Mechanism of chloride channeling by Excitatory Amino Acid Transporters

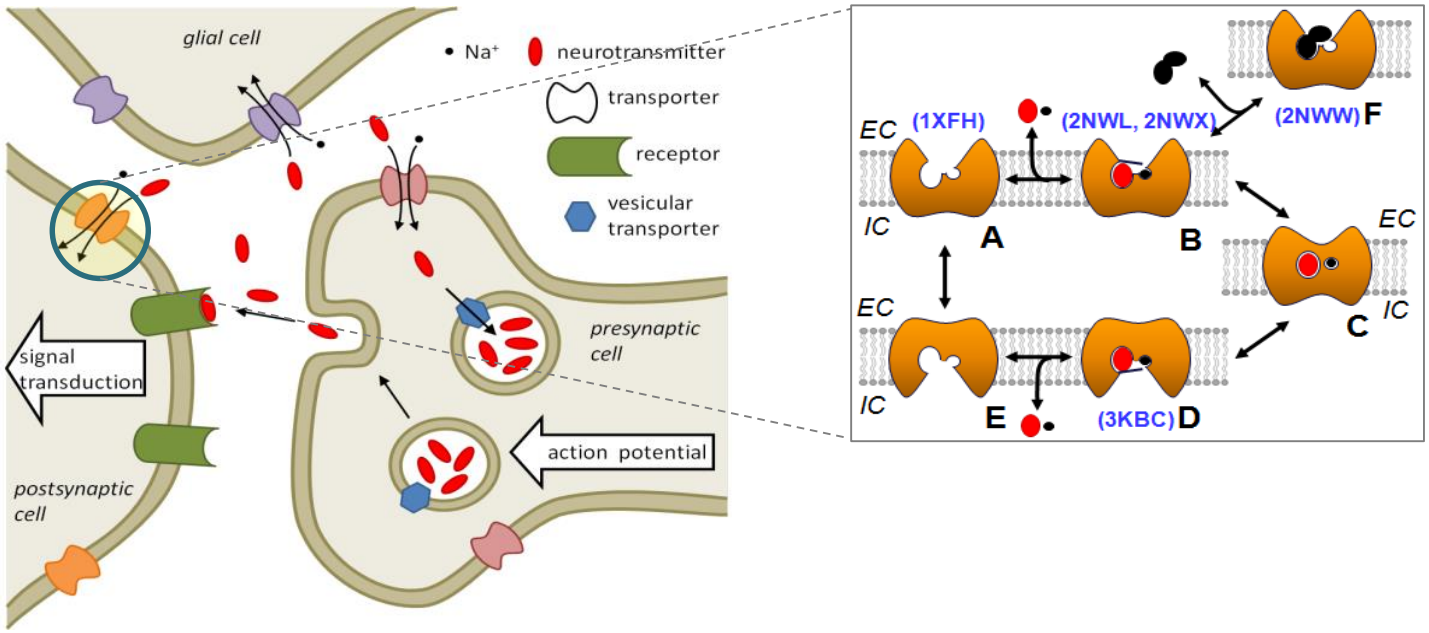
Mary Cheng, D. Torres-Salazar, S. Amara & I. Bahar

Department of Computational & Systems Biology

School of Medicine, University of Pittsburgh

Susan Amara

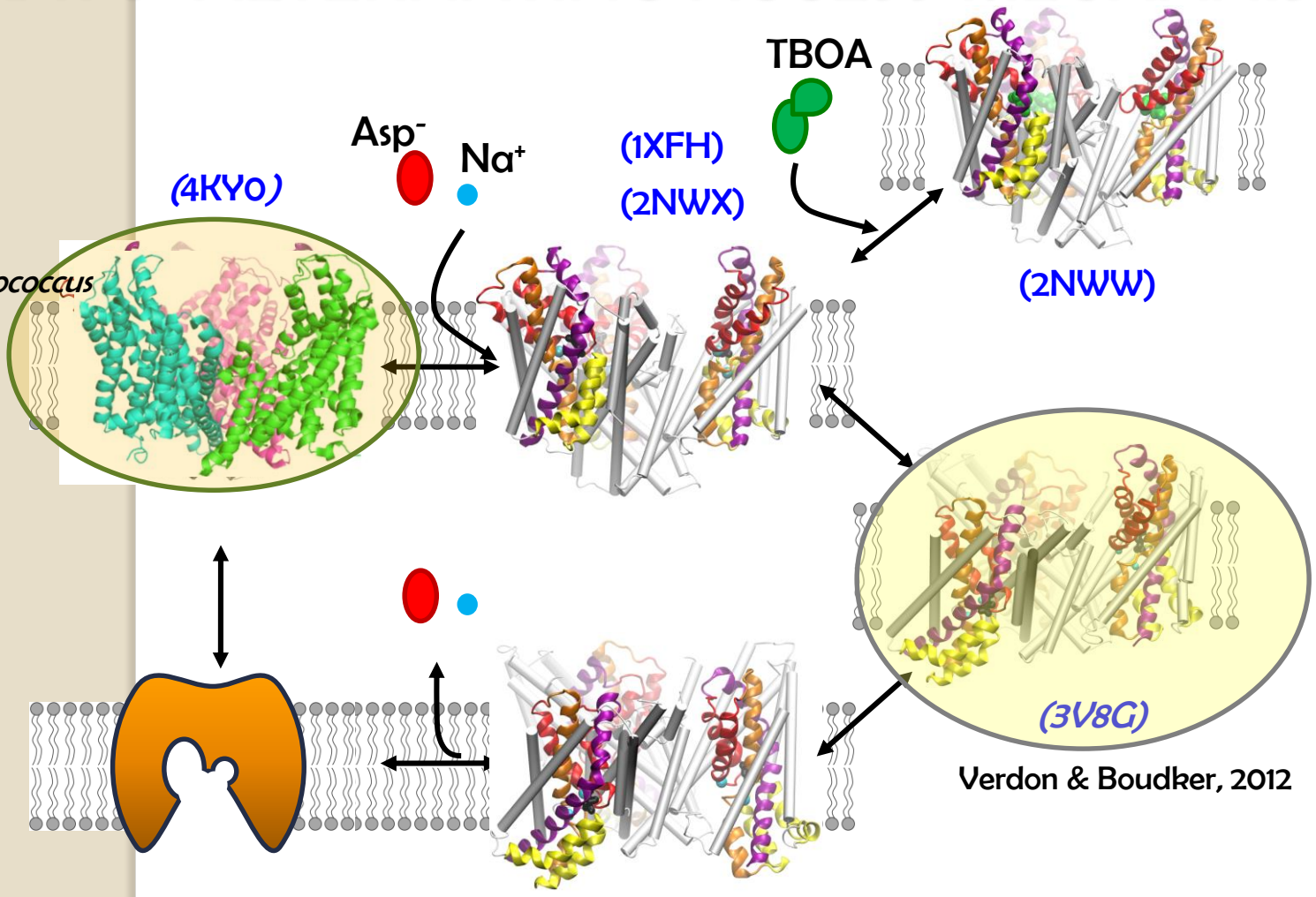
GLUTAMATERGIC SIGNALING



References

Gu et al (2009) *Proc Natl Acad Sci USA* 106: 2589-94; Shrivastava et al (2008) *J Biol Chem* 283: 28680-90

