

Evaluating translational capabilities of Caco-2 derived permeability with mechanistic modeling in PK-Sim®

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BACKGROUND

- In vitro Caco-2 assay** derived permeability supports
 - Early compound selection and ranking in drug research based on **expected absorption properties**^[1]
 - Assessment of drug products as BCS-based biowaivers^[2]
- Common Caco-2 assay readouts are **apparent permeability coefficient (P_{app})**, efflux ratio and recovery^[3]
- Assay readouts serve as **classification tools** and are **not optimized for translational modeling approaches**

Objective

Investigate applicability of **Caco-2 derived P_{app}** values for **translational modeling approaches** by extrapolating human fraction absorbed (F_a)

METHODS

- Data acquisition** and **literature search** performed with **Pubmed**^[4]
- Intestinal permeability calculation** based on **physico-chemical** properties ($P_{int,PC}$) and estimation of **optimal values** ($P_{int,opt}$) accomplished using **PK-Sim® V11**^[5], **R 4.2.0**^[6] and the **OSPsuite-R package**^[7]

Status quo

- Challenging **in vitro-to-in vivo extrapolation (IVIVE)** of **human intestinal permeability**
- Using **Caco-2 P_{app}** for **intestinal permeability** in **PK-Sim®** shows poor predictability

Assumptions

- $F_{oral} = F_a * F_g * F_h$ ^[8]
- F_a is the sum of **passive** and **transporter-mediated** membrane permeation (influx and/or efflux)

Benchmark dataset

- Human drug **absorption**
- Physico-chemical** properties
- Intestinal permeability**
- Active **transport** and **metabolism** in gut

$P_{app} \rightarrow P_{int,opt}$

- Determination of **optimal permeability** to simulate F_a
- Investigation of **compound properties** affecting IVIVE, e.g., lipophilicity, solubility and active **ADME** processes

RESULTS

Benchmark dataset

Table 1. Establishment of a diverse benchmark dataset including 138 drug compounds

General	Physico-chemical	In vivo ^[8]	Intestinal permeability ^a	ADME ^b
- Molecular weight - Indication - Therapeutic dose	- logP, logD_{7.4} - Solubility - BDDCS class^[9]	F_{oral} F_a^c F_g	P_{app}^d P_{eff}^e (n = 14)^e $P_{int,PC}^f$	- Gut metabolism - Active transport in gut

^ain cm/s. ^bProtein expression informed with PK-Sim® expression database. ^cExclusion of compounds with reported F_a of 100%. ^d*in vitro* determined apparent permeability coefficient (Caco-2). ^e*in situ* determined effective human intestinal permeability^[10]. ^f*in silico* transcellular intestinal permeability calculated from physico-chemical properties^[11]

