MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE BERLIN-BUCH

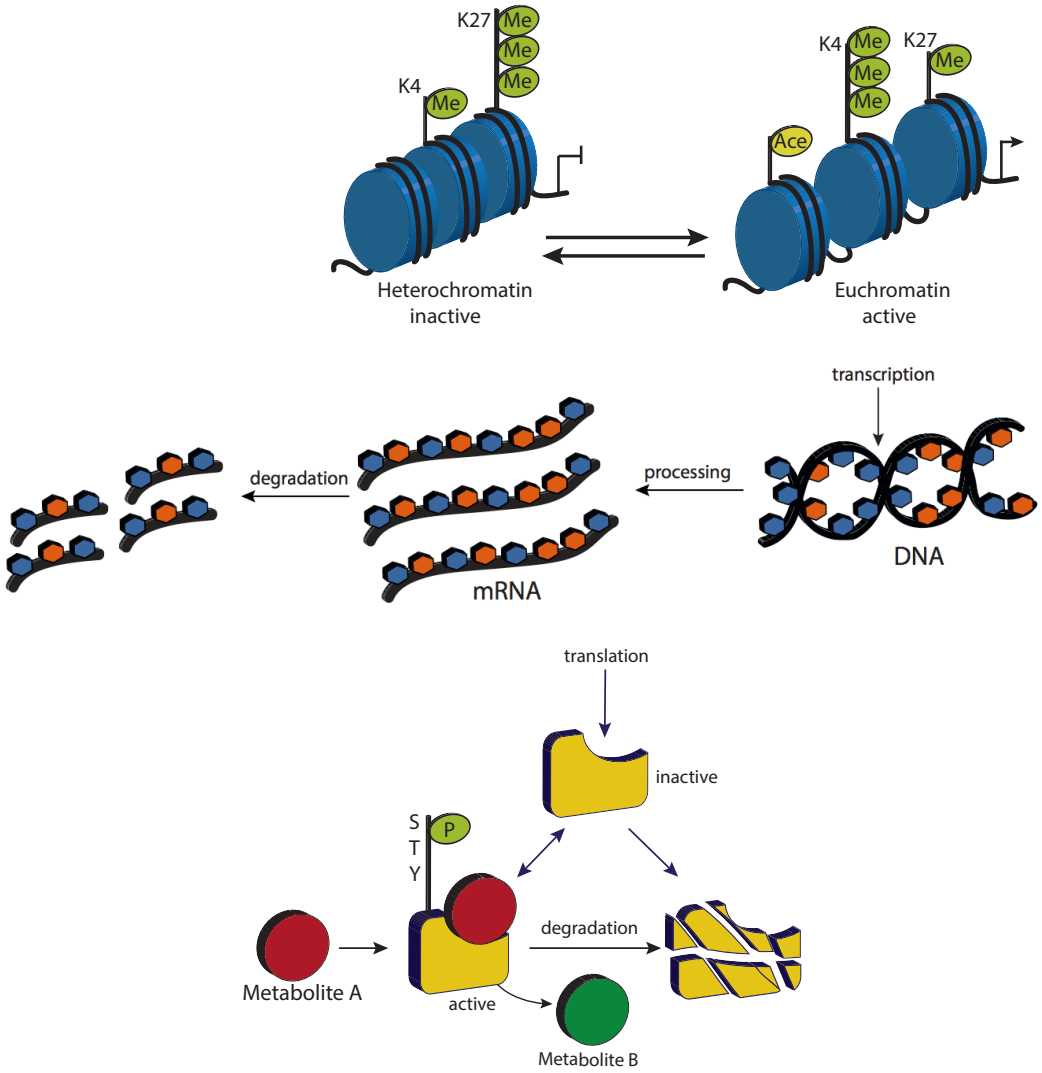
BIMSB THE BERLIN INSTITUTE FOR MEDICAL SYSTEMS BIOLOGY

QUANTIFYING METABOLIC FLUXES IN CANCER AND STEM CELLS

An integrative approach monitoring metabolic reprogramming

Christin Zasada
Kempa Lab - Integrative Proteomics & Metabolomics

Genome-regulatory code



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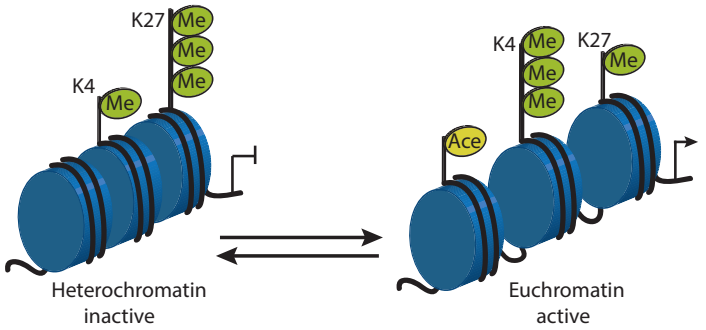
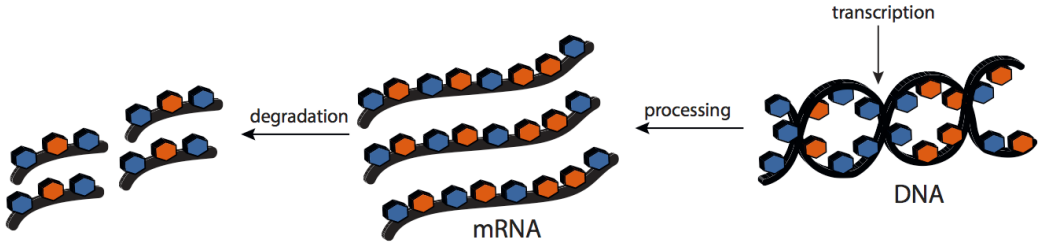
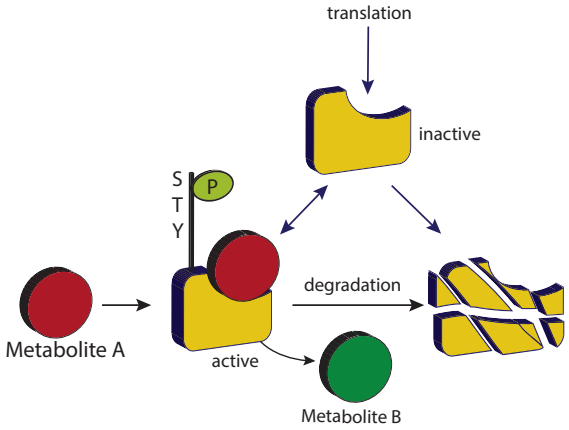


