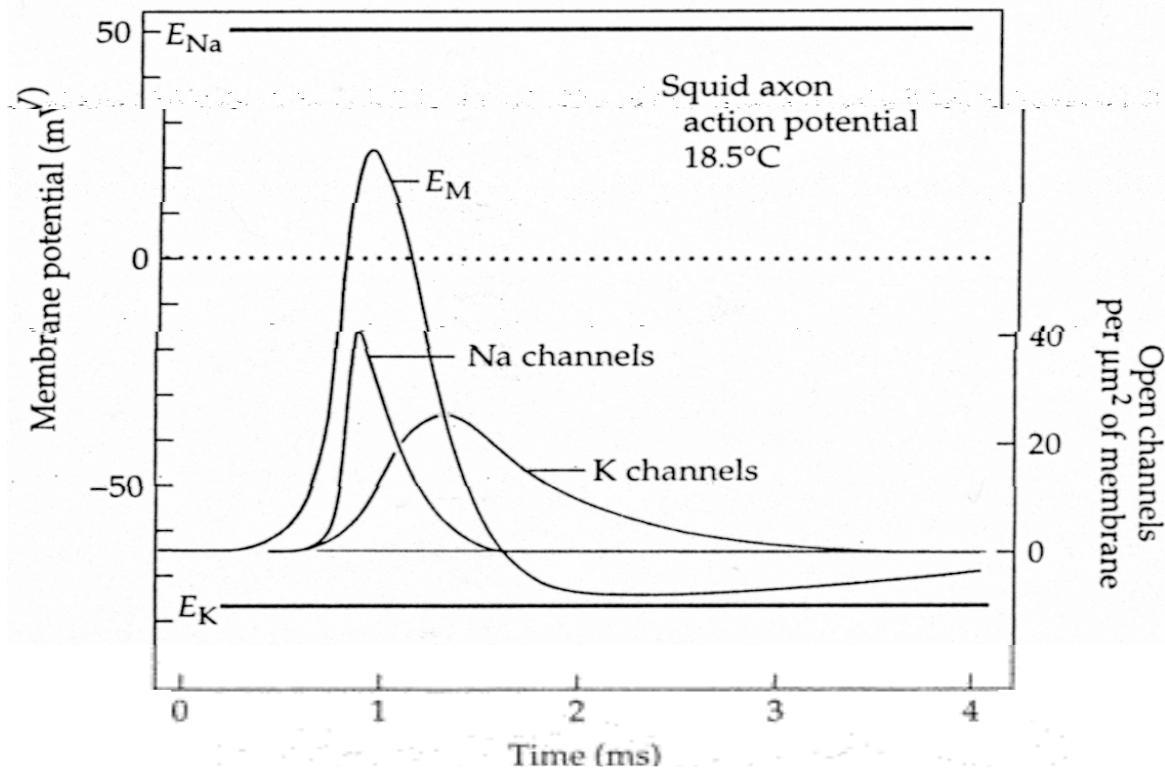


Le potentiel d'action résulte de l'ouverture séquentielle de canaux sodiques et potassiques



Deux questions fondamentales :

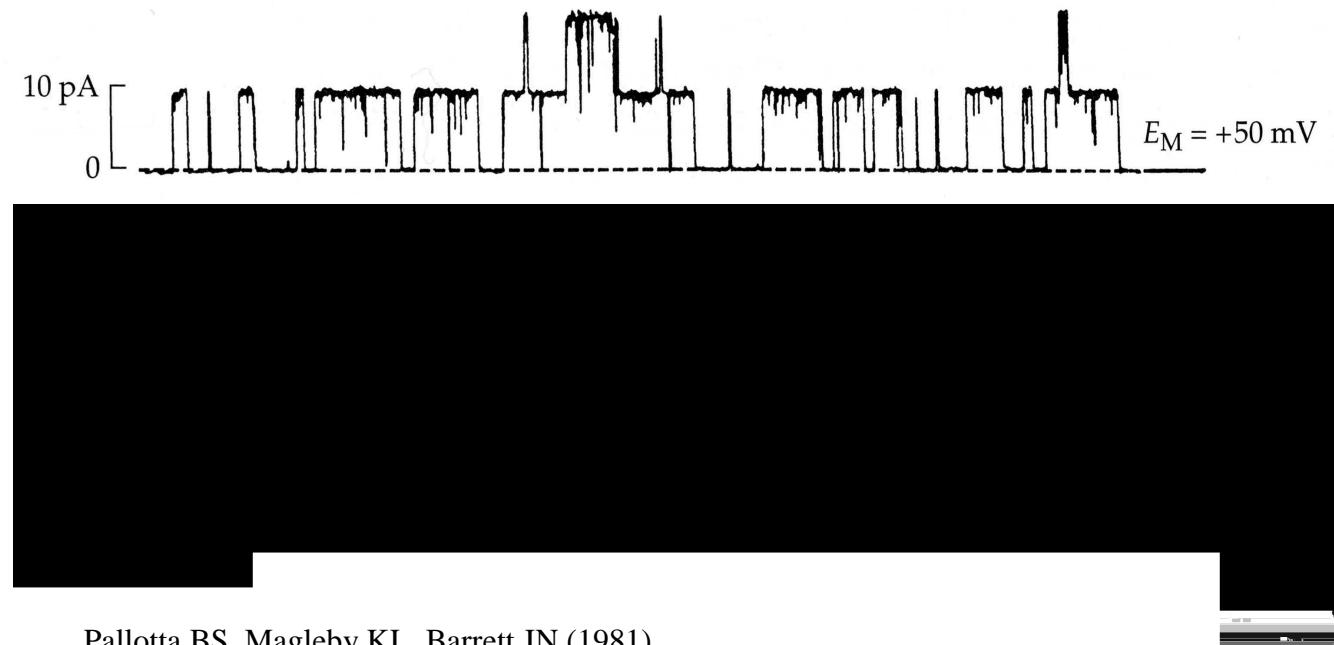
- 1) comment est obtenue la sélectivité des canaux ?
- 2) comment est contrôlée leur ouverture ?

Hodgkin AL, Huxley AF (1952)
A quantitative description of membrane current and its application to conduction and excitation in nerve.

J Physiol Lond 117: 500-544.

perméation et sélectivité dans les canaux potassiques

Les canaux ioniques sont des protéines transmembranaires qui catalysent le transport des ions à des vitesses très élevées



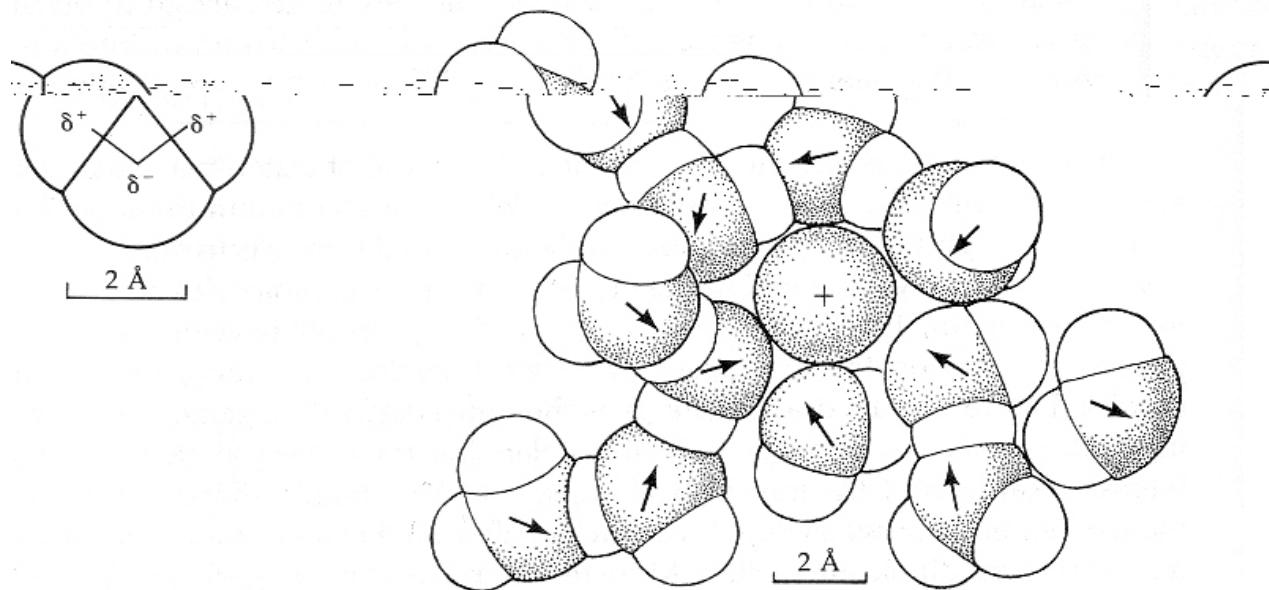
Pallotta BS, Magleby KL, Barrett JN (1981)
Single channel recordings of Ca^{2+} -activated K^+ currents in rat muscle cell culture.
Nature 293: 471-474.

$$10 \text{ pA} = 10^{-11} \text{ Cs}^{-1} = 10^{-11} \times 6.3 \times 10^{18} \text{ q}_e \text{s}^{-1} = 6.3 \times 10^7 \text{ q}_e \text{s}^{-1}$$

O

(B) Rb⁺ ION IN WATER

(A) H₂



* WATER MOLECULES AND ION HYDRATION.

(A) The water molecule is a dipole with partial negative charge on the oxygen and partial positive charges on the two hydrogens. (B) A hypothetical instantaneous snapshot of the rapidly changing organization of water molecules near a Rb⁺ ion. The negative ends of the H₂O dipoles (arrows) tend to point in the direction of the ion and most molecules make several —OH...O hydrogen bonds to neighbors. Oxygen atoms are stippled. All the molecular orientations change in a few picoseconds. [From Hille, 1975c.]

Hille B (2001)

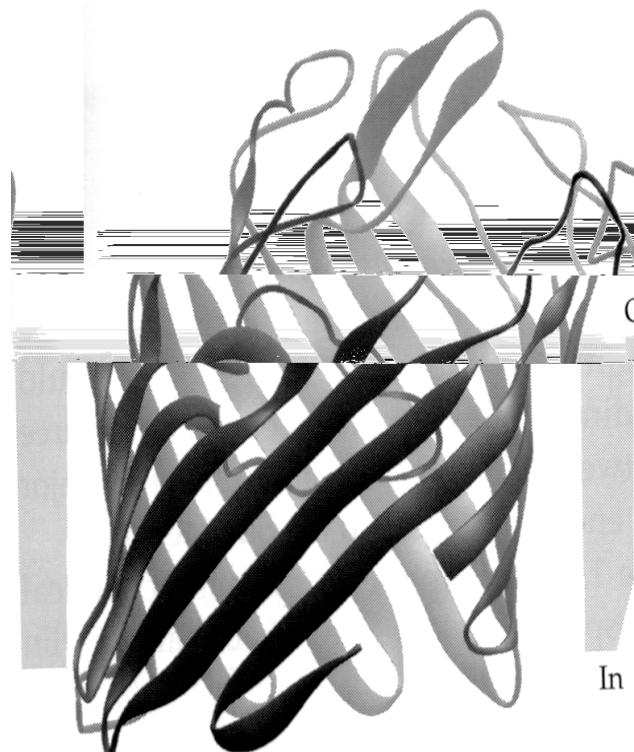
Ionic Channels of Excitable Membranes

Rayons de Pauling et coefficients de diffusion

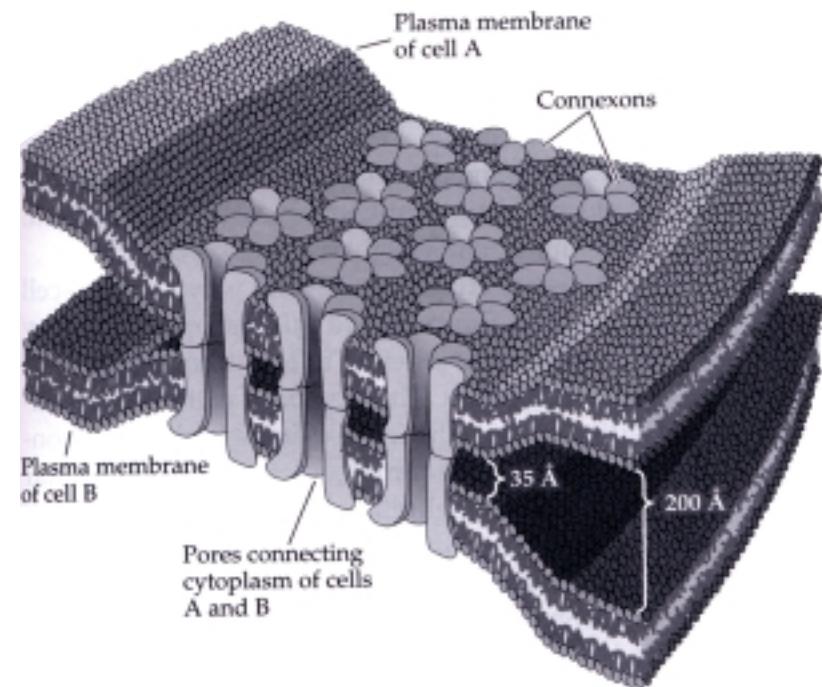
Atome	Rayon (A)	Coef. Dif. ($10^{-5}\text{cm}^2/\text{s}$)
H ⁺	--	9.31
Na ⁺	0.95	1.33
K ⁺	1.33	1.96
Mg ²⁺	0.65	0.71
Ca ²⁺	0.99	0.79
Cl ⁻	1.81	2.03

Exemples de canaux de large diamètre où les ions passent sous forme hydratée

Porin in the outer membrane
of Gram⁻ bacteria



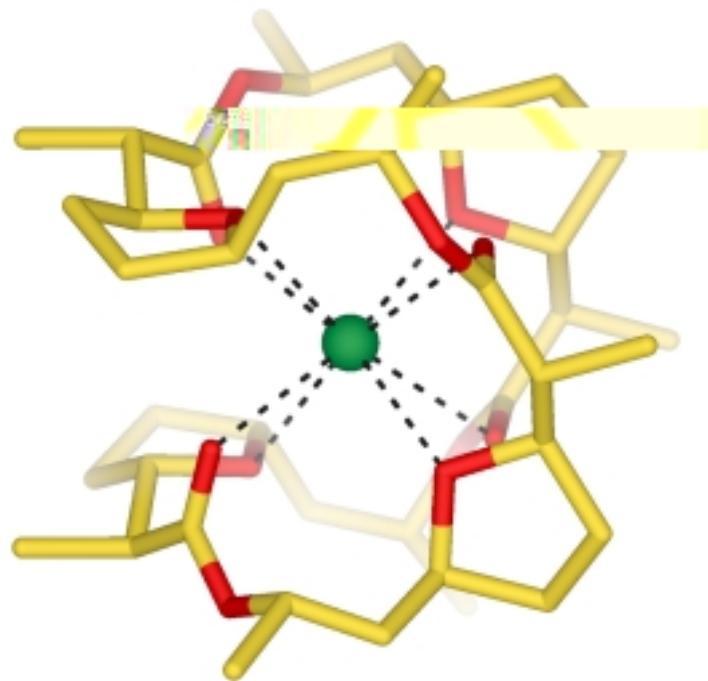
Gap-junctions between
eukaryotic cells



Rayons de Pauling, coefficients de diffusion et énergies d'hydratation des ions

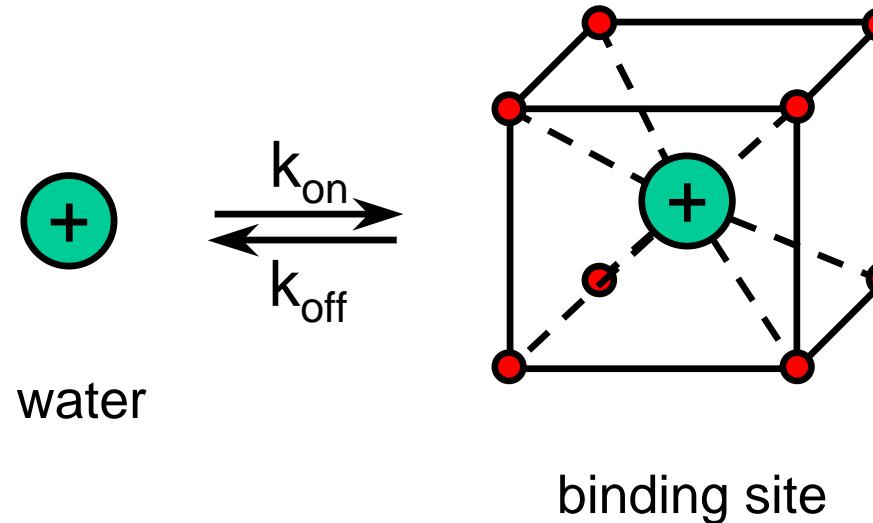
Atome	Rayon (A)	Coef. Dif. ($10^{-5}\text{cm}^2/\text{s}$)	$\Delta H^\circ\text{hydratation}$ (kcal/mol)
H ⁺	--	9.31	-269
Na ⁺	0.95	1.33	-105
K ⁺	1.33	1.96	-85
Mg ²⁺	0.65	0.71	-476
Ca ²⁺	0.99	0.79	-397
Cl ⁻	1.81	2.03	-82

Sélectivité ionique dans le macrocycle
de la molécule du ionophore nonactine



$\langle \text{distance } K^+ - O \rangle = 2.80 \text{ \AA}$

Avec quelle vitesse maximale la nonactine peut-elle transférer les ions K⁺ ?

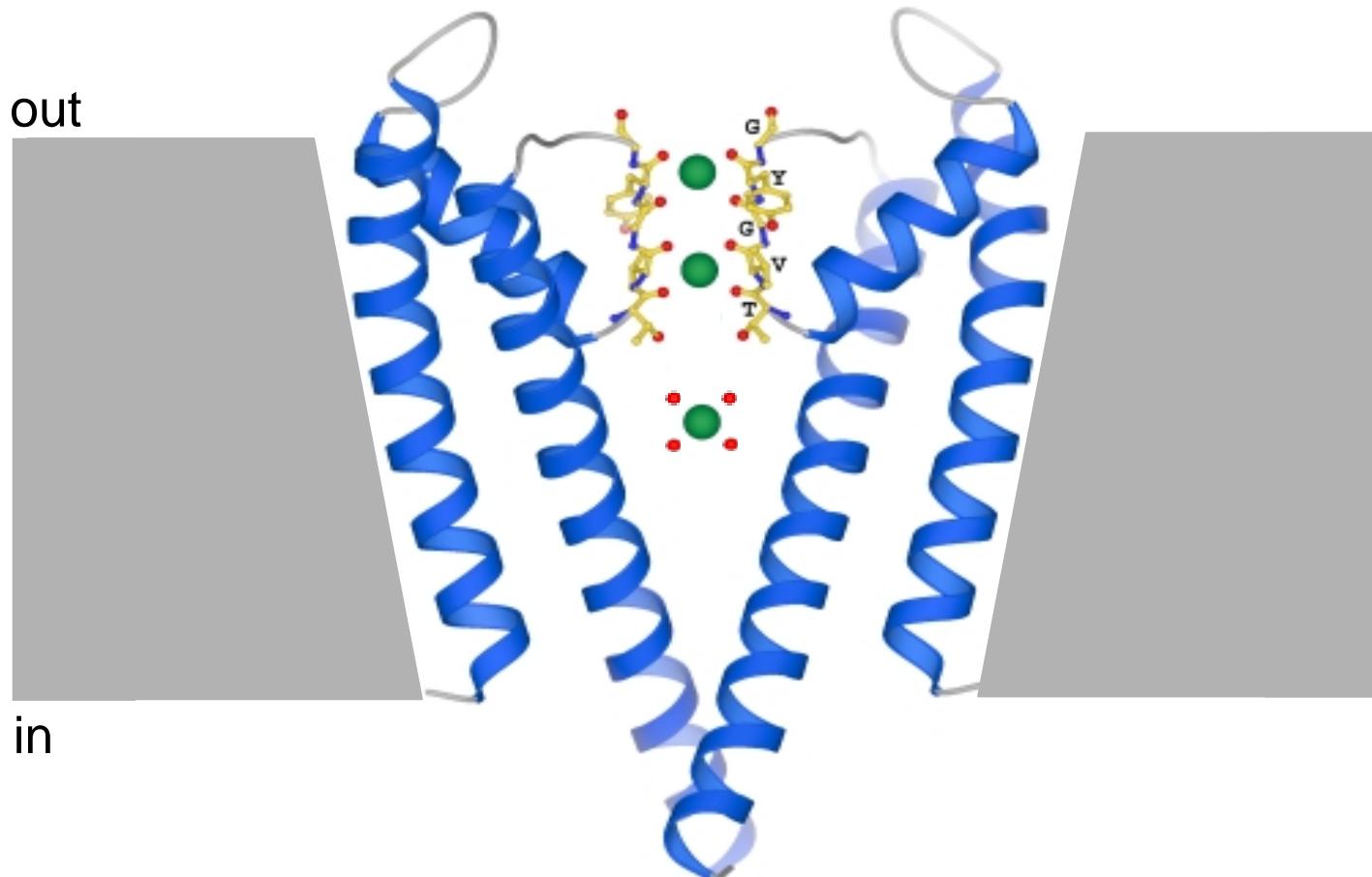


$$K_d = \frac{k_{\text{off}}}{k_{\text{on}}} = 10^{-4} \text{ M}$$

$$\begin{aligned} k_{\text{off}} &= K_d * k_{\text{on}} \\ &= (10^{-4} \text{ M}) * (10^8 \text{ M}^{-1}\text{s}^{-1}) = 10^4 \text{ s}^{-1} \end{aligned}$$

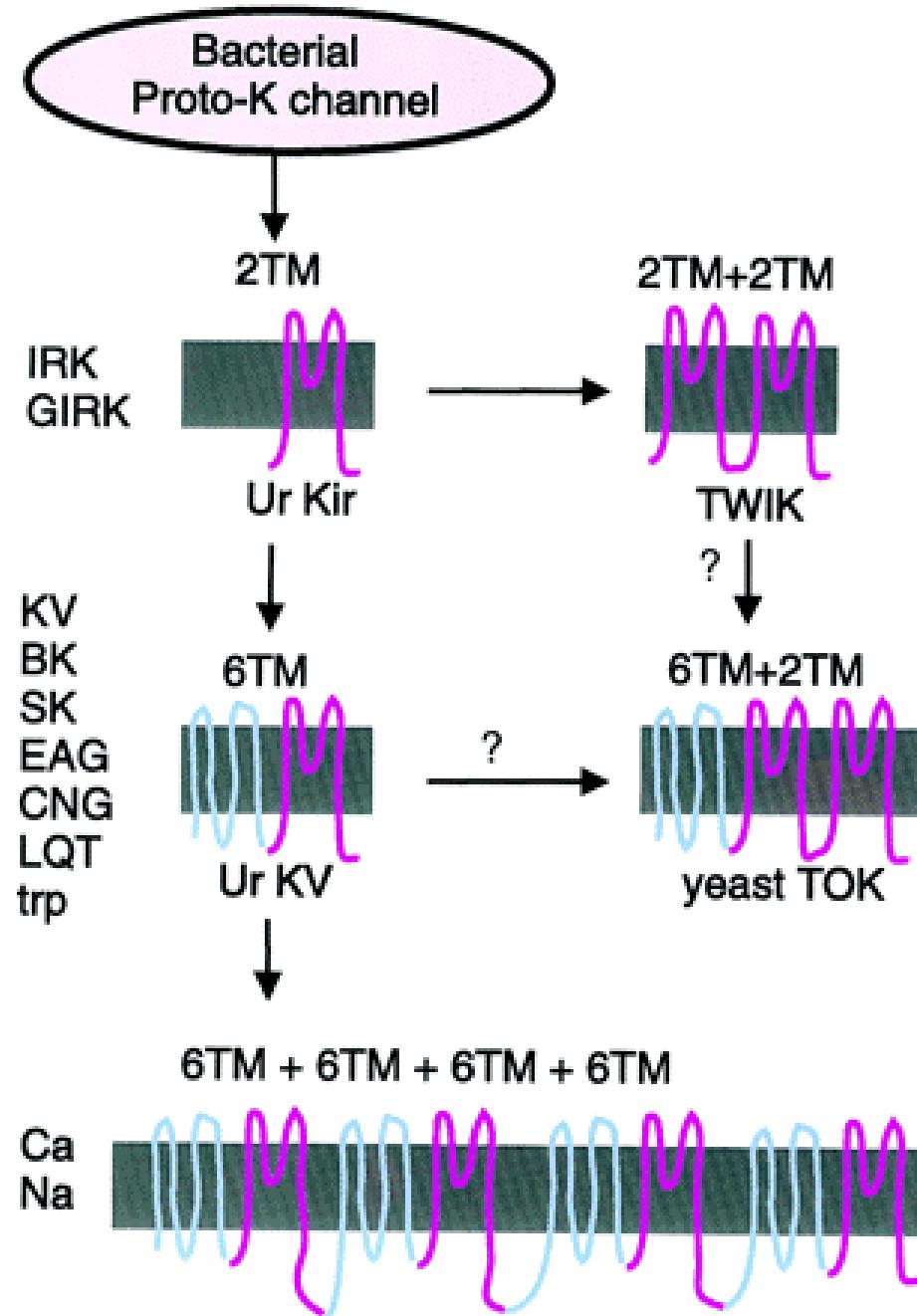
Paradoxe de la notion de canal ionique sélectif :
comment concilier vitesse de transport élevée
et discrimination fine du substrat ?

