Ions in solution

Properties of ions in solution

To make up some form of physiological saline you would dissolve salts such as KCl, NaCl, $CaCl_2$ etc in water.

Salts are ionic compounds containing two species of ions defined as cations (+) and anions (-) on the basis of their attraction to either a negatively charged cathode or positively charged anode.

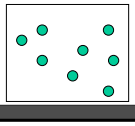
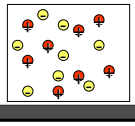
Properties of ions in solution

In dilute solutions salts dissociate fully.

Component ions are for the most part independent of each other.

Each ion will have characteristic properties irrespective of the oppositely charged ion from the same molecule.

Dissociation of component ions of salts explains the differences in physical characteristics of solutions of electrolytes and non-electrolytes. The relative difference in properties such as osmotic pressure arise from the contribution of both component ions to the property of the solution of an electrolyte.

10 mM sucrose   10 mM KCl

Properties of ions in solution

Ions move in solution by diffusion

Einstein (1905) demonstrated that diffusion could be described as a microscopic random walk of particles analogous to Brownian motion.

As a result of thermal agitation all particles will be in motion. Particles will travel in a straight line until they collide with other particles and change their direction of travel.

The random motion of particles such as ions accounts for diffusion down a concentration gradient and requires no force to drive the process.

Properties of ions in solution

Electrodiffusion

The motion of an ion in solution can be described by diffusion.

However when considering the movement of ions in biological systems we have to take into account an additional influence.

The movement of an ion across a biological membrane may be influenced by an electric field generated across the membrane.

The movement of an ion in solution within an electric field is described by electrodiffusion.

Properties of ions in solution

Electrodiffusion

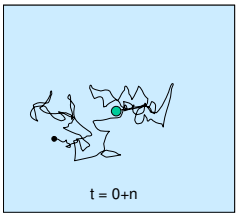
Electrodiffusion of an ion can be considered as the sum of diffusional and electrophoretic motions, a movement of ions along an electric field.

The ions have a net drift down a potential gradient whilst simultaneously spreading as a result of thermal agitation.

Alan Hodgkin described diffusion as being equivalent to a flea hopping and electrodiffusion as being equivalent to a flea hopping in a breeze.

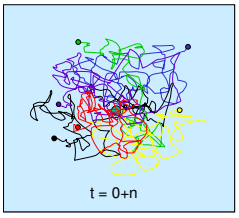
Properties of ions in solution

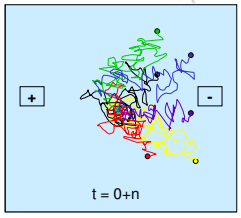
A comparison of diffusion & electrodiffusion using fleas



Properties of ions in solution

A comparison of diffusion & electrodiffusion using fleas





Diffusion: As a result of thermal agitation all particles will be in motion and will travel in a straight line until they collide with other particles and change their direction of travel. Diffusion down a concentration gradient requires no force to drive the process.

Electrodiffusion: Electrodiffusion of an ion can be considered as the sum of diffusional and electrophoretic motions. The ions have a net drift down a potential gradient whilst simultaneously spreading as a result of thermal agitation.

Properties of ions in solution

Interim summary (1.)

- IONS IN SOLUTION ARE INDEPENDENT.
- IONS MOVE IN SOLUTION BY DIFFUSION.
- IN AN ELECTRIC FIELD IONS MOVE BY ELECTRODIFFUSION.

Properties of ions in solution

Ions interact with water

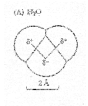
We have noted that ions of salts such as KCl and NaCl fully dissociate in dilute aqueous solution.

This happens because in a polar solvent such as water the solvent molecules are so strongly attracted that the ions loose association with each other and become free.

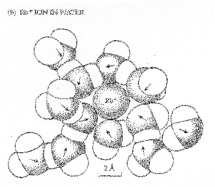
Properties of ions in solution

Interactions between ions and water are strong

Water molecules are strong permanent dipoles.



Hydration energy is the stabilisation gained by orientating water molecules appropriately and polarising their electron clouds in the intense local field of the ion.



From: Hille (2001)

Properties of ions in solution

Interactions between ions and water are strong

Ionic hydration energies are large. They are comparable with the energies holding salt crystals together.

Hydration energies are highest for small ions and for ions with large ionic charge.

TABLE 10.4 Pauling Radii and Ionic Hydration Energies

Anion or group	Radius (Å)	$\Delta H^\circ_{hydration}$ (kcal/mol)
H^+	--	-269
Li^+	0.60	-131
Na^+	0.95	-105
K^+	1.33	-85
Rb^+	1.48	-79
Cs^+	1.69	-71
Tl^+	1.49	---
Mg^{2+}	0.65	-476
Ca^{2+}	0.99	-397
Sr^{2+}	1.13	-361
Ba^{2+}	1.35	-328
Mn^{2+}	0.80	-456
Cu^{2+}	0.72	-503
Ni^{2+}	0.72	-517
Zn^{2+}	0.74	-503
F^-	1.36	-114
Cl^-	1.81	-82
Br^-	1.95	-79
I^-	2.16	-65
H	1.20	---
Methyl	2.0	---
N	1.5	---
O	1.40	---

Radii from Pauling (1960). Standard enthalpies of hydration at 25°C are taken from Edsall and McKenzie (1978), who also give entropies and free energies of hydration.

