University of Glasgow

Drug transporters in trypanosomes

Harry de Koning

Drug uptake: transporters, not diffusion

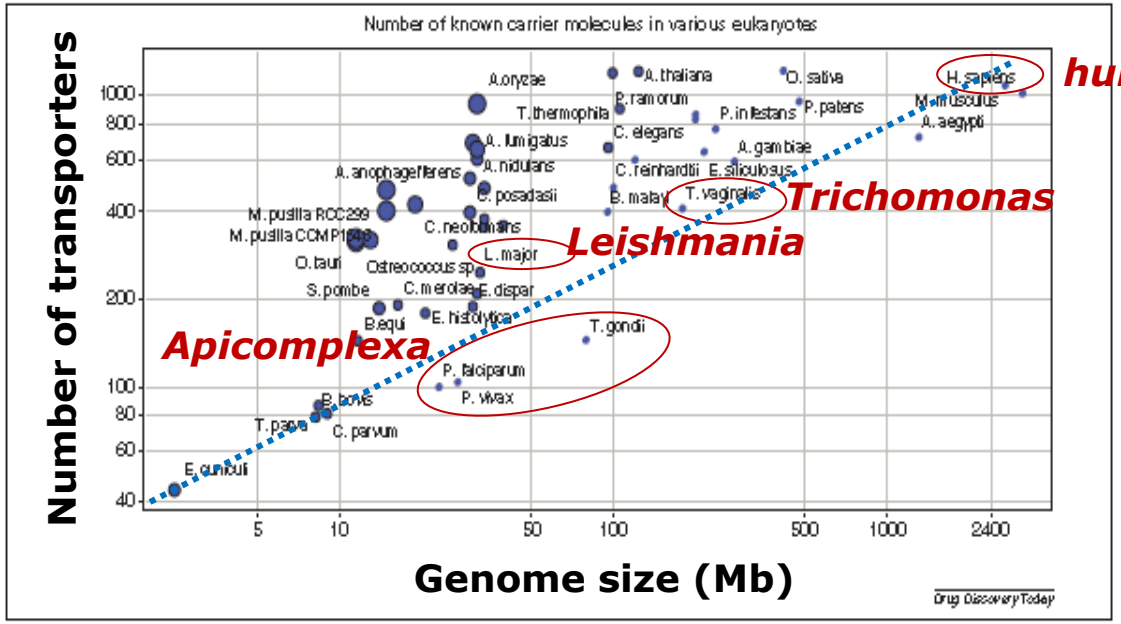
REVIEWS Drug Discovery Today • Volume 16, Numbers 15/16 • August 2011

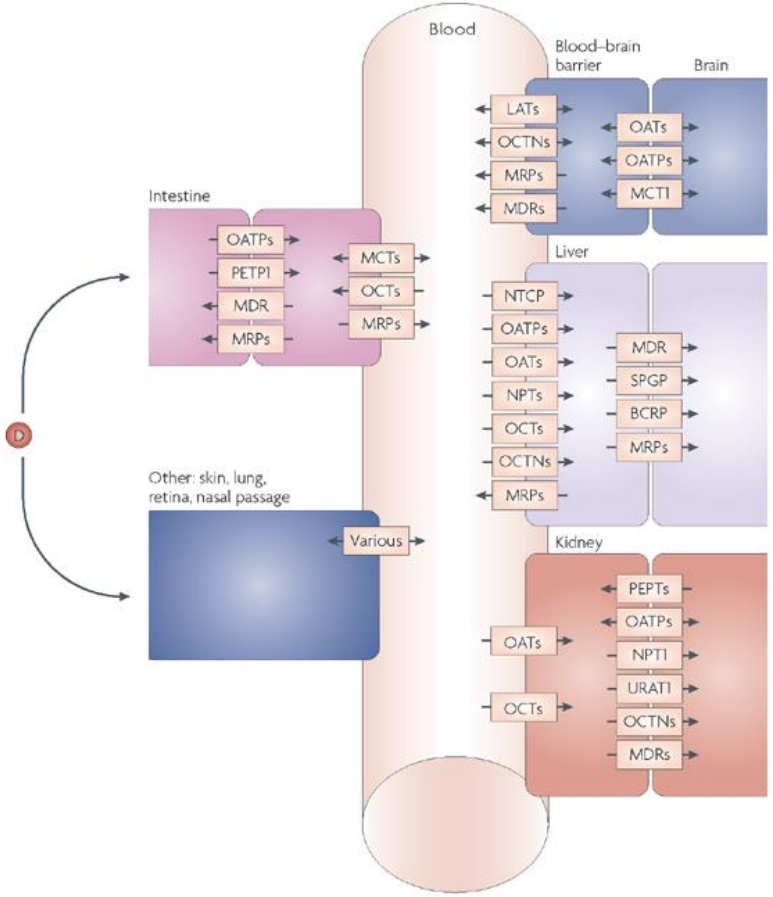
ELSEVIER

Reviews • POST SCREEN

Pharmaceutical drug transport: The issues and the implications that it is essentially carrier-mediated only

Douglas B. Kell[1,2], Paul D. Dobson[1,2,3] and Stephen G. Oliver[4,5]





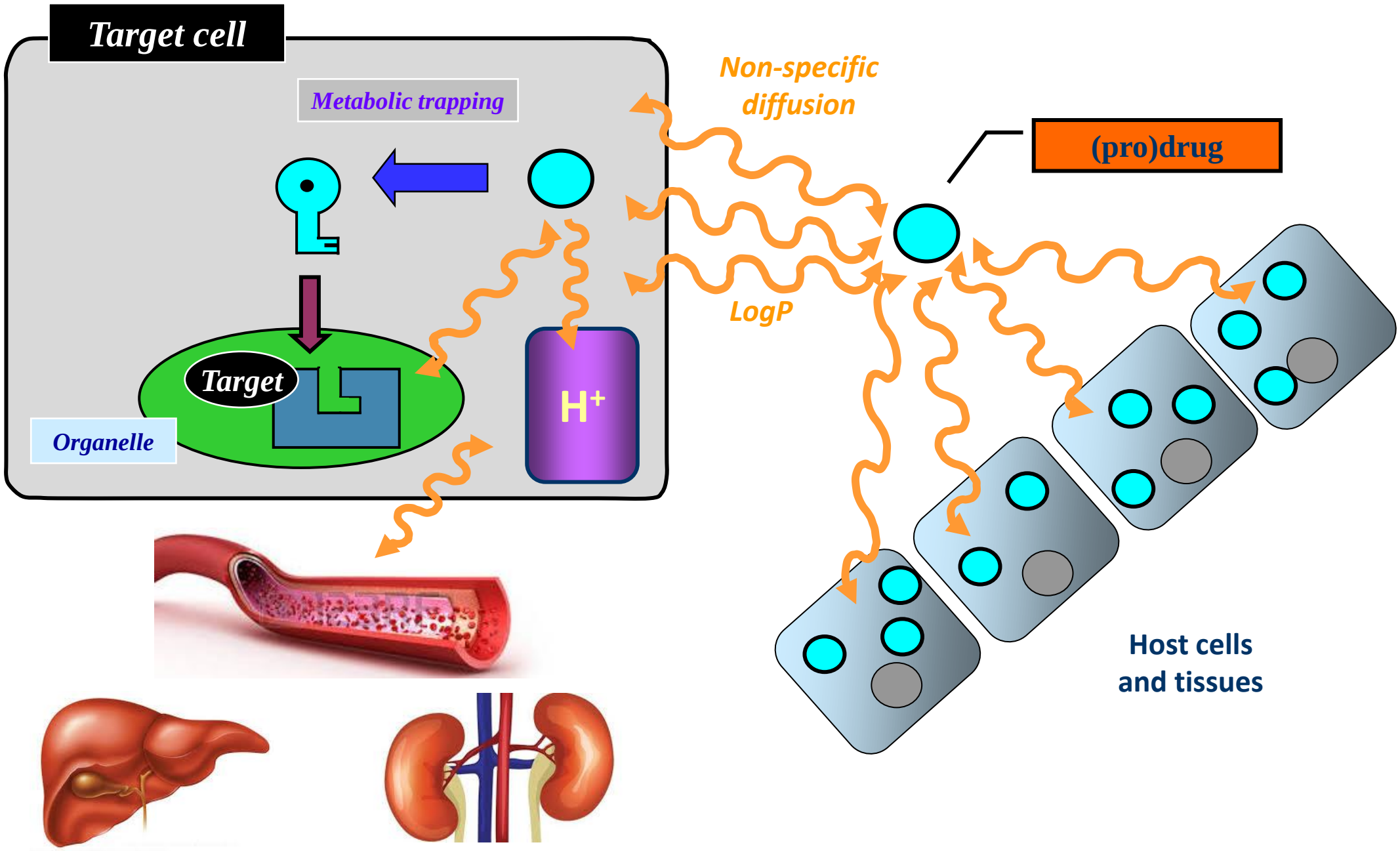
Nature Reviews | Drug Discovery

OPINION

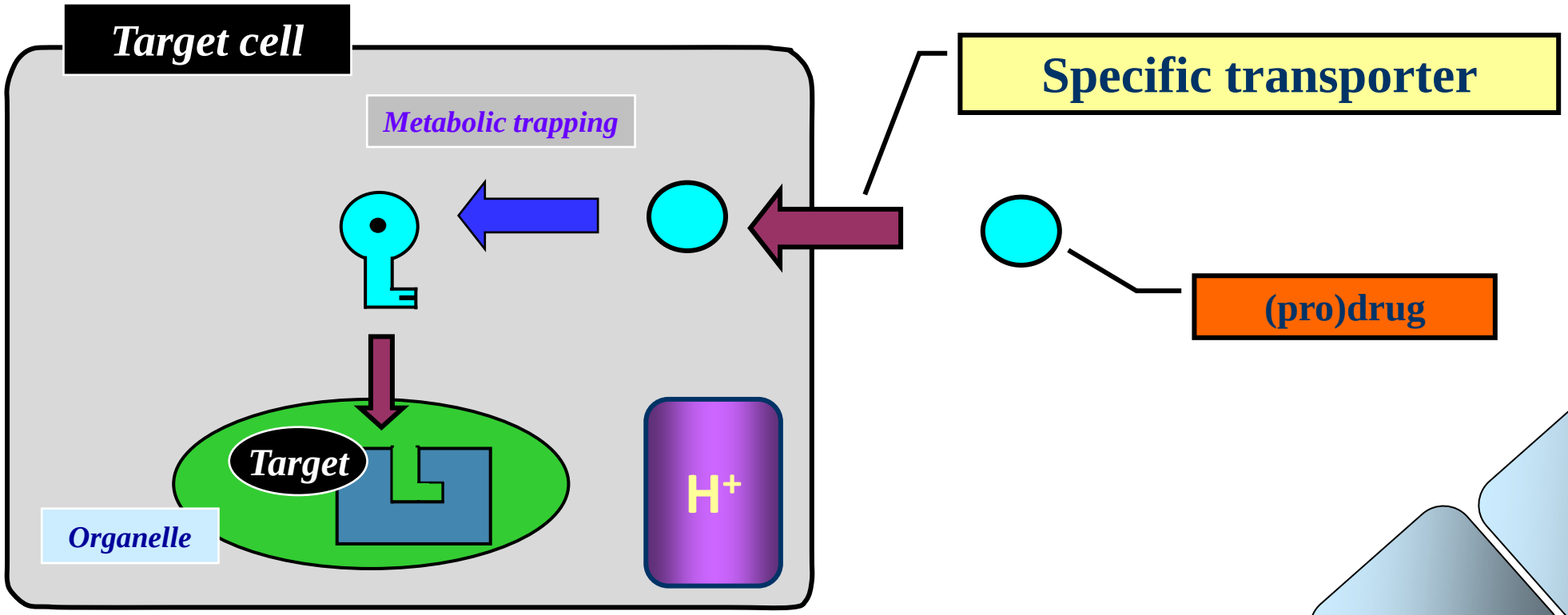
Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule?

