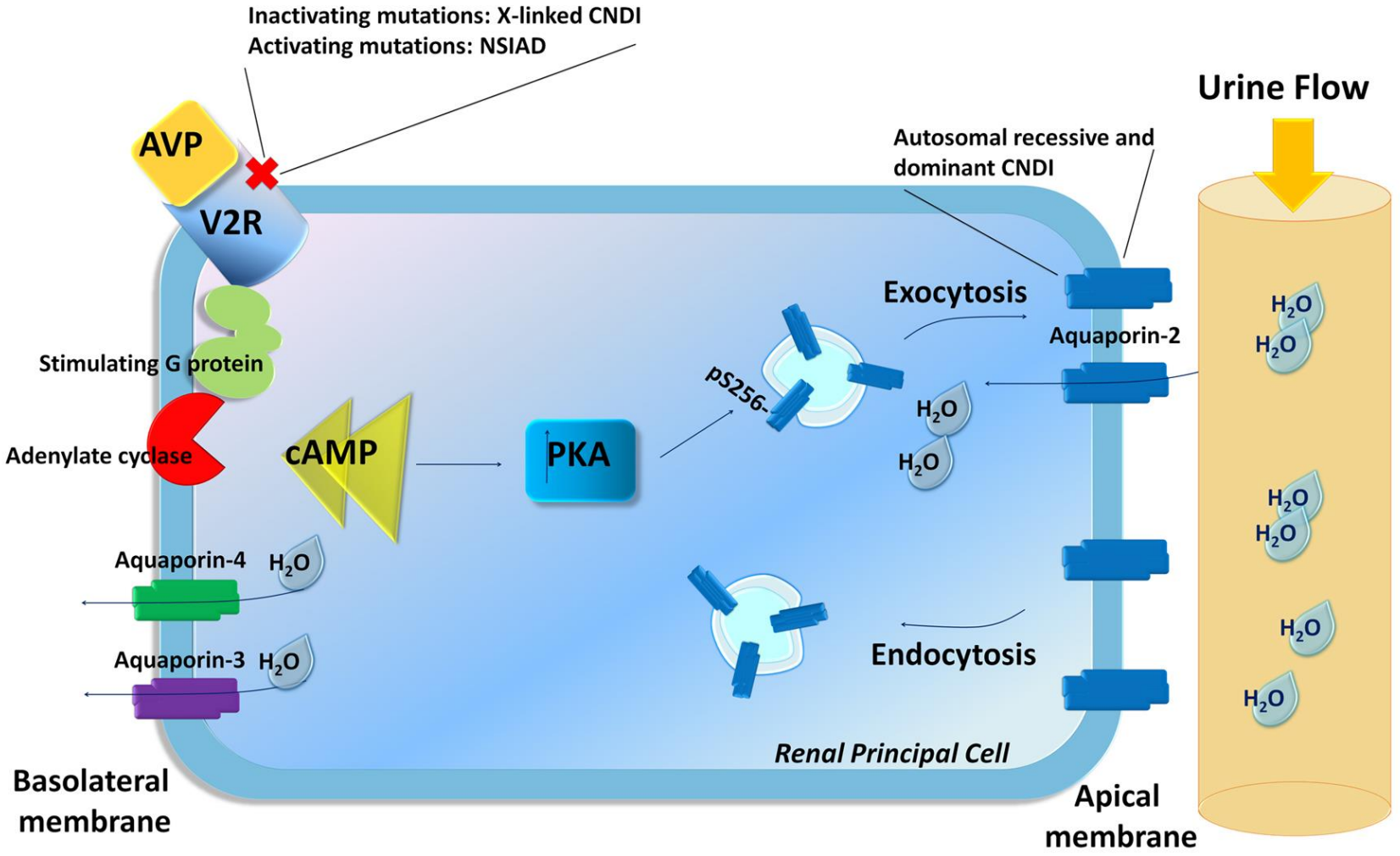
EPTRI General Assembly and Scientific Meeting 2024

AI-assisted drug repurposing studies for the identification of new promising vasopressin V2 receptor ligands for pharmacotherapy in pediatric nephrology

Grazia Tamma

Department of Biosciences, Biotechnologies and Environment
University of Bari Aldo Moro

Vasopressin activates renal water reabsorption acting on renal principal cells



Valenti &Tamma GIN 2016

Diseases linked to abnormal V2R pathways

Nephrogenic diabetes insipidus (NDI)

Etiology	Hereditary lack-of-funtion V2R gene mutation Electrolytes abnormalities (hypercalcemia, hypokalemia)
Clinical features	Polyuria- Water loss - Nocturia Polydipsia (intact thirst mechanism)

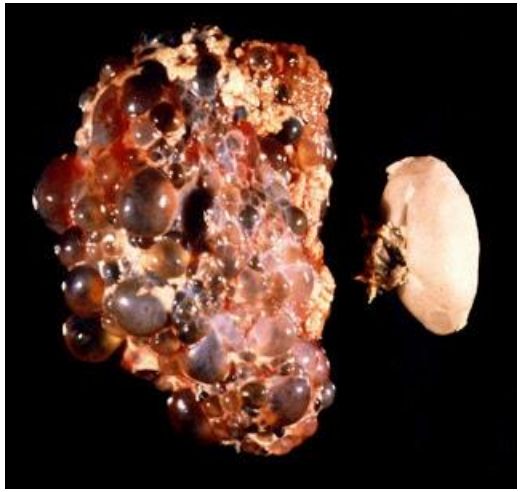
Nephrogenic Syndrome of inappropriate antidiuretic hormone secretion (NSIADH)

Etiology	Hereditary activating V2R gene mutation Malignancies Pulmonary or neurological disorders
Clinical features	Hyponatremia Oliguria – Water retention - Edema Weight gain Anorexia - Nausea - Vomiting Muscle weakness Seizures, Lethargy

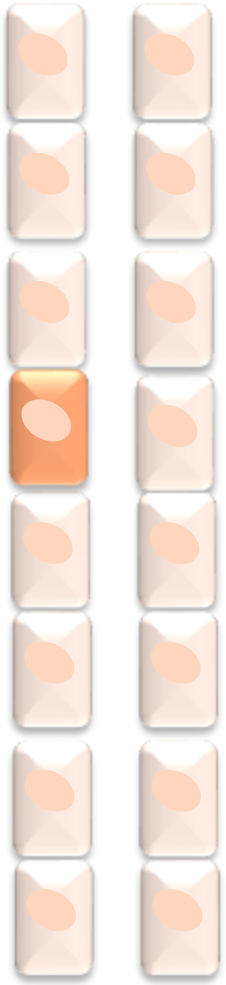
Diseases linked to abnormal V2R pathways

Polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD)	*Autosomal recessive polycystic kidney disease (ARPKD)*
Incidence: 1:400 – 1.1000	1.10000 – 1: 40000
Involved genes: PKD1 (on chromosome 16); PKD2 (on chromosome 4)	PKHD1 (on chromosome 6), *DZIP1L (on chromosome 3)*
Protein defects: Polycistin-1; Polycistin-2	Fibrocystin, DAZ Interacting Zinc Finger Protein 1 Like
Histological appearance: cystic dilation of all parts of the involved nephron	Renal and liver cystic disease
Age at symptoms onset: Middle-aged adulthood	Infancy
Clinical features: Renal failure	In utero demise Severe Oligohydramnios, Neonatal respiratory distress Arterial hypertension Renal failure Liver failure Neuro abnormalities

