

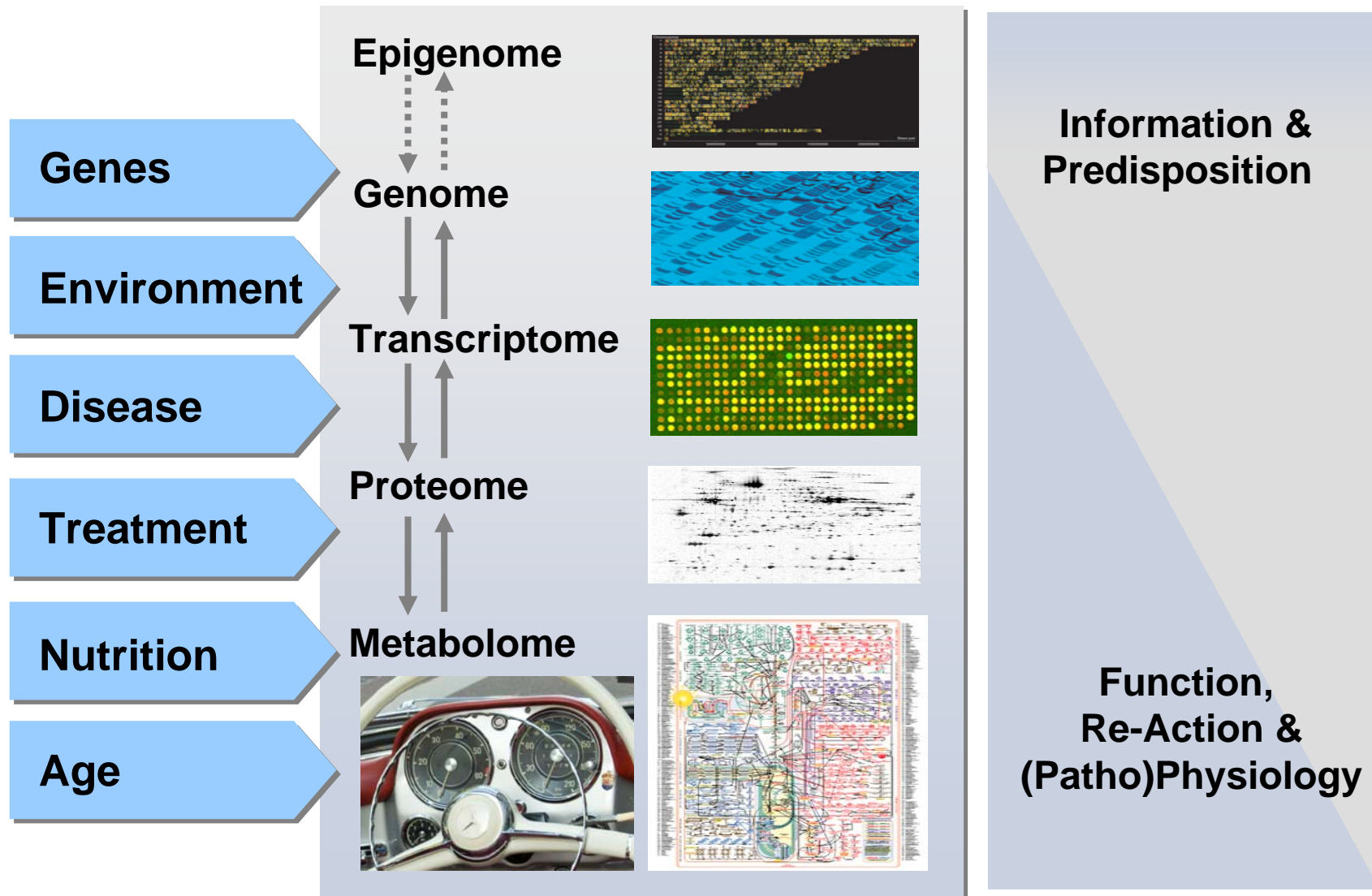


Comprehensive Metabolite Profiling: A Promising Tool for Identification and Validation of Novel Biomarkers

