Modeling the genetics and metabolism of heart failure

A multi-omics story in two parts

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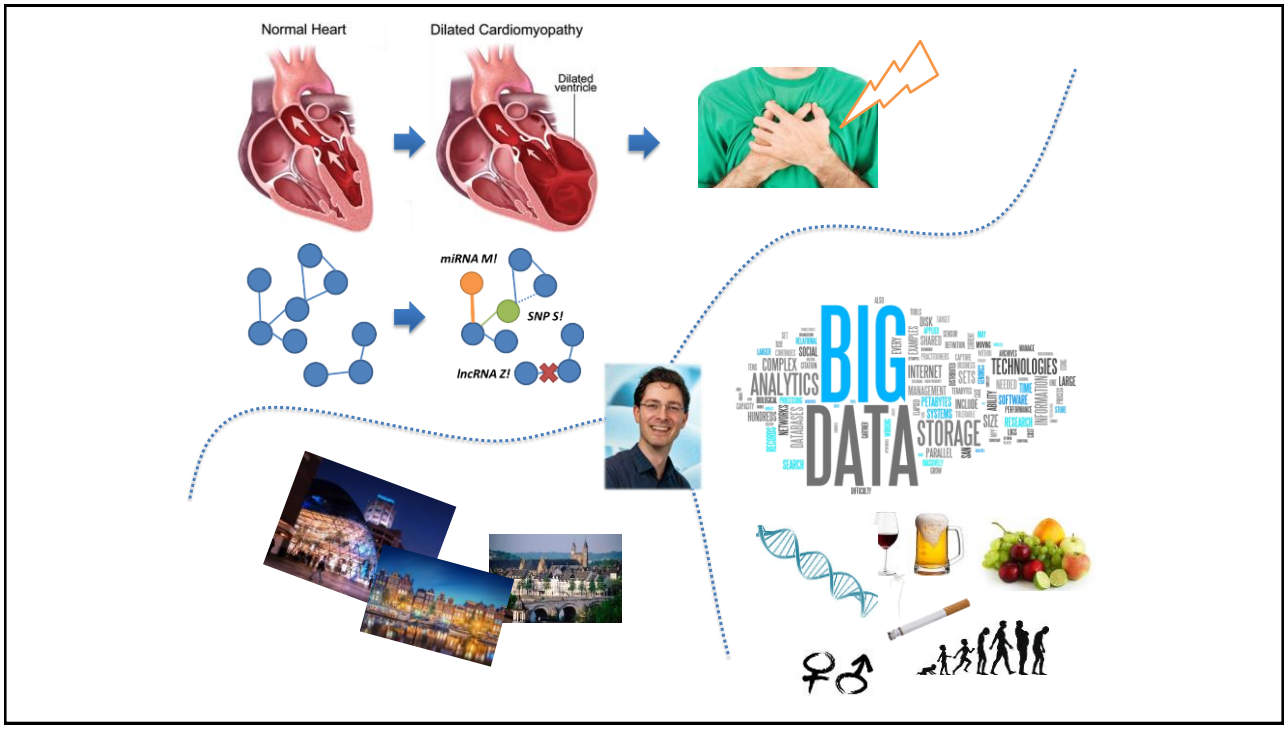

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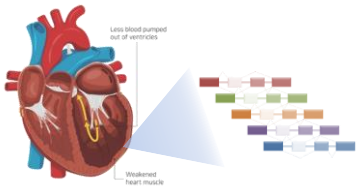
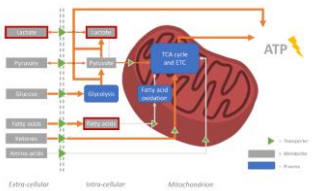
Outline

1. Genetic control of gene transcription in heart failure



2. Modeling the metabolism of the failing heart



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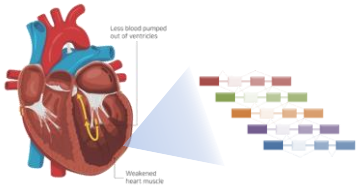

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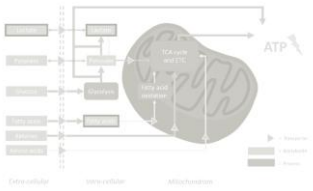

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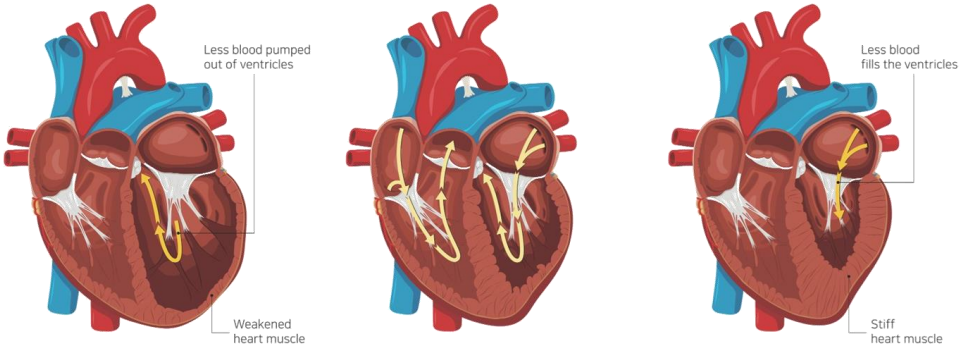
What is heart failure?



Systolic Dysfunction | Normal | Diastolic Dysfunction

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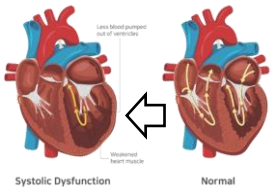
Heart failure ↔ complications

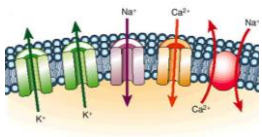
Structural remodeling



Heart Failure

Arrhythmias
Sudden cardiac death
Metabolic impairments

Electrical remodeling



- *Complications are patient specific*
- *What explains this difference?*

Genetics?

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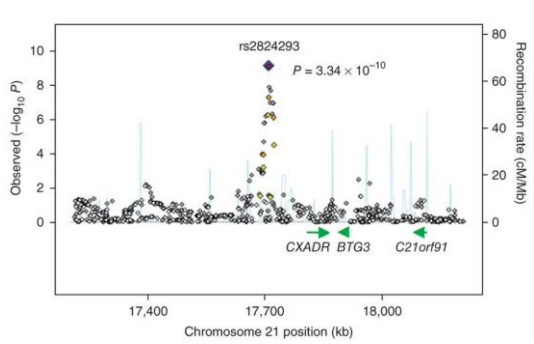
Genome-wide association studies

- **GWAS** have identified many genetic variants associated with complex traits and diseases
  - *Example below: susceptibility to arrhythmias after MI*



Genes?
Mechanism?

Bezzina, Pazoki, et al., Nat Gen (2010)

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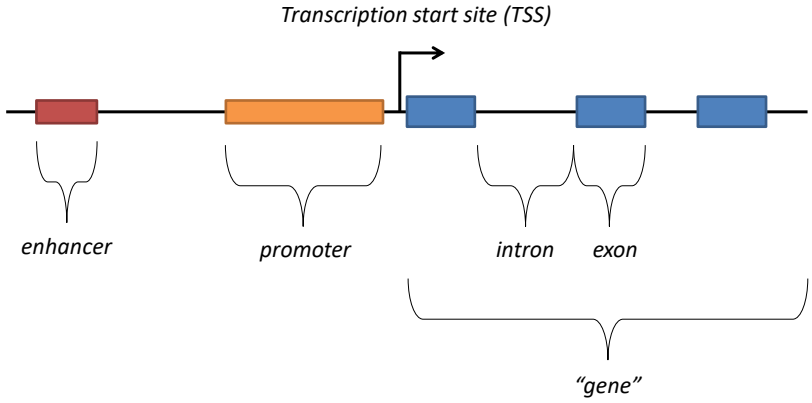
Genetic control of gene transcription



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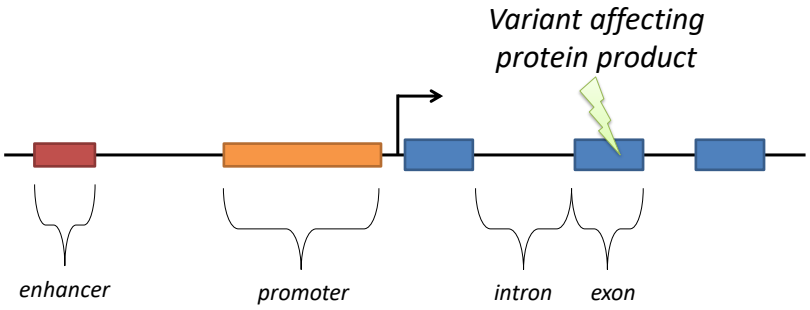
Genetic control of gene transcription



