

¹ Christian Doppler Laboratory for production of next-level biopharmaceuticals in *E. coli*, University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Bioprocess Science and Engineering, Muthgasse 18, 1190 Vienna, Austria

² University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Computational Biology, Muthgasse 18, 1190 Vienna, Austria

³ Boehringer Ingelheim RCV GmbH & Co KG, Dr.-Boehringer-Gasse 5-11, 1120 Vienna, Austria

Table S1: Selection of genes differentially expressed in fed-batch cultivations after 12 h of induction (n = 3, $\alpha \leq 0.05$).

Gene	Strain	Log2FC	P value
<i>ubiC</i>	BL21(DE3)	-1.06	6.31*10 ⁻¹⁶
	B<oFabx>	-1.17	1.91*10 ⁻⁷
	B<oBIBH1>	-1.31	1.05*10 ⁻¹⁰
	B<oBIWA4>	-0.89	3.25*10 ⁻⁶
	B<oFTN2>	-1.24	4.17*10 ⁻¹²
<i>ubiA</i>	BL21(DE3)	-0.81	2.24*10 ⁻¹²
	B<oFabx>	-0.89	2.00*10 ⁻⁴
	B<oBIBH1>	-0.92	2.07*10 ⁻⁶
	B<oBIWA4>	-0.74	1.77*10 ⁻⁶
	B<oFTN2>	-0.93	8.06*10 ⁻¹⁰
<i>soxS</i>	B<oFabx>	4.15	4.98*10 ⁻⁵²
	B<oBIBH1>	2.36	1.07*10 ⁻¹⁶
	B<oBIWA4>	3.39	1.96*10 ⁻⁶⁷
	B<oFTN2>	3.23	1.50*10 ⁻²⁶
<i>sodA</i>	BL21(DE3)	-0.90	3.19*10 ⁻⁸
	B<oFabx>	0.56	7.51*10 ⁻³
	B<oBIBH1>	-0.78	2.14*10 ⁻³
	B<oBIWA4>	-0.48	3.18*10 ⁻³
	B<oFTN2>	-0.91	2.14*10 ⁻⁴
<i>acrA</i>	B<oFabx>	0.58	1.18*10 ⁻²
<i>inaA</i>	B<oFabx>	0.70	2.66*10 ⁻³
<i>rimK</i>	B<oFabx>	0.82	4.64*10 ⁻⁴
<i>fumC</i>	BL21(DE3)	-0.92	3.30*10 ⁻¹⁴
	B<oFabx>	-1.34	5.73*10 ⁻⁹
	B<oBIBH1>	-1.53	6.77*10 ⁻¹¹
	B<oBIWA4>	-1.25	9.98*10 ⁻¹⁶
	B<oFTN2>	-1.43	1.02*10 ⁻¹⁰
<i>acnA</i>	BL21(DE3)	-0.86	1.56*10 ⁻¹⁶
	B<oFabx>	-1.91	1.16*10 ⁻¹⁴
	B<oBIBH1>	-1.62	1.94*10 ⁻¹⁸
	B<oBIWA4>	-1.42	1.28*10 ⁻²⁴
	B<oFTN2>	-1.55	2.55*10 ⁻²⁹
<i>marR</i>	B<oFabx>	2.75	1.73*10 ⁻¹⁸
	B<oBIBH1>	1.40	4.23*10 ⁻⁵
	B<oBIWA4>	1.78	2.52*10 ⁻¹⁶
	B<oFTN2>	1.65	2.21*10 ⁻¹⁰
<i>marA</i>	B<oFabx>	3.06	2.34*10 ⁻²⁶
	B<oBIBH1>	1.18	7.80*10 ⁻⁶
	B<oBIWA4>	1.87	3.57*10 ⁻³⁰
	B<oFTN2>	1.19	3.64*10 ⁻⁹

Interaction of periplasmic Fab production and intracellular redox balance in *Escherichia coli* affects product yield

Sophie Vazulka¹, Matteo Schiavinato², Martin Wagenknecht³, Monika Cserjan-Puschmann^{1*}, Gerald Striedner¹

¹ Christian Doppler Laboratory for production of next-level biopharmaceuticals in *E. coli*, University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Bioprocess Science and Engineering, Muthgasse 18, 1190 Vienna, Austria

