



# The Analysis of Structure and Function on Human TRPM7 Channel

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Group 16

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# Overview

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- Background of TRPM7
- General gene and protein analysis on TRPM7
- Crystal analysis on TRPM7
- Summary

Most analysis done with Mega 6.0 and Pymol 0.99

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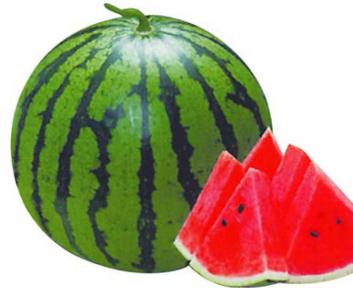
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## **TRPM7:**

Transient receptor potential (TRP) cation channel,  
subfamily M, member 7

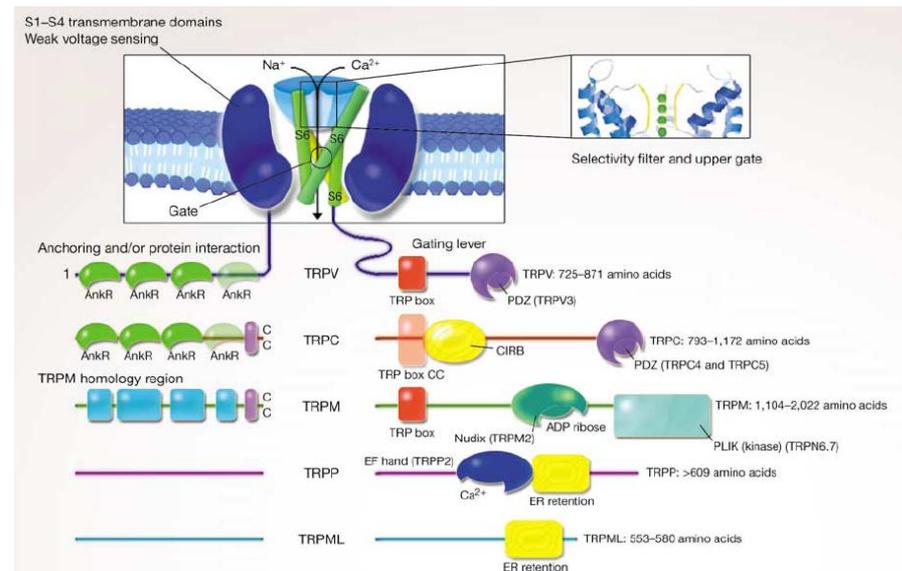
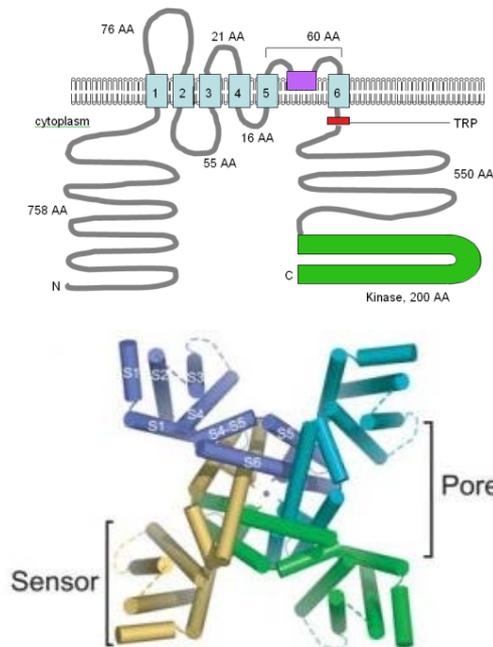
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# Our Senses Detected by TRP Channel



# TRP Introduction

- TRP channel proteins were first discovered in *Drosophila melanogaster*.
- The origin of TRP channels predates the emergence of metazoan organisms.

