FORTH
INSTITUTE OF CHEMICAL ENGINEERING SCIENCES

Therapeutic Application V: Metabolic & Protein Networks

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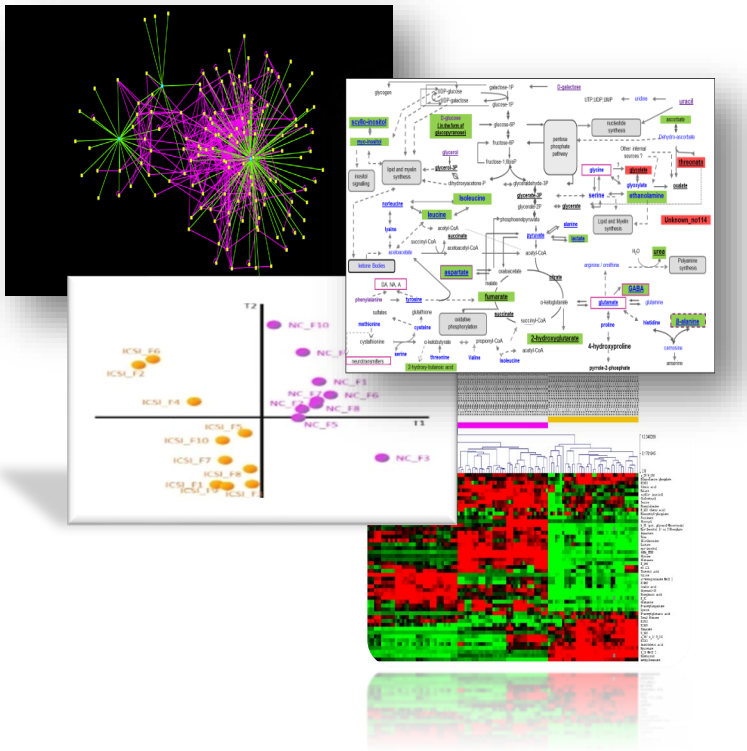
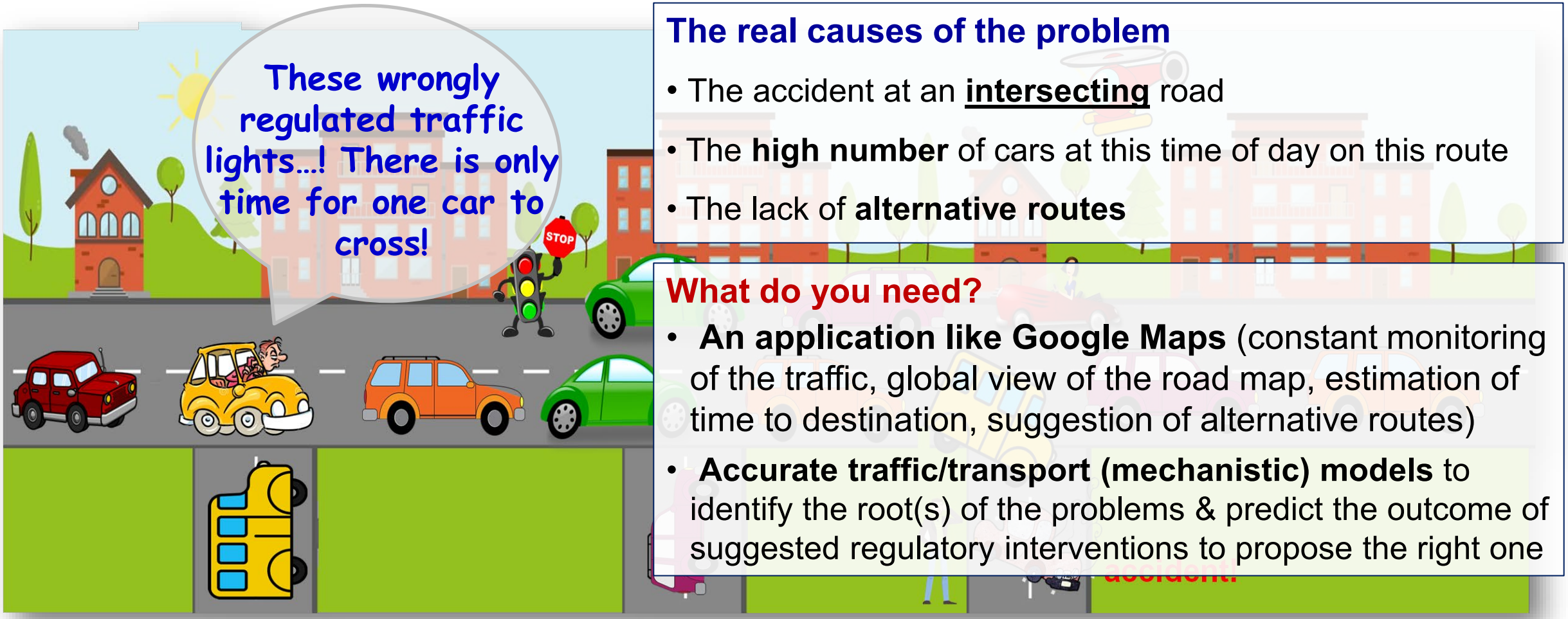


Virtual Human Global Summit II
Session B - Applications of Virtual Human Models and Digital Twins
Thursday, 23/10/2025

The value of the “big picture”: The network perspective

When you try to guess what the root of the heavy traffic problem is based on your **limited view** of the road without considering the complexity & interconnectivity of the road network