Perméation sélective et rapide dans les canaux K⁺:
liaison simultanée de plusieurs ions sur des sites
sélectifs de haute affinité et proches dans l'espace



Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998)
The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.
Science 280: 69-77.

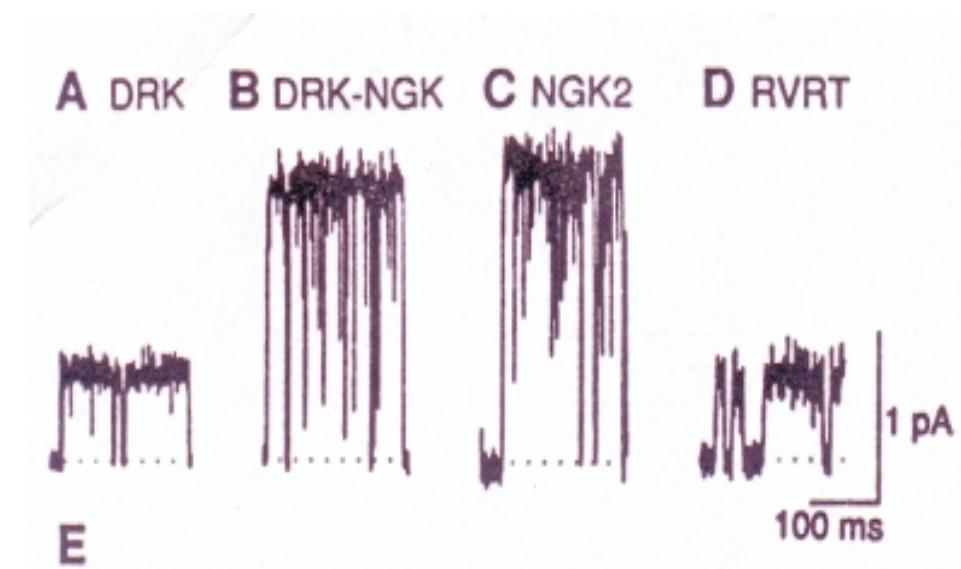
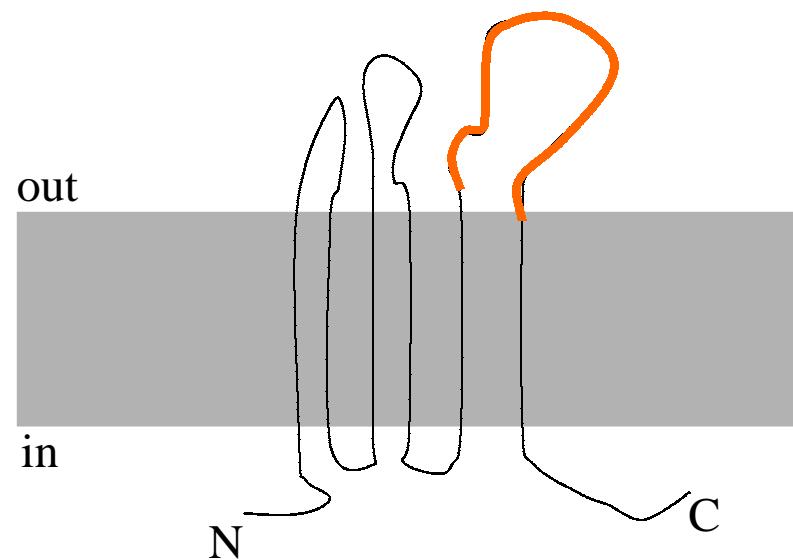
La topologie membranaire des canaux sensibles au potentiel est très variée



Armstrong CM, Hille B (1998)
Voltage-gated ion channels and electrical excitability.
Neuron 20: 371-380.

Identification de la région formant le pore dans les canaux K⁺ à 6 TM

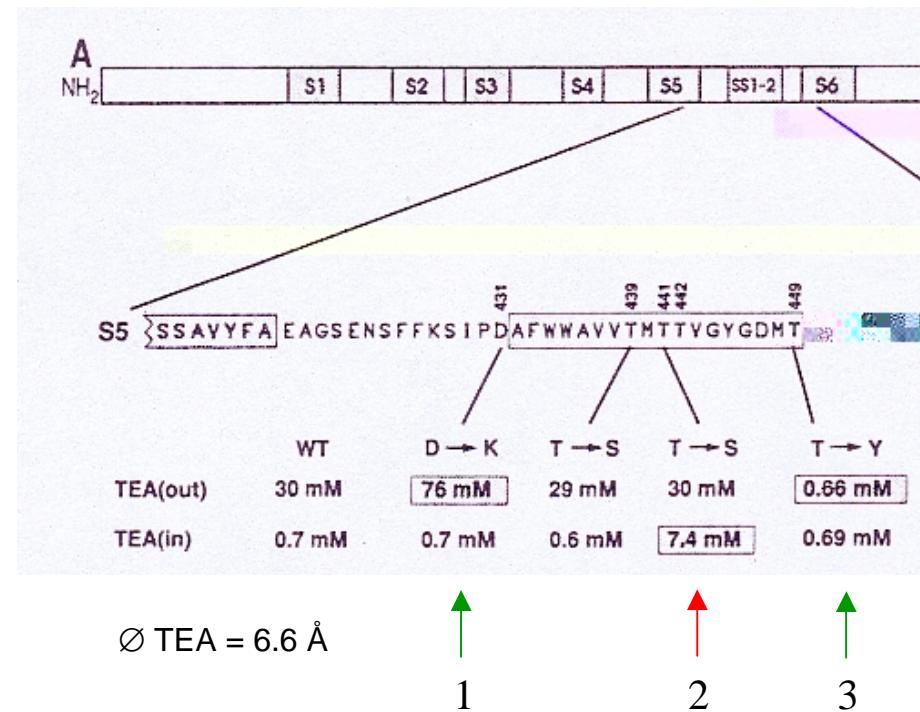
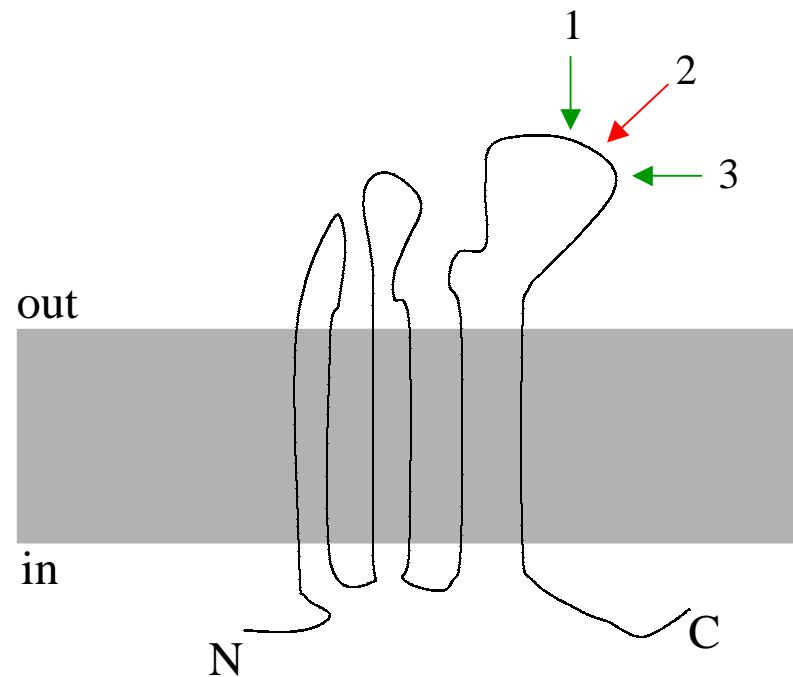
1) l'approche chimères



Hartmann HA, Kirsch GE, Drewe JA, Taglialatela M, Joho RH, Brown AM (1991)
Exchange of conduction pathways between two related K⁺ channels.
Science 251: 942-944.

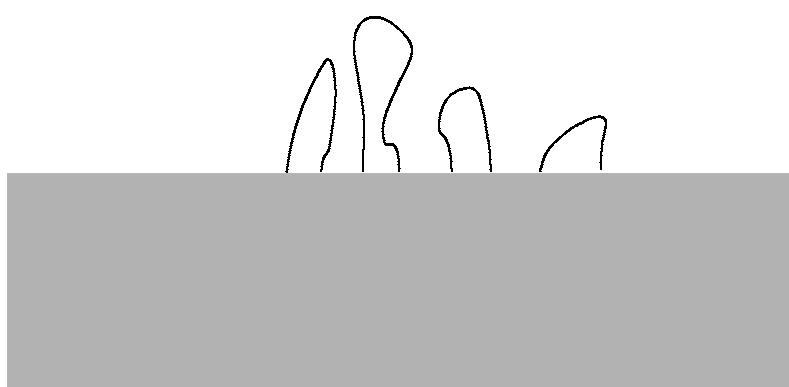
Identification de la région formant le pore dans les canaux K⁺ à 6 TM

2) l'approche par mutagenèse ponctuelle



Yellen G, Jurman ME, Abramson T, MacKinnon R (1991)

Mutations affecting internal TEA blockade identify the probable pore-forming region of a K⁺ channel.
Science 251: 939-942.



Le motif GYG dans la ‘P-loop’ contrôle la sélectivité des canaux K⁺

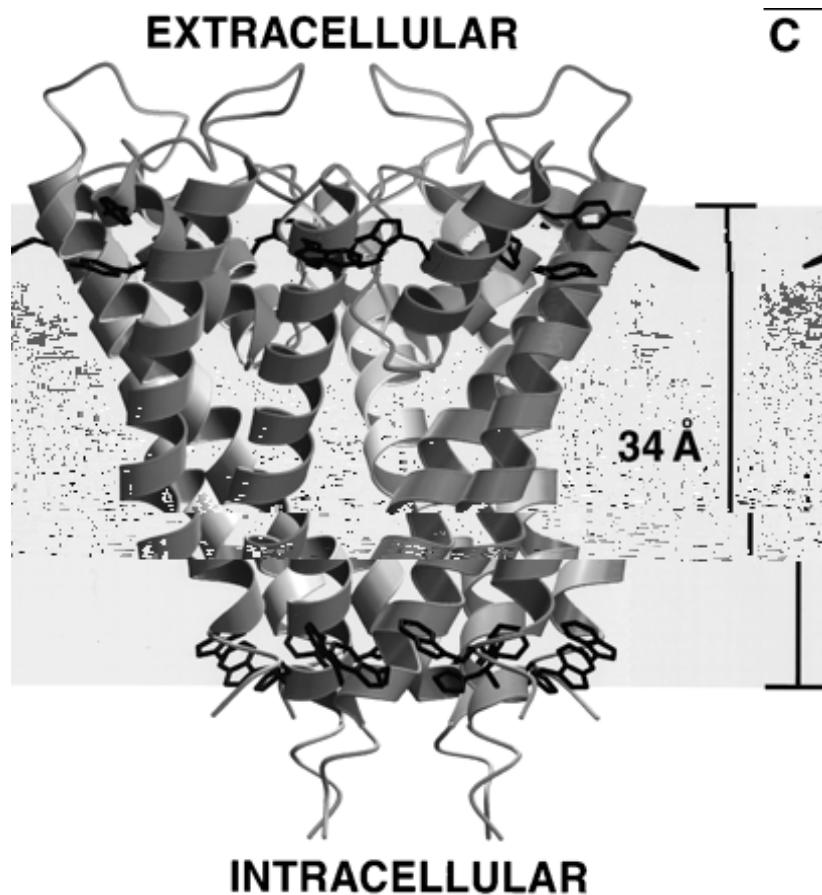
The sequence logo illustrates the conservation of amino acids at the three positions of the P-loop motif (GYG) across various K⁺ channel proteins. The x-axis represents the sequence position (1, 2, 3), and the y-axis lists the proteins. The color scale indicates conservation: red for G, green for Y, and blue for G.

Protein	Position 1 (Red)	Position 2 (Green)	Position 3 (Blue)
Shaker	S	I	P
hKv1.1	S	I	P
hDRK	S	I	P
Parame	Q	T	L
Celegans	S	I	P
mSlo	T	Y	E
cal_act	N	F	L
AKT1	R	I	V
berg	K	T	A
romk	G	M	T
hgirk	G	F	V
o1CNG	E	I	I
rodCNG	K	V	S

Heginbotham L, Abramson T, MacKinnon R (1992)
A functional connection between the pores of distantly related
ion channels as revealed by mutant K⁺ channels.
Science 258: 1152-1155.

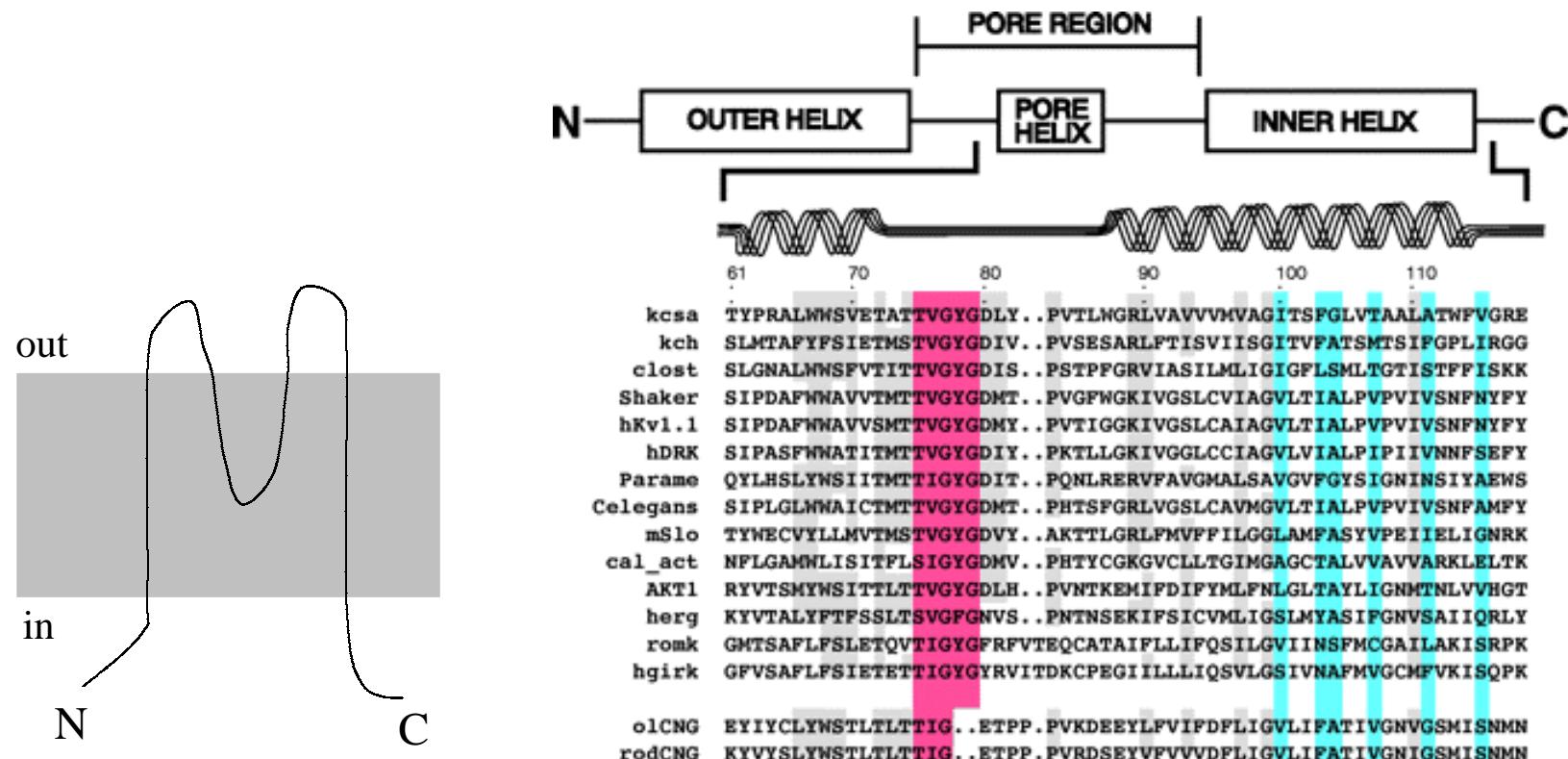
Heginbotham L, Lu Z, Abramson T, MacKinnon R (1994)
Mutations in the K⁺ channel signature sequence.
Biophys J 66: 1061-1067.

Détermination de la structure 3D du canal bactérien KcsA par cristallographie

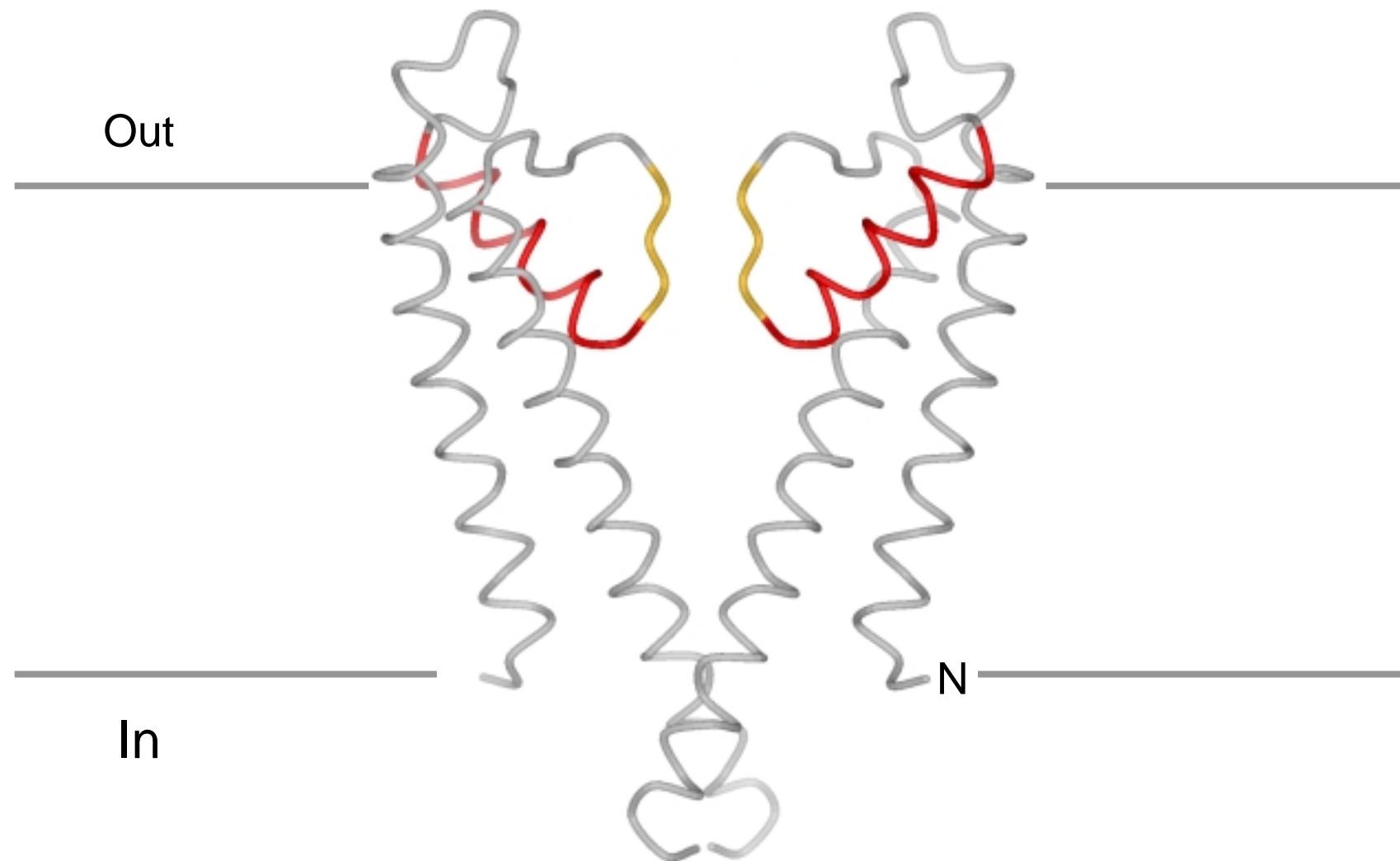


Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998)
The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.
Science 280: 69-77.

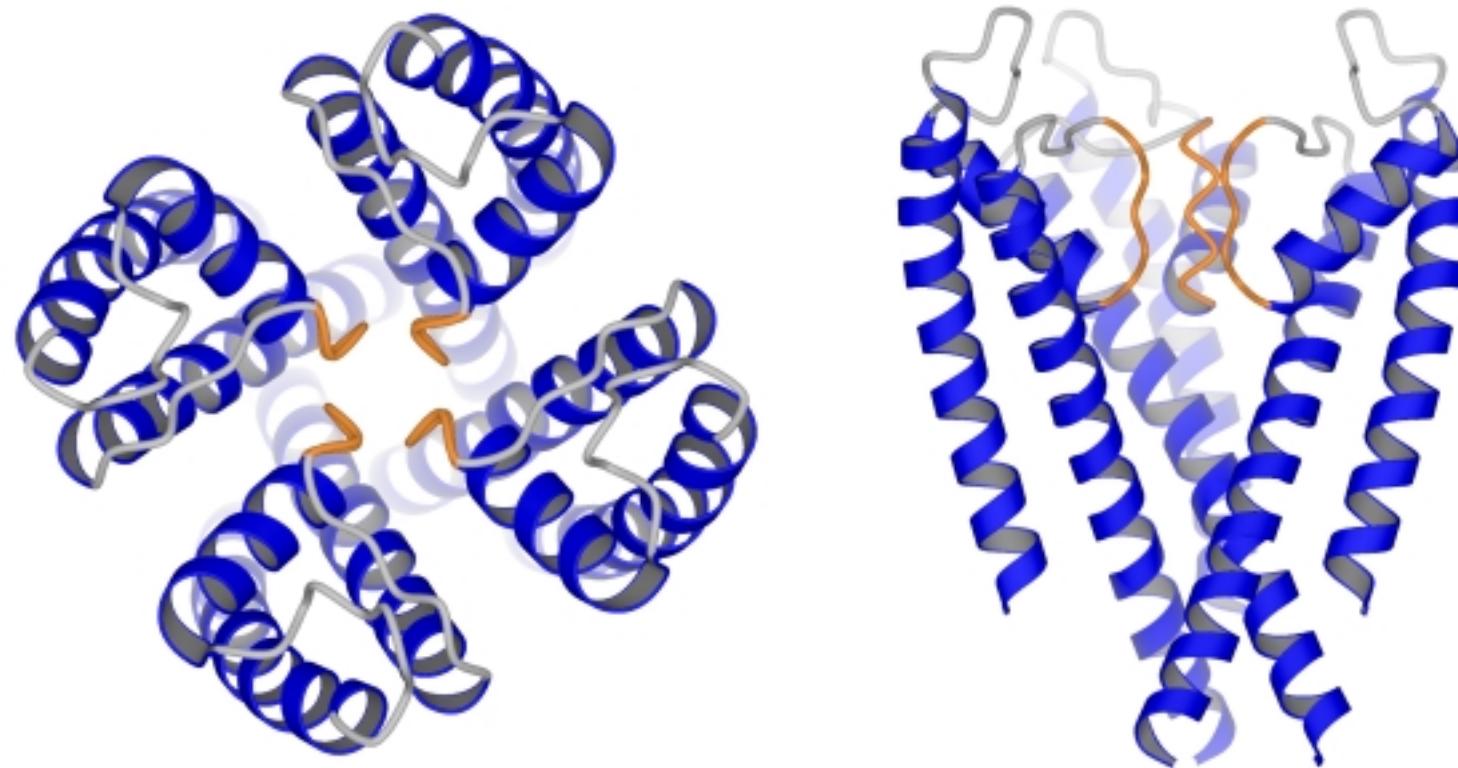
KcsA, un canal potassique de Streptomyces lividans



