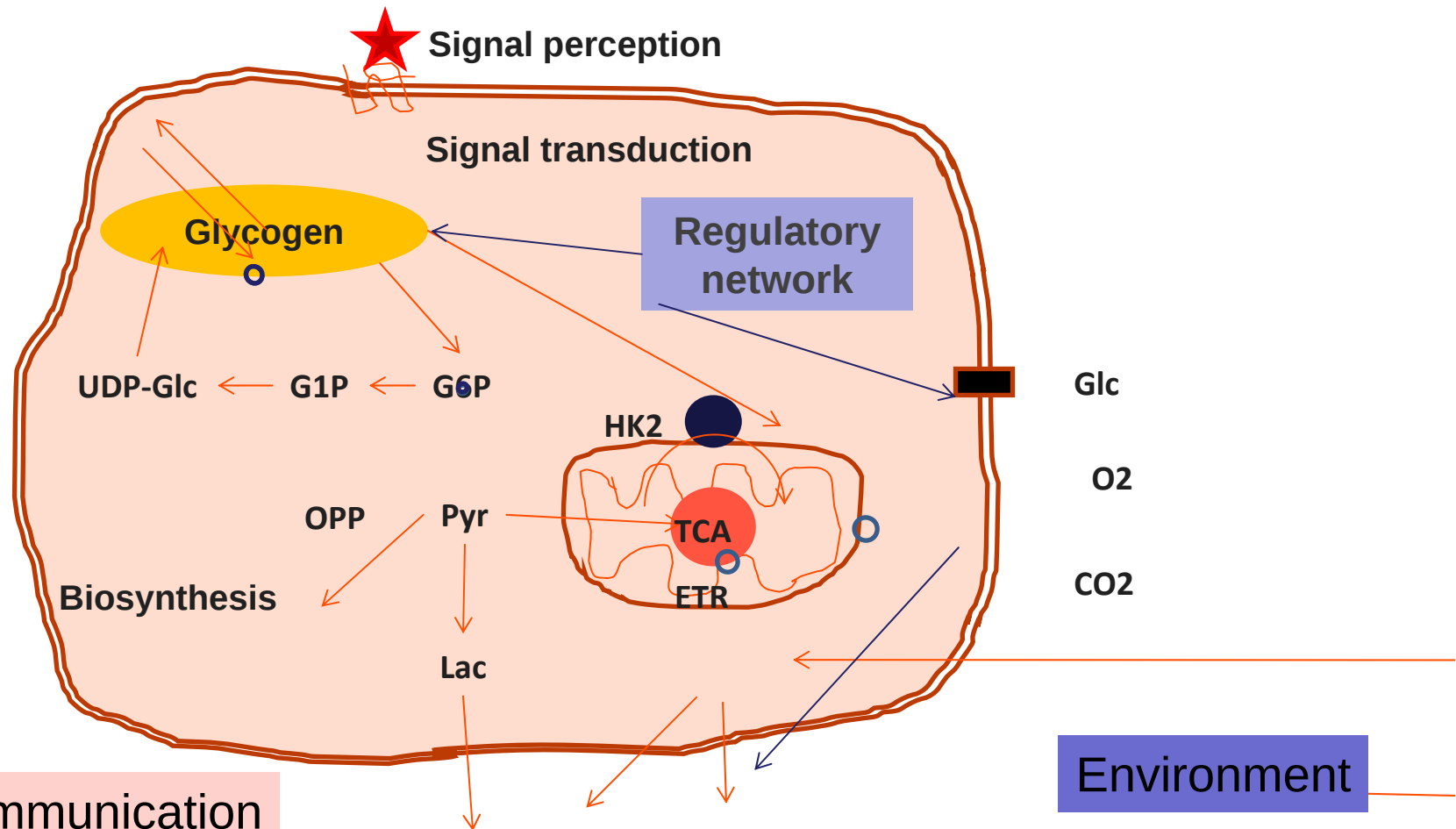


Integrative metabolomics and proteomics

Stefan Kempa BIMSB

- Chemical space of biomolecules
- Integrative analyses of metabolic regulation

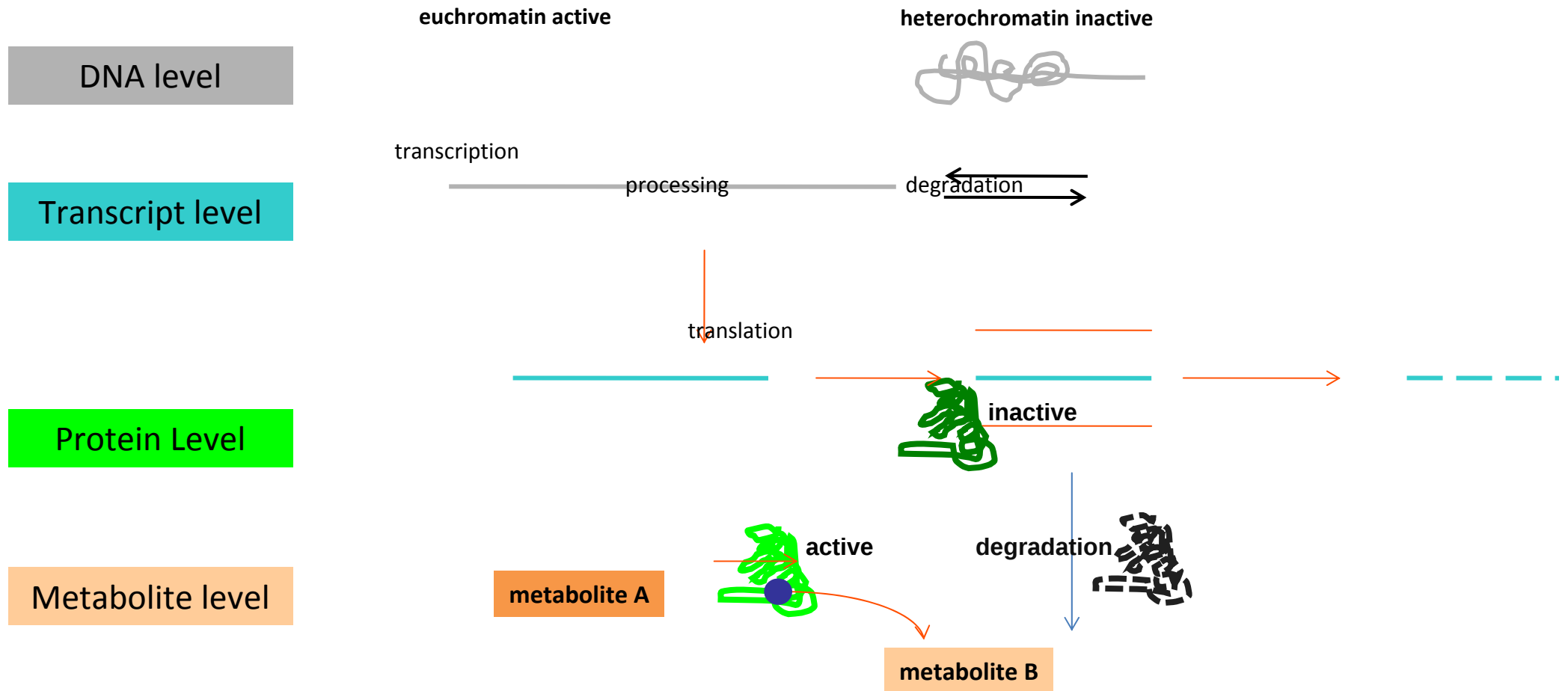
System – cell communication



Cell – cell communication

Gene activation (regulation)

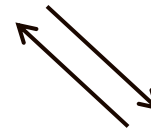
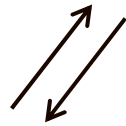
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at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch

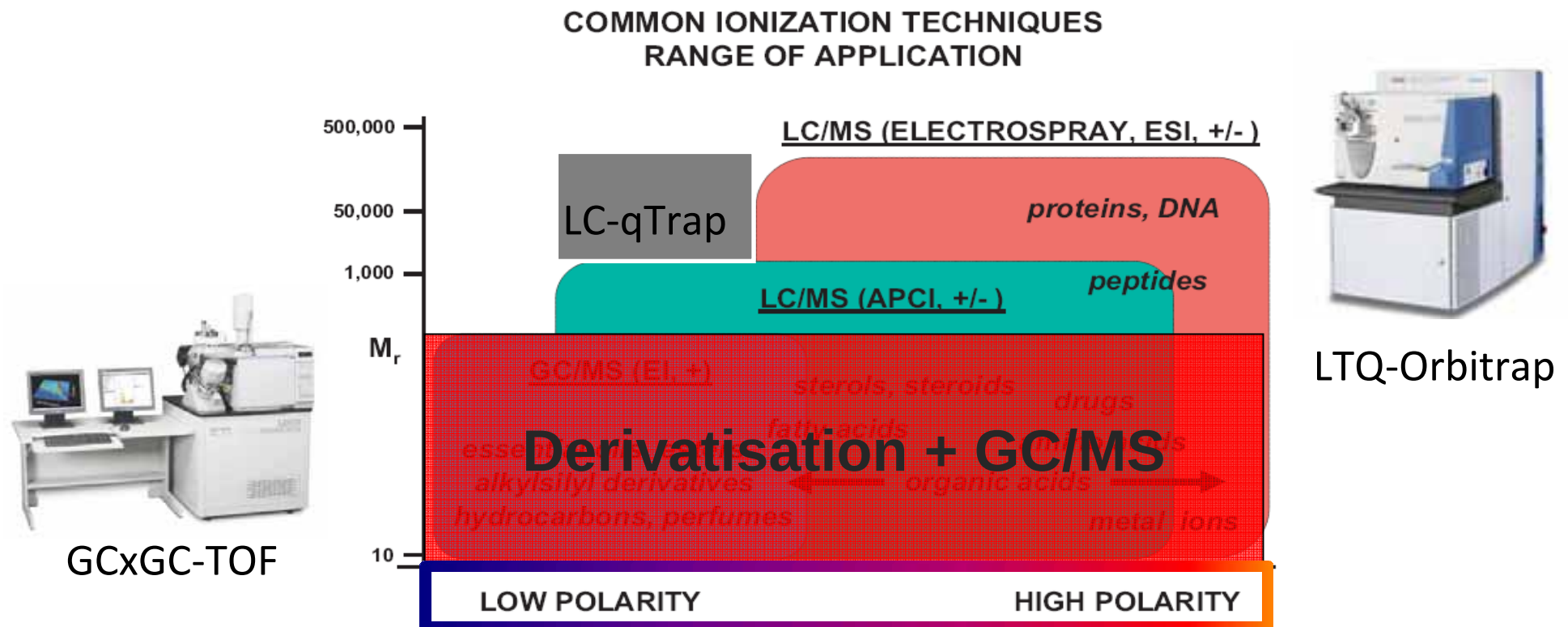
transcriptome



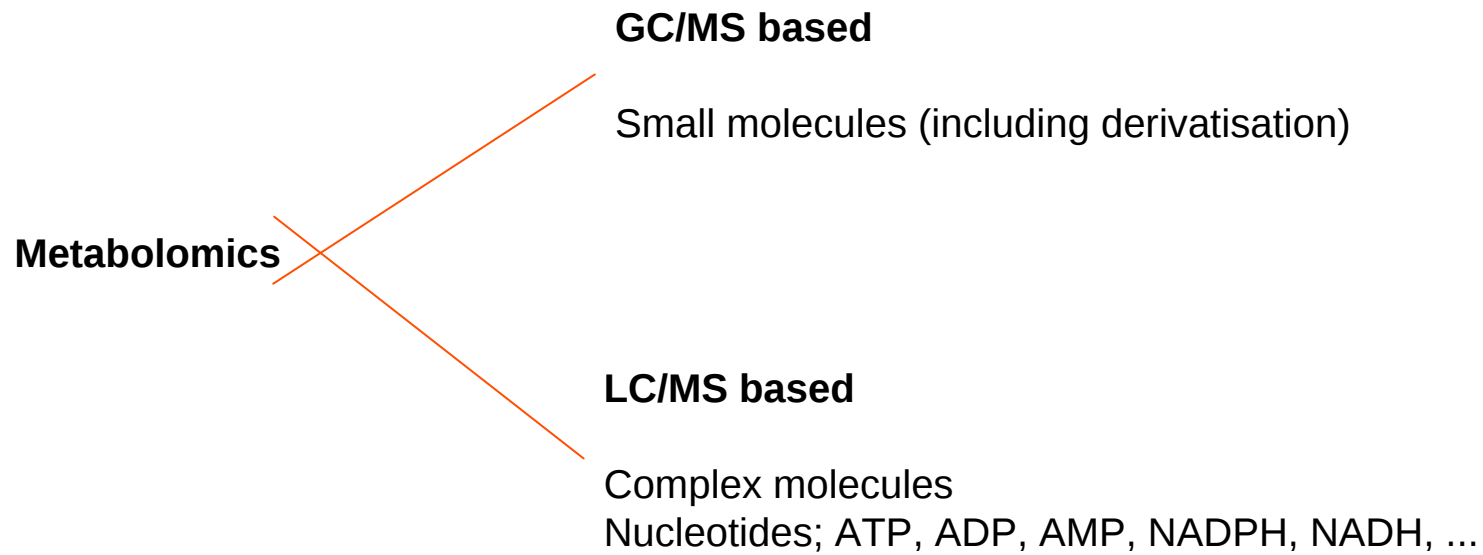
proteome

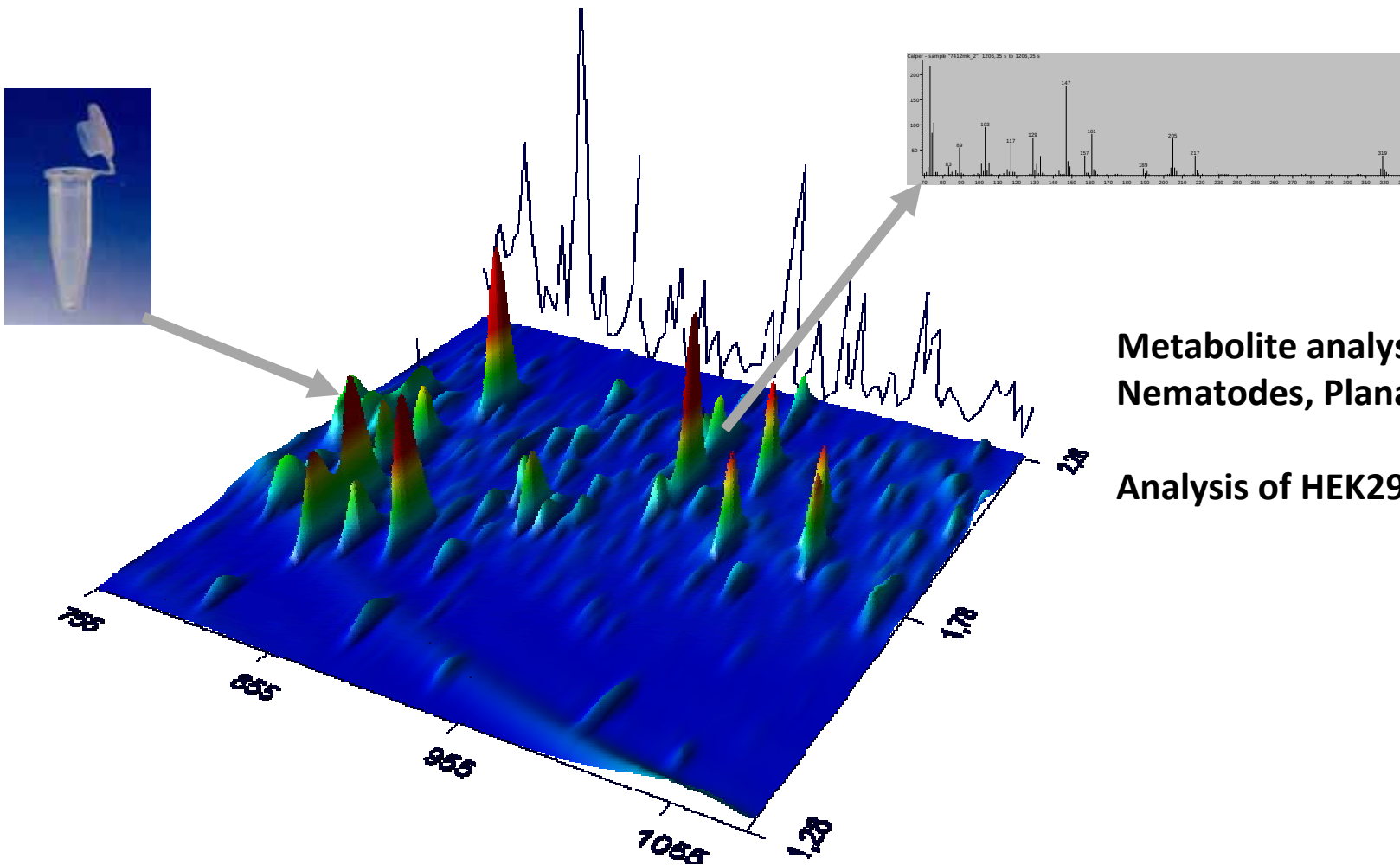


metabolome



Halket et al. 2005



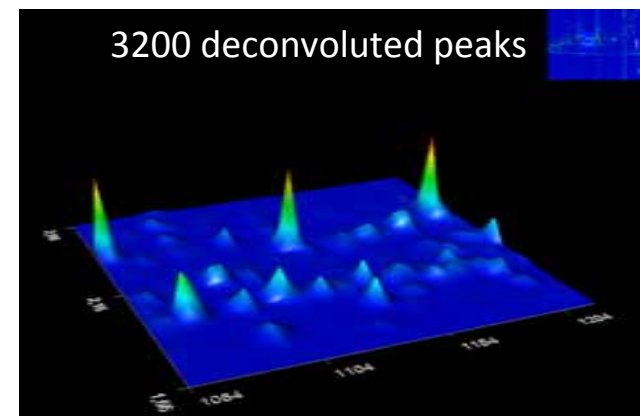
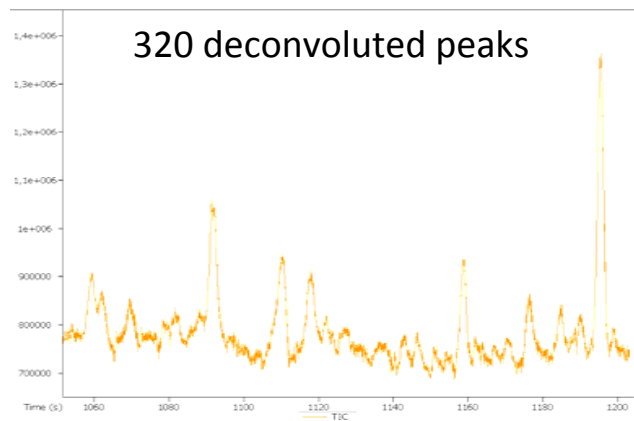


**Metabolite analyses of BIMSB MO's
Nematodes, Planaria, cell cultures**

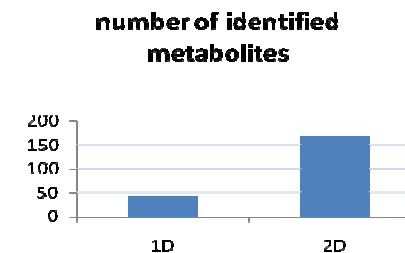
Analysis of HEK293 cell culture

Analysing biological samples using GCxGC/MS

BIMSB



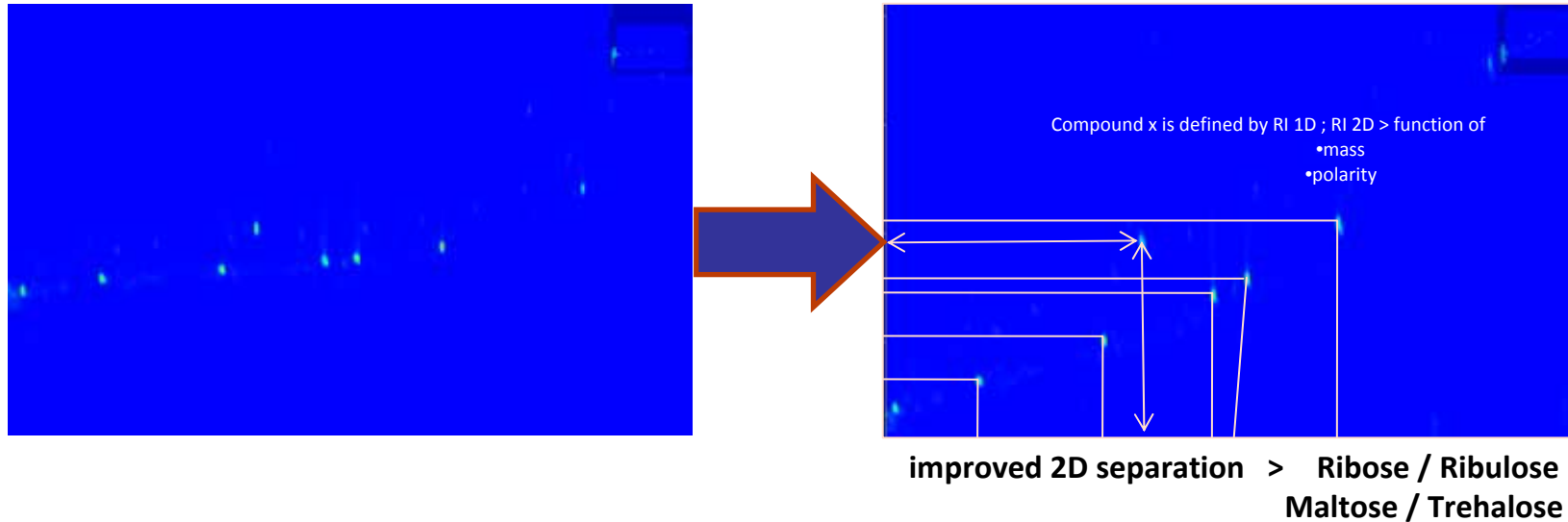
GC-mode	1D	2D
number of identified metabolites	42	168
similarity	893	892



calculation of 2D retention index

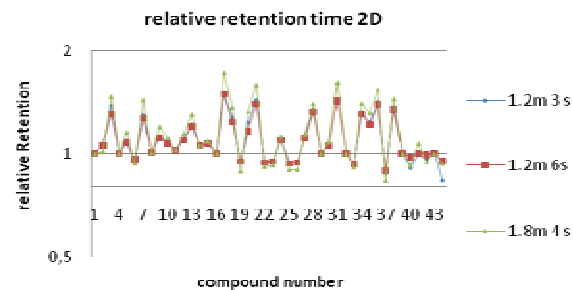
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1. Adaptation of 2D separation