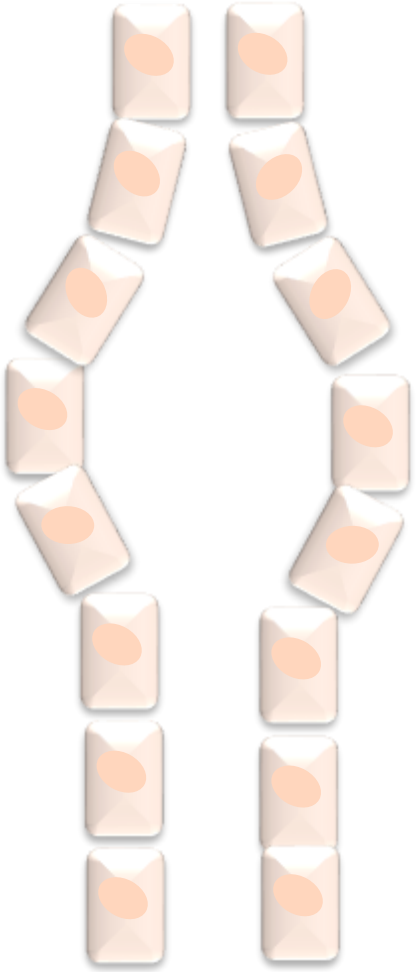


Process of cyst formation

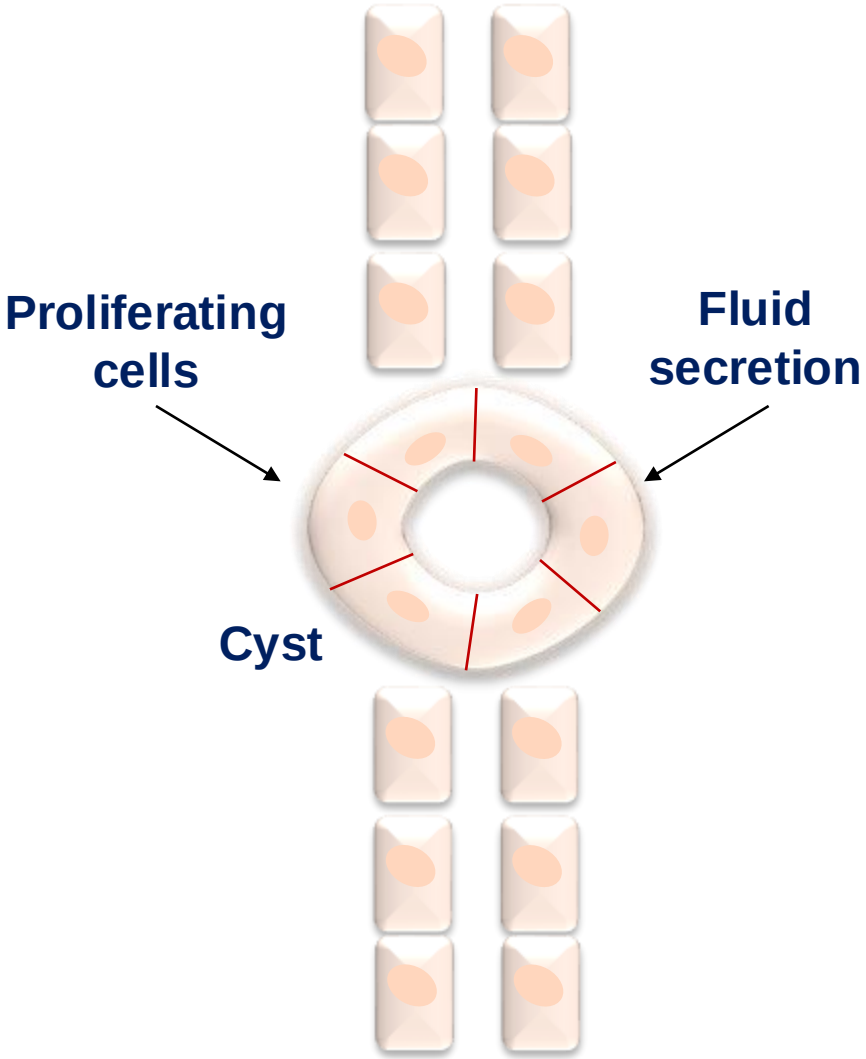


Normal Tubule

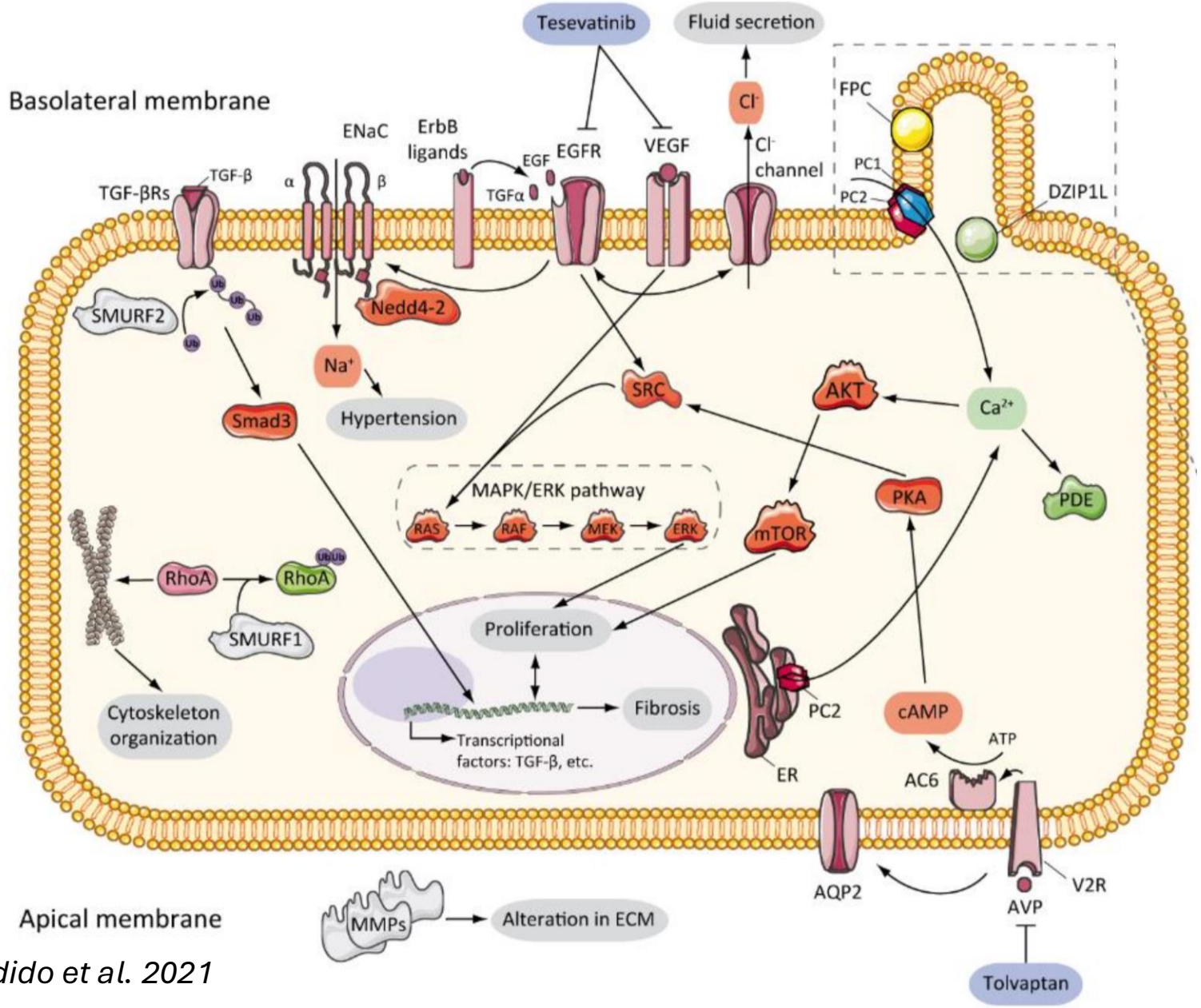
Abnormal cell proliferation



DilatingTubule



PKD molecular pathogenesis



Cordido et al. 2021

ARPKD and ADPKD share many similarities:

- ✓ Altered intracellular Ca^{2+}
- ✓ Increased cyclic adenosine monophosphate (cAMP) concentrations

The vasopressin V2 receptor (V2R) antagonist tolvaptan lowers cAMP in cystic tissues and slows renal cystic progression.

Tolvaptan is currently approved for the treatment of rapidly progressive disease in adult ADPKD patients.