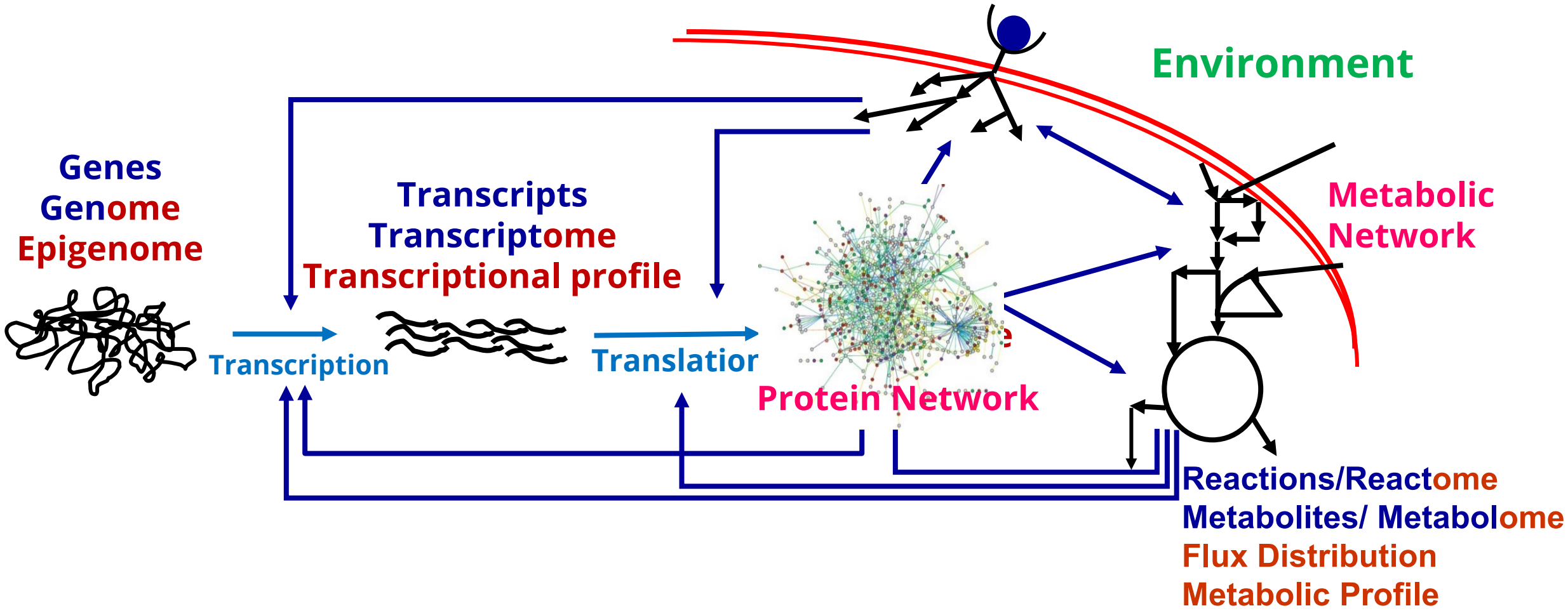
The real causes of the problem

- The accident at an **intersecting** road
- The **high number** of cars at this time of day on this route
- The lack of **alternative routes**

What do you need?

- **An application like Google Maps** (constant monitoring of the traffic, global view of the road map, estimation of time to destination, suggestion of alternative routes)
- **Accurate traffic/transport (mechanistic) models** to identify the root(s) of the problems & predict the outcome of suggested regulatory interventions to propose the right one

Extrapolation to the molecular physiology: The cell as a network of biomolecular networks



*The regulation of gene expression and protein activity is **NEITHER** linear **NOR** unidirectional*

The revolution of **high-throughput** biomolecular analyses (omics) →
Biology becomes a Quantitative Data Science / From Static to Dynamic Data

