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

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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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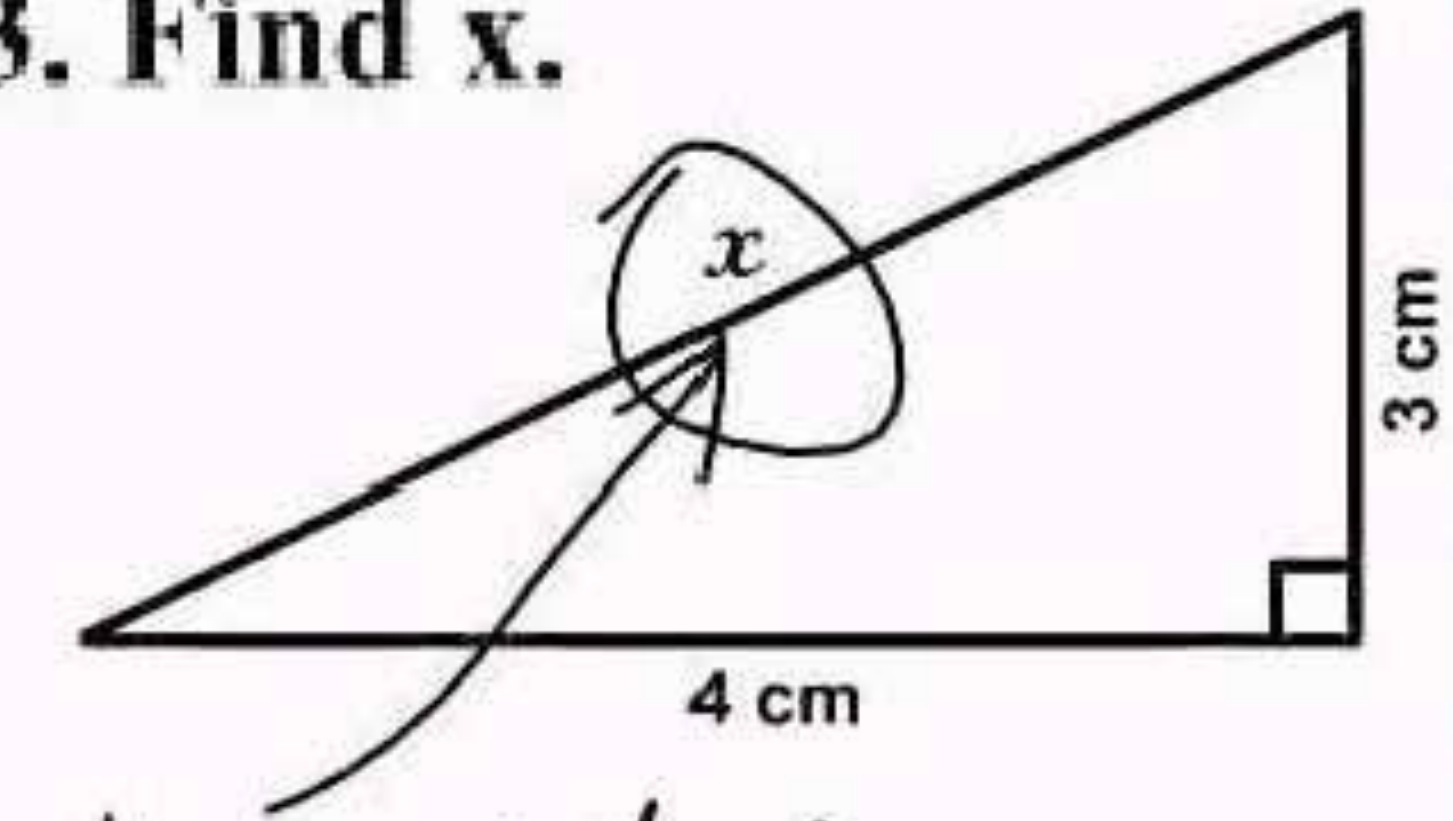
There are easier ways…

3. Find x.



Here it is

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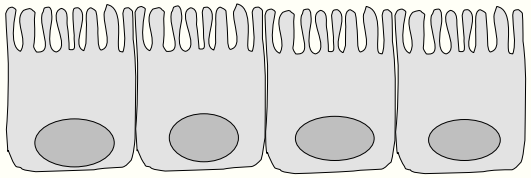
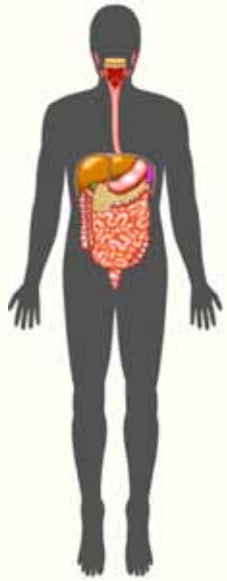
Outline

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





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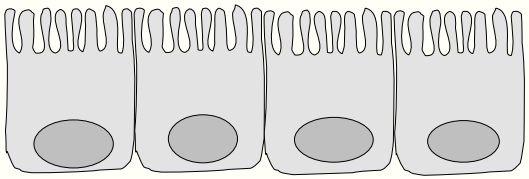
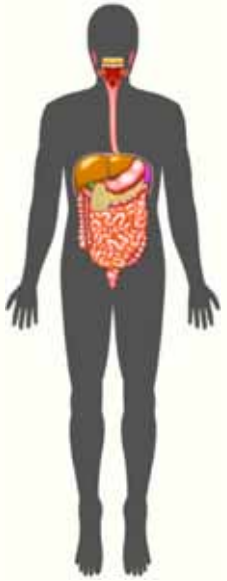
We develop in vitro and in silico assays that predict drug absorption and transport

In vitro screening



Hypothesis



In vivo confirmation

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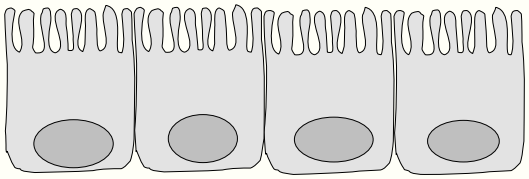
Sometimes we help drug and biotech companies

In vivo findings

Hypothesis

In vitro confirmation



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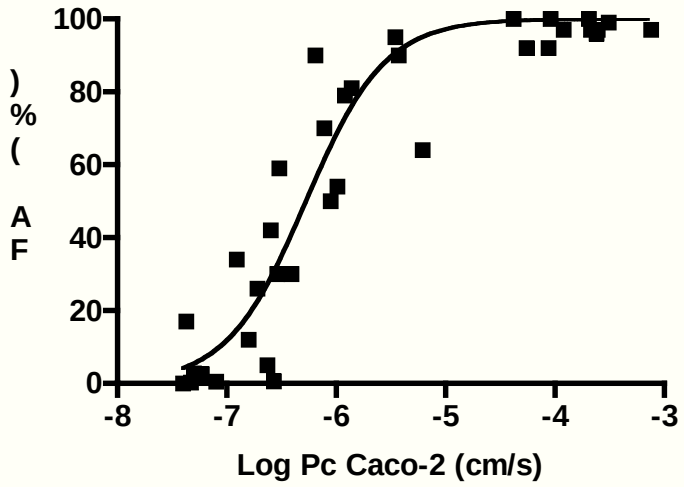
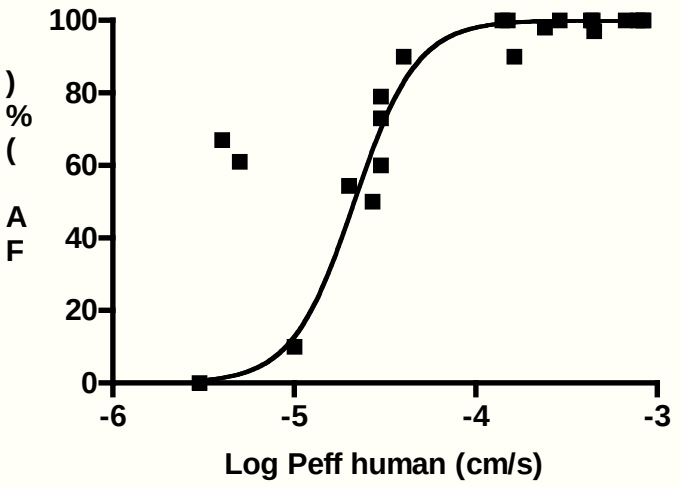
Prediction of oral absorption from in vitro permeability studies

Caco-2



Data compiled from Arturssons' laboratory

Human perfusion



Data compiled from Lennernäs' laboratory

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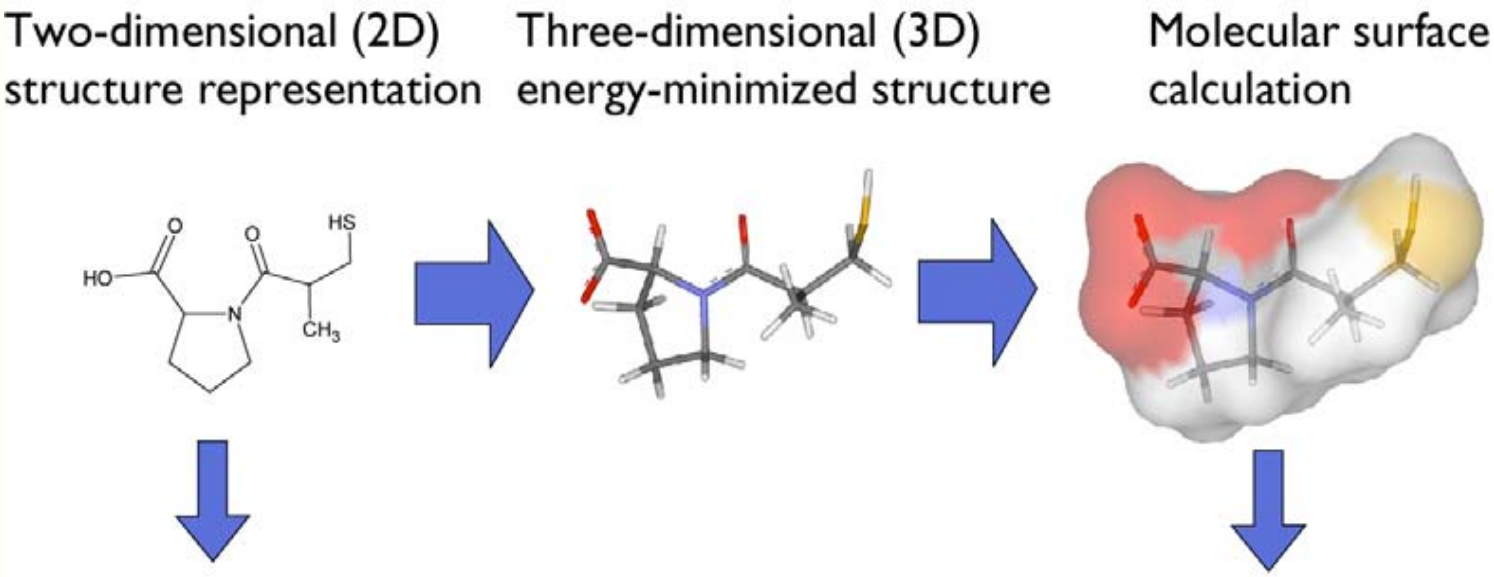
Computational models
Generation of molecular descriptors



Molecular weight
# of H-bond donors
# of H-bond acceptors
etc…

PSA
NPSA

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

Pär Matsson

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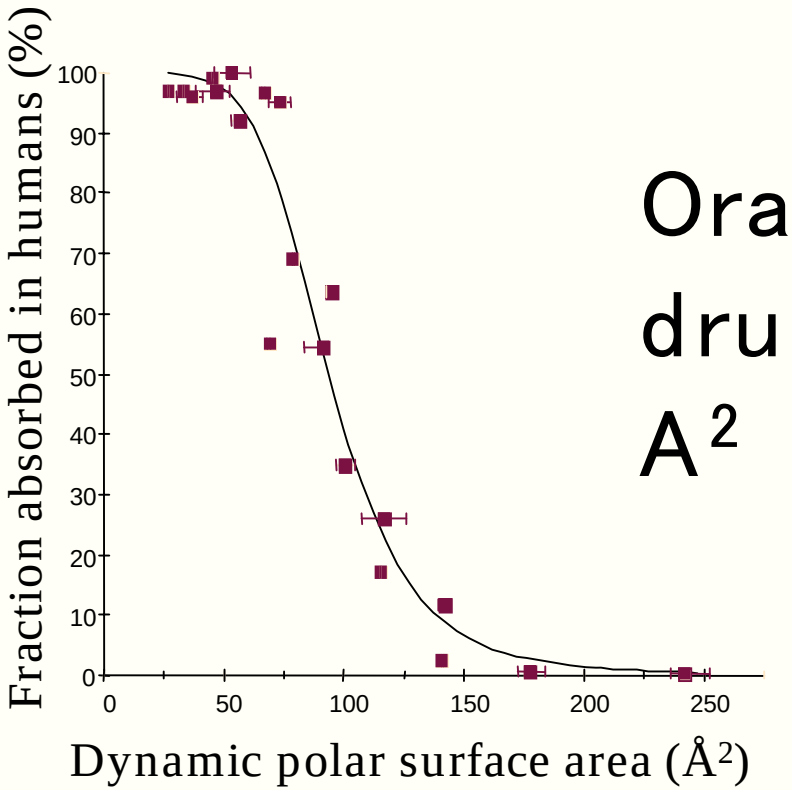
Polar Surface Area vs. drug absorption in humans



Orally available drugs: < 120 A^2

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

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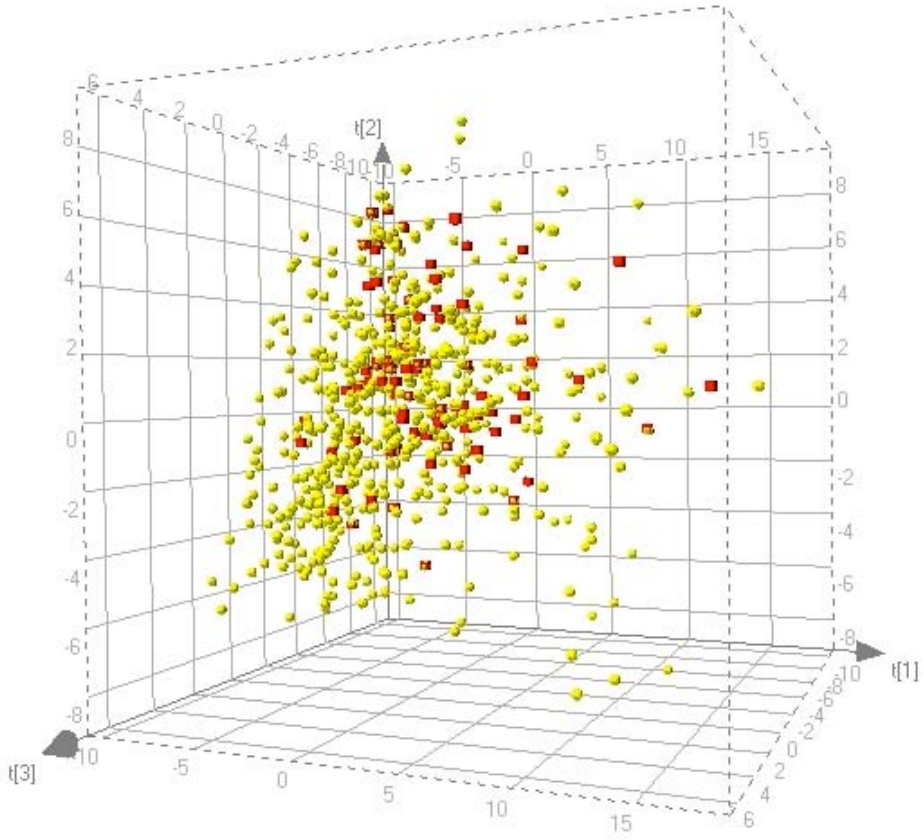
We work in the oral drug space



- 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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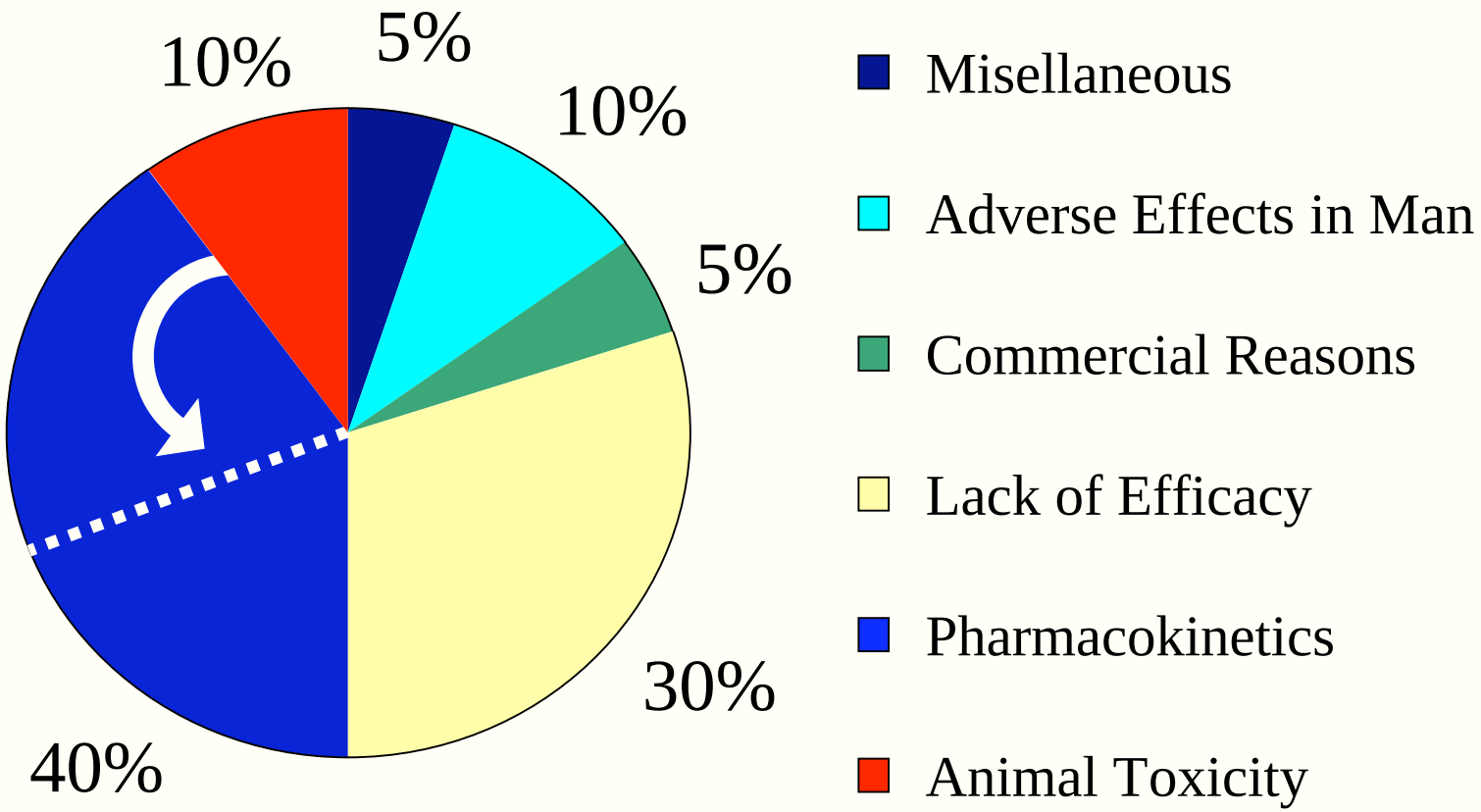
Reasons for Discontinuation of Clinical Development



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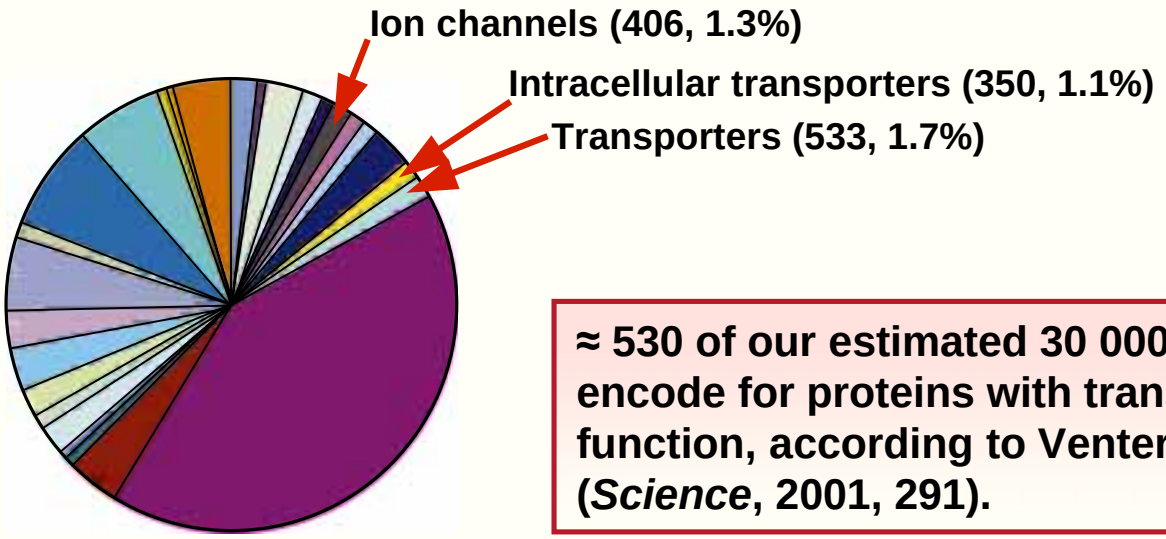
Transport proteins and the human genome



≈ 530 of our estimated 30 000 genes encode for proteins with transport function, according to Venter et al (*Science*, 2001, 291).

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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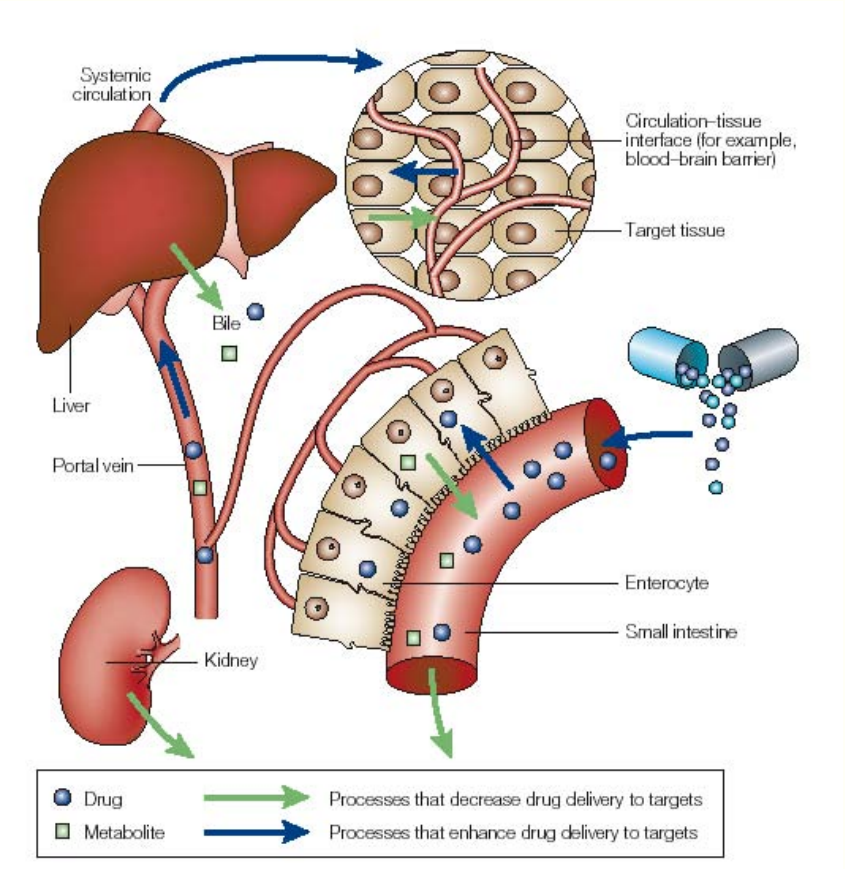
Importance of transporters

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



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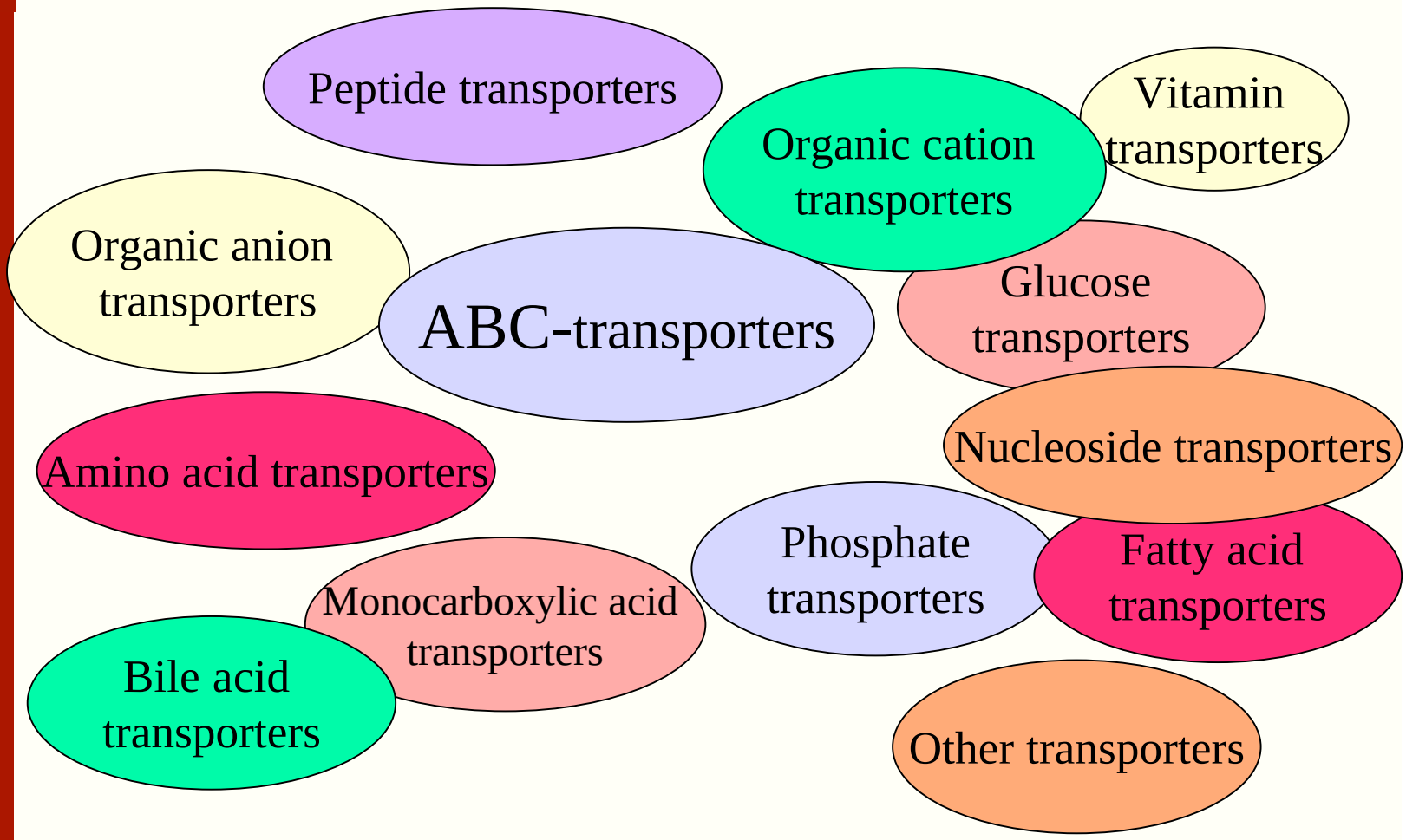
Functional names on transporter families



- Pharmaceutical relevance?

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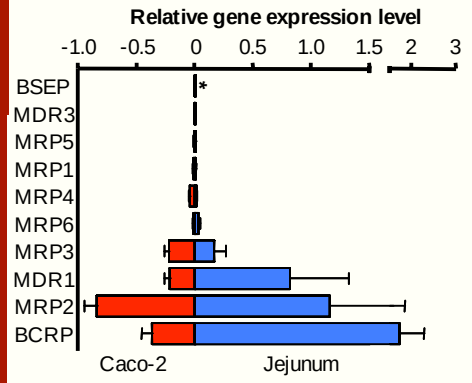
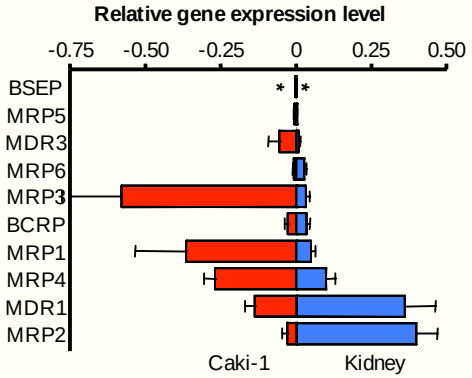
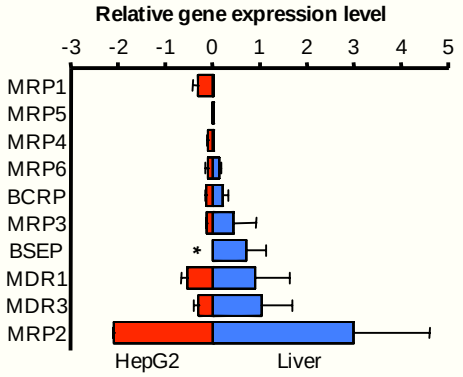
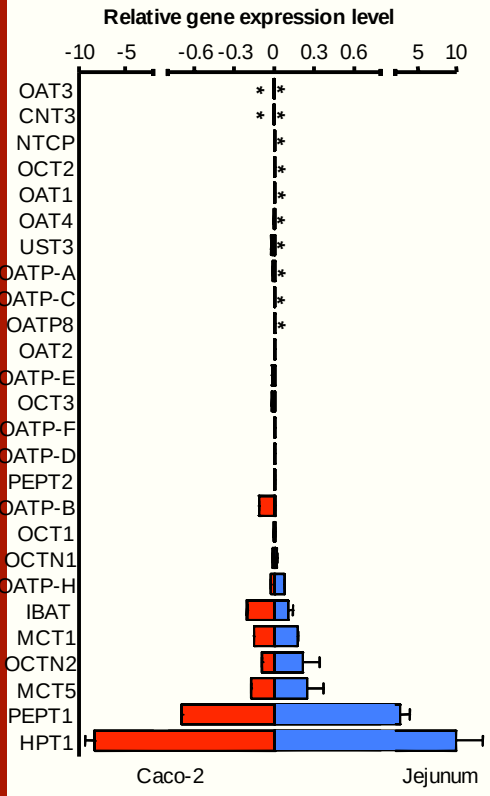
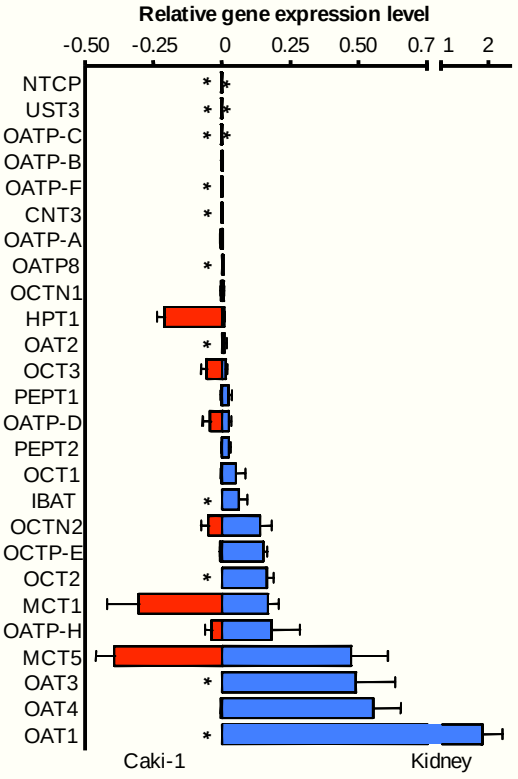
Expression of transport proteins in the human liver, kidney and intestine *(Hilgendorf, C. et al., in manuscript, 2006)*

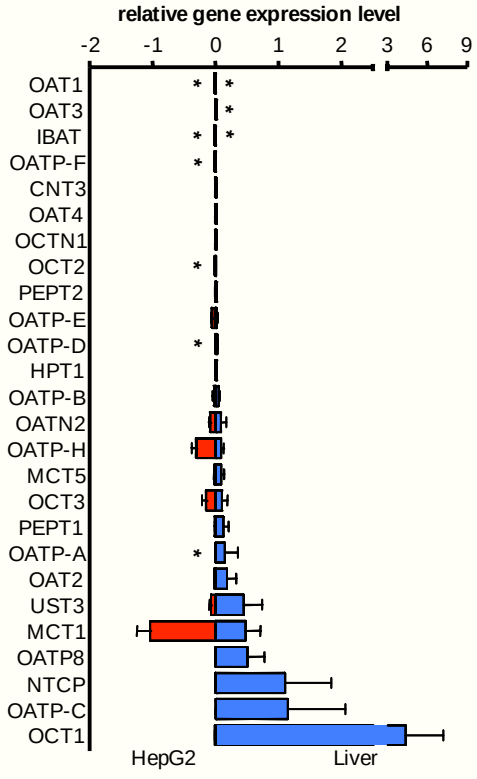












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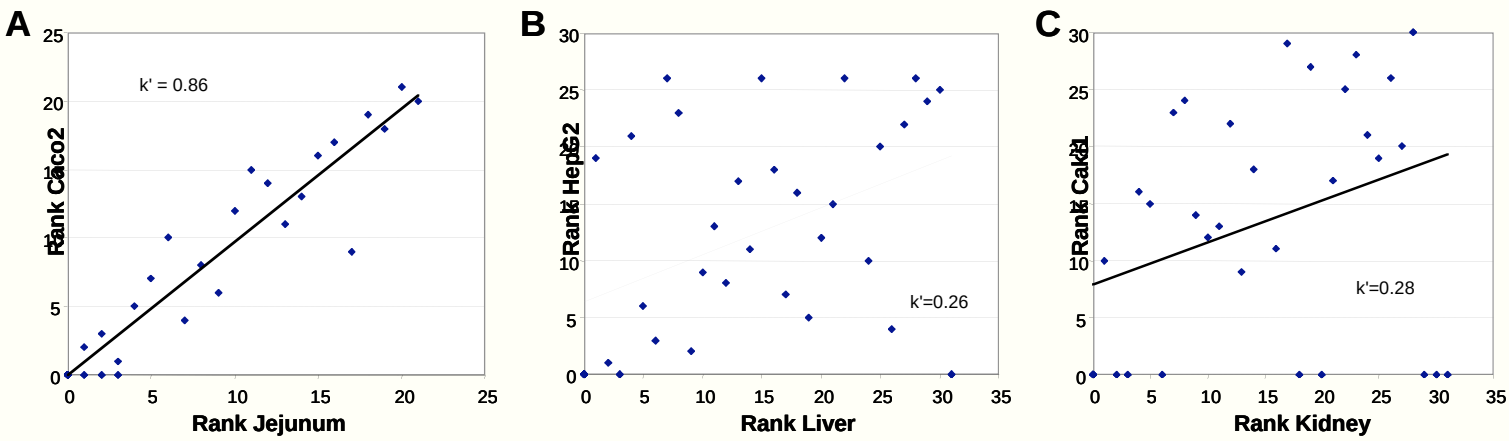
Tissue–cell line correlations



Human cell lines vs. Human tissue

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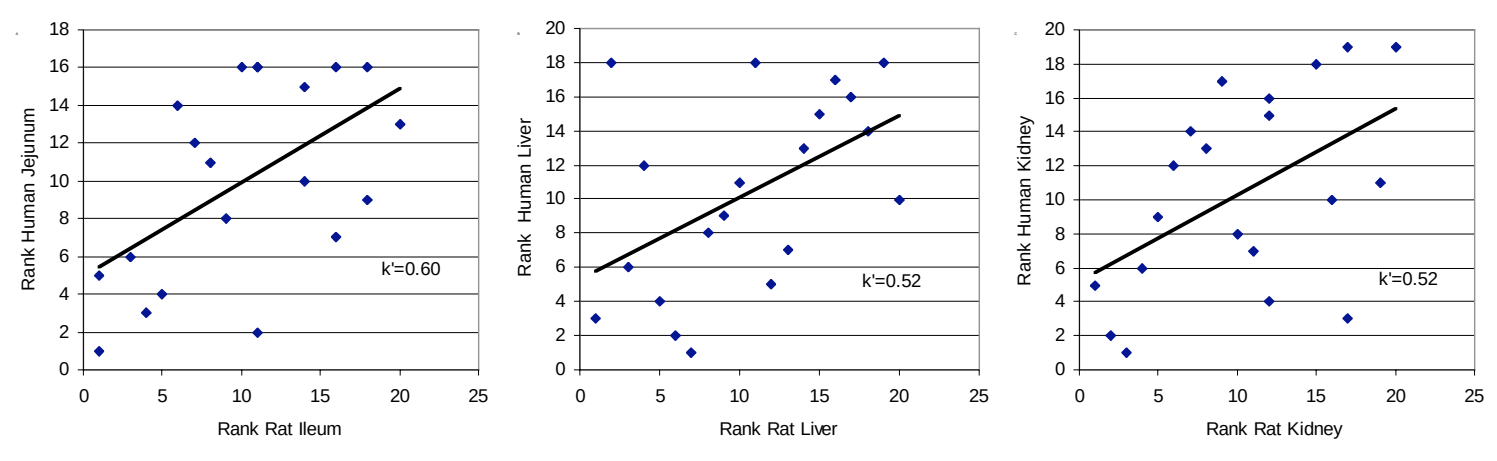
Tissue–tissue correlations



Rat tissue vs. Human tissue

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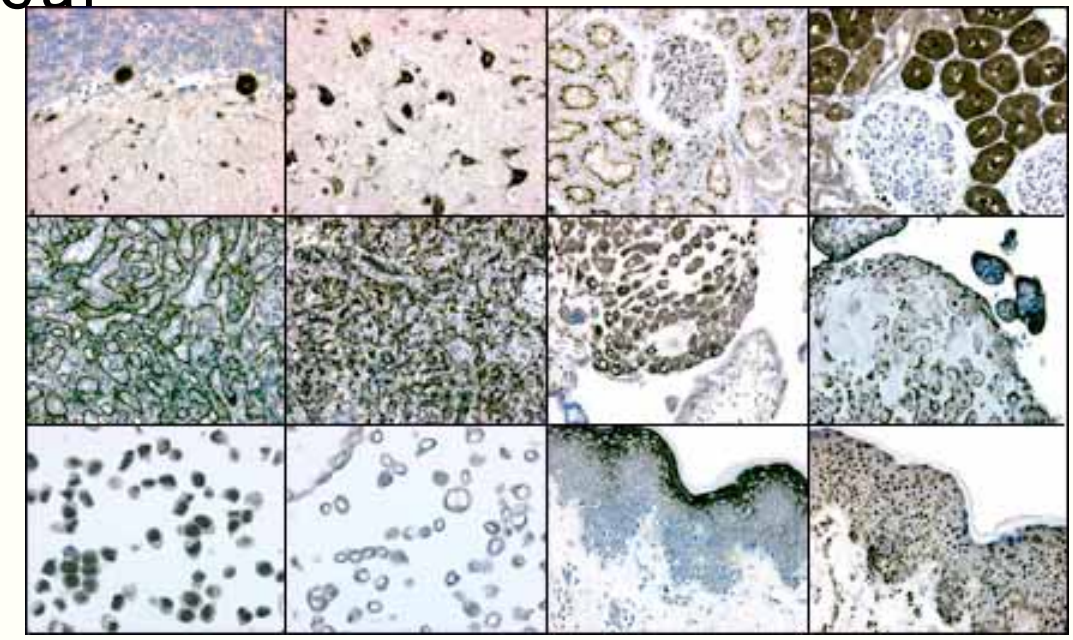
Human transporter proteome

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



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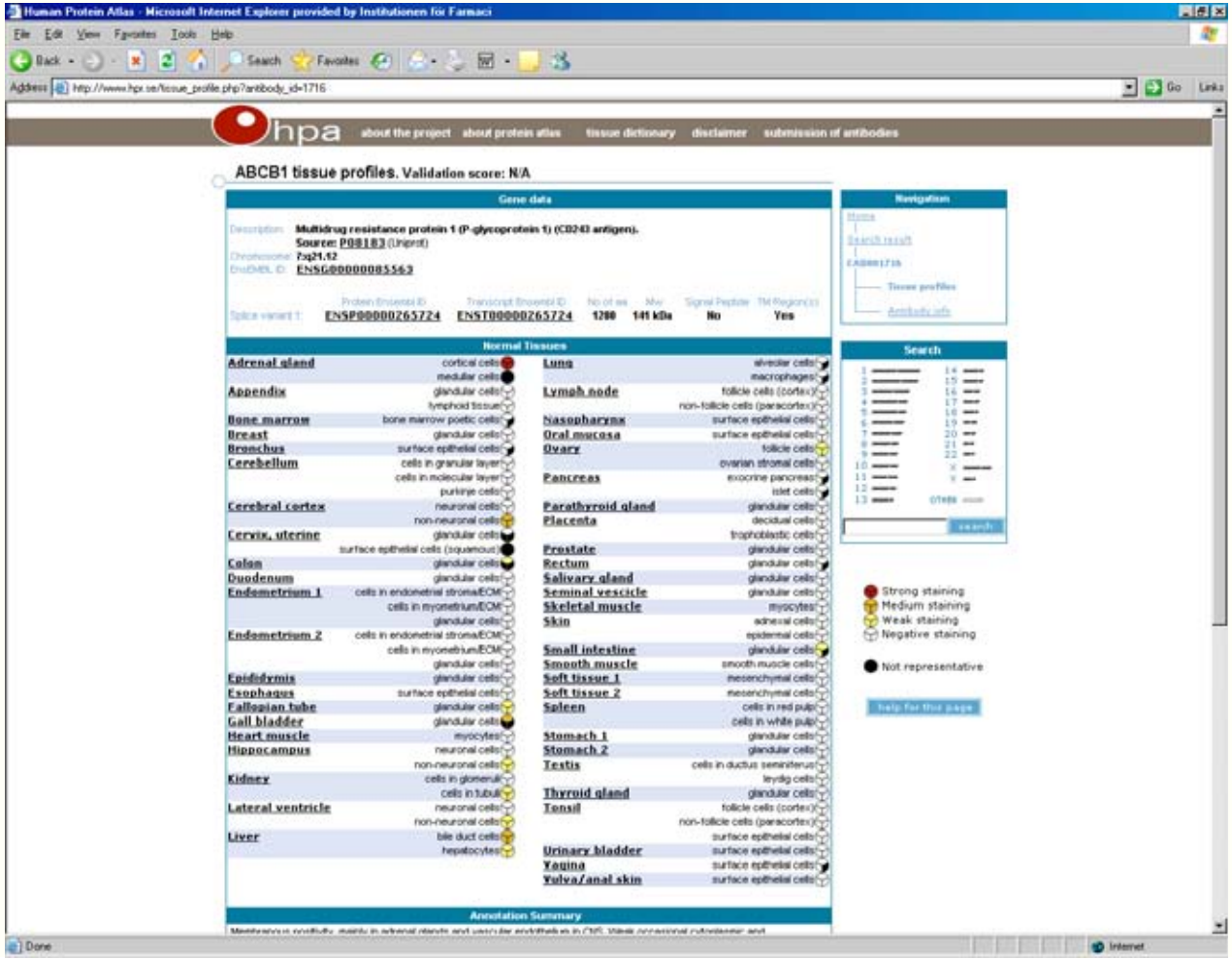
The human protein atlas



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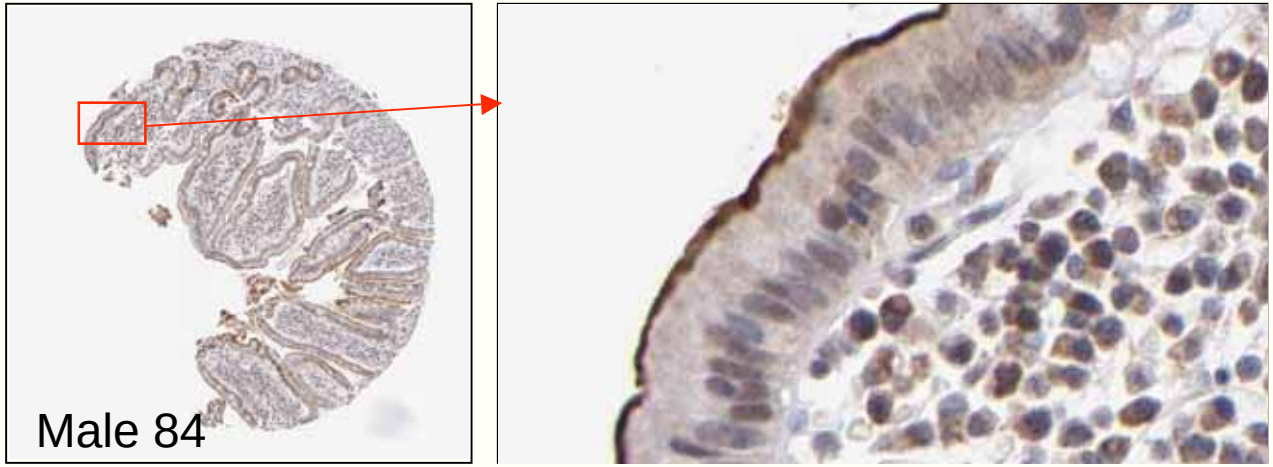
Apical expression of PEPT1 in human duodenal enterocytes



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

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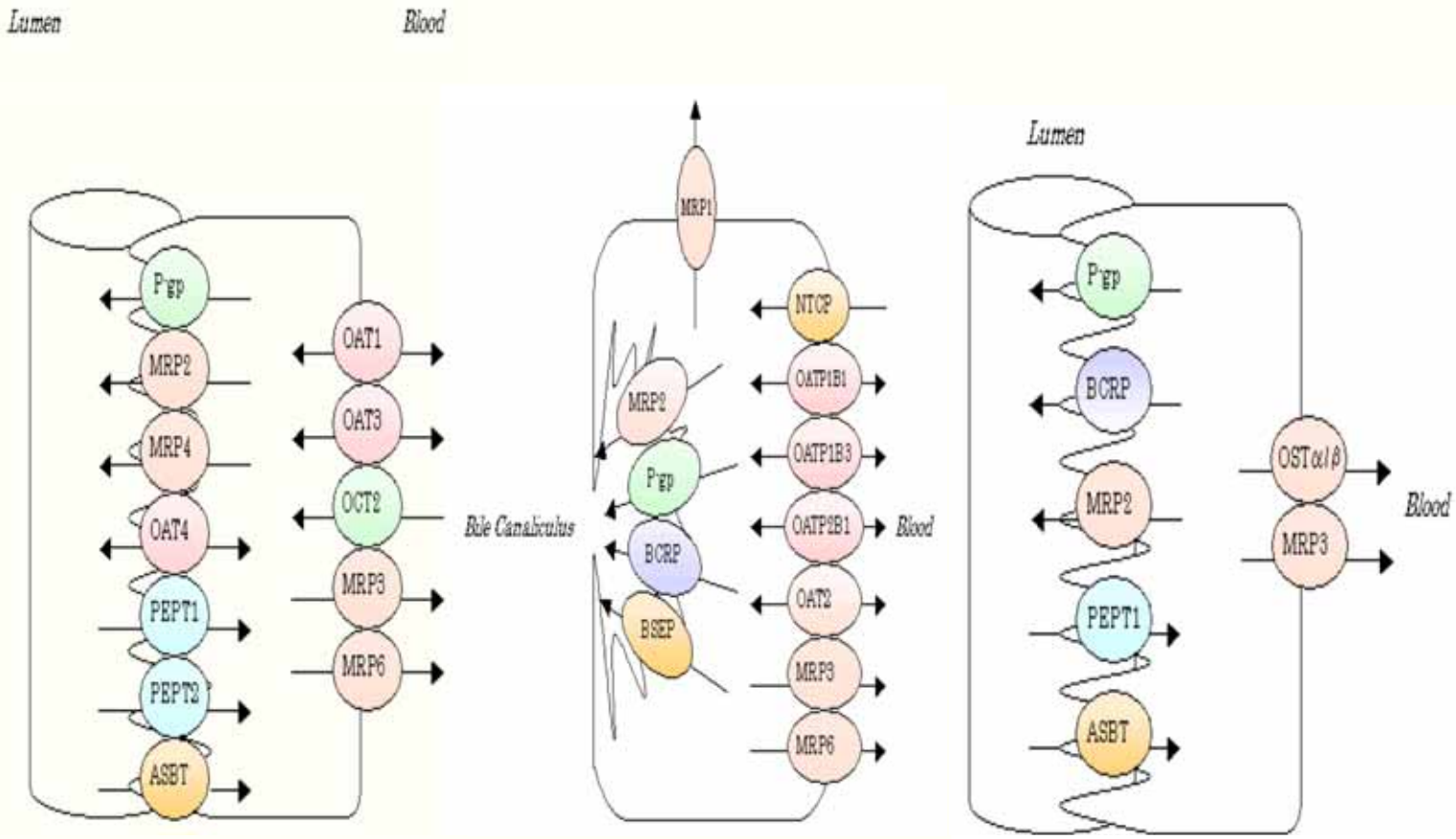
Own data combined with literature data identifies important transporters in each organ



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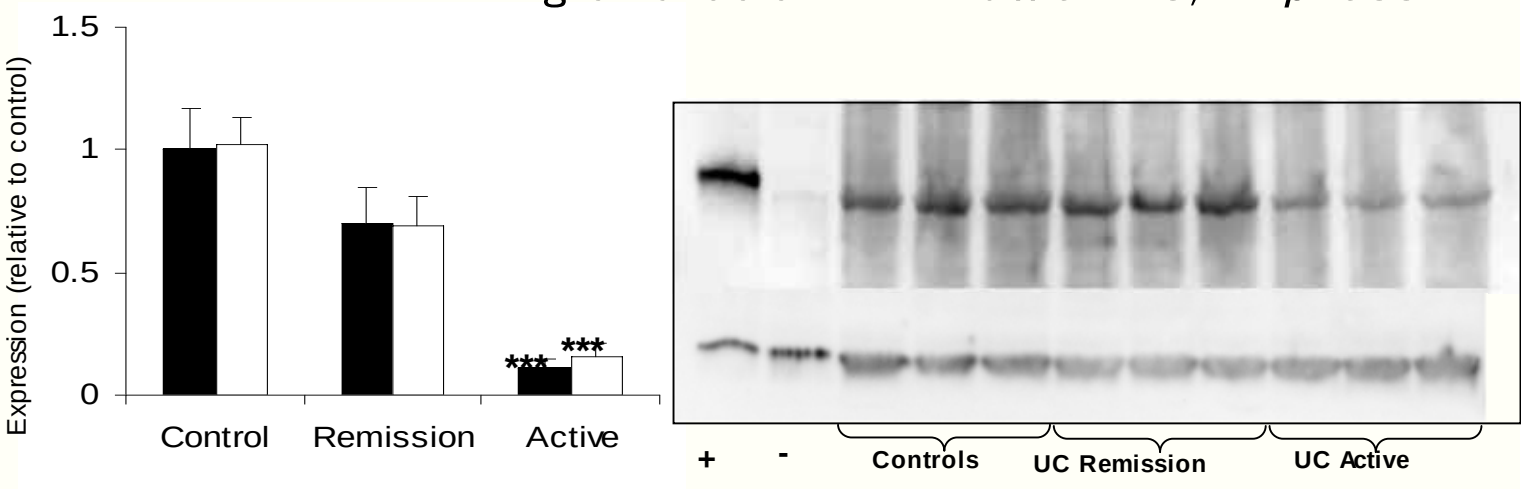
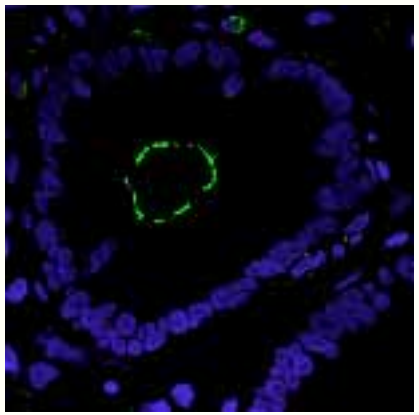
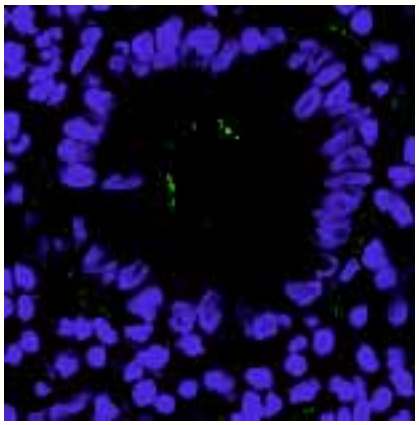
Altered expression of transportes in disease – BCRP in IBD

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



Control





Active.

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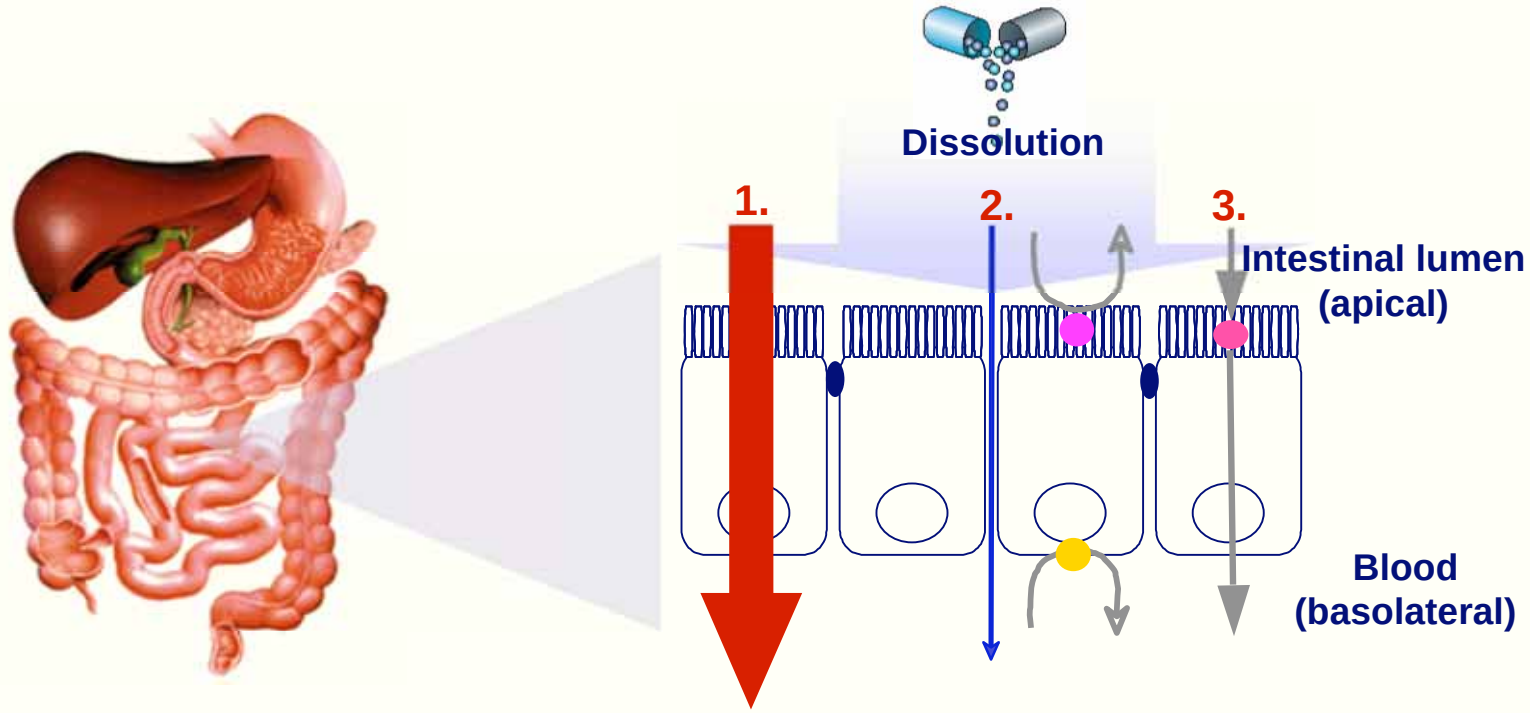
It is now well recognized that absorption of drugs often involves one or more active transport mechanisms



Drugs cross the intestinal epithelium by:

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

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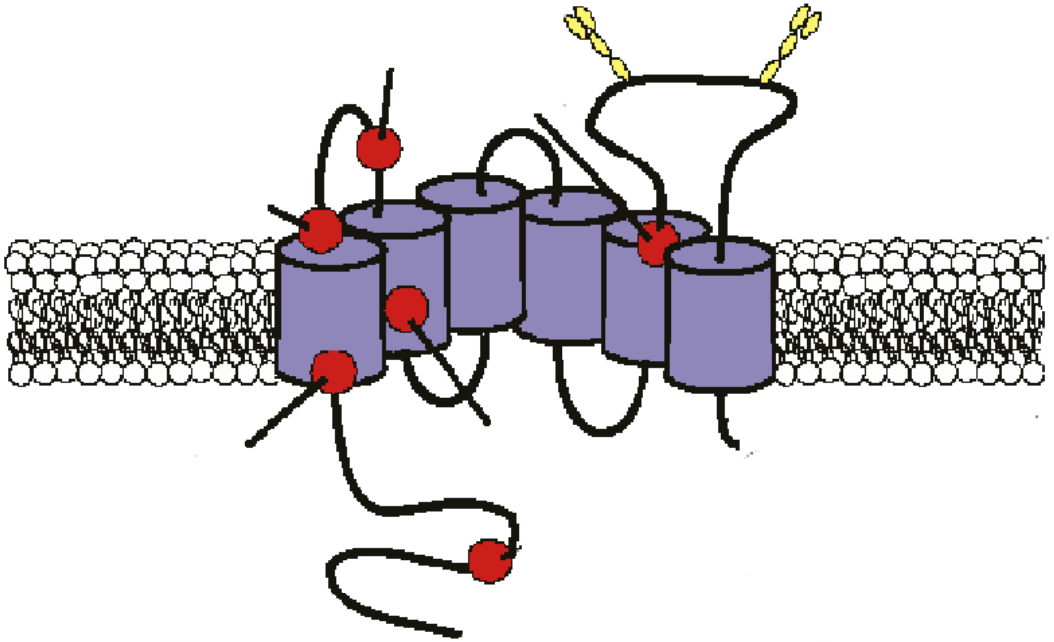
Role of active transport in drug absorption



Patient studies

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

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Digoxin – a Pgp-substrate with a narrc therapeutic range

- At Uppsala University Hospital ~2000 digoxin analysis 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 dr increase the plasma concentration of digoxin?

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

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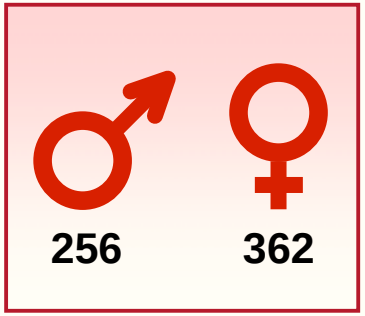
The study population was very heterogenous, including patients with heart failure, atrial flutter and fibrillation

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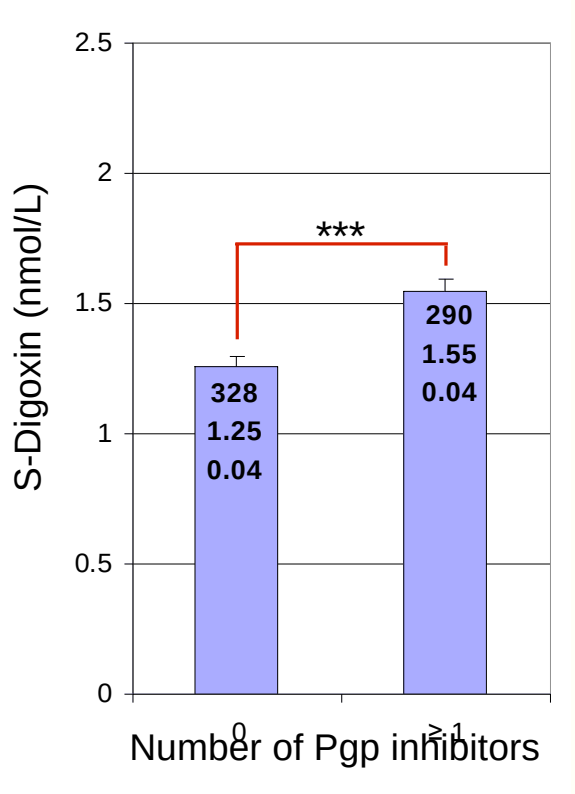
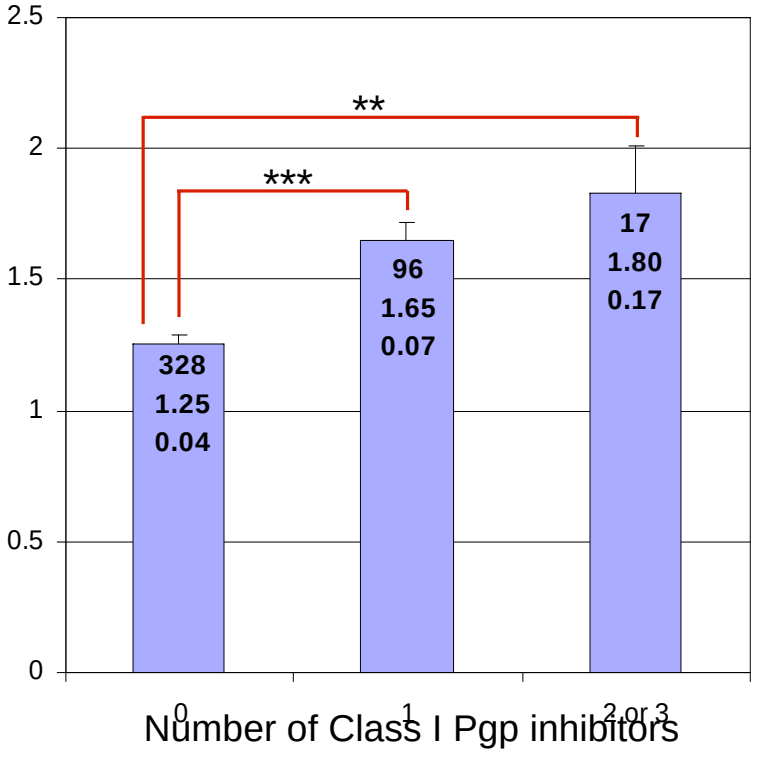
Patients with any Pgp inhibitor prescribed had higher s-digoxin levels…





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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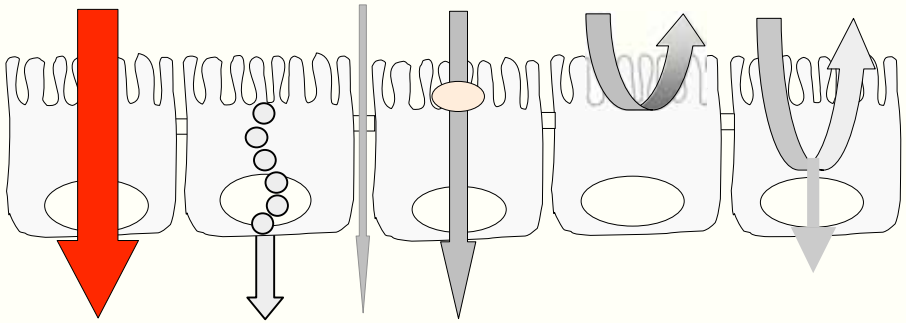
Is digoxin an exception?



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

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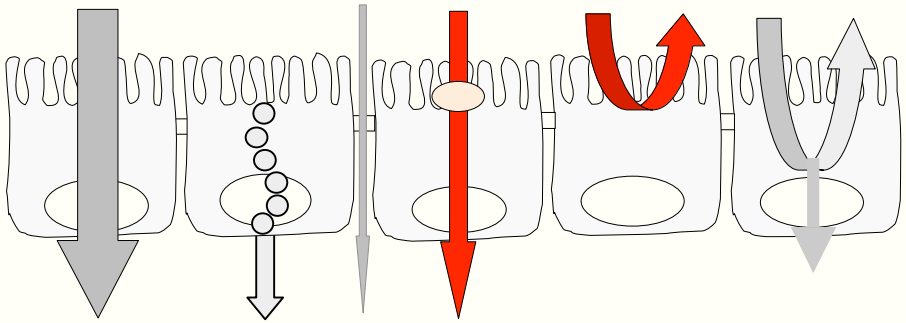
Role of different transport routes



How important are passive and active transport routes, respectively?

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Data set

- N = 30
- 16 compounds: passive transport mechanisms
- 14 compounds were reported to be at lease 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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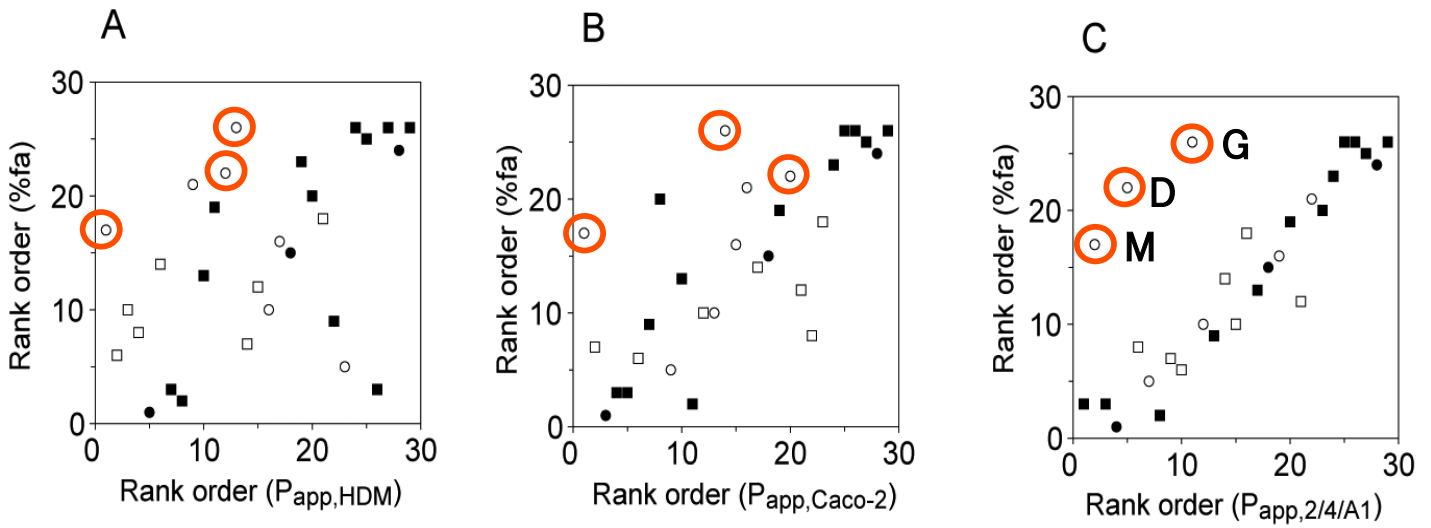
Results:
Rank order correlations Papp vs FA



- **HDM** **Caco-2** **2/4/A1**
- r_s= 0.47 r_s= 0.73 r_s= 0.74
  (0.57) (0.82) (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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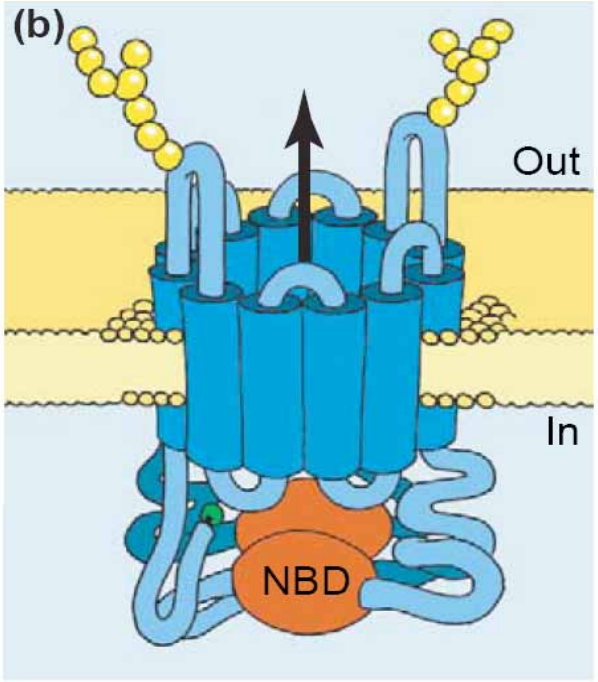
Mapping of transporter interactions - the first example

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



TIPS, 2005.

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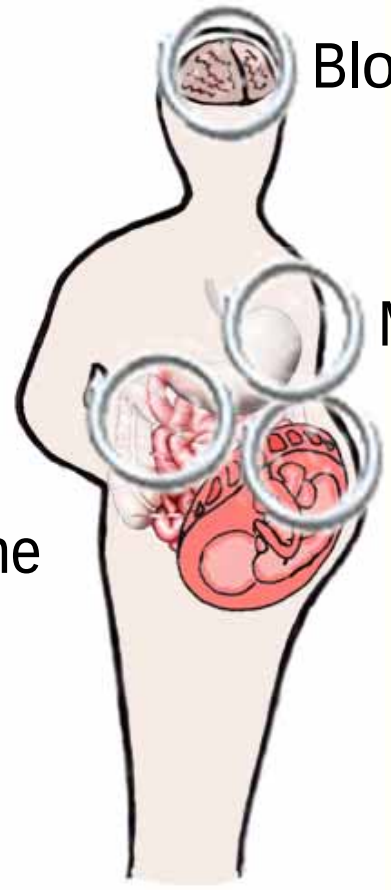
ABCG2: Physiological function



Blood-brain barrier

Mammary gland

Blood-placenta barrier

Small intestine

- Liver
- Kidney
- Stem cells

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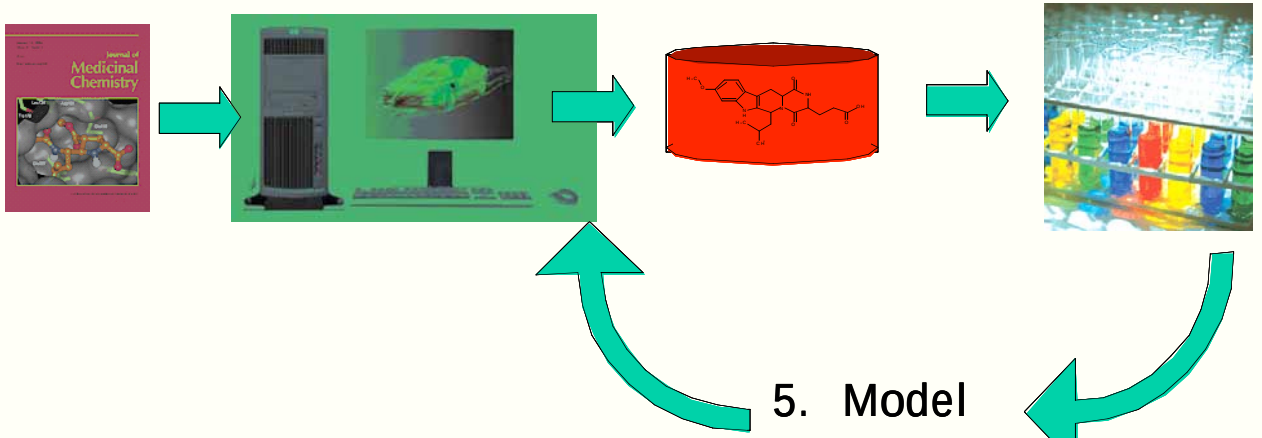
Aim

- To predict ABCG2–drug interaction

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



Matsson et al., in manuscript

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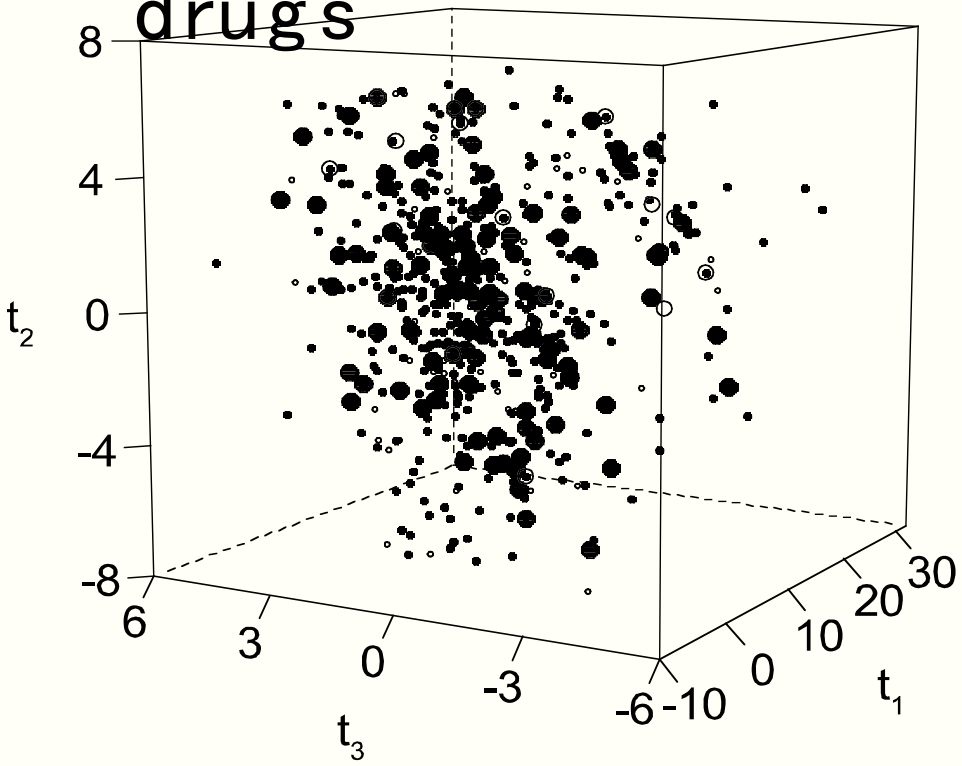
ChemGPS

- The dataset is representative of registered low-molecular drugs



- Training set
- Test set
- Reference drugs from the Physician's desk reference (PDR, 2005)

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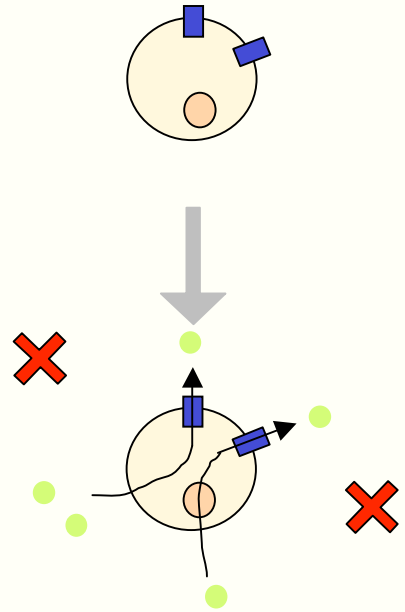
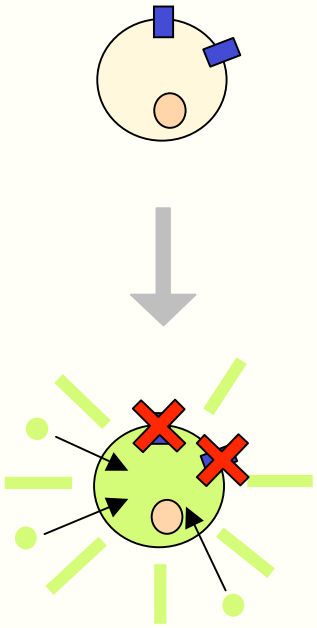
Experimental assay



No inhibition:
substrate is effluxed
➔ low intracellular fluorescence



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

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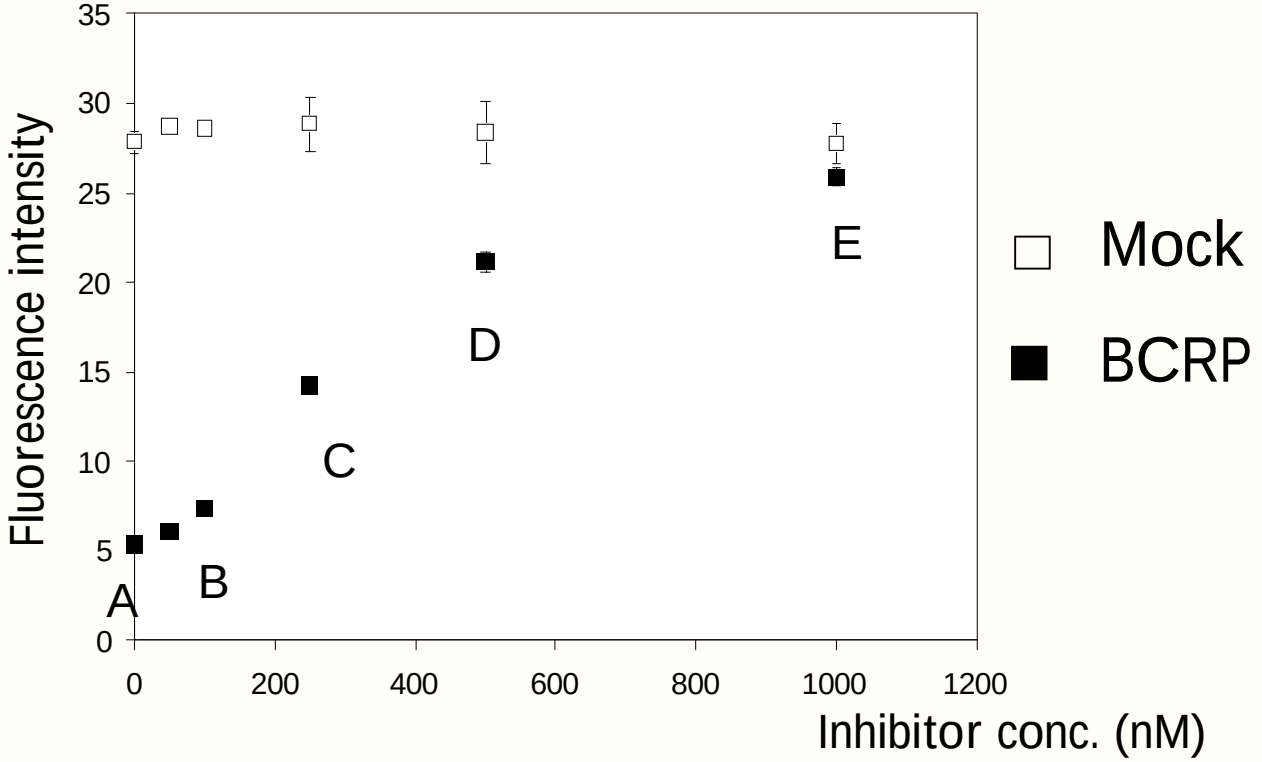
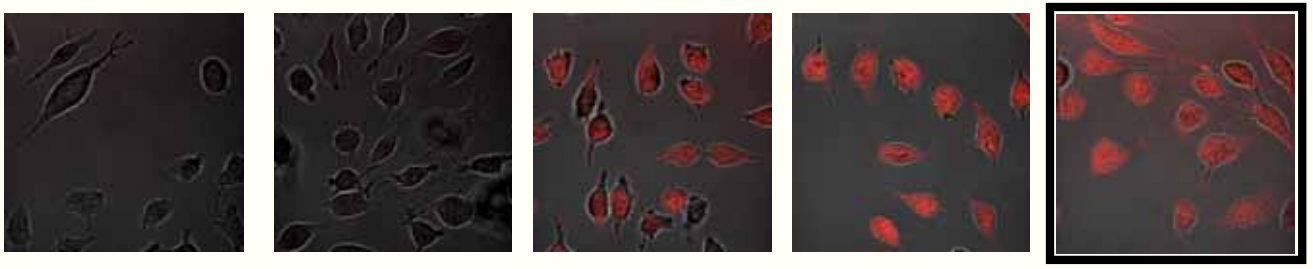
BCRP efflux inhibition: Ko143





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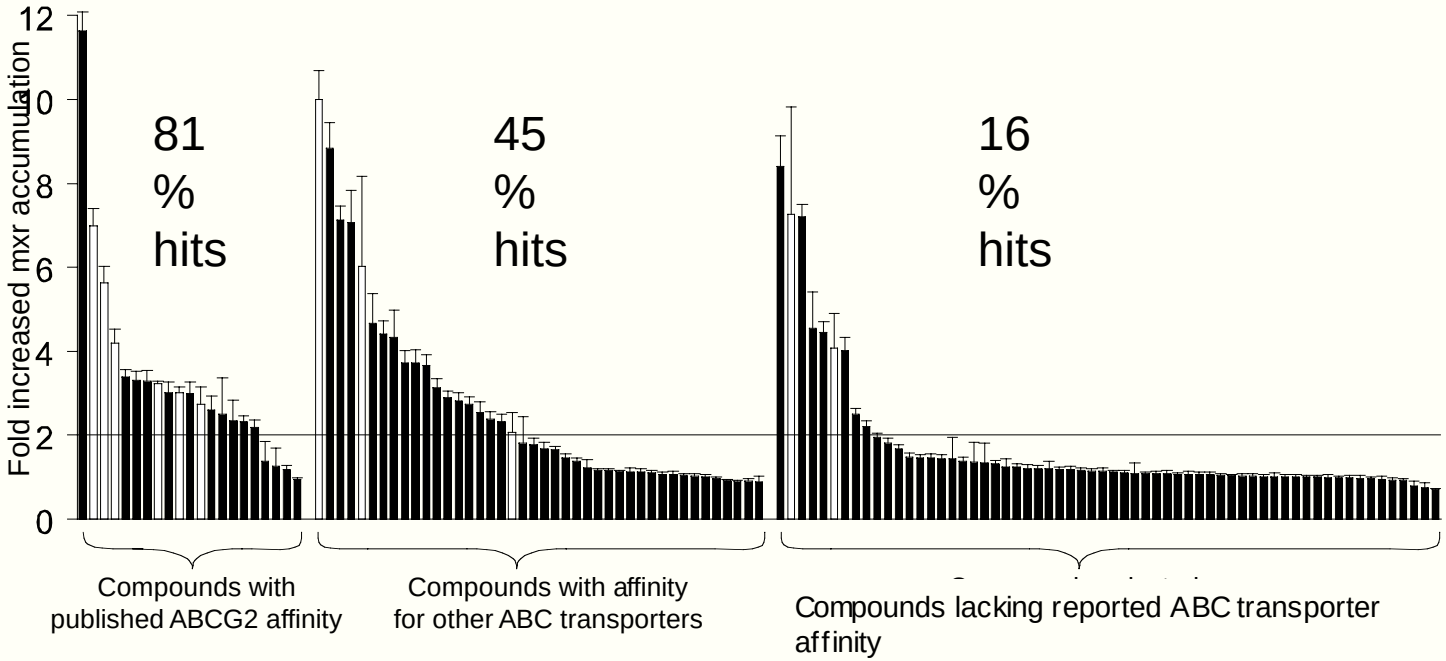
BCRP inhibition



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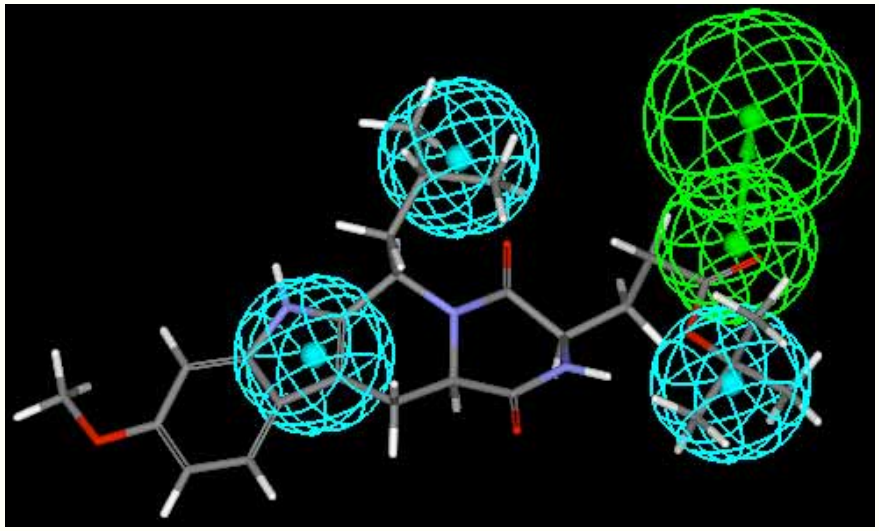
Pharmacophore modelling

- Identification of all BCRP-interacting compounds, but…
- **also many false positives!**
- Hypothesis: influence of **passive permeability** should be



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

Mateson et al, in manuscript

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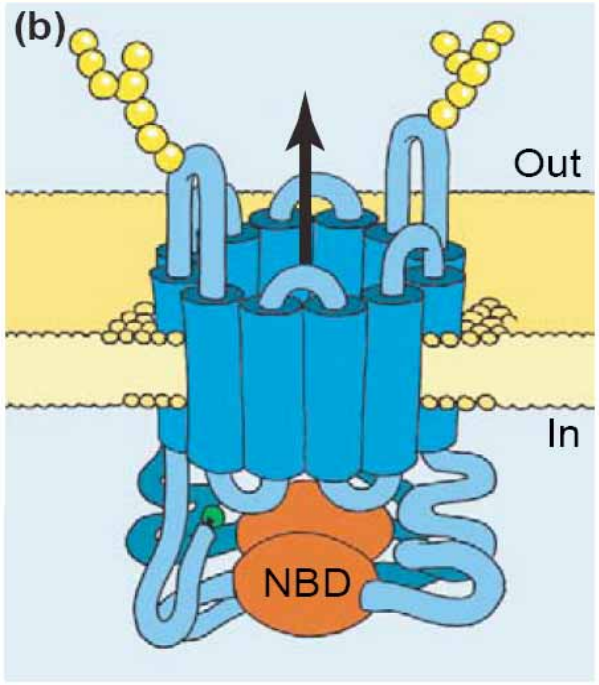
Influence of lipophilicity



TIPS, 2005.

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

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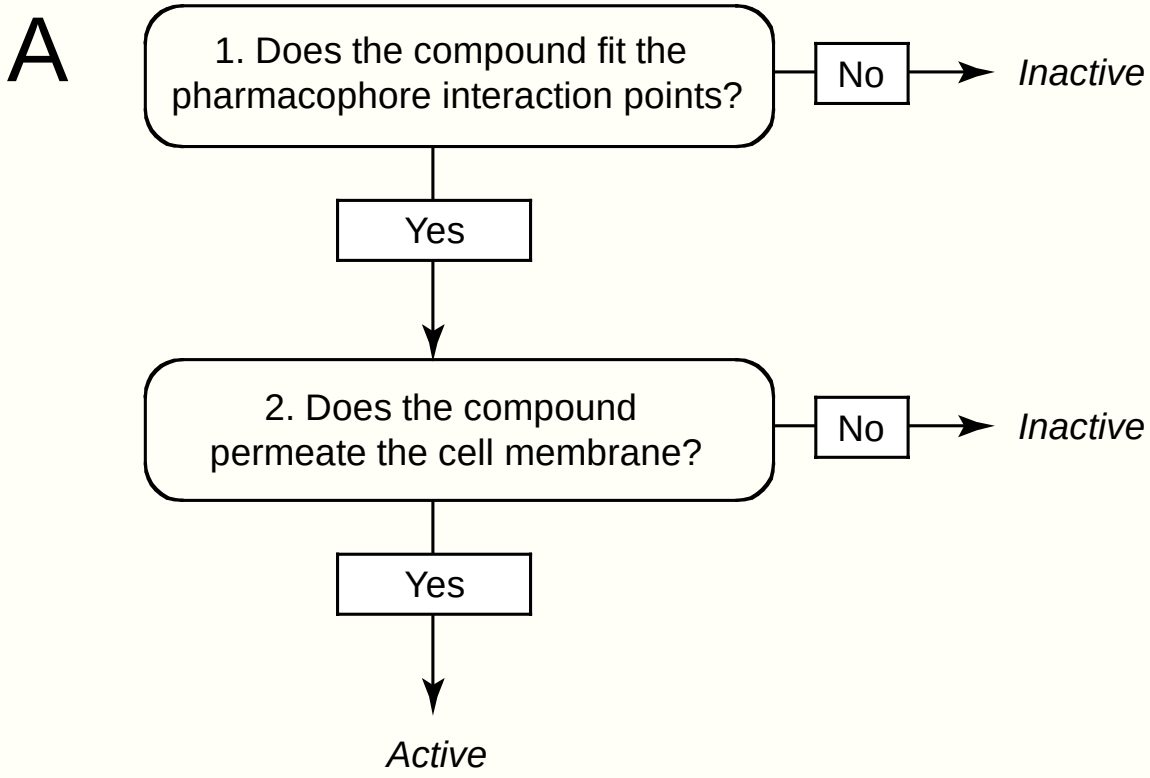
Augumented pharmacophore



Step 1: Catalyst common features pharmacophore
Step 2: PLS-discriminant analysis

Matsson et al., in manuscript

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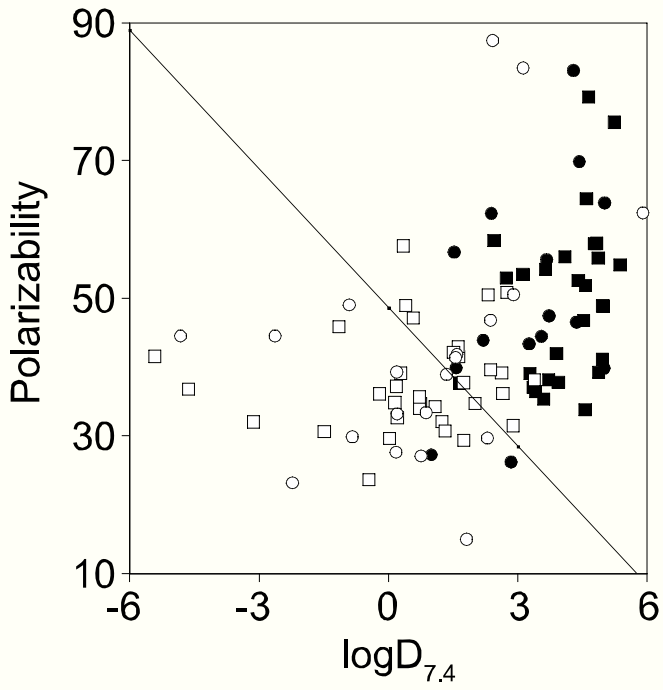
Results - augumented pharmacophore

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



Matsson et al., in manuscript

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Conclusion

- The augumented 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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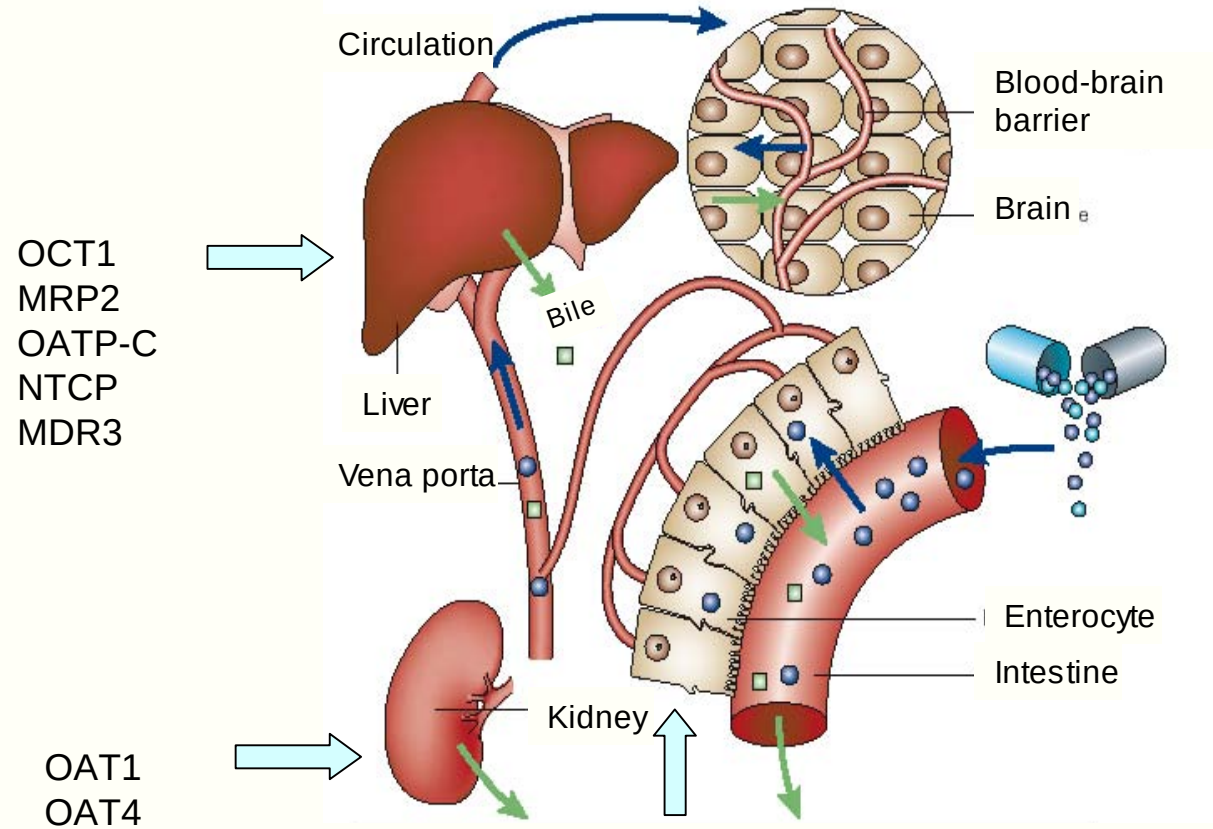
Our research agenda:
1. Mapping of transporter interactions



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Transporter interaction maps

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

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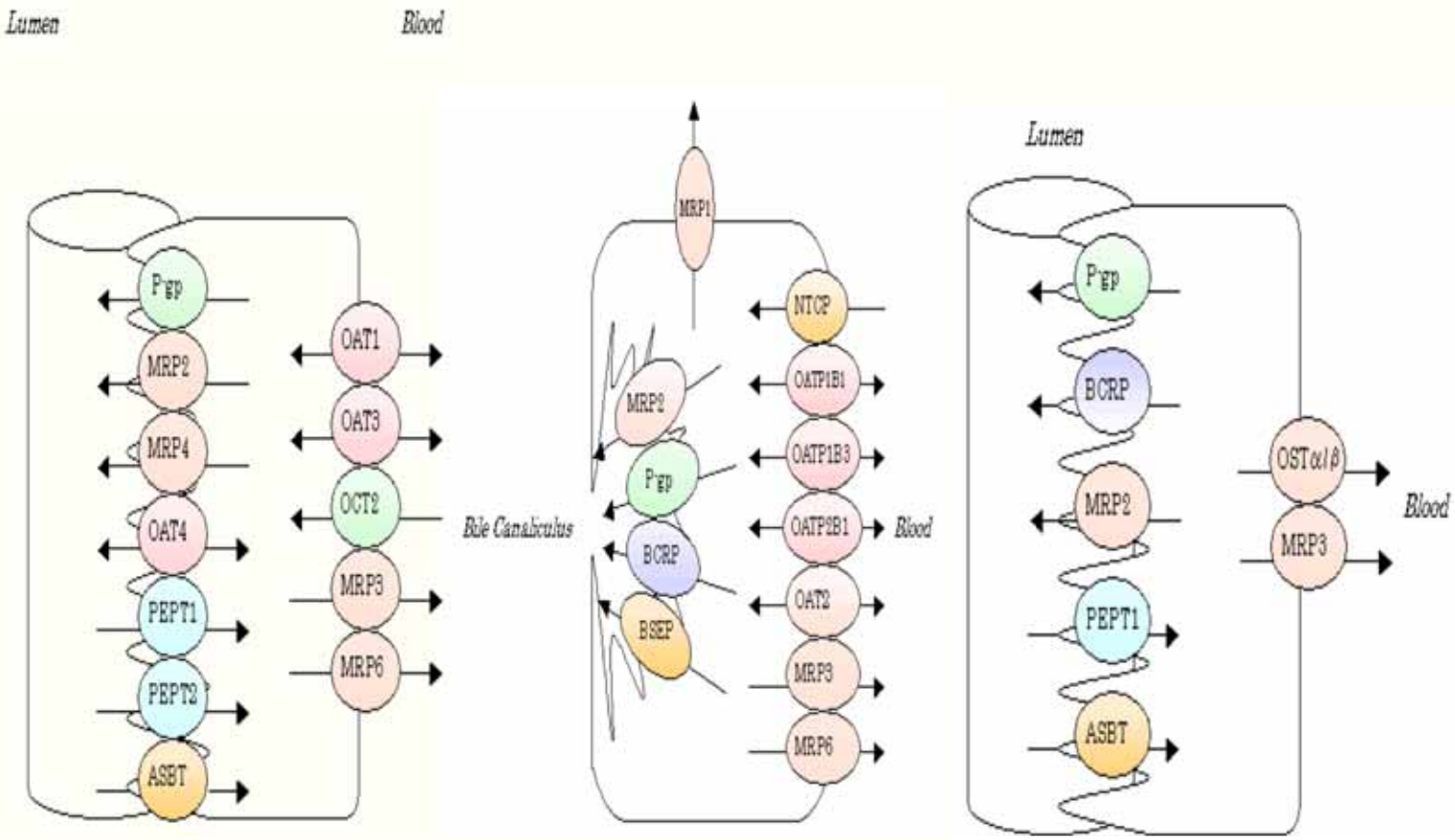
Another goal: Study transport kinetics and assemble the data to kinetic models for organotypic cell-kinetic models



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Thank you!