UNDERSTANDING AMPHETAMINES:

modeling the interaction of MDMA with the serotonin transporter from *C. elegans*.

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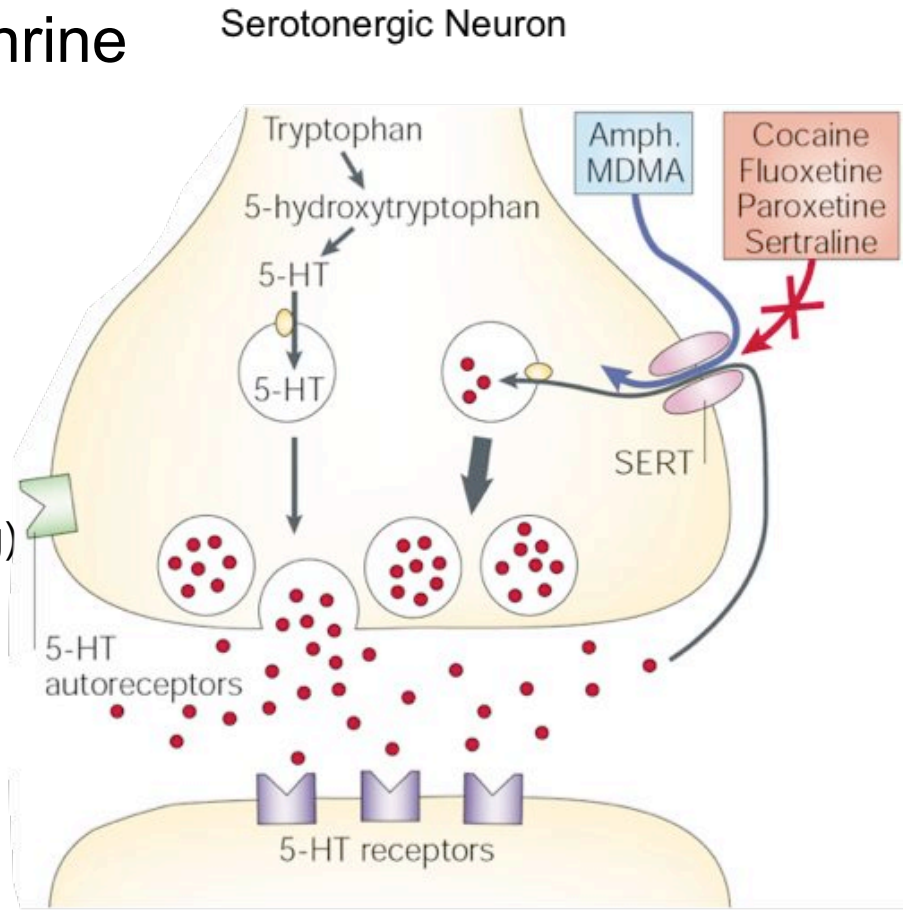
Clinical relevance- it's hard being number 1

Neurotransmitter transporters (monoamine)

- Serotonin, dopamine, norepinephrine
  - Molecular target

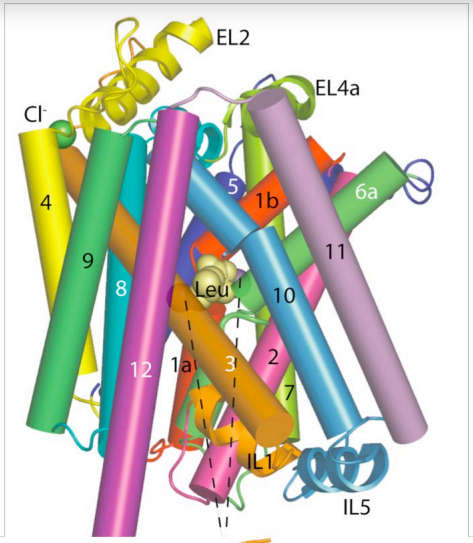
    Antidepressants – Cocaine – Ecstasy – Methamphetamine – neurodegenerative compounds
  - Diseases/disorders
    - Depression, anxiety, PTSD
    - Addiction (reward, craving, drug seeking)
    - ADHD (in reverse)
    - Autism (rigidity spectrum)
    - Neuropathic pain
    - OCD
  - Antidepressants
    - - #1 prescribed medication

