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VirCAD: bioCAD for Viral Engineering

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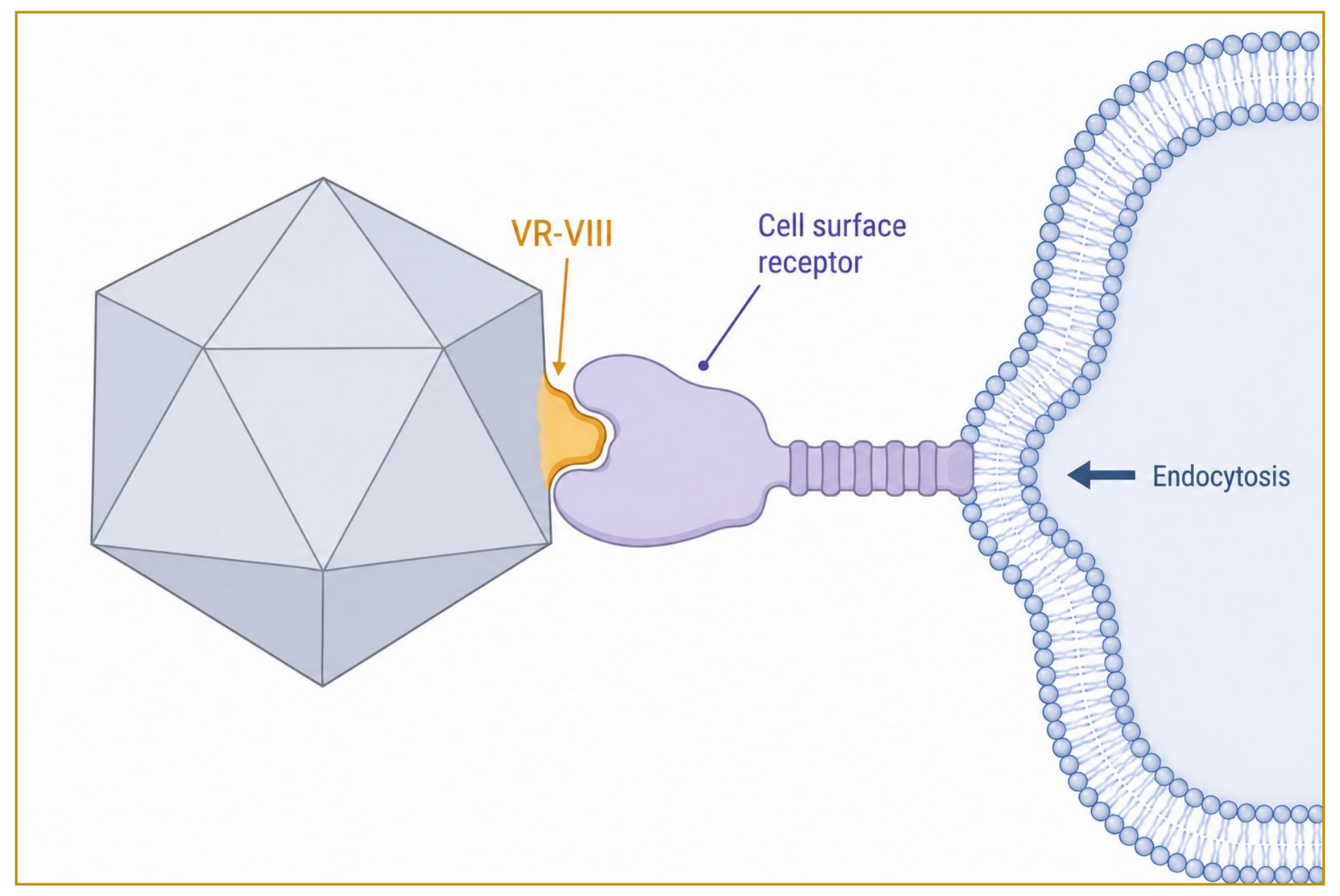
Background