Review › Pediatr Nephrol. 2018 Mar;33(3):395-408. doi: 10.1007/s00467-017-3672-x. Epub 2017 Apr 28.

Expanding the role of vasopressin antagonism in polycystic kidney diseases: From adults to children?

Janssens [1 2], Caroline Weydert [3], Stephanie De Rechter [3 4], Karl Martin Wissing [5], Christoph Liebau [6 7 8], Djalila Mekahli [3 4]

Review › Curr Opin Nephrol Hypertens. 2013 Jul;22(4):459-70. doi: 10.1097/MNH.0b013e3283621510.

Osmoregulation, vasopressin, and cAMP signaling in autosomal dominant polycystic kidney disease

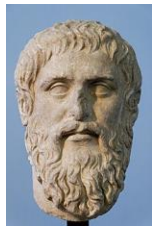
Olivier Devuyst [1], Vicente E Torres

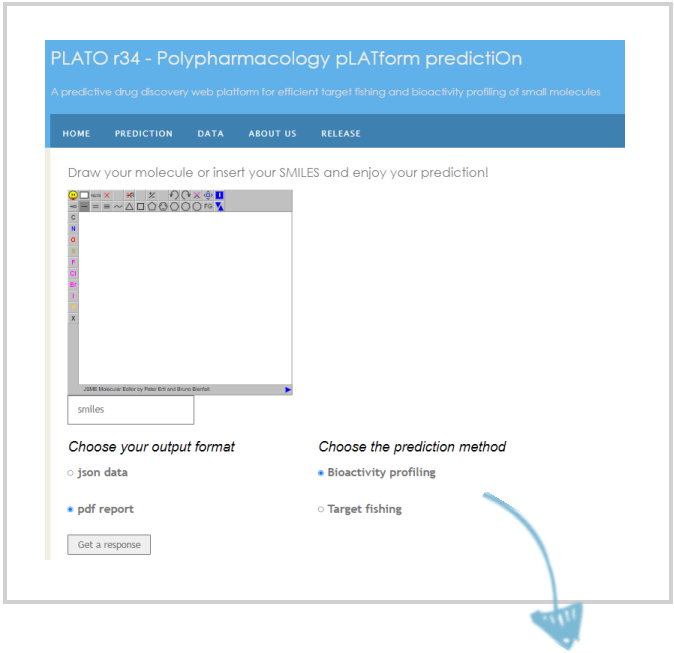
Aim of work

Identification of novel V2R ligands that could modulate various receptor-mediated effects through the application of an AI-based drug repurposing approach.

In silico reverse screening with PLATO platform

- PLATO is a user-friendly web platform free for target fishing and bioactivity prediction by using a similarity approach
- Reverse screening is capable of predicting the bioactivity of molecules towards a biological target (V2R) in order to repurpose well-known drugs
- A total number of five promising drugs as potential V2R modulators (DRUG 1 DRUG 2, DRUG 3, DRUG 4 and F2544) has been collected as potential modulators of V2R to be validated by molecular docking simulations.





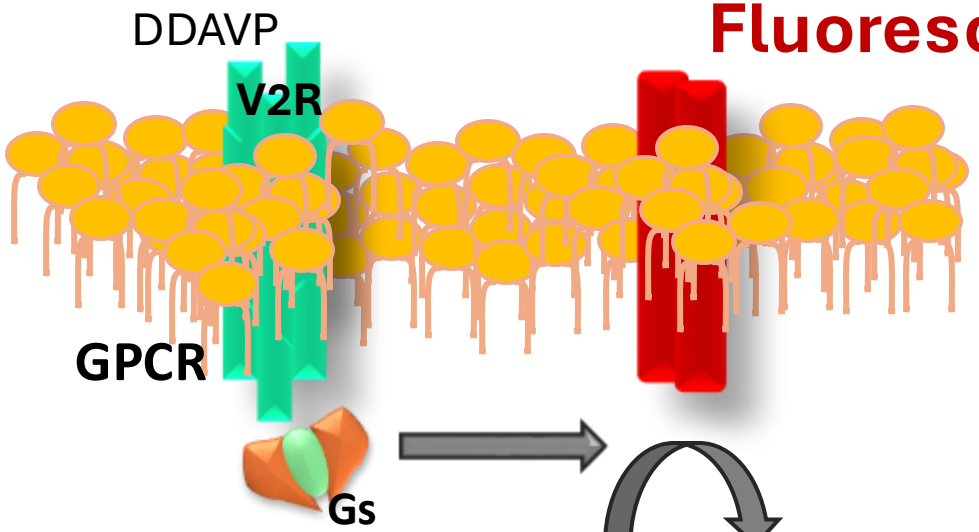
SCAN ME

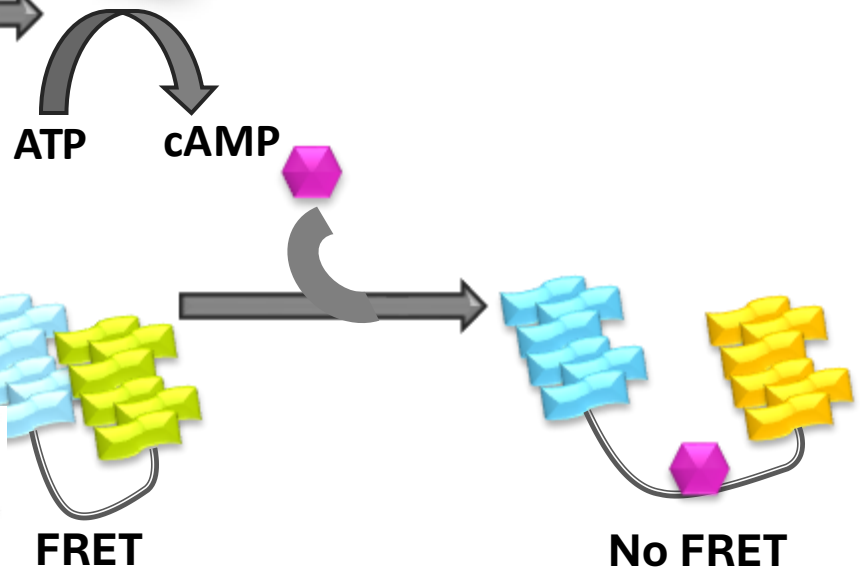
https://prometheus.farmacia.uniba.it/plato/

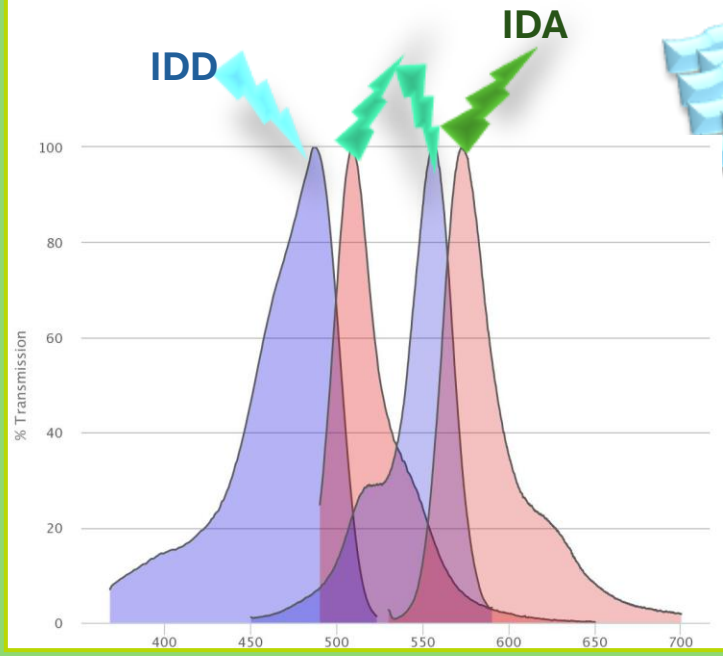
Plato bioactivity values (pred IC_{50})	
DRUG 1	33,58 nM
DRUG 2	34,17 nM
DRUG 3	33,46 nM
DRUG 4	33,90 nM
F2544	29,20 nM

