MAX PLANCK INSTITUTE FOR DYNAMICS OF COMPLEX TECHNICAL SYSTEMS MAGDEBURG

ARB ANALYSIS AND REDESIGN OF BIOLOGICAL NETWORKS

Network-wide Thermodynamic Constraints Shape NAD(P)H Cofactor Specificity of Metabolic Reactions

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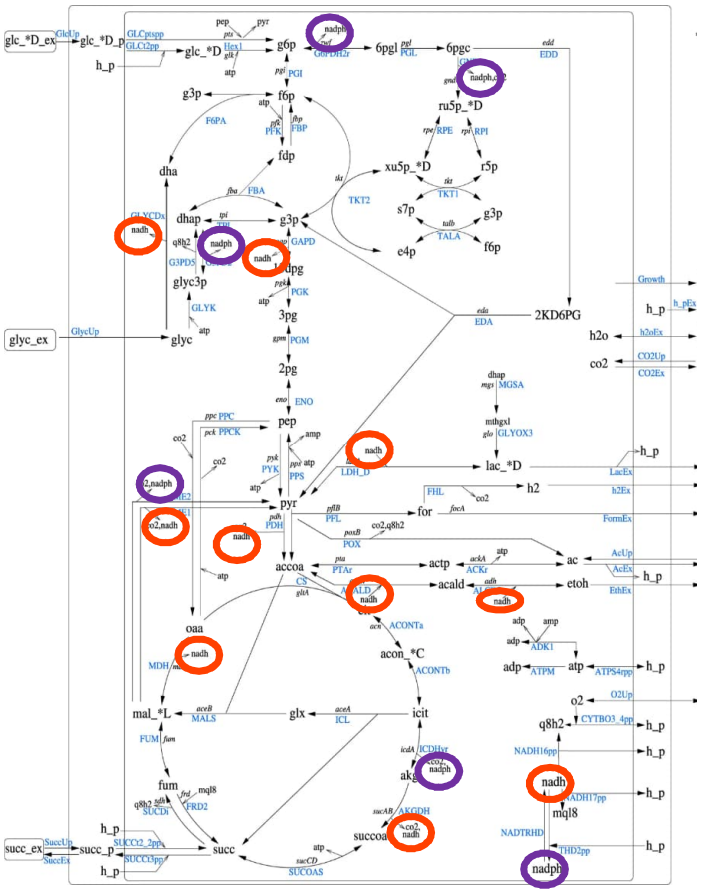
Max Planck Institute for Dynamics of Complex Technical Systems
Research Group „Analysis and Redesign of Biological Networks“

11/15/2023

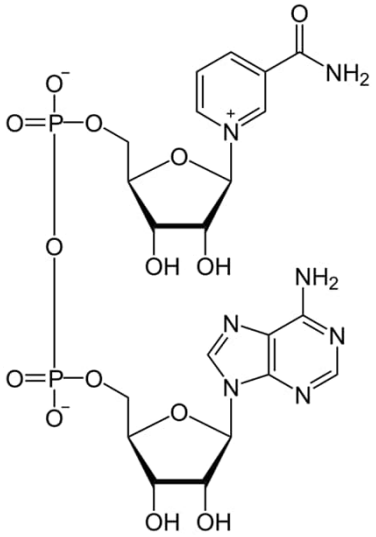
NADH / NADPH: Two ubiquitous redox cofactors

Central metabolism of E. coli:



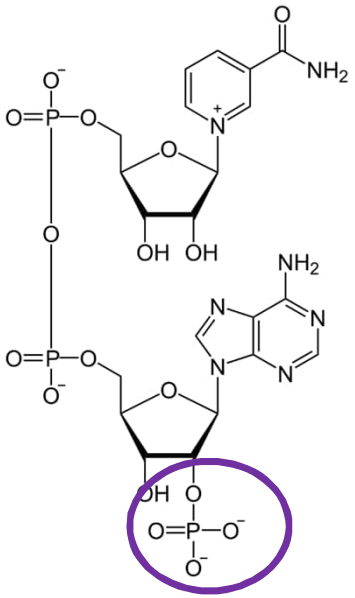
E. coli (iML1515): 128 reactions use NAD(H), 110 reactions use NADP(H), 6 reactions use both.

Nicotinamide adenine dinucleotide (NAD^+)



$NAD^+ + 2\ e^- + H^+ \leftrightarrow NADH$
$(\Delta E'° = -320\ mV)$

Nicotinamide adenine dinucleotide phoshpate ($NADP^+$)



$NADP^+ + 2\ e^- + H^+ \leftrightarrow NADPH$
$(\Delta E'° = -320\ mV)$

NADH / NADPH: Two ubiquitous redox cofactors

Why two pools of (very similar) redox cofactors?

Simultaneous operation of oxidation and reduction reactions!

$[NAD^+] \gg [NADH]$

in vivo $NADH/NAD^+$ **ratio of ≈0.03**
in *E. coli* (aerobic, Bennet et al., 2009)

Catabolism:

$A + NAD^+ \xrightarrow{favored} B + NADH$

$D + NAD^+ \xleftarrow[less\ favored!]{} C + NADH$

$[NADP^+] \ll [NADPH]$

in vivo $NADPH/NADP^+$ **ratio of ≈57**
in *E. coli* (aerobic, Bennet et al., 2009)

Anabolism:

$V + NADPH \xrightarrow{favored} X + NADP^+$

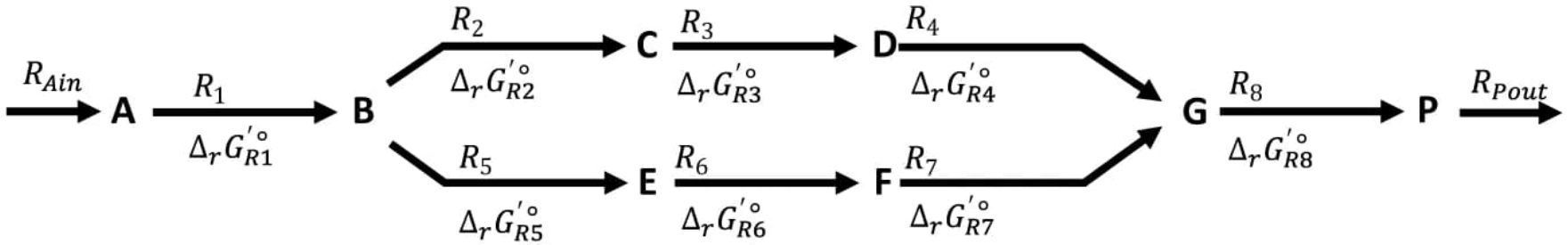
$Z + NADPH \xleftarrow[less\ favored!]{} Y + NADP^+$

- Are two pools of redox cofactors really advantageous?
- What shapes the NAD(P)(H) reaction specificities in the network?

→ *Hypothesis*: **NAD(H)** and **NADP(H)** reaction specificities are distributed such that the **network-wide thermodynamic driving force** for growth is optimized.