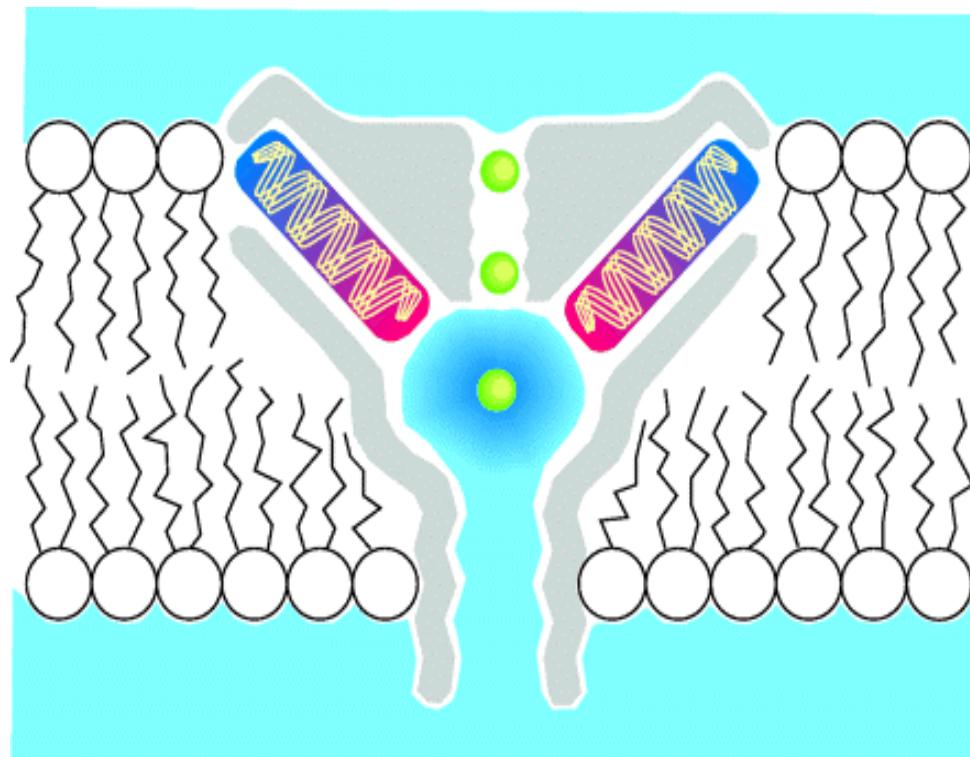
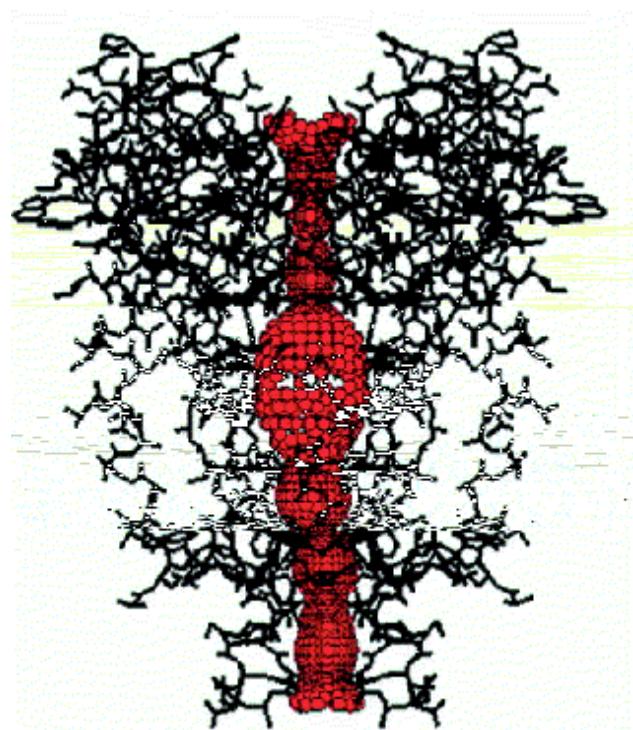
Structure de KcsA



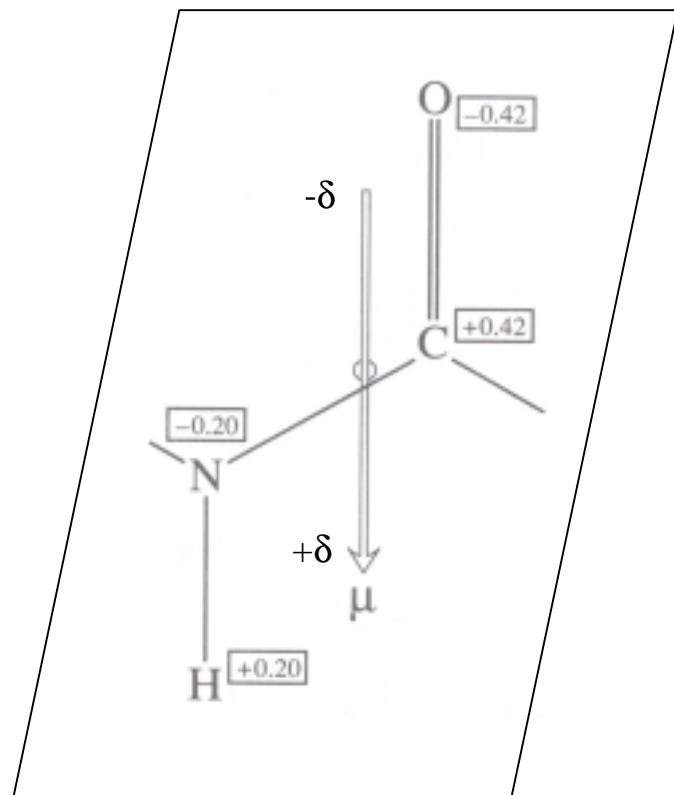
KcsA est un tétramère



Région accessible à l'eau dans le canal KcsA et
stabilisation d'un ion K⁺ dans la cavité centrale

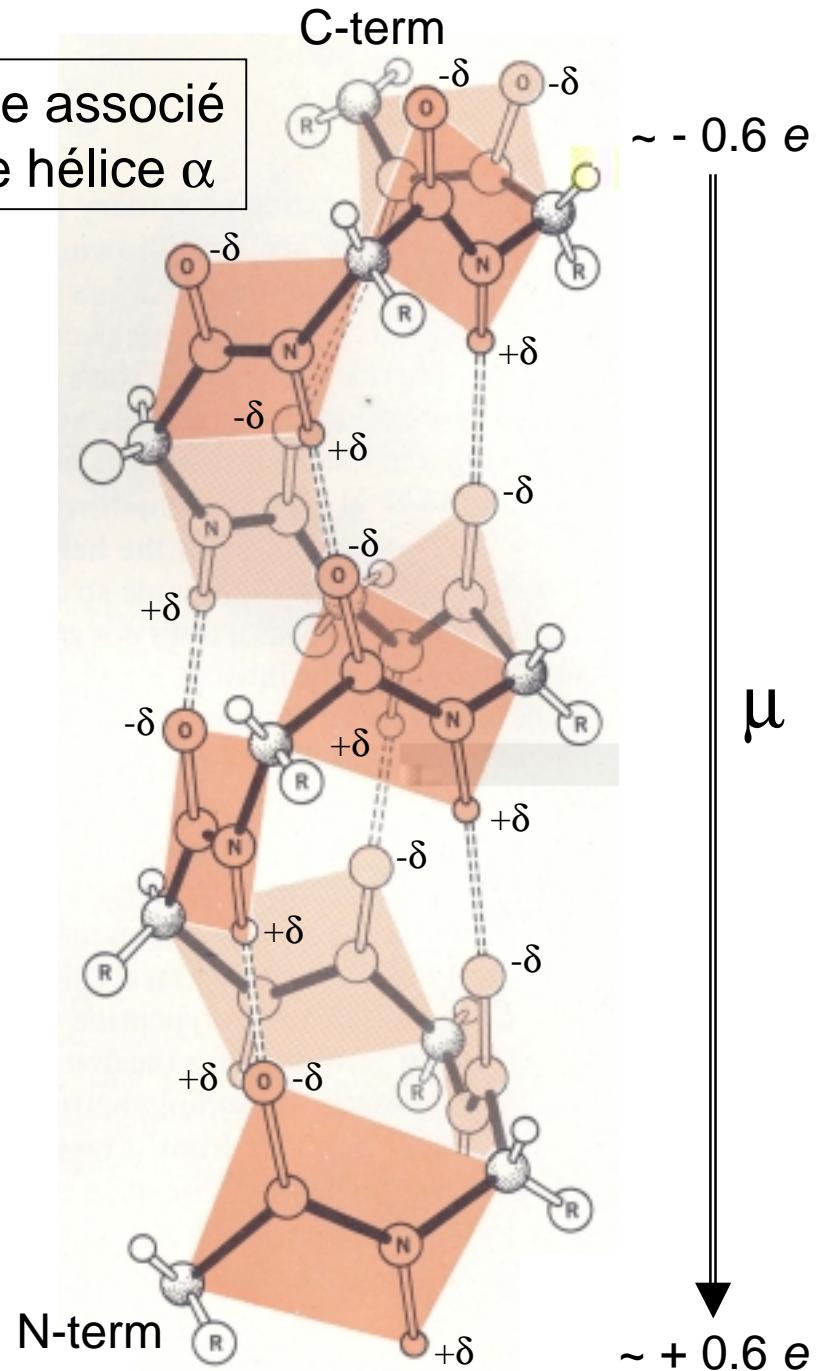


Dipole associé à la liaison peptidique

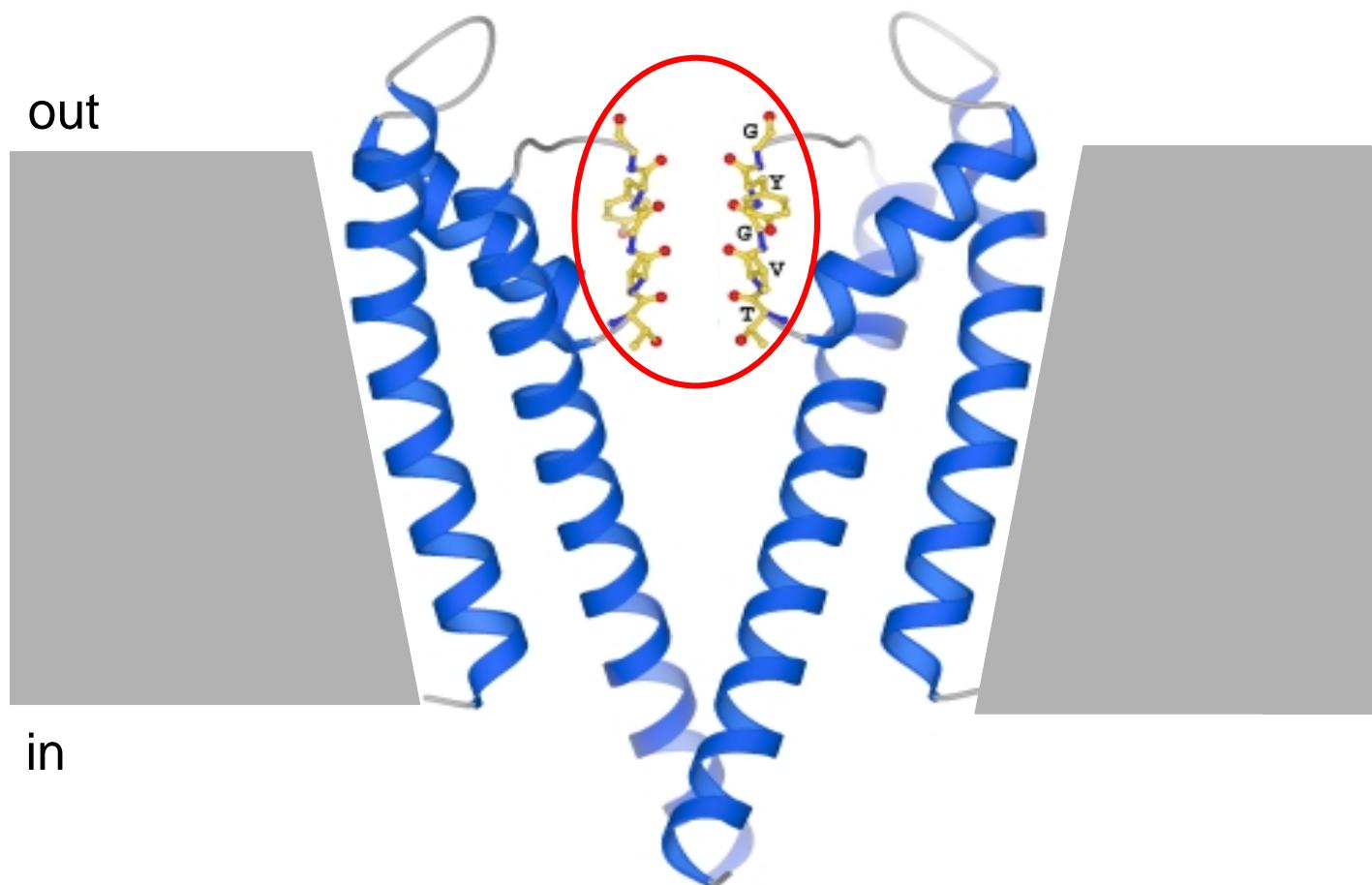


$$\begin{aligned}\mu_{\text{lp}} &= 3.50 \text{ D} \\ \mu_{\text{H}_2\text{O}} &= 1.85 \text{ D}\end{aligned}$$

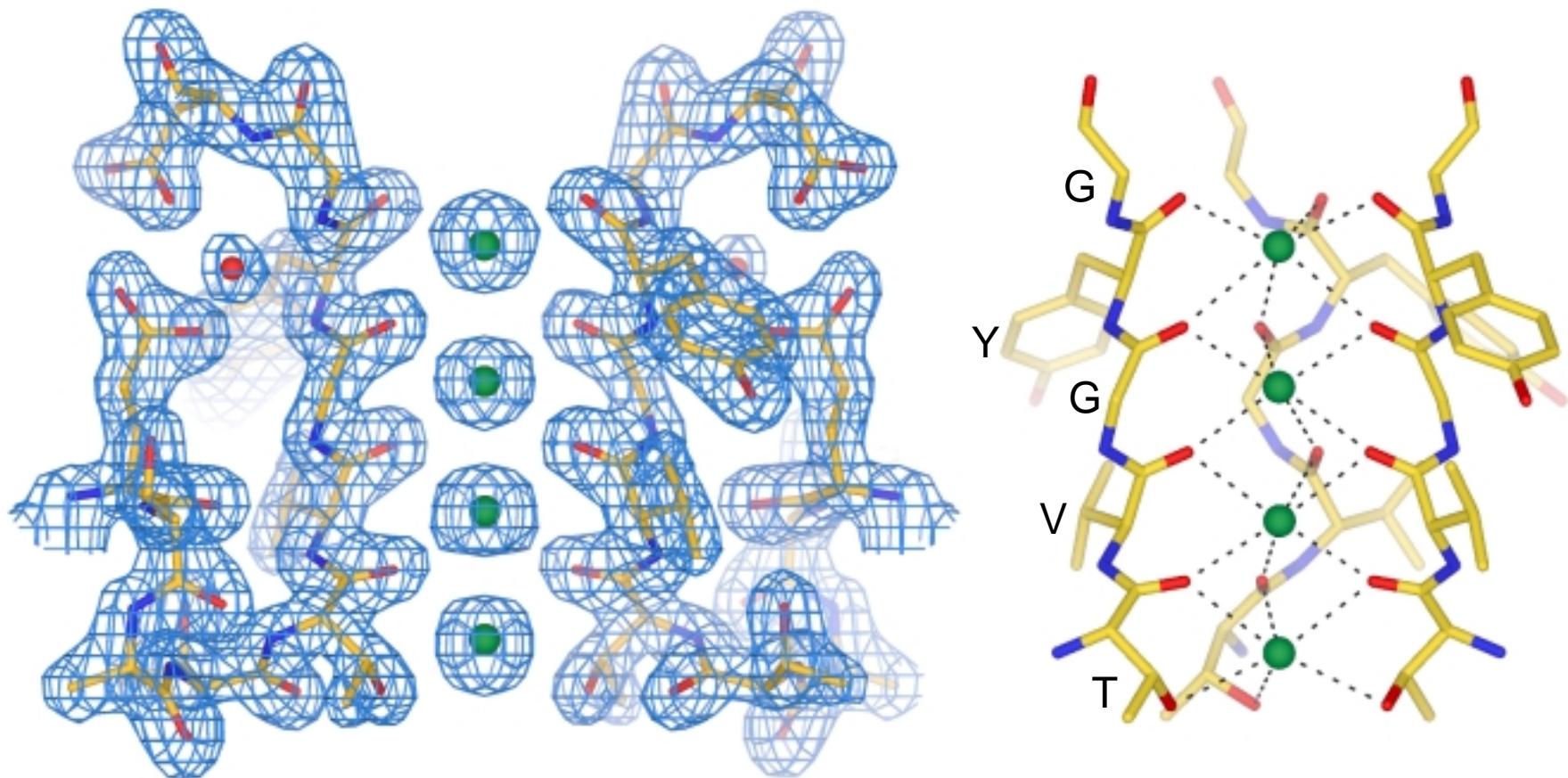
Dipole associé à une hélice α



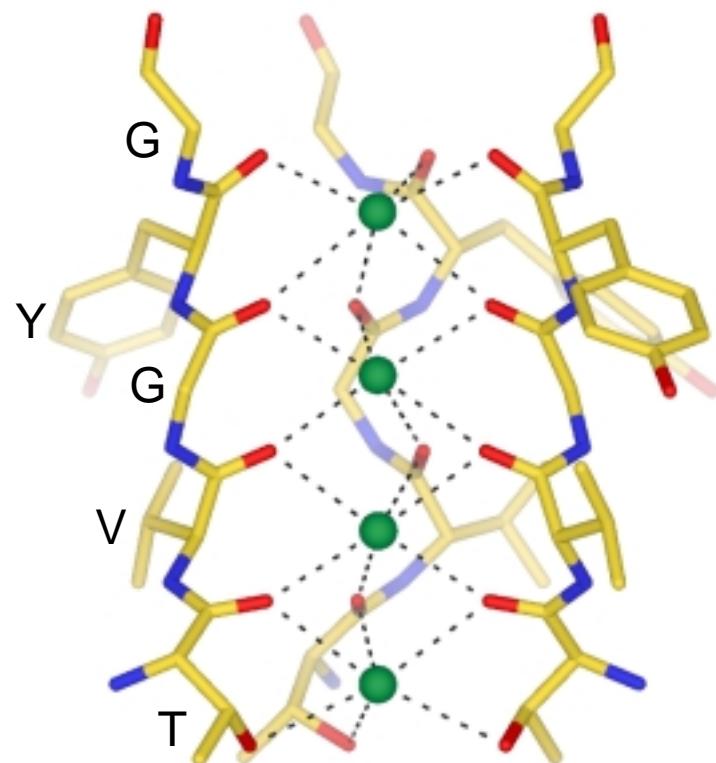
Le filtre de sélectivité des canaux potassiques



Le filtre de sélectivité des canaux potassiques

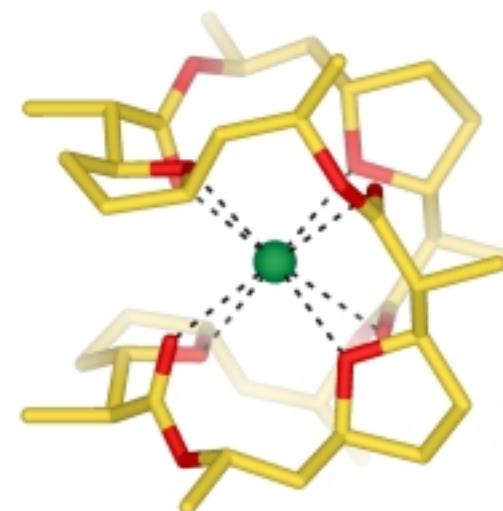


KcsA filter



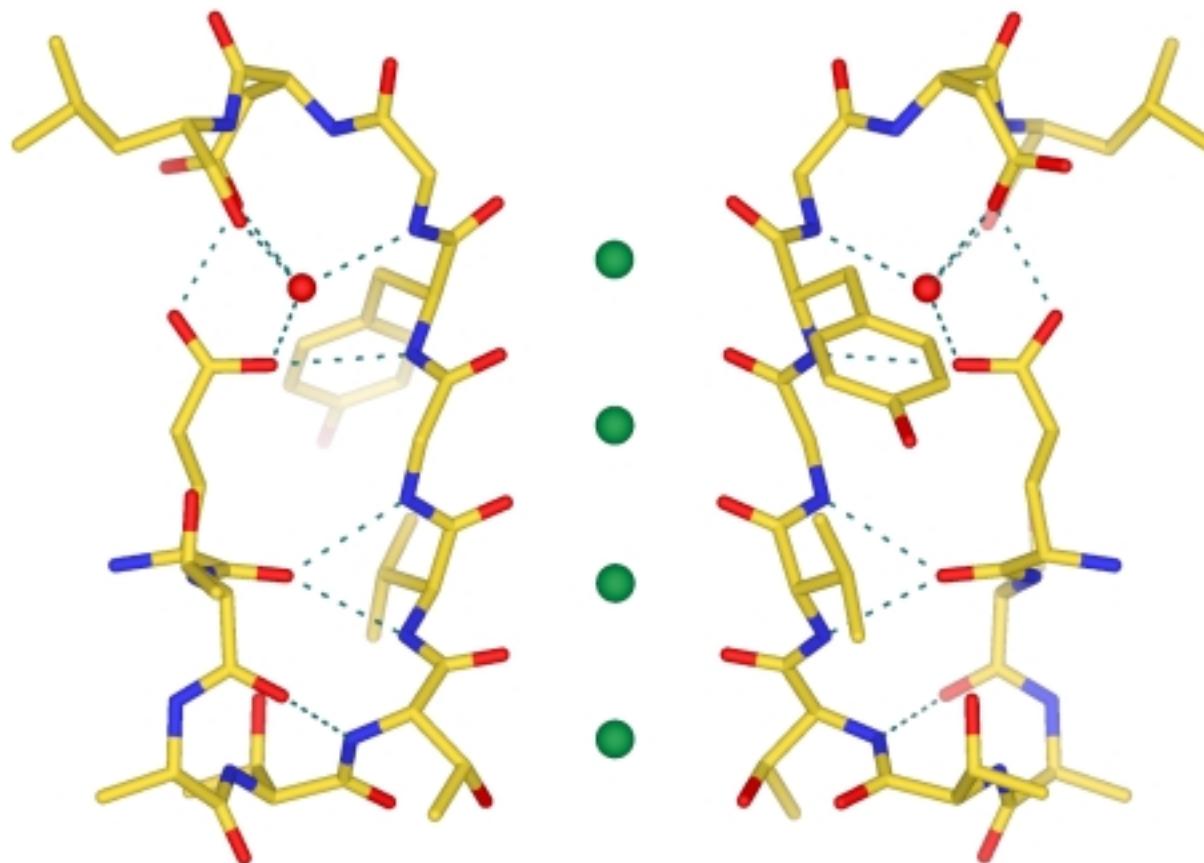
$\langle \text{K}^+ - \text{O distance} \rangle = 2.85\text{\AA}$

Nonactin



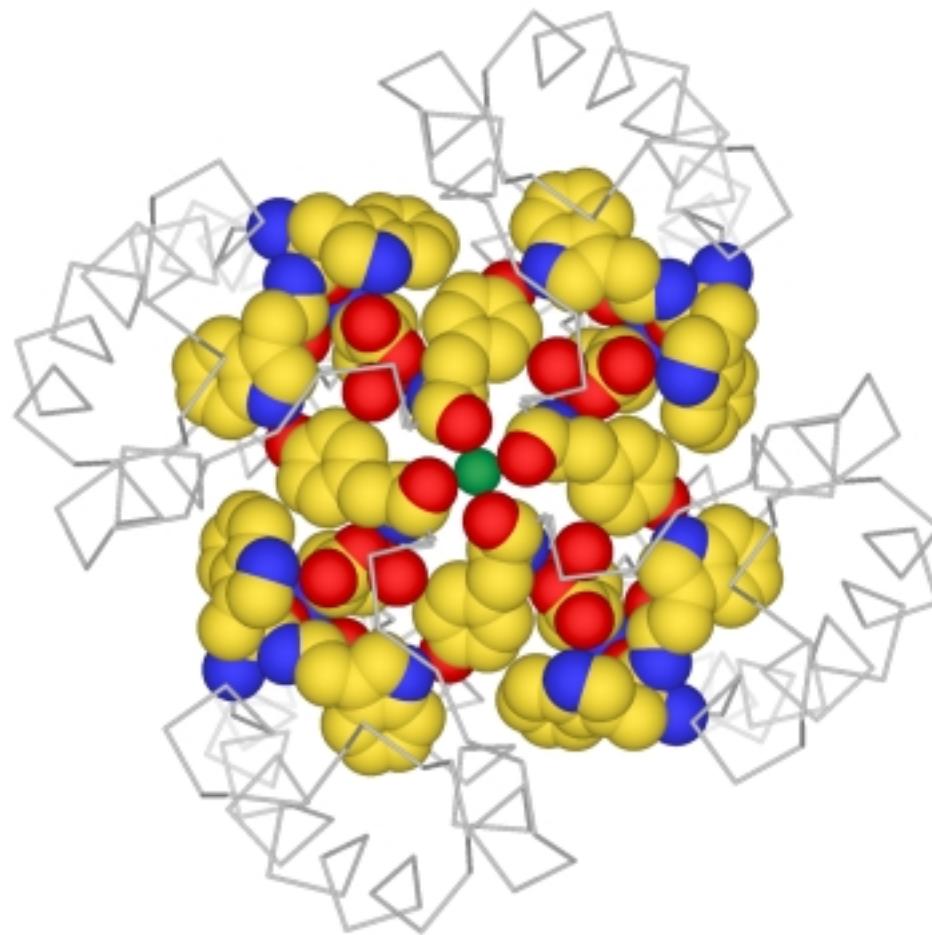
$\langle \text{K}^+ - \text{O distance} \rangle = 2.80\text{\AA}$

