High-throughput proteomics in Epidemiology and the genotype-phenotype problem

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Central Dogma

James Watson

Francis Crick

DNA

RNA

PROTEIN

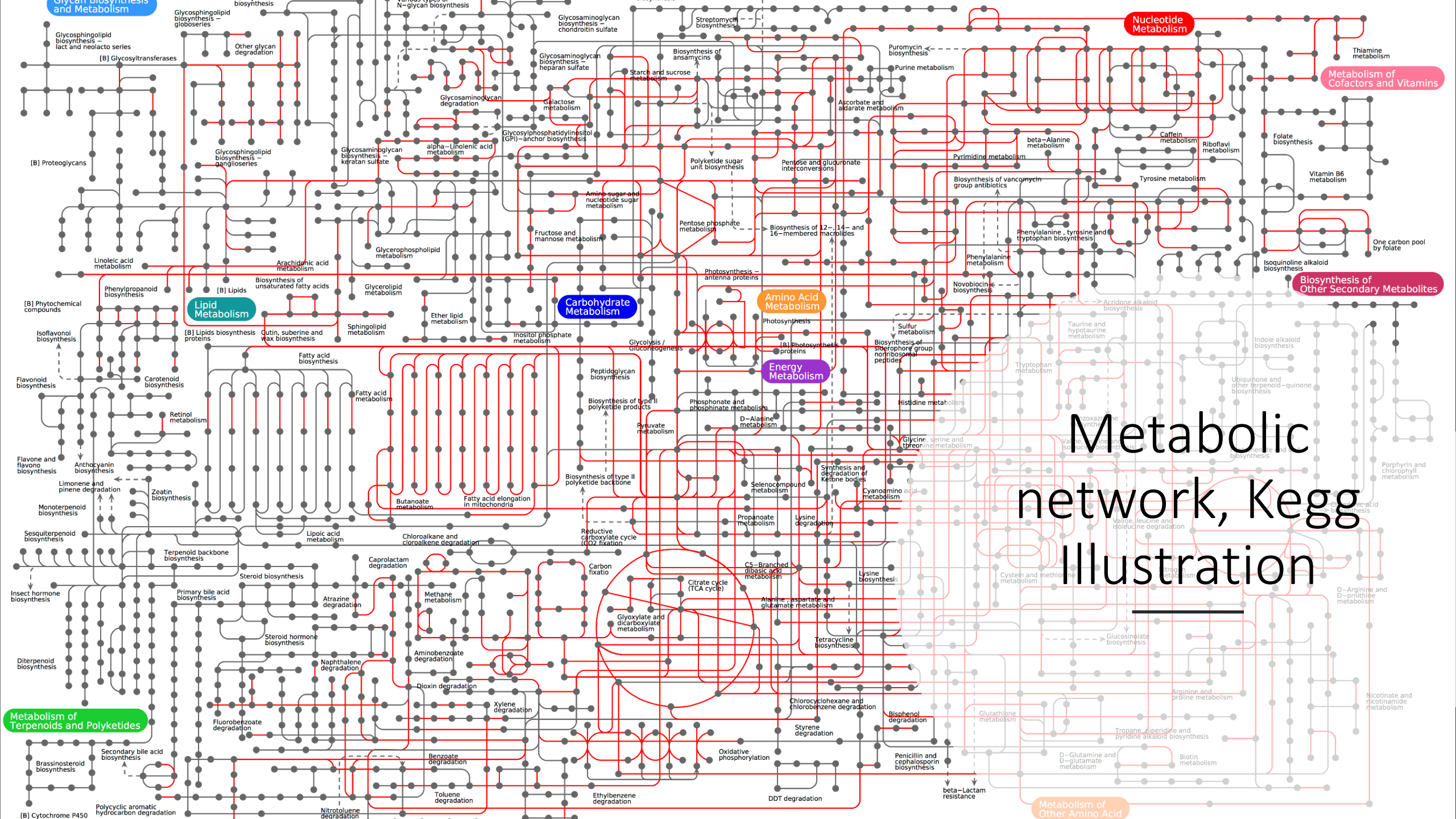
replication

transcription

translation

nucleic acids

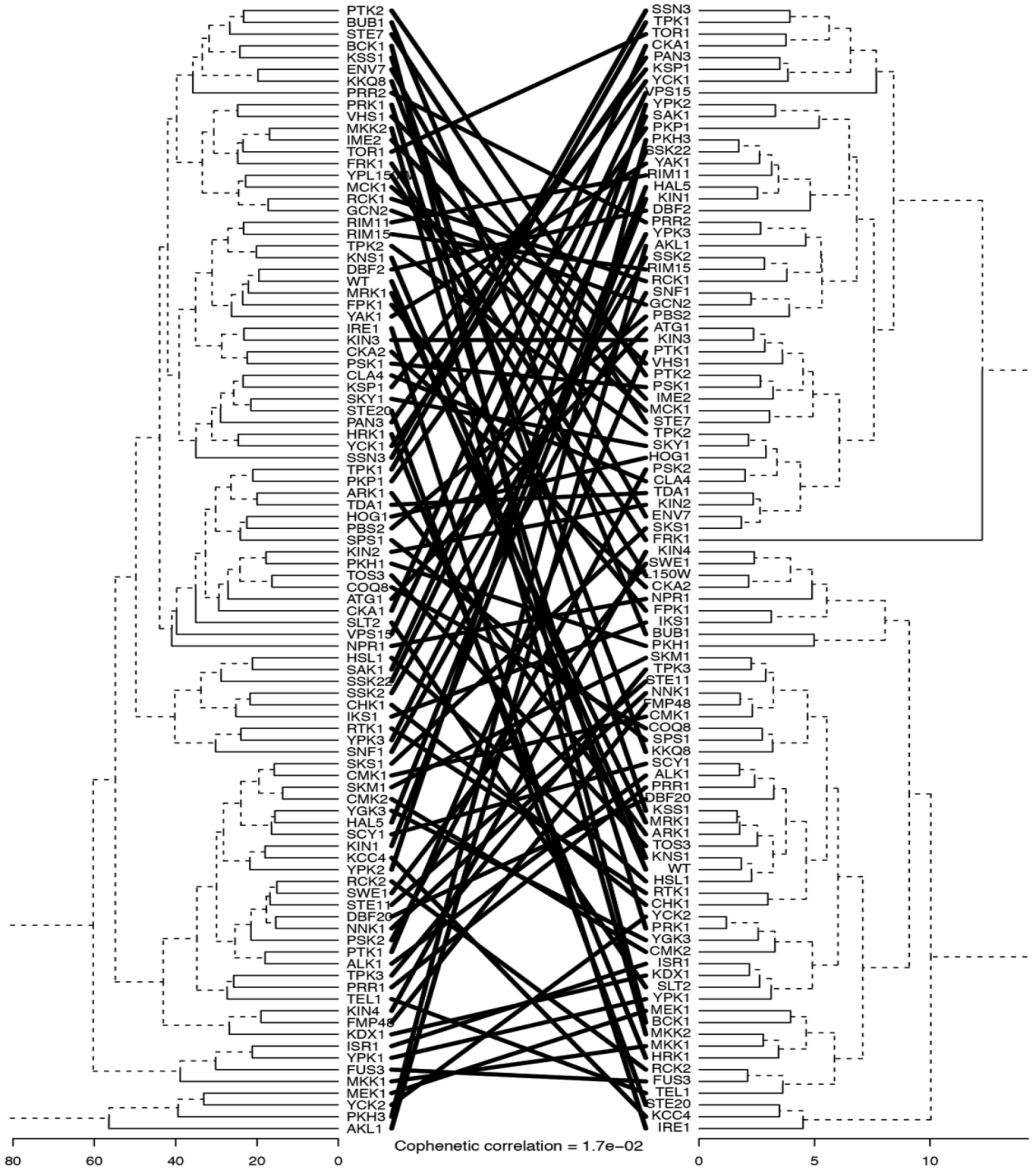
amino acids



Metabolic network, Kegg Illustration

The genotype-phenotype problem

gets very complicated at the step from proteome to metabolome

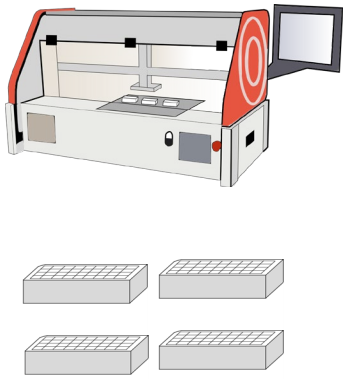


Proteome

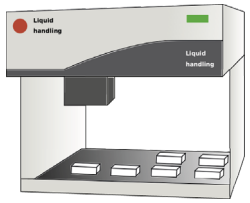
Metabolome

Scaling to thousands of LC-MS proteomes

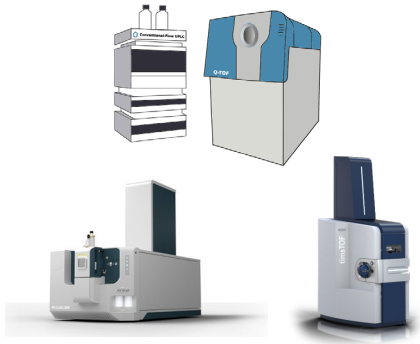
Sample preparation, high-flow LCs, Scanning SWATH acquisition, DIA-NN



All steps from cells to digests in 96 well plate format



Sample peparation:
96-well based semi-automated Protein extraction, digestion and SPE clean-up



Data acquisition:
- **Analytical LC or micro LC**
- 1- 5min gradients, 800µL/min
- SWATH acquisition
- **Scanning SWATH**
- **DIA-PASEF**
- **ZenoSWATH**

Data-processing

DIA-NN

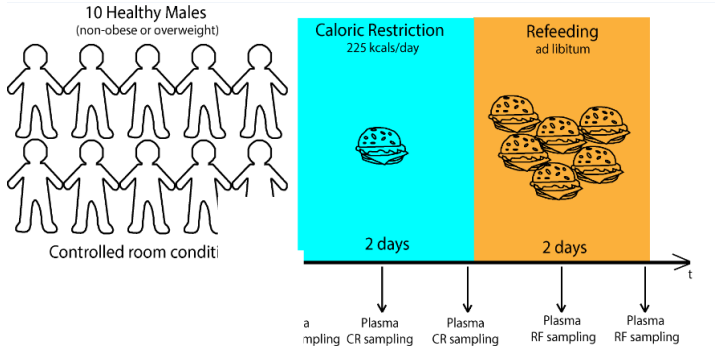
> 384 samples/batch

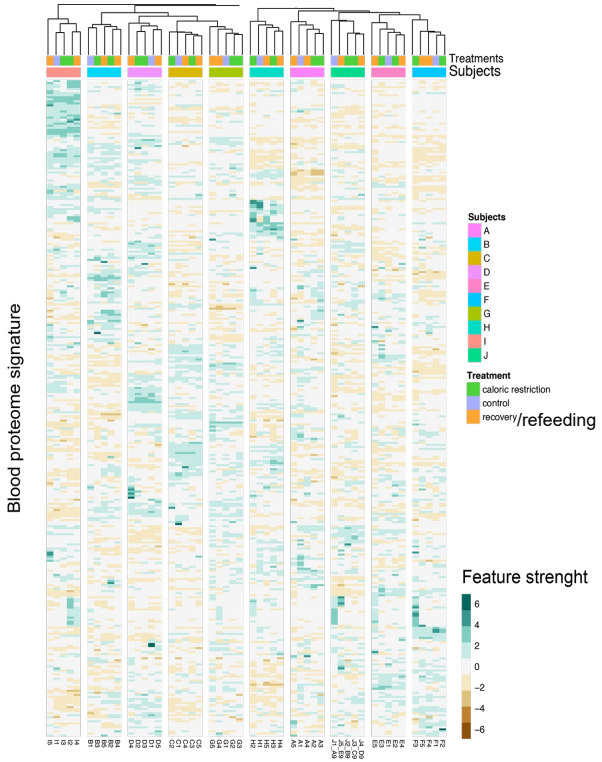
Up to 1800 proteomes per week per mass spectrometer

Scales with network & cluster >100s samples per day

Messner et al, Cell Systems, 2020
Demichev et al, Nature Methods, 2020
Messner et al, Nature Biotechnology, 2021

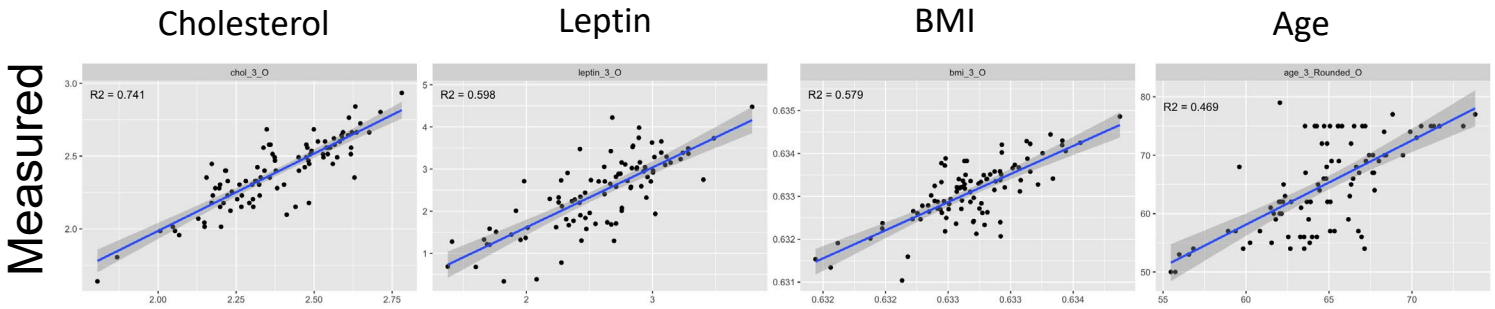
The proteome efficiently captures human physiology





Young, healthy individuals were exposed to extreme fasting and re-feeding over several days

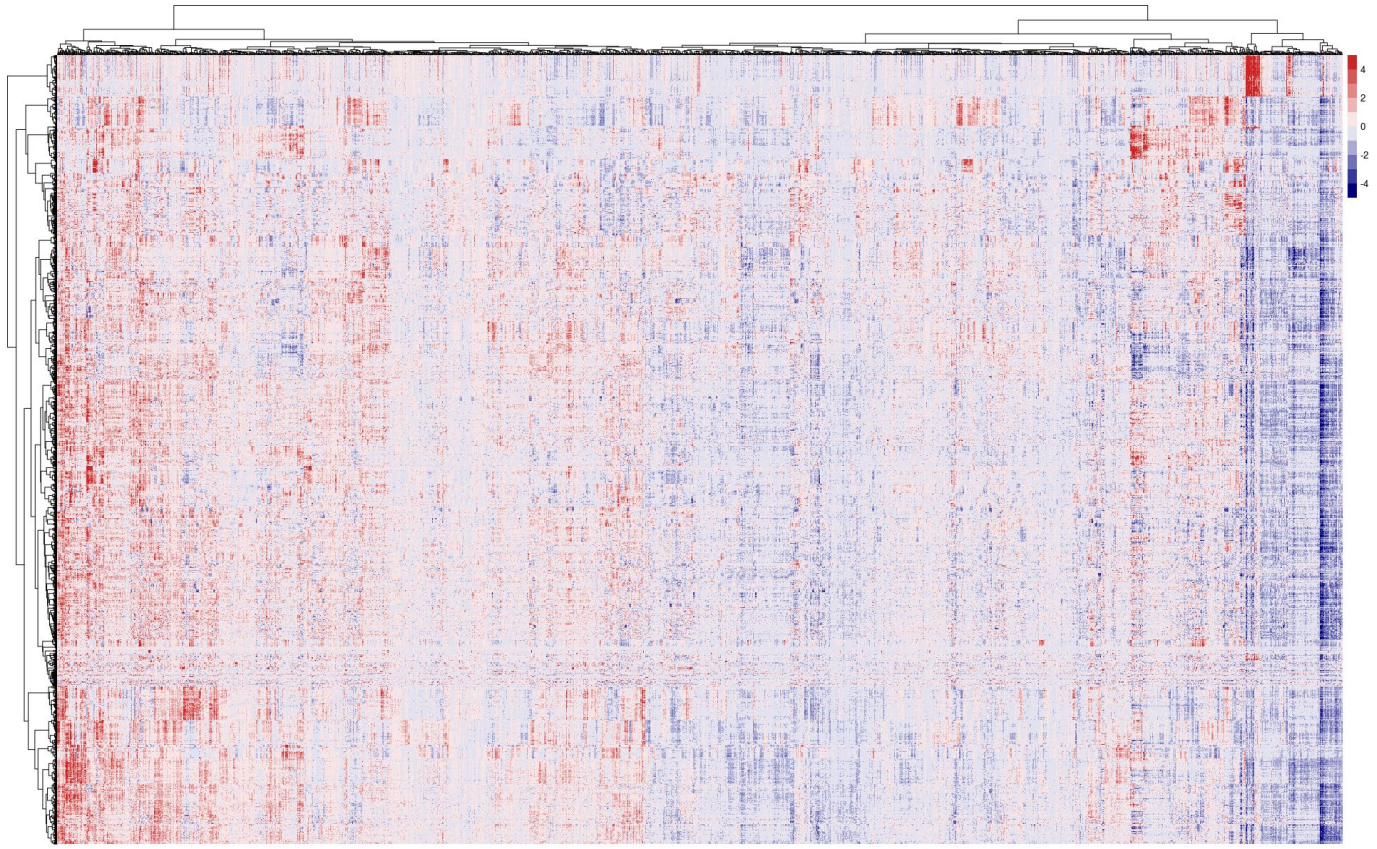
- Plasma samples were collected over time and measured with our platform, requiring 1-5µL of the samples
- The proteomes identify **the individual, the response to the nutritional intervention and, the inter-individual differences →** ***capturing all aspects of personalized medicine***
- **Trained machine learning algorithms derive a compendium of physiological parameters from the single blood proteome**



Predicted using machine learning from proteomes

Controlled human intervention study, with S. Farooqi, CIMR, Cambridge

Recording large human population baselines for genotype-phenotype mapping

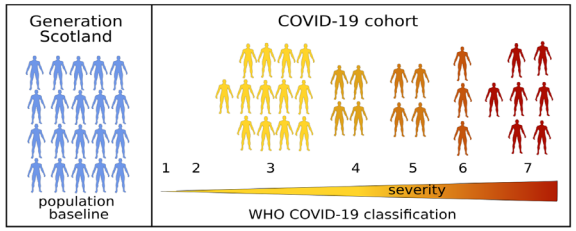


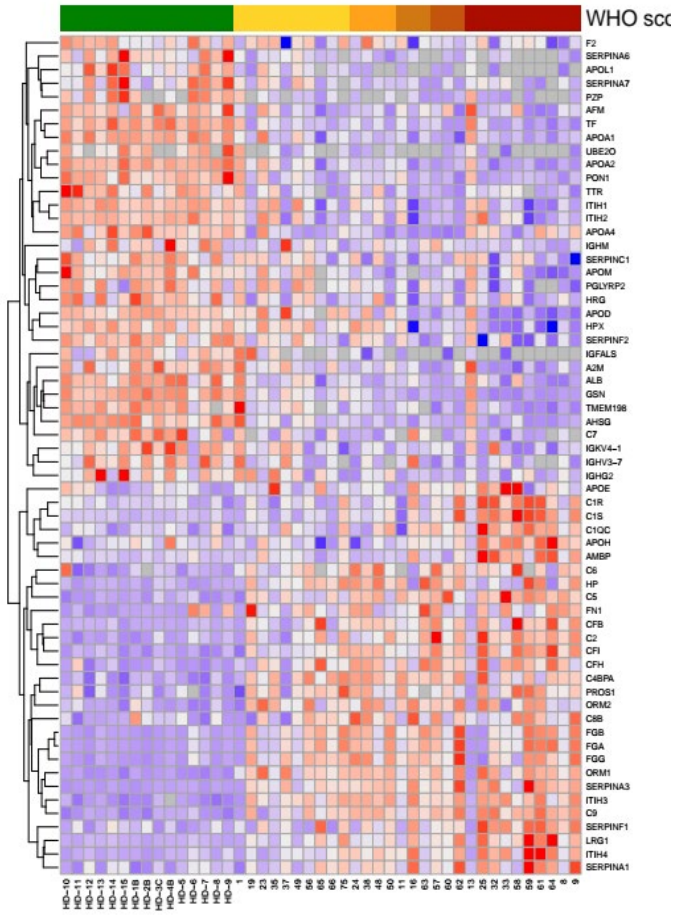
Population Structure
Of a on 2,000 individuals Fenland pilot

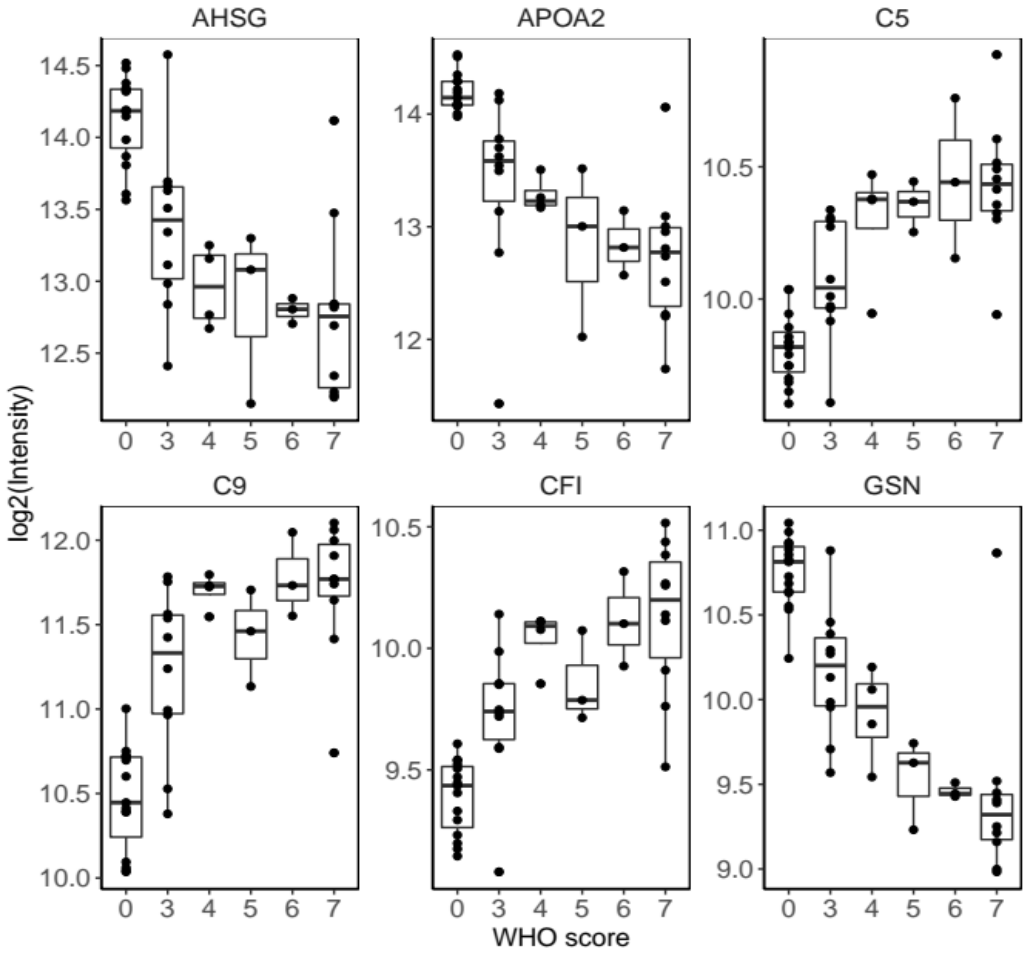
- Generation Scotland (~24,000 samples)
- Fenland study (~12,500 samples)
- eurac CHRIS (~5,000 samples)
- Genomics England rare disease (~5,000 samples)
- Vivaldi Carehome study (~7,000 samples)
- COVID study cohorts (>5,000 samples)
- Targeting >100,000 proteomes

FENLAND STUDY
generation scotland
Genomics england
MRC Epidemiology Unit
eurac research

Fast plasma proteomes classify COVID19 patients



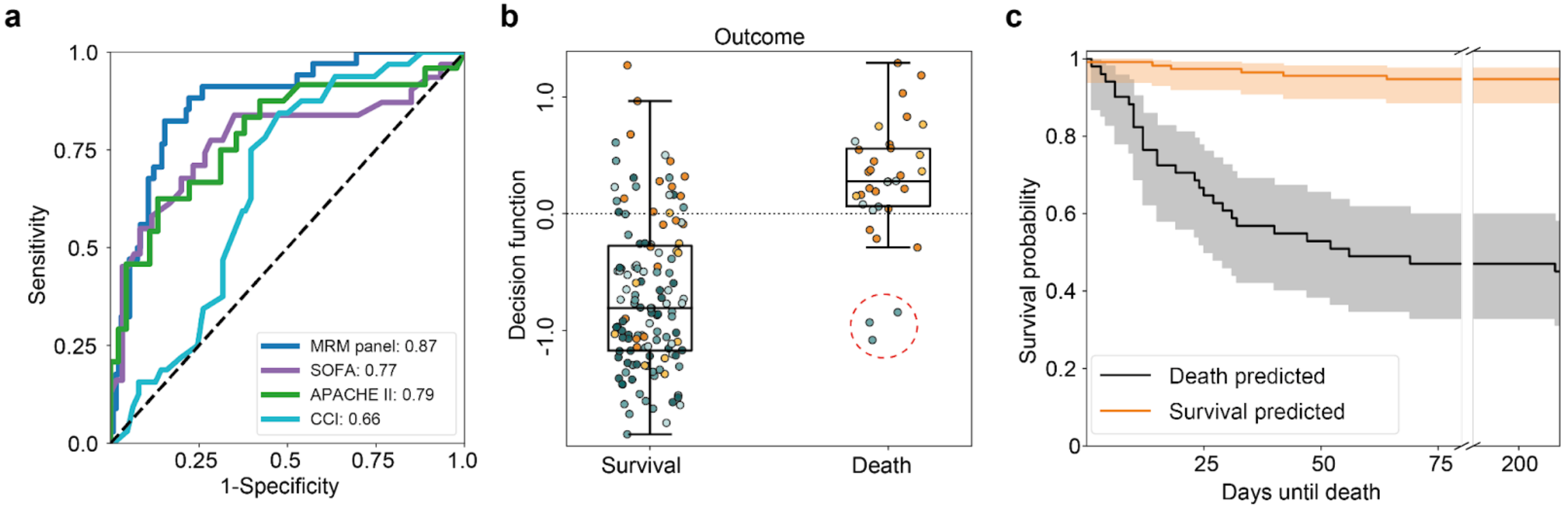




Florian Kurth, Pinkus Tober-Lau, Leif Sander, PA-COVID19 StdGrp

Messner et al, Cell systems, 2020
Messner, Demichev, et al, et al, Nature Biotechnology, 2021

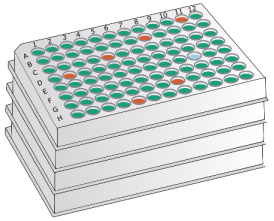
A COVID 19 peptide panel panel assay predicts outcome in severely affected COVID19 patients



Collaborations with Inoviv, Agilent, SCIEX. Wang, Hartl, Sirka et al, *medRxiv*
Panel is now implemented at Charité's routine lab and can be requested by physicians as part of care

A proteome for 5k yeast gene deletions

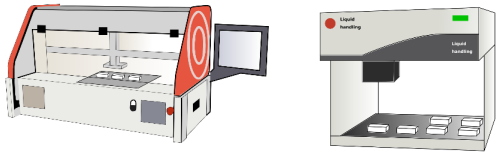
a **Gene deletion library**



WT strains (>6 per plate)
single knock-out strain
process blank (1 per plate)

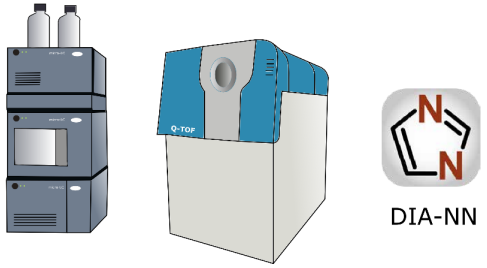
(57 plates - 4,567 KO strains)

b **Yeast growth and protein extraction**



Semiautomated and 96-well based

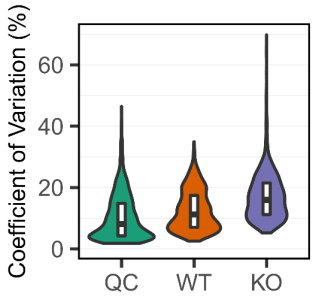
c **Micro-flow liquid chromatography and SWATH-MS**



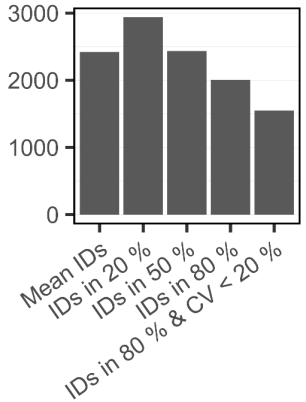
DIA-NN

29-minute measurement/sample

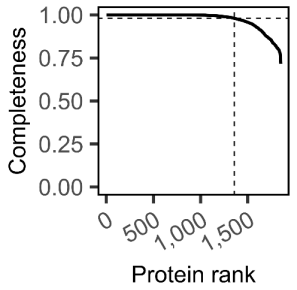
d **Reproducibility**



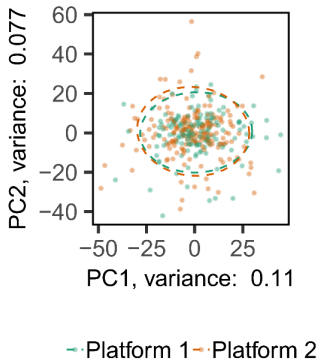
e **Identifications**

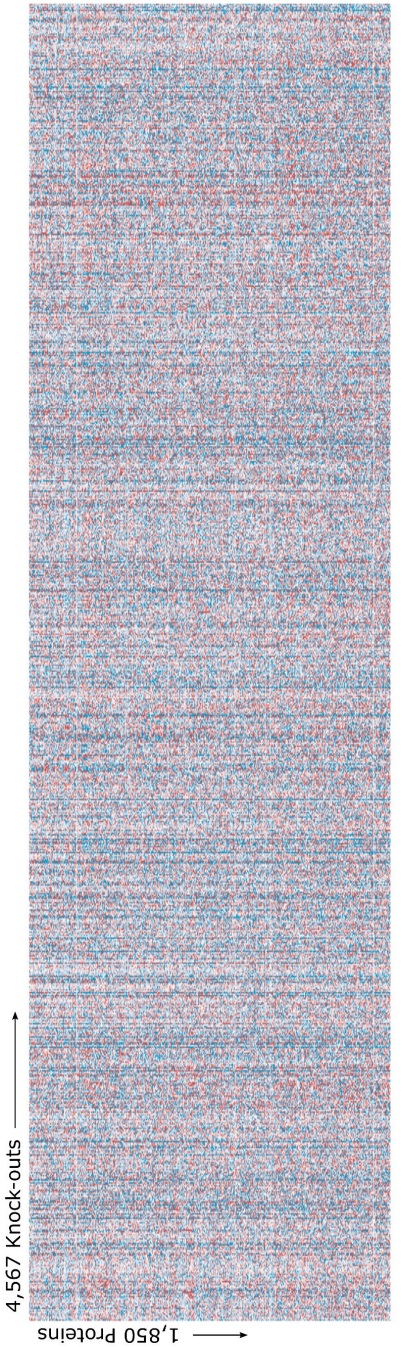


f **Data completeness**



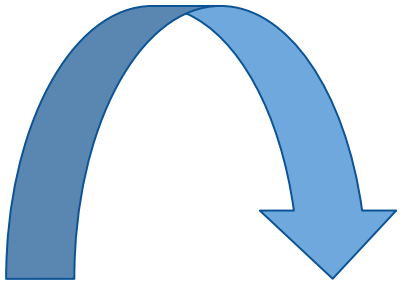
g **Parallelisation**





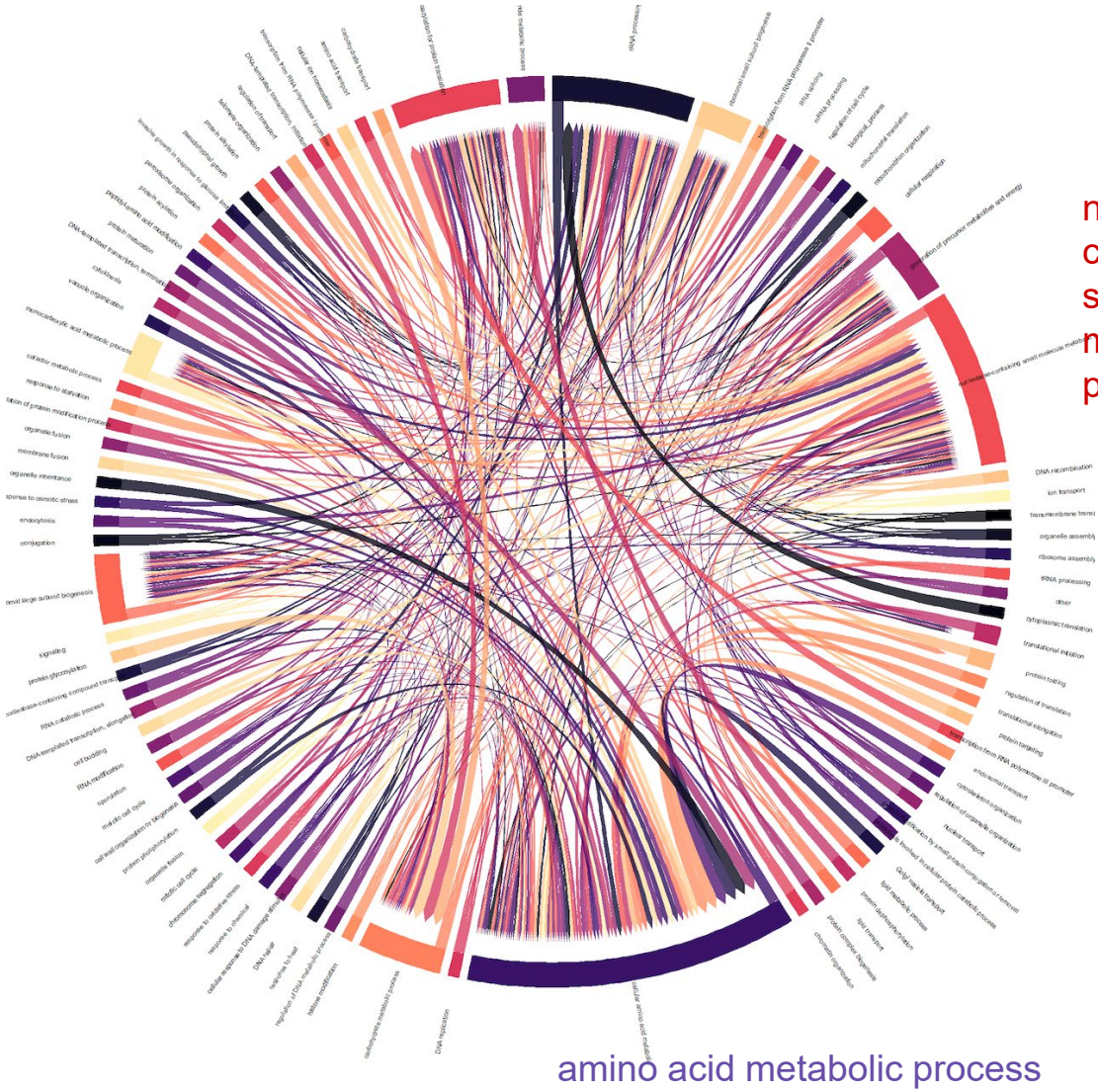
Christoph Messner

Proteome-Scale ‘Cause and Consequence’ Maps



Gset Perturbed

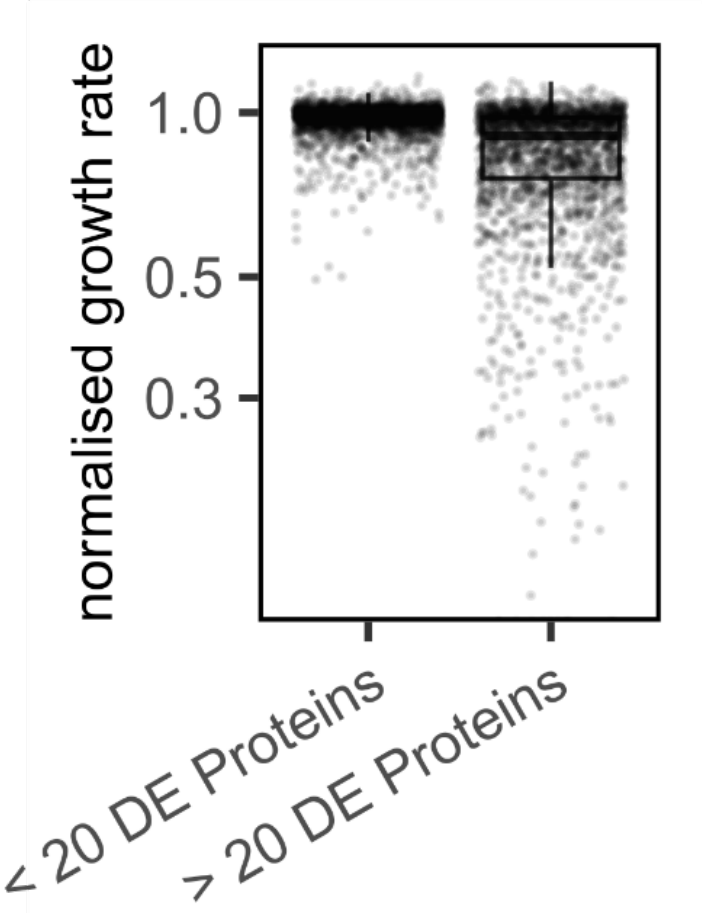
Gset Enriched

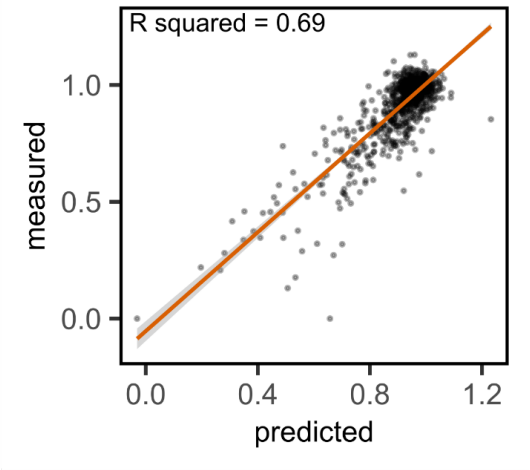


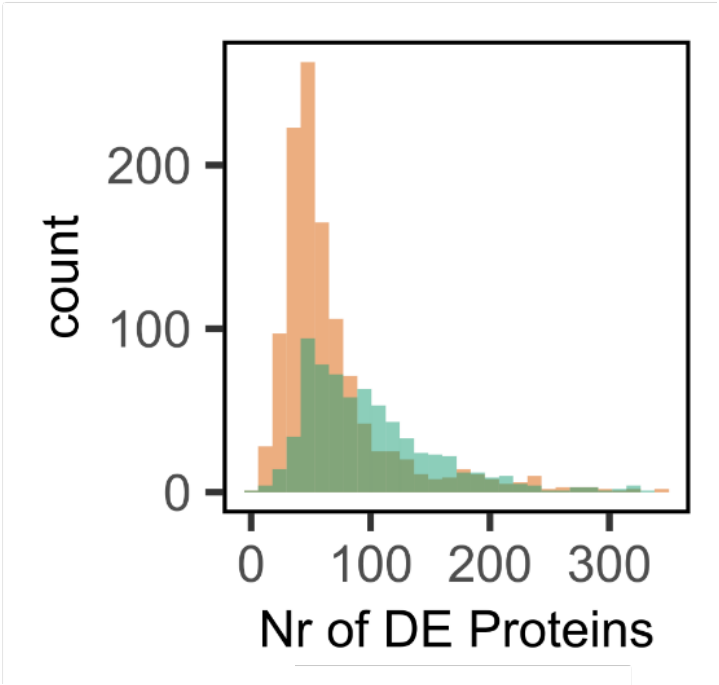
Metabolism and translation are the biggest responders to gene deletions

C. Messner, S. Aulakh

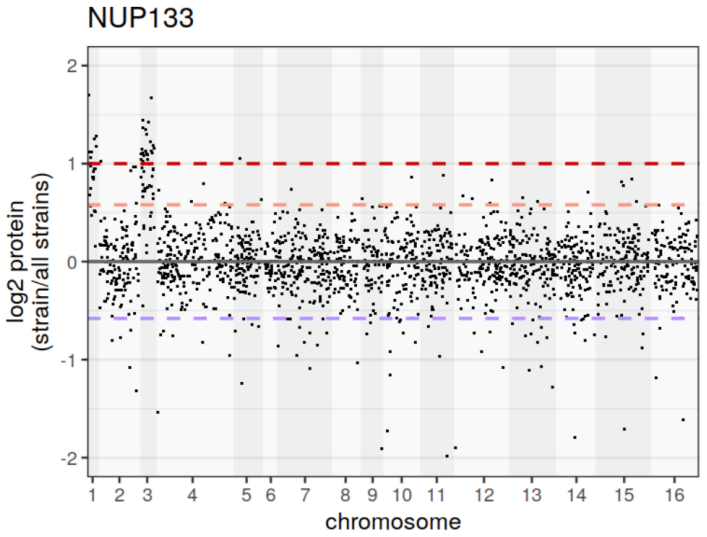
The relationship of growth rate and proteome

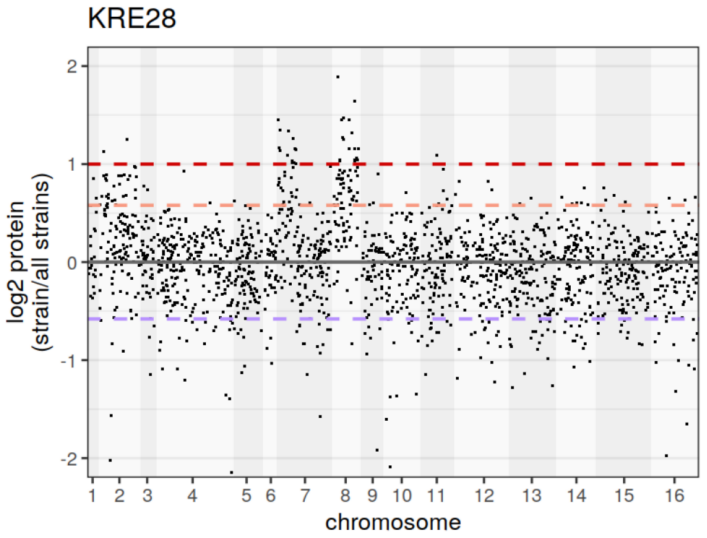


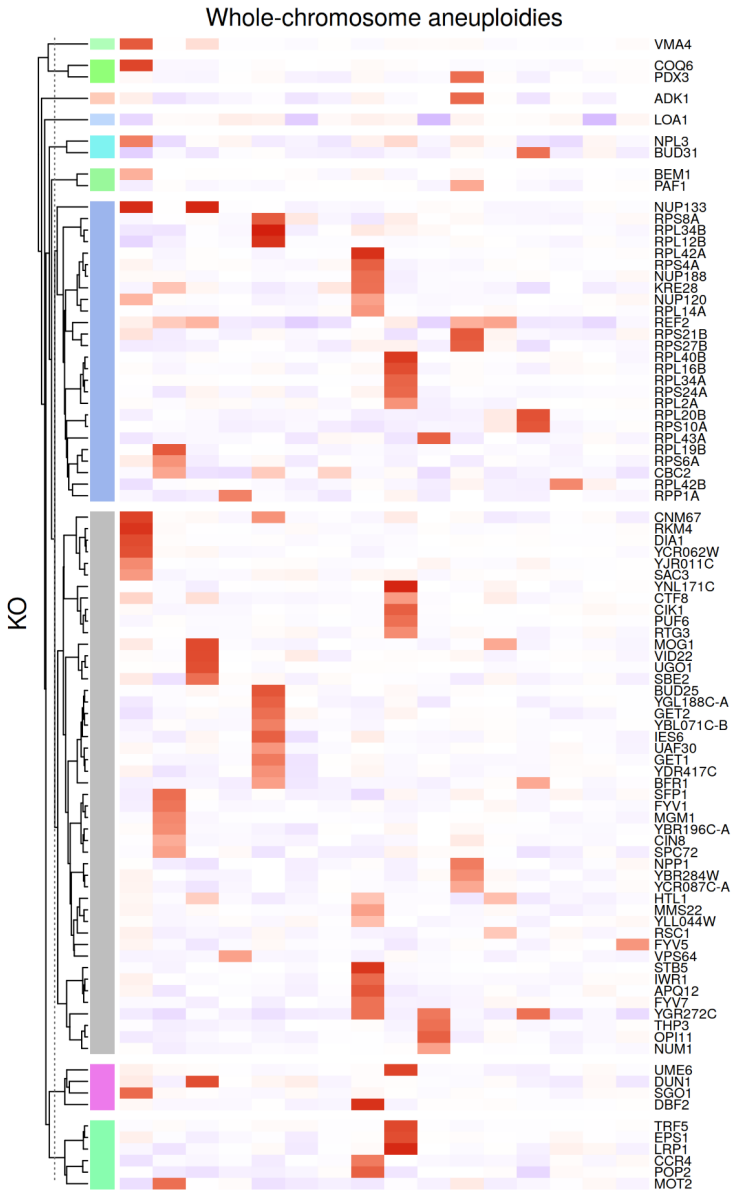


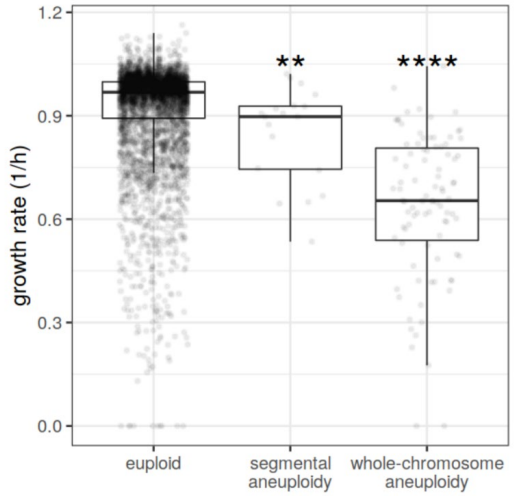


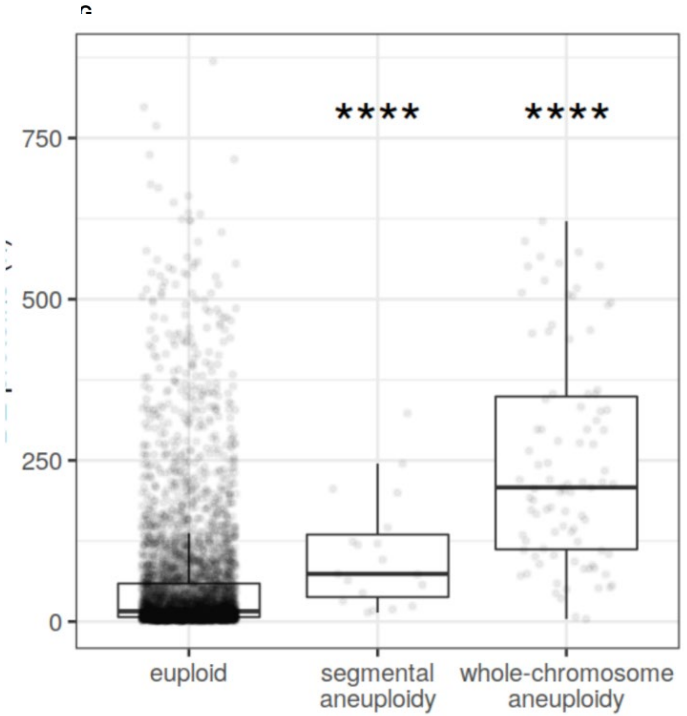
Aneuploidies explain strong proteomic changes in many slow growing cells



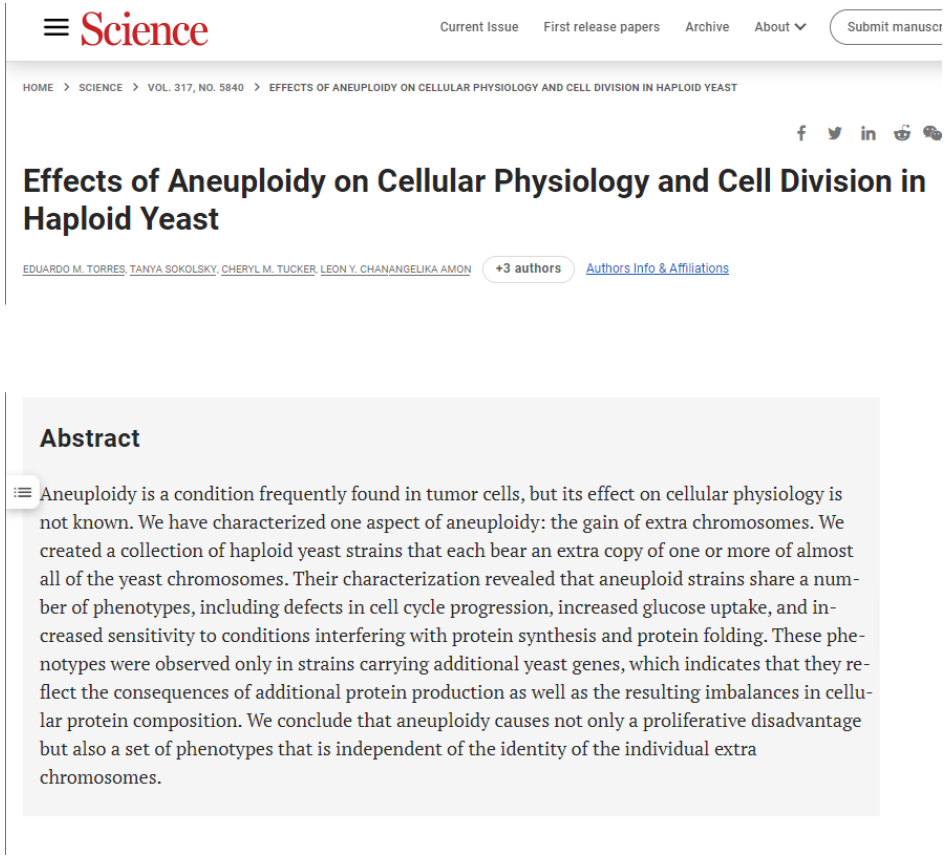








The unsolved problem of aneuploid dosage compensation



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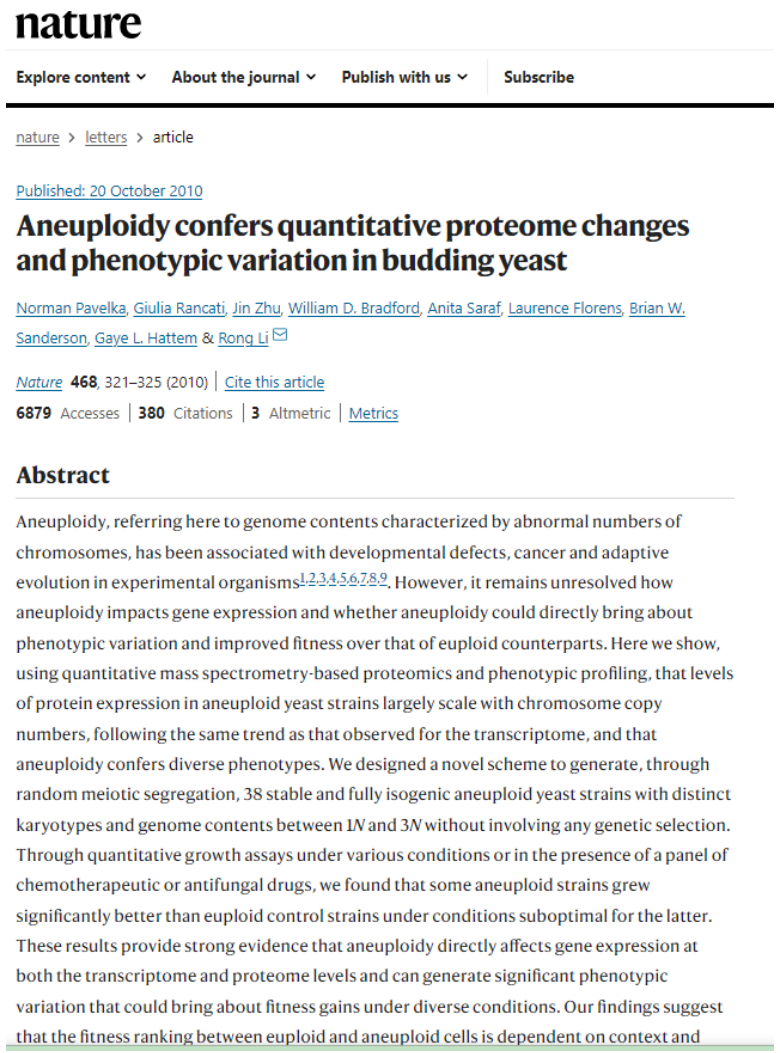
HOME > SCIENCE > VOL. 317, NO. 5840 > EFFECTS OF ANEUPLOIDY ON CELLULAR PHYSIOLOGY AND CELL DIVISION IN HAPLOID YEAST

Effects of Aneuploidy on Cellular Physiology and Cell Division in Haploid Yeast

EDUARDO M. TORRES, TANYA SOKOLSKY, CHERYL M. TUCKER, LEON Y. CHAN, ANGELIKA AMON +3 authors Authors Info & Affiliations

Abstract

Aneuploidy is a condition frequently found in tumor cells, but its effect on cellular physiology is not known. We have characterized one aspect of aneuploidy: the gain of extra chromosomes. We created a collection of haploid yeast strains that each bear an extra copy of one or more of almost all of the yeast chromosomes. Their characterization revealed that aneuploid strains share a number of phenotypes, including defects in cell cycle progression, increased glucose uptake, and increased sensitivity to conditions interfering with protein synthesis and protein folding. These phenotypes were observed only in strains carrying additional yeast genes, which indicates that they reflect the consequences of additional protein production as well as the resulting imbalances in cellular protein composition. We conclude that aneuploidy causes not only a proliferative disadvantage but also a set of phenotypes that is independent of the identity of the individual extra chromosomes.



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Published: 20 October 2010

Aneuploidy confers quantitative proteome changes and phenotypic variation in budding yeast

Norman Pavelka, Giulia Rancati, Jin Zhu, William D. Bradford, Anita Saraf, Laurence Florens, Brian W. Sanderson, Gaye L. Hattem & Rong Li

Nature **468**, 321–325 (2010) | Cite this article

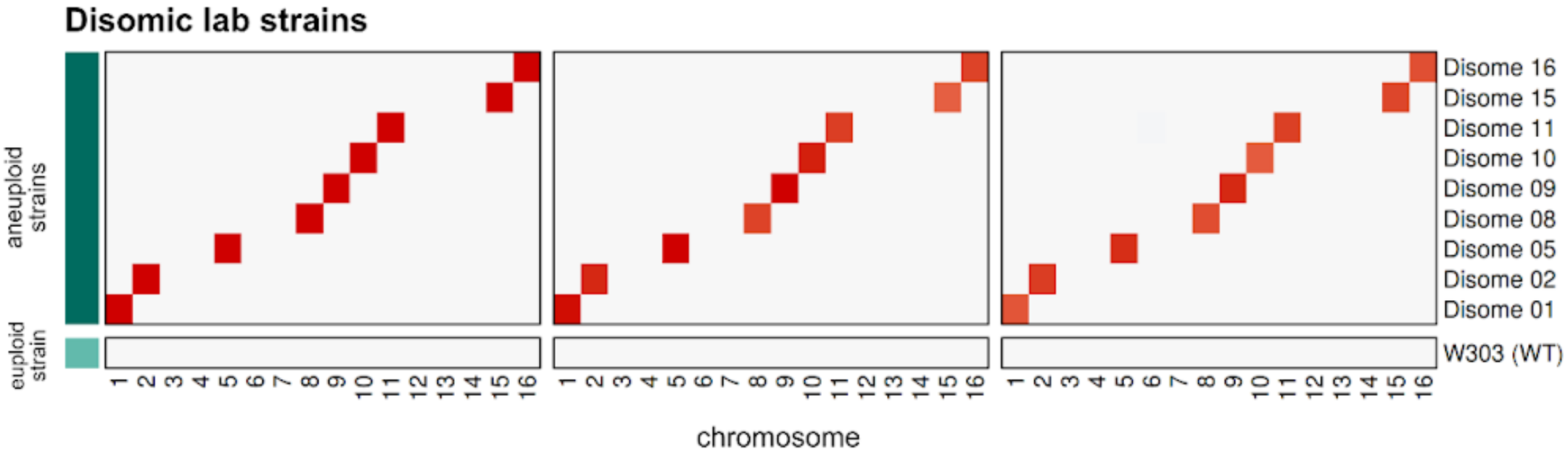
6879 Accesses | **380** Citations | **3** Altmetric | Metrics

Abstract

Aneuploidy, referring here to genome contents characterized by abnormal numbers of chromosomes, has been associated with developmental defects, cancer and adaptive evolution in experimental organisms[1,2,3,4,5,6,7,8,9]. However, it remains unresolved how aneuploidy impacts gene expression and whether aneuploidy could directly bring about phenotypic variation and improved fitness over that of euploid counterparts. Here we show, using quantitative mass spectrometry-based proteomics and phenotypic profiling, that levels of protein expression in aneuploid yeast strains largely scale with chromosome copy numbers, following the same trend as that observed for the transcriptome, and that aneuploidy confers diverse phenotypes. We designed a novel scheme to generate, through random meiotic segregation, 38 stable and fully isogenic aneuploid yeast strains with distinct karyotypes and genome contents between 1*N* and 3*N* without involving any genetic selection. Through quantitative growth assays under various conditions or in the presence of a panel of chemotherapeutic or antifungal drugs, we found that some aneuploid strains grew significantly better than euploid control strains under conditions suboptimal for the latter. These results provide strong evidence that aneuploidy directly affects gene expression at both the transcriptome and proteome levels and can generate significant phenotypic variation that could bring about fitness gains under diverse conditions. Our findings suggest that the fitness ranking between euploid and aneuploid cells is dependent on context and

Torres et al, 2007 (A. Amon Lab)
Pavelka et al, 2010 (R. Li lab)

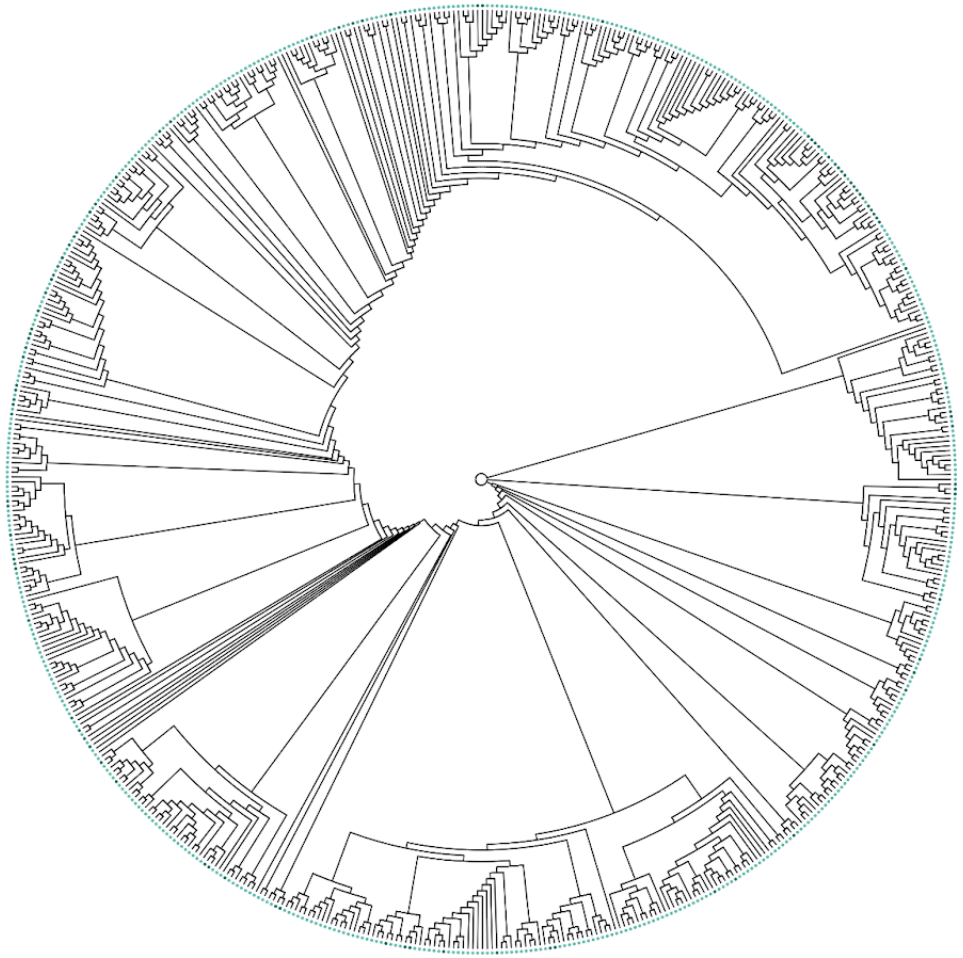
Essentially no chromosome-wide dosage compensation in synthetic aneuploids

