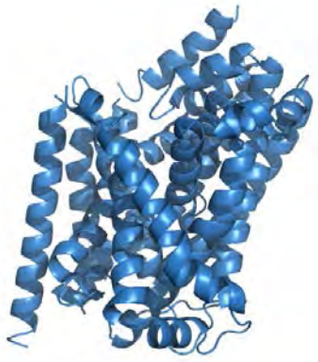

Inhibitor Discovery for GLUT1 from Homology Modeling and Virtual Screening

Avner Schlessinger
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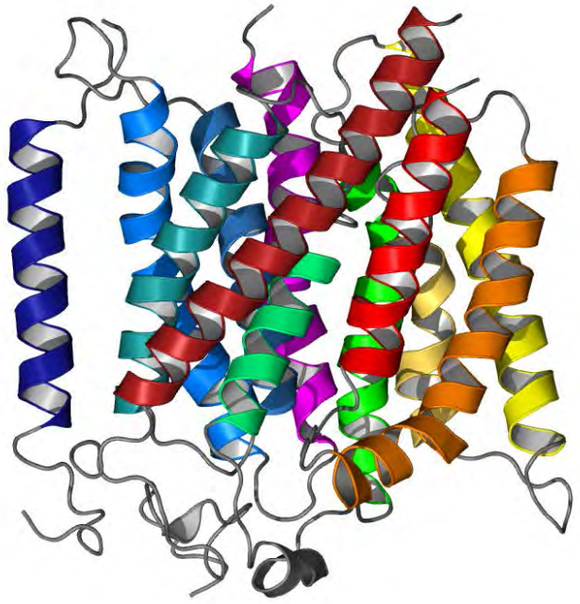
ITCW 3, ASCPT, Washington, DC
March 13, 2017

Mount Sinai

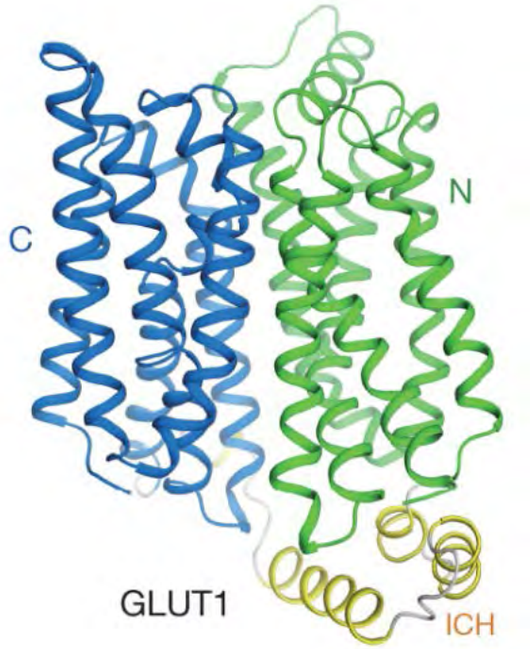
Small number of human SLC structures

- **<u>Six</u> structures of human SLCs were determined in atomic resolution**



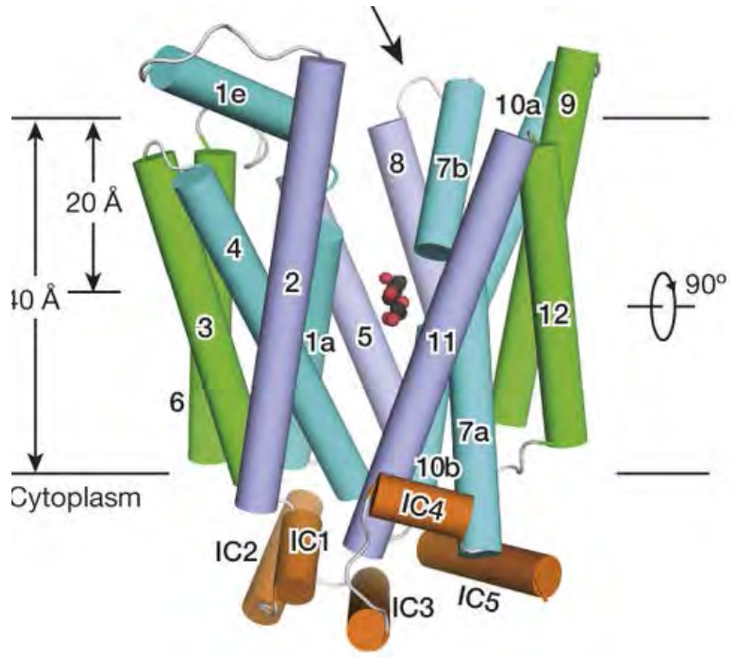
SLC42A3

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



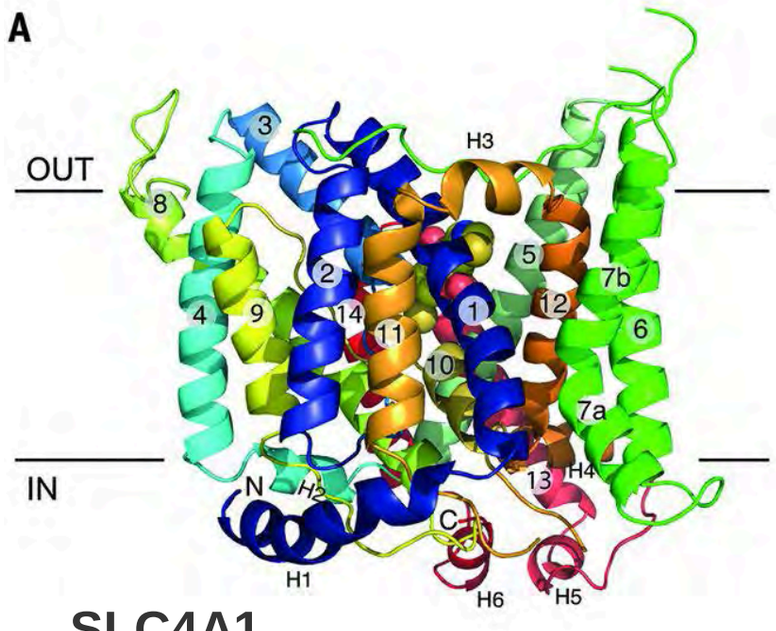
SLC2A1

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



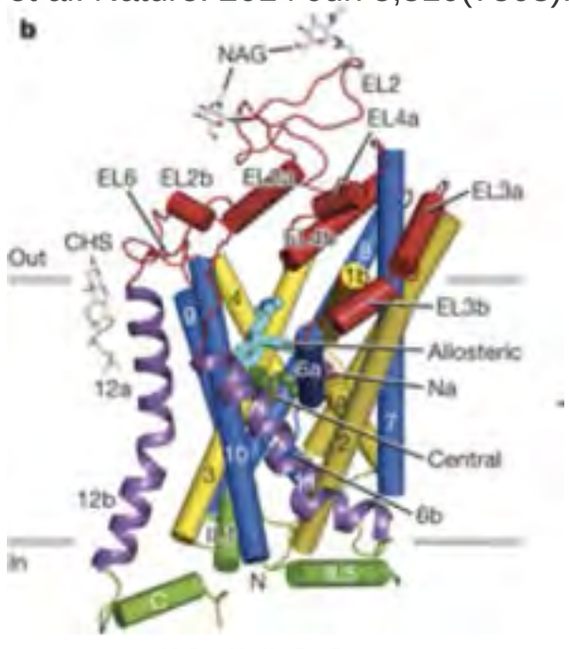
SLC2A3

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



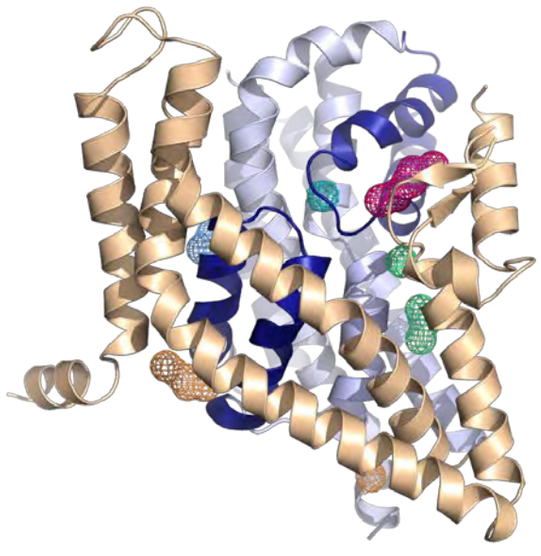
SLC4A1

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



SLC6A4

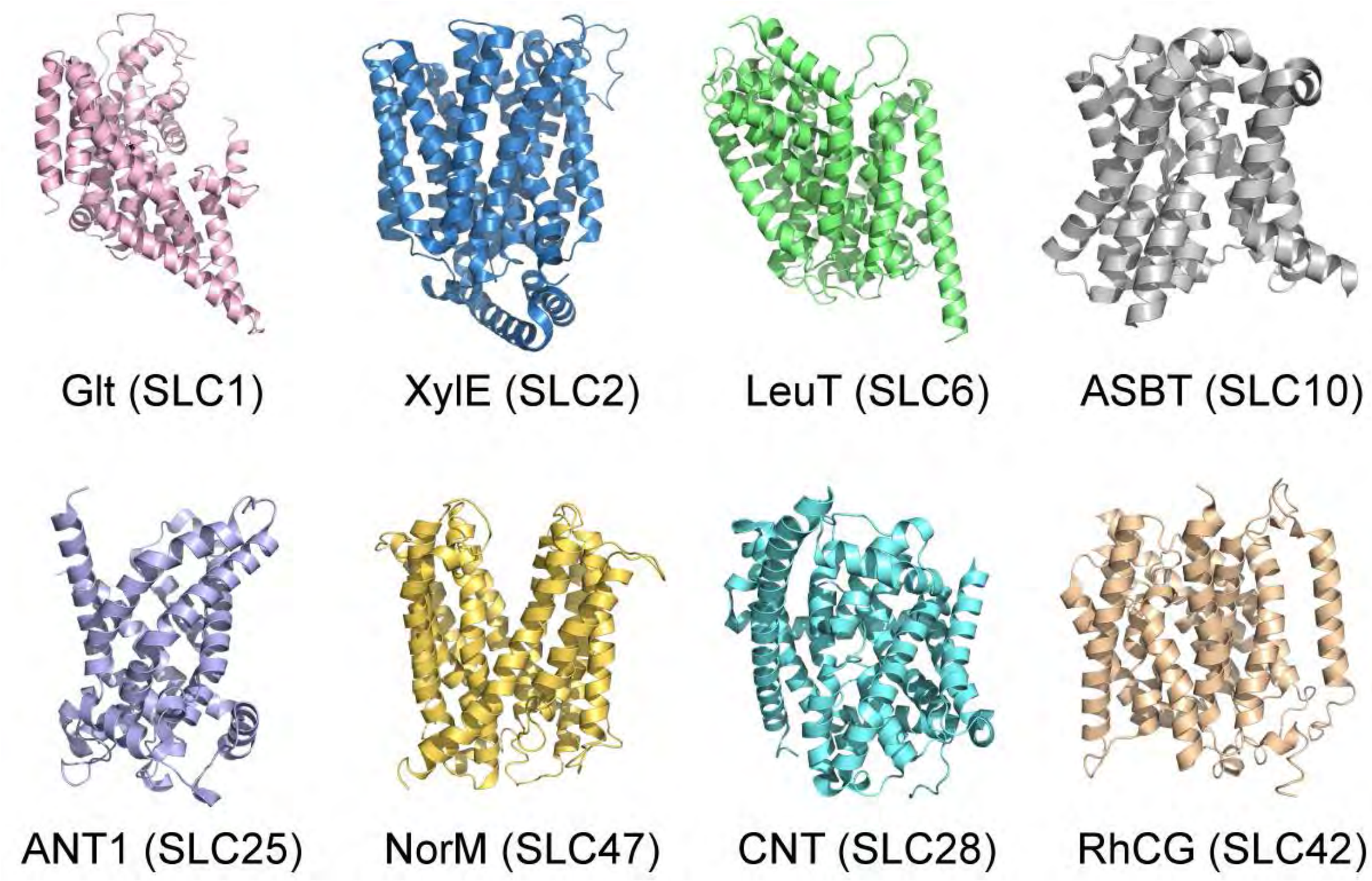
Coleman et al. Nature. 2016 Apr 21;532(7599):334-339



SLC1A3

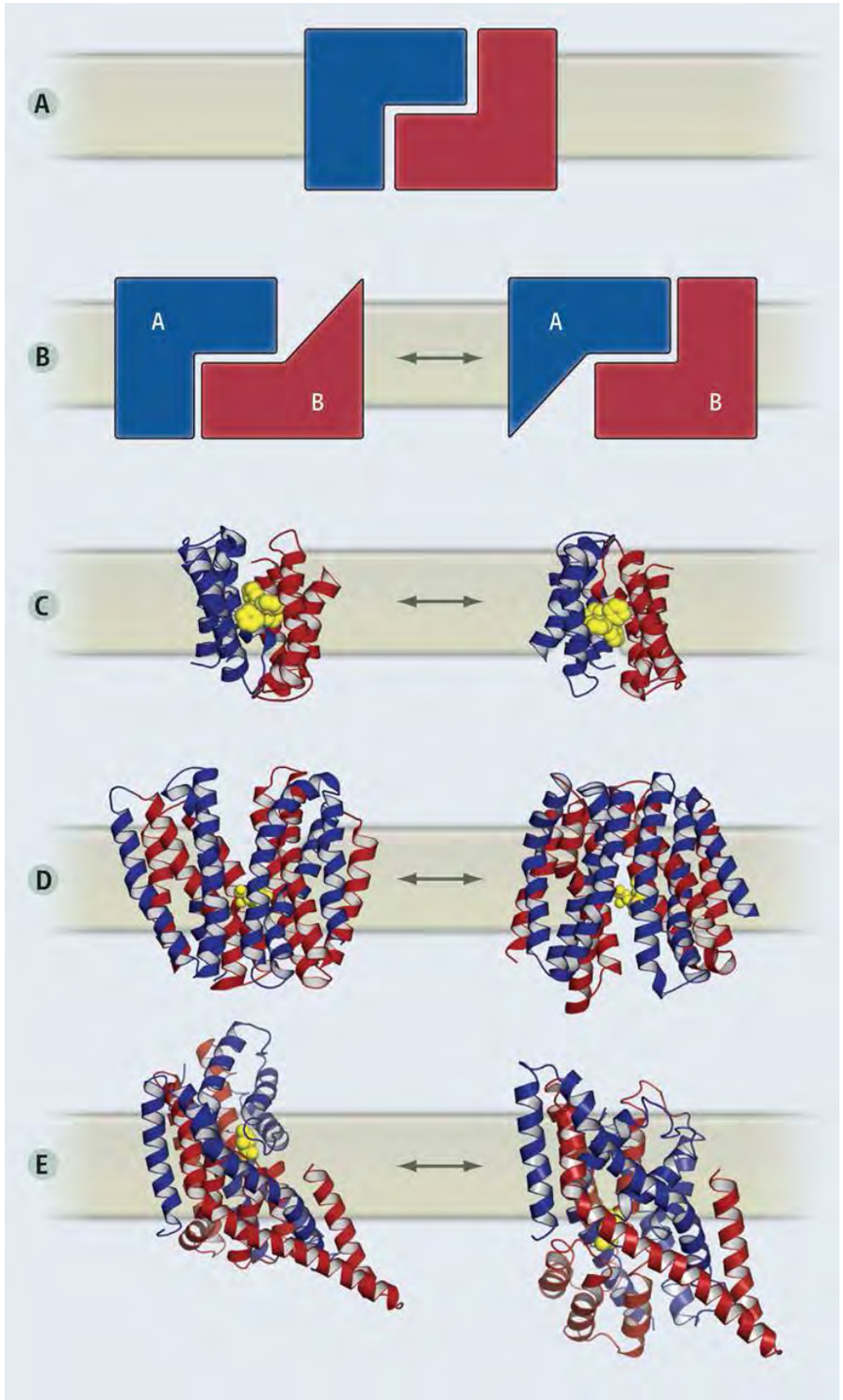
Reyes et al. Nature. in press.