Genomics
three billion DNA base pairs



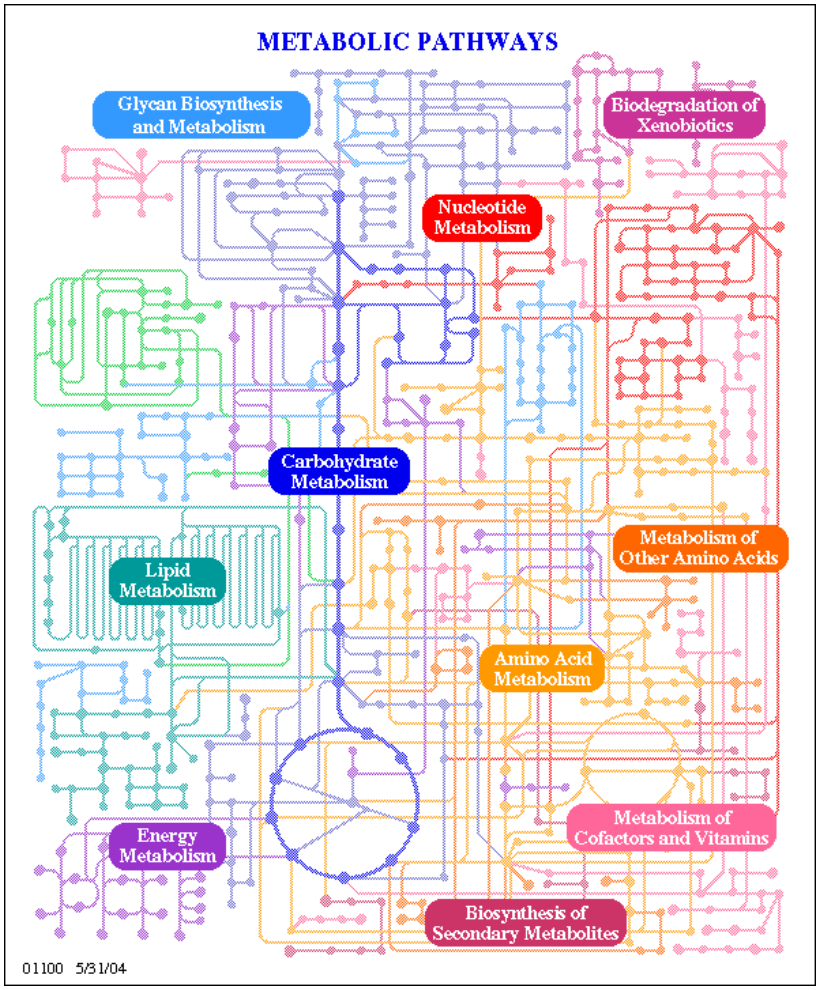
Transcriptomics
2 % of the genome



Proteomics
20’000 - 25’000 protein-coding genes

Metabolomics
~ 5 000

Reprogramming of metabolism



www.kegg.org

Reprogramming of metabolism

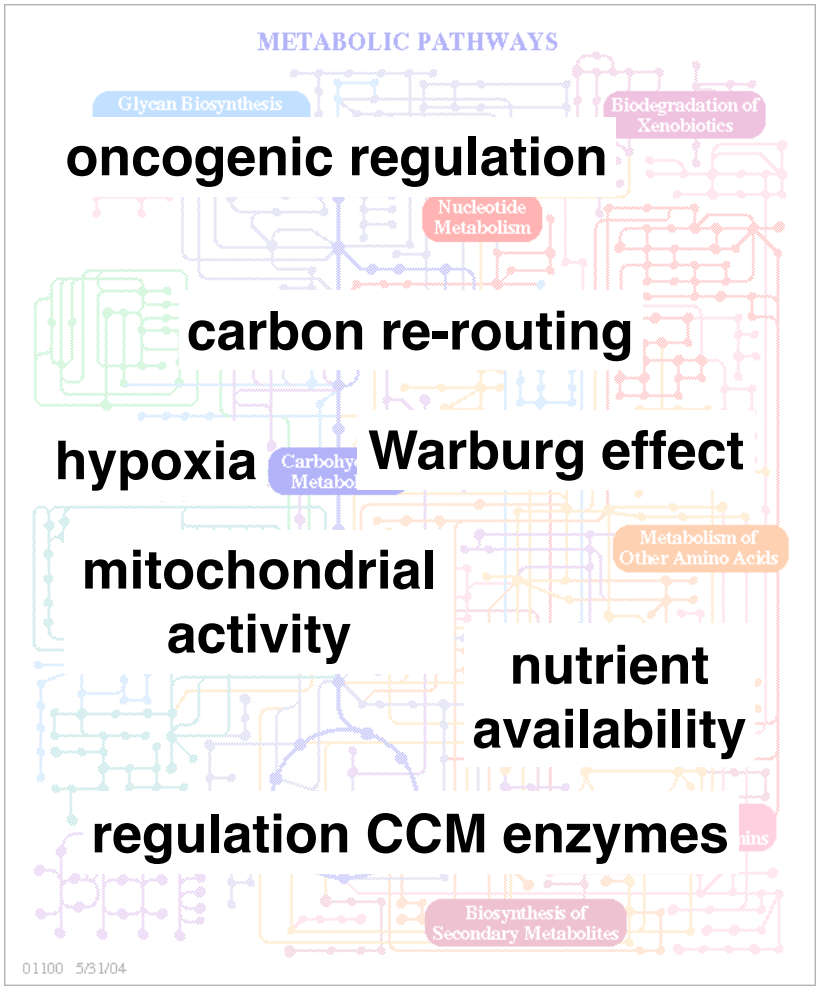
OXPHOS
Phenotype
finite life-span

DIFFERENTIATED CELL

REPROGRAMMING

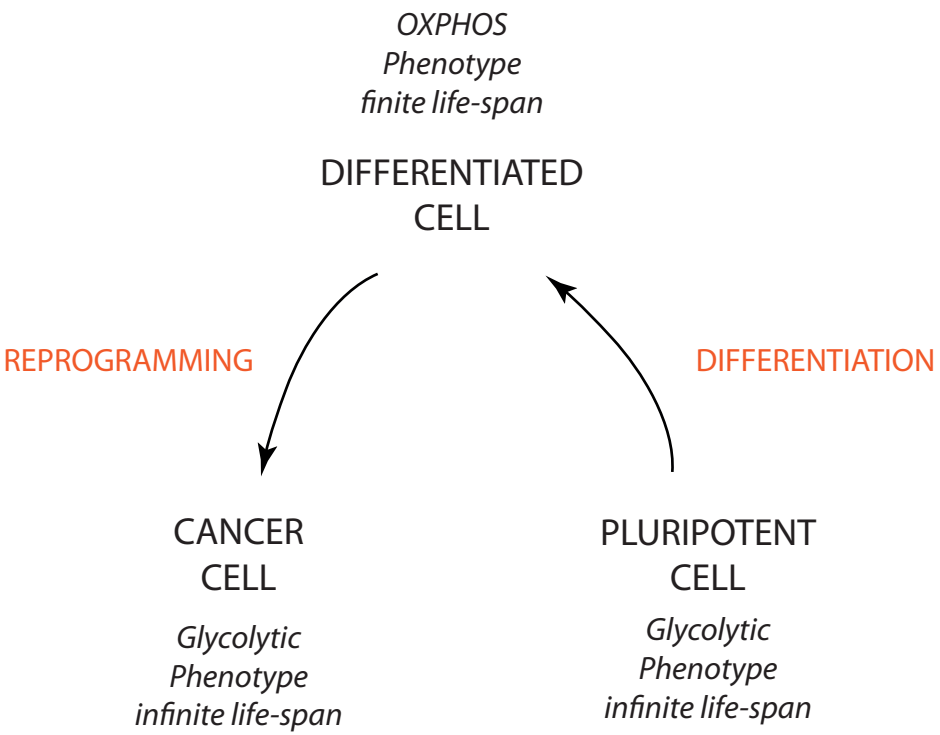
CANCER CELL

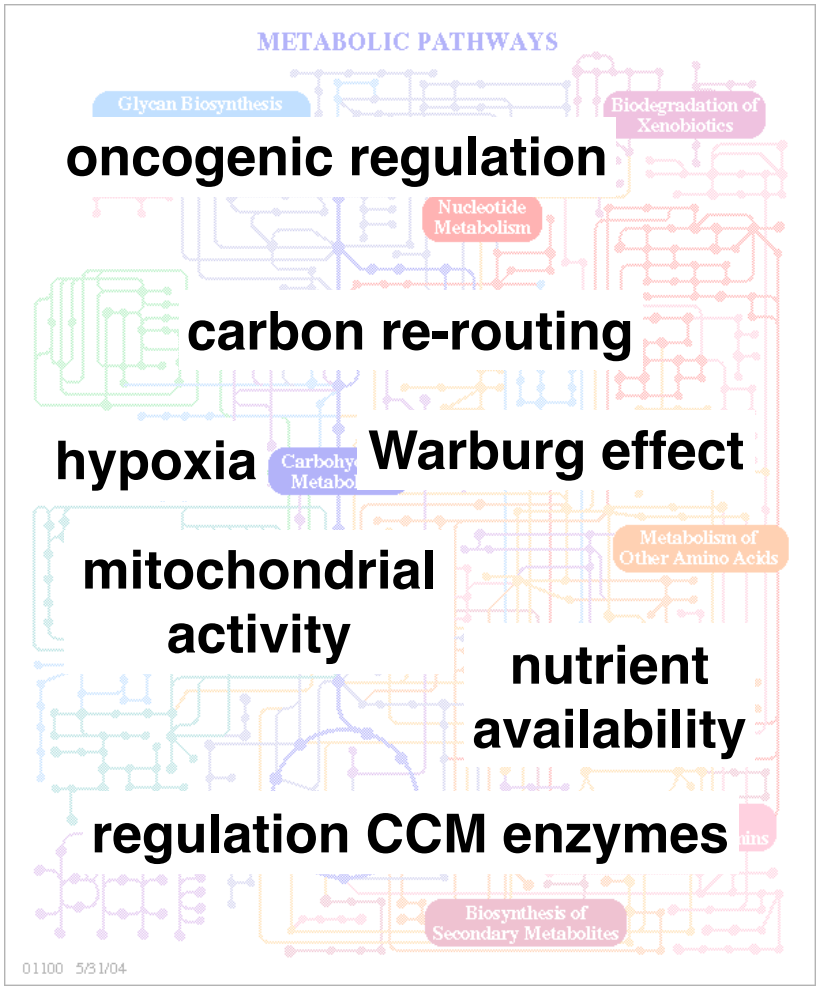
Glycolytic
Phenotype
infinite life-span



www.kegg.org

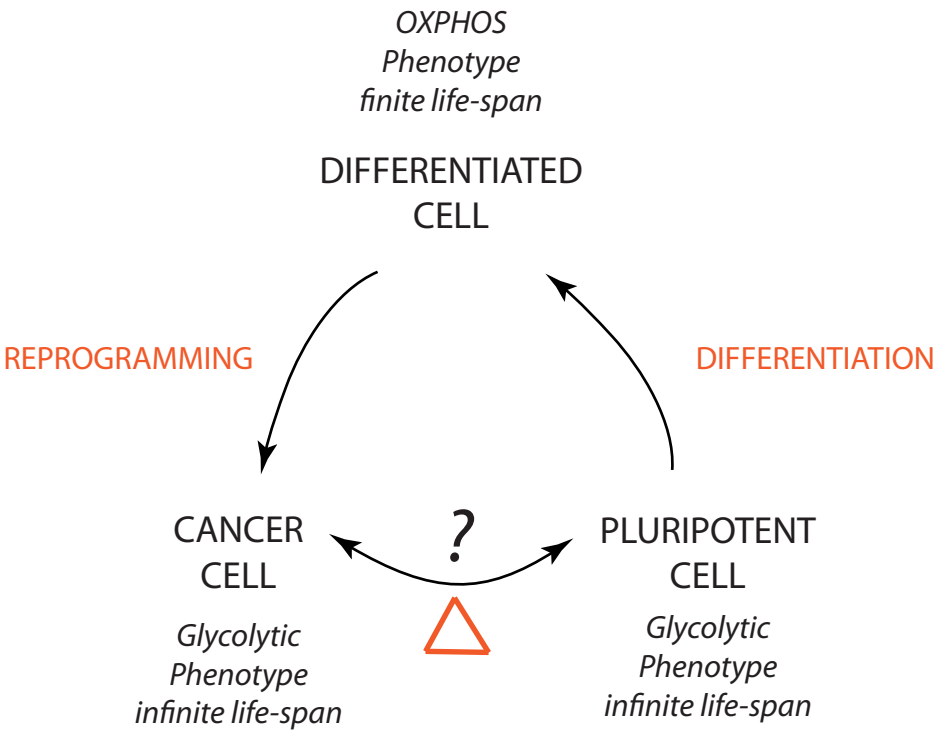
Reprogramming of metabolism