EAAT'S ALTERNATING ACCESS MECHANISM



Jensen et al., 2013
GltTk from *Thermococcus kodakarensis*

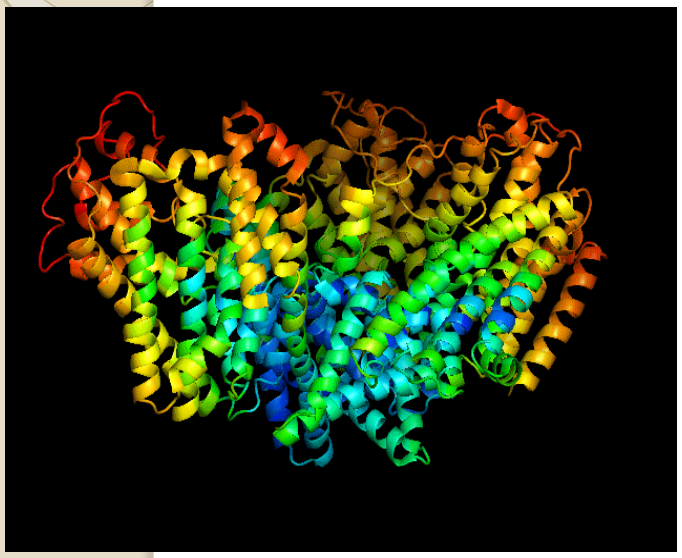
Verdon & Boudker, 2012

Gouaux, Boudker and Slotboom labs

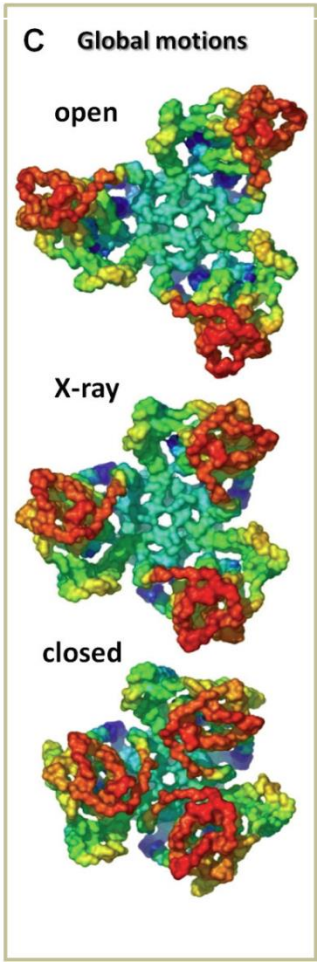
References

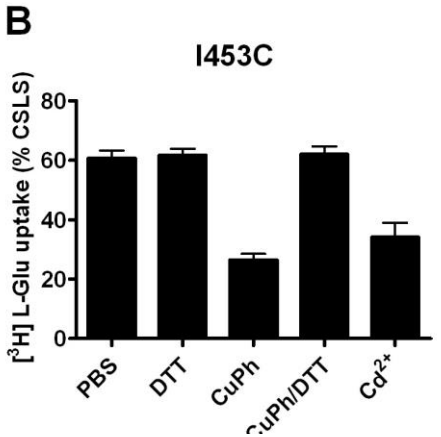
Lezon & Bahar (2012) *Biophys J* 102: 1331-40; Jiang et al (2011) *Proc Natl Acad Sci USA* 108: 15141-6
DeChancie et al (2011) *Mol. BioSyst* 7:832-42;

Cooperative Intersubunit Motions are Essential to Function

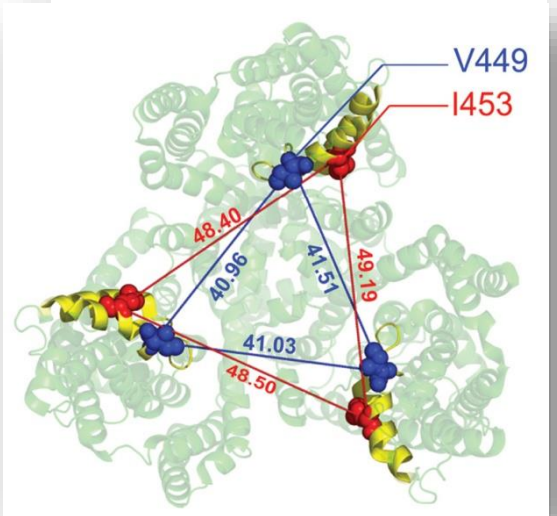


Jiang et al., *PNAS* 2011





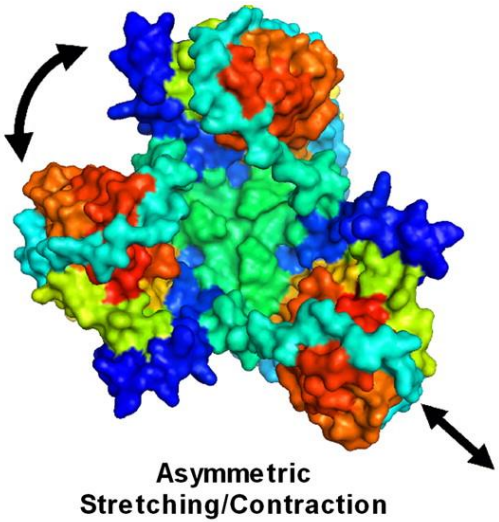
Residues mapped onto EAAT1

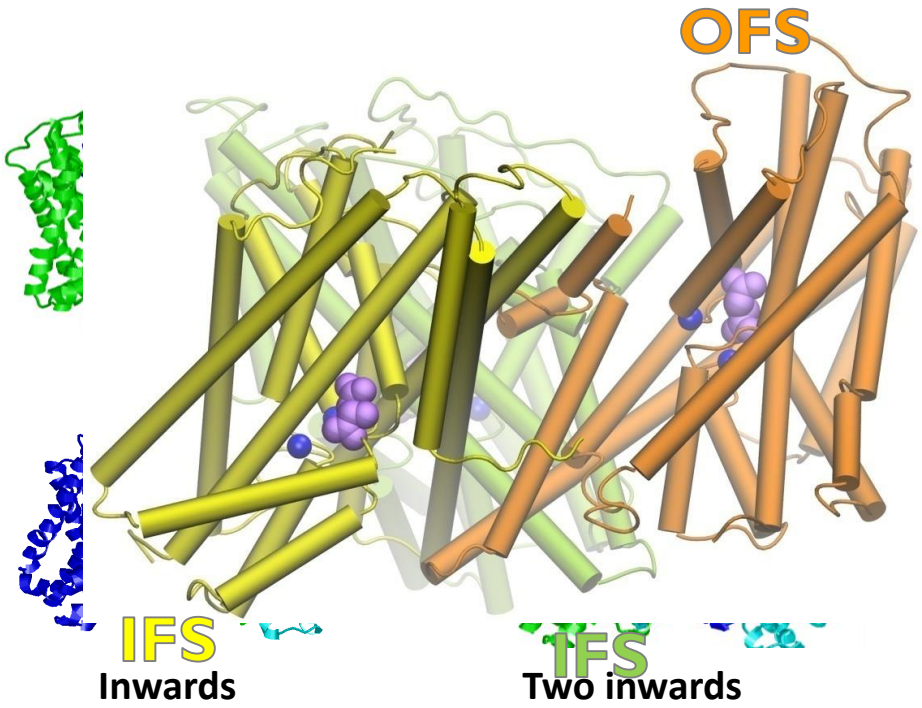


Glutamate uptake blocked by disulfide cross-linking between 'distant' pairs

Transition of one subunit at a time:

a mechanism revealed by our experiments & computations





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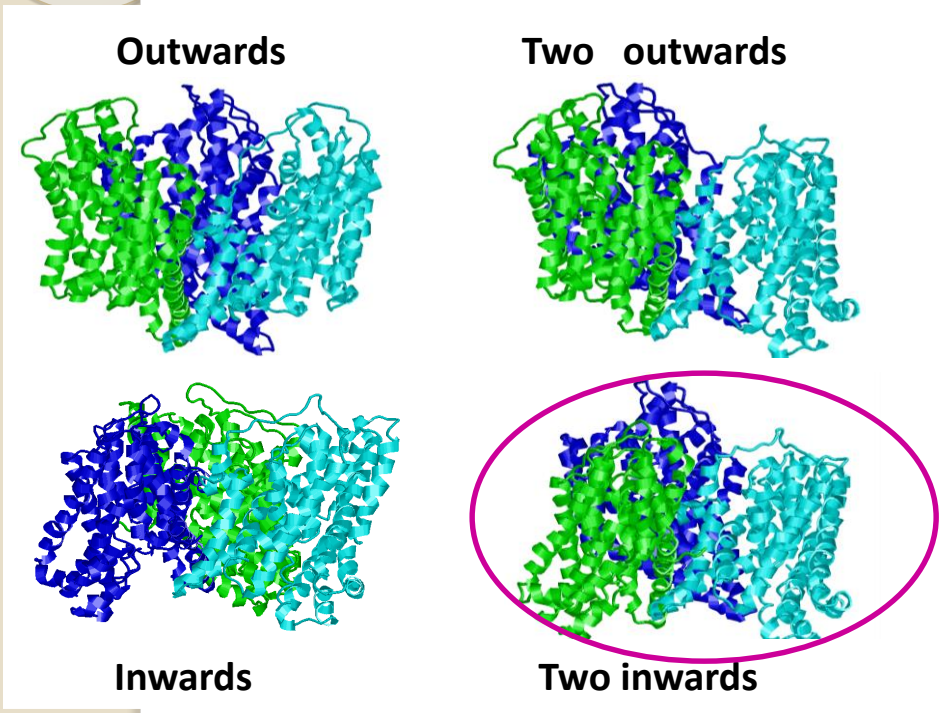
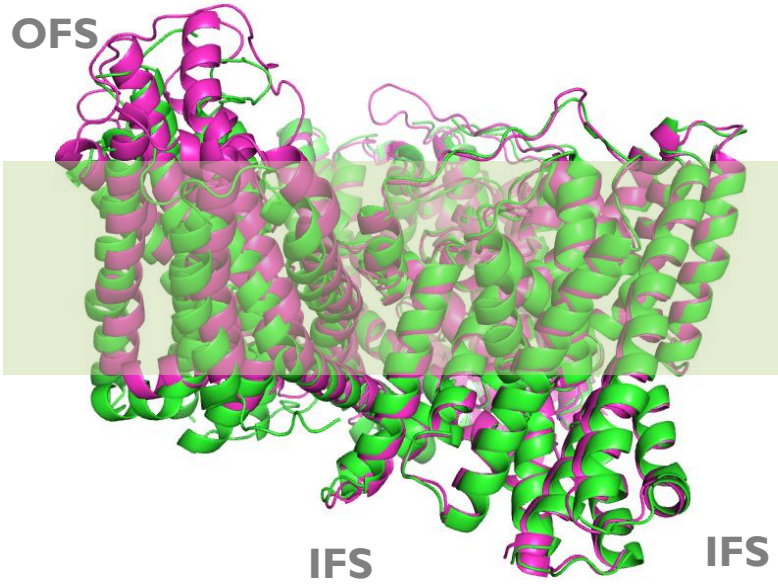
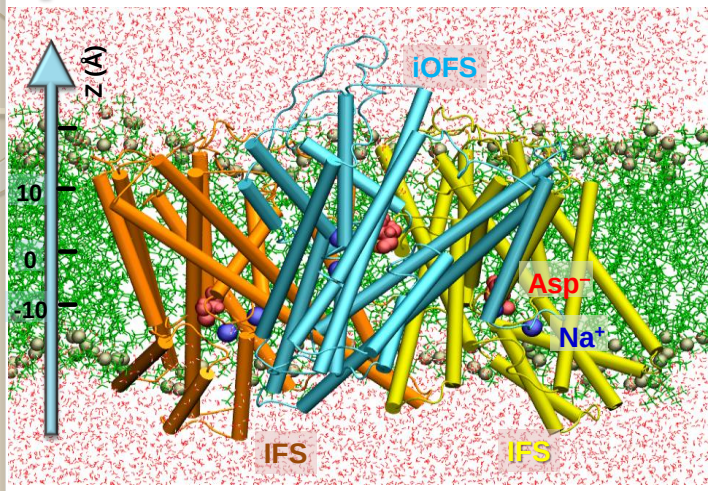


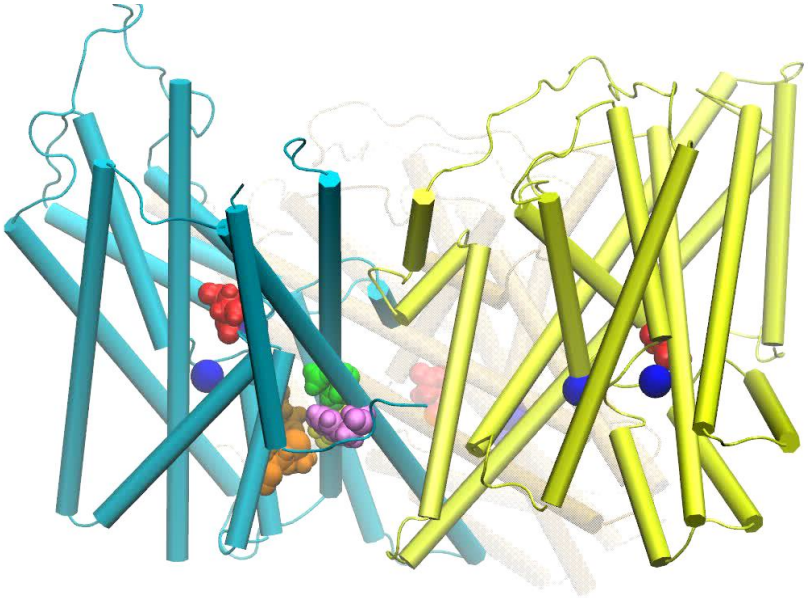


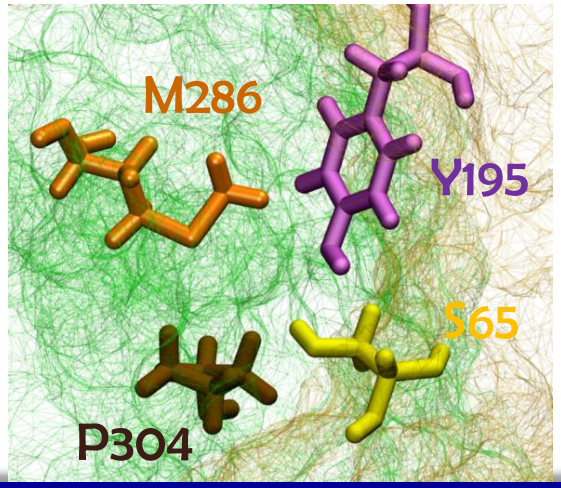
RMSD = 1.382 Å

Intermediates mediate permeation of polar solutes



MD setup for Glt_{Ph} (PDB:3V8G)