Tim P. Bölke
Managing Director
metanomics Health, Berlin

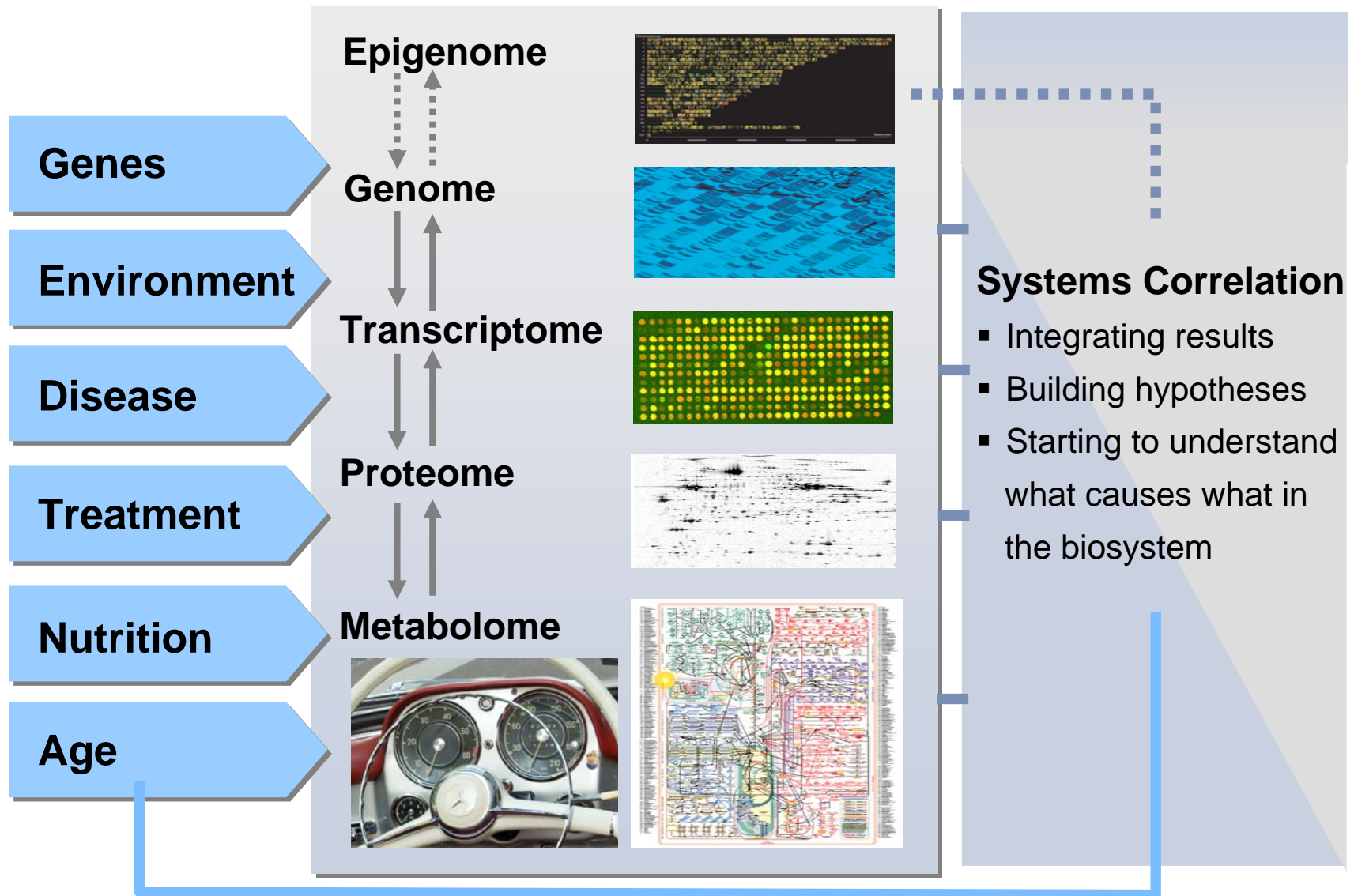
Berlin
June 17th, 2010

The Metabolome - Highly Responsive and Revealing



Phenotype Dashboard
of our "Bio-Engine"

