Models of Redox and Carbon Metabolism in *Escherichia coli:* Integrating Different Types of Data

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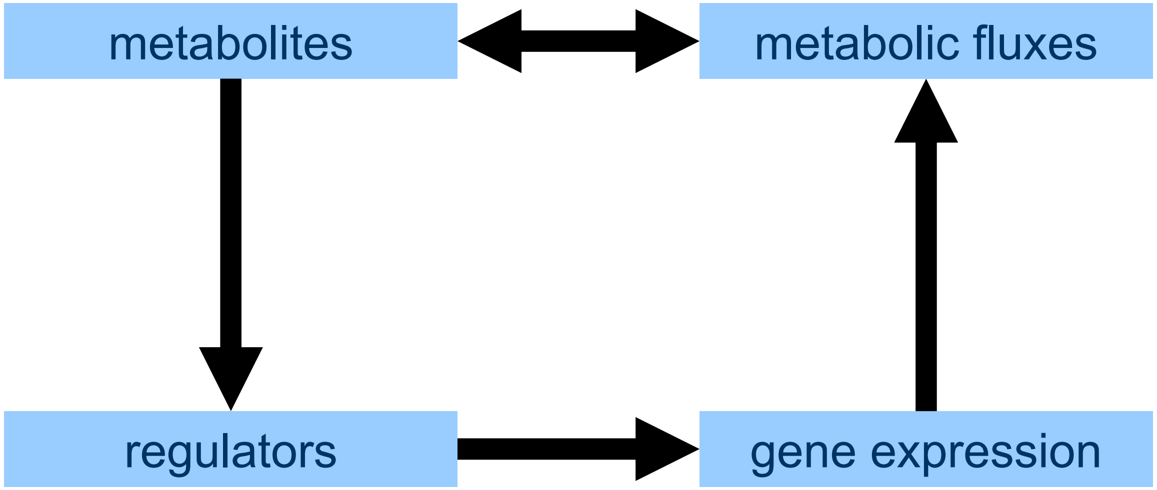
Michael Ederer, Klaas Hellingwerf, Robert Poole

and all of SUMO

SysMO Conference, Feb 2013

Model-based Integration of Data



metabolites

metabolic fluxes

regulators

gene expression

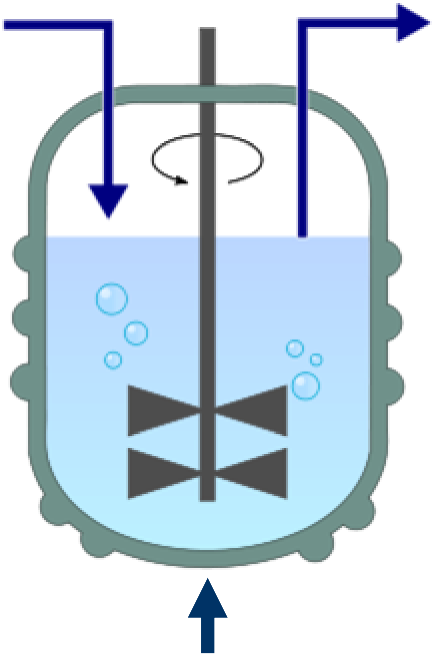
Idea of SUMO

Carbon-, Redox- and Energy Metabolism of *Escherichia coli* is sufficiently well understood, to develop mechanism-based, mathematical models

- describing the closed loop of metabolic regulation
- describing the closed loop of energy and redox equivalents

Reminder: The Aerobiosis Scale

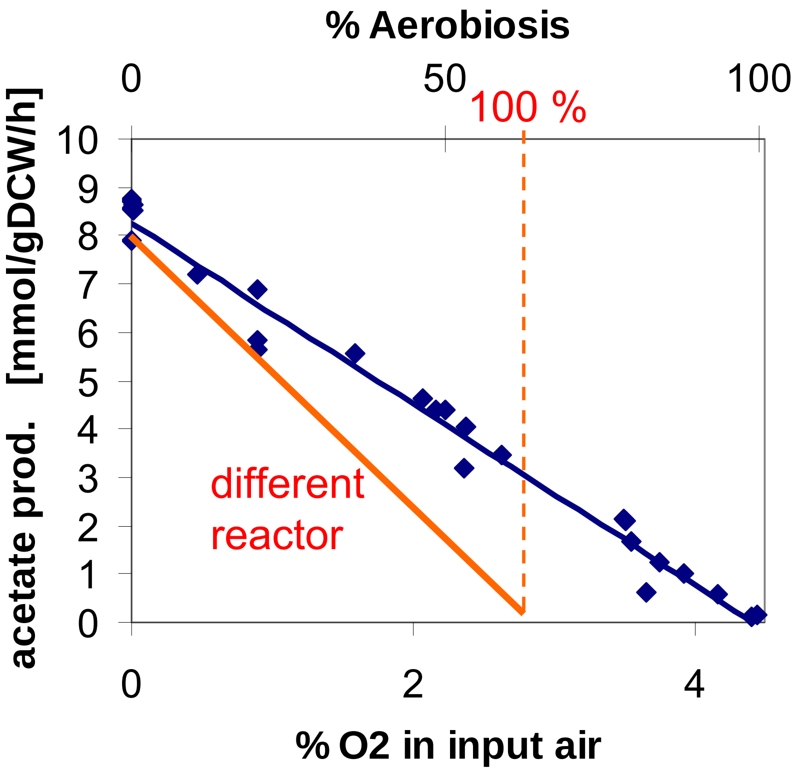
Glucose-limited chemostat



fresh medium

medium + cells

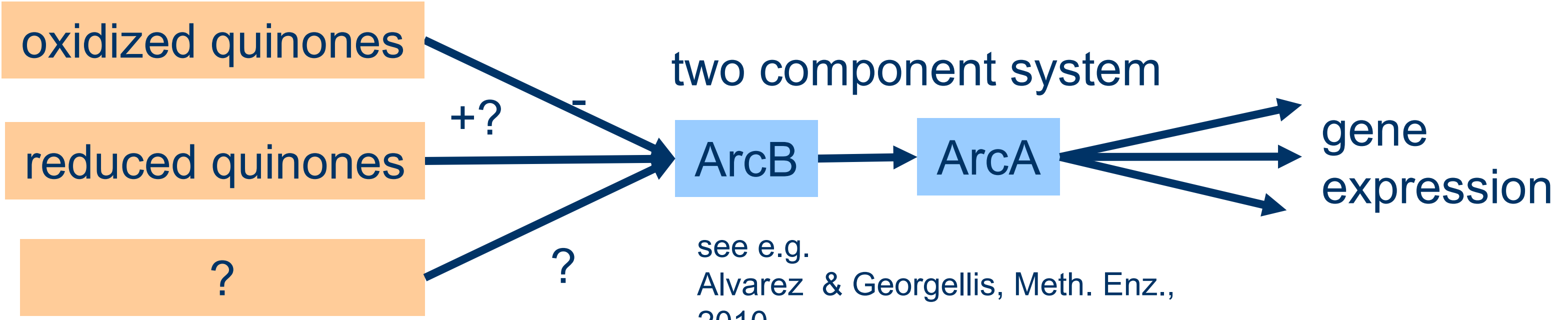
adjustable O_2 + N_2 mixture



[1] S. Alexeeva et al., J Bacteriol, vol. 182, no. 17, pp. 4934–4940, Sep 2000.
[2] S. Alexeeva et al., J Bacteriol, vol. 184, no. 5, pp. 1402–1406, Mar 2002.
[3] S. Alexeeva et al., J Bacteriol, vol. 185, no. 1, pp. 204–209, Jan 2003

Aerobiosis scale enables cross-lab reproducibility!

The ArcA Riddle



oxidized quinones

reduced quinones

?

+?