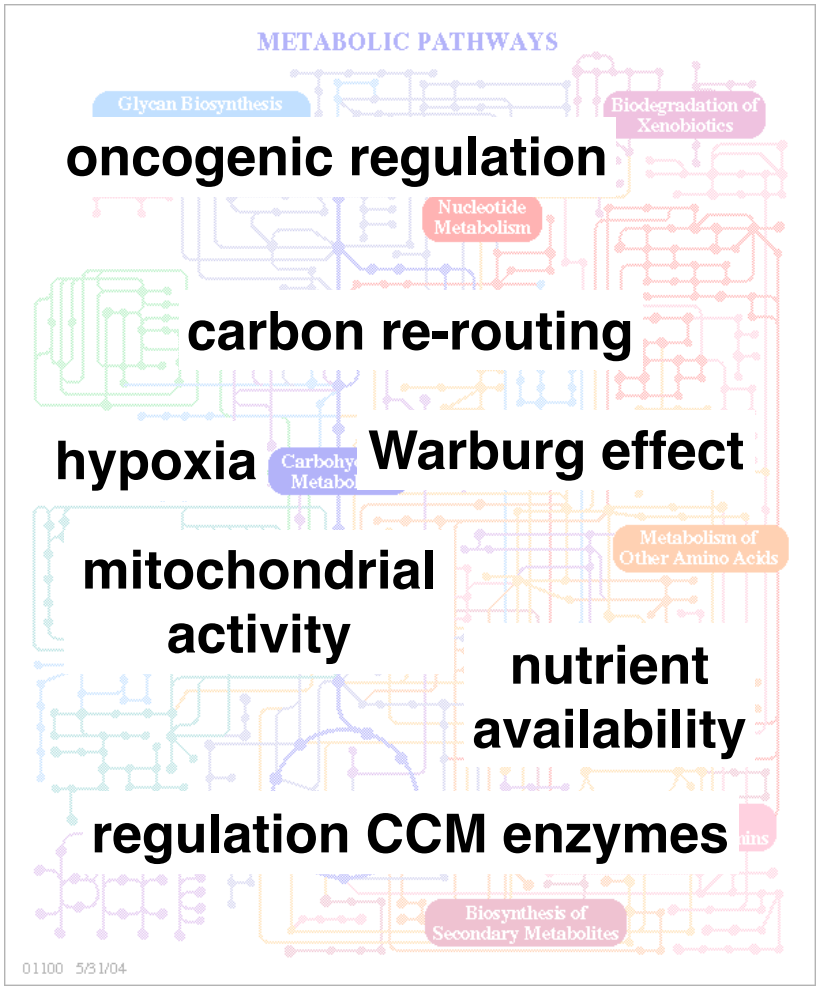




www.kegg.org

Reprogramming of metabolism





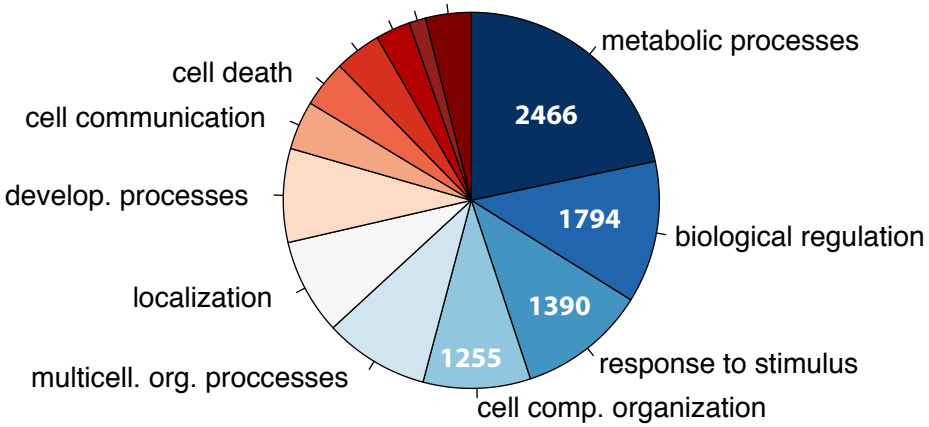
www.kegg.org

Quantitative information turns functional

Quantitative information turns functional

Proteomics

LC-MS/MS
label-free, SILAC quantification
~ 3500 proteins



Quantitative information turns functional

Proteomics

LC-MS/MS
label-free, SILAC quantification
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pulsed stable isotope resolved metabolomics (pSIRM)

GC-MS approach
identification
~ 100 metabolites
absolute quantification
~ 40 metabolites

