### what do you want? oder: wie sich die ergebnisse interpretieren ?!

#### Steffen Neumann

Institute for Plant Biochemistry Halle – A Leibniz Institute Bioinformatics Center Gatersleben-Halle

ESCEC'06, March 22nd 2006



Steffen Neumann (IPB-Halle.DE)

... everything looks like a nail

- We'd like to find out about Metabolomics
- We know Petri Nets
- We know Bayesian Nets

Can we do ① using ②+ ③ ??





#### Mass Spectrometry

- GC / LC Separation
- Ionisation & Acceleration
- Mass Detection
- Peaks with retention time and m/z Value

Fingerprinting Phenotypic Features Target Analysis Individual Metabolites Metabolite Profiling Quantification of Metabolite subset Metabolomics "Comprehensive" Quantification



(Fiehn, 2002)



#### Mass Spectrometry

- GC / LC Separation
- Ionisation & Acceleration
- Mass Detection
- Peaks with retention time and m/z Value

Fingerprinting Phenotypic Features

Target Analysis Individual Metabolites

Metabolite Profiling Quantification of Metabolite subset

Metabolomics "Comprehensive" Quantification



(Fiehn, 2002)



#### Mass Spectrometry

- GC / LC Separation
- Ionisation & Acceleration
- Mass Detection
- Peaks with retention time and m/z Value

Fingerprinting Phenotypic Features Target Analysis Individual Metabolites Metabolite Profiling Quantification of Metabolite subset

Metabolomics "Comprehensive" Quantification





(Fiehn, 2002)

- Siuzdak&Abagyan @ Scripps
- Bioconductor (optimised C)
- Peak alignment
- Peak integration
- "Differential" metabolites
- RServe Web Frontend underway





- Siuzdak&Abagyan @ Scripps
- Bioconductor (optimised C)
- Peak alignment
- Peak integration
- "Differential" metabolites
- RServe Web Frontend underway







- Siuzdak&Abagyan @ Scripps
- Bioconductor (optimised C)
- Peak alignment
- Peak integration
- "Differential" metabolites
- RServe Web Frontend underway







- Siuzdak&Abagyan @ Scripps
- Bioconductor (optimised C)
- Peak alignment
- Peak integration
- "Differential" metabolites
- RServe Web Frontend underway







- Siuzdak&Abagyan @ Scripps
- Bioconductor (optimised C)
- Peak alignment
- Peak integration
- "Differential" metabolites
- RServe Web Frontend underway

