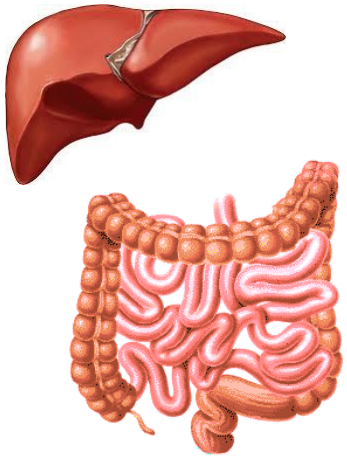
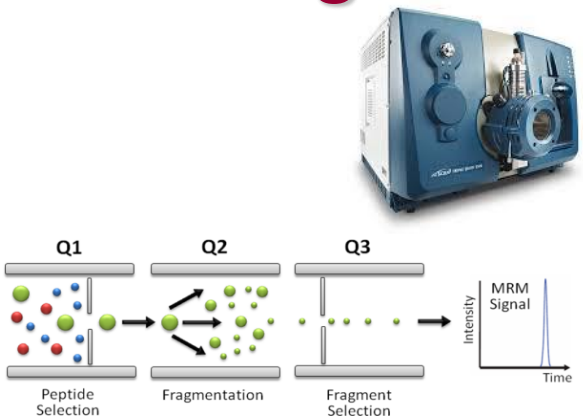
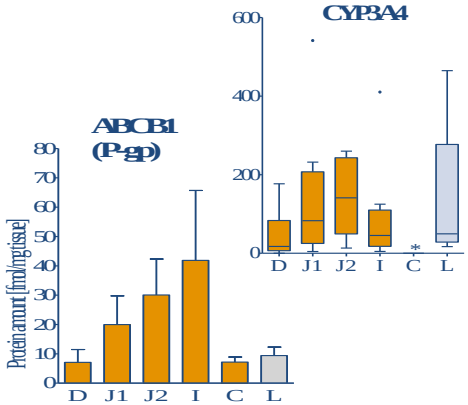
Mass spectrometry-based quantification of transporter proteins and metabolizing enzymes: an update on advantages and challenges



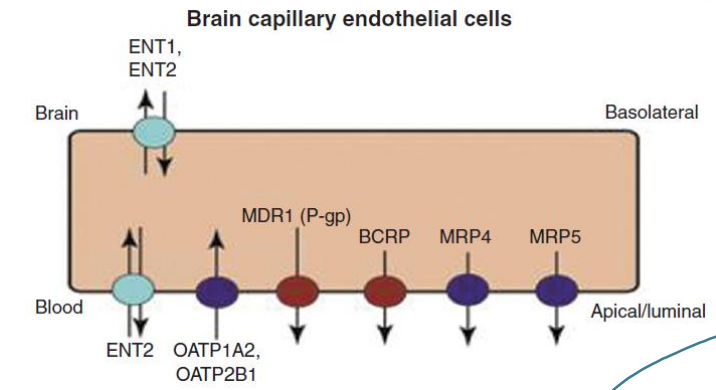


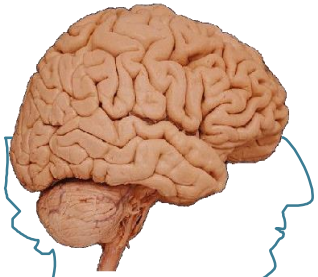


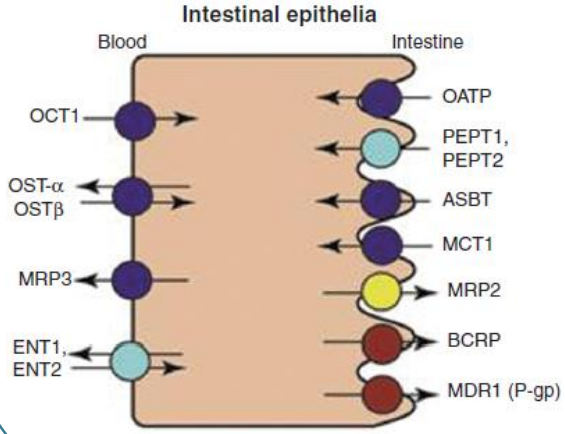
Stefan Oswald, PhD

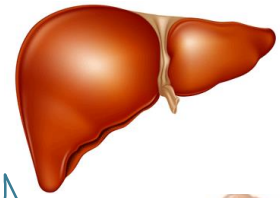
Department of Clinical Pharmacology
Center of Drug Absorption and Transport (C_DAT)
University Medicine of Greifswald, Germany

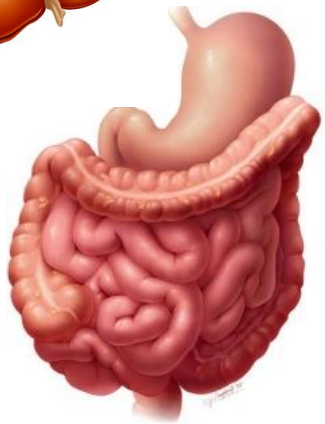
Clinically relevant transporters

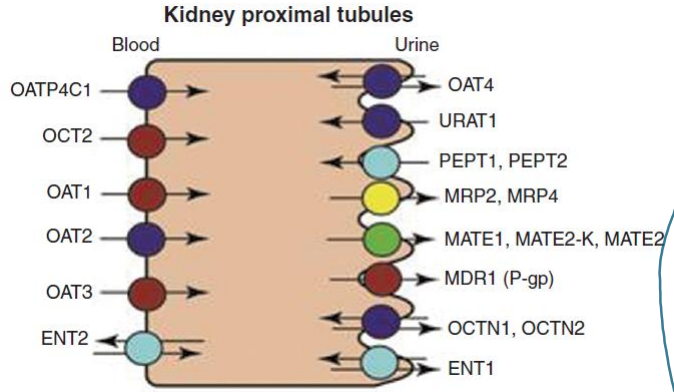


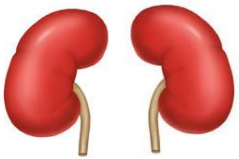


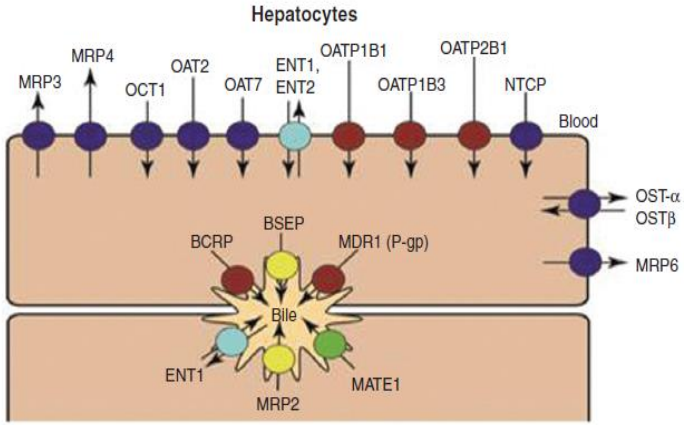








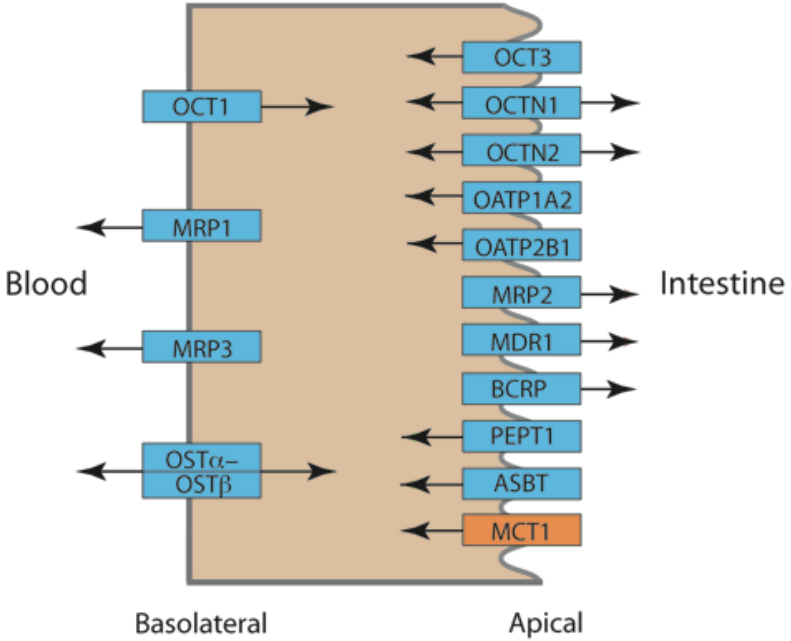




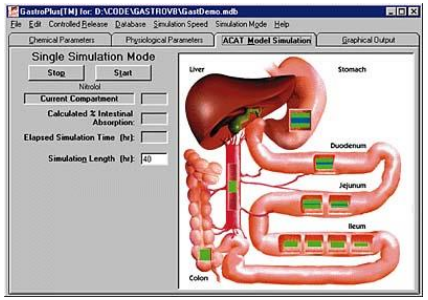
The International Transporter Consortium, Nature Reviews Drug Discovery 2010/ Clin Pharmacol Ther 2013

Why do we need intestinal expression data?

- To know which transporters are expressed in a certain tissue
- To know the absolute abundance of enzymes / transporters
- To estimate / predict the oral absorption of drugs
- To estimate / predict the potential contribution of each protein to drug-drug / drug-food interactions
- To characterize cellular transporter models or animal models

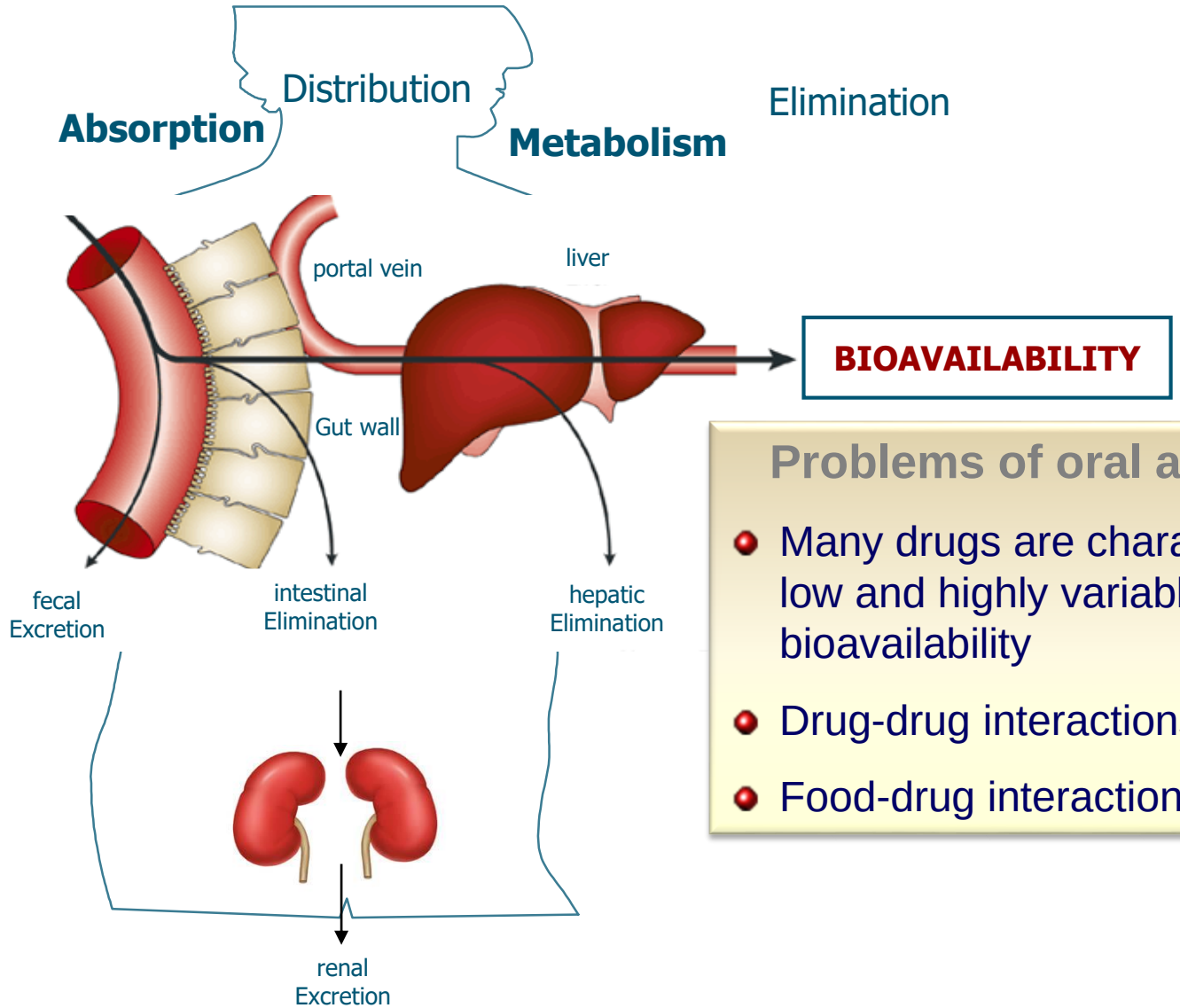


source: UCSF-FDA TransPortal



GastroPlus

First pass-route of drugs



Problems of oral absorption

- Many drugs are characterized by low and highly variable oral bioavailability
- Drug-drug interactions
- Food-drug interactions

Intestinal metabolism and transport

small intestinal expression

- Small number of genes / intestinal samples
- Merging of data from different donors
- Tissue from segments assessable by biopsies (duodenum, terminal ileum, colon)
- Samples from patients
- Mostly mRNA expression data

mal

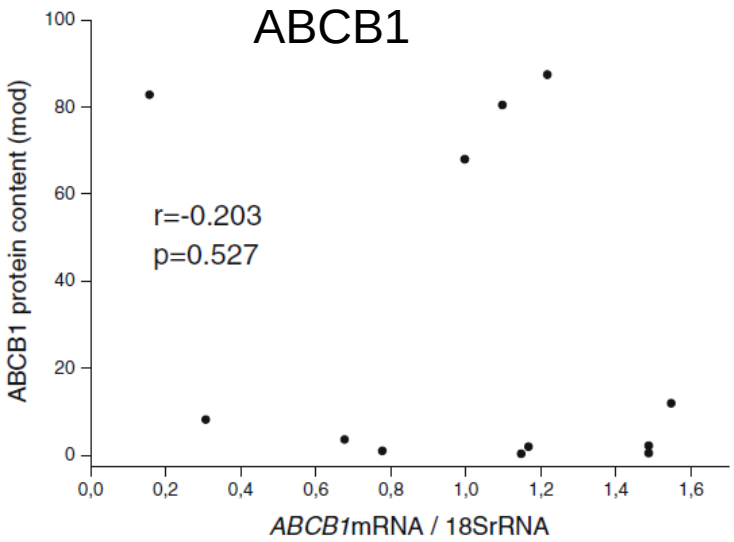
l

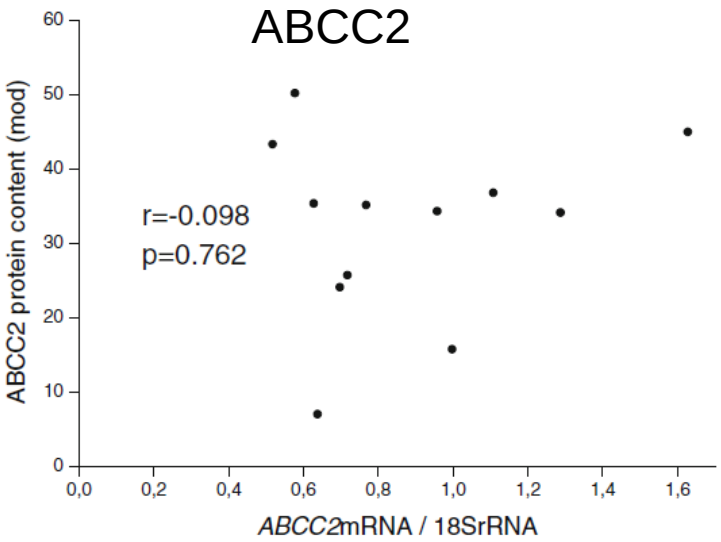
Müller et al. 2017, Update on intestinal transporters (Biol. Chem; 398(2):175-92)

Limitations of mRNA data

mRNA expression is not necessarily correlated to protein expression!

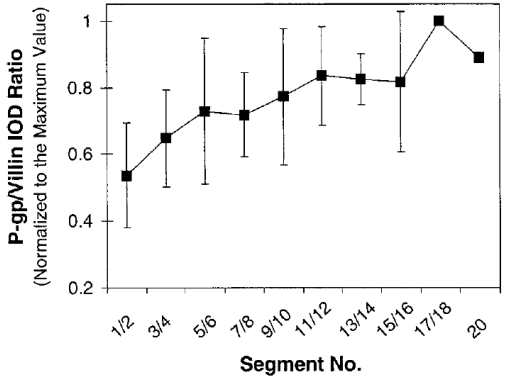
mRNA-Arrays

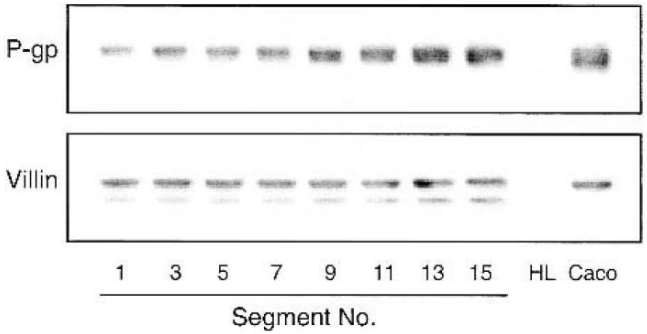




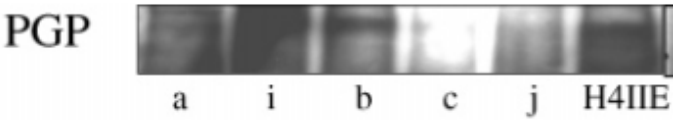
Oswald et al. 2013, AAPS Journal: 15(4):1128-40

All available protein data were generated via immunoblotting



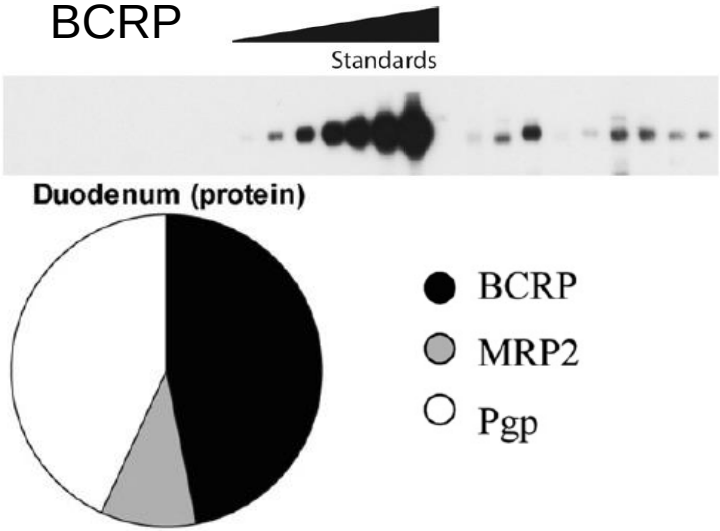


Mouly et al. 2003, Pharm Res



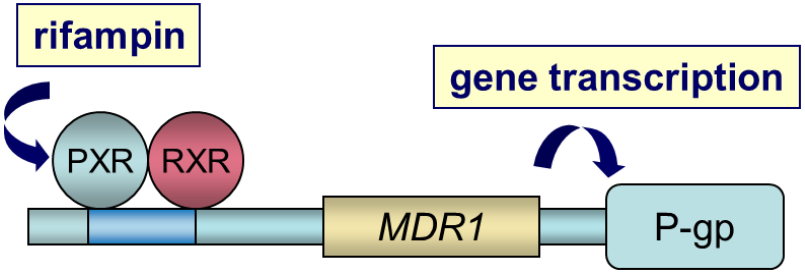
	protein		
	CYP3A4	Pgp	MRP2
jejunum	12.00	8.00	10.33
ileum	5.38	5.25	5.63
colon	5.58	7.67	4.90
P-value	0.0397	0.5485	0.0925

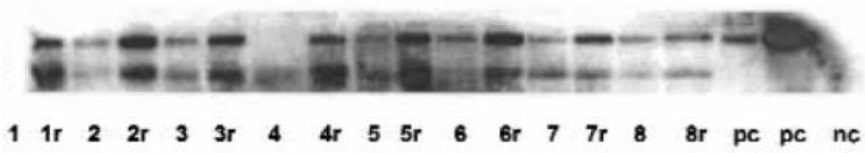
Berggreen et al. 2007, Mol Pharm



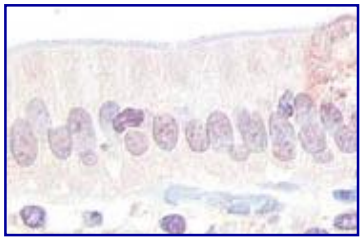
Tucker et al. 2012, Biochem Pharmacol

Limitations of immunoblotting

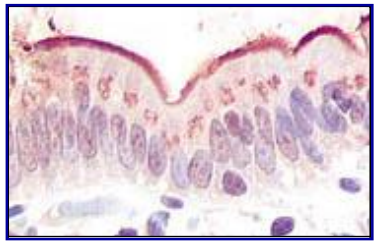




Impact of rifampin (600 mg, 6-8 d) on duodenal mRNA and protein expression of P-gp / *MDR1*



before rifampin



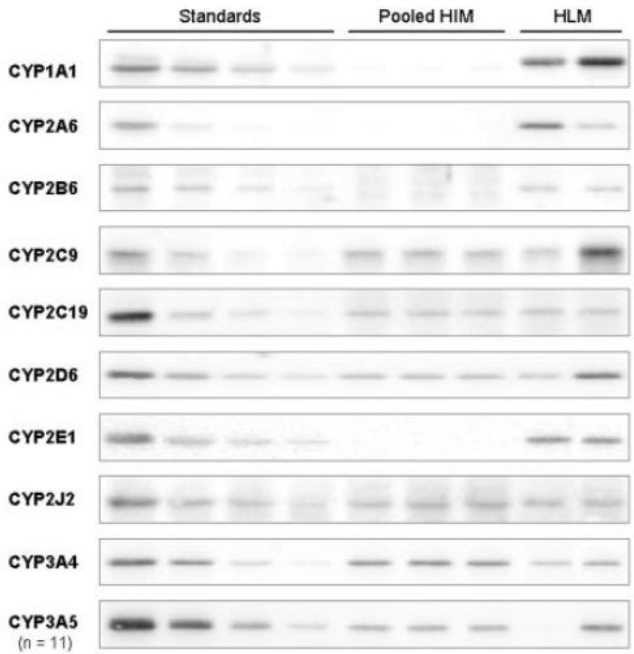
after rifampin

protein	mRNA	reference
8.3-fold	3-fold	Giessmann et al. 2004, CPT
1.5-fold	4.3-fold	Oswald et al. 2006, CPT
1.4- / 3.5*-fold	-	Greiner et al. 1999, JCI
1.1- / 4.2*-fold	2.4-fold	Westphal et al. 2000, CPT

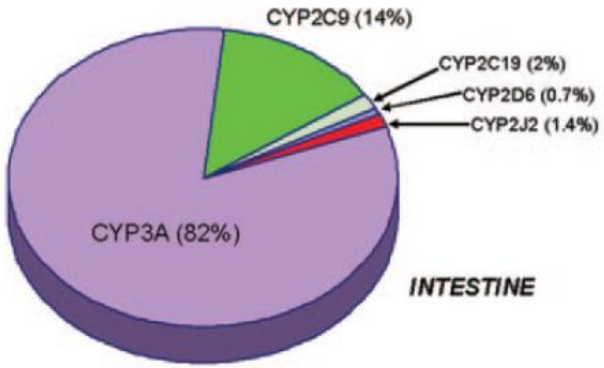
*Western blotting

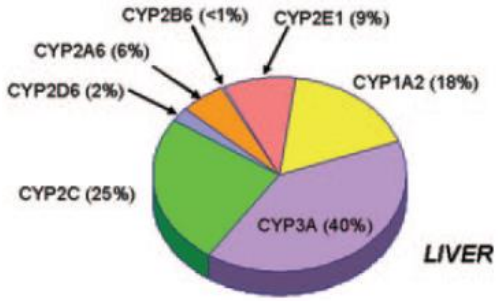
Question: Are the observed differences real or due tue methological issues?

