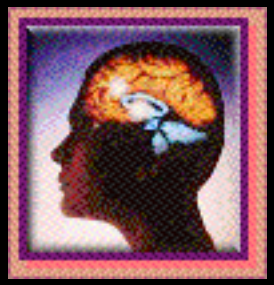
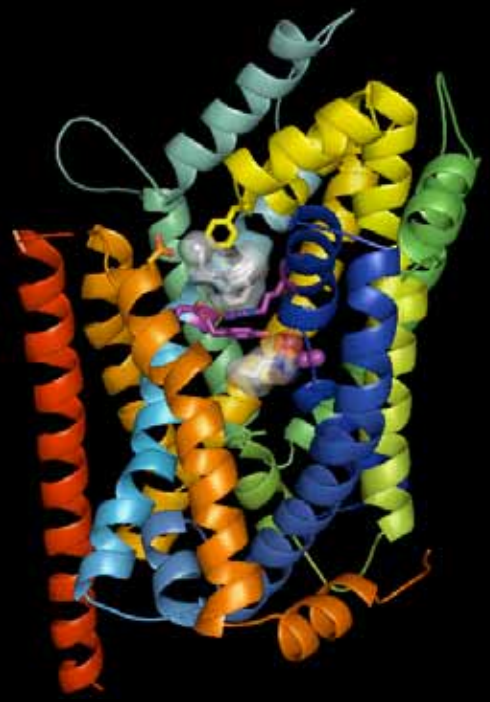
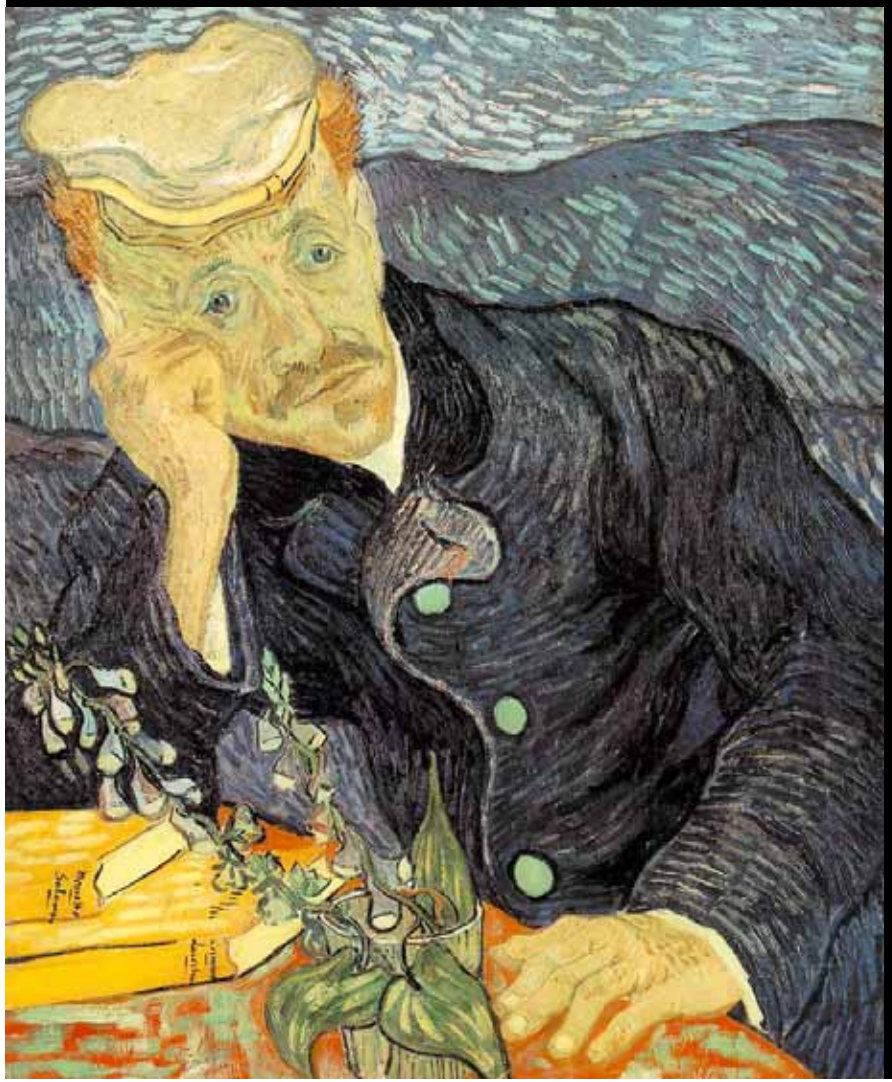
Structure of the LeuT-desipramine complex suggests how antidepressants inhibits neurotransmitter reuptake (*R21-GM075936)*