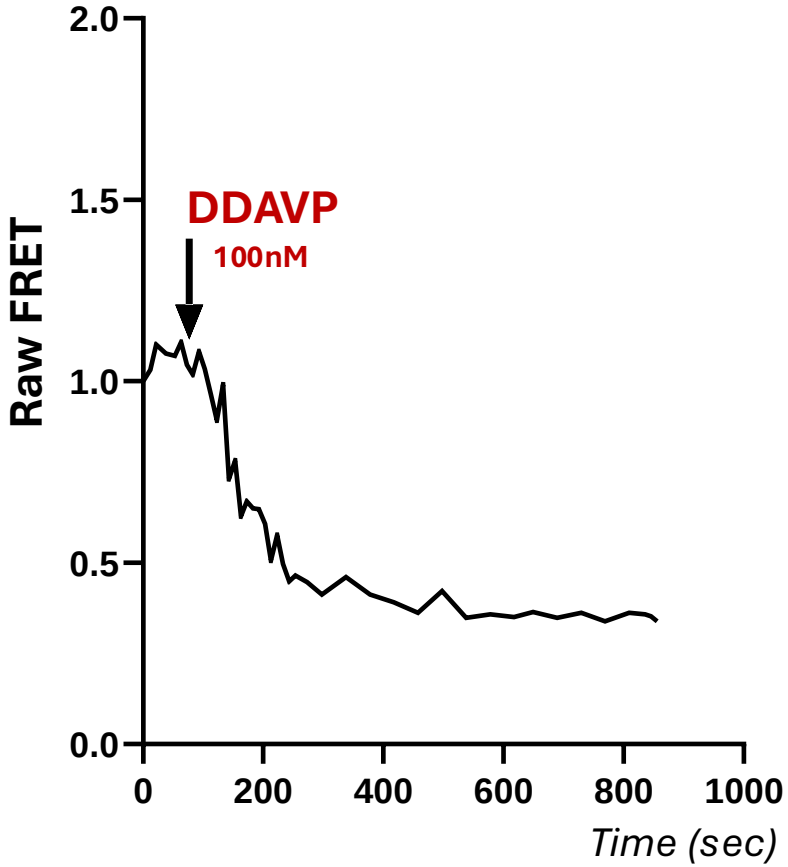
Ciriaco, F; Gambacorta, N.; Trisciuzzi, D.; Nicolotti O. PLATO: A Predictive Drug Discovery Web Platform for Efficient Target Fishing and Bioactivity Profiling of Small Molecules. *Int J Mol Sci*. **2022**,8, 5245.

Fluorescence Resonance Energy Transfer (FRET)

