St. Petersburg State Institute of Technology,
Laboratory of Molecular Pharmacology

A Fresh Angle on P-Glycoprotein to Overcome Tumor Chemoresistance

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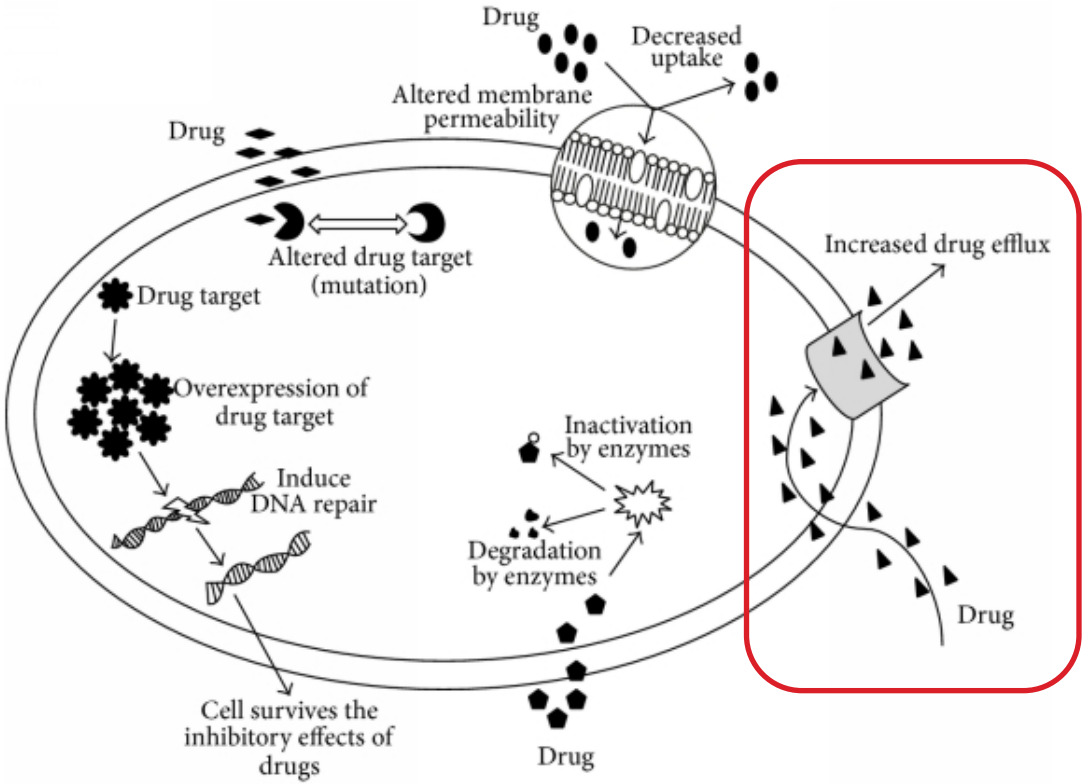
XXIX Symposium on Bioinformatics and Computer-Aided Drug Discovery

MDR – MultiDrug Resistance



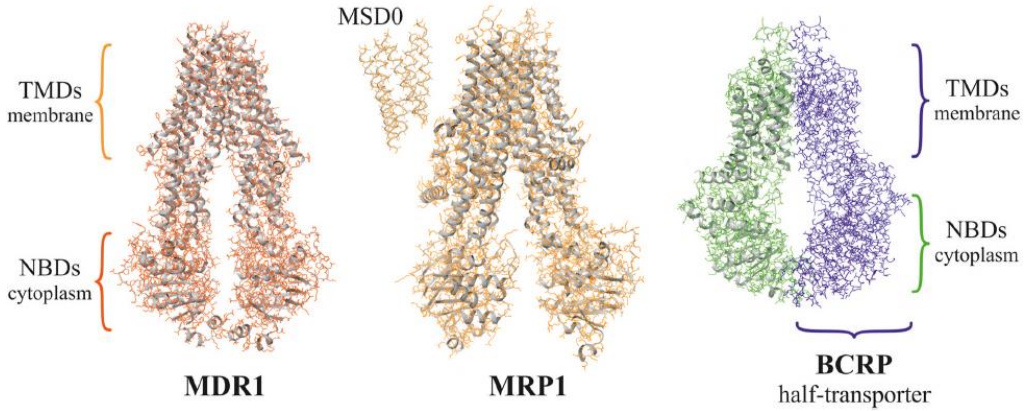
Tanwar et al. *Interdiscip. Perspect. Infect. Dis.* **2014**, *2014*, 541340.

