Case study 1. Prediction of Biological Activity

Neural Network Models for Prediction of Biological Activity using Molecular Dynamics Data: A Case of Photoswitchable Peptides

https://doi.org/10.1002/minf.70001
Molecular Informatics

Issue

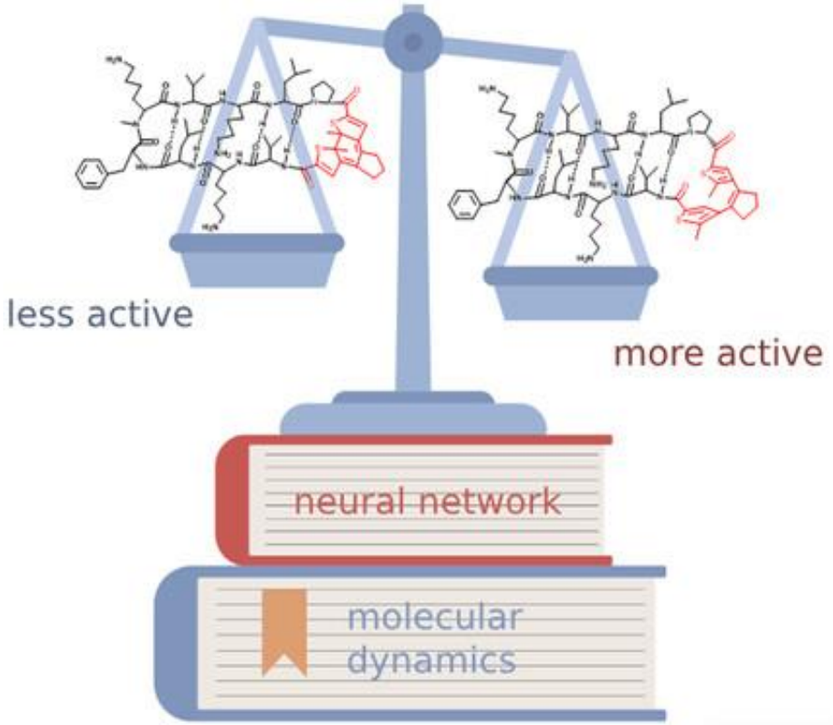
Conventional NN models based on static 2D/3D descriptors fail for large, flexible molecules.

This limitation is critical for photoswitchable macrocyclic peptides containing a diarylethene (DAE) photoswitch, where open and closed isomers exhibit distinct biological activities.

Methods

Molecular dynamics (MD) simulations were used to generate dynamic, 3D features for each peptide and its photoisomeric forms.

Two neural network models were developed:
- a Cytotoxicity Prediction Model (IC_{50} regression),
- an Isoform Similarity Model (binary classification of activity differences between isomers)



Result

MD-based features enabled the development of two NN models for complex photoswitchable peptides:
- Cytotoxicity Prediction Model accurately predicts IC_{50} values for gramicidin S analogs and distinguishes photoisomer activities.
- Isoform Similarity Model reliably identifies whether open and closed isomers differ significantly in activity, even beyond the training mechanisms.

Overall, incorporating molecular dynamics generalizes ligand-based NN activity prediction to large, flexible molecules not tractable by static descriptors.

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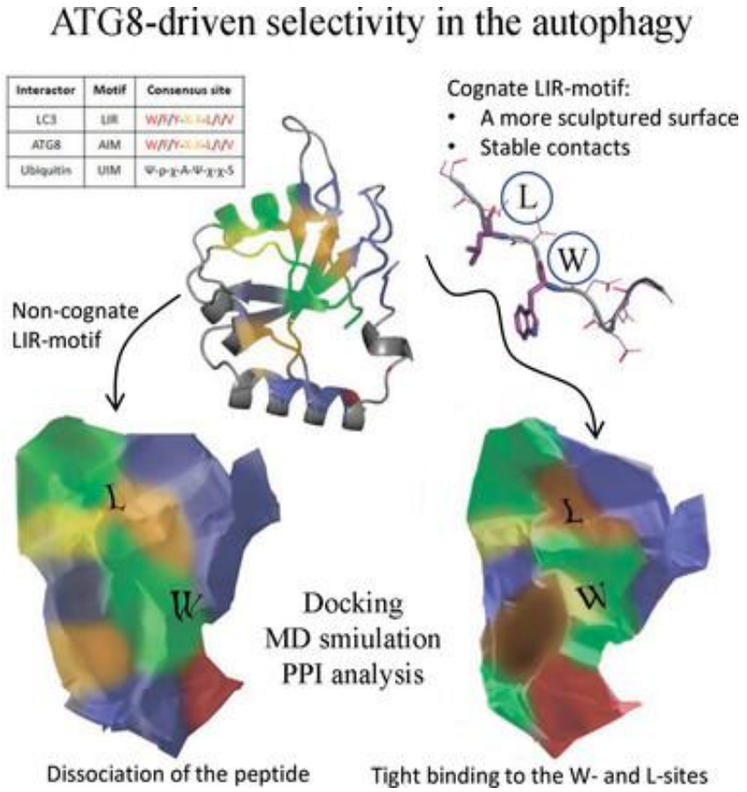
Case study 2. ATG8 Isoform Specificity and Structural Flexibility

Structural flexibility and shape similarity contribute to exclusive functions of certain ATG8 isoforms in the autophagy process

https://doi.org/10.21203/rs.3.rs-4831294/v1
Molecular Informatics

Issue

Although ATG8 proteins in plants share a highly conserved secondary structure and nearly identical protein–protein interaction interfaces, the molecular basis of isoform-specific selectivity remains unclear. In particular, the contribution of structural dynamics and binding-site mobility to the stage-dependent functions of ATG8 isoforms during autophagy is not well understood



Methods

- ATG8 isoforms from *Arabidopsis thaliana* were classified into three sequence-similarity groups using bioinformatic analysis. Structural models obtained from AlphaFold were subjected to molecular dynamics simulations (GROMACS, CHARMM36, explicit solvent).
- The dynamics of the LIR docking site (LDS) were analyzed using RMSD-based clustering to identify stable conformations and RMSF analysis to quantify isoform-dependent flexibility.
- Protein–peptide docking of ATG8–adaptor complexes was performed with HADDOCK using experimentally derived restraints.