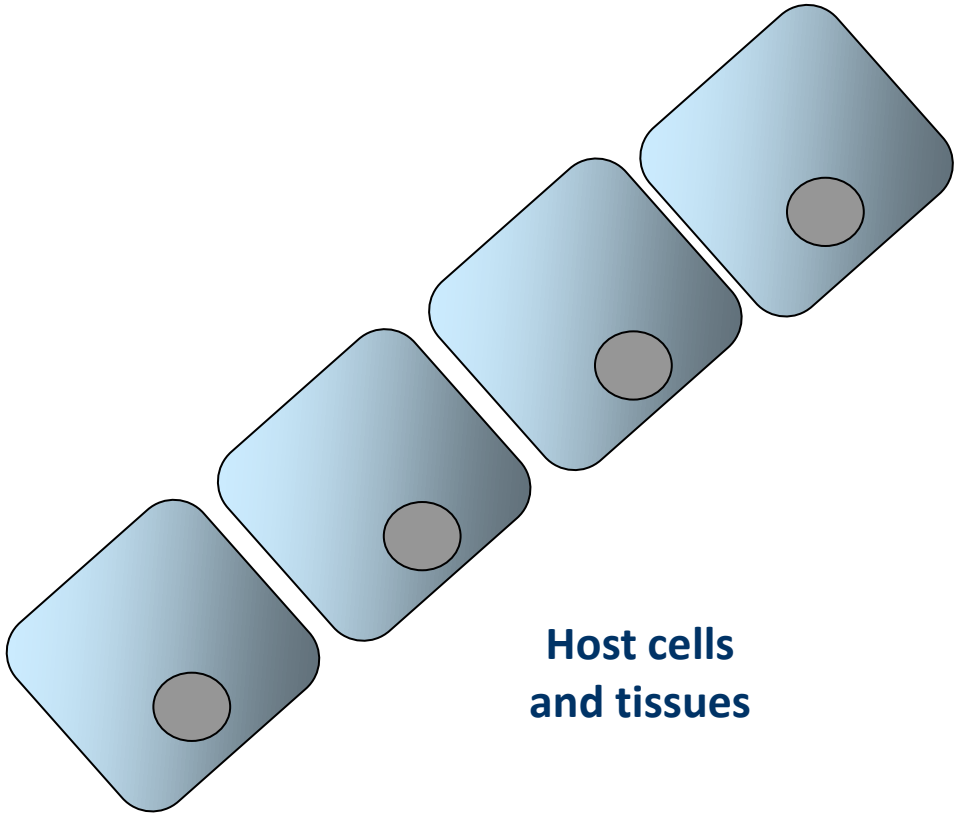
Paul D. Dobson and Douglas B. Kell

Diffusion of drugs across membranes



Drug Transporters

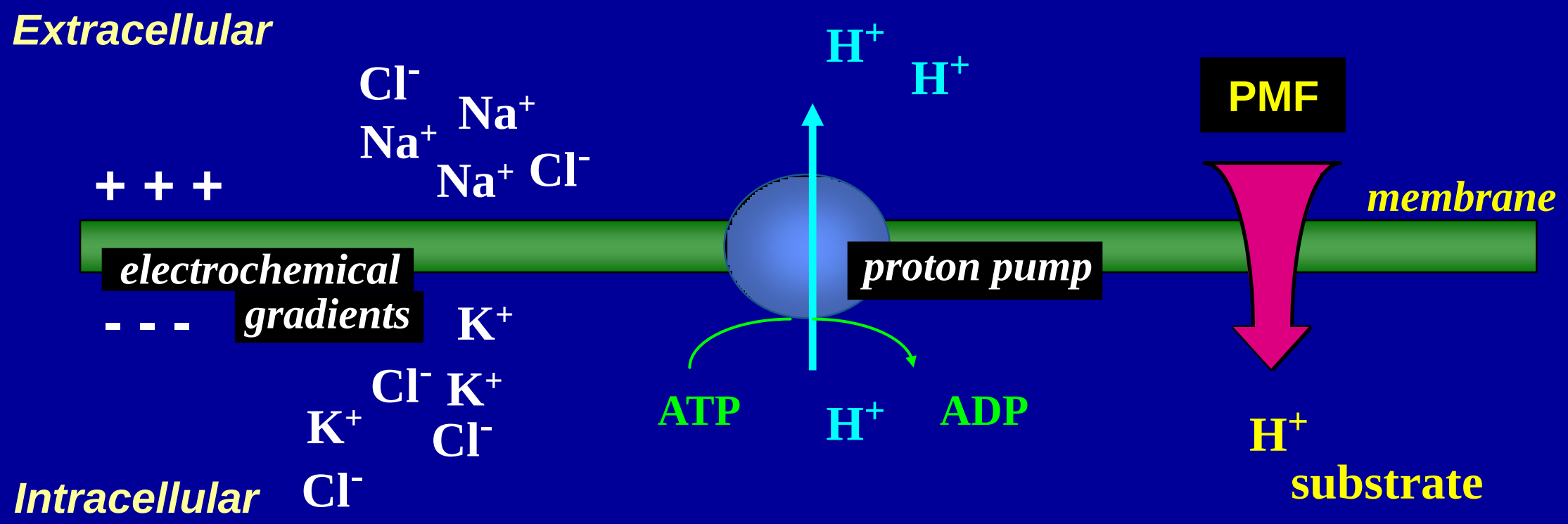




- Entry only to cells expressing the transporter
- Drug less likely to be in wrong cell or compartment
- Lower volume of distribution
- Lower dosage, less toxicity
- Reversible or concentrative

Active transport in protozoa is driven by the protonmotive force

consists of membrane potential (V_m) and proton gradient (ΔpH)

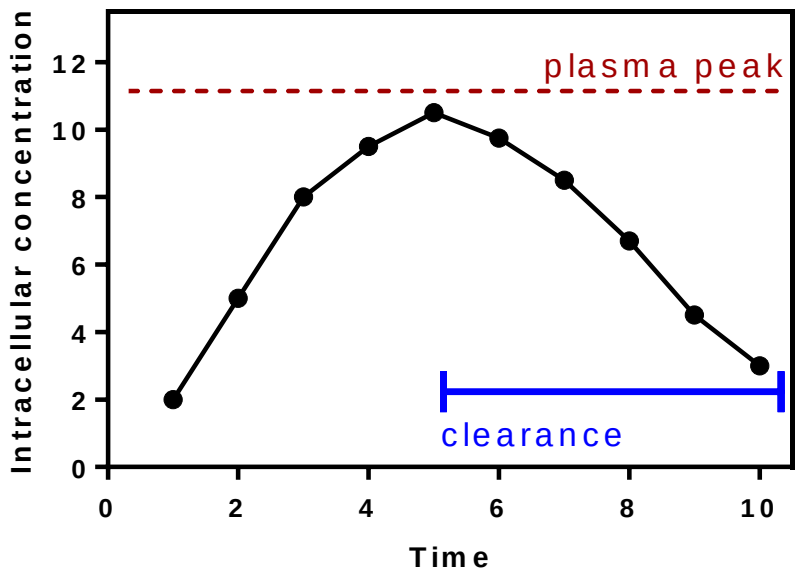


V_m + ΔpH = PMF

Intracellular drug levels from diffusion and active transport

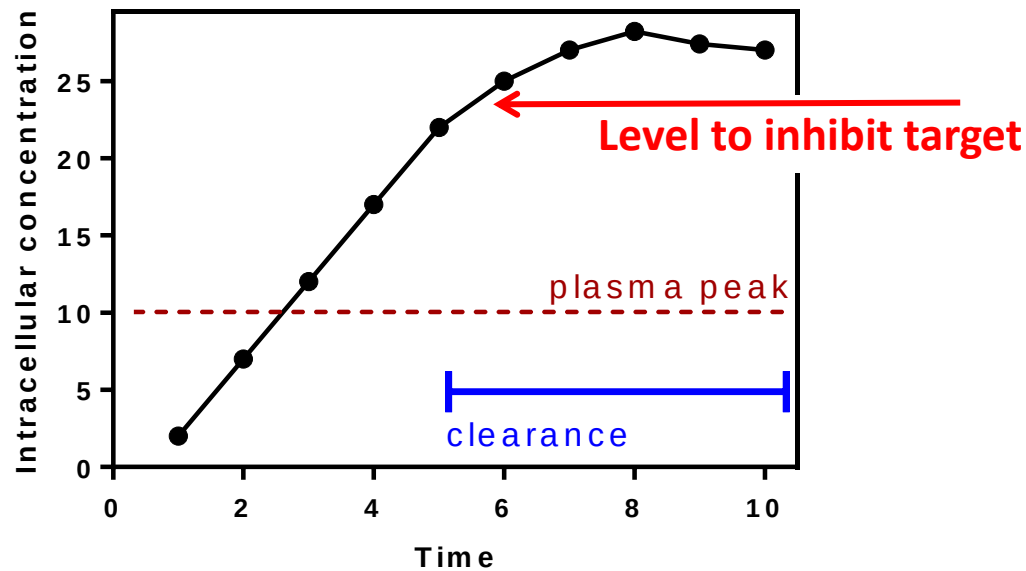
Diffusion

Cellular level follows plasma



Active transport

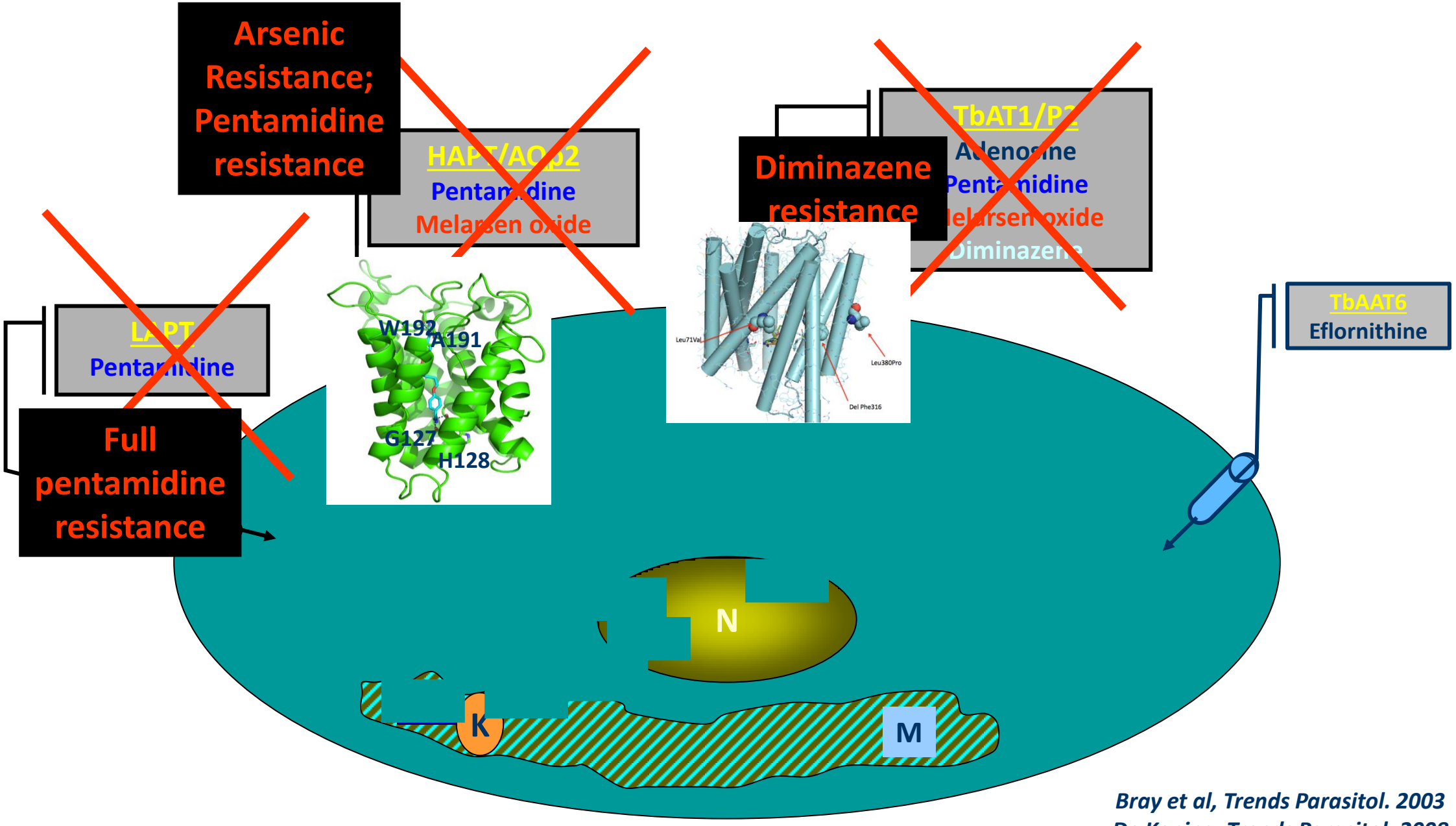
Accumulates above plasma



Transporters define…

- **What molecules reach the intracellular target**
- **What drug concentrations are achievable in the target cell**
- **How fast the drug is taken up**
- **Which cells are affected by the drug**
- **The distribution of a drug within the body**
- **Drug resistance and cross-resistance**