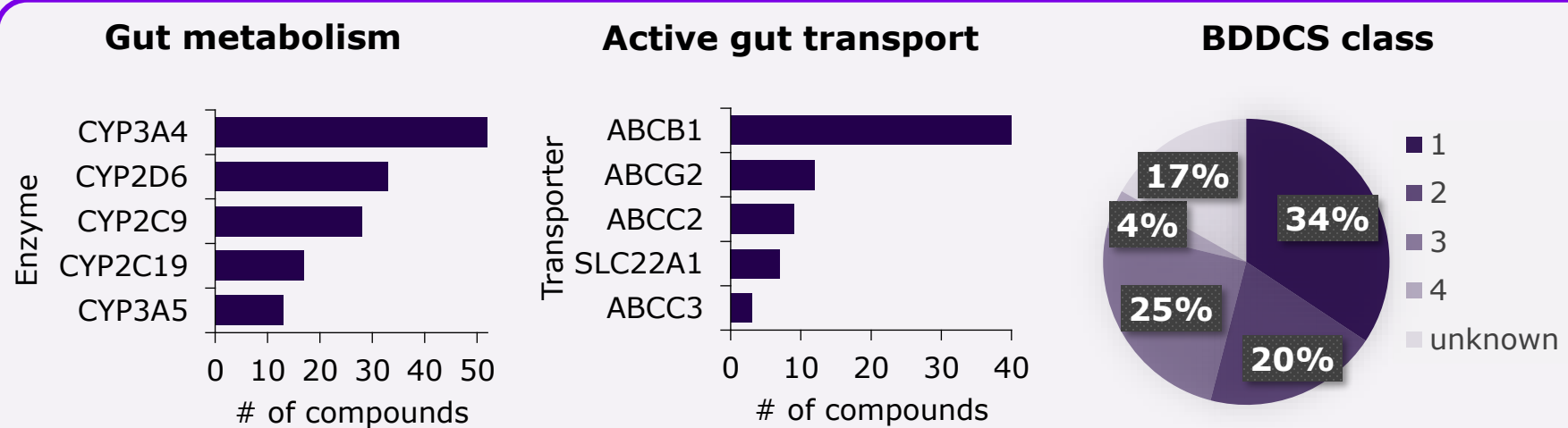


Fig. 1. Involved gut enzymes, gut transporters and compound BDDCS classes

Compound groups

- Compounds can be assigned to **four ADME-related groups**, while most compounds (41%) are liable to intestinal metabolism (**Fig. 2**)
- F_a shows different correlation with P_{app} , P_{eff} or $P_{int,PC}$ (**Fig. 3a**) while F_a vs. P_{app} correlation improves for compounds with passive permeability only (**Fig. 3b**)

