

Supplementary Information for

HrrSA orchestrates a systemic response to heme and determines prioritisation of terminal cytochrome oxidase expression

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Other supplementary materials for this manuscript include the following separate files:

Table S3: Full Dataset, binding peaks HrrA and transcriptome analysis of *C. glutamicum* wild type and $\Delta hrrA$.

Table S4: Filtered dataset of time resolved transcriptome analysis of *C. glutamicum* wild type and $\Delta hrrA$ with genes showing at least two-fold alteration.

Supplementary Figures

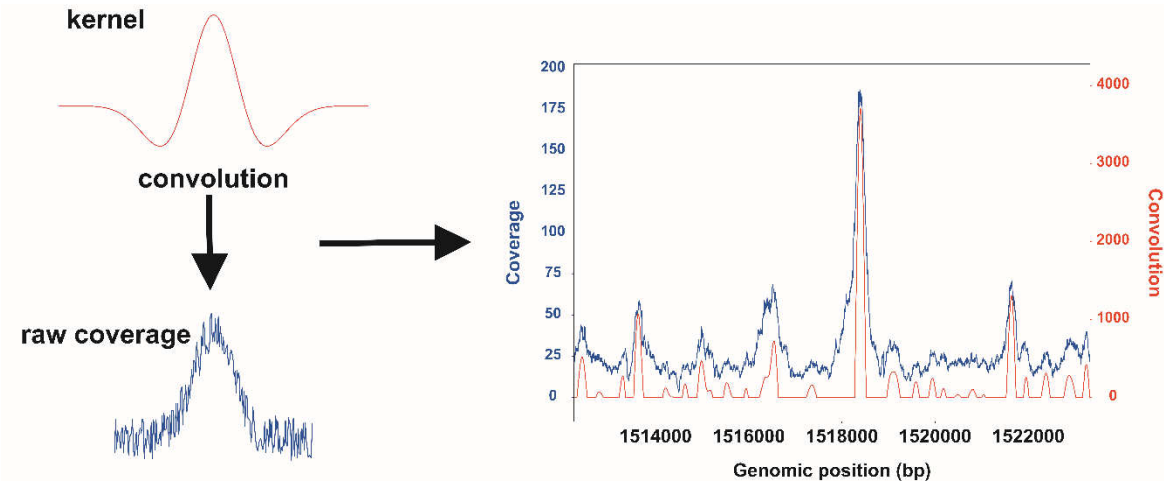


Figure S1: Schematic overview of the convolution profiling. Read coverage was convolved with negative second order Gaussian kernel. The convolved read coverage was then scanned to discover the local maxima (peaks).

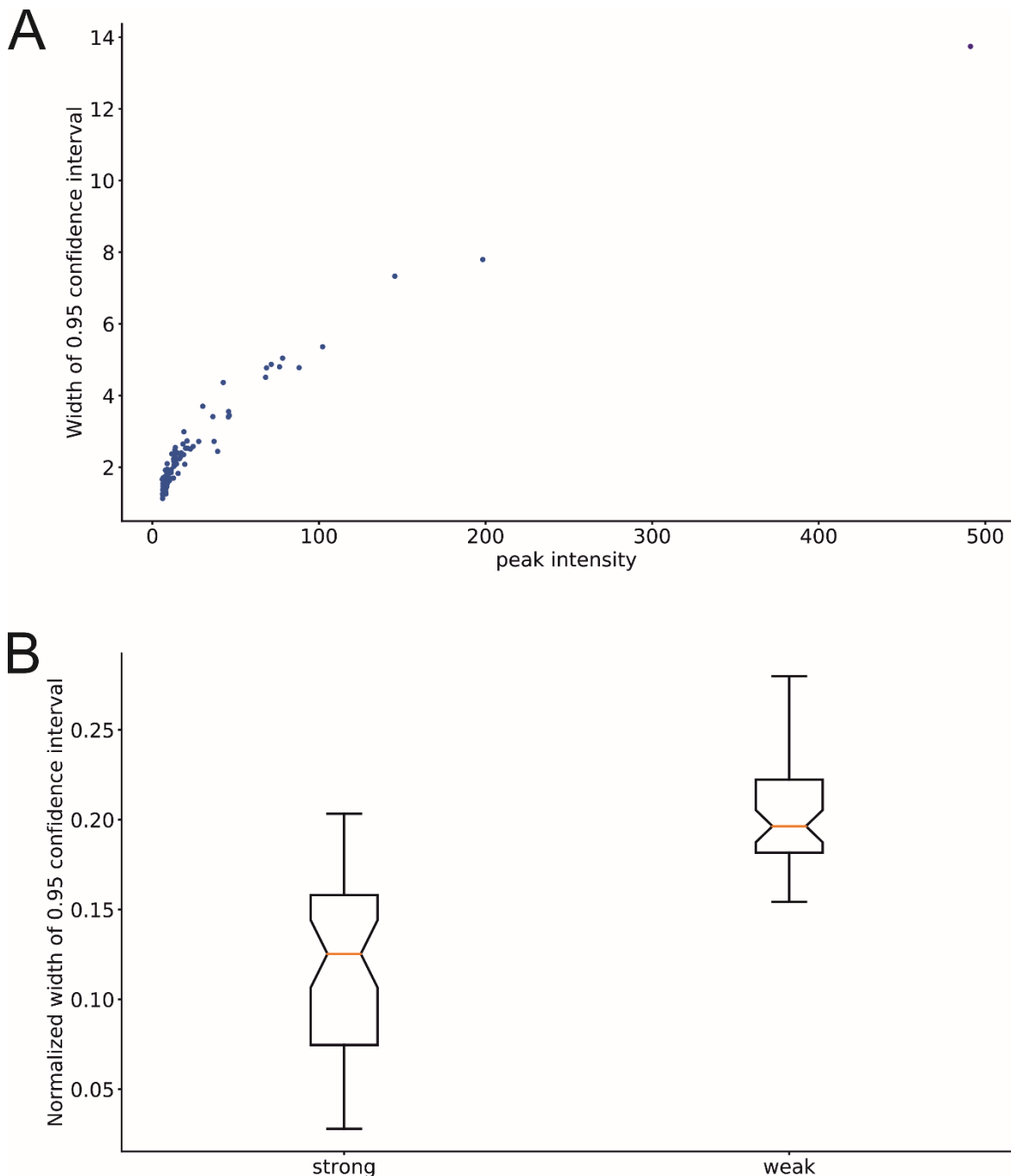
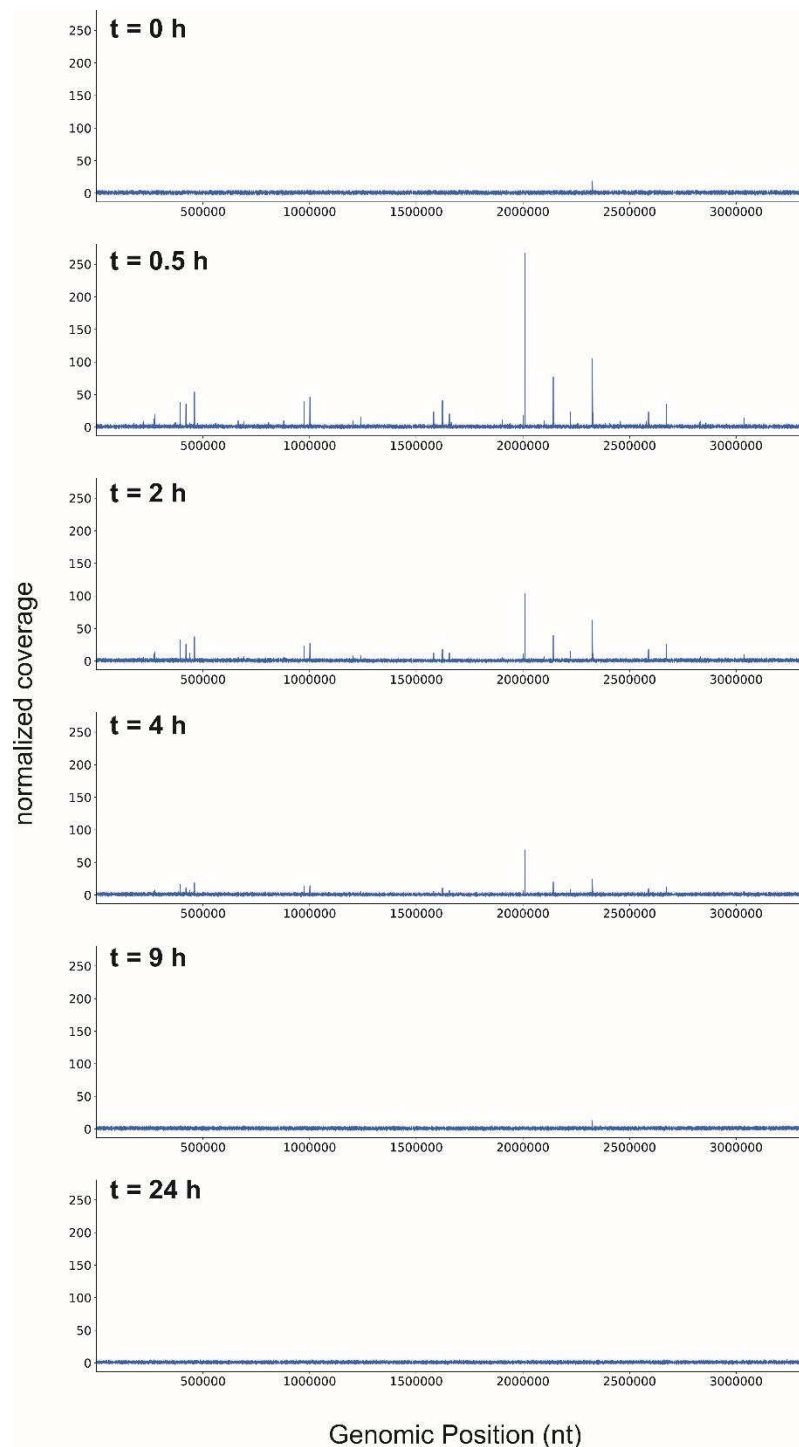


Figure S2: Assessment of significance for the reported peak intensity values. (A) The error of peak intensity value (0.95 confidence intervals width) linearly depends on its absolute value. The stronger the peak the less the confidence in its absolute value. In contrast, the relative error normalized to peak intensity is similar for peaks of various strength, hence can be used as universal measure for significance assessment. (B) Distribution of the normalized confidence intervals width (NCIW) among the detected peaks. The distribution is represented as box plots with box edges at 1st and 3rd quantiles and box whiskers at minimum and maximum values. For the weak peaks (peak intensity <10) average NCIW is around 0.2 and limited by 0.28, while for the strong ones the average NCIW is around 0.13 and limited by 0.2. The upper limits were taken for the final estimation, as the most conservative confidence evaluation was pursued.



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Figure S3: Global binding pattern of HrrA in the *C. glutamicum* genome in response to hemin addition. Genomic coverage (number of reads covering a particular genomic position) was normalized to the average coverage of the regions not harbouring binding peaks. Thus, depicted peak intensities are comparable between different time points. The strain *C. glutamicum::hrrA-C-twinstrep* was cultivated in CGXII minimal medium (lacking FeSO₄) supplemented with 2% (w/v) glucose and 4 µM hemin was added at 0 h. Cells were harvested at different time points as described in Figure 1.

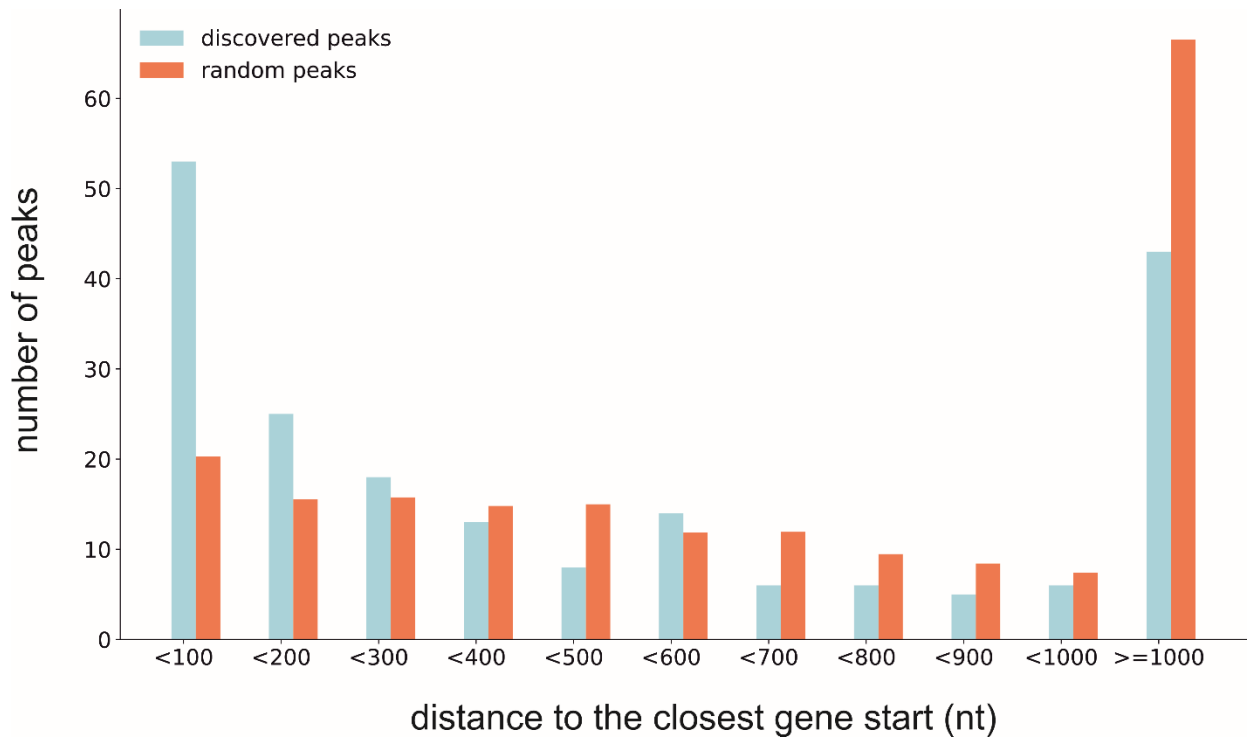


Figure S4: Distribution of distances from HrrA binding peaks centres to the closest downstream gene start site (transcription start site, TSS). As a background (red color), random peaks of the same width as real ones were generated. Random peak generation was performed 100 times and resulting distance distributions were then averaged into a single background distribution. Peaks with a distance of <100 nt can also be found up to 60 nt downstream of the TSS.

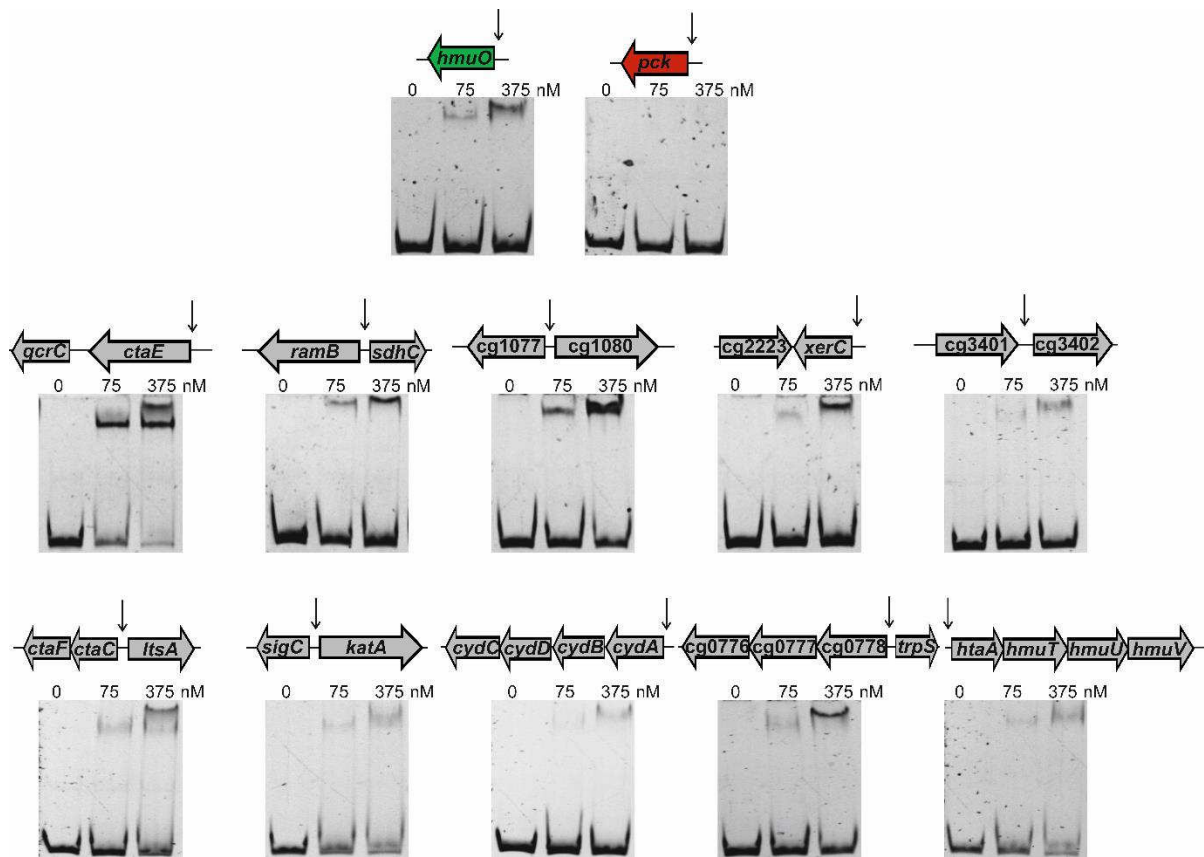


Figure S5: HrrA binding to selected target promoter regions. Protein-DNA interactions were validated by electrophoretic mobility shift assays (EMSA) using 15 nM DNA fragments covering 50 bp up- and downstream of the maximal ChAP-Seq peak height and an increasing protein monomer concentration of 0, 75 and 375 nM. The genomic location of the maximal peak height found in the ChAP-Seq experiments is indicated by an arrow. As control, the promoter regions of *hmuO* (positive control) and *pck* (negative control) were used.

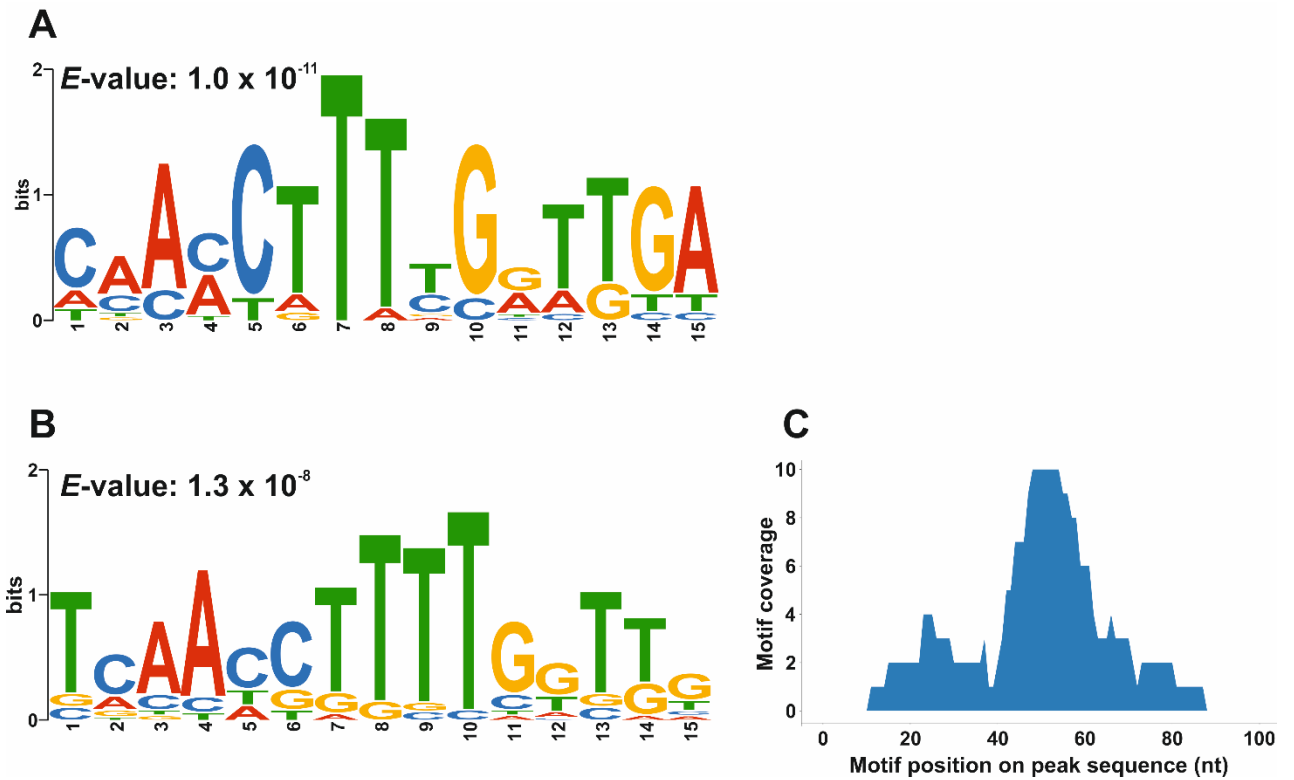


Figure S6: Derivation of a HrrA binding motif revealed a weakly conserved palindromic sequence. Sequences of all peaks with at least two-fold increased coverage (T_0) (A) or 100 bp of the tested EMSA DNA fragments (Figure S5) (B) were used for a MEME v.5 analysis (<http://meme-suite.org>). (C) Shown is the position of identified motif sequences within the analysed peak sequences used in (B). The majority of HrrA motifs centre at the position of the peak maximum (at 50 nt).

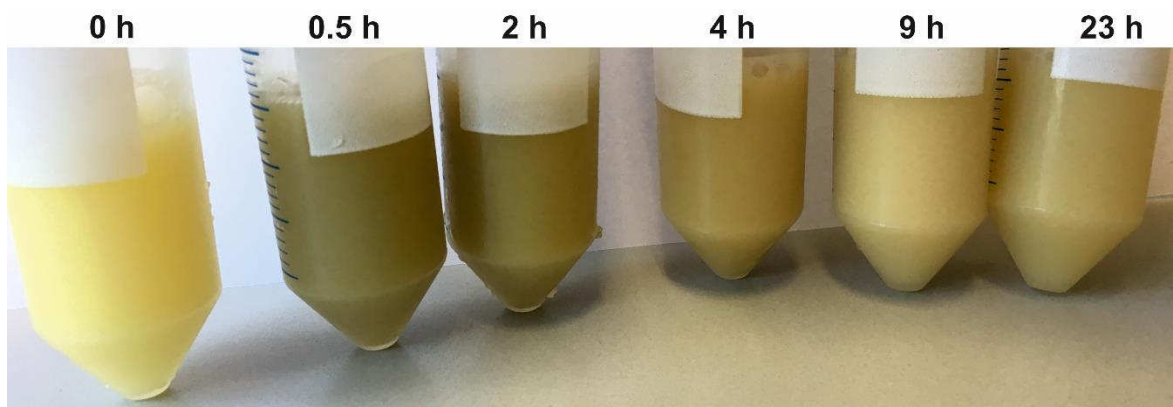


Figure S7: Visual inspection of *C. glutamicum* cells before and after addition of heme. Iron-starved *C. glutamicum* wild type cells were cultivated in CGXII medium (2 % (w/v) glucose, without FeSO_4) and cells were harvested at different time points before and after the addition of 4 μM heme. Cell pellets were subsequently resuspended in Tris buffer (100 mM Tris-HCl, 1 mM EDTA, pH 8.0) and adjusted to an OD_{600} of 3.5.

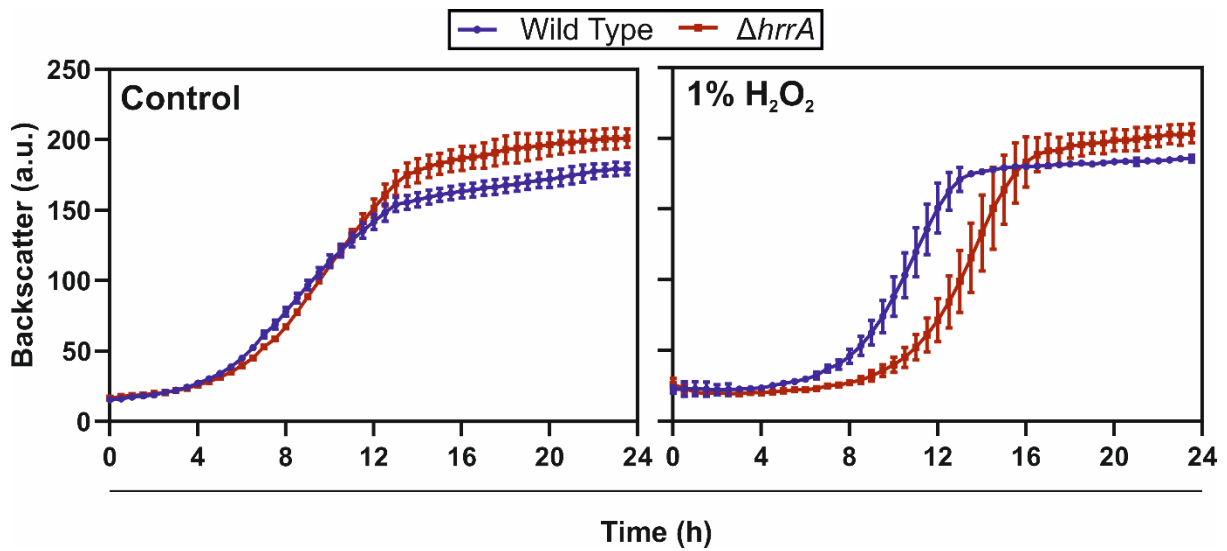


Figure S8: Growth assays revealed an increased sensitivity of *C. glutamicum* $\Delta hrrA$ against oxidative stress. Iron-starved *C. glutamicum* wild type as well as the mutant strain $\Delta hrrA$ were inoculated to an OD_{600} of 1 in CGXII medium (2% (w/v) glucose, 4 μ M hemin, without $FeSO_4$) and subsequently incubated for 15 min at RT either with 1% (v/v) H_2O_2 or without. This incubation time of 15 min served as avoidance of misleading backscatter measurements due to foam generation. After the incubation, cells were transferred to microtiter plates and cultivated in a microbioreactor cultivation system. Growth curves shown are based on backscatter measurements (expressed in arbitrary units (a.u.)) of three biological replicates. The error bars represent the standard deviation of these replicates.