-

?

two component system

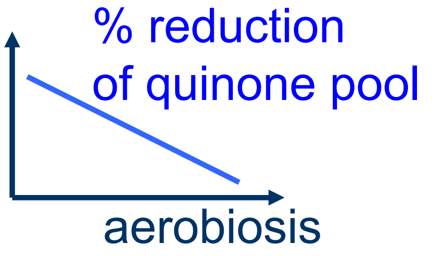
ArcB

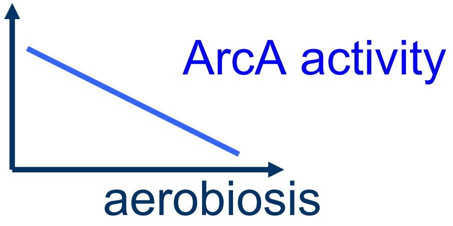
ArcA

gene expression

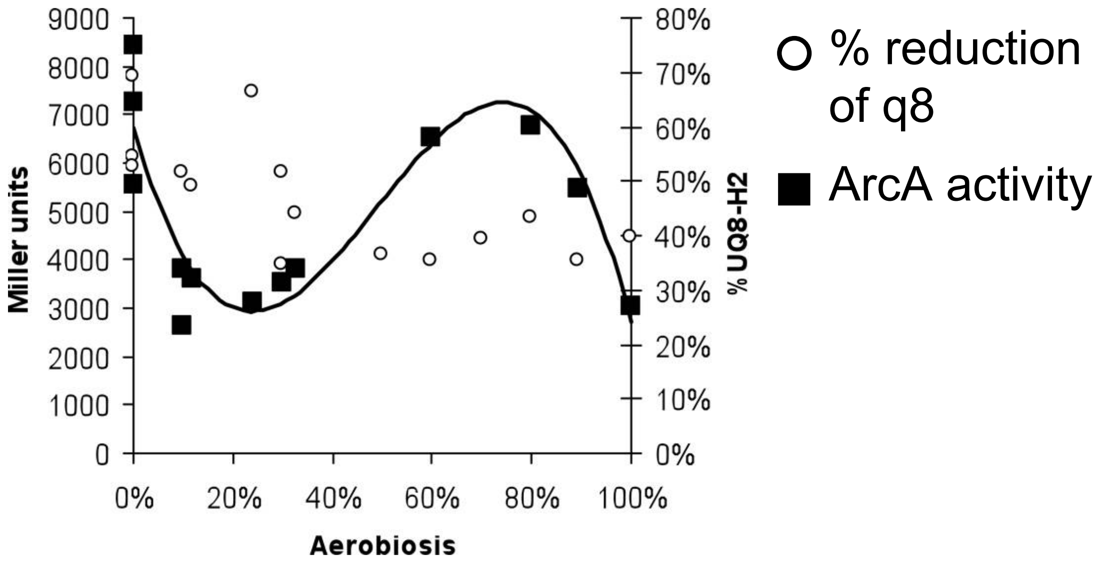
see e.g.
Alvarez & Georgellis, Meth. Enz., 2010.

Expected behaviour:



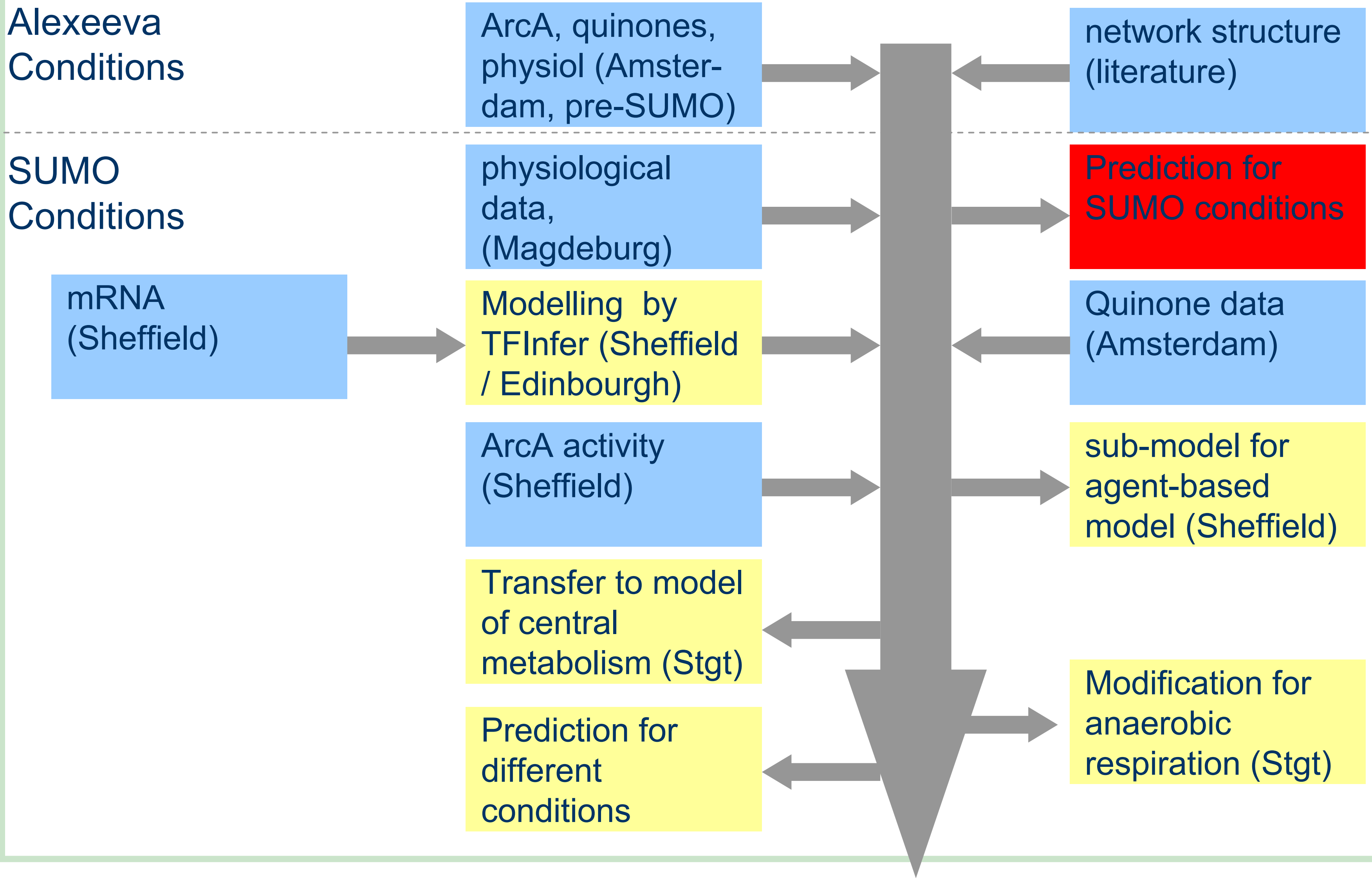


Measured behaviour:



(Bekker et al. J. Bac, 2010)

Modelling the Regulatory Structure of the ETC



Alexeeva Conditions

ArcA, quinones, physiol (Amsterdam, pre-SUMO)

network structure (literature)

SUMO Conditions

physiological data, (Magdeburg)

Prediction for SUMO conditions

mRNA (Sheffield)

Modelling by TFInfer (Sheffield / Edinbourgh)

Quinone data (Amsterdam)

ArcA activity (Sheffield)

sub-model for agent-based model (Sheffield)

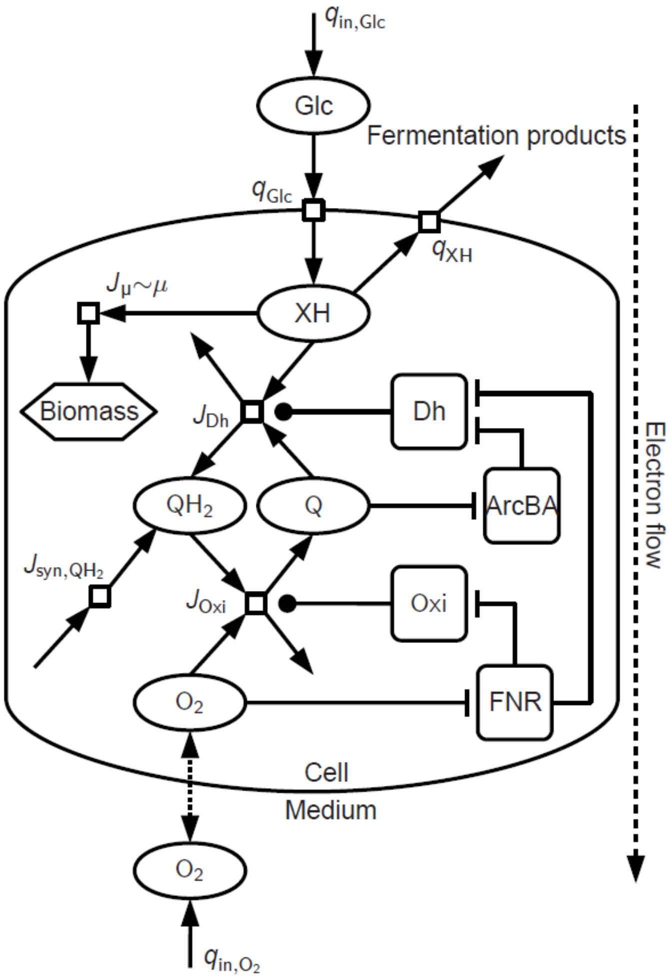
Transfer to model of central metabolism (Stgt)

Modification for anaerobic respiration (Stgt)