Stabilisation du filtre de sélectivité de KcsA par un réseau de liaisons hydrogène

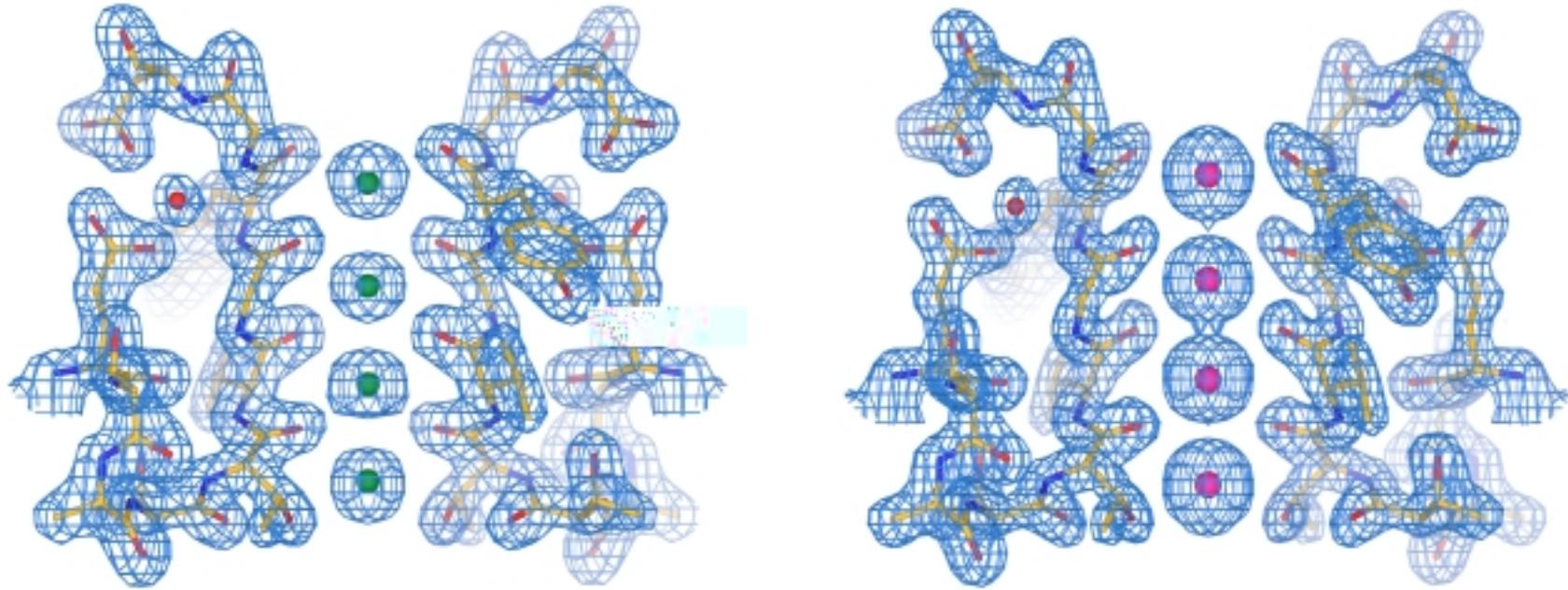


Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001)
Chemistry of ion coordination and hydration revealed by a K⁺ channel-Fab complex at 2.0 Å resolution.
Nature 414: 43-48.

Stabilisation du filtre de sélectivité de KcsA
par ‘packing’ hydrophobe



Combien d'ions K⁺ dans le filtre de sélectivité de KcsA ?



K⁺ radius = 1.33 Å

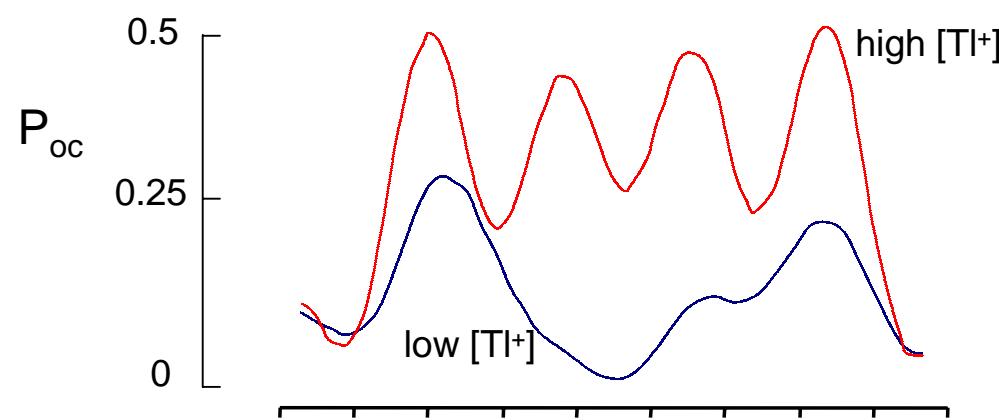
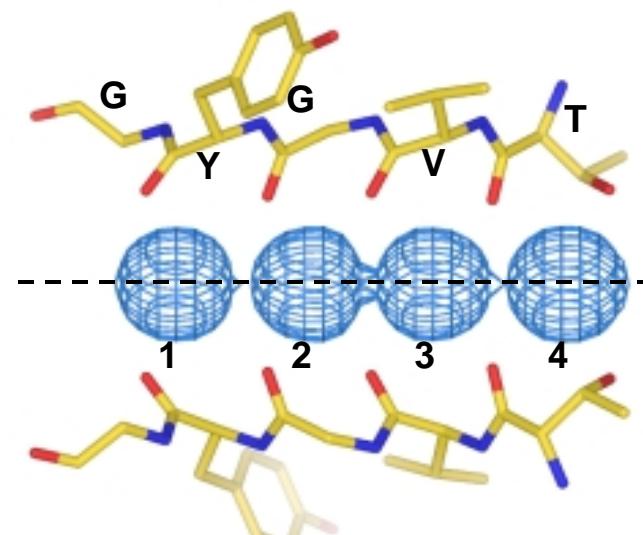
18 e⁻

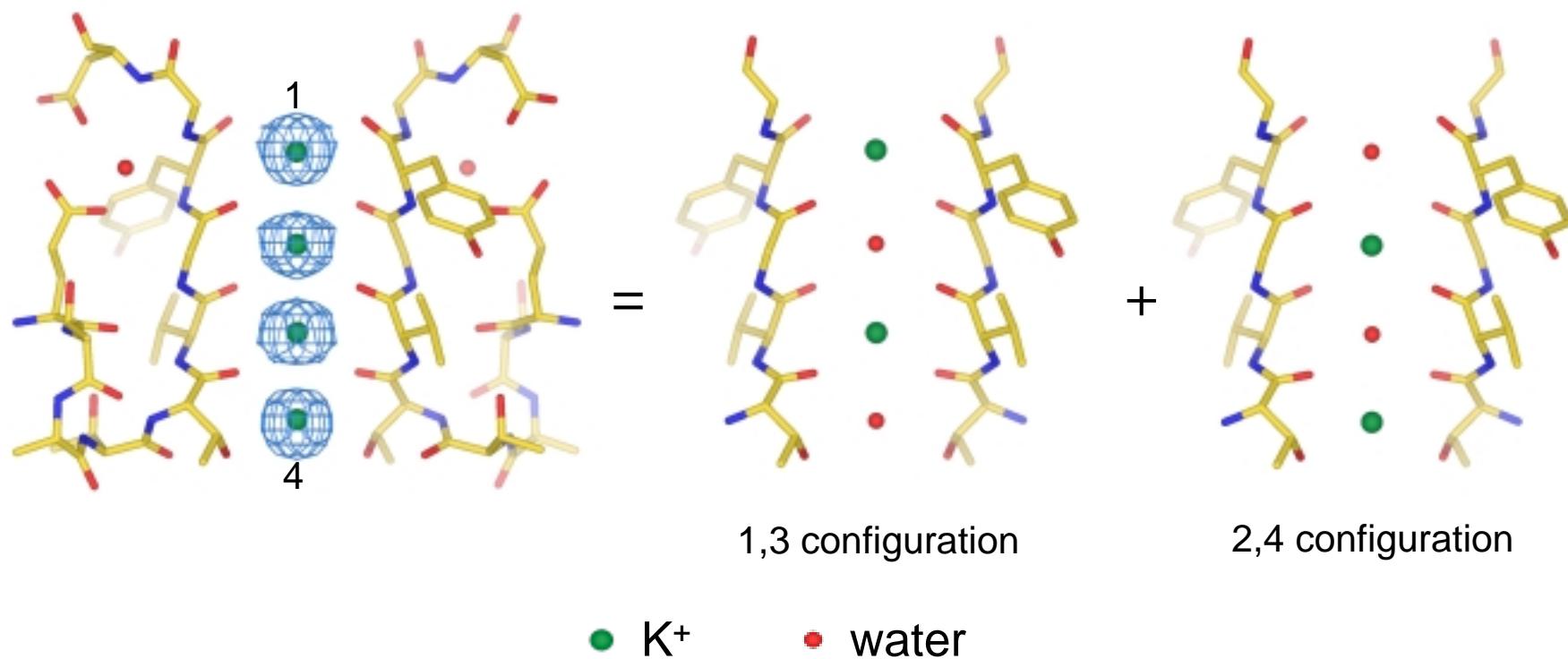
(O radius = 1.4 Å , 8 e⁻)

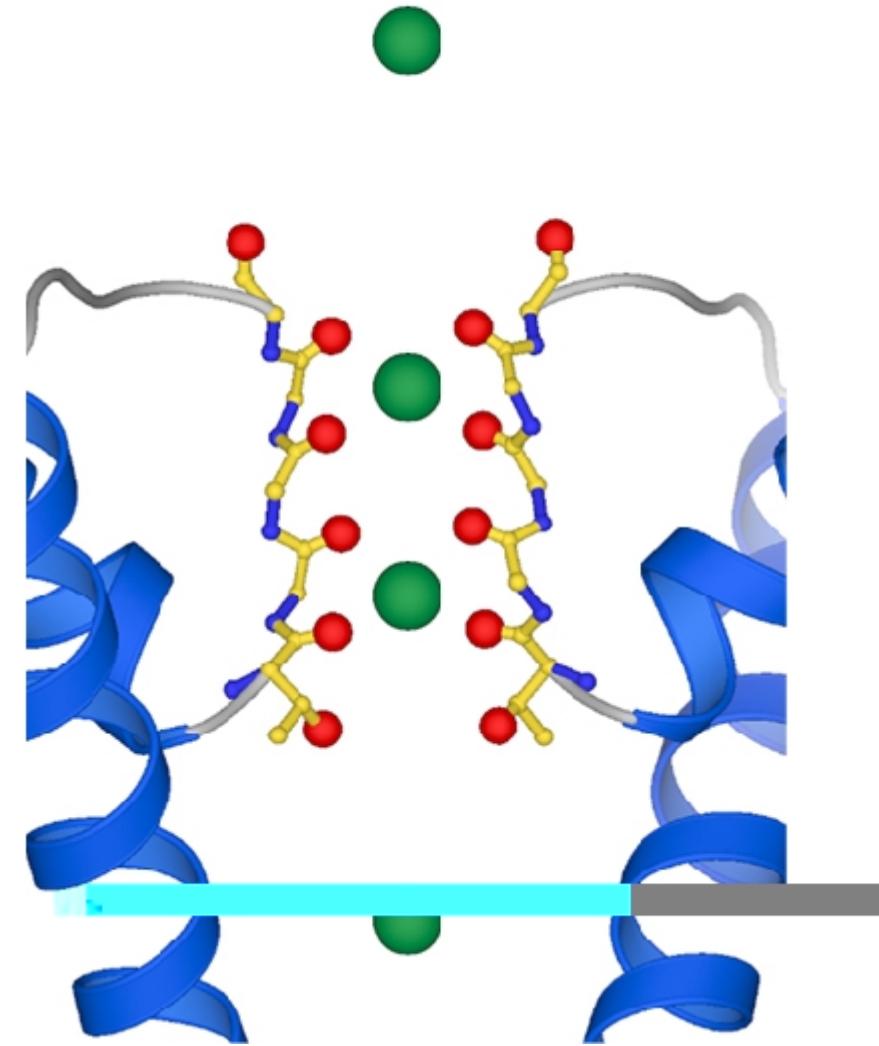
Tl⁺ radius = 1.40 Å

80 e⁻

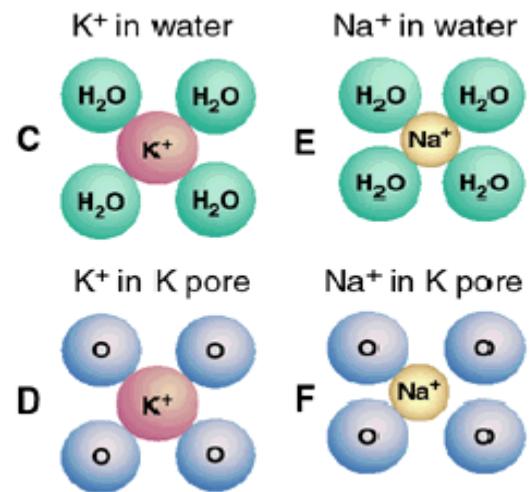
Morais-Cabral JH, Zhou Y, MacKinnon R (2001)
Energetic optimization of ion conduction rate by the K⁺ selectivity filter.
Nature 414: 37-42.





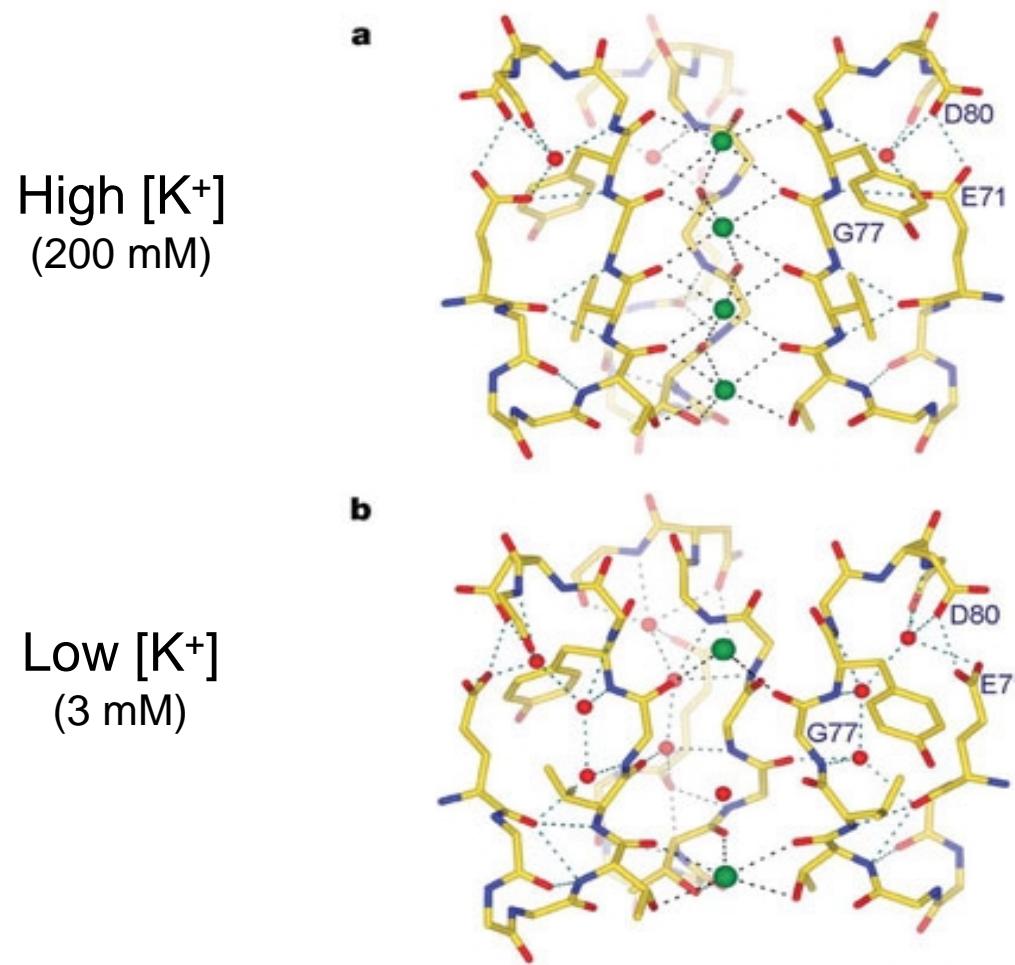


Sélectivité pour K^+ dans les canaux potassiques l'hypothèse du fit parfait



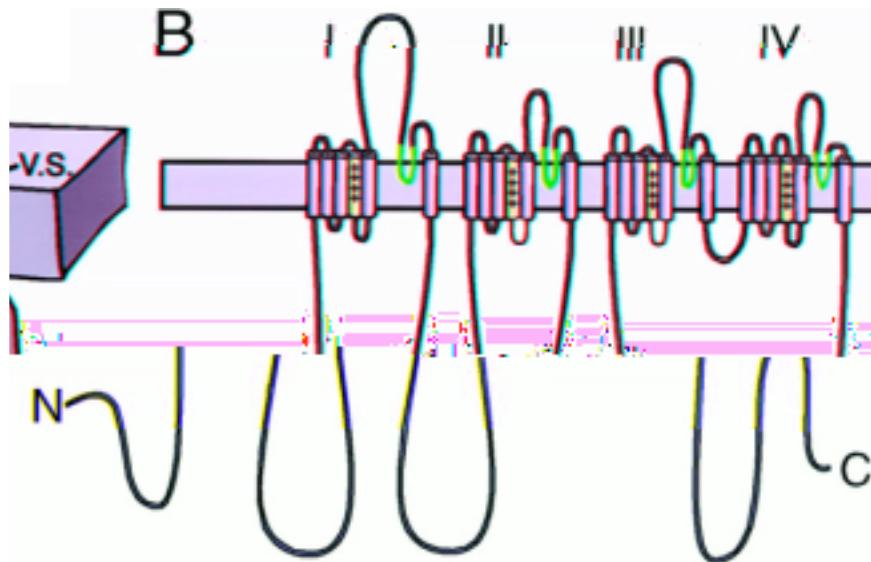
Armstrong C (1998)
The vision of the pore.
Science 280: 56-57

Fermeture du filtre de sélectivité de KcsA en $[K^+]$ faible



Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001)
Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution.
Nature 414: 43-48.

Conservations de séquence dans la ‘P-loop’ des canaux Na et Ca



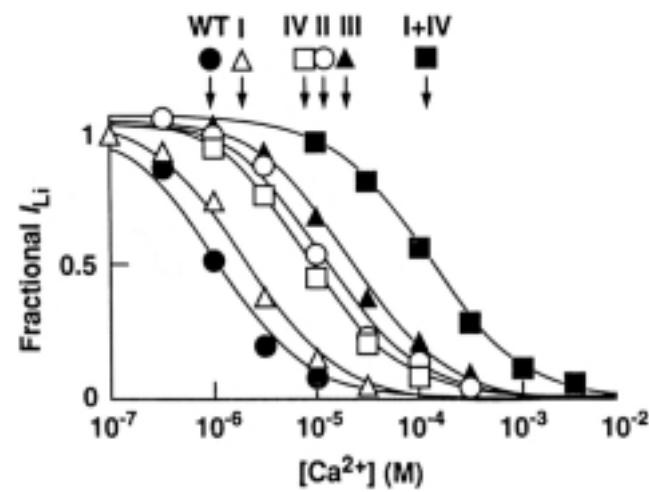
Na_v 1.2

	-3'	-2'	-1'	0'	1'	2'	3'	4'	5'	6'							
Na I	L	F	R	L	M	T	C	D	F	W	Y	N	L	Y	Q	L	T
Na II	V	F	R	V	I	C	C	W	W	W	M	W	D	C	M		
Na III	L	L	Q	V	A	T	F	K	G	W	M	D	I	M	Y	A	A
Na IV	L	F	Q	I	T	T	S	A	G	W	D	G	L	L	A	P	I

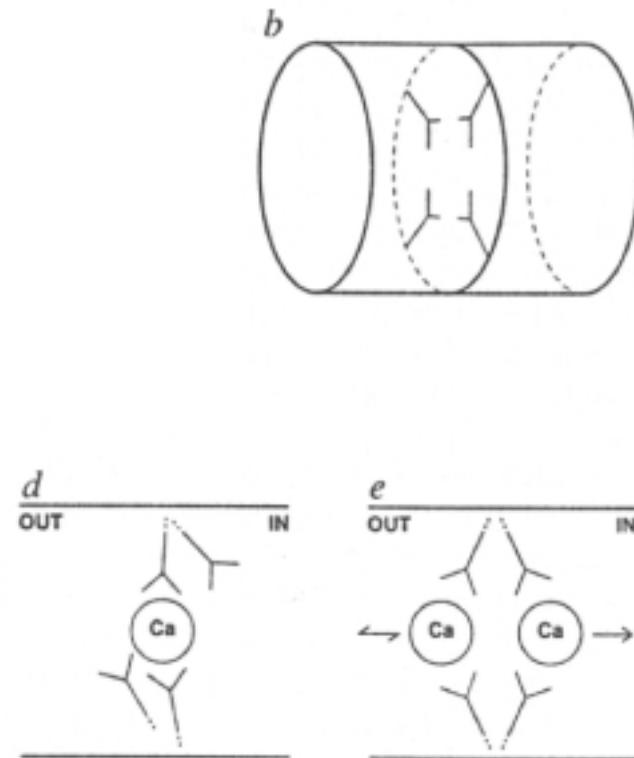
Ca_v 1.1

Ca I	V	F	Q	C	I	T	M	E	G	W	T	D	V	L	Y	W	N
Ca II	V	F	Q	I	I	T	C	R	D	W	N	C	V	M	Y	D	G
Ca III	L	F	T	V	S	T	F	E	G	W	P	E	L	L	Y	R	S
Ca IV	L	F	R	C	A	T	G	E	A	W	Q	D	I	M	L	A	C

2^{ème} modèle de perméation et sélectivité dans les canaux Ca
1 site capable d'accommorder 2 ions Ca

