Insights into cellular dynamics by quantitative proteomics and metabolomics

Guido Mastrobuoni,
AG Kempa – Integrative Metabolomics and Proteomics

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OVERVIEW:

- Introduction
- What is it Systems Biology?
- The systems biology approach
- Current projects at BIMSB
- Integrative analysis of salt stress in C. reinhardtii

WHAT IS SYSTEMS BIOLOGY?

Systems Biology aims at a (sub)system level understanding of biological processes and networks.

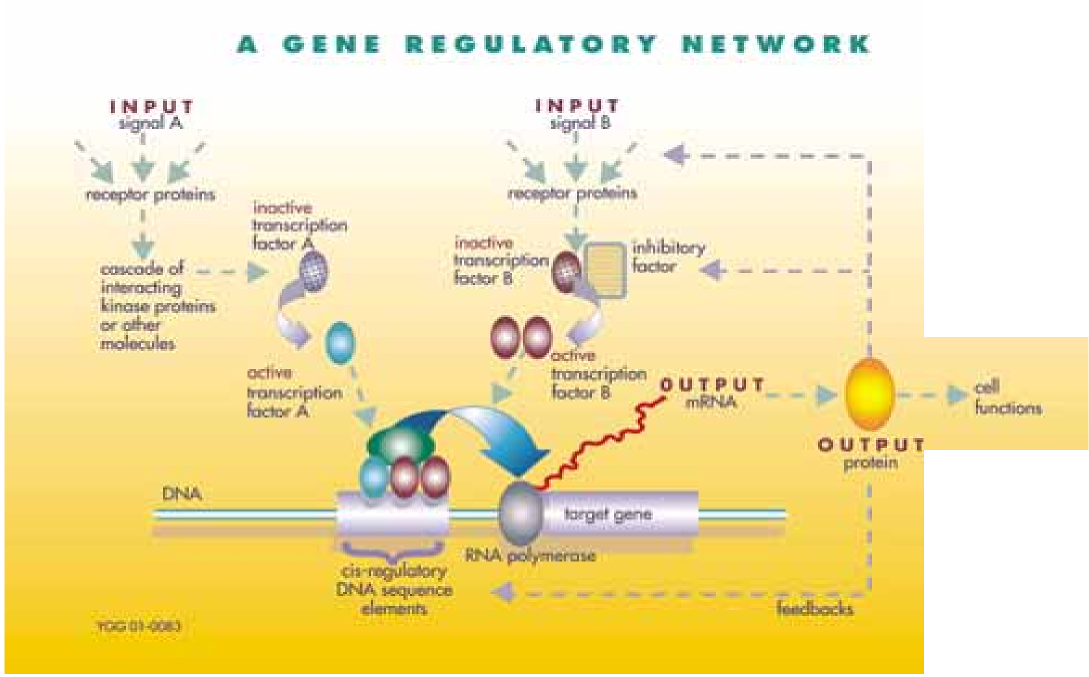
Systems Biology is the **quantitative** (thus modellable) study of biological processes as **whole systems** instead of isolate parts.

The goal of Systems Biology is the construction and experimental validation of model that explain and predict the behavior of biological systems

WHAT IS SYSTEMS BIOLOGY?

The Holy Grail of System Biology:

For any biological process, we want to know the whole picture from input stimuli to output response.



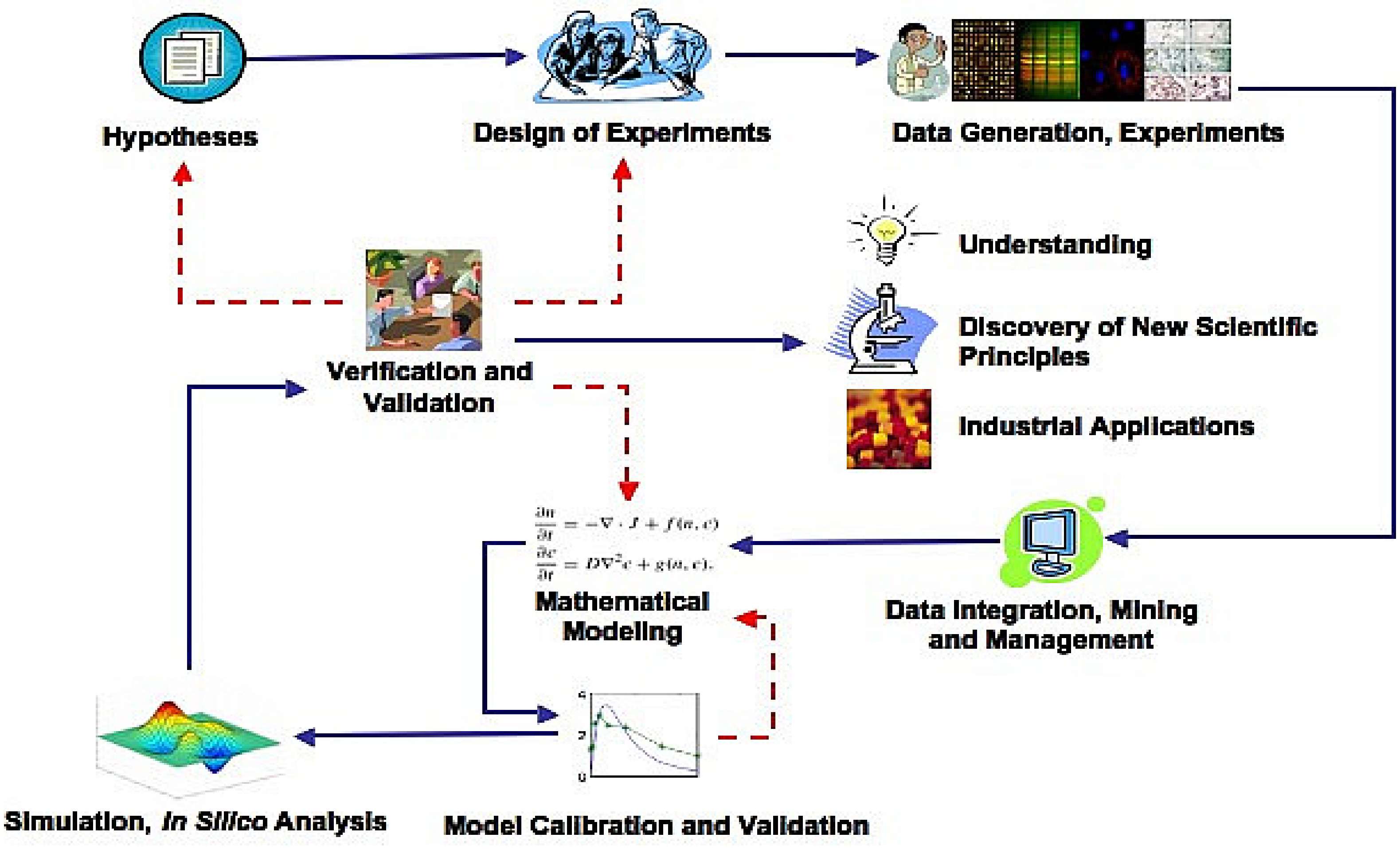
WHAT IS SYSTEMS BIOLOGY?

Systems Biology is a biology-based **interdisciplinary** study field that focuses on the systematic study of complex interactions in biological systems, thus using a new perspective (**holism** instead of reduction) for their study.

Cross-disciplinary projects involving biologists, computer scientist, chemists, engineers, mathematicians and physicists.

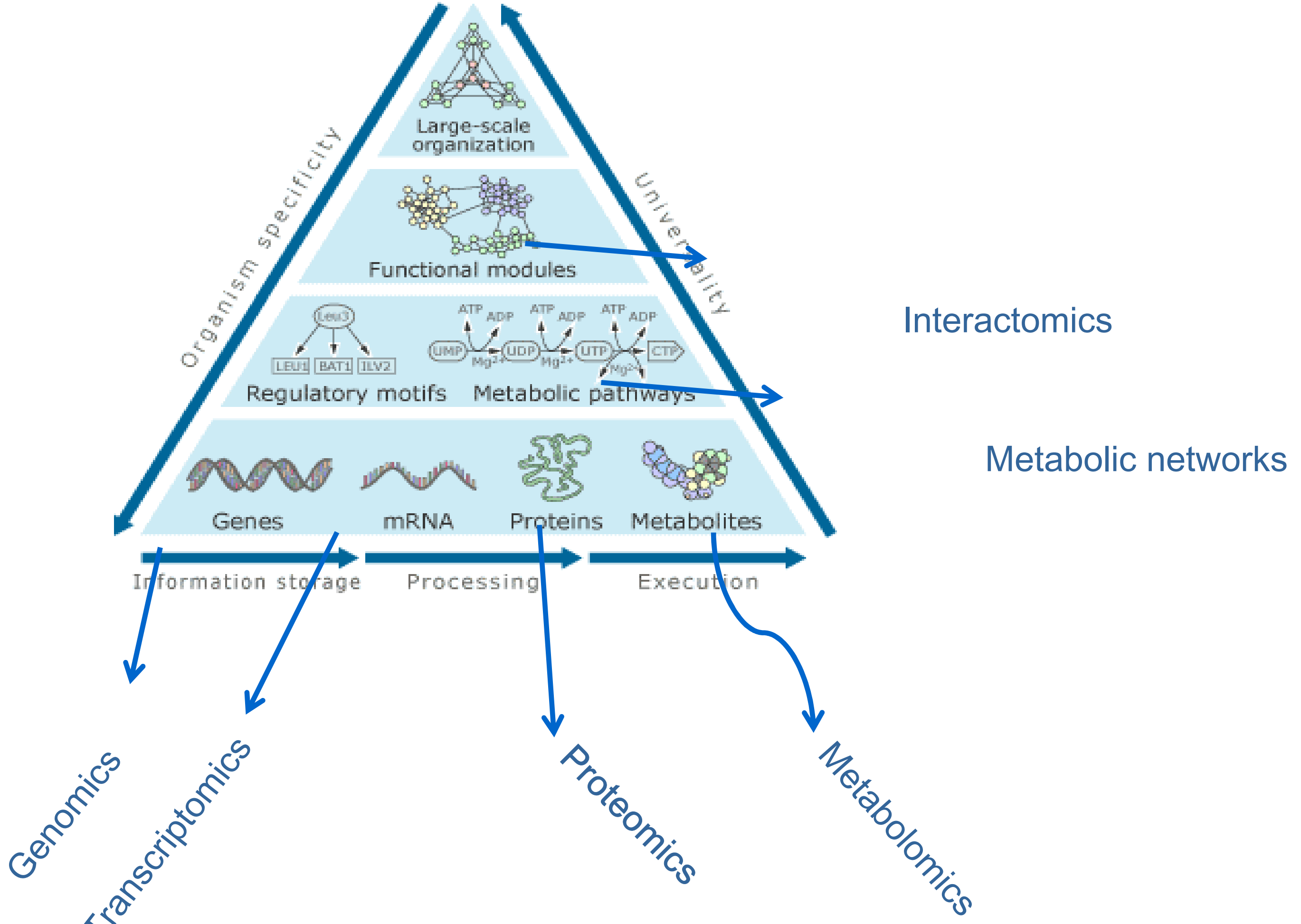
The reductionist approach has successfully identified most of the components and many of the interactions, but, unfortunately, offers no convincing concepts or methods to understand **how system properties emerge**... the pluralism of causes and effects in biological networks is better addressed by observing, through **quantitative measures**, multiple components simultaneously and by rigorous data integration with mathematical models (Sauer et al., Science 316:550).

THE SYSTEMS BIOLOGY APPROACH



THE SYSTEMS BIOLOGY APPROACH

BIMSB



Interactomics

Metabolic networks

Genomics

Transcriptomics

Proteomics

Metabolomics

Tools for Systems Biology at BIMSB/MDC

Need of cutting-edge technology to study a biological system at each level:

- Next generation sequencing (genomics and transcriptomics)
- High-throughput metabolomics (fast and reliable GC-MS)
- High-throughput proteomics (high resolution mass spectrometers)
- Bioinformatics (cluster)

AG Kempa – Integrative metabolomics and proteomics

GCxGC-TOF LECO

LTQ Obritrap Velos

UPLC Agilent 1290

2x UPLC Eksigent 1Dplus

LTQ Obritrap Velos ETD

Current projects

Study of cancer metabolism (‘Warburg effect’?) and metabolic (mis)regulation in cancer

miRNA influence on cell metabolism

Genome annotation of *Schmidtea mediterranea*

Cross-species comparison of Dauer and Mixed larval stage in nematodes

Functional characterization of RNA-binding proteins

Modeling of Wnt signaling pathway

Salt stress in *Chlamydomonas reinhardtii*

Why Chlamydomonas?