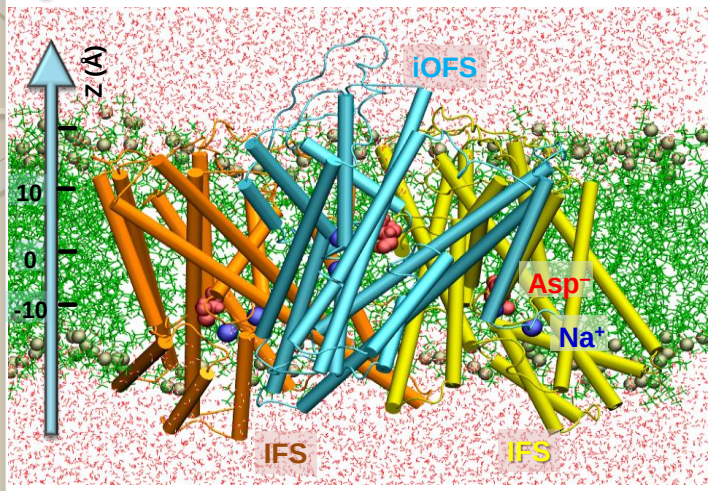




The putative anion pathway suggested by Verdon and Boudker shows a constriction zone near S65, Y195, M286 and P304

Verdon and Boudker , NSMB 2012: 19;355

Intermediates mediate permeation of polar solutes



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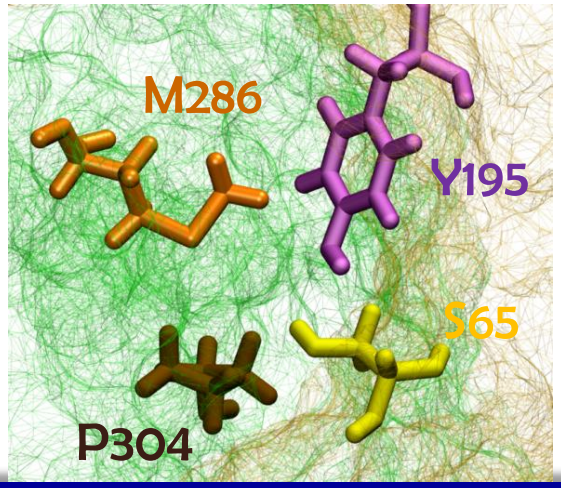
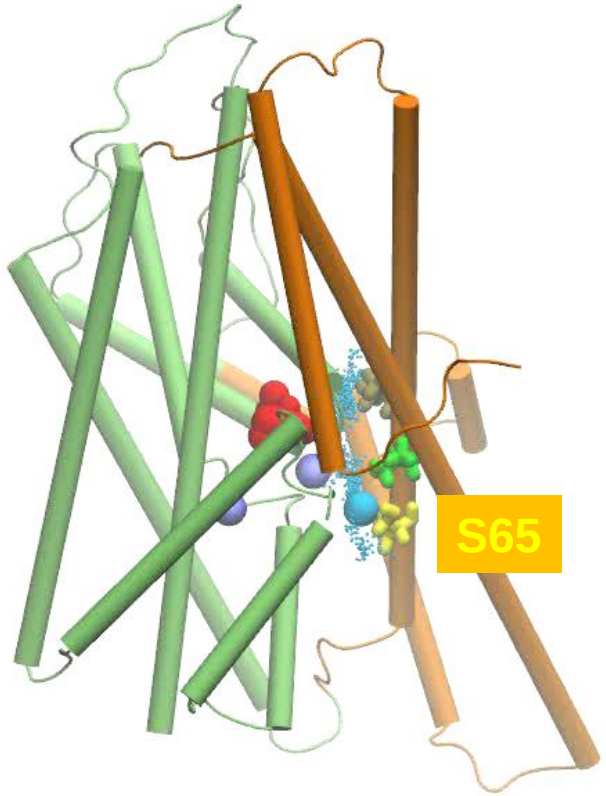
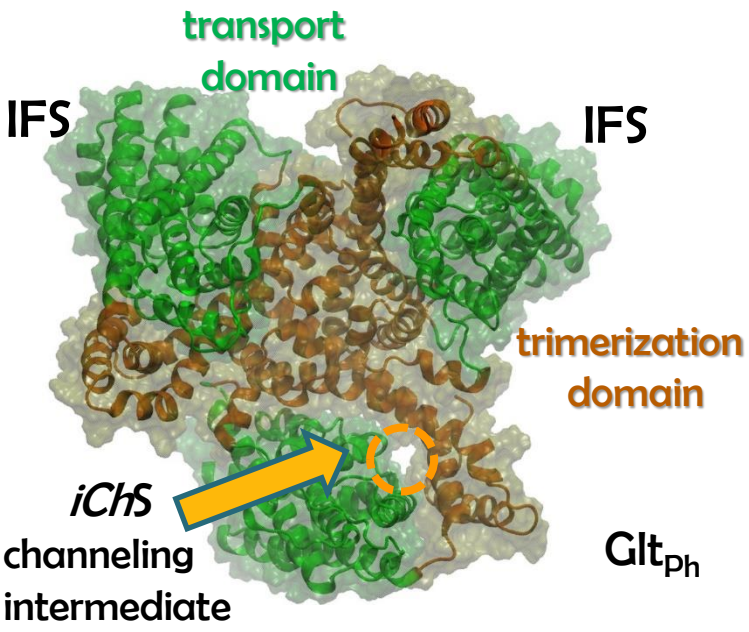


