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# Characterization of hepatocyte subpopulations by global proteomics

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# Global proteomics

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- MS-based proteomics: quantitative analysis of almost **entire proteomes**
- Detailed understanding of **cellular phenotype, function, and status**
- Basic workflow of bottom-up proteomics
  - Sample lysis
  - Proteolytic digestion of proteins into peptides
  - Analysis of peptide mixture on high-resolution MS
  - Data processing to identify and quantify proteins
  - Bioinformatics and pathway analysis

  - **Recent application**: Ölander, M. et al, **A simple approach for restoration of differentiation and function in cryopreserved human hepatocytes**. *Arch Toxicol.* 93,:819-829, 2019. PMID30971754

  - **White paper:** Prasad B. et al. Toward a Consensus on Applying Quantitative Liquid Chromatography-Tandem Mass Spectrometry Proteomics in Translational Pharmacology Research: A White Paper. *Clin Pharmacol Ther*. 106, 525-543, 2019. PMID: 31175671

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# Human hepatocyte research

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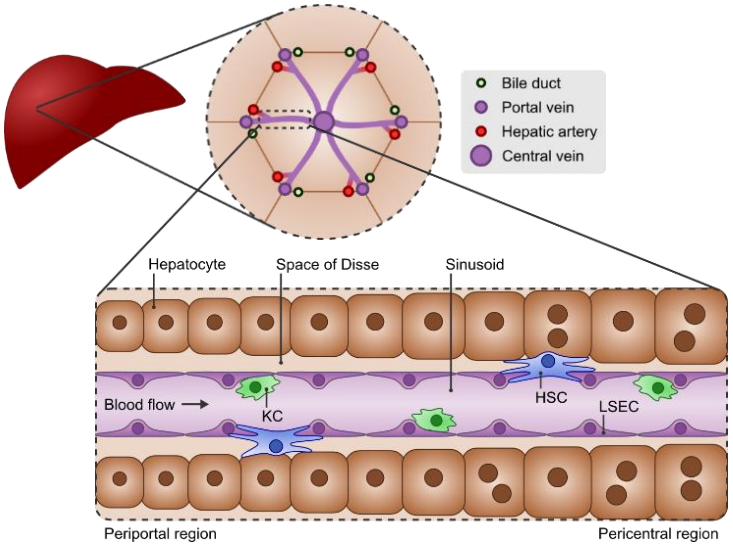
| | |
|---|---|
| Intracellular concentrations | Effects of transporters, enzymes; in predictions of DDIs |
| Model development | 3D spheroid cultures in 384 well format, 5s hepatocytes |
| Cell populations in the human liver | **Zone-specific hepatocytes**, hepatocytes and **three Non Parenchymal Cell populations** (from the same donor) |

Ölander, M., Wegler, C., Treyer, A., Flörkemeier, I., Handin, N., Pedersen, J.M., Vildhede, A., Mateus, A., LeCluyse, E.L., Urdzik, J., Artursson, P. *In manuscript.*

# Dissection of human hepatocyte zonation
# Background

- Differences in expression and function across lobules: liver zonation
  - Established by concentration gradients of oxygen, morphogens, hormones, etc.
  - **Liver Zonulation correlates with differences in cell size**




Ölander, M., PhD dissertation, Acta Universitatis Upsaliensis, 2019

**Aim:**

- To investigate the size characteristics of isolated and cryopreserved human hepatocytes
- To assess whether size separation can be used to study zonated liver functions *in vitro*.

