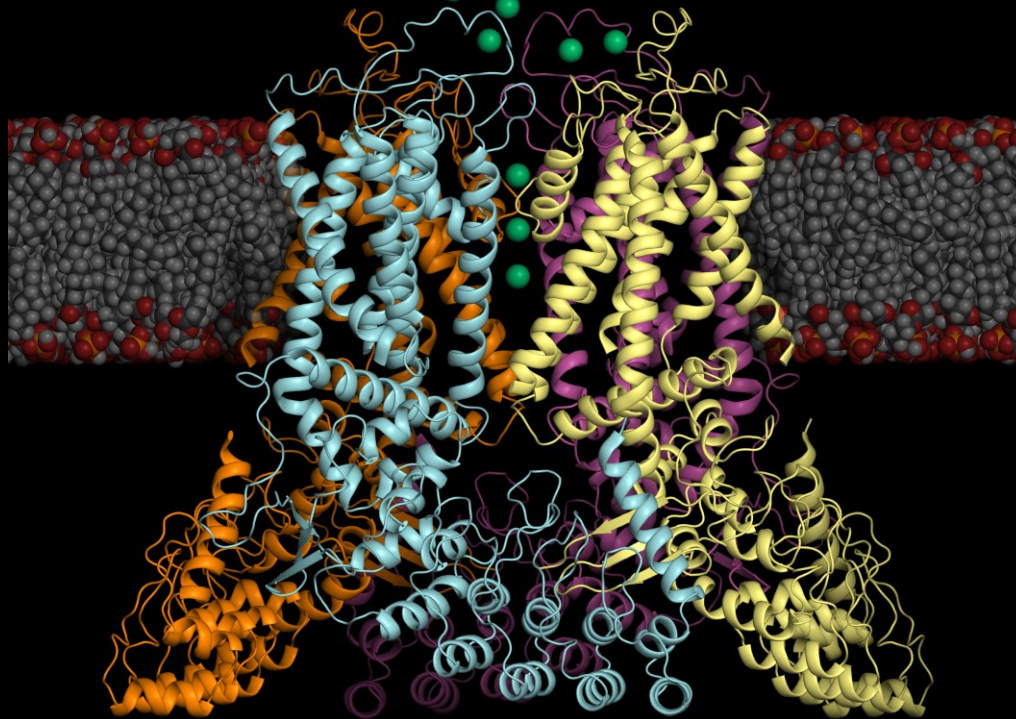


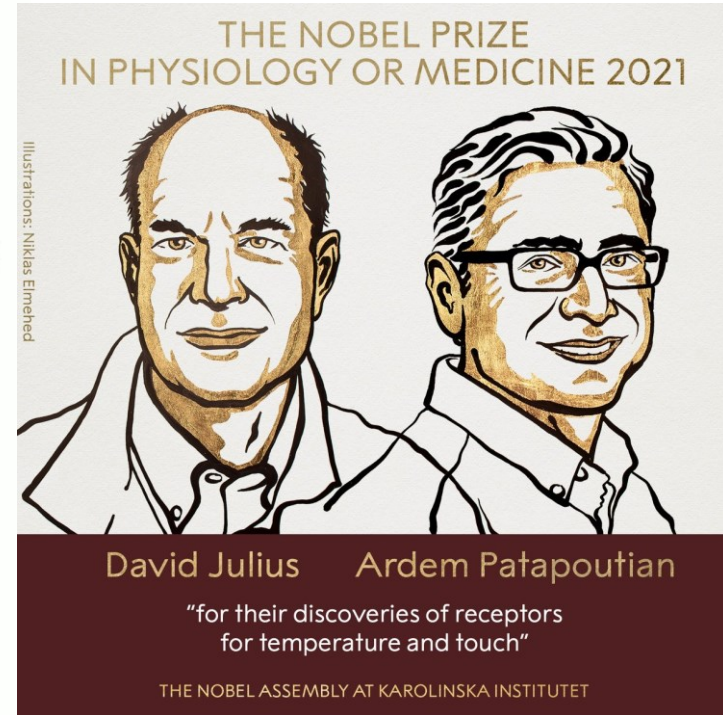
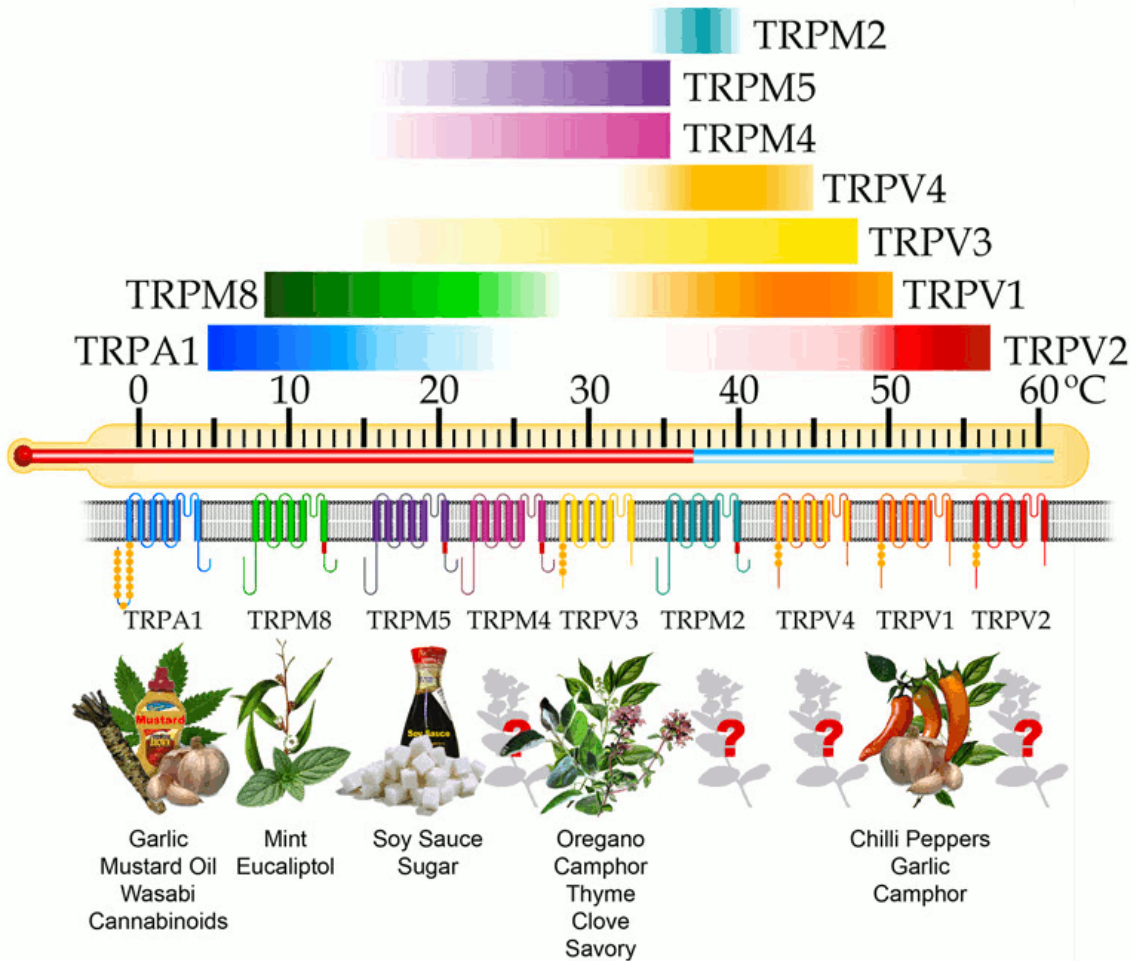
# Gating and molecular pharmacology of TRP Channels



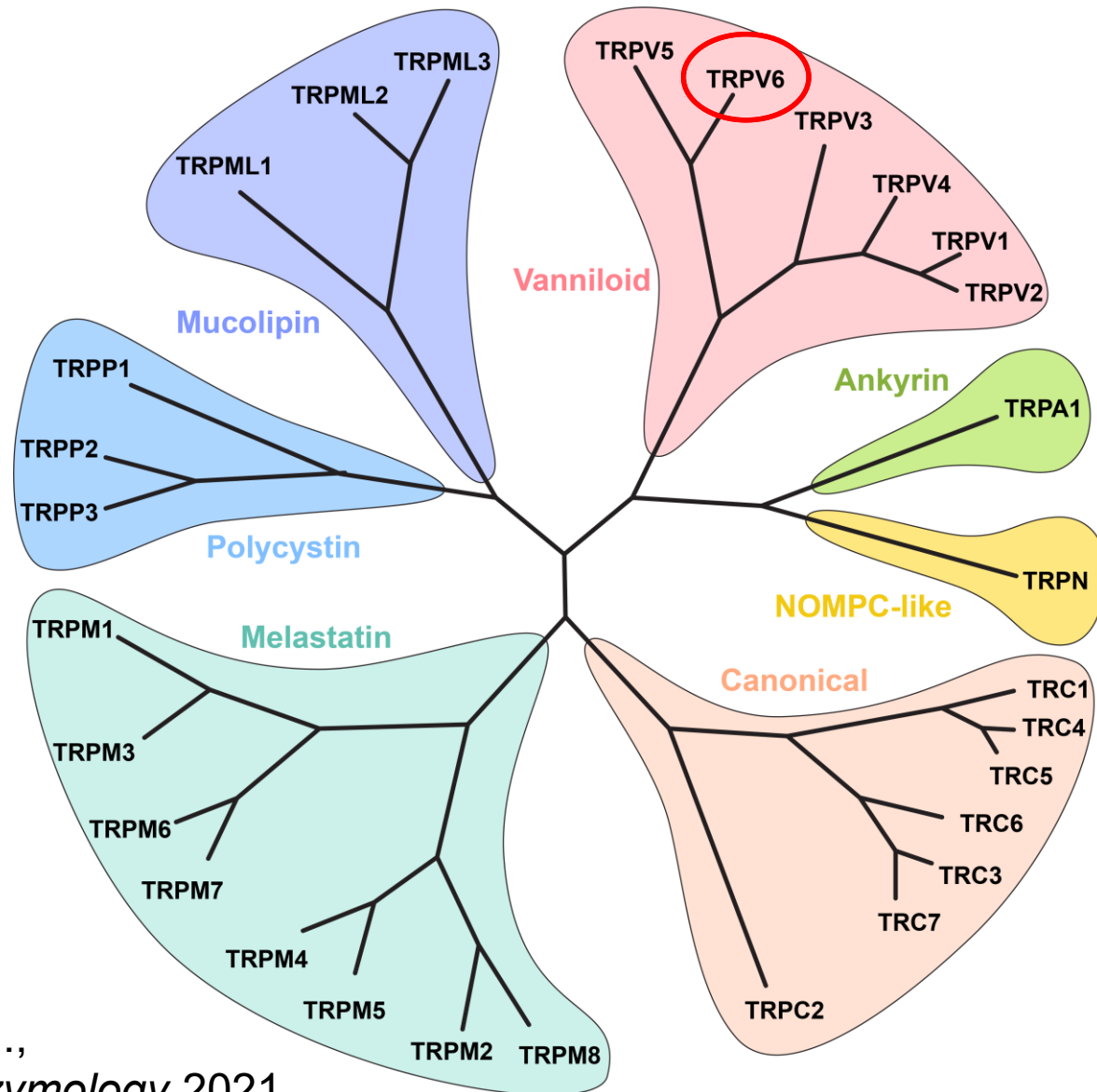
Alexander Sobolevsky

*Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center*

# TRP channels

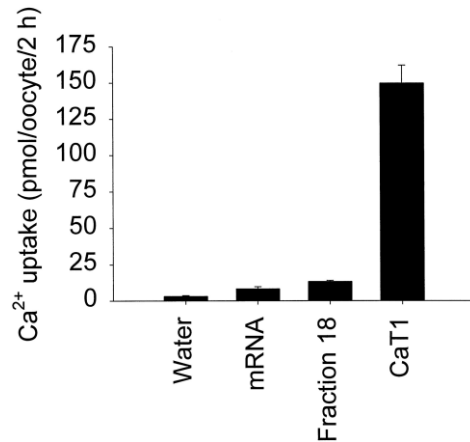


# TRP channels



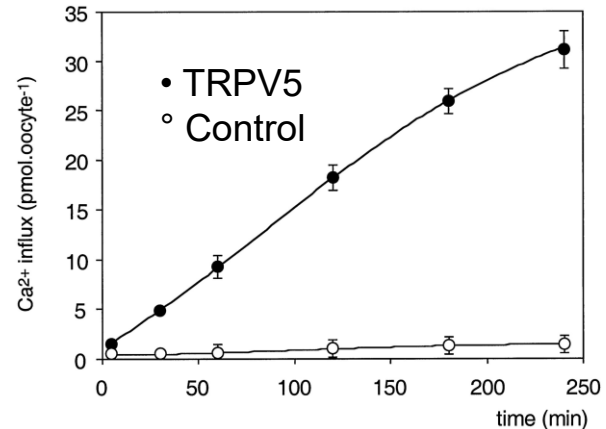
# TRPV5 (ECaC) and TRPV6 (CaT1) are $\text{Ca}^{2+}$ entry channels in epithelial cells

TRPV6 cloned from rat duodenal cDNA



Peng et al *J. Biol. Chem.* 1999

TRPV5 cloned from rabbit kidney cDNA



Hoenderop et al *J. Biol. Chem.* 1999

## TRPV5/6 KO mice:

- Defective intestinal  $\text{Ca}^{2+}$  absorption
- Increased urinary  $\text{Ca}^{2+}$  excretion
- Decreased femoral bone mineral density
- Lower body weight
- Impaired fertility
- Alopecia and dermatitis

## Expression changes (mouse models):

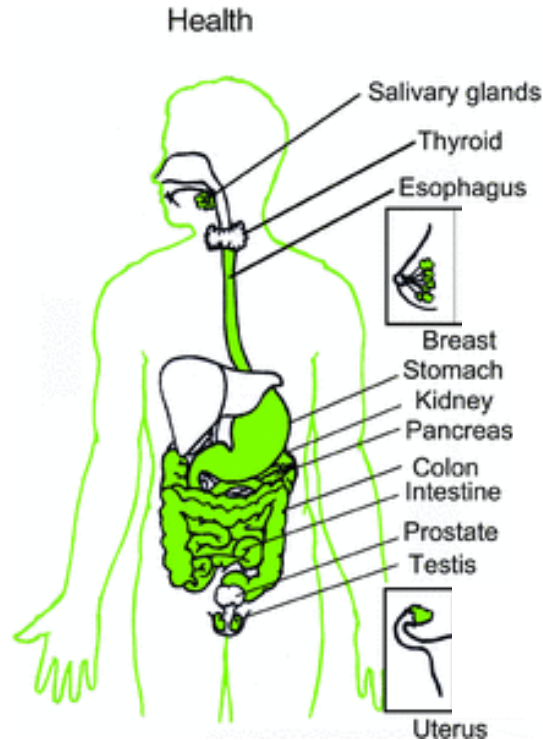
- Pendred syndrome
- Crohn's-like disease
- Lowe syndrome
- Dent disease
- Gitelman syndrome
- Kidney stone disease



# TRPV6 in Health

## Expressed in:

- Duodenum
- Pancreas
- Prostate
- Placenta
- Salivary gland
- Liver
- Stomach
- Kidney
- Testis
- Bone
- Parathyroid glands
- Blood cells