Da-Neng Wang
Skirball Institute
New York University School of Medicine

Depression

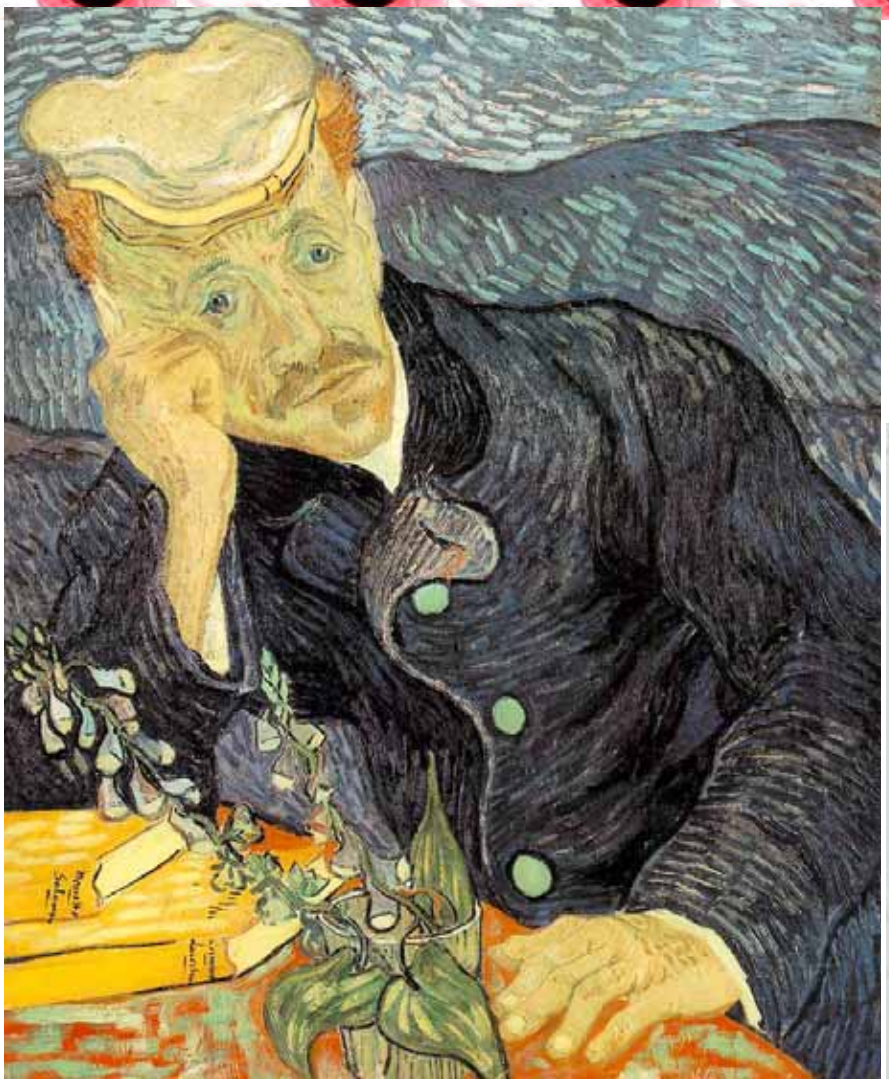


Most common psychiatric disorder

17 % prevalence rate

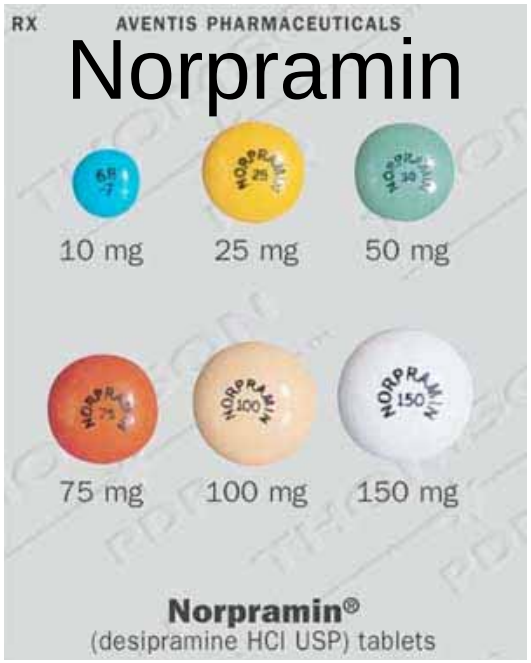
120 million patients world wide

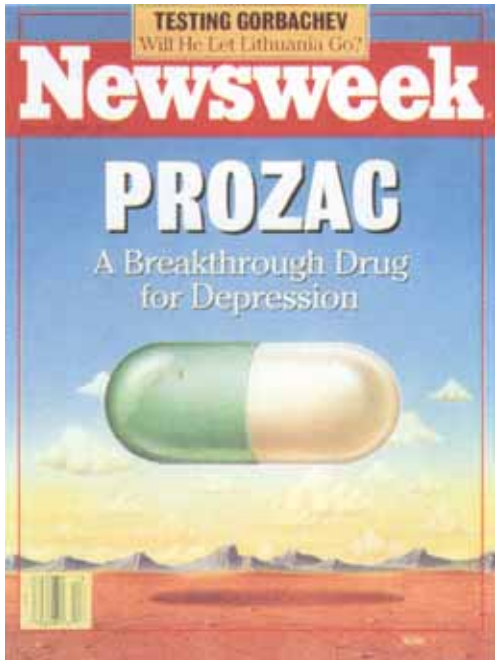
Antidepressants as major medication



Response rate ~70%

Annual sales US$ 16.7 billion

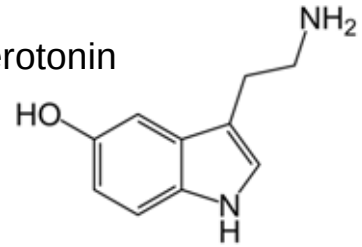




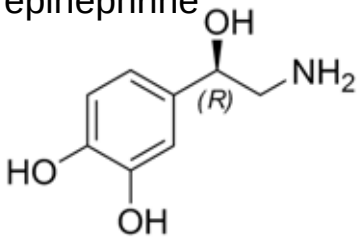
Neurotransmitter reuptake and its inhibition

Neurotransmitters

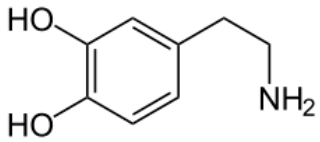
Serotonin

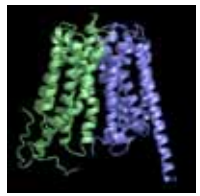


Norepinephrine



Dopamine

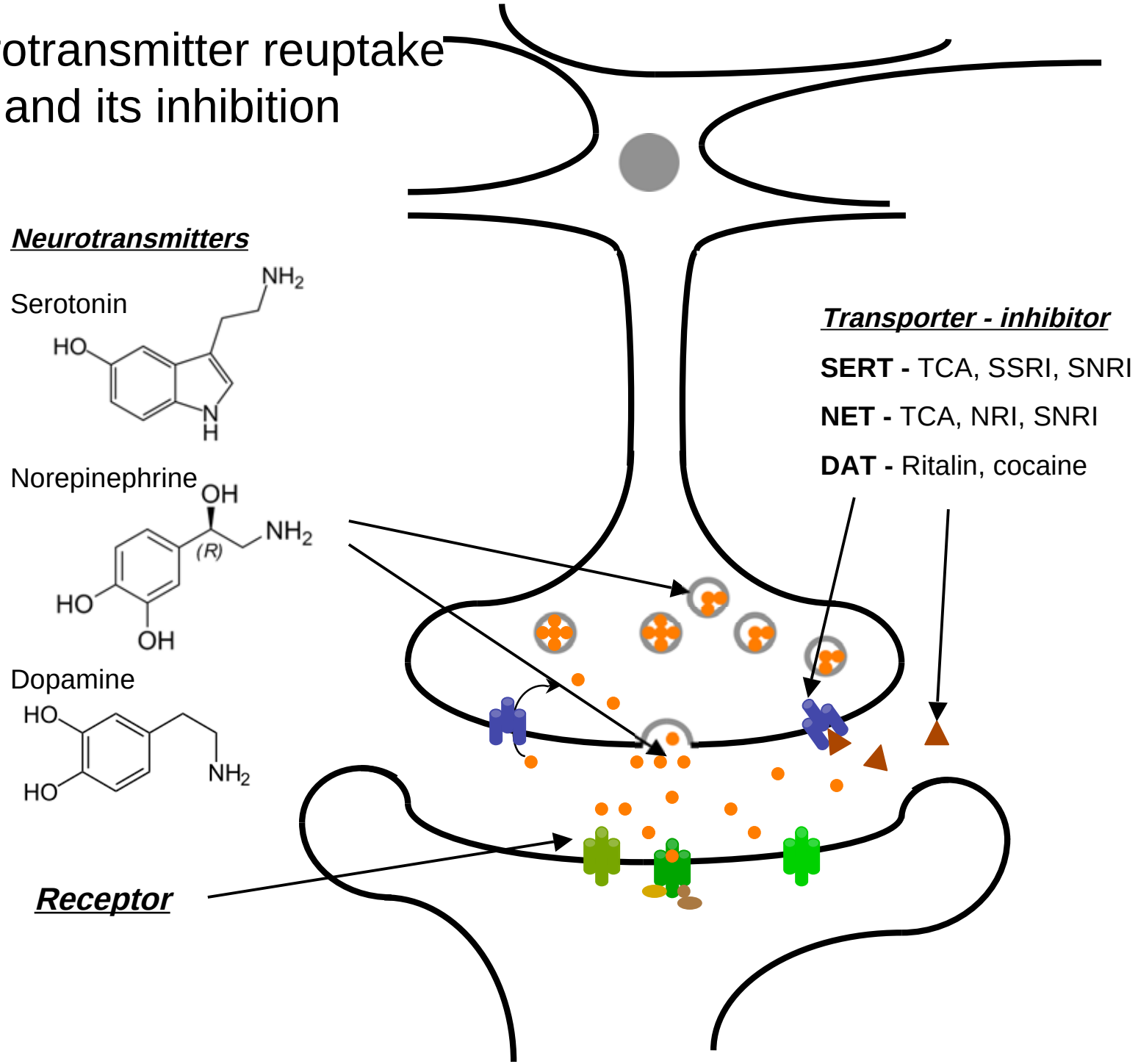




VMAT - GlpT(2003)

Receptor

Neurotransmitter reuptake and its inhibition



Competitive inhibition of human neurotransmitter transporters

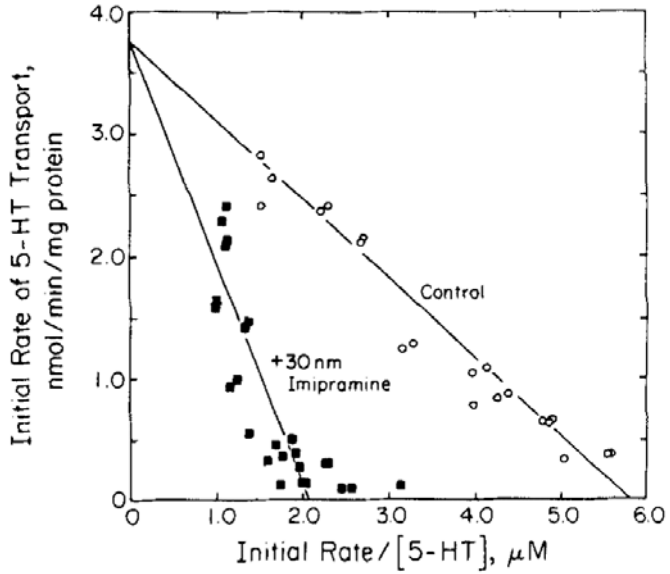
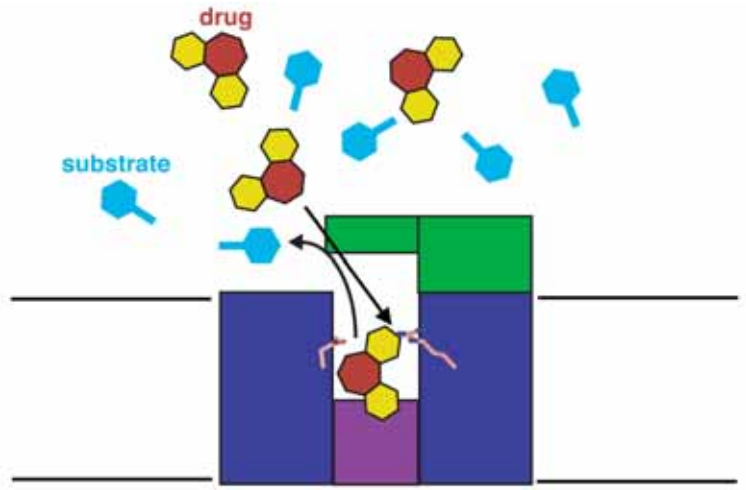


FIG. 1. Inhibition of 5-hydroxytryptamine (*5-HT*) transport by imipramine. Initial rates of 5-hydroxytryptamine transport were measured using a 10-s incubation at 37°C as described previously (5) in the presence and absence of 30 nM imipramine. The data are presented according to the method of Hofstee (17). ○—○, control; ■—■, imipramine. The lines were calculated from a linear least squares fit of the data.