Chlamydomonas is a model organism for plant physiology

Simple organism

Easy to grow in an inexpensive medium

Haploid genome – easy to get mutants

Complete nuclear genome sequence published in 2007

The salt stress experiment

Many questions to answer regarding this system:

How does the system (C. reinhardtii) respond to an excess of salt in the culture medium?

How is the dynamic cell response to that stress and which direction does it takes?

Which protein and metabolites show the biggest change?

How do transcript changes relate to protein changes?

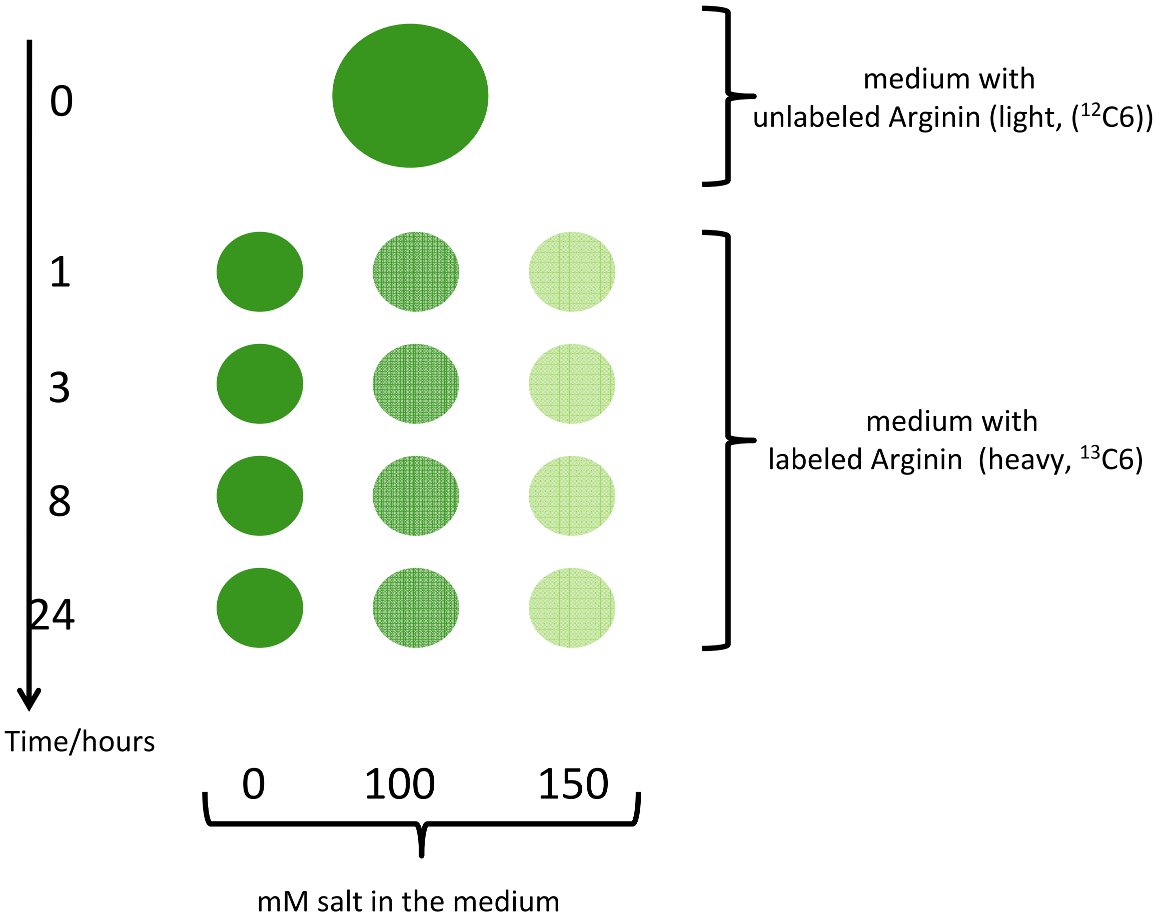
……

EXPERIMENTAL DESIGN

Auxotrofic strain (Arg$^-$)

3 different NaCl concentrations: 0mM, 100mM, 150mM

4 timepoints: 1h, 3h, 8h, 24h



0

1

3

8

24

Time/hours

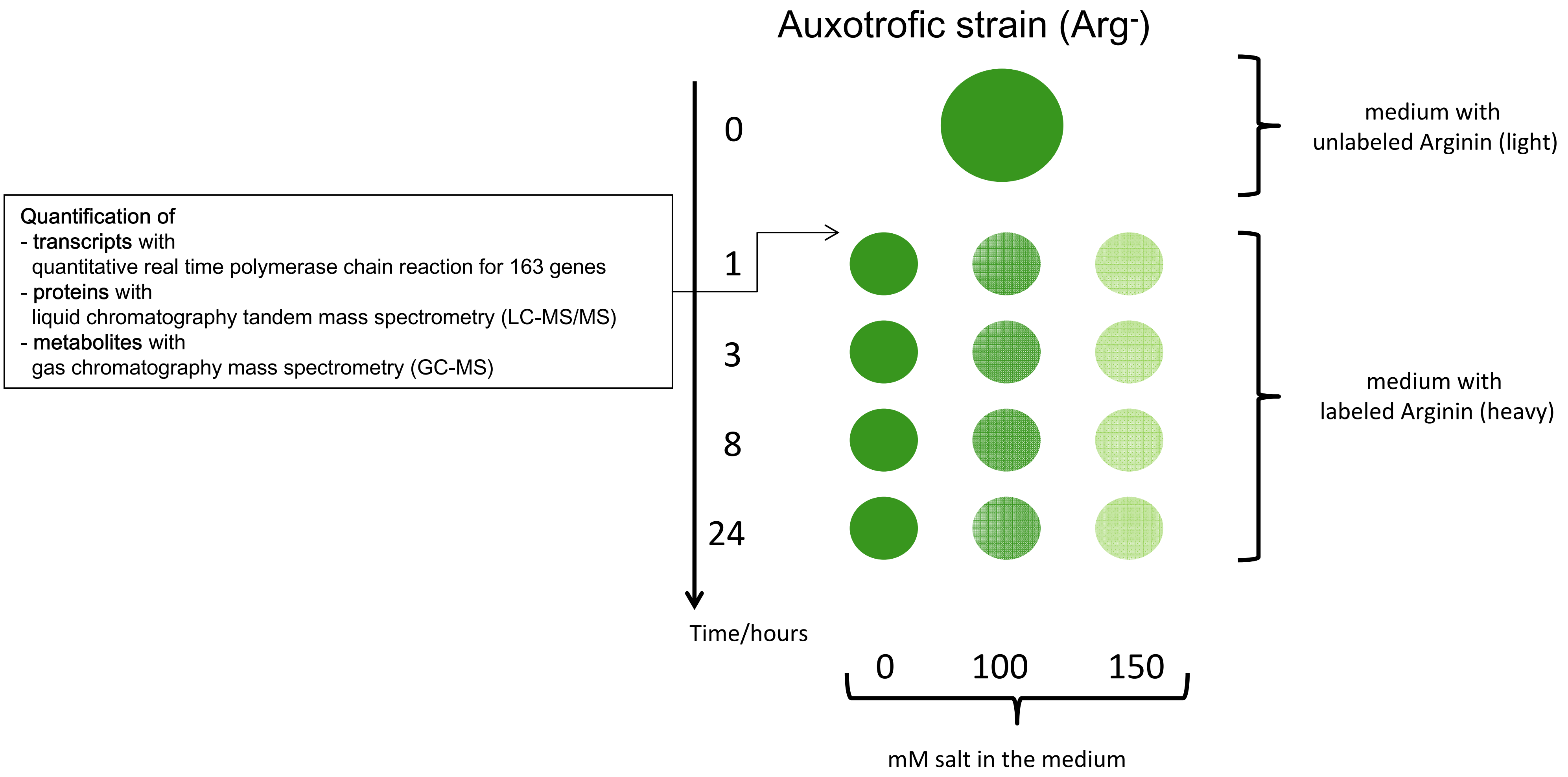
medium with
unlabeled Arginin (light, (^{12}C6))

medium with
labeled Arginin (heavy, ^{13}C6)

0 100 150

mM salt in the medium

EXPERIMENTAL DESIGN



Quantification of
- **transcripts** with
  quantitative real time polymerase chain reaction for 163 genes
- **proteins** with
  liquid chromatography tandem mass spectrometry (LC-MS/MS)
- **metabolites** with
  gas chromatography mass spectrometry (GC-MS)

Auxotrofic strain (Arg$^-$)

medium with
unlabeled Arginin (light)

medium with
labeled Arginin (heavy)

mM salt in the medium

PROTEOMIC WORKFLOW

The most updated protocol for in-depth protein identification:

Sample extraction and 1D SDS gel	~ 4h
15 fractions excided	
in gel enzymatic digestion	~ 16h
peptide extraction and desalting	~ 3h
LC-MSMS analysis (2 replicates per sample) with 2.5 h gradient	~2.5h * 2 * 12 = 60 h

Impossible!

Unfortunately the required time for our dataset (12 samples) is enormous:

~ 750 hours (31.5 days)!!

PROTEOMIC WORKFLOW

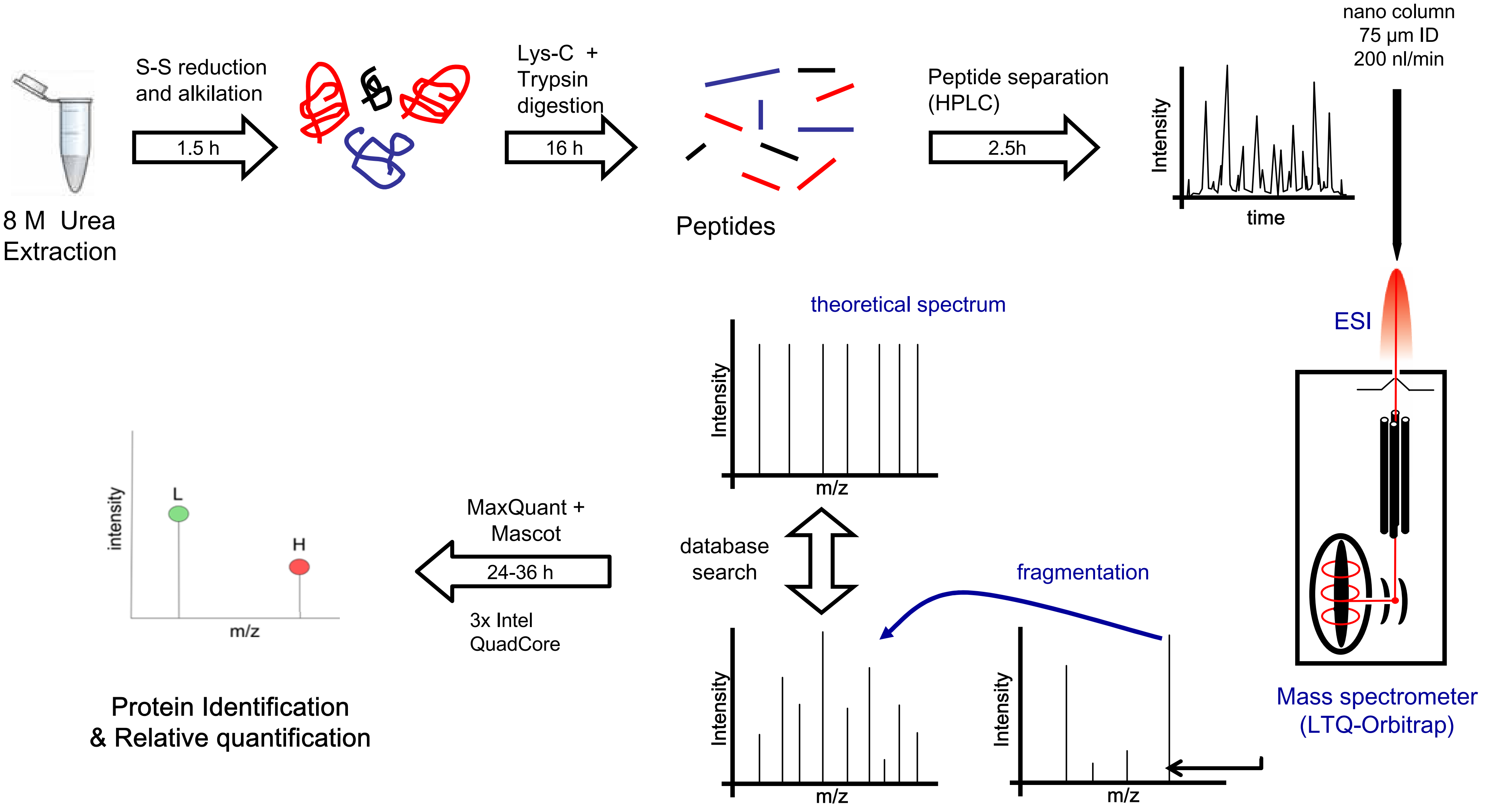
Our new workflow:

Sample extraction and in solution digestion	~ 22h
peptide desalting	~ 2h
LC-MSMS analysis (2 replicates per sample) with 4.5 h gradient	~4.5h * 2 = 9 h

New required time for our dataset (12 samples):

~ 132 hours (5.5 days)!!