Results

MD simulations revealed distinct conformational stability and mobility of the LDS among ATG8 isoform groups, despite high sequence similarity.
Docking results showed isoform-specific binding preferences and scoring differences for adaptor peptides.
Peptide binding induces local interface rearrangements, including hydrophobic pocket immersion and hydrogen bond formation, with binding strength correlating with overall protein flexibility rather than LDS sequence similarity alone.

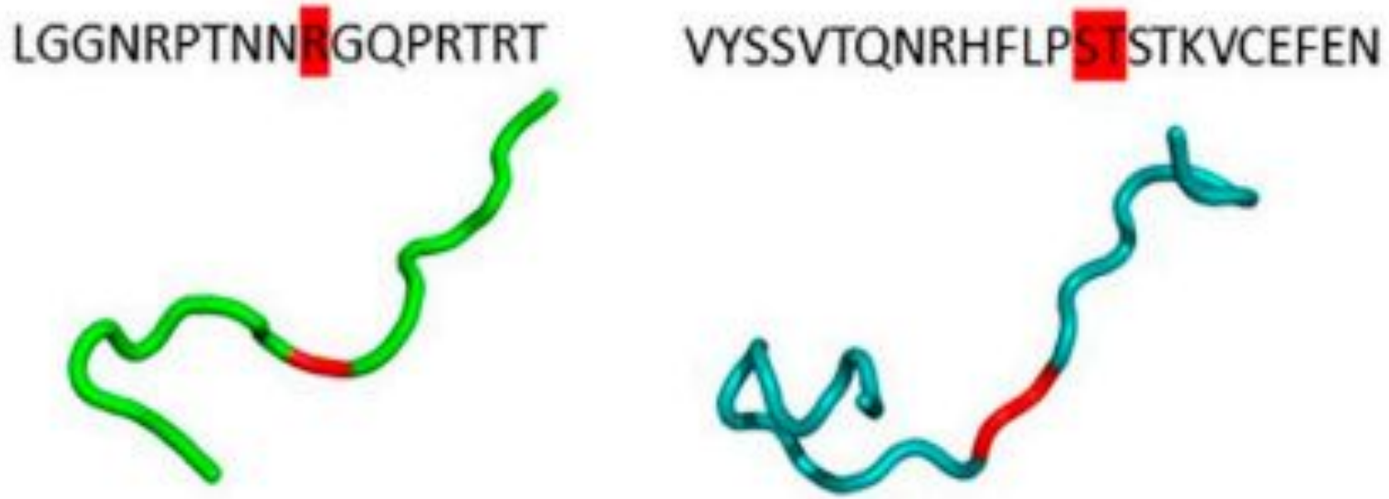
Case study 3. Novel Genetic Candidates in 46,XY Gonadal Dysgenesis

STARD9 and CDK5RAP2—Novel Candidate Genes for 46,XY Complete Gonadal Dysgenesis

https://doi.org/10.1002/jev2.12039
Int. J. Mol. Sci

Issue

The genetic basis of many cases of 46,XY gonadal dysgenesis remains unknown, suggesting the involvement of novel or oligogenic factors beyond known disease-associated genes.



Methods

Whole-exome sequencing of a 46,XY CGD patient, gene expression analysis of embryonic gonads, and in silico structural modelling and molecular dynamics simulations of STARD9 and CDK5RAP2 variants.

Results

Rare variants in STARD9 and CDK5RAP2 were identified; both genes are expressed in embryonic Sertoli cells, and molecular dynamics analyses suggest that the variants may impair protein structure and/or function, implicating these genes in 46,XY gonadal dysgenesis.

Case 4. Inhibitors for Mg^{2+}-ATPases

STRUCTURAL INSIGHT ON THE SELECTIVITY OF CALYX[4]ARENE-BASED INHIBITORS OF Mg^{2+}-DEPENDENT ATP-HYDROLASES

https://doi.org/10.1002/minf.202400200
Molecular Informatics

Issue

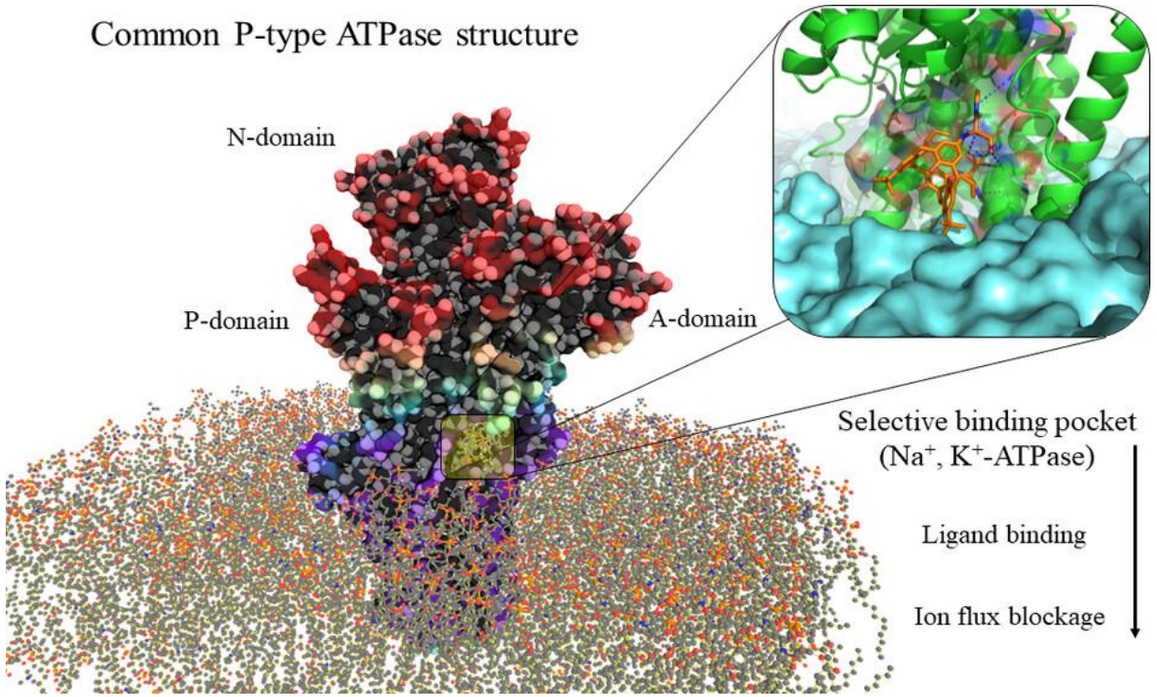
Selective modulation of Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase remains poorly understood, and effective inhibitors of Mg^{2+},ATP-dependent ion pumps are largely unknown. Understanding the structural basis of inhibition is required for rational drug design.