Talvenheimo, (1979)

Displacement model



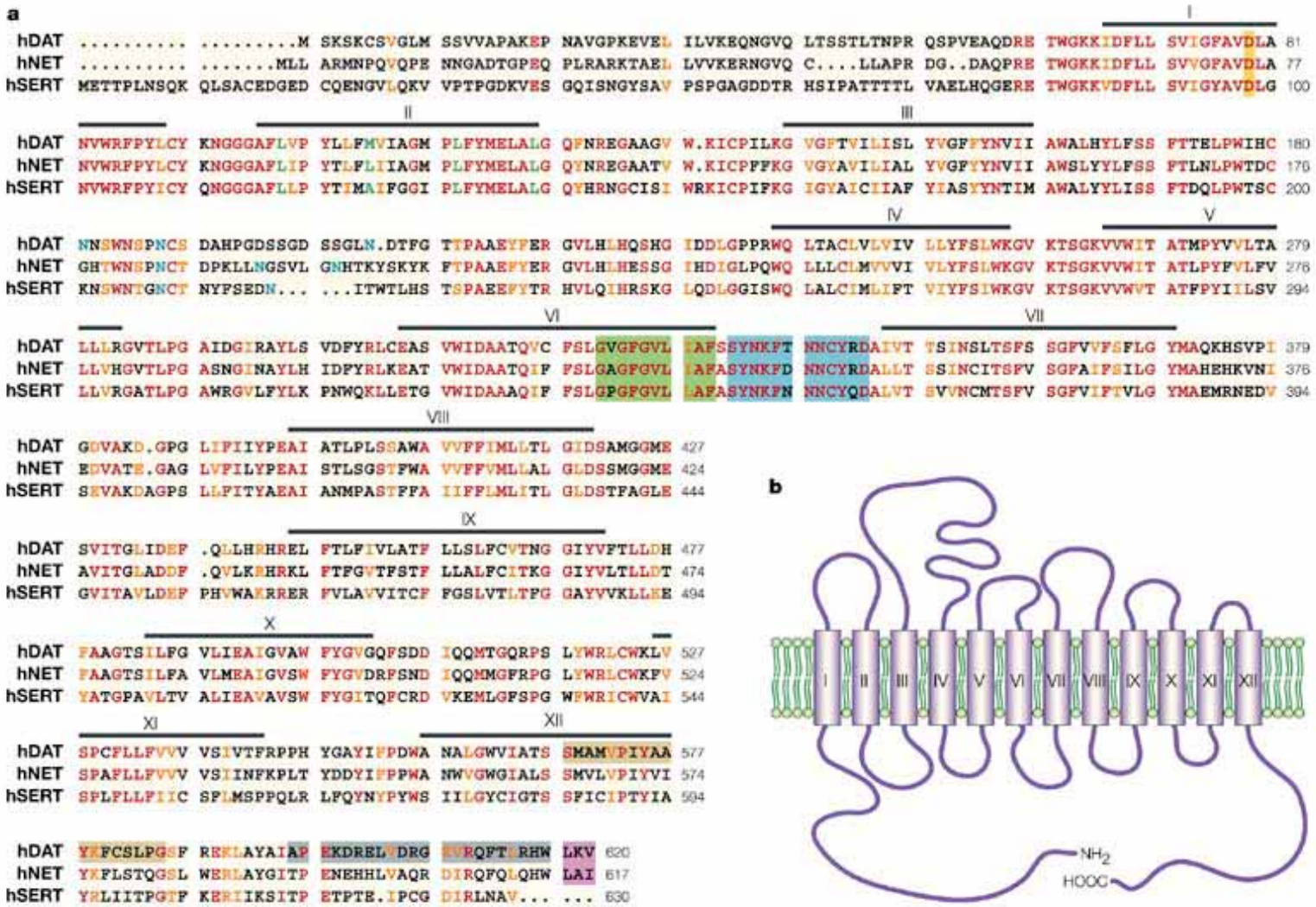
Key questions

Where is the drug-binding site in the protein?

Are the drug-binding site and the substrate-binding site overlapping?

What is the nature of the competitive inhibition?

Neurotransmitter:sodium symporters (NSS)



And bacterial NSS proteins

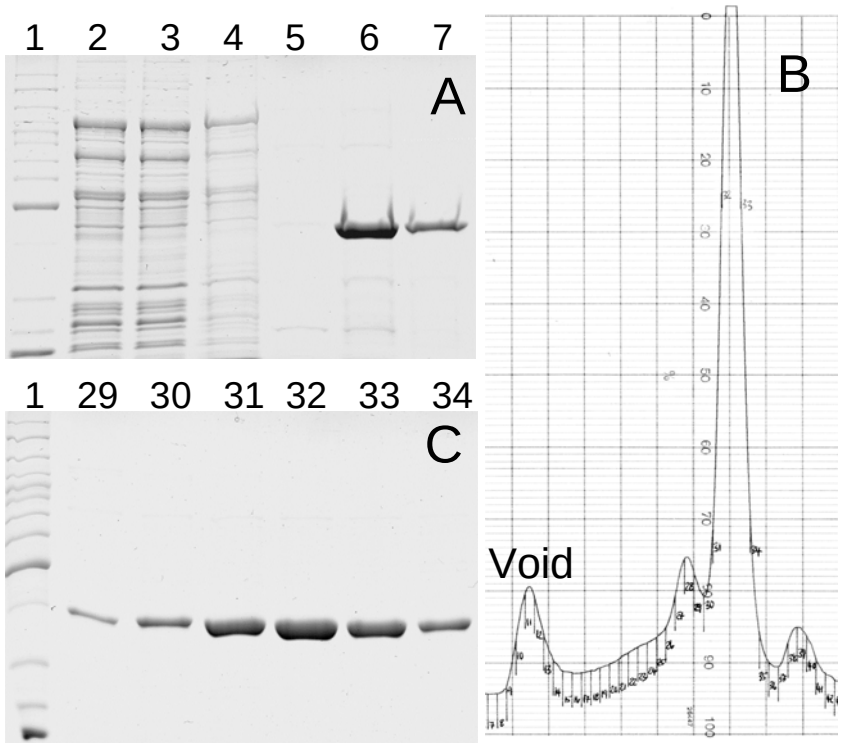
Drug binding to SERT, NET and DAT (IC_{50}, nM)

Drug	hSERT	hNET	hDAT	*Drosophila* DAT	*C. elegans* DAT
Desipramine	64	4.2	82,000	18	3
Imipramine	8.0	70	25,600	30	1
Fluoxetine	7.3	1,020	19,500	240	170
Sertraline	58	1,200	1,100		
Citalopram	5.4	>1,000	10,000		
Paroxetine	0.25	312			
Nisoxetine	400	5.3	497		
Reboxetine	129	1.1			
Venlafaxine	39	210	9,300		
Methylphenidate	>50,000	514	84		
Cocaine	410	910	278	2,660	5,000

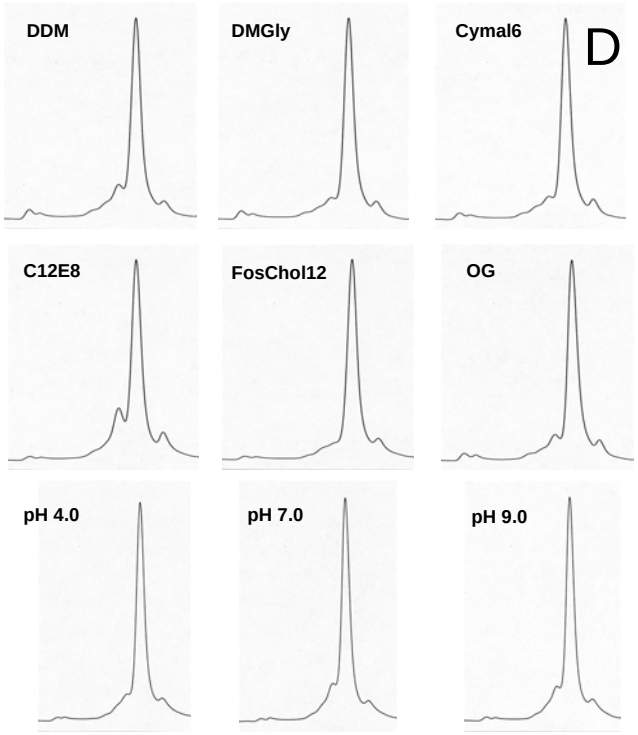
Leucine transporter LeuT from *Aquifex aeolicus*

