

# Principles of Clinical Pharmacology

January 10, 2008

Module 2: Drug Metabolism and Transport

Unit 6:        Equilibrative and Concentrative  
                  Drug Transport

Peter C. Preusch, Ph.D.

Pharmacology, Physiology, and

Biological Chemistry Division

National Institute of General Medical Sciences

# Objectives

- Vision, reality, and the path between
- Methods of measuring drug transport *in vitro* and *in vivo*
- Mechanisms of drug transport
- Recent advances in understanding the role and structure of membrane transport proteins
- Clinically important transporters
- Pharmacogenetics & pharmacogenomics of transporters

# Measurements of Drug Distribution Reflect Membrane Transport *In Vivo*

- Blood/tissue Samples, Biopsies, and Assays
- Autoradiography
- Perfusion/Cannulation Methods
- Radiology - x-ray, PET, SPECT
- Magnetic Resonance Imaging
- Microdialysis

# Apparatus for In Vivo GI Permeability Studies

Petri, et al. (Lennernäs) Drug Metab & Disposition 31(6), 805-813, 2003.

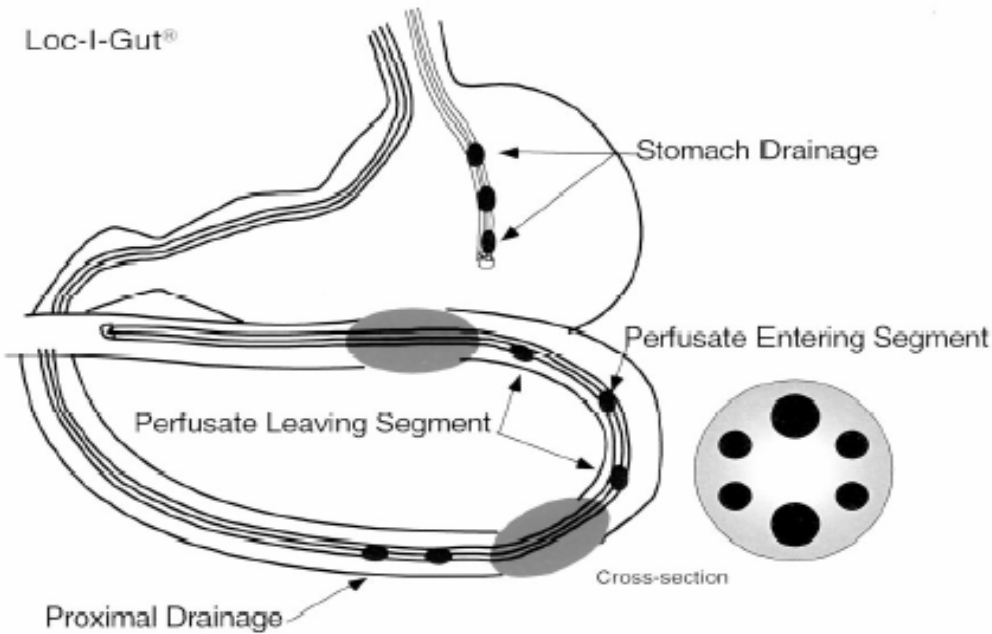


FIG. 1. Loc-I-Gut is a perfusion technique for the proximal region of the human jejunum.

Six healthy volunteers

Proximal jejunum segment

Single pass  $Q_i = 2 \text{ mL/min}$

$$P_{\text{eff}} = (C_i - C_o)Q_i / 2\pi LC_o$$

PEG 4000 non-absorbable  
marker => net water flux

$$F_{\text{abs}} = 1 - (C_o \text{PEG}_i / C_i \text{PEG}_o)$$

mRNA in shed enterocytes

GSTA1 and UGT1A1

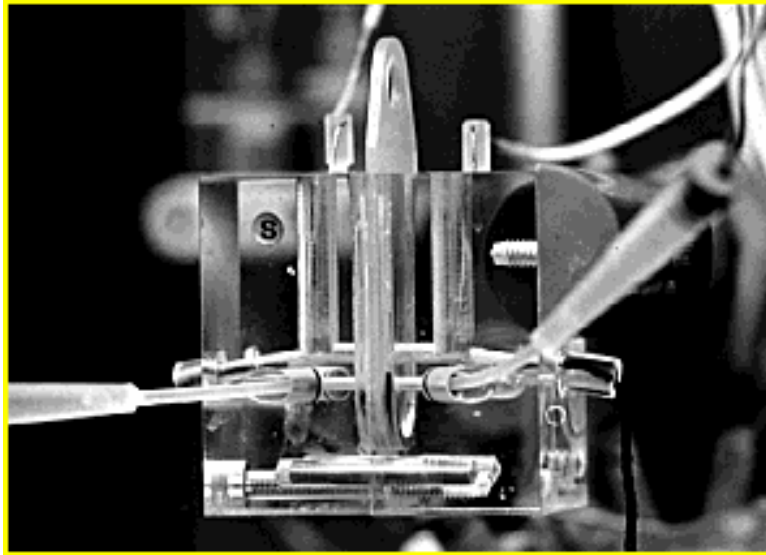
## Absorption of Phytochemicals from Onion and Broccoli Extract

<u>Compound</u>	<u>Permeability (cm/sec)</u>	<u>Absorbed</u>	<u>Metabolite</u>	<u>Induction</u>
Sulforaphane	$18.7 \pm 12.6 \times 10^{-4}$	$74 \pm 29\%$	GSH-conjugate	$2.0 \pm 0.4x$
Quercetin -3,4-glucoside	$8.9 \pm 7.1 \times 10^{-4}$	$60 \pm 31\%$	-3'-glucoronide	$2.4 \pm 1.2x$

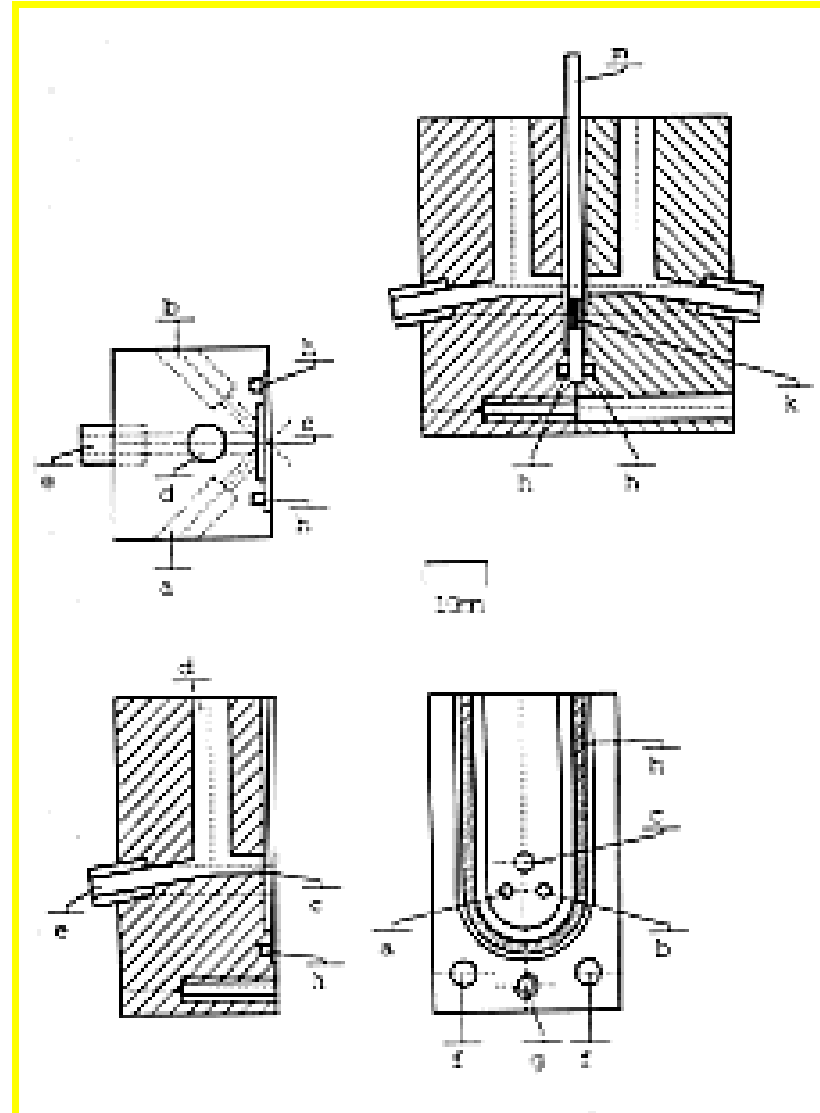
# Measurements of Membrane Transport *In Vitro*

- Ussing chamber - excised tissue samples
- Everted gut sac - uptake from medium
- Uptake/efflux by membrane vesicles, liposomes, BLM, PAMPA, cells in culture (CHO)
  - filtration, centrifugation, oil-stop separatory assays
- Fluorescent (confocal) microscopy of cultured cells fluorescent drugs (mitoxantrone, rhodamine)
- Electrophysiology in cells (e.g., oocytes)
- Monolayer cell cultures on permeable supports
  - Caco-2 cells, MDCKII, brain MVECs

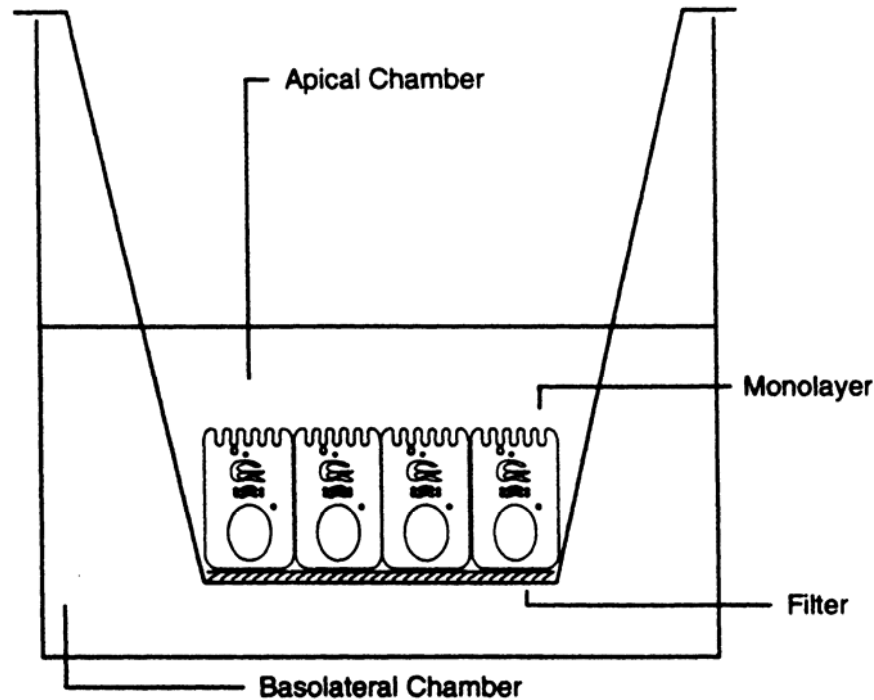
# Measurement of Transport in Excised Tissue Samples



Modified Ussing-chamber allows perfusion of solutions on both sides of membrane holder, control of pressure differential, measurement of potential, conductivity, pH.



# Monolayer Epithelial Cell Culture



**Figure 1.** Cell monolayer cultured on Transwell polycarbonate filters. Caco-2 cells are seeded on polycarbonate filters at a density of approximately 60,000 cells/cm<sup>2</sup>. Common insert diameters are 6.5, 12, and 24.5 mm. Pore sizes are 0.45 and 3.0  $\mu$ m. The volume of media used to feed cells grown on 24.5-mm inserts are 1.5 ml in the apical side and 2.6 ml in the basolateral side.

I.J. Hidalgo in *Models for Assessing Drug Absorption and Metabolism* (Borchardt, et al., Eds.) Plenum Press, NY, 1996, p. 38.

# Mechanisms of Transport Across Biological Membranes

## Diffusion Mechanisms: Equilibrative

- Passive (self) diffusion across the lipid bilayer
  - fluoroquinolones, tetracycline (hydrophobic)
- Diffusion through non-selective OM channels and porins
  - B-lactams, tetracyclins (hydrophilic, charged)
- Facilitated diffusion through selective channels and equilibrative transporters
  - imipenem, catechols, albomycin, albicin
- Carrier-mediated transport
  - Ion transport by valinomycin, host-guest delivery agents
- Paracellular transport – ions, mannitol, polymers

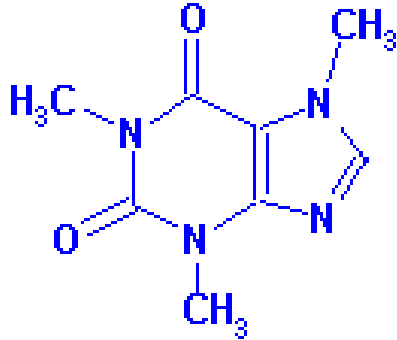
## Energy-requiring Mechanisms: Concentrative

# Examples of Transport

## Driving Force/Drug/Compartment

Diffusion	caffeine	total body water
Ion trapping	Tc-Sestamibi	heart mitochondria
pH trapping	quinidine	renal excretion
Binding	warfarin	plasma/liver ratio
Active	captopril	GI absorption
Group transfer	nucleosides	white cell uptake
Cytoskeletal	gentamicin	renal resorption

# Thermodynamics of Transport I: Equilibrative Diffusion of a Neutral Compound

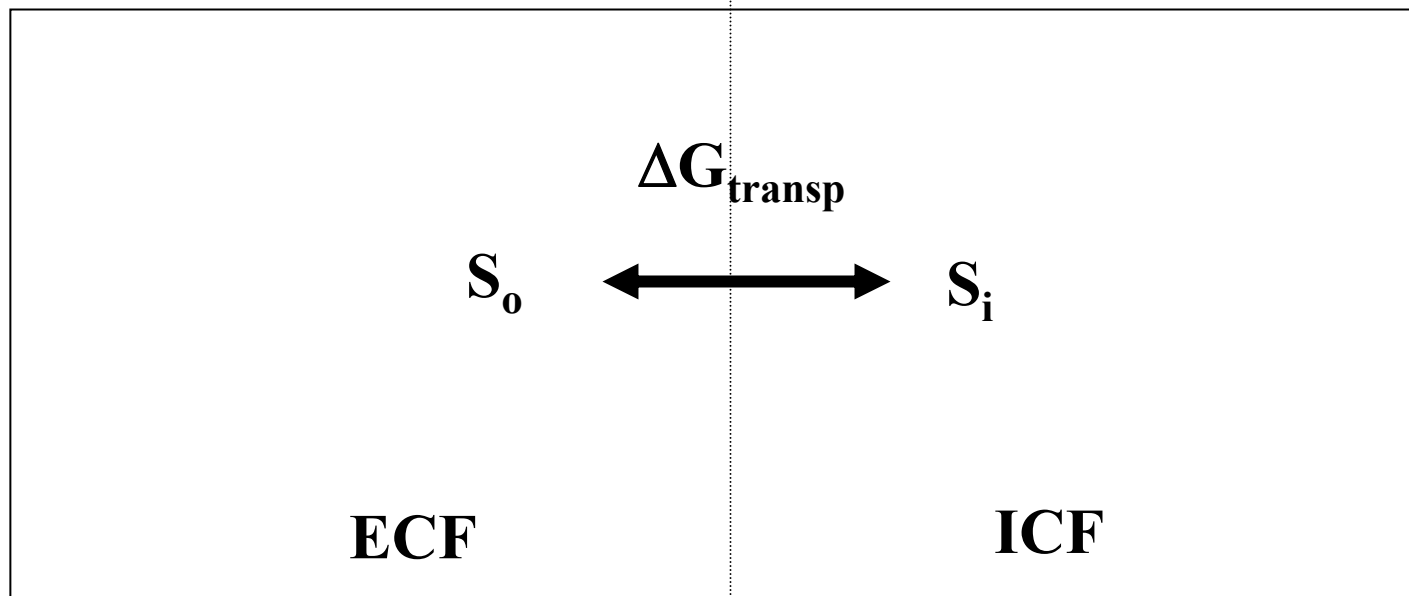


Caffeine

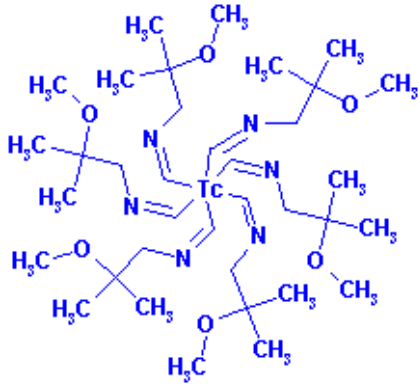
$$\Delta G_{\text{transp}} = 2.303RT \log[S_i]/[S_o]$$

$$= \Delta H - T\Delta S$$

$$\Delta H \approx 0, \Delta S > 0, \Delta G < 0$$



# Thermodynamics of Transport II: Equilibrative Diffusion of a Charged Compound

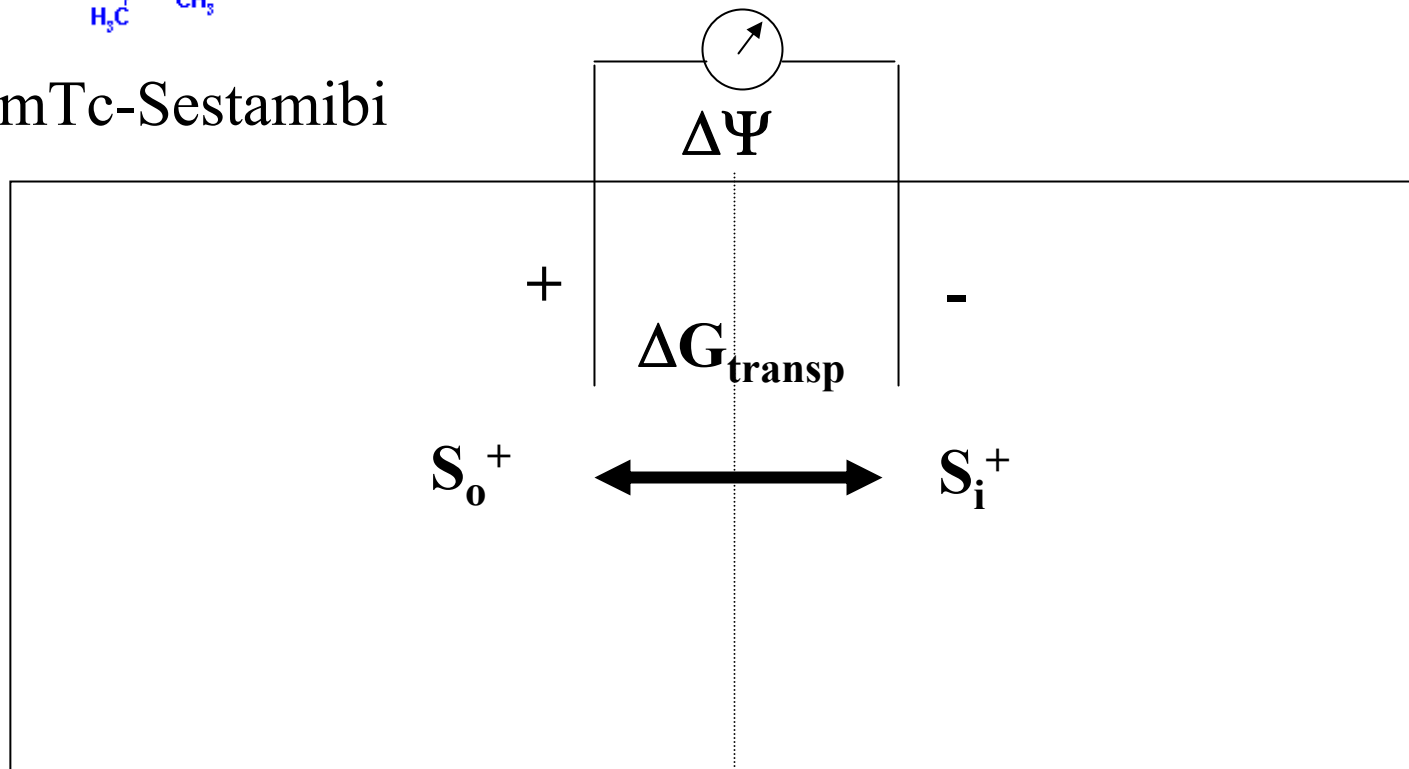


$$\Delta G_{\text{transp}} = 2.303RT \log[S_i]/[S_o] + nF\Delta\Psi$$

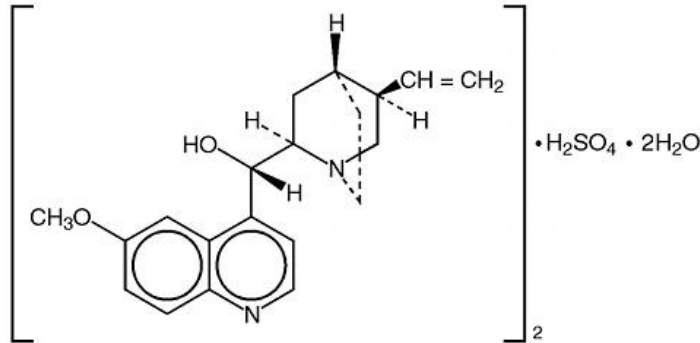
$$\Delta\Psi_{\text{cell}} = 60 \text{ mV} = 1.38 \text{ kcal} \Rightarrow S_i/S_o = 10x$$

$$\Delta\Psi_{\text{mito}} = 168 \text{ mV} = 3.87 \text{ kcal} \Rightarrow S_i/S_o = 622x$$

99mTc-Sestamibi



# Thermodynamics of Transport III: Equilibrative Diffusion – pH Trapping



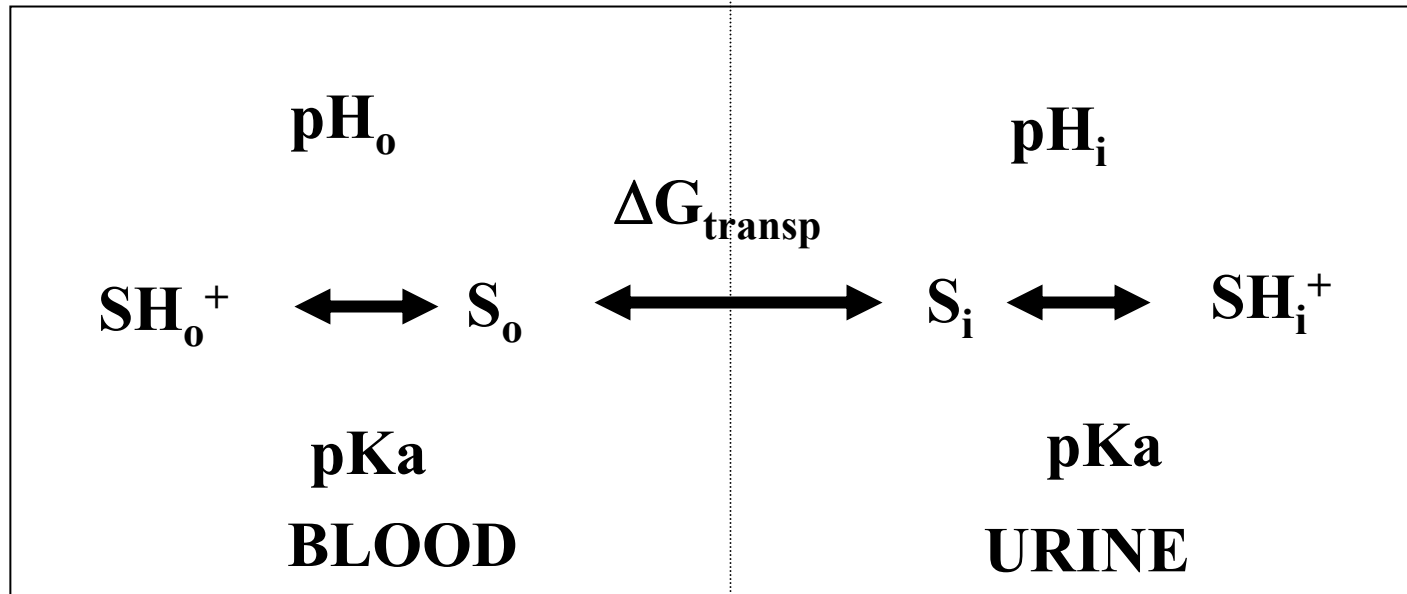
Quinidine sulfate  
 $pK_{a1} = 5.4$ ,  $pK_{a2} = 10$

$$pH = pK_a + \log[S]/[SH]$$

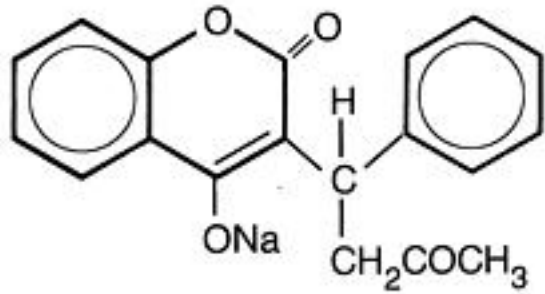
For  $pK_a = 7$ ,  $pH_o = 8$ ,  $pH_i = 6$ ,

$$S_o/S_{H_o} = 10 \quad S_i/S_{H_i} = 1/10$$

$$SH_i = 100x SH_o$$



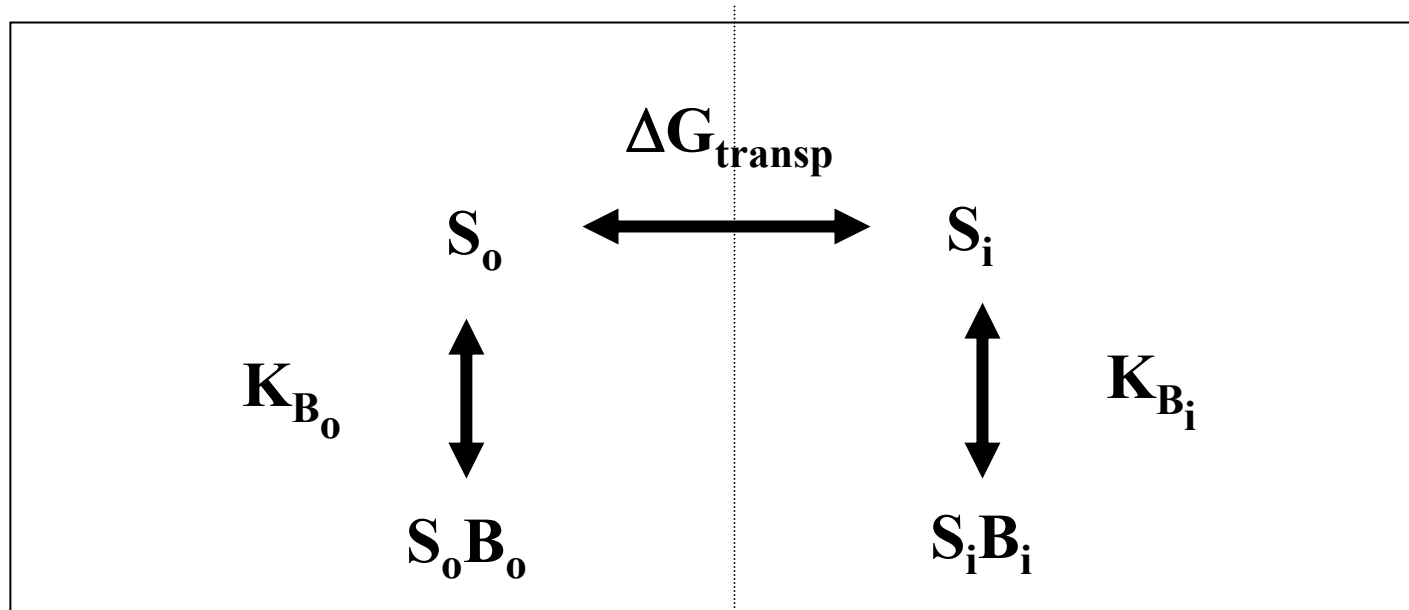
# Thermodynamics of Transport III: Equilibrative Diffusion – Protein Binding



$$K_B = \frac{[SB]}{[S][B]}$$

$$K_{B_0} \neq K_{B_i}$$

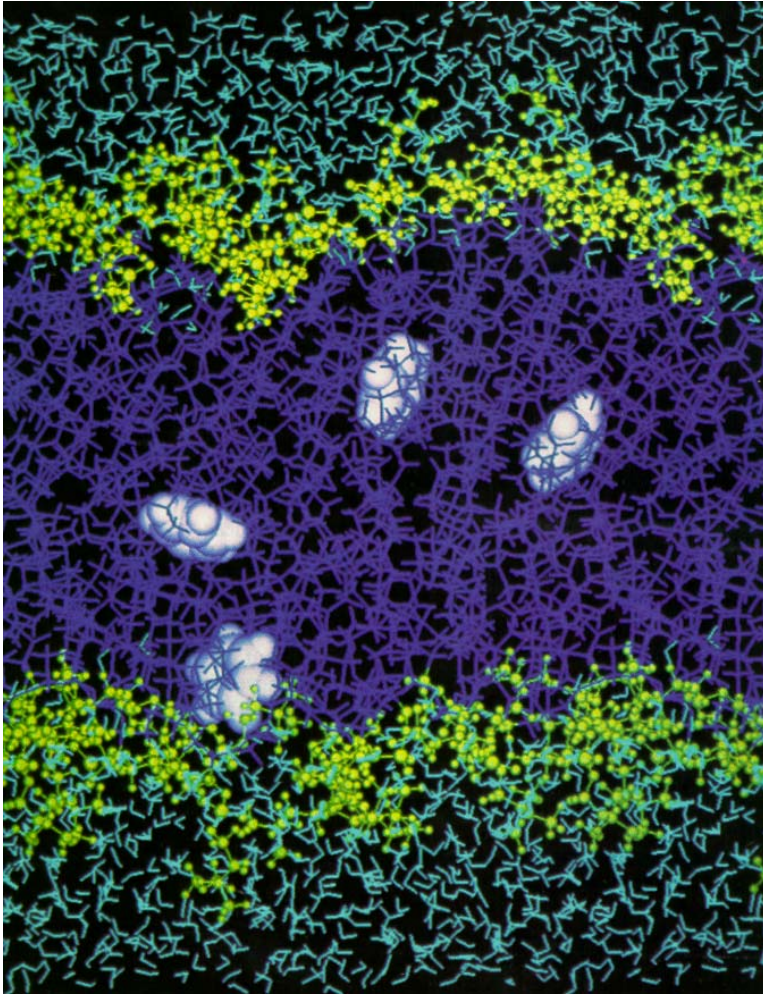
Warfarin



# Passive Diffusion

- Characteristics of passive diffusion
  - $k_{in} = k_{out}$ , net rate =  $k([S_o] - [S_i])$ , non-selective
- Model Membranes (experimental systems)
  - liposomes, BLMs, IAMs, PAMPA
- Membrane Models (functional/mathematical)
  - structural, electrical, single/multiple barrier, partition adsorption/diffusive, unstirred layers
- Simulation of bilayers and transport
  - molecular dynamics - diffusion within bilayer
- QSAR - structure/transport correlations

# Molecular Dynamics Simulation of Membrane Diffusion



From: Bassolino-Klimas, Alper and Stouch..

Snapshot from 10 nsec MD simulation in 100 fs steps. Showed hopping motions of 8 Å over ca 5 psec vs RMS motions of 1.5 Å. Motions differ in center and near surface, both differ from bulk organic. Rotational isomerizations (gauche/trans) gate channels between voids. Differing motions available to adamantane, nifedipine.

# QSAR of Transport

- Hansch Equation

- $\log (1/C) = -k(\log P)^2 + k'(\log P) + \rho\sigma + k''$
- $C = \text{dose or } [S] \text{ for effect (ED}_{50}, \text{IC}_{50}, \text{rate)}$
- $\log P = \text{partition coef or } \pi = \text{lipophilicity factor}$
- $\sigma = \text{Hammett electronic substituent effects}$
- $k, k', k'', \rho = \text{regression coefficients}$

# QSAR Conclusions

Passive Diffusion is a function of:

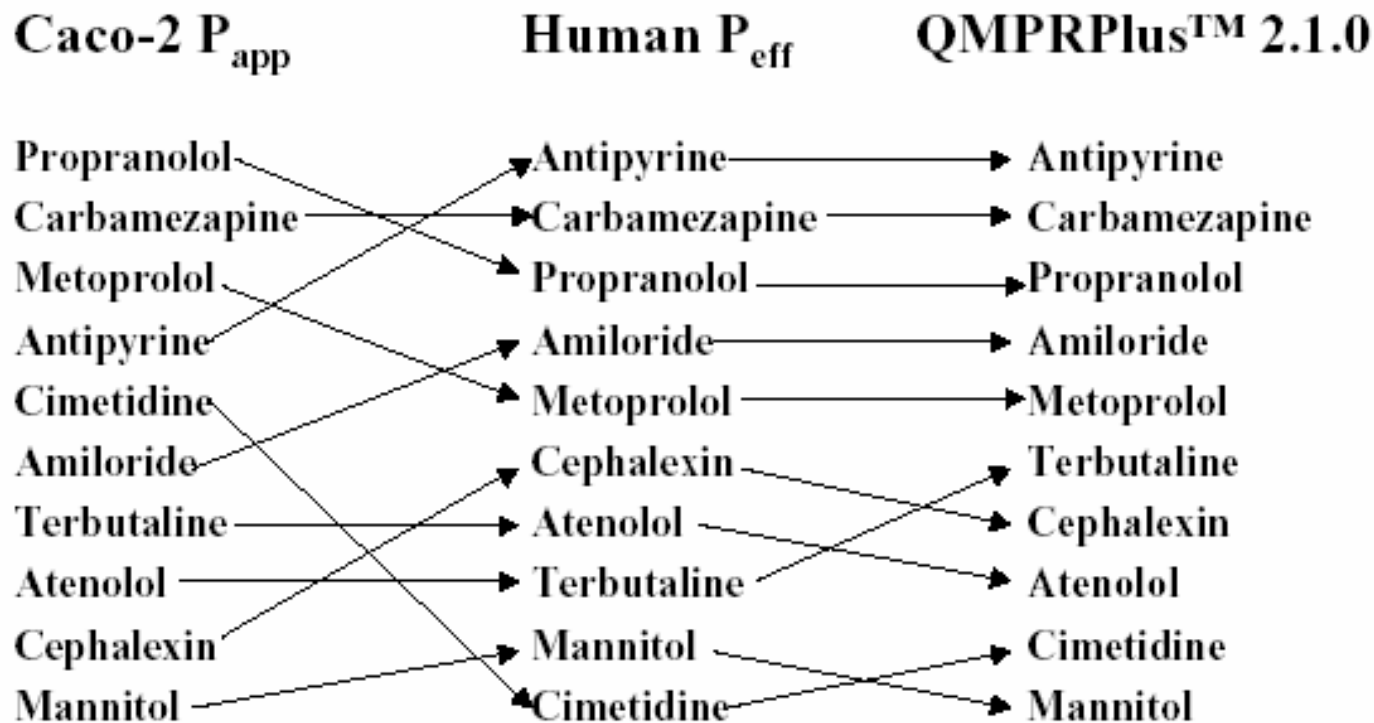
- Lipophilicity ( $\log P_{o/w}$  or CLOGP)
  - GI (0.5-2.0), buccal (4-4.5), topical ( $>2.0$ )
- Hydrogen bond donors/acceptors, polarity/charge
- Water solubility (measured or calculated)
  - melting point, solvation energy, pH/buffers
- pKa - fraction of neutral species available
- mw -  $D \propto 1/\sqrt{mw}$ ;  $mw < 500$  Da
- Confounding factors - inaccurate data, paracellular transport, mediated transport

# Lipinski Rules of Five

Based on analysis of human clinical data for 2245 compounds in the World Drug Index

- H-bond donors – no more than five
- H-bond acceptors – no more than five
- N plus O atoms – no more than ten
- MW no more than 500 dalton
- CLOGP no more than 5 (or  $\log P > 4.15$ )

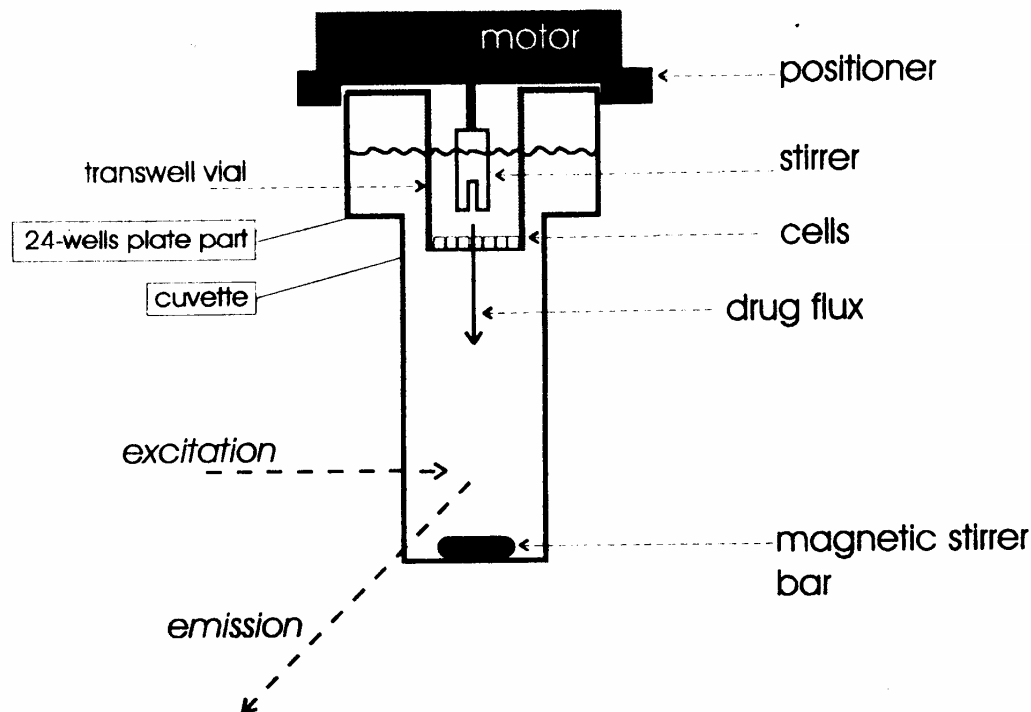
# How well do *in silico* results compare with observations in human?



Bolger; Copyright © Simulations Plus, Inc, 2000

[http://www.simulations-plus.com/pdf\\_files/aaps\\_2000\\_report.pdf](http://www.simulations-plus.com/pdf_files/aaps_2000_report.pdf)  
Neural Net models trained on up to 1337 compounds.

# Apparatus for On-Line Fluorescence Measurement of Transport in Epithelial Cell Cultures



MDCKII  $\pm$  MDR1

$\pm$  SDZ PSC 833

Daunorubicin

$\lambda_{\text{ex}} = 480, \lambda_{\text{em}} = 590$

FITC-dextran

$\lambda_{\text{ex}} = 480, \lambda_{\text{em}} = 525$

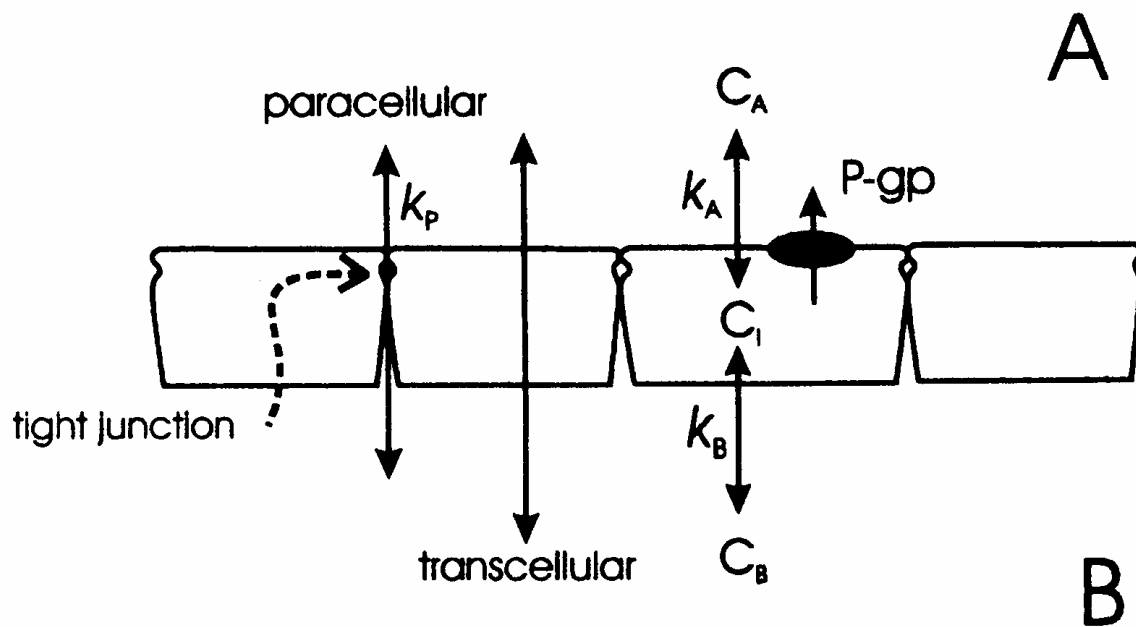
Trans Epithelial

Resistance (TER) =

300 - 600  $\Omega \cdot \text{mm}^2$

Wielinga, et al., J. Pharm. Sci, 88(12), 1340, 1999.

# Paracellular versus Transcellular Transport



Wielinga, et al., J. Pharm. Sci, 88(12), 1340, 1999.

# Paracellular Permeability Enhancers

- Examples:  $\text{Ca}^{++}$  chelators, bile salts, anionic surfactants, medium chain FAs, alkyl glycerols, cationic polymers, cytochalsin D, hormones,  $\text{TNF-}\alpha$ , enterotoxins, zonula occludens toxin (*V. cholerae*)
- Substrates: Ions, mannitol, ceftoxin, dextrans, proteins
- Advantages:
  - hydrophilic & macromolecular substrates
  - avoids intracellular degradation
- Disadvantages:
  - toxicity due high mM concentrations needed
  - non-selectivity of substrate transport
- Concern: systemic toxicity of lumenal contents, blood brain barrier effects (intended and/or not)

# Mechanisms of Transmembrane Drug Transport

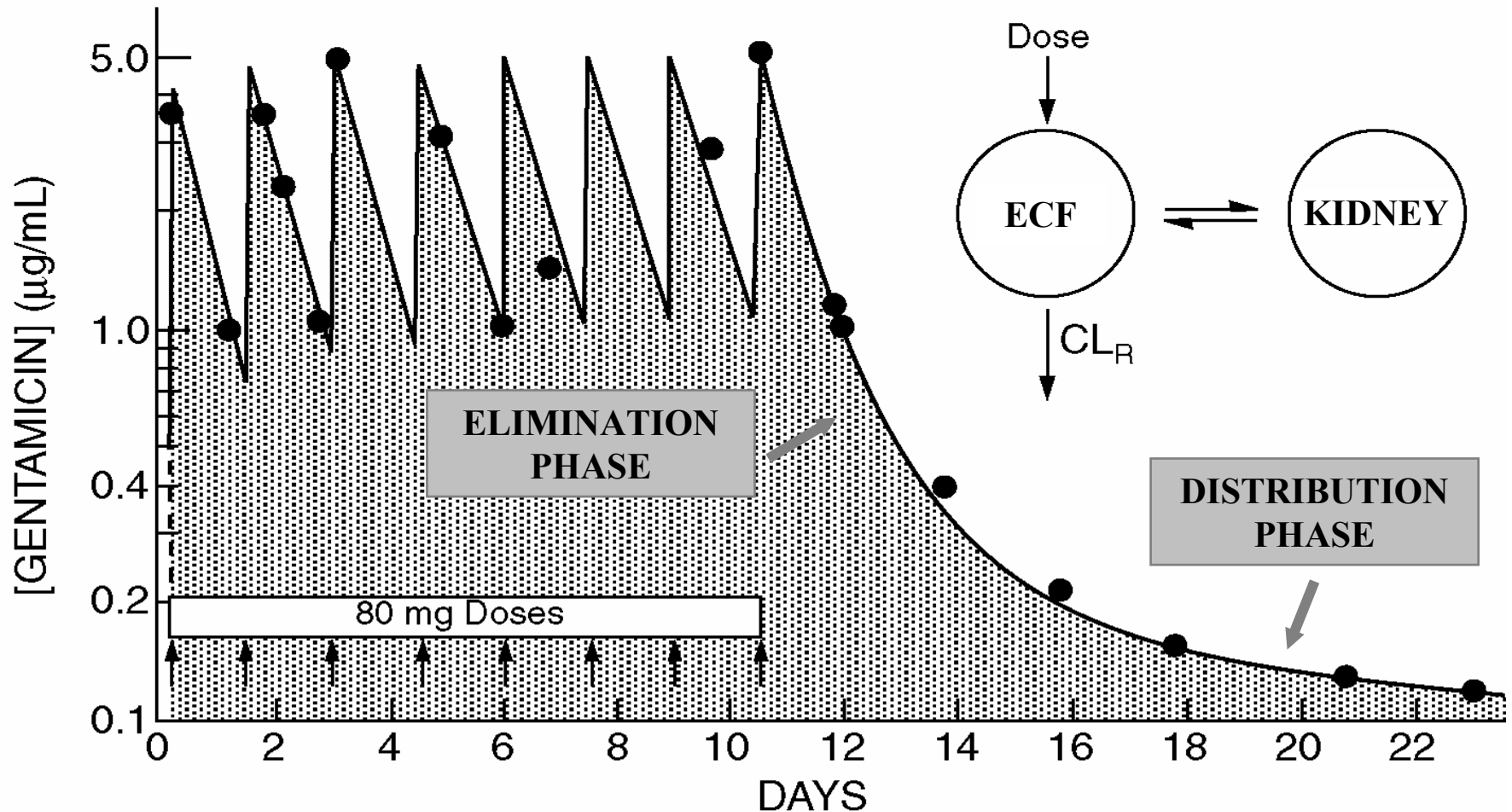
## Diffusion Mechanisms:

## Energy-requiring Mechanisms:

- Endocytosis - receptor mediated
  - aminoglycosides (renal tubule), polymers, peptide hormones, targeted delivery, prodrugs, proteins
- Transcytosis
  - Drug, macromolecule, particle delivery across GI, BBB, lung
- Protein transduction
  - HIV TAT, HSV VP22, antennapedia, other amphipathic peptides
- Active transport via membrane transport proteins
  - aminoglycosides (bacteria), cycloserine, phosphomycin, alaphosphin, others

# Endocytosis of Aminoglycosides

## Gentamicin Elimination Phase Precedes Its Distribution Phase (Flip/Flop)

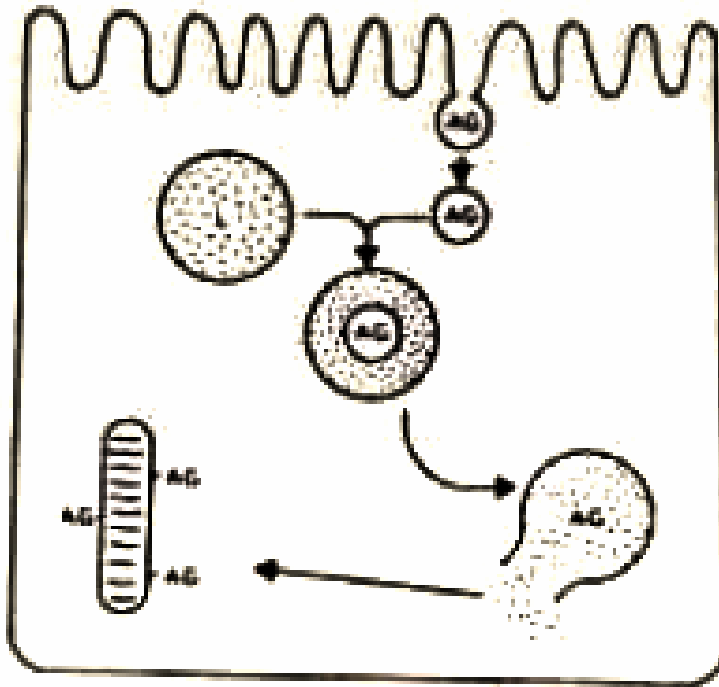


\* From Schentag JJ, et al. JAMA 1977;238:327-9.

# Receptor-Mediated Endocytosis

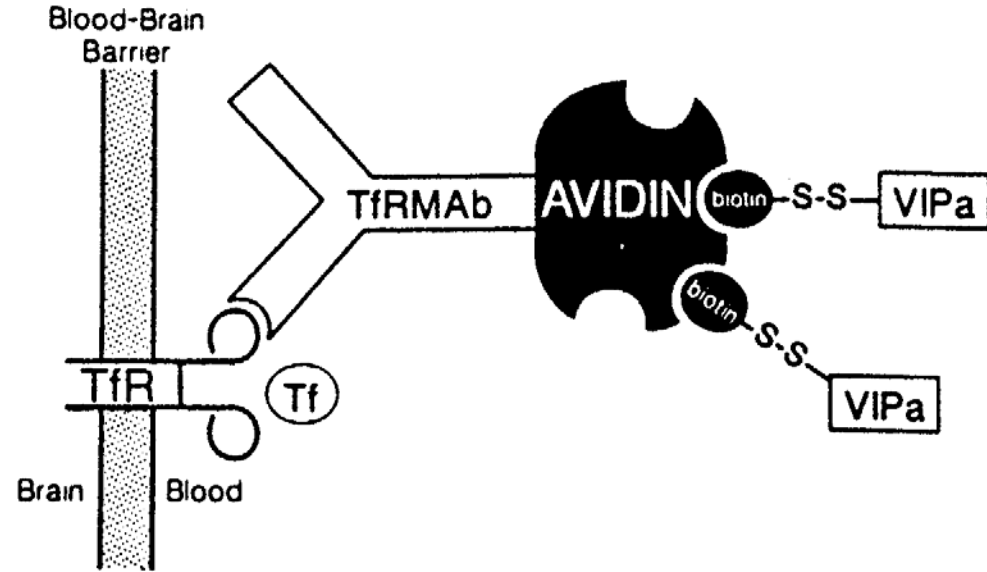
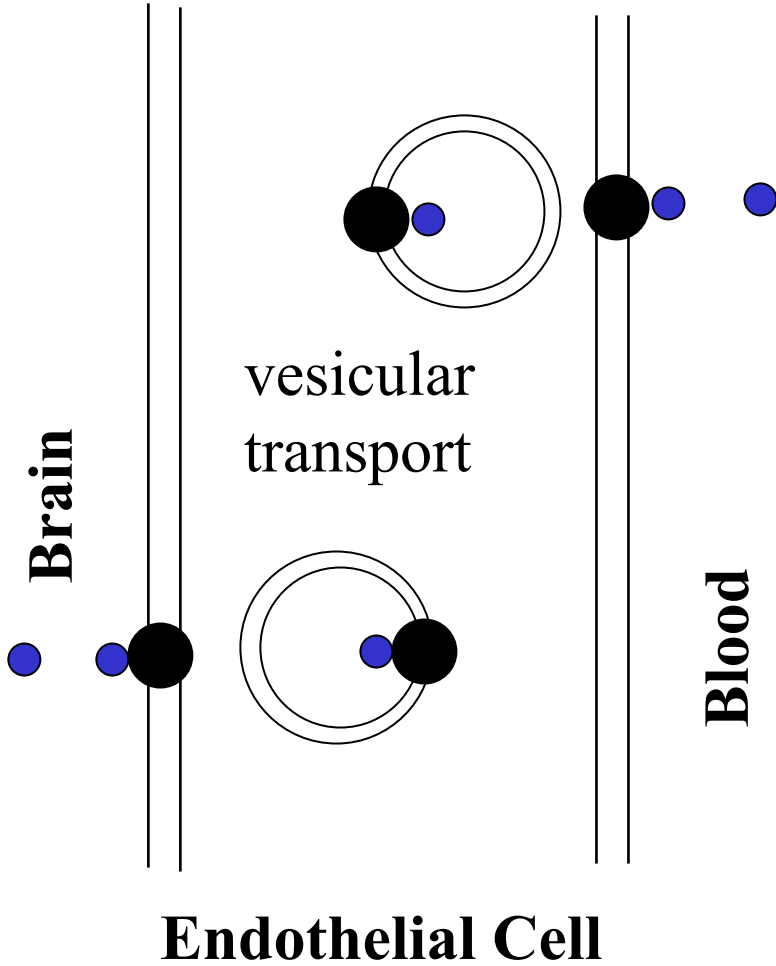
## Aminoglycoside Nephrotoxicity

### Proximal Renal Tubule



- Aminoglycosides (++++) bind to anionic phospholipids
- Endocytosis via clathrin-coated pits into lysosomes
- Reduced by 95% in megalin (gp330/LDL-receptor related protein-2) KO mice
- Uptake of proteins and Ca<sup>++</sup>
- Intracellular release leads to selective mitochondrial damage in kidney
- Epithelium of inner ear also sensitive (ototoxicity), but see also rRNA polymorphism.

# Transcytosis Delivery of Prodrug



From: Bickel & Pardridge.  
Transferrin receptor-mediated transcytosis of an mAb-avidin-biotin-disulfide cross-linked vasoactive intestinal peptide.

TfR, VitB12R, FcRn, PigR are under commercial development.

# Protein Transduction by Cell Penetrating Peptides

- Non-receptor mediated uptake (and subcellular targeting)
  - Self-inserting amphipathic peptides
  - Energy dependent (or not) internalization NOT via clathrin coated pits
  - Mediated by charge interaction with glycosaminoglycans on cell surface
  - D-enantiomers and inverted sequences are active
  - Cargos are synthetic or biosynthetically linked or fused peptides, proteins, small molecules, nucleic acids, vesicles, nanoparticles
  - Delivery to cells, perfused tissues, organism, expression in situ-gene therapy
- HIV transactivator of transcription (TAT)
  - Nuclear localization sequence Tat48-60
- Drosophila antennapedia transcription factor homeodomain
  - Penetratin = Antp43-58 homeodomain 3<sup>rd</sup> helix
- SynB vectors from protegrin-1 (18 a.a. peptide from porcine leukocytes)
- Transportan – synthetic 27 aa chimera of galanin and mastoparin-X
- Amphipathic model peptides, signal sequence peptides, homo-arginine polyers
- Example: Arg7 peptide-PKC- $\epsilon$  agonist protection of ischemic rat heart
- Example: SynB-doxorubicin delivery across BBB bypasses PgP

# Active Transport

- Rates  $>$  passive, solute specific, high  $Q_{10}$
- Non-symmetrical ( $k_{in} \neq k_{out}$  at  $[S_i] = [S_o]$ )
- Saturable transport - Michaelis-Menten
- Inhibitable - competitive, non-competitive
- Regulated - inducibility & repression
- Tissue specific- differential expression
- Energy dependent - active transport
  - primary pumps - respiration, photosyn, ATPase
  - secondary transporters (coupled to  $H^+$ ,  $Na^+$  etc.)

# Biochemistry of Transporters

- Discovery and functional definition in vivo and in vitro
- Genetic definition by cloning and sequencing
- Confirmation by expression of transport activity in vitro
- Substrate structure/activity profiles and co-substrates (GSH, ATP, H<sup>+</sup>, Na<sup>+</sup>), uncouplers
- Tissue distribution - EST database, RNA expression levels, antibodies, in situ methods
- Phenotypes in Knock Out Rodents
- Subcellular localization microscopy
- Isolation, purification, reconstitution
- Structural biology - EM, X-ray, NMR
- Mechanism of substrate transport and energy coupling - enzymology, inhibition, drug design

# Membrane Transporter Families

## ABC Superfamily

ABC peptide transporter family

P-glycoprotein (MDR) family

MDR1a,1b,2,3 - organic cations, lipids (PC)

MRP1,2,3 - organic anions, GSX conjugates

cMOAT - canalicular multispecific organic anion transporter = MRP2

cBAT - canalicular bile acid transporter

## Porins & Channels

## Major Facilitator

Superfamily (>1,000)

POT - proton coupled oligopeptide transporter

NT - Na<sup>+</sup> coupled nucleotide transporter

NTCP - N<sup>+</sup> coupled taurocholate protein

OATP - polyspecific organic anion transport protein

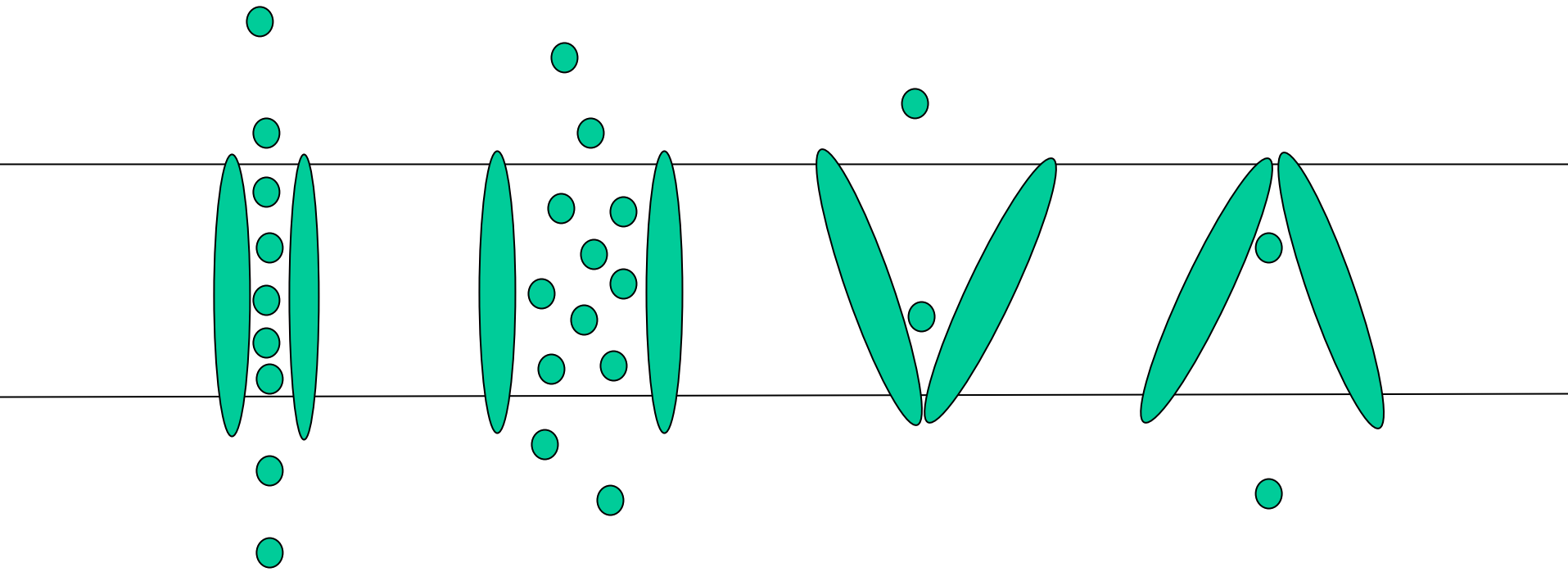
OAT-K1 - renal methotrexate transporter

OCT - organic cation transporter - electrogenic

RFC - reduced folate carrier

sGSHT - glutathione conjugate transporter

# Membrane Transporter Models Circa 1991

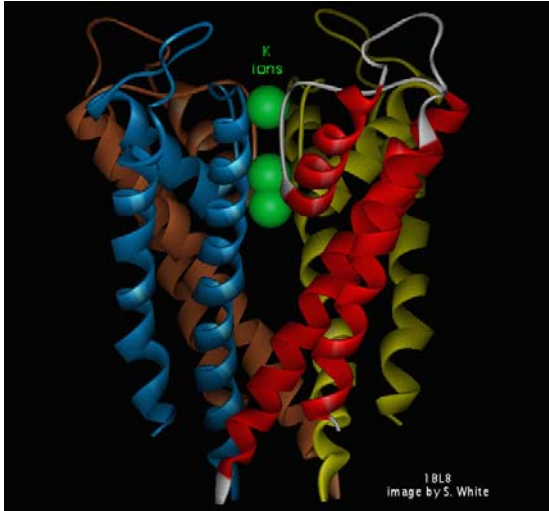


Channel

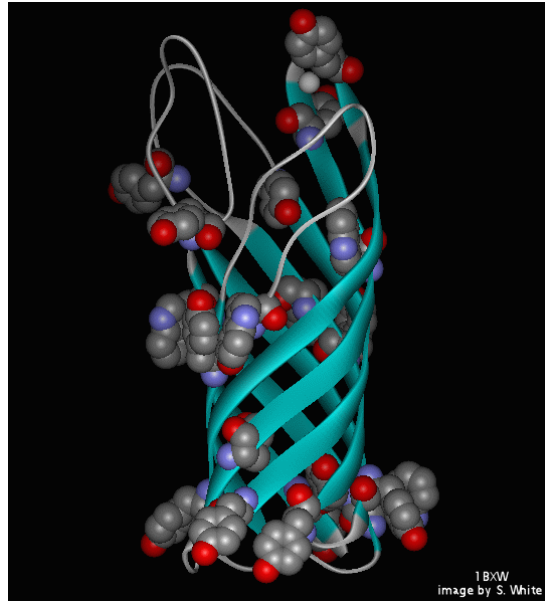
Pore

Transporter

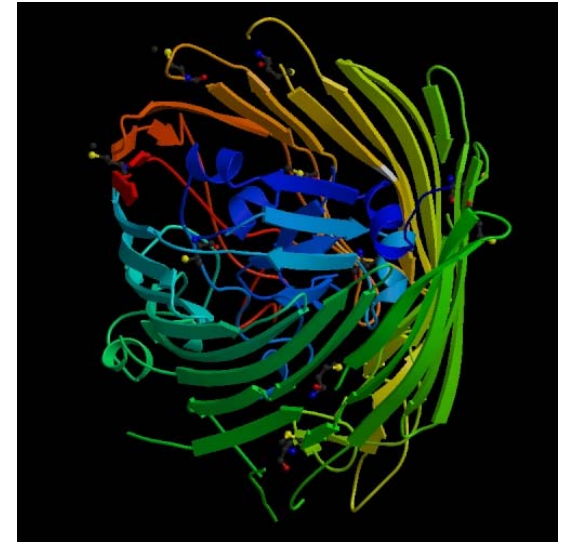
# Membrane Transporter Models Circa 2001



KscA



OmpA



FepA

Membrane Protein Resources web site by Stephen White lab.  
[http://blanco.biomol.uci.edu/MemPro\\_resources.html](http://blanco.biomol.uci.edu/MemPro_resources.html)



# Structure of bacterial oxalate transporter: a paradigm for the multifacilitator superfamily.

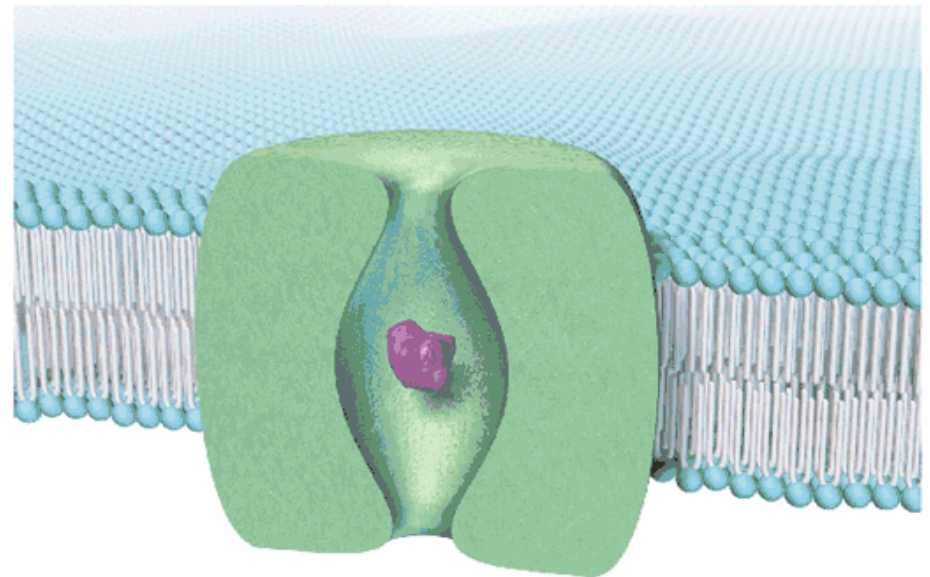
T. Hirai, et al. (Subramaniam lab at NIH), Nature Structural Biology 9(8): 597-600. Low (6.5 Å) resolution based on EM of 2D crystals.



*b*

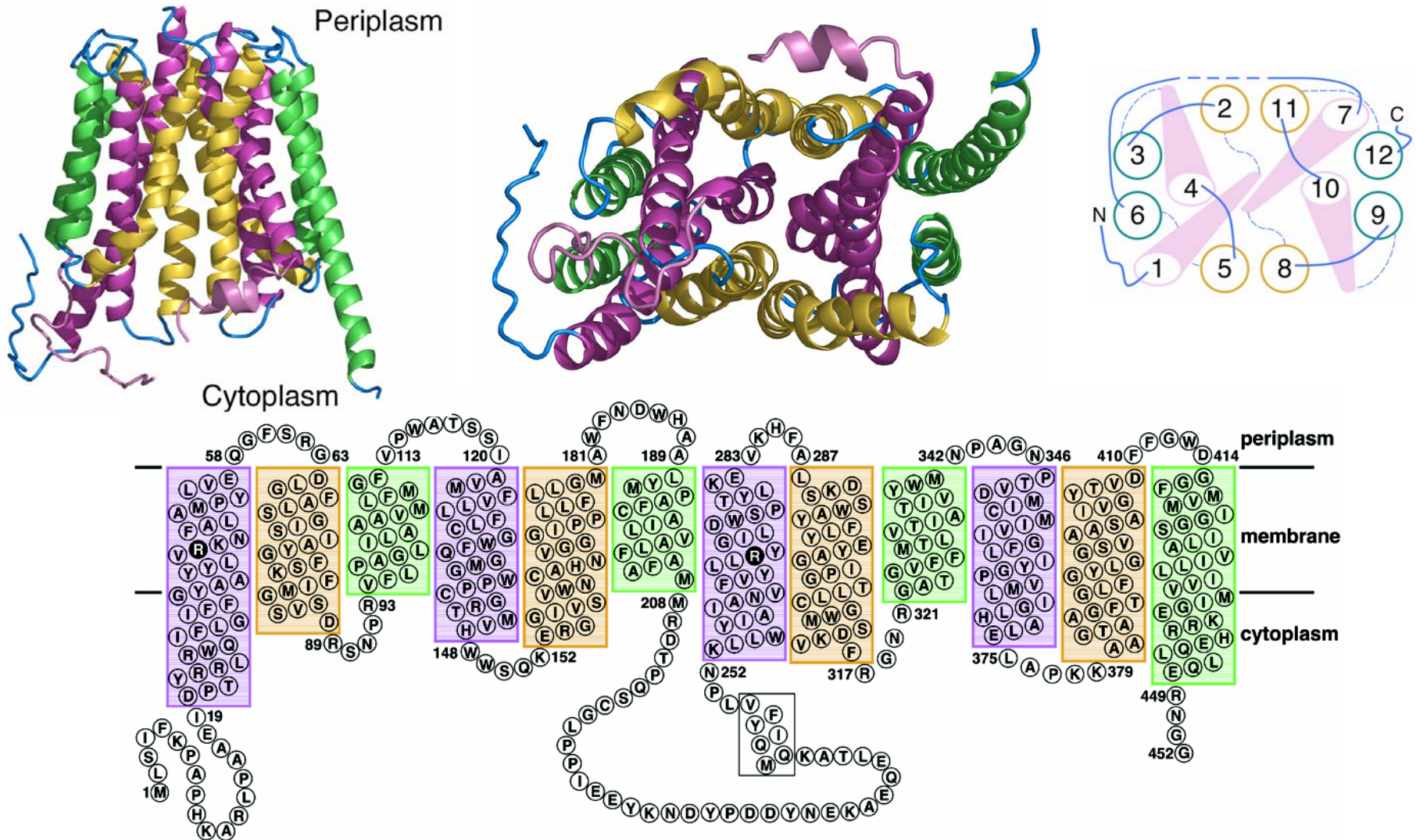


*c*

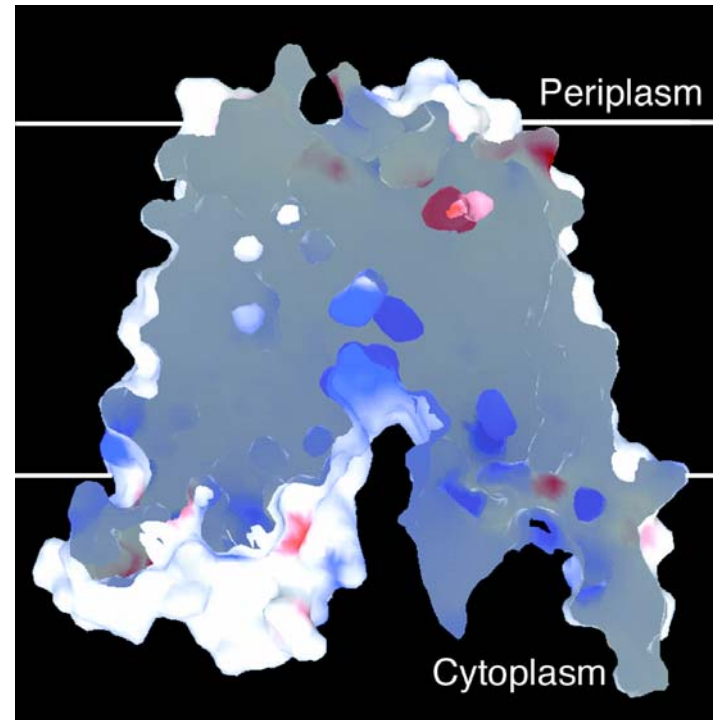
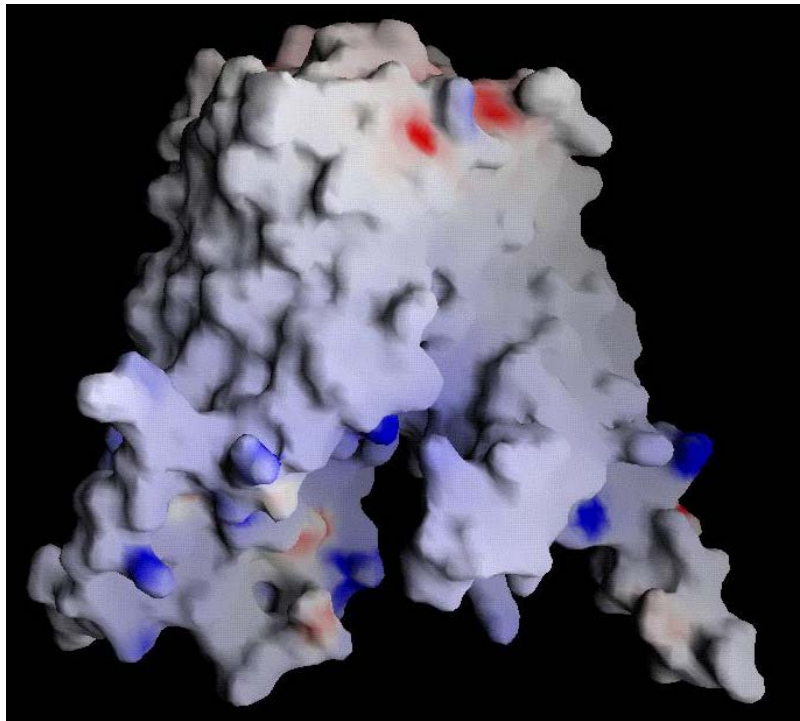


# Structure and Mechanism of the Glycerol-3-Phosphate Transporter from *Escherichia coli*. [G-3-P/ $P_i$ exchange $\Delta P_i$ driven]

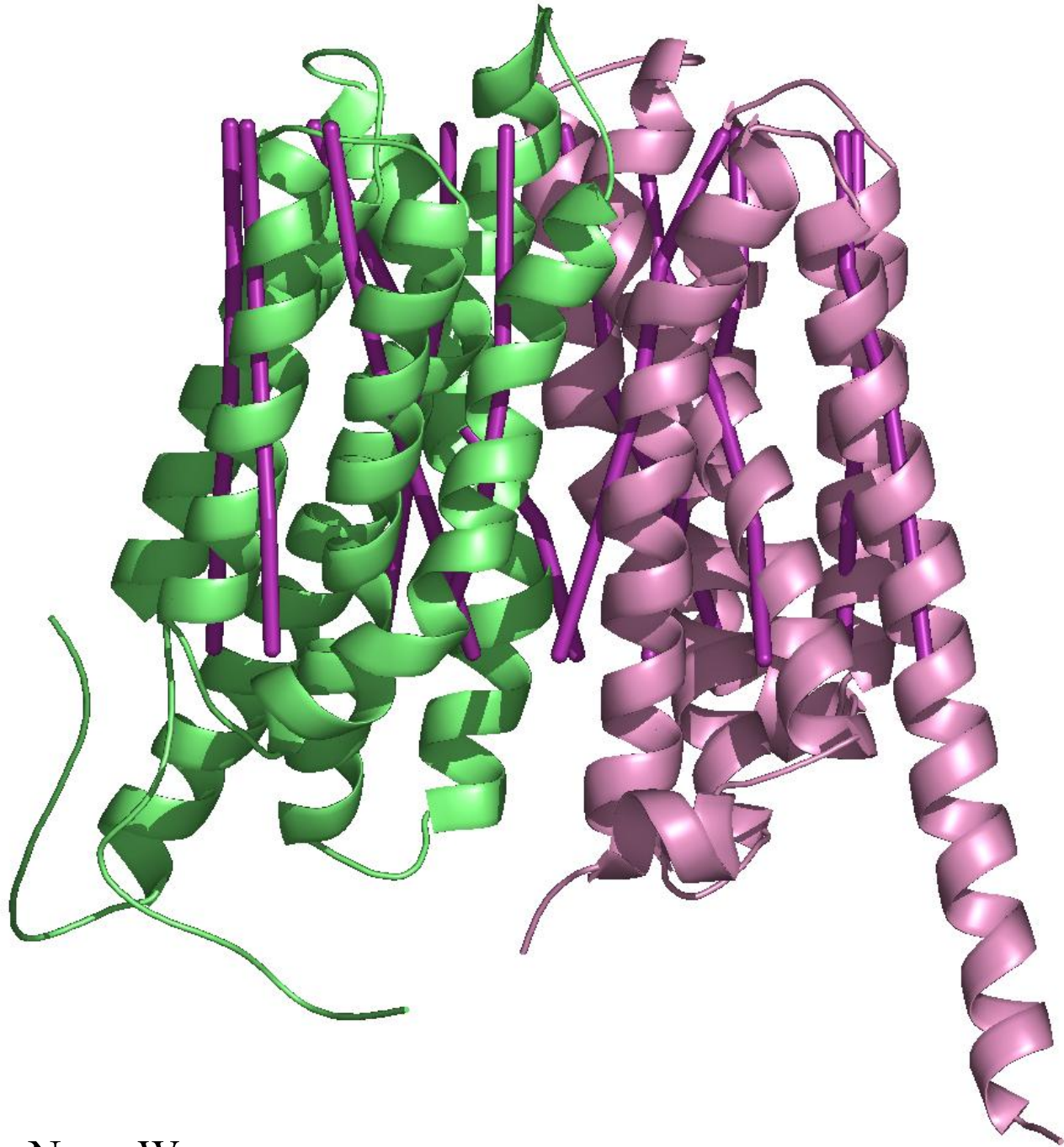
Y. Huang, et al. (Da-Neng Wang lab at NYU) *Science* 301, 616-620,  
Aug 1, 2003. High resolution (3.3 Å) based on x-ray crystallography.



Proposed transport mechanism: i) translocation pathway between N- and C-terminal halves; ii) binding of G-3-P between R45(H1) and R269(H7); iii) binding lowers barrier for conformational exchange; iv) rocking motion exposes binding site to alternate membrane faces; v)  $P_i$  gradient drives conformational return.

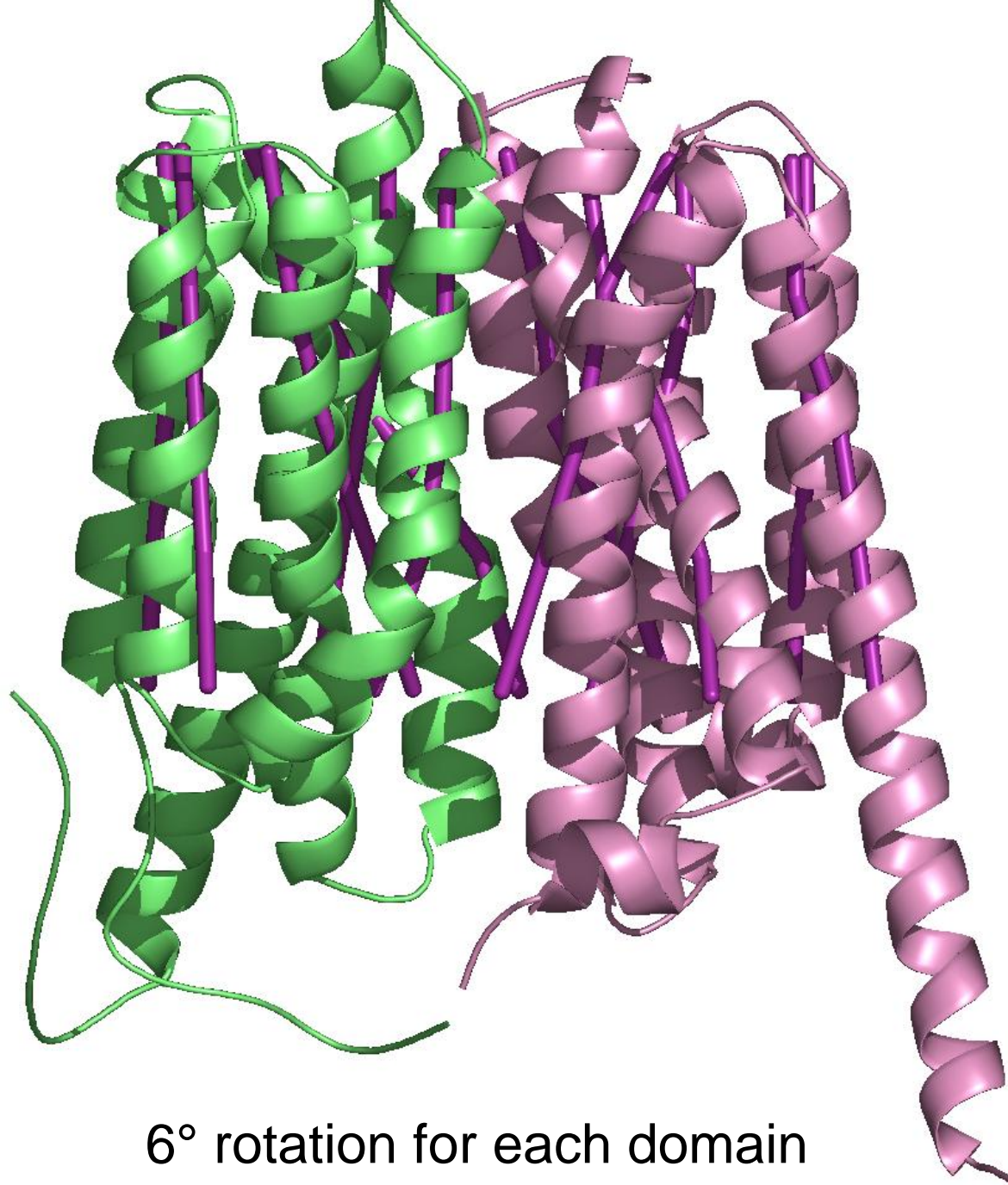


C<sub>i</sub>



Courtesy of Da-Neng Wang

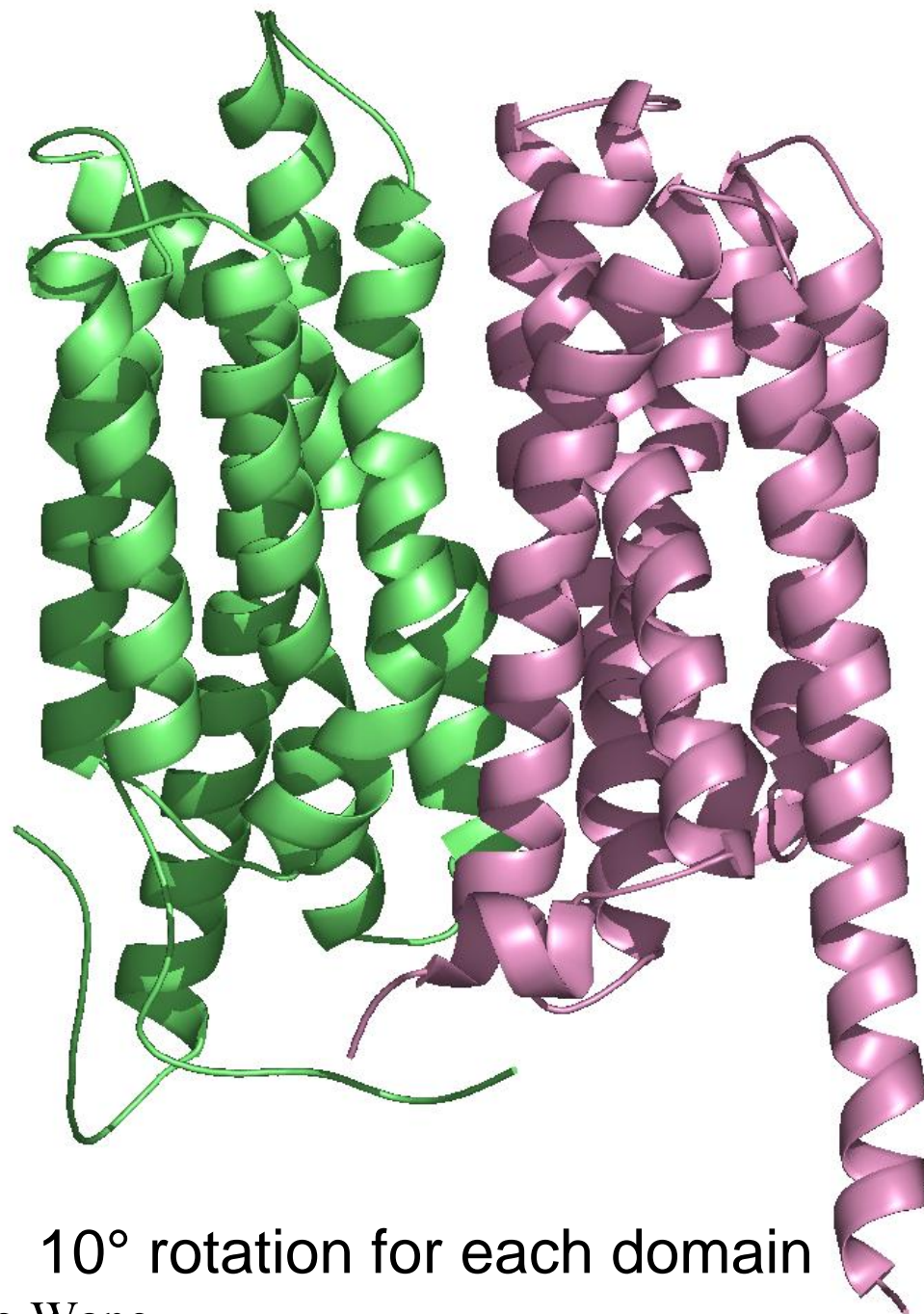
$C_0-S$



$6^\circ$  rotation for each domain

Courtesy of Da-Neng Wang

$C_0$



10° rotation for each domain

Courtesy of Da-Neng Wang

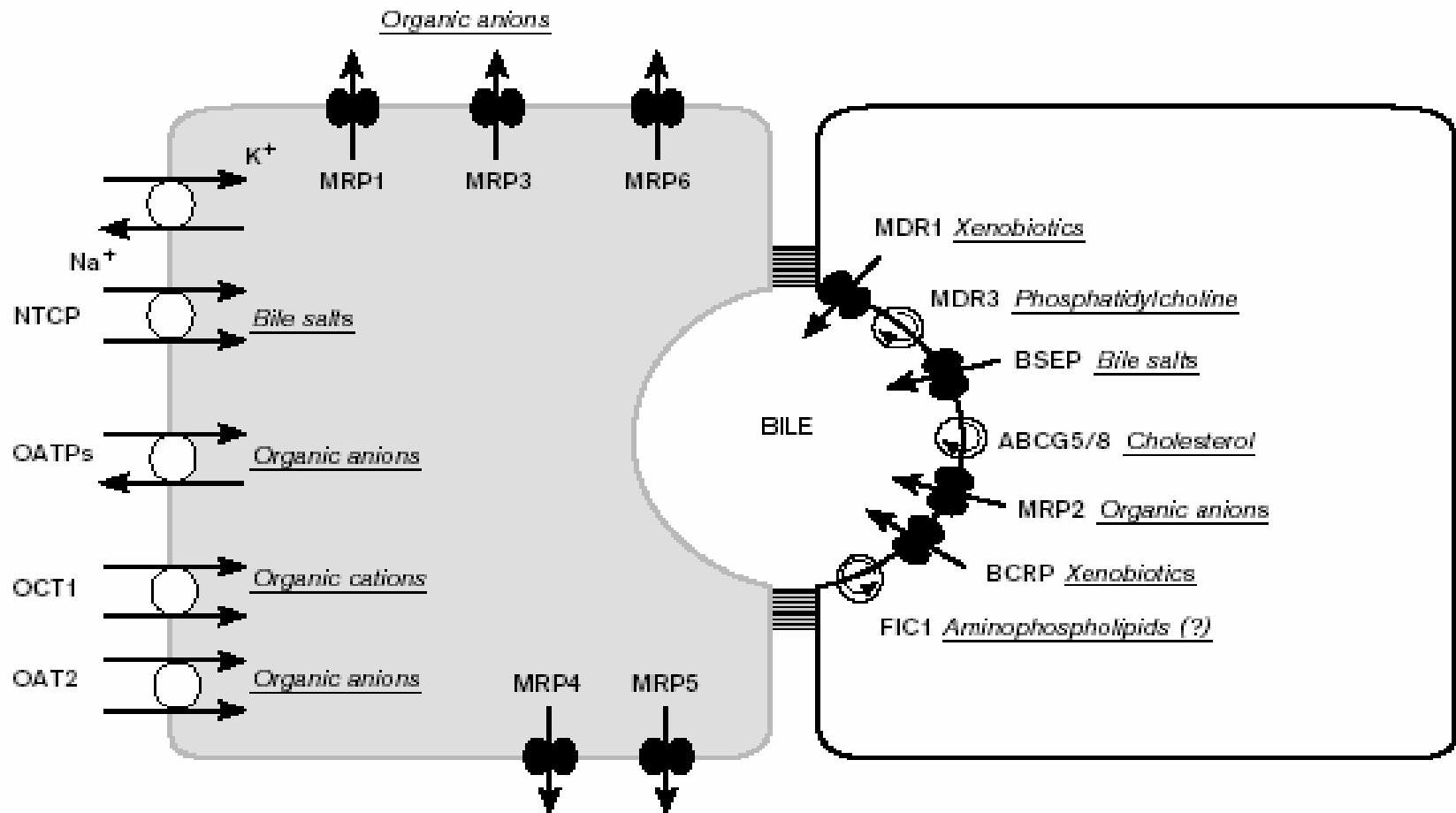
# Drug Uptake by Endogenous Transporters in the Small Intestine

Lee, et al., Adv. Drug Delivery Reviews, 2001. Table 1.

Transporter	Substrates
Amino Acid	L-DOPA, gabapentin
Organic Anion	Captopril, acyclovir
Nucleoside	Didanosine, idoxuridine
Oligopeptide	B-Lactam antibiotics
Monocarboxylic Acid	Valproic acid, pravastatin
Organic Cation	Cimetidine, verapamil

# Hepatic transporters *circa* 2003

C. Pauli-Magnus & P.J. Meier, Pharmacogenetics (2003) Apr;13(4):189-98.



The transport polarity of hepatocytes. See text for a detailed explanation. ○ Basolateral uptake systems. ↓ ATP-binding cassette (ABC) transporters ('efflux pumps'). ⊙ Presumptive ABC lipid translocases ('flippases').

# Substrates of cMOAT (MRP2)

(canalicular multispecific organic anion transporter)

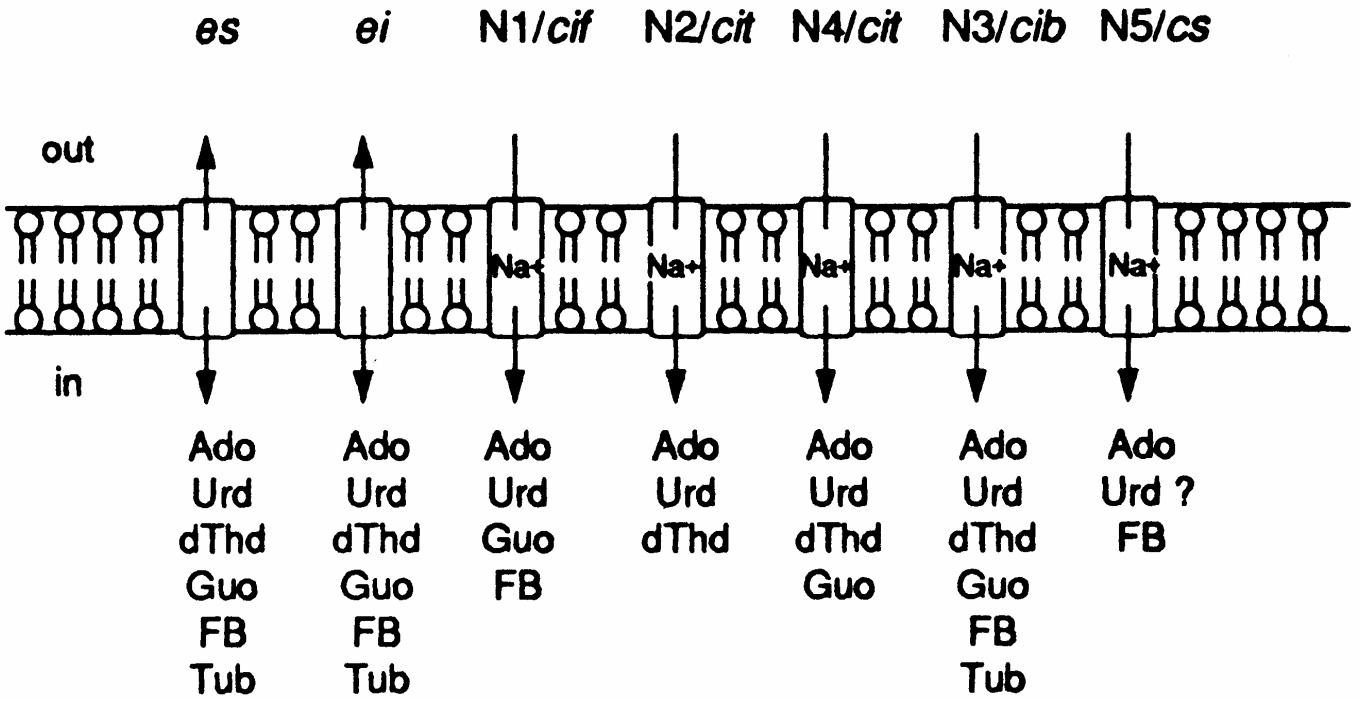
Selected from Table IV in Chap. 14 of Amidon & Sadee.

- glutathione disulfide
- leukotrienes (C4, D4, E4, N-acetyl-E4)
- glutathione conjugates (e.g., DNP, bromosulfophthalein, metals Sb, As, Bi, Cd, Cu, Ag, Zn)
- glucuronide conjugates (bilirubin, T3, p-nitrophenol, grepafloxacin)
- bile acid conjugates (glucuronides and sulfates)
- organic anions (folates, methotrexate, ampicillin, ceftiaxone, cefadizime, grepafloxacin, prevastatin, temocaprilate)

# Nucleotide Transporters of Mammalian Cells

From: C.E. Cass

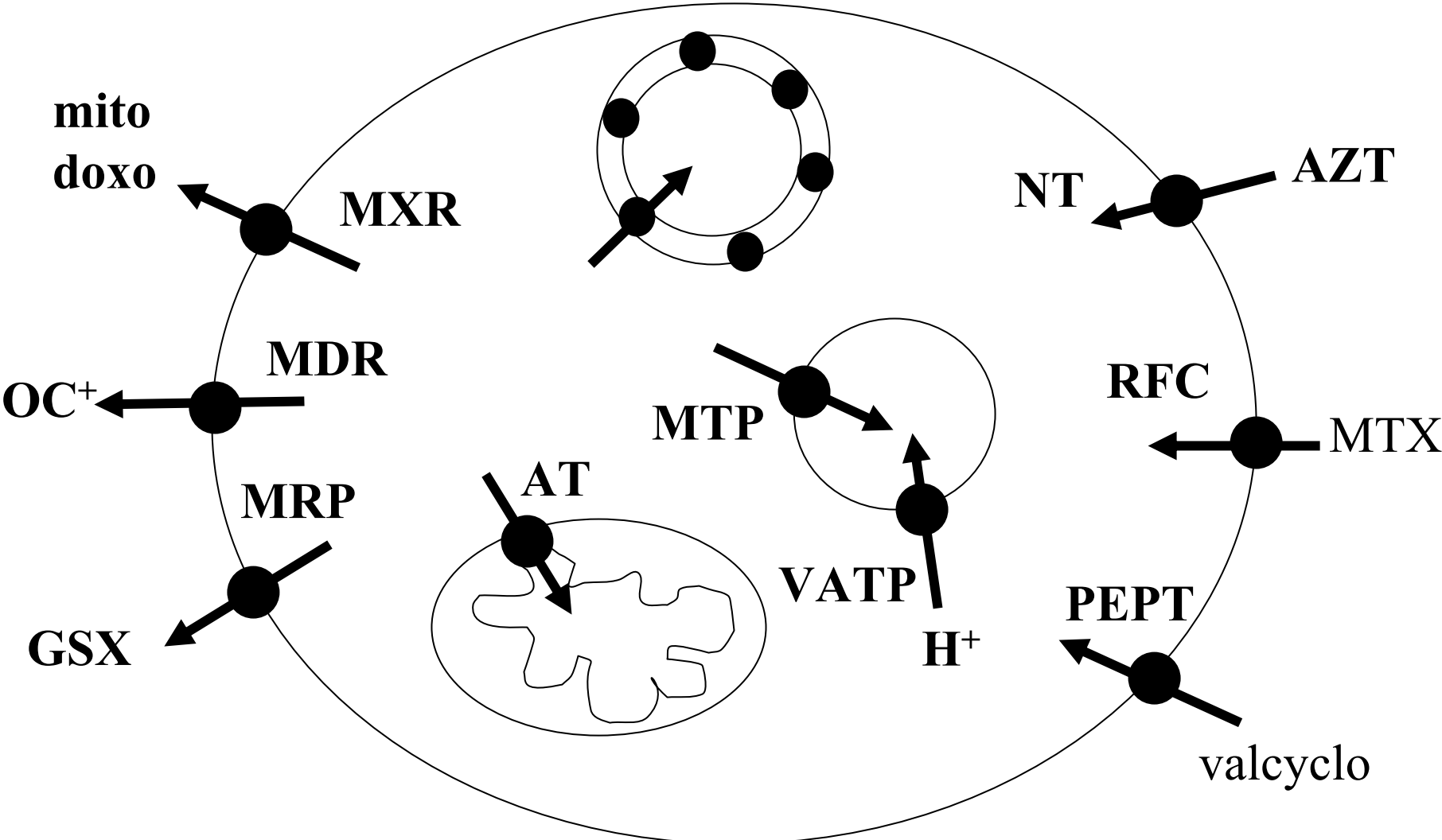
es,ei = sensitivity versus nitrobenzylthioinosine



ENT1 ENT2 CNT1 CNT2 CNT3  
 SLC29A1 A2 SLC28A1-A3 Cloned Transporters  
 Basolateral Apical in kidney

Mangravite (Giacomini) EJ Pharm 479 (2003), 269-281.

# Tissue Uptake and Intracellular Drug Transport (subcellular PK)





# Mitochondrial toxicity depends on differences in intracellular transport

- Nucleoside drugs target DNA replication
- Inhibition of poly leads to mtDNA loss
- AZT, ddC, ddI, d4t are known mito toxins
  - hepatotoxicity, pancreatitis, neuropathy, myopathy
  - but rarely fatal
- Fialuridine trial for hepatitis B at NIH resulted in hepatic failure in 7/14 (5 died)
  - Human ENT1 and ENT2 are expressed in mito
  - Mouse ENT1 and ENT2 are NOT
  - Drugs differ in rates of transport and activation

# Exploiting Nutrient Transporters to Enhance Drug Bioavailability

- Valacyclovir is an amino acid ester prodrug of the antiviral drug acyclovir.
- Oral bioavailability (AUC) is increased in humans 3-5x.
- Intestinal permeability in a rat perfusion model is increased 3-10x. Effect is specific (SAR), stereospecific (L), saturable, and inhibitable by PEPT1 substrates (cephalexin, dipeptides), and by gly-acyclovir, val-AZT.
- Competitive with  $^3\text{H}$ -gly-sarc in CHO/hPEPT1 cells.
- Enhanced, saturable, inhibitable mucosal to serosal transport demonstrated in CACO-2 cells and accompanied by hydrolysis. Serosal to mucosal transport is passive.
- Rationale applied by Roche to design of valgancyclovir.
- XenoPort, Inc. ([www.xenoport.com](http://www.xenoport.com)) gabapeptin-XP Pfizer

# Drug Interactions & Drug Transport

- Digoxin - non-metabolized substrate for Pgp
  - Verapamil, amiodarone, and quinidine increase plasma levels, reduce renal and non-renal clearance, increase blood/brain barrier transport.
  - Dose adjustment may be needed in 50% of cases.
  - St. John's wort (*Hypericum perforatum*) decreased digoxin AUC by 25% after 10 days treatment through induction of Pgp.
- HIV Protease Inhibitors
  - Amprenavir clearance reduced by nelfinavir (-41%) and by indinavir (-54%), but not saquinavir.
  - FDA warning against *Hypericum* supplements

# Drug Resistance & Reversal

- **MDR1 (P-glycoprotein) – drug efflux pump**
  - Multiple trials of multiple agents – recent efforts at inhibiting transcription
  - Steady state digoxin therapy was established in normal healthy volunteers (1 mg then 0.125 mg/day). Initiation of valsopodar (400 mg followed by 200 mg twice per day) caused immediate and progressive increases in digoxin AUC (+211%) and decreases in total body, renal, and non-renal clearance (-67%, -73%, -58%) after 5 days.
- **BCRP (breast cancer resistance protein or ABCG2)**
  - Inhibited by fungal toxin fumitremorgin C, but neurotoxic side effects
  - Koll143 and other derived analogs developed inhibit BCRP, but not Pgp or MRP
  - Non-toxic in mice, increased oral availability of topotecan in mice
- **RFC (reduced folate carrier) - antifolate drugs (methotrexate)**
  - Resistant leukemia cell lines were selected by stepwise doses
  - Cross resistance (>2000x) to five novel hydrophilic antifolates shown
  - Intracellular folate levels reduced, increased requirement 42x
  - Hypersensitive to hydrophobic antifolates
  - Mutations clustered in exons 2 and 3, TMD1

# Pharmacogenetics of Transport (I)

Estimating contribution of genes to variation in renal drug clearance.

Leabman & Giacomini, Pharmacogenetics 13(9), 581-4, 2003.

Based on Repeated Drug Administration Literature Data

Comparison of variation between individuals and variation in response for a given individual.

$$r_{GC} = (SD_{\text{between}}^2 - SD_{\text{within}}^2) / SD_{\text{between}}^2$$

Drug	CL <sub>mean</sub>	SD <sup>2</sup> <sub>between</sub>	SD <sup>2</sup> <sub>within</sub>	P <sub>between≠within</sub>	
Metformin	450	5343	299	<0.01	Mediated*
Amoxicillin	154	476	44	<0.01	Mediated*
Ampicillin	165	919	334	<0.01	Mediated*
Terodiline	11.3	6.4	4	>0.05	Passive
Iohexol	115	110	88	>0.05	Passive
Digoxin	150	1093	958	>0.05	Passive

Mediated by variations in OAT1, OT2, PEPT2, Npt1.

# Pharmacogenetics of Transport (II)

- OATP-C (organic anion transporting polypeptide-C)
  - liver specific uptake transport – bile salts, estrone sulfate, estradiol-glucuronide
  - multiple SNPs detected, including 14 non-synonymous, gene frequency depends on race. Tirona et al. JBC 276(38), 35669-75, 2001.
  - 16 assessed in vitro, 8 result in reduced transport, esp. T521C (val174ala) occurs in 14% European- and G146C (gly488ala) in 9% of African-Americans
  - Effects on pravastatin pharmacokinetics noted for OATP-C 15 allele (Asp130/Ala174) versus OATP-C 1b allele (Asp130/Val174). Nishizato et al. Clin Pharm Ther. 73(6), 554-65, 2003.

Non-renal clearances (l/kg/hr):

1b/1b =  $2.01 \pm 0.42$  n = 4 P < 0.05

1b/15 =  $1.11 \pm 0.34$  n = 9 P < 0.05

15/15 = 0.29

# Pharmacogenetics of Transport (III)

## Pharmacogenetics Network - UCSF Project

<http://pharmacogenetics.ucsf.edu>

OCT2 Transporter - renal tubule basolateral

Adverse Effects - procainamide, clonidine

Chromosome locus 6q26

Aliases

- Organic Cation Transporter 2
- Solute Carrier Family 22, Member 2
- SLC22A2

Links to NCBI Data

OMIM On-line Mendelian Inheritance in Man

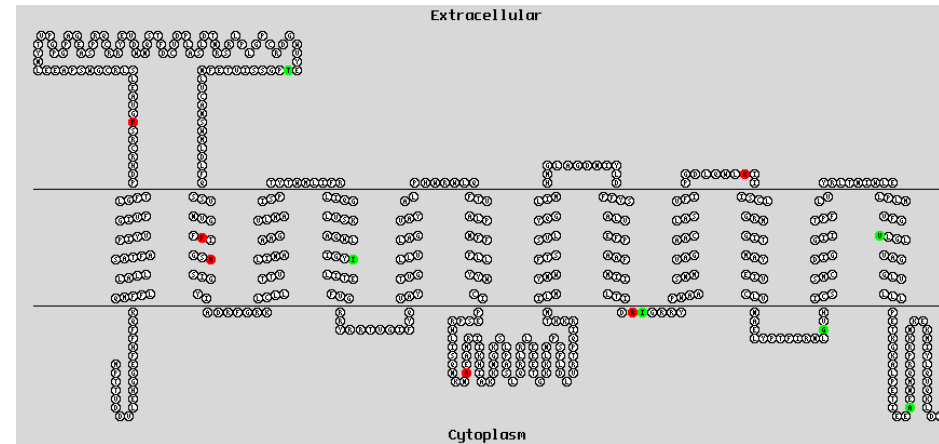
LocusLink Data

Reference Sequence:

Homo Sapiens mRNA for OCT2 from kidney.

Gene Structure Introns/Exons

Transmembrane Topology Prediction



Variants occur with frequency of 15%

Coding regions and Exon/Intron boundaries

For 247 DNA samples from Coriell Institute

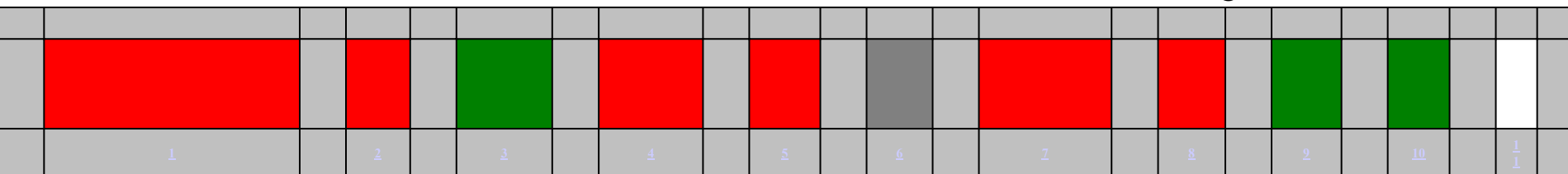
SNPs found at:

Synonymous: 130, 223, 297, 401, 466, 502, 529

Non-Syn: 54, 161, 165 (2), 270 (2), 400, 432

Cellular phenotyping: Data to be gathered.

Clinical studies: Data to be gathered



# Pharmacogenomics of Transport (I)

- Classification by mechanism, origin, topology, domain structure, energetics, energy source, substrate specificity, sequences, 3D structures, organisms, tissue localization, etc.
- BLAST (Basic Local Alignment Search Tool – NLM)
- INCA (Integrative Neighborhood Cluster Analysis – W. Saidee)
- T.C.# W.X.Y.Z (Saier et al)., e.g., MDR1 = 3.A.1.201
  - W = type and energy source (3 = primary transporter)
  - Z = transporter family or superfamily(3.A = P-P cleavage)
  - Y = transporter subfamily (3.A.1 = ABC family)
  - Z = substrate transported (3.A.1.201 = multiple drugs)
- **<http://www/biology.ucsd.edu/~msaier/transport/>**
- Recent Review: The ABCs of Solute Carriers. M. Hediger, Pflugers Archiv – EJ Physiol. See also:  
**<http://www.bioparadigms.org/>**

# Pharmacogenomics of Transport (II)

## Expression Patterns using MicroArray Chips

In vivo permeabilities measured in human duodenum using perfusion methods. In vitro permeabilities measured using Caco-2 cells. Expression patterns of 12,599 gene sequences analyzed using GeneChip (including 443 expected ADME genes). Sun, et al., 2002.

### Results: Functional Genomics

- 1) 37-47% of genes (26-44% of ADME genes) expressed in both cell types, but >1,000 sequences showed >5x variation between cell types. Variation >3x for >70 transporters detected.
- 2) In vivo/in vitro permeability correlated well ( $R^2 = 85\%$ ) for passively absorbed drugs.
- 3) Variations (3-35x) above expected passive values were observed for mediated absorption and correlated with differences (2-595x) in gene expression.
- 4) Interhuman variability (3-294% of mean) for 31% of genes.

# Conclusions

- We have been lucky in the past
- We have selected for drugs that are readily transported by passive diffusion – many of which act extracellularly
- We are just beginning to understand other transport processes and their consequences
- We are just beginning to understand the interindividual variations of transport
- We are just beginning to exploit that knowledge to design drugs for transport