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The role of drug transport proteins in drug absorption and tissue distribution

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Pharmacy
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Sweden

Reaching the Young
Scientist eSI workshop,
29–30 September,
2006, Alicante, Spain





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To follow your peer...





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...can be risky...





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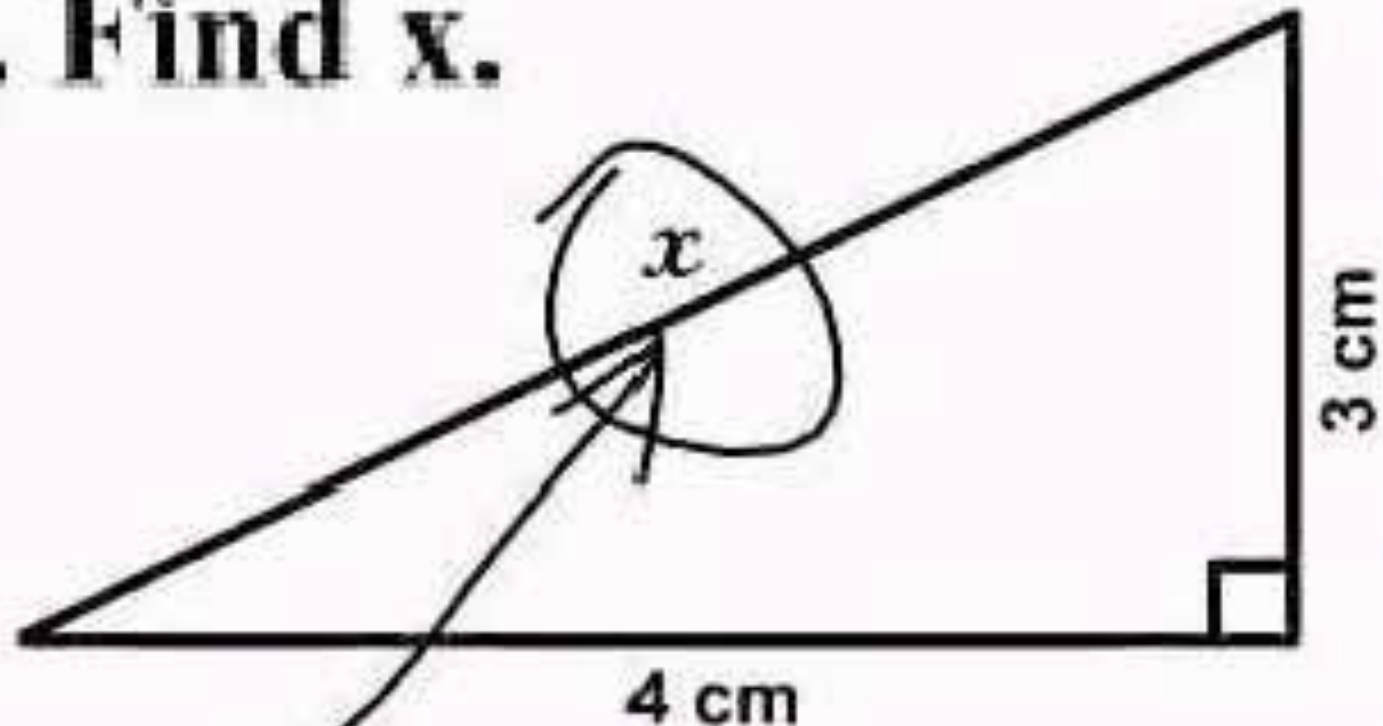




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There are easier ways...

3. Find x .



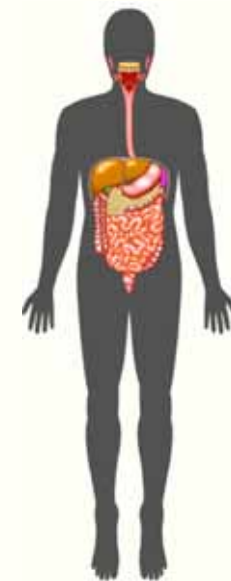
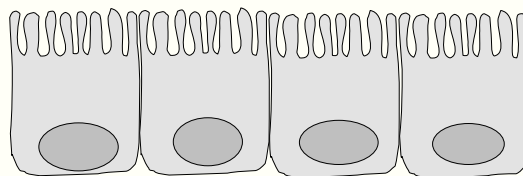
Here it is



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Outline

- Background
- Mapping of transporter distribution
- Transport proteins and drug absorption
- Mapping of drug-transporter interactions

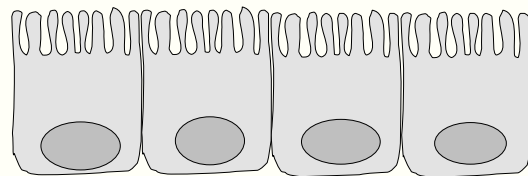




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We develop in vitro and in silico assays that predict drug absorption and transport

**In vitro
screening**



Hypothesis

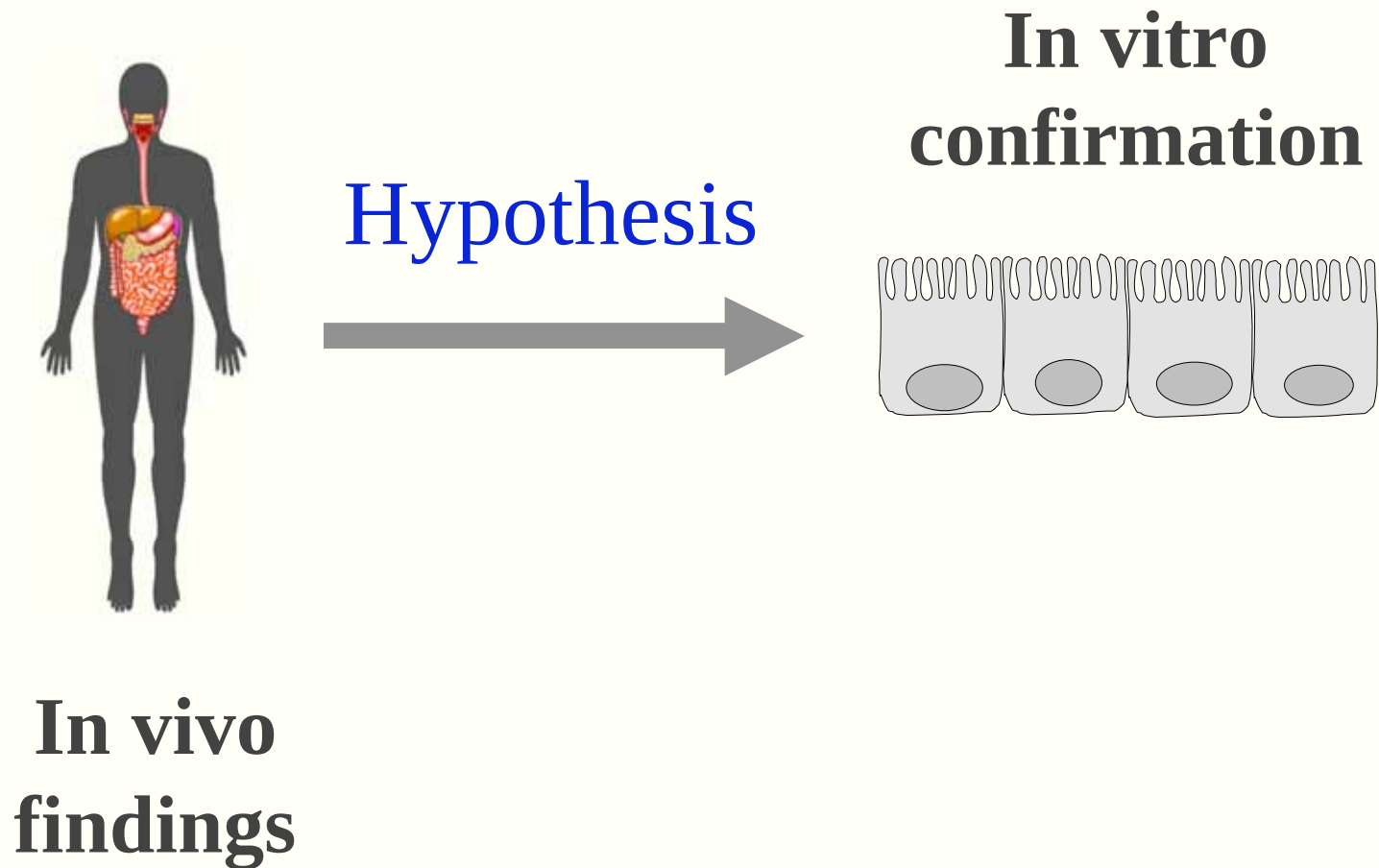


**In vivo
confirmation**



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Sometimes we help drug and biotech companies

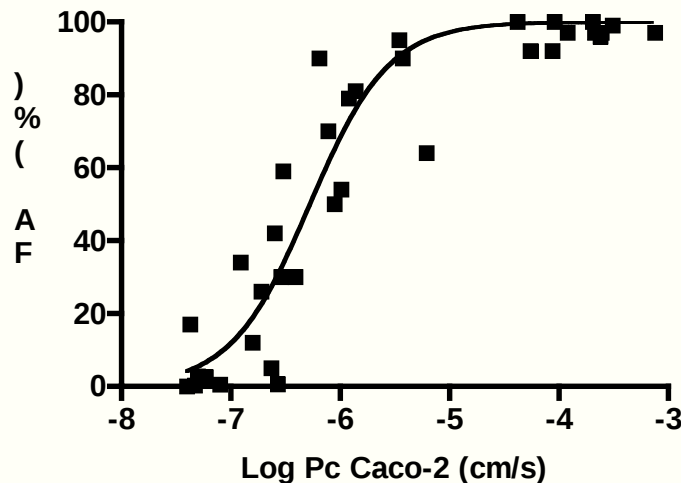




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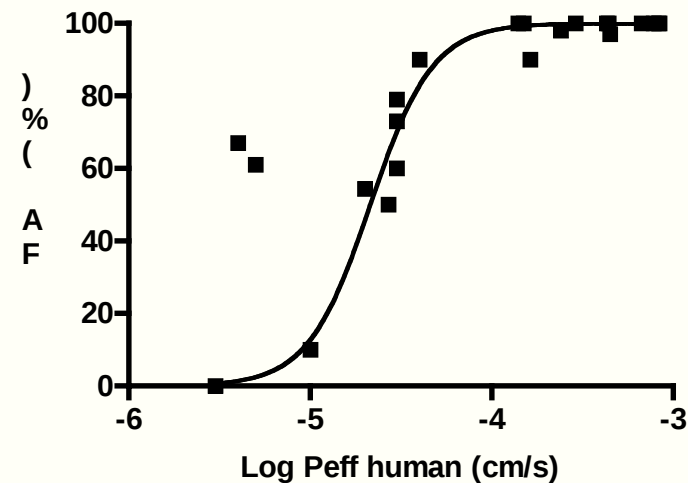
Prediction of oral absorption from in vitro permeability studies

Caco-2



Data compiled from
Arturssons' laboratory

Human perfusion



Data compiled from
Lennernäs' laboratory

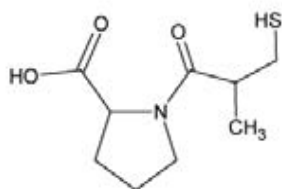


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Computational models

Generation of molecular descriptors

Two-dimensional (2D)
structure representation

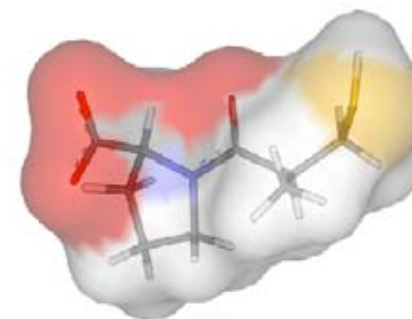


Molecular weight
of H-bond donors
of H-bond acceptors
etc...

Three-dimensional (3D)
energy-minimized structure



Molecular surface
calculation



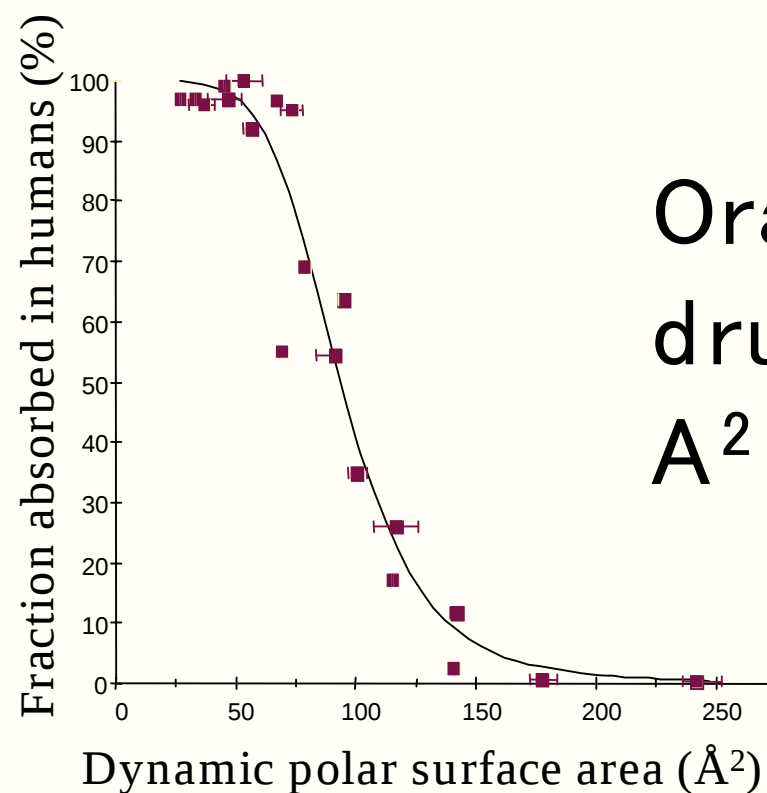
PSA
NPSA

PTSAs: C-sp², C-sp³, double bonded oxygens, etc...



