



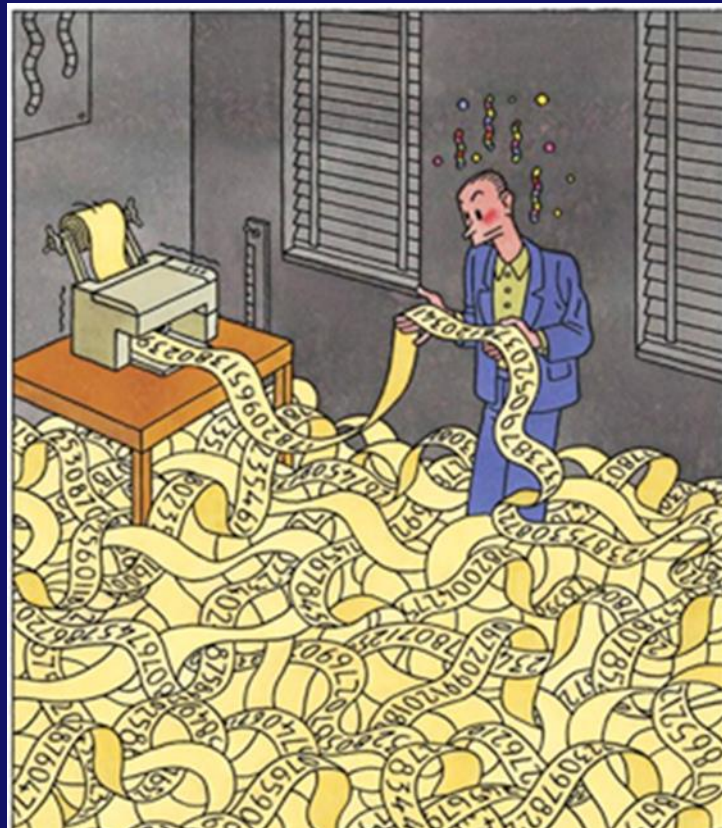
LEIDEN UNIVERSITY MEDICAL CENTER

Elementary Flux Mode analysis: a novel mathematical approach for metabolic pathway analysis to gain insight in gene-metabolite interactions

Ko Willems van Dijk



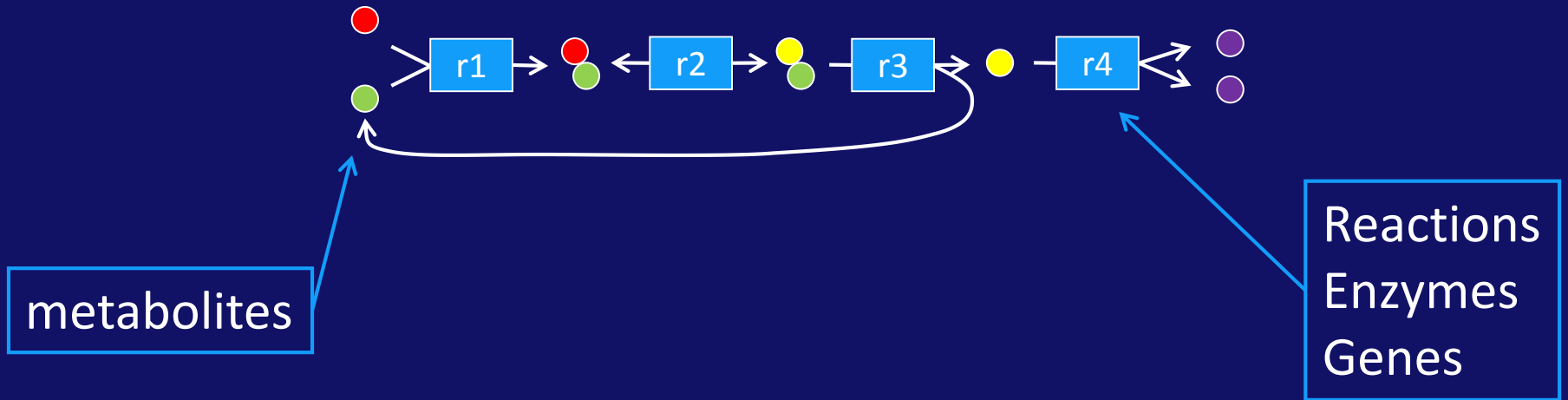
Link SNPs to genes and metabolites to genes/pathways



Pathway analyses

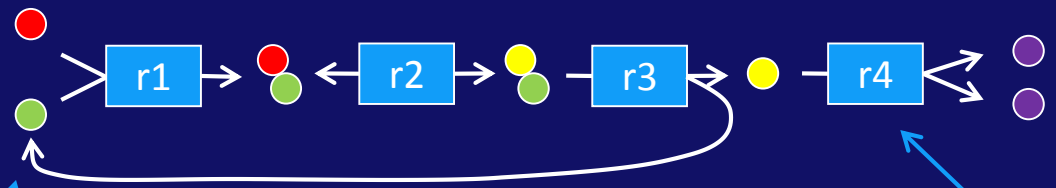
- Traditional pathway analyses:
 using KEGG, BioCyc
- Statistical pathway analysis:
 Gaussian Graphical Modeling (GGM)
- Mathematical pathway analysis:
 Using Genome Scale Models (GSM)