PROTEOMIC WORKFLOW



8 M Urea Extraction

S-S reduction and alkilation

1.5 h

Lys-C + Trypsin digestion

16 h

Peptides

Peptide separation (HPLC)

2.5h

nano column
75 µm ID
200 nl/min

ESI

Mass spectrometer (LTQ-Orbitrap)

fragmentation

theoretical spectrum

database search

MaxQuant + Mascot

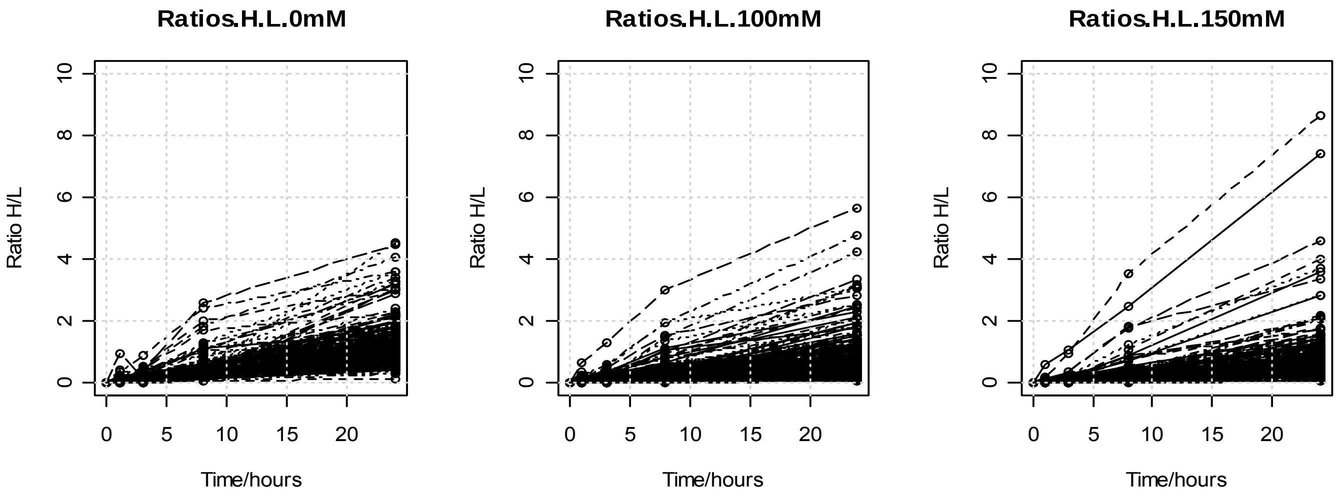
24-36 h

3x Intel QuadCore

Protein Identification & Relative quantification

The salt stress experiment – Proteomics

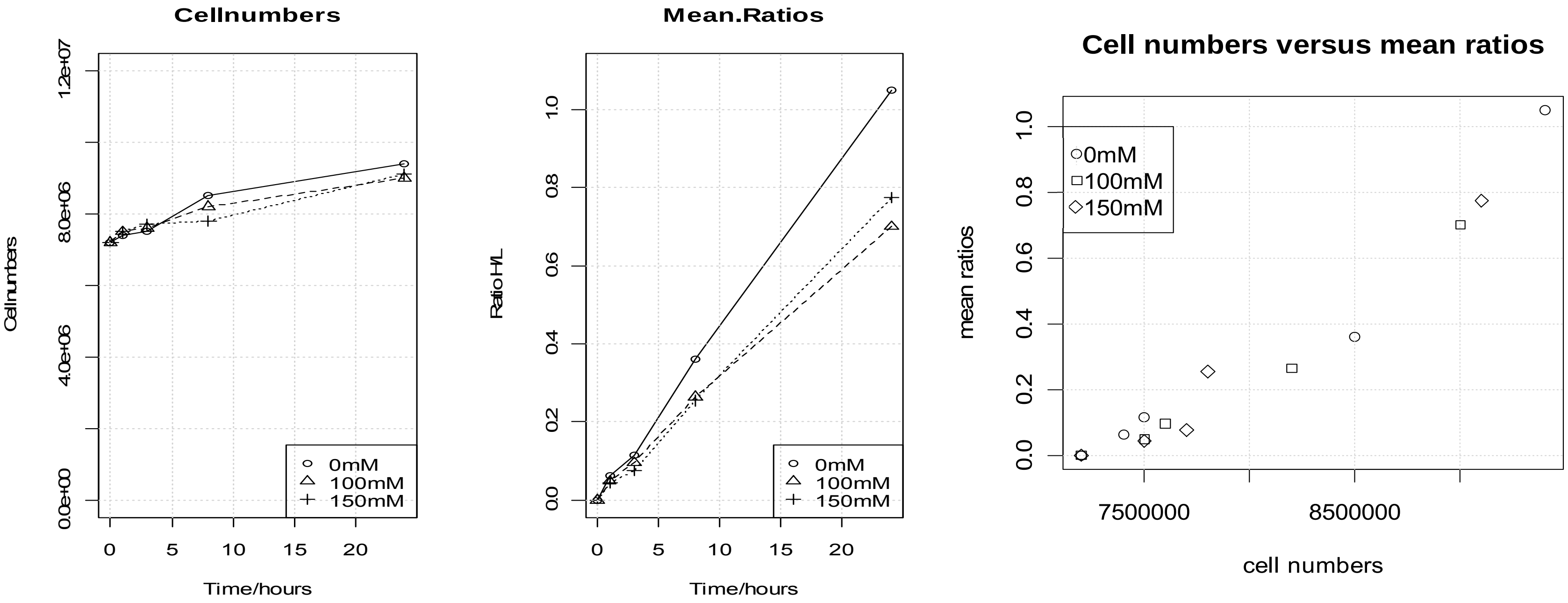
The dataset



241 proteins with a reliable measured ratio (at least three quantitaion events)in all the three tested conditions at all time points (out of 2564 identified proteins)

The salt stress experiment – Proteomics

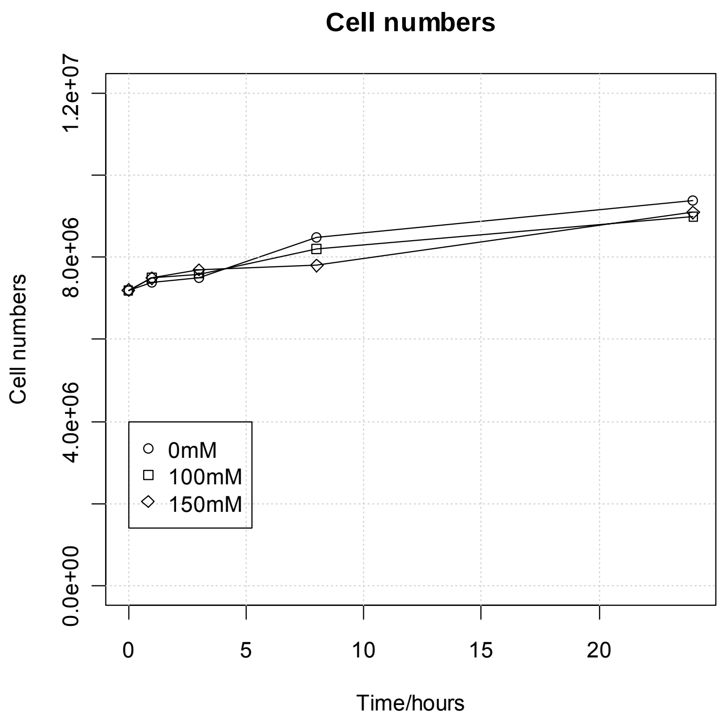
How do the protein synthesis relate to cell growth?

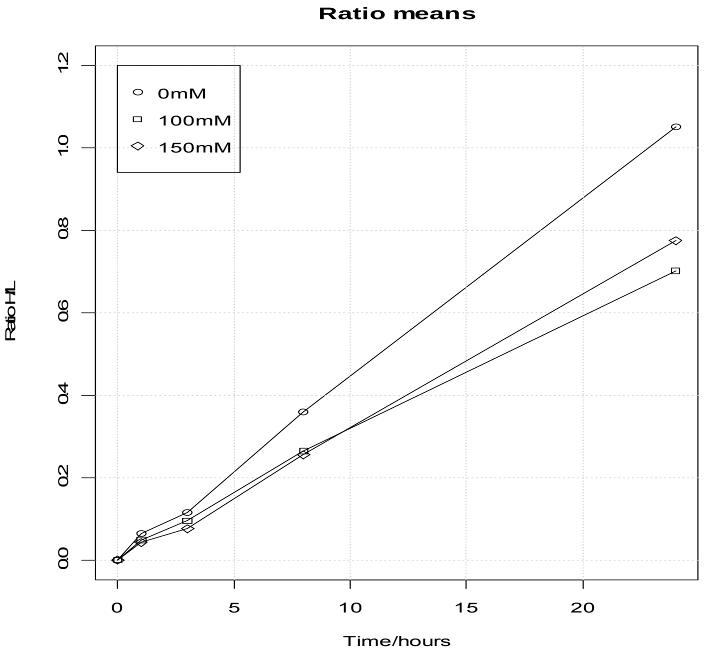


The salt stress experiment – Proteomics

BIMSB

SILAC ratios increase because of the biomass production...

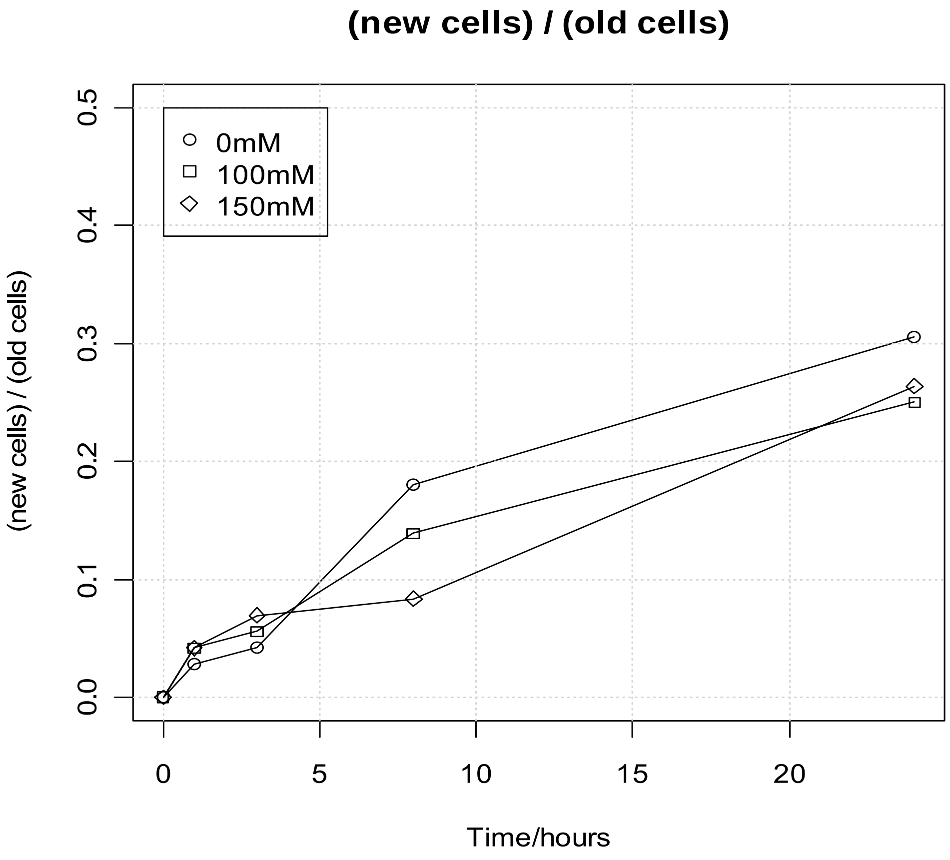




The salt stress experiment – Proteomics

SILAC ratios increase because of the biomass production...

