

Rational Design of Small Molecule Ligands for Nutrient SLC Transporters

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www.schlessingerlab.org

 @SchlessingerLab

Meet the Experts, Cambridge, MA

Sep 5, 2019.



**Mount
Sinai**

Disclaimer

Co-founder of AIchemy, LLC



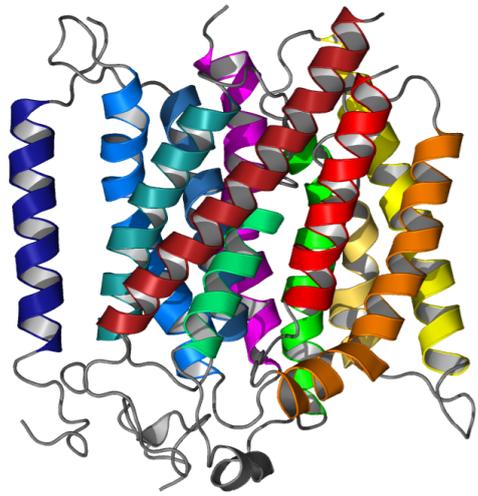
Structure and function SLC transporters guided by computational biology

- **Can we identify new SLC substrates and inhibitors?**
 - Deorphanization
 - Drug repurposing
 - DDI studies
 - Lead compounds
- **Can we develop rules to distinguish between substrates and inhibitors?**
- **Can we design inhibitors to deprive cancer cells from nutrients and substrates that serve as prodrugs?**

Schlessinger et al, CPT 2013, 2018; Garib Singh & Schlessinger, TIPS. In press

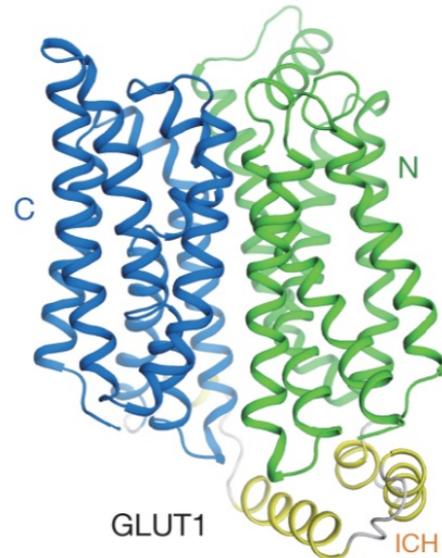


Growing number of human SLC structures



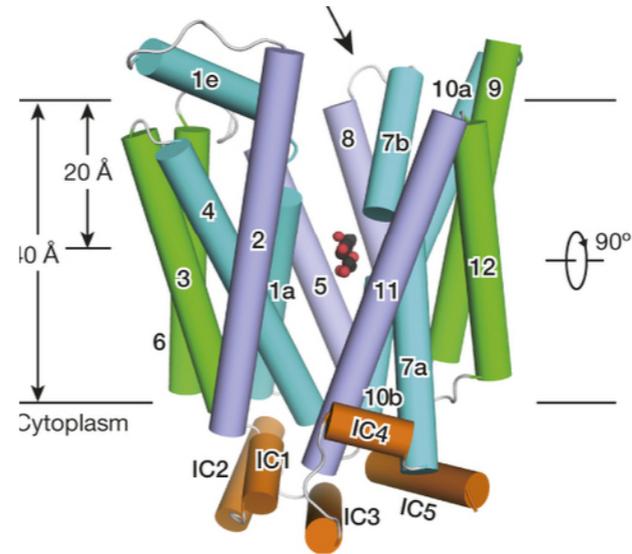
SLC42A3

Gruswitz et al. PNAS. 2010 May 25;107(21):9638-43.



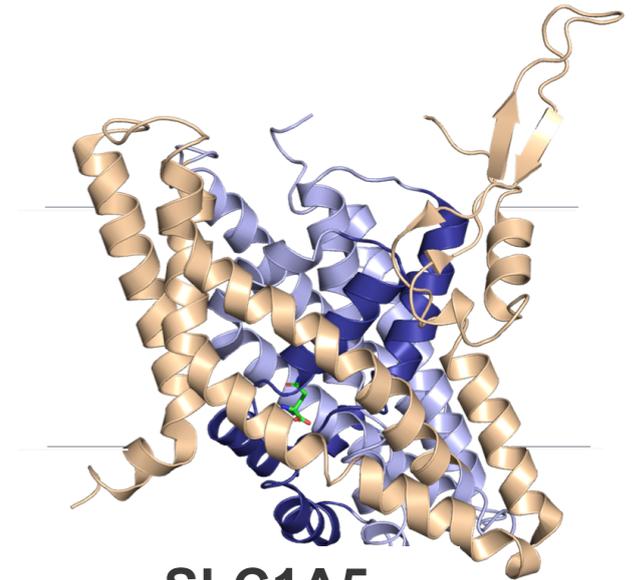
SLC2A1

Deng et al. Nature. 2014 Jun 5;510(7503):121-5.



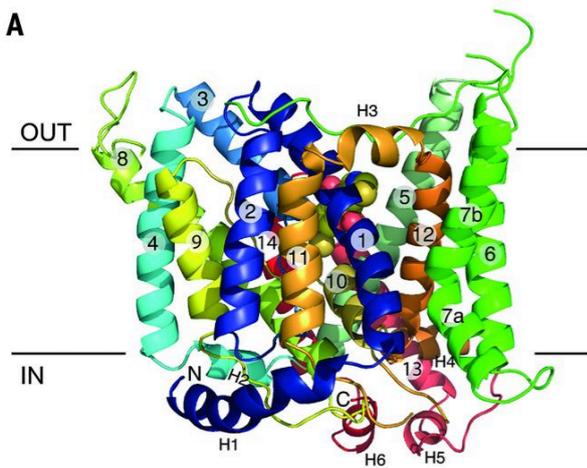
SLC2A3

Deng et al. Nature. 2015 Oct 15;526(7573):391-6.



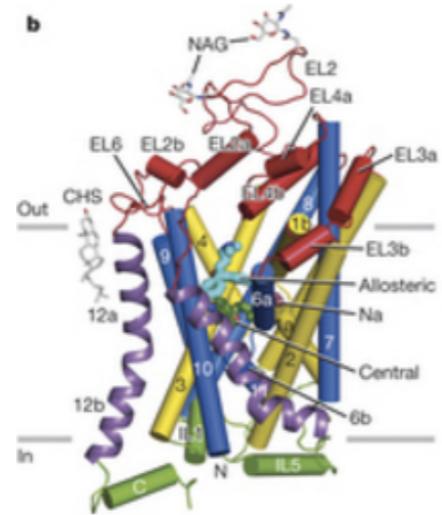
SLC1A5

Garaeva et al. Nat Struct Mol Biol. 2018 Jun;25(6):515-521.



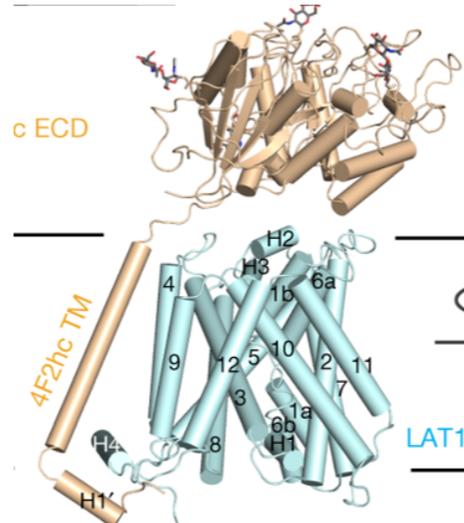
SLC4A1

Arakawa et al. Science. 2015 Nov 6;350(6261):680-4.



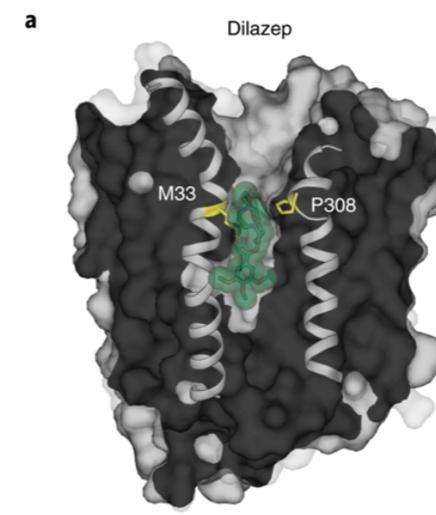
SLC6A4

Coleman et al. Nature. 2016 Apr 21;532(7599):334-339



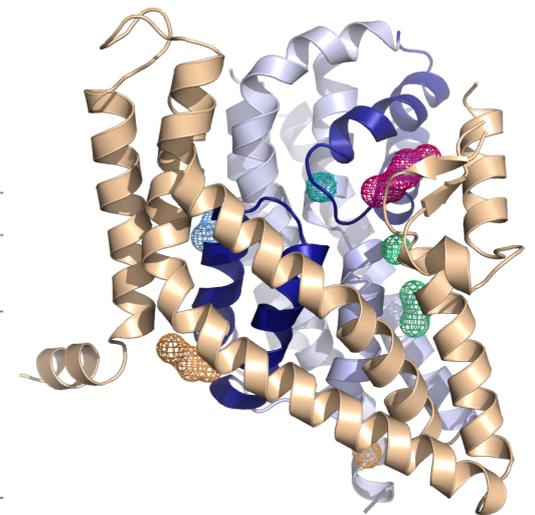
**SLC7A5
/SLC3A2**

Yan et al. Nature. 2019 Apr;568(7750):127-130.



SLC29A1

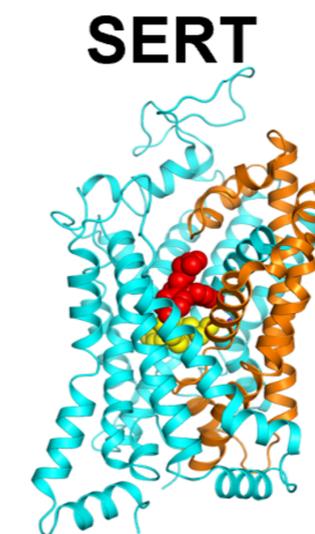
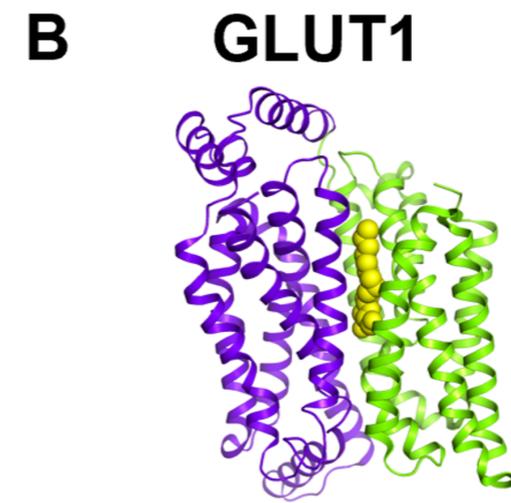
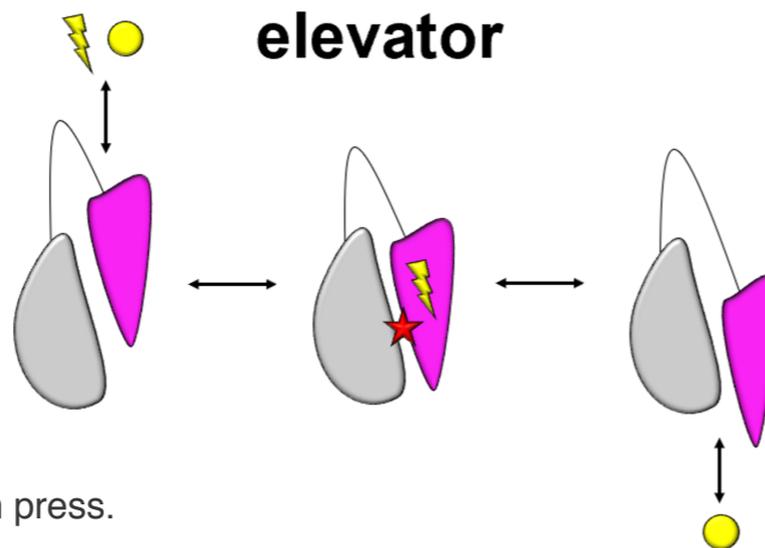
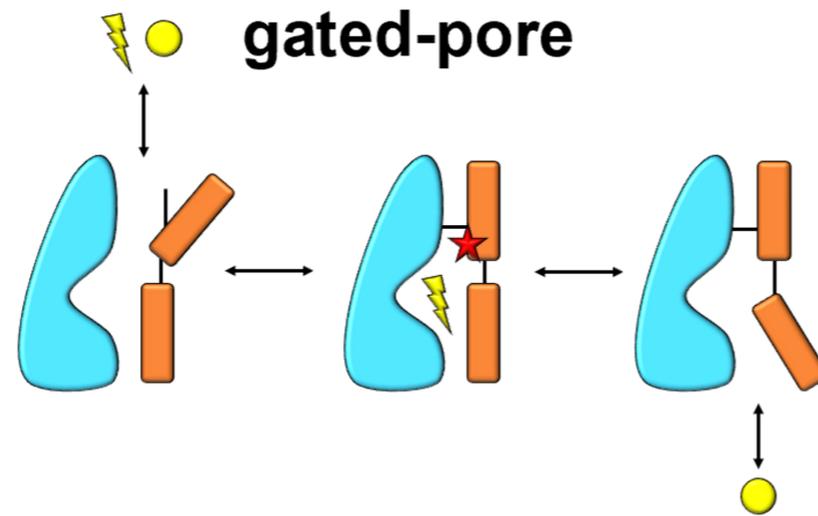
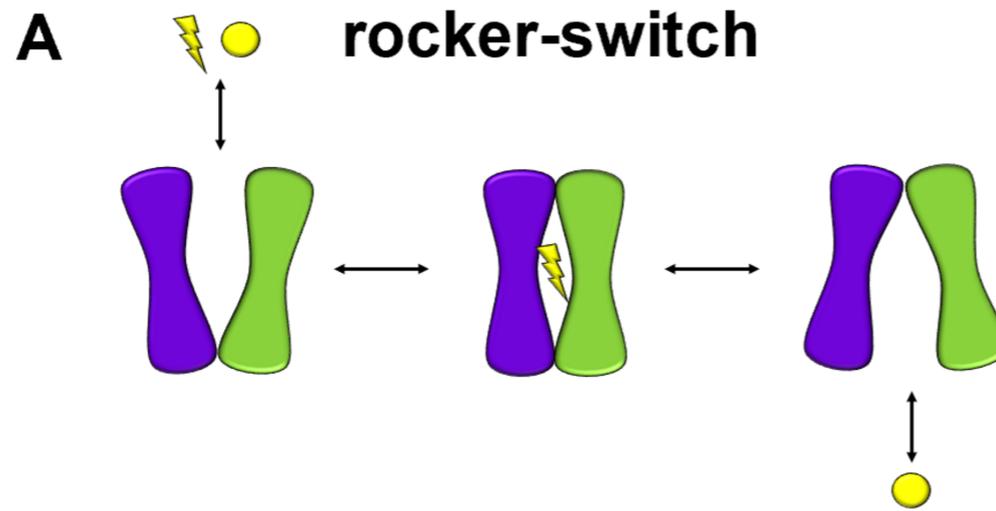
Wright et al. Nat Struct Mol Biol. 2019 Jul;26(7):599-606.



SLC1A3

Canul-Tec et al. Nature. 2017 Apr 27;544(7651):446-451.

Many SLCs use alternating access transport



Garib Singh and Schlessinger. *TIPS*. In press.

 Substrate binding site inhibitor  Substrate  Allosteric binding site inhibitor

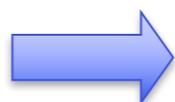
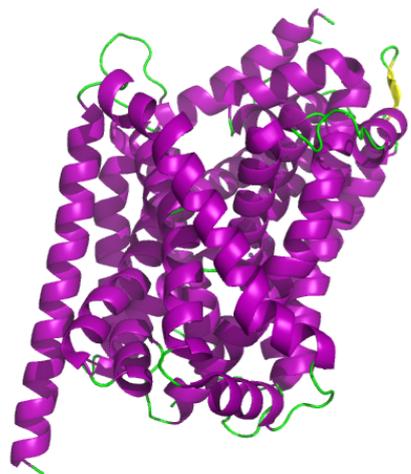
Homology modeling and virtual screening

1. Search for template
PDB, OPM

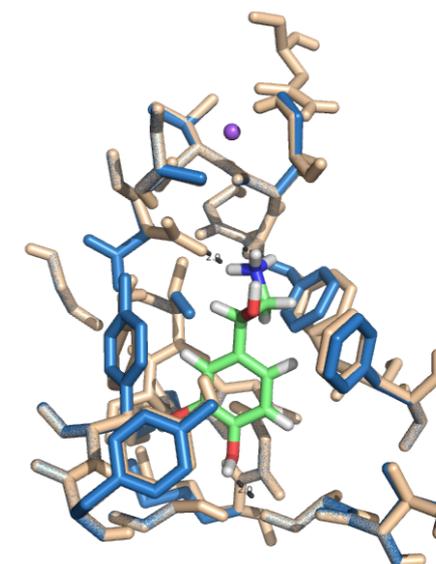
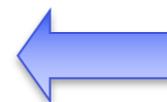
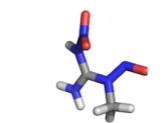
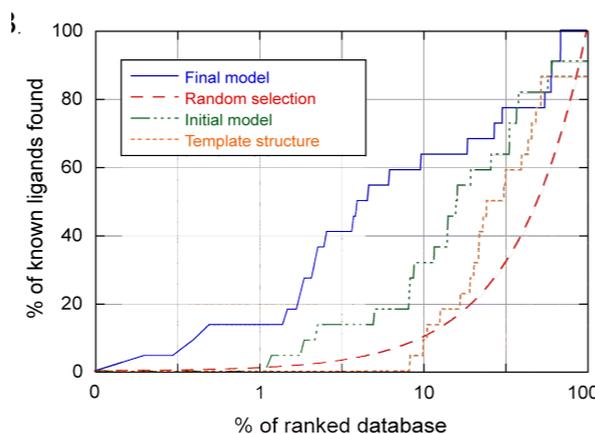
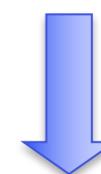
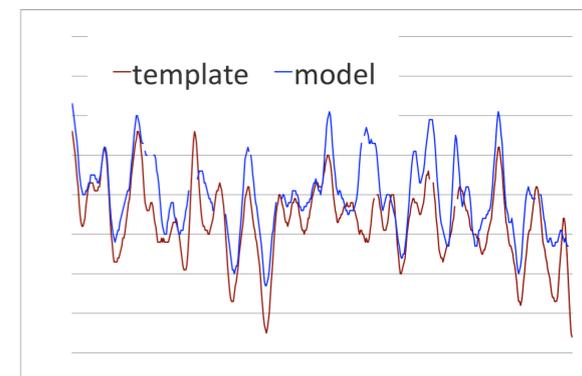
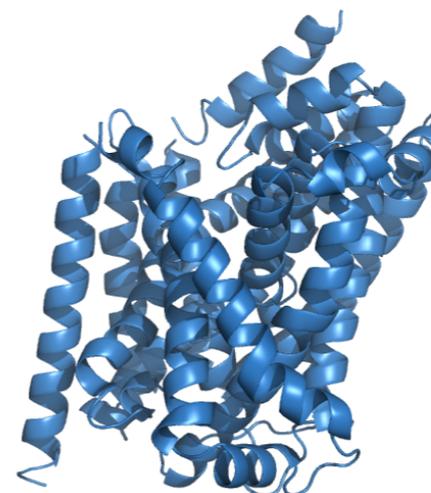
2. Align target and template
SALIGN, PROMALS3D