From: Hille (2001)

Properties of ions in solution

Interim summary (2.)

- IONS IN SOLUTION ARE INDEPENDENT.
- IONS MOVE IN SOLUTION BY DIFFUSION.
- IN AN ELECTRIC FIELD IONS MOVE BY ELECTRODIFFUSION.
- IN SOLUTION IONS INTERACT STRONGLY WITH WATER.
- THE STRENGTH OF THESE INTERACTIONS VARIES WITH THE CHARGE AND SIZE OF THE ION.

How are ions transported across membranes?

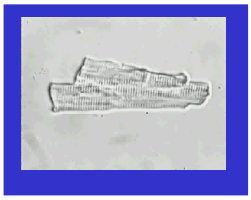
It is clear from the preceding discussion that a major energetic obstacle to the movement of an ion from solution into, and eventually across, a hydrophobic membrane would be the need to overcome the stabilisation of an ion by water.

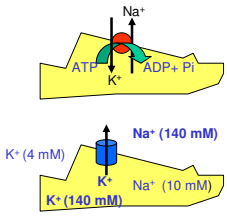
Biological membranes contain two systems that enable this to happen.

- Transport proteins that bind ions on one side of the membrane and use the energy provided by ATP or an electrochemical gradient to deliver the ion to the other side of the membrane.
- Channel proteins that are, in essence, aqueous pores spanning the membrane.

Cardiac Electrophysiology & Arrhythmias

The electrical and mechanical activities of cardiac muscle cells depend upon the differential separation of ions across the cell membrane.





How are ions transported across membranes?

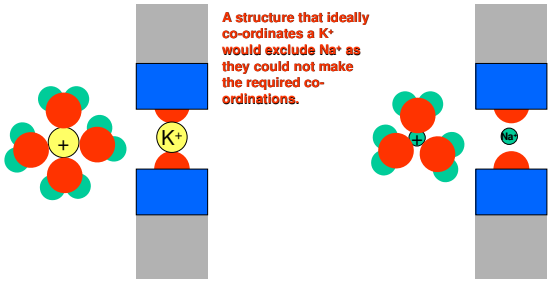
The most obvious feature distinguishing these two systems is the rate at which ions are translocated. A typical ATP-driven ion pump such as the Na/K ATPase can transport up to 500 ions per second.

In the same time a typical channel can allow in excess of 10^6 ions to cross a membrane.

How can an ion channel maintain such enormous rates of ion translocation? To make matters worse, ion channels can translocate 10^8 ions per second and simultaneously discriminate between cations as closely related as K^+ and Na^+.

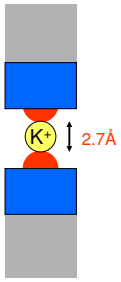
Mechanisms of ion discrimination in ion channels

Pores of selective ion channels provide structures that substitute for some or all of the hydration shell of ions.



Mechanisms of ion discrimination in ion channels

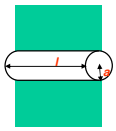
Channel pores of this nature would be very narrow. To co-ordinate a dehydrated K^+ a pore would have a radius of 1-2 Å.



Could a pore with a radius of 1-2 Å translocate 10^6 ions per second??

Rates of ion translocation in ion channels.

We can estimate this by constructing a model pore consisting of a cylinder with an appropriate radius and a length equivalent to the thickness of a typical phospholipid bilayer membrane.



The resistance in such a structure filled with Ringer's solution with l = 50Å and a=1.5Å, R_{pore}=1.4GΩ and conductance (1/ R_{pore}) is 14pS

which is equivalent to a flux of approximately 8.5×10^6 ions per second.

Rates of ion translocation in ion channels.

The rates of ion translocation calculated for our model pore are impressive, however a conductance of 14 pS is considerably less than values achieved by many selective ion channels .

How could the conductance of a channel be increased still further whilst retaining selectivity?

- ***The maximal conductance of a channel will be limited by the rate of exit of ions from the channel.***

The rate of exit of an ion from the channel.

If we accept that pores must be narrow to permit discrimination between similar ions there is a very obvious structural modification that would increase rates of ion exit from the pore. Based on our earlier calculations with a model pore rates of translocation could be enhanced by shortening the pore.

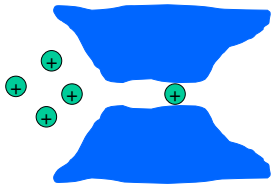
The rate of exit of an ion from the channel.