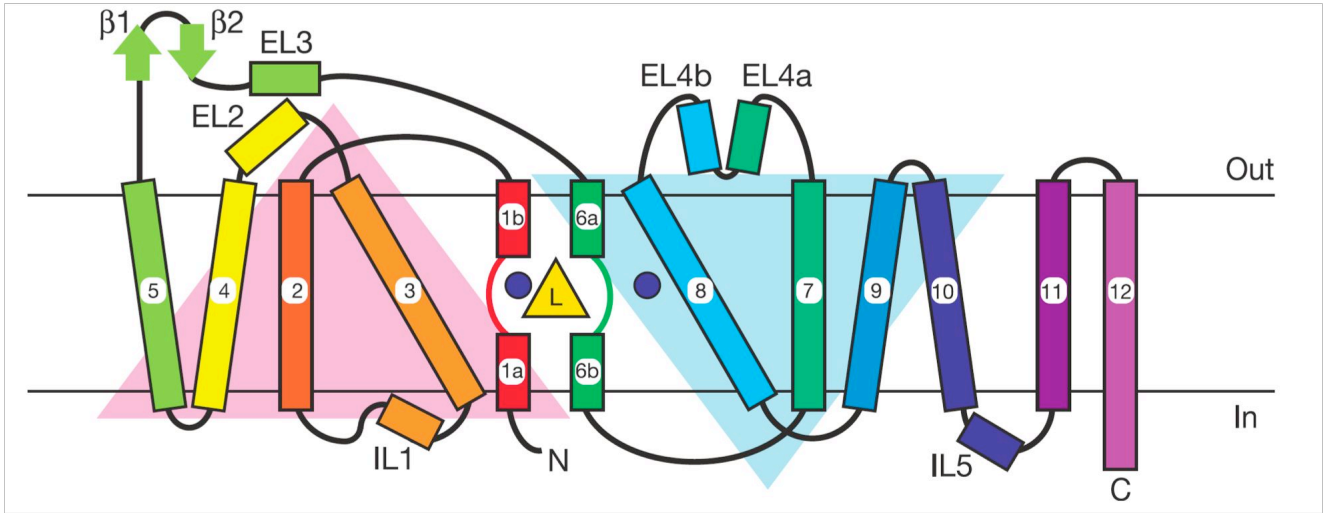


Torres *et al.*, 2003

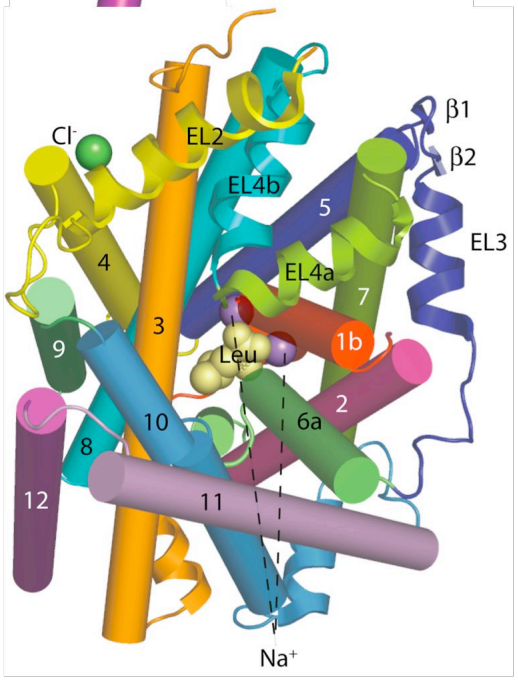
Bacterial homolog of NSS family is crystallized at high resolution

- LeuTAa - Yamashita *et al.*, 2005
  - leucine transporter from *Aquifex aeolicus*
  - ~21% homology to mammalian NSS transporters
  - 1.65 Å resolution
  - Na dependent
  - Cl independent
  - pseudo two-fold axis of symmetry





Yamashita *et al.*, 2005



Henry *et al.*, 2006

Understanding molecular contacts for substrates and antagonists

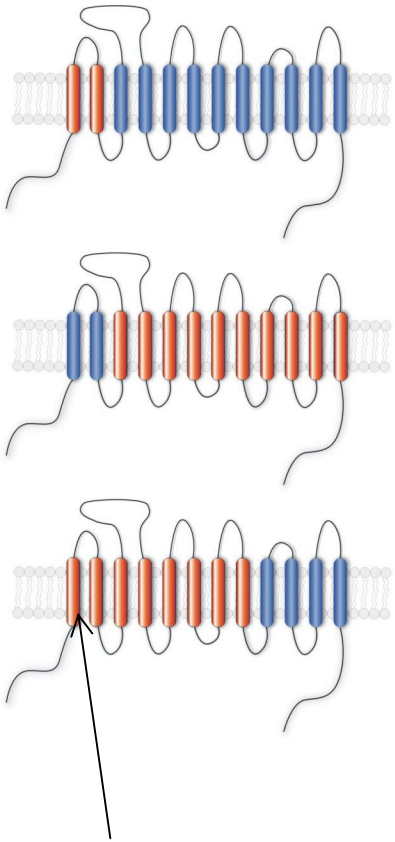
Integration of biochemistry, pharmacology and computational biology

PROTEINS
STRUCTURE ■ FUNCTION ■ BIOINFORMATICS

Structural determinants of species-selective substrate recognition in human and *Drosophila* serotonin transporters revealed through computational docking studies

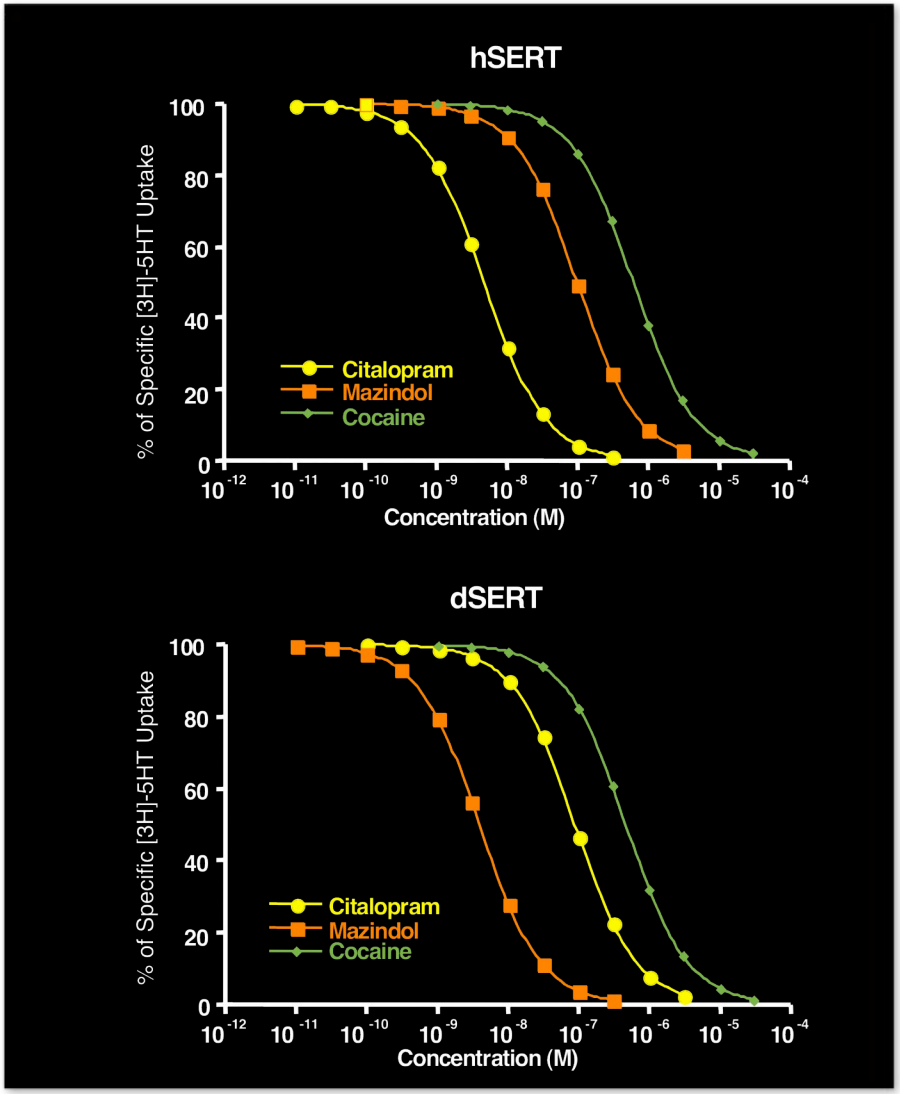
Kristian W. Kaufmann,[1] Eric S. Dawson,[2,3] L. Keith Henry,[4] Julie R. Field,[4] Randy D. Blakely,[4,5,6] and Jens Meiler[1,4,3]*