ATP-binding cassette (ABC) transporter family

Human genome contains 49 ABC genes organized into 7 subfamilies (*ABCA*–*ABCG*)

Clinically relevant ABC transporter for anticancer therapy:

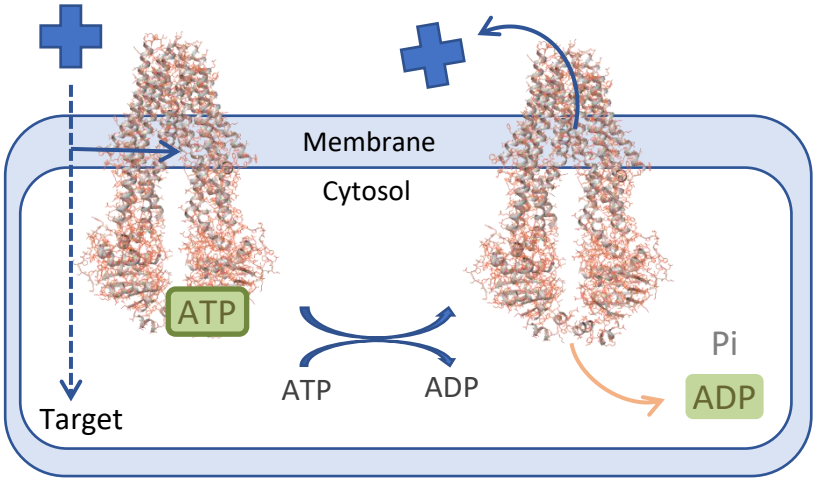
- **MDR1** (Multidrug resistance protein 1; also known as **P-glycoprotein** or **P-gp**) encoded by the *ABCB1* gene;
- **MRP1** (Multidrug resistance-associated protein 1) encoded by the *ABCC1* gene;
- **BCRP** (Breast cancer resistance protein) encoded by the *ABCG2* gene.

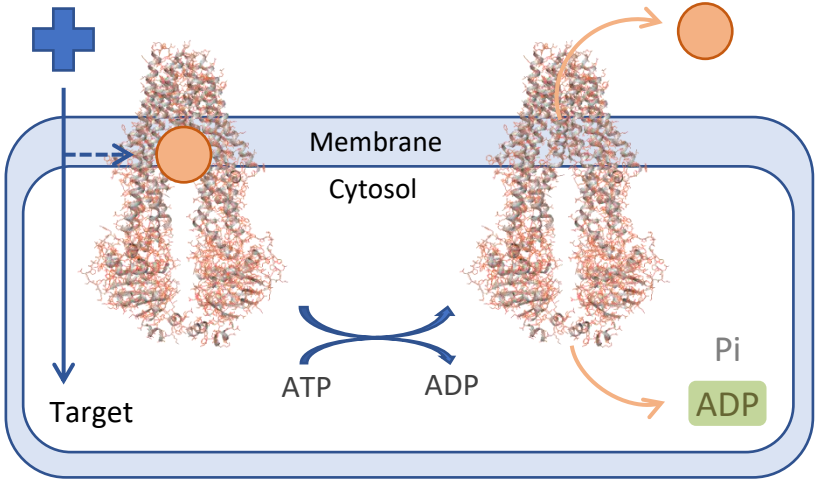


Grigoreva et al. *ACS Omega*. **2022**, *7*, 42835–42844.

