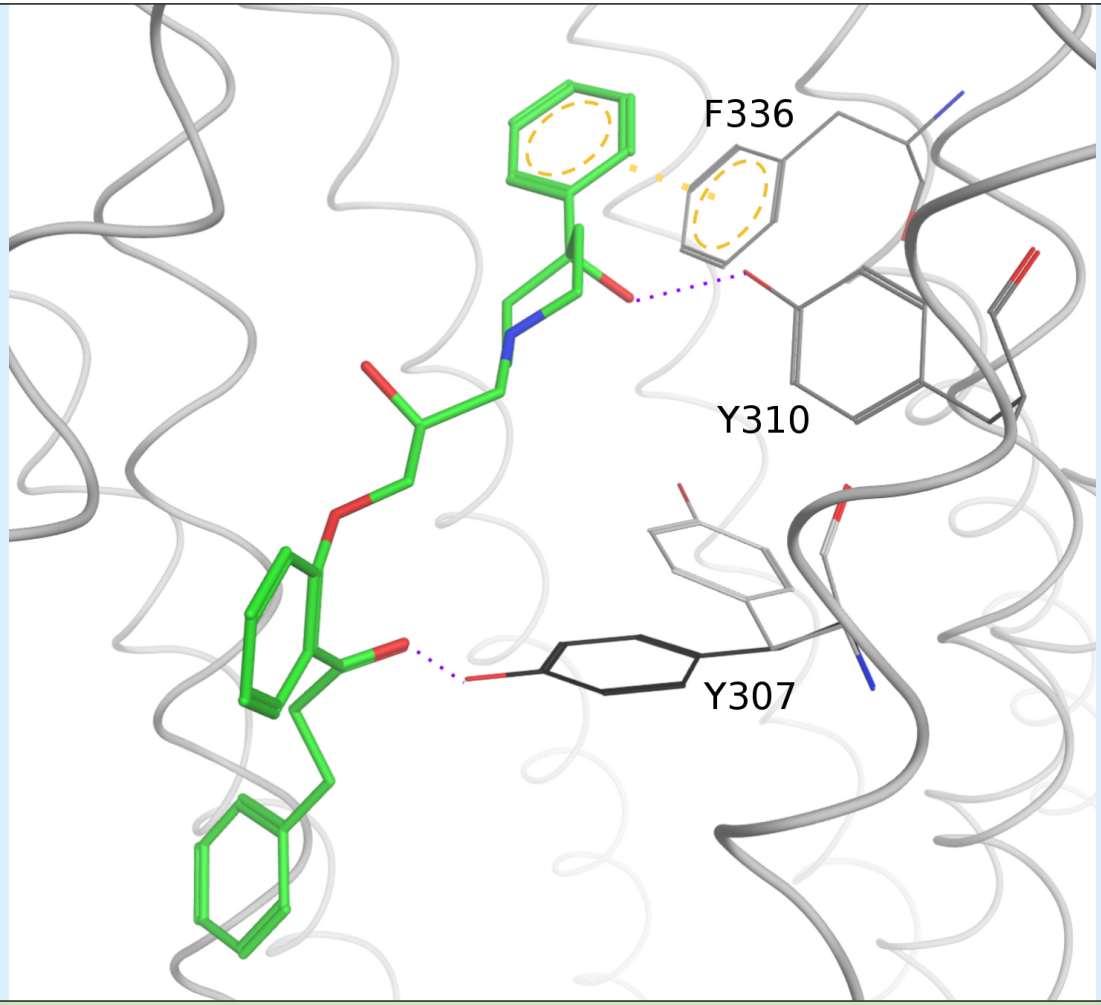


Determination of P-gp – Propafenone interaction by structure based methods

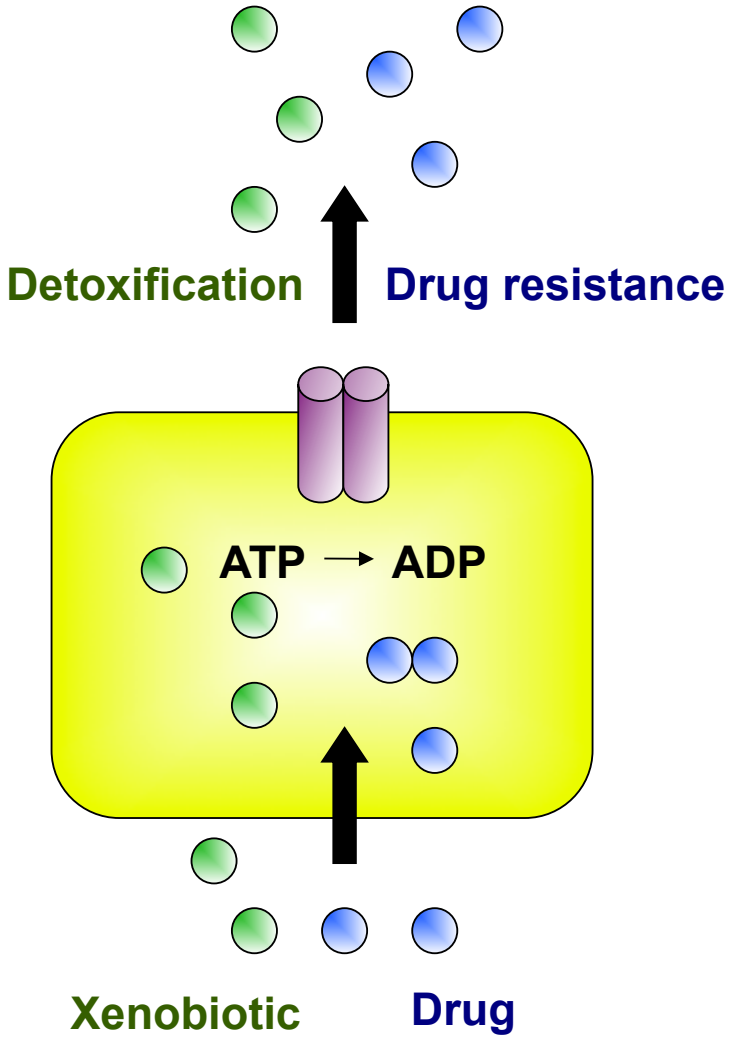
Freya Klepsch

Supervisor: Prof. Ecker

February 3rd 2011

P-glycoprotein (P-gp)