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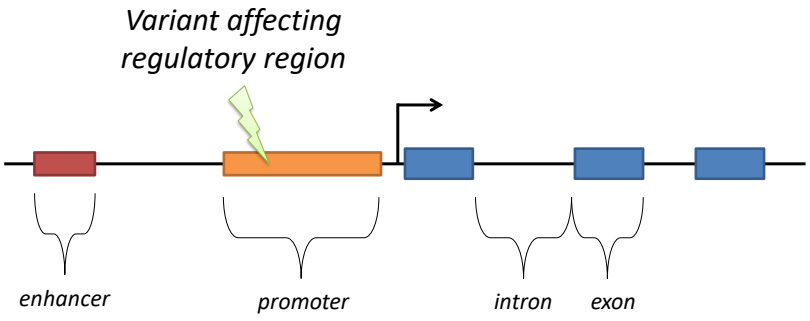
Genetic control of gene transcription



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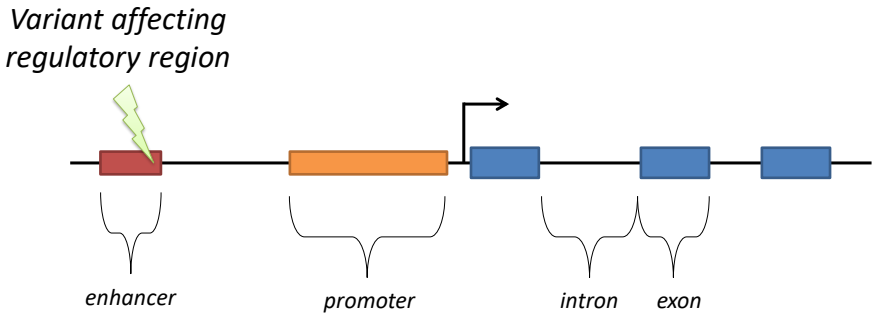
Genetic control of gene transcription



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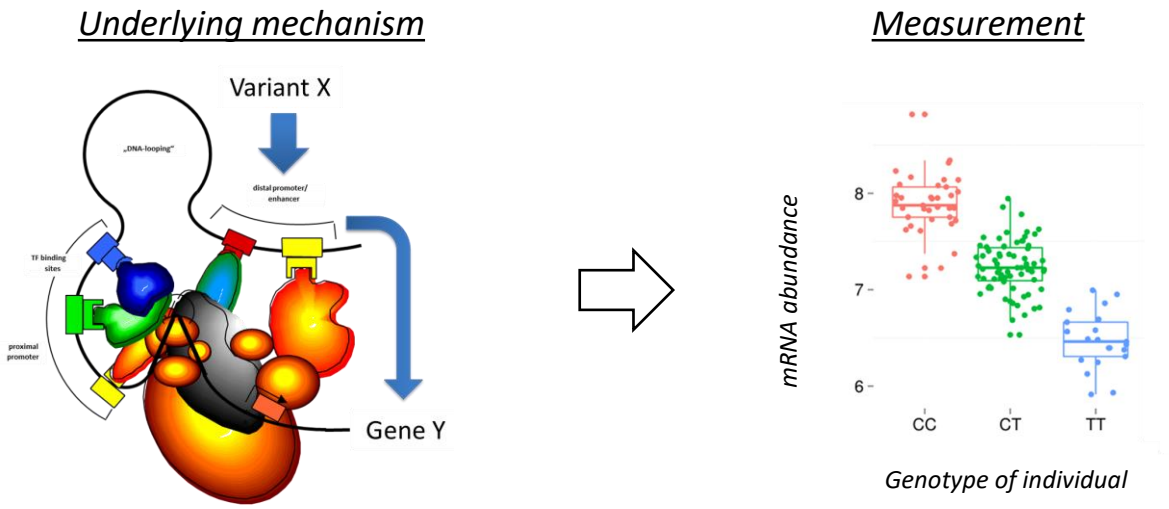
Genetic variant modulating expression levels



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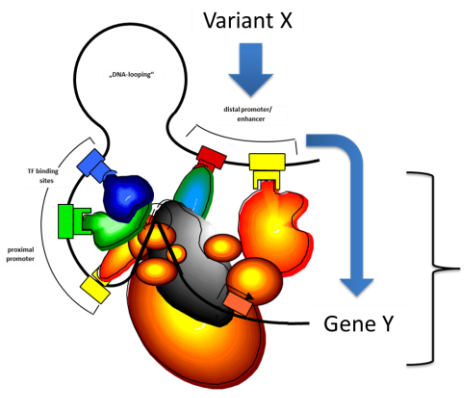
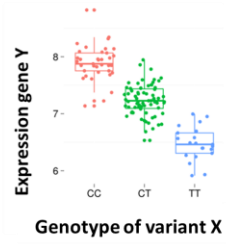
Genetic variant modulating expression levels





Expression quantitative trait locus
(eQTL)
=
in silico association between genotype and gene expression level within a specific population

- Method: Linear regression (*GenABEL, MATRIXEQTL* R)
- *cis* (= local) effects focused (sample size)

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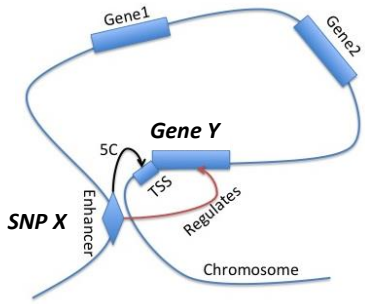
What are *cis* & *trans* eQTLs

- *trans* eQTL: **SNP X** with **Gene Y**
  - **SNP X** not within 1 megabase of **Gene Y**
  - **SNP X** and **Gene Y** on different chromosomes
- Distant interactions
  - **A.** **SNP X** could be in a distant regulatory element (interactions between chromosomes)
  - **B.** **SNP X** linked to a transcription factor
- Expect small effect sizes → power issues in all but the largest studies

A



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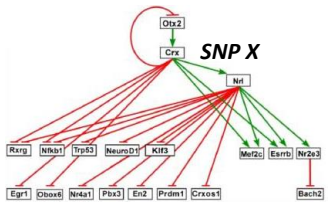

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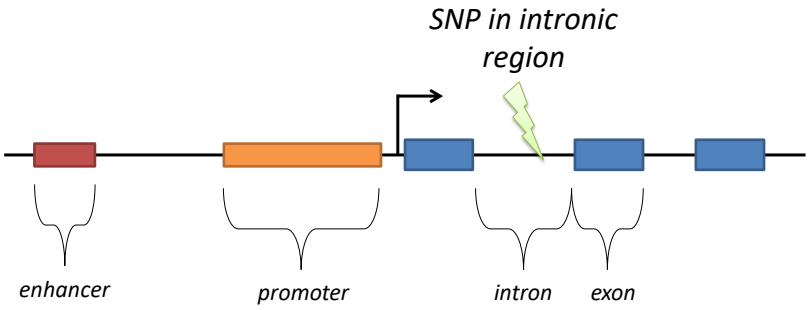
Genetic control of gene transcription



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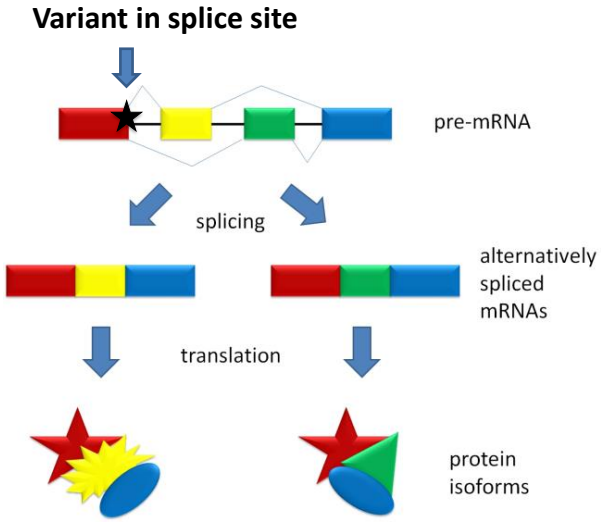
Genetic variants regulate exon usage



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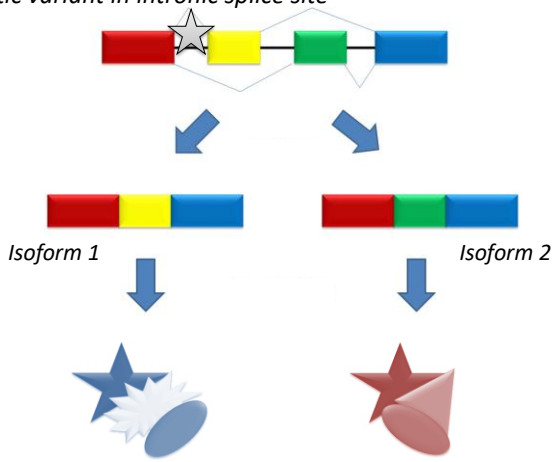
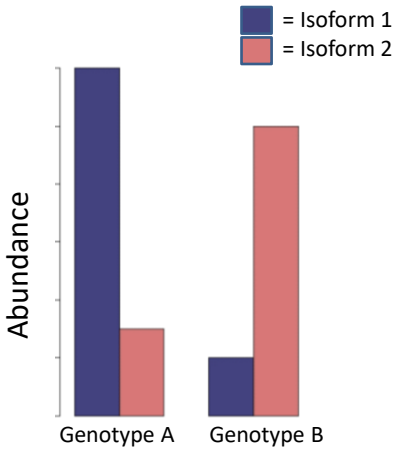
Genetic variants regulate exon usage