3. Construct and assess model
MODELLER, DOPE

Target sequence:
GGMEAVITGLADDFQAA



GGMEAVITGLADDFQAA
AIM--QPMIAFLEDELKL-

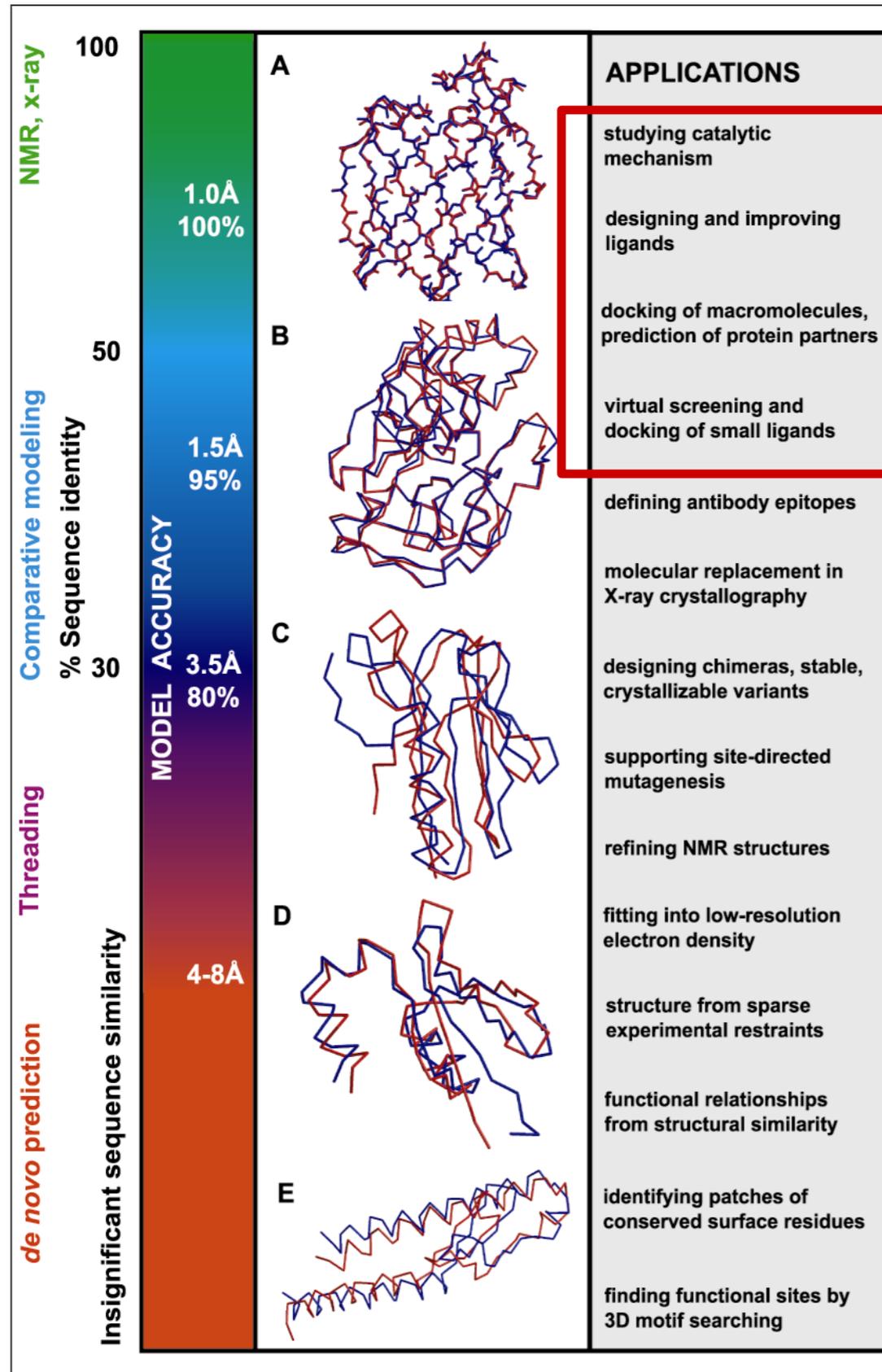


6. Virtual screening
Glide, FRED

5. Validate binding site
Glide, FRED, DUD

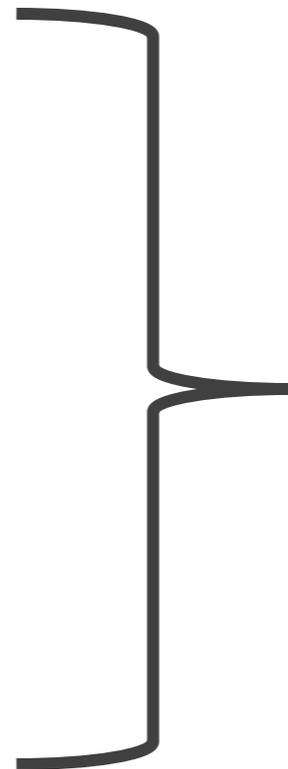
4. Refine model
SCWRL4, GROMACS

The human SLC transporters are technically challenging targets



Applications:

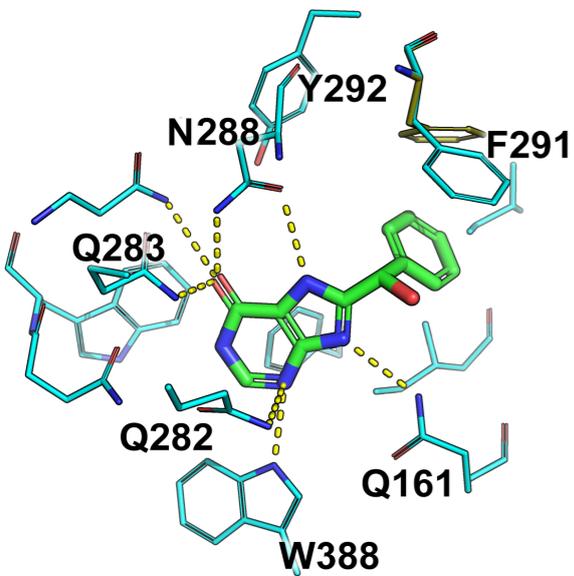
- Studying catalytic mechanism (e.g., transport)
- Designing and improving ligands
- Virtual screening and docking of small molecule ligands
- Modeling membrane proteins



Human SLC transporters

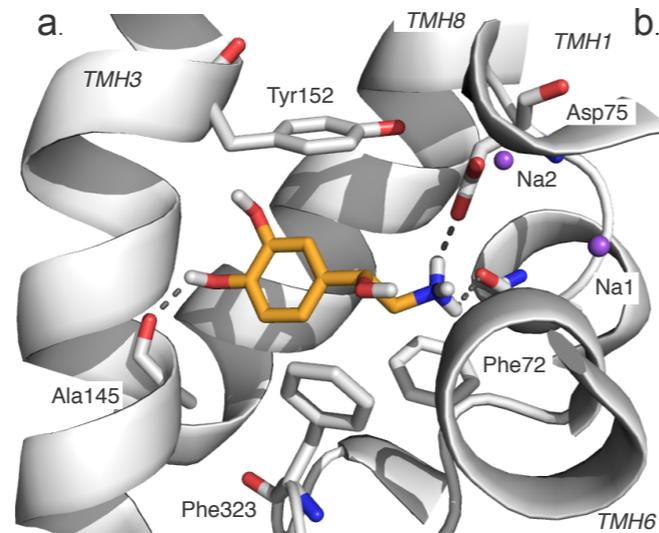
Structure-based ligand discovery for technically challenging membrane protein targets

The glucose transporter (GLUT1, SLC2A1)



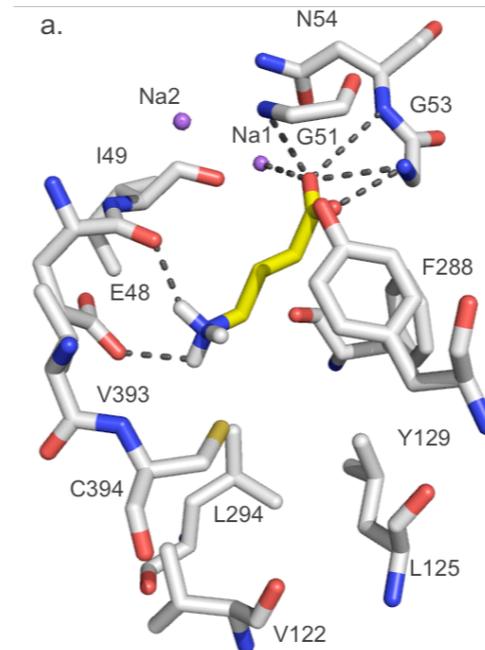
Ung *et al.* ACS Chem Bio. 2016 Jul 15;11(7):1908-16.

The norepinephrine transporter (NET, SLC6A2) (GAT2, SLC6A13)



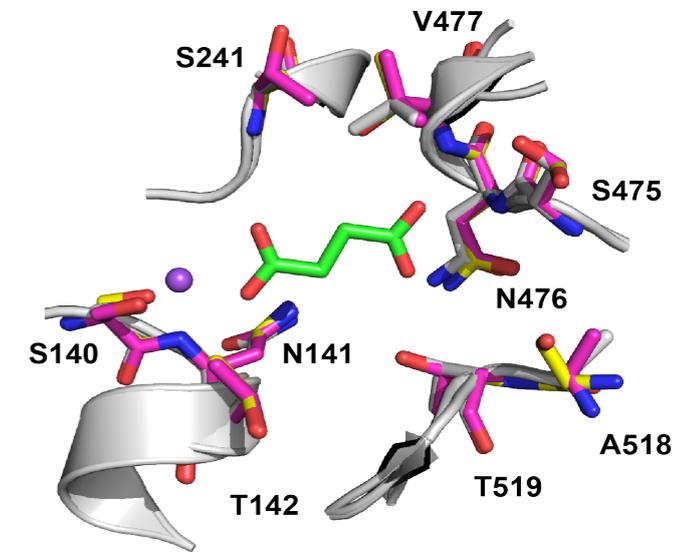
Schlessinger *et al.* PNAS 2011 Sep 20;108(38):15810-5.

The GABA transporter 2 (GAT2, SLC6A13)



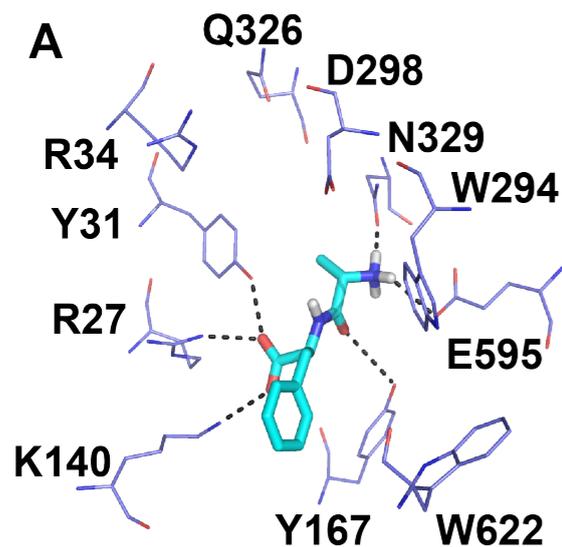
Schlessinger *et al.* JBC 2012 Nov 2;287(45):37745-56.

The SLC13 transporters of citric acid cycle metabolites



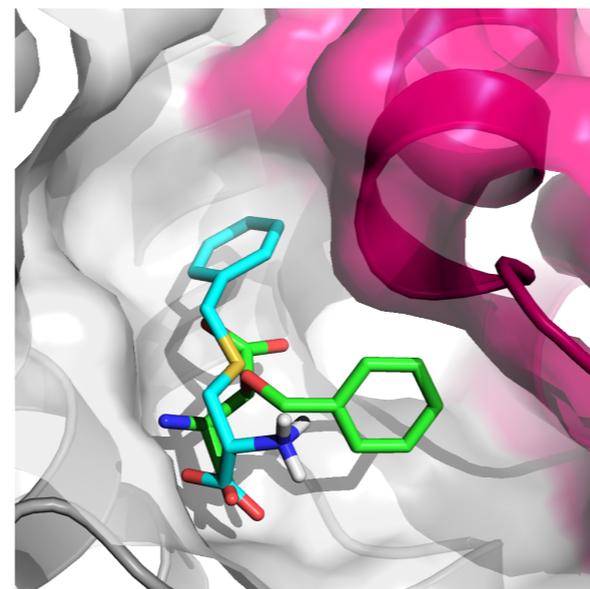
Schlessinger *et al.* JBC 2014 Jun 13;289(24):16998-7008
Colas *et al.* Biochemistry. 2015 Aug 11;54(31):4900-8.

The oligopeptide transporter 1 (PepT1/SLC15A1)



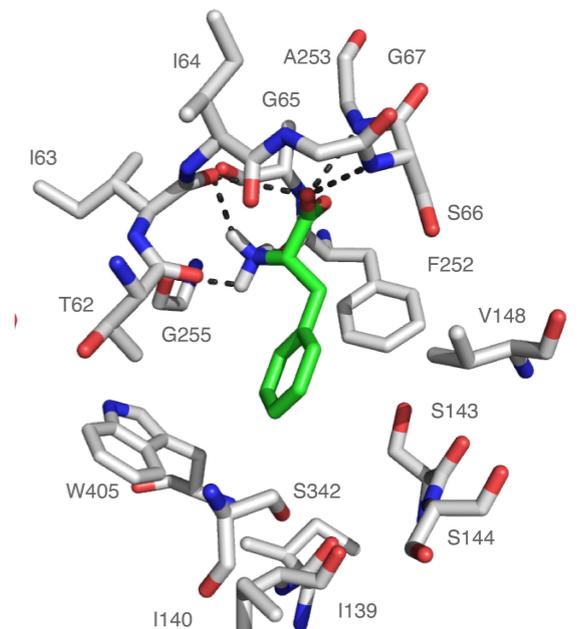
Colas *et al.* Mol Pharm. 2017 Dec 4;14(12):4685-4693.

The alanine-serine-cysteine amino acid transporter-2 (ASCT2/SLC1A5)



Colas *et al.* PLOS Comp Bio. 2015 Oct 7;11(10):e1004477.

The L-type amino acid transporter (LAT-1, SLC7A5)



Geier* and Schlessinger* *et al.* PNAS 2013 Apr 2;110(14):5480-5

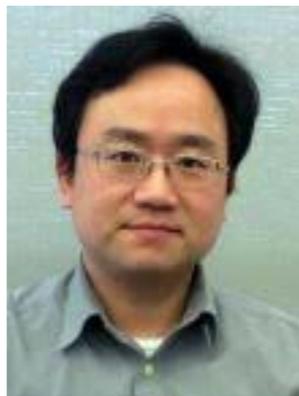
Cancer metabolism is supported by nutrient transporters

- Cancer cells undergo a metabolic shift, resulting in increased reliance on amino acids.
- ASCT2 and LAT-1 control cell cycle progression, mTORC1 pathway activation, cell growth and tumor development for both primary tumors and metastases.
- T cells accelerate essential amino acid uptake upon activation and adapt to essential amino acid starvation

Kolev *et al* Immunity. 2015 Jun 16;42(6):1033-47



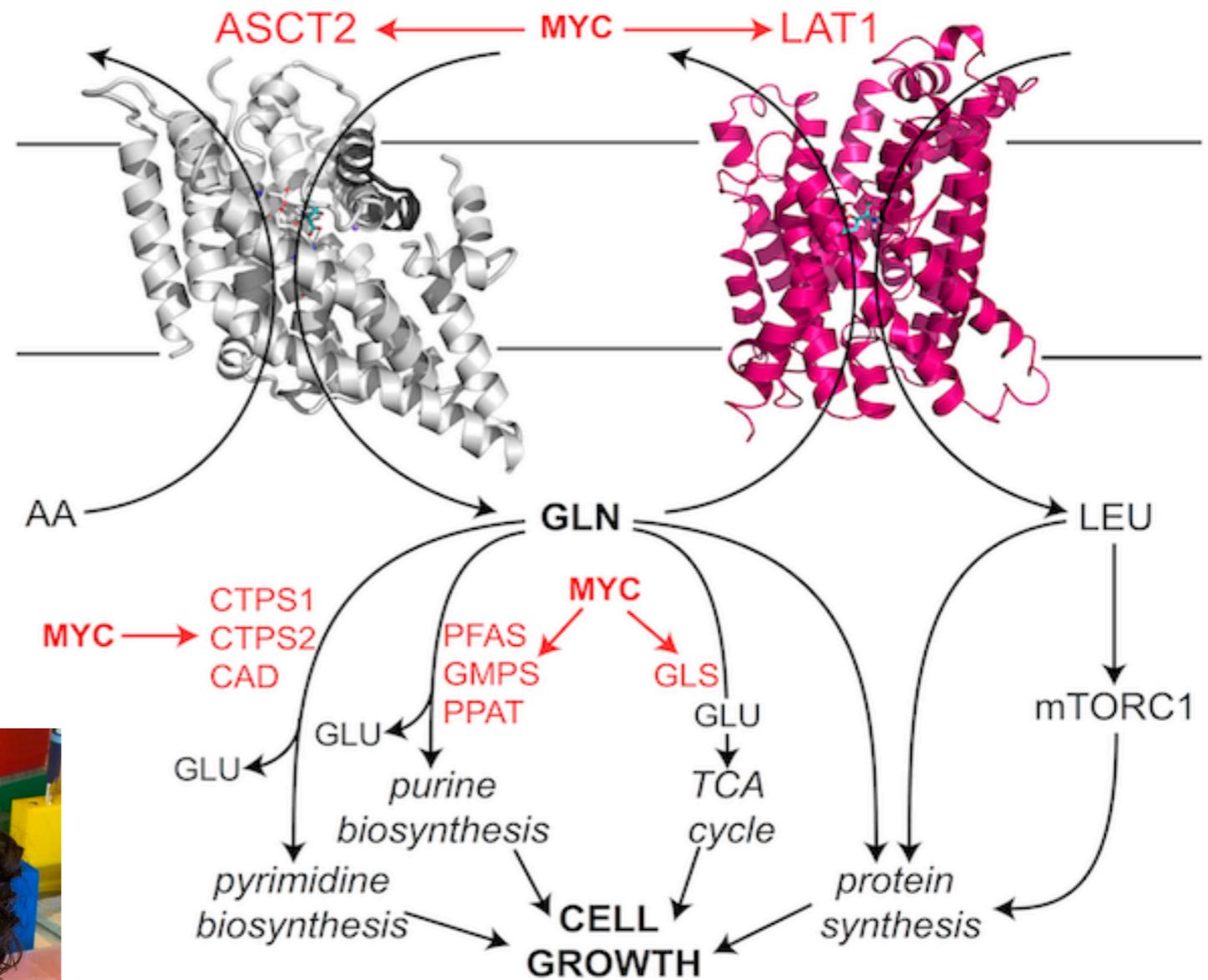
Claire Colas (Mt. Sinai and now Vienna)



James Huan-Chieh Chien (UCSF)



Kathy Giacomini (UCSF)



Allen Thomas (UNK – Kearny)

