Человек и Лекарство'2020

Температурный сенсор TRPV1: компьютерные и биохимические эксперименты

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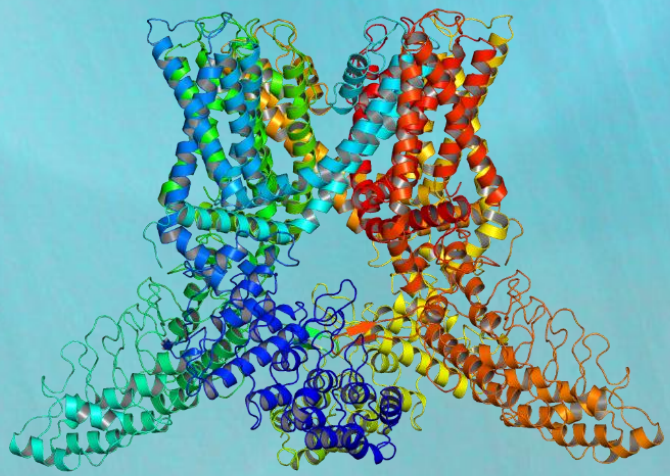
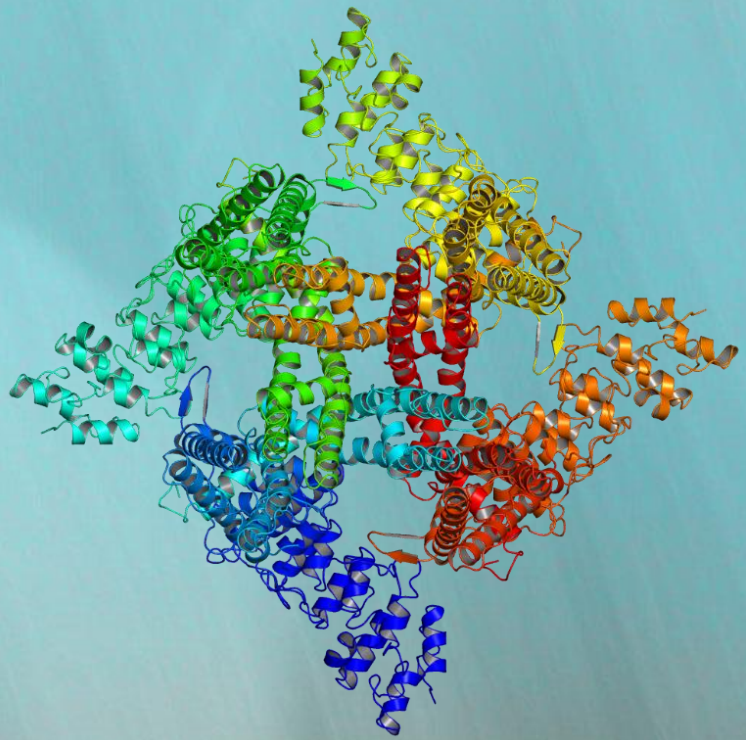
Красные виноградники в Арле (1888)
Винсент Ван Гог

TRPV1 — heat and capsaicin receptor

- Non-selective tetrameric cation channel
- Multi-modal activation:
  - Heat >43 °C
  - Low pH
  - “Hot” pepper (capsaicin)
- Cryo-EM spatial structures:
  - Open-state (3J5Q)
  - Closed-state (3J5P)

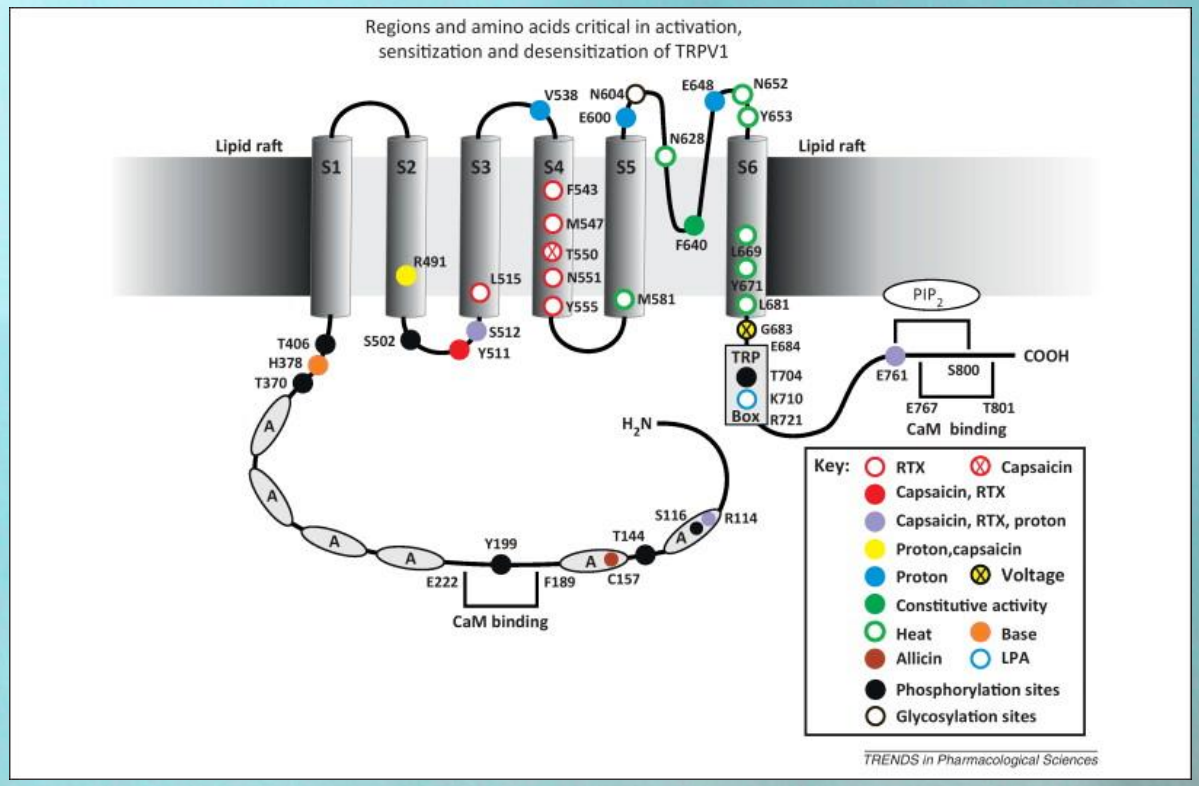
  Liao *et al.* (2013). *Nature* **504**, 107-112
- *Exact* activation mechanism is unknown





