

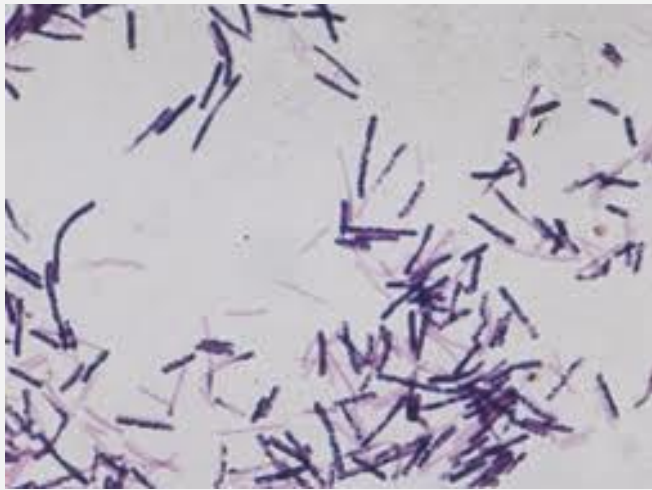
Impact of Clostridial Glucosylating Toxins on the Proteome of Colonocytes

Andreas Pich

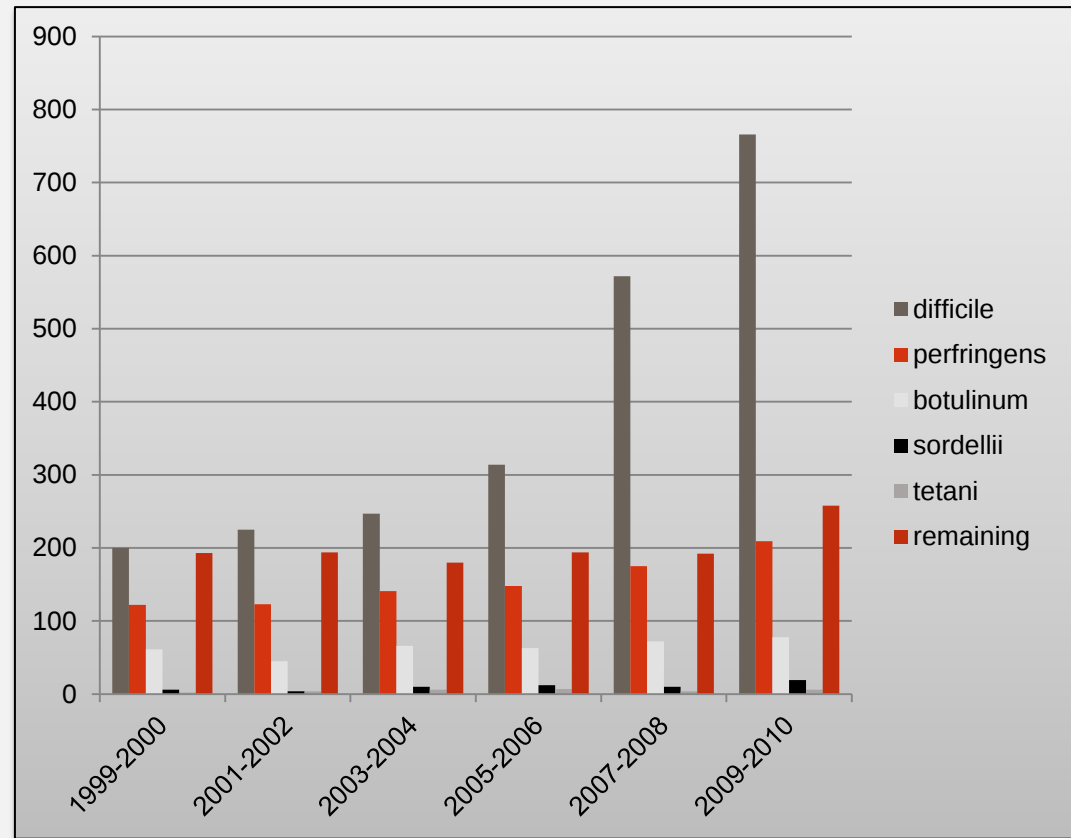


Hannover Medical School
Institut of Toxicology

Clostridium difficile



Gram-stain of *C. difficile*



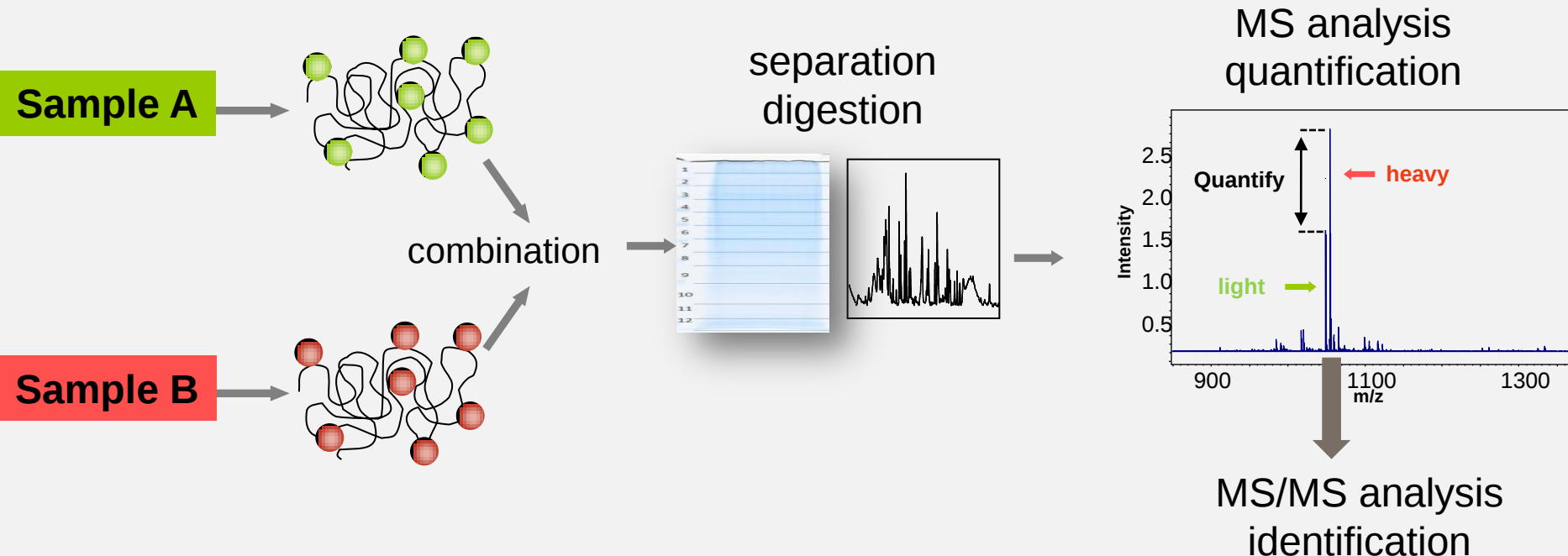
Proteome analysis of ToxinA treated Caco-2 cells

- Quantitative MS-analyses
 - stable isotope labeling (SILAC)
- Verification
 - label free MRM analysis
 - Western blot analysis
- Data mining
 - GO annotations, DAVID
 - interaction analysis STRING
- Objectives
 - new aspects of CGT
 - effects of inactive **gdTcdA** mutant