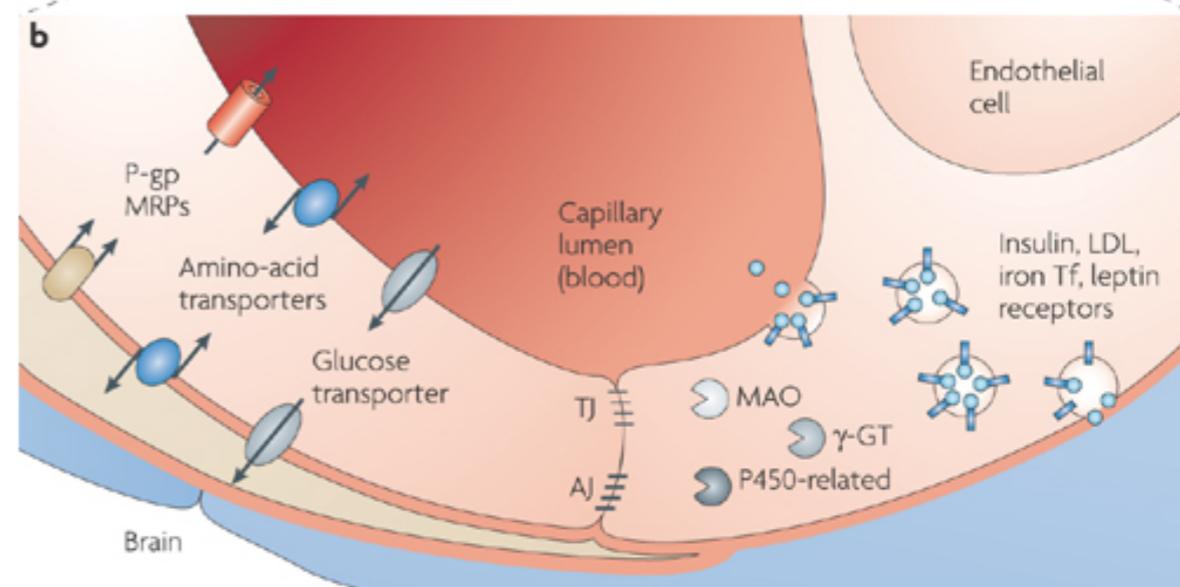
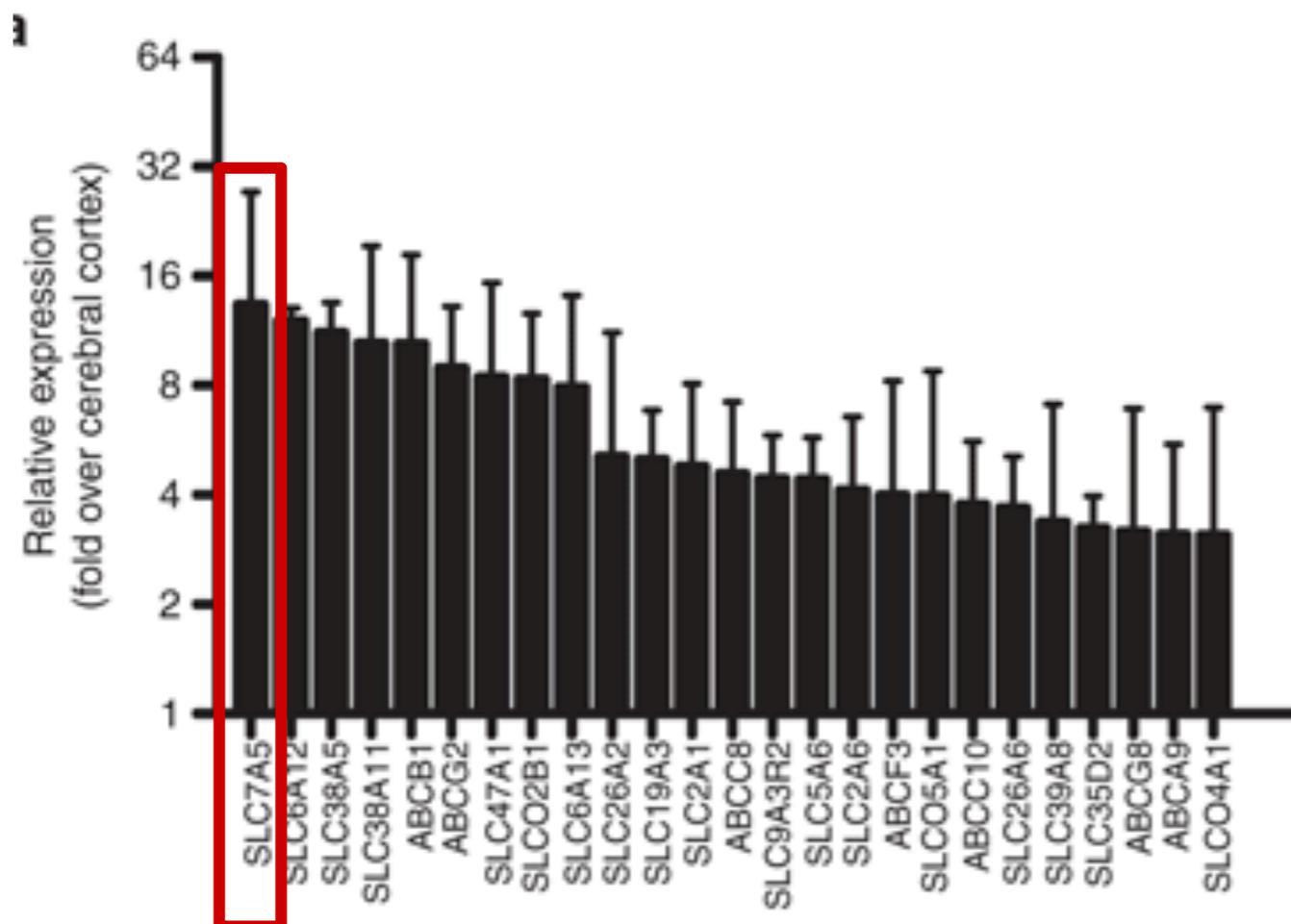
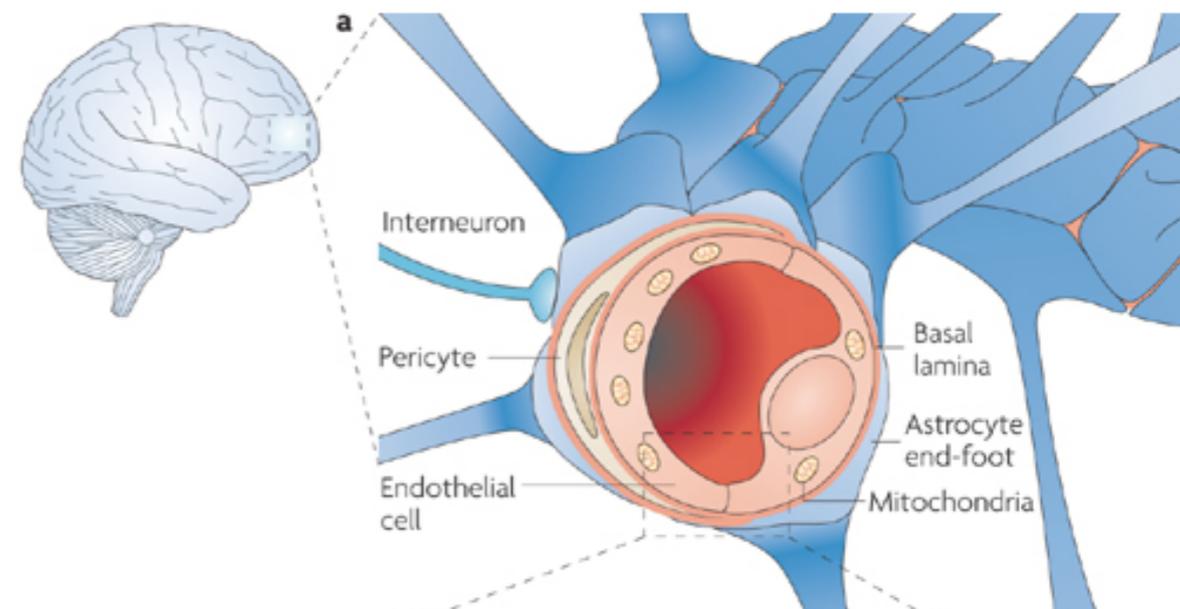
Possemato R. Nat Metabolism (2019)

Altman et al. Nat Rev Cancer. 2016 Jul 29.

Nicklin *et al*. Cell. 2009 Feb 6;136(3):521-34.

L-type amino acid transporter 1 (LAT-1, SLC7A5)

- Found in the blood-brain barrier (BBB), testis, placenta, and bone marrow
- Transports neutral amino acids (e.g., Trp and Leu), thyroid hormones (e.g., T₃, T₄), and xenobiotics (e.g., L-Dopa, gabapentin)
- Stereoselective; both carboxy- and amino- groups are needed for transported ligands.
- Heterodimerize with SLC3A2 to form an active transporter



Nature Reviews | Drug Discovery

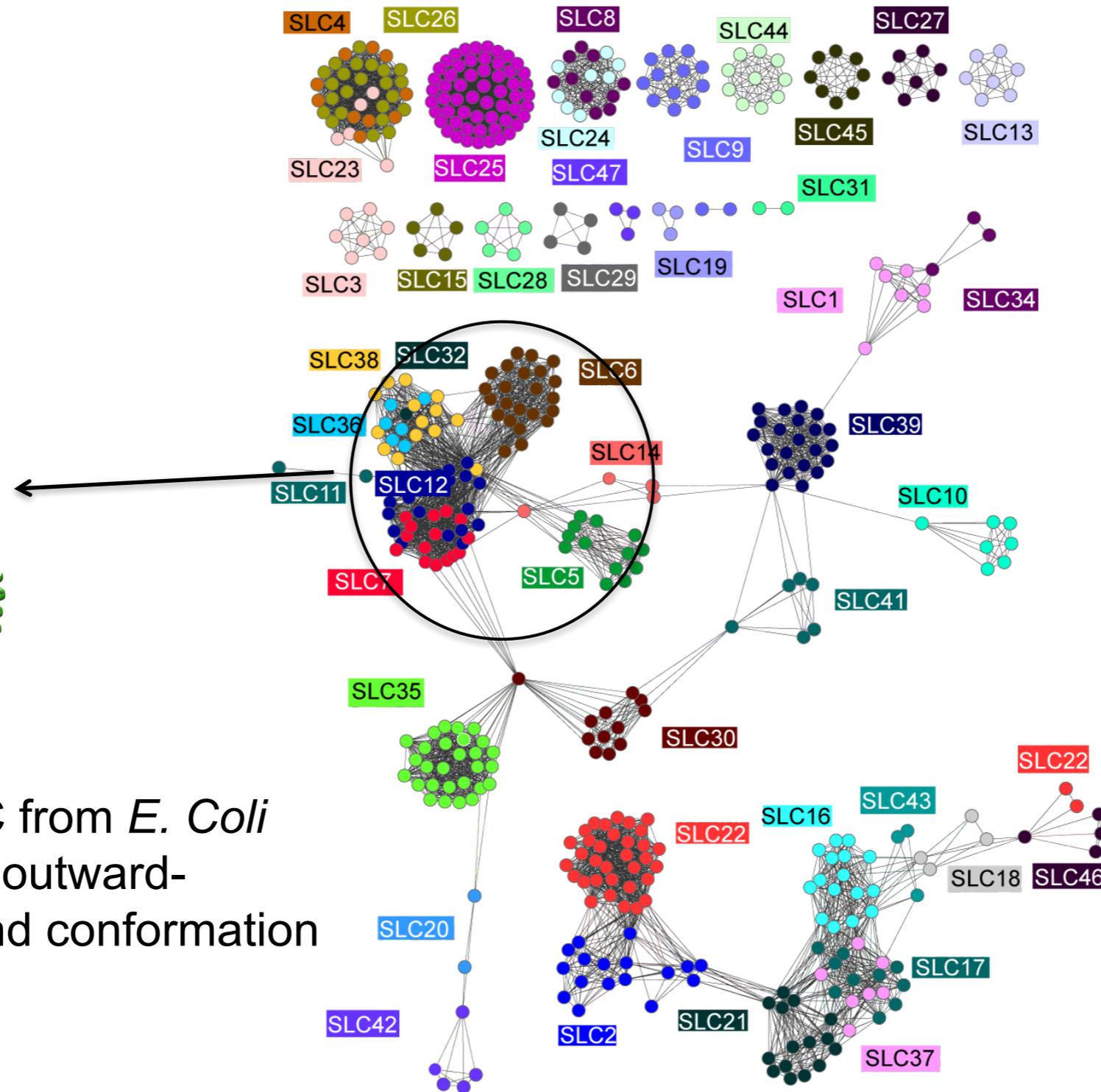
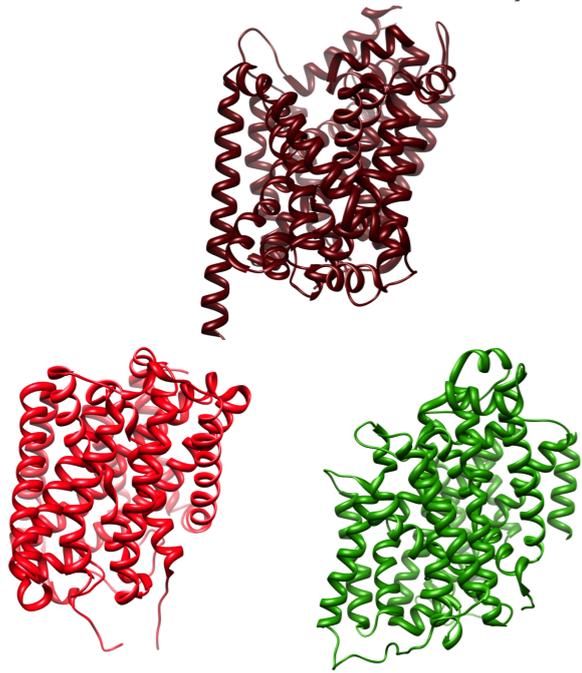
Cecchelli et al. Nat Rev Drug Discov. 2007 Aug;6(8):650-61

Geier et al. Clin Pharmacol Ther. 2013 Dec;94(6):636-9

Geier et al. Clin Pharmacol Ther. 2013 Dec;94(6):636-9.

Sequence-based network guides structural modeling of SLCs

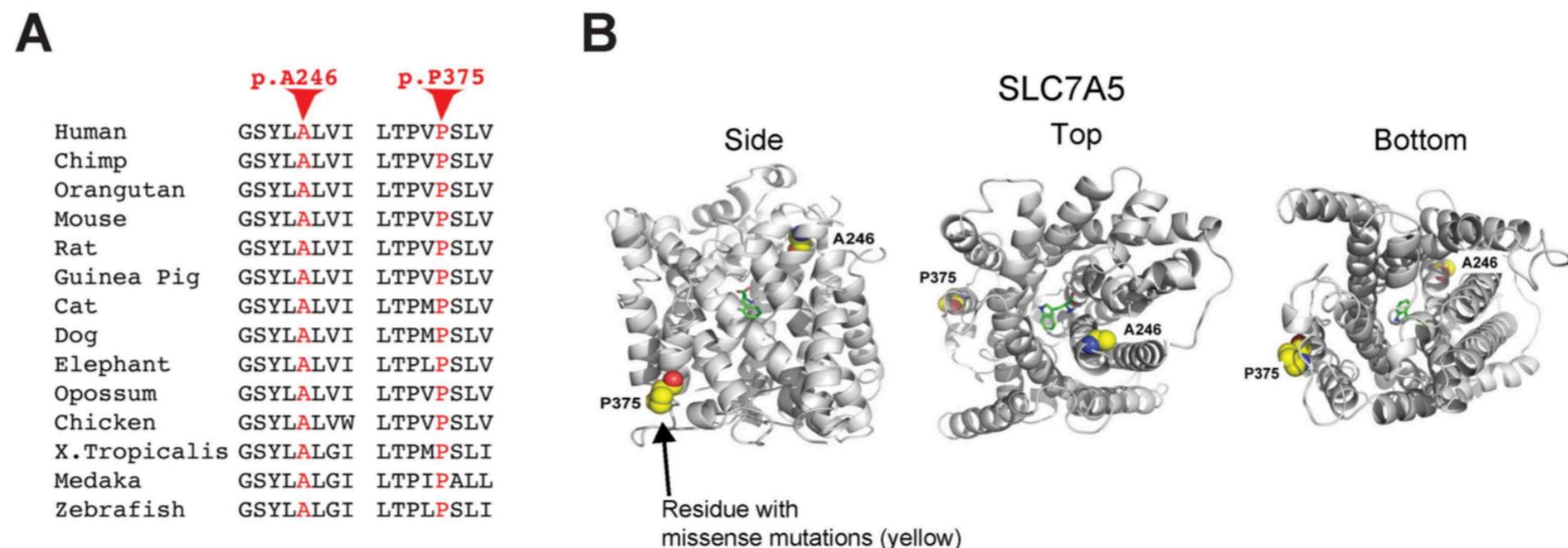
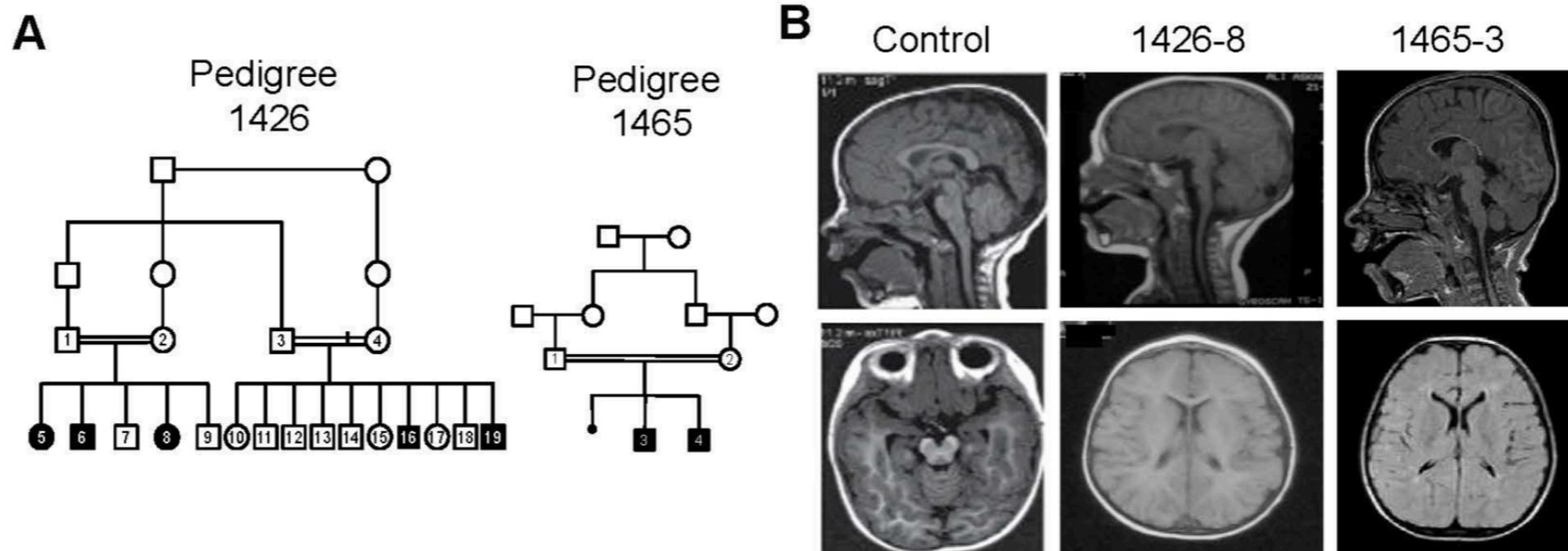
LeuT-like fold
(SLC5/6/7 in
one cluster)



Model based on AdiC from *E. Coli*
(~25% identity) in an outward-
occluded ligand-bound conformation