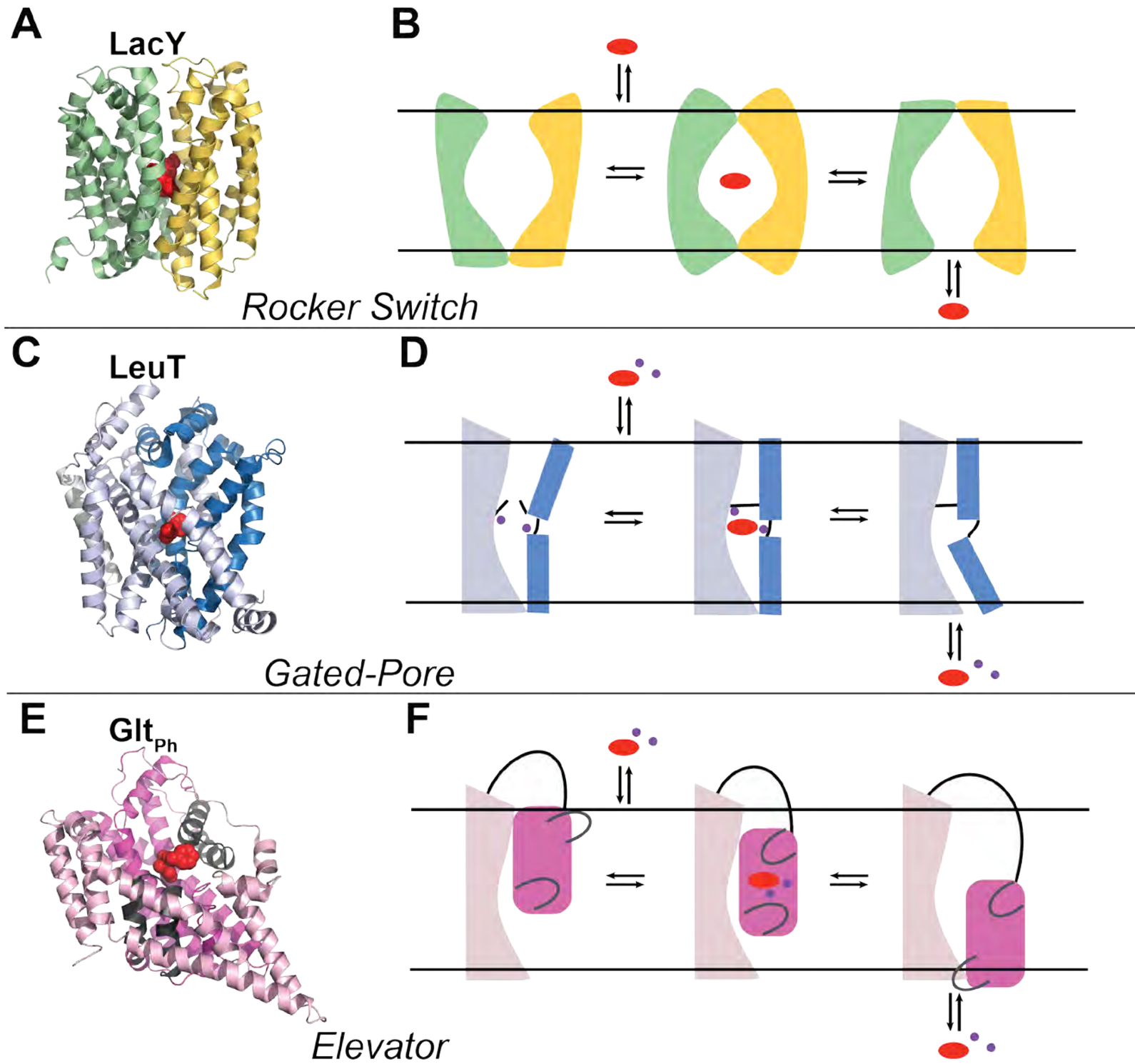
Structures of homologs reveal a highly diverse superfamily



Schlessinger A, Khuri N, Giacomini KM, Sali A. Current Topics in Medicinal Chemistry. 2013 Apr 1;13(7):843-56.

Many SLCs use alternating access transport





Forrest. Science. 2013 Jan 25;339(6118):399-401.

Colas C, Ung PMU, Schlessinger A. Medchemcomm. 2016 Jun 1;7(6):1069-1081

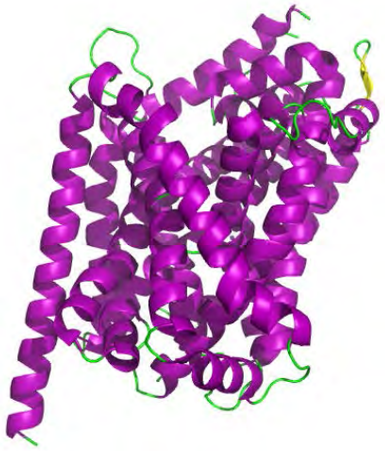
Homology modeling and virtual screening

1. Search for template
PDB, OPM

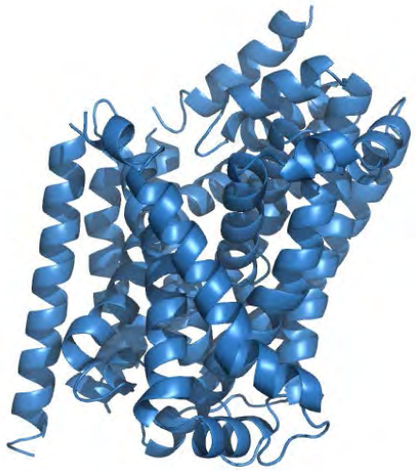
2. Align target and template
SALIGN, PROMALS3D

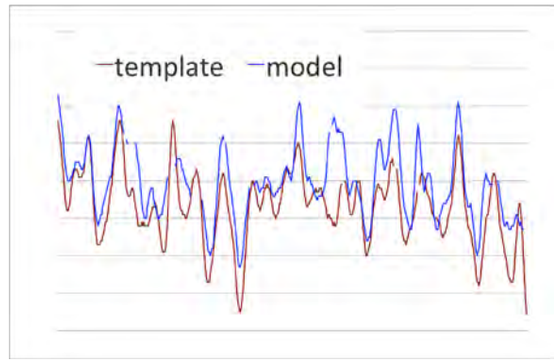
3. Construct and assess model
MODELLER, DOPE

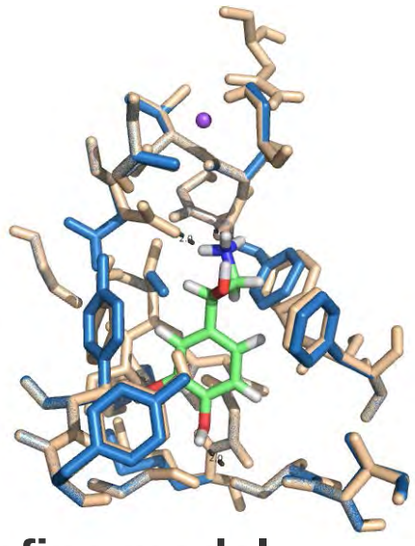
Target sequence:
GGMEAVITGLADDFQAA



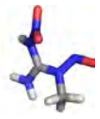
GGMEAVITGLADDFQAA
AIM--QPMIAFLEDELKL-

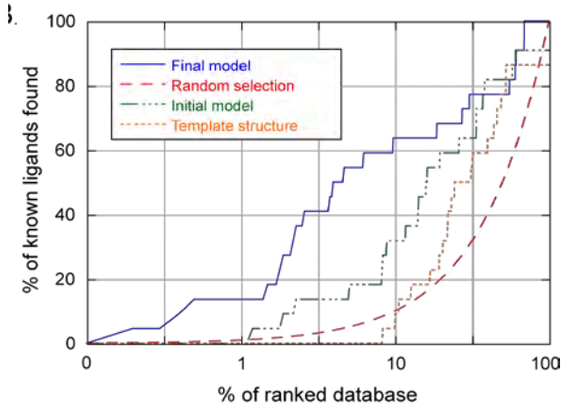






4. Refine model
SCWRL4, GROMACS





5. Validate binding site
Glide, FRED, DUD

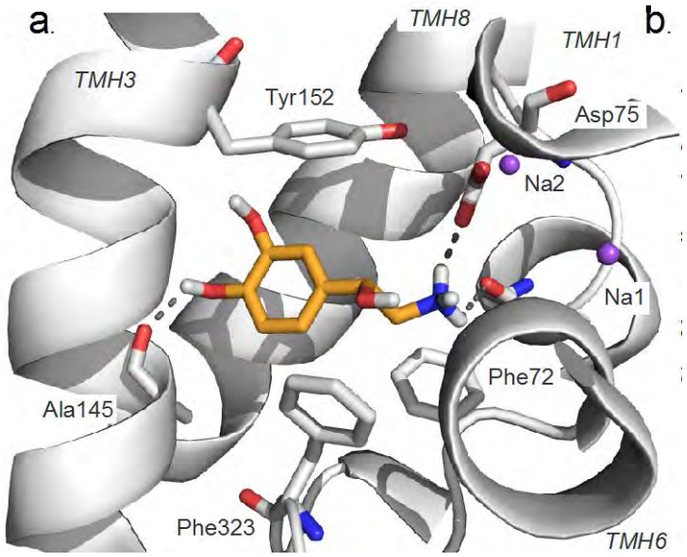
6. Virtual screening
Glide, FRED

Schlessinger *et al*. PNAS 2011 Sep 20;108(38):15810-5.
Schlessinger & Wittwer *et al*. JBC 2012 Nov 2;287(45):37745-56.

Carlsson *et al*. Nat Chem Biol. 2011 Sep 18;7(11):769-78

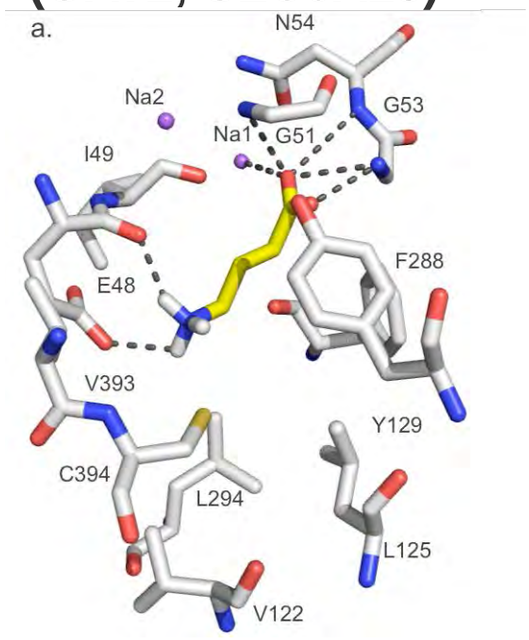
Structure based ligand discovery for human SLC transporters

The norepinephrine transporter (NET, SLC6A2)



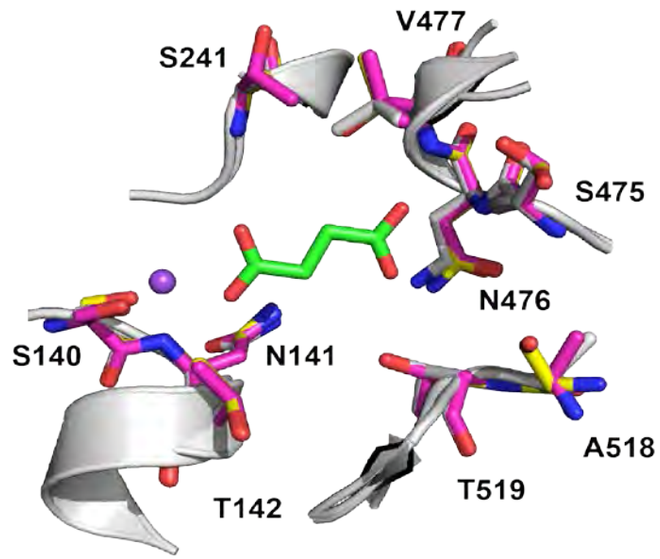
Schlessinger *et al*. PNAS 2011 Sep 20;108(38):15810-5.

The GABA transporter 2 (GAT2, SLC6A13)



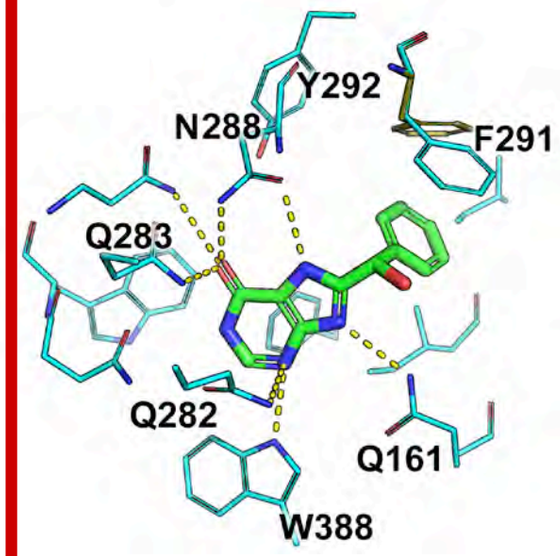
Schlessinger & Wittwer *et al*. JBC 2012 Nov 2;287(45):37745-56.

