

Daan W. van Valkengoed¹, Vivi Rottschäfer^{2,3}, Elizabeth C.M. de Lange¹

¹Division of Systems Pharmacology and Pharmacy, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands
²Mathematical Institute, Leiden University, Leiden, The Netherlands
³Korteweg-de Vries Institute for Mathematics, University of Amsterdam, Amsterdam, The Netherlands



Introduction

- Active transporters are important for determining the pharmacokinetics (PK) of a drug, for example at the Blood-Brain-Barrier (BBB)¹
- The transporter activity at the BBB makes PK in the brain commonly unknown, leading to high attrition rates of CNS drugs²
- In vitro methods to predict drug PK in vivo are sought-after
- P-glycoprotein (P-gp) is the most important efflux transporter at the BBB, reducing CNS concentrations of many drugs
- In vitro P-gp activity is translated to in vivo P-gp activity using differences in P-gp protein expression³
- This translation assumes that the P-gp expression-activity relationship (EAR) is linear, in a drug-independent manner⁴
- There is conflicting information regarding this crucial relationship⁵⁻⁷

Aims

- To enhance theoretical understanding of the P-gp expression-activity relationship (EAR) through simulations of a P-gp kinetic binding model
- To identify if, and under which conditions, P-gp activity can be assumed to scale linearly with its expression

Methods

- A P-gp kinetic binding model⁸ was used to simulate the interaction between P-gp substrates and P-gp (Figure 1)