Available protein data on intestinal metabolizing enzymes

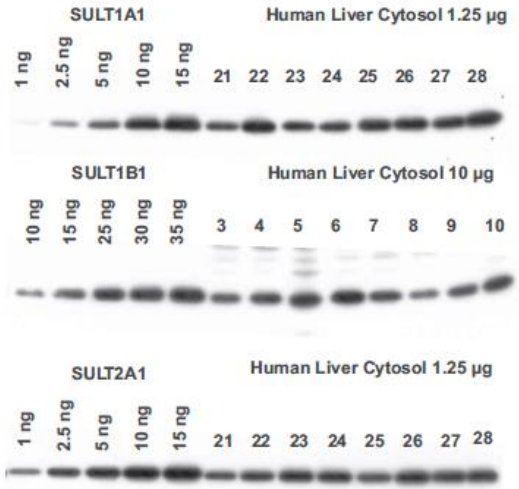


Paine et al. 2006





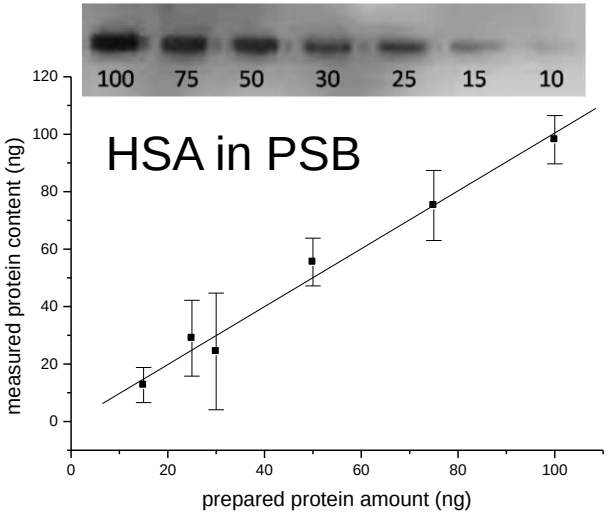
Shimada et al. 1994

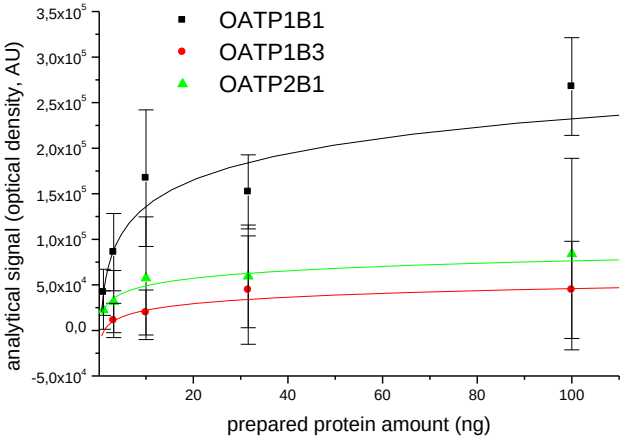


Riches et al. 2009

Limitations of immunoblotting

1. Antibody required (availability?, expensive)
2. Functionality uncertain (specificity?)
3. Only few protein simultaneously detectable
4. Poor reproducibility
5. Semiquantitative protein determination
6. Data about analytical quality often not available (e.g. limit of quantification, accuracy, precision)





protein	OATP1B1	OATP1B3	OATP2B1
range (µg)	1 - 100	3,16 - 100	1 - 100
outliner (%)	16,6	20,8	13,3
CV (%)	9,6 - 94,6	38,5 - 70,7	33,6 - 87,8
rel. error (%)	-33,5 - 101	-24 - 36	-13,1 - 108

Oswald et al. 2013, AAPS Journal: 15(4):1128-40

Promises of LC-MS/MS based protein quantification

- High specificity
- High accuracy and precision
- High sensitivity and wide analytical range
- High throughput and automatable technique
- Analytical quality parameters assessable (→ validation)

Mass spectrometry based protein quantification: applications

- Dass C et al. 1989: beta endorphine
- Barr JR et al. 1996: apolipoprotein A-I
- Gerber SA et al. 2003: yeast proteins
- Barnidge DR et al. 2003: rhodopsine
- Barnidge DR et al. 2004: PSA
- Anderson L et al. 2006: 53 plasma proteins
- Since 2008: transporters, enzymes

(established groups: Terasaki lab (Japan), Smith lab (USA), Unadkat / Prasad lab (USA), Oswald lab (Germany), Artursson lab (Sweden))

Mass spectrometry based protein quantification: applications

Simultaneous Absolute Quantification of 11 Cytochrome P450 Isoforms in Human Liver Microsomes by Liquid Chromatography Tandem Mass Spectrometry with *In Silico* Target Peptide Selection

HIROTAKA KAWAKAMI,[1] SUMIO OHTSUKI,[1] JUNICHI KAMIIE,[2] TAKASHI SUZUKI,[3] TAKAAKI ABE,[4] TETSUYA TERASAKI[1]

[1]Division of Membrane Transport and Drug Targeting, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan

Proceomics 2011, *11*, 33–41 DOI 10.1002/pmic.201000456

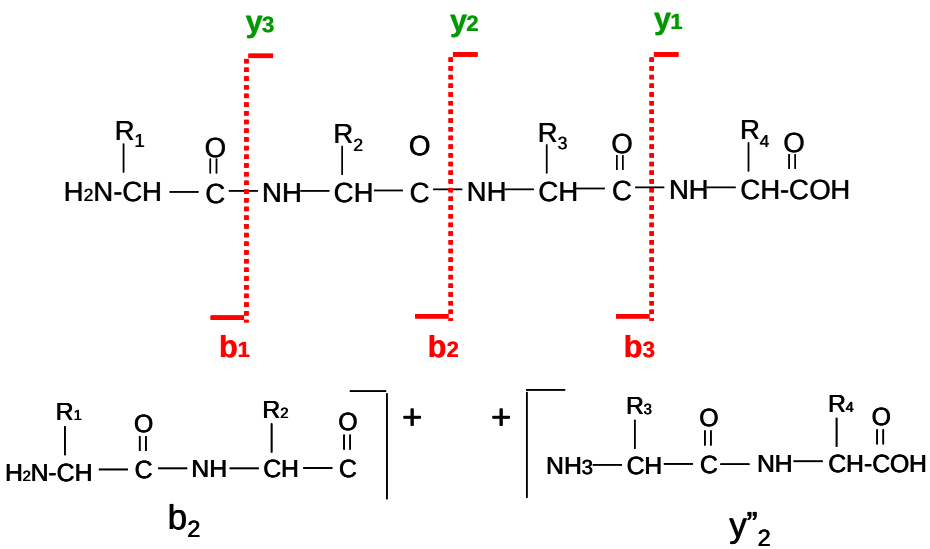
RESEARCH ARTICLE

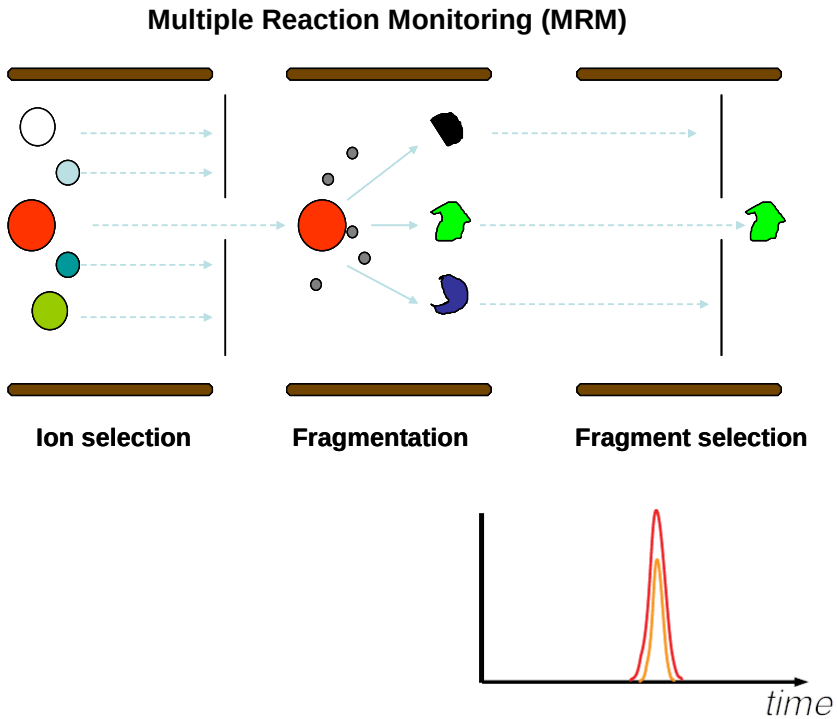
Quantitative protein determination for CYP induction via LC-MS/MS

Brian L. Williamson[1], Subhasish Purkayastha[1], Christie L. Hunter[2], Lydia Nuwaysir[2], James Hill[3], LaHoma Easterwood[3] and Jeanette Hill[3]

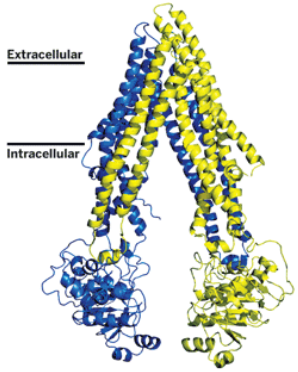
Mass spectrometry based protein quantification

- Protein is enzymatically digested (trypsin) → proteospecific peptides
- Peptides are separated via a HPLC and detected with mass spectrometry
- Protein identification / quantification by MRM experiment

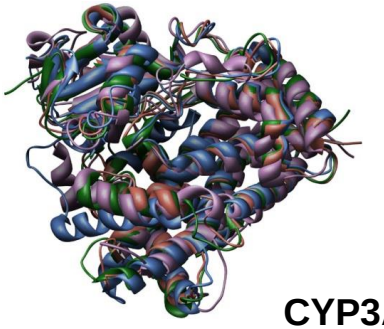




Mass spectrometry based protein quantification



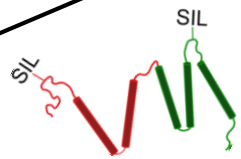
ABCB1 (P-gp)



CYP3A4

Label-free absolute quantification

Targeted proteomics



QconCAT proteomics

Global proteomics
(*shotgun* approach)

- Absolute quantification of pre-defined proteins using proteospecific peptides and stable-labelled internal standard peptides (~1-50 proteins)

- Absolute / relative quantification of proteins without internal standards using protein databases (thousands of proteins)

General assumption: amount of peptides equal the amount of proteins (complete digestion)

Targeted or global proteomics?

- targeted analysis vs. non-targeted screening

What is in the box?

targeted analysis:

Grape?

Apple?

Cherry?

Banana?

Targeted or global proteomics?

- targeted analysis vs. non-targeted screening

What is in the box?

targeted analysis:

Grape? no

Apple? no

Cherry? no

Banana? yes

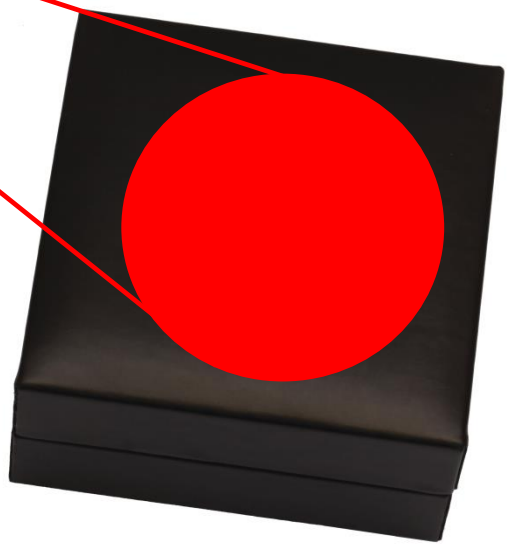
Targeted or global proteomics?

- targeted analysis vs. non-targeted screening

What is in the box?

Non-targeted screening:

M	I	N	I	O	N	S
H	B	E	L	K	R	T
D	T	A	B	D	E	K
O	G	U	N	C	P	J
G	F	I	M	A	W	V
C	V	F	W	L	N	A
D	H	Z	A	T	X	A



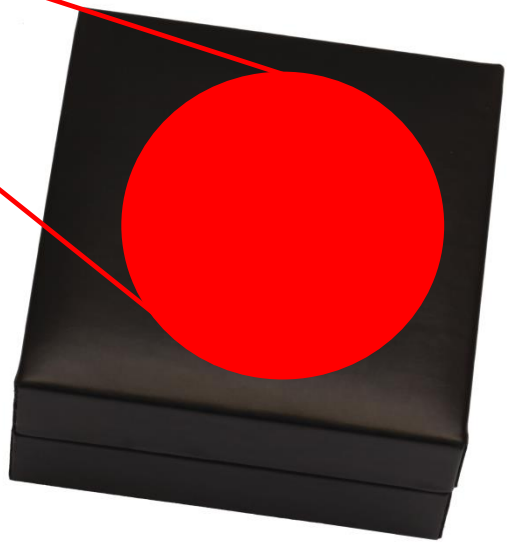
Targeted or global proteomics?

- targeted analysis vs. non-targeted screening

What is in the box?

Non-targeted screening:

M	I	N	I	O	N	S
H	**B**	E	L	K	R	T
D	T	**A**	B	D	E	K
O	G	U	**N**	C	P	J
G	F	I	M	**A**	W	V
C	V	F	W	L	**N**	A
D	H	Z	A	T	X	**A**



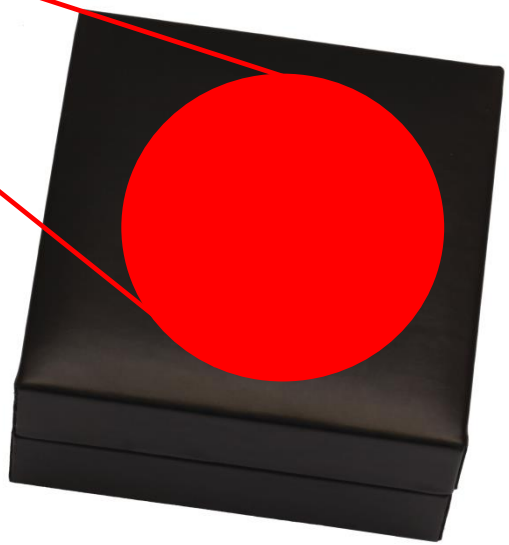
Targeted or global proteomics?

- targeted analysis vs. non-targeted screening

What is in the box?

Non-targeted screening:

M	**I**	**N**	**I**	**O**	**N**	**S**
H	**B**	E	L	K	R	T
D	T	**A**	B	D	E	K
O	G	U	**N**	C	P	J
G	F	I	M	**A**	W	V
C	V	F	W	L	**N**	A
D	H	Z	A	T	X	**A**



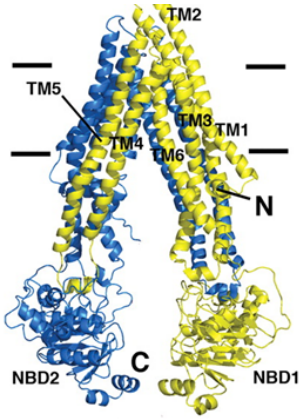
Targeted or global proteomics?

targeted		Non-targeted (global)	
Only expected peptides detected	✗	Vast amount of present peptides detected	✓
No modifications detected	✗	modifications detectable	✓
Absolute quantification possible	✓	Relative quantification	✗
Validation possible (accuracy, precision, stability)	✓	Validation not possible	✗
High sensitivity & accuracy	✓	Low abundant proteins may not be detected	✗
Fair Throughput (run time ~1 h)	✓	Low Throughput (run time ~3 h)	✗

Targeted or global proteomics?

targeted		Non-targeted (global)	
Only expected peptides detected	✗	Vast amount of present peptides detected	✓
No modifications detected	✗	modifications detectable	✓
Absolute quantification possible	✓	Relative quantification	✗
Validation possible (accuracy, precision, stability)	✓	Validation not possible	✗
High sensitivity & accuracy	✓	Low abundant proteins may not be detected	✗
Fair Throughput (run time ~1 h)	✓	Low Throughput (run time ~3 h)	✗

LC-MS/MS-based protein quantification



P-gp (ABCB1)

diges

Criteria for selectior
Length between 7-25
No posttranslational r
No single nucleotide p
No repeated sequenc
No methionine or cyst
Check for protein spe

Available Assays	
Transporter	**Enzymes**
ABCB1	CYP1A2
ABCC1	CYP2B6
ABCC2	CYP2C8
ABCC3	CYP2C9
ABCC4	CYP2C19
ABCG2	CYP2D6
ASBT	CYP2E1
MATE1	CYP2J2
Na/K-ATPase	CYP3A4
NTCP	CYP3A7
OAT1-3	CYP4A11
OATP1A2	CYP4F2
OATP1B1 / 1B3	UGT1A1
OATP2B1	UGT1A3
OCT1-3	UGT2B7
OCTN2	UGT2B15
PEPT1-2	UGT2B17

Q1 mass	Q3.1	Q3.2	Q3.3	Q3.4
947,9	889,4	1176,6	988,5	588,3
831,4	1149,6	1078,5	1262,7	644,4
823,4	888,5	1088,6	1001,6	791,4
635,4	971,6	900,4	771,5	642,4
618,8	621,3	894,4	807,4	993,5
601,3	816,4	653,3	887,4	1001,5
582,3	749,4	979,5	678,4	565,3

modified according to Oswald et al. 2013, AAPS Journal

Critical aspects of mass spectrometry based proteomics

Protein

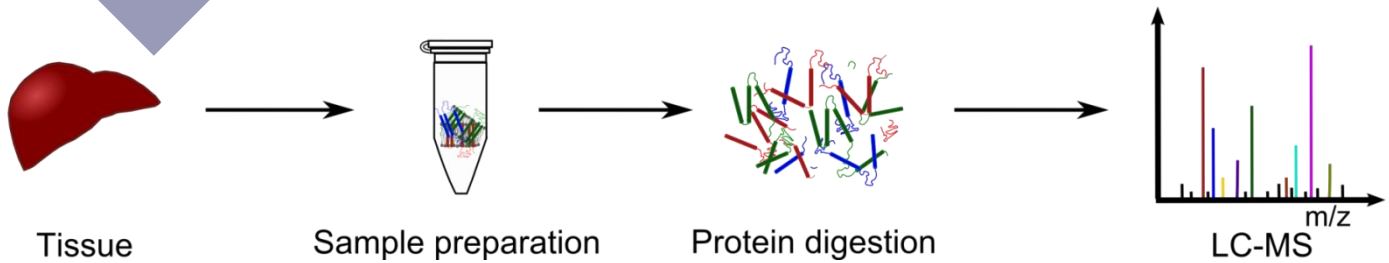
- Biological / Clinical Question
- Protein sequence data (consider SNPs, PTMs)
- Sample preparation (fractionation, whole lysate)
- Protein determination / digestion

