

# Fitness Profiles Identify Enzyme Capacities

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## 1 An algorithm to identify enzyme capacities

Constraint-based models (CBMs) successfully characterize metabolism at the genome-scale by incorporating experimentally validated constraints. Enzymatic capacity constraints (ECCs) are currently lacking in CBMs as measurement of these is difficult. Meanwhile, fitness competition assays provide precise measurements to understand metabolism. Here, we develop an algorithm that utilizes such fitness measurements from Zhu et al. (2005) to identify ECCs that reconcile model predictions with observed growth phenotypes. Simulation of fitness and flux distributions with these ECCs supports hypotheses of Zhu et al. (2005) and generates new, testable hypotheses.

## 2 The algorithm reconciles model predictions with measurements

We developed the optimal capacity constraint identification (OCCI) algorithm that utilizes fitness measurements to identify ECCs, allowing CBMs to accurately predict growth phenotypes. OCCI first identified ECCs using a training set of fitness measurements from data by Zhu et al. (2005). By incorporating these ECCs, model predictions agreed well with the validation set of fitness measurements (Figure 1).

The soluble transhydrogenase, UdhA, was identified as a capacity constraint that persistently had flux unless its corresponding gene was knocked out. This result was significant in two ways: first, it confirmed observations by Zhu et al. (2005); Zhao et al. (2004) that under abundance of NADPH, such as for growth on acetate, UdhA catalyzes the forward reaction, and that *udhA* expression is modulated by levels of NADPH (Sauer et al., 2004). Second, it confirmed the hypothesis of Zhu et al. (2005) that under a  $\Delta udhA$  genetic background, the *icd*<sup>NAD</sup> strain attains a fitness advantage over the *icd*<sup>NADP</sup> strain, as NADH rather than NADPH becomes the major bottleneck to growth.

## 3 Identified ECCs are used to design experiments for further hypothesis testing

The identified ECCs were then used to simulate relative fitness under additional genetic backgrounds that all included deletion of *gnd*. The 6-phosphogluconate dehydrogenase (GND)-catalyzed reaction constitutes part of the oxidative pentose phosphate pathway (PPP), which generates NADPH. Hence, we expect selection against the *icd*<sup>NAD</sup> strain to intensify under

a  $\Delta gnd$  background when lack of NADPH is a bottleneck to growth. Predictions of relative fitness supported this hypothesis (Figure 2). Furthermore, Zhao et al. (2004) show that *Escherichia coli* strains grown on acetate that lack the oxidative PPP exhibit reversed non-oxidative PPP flux. Using the ECCs, flux distribution simulations agreed with these observations.

## 4 Conclusions

Enzyme capacities can strongly affect metabolic flux distributions but can be difficult to characterize in the absence of measurements. The OCCI algorithm was developed to infer unknown constraints from other, abundant measurements and demonstrated promising capabilities using data of Zhu et al. (2005). Hence, OCCI can be used to gain additional insight for well-characterized organisms such as *E. coli*, or to identify engineering bottlenecks for less studied but practically relevant organisms such as the *Geobacter* species (Mahadevan et al., 2006).

## References

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Genetic background of acetate-grown strains	Relative fitness = ( $\mu^{\text{NAD}} / \mu^{\text{NADP}}$ )*				
	Exp **	FBA	FBA with capacity constraints from OCCL		
			A	B	C
Wild type	<b>0.96</b> ± 0.02	1.00	<b>0.96</b>	<b>0.96</b>	<b>0.96</b>
$\Delta pntAB$	<b>0.73</b> ± 0.04	1.00	0.77	0.77	0.73
$\Delta maeB$	<b>0.63</b> ± 0.04	1.00	<b>0.63</b>	<b>0.63</b>	0.67
$\Delta pntAB, \Delta maeB$	<b>0.44</b> ± 0.03	1.00	<b>0.44</b>	<b>0.44</b>	<b>0.44</b>
$\Delta udhA$	<b>1.46</b> ± 0.03	1.01	<b>1.46</b>	<b>1.46</b>	1.65
$\Delta udhA, \Delta pntAB$	1.65 ± 0.09	1.01	1.46	1.37	<b>1.65</b>
$\Delta udhA, \Delta maeB$	<b>0.95</b> ± 0.11	1.00	<b>0.95</b>	<b>0.95</b>	<b>0.95</b>

\*  $\mu^{\text{NAD}}$  &  $\mu^{\text{NADP}}$  are growth rates of  $icd^{\text{NAD}}$  &  $icd^{\text{NADP}}$  strains, respectively  
\*\* Experimental data from Zhu et al. (2005)

Figure 1: A training set is selected from relative fitness measurements of Zhu et al. (2005) to identify enzymatic capacity constraints and to accurately predict the rest of the data. Grey boxes indicate data used for validation. Set B uses identical validation data as Set A but a flux is forced through the *pntAB*-encoded transhydrogenase. Bold-face predictions fit well with the experimental data.

Genetic background of <i>in silico</i> acetate-grown strains	Relative fitness = ( $\mu^{\text{NAD}} / \mu^{\text{NADP}}$ )*			
	FBA	FBA with capacity constraints from OCCL		
		A	B	C
$\Delta gnd$	1.00	0.21	0.24	0.24
$\Delta gnd, \Delta pntAB$	1.00	0.011	0.016	0.010
$\Delta gnd, \Delta udhA$	1.01	0.54	0.65	0.86
$\Delta gnd, \Delta udhA, \Delta pntAB$	0.97	0.34	0.39	0.48
$\Delta gnd, \Delta udhA, \Delta maeB$	1.00	0.20	0.23	0.23

\*  $\mu^{\text{NAD}}$  &  $\mu^{\text{NADP}}$  are growth rates of  $icd^{\text{NAD}}$  &  $icd^{\text{NADP}}$  strains, respectively

Figure 2: The capacity constraints identified from results of Figure 1 (Set A, B, and C) are incorporated into simulations of the relative fitness of additional deletion strains that all involve a *gnd* knockout.