**Supporting Information for**

# Regulation of CsrB/C sRNA decay by EIIA$^{Glc}$ of the phosphoenolpyruvate: carbohydrate phosphotransferase system

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## Supplementary experimental procedures

*Gel mobility shift assay*

RNAs (CsrB, CsrC, *rpsT* and GlmZ) were synthesized and end-labeled as previously described (Suzuki *et al.*, 2006). RNAs were gel purified, suspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and renatured by heating to 70°C for 5 min and slow cooling to room temperature. Binding reactions (10 µl) contained 10 mM Tris-HCl, pH7.5, 125 mM KCl, and 2 mM $MgCl_2$, 32.5 ng of yeast RNA, 7.5% glycerol, 1 mM dithiothreitol (DTT), 4 U of RNase inhibitor (Ambion), 0.6nM CsrB RNA, purified MBP-tagged $CsrD^{\Delta TM}$ and/or His-tagged $EIIA^{Glc}$ and 0.1 mg $ml^{-1}$ xylene cyanol. For competition studies, assays were carried out with unlabeled RNA competitors (CsrC, *rpsT* and GlmZ). Reaction mixtures were incubated for 30 min at 37°C to allow protein–RNA complex formation. Samples were then fractionated on 5% native Bis-Tris gels. Radioactive bands were visualized and quantified using a phosphorimager and Image Quant software.

## Supplementary figures

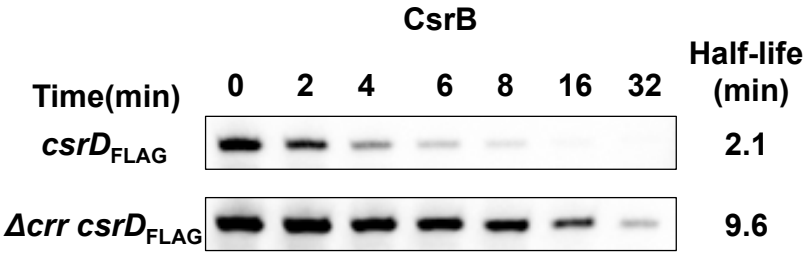


Figure S1. EIIA$^{Glc}$ stimulates CsrB decay in the MG1655 *csrD$_{FLAG}$* strain.

Northern blots depicting the effect of EIIA$^{Glc}$ on CsrB decay in strains MG1655 *csrD$_{FLAG}$* and MG1655 *Δcrr* csrD$_{FLAG}$. Half-lives were determined as in Figure 1.

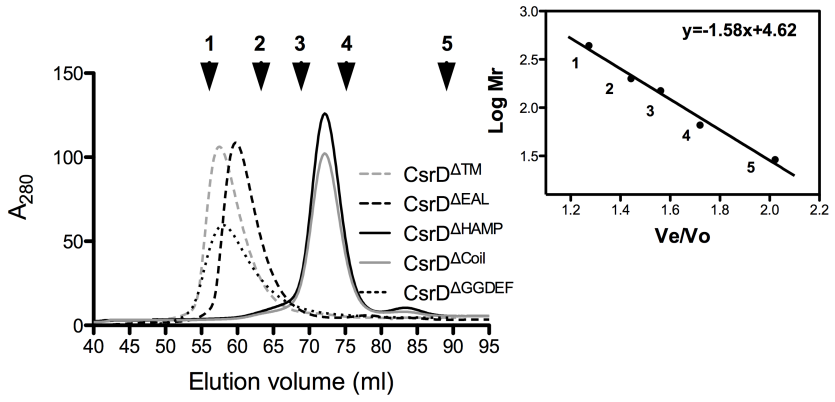


Figure S2. Gel filtration chromatography of CsrD variants.

Each CsrD variant (1 mg) was passed through a Superdex 200 column (HiLoad$^{TM}$ 16/60, 120 ml). Arrows indicate elution volumes of molecular weight markers used to calibrate the column (Experimental procedures).