Drug transporters of *Trypanosoma brucei*

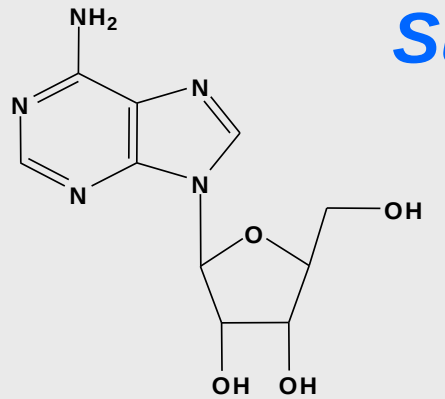


Bray et al, Trends Parasitol. 2003
De Koning, Trends Parasitol. 2008

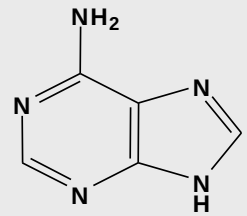
Substrates of P2

Purines

Substrate

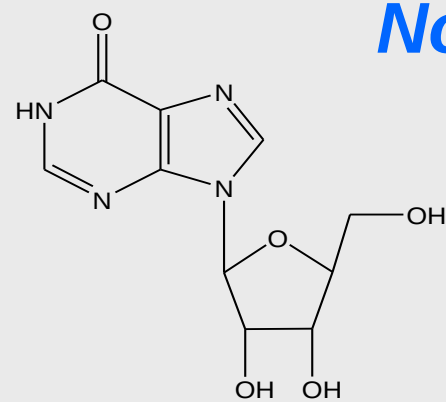


Adenosine

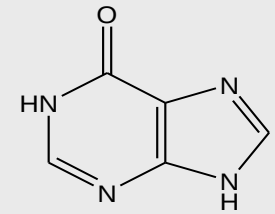


Adenine

Not substrate



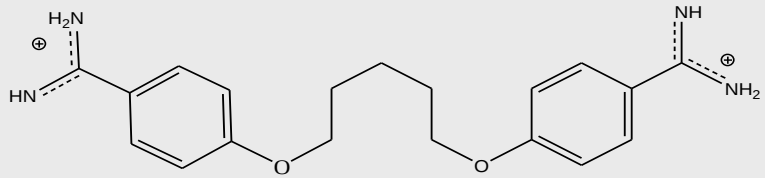
Inosine



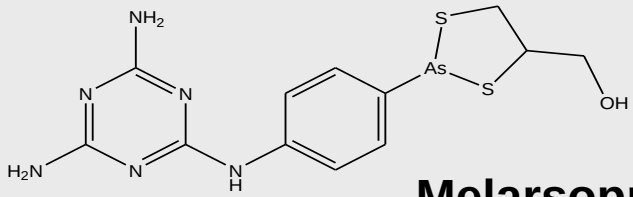
Hypoxanthine

Trypanocides

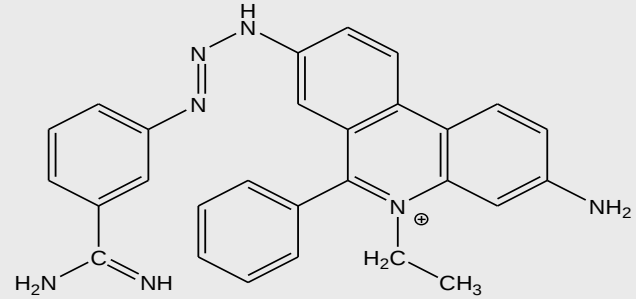
High affinity substrates



Pentamidine



Melarsoprol



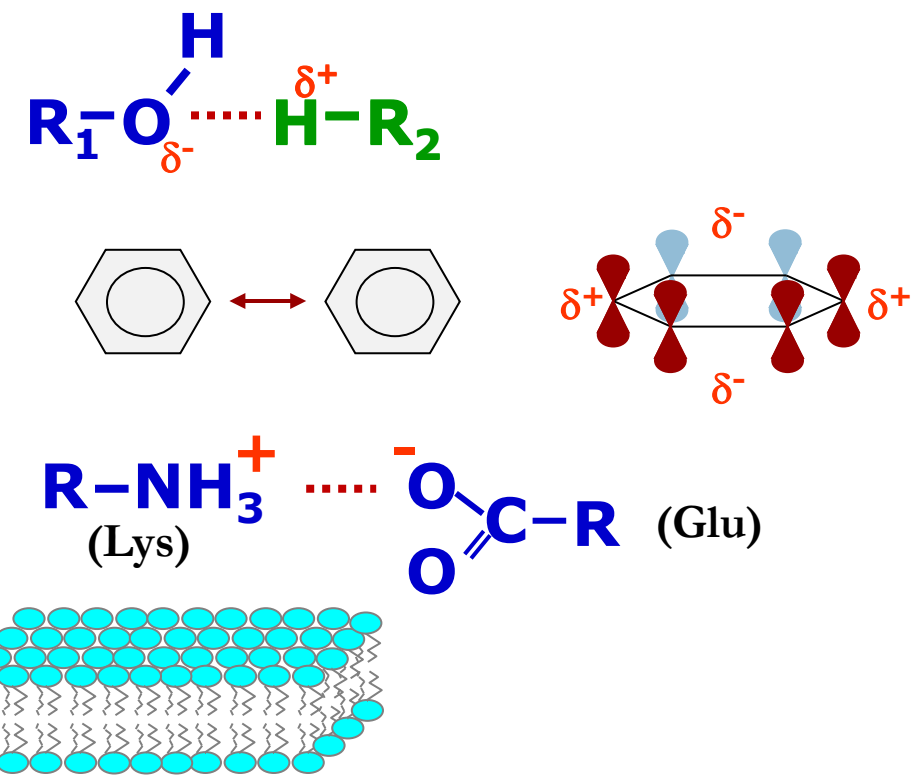
(Isometamidium)

Carter and Fairlamb, 1993, 1995
De Koning and Jarvis, 1999; De Koning, 2001

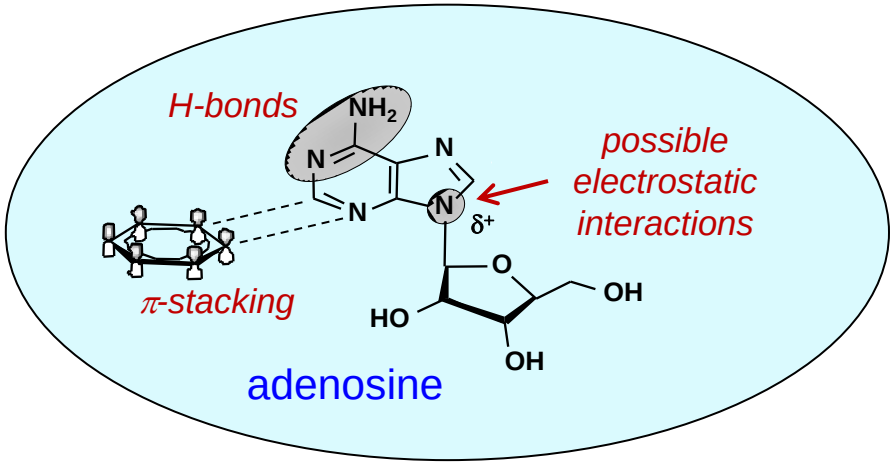
High affinity / selectivity

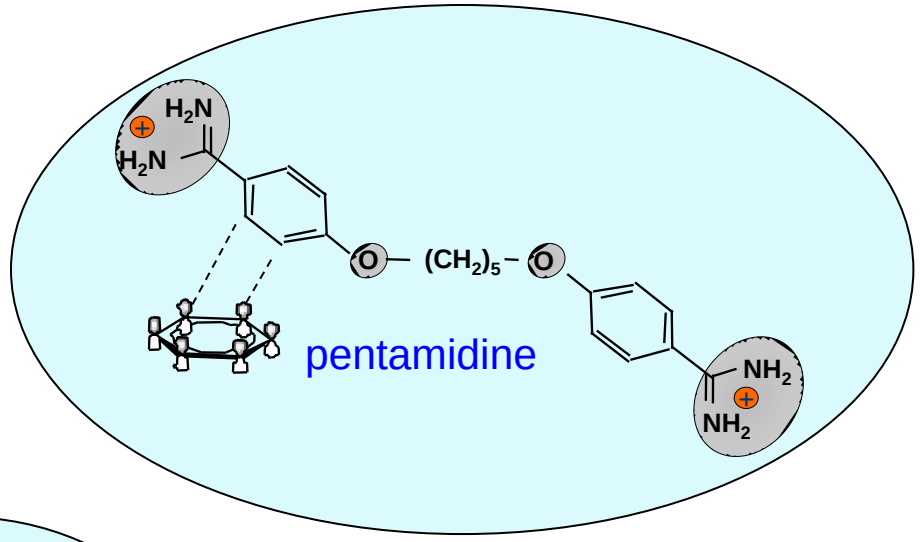
The affinity and and selectivity of a transporter are determined by the interactions between the substrate and the transporter binding site.

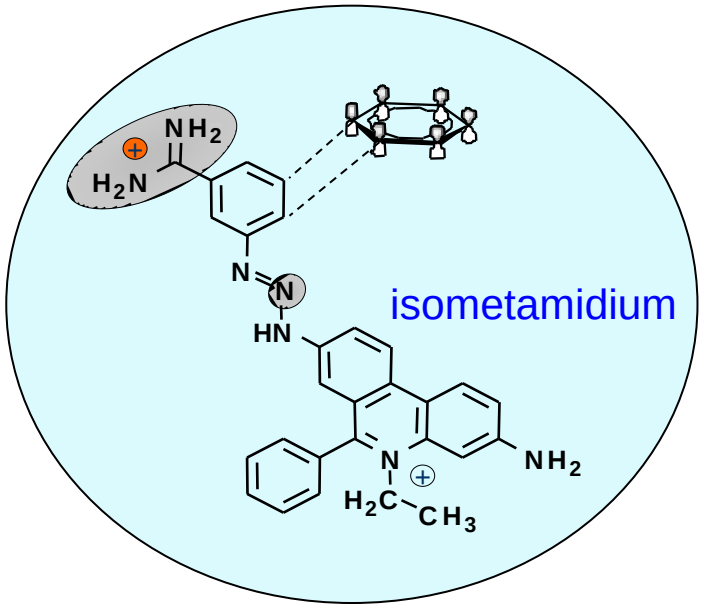
- **Hydrogen bonds**
- **π-orbital stacking**
- **Electrostatic interactions**
- **Hydrophobic interactions**