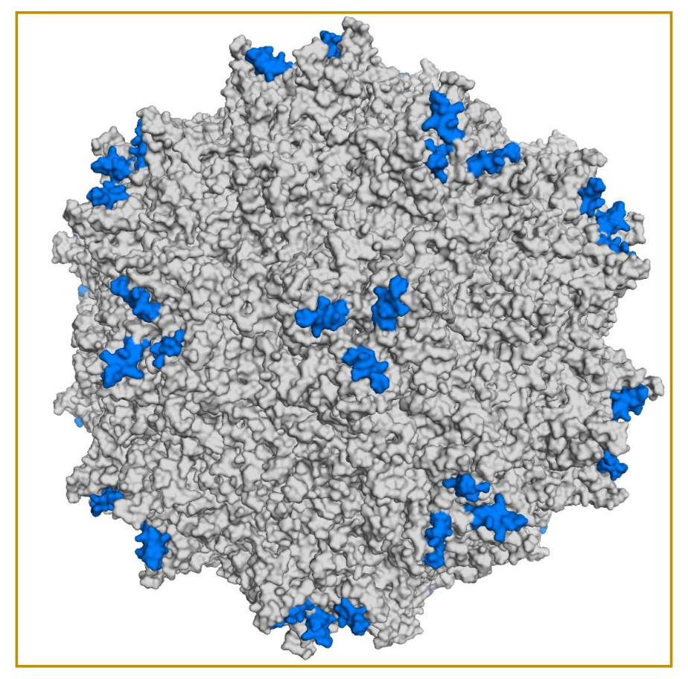
Vircad

- A comprehensive computational drug discovery platform
- Makes advanced bioinformatics tools accessible to researchers without coding experience

AAV vectors

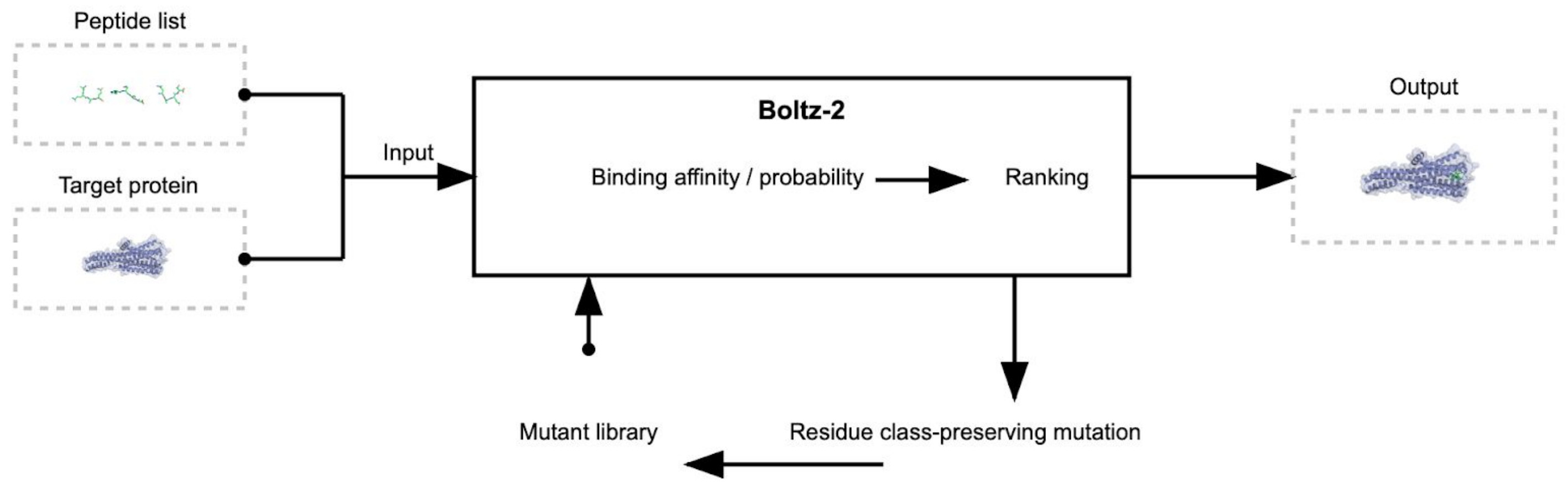
- enable CNS gene replacement therapy but require capsid engineering for efficient receptor targeting