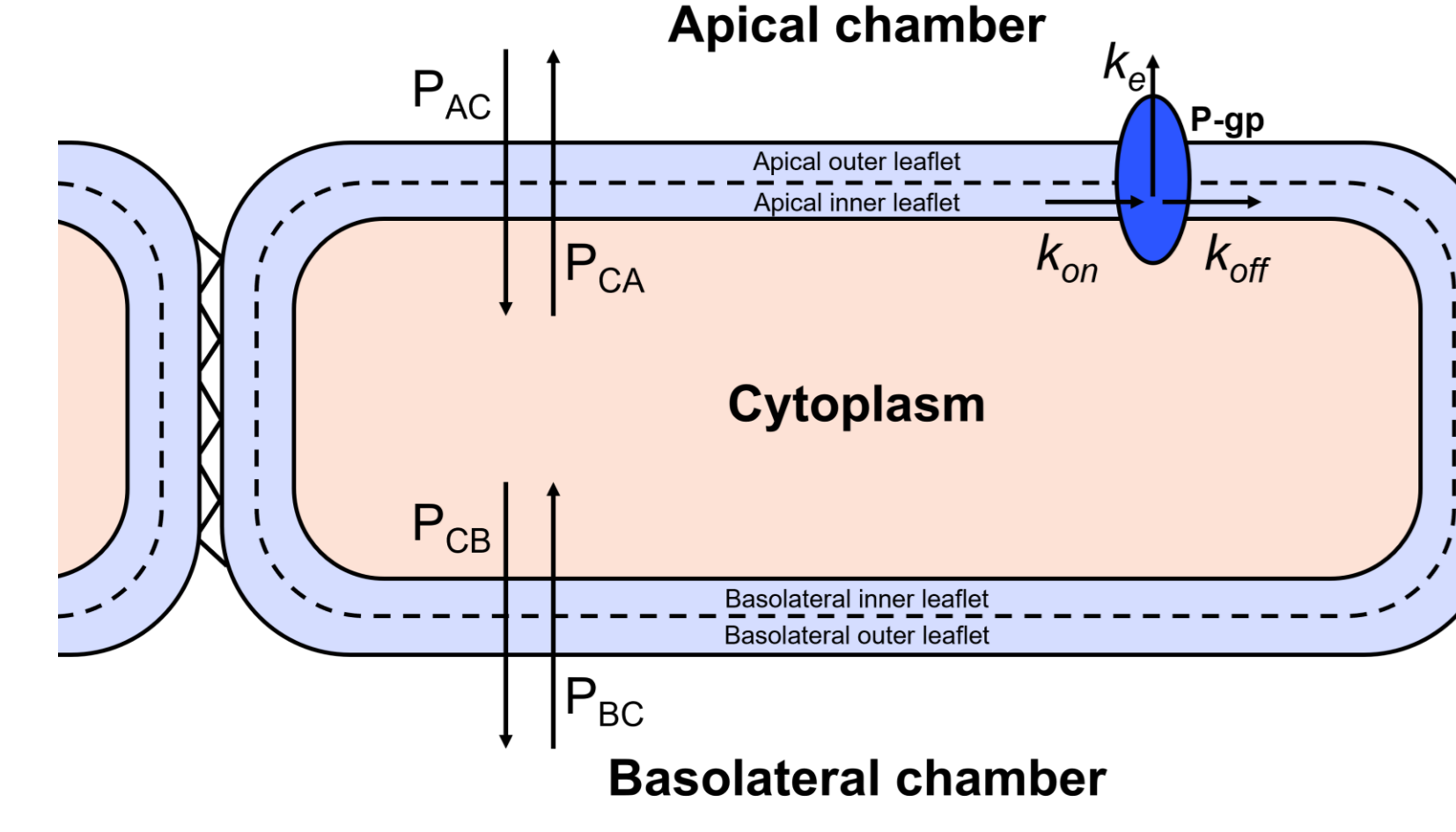


Figure 1: Schematic of the P-gp kinetic binding model.
The model resembles the in vitro setup commonly used for transwell permeability assays: a drug can passively diffuse across the membrane given by the passive permeability rate constants P_{AC} , P_{CA} , P_{BC} and P_{CB} . Drug in the inner leaflet of the apical membrane may bind to P-gp with the association rate constant k_{on} (fixed to $10^8 \text{ M}^{-1}\text{s}^{-1}$) and then be effluxed with efflux rate constant k_e or dissociate back into the inner leaflet by dissociation rate constant k_{off} .

- The relative EAR (rEAR) at different P-gp expressions of x% compared to a reference expression (100%) was calculated as:

$$rEAR_{x\%} = \frac{A_{eff,cum,x\%}}{A_{eff,cum,100\%}} * 100\%$$

with $A_{eff,cum}$ as total amount of drug effluxed by P-gp at a given P-gp expression from the start to the end of the simulation (6 hours)

- P-gp rEAR_{x%} was determined for 7 known P-gp substrates at 2%-300% of a reference P-gp expression of $10^3 \mu\text{M}$ after $1 \mu\text{M}$ apical administration
- rEAR_{50%} was subsequently determined for virtual drugs ranging in k_{off} ($10^3 - 10^7 \text{ s}^{-1}$) and k_e ($0.1 - 30 \text{ s}^{-1}$), also simulating different reference P-gp expressions ($10^1 - 10^5 \mu\text{M}$) and drug concentrations ($0.1 - 100 \mu\text{M}$)

Results

EAR of known P-gp substrates

- Simulations of 7 P-gp substrates (same drug dose and reference P-gp expression) show that the P-gp EAR can be linear and non-linear (Figure 2)