The network perspective is focal in Systems – Precision Medicine

ALL POTENTIAL PHENOTYPES
PHENOME/DISEASOME

↑

METABOLOME

↑

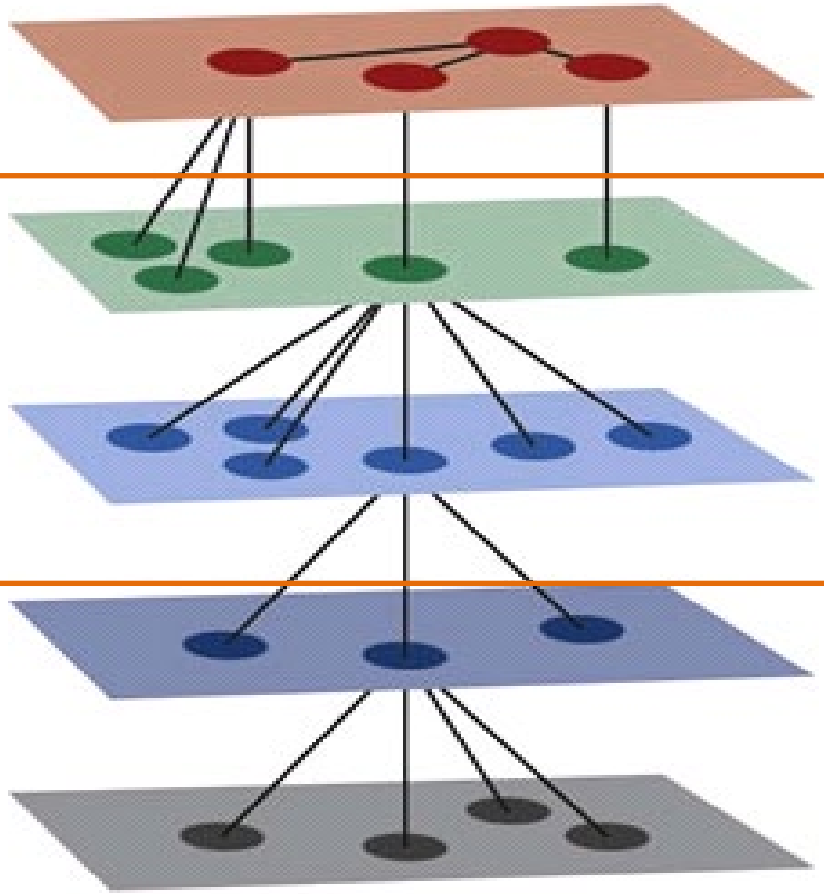
PROTEOME

↑

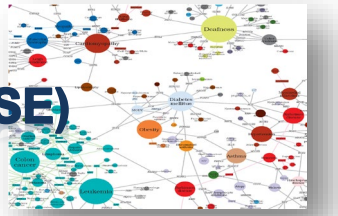
TRANSCRIPTOME
/ EPIGENOME

↑

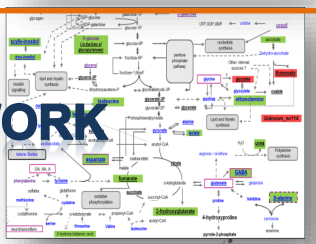
GENOME



PHENOTYPE (DISEASE) NETWORK

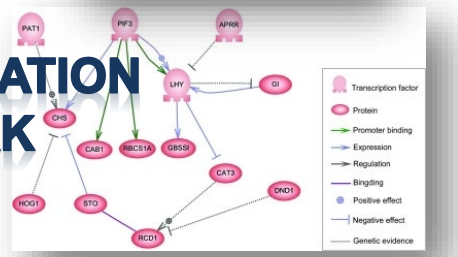


METABOLIC NETWORK



PROTEIN INTERACTION NETWORK

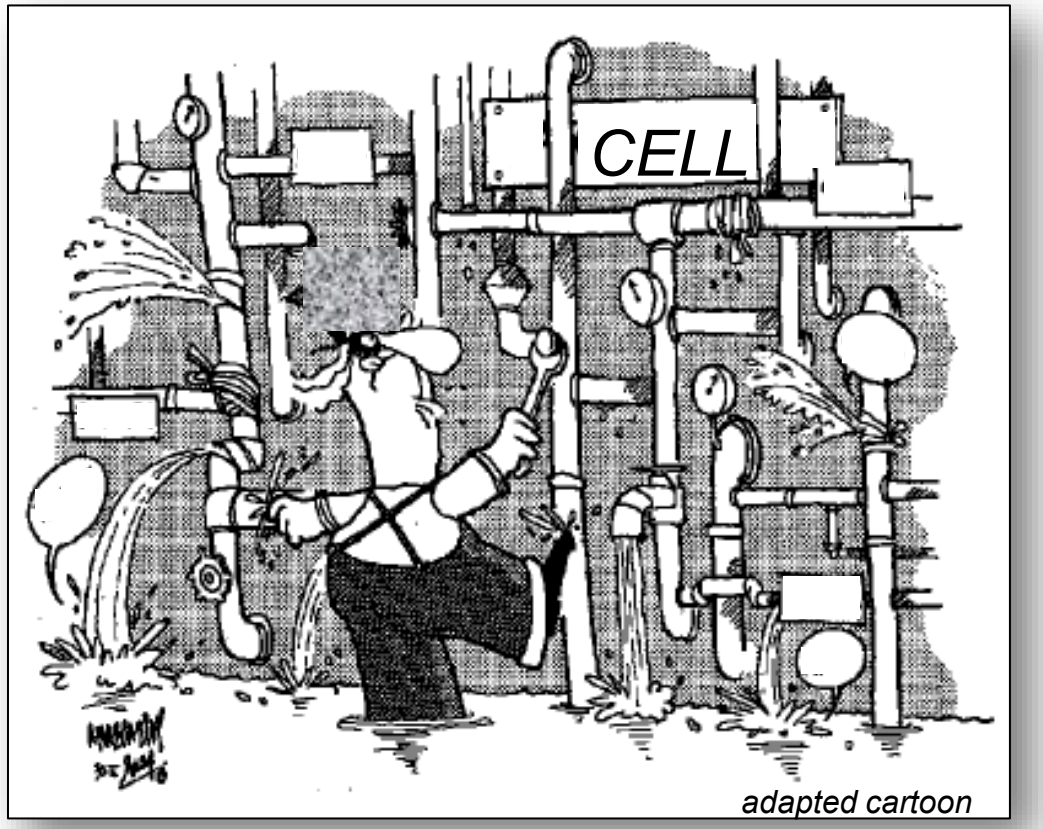
GENE REGULATION NETWORK



Moving away **from** the **gene-centric** approach **to** identifying the **genetic→ physiological architecture** of diseases/phenotypes as **new properties emerge through the biomolecular interactions**

Biomolecular Disease Modules / Related Phenotypes- Diseases / Treatment – Drug Repurposing

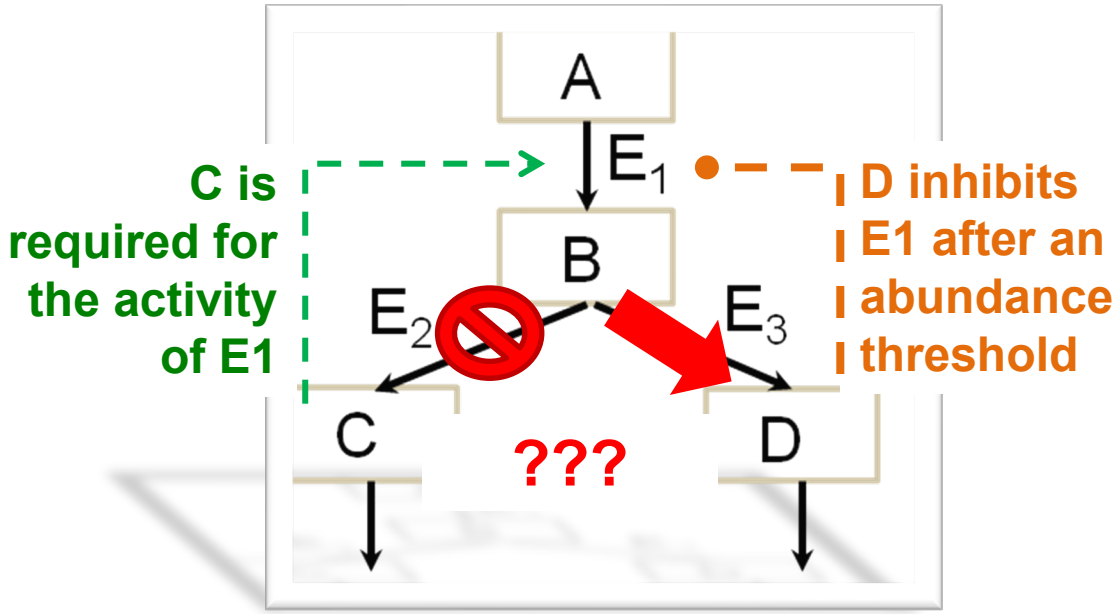
Do not forget the bioprocess dynamics! : The contribution of Metabolic Engineering