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Polar Surface Area vs. drug absorption in humans



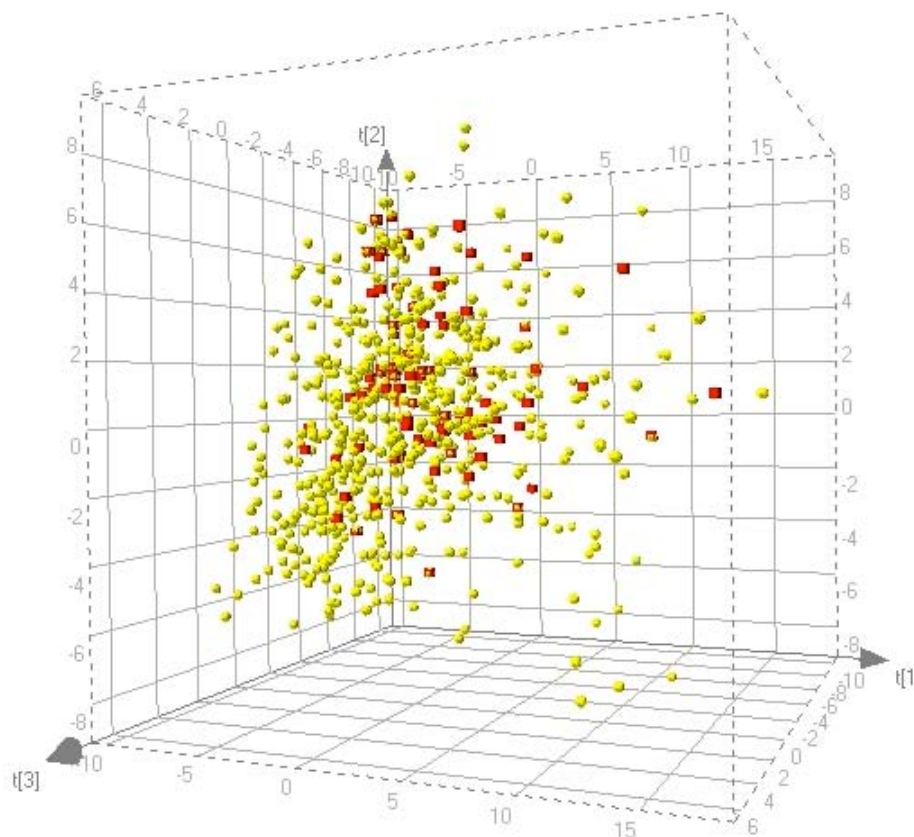
Orally available
drugs: < 120
 $\text{\AA}^2$

Palm et al. *Pharm. Res.* 1997; *J Med Chem* 1998
Stenberg et al. *J Med Chem* 2001



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We work in the oral drug space



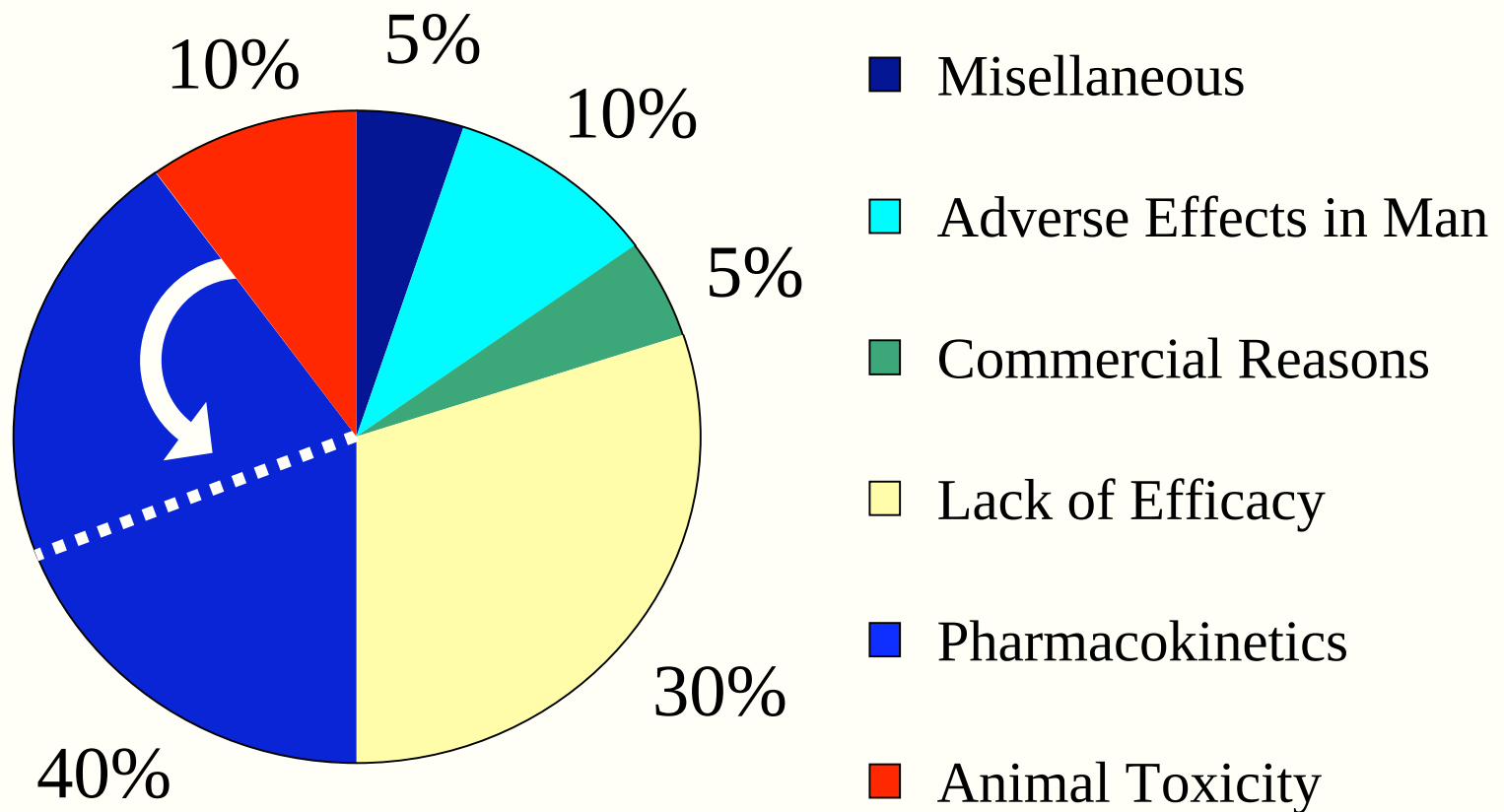
- Property space of oral drugs
- Property space of data set of drugs

Bergström C.A.S *et al.*, *J. Chem. Inf. Comput. Sci.*, 2004
Matsson *et al.* *J. Med. Chem.*, 2005



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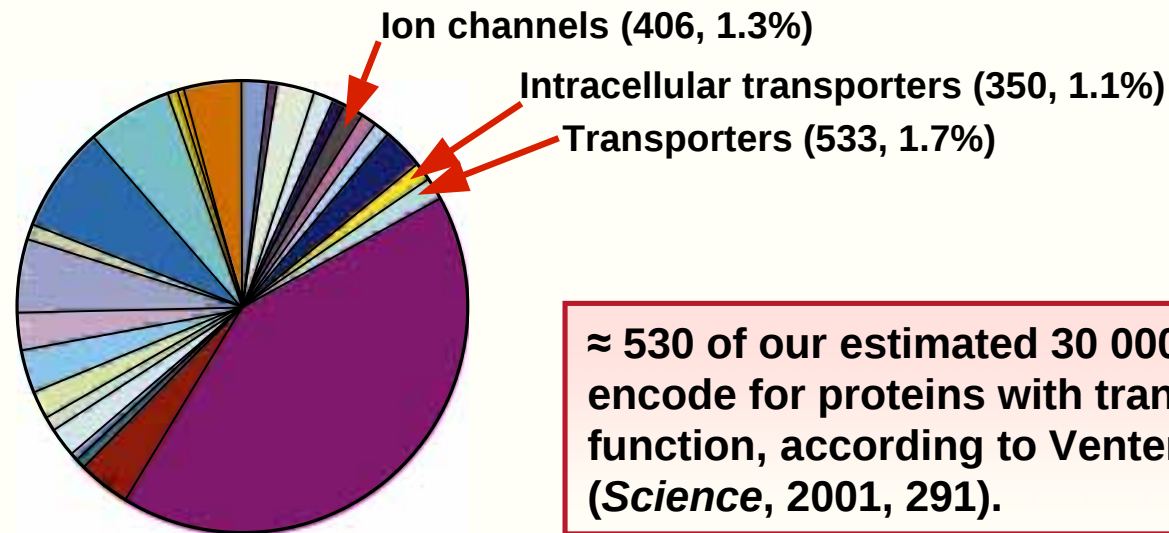
Reasons for Discontinuation of Clinical Development





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Transport proteins and the human genome



Why do we need to consider transport proteins?

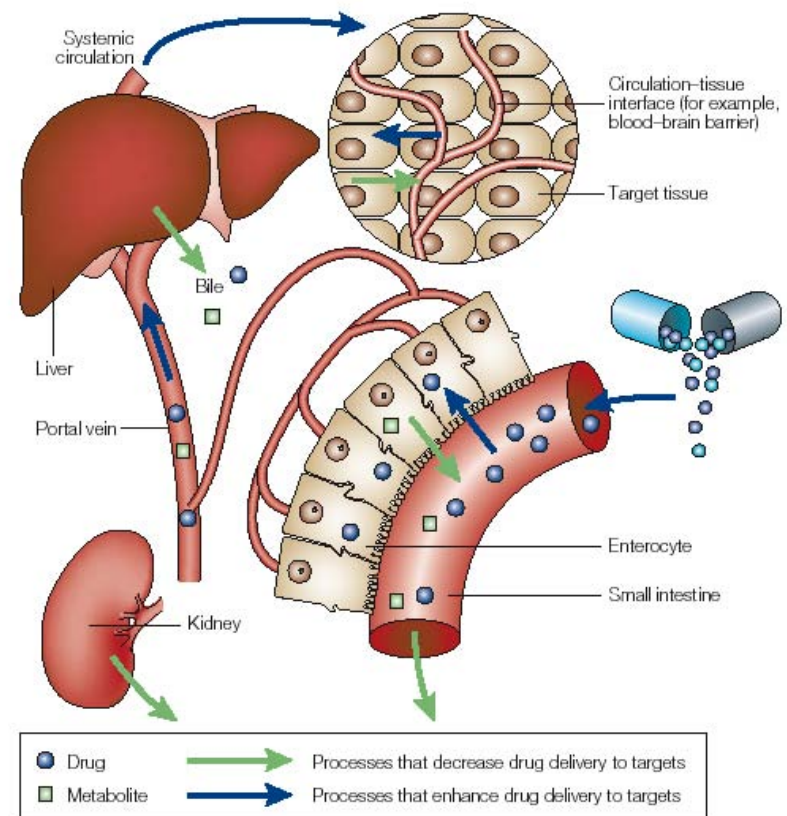
- ✓ Transport proteins are expressed in tissues involved in drug disposition
- ✓ A vast number of drugs are substrates for transport proteins
- ✓ Genetic variation in genes encoding transport proteins can affect functionality and/or expression levels
- ✓ Transport proteins can be inhibited or induced by drugs