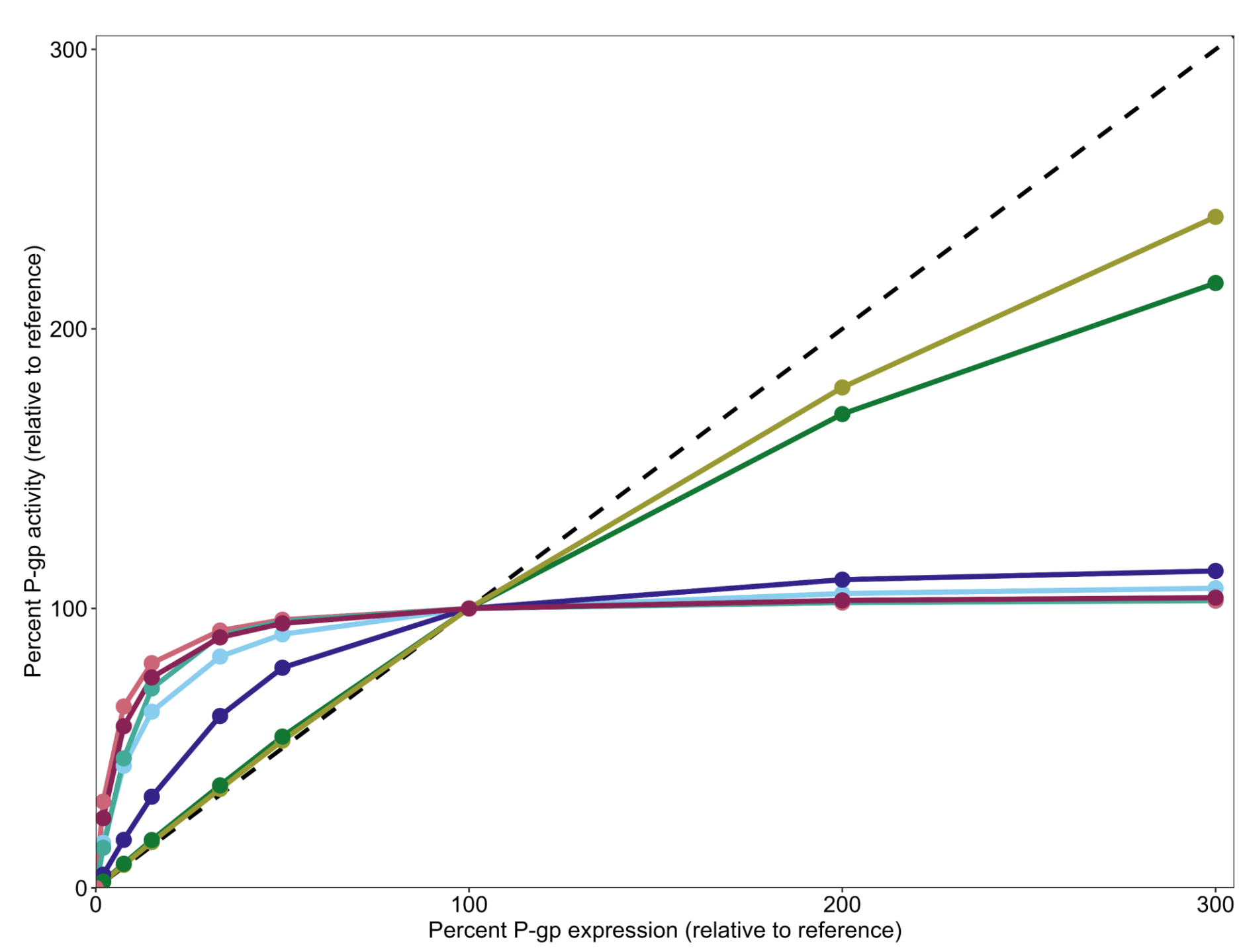


Figure 2: Expression-activity relationship (EAR) of seven P-gp substrates. Simulations started with a reference (100%) P-gp expression of $10^3 \mu\text{M}$ and the drug concentration in the apical chamber at $t = 0$ was $1 \mu\text{M}$. P-gp expression was subsequently changed (2%-300%), and the change in activity compared to the reference simulation was calculated. The black dashed line represents a directly proportional relationship between P-gp expression and activity of P-gp.

EAR of theoretical P-gp substrates

- Simulations of virtual drugs that vary in k_{off} and k_e show the importance of the ratio k_{off}/k_e in determining the EAR (Figure 4)
- Higher k_{off}/k_e ratios move the EAR towards linearity
- The ratio P-gp expression to initial drug concentration also influences the EAR: when P-gp is in excess, a non-linear EAR is expected