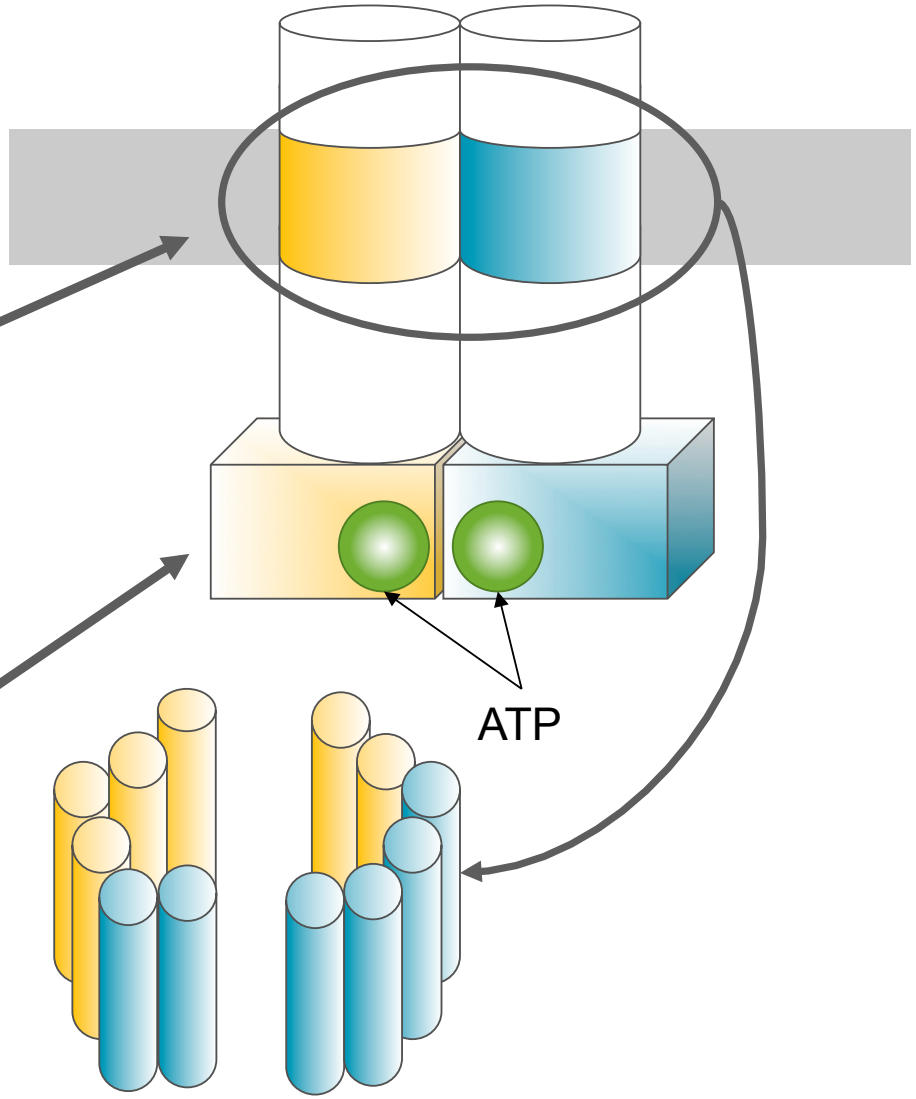
- Function
  - Active exporter
  - Hydrophobic vacuum cleaner
  - Xenobiotic efflux
    - Detoxification
    - Multidrug resistance (MDR)