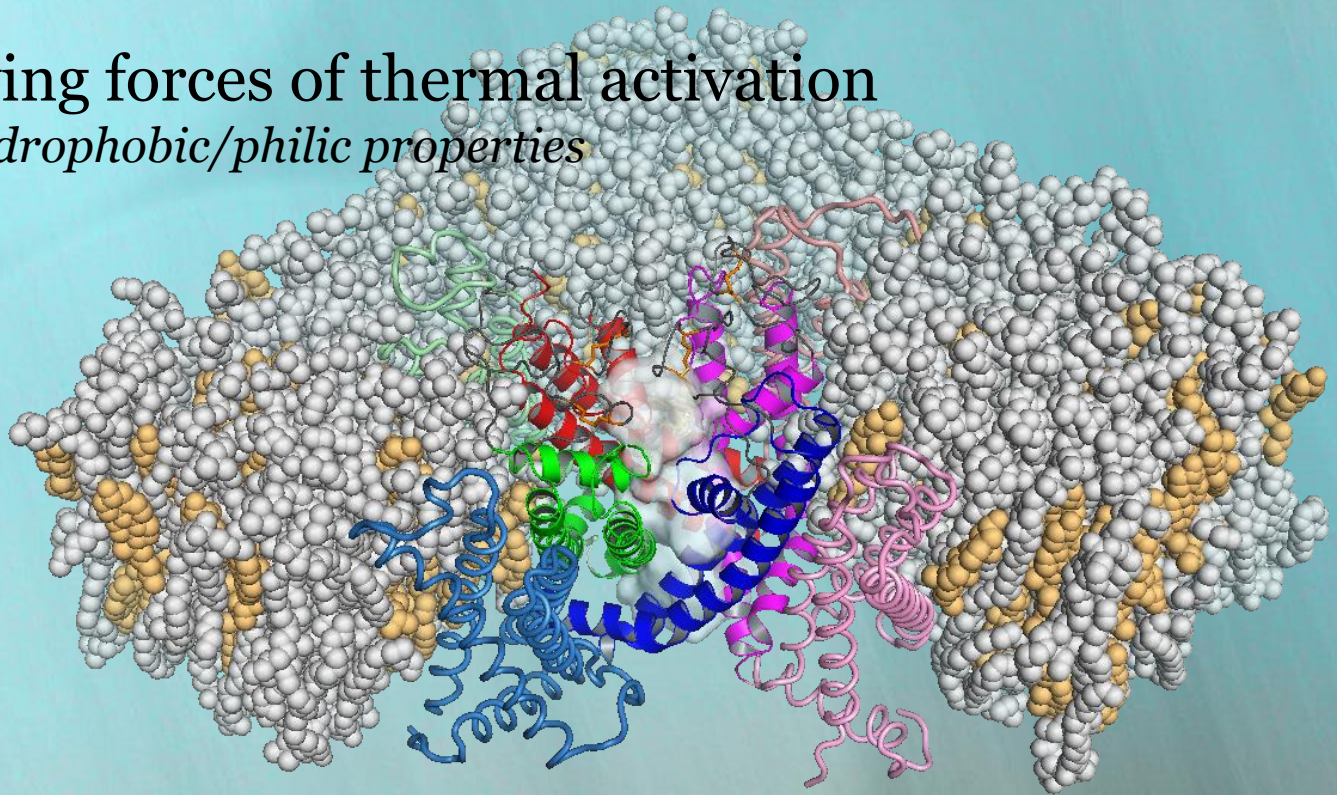
TRPV1 experiments

- Biochemistry (important residues)
- Computer simulations
  - Ion conductance
  - Capsaicin binding
- Cryo-electron microscopy (high-resolution structures in closed and open states)