Prediction for different conditions

Regulatory Structure of the ETC

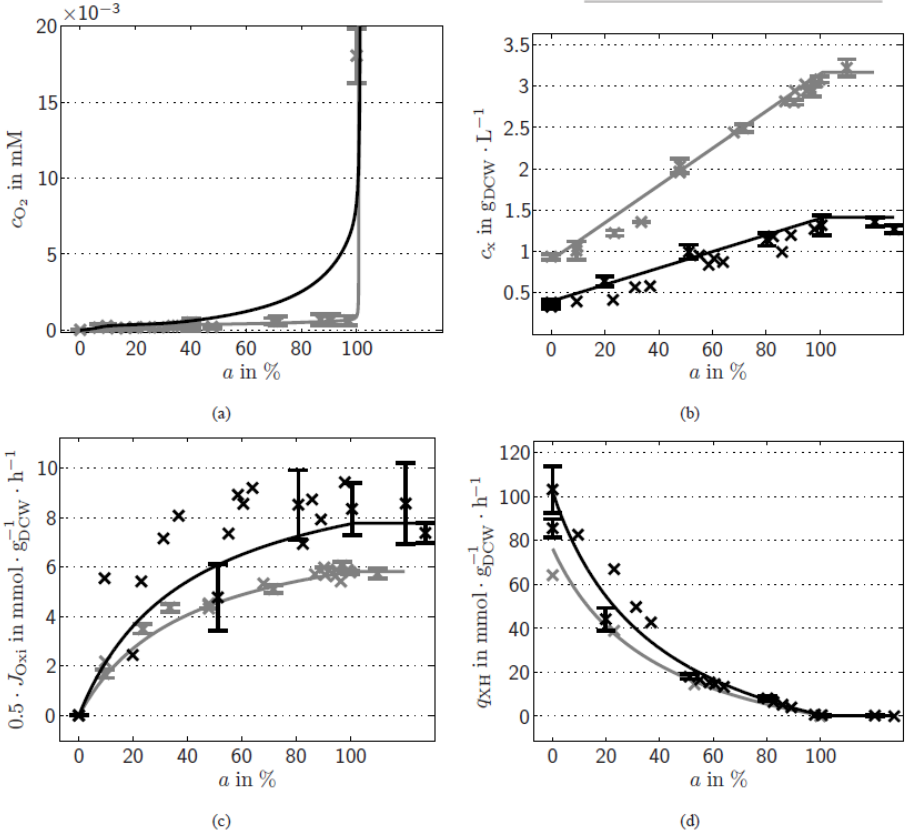
Differential equation model



ExpA: Alexeeva ▬ (grey)

ExpB: SUMO ▬

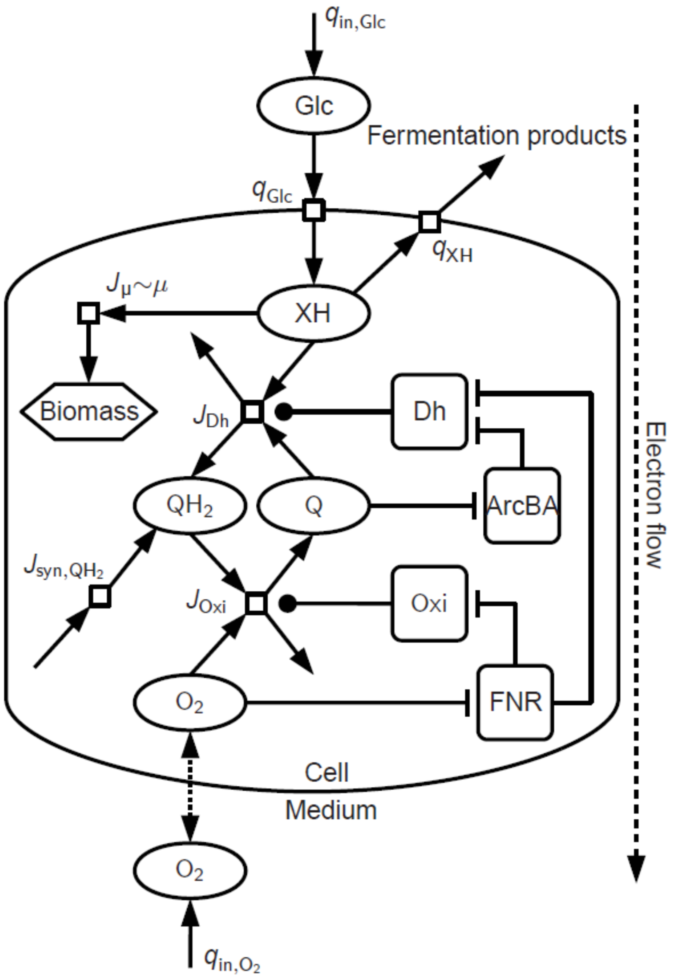
Variable	"ExpA"	"ExpB"
Strain	MC4100	MG1655
D	$0.15\,h^{-1}$	$0.2\,h^{-1}$
$c_{in,Glc}$	45 mM	20 mM
pH_{Medium}	7	6.9
θ_{Medium}	35 °C	37 °C



(a) (b) (c) (d)

Regulatory Structure of the ETC

Differential equation model



ExpA: Alexeeva —

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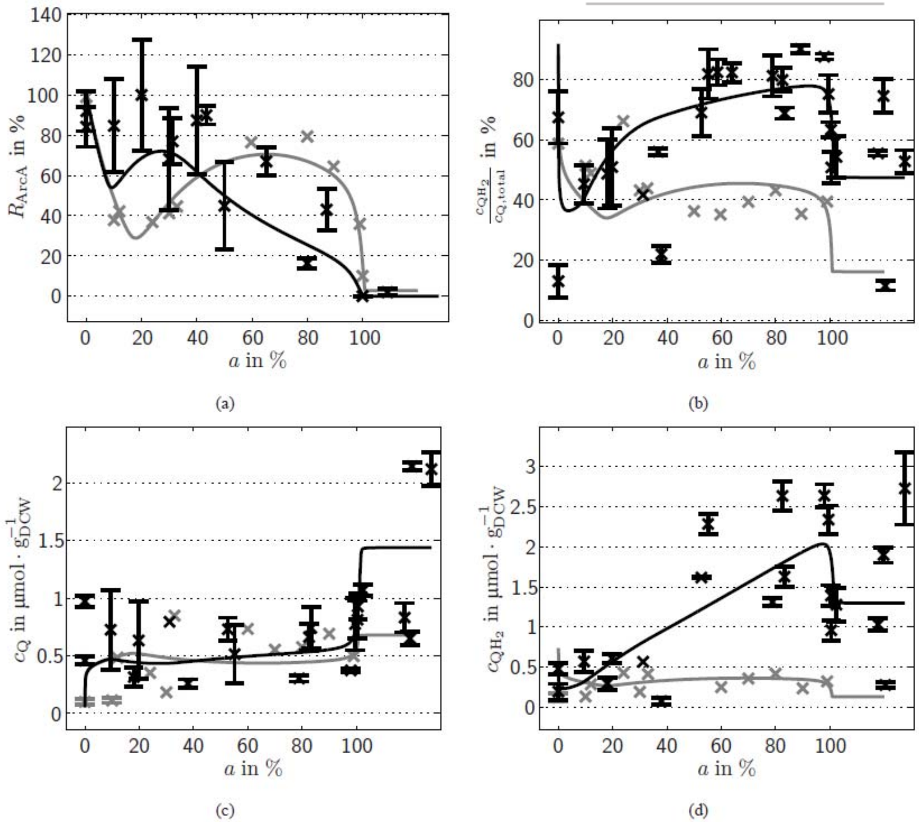
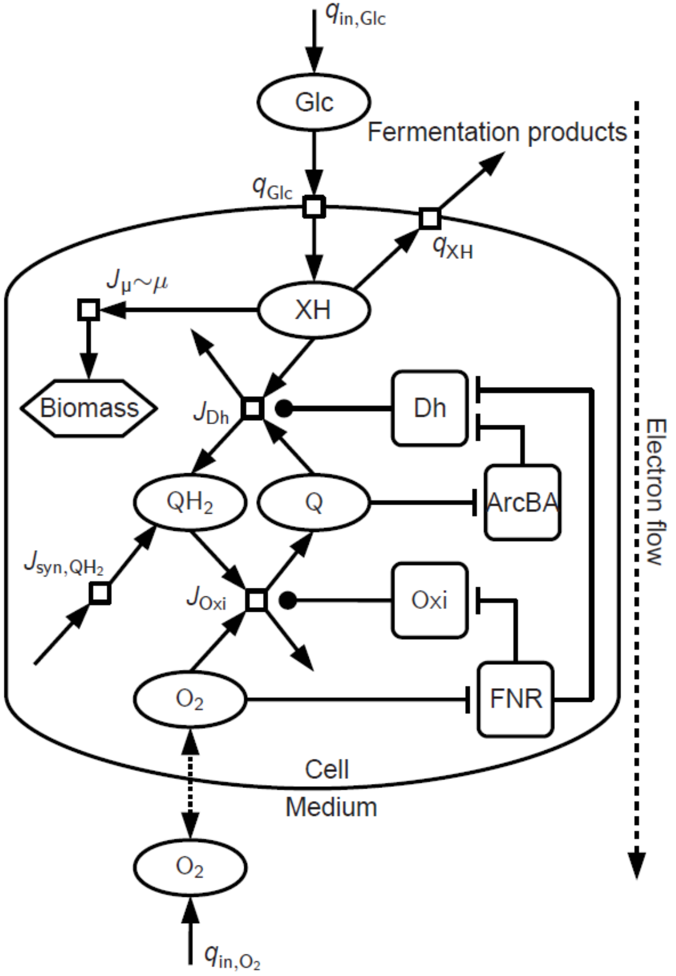