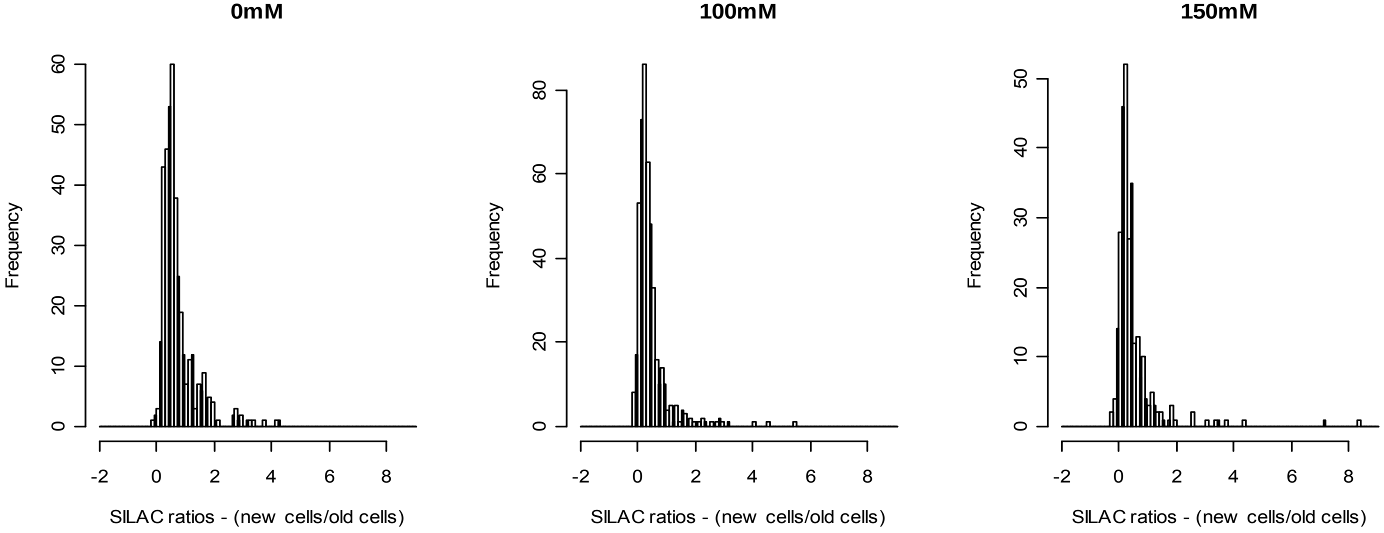
expected ratios

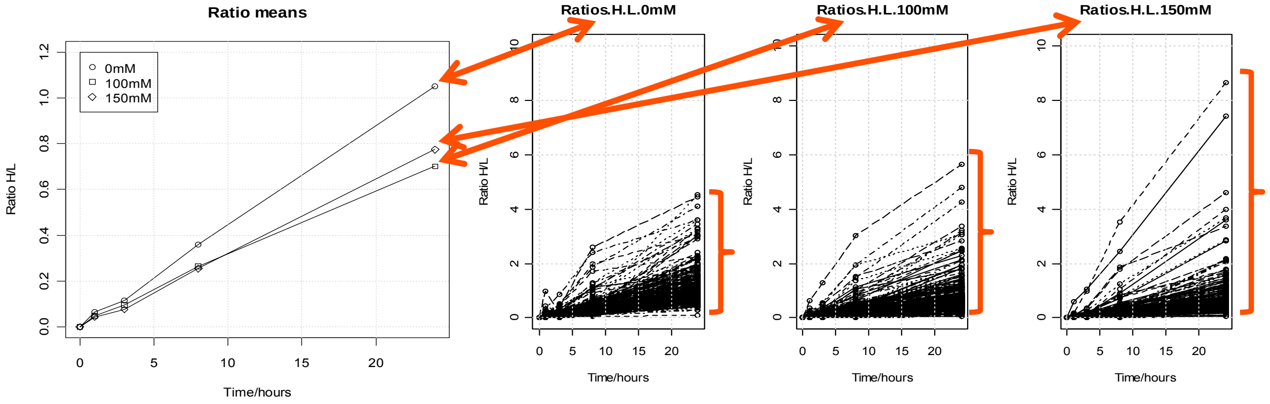


The salt stress experiment – Proteomics

...but also because of protein turnover

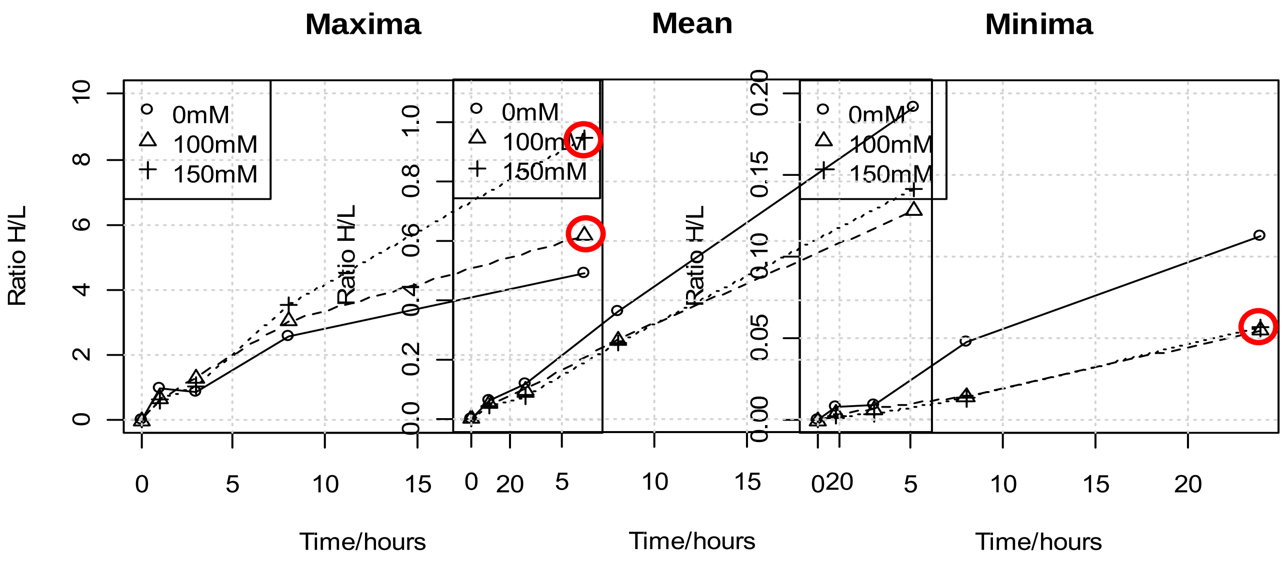
At 24 h all the proteins have a ratio higher than the minimum expectable one



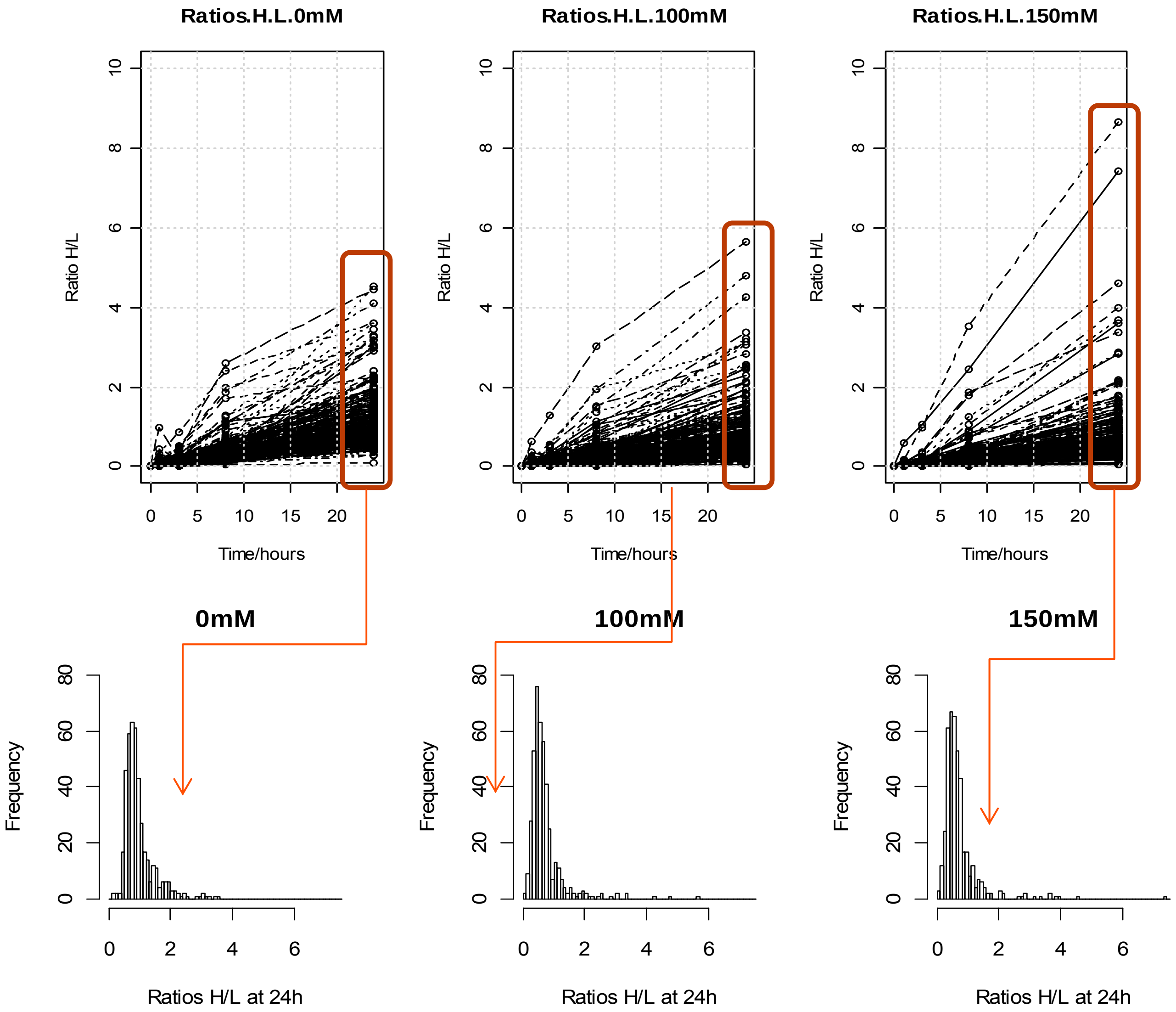


The salt stress experiment – Proteomics

Which proteins show the biggest change in their concentration when the cell responds to the salt stress?