Peptide

- Selection of appropriate peptides (in silico / wet-lab)
- Number of peptides / protein

LC-MS/MS-Analysis

- Global or targeted proteomic analysis
- Selection of mass transitions (non- / scheduled MRM)
- Data analysis (peptides, MRMs, normalization)
- Validation of the method



Critical aspects of mass spectrometry based proteomics

- Method Development -

Protein

- Biological / Clinical Question
- **Protein sequence data (consider SNPs, PTMs)**
- Sample preparation (fractionation, whole lysate)
- Protein determination / digestion

Peptide

- **Selection of appropriate peptides (in silico / wet-lab)**
- Number of peptides / protein

LC-MS/MS-Analysis

- Global or targeted proteomic analysis
- **Selection of mass transitions (non- / scheduled MRM)**
- Data analysis (peptides, MRMs, normalization)
- **Validation of the method**

Critical aspects of mass spectrometry based proteomics

- Method Development -

Protein

- Biological / Clinical Question
- **Protein sequence data (consider SNPs, PTMs)**
- Sample preparation (fractionation, whole lysate)
- Protein determination / digestion

Peptide

- **Selection of appropriate peptides (in silico / wet-lab)**
- Number of peptides / protein

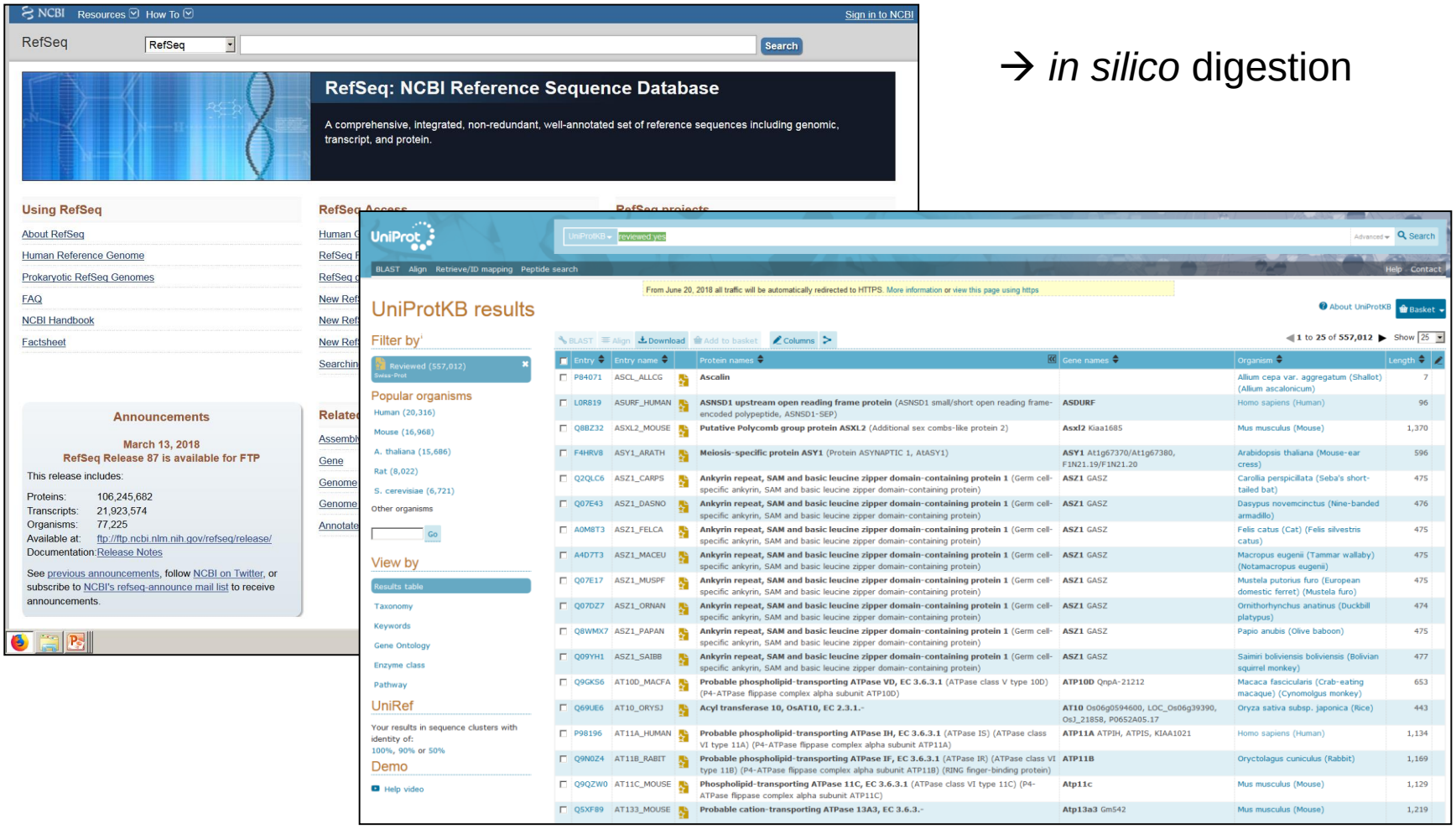
LC-MS/MS-Analysis

- Global or targeted proteomic analysis
- **Selection of mass transitions (non- / scheduled MRM)**
- Data analysis (peptides, MRMs, normalization)
- **Validation of the method**

Critical aspects of mass spectrometry based proteomics

- Method Development -

Protein sequence databases



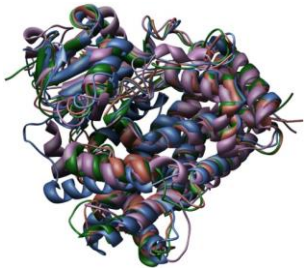
→ *in silico* digestion

Critical aspects of mass spectrometry based proteomics

- Method Development -

CYP3A4 (503 AA)

MALIPDLAMETWLLLAVSLVLLYLYG
THSHGLFKKLGIPGPTPLPFLGNILS
YHKGFCMFDMECHKKYGKVWGFY
DGQQPVLAITDPDMIKTVLVKECYSV
FTNRRPFGPVGFMKSAISIAEDEEW
KRLRSLLSPTFTSGKLKEMVPIIAQY
GDVLVRNLRREAETGKPVTLKDVFG
AYSMDVITSTSFGVNIDSLNNPQDPF
VENTKKLLRFDFLDPFFLSITVFPFLI
PILEVLNICVFPREVTNFLRKSVKRM
KESRLEDTQKHRVDFLQLMIDSQNS
KETESHKALSDLELVAQSIIFIFAGYE
TTSSVLSFIMYELATHPDVQQKLQE
EIDAVLPNKAPPTYDTVLQMEYLDM
VVNETLRLFPIAMRLERVCKKDVEIN
GMFIPKGVVVMIPSYALHRDPKYWT
EPEKFLPERFSKKNKDNIDPYIYTPF
GSGPRNCIGMRFALMNMKLALIRVL
QNFSFKPCKETQIPLKLSLGGLLQPE
KPVVLKVESRDGTVSGA



Selection criteria for peptides
Mass range of MS (~ 500-3500), ~7-25 AA
Not localized in transmembrane region (transporter)
No genetic polymorphisms (> 1%)
No posttranslational modifications
No repeated sequence of arginine and lysine
No cysteine, methionine or tryptophan
Protein (species) specific (BLAST search)

Critical aspects of mass spectrometry based proteomics

- Method Development -

CYP3A4 (503 AA)

MALIPDLAMETWLLLAVSLVLLYLYG
THSHGLFKKLGIPGPTPLPFLGNILS
YHKGFCMFDMECHKKYGKVWGFY
DGQQPVLAITDPDMIKTVLVKECYSV
FTNRRPFGPVGFMKSAISIAEDEEW
KRLRSLLSPTFTSGKLKEMVPIIAQY
GDVLVRNLRREAETGKPVTLKDVFG
AYSMDVITSTSFGVNIDSLNNPQDPF
VENTKKLLRFDFLDPFFLSITVFPFLI
PILEVLNICVFPREVTNFLRKSVKRM
KESRLEDTQKHRVDFLQLMIDSQNS
KETESHKALSDLELVAQSIIFIFAGYE
TTSSVLSFIMYELATHPDVQQKLQE
EIDAVLPNKAPPTYDTVLQMEYLDM
VVNETLRLFPIAMRLERVCKKDVEIN
GMFIPKGVVVMIPSYALHRDPKYWT
EPEKFLPERFSKKNKDNIDPYIYTPF
GSGPRNCIGMRFALMNMKLALIRVL
QNFSFKPCKETQIPLKLSLGGLLQPE
KPVVLKVESRDGTVSGA

mass	position	variants	peptide sequence
3821.7850	174-208	174:D->H 185:T->S 189:F->S	DVFGAYSMDVITSTSFGVNIDSLNNPQDPFVENTK
3698.0429	2-34	15:L->P	ALIPDLAMETWLLLAVSLVLLYLYGTHSHGLFK
3671.9989	213-243	218:P->R 222:S->P	FDFLDPFFLSITVFPFLIPILEVLNICVFPR
2698.3102	343-365	349:T->N 363:T->M	APPTYDTVLQMEYLDMVVNETLR
2393.1845	71-91		VWGFYDGQQPVLAITDPDMIK
2134.2059	36-55		LGIPGPTPLPFLGNILSYHK (Phosphorylated?, 74%)
1811.8598	425-440	431:I->T	DNIDPYIYTPFGSGPR (Phosphorylated?, ~80%)
1702.9196	144-158		EMVPIIAQYGDVLVR
1691.0465	477-492		LSLGGLLQPEKPVVLK
1637.8203	269-282		VDFLQLMIDSQNSK
1441.7984	391-403		GVVVMIPSYALHR
1377.6532	116-127	118:I->V	SAISIAEDEEWK
1368.7369	331-342		LQEEIDAVLPNK
1347.4989	56-66	56:G->D	GFCMFDMECHK
1310.6925	459-469	467:P->S	VLQNFSFKPCK
1262.6449	380-390		DVEINGMFIPK
1172.6521	163-173	170:V->I	EAETGKPVTLK
1137.6150	131-141		SLLSPTFTSGK
1135.6081	106-115		RPFGPVGFMK
1118.4935	97-105		ECYSVFTNR
952.4410	407-413		YWTEPEK
878.4730	244-250		EVTNFLR
854.4263	447-453		FALMNMK
847.4858	366-372		LFPIAMR
828.4825	470-476		ETQIPLK
733.3726	261-266		LEDTQK
730.3366	283-288		ETESHK
693.3171	441-446	445:M->T	NCIGMR
606.2729	497-503		DGTVSGA

Critical aspects of mass spectrometry based proteomics

- Method Development -

OCT1 (554 AA)

MPTVDDILEQVGESGWFQKQAFLIL
CLLSAAFAPICVGIVFLGFTPDHHCQ
SPGVAELSQRCGWSPAEELNYTVP
GLGPAGEAFLGQCRRYEVDWNQSA
LSCVDPLASLATNRSHLPLGPCQDG
WVYDTPGSSIVTEFNLVCADSWKLD
LFQSCLNAGFLFGSLGVGYFADRFG
RKLCLLGTVLVNAVSGVLMAFSPNY
MSMLLFRLLQGLVSKGNWMAGYTLI
TEFVGSGSRRTVAIMYQMAFTVGLV
ALTGLAYALPHWRWLQLAVSLPTFLF
LLYYWCVPESPRWLLSQKRNTEAIK
IMDHIAQKNGKLPPADLKMLSLEEDV
TEKLSPSFADLFRTPRLRKRTFILMY
LWFTDSVLYQGLILHMGATSGNLYL
DFLYSALVEIPGAFIALITIDRVGRIYP
MAMSNLLAGAACLVMIFISPDLHWL
NIIIMCVGRMGITIAIQMICLVNAELYP
TFVRNLGVMVCSSLCDIGGIITPFIVF
RLREVWQALPLILFAVLGLLAAGVTL
LLPETKGVALPETMKDAENLGRKAK
PKENTIYLKVQTSEPSGT

mass	position	modifications	variants	peptide sequence
3721.7301	114-147			SHLPLGPCQDGWVYDTPGSSIVTEFNLVCADSWK
3259.7113	177-206		189:S->L	LCLLGTVLVNAVSGVLMAFSPNYMSMLLFR
3093.6416	235-262			TVAIMYQMAFTVGLVALTGLAYALPHWR
3088.8423	489-517			EVWQALPLILFAVLGLLAAGVTLLLPETK
3041.5997	263-287		283:P->L 287:R->G	WLQLAVSLPTFLFLLYYWCVPESPR
2922.3549	62-89		85:L->F 88:C->R	CGWSPAEELNYTVPGLGPAGEAFLGQCR
2710.3333	148-172		160:L->F	LDLFQSCLNAGFLFGSLGVGYFADR
2596.3699	440-462		440:M->I 461:V->I	MGITIAIQMICLVNAELYPTFVR
2554.3230	463-486		465:G->R	NLGVMVCSSLCDIGGIITPFIVFR
2552.2085	91-113			YEVDWNQSALSCVDPLASLATNR
2179.0375	1-19		14:S->F	MPTVDDILEQVGESGWFQK
2045.9749	215-233		220:G->V	GNWMAGYTLITEFVGSGSR
1293.6242	319-329			MLSLEEDVTEK
1152.6047	330-339	PHOS: 333		LSPSFADLFR
955.5029	301-308			IMDHIAQK
945.5073	518-526			GVALPETMK
905.4210	546-554			VQTSEPSGT
880.4774	539-545	PHOS: 541		ENTIYLK
857.5454	207-214			LLQGLVSK
774.4508	288-293			WLLSQK
774.3740	527-533			DAENLGR
753.4505	312-318			LPPADLK
675.3672	295-300			NTEAIK

Critical aspects of mass spectrometry based proteomics

- Method Development -

Protein

- Biological / Clinical Question
- **Protein sequence data (consider SNPs, PTMs)**
- Sample preparation (fractionation, whole lysate)
- Protein determination / digestion

Peptide

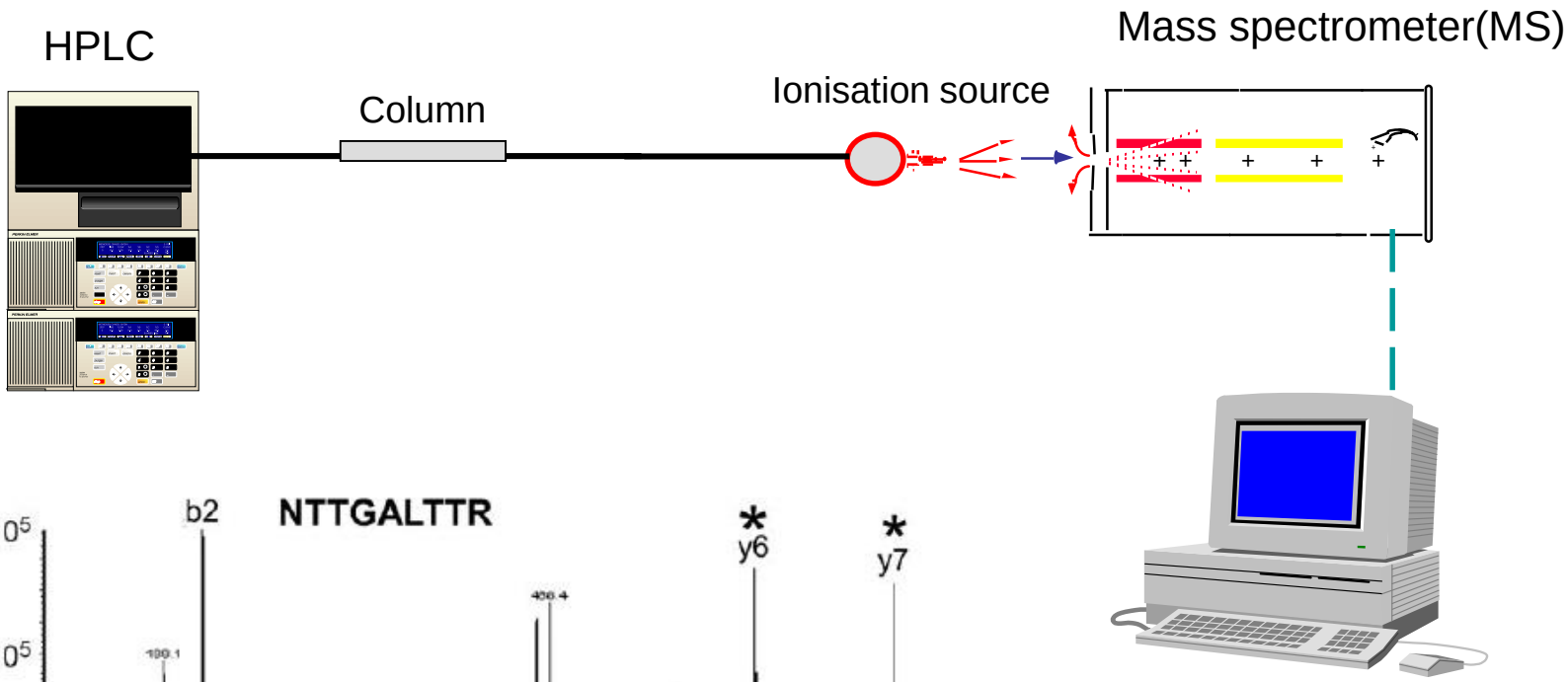
- **Selection of appropriate peptides (in silico / wet-lab)**
- Number of peptides / protein

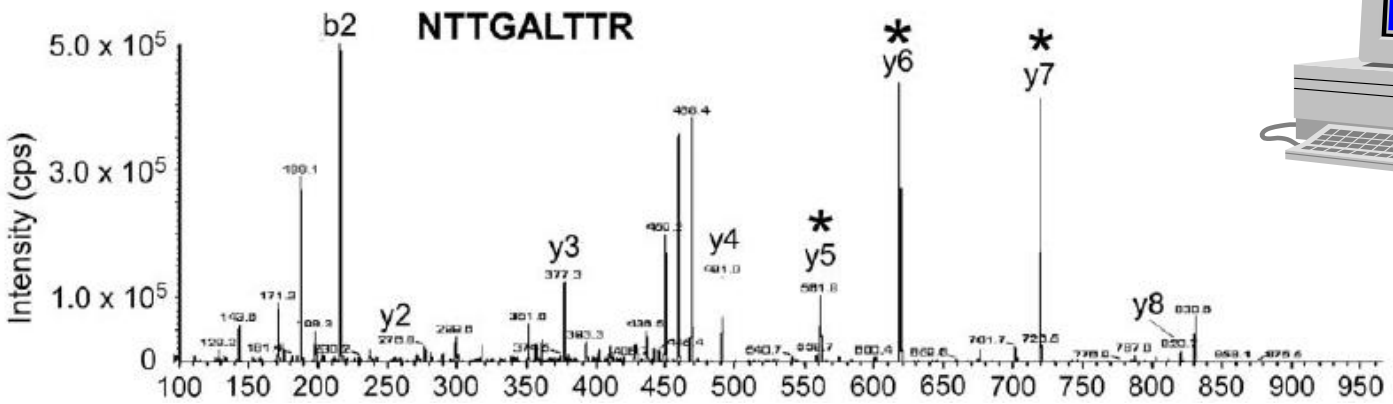
LC-MS/MS-Analysis

- Global or targeted proteomic analysis
- **Selection of mass transitions (non- / scheduled MRM)**
- Data analysis (peptides, MRMs, normalization)
- **Validation of the method**

Critical aspects of mass spectrometry based proteomics

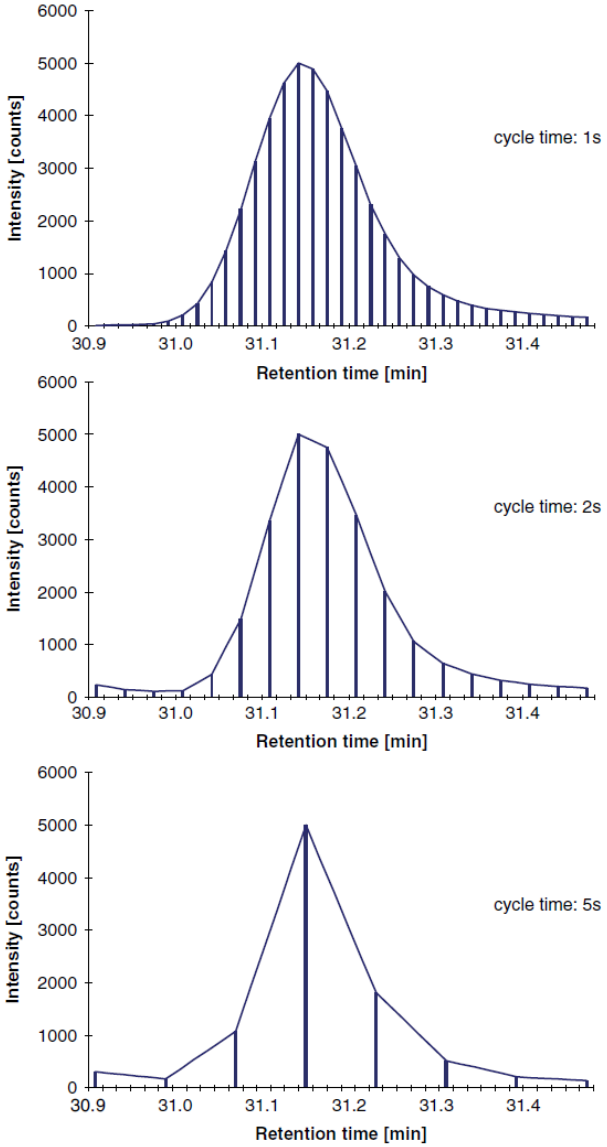
- Method Development -





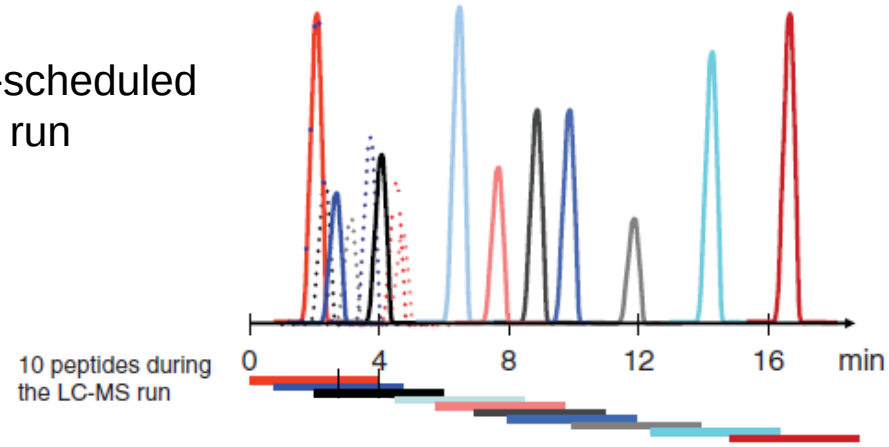
Critical aspects of mass spectrometry based proteomics