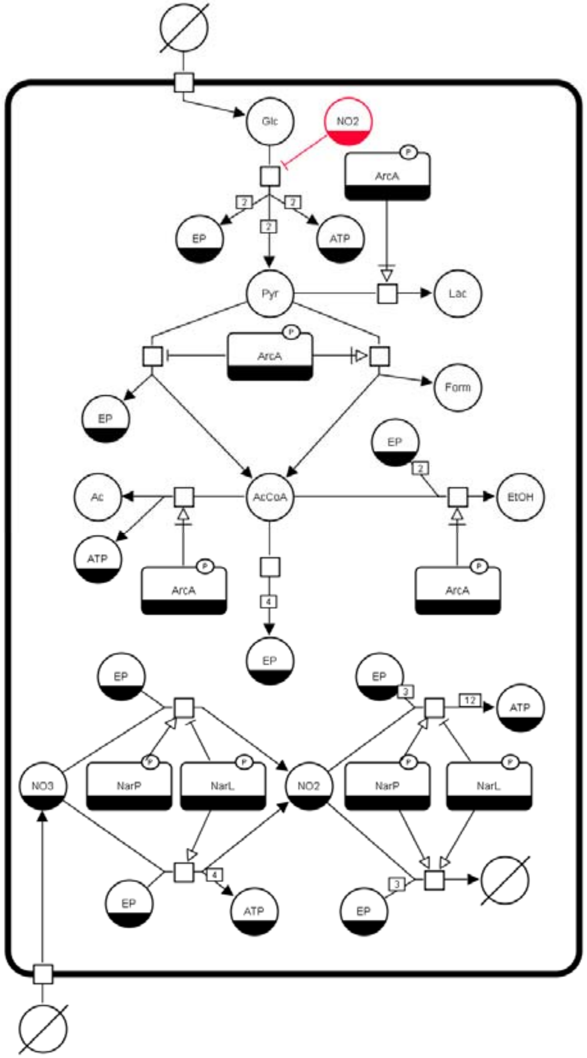
Regulatory Structure of the ETC



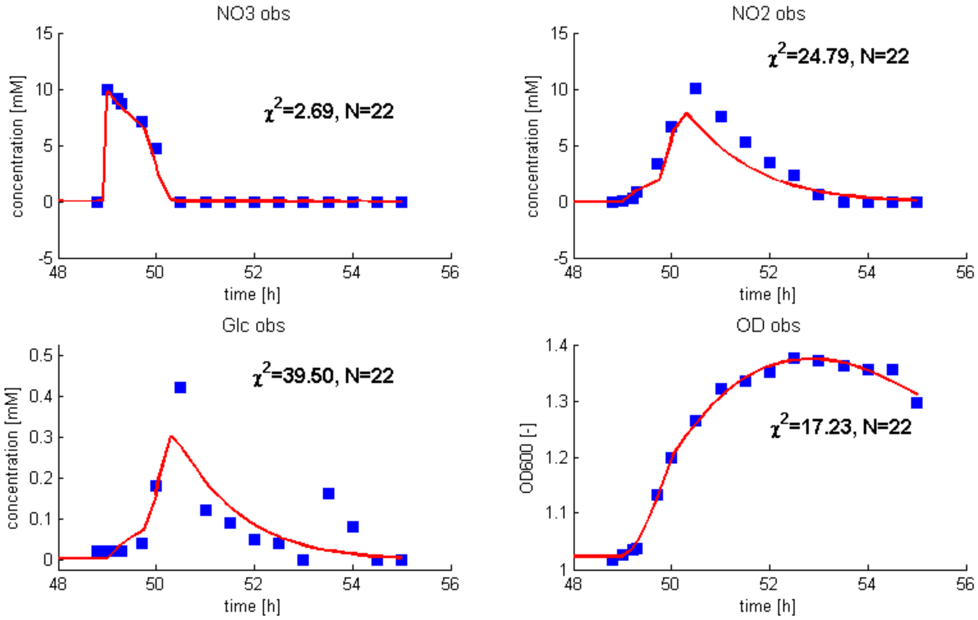
Conclusions

- Model compares the wild-type behaviour for Alexeeva vs SUMO conditions
- Experimental Data is consistent with the assumption oxidized quinones ─┤ ArcA (no influence of reduced quinones)
- Qualitative differences of quinone and ArcA data between growth conditions …
- … can be explained by quantitative differences in dilution rate and quinone synthesis rate

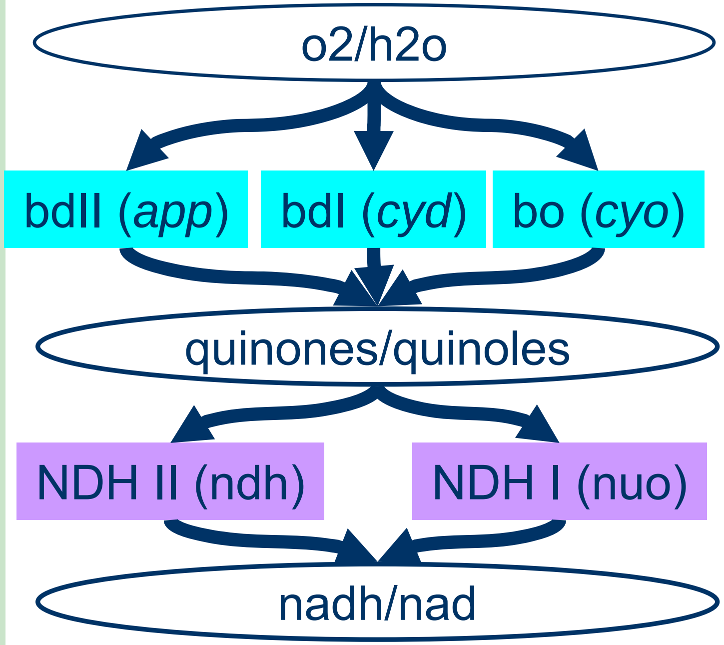
Nitrate and Nitrite Respiraton



- model structure adapted to nitrate/nitrite respiration
- nitrate pulse to anaerobic chemostat
- model-based analysis of different hypotheses



Linear Electron Transport Chains



o2/h2o

bdII (*app*)

bdI (*cyd*)

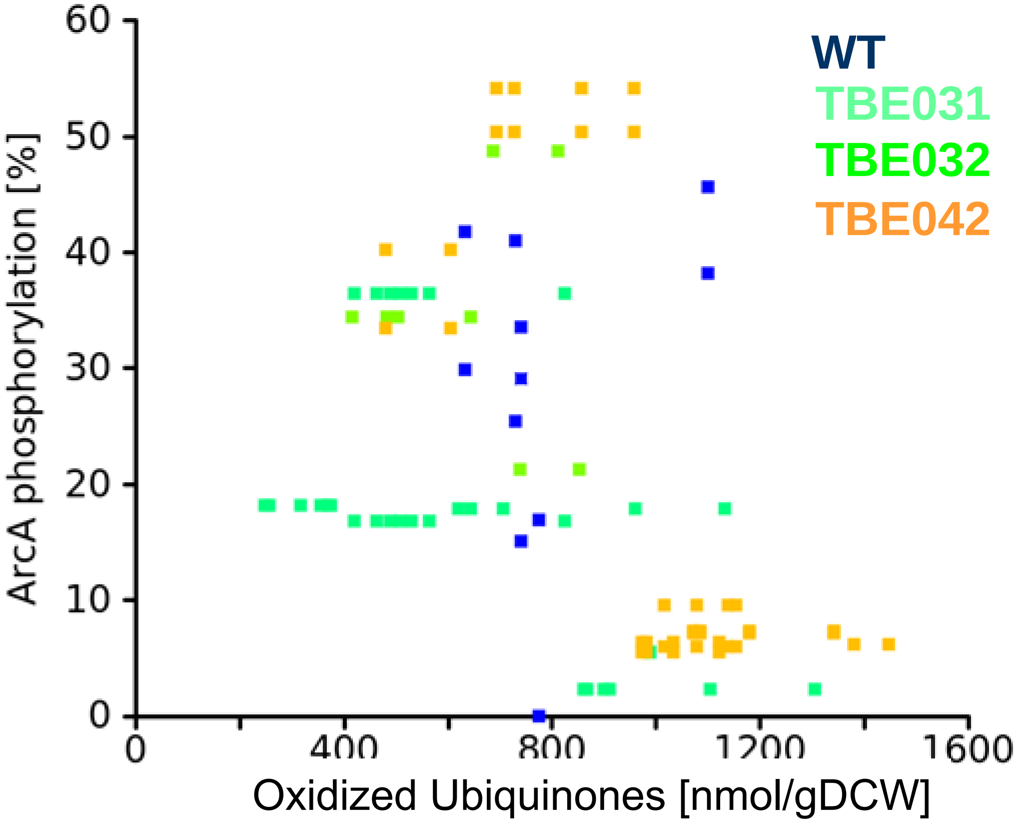
bo (*cyo*)

quinones/quinoles

NDH II (ndh)