Quantitative MS based on stable isotopes

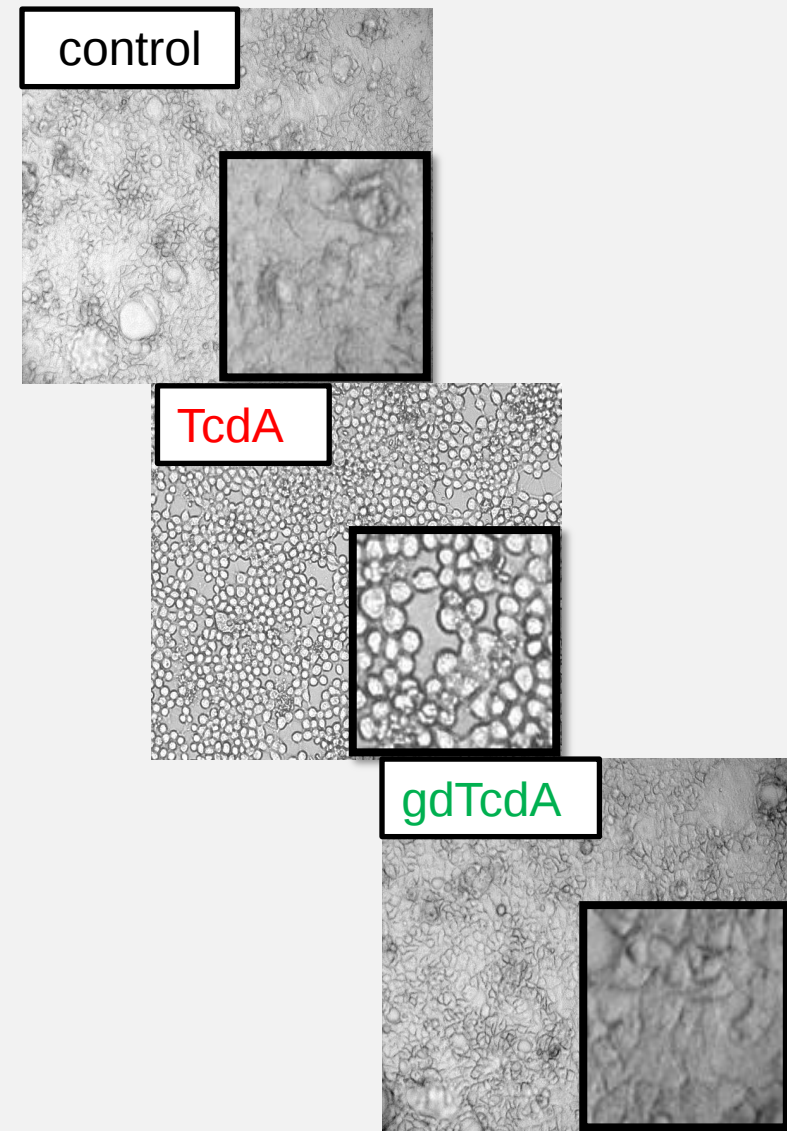
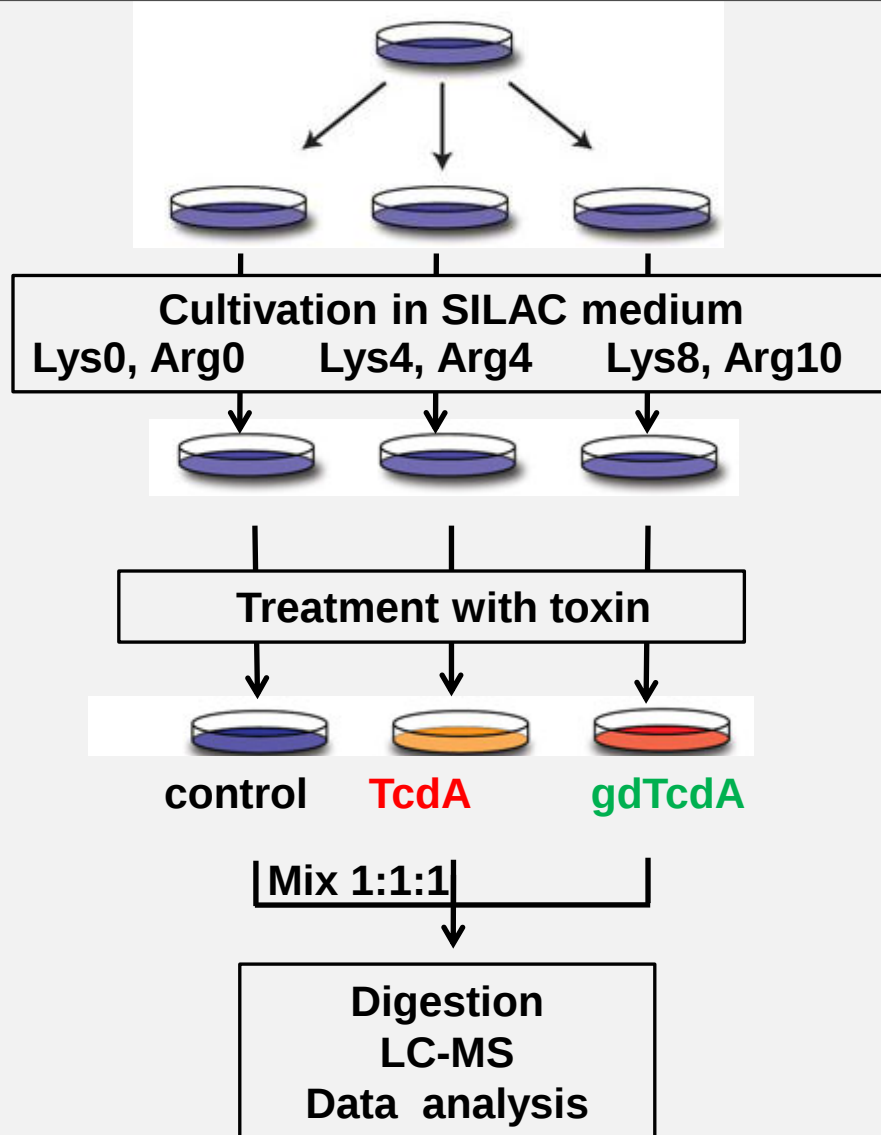
- protein labeling with heavy and light amino acids

• $^{12}\text{C}/^{13}\text{C}$ $\longrightarrow$ arginine, lysine



SILAC, Ong et al. 2002

Workflow toxin treatment and SILAC



LC-MS data analysis

peptides

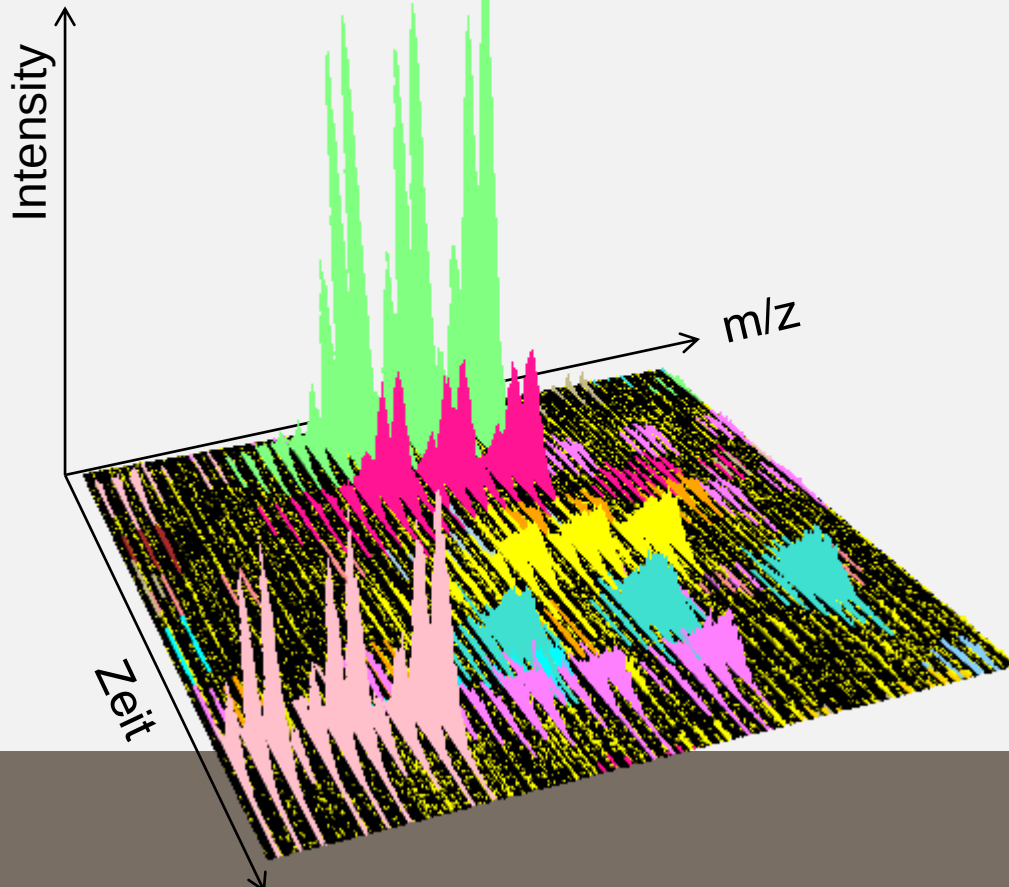
Chromatography

MS

MQ

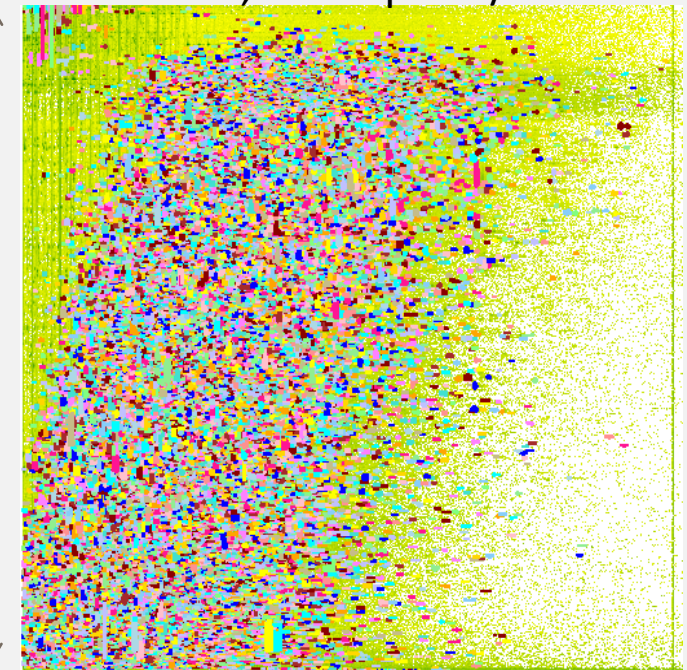
TcdA gdTcdA control

light | medium | heavy



140 min

20,000 Triplets /LC-MS run



m/z 300-1600

Number of identified proteins

Identified proteins

6643

- **Identification**

- triplicate analysis
- two peptides/protein
- false discovery rate < 0.01

Quantified proteins

5111

- **Quantification**

- triplicate analysis
- two peptides/protein L/M/H

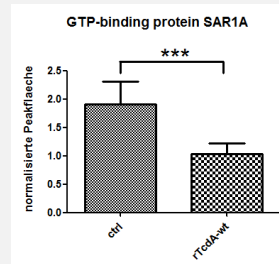
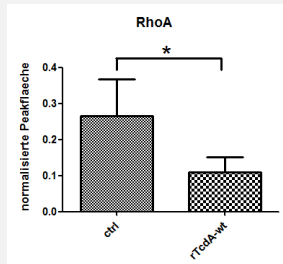
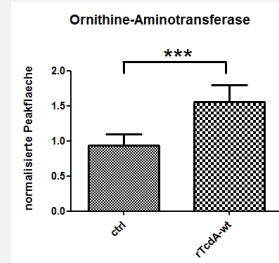
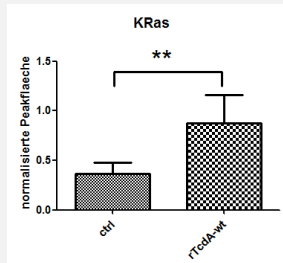
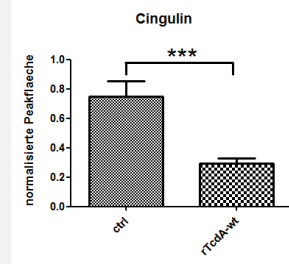
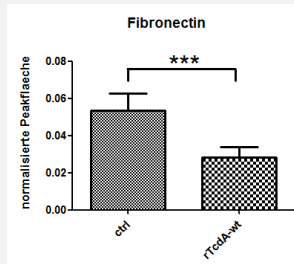
Regulated proteins