Toward a science of metabolic engineering
JE Bailey, *Science* 21 Jun 1991 252 pp. 1668-1675

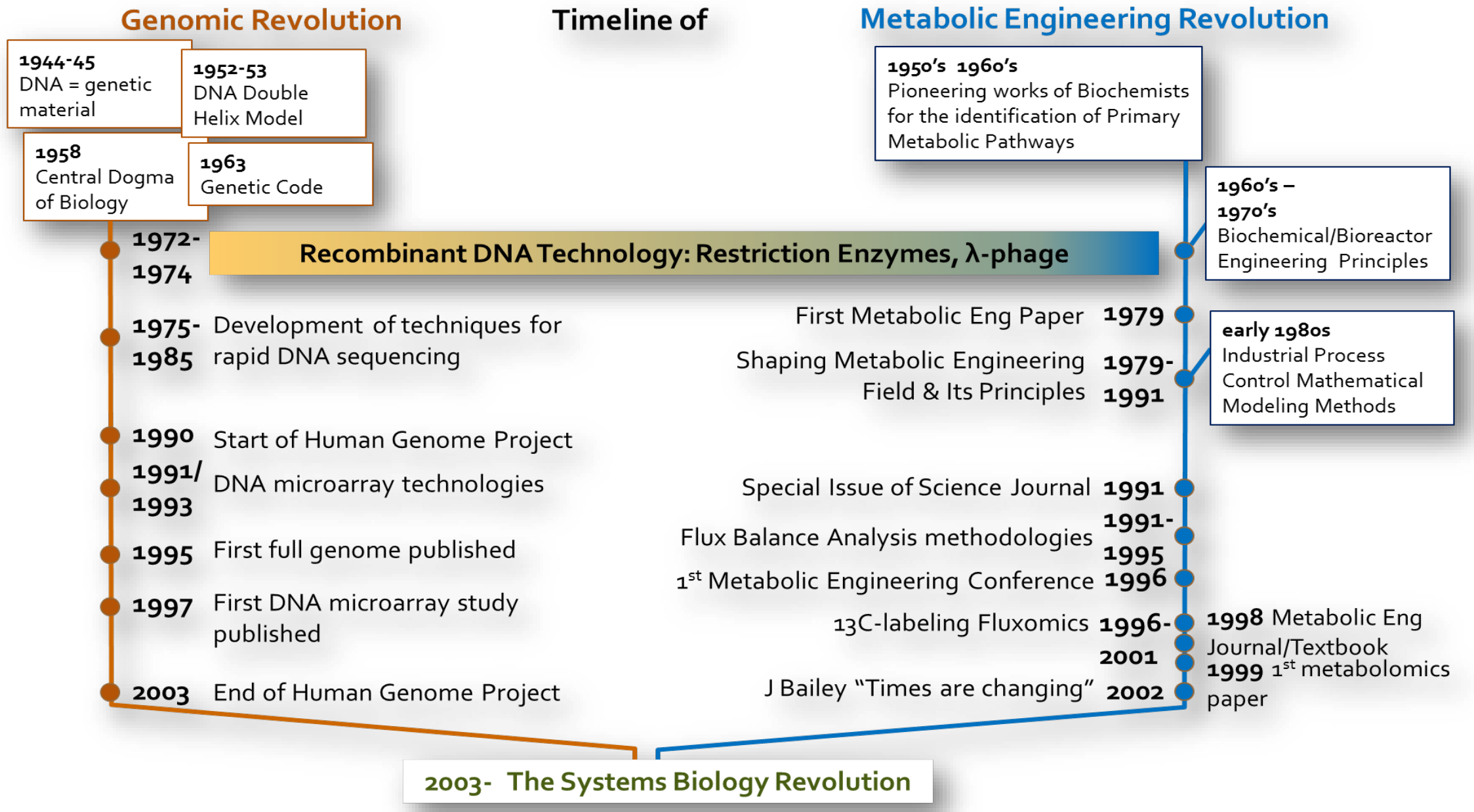
Although some of the experimental and mathematical tools required for rational metabolic engineering are available, **complex cellular responses to genetic perturbations can complicate predictive design**.

Is the knowledge of the metabolic network structure sufficient to propose its restructuring towards an increased production of D?



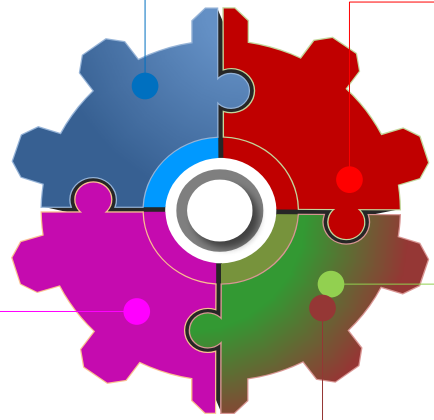
Holistic Metabolic Network Analysis
Metabolic Pathway Rate/Flux Analysis (MFA)
Process Dynamics
Flexible & Rigid Nodes
Mathematical Modeling of Biological Systems

Genomic Revolution & Metabolic Network Engineering merged in the era of Systems Biology → Network & Precision Medicine



What have we accomplished so far with Metabolic Network/Flux Analysis?