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Importance of transporters

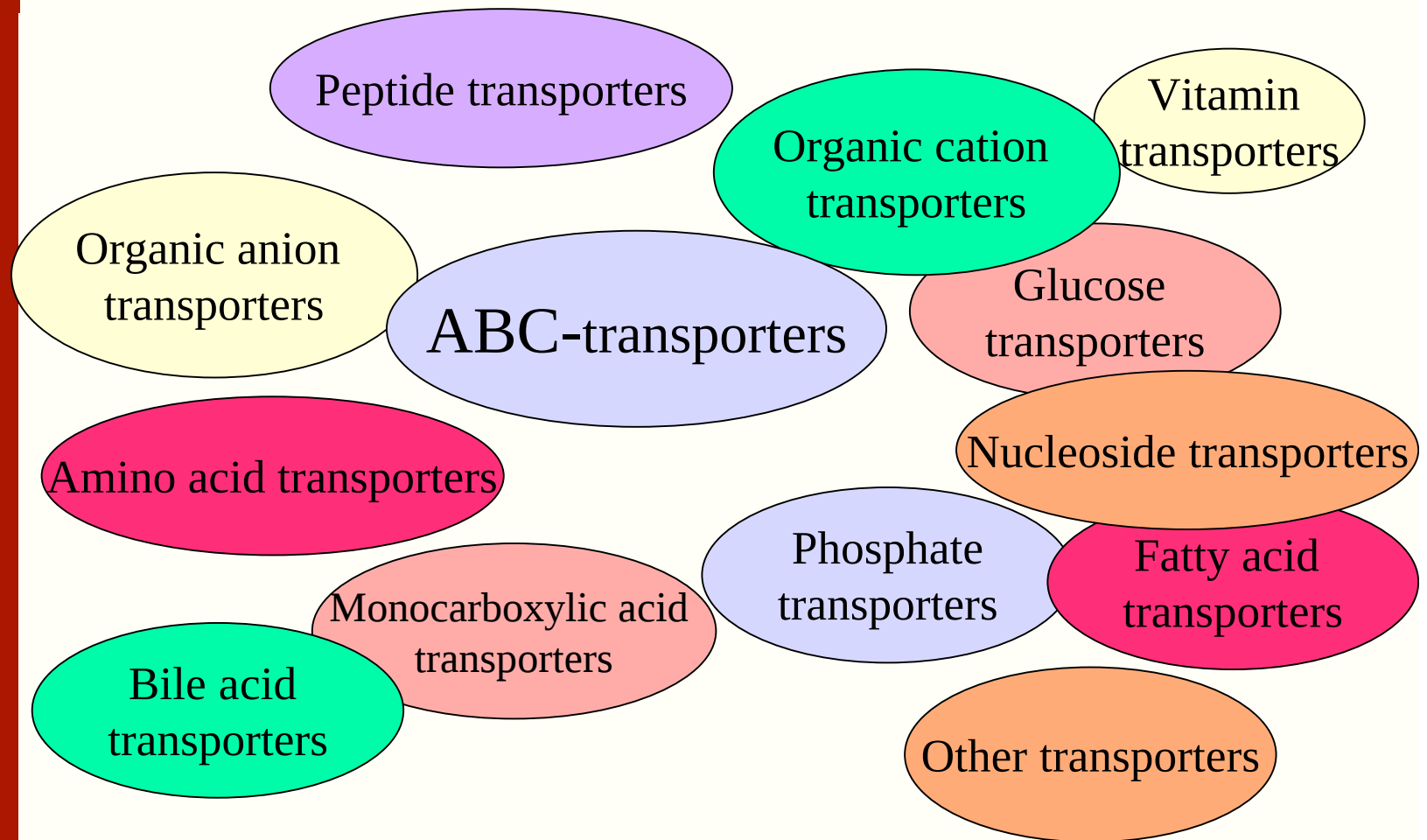
- A: Absorption
- D: Distribution
- M: Metabolism
- E: Elimination
- T: Toxicity





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Functional names on transporter families



- Pharmaceutical relevance?



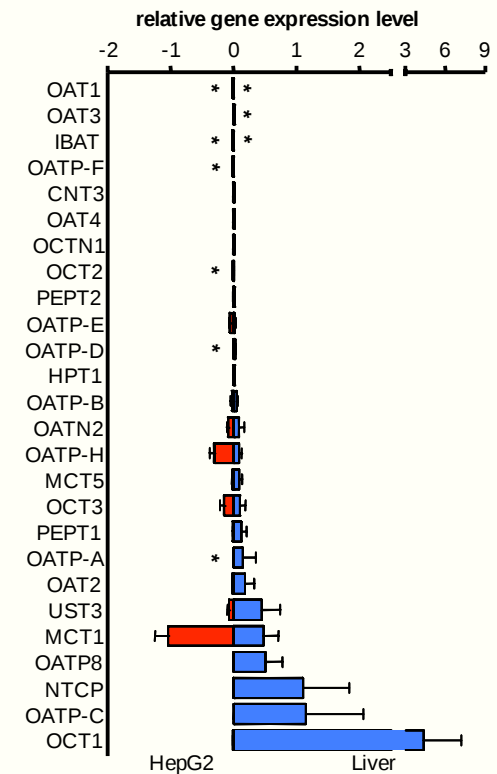
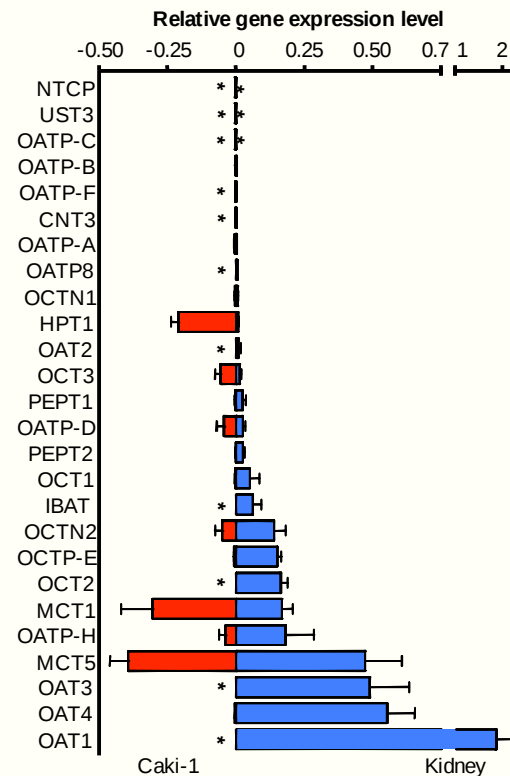
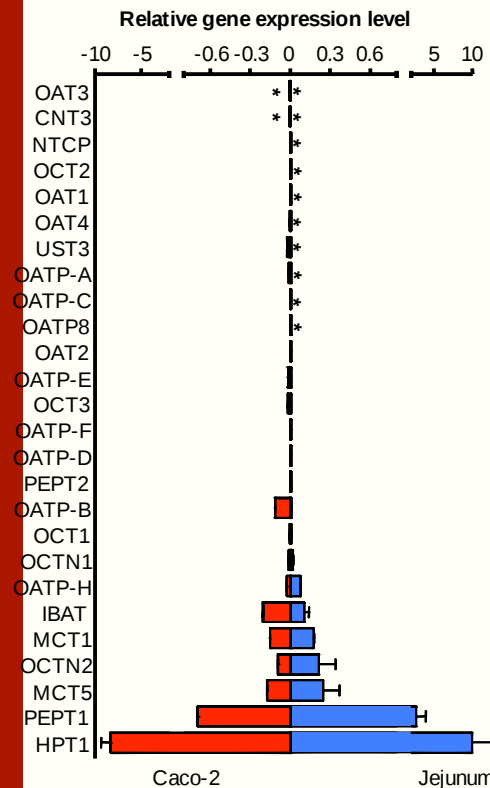
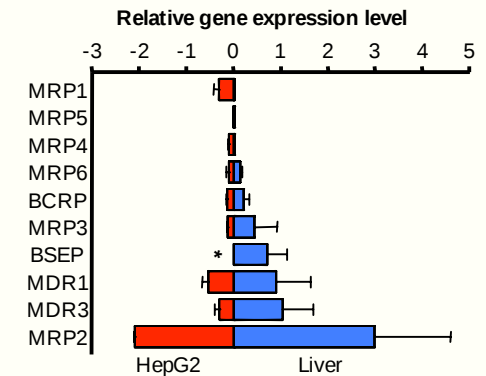
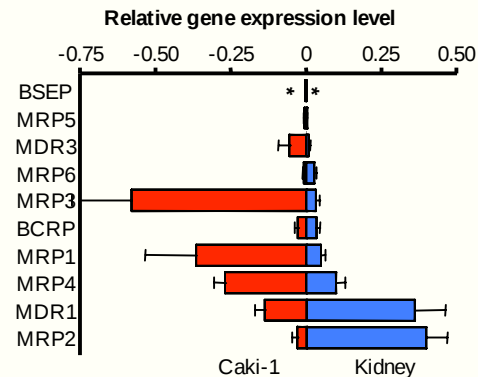
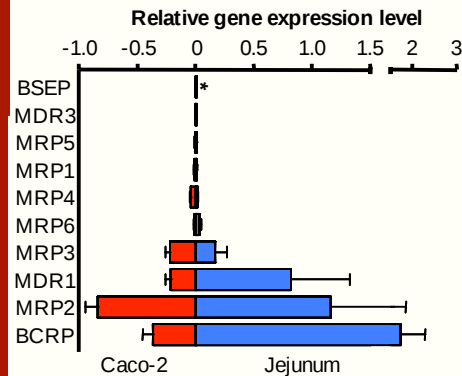
Transporter distribution?

- Quantitative gene expression of 36 transporters in human liver, kidney and intestine
- Gene expression was also investigated in organotypic human cell lines: Caco.2 (intestine), HepG2 (liver) and Caki-1 (kidney)
- Immunocytochemistry of 40 transporters in 50 human organs and 10 tumour tissues (9 delivered so far)



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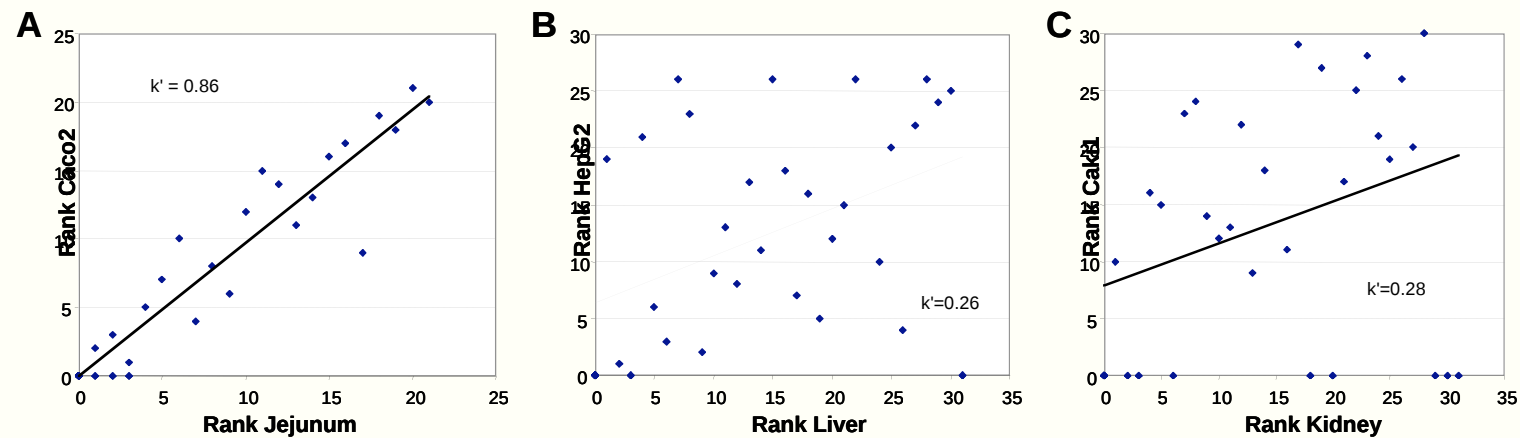
Expression of transport proteins in the human liver, kidney and intestine (*Hilgendorf, C. et al., in manuscript, 2006*)





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Tissue-cell line correlations

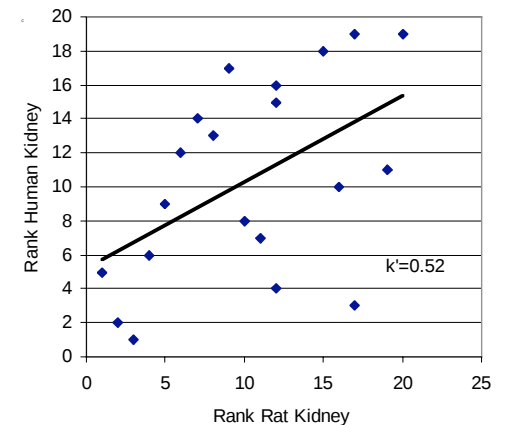
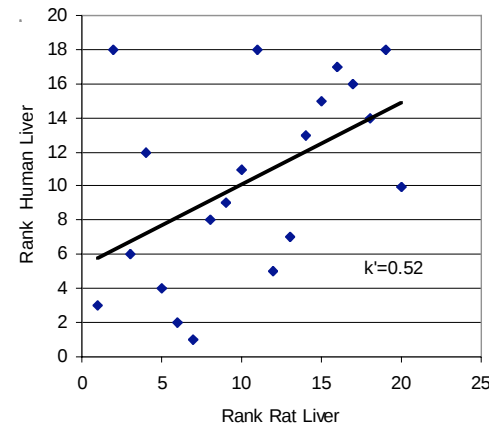
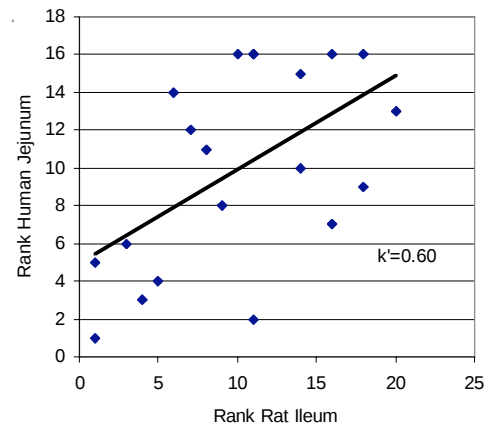