<u>*Underlying mechanism*</u>

Genetic variant in intronic splice site



<u>*Measurement*</u>



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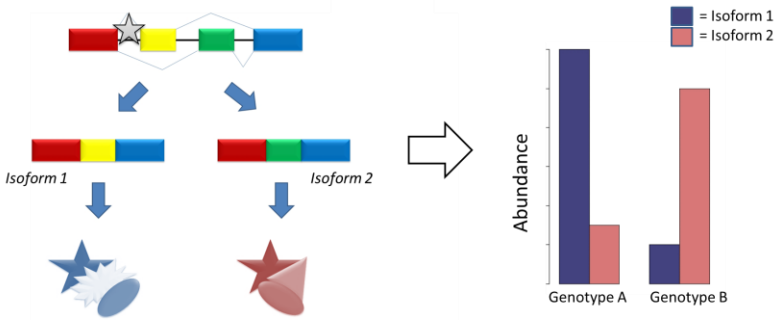

Splicing quantitative trait locus
(sQTL)
=
in silico association between genotype and alternative splicing <u>within a specific population</u>

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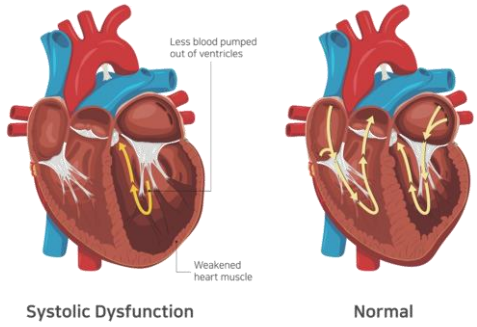
Research: genetics of transcription and splicing in DCM

Samples: Left ventricle
- **108** non-diseased donor hearts
- **97** dilated cardiomyopathy (DCM) hearts

Data:
- RNA-seq: 16,219 unique mRNA levels
- Genotyping: 2 million common variants (SNPs)



Adriaens, Koopmann et al. (2014)

Heinig, Adriaens, Schaefer et al. (2017)

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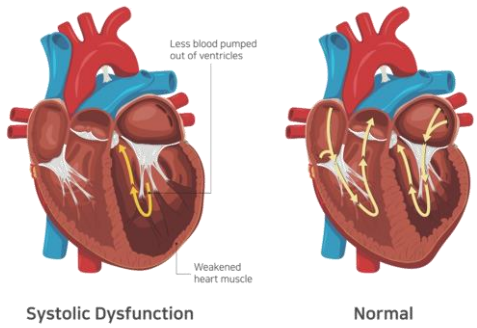
Research: genetics of transcription and splicing in DCM

Samples: Left ventricle
- **108** non-diseased donor hearts
- **97** dilated cardiomyopathy (DCM) hearts

Data:
- RNA-seq: 16,219 unique mRNA levels
- Genotyping: 2 million common variants (SNPs)

Research questions:
- **Which variants modulate gene expression? (eQTL)**
- **Which variants modulate splicing? (sQTL)**
- **Do these differ between DCM and controls?**



Adriaens, Koopmann et al. (2014)

Heinig, Adriaens, Schaefer et al. (2017)

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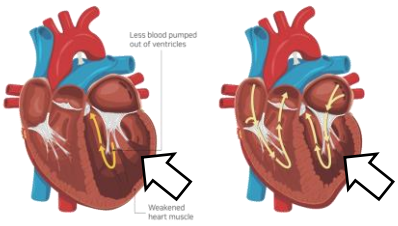
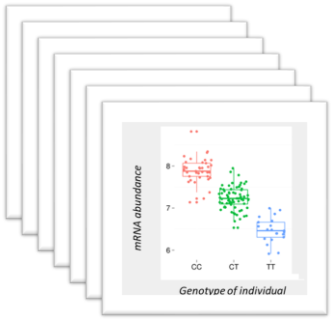
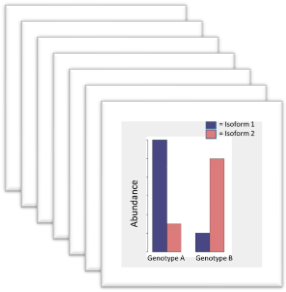
Research: regulatory genomics of dilated cardiomyopathy



Transcriptomics

Genomics





Conclusions:

- *Splicing and transcription is different in DCM*
- *Some of these differences are in-born*

Heinig, Adriaens, Schaefer et al. (2017)

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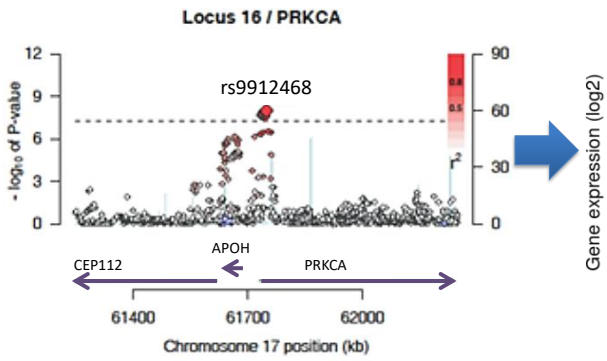
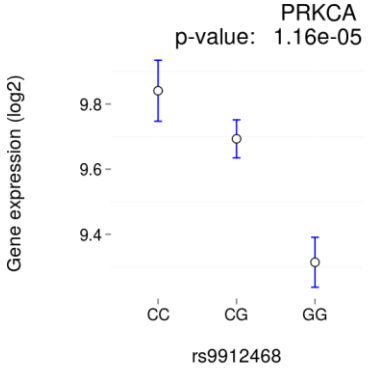
Usage example: eQTLs for known GWAS loci





GWAS result from Sotoodehnia et al. (2010)

- ***rs9912468***: associated with QRS prolongation (effect allele = G)
- **Protein kinase C alpha**: regulator of cardiac contractility and Ca^{2+} handling in myocytes

Adriaens, Koopmann et al. (2014)

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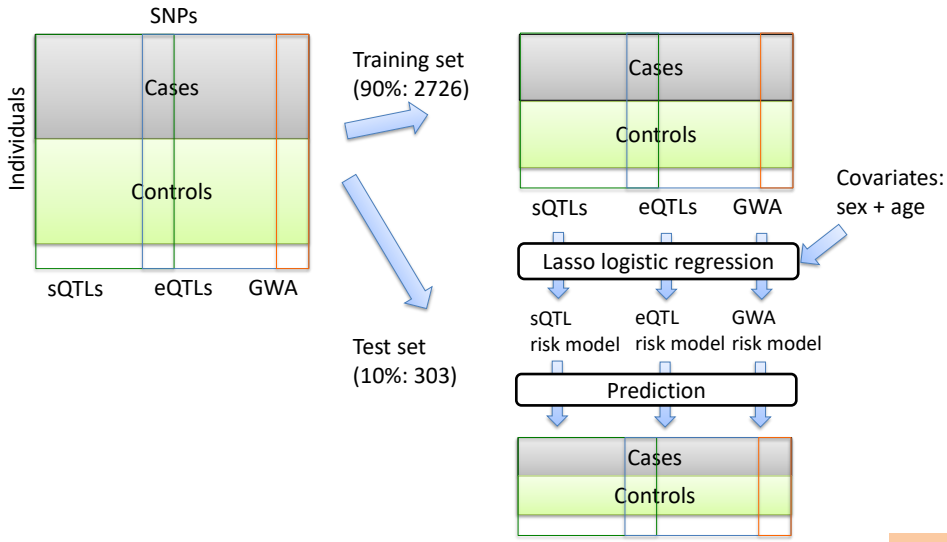
Usage example: genetic risk prediction



GWAS data from: Meder et al. Eur Heart J 2014

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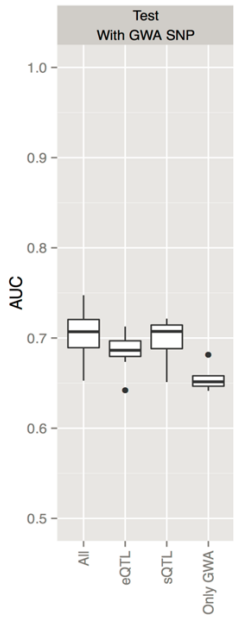
Usage example: genetic risk prediction