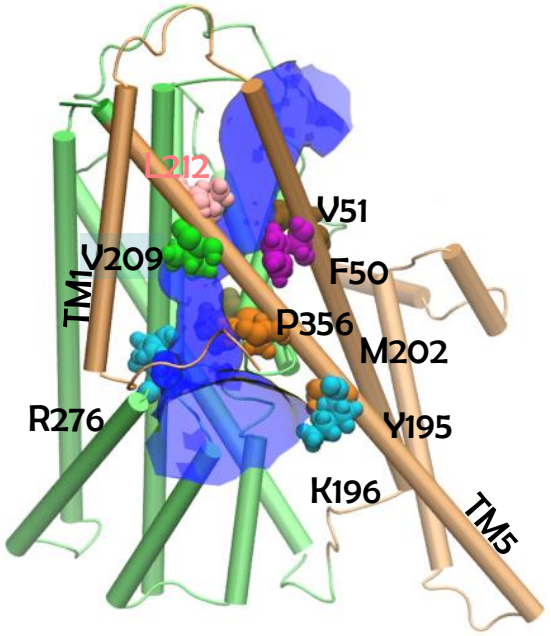


Energy barrier ~20 kcal/mol

*Ryan & Mindell *Nat Struct Mol Biol* **14**, 365-71 (2007)

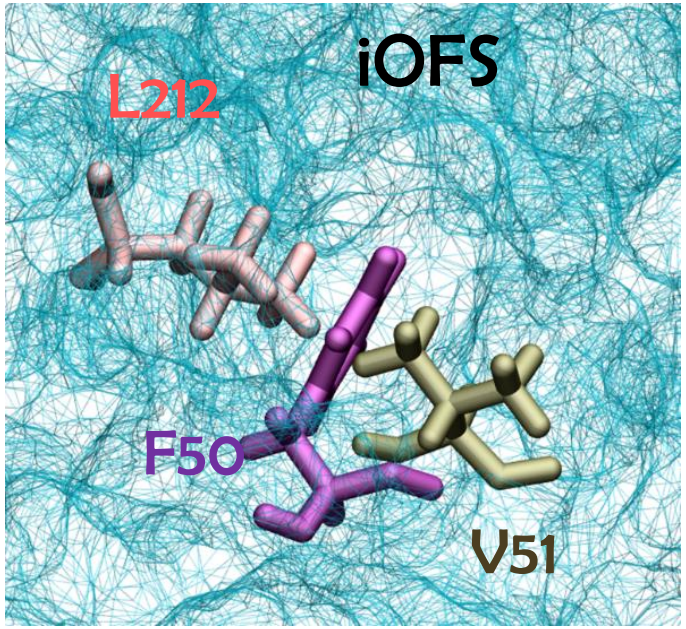
A intermediate water-channeling state iChS forms during the transition to inward-facing conformations



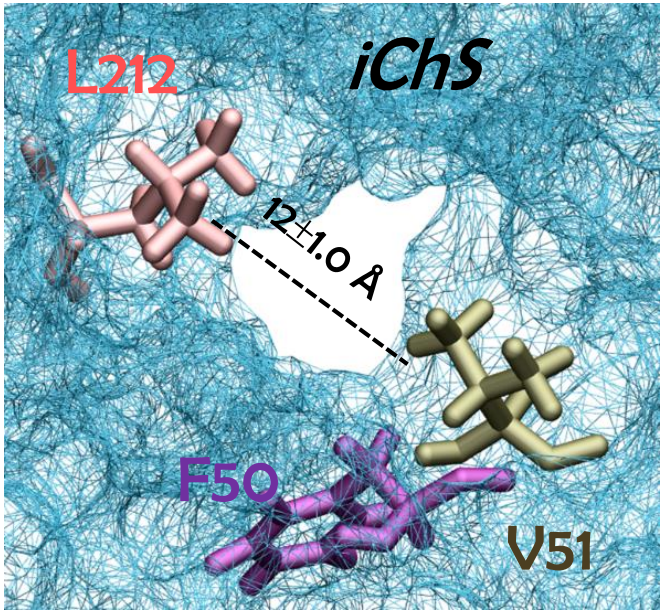


A intermediate water-channeling state iChS forms during the transition to inward-facing conformations