Driving Forces of Reactions and Pathways

Example network with (given) standard Gibbs free energies of reactions:



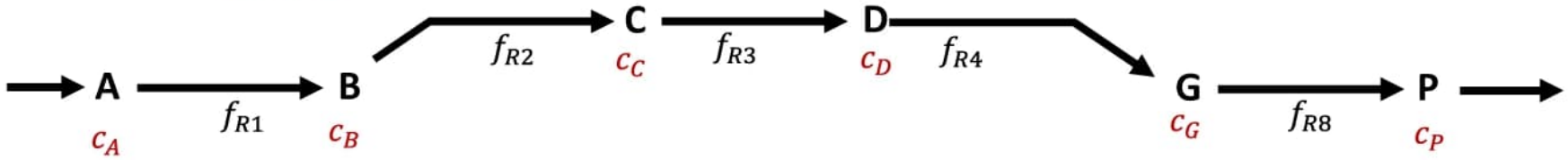
Driving force of a single reaction: $f_i = -\Delta_r G_i' = -\Delta_r G_i'^{\circ} - R \cdot T \cdot \sum_j N_{ji} \cdot \ln(c_j)$

Example: $R_1 (A \rightarrow B)$ with fixed concentrations: $f_{R1} = -\Delta_r G'^{\circ}_{R1} - R \cdot T \cdot (-\ln(c_A) + \ln(c_B))$

Red: given
Blue: calculated

Driving force of a specific pathway: minimum driving force of all reactions of the pathway

$f_{Pathway} = \min(f_{R1}, f_{R2}, f_{R3}, f_{R4}, f_{R8})$ with fixed concentrations

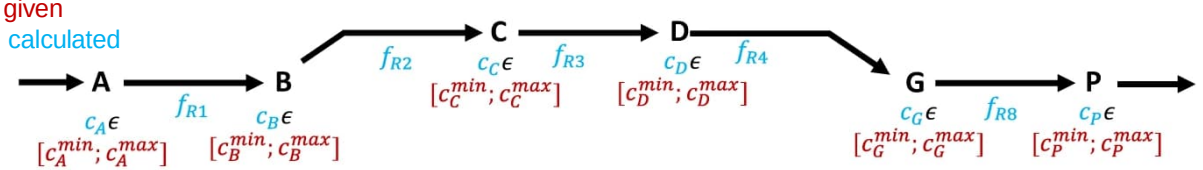


Max-min Driving Force (MDF) of a Pathway and Network

Max-min driving force (MDF) of a pathway: maximal achievable driving force of the pathway

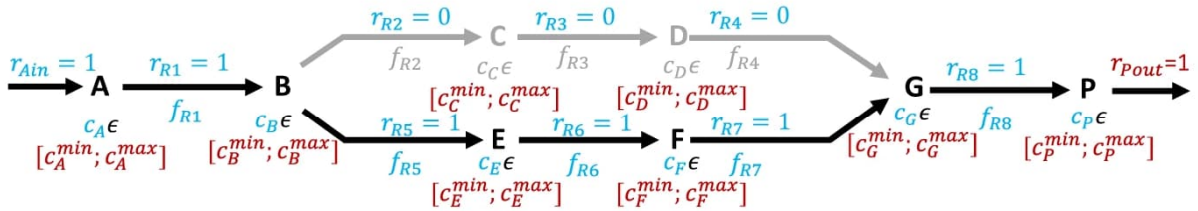
$MDF_{Pathway} = \max(f_{Pathway}) = \max(\min(f_{R1}, f_{R2}, f_{R3}, f_{R4}, f_{R8}))$ within given concentration ranges

Red: given
Blue: calculated



MDF of network (also called OptMDF): find flux distribution $\mathbf{r}$ maximizing MDF of active reactions

$MDF_{Network} = \max(MDF(\mathbf{r}))$ with given concentration ranges and flux constraints



(Noor et al., 2014)

(Hädicke et al., 2018)
→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

Max-min Driving Force (MDF) in a Network

MDF in a network (Hädicke et al., 2018)

→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

$$\underset{\mathbf{x},\mathbf{r},\mathbf{z}}{Maximize} \quad B$$

$$s.t.$$

$$\mathbf{Nr} = \mathbf{0}$$

$$\alpha_i \leq r_i \leq \beta_i$$

$$\ln(\mathbf{c_{min}}) \leq \mathbf{x} \leq \ln(\mathbf{c_{max}})$$

$$r_i \leq z_i \cdot \beta_i$$

$$z_i \in \{0,1\}$$

$$f_i = -\Delta_r G_i' = -\Delta_r G'^{\circ} - RT \cdot (\mathbf{N}_{*,i})^T \cdot \mathbf{x}$$

$$B \leq f_i + M \cdot (1 - z_i), \qquad (M\ very\ large)$$

Max-min Driving Force (MDF) of a Pathway and Network

Max-min driving force (MDF) of a pathway: maximal achievable driving force of the pathway

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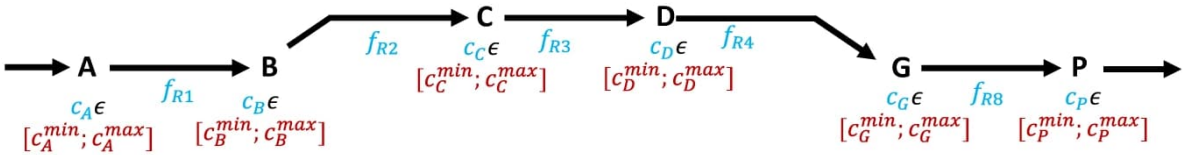

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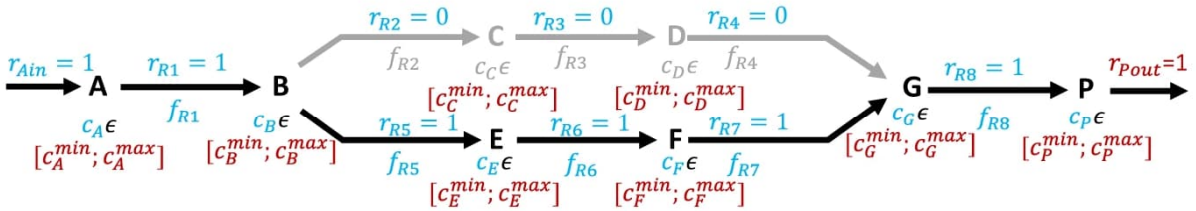
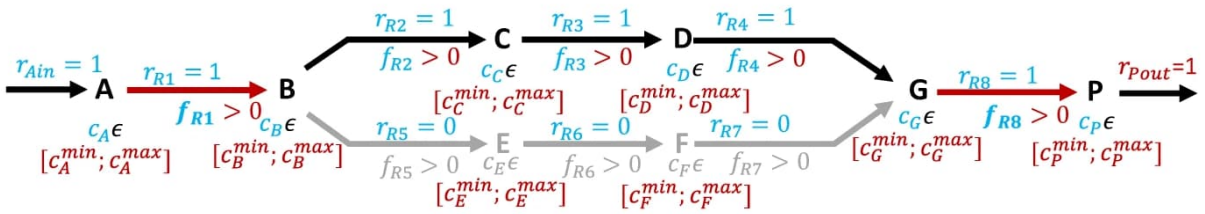