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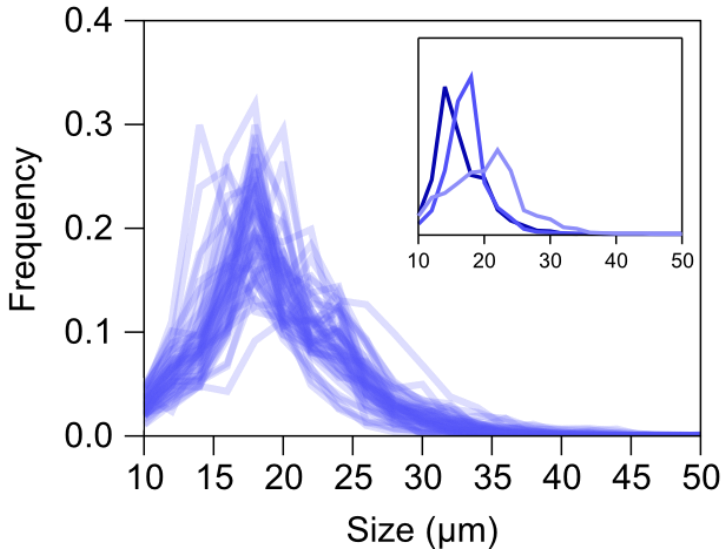
# Size distributions

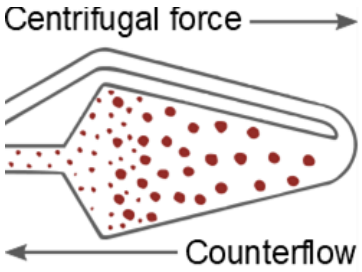
- Sizes of individual hepatocytes were determined with image cytometry
- Analyzed the size distributions of 48 human hepatocyte batches

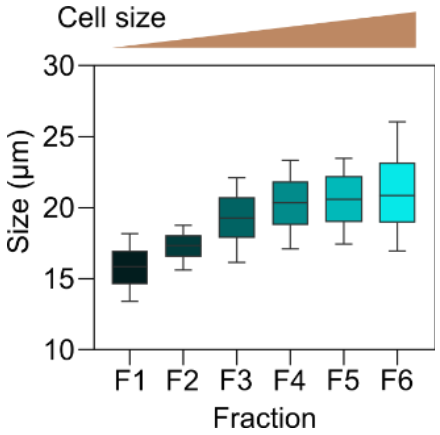



Size distributions varied between different cryopreserved batches

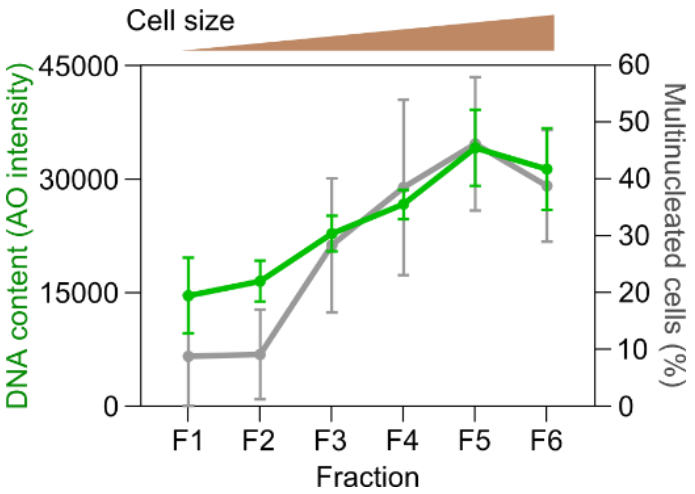
# Size separation

- Used counterflow centrifugal elutriation to separate hepatocytes into six size fractions