Quantitative information turns functional

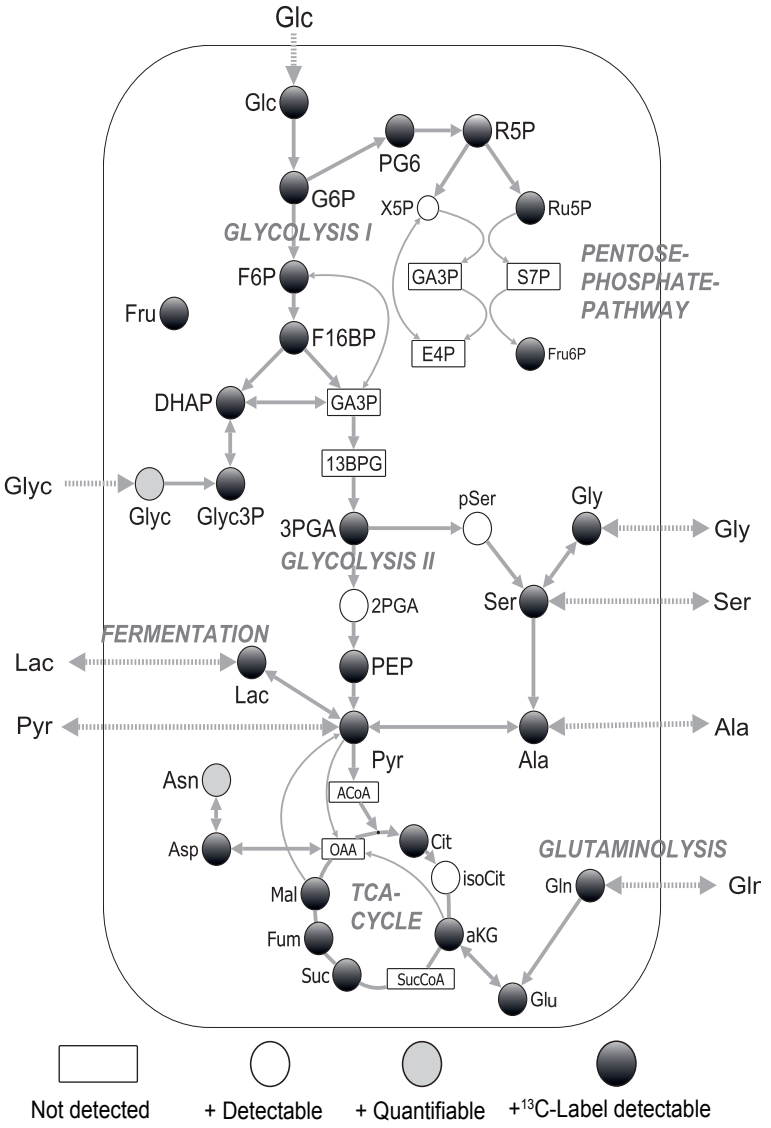
Proteomics

LC-MS/MS
label-free, SILAC quantification
~ 3500 proteins

pulsed stable isotope resolved metabolomics (pSIRM)

GC-MS approach
identification
~ 100 metabolites
absolute quantification
~ 40 metabolites

application of stable isotopes
—> time-resolved
—> position-specific
—> indirect measurement of flux



Pietzke & Zasada et al. Cancer & Metabolism 2014, 2:9

Non-stationary metabolic flux analysis

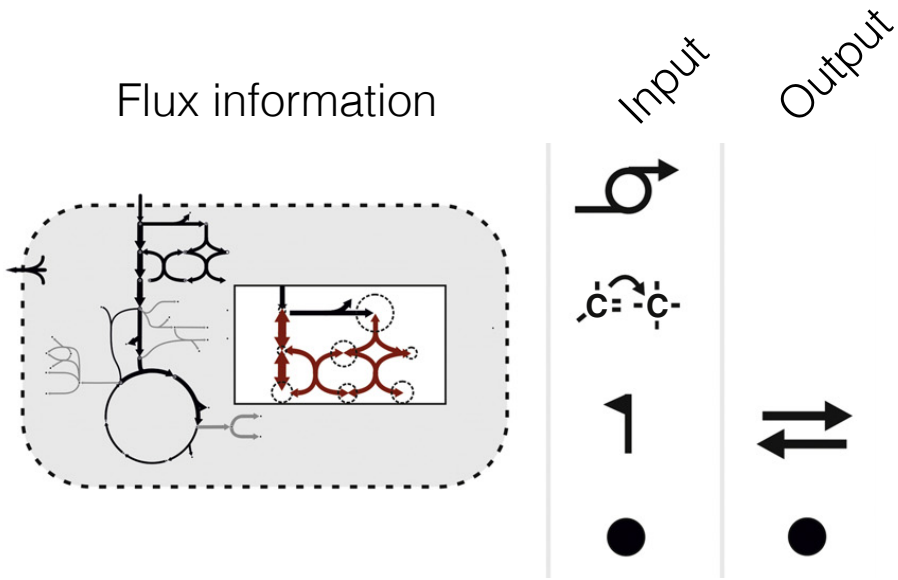
in cooperation with Katharina Nöh
and Sebastian Niedenführ
Forschungszentrum Jülich GmBH

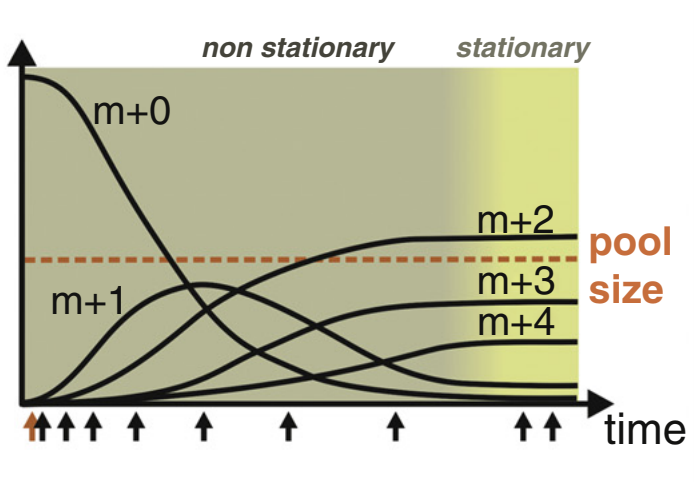
metabolic steady &
isotopic non-stationary

Time courses for:
Glucose
Glutamine

Model parameter
168 labeling data point
5 measured rates
14 measured pool sizes

Pathways coverage
Glycolysis
TCA-Cycle
Pentose-Phosphate Pathway
Glutaminolysis
Amino acid synthesis
Biosynthesis

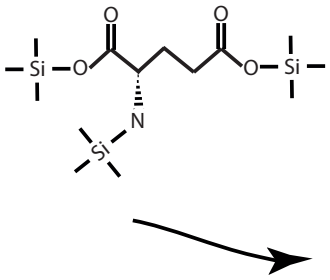




Niedenführ S, Nöh K, Wiechert W
Current Opinion in Biotechnology 2015, 34:82–90

GC-MS fragment mapping to molecule level

Glutamine DL (3 TMS)



modified from
S. Niedenführ

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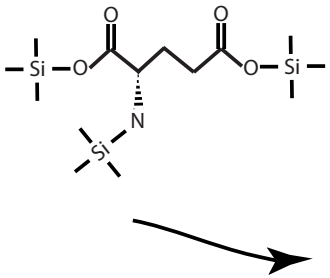

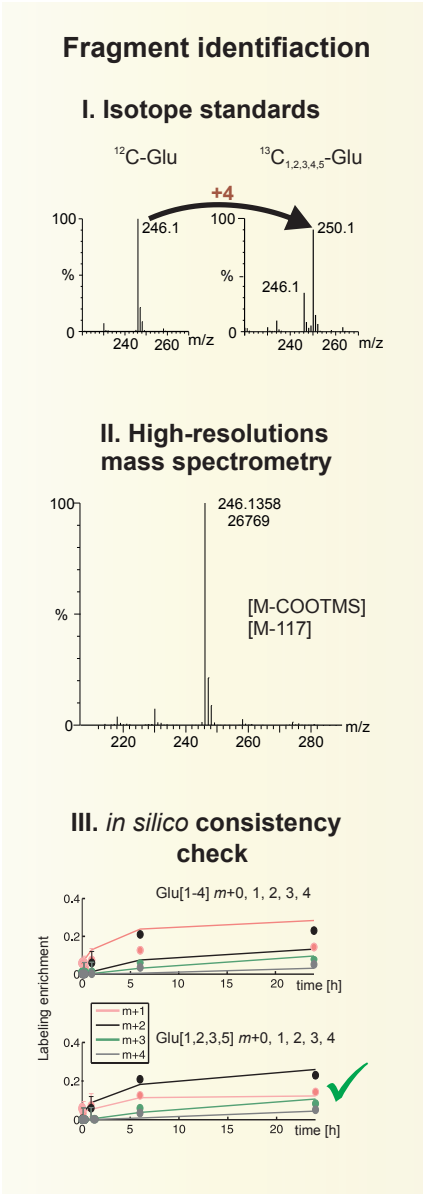

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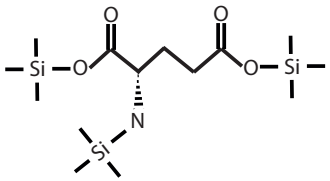
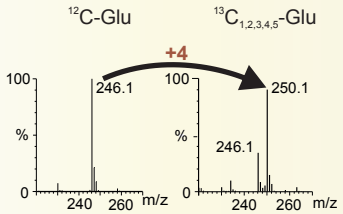
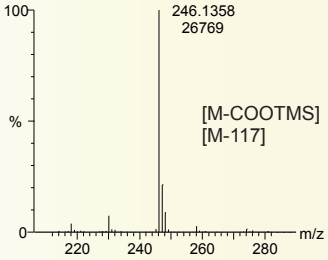