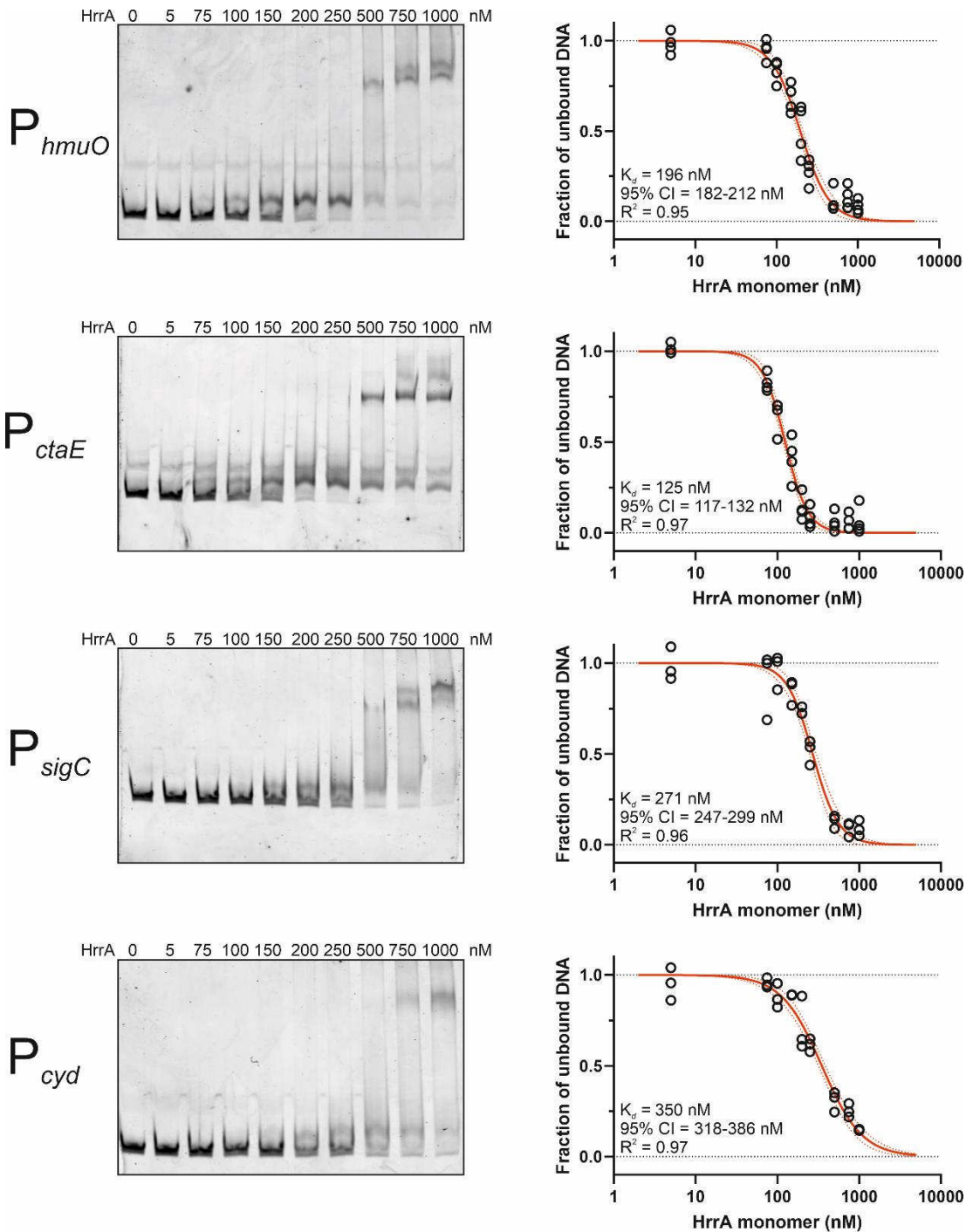


Figure S9: Binding affinity of HrrA to selected target promoters. Depicted are representative images of quantitative EMSAs used for analysis of protein-DNA interaction and the calculation of HrrA affinities to the different promoters. For the analysis, 10 nM Cy3-labelled 98-105 bp DNA fragments containing the maximal ChAP-Seq peak height were used with increasing amounts of HrrA (given as monomers). Determination of unbound DNA in EMSA studies allowed the calculation of HrrA binding affinities to different target promoters. Quantification of unbound DNA band intensities was performed using Image Studio Lite (Licor, Bad Homburg, Germany) and apparent K_d values were calculated using GraphPad Prism 7. The calculation of apparent K_d – values is based on 3-4 gels each. Black dotted lines represent top and bottom constraints for the fit. Red dotted lines represent the 95% confidence level. CI, confidence interval.

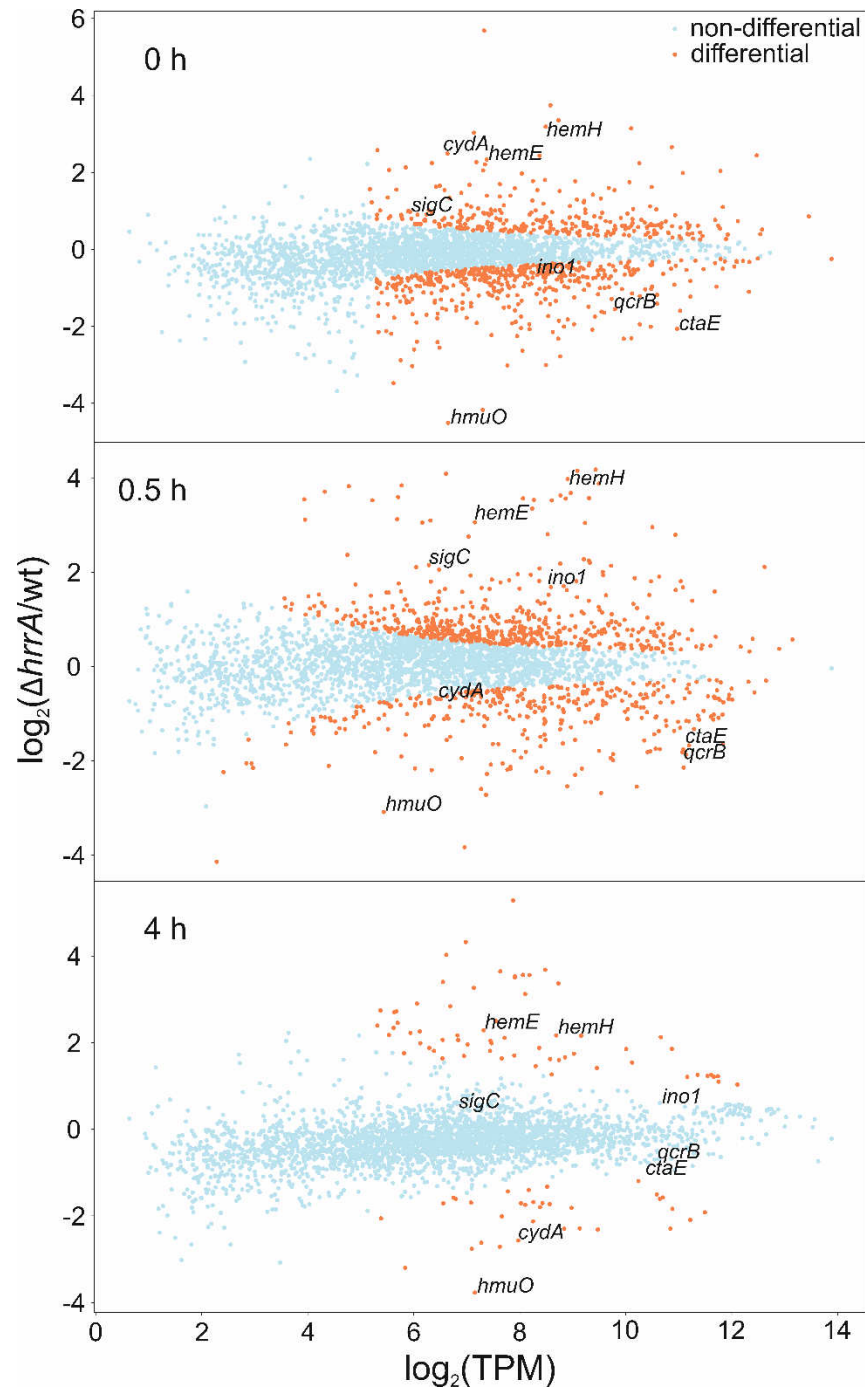


Figure S10: Time-resolved differential gene expression analysis. Shown is the log₂ fold change in gene expression ($\Delta hrrA$ versus wild type) along with a log₂ mean expression (expression averaged for $\Delta hrrA$ and WT samples) in transcripts per million (TPM). Orange dots represent significantly differentially expressed genes with an empirical FDR < 0.05 (see material and methods). Wild type and $\Delta hrrA$ *C. glutamicum* strains were grown in CGXII medium (without FeSO₄) supplemented with 2% (w/v) glucose and 4 μ M hemin (T_0 is prior addition of hemin; for details on cultivation and sample preparation see material and methods).

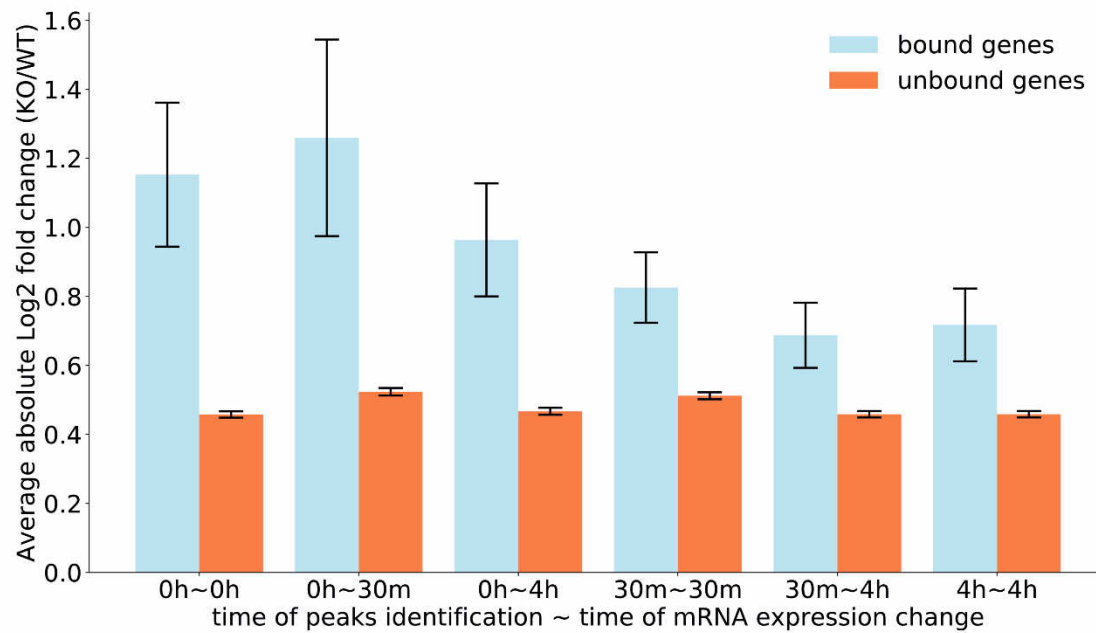


Figure S11: Correlation of HrrA binding and expression change. For the time-points 0h, 0.5h, 4h all the *C. glutamicum* protein-coding genes with decent expression (>10 TPM in $\Delta hrrA$ and WT samples) were split into groups: bound by HrrA (the ones which have an HrrA binding peak within 800 nt region upstream or 200 nt downstream to the transcription start site) and unbound. For these groups mean absolute log2 fold change ($\Delta hrrA$ /WT) was calculated for the time-points 0h, 0.5h, 4 h along with standard error of the mean.

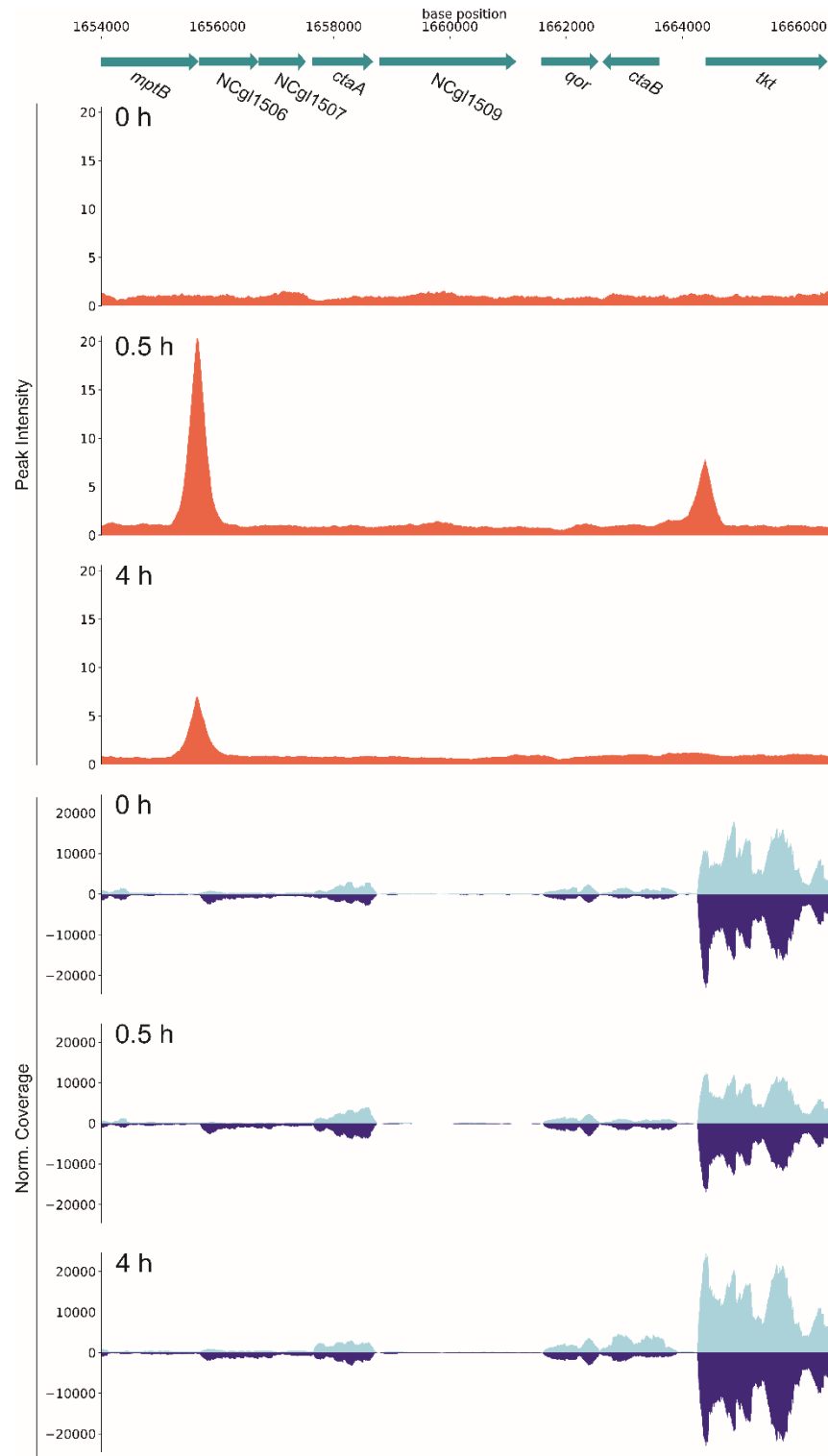


Figure S12: HrrA coordinates expression of *ctaA* and *ctaB* in response to heme. Shown are the ChAP-Seq (orange) and RNA-Seq (blue) results focusing on the *ctaA* and *ctaB* locus in the genome of *C. glutamicum*. Depicted is the genomic region between *mptb* (cg1766) and *tkf* (cg1774). For the cultivation, CGXII medium supplemented with 2% (w/v) glucose and 4 μ M hemin was inoculated with iron starved cells from a stationary culture and adjusted to an OD₆₀₀ of 3.5. Samples were analysed at the indicated time points as described in material and methods.

Supplementary Tables

Table S1: Bacterial strains and plasmids used in this study. Oligonucleotides used for the construction of the plasmids are listed in Table S2.

Strain	Relevant characteristics	Reference
<i>Escherichia coli</i>		
DH5α	<i>fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</i> ; for general cloning purposes	Invitrogen
BL21(DE3)	B F ⁻ <i>ompT gal dcm lon hsdS_B(r_B⁻m_B⁻) λ(DE3 [<i>lacI</i> <i>lacUV5-T7p07 ind1 sam7 nin5</i>] [<i>malB</i>⁺]_{K-12}(λ^S); overexpression of proteins.</i>	(1)
<i>Corynebacterium glutamicum</i>		
<i>C. glutamicum</i> ATCC 13032	Biotin-auxotrophic wild type strain	(2)
<i>C. glutamicum</i> Δ<i>hrrA</i>	Derivative of ATCC 13032 with in-frame deletion of the <i>hrrA</i> gene (cg3247).	(3)
<i>C. glutamicum</i>::<i>hrrA</i>-C-<i>twinstrep</i>	Derivative of ATCC 13032 encoding a C-terminally <i>twinstrep</i> -tagged version of <i>hrrA</i> (cg3247).	This study
Plasmids		
Name	Resistance	Source
pK19 <i>mob sacB</i>	Kanamycin	(4)
pK19 <i>mob sacB_hrrA</i>-C-<i>twinstrep</i>	Kanamycin	This study

Table S2: Oligonucleotides used in this study.

#	Name	Sequence
Construction of pK19 <i>mob sacB_hrrA-C-twinstrep</i>		
1	<i>hrrA</i> -LF-twin-strep_fw	CAAGCTTGCATGCCTGCAGGTCGACGCGGAATCGACGTCATCTTG
2	<i>hrrA</i> -LF-twin-strep_rv	ACCTAAAGCCTTGCAGCAACCCCGCTATTTTCGAACTGCGGGTGG
3	<i>hrrA</i> -RF_fw	GAGCCACCCGCAGTTCGAAAAATAGCGGGGGTTGCTGCAAGGC
4	<i>hrrA</i> -RF_rv	ATTCGAGCTCGGTACCCGGGGATCCCCGGAATCAATACACCGGC
Amplification of DNA probes for EMSAs		
5	<i>P_{hmuO}</i> (EMSA) fw	GAGAAATCCTCACGCTCAC
6	<i>P_{hmuO}</i> (EMSA) fw-Cy3	Cy3-GAGAAATCCTCACGCTCAC
7	<i>P_{hmuO}</i> (EMSA) rv	GGTGGGAGCCCCAAAGTTG
8	<i>P_{ctaE}</i> (EMSA) fw	CCCAAAGTGGTTTCCGCAGG
9	<i>P_{ctaE}</i> (EMSA) fw-Cy3	Cy3-CCCAAAGTGGTTTCCGCAGG
10	<i>P_{ctaE}</i> (EMSA) rv	ACGCCTTTTATTCGGGTTC
11	<i>P_{pck}</i> (EMSA) fw	CTTTCTATGGAGATGATCG
12	<i>P_{pck}</i> (EMSA) rv	CGATTAAATGGACCCTAAAC
13	<i>P_{ramB}</i> (EMSA) fw	CCTGCGCAAAGTTGCTCCCTG
14	<i>P_{ramB}</i> (EMSA) rv	CTCACAGGATACCGATCCGAAC
15	<i>P_{cg1080}</i> (EMSA) fw	CGCTCCTCTGTGGGATTTGTC
16	<i>P_{cg1080}</i> (EMSA) rv	GCCTTCACTCCCTCAAAC
17	<i>P_{xerC}</i> (EMSA) fw	CTTAGGCTTGCCTCACACAC
18	<i>P_{xerC}</i> (EMSA) rv	AATGCGGAAATGCCATAAAACC
19	<i>P_{cg3402}</i> (EMSA) fw	CATAGGGGTATAGCCTTGAG
20	<i>P_{cg3402}</i> (EMSA) rv	CAGTGTGCGCAGGTCATGCC
21	<i>P_{ctaC}</i> (EMSA) fw	GGAATACCTAAAGTCTAGGC
22	<i>P_{ctaC}</i> (EMSA) rv	GTAGGAACGTAGGGGGTAAG
23	<i>P_{sigC/katA}</i> (EMSA) fw	GGTCACCATAAAGGTGTGTAG
24	<i>P_{sigC/katA}</i> (EMSA) fw-Cy3	Cy3-GGTCACCATAAAGGTGTGTAG
25	<i>P_{sigC/katA}</i> (EMSA) rv	GCCACCAAATAATCAGCCC
26	<i>P_{cyd}</i> (EMSA) fw	GTTCCCGCTCACAGCTTAAC
27	<i>P_{cyd}</i> (EMSA) fw-Cy3	Cy3-GTTCCCGCTCACAGCTTAAC
28	<i>P_{cyd}</i> (EMSA) rv	GGTGACTTGTCACAAGGGG
29	<i>P_{trpS}</i> (EMSA) fw	GACTTGTTTACCCAAGCAATAC
30	<i>P_{trpS}</i> (EMSA) rv	CCGGTGAGGCAACATTACC
31	<i>P_{htaA}</i> (EMSA) fw	GTCATGATGGCGTCTCGGGC
32	<i>P_{htaA}</i> (EMSA) rv	GTAATCAACGCACAAATG

Table S5: Pearson correlation for the gene expression values (TPM) between the two biological replicates. Transcriptome expression estimates for all the three time-points and both KO and WT conditions show high reproducibility. The genes with low expression (combined expression in replicates < 5 TPM) were not included in this analysis.

Time Point	WT	KO
0 h	0.9987	0.9994
0.5 h	0.9990	0.9984
4 h	0.9974	0.9964

References

- Studier, F.W. and Moffatt, B.A. (1986) Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.*, **189**, 113-130.
- Kalinowski, J., Bathe, B., Bartels, D., Bischoff, N., Bott, M., Burkovski, A., Dusch, N., Eggeling, L., Eikmanns, B.J., Gaigalat, L. *et al.* (2003) The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *J Biotechnol*, **104**, 5-25.
- Frunzke, J., Gätgens, C., Brocker, M. and Bott, M. (2011) Control of heme homeostasis in *Corynebacterium glutamicum* by the two-component system HrrSA. *J Bacteriol*, **193**, 1212-1221.
- Schäfer, A., Tauch, A., Jäger, W., Kalinowski, J., Thierbach, G. and Pühler, A. (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene*, **145**, 69-73.

HrrSA orchestrates a systemic response to heme and determines prioritization of terminal cytochrome oxidase expression

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Keywords: Heme signaling, *Corynebacterium glutamicum*, *Corynebacterium diphtheriae*, two-component systems, bacterial transcription, regulatory networks, ChIP-Seq, HrrSA

Abstract

Heme is a multifaceted molecule. While serving as a prosthetic group for many important proteins, elevated levels are toxic to cells. The complexity of this stimulus has shaped bacterial network evolution. However, only a small number of targets controlled by heme-responsive regulators have been described to date. Here, we performed chromatin affinity purification and sequencing to provide genome-wide insights into *in vivo* promoter occupancy of HrrA, the response regulator of the heme-regulated two-component system HrrSA of *Corynebacterium glutamicum*. Time-resolved profiling revealed dynamic binding of HrrA to more than 200 different genomic targets encoding proteins associated with heme biosynthesis, the respiratory chain, oxidative stress response and cell envelope remodeling. By repression of the extracytoplasmic function sigma factor *sigC*, which activates the *cydABCD* operon, HrrA prioritizes the expression of genes encoding the cytochrome *bc₁-aa₃* supercomplex. This is also reflected by a significantly decreased activity of the cytochrome *aa₃* oxidase in the $\Delta hrrA$ mutant. Furthermore, our data reveal that HrrA also integrates the response to heme-induced oxidative stress by activating *kata* encoding the catalase. These data provide detailed insights in the systemic strategy that bacteria have evolved to respond to the versatile signaling molecule heme.

Introduction

Heme (iron bound protoporphyrin IX) is a versatile molecule that is synthesized and used by virtually all aerobic eukaryotic and prokaryotic cells (1). It serves as the prosthetic group of hemoglobins, hydroxylases, catalases, peroxidases, and cytochromes (2) and is therefore essential for many cellular processes, such as electron transfer, respiration and oxygen metabolism (3). Furthermore, salvaged heme represents the most important iron source for a variety of pathogenic bacteria (4,5), and also non-pathogenic bacteria can meet their iron demand by degradation of environmental heme. This becomes evident from the diverse set of heme uptake systems and heme oxygenases that catalyze the degradation of the protoporphyrin ring to biliverdin and the concomitant release of carbon monoxide and iron (6).

While heme represents an essential cofactor for a variety of proteins, this molecule also exhibits severe toxicity at high concentrations. Therefore, organisms have evolved sophisticated regulatory networks to tightly control heme uptake, detoxification (including export), synthesis and degradation (4). Several heme-regulated transcription factors have been described, including the heme activator protein (Hap) 1, which is an activator of genes required for aerobic growth of the yeast *Saccharomyces cerevisiae* (7); the transcription factor BACH1 (BTB and CNC homology 1), which is conserved in mammalian cells (8,9); and the rhizobial Irr protein, which is a heme-regulated member of the Fur family of transcriptional regulators (10-12).

In Gram-positive bacteria, two-component systems (TCSs) appear to play a prevalent role in heme-responsive signaling (13,14), as exemplified by the heme sensor system HssRS of *Staphylococcus aureus* and *Bacillus anthracis*, which controls the expression of the *hrtBA* operon, encoding a heme efflux system in both species (15,16). Remarkably, several members of the *Corynebacteriaceae* family, including the human pathogen *Corynebacterium diphtheriae* and the biotechnological platform strain *C. glutamicum*, have two paralogous TCSs, namely, HrrSA and ChrSA, dedicated to heme-responsive control of gene expression (17-20). The kinases HrrS and ChrS were recently shown to perceive transient changes in heme availability by direct intramembrane interactions with heme (21,22). Heme binding triggers autophosphorylation of the sensor kinase, followed by transfer of the phosphoryl group to the cognate response regulators HrrA and ChrA. In *C. glutamicum*, significant cross-phosphorylation was observed between the closely related systems; however, this crosstalk is proofread by a highly specific phosphatase activity of the kinases toward the cognate response regulators under non-inducing conditions (23). While the ChrSA system appears to be mainly involved in rapid activation of the HrtBA detoxification system (19), previous data suggest that HrrSA coordinates a homeostatic response to heme (18). In recent studies, six direct target operons have been described for HrrA, including genes encoding enzymes involved in heme synthesis (*hemE*, *hemA* and *hemH*), heme utilization (*hmuO*, encoding a heme oxygenase) and the *ctaE-qcrCAB* operon, encoding components of the heme-containing cytochrome *bc₁-aa₃* supercomplex of the respiratory chain (18). Expression of *hrrA* as well as *hmuO* is, furthermore, repressed by the global iron-dependent regulator DtxR in *C. glutamicum* under conditions of sufficient iron supply (24,25) thereby linking iron and heme regulatory networks in this organism.

