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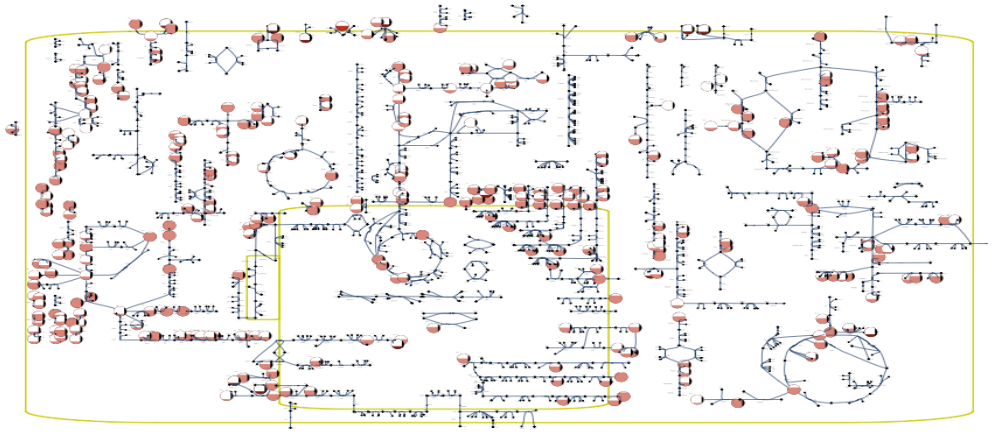
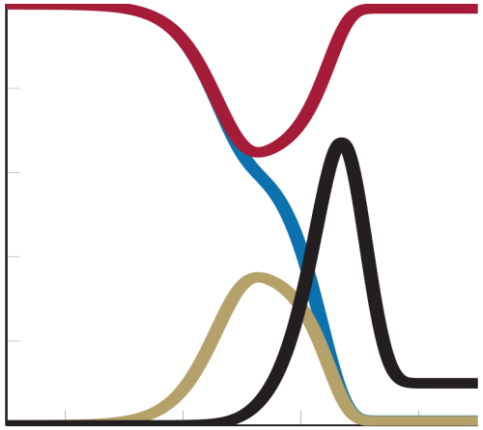
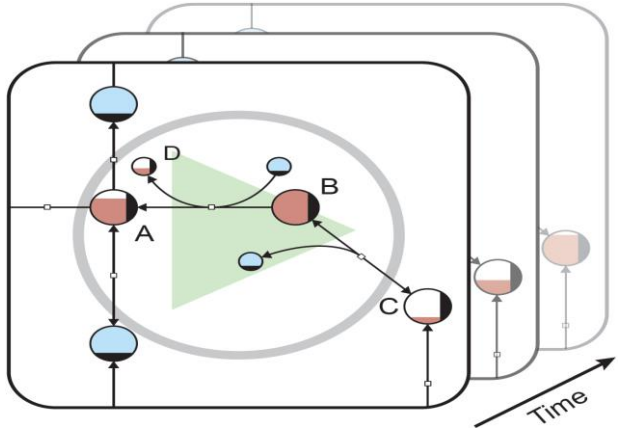
Faculty of Science
Center for Bioinformatics Tübingen (ZBIT)
Computational Systems Biology of Infection







Metabolic network reconstruction of the pathogen *Treponema pallidum* ssp. *pallidum*

Silvia Morini

csb.informatik.uni-tuebingen.de · github.com/draeger-lab · youtube.com/c/systemsbiology

Outline

- Syphilis and its etiologic agent
- Genome-scale metabolic models
- Genome-scale metabolic models *reconstruction process*
- Tools and software used
- Results: current state of the work
- What next?

Who is to blame?

„From the very beginning […] each country whose population was affected by the infection blamed the neighboring (and sometimes enemy) countries […]. So, the inhabitants of today's Italy, Germany and United Kingdom named syphilis 'the French disease', the French named it 'the Neapolitan disease', the Russians assigned the name of 'Polish disease', the Polish called it 'the German disease'. The Danish, the Portuguese and the inhabitants of Northern Africa named it 'the Spanish/Castilian disease' and the Turks coined the term 'Christian disease'. Moreover, in Northern India, the Muslims blamed the Hindu for the outbreak of the affliction. However, the Hindu blamed the Muslims and in the end everyone blamed the Europeans."

(Tampa et al., 2014)

Source: freeworldmaps.net

The *Aenigma* of Syphilis and *T. pallidum*

- ✓ Diagnostic tests
- ✓ Antibiotic therapy

T. pallidum:

- ✓ poorly tolerates desiccation, high temperature, oxygen
- ✓ is an obligate parasite – i.e., is entirely dependent upon the host for surviving
- ✓ has a 1,14 Mb genome (one of the smallest among bacteria) - does NOT encode for enzymes for *de novo* biosynthesis of nucleotides, fatty acids, vitamins, cofactors, amino acids, TCA (tricarboxylic acid/Krebs cycle), oxidative phosphorylation

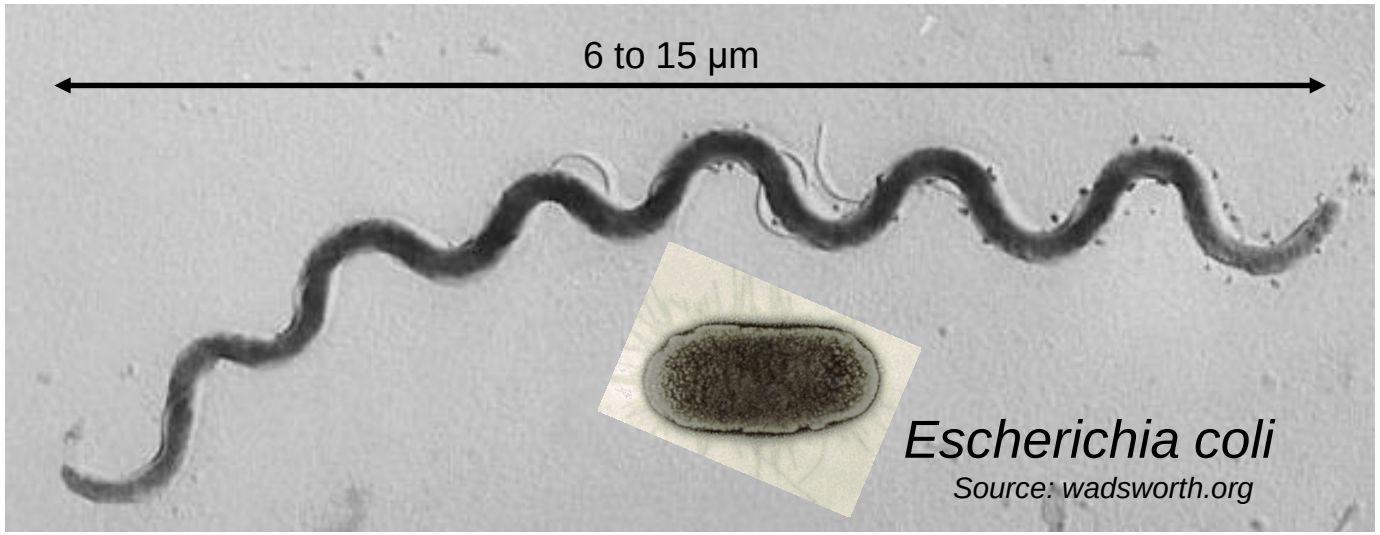
… but:

- No vaccine
- 2016: 8.7 cases per 100,000 population in the USA

… and moreover:

- early syphilis enhances the transmission of HIV

Treponema pallidum ssp. *pallidum*



Source: Public Health Image Library, Center for Disease Control, Susan Lindsley (1972)

- First isolated in 1912 (by Major J. H. Nichols of the US Army)
- Gram-negative
- One single circular chromosome with 1039 predicted ORFs
- Member of the Spirochetaceae family (phylum Spirochaetes)

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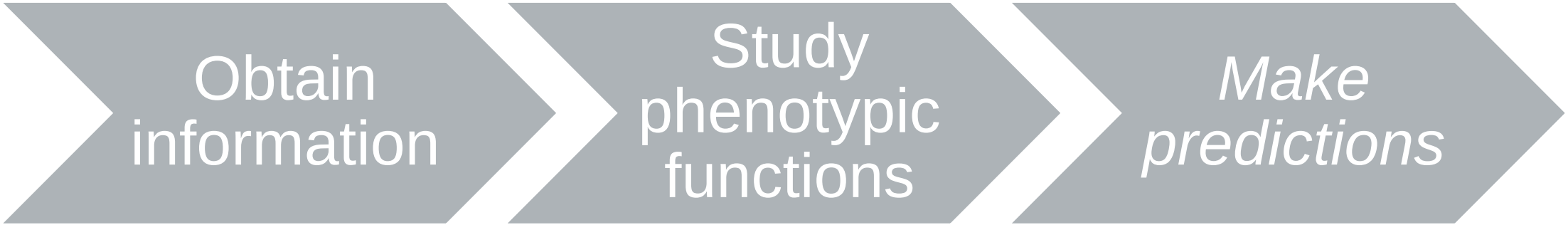
Why genome-scale modelling



- 113 predictive Genome-scale Models (GEMs) of *Bacteria*
- 57 predictive GEMs of *Eukaryota*
- 8 predictive GEMs of *Archea*

including multiple versions of GEMs of one same organism (e.g. 5 different GEMs are available for *E. coli*)

Why genome-scale modelling *of T. pallidum*?

- **fill** knowledge **gaps**: *how* does he make a living?
- identification of potential **drug targets**
- no **media for continuous culture** available means:

  - need for animals (*T. pallidum* has been propagated in rabbit testicles since 1912)