Profiling Diseases → Identifying combined biomarkers → Developing tools for early & accurate diagnosis



Identifying underlying mechanisms of disease including extrapolation from cell & animal models too

Identification of Drug Targets / Evaluation & Prediction of Treatment Outcomes – Survival Analysis – Gene Therapies

Identifying quality control biomarkers contributing to the standardization of tissue engineering & cell therapy processes

In development of Exposome – Lifestyle – Wellness // Nutrition & Toxicology Models

Challenges in/for Metabolic Network/Flux Analysis in the context of Virtual Human

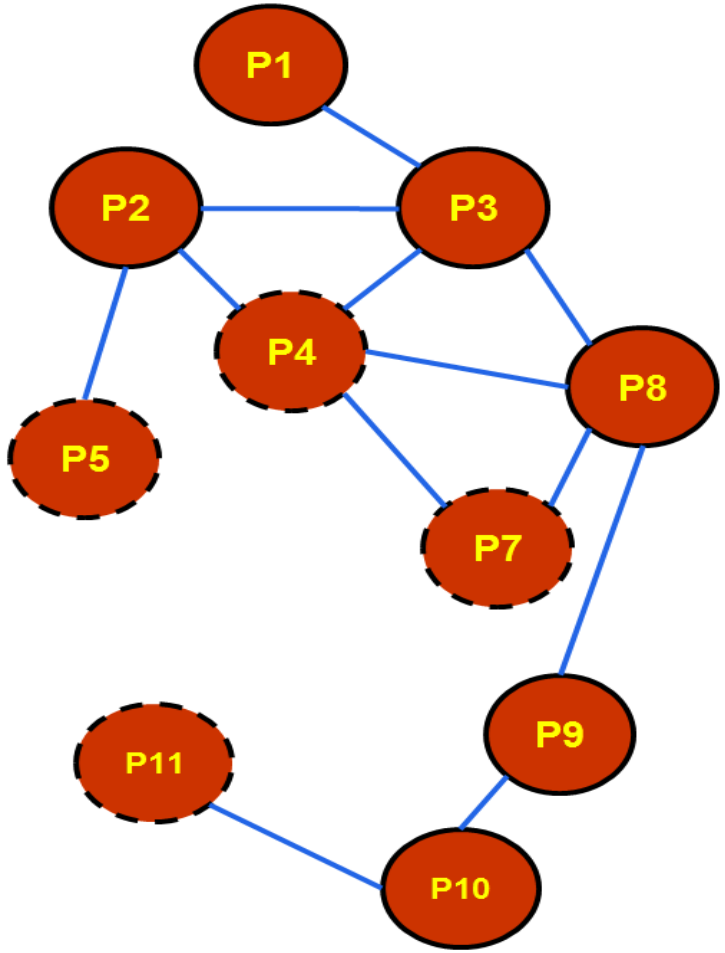
- **Limited metabolomic data (so far) & difficulty in collecting *in vivo* samples for certain phenotypes & tissues // lack of stratification between ancestries, sexes, age, lifestyle**
- Limitations in the harrmonization of sample collection & handling, and the metabolomic data acquisition protocols

- **Need for Standardized repositories for Metabolomic/Isotopomic Data**
  robust international effort so far; additional efforts needed for mandatory presentation & deposition of metabolomic data of published studies based on ***accepted standards***

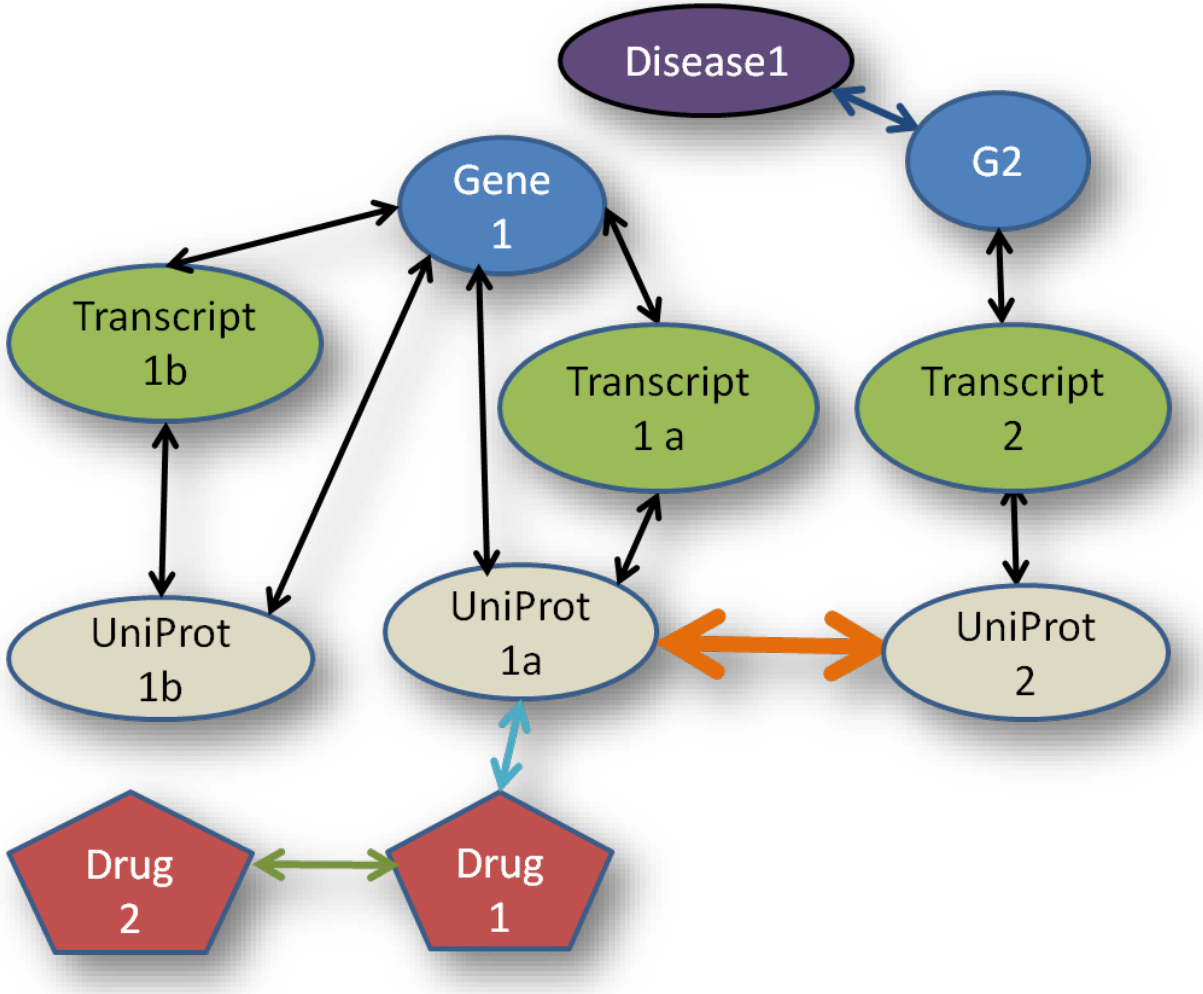
- **Lack of:**
  - ***standardized genome-wide & tissue-specific human metabolic models***
  - ***extensive in vivo kinetics data***
  - ***standardized metabolic flux analysis (fluxomic) workflows***
  - ***interoperability between metabolic databases & metabolic network analysis software tools (fragmented landscape)***