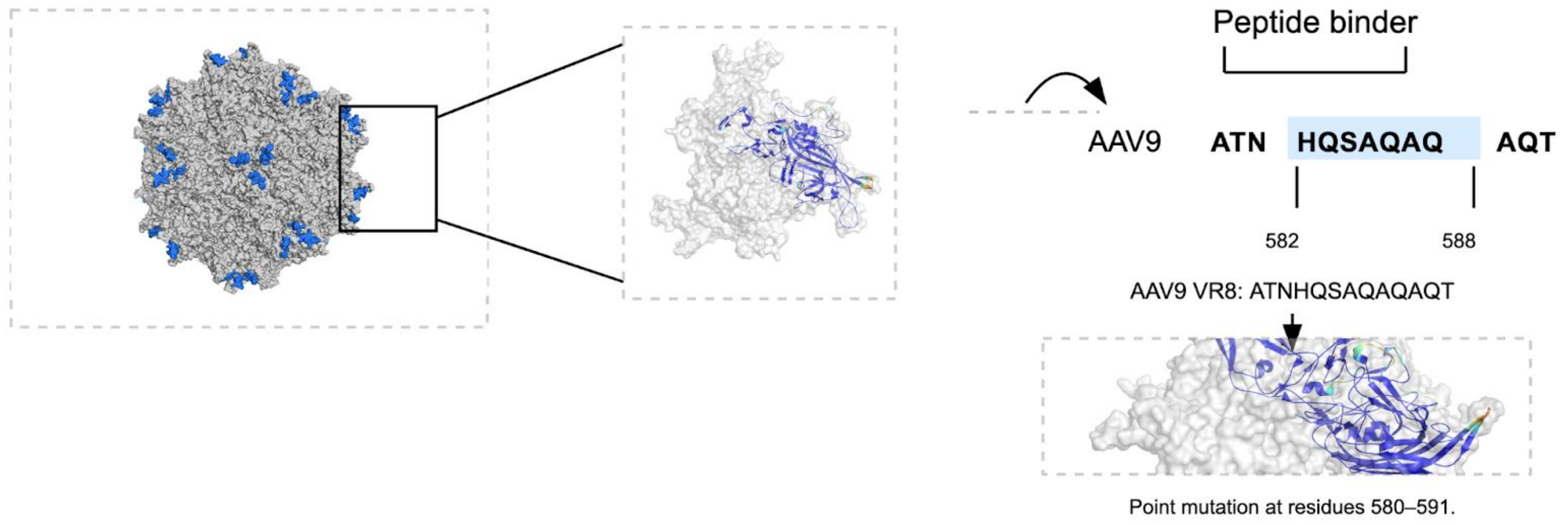




Engineer Variable Region of AAV to enable CNS specific endocytosis

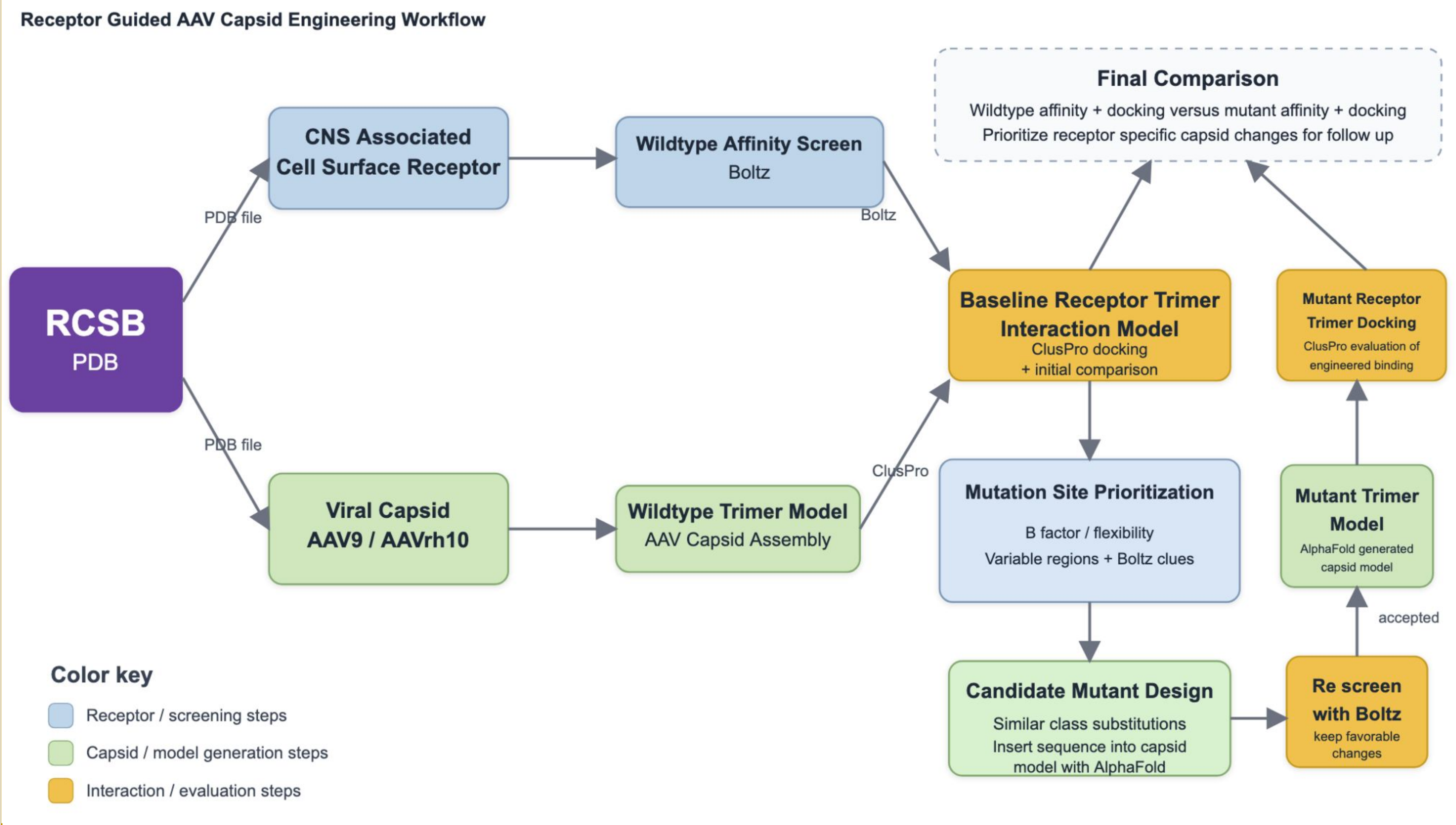
Workflow


Peptide list
Target protein
Input
Boltz-2
Binding affinity / probability
Ranking
Output
Mutant library
Residue class-preserving mutation


Peptide binder
AAV9 ATN **HQSAQAQ** AQT
582
588
AAV9 VR8: ATNHQSAQAQAQT
Point mutation at residues 580–591.