The LAT-1 model explains genetic variations that are associated with autism

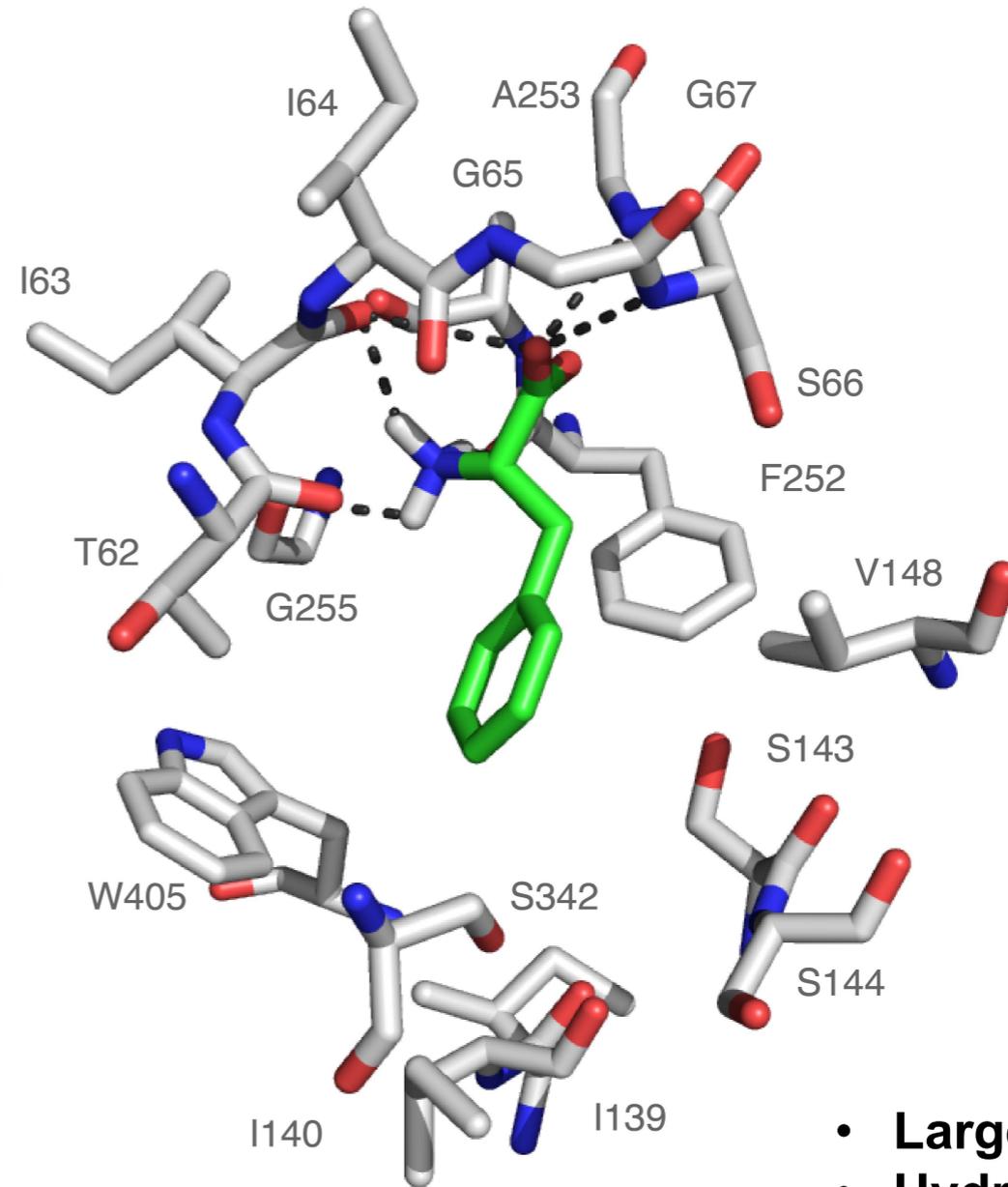
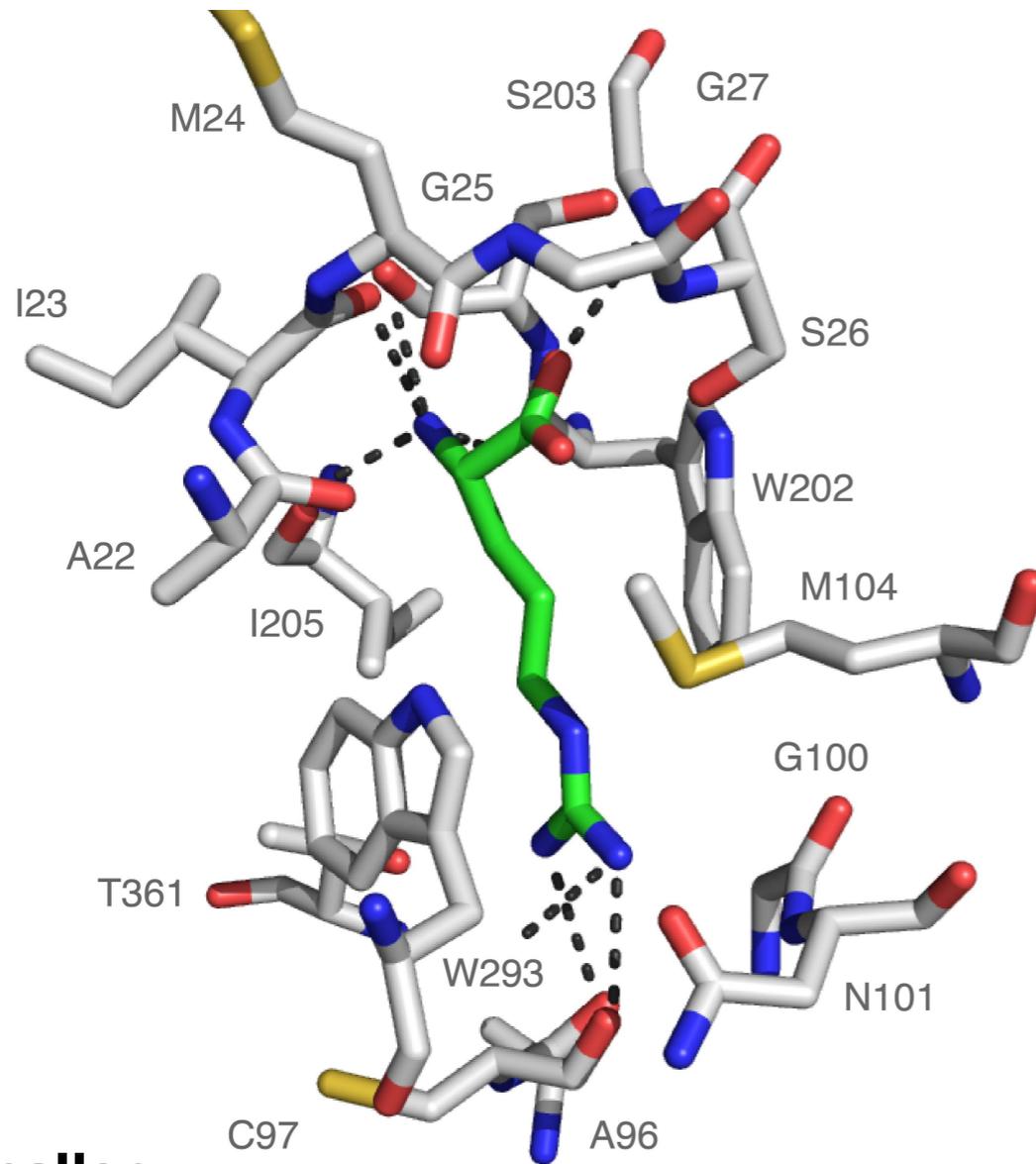
- Deficient of transport of branched chain amino acid at the blood brain barrier is a cause of autism and motor dysfunctions
- Leads to abnormal activation of the amino acid response (AAR) pathway and a corresponding reduction in mRNA translation along with neuronal activity imbalance and behavioral problems.



Comparison among amino acid transporters proposes substrate specificity determinants

**Arginine transporter
(template structure)**

LAT-1 (model)



- **Smaller**
- **Polar**

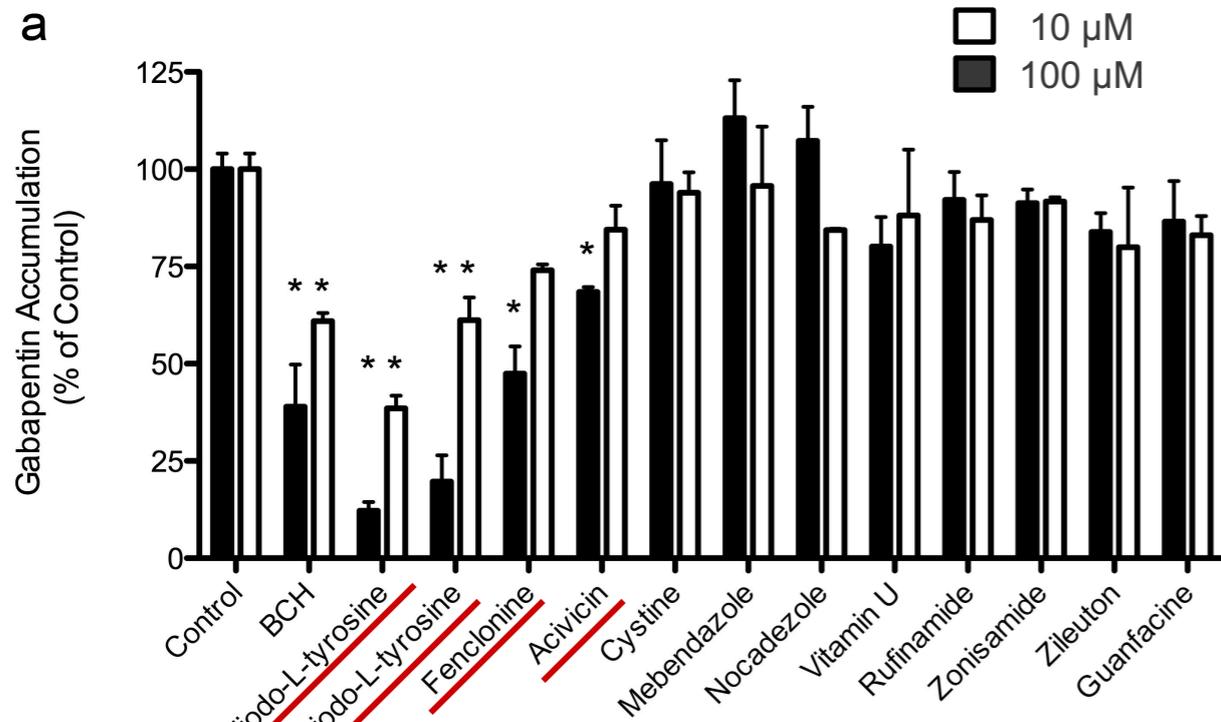
AdiC

LAT-1

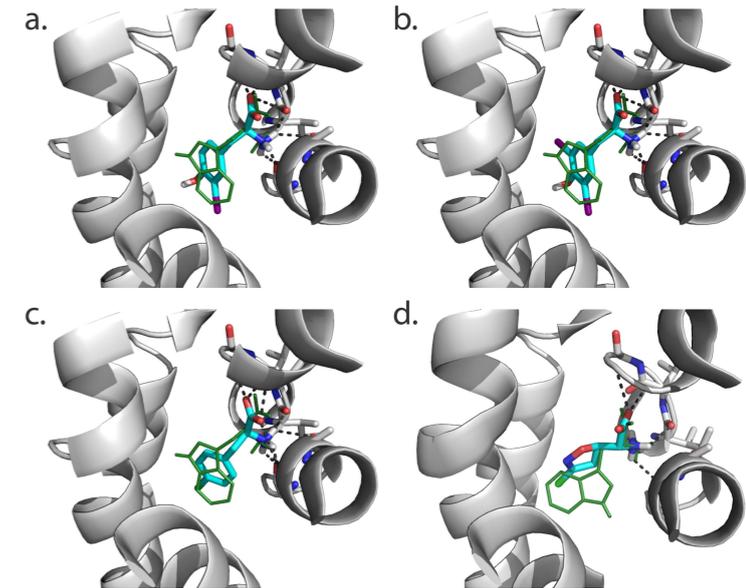
- **Larger**
- **Hydrophobic**

Experimental testing of 12 compounds reveals novel inhibitors and substrates

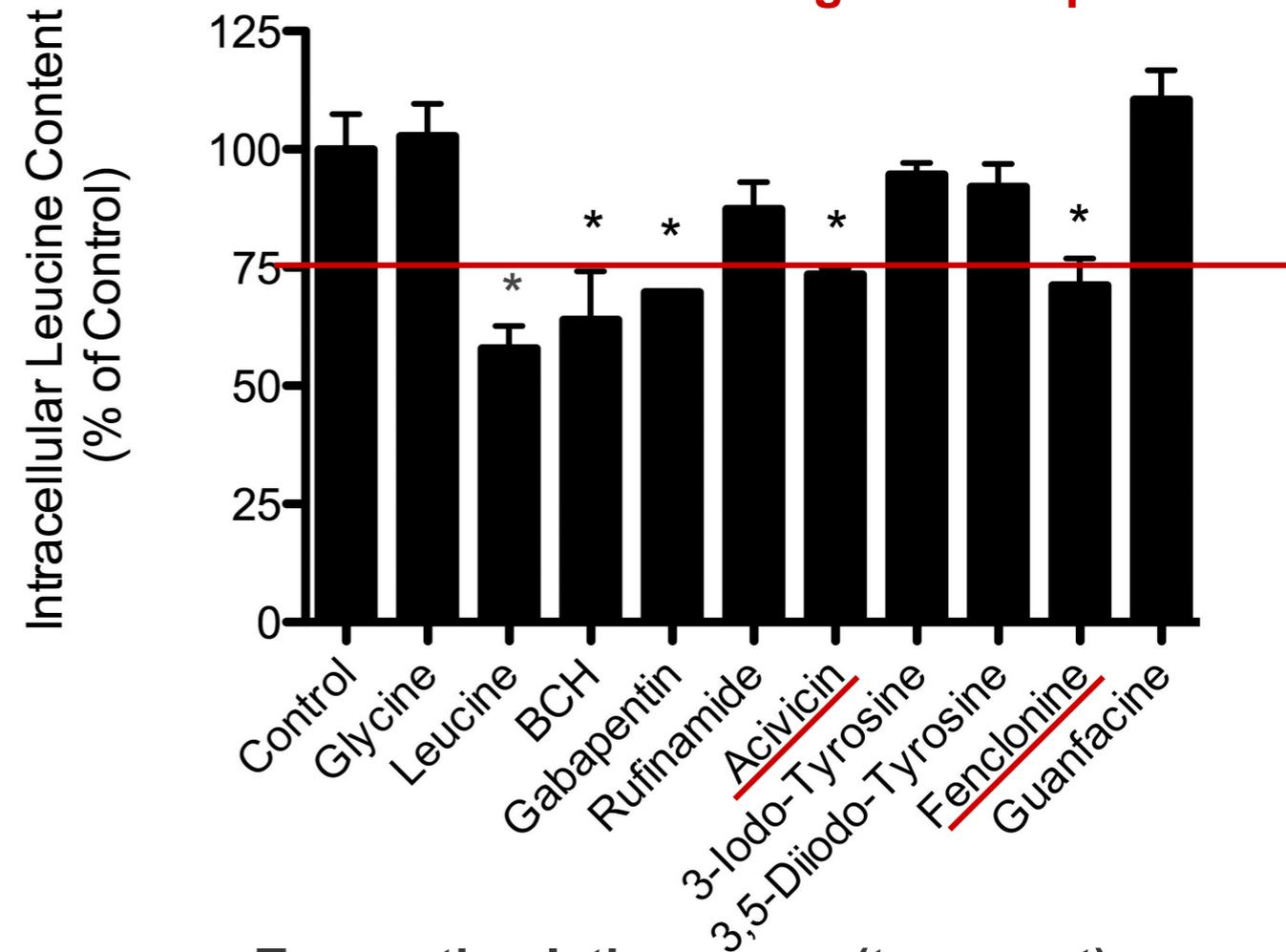
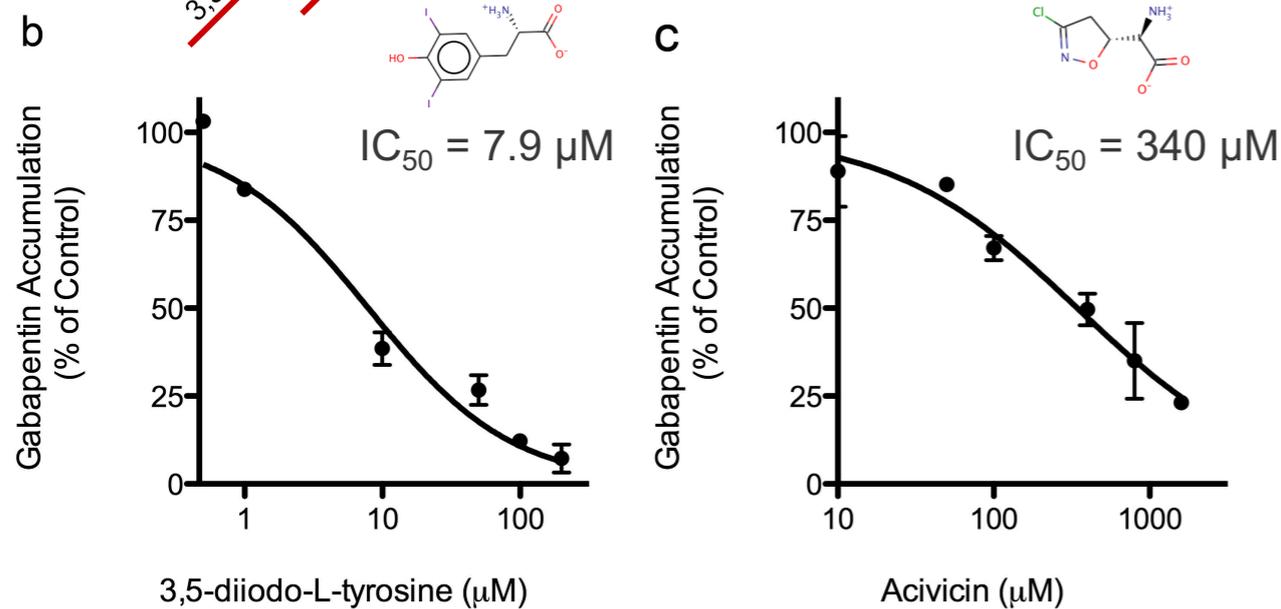
Four ligands: two metabolites and two drugs



Virtual screening of 19,166 prescription drugs and metabolites from KEGG



Two substrates: CNS active drug-like compounds



Inhibition of H³-Gabapentin Uptake (inhibition)