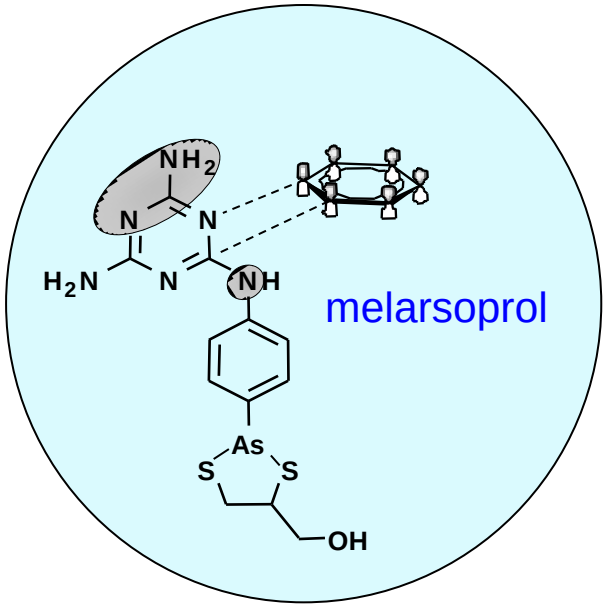


Recognition motif of P2



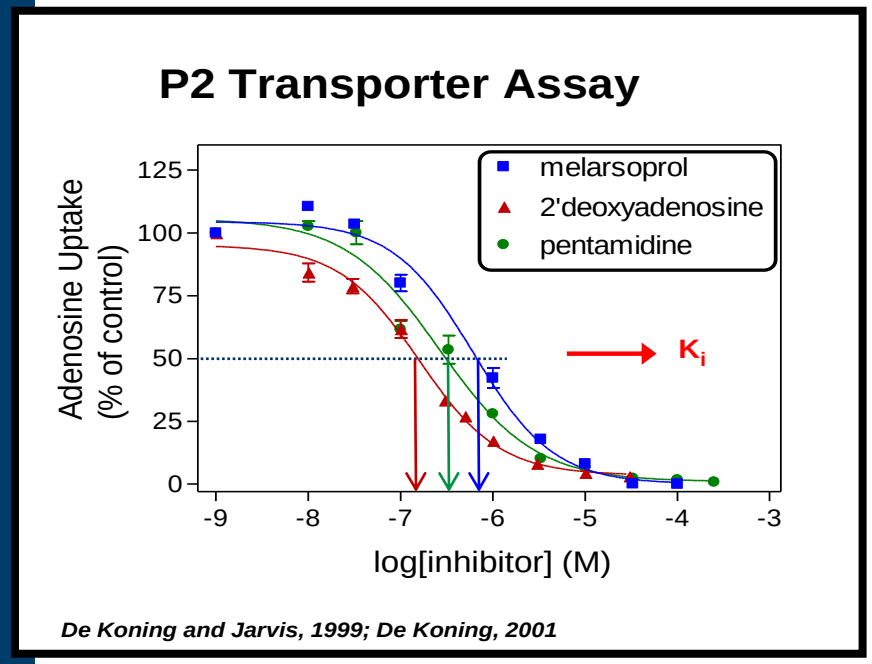




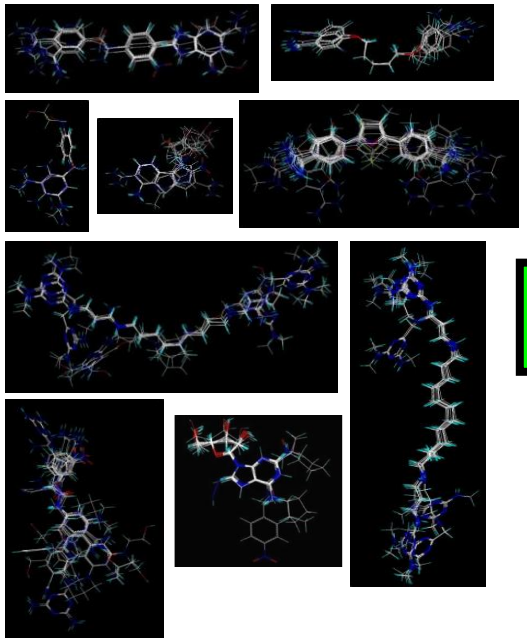


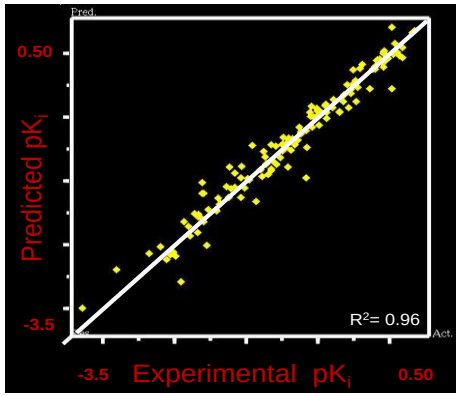
De Koning and Jarvis, Mol. Pharmacol. 1999
De Koning et al., FEMS Microbiology Reviews 2005

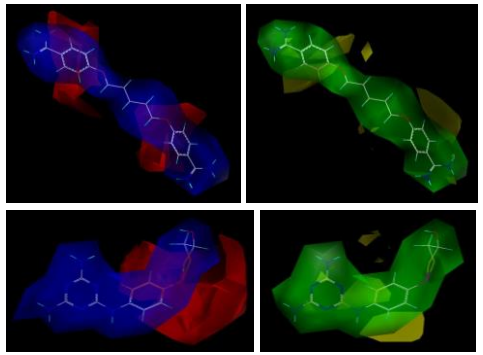
QSAR of the P2 transporter



De Koning and Jarvis, 1999; De Koning, 2001





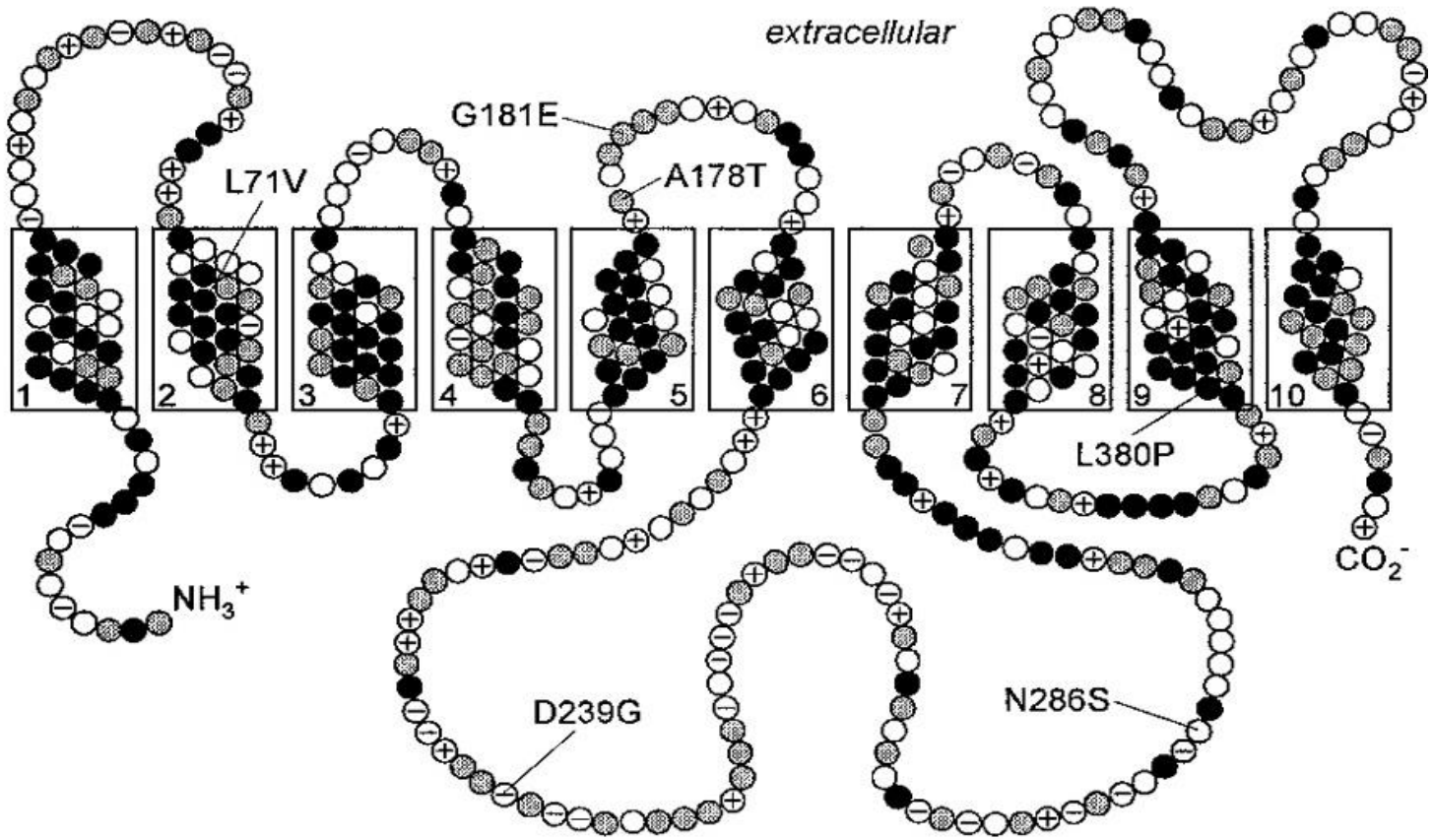


Comparative Molecular Field Analysis (CoMFA)
Comparative Similarity Indices Analysis (CoMSIA)

Collar et al., J. *Biol. Chem.* 2009

Predicted location of SNPs in TbAT1

From STIB777R:



Six possible resistance-associated mutations

Mäser et al., Science 1999

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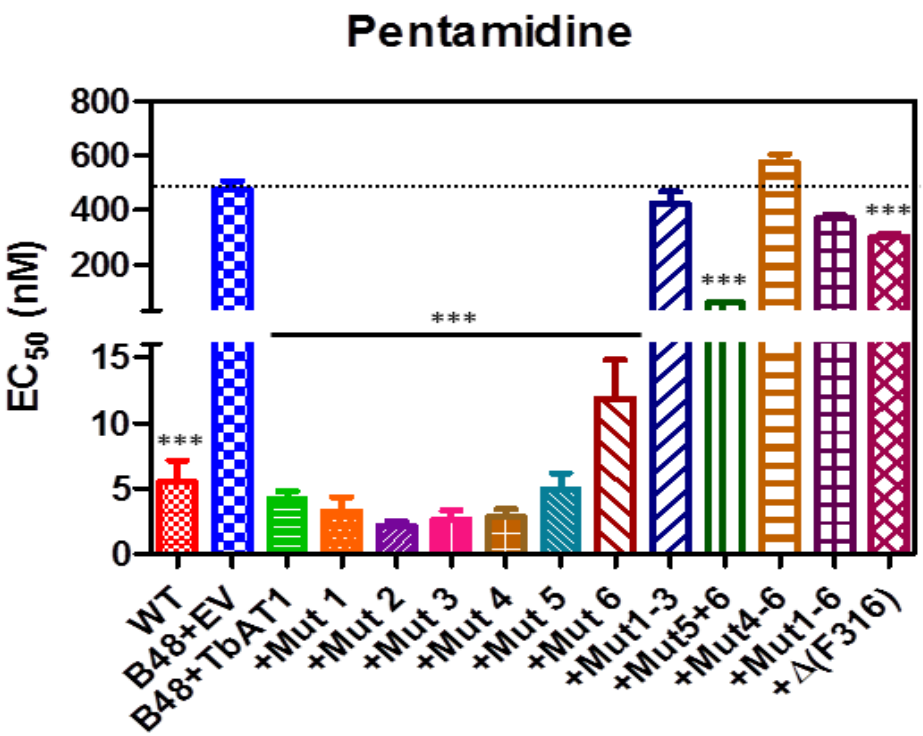
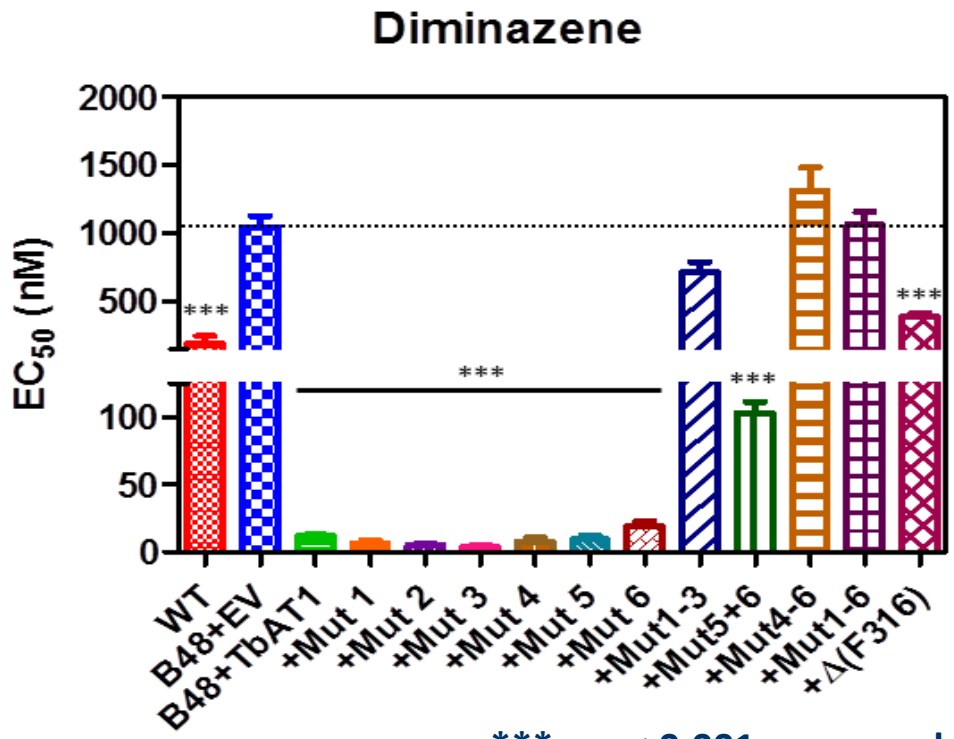
Systematic introduction of TbAT1 mutant alleles in the T brucei B48 line

Line	Mutation?
B48 + EV	B48 with empty vector – negative control
B48 + TbAT1	wild-type TbAT1 – positive control
B48 + Mut 1	L71V
B48 + Mut 2	A178T
B48 + Mut 3	G181E
B48 + Mut 4	D239G
B48 + Mut 5	N286S
B48 + Mut 6	L380P
B48 + Mut 1-3	L71V, A178T and G181E
B48 + Mut 5+6	N286S and L380P
B48 + Mut 4-6	D239G, N286S and L380P
B48 + Mut 1-6	L71V, A178T, G181E, D239G, N286S and L380P
B48 + ΔF316	ΔF316