Molecular Differences that Determine Drug Potency at the Species Level



dSERT
hSERT

Y95F



Methodology

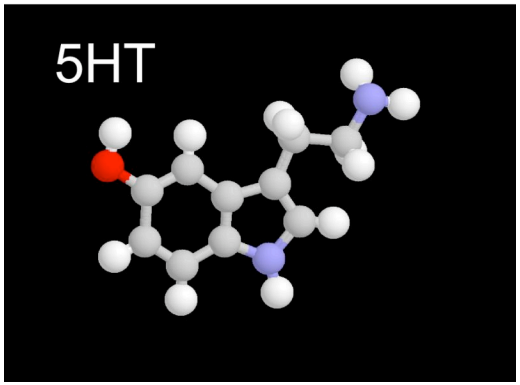
- Alignment
  - Based on LeuTAa and Eukaryotic NSS transporters (Beuming et al)
    - Overall homology 17%
  - TMs 1, 3, 6 and 8 comprise majority of binding site
    - 1st shell – residues with Cα ≤ 7Å
      - >50% homology
    - 2nd shell – residues with Cα ≤ 12Å
- Retained Backbone coordinates for hSERT & dSERT models

Methodology continued…

1. Ten models each for dSERT and hSERT
2. Repacking
   A. Eight cycles of side-chain repacking
   B. Gradient minimization of ϕ,ψ and χ angles

Ligand conformers

1. Used mmff94 (MOE) to generate 100 conformations of 5-HT
   A. Yielded ± gauche and trans configurations of amine tail
2. Created a 10Å cube centered where leucine lies in LeuTAa structure
3. Generated 13,000 docked structures for hSERT and dSERT



Filtering and Refinement of SERT models

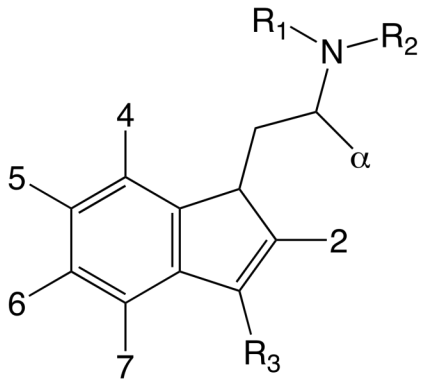
Filtering

1. Top 10% of best protein-ligand interactions based on energy
2. 3.6Å distance constrained between 5-HT amine tail and D98 (O)
3. 5-HT binding mode conservation (similar binding mode in hSERT and dSERT)

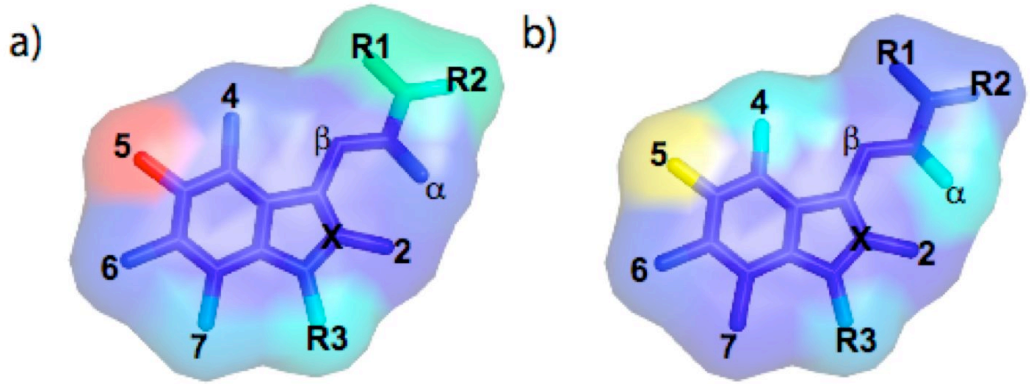
Refinement

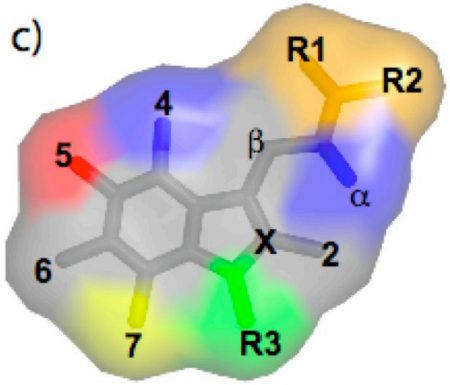
1. Placement of Na
2. Molecular dynamic simulation analysis of 5-HT docked structures

SVM QSAR Analysis of 5-HT in hSERT and dSERT



hSERT dSERT





difference

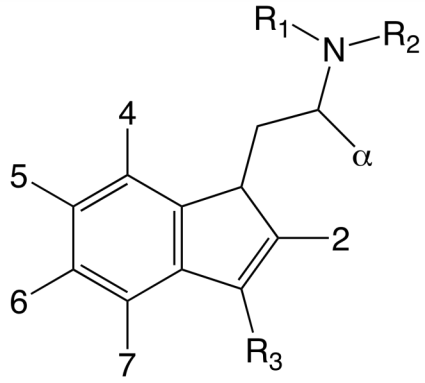
Blue = higher sensitivity dSERT

Green-red = higher sensitivity hSERT

Analog docking as readout for relevance of models

Tryptamine analogs:

1. 7-benzyloxy
2. 2-methyl
3. N-isopropyl
4. Tryptamine
5. 5-o-sulfate
6. 4-hydroxyl
7. 7-methyl
8. 5-hydroxy
9. 5-methoxy-N-isopropyl



Docking

1. Placement – Monte Carlo refinement and gradient minimization
2. 1° Top 9 lowest energy structures for each analog
3. 2° Top structure with lowest energy and indole ring < 1RMSD from starting position used for binding energy calculations
4. 7-benzyloxy was allowed to freely rotate due to size.
5. $\Delta E_{\text{ligand binding}} = \Delta E_{\text{protein bound state}} - \Delta E_{\text{protein unbound state}}$
6. $\Delta E < -1$ considered major contributors