Metabolism - Research Key for Systems Biology



metanomics and metanomics Health at Berlinbiotechpark



**Main Building
metanomics &
metanomics Health**



**Tegel International Airport
3 km**

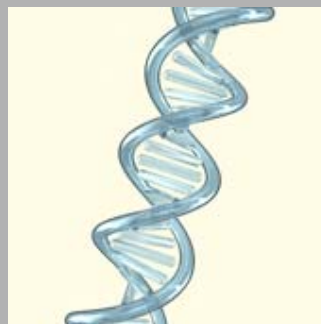
**Berlin Center
6 km**

Two Companies - One Common Focus



Plant Biotechnology

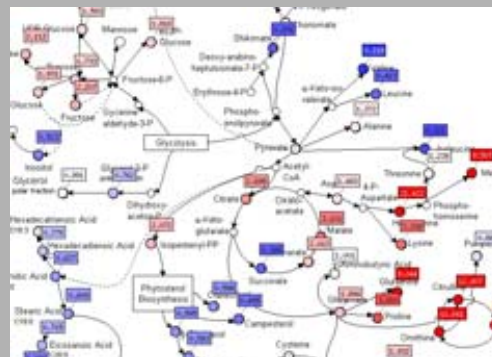
- founded 1998
- BASF Company
- Focus on plant biotechnology
- ca. 1/3 dedicated to pharma, diagnostics and nutrition
- Most comprehensive metabolite profiling platform worldwide
- >75 mass specs (GC-MS, LC-MS/MS etc.)
- Integrated IT infrastructure
- Largest database for gene functions in plants



GENOME



METABOLOME



Pharma & Diagnostics

- founded 2003
- BASF Company
- Biomarker research for pharma, nutrition and diagnostics companies
- Proprietary biomarker development program in various indications
- Technical work performed by metanomics
- metanomics Health benefits from technology and know how by metanomics



Comprehensive Profiling Platform & Process

SAMPLES

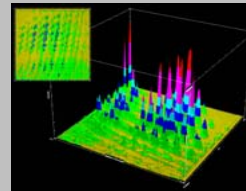
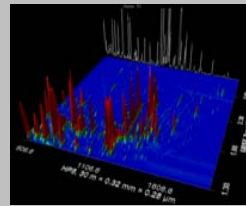
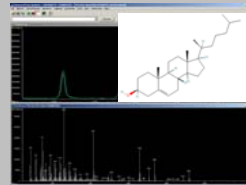


METABOLITE PROFILING



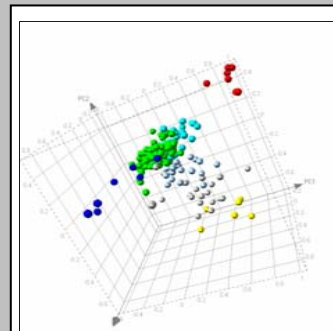
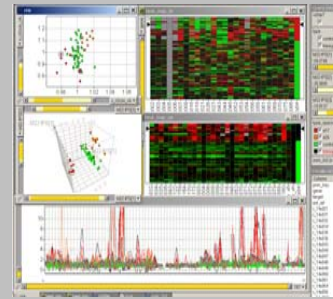
>75 MS instruments
400,000 analyses p.a.