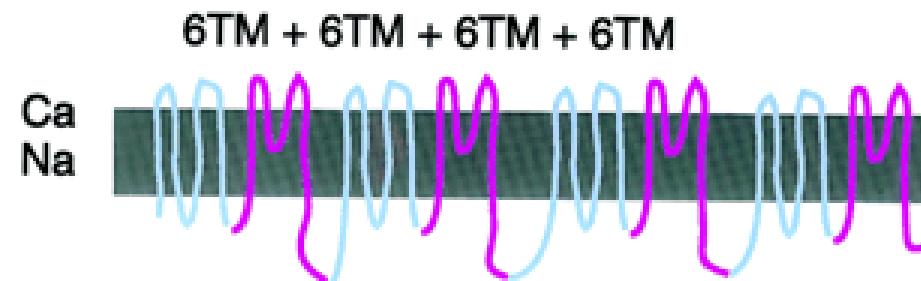
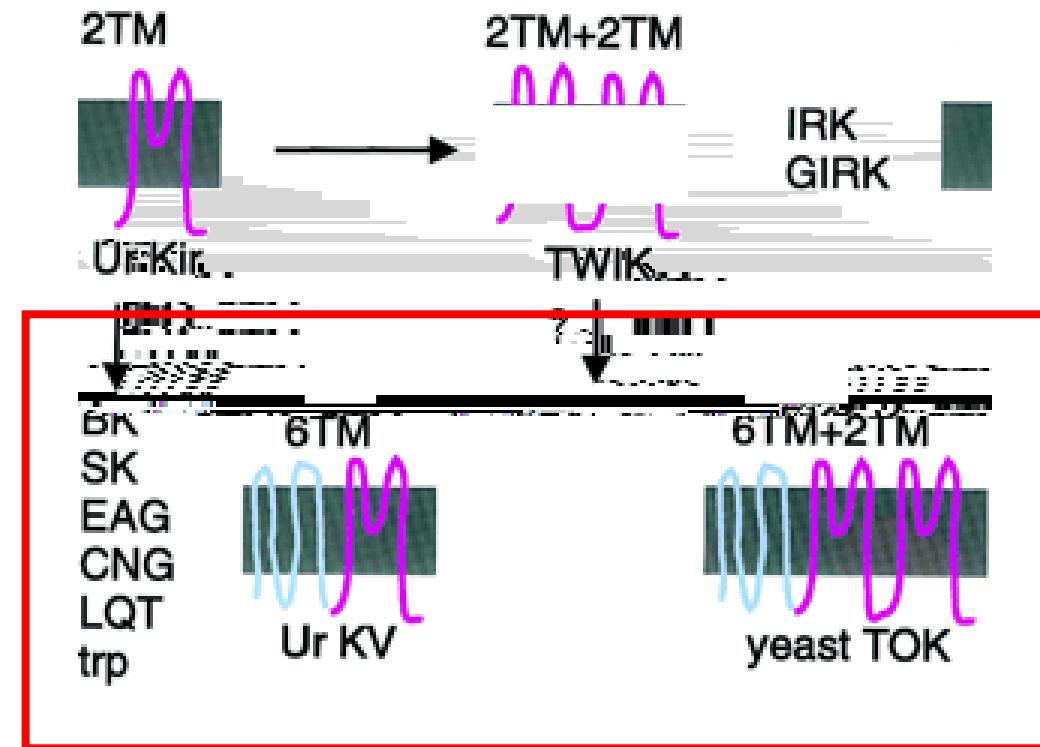


Yang J, Ellinor PT, Sather WA, Zhang JF, Tsien RW (1993)
Molecular determinants of Ca^{2+} channel selectivity and ion
permeation in L-type Ca^{2+} channels.
Nature 366: 158-161



‘gating’ des canaux potassiques

canaux potassiques dits
'voltage-dépendants'

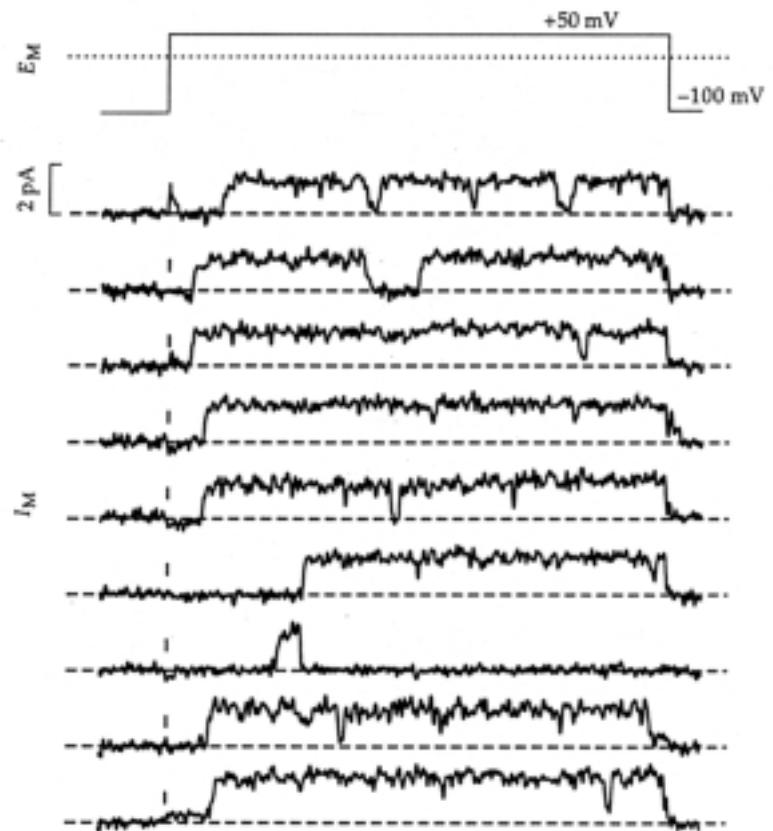


Activation dans les canaux potassiques axonaux

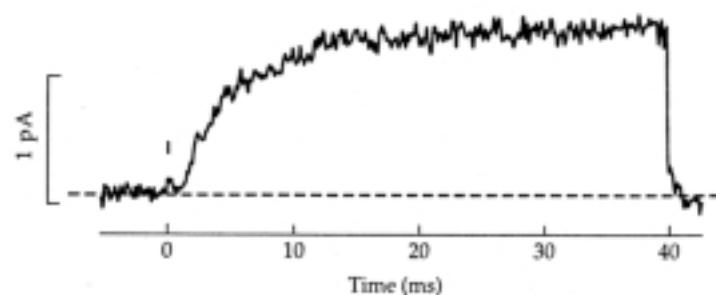
Gating in Single K Channels Patch-clamp recording of unitary K currents in a squid giant axon during voltage steps from -100 to +50 mV. To avoid the overlying Schwann cells, the axon was cut open and the patch electrode sealed against the *cytoplasmic* face of the membrane. (A) Nine consecutive trials showing channels of 20-pS conductance filtered at 2-kHz bandwidth. (B) Ensemble mean of 40 repeats. $T = 20^\circ\text{C}$. [Kindly provided by F. Bezanilla and C. K. Augustine; see Llano et al. 1988.]

Llano I, Webb CK, Bezanilla F (1988)
Potassium conductance of the squid giant axon.
Single-channel studies.
J Gen Physiol 92: 179-196.

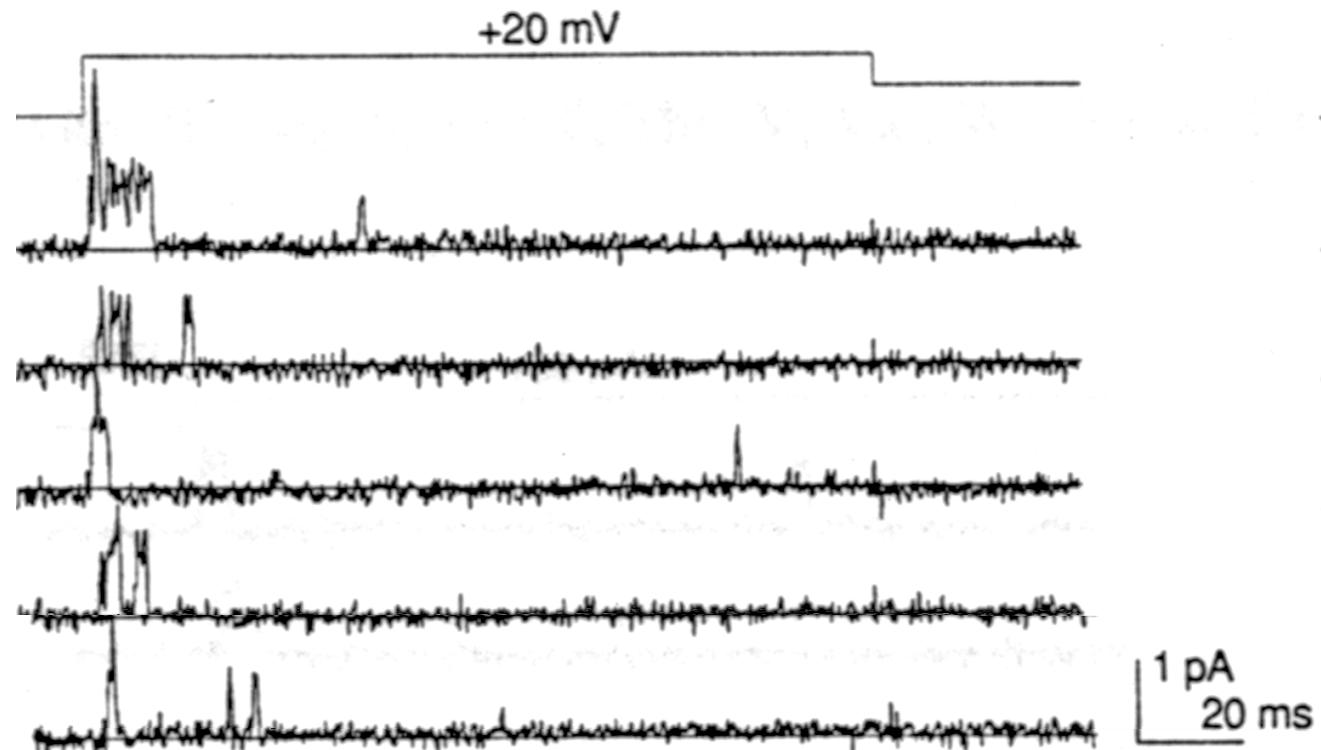
(A) UNITARY K^+ CURRENTS



(B) ENSEMBLE AVERAGE



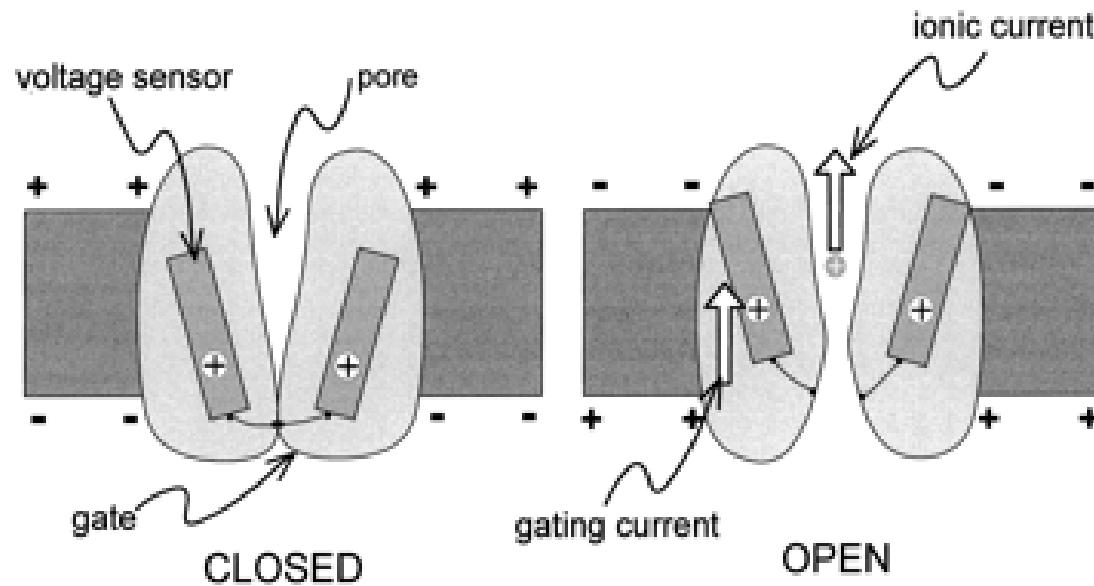
Inactivation dans les canaux potassiques Shaker



Hoshi T, Zagotta WN, Aldrich RW (1990)
Biophysical and molecular mechanisms of Shaker potassium channel inactivation.
Science 250: 533-538.

mécanisme de l'activation

Le ‘gating’ des canaux par le potentiel requiert
un mouvement de charge dans la protéine



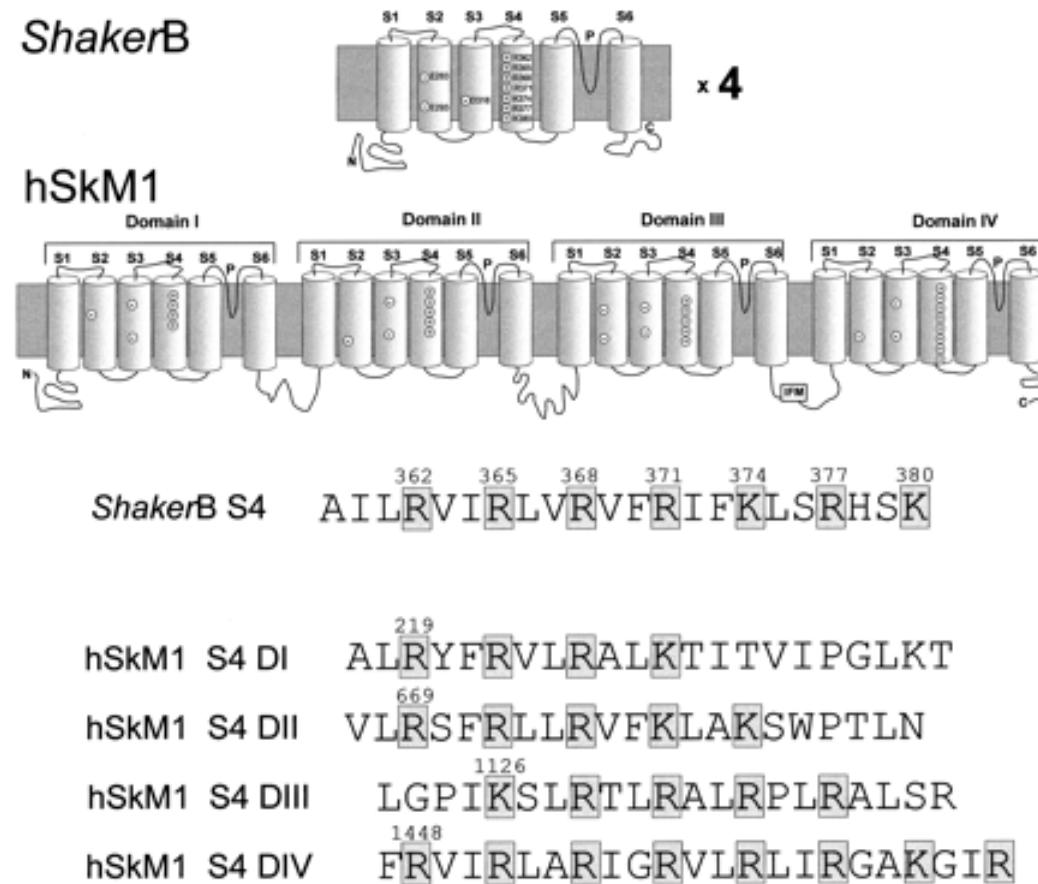
Bezanilla F (2000)

The Voltage Sensor in Voltage-Dependent Ion Channels

Physiol Rev 80: 555-592.

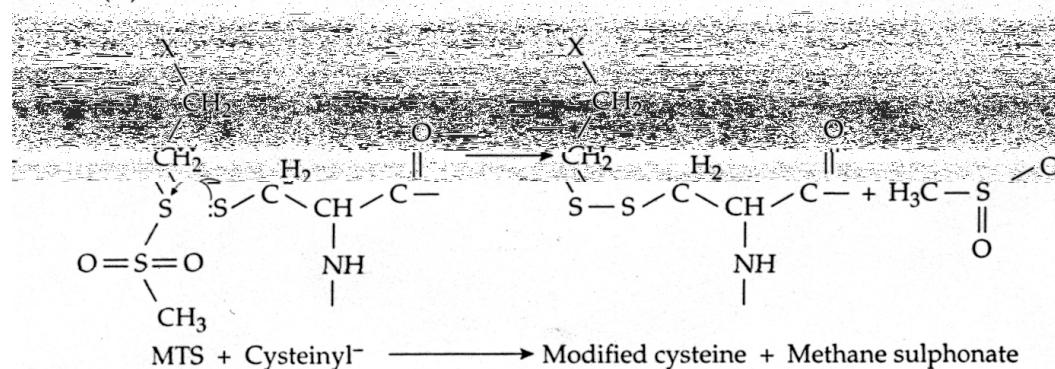
le senseur de potentiel ('voltage-sensor')

Le segment S4 bon candidat pour le senseur de potentiel

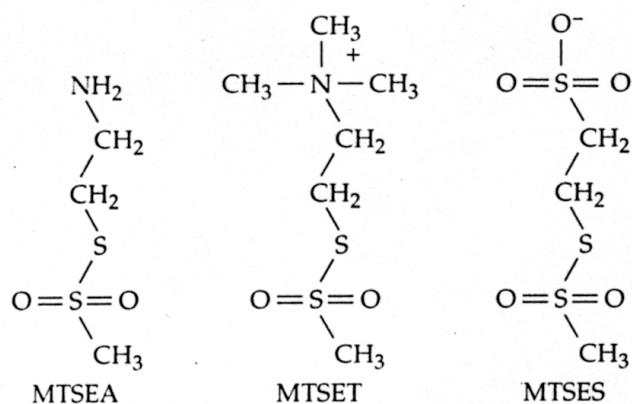


La méthode SCAM (substituted cystein accessibility method) permet de sonder les changements de conformation des protéines membranaires à l'aide de composés methane-thio-sulfonate (MTS)

(A) REACTION

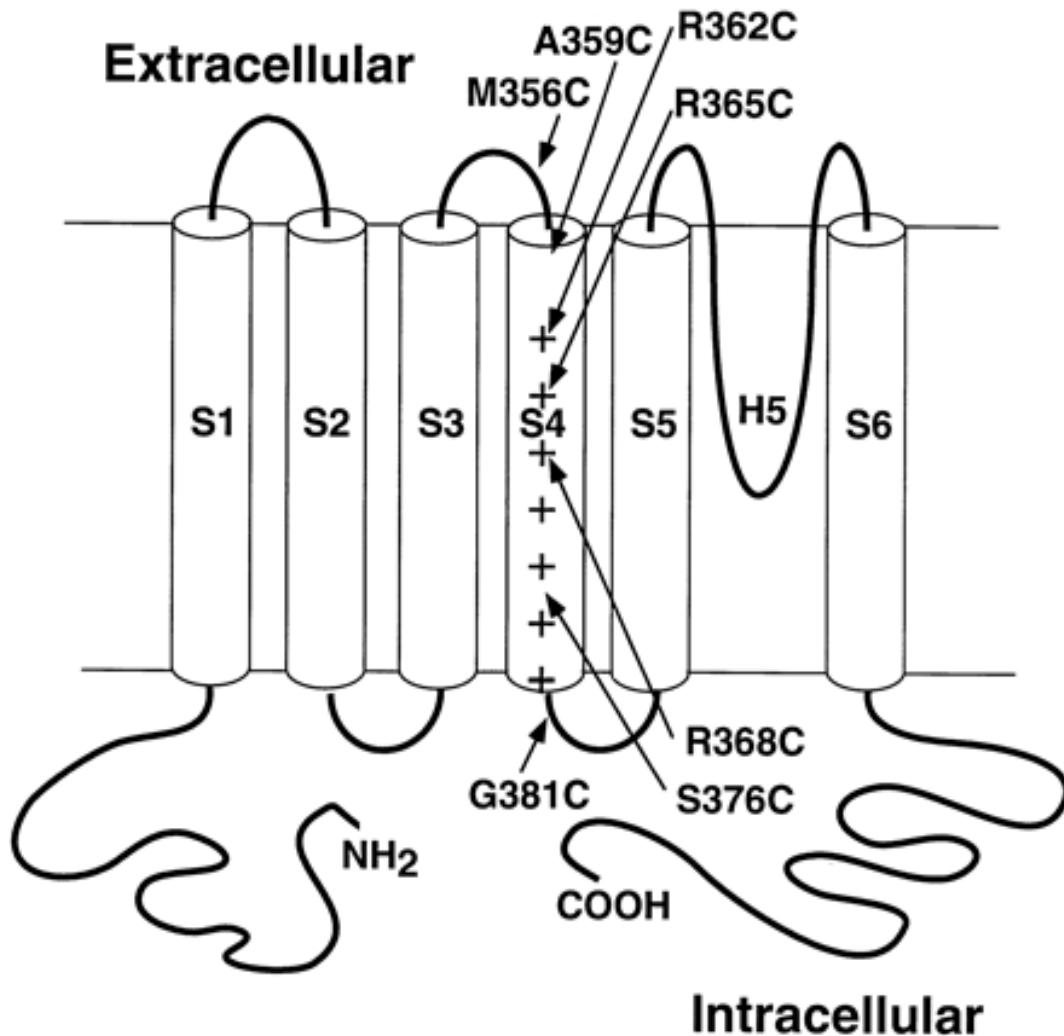


(B) MTS REAGENTS

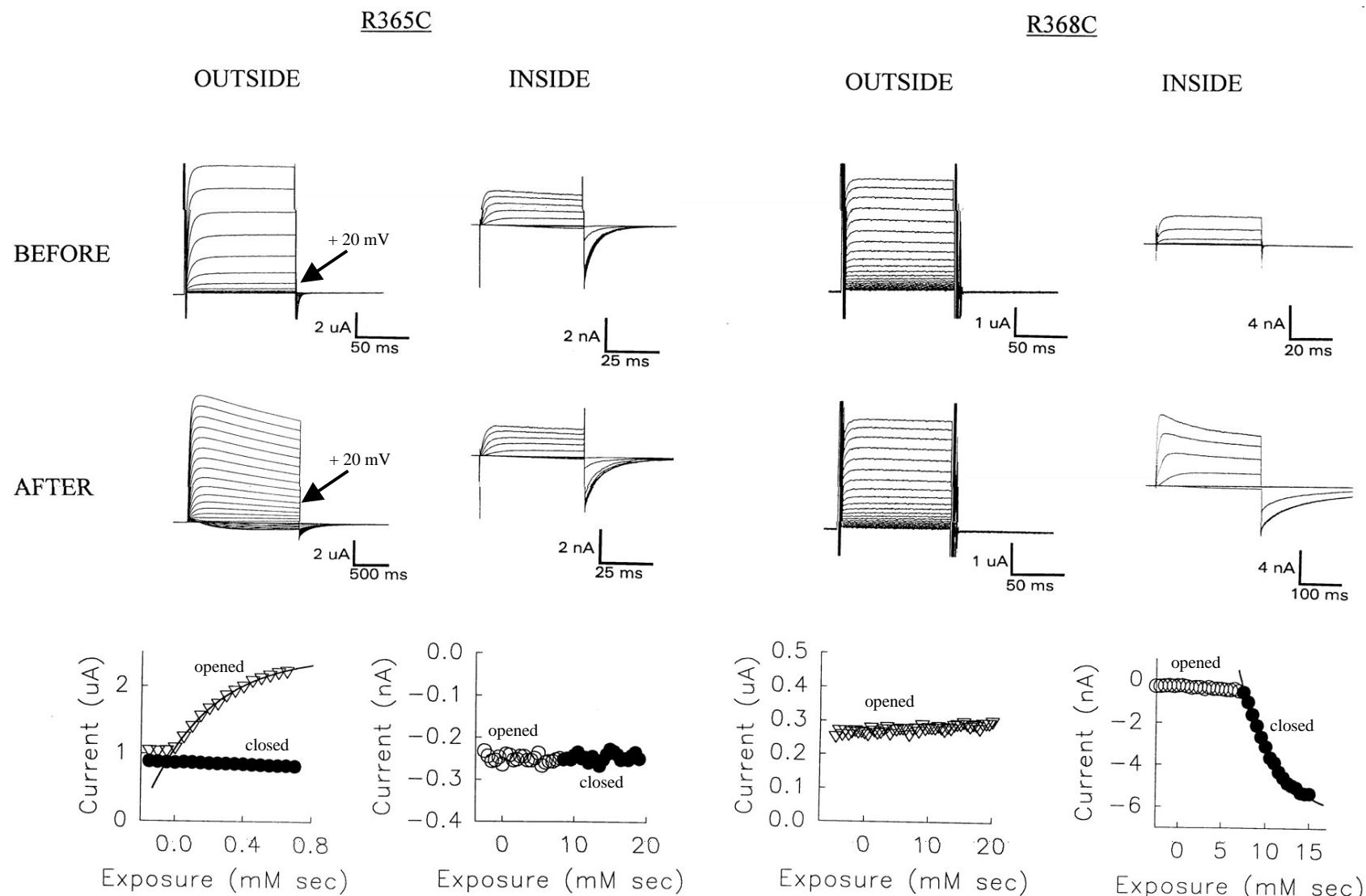


Karlin A, Akabas MH (1998)
Substituted-cysteine accessibility method.
Methods Enzymol 293: 123-145

Analyse par SCAM du mouvement de S4 dans les canaux Shaker

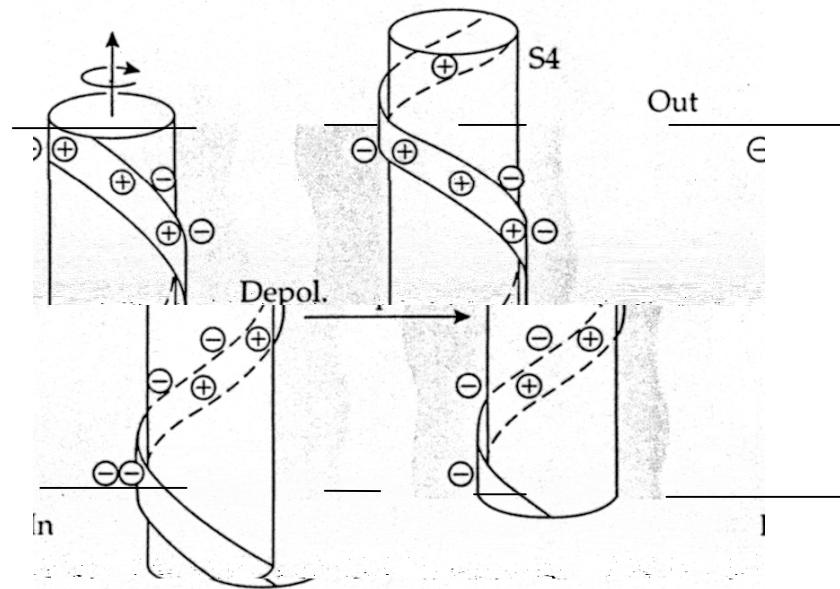


Larsson HP, Baker OS, Dhillon DS,
Isacoff EY (1996) Transmembrane
movement of the shaker K⁺ channel S4.
Neuron 16: 387-397.