  → *Need to combine forces at the international level*

Protein Networks
provide a valid representation of molecular (patho)physiology enabling the investigation of a disease through graph theory

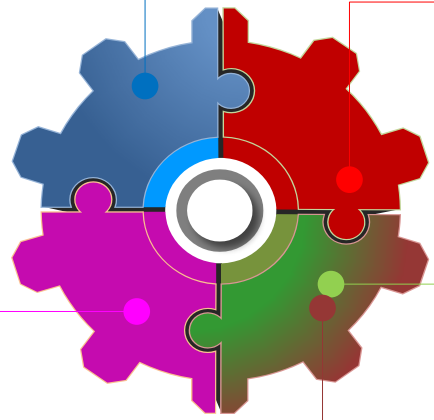


Protein Networks
can be used to integrate genomic/transcriptomic/proteomic data & investigate drug effect and/or repurposing through disease commorbidities



What have we accomplished so far with Protein Network Analysis?

Reconstructed the protein network of diseases based on genetic and phenotypic data



Furthered our understanding of pathophysiologies through connectivity & topology analysis

Suggested new genes/proteins associated with specific diseases ("guilt by association")

Drug repurposing through identification of commorbidities

Prioritized pathways and genes/proteins through the integration of network analysis with functional data

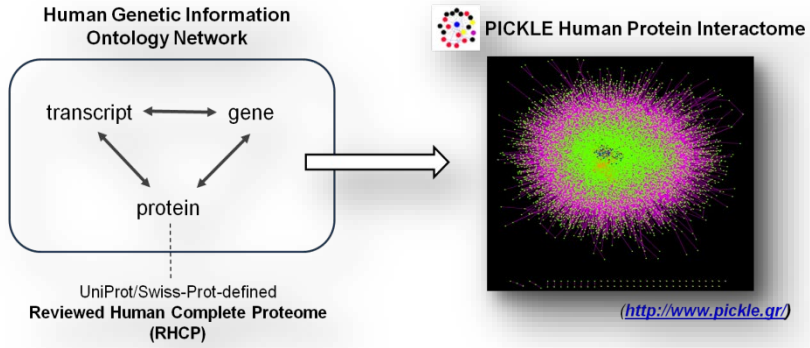
Challenges in/for Protein Network Analysis/Modeling in the context of Virtual Human

- **Inherent errors in the protein network reconstruction due to the nature of the available experimental methods**

- **Protein-protein interaction information is stored in primary databases at different levels of the genetic information flow (gene, RNA, UniProt/protein)**

- **Need for standardized meta-databases that appropriately integrate the primary datasets & evaluate protein-protein interaction reliability**

Dimitrakopoulos *et al*., *Biomolecule*s (2022), *Bioinformatics* (2021)
Gioutlakis *et al*.,*PLoS ONE* (2017) Klapa *et al*., BMC Syst. Biol. (2013)



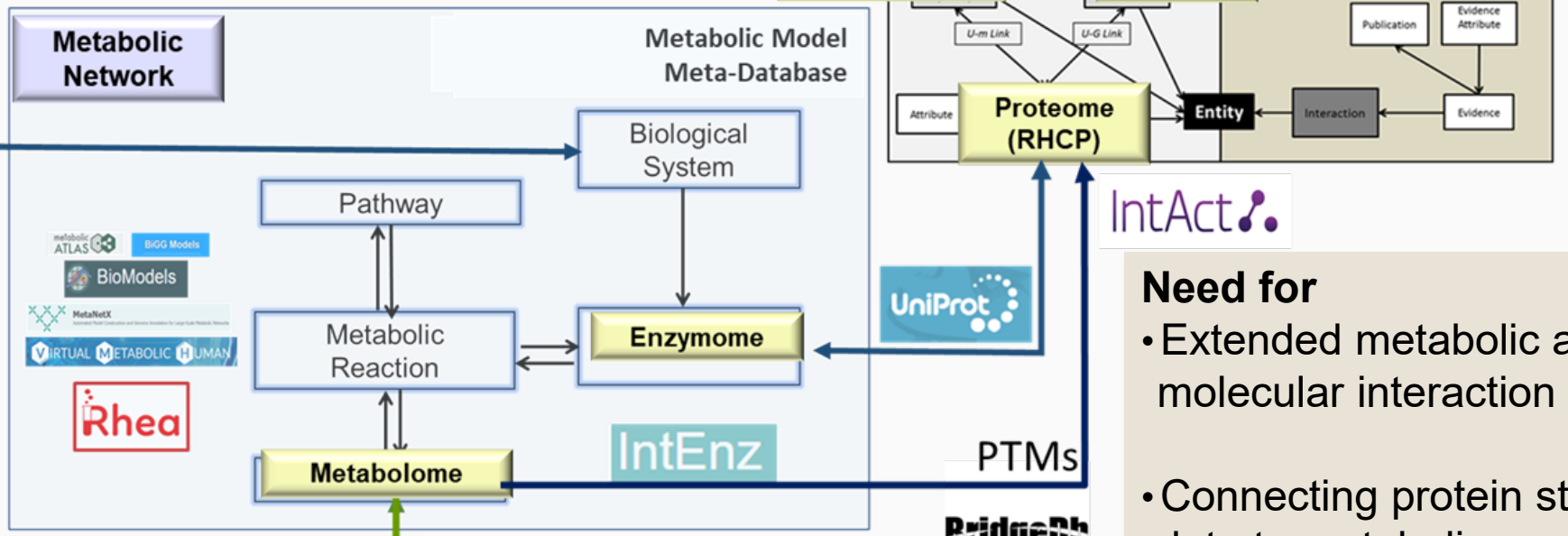
- **Lack of physical meaning of most of the experimentally identified protein-protein interactions → need for their connection with other biological data**