DATA VALIDATION



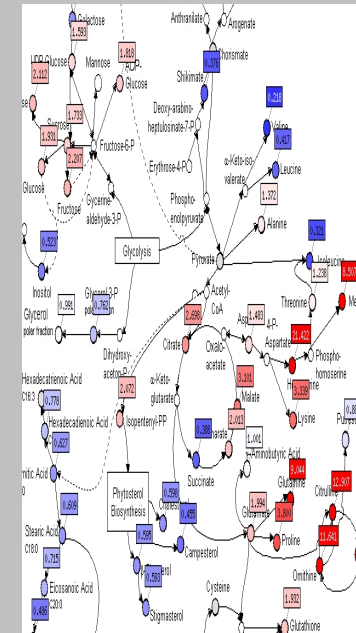
Correctness
Identification

METABOLITE CORRELATION



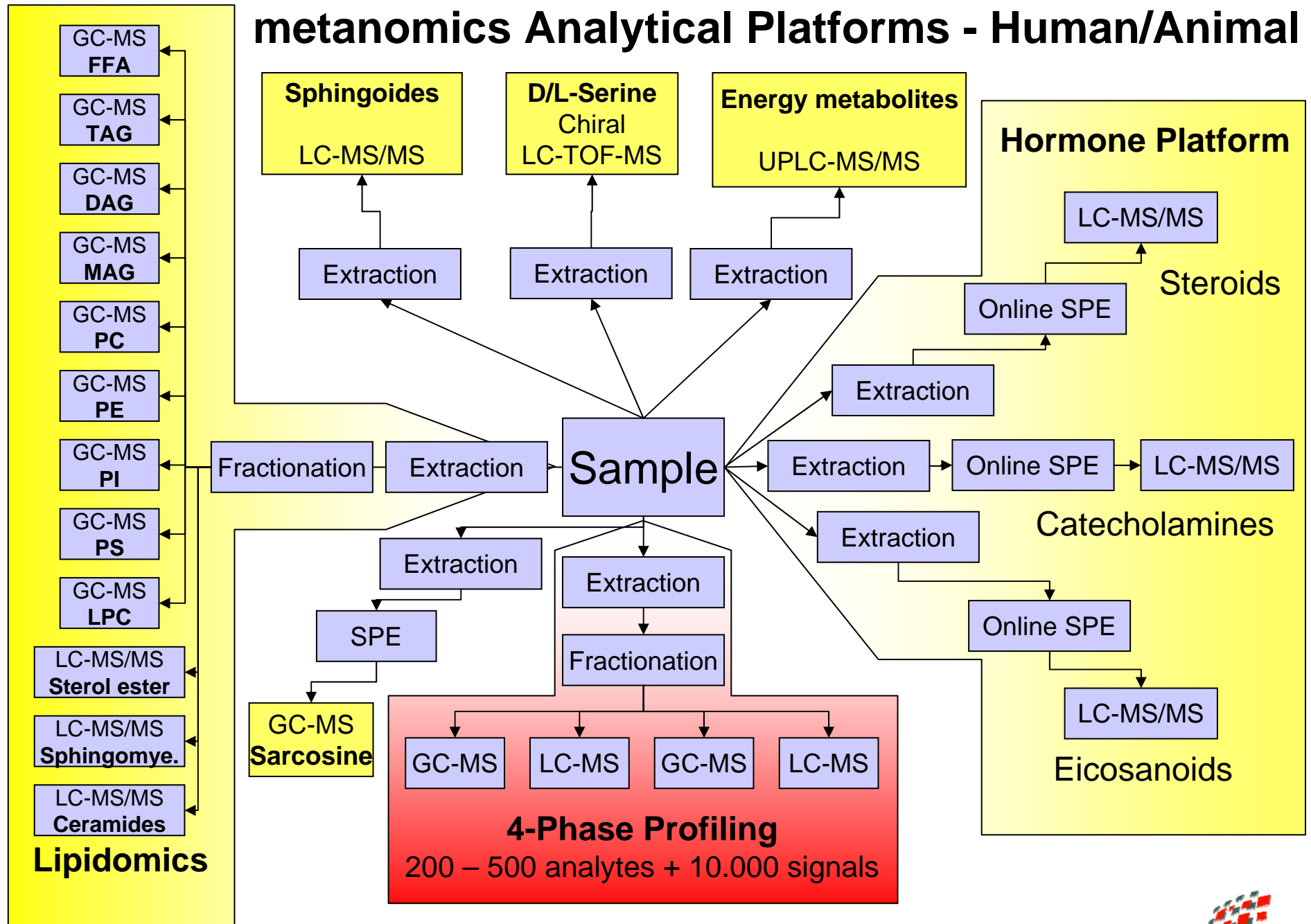
Change & Regulation
Simple or Complex

INTERPRETATION & BIOMARKERS

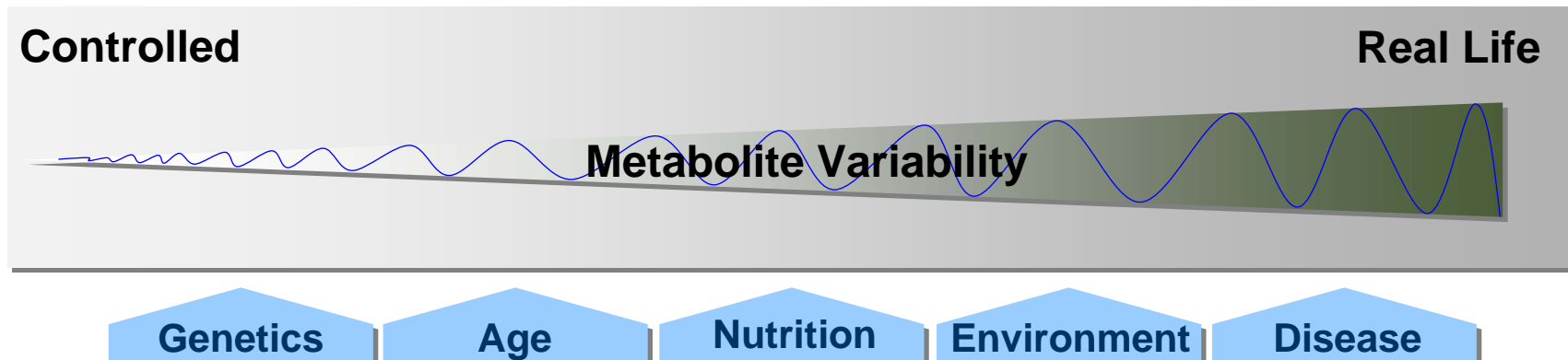


Data Integration &
Correlation

metanomics Analytical Platforms - Human/Animal



The Metabolome is highly responsive ☺ *Friend or*
The Metabolome is highly responsive ☹ *Foe*



Dealing with variability during identification & validation:

- Stringent control of process & analytical variability
- Controlled study design
- Information on/avoidance or correction of confounding factors