Better prediction

Random prediction



Combining

1. Co-variates (age, sex)
2. Genotype of DCM GWA SNP (rs9262636)
3. Genotypes of SNPs modulating expression (eQTLs)
4. Genotypes of SNPs modulating splicing (sQTLs)

All variants taken together predict DCM status better

DCM: unfortunate combination of small complex genetic effects

Heinig, Adriaens, Schaefer et al. (2017)

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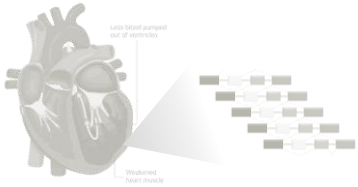
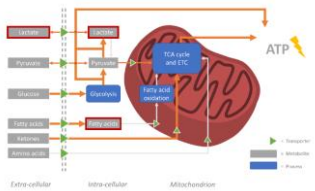


2. Modeling the metabolism of the failing heart



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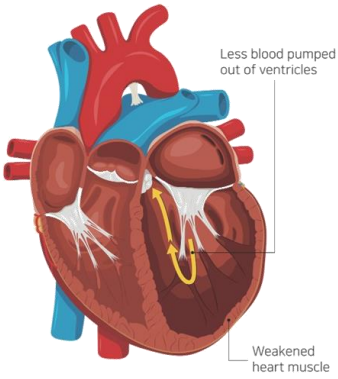
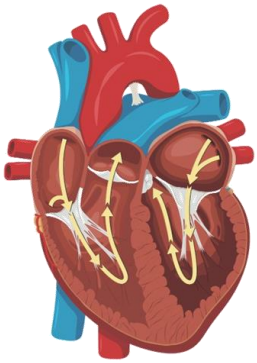
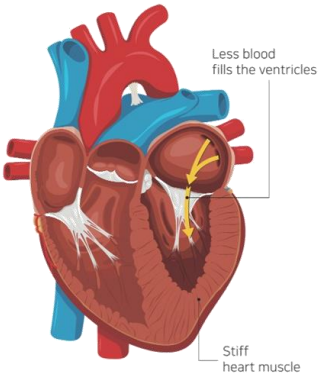
Recap: what is heart failure?



Systolic Dysfunction



Normal



Diastolic Dysfunction

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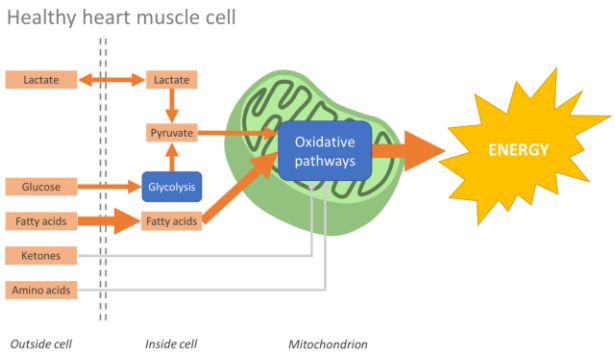
Loss of metabolic flexibility in DCM



Healthy heart

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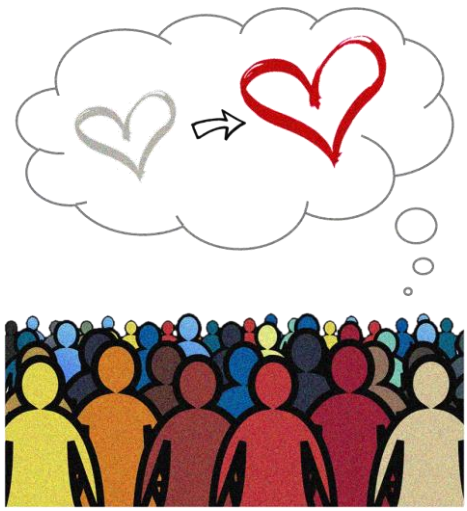
Restoring metabolic flexibility?

- Clinical trials aimed at restoring metabolic flexibility have so far led to mixed results
- Patient-to-patient differences are currently poorly understood

→ Targeted metabolic therapies have therefore not seen clinical implementation yet



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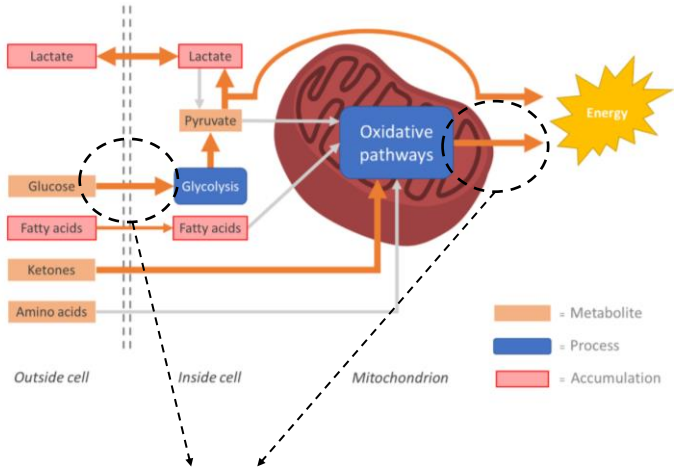
Diagnosing loss of metabolic flexibility

- To diagnose, we need to determine **metabolic fluxes**
  - **Fluxomics: reaction fluxes of all known metabolic reactions**
  - Identify which pathways differ between patients
- Ideally: *in vivo* tracer studies to measure metabolic fluxes:
  - Problem 1: expensive and low sensitivity
  - Problem 2: some impairments only appear under stress



How to quantify? How to place in context?

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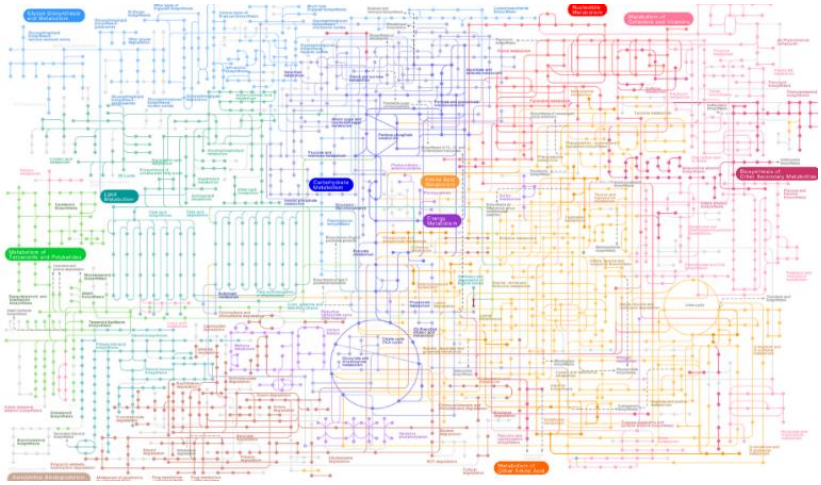
Genome-scale metabolic model (GEM)

- Contains all know metabolic reactions including:
  - Transport reactions
  - Enzymatic reactions
- Derived from existing knowledge:
  - Pathway databases
  - Literature
- Creating and curating such a network is a lot of work:
  - Only a few dedicated groups world-wide



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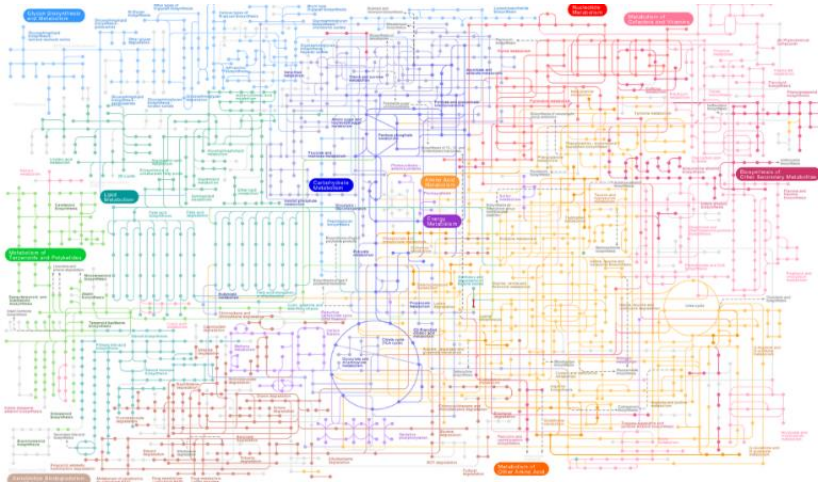
Genome-scale metabolic model (GEM)

- Metabolites
- Reactions
- Stoichiometry
- Directionality of reactions (thermodynamic considerations)
- Enzymes (catalysing a reaction)
- Gene-Protein-Reaction rules
- Mass- and charge-balance of reactions
- Compartmentalisation