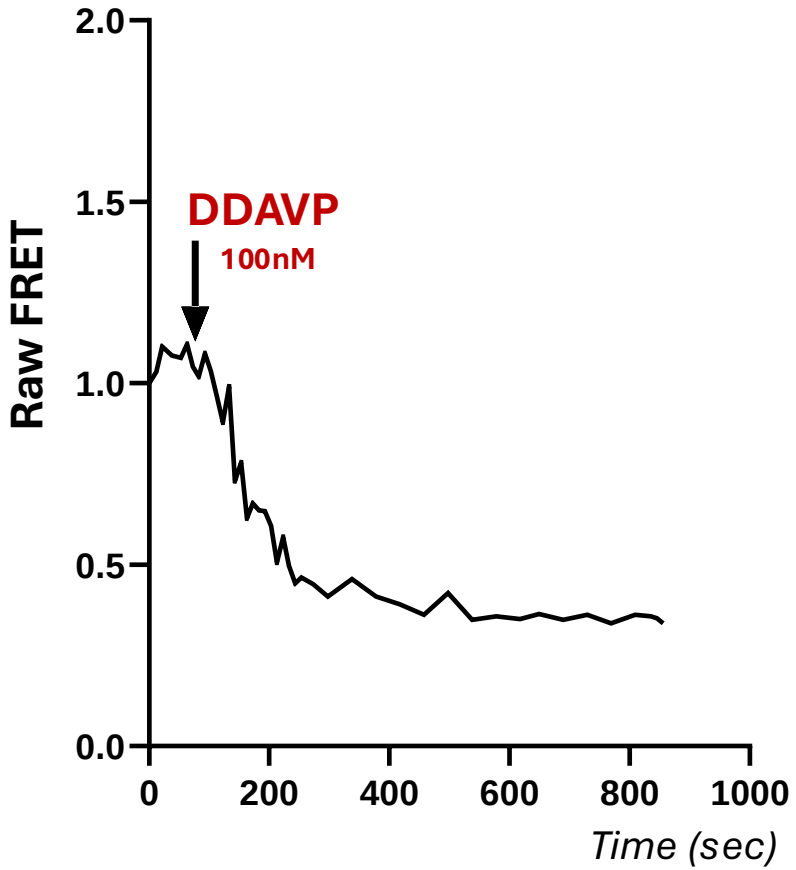






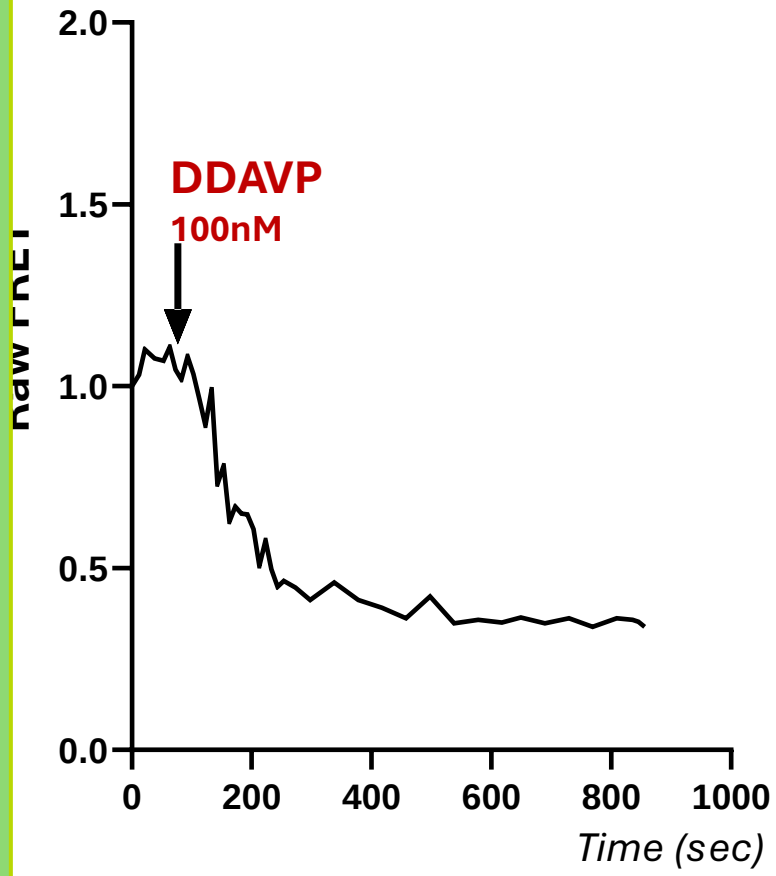


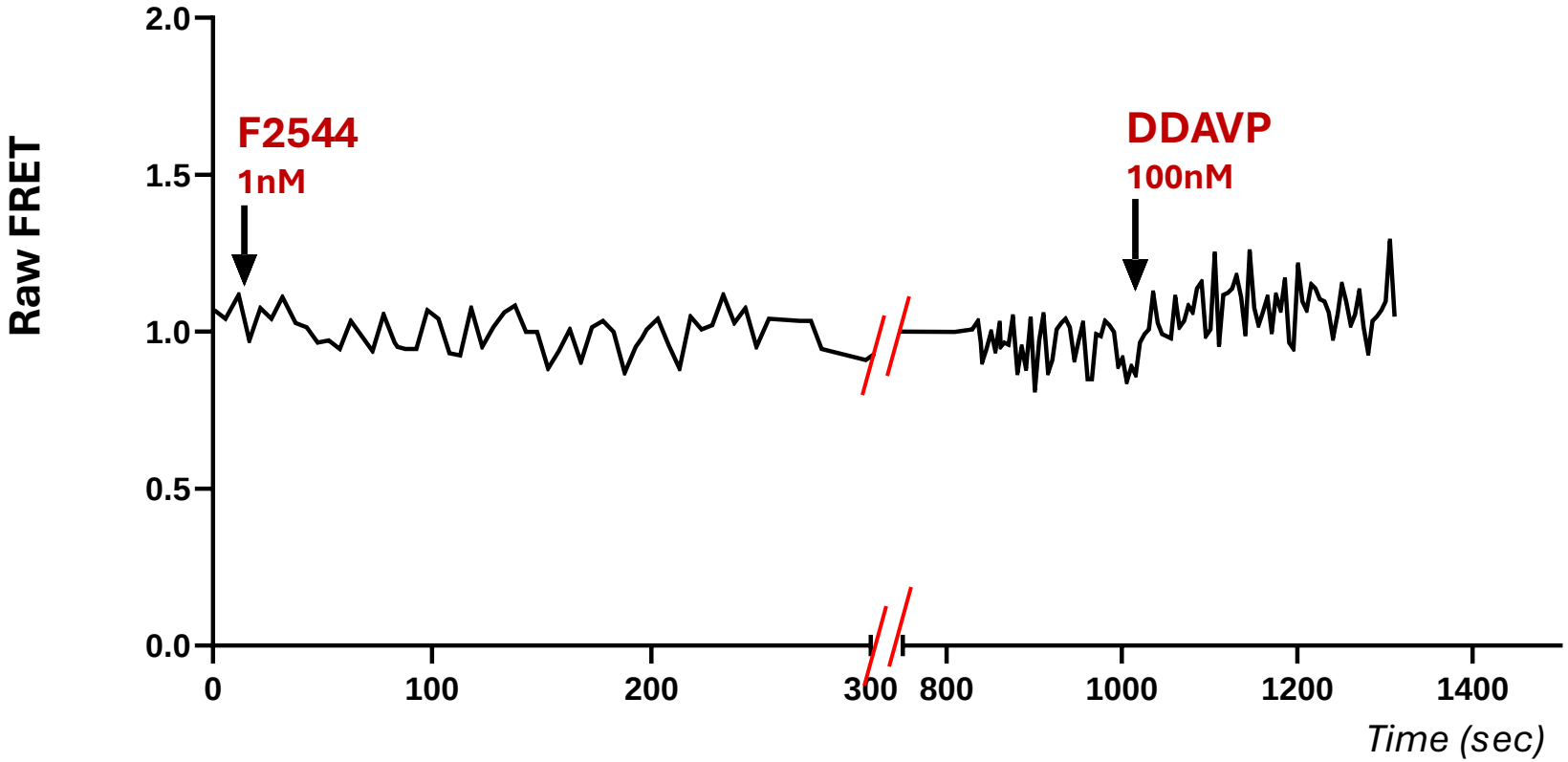
cAMP changes measured by FRET studies



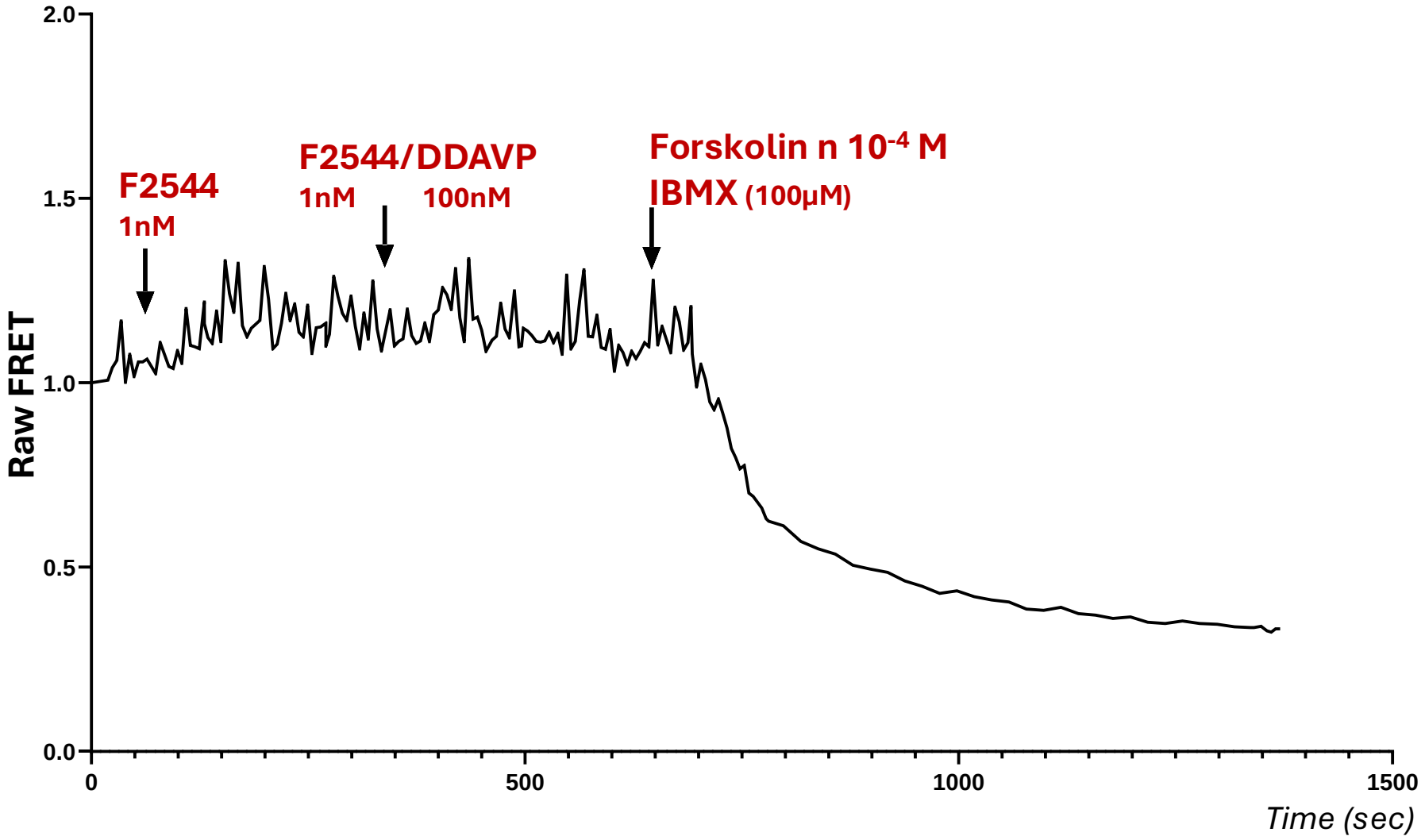
FRET measurements	basal	DDAVP
DDAVP	1,00	0,66 ± 0,03
Drug 1	0,9 ± 0,02	0,54 ± 0,03
Drug 2	0,9 ± 0,01	0,42 ± 0,03
Drug 3	0,99 ± 0,03	0,56 ± 0,04
Drug 4	1,01 ± 0,02	0,81 ± 0,06
F2544	**1,11 ± 0,07**	**1,02 ± 0,03**