- Method Development -



- Mass transitions are not measured simultaneously but consecutively → not unlimited

Time-scheduled MRM run



proteins	peptides / protein	transitions / peptide	sum of transitions*	cycle time (s)
2	2	3	24	0.36
2	3	3	36	0.45
5	2	3	60	1.8
5	3	3	90	1.2
10	2	3	120	3.6
10	3	3	180	5.4
20	2	3	240	7.2
20	3	3	360	10.8

*including internal standard peptides; assumed dwell time: 30 ms

Gallien et al., J Mass. Spectrom. 2011

Critical aspects of mass spectrometry based proteomics

- Method Development -

Protein

- Biological / Clinical Question
- **Protein sequence data (consider SNPs, PTMs)**
- Sample preparation (fractionation, whole lysate)
- Protein determination / digestion

Peptide

- **Selection of appropriate peptides (in silico / wet-lab)**
- Number of peptides / protein

LC-MS/MS-Analysis

- Global or targeted proteomic analysis
- **Selection of mass transitions (non- / scheduled MRM)**
- Data analysis (peptides, MRMs, normalization)
- **Validation of the method**

Method validation

Guidance for Industry

Bioanalytical Method Validation

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

September 2013
Biopharmaceutics

Revision 1

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

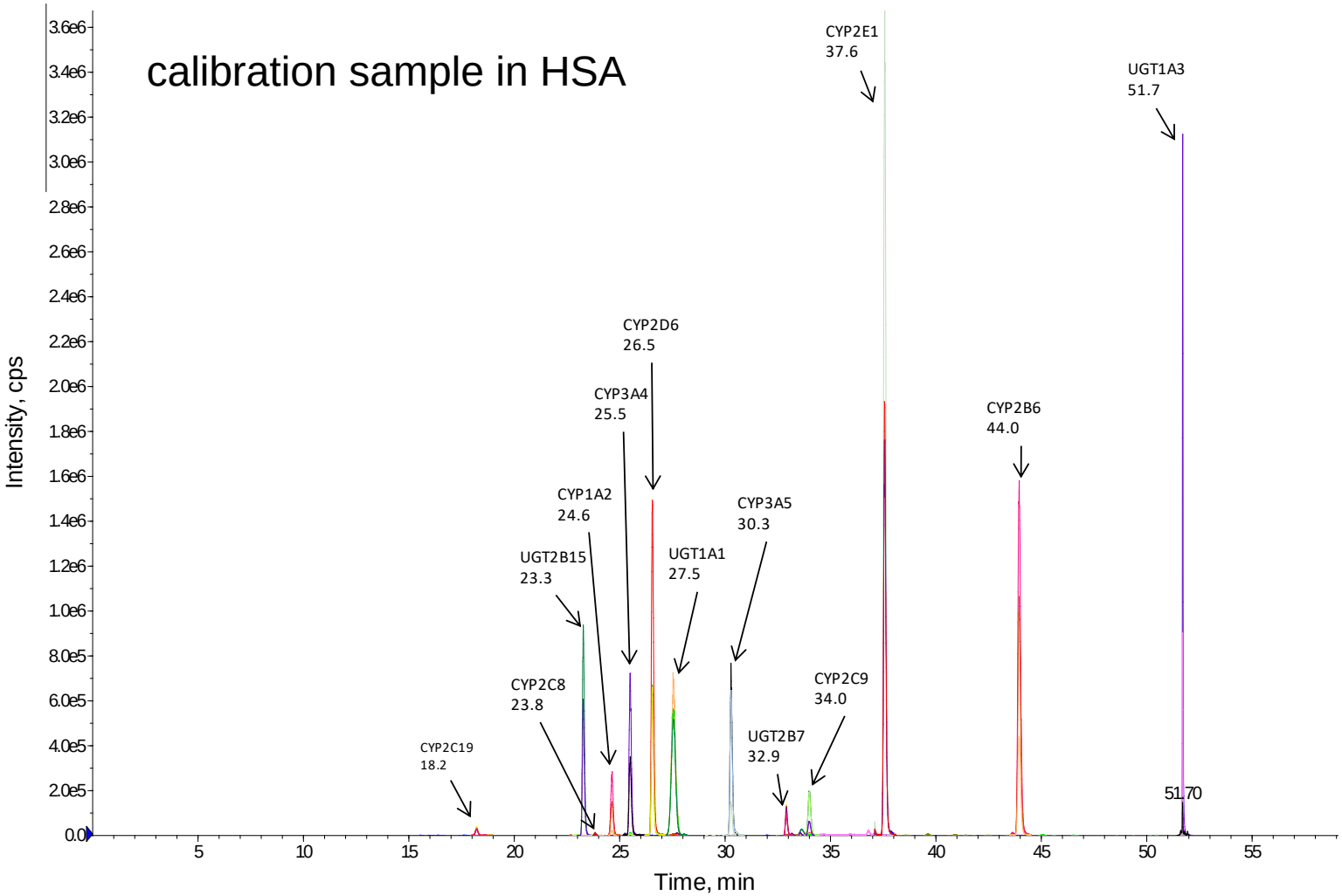
21 July 2011
EMEA/CHMP/EWP/192217/2009
Committee for Medicinal Products for Human Use (CHMP)

Guideline on bioanalytical method validation

Draft agreed by the Efficacy Working Party	September 2009
Adoption by CHMP for release for consultation	19 November 2009
End of consultation (deadline for comments)	31 May 2010
Agreed by Pharmacokinetics Working Party (PKWP)	June 2011
Adoption by CHMP	21 July 2011
Date for coming into effect	1 February 2012

Important parameters: specificity, linearity, range, LLOQ, accuracy and precision (within- and between-day), stability (short-term, post-preparative, long-term, freeze-and-thaw), matrix effects

Method validation: specificity



Gröer et al. 2014, J Pharm Biomed Anal

Method validation: specificity

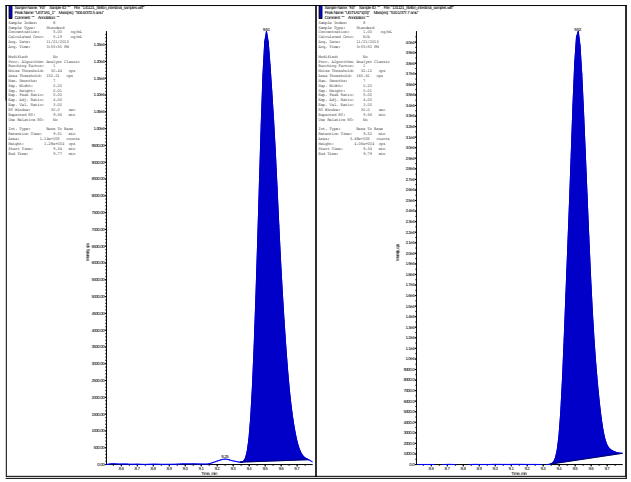
Protein	Peptide	mass	Q1	Q3.1	Q3.2	Q3.3
CYP1A2	YLPNPNALQR	1071.0	536.6	398.7	584.8	795.9
	YLPNPNALQR*	1081.0	541.7	403.7	594.8	805.9
CYP3A4	EVTNFLR	878.4	463.9	435.5	650.7	549.6
	EVTNFLR*	888.0	468.8	445.3	660.6	559.6
CYP3A5	DTINFLSK	937.5	648.2	608.7	721.8	494.7
	DTINFLSK*	944.0	653.3	616.3	729.9	502.6
CYP2B6	TAEIPFSLGK	1209.6	451.9	648.7	980.9	909.9
	TAEIPFSLGK*	1217.0	456.8	656.9	989.0	918.1
CYP2C19	GHFPLAER	926.5	482.9	585.7	342.4	732.8
	GHFPLAER*	936.0	487.8	595.7	342.5	742.9
CYP2C8	VQEEIDHVIGR	1294.6	606.3	1067.9	581.8	345.6
	VQEEIDHVIGR*	1304.0	610.3	1078.0	591.8	355.6
CYP2C9	GIFPLAER	902.5	440.2	367.1	585.8	293.5
	GIFPLAER*	912.0	445.3	371.9	595.7	298.4
CYP2D6	DIEVQGFR	963.5	469.5	507.4	379.4	606.7
	DIEVQGFR*	973.0	473.9	517.5	389.5	616.8
CYP2E1	FITLVPSNLPHEATR	1695.0	566.4	562.0	718.6	611.5
	FITLVPSNLPHEATR*	1705.0	570.1	567.3	724.2	616.8
UGT1A1	TYPVPFQR	1007.0	504.6	372.5	547.6	743.8
	TYPVPFQR*	1017.0	510.1	377.7	557.7	753.8
UGT1A3	YLSIPTVFFLR	1355.7	678.8	879.9	364.5	1080.2
	YLSIPTVFFLR*	1366.0	684.4	890.1	364.5	1090.1
UGT2B7	IEIYPTSLTK	1164.6	583.3	646.8	922.9	356.6
	IEIYPTSLTK*	1172.0	587.8	654.8	930.9	356.6
UGT2B15	SVINDPVYK	1034.5	518.1	425.0	848.9	506.7
	SVINDPVYK*	1042.0	522.4	428.9	856.9	514.8

Gröer et al. 2014, J Pharm Biomed Anal

Method validation: specificity

TYPVPFQR

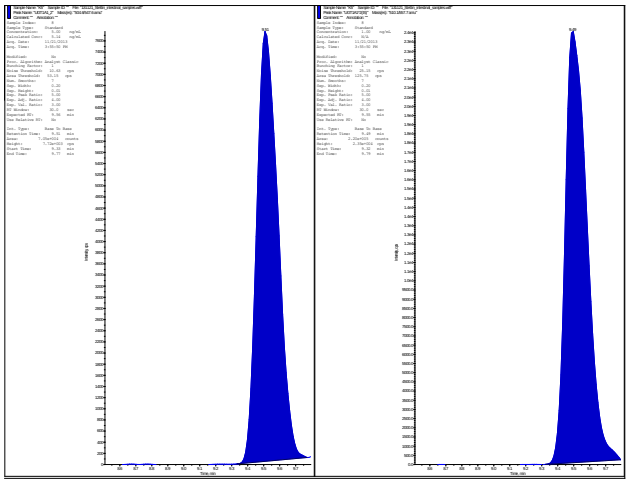
504.6 / 372.5 510.1 / 377.7



t=9.51 min t=9.52 min

TYPVPFQR

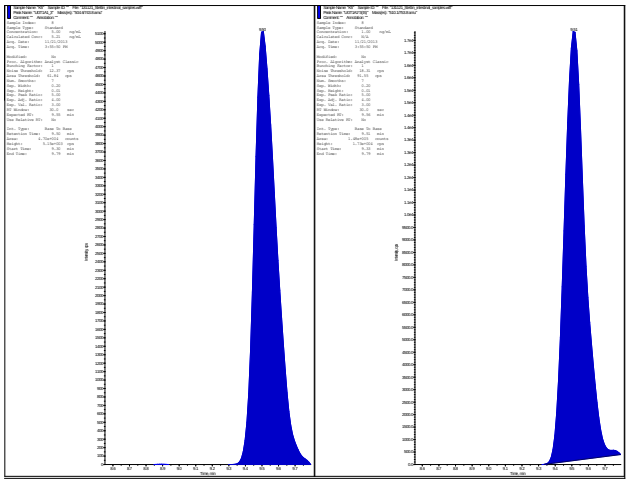
504.6 / 547.6 510.1 / 557.7



t=9.51 min t=9.50 min

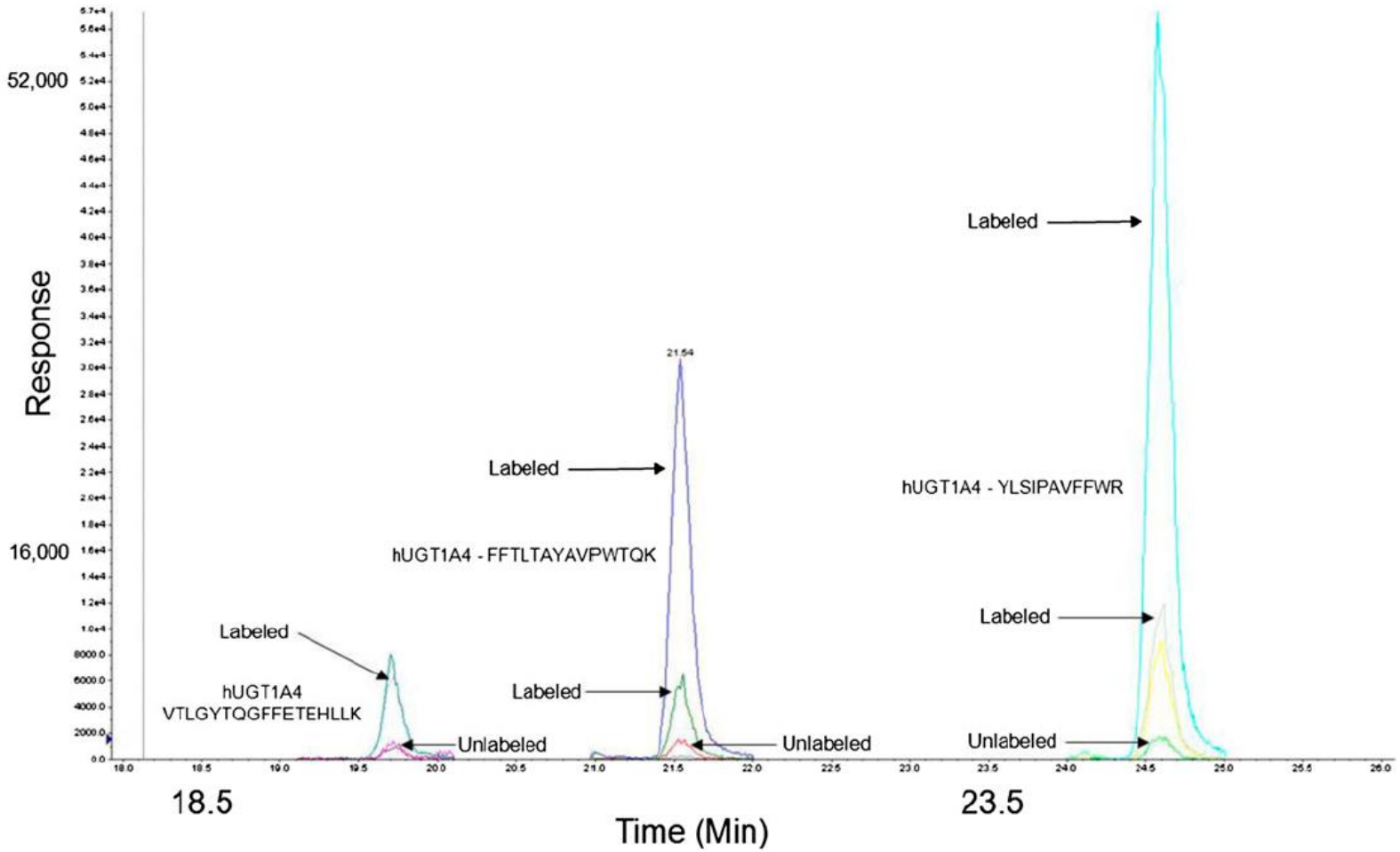
TYPVPFQR

504.6 / 743.8 510.1 / 753.8



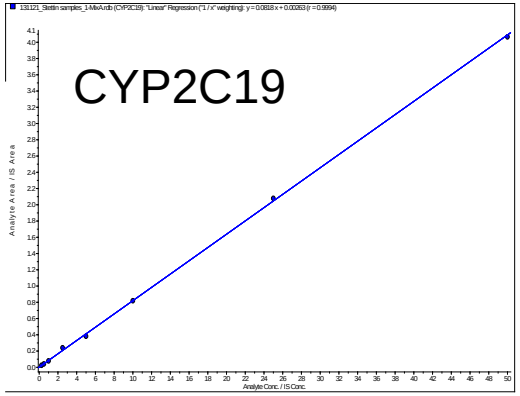
t=9.50 min t=9.51 min

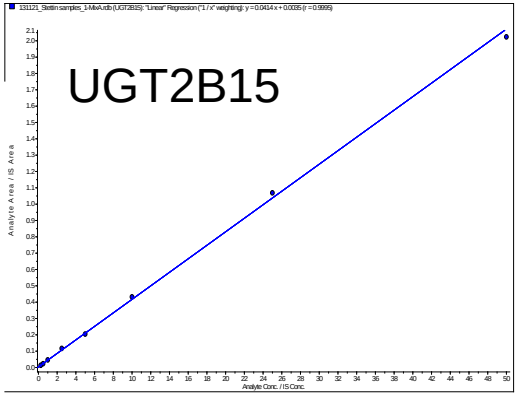
Method validation: specificity

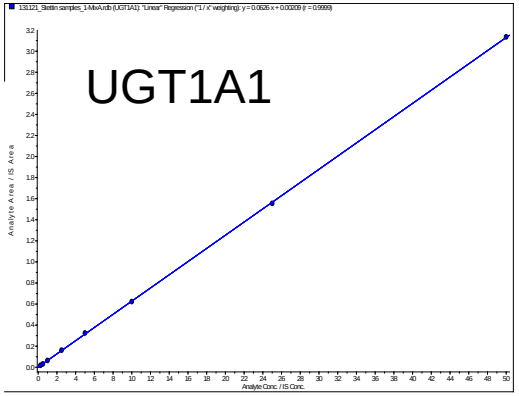


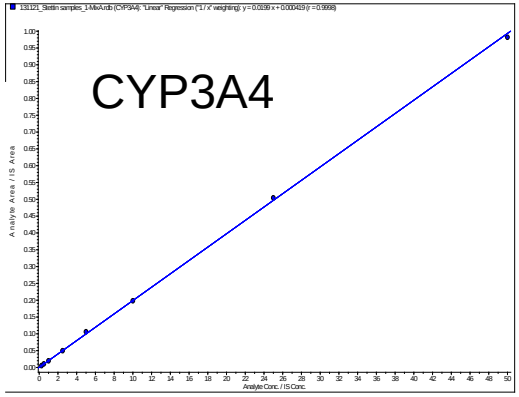
Fallon et al. 2013, DMD

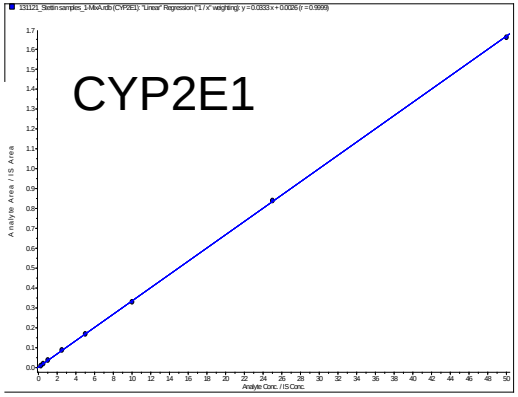
Method validation: linearity & range

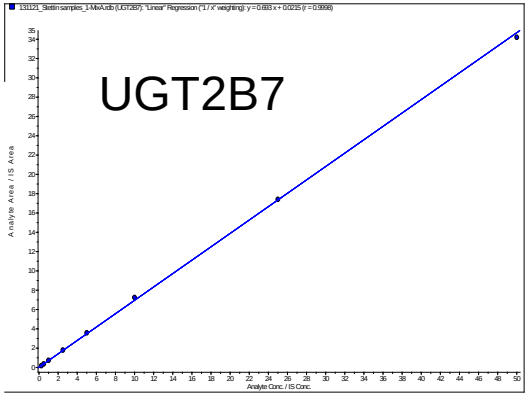












- **Range for all proteins:** 0.25 – 50 nmol/l (LLOQ = 3.75 fmol per injection)
- Linearity proven for all 13 peptides (from at least N=6 calibration rows)