- Correction by e.g. normalization strategies and linear modelling
- Use what the „normal“ metabolome tells us
- Cross study normalization through plasma mother pool

Avoidance or Living with Confounding Factors

Related to donor (patient)

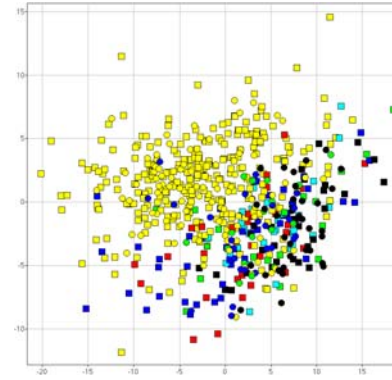
- Age; Gender
- Medication; Excipients
- Clinical history
- Diet; Life style; Smoking
- Gut microflora (leads to significant inter-subject variability)

Sample collection and handling

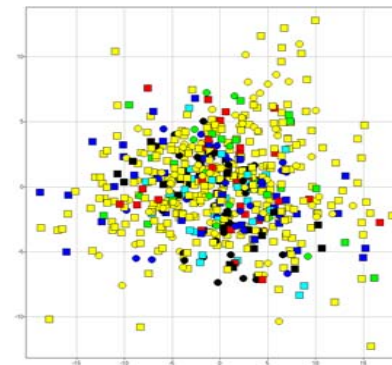
- **Center dependencies**
- Diurnal (circadian) variation
- Storage of samples (time of storage, temperature, oxidation)
- Shipping