NDH I (nuo)

nadh/nad

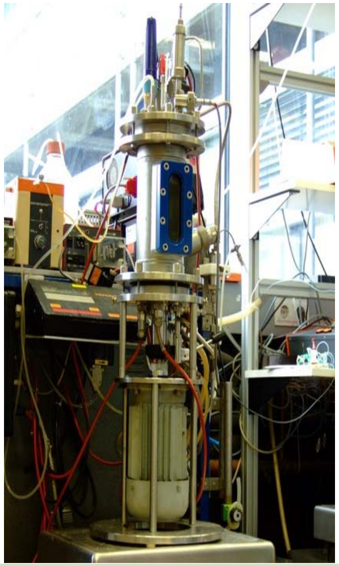


	NDH I	NDH II	bo	bdI	bdII
WT	+	+	+	+	+
TBE031	-	+	+	-	-
TBE032	-	+	-	-	+
TBE042	-	+	-	+	-
TBE029	-	+	+	+	+

- ArcA signal is clearly more complex than just q8.
- Bekker et al. (J. Bac, 2010) show involvement of menaquinones
- Involvement of metabolites (e.g. acetate) was hypothesized in the literature

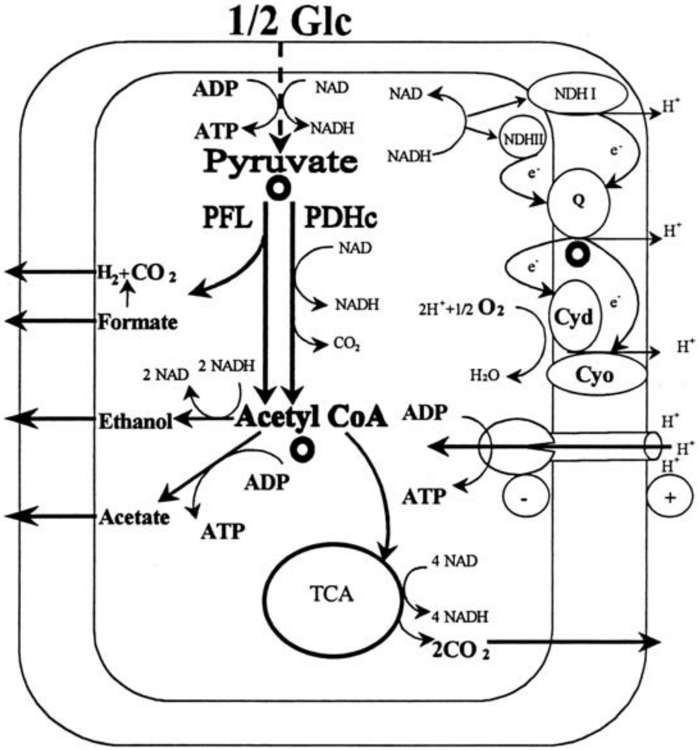
Modelling the Central Metabolism

- Goal:
  to provide a physically and logically coherent framework in order to check the consistency of different types of measurement data and our understanding of the network.



Scope of model:

- Central metabolism (Glycolysis, ETC, PPP, TCA, fermentation pathways)
- Regulation (enzymatic, genetic)
- Cellular Growth overall reaction: precursor → biomass
- Chemostat dynamics



(Source: Alexeeva et al., 2000)

Thermodynamic Consistency

- Thermodynamically consistent modelling is crucial to avoid spurious model variants
- Thermodynamic-Kinetic Modeling
  (TKM, Ederer & Gilles, Biophys J, 2007)
- Thermodynamic Data (Alberty, Thermodyn. of Bioch. Reac.,2003)

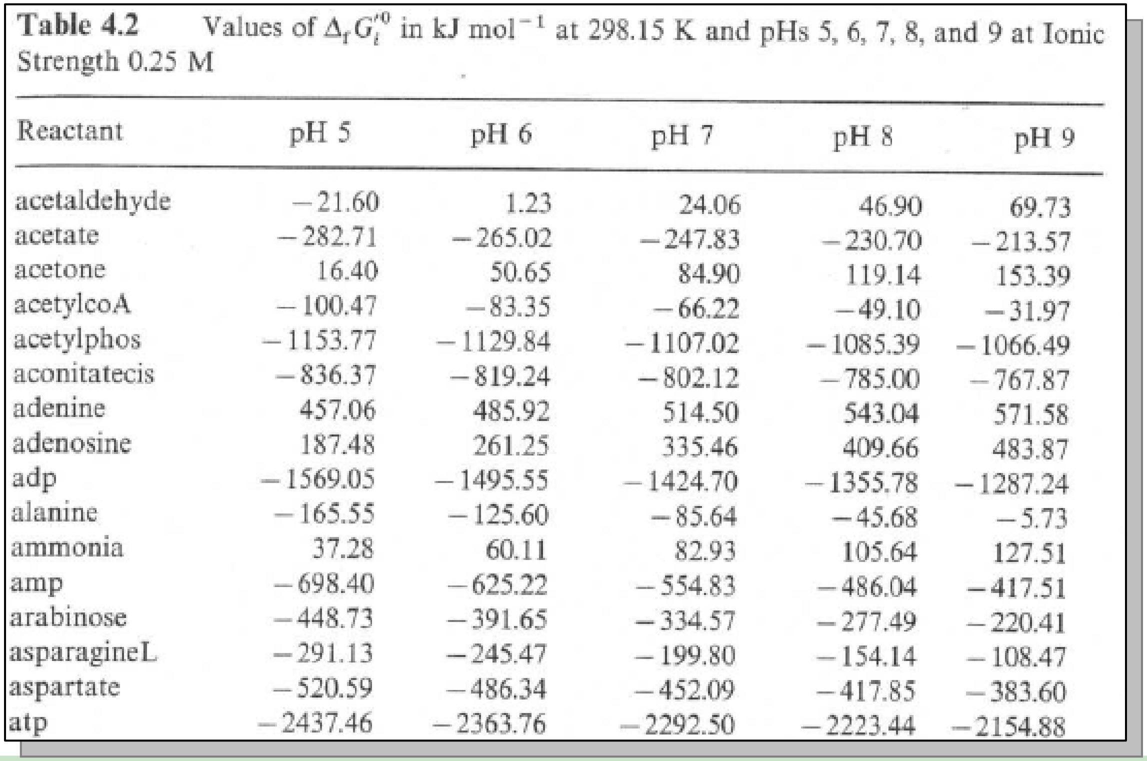


Table 4.2 Values of $\Delta_f G_i'^0$ in kJ mol^{-1} at 298.15 K and pHs 5, 6, 7, 8, and 9 at Ionic Strength 0.25 M

Reactant	pH 5	pH 6	pH 7	pH 8	pH 9
acetaldehyde	−21.60	1.23	24.06	46.90	69.73
acetate	−282.71	−265.02	−247.83	−230.70	−213.57
acetone	16.40	50.65	84.90	119.14	153.39
acetylcoA	−100.47	−83.35	−66.22	−49.10	−31.97
acetylphos	−1153.77	−1129.84	−1107.02	−1085.39	−1066.49
aconitatecis	−836.37	−819.24	−802.12	−785.00	−767.87
adenine	457.06	485.92	514.50	543.04	571.58
adenosine	187.48	261.25	335.46	409.66	483.87
adp	−1569.05	−1495.55	−1424.70	−1355.78	−1287.24
alanine	−165.55	−125.60	−85.64	−45.68	−5.73
ammonia	37.28	60.11	82.93	105.64	127.51
amp	−698.40	−625.22	−554.83	−486.04	−417.51
arabinose	−448.73	−391.65	−334.57	−277.49	−220.41
asparagineL	−291.13	−245.47	−199.80	−154.14	−108.47
aspartate	−520.59	−486.34	−452.09	−417.85	−383.60
atp	−2437.46	−2363.76	−2292.50	−2223.44	−2154.88