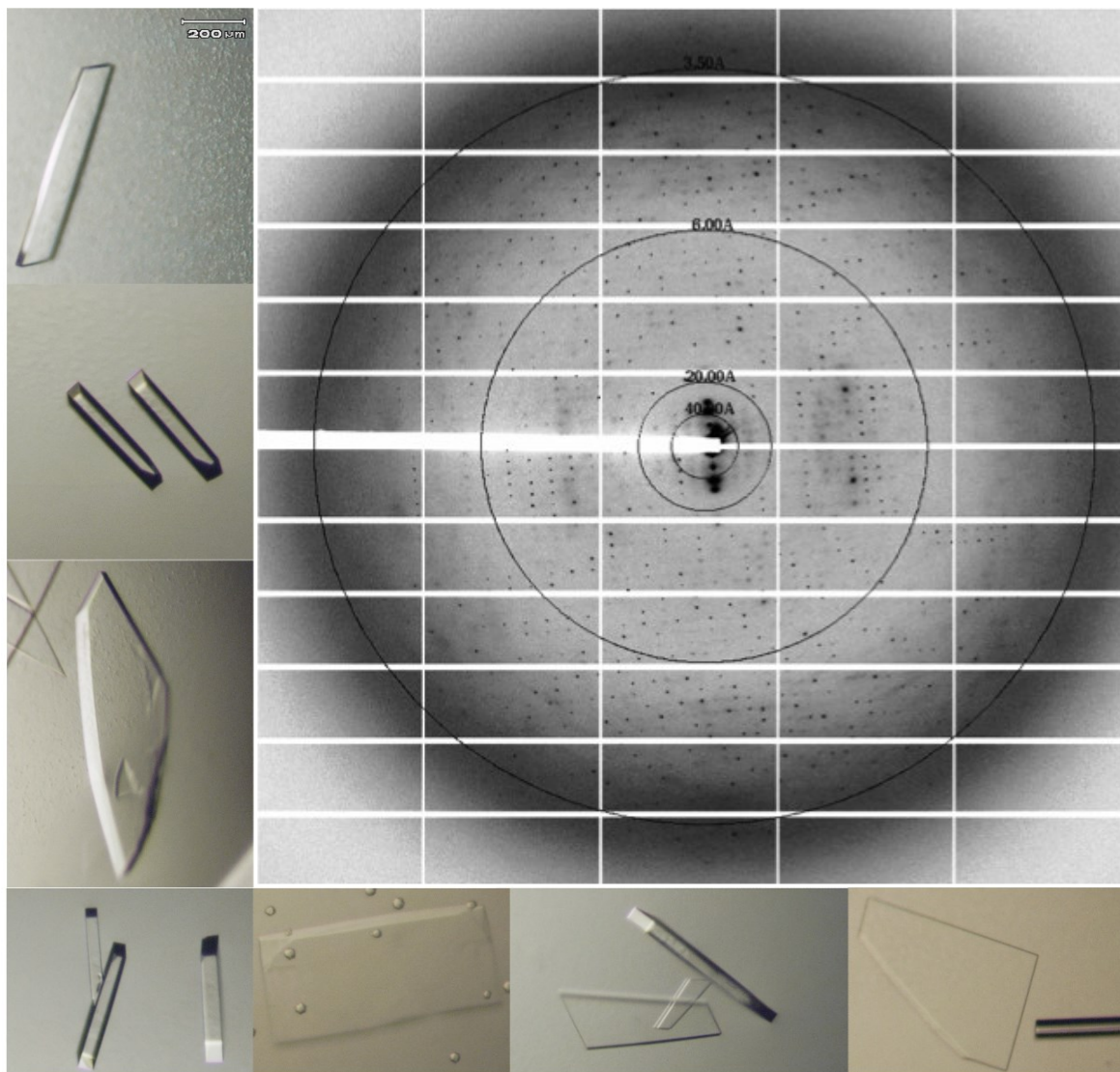
Lehen'kyi et al, J. Physiol. 2012

## Overexpressed in human cancers:

- Breast
- Prostate
- Colon
- Ovarian
- Thyroid
- Endometrial
- Leukemia

**Overexpression pattern correlates with the aggressiveness of the disease !**

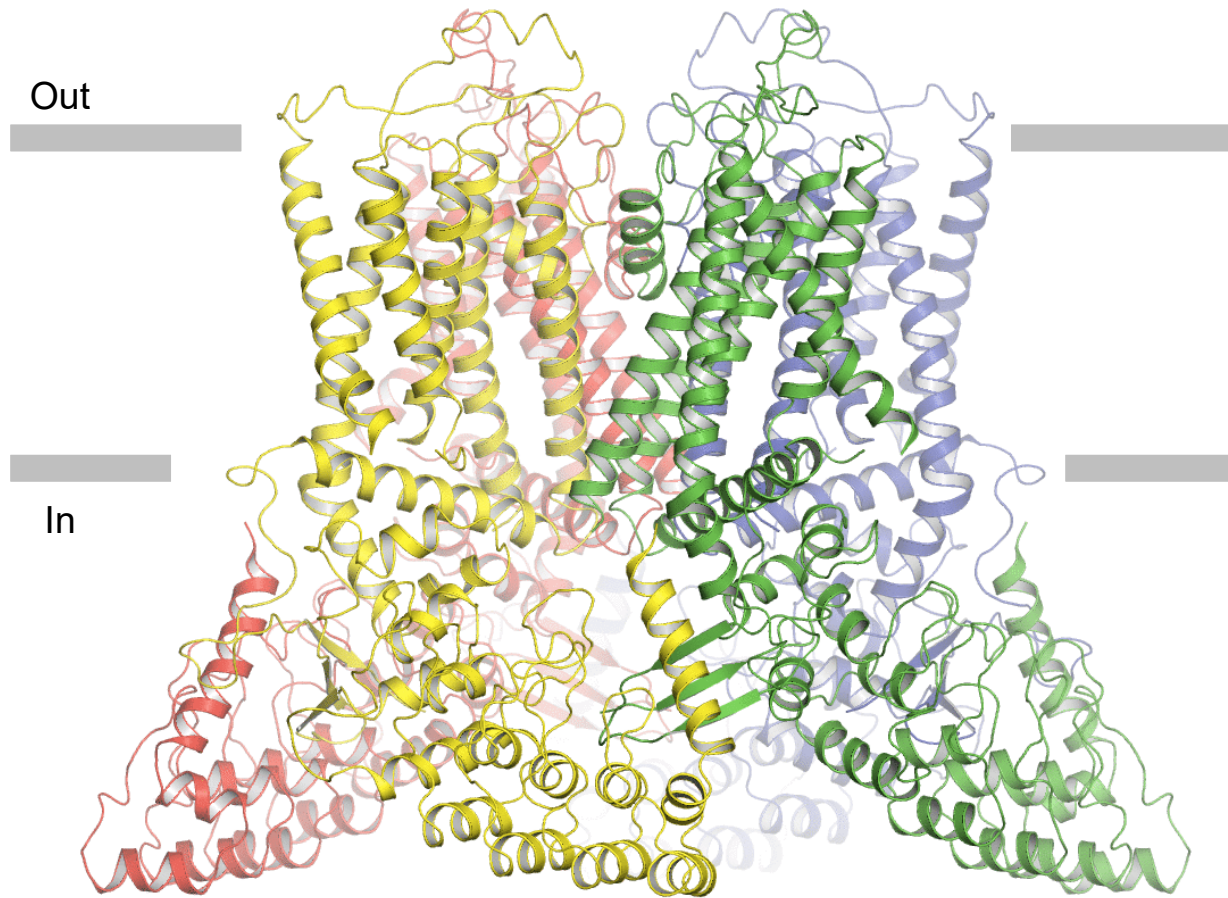
# Crystallization and structure solution



Data Collection	
Beamline	APS-24ID-C
Space group	P4 <sub>2</sub> ,2
Cell dimensions: a, b, c, (Å)	143.81, 143.81, 113.22
α, β, γ (°)	90 90 90
Wavelength (Å)	0.9791
Resolution (Å)*	44.48 - 3.25 (3.36 - 3.25)
Completeness (%)*	96.0 (94.7)
Redundancy*	8.7 (9.2)
$ I/\sigma $ *	16.9 (1.3)
$R_{meas}$ (%)*	9.8 (132.6)
$CC_{1/2}$	99.8 (85.7)
Structure solution	
Method	Molecular Replacement
Search probe	TRPV6 ARD (2RFA)
Refinement	
Resolution (Å)*	44.48 - 3.25 (3.36 - 3.25)
Completeness (%)	96 (93.8)
Number of reflections	18531 (1724)
$R_{work}/R_{free}$	0.273/0.287
Number of atoms: Total	4747
Ligand	16
B-factor (Å <sup>2</sup> )	
Protein	120.5
Ligand	77.27
RMS deviations	
Bond length (Å)	0.003
Bond angles (°)	0.7
Ramachandran	
Favored (%)	94.6
Allowed (%)	5.4
Disallowed (%)	0.0

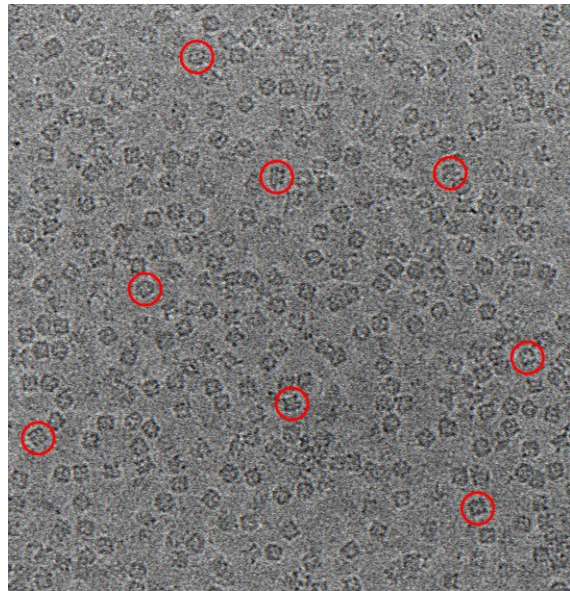
\*Highest resolution shell in parentheses  
5% reflections were used for calculation of  $R_{free}$

# Rat TRPV6 structure

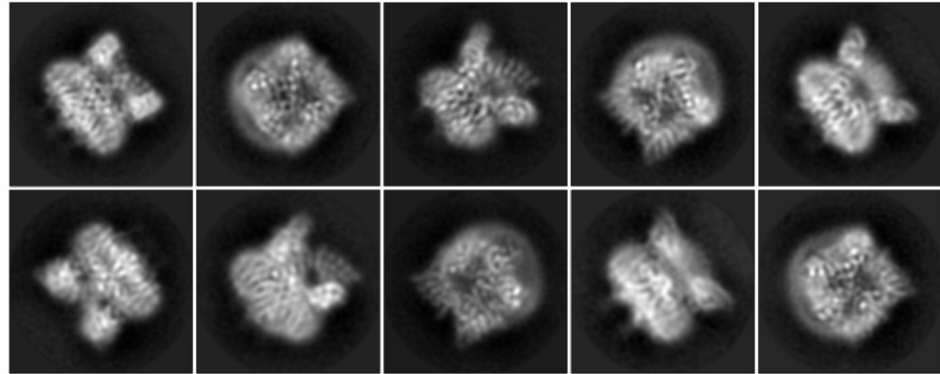




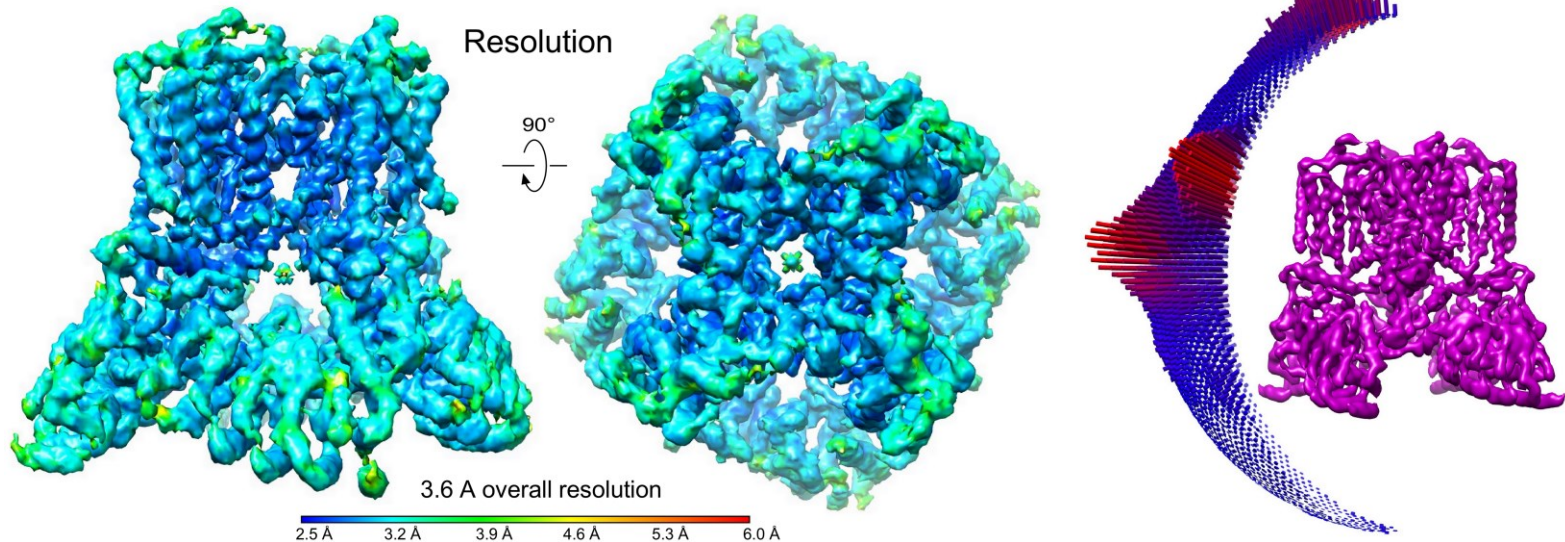
# Cryo-EM structure of human TRPV6



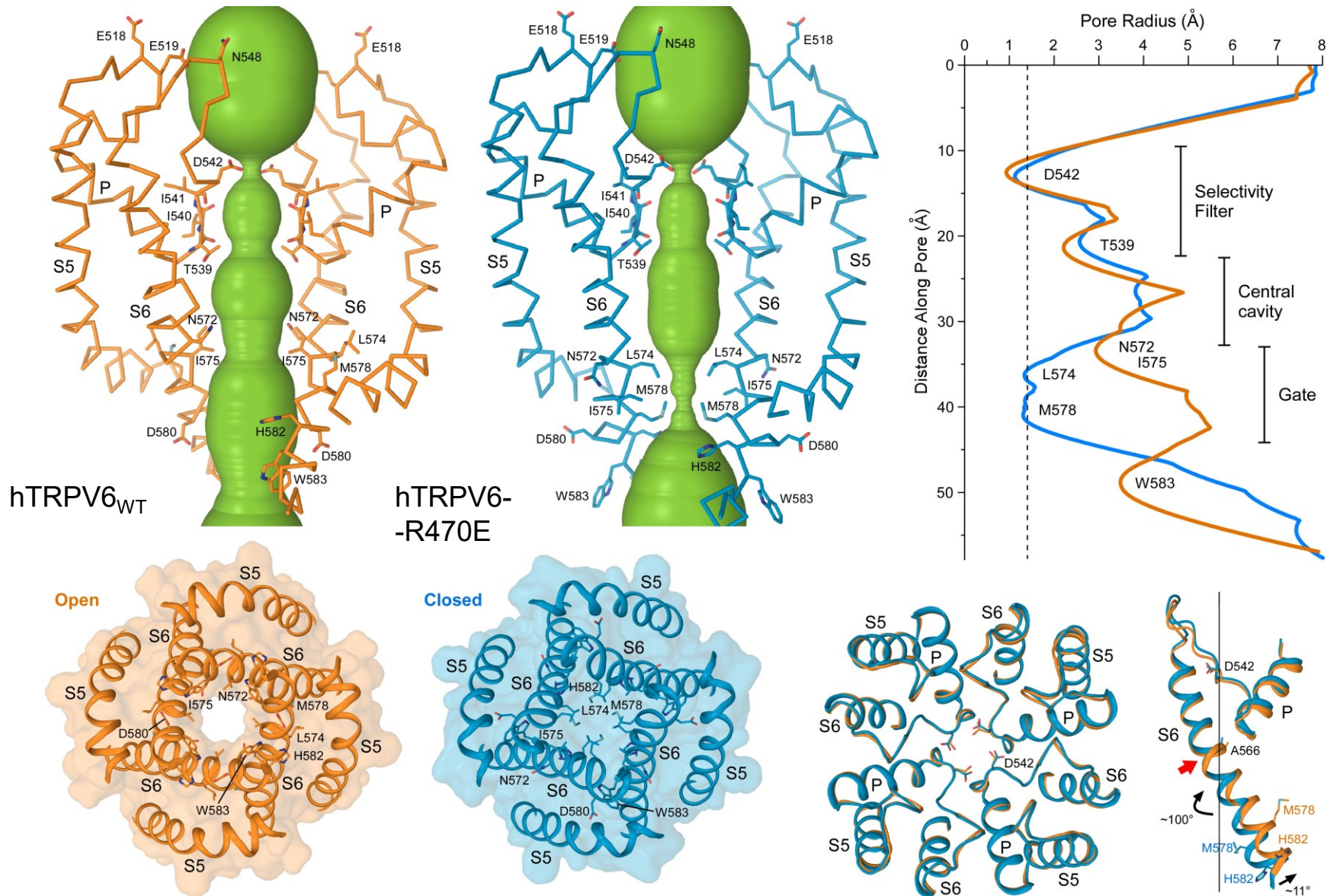
2D class averages



Particle orientations

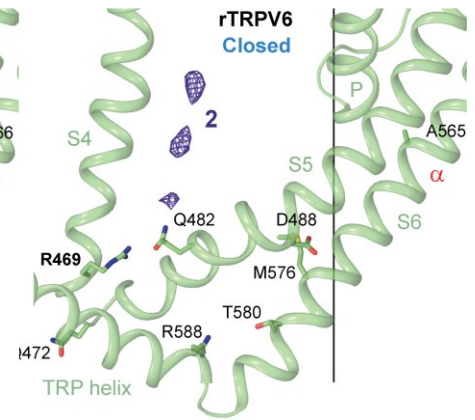
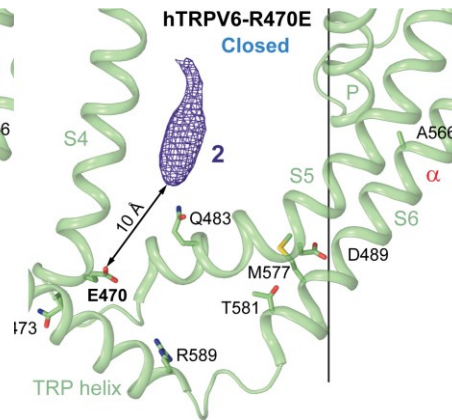
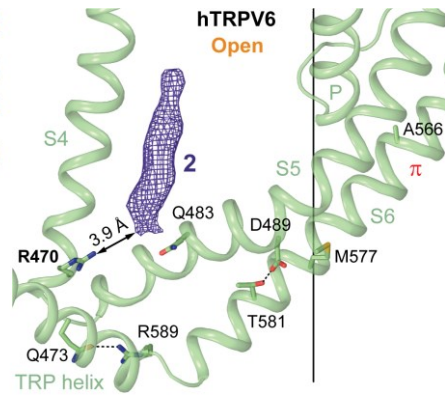
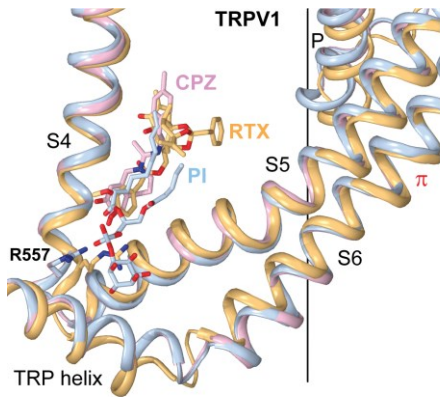
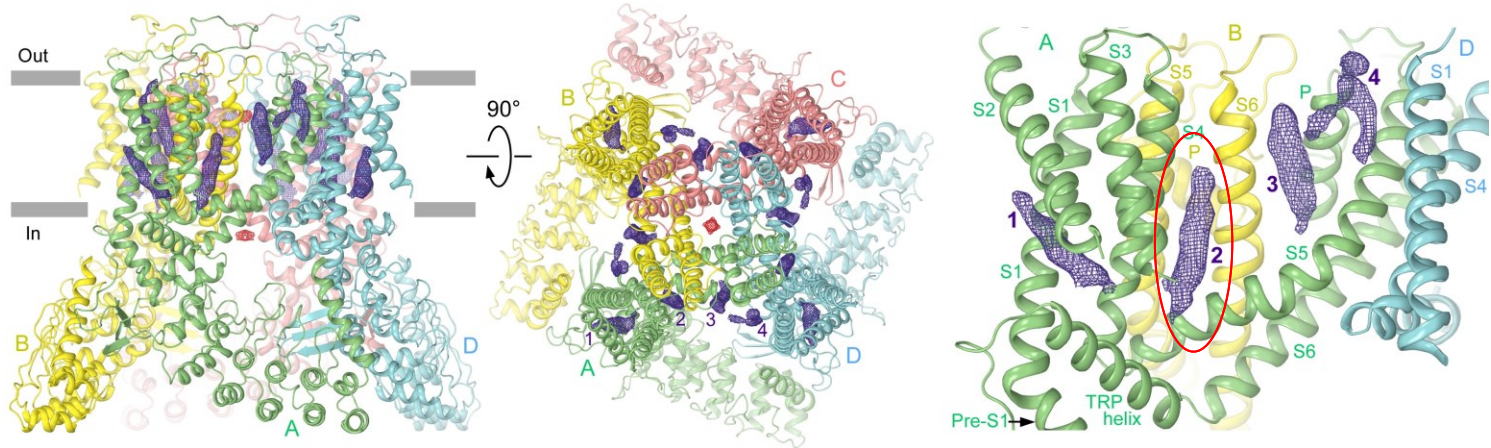


# Open ion channel pore

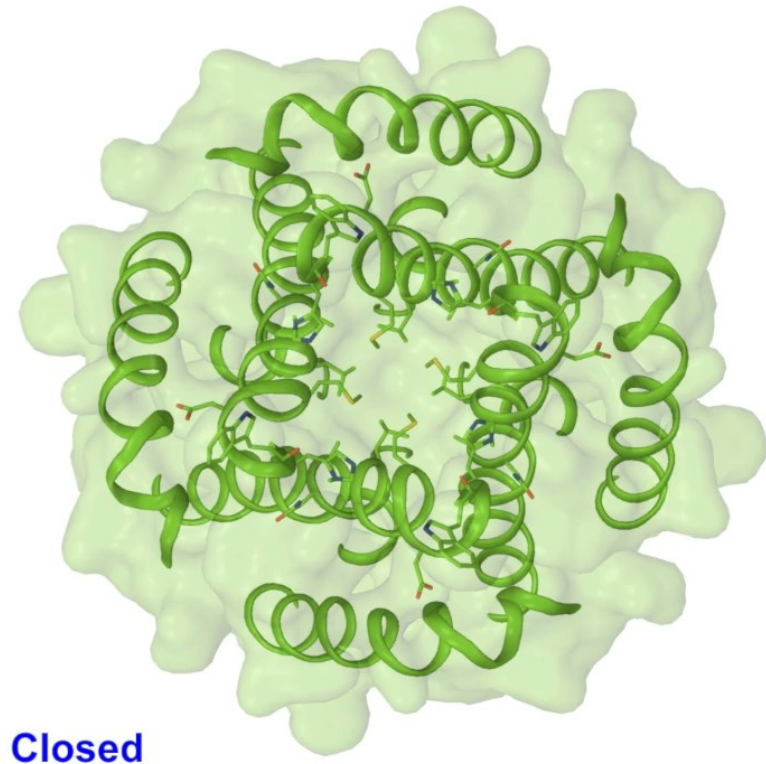
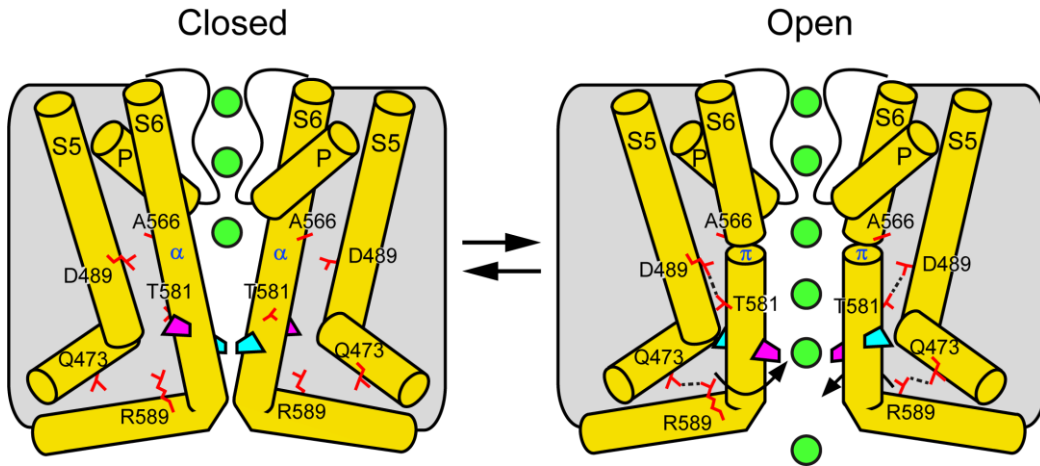