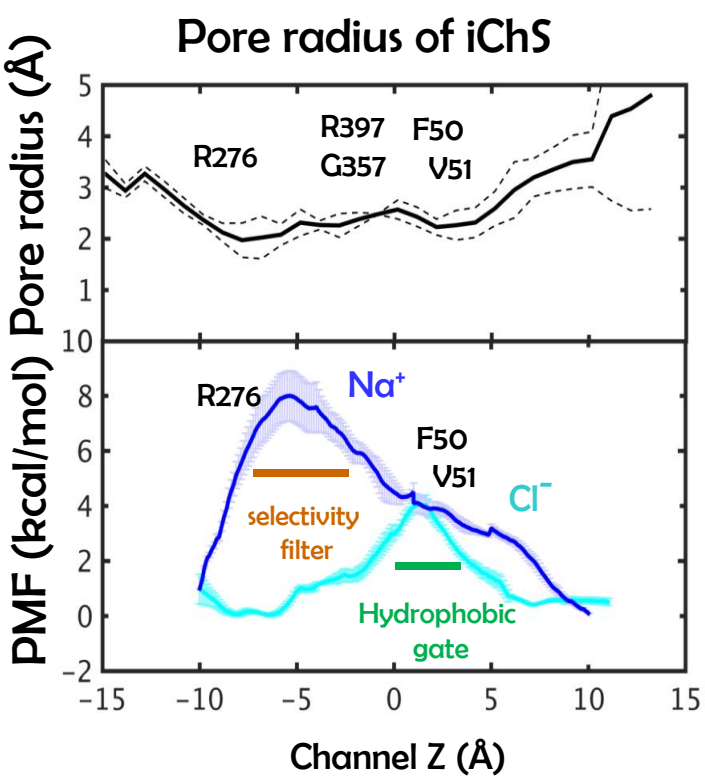
Closed state



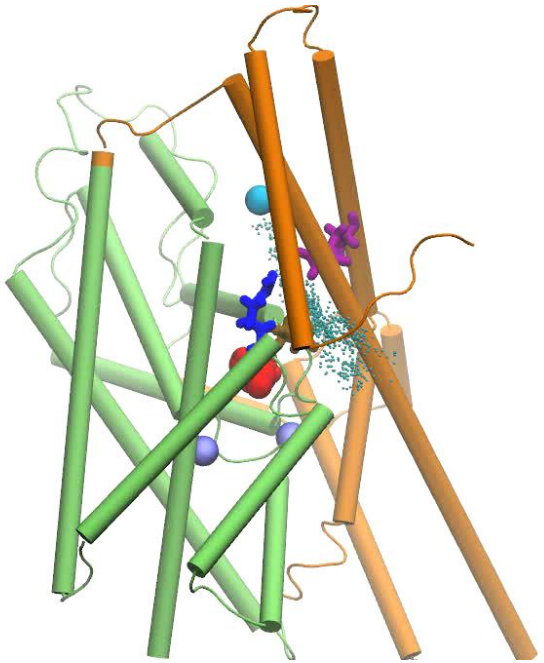
Open state



iChS favors anion permeation

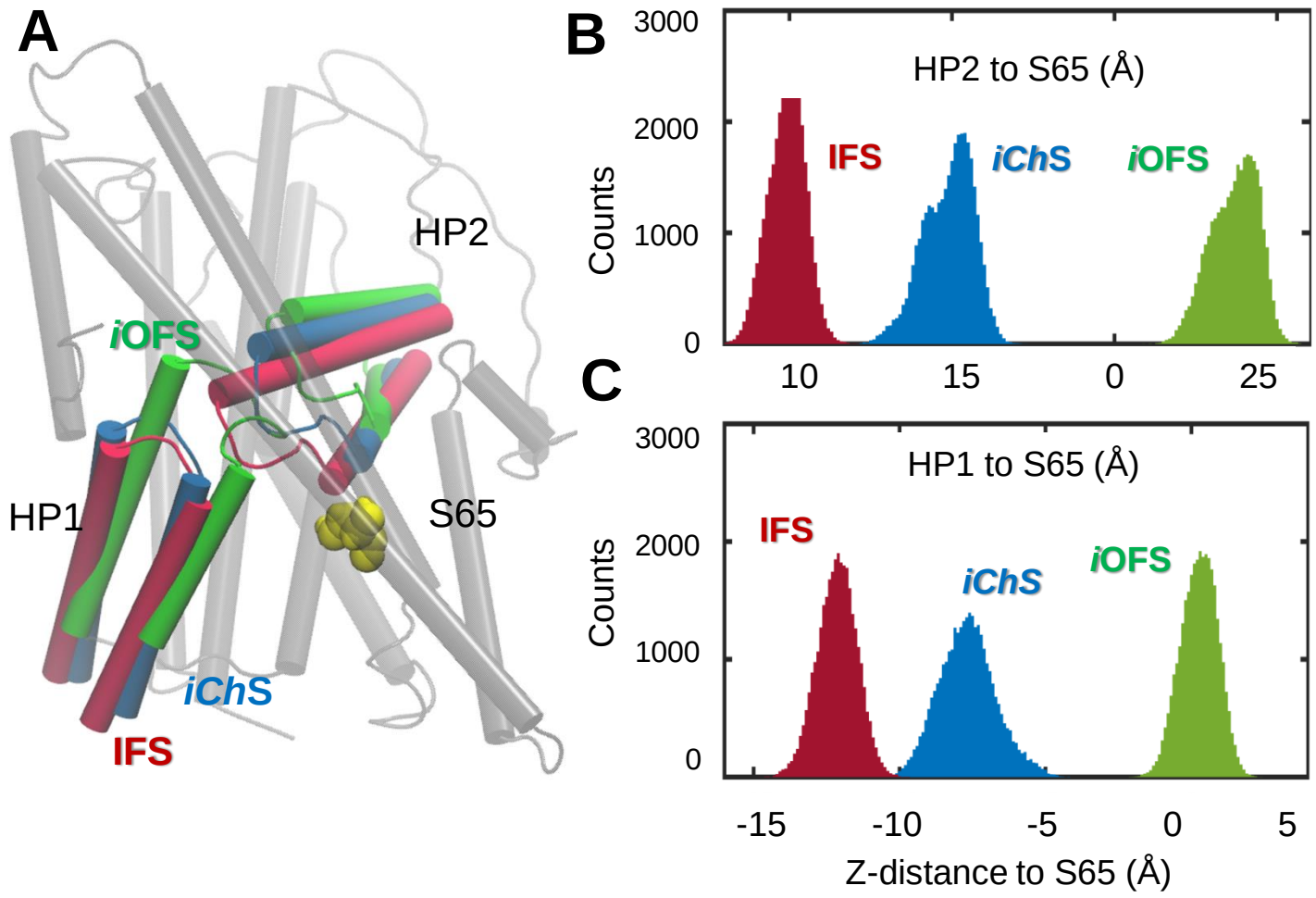


iChS

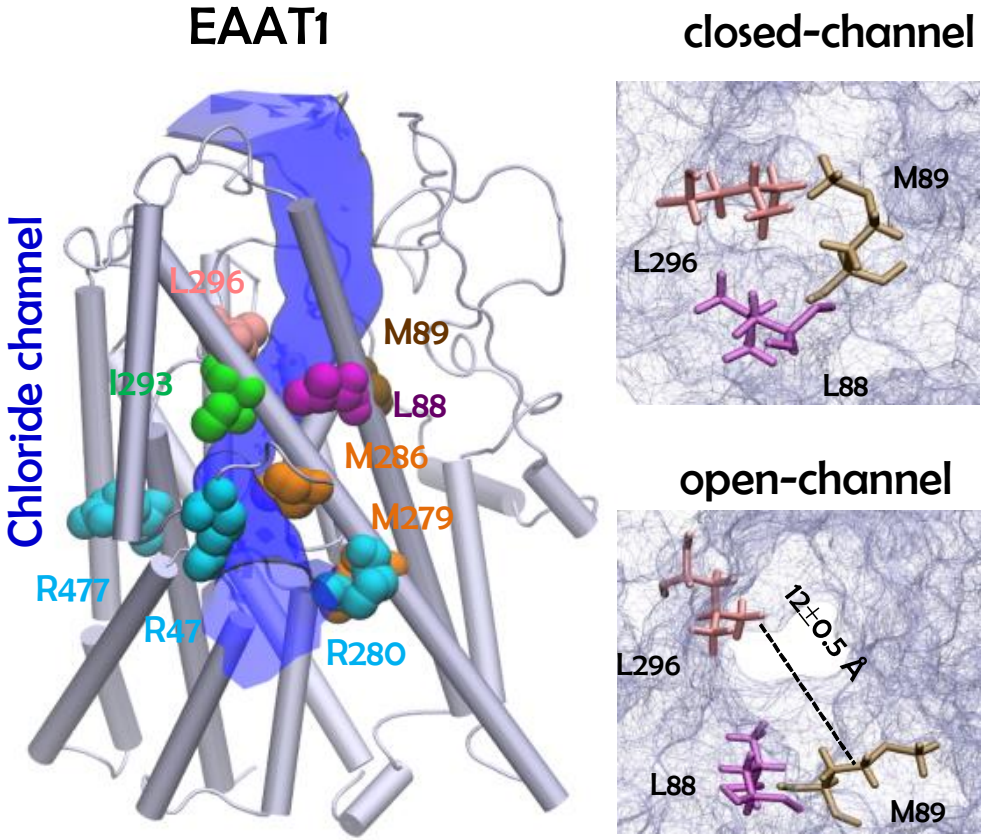


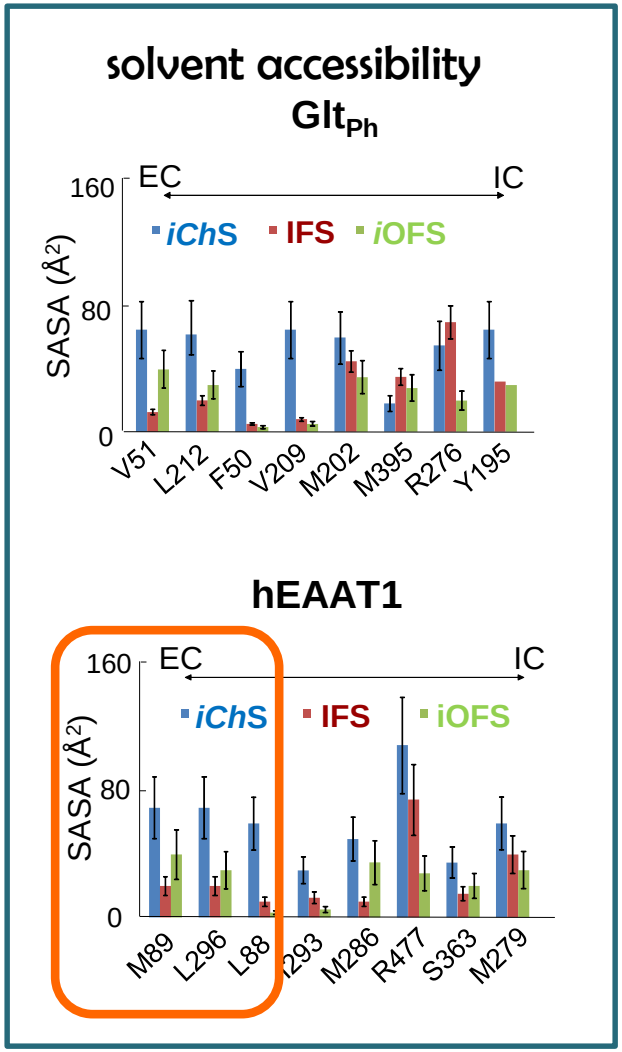
Energy barrier < 5 kcal/mol

iChS distinct from OF and IF states



Same channel confirmed in EAAT1





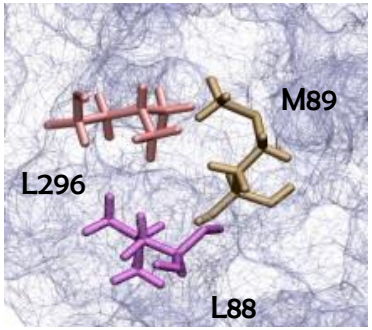
Hypothesis:

Three residues are found to control the channel opening or closure, and they are solvent-exposed prior to transitioning to closed state.

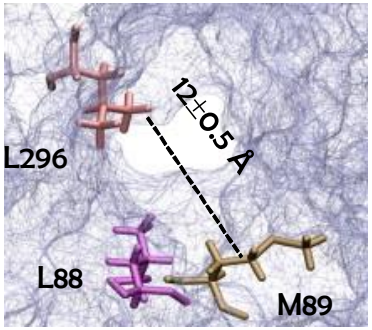
Suppose we bind at those positions a bulky molecule at those positions.

Do we observe an effect on channel permeability?