Human cell lines vs. Human tissue



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Tissue-tissue correlations



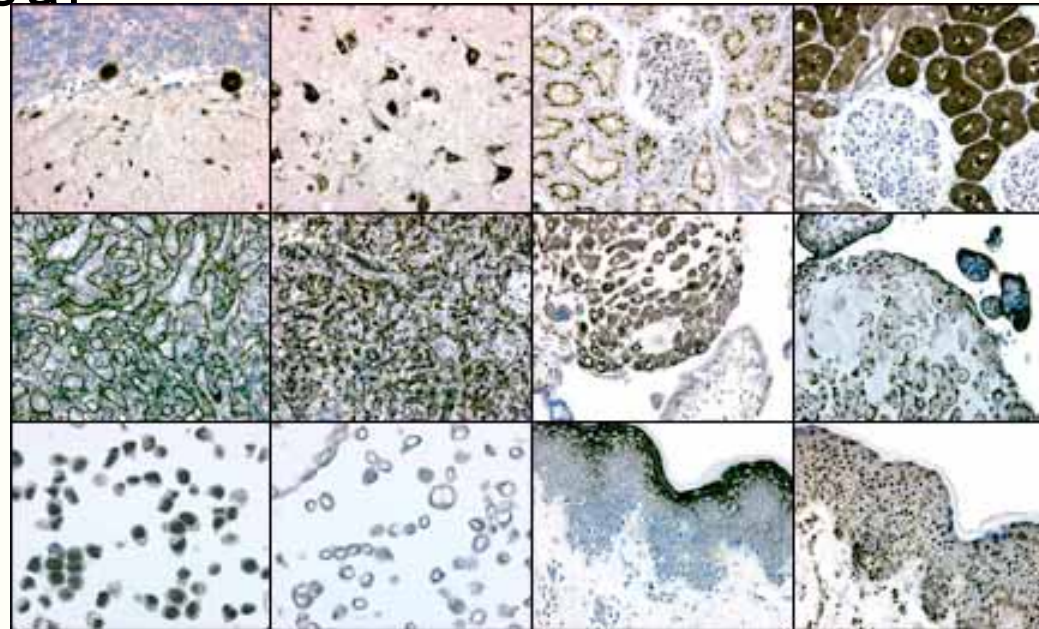
Rat tissue vs. Human tissue



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Human transporter proteome

- Antibodies generated to 40 human drug transporters
- Tissue array expression data within a year





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The human protein atlas

Human Protein Atlas - Microsoft Internet Explorer provided by Institutionen för Farmaci

File Edit View Favorites Tools Help

Back Forward Stop Search Favorites Home

Address http://www.hpa.se/tissue_profile.php?antibody_id=1716 Go Links

hpa about the project about protein atlas tissue dictionary disclaimer submission of antibodies

ABCB1 tissue profiles. Validation score: N/A

Gene data

Description: **Multidrug resistance protein 1 (P-glycoprotein 1) (CD243 antigen).**
Source: **P98183 (Uniprot)**
Chromosome: **7p21.12**
Ensembl ID: **ENSG0000005563**

Protein ID: **ENSP00000265724** Transcript ID: **ENST00000265724** No. of ex: **1200** Size: **141 kDa** Signal Peptide: **No** TM Region(s): **Yes**

Splice variant 1:

Normal Tissues

Tissue	Staining	Cell Type
Adrenal gland	Strong staining	cortical cells
Appendix	Medium staining	glandular cells
Bone marrow	Medium staining	bone marrow poetic cells
Breast	Medium staining	glandular cells
Bronchus	Medium staining	surface epithelial cells
Cerebellum	Medium staining	cells in granular layer
Cerebral cortex	Medium staining	pyramidal cells
Cervix, uterine	Medium staining	non-neuronal cells
Colon	Medium staining	glandular cells
Duodenum	Medium staining	glandular cells
Endometrium 1	Medium staining	cells in endometrial stroma/ECM
Endometrium 2	Medium staining	cells in myometrium/ECM
Endometrium 3	Medium staining	cells in endometrial stroma/ECM
Epididymis	Medium staining	glandular cells
Esophagus	Medium staining	surface epithelial cells
Fallopian tube	Medium staining	glandular cells
Gall bladder	Medium staining	myocytes
Heart muscle	Medium staining	myocytes
Hippocampus	Medium staining	non-neuronal cells
Kidney	Medium staining	cells in glomeruli
Lateral ventricle	Medium staining	cells in tubuli
Liver	Medium staining	non-neuronal cells
Lung	Medium staining	alveolar cells
Lymph node	Medium staining	follicle cells (cortex)
Nasopharynx	Medium staining	non-follicle cells (paracortex)
Oral mucosa	Medium staining	surface epithelial cells
Ovary	Medium staining	follicle cells
Pancreas	Medium staining	ovarian stromal cells
Parathyroid gland	Medium staining	endocrine pancreas
Placenta	Medium staining	islet cells
Prostate	Medium staining	glandular cells
Rectum	Medium staining	glandular cells
Salivary gland	Medium staining	glandular cells
Seminal vesicle	Medium staining	glandular cells
Skeletal muscle	Medium staining	myocytes
Skin	Medium staining	adipose cells
Small intestine	Medium staining	epithelial cells
Smooth muscle	Medium staining	glandular cells
Soft tissue 1	Medium staining	smooth muscle cells
Soft tissue 2	Medium staining	mesenchymal cells
Spleen	Medium staining	mesenchymal cells
Stomach 1	Medium staining	cells in red pulp
Stomach 2	Medium staining	cells in white pulp
Testis	Medium staining	glandular cells
Thyroid gland	Medium staining	cells in ductus semisterus
Tonsil	Medium staining	lymphoid cells
Urinary bladder	Medium staining	glandular cells
Uterus	Medium staining	follicle cells (cortex)
Vagina	Medium staining	non-follicle cells (paracortex)
Vulva/anal skin	Medium staining	surface epithelial cells

Navigation

Home

Search results

ABCB1 tissue profiles

Antibody info

Search

1 1.4

2 1.5

3 1.6

4 1.7

5 1.8

6 1.9

7 2.0

8 2.1

9 2.2

10 2.3

11 2.4

12 2.5

13 2.6

0 tests

search

Strong staining

Medium staining

Weak staining

Negative staining

Not representative

help for this page

Annotation Summary

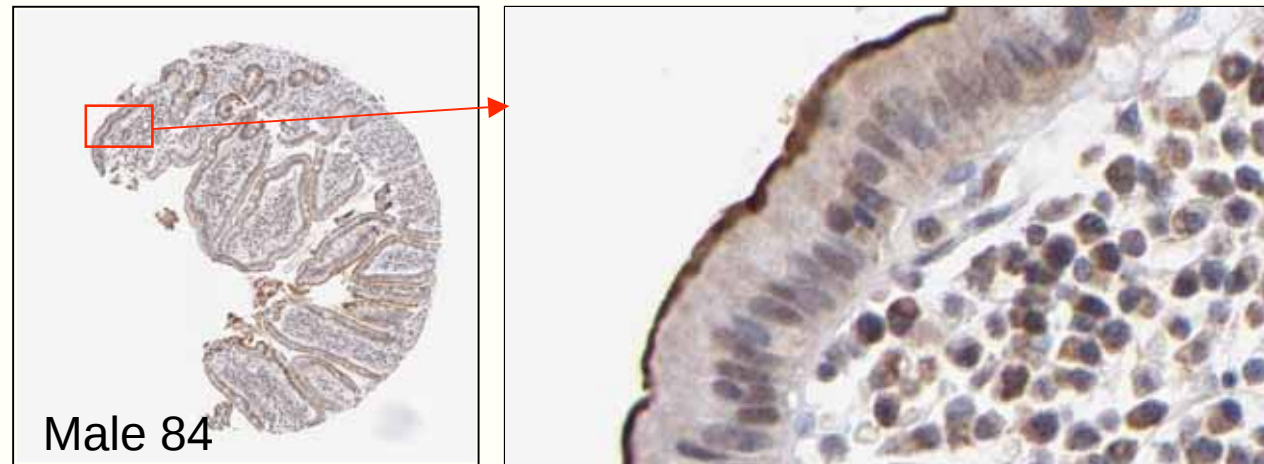
Monoclonal antibody data is shown in red and polyclonal antibodies in green. Black non-neuronal antibodies are not shown.

Done Internet



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Apical expression of PEPT1 in human duodenal enterocytes

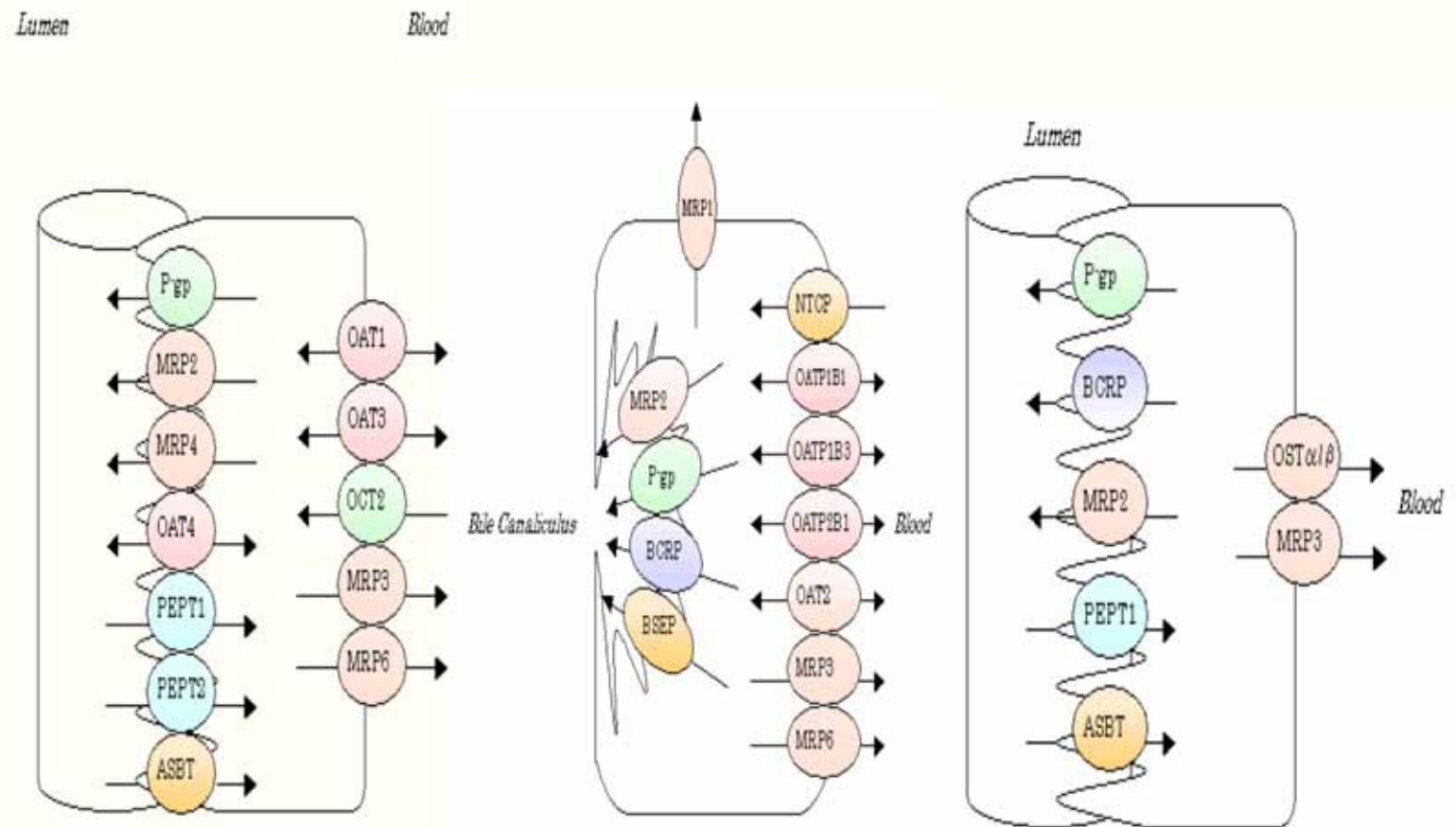


- Moderate intensity
- >75% cells stained
- Localization:
 - Cytoplasmic/Membranous



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Own data combined with literature data identifies important transporters in each organ

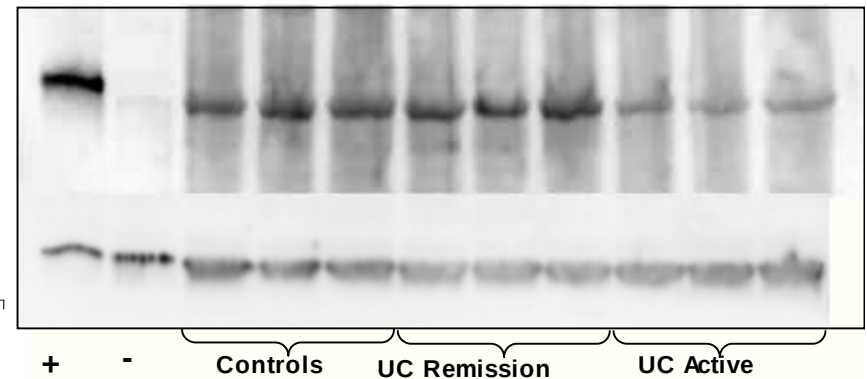
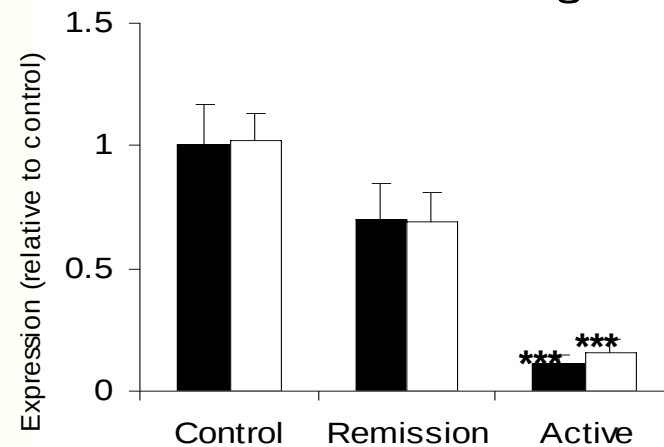