F2544 impaired the increase of the DDAVP-induced cAMP

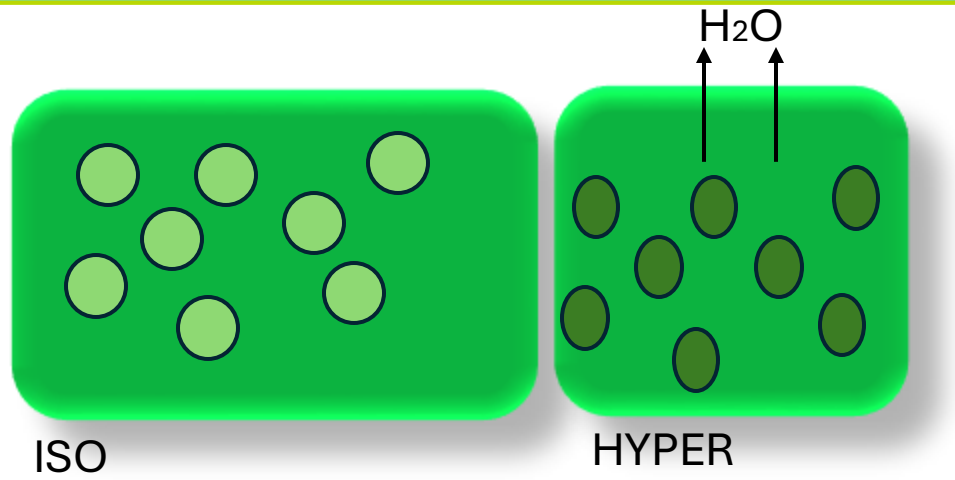


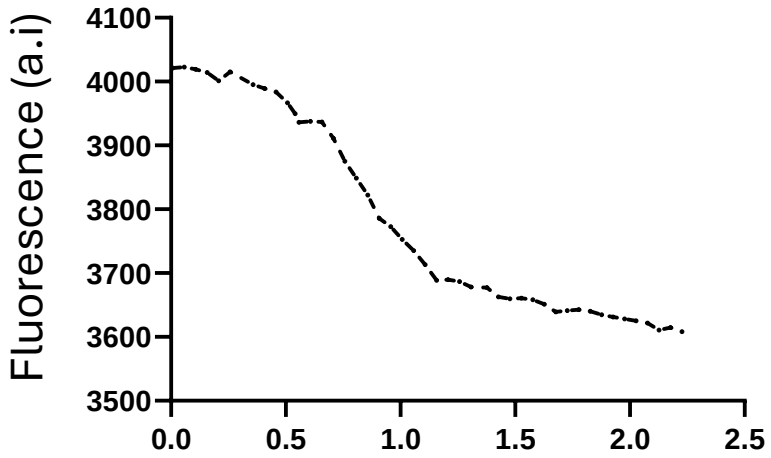


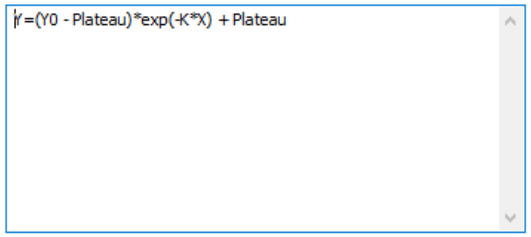
F2544 impaired the increase of the DDAVP-induced cAMP

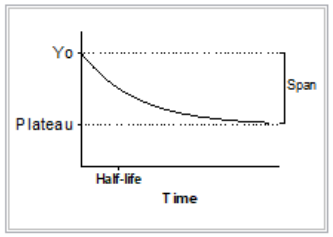


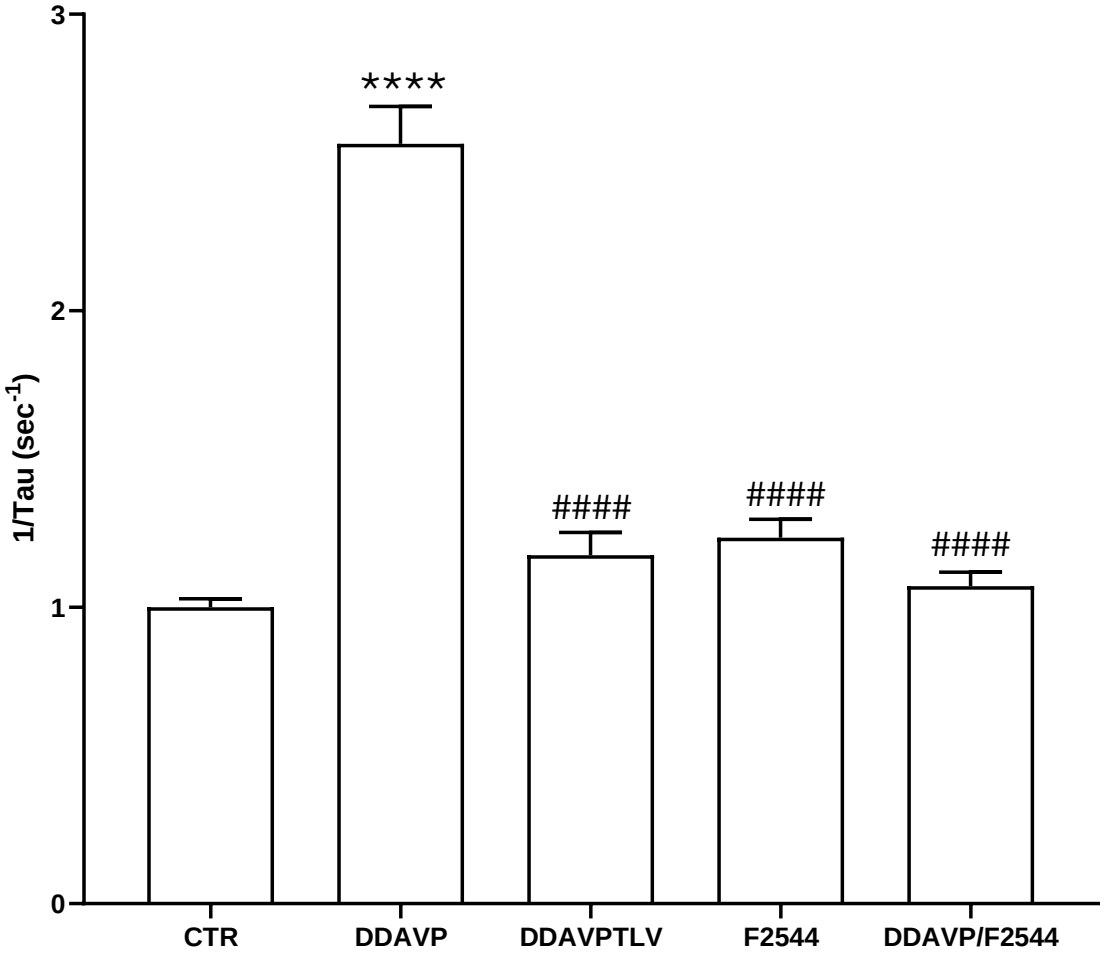
F2544 impaired the water transport induced by DDAVP