Methods

Homology models of Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase were constructed based on available crystal structures. Molecular dynamics simulations in a lipid bilayer, binding site identification, and molecular docking of calix[4]arene derivatives were performed to analyse ligand–protein interactions.

Result

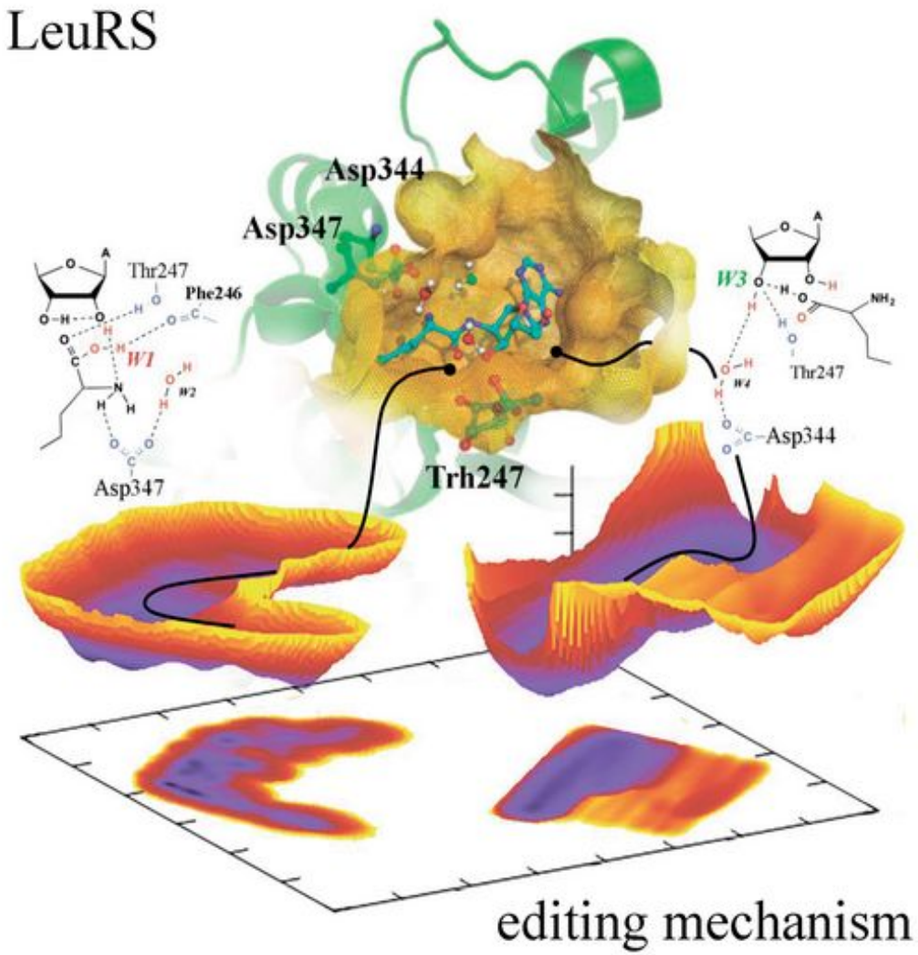
Calix[4]arenes C-97 and C-107 showed selective and strong inhibition of Na^+,K^+-ATPase ($I_{0.5}$ < 100 nM), while Ca^{2+},Mg^{2+}-ATPase activity was affected to a lesser extent. Docking and molecular dynamics simulations identified preferred binding sites and suggested a mechanism of selective enzyme inactivation.



Case 5. Alternative Hydrolysis Pathways

Alternative substrate-assisted hydrolysis pathways of posttransfer editing by prokaryotic leucyltRNA synthetase

https://doi.org/10.1111/febs.70153
The FEBS Journal



Issue

The molecular mechanisms of substrate-assisted hydrolysis in enzymatic reactions are not fully understood, particularly the existence of alternative reaction pathways beyond classical catalytic mechanisms.

Methods

Quantum-chemical calculations and computational modelling of reaction pathways were used to analyse substrate-assisted hydrolysis mechanisms and compare alternative catalytic routes.

Result

The study identified alternative substrate-assisted hydrolysis pathways that can operate alongside canonical mechanisms. These pathways differ in proton transfer and transition-state organisation, potentially contributing to enzymatic efficiency and specificity.

Case study 6. Lysine Acetylation Controls Plant Microtubule Dynamics

Lysine Acetylation of Plant α-Tubulins: Scaling Up the Local Effect to Large System Transformations

