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两种膜通道蛋白的序列、结构与功能分析

Sequence, structure and function analysis of two membrane channel proteins

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2021/01/23

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PART 01

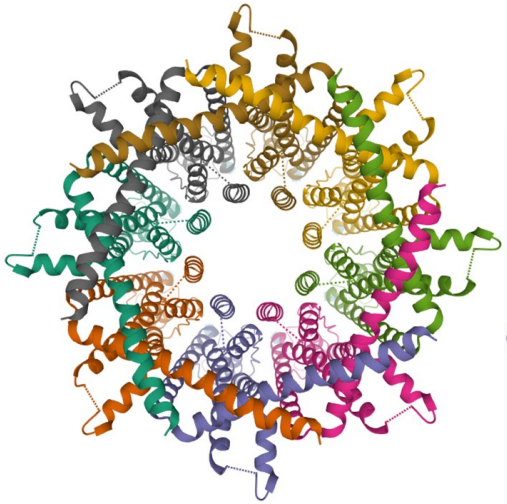
钙稳态调节蛋白2（CALHM2)

CALHM family

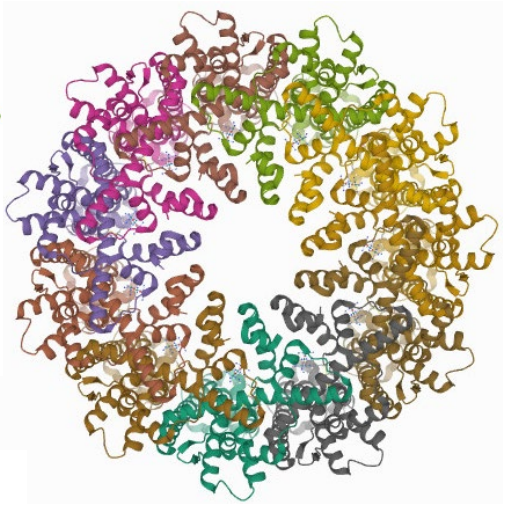
- **CALHM1** 电压门控的膜通道蛋白
  被去极化和细胞外钙离子浓度降低激活
  通透Ca^{2+}, K^+, Cl^-和ATP等
- **CALHM2** Ca^{2+}抑制性通道蛋白
  通透Ca^{2+}, ATP/ADP等离子和小分子
- **CALHM3** 可能与CALHM1形成异聚化通道
  介导ATP的释放
- **CALHM4**
- **CALHM5**
- **CALHM6**

  目前功能未知

CALHM1



CALHM2



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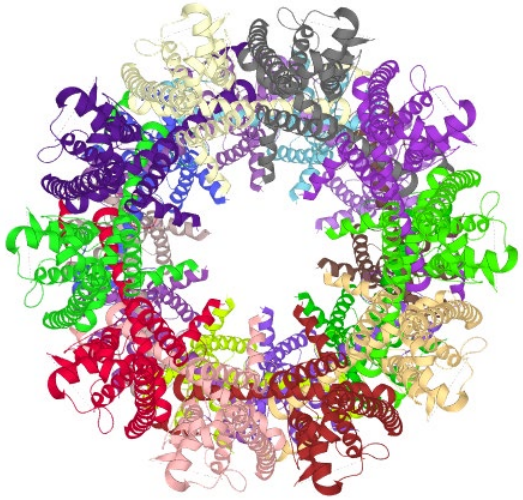

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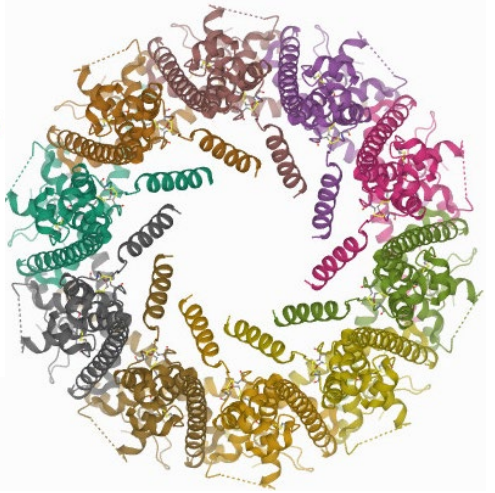
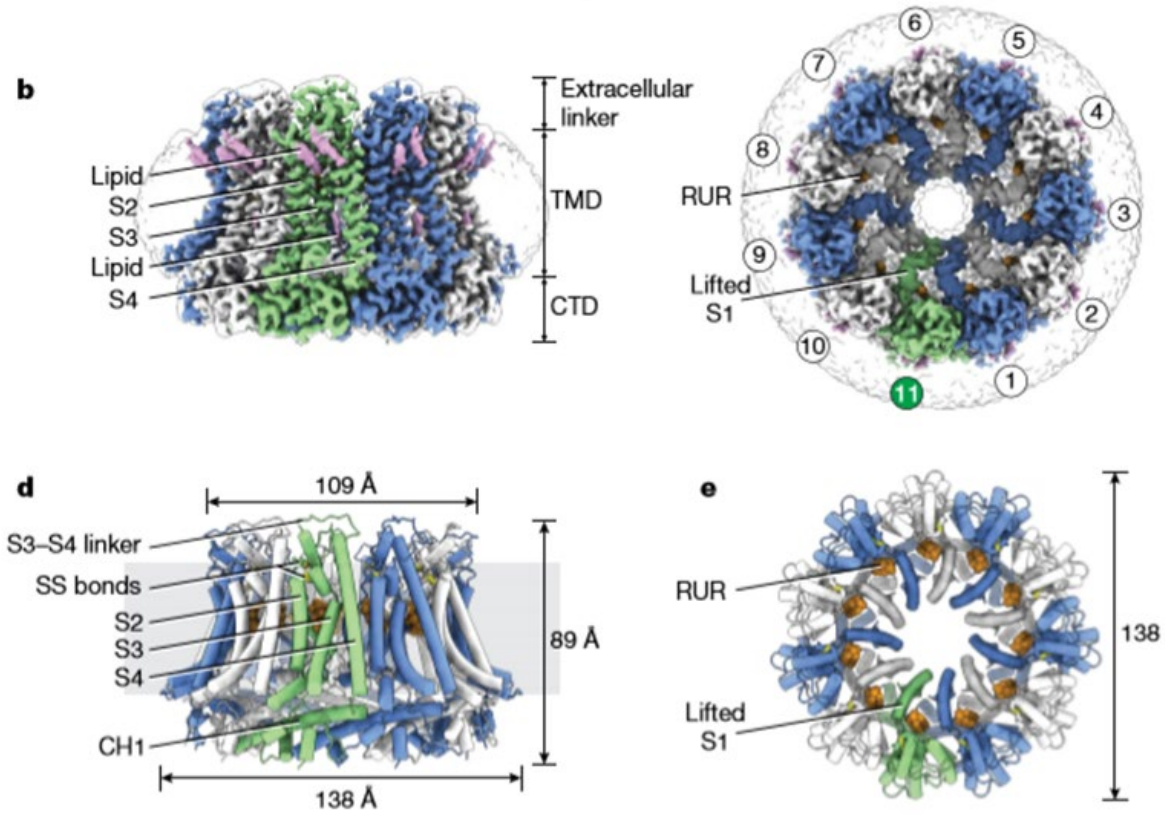


Overall architecture

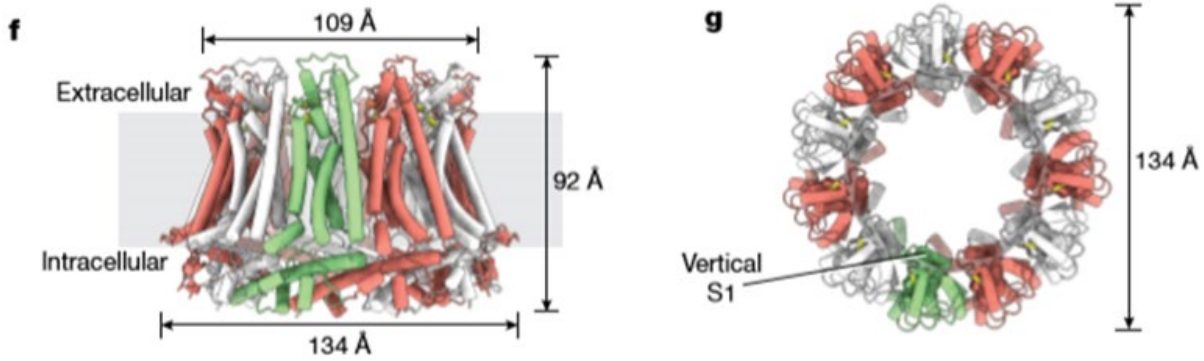
(Choi et al. 2019)