25% sequence identity, ~45% similarity to human NSS proteins

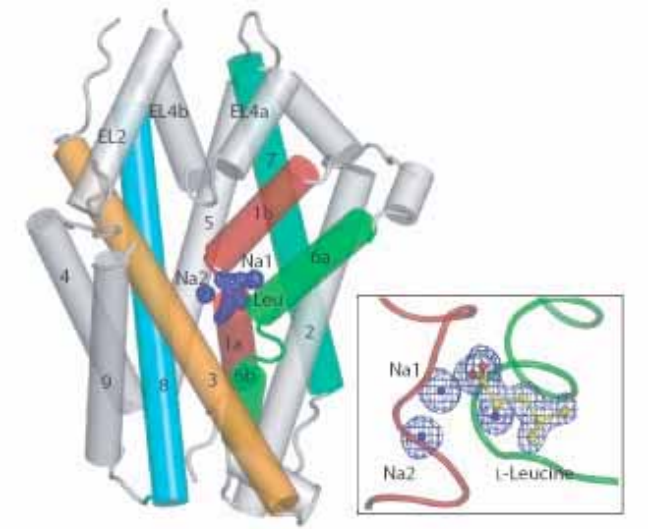
LeuT expression & purification



LeuT stability

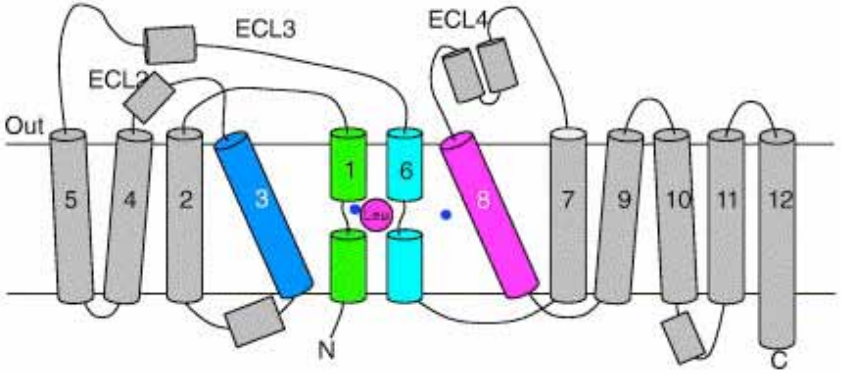


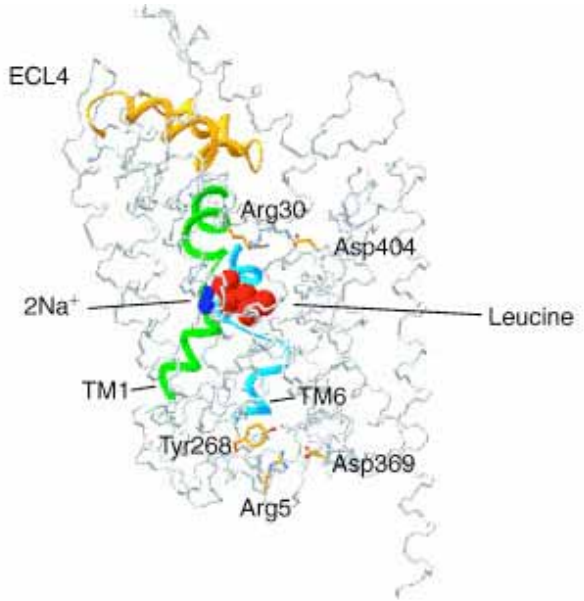
1.65 Å structure of LeuT in drug-free form



Structure solved by the Gouaux lab in 2005

LeuT + 1 Luecine + 2 Na^+

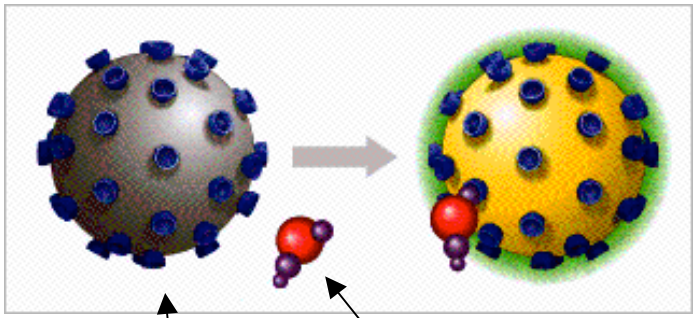




Yamashita, 2005

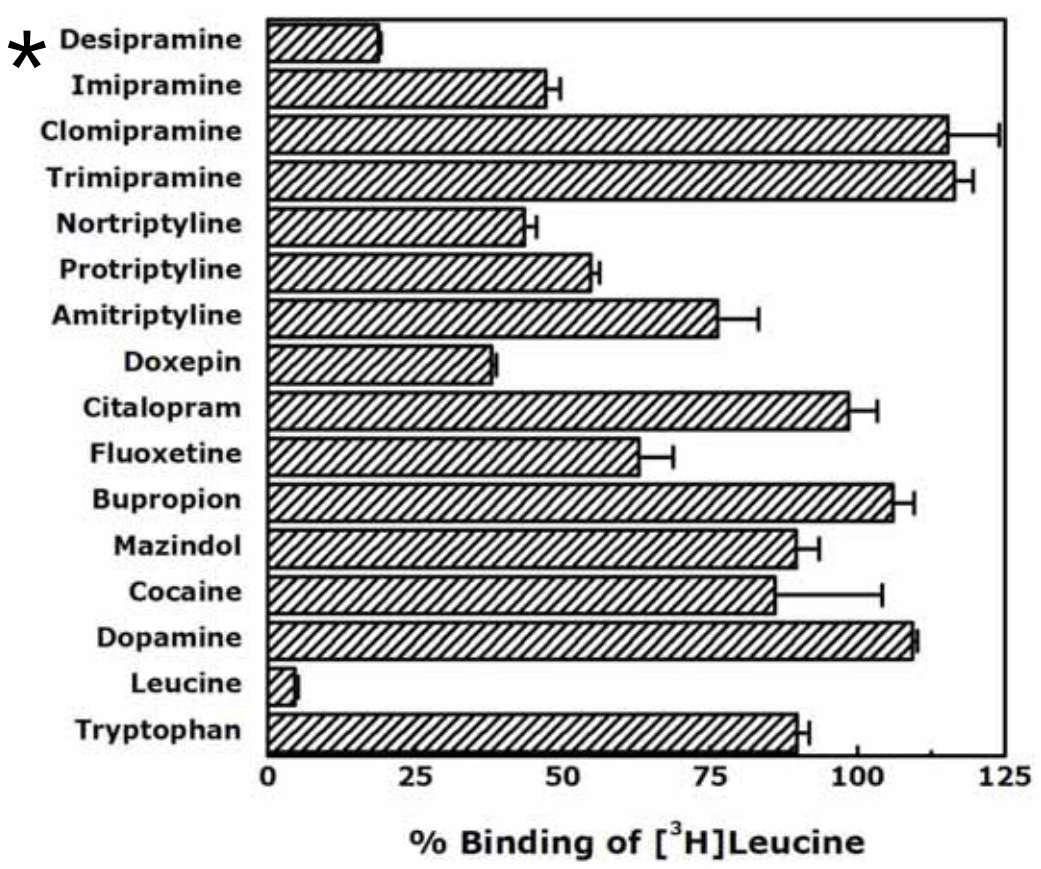
Measurements of antidepressant binding to LeuT