Fragment identifiaction

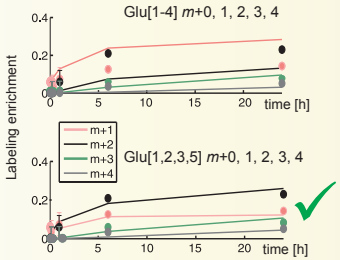
I. Isotope standards



II. High-resolutions mass spectrometry



III. *in silico* consistency check

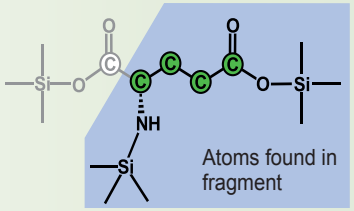


Identified fragment

I. Chemical formula

$[C_{10}H_{24}NO_2Si_2]^+$

II. Fragment and precursor mapping



modified from S. Niedenführ

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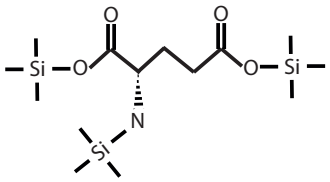

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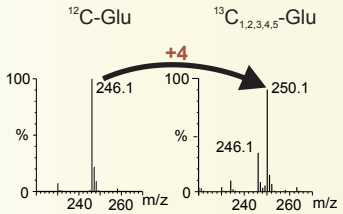

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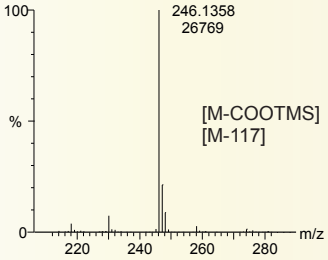

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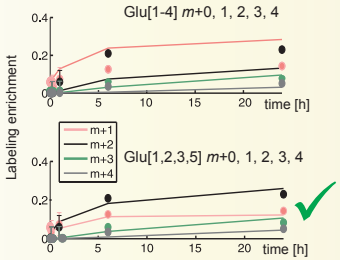

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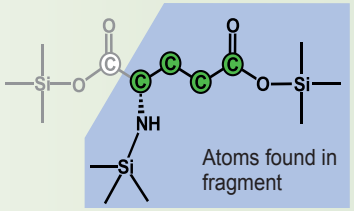
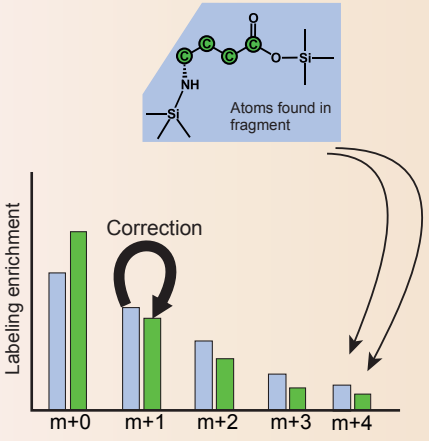


Correction for natural abundance



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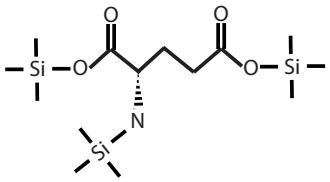

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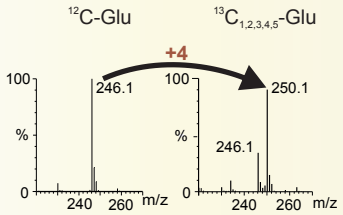

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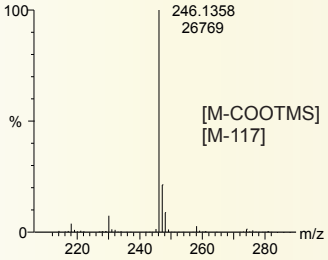

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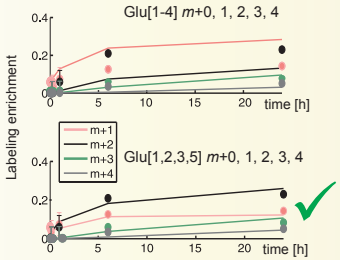

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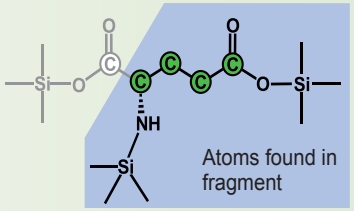

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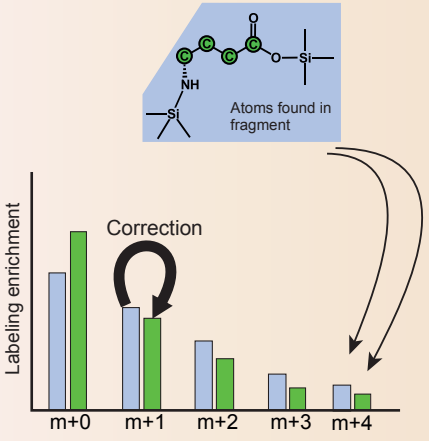

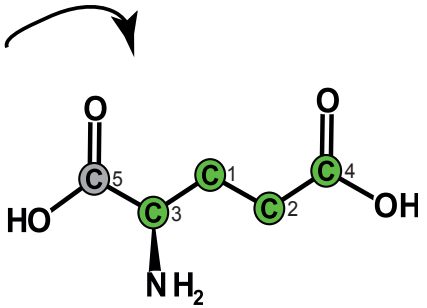

Measurement specification

Glu[1-4] *m*+0, 1, 2, 3, 4

Measured atom

Not measured atom

modified from S. Niedenführ

Monitoring metabolic reprogramming - experimental setup

in cooperation with R. Bucowiecki and A. Prigione (MDC, Berlin, Germany)

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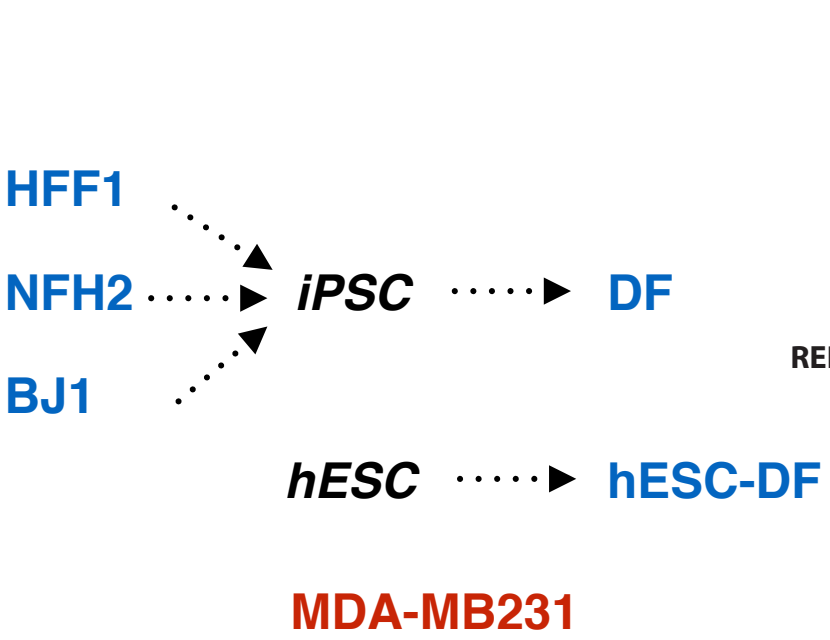