Side view　　Top view



RUR抑制状态（通道关闭）

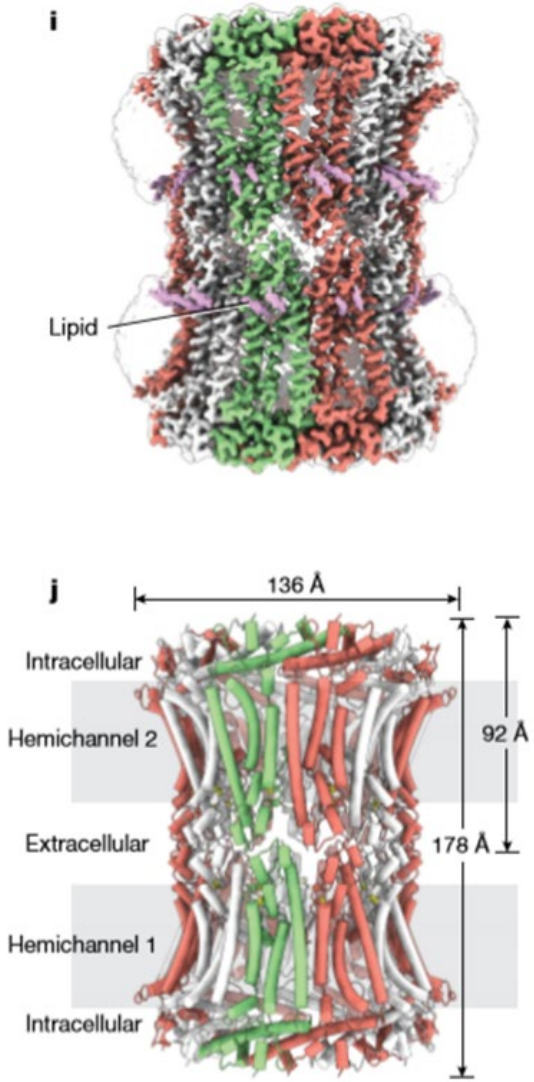
Side view　　Top view

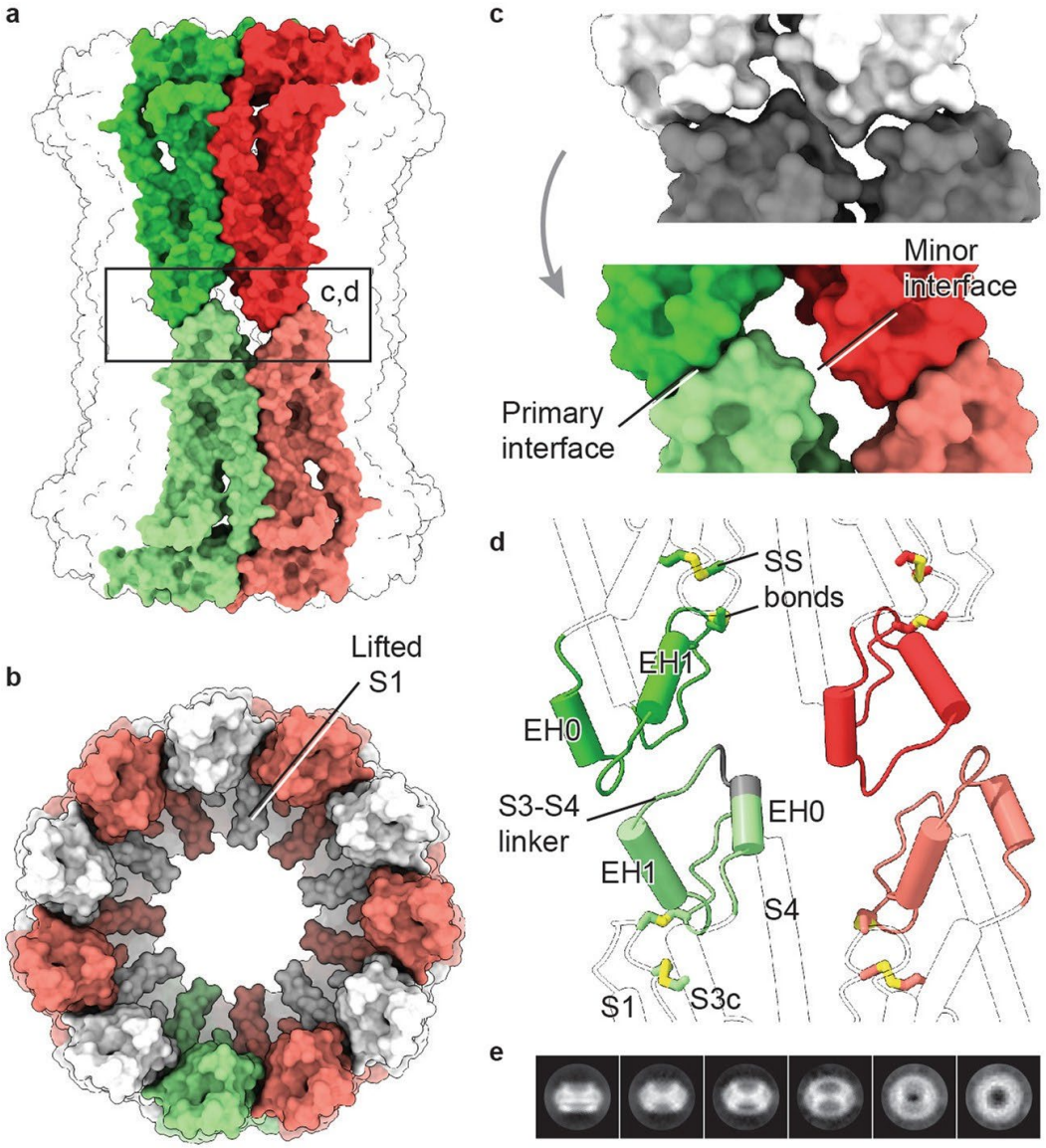


通道开放状态

CALHM2 gap junction

(Choi et al. 2019)





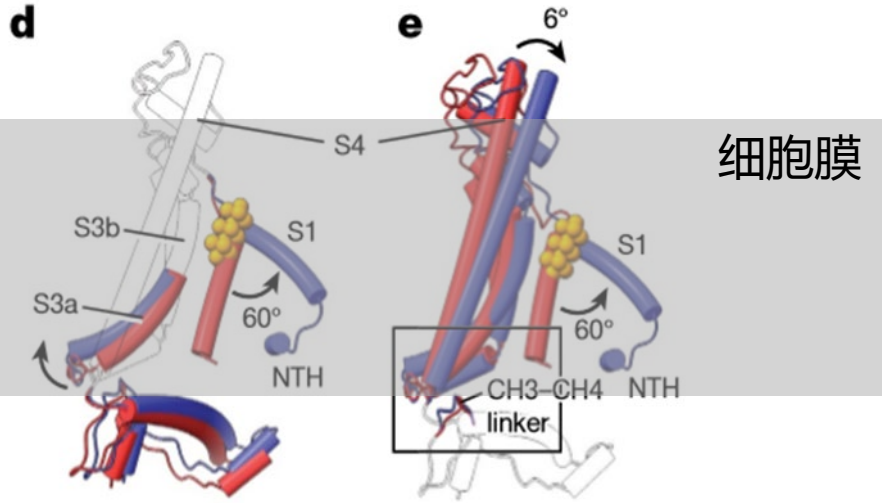
Subunit structure and RUR-binding site

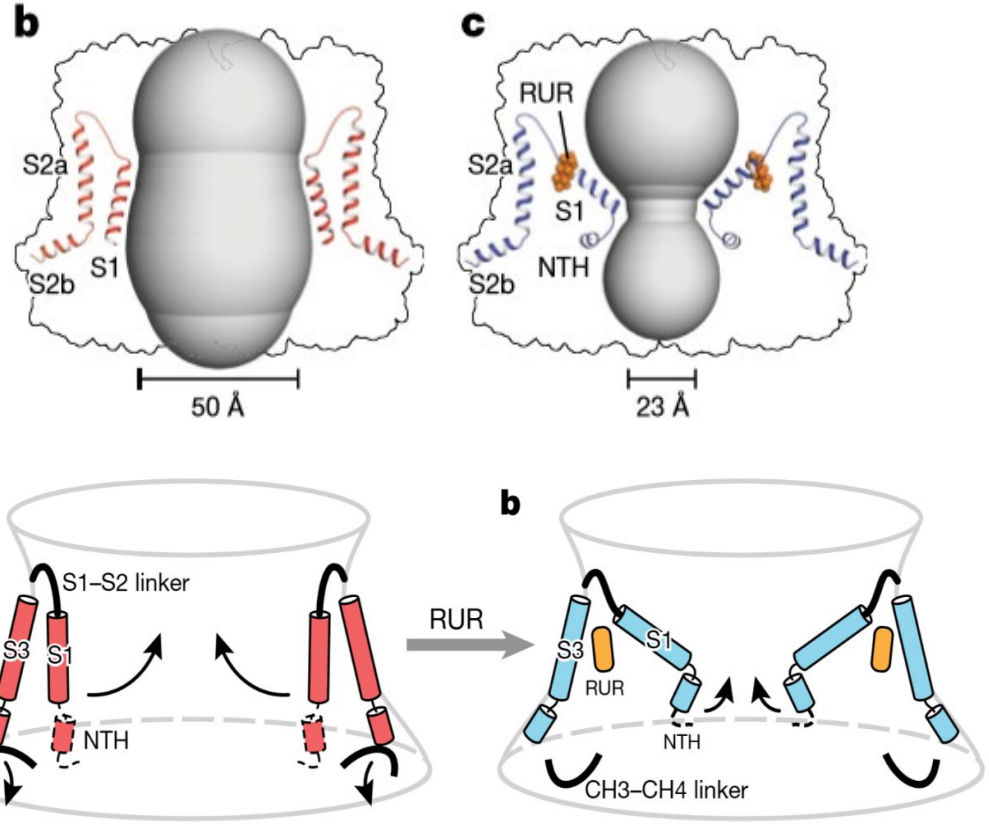
(Choi et al. 2019)