874

- **Regulation**

- p-value < 0.05
- threshold >1.5

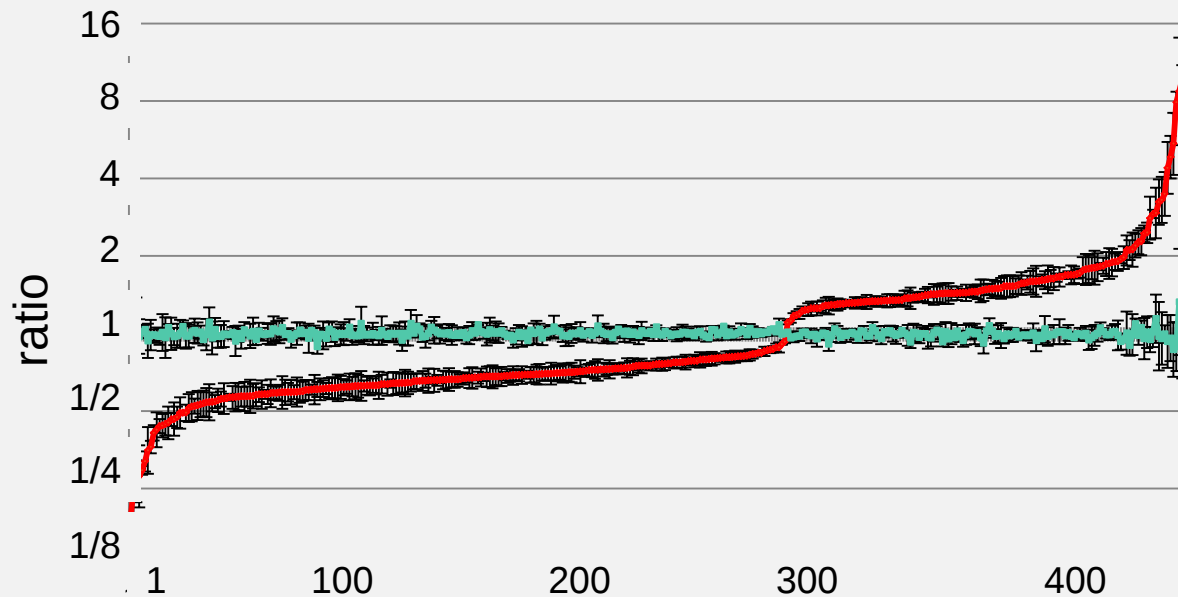
Verification by Western blot and label-free MRM analysis



Protein	label free	isotope label
Fibronectin	0.52	0,58
Cingulin	0,39	0,48
Ornithine-Aminotransferase	1,66	1,88
GTPase KRas	2,38	1,72
RhoA	0,41	0,42
GTP-binding protein SAR1A	0,54	0,59

TcdA vs gdTcdA effects (24 h)

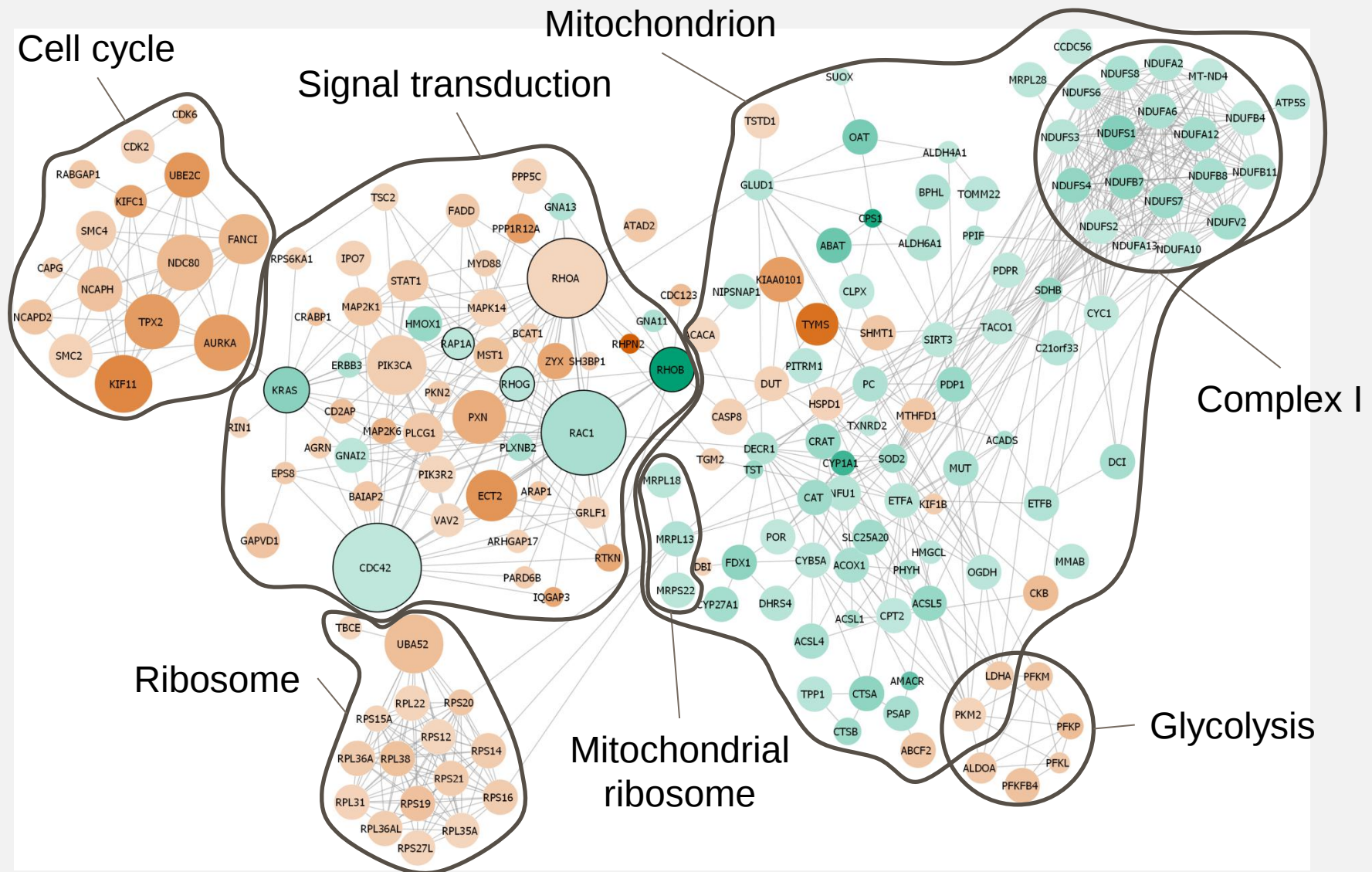
874 Proteins with different abundance
($p < 0.05$; threshold regulation factor 1.4)