MDF of subnetwork (SubMDF): find flux distribution $\mathbf{r}$ maximizing MDF of selected set of reactions

e.g., SubMDF = MDF(R_1, R_8) with given concentration ranges and flux constraints
and demanded minimal MDF (e.g., >0 kJ/mol) of other active reactions



(Noor et al., 2013)

Metabolite concentrations under MDF minimize enzyme costs (neglecting saturation effects)

(Hädicke et al., 2018)
→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

(This work, 2023)
→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

Here: SubMDF with respect to NAD(P)(H)-dependent reactions

Max-min Driving Force in a Subnetwork (SubMDF)

MDF in a network (Hädicke et al., 2018)

→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

$$\underset{\mathbf{x},\mathbf{r},\mathbf{z}}{Maximize} \quad B$$

$$s.t.$$

$$\mathbf{Nr} = \mathbf{0}$$

$$\alpha_i \leq r_i \leq \beta_i$$

$$\ln(\mathbf{c_{min}}) \leq \mathbf{x} \leq \ln(\mathbf{c_{max}})$$

$$r_i \leq z_i \cdot \beta_i$$

$$z_i \in \{0,1\}$$

$$f_i = -\Delta_r G_i' = -\Delta_r G'^{\circ} - RT \cdot (\mathbf{N}_{*,i})^T \cdot \mathbf{x}$$

$$B \leq f_i + M \cdot (1 - z_i), \qquad (M\ very\ large)$$

SubMDF in a network (Bekiaris and Klamt, 2023)

→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

Maximize MDF for selected reactions → $\underset{\mathbf{x},\mathbf{r},\mathbf{z},B}{Maximize} \quad B_{sub}$

$$s.t.$$

$$\mathbf{Nr} = \mathbf{0}.$$

$$\alpha_i \leq r_i \leq \beta_i$$

$$\ln(\mathbf{c_{min}}) \leq \mathbf{x} \leq \ln(\mathbf{c_{max}})$$

$$r_i \leq z_i \cdot \beta_i$$

$$z_i \in \{0,1\}$$

$$f_i = -\Delta_r G_i' = -\Delta_r G'^{\circ} - RT \cdot (\mathbf{N}_{*,i})^T \cdot \mathbf{x}$$

$$B \leq f_i + M \cdot (1 - z_i), \qquad (M\ very\ large)$$

Thermodynamic feasibility of entire flux vector → $B \geq 0.1$

Minimum Driving force of the subset *S* of selected reactions → $B_{sub} \leq f_j + M \cdot (1 - z_j), \qquad \forall j \in S$

Max-min Driving Force (MDF) of a Pathway and Network

Max-min driving force (MDF) of a pathway: maximal achievable driving force of the pathway

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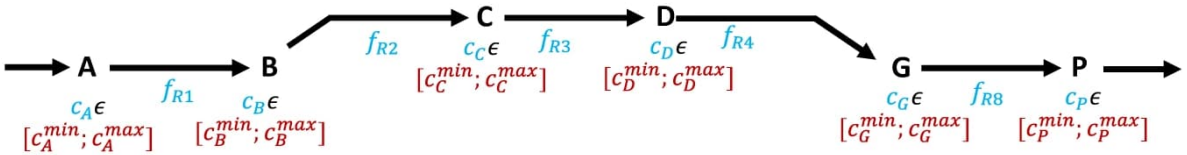

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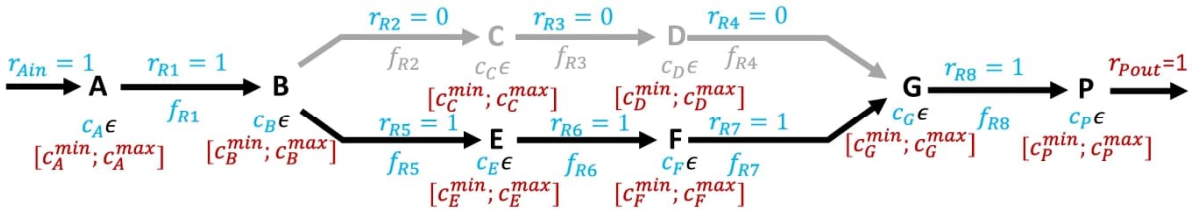

MDF of subnetwork (SubMDF): find flux distribution $\mathbf{r}$ maximizing MDF of selected set of reactions

e.g., SubMDF = MDF(R_1, R_8) with given concentration ranges and flux constraints
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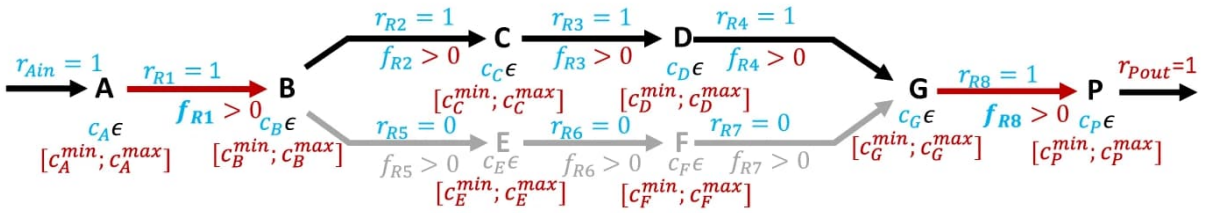


(Noor et al., 2013)

Metabolite concentrations under MDF minimize enzyme costs (neglecting saturation effects)

(Hädicke et al., 2018)
→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

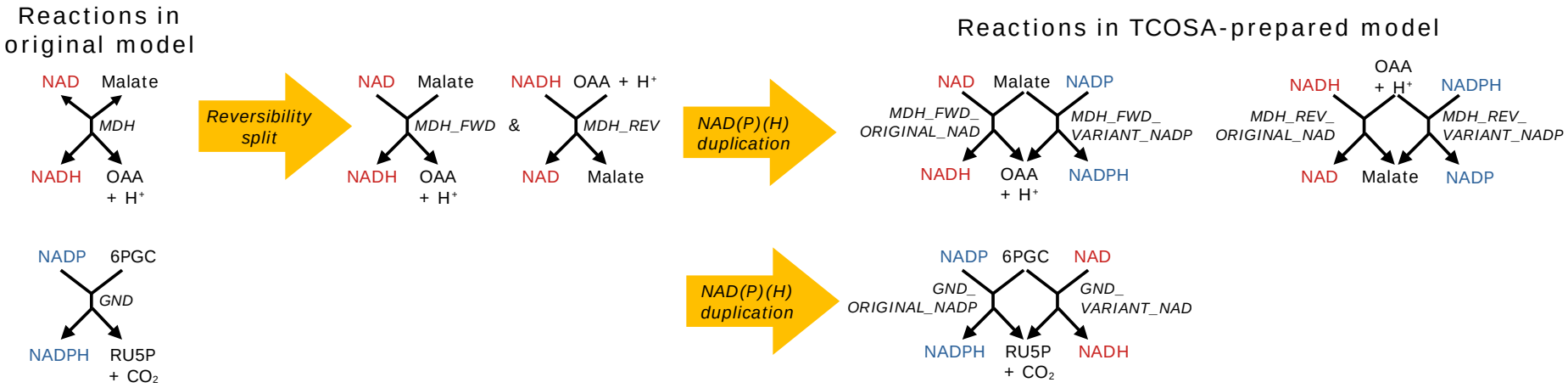
What NAD(P)(H) specificities maximize the MDF/SubMDF for growth-related flux distributions and how close is the wild-type specificity to this optimal specificty?