² University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Computational Biology, Muthgasse 18, 1190 Vienna, Austria

³ Boehringer Ingelheim RCV GmbH & Co KG, Dr.-Boehringer-Gasse 5-11, 1120 Vienna, Austria

<i>marB</i>	B<oFabx>	2.79	1.32*10 ⁻¹⁶
	B<oBIWA4>	1.79	9.60*10 ⁻¹⁸
	B<oFTN2>	1.01	6.13*10 ⁻⁴
<i>ybjC</i>	B<oFabx>	1.04	1.85*10 ⁻⁴
<i>katG</i>	BL21(DE3)	-0.34	2.57*10 ⁻³
	B<oFabx>	-0.71	1.24*10 ⁻³
	B<oBIBH1>	-0.88	4.52*10 ⁻⁵
	B<oBIWA4>	-0.52	1.29*10 ⁻³
	B<oFTN2>	-0.72	2.00*10 ⁻⁷
<i>dps</i>	BL21(DE3)	-2.03	7.99*10 ⁻⁶⁰
	B<oFabx>	-2.87	5.45*10 ⁻³⁰
	B<oBIBH1>	-2.42	7.98*10 ⁻²⁰
	B<oBIWA4>	-2.95	1.94*10 ⁻¹⁰⁹
	B<oFTN2>	-2.88	1.17*10 ⁻⁷²
<i>sufAB</i>	BL21(DE3)	-1.32	2.07*10 ⁻²⁵
	B<oFabx>	-1.17	2.14*10 ⁻⁶
	B<oBIBH1>	-1.67	2.95*10 ⁻¹⁵
	B<oBIWA4>	-1.67	7.25*10 ⁻²⁷
	B<oFTN2>	-1.86	1.25*10 ⁻²⁶
<i>sufC</i>	BL21(DE3)	-1.04	2.35*10 ⁻¹³
	B<oFabx>	-1.25	4.34*10 ⁻⁷
	B<oBIBH1>	-1.66	1.07*10 ⁻¹⁵
	B<oBIWA4>	-1.67	6.05*10 ⁻²⁸
	B<oFTN2>	-1.54	3.80*10 ⁻²²
<i>sufD</i>	BL21(DE3)	-1.02	4.70*10 ⁻¹⁶
	B<oFabx>	-1.40	2.76*10 ⁻¹⁰
	B<oBIBH1>	-1.58	5.45*10 ⁻¹⁶
	B<oBIWA4>	-1.54	1.55*10 ⁻²³
	B<oFTN2>	-1.64	1.86*10 ⁻²⁰
<i>mntH</i>	BL21(DE3)	-0.63	2.20*10 ⁻⁶
	B<oFabx>	-1.26	1.96*10 ⁻⁸
	B<oBIBH1>	-1.14	8.54*10 ⁻¹⁰
	B<oBIWA4>	-1.42	1.50*10 ⁻²⁰
	B<oFTN2>	-1.24	2.76*10 ⁻¹⁴
<i>grxA</i>	B<oFabx>	1.47	9.85*10 ⁻⁷
	B<oBIBH1>	0.82	8.06*10 ⁻³
	B<oBIWA4>	0.86	8.91*10 ⁻⁵
	B<oFTN2>	1.04	5.80*10 ⁻⁴
<i>sodB</i>	B<oFabx>	0.60	8.14*10 ⁻³
	B<oBIBH1>	0.85	3.10*10 ⁻⁴
	B<oBIWA4>	1.00	5.73*10 ⁻¹²
	B<oFTN2>	0.59	8.71*10 ⁻⁵
<i>sodC</i>	BL21(DE3)	-1.63	4.12*10 ⁻³²

Interaction of periplasmic Fab production and intracellular redox balance in *Escherichia coli* affects product yield

Sophie Vazulka¹, Matteo Schiavinato², Martin Wagenknecht³, Monika Cserjan-Puschmann^{1*}, Gerald Striedner¹

¹ Christian Doppler Laboratory for production of next-level biopharmaceuticals in *E. coli*, University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Bioprocess Science and Engineering, Muthgasse 18, 1190 Vienna, Austria

² University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Computational Biology, Muthgasse 18, 1190 Vienna, Austria

³ Boehringer Ingelheim RCV GmbH & Co KG, Dr.-Boehringer-Gasse 5-11, 1120 Vienna, Austria