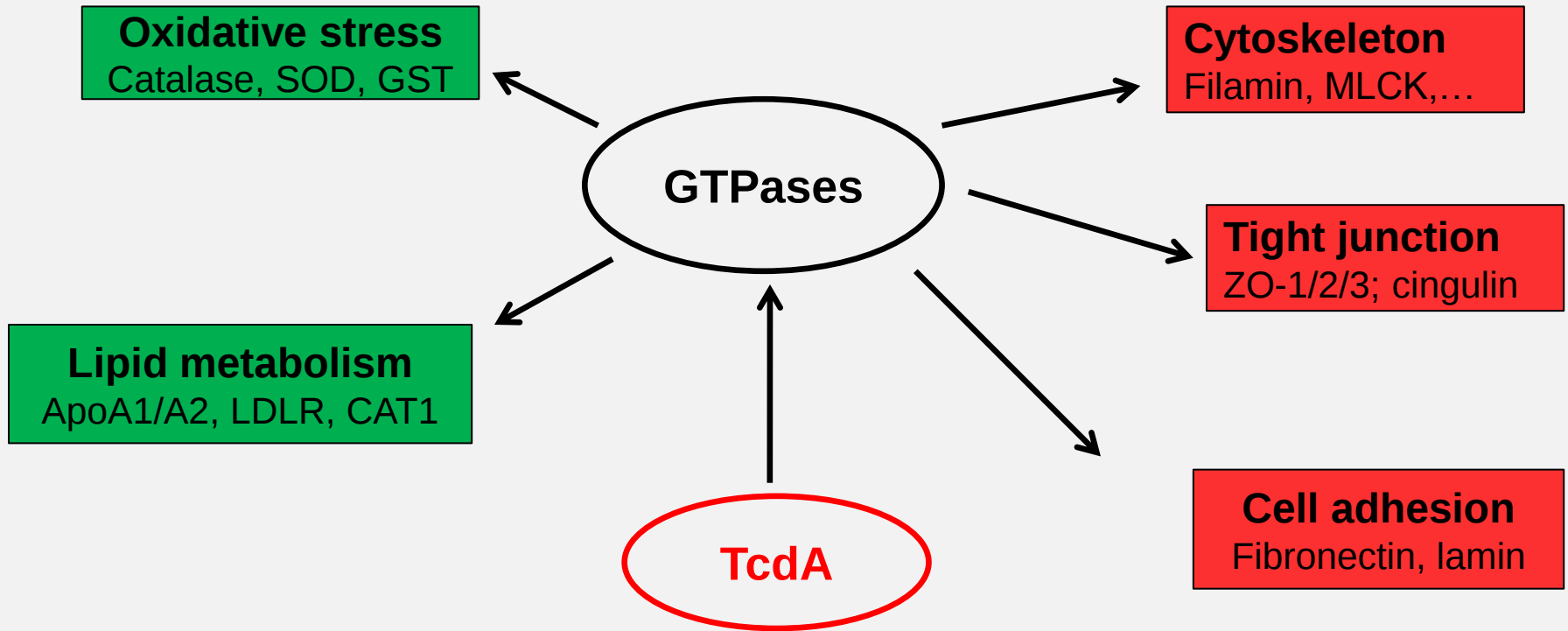
(+) TcdA effects

(-) gdTcdA: no effect

TcdA-responsive proteins

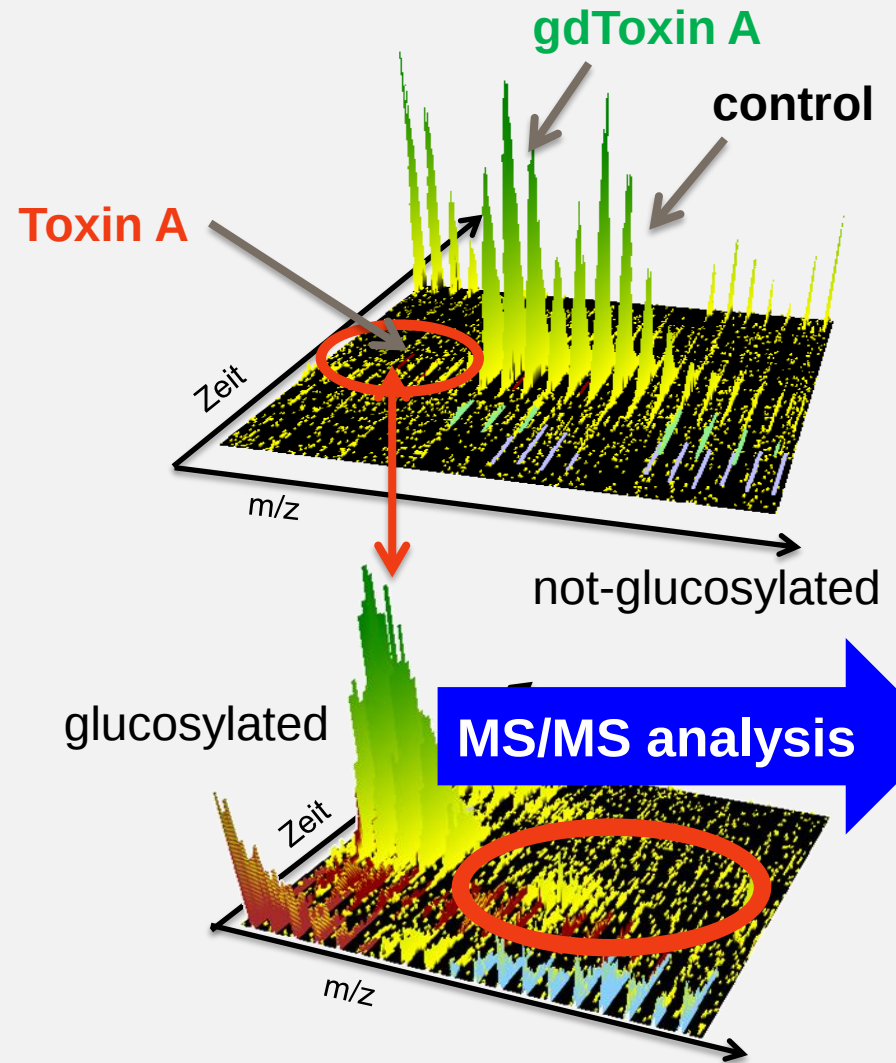
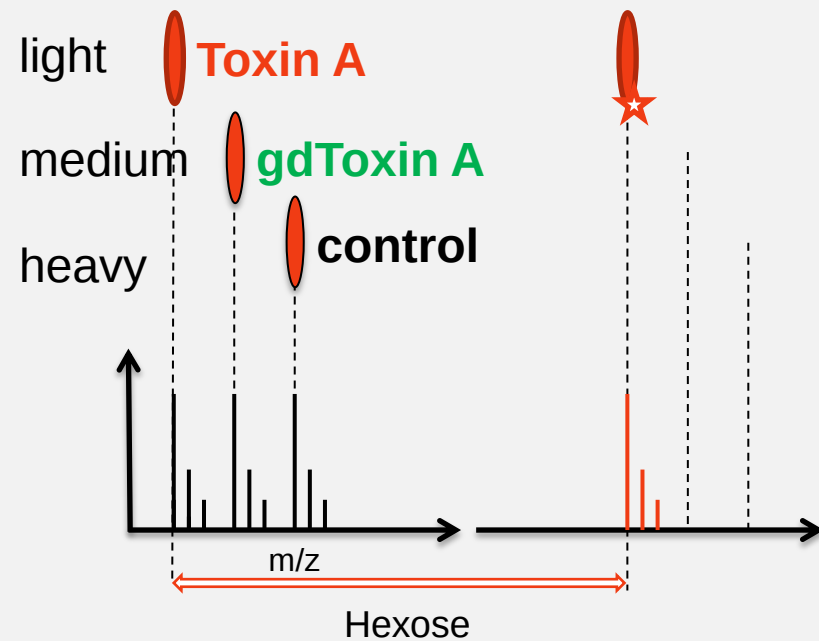