Current strategy to overcome tumor resistance

Combined treatment with anticancer drugs
and P-gp inhibitors





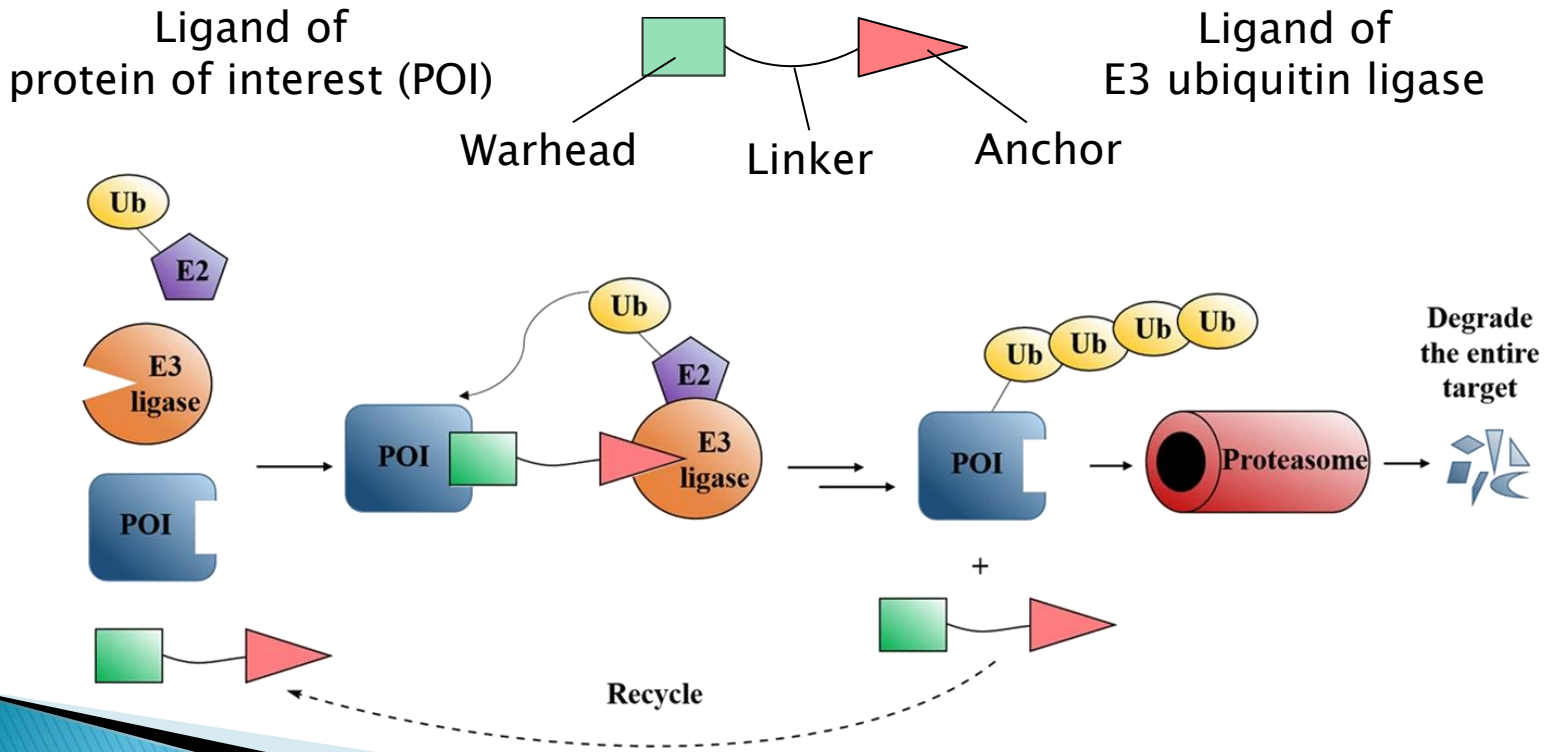
= drug = inhibitor

Efficiency of P-gp inhibitors in patients

ClinicalTrials.gov Identifier	Drug	Study completion	Phase	Status
NCT00001302	Valspodar + Vinblastine	2002	I	Completed
NCT00001383	Valspodar + Paclitaxel	2001	I	Completed
NCT00001944	Tariquidar + Vinorelbine	2001	I	Completed
NCT00011414	Tariquidar + Doxorubicin, Vinorelbine, or Docetaxel	2016	I	Completed
NCT00028873	Laniquidar + paclitaxel or docetaxel	2002	II	Completed
NCT00042302	Tariquidar + Paclitaxel/Carboplatin	2003	III/IV	Terminated
NCT00042315	Tariquidar + Vinorelbine	2003	III/IV	Terminated
NCT00046930	Zosuquidar + Daunorubicin and Cytarabine	2010	III	Completed
NCT00048633	Tariquidar + taxane or anthracycline	2003	II	Completed
NCT00069160	Tariquidar + Docetaxel	2009	II	Completed
NCT00071058	Tariquidar + Surgery Plus Chemotherapy (Doxorubicin, Vincristine and Etoposide)	2009	II	Completed
NCT00129168	Zosuquidar + Daunorubicin and Cytarabine	2008	I/II	Completed
NCT00233909	Zosuquidar + Gemtuzumab ozogamicin	2008	I/II	Completed
NCT04603066	Tariquidar + 5-HT3R antagonist ondansetron, in patients with neuropathic pain		I/II	RECRUITING

PROTAC concept

PROTAC = **PRO**teolysis **TA**rgeting **C**himera



Sun et al. *Signal Transduct. Target Ther.*, **2019**, *4*, 64.

PROTAC for membrane protein

Why not?!

- some evidence that the stability of the MDR1 gene product (P-gp) could be regulated by ubiquitination;

Zhang et al. *Mol. Pharmacol.,* **2004**, *66*, 395-403.

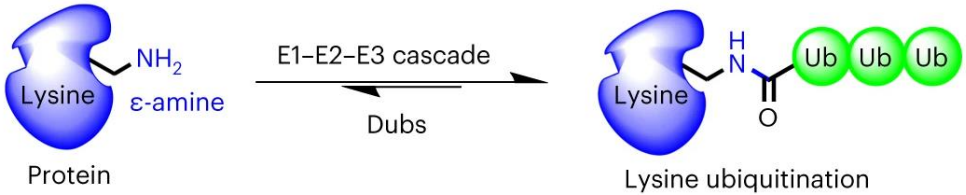
- some evidence that increased ubiquitination level of P-gp is accompanied by decreased P-gp protein expression;

Nawa et al. *Drug Metab. Pharmacokinet.,* **2012**, *27*, 548-552.

- some evidence that ubiquitination of transmembrane proteins promotes release of the protein from the membrane.