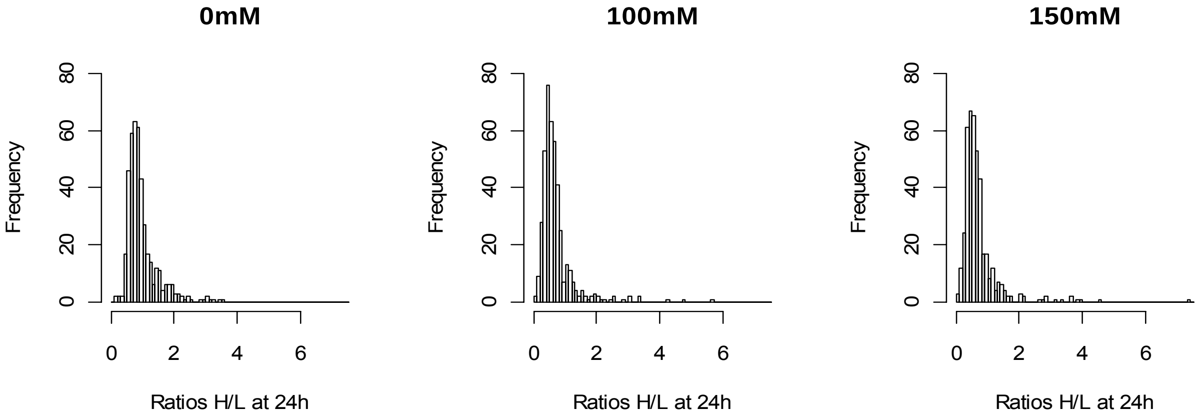


The salt stress experiment – Proteomics

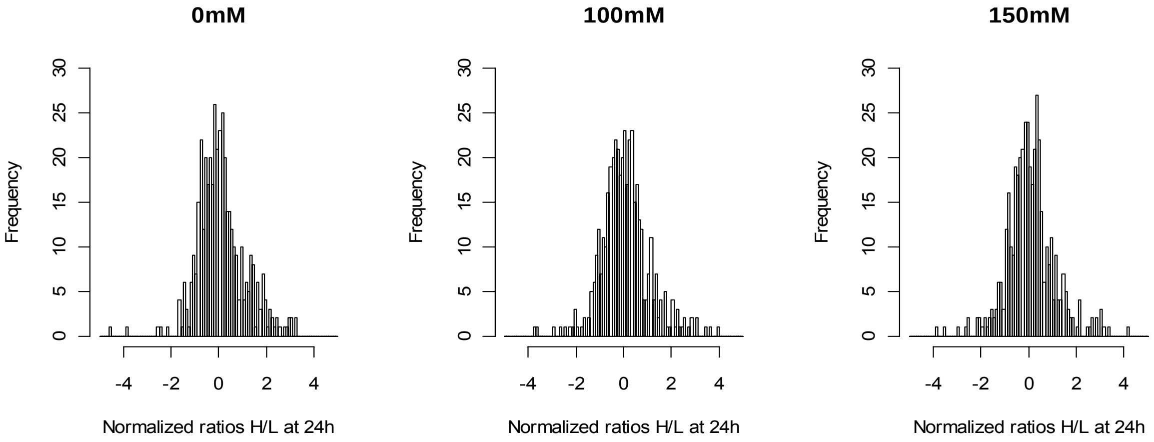


The salt stress experiment – Proteomics

Some normalization to make the data comparable…



log transformation,
median subtraction,
division by standard deviation



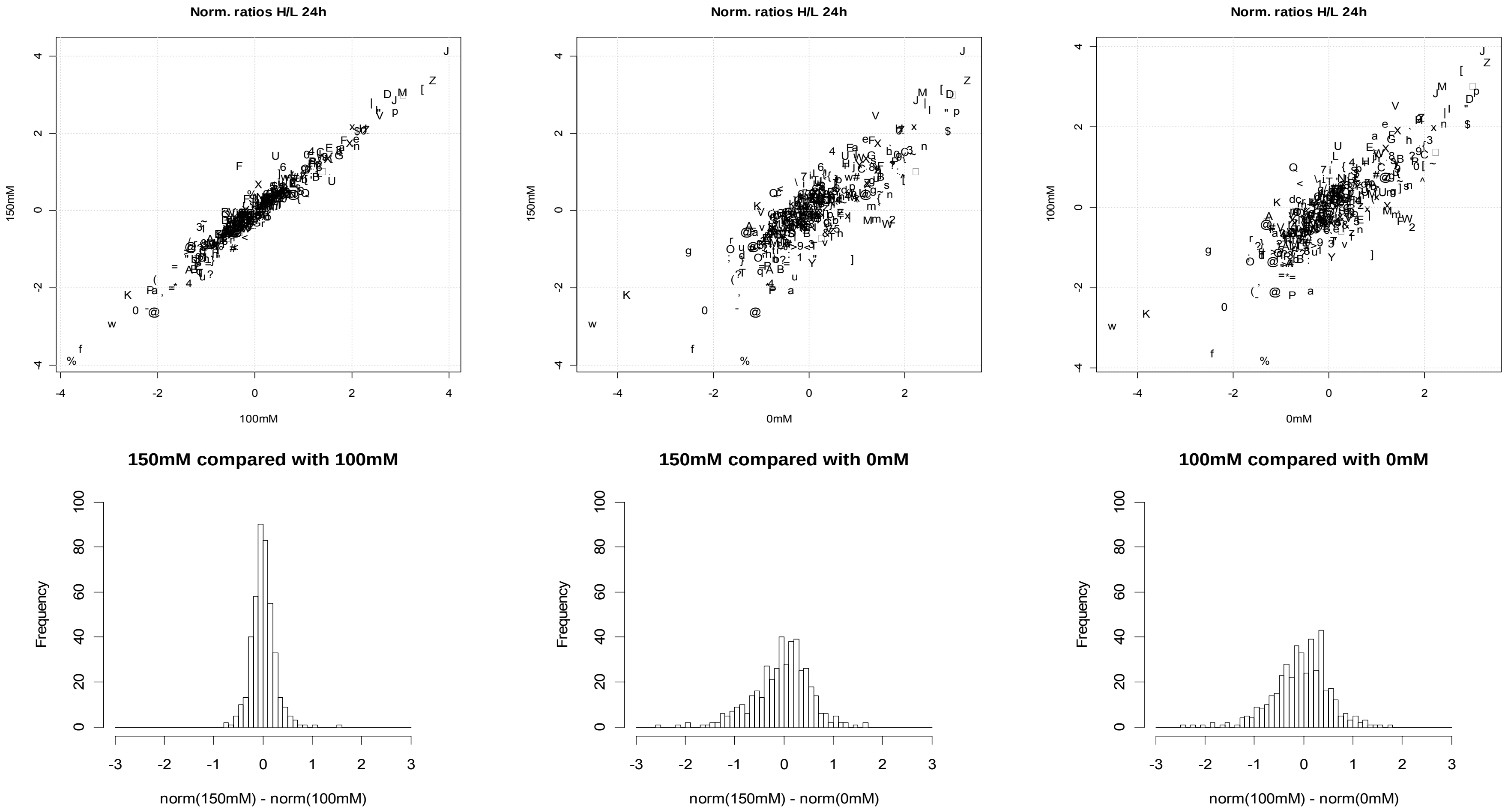
The salt stress experiment – Proteomics

The ten proteins with the highest turnover are the same in all the three conditions, but the salt stress speeds up this process

GeneName	Annotation	normRatio H/L 0mM 24h	normRatio H/L 100mM 24h	normRatio H/L 150mM 24h
e_gwH.18.127.1	protein synthesis initiation	3.21	3.93	4.17
e_gwH.1.136.1	Arginine metabolism	3.30	3.64	3.40
e_gwH.34.63.1	Arginine and proline metabolism	2.76	3.44	3.17
e_gwH.79.31.1	Photosynthesis - calvin cyle rubisco small subunit	2.37	3.03	3.09
fgenesh1_pg.C_scaffold_11000208	protein degradation	2.94	2.74	3.04
NA	NA	3.01	3.04	3.01
e_gwH.367.7.1	DNA synthesis/chromatin structure - histone	2.24	2.87	2.89
e_gwH.38.29.1	protein postranslational modification	2.42	2.38	2.81
NA	NA	2.88	2.55	2.63
e_gwH.38.2.1	cell organisation	2.51	2.48	2.62
e_gwH.18.93.1	glutathione S transferases	3.09	2.89	2.56

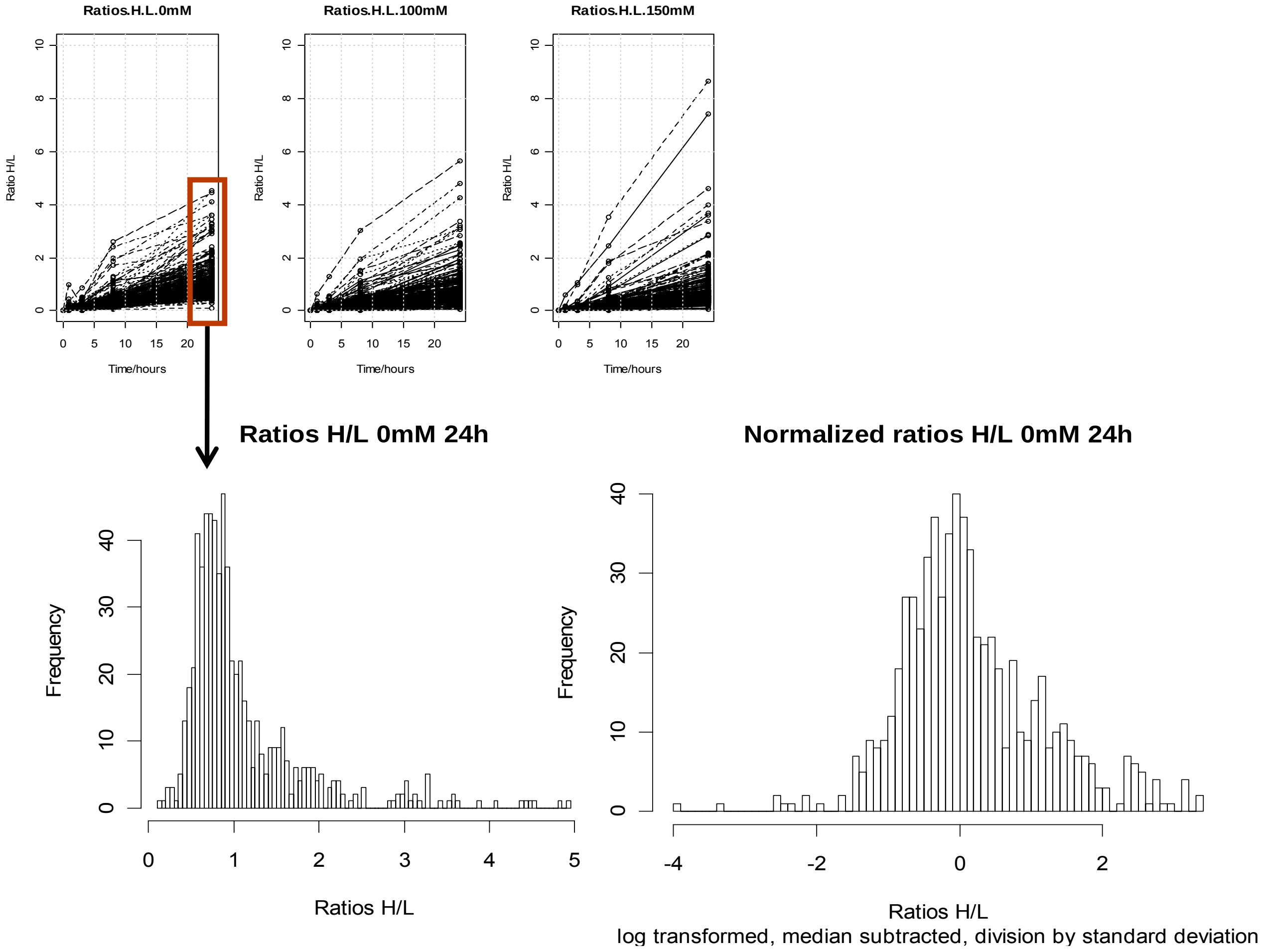
The salt stress experiment – Proteomics

The overall salt stress response follow a common pattern at both concentration



The salt stress experiment – Proteomics

In normal condition, which proteins have highest and the lowest turnover?