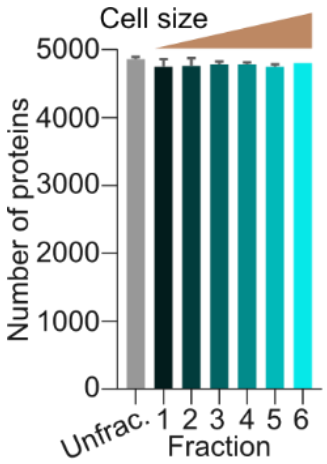

Cell size increased from F1 to F6



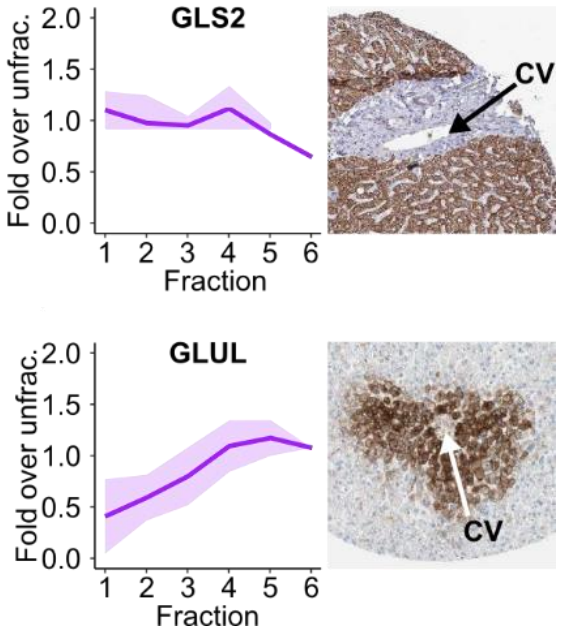

DNA content and multinucleation increased with increasing size

# Proteomic analysis

- Global proteomic analysis of all size fractions




5163 proteins detected




Histological images from the Human Protein Atlas

Zonal marker expression across fractions matched *in vivo* patterns

**Small cells**

| Annotation cluster | | Enrichment score |
|---|---|---|
| S1 | Citric acid cycle | 2.55 |
| S2 | Mitochondrial translation | 2.37 |
| S3 | Oxidative phosphorylation | 2.35 |
| S4 | Immune response | 1.49 |

**Large cells**

| Annotation cluster | | Enrichment score |
|---|---|---|
| L1 | Drug metabolism | 7.94 |
| L2 | Translation | 6.14 |
| L3 | Proteasomal processing | 4.72 |
| L4 | Translation initiation | 4.46 |
| L5 | Retinol metabolism | 4.38 |
| L6 | Peroxisome | 3.85 |
| L7 | mRNA splicing | 3.75 |
| L8 | mRNA export | 3.07 |
| L9 | Bile acid biosynthesis | 2.72 |
| L10 | Early endosome | 2.70 |
| L11 | Glutathione metabolism | 2.56 |
| L12 | Nuclear transport | 2.53 |
| L13 | Xenobiotic metabolism | 2.15 |
| L14 | Nucleotide metabolism | 1.99 |
| L15 | Late endosome | 1.67 |
| L16 | Membrane organization | 1.48 |
| L17 | Oxidative stress response | 1.40 |

Proteins with sequential enrichment across fractions largely represented zonated biological functions