	B<oFabx>	-2.48	3.96*10 ⁻²⁷
	B<oBIBH1>	-2.33	3.53*10 ⁻²⁶
	B<oBIWA4>	-2.37	3.94*10 ⁻⁵⁷
	B<oFTN2>	-2.41	1.42*10 ⁻⁵⁵
<i>nuoA</i>	B<oFabx>	-1.44	5.18*10 ⁻⁷
	B<oBIBH1>	-0.58	2.00*10 ⁻³
	B<oFTN2>	-0.37	8.19*10 ⁻³
<i>nuoB</i>	B<oFabx>	-1.50	6.65*10 ⁻⁸
	B<oBIBH1>	-0.93	6.57*10 ⁻⁷
	B<oBIWA4>	-0.52	1.67*10 ⁻³
	B<oFTN2>	-0.58	1.06*10 ⁻⁴
<i>nuoC</i>	B<oFabx>	-1.61	6.75*10 ⁻⁷
	B<oBIBH1>	-1.02	2.08*10 ⁻⁹
	B<oBIWA4>	-0.47	8.00*10 ⁻³
	B<oFTN2>	-0.59	1.58*10 ⁻³
<i>nuoE</i>	B<oFabx>	-1.60	4.64*10 ⁻⁷
	B<oBIBH1>	-1.12	6.13*10 ⁻¹⁰
	B<oBIWA4>	-0.54	1.70*10 ⁻³
	B<oFTN2>	-0.67	8.21*10 ⁻⁶
<i>nuoF</i>	B<oFabx>	-1.57	6.52*10 ⁻⁷
	B<oBIBH1>	-1.18	1.03*10 ⁻¹¹
	B<oBIWA4>	-0.55	2.16*10 ⁻³
	B<oFTN2>	-0.62	7.68*10 ⁻⁵
<i>nuoG</i>	B<oFabx>	-1.57	3.35*10 ⁻⁷
	B<oBIBH1>	-1.02	3.06*10 ⁻⁸
	B<oBIWA4>	-0.52	3.90*10 ⁻³
	B<oFTN2>	-0.55	1.49*10 ⁻³
<i>nuoH</i>	B<oFabx>	-1.69	5.10*10 ⁻⁸
	B<oBIBH1>	-1.07	1.04*10 ⁻⁸
	B<oBIWA4>	-0.59	1.72*10 ⁻³
	B<oFTN2>	-0.59	1.35*10 ⁻³
<i>nuoI</i>	B<oFabx>	-1.53	3.44*10 ⁻⁷
	B<oBIBH1>	-0.95	1.29*10 ⁻⁵
	B<oBIWA4>	-0.59	5.48*10 ⁻⁴
	B<oFTN2>	-0.65	2.32*10 ⁻⁴
<i>nuoJ</i>	B<oFabx>	-1.65	2.46*10 ⁻⁸
	B<oBIBH1>	-1.04	6.00*10 ⁻⁸
	B<oBIWA4>	-0.64	3.34*10 ⁻⁴
	B<oFTN2>	-0.59	1.26*10 ⁻³
<i>nuoK</i>	B<oFabx>	-1.50	7.46*10 ⁻⁷
	B<oBIBH1>	-0.87	1.72*10 ⁻⁵
	B<oBIWA4>	-0.46	1.17*10 ⁻²
	B<oFTN2>	-0.51	6.52*10 ⁻³

¹ Christian Doppler Laboratory for production of next-level biopharmaceuticals in *E. coli*, University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Bioprocess Science and Engineering, Muthgasse 18, 1190 Vienna, Austria

² University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Computational Biology, Muthgasse 18, 1190 Vienna, Austria

³ Boehringer Ingelheim RCV GmbH & Co KG, Dr.-Boehringer-Gasse 5-11, 1120 Vienna, Austria

<i>nuoL</i>	B<oFabx>	-1.50	5.20*10 ⁻⁷
	B<oBIBH1>	-0.99	4.54*10 ⁻⁷
	B<oBIWA4>	-0.58	1.07*10 ⁻³
	B<oFTN2>	-0.56	1.78*10 ⁻³
<i>nuoM</i>	B<oFabx>	-1.27	5.85*10 ⁻⁶
	B<oBIBH1>	-0.79	4.05*10 ⁻⁵
	B<oFTN2>	-0.41	4.78*10 ⁻³
<i>nuoN</i>	B<oFabx>	-0.83	6.19*10 ⁻⁴
	B<oBIBH1>	-0.67	3.03*10 ⁻⁴
<i>ndh</i>	B<oFabx>	1.73	9.46*10 ⁻¹²
	B<oBIWA4>	0.56	5.52*10 ⁻⁴

Table S2: GO terms enriched (level 5) in B<oFabx> after 12 h of production in fed-batch processes.