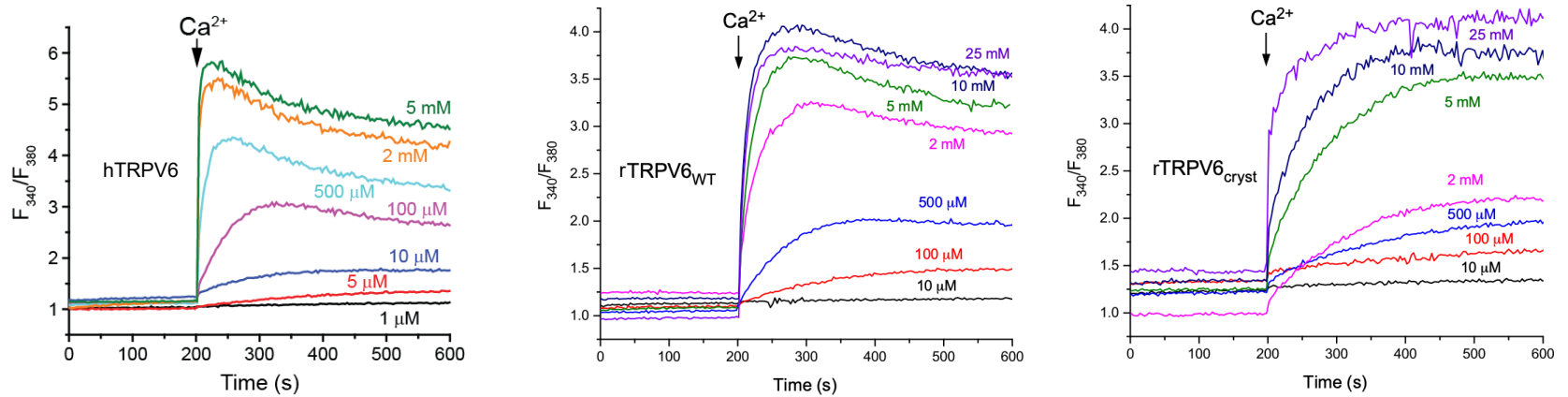
# Lipids in human TRPV6 structure



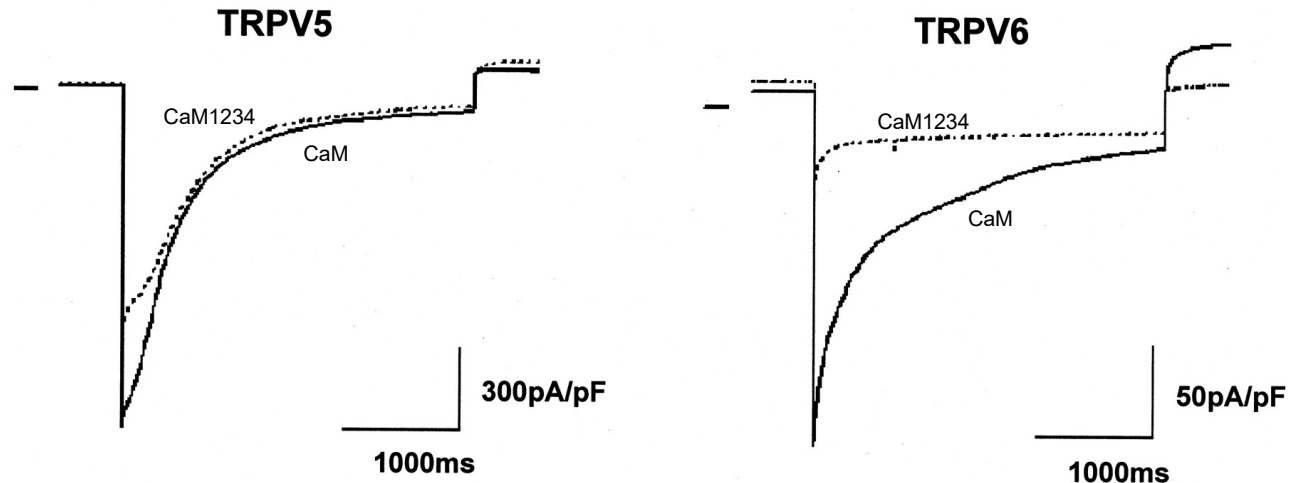
# TRPV6 gating mechanism



# Ca<sup>2+</sup>-induced inactivation

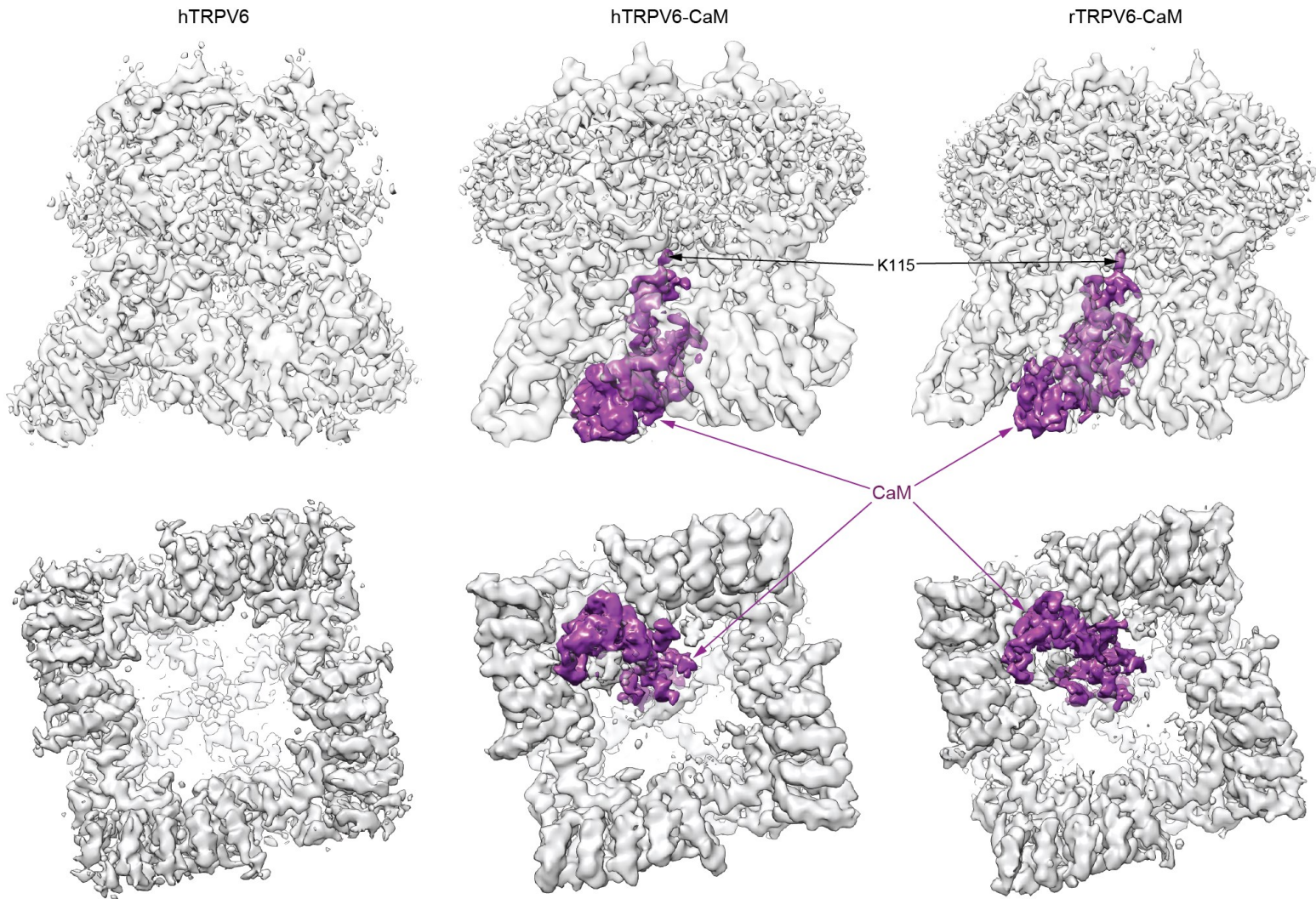


Effect of Ca<sup>2+</sup>-insensitive CaM (CaM1234) on TRPV5 and TRPV6 Ca<sup>2+</sup> current kinetics

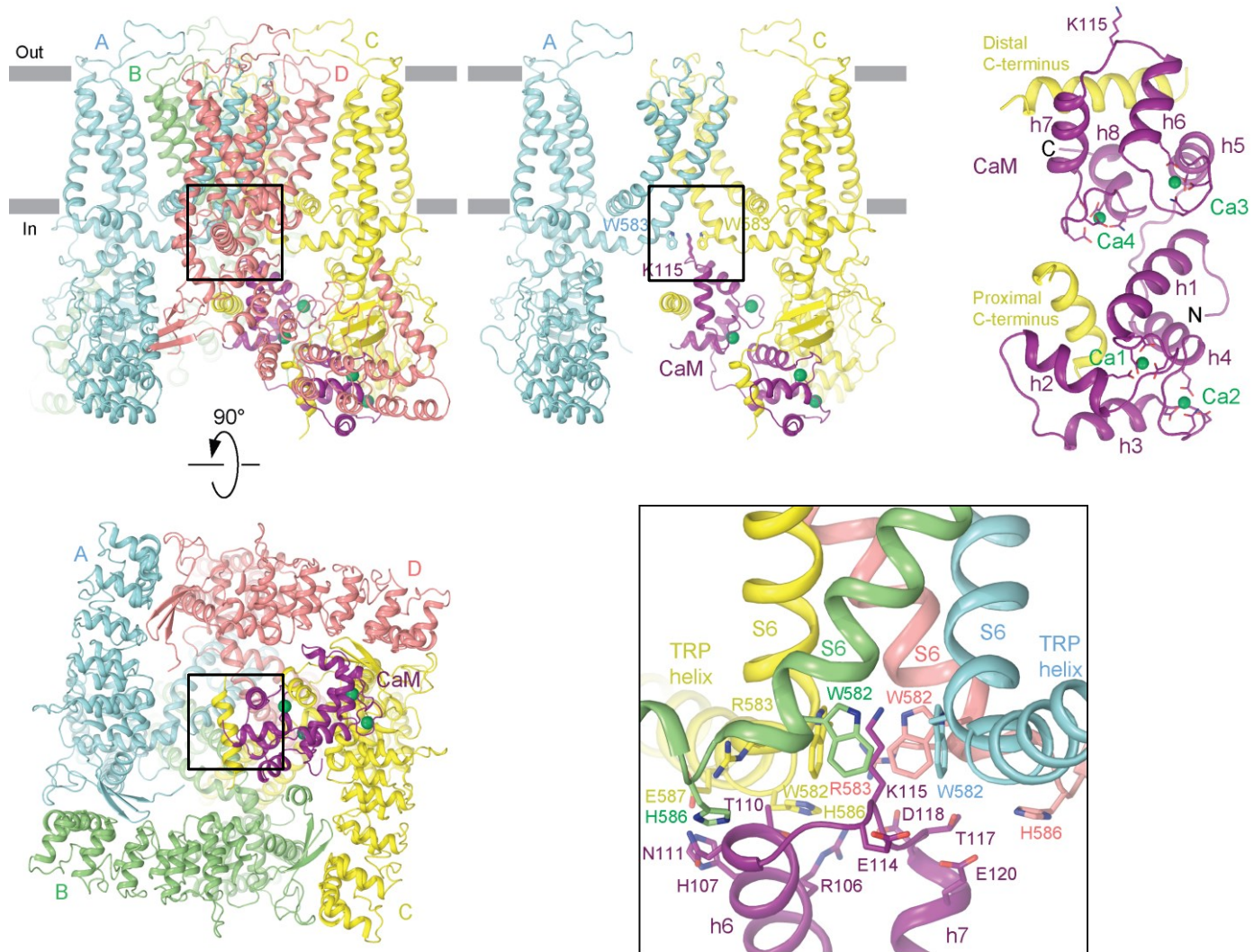




# Cryo-EM structures of CaM-bound TRPV6

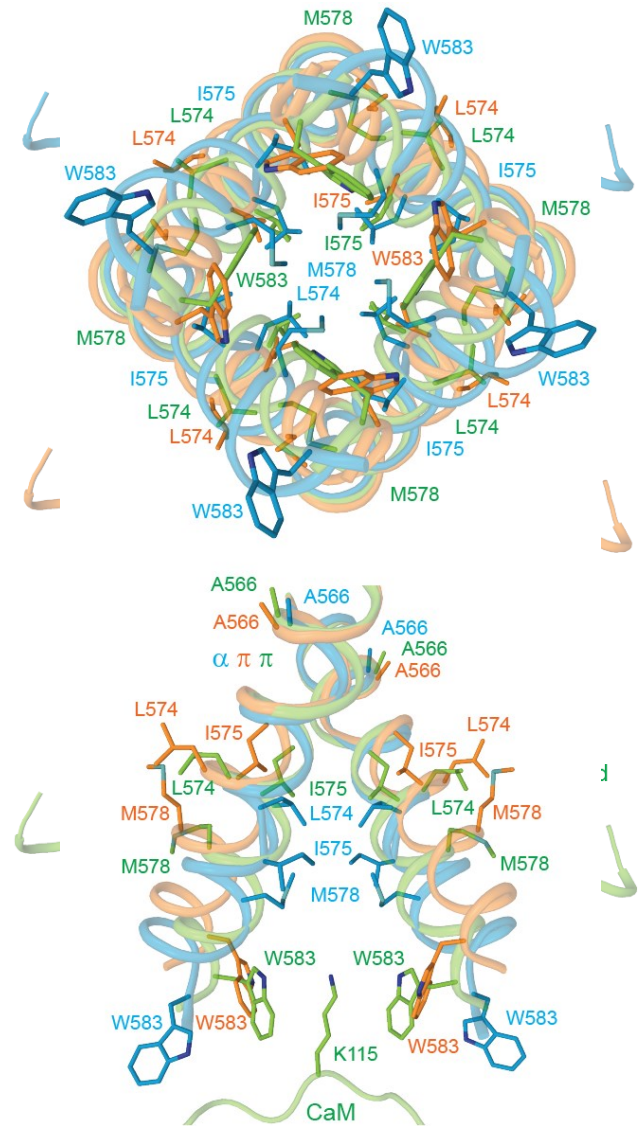
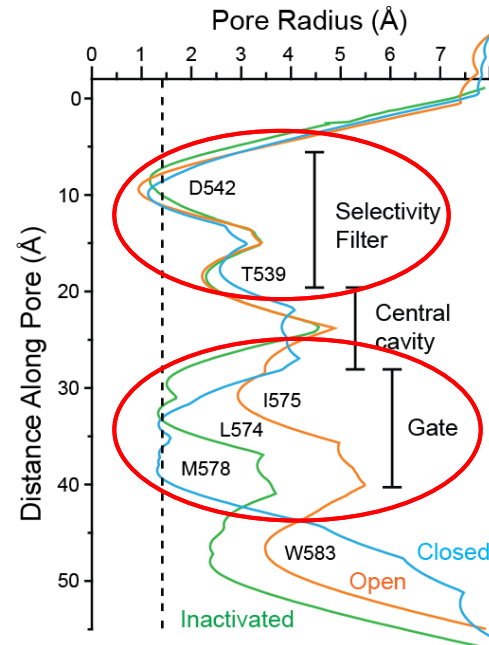
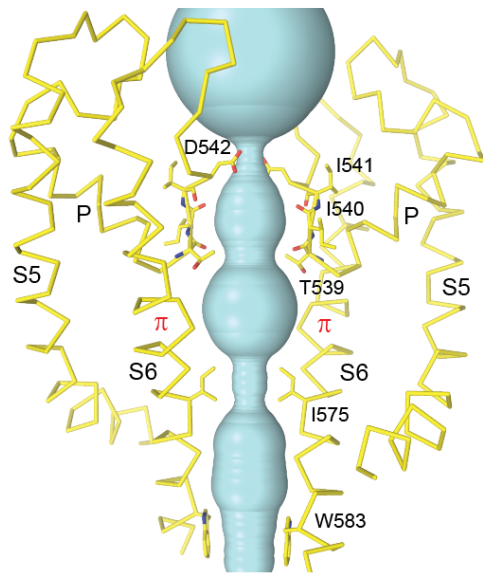