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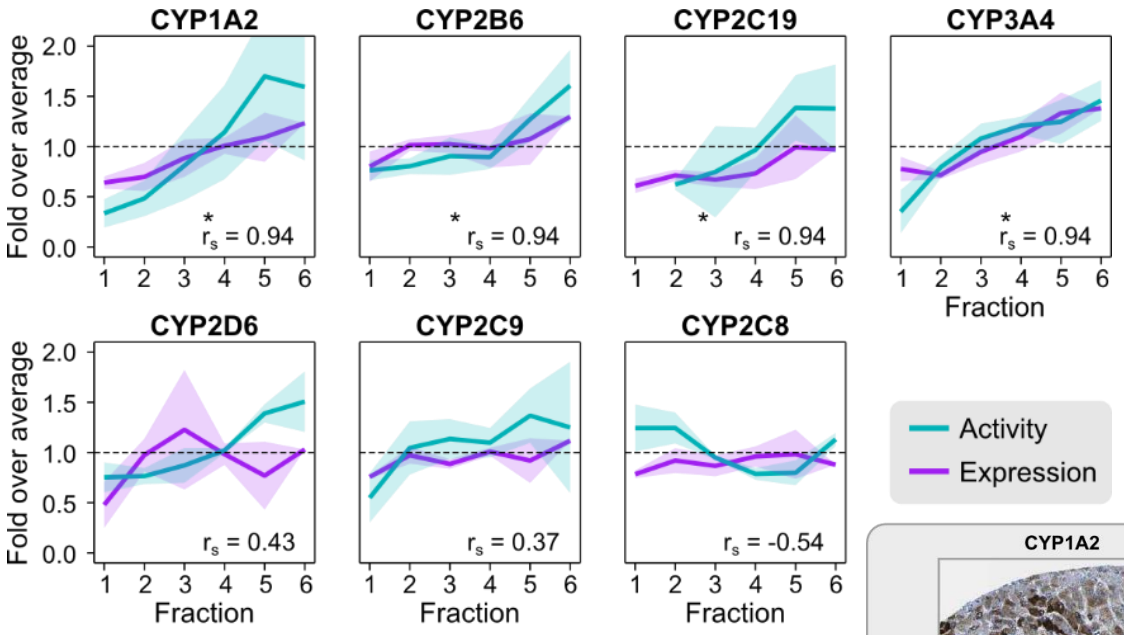
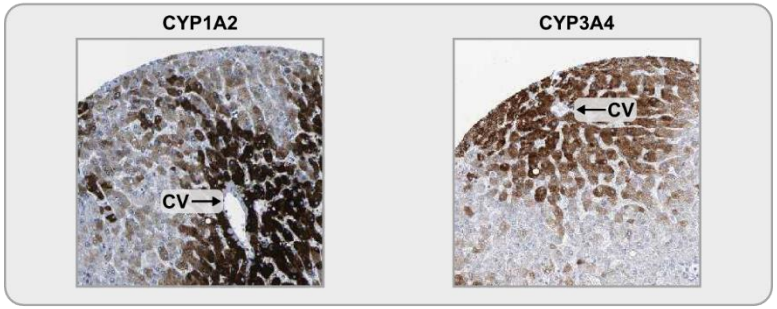
# Correlation between zone-specific CYP expression and metabolic function

- Metabolic activity of important CYP enzymes
  - Expression differences reflected by functional differences?
  - CYP substrate cocktail – metabolite formation



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# Zonal expression of SLC and ABC transporters is generally not zone-specific

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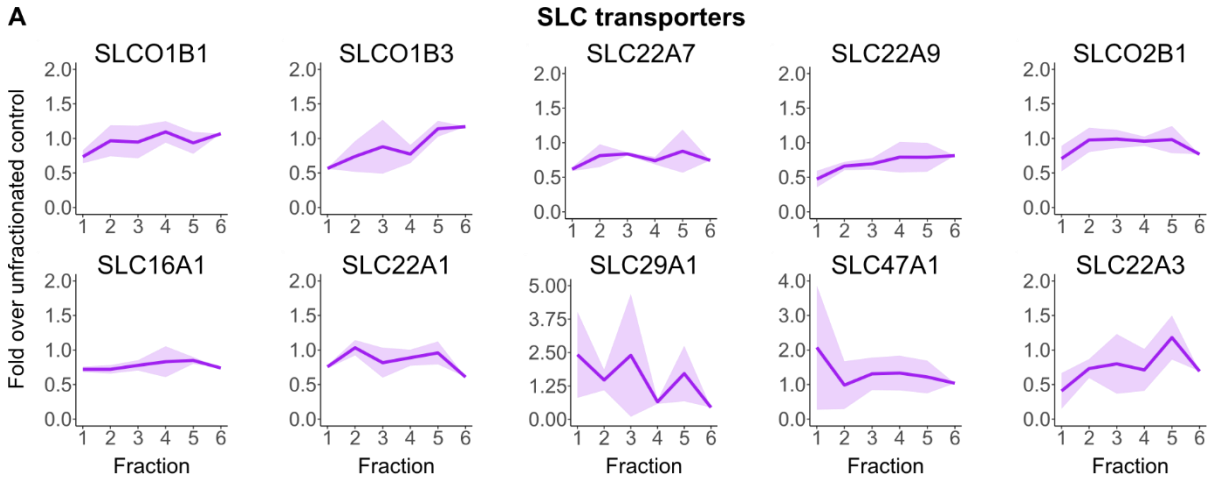

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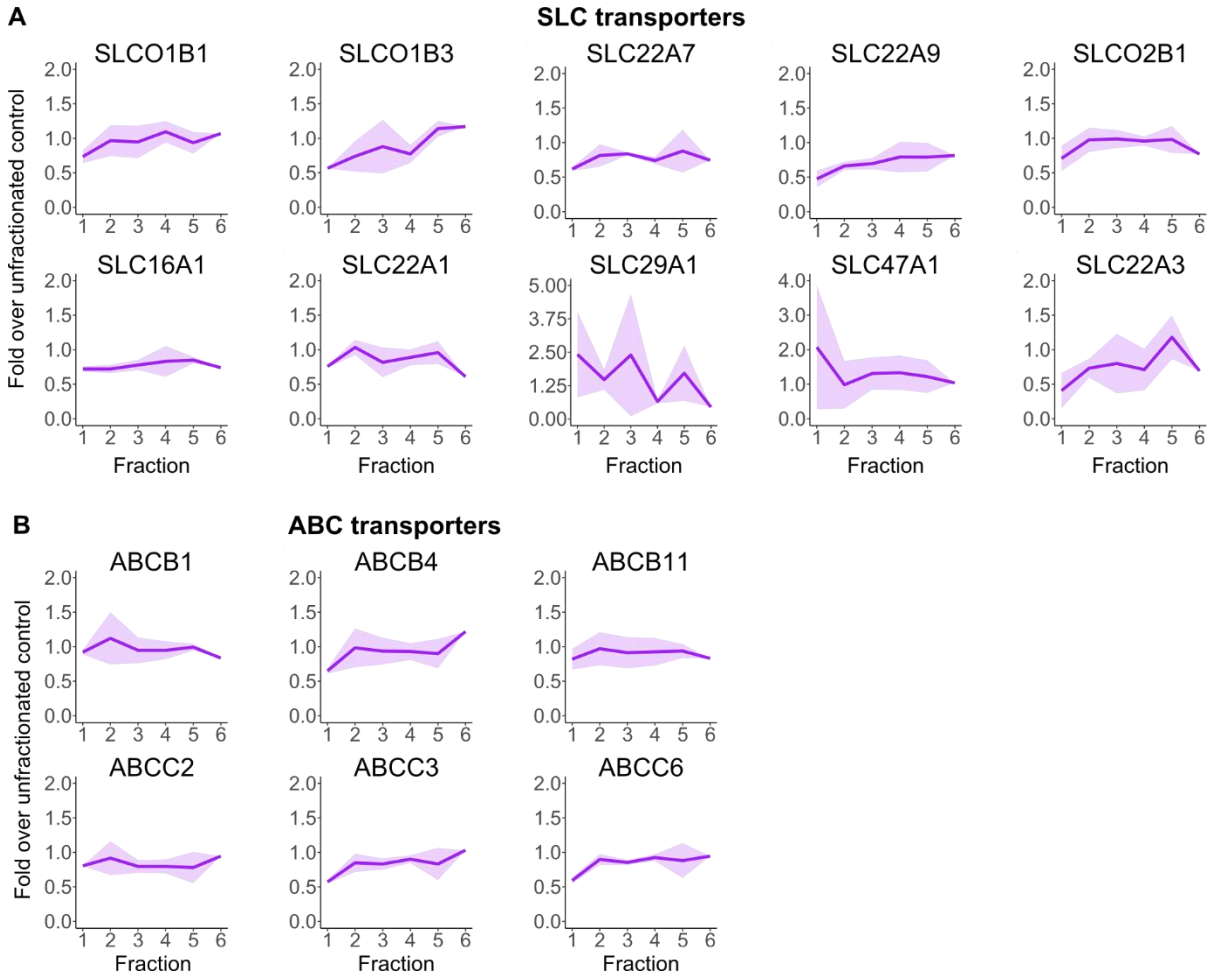

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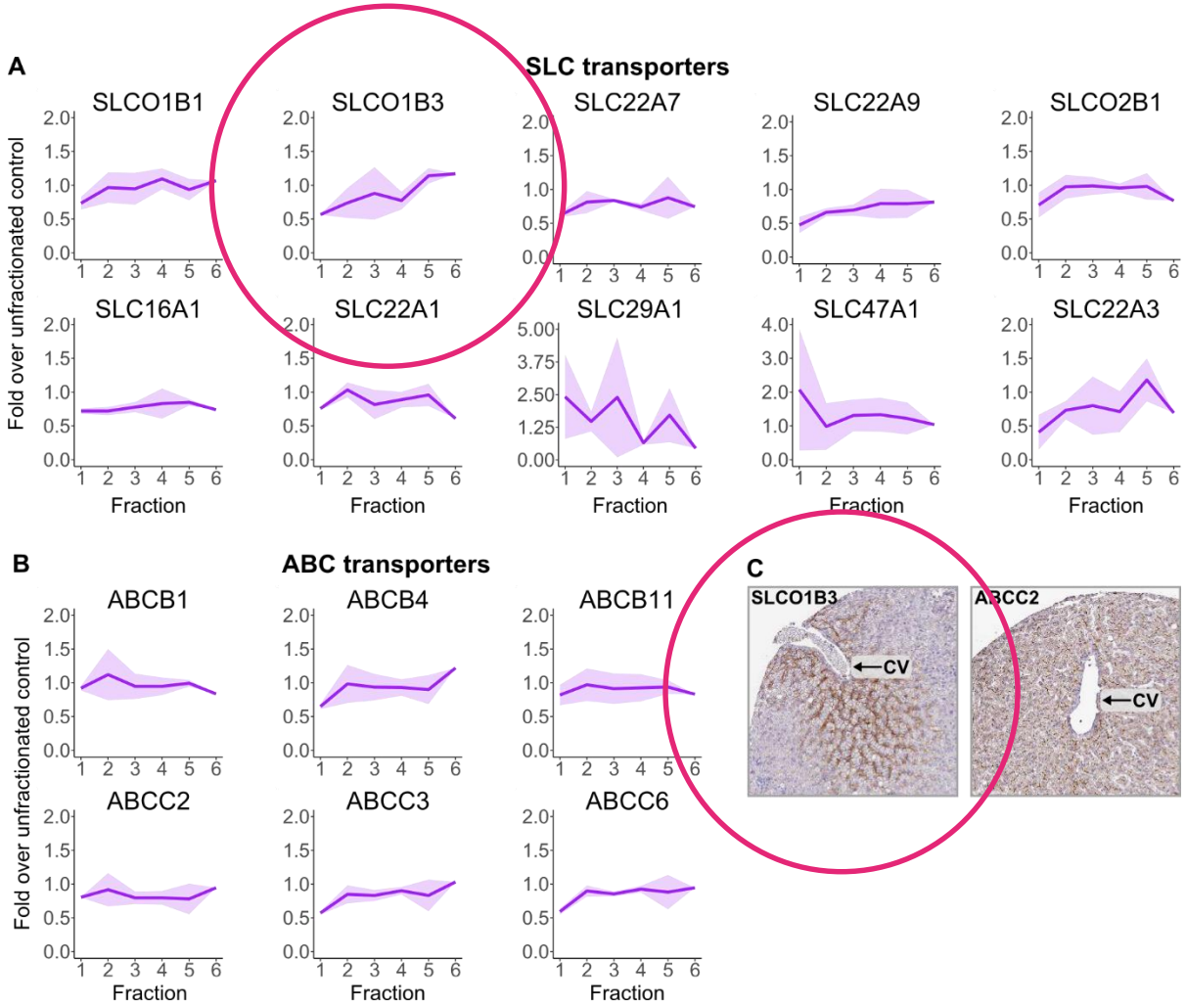
# SLCO1B3 (OATP1B3) is the exception



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# Conclusions

- Human hepatocyte batches show considerable variability in size distributions
- Zonal differences in hepatocyte function are retained after isolation
- Size separation of human hepatocytes can be used to study zone-specific functions in a way that cannot be done with whole-batch experiments
- CYPs and UGTs are preferentially expressed in zone 3 hepatocytes while transporters are more evenly distributed across the zones

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# Proteomic analysis of major cell types in the human liver

Ölander, M., Wiśniewski, J.R., Artursson, P. *In manuscript.*

**Aim:** To analyze the proteomes of human hepatocytes and the major types of liver NPCs, for improved understanding of the functional roles of different cell types.