	• g	• 🖬	0	1 😼 1		"V "V	2 8	) 🖺 🗳	9.1	e - 🔊	i în	<u>\$</u> .	ø	7 3	÷	B 200%	- 🔯 ,			
Weinstation: • μ • A A Σ Σ Σ Ξ Ξ Δ Φ Σ .• M • A •     T = μ Φ Σ = μ Σ σ σ σ σ σ σ σ σ σ σ σ σ σ σ σ σ σ σ															1					
															-					
		8		D			0			1			м	N			q	н	3	
1		fold	Istat	make	maned	marnin		stored	dmin	rtmax	npe aks	BC.	Gin	E258C1- R1	E258C1- R2	E258C1- R3	E158C4- R1	E158C4- R2	E258C4- R4	. 8
2	1	5.22	-58.12	0.00	489.67	489.66	489.74	267.62	267.4	268.04	6	6	0	96.02	102.01	102.01	102.02	102.01	104.62	
2	2	3.19	23.24	0.00	357.95	357.89	357.95	253.16	267.31	203.46	2	6	3	254.04	240.00	148.00	238.04	234.04	218.00	
4	3	1.8	22.67	0.00	363.09	353.09	363.14	2072.25	2069.37	2074.27	3	0	3	82	95.73	98,26	87.84	85.92	82.19	
5	4	1.67	-27.26	0.00	212.84	212.84	212.85	268.04	267.47	268.46	9	6	3	1944.38	1896.25	1890.21	2068.31	2012.22	1956.34	T
6	5	1.91	20.51	0.00	592.16	592.15	592.16	1241.43	1224.12	1245.06	9	6	3	1046.14	1058.17	1068.18	1002.17	1030.22	808.09	
	6	1.52	18.07	0.00	473.05	473.05	473.13	1232.09	1325.45	1237.69	9	6	3	165.04	163.03	168.02	144.03	158.02	154.03	
	7	1.35	18.39	0.00	155.08	155.08	155.08	1330.06	1329.48	1330.12	3	0	3	712.71	784.22	753.02	729.2	699.6	731.57	
5	8	2.65	19	0.00	189.2	189.2	189.21	263.15	251.96	264.41	4	1	3	143.29	134.7	158.02	143.34	208.02	153.67	
LO	8	2.4	40.62	0.00	155.05	155.06	155.05	1566.69	1588.11	1568.25	3	0	3	493.49	478.03	451.3	512.93	455.05	500.63	
	10	4.13	16.28	0.00	156.07	155.97	156.08	1568.47	1568.1	1573.13	4	1	3	24.12	78.01	56.16	64.12	29.94	64.33	
12	11	8.06	14.88	0.00	486.69	488.59	486.73	2073.95	2073.32	2075.28	6	2	3	24	54.01	4.01	3.99	46.01	8.02	
13	12	1.74	14.51	0.00	\$00.25	900.24	\$00.25	1529.76	1324.04	1335.7	4	1	3	62.23	60.32	50.2	60.01	61.61	69.5	
	13	3.6	14.15	0.00	603.18	603.17	603.24	2074.24	2071.35	2078.28	3	0	3	20	17.95	8.03	23.95	27.97	32.08	
15	54	4.43	-27.61	0.00	487.66	487.59	487.74	267.47	266.34	268.04	7	6	0	148.02	166.02	164.03	166.03	160.03	158.02	
16	15	2.48	13.3	0.00	450.65	430.64	450.74	1238.41	1551,49	1341.66	8	5	3	122.02	122.02	124.02	118.02	120.02	57.08	
	16	4.03	15.19	0.00	\$52.15	552.14	\$52.15	2074.24	2073.32	2076.28	3	0	3	32	37.89	16.05	33.93	43.96	18.04	
18	17	4.02	+14.05	0.00	499.76	499.75	499.84	269.02	267.55	269.46	6	6	0	90.02	76.01	84.02	105.01	84.01	82.02	
19	18	3.63	13.22	0.00	535.22	535.22	535.24	2074.24	2073.32	2076.28	3	0	3	35	23.93	8.03	25.95	37.96	26.06	
20	19	2.56	16.57	0.00	236.12	235.09	236.12	2074.24	2073.32	2076.28	3	0	3	298.05	299.12	240.89	251.5	291.69	190.46	
21	20	6.72	14.69	0.00	784.25	784.25	784.25	2074.24	2073.32	2076.28	3	0	3	0	13.95	4.01	7.98	4	20.05	1



### Metabolomics: Ingredients

- Metabolites
- Proteins/Enzymes/Genes
- Reactions:
  - Stoichiometry
  - Kinetics
    - Mass Action
    - Michaelis Menten
    - Hill-type kinetics
    - Kinetic Constants

What we measure:

- Metabolite Profiles
- Fluxes
- Proteomics
- Enzyme Activity
- Transcripts



tinyhttp://eot.bu.edu/ccb/Kinetics/index.htm
Mass action kinetics state:

$$r = k_{\rm S} \times {\rm S}$$
 (1)

Hill type

Michaelis-Menten:

- reversible or irreversible reactions
- more than one substrate
- competitive inhibitors
- non competitive inhibitors

$$v = \frac{V_{max}S}{K_m + S}$$
(2)  
$$r = \frac{k_{cat}ES}{K_m + S}$$
(3)



A riboswitch is a part of an mRNA molecule that can directly bind a small target molecule, and affects the gene's activity. An mRNA that contains a riboswitch is directly involved in regulating its own activity.

http://en.wikipedia.org/wiki/Riboswitch

- activation / repression
- transcription termination
- translation initiation
- self-cleavage (ribozyme that cleaves itself)
- mostly eubacteria
- some in eukaryotes
- similar in archaea



The only thing we measure are Metabolite-"concentrations" under various conditions:

- Is there a way to infer Pathways ?
- Is there a way to infer Activation / Inhibition of Reactions ?
- What experiments are needed to infer these ?
  - Time Series ?
  - Knock-Outs ?
- Can we find Gene Regulation by Metabolites ?