Mathematical pathway analysis



- Convert metabolic pathway to stoichiometric matrix

Mathematical pathway analysis



metabolites

$$\begin{matrix}
 \text{red} \\
 \text{green} \\
 \text{red} \\
 \text{green} \\
 \text{yellow} \\
 \text{yellow} \\
 \text{purple}
 \end{matrix}
 \begin{bmatrix}
 -1 & 0 & 0 & 0 & 0 \\
 -1 & 0 & 1 & 0 & 0 \\
 1 & -1 & 0 & 0 & 0 \\
 0 & 1 & -1 & 0 & 0 \\
 0 & 0 & 1 & -1 & 0 \\
 0 & 0 & 0 & 0 & 2
 \end{bmatrix}
 = \mathbf{S}$$

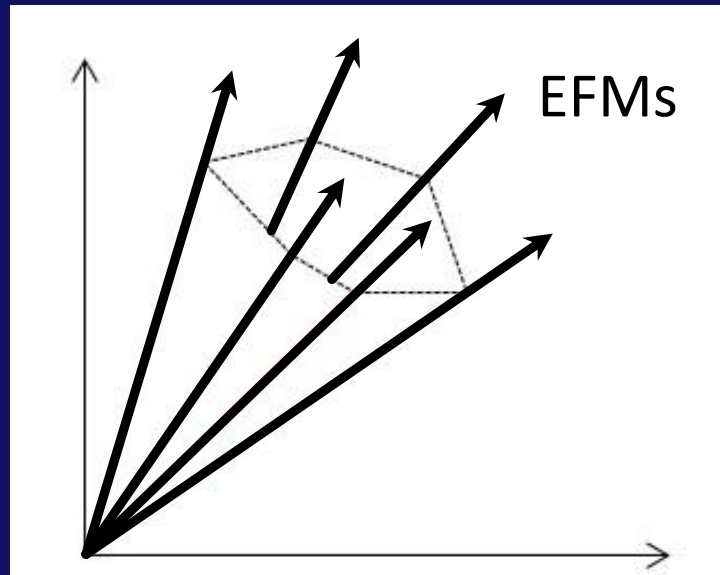
Reactions
Enzymes
Genes

stoichiometric matrix

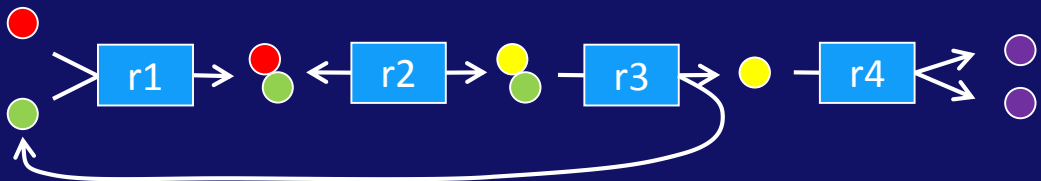
Mathematical formalization

Formally, EFMs correspond to the extreme rays of the polyhedral cone P that results from constraining the right null space of the stoichiometric matrix S to the positive orthant

$$P(S) = \left\{ \mathbf{x} \in R^n \mid S \cdot \mathbf{x} = \mathbf{0} \text{ and } \mathbf{x} \geq \mathbf{0} \right\}$$