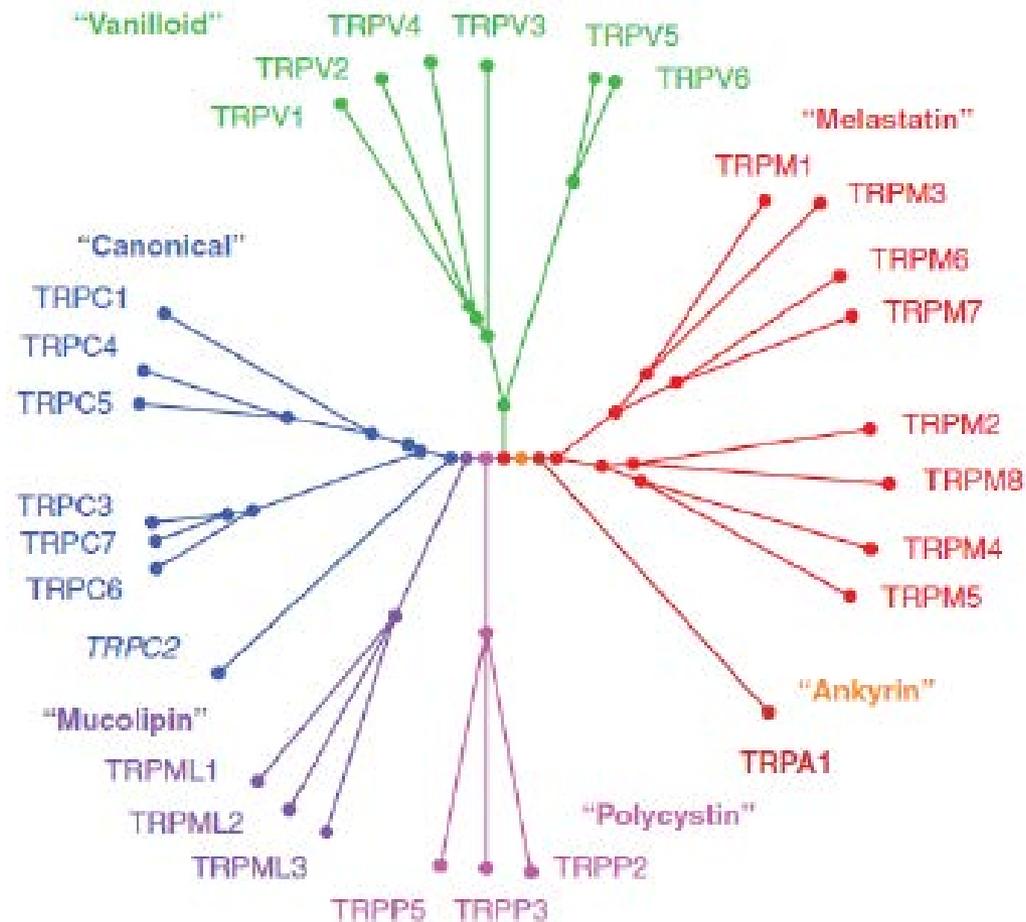


- Kartik Venkatachalam and Craig Montell, TRP Channels, *Annu. Rev. Biochem.* 76:387–417. 2007
- Wolfgang B Liedtke and Stefan Heller, TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades, Chapter 25. 2007
- David E. Clapham, TRP channels as cellular sensors, *Nature*, 426: 571-524. 2003

# TRP Classification

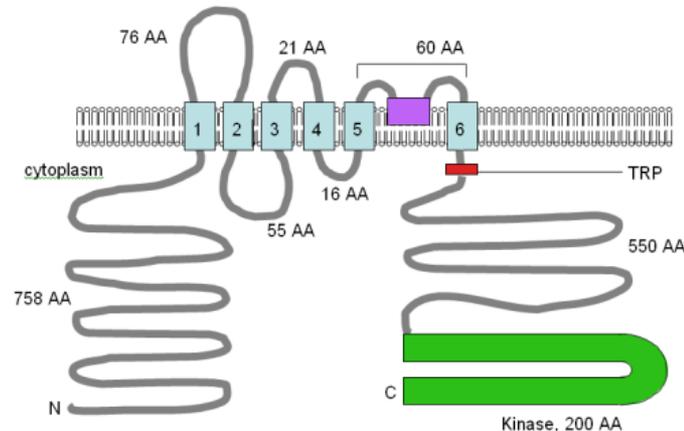
## Classification Based on Sequence Similarity

28 mammalian members (6 subfamilies)

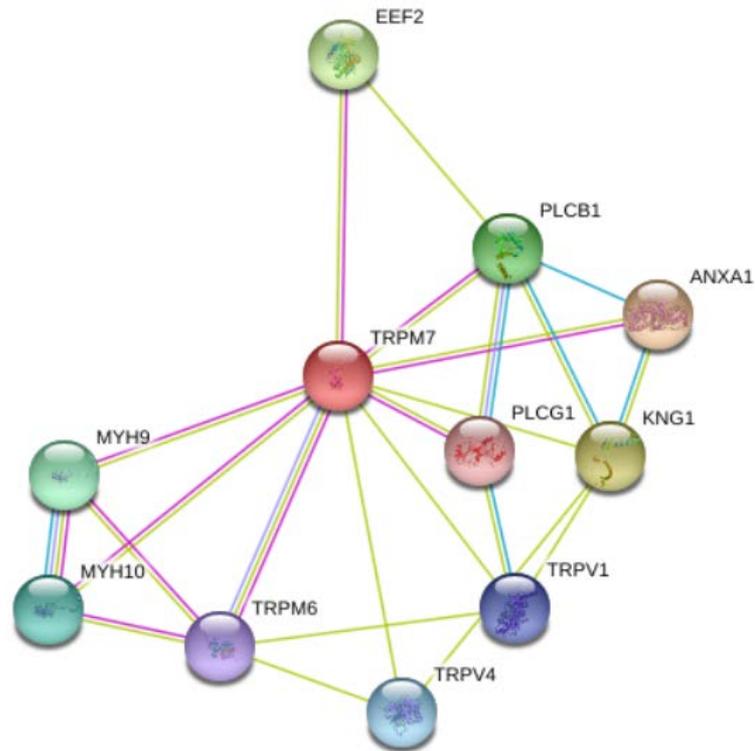


# TRPM7 Introduction

- TRPM7 is expressed in virtual all cell types
- TRPM7 is unspecific divalent anion channel. It can increase intracellular  $\text{Ca}^{2+}$  level, help to keep  $\text{Mg}^{2+}$  Homeostasis
- TRPM7 activity is regulated by pH, ATP, lipids, and translocation
- TRPM7 is relative with AD. More and more evidences show TRPM7 plays role in cancer proliferation, migration and invasion