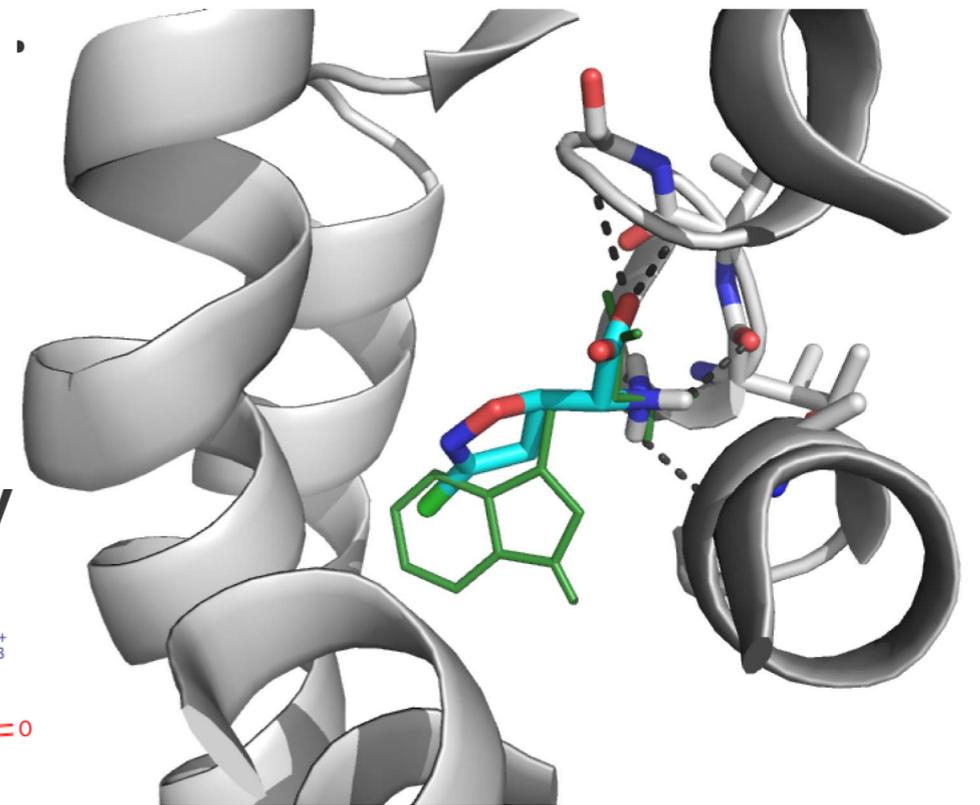
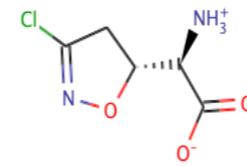
Geier* and Schlessinger* *et al.* PNAS 2013 Apr 2;110(14):5480-5

Trans-stimulation assay (transport)
Ethan Geier / Kathy Giacomini

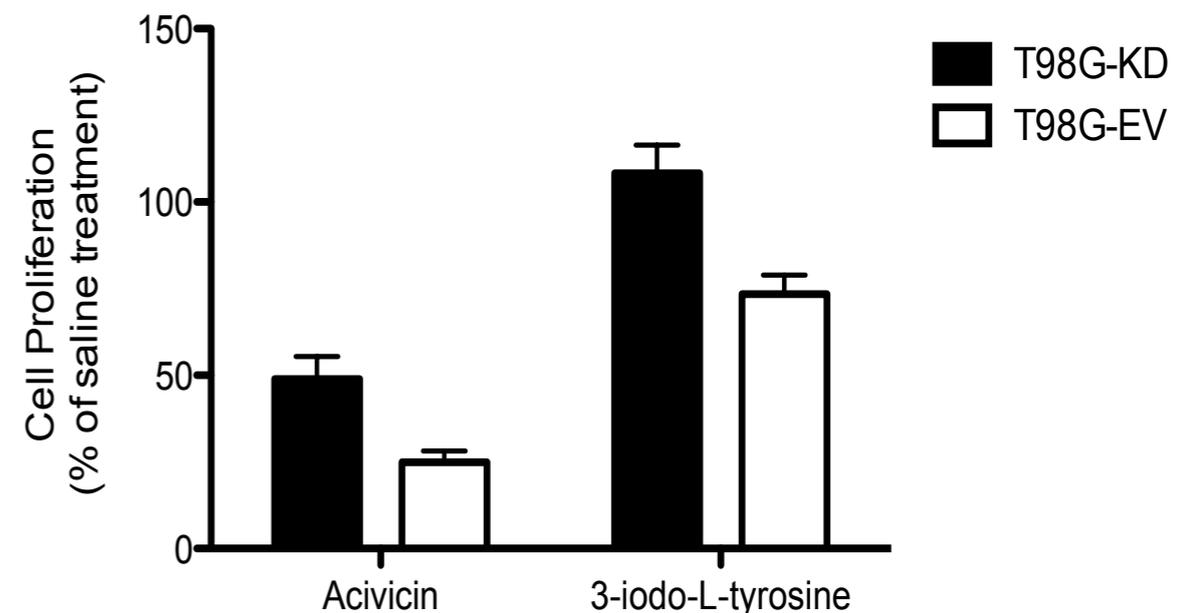
Structure-based discovery of enhanced blood-brain-barrier permeability of a drug candidate

- **Acivicin** is an **antimetabolite** that disrupts cellular glutamine metabolism
- Clinical utility is limited by **central nervous system-related side effects** (hallucinations, anxiety, confusion, psychoses, and lethargy)
- Crosses the BBB *via* active transport
- Transport by LAT-1 might contribute to **Drug efficacy** (crosses the BBB) and **Side effects** (e.g., sleepiness)

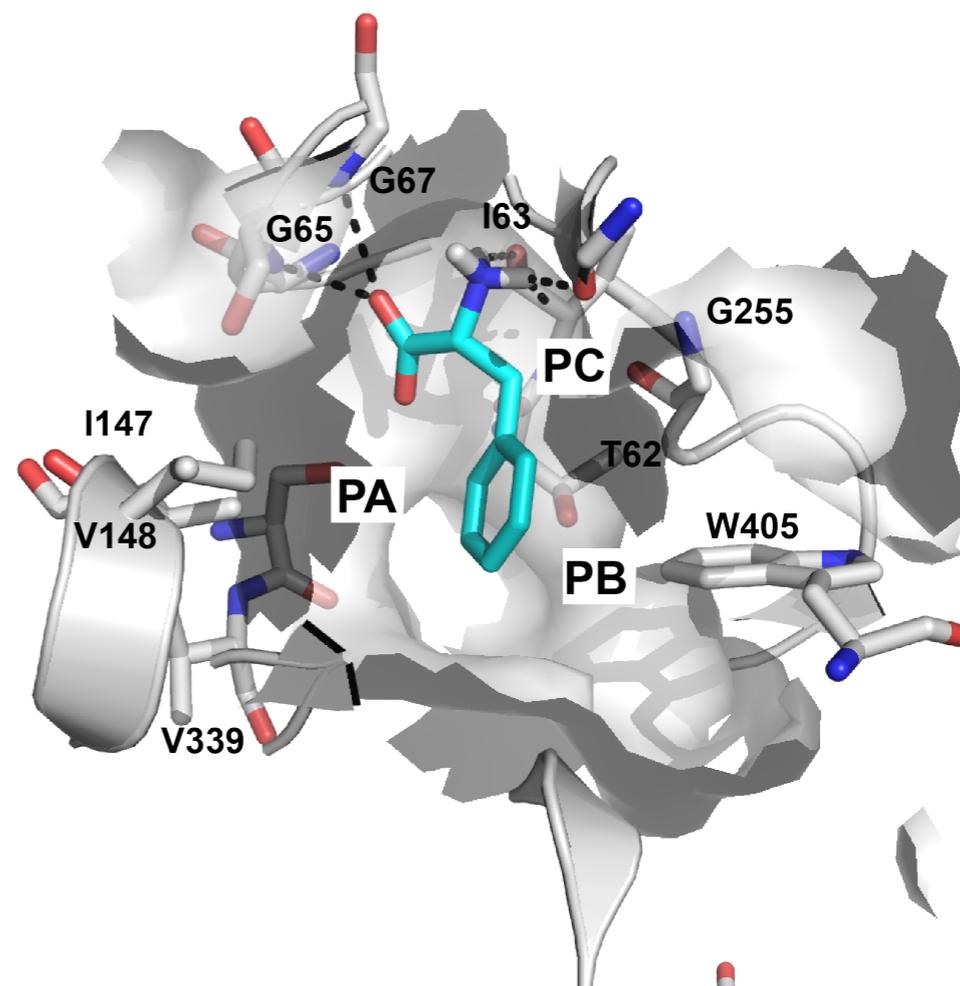
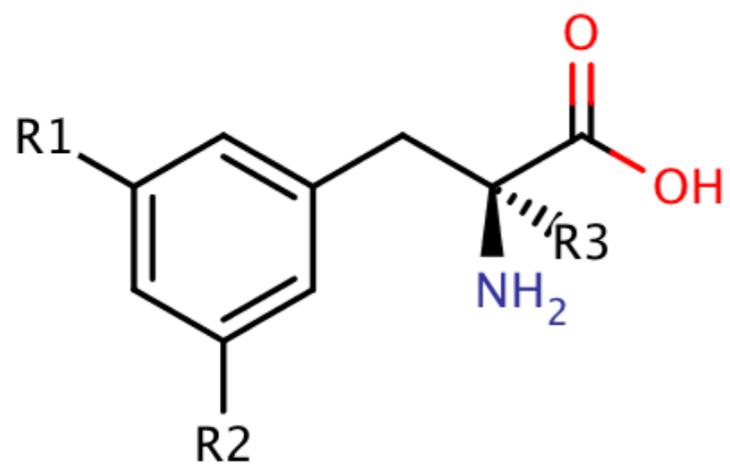
Chikhale *et al.*, 1994; Olver *et al.*, 1998; McGovern *et al.*, 1985
Geier and Schlessinger *et al.* PNAS 2013



- **Acivicin**: Cytotoxic substrate of LAT-1
- **3-iodo-L-tyrosine**: Inhibitor of LAT-1

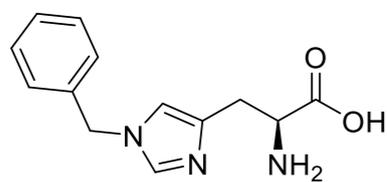


Can we use the structural model to optimize inhibitors and substrates?



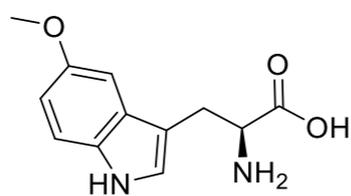
Augustyn et al. *Bioorg Med Chem Lett.* 2016 Jun 1;26(11):2616-21

Zur et al. *Bioorg Med Chem Lett.* 2016 Oct 15;26(20):5000-5006.



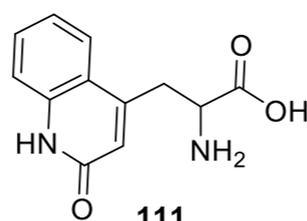
59

Efflux Rate (fmol/min): 2.2
%Inhibition at 200 μ M: 74



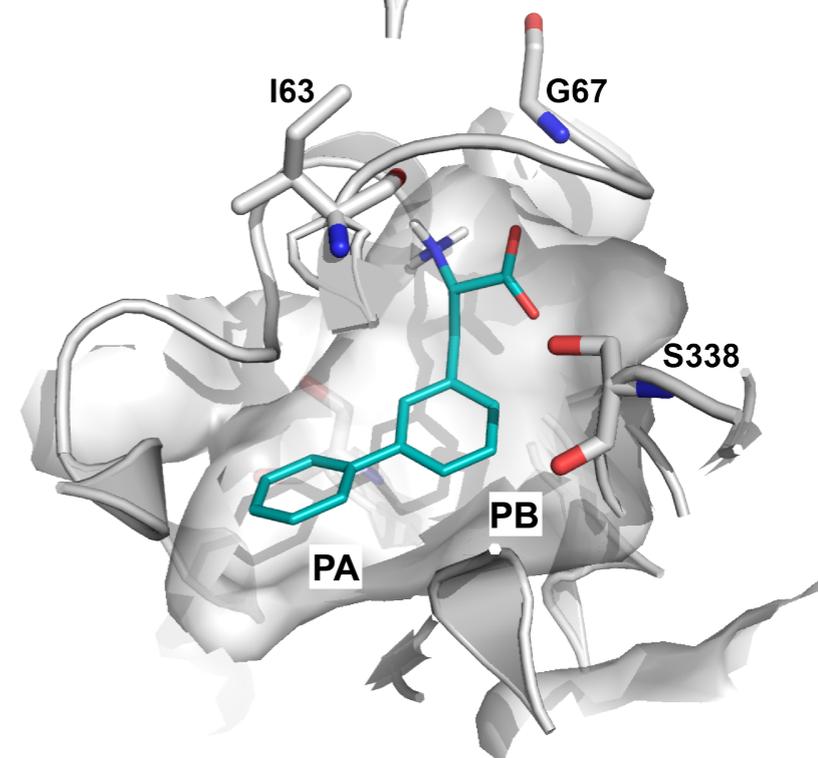
110

2.3
72



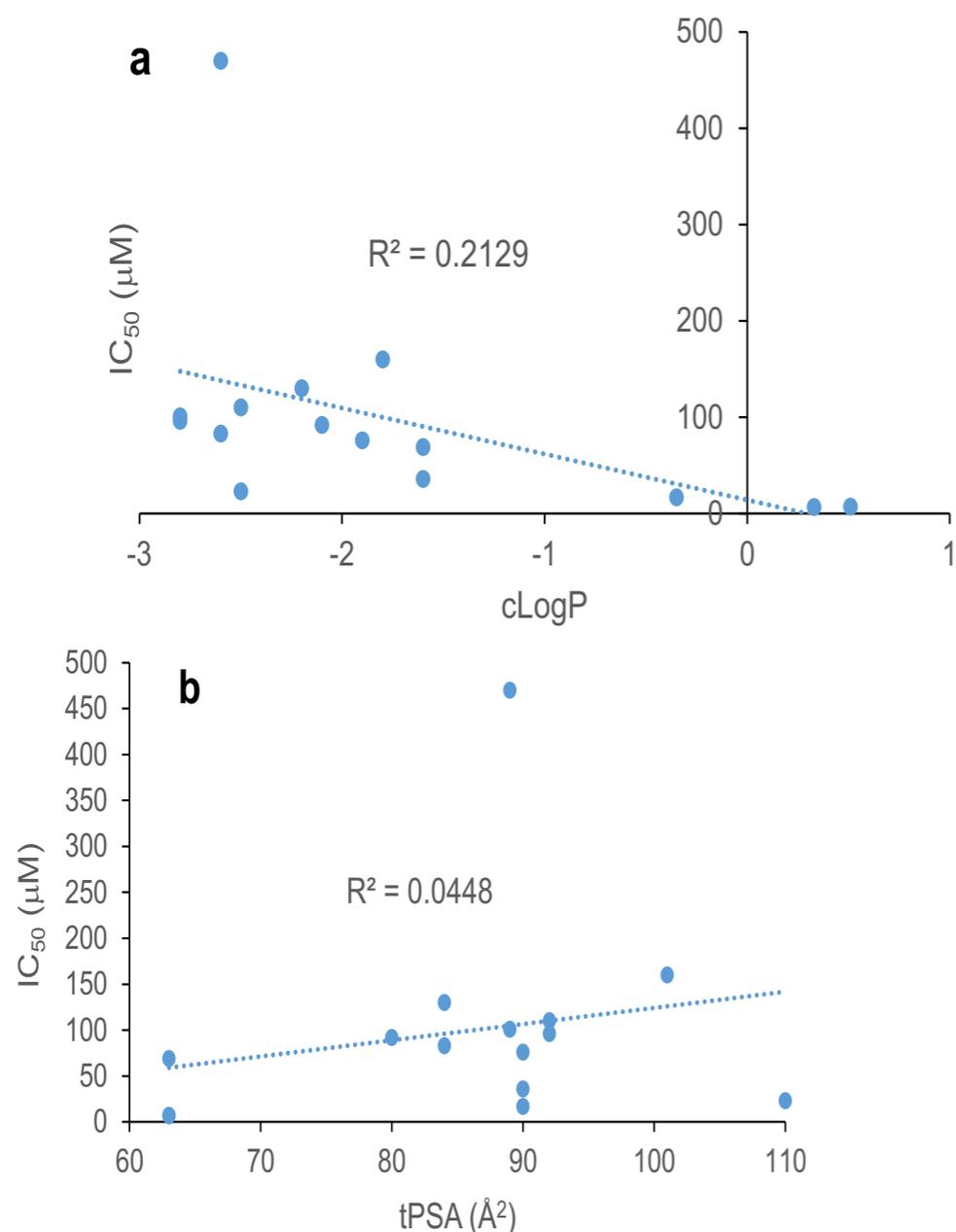
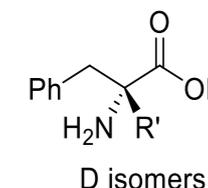
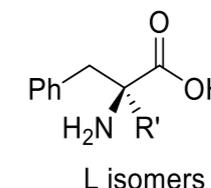
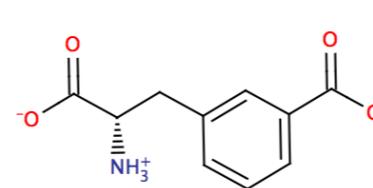
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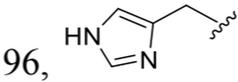
2.4
63



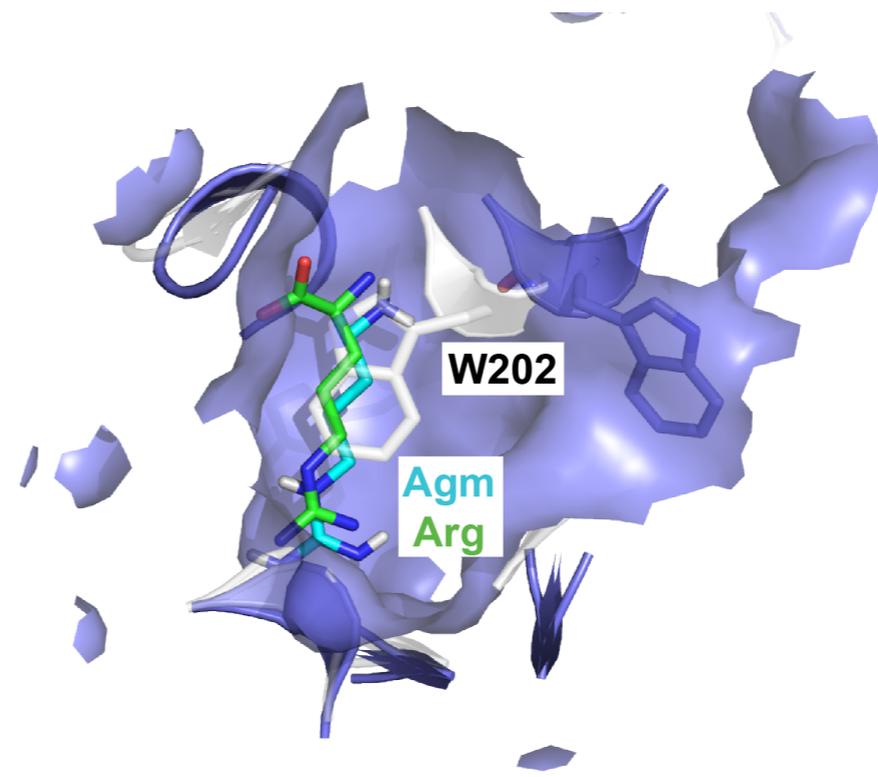
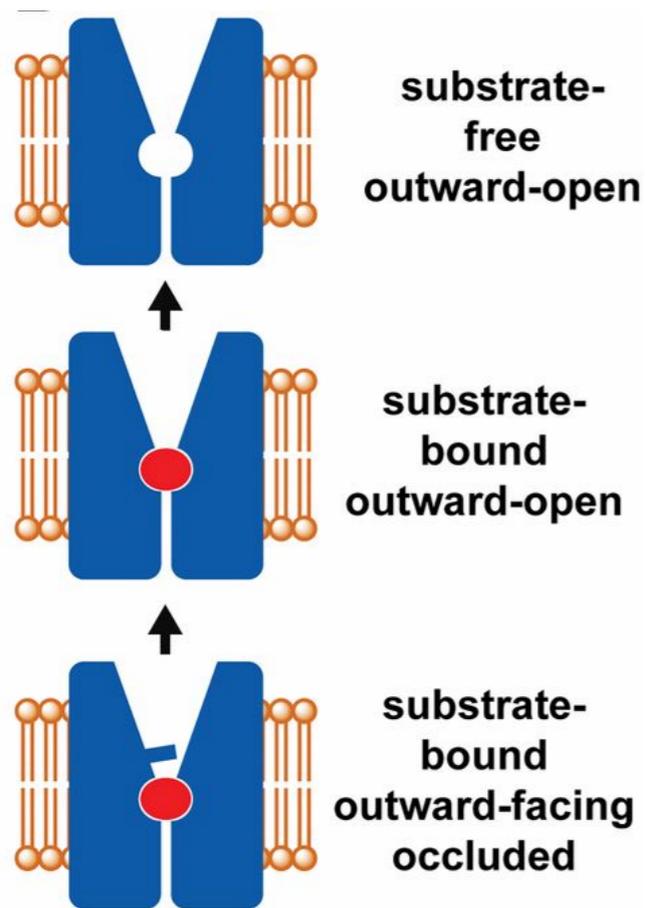
Deorphanizing the “L-Type Amino Acid Transporter 1”