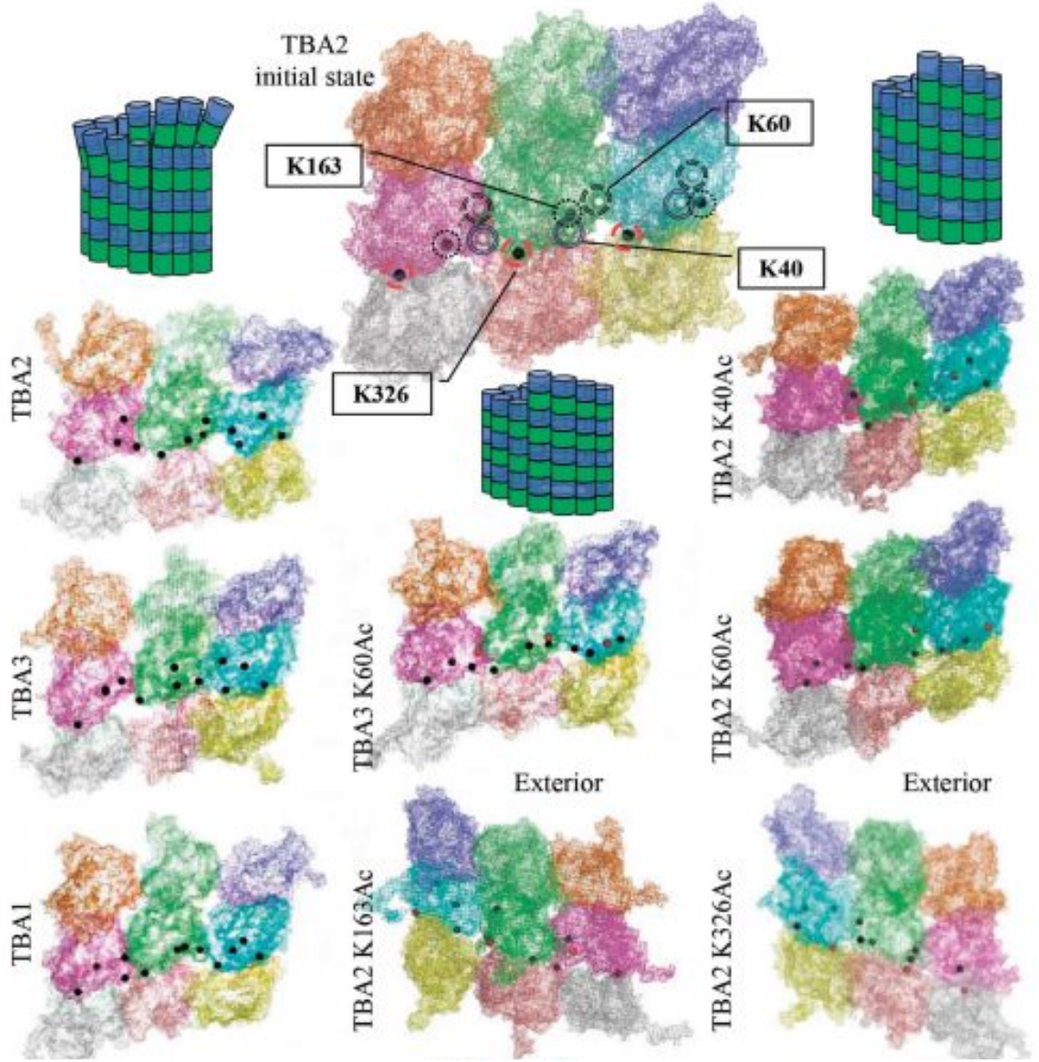
https://doi.org/10.1002/prot.26846
Proteins: Structure, Function, and Bioinformatics

Issue

The role of lysine acetylation in regulating plant α-tubulin structure, interactions, and microtubule stability is not fully understood, particularly how local acetylation events scale to large cytoskeletal rearrangements.

Methods

Comprehensive analysis of plant acetylomes, sequence conservation and phylogeny of α-tubulins, structural modelling, and molecular dynamics simulations of acetylated tubulin monomers, heterodimers, and microtubule lattice fragments.



Result

Multiple conserved and novel acetylation sites in plant α-tubulins were identified. Acetylation at K60, K163, and K326 differentially altered inter-subunit interactions, hydrogen bonding, and microtubule stability, indicating site-specific effects on microtubule assembly, function, and lifespan.

Case study 7. Identification of αTAT1 Modulators

Computational and experimental identification of putative αTAT1 modulators: implications for nervous system function

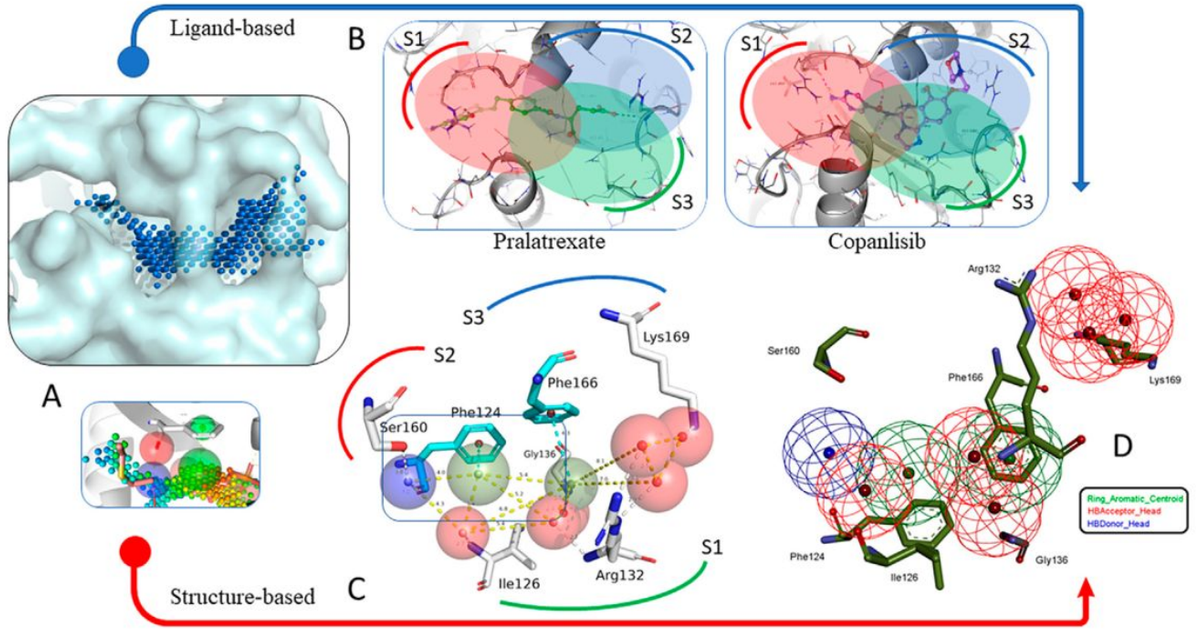
https://doi.org/10.3389/fphar.2025.1654114
Frontiers in Pharmacology

Issue

αTAT1 is the only mammalian enzyme responsible for microtubule acetylation and plays a key role in cytoskeletal stability and nervous system function. Despite its biological importance, no well-characterized small-molecule modulators of αTAT1 are available.