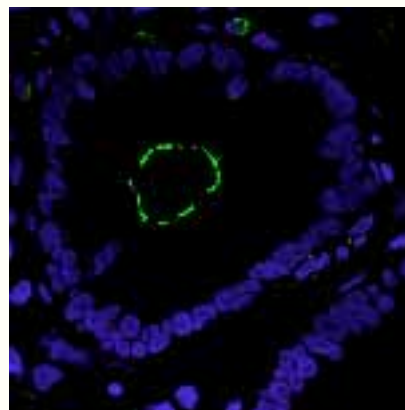
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Altered expression of transporters in disease – BCRP in IBD

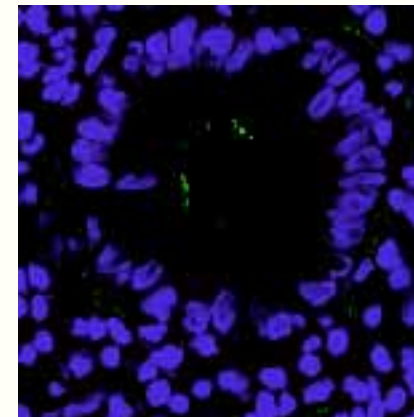
Englund et al. *Inf/Bowel Dis*, in press



Control



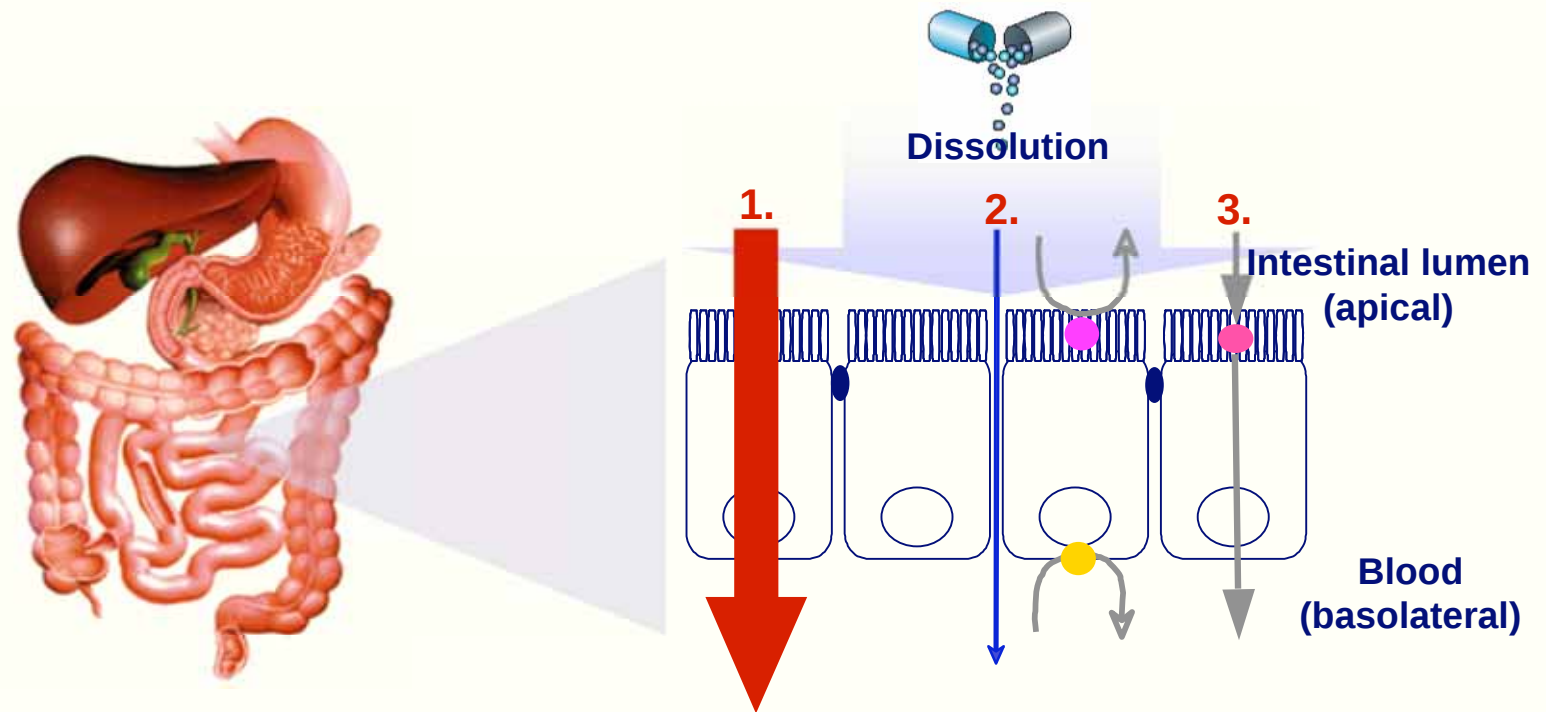
Active.





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It is now well recognized that absorption of drugs often involves one or more **active transport** mechanisms



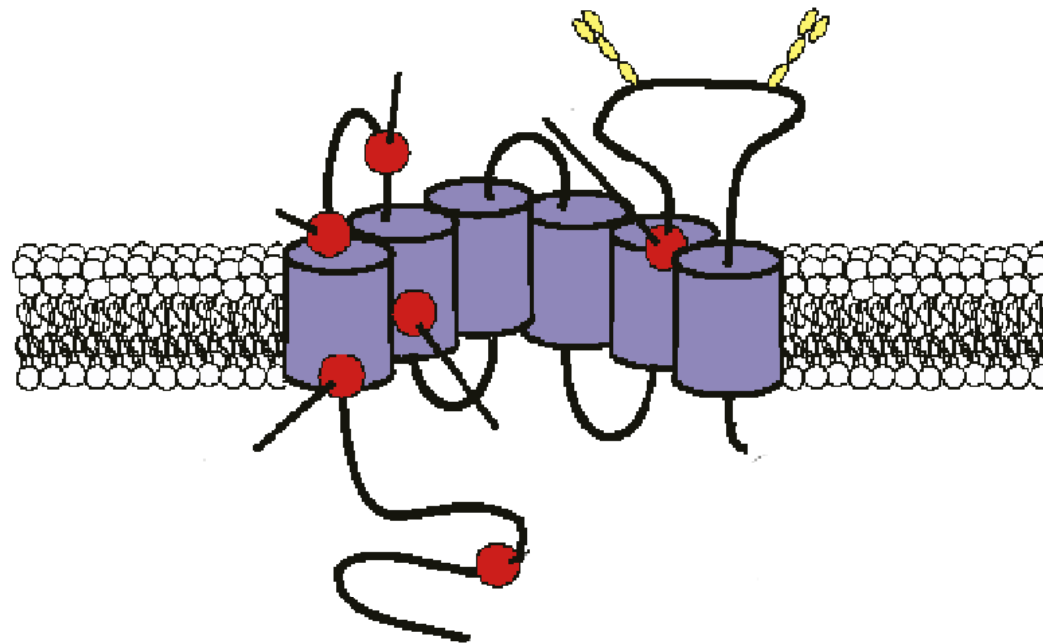
Drugs cross the intestinal epithelium by:

1. Passive transcellular diffusion
2. Passive paracellular diffusion
3. Active transport mechanisms



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Role of active transport in drug absorption



Patient studies

Englund et al. *BMC Med.* 2004, 2:8



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Digoxin – a Pgp-substrate with a narrow therapeutic range



- At Uppsala University Hospital ~2000 digoxin analyses are performed in therapeutic drug monitoring (TDM) every year.
- Digoxin is a good substrate for Pgp (Tanigawara Y et al., *J Pharmacol Exp Ther* 1992; 263: 840-845).

Does co-administration of other Pgp-interacting drugs increase the plasma concentration of digoxin?

Englund et al. *BMC Med.*, 2004, 2:8



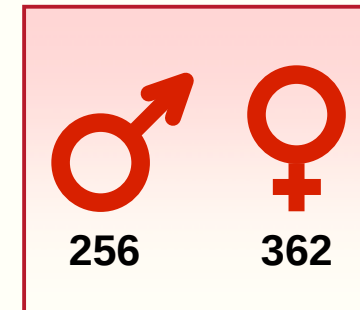
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The study population was very heterogenous,
including patients with heart failure, atrial flutter
and fibrillation

CHARACTERISTIC	TDM PATIENTS
Age (years)*	84 (24-99)
P-creatinine (mmol/L)*	100 (36-598)
Daily digoxin dose (mg)*	0.13 (0.04-0.5)
Concomitant drugs*	5 (1-21)

* Median (range)

Patients below therapeutic range (<1.2 nmol/L)	263
Patients within therapeutic range (1.2 – 2.5 nmol/L)	
317 Patients above therapeutic range (>2.5 nmol/L)	38



Will the recommended digoxin levels be revised downwards?

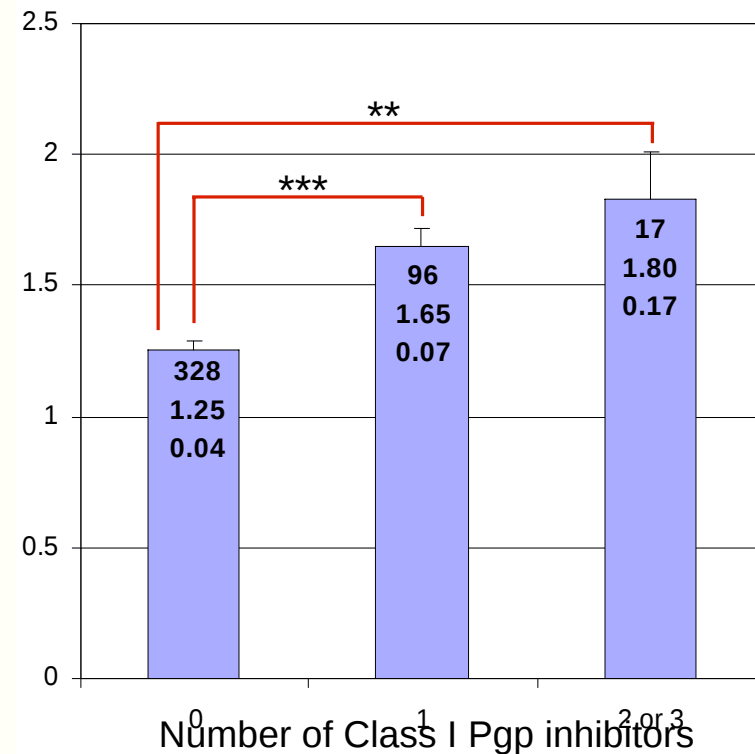
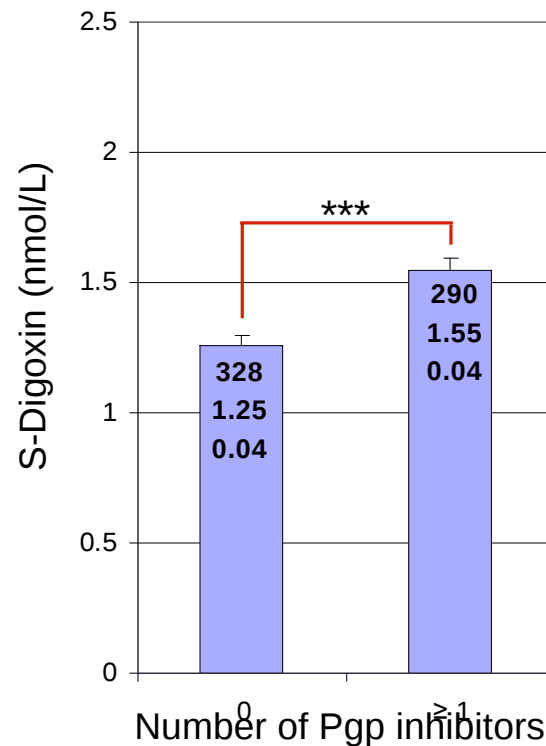
For men with heart failure the mortality rate increases for s-digoxin levels > 1.5 nmol/L. For this patient group the therapeutic range is suggested to be 0.6 - 1 nmol/L (Rathore SS *et al.*, *Jama* 2003; 289: 871-878).

Englund *et al.* *BMC Medicine*, 2004, 2:8



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Patients with any Pgp inhibitor prescribed had higher s-digoxin levels...



Values in bars Top: Patient frequency
Middle: S-Digoxin adjusted estimated mean
Bottom: SE

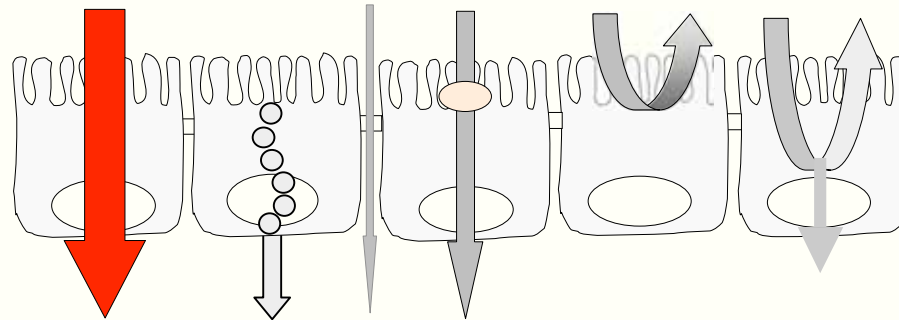
...and the s-digoxin level increased step-wise with an increasing number of Class I Pgp inhibitors

Englund et al. *BMC Medicine*, 2004, 2:8



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Is digoxin an exception?