* vs CTR

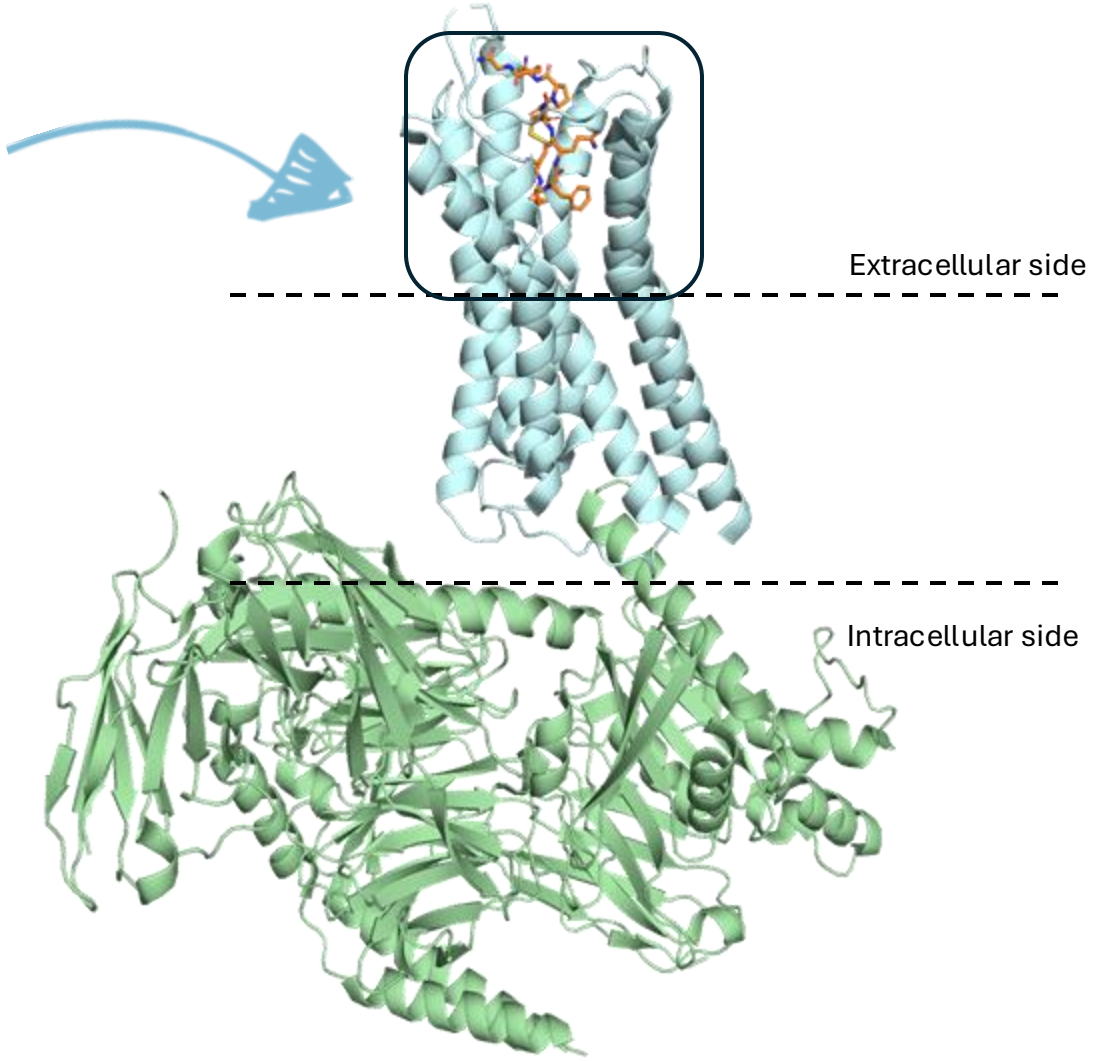
\# vs DDAVP

Cryo-EM structure of human V2 vasopressin

The Cryo-EM structure of human V2 vasopressin receptor in complex with a Gs protein is available on Protein Data Bank

The idea is to further investigate the candidate drugs by using structures-based approaches

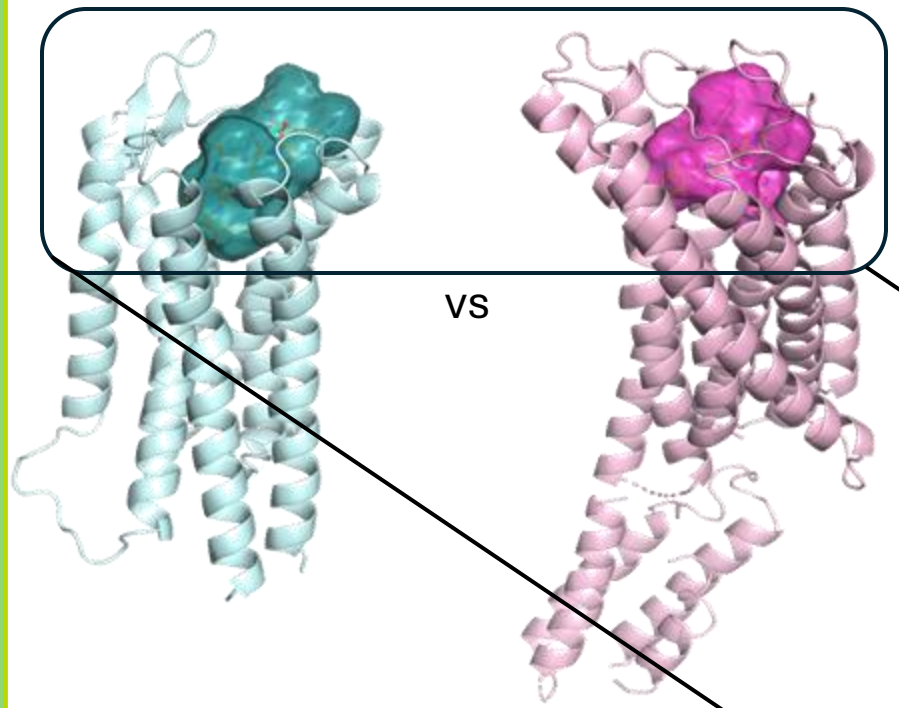
Plato bioactivity values (pred IC_{50})	
DRUG 1	33,58 nM
DRUG 2	34,17 nM
DRUG 3	33,46 nM
DRUG 4	33,90 nM
F2544	29,20 nM



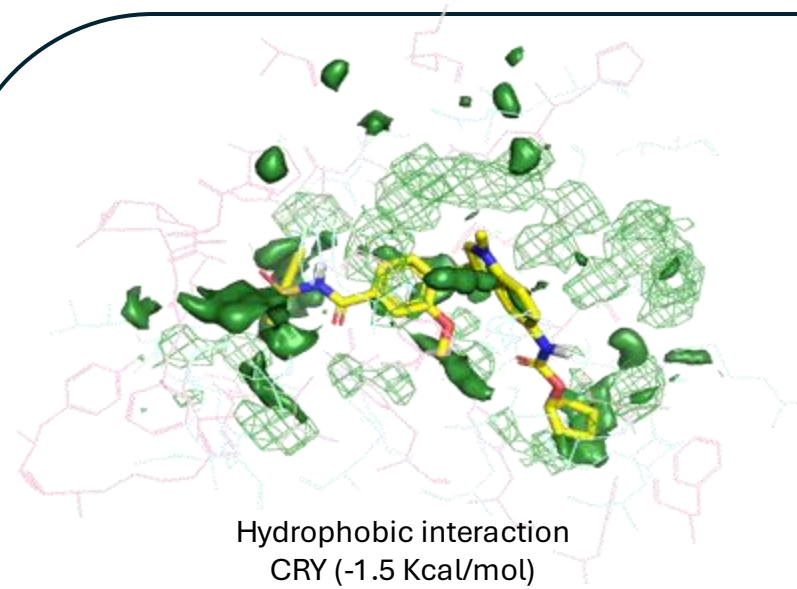
PDB = 7DW9, Resolution = 2.60 Å

Cavities comparison

We noted a good level of similarity among V2R and F2544-biasing therapeutic target binding sites by using GRID energetic studies