TcdA-responsive proteins

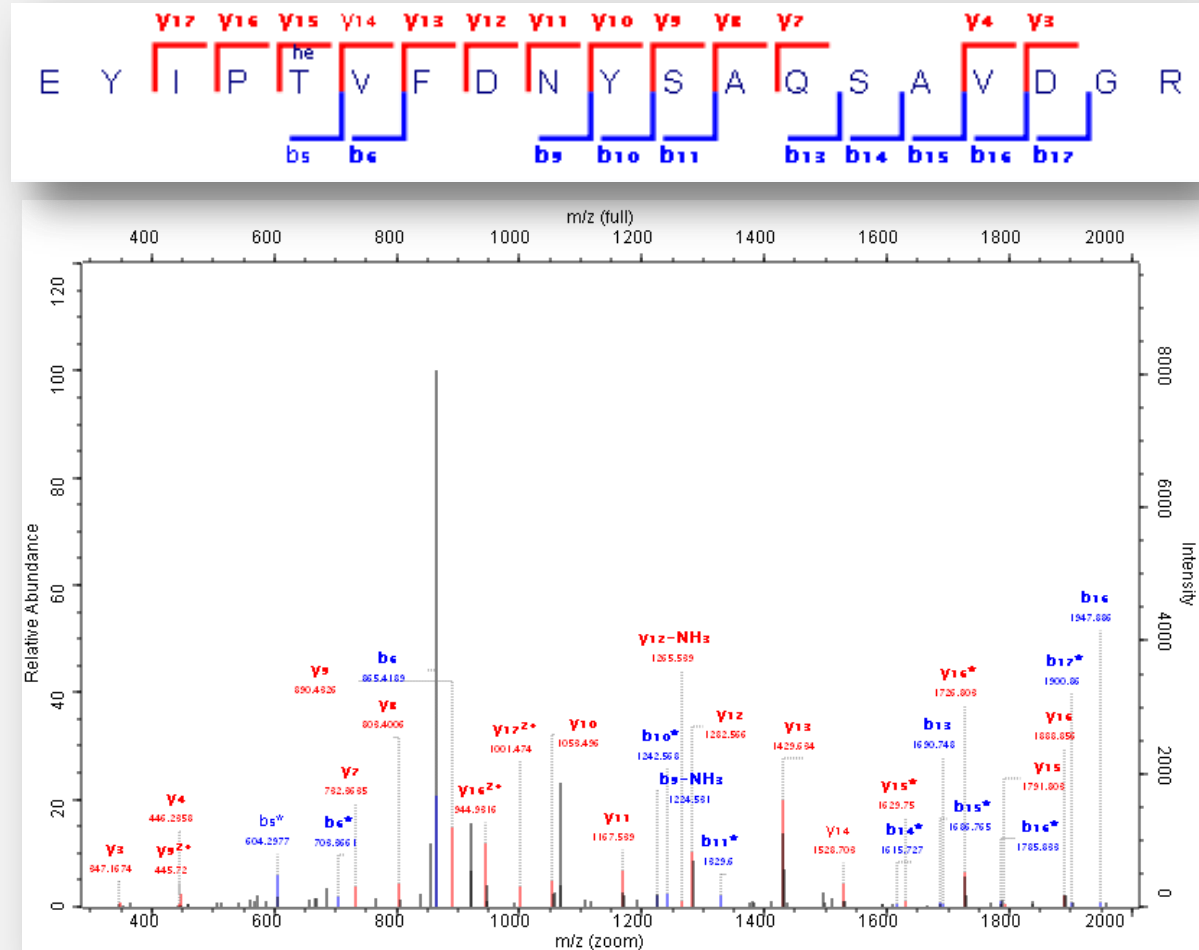


In vivo detection of glucosylation

triplex SILAC LC-MS

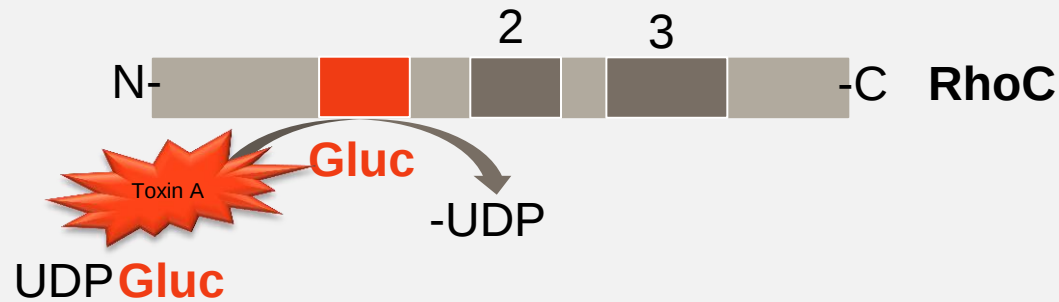


In vivo detection of glucosylation

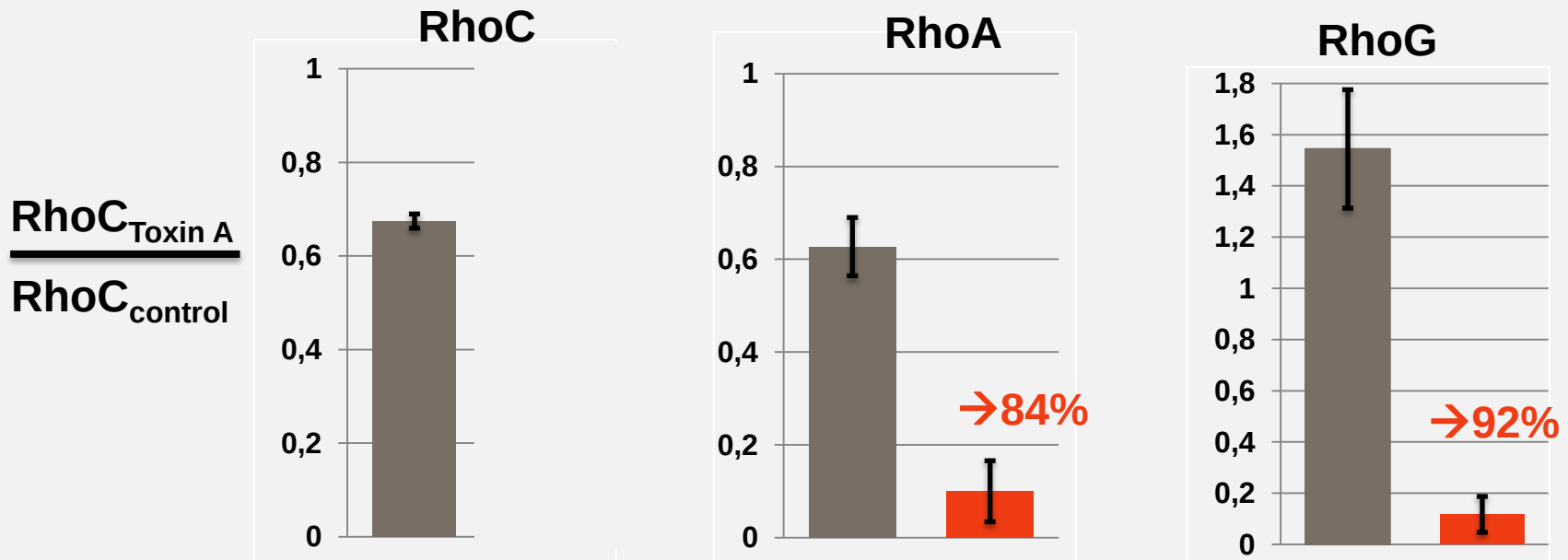


MS/MS analysis

In vivo quantification of glucosylation



peptide 2+3 **Non gluc peptide**



In vivo glucosylation of small GTPases

GTPase	TcdA <i>C. difficile</i>		TcdB <i>C. difficile</i>		Lethal Toxin <i>C. sordellii</i>	
	known	MS	known	MS	known	MS
RhoA	+	84 %	+	91 %	-	-
RhoB	+	>99 %	+	>99 %	-	-
RhoC	+	90 %	+	93 %	-	-
Rac1	+	-	+	-	+	-
RhoG	+	92 %	+	87 %	+	94 %
Cdc42	+	-	+	-	+	-
Rap1	-	97 %	-	94 %	+	91 %
Rap2	+	93 %	-	96 %	+	95 %
Ras	-	72 %	-	84 %	+	88 %
RalA/B	-	27 %	-	43 %	+	-

Conclusion

- **874 TcdA-responsive proteins identified**
 - Expected: cytoskeleton, adhesion, tight junctions cell cycle
 - Unexpected: mitochondria, ribosomes, metabolic processes
- **no gdTcdA-responsive proteins (24 h treatment)**
 - all observed changes were due to glucosyltransferase activity
- ***in vivo* detection and quantification of GTPase glucosylation**
 - new targets: Ras Ral Rap

Thanks to:

Nelli Jochim
Johannes Zeiser
Ralf Gerhard
Ingo Just

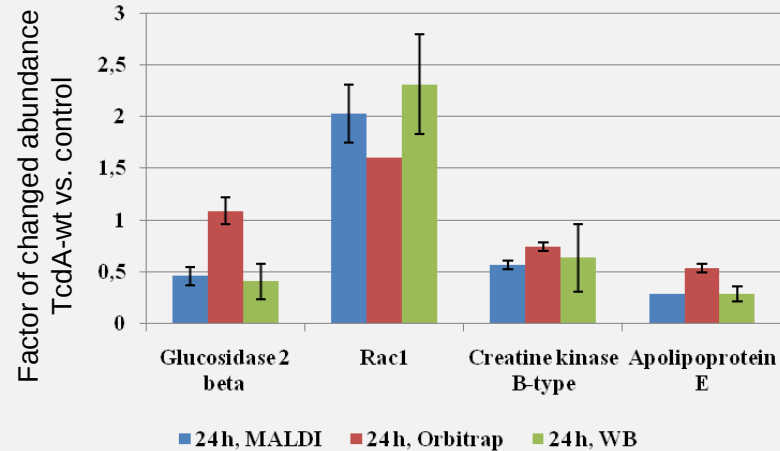
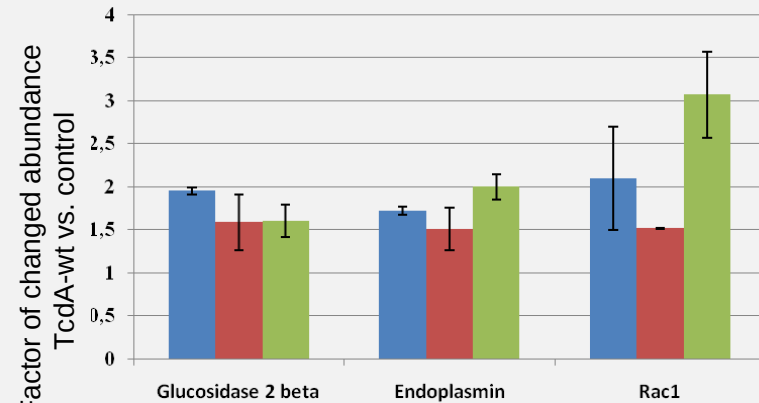
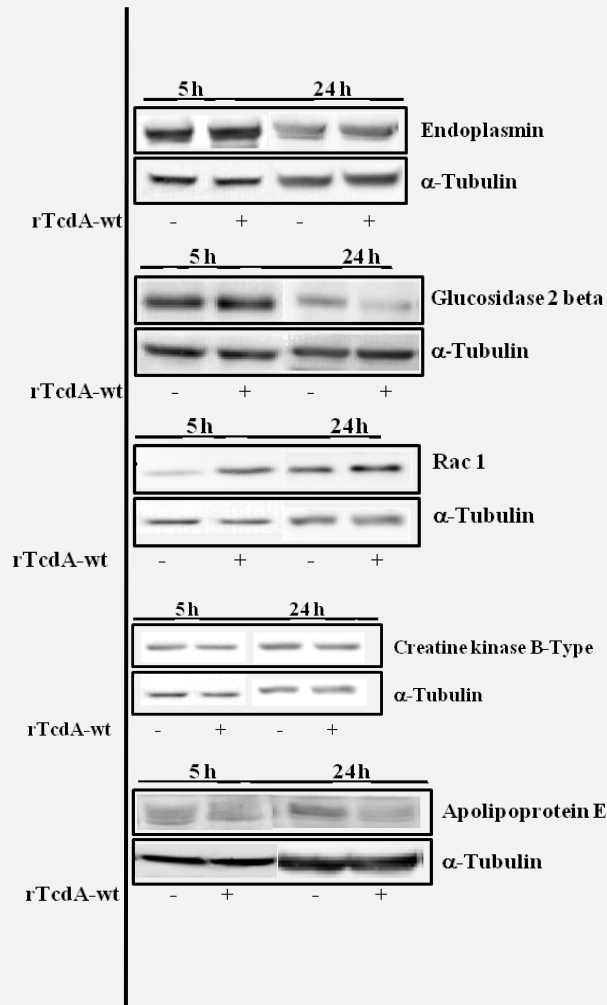
MS-Core Facility
Bijon Chatterji
Anke Schröder
Karin Agternkamp
Karsten Heidrich
Jonas Krüger
Sebastian Doberenz

Institute of Toxicology
Helma Tatge
Harald Genth
Astrid Rohrbeck
Andreas Rummel
Alexandra Olling



Institute of Toxicology - MHH campus

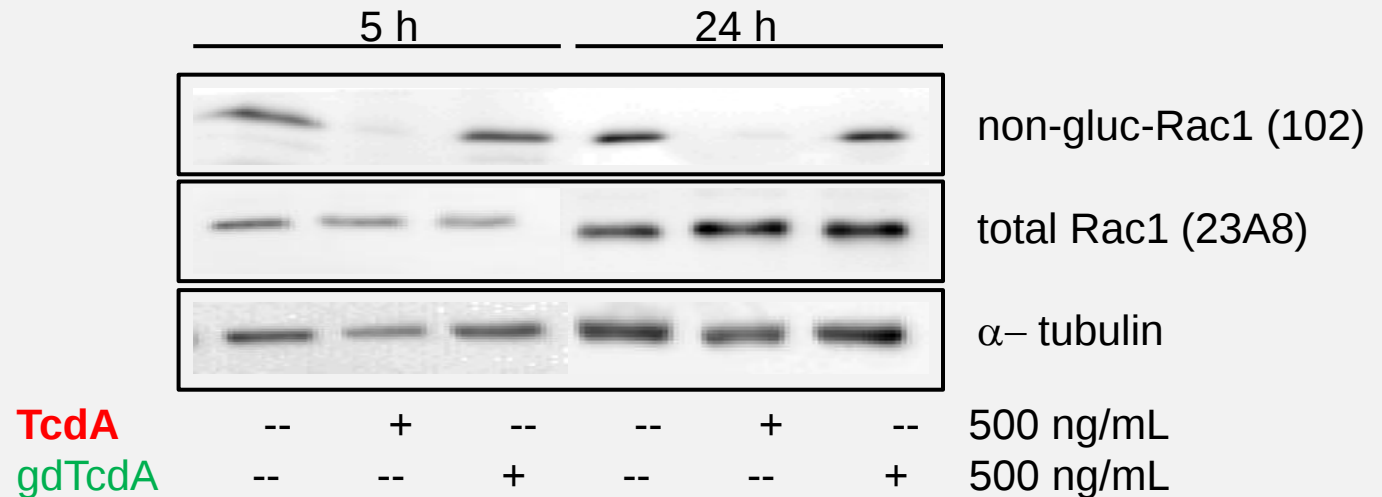
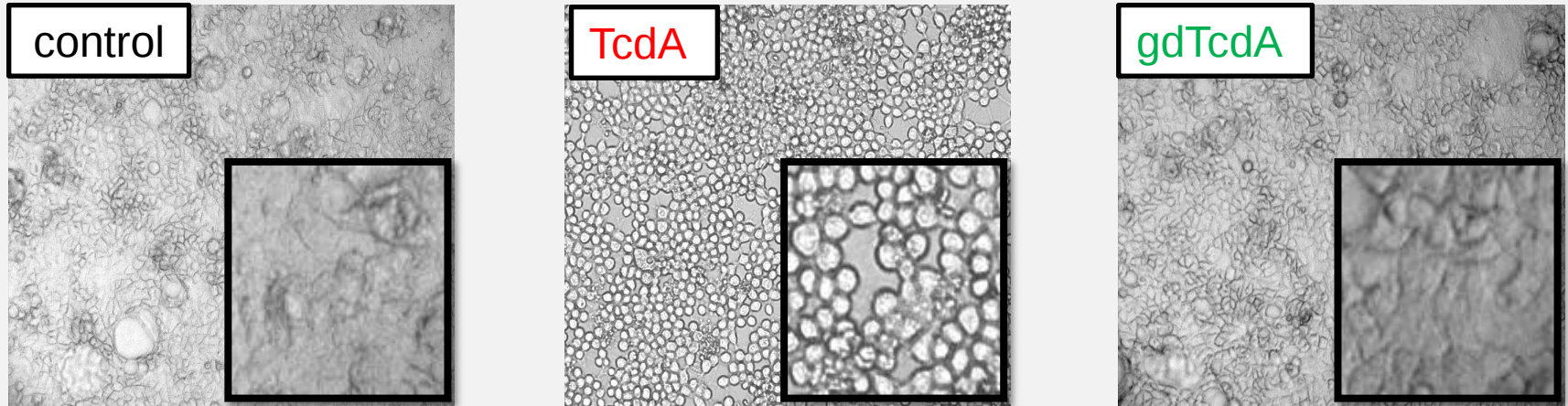
Verification by Western blot analysis