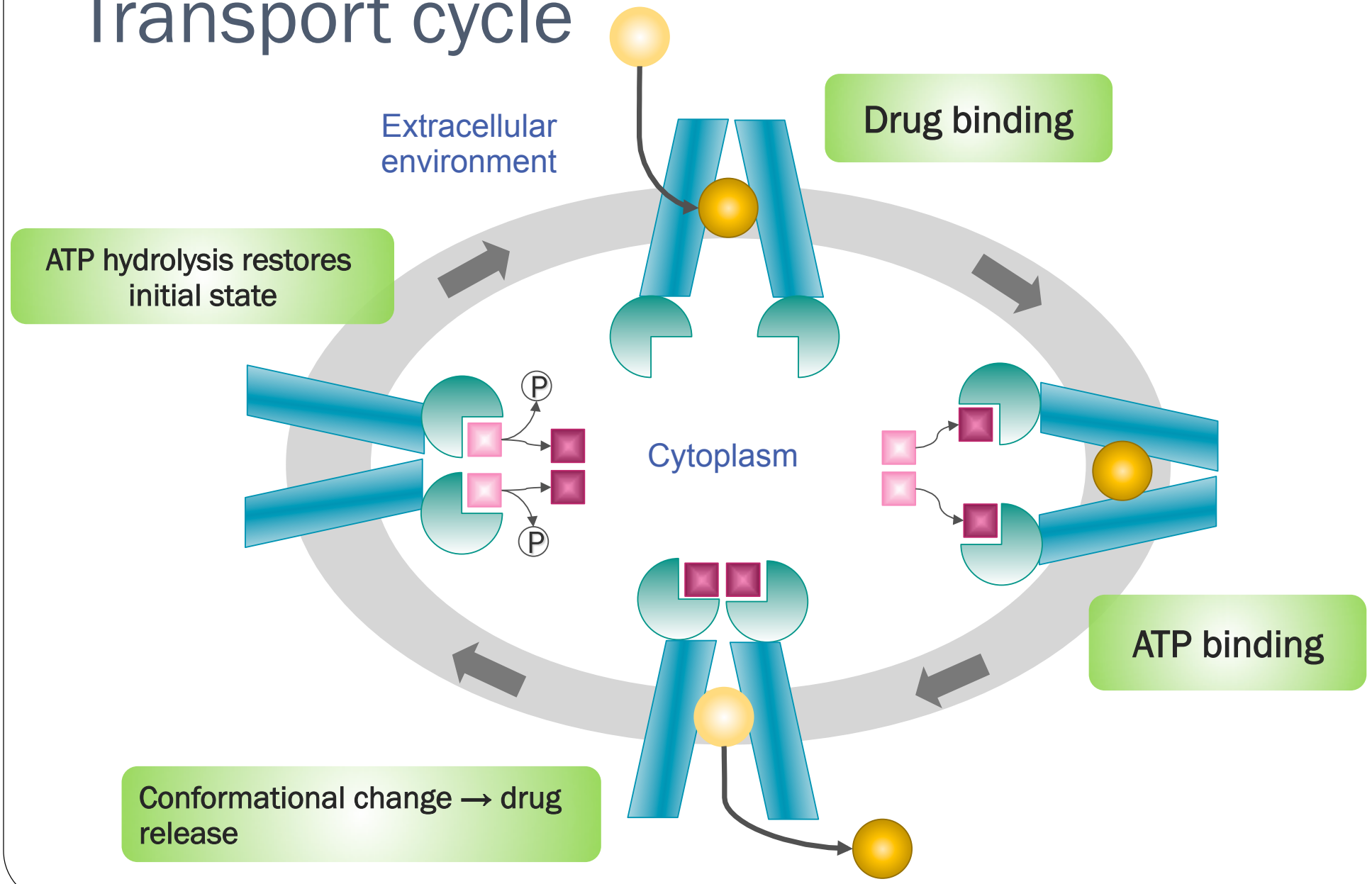
adapted from Fletcher et al. *Nat Rev Cancer* 2010
doi:10.1038/nrc2789

P-glycoprotein (P-gp)

- Structure
  - Heterodimer
    - Transmembrane domain (TMD)
      - 6 TM-helices each
      - Binding of drugs
    - Nucleotide binding domain (NBD)
      - Binding and hydrolysis of ATP



Transport cycle

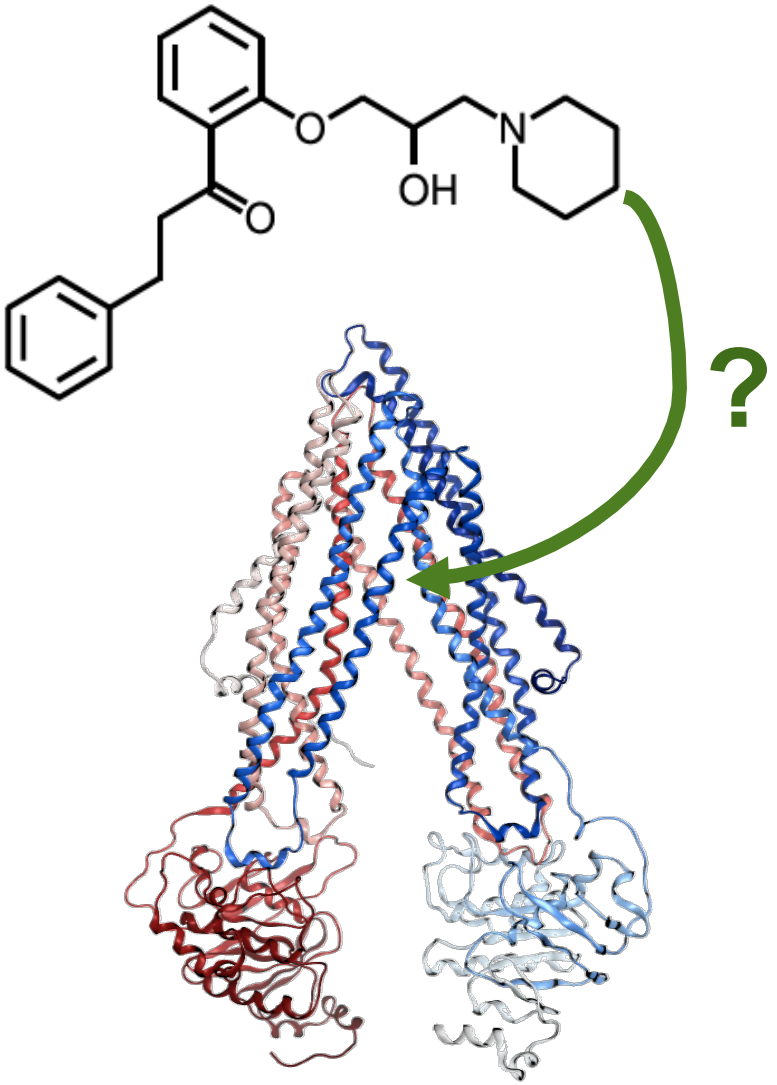


Propafenones

- Show P-gp activity
- Database of 400 analogs
- Clear SAR
- Concrete binding mode still unclear