Metabolite Profiling

- Sample preparation
- Analysis of samples through metabolite profiling platform



Linear modelling to correct for center dependencies



Start of Biomarker ID in „Balanced Population“

Avoidance or Living with Confounding Factors

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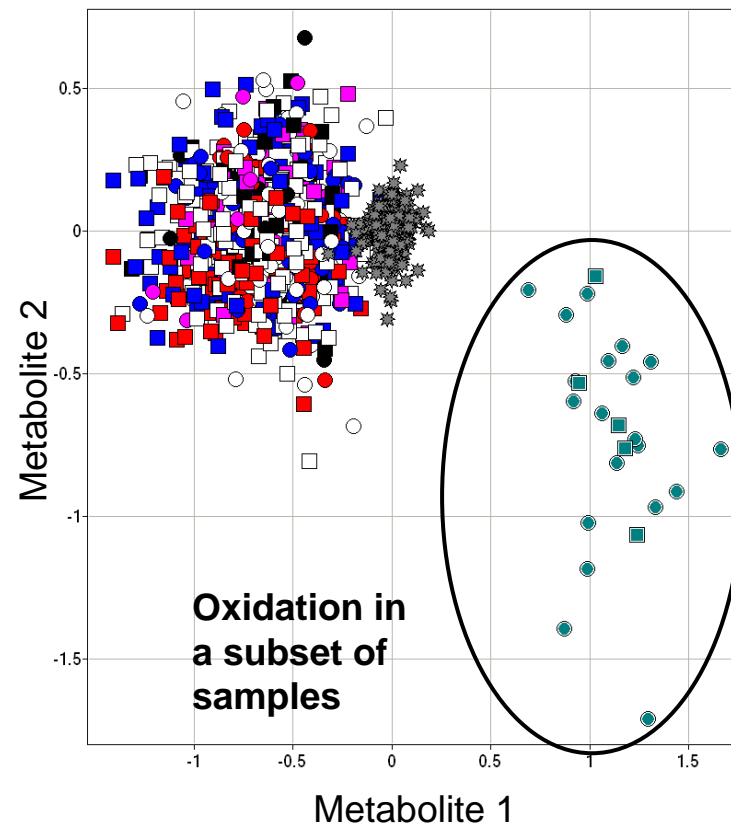
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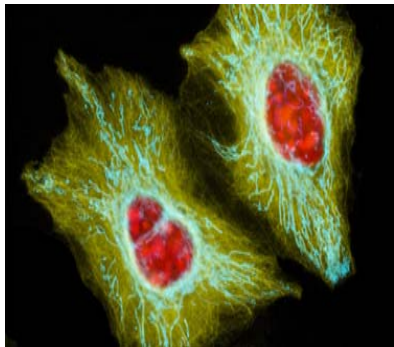
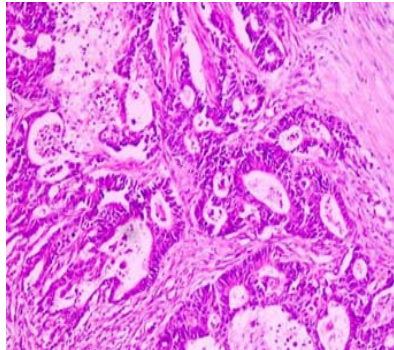
Metabolite Profiling

- Sample preparation
- Analysis of samples through metabolite profiling platform

Pre-Analytics Artefact Sub-optimal sample handling



Mode-of-Action Case Study - Anticancer Agents



Testsystem & Metabolite Profiling

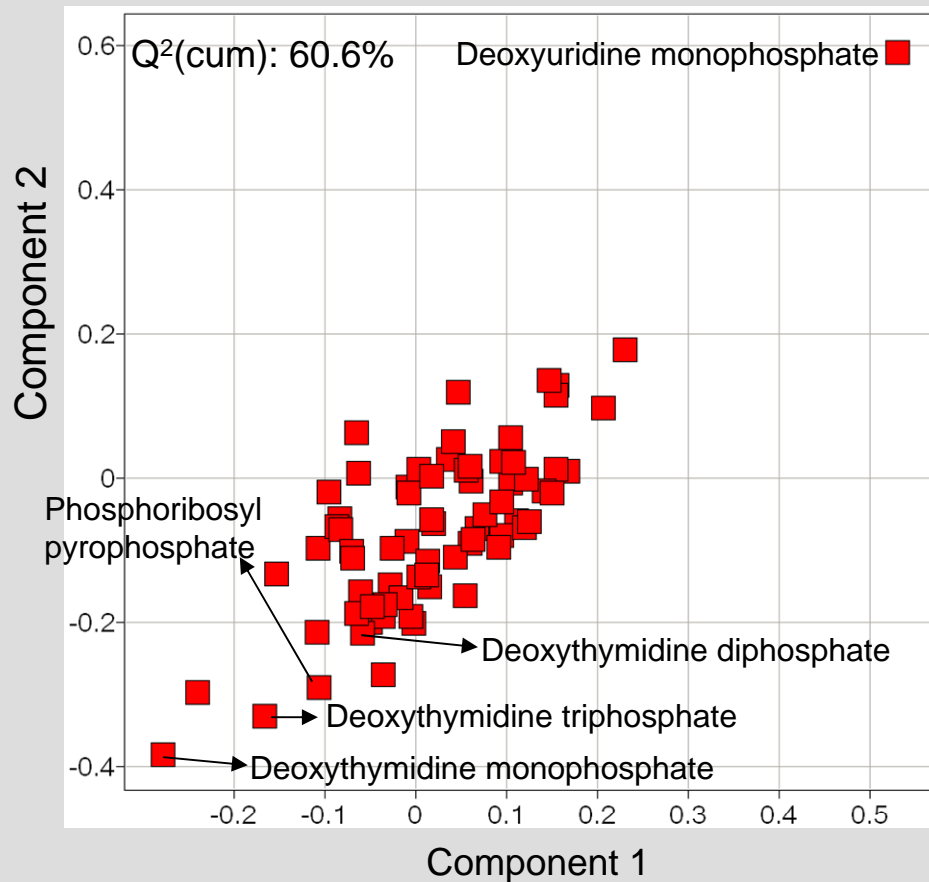
- Human adenocarcinoma cell lines (HCT116 (colon) and Du145 (prostate))
- Developmental active, positive control, vehicle control (DMSO)
- Profiling: Energy Platform and non-biased 4 Phase Profiling Platform
- 72 metabolites evaluated from Energy Platform
- > 200 analytes from 4 Phase Metabolite Profiling Platform