If we accept that pores must be narrow to permit discrimination between similar ions there is a very obvious structural modification that would increase rates of ion exit from the pore. Based on our earlier calculations with a model pore rates of translocation could be enhanced by shortening the pore.

Shortening the pore effectively brings the bulk solution into the membrane and creates a short "selectivity filter" where ion discrimination can take place and through which ions cross the membrane.

The rate of exit of an ion from the channel.

Functional evidence indicates that most cation-selective channels can contain more than one ion simultaneously in the channel pore. Such a mechanism may contribute to high rates of ion exit as a result of electrostatic repulsion.



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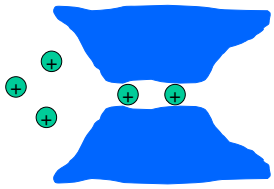

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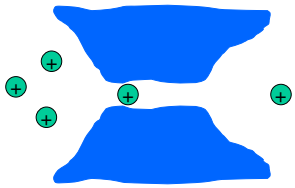
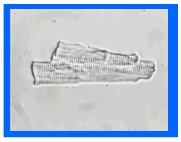
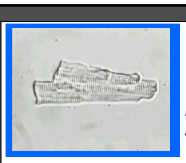




SUMMARY (1)

The electrical and mechanical activities of cardiac muscle cells depend upon the differential separation of ions across the cell membrane.

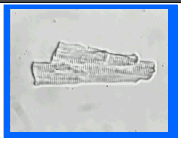
- IONS IN SOLUTION ARE INDEPENDENT.
- IONS MOVE IN SOLUTION BY DIFFUSION.
- IN AN ELECTRIC FIELD IONS MOVE BY ELECTRODIFFUSION.



SUMMARY (2)

The electrical and mechanical activities of cardiac muscle cells depend upon the differential separation of ions across the cell membrane.

- IN SOLUTION IONS INTERACT STRONGLY WITH WATER.
- THE STRENGTH OF THESE INTERACTIONS VARIES WITH THE CHARGE AND SIZE OF THE ION.
- ION CHANNELS ARE AQUEOUS PORES.
- RATES OF ION TRANSLOCATION ARE VERY HIGH.
- ION DISCRIMINATION IN CHANNELS INVOLVES SUBSTITUTION OF HYDRATION SHELL BY COMPONENTS OF PORE.



SUMMARY (3)

The electrical and mechanical activities of cardiac muscle cells depend upon the differential separation of ions across the cell membrane.

- THE ABILITY OF ION CHANNELS TO MAINTAIN VERY HIGH RATES OF ION TRANSLOCATION WHILST DISCRIMINATING BETWEEN IONS OF SIMILAR SIZE MAY REFLECT STRUCTURAL SPECIALISATIONS.

THESE ARE LIKELY TO INCLUDE:

- WIDE MOUTHS THAT SHORTEN THE PORE (BRING THE BULK SOLUTION INTO THE MEMBRANE).
- A NARROW SELECTIVITY FILTER (MAY CONTAIN MORE THAN ONE ION AT A TIME) OVER WHICH TRANS-MEMBRANE POTENTIAL FALLS.

Resource material

The topics presented in this lecture are covered in considerably more detail in:

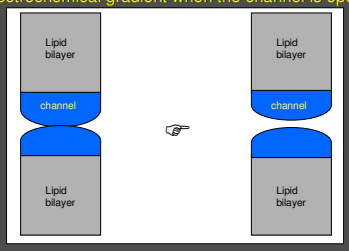
Ion Channels of Excitable Membranes. Hille, B.

Chapters on Elementary properties of Ions in Solution & Elementary Properties of Pores can be found in all three editions of this excellent book (published respectively in 1984,1992 & 2001 by Sinauer).

The structure of ion channels

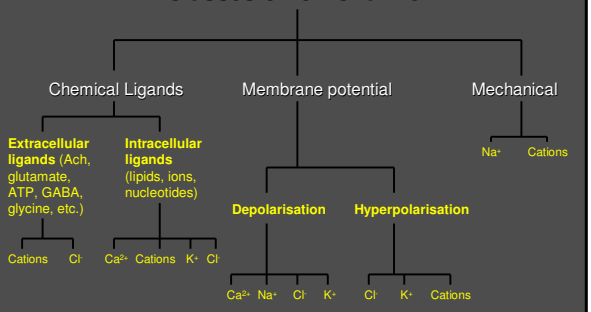
The Structure of Ion Channels

Theoretical and experimental evidence supports the proposal that ion channels are membrane spanning structures with an axial aqueous pore in which is located a structure that discriminates between ions. Ions move down an electrochemical gradient when the channel is open.



A complete understanding of the mechanisms governing ion translocation, ion discrimination and gating will require knowledge of the structure of channel proteins at atomic resolution.