→ Structure based design

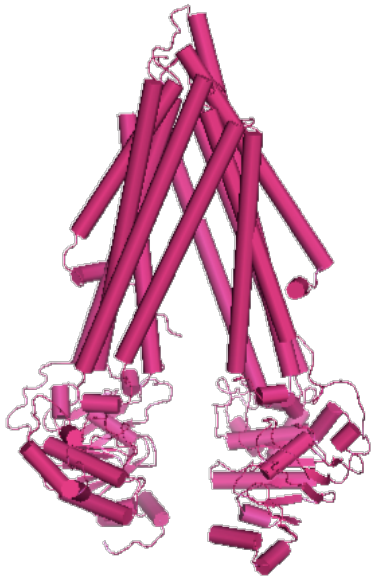
- Homology Modeling
- Docking



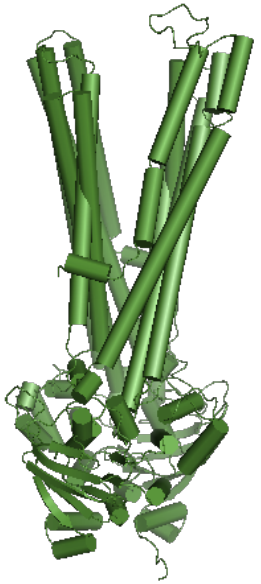
Homology Modeling

- Human P-gp sequence
- 2 templates

- Apo structure from mouse P-gp, (PDB code: 3G5U)
  - high-affinity conformation

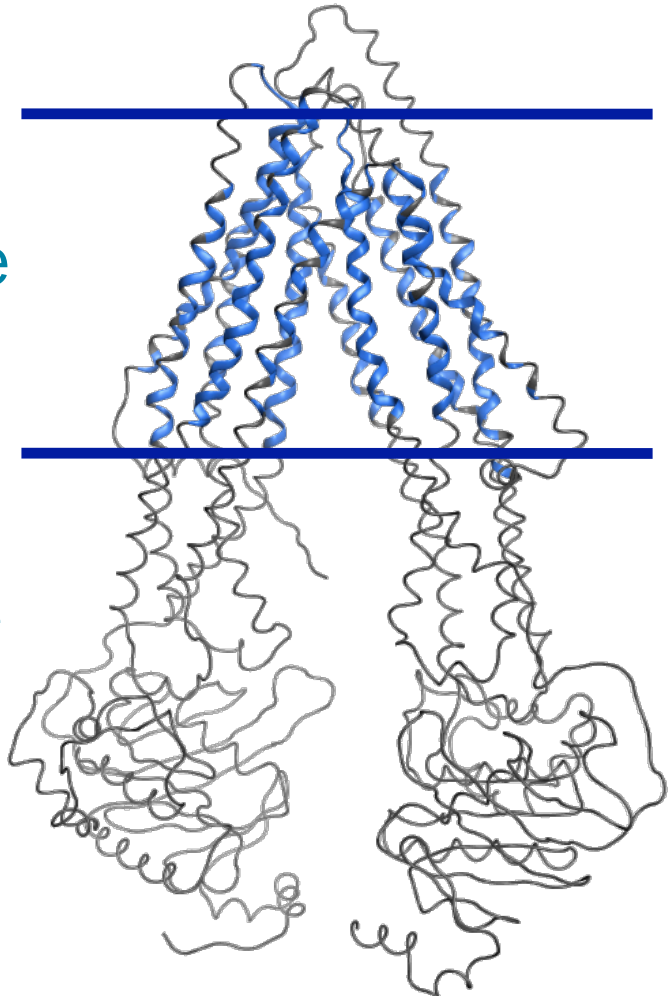


- Nucleotide bound structure from Sav1866, (PDB code: 2HYD)
  - low-affinity conformation