Ex.:

$$A + B \underset{}{\overset{J}{\rightleftharpoons}} C$$

$$C_i \xi_i = c_i$$

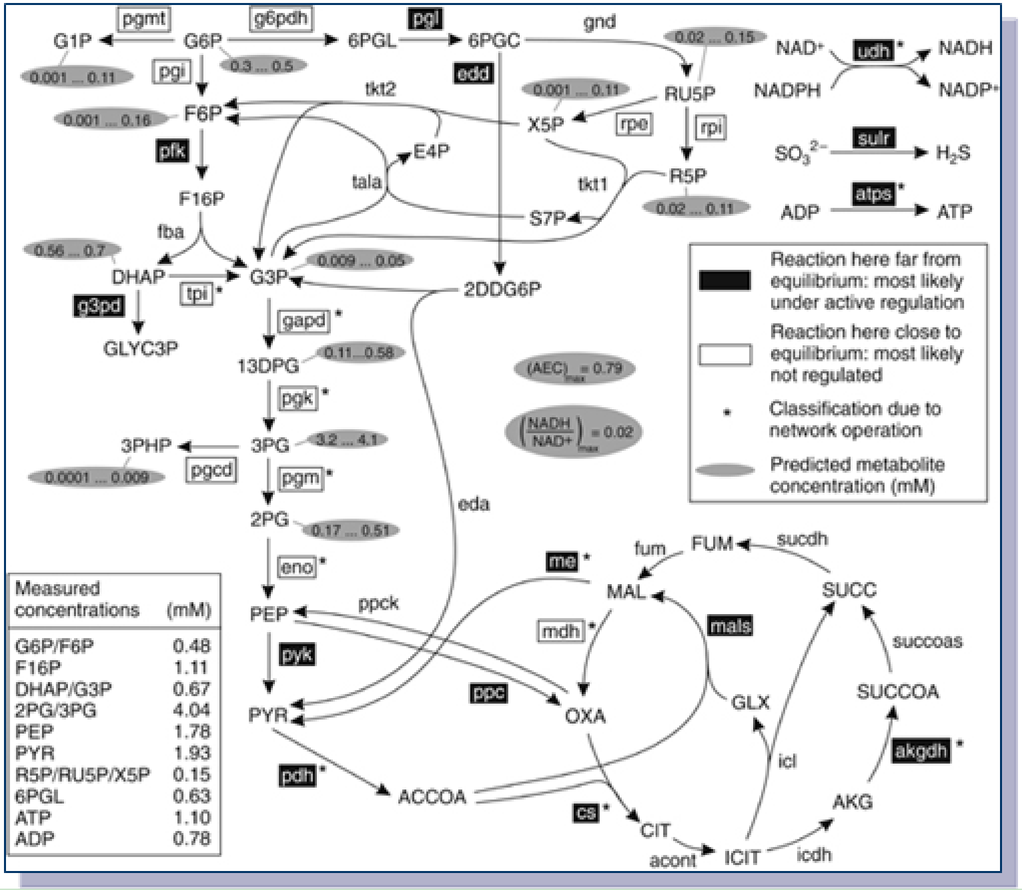
$$R_j J_j = \xi_A \xi_B - \xi_C$$

for $i \in \{A, B, C\}$:

$$C_i = c^o \exp\left(-\frac{\Delta_f G_i'^o}{R^* T} \right)$$

Model-Reduction by Rapid Equilibrium

- Stiffness of the model → slow simulation & numerical instability
- Model reduction by separation of time scales reduces stiffness and numbers of variables and parameters



■ Reaction here far from equilibrium: most likely under active regulation

□ Reaction here close to equilibrium: most likely not regulated

* Classification due to network operation

● Predicted metabolite concentration (mM)

Measured concentrations	(mM)
G6P/F6P	0.48
F16P	1.11
DHAP/G3P	0.67
2PG/3PG	4.04
PEP	1.78
PYR	1.93
R5P/RU5P/X5P	0.15
6PGL	0.63
ATP	1.10
ADP	0.78

Kümmel et al., MSB, 2006:

Classification of reactions:

- ➢ near (open boxes)
- ➢ far (filled boxes)

from equilibrium

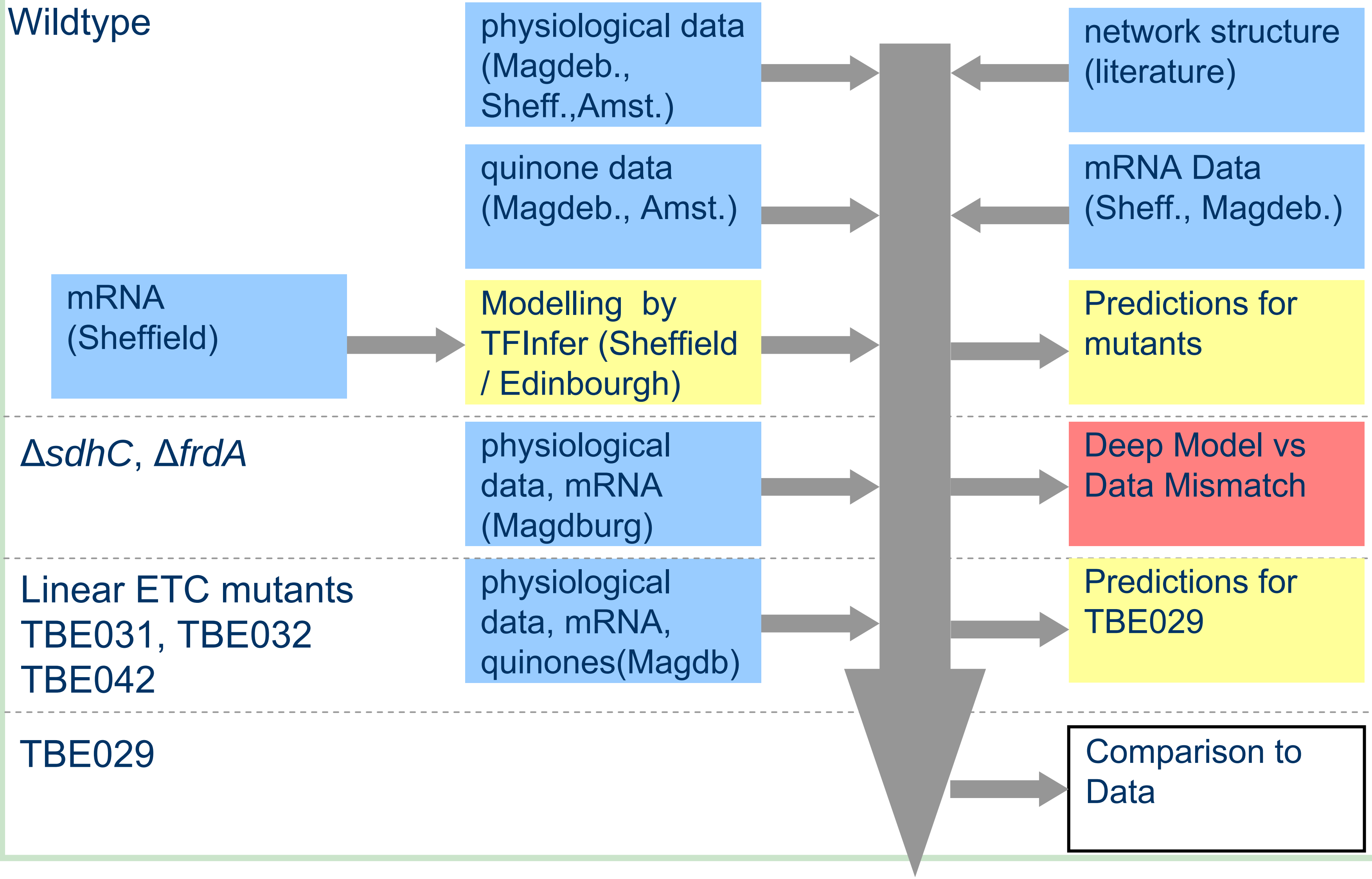
A Global Model of Central Metabolism Regulation

Mathematical Form

1. Textual model description file
2. Symbolic Preprocessing (Model Reduction)
3. Translation into differential-algebraic equations
4. Simulation with DASKR (FORTRAN)

```
Flux["CYTBD2",
     "Educts"           -> 2*P["BD2_H","Value"->2] "h" + "o2" + 2 "q8h2",
     "Products"         -> 2*P["BD2_H"] "h(p+)"+  2 * "h2o" + 2* "q8",
     "Name"             -> "cytochrome oxidase bd2",
     "Subsystem"        -> "Oxidative phosphorylation",
     "Constraint"       -> J[]>=0,
     "EC"               -> "1.10.3.-",
     "Link"             -> {"EcoCyc"->"http://www.ecocyc.org/ECOLI/NEW-IMAGE?type=REACTION&obj
     "Compartment"      -> "cm",
     "Resistance"       -> P["CYTBD2_R","Value"->1]/c["E-CYTBD2"][t]/(Cap["h"]^P["BD2_H"]*Cap[
Compound["E-CYTBD2",
         "Compartment"         -> "cm",
         "Name"                -> "enzyme of bd2",
         "Link"             -> {"EcoCyc"->"http://www.ecocyc.org/ECOLI/NEW-IMAGE?type=ENZYME&o
         "Capacity"            -> 1,
         "Potential"           -> P["E-CYTBD2_IC","Value"->1.0],
         "Scale"               -> 1],
Flux["E-CYTBD2-syn",
     "Educts"           -> 0 ,
     "Products"         -> "E-CYTBD2",
     "Name"                -> "enzyme synthesis",
     "Compartment"         -> "c", (*FNR dependency not in literature*)
     "Flux"                -> s[P["CYTBD2_ArcA","Value"->1],c["ArcA"][t]]*s[P["CYTBD2_AppY","V
     "Clamped"             -> True],
```