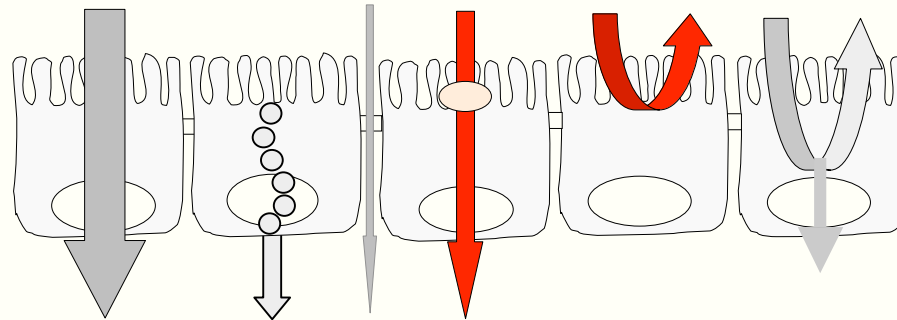


Orally administered drugs are mainly absorbed by the passive transcellular route.



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Role of different transport routes



How important are passive and
active transport routes,
respectively?



Data set

- $N = 30$
- 16 compounds: passive transport mechanisms
- 14 compounds were reported to be at least partly actively transported
- Substrates for "importers" and "exporters"
- PEPT1 (2), Pgp (6), OCT1, RFC, ENT2 (2), NAP1, MRP2, OAT, BCRP

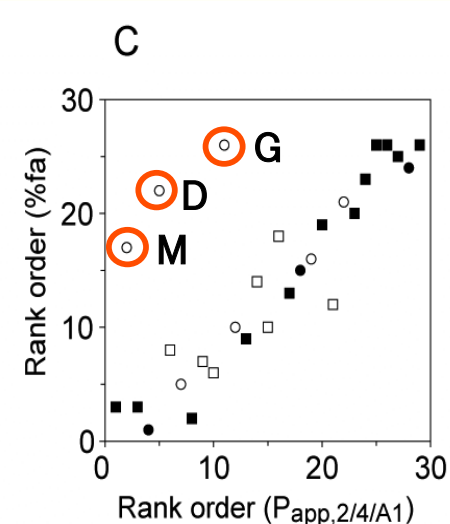
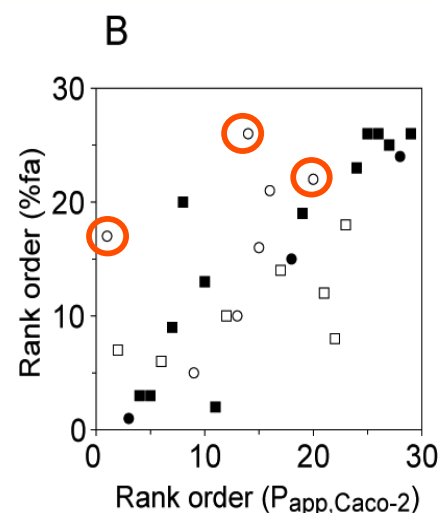
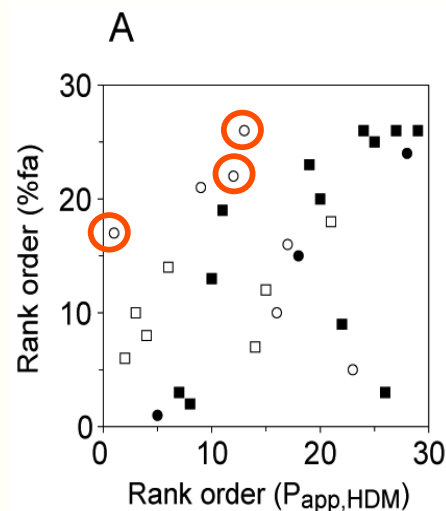
Matsson et al. *J.Med.Chem.* 2005, 48, 604–13



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Results:

Rank order correlations Papp vs FA



- HDM**
2/4/A1

- $r_s = 0.47$
(0.57)

- Caco-2**

- $r_s = 0.73$
(0.82)

- $r_s = 0.74$
(0.95)

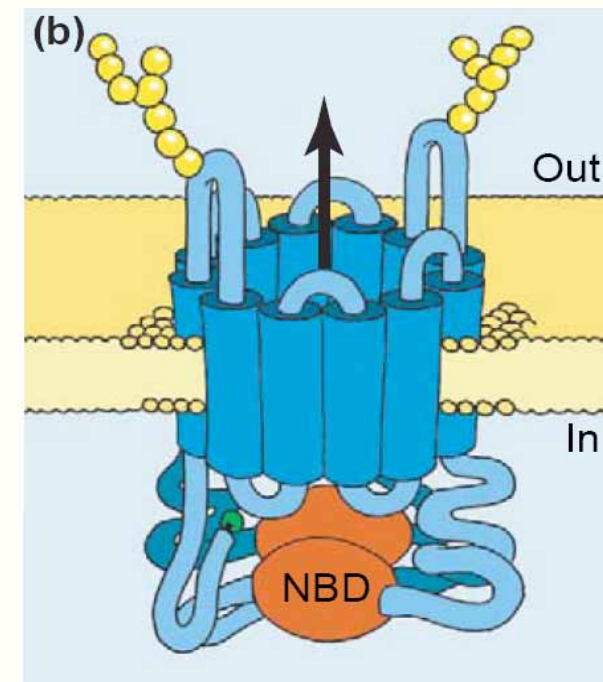
Figures in parentheses are correlation coefficients with outliers removed: Matsson et al. *J.Med.Chem.* 48, 604-613, 2005



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Mapping of transporter interactions - the first example

- ABCG2 (BCRP)
- ATP-binding cassette (ABC) transporter family
- Affects drug and toxin absorption, distribution etc

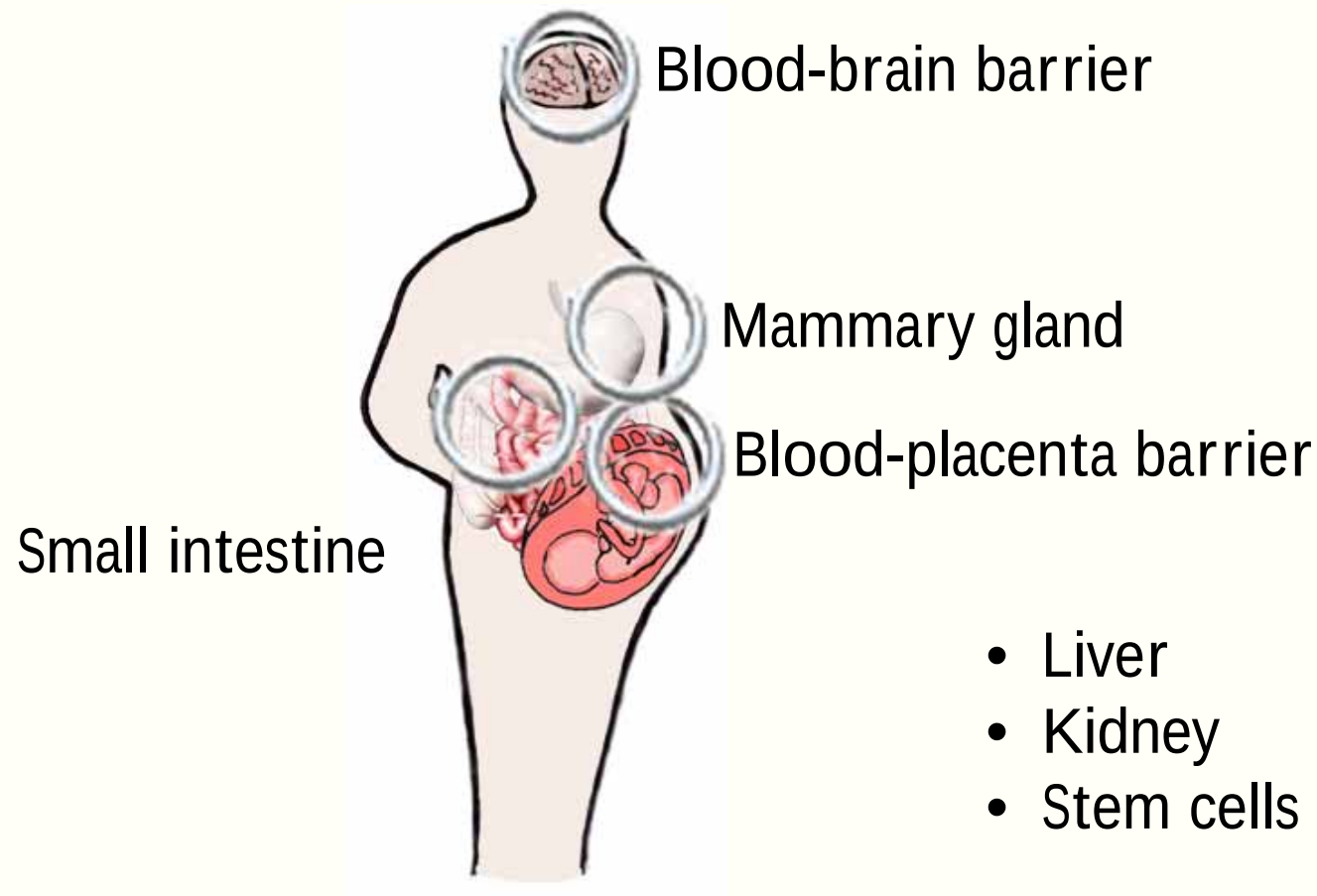


TIPS, 2005.



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ABCG2: Physiological function