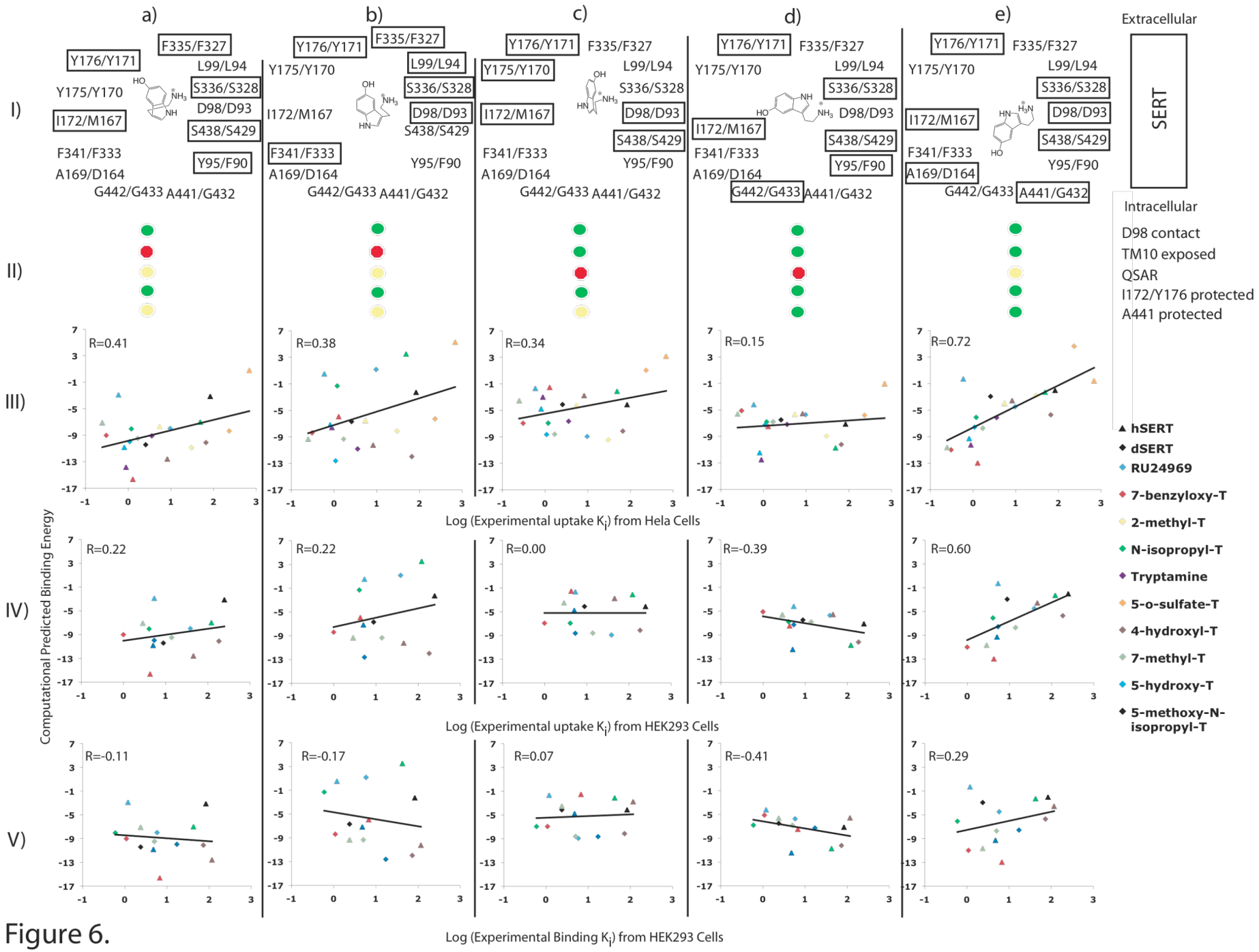
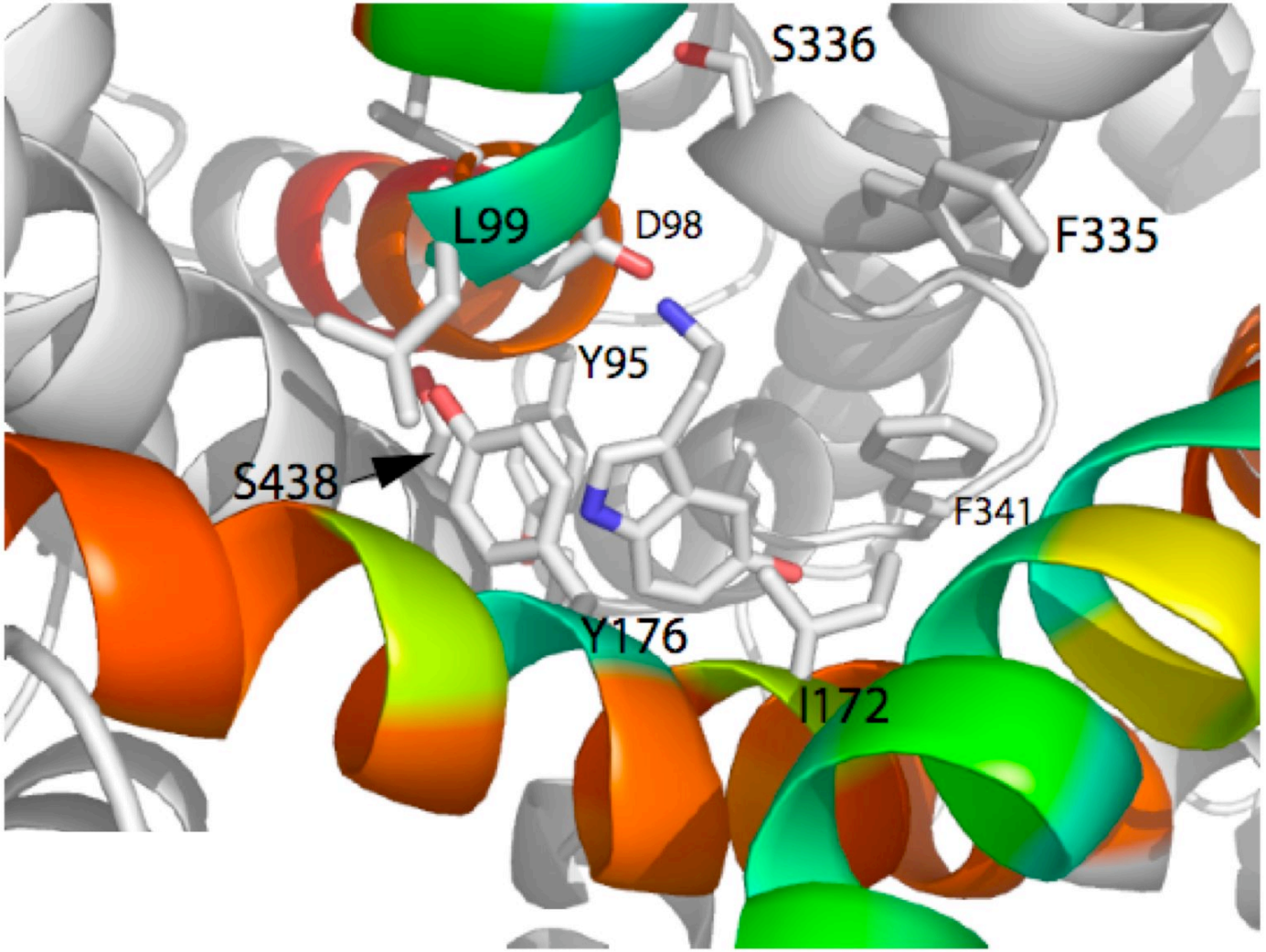