  - no genetic manipulation possible

source: care2.com

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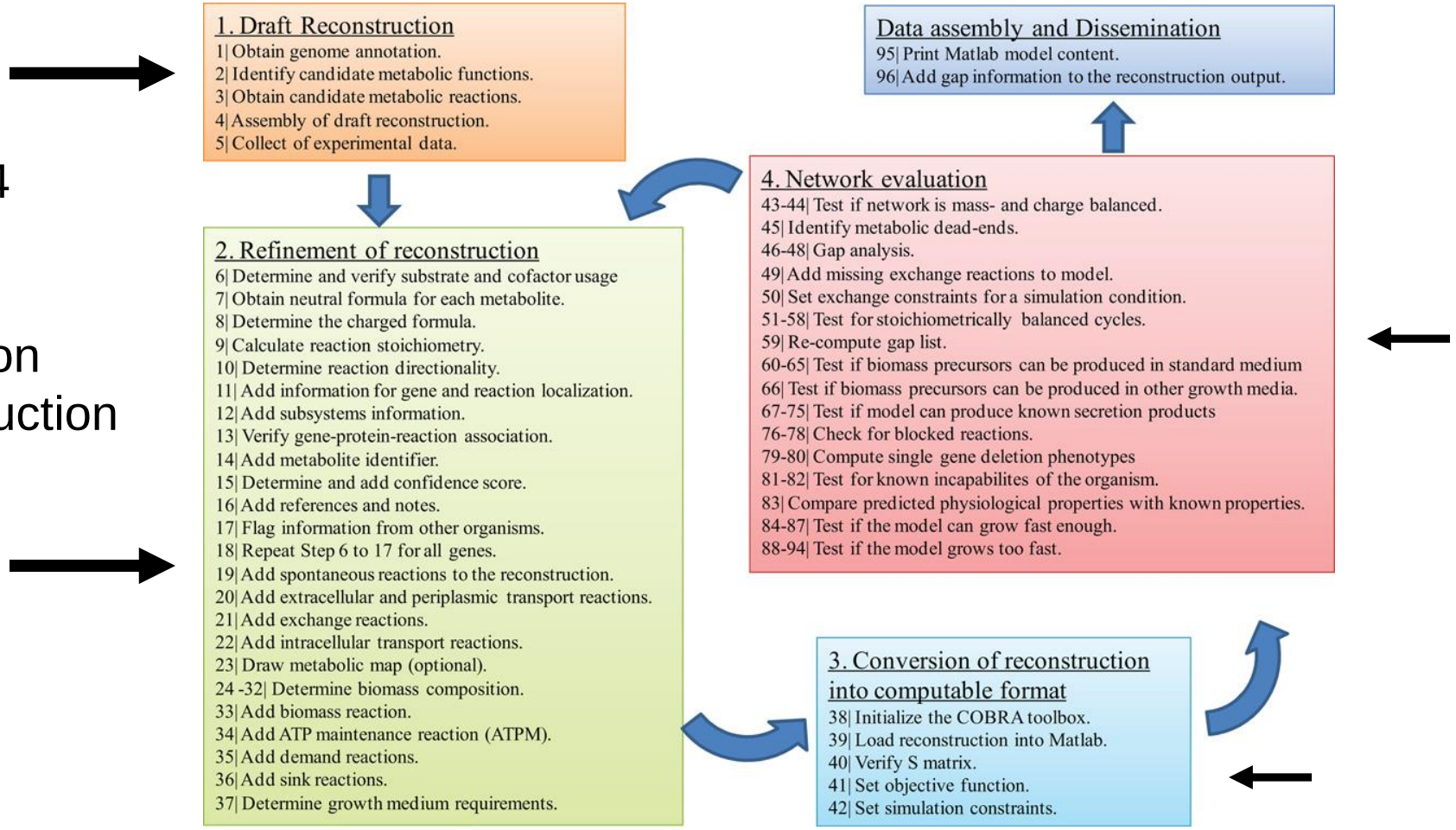
A protocol for generating a high-quality genome-scale metabolic reconstruction

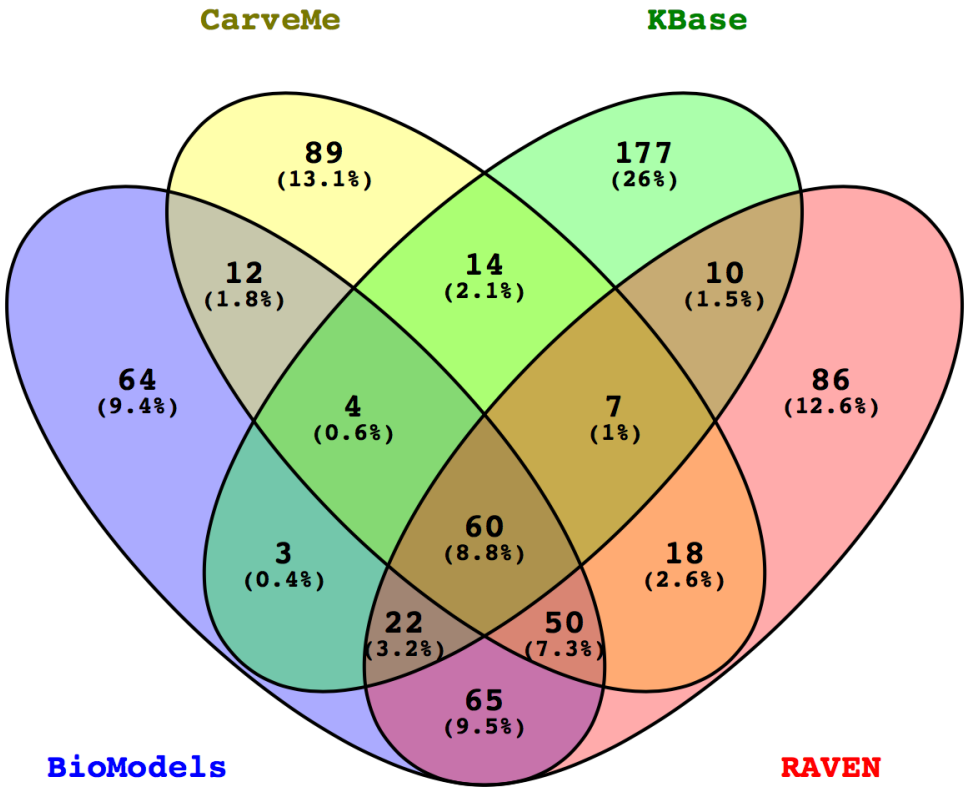
Ines Thiele[1,2] & Bernhard Ø Palsson[1]

How to do it, in 96 steps and 4 stages:

1. Draft Reconstruction
2. Refinement of reconstruction
3. Conversion of the reconstruction into computable format
4. Network evaluation



Stage 1: *obtaining draft reconstruction*



Tool	Reactions	Metabolites	Reaction ID
CarveMe	723	615	BiGG
RAVEN toolbox	318	402	KEGG
ModelSEED	599	738	ModelSEED
Kbase	526	685	ModelSEED
BIOMODELS	649	829	BIOMODELS

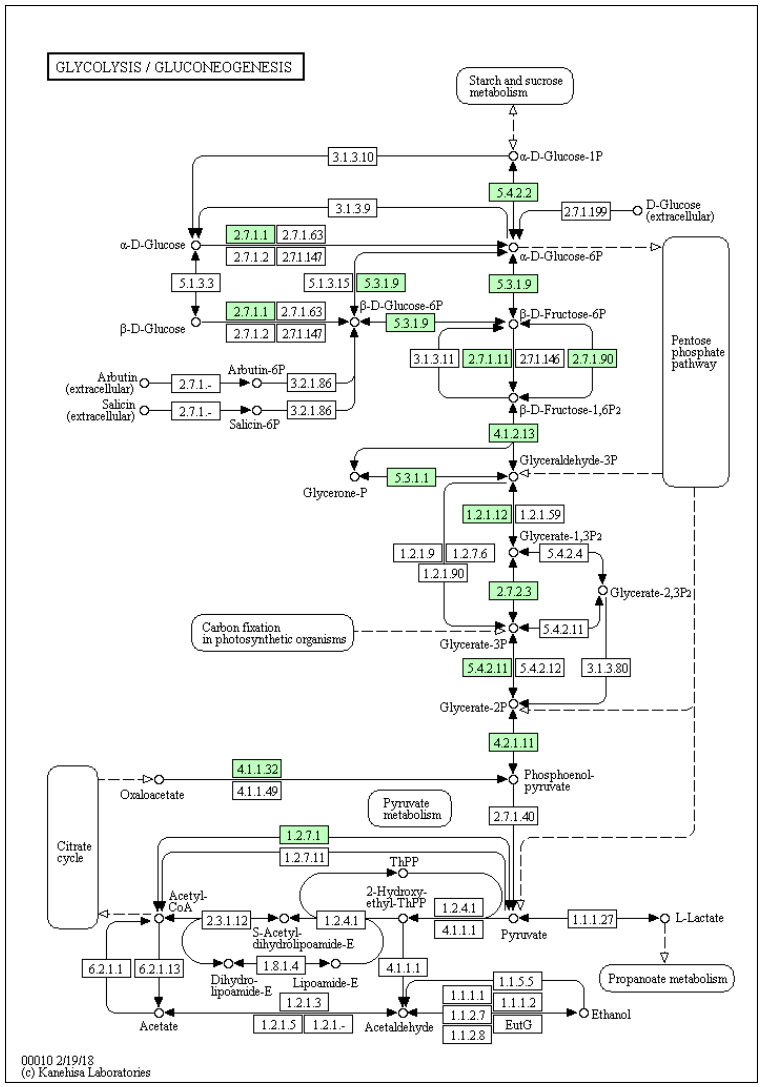
Number of reactions compared for each draft			
CarveMe	RAVEN	Kbase	BioModels
254	318	297	280

The drafts are diverse, but how?

Found a common core of 60 reactions, not all of which were organism-specific.

Conclusion? *"[. . .] it is both wise and necessary to not always trust the databases."* (Santos et al.)

Stage 2: *manual reconstruction and refinement*



UniProt

KEGG Kyoto Encyclopedia of Genes and Genomes

PubChem

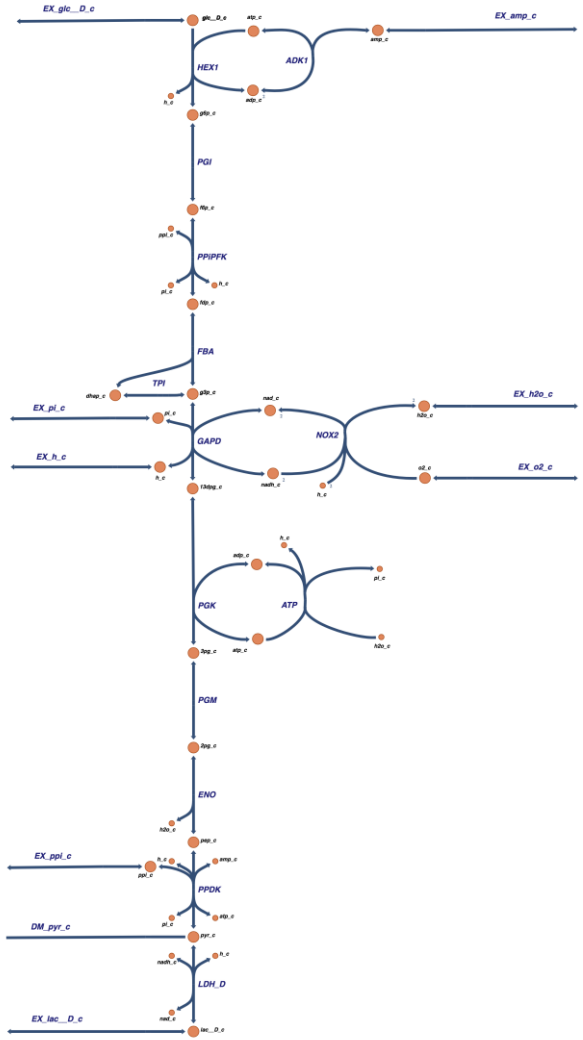
BRENDA 1987 2015
The Comprehensive Enzyme Information System

MavinSketch

source: genengnews.com

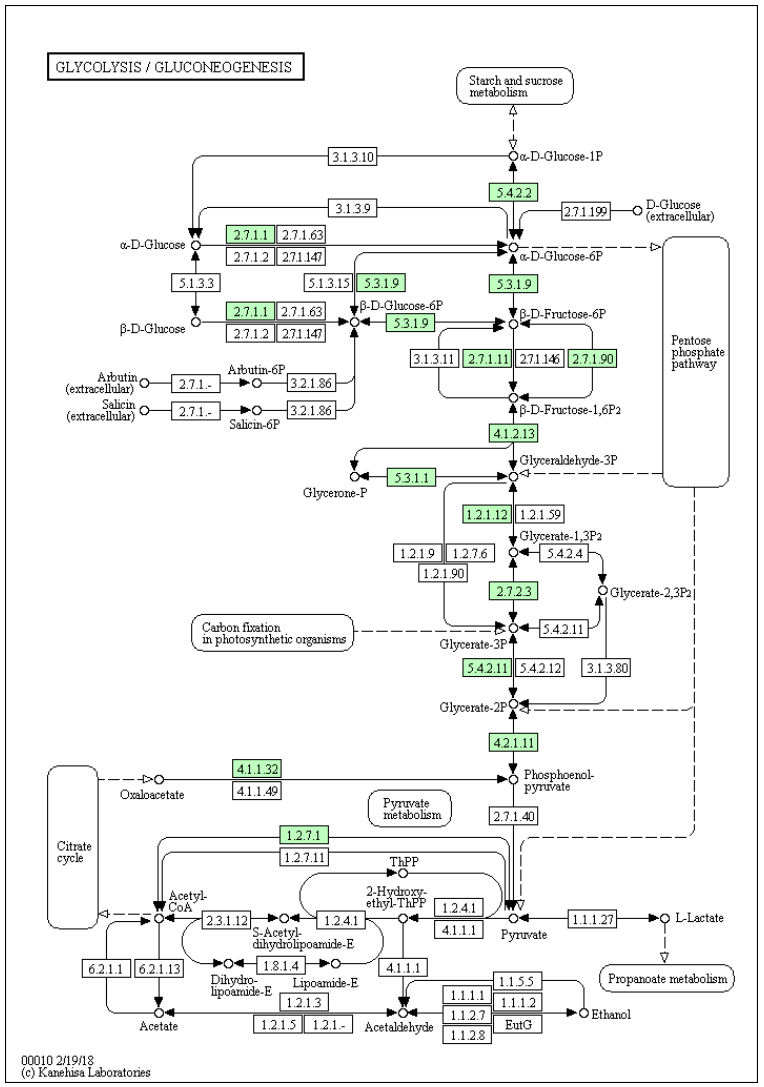
source: videoblocks.com

!ID
R_PGMT
R_HEX1
R_GLUK_syn
R_G6PI3
R_G6PI
R_PGI
R_PPiPFK
R_FBA
R_TPI
R_GAPD
R_PGK
R_PGM
R_ENO
R_PEPCK
R_POR
%technically in K
R_OAADC
R_PPDK
R_IPDPS
%spontaneous reactio
R_G5SADs
R_GTPHs
% I did not add to this



GPR
TP_0413
TP_0505
TP_0505
TP_0475
TP_0475
TP_0475
TP_0542
TP_0662
TP_0537
TP_0844
TP_0538
TP_0168
TP_0817
TP_0122
TP_0939
A TP_0056 A
TP_0746
TP_0547
n't be riconverted

Stage 2: *manual reconstruction and refinement*

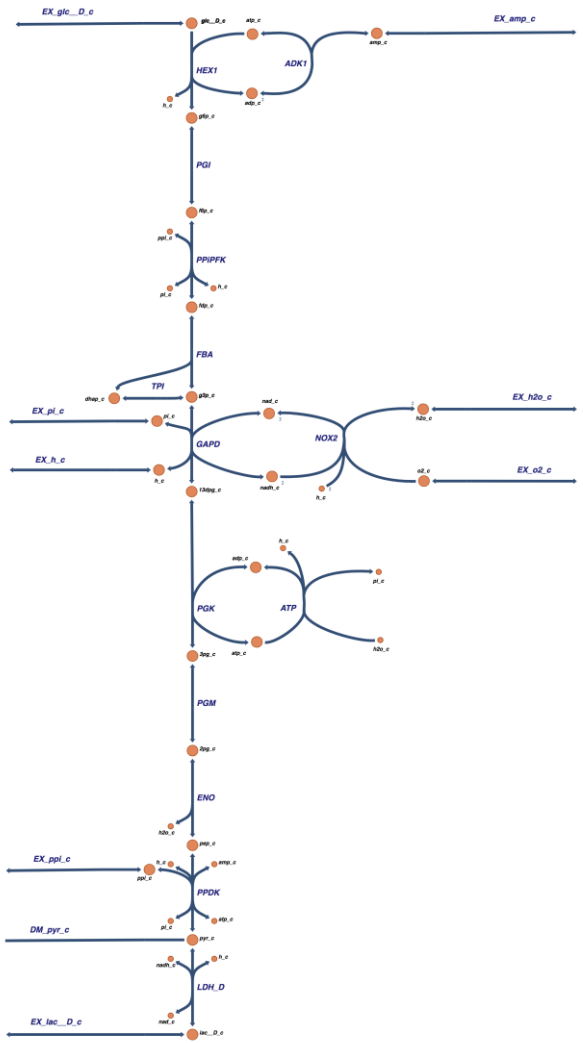


Issues (among others):

- guarantee reaction specificity
- detect transport reactions
- identify cofactor usage

source: genengnews.com

source: videoblocks.com



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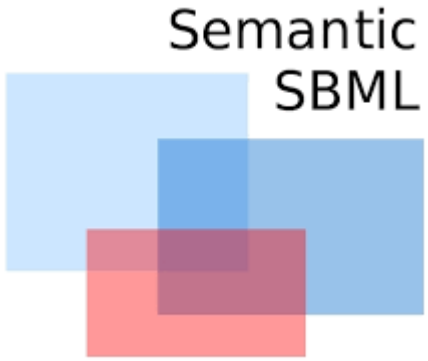
Stage 2: *manual reconstruction and refinement*

Consistent way of storing information

!!SBtab SBtabVersion="1.0" TableType="Reaction" TableName="Treponema Reactions"			
!ID	EC	!Enzyme:Name	!ReactionFormula
%Glycolysis			
R_HEX1	2.7.1.1	Hexokinase (D-glucose:ATP)	M_atp_c + M_glc__D_c <=> M_adp_c + M_h_c + M_g6p_c

KEGG translator

Automatically retrieving information from KEGG PATHWAYS



& **ModelPolisher**

Annotation of the model

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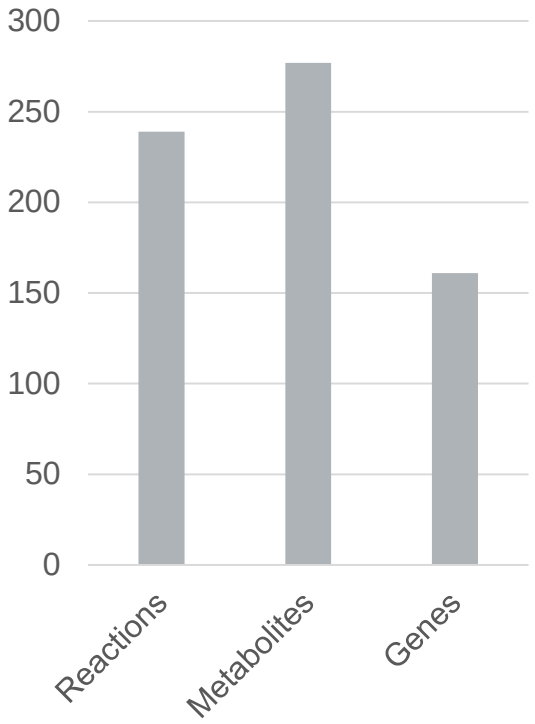