Methods

A ligand-dependent pharmacophore model was designed using available αTAT1 crystal structures and molecular interaction analysis. Virtual screening was performed to identify compounds matching the pharmacophore hypothesis. The resulting hits were further refined, and selected compounds were experimentally evaluated using confocal microscopy to assess cytoskeletal changes in neural stem cells.



Result

Screening identified a set of putative αTAT1 modulators consistent with the proposed pharmacophore model. Experimental validation by confocal microscopy confirmed the biological activity of selected hits. These results enabled refinement of the computational screening models and supported the generation of new focused compound subsets for further optimization.

Case study 8. PPI library: PDZ-domains

Collaboration with Institute of Molecular Pharmacology Berlin, Germany
Centre de Recherche en Cancerologie de Marseille (CRCM), Aix-Marseille Universite, Inserm, CNRS, Institut Paoli-Calmettes, Marseille, France
Department of Human Genetics, K. U. Leuven, Leuven, Belgium

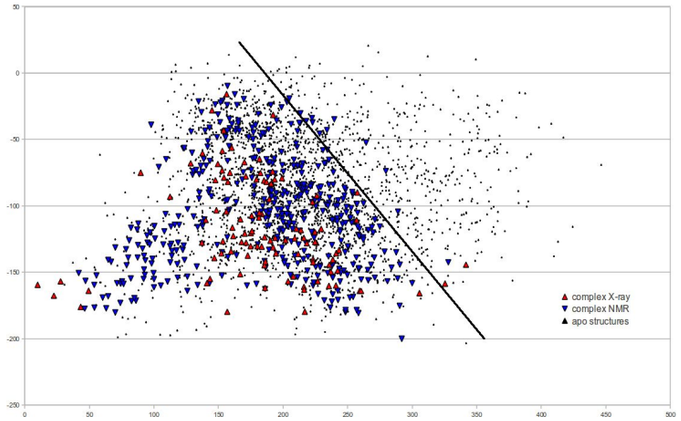
Issue

Lack of PPI library: PDZ-domains

Methods

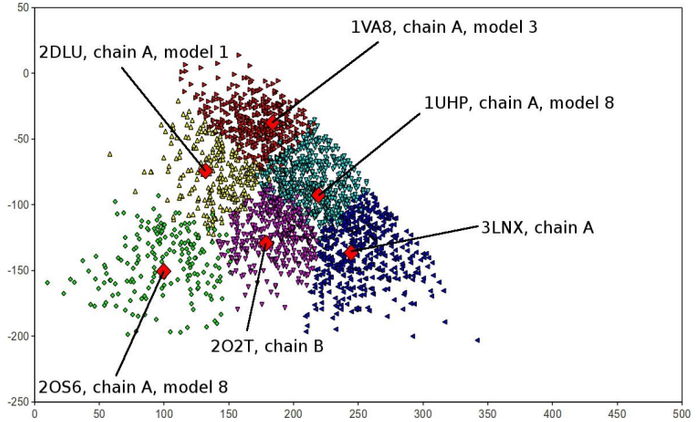
2,800 conformations from 163 PDZ domains

Space of shape descriptors: undruggable conformations



Space of shape descriptors: Alignment and analysis of protein binding sites Clustering

6 conformations selected for virtual screening



Virtual screening (Enamine's 1.8M cmpds) Processing

Results

Library of 1,000 cmpds validated activity

Method of shape-based library design for a class of PPI domains was further used for other targets because of its effectiveness and high success rate

Case study 9. Inhibition of syntenin PDZ2

Pharmacological inhibition of syntenin PDZ2 domain impairs breast cancer cell activities and exosome loading with syndecan and EpCAM cargo (supervised hit optimization)

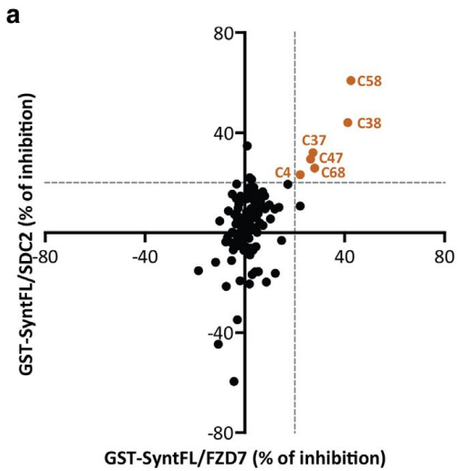
https://doi.org/10.1002/jev2.12039
Journal of Extracellular Vesicles

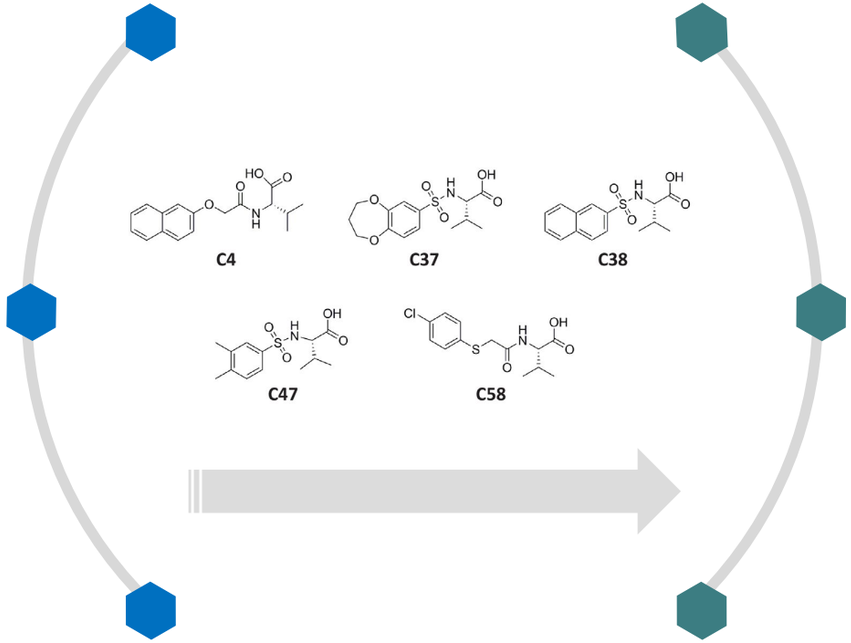
Issue

To develop a novel syntenin inhibitor

Input

As a source of screening compounds, we used the PDZ targeted library (previous slide) developed at Enamine to cover all PDZ domains with published structures