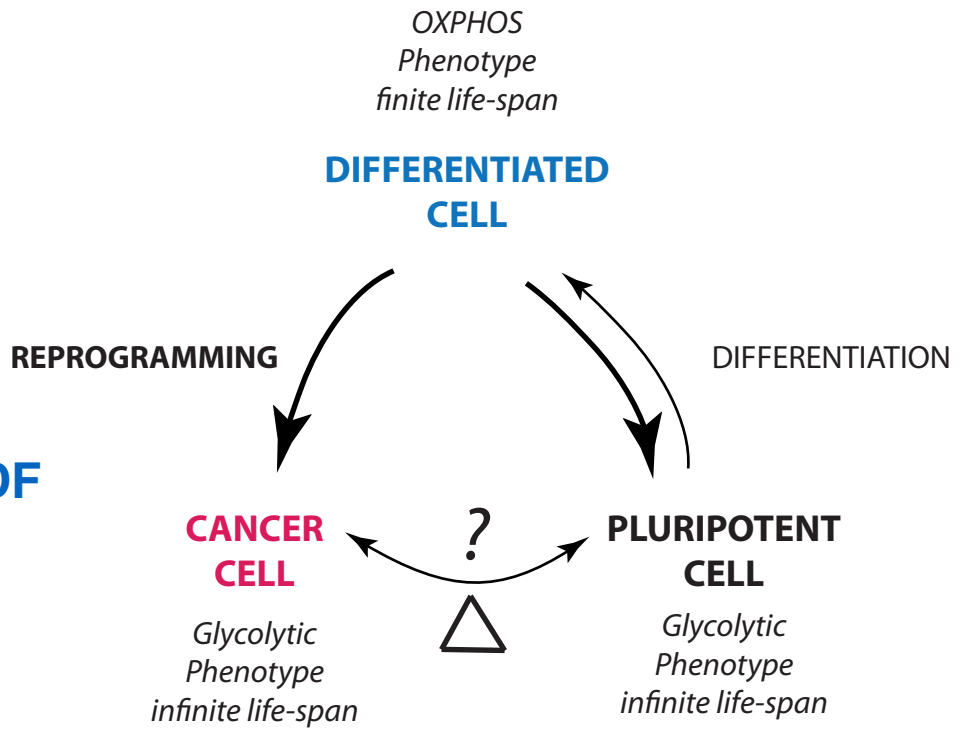

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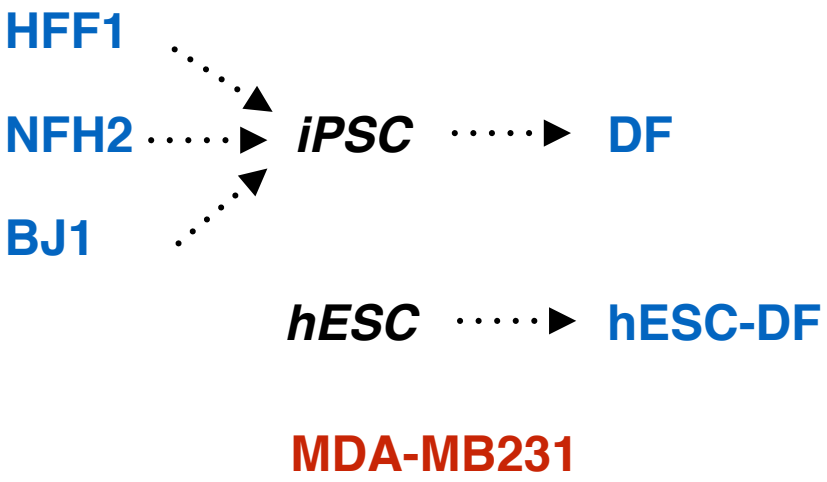

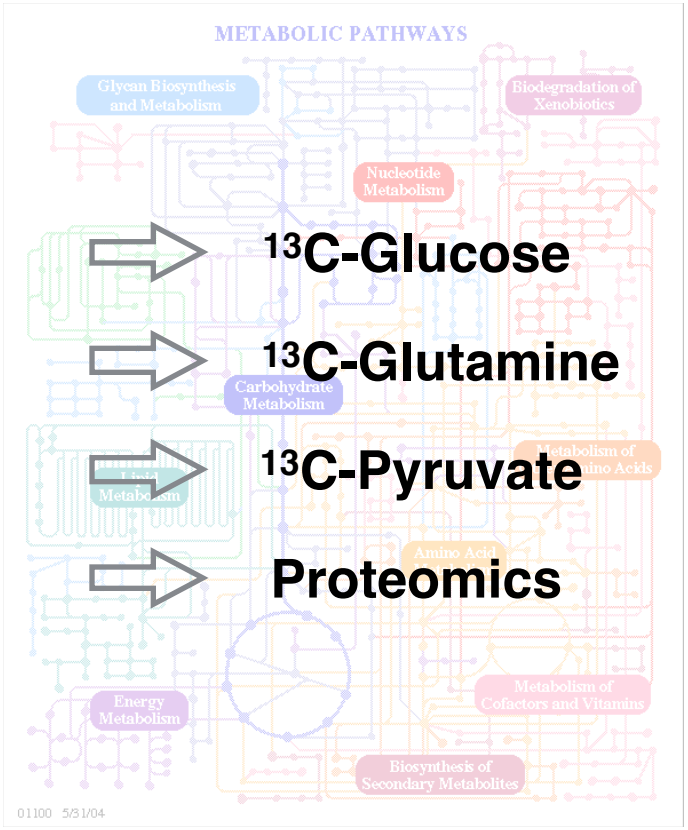

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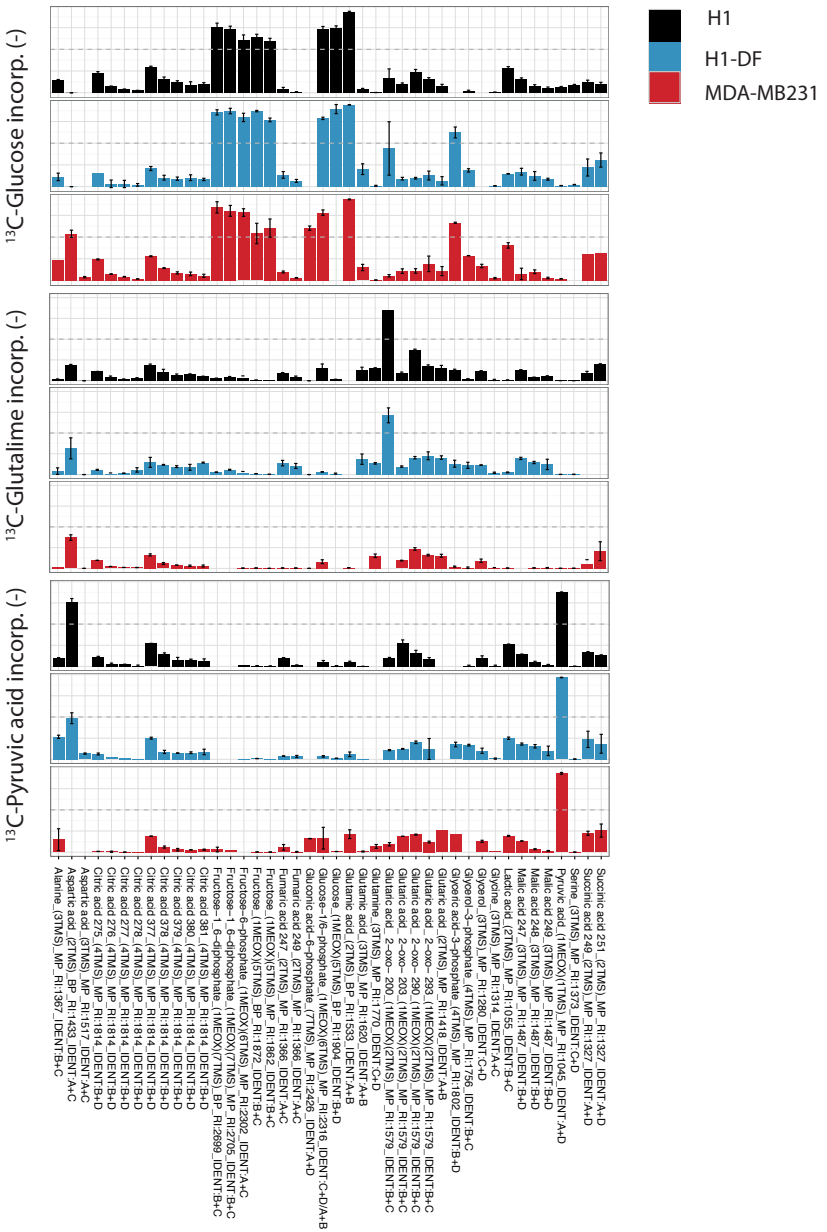
Re-arrangement of carbon fate