Build a proof of concept - ReNU Syndrome
End-to-end AAV caspid design pipeline

Methods


Receptor Guided AAV Capsid Engineering Workflow

RCSB
PDB

PDB file
CNS Associated Cell Surface Receptor
Wildtype Affinity Screen
Boltz

PDB file
Viral Capsid
AAV9 / AAVrh10
Wildtype Trimer Model
AAV Capsid Assembly

Boltz
ClusPro
Baseline Receptor Trimer Interaction Model
ClusPro docking
+ initial comparison

Mutation Site Prioritization
B factor / flexibility
Variable regions + Boltz clues

Candidate Mutant Design
Similar class substitutions
Insert sequence into capsid model with AlphaFold

Re screen with Boltz
keep favorable changes

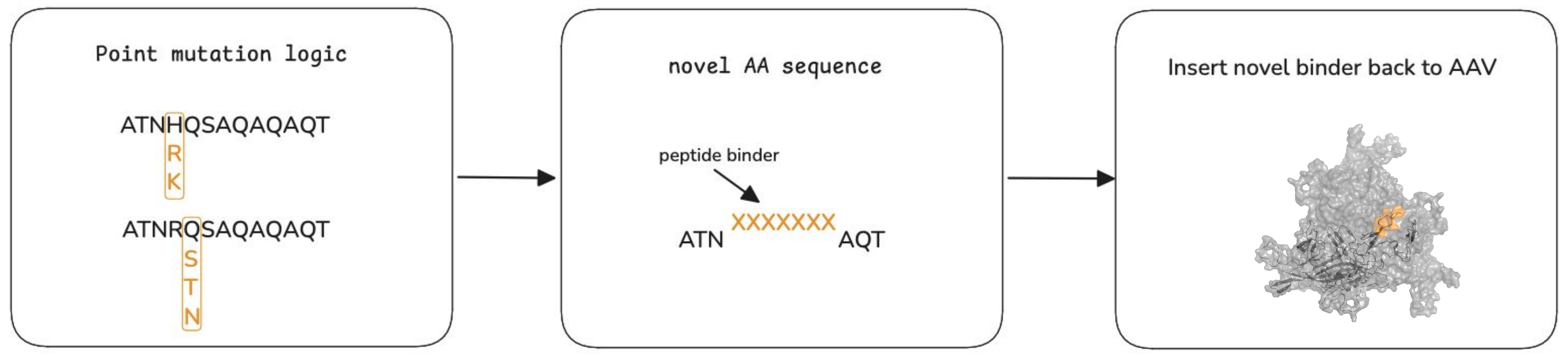
accepted

Mutant Trimer Model
AlphaFold generated capsid model

Mutant Receptor Trimer Docking
ClusPro evaluation of engineered binding

Final Comparison
Wildtype affinity + docking versus mutant affinity + docking
Prioritize receptor specific capsid changes for follow up

Color key
- 🟦 Receptor / screening steps
- 🟩 Capsid / model generation steps
- 🟨 Interaction / evaluation steps