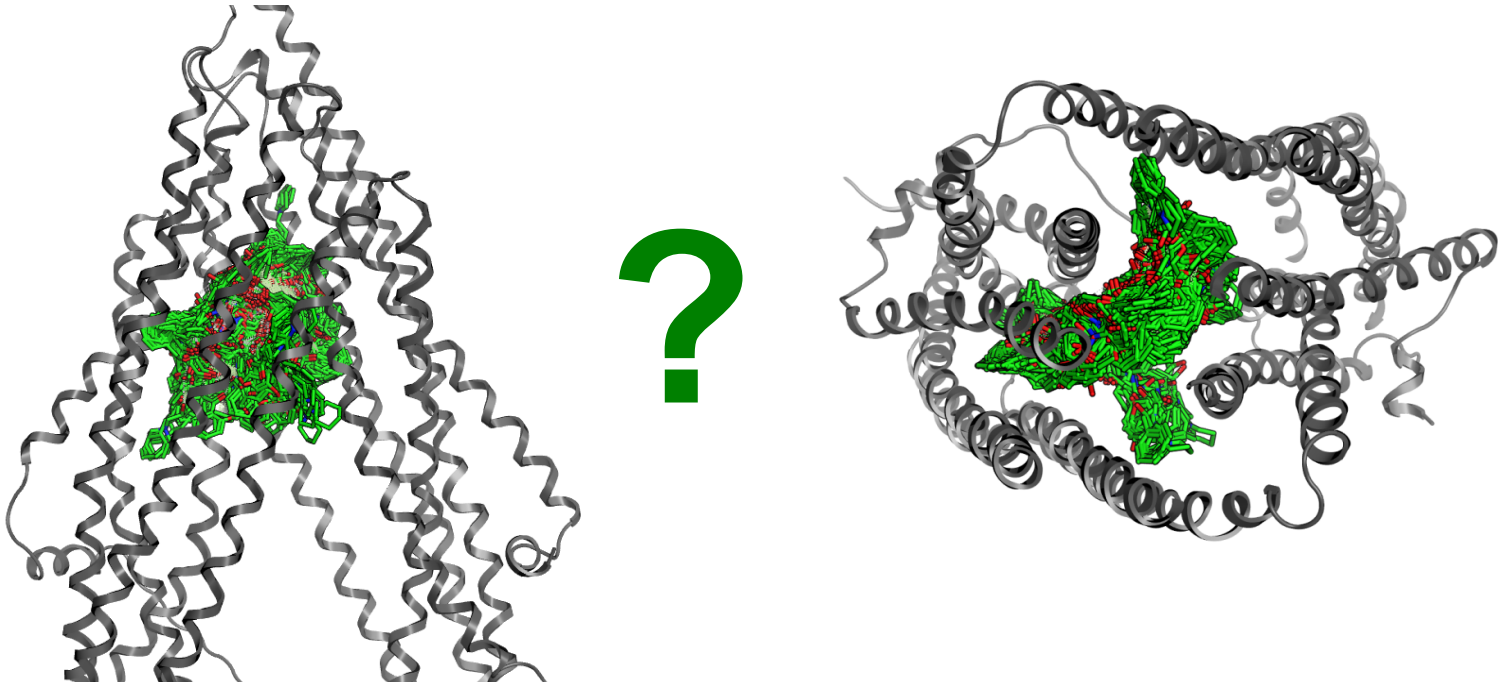
Docking

- Program: GOLD
- Placement of 5 different propafenone analogs in binding site
  - Sampling of ligand conformations
  - Sampling of orientations in binding site
- Binding site: whole transmembrane region
- 100 docking poses/ligand

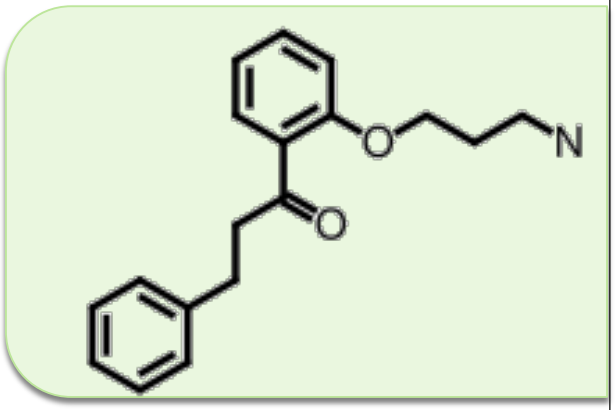


Analysis

- Pose selection?
  - Scoring function → problem: no evaluation for this problem
  - Information from ligand-based studies