Method validation: accuracy & precision

Protein	Within-day data		Between-day data		Range
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	(nmol/L)
CYP1A2	1.5 - 2.1	2.3 - 4.4	-5.3 - 6.3	1.1 - 5.2	0.25 - 50
CYP3A4	-1.1 - -4.6	2.4 - 5.1	-4.4 - 4.0	2.8 - 11.5	0.25 - 50
CYP3A5	-2.9 - 2.6	3.2 - -5.4	-2.9 - 4.2	1.4 - 3.8	0.25 - 50
CYP2B6	-1.6 - 9.2	2.7 - 13.6	-2.8 -4.0	2.0 - 14.2	0.25 - 50
CYP2C19	-13.1 - 2.7	4.9 - 9.9	-6.2 - 2.5	2.9 - 9.9	0.25 - 50
CYP2C8	-0.7 - 6.1	4.6 - 11.4	-6.1 - 8.5	3.5 - 14.8	0.25 - 50
CYP2C9	-0.9 - 0.1	1.6 - 3.5	-2.9 - -2.4	1.1 - 10.4	0.25 - 50
CYP2D6	-10.1 - 1.8	3.1 - 9.4	-3.2 - 2.3	1.6 - 13.6	0.25 - 50
CYP2E1	-0.8 - 12.5	4.5 - 7.0	-8.2 - 7.4	2.5 - 10.6	0.25 - 50
UGT1A1	1.2 - 4.7	1.2 - 2.7	-1.8 - 2.3	1.7 - 6.6	0.25 - 50
UGT1A3	-5.6 - 6.2	4.5 - 6.7	-4.2 - 2.2	4.0 - 11.4	0.25 - 50
UGT2B7	7.5 - 9.6	2.5 - 3.9	-3.0 - 4.2	1.9 - 7.6	0.25 - 50
UGT2B15	-0.7 - -2.8	2.2 - 5.3	-2.3 - 2.2	1.5 - 6.7	0.25 - 50

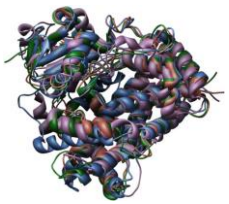
Gröer et al. 2014, J Pharm Biomed Anal

Method validation: stability

Parameter	short-term (bench-top)stability (%)	Post-preparative (rack) stability (%)	Digestion stability (%)
Protein	2 h @ room temperature	24 h @ 5°C (autosampler)	16 h @ 37°C
CYP1A2	92.7 - 104.7	100.3 - 101.1	87.6 - 105.9
CYP3A4	85.1 - 103.7	101.8 - 105.6	87.7 - 104.2
CYP3A5	92.0 - 105.1	101.2 - 103.1	91.0 - 103.3
CYP2B6	87.8 - 106.4	99.2 - 101.6	88.1 - 104.4
CYP2C19	89.9 - 97.2	93.5 - 111.2	106.4 - 115.4
CYP2C8	101.3 - 111.4	86.0 - 105.0	99.6 - 115.9
CYP2C9	91.9 - 103.6	100.7 - 102.3	90.4 - 103.6
CYP2D6	89.2 - 102.1	97.5 - 110.1	91.2 - 103.5
CYP2E1	92.2 - 102.8	93.1 - 98.9	102.3 - 105.9
UGT1A1	89.5 - 98.4	99.5 - 101.7	93.3 - 100.0
UGT1A3	94.9 - 104.0	92.3 - 101.9	99.7 - 106.6
UGT2B7	93.8 - 104.2	90.5 - 97.1	96.3 - 102.7
UGT2B15	86.8 - 102.6	100.3 - 103.0	85.2 - 88.59

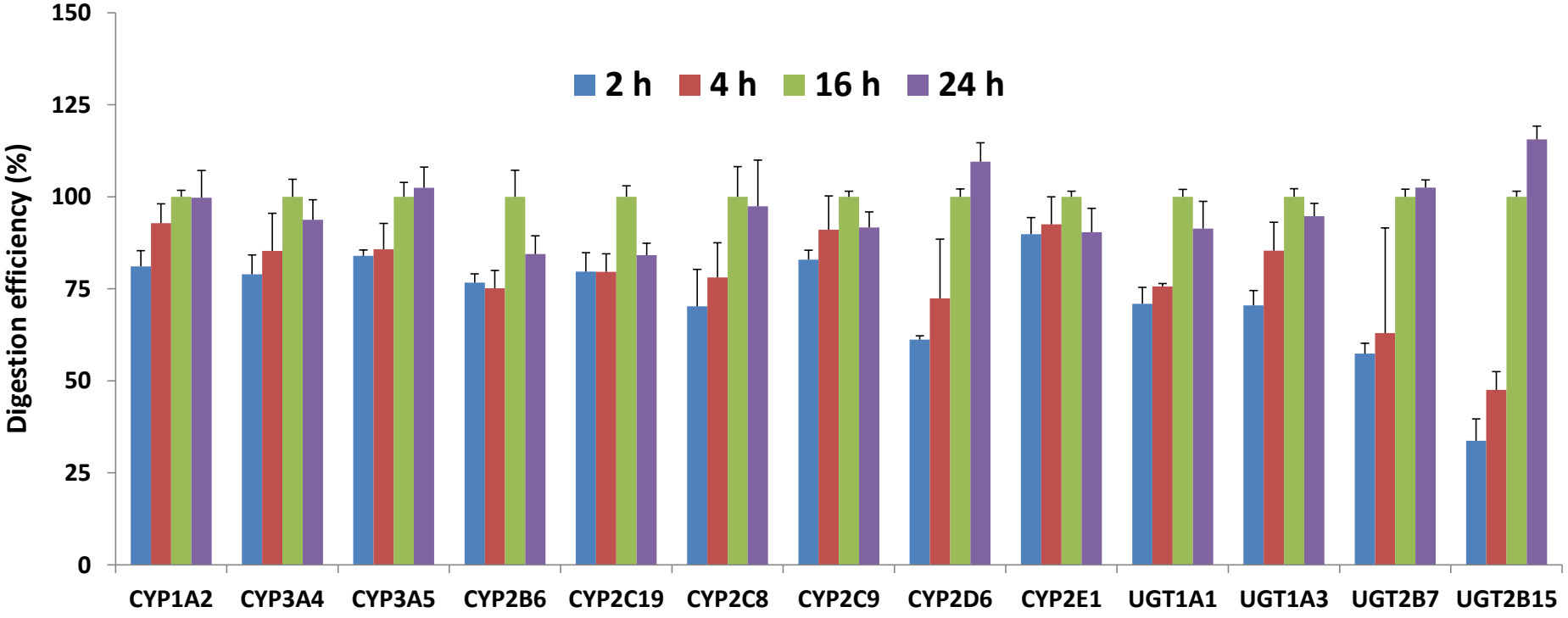
Gröer et al. 2014, J Pharm Biomed Anal

Method validation: digestion



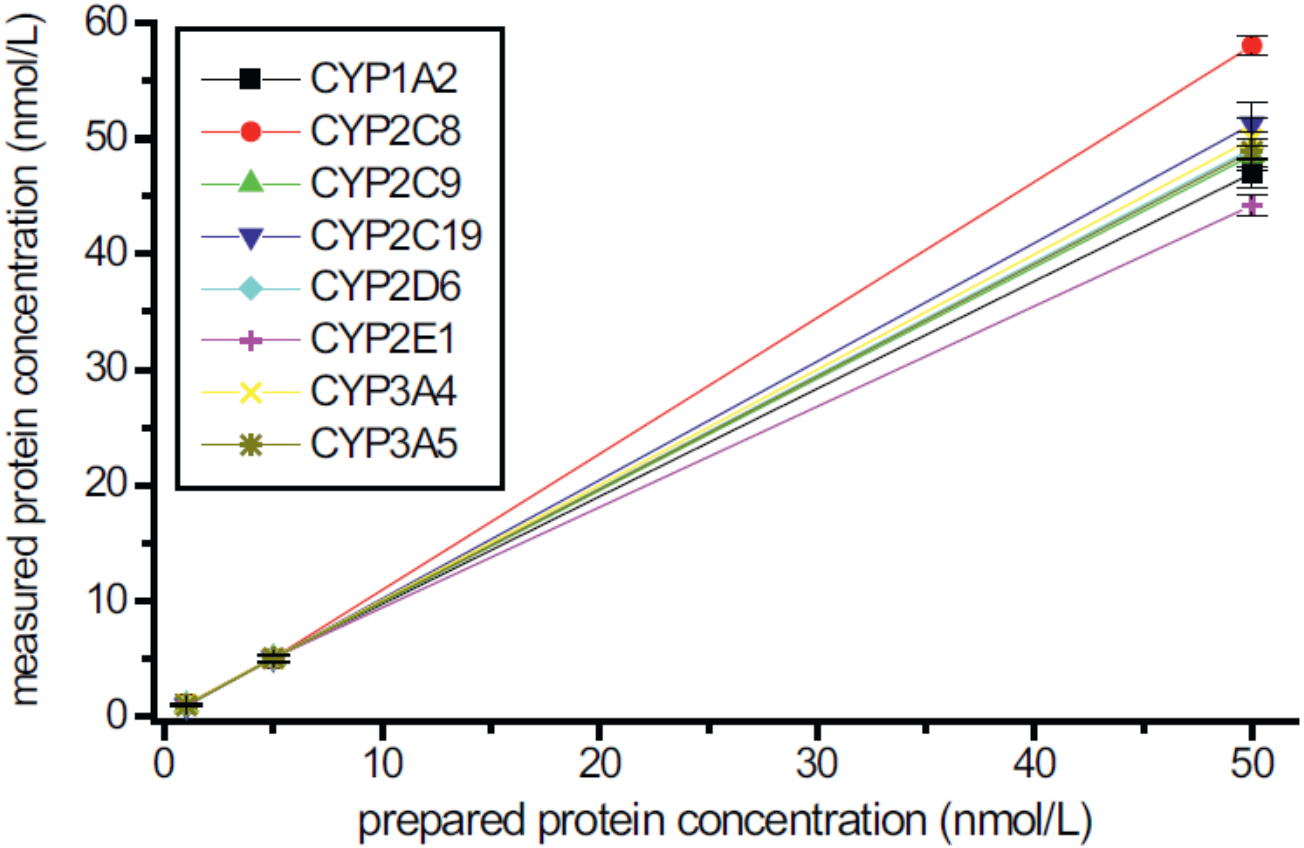
protein

trypsin



Gröer et al. 2014, J Pharm Biomed Anal

Accuracy of the entire analytical process



Gröer et al. 2014, J Pharm Biomed Anal

Validation of LC-MS/MS assays

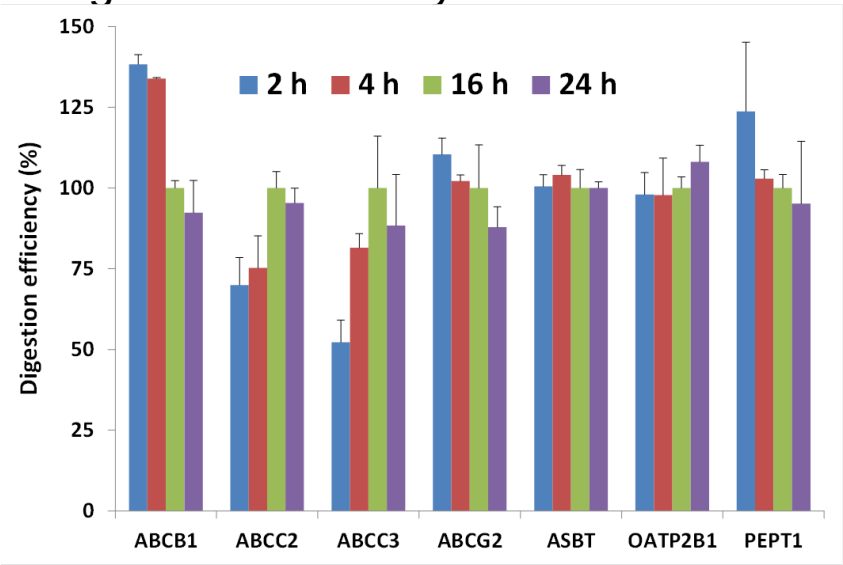
accuracy and precision

Protein	Within-day data		Between-day data		Range
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)	(nmol/L)
ABCB1	1.1 - 8.1	1.5 - 3.3	-1.9 - 8.1	3.7 - 13.3	0.1 - 25
ABCC2	-3.6 - 7.3	5.2 - 13.4	-6.2 - 6.4	1.7 - 17.2	0.1 - 25
ABCC3	-0.3 - 3.7	3.1 - 6.5	-1.3 - 7.1	3.6 - 10.8	0.25 - 25
ABCG2	-14.1 - -0.9	2.5 - 4.4	-9.7 - 9.7	1.4 - 8.8	0.1 - 25
OATP1A2	-3.5 - 2.5	4.1 - 6.5	-2.7 - 3.3	3.6 - 9.3	0.1 - 25
OATP2B1	-4.5 - 4.4	8.7 - 13.5	-5.5 - 5.2	2.2 - 8.9	0.1 - 25
OCT1	-3.0 - 5.5	5.8 - 13.9	-3.9 - 5.7	3.2 - 13.4	0.1 - 25
OCT3	-2.2 - 1.5	4.2 - 6.0	-2.7 - 12.1	4.9 - 10.5	0.25 - 25
PEPT1	-6.1 - -1.5	4.7 - 6.0	-8.0 - 7.0	4.3 - 10.2	0.1 - 25
ASBT	-0.2 - 12.3	1.6 - 3.8	-3.7 - 7.4	3.4 - 11.8	0.1 - 25

stability

Parameter	short-term (bench-top)stability (%)	Post-preparative (rack) stability (%)	Digestion stability (%)
Protein	2 h @ room temperature	24 h @ 5°C (autosampler)	16 h @ 37°C
ABCB1	98.7 - 96.8	100.0 - 106.4	100.4 - 110.5
ABCC2	91.1 - 103.2	92.2 - 98.5	85.0 - 104.9
ABCC3	90.8 - 109.3	100.9 - 111.6	94.4 - 108.2
ABCG2	89.3 - 99.9	99.0 - 100.4	94.4 - 108.5
OATP1A2	97.1 - 106.9	100.0 - 102.9	92.6 - 94.8
OATP2B1	93.1 - 100.7	97.4 - 105.5	92.0 - 102.1
OCT1	80.2 - 113.9	99.5 - 118.9	99.5 - 105.4
OCT3	100.3 - 114.4	100.5 - 109.0	89.1 - 111.3
PEPT1	84.8 - 102.5	97.3 - 106.0	93.9 - 97.1
ASBT	93.2 - 97.4	97.1 - 104.3	88.7 - 114.9

digestion efficiency



species specificity

Protein	Human (homo sapiens)	Mouse (mus musculus)	Rat (rattus norvegicus)	Dog (canis lupus)
ABCB1	x	x	x	x
ABCC2	x	–	x	x
ABCC3	x	–	–	x
ABCG2	x	x	x	x
OATP1A2	x	–	–	–
OATP2B1	x	–	–	–
OCT1	x	–	–	–
OCT3	x	–	–	x
PEPT1	x	–	–	–
ASBT	x	–	–	–

Gröer et al. 2013, J Pharm Biomed Anal

Gröer et al. 2014, J Pharm Biomed Anal

Critical aspects of mass spectrometry based proteomics

- Application of the Method -

Protein

- Biological / Clinical Question
- Protein sequence data (consider SNPs, PTMs)
- **Sample preparation (fractionation, whole lysate)**
- **Protein determination / digestion**

Peptide

- Selection of appropriate peptides (in silico / wet-lab)
- **Number of peptides / protein**

LC-MS/MS-Analysis

- **Global or targeted proteomic analysis**
- Selection of mass transitions (non- / scheduled MRM)
- **Data analysis (peptides, MRMs, normalization)**
- Validation of the method

Universitätsmedizin GREIFSWALD

C_DAT Center of Drug Absorption and Transport

UPPSALA UNIVERSITET

TNO innovation for life

MANCHESTER 1824 The University of Manchester

Universitätsmedizin GREIFSWALD

max-planck-institut für biochemie

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

Variability in Mass Spectrometry-based Quantification of Clinically Relevant Drug Transporters and Drug Metabolizing Enzymes

Christine Wegler,[†,‡] Fabienne Z. Gaugaz,[†] Tommy B. Andersson,[‡] Jacek R. Wiśniewski,[§] Diana Busch,[∥] Christian Gröer,[∥] Stefan Oswald,[∥] Agneta Norén,[⊥] Frederik Weiss,[#] Helen S. Hammer,[#] Thomas O. Joos,[#] Oliver Poetz,[#] Brahim Achour,[▽] Amin Rostami-Hodjegan,[▽] Evita van de Steeg,[⬢] Heleen M. Wortelboer,[⬢] and Per Artursson[*,†]

Mol Pharm. 2017; 4(9):3142-3151.

- European inter-laboratory comparison
- Analysis of 10 identical human livers → quantification of clinically relevant enzymes (N=13) and transporters (N=10)
- Use of different sample preparation procedures and MS techniques

Critical aspects of mass spectrometry based proteomics

- Application of the Method -

Protein

- Biological / Clinical Question
- Protein sequence data (consider SNPs, PTMs)
- **Sample preparation (fractionation, whole lysate)**
- **Protein determination / digestion**

Peptide

- Selection of appropriate peptides (in silico / wet-lab)
- **Number of peptides / protein**

LC-MS/MS-Analysis

- **Global or targeted proteomic analysis**
- Selection of mass transitions (non- / scheduled MRM)
- **Data analysis (peptides, MRMs, normalization)**
- Validation of the method

Critical aspects of mass spectrometry based proteomics

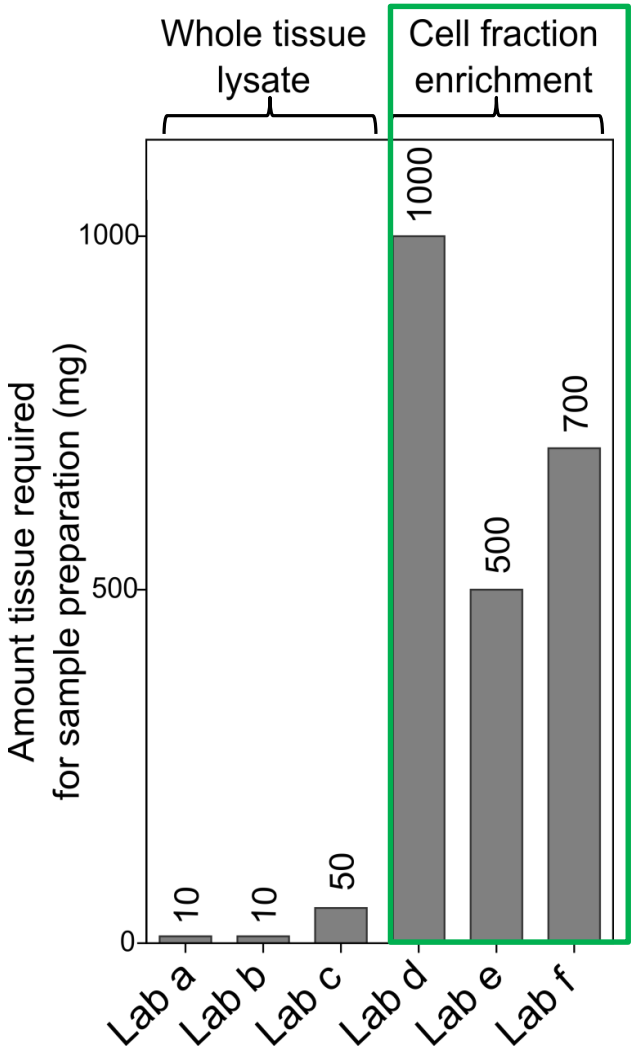
- Application of the Method -

	Tissue preparation	Subcellular fractionation	Protein extraction	Protein digestion	Peptide standards	Peptide enrichment	LC-MS/MS analysis
Whole tissue lysate: Lab a	Homogenization Lysis	—	FASP	Trypsin	SIL peptides	—	MRM (m/z)
Whole tissue lysate: Lab b	Homogenization Lysis	—	MED-FASP	Trypsin LysC	—	—	Label-free (m/z)
Whole tissue lysate: Lab c	Homogenization Lysis	—	—	Trypsin	SIL peptides	Multi-specific antibody	tSIM (m/z)
Subcellular fraction: Lab d	Homogenization Lysis	Microsomes/ Crude membrane	—	Trypsin LysC	SIL (QconCAT derived) peptides	—	MRM (m/z)
Subcellular fraction: Lab e	Homogenization Lysis	Microsomes/ Crude membrane (Enzymes); Crude membrane (Transporters)	—	Trypsin	SIL peptides	—	MRM (m/z)
Subcellular fraction: Lab f	Homogenization Lysis	Plasma membrane	—	Trypsin	SIL peptides	—	MRM (m/z)

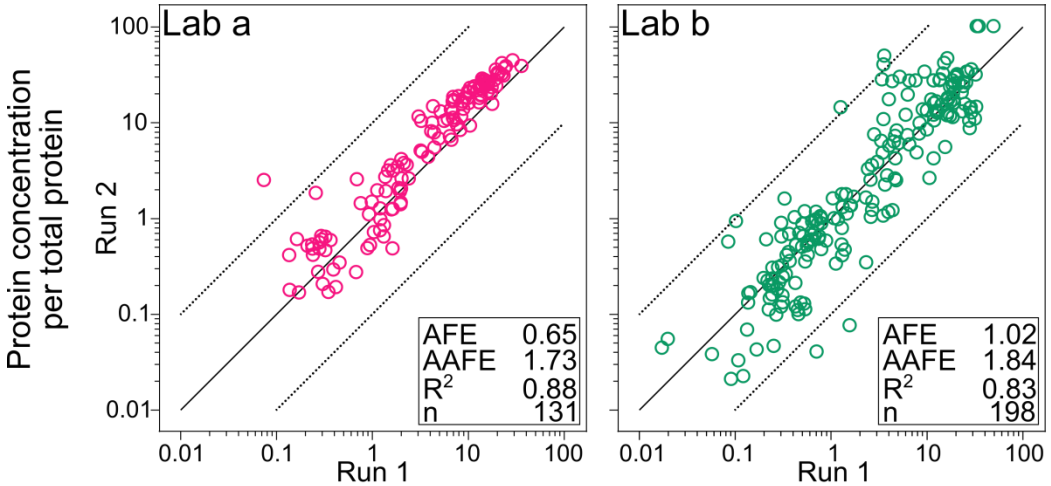
Mol Pharm. 2017;14(9):3142-3151.