# TRPM7 Interaction with STRING



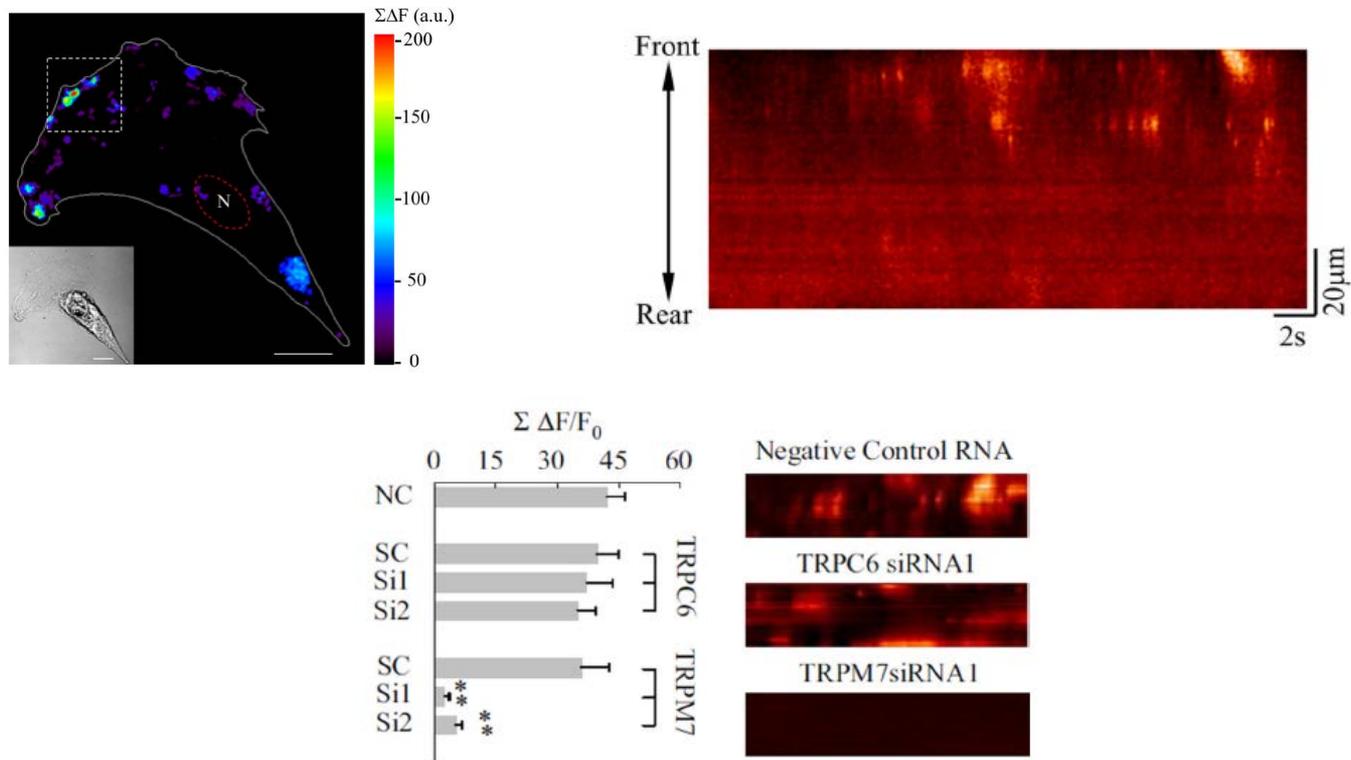
Neighborhood  
Gene Fusion  
Cooccurrence  
Coexpression  
Experiments  
Databases  
Textmining  
[Homology]

**Score**

## Predicted Functional Partners:

ANXA1	annexin A1; Calcium/phospholipid-binding protein which promotes membrane fusion and is involved [...]	(346 aa)					●	●	0.946
KNG1	kininogen 1; (1) Kininogens are inhibitors of thiol proteases; (2) HMW-kininogen plays an impor [...]	(644 aa)					●	●	0.860
EEF2	eukaryotic translation elongation factor 2; This protein promotes the GTP-dependent translocati [...]	(858 aa)					●	●	0.782
PLCB1	phospholipase C, beta 1 (phosphoinositide-specific); The production of the second messenger mol [...]	(1216 aa)					●	●	0.767
MYH9	myosin, heavy chain 9, non-muscle; Cellular myosin that appears to play a role in cytokinesis, [...]	(1960 aa)					●	●	0.753
MYH10	myosin, heavy chain 10, non-muscle; Cellular myosin that appears to play a role in cytokinesis, [...]	(1976 aa)					●	●	0.698
TRPV4	transient receptor potential cation channel, subfamily V, member 4; Non-selective calcium perme [...]	(871 aa)					●	●	0.659
TRPV1	transient receptor potential cation channel, subfamily V, member 1; Receptor-activated non-sele [...]	(839 aa)					●	●	0.659
TRPM6	transient receptor potential cation channel, subfamily M, member 6; Essential ion channel and s [...]	(2022 aa)					●	●	0.640
PLCG1	phospholipase C, gamma 1; PLC-gamma is a major substrate for heparin-binding growth factor 1 (a [...]	(1291 aa)					●	●	0.631