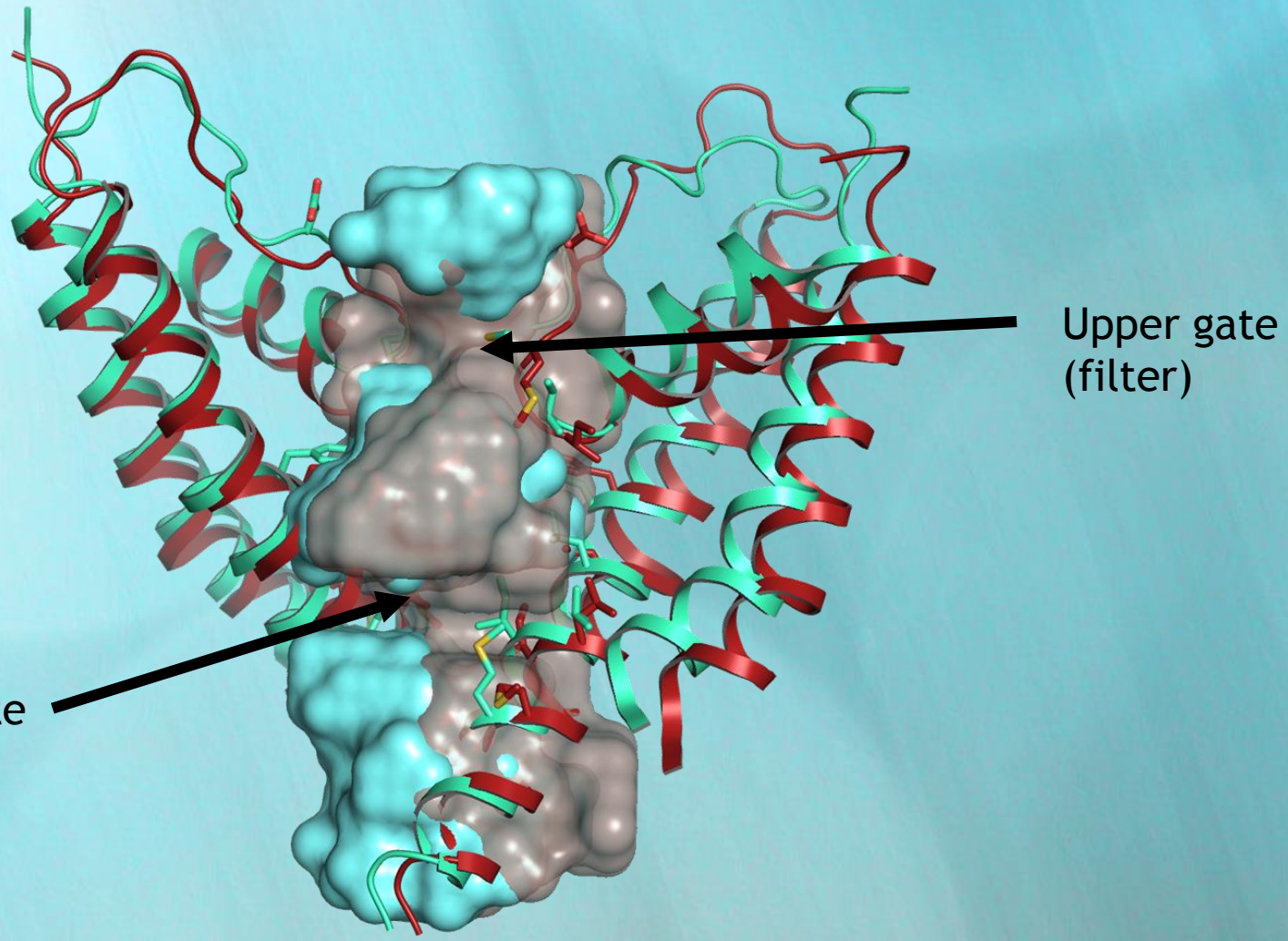


Computational study of TRPV1 receptor

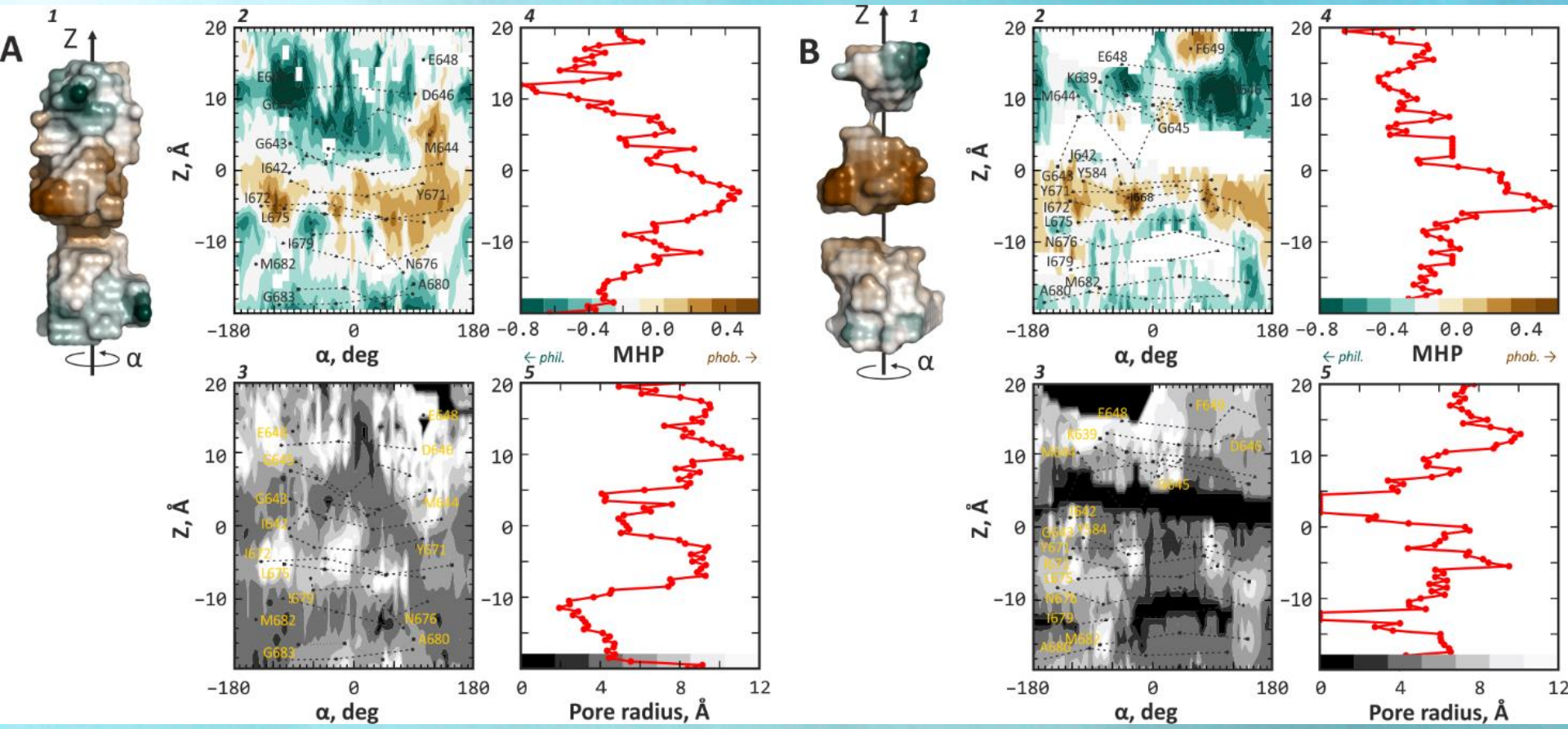
- Closed vs. open: what's the difference?
  Channel's pore mapping
- Simulation of thermal sensitivity
  Molecular dynamics at different temperatures
- Discover driving forces of thermal activation
  Assessment of hydrophobic/philic properties



Closed vs. open: what's the difference?



Closed vs. open: what's the difference?



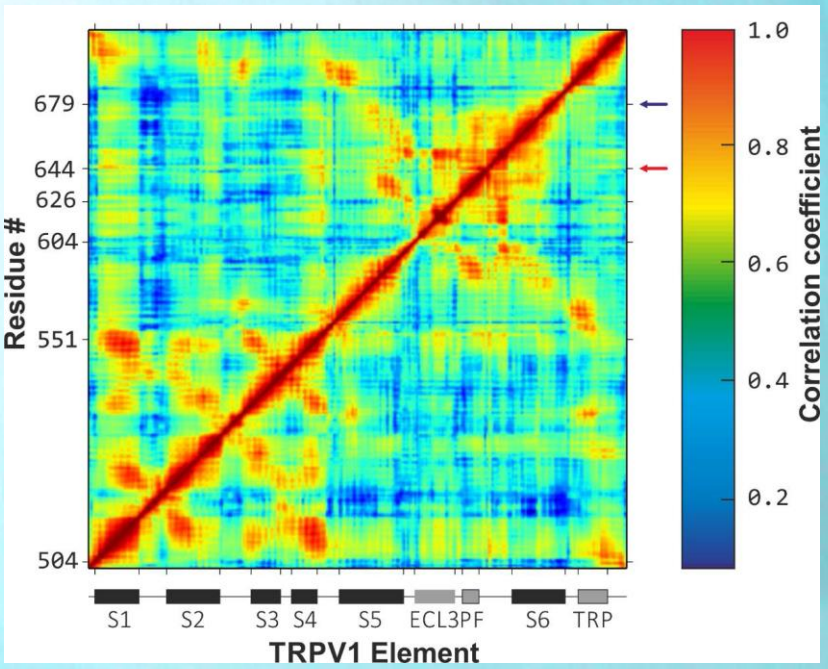
- A "two-gate" mechanism
- Upper gate: a "filter"
- Lower gate: "hydrophobic belt"