Enriched GO term	Q value	Gene count	
Cellular component	GO:0022626 cytosolic_ribosome	1.85*10 ⁻²⁰	55
	GO:0044391 ribosomal_subunit	1.85*10 ⁻²⁰	55
	GO:0043232 intracellular_non-membrane-bounded_organelle	2.87*10 ⁻¹⁸	67
	GO:0044445 cytosolic_part	2.54*10 ⁻¹³	60
	GO:0030964 NADH_dehydrogenase_complex	1.16*10 ⁻⁴	14
	GO:0070470 plasma_membrane_respiratory_chain	1.14*10 ⁻⁴	18
	GO:0098803 respiratory_chain_complex	1.14*10 ⁻⁴	18
	GO:0045272 plasma_membrane_respiratory_chain_complex_I	2.08*10 ⁻⁴	13
	GO:0098797 plasma_membrane_protein_complex	2.34*10 ⁻²	64
Molecular function	GO:0003723 RNA_binding	4.33*10 ⁻⁶	60
	GO:0003954 NADH_dehydrogenase_activity	2.02*10 ⁻³	16
Biological process	GO:0022618 ribonucleoprotein_complex_assembly	3.68*10 ⁻¹³	47
	GO:0071826 ribonucleoprotein_complex_subunit_organization	3.68*10 ⁻¹³	48
	GO:0070925 organelle_assembly	7.75*10 ⁻¹³	52
	GO:0008652 cellular_amino_acid_biosynthetic_process	3.65*10 ⁻⁵	65
	GO:1901605 alpha-amino_acid_metabolic_process	9.65*10 ⁻⁴	83
	GO:0009408 response_to_heat	6.30*10 ⁻³	36
	GO:0044275 cellular_carbohydrate_catabolic_process	2.73*10 ⁻²	27
GO:0097237 cellular_response_to_toxic_substance	3.70*10 ⁻²	12	

Table S3: GO terms enriched (level 5) in B<oBIBH1> after 12 h of production in fed-batch processes.

Enriched GO term	Q value	Gene count	
Cellular component	GO:0022626 cytosolic_ribosome	9.01*10 ⁻¹⁴	44
	GO:0044391 ribosomal_subunit	9.01*10 ⁻¹⁴	44
	GO:0044446 intracellular_organelle_part	8.69*10 ⁻¹²	46
	GO:0044445 cytosolic_part	2.23*10 ⁻¹⁰	48
	GO:0070470 plasma_membrane_respiratory_chain	1.19*10 ⁻⁶	18

¹ Christian Doppler Laboratory for production of next-level biopharmaceuticals in *E. coli*, University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Bioprocess Science and Engineering, Muthgasse 18, 1190 Vienna, Austria

² University of Natural Resources and Life Sciences, Vienna, Department of Biotechnology, Institute of Computational Biology, Muthgasse 18, 1190 Vienna, Austria

³ Boehringer Ingelheim RCV GmbH & Co KG, Dr.-Boehringer-Gasse 5-11, 1120 Vienna, Austria

	GO:0098803 respiratory_chain_complex	1.19*10 ⁻⁶	18
	GO:0045271 respiratory_chain_complex_I	5.33*10 ⁻⁶	13
	GO:0045272 plasma_membrane_respiratory_chain_complex_I	5.33*10 ⁻⁶	13
	GO:0098797 plasma_membrane_protein_complex	1.17*10 ⁻²	51
	GO:0030288 outer_membrane-bounded_periplasmic_space	2.02*10 ⁻²	54
Molecular function	GO:0003954 NADH_dehydrogenase_activity	5.42*10 ⁻⁴	15
	GO:0070925 organelle_assembly	3.27*10 ⁻⁷	40
Biological process	GO:0071826 ribonucleoprotein_complex_subunit_organization	7.73*10 ⁻⁶	34
	GO:0042273 ribosomal_large_subunit_biogenesis	1.42*10 ⁻⁴	20
	GO:0034599 cellular_response_to_oxidative_stress	2.85*10 ⁻³	18
	GO:0097237 cellular_response_to_toxic_substance	2.58*10 ⁻²	11

Table S4: GO terms enriched (level 5) in B<BIWA4> after 12 h of production in fed-batch processes.