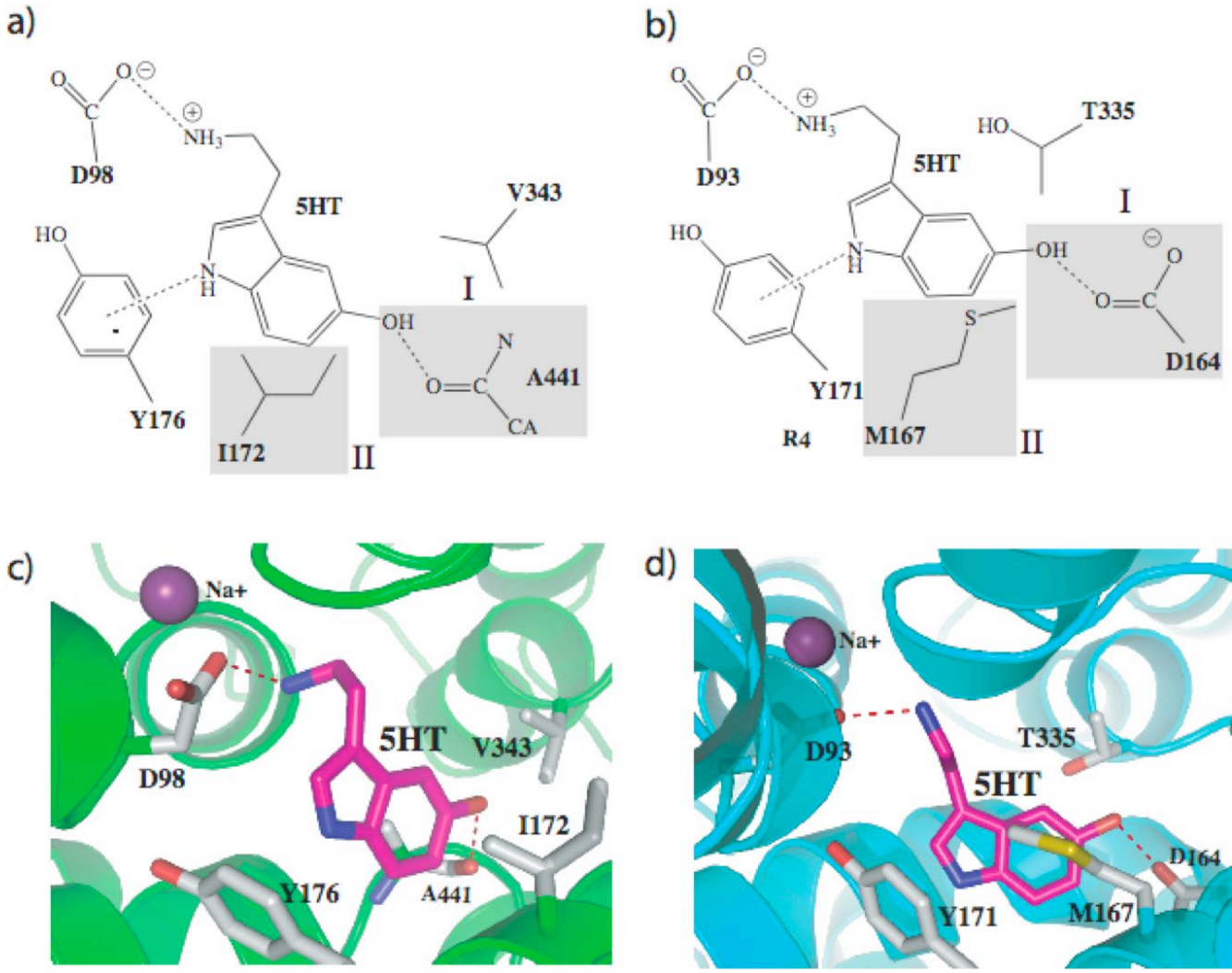