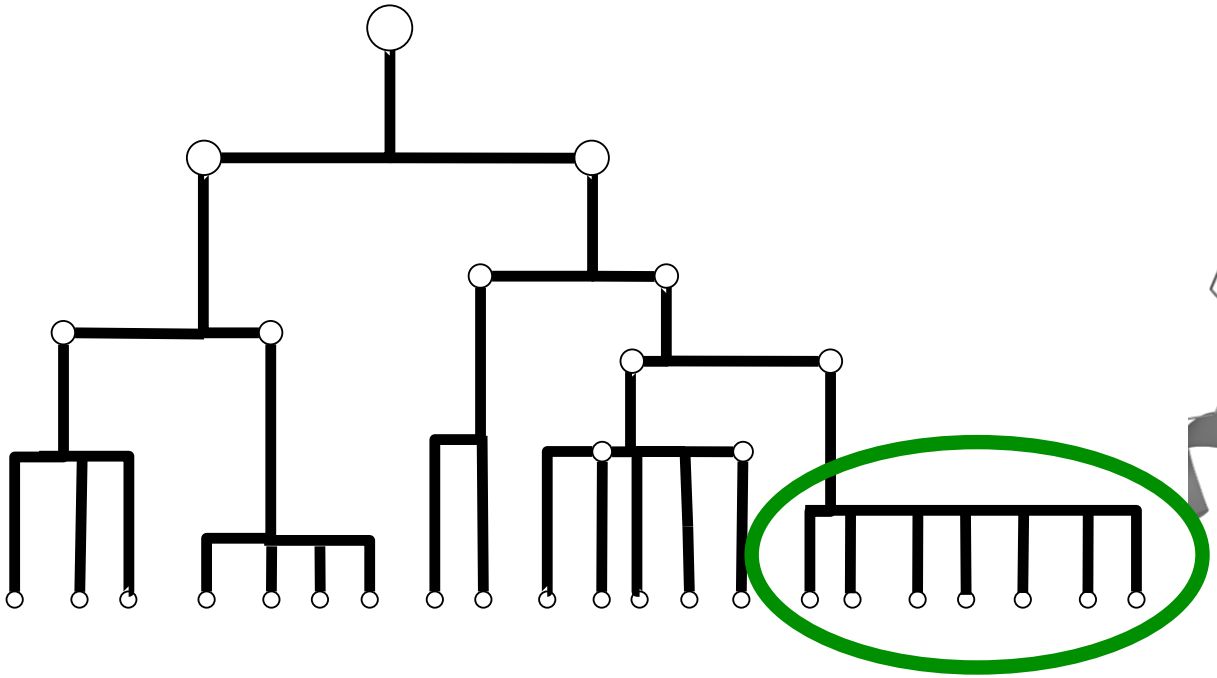
SAR guided pose selection

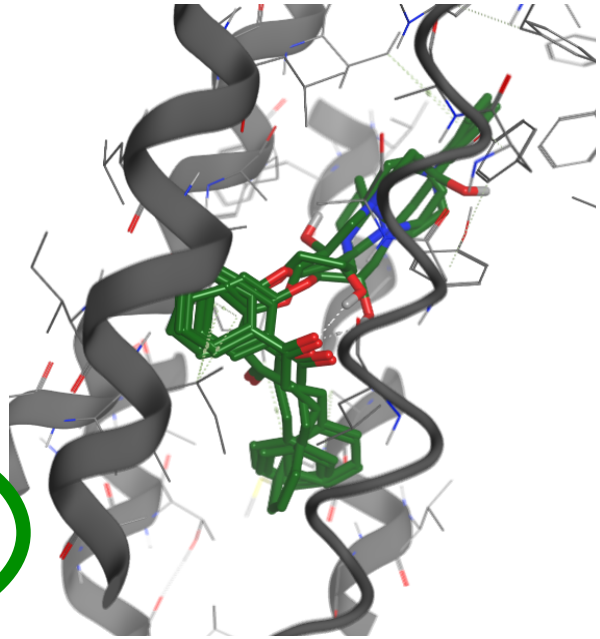


- Common binding mode

→ RMSD clustering of poses according the common scaffold

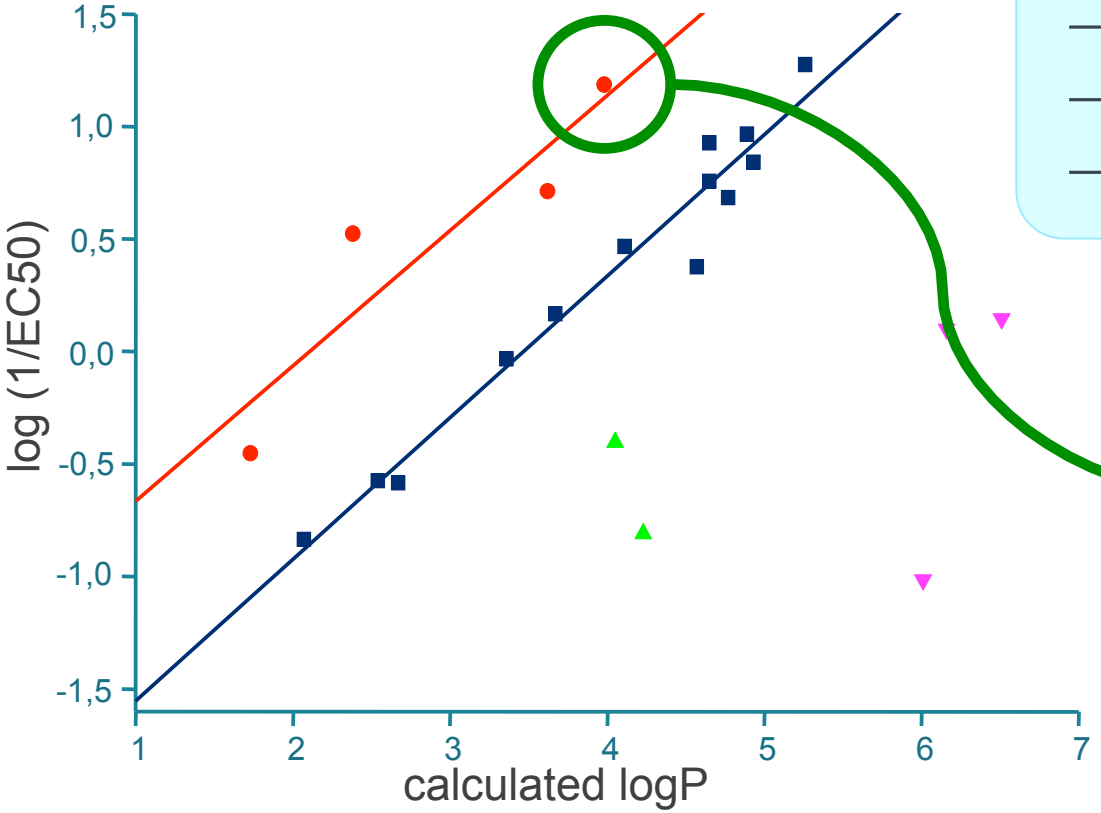
→ common scaffold clusters (CSC): min 4 different ligands



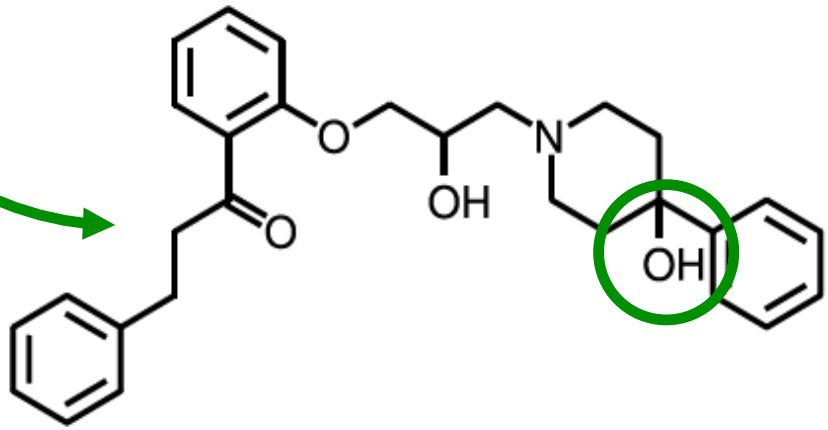


SAR guided pose selection

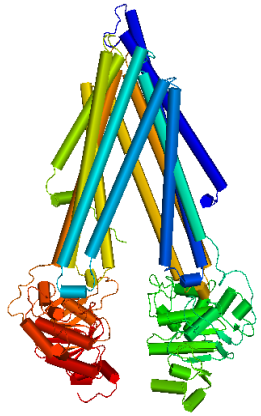
- Specific interactions
  - Propafenone-Pgp activity correlates with lipophilicity
  - Outlier: GP062