# Architecture of TRPV6-CaM complex

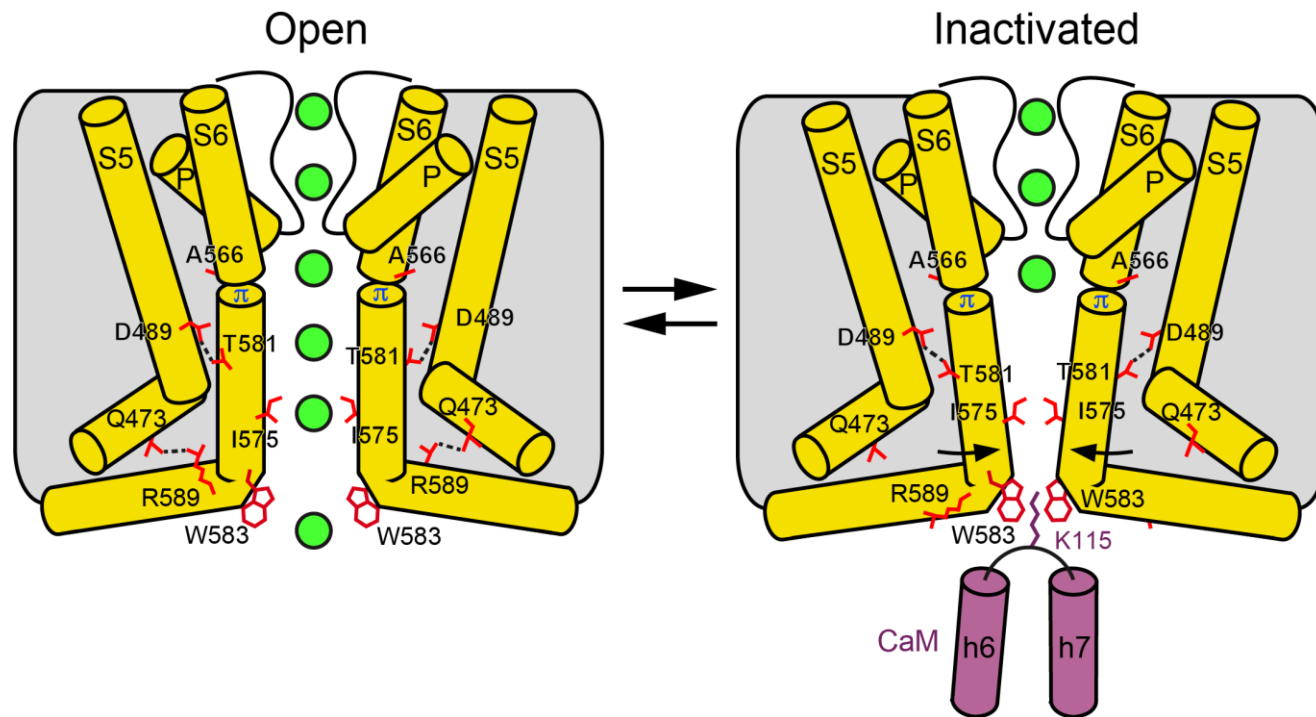




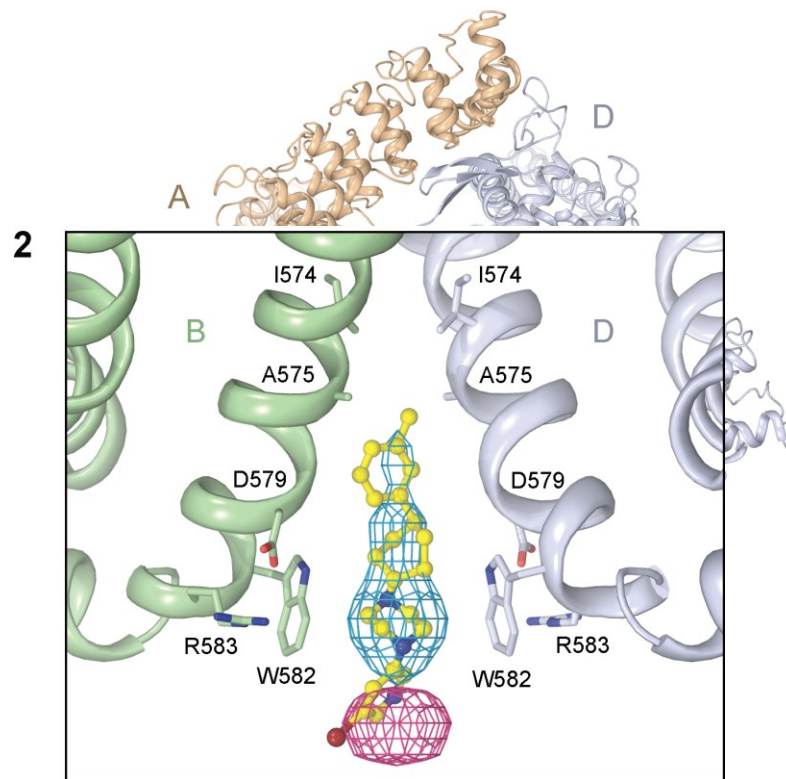
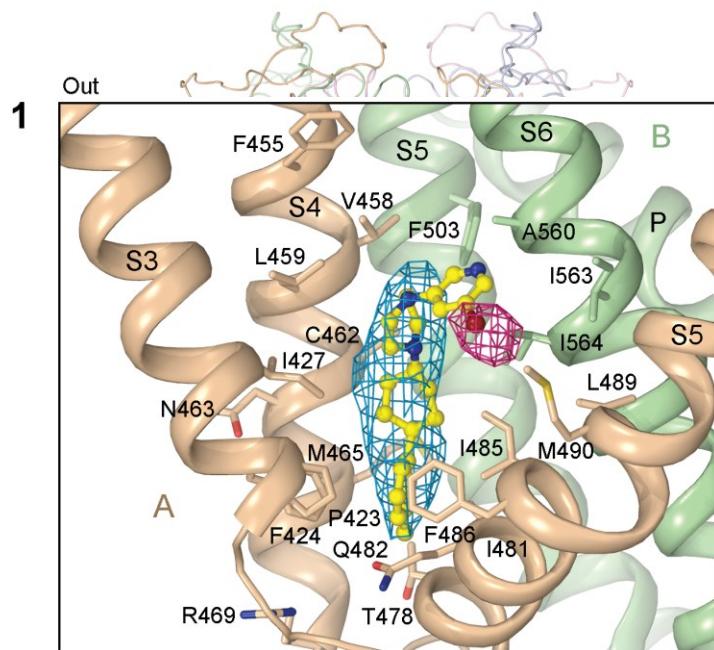
# Inactivated TRPV6 pore



# Mechanism of TRPV6 inactivation

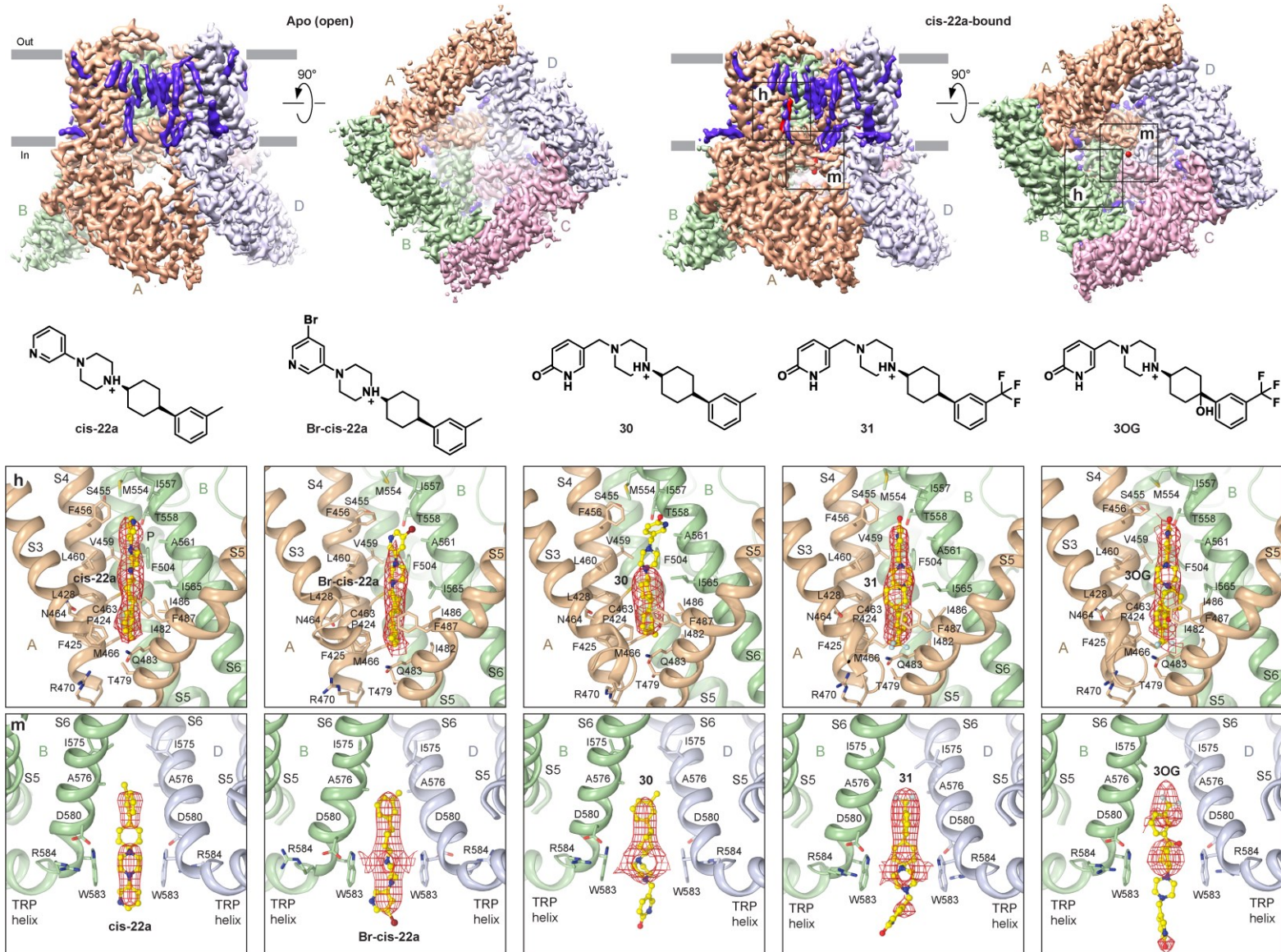


# Inhibition of TRPV6 by PCHPDs (4-phenylcyclohexyl) piperazine derivatives

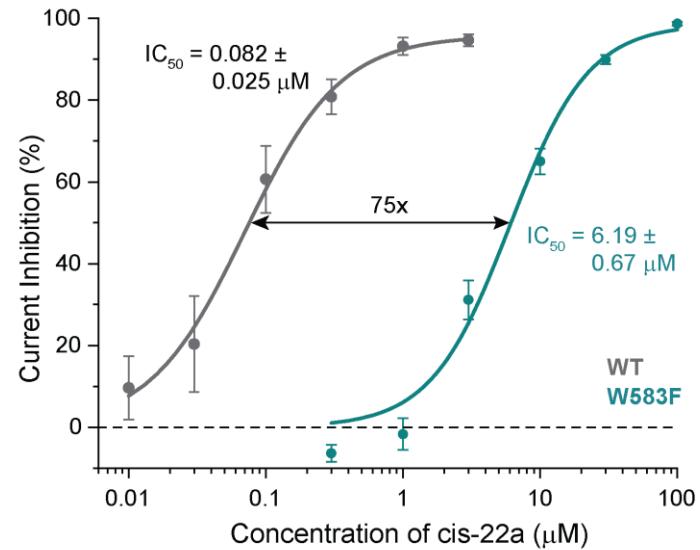
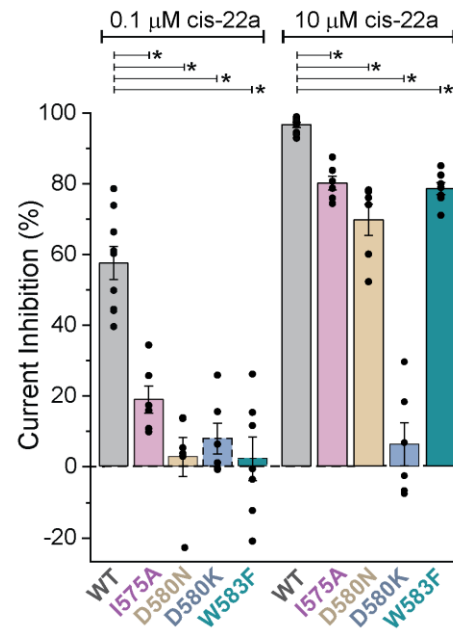
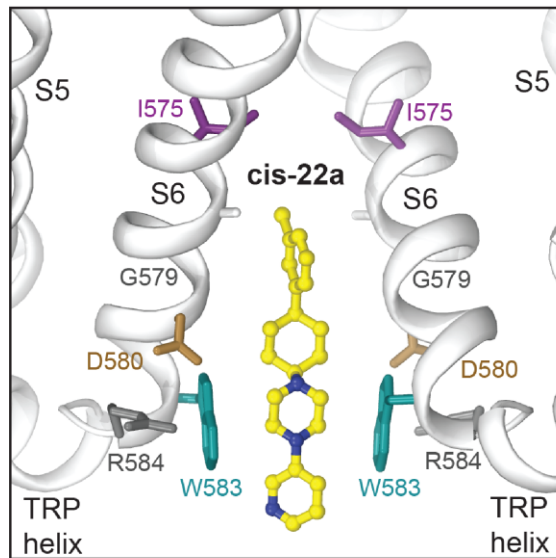




# Inhibition of TRPV6 by (4-phenylcyclohexyl) piperazine derivatives (PCHPDs)

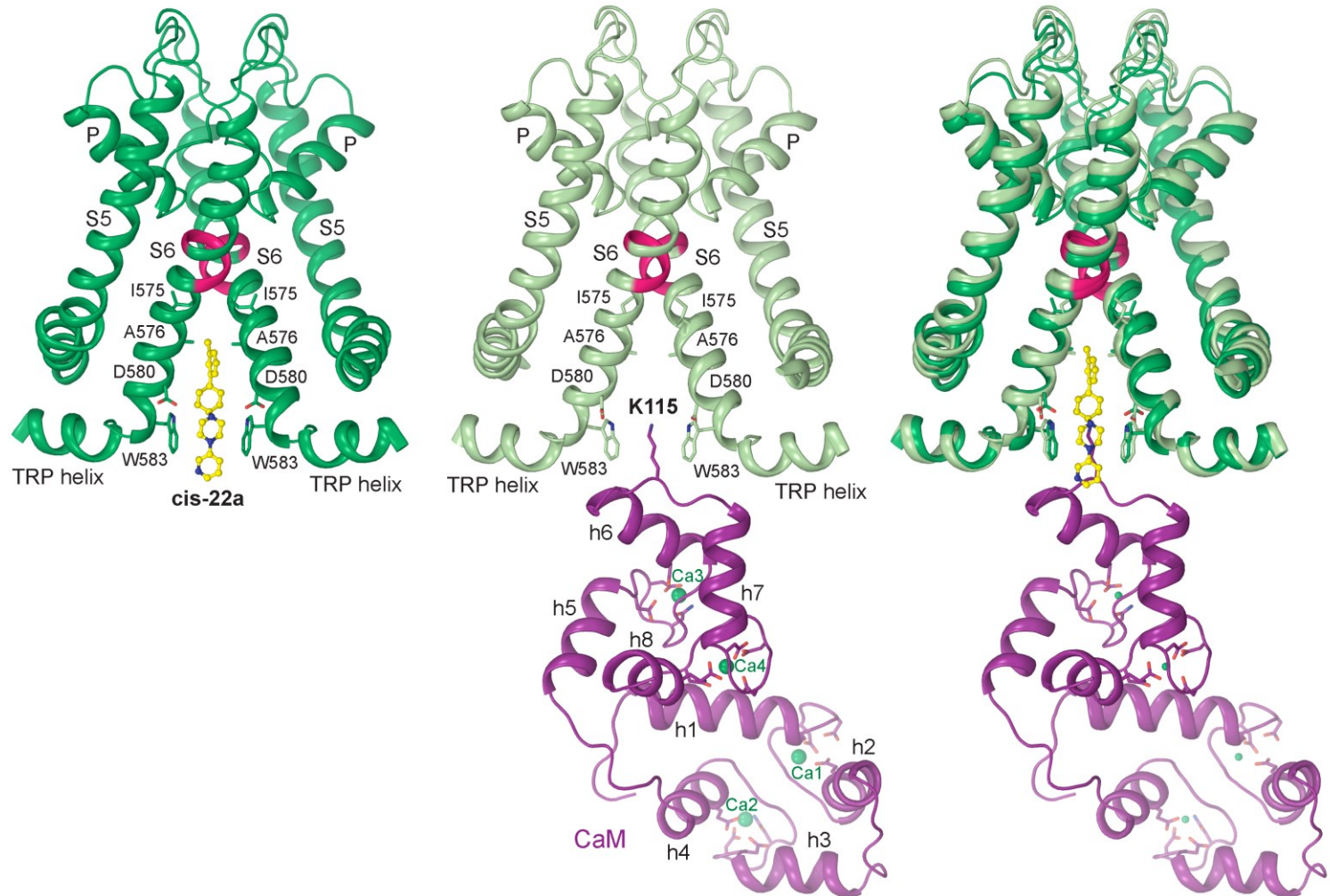


# Functional analysis of the PCHPD pore binding site

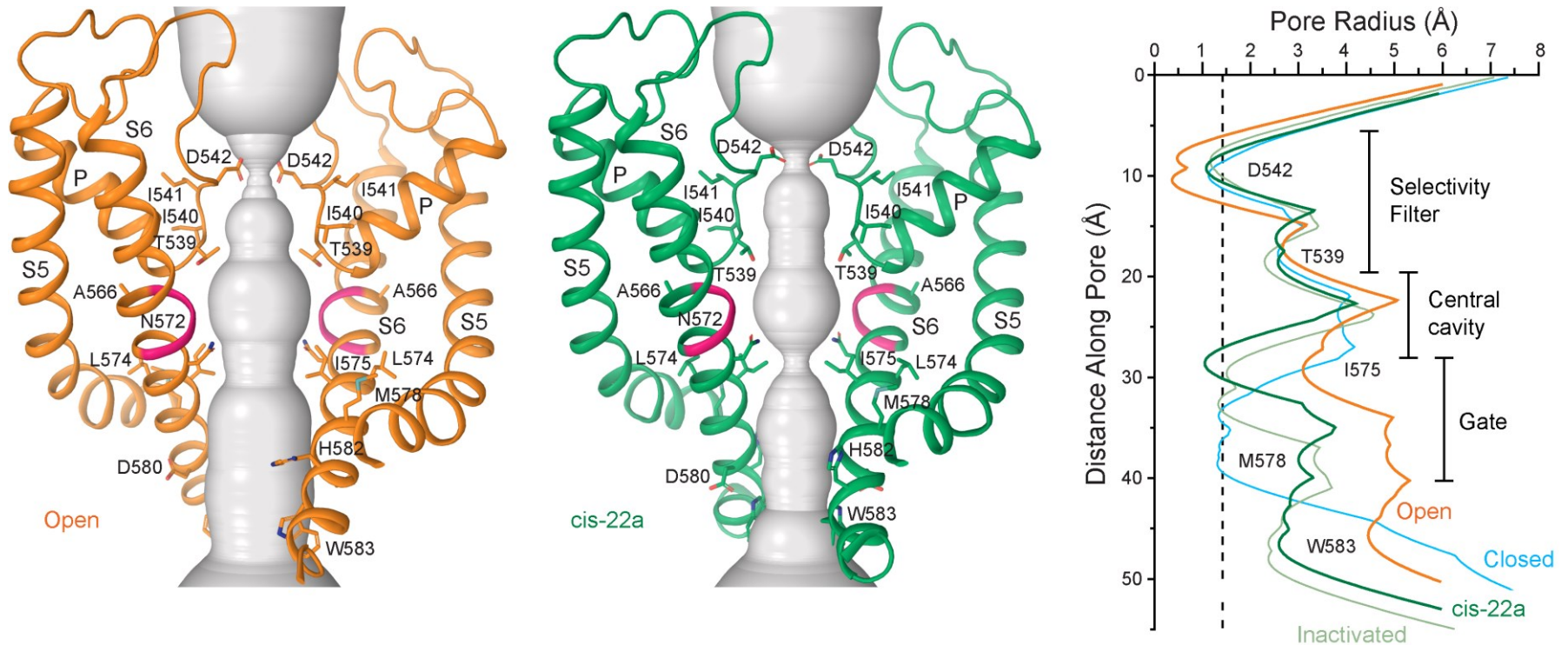




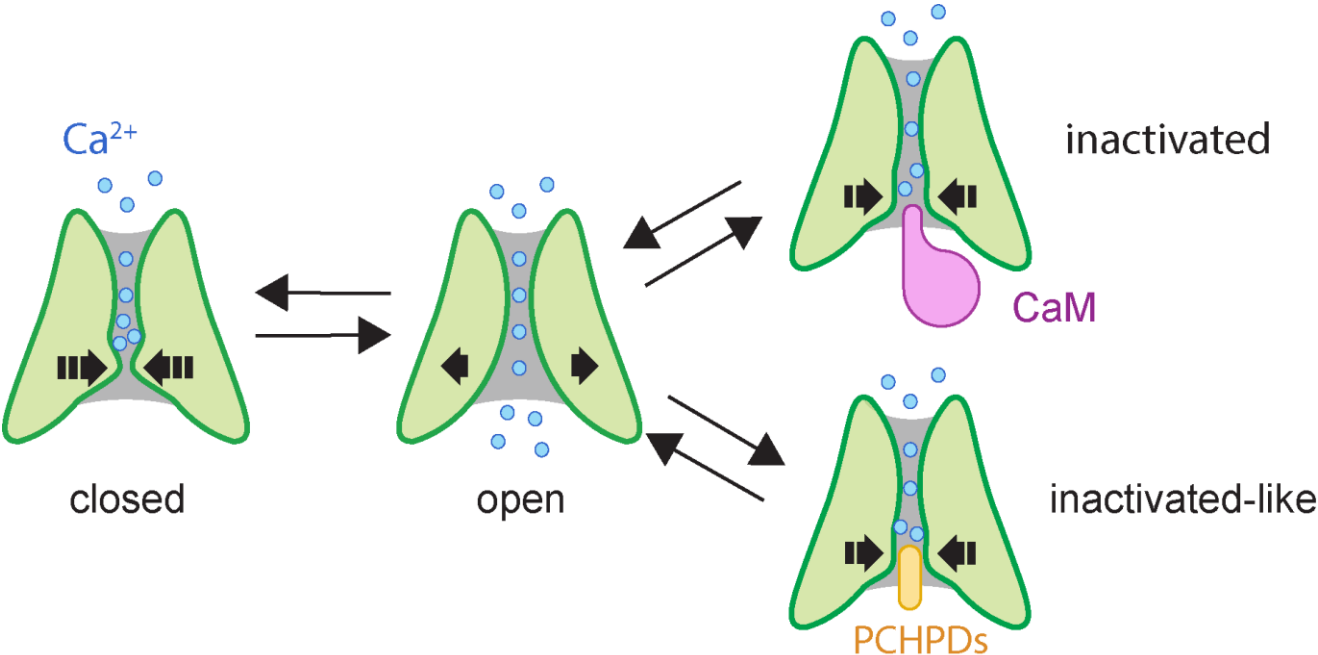
# Comparison of TRPV6 structures inhibited by cis-22a and inactivated by CaM