a)
b)
c)
d)
e)
I)
II)
III)
IV)
V)
Y176/Y171
F335/F327
L99/L94
Y175/Y170
S336/S328
I172/M167
D98/D93
S438/S429
F341/F333
Y95/F90
A169/D164
G442/G433
A441/G432
Extracellular
SERT
Intracellular
D98 contact
TM10 exposed
QSAR
I172/Y176 protected
A441 protected
R=0.41
R=0.38
R=0.34
R=0.15
R=0.72
R=0.22
R=0.22
R=0.00
R=-0.39
R=0.60
R=-0.11
R=-0.17
R=0.07
R=-0.41
R=0.29
Computational Predicted Binding Energy
Log (Experimental uptake Ki) from Hela Cells
Log (Experimental uptake Ki) from HEK293 Cells
Log (Experimental Binding Ki) from HEK293 Cells
hSERT
dSERT
RU24969
7-benzyloxy-T
2-methyl-T
N-isopropyl-T
Tryptamine
5-o-sulfate-T
4-hydroxyl-T
7-methyl-T
5-hydroxy-T
5-methoxy-N-isopropyl-T

Figure 6.

5-HT down binding mode (hSERT)

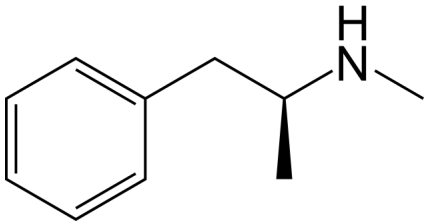


Down binding mode

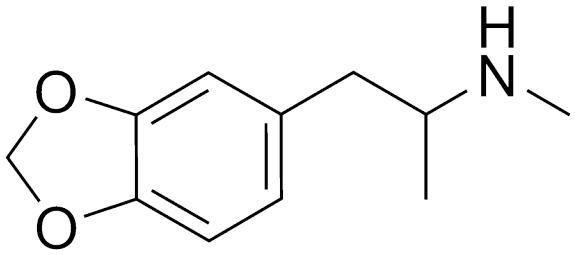


hSERT dSERT

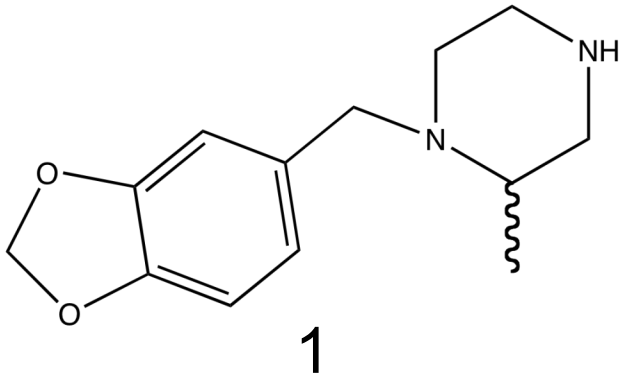
Putting the transporter in reverse and revving the engine



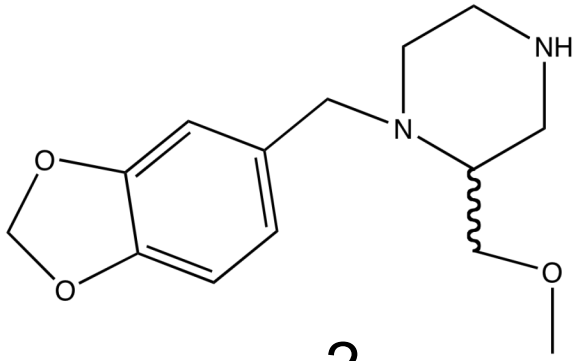
amphetamine



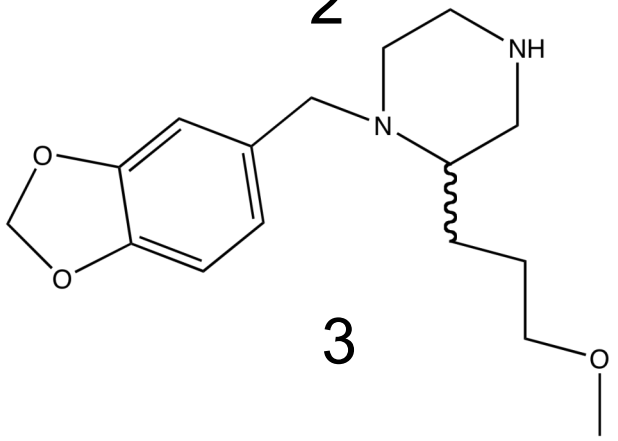
MDMA



1



2



3

My processor may be slow but its been going a LONG time

C. elegans SERT is more distant from hSERT than hSERT is from hNET or hDAT

Acknowledgements

VANDERBILT
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ROSETTACO
N

THE UNIVERSITY OF NORTH DAKOTA

Benchmarking

Benchmarking

1. LeuT crystal structure
2. Re-dock leucine using ROSETTALIGAND

RESULT: The lowest energy structure recaptured the native binding mode (RMSD 0.81 Å)

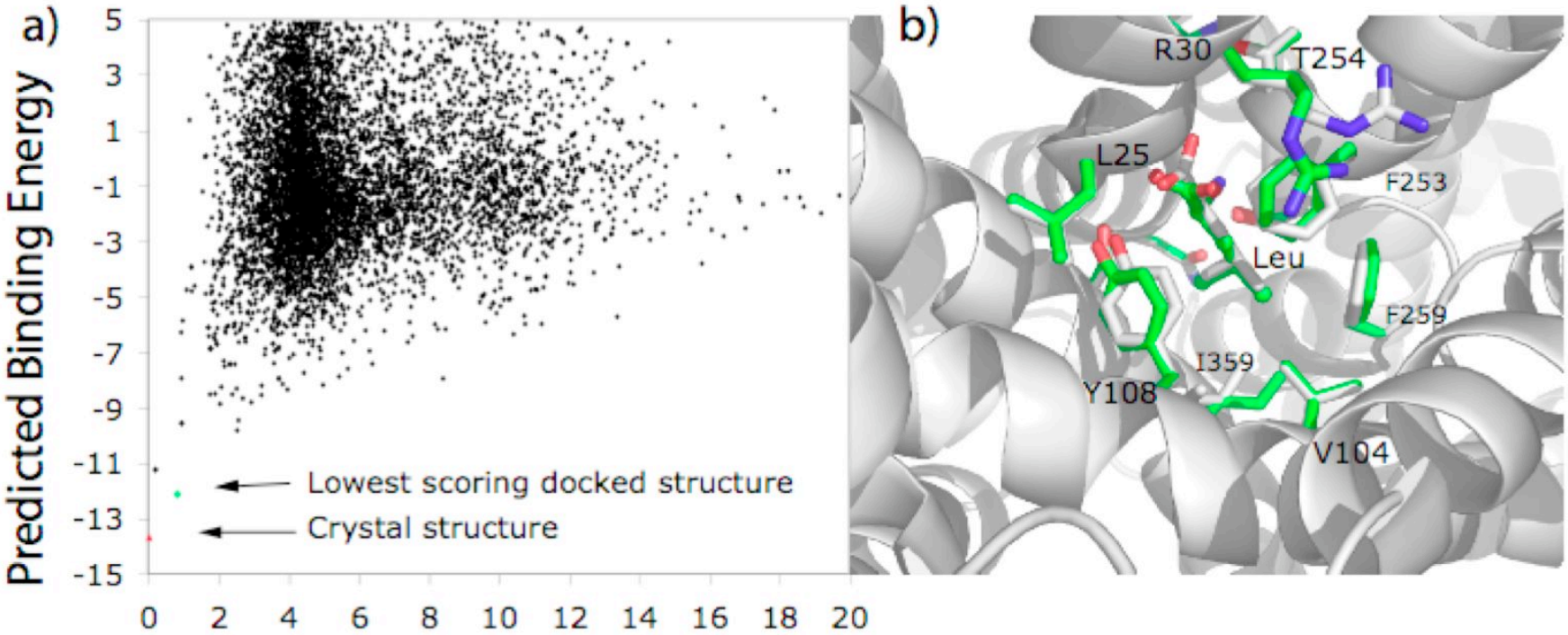

Figure 2.