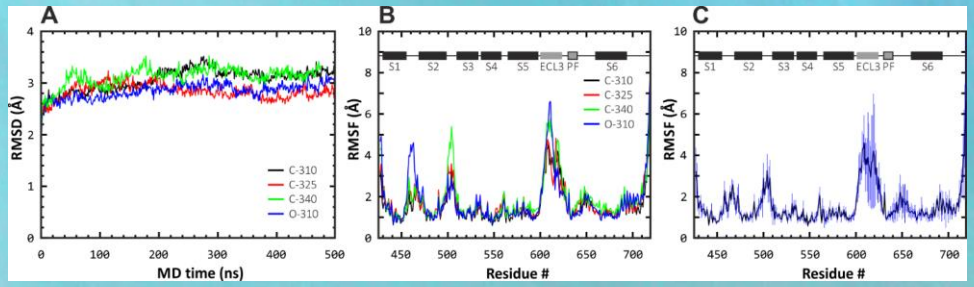
Computational set-up

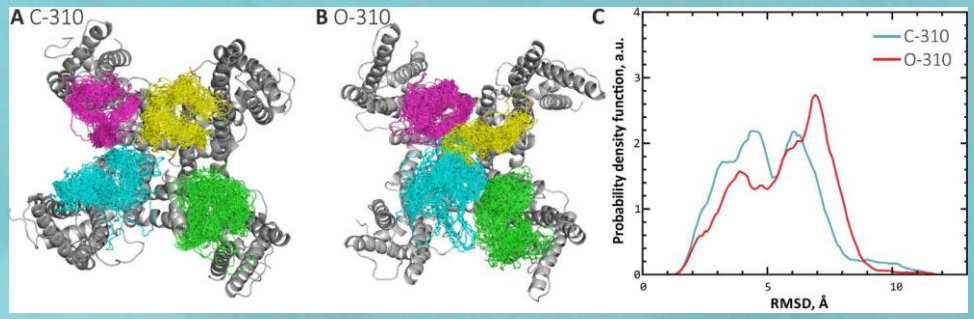
Force field: Amber-ildn + Slipids; tip3p water
Protocol: Gromacs; NPT with semiisotropic Parinello-Rahman barostat
Parameters: VdW: Cutoff 1.4 nm; Electrostatics: PME; Timestep: 2 fs
TRPV1 segment: 427-719

ID	Starting structure	Temperature, K	MD length, ns	System composition
c-280.1 c-310.1 c-310.2 c-325.1 c-325.2 c-340.1 c-340.2	**3J5P**	280 310 310 325 325 340 340	500 500 500 500 500 1000 500	Protein 4 POPC 256 POPE 121 CHOL 148 Water 43655 Cl^- 40
c-340 → 310	c-340.1	310	500	
o-280.1 o-310.1 o-310.2	**3J5Q**	280 310 310	500 1000 500	≈ the same
Several TRPV1 mutants: G643A, I679A+A680G, K688G/P				

TRPV1 "domain portrait"