The branched electron transport chain of *C. glutamicum* consists of the cytochrome *bc₁-aa₃* supercomplex (encoded by *ctaD*, the *ctaCF* operon, and the *ctaE-qcrCAB* operon) and the

cytochrome *bd* oxidase, encoded by the first two genes of the *cydABDC* operon (26). Although both the cytochrome *aa₃* oxidase and the *bd* oxidase are involved in the establishment of a proton-motive force (PMF), the *aa₃* oxidase is an active proton pump that is responsible for the increased proton translocation number ($6\text{ H}^+/2\text{ e}^-$) of the cytochrome *bc₁-aa₃* supercomplex compared to that of the *bd* oxidase ($2\text{ H}^+/2\text{ e}^-$) (26). The presence of the cytochrome *bc₁-aa₃* supercomplex is a characteristic feature of almost all actinobacteria, because members of this phylum lack a soluble cytochrome *c* and instead harbor a diheme cytochrome *c₁* that directly shuttles electrons from the *bc₁* complex to the *aa₃* oxidase (27-32). Furthermore, both terminal oxidases differ in heme content, as the *bc₁-aa₃* supercomplex harbors six heme molecules, while the *bd* oxidase harbors only three. Surprisingly, not much is known about the regulation of terminal oxidases in *C. glutamicum*. In addition to the described activation of the *ctaE-qcr* operon by HrrA, the hydrogen peroxide-sensitive regulator OxyR was described as a repressor of the *cydABCD* operon (33,34). Furthermore, the ECF sigma factor SigC (σ^C) activates expression of the *cydABCD* operon (33,35). For σ^C , a speculated stimulus is a defective electron transfer in the *aa₃* oxidase (35) and such a defect was observed under copper-deprivation or when heme *a* insertion was disturbed, which resulted in activation of the σ^C regulon (36,37).

Interestingly, the regulons of prokaryotic heme regulators described thus far comprise only a low number of direct target genes, which are mostly involved in heme export (e.g., *hrtBA*) or degradation (*hmuO*). This current picture of prokaryotic heme signaling, however, does not match the complexity of the cellular processes influenced by heme. In this study, we performed a time-resolved and genome-wide binding profiling of HrrA in *C. glutamicum* using chromatin affinity purification and sequencing (ChAP-Seq) of HrrA in *C. glutamicum* showing the transient HrrA promoter occupancy of more than 200 genomic targets in response to heme. The obtained results emphasize that HrrA is a global regulator of heme homeostasis, which also integrates the response to oxidative stress and cell envelope remodeling. Transcriptome analysis (RNA-Seq) at different time points after heme induction revealed HrrA to be an important regulator of the respiratory chain by coordinating the expression of components of both quinol oxidation branches as well as menaquinol reduction. Remarkably, HrrA was found to prioritize the expression of operons encoding the cytochrome *bc₁-aa₃* supercomplex by repressing *sigC* expression.

Methods

Bacterial strains and growth conditions

Bacterial strains used in this study are listed in Table S1. The *C. glutamicum* strain ATCC 13032 was used as wild type (29) and cultivations were performed in liquid BHI (brain heart infusion, Difco BHI, BD, Heidelberg, Germany), as complex medium or CGXII (38) containing 2 % (w/v) glucose as minimal medium. The cells were cultivated at 30°C; if appropriate, 25 µg/ml kanamycin was added. *E. coli* (DH5α and BL21 (DE3)) was cultivated in Lysogeny Broth (Difco LB, BD, Heidelberg, Germany) medium at 37°C in a rotary shaker and for selection, 50 µg/ml kanamycin was added to the medium.

Recombinant DNA work and cloning techniques

Cloning and other molecular methods were performed according to standard protocols (39). As template, chromosomal DNA of *C. glutamicum* ATCC 13032 was used for PCR amplification of DNA fragments and was prepared as described previously (40). All sequencing and synthesis of oligonucleotides was performed by Eurofins Genomics (Ebersberg, Germany). For ChAP sequencing, the native *hrrA* was replaced with a twin-strep-tagged version of this gene using a two-step homologous recombination system. This system is based on the suicide vector pK19 *mob-sacB* (41,42), containing 500 bps flanking each site of the targeted sequence inside the *C. glutamicum* genome. The pK19*mob-sacB hrrA-C-twinstrep* plasmid was constructed using Gibson assembly of PCR products (primers indicated in Table S2) and the cut pK19 vector (43).

ChAP Sequencing – Sample preparation

The preparation of DNA for ChAP sequencing was adapted from (44). The *C. glutamicum* strain ATCC 13032::*hrrA-C-twinstrep* was used for the time series experiment. A preculture was inoculated in liquid BHI medium from a fresh BHI agar plate and incubated for 8-10 h at 30°C in a rotary shaker. Subsequently, cells were transferred into a second preculture in CGXII medium containing 2 % (w/v) glucose and 0 µM FeSO₄ to starve the cells from iron. Protocatechuic acid (PCA), which was added to the medium, allowed the uptake of trace amounts of iron. From an overnight culture, six main cultures were inoculated to an OD₆₀₀ of 3.0 in 1 l CGXII medium containing 4 µM hemin as sole iron source. For the time point t = 0, the cells were added to 1 l fresh CGXII containing no additional iron source. After 0 h, 0.5 h, 4 h, 9 h and 24 h, cells corresponding to an OD₆₀₀ of 3.5 in 1 l were harvested by centrifugation at 4 °C, 5000 x g and washed once in 20 ml CGXII. Subsequently, the cell pellet was resuspended in 20 ml CGXII containing 1 % (v/v) formaldehyde to crosslink the regulator protein to the DNA. After incubation for 20 min at RT, the cross linking was stopped by addition of glycine (125 mM), followed by additional incubation of 5 min at RT. After that, the cells were washed three times in buffer A (100 mM Tris-HCl, 1 mM EDTA, pH=8.0) and the pellets stored overnight at -80 °C. For cell disruption, the pellet was resuspended buffer A containing “cOmplete” protease inhibitor cocktail (Roche, Germany) and disrupted using a French press cell (SLM Aisco, Spectronic Instruments, Rochester, NY) five times at 207 MPa. The DNA was fragmented to ~500 bp by sonication (Branson Sonifier 250, Branson Ultrasonics Corporation, Connecticut, USA) and the supernatant was collected after ultra-centrifugation (150.000 x g, 4 °C, 1 h). The DNA bound by the twin-Strep-

tagged HrrA protein was purified using Strep-Tactin XT Superflow column material (IBA Lifesciences, Göttingen, Germany) according to the supplier's manual (applying the gravity flow protocol, 1.5 ml column volume). Washing of the column was performed with buffer W (100 mM Tris-HCl, 1 mM EDTA, 150 mM NaCl, pH 8.0) and the tagged protein was eluted with buffer E (100 mM Tris-HCl, 1 mM EDTA, 150 mM NaCl, pH 8.0, added 50 mM D-Biotin). After purification, 1 % (w/v) SDS was added to the elution fractions and the samples were incubated overnight at 65°C. For the digestion of protein, 400 µg/ml Proteinase K (AppliChem GmbH, Darmstadt, Germany) was added and incubated for 3 h at 55 °C. Subsequently, the DNA was purified as following: Roti-Phenol/Chloroform/Isoamyl alcohol (Carl Roth GmbH, Karlsruhe, Germany) was added to the samples in a 1:1 ratio and the organic phase was separated using Phase Lock Gel (PLG) tubes (VWR International GmbH, Darmstadt, Germany) according to the supplier's manual. Afterwards, the DNA was precipitated by adding ice-cold ethanol (to a conc. of 70 % (v/v) and centrifugation at 16.000 x g, 4 °C for 10 min. The DNA was washed with ice-cold 70 % (v/v) ethanol, then dried for 3 h at 50 °C and eluted in dH₂O.

ChAP-Seq analysis - Sequencing

The obtained DNA fragments of each sample (up to 2 µg) were used for library preparation and indexing using the TruSeq DNA PCR-free sample preparation kit according to the manufacturer's instruction, yet skipping fragmentation of the DNA and omitting the DNA size selection steps (Illumina, Chesterford, UK). The resulting libraries were quantified using the KAPA library quant kit (Peqlab, Bonn, Germany) and normalized for pooling. Sequencing of pooled libraries was performed on a MiSeq (Illumina) using paired-end sequencing with a read-length of 2 x 150 bases. Data analysis and base calling were accomplished with the Illumina instrument software and stored as fastq output files. The sequencing data obtained for each sample were imported into CLC Genomics Workbench (Version 9, Qiagen Aarhus A/S) for trimming and base quality filtering. The output was mapped to accession NC_003450.3 BX927147 as *C. glutamicum* reference genome (www.ncbi.nlm.nih.gov/pubmed/12948626). Genomic coverage was convoluted with second order Gaussian kernel. The kernel was truncated at 4 sigmas (that is all kernel values positioned further then 4 sigmas from the center were set to zero) and expanded to the "expected peak width". The expected peak width was estimated *via* the following procedure: 1) all the peaks higher than 3-fold mean coverage were detected. 2) Points at which their coverage dropped below ½ of the maximal peak height were found and the distance between them was considered as a peak width. 3) The "estimated peak width" was set equal to the median peak width. The convolution profile was scanned in order to find points where first derivative changes its sign from positive to negative (Figure S1). Each such point was considered as a potential peak and was assigned with a convolution score (that is convolution with second order Gaussian kernel centred at the peak position). Furthermore, we explored the distribution of the convolution scores. It appeared to resemble normal distribution, but with a heavy right tail. We assumed that this distribution is indeed bimodal of normal distribution (relatively low scores) representing 'noise' and a distribution of 'signal' (relatively high scores). We fit the Gaussian curve to the whole distribution (via optimize.fit function from SciPy package (45)) and set a score thresholds equal mean + 4 sigmas of the fitted distribution. Further filtering with this threshold provided estimated FDR (false discovery rate) of 0.004-0.013 depending on a sample. Filtered peaks were normalized to allow inter-sample comparisons. Sum of coverages of the detected peaks was negated from

the total genomic coverage. The resulting difference was used as normalization coefficient; that is peak intensities were divided by this coefficient.

ChAP-Seq analysis – Estimation of confidence intervals

To compare peak intensities between the samples, we assessed the significance levels of the detected intensity values by an extensive *in silico* simulation of ChAP-seq experiments along with further peak-detection analyses.

The simulation consisted of the following steps: The reads were artificially generated from *C. glutamicum* genome (NC003450.3) with the error rate (number of nucleotide mismatches) equal to the average error rate of the real HrrA ChAP-seq reads (estimated from the mapping statistics). The reads were taken from randomly selected spots in the genome (simulation of the non-peak coverage) and from the regions of the detected HrrA binding peaks with the probabilities proportional to the original peak intensities. Thus, we tried to emulate the original binding architecture. We also added a small amount (10% of the total simulated reads) of the sequences heavily affected by mismatches (25% mismatches for the original *C. glutamicum* sequences), as we wanted to account for around 10% of the unmapped reads in the original HrrA ChAP-seq experiments. Finally, the simulated reads were subjected to the computational peak-detection pipeline with the same parameters as in the original analyses. As a result, we obtained the peak intensity values for the detected peaks.

In total, we simulated 200 ChAP-seq samples, each containing 1.14M reads (the average amount of reads in the original samples). For each of the detected peaks we estimated the variation of the reported peak intensity among all the simulations. That is, for each peak intensity we estimated 0.95 confidence interval, as a difference between 97.5 and 2.5 percentiles. We discovered a strong positive correlation (0.94 Pearson) between the width of the confidence intervals and mean intensity (Figure S2A). Therefore, we then normalized the width of the confidence intervals to the mean intensity values. The normalized confidence interval width (NCIW) appears to be a convenient metric as it is similar for all peaks, weakly dependent on their intensity. However, for the strongest peaks (peak intensity > 10) the NCIW is limited by 0.2, while for the weaker ones by 0.28 (Figure S2B). Then we convert NCIW upper limits to the minimum confident fold changes by the following rule: $\text{min_fold} = (1 + \text{NCIW}/2) / (1 - \text{NCIW}/2)$. Thus, we conclude that for the stronger peaks minimum confident fold change (p-value < 0.05) is ~1.23, while for the weaker peaks - ~1.33.

RNA Sequencing – Sample preparation

For RNA sequencing, *C. glutamicum* wild type and the ΔhrrA mutant strain were cultivated under the same conditions as described for ChAP Sequencing. Both strains did not contain any plasmids and, hence, were cultivated without addition of antibiotics in biological duplicates. After 0 h (no heme), 0.5 h and 4 h, cells corresponding to an OD₆₀₀ of 3 in 0.1 l were harvested in falcon tubes filled with ice by centrifugation at 4 °C and 5000 x g for 10 minutes and the pellets were stored at -80 °C. For the preparation of the RNA, the pellets were resuspended in 800 µl RTL buffer (QIAGEN GmbH, Hilden, Germany) and the cells disrupted by 3 x 30 s silica bead beating, 6000 rt/min (Precellys 24, VWR International GmbH, Darmstadt, Germany). After ultra-centrifugation

(150.000 x g, 4 °C, 1 h), the RNA was purified using the RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the supplier's manual. Subsequently, the ribosomal RNA was removed by running twice the workflow of the Ribo-Zero rRNA Removal Kit [Bacteria] (Illumina, California, USA) in succession. Between steps, the depletion of rRNA as well as the mRNA quality was analysed using the TapeStation 4200 (Agilent Technologies Inc, Santa Clara, USA). After removal of rRNA, the fragmentation of RNA, cDNA strand synthesis and indexing was carried out using the TruSeq Stranded mRNA Library Prep Kit (Illumina, California, USA) according to the supplier's manual. Afterwards, the cDNA was purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, USA). The resulting libraries were quantified using the KAPA library quant kit (Peqlab, Bonn, Germany) and normalized for pooling.

RNA-seq analysis

Sequencing reads quality was explored with the FastQC (46) tool. Since reads appeared to be of a good quality and did not harbor significant fraction of adapters or overrepresented sequences, no pre-processing was undertaken. Identical reads were collapsed with a custom script in order to prevent gene levels' misquantification caused by PCR overamplification. Reads were mapped to the *Corynebacterium glutamicum* genome (BX927147) with Bowtie2 (47). Bowtie2 was run with the following parameters: bowtie2 -1 [path to the reads, 1st mate] -2 [path to the reads, 2nd mate] -S [path to the mappings] -phred33 -sensitive-local -local -score-min C,90 -rdg 9,5 -rfg 9,5 -a -no-unal -I 40 -X 400 -no-mixed -ignore-quals.

The reads mapped to multiple locations were split proportionally between parental genes. That is if 3 reads are mapped to gene A and gene B, expression of gene A is 10 and expression of gene B is 5, then 2 reads will go to gene A and 1 read to gene B. For each *C. glutamicum* gene (48) we assigned an expression value equal to the average read coverage over the gene region. These expression values were then normalized to TPM (transcripts per million) values (49).

Furthermore, we analyzed which genes are significantly differentially expressed between conditions. We set combinatorial thresholds on normalized GEC (gene expression change) $[|\text{expr1} - \text{expr2}| / (\text{expr1} + \text{expr2})]$ and MGE (mean gene expression) $[\log_2((\text{expr1} + \text{expr2})/2)]$ where "expr1" is gene expression for the first condition and "expr2" for the second. Thresholds were set in a way to achieve maximal sensitivity while keeping FDR (false discovery rate) less than 0.05. FDR was estimated as $\text{GEC}_{\text{intra}} / (\text{GEC}_{\text{intra}} + \text{GEC}_{\text{inter}})$; where $\text{GEC}_{\text{intra}}$ is a number of genes passed the thresholds based on intrasample GEC (that is gene expression change between the replicates for the same condition), $\text{GEC}_{\text{inter}}$ is a number of genes passed the thresholds based on intersample GEC (that is gene expression change between two different conditions). Threshold function for GEC was defined as: $1 \mid \text{if } \text{MGE} < C; 2^{*-}(-A * \text{MGE}) + B \mid \text{if } \text{MGE} \geq C$; where A, B, C are parameters to be adjusted. Parameters A, B, C were adjusted with genetic algorithm optimization approach to achieve maximal sensitivity in discovery of differentially expressed genes while keeping FDR below 0.05.

Sequencing and sequence analysis

Pooled libraries were sequenced on a MiSeq (Illumina, California, USA) generating paired-end reads with a length of 2 x 75 bases. Data analysis and base calling were performed with the Illumina instrument software and stored as fastq output files.

Measurement of cell-associated hemin

C. glutamicum was cultivated in 4 μM hemin as described above (see ChAP Sequencing). To measure the cell-associated heme pool, CGXII minimal medium supplemented with 2 % (w/v) glucose and 4 μM heme was inoculated to an OD_{600} of 3.5. Samples were taken 0.5, 2, 4, 9 and 24 hours after addition of heme. Cells were harvested, resuspended in 100 mM Tris-HCl (pH 8) and adjusted to an OD_{600} of 100. Cells cultivated in 4 μM FeSO_4 supplemented medium were taken as a control and harvested at the same time points. Absolute spectra of cells reduced with a spatula tip of sodium dithionite were measured at room temperature using the Jasco V560 with a silicon photodiode detector in combination with 5 mm light path cuvettes. Absorption values at 406 nm were normalized by subtracting the measured absorption values of Fe-cultivated cells.

Electrophoretic mobility shift assays (EMSA)

The promotor regions of HrrA target genes (100 bp) were chosen based on the ChAP-Seq analyses and covered the maximal HrrA peak area (for primers see Table S2). For quantitative measurements, Cy3-labelled oligonucleotides were used for the generation of the DNA fragments. Before addition of the DNA, HrrA was phosphorylated by incubation for 60 min with MBP-HrrSA1-248 in a ratio of 2:1 and 5 mM ATP. Binding assays were performed in a total volume of 20 μl using 15 nM DNA and increasing HrrA concentrations (75 nM and 375 nM) for the qualitative analyses and 10 nM DNA with increasing HrrA concentrations from 5-1000 nM for quantitative analyses, respectively. The binding buffer contained 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM MgCl_2 , 5% (v/v) glycerol, 0.5 mM EDTA and 0.005% (w/v) Triton X-100. After incubation for 20 min at room temperature, the reaction mixtures were loaded onto a 10 % native polyacrylamide gel and subsequently separated and documented using a Typhoon TrioTM scanner (GE healthcare). The band intensities of unbound DNA were quantified using Image Studio Lite (Licor, Bad Homburg, Germany). The band intensities were normalized to the lane containing no DNA and plotted against the HrrA concentration in \log_{10} scale. Apparent K_d values were calculated based on at least 3 gels each using a sigmoidal fit and the software GraphPad Prism 8. For the sigmoidal fit, $Y=0$ and $Y=1$ were set as top and bottom constraints. The turning point of the curve was defined as the apparent K_d .

TMPD oxidase assay

C. glutamicum wild type strain and the ΔhrrA mutant were cultivated to an OD_{600} of 4 in CGXII minimal with or without the addition of 4 μM hemin. Subsequently, cells were disrupted in a homogenisator Precellys[®] (VWR International GmbH, Darmstadt, Germany) using zirconia/silica-beads (\varnothing 0.1 mm, Roth, Karlsruhe) in 100 mM Tris-HCl (pH 7.5) buffer. Ultracentrifugation at 200,000 $\times g$ for 1 h was used for membrane isolation. The pellet was resuspended in 100 mM Tris-HCl buffer and the protein concentration was determined using a BCA assay. The N,N,N',N'-Tetramethyl-*p*-phenylenediamine (TMPD) oxidase activity in the membrane fraction was measured spectrophotometrically at 562 nm in a TECAN Reader (Thermo Fisher Scientific, Massachusetts, US) by injecting 200 μM TMPD (37). An extinction coefficient of 10.5 $\text{mM}^{-1} \text{cm}^{-1}$ was used (50). One unit of activity was defined as 1 μmol of TMPD oxidized per minute. As a control for autooxidation, a sample containing only 100 mM Tris-HCl buffer was recorded after

TMPD addition and subtracted from the actual rates. Significance was evaluated by an unpaired t-test with a 95% confidence interval.

Results

Genome-wide profiling of HrrA promoter occupancy

In previous studies, a number of direct HrrA target operons were described in *C. glutamicum* and *C. diphtheriae*, suggesting an important role of the HrrSA TCS in the control of heme homeostasis (17-20). It has to be noted, that the membrane embedded HrrS sensor kinase is also activated by endogenously synthesized heme (21) and that the addition of external heme leads to a boost of the HrrSA response. In this study, we investigated the genome-wide binding profile of HrrA using chromatin affinity purification of twin-Strep-tagged HrrA combined with DNA sequencing (ChAP-Seq). Importantly, qPCR experiments confirmed wild-type level expression of the twin-Strep-tagged version of HrrA.

To obtain insights into the stimulus-dependent DNA association and dissociation, *C. glutamicum* cells were grown in iron-depleted glucose minimal medium, and samples were obtained before (T_0) and 0.5, 2, 4, 9 and 24 h after the addition of 4 μ M hemin. HrrA was purified, and the bound DNA fragments were sequenced (Figure 1A). We obtained substantial enrichment of known HrrA targets in response to heme (e.g. after 0.5 hours: 5-fold *hmuO*, 54-fold *hemE*, 105-fold *ctaE*; Figure 1B, C, D, respectively) and identified more than 200 previously unknown HrrA-bound regions in the *C. glutamicum* genome (Table S3).

As expected, the highest number of peaks was identified at the first time point after the heme pulse (0.5 h), with 199 peaks meeting our applied threshold (distance of <800 bp to the closest downstream or <200 bp to the closest upstream transcription start site (TSS)). In comparison, only 15 peaks showed a more than two-fold enrichment before hemin addition (T_0 , Table S3 and Figure S3). It has to be noted, that these 15 peaks detected at T_0 appear to be specific HrrA targets, since none of them was detected in an input control sample. Overall, these data illustrate the fast and transient DNA binding by HrrA in response to heme. In general, the majority of the discovered HrrA binding sites were close to TSSs (Figure S4). The binding of HrrA to 11 selected targets was confirmed by electrophoretic mobility shift assays (Figure S5), and a palindromic binding motif was deduced (Figure 2B and Figure S6).

The HrrA binding patterns depicted in Figure 1B-D are representative of many bound regions. Thirty minutes after the heme pulse, the average peak intensities increased approximately 2.5-fold in comparison to those at T_0 (Figure 2A). After 2 h of cultivation in hemin, the average peak intensity is declining and is, after 9 hours, already below the starting level at T_0 reaching a minimum in stationary phase (24 h). This is likely the result of the pre-cultivation and main cultivation under iron starvation conditions leading to a lowered intracellular heme pool. The dissociation of HrrA from its target promoters is, consequently, caused by rapid depletion of heme and a switch of HrrS from kinase to phosphatase state (23). Heme depletion was confirmed by spectroscopy of *C. glutamicum* cells (Figure 2A, dashed line) and was also obvious upon visual inspection (Figure S7). Of all peaks, that passed our threshold, 128 were upstream of genes encoding hypothetical proteins, while 150 could be assigned to genes with known or predicted function (Figure 2C). Furthermore, we assessed the significance levels of HrrA binding changes between samples from different conditions or/and time-points. It turned out, that for the

stronger peaks (peak intensity > 10) the minimum significant fold change (p-value < 0.05) is ~1.23, while for the weaker peaks (peak intensity < 10) it is ~1.33 (see Material and Methods).

To analyze the synchronicity in the HrrA regulon, peak intensities were correlated over time. A relatively high correlation between peak intensities for the time points 0.5, 2 and 4 h (Figure 2D) showed that, the system reacted proportionally for a majority of the binding sites and the strength of HrrA binding changed in response to heme availability. Relaxation of the system was observed after 9 h were peak intensities correlated well with T_0 .

The HrrSA TCS coordinates heme homeostasis by integrating the response to oxidative stress and cell envelope remodeling

Our dataset confirmed the binding of HrrA to all previously known targets, including genes encoding components of heme biosynthesis (*hemE*, *hemH* and *hemA*), degradation (*hmuO*), and export (*hrtBA*) pathways and heme-containing complexes of the respiratory chain (*ctaE-qcrCAB* operon and *ctaD*). A comprehensive overview of all identified HrrA targets is presented in Table S3; selected target genes are listed in Table 1. Among the more than 180 novel targets identified in this study, we observed HrrA binding upstream of *ctaB*, which encodes a protoheme IX farnesyltransferase that catalyzes the conversion of heme *b* to heme *o* (26) and upstream of *ctaC*, which encodes subunit 2 of the cytochrome *aa₃* oxidase. Remarkably, HrrA binding was also observed upstream of the *cydABDC* operon, which encodes the cytochrome *bd* oxidase of the respiratory chain. Altogether, this set of target genes highlights the global role of the HrrSA system in heme-dependent coordination of both branches of the respiratory chain. The HrrA regulon appeared to cover also the aspect of cofactor supply for the respiratory chain, as several HrrA targets encode enzymes involved in menaquinone reduction (*sdhCD*, *lldD* and *dld*).

Besides the known heme biosynthesis targets (*hemE*, *hemH* and *hemA*), HrrA binding was also observed upstream of a gene (*hemQ*) encoding a putative dismutase-family protein. However, in actinobacteria, it was proposed that these proteins do not possess chlorite dismutase activity but instead are essential for heme synthesis (51). Furthermore, we observed binding of HrrA upstream of the *chrSA* operon encoding the second TCS involved in heme-dependent regulation in *C. glutamicum*. This finding therefore confirmed the previously postulated cross-regulation of these TCS at the level of transcription (24,52).

Furthermore, HrrA binding was also observed upstream of several genes involved in the oxidative stress response, including *kata*, encoding catalase, *tusG*, encoding a trehalose uptake system (53), and upstream of *gapA* and *gapB* (glyceraldehyde-3-phosphate dehydrogenase, glycolytic and gluconeogenic, respectively) (54,55). In line with these findings, the phenotypic analysis of a *C. glutamicum hrrA* mutant revealed a significantly higher sensitivity to oxidative stress (treatment with H_2O_2) in comparison to the wild type (Figure S8). These findings suggest that the HrrSA system not only controls heme biosynthesis and degradation but also integrates the response to heme-induced oxidative stress.

A further important class of HrrA targets is represented by genes associated with the regulation or maintenance of the *C. glutamicum* cell envelope. The gene products of these previously unknown HrrA targets are, for instance, involved in the synthesis of peptidoglycan (*murA*), the peptidoglycan precursor meso-2,6-diaminopimelate (mDAP), inositol-derived lipids (*ino1*) and arabinogalactan (*aftC*). Furthermore, HrrA binding was revealed upstream of a number of genes encoding global transcriptional regulators (e.g., *ramA*, *ramB*, and *amtR*), adding a further level of complexity to this systemic response to heme.

Temporal dynamics of promoter occupancy reveal hierarchy in the HrrA regulon

With the time-resolved and genome-wide analysis of HrrA binding, we were also able to visualize distinct binding patterns of HrrA in response to addition and depletion of heme. Consequently, we asked whether the binding patterns (ChAP-Seq coverage) could provide information regarding the apparent dissociation constant (K_d) of HrrA to specific genomic targets. We compared the *in vivo* binding patterns of HrrA to *ctaE*, *hmuO* and *cydAB* (Figure 1, Table S3). While a comparably high peak was observed upstream of the *ctaE* promoter – even before the addition of heme (T_0) – the binding of HrrA to the promoter of *hmuO* occurred with apparently high stimulus dependency and appeared to be rather transient, as HrrA was fully dissociated from this promoter 9 h after the addition of hemin (Figure 1BC).