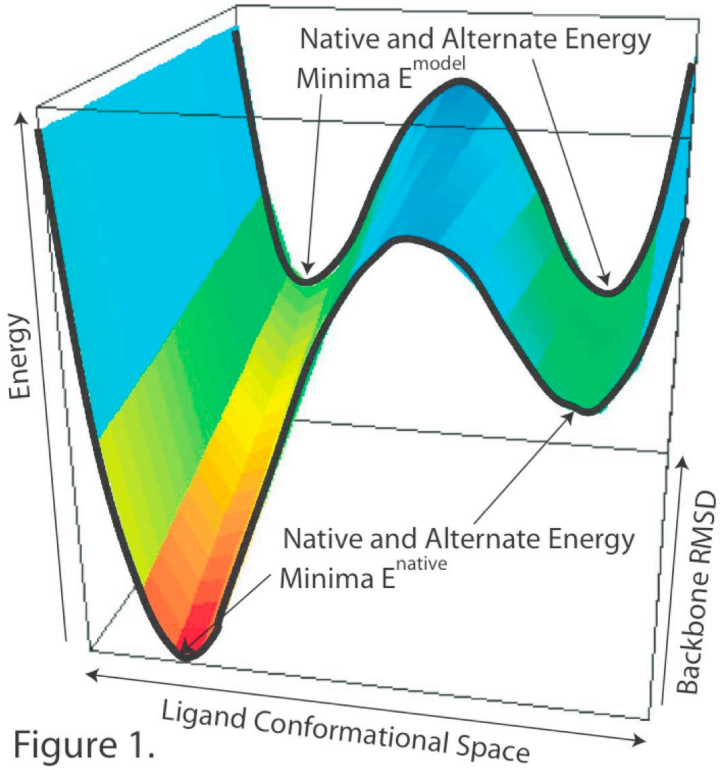
Native and Alternate Energy
Minima E^model
Energy
Native and Alternate Energy
Minima E^native
Backbone RMSD
Ligand Conformational Space

Figure 1.

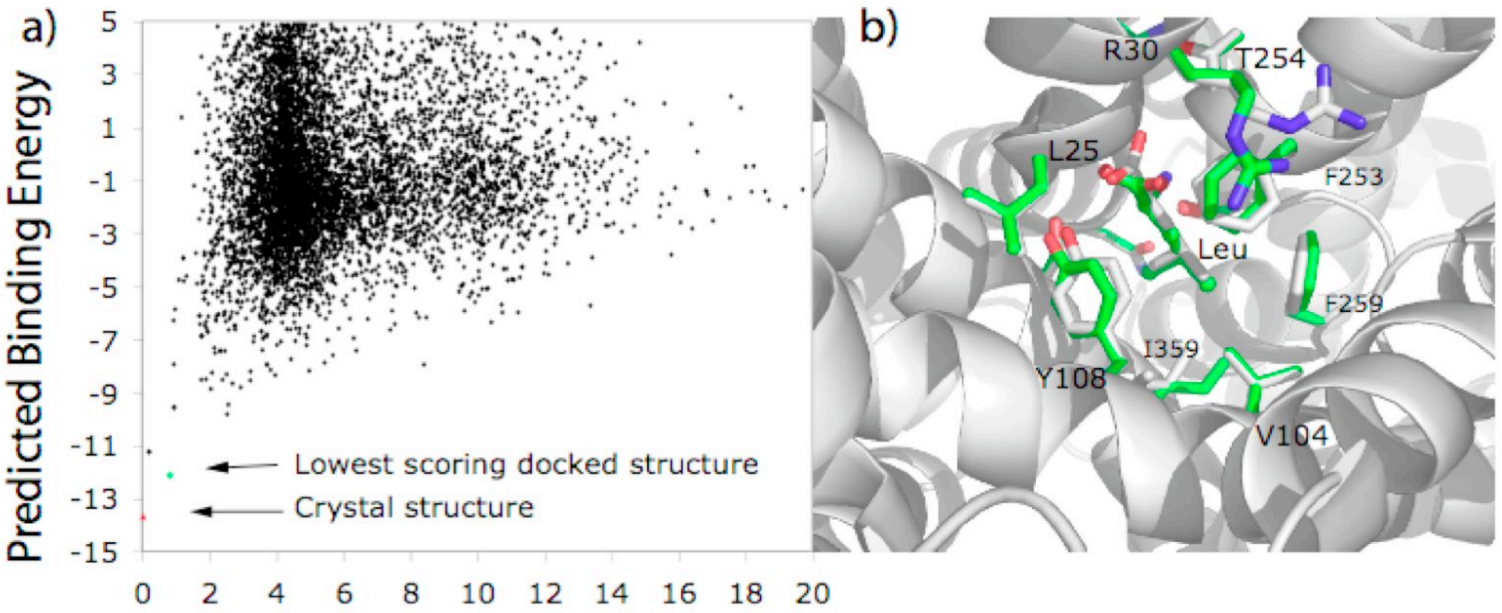
a)
Predicted Binding Energy
5
3
1
-1
-3
-5
-7
-9
-11
-13
-15
0
2
4
6
8
10
12
14
16
18
20
RMSD
Lowest scoring docked structure
Crystal structure
b)
R30
T254
L25
F253
Leu
F259
I359
Y108
V104

Figure 2.