Reactions	239
Metabolites	277
Genes	161
Subsystems	18
Pseudoreactions	53

```
9067    <reaction fast="false" fbc:lowerFluxBound="cobra_default_lb" fbc:upperFluxBound="cobra_default_ub" id="R_OAADC" metaid="R_OAADC" name="
            Oxaloacetate decarboxylase" reversible="true"
9068    sboTerm="SBO:0000375">
9069      <annotation xmlns:sbml="http://www.sbml.org/sbml/level3/version1/core">
9070        <rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#" xmlns:bqbiol="http://biomodels.net/biology-qualifiers/">
9071          <rdf:Description rdf:about="#R_OAADC">
9072            <bqbiol:is>
9073              <rdf:Bag>
9074                <rdf:li rdf:resource="http://identifiers.org/bigg.reaction/OAADC" />
9075                <rdf:li rdf:resource="http://identifiers.org/biocyc/META:OXALODECARB-RXN" />
9076                <rdf:li rdf:resource="http://identifiers.org/ec-code/4.1.1.3" />
9077                <rdf:li rdf:resource="http://identifiers.org/kegg.reaction/R00217" />
9078                <rdf:li rdf:resource="http://identifiers.org/kegg.reaction/R00219" />
9079                <rdf:li rdf:resource="http://identifiers.org/metanetx.reaction/MNXR3932" />
9080                <rdf:li rdf:resource="http://identifiers.org/rhea/15641" />
9081                <rdf:li rdf:resource="http://identifiers.org/rhea/15642" />
9082                <rdf:li rdf:resource="http://identifiers.org/rhea/15643" />
9083                <rdf:li rdf:resource="http://identifiers.org/rhea/15644" />
9084              </rdf:Bag>
9085            </bqbiol:is>
9086          </rdf:Description>
9087        </rdf:RDF>
9088      </annotation>
9089      <fbc:geneProductAssociation xmlns:fbc="http://www.sbml.org/sbml/level3/version1/fbc/version2">
9090        <fbc:and>
9091          <fbc:geneProductAssociation xmlns:geneProduct="G_TP_0055" />
9092          <fbc:geneProductAssociation xmlns:geneProduct="G_TP_0056" />
9093          <fbc:geneProductAssociation xmlns:geneProduct="G_TP_0057" />
9094        </fbc:and>
9095      </fbc:geneProductAssociation>
9096      <listOfReactants>
9097        <speciesReference constant="true" sboTerm="SBO:0000010" species="M_h_c" stoichiometry="1" />
9098        <speciesReference constant="true" sboTerm="SBO:0000010" species="M_oaa_c" stoichiometry="1" />
9099      </listOfReactants>
9100      <listOfProducts>
9101        <speciesReference constant="true" sboTerm="SBO:0000011" species="M_co2_c" stoichiometry="1" />
9102        <speciesReference constant="true" sboTerm="SBO:0000011" species="M_pyr_c" stoichiometry="1" />
9103      </listOfProducts>
9104    </reaction>
```

Stage 3 and 4: *conversion of reconstruction into computable format and network evaluation*

The network has been transformed into an SBML model has been written with COBRApy and libSBML functions.

libSBML

cobrapy

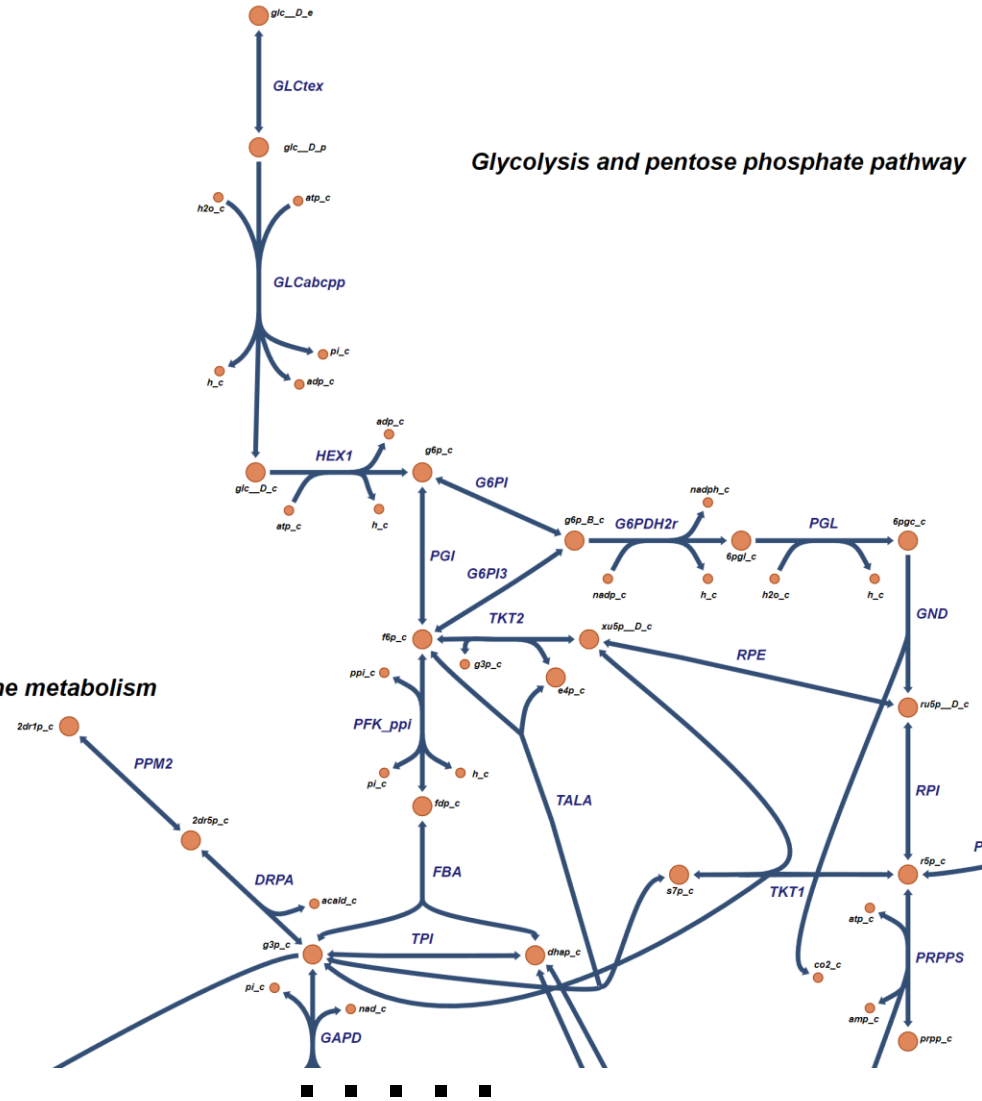
Building the stoichiometric matrix of the network allowed detection (and resolution) of dead-end metabolites.

	HEX1	PGI	PPiPFK	FBA	TPI	GAPD	PGK	PGM	ENO	PPDK
AMP	0	0	0	0	0	0	0	0	0	-1
ATP	-1	0	0	0	0	0	1	0	0	1
GLC	-1	0	0	0	0	0	0	0	0	0
ADP	1	0	0	0	0	0	-1	0	0	0
G6P	1	-1	0	0	0	0	0	0	0	0
H^+	1	0	1	0	0	1	0	0	0	-1
F6P	0	1	-1	0	0	0	0	0	0	0
FDP	0	0	1	-1	0	0	0	0	0	0
DHAP	0	0	0	1	-1	0	0	0	0	0
G3P	0	0	0	1	1	-1	0	0	0	0
NAD	0	0	0	0	0	-1	0	0	0	0
Pi_i	0	0	1	0	0	-1	0	0	0	1
PPi_i	0	0	-1	0	0	0	0	0	0	0
13DPG	0	0	0	0	0	1	-1	0	0	0
NADH	0	0	0	0	0	1	0	0	0	0
3PG	0	0	0	0	0	0	1	-1	0	0
2PG	0	0	0	0	0	0	0	1	-1	0
PEP	0	0	0	0	0	0	0	0	1	-1
H_2O	0	0	0	0	0	0	0	0	1	0
PYR	0	0	0	0	0	0	0	0	0	1

Stoichiometric matrix of glycolysis in T. pallidum

Stage 3 and 4: *conversion of reconstruction into computable format and network evaluation*

SBML ⟶ JSON ⟶



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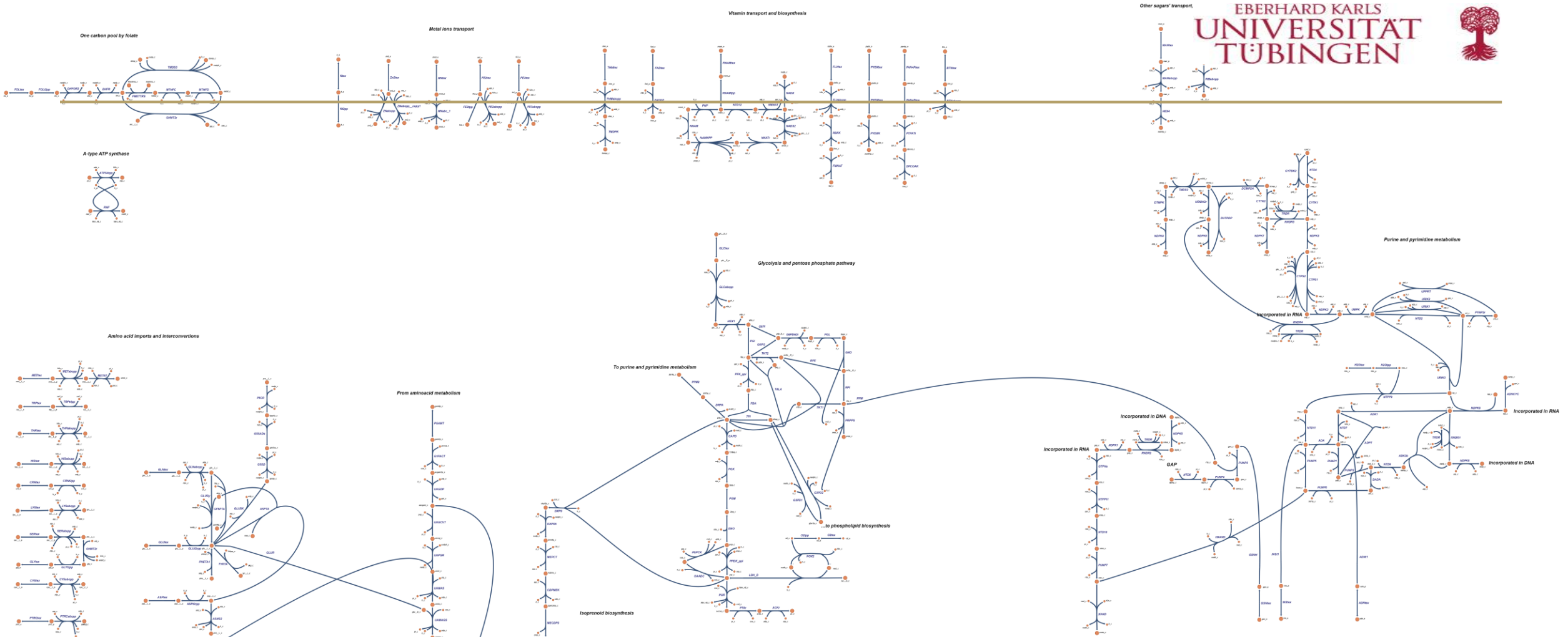
Next steps:

Stage 4: *Network evaluation: retrieve biomass composition to formulate a biomass objective function to evaluate the model. Eventually compare in silico results with experimental data.*



Thank you!