Mathematical pathway analysis



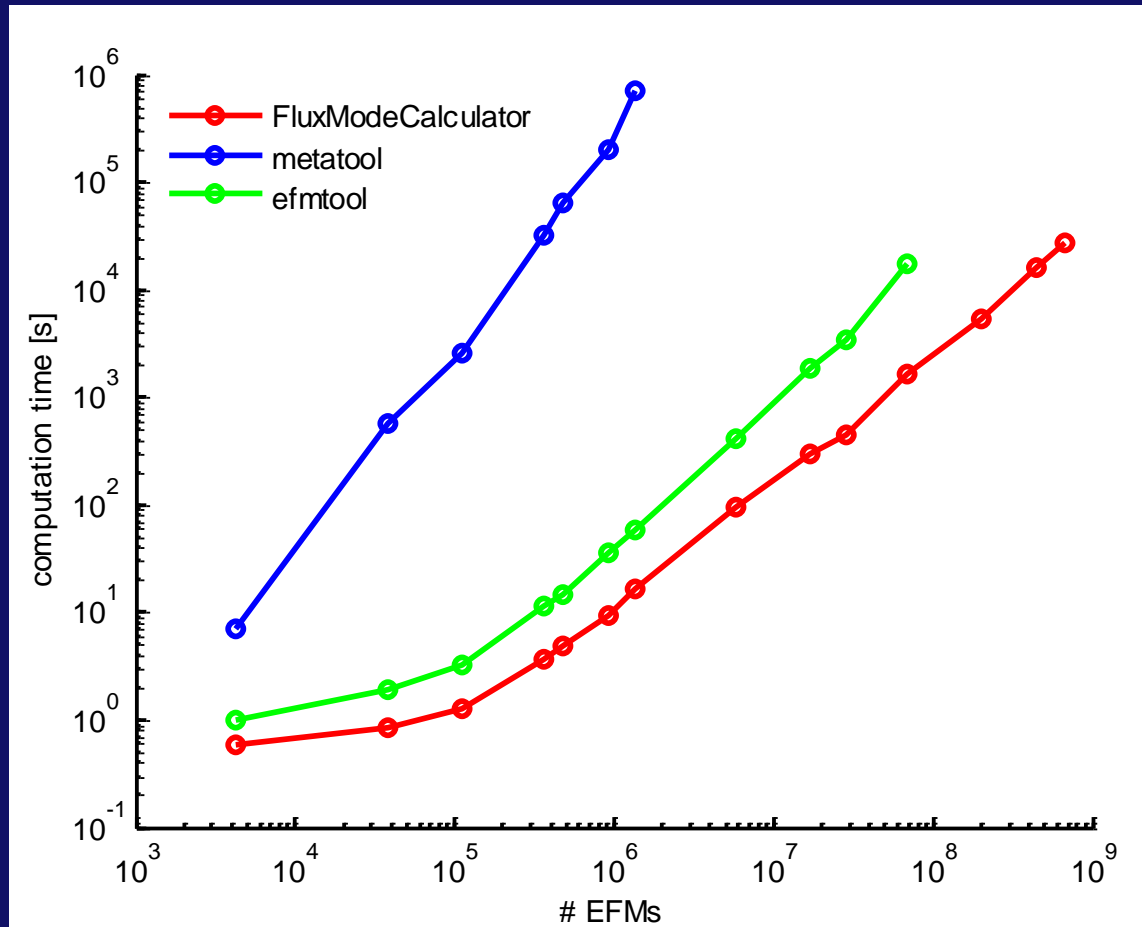
$$\begin{matrix}
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 \begin{bmatrix}
 -1 & 0 & 0 & 0 & 0 \\
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 \end{bmatrix}
 = \mathbf{S}$$

$$\begin{matrix}
 \bullet \\
 \bullet \\
 \bullet \\
 \bullet \\
 \bullet
 \end{matrix}
 \begin{bmatrix}
 -1 \\
 0 \\
 0 \\
 0 \\
 0 \\
 2
 \end{bmatrix}$$

EFM based pathway analysis

1. Start with Genome Scale Metabolic Model, i.e. **HepatoNet1** (Gille et al, Mol Sys Biol, 2010)
 - all reactions are mass balanced (2539 reactions, 1298 metabolites)
 - model is functionally validated
2. Calculate EFMs
 - apply FluxModeCalculator
3. Interrogate EFM database

Performance comparison of *FluxModeCalculator*, *metatool* and *efmtool* on a set of random stoichiometric matrices



Intel Core i3
16 GB RAM

Analyze flexibility of cholesterol synthesis from acetyl-CoA

total: 20332 EFMs

	# routes	# reac	Essential genes
3 acetyl-CoA(m) -> acetoacetyl-CoA(c) + acetyl-CoA(c)	5083	283	
6 acetoacetyl-CoA(c) + acetyl-CoA(c) -> zymosterol(r)	1	27	<i>HMGCS1/2 & HMGCR & MVK & PMVK & MVD & FDPS & GGPS1 & FDFT1 & SQLE & LSS & CYP51A1 & TM7SF2 & FAXDC2 & MSMO1 & NSDHL & HSD17B7</i>
zymosterol(r) -> cholesterol(r)	4	9	<i>DHCR7 & DHCR24 & SC5D & EBP</i>

- EFM analysis is a powerful tool for investigating the flexibility/redundancy of metabolic pathways
- *FluxModeCalculator* enables large-scale EFM enumeration on ordinary desktop computers
 - more time and memory efficient than existing tools
 - scales well to large models
- EFM analysis on metabolite GWAs results are ongoing

A beta version of *FluxModeCalculator* for Windows
can be downloaded from

<https://www.lumc.nl/org/bioinformatica/medewerkers/903200516582525>

Acknowledgements:

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Aaron Isaacs

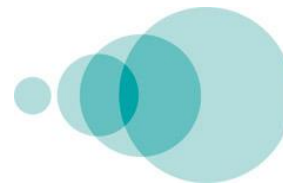
Cornelia van Duijn

Peter-Bram 't Hoen



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