Larsson HP, Baker OS, Dhillon DS, Isacoff EY (1996)
 Transmembrane movement of the shaker K⁺ channel S4.
Neuron 16: 387-397.

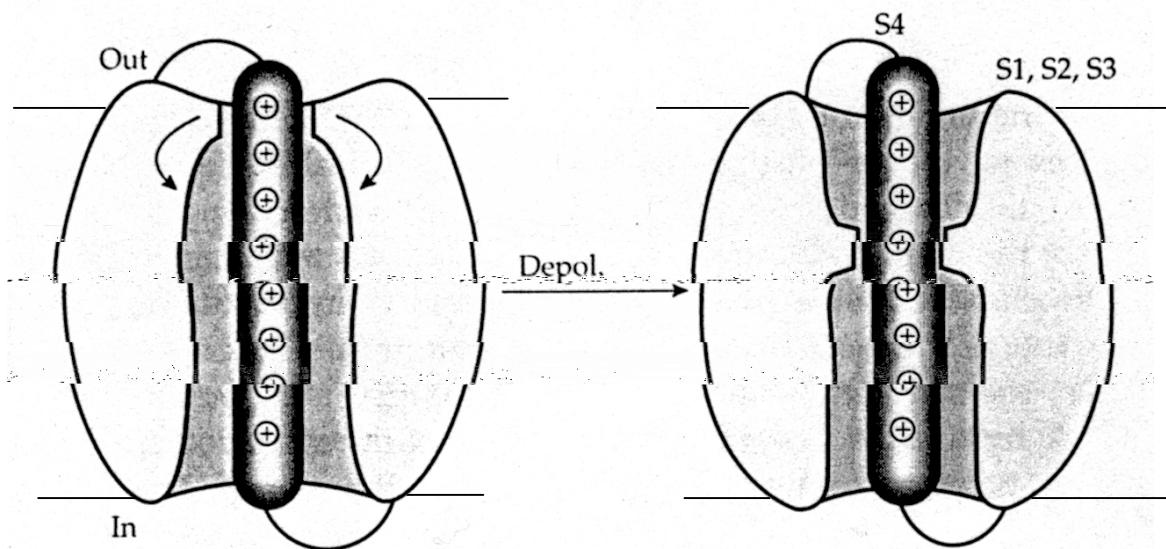
NG HELIX



(B) SLIDI

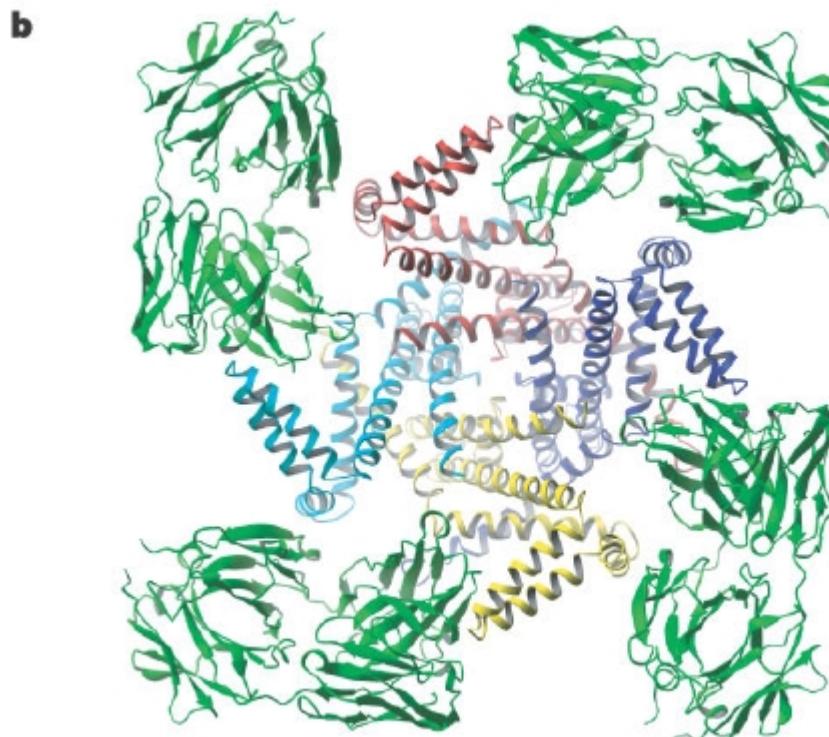
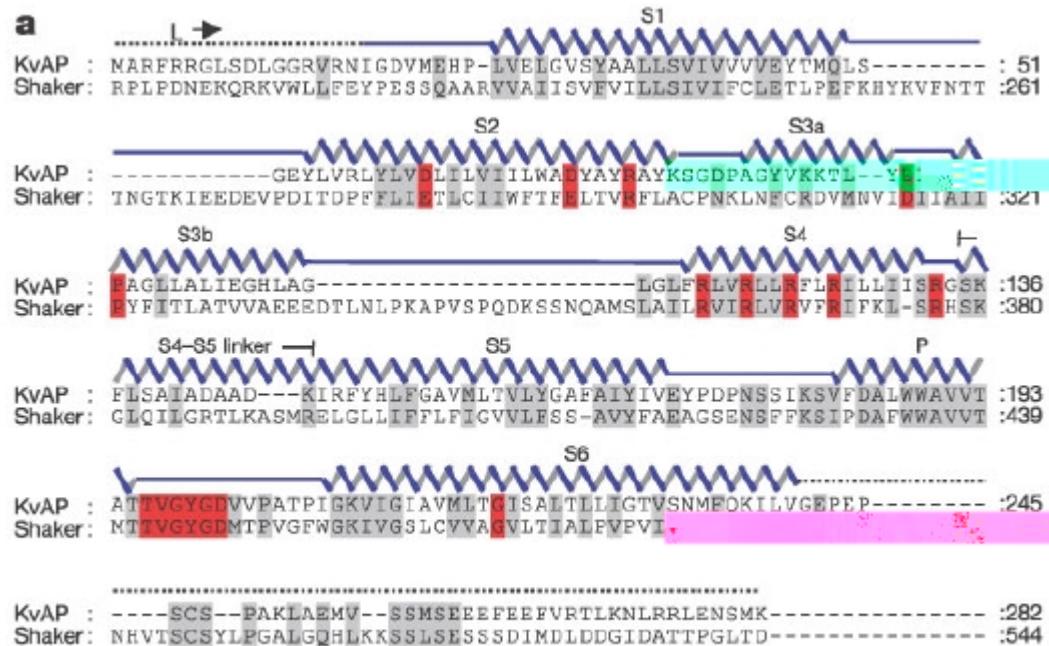
Out
 ϵ

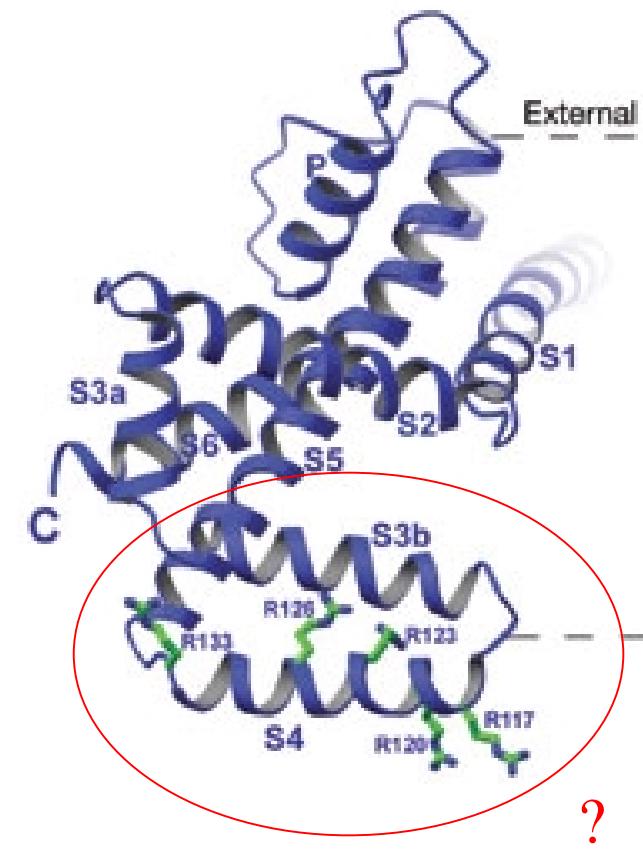
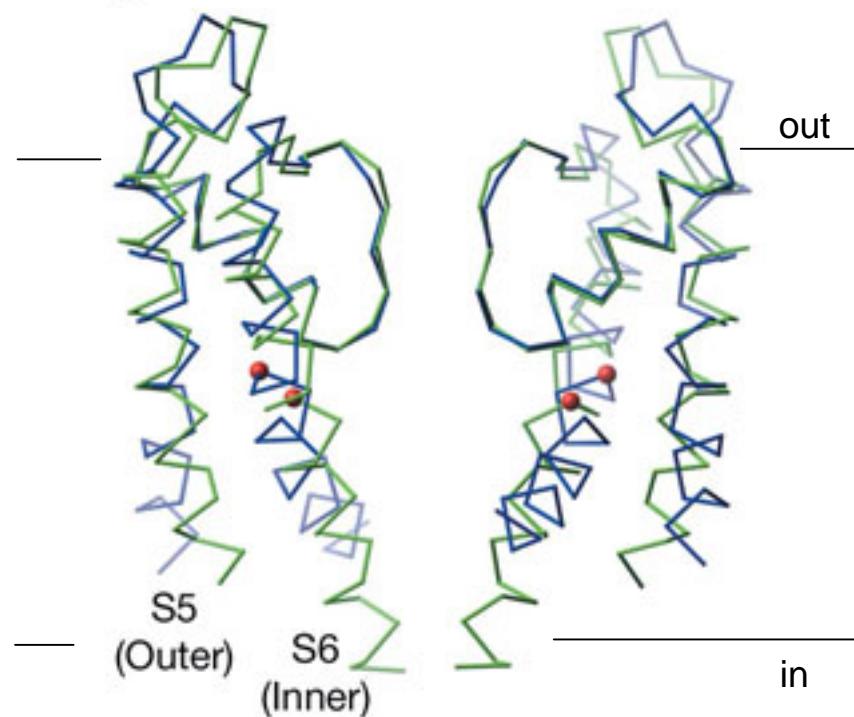
(C) MOVING ORIFICE



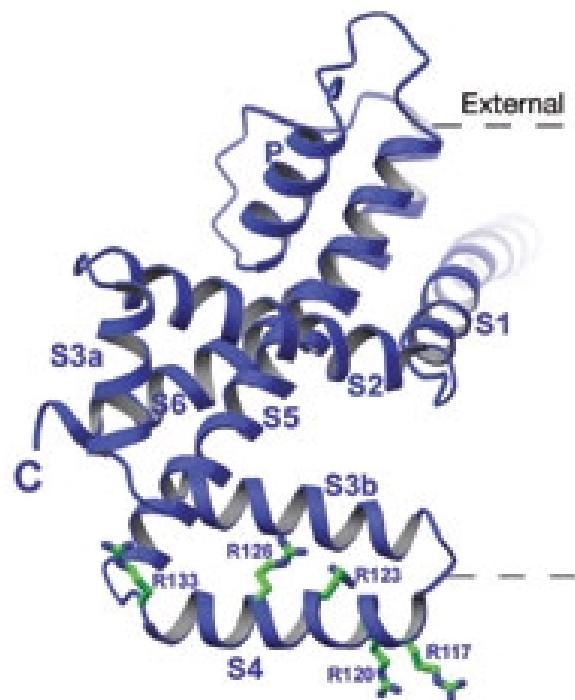
Structure 3D du canal KvAP de la bactérie thermophile Aeropyrum pernix

Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, MacKinnon R (2003)
 X-ray structure of a voltage-dependent K⁺ channel. *Nature* 423: 33-41.

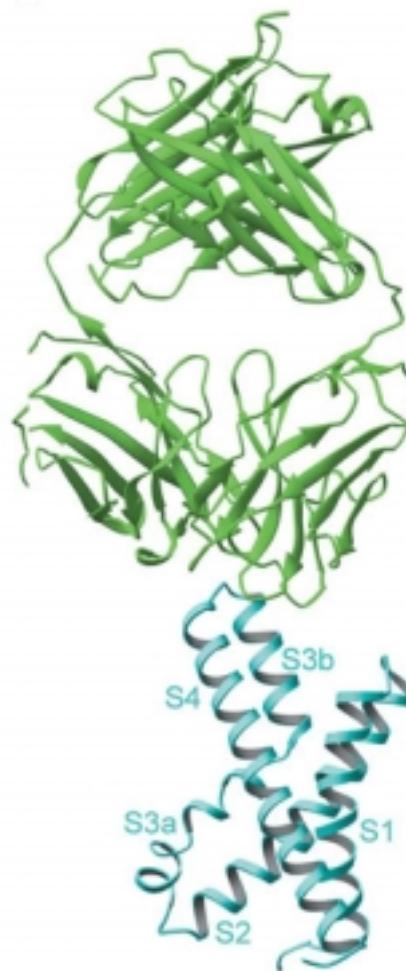




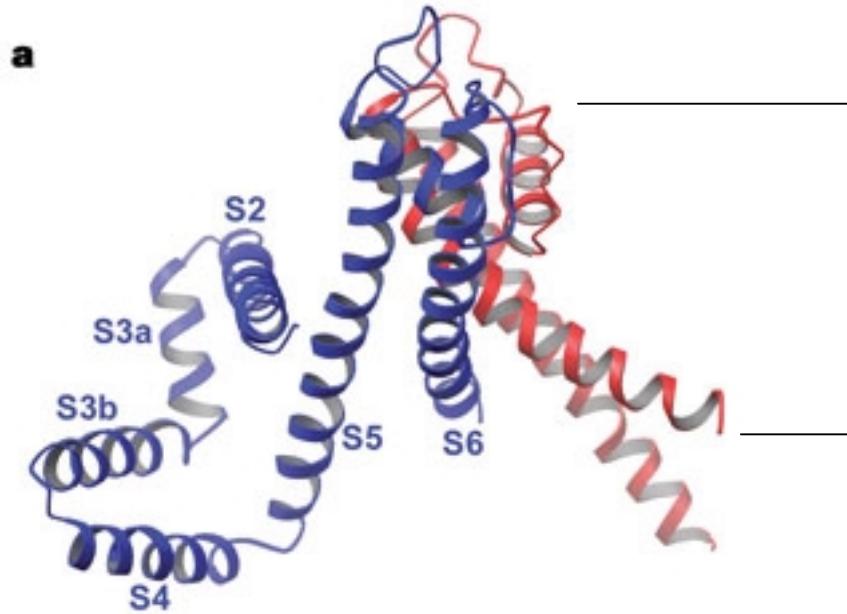
canal entier



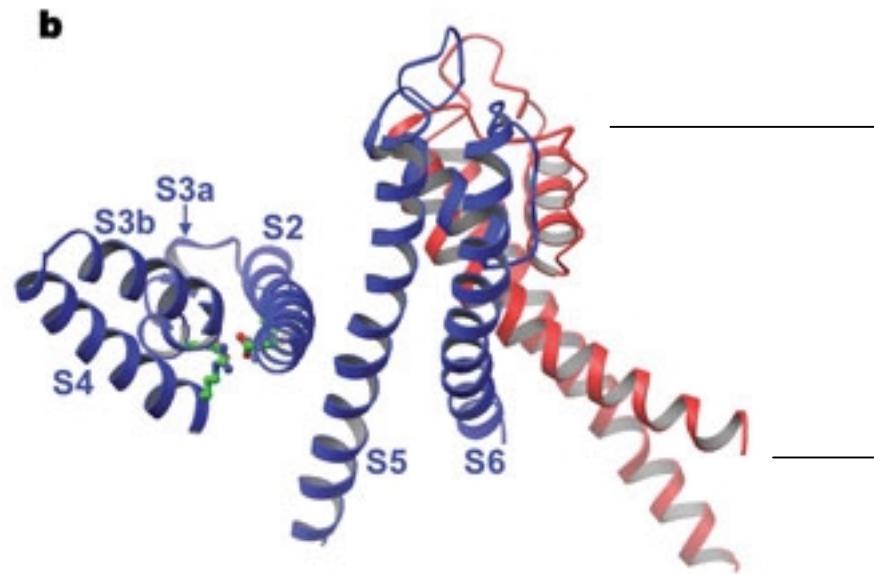
senseur de potentiel



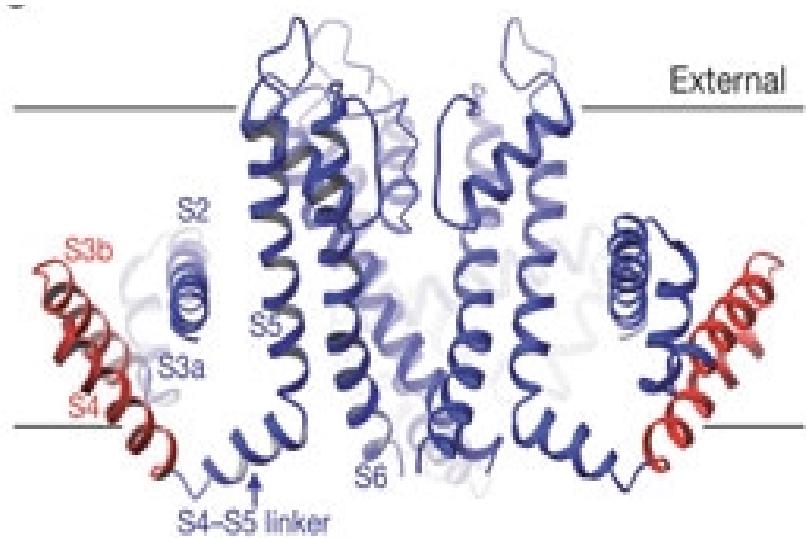
dans le cristal



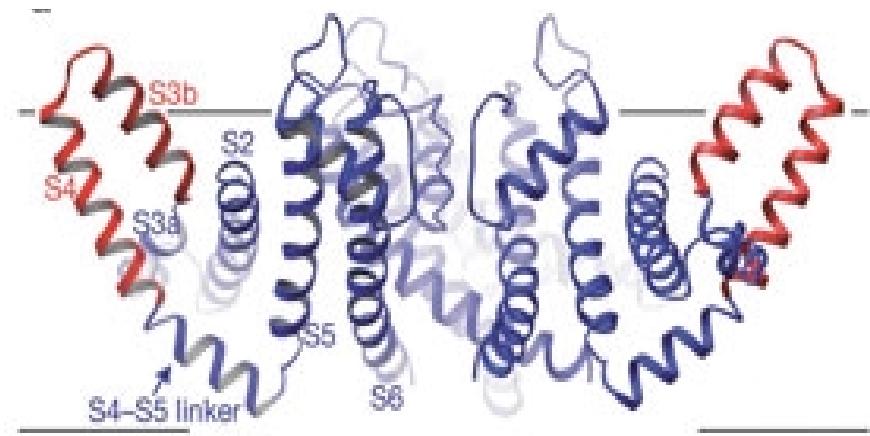
dans la membrane



Modèle ‘paddle’ pour le mouvement du senseur de potentiel



resting (hyperpolarized)

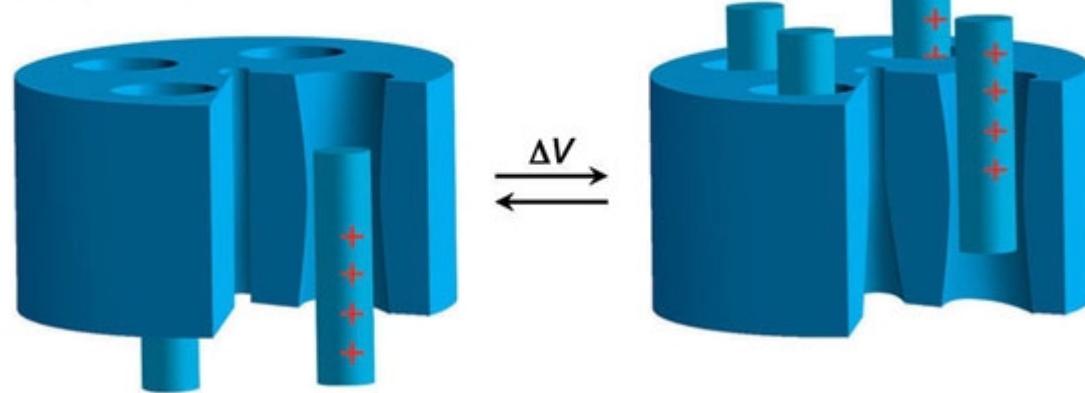


activated (depolarized)

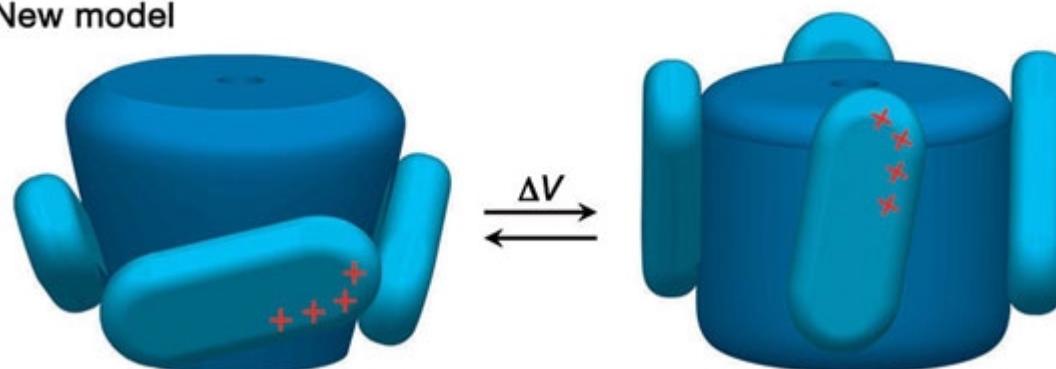
Jiang Y, Ruta V, Chen J, Lee A, MacKinnon R (2003)
The principle of gating charge movement in a voltage-dependent K⁺ channel.