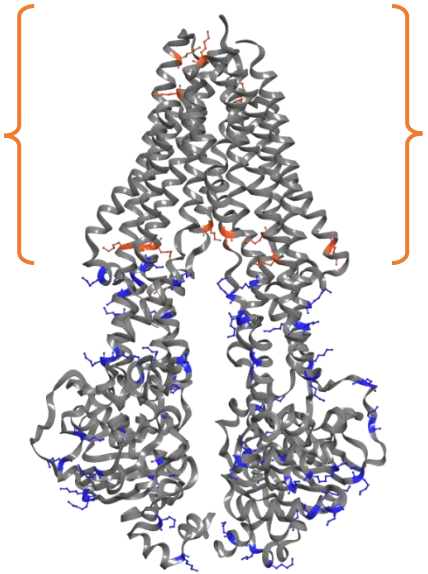
Liu et al. *Oncogene* **2013**, *32*, 1660-1669.

Ubiquitination of proteins



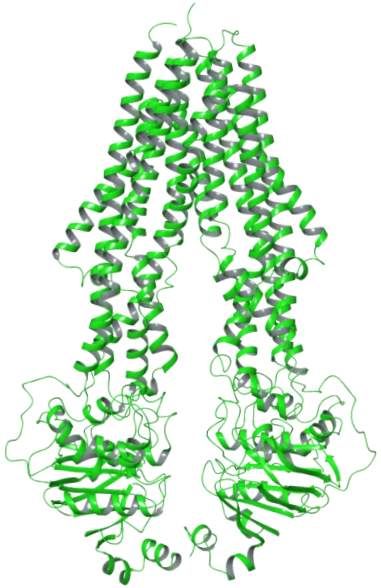
Zang et al. *Nat. Struct. Mol. Biol.*, **2023**, *30*, 62–71

TMDs
membrane



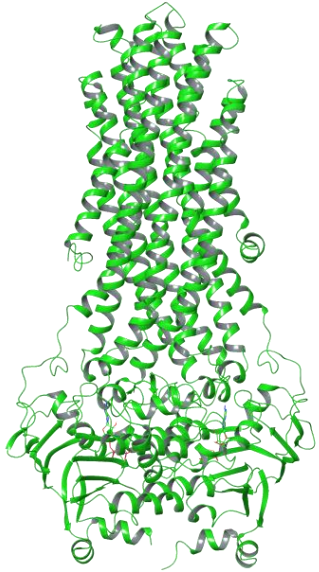
68 lysine
residues

Used P-gp models



inward-facing conformation

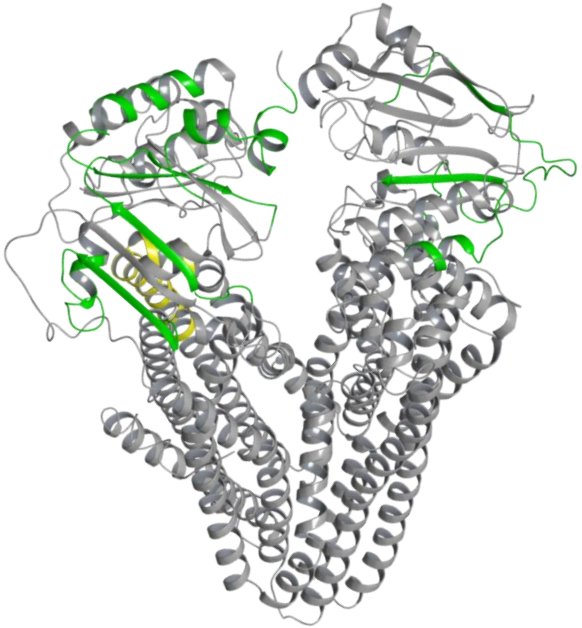
PDB id 7A65



outward-facing conformation

PDB id 6C0V

Choice of E3 ligase



GPS-Uber

(**ub**iquitin-protein ligase **e**nzymes-substrate **r**elationship prediction)

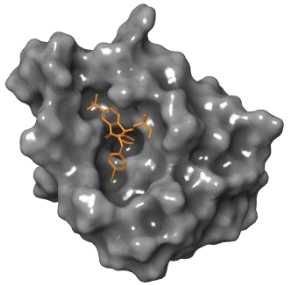
- CRBN sites (not found);
- VHL sites (yellow);
- …
- Mdm2 (green).

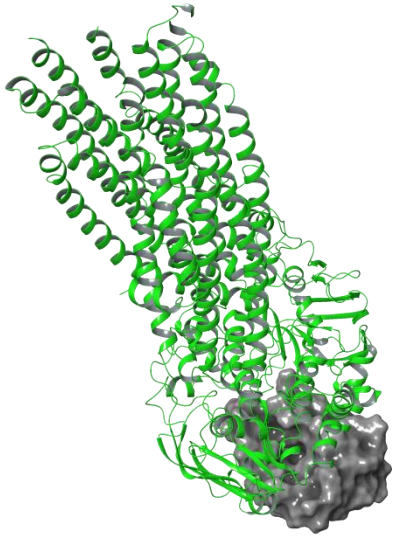
Wang et al. *Brief. Bioinformatics*, **2022**, *23*, bbab574.