Modelling the Central Metabolism



Wildtype

physiological data (Magdeb., Sheff.,Amst.)

network structure (literature)

quinone data (Magdeb., Amst.)

mRNA Data (Sheff., Magdeb.)

mRNA (Sheffield)

Modelling by TFInfer (Sheffield / Edinbourgh)

Predictions for mutants

ΔsdhC, *ΔfrdA*

physiological data, mRNA (Magdburg)

Deep Model vs Data Mismatch

Linear ETC mutants TBE031, TBE032 TBE042

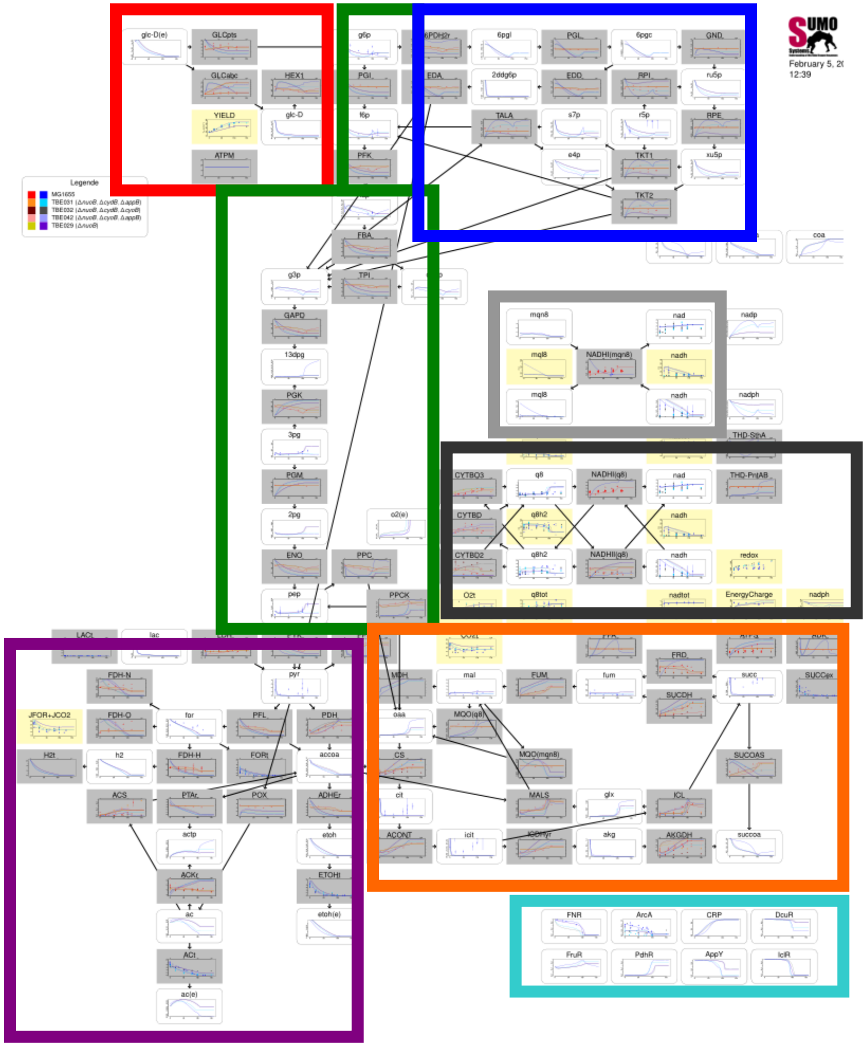
physiological data, mRNA, quinones(Magdb)

Predictions for TBE029

TBE029

Comparison to Data

Comprehensive Metabolism Model Overview



Glucose Uptake

Glycolysis

Pentosephosphate Pathway

Fermentation Pathways

Citric Acid Cycle

Electron Transport Chain (ubiquinone)

Electron Transport Chain (menaquinone)

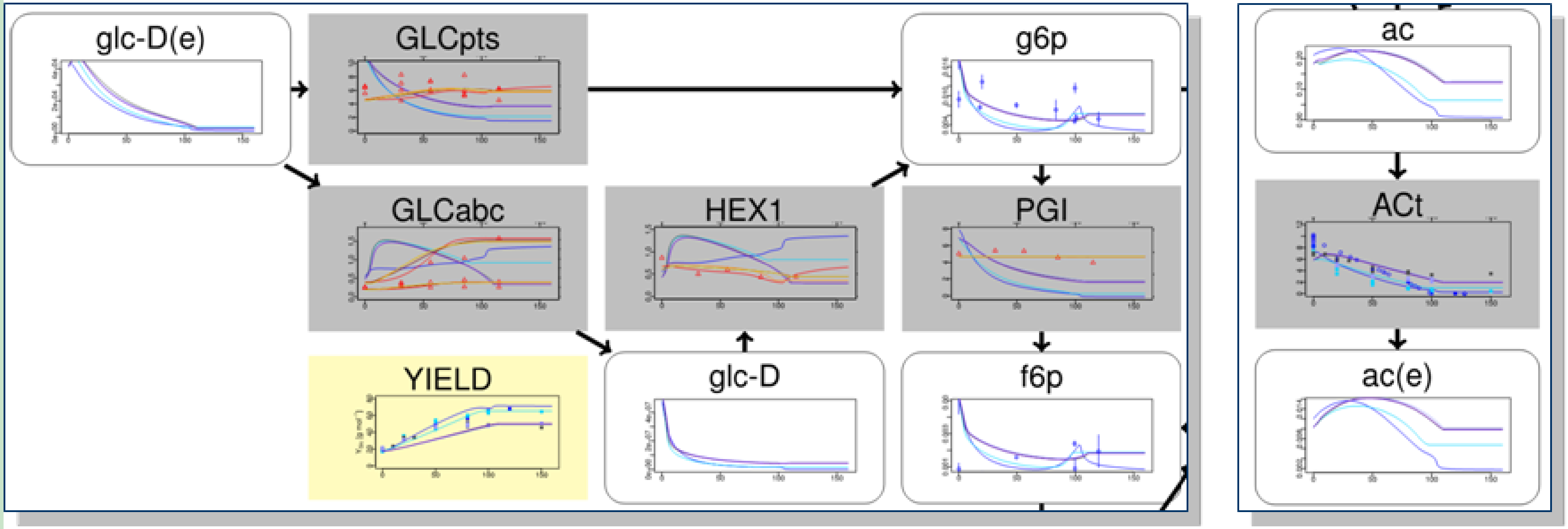
Transcription Factor Activities

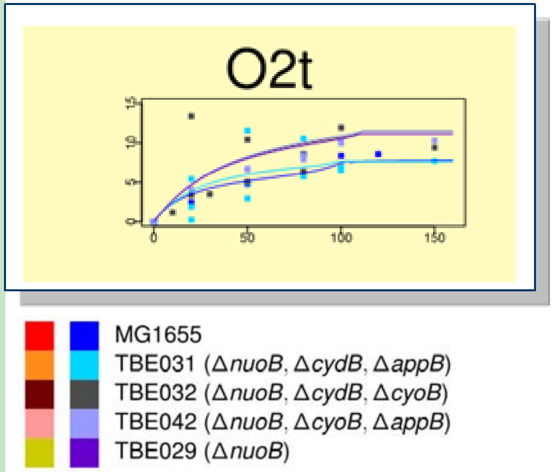
gene expression

Concentrations & Fluxes

gene expression	Concentrations & Fluxes	
🟥	🟦	MG1655
🟧	🟦	TBE031 (Δ*nuoB*, Δ*cydB*, Δ*appB*)
🟫	⬛	TBE032 (Δ*nuoB*, Δ*cydB*, Δ*cyoB*)
🟥	🟪	TBE042 (Δ*nuoB*, Δ*cyoB*, Δ*appB*)
🟨	🟪	TBE029 (Δ*nuoB*)