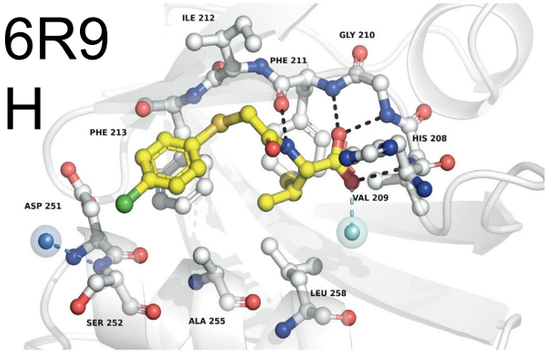




Methods and results

After docking of the PDZ library – 139 compounds were selected and Five structurally related compounds were obtained as hits after biology validation

X-ray crystal structure of a complex syntenin-PDZ2 domain with one hit (C58) was resolved (PDB code 6R9H)



Case study 9 continued. Inhibition of syntenin PDZ2

Pharmacological inhibition of syntenin PDZ2 domain impairs breast cancer cell activities and exosome loading with syndecan and EpCAM cargo (supervised hit optimization)

https://doi.org/10.1002/jev2.12039
Journal of Extracellular Vesicles

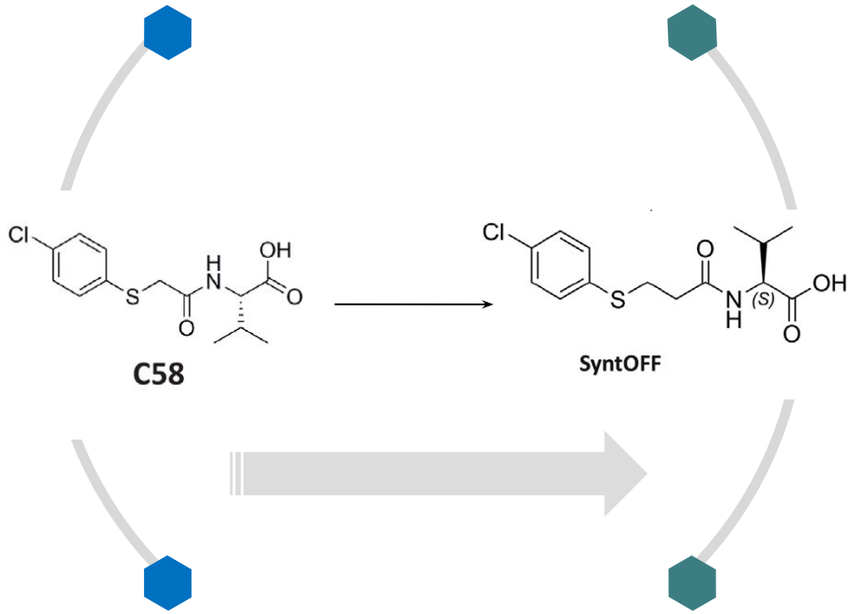
Issue

To develop a novel syntenin inhibitor

Methods

Molecular modelling studies, relying on the X-ray structure of C58 and MOE/Pymol tools, suggested several changes to increase activity :

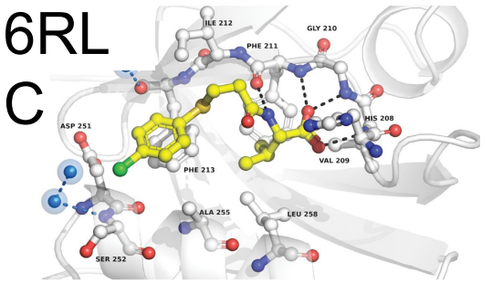
- addition of one carbon spacer in the compound structure could maintain both the canonical binding mode
- the face-to-face π-stacking with Phe213, while slightly increasing the distance between the hydrophobic thioether-chlorophenyl moiety and the polar protein backbone
- better torsion angles, closer to optimal geometry (60◦), with favourable staggered conformation on the flexible carbohydrate moiety



Results

An optimization strategy, based on crystallographic data, enabled the development of SyntOFF where the introduction of an additional methylene group improved the affinity 10-fold compared to C58 (IC50 = 37 μM)

The X-ray crystal structure of compound SyntOFF in complex with syntenin was also resolved (PDB code 6RLC) indicating



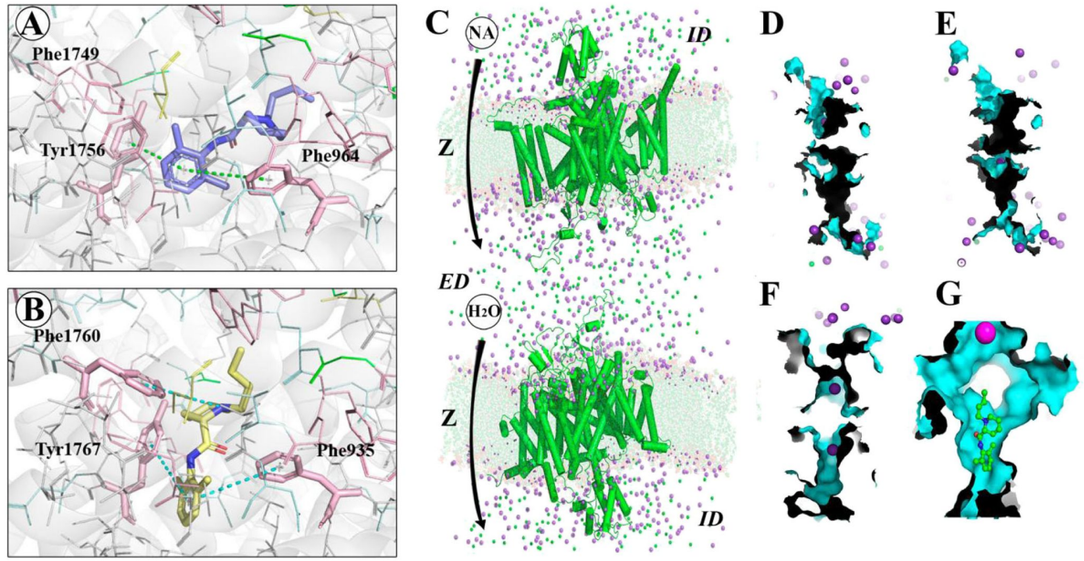
Case 10. The mechanism of the ion channel