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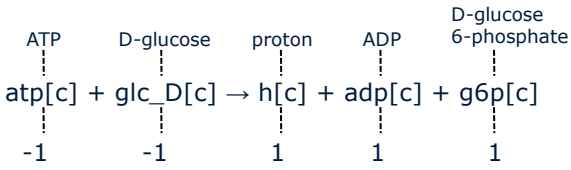
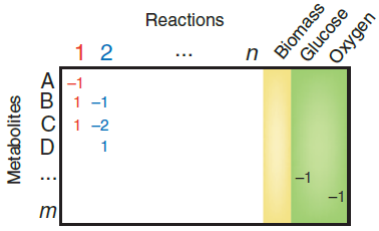
Genome-scale metabolic model (GEM)

- Metabolites
- Reactions
- Stoichiometry

Reaction: HEX1: hexokinase



Stoichiometric matrix S:



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Genome-scale metabolic model (GEM)

Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c]	+ glc_D[c] →	h[c]	+ adp[c]	+ g6p[c]
-1	-1	1	1	1

c = cytoplasm
e = extracellular space
g = Golgi apparatus
l = lysosome
m = mitochondrion
n = nucleus
r = endoplasmic reticulum
x = peroxisome

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Genome-scale metabolic model (GEM)

Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c]	+ glc_D[c] →	h[c]	+ adp[c]	+ g6p[c]
-1	-1	1	1	1

Flux of reaction
- *has upper and lower bound*
- *often expressed in mmol/gDW/s*
- *gDW = gram dry weight*

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Genome-scale metabolic model (GEM)

Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c]	+ glc_D[c]	→ h[c]	+ adp[c]	+ g6p[c]
-1	-1	1	1	1

Positive flux

Flux of reaction

- *has upper and lower bound*
- *often expressed in mmol/gDW/s*
- *gDW = gram dry weight*

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Genome-scale metabolic model (GEM)

Metabolites

Reactions

Stoichiometry

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c]	+ glc_D[c]	→ h[c]	+ adp[c]	+ g6p[c]
-1	-1	1	1	1

Negative flux

Flux of reaction

- *has upper and lower bound*
- *often expressed in mmol/gDW/s*
- *gDW = gram dry weight*

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Genome-scale metabolic model (GEM)

- Metabolites
- Reactions
- Stoichiometry
- Directionality of reactions (thermodynamic considerations)
- Enzymes (catalysing a reaction)
- Gene-Protein-Reaction rules
- Mass- and charge-balance of reactions
- Compartmentalisation

Reaction: HEX1: hexokinase

ATP	D-glucose	proton	ADP	D-glucose 6-phosphate
atp[c] +	glc_D[c] →	h[c] +	adp[c] +	g6p[c]
-1	-1	1	1	1

→ vs. ↔, irreversible vs. reversible

Hexokinase 1, 2, 3, or 4 (glucokinase) catalyze the reaction

(3098) or (3099) or (3101) or (2645)…

Gene number for hexokinase 1

glc_D[e] <=> glc_D[c]

Glucose transport from extracellular space to cytosol

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The aim of a model is context specific

- GEMs are often organism-specific, but not tissue/cell type specific
- Tissue-specific models include only reactions that are active in the respective tissue
- Two "static" omics types can inform this modeling process:
  - Transcriptomics
  - Metabolomics

- *Transcriptomics: reaction is inactive if catalyzing enzyme is not expressed*
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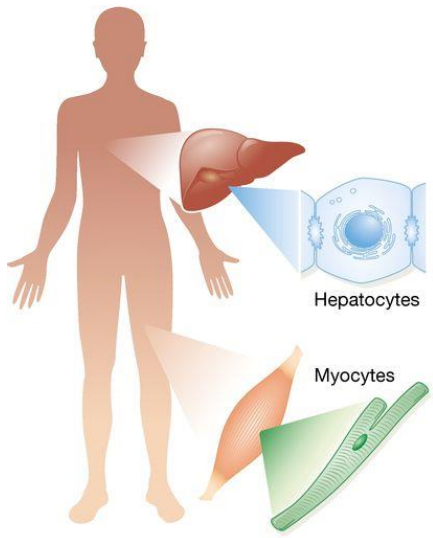

Uhlen, M et al. Mol Sys Bio, 12:862 (2016)

38

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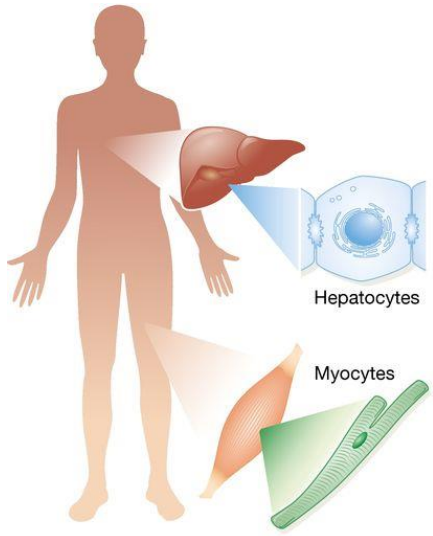

Uhlen, M et al. Mol Sys Bio, 12:862 (2016)

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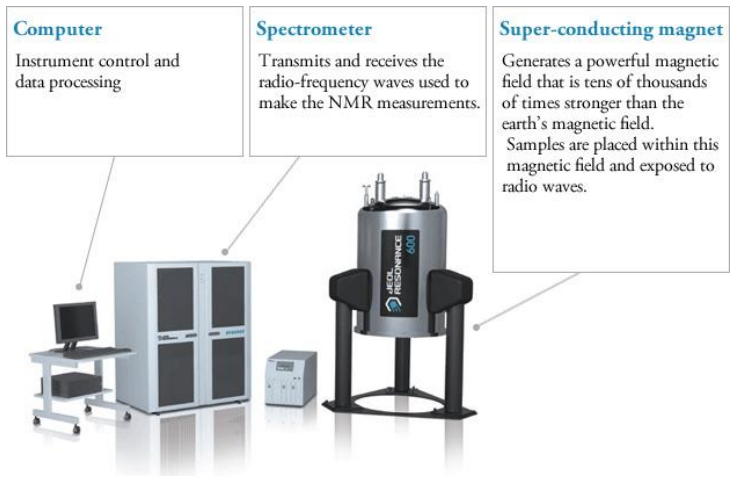
NMR metabolomics

- NMR is an abbreviation for Nuclear Magnetic Resonance:
  - allows the molecular structure of a sample to be analyzed by observing and measuring the interaction of nuclear spins when placed in a powerful magnetic field

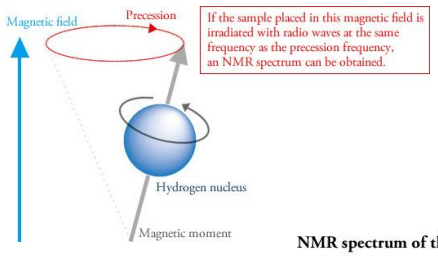


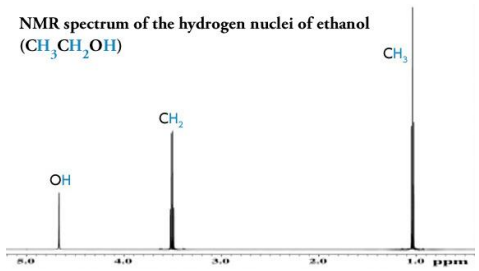
https://www.jeol.co.jp/en/products/nmr/basics.html

40

NMR metabolomics

- NMR is an abbreviation for Nuclear Magnetic Resonance:
  - allows the molecular structure of a sample to be analyzed by observing and measuring the interaction of nuclear spins when placed in a powerful magnetic field
- When a nucleus that possesses a magnetic moment is placed in a strong magnetic field, it will begin to precess, like a spinning top





Known "spectral fingerprint" → identify molecule(s)

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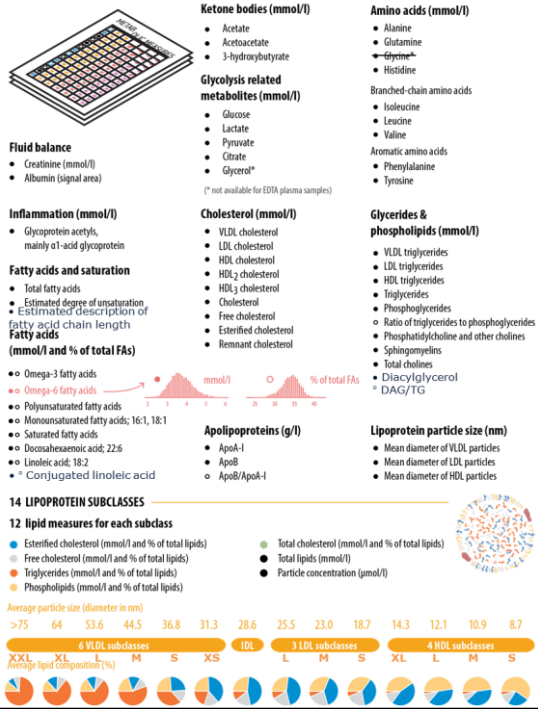
NMR metabolomics

- Nightingale's technology utilizes NMR and proprietary software to provide metabolome profiles
  - Consists of 231 metabolites
  - Quite low compared to total number of known metabolites: 220945
    - We measure only 0.1%!



42

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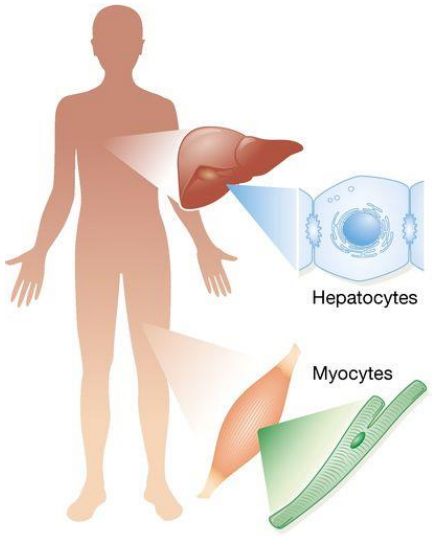
  - *So mostly useful for validating excretion to the circulation*



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43

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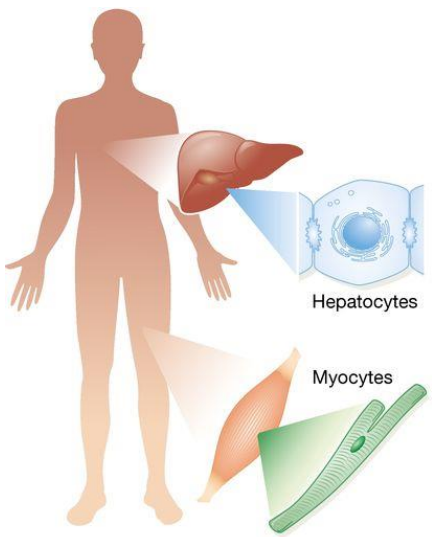

Uhlen, M et al. Mol Sys Bio, 12:862 (2016)

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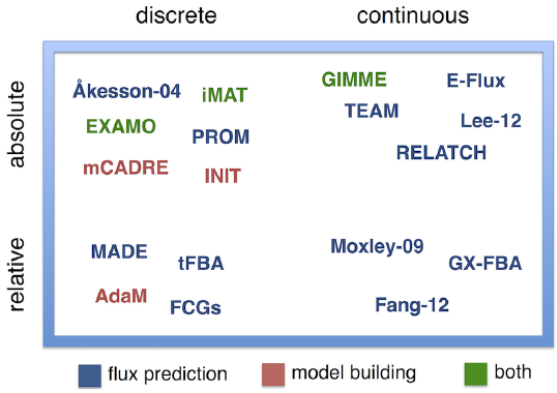
The aim of a model is context specific

- Many algorithms have been proposed for building tissue-specific models based on generic models
- Simplest approach: delete reactions of genes that are not expressed
  - Typically based on tissue-specific transcriptomics data
- Problems:
  - Cutoff for being not expressed
  - Orphan reactions & dead-ends
  - Need to check metabolic functions

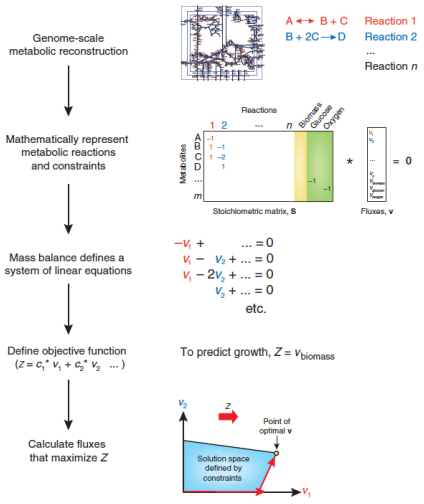


Machado, D et al. PLoS Comp Bio, 10(4):e1003580 (2014)

45

Flux balance analysis

- Used to calculate flow of metabolites through metabolic network
- Predict growth rate of organism or rate of production of given metabolite
- Assumes steady state
- Optimizes a given objective function



Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

46

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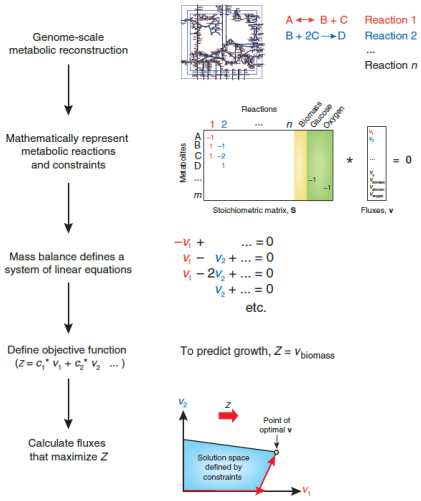

Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

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Objective function - Examples

The objective function ≈ the aim of the model

- Biomass reaction (e.g. plants for consumption)
- ATP production (ATP demand reaction)
- Maximize a product of interest (e.g. lysine production)
- …

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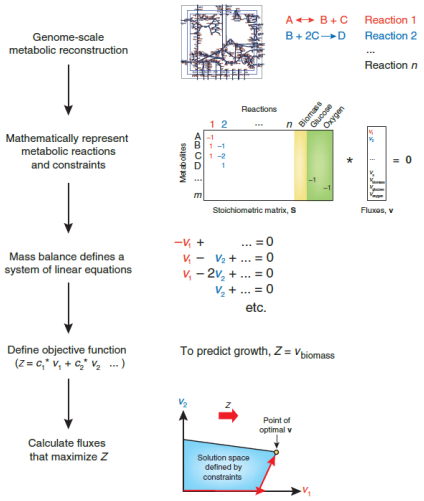

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49

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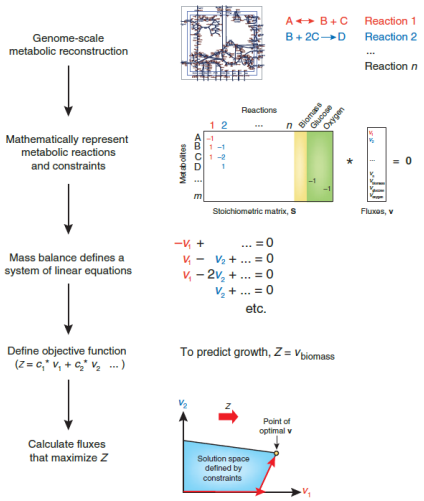

Orth JD, et al. Nat Biotech, 28(3):245-8 (2010)

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Steady-state assumption

Assumption to reduce model complexity:

Metabolite concentrations and reaction rates stay constant over time (steady-state)

Benefit:

1. We have to estimate only one value (reaction rate/flux) per reaction instead of a function over time
2. We do not have to care about different metabolite concentrations
3. Introduces a direct dependence between reactions: Production and consumption of each metabolite cancel out

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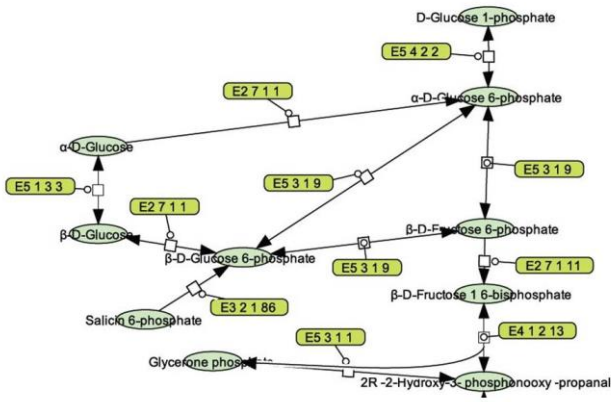
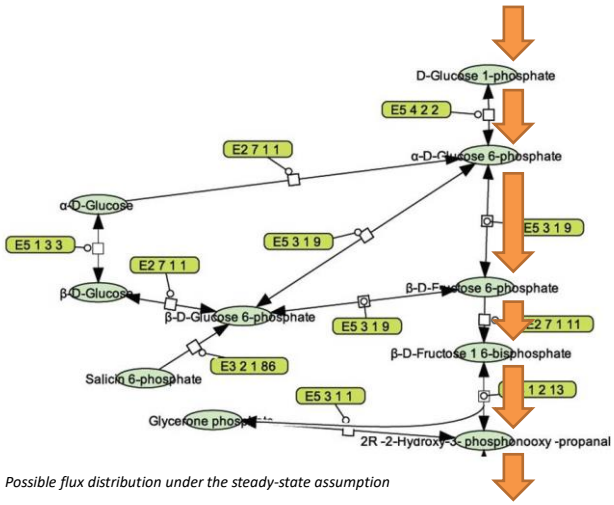
Steady-state assumption visualized