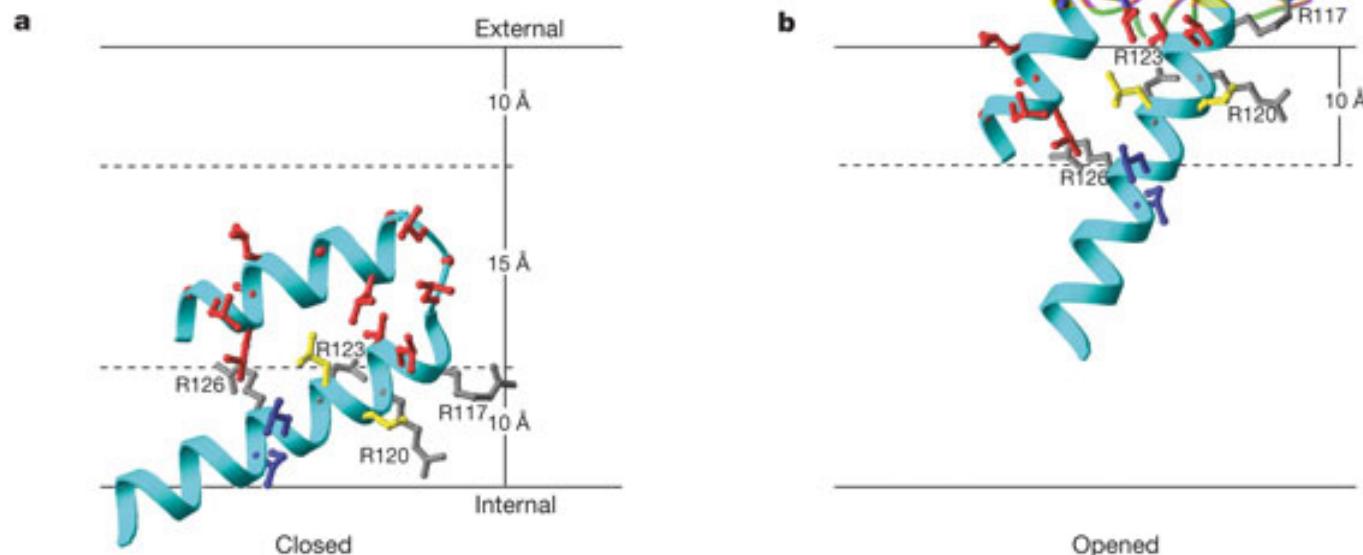
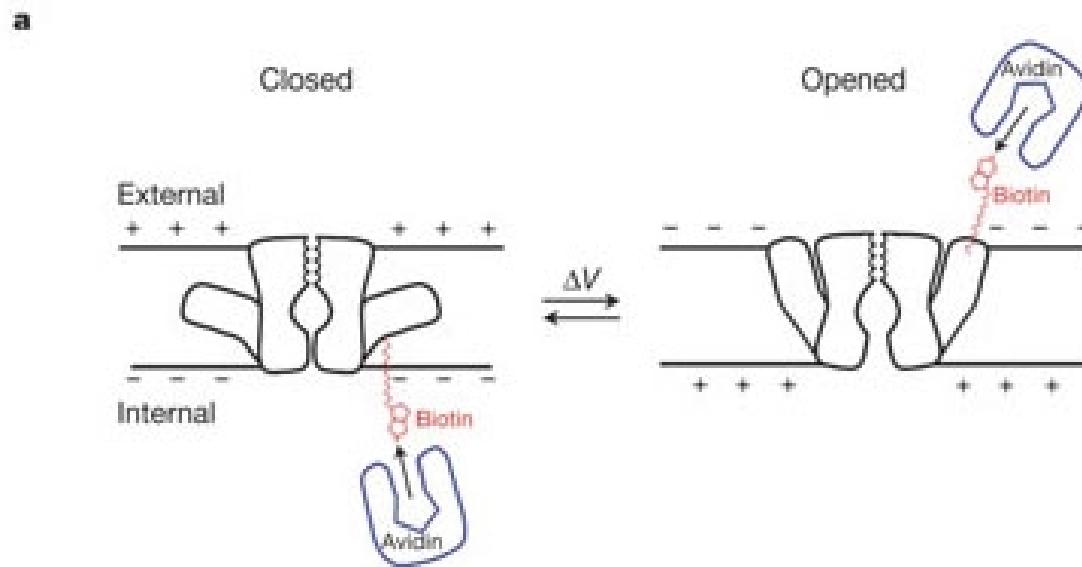
Nature 423: 42-48.

a Conventional model



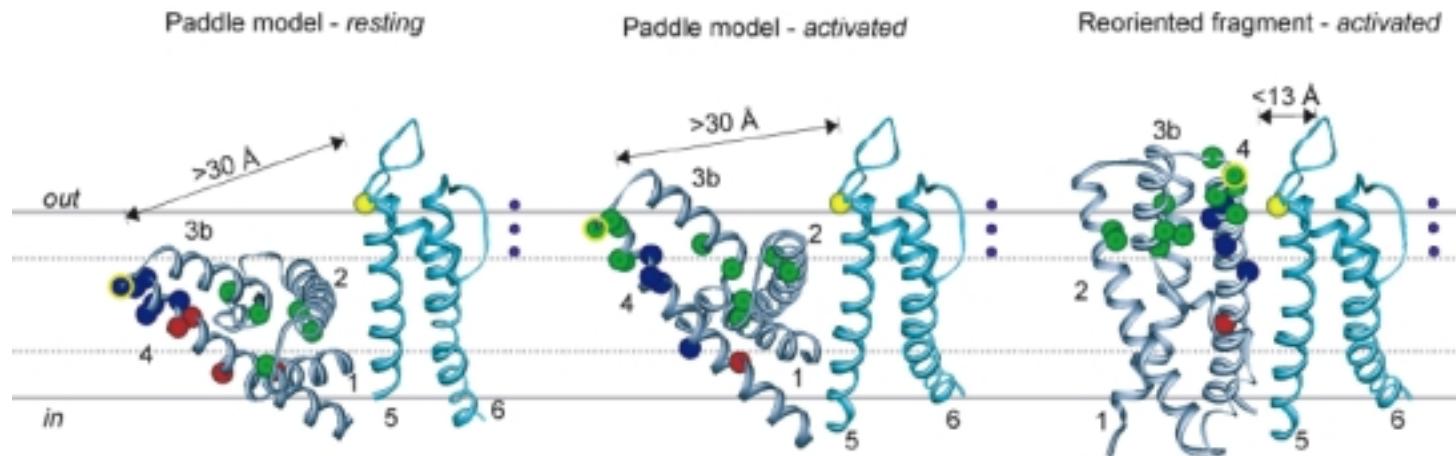
b New model



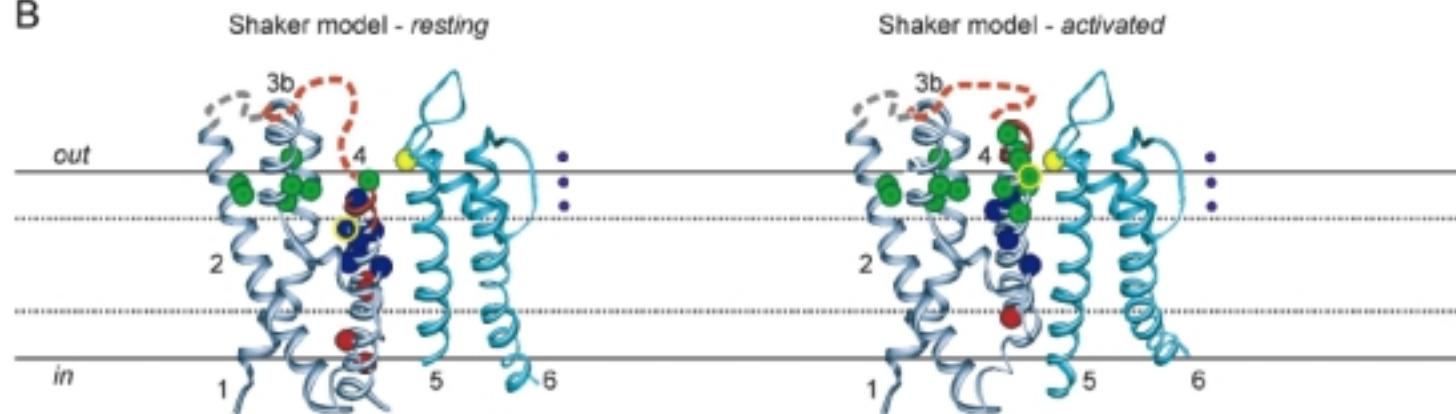


Le modèle ‘paddle’ est très controversé

A

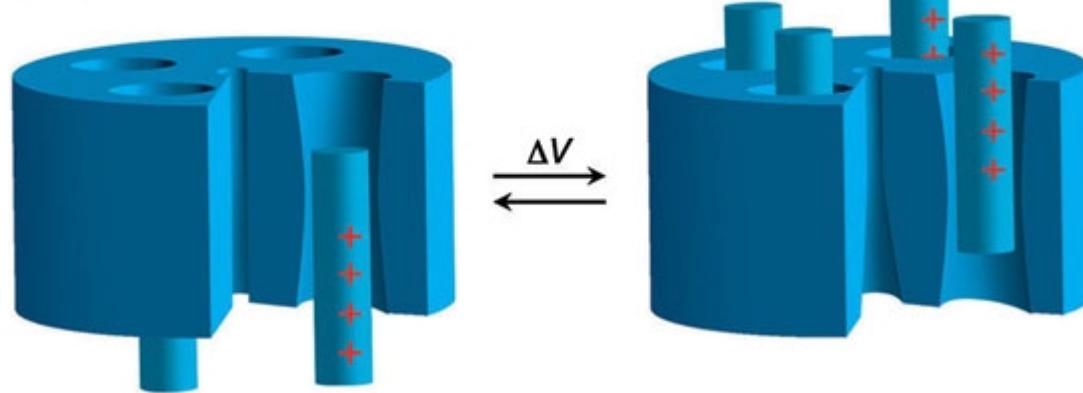


B

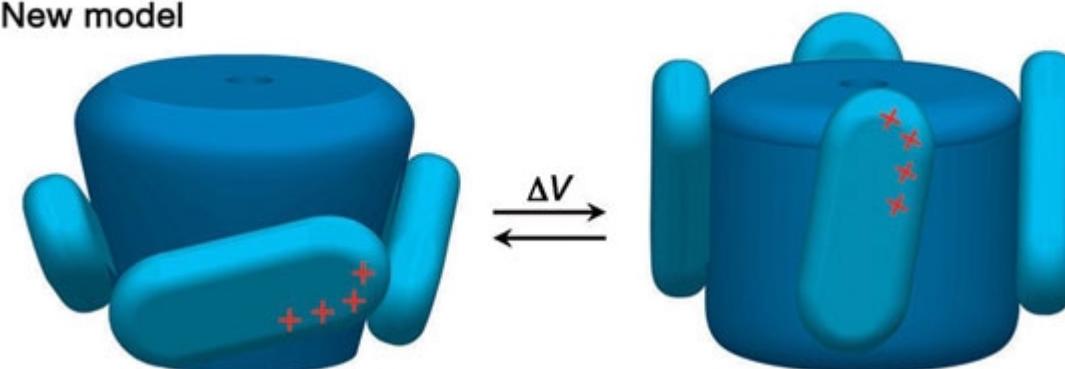


Gandhi CS, Clark E, Loots E, Pralle A, Isacoff EY (2003)
The Orientation and Molecular Movement of a K⁺ Channel Voltage-Sensing Domain.
Neuron 40: 515-525

a Conventional model

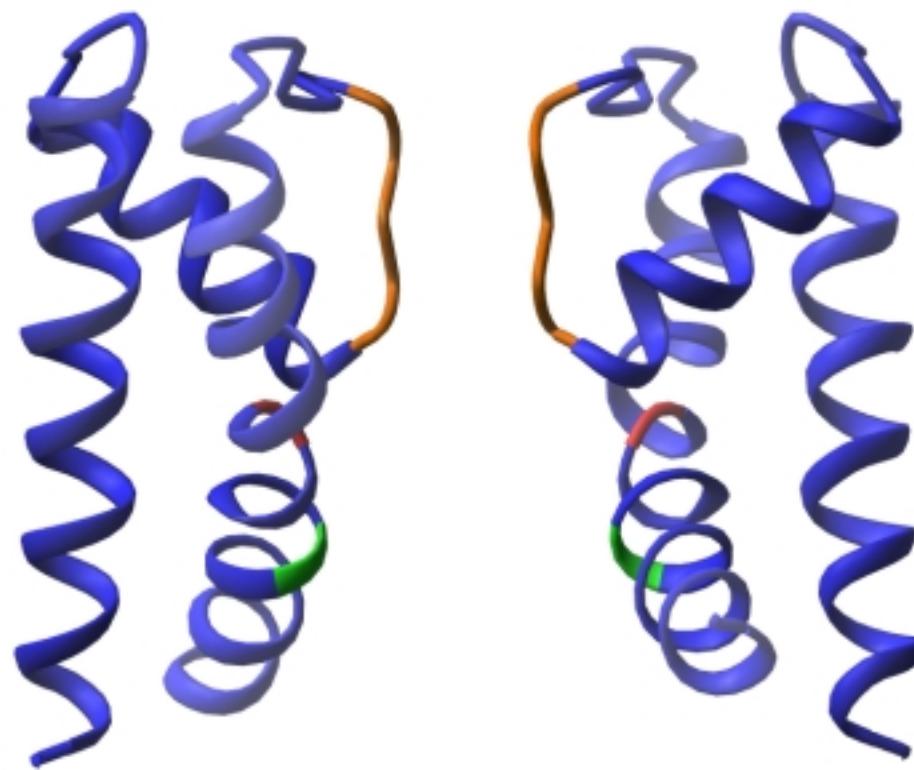


b New model



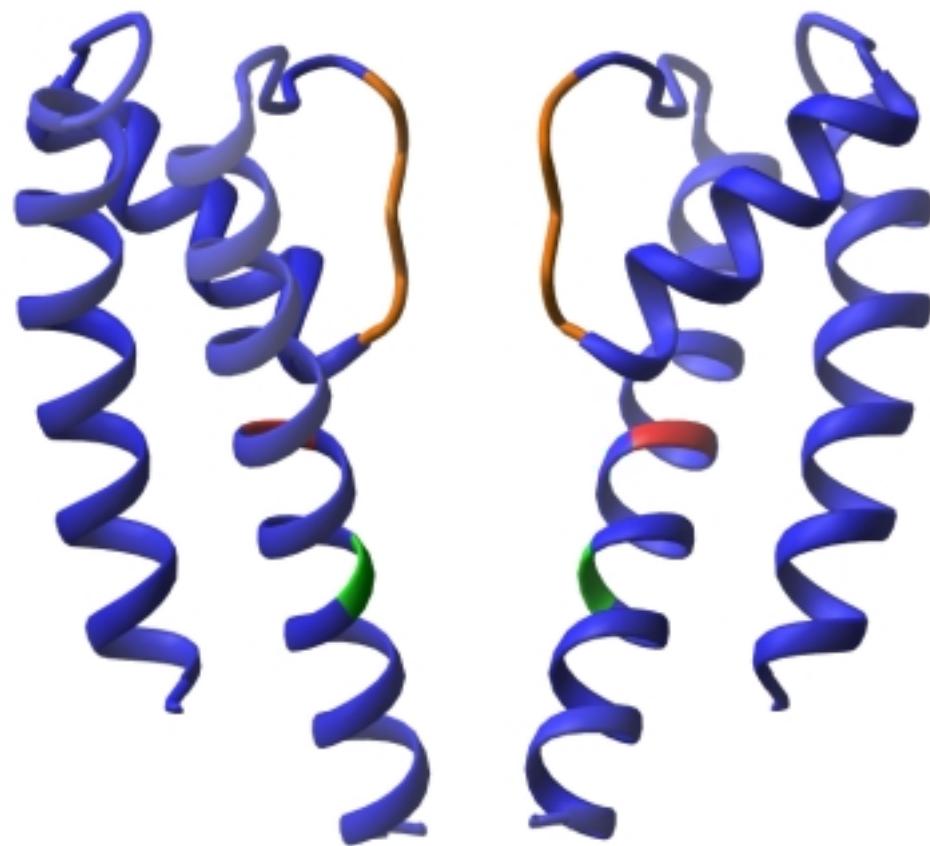
la porte (gate)

Structure 3D du canal MthK de
Methanobacterium thermoautotrophicum



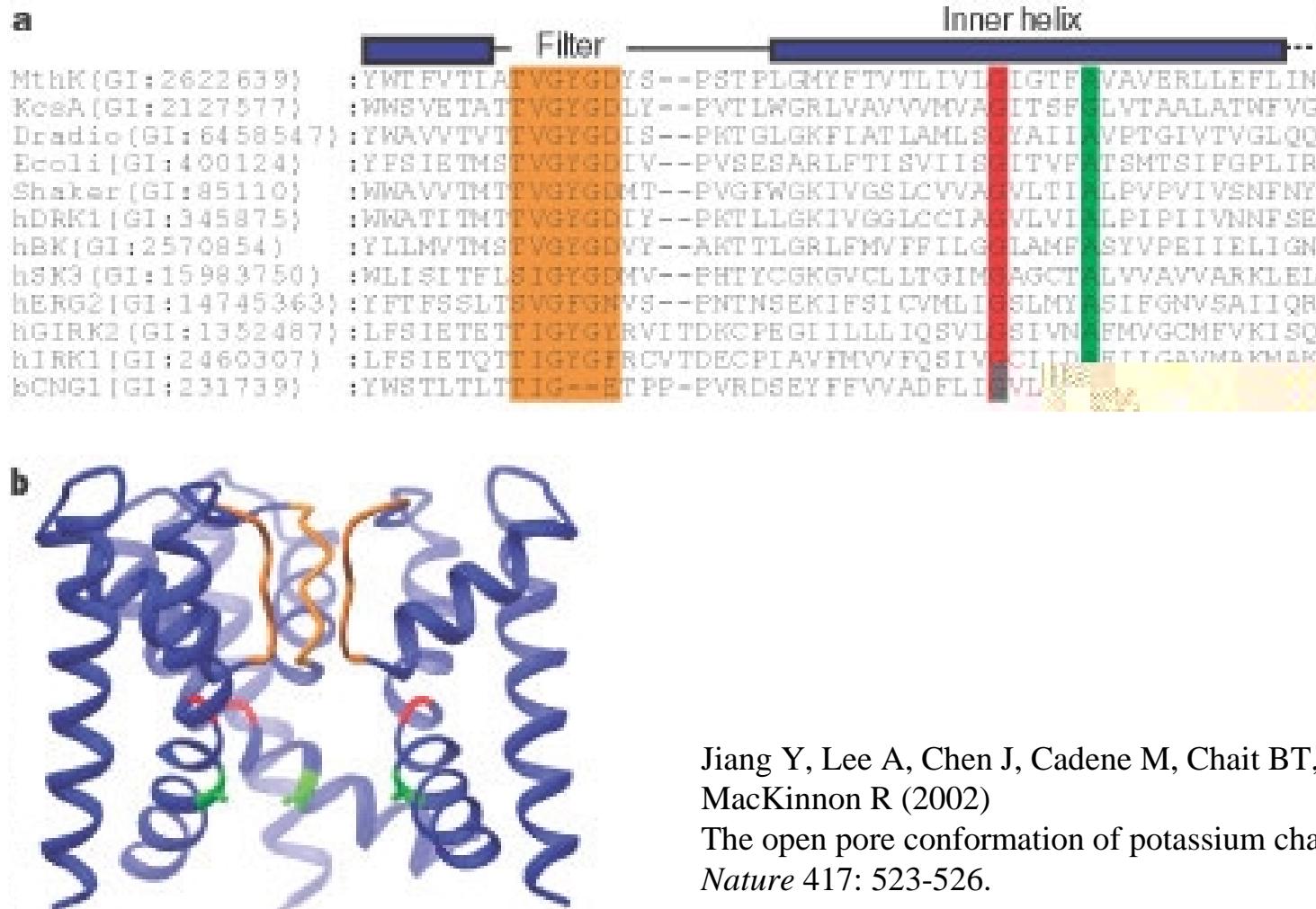
Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R (2002)
The open pore conformation of potassium channels.
Nature 417: 523-526.

Structure 3D de KcsA
(probablement dans l'état fermé)



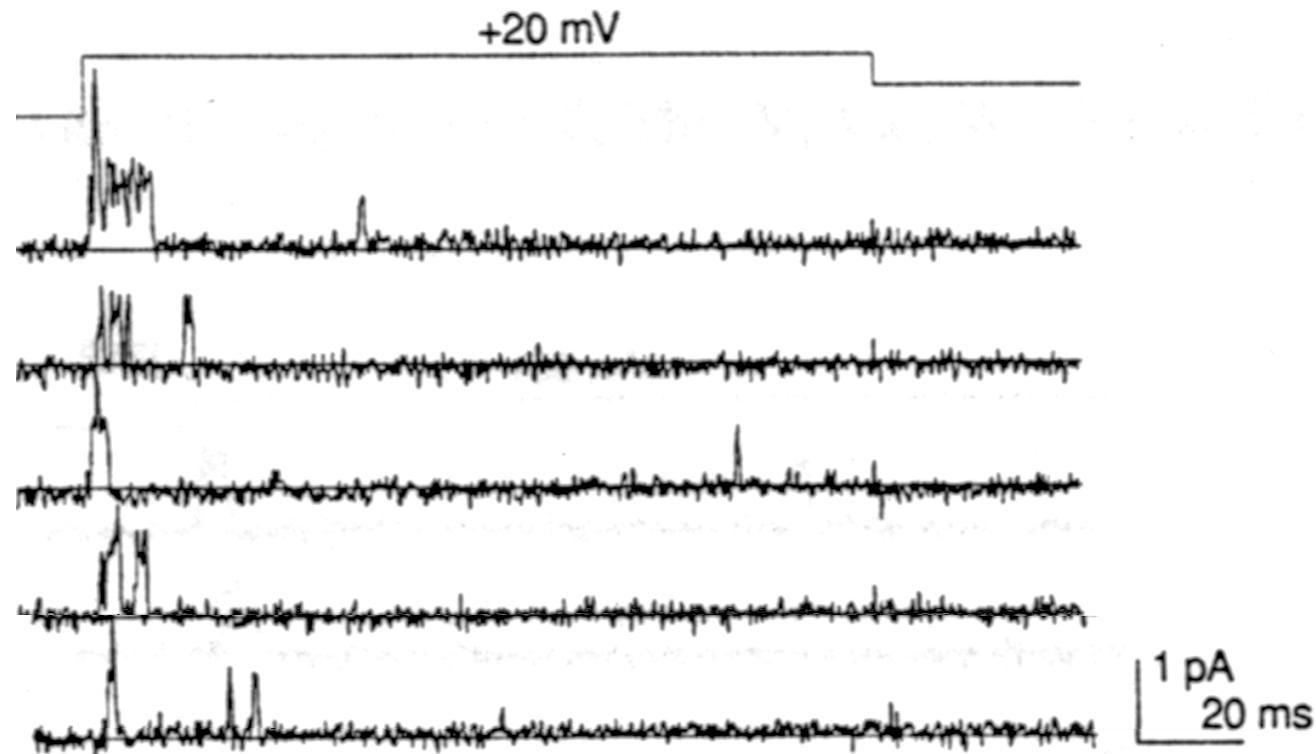
Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998)
The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.
Science 280: 69-77.

Cassure de l'hélice S6, un mécanisme de 'gating' conservé dans les canaux potassiques ?



mécanismes d'inactivation

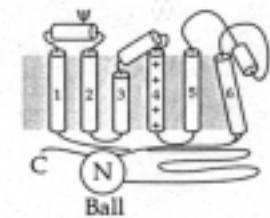
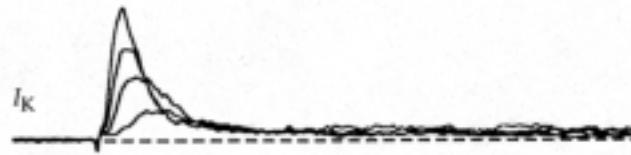
Inactivation dans les canaux potassiques Shaker



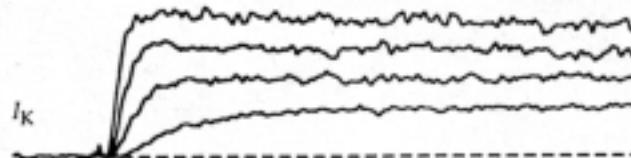
Hoshi T, Zagotta WN, Aldrich RW (1990)
Biophysical and molecular mechanisms of Shaker potassium channel inactivation.
Science 250: 533-538.

Suppression et reconstitution de l'inactivation dans les canaux potassiques Shaker

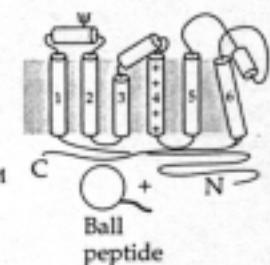
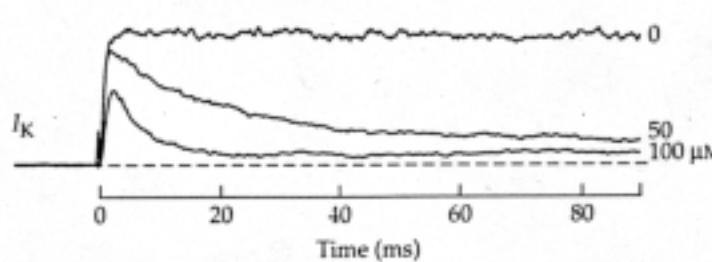
(A) WILD-TYPE ShB



(B) DELETION MUTANT $\Delta 6-46$



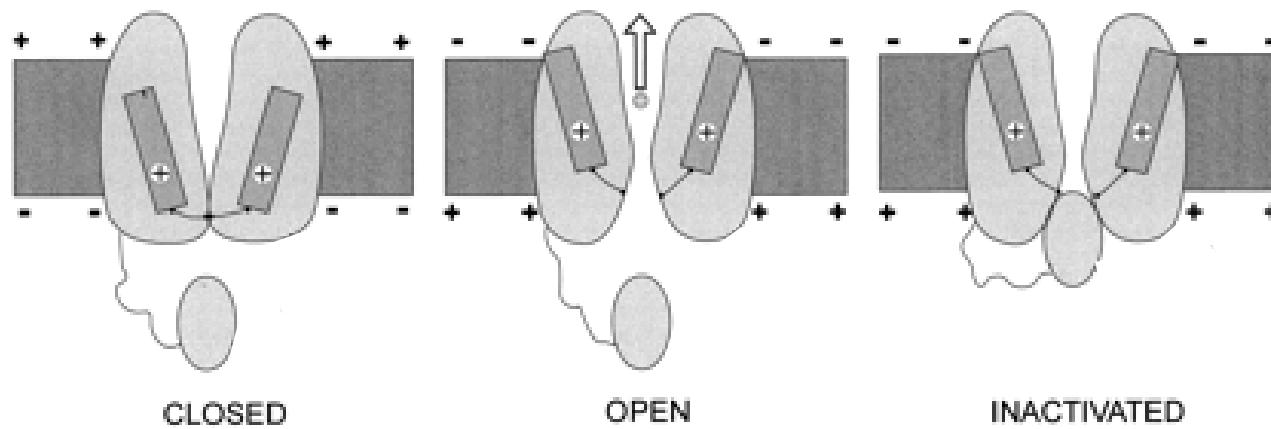
(C) MUTANT + ShB PEPTIDE



Zagotta WN, Hoshi T, Aldrich RW (1990)

Restoration of inactivation in mutants of Shaker potassium channels by a peptide derived from ShB.
Science 250: 568-571.