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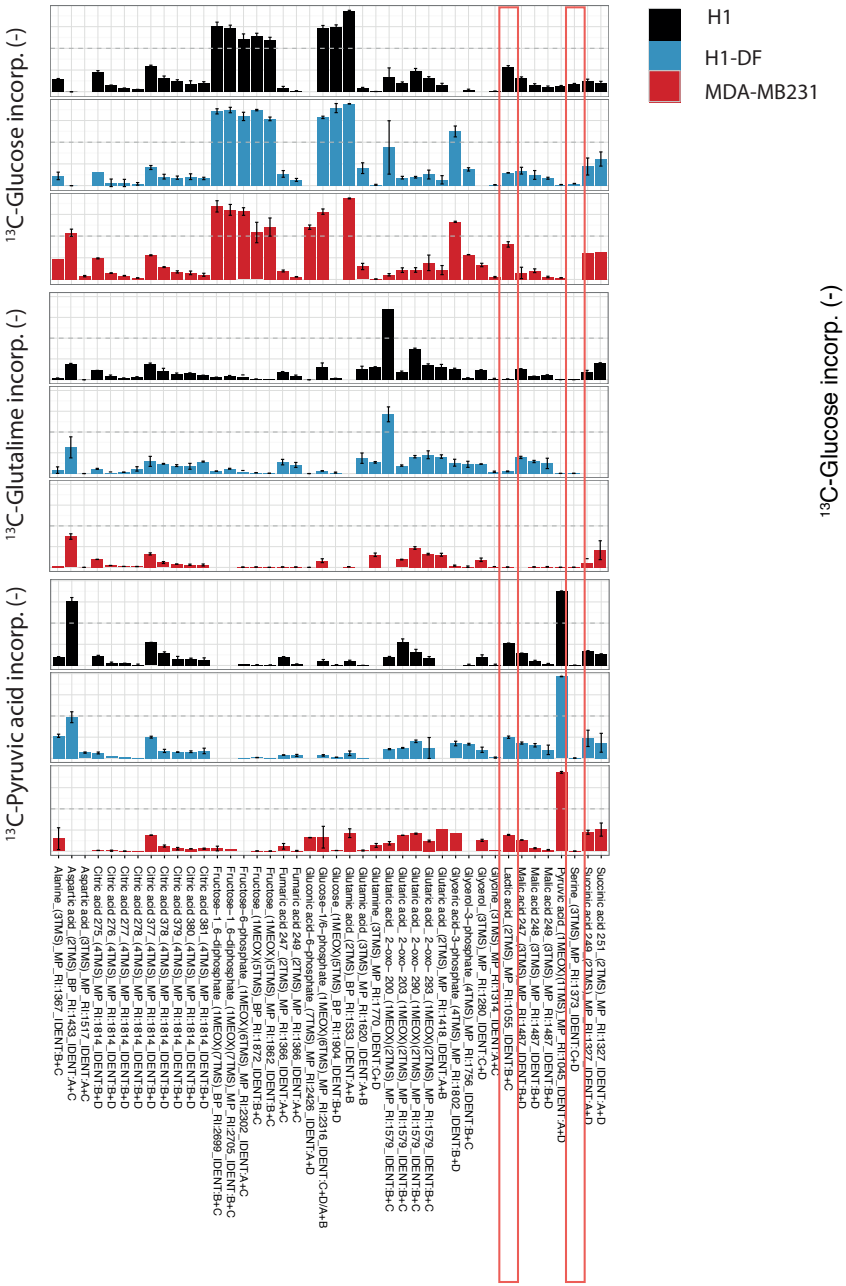

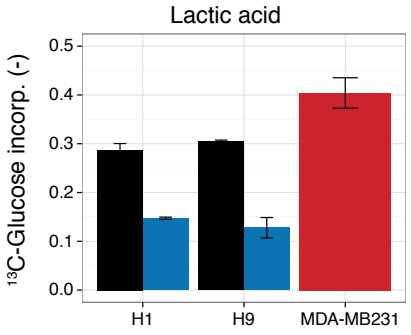

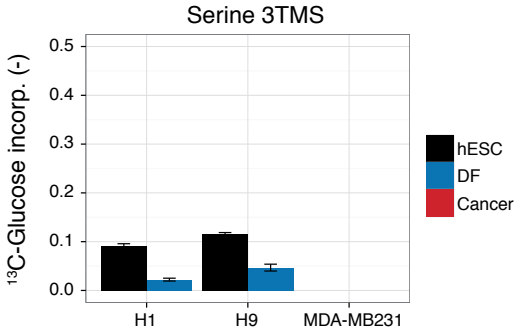

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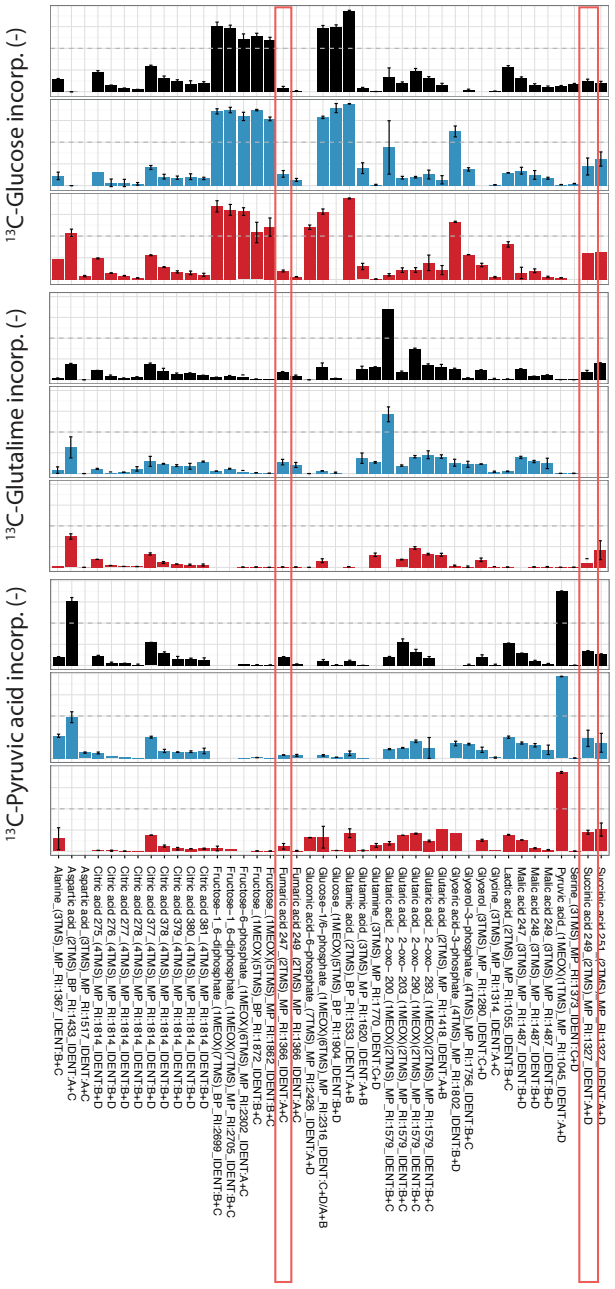
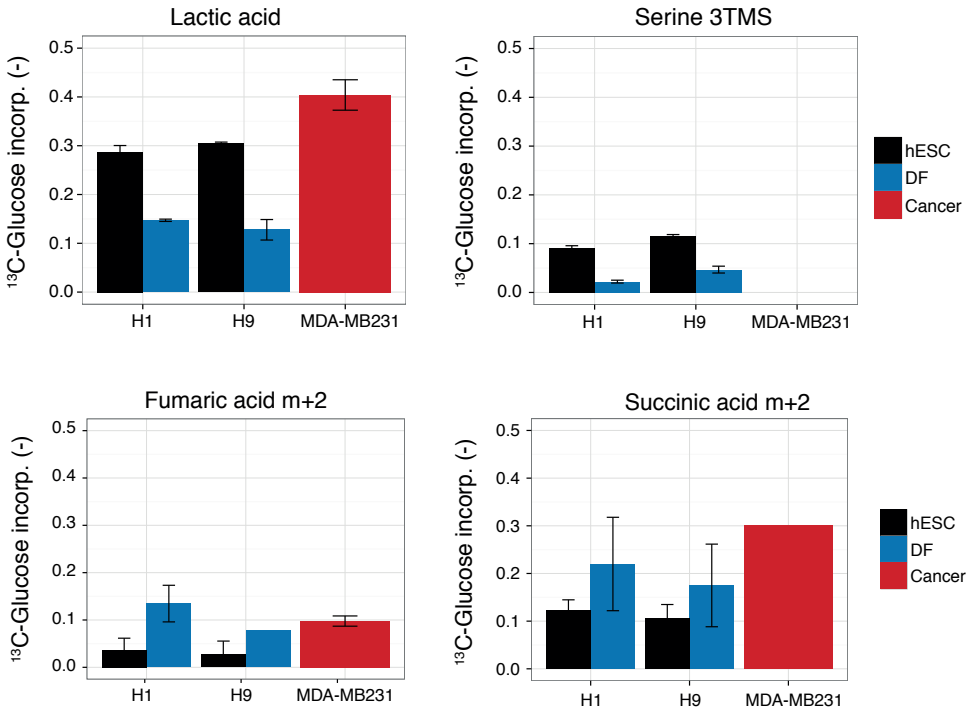
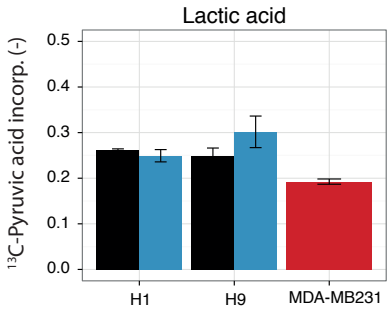
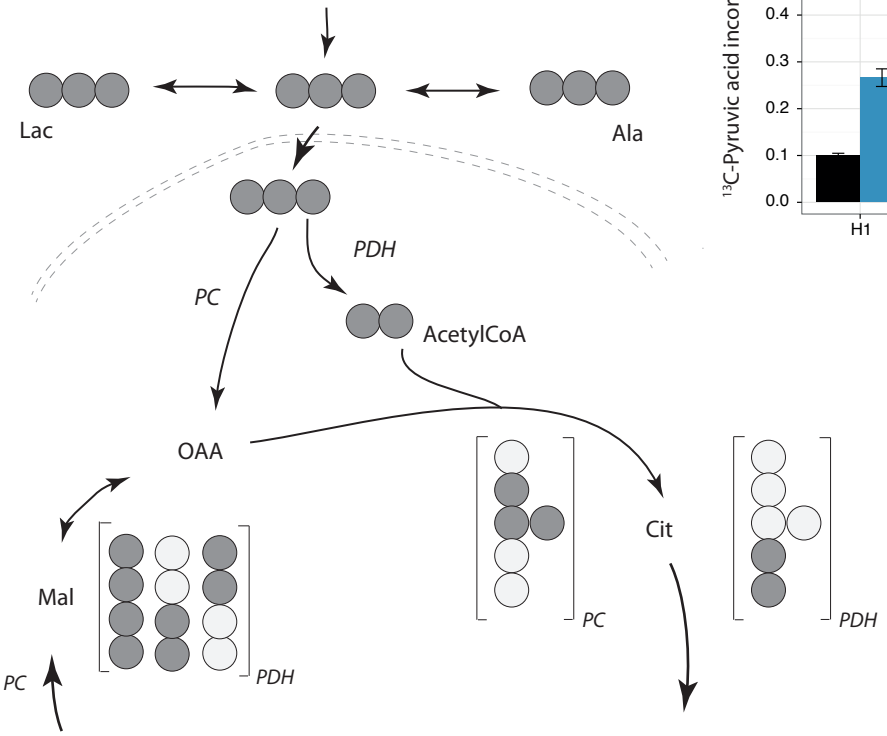
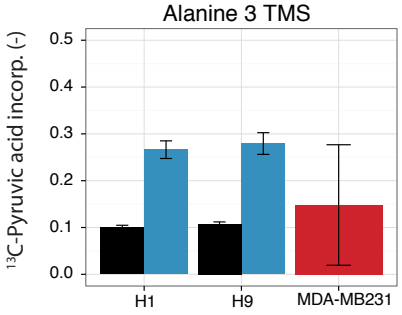


