Challenges of Single Ion-Channel Biosensors

Fredrick Sachs (SUNY Buffalo)
John P. Wikswo (Vanderbilt)

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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

From Ionic Channels of Excitable Membranes, B. Hille, 1992
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.

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

\[ I = g \times V \quad Q = I \times t \quad N = \frac{Q}{z \times e} \quad N = \frac{g \times V \times t_{\text{bound}}}{z \times e} \]

- \(1 \text{ ms} < t_{\text{bound}} < 10 \text{ ms}\)
- \(10^4 < \text{flux} < 10^7\) ions per second
- \(10 < \text{Gain} < 10^5\).

- Large channels like gluR0 in normal \([\text{K}^+]\) pass about \(10^7\) ions/s at a 100 mV driving force. In higher \([\text{K}^+]\) or \(V_m\) 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?

\[ L + R_0 \xrightleftharpoons[k]{\text{Unbound}} k \xrightarrow{\text{Bound}} R_1 \]

Free Ligand + Unbound \rightarrow Bound

Tense (Closed) \rightarrow Relaxed (Open)

“Allosteric Mechanisms in the Activation of Ligand-Gated Channels,” Meyer B. Jackson, University of Wisconsin - Madison
Ligand binding is a bimolecular reaction

- Unbound Receptor plus Ligand gives Bound Receptor

\[ R_0 + L \overset{k}{\longrightarrow} R_1 \]

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

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

- There is an energy associated with binding

\[ \Delta G = -RT \ln(k) \]
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
- \( [R] = 1.3 \times 10^{-24} \text{ mole/femtoliter} = 1.3 \text{ nM} \)
- 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

  \[ K_{diff} \approx 10^8 \text{ M}^{-1}\text{sec}^{-1} \text{ so } K_{on} \approx K_{diff} [L] \]

• If \([L] = 1 \text{ nM}, 1/K_{on} \approx 0.1 \text{ binding event/second} \]

<table>
<thead>
<tr>
<th>1 mM</th>
<th>1 µm</th>
<th>1 nM</th>
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<tbody>
<tr>
<td>10 µs</td>
<td>10 ms</td>
<td>10 s</td>
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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 $[L] \approx 1$ 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

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Ligand Position

Channel Current

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

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

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 \( \mu \text{m} \))^3 box to get \([L] = 1 \mu\text{M}\). 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.