52

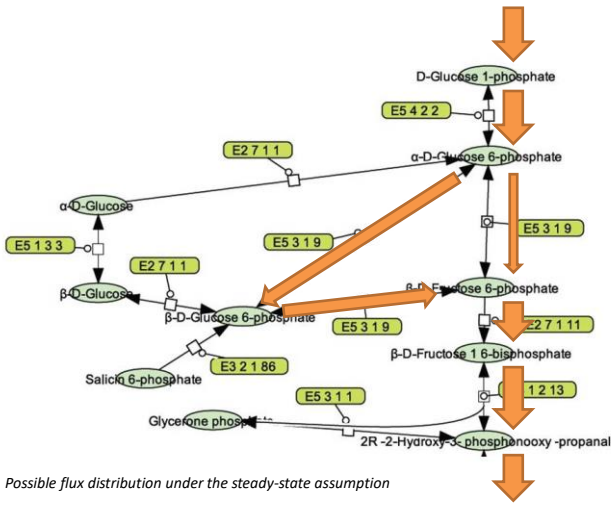
Steady-state assumption visualized



Possible flux distribution under the steady-state assumption

53

Steady-state assumption visualized



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54

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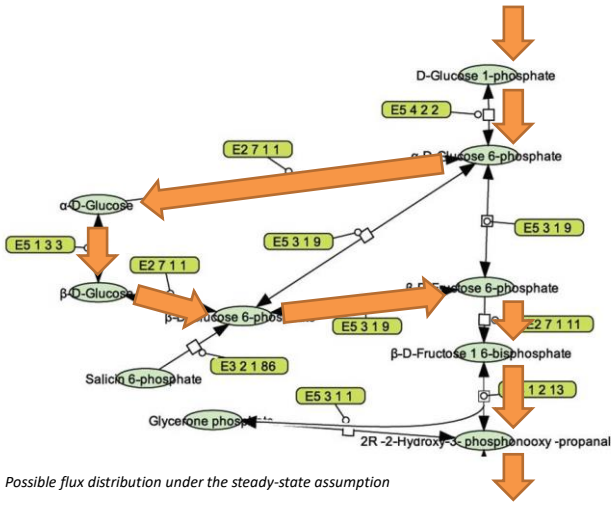

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55

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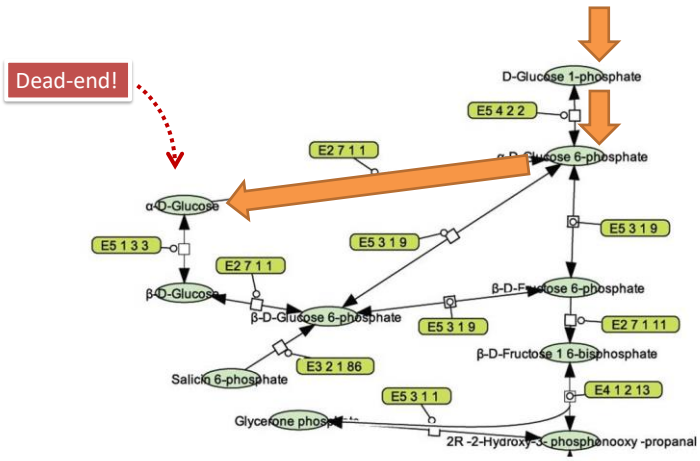

*Flux distribution **not** possible under the steady-state assumption*

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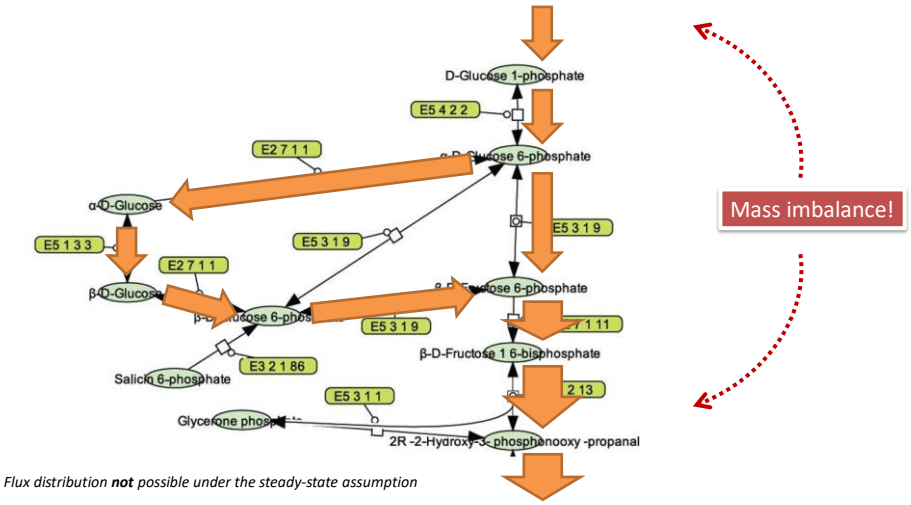
Steady-state assumption visualized



*Flux distribution **not** possible under the steady-state assumption*

57

Steady-state assumption visualized

Like a water supply network!

- *Some places might use more than others*
- *But what goes in, must come out*

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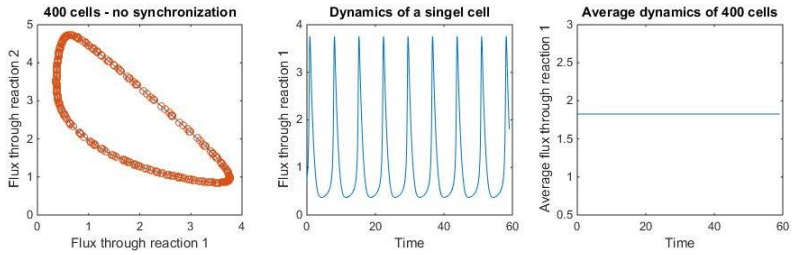
Validity of the steady state assumption

- Cyclic behavior (e.g. limit cycles/periodic fixed points)
- No steady state for single cells
- Consider average of many cells (no synchronization) → steady state reasonable



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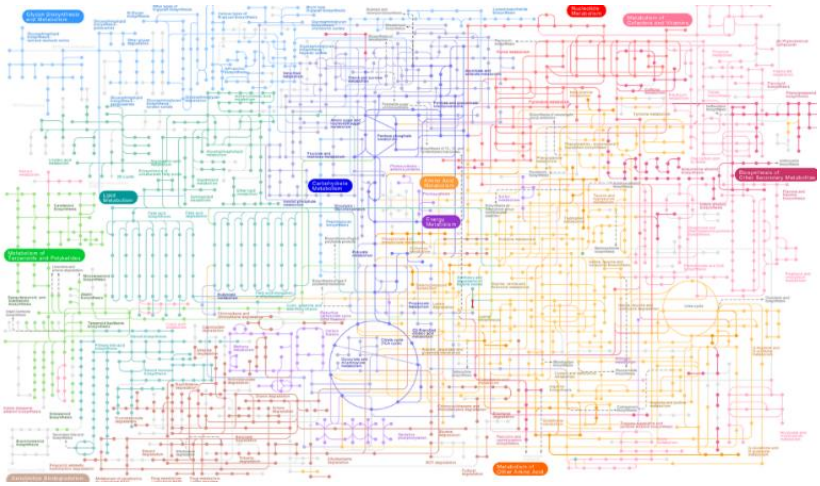
Recap: modeling loss of metabolic flexibility



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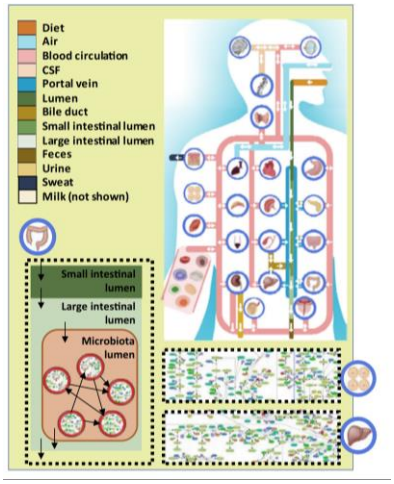
Metabolic model and patient data

- Model: heart model from Harvey and Harvetta
  - Sex specific whole-body metabolic reconstructions
  - 26 organs and 6 blood cell types
  - Organ resolved → isolated organ models available as well
- Data: left ventricular RNA-seq data from MAGNet consortium
  - DCM, HCM, PPCM and healthy controls
- Constrain influx of substrates:
  - "Fed" a standard western-European diet



Transcriptomics: MAGNet (GSE141910)