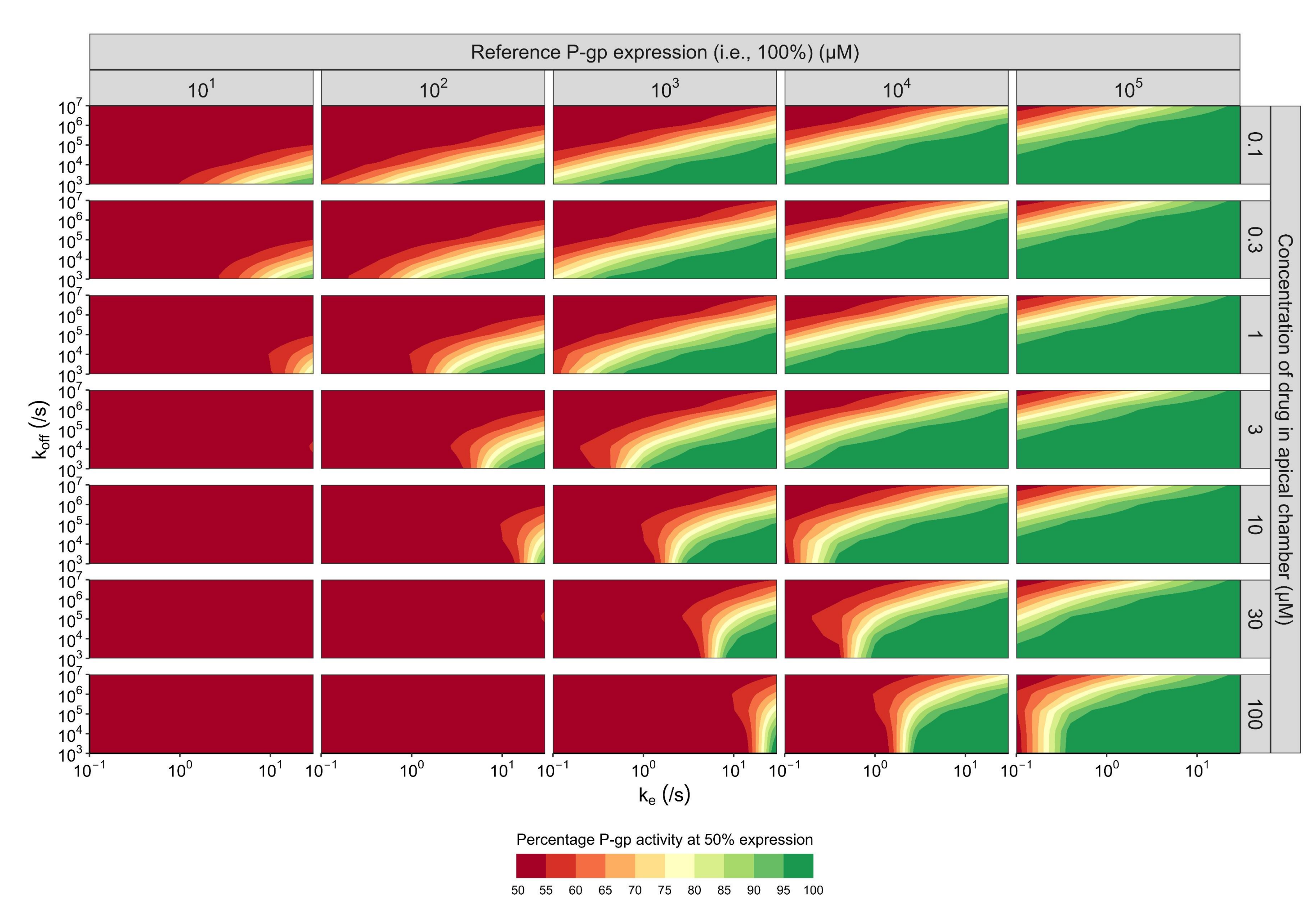


Figure 4: Heatmap of the rEAR_{50%} of virtual drugs with different combinations of k_{off} and k_e (axes), under different initial drug concentrations (rows) and reference P-gp expressions (columns). In every figure, the value of k_e is plotted on the horizontal axis and the value of k_{off} is plotted on the vertical axis. The columns indicate the reference P-gp expression (μM), whereas the rows indicate the drug concentration in the apical chamber at $t=0$ (μM). Red colours indicate a linear response of P-gp activity to 50% reduction in P-gp expression ($rEAR_{50\%} \approx 50\%$), while green corresponds to non-linear changes in activity ($rEAR_{50\%} \approx 100\%$).

Relationship between drug properties and EAR

- For 4 characteristics of the drugs we explored the relationship to their EAR: passive permeability (P_{AC}), partitioning coefficient (K_{IL}), P-gp efflux efficiency (ratio k_{off}/k_e) and lipophilicity ($\log P$) (Figure 3)
- Only the ratio k_{off}/k_e shows a clear relationship to the P-gp EAR