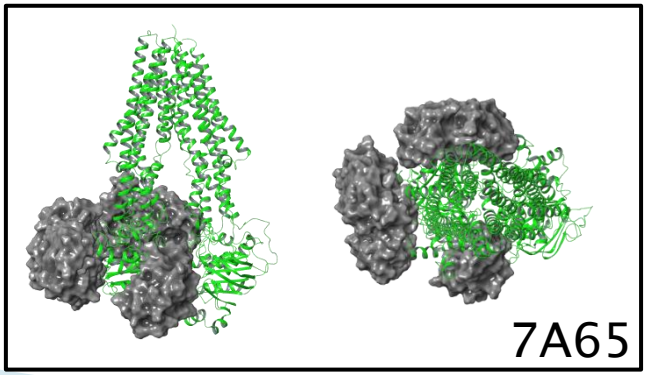
gpsuber.biocuckoo.cn/

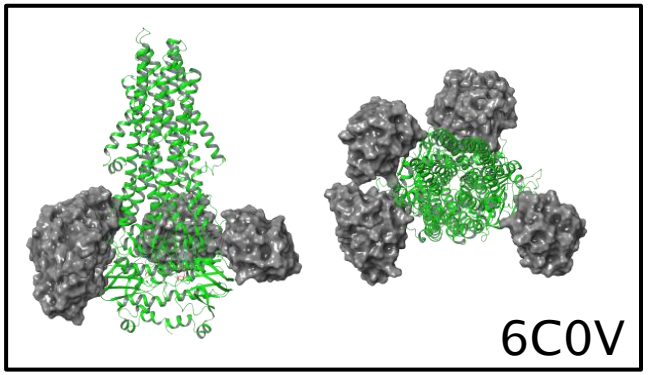
Protein–protein docking

Mdm2 complex with isoindolinone inhibitor (PDB id 7BMG)