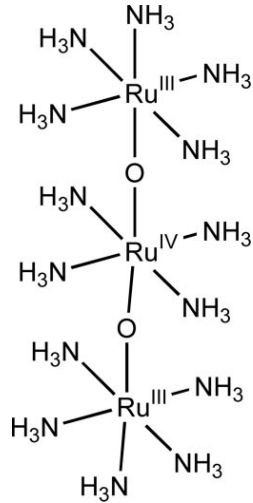
# Pore in TRPV6 structure inhibited by cis-22a compared to other states



# Mechanism of TRPV6 inhibition by PCHPDs

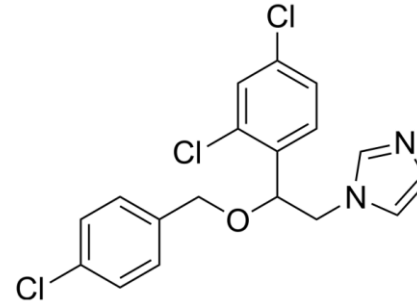


# Ruthenium Red



The inorganic dye ammoniated ruthenium oxychloride, also known as ruthenium red, is used in histology to stain aldehyde fixed mucopolysaccharides. Inhibitor of different ion channels.

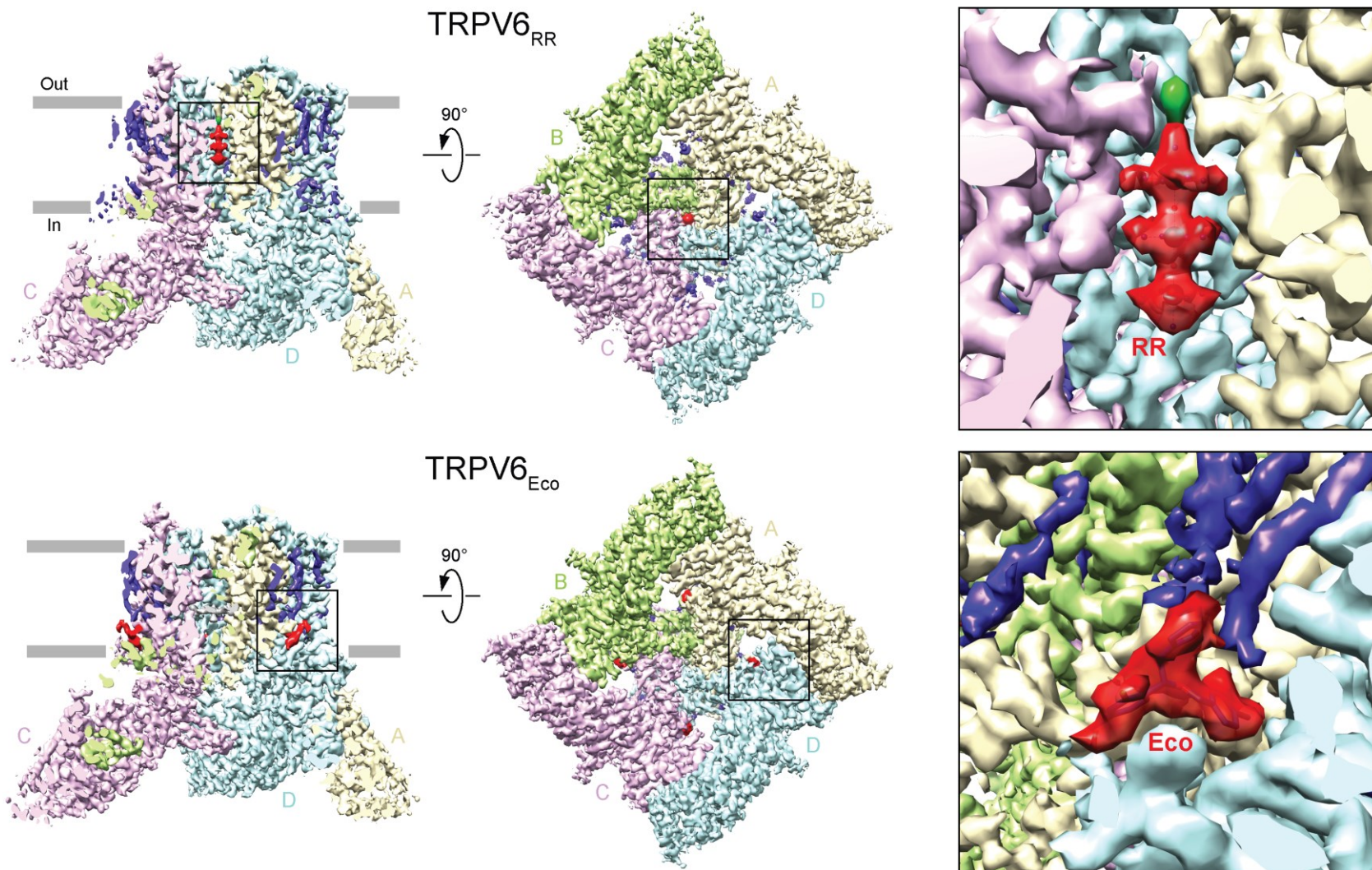
# Econazole



Econazole is used as a cream to treat infections caused by a fungus or yeast, such as athlete's foot, tinea, pityriasis versicolor, ringworm, and jock itch.

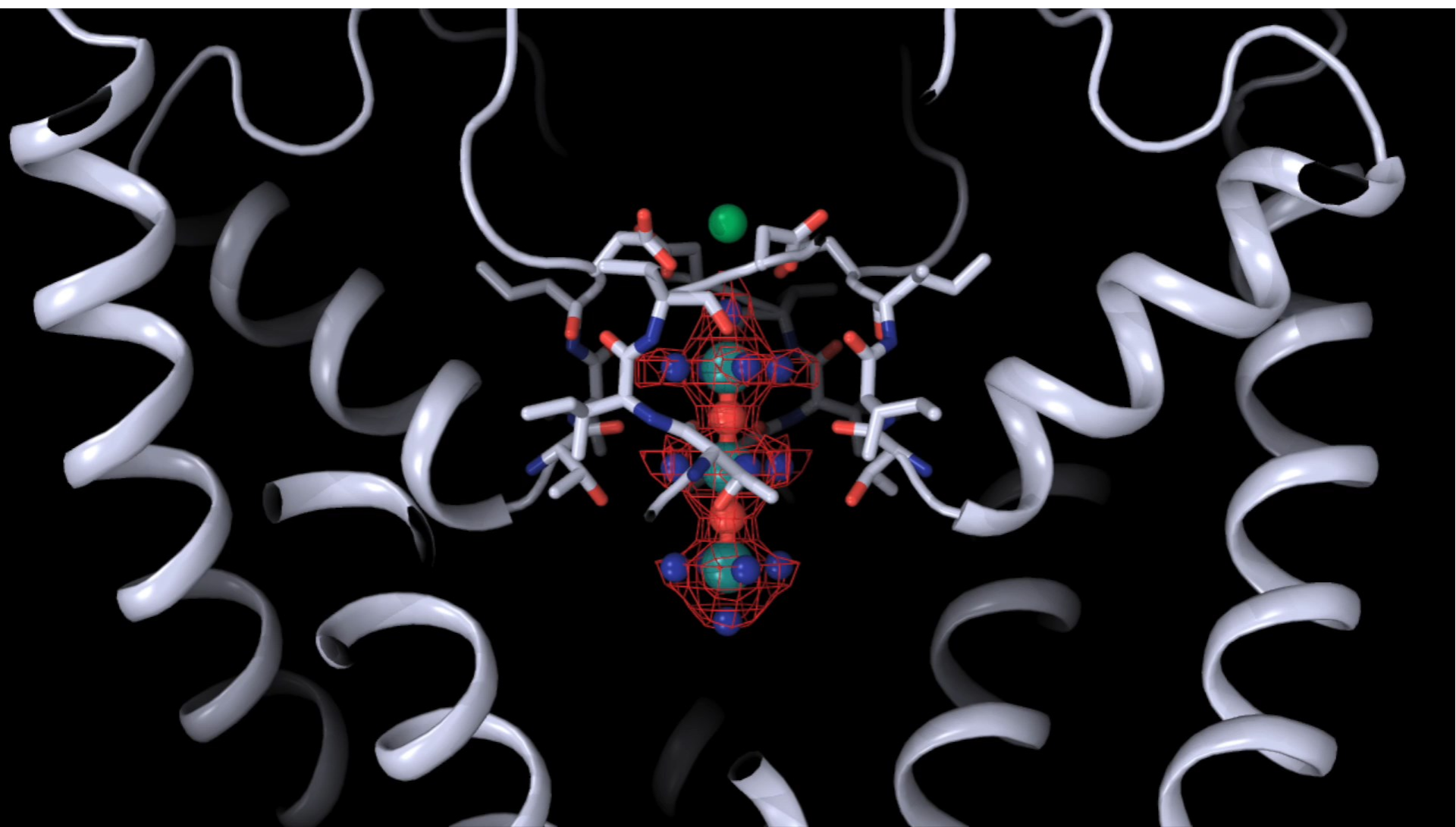


# TRPV6 in complex with Ruthenium Red and Econazole

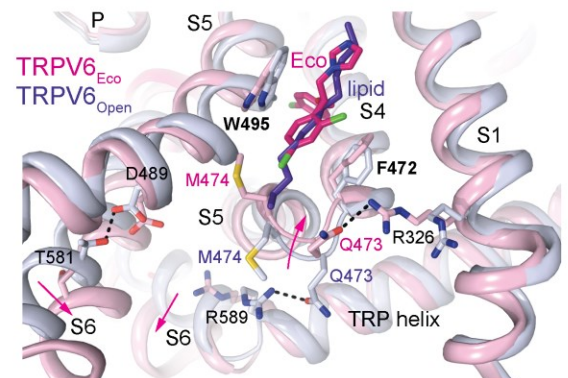
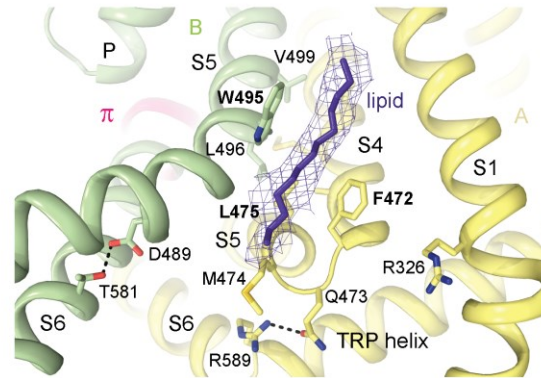
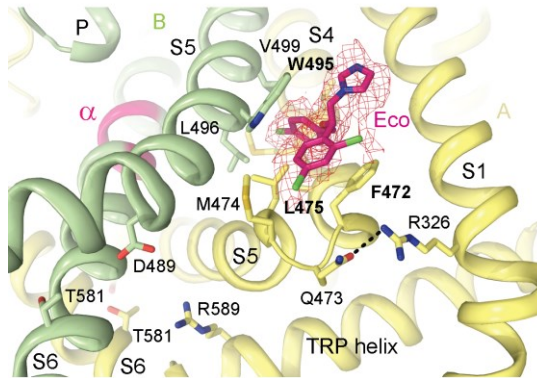
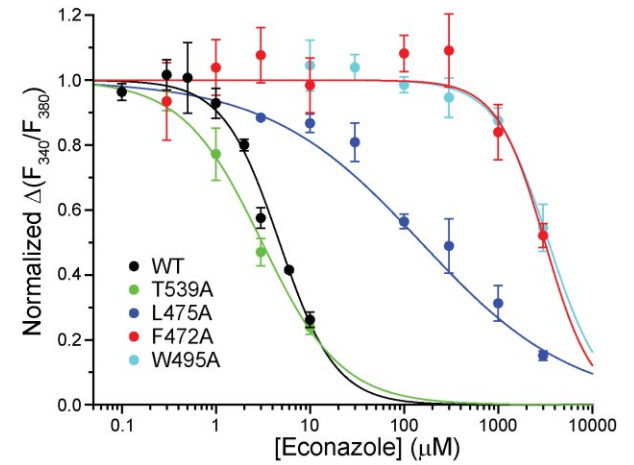
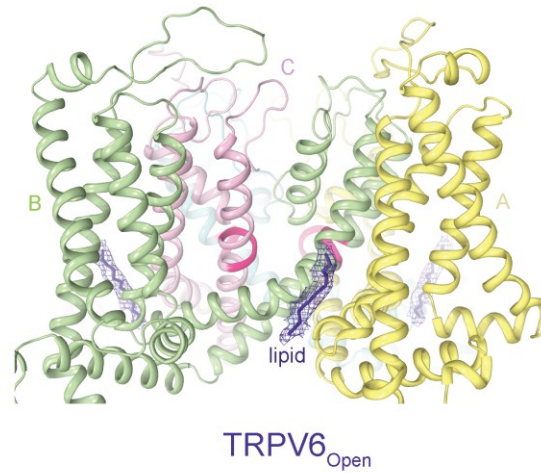
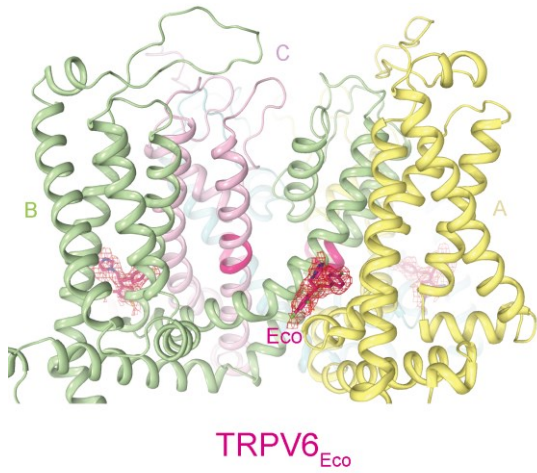




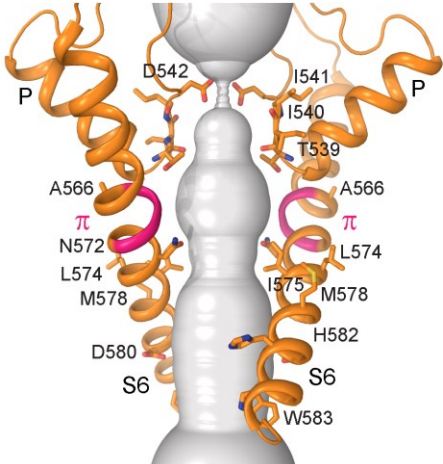
# Binding site of RR in the channel pore