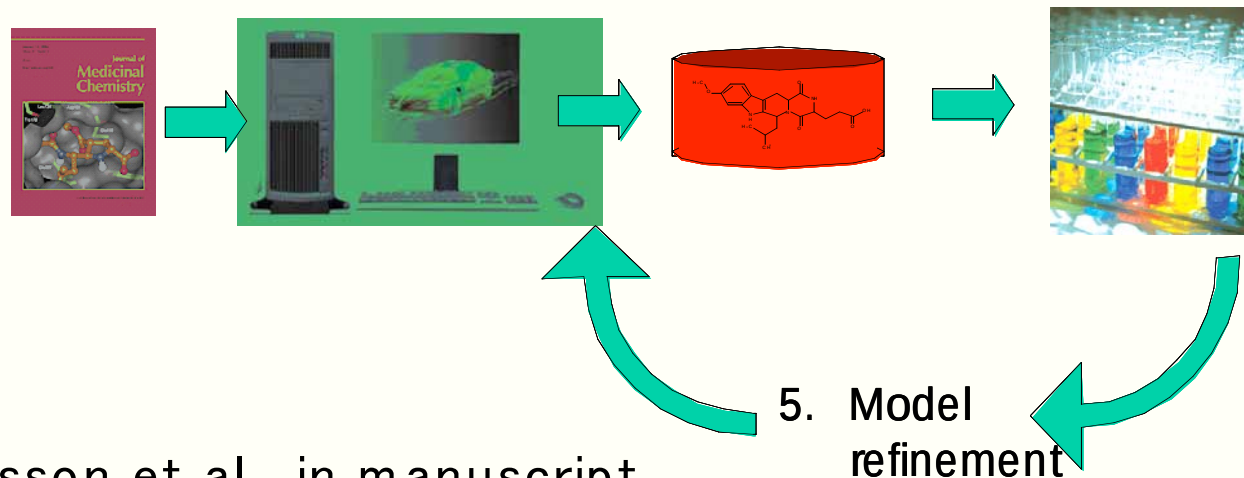


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Aim

- To predict ABCG2–drug interaction

1. Literature search
2. Initial 'crude' model
3. Database search
4. Experiments



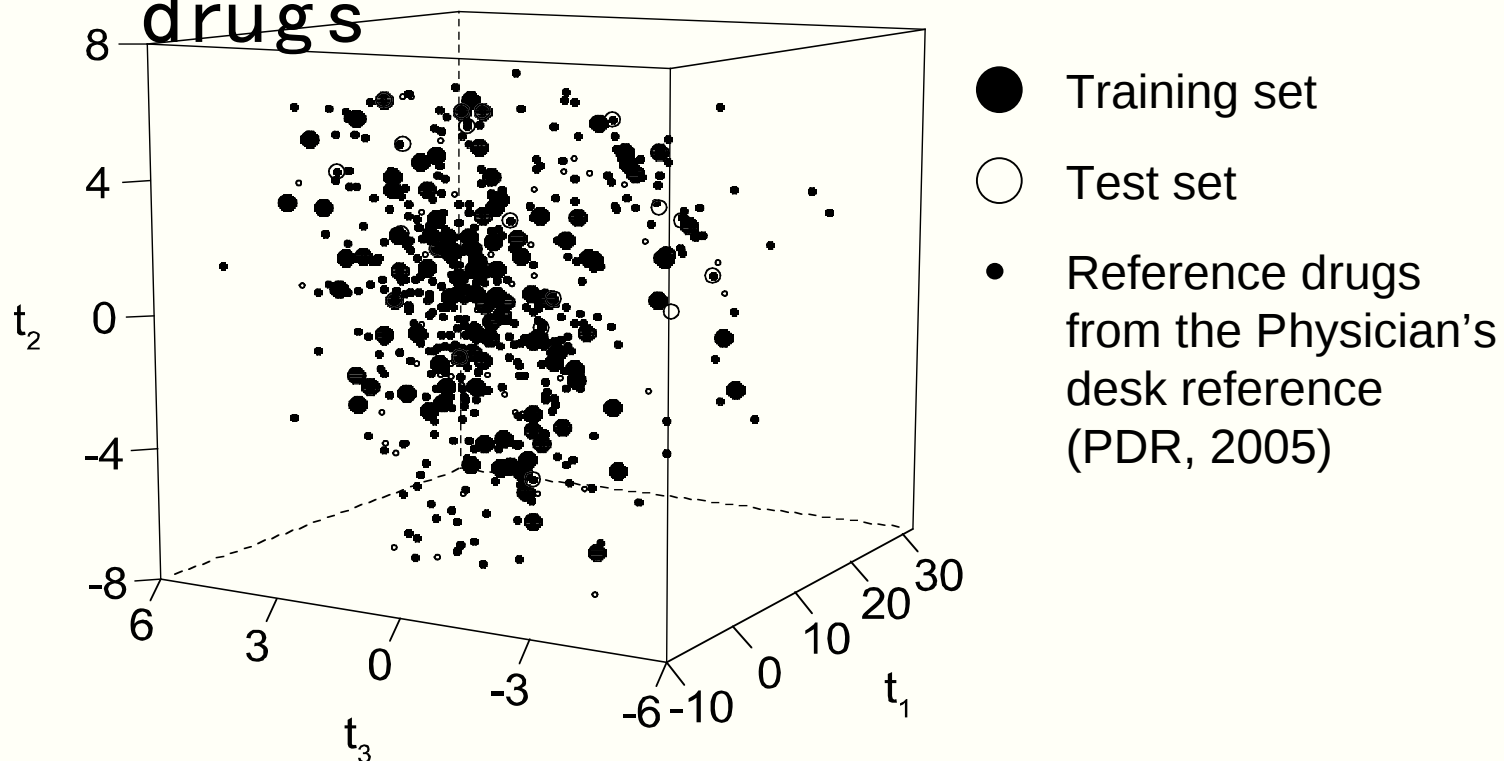
Matsson et al., in manuscript



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Chem GPS

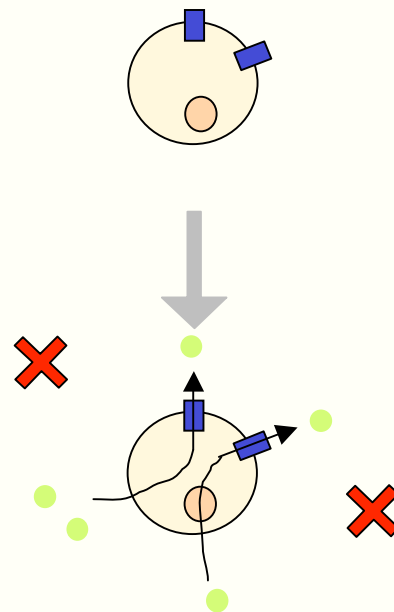
- The dataset is representative of registered low-molecular drugs



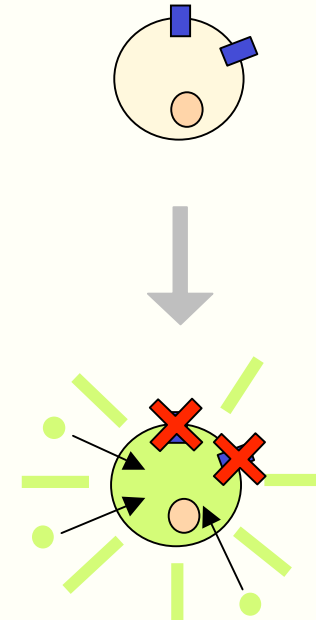


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Experimental assay



No inhibition:
substrate is effluxed
→ low intracellular
fluorescence

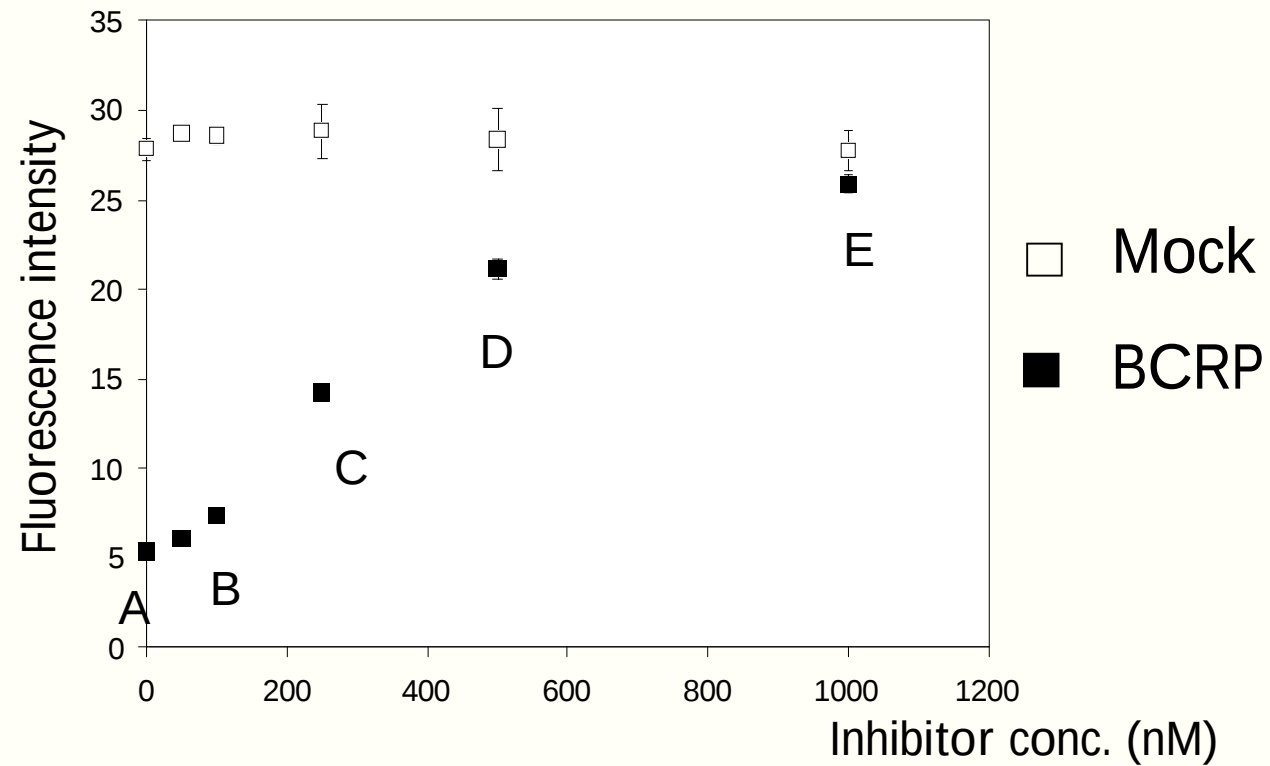


Inhibition:
substrate is retained in the
cells → high intracellular
fluorescence

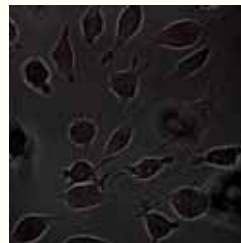


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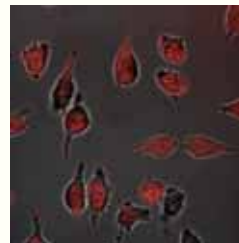
BCRP efflux inhibition: Ko143



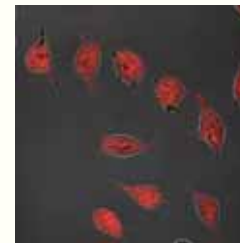
A



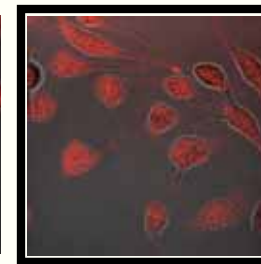
B



C



D

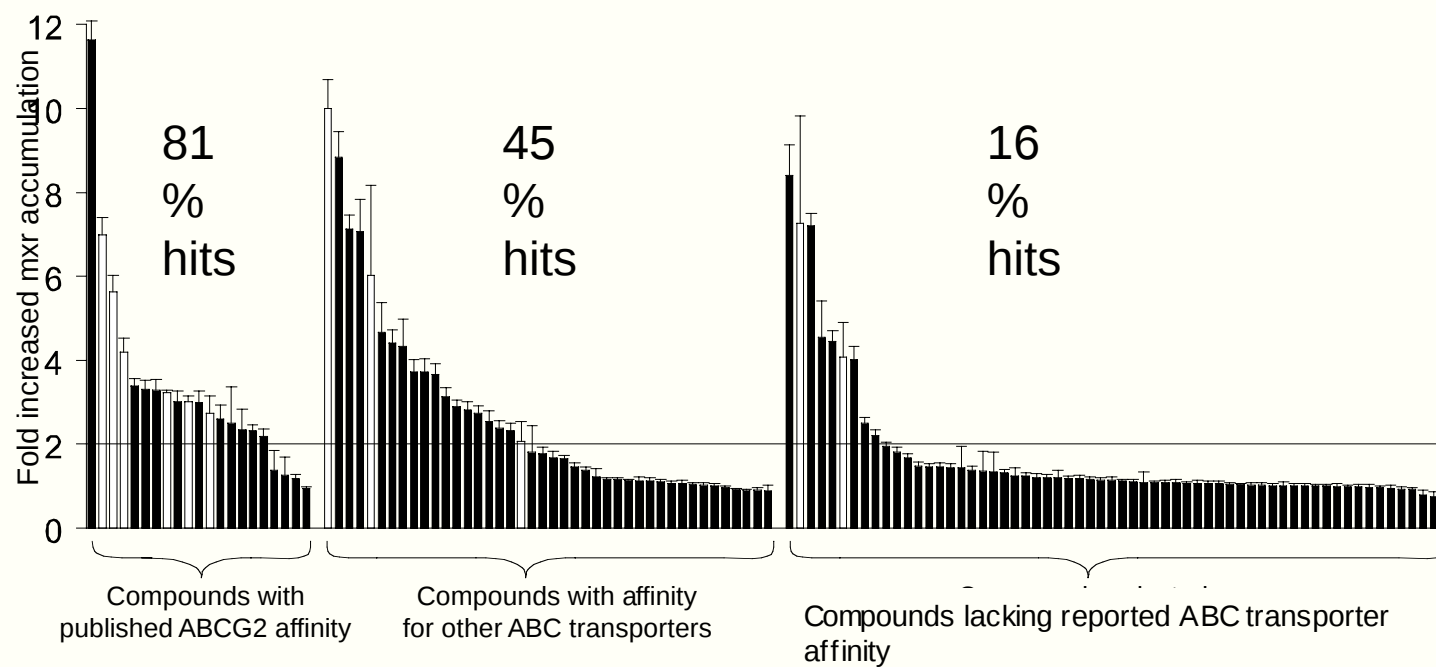


E



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BCRP inhibition



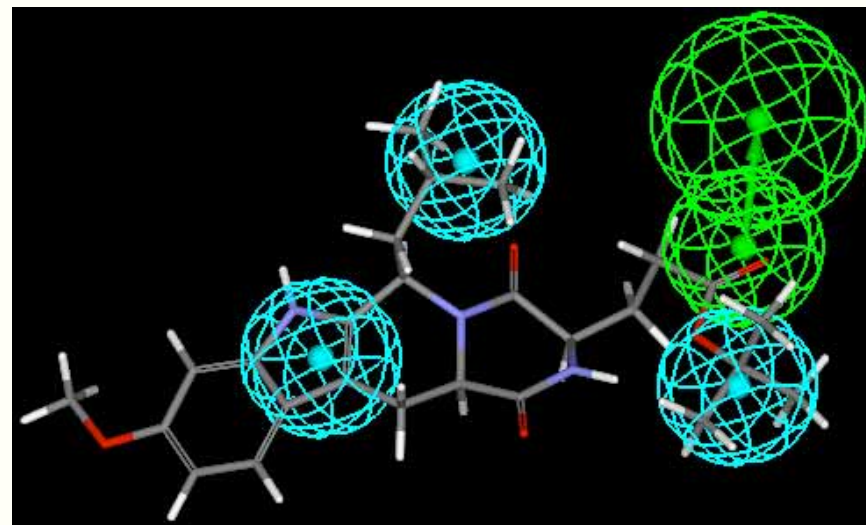


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Pharmacophore modelling

- Identification of all BCRP-interacting compounds, but...
- **also many false positives!**
- Hypothesis: influence of **passive**