- **Have we identified the complete human interactome?**

Dimitrakopoulos G, Klapa MK, Moschonas NK. Biomolecules 12:140 (2022)

- **There has not (yet?) been incorporated any "flux" in the protein-protein interactions**

Towards the Development of a Framework for Multi-omic Data Analysis:
Metabolic Network as a focal part



MetaboLights

https://www.ebi.ac.uk/metabolights/

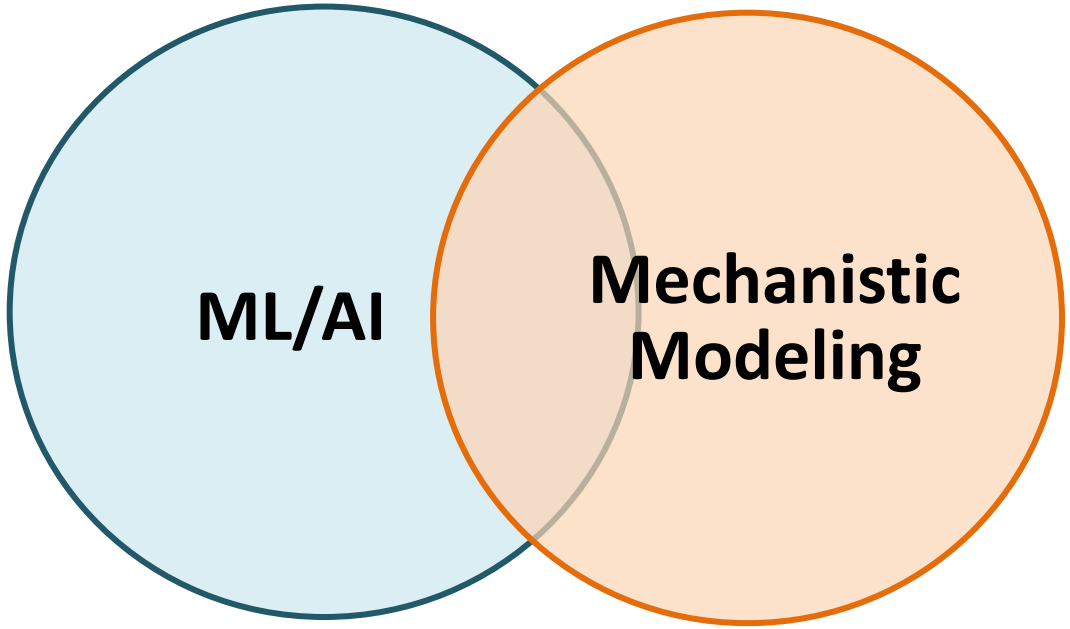
Need for

- Extended metabolic and molecular interaction databases
- Connecting protein structure data to metabolic models
- Standardized / Connected IDs
- Standardized workflows from metabolome to genome & *vice versa*

The vision: How do we incorporate the biomolecular networks into the Virtual Human?

Biomolecules + Networks + Dynamics (Time)

Medical + Multi-omic Data → Networks → Mechanistic Insights → Models →
Accurate Diagnosis → Prediction of Outcome → Digital Twins → Virtual Patients →
Precision & Personalized Treatment



Integration/Interplay of AI/ML and Mechanistic Modeling

Thank you!