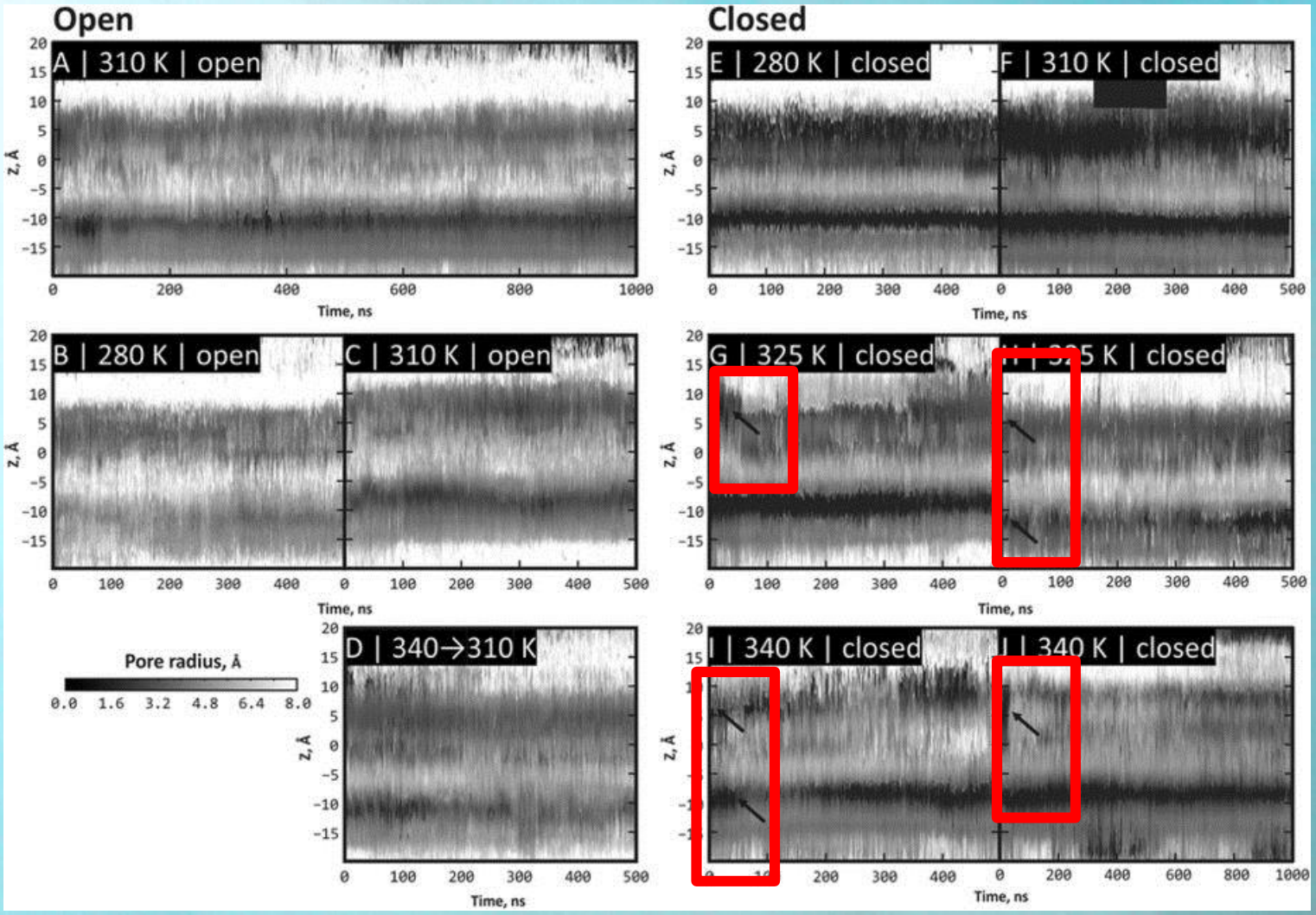






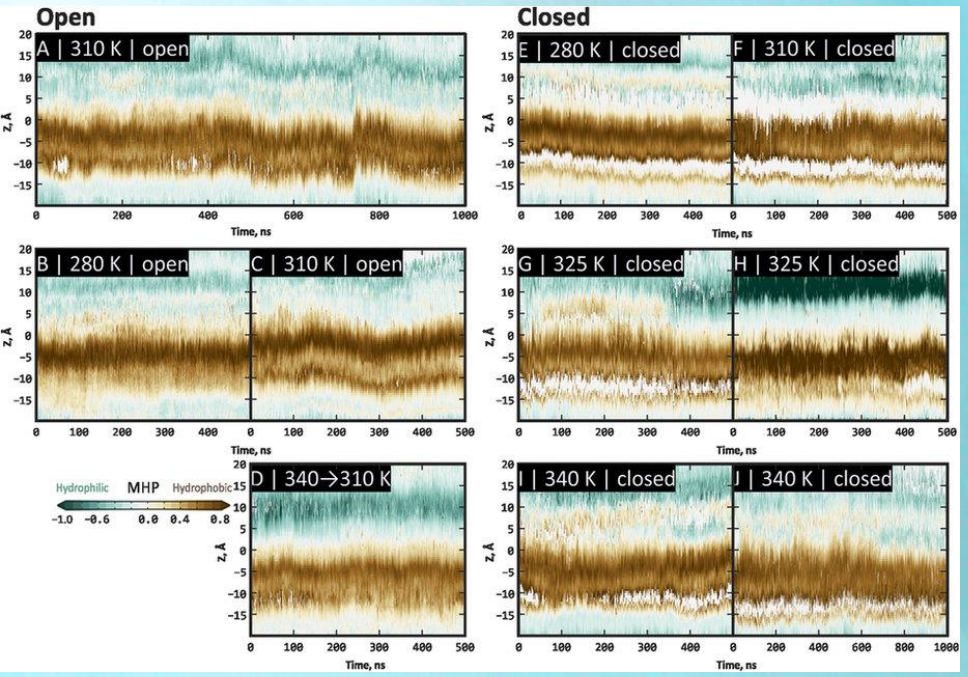
- S1–S4 (ligand-binding) domain remain stationary
- ECL3 is conformationally heterogeneous
- Pore domain is most flexible

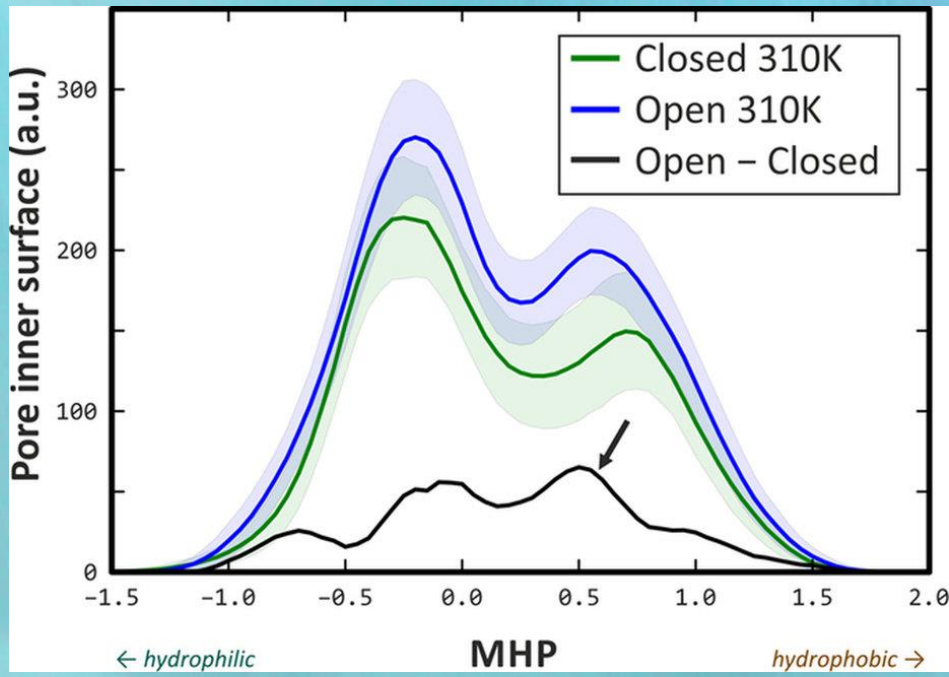
Heating: open the pore!



- Closed state (partially) opens upon heating
- Open state *does not* close upon cooling

TRPV1 opening: being hydrophobic!