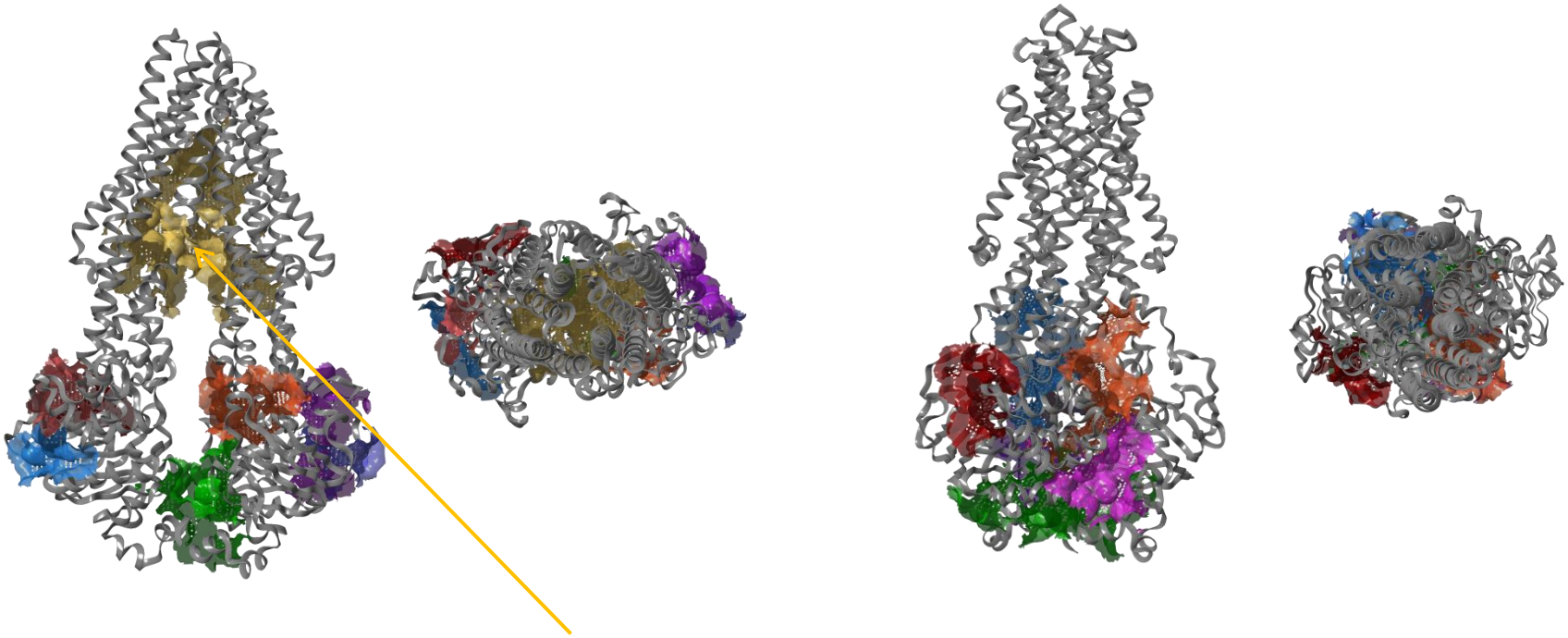








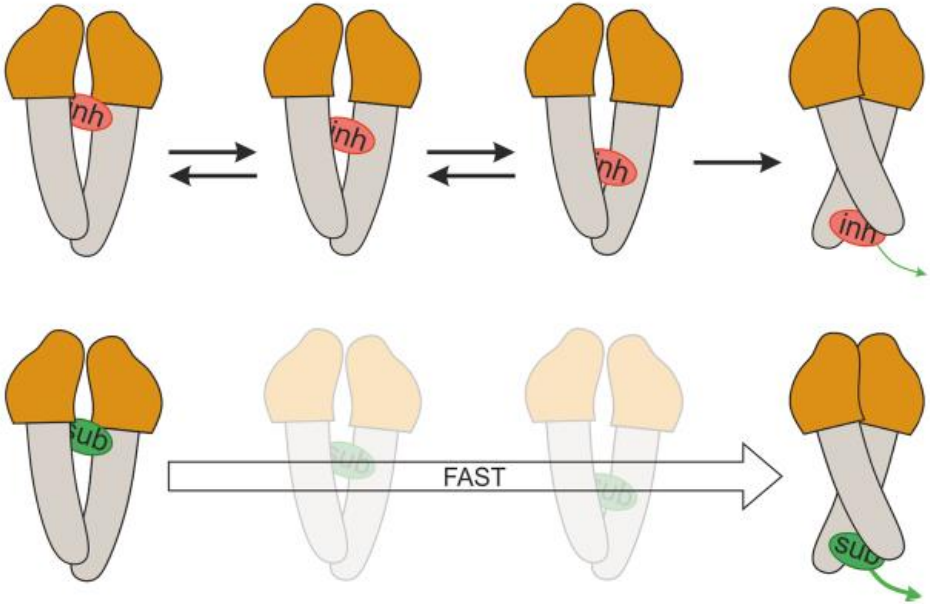
Search for “PROTAC” site



Commonly used for search and development of P-gp inhibitors

Groundwork

Working within TMD:
transported substance or inhibitor?

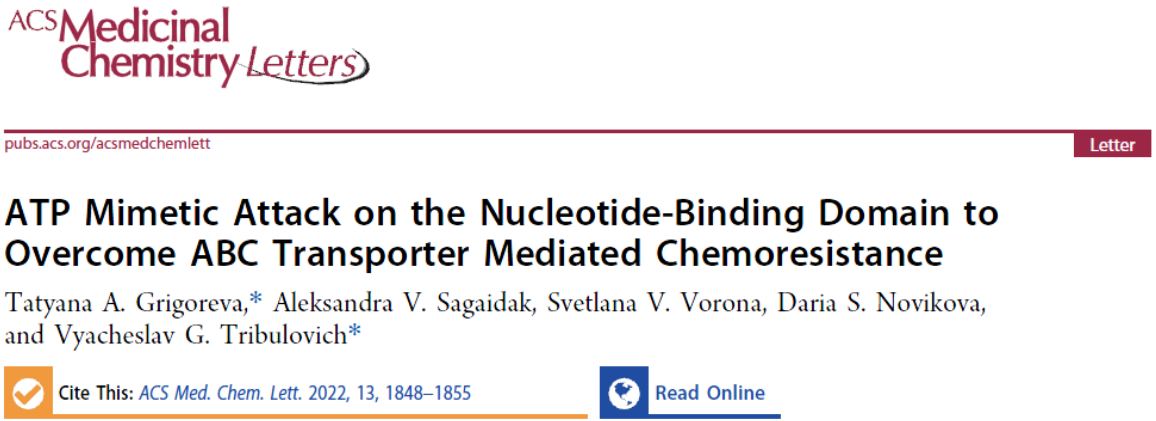


Grigoreva et al. *ACS Omega*. **2022**, *7*, 42835–42844.