Effect of mutations on sensitivity to pentamidine and diminazene





1. L71V
2. A178T
3. G181E
4. D239G
5. N286S
6. L380P

*** = $p < 0.001$ compared to B48+EV

- **On its own, only mutation 6 (L380P) caused a slight decrease in drug susceptibility.**
- **Susceptibility was further reduced by additional SNPs**
- **ΔF316 also almost completely reduced the sensitivity to both drugs.**

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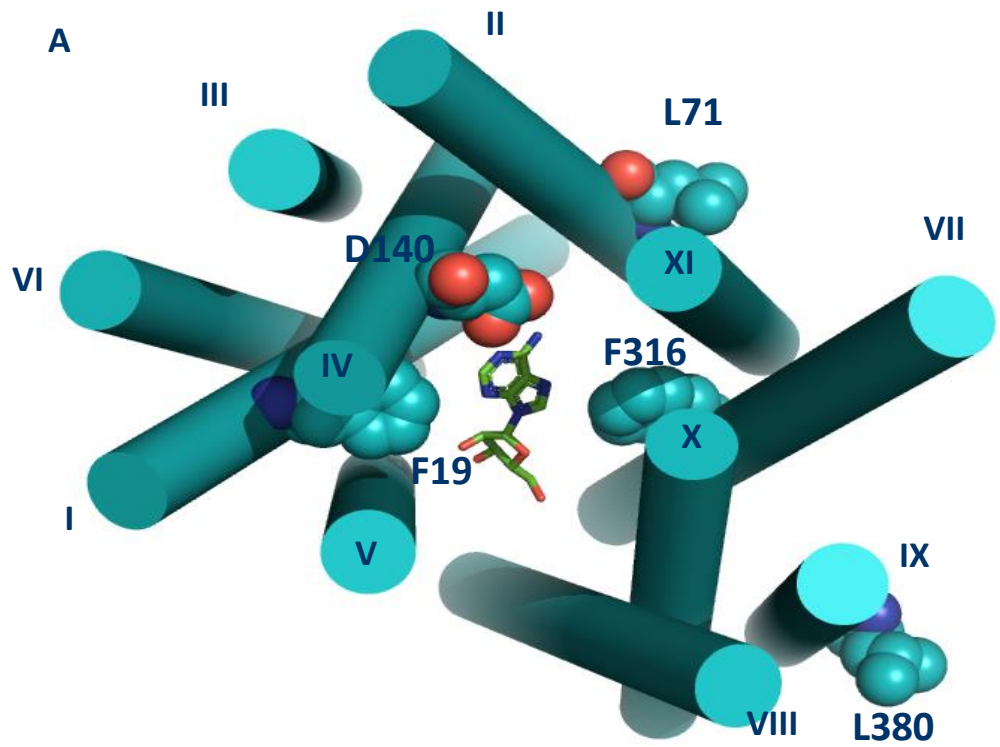
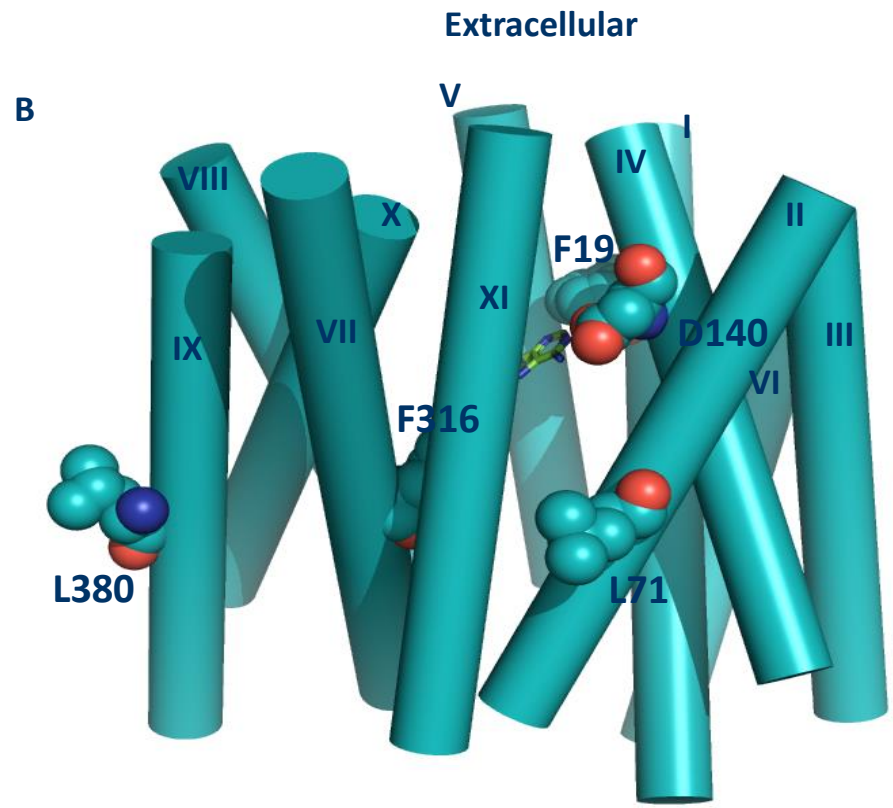
Some of the mutated residues mapped close to the expected binding site



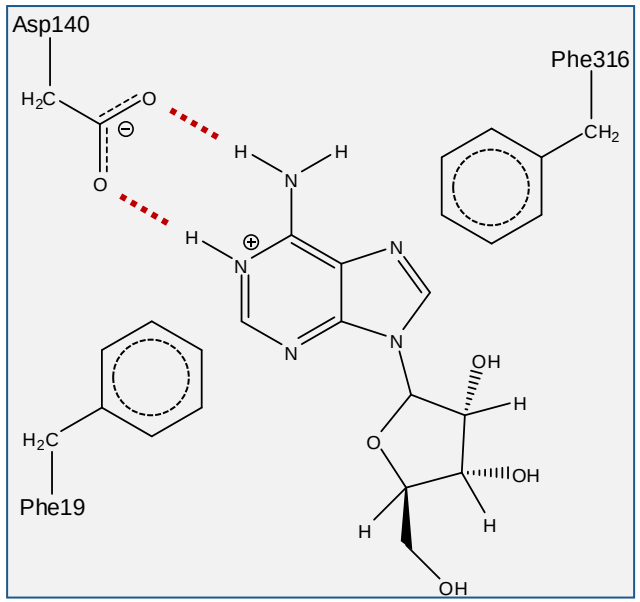
Extracellular view

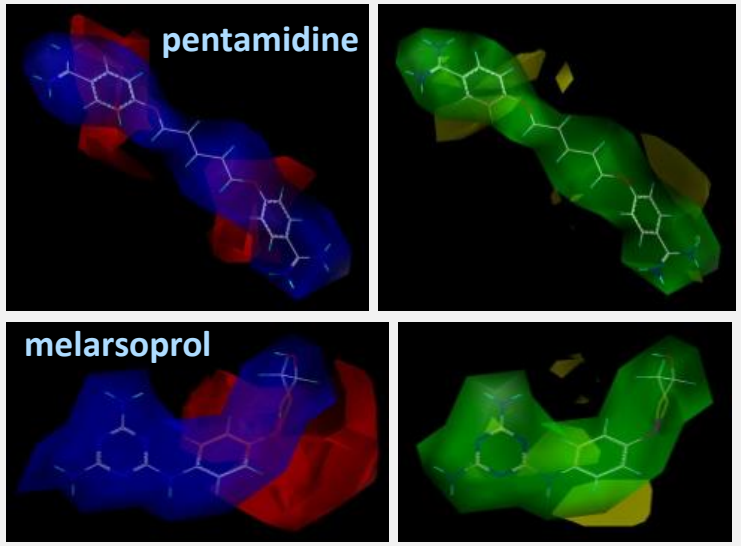


Cytoplasmic

Munday et al., Mol. Microbiol. 2015

P2 structure and substrate binding





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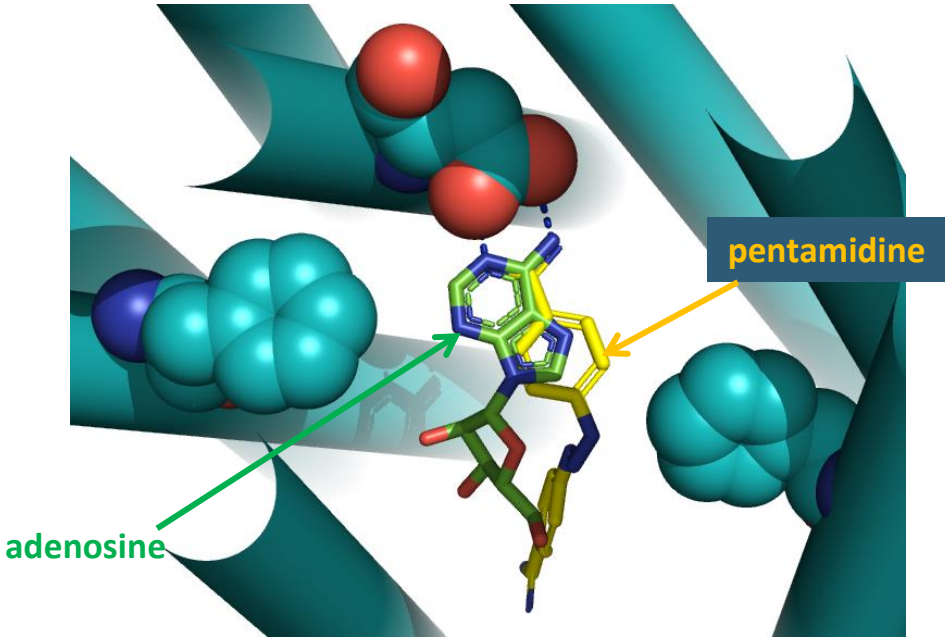
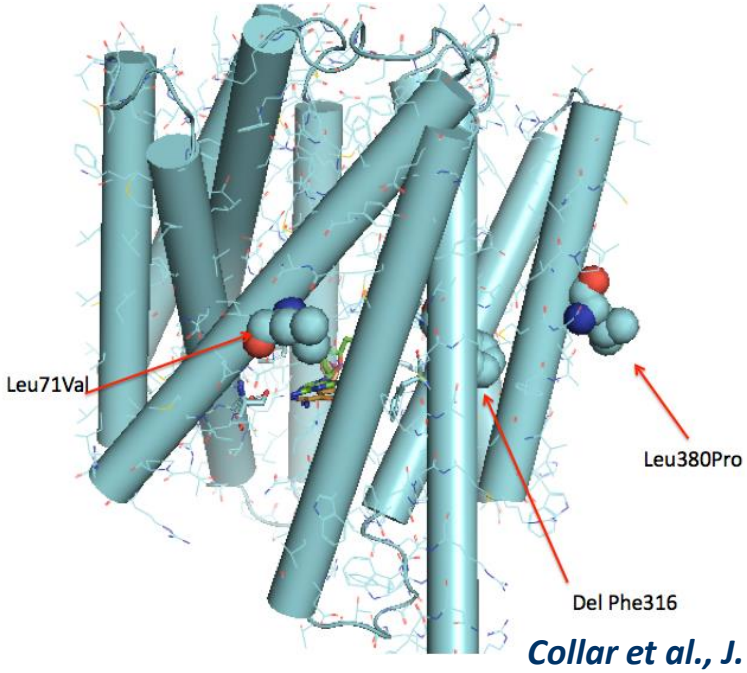
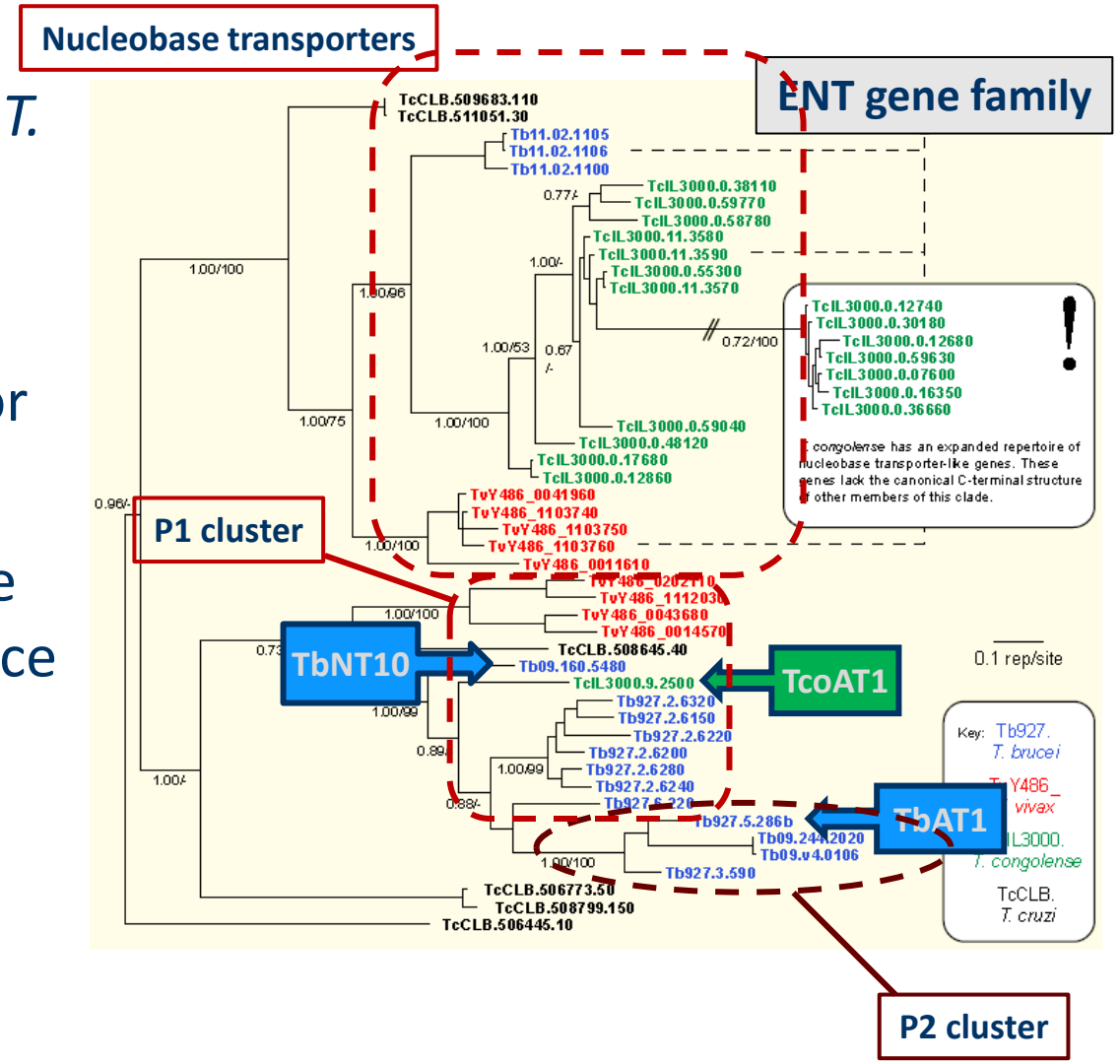