Classes of Ion Channel



Structure of Ion Channels

- Ion channels are membrane proteins. The first step in determining structure is the purification of the channel.
- Channel proteins must be liberated from membranes by an appropriate detergent.
- The channel of interest is then separated from other solubilised membrane proteins and its composition assessed by SDS PAGE.
- Many ion channels are multimeric complexes.

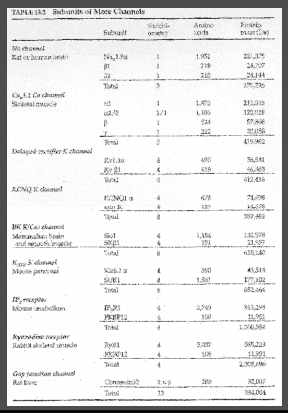
Sub-unit composition of channels (I).

TABLE 13.1 Subunits of Some Ligand-Gated Channels

	Subunit	Stoichiometry	Amino acids	Protein mass[a] (Da)
Nicotinic ACh receptor				
Torpedo electric organ	α	2	437	50,116
	β	1	469	53,681
	γ	1	489	56,279
	δ	1	501	57,565
	Total	5		267,757
Glycine receptor				
Rat spinal cord	α1	*n*	421	48,383
	β	5 – *n*	474	53,428
$GABA_A$ receptor				
Bovine brain	α1	2	429	48,800
	β1	2	449	51,400
	γ2	1	442	50,400
	Total	5		250,800
Glutamate (AMPA) receptor				
Rat brain	GluR1	2	889	99,769
	GluR2	2	862	96,400
	Total	4		392,338
cAMP-gated channel				
Human retinal rods	CNG1	2	690	79,760
	CNG4	2	909	102,337
	Total	4		364,194

[a]The molecular weight is for the predicted protein part of the mature subunit. All channels have 5–30% additional weight from sugar residues.

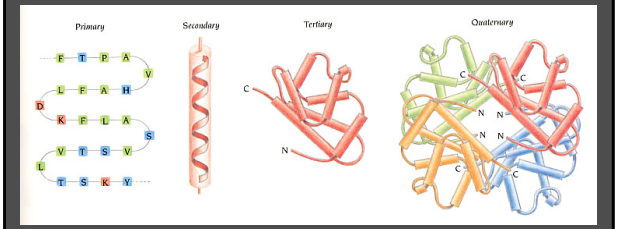
Sub-unit composition of channels (II).



Structure of Ion Channels

Having established the sub-unit composition of an ion channel the "structure" of the various components can be investigated.

As with all proteins the structure of a channel protein can be examined at various levels of complexity.



Determination of the primary sequence of an Ion Channel sub-unit.

The primary sequence of a purified sub-unit is generally determined by a combination of protein sequencing and molecular biological techniques.

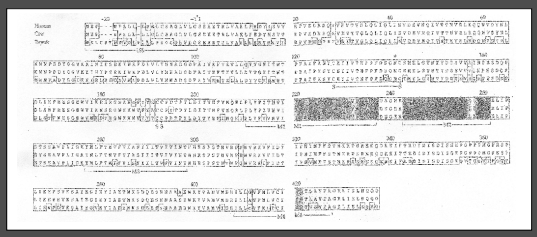
Sequencing of the N-terminus of the polypeptide permits the synthesis of DNA probes that are used to screen cDNA libraries.

The isolation of overlapping cDNA clones comprising the complete coding sequence of the protein.

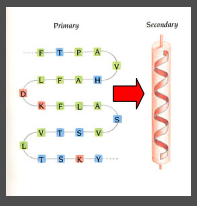
The primary amino acid sequence of the protein can then be deduced from the nucleotide sequence.

The size of Ion Channel sub-units varies.

Primary sequence of α subunit of nACh receptor from three species. Each contains less than 450 residues.



Predictions of secondary structure & topology

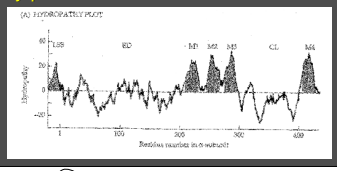


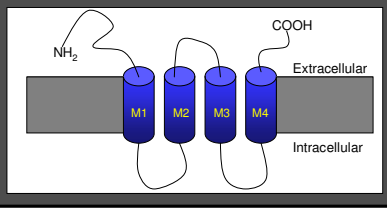
The primary sequence of amino acids of a protein determines its secondary structure. Armed with the primary sequence it is possible to predict regions of the protein molecule that are likely to form an α-helix or a β-sheet.

Similarly, assessment of the relative hydrophobicity of sequences of amino acid residues allows for the prediction of the probability of an α-helix or β-sheet forming a trans-membrane domain.