- Little correlation between LogP or polar surface area (PSA) with activity
- Some sidechains had negative charge
- No significant stereoselectivity

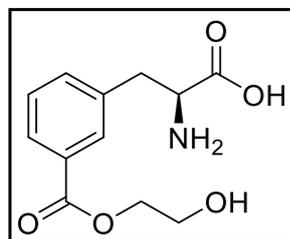


Compound ^a	AT#, R'	Efflux Rate ^b	% Inhibition ^c	IC ₅₀ (μM) ^d
50-L (L-Phe)	34, H	3.6	85	69 ± 29
50-D (D-Phe)	68, H	2.6	74	46 ± 17
92-L	42, Me-	2.8	-	130 ± 22
92-D	43, Me-	2.7	-	810 ± 160
93	84, PhCH ₂ (achiral)	0.61	11	-
94-L	92, (CH ₃) ₂ CHCH ₂ -	0.50	15	-
94-D	93, (CH ₃) ₂ CHCH ₂ -	0.72	2.1	-
98-L	94, CH ₃ S(CH ₂) ₂ -	1.5	21	240 ± 89
98-D	95, CH ₃ S(CH ₂) ₂ -	0.73	-0.20	-
107-L	96, 	0.35	19	-

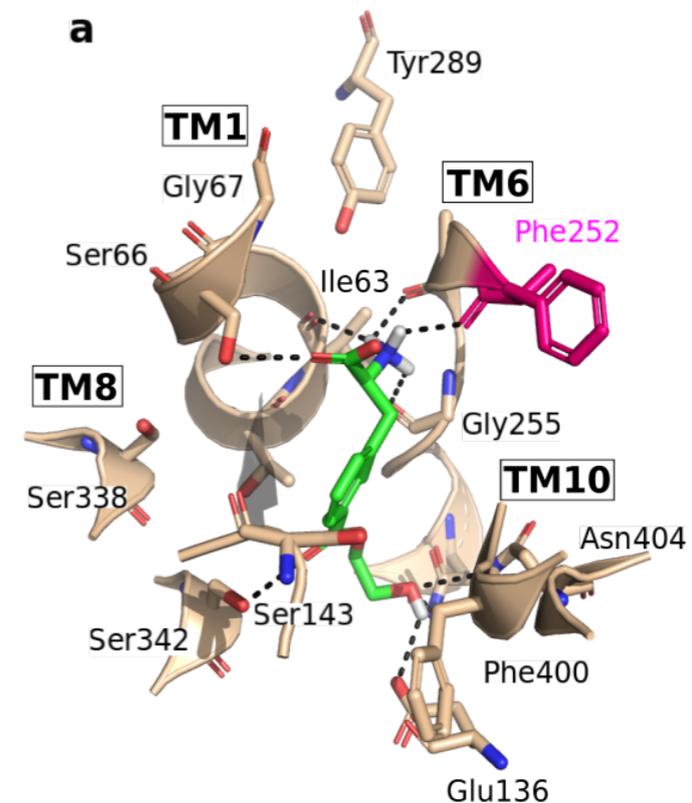
Targeting the outward-facing conformation with non-amino acids



Ilgü H, Jeckelmann JM, Gapsys V, Ucurum Z, de Groot BL, Fotiadis D. PNAS 2016.



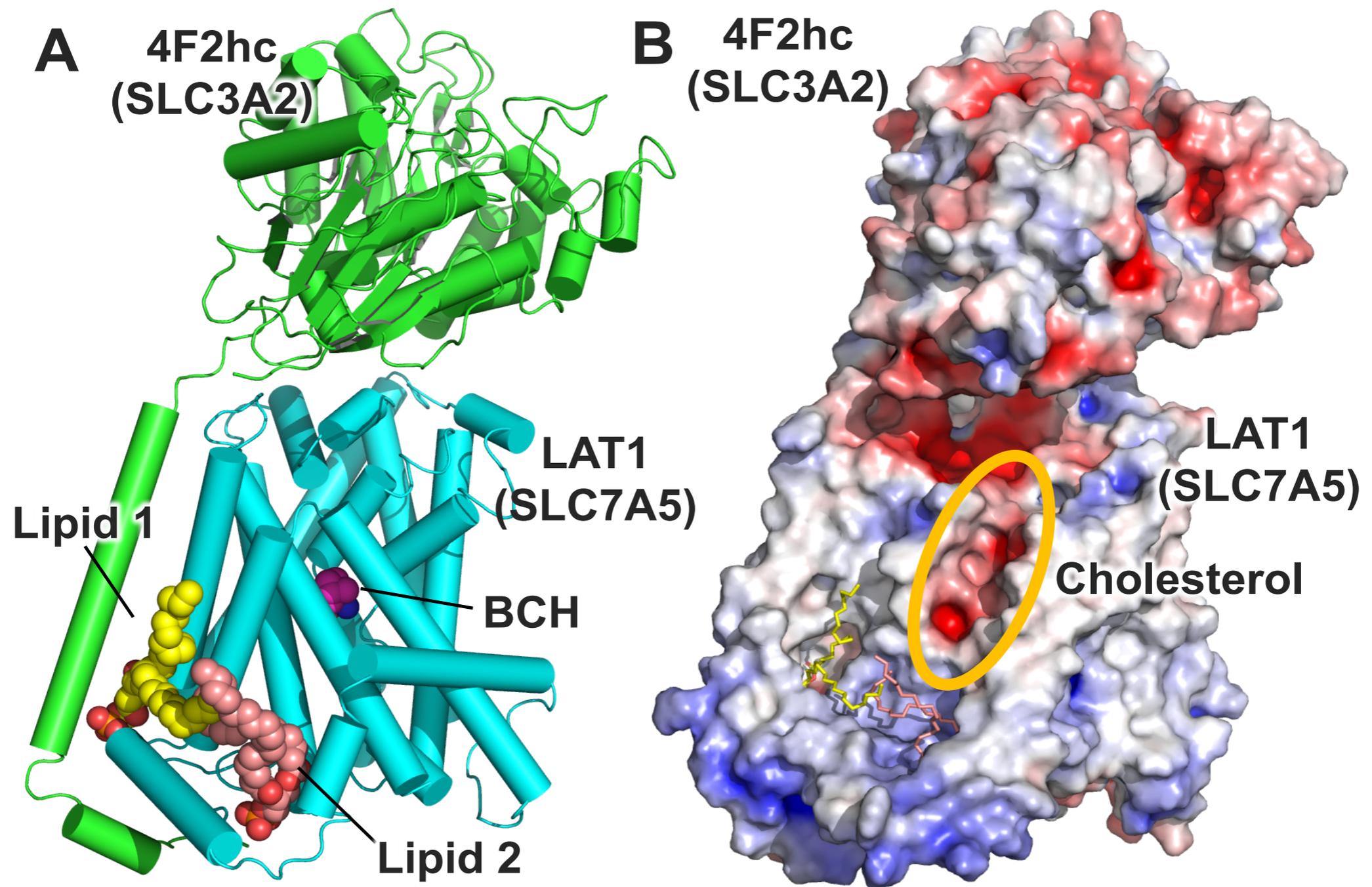
IC_{50} $23 \pm 9 \mu M$
 Efflux rate 2.0 (fmol/min)



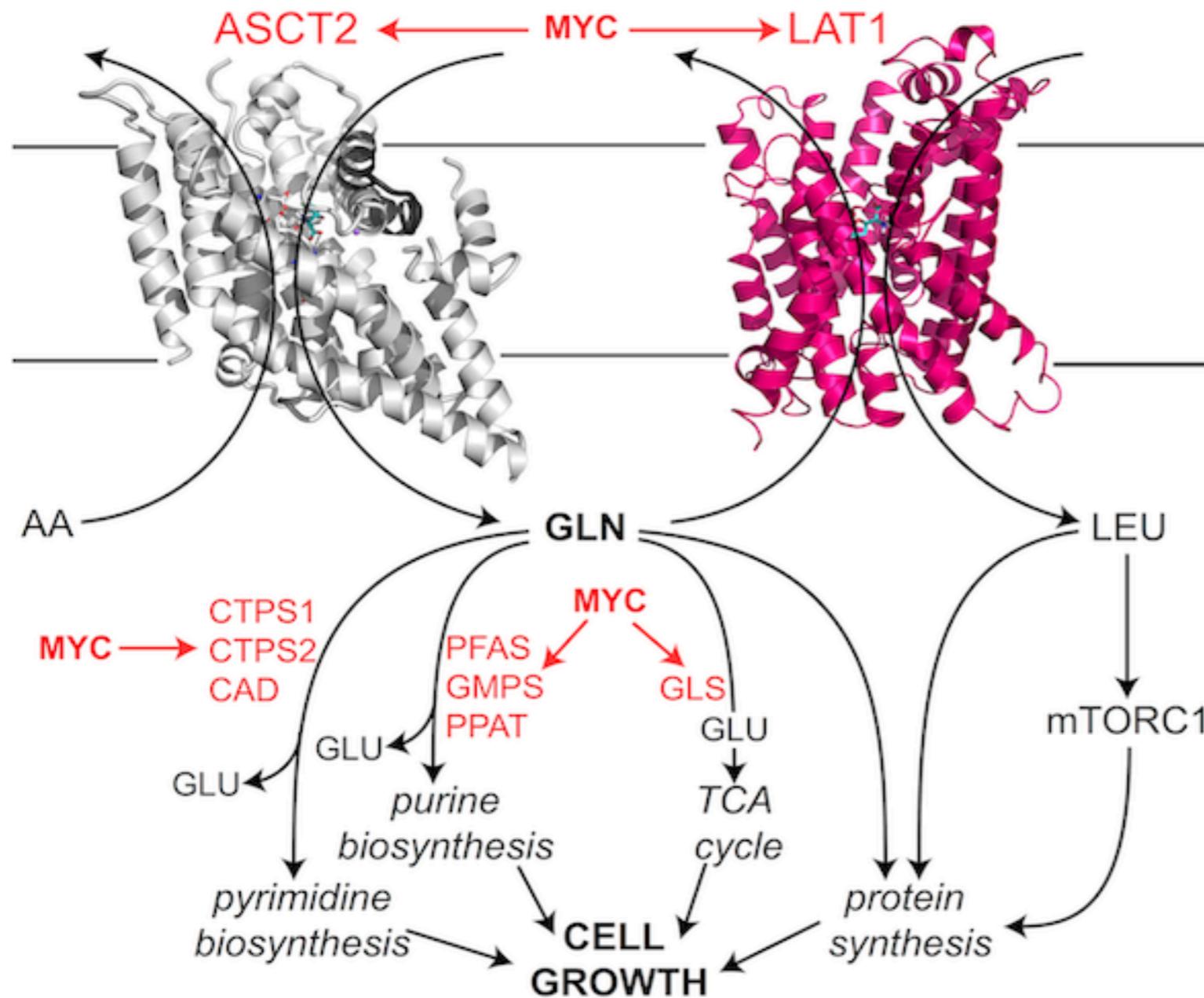
Chien HC* and Colas C* et al. J Med Chem 2018 Aug 23;61(16):7358-7373..

Human structures confirm specificity determinants and provide new opportunities

Can we target allosteric pockets with activators and inhibitors?



Cancer metabolism is supported by nutrient transporters



Rachel-Ann Garibsingh



Claire Colas



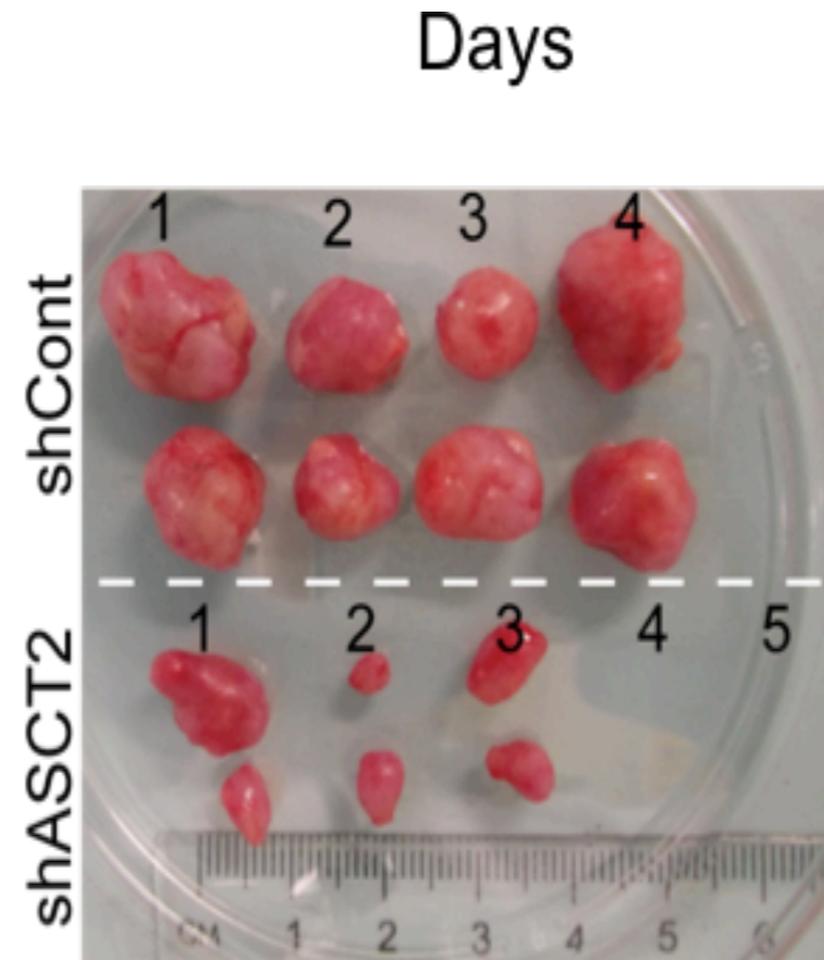
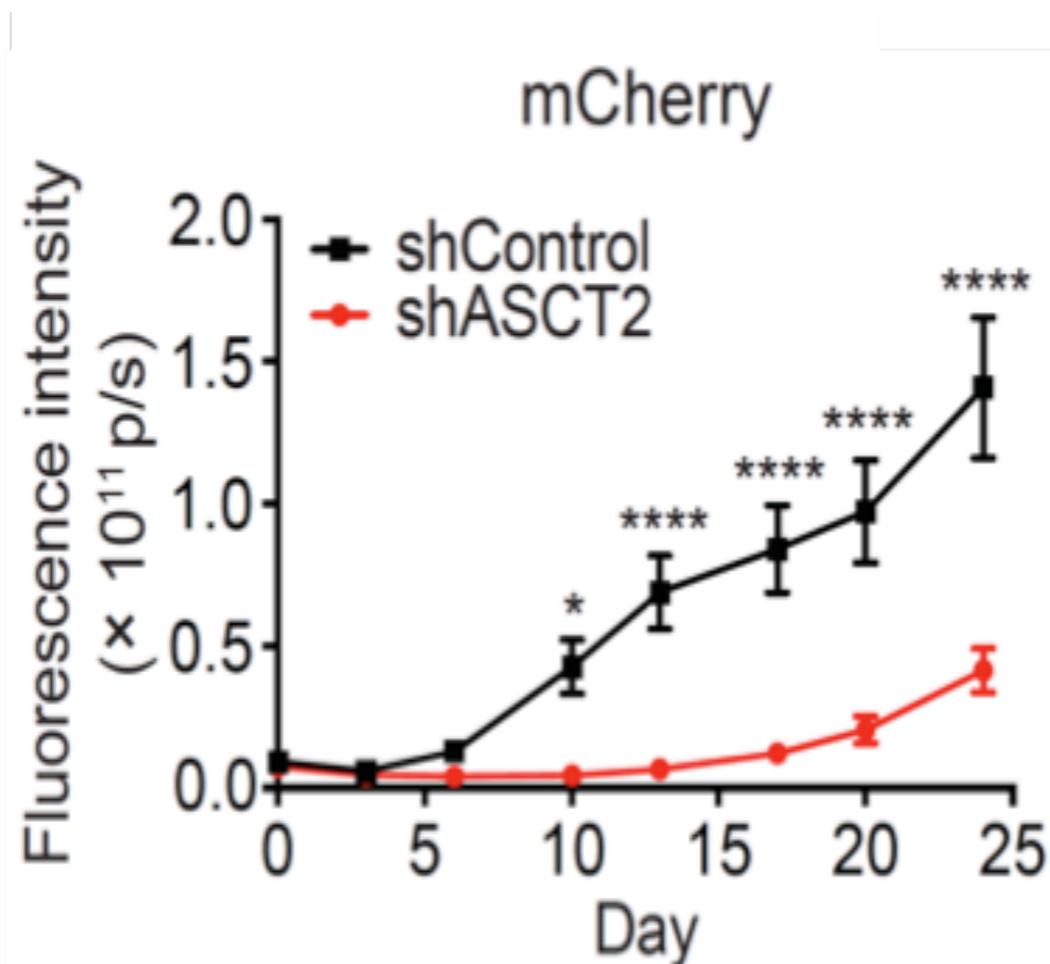
Jeff Holst (UNSW, Australia)



Christof Grewer (SUNY Binghamton)

The alanine-serine-cysteine transporter-2 (ASCT2/SLC1A5)

- ASCT2 is a peripheral sodium dependent neutral amino transporter.
- ASCT2 gets upregulated in certain cancers, such as TNBC, melanoma, prostate cancer, hepatocellular carcinoma.
- ASCT2 is the primary glutamine transporter in specific tumor types.