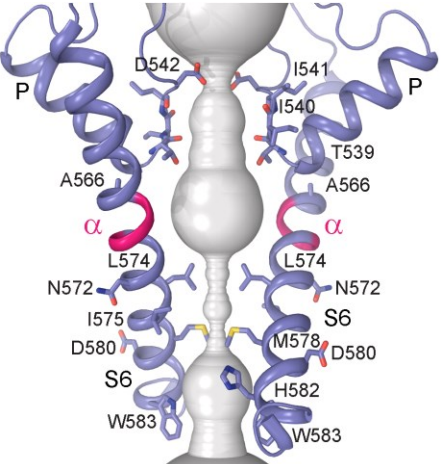
# Allosteric binding site of econazole



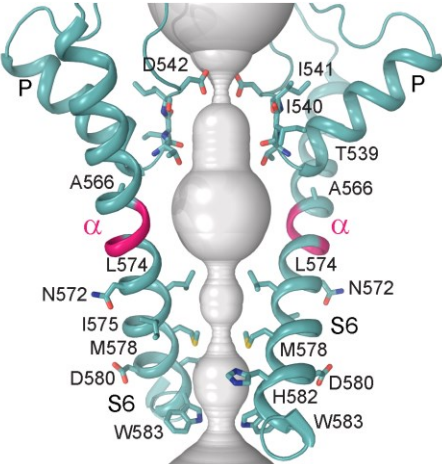
# Ion channel pore



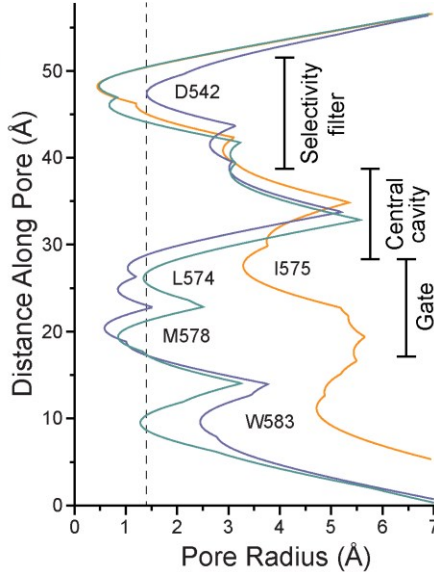
TRPV6<sub>Open</sub>



TRPV6<sub>RR</sub>

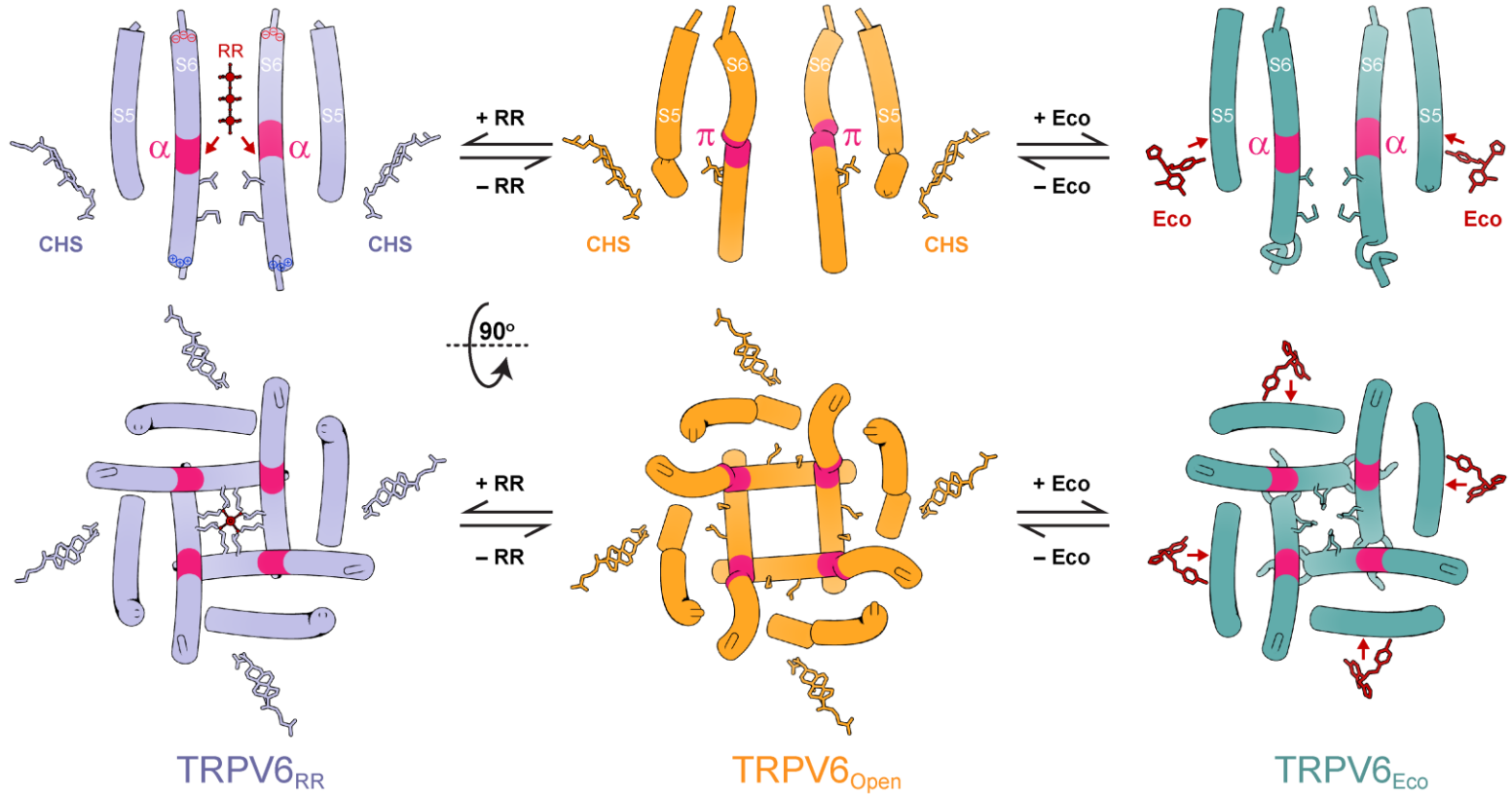


TRPV6<sub>Eco</sub>

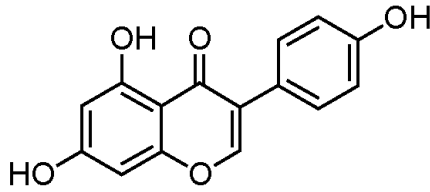




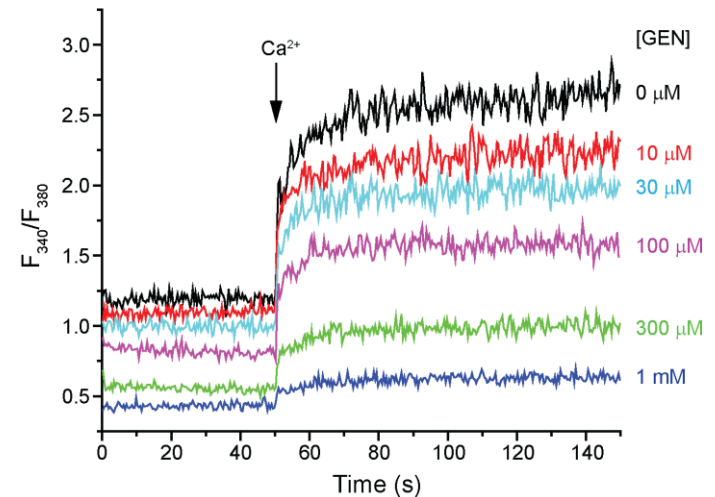
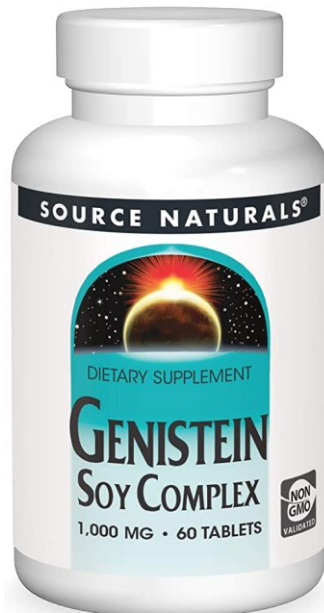
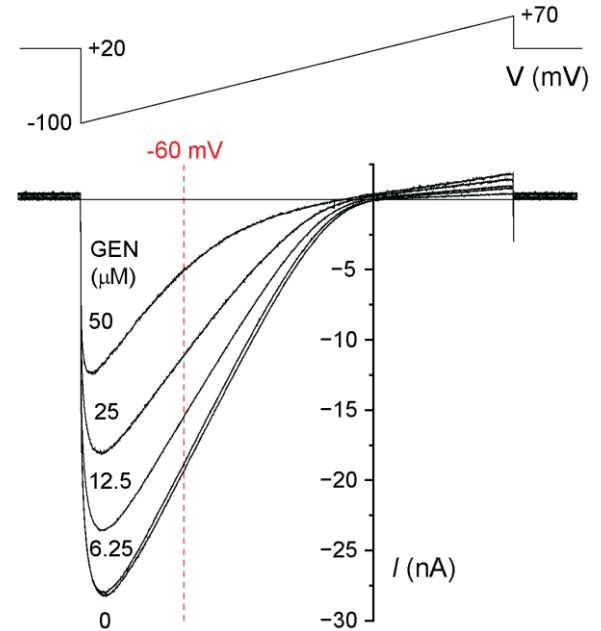
# Mechanism of TRPV6 inhibition by RR and econazole



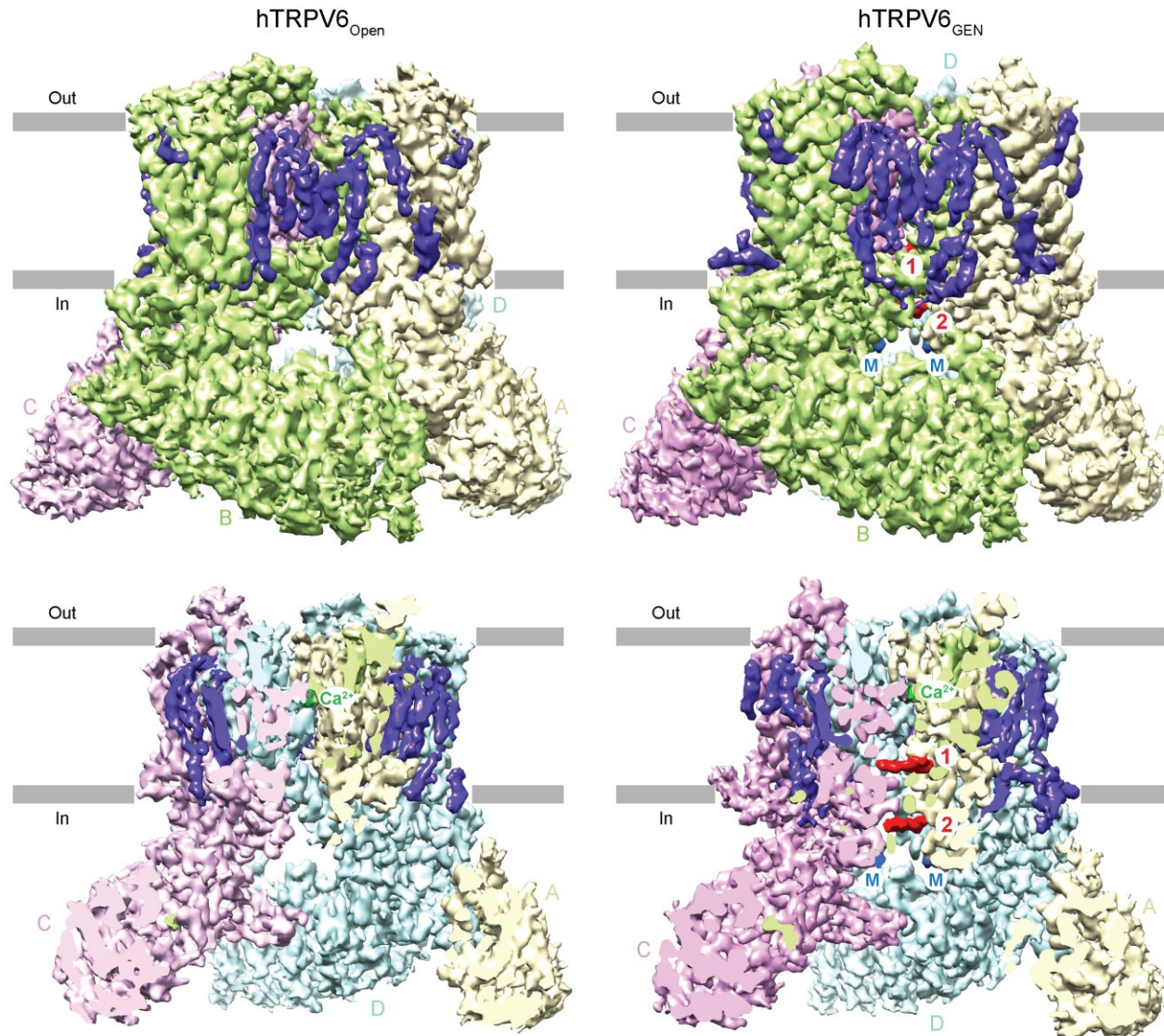
# Genistein



Genistein is a **polyphenolic isoflavone** that belongs to the flavonoid group and is commonly found in various dietary vegetables, such as **soy beans** and fava beans. Epidemiological studies have shown that the intake of dietary-rich isoflavones **reduces the risk of various human cancers**.

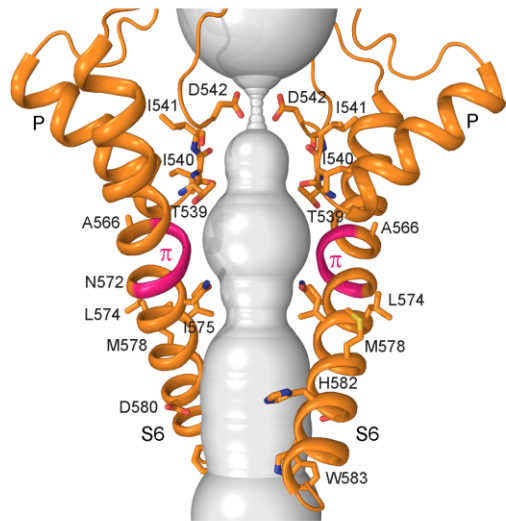
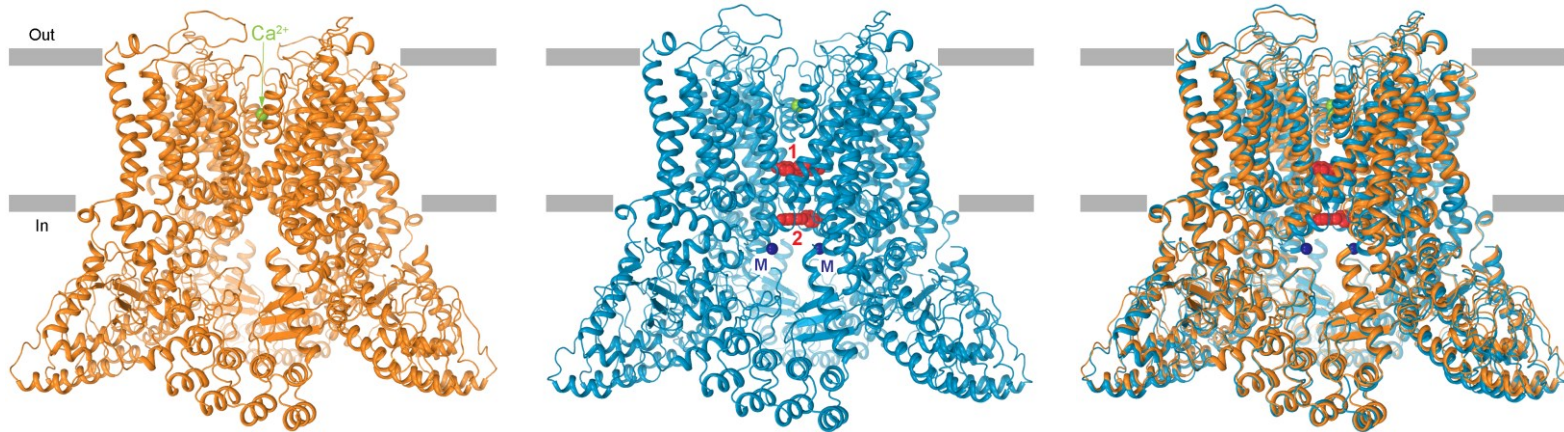


# Cryo-EM of hTRPV6 in the presence of genistein

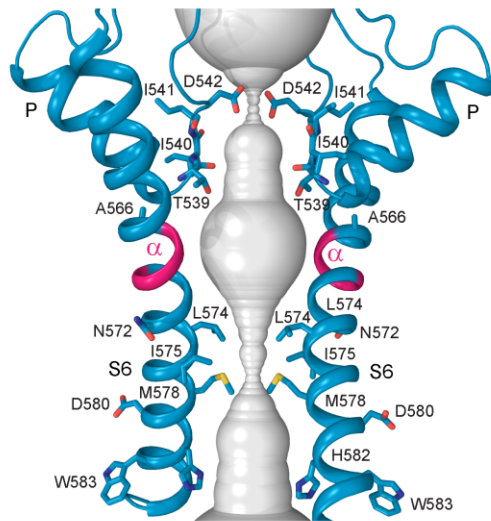




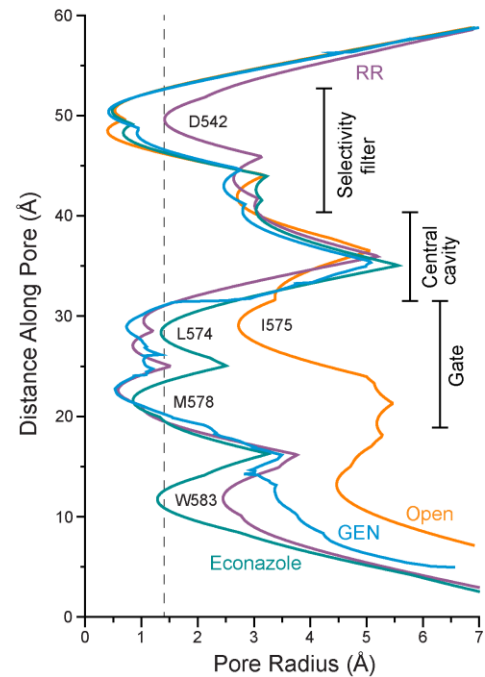
# Structures of hTRPV6 in the presence of genistein



