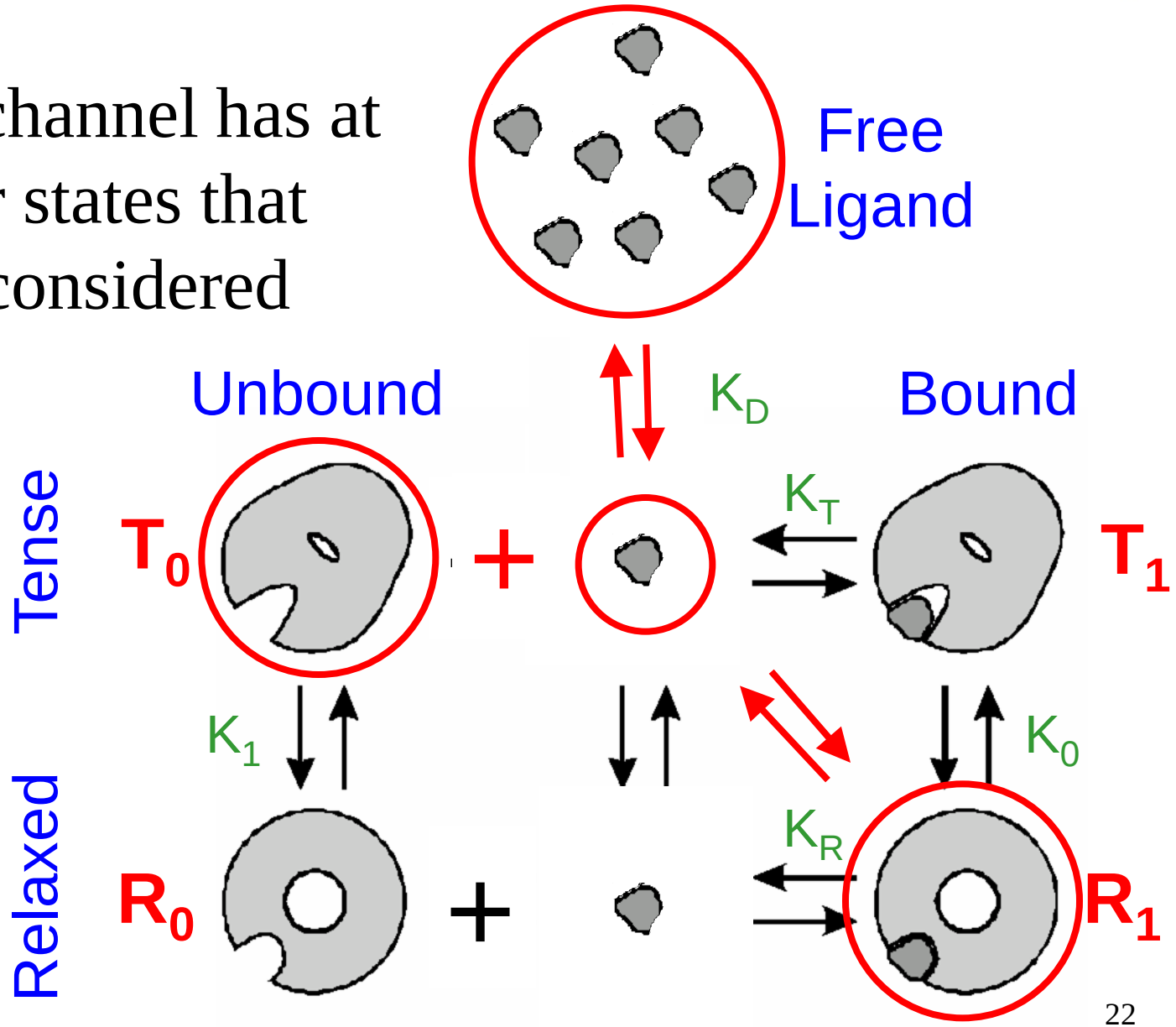
http://www.biophysics.org/btol/img/Jackson.M.pdf

What you need

- You want the ligand to stick well
- You don’t want the ligand to stick so well that you can’t reuse the channel
- You have to measure for a long time to get enough data to get a concentration
- You want the ligand to stick well enough to minimize blinking