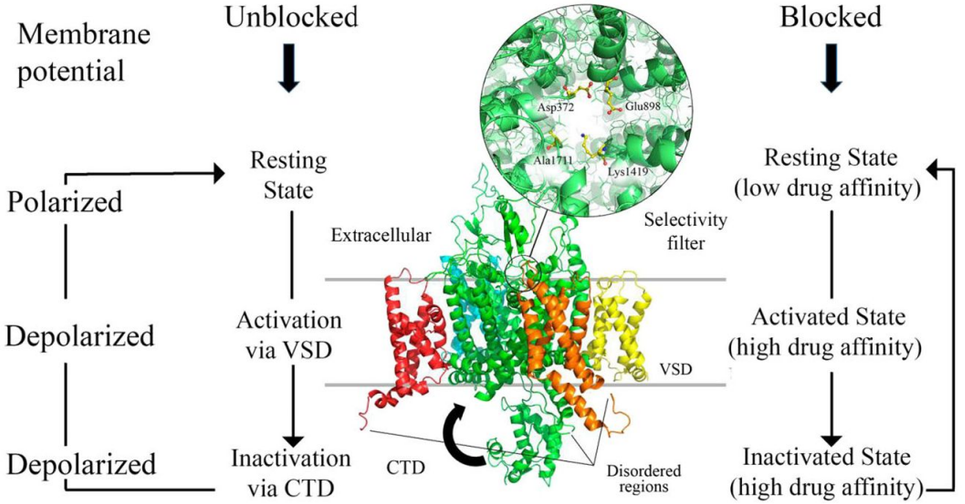
Study of the effect of inhibitors on the mechanism of the ion channel

doi.org/10.1007/s00894-021-04799-w
Journal of Molecular Modeling

Methods

To prevent a collapse of the system, a trans-membrane protein was embedded in a lipid bilayer. A double membrane system was generated, implying a free of ligand Nav1.5 protein and on the opposite side its copy containing a docked bupivacaine molecule inside the pore channel





Result

Developing the CompEI swapping protocol to allow understanding molecular binding details and subsequent structural rearrangements, that could give a chance to increase the potency of novel compounds against Nav1.7and avoid the influence on the Nav1.5

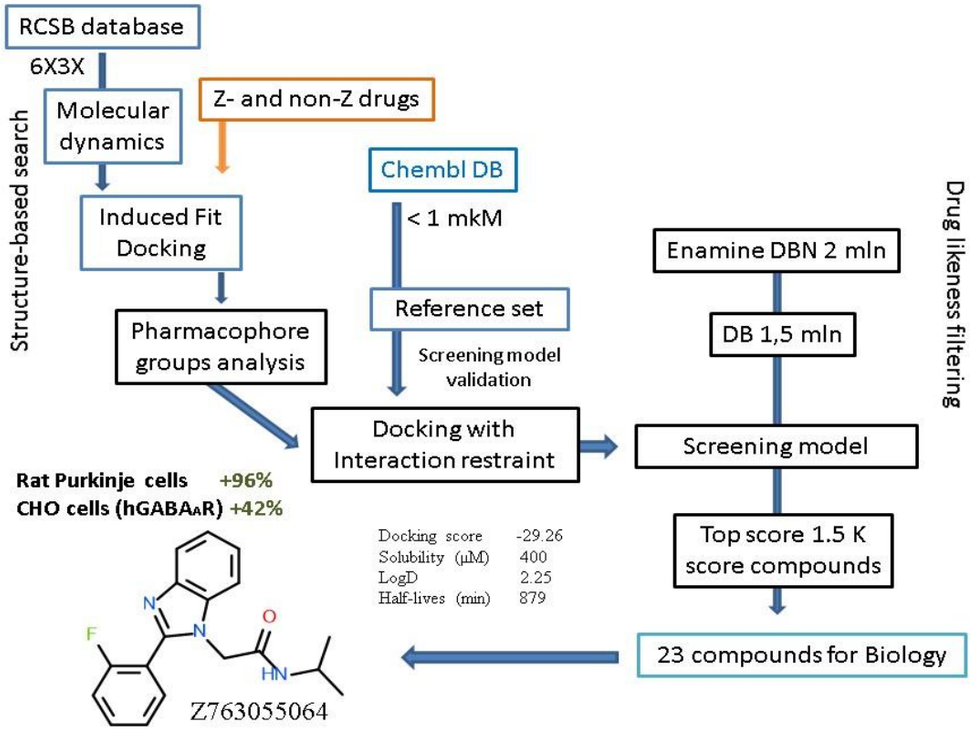
Case 11. Identification of new GABA modulators