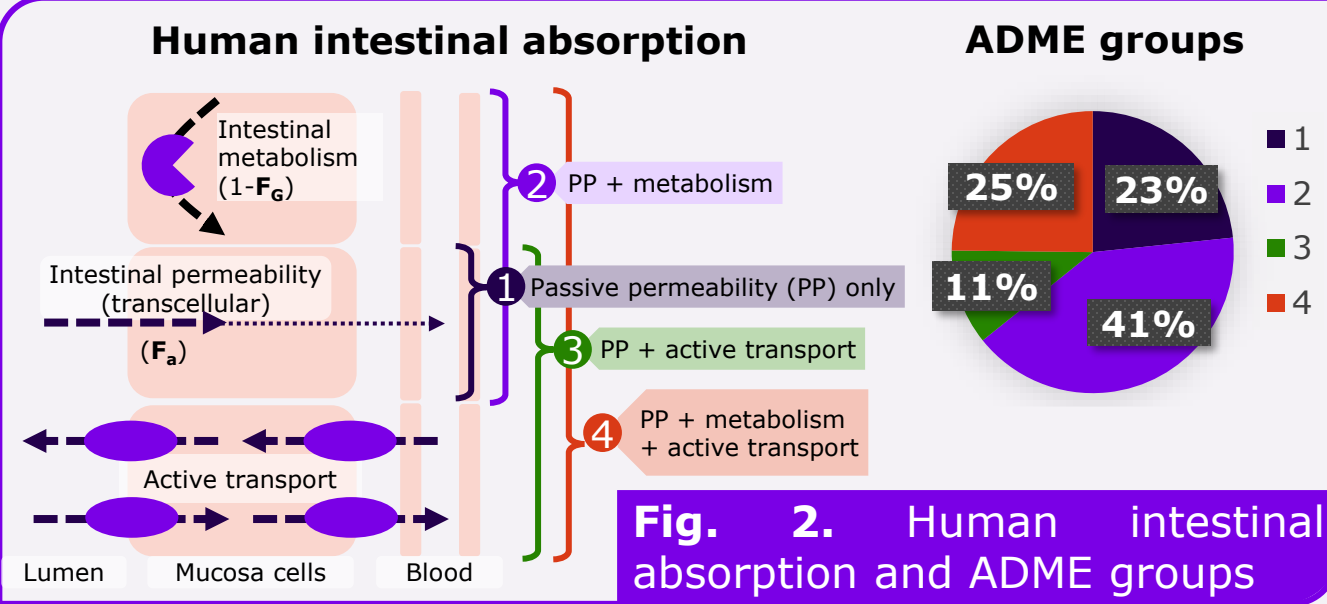


Fig. 2. Human intestinal absorption and ADME groups

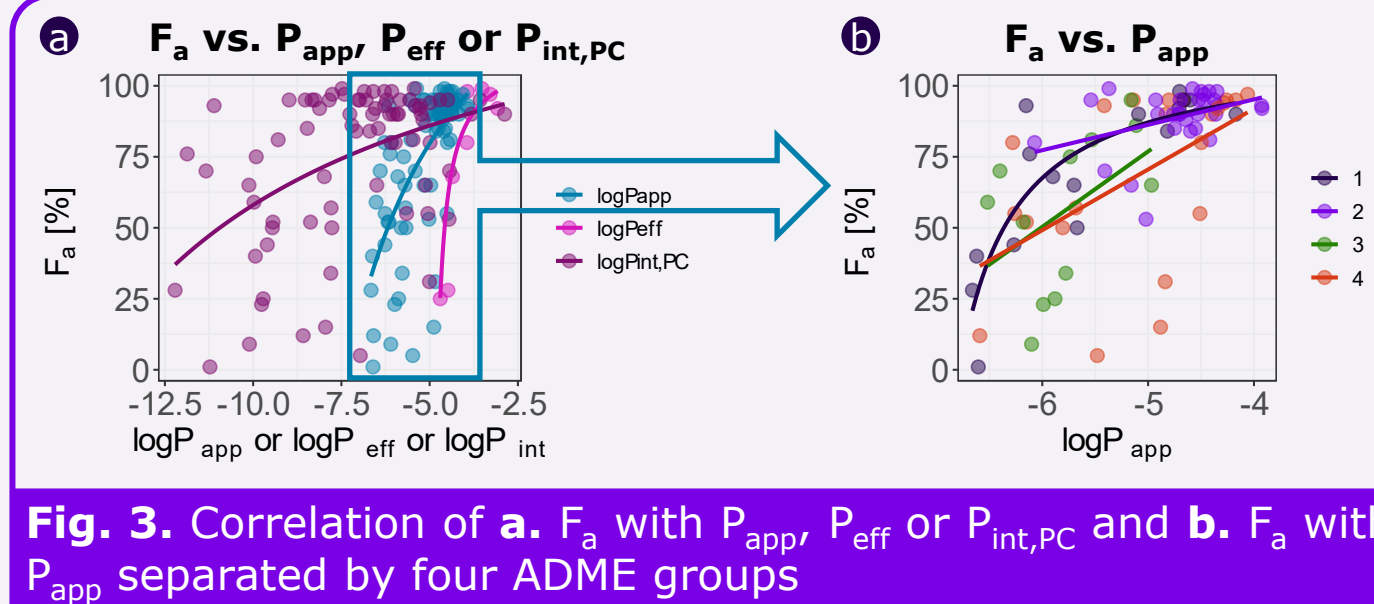


Fig. 3. Correlation of a. F_a with P_{app} , P_{eff} or $P_{int,PC}$ and b. F_a with P_{app} separated by four ADME groups

Translational capabilities of Caco-2 permeability

- F_a cannot be predicted using P_{app} , P_{eff} or $P_{int,PC}$ as input for **intestinal permeability** (**Fig. 4a**), while potential mapping function for F_a and $P_{int,opt}$ follows sigmoidal shape (**Fig. 4b**)
- No pattern of permeability ratio ($P_{int,opt}/P_{app}$)** observed for **passively permeable compounds** (ADME group 1, **Fig. 5a**)
- Improved prediction for ADME group 2 and BDDCS 1/2** (metabolized compounds) using **correction factor** (**Fig. 5 a, b**)
- Trend for **larger gap** between $P_{int,opt}$ and P_{app} identified for compounds with **high P_{app}** , supporting more **precise prediction** for **metabolized compounds** (**Fig. 5c**)

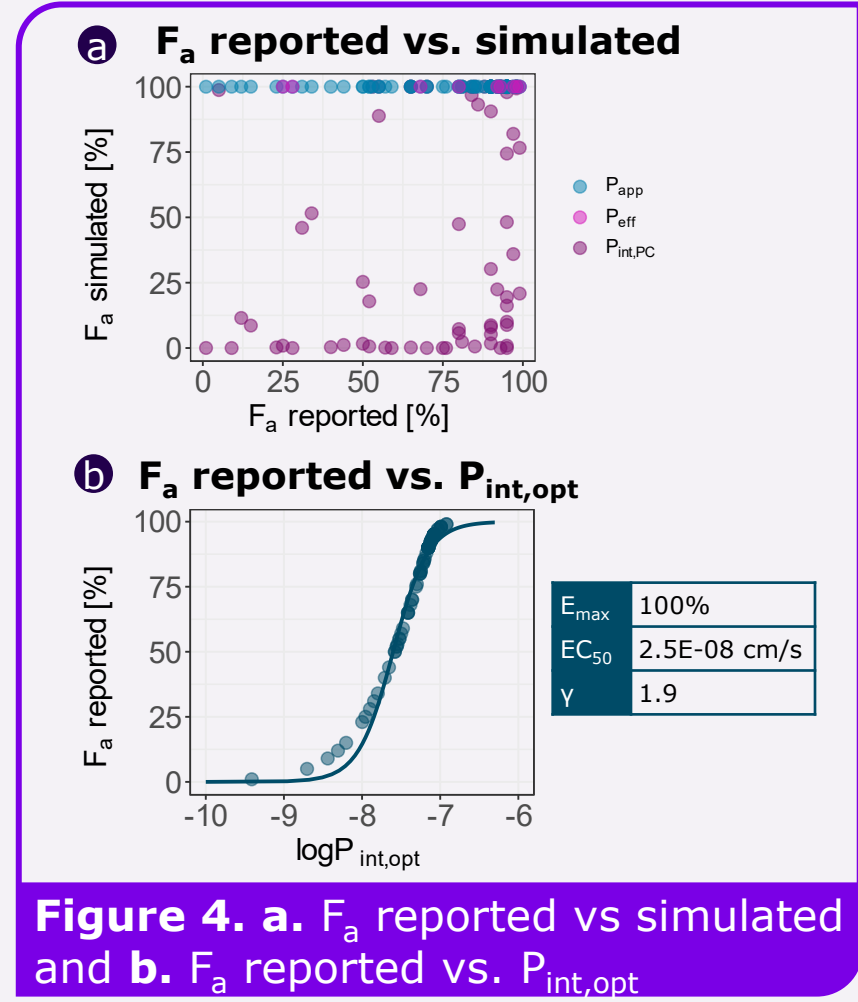


Figure 4. a. F_a reported vs simulated and b. F_a reported vs. $P_{int,opt}$

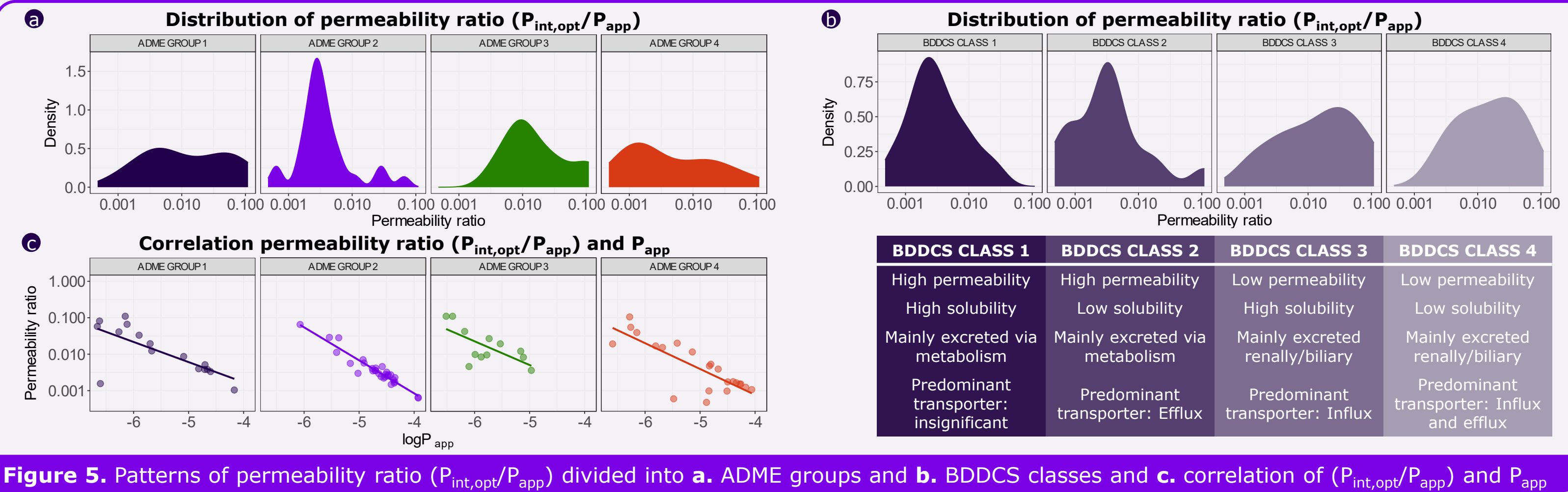


Figure 5. Patterns of permeability ratio ($P_{int,opt}/P_{app}$) divided into a. ADME groups and b. BDDCS classes and c. correlation of ($P_{int,opt}/P_{app}$) and P_{app}

DISCUSSION AND CONCLUSION

The **Caco-2 in vitro permeability assay** is designed to support **compound classification** and **ranking**. **Assay readouts, design and procedure** need to be **adjusted** for application in **translational models**, e.g., due to **high assay complexity** and non-isolated assessment of different processes. Our analysis shows that **correction factors** based on **compound properties** (e.g., involvement of active ADME processes) can **improve utilization of Caco-2 permeability** for complex mechanistic modeling approaches and IVIVE. Next steps are **inclusion of further compounds** aiming for an **unbiased dataset** (e.g., most drugs show a high fraction absorbed) as well as **prediction of relevant ADME mechanisms applying AI technologies**, to enable translation to **mechanistic modeling approaches** and assess **predictivity of $P_{int,opt}$ vs. P_{app}** based on **ADME groups**.