Figure 1B, Thiele et al. 2020

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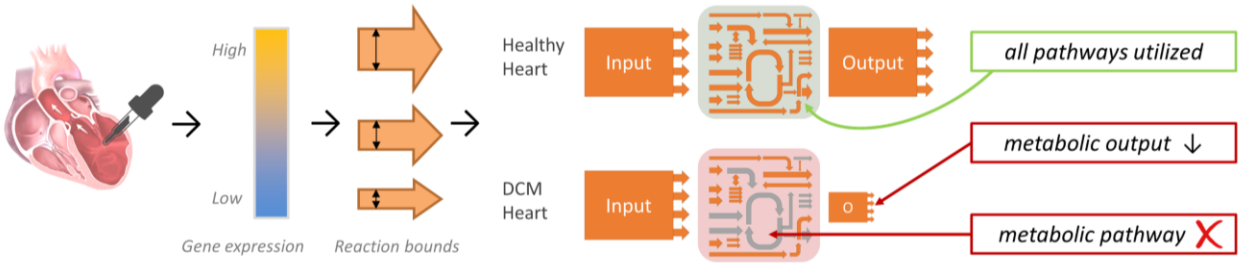
Modeling loss of metabolic flexibility: setup



Bastien Nihant

Dr. Marian Breuer

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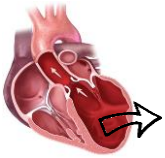
Modeling loss of metabolic flexibility

- Genome-scale metabolic models
- Activate and deactivate reactions based on **gene activity**

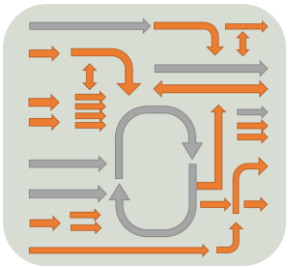


Measure gene activity as proxy for metabolic enzyme activity

Active gene =

Inactive gene =

Personalized model



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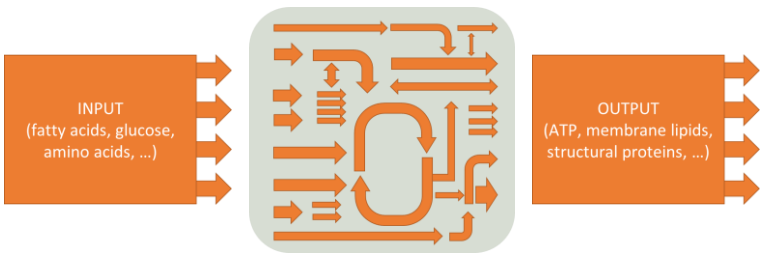
Modeling loss of metabolic flexibility

- Genome-scale metabolic models
- Activate and deactivate reactions based on **gene activity**
- Simulate metabolism for individual
  - Choose objective
  - Find optimal combination of fluxes to maximize objective
  - High flux pathway = active
  - Low flux pathway = less active

Healthy



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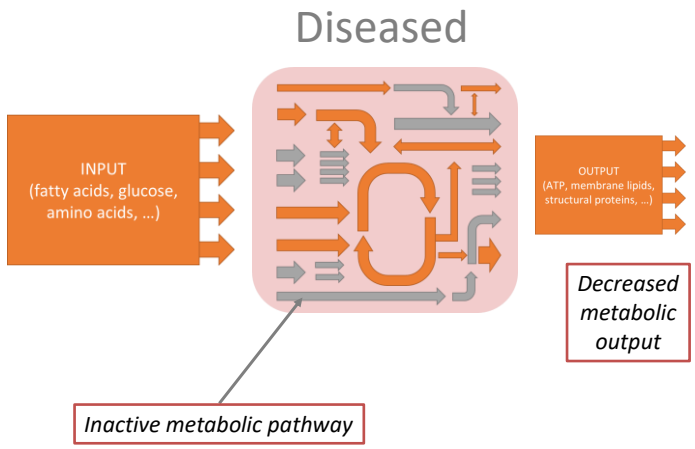

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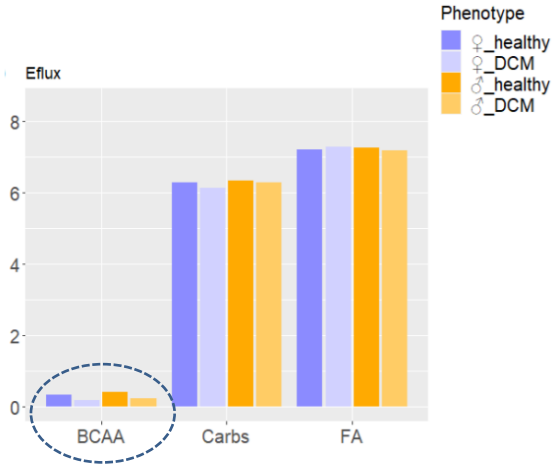
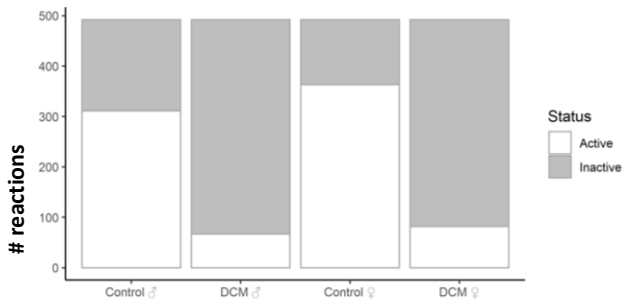
Pilot results: models capture broad trends

Branched chain amino-acids contribute less to mitochondrial energy production in DCM



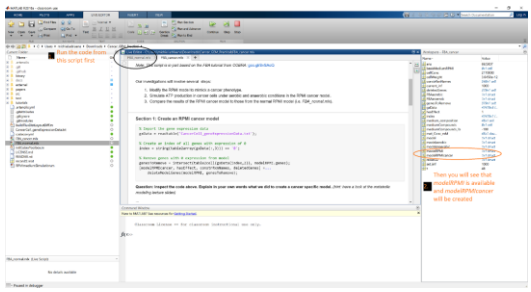
The fatty acid oxidation pathways have many inactive reactions in DCM

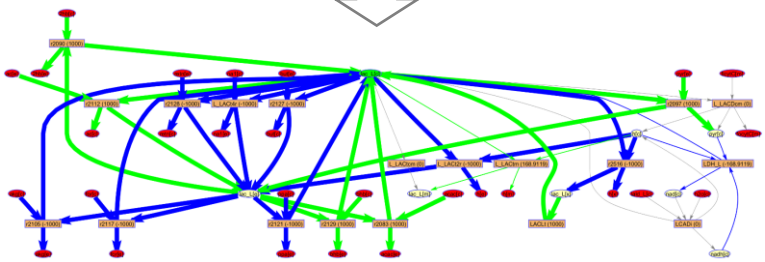


66

Software & tools

- Matlab
  - Python can be an alternative open-source solution for GEM analysis
- CobraToolbox
  - https://opencobra.github.io/cobratoolbox/stable/
  - Model extraction methods
    - Transcriptomics data integration
  - Flux balance analysis





Aerobic glycolysis in cancer

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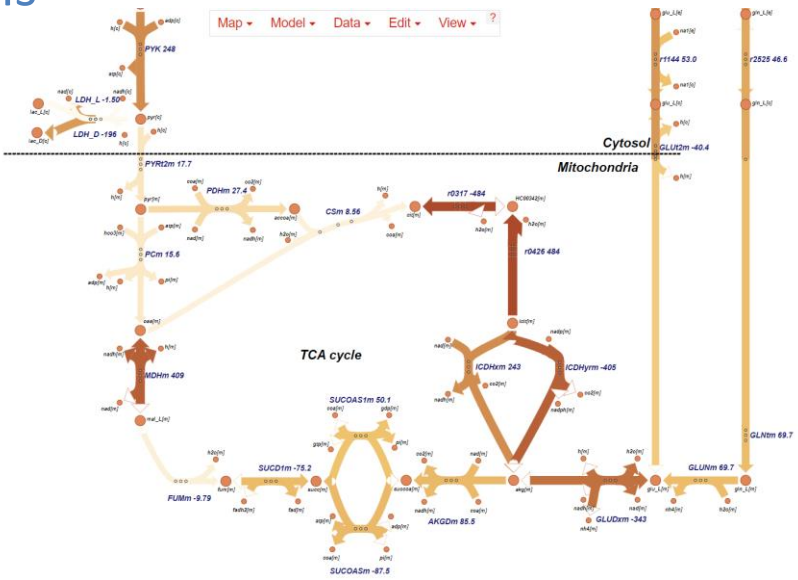
Software & tools

- Escher maps:
  - Demo:

https://sbrg.github.io/escher-fba



Male DCM cardiomyocyte metabolism simulation

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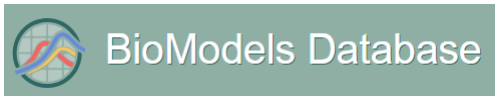
Model databases



https://www.ebi.ac.uk/biomodels-main/

https://vmh.uni.lu/

http://www.metabolicatlas.org/

http://bigg.ucsd.edu/

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Advantages & limitations of GEMs

+ Relatively little information needed
+ Applicable to large networks
+ Quantitative flux estimations

- Only steady state estimation
- Often no unique solutions (large solution space)
- Optimization assumptions (FBA) critical

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