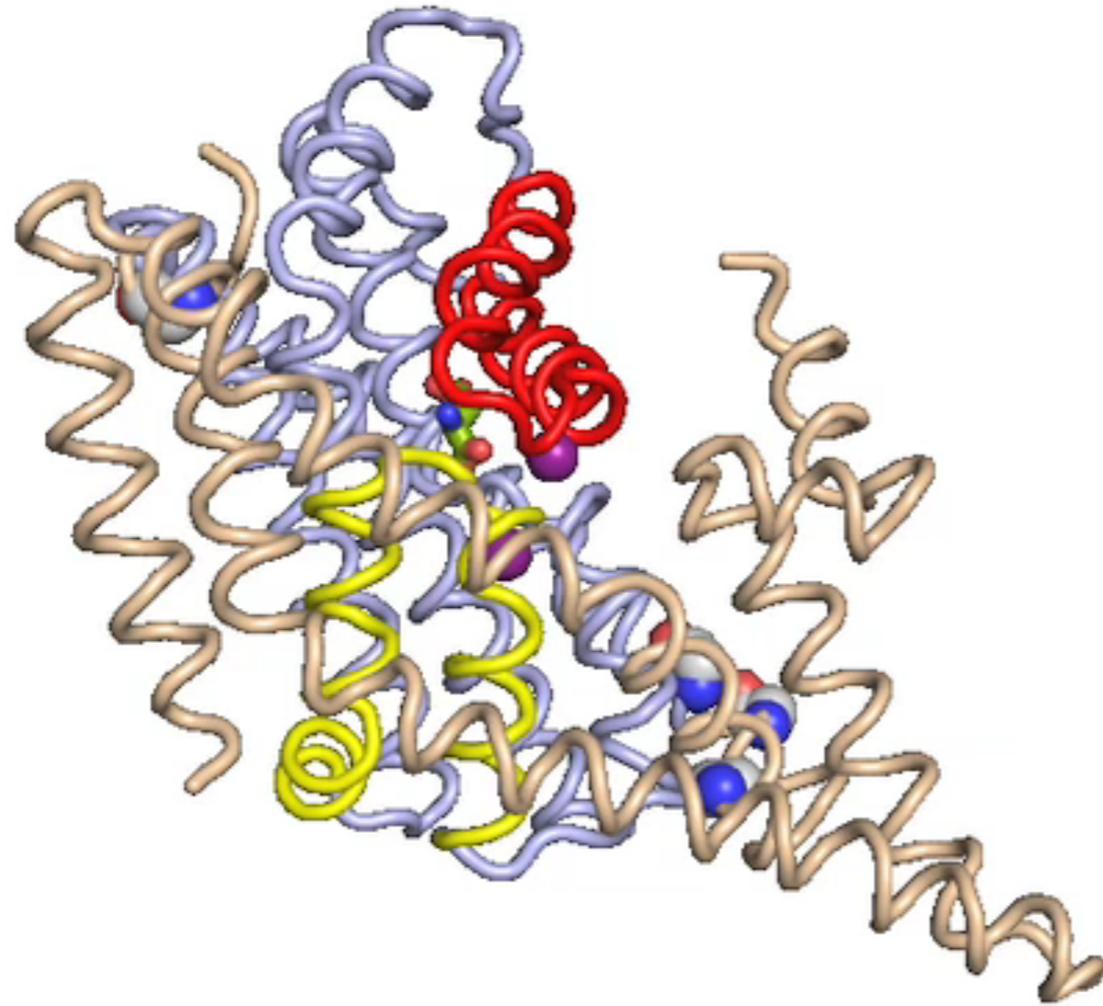
van Geldermalsen *et al.* Oncogene. 2016 Jun 16;35(24):3201-8.

Wang *et al.* Int J Cancer. 2013, 135, 1060-1071.

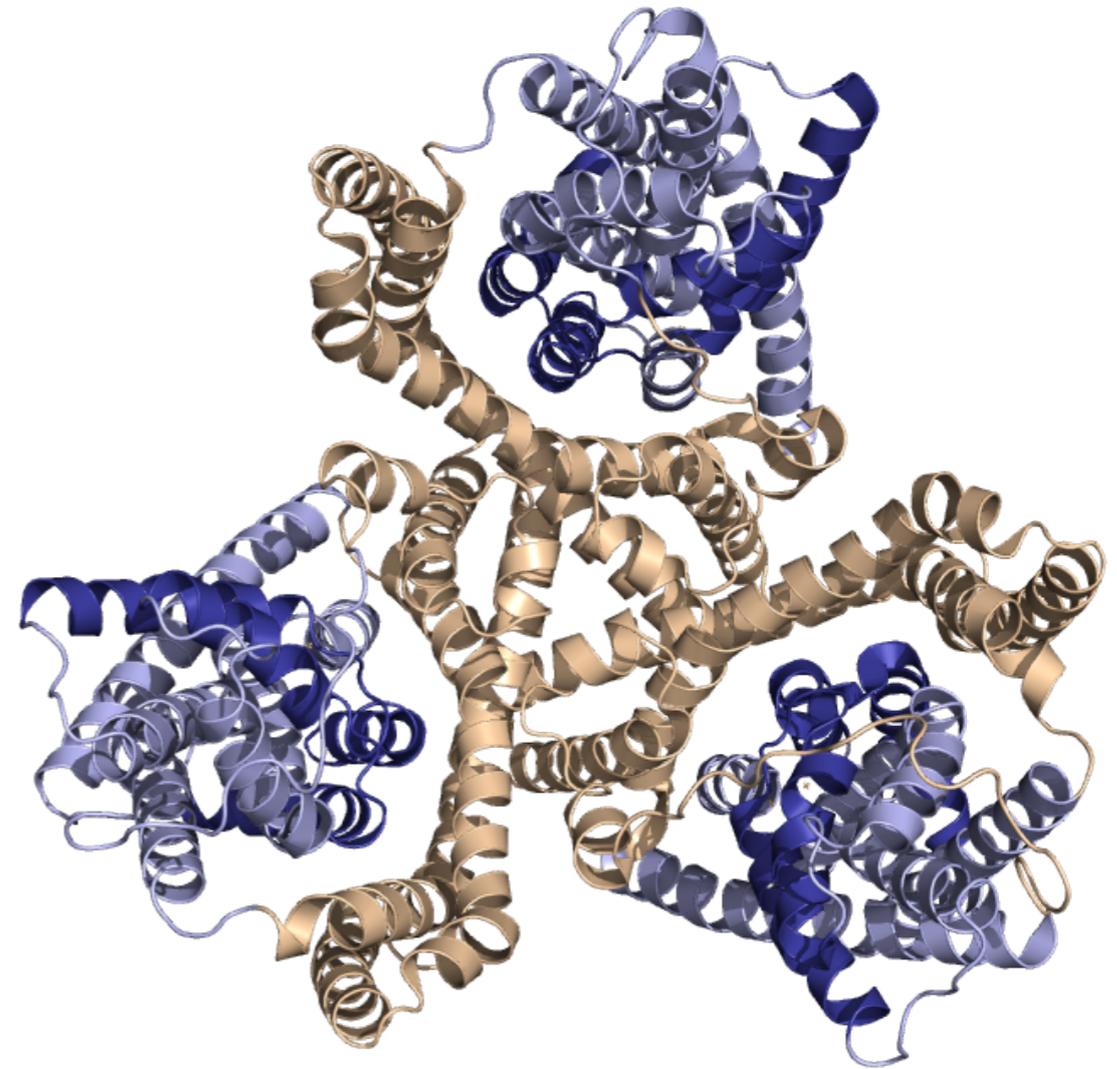
Wang *et al.* J Pathol. 2015 236, 278-289.

Schulte, *et al.* Nat Med. 2018 Feb;24(2):194-202.

Prokaryotic homolog structures reveal key mechanistic insights



Structures of the prokaryotic aspartate transporter
 Glt_{PH} from *Pyrococcus horikoshii*



Top view of the Glt_{PH} trimer

Boudker *et al.* Nature. 2007 Jan 25;445(7126):387-93.

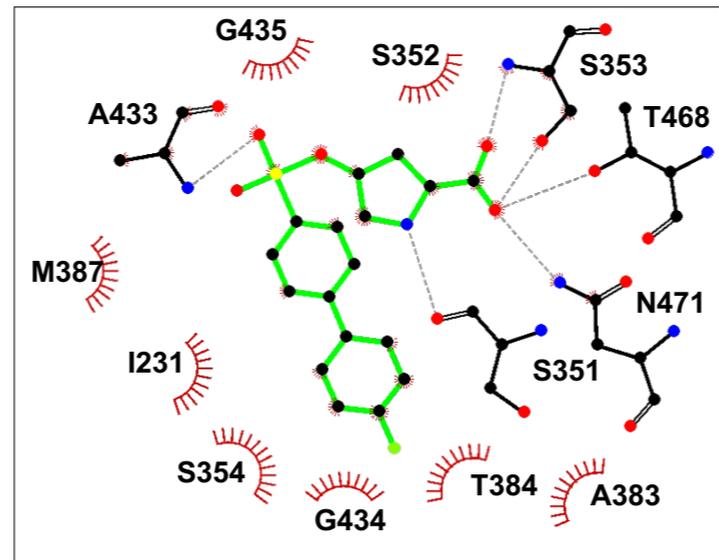
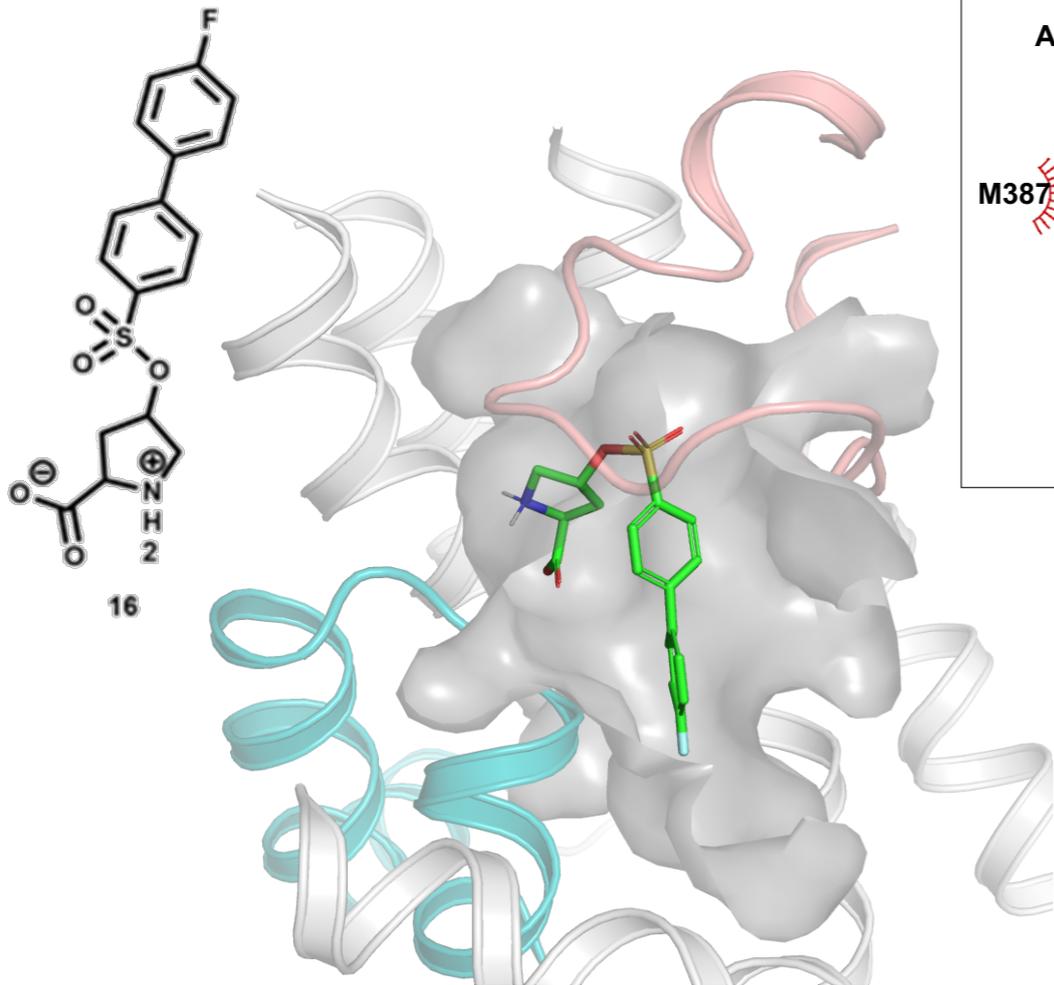
Reyes N, Ginter C, Boudker O. Nature. 2009 Dec 17;462(7275):880-5.

Akyuz *et al.* Nature. 2013 Oct 3;502(7469):114-8.

Newer models based on hEAAT1 allow further optimization

Novel scaffolds

Potent inhibitors

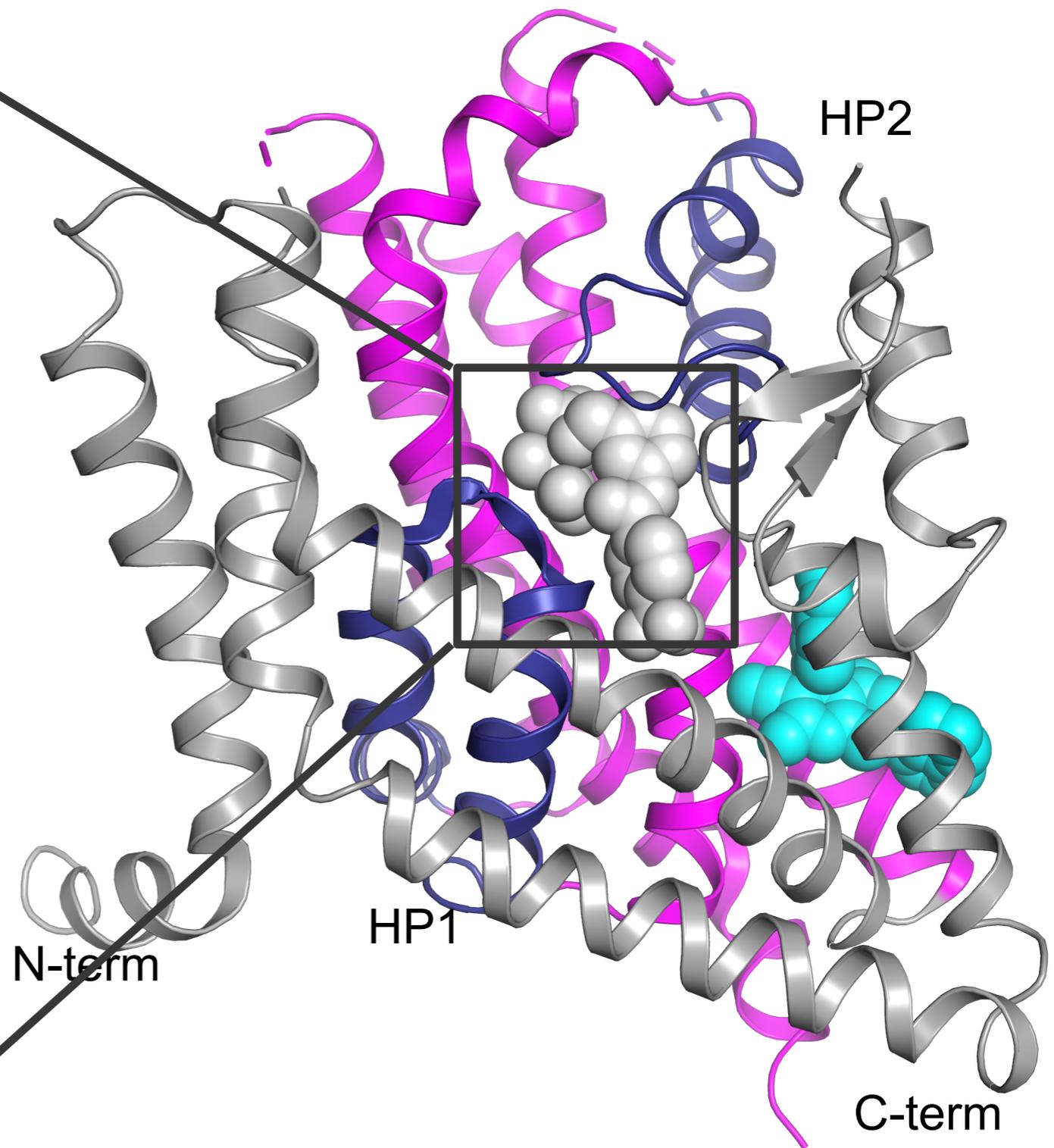
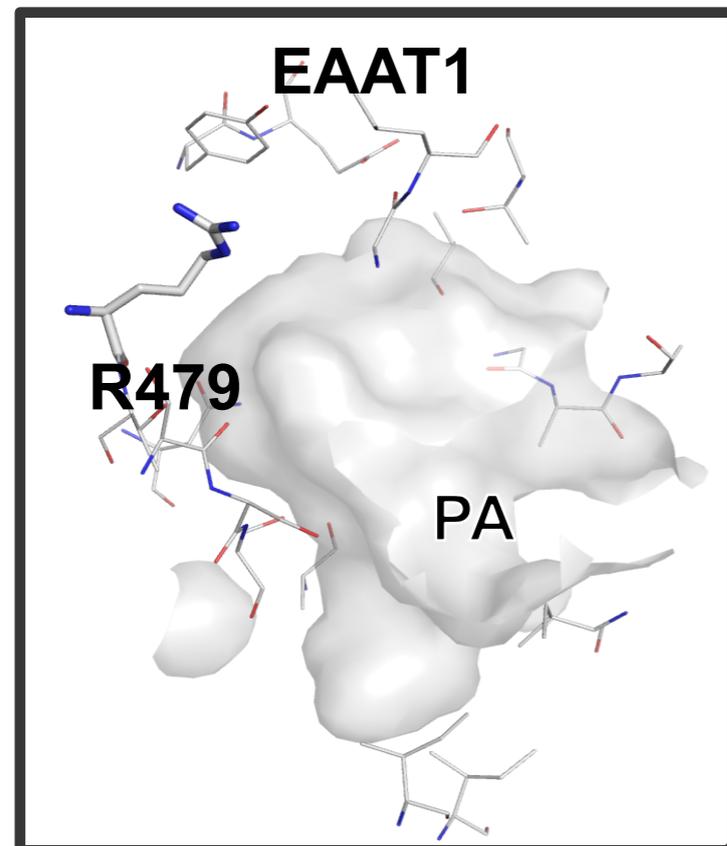
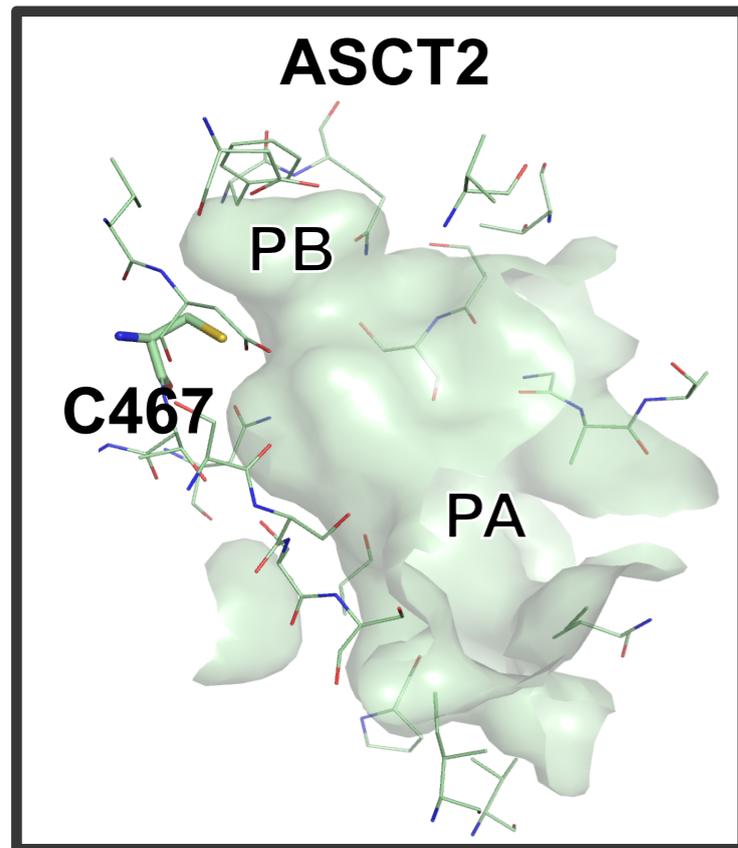