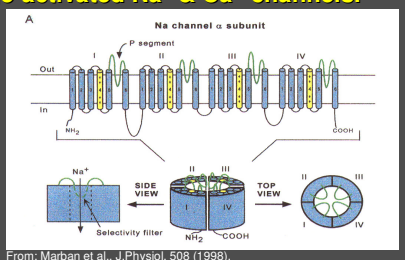
Predictions of secondary structure & topology

Hydropathy plot for the α-subunit of the nACh receptor .





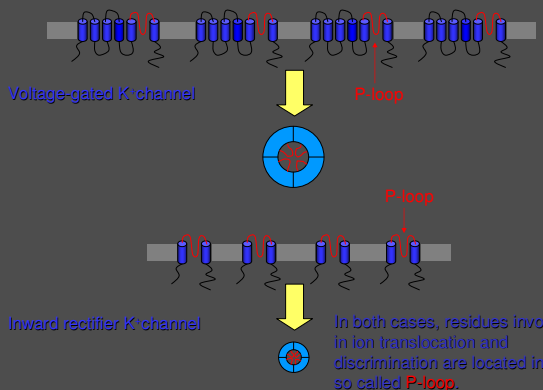
Secondary structure & topology predictions for voltage-activated Na^+ & Ca^{2+}channels.



From: Marban et al., J.Physiol. 508 (1998).

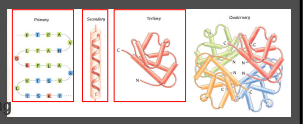
Voltage-gated Na^+ and Ca^{2+}channels have 4 repeat domains (I-IV). In both cases the fourth TM-helix in each repeat has an excess of positively charged residues (voltage sensor) and the extracellular loop linking TM-5 and TM-6 contains residues known to be involved in ion conduction and discrimination.

K^+channels are homotetramers.



In both cases, residues involved in ion translocation and discrimination are located in the so called P-loop.

Structure of Ion Channels



Summary

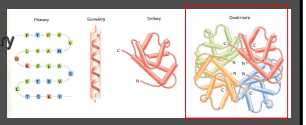
Ion Channels are membrane spanning multimeric proteins.

The primary structure of a wide range of channel proteins has been deduced.

The secondary structure of the membrane spanning regions of various of these channels has been predicted.

Amino acid residues involved in various aspects of channel function have been identified.

Structure of Ion Channels



The gross morphology of the quaternary structure of channel proteins can be determined using cryo-electron microscopy.

Individual channel proteins visualised and 3D structure obtained by angular reconstitution. Resolution ~25Å.

Sarcoplasmic reticulum Ca^{2+}-release channel (RyR).

Homotetramer - monomer ~ 550kDa.

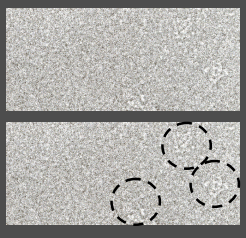
Dihydropyridine-sensitive Ca^{2+} channel (L-type).

Shaker K^+ channel (Kv1.1).

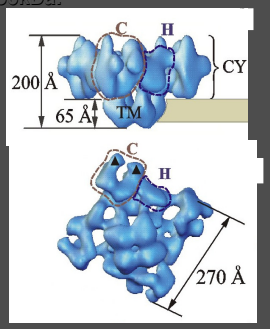
Homotetramer - monomer ~ 74kDa.

Sarcoplasmic reticulum Ca^{2+}-release channel (RyR).

Homotetramer - monomer ~ 550kDa.



Images of individual homotetramers in cryoEM.



3D structure of individual homotetramer obtained by angular reconstitution.

Resolution ~25Å.

From: Orlova et al., (1999)

Dihydropyridine-sensitive Ca^{2+}channel (L-type).

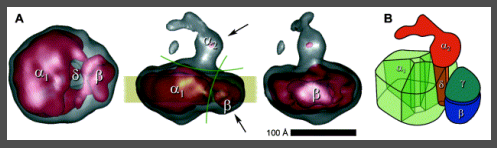


Figure 6. A, Three orthogonal views of the refined 3D structure of the dihydropyridine receptor, filtered at 23 Å resolution. The reconstruction was corrected for the contrast transfer function of the electron microscope. The volume in gray encloses approximately 550 kDa. When contoured at a higher density level (red volume), the globular density is separated by a gap into a larger and smaller density, indicating possible boundaries of channel subunits. Labels indicate the putative locations of individual subunits. Left: view facing the cytosolic side. Middle: view along the membrane plane (depicted by a yellow bar) with the extracellular $_2$ subunit facing upwards. The green arcs indicate how the structure was divided to estimate the molecular masses of individual subunits (see discussion). The two arrows point to approximate binding locations of the respective monoclonal antibodies. The size of the subunit is most likely underestimated in this rendering (see discussion). Right: view along the membrane plane after rotating the structure by 90 °from its orientation shown in the middle panel. The surface views were generated with the molecular viewer CHIMERA (http://www.cgl.ucsf.edu/chimera/) [84]. B, Cartoon of the L-type calcium channel, summarizing our results together with previously published data on subunit interactions. It assumes pseudo-4-fold symmetry of the $_1$ subunit. The view shows the extracellular side with the $_1$ subunit. The $_2$, and subunits are embedded into the lipid membrane (not shown), which separates the extracellular $_2$ subunit from the cytosol. $_2$ is anchored via the disulfide-linked subunit within the $_1$ subunit. The proposed model allows for a tight interaction between , and as well as and .