# Our Previous Work on TRPM7 Before

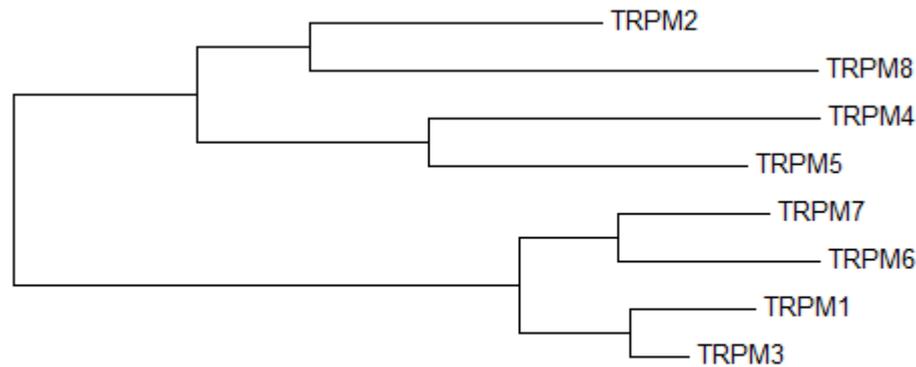


TRPM7 is important in cell migration

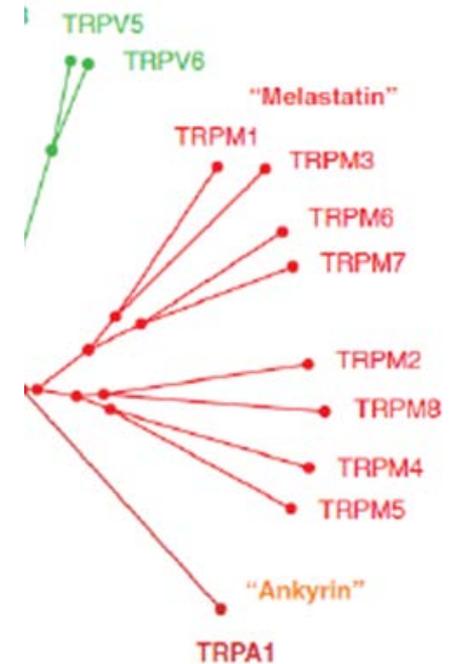
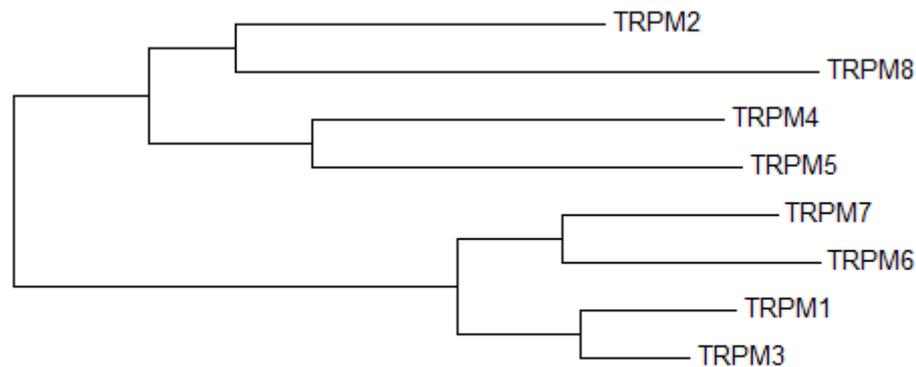
- Wei C, et al, Calcium flickers steer cell migration, Nature, 457:901-905. 2009

# TRPM Subfamily Phylogeny Tree

With maximum likelihood tree



With neighbor-joining tree



# Similarity of TRPM7 and TRPM6

With Needleman-Wunsch method

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
6261	17663.0	3637/6261 (58.1%)	3637/6261 (58.1%)	855/6261 (13.7%)

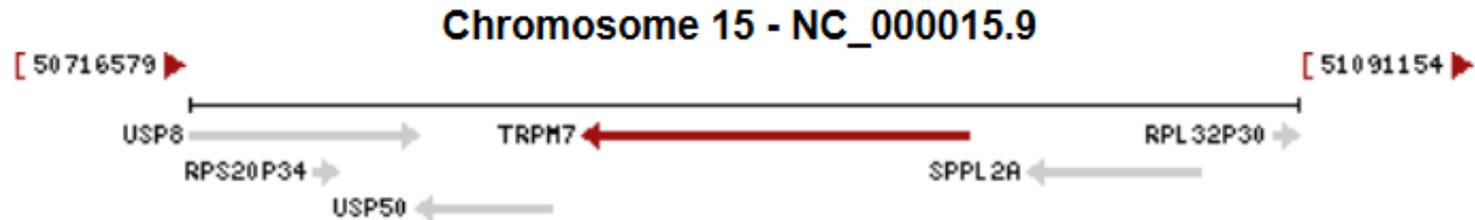
- TRPM7 nucleotide length 5598
- TRPM6 nucleotide length 6069

LENGTH	SCORE	IDENTITY	SIMILARITY	GAPS
2096	5079.0	1063/2096 (50.7%)	1331/2096 (63.5%)	305/2096 (14.6%)

- TRPM7 amino acid length 1865
  - TRPM6 amino acid length 2022
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# TRPM7 Gene Sequence

- NCBI Reference Sequence: NM\_017672.4



RefSeq status	<i>REVIEWED</i>
location	<i>Chromosome: 15, 15q21</i>
Remark	<i>TRPM7 contribute to the anti-proliferative characteristics in cancer</i>
gene	<i>1..10404bp; GeneID:54822;</i>
Sequence features	<i>Exon 38; polyA_signal 5; polyA_site 4; misc_feature 9</i>
CDS	<i>283..5880</i>