16b

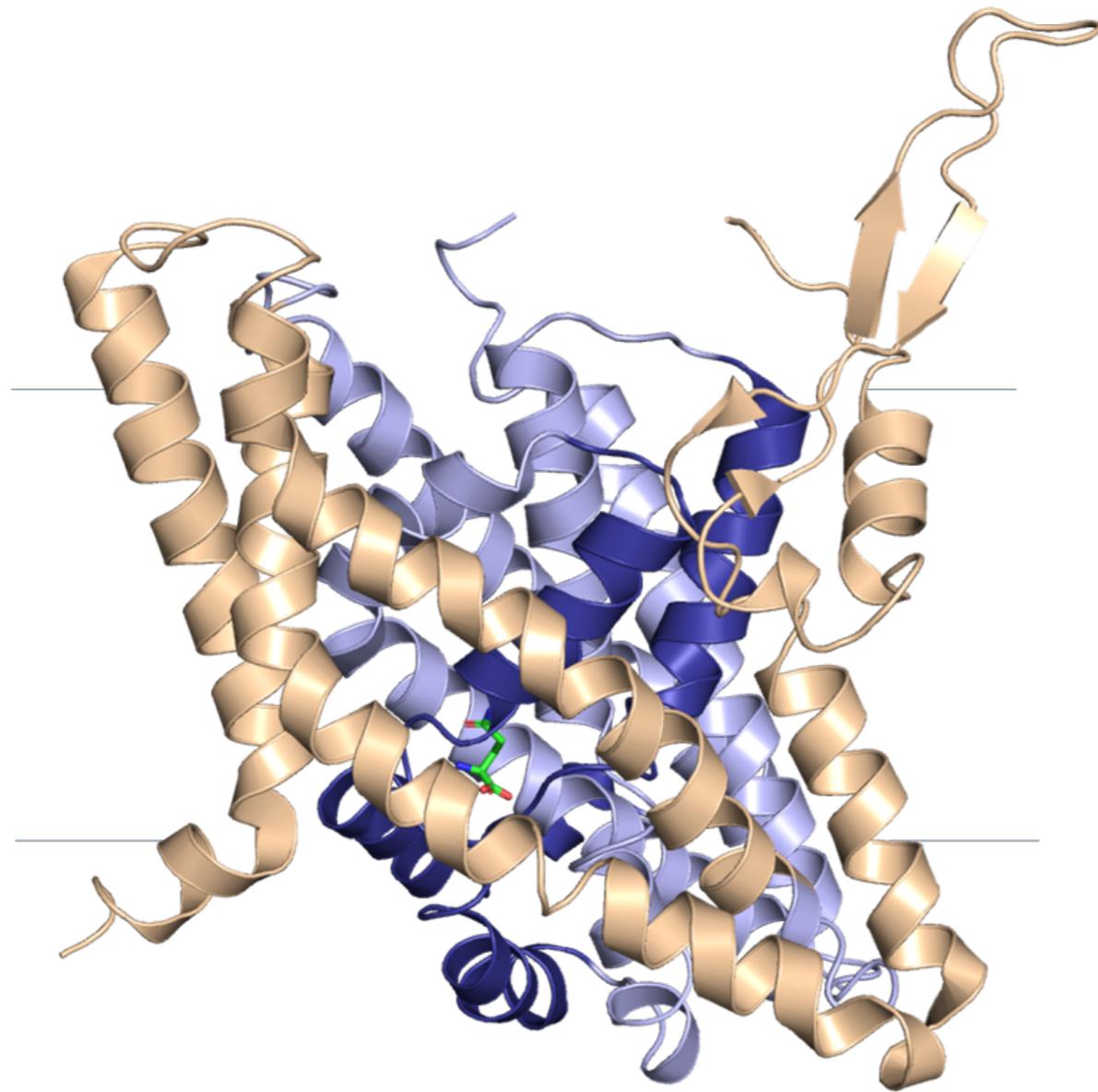
$$K_i = 8.07 \pm 4 \mu\text{M}$$

$$K_i = 0.7 \mu\text{M}$$

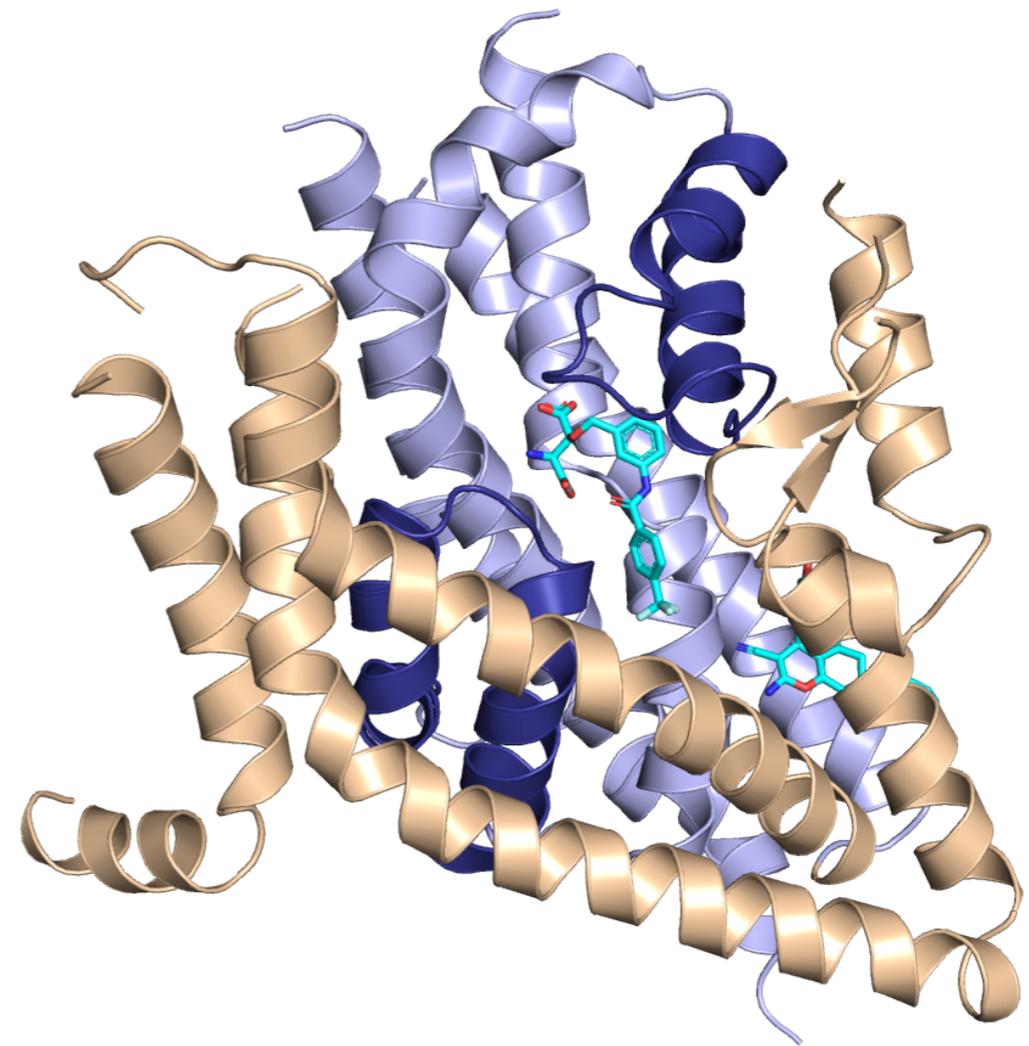
Amino acid selectivity in the SLC1 family



New structures confirm specificity determinants and provide additional structural insights



ASCT2 monomer

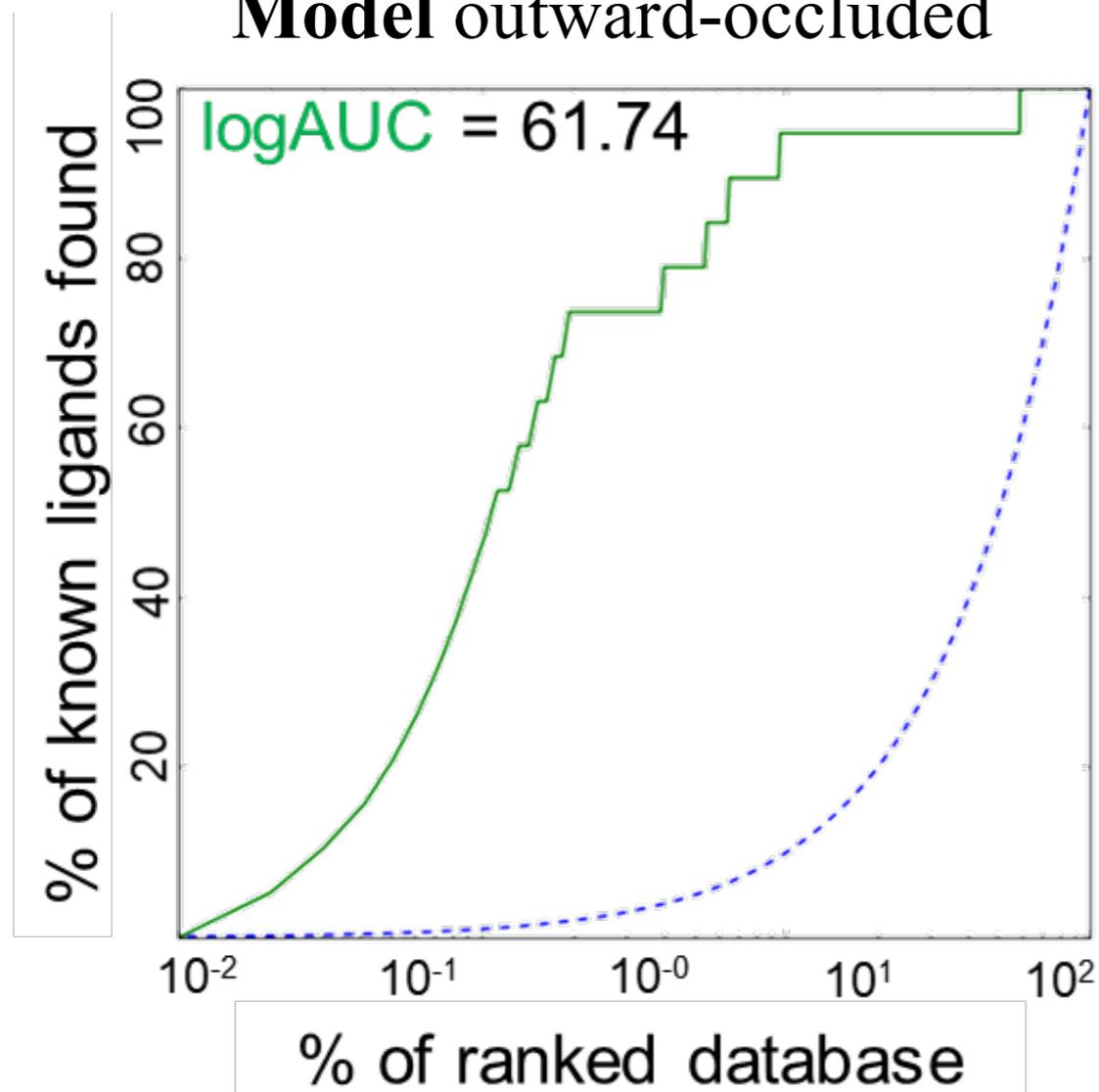


ASCT2 outward open model based on hEAAT1

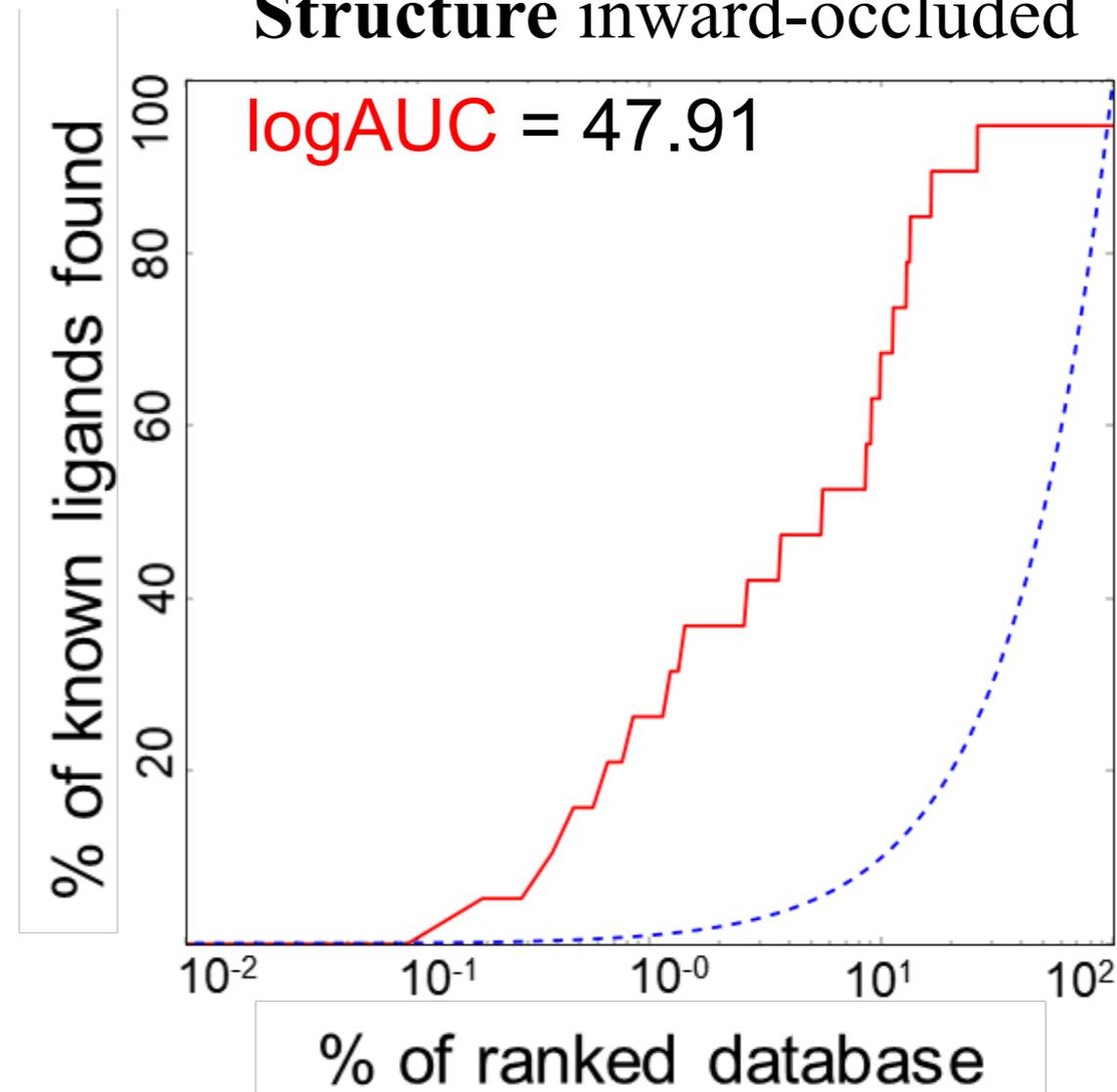
Do we still need ASCT2 models?

Model can capture pharmacologically relevant conformations

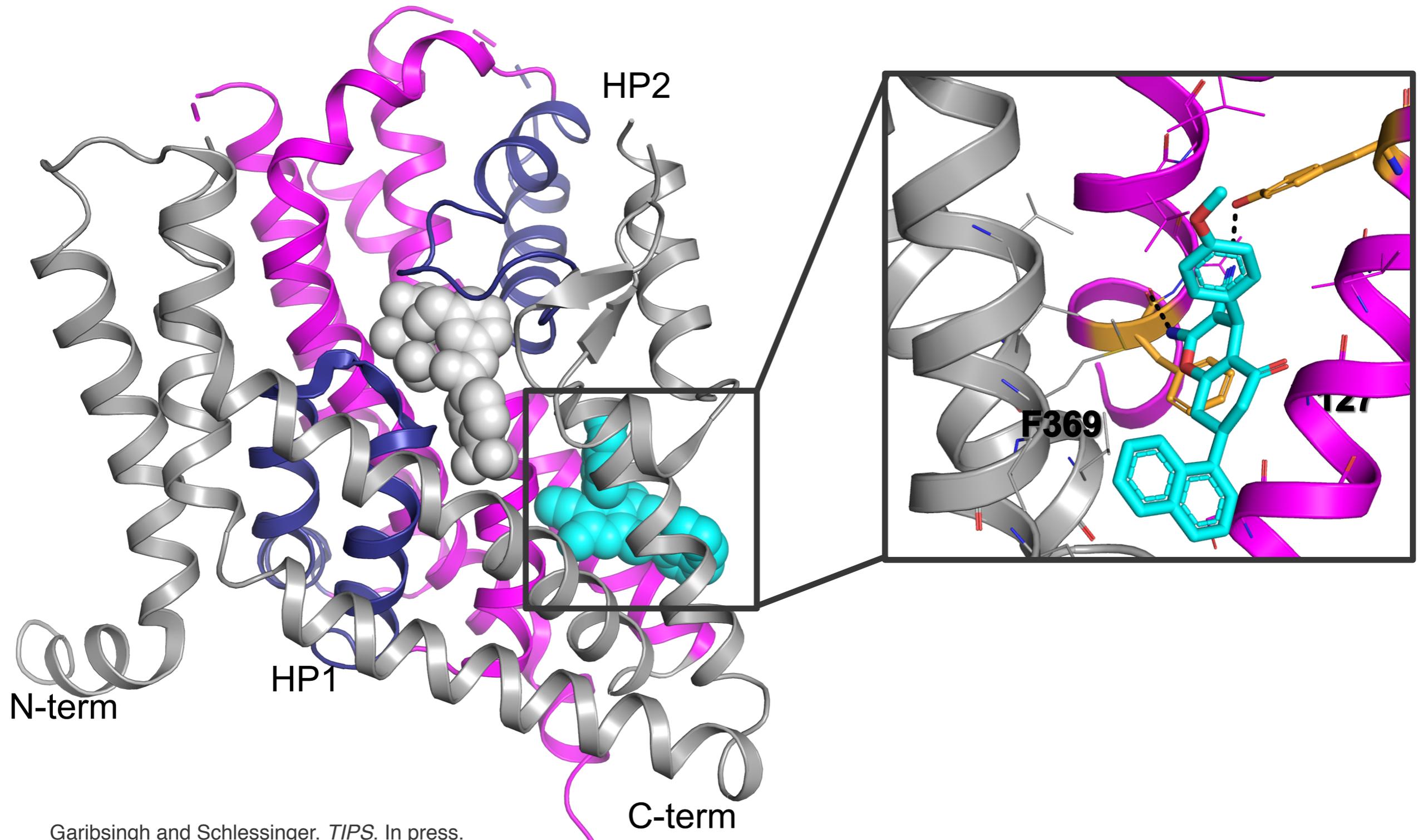
Model outward-occluded



Structure inward-occluded



EAAAT1 based model provides a framework for allosteric inhibitor design



Summary: An integrated structure-based approach to identify ligands for Solute Carriers

- **Deorphanized a challenging membrane protein target**
- **Rationalized the effect of disease variants on function**
- **Compounds explain efficacy and side effects of drugs and exhibit novel scaffolds**
- **Improve our understanding of how amino acid specificity is achieved**
- **We need more structures in new conformations**

Outstanding questions

- **Is there sufficient coverage of SLC structures and conformations to describe the effects of disease-related mutations and to design useful tool compounds or future therapeutics?**
- **Can computational methods can be improved to distinguish between substrates, inhibitors, and activators of SLC transporters?**
- **Can we use protein structure and rational design to predict transporter-mediated drug–drug interactions, drug distribution, and differential drug response among patients?**

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