Point mutation logic
ATNHQSAQAQAQT
R
K
ATNRQSAQAQAQT
S
T
N

novel AA sequence
peptide binder
ATN XXXXXXX AQT

Insert novel binder back to AAV

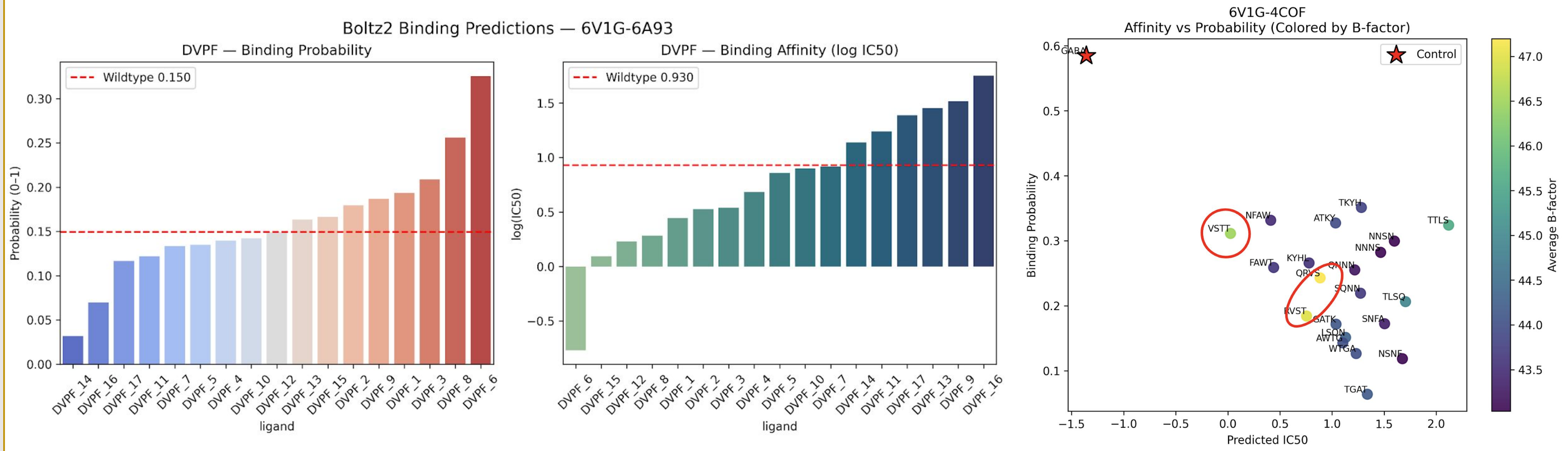
Capsid selection criteria

- High B-factor values - indicates structural flexibility
- Low predicted IC50 - reflects favorable binding affinity
- High binding probability - indicates a higher likelihood of receptor interaction