(This work, 2023)
→ Mixed-Integer Linear Program (MILP) within constraint-based metabolic model

Here: SubMDF with respect to NAD(P)(H)-dependent reactions

TCOSA: Thermodynamic Cofactor Swapping Analysis

Reconfiguration of a given (stoichiometric) metabolic model for TCOSA:



Application to *E. coli*

Resulting model: iML1515_TCOSA (derived from genome-scale *E. coli* model *i*ML1515; Monk *et al.*, 2017).

- Substrate: glucose. Aerobic ($+O_2$) and anaerobic ($-O_2$) conditions.
- Metabolite concentration ranges: [10^{-6}… 0.02 M]
- $\Delta_rG'^{\circ}$ values: from eQuilibrator (Flamholz *et al.*, 2012) via its Python API (Beber *et al.*, 2021)

Computing (Sub)MDF with Different NAD(P) Specificity Scenarios

Wild-type specificity: Use original NAD(P)(H) specificity for the NAD(P)(H)-dependent reactions

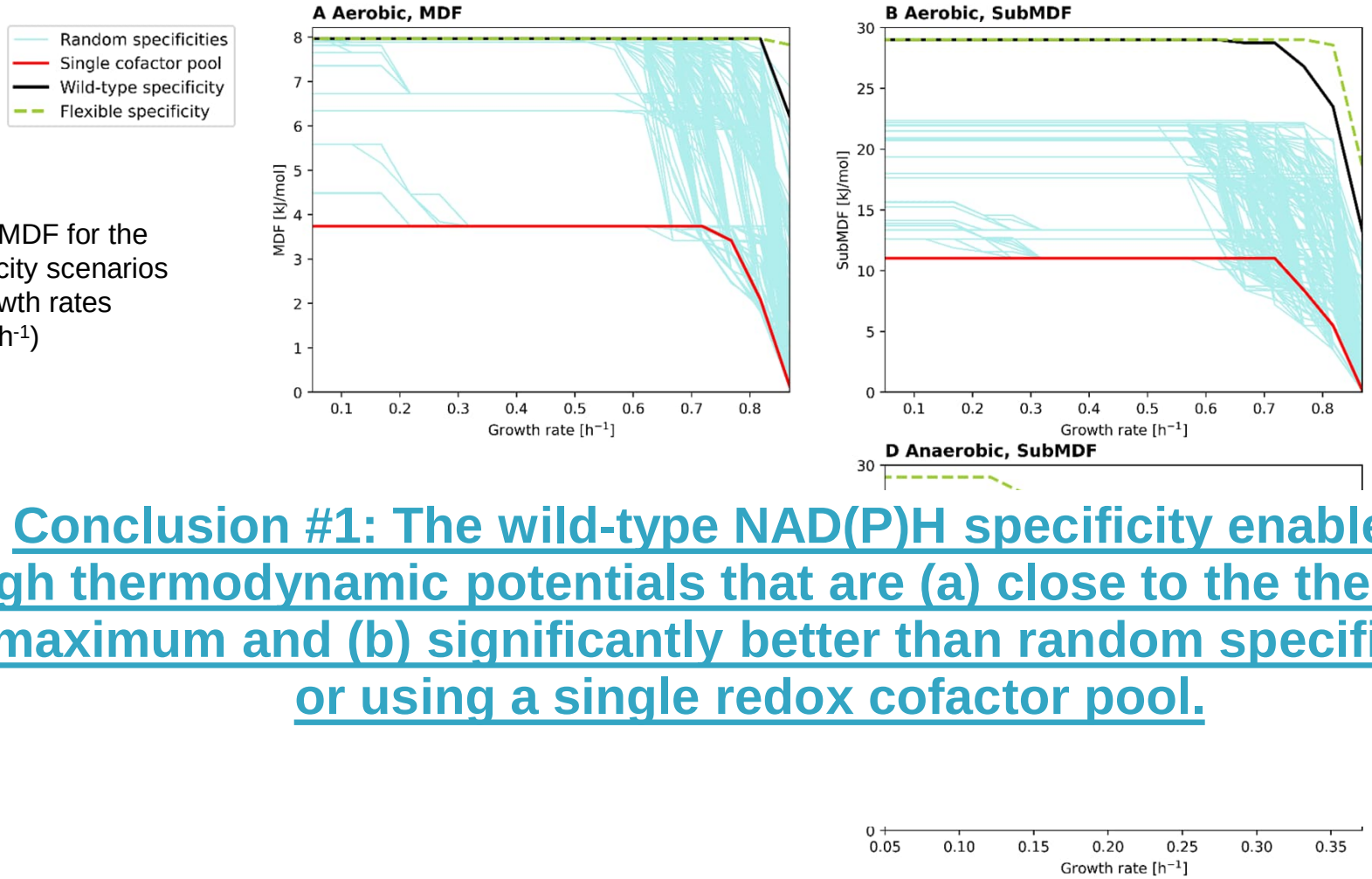
Flexible specificity: NAD(P)(H) specificity can be freely selected for each reaction (but only one at a time for each reaction)

Single cofactor pool: Only NAD(H)-dependent reactions can be used (NADP(H) not allowed)

Random specificity: 1'000 random specificities (stochastic coin flip to select NAD(H) or NADP(H) specificity for each redox-cofactor-dependent reaction)

Analysis 1: (Sub)MDF Results for the Different Specificity Scenarios

Compute (Sub)MDF for the different specificity scenarios for different growth rates (step size 0.05 h^{-1})



Conclusion #1: The wild-type NAD(P)H specificity enables high thermodynamic potentials that are (a) close to the theoretical maximum and (b) significantly better than random specificities or using a single redox cofactor pool.