Scintillation proximity assay



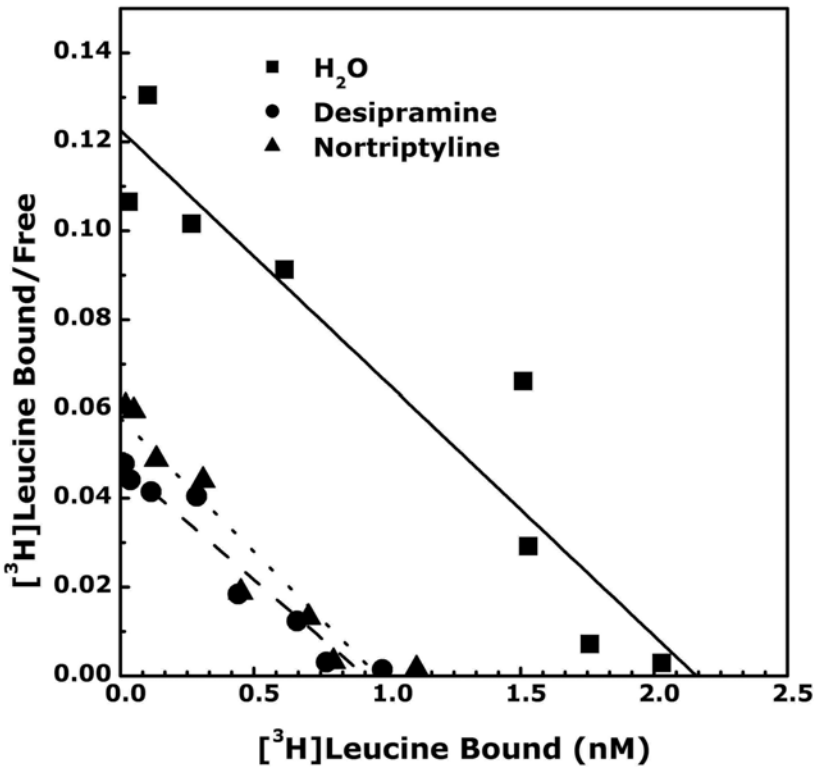
Purified LeuT bound with radioactive substrate

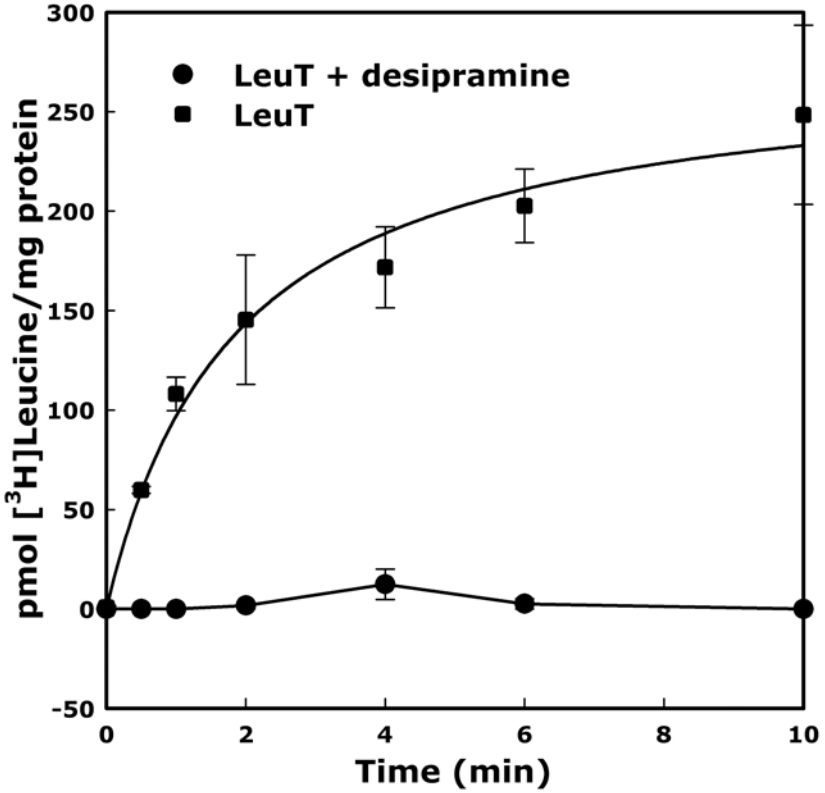
Scintillation beads



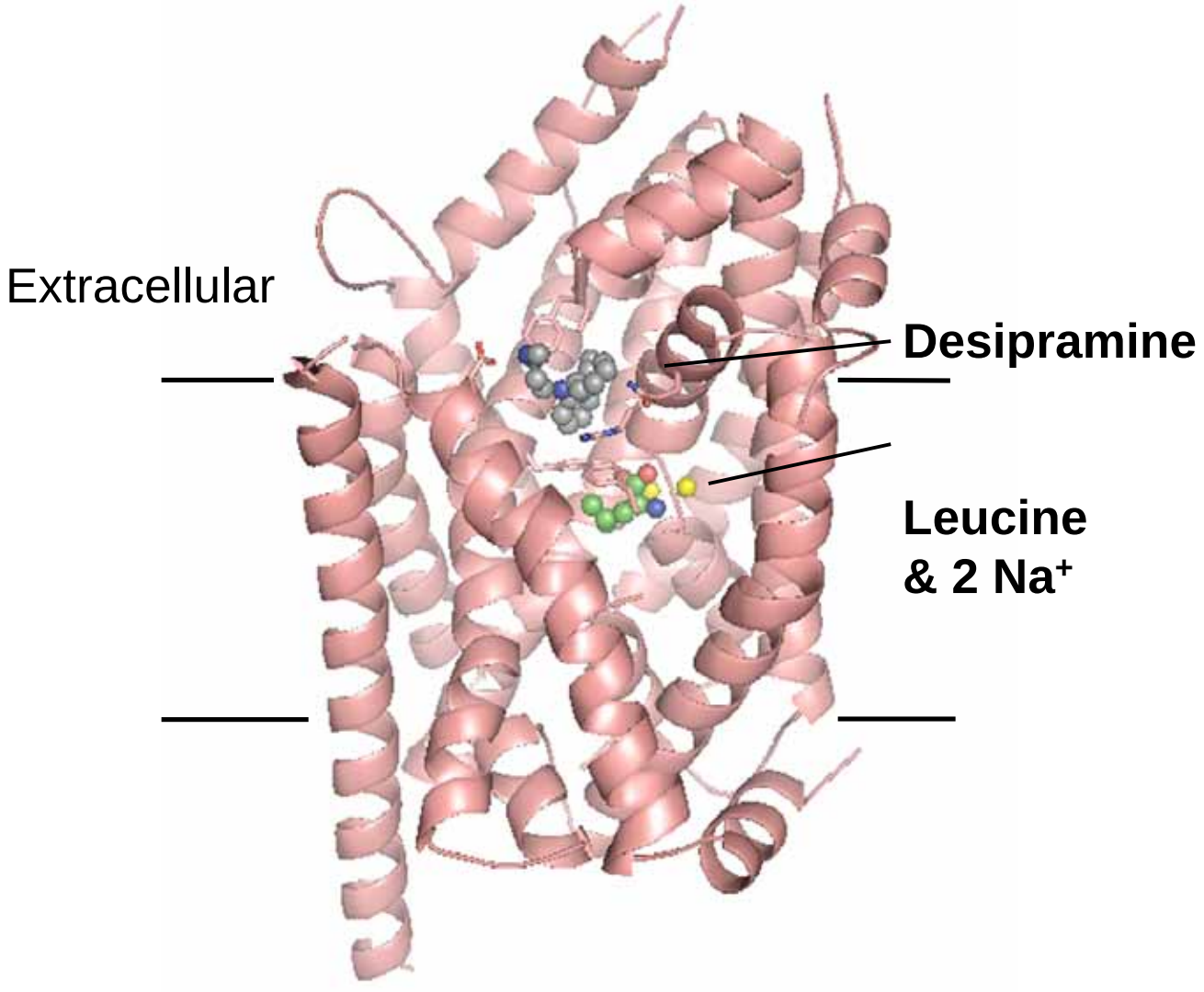
Quick & Javitch, (2007)

Desipramine binding to LeuT is not competitive

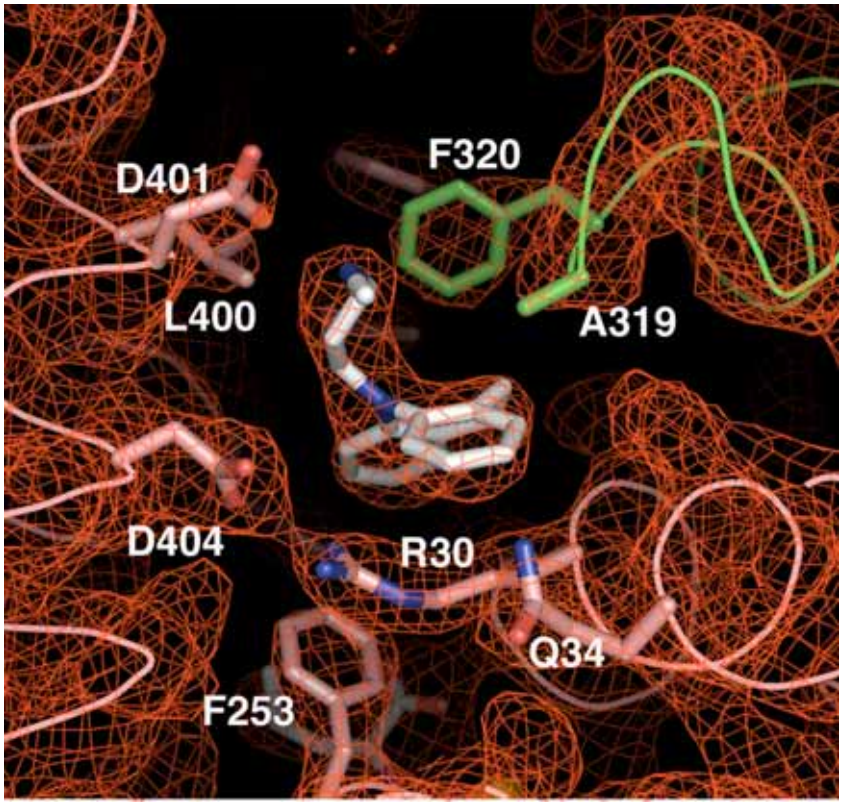


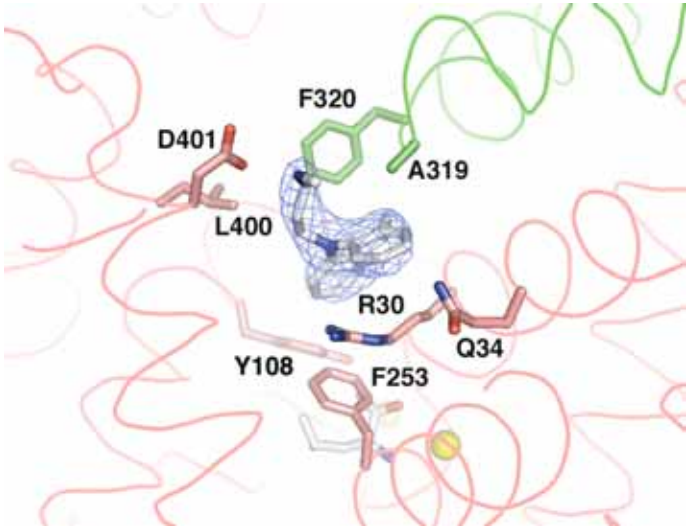


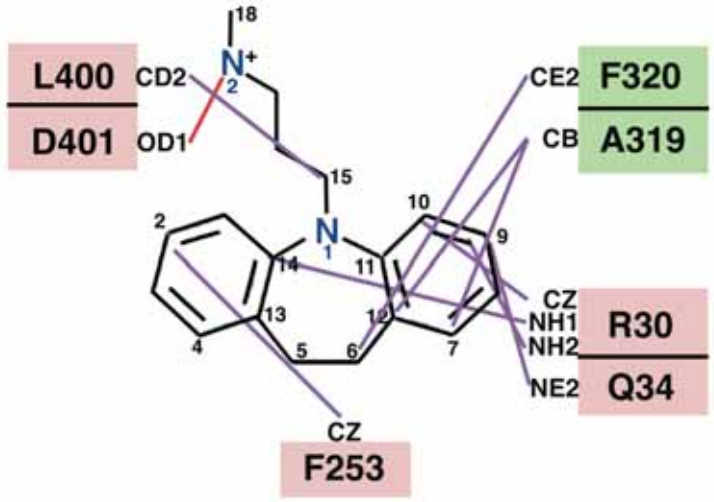
Desipramine-binding site and the leucine-binding site are separated by the extracellular gate



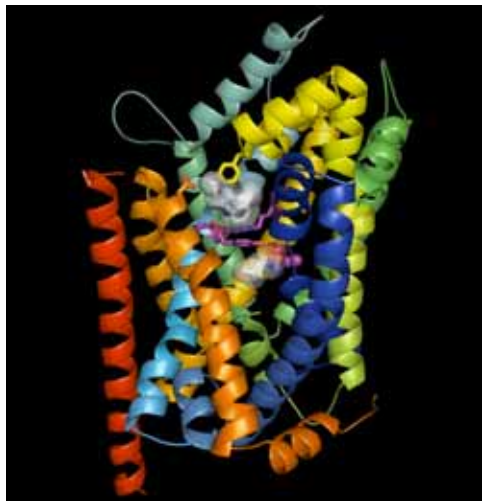
Binding pocket for desipramine in LeuT

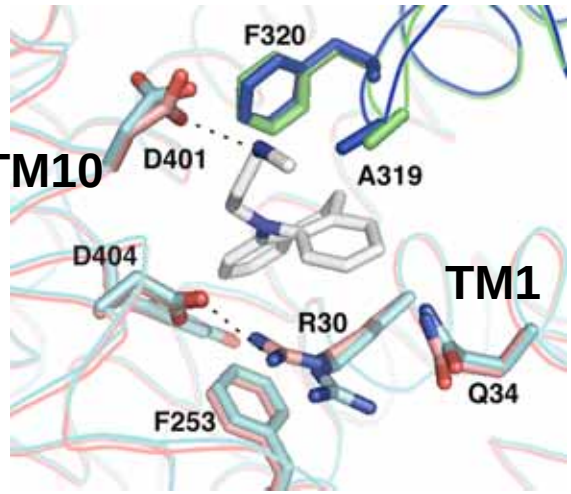


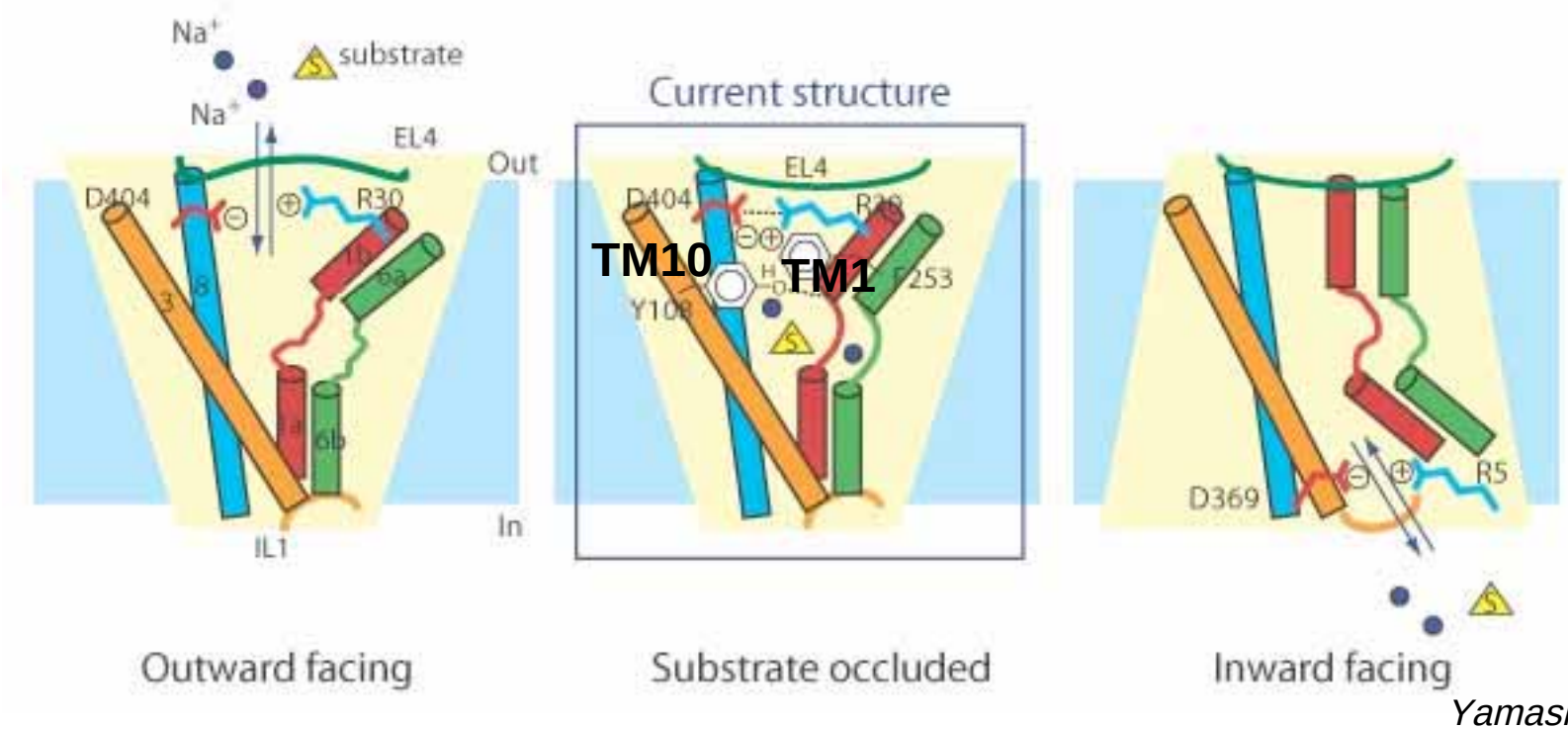




Desipramine binding slows down substrate release

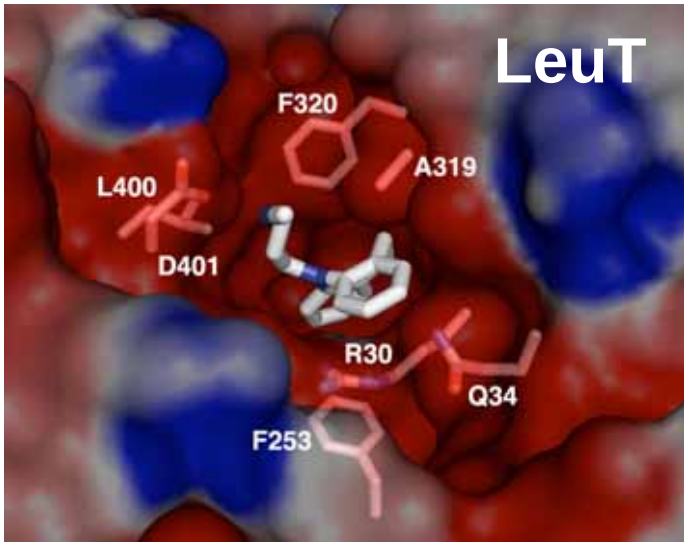


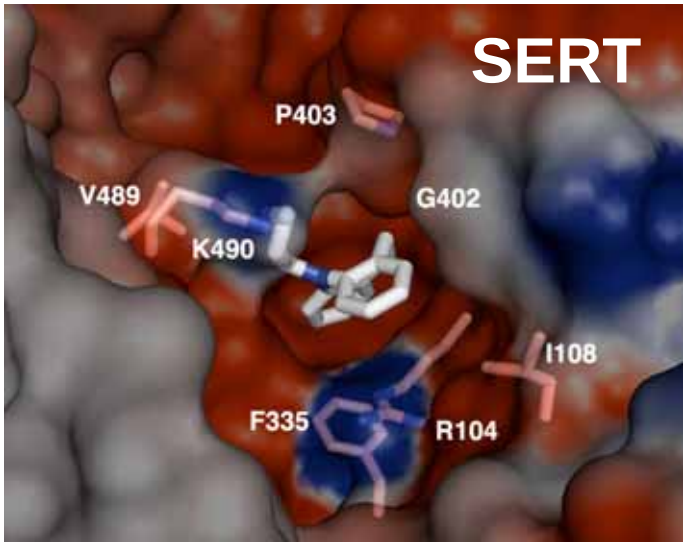


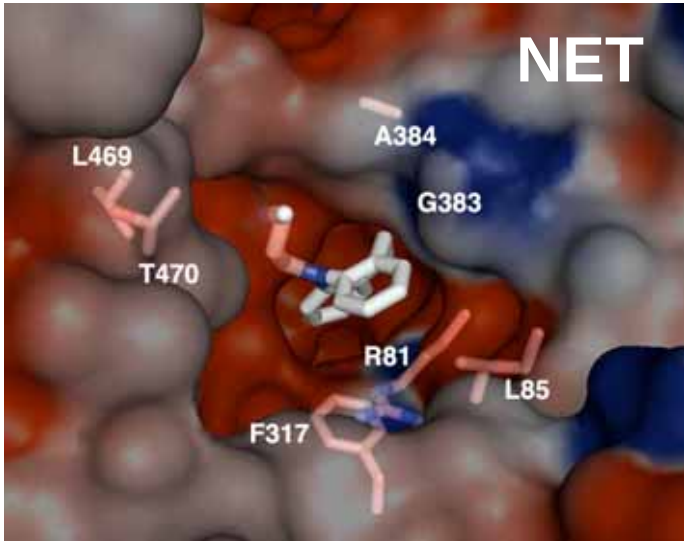


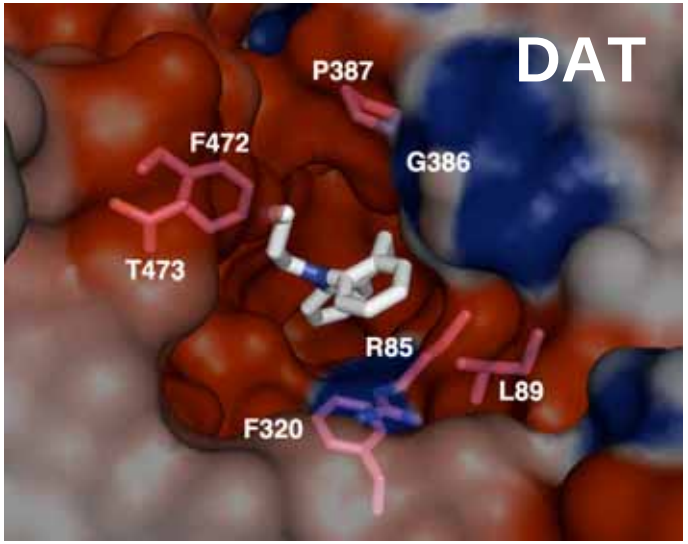
Yamashita, 2005

Homology modeling of SERT, NET and DAT









Probing desipramine-binding site in human proteins

Affinity of human NSS transporters
to desipramine (IC_{50})

Human NET	4.2 nM
Human SERT	64 nM
Human DAT	82,000 nM

NET
SERT
DAT

Probing desipramine-binding site in human proteins

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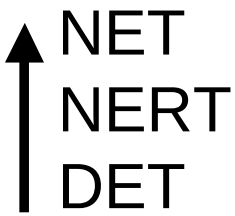
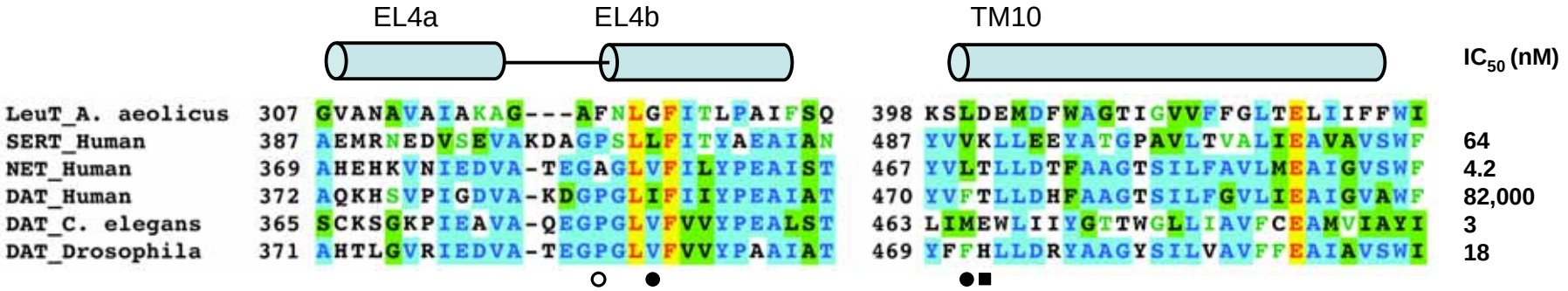
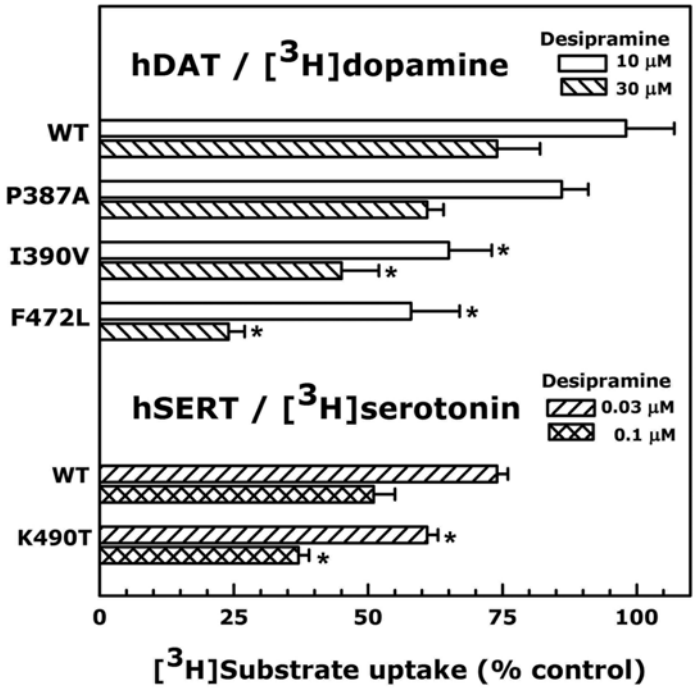
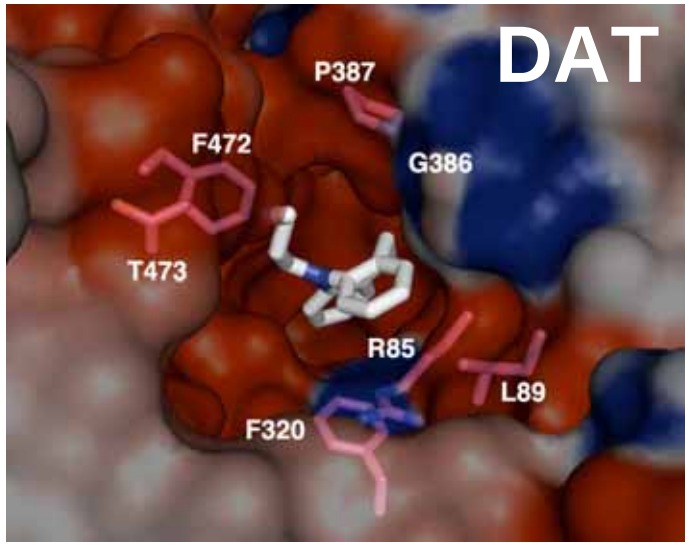