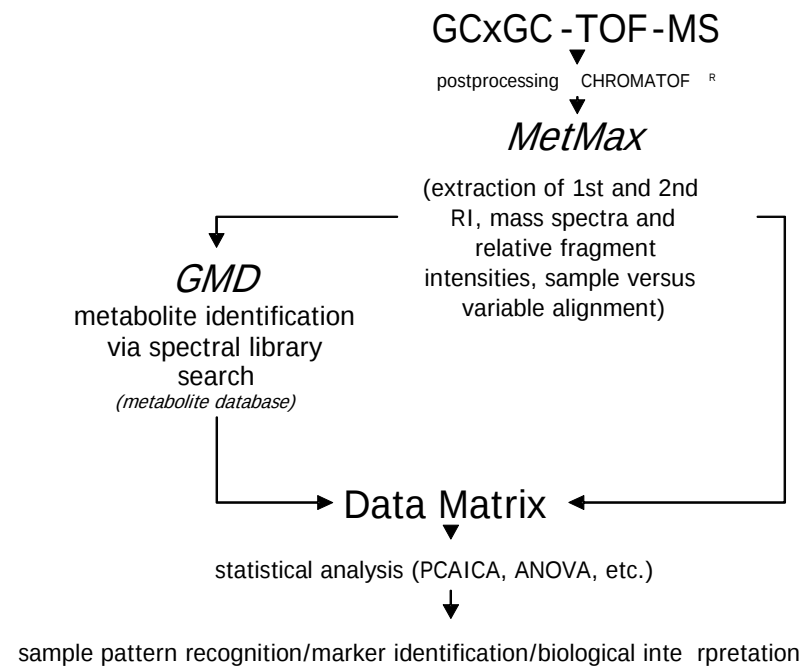
2. stability of relative retention

(various modulation times and column length)



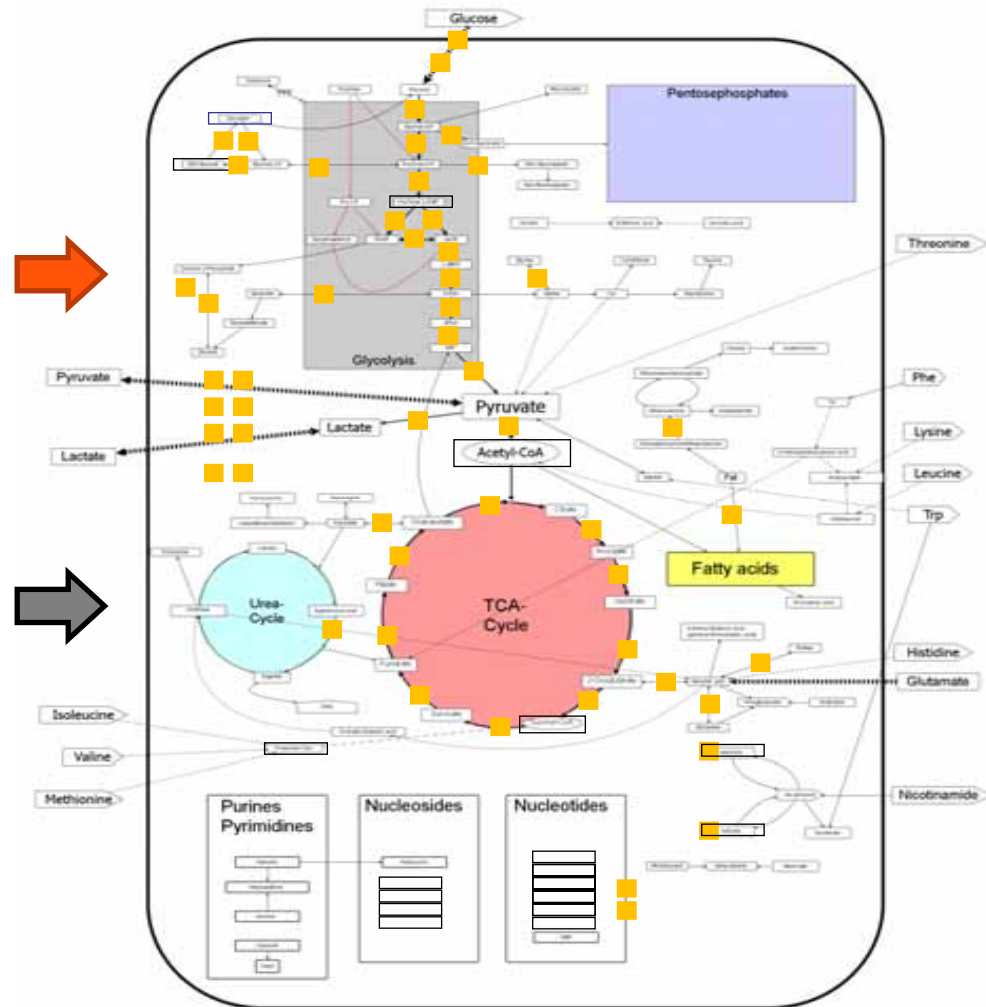
GCxGC metabolite matrix builder > MetMax

- Calculation of 2D retention index
- Peak alignment – matrix building
- Export of retention information
- Export of isotopomer matrix
- Export of quantitative information
- Library search based on GMD information
- Platform to enter new compounds to GMD



Kempa et al. JBM 2009

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[illegible]

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at the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch

Analysis of salt stress response of Arabidopsis

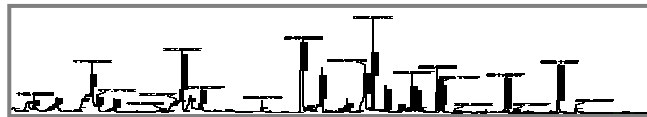
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salt stress

experiment 1: time course 0h – 5d

experiment 2: time course 0h – 5d

metabolite profiling
identification of altered metabolites



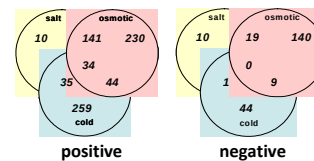
log2 transformed

to analyse metabolic reaction

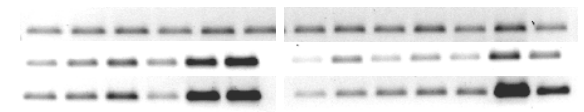
z-transformed

to compare metabolite levels of exp1 and exp2

Public micro array data
cold, salt and osmotic stress



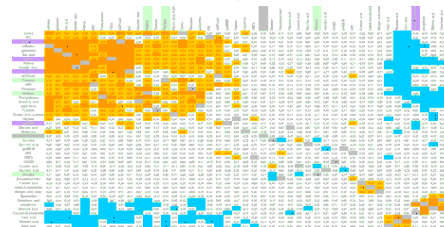
selected genes were tested
in both experiments



z-transformed

Transformed data

to compare metabolites and transcripts



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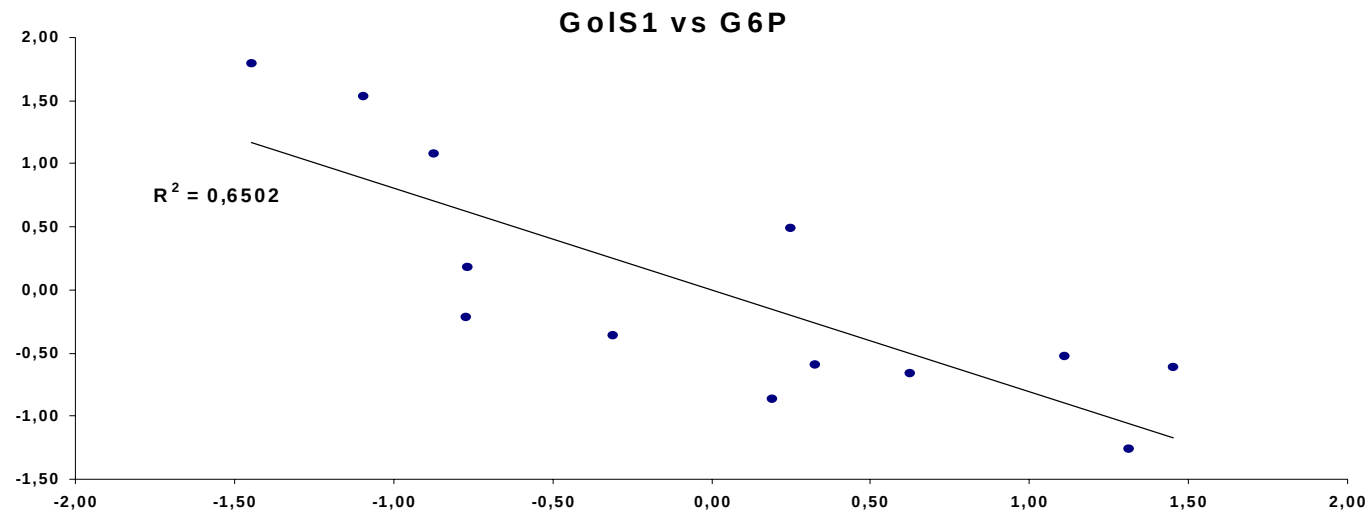
Correlation matrix of metabolite and transcript data

