

## CHARACTERIZATION OF IN VITRO MODELS TO DETERMINE THE PRE-SYSTEMIC METABOLISM OF THE HUMAN INTESTINE FOLLOWING ORAL ADMINISTRATION

Patrik Lundquist<sup>1</sup>, Merve Ceylan<sup>1</sup>, Rebekah Hammar<sup>1</sup>, Ana C.C. Lopes<sup>2</sup>, Mikael E. Sellin<sup>2</sup>, Helen Sophie Hammer<sup>3</sup>, Oliver Poetz<sup>3,4</sup>, Per Artursson<sup>1</sup>

<sup>1</sup>Department of Pharmacy, Uppsala University, Uppsala, Sweden <sup>2</sup>Department of Medical Biochemistry and Microbiology, Infection and Immunity, Uppsala University, Uppsala, Sweden <sup>3</sup>SIGNATOPE GmbH, Reutlingen, Germany <sup>4</sup>NMI Natural and Medical Sciences Institute, University of Tuebingen, Reutlingen, Germany

### ABSTRACT

The EU-funded RISK-HUNT3R project aims to improve the assessment of human exposure to chemicals and pharmaceuticals via the lungs, skin and gastrointestinal tract. A key objective of the project is to develop new approach methods (NAMs) that more accurately replicate human absorption barriers and generate high-quality input data for predictive toxicokinetic models.

For the assessment of oral exposure, intestinal permeability through epithelial cells is a crucial factor for systemic availability after ingestion. Although permeability measurements provide valuable information, a realistic assessment of oral absorption also requires the quantification of intestinal first-pass metabolism, i.e. the proportion of a compound that is metabolized by enterocytes in the small intestine. Existing in vitro models, particularly Caco-2 cells, are of limited use in this regard due to their insufficient expression of relevant metabolizing enzymes. Therefore, we focused on the development of three advanced human intestinal models that capture both permeability and metabolic capacity.

### DEVELOPMENT OF INTESTINE CELL MODELS

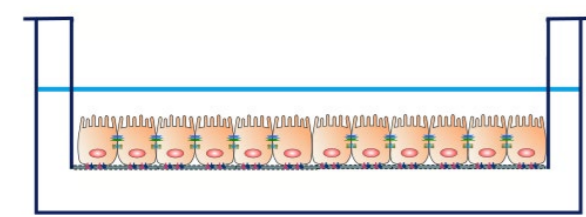


Figure 1: Caco-2



Figure 2: Primary human jejunal enterocytes

- Good correlation  
Permeability ↔ fraction absorbed
- No mucus barrier
- Low expression of major metabolic enzymes

- Primary human jejunal enterocyte isolation yielded stable cell viability (>85%), indicating robust isolation and culture conditions
- Cell yield consistently exceeded 30 million cells per gram of mucosa
- Major intestinal CYP enzymes (CYP3A4, CYP2C9, CYP2D6) and Phase II enzymes were functionally active
- High CYP3A4 and carboxylesterase (CES) activity highlights strong potential for accurate modeling of intestinal metabolism

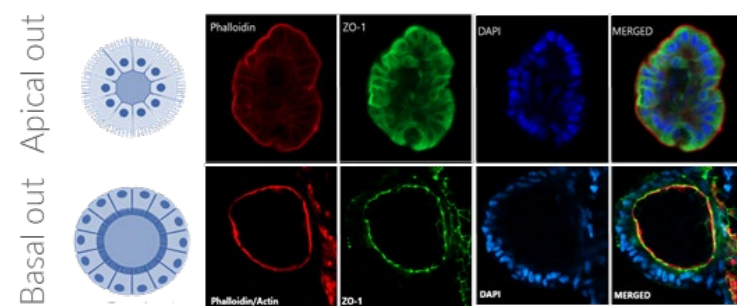


Figure 3: Intestinal organoids

- Enteroids can be cultured in apical-out or basal-out configurations, offering experimental flexibility
- Show in vivo-like expression of ADME proteins, supporting physiological relevance
- Suitable for metabolism studies
- Primary enterocytes generally provide more robust metabolic results
- Permeability measurements remain difficult in these systems



Figure 4: 2D enteroids – Air-Liquid Interface (ALI) cultures

- 2D Air-Liquid Interface (ALI) cultures show high cellular differentiation and good morphology
- Mucus production indicates functional epithelial characteristics
- High expression of differentiation markers and ADME proteins
- Presence of goblet, Paneth, and enteroendocrine cell markers reflects epithelial diversity
- Functional studies are ongoing to further characterize the model

### QUANTIFICATION OF ADME PROTEIN EXPRESSION

The abundance of cytochrome P450 (CYP450) enzymes, transporters, and other drug-metabolizing enzymes in the intestine plays a key role in intestinal first-pass metabolism, contributing to the pre-systemic breakdown of drugs in enterocytes before reaching the liver and systemic circulation. Over the past decade, mass spectrometry-based methods have been developed to directly quantify such proteins by analyzing proteotypic peptides generated through enzymatic digestion. Quantification is typically achieved by adding defined amounts of corresponding <sup>13</sup>C/<sup>15</sup>N-labeled peptide standards. However, these approaches require relatively large sample amounts, as labor-intensive microsomal or membrane preparation is often needed for sufficient sensitivity. To address this, we combined immunoprecipitation of CYP isoform- and transporter-specific peptides with mass spectrometry, enabling protein quantification from very small samples. Antibodies targeting conserved peptide motifs can capture multiple peptides, providing a sensitive method to quantify intestinal CYP enzymes, transporters, and other drug-metabolizing proteins in limited biological material (see Figure 5).

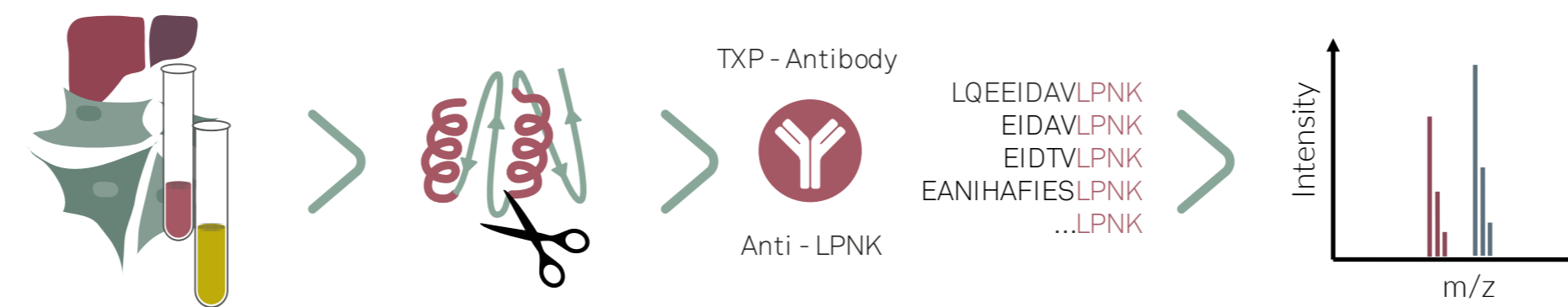


Figure 5: Workflow of immunoaffinity-MS. Proteins are typically digested and isotopically labeled standard peptides are added (I). Group-specific antibodies targeting C-terminal or common sequence motifs are employed in an automated immunoprecipitation workflow to enrich peptides derived from proteins of interest and standards (II). Finally unique peptides derived from the protein biomarkers are quantified via reference peptides and targeted nLC-mass spectrometry (III).

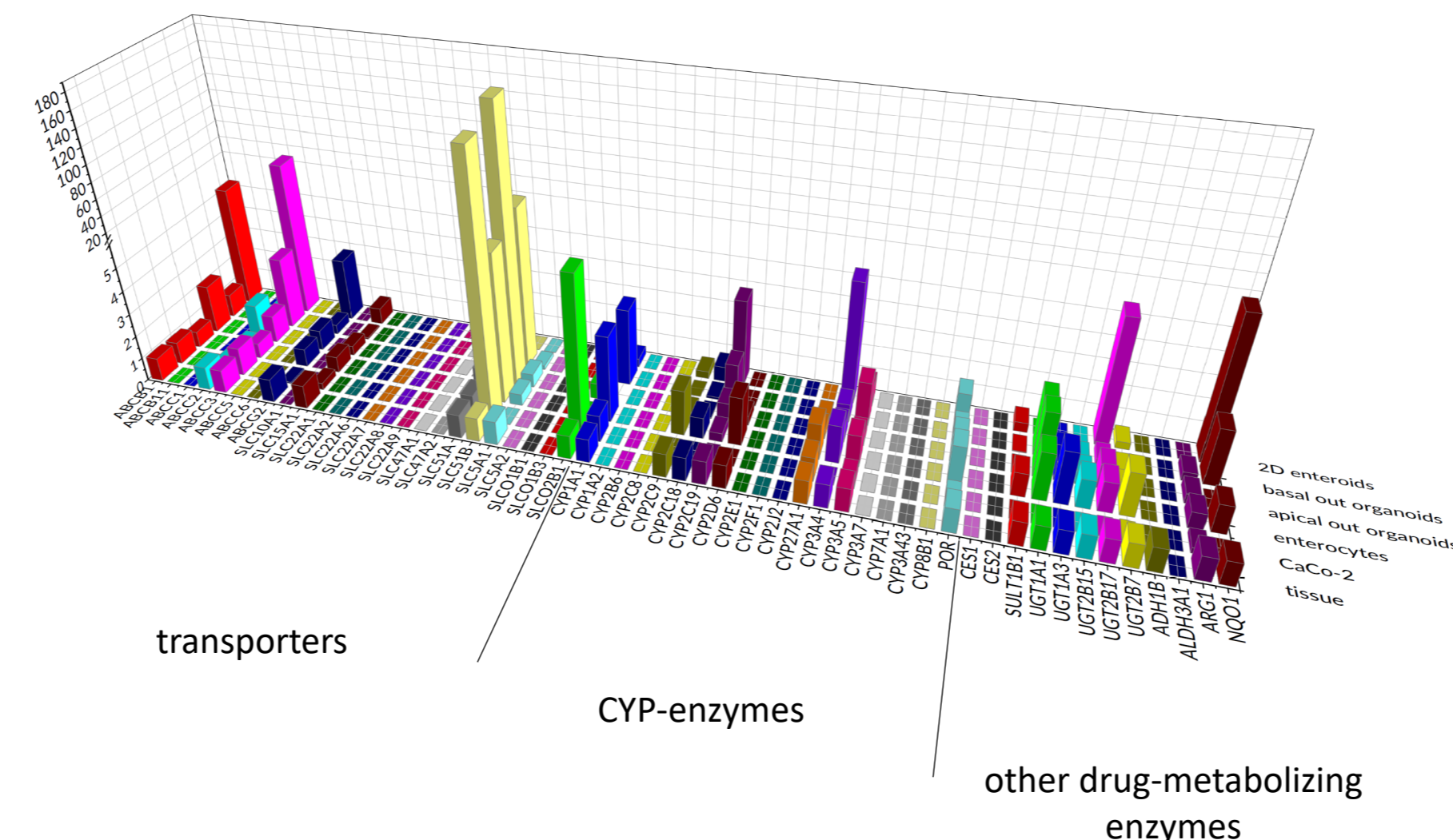


Figure 6: ADME protein abundance has been determined by immunoaffinity-LC-MS/MS and normalized by protein amount in tissue, CACO2, enterocytes, apical and basal out organoids, 2-D enteroids. Results were normalized to tissue expression.

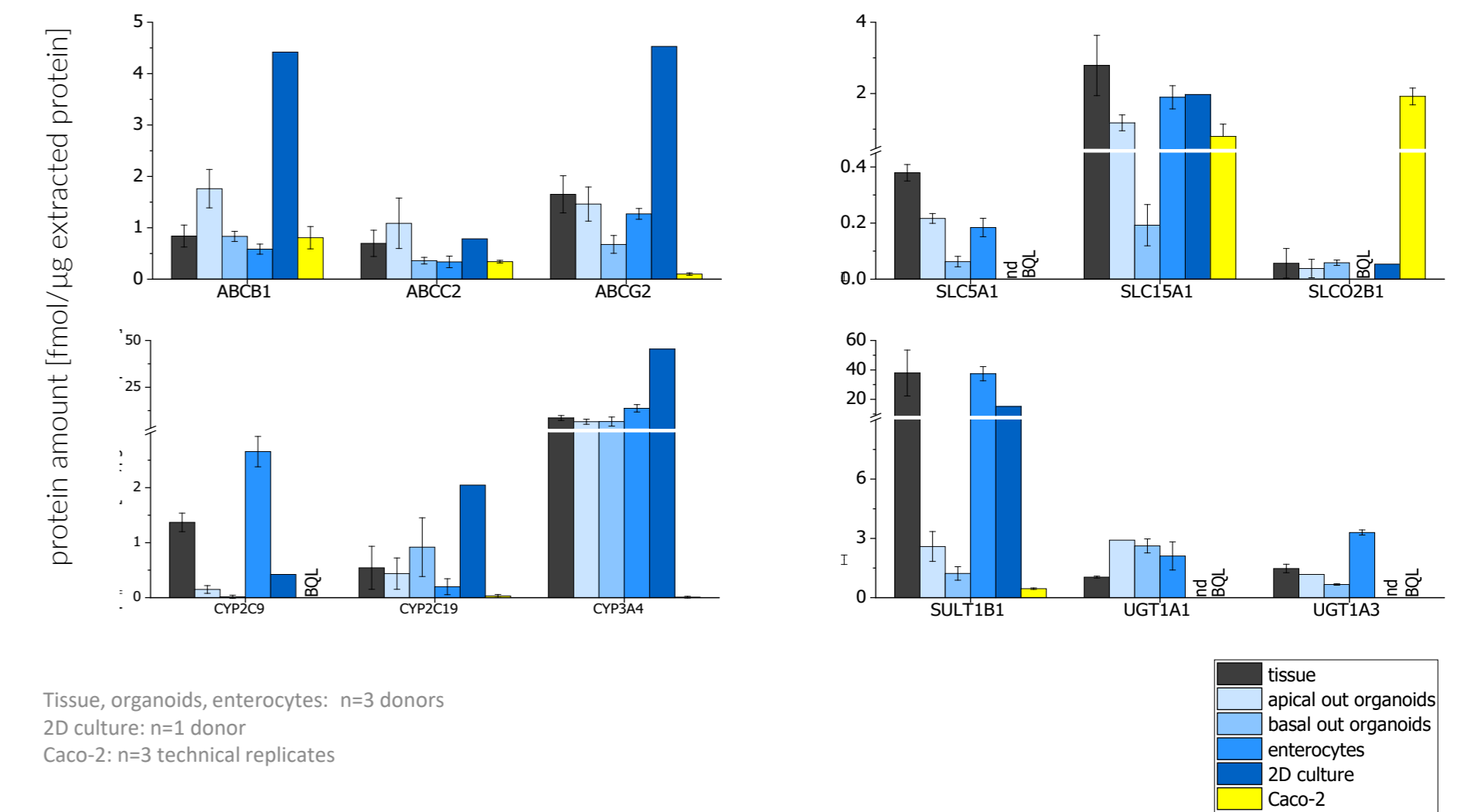


Figure 7: Protein abundance of selected ADME Proteins in tissue, enterocytes, apical and basal out organoids, 2-D enteroids and Caco-2 normalized to tissue expression.

### SUMMARY

Significant progress has been achieved in the development of intestinal new approach methodologies (NAMs). Across all models, the expression of ADME proteins closely resembles in vivo conditions, demonstrating their physiological relevance and suitability for drug metabolism and transport studies. Primary human enterocytes have been successfully isolated and established as a model system suitable for metabolism studies. In addition, apical-out and basal-out enteroids have been cultivated. These models are also suitable for metabolism investigations; however, measuring permeability in these systems remains challenging due to their structural characteristics. For permeability studies, two-dimensional (2D) enteroid models have been developed. These models exhibit a high expression of differentiation markers, show good morphological characteristics, and produce mucus, indicating a well-differentiated intestinal phenotype. Functional studies with these 2D enteroids are currently ongoing to further validate their applicability.

### ACKNOWLEDGMENT

This project receives funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No. 964537 (RISK-HUNT3R), which is part of the ASPIS cluster.

### CONTACT

Protein analysis

Oliver Poetz  
SIGNATOPE GmbH  
Markwiesenstr. 55  
72770 Reutlingen, Germany  
poetz@signatope.com

Intestine cell models

Patrik Lundquist  
Uppsala University  
Department of Pharmacy  
Uppsala, Sweden  
patrik.lundquist@uu.se

For poster  
download and  
further information  
follow the QR Code