Subsequently, we determined the *in vitro* affinity of phosphorylated HrrA to the promoter regions of *ctaE*, *cydAB* and *hmuO* (Table 2, Figure S9). Consistent with the ChAP-Seq data, we measured the highest affinity of HrrA to P_{ctaE} with an apparent K_d of 125 nM. We therefore hypothesize that the *ctaE* promoter is a prime target that is constitutively activated by HrrA (Table S3) to maintain high gene expression of the operon encoding the *bc₁-aa₃* supercomplex. In line with this hypothesis, we also found a high HrrA binding peak upstream of the other operons encoding components of the *bc₁-aa₃* supercomplex (*ctaD* and *ctaCF*, Table 1 and Table S3).

In contrast, we measured an almost 3-fold higher apparent K_d (350 nM) for P_{cydAB} , which was consistent with the relatively transient binding pattern observed for this target. With an apparent K_d of 196 nM, the *in vitro* binding affinity of HrrA to the *hmuO* promoter was rather high considering the genomic coverage measured in the ChAP-Seq analysis. However, *in vitro* analysis does not account for the widespread interference among regulatory networks *in vivo*. In the particular example of *hmuO*, the pattern of HrrA binding was likely the result of interference with the global regulator of iron homeostasis, DtxR, which has previously been described to repress *hmuO* expression by binding to adjacent sites (56). Taken together, these results suggest that *in vivo* promoter occupancy is not only influenced by the binding affinity of the regulator to the particular target, but also significantly shaped by network interference. Consequently, high *in vivo* promoter occupancy indicates high binding affinity, but conclusions based on weakly bound regions may be confounded by competition with other binding factors.

HrrA activates the expression of genes encoding components of both branches of the quinol oxidation pathway

To evaluate how HrrA binding affects the expression of individual target genes, we analyzed the transcriptome (RNA-Seq) of the *C. glutamicum* wild type strain (ATCC 13032) as well as a $\Delta hrrA$ mutant (Table S4). Analogous to the ChAP-Seq experiments, RNA-Seq analysis was performed prior to the addition of heme (T_0) as well as 0.5 and 4 h after the heme pulse (in medium containing no other iron source). The RNA-Seq analysis was performed in two independent biological replicates (for inter-replicate, see Table S5).

At T_0 , before the addition of heme, already 212 genes showed a more than 2-fold altered expression level in $\Delta hrrA$ cells compared to wild type cells ($\Delta hrrA$ /wt). Directly after the addition of heme (0.5 h), the expression of 309 genes changed more than 2-fold. (Table S4, Figure 3A, orange dots). Of these genes, 174 were upregulated and 135 were downregulated in the *hrrA* deletion strain. 4 h after addition of heme, only 167 genes exhibited a greater than 2-fold increase or decrease (scatter plots for additional time points are presented in Figure S10).

The *hrrA* expression decreased after 0.5 h upon the addition of heme, which was likely caused by DtxR repression in response to increased intracellular iron levels (Figure 3B) (24). In contrast, after 4 h of cultivation, *hrrA* levels significantly increased, reflecting the depletion of heme as an alternative iron source and dissociation of DtxR. Furthermore, differential gene expression analysis revealed HrrA to be an activator of all genes encoding components of the respiratory chain (*ctaE*, *ctaD*, *ctaF* and *cydAB*) and as a repressor of heme biosynthesis (*hemA*, *hemE* and *hemH*) (Figure 3C). The impact on the cytochrome *bc₁-aa₃* supercomplex was also confirmed by measuring the activity of the *aa₃* oxidase, which was about 2-fold reduced in a *hrrA* mutant in comparison to the wild type when grown on heme (Figure 3D). Additionally, expression of *lldD* (L-lactate dehydrogenase) as well as *sdhCD* (succinate dehydrogenase) contributing to the reduced menaquinone pool was downregulated more than three-fold upon deletion of *hrrA*. In addition to these considerable differences between the wild type and the $\Delta hrrA$ mutant, we also observed decreased mRNA levels of genes involved in the oxidative stress response (e.g. *katA*) or cell envelope remodeling (e.g. *murA*) in the $\Delta hrrA$ mutant, suggesting HrrA to be an activator of these targets.

In some cases, promoter occupancy by HrrA did not result in altered expression levels of the particular target gene in a $\Delta hrrA$ mutant under the tested conditions (Table 1, Table S3). This finding is, however, not surprising considering the multiplicity of signals and regulators affecting gene expression. Under changing environmental conditions, transcription factor binding will not necessarily always be translated in an altered gene expression of the respective target. When we compare the results obtained from RNA-Seq and ChAP-Seq analysis, 269 genes out of 309 genes featuring an >2-fold change in gene expression did not show HrrA binding in their upstream promoter region. Looking at all HrrA targets (ChAP-Seq analysis) on a global scale, there is, nevertheless, a significantly higher impact on gene expression in a strain lacking *hrrA* for all targets bound by HrrA in comparison to non-targets (unbound, Figure S11). Overall, 109 out of 228 HrrA targets featured a significantly altered gene expression in the *hrrA* mutant (64 increased and 55 decreased).

HrrA determines the prioritization of terminal cytochrome oxidases by repression of *sigC*

The results from ChAP-Seq and RNA-Seq experiments highlight the important role of HrrA in the control of the respiratory chain, including cofactor supply. Our data revealed that HrrA activates the expression of genes encoding the cytochrome *bc₁-aa₃* supercomplex (*ctaE-qcrCAB*, *ctaD*, *ctaCF*) and of *cydAB*, encoding the cytochrome *bd* branch of the respiratory chain (Figure 4, Table S3). Remarkably, the mRNA profiles of the corresponding operons exhibited significantly delayed activation of *cydAB* in response to heme, which was abolished in the $\Delta hrrA$ mutant (Figure 4). In contrast, *ctaE* expression was significantly higher in wild type cells, even before hemin addition (T_0), but showed a further induction after stimulus addition (T 0.5 h, Table S4). Notably, we also observed binding of HrrA upstream of *sigC*, encoding an ECF sigma factor that was shown to be involved in the activation of the *cydABDC* operon (35). The mRNA level of *sigC* increased more than two-fold in the $\Delta hrrA$ mutant, indicating HrrA to be a repressor of this gene (Figure 4). Consistent with this hypothesis, *sigC* expression was slightly decreased in response to the addition of heme, which correlated with increased HrrA peak intensity (Figure 4F). Additionally, the higher *cydAB* expression, observed in the $\Delta hrrA$ strain before addition of stimulus (Figure 4B) is likely the effect of increased *sigC* expression (Figure 4C). Dissociation of HrrA from P_{sigC} at a later time point (4 h after heme pulse) led to derepression of *sigC* coinciding with an increased expression of *cydAB* in the wild type. Because *cydAB* levels were constitutively low in the $\Delta hrrA$ mutant in response to heme, we hypothesized that activation by HrrA together with an additional boost by SigC (Figure 5) leads to delayed activation of *cydAB* after the heme pulse. This regulation enables cells to channel most of the available heme pool into the more efficient cytochrome *bc₁-aa₃* supercomplex. The lower apparent K_d of HrrA for the *ctaE* promoter (125 nM) compared to P_{cydAB} (350 nM) or P_{sigC} (270 μ M) also reflects this prioritization of HrrA targets (Table 2). Consequently, this almost 3-fold decrease in affinity (apparent K_d) increases the threshold for HrrSA activity to control these targets.

HrrA activates PTS-dependent and –independent glucose uptake

Besides the activation of all components constituting the respiratory chain, ChAP-Seq experiments and transcriptome analysis revealed HrrA as a direct activator of genes encoding components of the phosphotransferase (PTS) system (*ptsH* and *ptsG*) and of *iolT1* encoding inositol permease with a reported function as a PTS-independent glucose uptake system (57). Remarkably, the gene *ppgK*, encoding the polyphosphate glucokinase was among the targets with the highest HrrA peak and showed reduced expression in the 4 h sample (Table 1 and Table S4). These results emphasize that cellular respiration and glucose uptake is coordinated via the HrrSA system in response to cellular heme levels.

Discussion

In this work, we applied a genome-wide approach to study the “heme-responsive regulator” HrrA in *C. glutamicum* and identified more than 200 genomic target regions of this response regulator. This intriguingly diverse set of target genes, encoding enzymes involved in heme biosynthesis, heme-containing proteins, and components of the respiratory chain as well as proteins involved in oxidative stress response, glucose uptake and cell envelope remodeling, provided unprecedented insight into the systemic response to heme coordinated by the TCS HrrSA.

In Gram-positive bacteria, TCSs appear to play a central role in transient heme sensing, and heme-responsive systems have been described in several prominent pathogens, including *C. diphtheriae*, *S. aureus* and *B. anthracis* (15-18). However, for all prokaryotic heme regulatory systems, only a small number of target genes have been described to date, focusing on targets involved in degradation (*hmuO* (18,58)), heme export (*hrtBA* (19,59)) or heme biosynthesis (*hemA* (18,20)). Systems orthologous to HrrSA are present in almost all corynebacterial species and the high amino acid sequence identity shared by the response regulators (87 %, between *C. glutamicum* and *C. diphtheriae* HrrA) suggests that the important role of HrrSA in the control of heme homeostasis is conserved. In many corynebacteria, including *C. diphtheriae*, control of heme homeostasis is shaped by the tight interplay of HrrSA with a second heme-dependent system, ChrSA. While the present study emphasized that HrrSA governs a large and complex homeostatic response, the only known target of the response regulator ChrA in *C. glutamicum* is the divergently located operon *hrtBA* encoding a heme export system. There is, however, also evidence for a cross-regulation between the TCSs, not only by cross-phosphorylation but also on the transcriptional level (23,24). In *C. diphtheria*, evidence for more overlap between the regulons of the TCSs has been provided, since both response regulators were shown to control a common set of target genes including *hrtBA*, *hemA* and *hmuO* (20,60). Genome-wide analysis of these systems have, however, not been performed so far and in vitro protein-DNA interaction studies may not necessarily reflect the in vivo promoter preferences of these highly similar systems.

Coping with heme stress

While being an essential cofactor for many proteins, heme causes severe toxicity to cells at high levels (4). In mammalian cells, the BACH1 regulator is inactivated by heme binding and plays a key role in maintaining the balance of the cellular heme pool (8,61). Heme oxygenases are targets of various heme-dependent regulators (18,62,63), and consistent with this principle, the mammalian *HMOX1* gene, encoding an NADPH-dependent oxygenase, is regulated by BACH1 (61). Other identified BACH1 targets are involved in redox regulation, the cell cycle, and apoptosis as well as subcellular transport processes (9,64,65).

Although neither the regulator nor the constitution of the regulon is conserved, the responses of BACH1 and HrrSA share a similar logic. Analogous to eukaryotic BACH1, we observed HrrA-mediated activation of genes involved in the oxidative stress response, including *kataA*, which appears to be required to counteract oxidative stress caused by elevated heme levels (Figure S8).

Remarkably, HrrA binding was also observed upstream of both *gapA* and *gapB*, which encode glyceraldehyde-3-phosphate dehydrogenases (GapDHs) involved in glycolysis and

gluconeogenesis, respectively. Previous studies in baker's yeast and mammalian cells have revealed that oxidative stress may block glycolysis by inhibiting GapDH (55,66). Furthermore, GapDH of *C. diphtheriae* was recently shown to be redox-controlled by S-mycothiolation (67). Slight activation of *gapA* by HrrA may thus counteract an impaired glycolytic flux under conditions of heme stress.

Furthermore, several HrrA targets play a role in the biosynthesis and remodeling of the corynebacterial cell envelope, including *ino1*, which is required for the synthesis of inositol-derived lipids (68), *lysC*, providing the peptidoglycan precursor meso-2,6-diaminopimelate (mDAP), and *murA* (Table 1). Taken together, these insights emphasize the important role of the HrrSA system in the control of heme stress responses.

From networks to function

Genome-wide analysis of regulatory networks may provide important hints towards the physiological function of genes. An example is provided by the HrrA-dependent regulation of *cg2079* (*hemQ*), described in this study (Table 1). In actinobacteria, it was recently proposed that these proteins inherit an essential role in heme biosynthesis (51,69). The finding that HrrA binds to the promoter of this gene and represses its expression supports a role of HemQ in heme biosynthesis in *C. glutamicum*. Among the direct targets of HrrA are many further targets encoding proteins of unknown function, including several ABC transport systems with a potential role in heme uptake or export. Therefore, this dataset provides guidance for further functional analysis of these HrrA targets to decipher their role in heme homeostasis.

Coordinated control of the respiratory chain

Among the most significantly affected targets in the $\Delta hrrA$ mutant were many genes encoding components of the respiratory chain (26). These genes comprise all the genes constituting the cytochrome *bc*₁-*aa*₃ branch of the respiratory chain (*ctaE-qcrCAB*, *ctaCF* and *ctaD*) (70); genes encoding the cytochrome *bd* branch (*cydAB* (26)); *ctaA* (71) and *ctaB* (72), encoding enzymes responsible for heme *a* synthesis; and *lldD* and *dld*, encoding lactate dehydrogenases that contribute to the reduced menaquinone pool (26) (Figure 4, Figure S12 and Table S3).

In a recent study, Toyoda and Inui described the ECF sigma factor σ^C to be an important regulator of both branches of the *C. glutamicum* respiratory chain. The *ctaE-qcrCAB* operon was shown to be significantly downregulated after σ^C overexpression due to binding of the sigma factor to the antisense strand of the promoter (35). Here, we demonstrated that this repression is counteracted by HrrA, which not only represses *sigC* but also activates *ctaE-qcrCAB* expression. While the two proteins have antagonistic effects on the expression of the supercomplex, both σ^C and HrrA positively regulate the *cyd* operon, encoding the cytochrome *bd* branch of the respiratory chain (Figure 5).

Interestingly, a hierarchy in the regulon was reflected by the differences in the apparent *K_d* values of HrrA with *P_{cydA}* and *P_{sigC}*, which were two-fold lower than those with the promoter of *ctaE*. These findings were also consistent with the ChAP-Seq experiments, where the peaks upstream of *ctaE* and *ctaD* were among the highest peaks at T₀ and after 0.5 h (Figure 4A). These data suggest that under conditions of sufficient heme supply, production of the cytochrome *bc*₁-*aa*₃

supercomplex is preferred, which is highly effective but requires the incorporation of six heme molecules (in contrast to only three molecules for the synthesis of the *bd* oxidase). Repression of *sigC* by HrrA and the relatively low affinity to the *cydAB* promoter results in delayed production of the *bd* branch. Under the applied aerobic conditions, available heme is thus first channeled to the cytochrome *bc₁-aa₃* supercomplex before the cytochrome *bd* oxidase is used, which is less efficient but has a higher oxygen affinity. Remarkably, HrrA was also found to activate expression of genes involved in PTS-dependent (*ptsH* and *ptsG*) and –independent (*ioIT1*) glucose uptake thereby ensuring a high glucose uptake rate under conditions of active cellular respiration.

Interference with other regulatory networks

Deletion of the *hrrA* gene led to more than 2-fold upregulation of 174 genes, while 135 genes were downregulated after the addition of heme. Several other genes were significantly affected but to a lesser extent. Remarkably, among the direct target genes controlled by HrrA, we identified several prominent global regulators, including the regulators of acetate metabolism *ramA* and *ramB* (73,74), and *amtR* encoding the master regulator of nitrogen control (75). Furthermore, *cpdA* encoding a cAMP phosphodiesterase playing a key role in the control of cellular cAMP levels in *C. glutamicum* (76) was found to be under direct control of HrrA. These examples illustrate the profound influence of HrrA on cellular networks and the systemic response cells have programmed to respond to heme availability.

Conclusion

Genome-wide analyses of targets controlled by prokaryotic transcription factors will change our view on many systems we believe to know. In this study, we provide an unprecedented insight into the systemic response to heme coordinated by the TCS HrrSA. Given the many properties of this molecule, the complexity of this response is actually not surprising but paves the way for further functional analysis of HrrA targets with so far unknown functions in heme homeostasis.

Data availability

The custom-developed software used in this study is publicly available at GitHub repository under the link <https://github.com/afilipch/afp>.

All sequencing data were deposited in the GEO database under the accession numbers GSE121962 (ChAP-Seq) and GSE120924 (RNA-Seq).

Supplementary Data

Supplementary Data are available at NAR online.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Ponka, P. (1999) Cell biology of heme. *The American journal of the medical sciences*, **318**, 241-256.
2. Layer, G., Reichelt, J., Jahn, D. and Heinz, D.W. (2010) Structure and function of enzymes in heme biosynthesis. *Protein science : a publication of the Protein Society*, **19**, 1137-1161.
3. Ajioka, R.S., Phillips, J.D. and Kushner, J.P. (2006) Biosynthesis of heme in mammals. *Biochimica et biophysica acta*, **1763**, 723-736.
4. Anzaldi, L.L. and Skaar, E.P. (2010) Overcoming the heme paradox: heme toxicity and tolerance in bacterial pathogens. *Infection and immunity*, **78**, 4977-4989.
5. Huang, W. and Wilks, A. (2017) Extracellular Heme Uptake and the Challenge of Bacterial Cell Membranes. *Annual review of biochemistry*, **86**, 799-823.
6. Wilks, A. (2002) Heme oxygenase: evolution, structure, and mechanism. *Antioxid Redox Signal*, **4**, 603-614.
7. Hickman, M.J. and Winston, F. (2007) Heme levels switch the function of Hap1 of *Saccharomyces cerevisiae* between transcriptional activator and transcriptional repressor. *Molecular and cellular biology*, **27**, 7414-7424.
8. Ogawa, K., Sun, J., Taketani, S., Nakajima, O., Nishitani, C., Sassa, S., Hayashi, N., Yamamoto, M., Shibahara, S., Fujita, H. *et al.* (2001) Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *The EMBO journal*, **20**, 2835-43.
9. Warnatz, H.J., Schmidt, D., Manke, T., Piccini, I., Sultan, M., Borodina, T., Balzereit, D., Wruck, W., Soldatov, A., Vingron, M. *et al.* (2011) The BTB and CNC homology 1 (BACH1) target genes are involved in the oxidative stress response and in control of the cell cycle. *J Biol Chem*, **286**, 23521-23532.
10. Qi, Z., Hamza, I. and O'Brian, M.R. (1999) Heme is an effector molecule for iron-dependent degradation of the bacterial iron response regulator (Irr) protein. *Proc Natl Acad Sci U S A*, **96**, 13056-13061.
11. Qi, Z. and O'Brian, M.R. (2002) Interaction between the bacterial iron response regulator and ferrochelatase mediates genetic control of heme biosynthesis. *Mol Cell*, **9**, 155-162.
12. O'Brian, M.R. (2015) Perception and Homeostatic Control of Iron in the Rhizobia and Related Bacteria. *Annual review of microbiology*, **69**, 229-245.

13. Torres, V.J., Stauff, D.L., Pishchany, G., Bezbradica, J.S., Gordy, L.E., Iturregui, J., Anderson, K.L., Dunman, P.M., Joyce, S. and Skaar, E.P. (2007) A *Staphylococcus aureus* regulatory system that responds to host heme and modulates virulence. *Cell host & microbe*, **1**, 109-119.
14. Mike, L.A., Choby, J.E., Brinkman, P.R., Olive, L.Q., Dutter, B.F., Ivan, S.J., Gibbs, C.M., Sulikowski, G.A., Stauff, D.L. and Skaar, E.P. (2014) Two-component system cross-regulation integrates *Bacillus anthracis* response to heme and cell envelope stress. *PLoS Pathog*, **10**, e1004044.
15. Stauff, D.L. and Skaar, E.P. (2009) The heme sensor system of *Staphylococcus aureus*. *Contributions to microbiology*, **16**, 120-135.
16. Stauff, D.L. and Skaar, E.P. (2009) *Bacillus anthracis* HssRS signalling to HrtAB regulates haem resistance during infection. *Mol Microbiol*, **72**, 763-778.
17. Bibb, L.A., Kunkle, C.A. and Schmitt, M.P. (2007) The ChrA-ChrS and HrrA-HrrS signal transduction systems are required for activation of the *hmuO* promoter and repression of the *hemA* promoter in *Corynebacterium diphtheriae*. *Infection and immunity*, **75**, 2421-2431.
18. Frunzke, J., Gatgens, C., Brocker, M. and Bott, M. (2011) Control of heme homeostasis in *Corynebacterium glutamicum* by the two-component system HrrSA. *Journal of bacteriology*, **193**, 1212-1221.
19. Heyer, A., Gatgens, C., Hentschel, E., Kalinowski, J., Bott, M. and Frunzke, J. (2012) The two-component system ChrSA is crucial for haem tolerance and interferes with HrrSA in haem-dependent gene regulation in *Corynebacterium glutamicum*. *Microbiology (Reading, England)*, **158**, 3020-3031.
20. Burgos, J.M. and Schmitt, M.P. (2016) The ChrSA and HrrSA Two-Component Systems Are Required for Transcriptional Regulation of the *hemA* Promoter in *Corynebacterium diphtheriae*. *J Bacteriol*, **198**, 2419-2430.
21. Keppel, M., Davoudi, E., Gätgens, C. and Frunzke, J. (2018) Membrane Topology and Heme Binding of the Histidine Kinases HrrS and ChrS in *Corynebacterium glutamicum*. *Front Microbiol*, **9**.
22. Ito, Y., Nakagawa, S., Komagata, A., Ikeda-Saito, M., Shiro, Y. and Nakamura, H. (2009) Heme-dependent autophosphorylation of a heme sensor kinase, ChrS, from *Corynebacterium diphtheriae* reconstituted in proteoliposomes. *FEBS letters*, **583**, 2244-2248.
23. Hentschel, E., Mack, C., Gatgens, C., Bott, M., Brocker, M. and Frunzke, J. (2014) Phosphatase activity of the histidine kinases ensures pathway specificity of the ChrSA and HrrSA two-component systems in *Corynebacterium glutamicum*. *Mol Microbiol*, **92**, 1326-1342.
24. Keppel, M., Piepenbreier, H., Gatgens, C., Fritz, G. and Frunzke, J. (2019) Toxic but tasty - temporal dynamics and network architecture of heme-responsive two-component signaling in *Corynebacterium glutamicum*. *Mol Microbiol*, **111**, 1367-1381.
25. Wennerhold, J. and Bott, M. (2006) The DtxR regulon of *Corynebacterium glutamicum*. *J. Bacteriol.*, **188**, 2907-2918.
26. Bott, M. and Niebisch, A. (2003) The respiratory chain of *Corynebacterium glutamicum*. *Journal of biotechnology*, **104**, 129-153.
27. Kao, W.C., Kleinschroth, T., Nitschke, W., Baymann, F., Neehaul, Y., Hellwig, P., Richers, S., Vonck, J., Bott, M. and Hunte, C. (2016) The obligate respiratory supercomplex from Actinobacteria. *Biochimica et biophysica acta*, **1857**, 1705-1714.
28. Niebisch, A. and Bott, M. (2001) Molecular analysis of the cytochrome *bc₁-aa₃* branch of the *Corynebacterium glutamicum* respiratory chain containing an unusual diheme cytochrome *c₁*. *Archives of microbiology*, **175**, 282-294.
29. Kalinowski, J., Bathe, B., Bartels, D., Bischoff, N., Bott, M., Burkovski, A., Dusch, N., Eggeling, L., Eikmanns, B.J., Gaigalat, L. et al. (2003) The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *Journal of biotechnology*, **104**, 5-25.

30. Ikeda, M. and Nakagawa, S. (2003) The *Corynebacterium glutamicum* genome: features and impacts on biotechnological processes. *Appl Microbiol Biotechnol*, **62**, 99-109.
31. Sone, N., Nagata, K., Kojima, H., Tajima, J., Koder, Y., Kanamaru, T., Noguchi, S. and Sakamoto, J. (2001) A novel hydrophobic diheme c-type cytochrome. Purification from *Corynebacterium glutamicum* and analysis of the QcrCBA operon encoding three subunit proteins of a putative cytochrome reductase complex. *Biochimica et biophysica acta*, **1503**, 279-290.
32. Niebisch, A. and Bott, M. (2003) Purification of a cytochrome *bc-aa₃* supercomplex with quinol oxidase activity from *Corynebacterium glutamicum*. Identification of a fourth subunit of cytochrome *aa₃* oxidase and mutational analysis of diheme cytochrome *c₁*. *J. Biol. Chem.*, **278**, 4339-4346.
33. Teramoto, H., Inui, M. and Yukawa, H. (2013) OxyR acts as a transcriptional repressor of hydrogen peroxide-inducible antioxidant genes in *Corynebacterium glutamicum* R. *Febs J*, **280**, 3298-3312.
34. Milse, J., Petri, K., Ruckert, C. and Kalinowski, J. (2014) Transcriptional response of *Corynebacterium glutamicum* ATCC 13032 to hydrogen peroxide stress and characterization of the OxyR regulon. *J Biotechnol*, **190**, 40-54.
35. Toyoda, K. and Inui, M. (2016) The extracytoplasmic function sigma factor sigma(C) regulates expression of a branched quinol oxidation pathway in *Corynebacterium glutamicum*. *Molecular microbiology*, **100**, 486-509.
36. Morosov, X., Davoudi, C.F., Baumgart, M., Brocker, M. and Bott, M. (2018) The copper-deprivation stimulon of *Corynebacterium glutamicum* comprises proteins for biogenesis of the actinobacterial cytochrome *bc₁-aa₃* supercomplex. *J Biol Chem*.
37. Davoudi, C.F., Ramp, P., Baumgart, M. and Bott, M. (2019) Identification of Surf1 as an assembly factor of the cytochrome *bc₁-aa₃* supercomplex of Actinobacteria. *Biochimica et biophysica acta. Bioenergetics*, **1860**, 148033.
38. Keilhauer, C., Eggeling, L. and Sahm, H. (1993) Isoleucine synthesis in *Corynebacterium glutamicum*: molecular analysis of the *ilvB-ilvN-ilvC* operon. *Journal of bacteriology*, **175**, 5595-5603.
39. Sambrook J., R.D. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edn. . NY: Cold Spring Harbor Laboratory Press.
40. Eikmanns, B.J., Thum-Schmitz, N., Eggeling, L., Ludtke, K.U. and Sahm, H. (1994) Nucleotide sequence, expression and transcriptional analysis of the *Corynebacterium glutamicum* *gltA* gene encoding citrate synthase. *Microbiology*, **140 (Pt 8)**, 1817-1828.
41. Niebisch, A. and Bott, M. (2001) Molecular analysis of the cytochrome *bc₁-aa₃* branch of the *Corynebacterium glutamicum* respiratory chain containing an unusual diheme cytochrome *c₁*. *Arch. Microbiol.*, **175**, 282-294.
42. Schäfer, A., Tauch, A., Jager, W., Kalinowski, J., Thierbach, G. and Pühler, A. (1994) Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene*, **145**, 69-73.
43. Gibson, D.G., Young, L., Chuang, R.Y., Venter, J.C., Hutchison, C.A., 3rd and Smith, H.O. (2009) Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nature methods*, **6**, 343-345.
44. Pfeifer, E., Hünnefeld, M., Popa, O., Polen, T., Kohlheyer, D., Baumgart, M. and Frunzke, J. (2016) Silencing of cryptic prophages in *Corynebacterium glutamicum*. *Nucleic acids research*, **44**, 10117-10131.
45. Jones, E., Oliphant, T. and Peterson, P. (2001) SciPy: Open source scientific tools for Python <http://www.scipy.org/>.
46. Andrews, S. (2010) FASTQC. A quality control tool for high throughput sequence data.