Critical aspects of mass spectrometry based proteomics

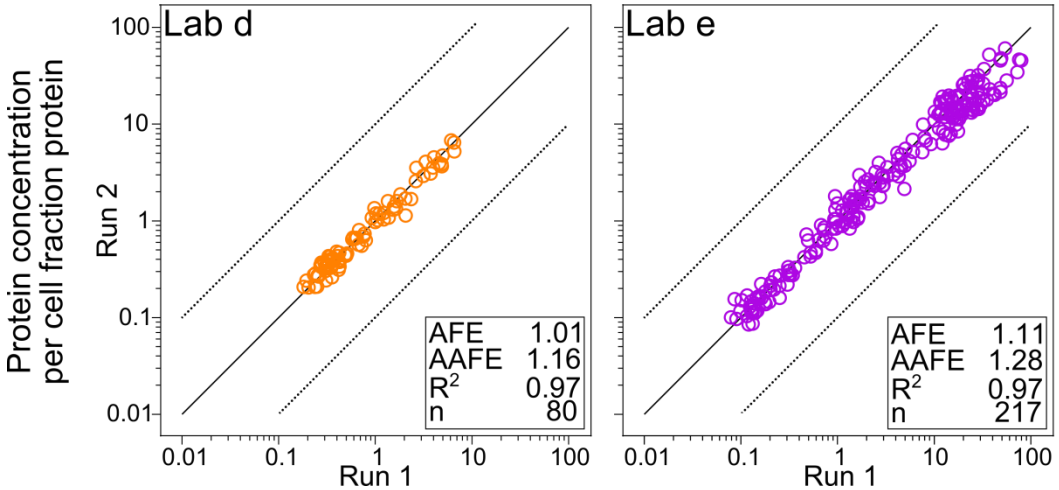
- Application of the Method -



Whole tissue lysates



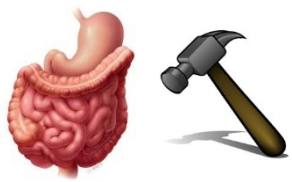
Cell fraction enrichment



Mol Pharm. 2017;14(9):3142-3151.

Sample preparation: transporters

disruption of frozen tissue



tissue homogenization

cell lysis

ProteoExtract®
Native Membrane
Protein Extraction Kit

extraction of membrane proteins

ProteoExtract®
Native Membrane
Protein Extraction Kit

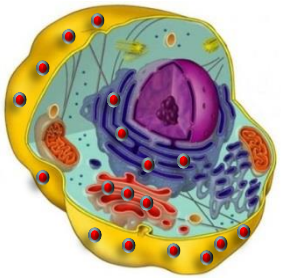
determine protein concentration (BCA)

adjust concentration, denaturation, reduction, alkylation, trypsin digestion

LC-MS/MS analysis (QTRAP5500)

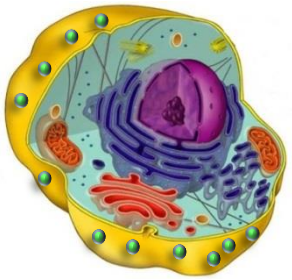
Limitation:

- Isolation of crude membrane (CM)
- Only transporters in plasma membrane determine function
- Plasma membrane ~10% of CM → risk of over- or underestimation



transporter protein in crude membrane

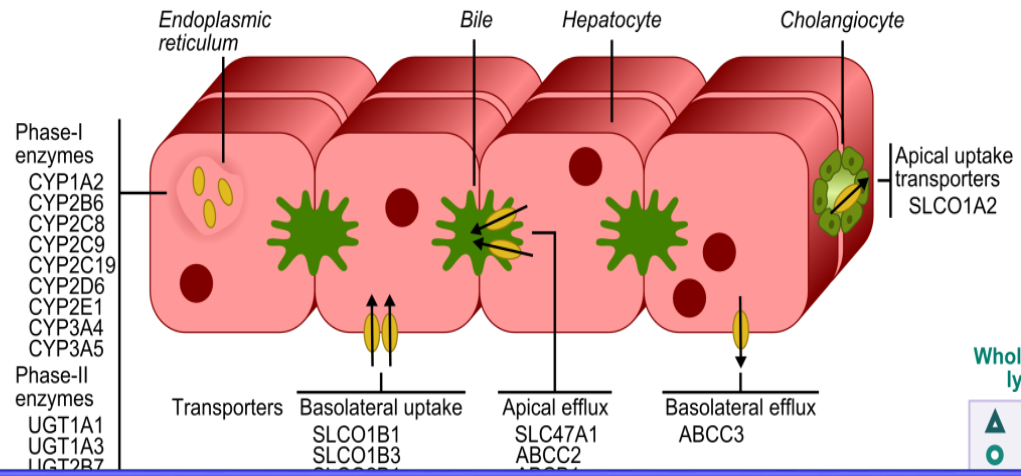
vs.



transporter protein in plasma membrane

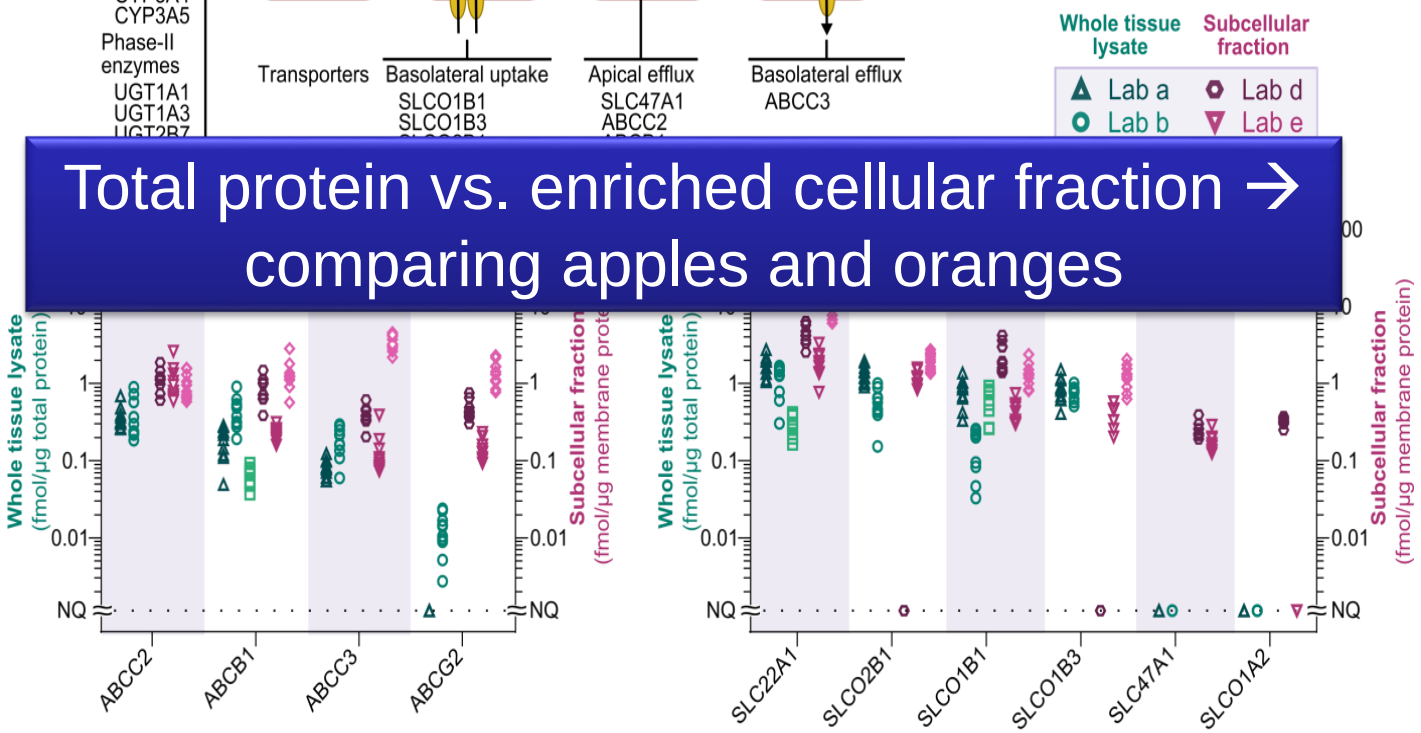
Critical aspects of mass spectrometry based proteomics

- Application of the Method -

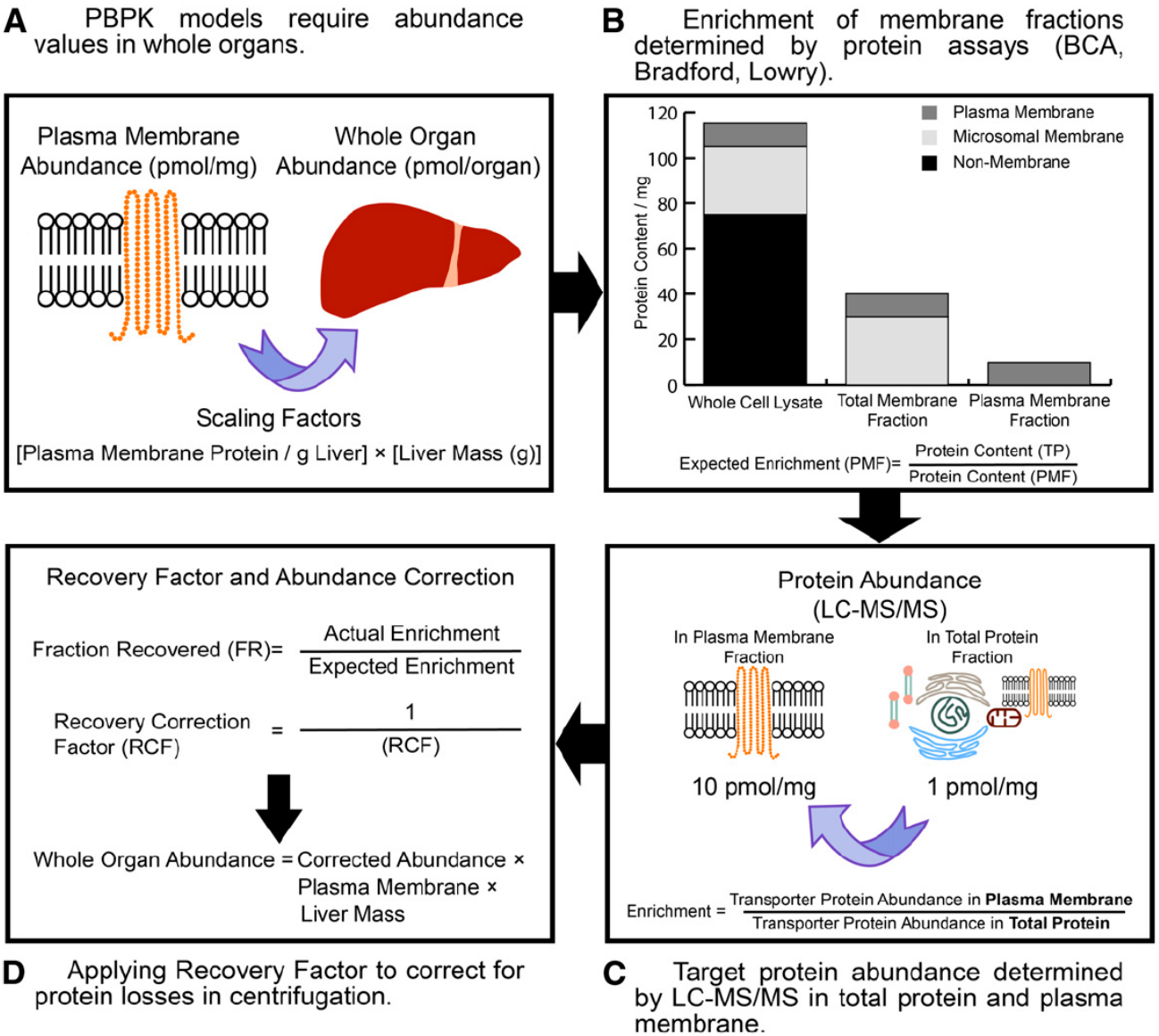


1 to 100-fold difference between groups

Total protein vs. enriched cellular fraction → comparing apples and oranges

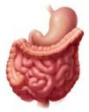


Wegler et al. 2017, Mol Pharm

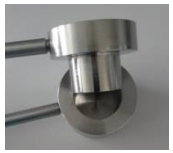


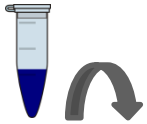
Harwood et al. 2014, DMD

Sample preparation: enzymes

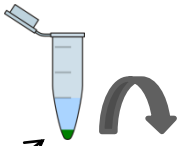


tissue disruption and homogenization





centrifugation
9,000 *g*

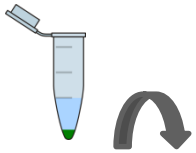


supernatent: S9 fraction

centrifugation of supernatent
100,000 *g*

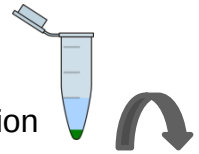
cell debris

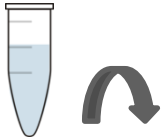
supernatent: cytosolic fraction



wash pellet
centrifugation (100,000 *g)*

pellet: microsomal fraction





resuspension of pellet,
adjust concentration,
denaturation,
reduction,
alkylation,
trypsin digestion

1521-009X/42/10/1766–1772$25.00
Drug Metabolism and Disposition
Copyright © 2014 by The American Society for Pharmacology and Experimental Therapeutics

http://dx.doi.org/10.1124/dmd.114.058446
Drug Metab Dispos 42:1766–1772, October 2014

Perspective

Lost in Centrifugation: Accounting for Transporter Protein Losses in Quantitative Targeted Absolute Proteomics

Matthew D Harwood, Matthew R Russell, Sibylle Neuhoff, Geoffrey Warhurst, and Amin Rostami-Hodjegan

Gut Barrier Group, Inflammation & Repair, University of Manchester, Salford Royal NHS Trust, Salford (M.D.H., G.W.), Simcyp Limited (a Certara Company), Blades Enterprise Centre, Sheffield, (M.D.H., S.N., A.R-H.), and Centre for Applied Pharmacokinetic Research, Manchester Pharmacy School, Manchester (M.R.R., A.R-H.), United Kingdom

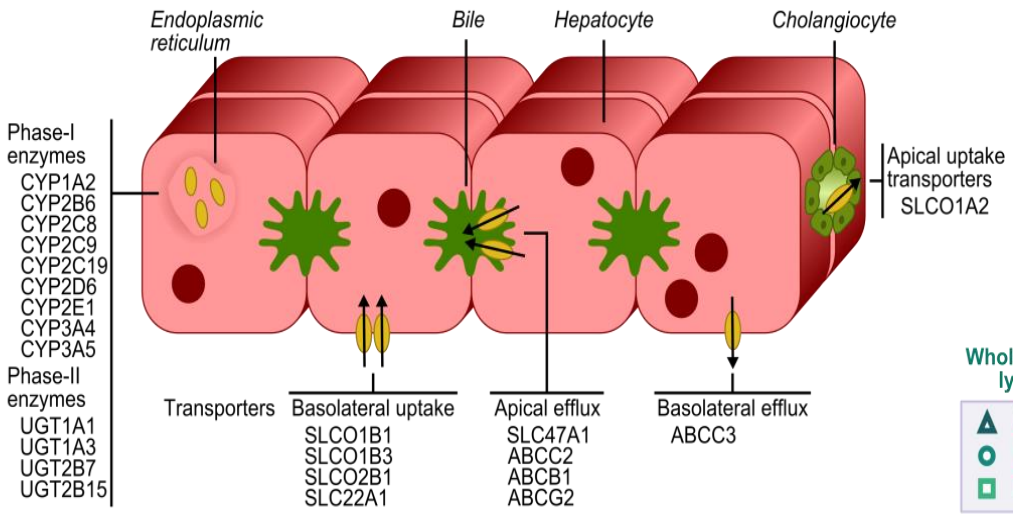
Received April 4, 2014; accepted July 24, 2014

Limitation:

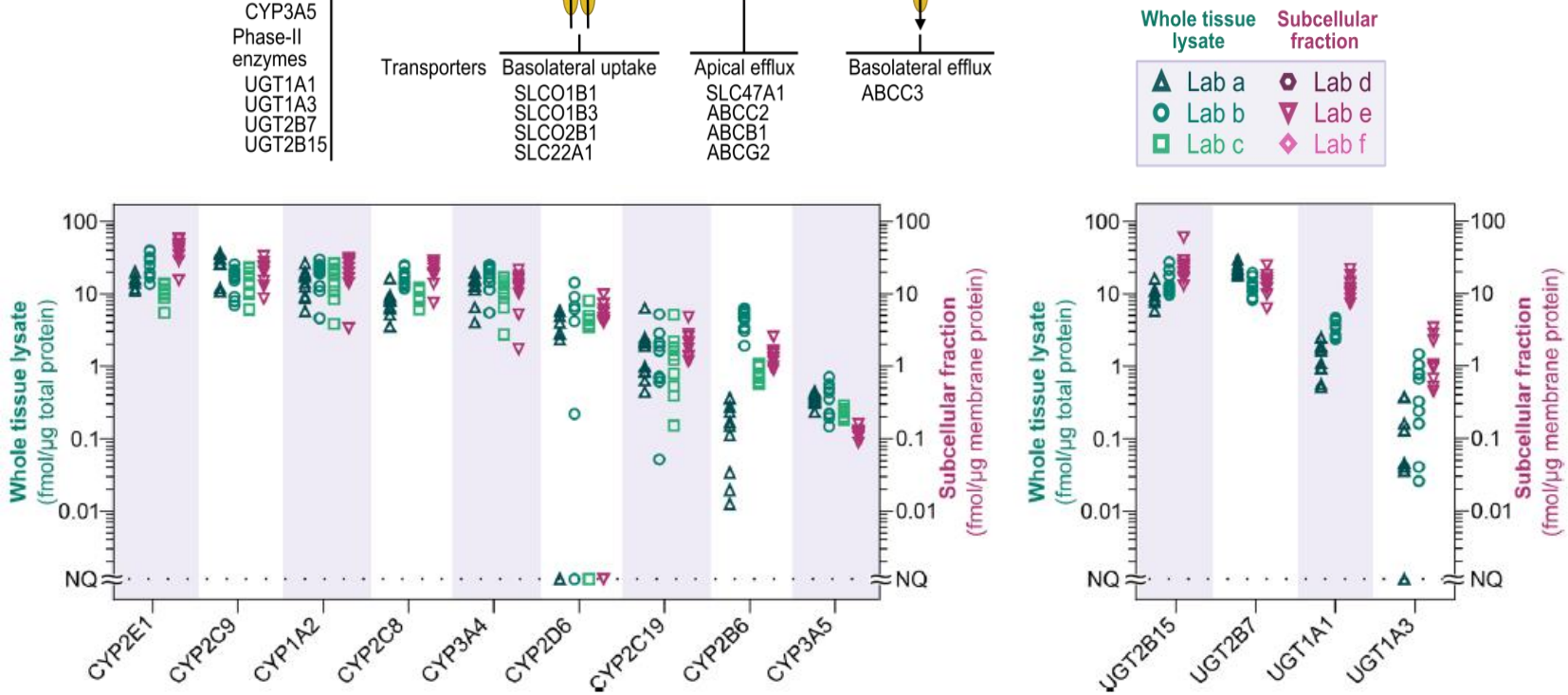
- There are no microsomes in vivo, they are an artificial product
- Yield of microsomes is highly variable (in dependence on prep.)
- Not surprising: highly variable data
  → **better:** whole tissue lysate, but…

Critical aspects of mass spectrometry based proteomics

- Application of the Method -



1 to 6-fold difference between groups



Wegler et al. 2017, Mol Pharm

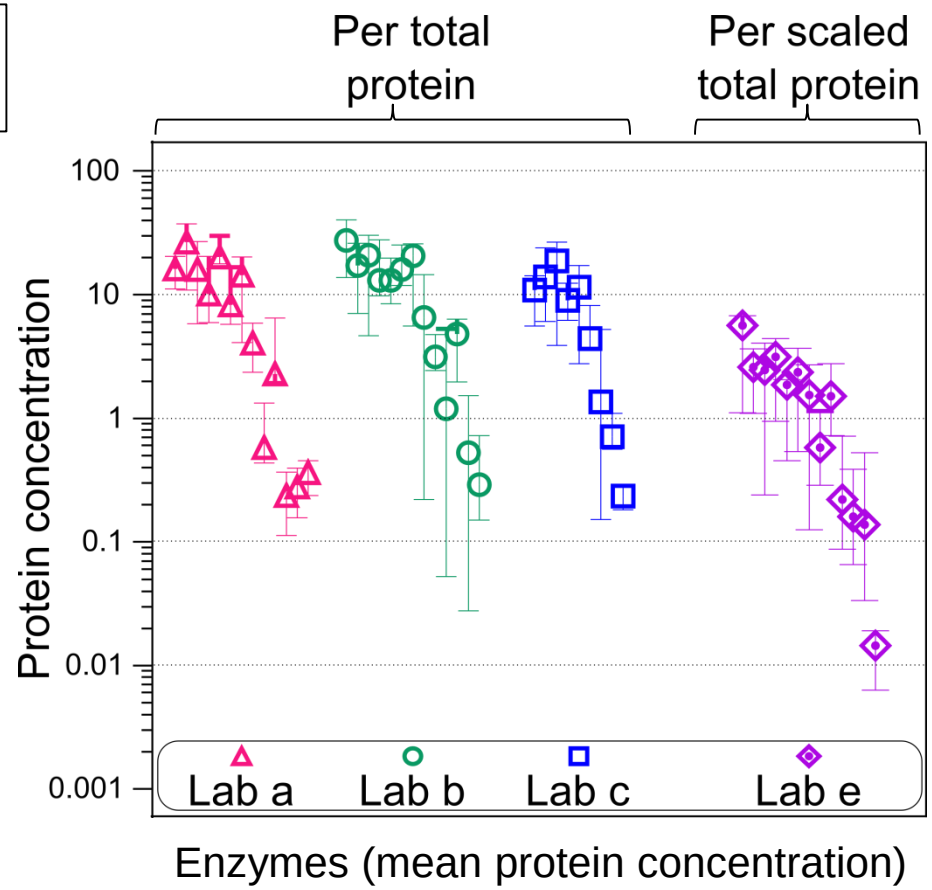
Critical aspects of mass spectrometry based proteomics

- Application of the Method -

Scaling enriched cell fraction → total protein

3 to 30-fold difference between groups

$$\text{Scaling factor} = \frac{[\text{Protein}]\ \text{cell fraction}}{[\text{Protein}]\ \text{tissue lysate}}$$



Sample preparation for enzymes and transporters: FASP

Universal sample preparation method for proteome analysis

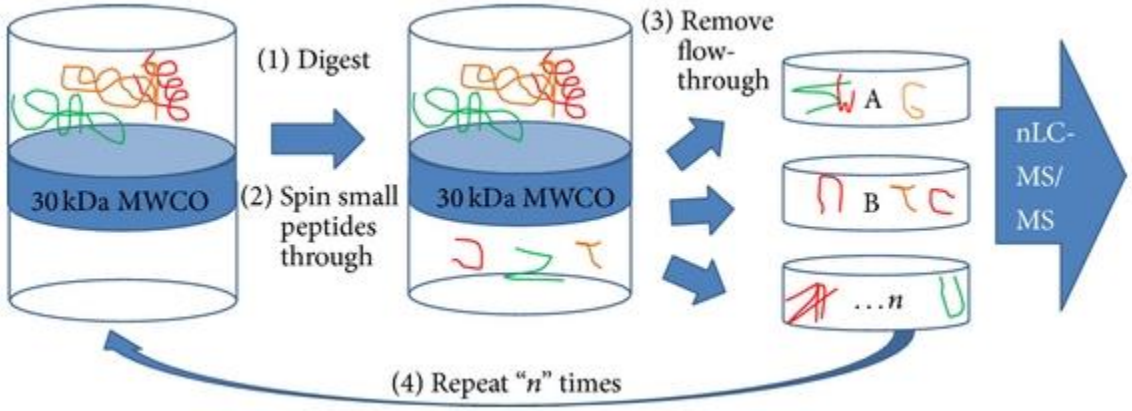
Jacek R Wiśniewski, Alexandre Zougman, Nagarjuna Nagaraj & Matthias Mann

We describe a method, filter-aided sample preparation (FASP), which combines the advantages of in-gel and in-solution digestion for mass spectrometry–based proteomics. We completely solubilized the proteome in sodium dodecyl sulfate, which we then exchanged by urea on a standard filtration device. Peptides eluted after digestion on the filter were pure, allowing single-run analyses of organelles and an unprecedented depth of proteome coverage.