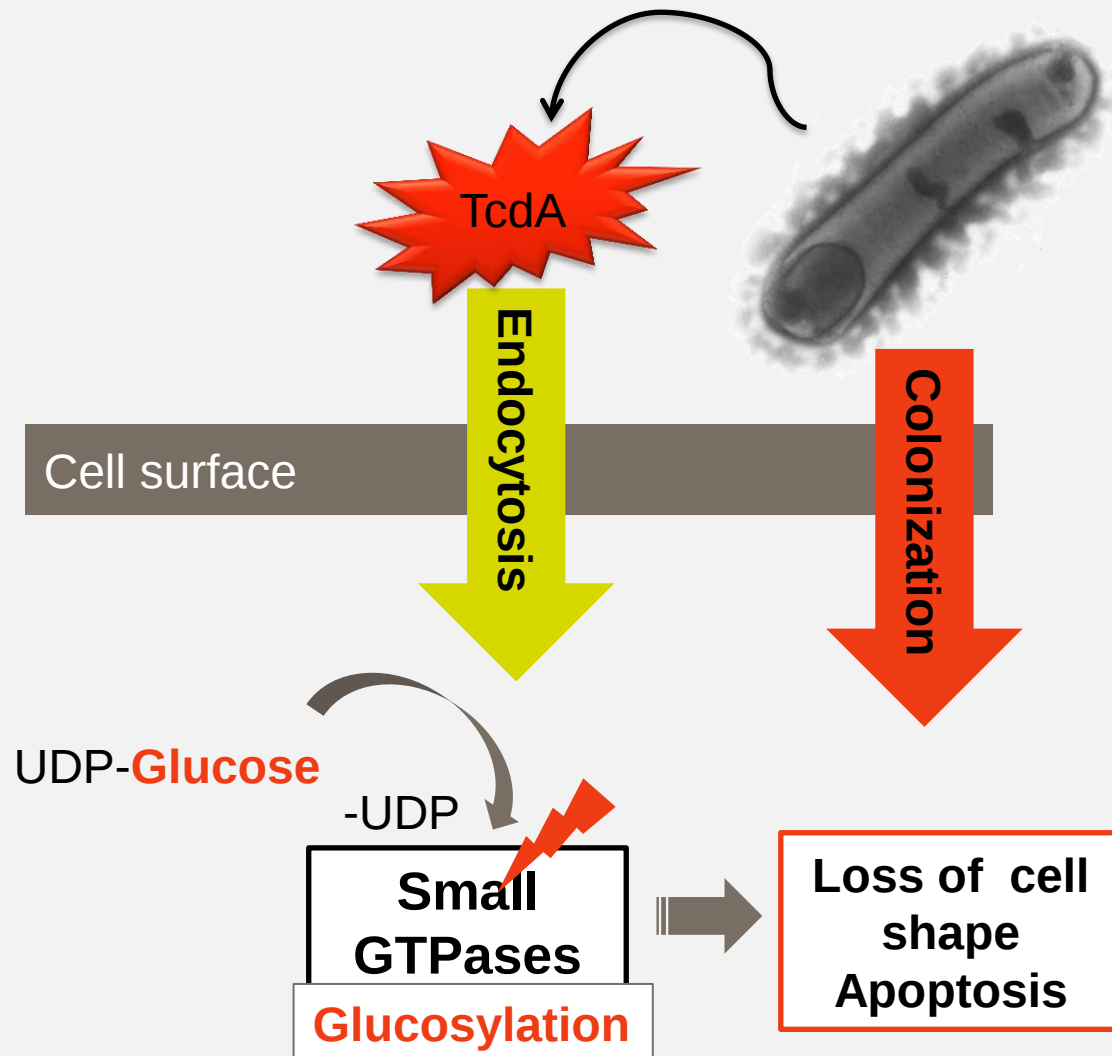
5 h

24 h

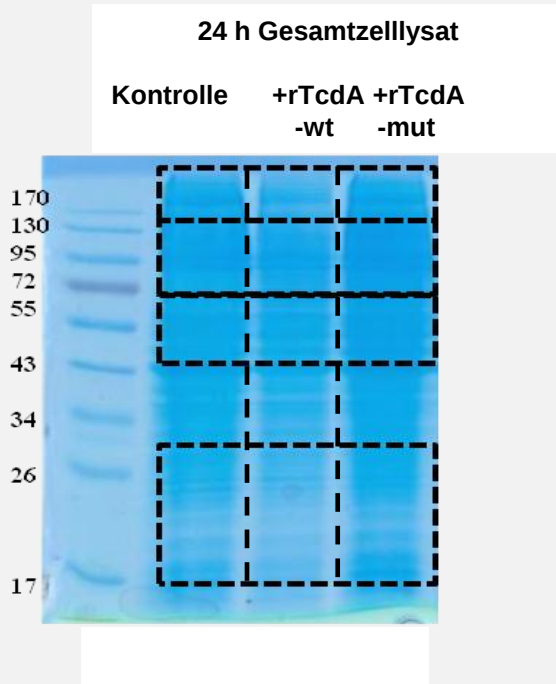
Effects of toxinA on Caco-2 cells



Clostridium difficile infection



MRM-Experimente



Entwicklung von MRM-Methoden für:

8 regulierte Proteine

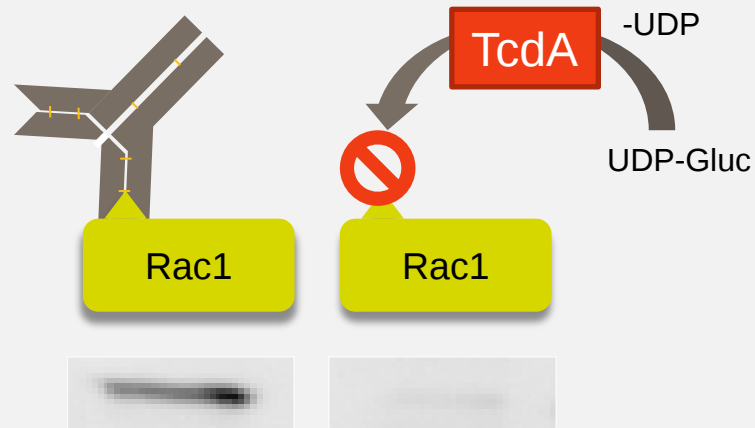
6 Kontrollproteine

→ Interne Standards zur Normalisierung

→ 2 Peptide/Protein

→ 2 Fragmente/Peptid

In vivo detection of glucosylation



TcdA-responsive proteins (24 h)