A more detailed view

- The ion channel has at least four states that must be considered

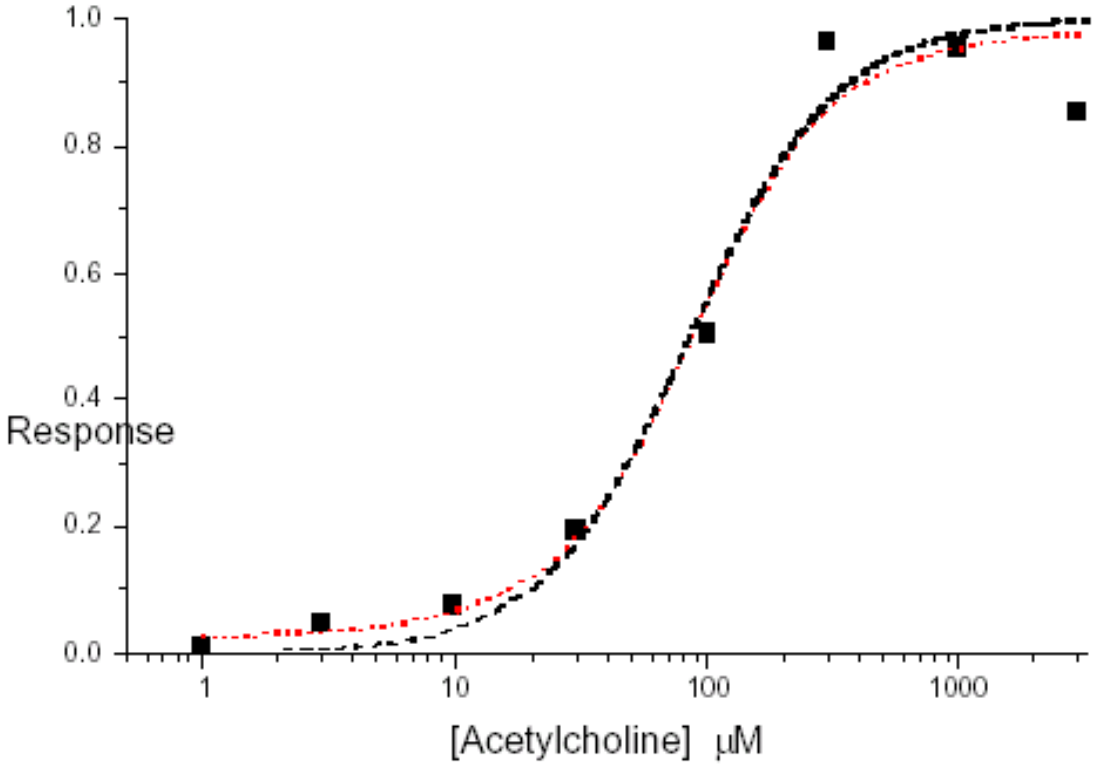


What you need

- You want the ligand to stick well
- You don’t want the ligand to stick so well that you can’t reuse the channel
- You have to measure for a long time to get enough data to get a concentration
- You want the ligand to stick well enough to minimize blinking
- You need to measure a long time to get the channel kinetics

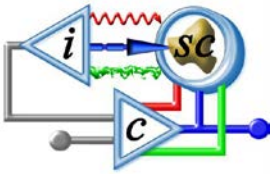
Other problems

- Binding is not a binary event
- Binding is not perfectly specific
- As we said, many channels have multiple binding sites and cooperative binding



http://www.biophysics.org/btol/img/Jackson.M.pdf

Solutions to get faster response and bigger signals



- A single ion channel is infinitely sensitive if you wait infinitely long, but you then couldn't measure the concentration…
- Put the ligand molecule in a $(0.1\ \mu m)^3$ box to get [L] = 1 μM. Detection straightforward thereafter
- Put multiple channels in the patch (~500)
  - Increase the current
  - Increase the probability of getting a binding event
  - Loose information about channel binding dynamics
- Use a massively parallel array of ion channels

Conclusions

- Single channels have a very high internal gain as detectors where binding of one molecule can result in the transport of > 10^7 ions.
- A single-channel chemical detector is not a single molecule detector: it runs on a bimolecular reaction with [R][L].
- Single molecule sensors take time to respond that is dependent upon concentration in a diffusion-limited manner.
- To detect concentration, channel detectors must make repeated cycles of binding and unbinding since concentration is inferred from the time between binding events.
- While channels can be engineered to improve selectivity and responsiveness, diffusion places limits on the maximum speed of response.
- The use of channels as detectors requires the ability to distinguish different compounds in mixtures of different concentrations. This requires large parallel arrays.