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KcsA蛋白的生物信息学分析

Bioinformatics Analysis of KcsA Protein

实用生物信息学课程汇报 G14组

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致谢

Acknowledgement





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第零部分

Background

背景



- KcsA是第一个在1998年由Roderick MacKinnon及其同事用X射线晶体学表征的钾离子通道。
- KcsA是钾离子通道蛋白中结构较为简单的一种，而且是原核生物体内的，相比于真核生物内的蛋白，更容易研究
- KcsA蛋白与Kv类型的通道蛋白具有强序列相似性，因此对KcsA的生物信息学研究可以为后者的研究提供参考
- 目前，KcsA研究的重点是利用原核通道作为大型真核K⁺通道（包括hERG）的通道动力学模型

Uysal S , Cuello L G , Cortes D M , et al. Mechanism of activation gating in the full-length KcsA K⁺ channel[J]. Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(29):11896-11899.



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第一部分

Transmembrane structure predication

预测跨膜结构



- WebLab 中Tmap 程序
- ExPASy上预测跨膜结构的软件

DAS—— Prediction of transmembrane regions

HMMTOP——Prediction of transmembranes helices and topology

Phobius—— Predict transmembrane topology and signal peptides

PredictProtein——Prediction of physico-chemical protein properties

SOSUI——Classification and secondary structure prediction

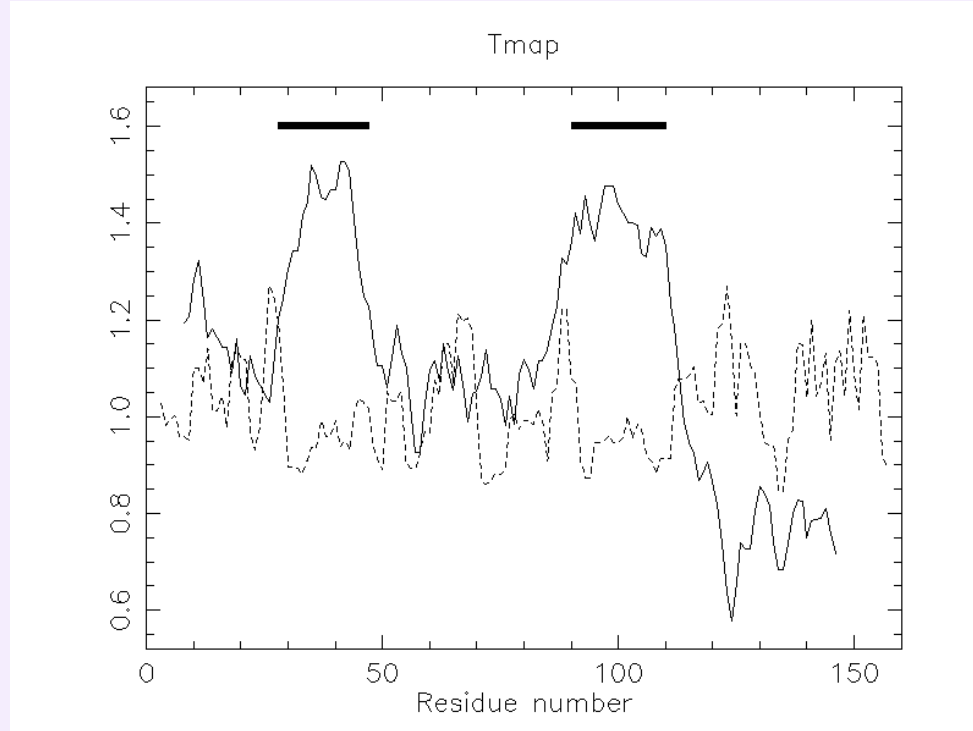
TMHMM——Prediction of transmembrane helices in proteins