Ratios.H.L.0mM

Ratios.H.L.100mM

Ratios.H.L.150mM

Ratios H/L 0mM 24h

Normalized ratios H/L 0mM 24h

The salt stress experiment – Proteomics

Specific enrichment of certain protein classes

Top 50
highest turnover at 0mM)

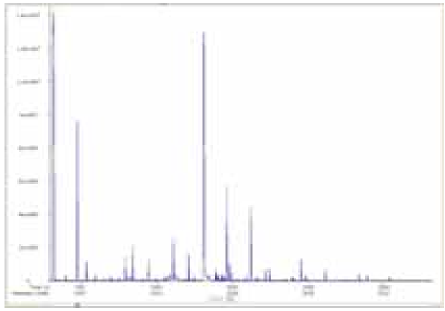
- Tetrapyrrole synthesis
- secondary metabolism –isoprenoids non-mevalonate pathway
- Protein synthesis
- Protein degradation

Bottom 50
(lowest turnover at 0 mM)

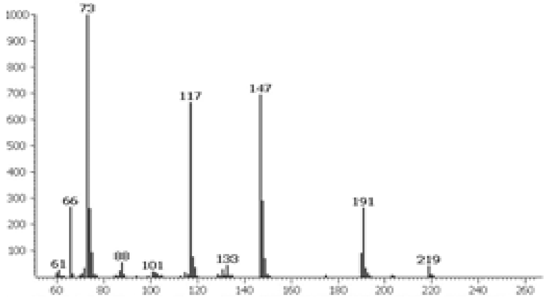
- Photosystem
- ATP synthesis

The salt stress experiment – Metabolomics

5 h



+



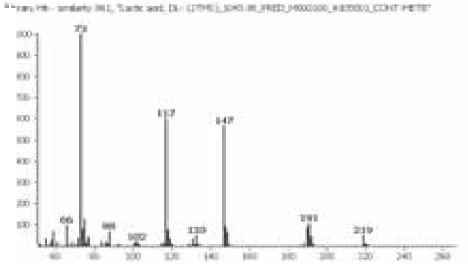
1) Methanol-Chloroform Water Extraction + internal standard ^{13}C-Sorbitol
2) Derivatization

GC Chromatography

TOF Mass Spectrometry

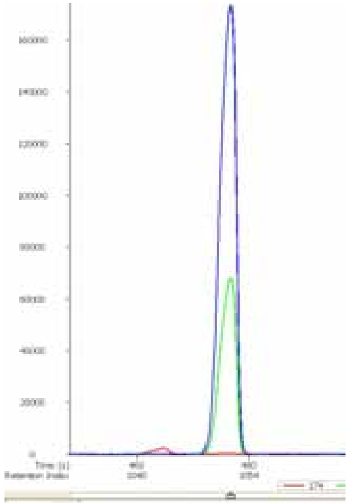
3 d

Library search

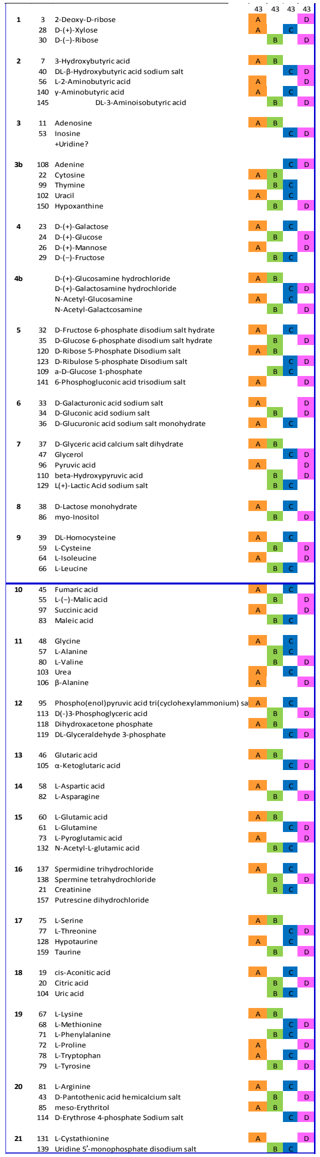


Quant + Ident Mix

Annotation + Quantification



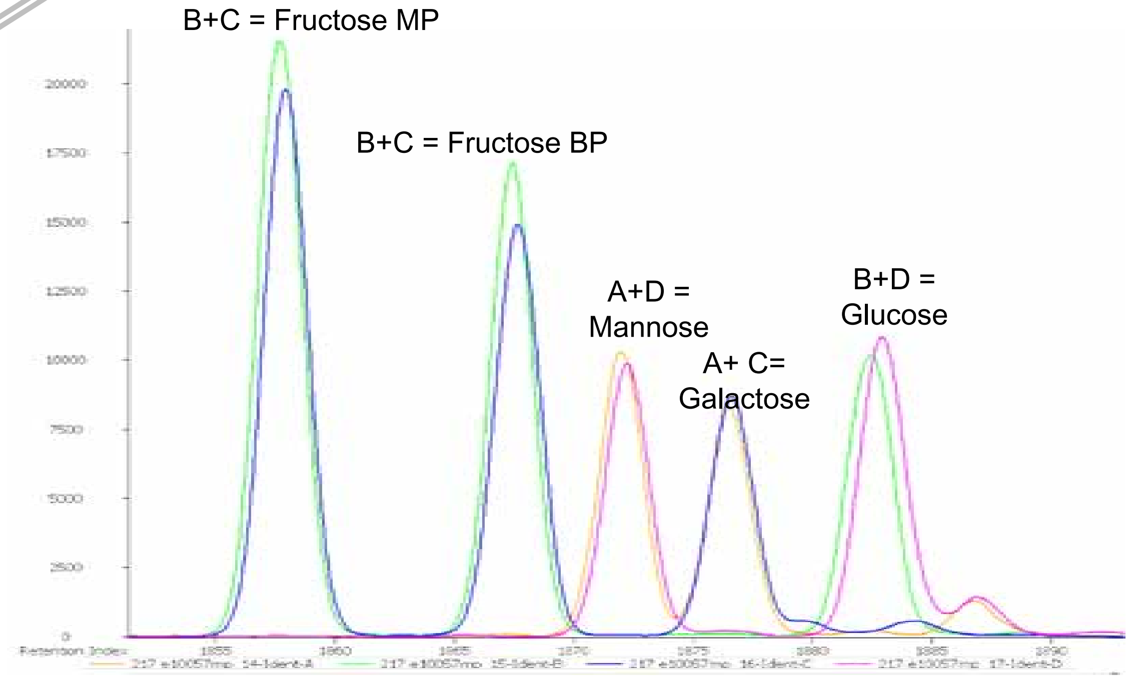
The salt stress experiment – Metabolomics



			43	43	43	43
1	3	2-Deoxy-D-ribose	A			D
	28	D-(+)-Xylose	A		C	
	30	D-(−)-Ribose		B		D
2	7	3-Hydroxybutyric acid	A	B		
	40	DL-β-Hydroxybutyric acid sodium salt			C	D
	56	L-2-Aminobutyric acid	A			D
	140	γ-Aminobutyric acid	A		C	
	145	DL-3-Aminoisobutyric acid		B		D
3	11	Adenosine	A	B		
	53	Inosine			C	D
		+Uridine?				
3b	108	Adenine			C	D
	22	Cytosine	A	B		
	99	Thymine		B	C	
	102	Uracil	A		C	
	150	Hypoxanthine		B		D
4	23	D-(+)-Galactose	A		C	
	24	D-(+)-Glucose		B		D
	26	D-(+)-Mannose	A			D
	29	D-(−)-Fructose		B	C	
4b		D-(+)-Glucosamine hydrochloride	A	B		
		D-(+)-Galactosamine hydrochloride			C	D
		N-Acetyl-Glucosamine	A		C	
		N-Acetyl-Galactcosamine		B		D
5	32	D-Fructose 6-phosphate disodium salt hydrate	A		C	
	35	D-Glucose 6-phosphate disodium salt hydrate		B		D
	120	D-Ribose 5-Phosphate Disodium salt	A	B		
	123	D-Ribulose 5-phosphate Disodium salt			C	D
	109	a-D-Glucose 1-phosphate		B	C	
	141	6-Phosphogluconic acid trisodium salt	A			D
6	33	D-Galacturonic acid sodium salt	A			D
	34	D-Gluconic acid sodium salt		B		D
	36	D-Glucuronic acid sodium salt monohydrate	A		C	
7	37	D-Glyceric acid calcium salt dihydrate	A	B		
	47	Glycerol			C	D
	96	Pyruvic acid	A			D
	110	beta-Hydroxypyruvic acid		B		D
	129	L(+)-Lactic Acid sodium salt		B	C	
8	38	D-Lactose monohydrate	A		C	
	86	myo-Inositol		B		D
9	39	DL-Homocysteine	A		C	
	59	L-Cysteine		B		D
	64	L-Isoleucine	A			D
	66	L-Leucine		B	C	
10	45	Fumaric acid	A		C	
	55	L-(−)-Malic acid		B		D
	97	Succinic acid	A			D
	83	Maleic acid		B	C	
11	48	Glycine	A		C	
	57	L-Alanine		B	C	
	80	L-Valine		B		D
	103	Urea	A		C	
	106	β-Alanine	A			D
12	95	Phospho(enol)pyruvic acid tri(cyclohexylammonium) sal	A		C	
	113	D(-)3-Phosphoglyceric acid		B		D
	118	Dihydroxacetone phosphate	A	B		
	119	DL-Glyceraldehyde 3-phosphate			C	D
13	46	Glutaric acid	A	B		
	105	α-Ketoglutaric acid			C	D
14	58	L-Aspartic acid	A		C	
	82	L-Asparagine		B		D
15	60	L-Glutamic acid	A	B		
	61	L-Glutamine			C	D
	73	L-Pyroglutamic acid	A			D
	132	N-Acetyl-L-glutamic acid		B	C	
16	137	Spermidine trihydrochloride	A		C	
	138	Spermine tetrahydrochloride		B		D
	21	Creatinine		B	C	
	157	Putrescine dihydrochloride				
17	75	L-Serine	A	B		
	77	L-Threonine			C	D
	128	Hypotaurine	A		C	
	159	Taurine		B		D
18	19	cis-Aconitic acid	A		C	
	20	Citric acid		B		D
	104	Uric acid		B	C	
19	67	L-Lysine	A	B		
	68	L-Methionine			C	D
	71	L-Phenylalanine		B	C	
	72	L-Proline	A			D
	78	L-Tryptophan	A		C	
	79	L-Tyrosine		B		D
20	81	L-Arginine	A		C	
	43	D-Pantothenic acid hemicalcium salt		B		D
	85	meso-Erythritol	A	B		
	114	D-Erythrose 4-phosphate Sodium salt			C	D
21	131	L-Cystathionine	A			D
	139	Uridine 5′-monophosphate disodium salt		B	C	

IdentMix with 88 standard substances to discriminate between very similar analytes

D-(+)-Galactose	A		C	
D-(+)-Glucose		B		D
D-(+)-Mannose	A			D
D-(−)-Fructose		B	C	