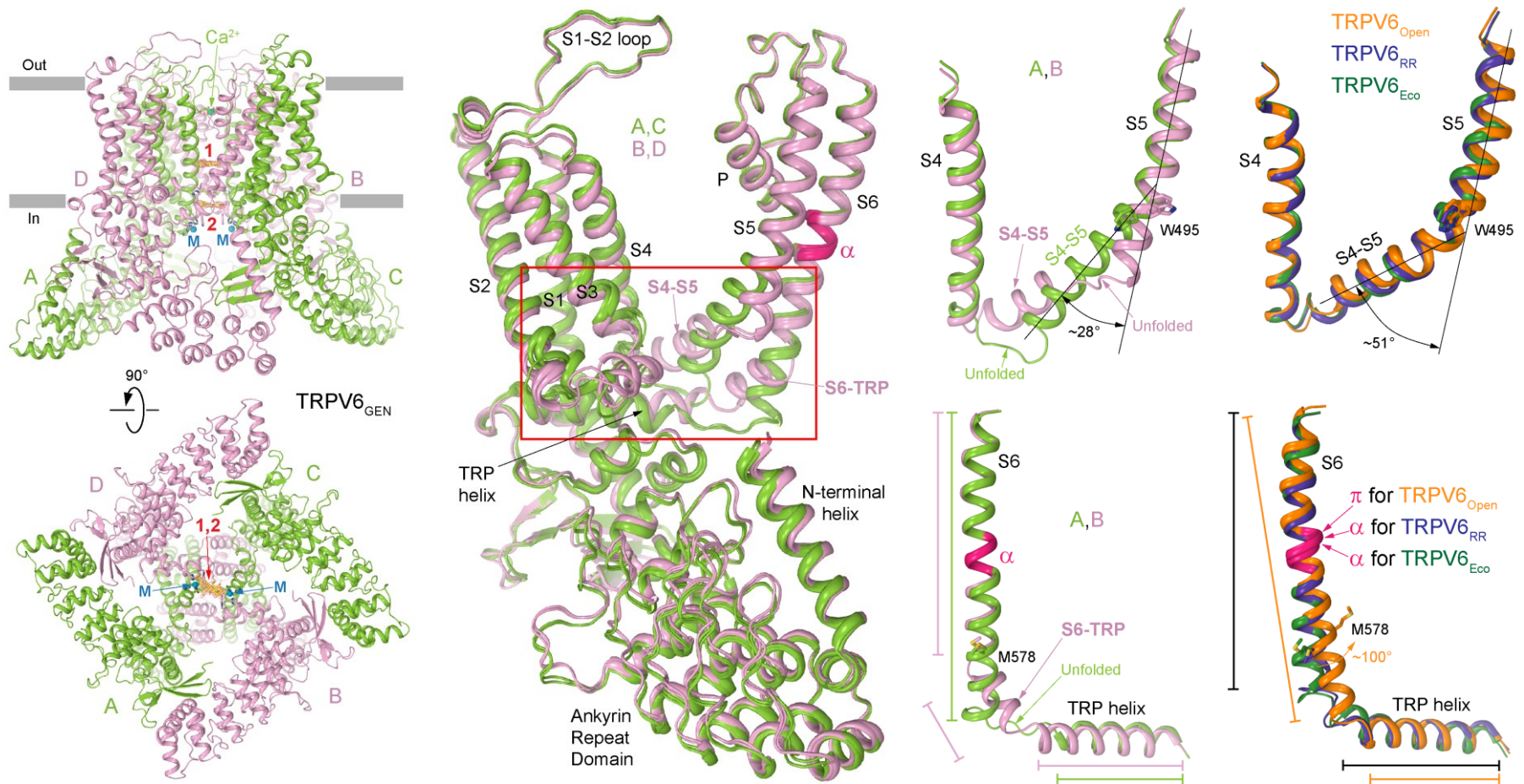
TRPV6<sub>Open</sub>



TRPV6<sub>GEN</sub>

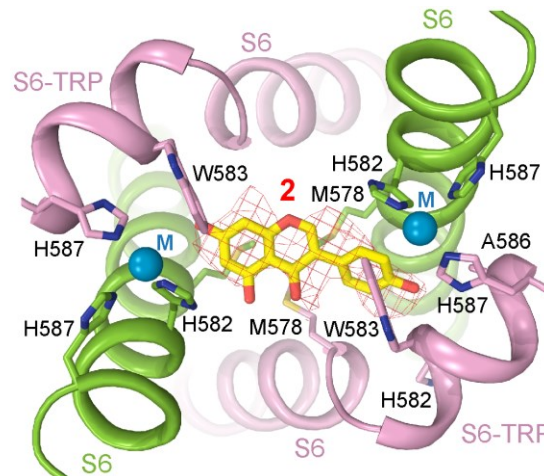
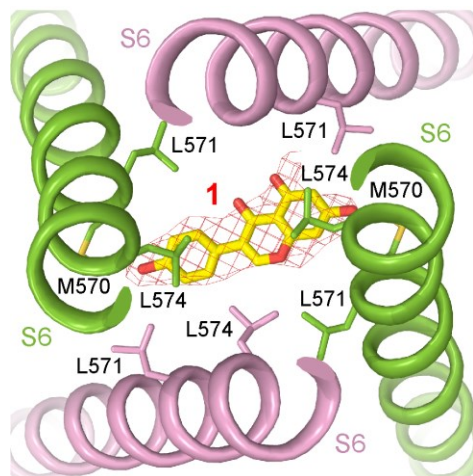
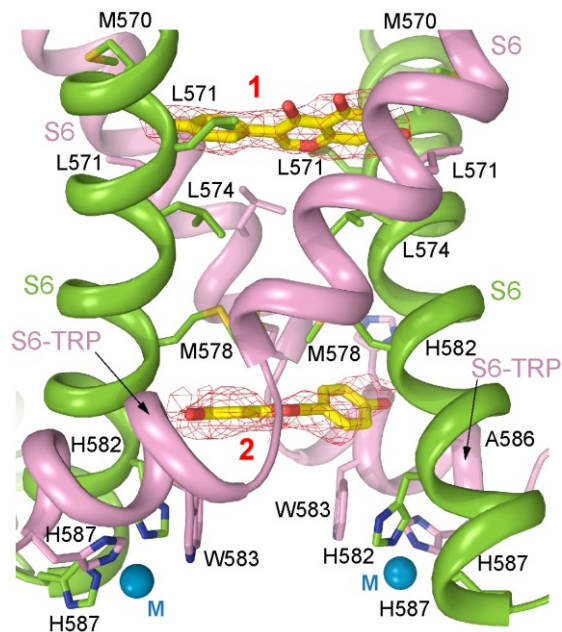
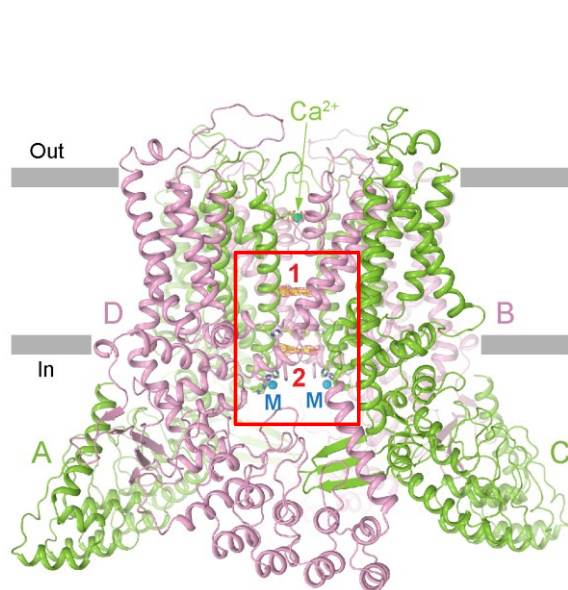


# Structures of hTRPV6 in the presence of genistein



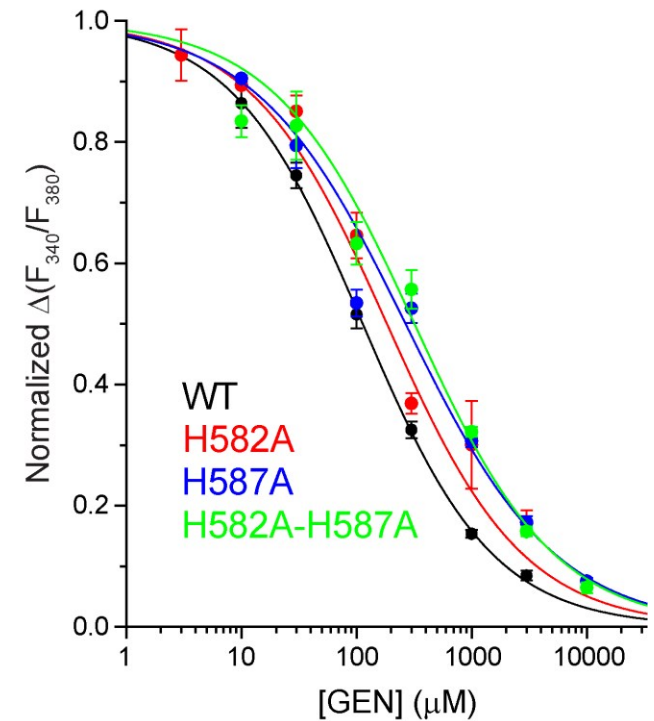
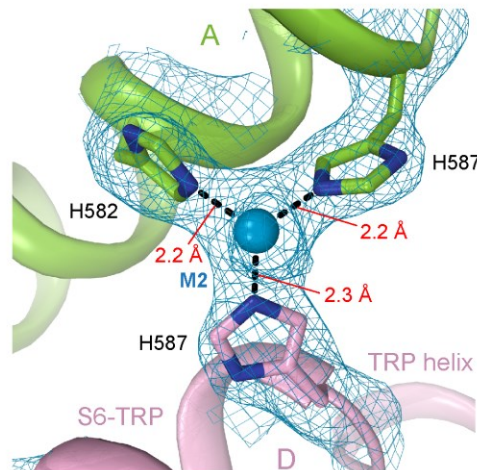
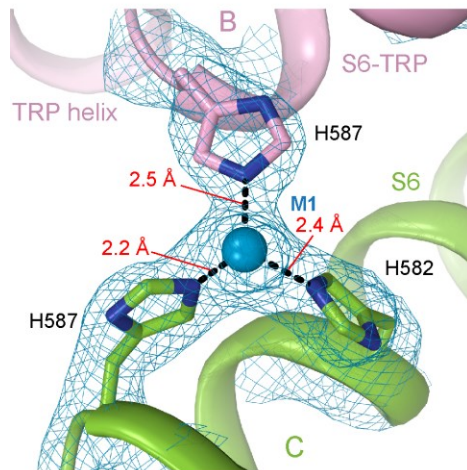
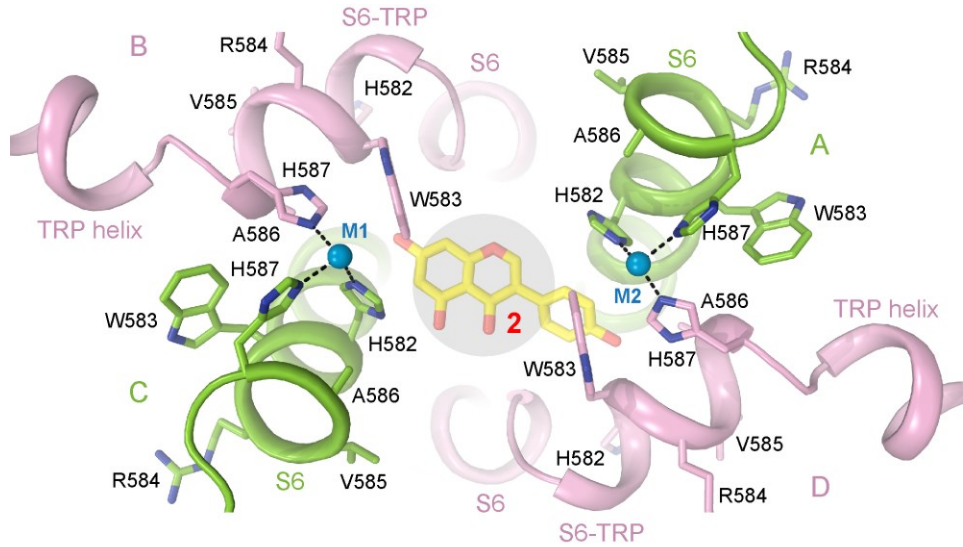


# Genistein binding sites



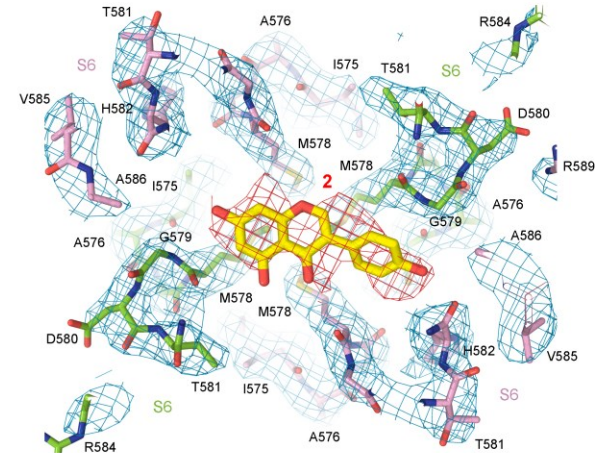
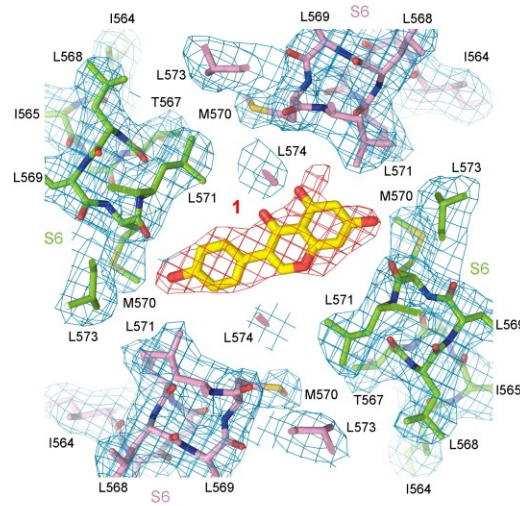


# Binding sites of metal ions



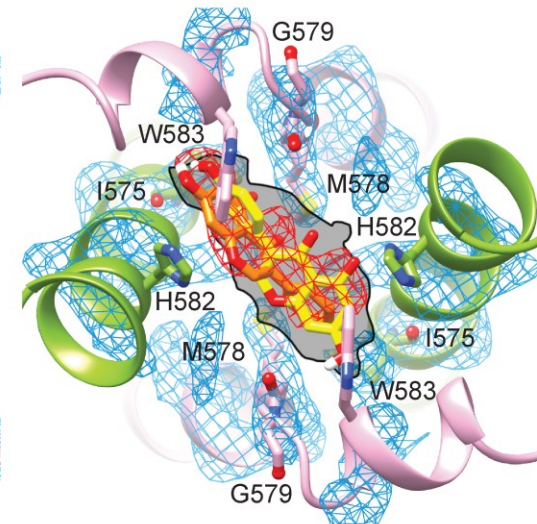
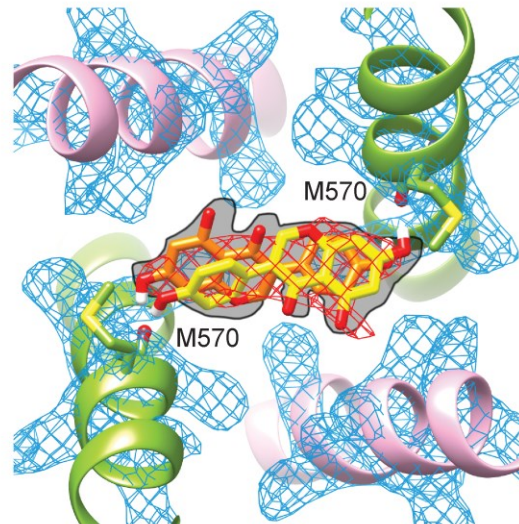
# MD simulations of genistein binding sites

Cryo-EM



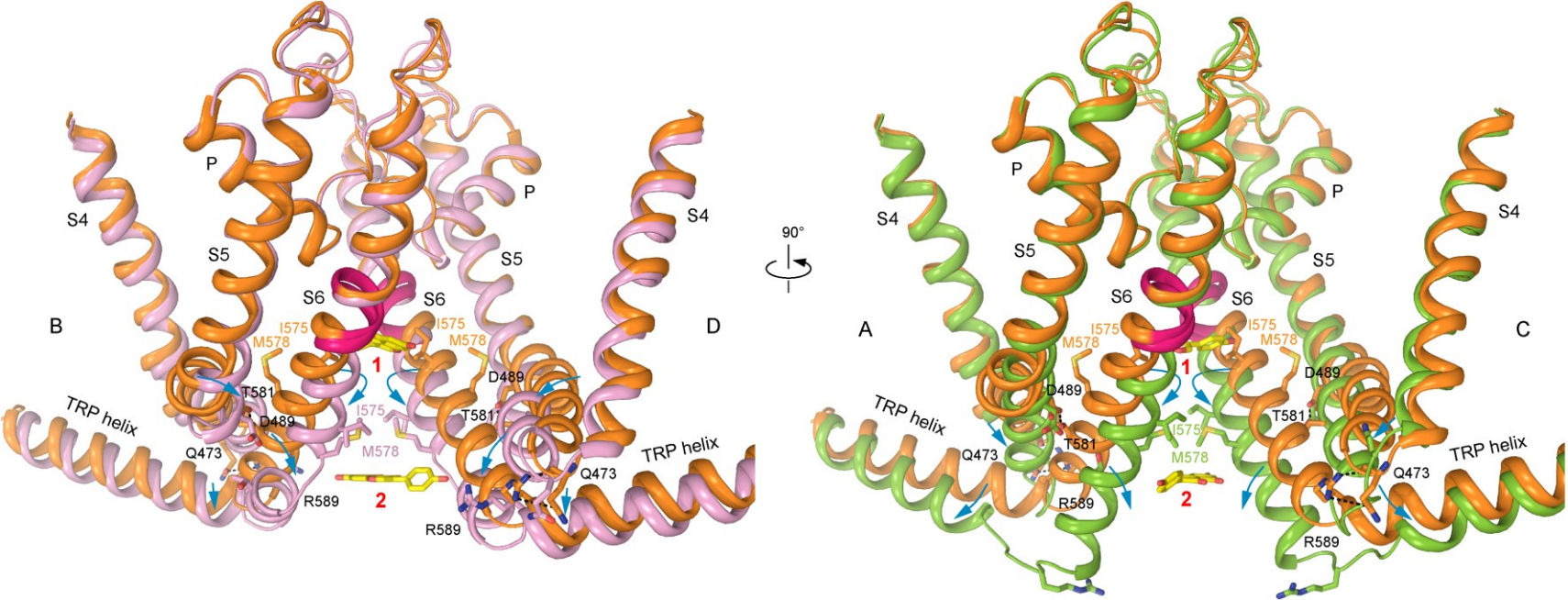
Roman Efremov  
Yuri Trofimov  
Nikolay Krylov

MD



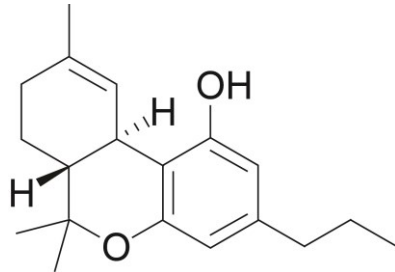


# Mechanism of TRPV6 inhibition by genistein



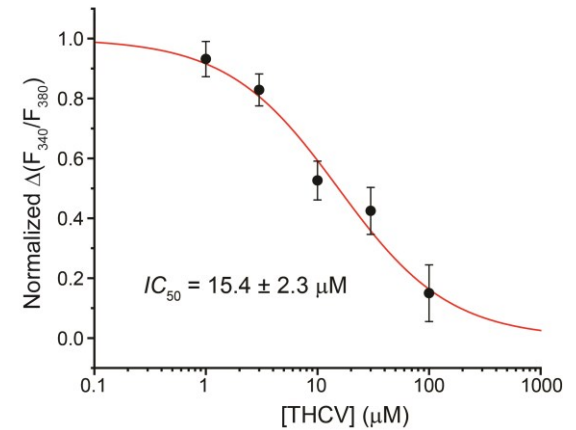
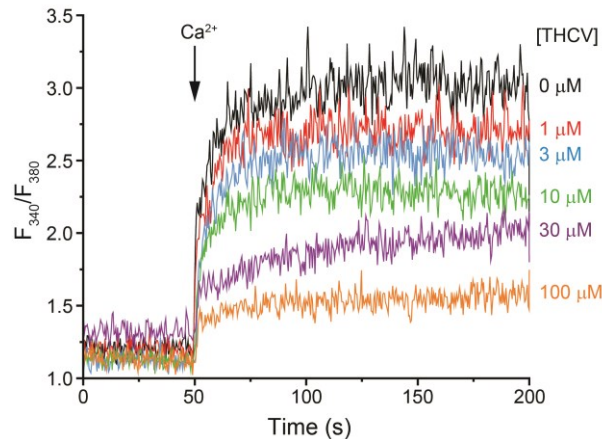
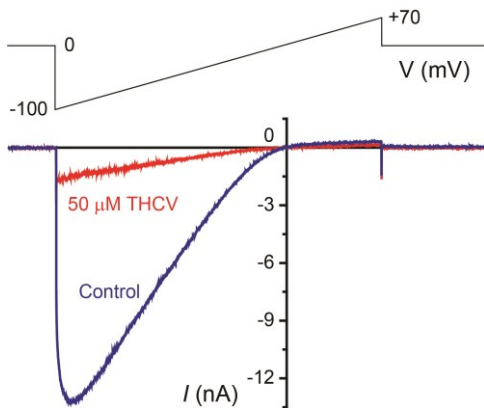