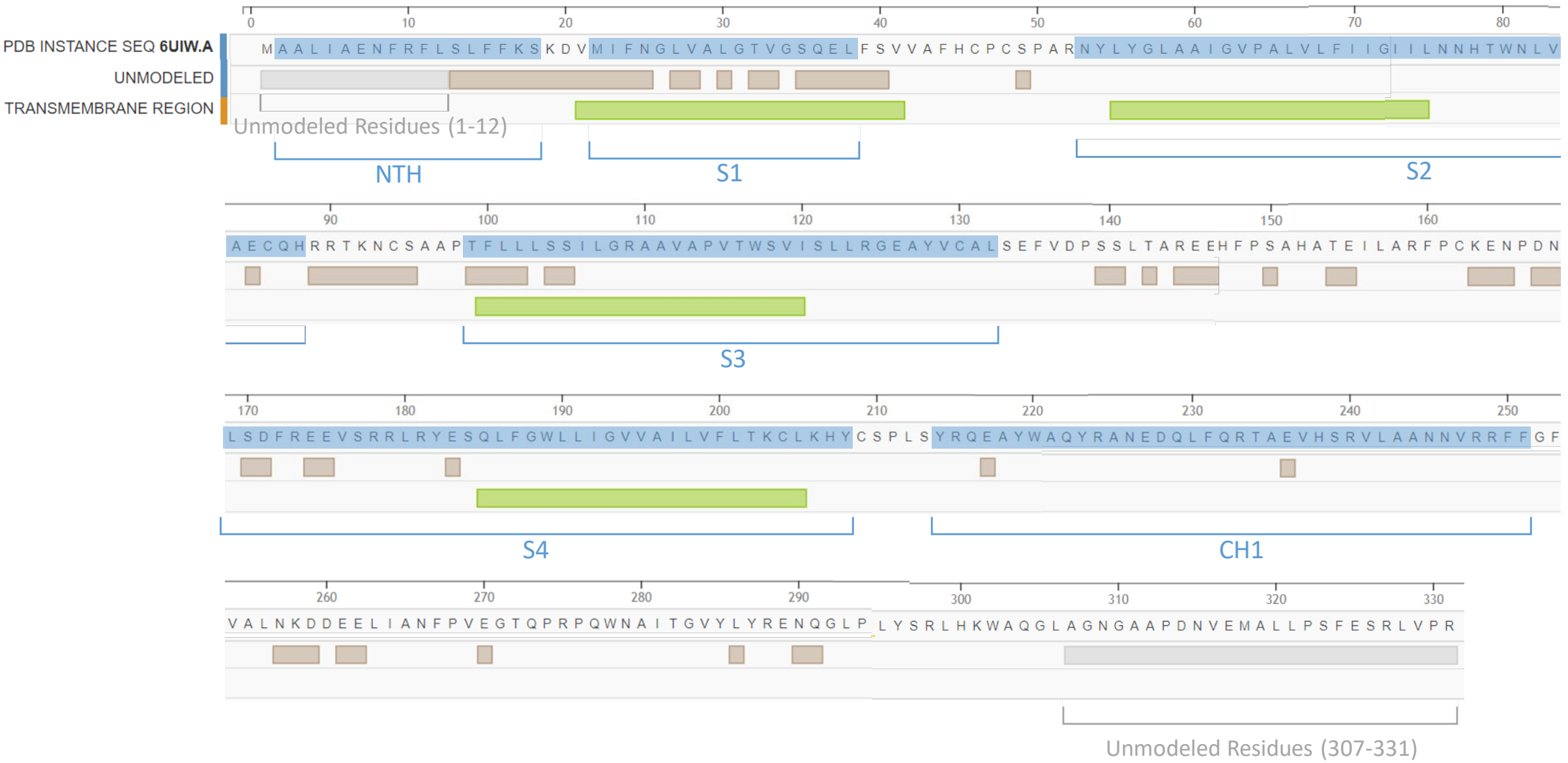
细胞外连接区

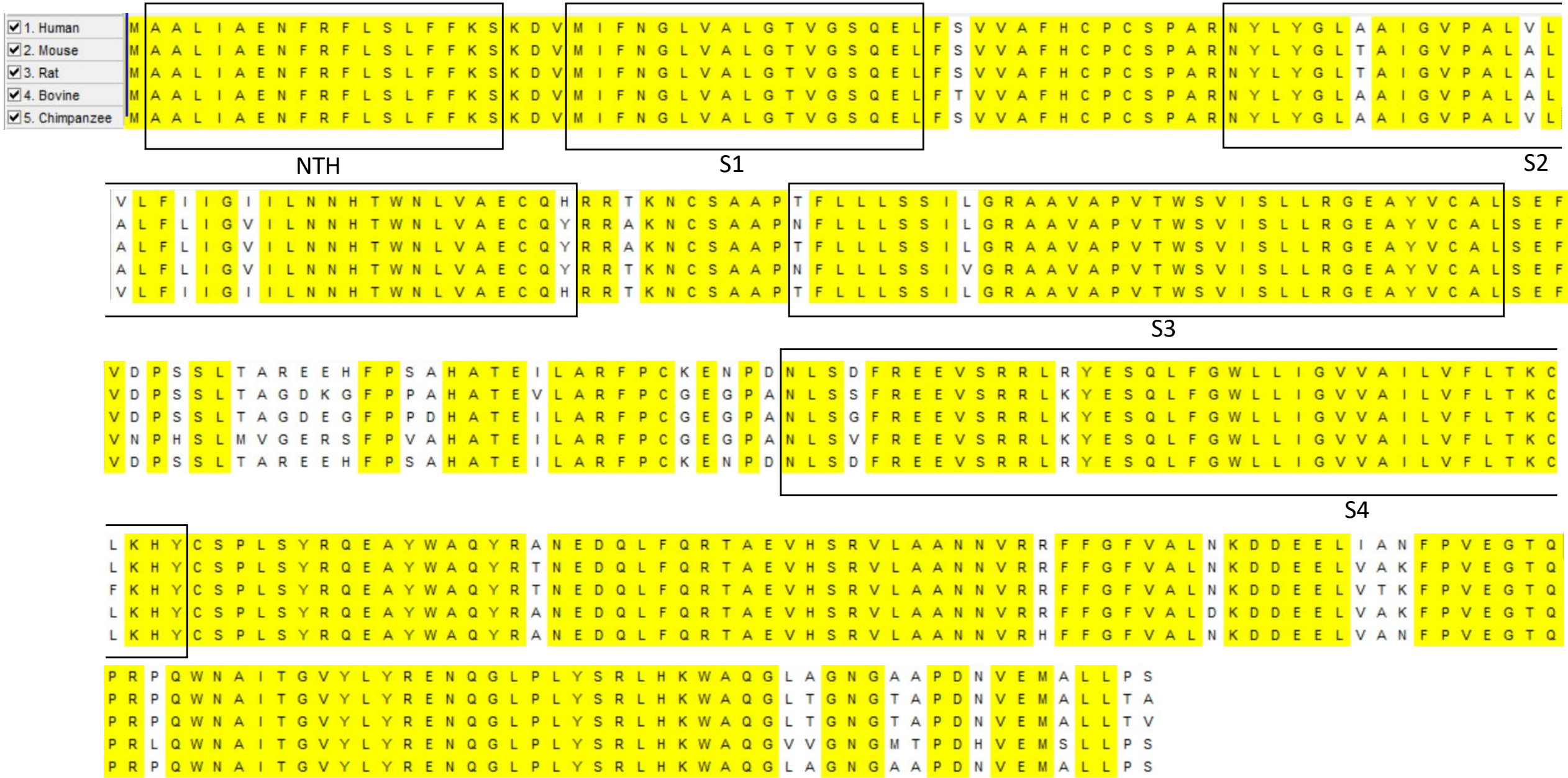
跨膜螺旋
S1 – S4

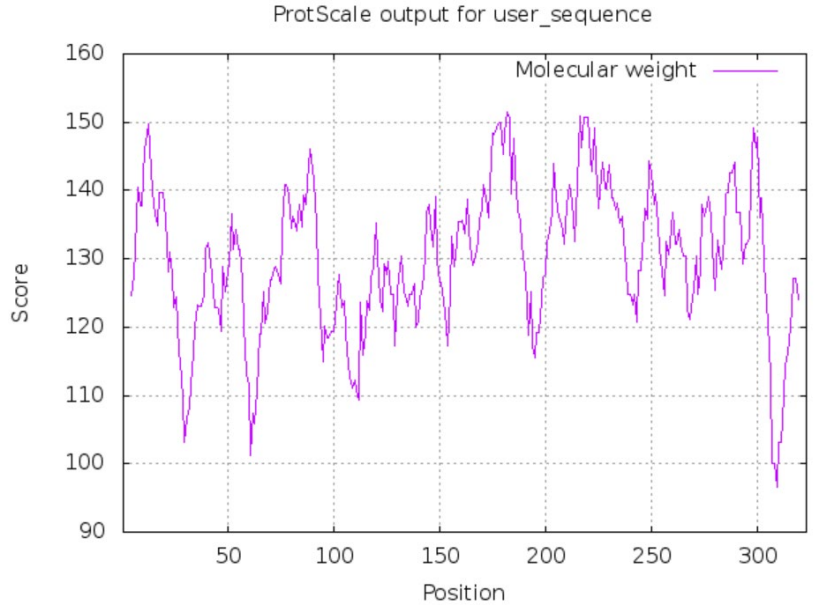
细胞内
C端长螺旋
CH1

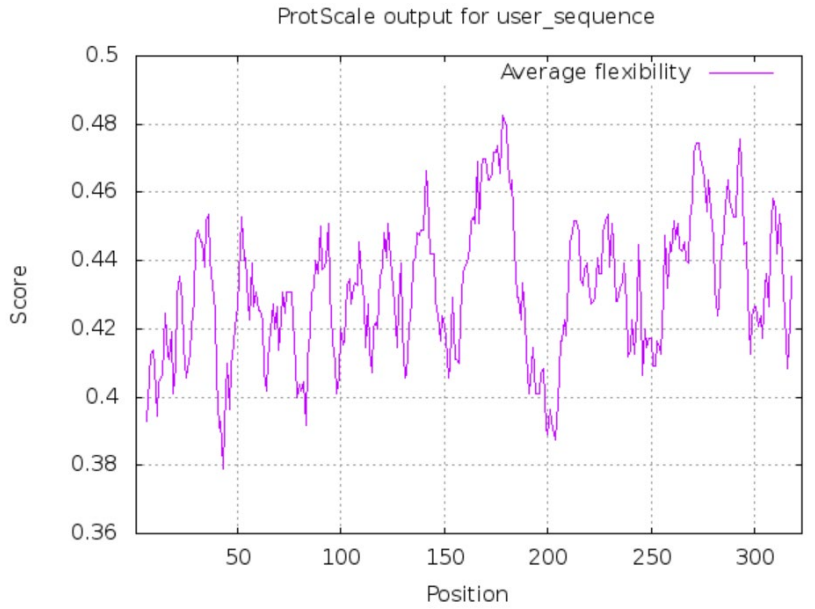


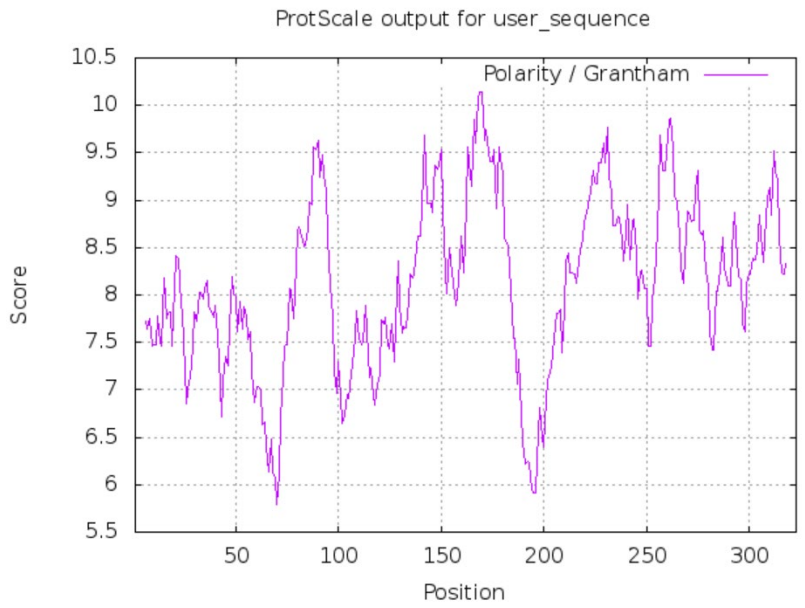


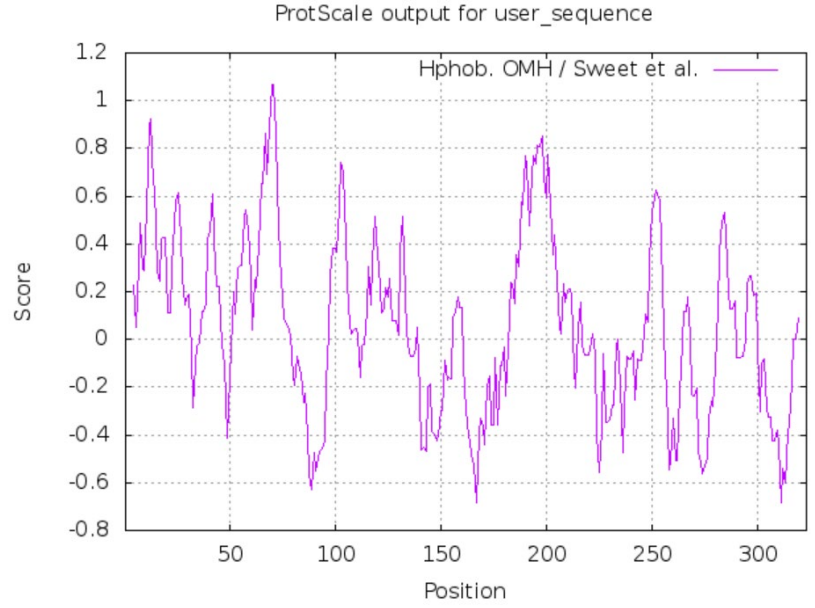

PDB INSTANCE SEQ 6UIW.A
UNMODELED
TRANSMEMBRANE REGION
Unmodeled Residues (1-12)
NTH
S1
S2
S3
S4
CH1
Unmodeled Residues (307-331)


1. Human
2. Mouse
3. Rat
4. Bovine
5. Chimpanzee
NTH
S1
S2
S3
S4





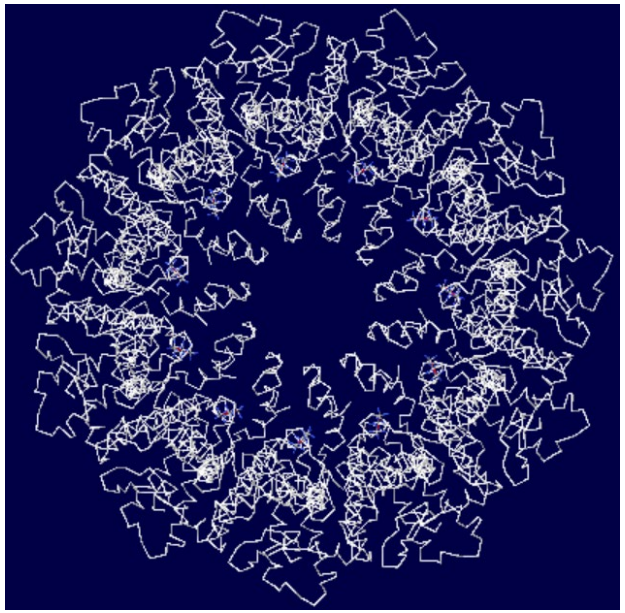




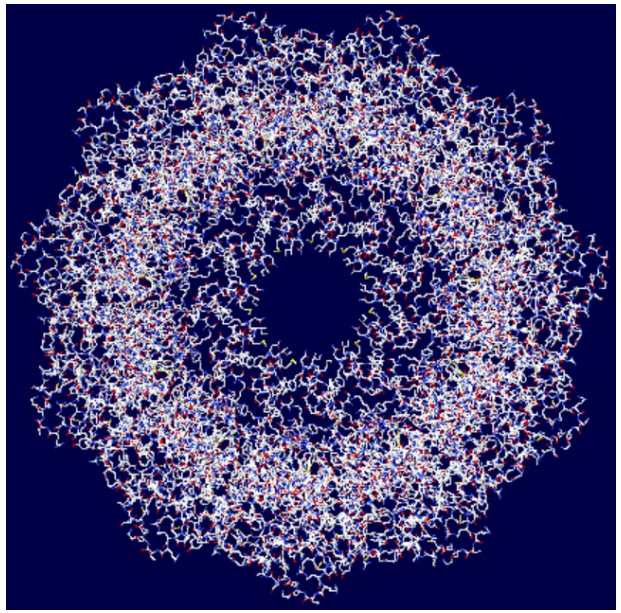
NTH (2 - 18)
S1 (22 - 38)
S2 (53 - 88)
S3 (99 - 132)
S4 (169 - 208)

Entry	Entry name		Protein names	Gene names	Organism	Length
Q9HA72	CAHM2_HUMAN		**Calcium homeostasis**	**CALHM2** FAM26B	Homo sapiens (Human)	323

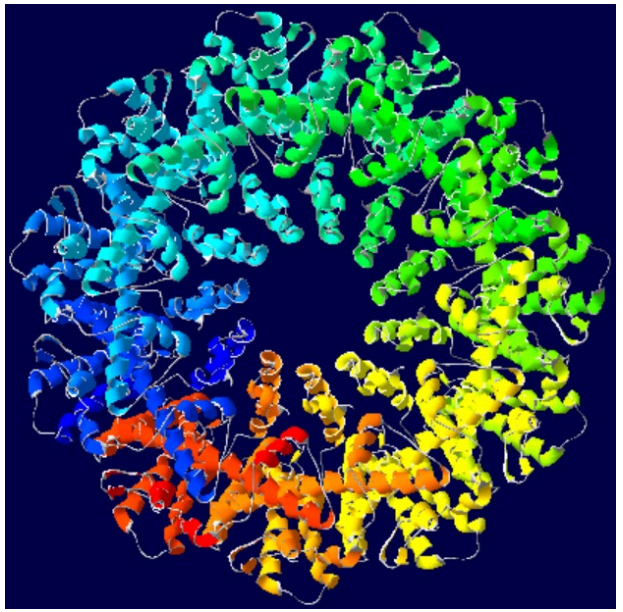
PDB ID: 6UIW



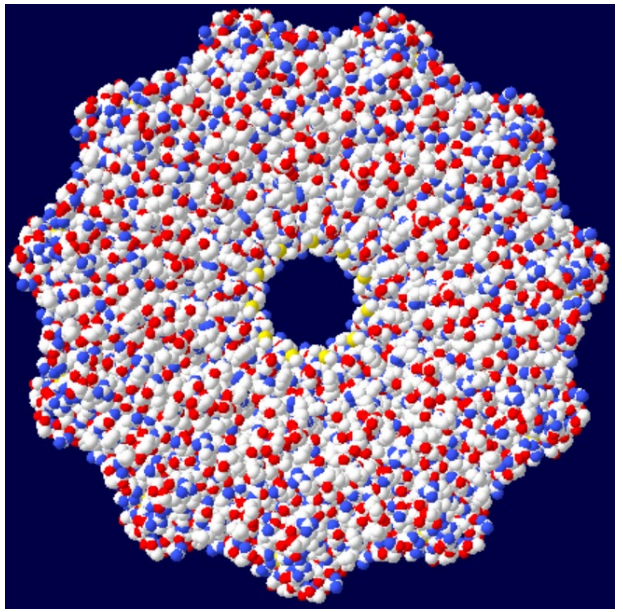
Carbon alpha trace



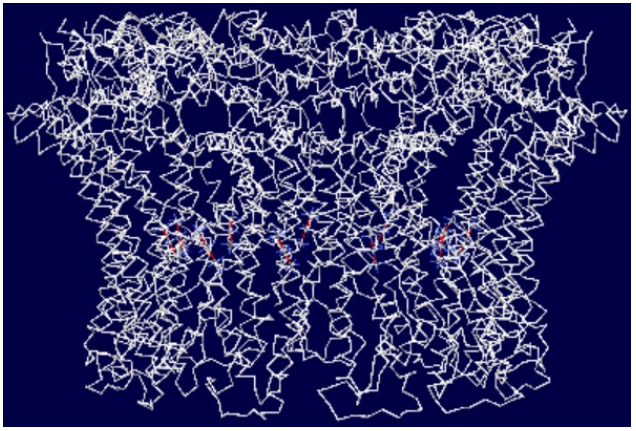
Backbone + Sidechains

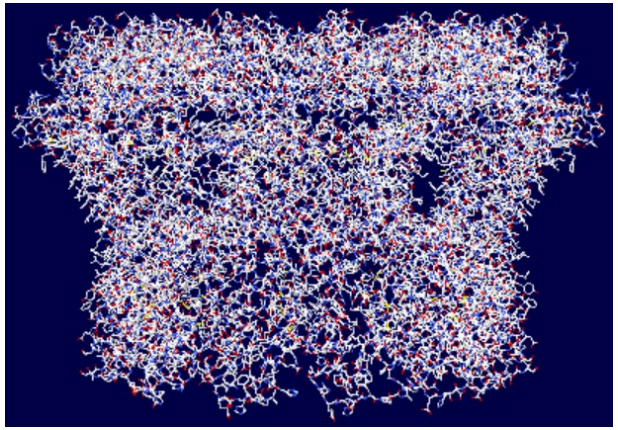


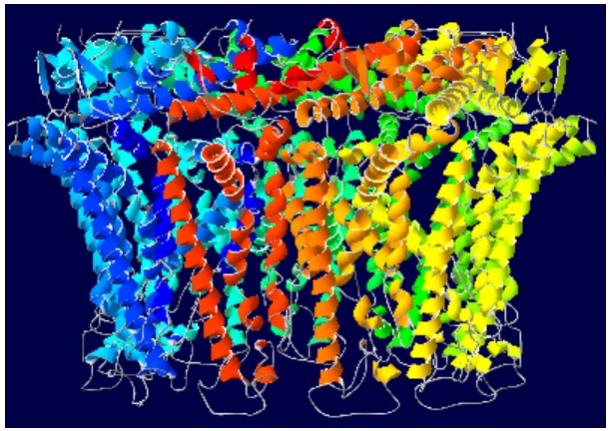
Ribbon

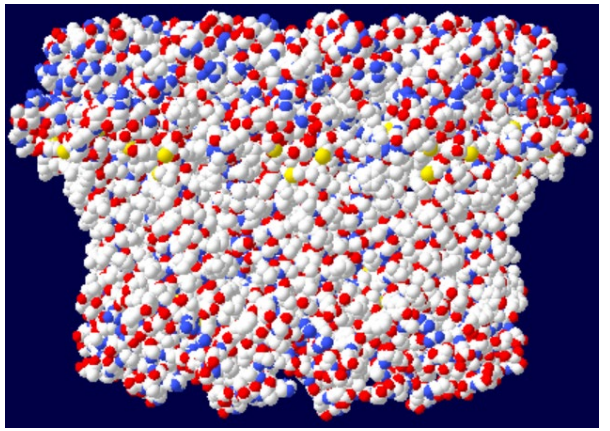


Spheres

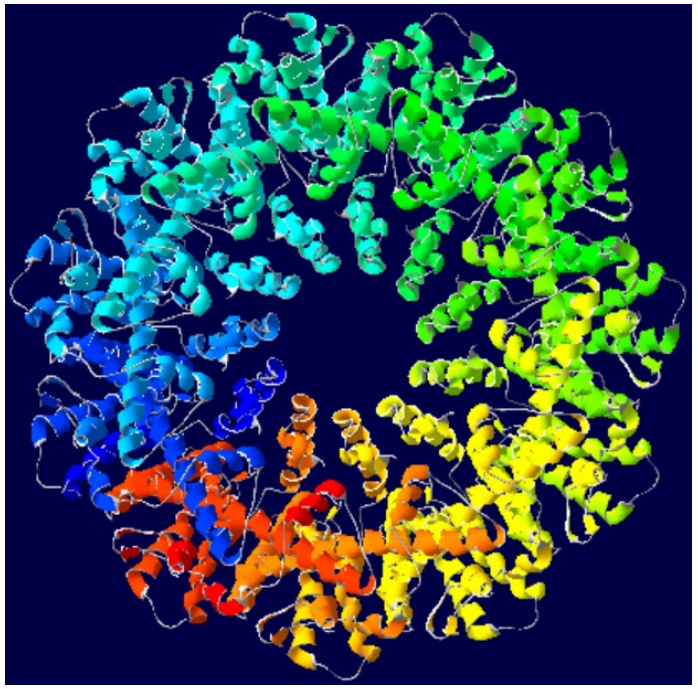


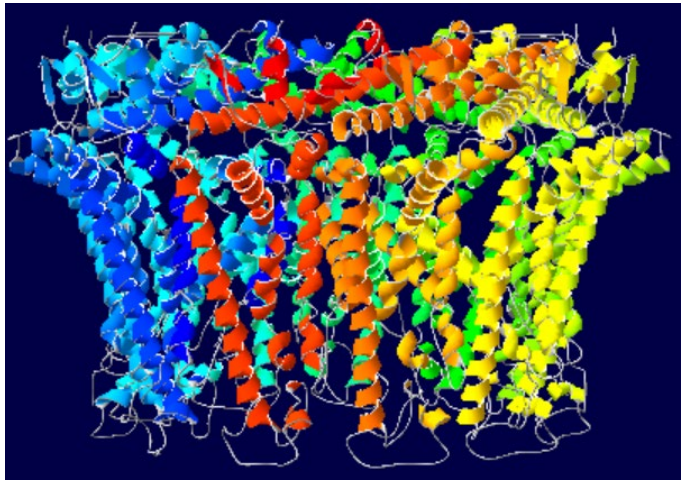




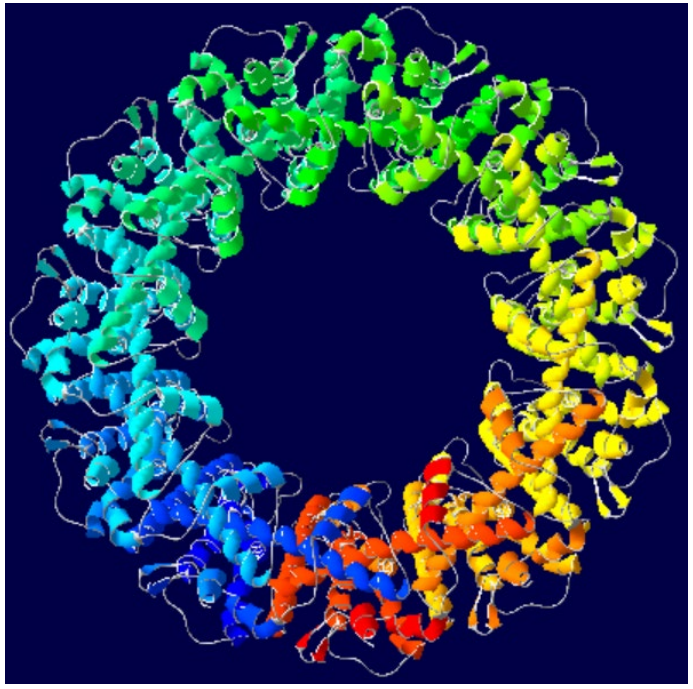


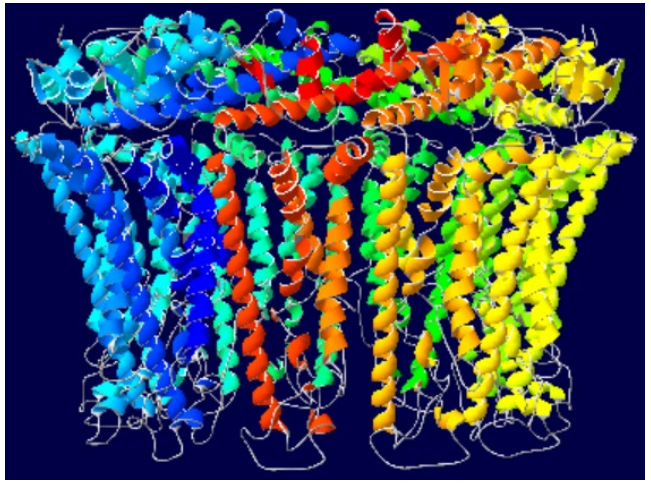
ruthenium red-bound inhibited state (6UIW)



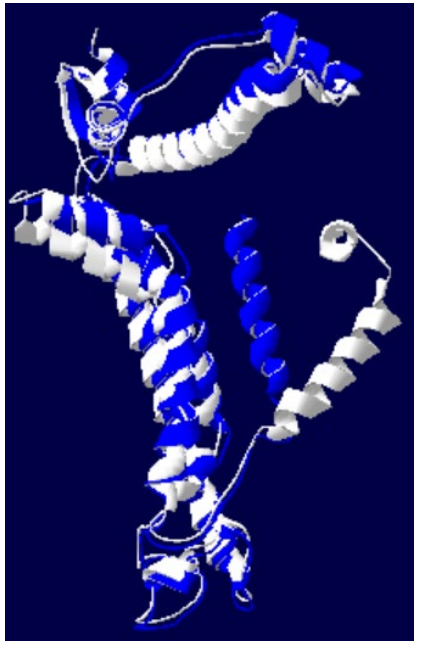


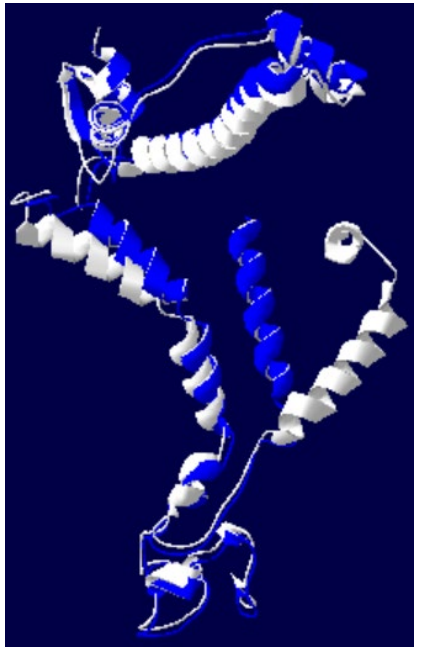
active/open state (6UIV)





Magic Fit – All atoms



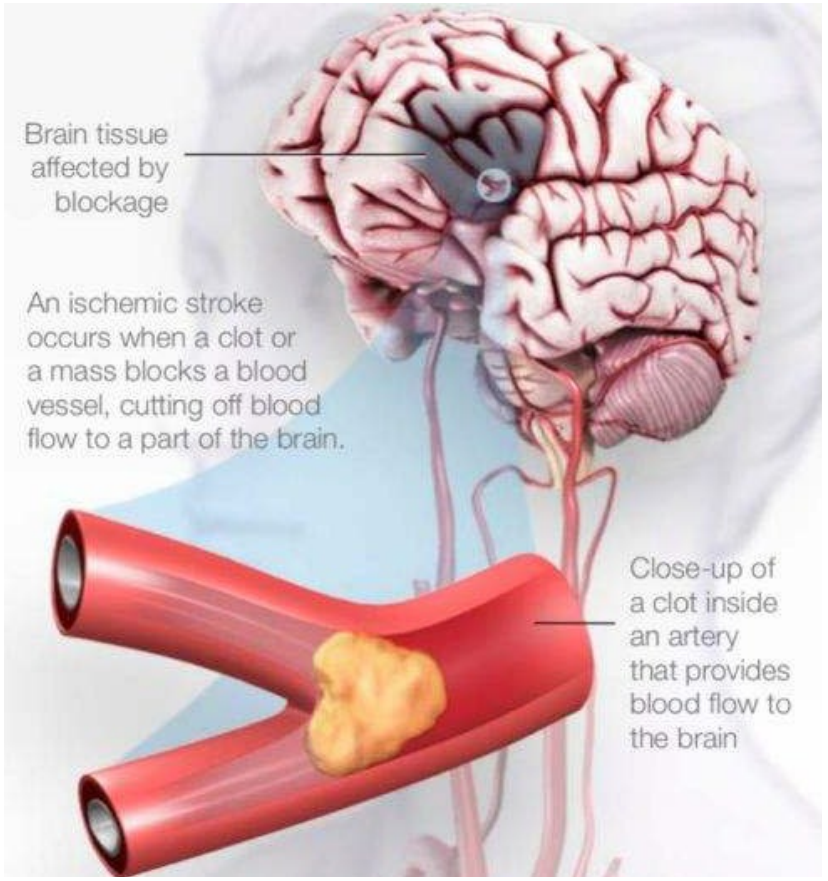


Inhibited state
Open state

PART 02

质子激活的氯离子通道(PACC1)

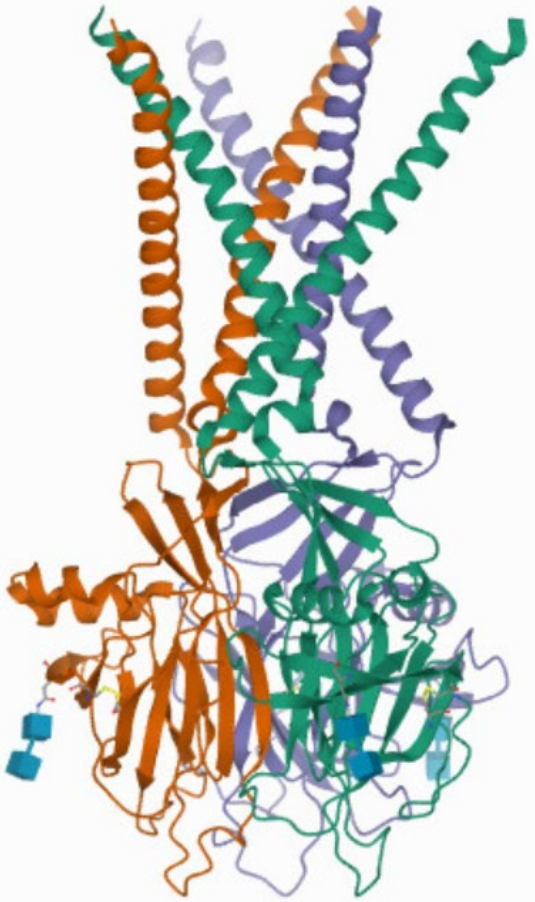
酸与缺血性脑中风



- 血管阻塞
- 细胞内的有氧代谢转换为无氧代谢，导致大量乳酸堆积，引起胞内外环境的酸化，导致神经元死亡
- 最近发现，PACC1是一个质子激活的氯离子通道，在神经元中敲除PACC1对缺血损伤有一定的挽救作用
- 利用生物信息学的方法对PACC1蛋白结构进行预测与分析

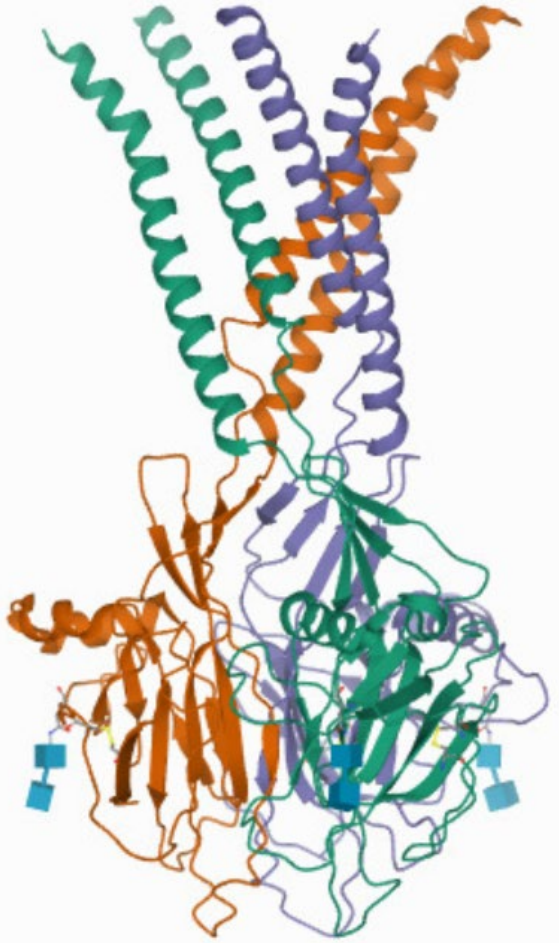
人类PACC1蛋白结构

PDB ID：7JNC



pH=4

PDB ID：7JNA



pH=8

人类PACC1蛋白结构

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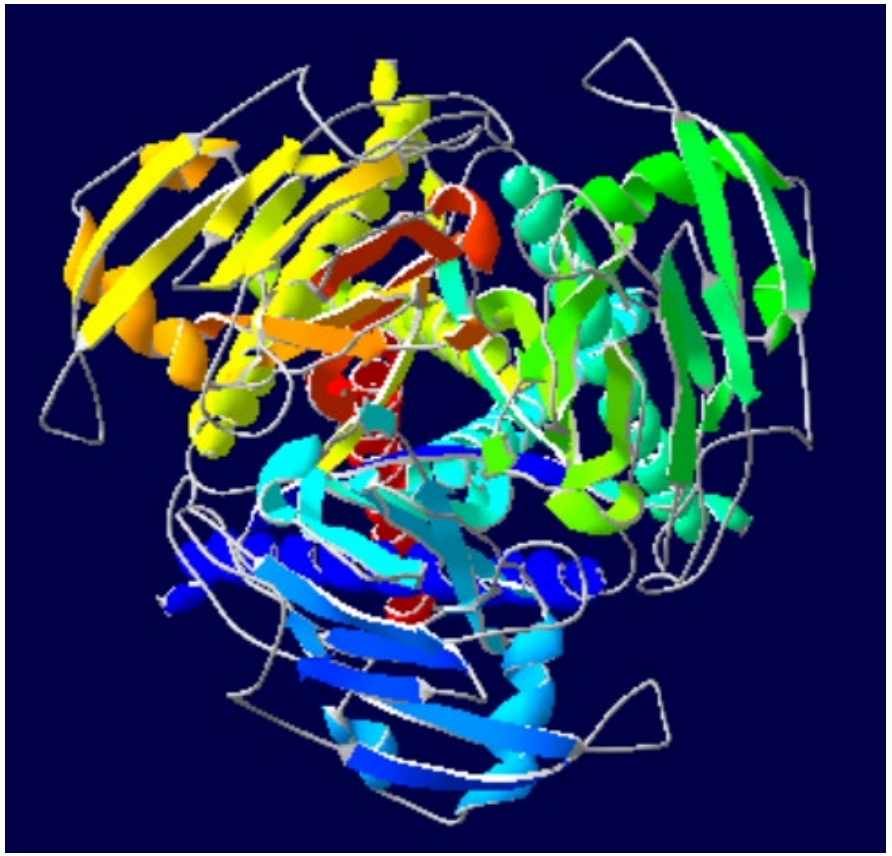
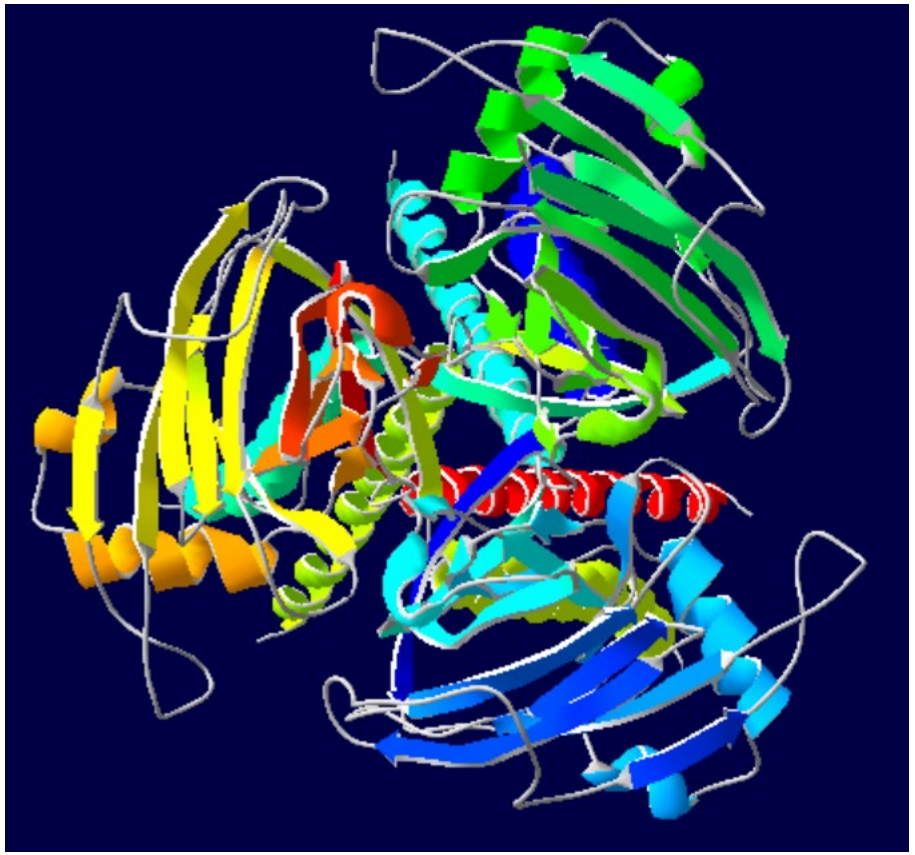


pH=4

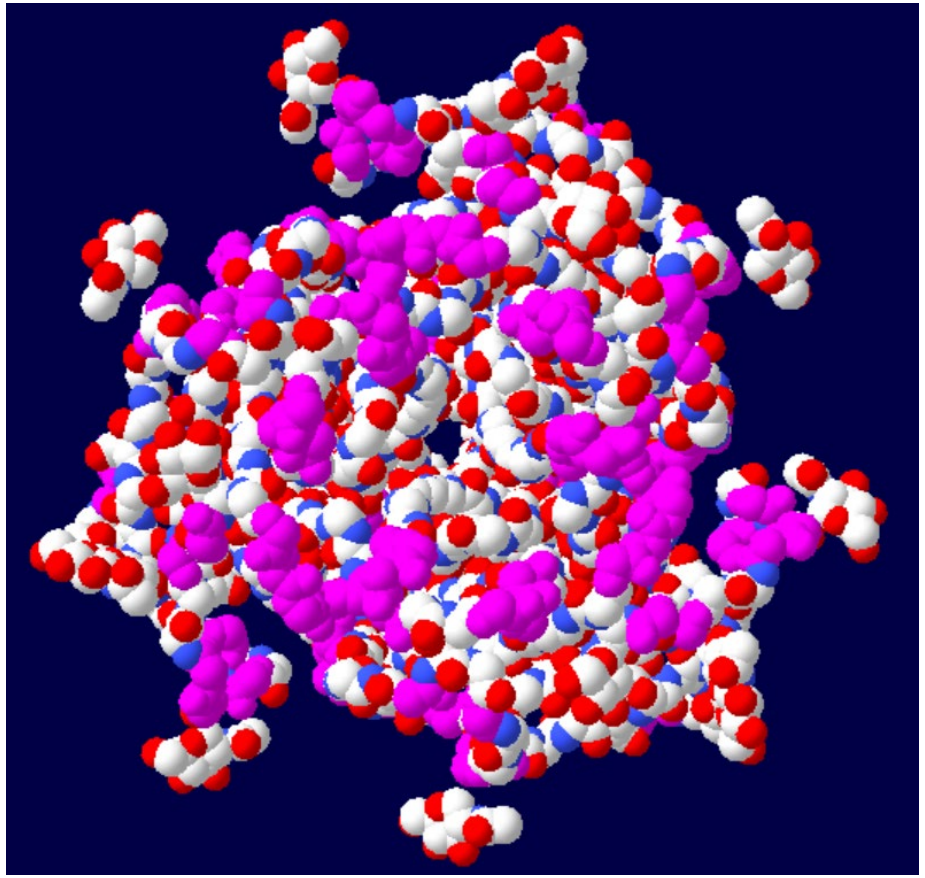
PDB ID：7JNA



pH=8

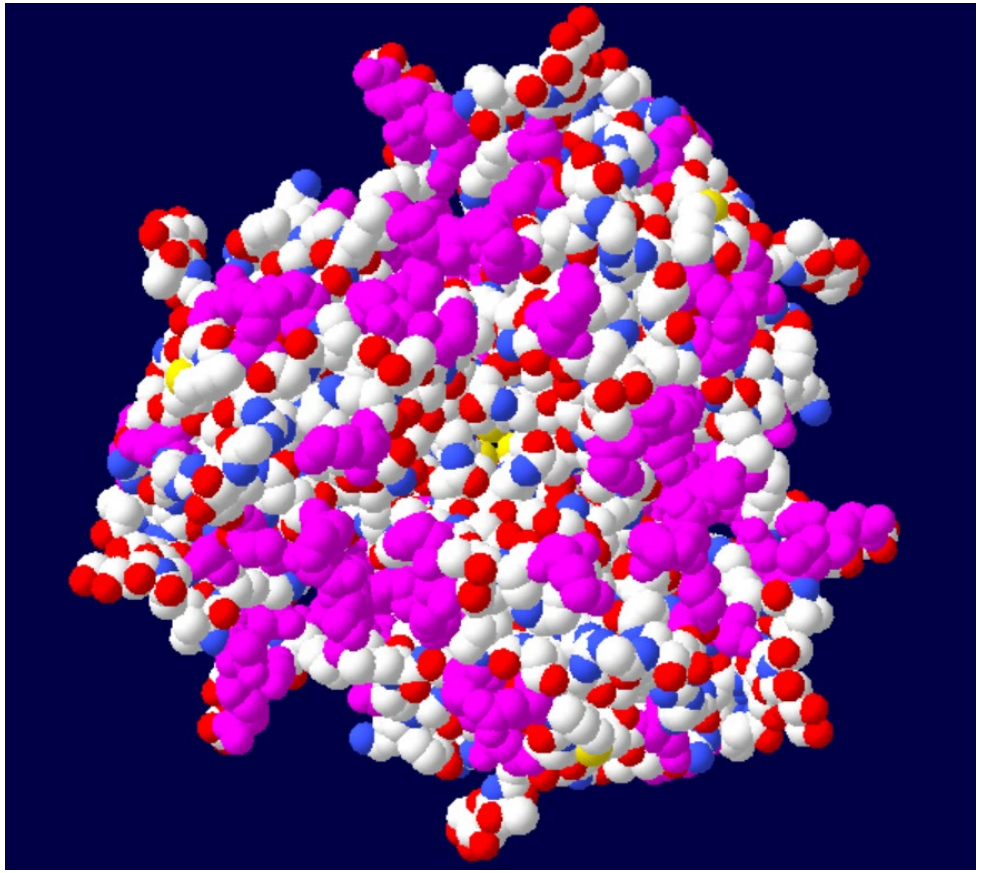
人类PACC1蛋白结构

PDB ID：7JNC



pH=4

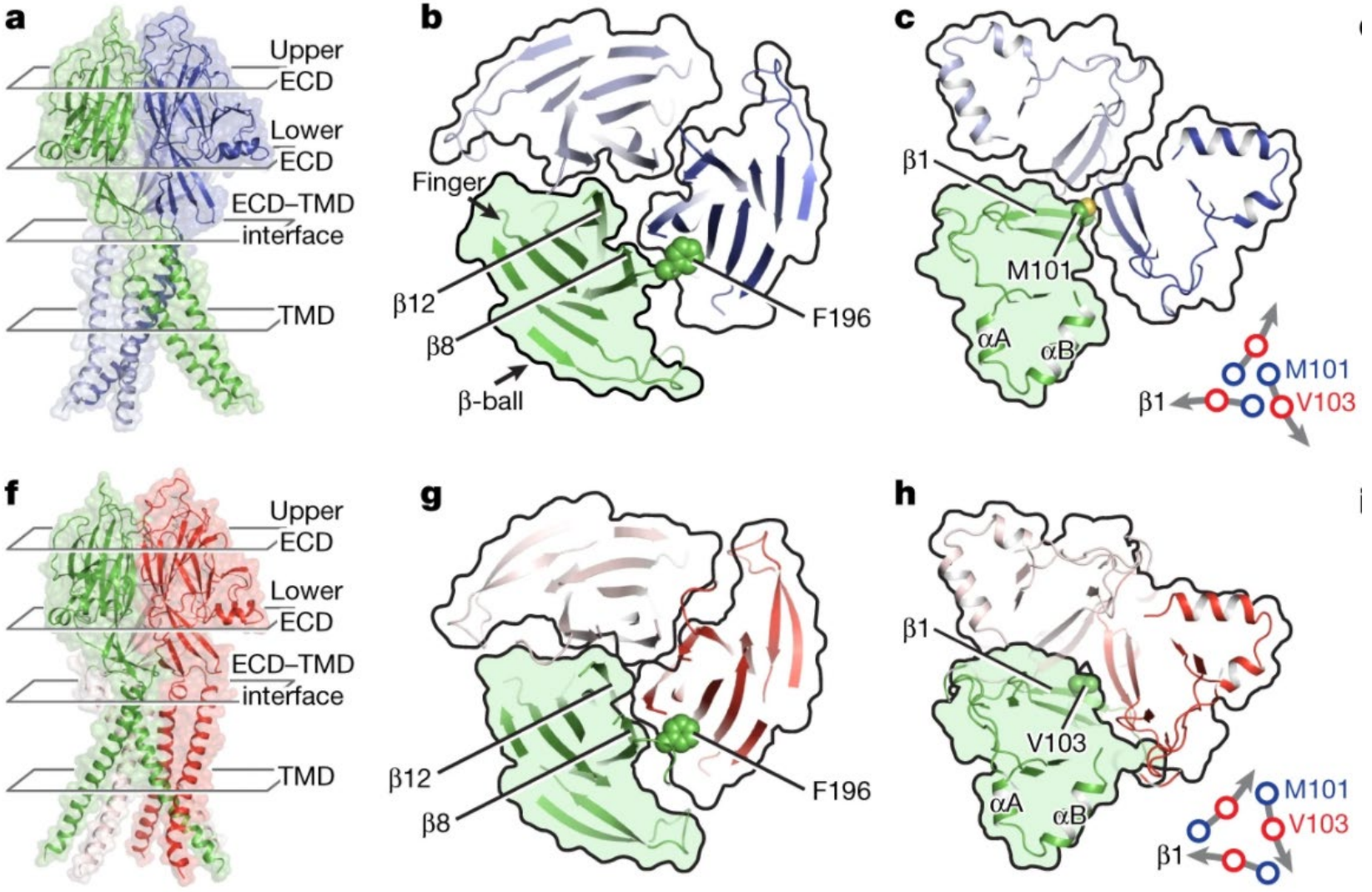
PDB ID：7JNA



pH=8

人类PACC1蛋白结构

- PACC1的开放状态/关闭状态
- 苯丙氨酸（F196），调节亚基间的相互作用
- 在 pH 8 时，α1 链的 Met101 位于Lower ECD 的中心（c，右下角）。
- 在 pH 4 下，Lower ECD 进行顺时针向内旋转，使 α1 链中间的 Val103 移动到Lower ECD 的中心

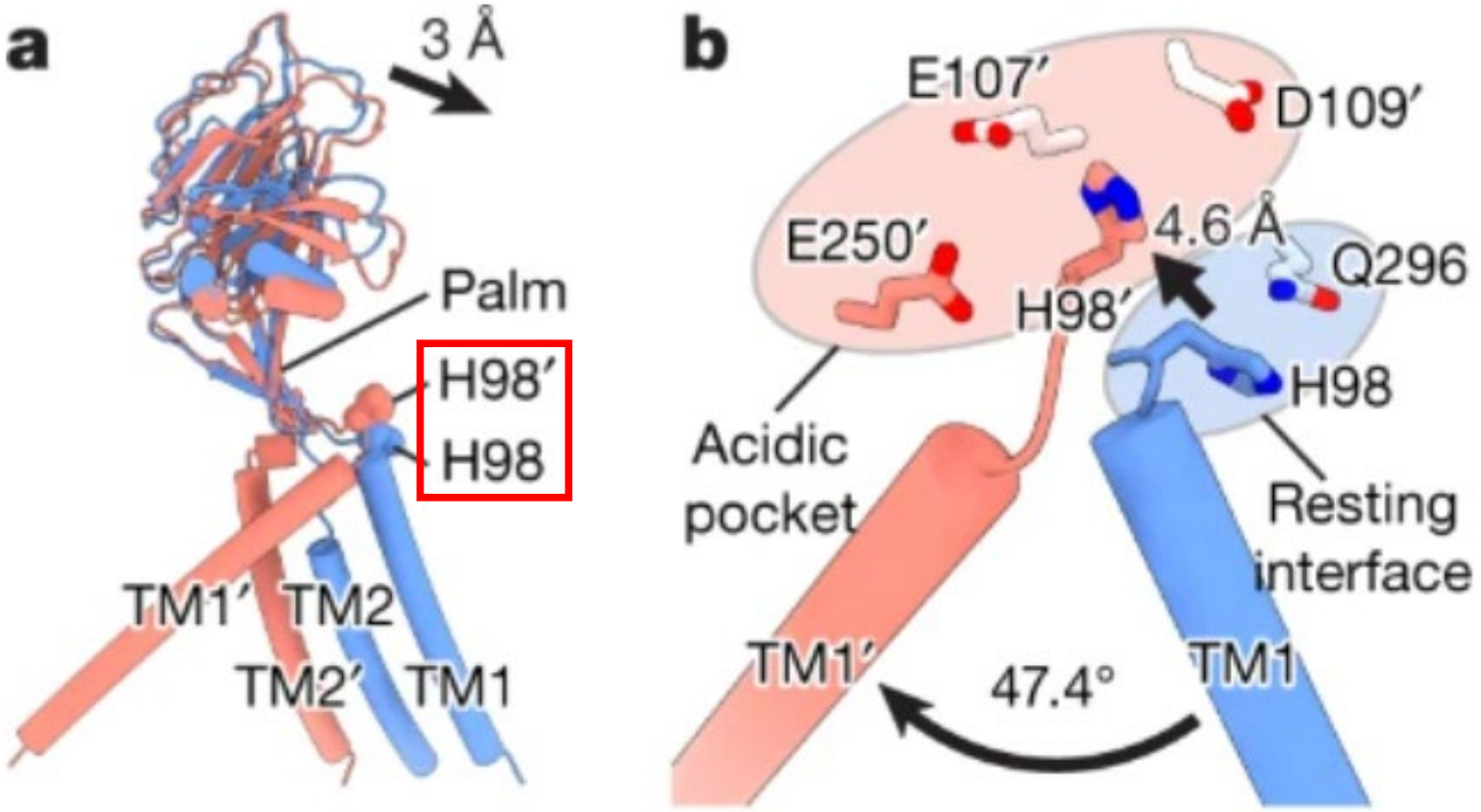


Ruan, Z. et al. *Nature*, 2020.

人类PACC1蛋白结构

红色：pH=4
蓝色：pH=8

- PACC1的开放状态/关闭状态
- 依赖98位的组氨酸的结合位点的改变控制通道构象变化



Ruan, Z. et al. *Nature*, 2020.

双序列比对

相似性：96.6%
一致性：90.9%

高度相似性

在人类PACC1蛋白中调节构象的关键氨基酸在大鼠中都是保守的

差异氨基酸：

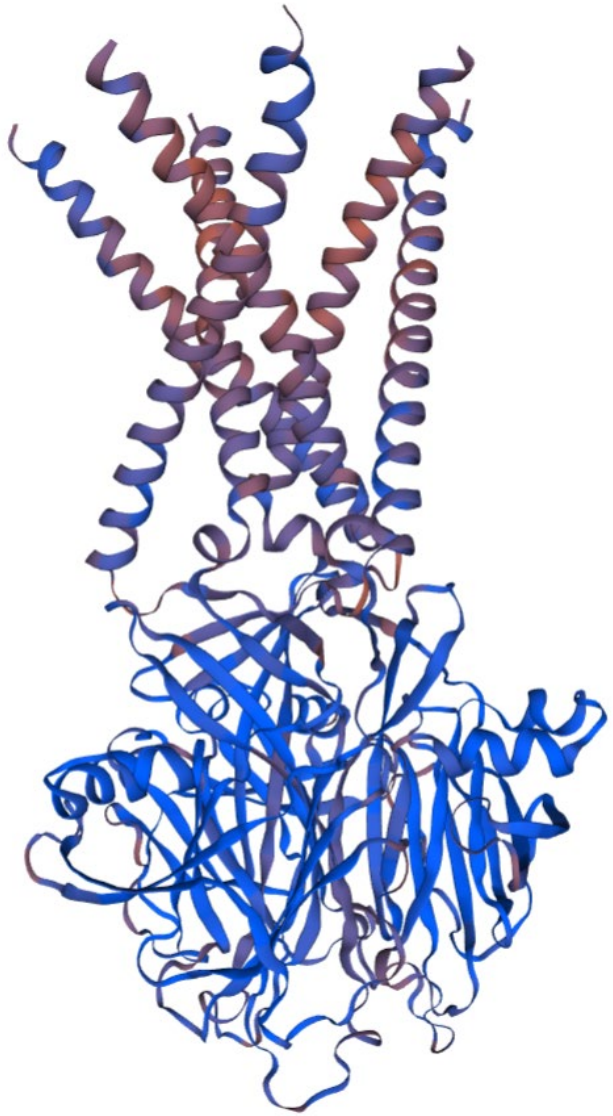
- 亲疏水性的差异：6，18，26，34，139，147，278位氨基酸残基）
- 电荷差异（158,163位氨基酸残基）
  —造成局部构象的差异

```
#
# Aligned_sequences: 2
# 1: PACC1_RAT
# 2: PACC1_HUMAN
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 350
# Identity:     318/350 (90.9%)
# Similarity:   338/350 (96.6%)
# Gaps:           0/350 ( 0.0%)
# Score: 1662.0
#
#
#=======================================

PACC1_RAT         1 MIRQDLSTSYQELSEELEQVVENSEQADERDKELVQVQGPGVVPGVDNES     50
                    ||||:.|||||||||.|||||||.|||:|||.:|||||::||:|:||
PACC1_HUMAN       1 MIRQERSTSYQELSEELVQVVENSELADEQDKETVRVQGPGILPGLDSES     50

PACC1_RAT        51 ASSSIRFSKACLKNVFSVLLILIYLLLMAVAVFLVYQTITDFREKLKHPV    100
                    ||||||||||||||||||||.|||||||||||||||:||||||||||||||
PACC1_HUMAN      51 ASSSIRFSKACLKNVFSVLLIFIYLLLMAVAVFLVYRTITDFREKLKHPV    100

PACC1_RAT       101 MSVSYKEVDRYDAPGIALYPGQAQLLSCKHHYEVIPPLASPGQPGDRNCT    150
                    |||||||||||||||||||||||||||||||||||||||.||||||.|||
PACC1_HUMAN     101 MSVSYKEVDRYDAPGIALYPGQAQLLSCKHHYEVIPPLTSPGQPGDMNCT    150

PACC1_RAT       151 TQRINYTHPFSNHTMQSALIVQGPQEVKKRELVFLQFRLNQSDEDFSAID    200
                    ||||||.||||. ::||||||:|||||||||||||||:|.|||||||||
PACC1_HUMAN     151 TQRINYTDPFSNQTVKSALIVQGPREVKKRELVFLQFRLNKSSEDFSAID    200

PACC1_RAT       201 YLLFSSFREFMQSPDKAGFMQACESAYSSWKFSGGFRTWVKMSLVKTKEE    250
                    |||||||:||:|||::.||||||||||||||||||||||||||||||||
PACC1_HUMAN     201 YLLFSSFQEFLQSPNRVGFMQACESAYSSWKFSGGFRTWVKMSLVKTKEE    250

PACC1_RAT       251 DGREAVEFRQETSVVNYIDQRPAAEKSTQLFFVVFEWKDPFIQKVQDIIT    300
                    ||||||||||||||||||||||||:||.||||||||||||||||||||:|
PACC1_HUMAN     251 DGREAVEFRQETSVVNYIDQRPAAKKSAQLFFVVFEWKDPFIQKVQDIVT    300

PACC1_RAT       301 ANPWNTIALLCGAFLALFKAAEFAKLSVKWMIKIRKRYLKRRGQATNHIS    350
                    ||||||||||||||||||||||||||:||||||||||||||||||:|||
PACC1_HUMAN     301 ANPWNTIALLCGAFLALFKAAEFAKLSIKWMIKIRKRYLKRRGQATSHIS    350
```