Tmpred——membrane-spanning region prediction

TopPred——Topology prediction of membrane proteins



Tmap

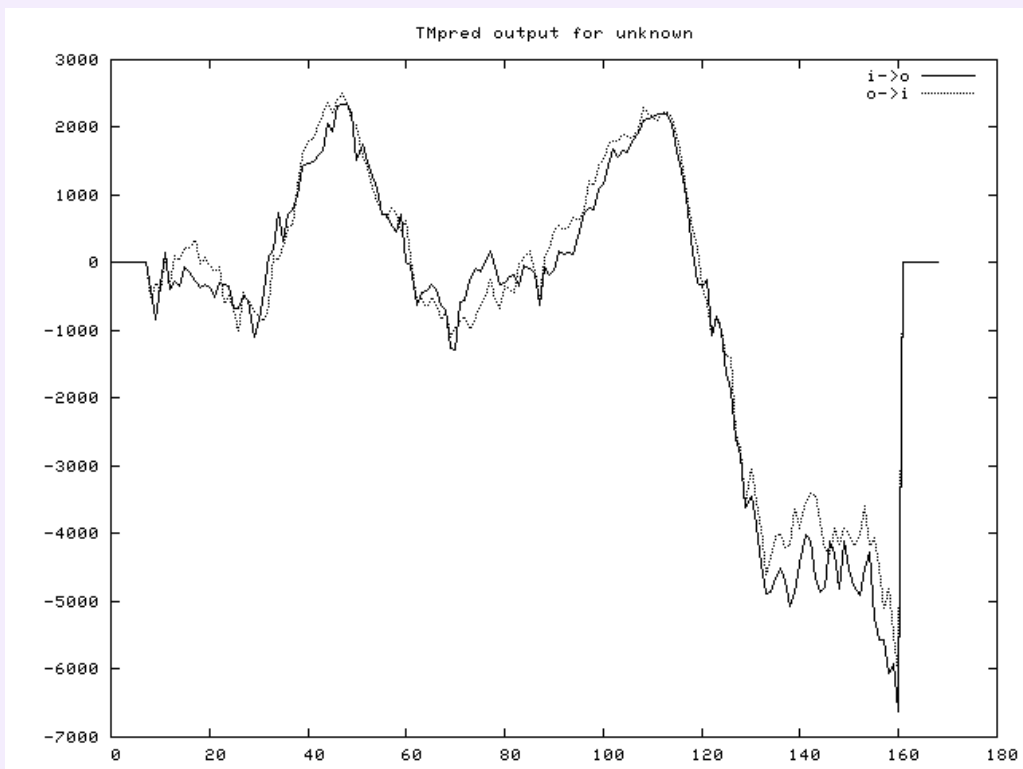


HMMTOP

No	From	To	Length	Orientation
1	37	59	23	i→o
2	99	121	23	o→i



TMpred

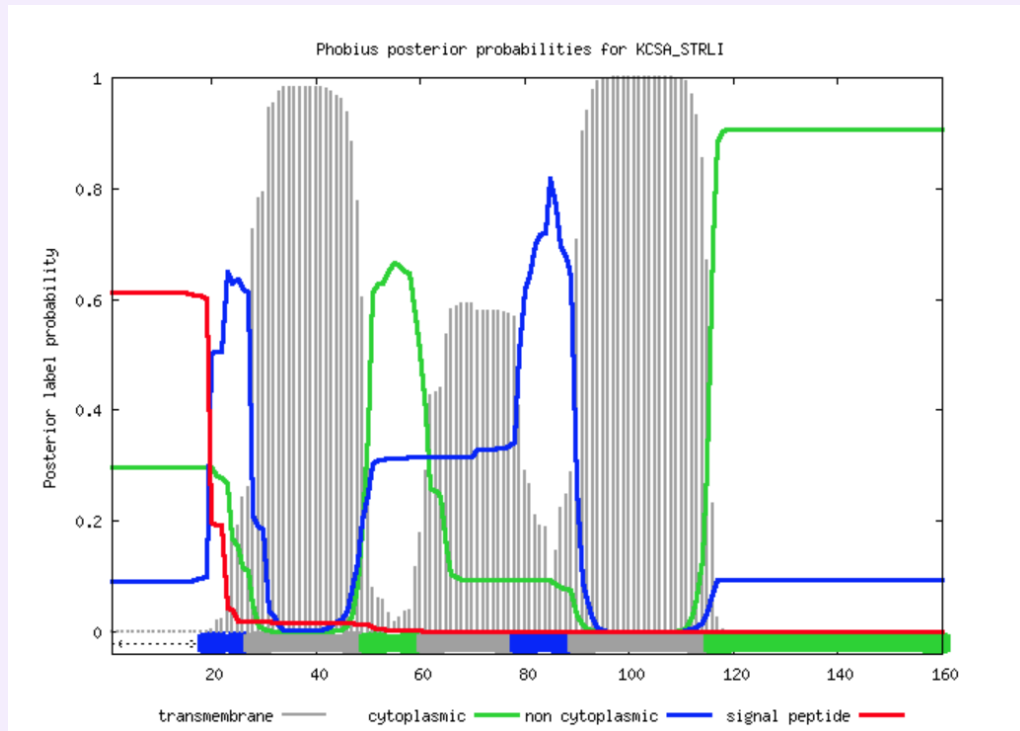


No	From	To	Length	Score	Orientation
1	37	59	23	2507	o->i
2	99	124	26	2204	i->o

No	From	To	Length	Score	Orientation
1	37	59	23	2351	i->o
2	99	124	26	2288	o->i



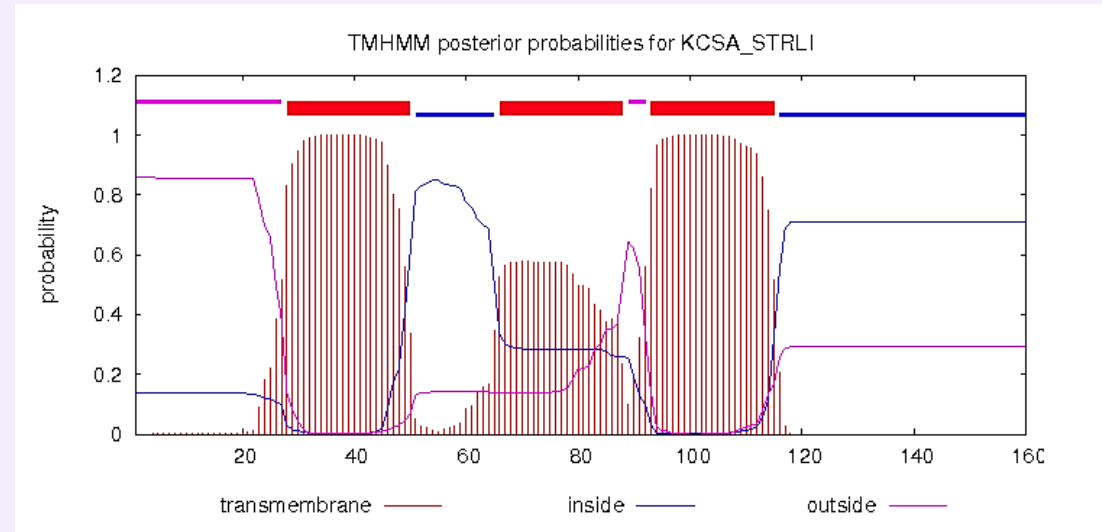
Phobius



No	From	To	Length	Orientation
1	28	49	22	o->i
2	61	78	18	i->o
3	90	115	26	o->i
Signal	1	18		18
Region	1	4	4	N-Region
Region	5	13	9	H-Region
Region	14	18	5	C-Region



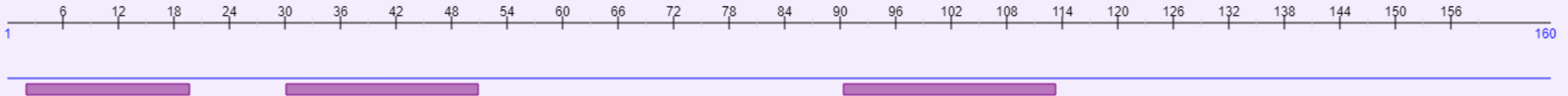
TMHMM



No	From	To	Length	Orientation
1	28	50	23	o—>i
2	66	88	23	i—>o
3	93	115	23	o—>i



PredictProtein



No	From	To	Length
1	2	19	18
2	29	49	21
3	87	109	23



DAS

No	From	To	Length	Qvalue	Note
1	8	17	10	8.980e-04	Potential Signal peptide
2	29	49	21	4.077e-11	--
3	83	112	30	7.083e-08	--



跨膜结构预测总结

软件	结果	评注
Tmap	2个	--
HMMTOP	2个	--
TMpred	2个	--
Phobius	3个	外加1个信号肽
TMHMM	3个	外加1个信号肽
PredictProtein	3个	--
DAS	3个	第1个跨膜螺旋可能为信号肽



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第二部分

Sequence alignment

序列比对



Alignment overview	Info	Status
Query: sp P0A334 KCSA_STRLI_B201901103707B37BB34871DC742D8A439176A1E105489EA		
<input type="checkbox"/> KCSA_STRLI - pH-gated potassium channel KcsA - Streptomyces liv... - View alignment	E-value: 2.8e-110 Score: 817 Ident.: 100.0%	
<input type="checkbox"/> KCSA_STRCO - pH-gated potassium channel KcsA - Streptomyces coe... - View alignment	E-value: 2.8e-110 Score: 817 Ident.: 100.0%	
<input type="checkbox"/> A0A1H2DGU0_9ACTN - Voltage-gated potassium channel - Streptomyces sp.... - View alignment	E-value: 2e-107 Score: 798 Ident.: 100.0%	
<input type="checkbox"/> A0A076LWY9_STRLI - Voltage-gated potassium channel - Streptomyces liv... - View alignment	E-value: 2e-107 Score: 798 Ident.: 100.0%	
<input type="checkbox"/> A0A397QGE6_9ACTN - Voltage-gated potassium channel Streptomyces sp. 19 - View alignment	E-value: 2e-107 Score: 798 Ident.: 100.0%	
<input type="checkbox"/> A0A2P8ACG2_9ACTN - pH-gated potassium channel KcsA - Streptomyces sp.... - View alignment	E-value: 6.6e-106 Score: 788 Ident.: 98.1%	
<input type="checkbox"/> A0A346CBS0_9ACTN - Voltage-gated potassium channel - Streptomyces sp.... - View alignment	E-value: 3e-105 Score: 784	

- 利用Uniprot内置的blast功能寻找同源蛋白，发现有大量序列高度一致的蛋白质，但基本都是细菌内的
- 在人和哺乳动物体内没有发现高度同源的蛋白质



- 人工检索了人类的钾离子通道和人类的钠离子通道蛋白，并分别通过Uniprot及NCBI blast工具进行了序列比对
- 右图为uniprot的序列比对结果
- 其中前两个蛋白分别是人类的钾离子通道蛋白和目标蛋白KcsA
- 最后一个蛋白是人类的钠离子通道蛋白
- 研究这三个蛋白是希望看不同物种的同类离子通道与相同物种的不同离子通道之间的差别

Alignment

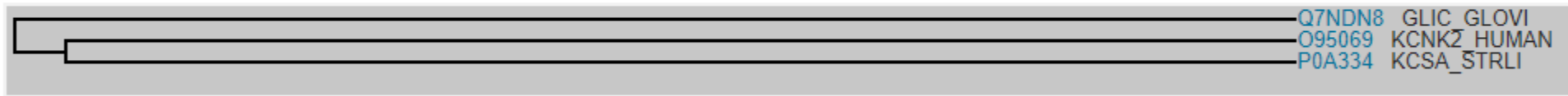
[How to print an alignment in color](#)

095069	KCNK2_HUMAN	1	MLPSASRERPGYRAGVA-----APDLLPKSAAQNSKP	33
POA334	KCSA_STRLI	1	-----	0
Q7NDN8	GLIC_GLOVI	1	MFPFTGWRP--KLSESTAASRMLWQPMAAVAVWVQIGLLWFSPPVWQDMVSPPPPIADE-P	57
095069	KCNK2_HUMAN	34	RLSFSSTKPTV---LASRVE--SDTTINVMKWKTVSTIPLVVVLYL-----IIG	76
POA334	KCSA_STRLI	1	-----	0
Q7NDN8	GLIC_GLOVI	58	-LTVMTGTYLIBCYSLDDKAEITFKVNAFLSLSWKDRRLAFDPVRSQVVKTYEPEATWIP	116
095069	KCNK2_HUMAN	77	ATVFKALEQPHEI-----SQRT--TIVIQKQTFISQHSK---WNS	111
POA334	KCSA_STRLI	1	-----	0
Q7NDN8	GLIC_GLOVI	117	EIRFWNVENARDADVDVDSVSPDGTWQYLERFSARVLSPLDFRRYPPFDSQTLHIYLIWRS	176
095069	KCNK2_HUMAN	112	TELDELIQQIVAAINAGIIPLG-NTSNQISHWDLGSSFFAGTWTITIGFGNISPRTEG-	169
POA334	KCSA_STRLI	1	-----	0
Q7NDN8	GLIC_GLOVI	177	VDT---RNIVLAV--DLEKVGKNDVFLTGWDIESFTAV---VKPANFA-LEDRLSEK	225
095069	KCNK2_HUMAN	170	-----GKIFCIYALLGIPLFGFLLAGVGDQLGTIFGKGIKVEDTFIKWNV---S	217
POA334	KCSA_STRLI	1	-----MPPMLSGLLARL---VKLLL---GRHGSALHWRA---A	29
Q7NDN8	GLIC_GLOVI	226	LDYQLRISRQYFSYIPNI-ILPMLFI-----LFSWTFWFSTIS	262
			*::	
095069	KCNK2_HUMAN	218	QTKIRIISTIIIFILFGCVLFVALPAIIFKHIEGWSALDAIYFVVI TL---TIGFGDY	272
POA334	KCSA_STRLI	30	GAATVLL--VIVLAGSYLAVL-AERGAQAQLITYPRALWVSVETA---ITVGYGD-	80
Q7NDN8	GLIC_GLOVI	263	YEANVIL--VWSTLTAHIAFNILVEINLPKTPYMTYTGAIIFMIYLFYFVAVIEVTVQHY	320
			: : : *	
095069	KCNK2_HUMAN	273	VAGGS-----DIEY-LD-FYKPVWFVW----ILVGLAYFAAWLSMIGDWLKVSKKT	318
POA334	KCSA_STRLI	81	-----LYPVILWRLVAVVVMVAGITSFGLVTAALATWFGR---E	118
Q7NDN8	GLIC_GLOVI	321	LKVESQPARAASTIRASRIAPPV-VFL---LANTILAFIFRFGF-----	359
			: : : *	
095069	KCNK2_HUMAN	319	KEEVEGFRHAHAEWIANVIAEFKETRRLSVEIYDKPQRATSIKRLSAELAGNHQELT	378
POA334	KCSA_STRLI	119	QERRGHFVRHSEKAAE--EAYTRTI-RALHERFDRLERMLDDNRR-	160
Q7NDN8	GLIC_GLOVI	360	-----	359
095069	KCNK2_HUMAN	379	PCRRILSVNHLTSERDVLPPLKTESIYLNGLTPHCAGEEIAVIENIK	426
POA334	KCSA_STRLI	161	-----	160
Q7NDN8	GLIC_GLOVI	360	-----	359

You may add additional sequences to this alignment (in FASTA format)



Tree



- 可以看到人类的钾离子通道蛋白和目标蛋白KcsA是在进化上更为接近的
- 人类的钠离子通道蛋白则与二者进化关系较远



Download Graphics Sort by: E value

sp|O95069|KCNK2_HUMAN Potassium channel subfamily K member 2 OS=Homo sapiens OX=9606 GN=KCNK2 PE=1 SV=2
Sequence ID: Query_97447 Length: 426 Number of Matches: 2

Range 1: 156 to 192 Graphics Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps
30.4 bits(67)	1e-05	Compositional matrix adjust.	12/37(32%)	23/37(62%)	0/37(0%)

Query 74 ITVGYGDLVFTLWGRLVAVVMMVAGITSFGLVTAAL 110
TT+G+G++ P T G++ ++ + GI FG + A +
Sbjct 156 TTIGFGNISFRTEGGKIFCIYALLGIPLFGFLLAGV 192

Range 2: 256 to 364 Graphics Next Match Previous Match First Match

Score	Expect	Method	Identities	Positives	Gaps
25.0 bits(53)	0.001	Compositional matrix adjust.	25/115(22%)	45/115(39%)	25/115(21%)

Query 65 ALWWSVETATTVGYG-----DLVPTLWGRLVAVVMMVAGITSFGLVTAALAT 112
A+++ V T TT+G+G D Y #H ++ G+ F V + +
Sbjct 256 AIYFVWITLTTIGFGDYVAGGSDIEYLDYKPVVW-----FWILVGLAYFAAVLSMIGD 309

Query 113 WF--VGREQERRGHFVRHSEK--AAEEAYTRTRTRALH-ERFDRLEMLDDNRR 160
W + +E G F H+ + + T R L E +D+ +R R+
Sbjct 310 WLRVISKKTKEEVGFRHAHAAEWTANVTAEFKETRRRLSVEIYDKFQRATSIKRR 364

- 上图为通过NCBI blast功能对人类钾离子通道和KcsA蛋白进行的双序列比对
- 二者显示了20-30%左右的序列一致性



```

How to print an alignment in color

POA334 KCSA_STRLI      1 -----                                0
Q9YDF8 KVAP_AERPE    1 MSVERWVFPGC SVMARFRRGLSDLGGRVNRNIGDVMEHPLVELGVSYAALLSVI VVVVEYT      60

POA334 KCSA_STRLI      1 -----MPPM-----LS                6
Q9YDF8 KVAP_AERPE    61 MQLSGEYLVRLYLVDLILV IILWADYAYRAYKSGDPAGYVKKTLYEIPALVPAGLLALIE      120

POA334 KCSA_STRLI      7 GLLA-----FLVK-LLLGRHG SALHWRAAGAATV-----LLVIVLLAGSYL          46
Q9YDF8 KVAP_AERPE   121 GHLAGLGLFRLVRLLRFLRILLIISRG SKFLSAIADAADKIRFYHLFGAVMLTVLYGAF      180
      *  **                               *::  **:   :**  :      *..**      :::: *  *::

POA334 KCSA_STRLI     47 AVL AERGAPGAQLITYPRALWWSVE TATTWG YGDL YPVTLWGRLVAVVVMVAG IT SFGLV      106
Q9YDF8 KVAP_AERPE   181 IYI VEYDPD PNSSIKS VFDALWVA VV TATTWG YGDVVPATPIGKVIGIAVMLTG ISAL TLL      240
      :..*      *..: :      ****:* *****: *..*      *:::..**::**::: *

POA334 KCSA_STRLI    107 TAALATW FV---GRQERRGHFVRHSEKAAEEAYTRITRALHERFDRLERMLDDNRR--      160
Q9YDF8 KVAP_AERPE   241 IGT VSNMFQKILVGEPEPSCSP---AKLAE---MVS SMSEEEFEEFVRILKNLRRL      291
      :::: *      *..: .      * **      :.      *.*..: * *.. **

POA334 KCSA_STRLI    161 ----                                160
Q9YDF8 KVAP_AERPE   292 NSMK                                295
  
```

- 上图为 KcsA蛋白与KVAP蛋白的双序列比对，是一类电压门控的钾离子通道蛋白，是一个原核内的通道蛋白



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第三部分

Phylogenetic tree
系统发生树



系统发生树的构建 Building a phylogenetic tree

- 选择KcsA与其他不同种类的钾离子通道蛋白进行多序列比对与系统发生树的构建。
- 电压门控钾离子通道 (Kv)
- 内向整流钾离子通道 (Kir)
- 双孔区域型钾离子通道 (K2P)
- 配体门控钾离子通道 (Kligand)

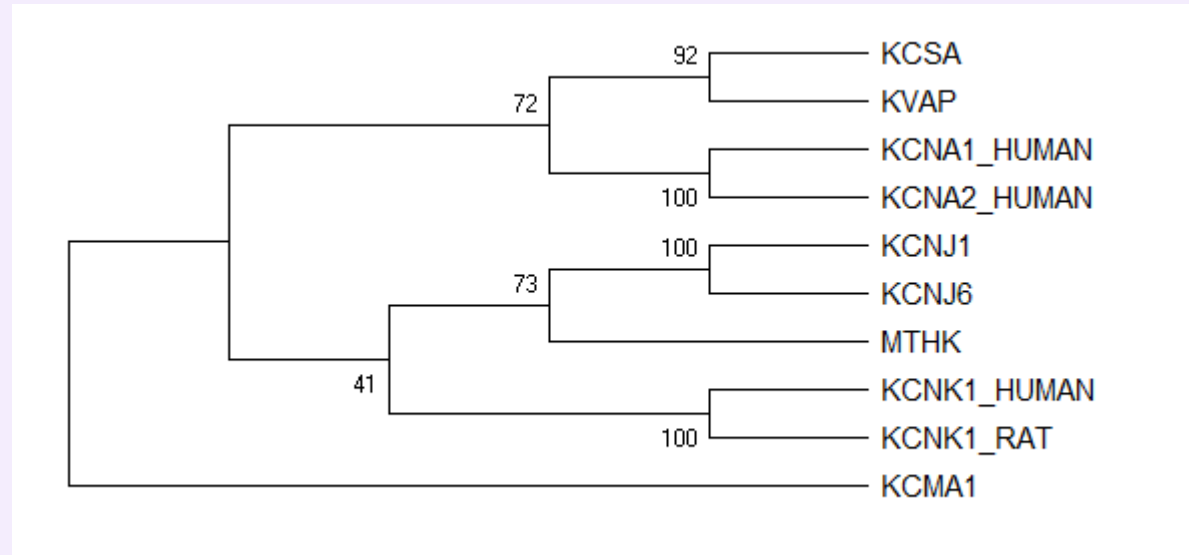


构建蛋白的选择

Channel Type	Name	Organism	Entry
K ⁺ channel pore	KcsA	Streptomyces lividans	P0A334
Kv	KCNA1	Human	Q09470
	KCNA2	Human	P16389
	KVAP	Aeropyrum pernix	Q9YDF8
Kir	KCNJ1	Human	P48048
	KCNJ6	Human	P48051
K2P	Kcnk1	Human	O00180
	Kcnk1	Rat	Q9Z2T2
Kligand	mthK	Methanothermobacter thermautotrophicus	O27564
	KCMA1	Human	Q12791



构建结果 |



- 我们可以看到我们的研究对象KcsA蛋白在系统发生树上与电压门控钾离子通道家族的蛋白相似性最高，可以理解为KV家族的蛋白与KcsA结构比较相似，甚至有可能都是从KcsA蛋白一步步发展出来的。
- 同时其他三类钾离子通道在树上与电压门控离子通道处于不同的枝上，相似性不高，据此推断它们的结构与实现功能的方式并不相同。



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第四部分

Structure predication

结构预测



Motif analysis

- UniProt
- InterPro
- Pfam
- HMM logo
- SMART
- TMHMM
- TMpred
- Motif Scan



UniProt

Subcellular locationⁱ

- Cell membrane ⁱ ; Multi-pass membrane protein ⁱ

Topology

Feature key	Position(s)	Description	Actions	Graphical view	Length
Topological domain ⁱ	1 – 27	Cytoplasmic	Add BLAST		27
Transmembrane ⁱ	28 – 50	Helical	Add BLAST		23
Topological domain ⁱ	51 – 61	Extracellular	Add BLAST		11
Intramembrane ⁱ	62 – 72	Helical; Pore-forming	Add BLAST		11
Intramembrane ⁱ	73 – 80	Pore-forming			8
Topological domain ⁱ	81 – 87	Extracellular			7
Transmembrane ⁱ	88 – 111	Helical	Add BLAST		24
Topological domain ⁱ	112 – 160	Cytoplasmic	Add BLAST		49

GO - Cellular componentⁱ

- voltage-gated potassium channel complex Source: InterPro

[View the complete GO annotation on QuickGO ...](#)

Keywords - Cellular componentⁱ

Cell membrane, Membrane



Motif, Tertiary structural analysis

InterPro

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EMBL-EBI Hinxton

InterPro

Protein sequence analysis & classification

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Overview
Similar proteins (21237)
Structures

Filter view on

Entry type
 Homologous superfamily
 Family
 Domains
 Repeats
 Site

Status
 Unintegrated

Per-residue features
 Residue annotation

Colour by [help](#)
 domain relationship
 source database

pH-gated potassium channel KcsA (P0A334)

Export TSV Export FASTA

Accession [P0A334](#) (KCSA_STRLI)
Species *Streptomyces lividans*
Length 160 amino acids (complete) Source: UniProtKB

Protein family membership

- Voltage-gated potassium channel (PRO28325)

Homologous superfamilies

None predicted

Domains and repeats

Detailed signature matches

- [IPR028325](#) Voltage-gated potassium channel
 - [PTHR11537](#)
 - [PRO1169](#) pCHANNEL
- [IPR013099](#) Potassium channel domain
 - [PF07885](#) (pH_gat_2)
- no IPR Unintegrated signatures
 - [G3DBA:1.1028...](#)
 - [G3DBA:1.205.110](#)
 - [G3DBA:1.205.440](#)
 - [PTHR11537_SF14](#)
 - [SSF81324](#)

Other features

- [CYTOPLASMIC_D...](#)
- [NON_CYTOPLASMI...](#)
- [SIGNAL_PEPTIDE](#)
- [SIGNAL_PEPTID...](#)
- [SIGNAL_PEPTID...](#)
- [SIGNAL_PEPTID...](#)
- [SIGNAL_PEPTID...](#)
- [TMhelix](#)
- [TRANSMEMBRANE](#)

GO term prediction

Biological Process
[GO:0006813](#) potassium ion transport

Molecular Function
[GO:0005240](#) voltage-gated potassium channel activity

Cellular Component
[GO:0008076](#) voltage-gated potassium channel complex



Motif, Tertiary structural analysis

Pfam

Protein: *KCSA_STRLI* (P0A334)

Summary

Sequence

Structures

TreeFam

Jump to...



Summary

This is the summary of UniProt entry [KCSA_STRLI](#) (P0A334).

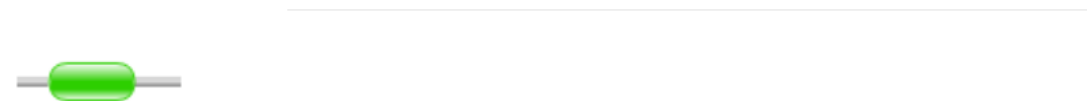
Description:	pH-gated potassium channel KcsA
Source organism:	Streptomyces lividans (NCBI taxonomy ID)
Length:	160 amino acids
Reference Proteome:	×

Please note: when we start each new Pfam data release, we take a copy of the UniProt sequence database. This snapshot of UniProt forms the basis of the overview that you see here. It is important to note that, although some UniProt entries may be removed *after* a Pfam release, these entries will not be removed from Pfam until the *next* Pfam data release.

Pfam domains

This image shows the arrangement of the Pfam domains that we found on this sequence. Clicking on a domain will take you to the page describing that Pfam entry. The table below gives the domain boundaries for each of the domains. [Less...](#)

E-values are based on searching the Pfam-A family against UniProtKB [2018_04](#) using hmmsearch.



[Download](#) the data used to generate the domain graphic in JSON format.

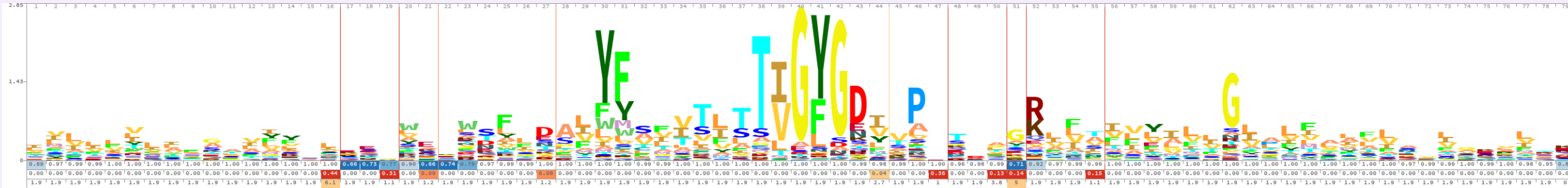
Source	Domain	Start	End	Gathering threshold (bits)		Score (bits)		E-value	
				Sequence	Domain	Sequence	Domain	Sequence	Domain
Pfam	Ion trans 2	33	116	22.50	22.50	53.90	53.40	8.6e-11	1.2e-10

[Show](#) or [hide](#) domain scores.



Motif, Tertiary structural analysis

HMM(hidden Markov model) logo



The HMM logo is a graphical representation of the Hidden Markov Model that describes the properties of that HMM. The differing heights and colors of the letters represent different properties of the positions in the HMM they represent.



Motif, Tertiary structural analysis

SMART

SMART SETUP FAQ ABOUT GLOSSARY WHAT'S NEW FEEDBACK keywords... Search SMART

Domains within *Streptomyces coelicolor* A3(2) protein KCSA_STRCO (P0A333)
pH-gated potassium channel KcsA

+ = - SAVE

Selected feature details

Transmembrane region

This is a transmembrane helix region, as detected by the TMHMM v2.0 program. The region starts at position 28 and ends at position 50.

Transmembrane region (23 aa):

[Submit to BLAST](#) [Copy to clipboard](#)

AAGAATVLLVIVLLAGSYLAVLA

Information Interactions Orthology

Length 160 aa

Source database UniProt

Identifiers KCSA_STRCO, P0A333

The SMART diagram above represents a summary of the results shown below. Domains with scores less significant than established cutoffs are not shown in the diagram. Features are also not shown when two or more occupy the same piece of sequence; the priority for display is given by SMART > PFAM > PROSPERO repeats > Signal peptide > Transmembrane > Coiled coil > Unstructured regions > Low complexity. In either case, features not shown in the above diagram are marked as 'overlap' in the right side table below.

Confidently predicted domains, repeats, motifs and features:

Name	Start ▲	End	E-value
low complexity	5	17	N/A
transmembrane region	28	50	N/A
transmembrane region	66	88	N/A
transmembrane region	93	115	N/A
low complexity	132	143	N/A

Click on a row to highlight the feature in the diagram above. Click the feature name for more information.

Features NOT shown in the diagram:

Name	Start ▲	End	E-value	Reason
low complexity	28	42	N/A	overlap

Click on a row to highlight the feature in the diagram above. Click the feature name for more information.



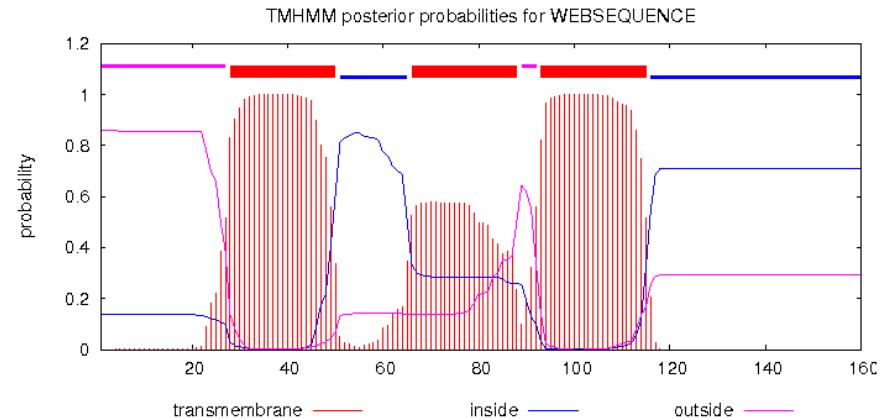
Motif, Tertiary structural analysis

TMHMM Server v. 2.0

TMHMM result

[HELP](#) with output formats

```
# WEBSEQUENCE Length: 160
# WEBSEQUENCE Number of predicted TMHs: 3
# WEBSEQUENCE Exp number of AAs in TMHs: 58.34819
# WEBSEQUENCE Exp number, first 60 AAs: 22.71345
# WEBSEQUENCE Total prob of N-in: 0.14030
# WEBSEQUENCE POSSIBLE N-term signal sequence
WEBSEQUENCE TMHMM2.0 outside 1 27
WEBSEQUENCE TMHMM2.0 TMhelix 28 50
WEBSEQUENCE TMHMM2.0 inside 51 65
WEBSEQUENCE TMHMM2.0 TMhelix 66 88
WEBSEQUENCE TMHMM2.0 outside 89 92
WEBSEQUENCE TMHMM2.0 TMhelix 93 115
WEBSEQUENCE TMHMM2.0 inside 116 160
```



[plot](#) in postscript, [script](#) for making the plot in gnuplot, [data](#) for plot



Motif, Tertiary structural analysis

TMpred

Possible transmembrane helices

=====

The sequence positions in brackets denominate the core region.

Only scores above 500 are considered significant.

Inside to outside helices : 2 found

from	to	score	center
28 (30)	50 (47)	2351	39
90 (90)	115 (111)	2204	103

Outside to inside helices : 3 found

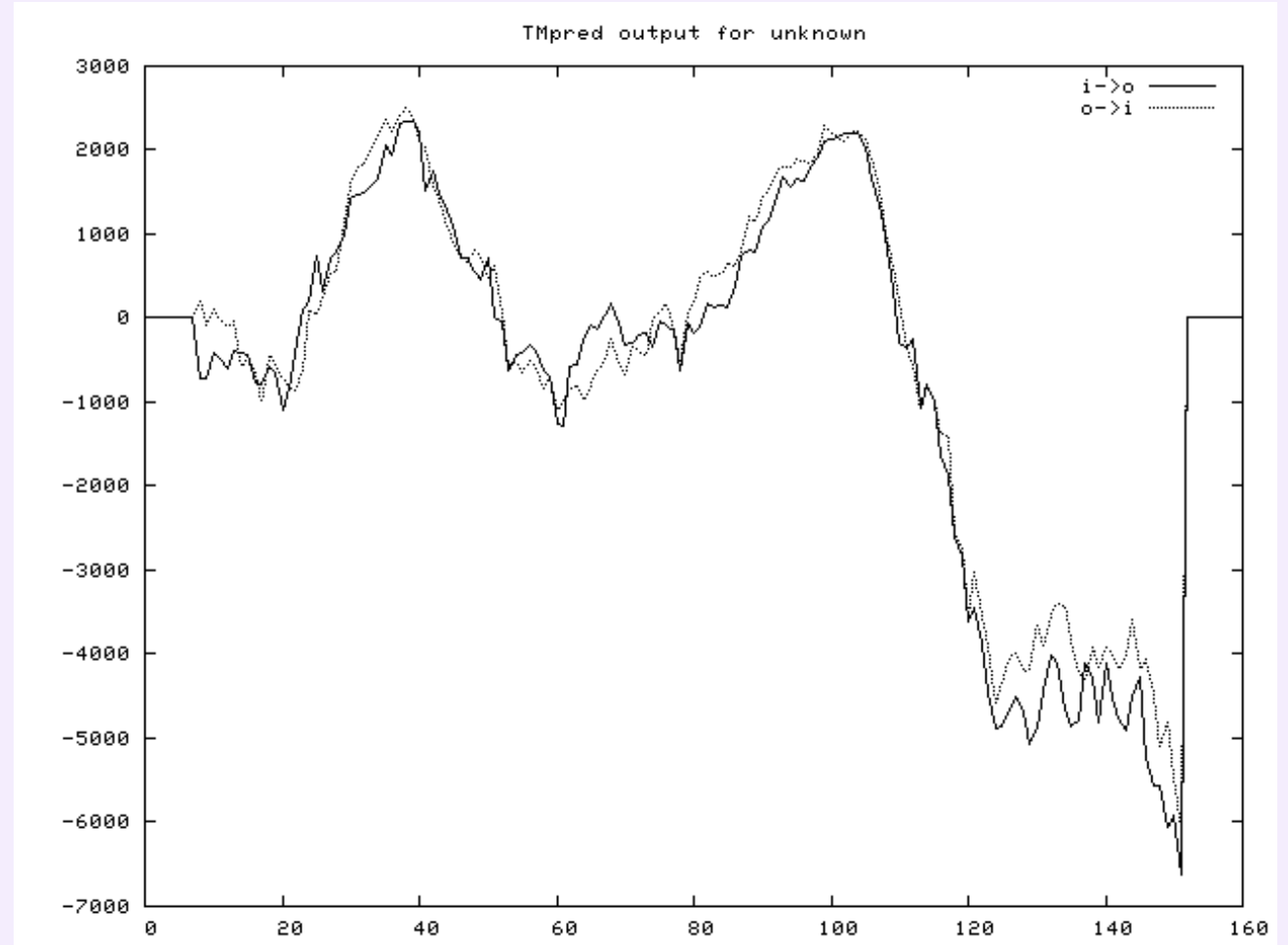
from	to	score	center
1 (1)	18 (18)	234	9
28 (31)	50 (47)	2507	39
90 (90)	116 (110)	2288	100

-----> STRONGLY preferred model: N-terminus outside

2 strong transmembrane helices, total score : 4711

from to length score orientation

1	28	50 (23)	2507	o-i
2	90	115 (26)	2204	i-o





Motif, Tertiary structural analysis

Motif Scan

SIB myhits

user: GUEST width: 600

Tools Hub

Results

- Stored results
- Private area
- Misc
- Deprecated
- Privacy notice

Motif Scan Results

Query Protein temporarily stored [here](#).

Database of motifs HAMAP profiles [hamap], PROSITE patterns [pat], More profiles [pre], PROSITE patterns (frequent match producers) [freq_pat], PROSITE profiles [prf].

searching HAMAP profiles
 searching PROSITE patterns
 searching PROSITE patterns (frequent match producers)
 searching More profiles
 searching PROSITE profiles
 postprocessing

Summary

Original output [hamap](#), [pat](#), [freq_pat](#), [pre](#), [prf](#).

Matches map
 (features from query are above the ruler, matches of the motif scan are below the ruler)

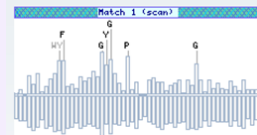
List of matches

FT	MYHIT	43	48	freq_pat:MYRISTYL [?]
FT	MYHIT	53	58	freq_pat:MYRISTYL [?]
FT	MYHIT	104	109	freq_pat:MYRISTYL [?]
FT	MYHIT	129	131	freq_pat:PKC_PHOSPHO_SITE [?]
FT	MYHIT	140	142	freq_pat:PKC_PHOSPHO_SITE [?]
FT	MYHIT	131	137	freq_pat:TYR_PHOSPHO_SITE [?]
FT	MYHIT	1	114	pre:CATION_CHANNEL_TM [?]
FT	MYHIT	57	114	pre:CHANNEL_PORE_K [!]

Graphics pre:CHANNEL_PORE_K

Query

ID CHANNEL_PORE_K; MATRIX.
 DE Pore region of potassium channels.
 CC The scoring system depicted below is approximate.



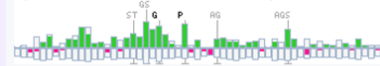
Subject

ID RAW_TEXT TEMPORARY; PRT; 160 AA.



Match 1

>pre:CHANNEL_PORE_K 17.550 729 pos. 57 - 114 [1, -1] PSS0265|pre:CHANNEL_PORE_K Pore region of potassium channels.



AQLITYPRLHWVSVEITATTVGVGDLVPVTLWGLVHVVMVHAGITSFGLVTRALATWF



Motif analysis conclusion

- Motif prediction gave us similar result:
kcsA has 3 transmembrane region, with 1 ion passing pore.
- Motif prediction also gave us cues for predicting its tertiary structure in vivo.



Tertiary structural analysis

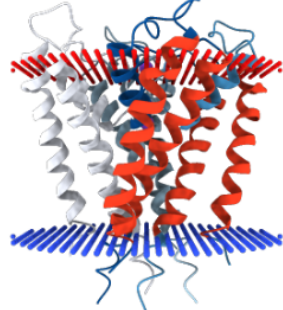
- Protein Data Bank (PDB)
- Literature
 - The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.



Protein Data Bank

Transmembrane View

transmembrane regions OPM



3D View: Structure | Electron Density | Ligand Interaction

Standalone Viewers
Protein Workshop | Ligand Explorer

Macromolecule Content

- Total Structure Weight: 41129.13
- Atom Count: 2823
- Residue Count: 388
- Unique protein chains: 1

1BL8

POTASSIUM CHANNEL (KCSA) FROM STREPTOMYCES LIVIDANS

DOI: [10.2210/pdb1BL8/pdb](https://doi.org/10.2210/pdb1BL8/pdb)

Classification: [MEMBRANE PROTEIN](#)

Organism(s): [Streptomyces lividans](#)

Expression System: [Escherichia coli](#)

Mutation(s): 1

Deposited: 1998-07-23 Released: 1998-07-29

Deposition Author(s): [Doyle, D.A.](#), [Cabral, J.M.](#), [Pfuetzner, R.A.](#), [Kuo, A.](#), [Gulbis, J.M.](#), [Cohen, S.L.](#), [Chait, B.T.](#), [Mackinnon, R.](#)

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 3.2 Å

R-Value Free: 0.290

R-Value Work: 0.280

wwPDB Validation

Metric	Percentile Ranks	Value
Rfree		0.799
Clashscore		58
Ramachandran outliers		8.4%
Sidechain outliers		23.1%
RSRZ outliers		0.3%

This is version 1.3 of the entry. See complete [history](#).

Literature

The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.

[Doyle, D.A.](#), [Morais Cabral, J.](#), [Pfuetzner, R.A.](#), [Kuo, A.](#), [Gulbis, J.M.](#), [Cohen, S.L.](#), [Chait, B.T.](#), [MacKinnon, R.](#)

(1998) Science **280**: 69-77

PubMed: [9525859](#) [Search on PubMed](#)



PDB-Protein Feature View

Macromolecules

Find similar proteins by: [Sequence](#) | [Structure](#)

Entity ID: 1

Molecule	Chains	Sequence Length	Organism	Details
PROTEIN (POTASSIUM CHANNEL PROTEIN)	A, B, C, D	97	Streptomyces lividans	Mutation(s): 1 ⓘ Gene Names: kcsA (skc1)
Membrane protein mpstruct	Group: TRANSMEMBRANE PROTEINS: ALPHA-HELICAL		Sub Group: Channels: Potassium, Sodium, & Proton Ion- Selective	Protein: KcsA Potassium channel, H ⁺ gated

Find proteins for [P0A334](#) (*Streptomyces lividans*) Go to UniProtKB: [P0A334](#)

Protein Feature View Full Protein Feature View for [P0A334](#)



PDB-sequence view

Mouse over an annotation to see more details. Click on any annotation to enable Jmol.

Add an Annotation

Select

Annotations	Details
Domain Assignment: SCOP [hide] [reference]	d1bl8a Potassium channel protein: 97 residues
Secondary Structure: DSSP [hide] [reference]	67% helical (3 helices; 65 residues)
Structural Feature: Site Record [hide] [reference]	1BL8 A AC2 9 BINDING SITE FOR RESIDUE K A 402 (Software) 1BL8 A SEL 4 FILTER TO SELECT FOR POTASSIUM IONS OVER OTHER MONOVALENT CATIONS. (Author) 1BL8 A AC1 8 BINDING SITE FOR RESIDUE K A 401 (Software)
Structural Feature: Protein Modification [hide] [reference]	350 potassium ion <i>PDB:K</i> 350 potassium ion <i>PDB:K</i>

Sequence Chain View

Protein Modification Legend

- * potassium ion
- potassium ion

Site Record Legend

- FILTER TO SELECT FOR POTASSIUM IONS OVER OTHER MONOVALENT CATIONS. (Author)
- BINDING SITE FOR RESIDUE K A 401 (Software)
- BINDING SITE FOR RESIDUE K A 402 (Software)

DSSP Legend

- empty: no secondary structure assigned
- S: bend
- T: turn
- H: alpha helix



Tertiary structural analysis

Swissmodel-homo-4-mer

BIOZENTRUM University of Basel The Center for Molecular Life Sciences **SWISS-MODEL** Modelling Repository Tools Documentation Log in Create Account

Search SWISS-MODEL Repository

P0A334 (KCSA_STRLI) *Streptomyces lividans*
pH-gated potassium channel KcsA UniProtKB[†] InterPro[†] Interactive Modelling

160 aa; Sequence (Fasta) Identical sequences: *Streptomyces coelicolor*: P0A333

P0A334
(X-RAY) heteromer, 22-160
(SOLUT) homo-4-mer, 1-160

PDB 1f6g[†] "POTASSIUM CHANNEL (KCSA) FULL-LENGTH FOLD"

Method: SOLUTION NMR
Released: 2001-02-21
Bioassembly File: [↓](#)
Ligands: None
RCSB[†] PDBsum[†] PDBe[†] SMTL[†]

Sequence Features

Transmembrane Intramembrane InterPro

Sequence Alignments ^

VOLTAGE-GATED POTASSIUM CHANNEL P0A334 UniProtKB[†] InterPro[†]

Toggle Identical (ABCD)

P0A334	MFPMLSGLLARLVKLLLGRHGSALHWRAAGAATVLLVIVLLAGSYLAVLAERGAFGAQLITYPRALWWSV	70
1f6g. (ABCD)	MFPMLSGLLARLVKLLLGRHGSALHWRAAGAATVLLVIVLLAGSYLAVLAERGAFGAQLITYPRALWWSV	70
P0A334	EIATTVGYGDLVPVILNGRLVAVVVMVAGITSFGLVTAALATWVFGREQERRGHFVRHSEKAAEEAYTR	140
1f6g. (ABCD)	EIATTVGYGDLVPVILNGRLVAVVVMVAGITSFGLVTAALATWVFGREQERRGHFVRHSEKAAEEAYTR	140
P0A334	TRALHERFDRLERMLDDNRR	160
1f6g. (ABCD)	TRALHERFDRLERMLDDNRR	160



Tertiary structural analysis

Swissmodel-heteromer

BIOZENTRUM University of Basel The Center for Molecular Life Sciences
SWISS-MODEL Modelling Repository Tools Documentation Log in Create Account

Search SWISS-MODEL Repository

P0A334 (KCSA_STRLI) *Streptomyces lividans*
pH-gated potassium channel KcsA UniProtKB[↗] InterPro[↗] [Interactive Modelling](#)

160 aa; Sequence (Fasta) Identical sequences: *Streptomyces coelicolor*: P0A333

P0A334
(X-RAY) heteromer; 22-160
(SOLUT) homo-4-mer; 1-160

PDB 3pjs[↗] "Mechanism of Activation Gating in the Full-Length KcsA K⁺ Channel"

Method: X-RAY DIFFRACTION 3.80Å
Released: 2011-07-06
Bioassembly File: [↓](#)
Ligands: None
RCSB[↗] PDBsum[↗] PDBe[↗] SMTL[↗]

Sequence Features

Transmembrane Intramembrane
InterPro

Colours NGL Cartoon

Sequence Alignments

Voltage-gated potassium channel P0A334 UniProtKB[↗] InterPro[↗]

[Toggle Identical \(EFGH\)](#)

```

P0A334  SALHWRAAGAATVLLVIVLLAGSYLAVLAERGAAPGAQLIITYPRALWWSVEITATVGYGDLVPVILWGRLV  91
3pjs.e. (EFGH)  SALHWRAAGAATVLLVIVLLAGSYLAVLAERGAAPGAQLIITYPRALWWSVEITATVGYGDLVPVILWGRLV  97
P0A334  AVVVMVAGITSFGLVIAALATWVFGREQERRGHFVRHSEKAAEEAYTRITRALHERFDLERMLDDNRR  160
3pjs.e. (EFGH)  AVVVMVAGITSFGLVIAALATWVFGREQERRGHFVRHSEKAAEEAYTRITRALHERFDLERMLDDNRR  166

```

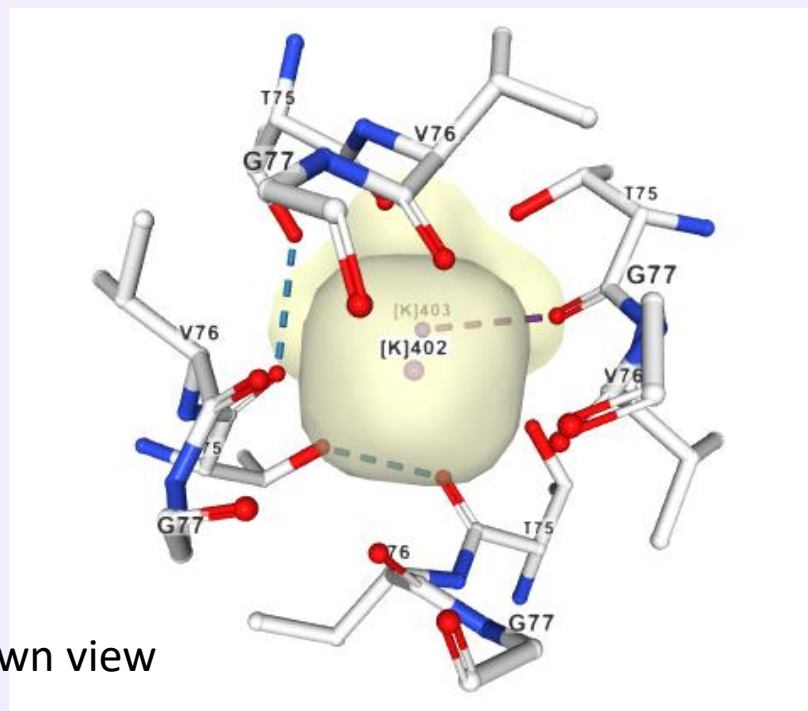



PDB-Ligand View

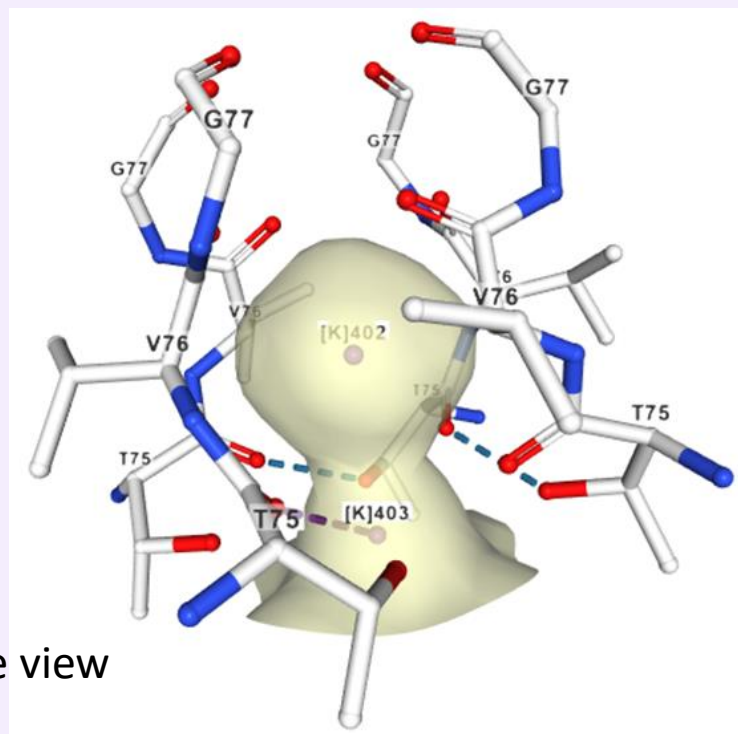
1BL8

POTASSIUM CHANNEL (KCSA) FROM STREPTOMYCES LIVIDANS

Note: Use your mouse to drag, rotate, and zoom in and out of the structure. Mouse-over to identify atoms and bonds. [Mouse controls documentation](#).



Up-down view



Side view

Structure View | Electron Density Maps | **Ligand View**

[Ligand View Documentation](#)

The ligand pocket is only visible if the opacity is set to greater than zero.

Ligand

Pocket

Opacity

Near Clipping

Radius Clipping

Color

Hydrogen Bonds (blue)

Halogen Bonds (turquoise)

Hydrophobic Contacts (grey)

Pi Interactions (orange, green)

Metal Interactions (purple)

Label

Polymer Display



Literature

The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity.

[Doyle DA](#)¹, [Morais Cabral J](#), [Pfuetzner RA](#), [Kuo A](#), [Gulbis JM](#), [Cohen SL](#), [Chait BT](#), [MacKinnon R](#).

⊕ Author information

Abstract

The potassium channel from *Streptomyces lividans* is an integral membrane protein with sequence similarity to all known K⁺ channels, particularly in the pore region. X-ray analysis with data to 3.2 angstroms reveals that four identical subunits create an inverted teepee, or cone, cradling the selectivity filter of the pore in its outer end. The narrow selectivity filter is only 12 angstroms long, whereas the remainder of the pore is wider and lined with hydrophobic amino acids. A large water-filled cavity and helix dipoles are positioned so as to overcome electrostatic destabilization of an ion in the pore at the center of the bilayer. Main chain carbonyl oxygen atoms from the K⁺ channel signature sequence line the selectivity filter, which is held open by structural constraints to coordinate K⁺ ions but not smaller Na⁺ ions. The selectivity filter contains two K⁺ ions about 7.5 angstroms apart. This configuration promotes ion conduction by exploiting electrostatic repulsive forces to overcome attractive forces between K⁺ ions and the selectivity filter. The architecture of the pore establishes the physical principles underlying selective K⁺ conduction.

Comment in

[The vision of the pore.](#) [Science. 1998]

Science. 1998 Aug 14;281(5379):883.

[A vision of the pore.](#) [Science. 1998]

PMID: 9525859

[Indexed for MEDLINE] **Free full text**



Tertiary structural analysis

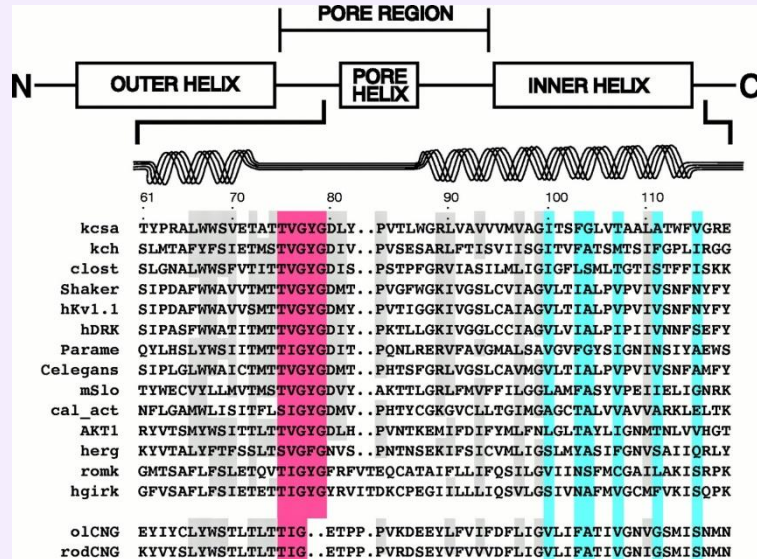


Figure 1 Sequence alignment of selected K⁺ channels and cyclic nucleotide-gated channels.