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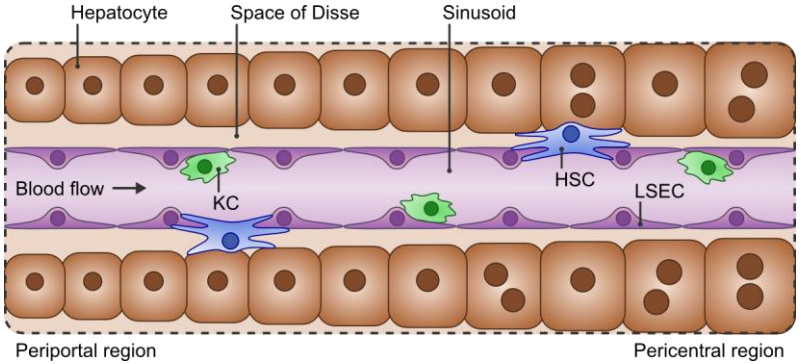
# Background

- The liver does not only contain hepatocytes

- Major types of non-parenchymal cells (NPCs):
  - Liver sinusoidal endothelial cells (LSEC)
  - Kupffer cells (KC)
  - Hepatic stellate cells (HSC)

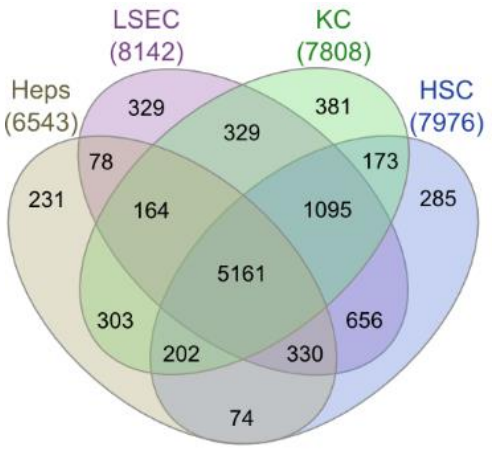



Ölander, M., PhD dissertation, Acta Universitatis Upsaliensis, 2019

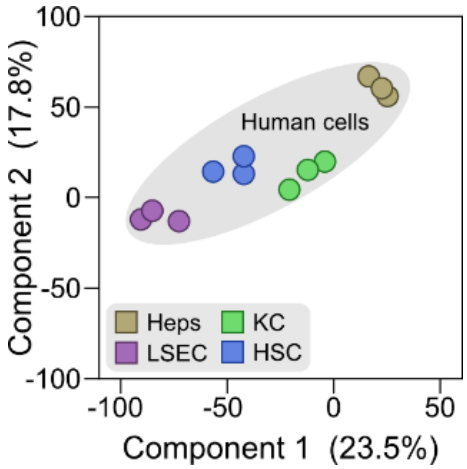
- The proteomes of murine NPCs have been analyzed, but not human
  - Mice are not necessarily representative of humans
  - Human data would be useful

# Liver cell proteomes

- Matched samples of hepatocytes, LSEC, KC, and HSC
  - Obtained from four histologically normal donors
- Global proteomic analysis




9791 proteins detected
(53% common to all cell types)