Collar et al., J. Biol. Chem. 2010
Munday et al., Mol. Microbiol. 2015

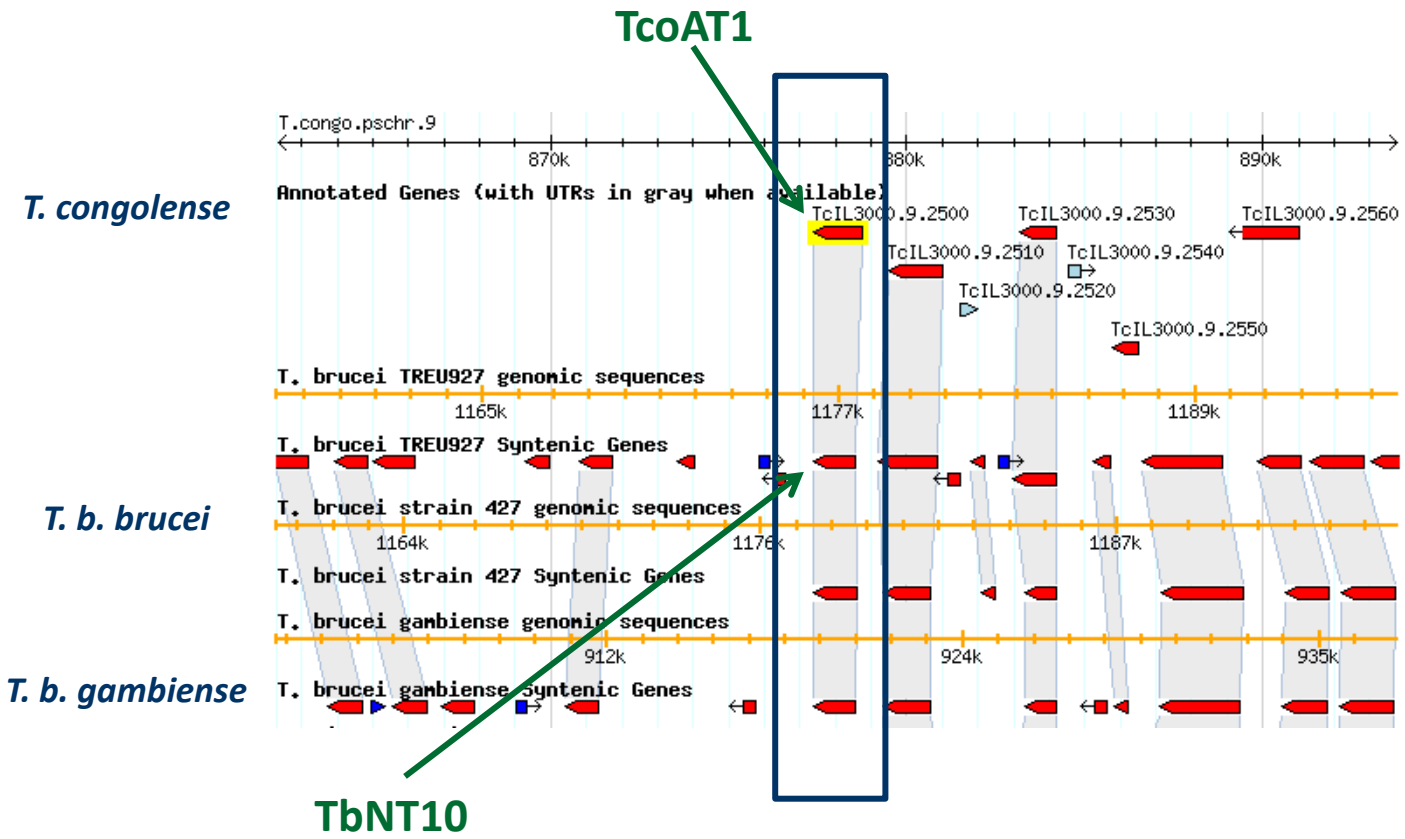
Is the same model applicable to *T. congolense*?

- BLAST searches identified a related *T. congolense* gene
- This was named *TcoAT1*
- It was assumed to be responsible for diminazene uptake in *T. congolense*
- A SNP in *TcoAT1* was proposed to be responsible for diminazene resistance



No P2 equivalent in *T. congolense*

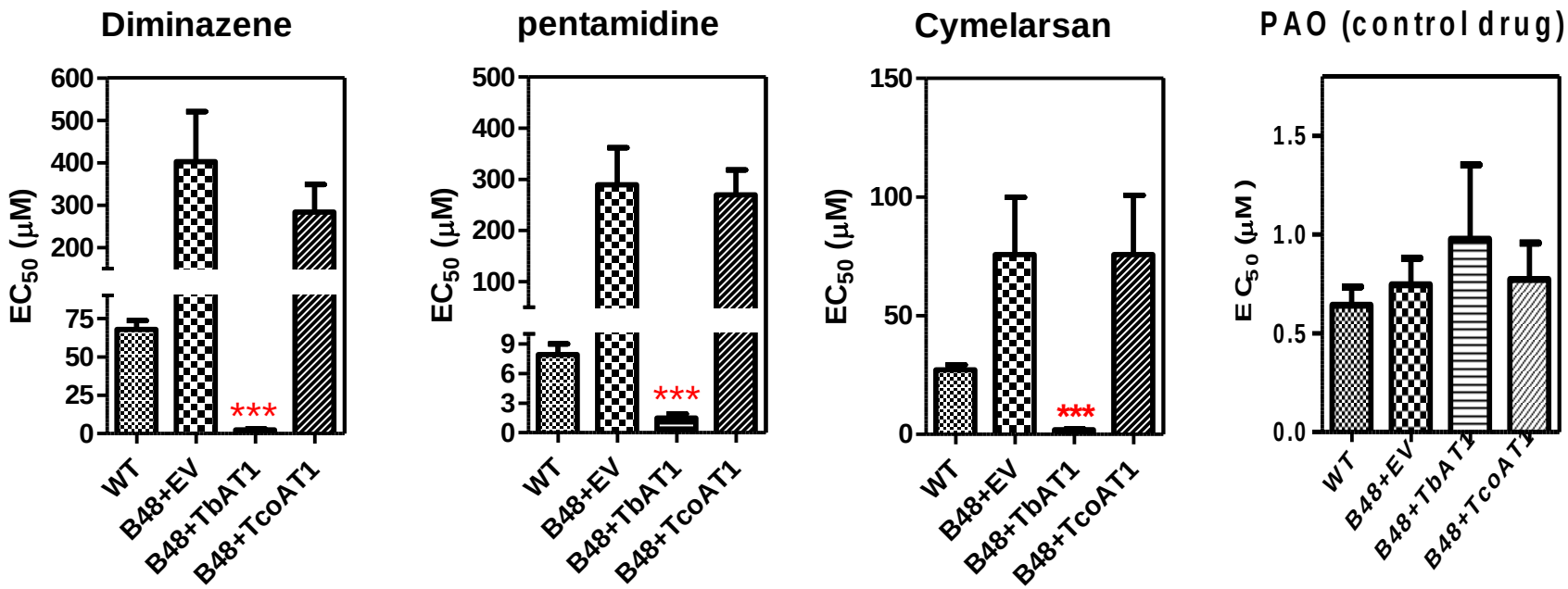
1. **There is no transporter in the syntenic position of TbAT1 in the T. congolense genome**
2. **The syntenic equivalent of TcoAT1 is T. brucei NT10, a P1-type nucleoside transporter**



Functional expression of TcoAT1 in *T brucei*

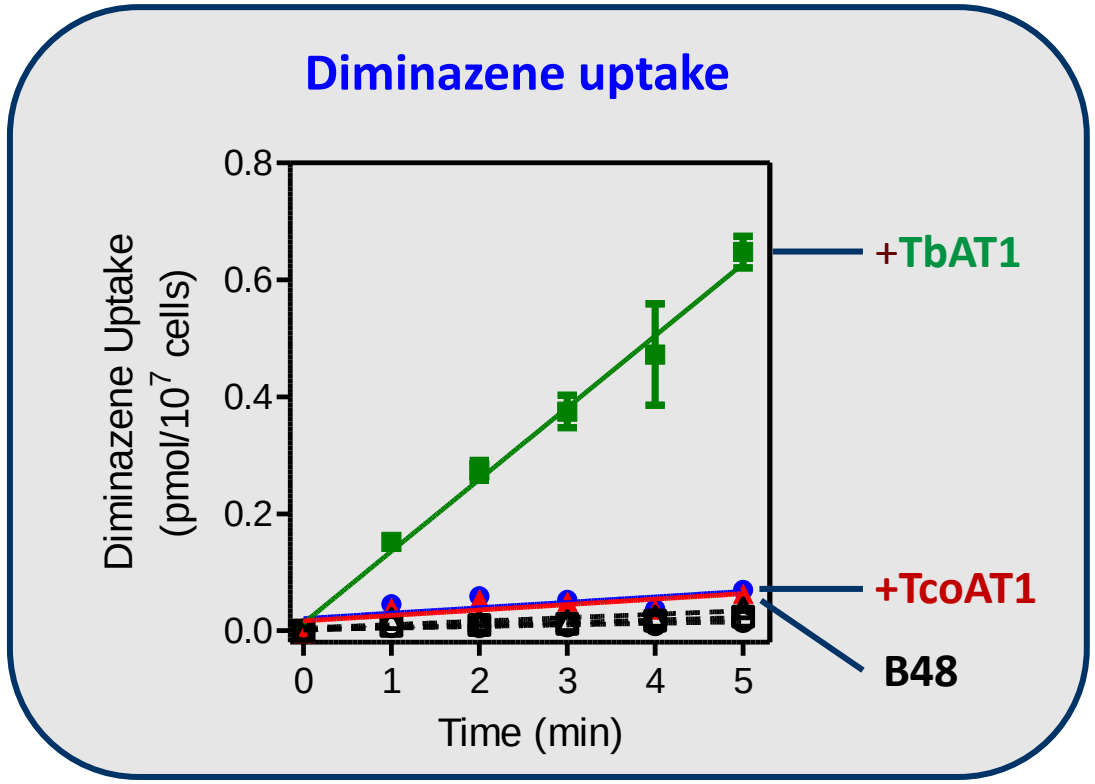
Choose *T. brucei* clone B48:

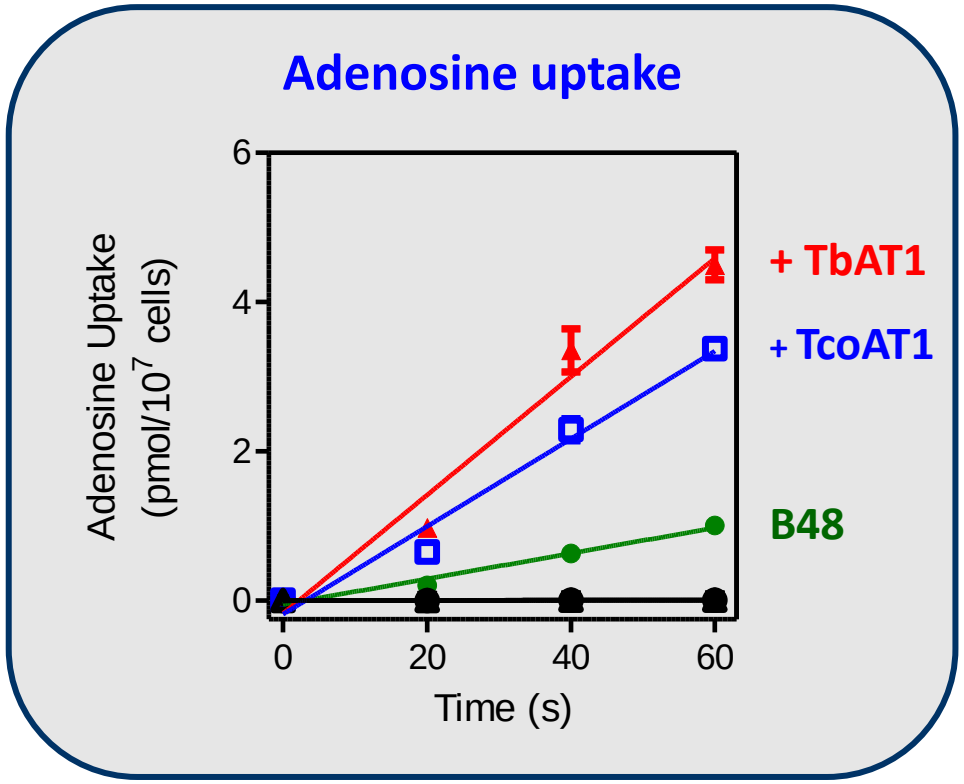
- Strongly resistant to diminazene, pentamidine and arsenicals
- Very well characterised
- Lacks TbAT1/P2 and HAPT transporters



- *Expression of TbAT1: sensitisation to diamidines and melaminophenyl arsenicals*
- *Expression of TcoAT1: no change in diamidine or arsenical sensitivity*

TcoAT1 does not mediate diminazene uptake



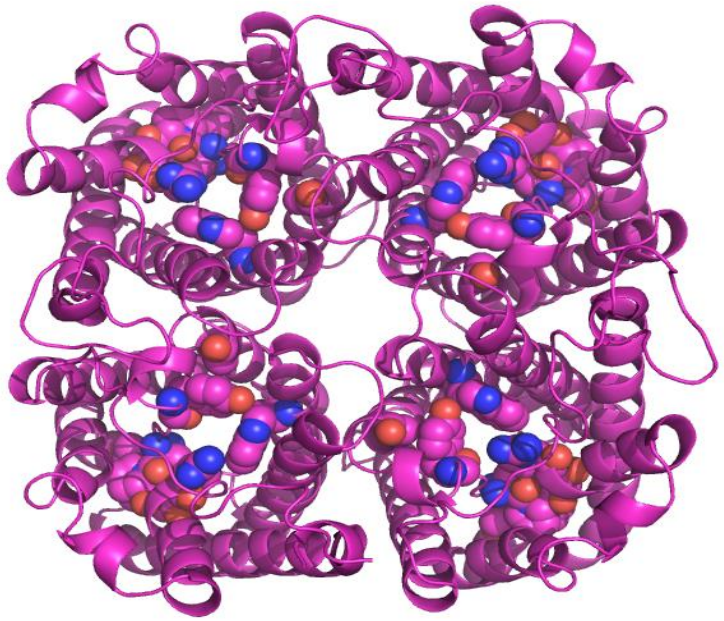


- **Expressing *TcoAT1* does not increase [^{3}H]-diminazene uptake**
- **It is a 'normal' purine nucleoside transporter (P1-type)**

Munday JC, Rojas López KE, Eze AA, Delespaux V, Van Den Abbeele J, Rowan T, Barrett MP, Morrison LJ, De Koning HP (2013) Functional expression of *TcoAT1* reveals it to be a P1-type nucleoside transporter with no capacity for diminazene uptake. Int J Parasitol Drugs Drug Resist 3:69-76.

Aquaporins

- **Tetrameric channels**
  - Water
  - Glycerol
  - Small sugars
  - Inorganic arsenic and antimony oxides
- **In *T. brucei*: AQP1-3**
  - Water, glycerol, dihydroxyacetone

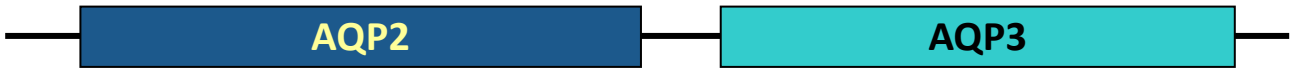


***Trypanosoma brucei* AQP3**
a classical aquaglyceroporin

Aquaporins in pentamidine sensitivity

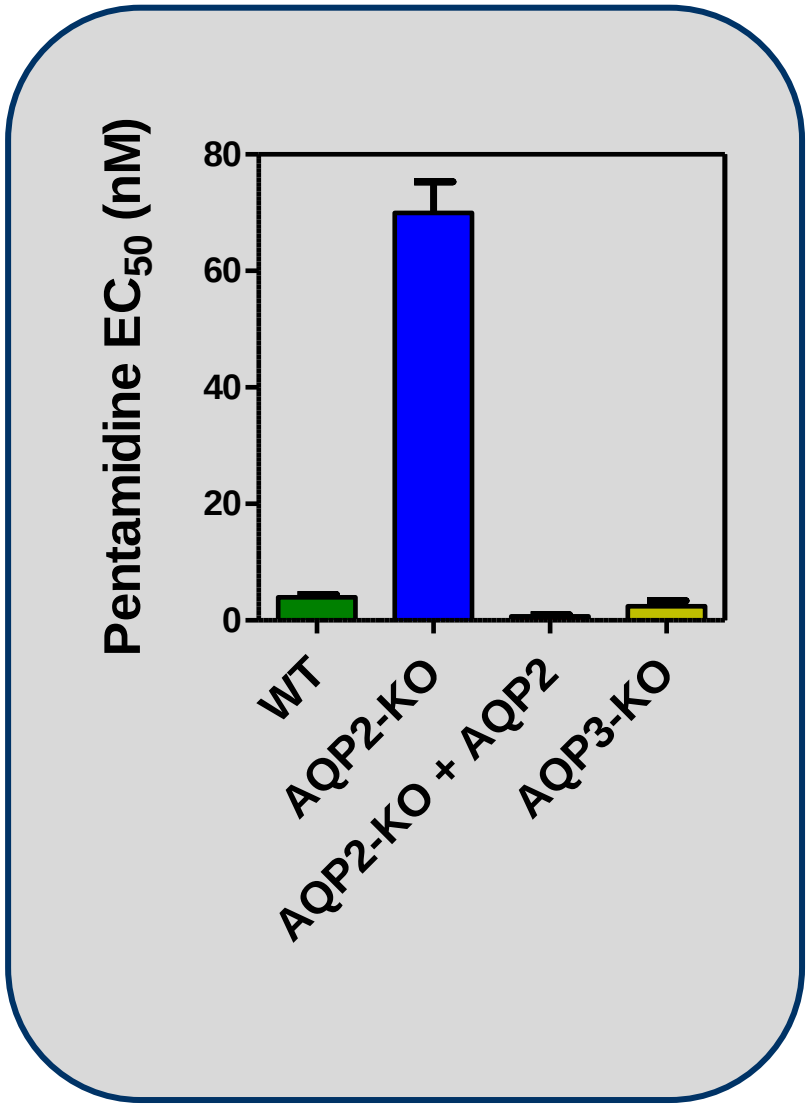
A genome-wide RNAi screen for pentamidine resistance identified the *T. brucei* AQP2 – AQP3 locus

***T. b. brucei* aquaporin locus**



Which one was linked to resistance?

- Knockout of TbAQP3 does not affect drug sensitivity
- Knockout of TbAQP2 gives pentamidine and melarsoprol resistance
- Re-introduction of WT AQP2 reverses resistance

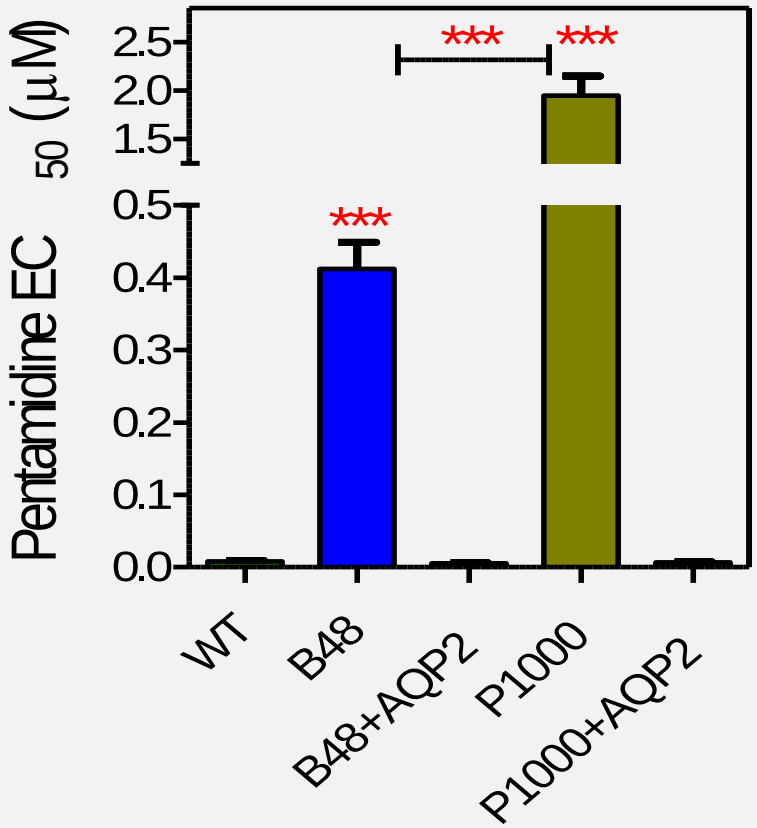


Baker et al Proc Natl Acad Sci USA, 2012

AQP2 determines pentamidine sensitivity

Expression of WT AQP2 in highly resistant lines results completely reverses resistance

Identical results with melaminophenyl arsenicals



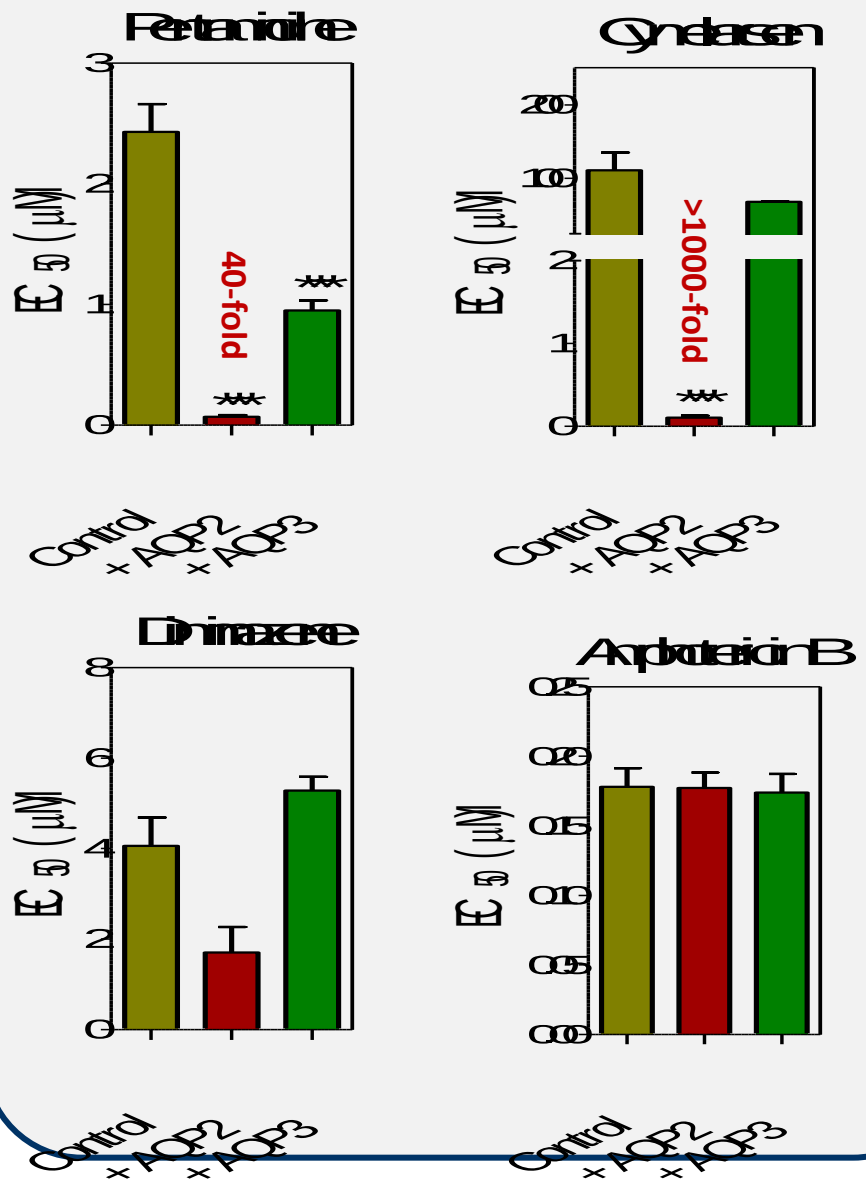
Conclusions:

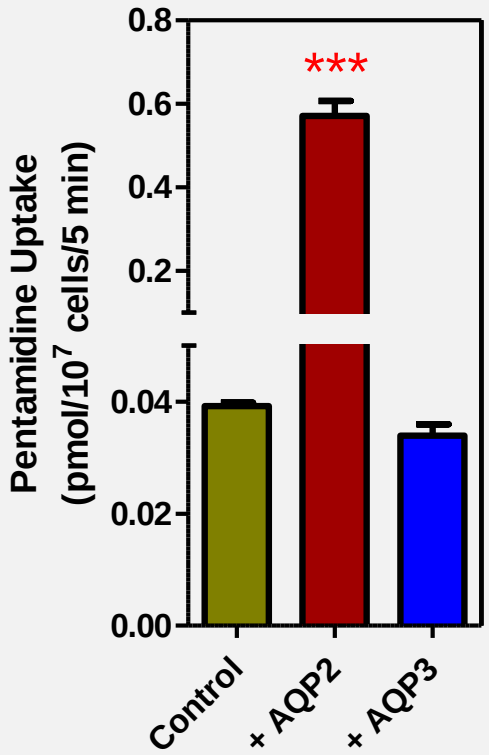
- **Pentamidine/Melarsoprol cross-resistance is related to transport**
- **The phenotype is determined by TbAQP2**

Munday et al. J. Antimicrob. Chemother. 2014

Expression of TbAQP2 in Leishmania

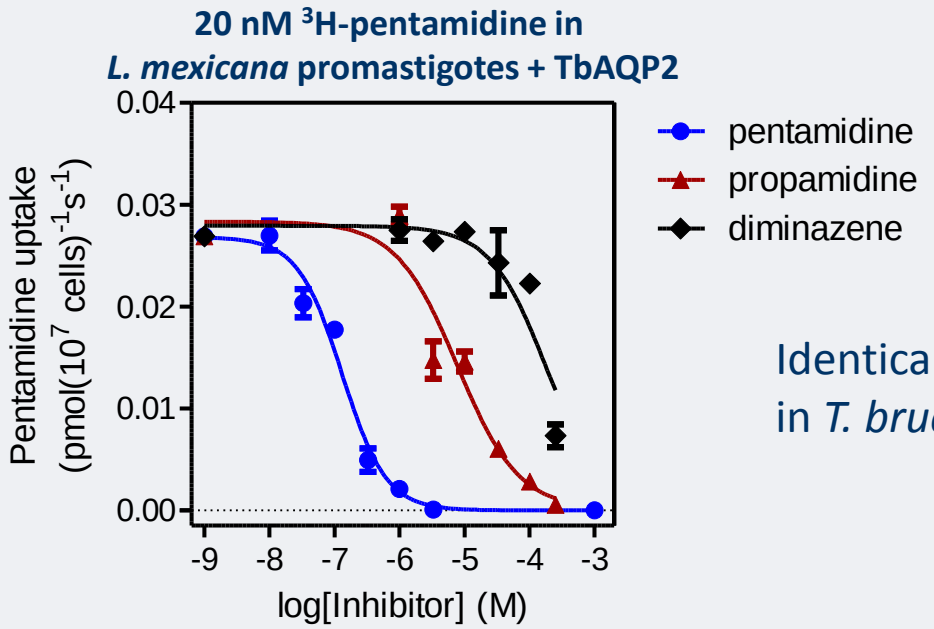
TbAQP2 sensitises Leishmania to Pentamidine and Cymelarsen



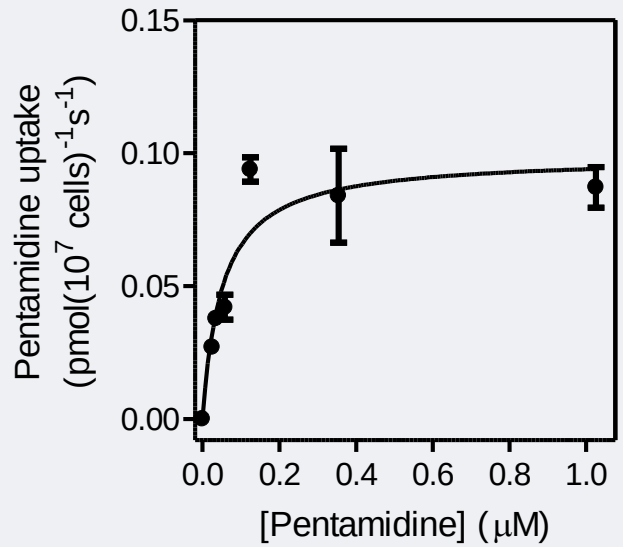


Uptake of 100 nM ^{3}H-pentamidine is 15-fold increased in *L. mexicana* promastigotes transformed with TbAQP2 but not with TbAQP3

Pentamidine uptake in Leishmania + TbAQP2



Identical inhibition profile to HAPT transport in *T. brucei* bloodstream forms



K_m = 55 ± 4 nM (n=5)

Almost identical to HAPT transport in *T. brucei* bloodstream forms (36 ± 6 nM)

Munday et al. J. Antimicrob. Chemother. 2014

University of Glasgow

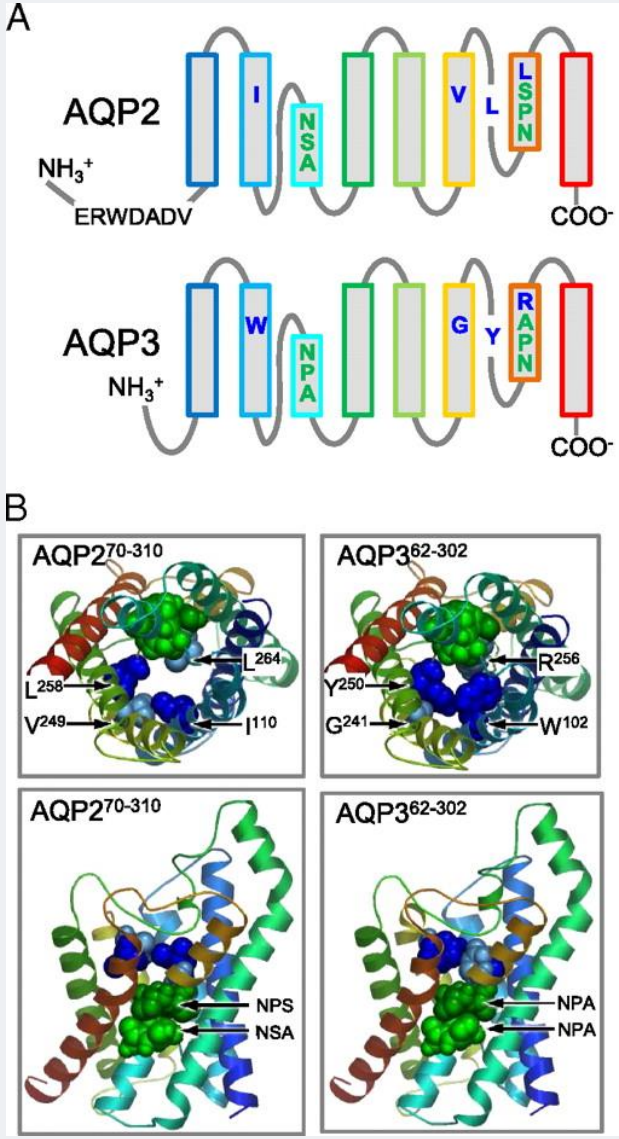
Conclusions:

- TbAQP2 is the High Affinity Pentamidine Transporter HAPT1
- Rate of transport does not correlate with endocytosis rate

Question:

- What makes AQP2 take up such large molecules as pentamidine?

TbAQP2 has a unique selectivity filter



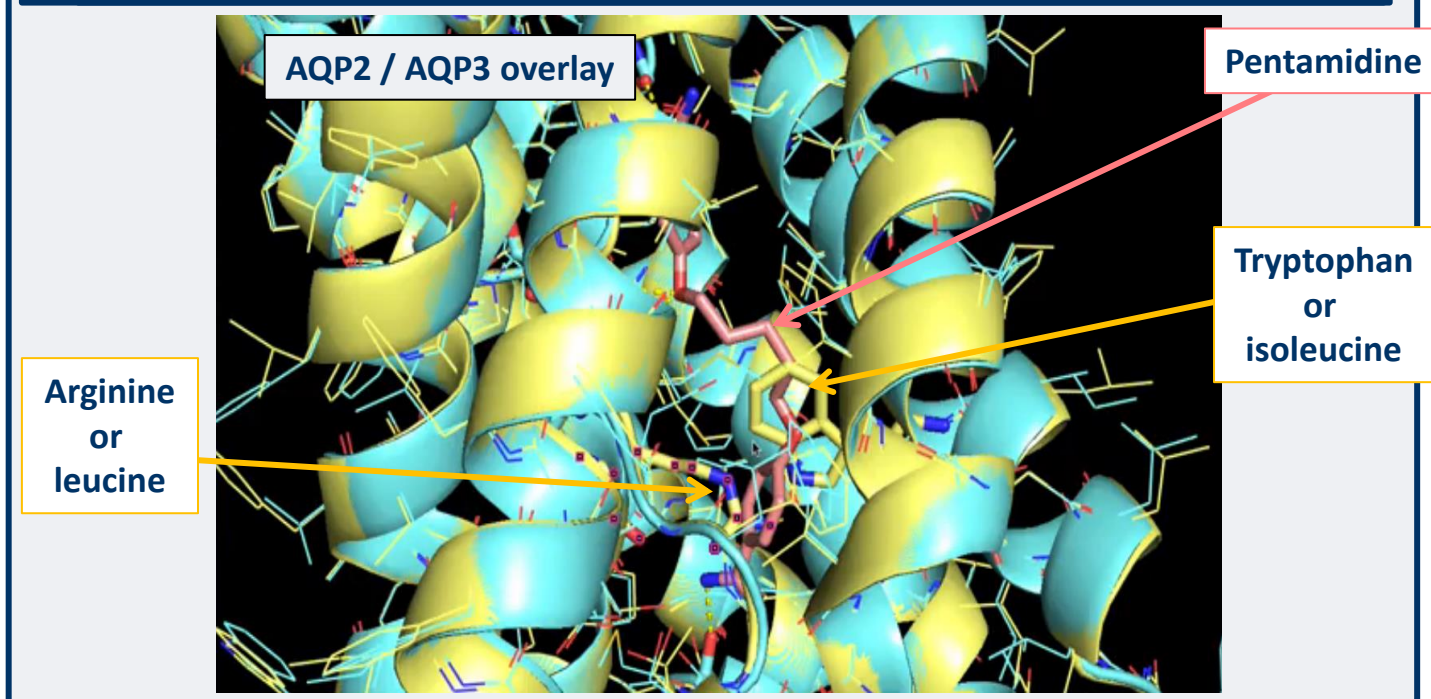
Baker et al. *Proc. Natl. Acad. Sci.* 2012

74% identity, 83% similarity

Yet, almost the entire selectivity filter has been changed to widen the pore and allow cations:

- **Tryptophan → Isoleucine**
- **Tyrosine → Leucine**
- **Arginine → Leucine**
- **Classical NPA/NPA → NSA/NPS**

Evidence of non-random adaptation



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