closed-channel

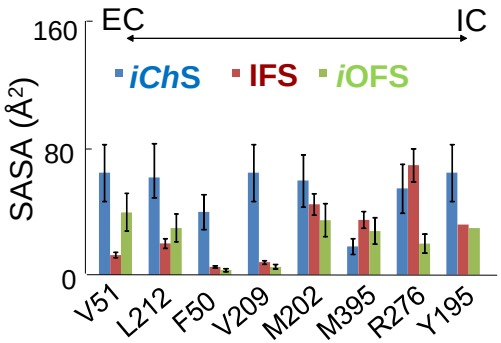


open-channel

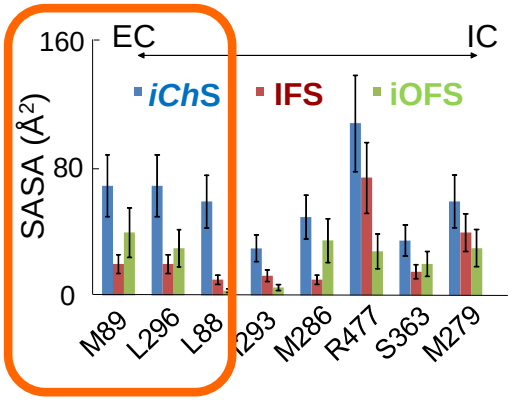


solvent accessibility

$GltPh$

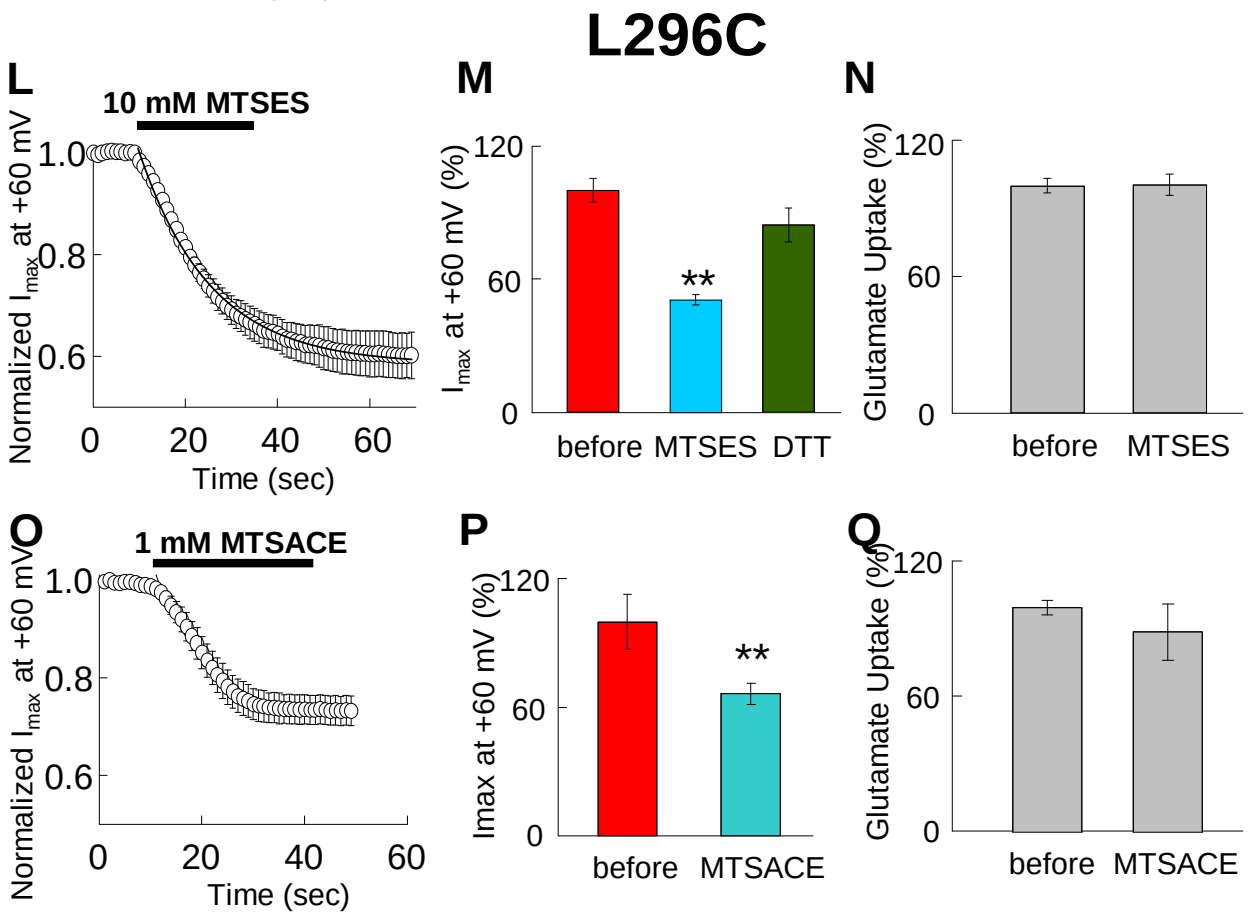


hEAAT1



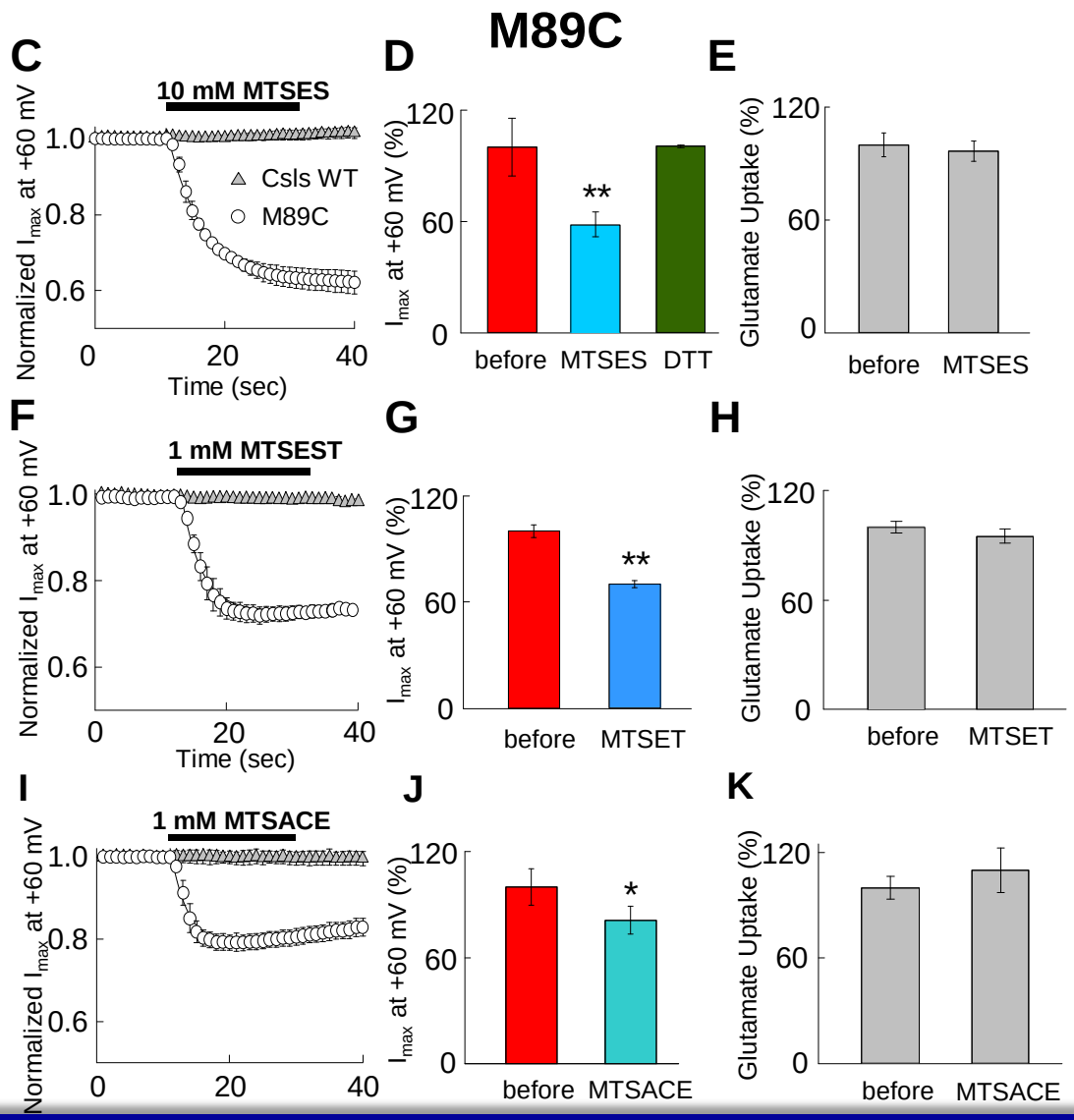
Experiments confirm the key role of L296

SCAM | Electrophysiology | Glu uptake



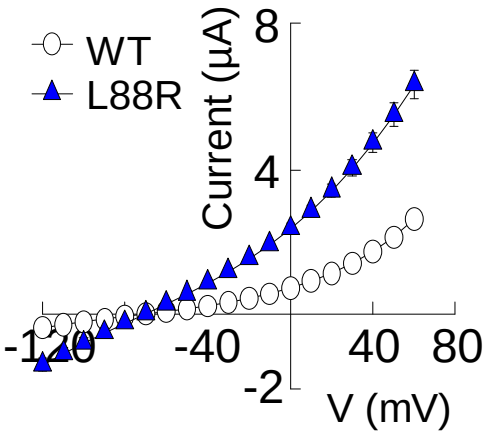
MTS-reagents that are covalently bound to cysteines: MTSES :negatively-charged; MTSET: positively-charged MTSACE : neutral DTT: reagent to reduce the disulfide bridge

M89 in EAAT1 also controls anion permeation

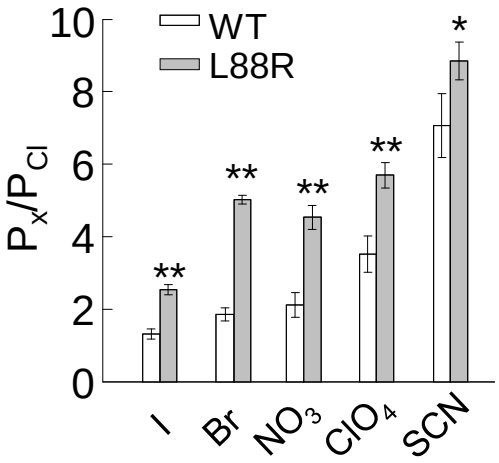


L88 controls both anion permeation and substrate transport

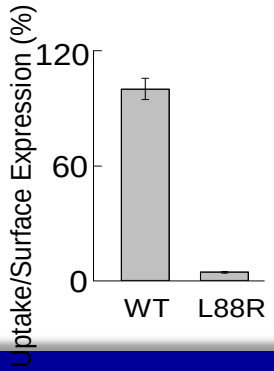
Current-voltage



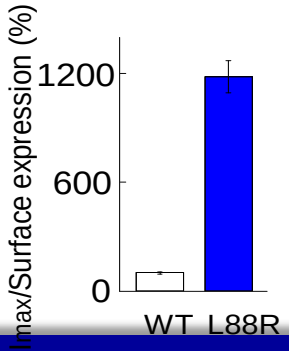
ion selectivity



Glu uptake



Normalized I_{max}



Conclusion

- Anion permeation takes place in the intermediate channeling state, *iChS*.
- Channel opening is enabled by
  - elevator-like downward movement of transport core in the substrate-loaded state
  - repacking of a cluster of hydrophobic residues (L88, M89 and L296 in EAAT1)
- Robustly shared by both the archaeal and mammalian transporters.