Un mécanisme ball and chain pour l'inactivation des canaux sensibles au potentiel



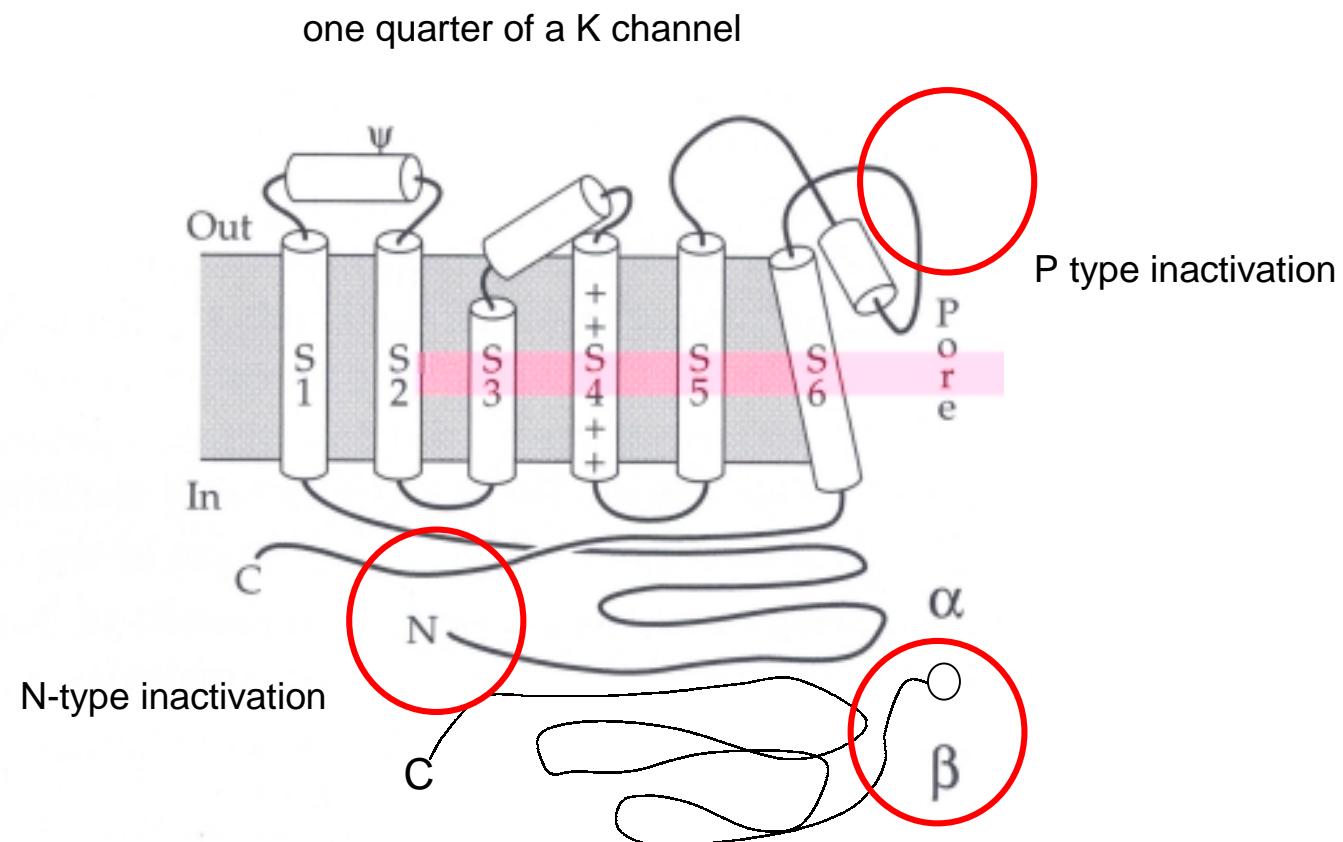
Armstrong CM, Bezanilla F, Rojas E (1973)

Destruction of sodium conductance inactivation in squid axons perused with pronase.
J Gen Physiol 62: 375-391

Hoshi T, Zagotta WN, Aldrich RW (1990)

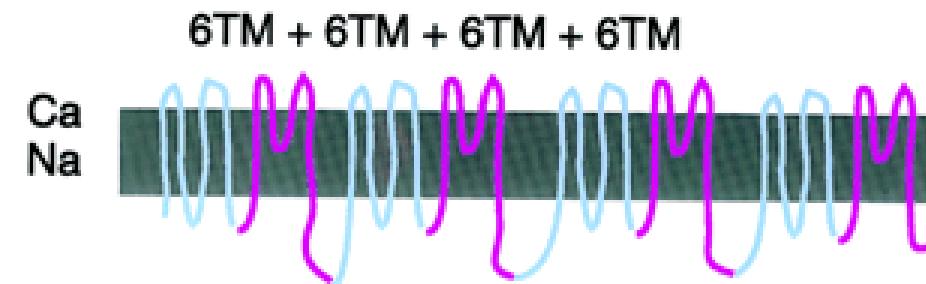
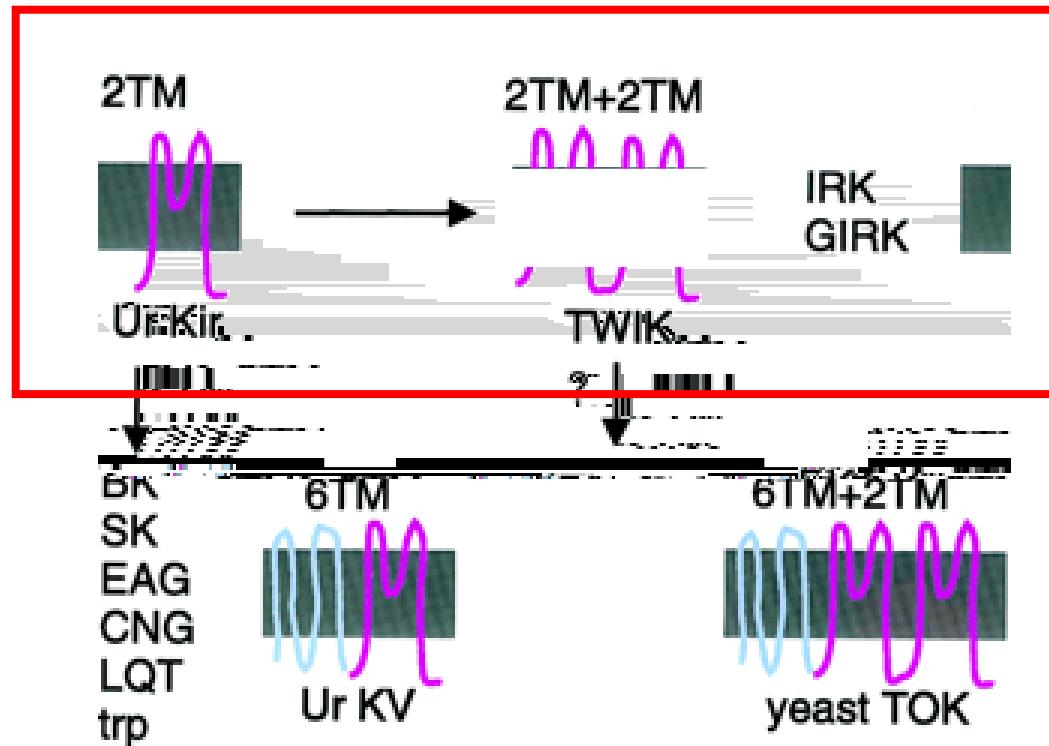
Biophysical and molecular mechanisms of Shaker potassium channel inactivation.
Science 250: 533-538

mécanismes d'inactivation

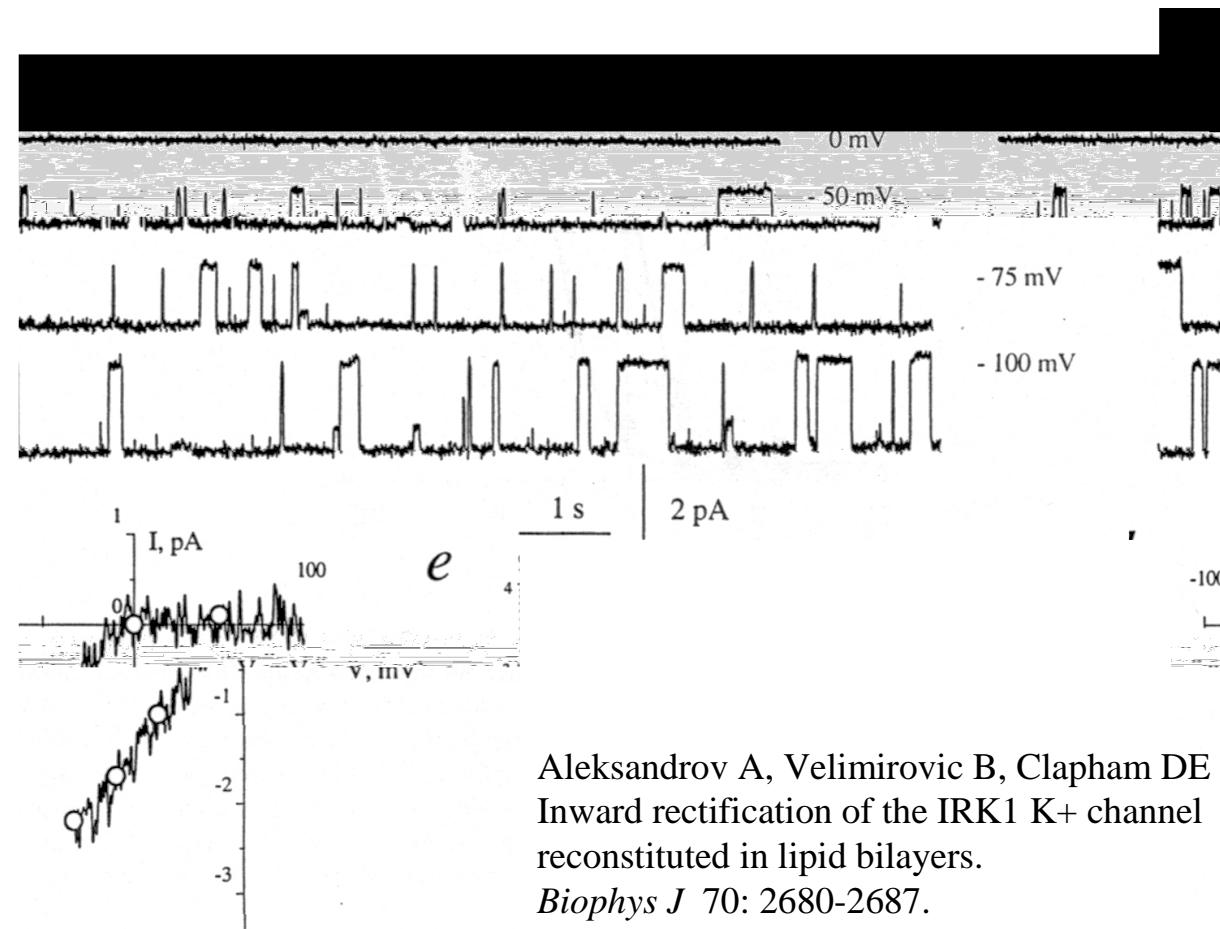


‘gating’ dans les canaux K⁺ ‘inward rectifier’

B ϵ
Proto



Le canal IRK1 (inward rectifier) est ouvert aux potentiels hyperpolarisés



Aleksandrov A, Velimirovic B, Clapham DE (1996)
Inward rectification of the IRK1 K⁺ channel
reconstituted in lipid bilayers.
Biophys J 70: 2680-2687.

Les ions Mg^{2+} et des polycations intracellulaires (famille de la spermine) bloquent IRK1, un canal de la famille des inward rectifiers à 2 TM

Putrescine



Spermidine



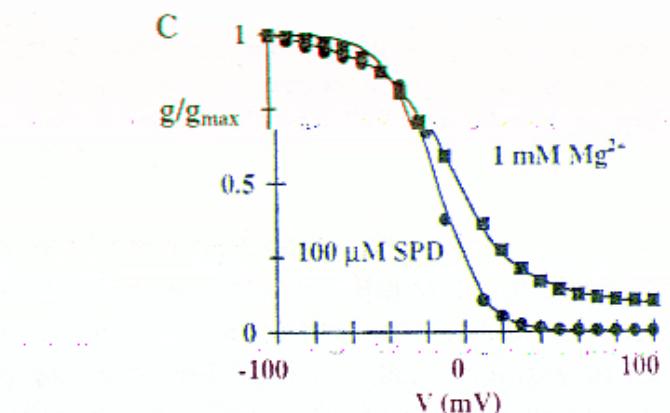
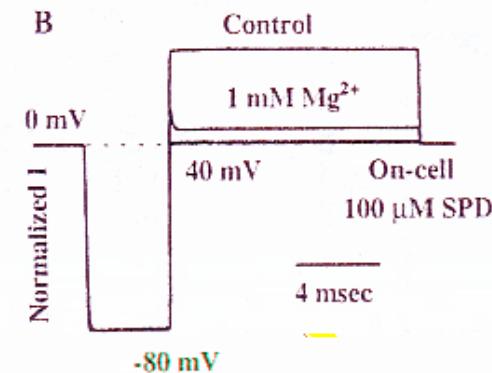
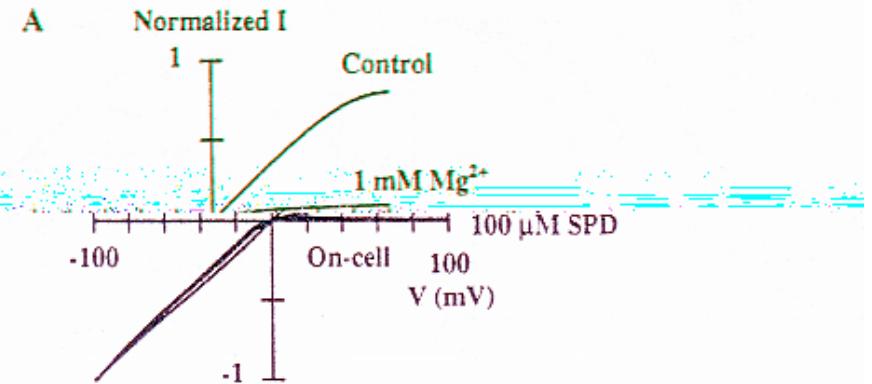
Spermine



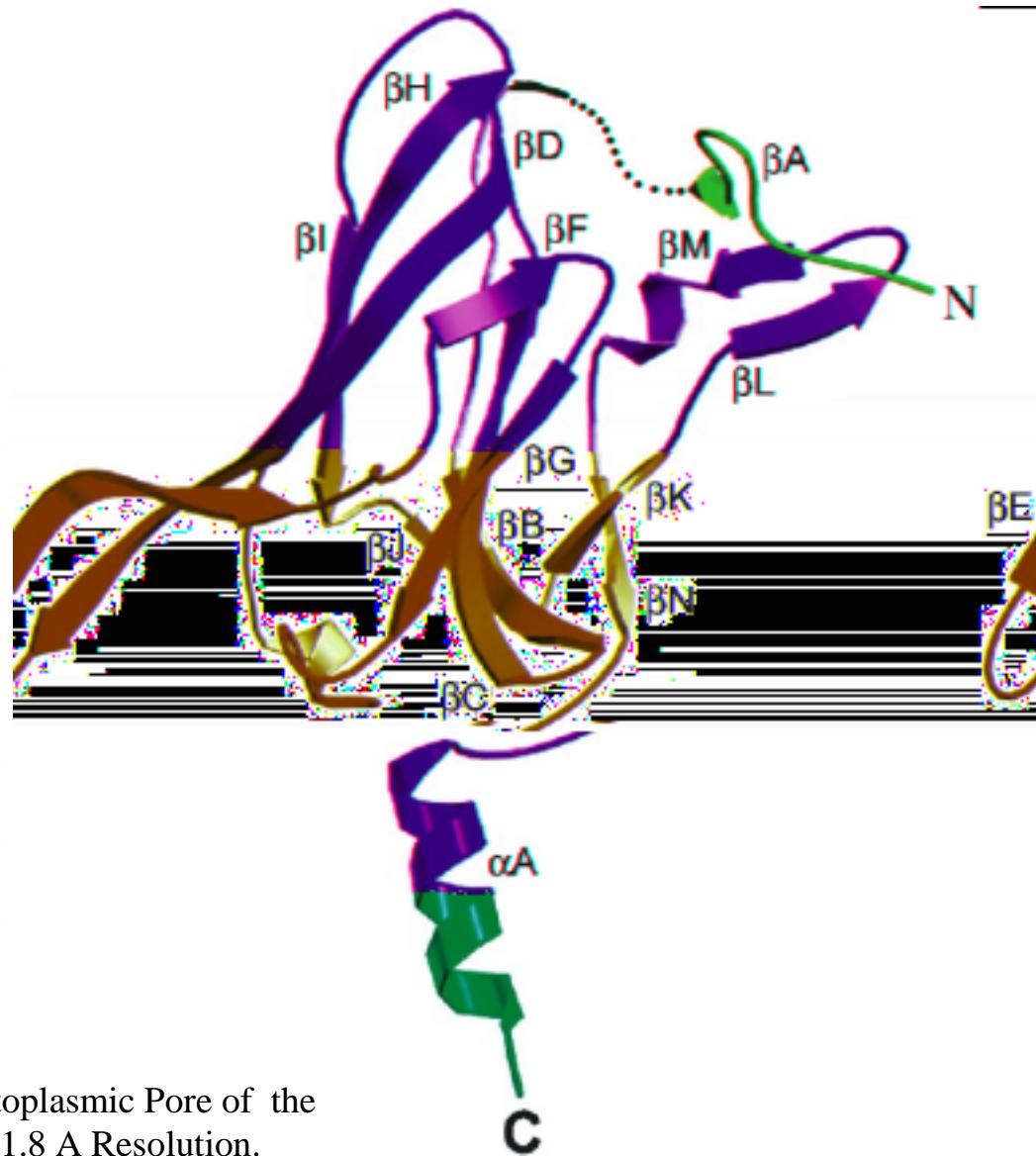
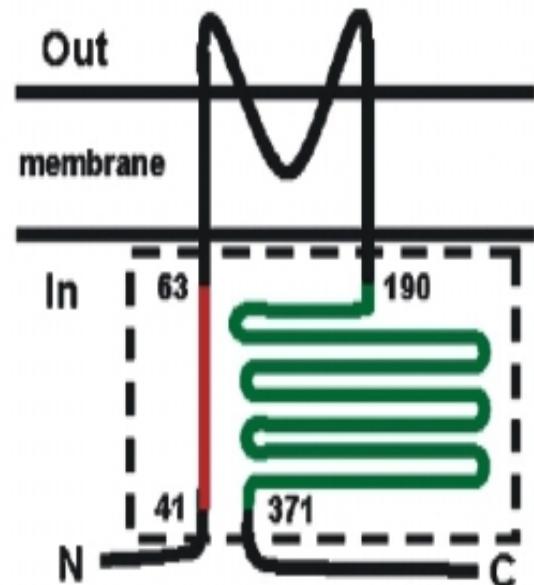
Yang J, Jan YN, Jan LY (1995)

Control of rectification and permeation by residues in two distinct domains in an inward rectifier K⁺ channel.

Neuron 14: 1047-1054.

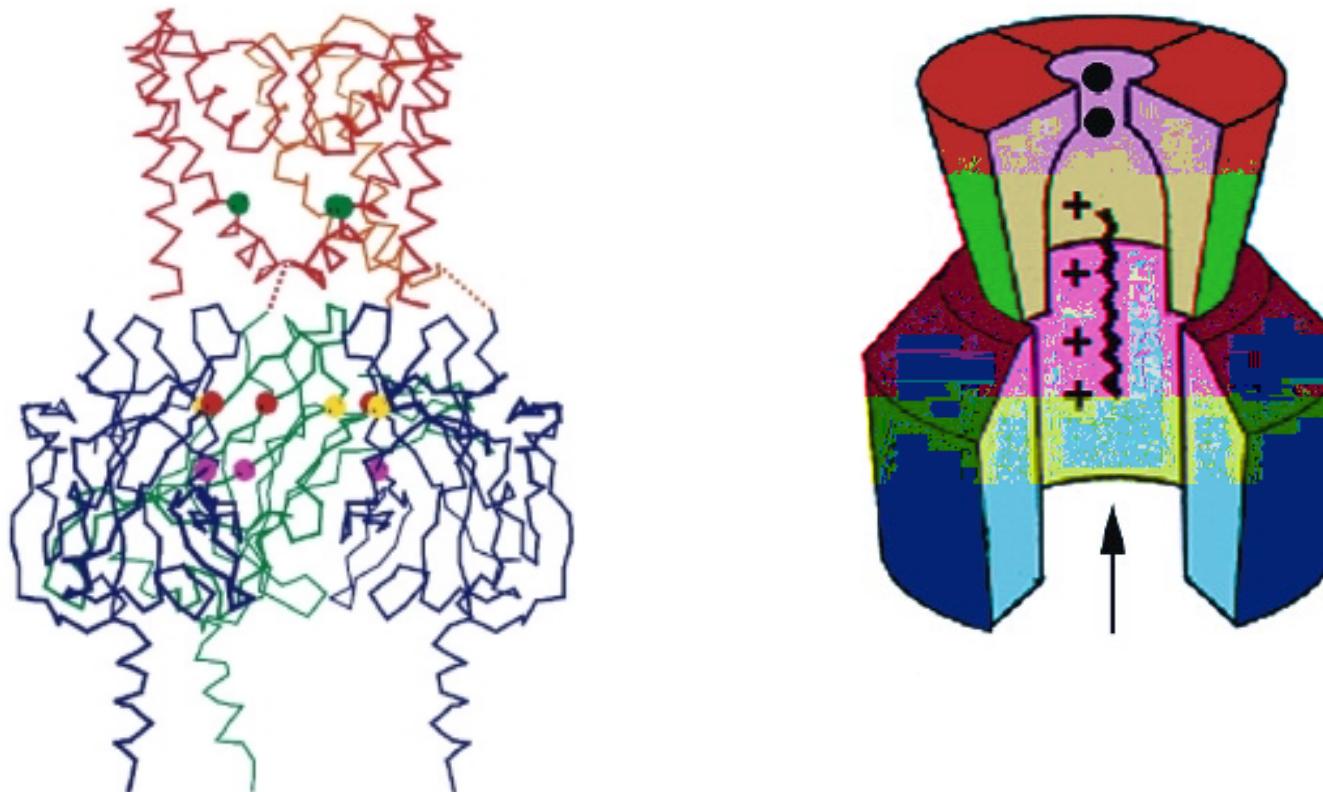


Structure cristallographique du domaine cytoplasmique du canal mGIRK1



Nishida M, MacKinnon R (2002)
Structural Basis of Inward Rectification. Cytoplasmic Pore of the
G Protein-Gated Inward Rectifier GIRK1 at 1.8 Å Resolution.
Cell 111: 957-965.

Le domaine cytoplasmique de mGIRK1 forme un pore disposé en registre avec le pore transmembranaire où se lient les polyamines



Nishida M, MacKinnon R (2002)
Structural Basis of Inward Rectification. Cytoplasmic Pore of the G Protein-Gated Inward Rectifier GIRK1 at 1.8 Å Resolution.
Cell 111: 957-965.