Signal
transduction

Cell
cycle

Ribosome

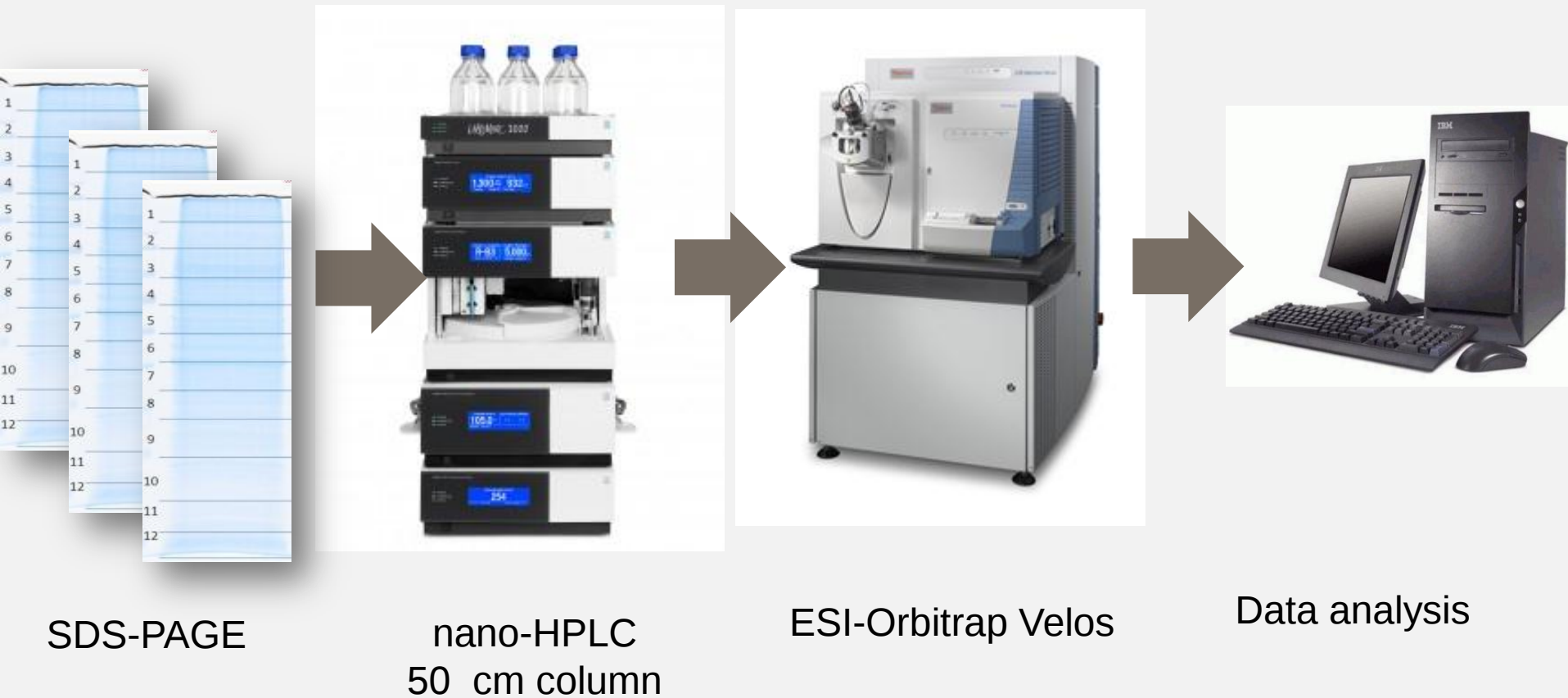
Mitochondrion

Lysosome

Respiratory chain,
mitochondrion



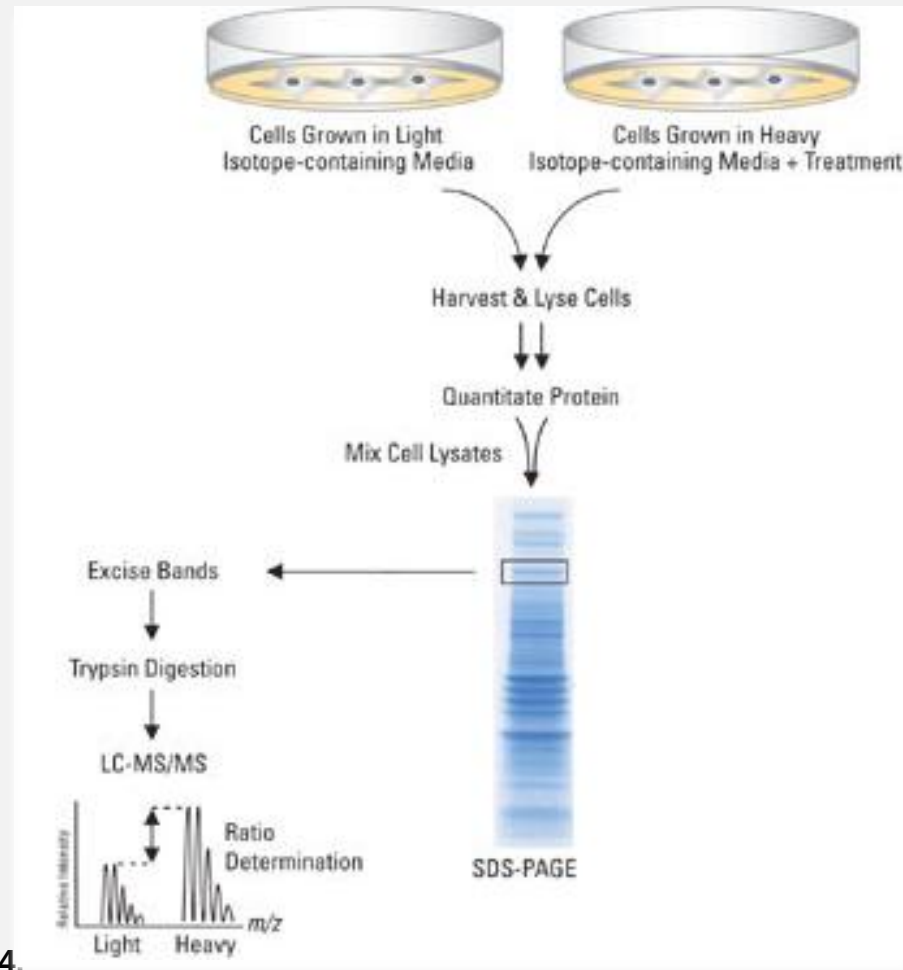
GeLC-MS-Method



Stable isotope labeling by amino acids in culture (SILAC)

^{13}C -Arginin

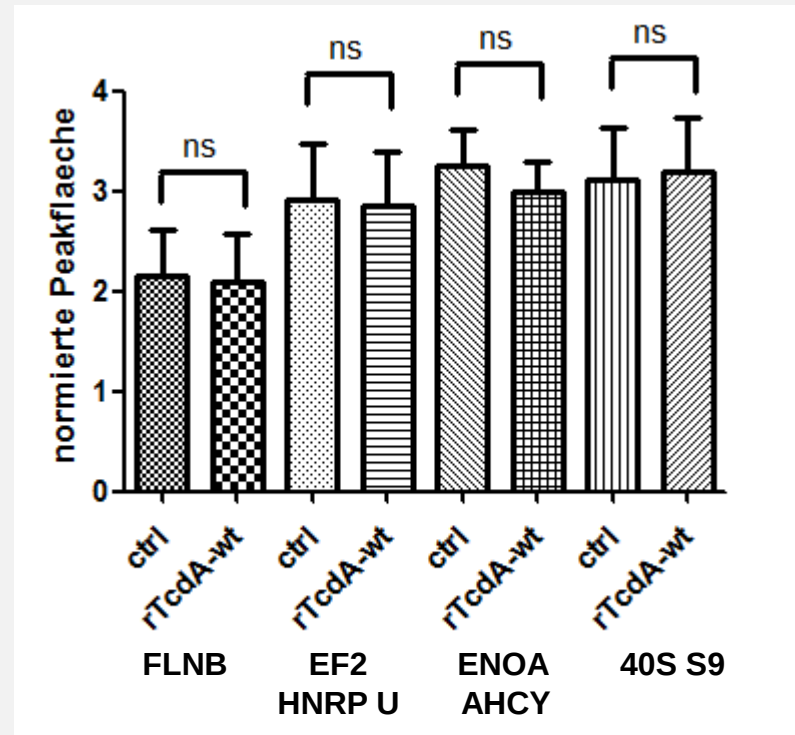
^{13}C -Lysin



Krüger M, et al., Mann M Cell. 2008 Jul 134:353-64.

Internal standard proteins

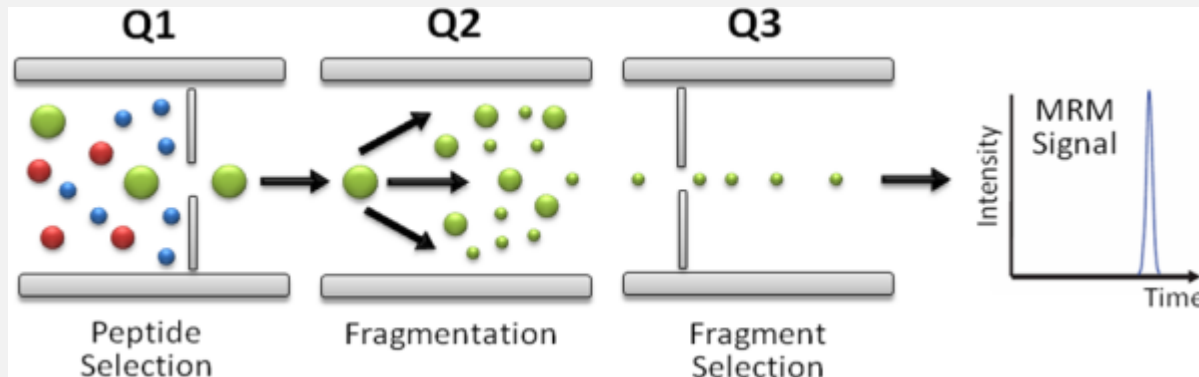
Standards	ICPL
Filamin B (FLNB)	$0,82 \pm 0,1$
Elongation factor 2 (EF2)	$0,82 \pm 0,12$
HNRP U	$1,27 \pm 0,01$
Alpha-Enolase (ENOA)	$0,90 \pm 0,02$
Adenosylhomocysteinase (AHCY)	$0,94 \pm 0,09$
40S S9	$0,94 \pm 0,07$



Verification of protein quantification

Protein	label free	isotope label
Fibronectin	0,52	0,58
Cingulin	0,39	0,48
Ornithine- Aminotransferase	1,66	1,88
Fuctose-P ₂ - Aldolase	0,68	0,66
Apolipoprotein A-I	0,30	0,41
GTPase KRas	2,38	1,72
RhoA	0,41	0,42
GTP-binding protein SAR1A	0,54	0,59