J Mol Biol. 2003 Sep 5;332(1):171-82.
Visualization of the domain structure of an L-type Ca^{2+} channel using electron cryo-microscopy.
Wolf M, Eberhart A, Glossmann H, Striessnig J, Grigorieff N.

Reconstruction of Dihydropyridine-sensitive Ca^{2+}channel-RyR Complex.

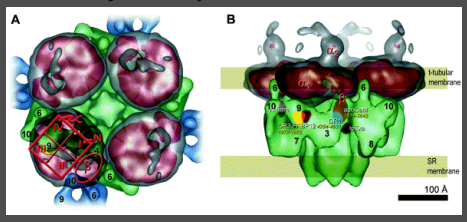
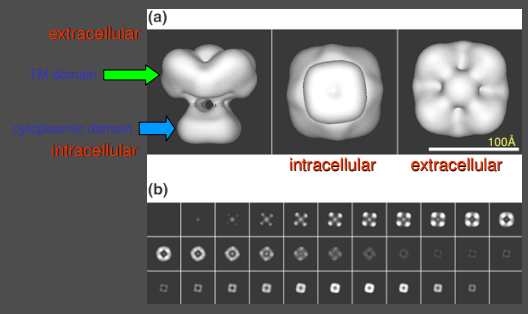


Figure 10. Model of DHPR–RyR complex. EC coupling in skeletal muscle is assumed to consist of a complex between the calcium release channel (RyR, green) and the voltage sensing L-type calcium channel (DHPR, grey and red). DHPRs are arranged in assemblies of four, called tetrads, on every other RyR. Views from the t-tubular side (A) and parallel (B) with the membranes. The red square in A indicates the putative $_1$ domain structure. Divergent region 3 (DR3) of the RyR (yellow in A and B) can interact with fragments of the DHPR II–III loop [28]. Fragments overlapping with the apo-calmodulin binding site (orange in B) were shown to bind to the DHPR subunit [26]. The N terminus and parts of the C terminus are shown to point into the center of the tetrad (see discussion). Inter-DHPR spacing is 16 nm. The height of the junctional gap as shown corresponds to 13.3 nm, consistent with the results of Takekura *et al.* [85]. The inter-RyR spacing between tetrad-occupied RyRs is 42.9 nm. If RyRs are arranged as indicated by a neighboring RyR (blue structure). The black numbers designate RyR regions as used by Wagenknecht *et al.* [42]. The yellow numbers indicate RyR amino acid residues in the identified regions or binding sites (white text). Indicated locations: N terminus, [86] and [81]; DR3, [28]; FKBP12, [82]; DR1, [83]; apoCaM, [26]; Ip3ka, [84]. The scale bar represents 100 Å.

J Mol Biol. 2003 Sep 5;332(1):171-82.
Visualization of the domain structure of an L-type Ca^{2+} channel using electron cryo-microscopy.
Wolf M, Eberhart A, Glossmann H, Striessnig J, Grigorieff N.

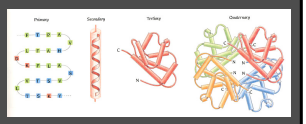
Shaker K+ channel (Kv1.1).

Homotetramer - monomer ~ 55kDa.



From Sokolova et al., Structure 9, 215 (2001)

Structure of Ion Channels

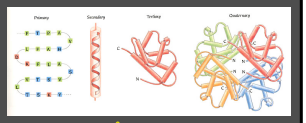


Gross morphology of the quaternary structure of channel proteins provides little or no information on the relationship between the structure of the protein and its function.

This information can only be provided by knowledge of the structure of a channel protein at atomic resolution.

This level of resolution can be reached by X-ray crystallography, which is a relatively straightforward procedure for soluble proteins, but much more difficult for membrane proteins.

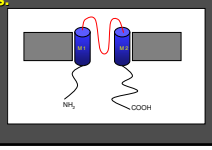
Structure of Ion Channels



The structure of a K^+ channel obtained at Å resolution!

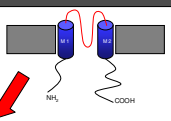
Rod MacKinnon produced 3D crystals of a bacterial K^+ channel (KcsA from *Streptomyces lividans*).

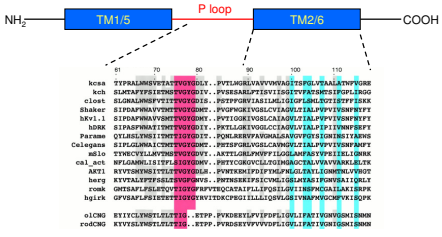
KcsA resembles the eukaryotic inward rectifier in having only 2 membrane-spanning domains.