Analysis 2: Necessary Swaps in Wild-type Specificity to Reach the Theoretical Maximal (Sub)MDF of the Flexible Specificity

Oxygen availability	Growth rate [h^{-1}]	Number of necessary swaps in wild type to reach (Sub)MDF of flexible specificity	
		MDF	SubMDF
Aerobic	0.868	6	2
Aerobic	0.818	0	3
Aerobic	0.768	0	2
Aerobic	0.718	0	2
Aerobic	0.668	0	0
Aerobic	0.618	0	0
Aerobic	0.568...0.518	0	0
Aerobic	0.468...0.118	0	0
Aerobic	0.068	0	0
Aerobic	0.05	0	0
Anaerobic	0.371	1	1
Anaerobic	0.321	0	0
Anaerobic	0.271	9	14
Anaerobic	0.221	0	2
Anaerobic	0.171	0	5
Anaerobic	0.121	0	4
Anaerobic	0.071	0	3
Anaerobic	0.05	0	3

Analysis 2: Necessary Swaps in Wild-type Specificity to Reach the Theoretical Maximal (Sub)MDF of the Flexible Specificity

Two frequently suggested cofactor swaps to increase (Sub)MDF:

1) Pyruvate dehydrogenase

CoA + pyruvate + NAD^+ → acetyl-CoA + CO_2 + NADH (ΔG'° of -34.37 kJ/mol)

⇨ CoA + pyruvate + $NADP^+$ → acetyl-CoA + CO_2 + NADPH

Synthesis of NADPH (thermodynamically unfavorable) in a reaction that has very negative ΔG'°.

2) Isocitrate dehydrogenase

isocitrate + $NADP^+$ → 2-oxoglutarate + CO_2 + NADPH (ΔG'° of +5.13 kJ/mol)

⇨ isocitrate + NAD^+ → 2-oxoglutarate + CO_2 + NADH

Use NAD^+ (thermodynamically favorable) instead of $NADP^+$ to overcome the positive ΔG'° of this reaction.
(**But: unfavorable when using acetate as substrate!**).

Analysis 3: Trends of NAD(P)(H) Concentration Ratios

Observed trends in *E. coli*:

$[NADH] << [NAD^+]$ $[NADPH] >> [NADP^+]$

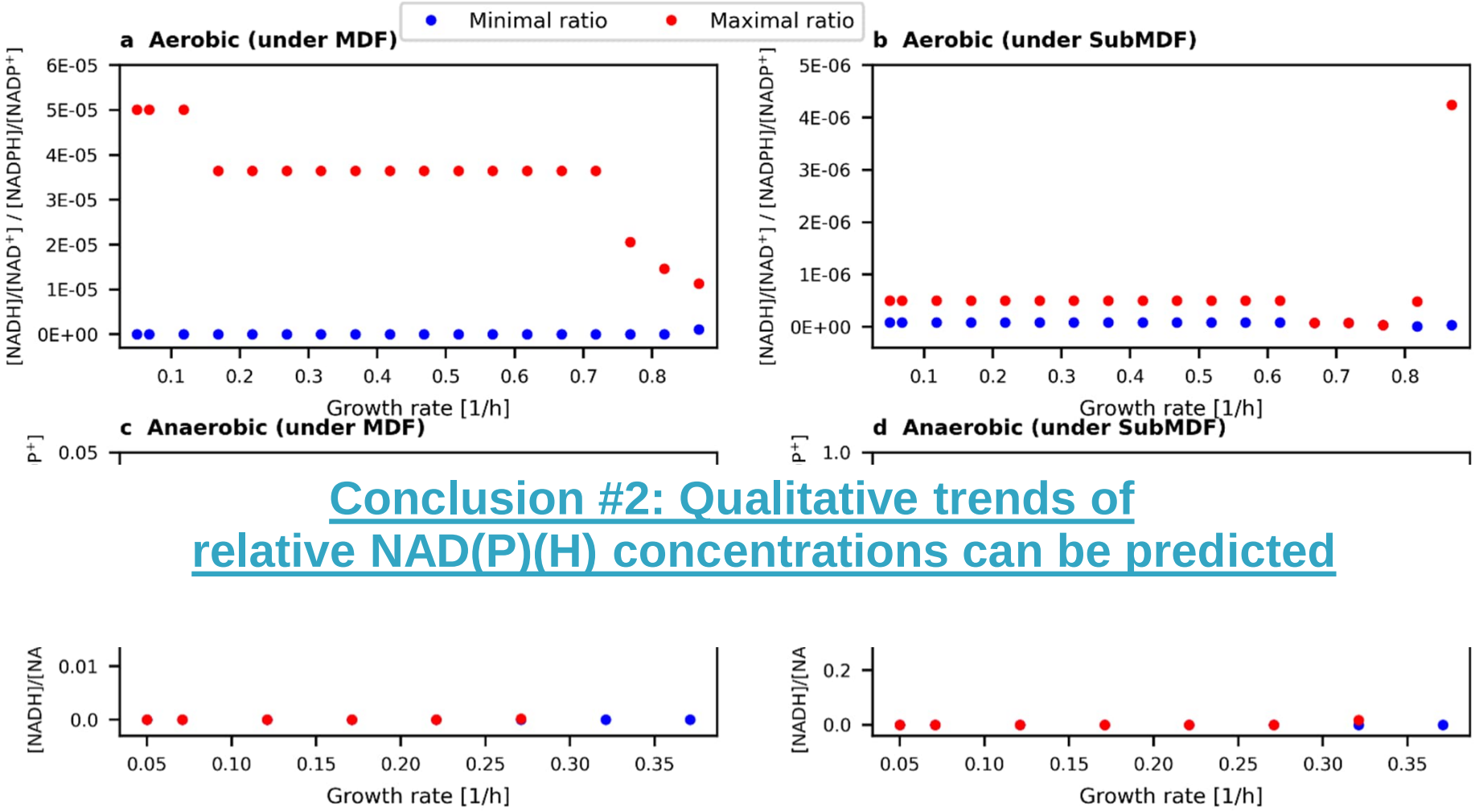
in vivo $NADH/NAD^+$ **ratio of ≈0.03** in *E. coli* (aerobic, Bennet et al., 2009)

in vivo $NADPH/NADP^+$ **ratio of ≈57** in *E. coli* (aerobic, Bennet et al., 2009)

$$Q = \frac{[NADH]/[NAD^+]}{[NADPH]/[NADP^+]} \ll 1$$

in vivo **≈0.00053** in *E. coli* (aerobic, Bennet et al., 2009)

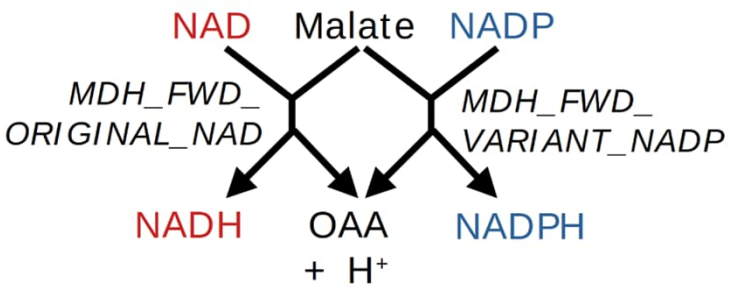
Analysis 3: Minimal/Maximal Concentration Ratios at Optimal (Sub)MDF (Wildtype Specificity)