Verification by MRM assay and label-free quantification



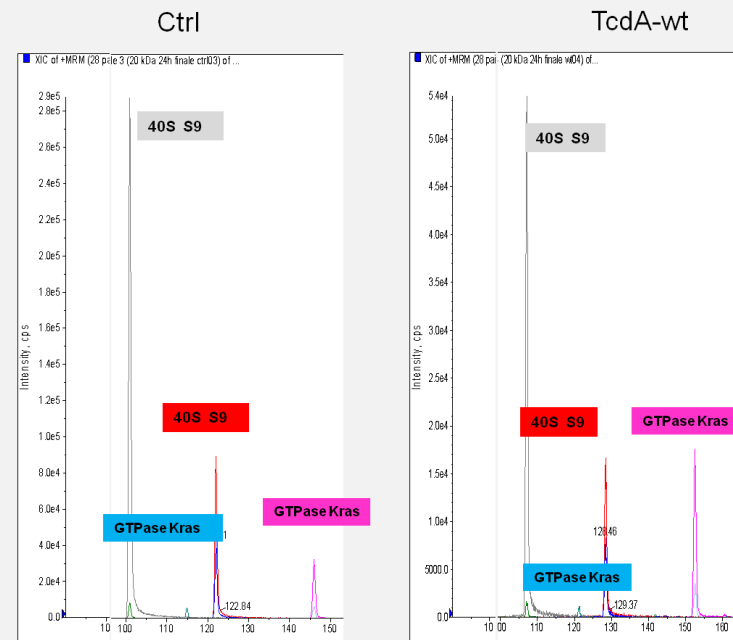
TcdA responsive proteins

House-keeping proteins

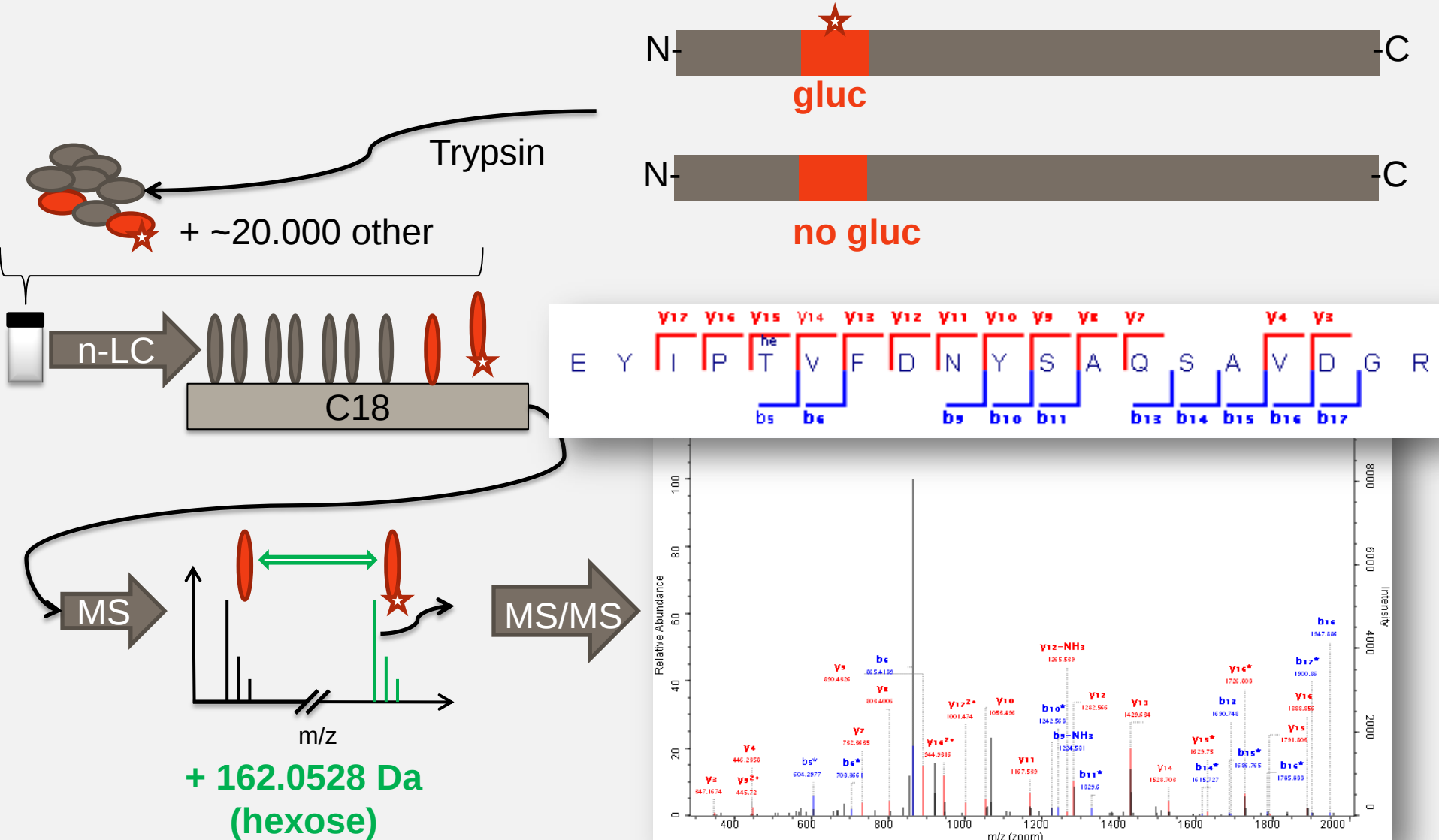
2 peptides/protein
2 fragments/peptide



4 transitions / protein

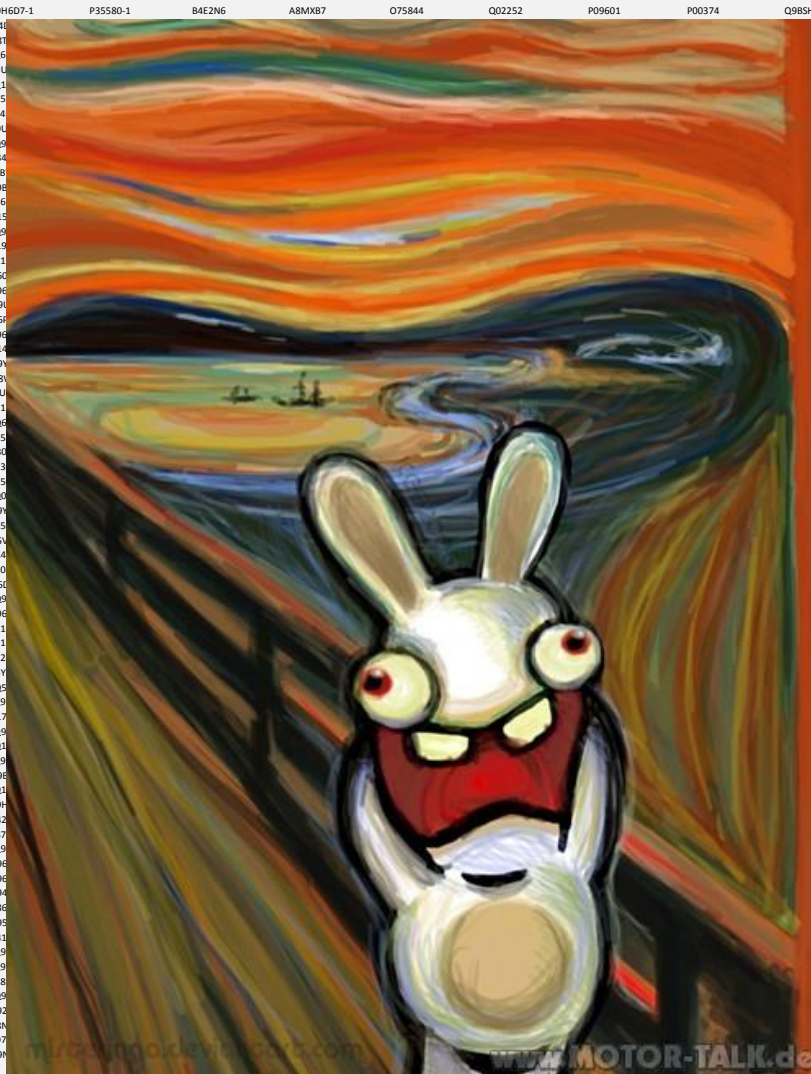


Detection of glucosylation



TcdA-responsive proteins

Q9NRR3	Q4VBZ6	Q9H6D7-1	P35580-1	B4E2N6	A8MXB7	O75844	Q02252	P09601	P00374	Q9BSH4	Q00534	P82675-1
C9JXC1	P22735	B4E2N6									Q8WW1-3	Q92823-5
Q8UUC4	Q15154-1	Q8T1									Q95425-1	B7Z6G5
P46937-1	Q9BVW2	Q6									P35754	P38117-1
P04183	A09176	Q91									Q9Y3D8	P14649
P04818	Q8N392-1	Q1									Q95359-4	Q6IA69
P14635	Q8IVD9	P5									Q07157-1	P17655
Q9NP08	Q8GK24	B4									Q96584-2	Q6NXE6
Q9Y6A5	Q8TEQ6	Q9L									Q86WV7	
Q14807	B4DZK4	Q9									Q13572-1	
P52732	P52701-1	P34									Q9NPH2-1	
Q9H4H8-1	D3DNT2	Q98									Q9BX9-1	
Q9H8V3-2	Q9H673-1	Q9E									Q96GA9	
Q9NQ57-1	Q8WUV3	P6									Q94763-1	
O00762	Q9Y5K6	Q15									Q15021	
P57739	Q14933-1	Q9									Q3YEC7-1	
O75330-3	Q9NCK3-2	P15									Q99700-1	
Q9P2M7-1	P05162	P1									Q9UHD1-1	
Q96R85	Q6FGM0	Q6C									Q9NSK7-4	
O14965	O60232	Q9K									Q98TE3-1	
Q96K85	Q15003	Q9L									Q8TEX9-2	
Q9H7C9-1	P39019	Q6F									Q9Y490	
Q15004	O14737	Q9E									Q8N6R0-5	
O14974-1	Q8N9N8	Q14									Q15417	
A8K024	P63173	Q9V									Q5T5C7	
Q9BW19	A8MUW3	Q8V									Q98QI0-2	
Q99661-1	Q13043-1	Q9U									P62837	
O43490-1	Q9YAC2-2	P1									O00468	
Q15942	P07311	Q8E									P08133	
Q96G20	P23588	P5									Q14353	
Q86V13	Q9HBM1	P3B									Q9BV86	
Q8WVK7	P57076	P3									Q14CX7-1	
Q98TS9-1	Q0QET7	P5									P18206-1	
Q14527-1	B4DQM4	Q0									P41252	
Q8TAP6-1	Q9NR09	Q9V									Q7L8W6	
Q9GHL8-1	Q14651	P5									Q96KG9-1	
Q5ISPO-1	O75157-1	Q5V									Q15785	
P49023-3	O60256	A4									B1ALK7	
P13797	C9JER1	P0									Q71UM5	
P49366-1	Q16877	Q6C									Q98RT3	
P23921	Q9NVW0-1	Q9									Q9P2J5	
P09493-4	Q9YV3-2	Q9E									Q15276-1	
Q9Y4C1	O75179-1	P1									Q15056-1	
Q98X66-9	P22681	B1									88Z287	
Q9Y613	Q8WWM7-1	P2									Q13085-4	
Q95832	Q9NUG6	Q6Y									P43246	
P49454	Q9BYB4-1	Q5									O75915	
P52564-1	Q9Y237-2	Q9									Q6DKK2	
P17540	Q9UHG0-1	P17									Q9Y2R0	
C9JF11	B3KX20	Q9									P35610	
Q86WQ0	P27708	Q1									Q9UIW2	
Q9NV11-3	Q9NVE7	Q9									P13804	
Q8N8T2	Q8NC51-1	Q9E									Q98T22-1	
Q5NV41-1	Q8NV29	Q1									O75489	
Q9BV54	Q9UDY2-1	Q9H									Q8NCC3	
Q5HY93	Q5TA95	P4Z									Q9IUM50	
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O75153	P29762	Q9E									O75298-1	
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Q9ULX3	Q93052	Q8E									Q9BSH5	
P60520	P60891	Q9S									P04179	
Q9COC9	Q86Y56-1	P41									Q86X29-1	
O14777	A8KZW7	Q9									O75907	
Q96897-1	P22102-1	Q9									P14410	
Q9H694-1	Q9BR40-1	P6									P08319-2	
Q9H900-1	Q16890-1	Q9									P02652	
B4DUT8	Q14C86-6	Q9D									P62745	
Q8NC51-3	Q9UN36-1	Q8N									C9IC84	
Q9R661	O43156	O7									P02771	
O75841	B4DHQ3	Q9H									B7Z8Q2	



Effects of TcdA-wt on colonic cells