4-mer testing

- Chain A of AAV mutated based on conservative amino acid substitutions

AAV10

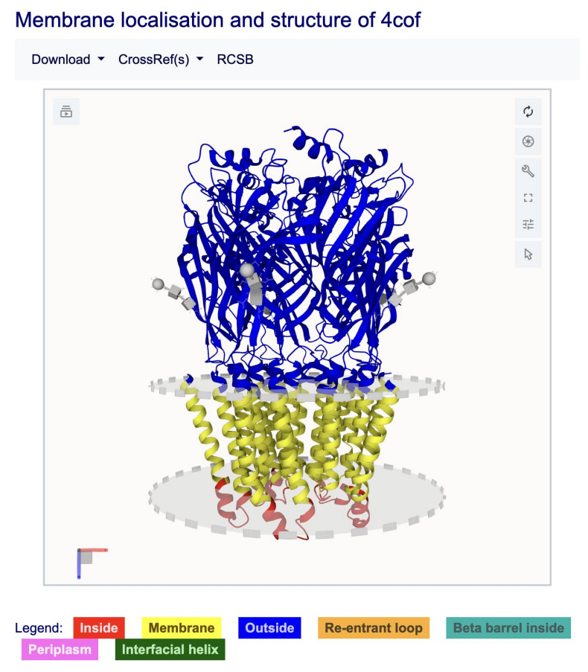


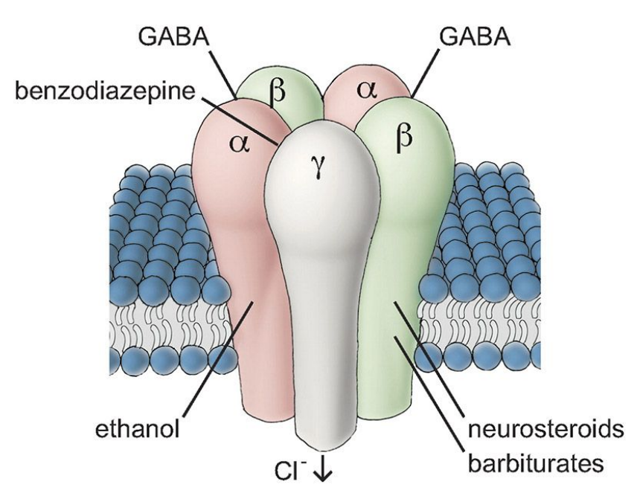
Results

Mutated Variants	Neuronal Receptors
- **DVPF3 (DYPF) and DVPF8 (DMPF)** - **Lower** predicted IC50 values + **higher** binding probabilities than WT across all neuronal receptors tested	- **1B8M and 4PE5** receptors showed the most favorable interaction patterns - Conducted one-tailed t-tests at the 0.05 significance level across all peptides - Statistically significantly LOWER affinity and HIGHER probability values compared to the WT 4-mers

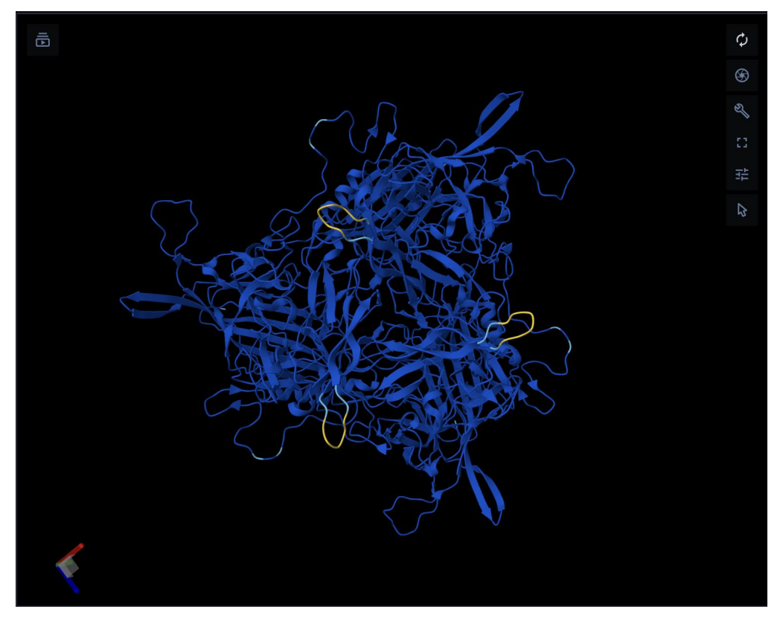
Structural Validation via Docking (AAV9)

- receptor ectodomain and AAV trimer as input from PDBTM database
- novel AAV variant: trimer representation from Alphafold