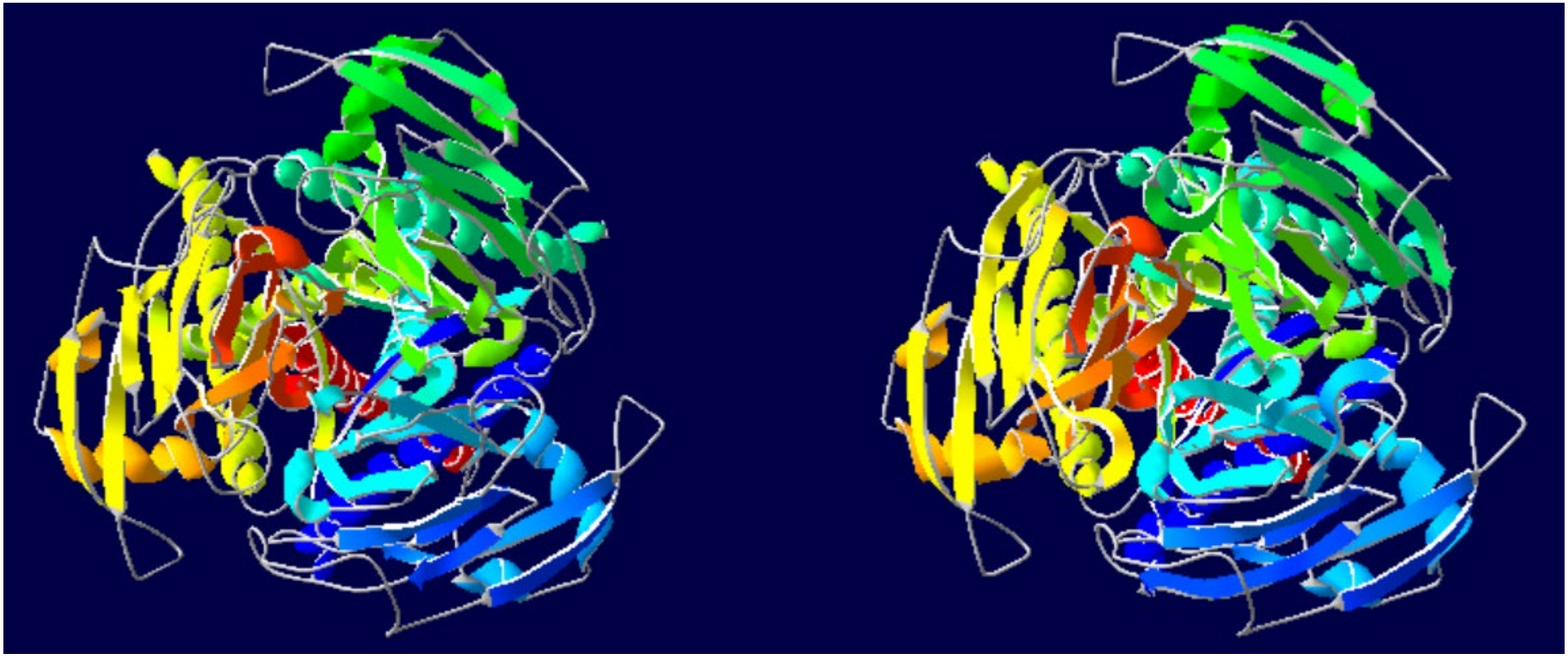
SWISS-MODEL预测结构

由两个结构域组成

- 跨膜区
- 质子激活区



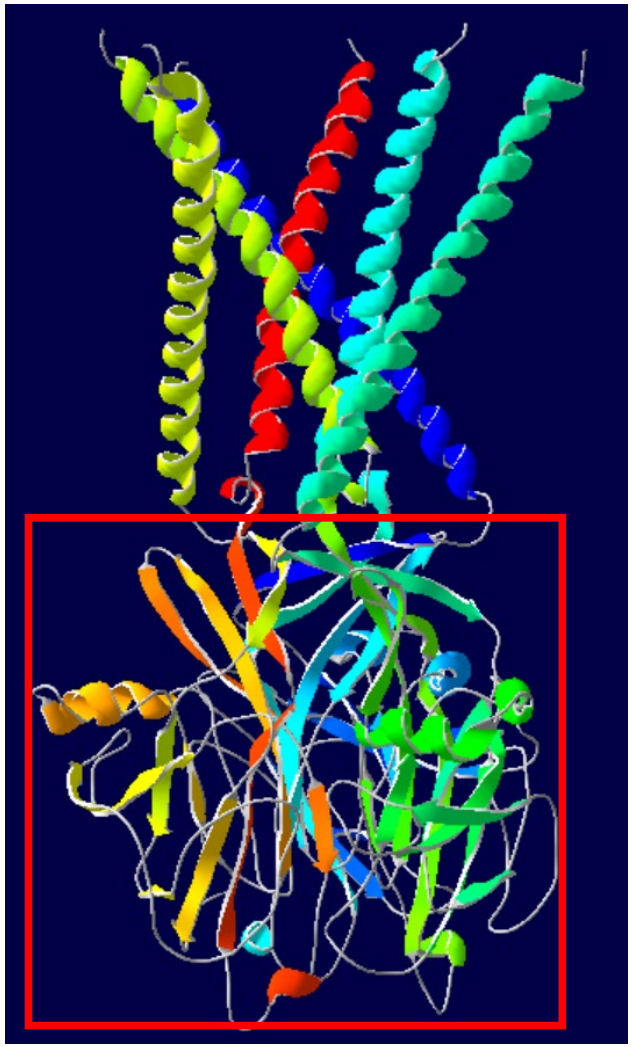
PDBviewer比较大鼠和人的PACC1（pH=4）



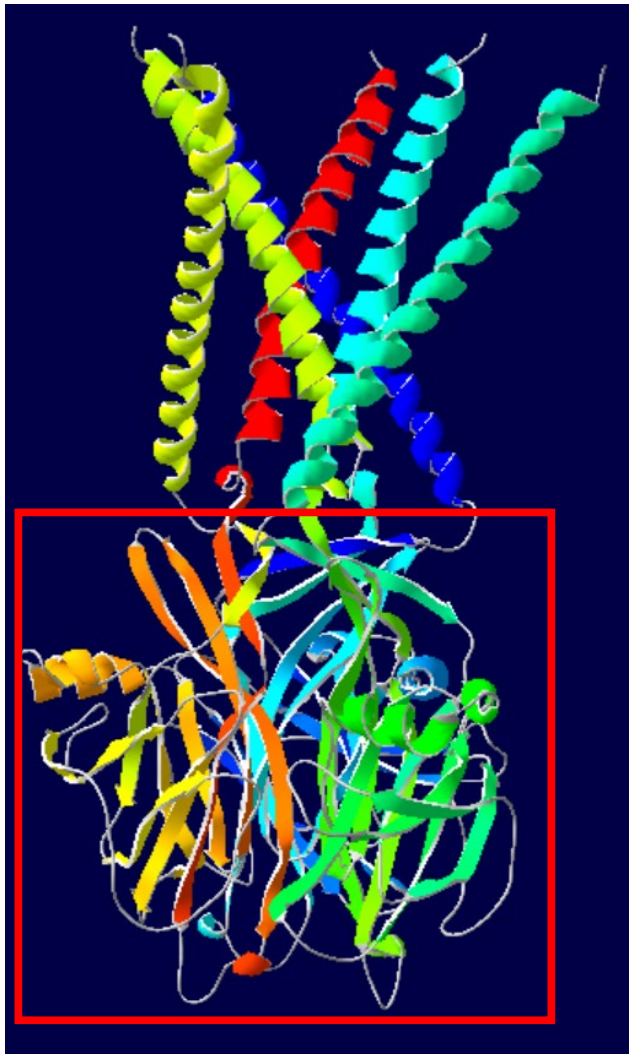
人类PACC1蛋白

大鼠PACC1蛋白

PDBviewer比较大鼠和人的PACC1（pH=4）



人类PACC1蛋白



大鼠PACC1蛋白

PDBviewer比较大鼠和人的PACC1（pH=4）

- 在胞外结构域感受外界环境pH值变化而结合质子时可能有所差异

- 通道被激活后，跨膜区构象变化可能相同

分析大鼠的PACC1蛋白的氨基酸突变敏感性，寻找关键的氨基酸位点，并构建突变蛋白，通过实验进一步研究在该蛋白中发挥关键作用的氨基酸残基

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致谢

ACKNOWLEGEMENTS

感谢罗老师的悉心指导！

感谢小组成员的支持与帮助！

感谢大家的聆听，请大家批评指正！

徐泽


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感谢您的聆听与指导

Thanks for your attention!

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