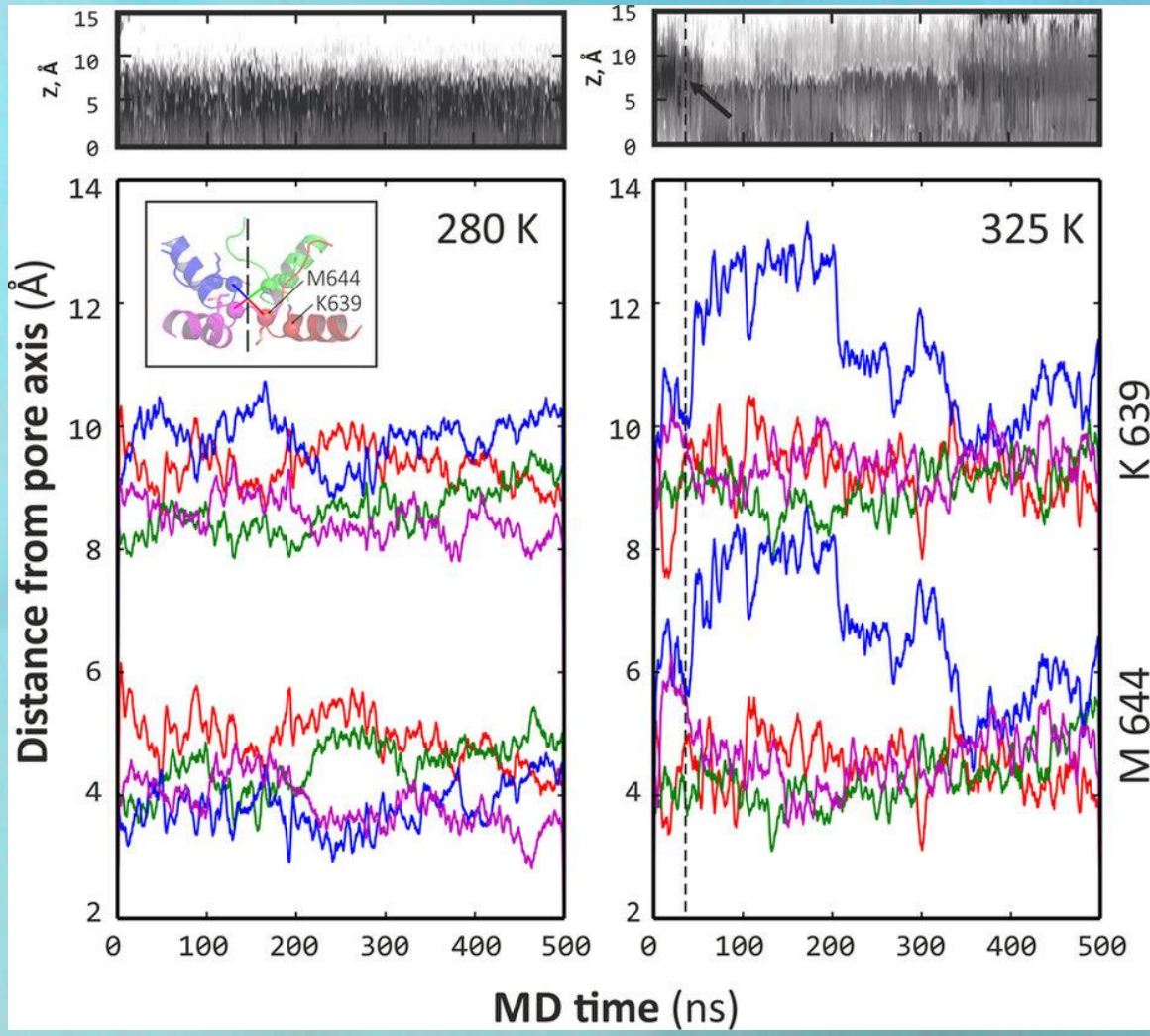


- In the open state pore surface is more hydrophobic
- This increases heat capacity (C_p) and entropy (S)
- This effect (partially) underlies temperature sensation

Clapham & Miller (2011). *Proc. Natl. Acad. Sci. U.S.A.* **108**, 19492–19497

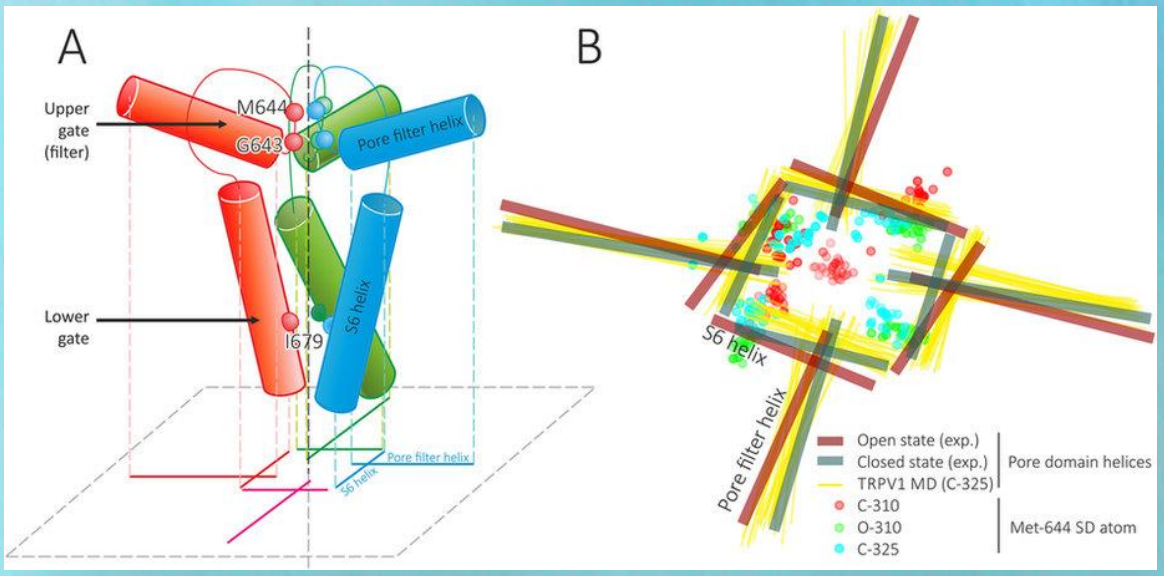
Upper gate opening

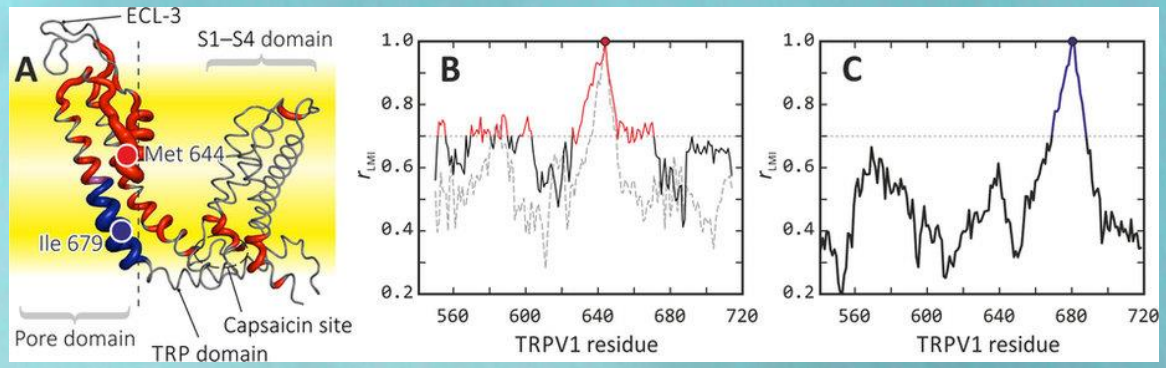
- TRPV1 opening: “filter” at the level of Met 644
- “Asymmetric” opening: one subunit goes first



Upper gate opening: a piston-like mechanism

- Met 644 plays crucial role
- Lots of correlated movements
- Opening of the lower gate is correlated with S6 fragment

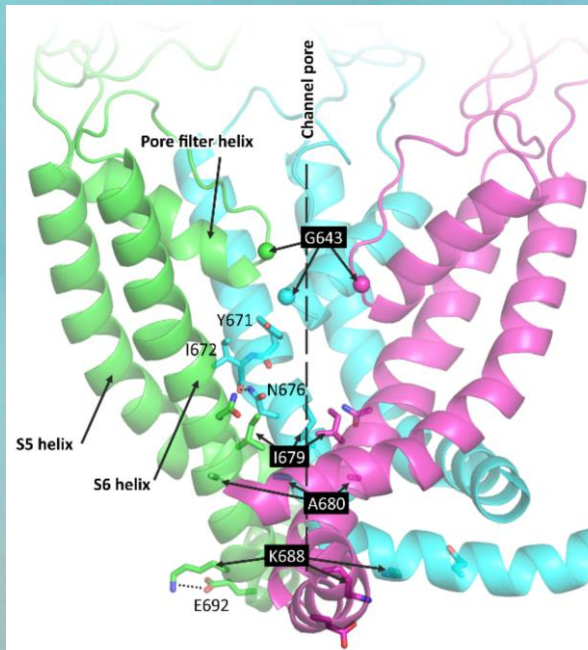




TRPV1 "hot spots":

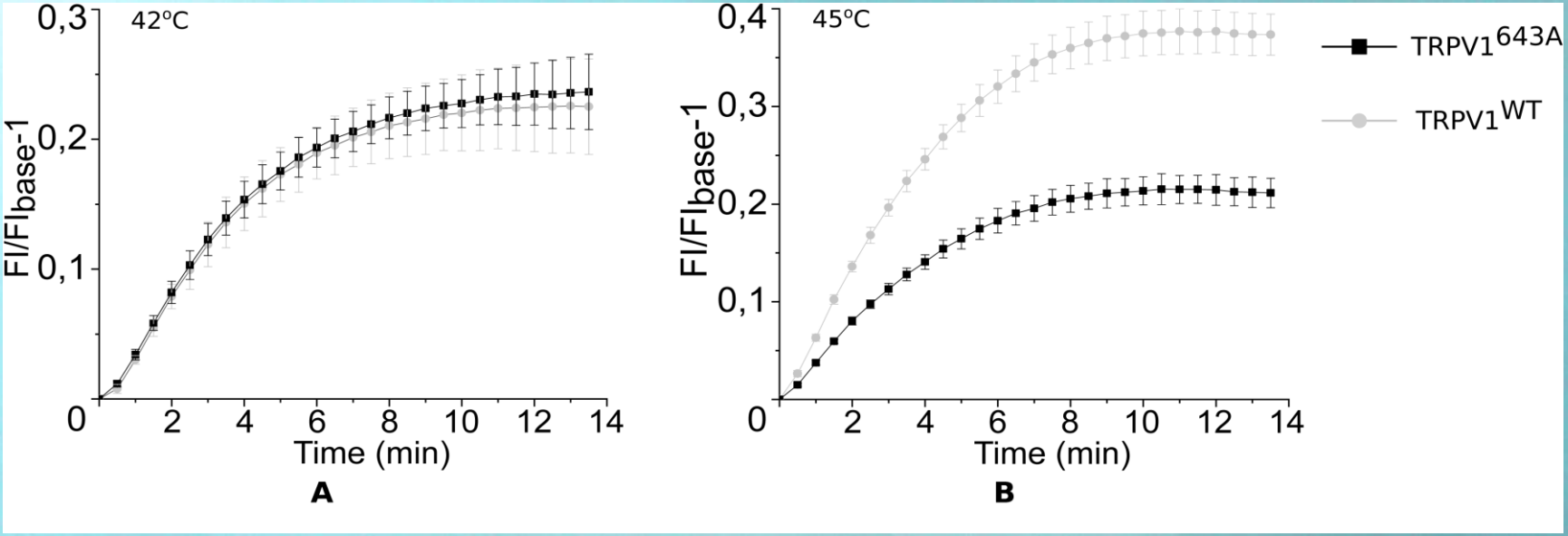
- G643: the upper gate "bottleneck"
- I679, A680: the lower gate "bottleneck"
- K688: the "hinge" of the TRP domain

We recombinantly produced and biochemically studied the following mutants:
G643A, I679A/A680G, K688P, K688G