→ OH-group decreases logP
→ but still high activity
→ H-bond with protein

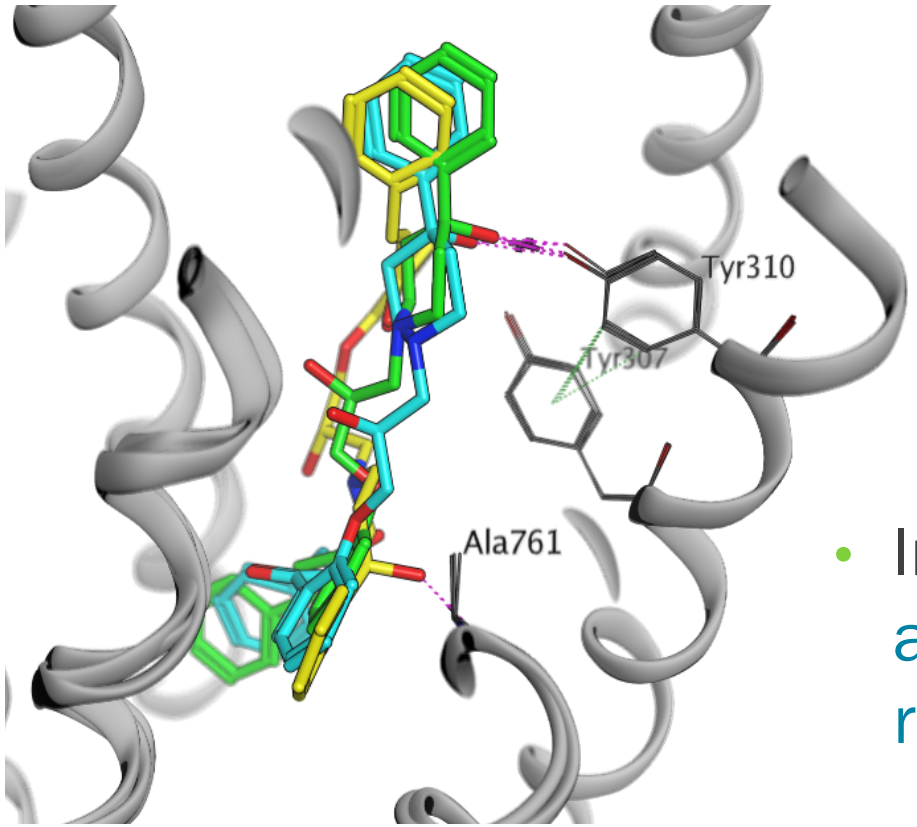


Docking results



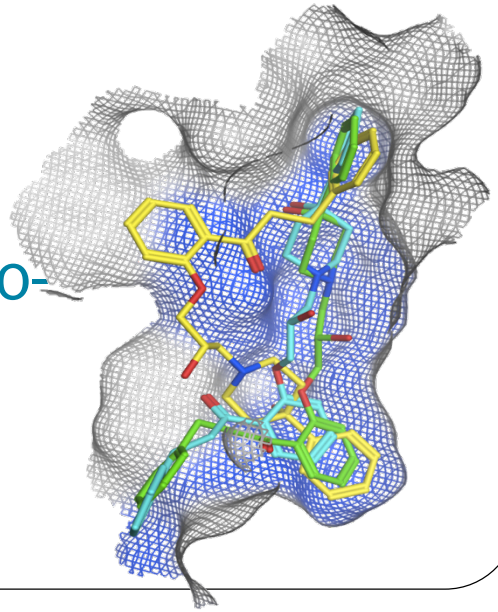
3G5U_Pgp (apo)