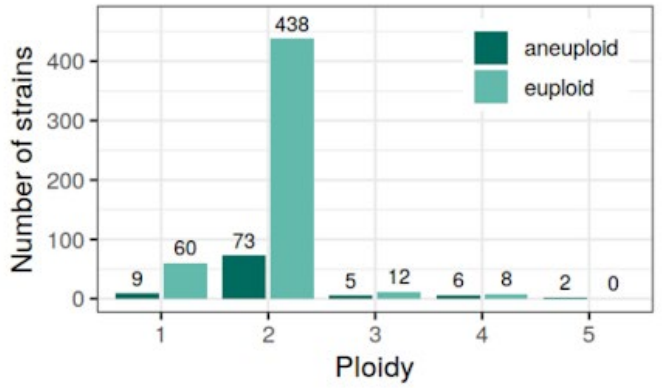


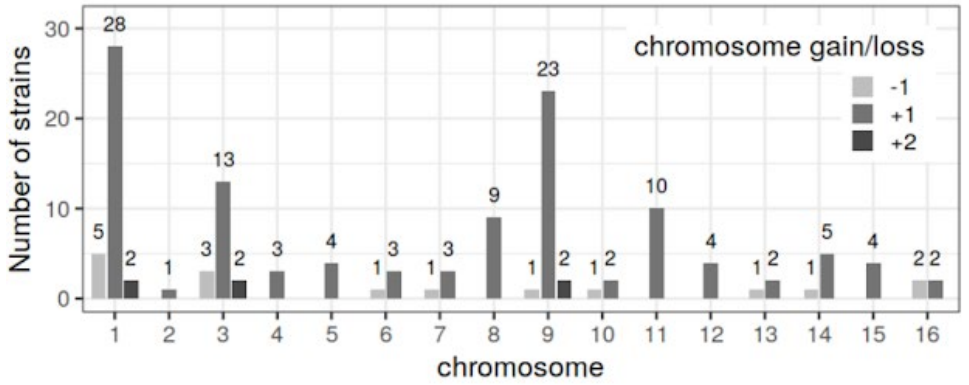
Strains & transcriptomes: Torres et al, 2007. Proteomes: Scanning SWATH

A proteome for ~900 natural yeast isolates

of which 19% carry natural aneuploidies







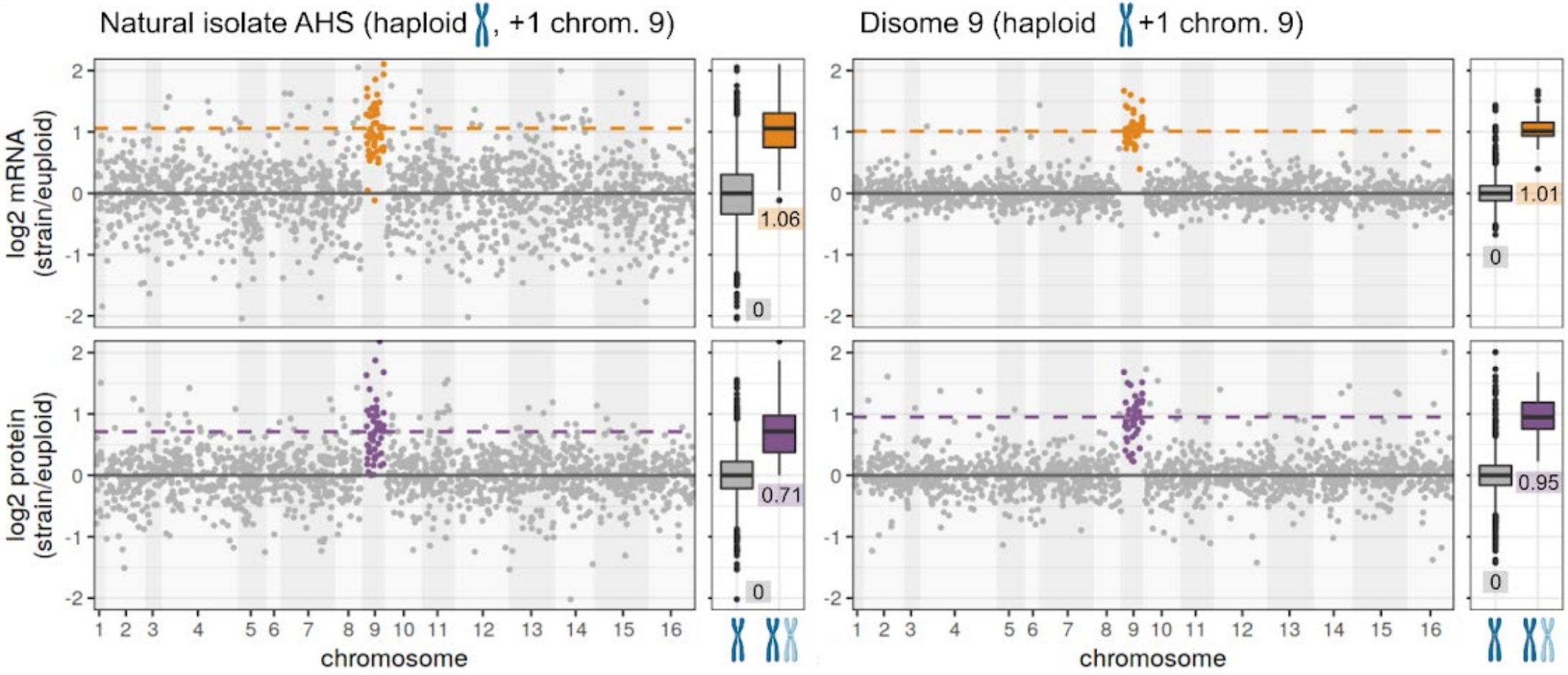
Gianni Liti, Joseph Schacherer, et al.

Peter et al, Nature, 2018

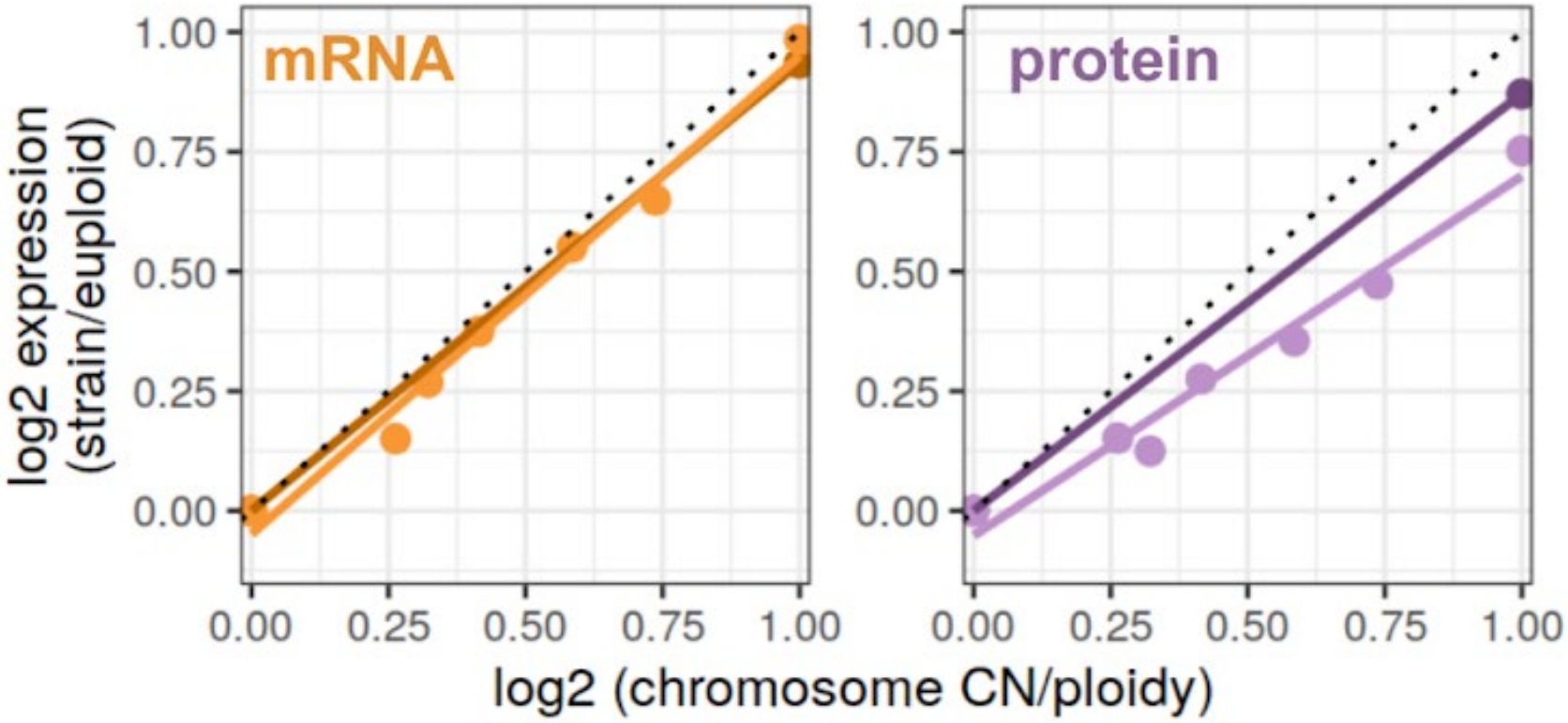
Aneuploidy is transmitted from genome, to transcriptome to proteome in natural strains, but…

Julia Münzner, Pauline Trebulle, Federica Agostini

Aneuploidy is dosage compensated at the chromosome wide-level, at the proteome.



Quantifying dosage-compensation across natural isolates and lab strains



Julia Münzner

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