permeability
Mansson et al manuscript
should be

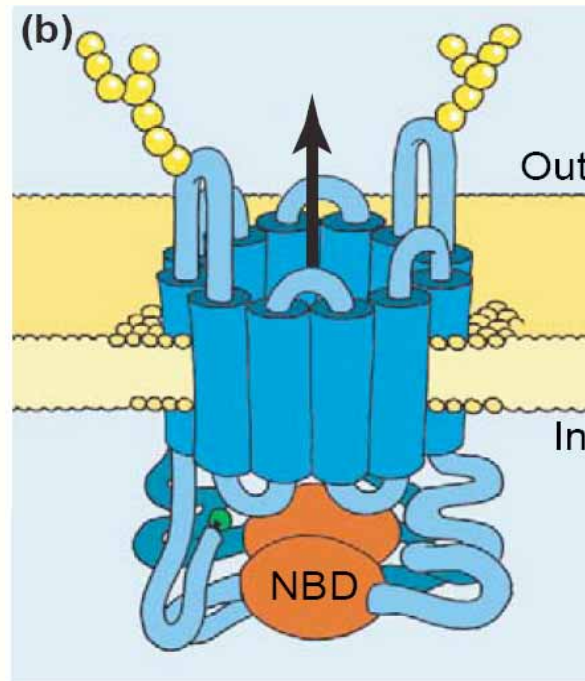


Ko143: Experimental result: 3.45x
Pharmacophore fit value: 3.45



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Influence of lipophilicity



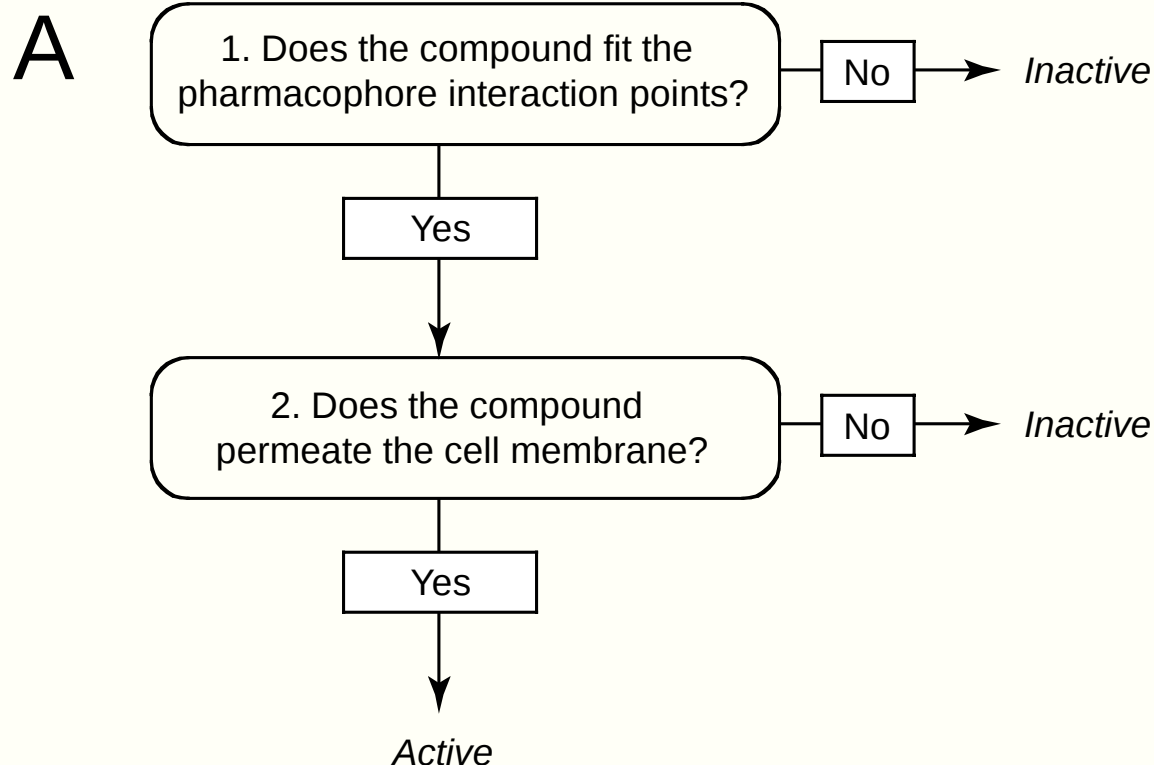
TIPS, 2005.

- Compounds need to enter the cell membrane to reach the intracellular or intramembraneous BCRP binding site



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Augumented pharmacophore



Step 1: Catalyst common features pharmacophore

Step 2: PLS-discriminant analysis

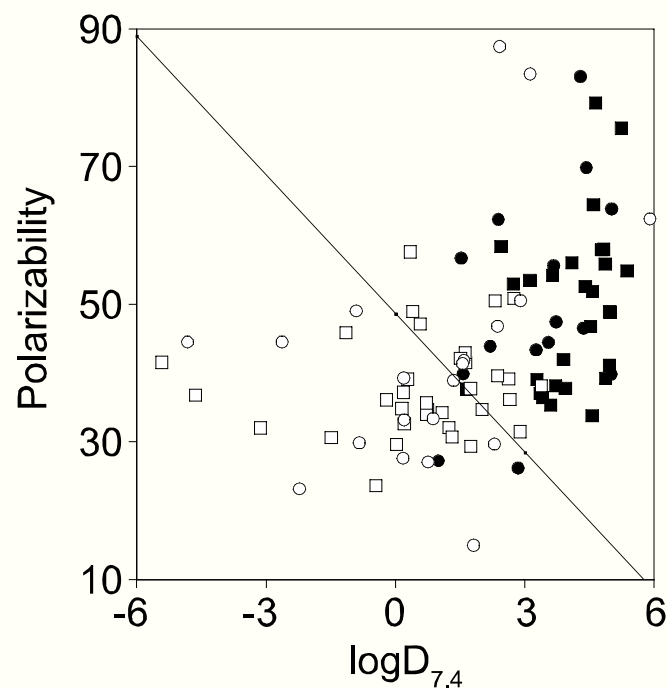
Matsson et al., in manuscript



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Results - augmented pharmacophore

- 93 % of the active and 88 % of the inactive compounds were correctly predicted
- Corresponding figures for test set: 72 and 81 %
- These results are better than those obtained



Matsson et al., in manuscript



Conclusion

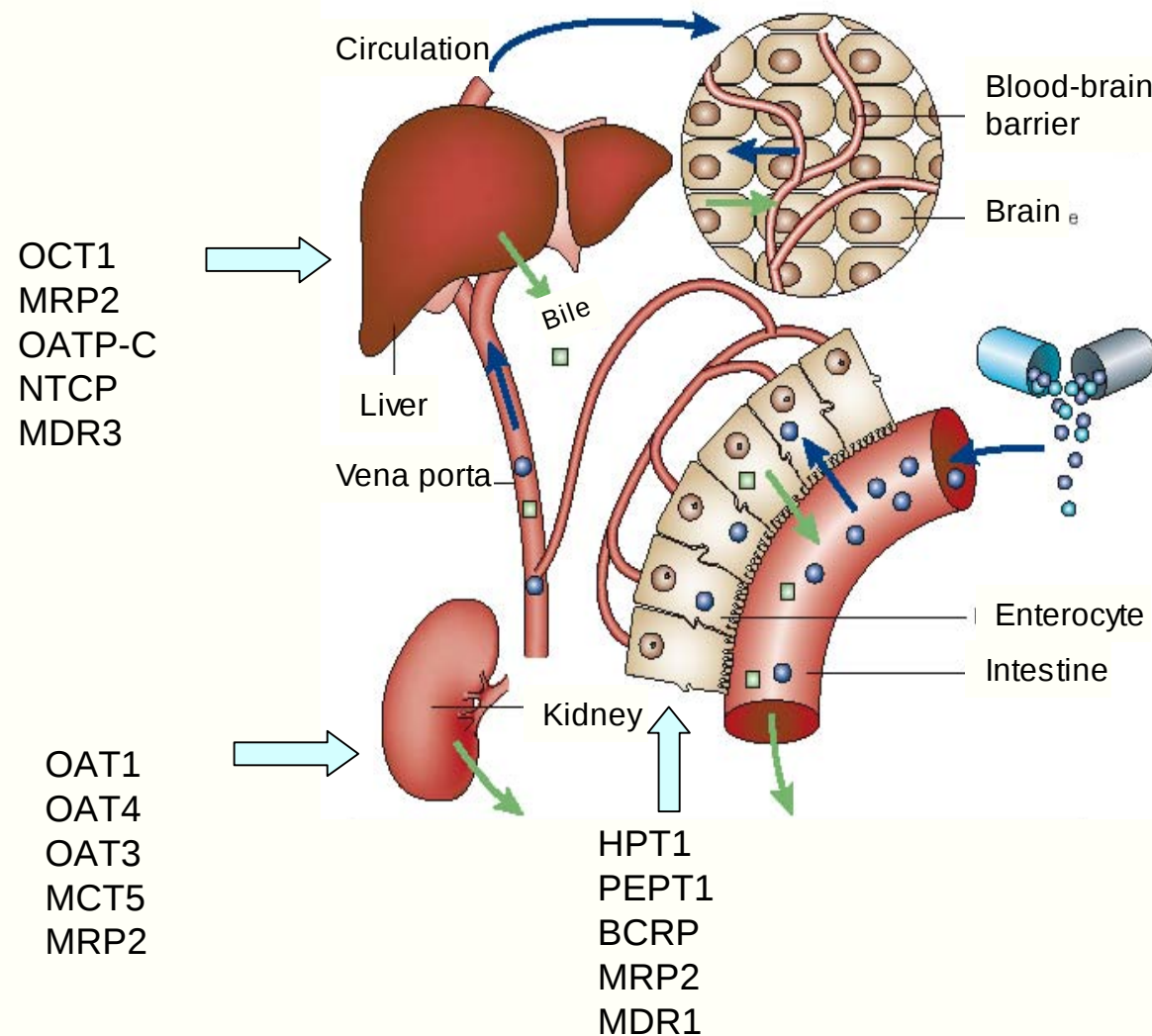
- The augmented pharmacophore gave predictions that are in good agreement with the experimental results
- Further analysis is required to elucidate the potential clinical relevance of drug-drug interactions at the transporter level



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Our research agenda:

1. Mapping of transporter interactions





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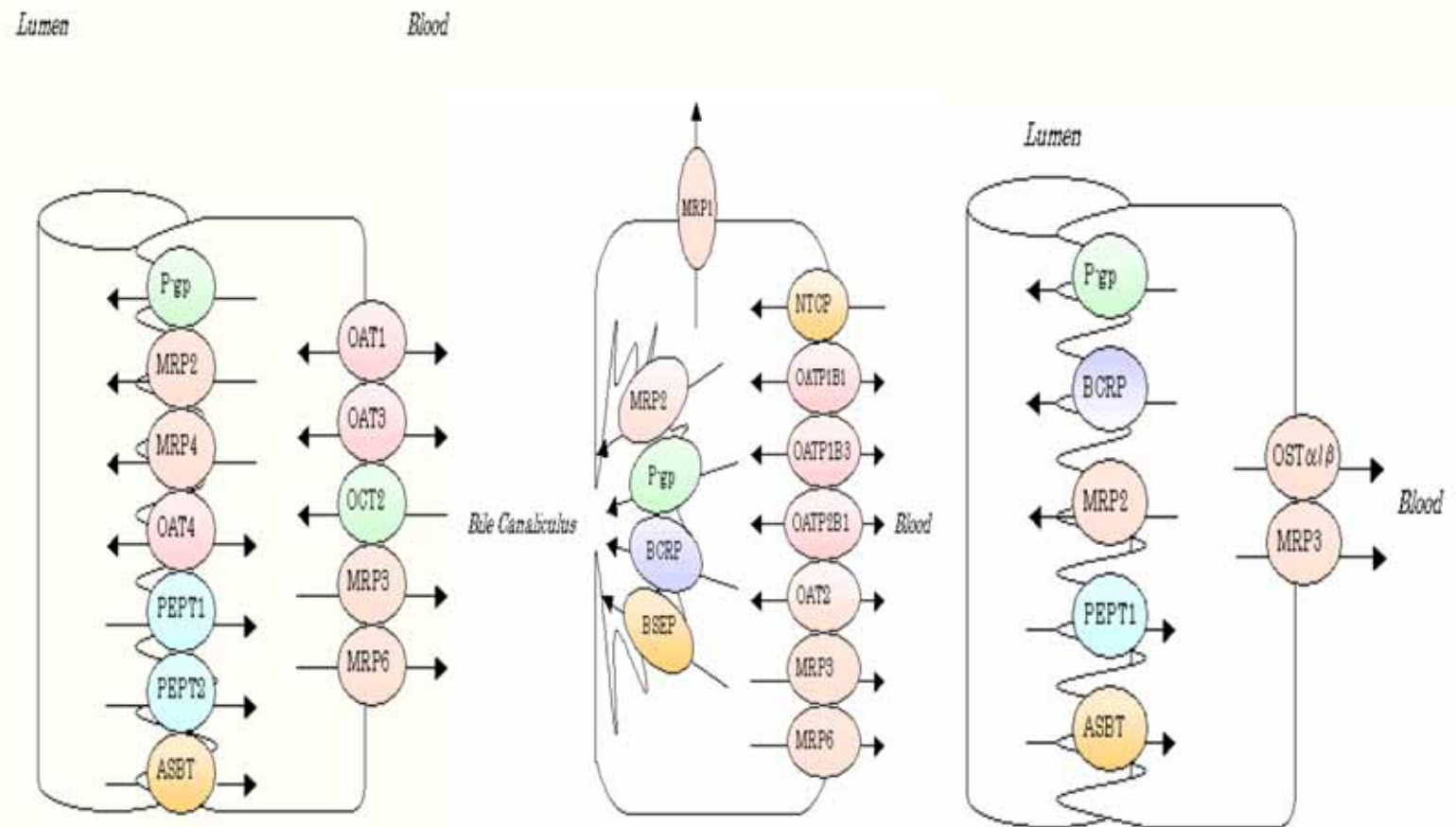
Transporter interaction maps

Drug	Trp 1	Trp2	Trp3		Trpn
1	+	+	-	-	-
2	+	-	-	+	-
3	-	+	-	-	-
4	+	+	+	-	-
...	+	-	-	+	+
n	+	+	+	+	-



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Another goal: Study transport kinetics and assemble the data to kinetic models for organotypic cell-kinetic models





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Thank you!