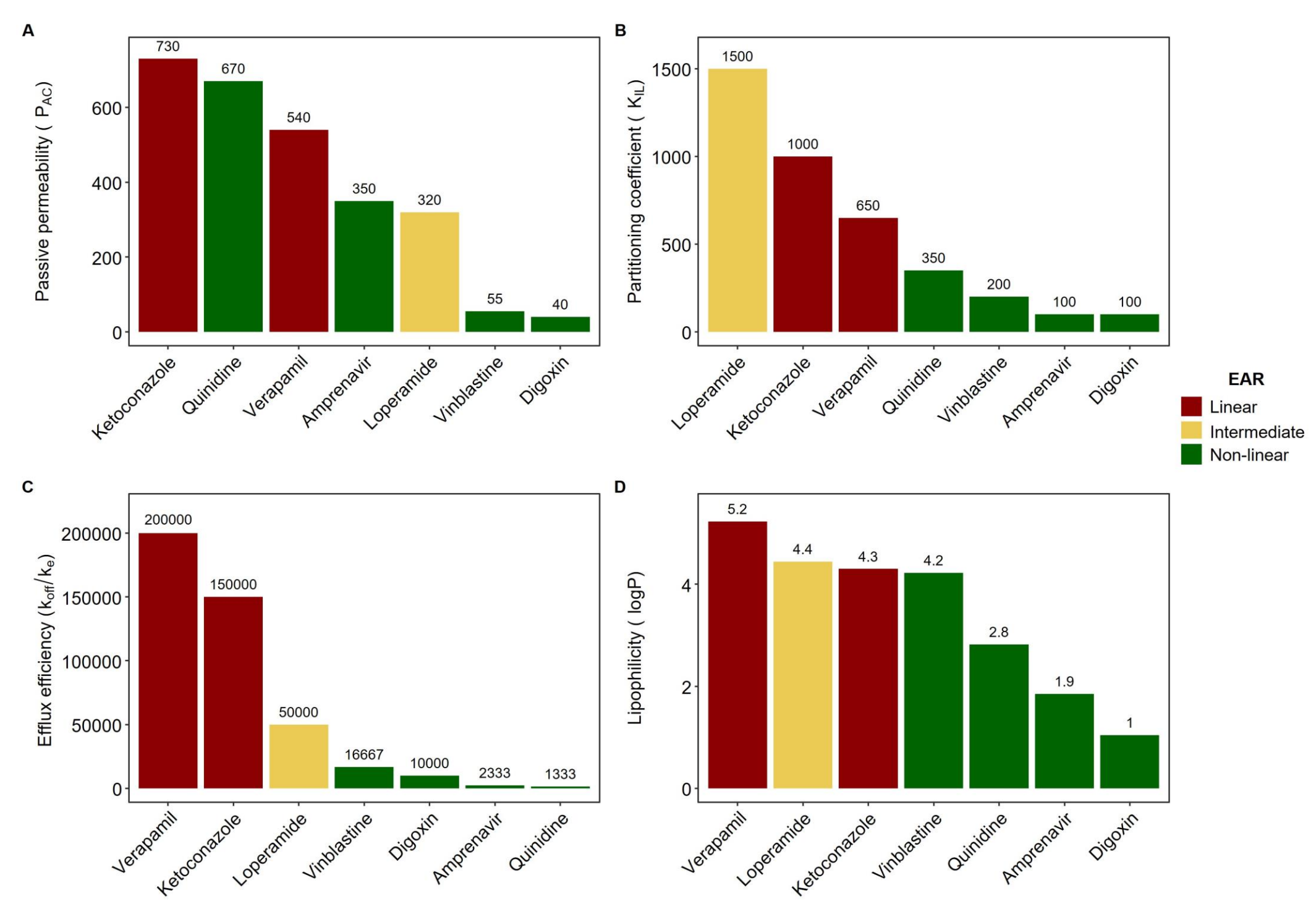


Figure 3: Relationship between the EAR of the seven P-gp substrates and their (A) P_{AC} (B) K_{IL} (C) ratio k_{off}/k_e , and (D) $\log P$. Red bars indicate drugs that show a linear EAR (verapamil and ketoconazole), green bars drugs with a non-linear EAR (amprenavir, digoxin, quinidine and vinblastine), and yellow bars a drug with an EAR in between the other two groups (loperamide).

Conclusions

- The P-gp EAR is not always linear: it depends on the drug and system and can vary between linearity and non-linearity
- A non-linear EAR is expected whenever a drug is efficiently effluxed (low ratio k_{off}/k_e)
- Non-linear EAR is also expected whenever P-gp is in excess to the drug concentration
- These results show a need to reconsider the widespread use of P-gp expression as single biomarker for its activity

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 d.w.van.valkenoed@lacdr.leidenuniv.nl