- 3 CSC showed interaction between GP062-OH and protein

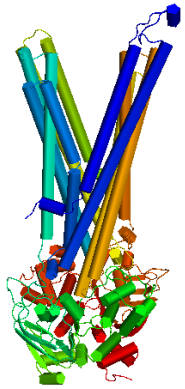


- Group A: H-bond with Y310
- Group B: H-bond with A761

- In vicinity of photo-affinity labeled residues

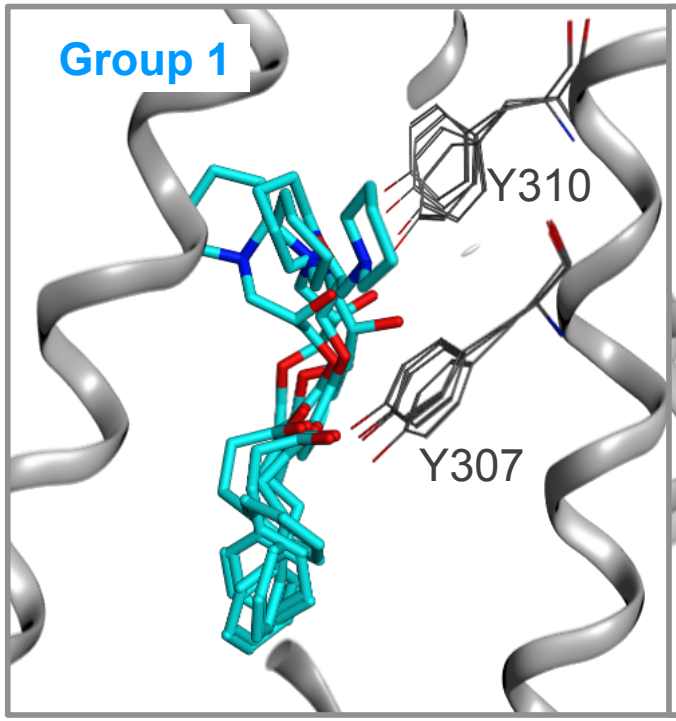


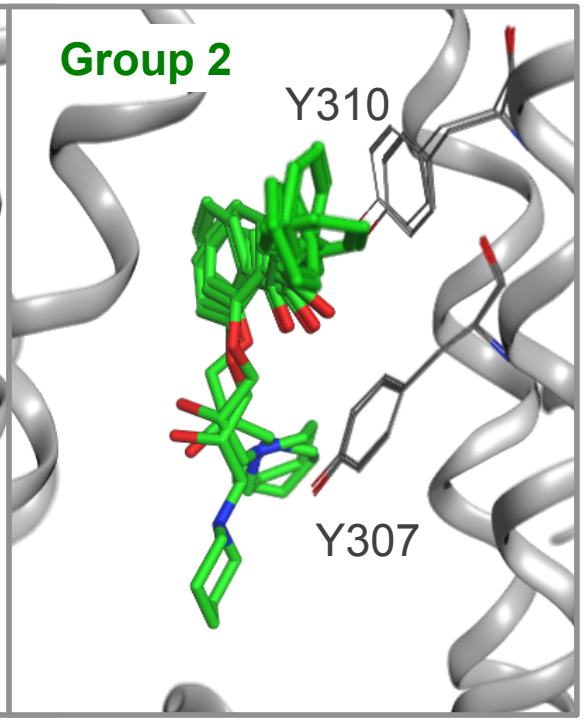
Docking results

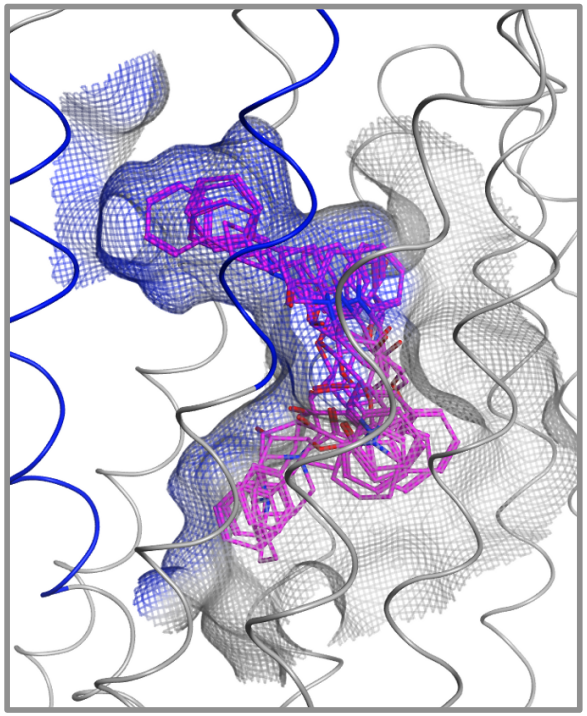


2HYD_Pgp (nucleotide bound)

- 8 CSC → 2 groups
- Group 1 ⬅➡ Group A (Y310)
- In vicinity of photo-affinity labeled residues



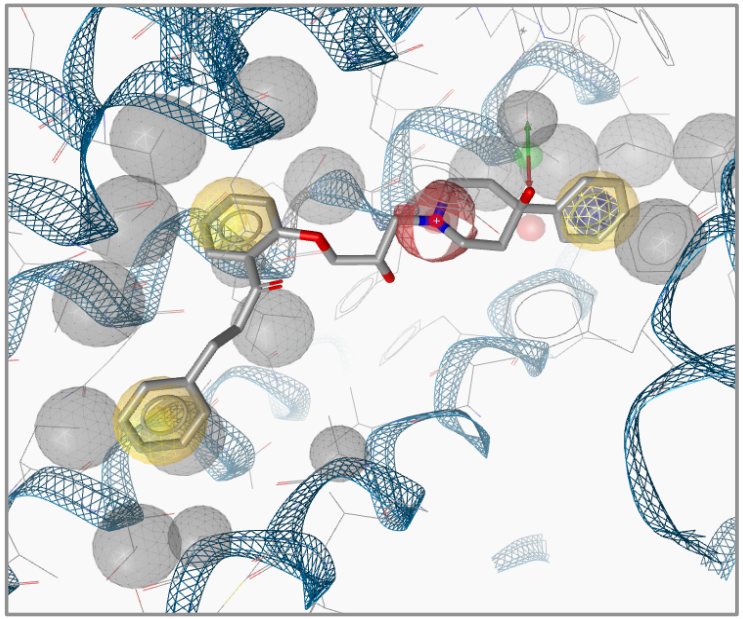


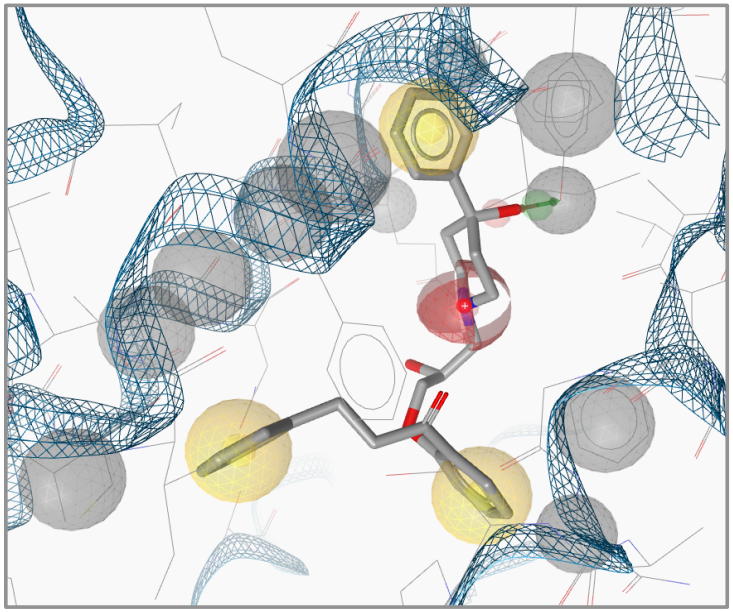


Pharmacophore modeling

Templates for pharmacophore modeling

- Pgp_apo: pose interacting with Y310
- Pgp_nbd: Group 1





→ Hits: structurally diverse P-gp substrates (verapamil)

Conclusions and Outlook

- Docking results are in agreement with ligand based studies (SAR) and experiments (photoaffinity labeled residues)

  ➔ Confirmation by MD simulations
- There are corresponding clusters for both models (apo and nucleotide bound) ⟶ propafenone transport?

  ➔ Steered MD simulation
- Pharmacophore model represents binding competent pose

  ➔ Identify new P-gp hits
- H-bond between Y310 and ligand

  ➔biochemical experiments in progress

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Thank you for your attention!

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FWF Der Wissenschaftsfonds.

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