B+C = Fructose MP

B+C = Fructose BP

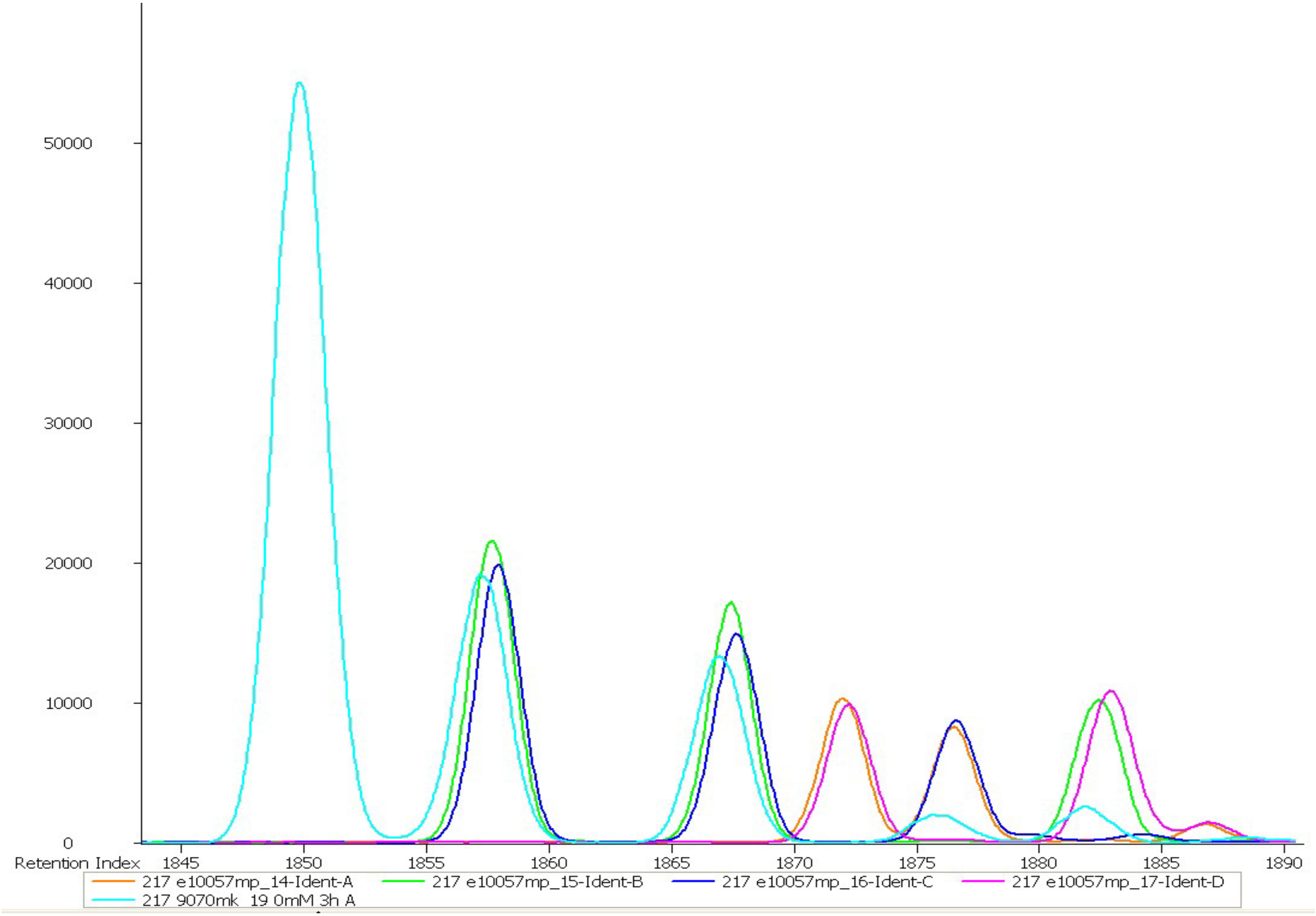
A+D = Mannose

A+ C= Galactose

B+D = Glucose

The salt stress experiment – Metabolomics

A real example – Unknown sugars mixture



The salt stress experiment – Metabolomics

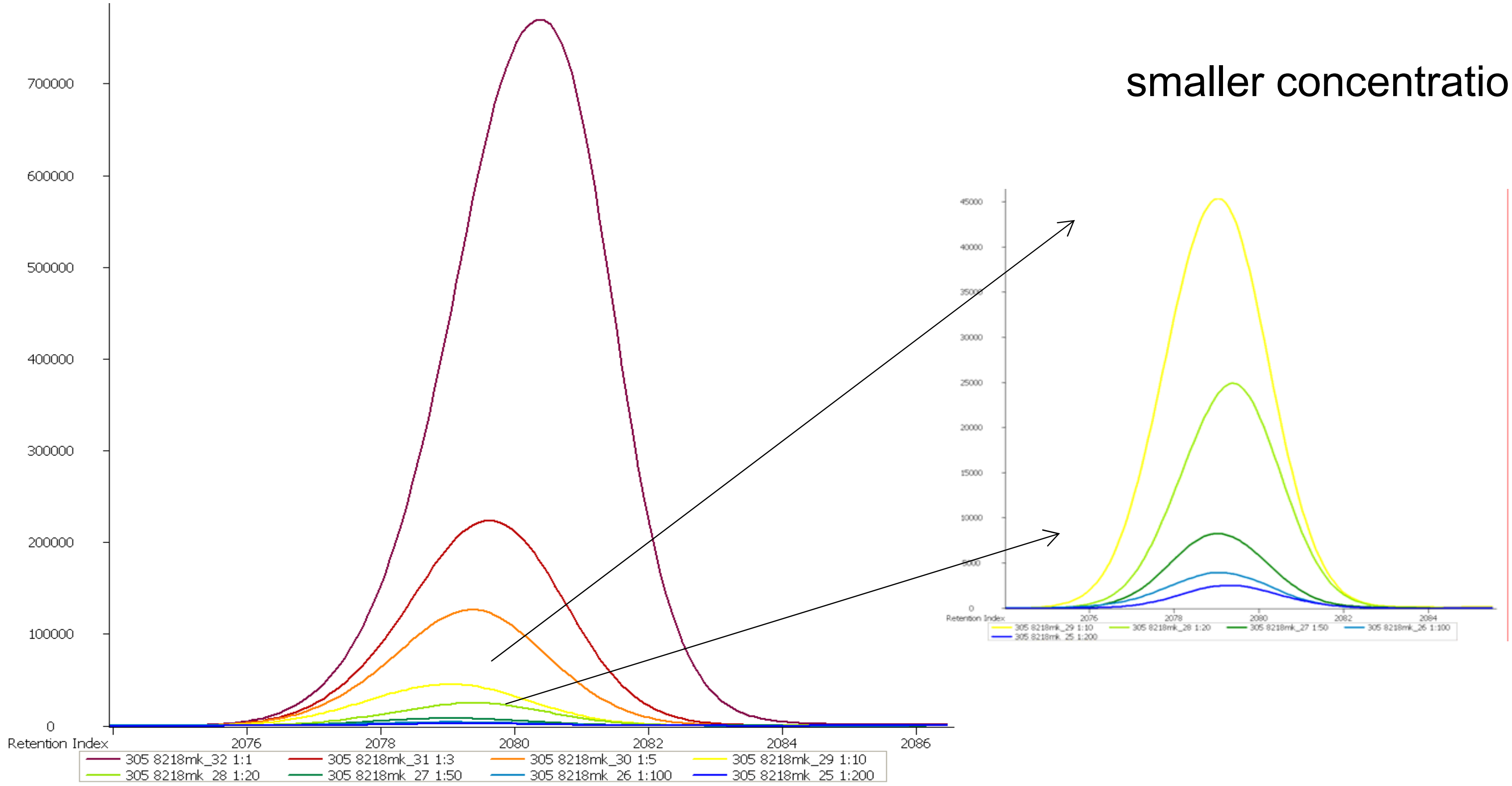
Calibration strategy:

- Using external calibration-curve.
- Mixture of 86 substances, 8 different concentrations over a factor of 200

  (1:1, 1:3, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200)
- Calibration-curves with LECO-ChromaTOF-procedure (built-in)
- Advantages:
  - no manipulation of samples (as with internal standard)
  - Quantification of many samples with 1 calibration curve.

The salt stress experiment – Metabolomics

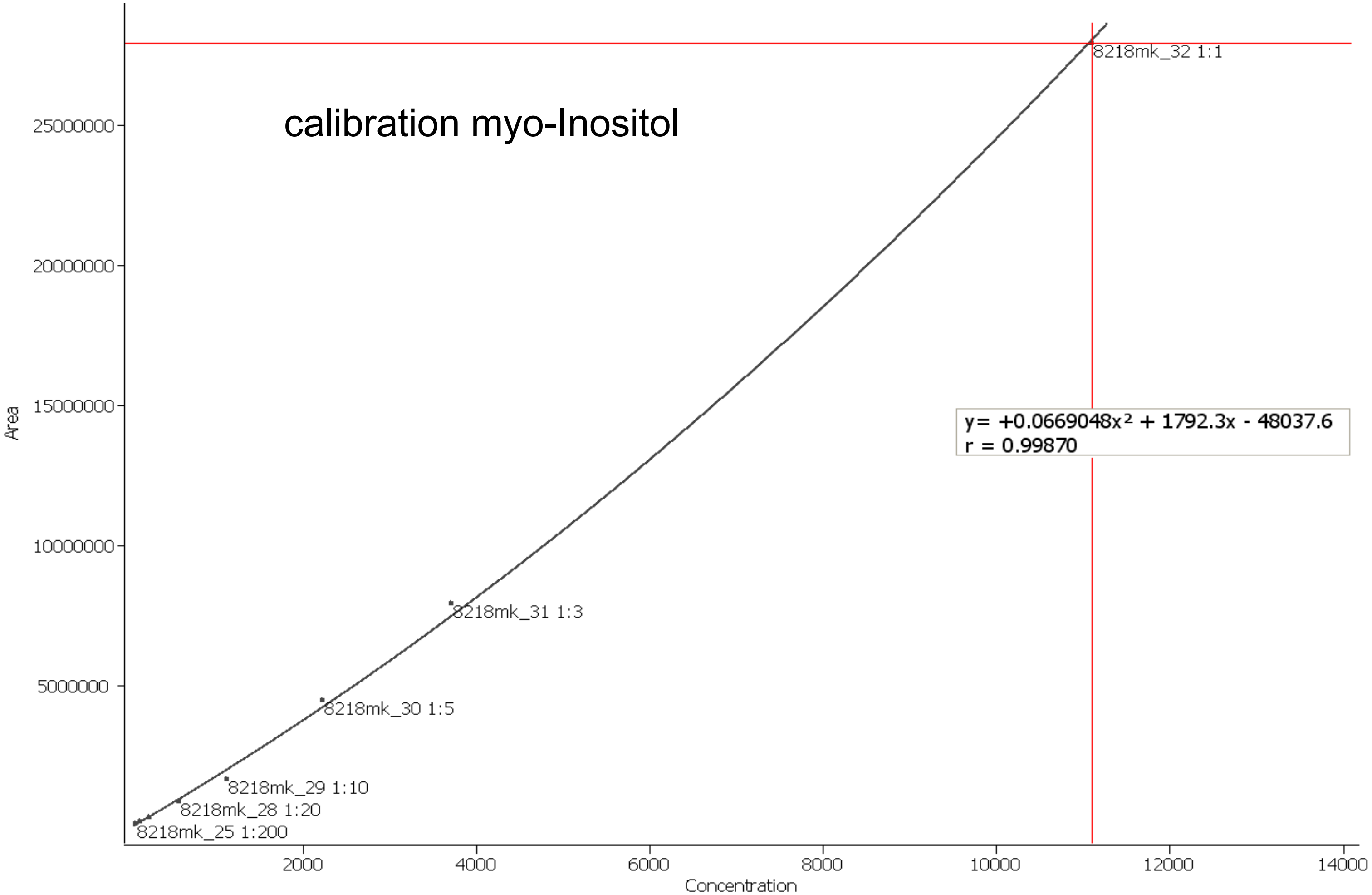
8 different concentration for each of the 86 substances from the **QuantMIx**



smaller concentrations

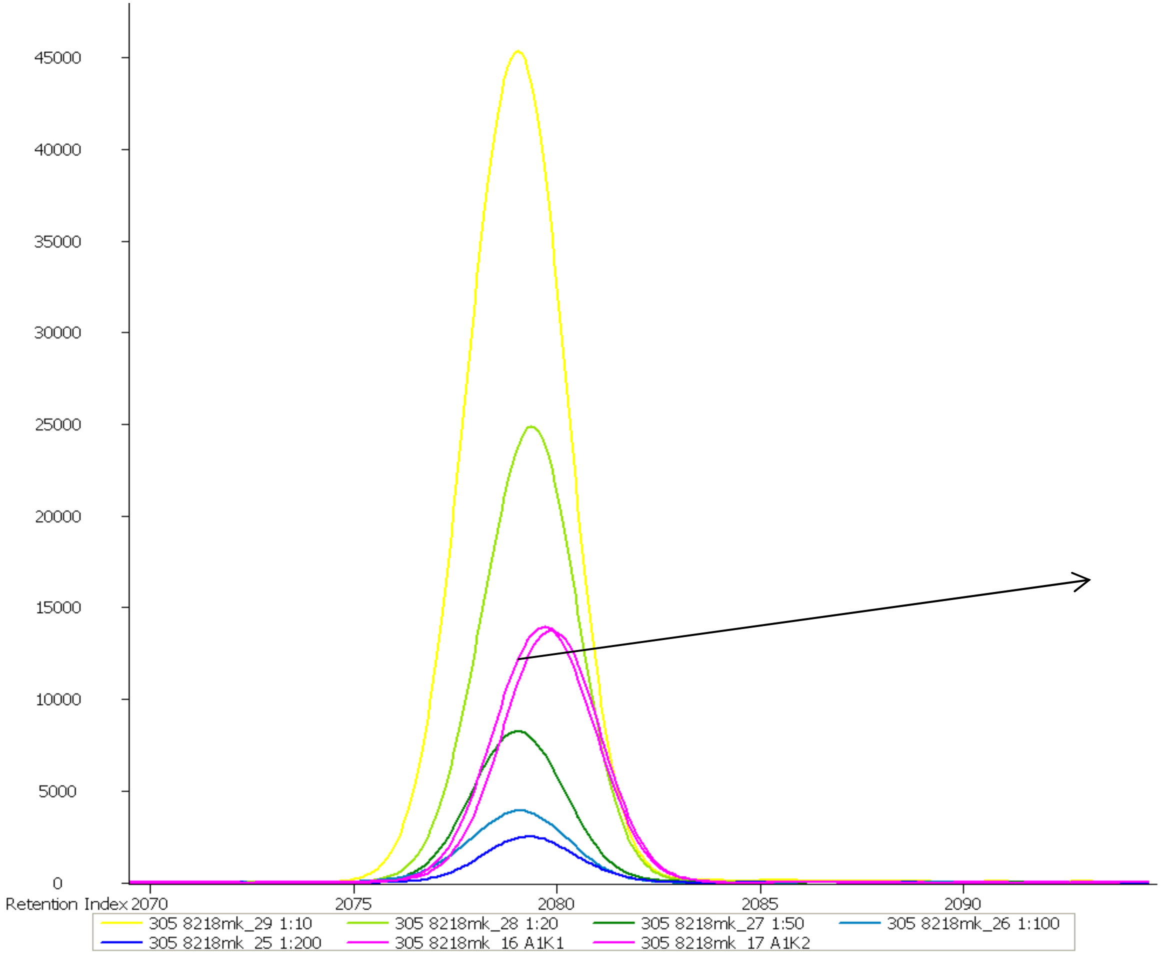
The salt stress experiment – Metabolomics