Comprehensive Metabolism Model – Transport & Growth

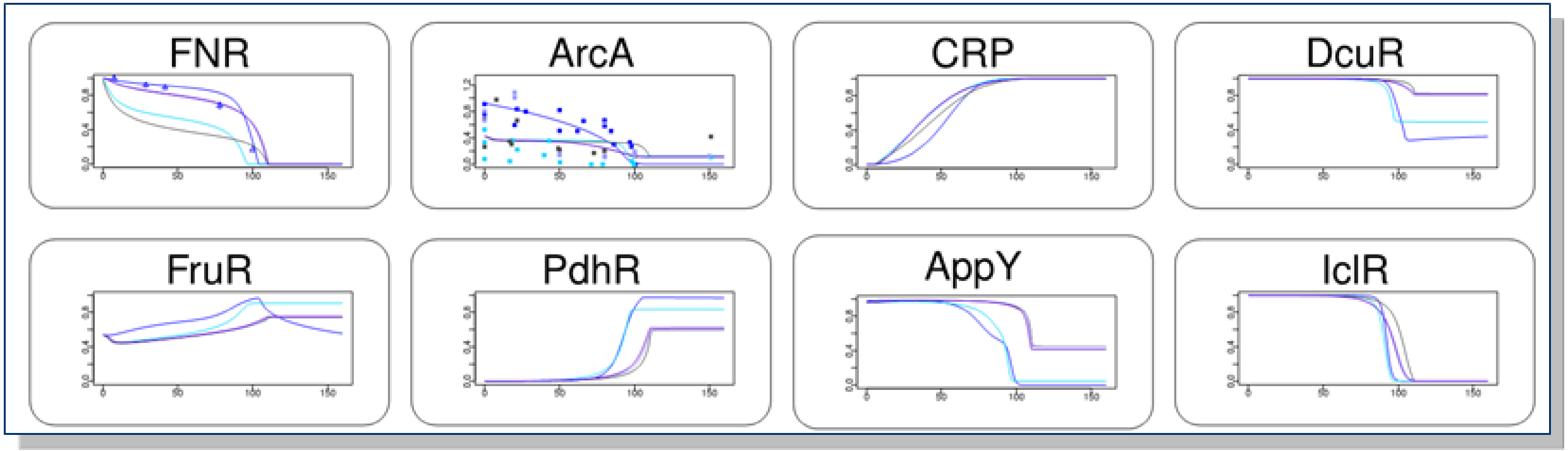




- Transport and growth fluxes can be reproduced qualitatively and to some extent also quantitatively

- Many features of these solutions are robust with respect to model assumptions (e.g. parameter values)

Comprehensive Metabolism Model – Genetic Regulation



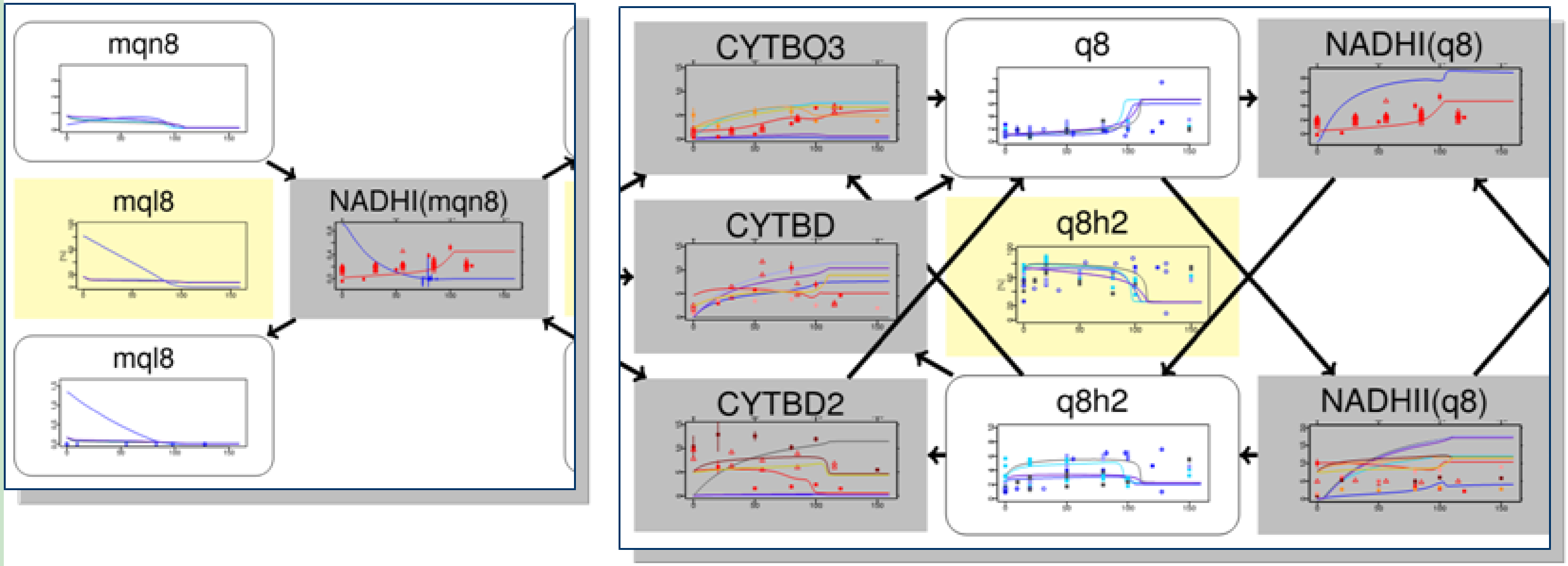
🟥 🟦 MG1655
🟧 🟦 TBE031 (Δ*nuoB*, Δ*cydB*, Δ*appB*)
🟫 ⬛ TBE032 (Δ*nuoB*, Δ*cydB*, Δ*cyoB*)
🟥 🟪 TBE042 (Δ*nuoB*, Δ*cyoB*, Δ*appB*)
🟨 🟪 TBE029 (Δ*nuoB*)

- Model provides (partly robust) predicitions on transcription factor activities

- In this model version: ArcA signal dependent on menaquinones and ubiquinones

- Measured ArcA activity remains a mystery (different anaerobic ArcA activity in strains differing only in oxidase)

Comprehensive Metabolism Model – Electron Transport Chains



🟥 🟦 MG1655
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🟫 ⬛ TBE032 (Δ*nuoB*, Δ*cydB*, Δ*cyoB*)
🟥 🟪 TBE042 (Δ*nuoB*, Δ*cyoB*, Δ*appB*)
🟨 🟪 TBE029 (Δ*nuoB*)

- Quinone and quinol pools can only partly be explained.
- This is related to our incomplete knowledge of the ArcA signal
- Ongoing work: Find a phenomenological ArcA model explaining the data.
  - influence of metabolites (e.g. acetate)
  - influence of oxidase concentrations

Conclusions

- Major components of redox regulation in *E. coli* are not well understood → SUMO models provide tools to tackle these questions in a systemic way

- Modelling in SUMO allows
  - integrating different data types (metabolites, mRNA, transcription factors, fluxes)
  - integrating information from different mutants

  into a physically and logically consistent framework.

- The modelling process
  - reveals inconsistencies and gaps in our understanding of the network
  - provides a framework for an consistent testing of hypotheses (e.g. the signal of ArcAB, nitrite inhibition of glucose transport)

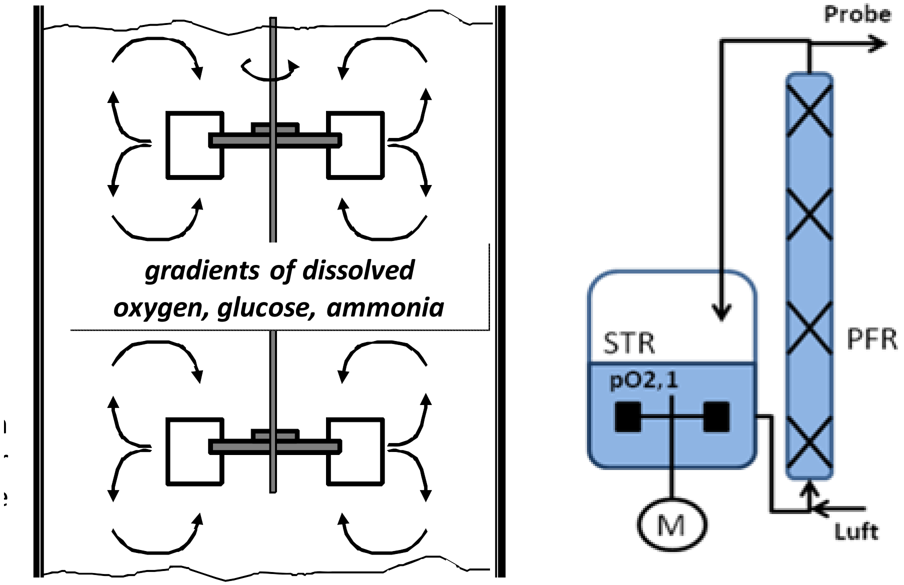
- The model suggested and suggests experiments to test the hypotheses and is able to evaluate the experimental data (e.g. new experimental data on menaquinone redox state is on the way)

Follow-up Project: RecogNize

- Models will be used in

  RecogNice: Modeling Regulation of Carbon, Oxygen and Nitrogen in Large-Scale Processes with E. coli

  Cooperation project with IBVT, IMB (University of Stuttgart) and IMG (University of Tübingen)



- Funded in BMBF initiative e:Bio

Projektträger Jülich
Forschungszentrum Jülich

Bundesministerium
für Bildung
und Forschung

SUMO Systems
Understanding of Microbial Oxygen Responses

Thank you!

SysMO
Systems Biology of Microorganisms

PtJ
Projektträger Jülich
Forschungszentrum Jülich

NWO
Netherlands Organisation for Scientific Research

Federal Ministry of Education and Research

BBSRC
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