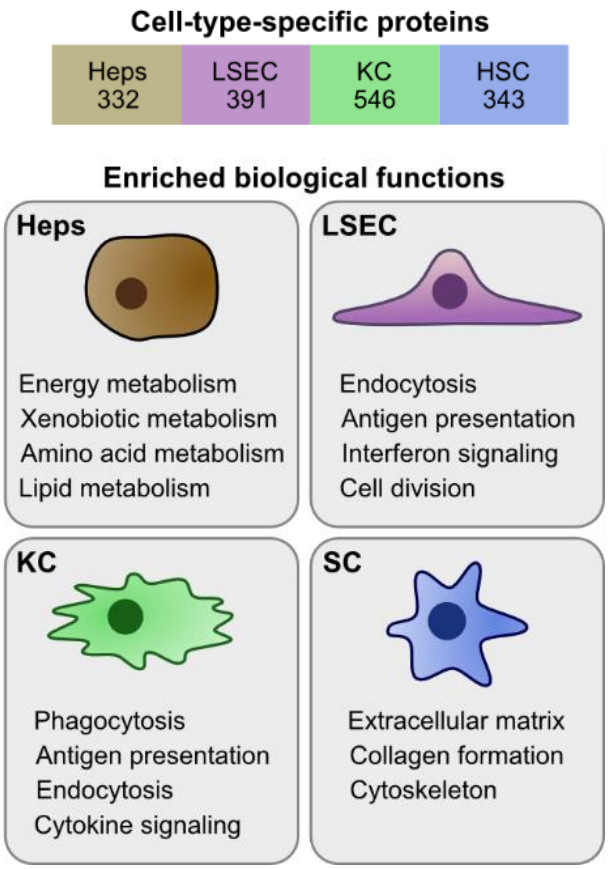



The four cell types had distinctly different proteomes

A comparison with murine data showed clear species differences

# Cell-type-specific proteins

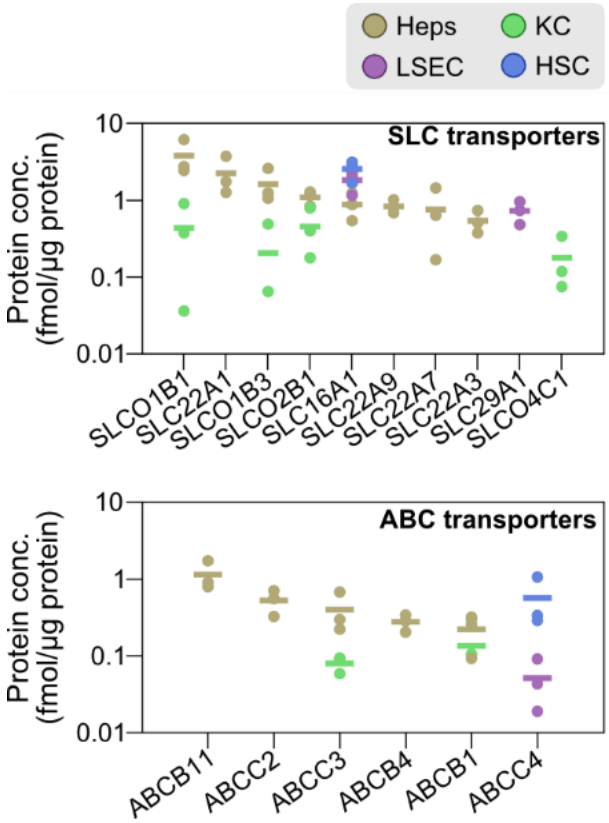
- Cell-type-specific proteins:
  - Uniquely expressed in one cell type, or
  - Over 50-fold higher levels in one cell type compared to the others




Cell-type-specific proteins were enriched for biological processes representative of cell-type-specific functions

# Proteins related to drug disposition

- Analyzed the expression of clinically relevant transport proteins and CYP enzymes in the different cell types




CYPs were mostly expressed in hepatocytes, but to some extent also in KC

Most transporters were predominantly expressed in hepatocytes, with some exceptions

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# Conclusions

- Human hepatocytes, LSEC, KC, and HSC all have unique proteomes
- NPCs may contribute in some aspects of drug disposition
- Our data can be used as a resource to explore various aspects of human liver biology

# Acknowledgements

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