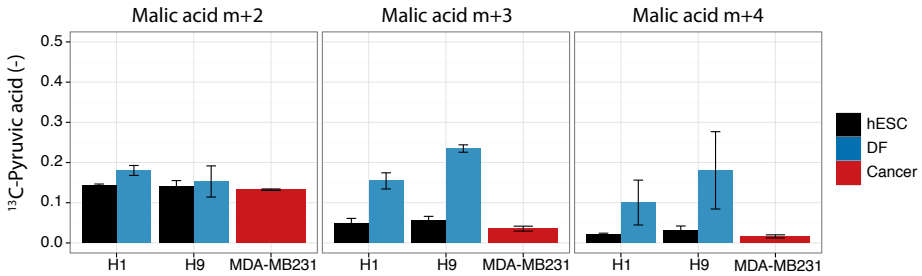


Routing of ^{13}C-Pyruvic acid

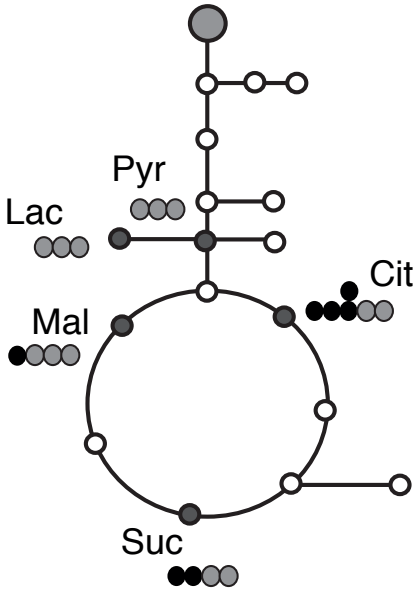




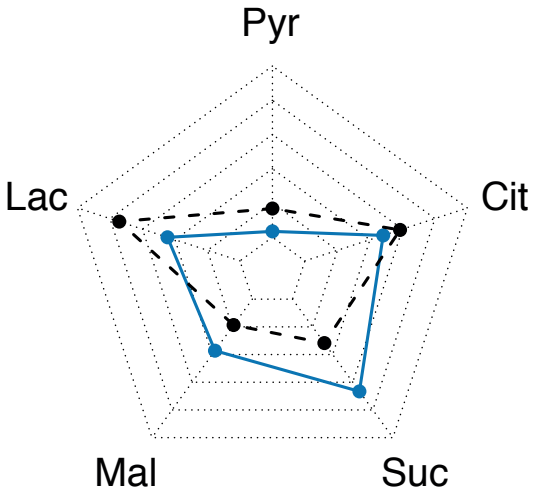




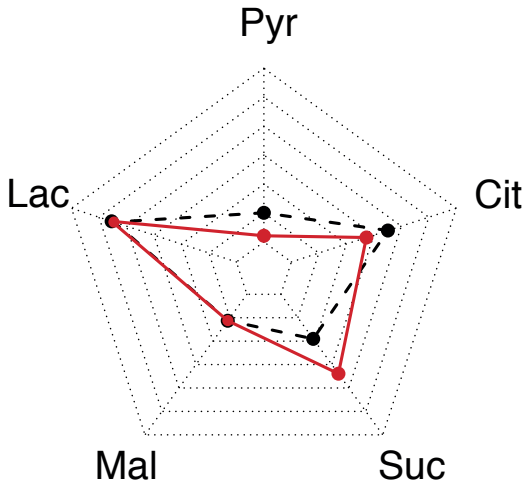
Summary

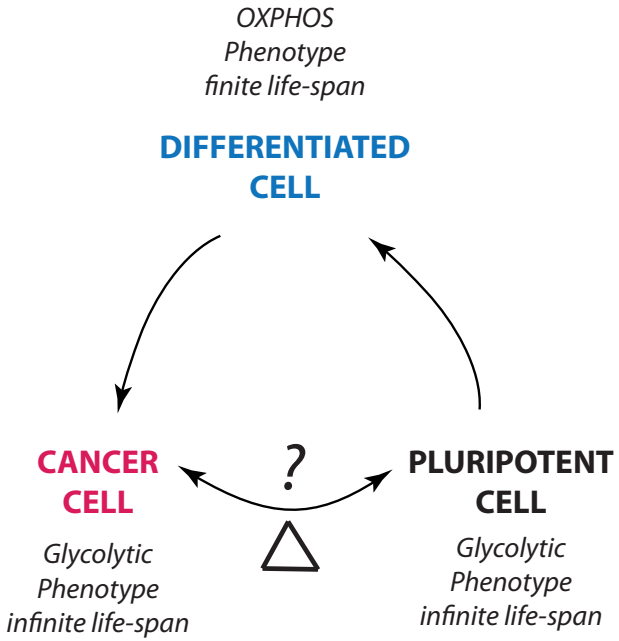


pluripotent vs. differentiated



pluripotent vs. tumorgenic





Acknowledgments

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Alessandro Prigione
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MDC Berlin-Buch, Germany

Katharina Nöh
Sebastian Niedenführ

Forschungszentrum Jülich
Germany

… thank you for your attention