Structure of Ion Channels

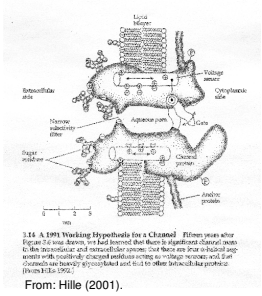
The primary sequence of KcsA closely resembles that of TM5-P loop-TM6 region of other K^+ channels.

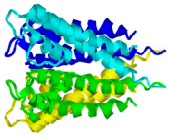




Structure of Ion Channels

The structure of a K^+ channel obtained at 3.2 Å resolution!

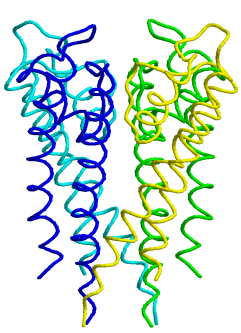




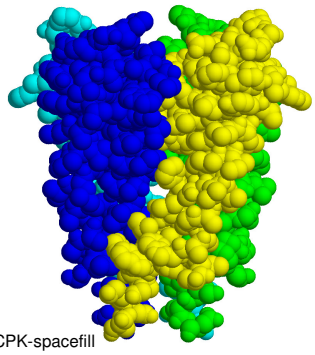
From: Hille (2001).

The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))



Backbone

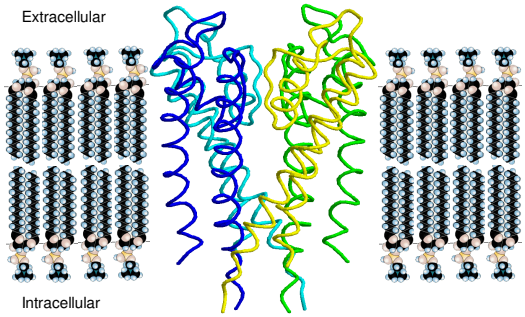


CPK-spacefill

The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))

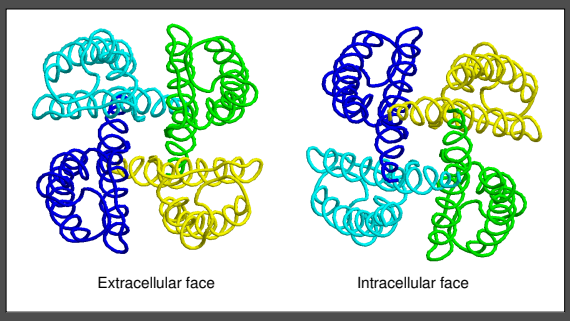
Extracellular



Intracellular

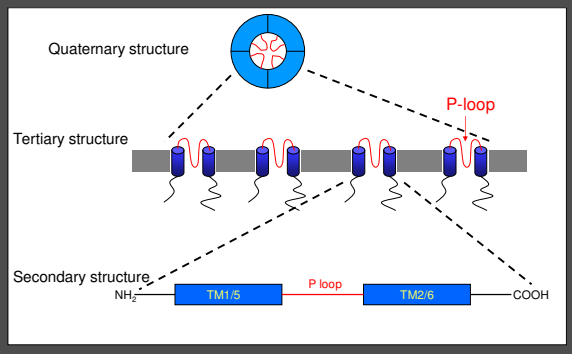
The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))



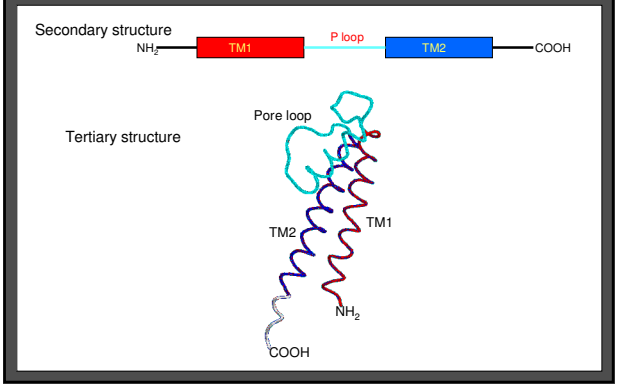
The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))



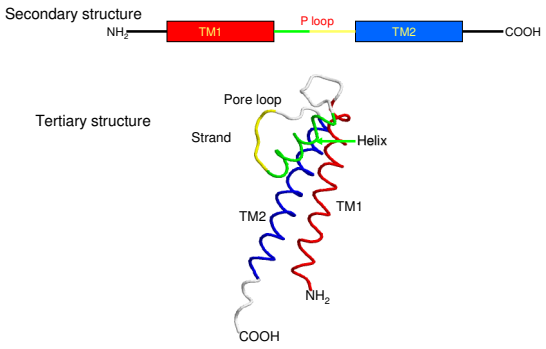
The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))