# Inhibition of TRPV6 by tetrahydrocannabivarin (THCV)

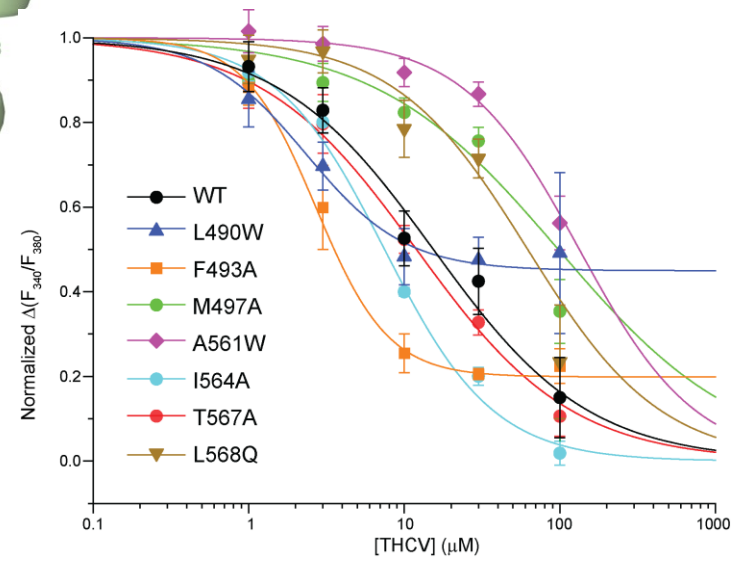
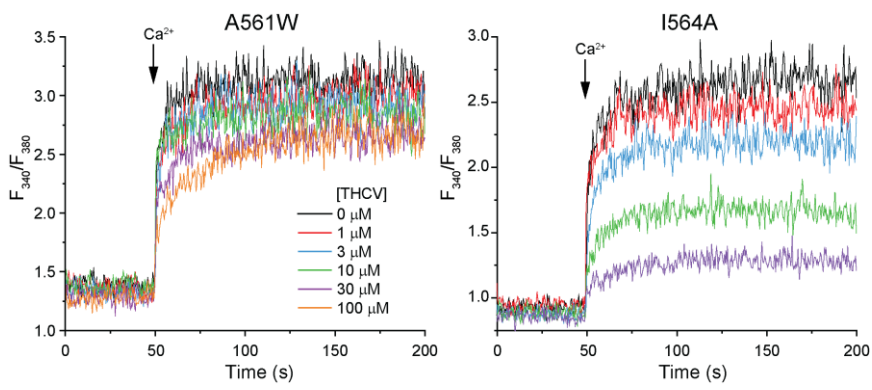
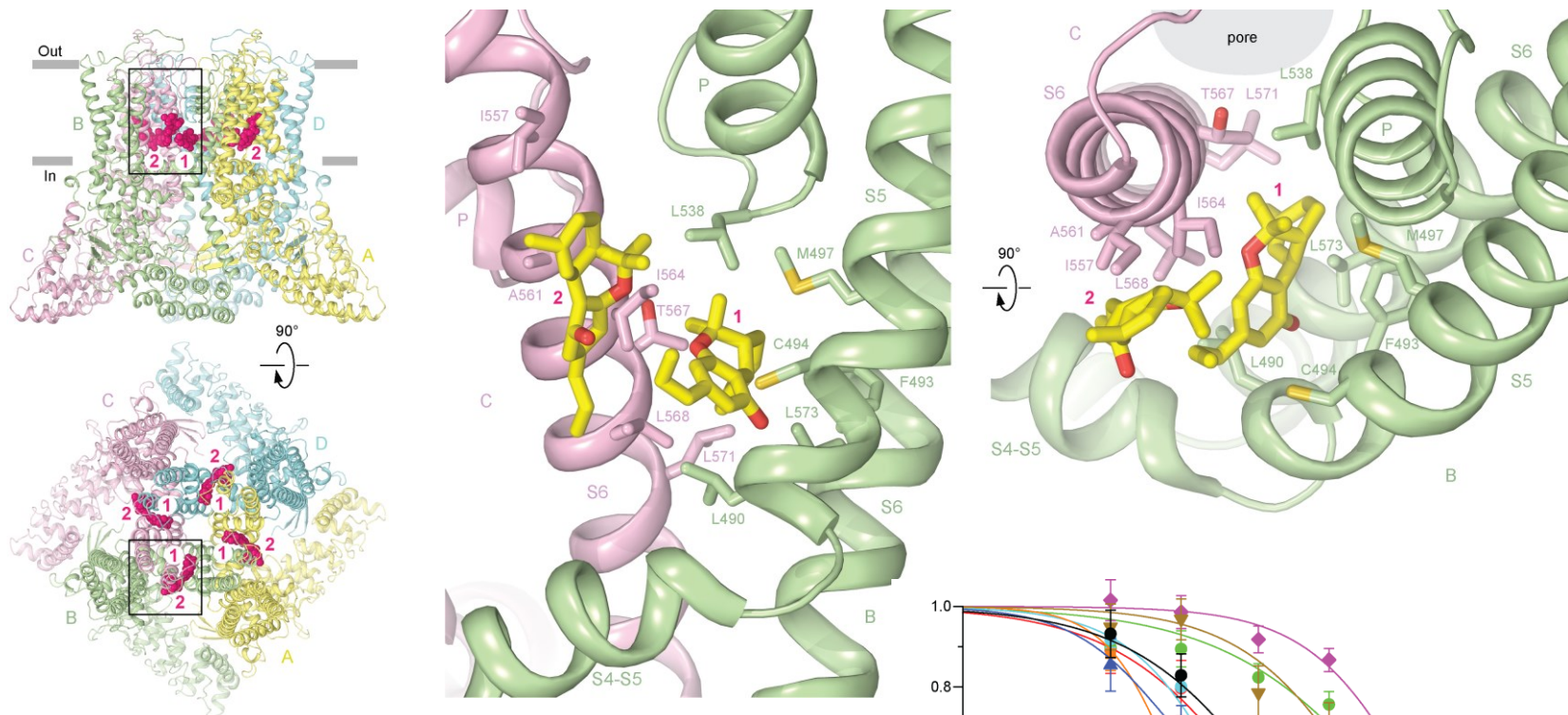


THCV

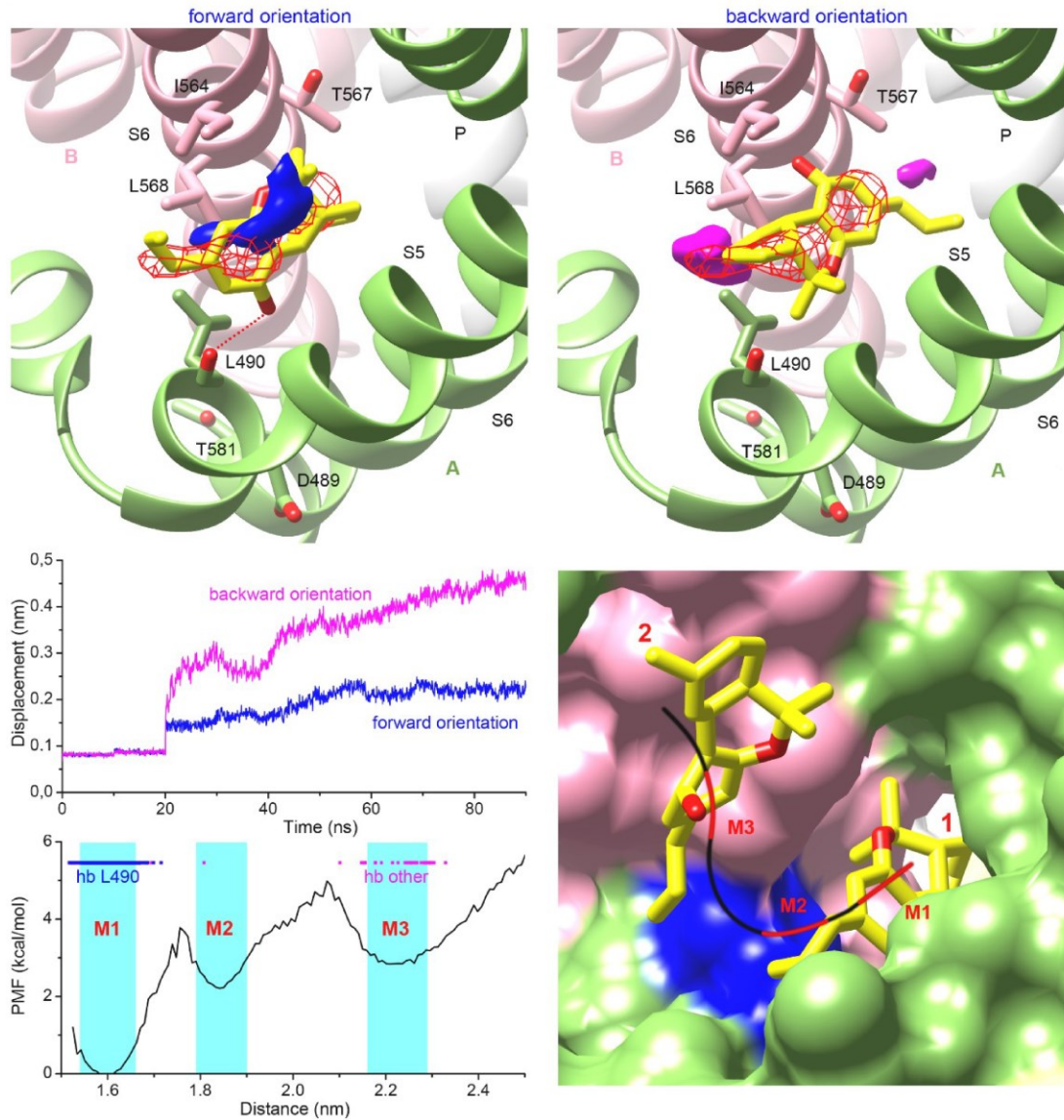
- Natural cannabinoid from *Cannabis sativa*
- Non-psychoactive, compared to CBD and THC
- Neuroprotective activity
- Appetite suppression
- Glycemic control
- Potential treatment for obesity and diabetes
- Anti-cancer properties



# Structure of TRPV6<sub>THCV</sub> and THCV binding sites



# MD simulation of THCV binding to TRPV6



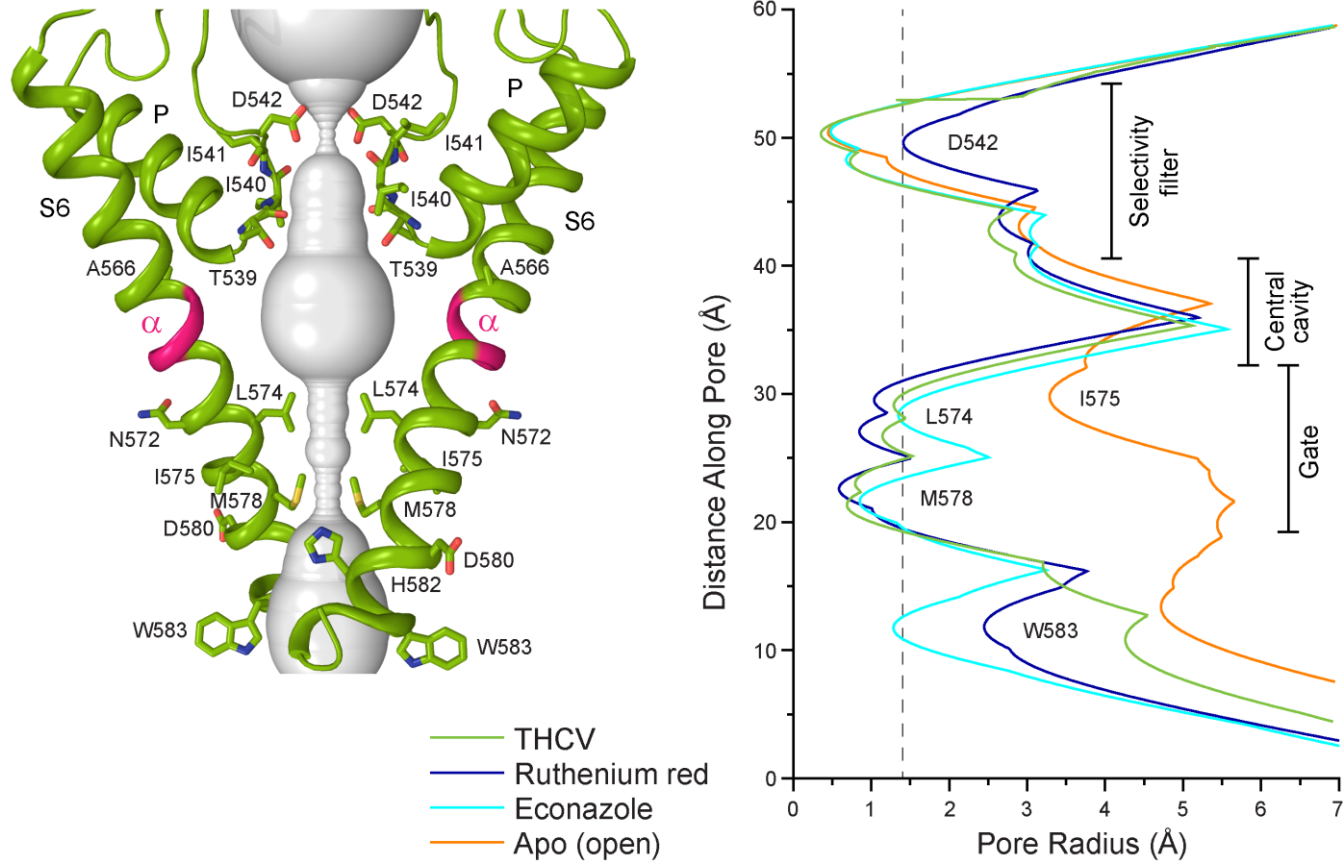
Roman Efremov

Yuri Trofimov

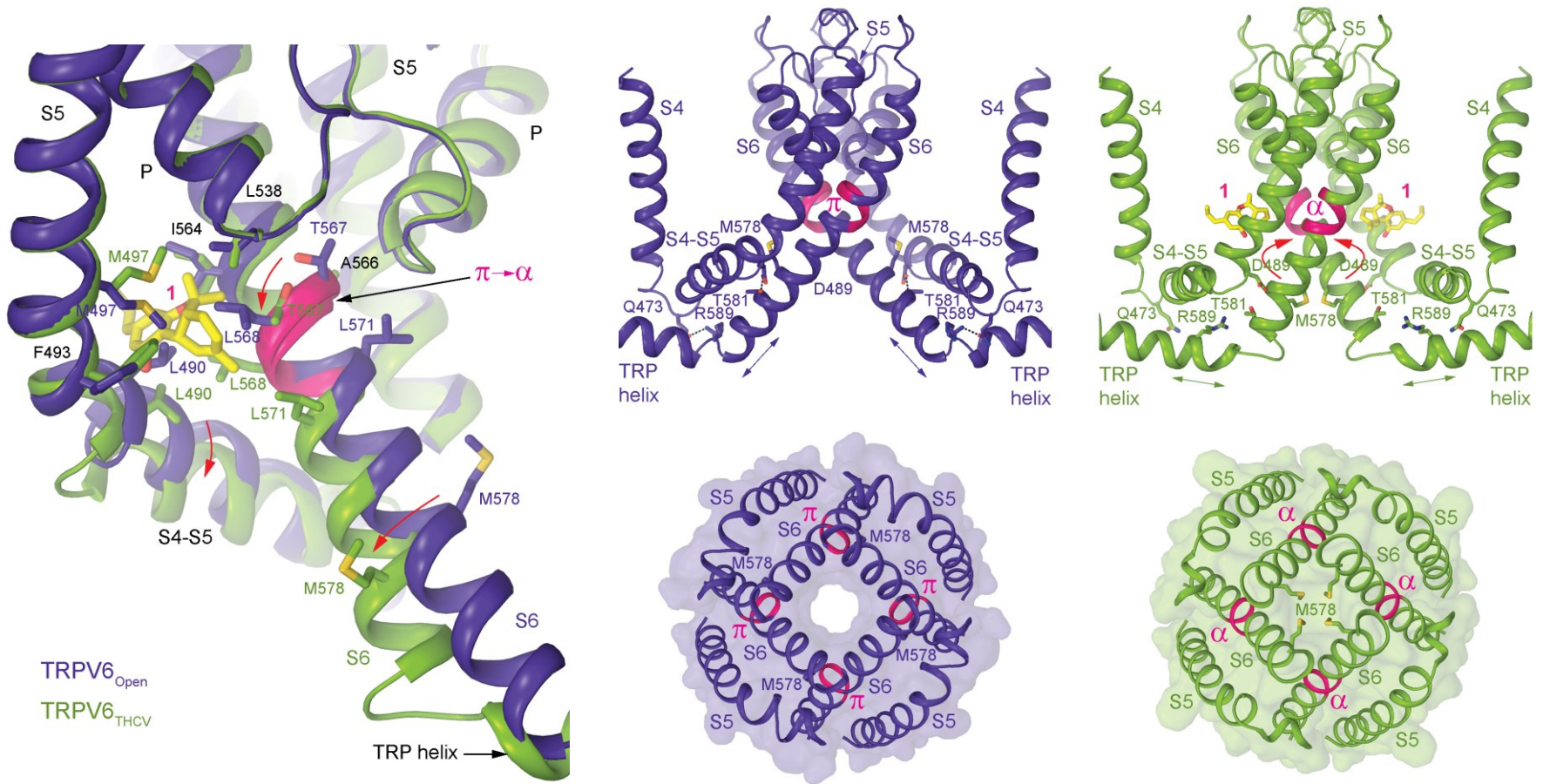
Nikolay Krylov



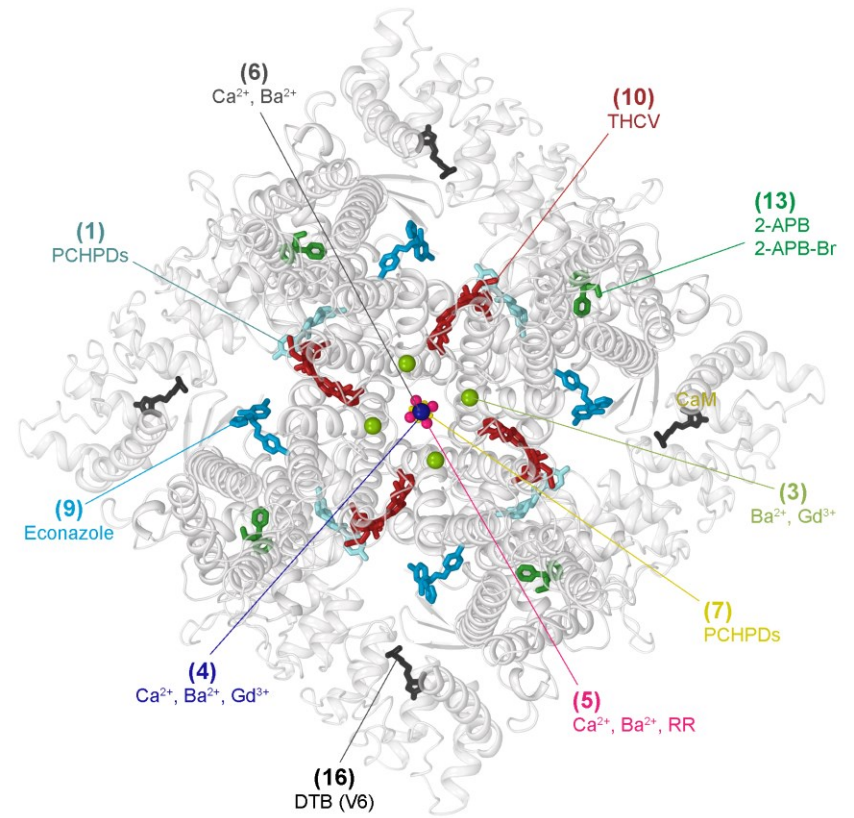
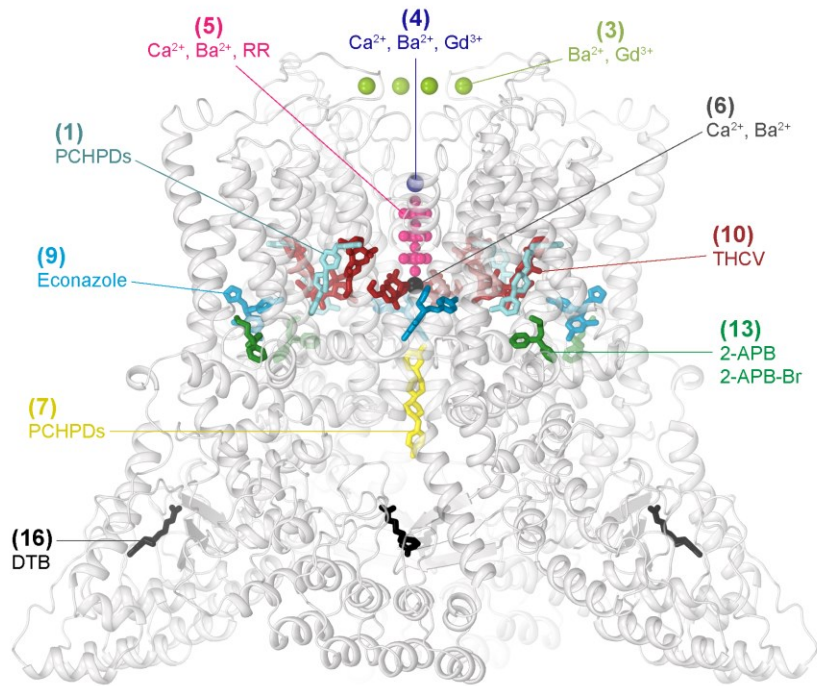
# TRPV6<sub>THCV</sub> pore



# Conformational changes and mechanism of THCV inhibition



# Binding sites in TRPV6





Kirill  
Nadezhdin



Arthur  
Neuberger

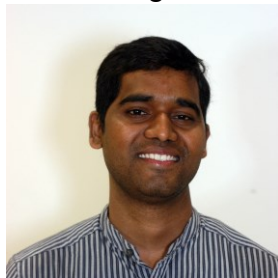


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Musculoskeletal and Skin Diseases

*Thank you!*