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.
Colas *et al*. Submitted.

The glucose transporter 1 (GLUT1, SLC2A1)



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

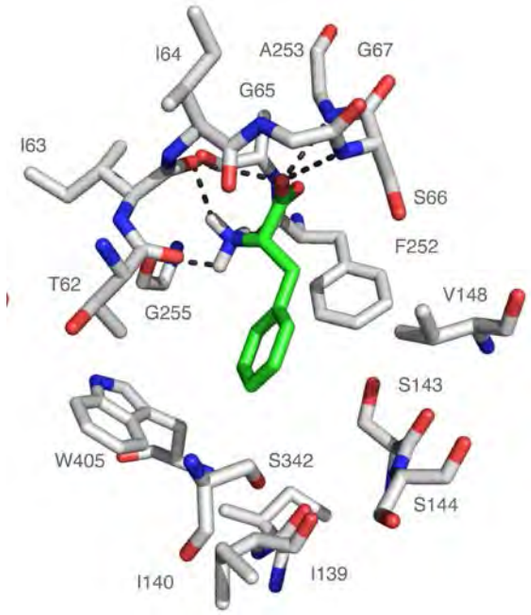
The alanine-serine-cysteine amino ac SLC1A5)

Peter Man-Un Ung

7;11(10):e0164477.

Oct

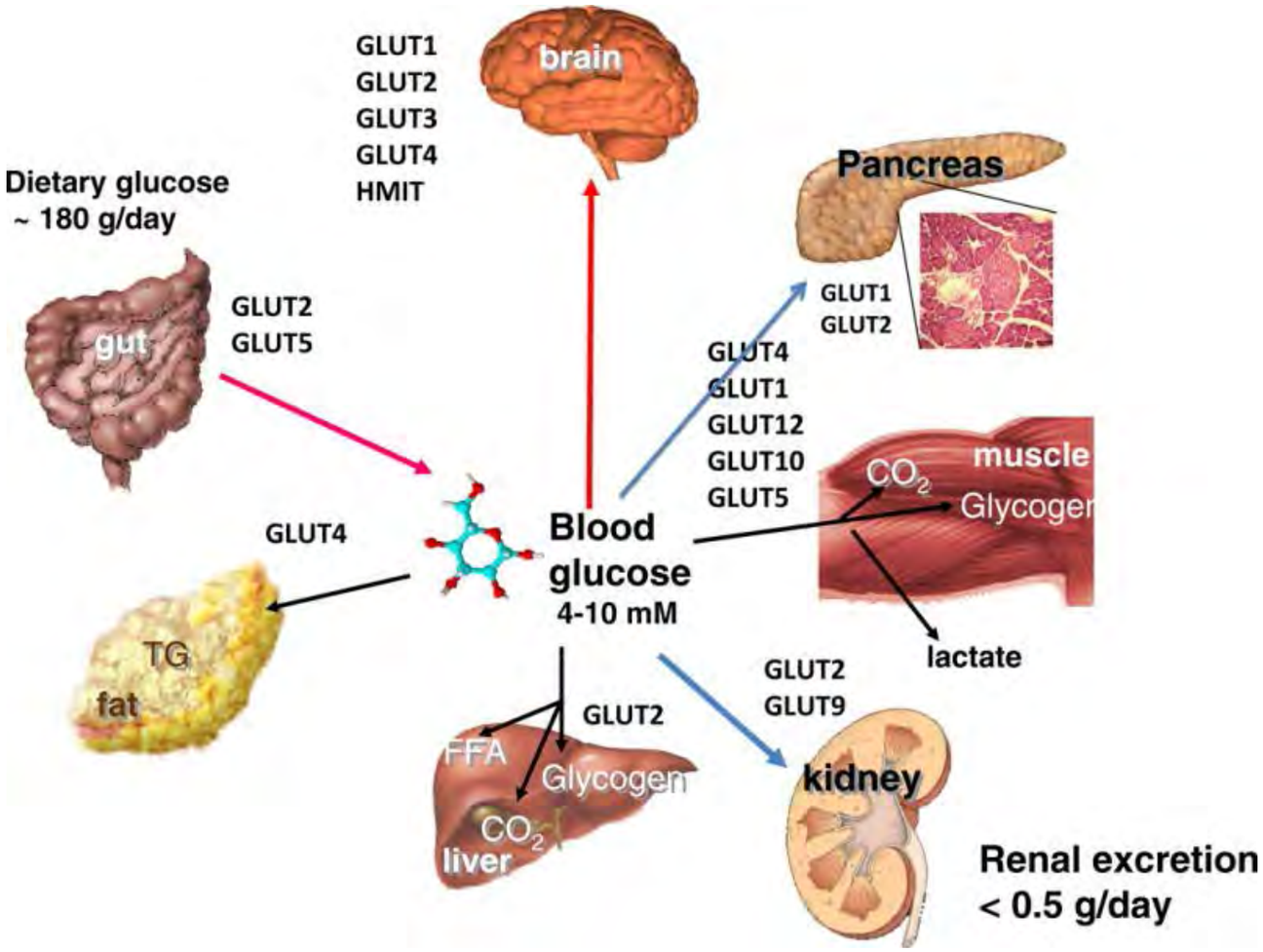
The L-type amino acid transporter (LAT-1, SLC7A5)



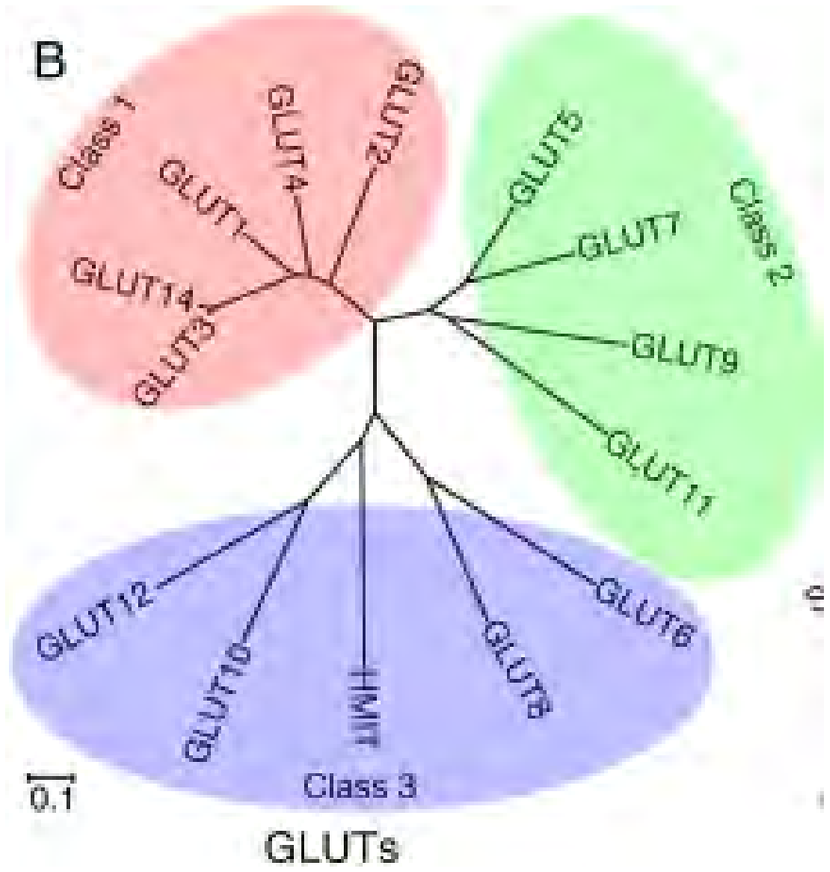
Geier* and Schlessinger* *et al*. PNAS 2013 Apr 2;110(14):5480-5

The SLC2 family of facilitative transporters

- The family includes 14 members, divided into three classes
- 11 of the 14 SLC2 members are capable of transporting glucose under some experimental conditions (GLUT1-4: glucose, GLUT5: fructose, GLUT9: urate, GLUT13: inositol)
- One or more SLC2 members are expressed in virtually every cell type of the human body
- Contain ~500 amino acids with 12 transmembrane helices



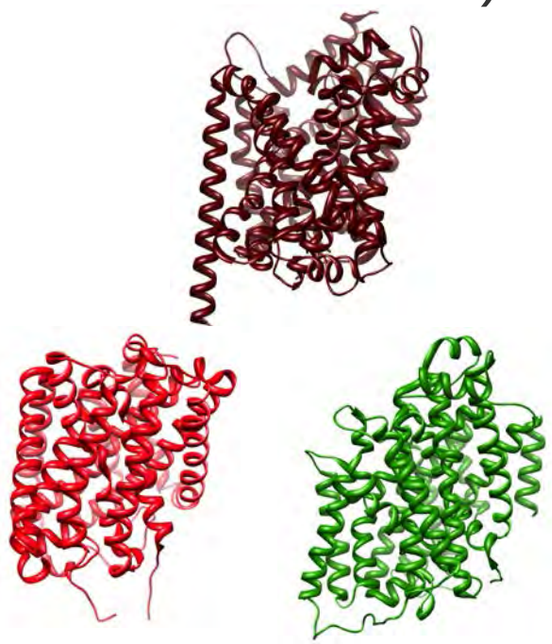
Mueckler & Thorens. Mol Aspects Med. 2013 Apr-Jun;34(2-3):121-38.

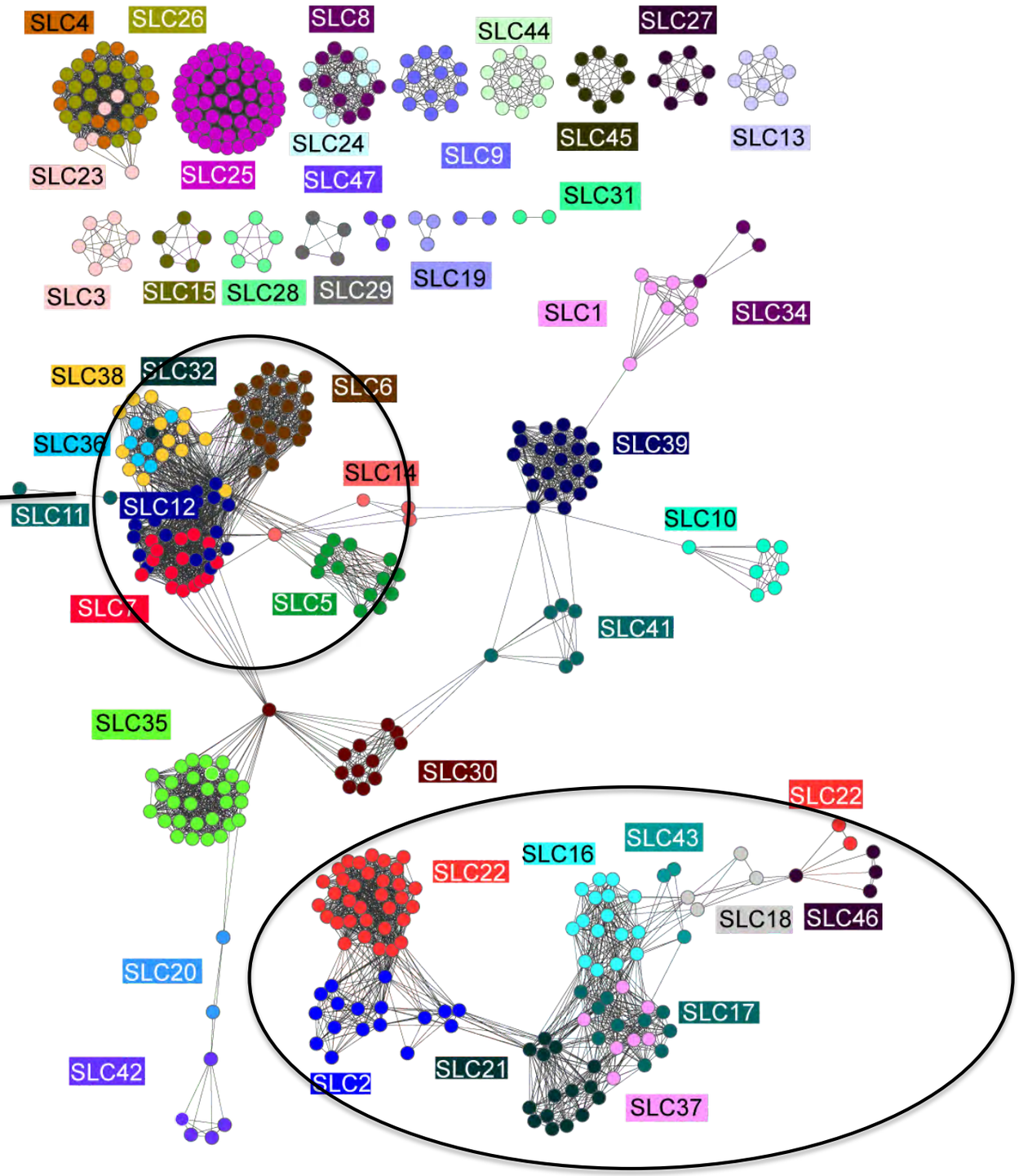


Deng & Yan. Protein Sci. 2016 Mar;25(3):546-58.

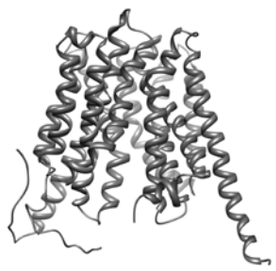
GLUTs adopt the MFS fold and are related to SLC22

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



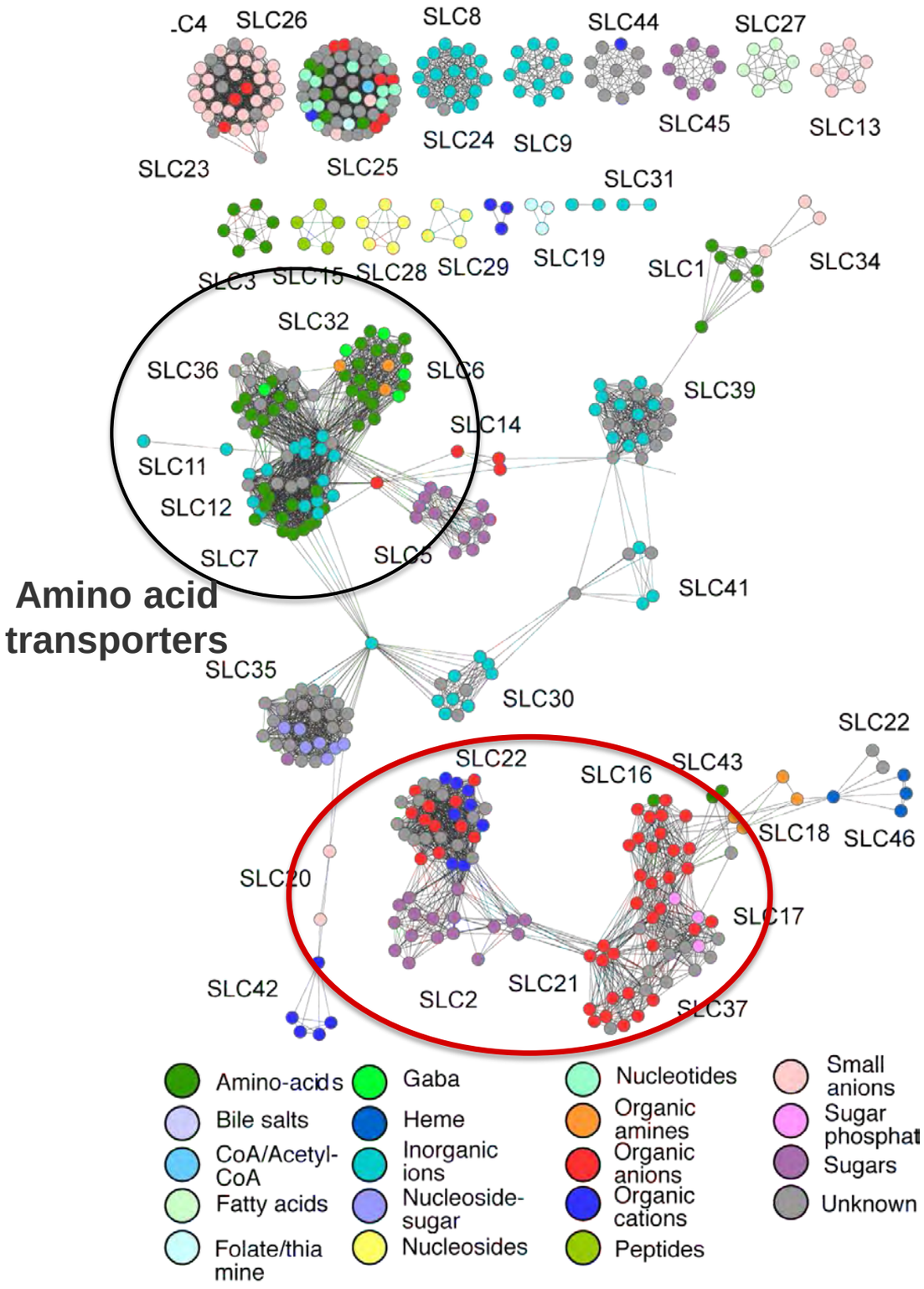


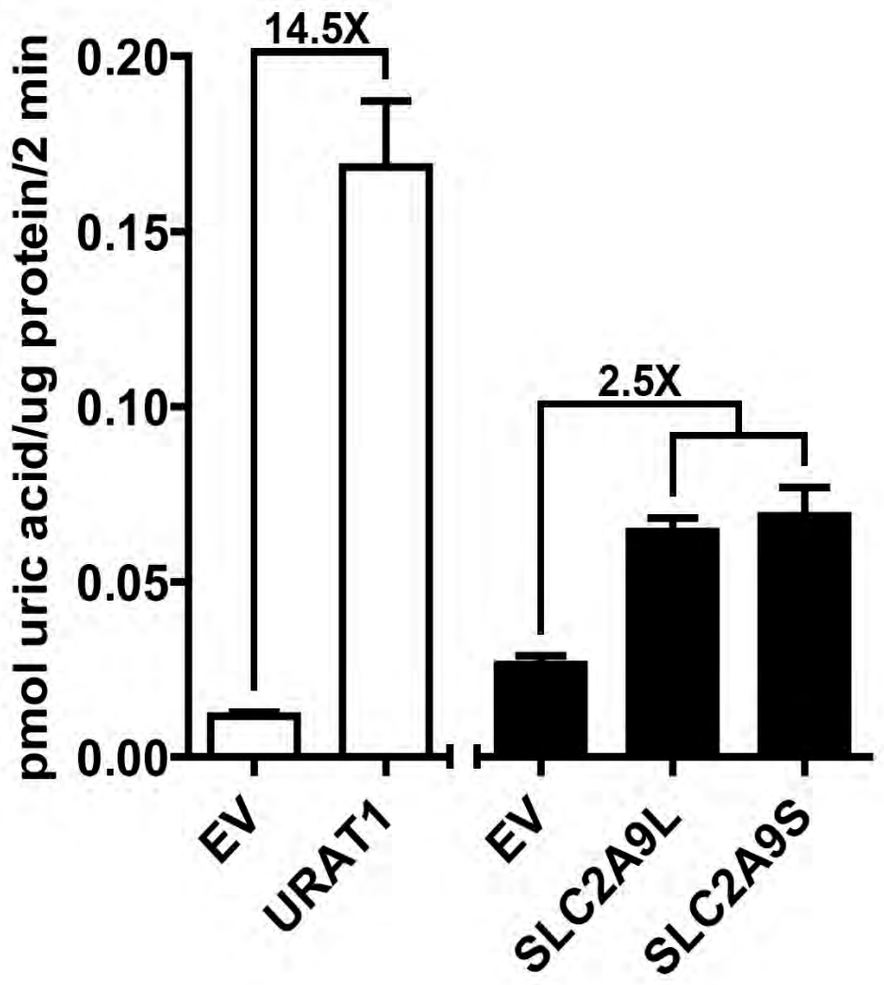
MFS fold



Schlessinger A, Matsson P, Shima JE, Pieper U, Yee SW, Kelly L, Apeltsin L, Stroud RM, Ferrin TE, Giacomini KM, Sali A. Protein Sci. 2010 Mar;19(3):412-28.

Function prediction based on similarity network



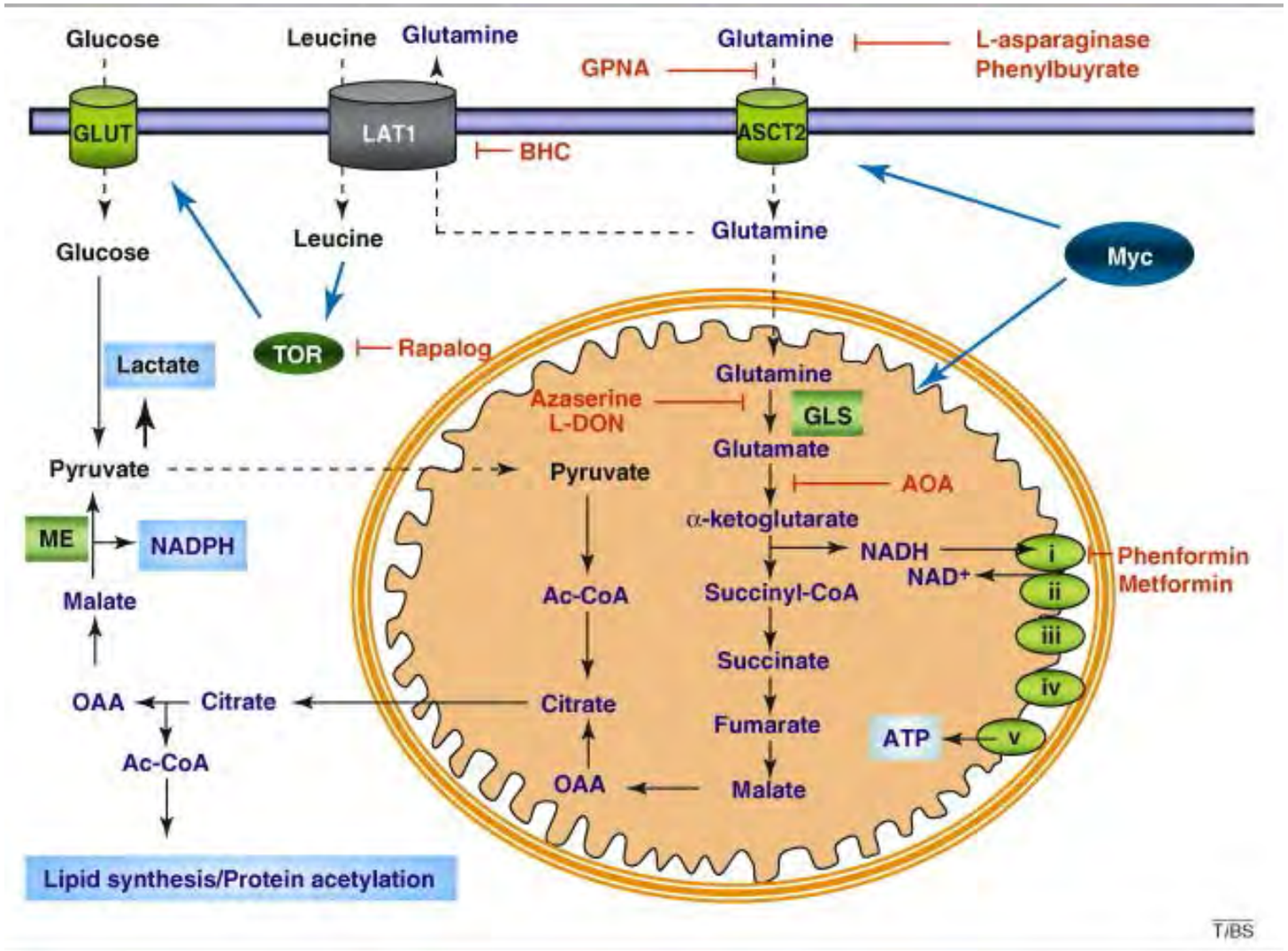


Functional overlap between a drug transporter (URAT1) and a glucose transporter (GLUT9)

Schlessinger A, Matsson P, Shima JE, Pieper U, Yee SW, Kelly L, Apeltsin L, Stroud RM, Ferrin TE, Giacomini KM, Sali A. Protein Sci. 2010 Mar;19(3):412-28.

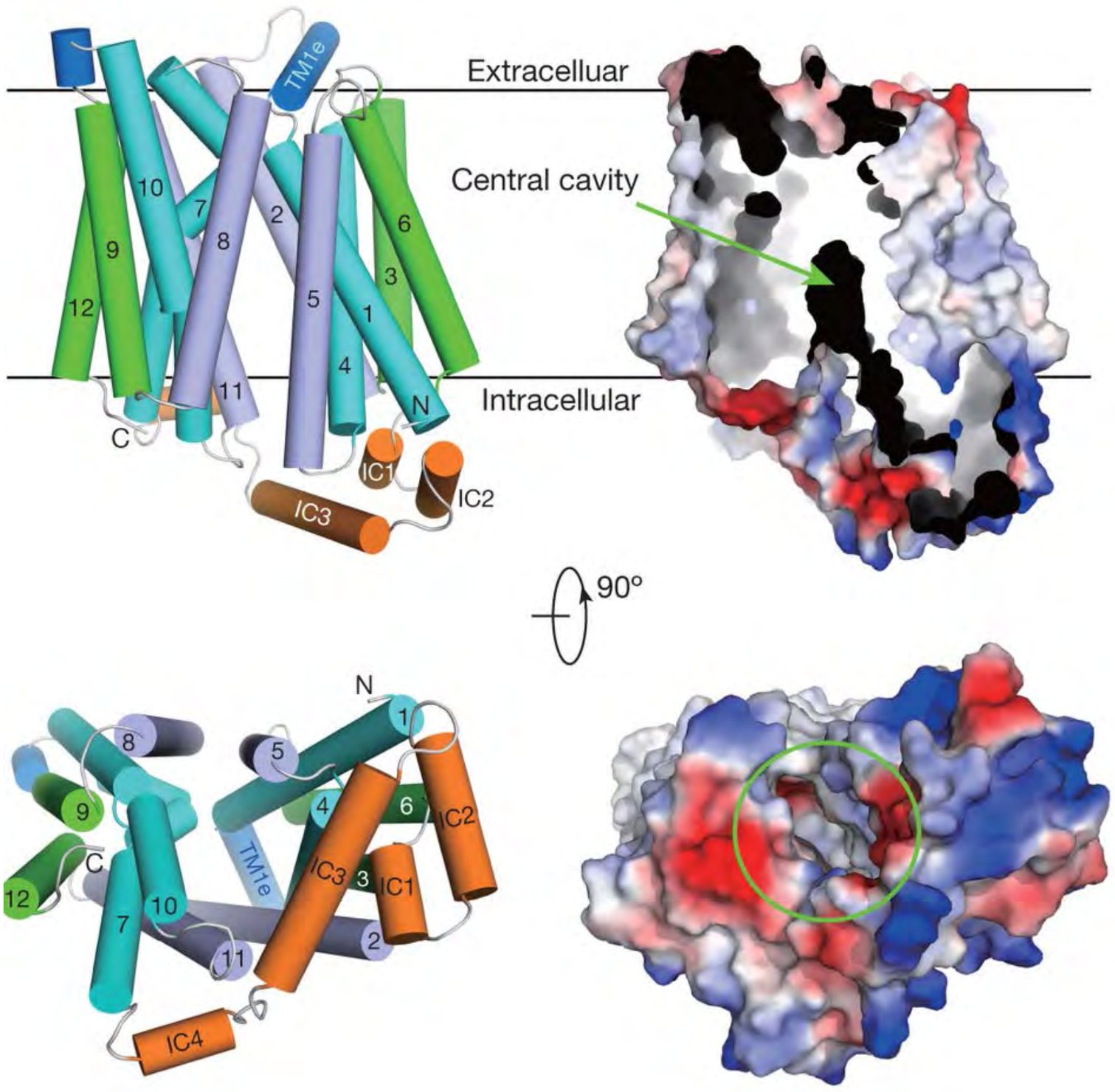
The human GLUT1

- GLUT1 transports glucose, galactose, glucosamine, and reduced ascorbate; inhibitors include cytochalasin B and phloretin
- GLUT1 is highly expressed in the endothelial cells, erythrocytes, and the blood-brain barrier
- Genetic variations are associated with GLUT1-deficiency syndrome (GLUT1-DS), an autosomal dominant haplo-insufficiency disorder characterized by a low glucose concentration in the cerebrospinal fluid
- Upregulated in multiple cancers, supporting the increased need for glycolysis and glucose uptake for ATP production, as well as for lactate secretion in cancer cells lacking of oxygen supply
- GLUT1 overexpression is associated with poor overall survival and tumor progression



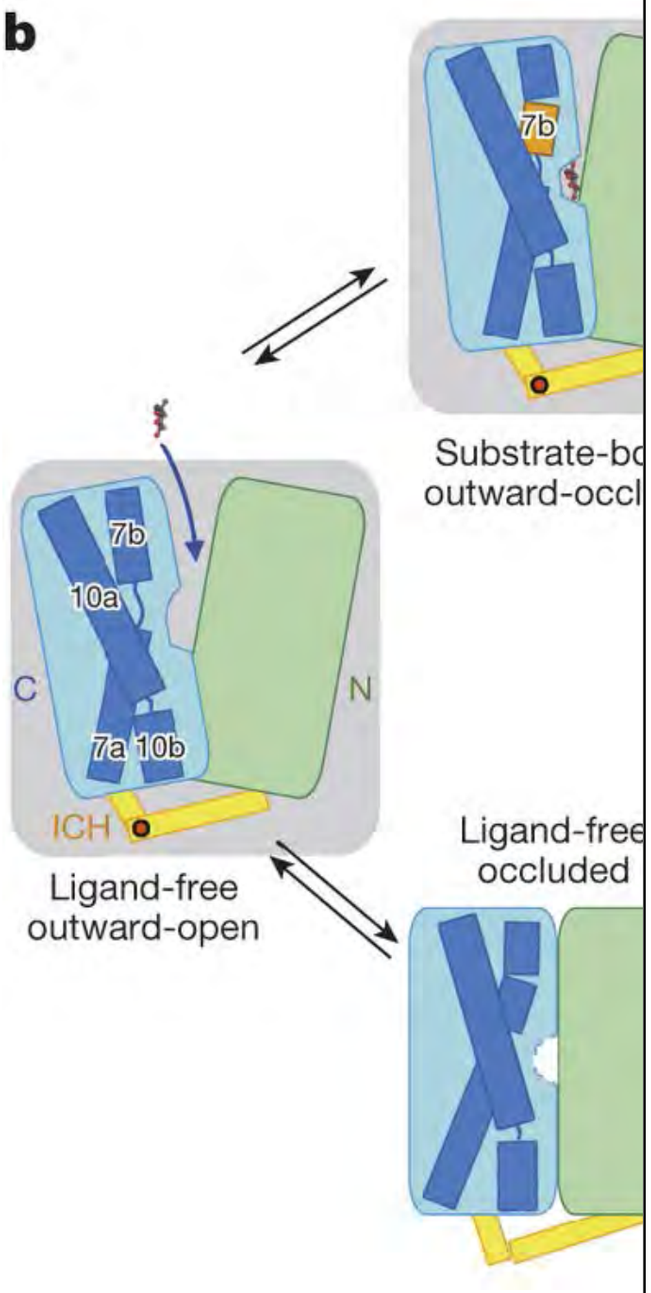
Wise & Thompson. Trends Biochem Sci. 2010 Aug;35(8):427-33.

GLUT1 structure in an inward conformation proposes the structural basis for GLUT1-sugar recognition



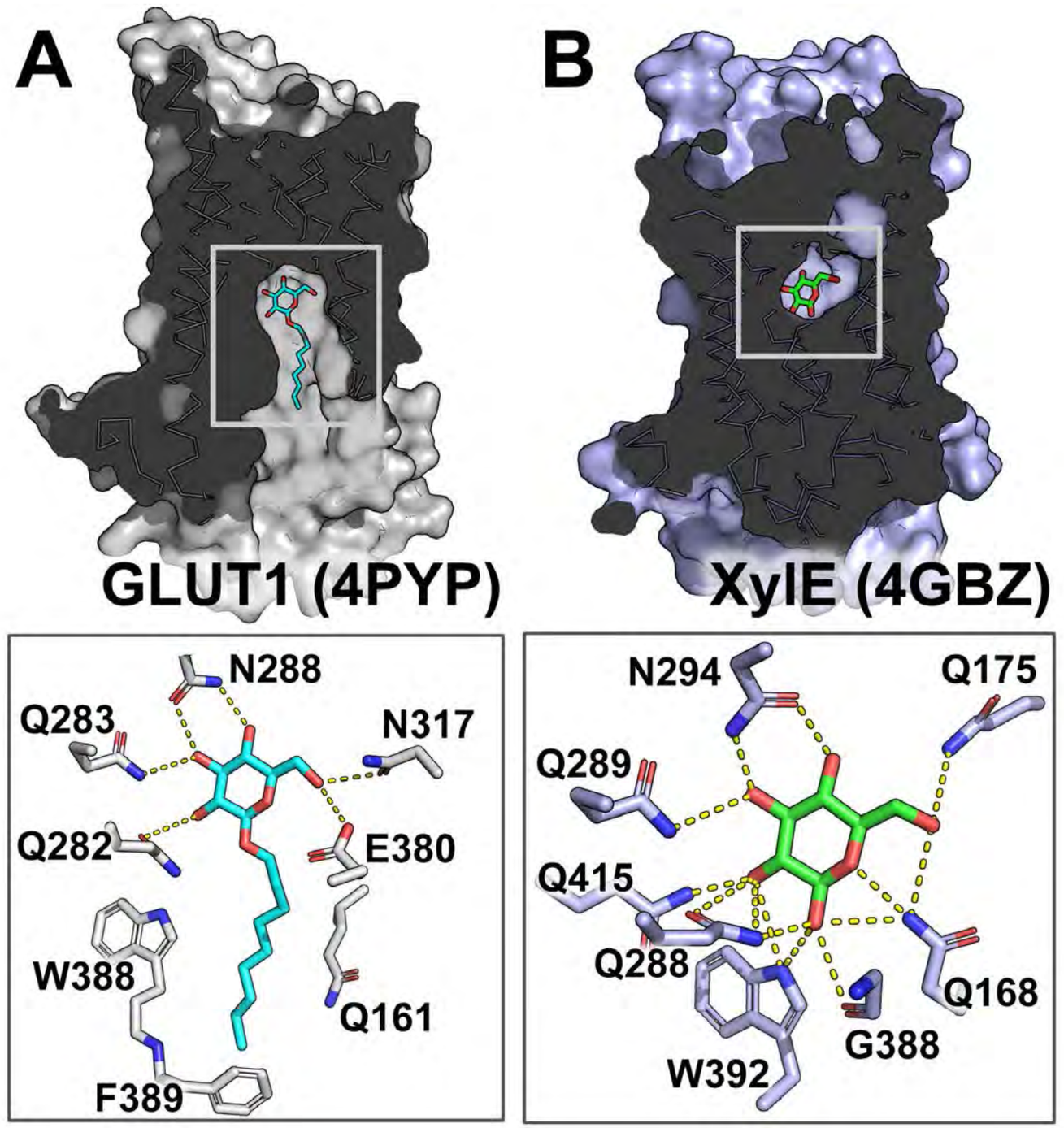
Deng et al. Nature. 2014 Jun 5;510(7503):121-5.

The ‘Rocker Switch’ mechanism



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

Different conformations of the GLUTs binding site



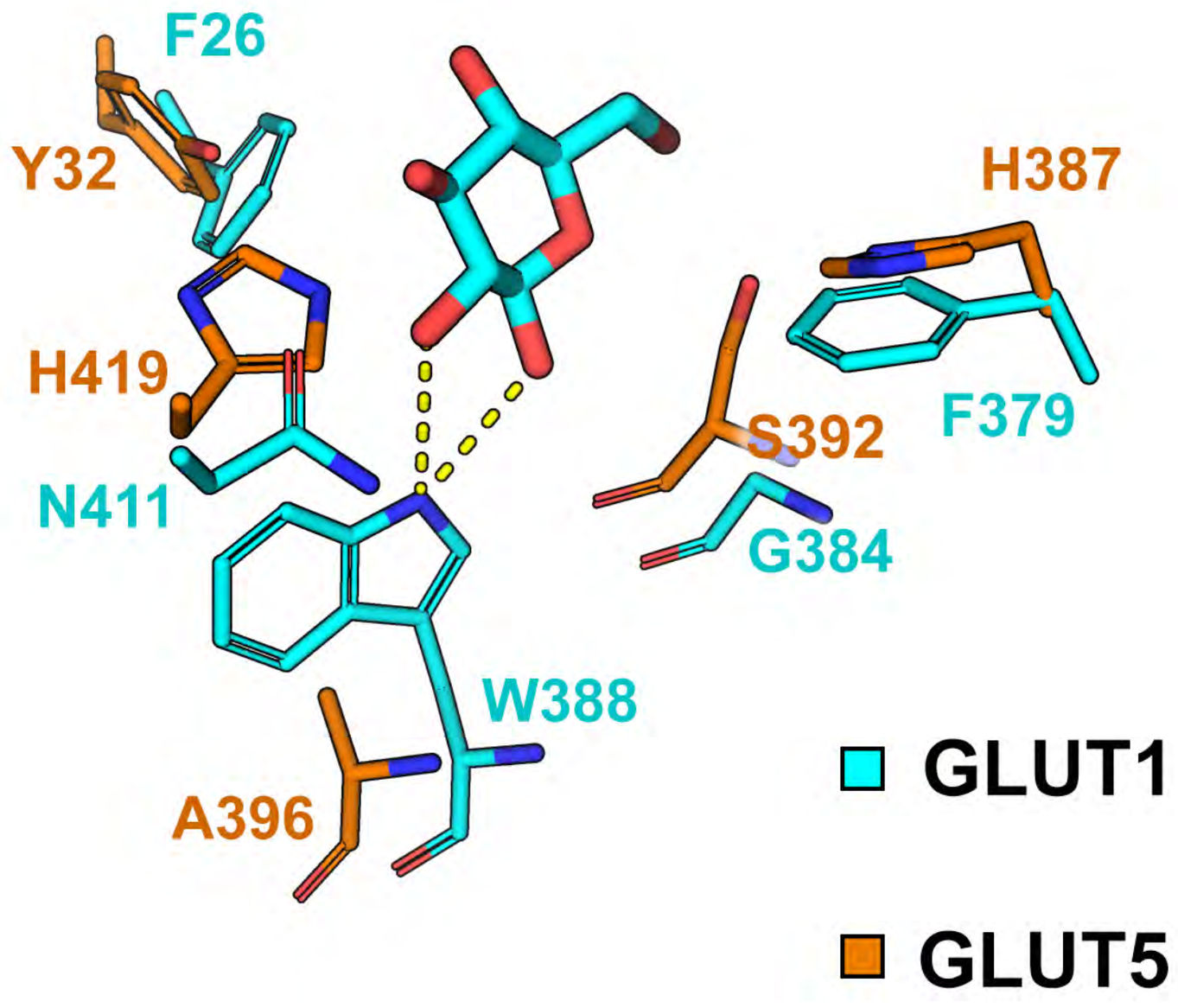
Different conformations capture different chemical space of ligands

Schlessinger and Wittwer *et al.* J Biol Chem. 2012 Nov 2;287(45):37745-56.

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

Is the mammalian GLUT occluded structure a better modeling template?

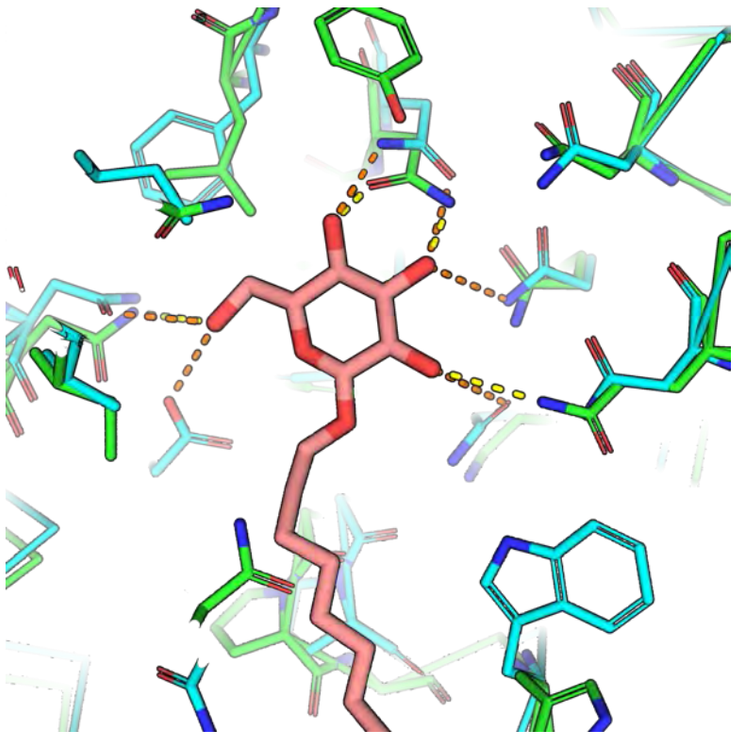
- **Rat GLUT5: seq identity 43%**
- ***E. Coli* XylE: seq identity 30%**

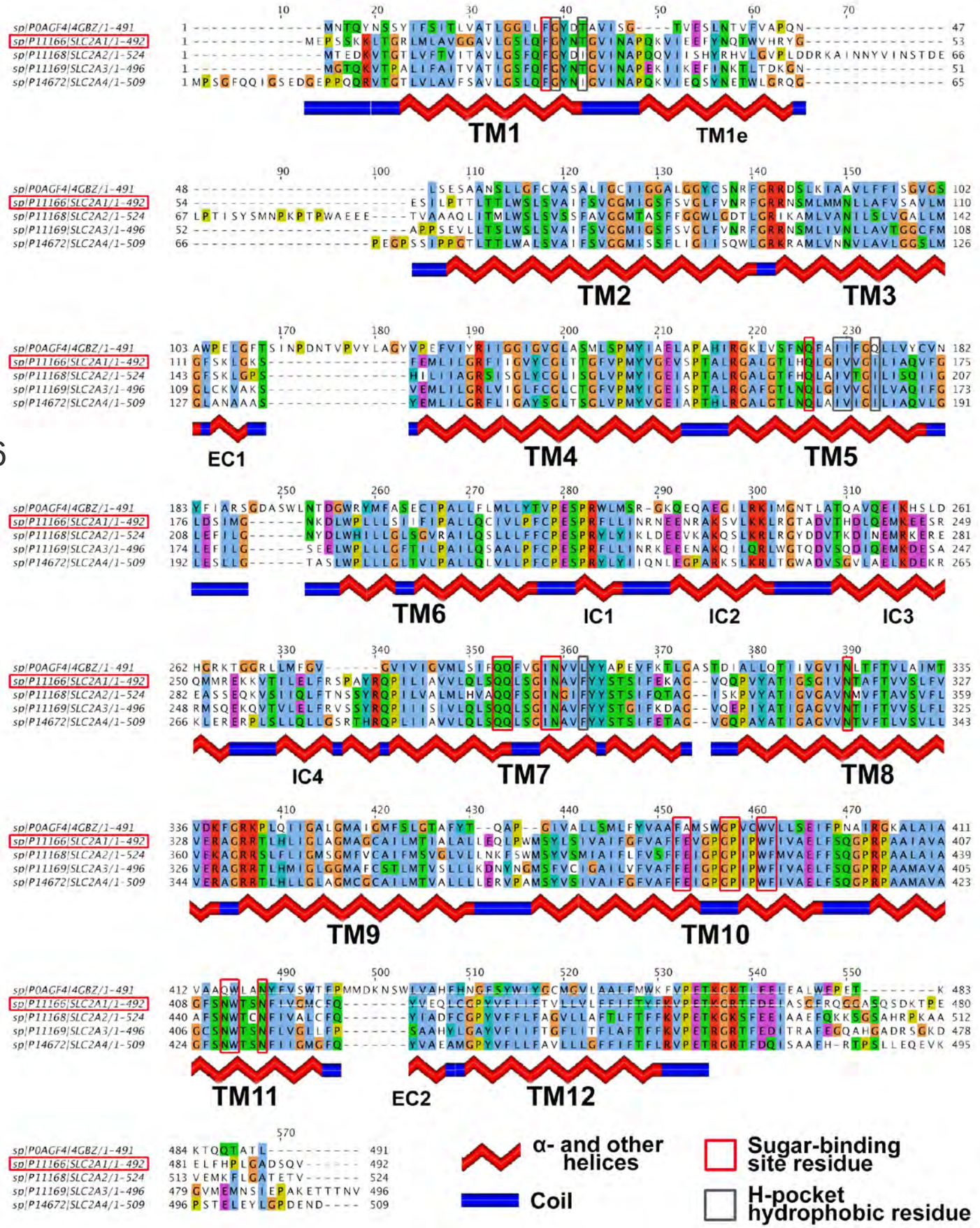


Ung *et al.* ACS Chem Bio. 2016 Jul 15;11(7):1908-16.

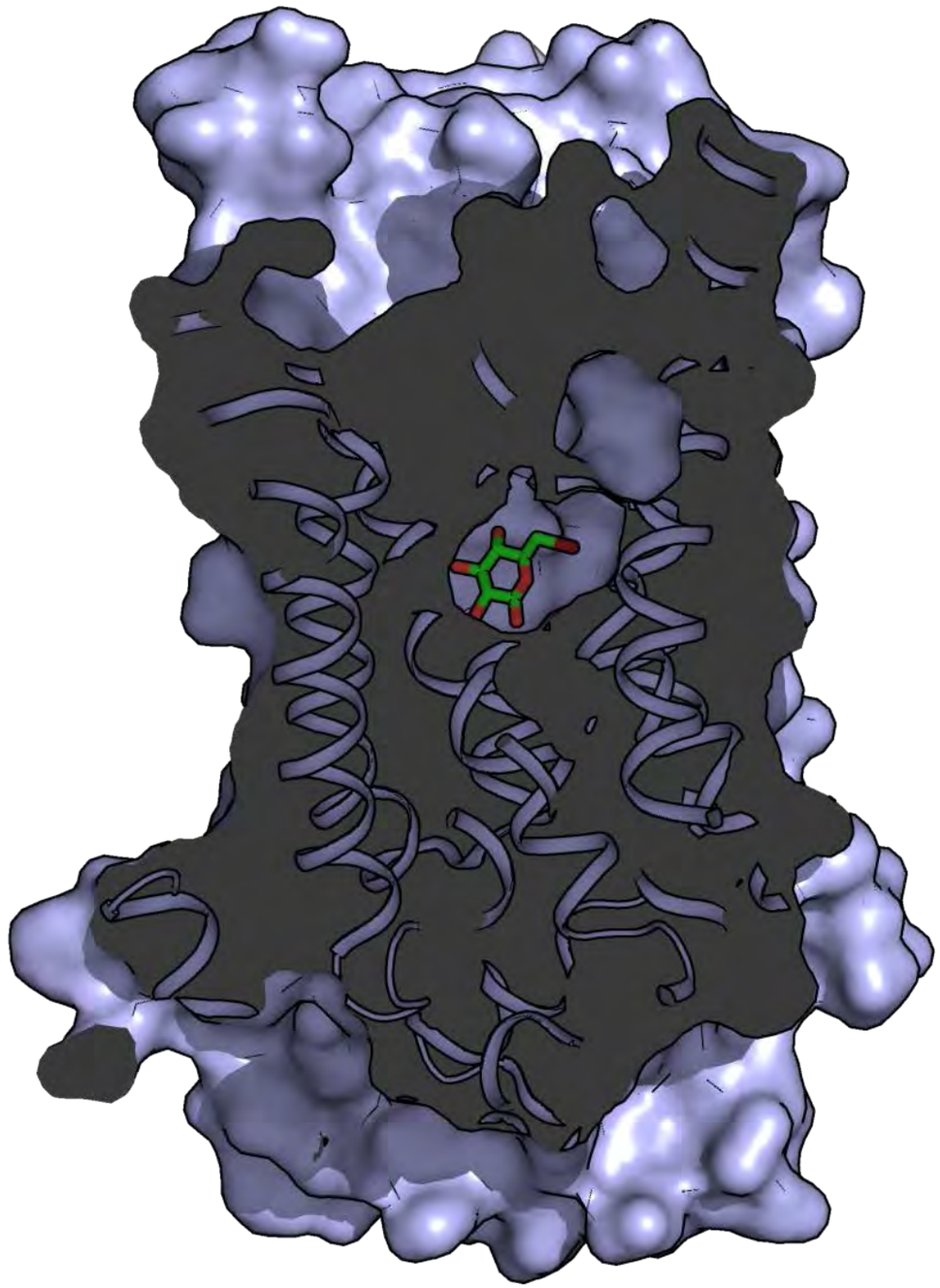
XylE provides an excellent modeling template

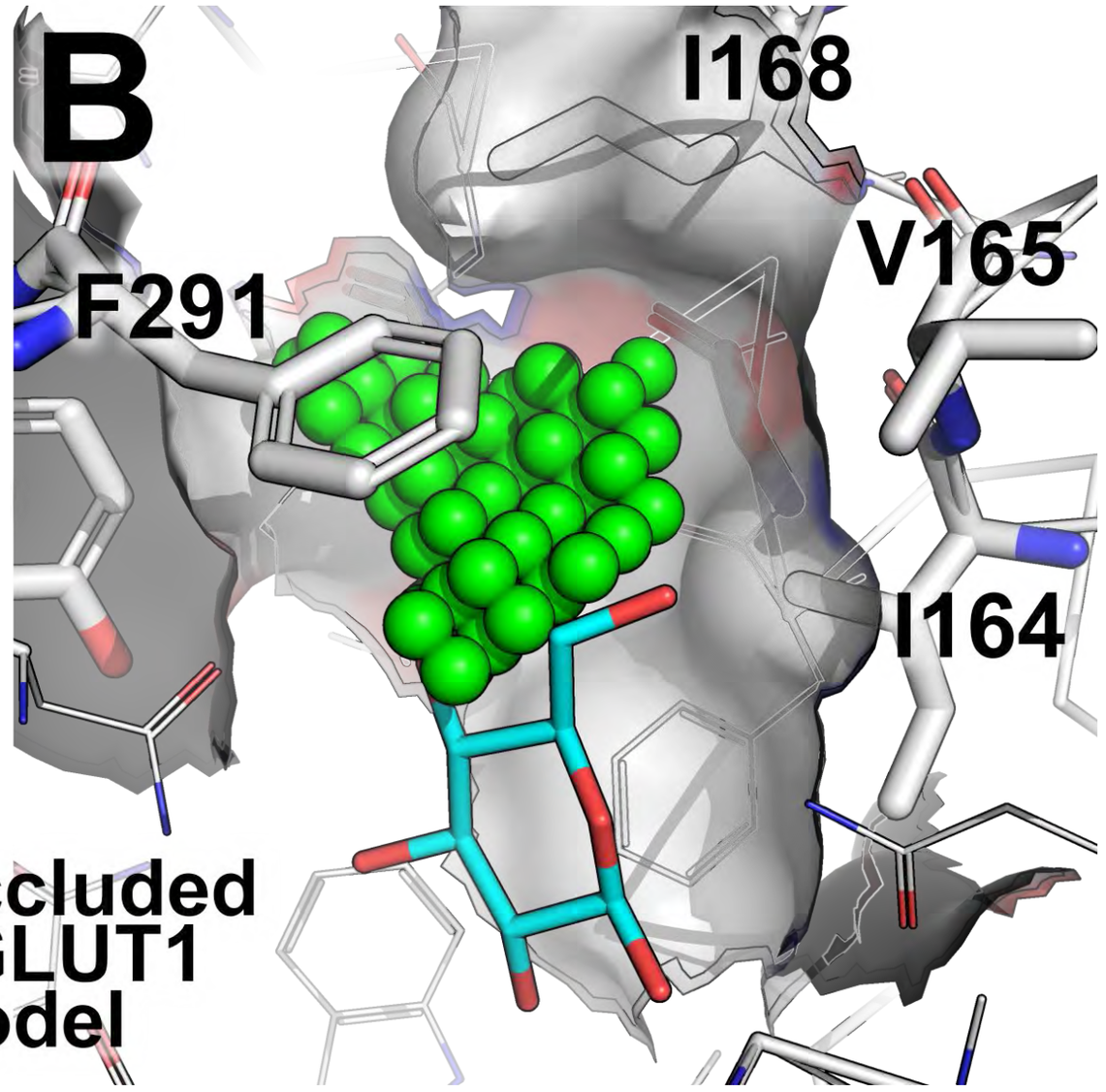
- Conserved topology: the transmembrane region is highly conserved
- Atomic structures of XylE and hGLUT1 in the inward-open conformation have an RMSD of 1.5 Å
- Binding site highly conserved (i.e., 12 of the 16 binding site residues are identical; 2 are similar)
- Highly similar substrate specificity
- XylE structures were solved with the natural substrates bound in a unique binding site conformation not available for any of the other hGLUT1 homologues



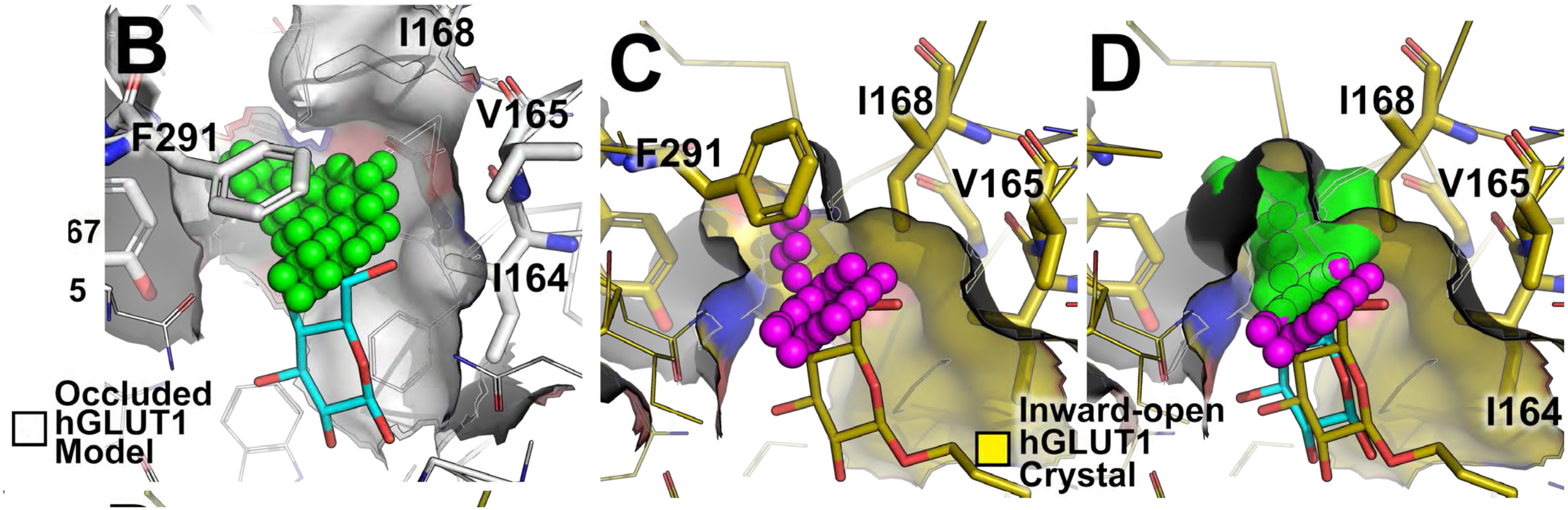


GLUT1 model reveals a putative pocket termed H-pocket

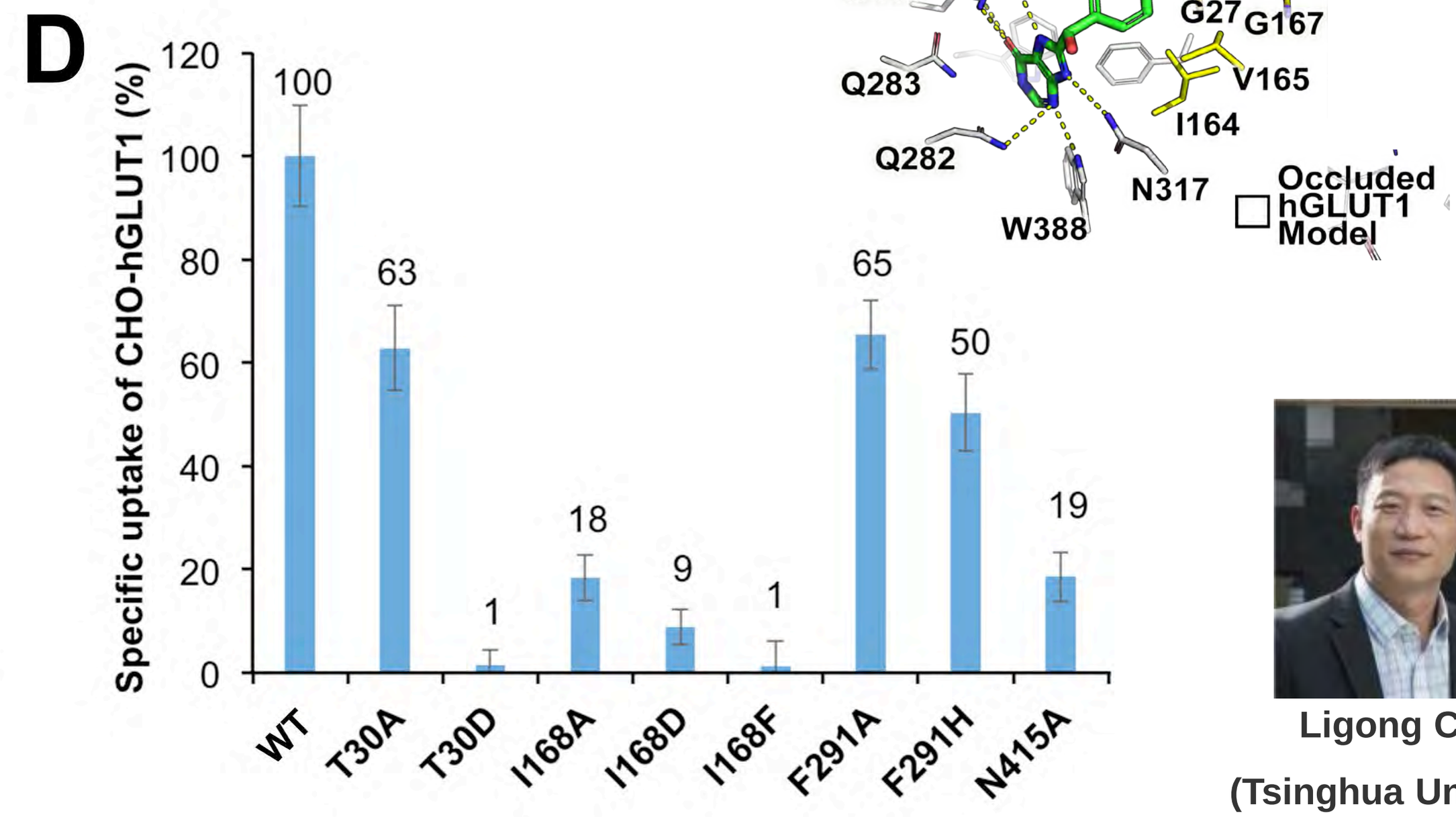




The putative H-pocket is conformation specific



H-pocket residues are tested with site-directed mutagenesis

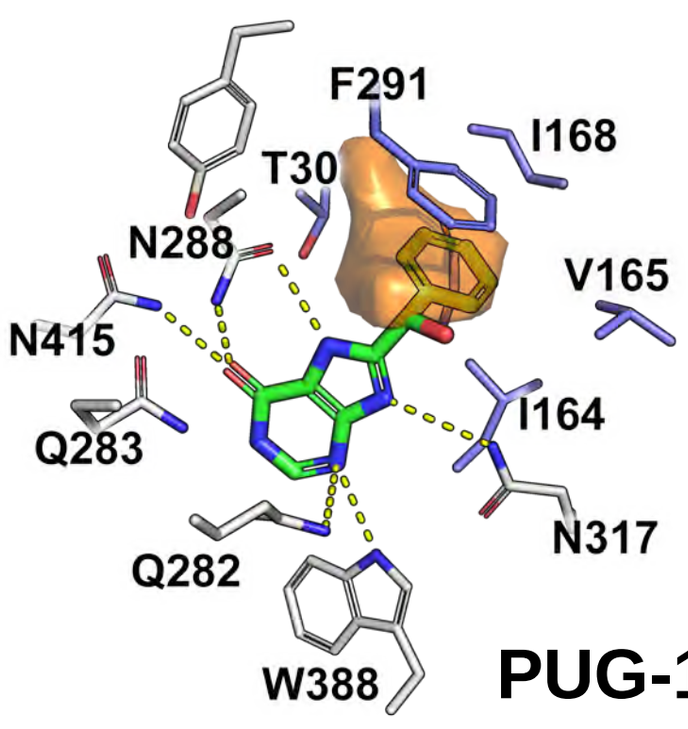


Ligong Chen

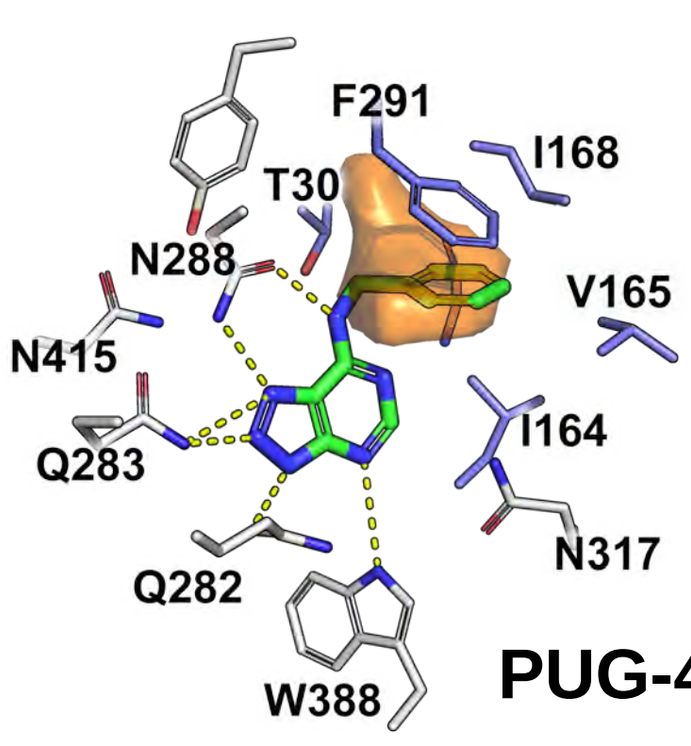
(Tsinghua University)

Virtual screening of small molecule libraries targeting the H-pocket

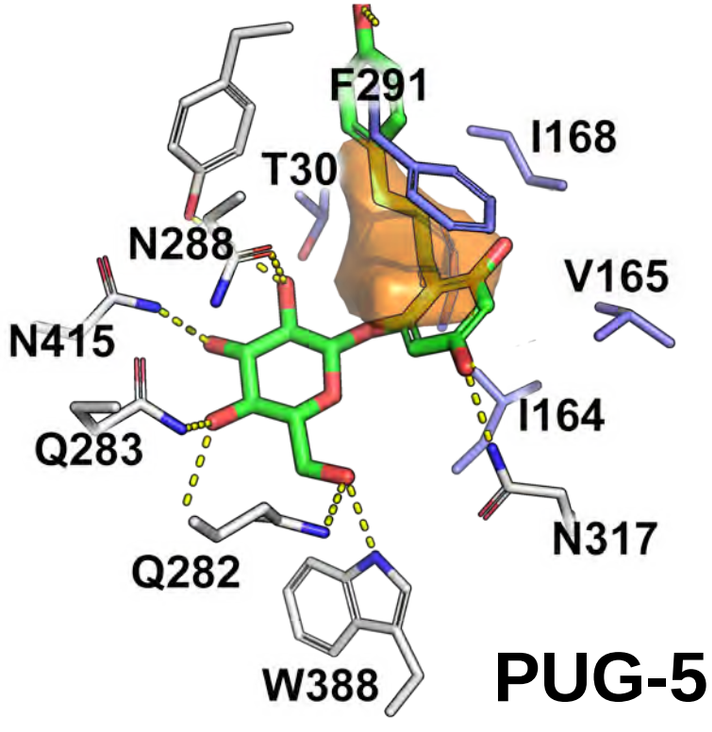
- Virtual screening of purchasable fragments from NCI and ZINC libraries
- Visual analysis the 250 top-scoring hits
- Focus on compounds with unique scaffolds that are predicted to access the H-pocket with their hydrophobic moiety
- 19 compounds were selected for experimental testing



PUG-1

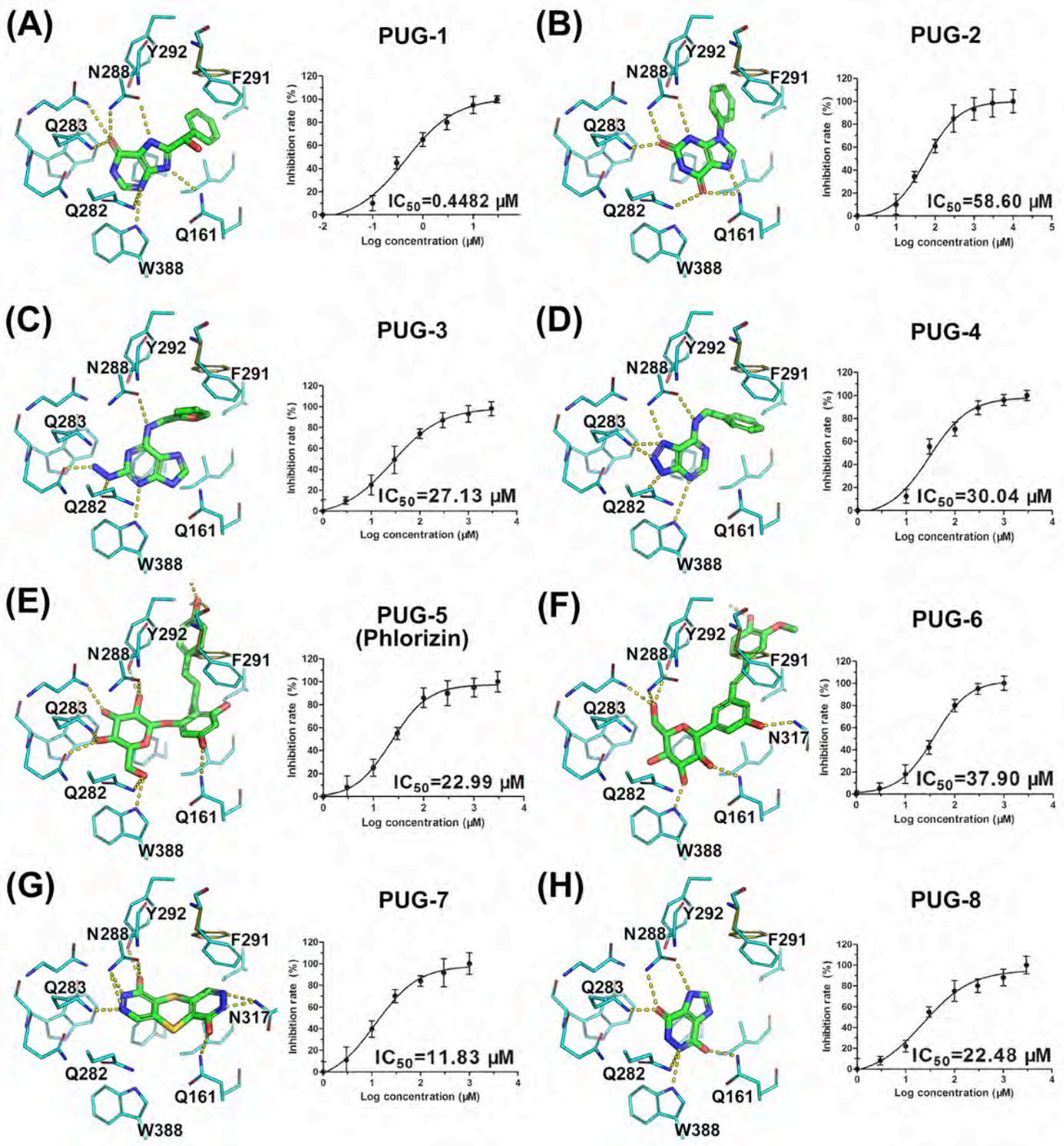


PUG-4



PUG-5

Cis-inhibition assays identify 8 new GLUT1 potent inhibitors

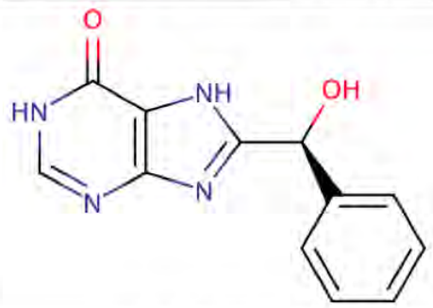
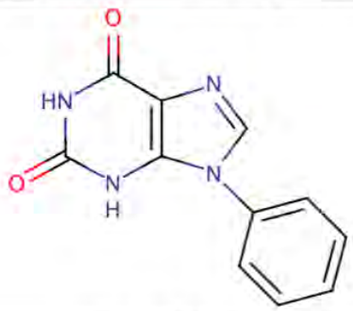
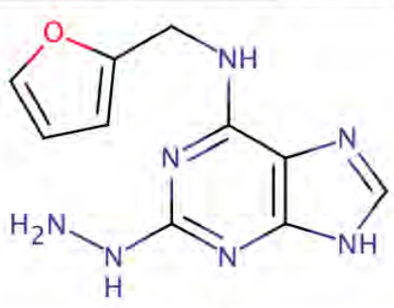
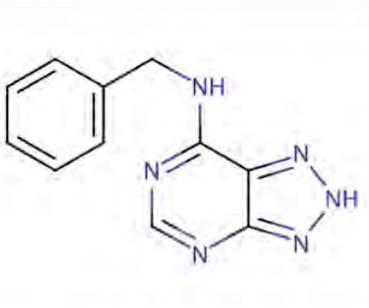


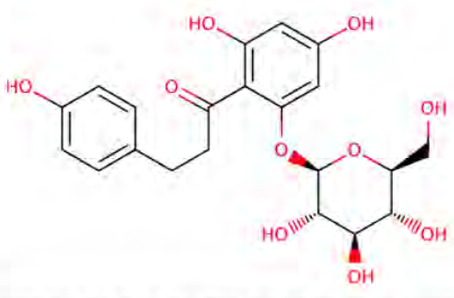
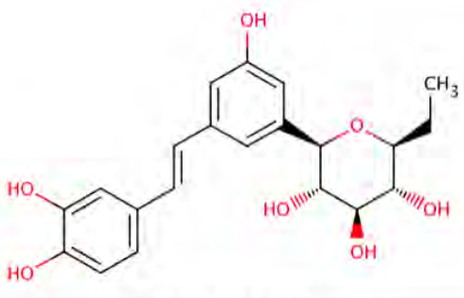
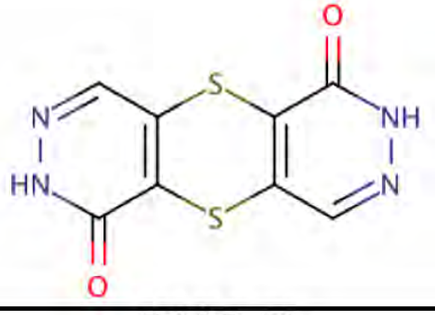
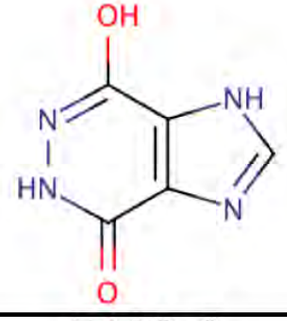
Ligong Chen

(Tsinghua University)

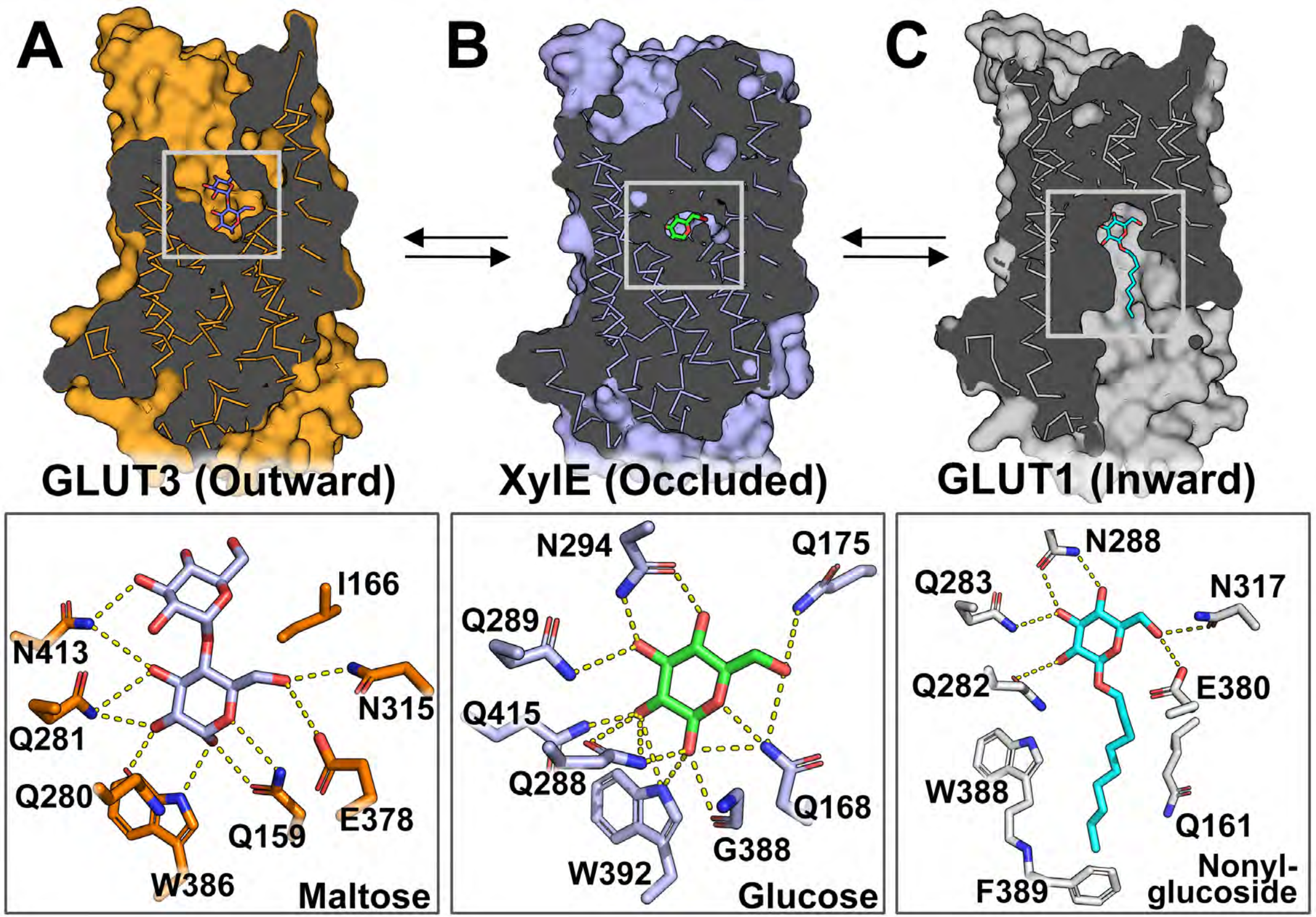
New ligands reveal new scaffolds and have improved Ligand Efficiency

Table 1. IC_{50} of screening hits in [^{3}H]-2-deoxy-D-glucose uptake assay.

				
Name	PUG-1	PUG-2	PUG-3	PUG-4
IC_{50}(μM)	0.45	58.6	27.1	30.0
LE[a]	0.481	0.339	0.346	0.363
Tc[b]	0.152	0.145	0.099	0.153

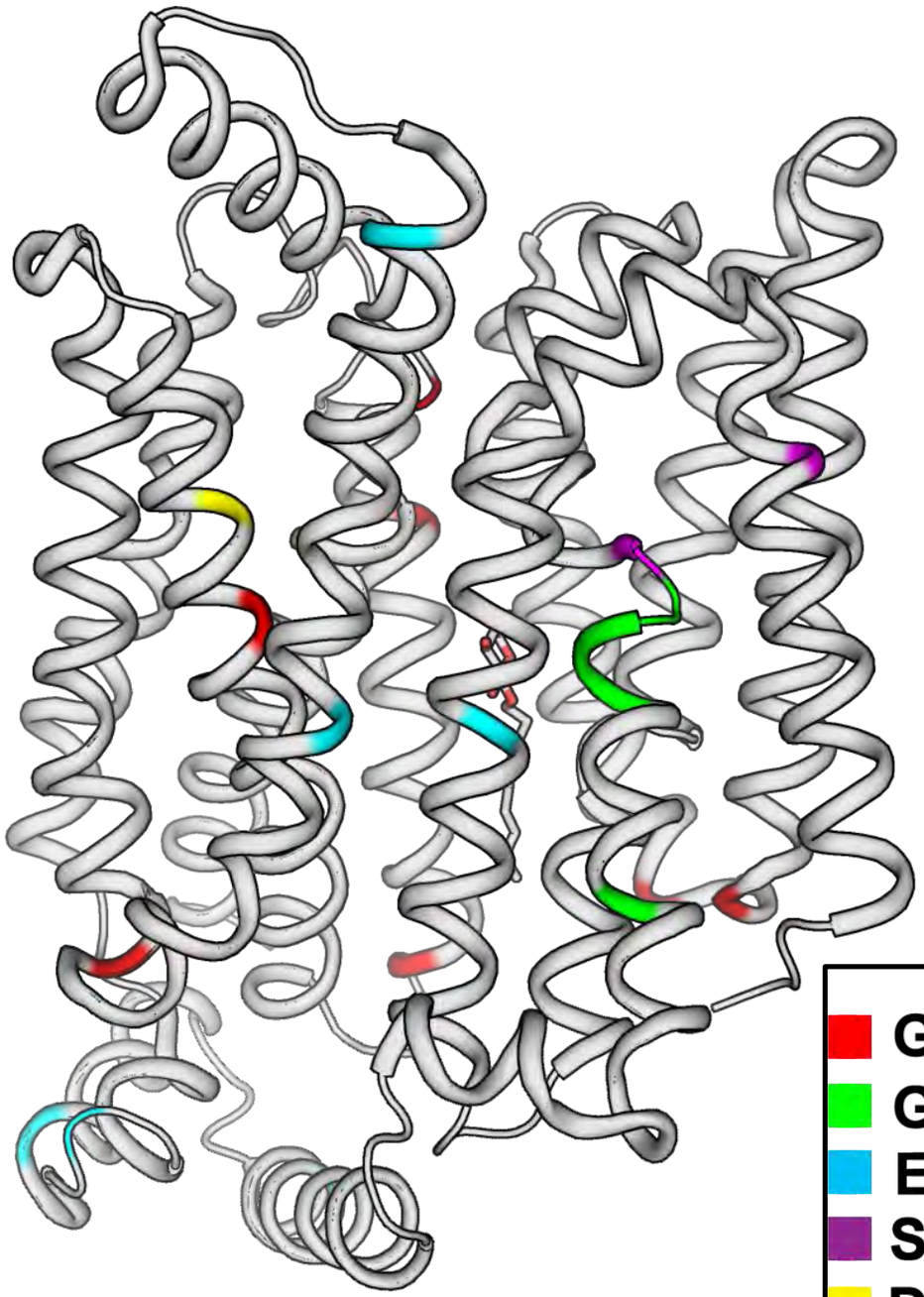
				
Name	PUG-5 (Phlorizin)	PUG-6	PUG-7	PUG-8
IC_{50}(μM)	23.0	37.9	11.8	22.5
LE	0.204	0.215	0.420	0.576
Tc	0.531 (Phloretin)	0.357 (Phloretin)	0.097	0.089

Different conformations of the GLUT binding site



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

Can disease-related mutations' effects be explained with the GLUT1 structures and models?



Epilepsy, idiopathic generalized 12 (EIG12)	GLUT1 deficiency syndrome 1 (GLUT1SD1)	GLUT1 deficiency syndrome 2 (GLUT1SD2)
T60M	N34S	R126C
M77T	G91D	A275T
R218S	R126H	Del282-285 – hemolytic anemia
R223P	R126C	G314S
E243V	G130S	
N411S	R153C	**Stomatin-deficient cryohydrocytosis with neuro defect (SDCHCN)**
R458W	Del169	
	E329Q	G286D
	R333W	Del435

- Almost all mutations occur in the interface with the membrane or between transmembrane helices
- Mutations are usually not found in the sugar binding site except for the deletion associated with anemia

Structure-based ligand discovery for GLUT1

- **The GLUT1 model is used to identify novel, potent, and efficient inhibitors**
- **The model characterizes an occluded-outward conformation, revealing a new binding sub pocket**
- **The GLUT1 model and previous structures explain some mutations' effect on function**
- **Future: can we design GLUT1-specific inhibitors?**
- **Can we apply this approach for targeting other transporters? For designing ligands with optimal ADME properties?**
- **Can we predict mutation effect on function for uncharacterized variants?**

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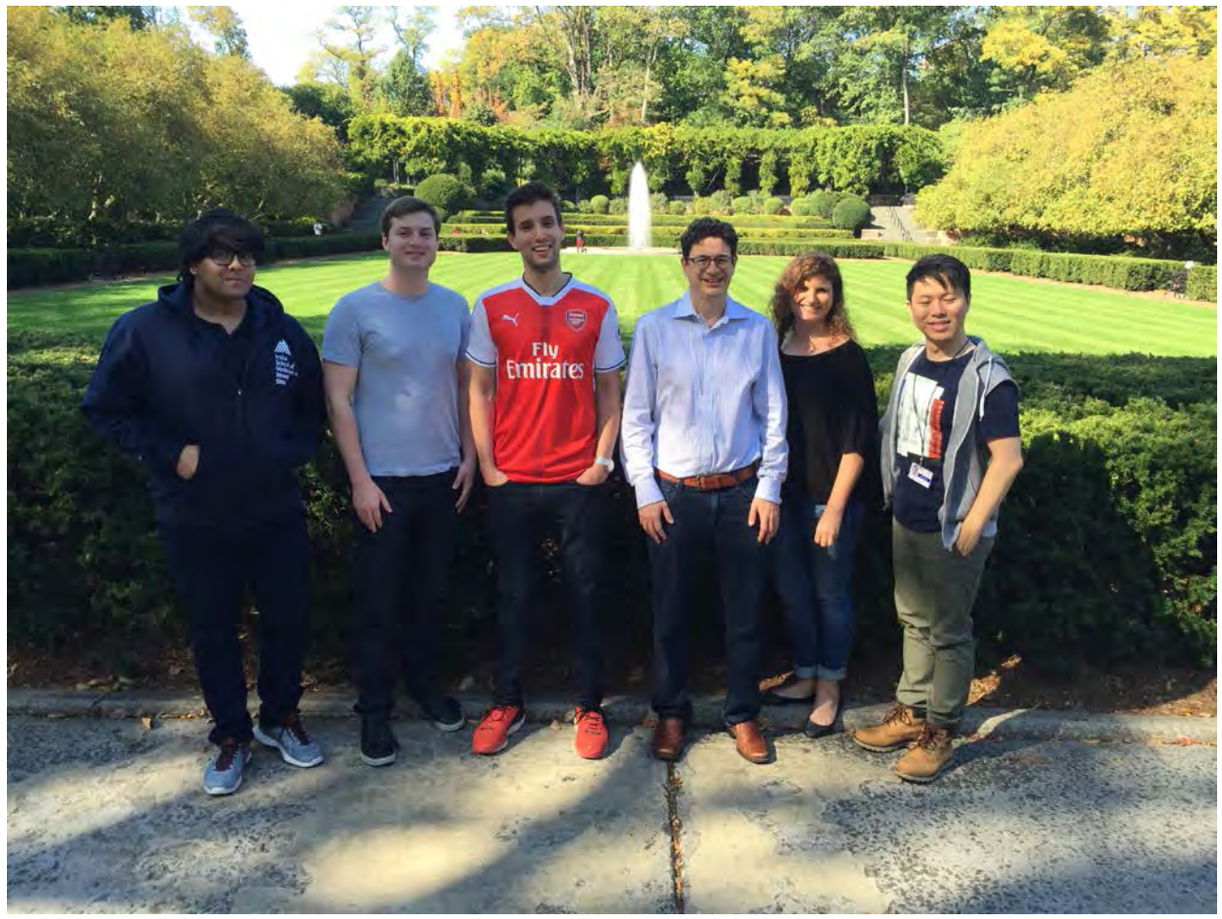

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