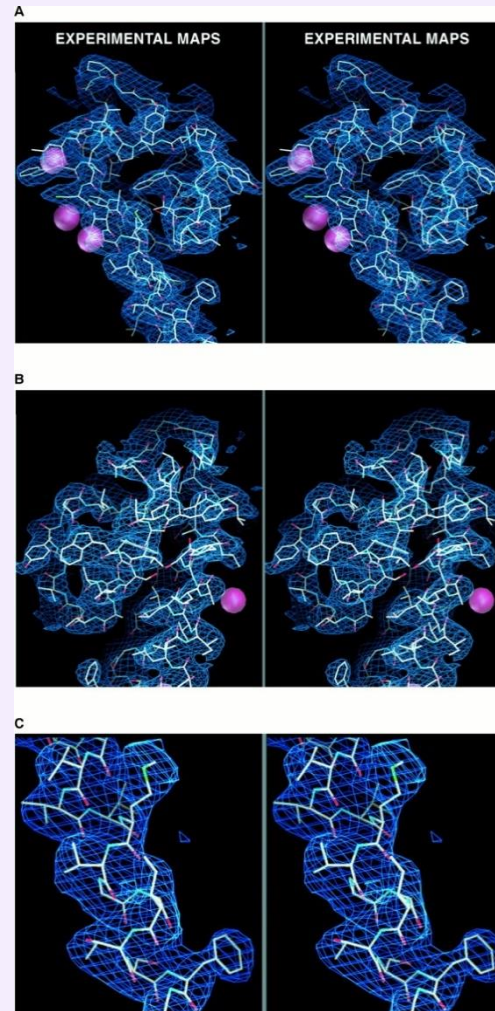


Figure 2 Experimental electron density map.

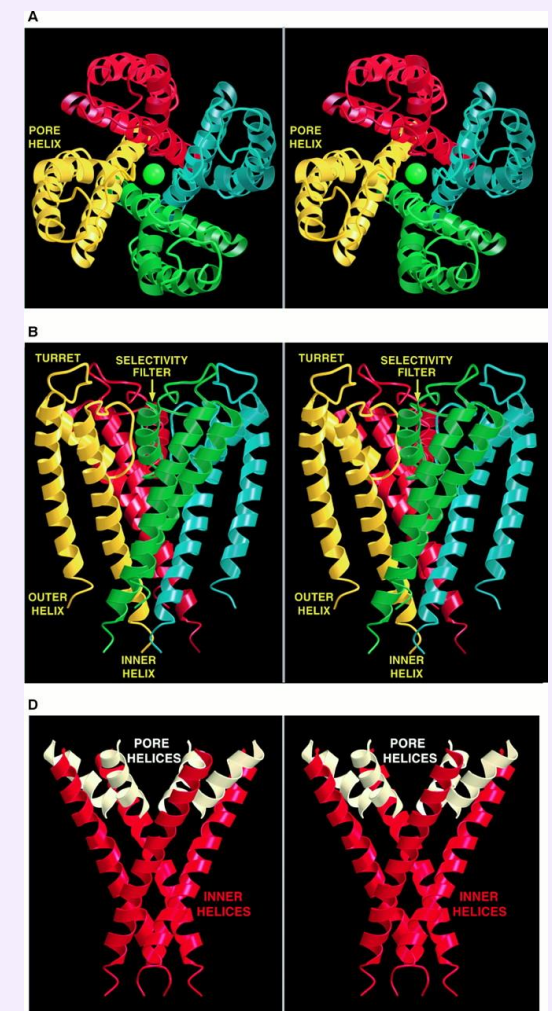


Figure 3 Views of the tetramer.



Tertiary structural analysis

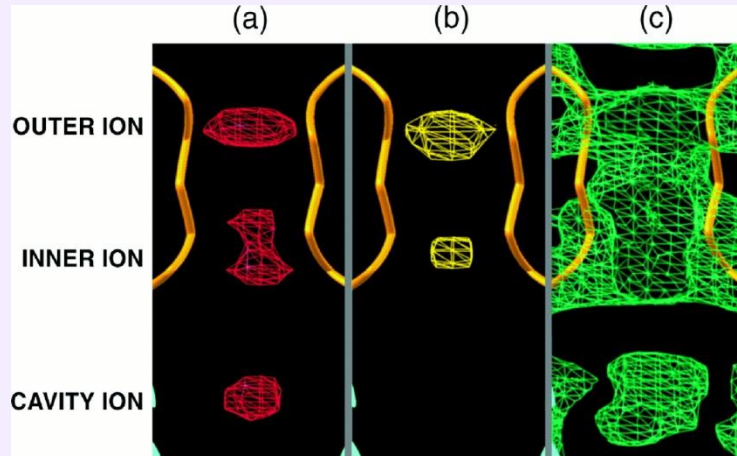


Figure 6 Identification of permeant ion positions in the pore.

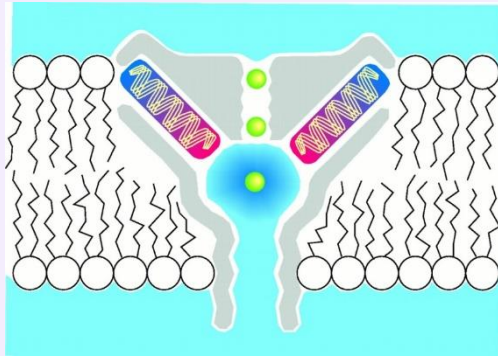


Figure 7 Two mechanisms by which the K^+ channel stabilizes a cation in the middle of the membrane.

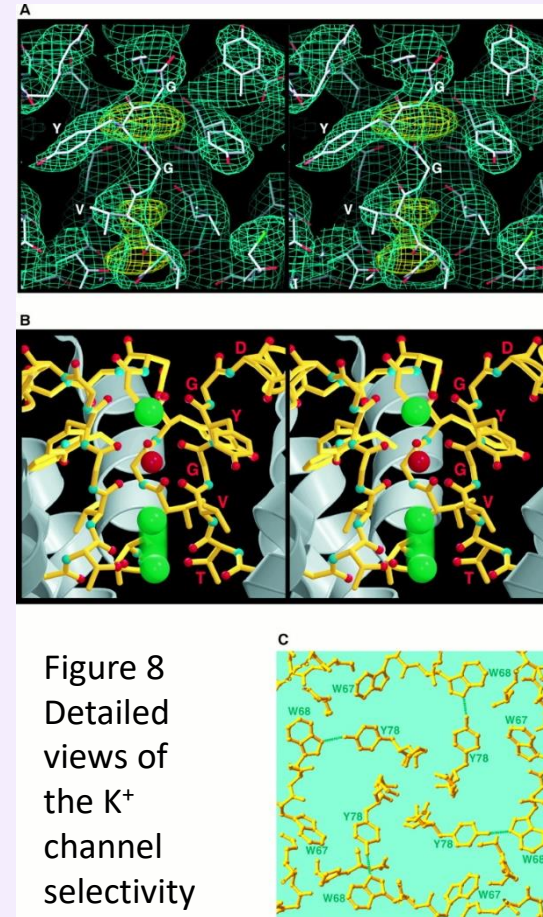


Figure 8 Detailed views of the K^+ channel selectivity filter.

Declan A. Doyle et al.
Science 1998;280:69-77



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第五部分

Functional analysis

功能分析



We propose that the following principles underlie the structure and operation of K^+ channels. (i) The pore is constructed of an inverted teepee, with the selectivity filter held at its wide end. This architecture also describes the pore of cyclic nucleotide-gated channels and probably Na^+ and Ca^{2+} channels as well. (ii) The narrow selectivity filter is only 12 Å long, whereas the remainder of the pore is wider and has a relatively inert hydrophobic lining. These structural and chemical properties favor a high K^+ throughput by minimizing the distance over which K^+ interacts strongly with the channel. (iii) A large water-filled cavity and helix dipoles help to overcome the high electrostatic energy barrier facing a cation in the low dielectric membrane center. (iv) The K^+ selectivity filter is lined by carbonyl oxygen atoms, which provide multiple closely spaced sites. The filter is constrained in an optimal geometry so that a dehydrated K^+ ion fits with proper coordination but the Na^+ ion is too small. (v) Two K^+ ions at close proximity in the selectivity filter repel each other. The repulsion overcomes the otherwise strong interaction between ion and protein and allows rapid conduction in the setting of high selectivity.



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第六部分

Acknowledgement

致谢



致谢

感谢罗老师一学期的辛勤指导~

感谢认真负责的助教师姐~

感谢在座各位的聆听~