Groundwork

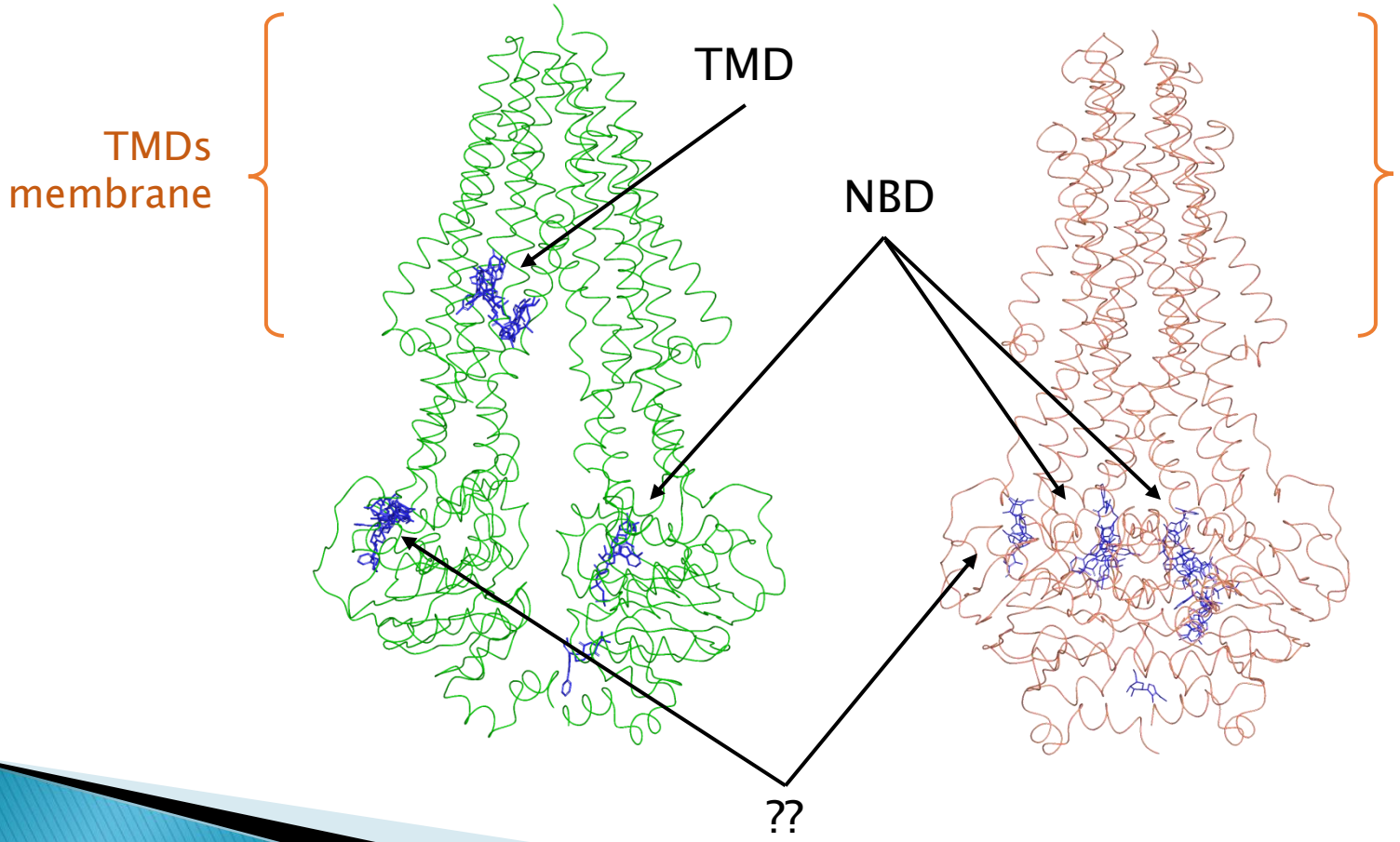
Move towards the nucleotide binding domain (NBD):

Talk by Aleksandra Sagaidak

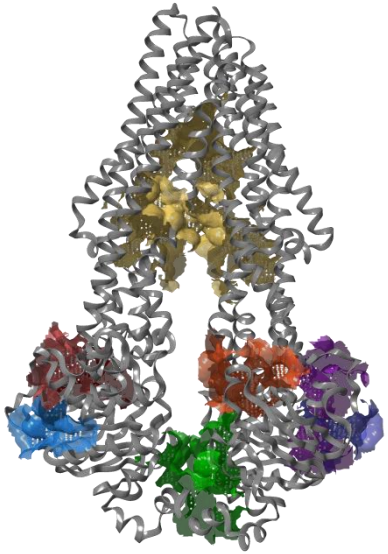


Library of ATP (AMP) mimetics

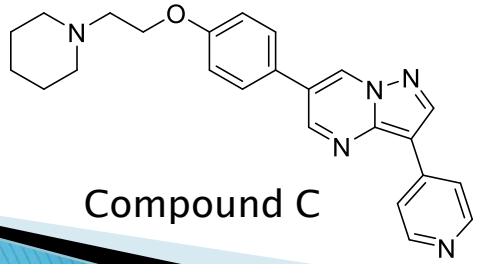
Docking of compound library



Docking results



Compound	Docking Score						
		NBD		new site			TMD
AICAR	-5.3	-5.3	-5.6	-7.0	-5.2	-4.9	-5.9
Cladribin	-6.0	-6.0	-6.2	-6.7	-4.6	-4.9	-6.1
AMP	-5.4	-3.8	-5.3	-5.5	-4.4	-4.0	-5.7
ATP	-6.1	-6.2	-6.0	-5.3	-4.4	-3.6	-7.0
CompC del	-6.2	-3.9	-5.4	**-7.3**	-4.6	-4.1	**-7.2**
CompC	-5.3	-2.5	-4.8	**-7.8**	-5.0	-3.2	-6.4
DKPP	-6.0	-4.2	-4.2	-5.4	-4.4	-4.1	**-7.7**
FM04	-5.2	-3.7	-4.7	**-7.4**	-4.7	-3.3	**-7.2**
PheCH$_3$Cl R	-4.5	-2.7	-3.4	-5.7	-3.6	-3.1	**-8.1**
PheCH$_3$Cl S	-4.8	-3.7	-3.3	-6.4	-4.5	-1.8	**-7.2**
Ribavirin	-5.0	-6.3	-4.7	**-7.4**	-5.1	-5.0	-5.8
SN-202	-5.0	-4.7	-5.1	-5.8	-4.1	-4.2	-4.7
ZMP	-5.4	-5.5	-5.2	-5.1	-4.4	-3.6	-6.9