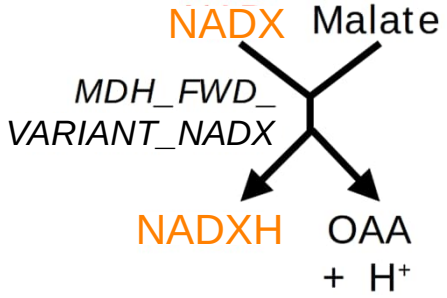
Conclusion #2: Qualitative trends of relative NAD(P)(H) concentrations can be predicted

Analysis 4: Effect of a Third Redox Cofactor Pool (Flexible Specificity)

NAD(H) NADP(H)



"NADX(H)"



NAD → NADH

Δ_fG'° difference of ca. 61 kJ/mol → Standard redox potential (ΔE'°) of -320 mV

NADP → NADPH

3 redox potential scenarios:

1) As for NAD(P)(H)

NADX → **ΔE'°=-320 mV** → NADXH

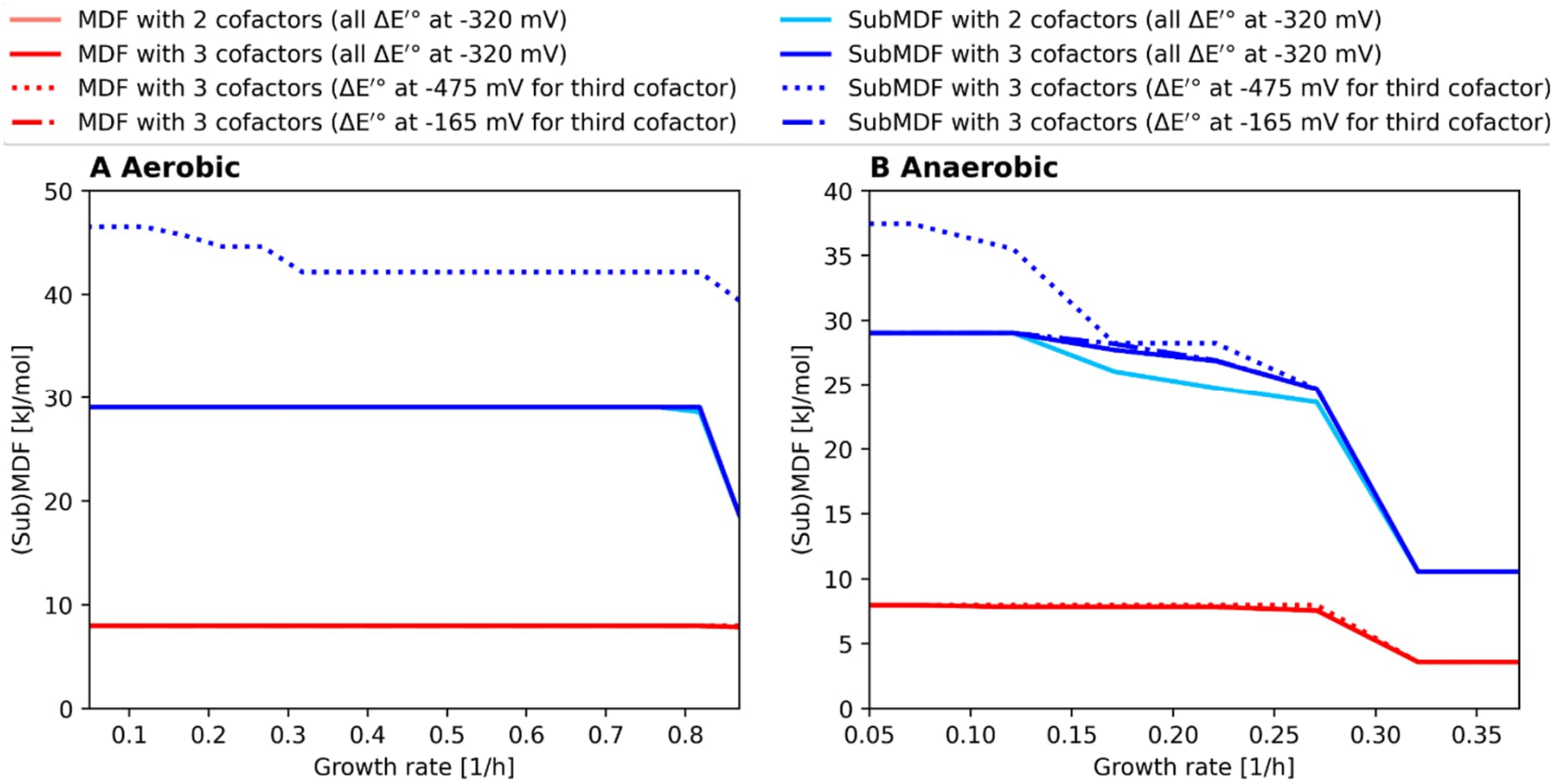
2) Lower potential

NADX → **ΔE'°=-475 mV** → NADXH

3) Higher potential

NADX → **ΔE'°=-165 mV** → NADXH

Analysis 4: (Sub)MDF Results with 3 Cofactors (Flexible Specificity)

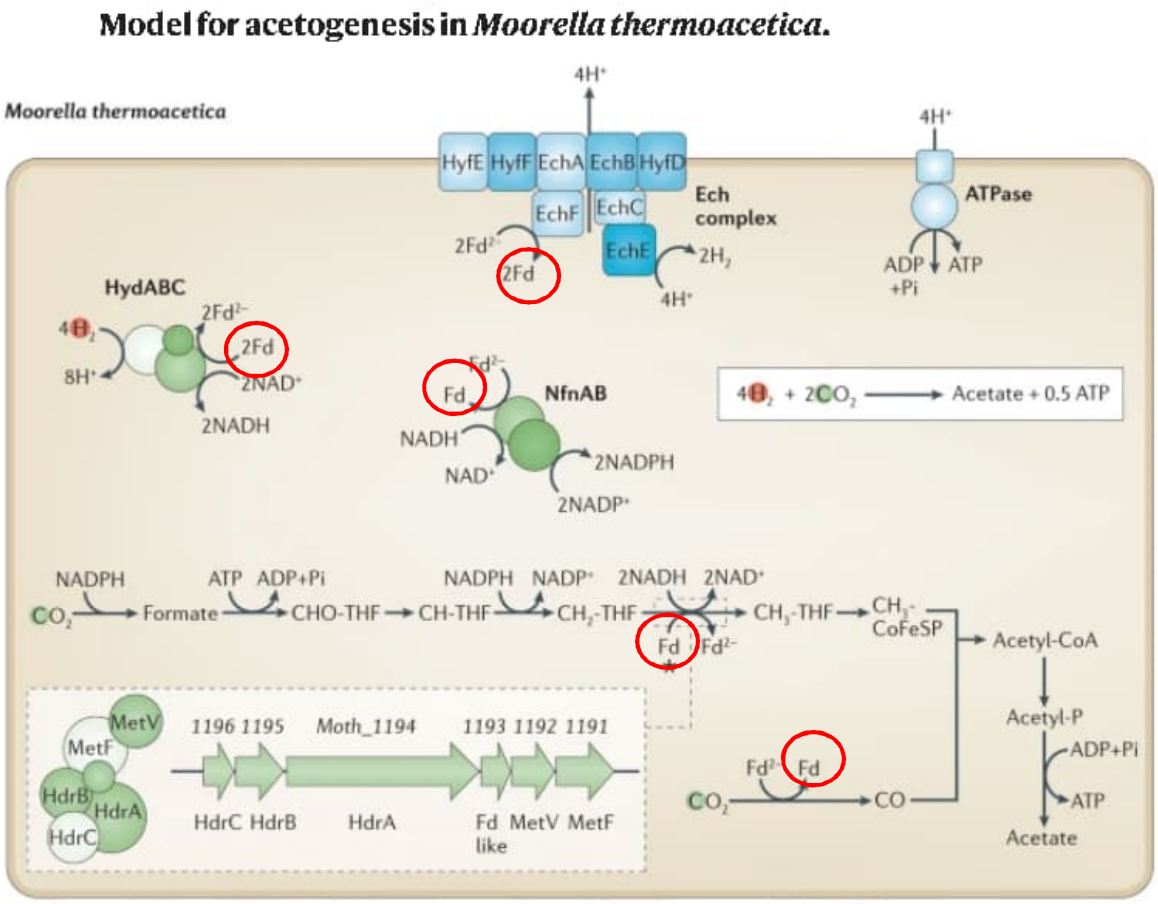


Conclusion #3: A third redox cofactor pool could be advantageous if it has a low standard redox potential!

Analysis 4: (Sub)MDF Results with 3 Cofactors (Flexible Specificity)

Several autotrophic organisms like acetogens use ferredoxin (ΔE'° of -420 mV) as a third major redox cofactor in many redox reactions.

→ Additional degree of freedom to maintain high thermodynamic driving forces in their complicated redox metabolism.

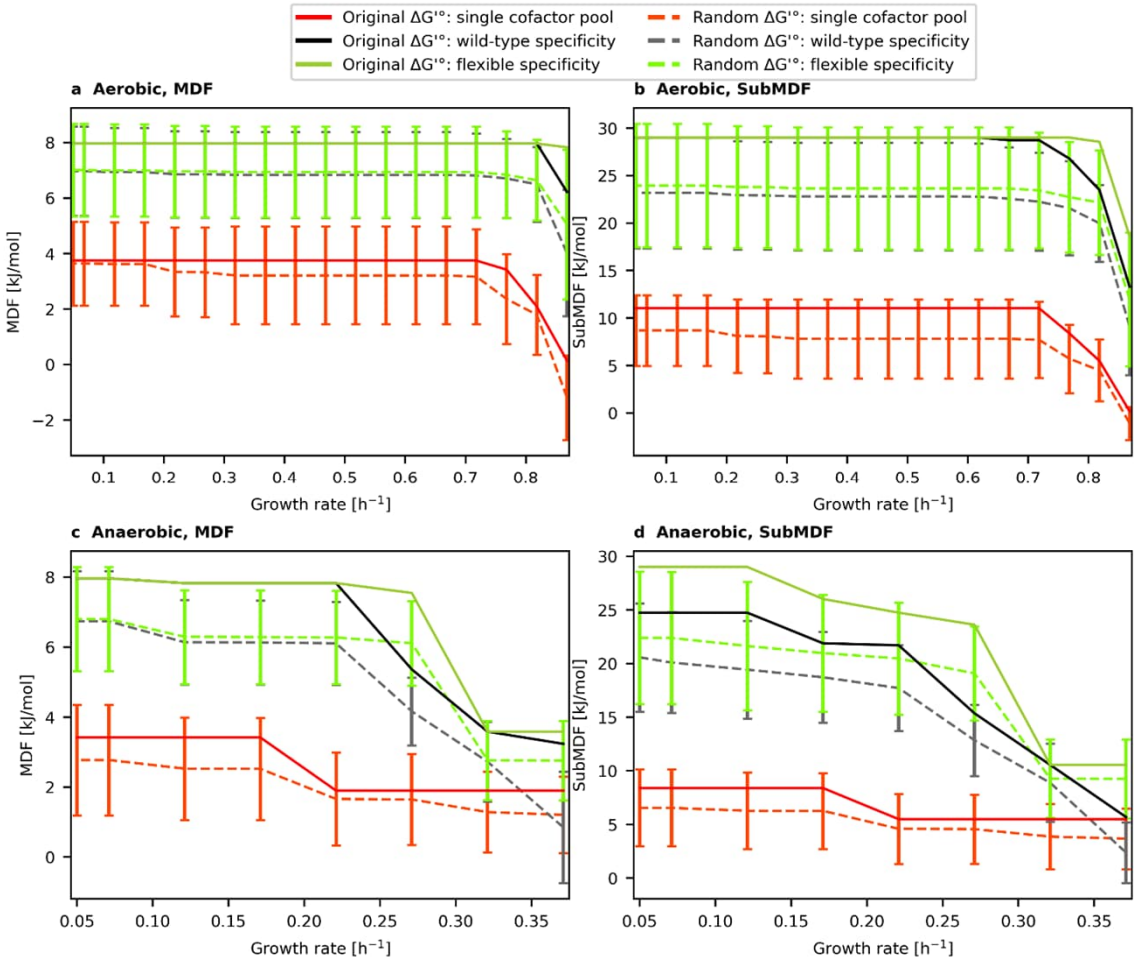


Schuchmann et al. *Nat Rev Microbiol* **12**, 809–821 (2014).

Analysis 5: Robustness of the Results

A) Robustness against random variations of $\Delta_r G'°$

(implemented by random variations of the $\Delta_f G'°$ of each metabolite)