Gain of function mutagenesis in DAT

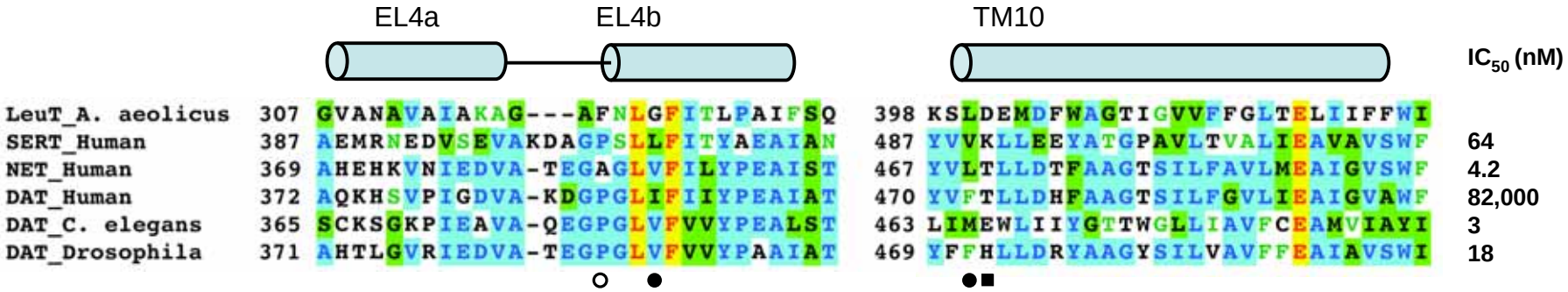


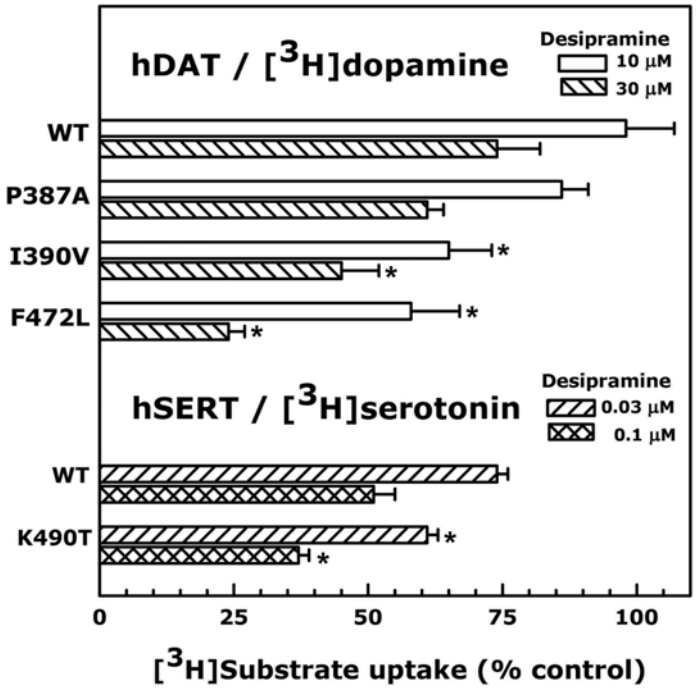


DAT-F472L: 79% decrease in IC_{50}

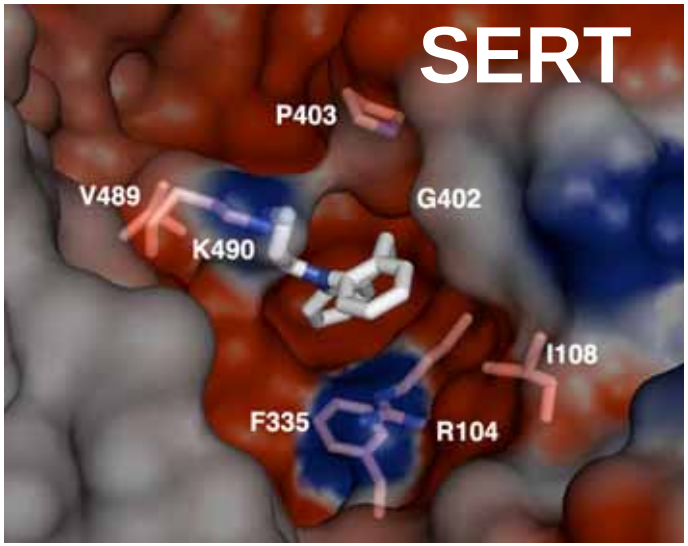


Gain-of-function mutagenesis in SERT

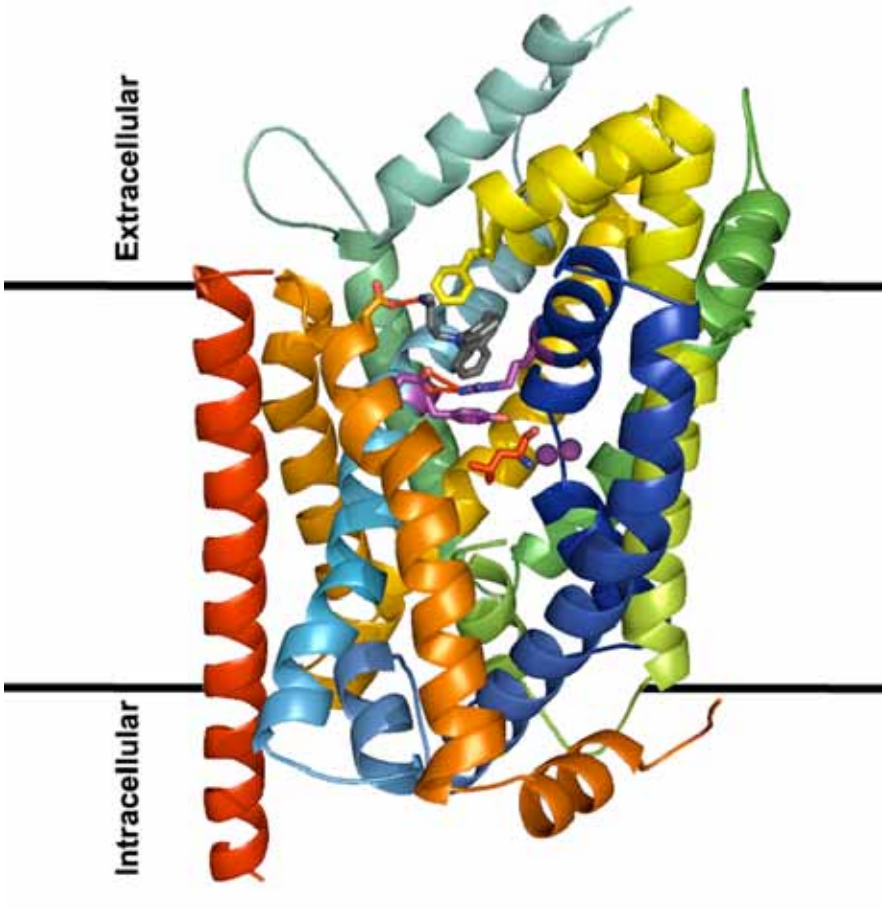




SERT-K490T: 51% decrease in IC_{50}



Summary



In LeuT, desipramine binding site at the extracellular gate, separated from the leucine binding site

Desipramine inhibits transport by preventing Substrate release to the cytosol

The drug binding site and inhibition mechanism are probably conserved in human SERT, NET and DAT

The structural information can help with drug design

Acknowledgements

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Christopher Law
Zheng Zhou

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Maarten Reith
Juan Zhen

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