Compound C

Library of its modifications

Independent confirmation of our results

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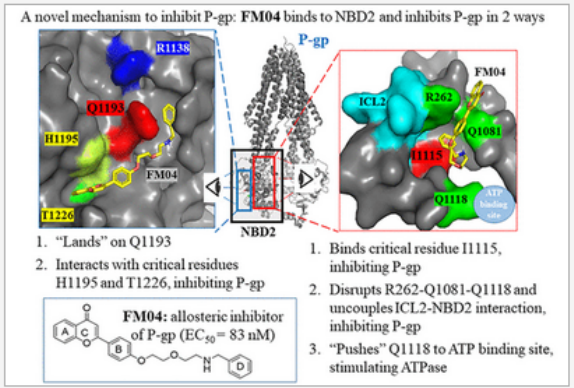
Identification of Binding Sites in the Nucleotide-Binding Domain of P-Glycoprotein for a Potent and Nontoxic Modulator, the Amine-Containing Monomeric Flavonoid FM04

Zhen Liu, Iris L. K. Wong, Jingcheng Sang, Fufeng Liu, Clare S. W. Yan, Jason W. Y. Kan, Tak Hang Chan*, and Larry M. C. Chow*

Cite this: *J. Med. Chem.* 2023, 66, 9, 6160–6183
Publication Date: April 25, 2023
https://doi.org/10.1021/acs.jmedchem.2c02005

Abstract

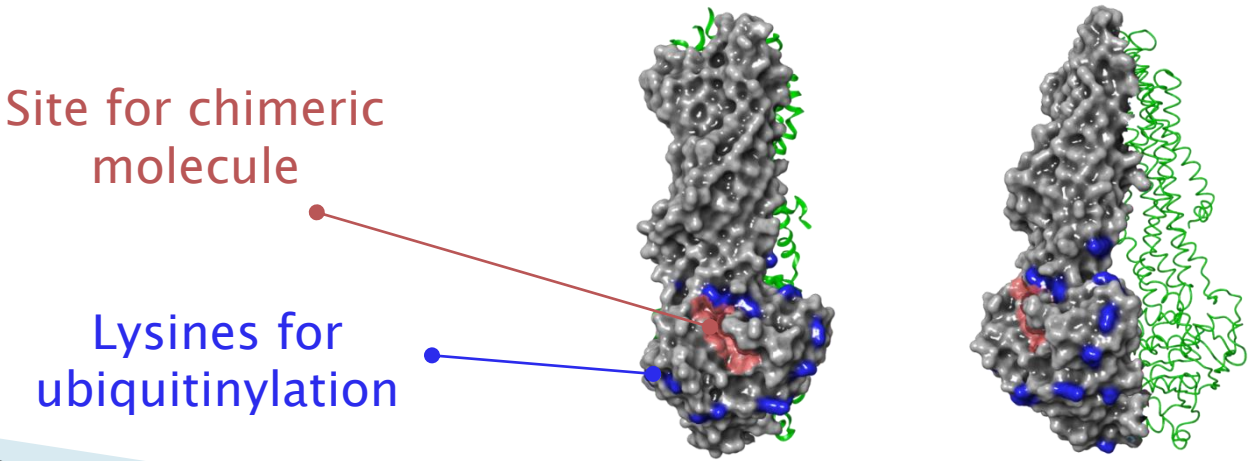
We have previously discovered an amine-containing flavonoid monomer **FM04** as a potent P-glycoprotein (P-gp) inhibitor (EC_{50} = 83 nM). Here, a series of photoactive **FM04** analogues were synthesized and used together with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify the **FM04**-binding sites on P-gp. Point mutations around the photo-crosslinked sites were made for verification. Together with the results from mutational studies, molecular docking, and molecular dynamics simulations, it was found that **FM04** can interact with Q1193 and I1115 in the nucleotide-binding domain 2 (NBD2) of human P-gp. It was proposed that **FM04** can inhibit P-gp in 2 novel mechanisms. **FM04** can either bind to (1) Q1193, followed by interacting with the functionally critical residues H1195 and T1226 or (2) I1115 (a functionally critical residue itself), disrupting the R262-Q1081-Q1118 interaction pocket and uncoupling ICL2–NBD2 interaction and thereby inhibiting P-gp. Q1118 would subsequently be pushed to the ATP-binding site and stimulate ATPase.



It is the right way to go on!

Conclusions

- We simulated the formation of P-gp complex with Mdm2;
- We identified P-gp site suitable for binding a chimeric molecule;
- We generated primary library of structures with high affinity to the site of interest.



This work was supported by the Russian Science Foundation (project no. 23-13-00344)

Thank you for attention!

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