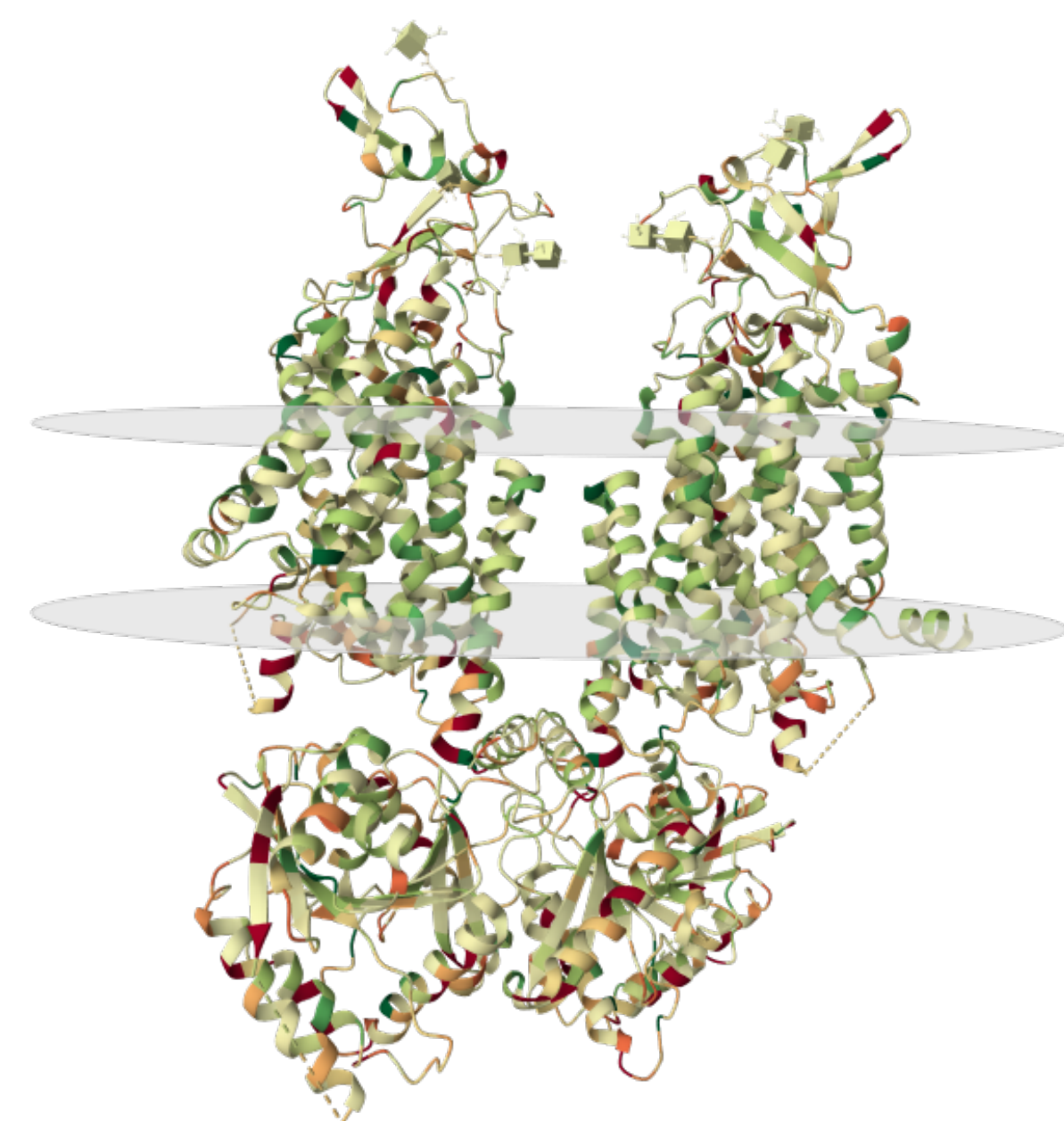


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## Background

- Potassium-Chloride Cotransporter 2 (KCC2) is a **neuronal membrane protein** specific to the central nervous system.
- It is responsible for removing Cl<sup>-</sup> ions from the intracellular space, **maintaining a normal Cl<sup>-</sup> gradient**. This is critical to the function of certain inhibitory synapses.
- Dysregulation causes an **upward shift in the Cl<sup>-</sup> reversal potential** resulting in a hyperexcitable state of the postsynaptic neuron.
- KCC2 has also been previously implicated in EtOH dependence.
- Several novel direct KCC2 agonists have been discovered [4]
- VU0500469, one of the recently identified agonists, was used for *in silico* modeling to identify possible KCC2 binding sites



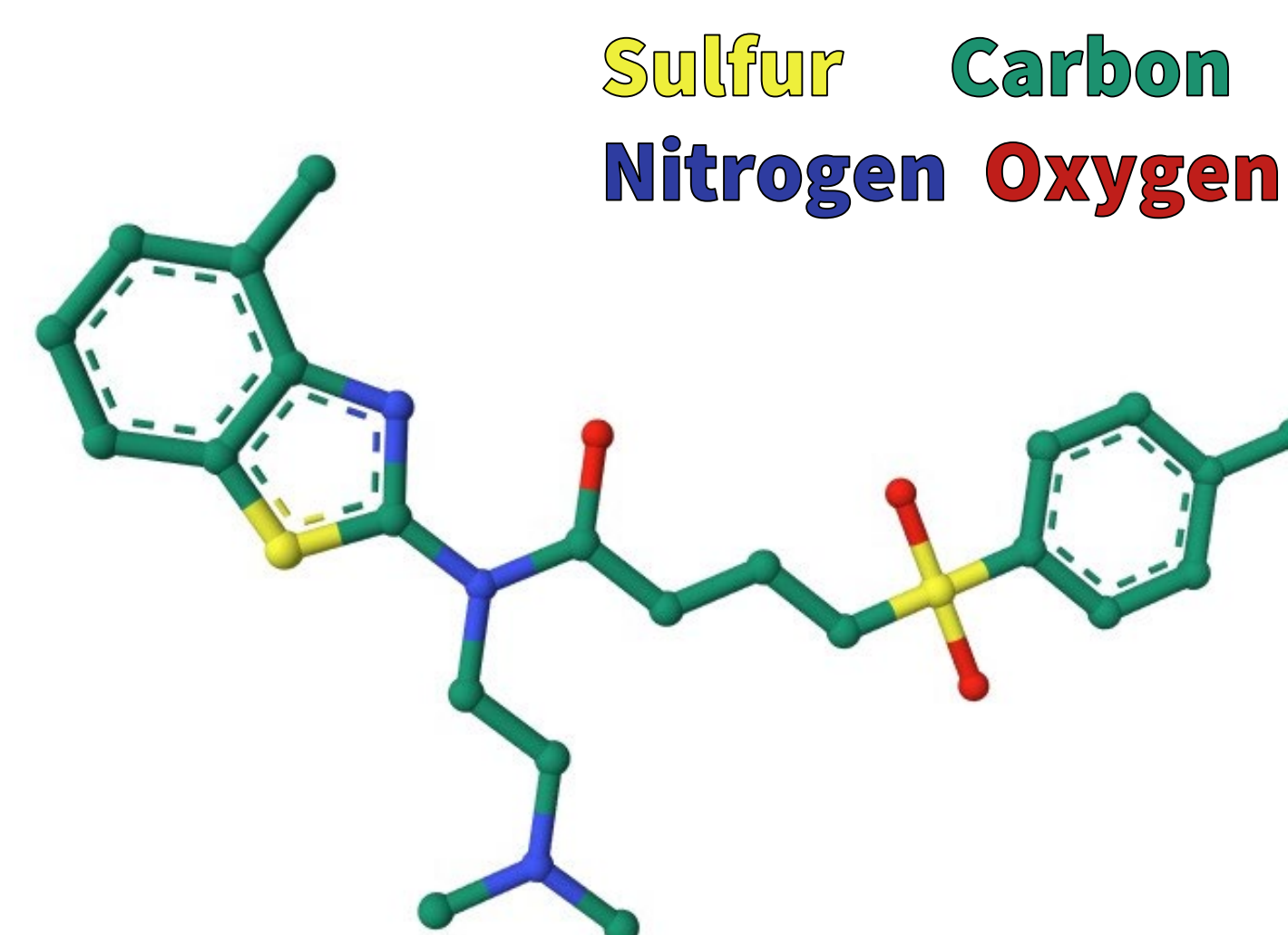
**Figure 1.** 3-D structure of human KCC2. Accessed from RCSB Protein Databank, including membrane prediction [1, 2, 3]

## Methods

### Preparation and Visualization

**Software:** AutoDock Tools, PyMOL

- 3-D structures of human KCC2 were obtained from RCSB Protein Databank.
- VU0500469 was recreated manually (Figure 2).
- PDB files were loaded into AutoDock tools and converted into .pdbqt files.



**Figure 2.** VU0500469. N-(2-(Dimethylamino)Ethyl)-N-(4-Methylbenzo[d]Thiazol-2-yl)-4-Tosylbutanamide. Formula: C<sub>21</sub>H<sub>26</sub>C<sub>1</sub>N<sub>3</sub>O<sub>3</sub>S<sub>2</sub>

### Modeling

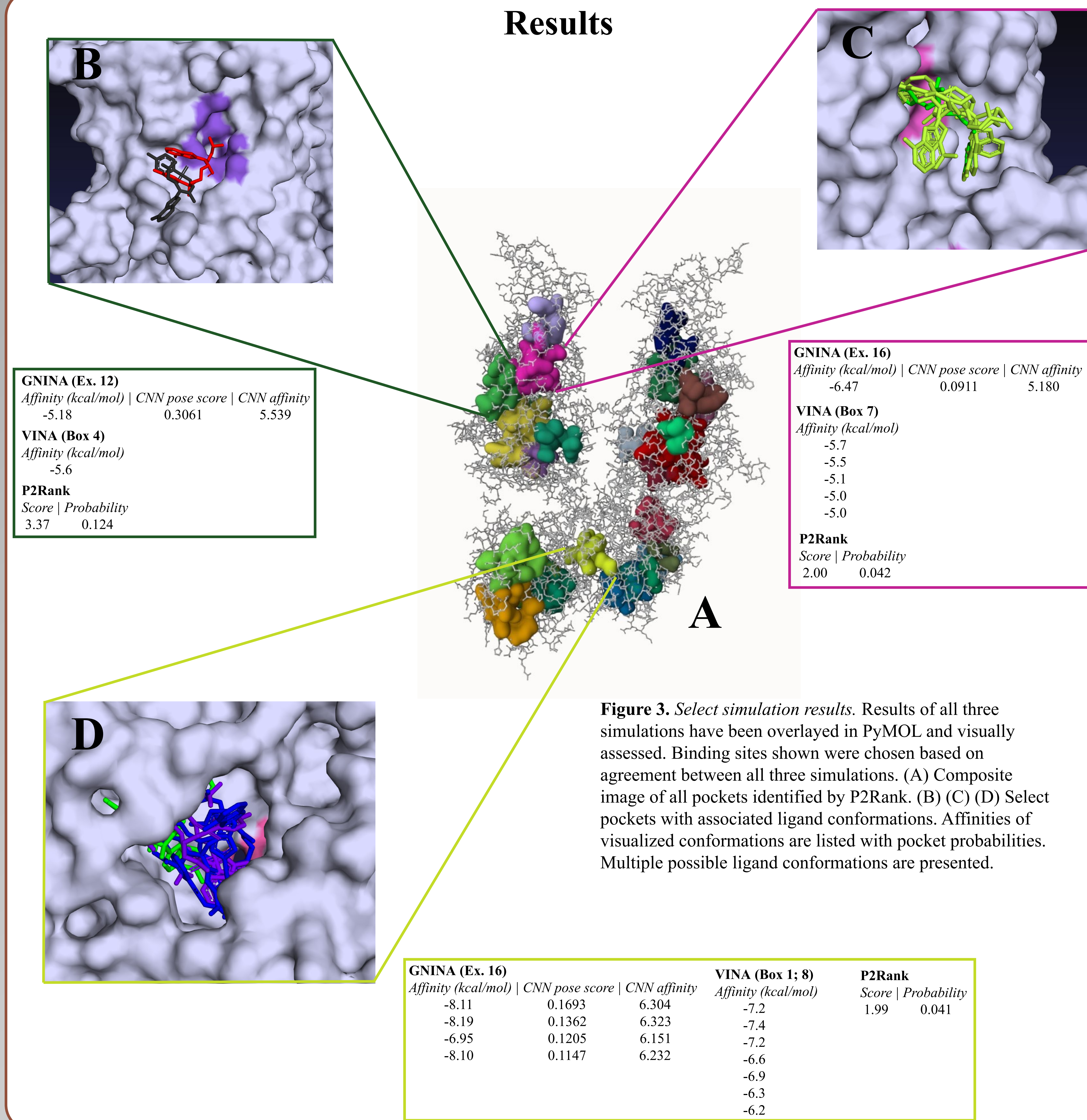
**Software:** AutoDock Vina [5], GNINA [6], P2Rank [7]

- Potential binding pockets for VU0500469 were identified using P2Rank.
- AutoDock Vina and GNINA simulations were used to identify optimal binding site location and conformation.
- Results from all three tools were compared to identify likely binding sites for further investigation.

LogP	TPSA
3.27	69.72

**Table 1.** Molecular properties calculations for VU0500469 [8]

## Results



**Figure 3.** Select simulation results. Results of all three simulations have been overlaid in PyMOL and visually assessed. Binding sites shown were chosen based on agreement between all three simulations. (A) Composite image of all pockets identified by P2Rank. (B) (C) (D) Select pockets with associated ligand conformations. Affinities of visualized conformations are listed with pocket probabilities. Multiple possible ligand conformations are presented.

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