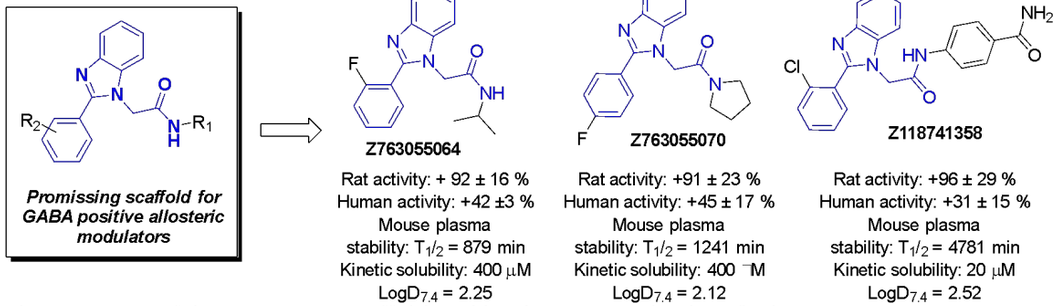
Integrated workflow for the identification of new GABA positive allosteric modulators based on the *in silico* screening with further in vitro validation

https://doi.org/10.1002/minf.202300156
Journal of Molecular Modeling

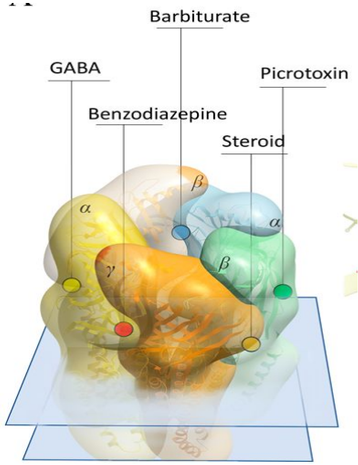
Methods

Our study represents a convenient and tunable model for the discovery of novel positive allosteric modulators of GABA receptors.





The structure of the promising scaffold and lead compounds identified.



Result

A High-throughput virtual screening of the largest available database of chemical compounds resulted in the selection of 23 compounds. Further electrophysiological tests allowed us to determine a set of 3 the most outstanding active compounds. Considering the structural features of leader compounds, the study can develop into the MedChem project soon.

Case 12. Novel Chemotype for ASIC1a Inhibitors

4-(Azolyl)-Benzamidines as a Novel Chemotype for ASIC1a Inhibitors

https://doi.org/10.3390/ijms25073584
IJMS

The process of channel inactivation was analyzed using MD simulation protocol of different functional states of ASIC1 . Then we applied a molecular docking procedure to predict the protein conformation suitable for the amiloride binding. To confirm the effect of its sole active blocker against the ASIC1 state transition route we studied the complex with another MD simulation run. Further experiments evaluated various compounds in the Enamine library that emerge with a detectable ASIC inhibitory activity. An artificial activation (otherwise, expansion of the central pore) causes a complex modification of the channel structure, namely its transmembrane domain. The output protein conformations were used as a set of docking models, suitable for a high-throughput virtual screening of the Enamine chemical library. The outcome of the virtual screening was confirmed by electrophysiological assays with the best results shown for three hit compounds.

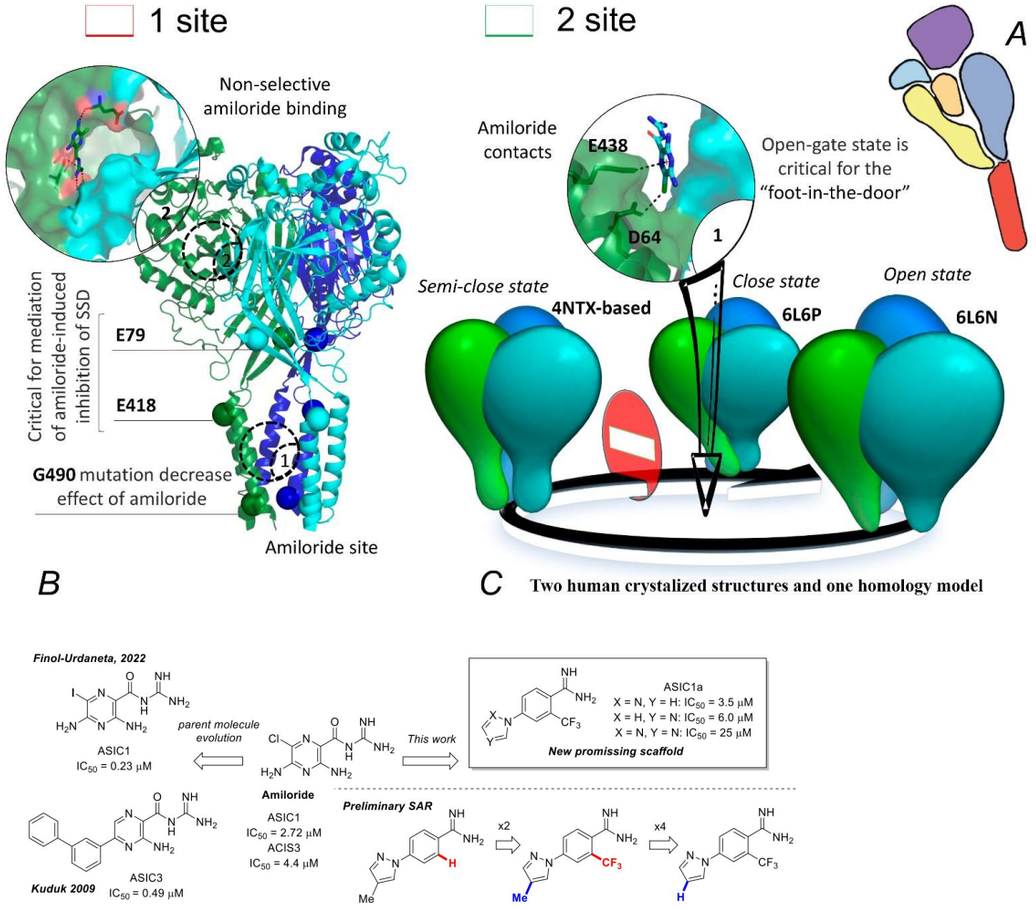


Figure 6. Finalized outcome of the study represented in the form of a chemical flow charts: initial ancestor and its evolution based on selected papers and our findings [51,74].

Case 12. Novel Chemotype for ASIC1a Inhibitors

4-(Azolyl)-Benzamidines as a Novel Chemotype for ASIC1a Inhibitors

https://doi.org/10.3390/ijms25073584
IJMS