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	ABI 1	RAB 18	ABI 2	Phenylalanine	GABA trans. 2	Maltose	Serine	P5CS	Proline	Raffinose	Invertase	GOL S2	Glutamate	Starch synth.	BMY 7	Galactinol	Na	BMY8	Threonine	AGPase	Raffinose synth.	G6P DH	Tyrosine	GABA trans. 1	Inositol	Nitrate reductas	Glucose	K	Shikimic acid	Galactonate	GAD2	Starch
ABI 1		0.98	0.97	0.96	0.95	0.93	0.93	0.92	0.91	0.91	0.89	0.88	0.87	0.87	0.86	0.86	0.85	0.81	0.80	0.78	0.78	0.74	0.72	0.72	0.68	0.68	-0.01	-0.14	-0.72	-0.75	-0.81	-0.83
RAB 18	0.98		0.96	0.93	0.93	0.91	0.91	0.90	0.92	0.93	0.87	0.89	0.83	0.87	0.84	0.88	0.84	0.79	0.77	0.82	0.75	0.73	0.67	0.67	0.72	0.66	-0.02	-0.18	-0.69	-0.71	-0.76	-0.85
ABI 2	0.97	0.96		0.92	0.97	0.95	0.89	0.95	0.88	0.84	0.91	0.80	0.81	0.89	0.90	0.77	0.79	0.85	0.75	0.73	0.86	0.81	0.69	0.77	0.61	0.68	-0.04	-0.21	-0.78	-0.78	-0.77	-0.85
Phenylalanine	0.96	0.93	0.92		0.92	0.87	0.89	0.88	0.90	0.87	0.84	0.88	0.87	0.81	0.81	0.81	0.74	0.74	0.78	0.70	0.74	0.73	0.63	0.74	0.61	0.67	-0.07	-0.22	-0.83	-0.82	-0.80	-0.79
GABA trans. 2	0.95	0.93	0.97	0.92		0.89	0.89	0.98	0.82	0.83	0.90	0.76	0.82	0.95	0.95	0.78	0.78	0.90	0.68	0.63	0.91	0.82	0.64	0.84	0.55	0.81	0.02	-0.15	-0.75	-0.78	-0.78	-0.82
Maltose	0.93	0.91	0.95	0.87	0.89		0.89	0.89	0.86	0.85	0.83	0.83	0.81	0.83	0.76	0.77	0.80	0.70	0.80	0.78	0.76	0.63	0.67	0.64	0.69	0.50	0.00	-0.23	-0.70	-0.71	-0.68	-0.83
Serine	0.93	0.91	0.89	0.89	0.89	0.89		0.84	0.86	0.90	0.75	0.80	0.96	0.82	0.72	0.82	0.85	0.69	0.88	0.71	0.66	0.58	0.80	0.58	0.69	0.54	0.01	-0.21	-0.66	-0.80	-0.74	-0.85
P5CS	0.92	0.90	0.95	0.88	0.98	0.89	0.84		0.78	0.80	0.93	0.76	0.76	0.97	0.95	0.75	0.74	0.91	0.59	0.60	0.93	0.83	0.60	0.88	0.55	0.82	0.03	-0.15	-0.75	-0.72	-0.77	-0.84
Proline	0.91	0.92	0.88	0.90	0.82	0.86	0.86	0.78		0.95	0.84	0.90	0.78	0.70	0.67	0.89	0.80	0.67	0.78	0.87	0.56	0.64	0.81	0.52	0.83	0.50	0.03	-0.19	-0.72	-0.63	-0.68	-0.88
Raffinose	0.91	0.93	0.84	0.87	0.83	0.85	0.90	0.80	0.95		0.81	0.93	0.81	0.80	0.67	0.98	0.88	0.68	0.77	0.88	0.57	0.56	0.85	0.51	0.90	0.56	0.17	-0.05	-0.58	-0.56	-0.66	-0.9
Invertase	0.89	0.87	0.91	0.84	0.90	0.83	0.75	0.93	0.84	0.81		0.79	0.65	0.88	0.89	0.77	0.76	0.92	0.50	0.69	0.81	0.90	0.65	0.80	0.64	0.76	-0.01	-0.10	-0.75	-0.54	-0.77	-0.88
GOL S2	0.88	0.89	0.80	0.88	0.76	0.83	0.80	0.76	0.90	0.93	0.79		0.73	0.73	0.61	0.92	0.82	0.59	0.74	0.90	0.55	0.55	0.67	0.52	0.84	0.51	0.09	-0.02	-0.62	-0.52	-0.64	-0.79
Glutamate	0.87	0.83	0.81	0.87	0.82	0.81	0.96	0.76	0.78	0.81	0.65	0.73		0.73	0.66	0.73	0.73	0.61	0.83	0.57	0.56	0.52	0.73	0.57	0.55	0.51	-0.12	-0.29	-0.61	-0.79	-0.82	-0.75
Starch synth.	0.87	0.87	0.89	0.81	0.95	0.83	0.82	0.97	0.70	0.80	0.88	0.73	0.73		0.92	0.78	0.78	0.91	0.55	0.58	0.91	0.77	0.61	0.84	0.57	0.85	0.14	-0.02	-0.62	-0.63	-0.71	-0.82
BMY 7	0.86	0.84	0.90	0.81	0.95	0.76	0.72	0.95	0.67	0.67	0.89	0.61	0.66	0.92		0.63	0.65	0.96	0.46	0.47	0.94	0.91	0.53	0.92	0.37	0.90	-0.01	-0.10	-0.70	-0.68	-0.79	-0.72
Galactinol	0.86	0.88	0.77	0.81	0.78	0.77	0.82	0.75	0.89	0.98	0.77	0.92	0.73	0.78	0.63		0.89	0.66	0.70	0.87	0.53	0.50	0.78	0.47	0.91	0.59	0.28	0.10	-0.47	-0.44	-0.59	-0.83
Na	0.85	0.84	0.79	0.74	0.78	0.80	0.85	0.74	0.80	0.88	0.76	0.82	0.73	0.78	0.65	0.89		0.69	0.79	0.85	0.60	0.51	0.76	0.46	0.83	0.53	0.29	0.25	-0.40	-0.44	-0.59	-0.76
BMY8	0.81	0.79	0.85	0.74	0.90	0.70	0.69	0.91	0.67	0.68	0.92	0.59	0.61	0.91	0.96	0.66	0.69		0.39	0.47	0.88	0.92	0.60	0.87	0.44	0.91	0.07	0.02	-0.62	-0.54	-0.78	-0.77
Threonine	0.80	0.77	0.75	0.78	0.68	0.80	0.88	0.59	0.78	0.77	0.50	0.74	0.83	0.55	0.46	0.70	0.79	0.39		0.76	0.45	0.30	0.65	0.30	0.65	0.22	0.08	-0.08	-0.52	-0.73	-0.50	-0.61
AGPase	0.78	0.82	0.73	0.70	0.63	0.78	0.71	0.60	0.87	0.88	0.69	0.90	0.57	0.58	0.47	0.87	0.85	0.47	0.76		0.41	0.41	0.72	0.26	0.91	0.28	0.20	0.08	-0.44	-0.36	-0.41	-0.72
Raffinose synth.	0.78	0.75	0.86	0.74	0.91	0.76	0.66	0.93	0.56	0.57	0.81	0.55	0.56	0.91	0.94	0.53	0.60	0.88	0.45	0.41		0.82	0.36	0.90	0.30	0.83	0.07	-0.05	-0.70	-0.69	-0.61	-0.63
G6P DH	0.74	0.73	0.81	0.73	0.82	0.63	0.58	0.83	0.64	0.56	0.90	0.55	0.52	0.77	0.91	0.50	0.51	0.92	0.30	0.41	0.82		0.49	0.86	0.29	0.80	-0.19	-0.17	-0.78	-0.58	-0.77	-0.71
Tyrosine	0.72	0.77	0.69	0.63	0.64	0.67	0.80	0.60	0.81	0.85	0.65	0.67	0.73	0.61	0.53	0.78	0.76	0.60	0.65	0.72	0.36	0.49		0.32	0.78	0.37	0.11	-0.12	-0.41	-0.44	-0.62	-0.87
GABA trans. 1	0.72	0.67	0.77	0.74	0.84	0.64	0.58	0.88	0.52	0.51	0.80	0.52	0.57	0.84	0.92	0.47	0.46	0.87	0.30	0.26	0.90	0.86	0.32		0.21	0.90	0.03	-0.06	-0.72	-0.63	-0.76	-0.61
Inositol	0.68	0.72	0.61	0.61	0.55	0.69	0.69	0.55	0.83	0.90	0.64	0.84	0.55	0.57	0.37	0.91	0.83	0.44	0.65	0.91	0.30	0.29	0.78	0.21		0.29	0.40	0.17	-0.34	-0.22	-0.36	-0.78
Nitrate reductase	0.68	0.66	0.68	0.67	0.81	0.50	0.54	0.82	0.50	0.56	0.76	0.51	0.51	0.85	0.90	0.59	0.53	0.91	0.22	0.28	0.83	0.80	0.37	0.90	0.29		0.19	0.15	-0.52	-0.47	-0.69	-0.57
Glucose	-0.01	-0.02	-0.04	-0.07	0.02	0.00	0.01	0.03	0.03	0.17	-0.01	0.09	-0.12	0.14	-0.01	0.28	0.29	0.07	0.08	0.20	0.07	-0.19	0.11	0.03	0.40	0.19		0.79	0.23	0.22	0.25	-0.05
K	-0.14	-0.18	-0.21	-0.22	-0.15	-0.23	-0.21	-0.15	-0.19	-0.05	-0.10	-0.02	-0.29	-0.02	-0.10	0.10	0.25	0.02	-0.08	0.08	-0.05	-0.17	-0.12	-0.06	0.17	0.15	0.79		0.43	0.45	0.22	0.22
Shikimic acid	-0.72	-0.69	-0.78	-0.83	-0.75	-0.70	-0.66	-0.75	-0.72	-0.58	-0.75	-0.62	-0.61	-0.62	-0.70	-0.47	-0.40	-0.62	-0.52	-0.44	-0.70	-0.78	-0.41	-0.72	-0.34	-0.52	0.23	0.43		0.81	0.61	0.71
Galactonate	-0.75	-0.71	-0.78	-0.82	-0.78	-0.71	-0.80	-0.72	-0.63	-0.56	-0.54	-0.52	-0.79	-0.63	-0.68	-0.44	-0.44	-0.54	-0.73	-0.36	-0.69	-0.58	-0.44	-0.63	-0.22	-0.47	0.22	0.45	0.81		0.59	0.57
GAD2	-0.81	-0.76	-0.77	-0.80	-0.78	-0.68	-0.74	-0.77	-0.68	-0.66	-0.77	-0.64	-0.82	-0.71	-0.79	-0.59	-0.59	-0.78	-0.50	-0.41	-0.61	-0.77	-0.62	-0.76	-0.36	-0.69	0.25	0.22	0.61	0.59		0.68
Starch	-0.83	-0.85	-0.85	-0.79	-0.82	-0.83	-0.85	-0.84	-0.88	-0.9	-0.88	-0.79	-0.75	-0.82	-0.72	-0.83	-0.76	-0.77	-0.61	-0.72	-0.63	-0.71	-0.87	-0.61	-0.78	-0.57	-0.05	0.22	0.71	0.57	0.68	

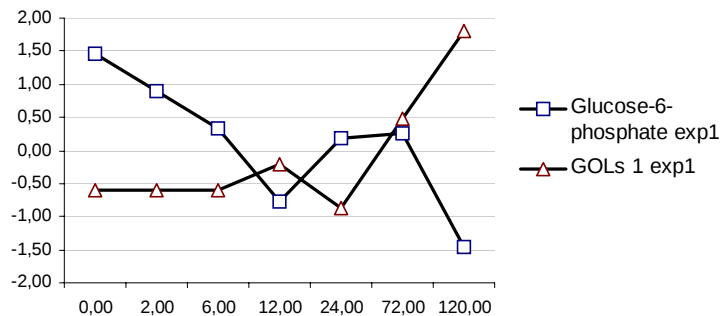
Correlation of glucose-6-phosphate and galactinol synthase