Enriched GO term	Q value	Gene count
GO:0022626 cytosolic_ribosome	2.76*10 ⁻¹⁹	55
GO:0044391 ribosomal_subunit	2.76*10 ⁻¹⁹	55
GO:0043232 intracellular_non-membrane-bounded_organelle	6.15*10 ⁻¹⁷	67
GO:0044445 cytosolic_part	9.02*10 ⁻¹⁴	62
GO:0070470 plasma_membrane_respiratory_chain	1.96*10 ⁻²	15
GO:0098803 respiratory_chain_complex	1.96*10 ⁻²	15
GO:0098797 plasma_membrane_protein_complex	2.35*10 ⁻²	67
GO:0030964 NADH_dehydrogenase_complex	2.59*10 ⁻²	11
GO:0022618 ribonucleoprotein_complex_assembly	3.78*10 ⁻¹¹	46
GO:0071826 ribonucleoprotein_complex_subunit_organization	3.78*10 ⁻¹¹	47
GO:0070925 organelle_assembly	6.52*10 ⁻¹¹	51
GO:0008652 cellular_amino_acid_biosynthetic_process	1.46*10 ⁻⁶	71
GO:1901605 alpha-amino_acid_metabolic_process	2.88*10 ⁻⁴	88
GO:0044275 cellular_carbohydrate_catabolic_process	8.23*10 ⁻³	29
GO:0009408 response_to_heat	1.23*10 ⁻²	36
GO:0097237 cellular_response_to_toxic_substance	1.23*10 ⁻²	13
GO:0010038 response_to_metal_ion	2.04*10 ⁻²	29

Table S5: GO terms enriched (level 5) in B<FTN2> after 12 h of production in fed-batch processes.

Enriched GO term	Q value	Gene count
GO:0022626 cytosolic_ribosome	4.54*10 ⁻²⁶	56
GO:0044391 ribosomal_subunit	4.54*10 ⁻²⁶	56
GO:0044446 intracellular_organelle_part	4.45*10 ⁻²¹	59
GO:0044445 cytosolic_part	9.54*10 ⁻¹⁹	62
GO:0045271 respiratory_chain_complex_I	3.60*10 ⁻⁴	12
GO:0045272 plasma_membrane_respiratory_chain_complex_I	3.60*10 ⁻⁴	12
GO:0070470 plasma_membrane_respiratory_chain	4.03*10 ⁻⁴	16

	GO:0098803 respiratory_chain_complex	4.03*10 ⁻⁴	16
	GO:0070925 organelle_assembly	7.11*10 ⁻¹⁵	51
	GO:0071826 ribonucleoprotein_complex_subunit_organization	3.64*10 ⁻¹³	45
	GO:0042273 ribosomal_large_subunit_biogenesis	2.48*10 ⁻⁷	24
	GO:1901605 alpha-amino_acid_metabolic_process	1.31*10 ⁻³	73
Biological process	GO:0097237 cellular_response_to_toxic_substance	1.04*10 ⁻²	12
	GO:0010038 response_to_metal_ion	1.26*10 ⁻²	26
	GO:0034599 cellular_response_to_oxidative_stress	2.37*10 ⁻²	17
	GO:0044275 cellular_carbohydrate_catabolic_process	3.08*10 ⁻²	24
	GO:0009308 amine_metabolic_process	3.08*10 ⁻²	18
	GO:0009408 response_to_heat	3.08*10 ⁻²	30

Table S6: Primers used for qPCR.

Target	Sequence of forward primer	Sequence of reverse primer	Amplicon length [bp]
<i>soxS</i>	5'-CAAGCGTCTGATGCGTCACC-3'	5'-CGAGCATATTGACCAGCCGC-3'	107
<i>cysG</i>	5'-GGTTGCTGTTAGACGCAGGC-3'	5'-CATCTGCCCATGCGGTGAAC-3'	78