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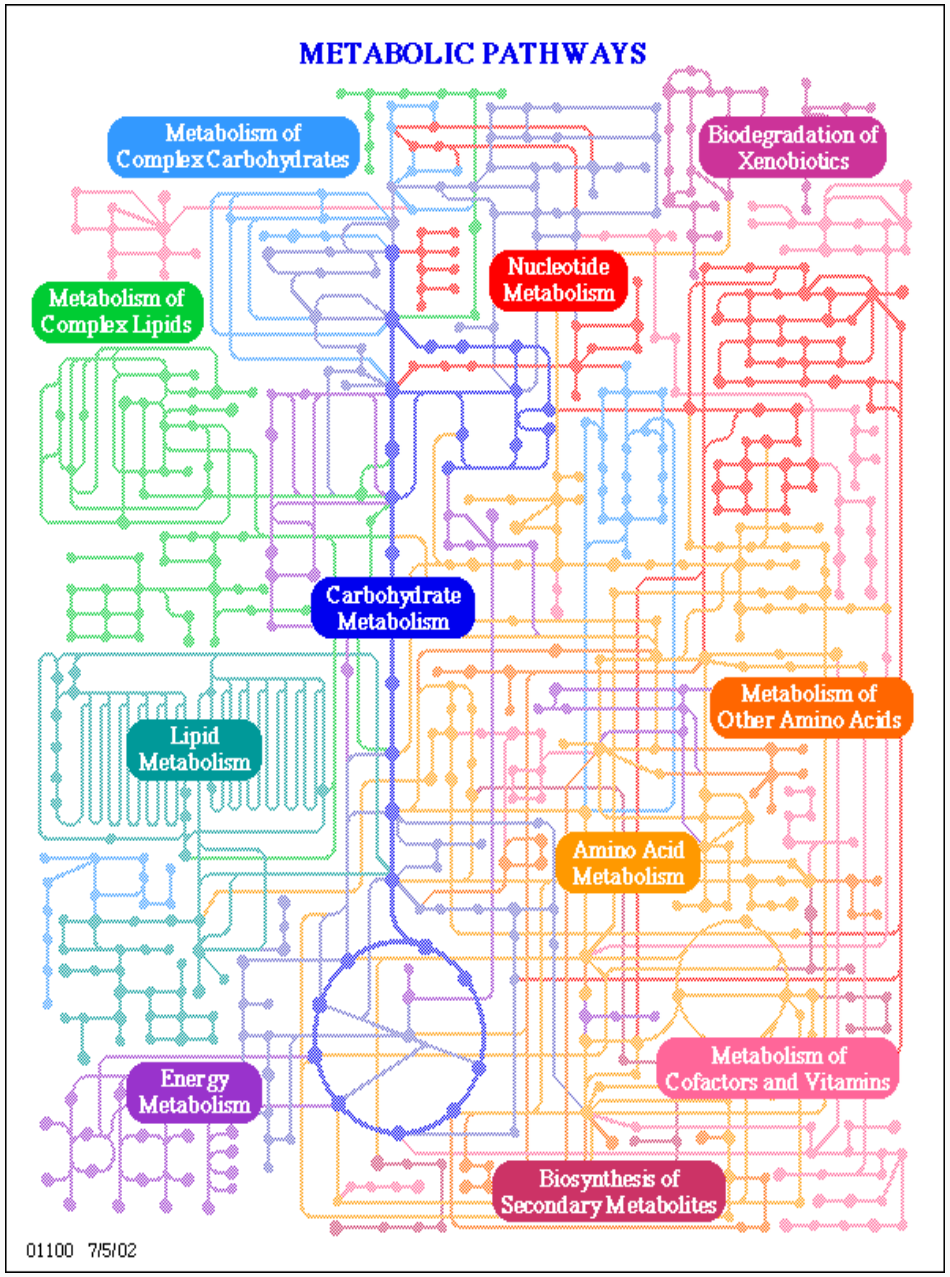
Metabolic Reaction Pathways…



… are sequences of *enzyme catalyzed* biochemical reactions,

converting substrates to a variety of products to meet the needs of the cells, including the formation of macromolecules and the cellular products/signaling molecules and the production of energy for all biological processes.

- Metabolic pathways can interact to create complex metabolic networks
- **Metabolites** are
  (i) the nodes of metabolic networks
  (ii) **regulatory molecules of proteins**

Metabolic Networks are complex but with a well-defined structure

- Metabolic reaction pathways are *stoichiometrically balanced*, their dynamics governed by the mass and energy conservation laws.
- If we know the enzyme we know the reaction that it catalyzes and *vice versa*
- Only 6 main enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases) + translocases (August 2018) □ only 6 types of chemical transformations
- Metabolism is a decentralized biological process

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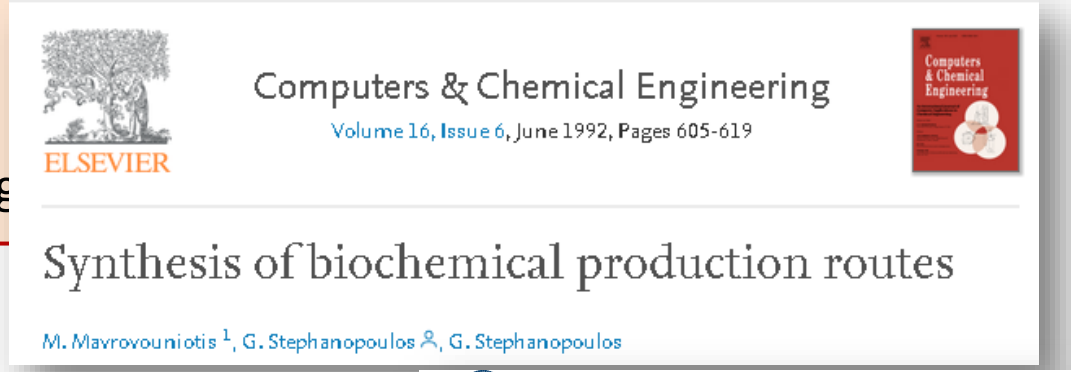


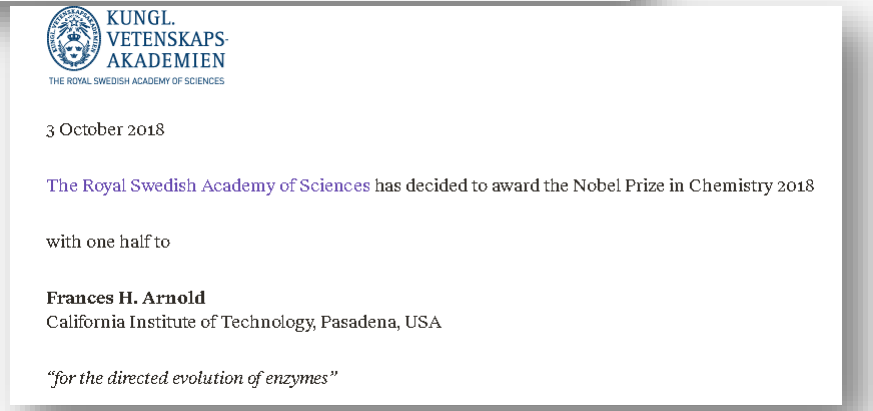
ELSEVIER

Computers & Chemical Engineering

Volume 16, Issue 6, June 1992, Pages 605-619

Synthesis of biochemical production routes

M. Mavrovouniotis [1], G. Stephanopoulos, G. Stephanopoulos



KUNGL. VETENSKAPS-AKADEMIEN

THE ROYAL SWEDISH ACADEMY OF SCIENCES

3 October 2018

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2018

with one half to

Frances H. Arnold
California Institute of Technology, Pasadena, USA

"for the directed evolution of enzymes"