BIMSB

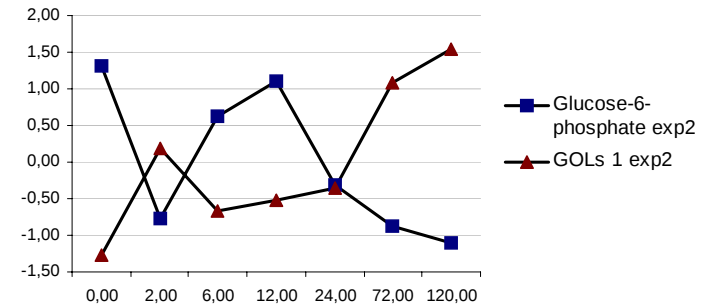


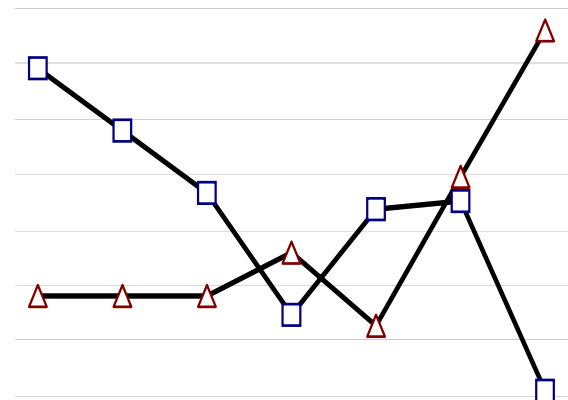
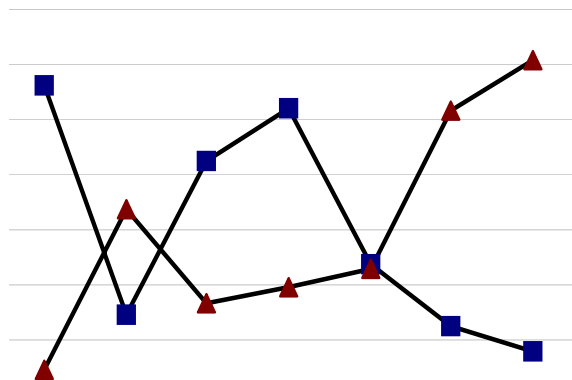
-time dependent behaviour of G6P and GoIS

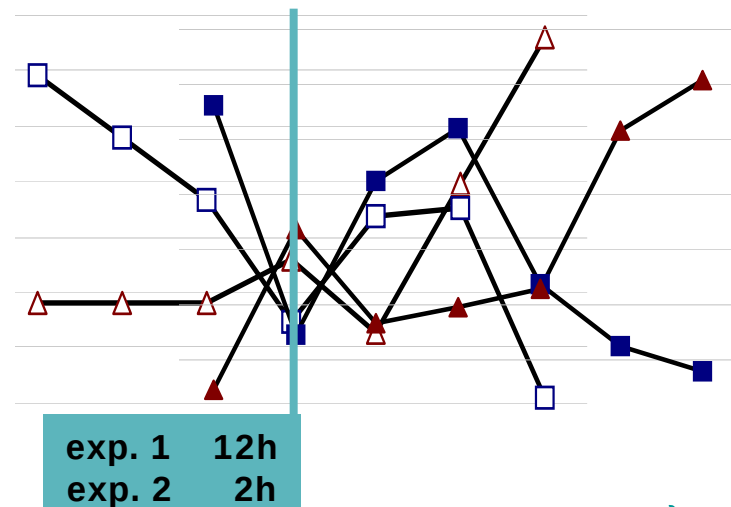
E1



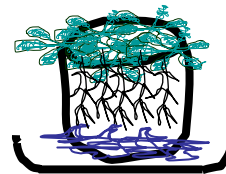
E2







time shift: exp. 1 big pots



exp. 2 small pots



salt at the roots



signal to the leaves
(ABA)



stomata closure

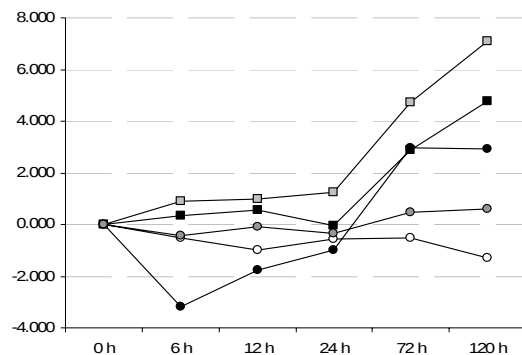


energy depletion



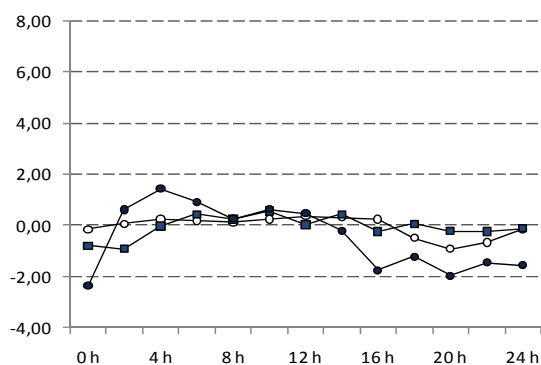
gene expression,
metabolic reaction
(G6P, Asc, ...)

3 data sets from *Arabidopsis thaliana* in a time course manner
analysed using GC/MS based metabolomics
analyses were performed on metabolite and transcript level



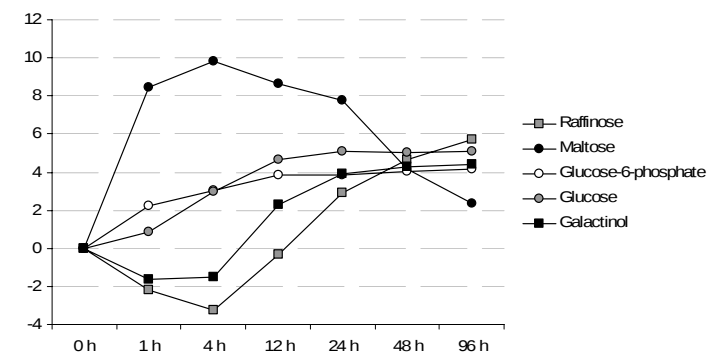
salt stress

Kempa et al. 2008



diurnal rhythm

Gibon et al. 2006



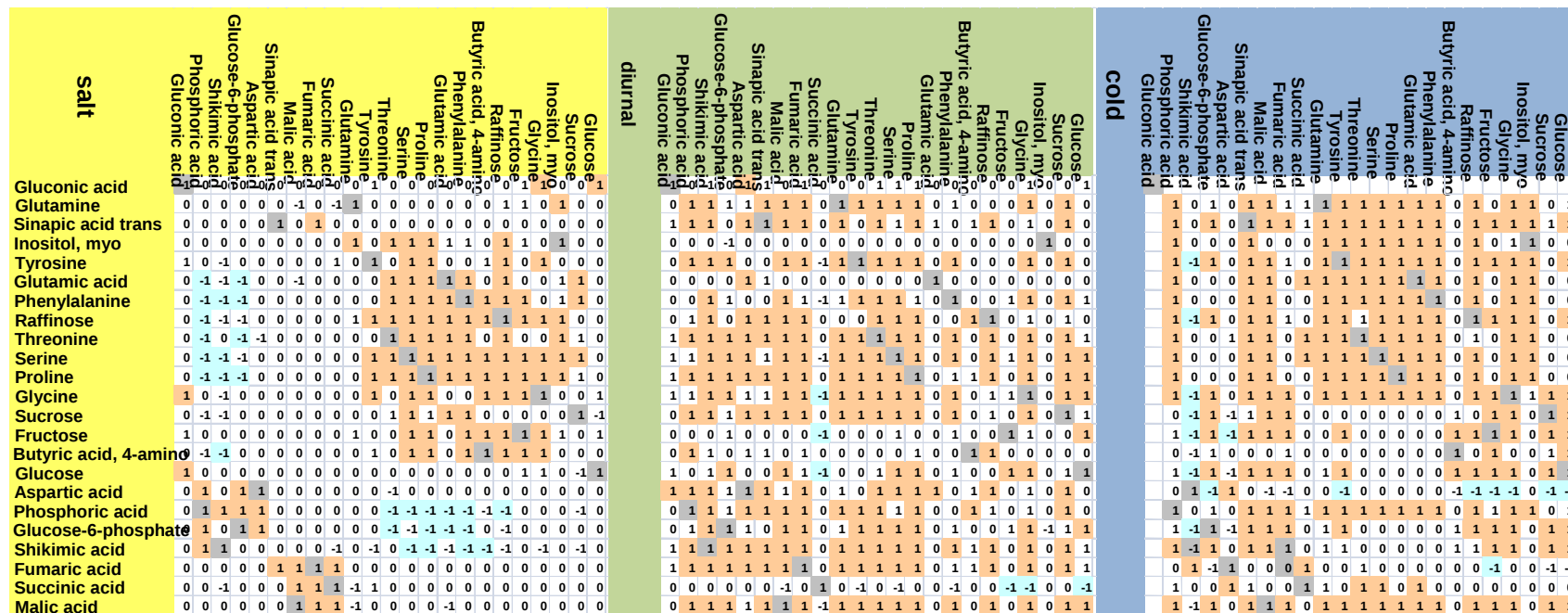
cold stress

Kaplan et al. 2004

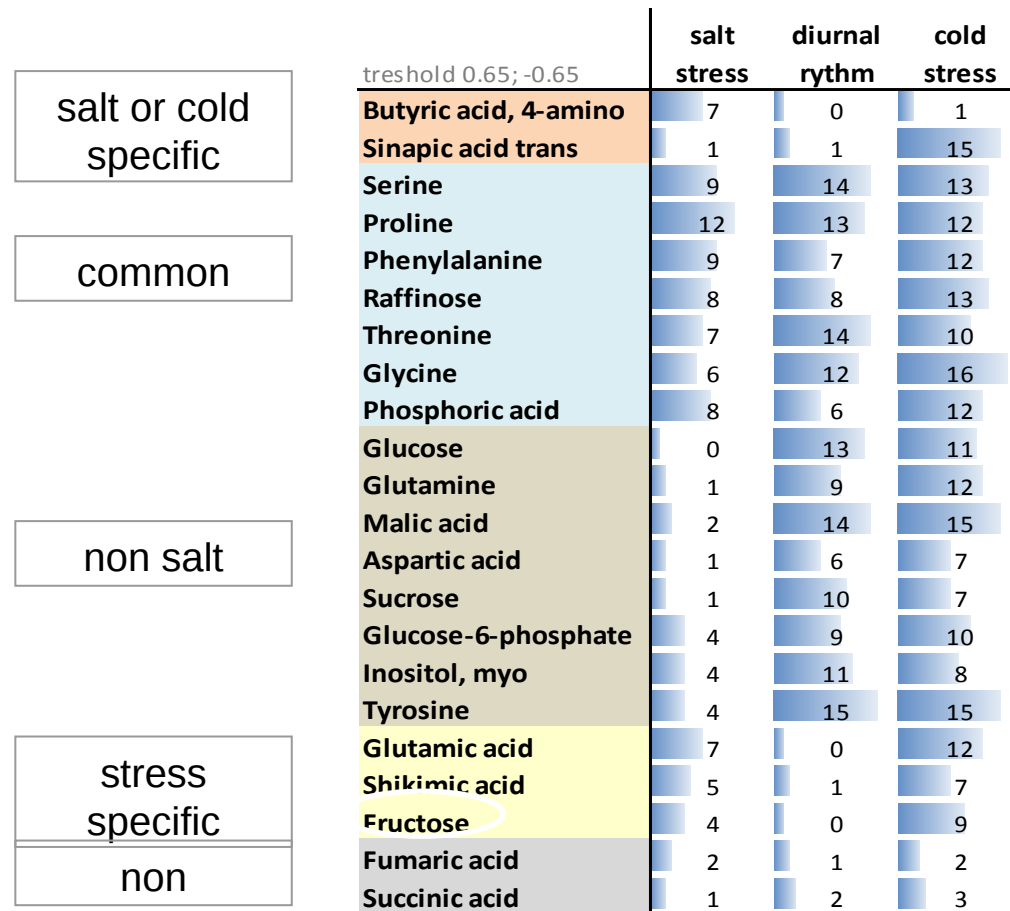
the data sets were aligned and pairwise metabolite metabolite correlations calculated