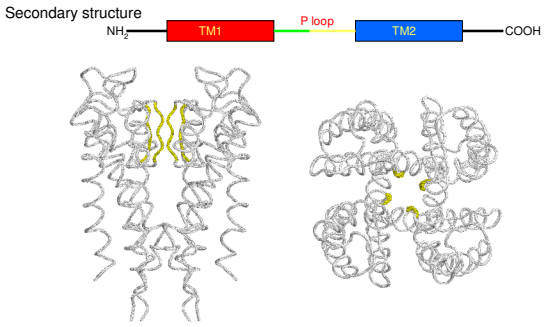
The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))



The structural elements of KcsA

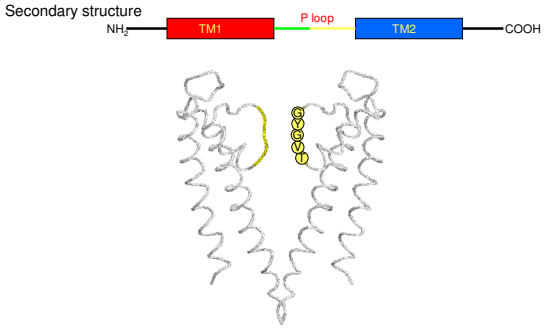
(Doyle et al. Science, 280 69-77 (1998))



The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))

Localisation of residues identified by mutagenesis of Shaker.

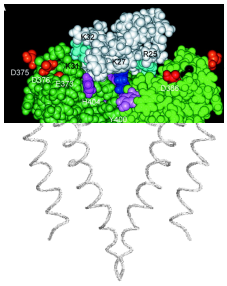


The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))

Localisation of residues identified by mutagenesis of Shaker.

Secondary structure



Residues involved in interaction of charybdotoxin.

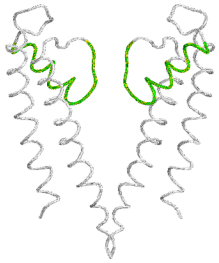
Rauer et al., J Biol Chem, Vol. 275, Issue 2, 1201-1208., 2000

The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))

Significance of the pore helix.

Secondary structure

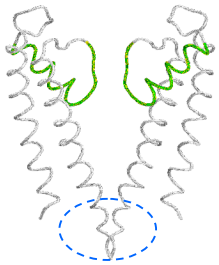


The structural elements of KcsA

(Doyle et al. Science, 280 69-77 (1998))

Significance of the inner helix crossover.

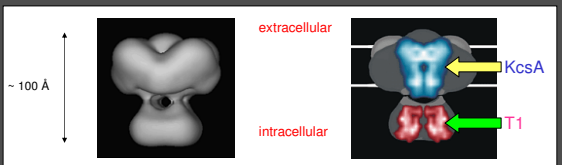
Secondary structure



It is probable that this structure represents KcsA in a closed conformation. (See Kuo, et al. (2003). Crystal Structure of the Potassium Channel KirBac1.1 in the Closed State. *Science* **300**, 1922-1926.)

Is there a correlation between the structure of K^+ channels at Å resolution and the gross morphology of channels in 3D reconstructions?

Shaker and KcsA



From Sokolova et al., Structure 9, 215 (2001)

Resource material

Ion Channels of Excitable Membranes. Hille, B.

Chapters on Elementary Properties of Pores and Channel Structure can be found in the 3rd edition of this book (published in 2001 by Sinauer).

Reviews & original papers:

Marban, E., Yamagishi, T., & Tomaselli, G. F. (1998). Structure and function of voltage-gated sodium channels. *Journal of Physiology* **508**, 647-657.

Hille, B., Armstrong, C. M., & MacKinnon, R. (1999). Ion channels: From idea to reality. *Nature Medicine* **5**, 1105-1109.

Gulbis, J. M. & Doyle, D. A. (2004). Potassium channel structures: do they conform? *Curr Opin Struct Biol* **14**, 440-446.

MacKinnon, R. (2003). Potassium channels. *FEBS Lett* **555**, 62-65.

MacKinnon, R. (2004). Potassium channels and the atomic basis of selective ion conduction (Nobel lecture). *Angew Chem Int Ed Engl* **43**, 4265-4277.

Sokolova, O., Kolmakova-Partensky, L., & Grigorieff, N. (2001). Three-Dimensional Structure of a Voltage-Gated Potassium Channel at 2.5 nm Resolution. *Structure.(Camb.)* **9**, 215-220.

Stokes, D. L. & Wagenknecht, T. (2000). Calcium transport across the sarcoplasmic reticulum - Structure and function of Ca^{2+}-ATPase and the ryanodine receptor. *European Journal of Biochemistry* **267**, 5274-5279.