47. Langmead, B. and Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nature methods*, **9**, 357-359.
48. Baumgart, M., Unthan, S., Kloss, R., Radek, A., Polen, T., Tenhaef, N., Muller, M.F., Kuberl, A., Siebert, D., Bruhl, N. *et al.* (2018) *Corynebacterium glutamicum* Chassis C1*: Building and Testing a Novel Platform Host for Synthetic Biology and Industrial Biotechnology. *ACS synthetic biology*, **7**, 132-144.
49. Pachter, L. (2011) Models for transcript quantification from RNA-Seq. *Conference Proceedings*.
50. Sakamoto, J., Shibata, T., Mine, T., Miyahara, R., Torigoe, T., Noguchi, S., Matsushita, K. and Sone, N. (2001) Cytochrome c oxidase contains an extra charged amino acid cluster in a new type of respiratory chain in the amino-acid-producing Gram-positive bacterium *Corynebacterium glutamicum*. *Microbiology*, **147**, 2865-2871.
51. Dailey, H.A. and Gerdes, S. (2015) HemQ: An iron-coproporphyrin oxidative decarboxylase for protoheme synthesis in Firmicutes and Actinobacteria. *Archives of biochemistry and biophysics*, **574**, 27-35.
52. Frunzke, J., Gätgens, C., Brocker, M. and Bott, M. (2011) Control of heme homeostasis in *Corynebacterium glutamicum* by the two-component system HrrSA. *J Bacteriol*, **193**, 1212-1221.
53. Alvarez-Peral, F.J., Zaragoza, O., Pedreno, Y. and Arguelles, J.C. (2002) Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*. *Microbiology*, **148**, 2599-2606.
54. Kuehne, A., Emmert, H., Soehle, J., Winnefeld, M., Fischer, F., Wenck, H., Gallinat, S., Terstegen, L., Lucius, R., Hildebrand, J. *et al.* (2015) Acute Activation of Oxidative Pentose Phosphate Pathway as First-Line Response to Oxidative Stress in Human Skin Cells. *Mol Cell*, **59**, 359-371.
55. Ralser, M., Wamelink, M.M., Latkolik, S., Jansen, E.E., Lehrach, H. and Jakobs, C. (2009) Metabolic reconfiguration precedes transcriptional regulation in the antioxidant response. *Nat Biotechnol*, **27**, 604-605.
56. Wennerhold, J. and Bott, M. (2006) The DtxR regulon of *Corynebacterium glutamicum*. *Journal of bacteriology*, **188**, 2907-2918.
57. Lindner, S.N., Seibold, G.M., Henrich, A., Kramer, R. and Wendisch, V.F. (2011) Phosphotransferase system-independent glucose utilization in *Corynebacterium glutamicum* by inositol permeases and glucokinases. *Appl Environ Microbiol*, **77**, 3571-3581.
58. Bibb, L.A., King, N.D., Kunkle, C.A. and Schmitt, M.P. (2005) Analysis of a heme-dependent signal transduction system in *Corynebacterium diphtheriae*: deletion of the *chrAS* genes results in heme sensitivity and diminished heme-dependent activation of the *hmuO* promoter. *Infection and immunity*, **73**, 7406-7412.
59. Bibb, L.A. and Schmitt, M.P. (2010) The ABC transporter HrtAB confers resistance to hemin toxicity and is regulated in a hemin-dependent manner by the ChrAS two-component system in *Corynebacterium diphtheriae*. *Journal of bacteriology*, **192**, 4606-4617.
60. Bibb, L.A. and Schmitt, M.P. (2010) The ABC transporter, HrtAB, confers resistance to hemin toxicity and is regulated in a hemin-dependent manner by the ChrAS two-component system in *Corynebacterium diphtheriae*. *J. Bacteriol*.
61. Sun, J., Hoshino, H., Takaku, K., Nakajima, O., Muto, A., Suzuki, H., Tashiro, S., Takahashi, S., Shibahara, S., Alam, J. *et al.* (2002) Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *The EMBO journal*, **21**.
62. Ratliff, M., Zhu, W., Deshmukh, R., Wilks, A. and Stojiljkovic, I. (2001) Homologues of neisserial heme oxygenase in gram-negative bacteria: degradation of heme by the product of the *pigA* gene of *Pseudomonas aeruginosa*. *J Bacteriol*, **183**, 6394-6403.
63. Schmitt, M.P. (1997) Transcription of the *Corynebacterium diphtheriae hmuO* gene is regulated by iron and heme. *Infection and immunity*, **65**, 4634-4641.

64. Hintze, K.J., Katoh, Y., Igarashi, K. and Theil, E.C. (2007) Bach1 repression of ferritin and thioredoxin reductase1 is heme-sensitive in cells and in vitro and coordinates expression with heme oxygenase1, beta-globin, and NAD(P)H quinone (oxido) reductase1. *J Biol Chem*, **282**, 34365-34371.
65. Dhakshinamoorthy, S., Jain, A.K., Bloom, D.A. and Jaiswal, A.K. (2005) Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. *J Biol Chem*, **280**, 16891-16900.
66. Kuehne, A., Emmert, H., Soehle, J., Winnefeld, M., Fischer, F., Wenck, H., Gallinat, S., Terstegen, L., Lucius, R., Hildebrand, J. *et al.* (2015) Acute Activation of Oxidative Pentose Phosphate Pathway as First-Line Response to Oxidative Stress in Human Skin Cells. *Molecular Cell*, **59**, 359-371.
67. Hillion, M., Imber, M., Pedre, B., Bernhardt, J., Saleh, M., Loi, V.V., Maaß, S., Becher, D., Astolfi Rosado, L., Adrian, L. *et al.* (2017) The glyceraldehyde-3-phosphate dehydrogenase GapDH of *Corynebacterium diphtheriae* is redox-controlled by protein S-mycothiolation under oxidative stress. *Scientific reports*, **7**, 5020.
68. Baumgart, M., Luder, K., Grover, S., Gatgens, C., Besra, G.S. and Frunzke, J. (2013) IpsA, a novel LacI-type regulator, is required for inositol-derived lipid formation in *Corynebacteria* and *Mycobacteria*. *BMC Biol*, **11**, 122.
69. Dailey, H.A., Dailey, T.A., Gerdes, S., Jahn, D., Jahn, M., O'Brian, M.R. and Warren, M.J. (2017) Prokaryotic Heme Biosynthesis: Multiple Pathways to a Common Essential Product. *Microbiology and molecular biology reviews : MMBR*, **81**.
70. Niebisch, A. and Bott, M. (2003) Purification of a cytochrome *bc-aa3* supercomplex with quinol oxidase activity from *Corynebacterium glutamicum*. Identification of a fourth subunit of cytochrome aa3 oxidase and mutational analysis of diheme cytochrome c1. *The Journal of biological chemistry*, **278**, 4339-4346.
71. Mueller, J.P. and Taber, H.W. (1989) Isolation and sequence of *ctaA*, a gene required for cytochrome *aa₃* biosynthesis and sporulation in *Bacillus subtilis*. *J Bacteriol*, **171**, 4967-4978.
72. Svensson, B., Lubben, M. and Hederstedt, L. (1993) *Bacillus subtilis* CtaA and CtaB function in haem A biosynthesis. *Mol Microbiol*, **10**, 193-201.
73. Auchter, M., Cramer, A., Huser, A., Ruckert, C., Emer, D., Schwarz, P., Arndt, A., Lange, C., Kalinowski, J., Wendisch, V.F. *et al.* (2011) RamA and RamB are global transcriptional regulators in *Corynebacterium glutamicum* and control genes for enzymes of the central metabolism. *J Biotechnol*, **154**, 126-139.
74. Shah, A., Blombach, B., Gauttam, R. and Eikmanns, B.J. (2018) The RamA regulon: complex regulatory interactions in relation to central metabolism in *Corynebacterium glutamicum*. *Appl Microbiol Biotechnol*, **102**, 5901-5910.
75. Burkovski, A. (2007) Nitrogen control in *Corynebacterium glutamicum*: proteins, mechanisms, signals. *Journal of microbiology and biotechnology*, **17**, 187-194.
76. Schulte, J., Baumgart, M. and Bott, M. (2017) Identification of the cAMP phosphodiesterase CpdA as novel key player in cAMP-dependent regulation in *Corynebacterium glutamicum*. *Mol Microbiol*, **103**, 534-552.

Tables

Table 1: Selected target genes of HrrA. This table summarizes results from the HrrA ChAP-Seq analysis of the *C. glutamicum* strain ATCC 13032::hrrA-C-twinstrep and the transcriptome analysis of *C. glutamicum* wild type and strain Δ hrrA (complete datasets are provided in Table S3 and S4, respectively). For both experiments, cells were grown on glucose minimal medium and 4 M heme (see Material and Methods).

Locus tag	Gene name	Annotation	Dist. TSS ^a	Peak intensity ^b	log ₂ (Δ hrrA/wt) ^c T 0.5h	log ₂ (Δ hrrA/wt) ^c T 4h
Heme homeostasis/metabolism						
cg2445	<i>hmuO</i>	Heme oxygenase	43	5.4	-3.1	-3.8
cg0516	<i>hemE</i>	Uroporphyrinogen decarboxylase	17	54	3.1	2.2
cg0497	<i>hemA</i>	Glutamyl-tRNA reductase	-162	13	0.7	1.0
cg0517	<i>hemY</i>	Protoporphyrinogen oxidase	429	3.0	2.8	1.6
cg2079	<i>hemQ</i> (?)	Putative chlorite dismutase-family protein, conserved		19	2.8	1.8
cg3156	<i>htaD</i>	Secreted heme transport-associated protein	-108	15	-0.3	-1.1
cg1734	<i>hemH</i>	Ferrochelatase	21	41	4.0	2.2
cg3247	<i>hrrA</i>	Heme-dependent response regulator	108	3.7	n.d.	n.d.
cg2201	<i>chrS</i>	Heme-dependent histidine kinase (<i>chrSA</i> operon)	32	2.5	-0.4	1.3
cg2202	<i>hrtB</i>	Heme exporter (<i>hrtBA</i> operon)	78	2.5	-1.0	4.3
Respiratory chain						
cg2406	<i>ctaE</i>	Cytochrome <i>aa</i> ₃ oxidase, subunit 3	307	105	-1.7	-0.8
cg2780	<i>ctaD</i>	Cytochrome <i>aa</i> ₃ oxidase, subunit 1	197	36	-1.1	-0.9
cg1301	<i>cydA</i>	Cytochrome <i>bd</i> oxidase	192	11	-0.7	-2.6
cg2409	<i>ctaC</i>	Cytochrome <i>aa</i> ₃ oxidase, subunit 2	47	22	-1.4	-1.0
cg1773	<i>ctaB</i>	Protoheme IX farnesyltransferase	667	7.9	0.4	-1.4
cg0445	<i>sdhC</i>	Succinate:menaquinone oxidoreductase, cytochrome b subunit	83	38	-1.7	-1.6
cg3226		L-lactate permease, operon with <i>lldD</i>	533	5.5	-1.7	0.9
Glucose uptake						
cg2121	<i>ptsH</i>	Phosphocarrier protein HPr, general component of PTS	-70	2.1	-1.2	-0.3
cg1537	<i>ptsG</i>	Glucose-specific EIIABC component EIIGlc of PTS	70	1.6	-1.1	-0.1
cg2091	<i>ppgG</i>	Polyphosphate glucokinase	199	266	0.2	-0.8
cg0223	<i>iolT1</i>	Myo-Inositol transporter 1, alternative glucose uptake system	73	2.0	-1.0	-0.7
Signal transduction						
cg0986	<i>amtR</i>	Master regulator of nitrogen control, repressor, TetR-family	366	1.8	0.3	0.1
Cg2461	<i>benR</i>	Transcriptional regulator, LuxR-family	229	5.6	-0.1	-1.2

cg2761	<i>cpdA</i>	cAMP phosphodiesterase	309	4.2	0.4	-0.5
cg0309	<i>sigC</i>	Extracytoplasmic-function σ factor, control of branched quinol oxidation pathway	29	19	2.1	0.6
cg0444	<i>ramB</i>	Transcriptional regulator, involved in acetate metabolism	83	38	-0.7	-0.6
cg2831	<i>ramA</i>	Transcriptional regulator, acetate metabolism, LuxR-family	-10	2.1	-0.5	0.6
Oxidative stress						
cg0310	<i>kata</i>	Catalase	132	19	-0.7	-1.2
cg0831	<i>tusG</i>	Trehalose uptake system, ABC-type, permease protein	-30	1.8	0.0	-0.2
cg1791	<i>gapA</i>	Glyceraldehyde-3-phos. dehydrogenase, glycolysis	86	3.9	-0.3	-0.4
cg1069	<i>gapB</i>	Glyceraldehyde-3-phos. dehydrogenase, gluconeogenesis	175	2.4	1.6	-0.1
Cell envelope						
cg2077	<i>aftC</i>	arabinofuranosyltransferase	271	3.0	-0.3	-0.2
cg3323	<i>ino1</i>	D-myo-inositol-1-phosphate synthase	-46	4.2	1.7	0.6
cg0337	<i>whcA</i>	WhiB homolog, role in SigH-mediated oxidative stress response	-21	2.1	-0.5	-0.7
cg0306	<i>lysC</i>	Aspartate kinase	32	13	0.7	0.1
cg0422	<i>murA</i>	UDP-N-acetylenolpyruvoylglucosamine reductase	591	3.5	-0.3	-0.1

a) Distance of the HrrA binding peak, identified via ChAP-Seq, to the start codon (transcription start site, TSS)

b) the corresponding peak intensity

c) Relative ratio of the transcript levels of the $\Delta hrrA$ deletion mutant compared to the wild type (\log_2 fold change). The values are derived from a comparison between the two strains 0.5 and 4 h after hemin addition. The $\log_2(\Delta hrrA/wt)$ value for was not determined for the deleted *hrrA* gene (n.d.).

Table 2: Apparent K_d values of HrrA to the promoters of *hmuO*, *ctaE*, *sigC* and *cydA*. The affinity of phosphorylated HrrA to the indicated regions was measured using purified protein in increasing concentrations and its ability to shift 10 nM DNA fragments of approximately 100 bp size covering the maximal ChAP-Seq peak (for detailed information, see Figure S9).

Promoter	Function	Apparent K_d value (nM)	95% confidence interval (nM)	R^2	Peak intensity after hemin addition (ChAP- Seq)
P_{hmuO}	Heme oxygenase	196	182-212	0.95	10
P_{ctaE}	Cytochrome <i>aa</i> ₃ oxidase	125	117-132	0.97	53
P_{sigC}	ECF sigma factor σ^C	271	247-299	0.96	25
P_{cydA}	Cytochrome <i>bd</i> oxidase	350	318-386	0.96	18

Figures and Figure legends

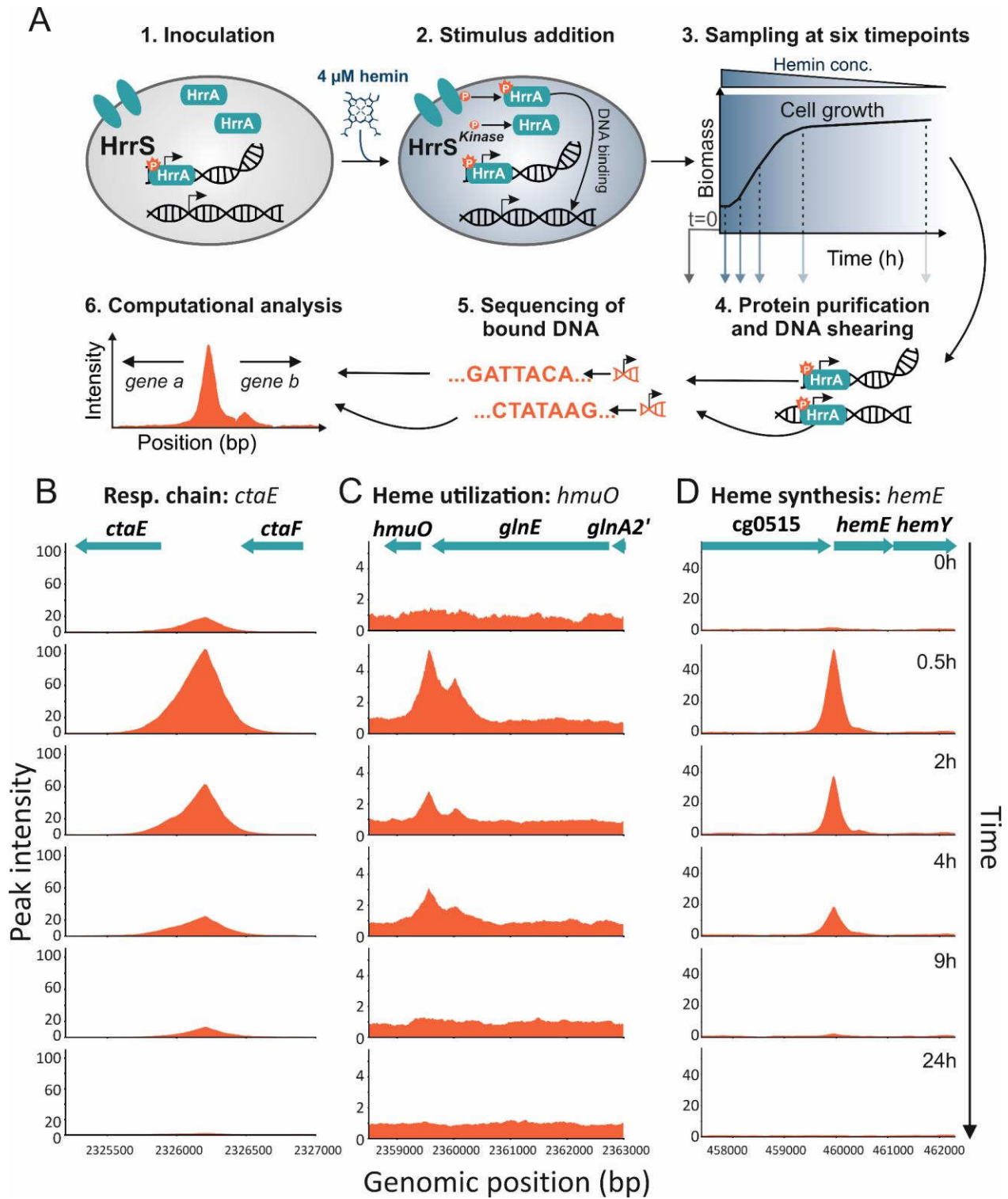


Figure 1: Genome-wide profiling of HrrA binding in response to addition of external heme. (A) ChAP-Seq analysis of the *C. glutamicum* strain ATCC 13032::*hrrA*-C-twinstrep grown in iron-depleted glucose minimal medium before and after addition of 4 μ M hemin. The experimental approach is briefly depicted: Cells were harvested at the indicated time points, twin-Strep tagged HrrA was purified and co-purified DNA was sequenced to identify HrrA genomic targets. This approach resulted in the identification of more than 200 genomic regions bound by HrrA upon addition of hemin after 30 minutes. Exemplarily shown is the HrrA binding to regions upstream of operons involved in (B) the respiratory chain (*ctaE*), (C) heme degradation (*hmuO*) and (D) heme biosynthesis (*hemE*).

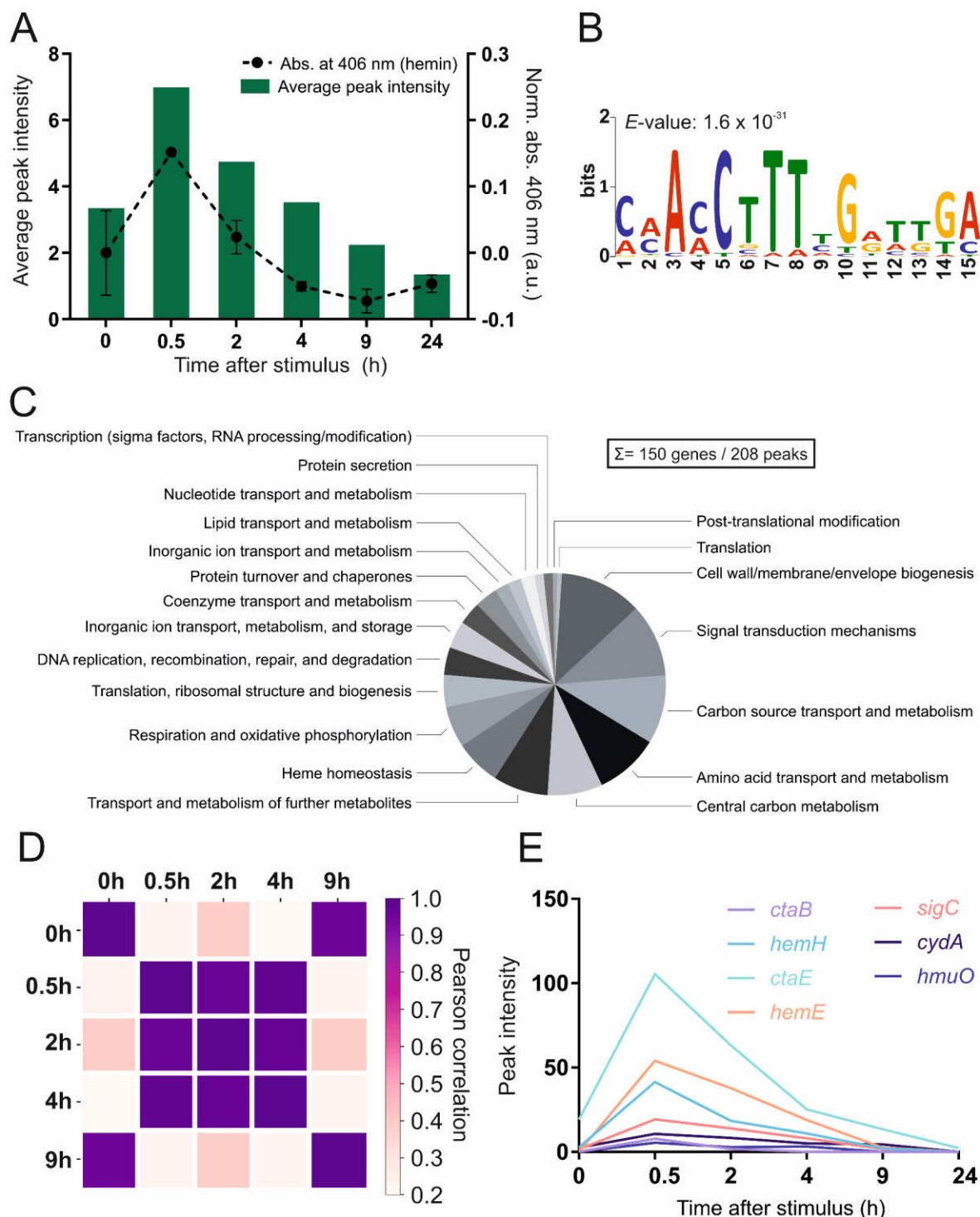


Figure 2: ChAP-Seq analysis revealed HrrA as a global regulator of heme homeostasis in *C. glutamicum*. (A) HrrA binding in response to the addition of hemin. The bar plot reflects the average peak intensities among detected peaks in ChAP-Seq experiments (<800 bp to the next TSS). The binding was correlated with the amount of cell-associated hemin (dashed line), measured at corresponding time points by spectroscopy as described in *Material and Methods*. (B) A binding motif was deduced from the sequences of the top 25 peaks ($T_{0.5}$) using MEME v.5 analysis (<http://meme-suite.org>). (C) Pie chart presenting HrrA targets, which can be attributed

to known functional categories (total of 272 genes, among which 128 encode proteins of unknown function, e.g., target genes within the CGP3 prophage region were excluded). For a complete overview of HrrA targets, see Table S3. (D) Proportional behavior of the HrrA regulon. For each peak that passed the threshold (distance of <800 bp to the closest downstream or <200 to the closest upstream transcription start site) at time point A, the highest peak in the same region (± 50 nucleotides from the center of the peak) was selected for time point B and *vice versa*. Thus, 'paired' peaks for these two time points were obtained, and the Pearson correlation of the intensities of all paired peaks was calculated for all six time points. (E) Peak intensities of selected HrrA targets over time, as identified by ChAP-Seq.