Data integration

pairwise metabolite-metabolite correlations (Pearson correlation coefficient)
correlation coefficients < -0.65 (blue) and > 0.65 (red) are indicated

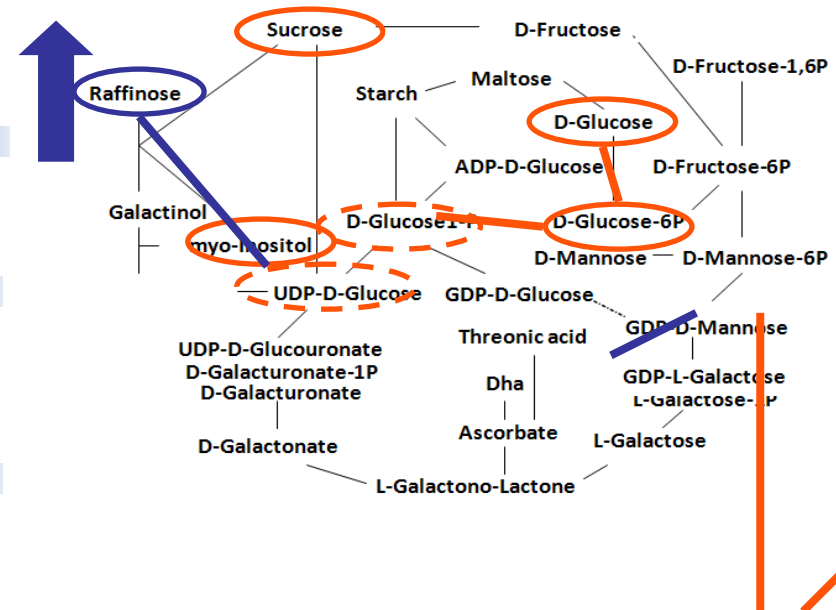


Number of associations between metabolites



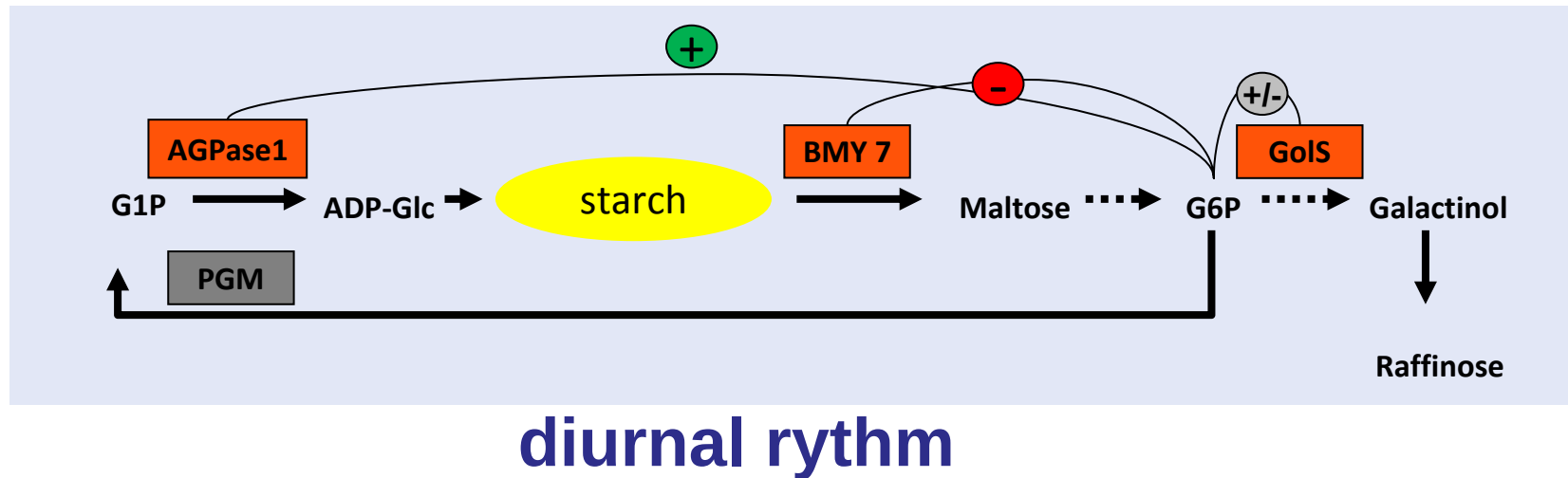
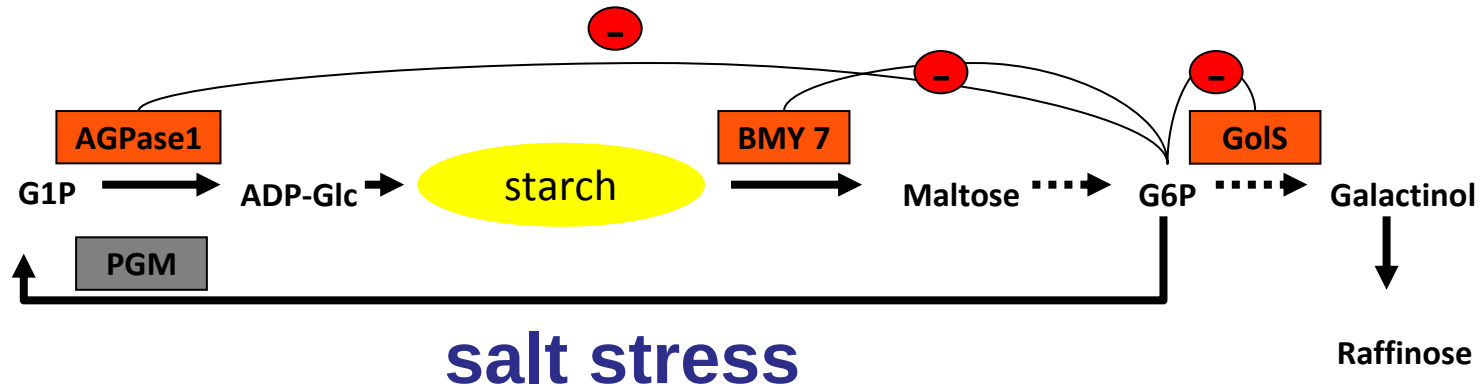
Number of associations between metabolites

		salt stress	diurnal rythm	cold stress
	threshold 0.65; -0.65			
salt or cold specific	Butyric acid, 4-amino	7	0	1
	Sinapic acid trans	1	1	15
	Serine	9	14	13
common	Proline	12	13	12
	Phenylalanine	9	7	12
	Raffinose	8	8	13
	Threonine	7	14	10
	Glycine	6	12	16
	Phosphoric acid	8	6	12
non salt	Glucose	0	13	11
	Glutamine	1	9	12
	Malic acid	2	14	15
	Aspartic acid	1	6	7
	Sucrose	1	10	7
	Glucose-6-phosphate	4	9	10
	Inositol, myo	4	11	8
	Tyrosine	4	15	15
	Glutamic acid	7	0	12
	Shikimic acid	5	1	7
stress specific	Fructose	4	0	9
	Fumaric acid	2	1	2
	Succinic acid	1	2	3

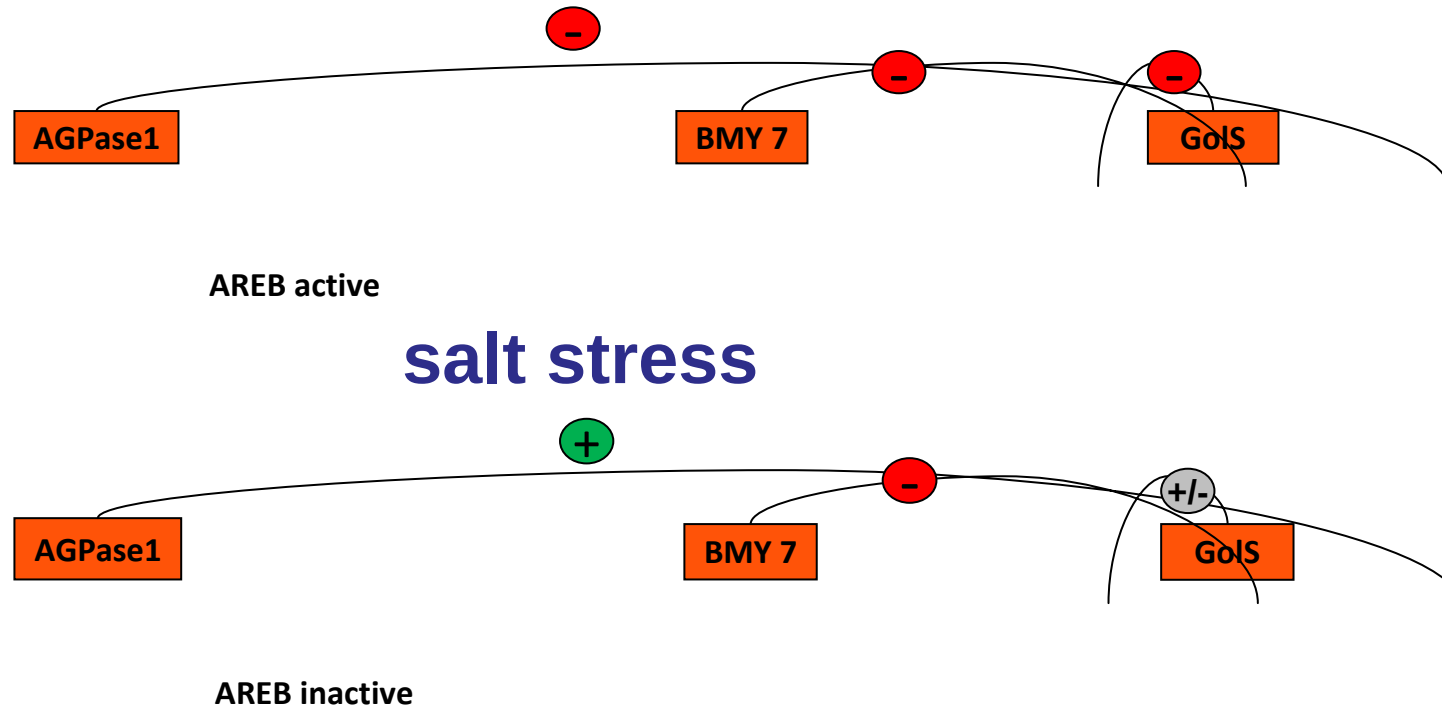


Correlation matrix of metabolite and transcript data

	ABI 1	RAB 18	ABI 2	Phenylalanine	GABA trans. 2	Maltose	Serine	P5CS	Proline	Raffinose	Invertase	GOL S2	Glutamate	Starch synth.	BM Y 7	Galactinol	Na	BM Y8	Threonine	AGPase	Raffinose synth.	G6P DH	Tyrosine	GABA trans. 1	Inositol	Nitrate reductas	Glucose	K	Shikimic acid	Galactonate	GAD2	Starch
ABI 1																																
RAB 18	0.98																															
ABI 2	0.97	0.96																														
Phenylalanine	0.96	0.93	0.92																													
GABA trans. 2	0.95	0.93	0.97	0.92																												
Maltose	0.93	0.91	0.95	0.87	0.89																											
Serine	0.93	0.91	0.89	0.89	0.89	0.89																										
P5CS	0.92	0.90	0.95	0.88	0.98	0.89	0.84																									
Proline	0.91	0.92	0.88	0.90	0.82	0.86	0.86	0.78																								
Raffinose	0.91	0.93	0.84	0.87	0.83	0.85	0.90	0.80	0.95																							
Invertase	0.89	0.87	0.91	0.84	0.90	0.83	0.75	0.93	0.84	0.81																						
GOL S2	0.88	0.89	0.80	0.88	0.76	0.83	0.80	0.76	0.90	0.93	0.79																					
Glutamate	0.87	0.83	0.81	0.87	0.82	0.81	0.96	0.76	0.78	0.81	0.65	0.73																				
Starch synth.	0.87	0.87	0.89	0.81	0.95	0.83	0.82	0.97	0.70	0.80	0.88	0.73	0.73																			
BM Y 7	0.86	0.84	0.90	0.81	0.95	0.76	0.72	0.95	0.67	0.67	0.89	0.61	0.66	0.92																		
Galactinol	0.86	0.88	0.77	0.81	0.78	0.77	0.82	0.75	0.89	0.98	0.77	0.92	0.73	0.78	0.63																	
Na	0.85	0.84	0.79	0.74	0.78	0.80	0.85	0.74	0.80	0.88	0.76	0.82	0.73	0.78	0.65	0.89																
BM Y8	0.81	0.79	0.85	0.74	0.90	0.70	0.69	0.91	0.67	0.68	0.92	0.59	0.61	0.91	0.96	0.66	0.69															
Threonine	0.80	0.77	0.75	0.78	0.68	0.80	0.88	0.59	0.78	0.77	0.50	0.74	0.83	0.55	0.46	0.70	0.79	0.39														
AGPase	0.78	0.82	0.73	0.70	0.63	0.78	0.71	0.60	0.87	0.88	0.69	0.90	0.57	0.58	0.47	0.87	0.85	0.47	0.76													
Raffinose synth.	0.78	0.75	0.86	0.74	0.91	0.76	0.66	0.93	0.56	0.57	0.81	0.55	0.56	0.91	0.94	0.53	0.60	0.88	0.45	0.41												
G6P DH	0.74	0.73	0.81	0.73	0.82	0.63	0.58	0.83	0.64	0.56	0.90	0.55	0.52	0.77	0.91	0.50	0.51	0.92	0.30	0.41	0.82											
Tyrosine	0.72	0.77	0.69	0.63	0.64	0.67	0.80	0.60	0.81	0.85	0.65	0.67	0.73	0.61	0.53	0.78	0.76	0.60	0.65	0.72	0.36	0.49										
GABA trans. 1	0.72	0.67	0.77	0.74	0.84	0.64	0.58	0.88	0.52	0.51	0.80	0.52	0.57	0.84	0.92	0.47	0.46	0.87	0.30	0.26	0.90	0.86										
Inositol	0.68	0.72	0.61	0.61	0.55	0.69	0.69	0.55	0.83	0.90	0.64	0.84	0.55	0.57	0.37	0.91	0.83	0.44	0.65	0.91	0.30	0.29	0.78	0.21								
Nitrate reductase	0.68	0.66	0.68	0.67	0.81	0.50	0.54	0.82	0.50	0.56	0.76	0.51	0.51	0.85	0.90	0.59	0.53	0.91	0.22	0.28	0.83	0.80	0.37	0.90	0.29							
Glucose	-0.01	-0.02	-0.04	-0.07	0.02	0.00	0.01	0.03	0.03	0.17	-0.01	0.09	-0.12	0.14	-0.01	0.28	0.29	0.07	0.08	0.20	0.07	-0.19	0.11	0.03	0.40	0.19						
K	-0.14	-0.18	-0.21	-0.22	-0.15	-0.23	-0.21	-0.15	-0.19	-0.05	-0.10	-0.02	-0.29	-0.02	-0.10	0.10	0.25	0.02	-0.08	0.08	-0.05	-0.17	-0.12	-0.06	0.17	0.15	0.79					
Shikimic acid	-0.72	-0.69	-0.78	-0.83	-0.75	-0.70	-0.66	-0.75	-0.72	-0.58	-0.75	-0.62	-0.61	-0.62	-0.70	-0.47	-0.40	-0.62	-0.52	-0.44	-0.70	-0.78	-0.41	-0.72	-0.34	-0.52	0.23	0.43				
Galactonate	-0.75	-0.71	-0.78	-0.82	-0.78	-0.71	-0.80	-0.72	-0.63	-0.56	-0.54	-0.52	-0.79	-0.63	-0.68	-0.44	-0.44	-0.54	-0.73	-0.36	-0.69	-0.58	-0.44	-0.63	-0.22	-0.47	0.22	0.45	0.81			
GAD2	-0.81	-0.76	-0.77	-0.80	-0.78	-0.68	-0.74	-0.77	-0.68	-0.66	-0.77	-0.64	-0.82	-0.71	-0.79	-0.59	-0.59	-0.78	-0.50	-0.41	-0.61	-0.77	-0.62	-0.76	-0.36	-0.69	0.25	0.22	0.61	0.59		
Starch	-0.83	-0.85	-0.85	-0.79	-0.82	-0.83	-0.85	-0.84	-0.88	-0.8	-0.88	-0.79	-0.75	-0.82	-0.72	-0.83	-0.76	-0.77	-0.61	-0.72	-0.63	-0.71	-0.87	-0.61	-0.78	-0.57	-0.05	0.22	0.71	0.57	0.68	



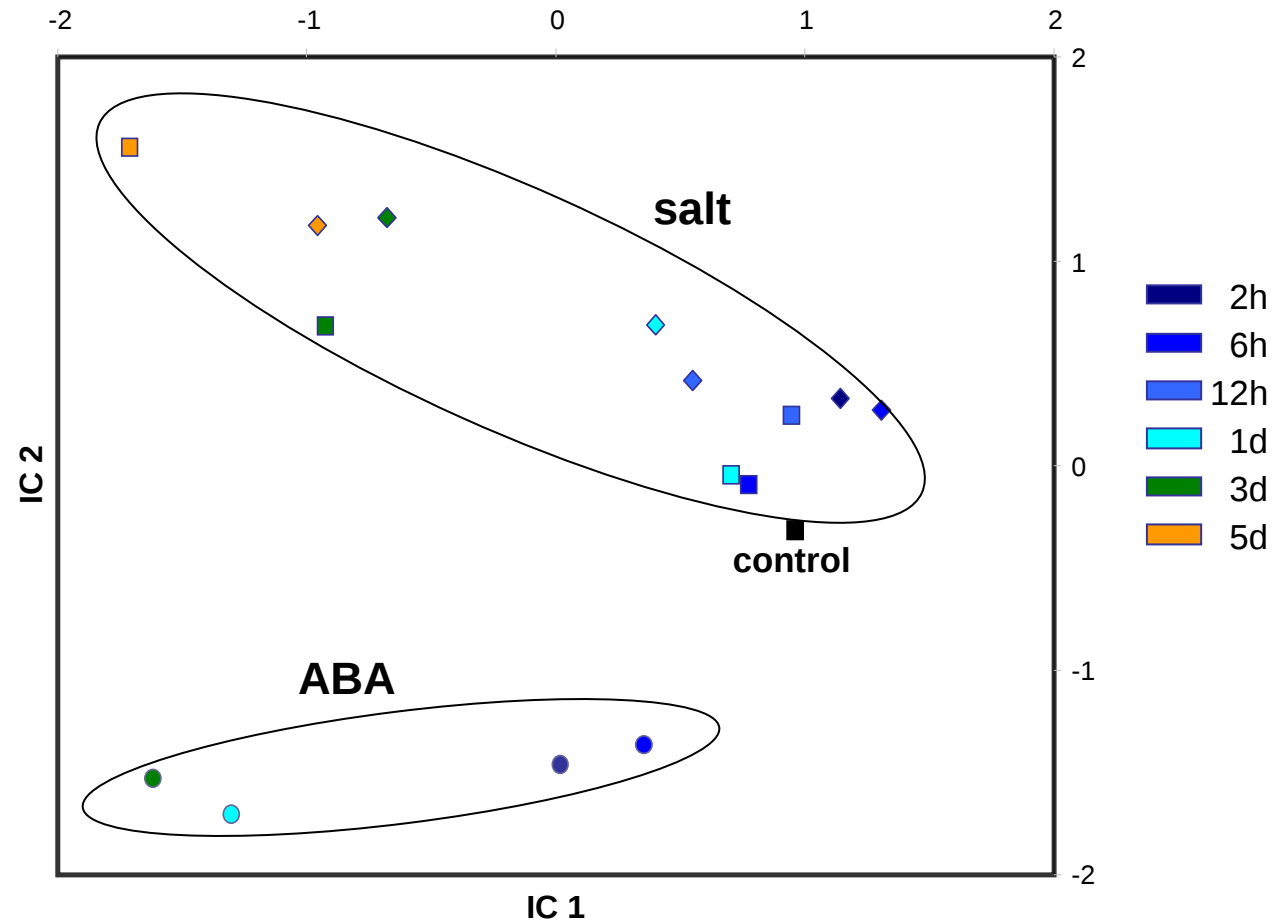
Genes involved in carbohydrate metabolism negatively co-regulated to G6P upon salt stress display a different behavior during extended night.



AGPase1; BMY7 and GoIS contain multiple copies of the promotor element (ARE), the activity of the ABA responsive element binding factor (AREB) over-writes the normal (diurnal) transcript behaviour upon salt stress (ABA induction).

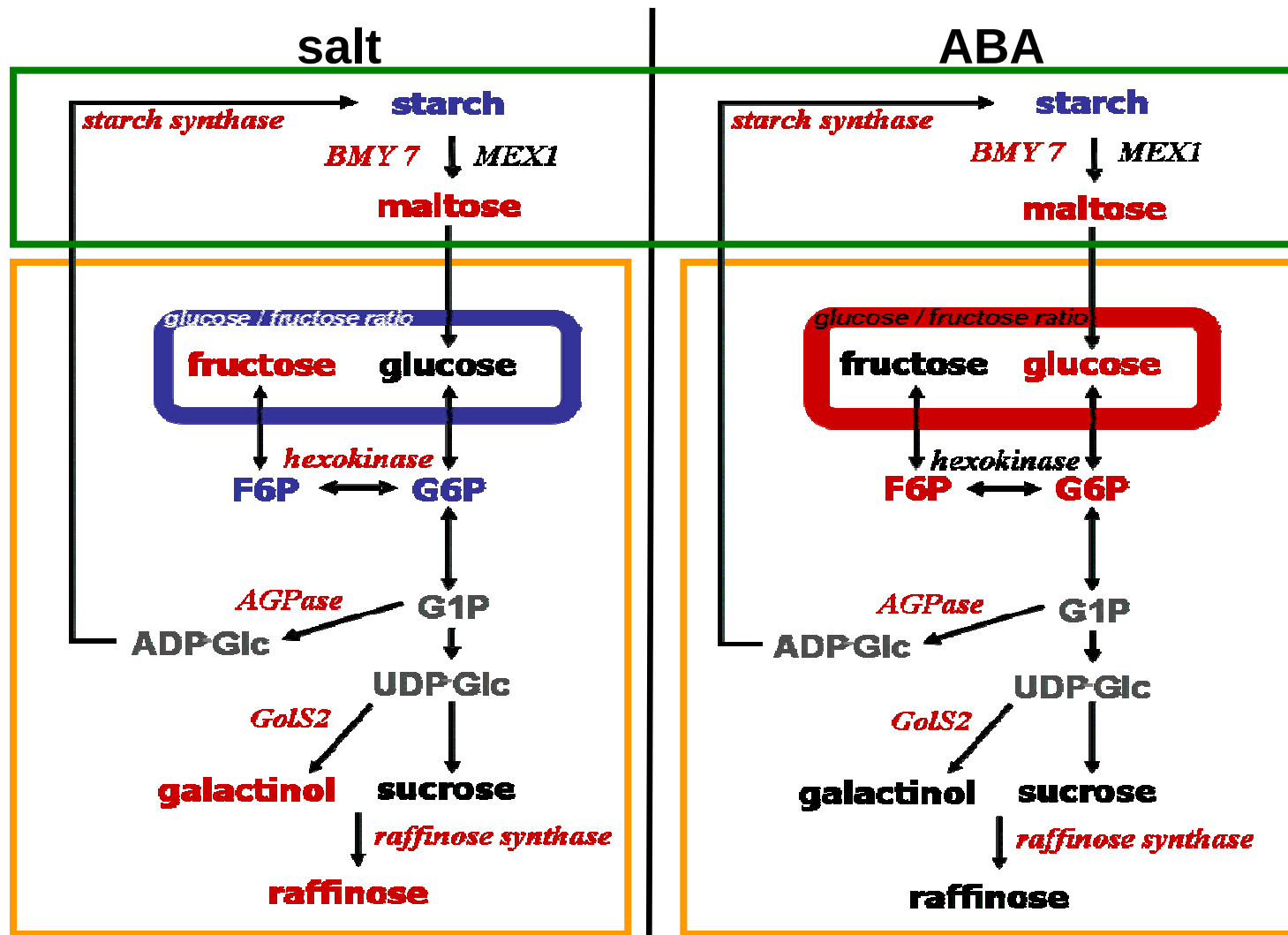
ICA of metabolite data from salt and ABA treated plants

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Overview

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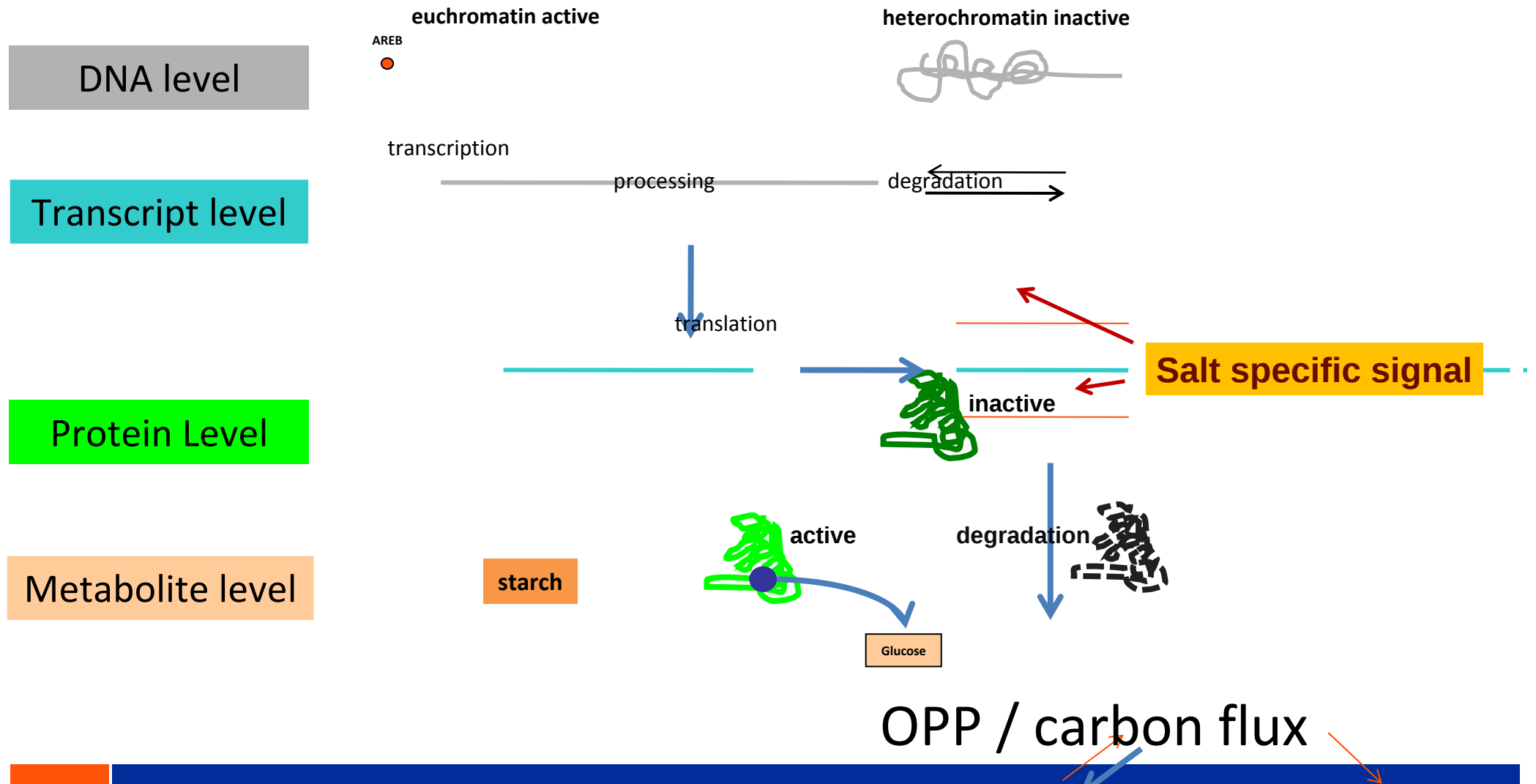
elevated levels reduced levels

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Gene activation

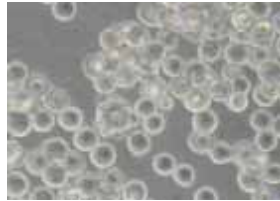
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Cells



Organs



Organisms



proteomics/metabolomics core & genomics core @ BIMSB

Metabolites



Proteins $\longleftrightarrow$ Transcripts

integrative analyses - bioinformatics core @ BIMSB

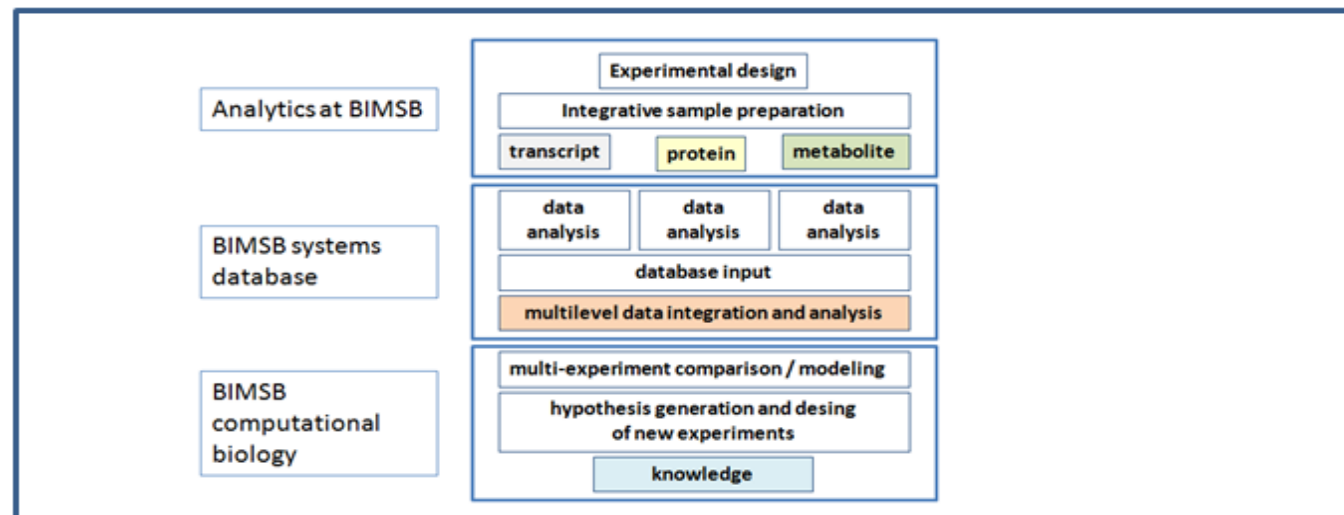
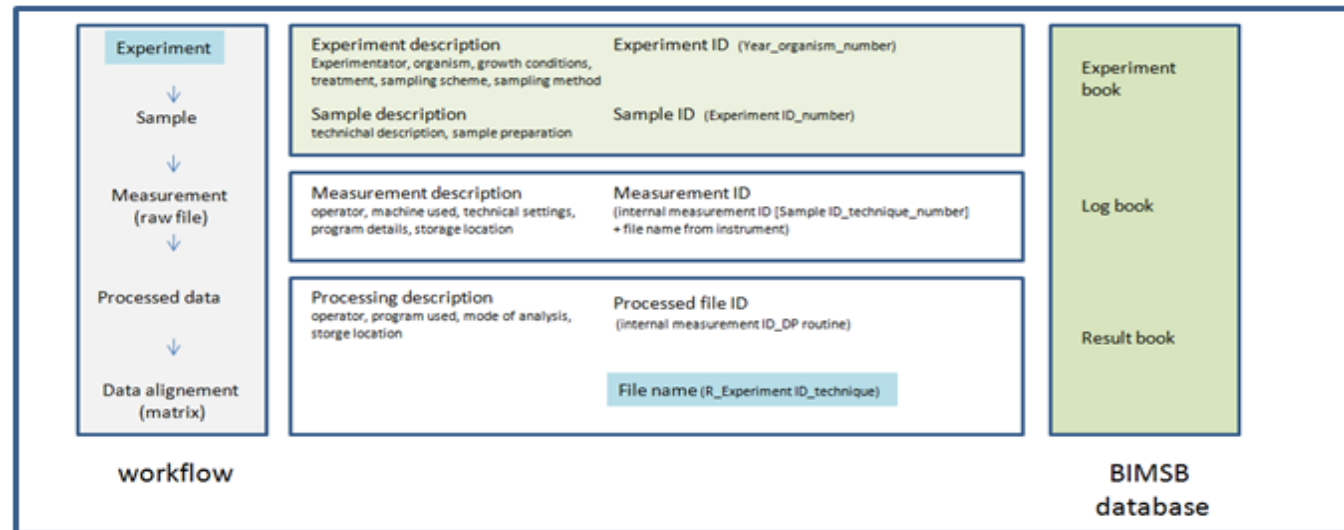


computational biology @ BIMSB



Data integration – BIMSB database

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Current research in the integrative metabolomics and proteomics group at BIMSB:

- We are running integrative analyses on proteomic and metabolomic (transcriptomic) level to understand metabolic regulation in cancer cells, during development and differentiation.
- We are running these projects in a cooperative way including several groups at BIMSB and MDC. Furthermore we are supporting projects of BIMSB and MDC groups with the cutting edge technologies which were established in our group.

Scientific members of the group:

Guido Mastrobouni
Gerd Anders
Matthias Pietzke
Christin Hess
Susann Mudrich
Markus Wenzel
Julia Diesbach