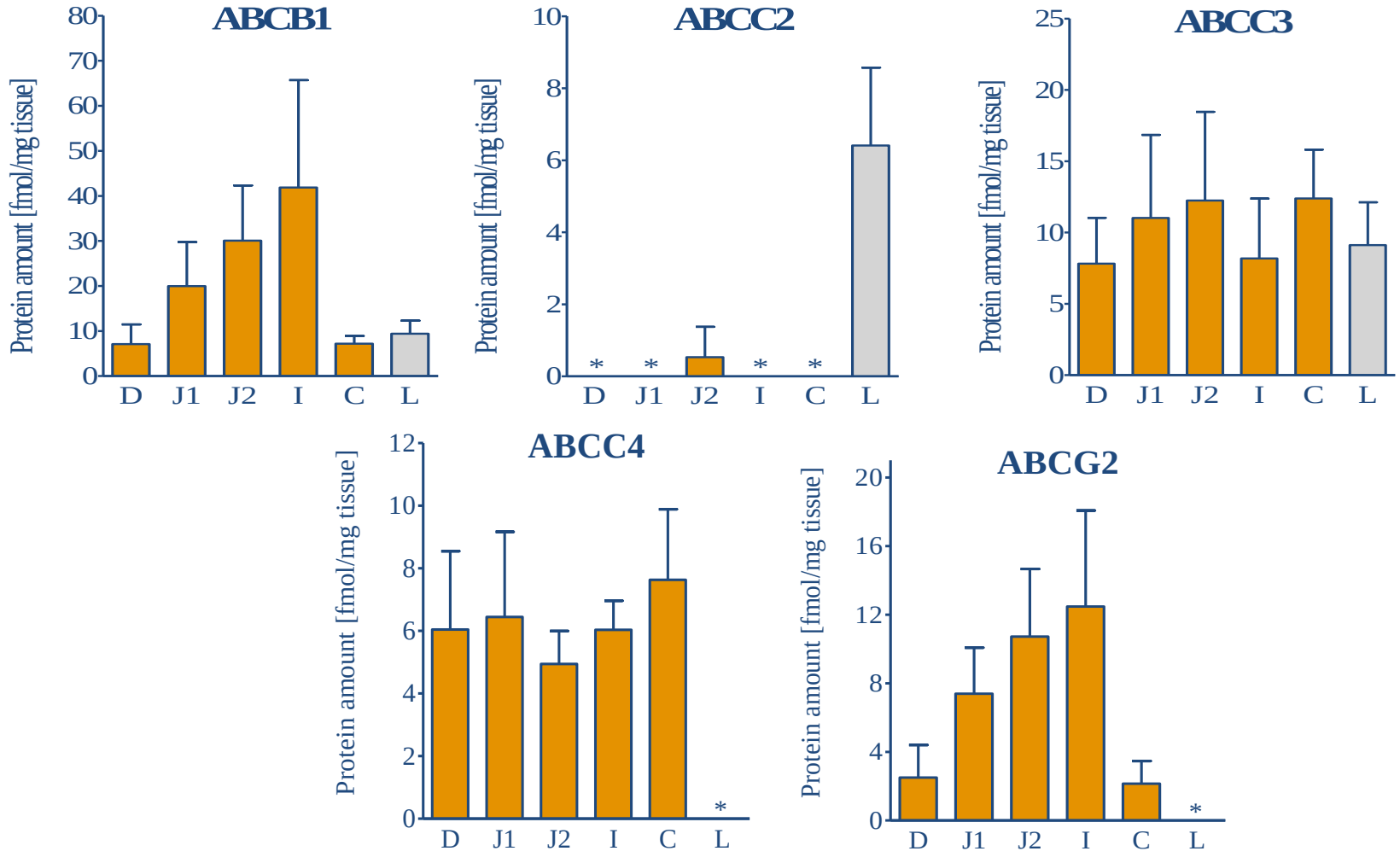
NATURE METHODS | VOL.6 NO.5 | MAY 2009

FASP:

- *Filter-aided sample preparation*
- Suitable for complex samples
- No loss of subcellular fractions → whole tissue lysate is used
- Enzymes and transporters can be measured in the same sample
- **but:** loss of sensitivity and enzymes function not assessable



Example: protein abundance of transporters in human intestine and liver



D: duodenum, J1: upper jejunum, J2: lower jejunum, I: ileum, C: colon, L: liver
* below limit of quantification (2.7 fmol/mg tissue)

Drozdzik et al., *unpublished*

Critical aspects of mass spectrometry based proteomics

- Application of the Method -

Protein

- Biological / Clinical Question
- Protein sequence data (consider SNPs, PTMs)
- **Sample preparation (fractionation, whole lysate)**
- **Protein determination / digestion**

Peptide

- Selection of appropriate peptides (in silico / wet-lab)
- **Number of peptides / protein**

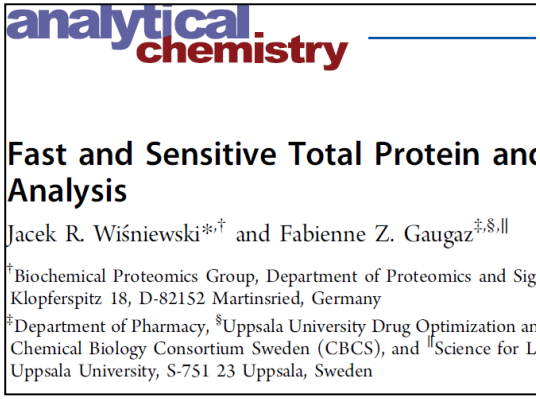
LC-MS/MS-Analysis

- **Global or targeted proteomic analysis**
- Selection of mass transitions (non- / scheduled MRM)
- **Data analysis (peptides, MRMs, normalization)**
- Validation of the method

Critical aspects of mass spectrometry based proteomics

- Application of the Method -

- Protein content of cell or tissue lysate samples for proteomic analysis is normally determined by unspecific methods (e.g. Lowry, Bradford, BCA)
- **Limitations:** highly variable, influenced by many factors (pH, buffer, detergents, temperature et cetera) → data obse
challenged by these



analytical chemistry

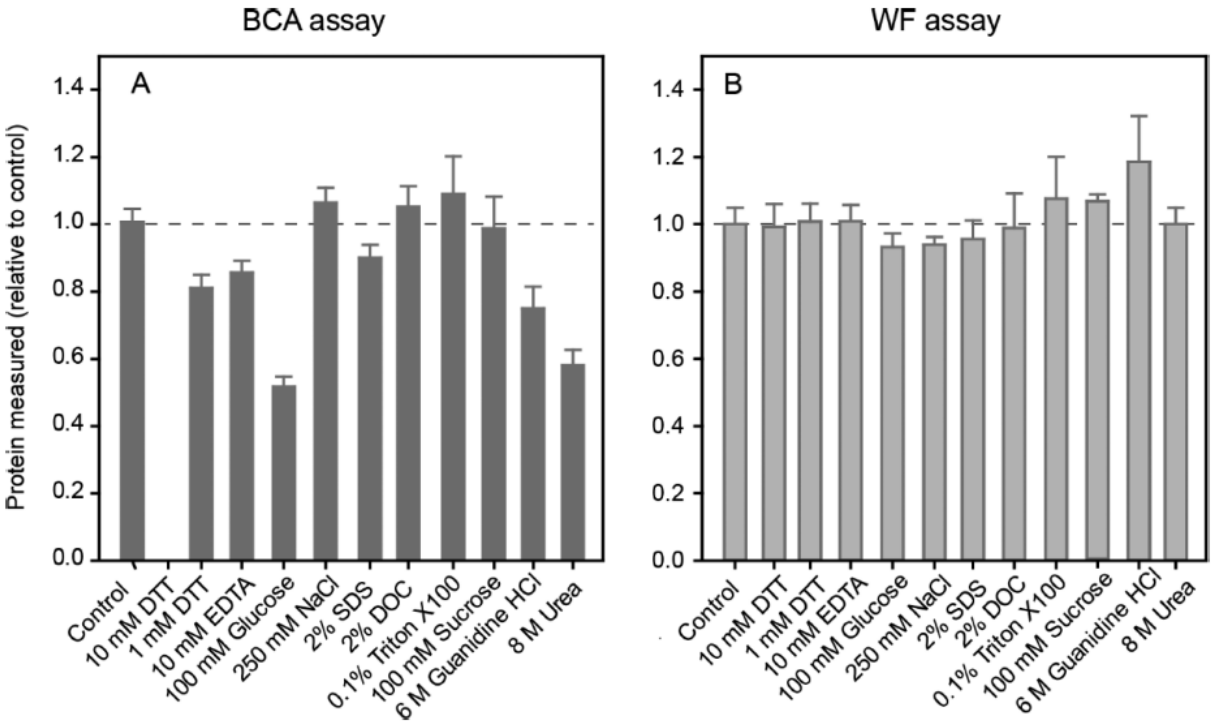
Fast and Sensitive Total Protein anc
Analysis

Jacek R. Wiśniewski*,† and Fabienne Z. Gaugaz‡,§,∥

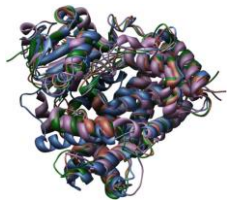
†Biochemical Proteomics Group, Department of Proteomics and Sig
Klopferspitz 18, D-82152 Martinsried, Germany

‡Department of Pharmacy, §Uppsala University Drug Optimization ar
Chemical Biology Consortium Sweden (CBCS), and ∥Science for L
Uppsala University, S-751 23 Uppsala, Sweden

Anal Chem. 2015 Apr 21;87(8):4110-

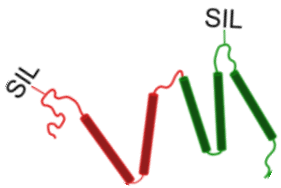


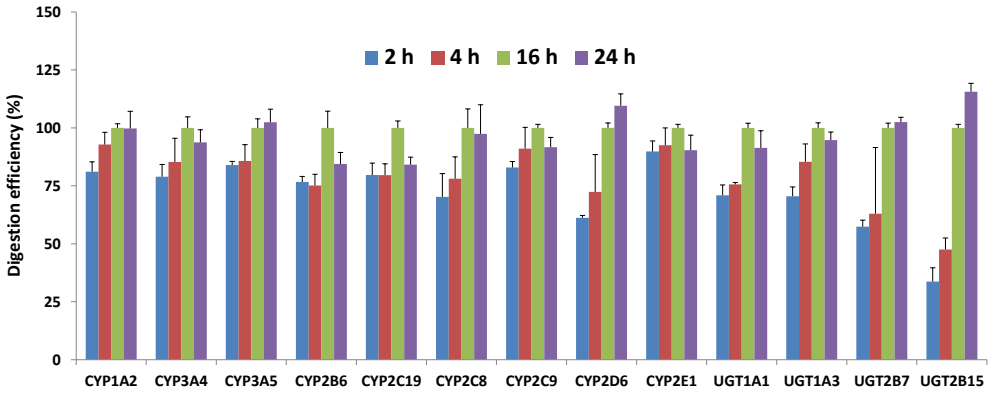
The Achilles Heel of proteomics: protein digestion



protein

trypsin





- Completeness of protein digestion is a prerequisite for assessing “absolute” protein abundance
- has to be determined for each protein and cannot be predicted
- QconCAT doe not overcome this issue as the resulting peptide chain is artificial
- SIL-proteins as internal standards may be an option (but: an in vitro model is needed, expensive)
- **Best practice so far:** characterize optimal digestion procedure (time / amount of trypsin), add marker protein as a surrogate for digestion (inter-sample variability can be minimized)

Gröer et al. 2014, J Pharm Biomed Anal

Critical aspects of mass spectrometry based proteomics

- Application of the Method -

Protein

- Biological / Clinical Question
- Protein sequence data (consider SNPs, PTMs)
- **Sample preparation (fractionation, whole lysate)**
- **Protein determination / digestion**

Peptide

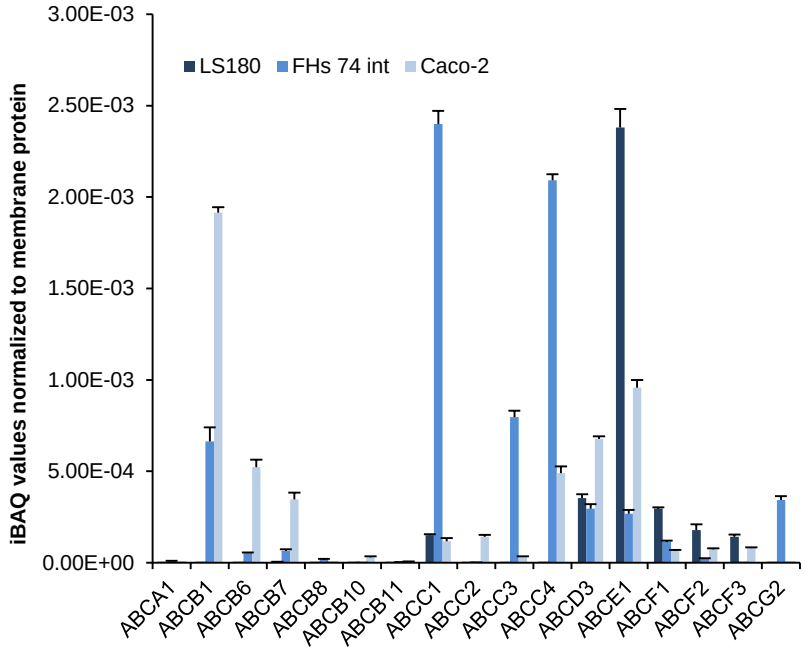
- Selection of appropriate peptides (in silico / wet-lab)
- **Number of peptides / protein**

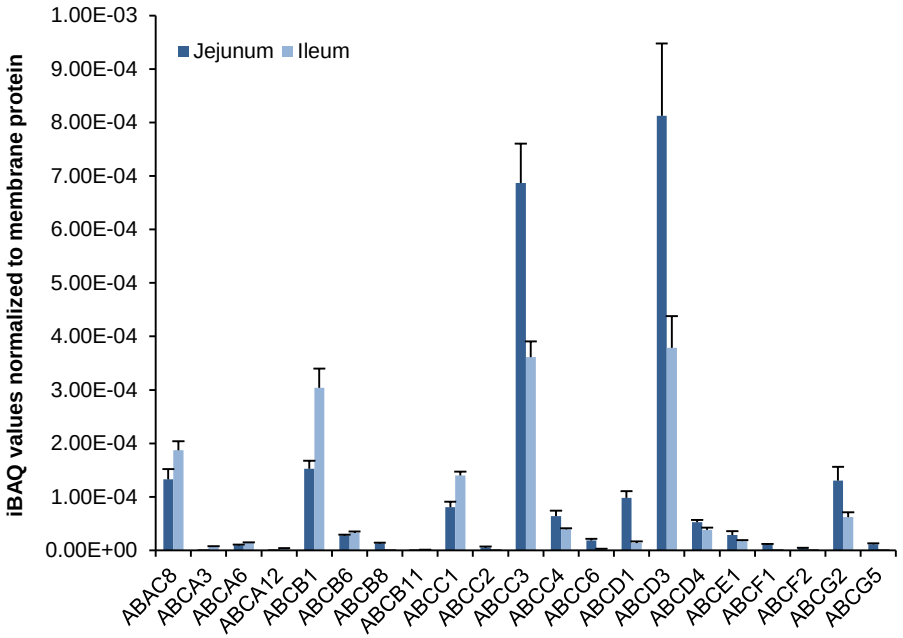
LC-MS/MS-Analysis

- **Global or targeted proteomic analysis**
- Selection of mass transitions (non- / scheduled MRM)
- **Data analysis (peptides, MRMs, normalization)**
- Validation of the method

Intestinal transporters: global proteomics

	total	Membrane proteins	ABC transporter	SLC transporter
Jejunum	3397	712	18	116
Ileum	3118	736	17	103
LS180	3993	420	7	47
FHs74	3457	473	14	80
Caco-2	3673	437	14	79





Absolute protein abundance by global proteomic data?

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

In-depth quantitative analysis and comparison of the human hepatocyte and hepatoma cell line HepG2 proteomes

Jacek R. Wiśniewski [a,*], Anna Vildhede [b], Agneta Norén [c], Per Artursson [b,d]

[a] Biochemical Proteomics Group, Department of Proteomics and Signal Transduction, Max
[b] Department of Pharmacy, Uppsala University, Uppsala, Sweden
[c] Department of Surgery, Uppsala University, Uppsala, Sweden
[d] Science for Life Laboratory, Uppsala, Sweden

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmacokinetics, Pharmacodynamics and Drug Transport and Metabolism

The Proteome of Filter-Grown Caco-2 Cells With a Focus on Proteins Involved in Drug Disposition

Magnus Ölander [1], Jacek R. Wiśniewski [2], Pär Matsson [1], Patrik Lundquist [1], Per Artursson [1, 3, 4, *]

[1] Department of Pharmacy, Uppsala University, 751 23 Uppsala, Sweden
[2] Biochemical Proteomics Group, Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
[3] Uppsala University Drug Optimization and Pharmaceutical Profiling Platform, Uppsala University, 751 23 Uppsala, Sweden
[4] Science for Life Laboratory Drug Discovery and Development Platform, Uppsala University, 751 23 Uppsala, Sweden

Journal of proteome research

Comparative Proteomic Analysis of Human Liver Tissue and Isolated Hepatocytes with a Focus on Proteins Determining Drug Exposure

Anna Vildhede,[†] Jacek R. Wiśniewski,[§] Agneta Norén,[‡] Maria Karlgren,[†] and Per Artursson[a,†,∥]

[†]Department of Pharmacy and [‡]Department of Surgery, Uppsala University, 751 05 Uppsala, Sweden
[§]Biochemical Proteomics Group, Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
[∥]Uppsala University Drug Optimization and Pharmaceutical Profiling Platform (UDOPP), Chemical Biology Consortium, Science for Life Laboratory, 750 03 Uppsala, Sweden

- Global proteomics is a powerful tool for high resolution identification of proteins (using many peptides, PTM also considered)
- no reference peptides are used → calculated "absolute" abundance is an estimation (assumption: each peptide generates the same intensity)
- **Limitations**: low abundance proteins may be overseen, same limitations as targeted proteomics

- more than 500 million Tweets per day
- People from Tibet contribute daily about 714286 tweets
- 330 million monthly active users → distribution around the world?

Critical aspects of mass spectrometry based proteomics

- Application of the Method -

Protein

- Biological / Clinical Question
- Protein sequence data (consider SNPs, PTMs)
- **Sample preparation (fractionation, whole lysate)**
- **Protein determination / digestion**

Peptide

- Selection of appropriate peptides (in silico / wet-lab)
- **Number of peptides / protein**

LC-MS/MS-Analysis

- **Global or targeted proteomic analysis**
- Selection of mass transitions (non- / scheduled MRM)
- **Data analysis (peptides, MRMs, normalization)**
- Validation of the method

Impact of different peptides, mass transitions and normalization

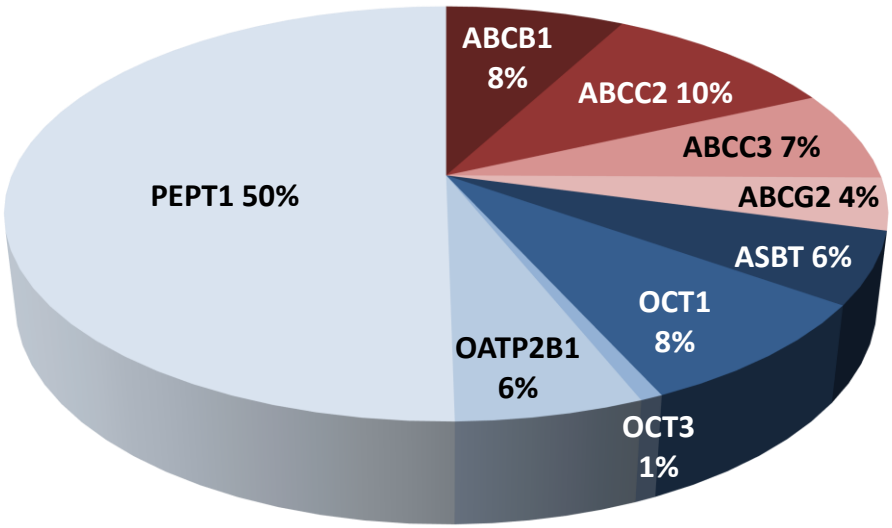
- **Different peptides** can result in different protein amounts → 2-3 peptides should be used
- **Different mass transitions** of each peptides should be separately analyzed (no sum)
- **Normalization**: very different → high variability of published data
  - to unspecific protein (BCA) or specific protein
  - to whole tissue / cell lysate or a certain fraction (membrane, microsomes)
  - Tissue samples represent a mixture of different cell types → housekeeper

UGT isoform	peptide ID	HLM 81 (pmol/mg) (n = 5)	% CV	HLM 74 (pmol/mg) (n = 5)	% CV
1A1	Peptide 1	3.4	4.2	46.1	8.0
1A1	Peptide 2	4.4	5.1	60.7	9.2
1A1	Peptide 66	7.3	21.2	82.4	9.9
1A3	Peptide 6	4.1	6.5	3.5	7.4
1A4	Peptide 15	4.2	7.3	7.3	13.1
1A4	Peptide 16	12.8	4.3	22.5	9.3
1A4	Peptide 67	33.5	8.4	53.8	5.8
1A5	Peptide 18	0	-	0	-
1A6	Peptide 3	11.0	4.6	13.5	8.9
1A6	Peptide 4	7.6	4.5	9.2	8.9
1A7[b]	Peptide 8	0	-	0	-
1A7	Peptide 47	0	-	0	-
1A8[b]	Peptide 25	0	-	0	-
1A9	Peptide 12	9.0	5.1	12.3	7.2
1A9	Peptide 27	13.8	7.1	16.9	9.0
1A10[b]	Peptide 13	0	-	0	-
1A10	Peptide 28	0	-	0	-
2B4	Peptide 54	20.8	7.5	43.2	7.2
2B4	Peptide 52	15.1	8.1	33.9	8.0
2B7	Peptide 34	26.0	4.6	47.2	8.7
2B7	Peptide 56	37.0	6.4	65.8	7.4
2B10	Peptide 37	6.5	9.4	8.1	12.5
2B15	Peptide 41	12.4	8.3	25.4	8.1
2B15	Peptide 61	13.7	4.9	29.2	8.0
2B17[c]	Peptide 42	0	-	0	-
2B17	Peptide 64	0	-	0	-

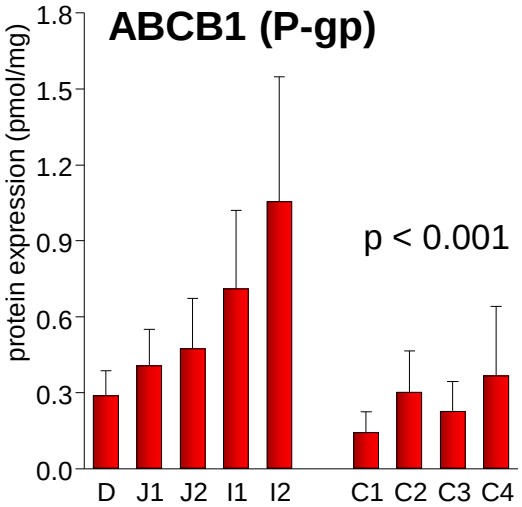
Fallon et al. 2013, J. Proteome Res.

Example: Intestinal transporter proteins

small intestine



no OATP1A2



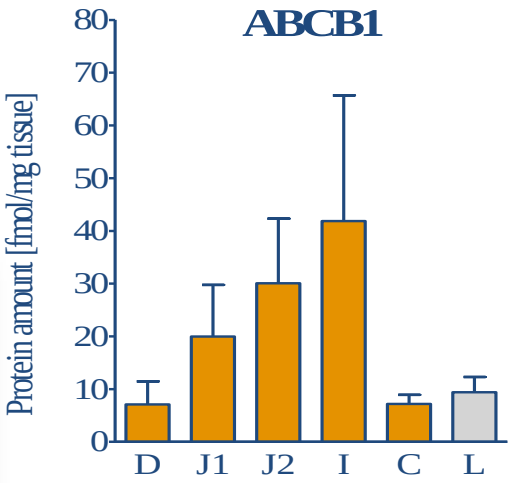
Limitations

- Protein determination in crude membrane fraction
- Only one peptide per protein
- No normalization on mucosal protein

Drozdzik, Mol Pharm. 2014 6;11:3547-55.

Example: protein abundance of transporters in human intestine and liver

Aim: To analyze protein abundance of of clinically relevant metabolizing enzymes (N=13) and transporters (N=20) along the entire human intestine and in the liver of 9 organ donors

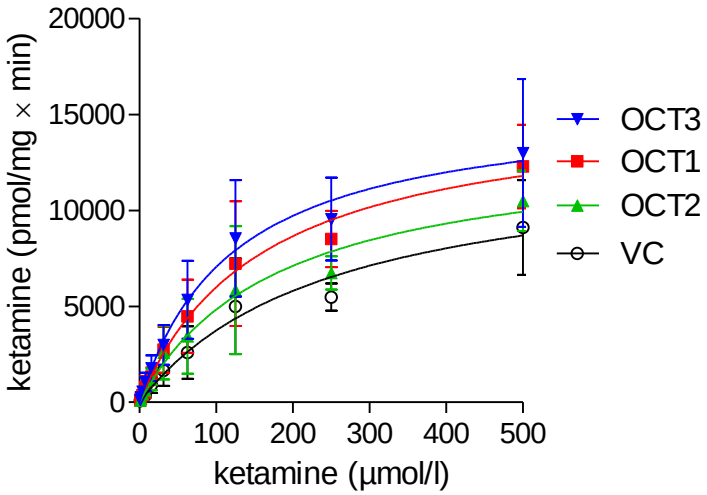


Limitations considered

- Protein determination in whole tissue lysate (FASP)
- 2-3 peptides per protein
- Normalization to villin-1 (enterocyte marker protein)

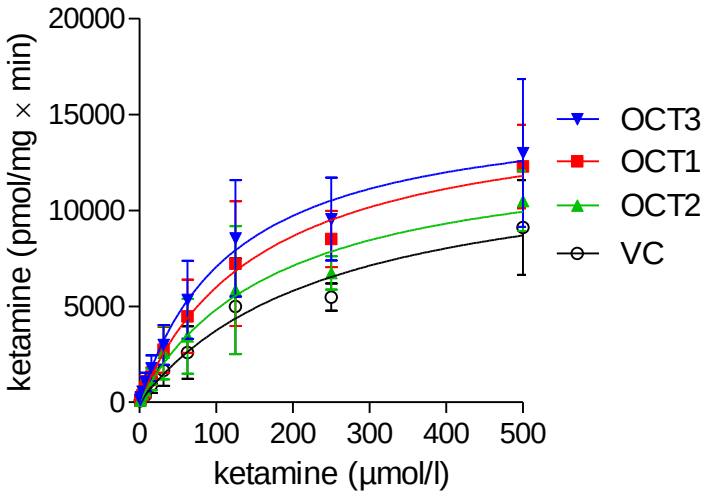
Drozdzik et al., *unpublished*

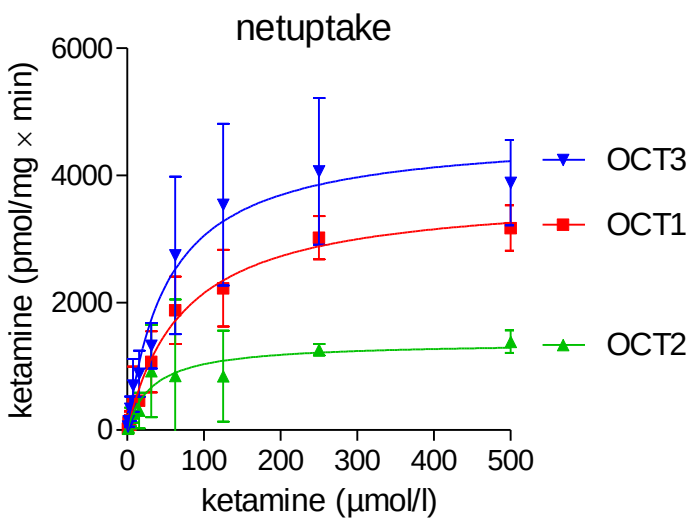
Example: in vitro transport of ketamine



Keiser, Mol Pharm. 2017

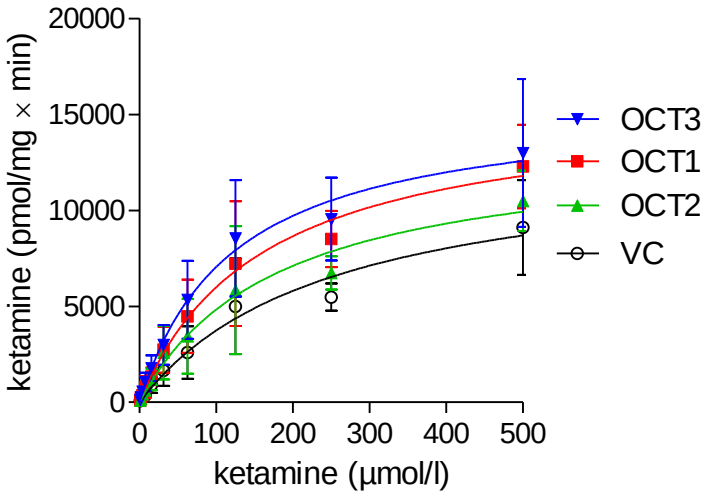
Example: in vitro transport of ketamine



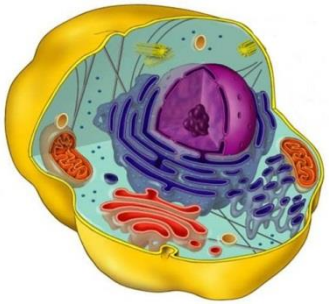


Keiser, Mol Pharm. 2017

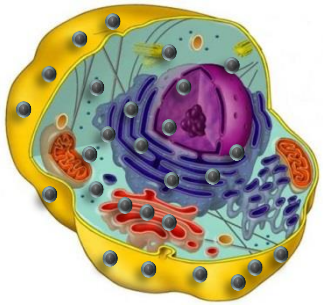
Example: in vitro transport of ketamine

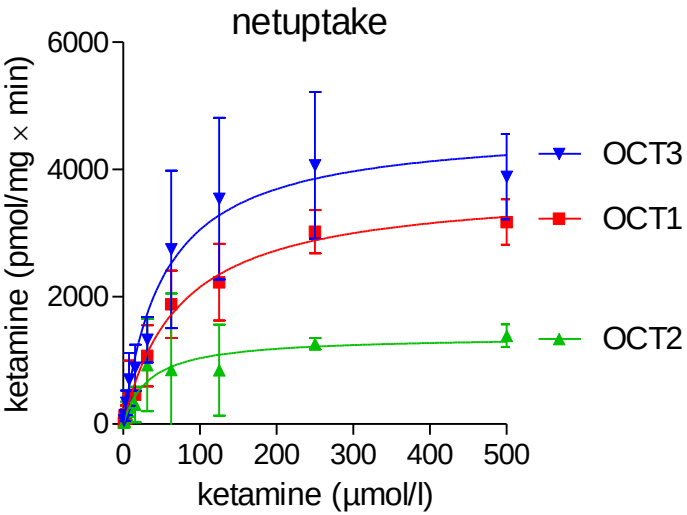


Total-Protein

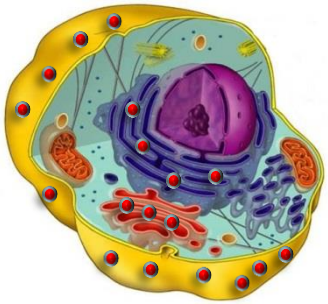


specific transporterprotein in total cell lysate

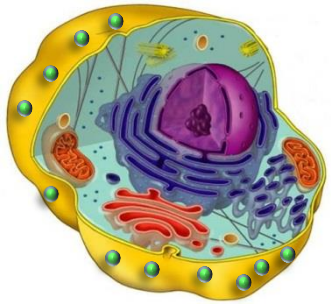




specific transporterprotein in crude membrane

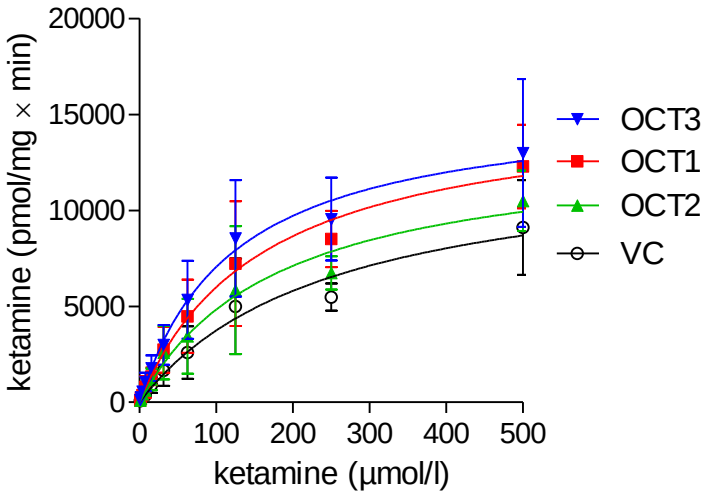


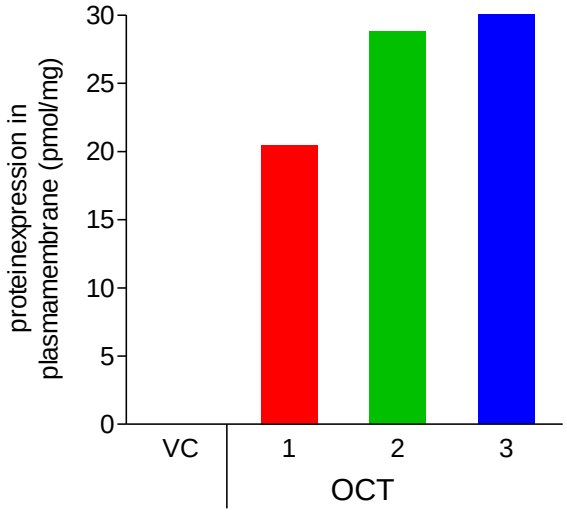
Specific transporterprotein in plasma membrane

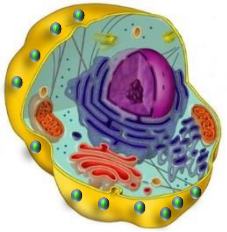


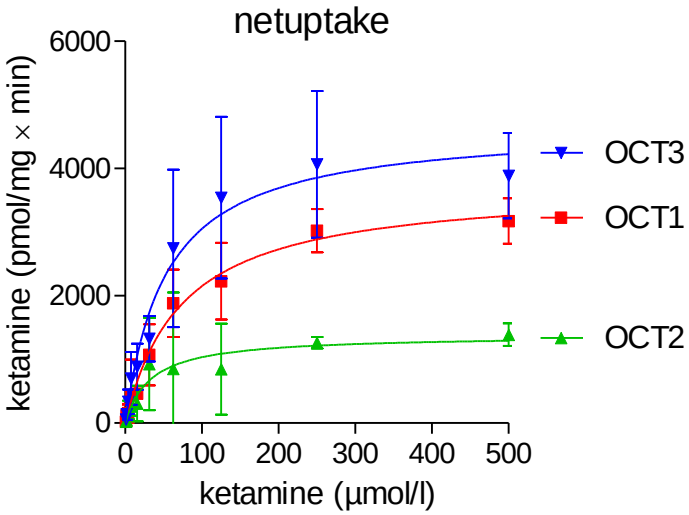
Keiser, Mol Pharm. 2017

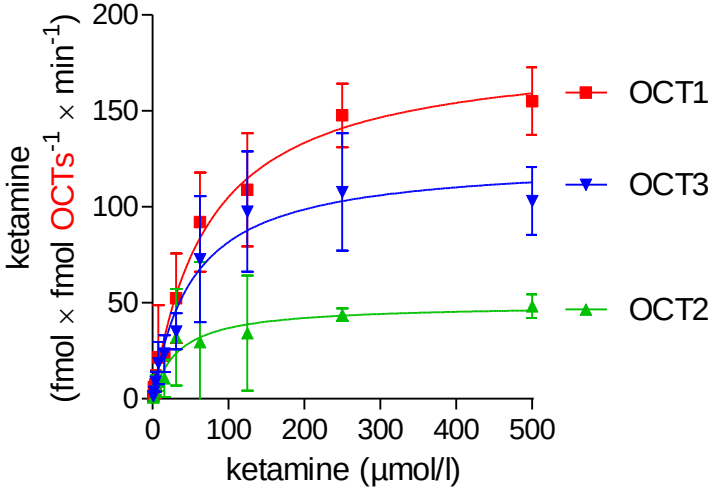
Example: in vitro transport of ketamine











Keiser, Mol Pharm. 2017

Summary & Conclusions

- LC-MS/MS-based targeted proteomics is a powerful tool to determine simultaneously absolute abundance of several ADME proteins
- High sensitivity, broad analytical range, high accuracy and precision and good reproducibility
- Limitation: completeness of protein digestion
- Sample preparation (e.g. fractionation) has a huge impact on the generated data (even higher than the used peptide)
- More than one peptide should be used (MRM analysis separately)
- Protein data should be generated from whole tisssue lysate (FASP) and not from enriched fractions
- Global proteomics is a complementary technique but provides per se no absolute data

Universitätsmedizin GREIFSWALD

C_DAT Center of Drug Absorption and Transport

editorial Journal of proteome research

Can Proteomics Retire the Western Blot?

Proteomics has made great strides toward biologically useful applications, but too often proteomics researchers and biologists still inhabit different worlds. Proteomics as a discipline grew up with the ambitions and pretensions of the genome projects, but biologists mostly want proteomics technology to solve their humdrum analytical problems. The truth is in between, and here I argue that true integration of proteomics technology into molecular biology laboratories could be a paradigm shift for all of biology and biomedicine.

Biologists currently are stuck in a time warp—they basically

the researcher the assurance that the investigated effect is one of the major ones. Proteomics also can reveal the modification status of the protein, which currently is seldom probed and, if so, only for specific modifications. In most cases, proteomics will reveal more changes than previously anticipated and provide a wealth of new discoveries and hypotheses to follow.

What do we have to do to make this happen? Basically, we have to make quantitative proteome measurements as accessible and convenient as western blots are now. This may seem like a tall order, but it may not be so for long. First, proteome

Matthias Mann, 2008

LC-MS/MS-based proteomics: need for a consensus

"*Towards Reaching a Consensus on Using Quantitative LC-MS/MS Proteomics in Translational DMPK/PD Research*"
ISSX Workshop, 27th -28th September 2018, Cambridge, MA

UGT1A3	SLCO1B3	ABCC2	
UGT2B7	SLCO2B1	ABCB1	
UGT2B15	SLC22A1	ABCG2	

molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

Variability in Mass Spectrometry-based Quantification of Clinically Relevant Drug Transporters and Drug Metabolizing Enzymes

Christine Wegler,[†,‡] Fabienne Z. Gaugaz,[†] Tommy B. Andersson,[‡] Jacek R. Wiśniewski,[§] Diana Busch,[∥] Christian Gröer,[∥] Stefan Oswald,[∥] Agneta Norén,[⊥] Frederik Weiss,[#] Helen S. Hammer,[#] Thomas O. Joos,[#] Oliver Poetz,[#] Brahim Achour,[▽] Amin Rostami-Hodjegan,[▽] Evita van de Steeg,[◆] Heleen M. Wortelboer,[◆] and Per Artursson[*,†]

Wegler et al. 2017, Mol Pharm

Acknowledgements

Bundesministerium für Bildung und Forschung

INNOPROFILE UNTERNEHMEN REGION
Die BMBF-Innovationsinitiative Neue Länder

University Medicine Greifswald, C_DAT

Clinical Pharmacology

Markus Keiser
Diana Busch
Janett Müller
Anja Fritz

University of Washington, Sweden

Department of Pharmaceutics

Per Artursson
Christine Wegeler

University of Washington, Seattle

Department of Pharmaceutics

Prof. Dr. Jashvant Unadkat
Dr. Bhagwat Prasad

University of Stettin, Poland

Clinical Pharmacology

Marek Drozdzik
Marek Ostrowski
Mateusz Kurzawski

Pfizer Inc., Groton, CT

Pharmacokinetics & Drug Metabolism

Yurong Lai
Larissa Balogh
Charles Rotter

Thank you for your attention