Membrane localisation and structure of 4cof

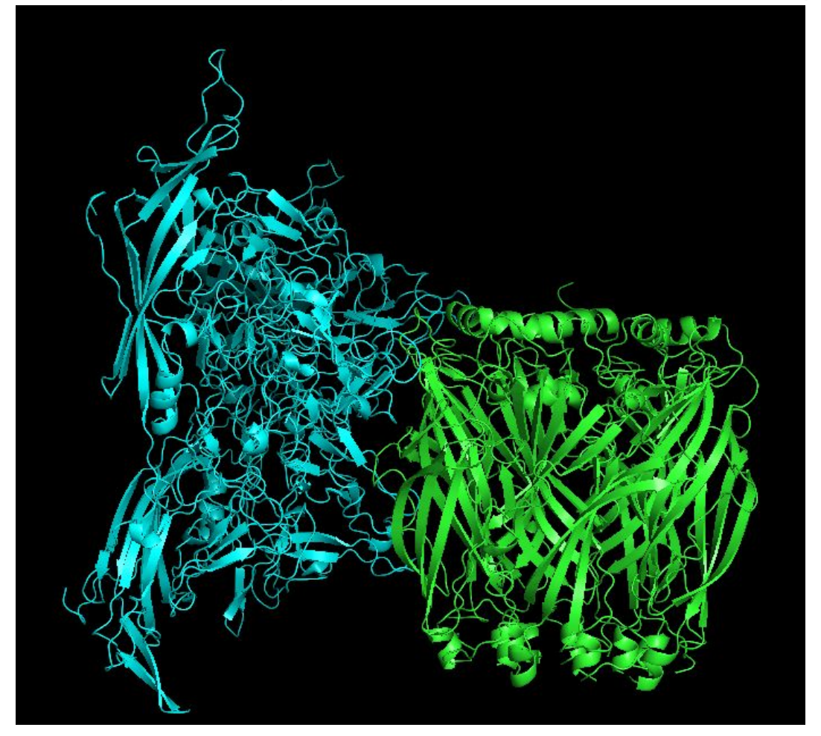


GABA receptor illustration

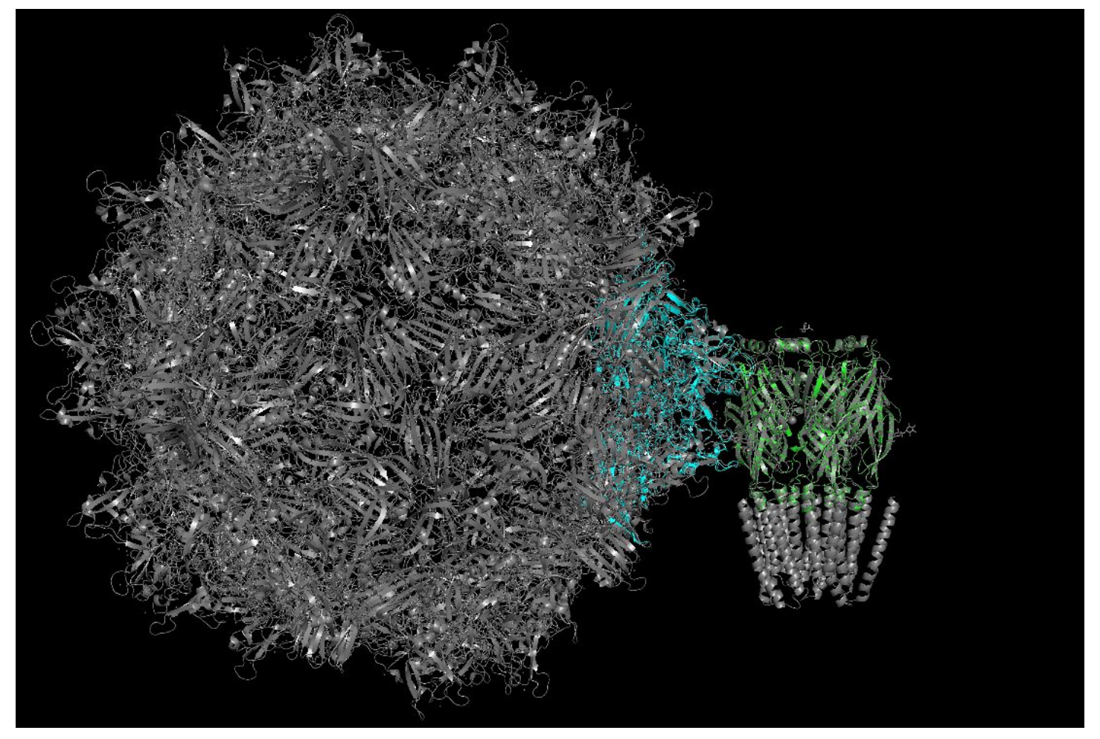


GABA receptor transmembrane region in blue

- Lowest-energy ClusPro cluster confirms VR-VIII loop docks into receptor binding cleft with optimal geometric complementarity



trimer with ectodomain docking result



docking result - aligned with capsid and receptor

Future Directions

- Immunogenesis: Engineer capsids to evade immune detection
- integrate our pipeline into Vircad GUI app

Acknowledgements & References

Salve Therapeutics

Thank you to Dr. Alyssa Taylor, Stefan N. Lukianov, Dr. Yu Ning Huang, and the rest of the senior design teaching team!