Objective

- Identification - possibly differentiation - of developmental active MoA
- Identification of metabolite changes: pathway, mechanism/drug target
- Identification of new biomarker candidates

Mode-of-Action Case Study - Anticancer Agents

PLS-DA Loadings Plot (Du145 cells)
Positive Control vs. DMSO



Results

- Steep increase of **dUMP**

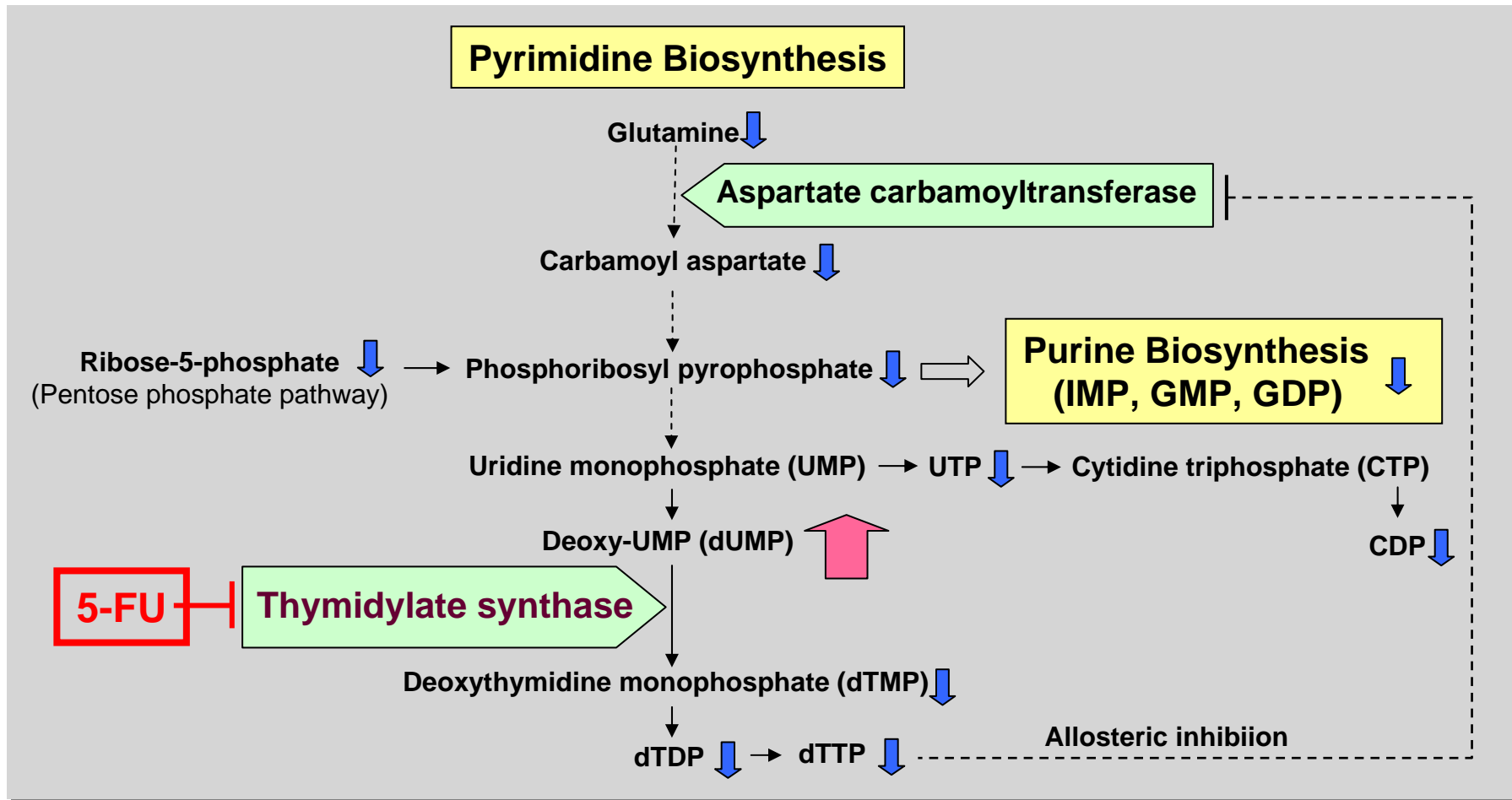
Combined with

- Decreases of **dTMP**, **dTDP**, and **dTTP**
- Decreased pyrimidine and purine biosynthesis
- Additional metabolic effects indicating reduced cell proliferation
- Metabolite changes were found within classes of phosphatidylcholines, sphingolipids and phospholipids
- Decrease of TCA cycle, glycolysis, and increase of fatty acids

MoA Hypothesis

- ▶ Positive Control = 5-Fluorouracil (5-FU) like mechanism

Mode-of-Action Case Study - Anticancer Agents



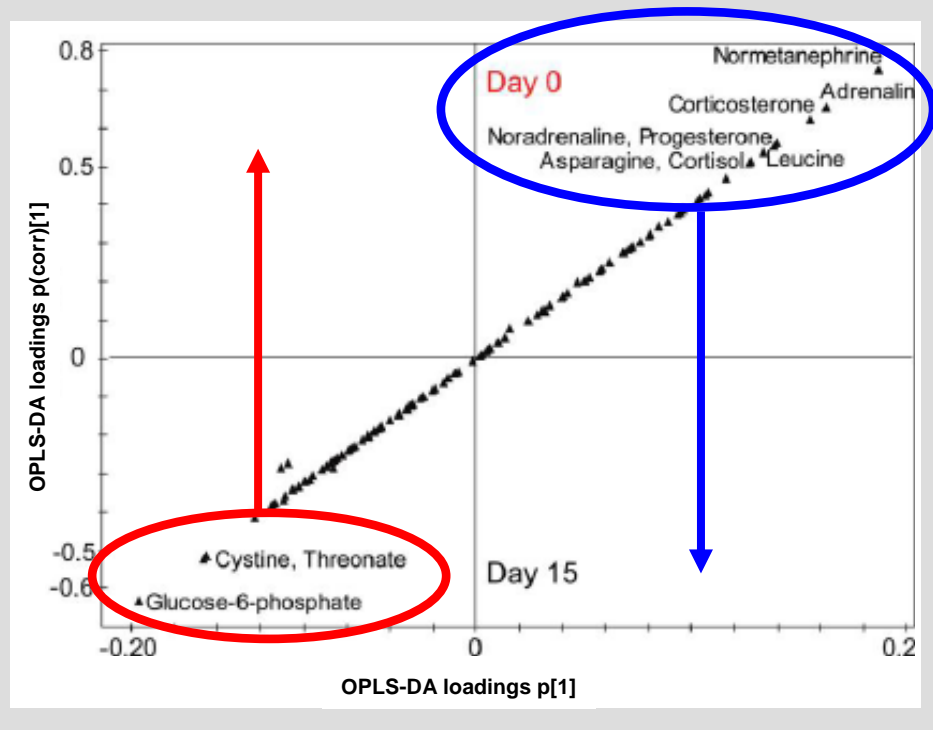
5-FU Mechanism

- Cytostatic agent; irreversibly inhibits **thymidylate synthase**
- Inhibition of DNA replication and repair, cell cycle arrest, and cell death

Stress Relief Through Chocolate - Metabolic Effects



Metabolic effects of 15 days of regular dark chocolate consumption (40 g / d) in healthy males



Nestlé – metanomics Health

- Chocolate has long been associated with effects on mood in humans
- Several components in chocolate act psychoactive and affect stress, mood and chocolate craving

Results

- Subjects with higher stress showed different energy homeostasis, hormonal metabolism and gut microbial activity
- Dark chocolate reduced stress associated energy and hormone metabolites mainly in healthy males
- 2 week consumption of dark chocolate reduced stress levels as indicated by reduced urinary cortisol and catecholamines

Martin et al. 2009, J. Proteome Res. 8: 5568-79



**This has been a joint team effort,
sincere thanks to ...**

- Our Pharma partners
- The Nestlé Nutrition Team
- Our dedicated and enthusiastic staff at metanomics Health and metanomics