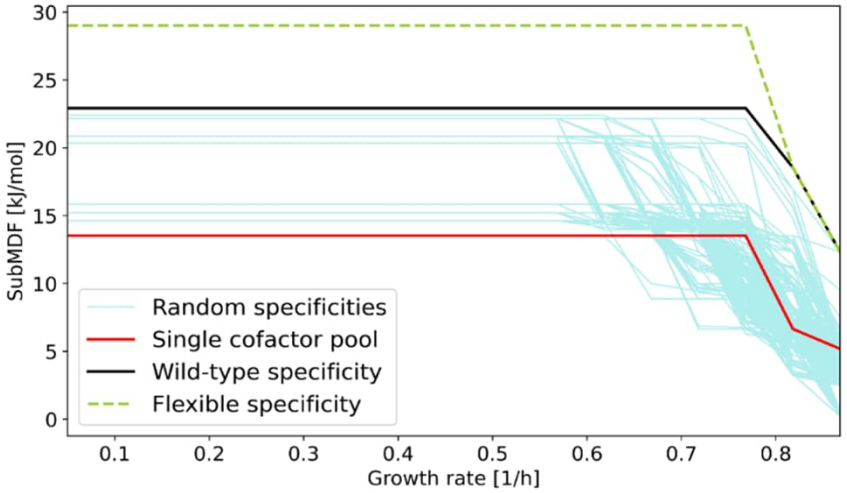


Analysis 5: Robustness of the Results

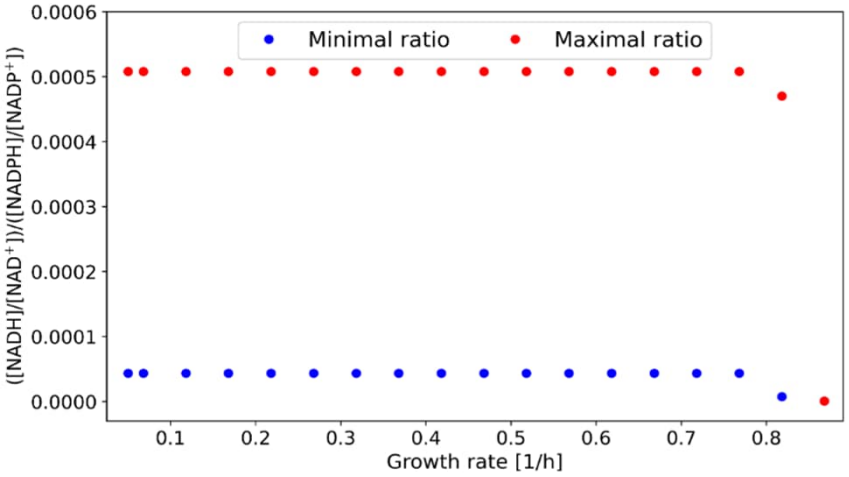
B) Robustness against assumed metabolite concentration ranges

→ *in vivo* concentration values from Bennett et al., 2009 (aerobic conditions)

SubMDF (aerobic)



Ratios under maximal SubMDF (aerobic)



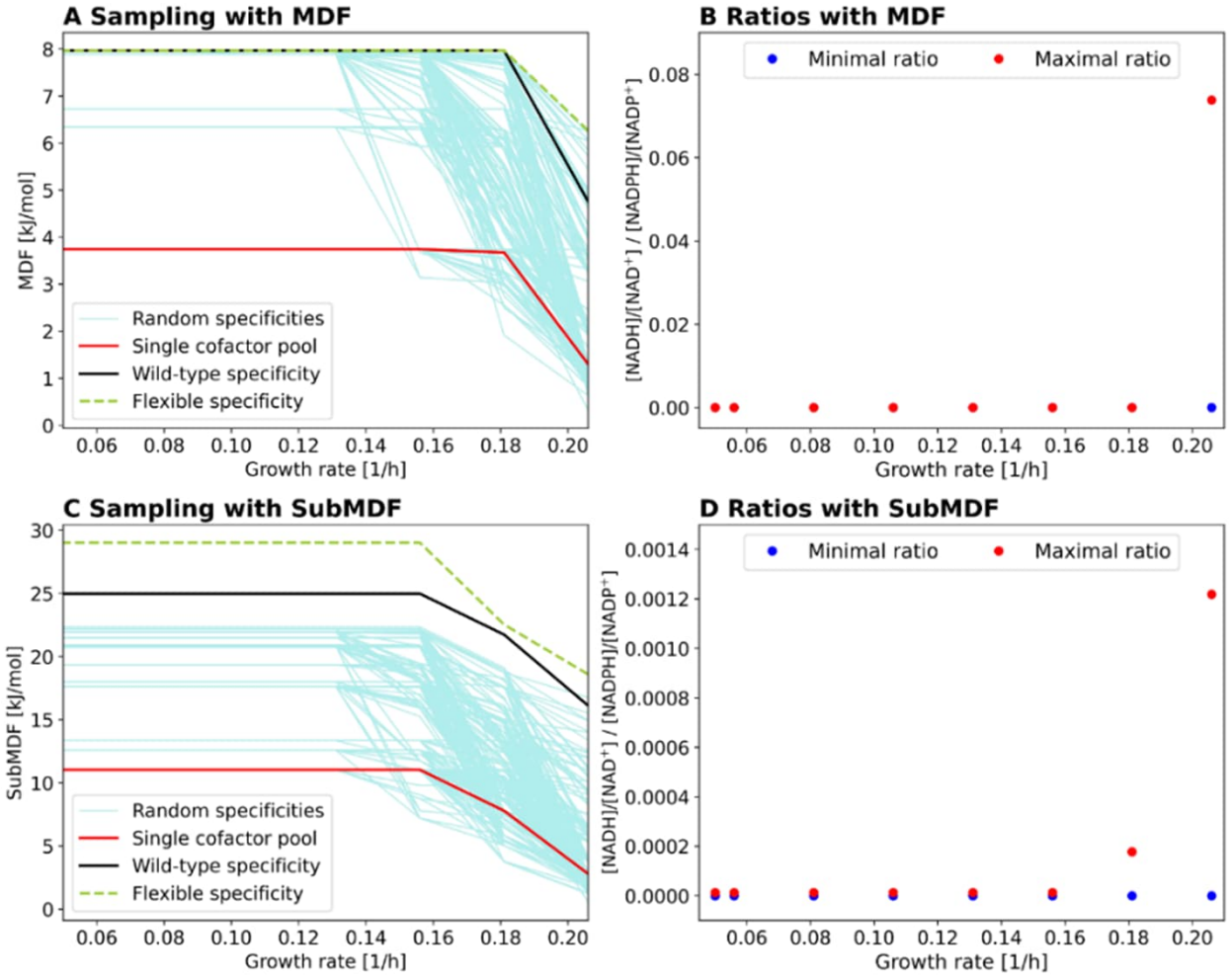
For MDF: single bottleneck
(independent of NAD(P(H) specificities)

Analysis 5: Robustness of the Results

C) Changing the substrate: acetate instead of glucose

(aerobic conditions only)

Conclusion #4:
Results are robust against different variations



Conclusion

- ✓ TCOSA framework for analyzing the thermodynamic effects of (redox) cofactor swaps.
- ✓ Our analysis indicates that evolution shaped **the NAD(P)(H) specificity of reactions to enable high thermodynamic potentials** in the metabolic network.
  - minimizes enzyme demand for redox reactions (cf. also Goldford et al., 2022)
- ✓ We used **MDF as a measure** for the (network-wide) thermodynamic **potential:**

  Caveat: A cell is likely not in a state close to a computed MDF (e.g., enzyme kinetics affects feasible metabolite concentrations and thus the MDF).

  But the higher the (theoretical) MDF, the larger **the thermodynamic flexibility** of the network (broader ranges of feasible metabolite concentration)!

- ✓ TCOSA can be used for other species and/or other cofactor pairs (e.g., ATP/GTP) and even for **predicting optimal cofactor specificities** (e.g. metabolic engineering).

Acknowledgements

MPI Magdeburg
Research Group Analysis and Redesign of Biological Networks

Thank you for your attention!

*Bekiaris PS, Klamt S (2023) Network-wide Thermodynamic Constraints Shape NAD(P)H Cofactor Specificity of Biochemical Reactions. Nature Communications **14**:4660.*