1. What are the different types of ion channels in the cell? How do they differ from one another? Give an example of each type.

- **Voltage-gated**, **Ligand-gated**, **Stretch or Pressure-gated**, **Phosphorylation gated**, **K⁺ leak channels.** Voltage gated Na⁺ channels open upon depolarization of the membrane containing the channel. Acetylcholine receptors at neuromuscular junction open after binding two acetylcholine (ACh) molecules. This in turn increases conductance of Na⁺ and K⁺.

2. What contributes to the ion selectivity of Na⁺ channels? Why can’t other ions pass through? The ion channel is size specific. Other ions, such as potassium, are too big to pass through the channel.

3. Explain how K⁺ channels show selectivity when they allow ions to pass through. Why can’t Na⁺ ions pass through the K⁺ channels if they’re smaller in size?

   K⁺ ions are coordinated by the carbonyl oxygen of the “signature sequence” of the potassium channel. The partial charge of the backbone carbonyl oxygen behave like surrogate water oxygen atoms. K⁺ ions sense little change as they move from bulk solvent (H₂O) to the selectivity filter. Na⁺ ions do not enter because the walls of the selectivity filter of K⁺ channels are too far apart to stabilize dehydrated Na⁺ ions.

4. You are given a chemically fixed neuron with all of the membrane proteins intact. How would you determine the number of sodium channels in the surface membrane?

   **Radiolabeled tetrodotoxin (TTX) has a high affinity and will bind to the sodium channels and autoradiography can be used to count the number of binding sites.**

5. (a) You are given a slice of brain tissue (all of the cells are alive and healthy) and asked to determine the number of active channels of a particular type in a particular neuron when it is clamped at a certain voltage. How would you do this? (hint: think about macroscopic and microscopic currents)

   Measure the macroscopic current (Iₘ) and the microscopic (iₘ) current at the same voltage. Find the probability of the ion channels being open at that particular voltage (P_open=(t_open)/total time). Iₘ/(iₘ*P_open)=N. N=the number of active channels.

   (b) Using patch recording, you determine γ for a specific Na⁺ channel at to be 30 pS (3.0 x 10⁻¹¹ Siemens). If the peak of the Na⁺ current in the whole cell at – 15 mV is measured to be 2 nA (2 x 10⁻⁹ A), what is the total number of Na⁺ channels in the whole cell? (Remember from lecture 3 that V_half in the Na⁺ channel activation curve is -15 mV and let’s assume that in our experiment E_Na = +50 mV).

   \[ i = \gamma(V_m-E_{Na}) = 30 \text{ pS} \times 65 \text{ mV} = 1950 \times 10^{-15} \text{ A} = 1.95 \text{ pA} \]

   Use the equation \( I = i \times N \). \( N \) is the number of active channels, \( i \) is the current through a single channel, and \( I \) is the total current. \( N_{tot} = N \times P_0 \); at \( V_{half} \) Po = 0.5; Therefore: 2 nA = 1.95 pA x N. Solving for N, we see that this cell has 1025 Na⁺ channels.

   \( N_{tot} = 2050 \text{ channels} \)

6. Explain the patch clamp technique.

7. What is the gating current? How would you measure it?

   The gating current is a small current across the membrane that occurs when a channel opens. It presumably arises because the conformation shift in the protein underlying the channel opening event results in charges being shifted across the membrane. To measure
the gating current, you must voltage clamp a membrane expressing several voltage gated
Na⁺ channels (e.g. the giant axon of a squid; the gating current is too small to be resolved in
an individual channels). Then, you must eliminate the ionic current due to the sodium ions.
Remove the sodium, then apply a voltage step. The current observed is the gating current.

8. If the N-terminus of the Na⁺ channel is cut, what happens to the Na⁺ channel? Why?
The current does not inactivate since inactivation depends on the N-terminal Ball-chain
mechanism.

9. What are the different methods for studying the structure and function of ion channels?
Sequence analysis (Hydropathy plots); Predict Structure from Amino Acids; Express in a
cell line of choice; Site-Specific Mutagenesis

10. After obtaining the DNA sequence for an ion channel, the amino acid sequence can be
deciphered. What can you do to see which specific sequences play a role in the function of the
channel?
The channel can be expressed in a cell line of choice. Then, studies can be made to observe
the characteristics that are inherent to that specific channel. Now, since you have the DNA
sequence, you can make site specific mutations so that the amino acid sequence differs from
your wild type. Perform the same tests, and compare and contrasts the results. Important
things to keep in mind are structure and function.

11. A channel is open ten times per second. Each opening lasts an average of 20 milliseconds.
What is the open probability of this channel?

.20

This channel is selective for a monovalent cation with an equilibrium potential of –100mV. The
conductance of the channel is 100 pS (1 pico siemens = 10⁻¹² S). How many charges pass through
the channel in one second when the membrane potential is held at –70mV?

Driving force=30mV
\[ \gamma = \frac{i}{(V_m - E_{eq})} \] (microscopic conductance= microscopic current/driving force)

100 pS=i/30 mV

\[ i = 3 \text{ pA (1 picoamper = } 10^{-12} \text{ A)} \]

\[ Q = i \cdot 0.2s \] (charge in coulombs (C) =current*seconds)

\[ Q = 3 \cdot 10^{-12} \text{ A} \cdot 0.2s = 6 \cdot 10^{-13} \text{ C} \]

1 coulombs contains \( 6.2 \cdot 10^{18} \) elementary charges

\[ 6 \cdot 10^{-13} \text{ C} \cdot 6.2 \cdot 10^{18} = 37.2 \times 10^5 \text{ ions in 200ms} \]

12. If a channel is ligand-gated, what are the factors that determine whether or not it’s open?
Presence (concentration) of the ligand
Activation and inactivation kinetics of the channel

13. What is the general structure of a voltage-gated channel?

Four subunits each with 6 transmembrane domains (S1 to S6; for the sodium channels and
the calcium channels the four subunits are linked together in a large alpha subunit). S4 is
the voltage sensor. The P-loop region, between S5 and S6 faces the poor and contains the
selectivity filter. In some channels the N-terminal is essential for the inactivation (ball-
chain)
14. What is the accessibility analysis and how was it used to study the structure of Na+ and K+ channels?

It addresses the accessibility of a reagent to specific residues of the protein. In the case of voltage gated channels, accessibility analysis (with the thiol reagent MTSET) allows to test for the possibilities that residues that are buried (and hence not exposed to the reagent) in the lipid bi-layer become exposed to the intracellular or extracellular bulk solution with changes in Vm. Since MTSET reacts with Cysteine residues, some of the S4 Arginines were mutated into Cysteines. This kind of experiment demonstrated that S4 moves with respect to the lipid bi-layer when Vm changes, and suggests that S4 is the voltage sensor.