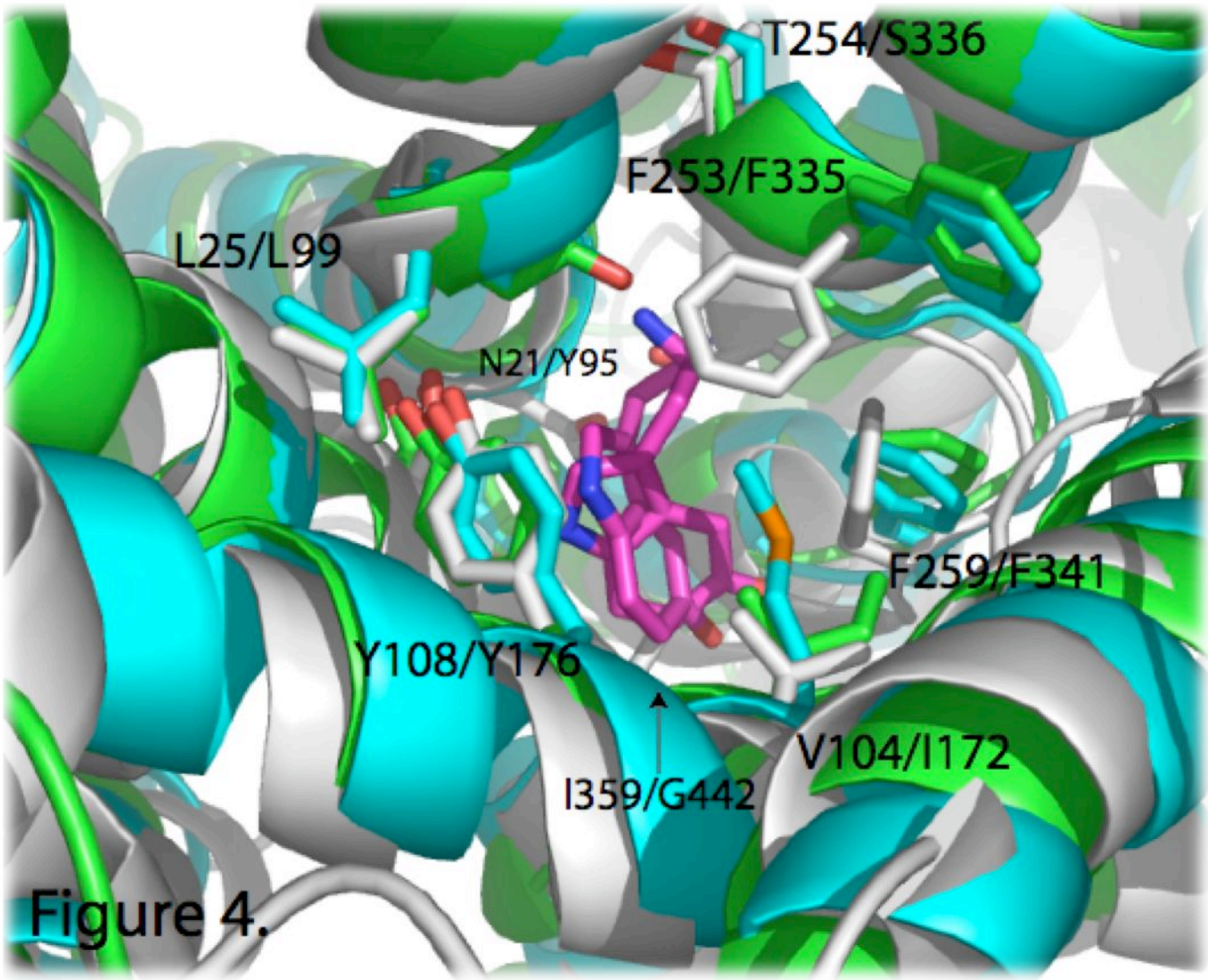
T254/S336
F253/F335
L25/L99
N21/Y95
F259/F341
Y108/Y176
V104/I172
I359/G442

Figure 4.

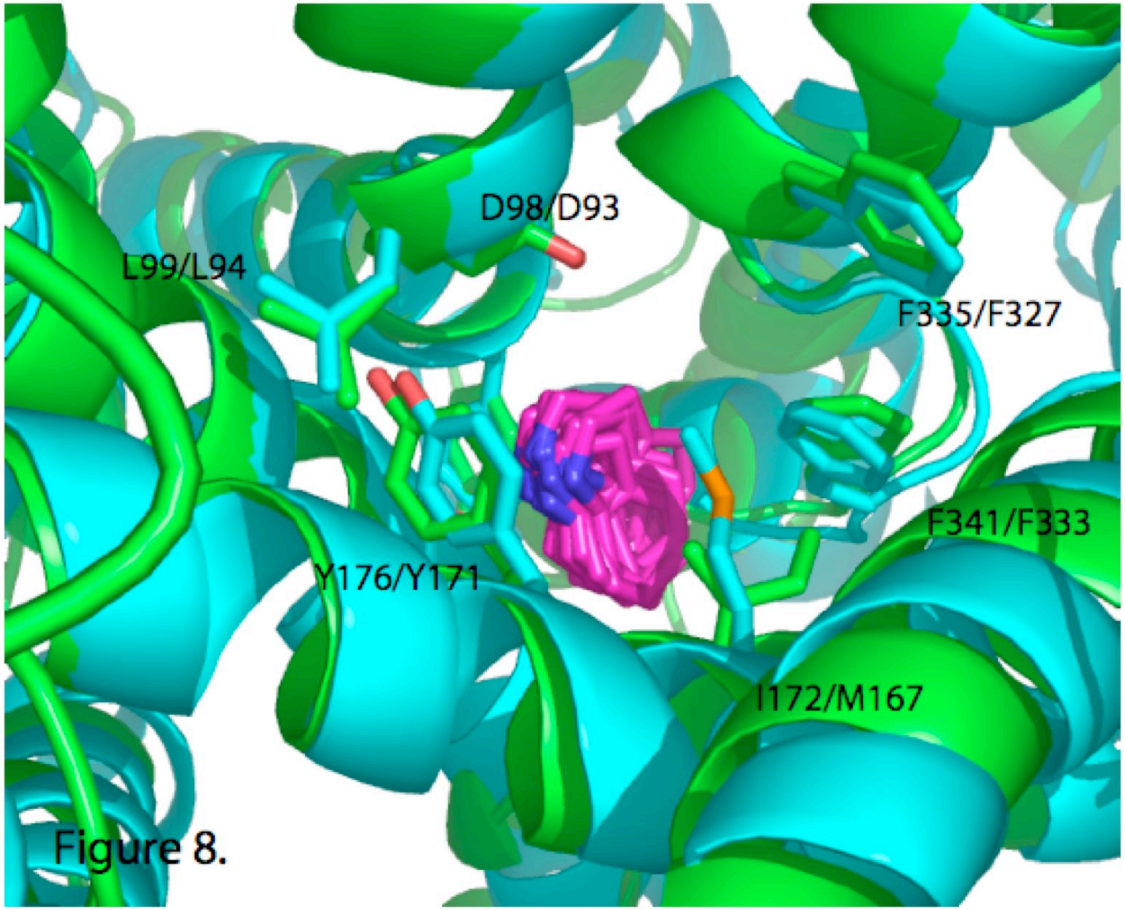

Figure 8.