# TRPM7 Codon Usage Composition

Codon	TRPM7	TRPM6	TRPC1	Codon	TRPM7	TRPM6	TRPC1	Codon	TRPM7	TRPM6	TRPC1	Codon	TRPM7	TRPM6	TRPC1
UUU(F)	1.6	0.97	1.42	UCU(S)	1.62	1.32	1.57	UAU(Y)	1.38	0.82	1.47	UGU(C)	1.3	1	1.05
UUC(F)	0.4	1.03	0.58	UCC(S)	1.17	1.15	1.11	UAC(Y)	0.62	1.18	0.53	UGC(C)	0.7	1	0.95
UUA(L)	1.2	0.67	0.91	UCA(S)	1.35	1.29	1.11	UAA(*)	3	3	3	UGA(*)	0	0	0
UUG(L)	0.91	1.08	1.2	UCG(S)	0.05	0.17	0.37	UAG(*)	0	0	0	UGG(W )	1	1	1
CUU(L)	2.04	0.94	1.66	CCU(P)	1.42	1.42	1.23	CAU(H)	1.63	1.13	1.75	CGU(R)	0.69	0.13	1.08
CUC(L)	0.39	1.02	0.46	CCC(P)	0.49	1.07	0.77	CAC(H)	0.37	0.87	0.25	CGC(R)	0.14	0.69	0.31
CUA(L)	0.45	0.35	0.69	CCA(P)	2	1.29	1.69	CAA(Q)	0.99	0.89	0.69	CGA(R)	0.83	0.76	1.08
CUG(L)	1.01	1.93	1.09	CCG(P)	0.09	0.22	0.31	CAG(Q)	1.01	1.11	1.31	CGG(R)	0.14	0.57	1.23
AUU(I)	1.6	1.15	1.53	ACU(T)	1.67	1.21	1.08	AAU(N)	1.41	1	1.08	AGU(S)	0.9	0.95	1.11
AUC(I)	0.55	1.13	0.67	ACC(T)	0.71	1.1	0.76	AAC(N)	0.59	1	0.92	AGC(S)	0.9	1.12	0.74
AUA(I)	0.85	0.72	0.8	ACA(T)	1.33	1.39	1.73	AAA(K)	1.35	1.09	1.13	AGA(R)	3.1	1.96	1.69
AUG(M)	1	1	1	ACG(T)	0.29	0.29	0.43	AAG(K)	0.65	0.91	0.87	AGG(R)	1.1	1.89	0.62
GUU(V)	1.56	0.88	1.19	GCU(A)	1.39	1.44	1.33	GAU(D)	1.65	1.16	1.3	GGU(G)	1.04	0.76	0.8
GUC(V)	0.49	0.91	0.68	GCC(A)	0.71	1.08	0.62	GAC(D)	0.35	0.84	0.7	GGC(G)	0.46	0.88	1.07
GUA(V)	1.07	0.35	0.51	GCA(A)	1.78	1.16	1.69	GAA(E)	1.58	0.93	1.35	GGA(G)	1.92	1.64	1.33
GUG(V)	0.88	1.86	1.62	GCG(A)	0.12	0.32	0.36	GAG(E)	0.42	1.07	0.65	GGG(G)	0.58	0.72	0.8

Species 1	Species 2	Dist. (P)	Std. Err
TRPM7_mRNA	TRPM6_transcript_variant_a_mRNA	0.000	-10.784
TRPM7_mRNA	TRPC1_transcript_variant_1_mRNA	0.010	-2.614
TRPM6_transcript_variant_a_mRNA	TRPC1_transcript_variant_1_mRNA	0.023	-2.309

# TRPM7 Amino Acid Sequence

- In UniProt, with human TRPM 40 sequences are reviewed
- Entry No. of human TRPM7 is Q96QT4

Sequence length	1865AA
Function	<i>Essential ion channel and serine/threonine-protein kinase</i>
Active site	1767
Natural variations	15
Catalytic activity	<i>ATP + a protein = ADP + a phosphoprotein</i>
Cofactor	<i>Binds 1 zinc ion per subunit</i>
Subcellular location	<i>Membrane; Multi-pass membrane protein</i>
Subunit structure	<i>Homodimer. Forms heterodimers with TRPM6</i>
Biological process	<i>Calcium transport, Ion transport, Transport</i>
Domain	<i>Coiled coil, Transmembrane, Transmembrane helix</i>
PTM	<i>Acetylation, Phosphoprotein</i>
3D structure	<i>Positions 1198-1249, 1551-1830</i>

# TRPM7 Amino Acid Composition

Amino Acid	Percentage (%)
Trp	1.50
Cys	1.98
His	2.90
Met	2.90
Tyr	3.27
Asp	4.56
Phe	4.56
Gln	4.56
Arg	4.66
Pro	4.83
Asn	4.93
Gly	5.15
Thr	5.15
Ala	5.42
Ile	6.43
Val	6.60
Glu	6.65
Lys	6.92
Ser	7.13
Leu	9.92

- Hydrophilic amino acid: 25.68%
- Hydrophobic amino acid: 42.14%

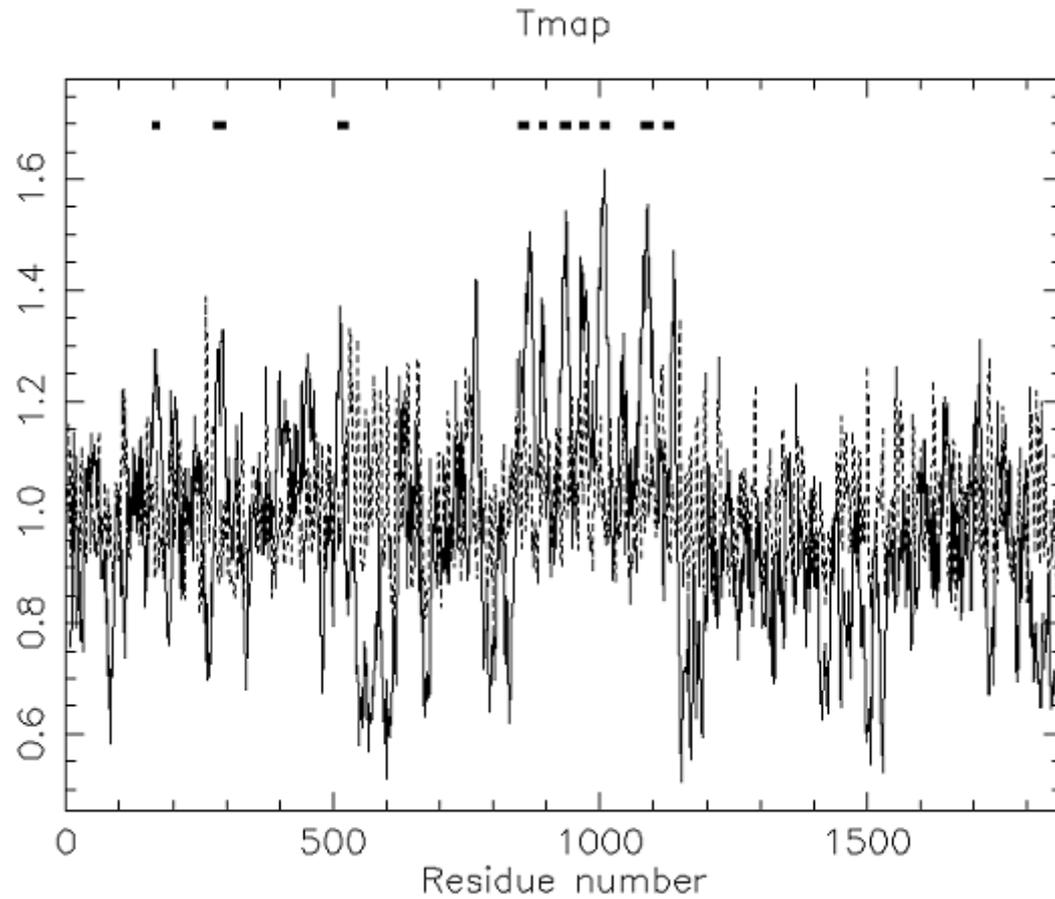
# TRPM7 Regions\_Amino Acid Sequence

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- 6 transmembrane regions  
756-776; 856-876; 919-939; 963-983; 996-1016; 1075-1095
  - Alpha-type protein kinase domain  
1594-1824
  - Nucleotide binding region  
1794-1800
  - Coiled coil region  
1198-1250
-

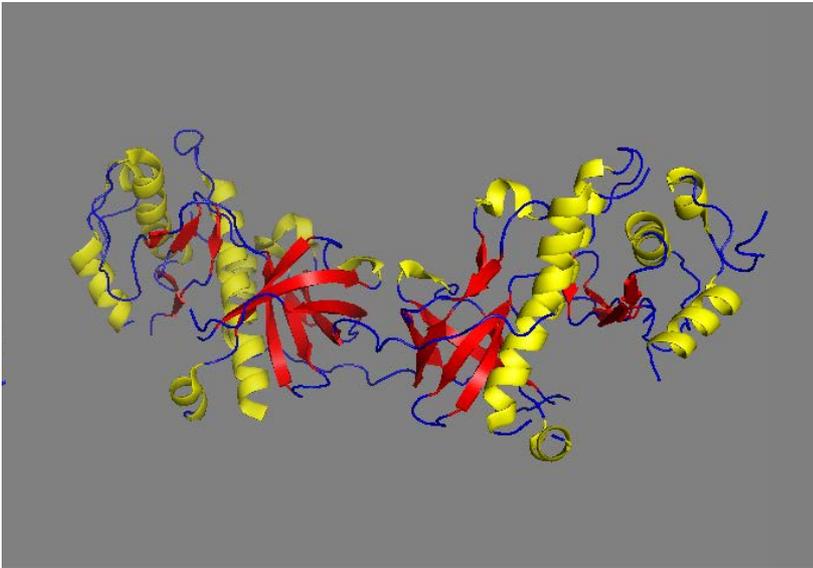
# Transmembrane Analysis

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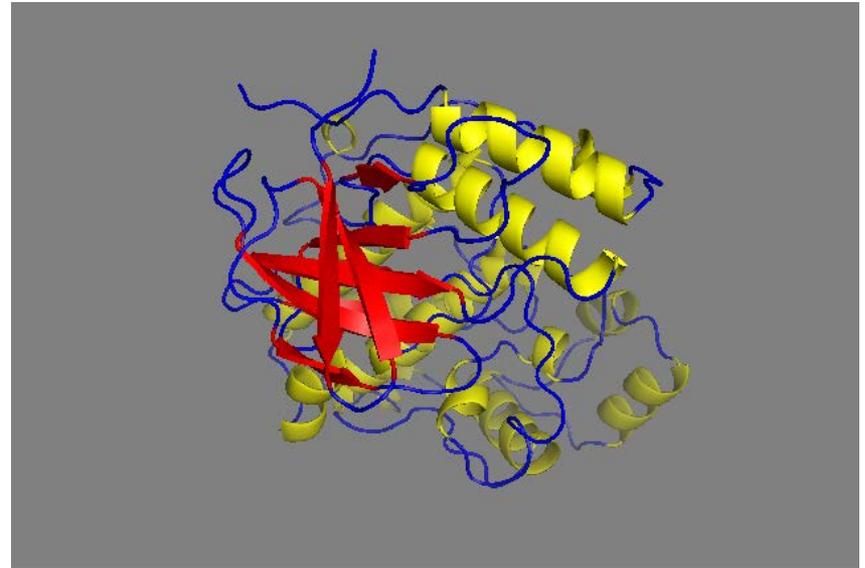


# TRPM7 Kinase Domain Crystal Structure

- It belongs to the atypical  $\alpha$ -kinase family. However, its central catalytic core structure resembles the classical protein kinases, despite a lack of sequence similarity between them.
- TRPM7 kinase domain forms intersubunit dimers in a domain swapping arrangement.

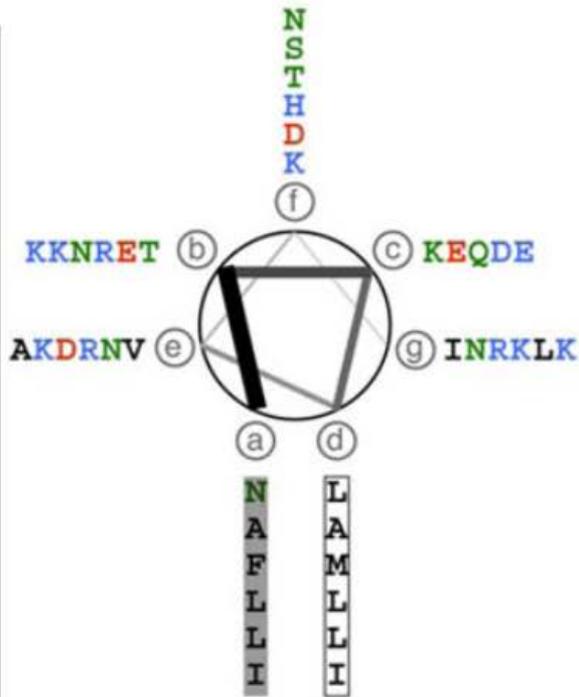


Structure of TRPM7 kinase



Structure of MAPK p38

# Coiled-coil Region



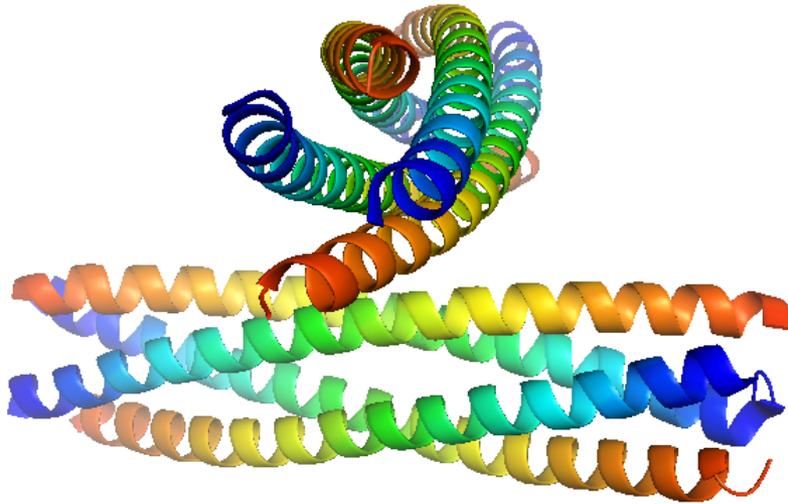
	7	6	5	4	3	2	1	1	2	3	4	5	6	7																																							
	bc	de	fg	ab	cd	ef	gh	ia	jb	kc	ld	me	nf	og																																							
TRPM7	R	V	T	F	E	R	V	E	Q	M	S	I	Q	I	K	E	V	G	D	R	V	N	I	K	R	S	L	Q	S	L	D	S	Q	I	G	H	L	Q	D	L	S	A	L	T	V	D	T	L	K	T	L	T	
TRPM6	R	A	T	S	E	R	V	S	E	M	F	F	Q	L	K	E	M	N	E	K	V	S	F	I	K	D	S	L	L	S	L	D	S	Q	V	G	H	L	Q	D	L	S	A	L	T	V	D	T	L	K	V	L	S
TRPM1	R	V	T	S	E	R	V	E	N	M	S	M	R	L	E	E	I	N	E	R	E	T	F	M	K	T	S	L	Q	T	V	D	L	R	L	A	Q	L	E	E	L	S	N	R	M	V	N	A	L	E	N	L	A
TRPM3	R	V	T	S	E	R	V	E	N	M	S	M	R	L	E	E	V	N	E	R	E	H	S	M	K	A	S	L	Q	T	V	D	I	R	L	A	Q	L	E	D	L	I	G	R	M	A	T	A	L	E	R	L	T
TRPM8	W	E	G	V	M	K	E	N	Y	L	V	K	I	N	T	K	A	N	D	N	A	E	E	M	R	H	R	F	R	Q	L	D	T	K	L	N	D	L	K	G	L	L	K	E	I	A	N	K	I	K	*		
TRPM2	G	K	H	G	R	V	D	A	M	V	D	L	L	D	L	D	P	L	K	R	S	G	S	M	E	Q	R	L	A	S	L	E	E	Q	V	A	Q	T	A	R	A	L	H	W	I	V	R	T	L	R	A	S	G
TRPM4	S	E	R	L	K	R	T	S	Q	K	V	D	T	A	L	K	Q	L	G	Q	I	R	E	Y	D	R	R	L	R	G	L	E	R	E	V	Q	H	C	S	R	V	L	T	W	M	A	E	A	L	S	H	S	A
TRPM5	E	E	V	L	R	K	T	A	H	R	V	D	L	I	A	K	Y	I	G	G	L	R	E	Q	E	K	R	I	K	C	L	E	S	Q	A	N	Y	C	M	L	L	L	S	S	M	T	D	T	L	A	P	G	G

- Based on coiled-coil prediction algorithm
- “a” and “d” core positions correspond predominantly to hydrophobic residues.

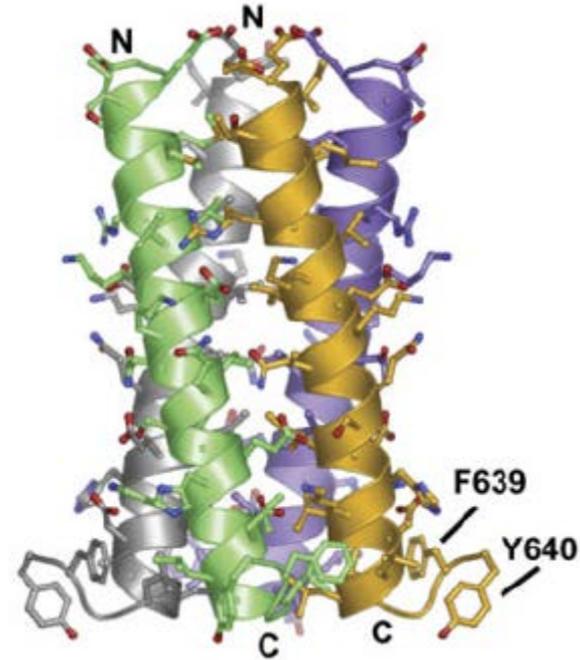
Pamela R. Tsuruda, et al, Coiled Coils Direct Assembly of a Cold-Activated TRP Channel, *Neuron*. 2006, 51(2): 201–212

# TRPM7 Coiled-coil Region Crystal Structure

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TRPM7 anti-parallel structure

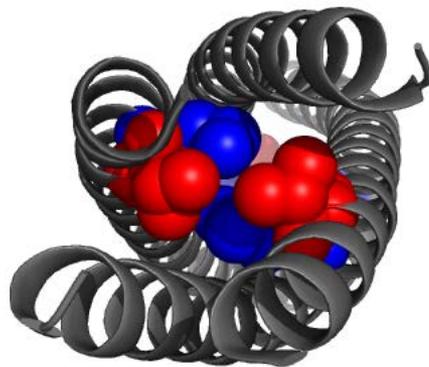
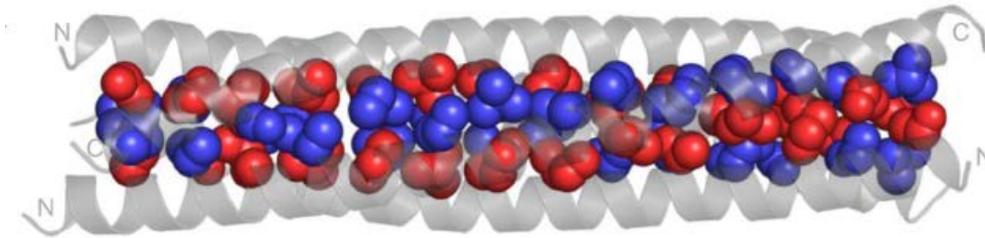


Kc7.4 parallel structure

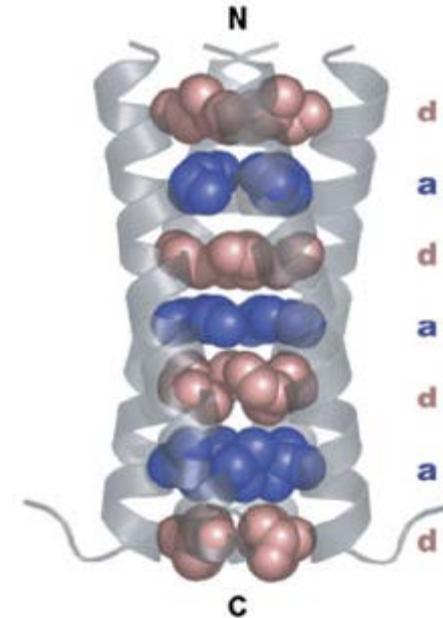
Rebecca J. Howard, et al. Structural Insight into KCNQ (Kv7) Channel Assembly and Channelopathy. *Neuron*. 53(5): 663–675. 2007

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# Position of Hydrophobic Amino Acids



TRPM7 anti-parallel structure



Kc7.4 parallel structure

Rebecca J. Howard, et al. Structural Insight into KCNQ (Kv7) Channel Assembly and Channelopathy. *Neuron*. 53(5): 663–675. 2007

Distance of a,d: 6.3Å

# Our Thoughts

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- Bioinformatics can tell us lots of valuable information, which can help to find structure and function of target protein, to direct our future work
  - However, all these must be based on full understanding of the protein and bioinformatics tools, and the analysis should be comprehensive
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# Summary

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- Being a member of TRP channel, TRPM7 has the typical TRP box and shows characteristic six transmembranes
  - TRPM7 owns kinase activity and antiparalleled coiled coil domain, which might contribute to their effect on cancer progress.
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*ABC Lesson*