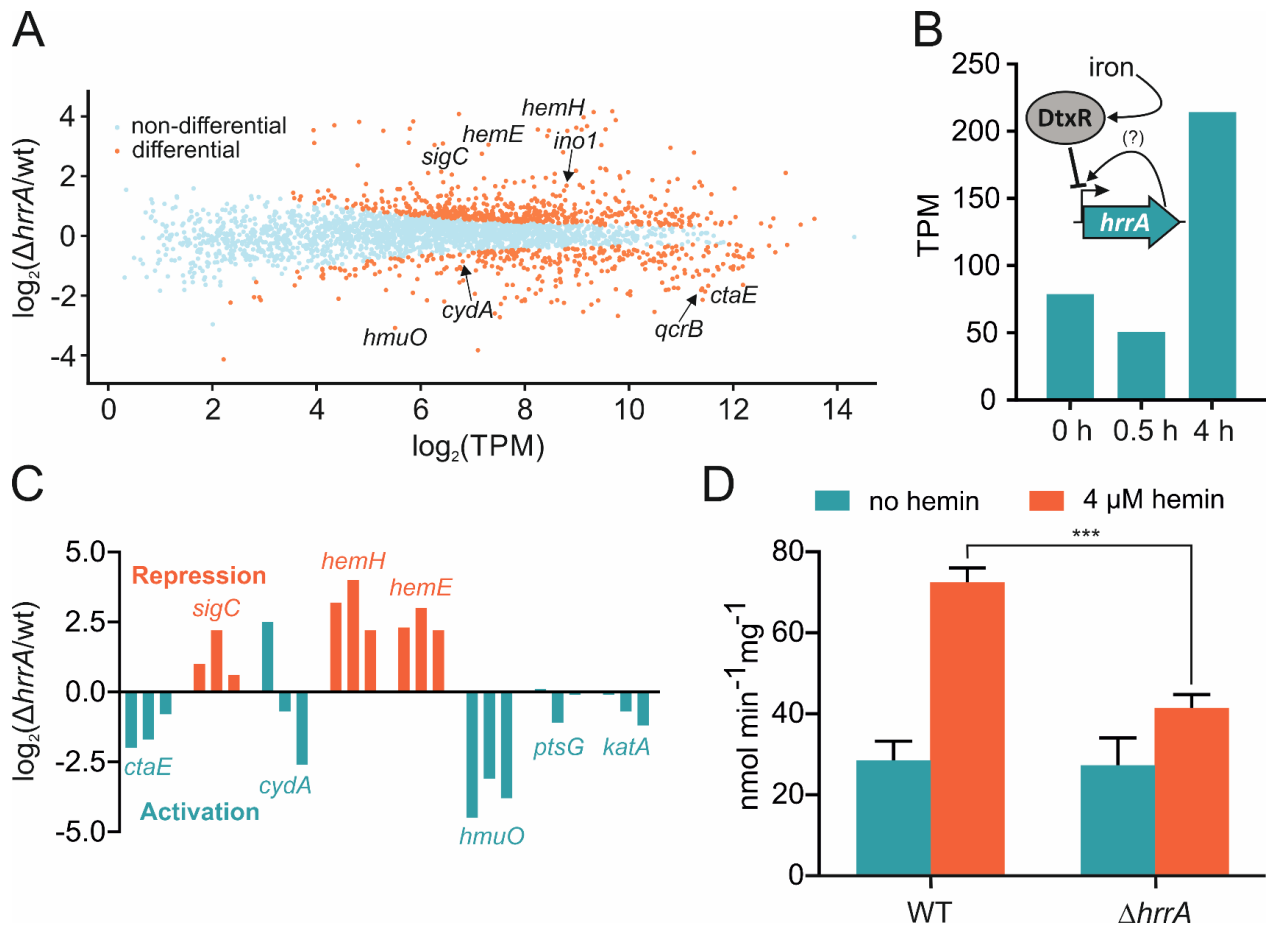


Figure 3: Differential gene expression analysis of wild type *C. glutamicum* and a $\Delta hrrA$ mutant. (A) Differential gene expression analysis (RNA-Seq) revealed 120 upregulated and 154 downregulated genes in the *hrrA* deletion strain compared to the wild type (in transcripts per million, TPM) after 30 minutes of cultivation in iron-depleted glucose minimal medium containing 4 μ M heme. (B) Expression levels of *hrrA* (TPM) 0, 0.5 and 4 h after the addition of hemin. A scheme depicts HrrA autoregulation and iron-dependent DtxR repression (24). (C) Impact of *hrrA* deletion on the transcript levels of six selected target genes at three different time points (0 h, 0.5 h, 4 h; orange: HrrA acts as a repressor, turquoise: HrrA acts as an activator). (D) Measurement

of cytochrome aa_3 oxidase activity using the TMPD oxidase assay in *C. glutamicum* wild type and $\Delta hrrA$ grown with or without 4 μ M heme.

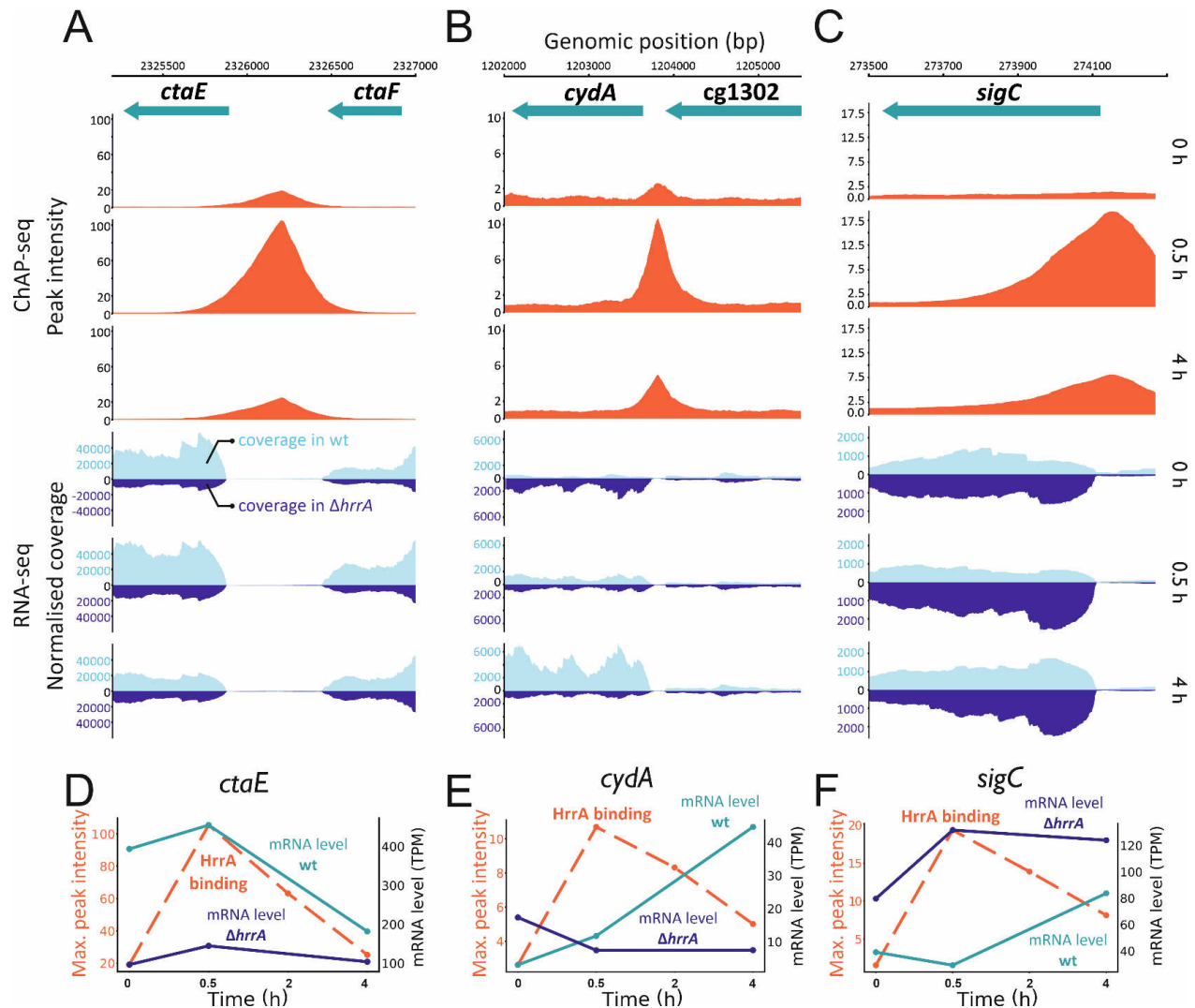


Figure 4: HrrA prioritizes the expression of genes encoding components of the bc_1 - aa_3 supercomplex. Depicted are HrrA binding peaks as identified by ChAP-Seq analysis (Figure 1 and 2) in comparison to the normalized coverage of RNA-Seq results (wild type and the $\Delta hrrA$ mutant) for the genomic loci of *ctaE* (A, D), *sigC* (B, E) and *cydA* (C, F). D-F: HrrA binding (max. peak intensities measured by ChAP-Seq experiments) and the mRNA levels (in transcripts per million, TPM) of the respective genes in the $\Delta hrrA$ strain as well as in wild type *C. glutamicum* cells 0, 0.5 and 4 h after the addition of heme.

gene Dataset of genome wide **H3K4me3** binding (ChIP-seq) and time resolved transcriptome analysis of *C. glutamicum* wild type and $\Delta hcrA$ RNA-seq. For ChIP-seq analysis, the strain *C. glutamicum* $\Delta hcrA$ *Cri-wt* was cultivated in CSXII minimal

medium with 2% (w/v) glucose and 4 μM hemin, was harvested at different time points as described in Figure 1. For RNA-Seq, wild type cells and a $\Delta hcrA$ strain were cultivated accordingly and harvested at 0 h, 0.5 h and 4 h after hemin addition. C show the peak name, the gene locus (ID) and hemin, was harvested at different time points as described in Figure 1. For RNA-Seq, wild type cells and a $\Delta hcrA$ strain were cultivated accordingly and harvested at 0 h, 0.5 h and 4 h after hemin addition.

The start site of the gene and column F indicates the distance to the transcription start site. In light grey (G-L), ChIP-seq peak intensities are indicated at 0 h, 0.5 h, 2 h, 4 h, 9 h and 24 h after hemin addition. In green (M-O) and red (P-R), the measured fold change of the corresponding genes in the wild type strain (green) and the $\Delta hcrA$ strain (red) are shown. In transcripts per million, mean of two biological replicates. Column S-U show the log2-fold change of $\Delta hcrA$ in comparison to the wild type after 0 h, 0.5

h, 2 h, 4 h, 9 h and 24 h after hemin addition.

Gene ID	Gene name	Annotation	Distance to ATG	CGAP-Seq1.0	T=0.5	T=2	T=4	T=9	T=24	mRNA wt t=0	mRNA wt t=0.5	mRNA wt t=2	mRNA wt t=4	mRNA wt t=9	mRNA wt t=24	Log2 DmrA/wt	Log2 DmrA/wt	Log2 DmrA/wt	
g0074	putative sulfurtransferase, RHO domain-containing protein		205	0.0	2.1	2.2	2.1	0.0	0.0	7.0	1.5	9.8	5.2	2.3	6.5	0.4	0.6	-0.6	
g0075	hypothetical protein, conserved		79	0.0	2.1	2.2	2.1	0.0	0.0	136.5	65.7	68.4	65.7	93.7	64.3	40.4	-0.5	0.7	
g0076	hypothetical protein		280	0.0	2.3	2.3	2.0	0.0	0.0	22.6	6.0	15.8	12.0	3.8	12.0	3.8	-0.9	-0.4	
g0113	urease Y subunit [EC:3.5.1.5]		557	0.0	1.8	1.6	0.0	0.0	0.0	406.2	363.9	116.3	165.2	216.3	93.4	11.3	0.4	-0.3	
g0128	putative secreted protein, signal peptide		166	0.0	1.7	1.9	1.6	0.0	0.0	10.6	5.9	17.4	5.8	12.5	13.0	12.5	-0.9	-0.4	
g0129	proline dehydratase (5-L-tryptone-5-carboxylate dehydratase [EC:1.5.99.8])		90	0.0	1.7	1.9	1.6	0.0	0.0	17.9	17.3	17.3	13.3	11.6	95.6	11.6	-0.4	-0.3	
g0153	putative esterase/lipase protein [EC:3.1.1.3]		302	0.0	3.8	3.7	2.5	0.0	0.0	20.5	30.1	30.1	21.3	21.3	25.9	21.3	0.1	0.3	
g0163	putative N-acetylglucosaminidase		153	0.0	4.9	4.9	4.6	0.0	0.0	35.9	35.9	35.9	35.9	35.9	35.9	35.9	0.0	0.0	
g0202	putative membrane protein		410	0.0	3.2	3.1	2.4	0.0	0.0	28.1	37.7	37.7	22.1	25.2	22.0	21.0	-0.2	0.8	
g0222	hypothetical protein		158	0.0	2.0	1.9	1.7	0.0	0.0	18.4	11.8	37.5	14.9	6.0	23.0	4.3	-1.0	0.7	
g0247	putative quinone oxidoreductase		180	0.0	2.2	1.7	1.5	0.0	0.0	40.4	21.2	38.5	37.2	34.6	23.3	4.3	-0.7	0.3	
g0251	putative quinone oxidoreductase [EC:1.6.5.15]		71	0.0	1.3	1.6	2.3	1.4	0.0	106.3	67.8	107.3	95.5	80.8	85.0	0.2	0.3	-0.3	
g0284	Associated with mycolic acid metabolism		122	0.0	1.8	2.0	0.0	0.0	0.0	95.7	76.4	103.8	109.7	85.9	88.3	0.2	0.2	-0.2	
NCg10236	putative transposase		169	1.0	1.3	1.3	1.3	1.2	1.0	0.0	0.1	0.1	0.1	0.1	0.3	0.0	-0.5	NA	
g00296	DNA polymerase II, subunits y and t (tau) [EC:2.7.7.7]		73	0.0	1.6	1.7	0.0	0.0	0.0	191.0	145.7	237.7	139.6	145.4	188.8	-0.5	0.0	-0.3	
g00304	putative membrane protein		214	0.0	1.3	1.1	5.9	0.0	0.0	96.2	39.6	71.9	90.0	67.5	53.8	0.1	0.8	-0.4	
g00306	aspartate kinase [EC:2.7.2.4], deletion causes lysine auxotrophy		32	0.0	1.3	1.1	5.9	0.0	0.0	601.5	598.3	692.6	722.9	970.1	764.5	0.3	0.7	0.1	
g00309	RNA polymerase σ factor, ECF-family, control of branched quinol oxidation pathway		29	1.5	1.9	1.4	8.1	1.7	0.0	39.1	29.2	83.7	79.7	131.6	124.0	1.0	2.2	0.6	
g00310	two component response regulator		175	1.32	1.5	1.9	1.4	8.1	1.7	0.0	1980.0	1980.0	1699.9	1847.3	3600.9	734.7	-0.1	0.7	
g00330	penicillin-binding protein 1A [EC:2.2.2.1]		24	0.0	3.0	2.7	1.9	0.0	0.0	25.8	35.3	19.7	13.8	19.7	16.5	-0.9	0.4	-0.3	
g00336	penicillin-binding protein 1A [EC:2.2.2.1]		533	0.0	2.1	2.0	1.6	0.0	0.0	41.5	22.9	22.6	22.4	15.7	19.4	-0.9	0.5	-0.2	
g00337	negative role in Sglp-mediated oxidative stress response, White homolog		133	0.0	2.1	2.0	1.6	0.0	0.0	119.5	102.4	111.8	25.4	74.4	67.5	-0.7	0.5	-0.7	
g00341	acyl-CoA ligase transmembrane protein, involved in degradation of aromatic compounds		175	0.0	2.6	2.7	2.0	1.5	0.0	5.0	9.8	9.8	4.2	3.7	4.2	0.6	1.4	0.6	
g00390	putative peroxidase, major facilitator family		350	0.0	3.4	3.6	3.0	0.0	0.0	95.4	96.1	95.0	99.1	90.1	43.3	-0.7	-0.1	-0.3	
g00420	putative glyoxylate transaminase, horizontally transferred		641	0.0	2.3	4.9	4.4	0.0	0.0	192.3	99.2	161.1	50.3	62.0	41.3	-1.9	0.7	-2.0	
g00420	putative glyoxylate transaminase, horizontally transferred		164	0.0	3.0	2.4	2.3	0.0	0.0	192.3	99.2	161.1	50.3	62.0	41.3	-1.9	0.7	-2.0	
g00422	UDP-N-acetylglucosamine 1-carboxyvinyltransferase [EC:2.5.1.7], horizontally transferred		675	0.0	3.5	2.4	2.5	0.0	0.0	230.1	157.2	218.4	237.6	144.1	205.0	0.0	-0.1	-0.1	
g00424	putative glyoxylate transaminase, horizontally transferred		89	0.0	2.2	1.5	0.0	0.0	0.0	194.0	149.5	254.4	183.3	135.3	216.8	-0.1	-0.1	-0.2	
g00437	putative membrane protein, involved in polysaccharide polymerization, horizontally transferred		312	60	0.0	4.0	3.4	2.7	0.0	154.8	119.9	212.2	88.7	124.0	160.7	-0.8	0.0	-0.4	
g00438	putative glyoxylate transaminase, horizontally transferred		35	0.0	2.3	1.7	0.0	0.0	0.0	416.0	360.2	458.3	385.8	399.8	263.8	-0.1	0.2	-0.8	
g00438	putative glyoxylate transaminase, horizontally transferred		79	0.0	2.3	1.7	1.8	0.0	0.0	416.0	360.2	458.3	385.8	399.8	263.8	-0.1	0.2	-0.8	
g00439	putative acetyl transferase [EC:3.1.1., horizontally transferred		326	0.0	2.3	1.7	0.0	0.0	0.0	194.3	209.8	254.1	169.3	227.8	203.2	-0.2	0.1	-0.3	
g00439	putative acetyl transferase [EC:3.1.1., horizontally transferred		281	0.0	2.3	1.7	1.8	0.0	0.0	194.3	209.8	254.1	169.3	227.8	203.2	-0.2	0.1	-0.3	
g00444	transcriptional regulator, involved in acetate metabolism, Merit family		213	1.98	3.1	3.8	3.3	1.6	0.0	79.0	29.4	47.9	353.7	439.9	319.0	-0.9	0.7	-0.6	
g00445	succinate:menquinone oxidoreductase, cytochrome b subunit		298	0.0	3.1	3.6	3.1	0.0	0.0	193.7	251.0	233.9	392.3	884.6	760.6	-2.3	3.7	1.6	
g00453	putative membrane protein, conserved		4	0.0	2.4	2.0	0.0	0.0	0.0	220.6	112.2	302.2	138.4	942.0	117.4	1.0	0.8	-0.3	
g00456	putative membrane protein, conserved		4	0.0	2.4	2.0	0.0	0.0	0.0	220.6	112.2	302.2	138.4	942.0	117.4	1.0	0.8	-0.3	
g00465	putative membrane protein, associated protein		115	0.0	2.8	2.3	2.2	0.0	0.0	5.6	11.1	87.7	3.9	1.1	11.3	0.5	0.1	0.4	
g00466	secreted heme transport-associated protein		185	43	0.0	2.8	2.3	2.2	0.0	19.4	8.6	3.0	72.5	2.7	1.5	-0.1	0.4	-0.1	
g00475	hypothetical protein, conserved		370	0.0	3.6	2.6	2.1	2.2	0.0	61.2	709.0	1603.6	1378.3	1607.6	1607.6	0.9	1.0	0.0	
g00495	putative phosphatase [EC:3.1.3.3]		434	0.0	7.1	13	8.1	0.0	0.0	39.2	24.8	46.5	30.3	33.6	33.7	-0.3	0.4	-0.5	
g00497	glutaryl-RNA reductase, involved in heme biosynthesis		34	162	0.0	7.1	13	8.1	0.0	115.4	487.8	1363.6	636.4	765.8	274.8	-2.5	0.7	1.0	
g00500	transcriptional activator of guaBDC genes, YnfK-family		16	40	0.0	4.8	4.1	2.9	0.0	38.8	25.7	20.4	22.2	20.1	14.0	-0.8	0.4	-0.6	
g00501	putative shikimate permease, MFS-type		260	80	0.0	4.8	4.1	2.9	0.0	59.3	25.4	20.4	22.2	20.1	14.0	-0.8	0.4	-0.6	
g00505	putative ribosomal protein L17/L12-family		7	7	0.0	1.9	1.6	1.4	0.0	79.4	99.2	81.2	126.7	93.2	41.2	0.7	0.7	-1.0	
g00516	hemE	uroporphyrinogen decarboxylase [EC:4.1.1.37], involved in heme biosynthesis	17	17	0.0	5.4	3.8	1.9	2.6	0.0	56.8	34.8	52.6	281.2	277.5	249.6	2.3	3.0	2.2
g00517	protoporphyrinogen oxidase [EC:1.3.3.4], involved in heme biosynthesis		624	429	0.0	0.0	3.0	0.0	0.0	65.7	37.6	96.6	215.2	258.1	297.0	1.7	2.8	1.6	
g00524	Cytochrome c assembly membrane protein, CcsA-family		38	38	0.0	2.2	2.2	1.5	0.0	95.5	234.0	292.9	112.7	300.6	375.5	0.2	0.4	0.9	
g00545	low-affinity phosphate transport protein, Ptc-family		3	158	0.0	1.6	1.4	0.0	0.0	105.9	51.6	206.8	138.9	81.9	289.6	0.4	0.7	0.5	
g00557	putative monooxygenase, FAD-binding		9	80	0.0	3.0	3.2	2.3	0.0	24.4	25.0	28.3	33.8	55.8	42.7	0.5	1.2	0.6	
g00559	putative octapylate-diphosphate synthase protein [EC:2.5.1.-]		154	80	0.0	3.0	3.2	2.3	0.0	271.6	230.9	253.5	213.1	263.4	273.8	-0.4	0.2	0.1	
g00566	4-aminobutyl-aminotransferase, AT class II [EC:2.6.1.19]		748	748	0.0	3.9	2.6	1.7	0.0	3.2	4.1	3.2	3.0	3.0	3.0	-0.1	0.3	-0.1	
g00614	hypothetical protein		654	654	0.0	2.1	1.5	1.6	0.0	78.5	37.6	53.7	73.5	14.3	33.6	-0.1	0.5	-0.7	
g00630	SOS ribosomal protein L18		441	441	0.0	2.8	0.0	0.0	0.0	1311.6	980.0	3685.9	1113.2	2620.0	97.8	1.8	0.7	0.4	
g00636	putative membrane protein		4	4	0.0	6.0	5.0	0.0	0.0	61.7	41.7	41.7	41.7	41.7	41.7	0.0	0.0	0.0	
g00636	putative membrane protein		402	402	0.0	3.5	2.5	0.0	0.0	50.9	50.2	48.7	31.1	31.1	31.1	-0.3	0.7	-0.5	
g00647	preprotein translocase subunit		708	595	0.0	1.8	1.4	0.0	0.0	652.3	537.1	587.1	551.5	477.7	563.0	0.0	0.2	-0.1	
g00656	SOS ribosomal protein L17		14	14	0.0	2.9	2.1	1.8	0.0	1374.9	760.8	3061.1	1423.6	1461.5	4076.1	0.1	0.2	0.4	
g00671	hypothetical protein, conserved		710	710	0.0	3.2	2.6	2.5	0.0	7.9	1.9	8.9	3.5	3.2	3.2	-1.2	1.1	-1.5	
g00673	SOS ribosomal protein L13		36	177	0.0	2.0	1.7	1.4	0.0	1752.3	1550.6	4184.4	2711.6	2511.2	5648.7	0.6	0.7	0.4	
g00688	putative protein, conserved, UPF0031-family		17	17	0.0	3.9	3.8	2.7	1.4	40.4	41.3	114.3	65.5	75.2	90.1	0.4	0.9	-0.3	
g00752	putative secreted or membrane protein		504	381	0.0	1.9	1.5	1.5	0.0	111.5	135.6</								

cg0876		54	8	0.0	4.5	3.4	2.1	0.0	0.0	0.0	3.8	4.0	3.5	2.2	3.2	1.7	4.8	0.3	1.1
cg0876	<i>sigH</i>	114	17	0.0	4.5	3.4	2.1	0.0	0.0	0.0	208.6	342.9	347.9	249.1	313.8	260.4	0.3	0.1	-0.4
cg0880		54	54	0.0	7.7	5.0	3.4	0.0	0.0	0.0	67.0	46.0	63.2	76.9	70.9	51.2	0.2	0.7	-0.3
cg0931		69	69	0.0	2.3	1.6	1.5	1.2	1.0	1.0	26.8	17.1	7.7	16.8	12.5	5.3	-0.7	-0.5	-0.5
cg0948	<i>serC</i>	207	147	0.0	2.8	2.3	1.7	0.0	0.0	0.0	800.8	553.5	595.8	662.8	839.1	927.2	-0.3	0.6	0.6
cg0949		519	140	0.0	2.8	2.3	1.7	0.0	0.0	0.0	4125.6	2270.2	2535.7	2959.2	1630.9	3228.6	-0.5	-0.5	0.3
cg0950	<i>gltA</i>	692	630	0.0	10	6.5	4.0	0.0	0.0	0.0	689.3	547.7	1184.1	718.0	952.9	1486.1	0.1	0.8	0.3
cg0951	<i>gltD3</i>	69	96	0.0	4.3	1.8	2.2	0.0	0.0	0.0	509.0	728.0	395.4	134.3	156.3	119.4	-1.9	-2.2	-1.7
cg0986	<i>amtR</i>	407	366	0.0	1.8	1.4	1.4	0.0	0.0	0.0	274.1	242.2	233.6	208.3	295.3	241.8	-0.4	0.3	0.1
cg0986		460	419	0.0	0.0	0.0	0.0	0.0	0.0	0.0	274.1	242.2	233.6	208.3	295.3	241.8	-0.4	0.3	0.1
cg1044		431	431	0.0	2.0	1.4	0.0	0.0	0.0	0.0	272.8	391.1	678.3	468.8	436.8	465.6	0.8	1.2	-0.5
cg1050		57	57	0.0	49	24	14	1.6	0.0	0.0	49.5	26.5	94.1	102.2	119.1	170.4	1.0	2.2	0.9
cg1051		159	159	0.0	2.8	2.1	1.7	0.0	0.0	0.0	25.8	28.8	88.1	49.4	69.8	159.0	0.9	1.3	-0.1
cg1052		332	332	0.0	2.8	2.1	1.7	0.0	0.0	0.0	25.8	28.8	88.1	49.4	69.8	159.0	0.9	1.3	-0.1
cg1052	<i>amt2</i>	277	233	0.0	2.8	2.1	1.7	0.0	0.0	0.0	220.6	155.6	176.1	172.6	220.6	96.2	-0.4	0.5	-0.9
cg1052	<i>amt3</i>	230	186	0.0	2.4	2.1	0.0	1.3	0.0	0.0	220.6	155.6	176.1	172.6	220.6	96.2	-0.4	0.5	-0.9
cg1069		295	215	0.0	2.4	2.1	1.4	0.0	0.0	0.0	75.8	96.1	538.2	89.4	304.8	503.9	0.2	1.7	-0.1
cg1069	<i>gpaB [gapX]</i>	255	175	0.0	2.4	2.1	1.4	0.0	0.0	0.0	75.8	96.1	538.2	89.4	304.8	503.9	0.2	1.7	-0.1
cg1077		33	33	4.1	47	28	15	2.4	0.0	0.0	4.4	2.8	7.2	23.0	25.6	21.1	2.4	3.2	1.5
cg1077	<i>gpaB [gapX]</i>	33	33	4.1	47	28	15	2.4	0.0	0.0	4.4	2.8	7.2	23.0	25.6	21.1	2.4	3.2	1.5
cg1080		50	17	4.1	47	28	15	2.4	0.0	0.0	26.9	12.1	52.1	130.4	206.2	200.5	2.3	4.1	1.9
cg1080	putative multicopper oxidase	50	17	4.1	47	28	15	2.4	0.0	0.0	26.9	12.1	52.1	130.4	206.2	200.5	2.3	4.1	1.9
NCgl0914	putative ABC transport system ATP-binding protein	450	382	0.0	3.4	2.3	1.9	0.0	0.0	0.0	33.5	24.2	100.2	51.3	44.2	71.0	0.6	0.9	-0.5

CEL105	lysE	lysine permease	553	553	0.0	3.8	2.5	2.1	0.0	0.0	23.3	14.4	13.7	20.3	17.9	11.2	4.2	0.3	4.3
CEL111	eno	enolase EC4.2.1.11, phosphoenolpyruvate hydratase	389	319	0.0	2.2	1.7	1.3	0.0	0.0	2231.6	1416.8	3740.6	2553.3	1758.9	3184.7	0.2	0.3	4.2
CEL145	fumC (fum)	fumarate fumarate hydratase EC4.2.1.21	115	78	0.0	2.2	1.3	1.3	0.0	0.0	664.9	719.6	1499.4	674.7	1209.2	1530.5	0.0	0.7	0.0
CEL147	sul	NaDPH-dependent FMN reductase [EC1.5.1.29] required for sulfonate and sulfonate ester utilization	203	17	0.0	2.2	1.3	1.3	0.0	0.0	416.4	258.7	1559.8	255.5	180.0	170.9	-0.7	-0.5	0.1
CEL157	fop	fructose 1,6 biphosphatase, class II [EC3.1.3.11] essential for gluconeogenesis	869	767	0.0	2.0	1.3	0.0	0.0	0.0	85.0	876.1	861.3	518.3	842.0	903.0	-0.7	-0.1	0.1
CEL179		putative sensory box/GGDEF-family membrane protein	782	740	0.0	2.2	1.7	1.3	0.0	0.0	82.5	114.7	85.8	101.9	105.8	60.6	0.3	-0.1	-0.5
CEL288		putative multidrug efflux permease of the major facilitator superfamily	775	666	0.0	1.9	0.0	1.3	0.0	0.0	25.6	62.2	19.7	16.6	43.4	19.0	-0.6	-0.5	-0.1
CEL289		putative multidrug efflux permease of the major facilitator superfamily	534	534	0.0	2.0	1.3	1.4	0.0	0.0	13.2	31.6	13.4	8.2	24.3	13.7	-0.7	-0.4	0.0
CEL292		putative flavin-containing monooxygenase 3 [EC1.14.13.8]	113	113	0.0	3.0	2.0	1.6	0.0	0.0	83.4	29.6	351.0	77.3	41.0	325.7	-0.1	-0.5	-0.1
CEL301	cydA	cytochrome bd oxidase, subunit I	192	192	2.6	1.1	8.3	5.0	4.5	0.0	30.3	117.6	446.8	173.5	74.3	745.5	2.5	-0.7	-2.6
CEL316		putative superfamily I DNA/RNA helicases, SPM2-family	443	383	0.0	1.8	1.3	0.0	0.0	0.0	94.7	62.3	100.3	85.5	68.2	91.2	-0.1	0.1	-0.1
CEL334	hxA	diaminopimelate decarboxylase [EC4.1.1.20]	114	54	2.2	1.6	9.0	8.4	1.8	0.0	326.0	330.5	350.0	273.3	337.0	388.8	-0.3	0.0	0.2
CEL355	p/pA	peptide chain release factor 1 [EC1.1.1.1]	67	159	0.0	1.2	1.4	1.2	0.0	0.0	222.0	322.0	338.5	244.1	250.2	372.0	-0.2	0.0	0.2
CEL359		glucosyltransferase 11, conserved, UDP-4'-acetyl(mu)amyl pentapeptide phosphotransferase/UDP-N-acetylglucosamine 1-phosphate	176	176	0.0	1.6	0.0	0.0	0.0	0.0	150.8	83.3	1359.7	133.3	112.9	139.3	-0.3	0.4	-0.2
CEL454		putative aliphatic sulfonates uptake ABC transporter secreted solute-binding protein	274	248	0.0	1.5	0.0	0.0	0.0	0.0	84.5	130.3	101.3	124.8	145.9	67.8	0.6	0.2	-0.6
CEL456		putative signal-transduction protein containing cAMP-binding and CBS domain, conserved	154	166	0.0	1.5	0.0	0.0	0.0	0.0	110.4	132.3	154.7	97.3	104.4	109.8	-0.2	-0.3	-0.5
CEL458	enr	oxaloacetate decarboxylase	144	144	0.0	2.1	1.4	0.0	0.0	0.0	238.6	153.4	371.2	212.6	253.8	418.5	-0.2	0.7	0.2
CEL467		putative transcriptional regulator, TetR-family	250	116	0.0	1.9	1.2	1.1	0.0	0.0	16.7	12.1	4.5	9.8	10.9	5.3	-0.8	-0.1	0.2
CEL474		putative ATP/GTP-binding protein, large untranslated regions besides	138	138	0.0	2.8	1.3	1.5	0.0	0.0	48.2	54.8	70.8	43.8	40.3	47.6	-0.1	-0.4	-0.6
CEL484	rpe	putative secreted protein	64	97	0.0	3.1	0.0	1.4	0.0	0.0	100.1	391.4	101.6	141.4	187.3	107.5	0.5	0.0	0.1
CEL484		putative secreted protein	20	53	0.0	3.1	1.9	1.4	0.0	0.0	100.1	391.4	101.6	141.4	187.3	107.5	0.5	0.0	0.1
CEL485		putative metal dependent phosphohydrolase, RelA/Spot homolog	254	187	0.0	2.4	1.6	0.0	0.0	0.0	50.5	40.6	26.7	34.3	35.1	22.3	-0.6	-0.2	-0.3
CEL485		putative metal dependent phosphohydrolase, RelA/Spot homolog	124	57	0.0	3.1	0.0	1.4	0.0	0.0	50.5	40.6	26.7	34.3	35.1	22.3	-0.6	-0.2	-0.3
CEL485		putative metal dependent phosphohydrolase, RelA/Spot homolog	80	13	0.0	3.1	1.9	1.4	0.0	0.0	50.5	40.6	26.7	34.3	35.1	22.3	-0.6	-0.2	-0.3
CEL526		putative multidrug efflux permease of the major facilitator superfamily	46	46	0.0	2.1	1.7	1.4	0.0	0.0	31.3	1.3	2.1	3.3	2.1	1.5	0.1	0.7	-0.6
CEL527	enrA	putative transcriptional regulator, TetR-family	85	109	0.0	1.1	1.7	1.4	0.0	0.0	27.3	1.3	2.1	3.3	2.1	1.5	0.1	0.7	-0.6
CEL527		glucosyltransferase 11, conserved, UDP-4'-acetyl(mu)amyl pentapeptide phosphotransferase/UDP-N-acetylglucosamine 1-phosphate	93	93	0.0	2.1	1.7	1.4	0.0	0.0	27.3	1.3	2.1	3.3	2.1	1.5	0.1	0.7	-0.6
CEL537	flaG	glucosyltransferase 11, conserved, UDP-4'-acetyl(mu)amyl pentapeptide phosphotransferase/UDP-N-acetylglucosamine 1-phosphate	69	70	0.0	1.6	1.4	0.0	0.0	0.0	2393.3	175.4	1855.8	2460.0	875.0	1779.5	0.1	1.1	-0.1
CEL538	enrE	dephospho-CoA kinase [EC2.7.1.24]	144	144	0.0	3.7	2.4	1.8	0.0	0.0	829.0	934.1	501.4	693.8	404.2	355.0	-0.3	-0.1	-0.5
CEL568	ugpA	ABC-type iron-glycero-3-phosphate transport system, permease protein, presumably regulated by RegX3	228	228	0.0	0.0	0.0	1.6	1.5	1.4	27.9	17.6	16.8	24.2	14.7	8.7	-0.3	-0.3	-1.0
CEL583	ergP	acyltolmidine aminotransferase, AT class II [EC2.6.1.11]	191	191	0.0	1.5	1.4	0.0	0.0	0.0	129.9	110.1	178.2	401.3	1329.5	356.0	1.6	3.6	1.0
CEL603		hypothetical protein, conserved	35	35	0.0	3.6	1.9	1.4	0.0	0.0	274.0	374.8	198.4	213.7	355.6	309.2	-0.4	-0.1	-0.1
CEL628	secA2	putative hydrolase of the GTP superfamily	13	62	0.0	4.9	2.9	2.0	0.0	0.0	60.3	198.4	19.6	41.0	362.6	31.7	-0.6	0.9	0.7
CEL629		preprotein translocase subunit SecY2, essential	225	193	0.0	4.9	2.9	2.0	0.0	0.0	119.5	101.9	164.4	94.7	127.1	143.8	-0.3	-0.3	-0.2
CEL636		putative secreted protein	14	110	0.0	4.1	1.7	1.3	0.0	0.0	86.6	330.8	152.6	88.4	147.2	132.1	0.0	0.4	-0.2
CEL639		putative membrane protein containing CBS domain	56	56	0.0	1.6	1.0	1.2	0.0	0.0	105.2	206.8	125.5	73.0	116.4	110.1	-0.3	0.1	-0.2
CEL668		putative membrane protein	155	155	0.0	2.0	1.4	1.2	0.0	0.0	155.9	23.3	164.7	200.6	213.3	238.0	0.4	0.8	0.5
CEL691	arc (mpa)	AAA-A'Pase forming ring-shaped complexes; recognizes pupylated proteins, homolog in M. tuberculosis. Interacts with proteasome	74	74	0.0	1.9	1.3	0.0	0.0	0.0	45.4	340.3	200.9	341.6	407.9	161.6	-0.3	-0.2	-0.3
CEL695		putative plasmid maintenance system antitoxin protein, HTX-mut1 XRE-family	210	37	0.0	2.4	1.3	0.8	0.0	0.0	30.1	434.4	172.6	171.1	314.4	62.1	-0.8	1.0	-1.5
CEL700	nerH	homologous protein to a putative protease, HTX-mut1 XRE-family	165	80	2.2	2.4	1.3	0.0	0.0	0.0	32.1	434.4	172.6	171.1	314.4	62.1	-0.8	1.0	-1.5
CEL701		homologous protein to a putative protease, HTX-mut1 XRE-family	218	180	0.0	2.2	1.3	1.4	0.0	0.0	1376.3	1206.0	102.7	1037.7	859.8	175.3	-0.4	-0.5	0.8
CEL731		putative membrane protein implicated in regulation of membrane protease activity	126	126	0.0	4.5	2.6	2.0	1.4	0.0	94.9	253.6	401.6	493.1	521.4	402	0.6	-0.5	-0.5
CEL734	hemH	ferrochelatase [EC4.99.1.1], essential	21	21	2.7	4.1	1.8	1.1	2.0	0.0	78.6	68.1	156.0	731.7	1086.0	692.5	3.2	4.0	2.2
CEL736		putative membrane protein	87	60	0.0	8.3	4.7	3.1	0.0	0.0	48.2	30.3	37.3	38.3	35.7	26.1	-0.3	-0.2	-0.5
CEL737	acn	aconitase aconitate hydratase [EC4.2.1.3], loss results in glutamate (glutamine auxotrophy	267	59	0.0	8.3	4.7	3.1	0.0	0.0	2242.6	3962.9	1878.7	1175.0	3469.1	1605.0	-0.9	-0.2	-0.2
CEL767	ctaB	putative ABC-type multidrug transport system, ATPase component	27	27	0.0	21	1.3	7.0	0.0	0.0	41.4	16.3	48.6	130.8	139.3	129.6	1.7	3.1	1.4
CEL773	gcpA (gcp)	polyphosphatase cytochrome oxidase assembly factor	789	667	0.0	7.9	1.8	0.0	0.0	0.0	96.0	73.4	313.9	73.8	101.1	115.2	-0.4	0.5	-1.4
CEL791	rpe	glyceraldehyde-3-phosphate dehydrogenase [glycolysis EC1.2.1.12	269	86	2.9	3.9	3.0	2.3	2.2	0.0	6401.7	3734.5	6577.6	9354.4	3023.4	5199.7	0.5	-0.3	-0.4
CEL801	ags	ribulose-phosphate 3-spiromerose [EC5.1.3.1]	67	67	0.0	2.6	1.6	1.2	0.0	0.0	350.8	379.0	445.3	356.6	384.5	414.8	0.0	0.0	-0.1
CEL841	ags	aspartyl-tRNA synthetase [EC-6.1.1.12]	189	79	0.0	1.7	0.0	0.0	0.0	0.0	264.9	264.9	550.0	258.2	268.4	550.7	-0.5	0.0	0.0
CEL842		putative secreted metalloprotease	43	43	0.0	1.7	0.0	0.0	0.0	0.0	542.0	357.4	234.1	400.4	374.9	223.1	-0.4	-0.1	-0.1
CEL893	ercD	putative N-acetyltransferase CGP3 region	462	367	0.0	2.2	1.4	1.3	0.0	0.0	139.7	98.1	103.4	122.9	101.9	75.5	-0.2	0.2	-0.5
CEL894		hypothetical protein CGP3 region	124	124	0.0	2.2	1.4	1.3	0.0	0.0	276.3	246.1	250.9	213.1	251.2	388.7	-0.4	0.0	-0.4
CEL896		hypothetical protein CGP3 region	143	143	0.0	2.9	1.9	1.7	0.0	0.0	127.3	103.3	103.3	103.3	103.3	103.3	0.8	-0.3	-0.4
CEL896		hypothetical protein CGP3 region	143	143	0.0	2.9	1.9	1.7	0.0	0.0	127.3	103.3	103.3	103.3	103.3	103.3	0.8	-0.3	-0.4
CEL1618		hypothetical protein CGP3 region	261	261	0.0	1.4	1.0	1.4	0.0	0.0	36.4	44.0	44.0	44.0	44.0	44.0	31.9	1.0	-0.5
CEL1918		putative secreted protein, CGP3 region	683	728	0.0	1.6	1.5	0.0	0.0	0.0	25.4	15.5	46.2	75.1	55.2	33.9	-0.2	-0.1	-0.5
CEL1923		hypothetical protein CGP3 region	156	156	0.0	1.8	1.1	0.0	0.0	0.0	140.4	93.9	103.1	75.1	55.2	70.8	-0.9	-0.8	-0.5
CEL1923		hypothetical protein CGP3 region	348	348	0.0	1.9	1.3	0.0	0.0	0.0	31.1	2.9	2.8	3.2	2.8	2.8	0.0	0.1	0.0
CEL1926		hypothetical protein CGP3 region	170	170	0.0	2.4	1.2	1.3	0.0	0.0	118.4	29.7	49.7	95.8	118.5	48.2	-0.3	0.6	0.0
CEL1926		hypothetical protein CGP3 region	130	130	0.0	2.0	1.4	0.0	0.0	0.0	47.7	38.5	29.6	25.1	52.5	23.4	-0.9	0.6	-0.3
CEL1959	prp	prophage DNA primase CGP3 region	16	180	0.0	2.0	1.4	0.0	0.0	0.0	47.7	38.5	29.6	25.1	52.5	23.4	-0.9	0.6	-0.3
CEL1981		hypothetical protein CGP3 region	556	556	0.0	2.4	0.0	1.8	0.0	0.0	4.8	2.6	2.7	3.6	2.6	2.0	-0.4	-0.5	-0.4
CEL1981		hypothetical protein CGP3 region	476	476	0.0	0.0	1.5	1.6	0.0	0.0	12.9	7.0	6.1	9.4	5.7	4.4	-0.5	-0.3	-0.5
CEL1981		hypothetical protein CGP3 region	438	438	0.0	0.0	1.5	1.6	0.0	0.0	12.9	7.0	6.1	9.4	5.7	4.4	-0.5	-0.3	-0.5
CEL1981		hypothetical protein CGP3 region	47	47	0.0	1.8	1.5	0.0	0.0	0.0	12.9	7.0	6.1	9.4	5.7	4.4	-0.5	-0.3	-0.5
CEL2005		putative protein-plasmin encoded, conserved CGP3 region	340	139	0.0	2.7	1.8	2.0	0.0	0.0	15.8	14.8	11.9	15.8	14.2	9.1	0.0	-0.1	-0.4
CEL2014		hypothetical protein CGP3 region	78	78	0.0														

cg0337		7	44		0.0	3.3	1.8	1.5	0.0	0.0	89.9	92.4	82.1	81.6	98.5	98.9	-0.1	0.1	-0.3
ncg1755		43	23		0.0	5.8	2.2	2.0	0.0	0.0	187.9	288.7	148.3	210.3	217.9	122.8	0.2	0.5	-0.3
ncg1787		44	42		0.0	2.8	2.1	1.3	0.0	0.0	8.3	9.7	17.1	9.7	12.6	9.5	0.2	0.4	-0.8
ncg1793		260	260		0.0	2.3	1.6	1.5	0.0	0.0	1.5	0.0	0.5	0.8	0.3	0.0	-1.0	NA	NA
ncg1815		507	507		0.0	1.6	1.2	1.1	1.0	1.1	9.2	5.4	4.1	6.8	4.6	3.4	-0.4	-0.2	-0.3
cg2077	gpcC	367	271		0.0	3.0	1.9	1.3	0.0	0.0	17.1	33.4	130.5	162.6	263.7	114.2	0.7	2.3	-0.2
cg2079	hemQ	133	13		2.0	19	11	6.9	1.7	0.0	69.7	109.0	460.8	630.7	773.5	1641.3	1.8	2.8	1.8
cg2080		160	67		2.0	19	11	6.9	1.7	0.0	69.7	109.0	460.8	630.7	773.5	1641.3	1.8	2.8	1.8
cg2085		164	96		0.0	0.0	1.5	1.4	0.0	0.0	281.8	252.3	351.3	259.5	252.7	257.4	-0.1	0.0	-0.4
cg2085		134	0.0		0.0	1.9	1.5	1.4	0.0	0.0	281.8	252.3	351.3	259.5	252.7	257.4	-0.1	0.0	-0.4
cg2090	sunB	129	129		2.3	266	104	7.0	1.6	0.0	166.2	117.0	181.7	287.2	242.2	164.6	0.8	1.0	-0.1
cg2091	gpcK	199	199		2.3	266	104	7.0	1.6	0.0	166.2	117.0	181.7	287.2	242.2	164.6	0.8	1.0	-0.1
cg2121	gpcH	25	70		0.0	2.1	3.2	0.0	0.0	0.0	2765.7	395.9	2443.1	2448.2	1299.4	498.5	0.1	0.2	-0.8
cg2135		326	326		0.0	2.6	1.9	0.0	0.0	0.0	46.4	171.5	356.7	444.8	640.5	128.9	0.5	0.8	-0.3
cg2135		326	326		0.0	2.6	1.9	0.0	0.0	0.0	46.4	171.5	356.7	444.8	640.5	128.9	0.5	0.8	-0.3
cg2187	gpcA	279	279		0.0	2.1	0.0	3.0	0.0	0.0	38.0	15.0	41.6	35.9	30.0	40.4	-1.1	0.5	0.0
cg2196		284	50		0.0	2.5	1.5	1.5	0.0	0.0	6624.2	8846.7	11679.9	8905.6	11651.9	11955.3	0.4	0.4	0.0
cg2201	chsS (cpi58)	4	4		0.0	2.5	1.4	1.0	0.0	0.0	36.8	490.1	23.7	11.6	363.7	57.5	-1.7	0.4	1.3
cg2202	hnbB	107	107		0.0	2.5	1.4	1.0	0.0	0.0	42.7	528.5	12.0	4.7	2771.7	240.2	-3.2	-1.0	4.3
cg2208	div	44	44		0.0	3.2	2.4	1.8	0.0	0.0	48.8	37.4	96.3	51.3	58.8	72.1	0.1	0.9	-0.4
cg2211		200	121		0.0	3.2	2.4	1.8	0.0	0.0	1747.7	2114.1	2363.5	808.6	1629.5	1453.7	-1.1	-0.4	-0.7
cg2224	xerC	129	26		2.4	78	40	20	1.8	0.0	42.7	26.5	31.3	43.0	42.1	33.3	0.0	0.7	0.1
cg2311		113	113		0.0	4.2	2.8	2.1	0.0	0.0	99.7	50.0	135.3	47.2	48.7	163.2	-1.1	0.0	0.3
cg2343	plcC	422	192		0.0	6.3	3.8	3.2	0.0	0.0	84.4	124.2	79.7	103.6	115.6	54.7	0.3	-0.1	-0.5
cg2398		153	75		0.0	2.3	1.8	1.4	0.0	0.0	441.4	656.1	375.5	243.1	242.3	147.8	-0.9	-1.4	-1.1
cg2406	cbtE	307	307		1.9	105	63	25	13	2.3	5935.0	4547.1	1818.0	965.4	1446.9	1038.4	-2.0	1.7	-0.8
cg2409	cbtC	233	47		1.9	22	12	6.3	2.1	0.0	2698.8	3552.2	2684.1	1037.3	1403.6	1371.5	-1.4	-1.3	-1.0
cg2409		270	84		1.9	22	12	6.3	2.1	0.0	2698.8	3552.2	2684.1	1037.3	1403.6	1371.5	-1.4	-1.3	-1.0
cg2410	ftsA	286	199		1.9	22	12	6.3	2.1	0.0	1533.0	136.3	205.1	135.0	142.7	168.0	-0.2	0.1	-0.3
cg2410		249	162		1.9	22	12	6.3	2.1	0.0	1533.0	136.3	205.1	135.0	142.7	168.0	-0.2	0.1	-0.3
cg2423	ftsA	327	33		0.0	3.3	2.3	1.9	0.0	0.0	677.5	1676.7	452.8	678.3	1702.6	462.5	0.0	0.0	0.0
cg2445	hmuO	149	43		0.0	5.4	2.8	3.1	0.0	0.0	185.3	80.4	263.1	8.3	19.1	4.5	-4.5	-3.1	-3.8
cg2445		625	519		0.0	5.4	2.8	3.1	0.0	0.0	185.3	80.4	263.1	8.3	19.1	4.5	-4.5	-3.1	-3.8
cg2473	acpM	586	586		0.0	6.6	3.7	2.6	0.0	0.0	188.3	140.2	247.5	253.0	278.1	175.4	0.4	1.0	-0.5
cg2495		43	43		0.0	6.7	4.3	3.4	0.0	0.0	90.2	69.8	104.0	91.0	70.3	67.5	0.0	0.0	-0.6
cg2495		-1	56		0.0	6.7	4.3	3.4	0.0	0.0	128.3	109.5	140.6	122.9	145.0	113.5	-0.1	0.4	-0.3
cg2521	ftsD15	184	15		0.0	1.3	1.6	1.5	0.0	0.0	284.8	254.5	209.6	250.1	244.5	204.5	-0.2	-0.1	0.0
cg2523	hmlQ	84	74		0.0	1.3	1.6	1.5	0.0	0.0	4629.8	1167.4	1339.1	1106.0	998.8	1089.7	-0.6	-0.2	-0.4
cg2537		345	235		0.0	3.9	2.9	2.0	1.5	0.0	178.7	133.9	150.6	158.3	187.0	169.8	-0.2	0.5	0.2
cg2546		183	162		0.0	8.6	5.3	3.5	1.3	0.0	5.3	4.0	12.1	3.2	3.7	16.2	-0.7	-0.1	0.4
cg2557		114	37		0.0	1.8	1.3	0.0	0.0	0.0	25.4	20.2	114.8	15.5	21.9	125.7	-0.7	0.1	0.1
cg2625	pcrP	219	219		0.0	2.0	1.8	1.6	0.0	0.0	817.0	233.1	19.9	207.6	77.2	17.5	2.0	2.1	-0.2
cg2641	benR	295	295		0.0	5.6	4.0	3.1	0.0	0.0	24.0	26.3	45.7	22.3	24.3	20.4	-0.1	0.1	-1.2

Table S4: Filtered dataset of time r
(w/v) glucose and 4 μ M hemin and
(red) are shown (in transcripts per r
fold altered fold-change in one of th

Gene ID	Gene name
cg0012	<i>ssuR</i>
cg0061	<i>rodA</i>
cg0160	
cg0161	
cg0162	
cg0163	
cg0165	
cg0230	<i>gltD</i>
cg0256	
cg0314	<i>brnF</i>
cg0315	<i>brnE</i>
cg0318	<i>arsC1 (arsB2)</i>
cg0319	<i>arsC2 (arsX)</i>
cg0421	<i>wzx</i>
cg0445	<i>sdhC sdhCD</i>
cg0446	<i>sdhA</i>
cg0447	<i>sdhB</i>
cg0455	
cg0456	
cg0463	<i>csaR</i>
cg0464	<i>copA (ctpA, ctpV)</i>
cg0466	<i>htaA</i>
cg0635	<i>creA</i>
cg0676	
cg0683	
cg0755	<i>metY</i>
cg0793	
cg0898	<i>pdxS</i>
cg0922	
cg0923	
cg0926	
cg0927	
cg0951	<i>accD3</i>
cg1120	<i>ripA</i>
cg1225	<i>benK3 (pcaK)</i>
cg1226	<i>pobB (pobA)</i>
cg1313	
cg1405	
cg1411	<i>rbsA</i>

cg1412	<i>rbsC</i>
cg1424	<i>lysE</i>
cg1425	<i>lysG</i>
cg1537	<i>ptsG</i>
cg1555	
cg1695	
cg1705	<i>arsB1 (arsC2)</i>
cg1738	<i>acnR</i>
cg1759	
cg1760	<i>sufU</i>
cg1761	<i>sufS</i>
cg1762	<i>sufC</i>
cg1763	<i>sufD</i>
cg1778	<i>zwf</i>
cg1779	<i>opcA</i>
cg1780	<i>pgi (devB)</i>
cg1787	<i>ppc</i>
cg1861	<i>rel</i>
cg1962	
cg2012	
cg2014	
NCgl1729	
cg2106	
cg2117	<i>ptsI</i>
cg2118	<i>fruR</i>
cg2119	<i>pfkB (fruK)</i>
cg2120	<i>ptsF</i>
cg2121	<i>ptsH</i>
cg2204	<i>hrtA</i>
cg2329	
cg2381	
cg2397	
cg2398	<i>plsC</i>
cg2403	<i>qcrB</i>
cg2404	<i>qcrA (qcrA1)</i>
cg2405	<i>qcrC</i>
cg2406	<i>ctaE</i>
cg2408	<i>ctaF</i>
cg2409	<i>ctaC</i>
cg2438	
cg2445	<i>hmuO</i>
cg2559	<i>aceB</i>
cg2560	<i>aceA</i>
cg2624	<i>pcaR</i>
cg2625	<i>pcaF</i>

cg2626	<i>pcaD</i>
cg2629	<i>pcaB</i>
cg2630	<i>pcaG</i>
cg2636	<i>catA1 (catA)</i>
cg2638	<i>benB</i>
cg2639	<i>benC</i>
cg2674	
cg2675	
cg2676	
cg2677	
cg2678	
cg2697	
cg2732	<i>gntV (gntK)</i>
cg2739	
cg2780	<i>ctaD</i>
cg2810	<i>cynT</i>
cg2833	<i>cysK</i>
cg2836	<i>sucD</i>
cg2838	
cg2867	<i>mpx</i>
cg2925	<i>ptsS</i>
cg2939	<i>siaG</i>
cg2940	<i>sial</i>
cg3101	
cg3109	
cg3112	<i>cysZ</i>
cg3141	<i>hmp</i>
cg3145	
cg3176	
cg3213	
cg3216	<i>gntP</i>
cg3226	
cg3227	<i>lldD</i>
cg3234	
NCgl2845	
cg3277	
cg3280	
NCgl2858a	
cg3281	<i>copB</i>
cg3282	
NCgl2861	
cg3284	<i>copS (cgtS9)</i>

cg3285	<i>copR (cgtR9)</i>
cg3286	
cg3287	<i>copO</i>
cg3334	<i>cepA</i>
cg3374	<i>cye1</i>
cg3385	<i>catA3 (rhcD2)</i>
cg3387	<i>iolT2</i>
cg3399	
cg3402	

resolved transcriptome analysis of *C. glutamicum* wild type and $\Delta hrrA$ with genes showing at least 2-fold change in expression at any of the time points harvested 0 h, 0.5 h and 4 h after hemin addition. Column A and B show the gene locus (ID) and gene name (ID, mean of two biological replicates). Column J-L show the log₂-fold change of $\Delta hrrA$ in comparison to the wild type at the measured time points and for a p-value <0.05.

Annotation
sulphonate sulphur utilization transcriptional regulator SsuR, activator of sulfonateester utilization, ROK-family, loss causes inability to utilize alkylsulfonates
putative FTSW/RODA/SPOVE-family cell cycle protein
hypothetical protein
putative membrane protein
putative membrane spanning protein
putative N-acetylglucosaminyltransferase
putative ABC-2-type transporter
glutamine 2-oxoglutarate aminotransferase NADPH small subunit, also glutamate synthase (EC:1.4.1.13)
putative protein, conserved
branched chain amino acid exporter Ile, Leu, Val, Met, large subunit
branched chain amino acid exporter Ile, Leu, Val, Met, small subunit
arsenite permease, arsenical resistance-3 (ACR3)-family
arsenate reductase, arsenical pump modifier (EC:1.20.4.1)
putative translocase involved in export of a cell surface polysaccharide, horizontally transferred
succinate:menaquinone oxidoreductase, cytochrome b subunit
succinate:menaquinone oxidoreductase, flavoprotein subunit
succinate:menaquinone oxidoreductase, iron-sulfur protein subunit
putative permease, major facilitator superfamily
putative permease, major facilitator superfamily
transcriptional repressor during copper starvation
copper-transporting P-type ATPase (EC:3.6.3.4)
secreted heme transport-associated protein
putative NAD ⁺ -dependent 4-hydroxybenzaldehyd dehydrogenase subunit (EC: 1.2.1.64), (N. Kallscheuer: why only 107 aa; too short)
hypothetical protein, conserved
putative permease
O-acetylhomoserine sulfhydrylase EC:2.5.1.49, loss causes methionine auxotrophy
putative secreted protein
pyridoxal 5-phosphate PLP synthase subunit
putative secreted siderophore-binding lipoprotein
putative membrane protein
putative putative iron-siderophore transporter, permease subunit
putative ABC-type putative iron-siderophore transporter, permease subunit
acetyl-coenzyme A carboxylase carboxyl transferase (EC:6.4.1.2)
transcriptional regulator of iron proteins and repressor of aconitase, AraC-family
putative benzoate transport transmembrane protein
4-hydroxybenzoate 3-monooxygenase (EC:1.14.13.2)
putative secreted lipoprotein
putative cytoplasmic siderophore-interacting protein
ribose/xylose transporter, ABC-type sugar aldose transport system, ATPase component (TC 3.A.1.2.1)

ribose/xylose transporter, ABC-type transport system, permease component (TC 3.A.1.2.1)

lysine efflux permease

transcriptional regulator of lysE, LysR-family

glucose-specific EIIABC component EIIGlc of PTS EC:2.7.1.69 fructose-specific enzyme II BC (EIIFru) component of PTS (EC:2.7.1.69)

putative superfamily I DNA or RNA helicase

putative plasmid maintenance system antidote protein, HTH-motif XRE-family

arsenite permease, arsenical resistance-3 ACR3-family

transcriptional regulator, represses aconitase, TetR-family

putative Fe-S cluster assembly protein, part of the sufBDCSU response

cysteine desulfhydrase

Fe-S cluster assembly protein

Fe-S cluster assembly ATPase

Fe-S cluster assembly membrane protein

glucose-6-phosphate dehydrogenase (EC:1.1.1.49)

putative subunit of glucose-6-phosphate dehydrogenase

6-phosphogluconolactonase (EC:3.1.1.31)

phosphoenolpyruvate carboxylase (EC:4.1.1.31)

ppGpp synthetase, ppGpp pyrophosphorylase (EC:2.7.6.5)

putative membrane protein CGP3 region

putative secreted protein CGP3 region

hypothetical protein CGP3 region

hypothetical protein CGP3 region

hypothetical protein, conserved

EI enzyme, general component of PTS (EC:2.7.3.9)

transcriptional regulator of sugar metabolism, presumably fructose responsive, DeoR-family

1-phosphofructokinase (EC:2.7.1.56)

fructose-specific enzyme II BC component of PTS (EC:2.7.1.69)

phosphocarrier protein HPr, general component of PTS

ABC-type heme transport system, ATPase component

putative coenzyme F420-dependent N5,N10-methylene tetrahydromethanopterin reductase or related flavin-dependent

hypothetical protein, conserved

putative membrane protein

1-acyl-sn-glycerol-3-phosphate acetyltransferase

cytochrome bc1 complex, cytochrome b subunit

cytochrome bc1 complex, Rieske iron-sulfur protein

cytochrome bc1 complex, diheme cytochrome c1 subunit

cytochrome aa3 oxidase, subunit 3

cytochrome aa3 oxidase, subunit 4

cytochrome aa3 oxidase, subunit 2

hypothetical protein

heme oxygenase

malate synthase (EC:2.3.3.9), part of glyoxylate shunt

isocitrate lyase (EC:4.1.3.1), part of glyoxylate shunt

transcriptional repressor involved in metabolism of 4-hydroxybenzoate, protocatechuate and p-cresol, IclR-family

β-ketoadipyl-CoA thiolase (EC:2.3.1.174)

β-ketoadipate enol-lactone hydrolase (EC:3.1.1.24)
β-carboxy-cis,cis-muconate cycloisomerase (EC:5.5.1.2)
protocatechuate dioxygenase α subunit (EC:1.13.11.3)
catechol 1,2-dioxygenase (EC:1.13.11.1)
benzoate dioxygenase small subunit (EC:1.14.12.10)
benzoate 1,2-dioxygenase ferredoxin reductase subunit (EC:1.18.1.3)
putative alkylhydroperoxidase AhpD-family core domain
putative ATPase component of ABC-type transport system, contains duplicated ATPase domains
putative ABC-type dipeptide/oligopeptide/nickel transport systems, permease component
putative ABC-type dipeptide/oligopeptide/nickel transport system, permease component
putative ABC-type dipeptide/oligopeptide/nickel transport systems, secreted component
putative single-strand DNA binding protein
putative gluconokinase (EC:2.7.1.12)
putative permease of the major facilitator superfamily
cytochrome aa3 oxidase, subunit 1
high affinity cysteine importer
O-acetylserine thiol-lyase, cysteine synthase (EC:2.5.1.47), loss causes cysteine auxotrophy
succinyl-CoA synthetase α subunit, ADP-forming (EC:6.2.1.5)
putative dithiol-disulfide isomerase
mycothiol peroxidase, GSH peroxidase-family (EC:1.11.1.9)
sucrose-specific EIIBC component EII _{Suc} of PTS fructose-specific enzyme II _{BC} (EII _{Fru})
component of PTS (EC:2.7.1.69)
ABC-Transporter for sialic acid, fused permease and ATPase components
ABC-Transporter for sialic acid, contain duplicated ATPase domains
putative permease
putative membrane protein
Sulfate transporter, loss causes sulfide/cysteine auxotrophy
flavohepoxypyrone synthase
BglG in CgR is 93 AA longer (belongs to BglG?), putative pseudo-gene
putative membrane protein
putative secreted protein
gluconate permease, gluconate:H ⁺ symporter GntP-family
L-lactate permease, operon with lldD, MFS-type
menaquinone-dependent L-lactate dehydrogenase operon with cg3226
putative metal-dependent amidase/aminoacylase/carboxypeptidase
hypothetical protein
putative protein, ACR, double-stranded β-helix domain
putative secreted protein, horizontally transferred gene
hypothetical protein
Cu ²⁺ /cation-transporting ATPase transmembrane protein, horizontally transferred gene
putative Cu ²⁺ /heavy metal binding transport protein, horizontally transferred gene
hypothetical protein
two component sensor kinase, copper homeostasis, horizontally transferred gene

two component response regulator, copper homeostasis, horizontally transferred gene

putative secreted protein of unknown function, horizontally transferred gene

secreted multicopper oxidase, horizontally transferred gene

putative toxine efflux permease, MFS-type

putative NADH-dependent flavin oxidoreductase, Old Yellow Enzyme family, probably involved in oxidative stress response

catechol 1,2-dioxygenase (EC:1.13.11.37)

myo-Inositol transporter 2, MFS-type

putative permease of the major facilitator superfamily

putative copper chaperone or Hg²⁺ permease, MerTP-family

at least two-fold alteration in gene expression. Wild type cells and a $\Delta hrrA$ strain were cultivated in CG medium. In green (D-F) and red (G-I), the measured mRNA levels of the corresponding genes in the $\Delta hrrA$ strain are compared to the wild type after 0 h, 0.5 h, or 4 h of incubation in hemin containing medium. The presence of hemin was confirmed by the color change of the medium from colorless to brown.

mRNA wt T=0h	mRNA wt T=30m	mRNA wt T=4h	mRNA DhrrA T=0h	mRNA DhrrA T=30m
783,2	319,1	5,9	160,5	103,2
146,9	344,0	222,8	169,4	166,3
7,8	2,0	3,6	2,4	0,6
494,5	397,9	287,3	80,6	88,9
306,1	327,4	201,3	66,6	50,4
165,9	302,2	181,6	56,6	50,7
31,6	114,3	88,2	26,0	26,2
57,0	427,7	8,4	147,6	96,4
42,1	5,4	39,3	9,4	0,3
37,5	47,5	14,2	29,1	19,8
50,5	39,6	16,2	34,2	19,4
39,6	90,2	19,4	29,6	32,9
86,2	116,4	34,9	77,2	50,3
69,7	36,7	60,2	20,7	14,8
1917,1	2911,0	2339,4	392,3	884,6
1156,2	2662,4	2224,9	278,2	768,3
458,1	2731,7	2527,3	229,8	798,8
634,7	578,7	24,9	364,1	261,3
276,6	580,5	17,8	320,0	253,6
537,5	3066,3	45,3	705,1	926,5
1863,7	4490,5	27,0	1442,6	1033,9
19,4	8,6	77,7	3,0	3,0
48,5	32,8	6,7	18,9	12,7
29,7	17,3	19,7	12,8	7,9
873,3	449,8	145,3	850,1	218,0
3615,4	2977,0	54,7	2088,5	1365,4
321,7	183,1	246,2	141,7	83,5
701,1	228,4	772,2	517,3	111,3
35,6	16,4	43,8	13,6	5,2
440,6	466,9	147,2	134,2	124,0
140,1	27,1	228,7	27,0	12,8
415,1	29,5	215,7	52,6	13,6
508,0	728,0	395,4	134,3	156,3
77,0	55,3	105,7	33,8	26,1
1672,9	600,3	42,2	1190,0	207,5
2600,5	682,1	60,3	1874,0	314,8
393,1	139,4	77,5	149,2	65,4
518,3	297,8	119,3	202,5	97,0
29,9	22,5	51,6	16,1	10,5

27,8	24,0	50,7	17,9	11,8
613,8	368,3	8,3	300,9	89,8
57,5	49,0	17,5	38,9	21,6
2393,3	1757,4	1855,8	2649,0	825,0
127,2	736,5	83,3	120,1	297,6
302,1	434,4	172,6	171,1	211,4
53,1	112,9	48,8	42,1	54,9
173,0	368,6	74,8	78,8	171,9
2544,1	4176,3	834,1	3478,9	1899,3
3043,1	4005,4	883,6	3669,2	1828,7
3417,4	3789,5	758,6	3412,6	1567,9
4638,9	4619,6	946,1	4230,5	1871,9
5218,2	4722,5	1051,6	4286,1	2202,4
411,2	728,0	294,8	316,8	321,9
382,6	823,1	302,5	338,8	342,5
184,5	399,9	181,9	208,7	198,5
1217,0	447,6	467,2	735,7	177,9
494,7	736,1	327,6	305,5	338,3
135,4	183,8	90,8	98,1	86,3
20,8	26,5	10,8	14,0	12,7
109,7	122,2	54,1	50,9	57,2
22,6	15,1	11,8	13,0	6,6
1096,6	3586,5	265,7	851,3	1615,4
1629,4	565,0	1067,6	1799,1	204,8
3069,0	1111,2	852,6	3750,8	251,3
2340,6	1037,4	848,4	2908,1	213,2
2834,9	2472,9	931,9	3460,9	431,2
2765,7	3056,9	2443,1	3448,2	1299,4
34,7	4329,7	11,2	2,8	2134,6
55,3	113,0	27,6	40,7	48,0
108,4	196,0	87,6	90,9	97,9
204,1	146,9	135,7	79,5	72,3
441,4	656,1	325,5	243,1	242,3
1531,9	4192,8	2001,6	533,1	1209,8
2308,3	4502,3	1893,6	612,3	1254,2
2729,9	4308,4	1782,9	692,9	1294,4
3935,0	4547,1	1818,0	965,4	1446,9
1375,0	2402,0	1615,9	575,2	824,0
2698,8	3552,2	2684,1	1037,3	1403,6
371,3	475,9	337,4	341,6	138,6
185,3	80,4	263,1	8,3	9,6
295,1	156,7	228,5	145,9	54,2
61,0	16,9	212,8	13,9	8,4
616,9	252,4	25,5	190,1	106,2
817,0	323,1	19,9	207,6	77,2

1150,5	606,2	36,7	349,9	132,0
2832,7	1115,4	156,2	2377,9	555,3
5111,6	2096,4	232,9	5046,4	999,5
143,3	298,3	821,6	49,7	62,5
4,4	6,6	7,3	3,9	1,6
5,2	6,7	5,9	1,8	1,5
349,7	1095,0	285,3	877,5	491,2
571,8	1357,7	18,0	859,1	399,3
891,9	1635,4	12,3	855,5	432,6
1213,2	1710,5	11,0	845,1	438,1
1362,4	1661,0	15,7	756,1	465,6
26,5	20,5	12,0	14,0	9,9
61,2	33,8	6,5	36,9	8,0
32,7	59,3	9,0	21,7	29,2
3107,7	5452,0	3251,3	1682,3	2653,4
212,1	944,4	26,1	94,1	164,4
8886,8	7276,4	611,9	4250,2	2372,2
45,7	35,7	576,0	87,0	16,6
283,1	1219,4	86,3	197,3	579,0
284,8	487,7	311,2	195,9	172,1
3076,8	629,6	1200,2	4006,3	144,1
35,4	22,2	36,7	41,0	9,9
19,8	23,7	33,5	31,4	9,4
393,2	430,1	327,3	106,2	126,7
12,4	12,0	4,8	8,2	5,2
4747,4	5718,7	15,8	4079,5	2666,5
825,6	454,8	8,7	238,8	170,1
7,5	9,4	5,8	5,7	2,2
430,2	422,9	189,2	92,0	98,6
74,6	260,6	15,5	104,4	122,7
383,5	166,1	178,6	212,9	60,1
2579,1	569,4	83,9	1161,8	178,5
4147,0	905,9	134,2	1808,7	311,1
129,2	557,6	59,9	89,6	262,7
1,5	7,1	10,4	1,5	2,4
181,8	804,8	57,2	198,6	265,1
1436,3	6136,9	43,1	1447,1	2940,8
459,2	4055,7	22,0	685,8	1736,3
1250,1	2882,3	27,1	647,3	1133,8
1870,4	2441,3	31,0	957,1	923,6
779,6	978,2	23,3	474,2	440,8
15,5	22,6	22,4	6,2	8,7

25,0	31,0	19,7	7,3	14,2
107,6	212,5	77,3	17,8	56,2
45,5	91,0	37,4	6,6	24,3
310,8	263,7	22,4	17,4	18,8
993,8	1177,7	27,2	610,7	554,7
94,1	35,1	49,3	86,0	16,5
127,1	33,4	56,0	62,0	13,6
1182,9	1521,5	10,6	602,5	241,8
1464,6	2591,0	28,3	1863,5	1236,0

XII minimal medium supplemented with 2%
 e wild type strain (green) and a $\Delta hrrA$ strain
 ented genes were filtered for at least two-

mRNA DhrrA T=4h	Log2 DhrrA/WT T=0h	Log2 DhrrA/WT T=30m	Log2 DhrrA/WT T=4h
20,4	-2,3	-1,6	1,8
155,6	0,2	-1,0	-0,5
18,7	-1,7	-1,7	2,4
133,9	-2,6	-2,2	-1,1
99,2	-2,2	-2,7	-1,0
87,6	-1,6	-2,6	-1,1
43,6	-0,3	-2,1	-1,0
6,4	1,4	-2,1	-0,4
20,8	-2,2	-4,1	-0,9
6,9	-0,4	-1,3	-1,0
11,0	-0,6	-1,0	-0,6
15,2	-0,4	-1,5	-0,4
25,5	-0,2	-1,2	-0,5
14,4	-1,8	-1,3	-2,1
760,6	-2,3	-1,7	-1,6
774,9	-2,1	-1,8	-1,5
839,3	-1,0	-1,8	-1,6
24,2	-0,8	-1,1	0,0
19,4	0,2	-1,2	0,1
39,9	0,4	-1,7	-0,2
30,1	-0,4	-2,1	0,2
72,5	-2,7	-1,5	-0,1
5,4	-1,4	-1,4	-0,3
17,5	-1,2	-1,1	-0,2
148,4	0,0	-1,0	0,0
304,6	-0,8	-1,1	2,5
153,9	-1,2	-1,1	-0,7
907,8	-0,4	-1,0	0,2
106,2	-1,4	-1,7	1,3
92,2	-1,7	-1,9	-0,7
718,5	-2,4	-1,1	1,7
644,6	-3,0	-1,1	1,6
119,4	-1,9	-2,2	-1,7
339,2	-1,2	-1,1	1,7
27,4	-0,5	-1,5	-0,6
37,4	-0,5	-1,1	-0,7
33,4	-1,4	-1,1	-1,2
135,6	-1,4	-1,6	0,2
61,5	-0,9	-1,1	0,3

69,2	-0,6	-1,0	0,4
9,1	-1,0	-2,0	0,1
14,7	-0,6	-1,2	-0,3
1779,5	0,1	-1,1	-0,1
71,6	-0,1	-1,3	-0,2
62,1	-0,8	-1,0	-1,5
45,0	-0,3	-1,0	-0,1
60,3	-1,1	-1,1	-0,3
830,8	0,5	-1,1	0,0
869,8	0,3	-1,1	0,0
766,3	0,0	-1,3	0,0
954,6	-0,1	-1,3	0,0
1067,8	-0,3	-1,1	0,0
243,7	-0,4	-1,2	-0,3
267,3	-0,2	-1,3	-0,2
157,0	0,2	-1,0	-0,2
332,6	-0,7	-1,3	-0,5
227,1	-0,7	-1,1	-0,5
63,5	-0,5	-1,1	-0,5
6,3	-0,6	-1,1	-0,8
33,9	-1,1	-1,1	-0,7
10,4	-0,8	-1,2	-0,2
206,4	-0,4	-1,2	-0,4
910,0	0,1	-1,5	-0,2
510,3	0,3	-2,1	-0,7
510,7	0,3	-2,3	-0,7
548,2	0,3	-2,5	-0,8
1981,3	0,3	-1,2	-0,3
185,5	-3,7	-1,0	4,0
14,6	-0,4	-1,2	-0,9
87,7	-0,3	-1,0	0,0
106,6	-1,4	-1,0	-0,3
147,8	-0,9	-1,4	-1,1
1227,5	-1,5	-1,8	-0,7
1153,9	-1,9	-1,8	-0,7
1034,6	-2,0	-1,7	-0,8
1039,4	-2,0	-1,7	-0,8
934,6	-1,3	-1,5	-0,8
1371,5	-1,4	-1,3	-1,0
169,3	-0,1	-1,8	-1,0
19,1	-4,5	-3,1	-3,8
159,1	-1,0	-1,5	-0,5
260,7	-2,1	-1,0	0,3
15,4	-1,7	-1,2	-0,7
17,5	-2,0	-2,1	-0,2

34,0	-1,7	-2,2	-0,1
60,4	-0,3	-1,0	-1,4
93,3	0,0	-1,1	-1,3
230,6	-1,5	-2,3	-1,8
1,1	-0,2	-2,0	-2,7
2,1	-1,6	-2,2	-1,5
225,6	1,3	-1,2	-0,3
61,0	0,6	-1,8	1,8
63,4	-0,1	-1,9	2,4
72,0	-0,5	-2,0	2,7
79,0	-0,8	-1,8	2,3
8,1	-0,9	-1,1	-0,6
10,6	-0,7	-2,1	0,7
9,0	-0,6	-1,0	0,0
1786,1	-0,9	-1,0	-0,9
35,7	-1,2	-2,5	0,5
2635,8	-1,1	-1,6	2,1
171,2	0,9	-1,1	-1,8
93,3	-0,5	-1,1	0,1
208,8	-0,5	-1,5	-0,6
674,8	0,4	-2,1	-0,8
26,8	0,2	-1,2	-0,5
25,5	0,7	-1,3	-0,4
178,9	-1,9	-1,8	-0,9
2,6	-0,6	-1,2	-0,9
166,0	-0,2	-1,1	3,4
12,5	-1,8	-1,4	0,5
2,2	-0,4	-2,1	-1,4
137,5	-2,2	-2,1	-0,5
9,7	0,5	-1,1	-0,7
89,0	-0,8	-1,5	-1,0
151,9	-1,2	-1,7	0,9
408,9	-1,2	-1,5	1,6
56,7	-0,5	-1,1	-0,1
14,2	-0,1	-1,6	0,4
43,7	0,1	-1,6	-0,4
26,3	0,0	-1,1	-0,7
13,0	0,6	-1,2	-0,8
16,0	-0,9	-1,3	-0,8
23,4	-1,0	-1,4	-0,4
20,7	-0,7	-1,2	-0,2
10,7	-1,3	-1,4	-1,1

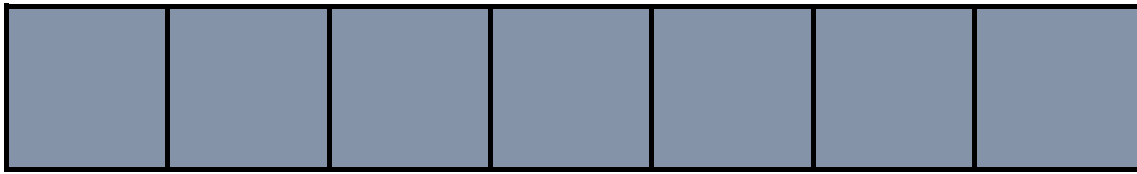
5,6	-1,8	-1,1	-1,8
59,4	-2,6	-1,9	-0,4
32,9	-2,8	-1,9	-0,2
6,8	-4,2	-3,8	-1,7
26,2	-0,7	-1,1	-0,1
30,8	-0,1	-1,1	-0,7
37,2	-1,0	-1,3	-0,6
47,0	-1,0	-2,7	2,1
24,2	0,3	-1,1	-0,2

Predicted function
Signal transduction mechanisms
Cell division, chromosome partitioning
Unknown function
Unknown function
Unknown function
General function prediction only
General function prediction only
Amino acid transport and metabolism
Unknown function
Amino acid transport and metabolism
Amino acid transport and metabolism
Inorganic ion transport, metabolism, and storage
Inorganic ion transport, metabolism, and storage
Cell wall/membrane/envelope biogenesis
Central carbon metabolism; Anaerobic metabolism; Respiration and oxidative phosphorylation
Central carbon metabolism; Anaerobic metabolism; Respiration and oxidative phosphorylation
Central carbon metabolism; Anaerobic metabolism; Respiration and oxidative phosphorylation
General function prediction only
General function prediction only
Signal transduction mechanisms; Inorganic ion transport, metabolism, and storage
Inorganic ion transport, metabolism, and storage
Transport and metabolism of further metabolites
General function prediction only
Unknown function
General function prediction only
Amino acid transport and metabolism
Unknown function
Coenzyme transport and metabolism
Inorganic ion transport, metabolism, and storage; Transport and metabolism of further metabolites
Unknown function
Inorganic ion transport, metabolism, and storage; Transport and metabolism of further metabolites
Inorganic ion transport, metabolism, and storage; Transport and metabolism of further metabolites
Lipid transport and metabolism
Signal transduction mechanisms
Carbon source transport and metabolism
Carbon source transport and metabolism
Unknown function
Transport and metabolism of further metabolites
Carbon source transport and metabolism

Carbon source transport and metabolism
Amino acid transport and metabolism
Signal transduction mechanisms
Carbon source transport and metabolism; signal transduction mechanisms
DNA replication, recombination, repair, and degradation; Transcription including sigma factors, RNA processing
General function prediction only
Inorganic ion transport, metabolism, and storage
Signal transduction mechanisms
Coenzyme transport and metabolism
Coenzyme transport and metabolism
Coenzyme transport and metabolism
Coenzyme transport and metabolism
Coenzyme transport and metabolism
Central carbon metabolism
Central carbon metabolism
Central carbon metabolism
Central carbon metabolism
Signal transduction mechanisms
Prophage genes
Prophage genes
Prophage genes
Prophage genes
Unknown function
Carbon source transport and metabolism
Signal transduction mechanisms
Central carbon metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism
Transport and metabolism of further metabolites
Coenzyme transport and metabolism
Unknown function
Unknown function
Cell wall/membrane/envelope biogenesis
Respiration and oxidative phosphorylation
Respiration and oxidative phosphorylation
Respiration and oxidative phosphorylation
Respiration and oxidative phosphorylation
Respiration and oxidative phosphorylation
Respiration and oxidative phosphorylation
Unknown function
Transport and metabolism of further metabolites
Central carbon metabolism
Central carbon metabolism
Signal transduction mechanisms
Carbon source transport and metabolism

Carbon source transport and metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism
General function prediction only
General function prediction only
General function prediction only
General function prediction only
General function prediction only
General function prediction only
DNA replication, recombination, repair, and degradation
Central carbon metabolism
General function prediction only
Respiration and oxidative phosphorylation
Amino acid transport and metabolism
Amino acid transport and metabolism
Central carbon metabolism
Protein turnover and chaperones
Transport and metabolism of further metabolites
Carbon source transport and metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism
General function prediction only
Unknown function
Amino acid transport and metabolism; Inorganic ion transport, metabolism, and storage
Inorganic ion transport, metabolism, and storage
Unknown function
Unknown function
Unknown function
Carbon source transport and metabolism
Carbon source transport and metabolism
Carbon source transport and metabolism; Respiration and oxidative phosphorylation
Protein turnover and chaperones
Unknown function
General function prediction only
Unknown function
Unknown function
Inorganic ion transport, metabolism, and storage
Inorganic ion transport, metabolism, and storage
Unknown function
Post-translational modification; Signal transduction mechanisms

Signal transduction mechanisms
Unknown function
Inorganic ion transport, metabolism, and storage
Transport and metabolism of further metabolites
General function prediction only
Transport and metabolism of further metabolites
Carbon source transport and metabolism
General function prediction only
Inorganic ion transport, metabolism, and storage



g and modification