Built-in calibration procedure



The salt stress experiment – Metabolomics

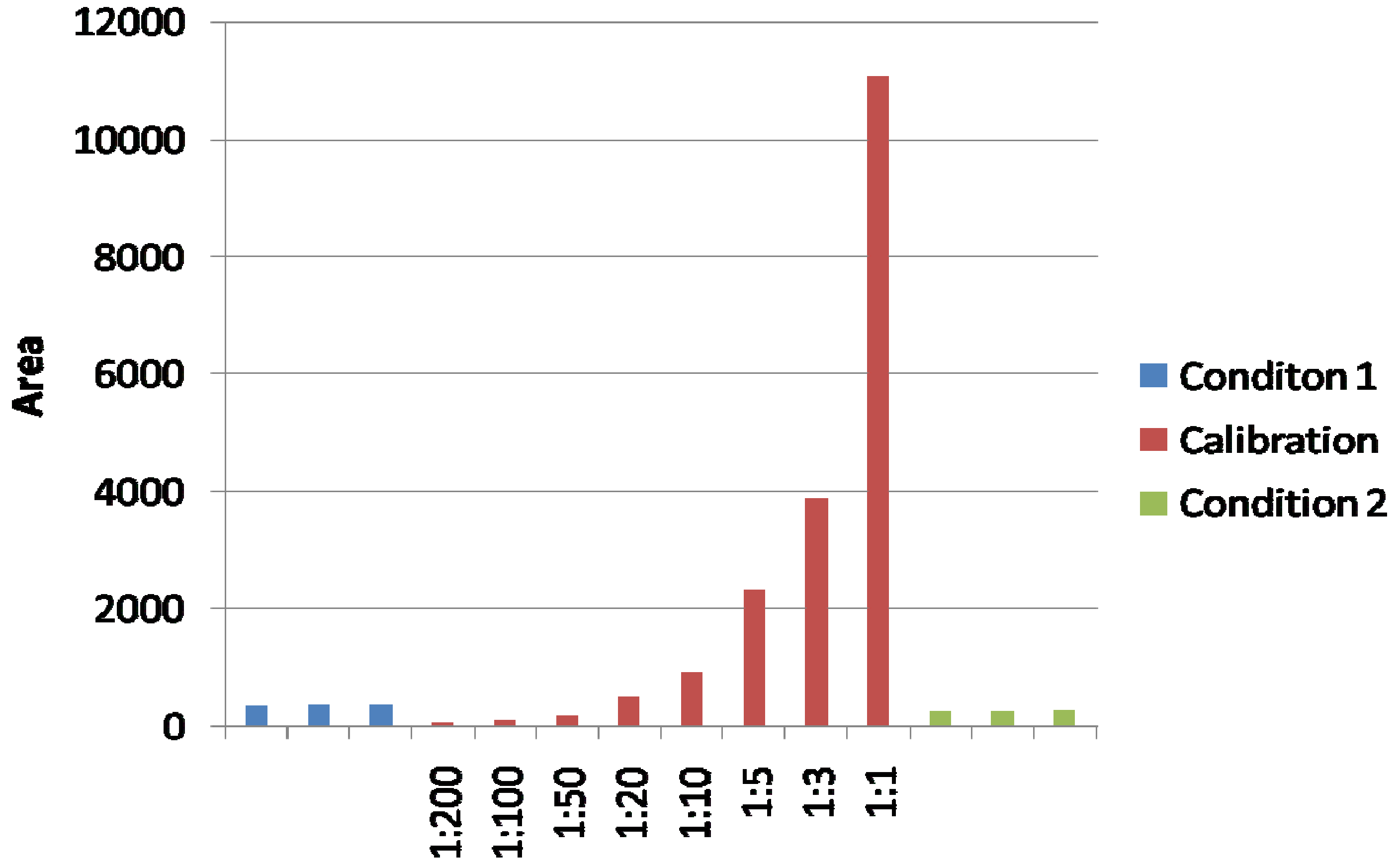
Built-in calibration procedure



sample

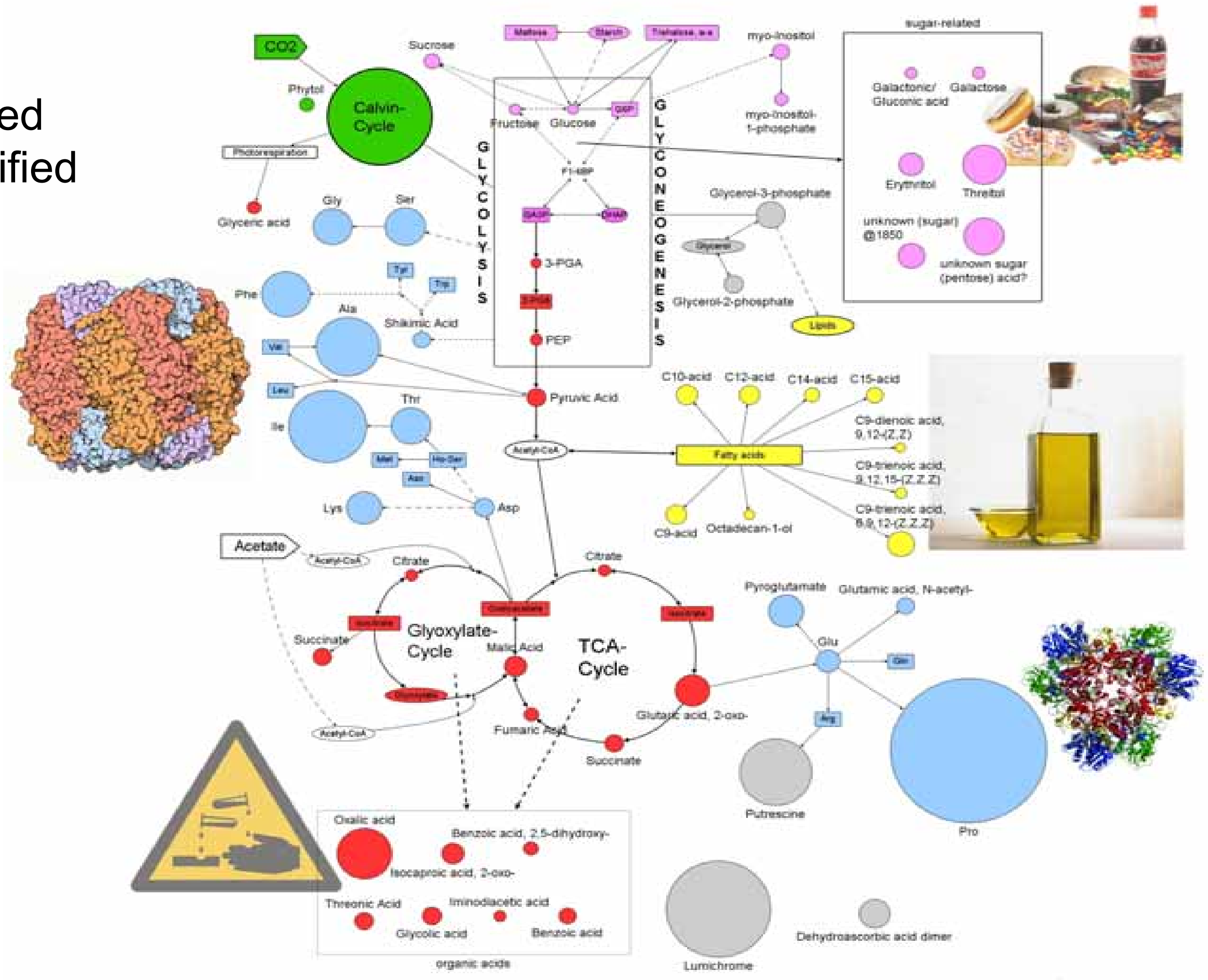
The salt stress experiment – Metabolomics

One calibration curve for ~ 20 samples



The salt stress experiment – Metabolomics

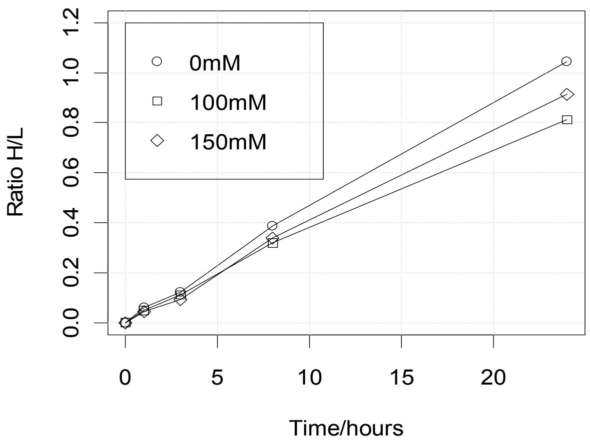
782 substances measured
214 identified and quantified



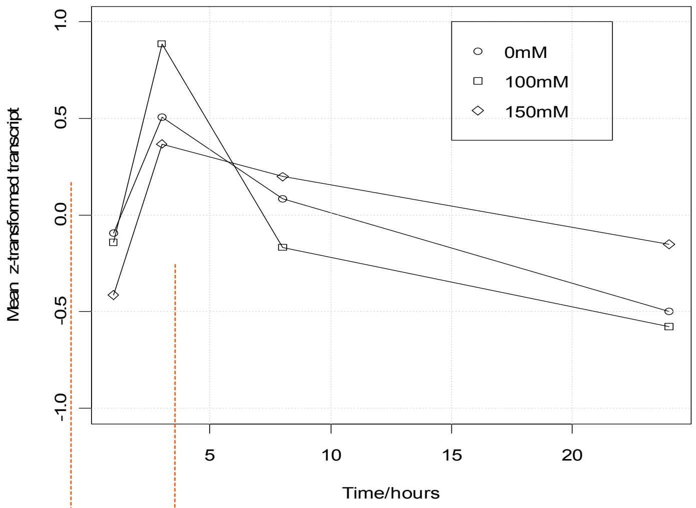
The salt stress experiment – Transcriptomics

164 transcripts quantified
~ 65 also with quantified protein

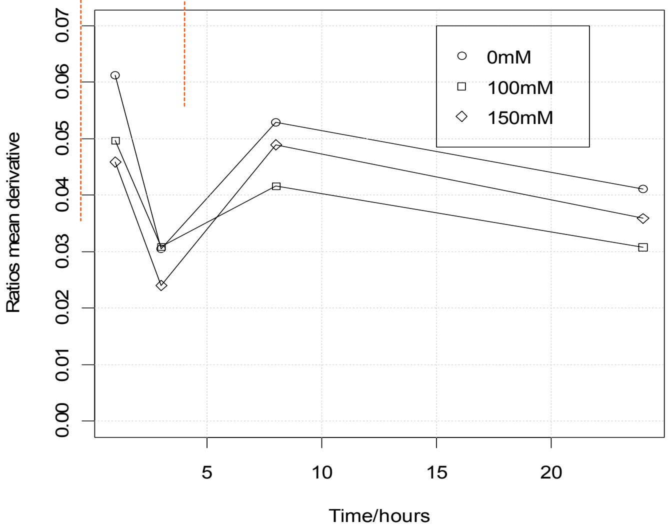
Ratio means (matching protein-transcript)



Mean z-transformed transcript (matching protein-transcript)



Ratios mean derivative (matching protein-transcript)



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