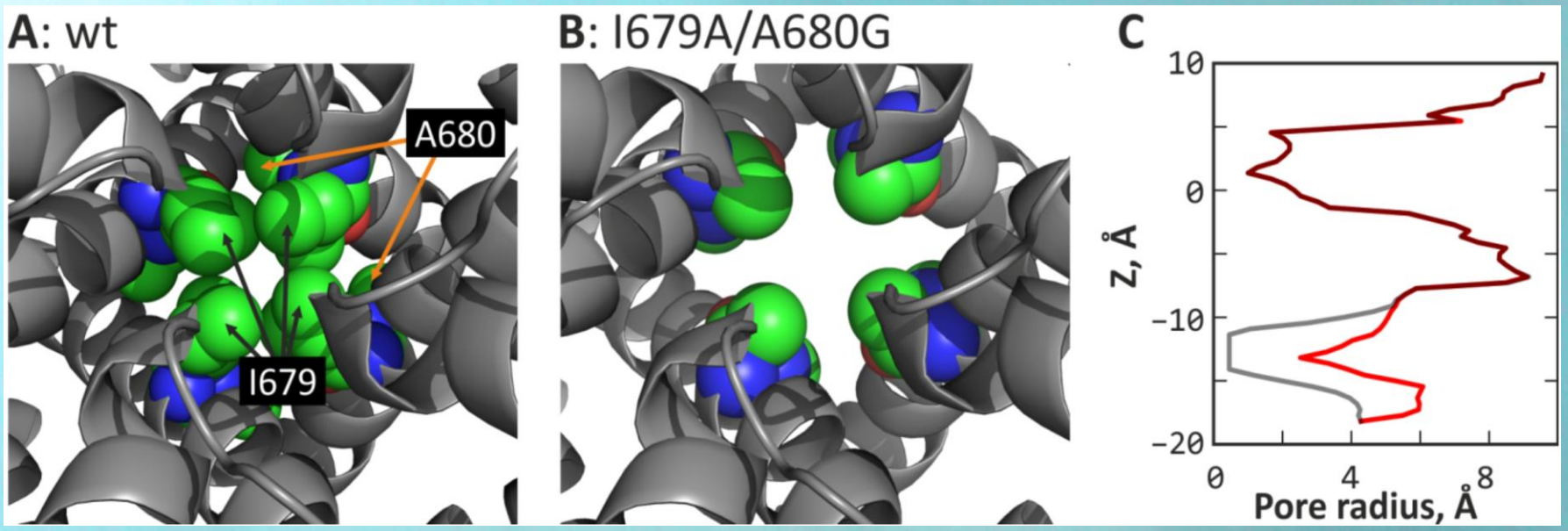
G643: the upper gate “bottleneck”

The substitution G643A reduced maximal conductivity that resulted in normal response to moderate stimuli, but relatively weak response to more intensive activation



I679, A680: the lower gate "bottleneck"

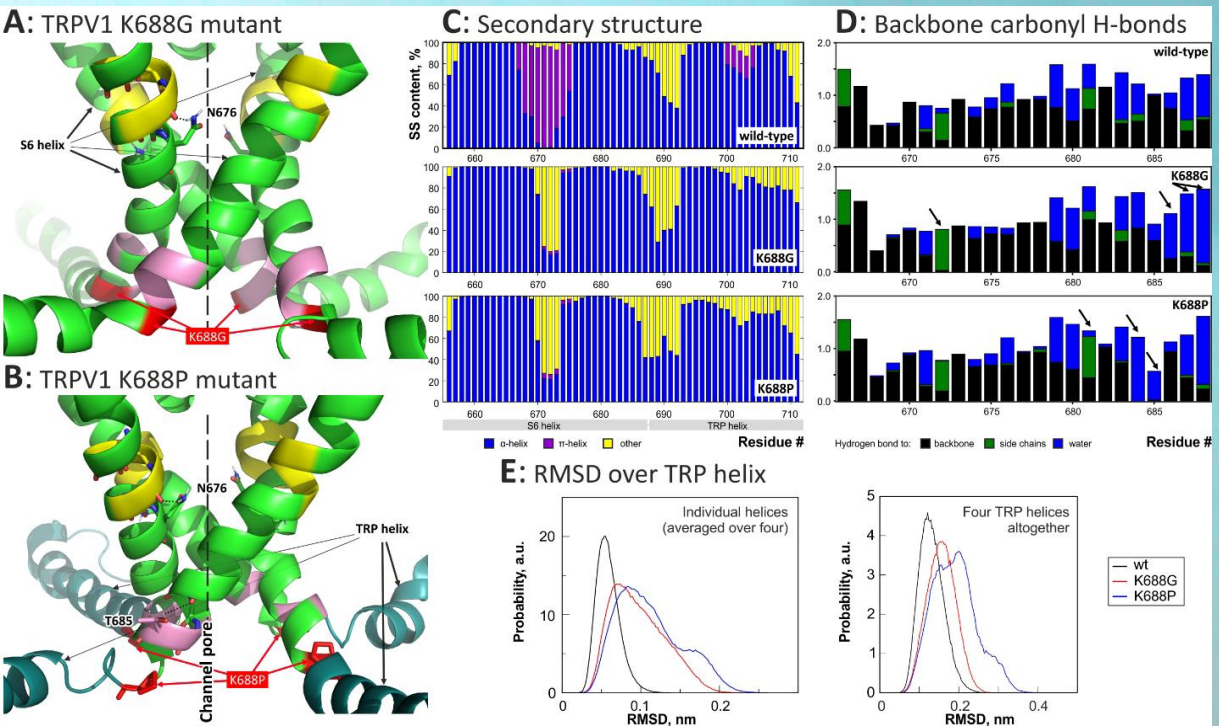
I679A+A680G channel was severely toxic for oocytes most probably due to "always open" phenotype

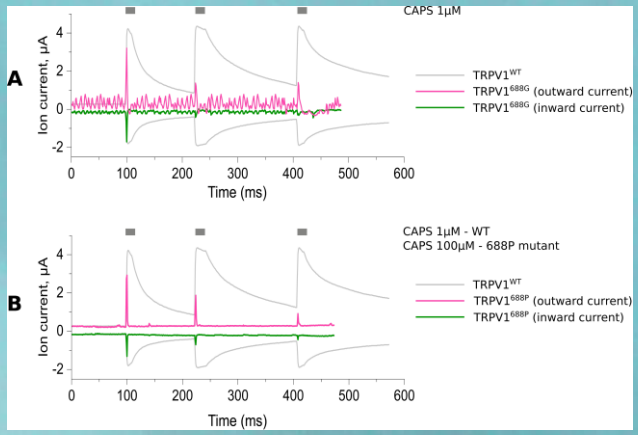


K688: the "hinge" of the TRP domain

K688P impairs TRP domain directed movement, shows ~100-fold less sensitivity to the capsaicin, enhanced desensitization and weaker activation by the heat.

K688G facilitates movements of TRP domain and disturbes its coupling to the pore, thus leading to spontaneous activation and enhanced desensitization of the channel.





Conclusions

1. Computations reveal temperature sensation in TRPV1 channel

2. Opening of the upper and the lower gates are independent and asymmetric

3. Upper gate: a piston-like mechanism

4. Lower gate: correlated motion of S6 and bending of TRP domain
5. Roles of residues G643, I679+A680, K688 are clarified

Publications: Chugunov *et al.* (2016). *Sci. Rep.* 33112
Lubova *et al.* (2020), submitted