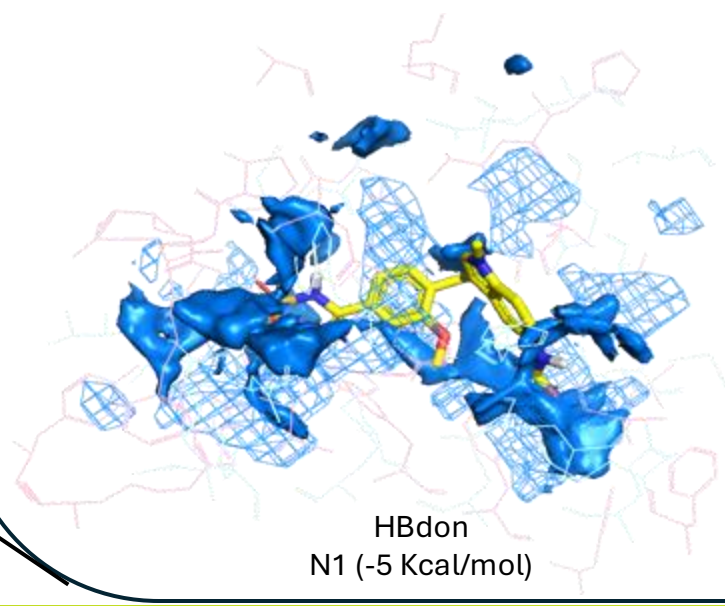


BioGPS software

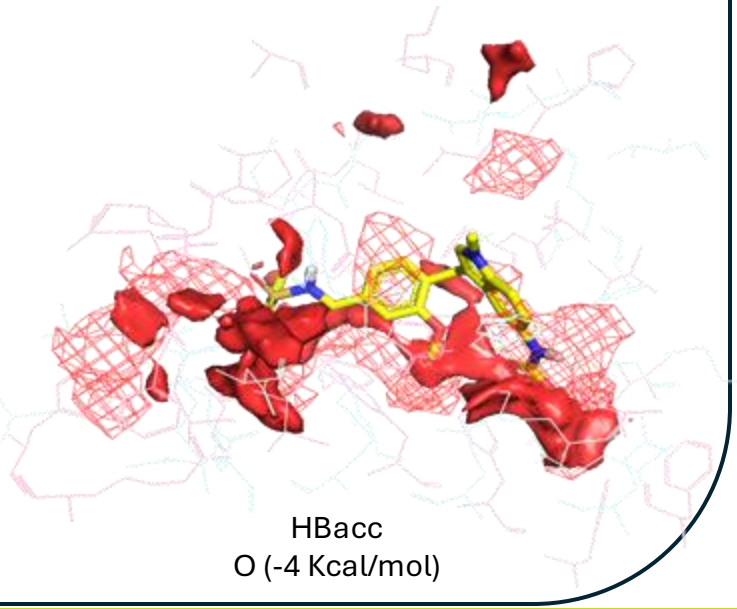


Hydrophobic interaction
CRY (-1.5 Kcal/mol)

Probe	GRID MIFs Score
H	0.793
N1	0.717
CRY	0.645
O	0.714
Glob-Prod	0.657
Glob-Sum	0.680
Glob-PSP	0.820



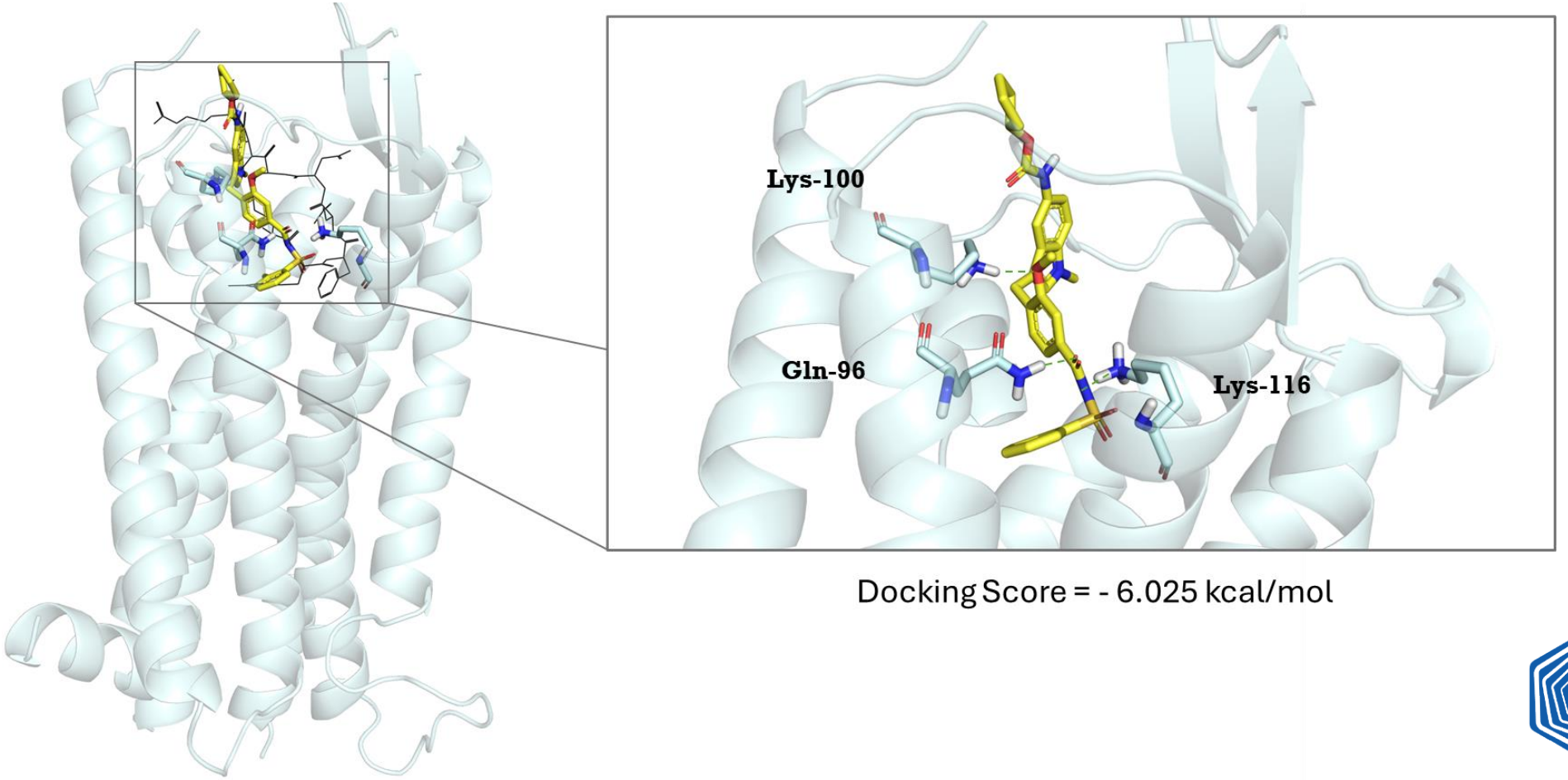
HBdon
N1 (-5 Kcal/mol)



HBacc
O (-4 Kcal/mol)

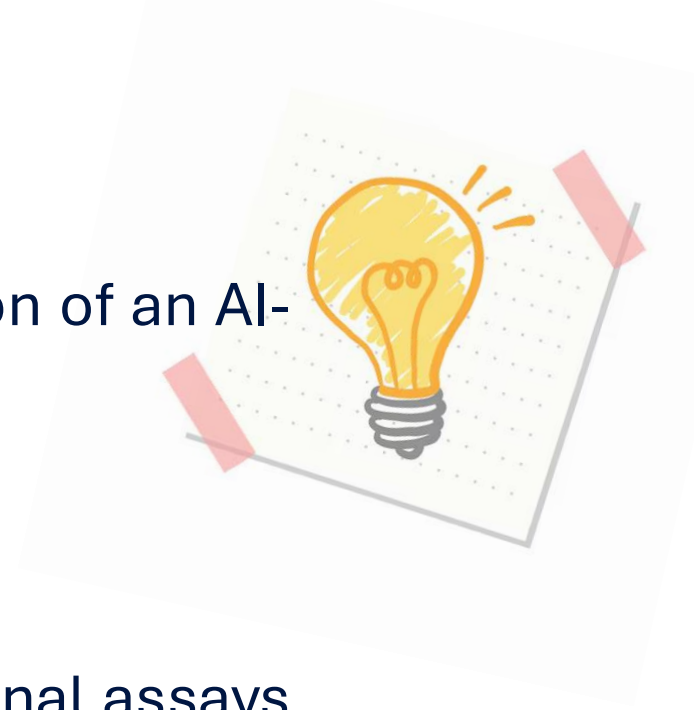
Drug repurposing of F2544 on V2R

The molecular docking simulations also confirmed the possibility of repurposing this approved drug towards V2R crystallographic structure as F2544 returned interesting results in terms of docking pose and scoring.



Docking Score = - 6.025 kcal/mol

Conclusions



- ✓ Identification of new V2R ligands through the application of an AI-based approach

- ✓ In-depth computational studies, combined with functional assays in renal collecting duct cells, suggest that F2544 should be prioritized for repurposing in treating diseases associated with abnormal V2R signaling.

ACKNOWLEDGMENTS

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Department of Pharmacy - Pharmaceutical Science,
University of Bari Aldo Moro

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO