Supplemental material

Table S1. Primers and probes used for RT-qPCR assays in this study.

Gene/ORF	Primer name	Sequence (5´➔3´)	Tm (°C)	Amplicon size (bp)
*crp*P	OD1-RT	GCTGGACAGACGACACTTCAA	57	114
	OR2-RT	CGCATCGCAGGATGACTGTAG	58	
	P-RT123	CCACCGGACTGTACGGGCTATTG	61	
16S RNA[a]	OD-P16S	TCGAAGCAACGCGAAGAAC	53	72
	OR-P16S	CGAAGGCACCAATCCATCTCT	52	
	P-P16S	TACCTGGCCTTGACATGCTGAGA	52	
16S RNA[b]	OD-E16S	TCGATGCAACGCGAAGAAC	53	72
	OR-E16S	CGAAGGCACCAATCCATCTCT	52	
	P-E16S	TACCTGGTCTTGACATCCACAGAAC	58	

a and b, primers and probe used to 16S RNA analysis expression from *P. aeruginosa* or *E. coli,* respectively.

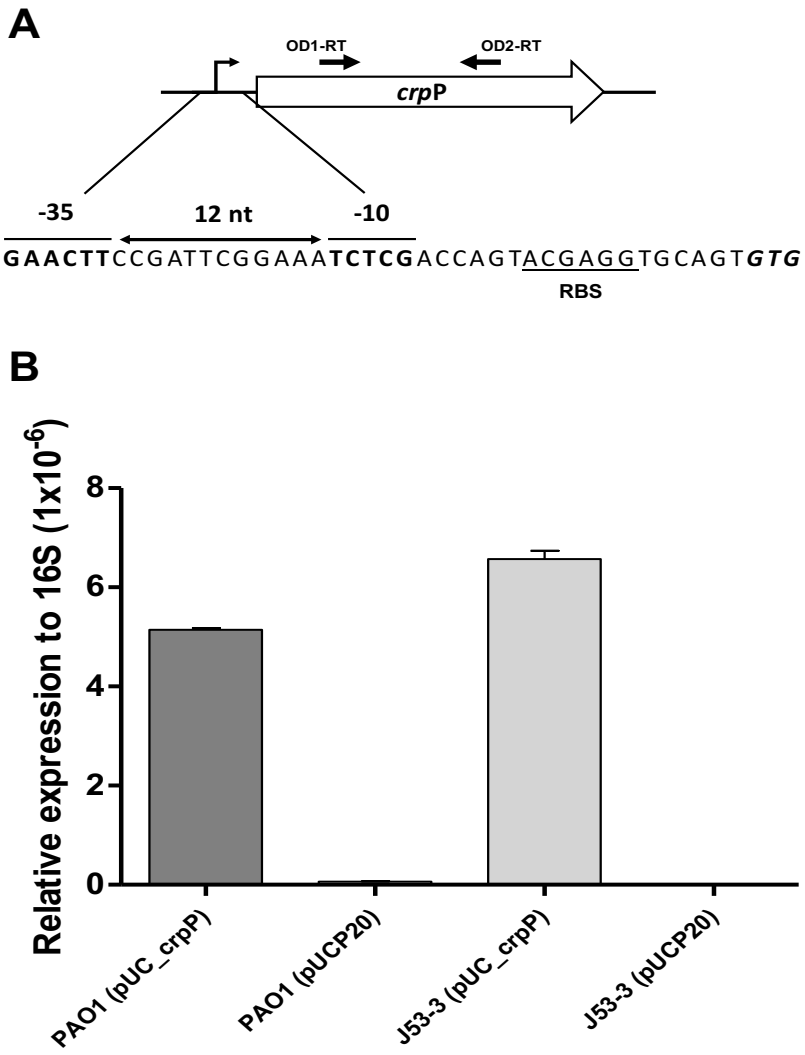

Figure S1. Analysis of functionality of the putative *crp*P promoter. A) The coding region of the *crp*P gene is depicted with an open arrow indicating transcription direction. Black arrows show positions of the oligonucleotides for RT-qPCR assays described in the text. Location of the possible promoter is indicated by (↱). The nucleotides (nt) sequence of the putative promoter is showed. The ribosome binding site (RBS) and the nt that encodes to the initial codon of the protein (bold and italics) are indicated. **B)** Expression levels of *crp*P from cultures of *P. aeruginosa* PAO1 or *E. coli* strains with or without the pUC-*crp*P plasmid determined by RT-qPCR. Values represent the means of two independent determinations in duplicate normalized with respect to transcription of the *16S* gene from *P. aeruginosa* or *E. coli*, respectively.

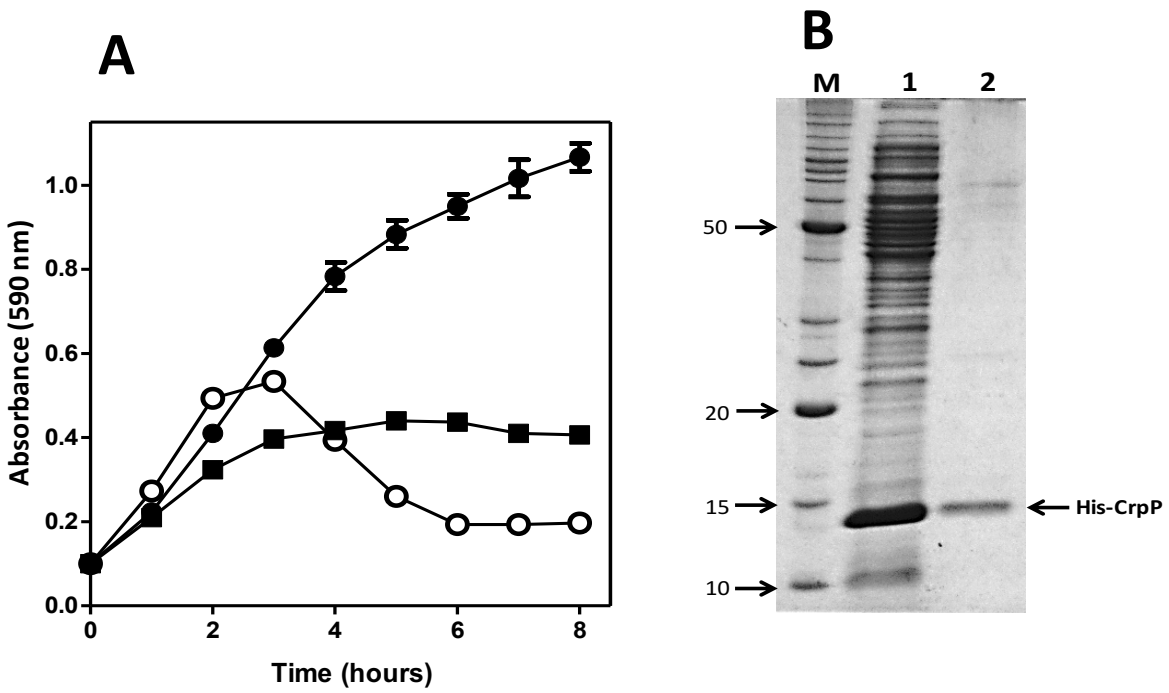

Figure S2. Role of His-CrpP in *E. coli* BL21 susceptibility to CIP. A) Growth of the *E. coli* BL21 (pTrcHis-*crp*P) strain under IPTG-induced (0.5 mM) (●, ■) or non-induced (○) conditions, in the presence (○, ■) or absence (●) of CIP (0.2 µg/mL). The data shown represent the mean and standard error (SE) of four independent duplicate assays. **B)** SDS-PAGE analysis of overexpression and purification of His-CrpP from the *E. coli* BL21*(*pTrcHis-*crp*P) strain. Lane 1, lysate from cells induced with 0.5 mM IPTG; 2, purification of His-CrpP. M, Molecular mass markers are shown on the left (in kDa).

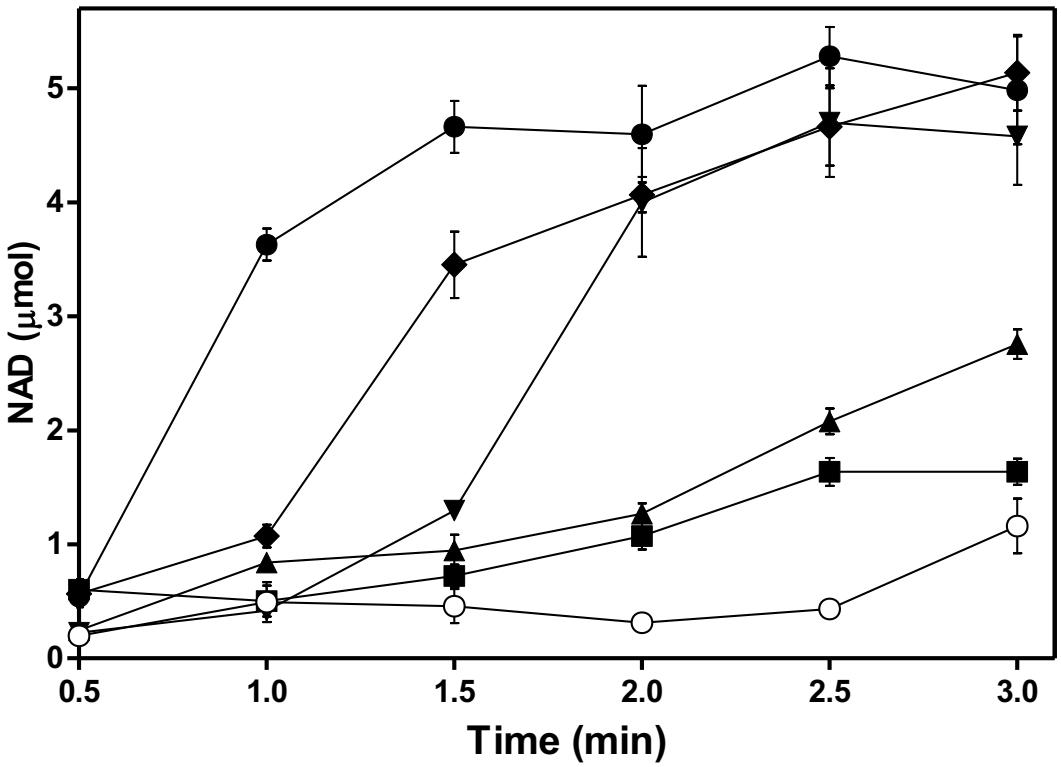

Figure S3. Comparison of the NADH-oxidation rate of native or denatured His-CrpP. NADH oxidation was measured in a reaction mix containing 2.0 mM CIP in the presence of 1.0 (■), 2.5 (▲), 5.0 (▼), 7.5 (◆), or 10 (●) μg/mL native or (○) 10 μg/mL denatured His-CrpP protein, as described in the Materials and Methods. The data shown represent the means and SEs of four independent duplicate assays.

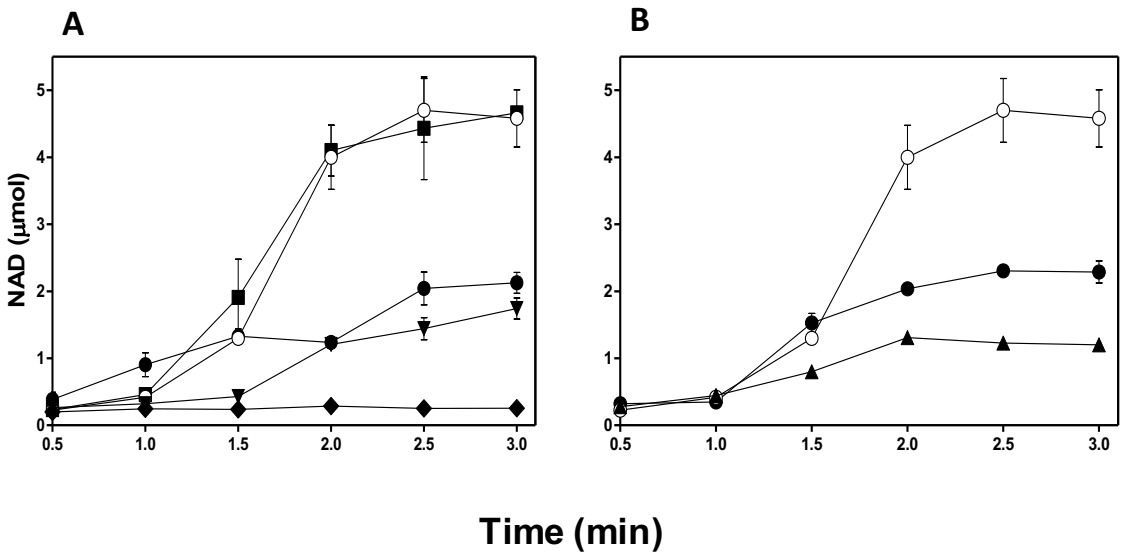

Figure S4. Effect of pH and Mg^{2+} on His-CrpP activity. CIP (2.0 mM) was incubated in the presence of 5.0 µg/mL of His-CrpP protein at different pH levels or $MgCl_2$ concentrations, at 37ºC for 15 min. NADH oxidation was measured spectrophotometrically at 390 nm, as described in the Materials and Methods section. **A)** Effect of pH on the NADH-oxidation rate. pH values are indicated with symbols: (●), 6.0; (■), 6.5; (○),7.0 (▼), 7.5, and (◆), 8.0. **B)** Effect of the $MgCl_2$ concentration on the NADH-oxidation rate. $MgCl_2$ concentrations are indicated with symbols: (●), 5.0; (○), 10 or (▲), 15 mM. The data shown represent the mean and SEs of four independent duplicate assays.

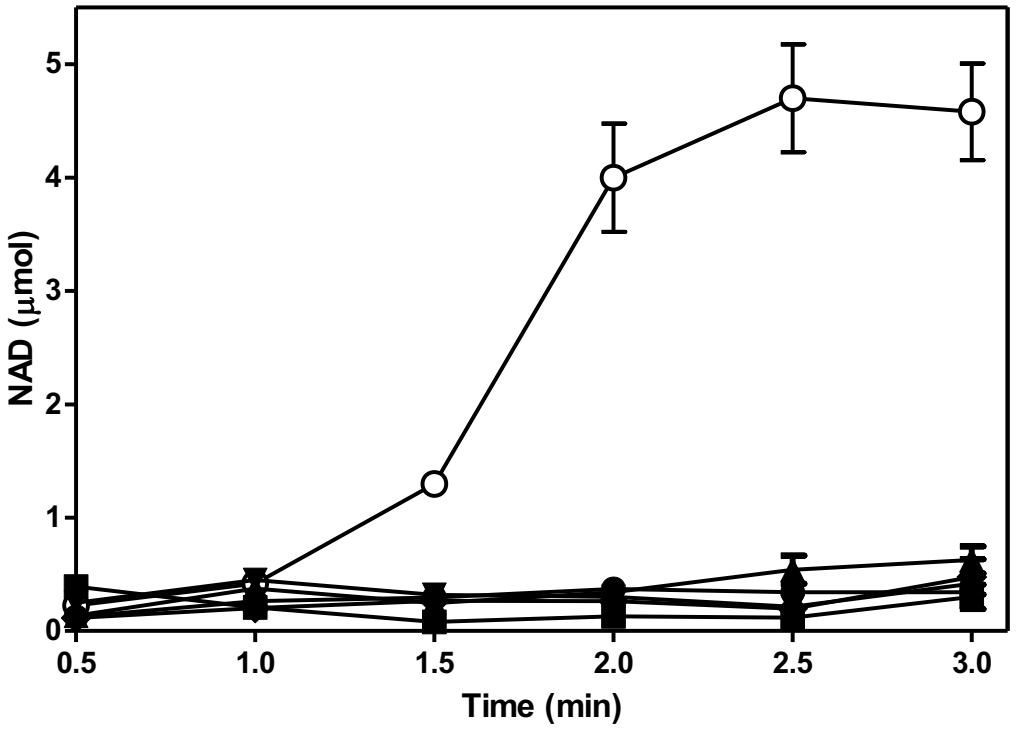

Figure S5. NADH-oxidation rate comparisons by His-CrpP on quinolones and aminoglycosides. NADH oxidation was measured in reaction mixtures containing 1 mM of each antibiotic in the presence of 5.0 μg/mL His-CrpP protein, as described in the Materials and Methods section. Symbols: (○), CIP; (▲), LVX; (▼), MXF; (●), NAL; (■), KAN and (◆), STR. The data shown represent the mean and SEs of four independent duplicate assays.

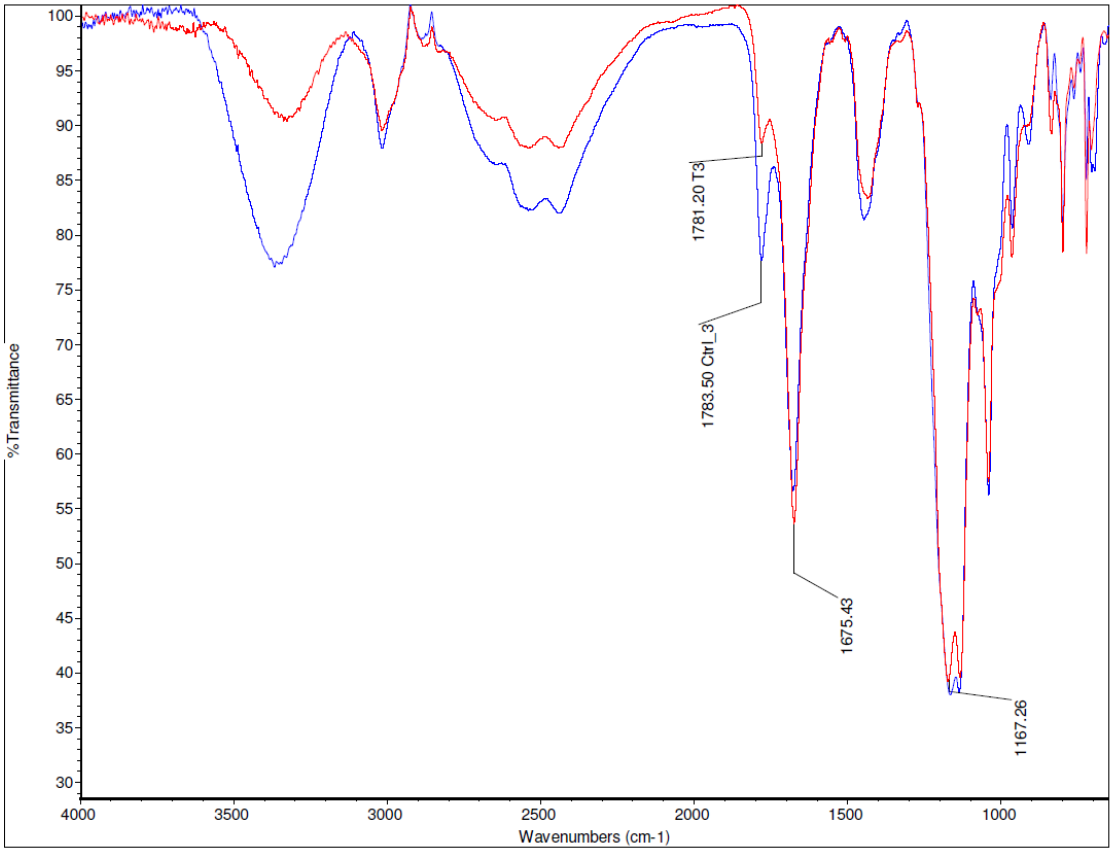

Figure S6. Infrared spectroscopy analysis of the modification of CIP by His-CrpP. Representative spectroscopy analysis of CIP after its incubation in the presence (red) or absence (blue) of 5.0 µg/mL His-CrpP protein in a reaction mixture containing 2.0 mM Mg-ATP, as described in the Materials and Methods section.

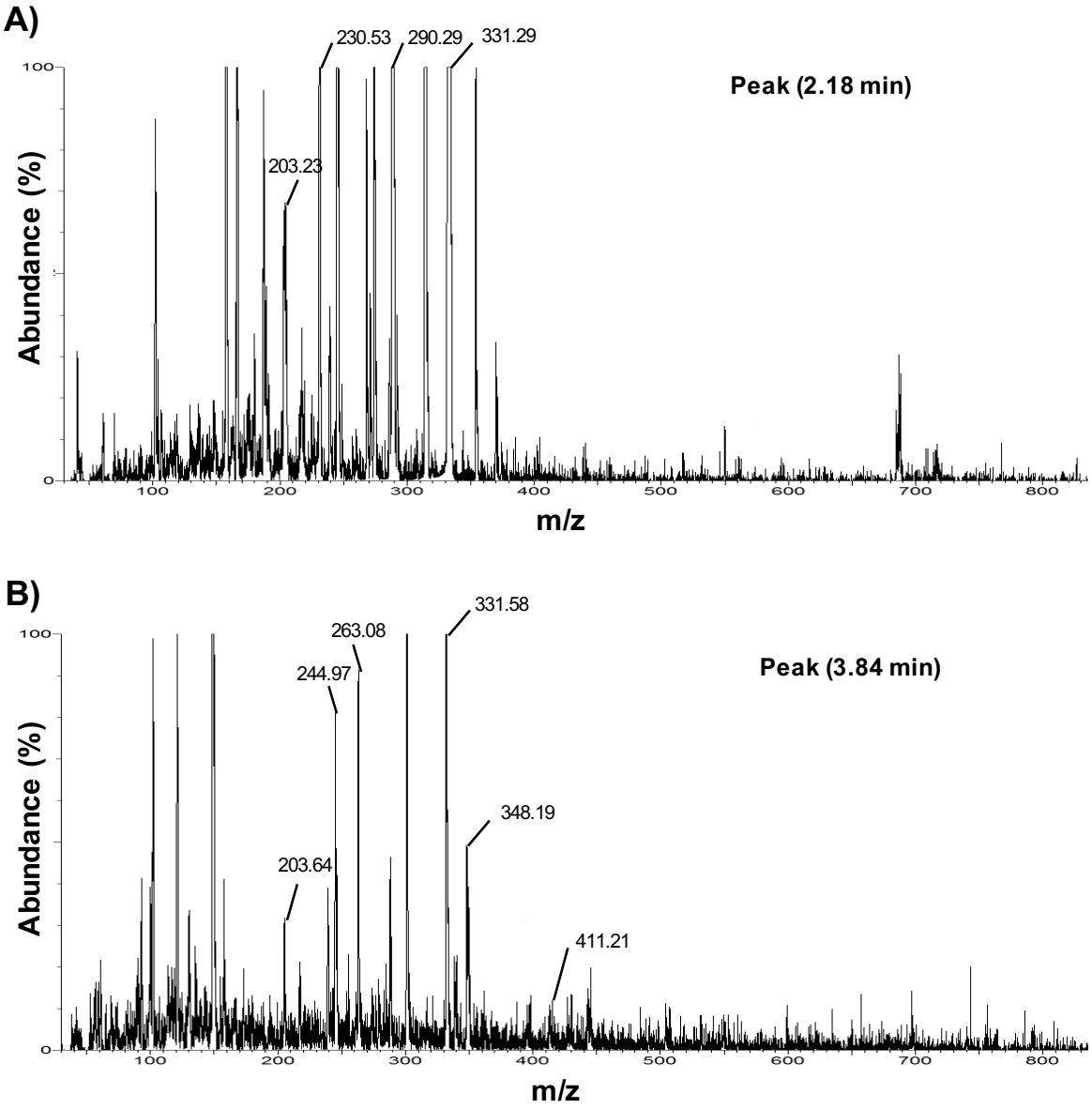

Figure S7. Fragment ion spectra of the retention pikes in LC-MS/MS metabolite analysis (CIP modification by His-CrpP). CIP inactivation by His-CrpP; representative mass-fragmentation profile corresponding to peaks with retention times of 2.18 **(A)** and 3.84 **(B)** min.