REFERENCES: [1] Sun et al. Expert Opin Drug Metab Toxicol. 2008;4(4):395-411. [2] US Food and Drug Administration. M9 Biopharmaceutics Classification System-Based Biowaivers. Guidance for Industry. 2021. [3] Hubatsch et al. Nat Protoc. 2007;2(9):2111-9. [4] National Library of Medicine. <https://pubmed.ncbi.nlm.nih.gov/>. [5] Open Systems Pharmacology Suite. <https://www.open-systems-pharmacology.org/>. [6] R Core Team 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. [7] Sevestre et al. 2023. ospsuite: R package to manipulate OSPsuite Models. <https://github.com/open-systems-pharmacology/ospsuite-r>. [8] Varma et al. J Med Chem. 2010;53(3):1098-108. [9] Bocci et al. AAPS J. 2022;24(2):37. [10] Avdeef et al. J Med Chem. 2010 May 13;53(9):3566-84. [11] Thelen et al. J Pharm Sci. 2011;100(12):5324-45. **ABBREVIATIONS:** ADME: absorption, distribution, metabolism and excretion, BDDCS: biopharmaceutics drug disposition classification system, F_a : fraction or drug absorbed from the gut lumen, F_g : fraction of drug that escapes gastric metabolism, F_h : fraction of drug that escapes hepatic metabolism, F_{oral} : oral bioavailability, IVIVE: *in vitro-to-in vivo* extrapolation, P_{app} : apparent permeability coefficient, P_{eff} : effective human intestinal permeability, $P_{int,opt}$: optimal transcellular intestinal permeability calculated to match F_a , $P_{int,PC}$: transcellular intestinal permeability calculated from physico-chemical properties. **FUNDING:** Study funded by Sanofi.