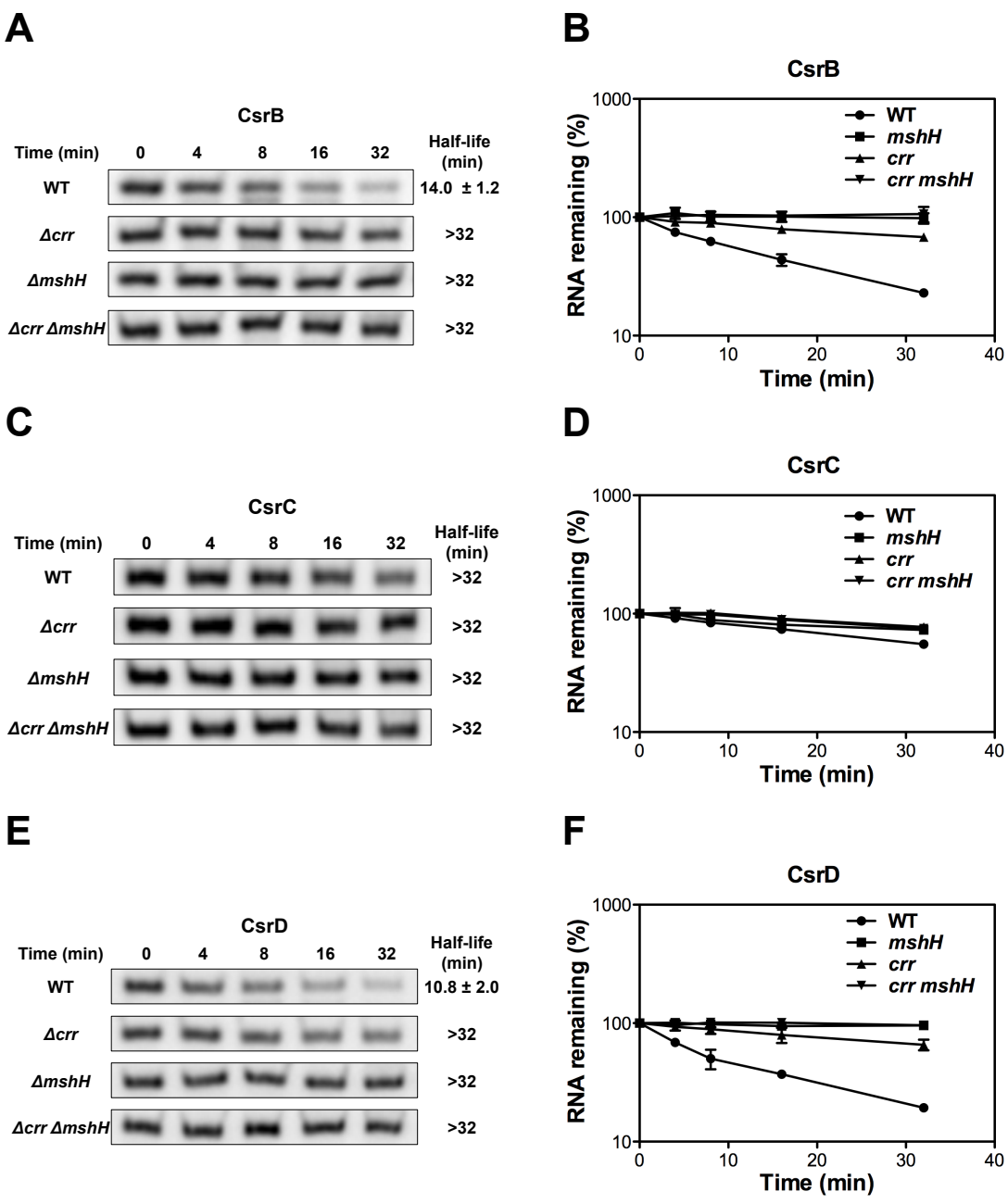


Figure S3. EIIA$^{Glc}$ and MshH (CsrD ortholog) affect CsrB and CsrD decay in *V. cholerae*.

A, C and E. Decay rates of CsrB/C/D sRNAs were determined by Northern blotting, after rifampicin addition (time points indicated) to 27°C exponentially growing ($OD_{600}$ ~0.5) cultures of *V. cholerae* 01 EI Tor (WT), *Δcrr*, *ΔmshH* and *Δcrr ΔmshH* strains. The RNA half-lives and standard derivations from duplicate experiments were determined as in Figure 1.

B, D and F. Quantification of signals obtained from two independent experiments, as presented in panels A, C and E. Standard deviations are indicated.

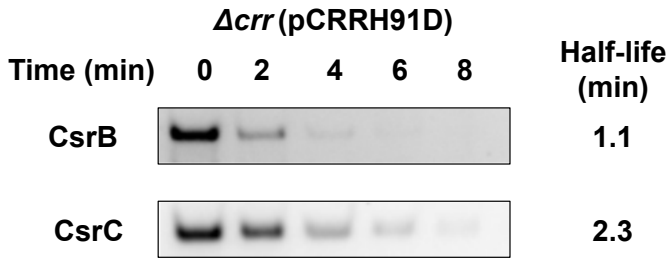


Figure S4. Decay of CsrB/C in a strain expressing $EIIA^{Glc}$H91D is similar to that of $EIIA^{Glc}$ H91A.

Decay rates of CsrB/C were determined in the *Δcrr* strain containing plasmid pCRRH91D, as described for the pCRRH91A-containing strain in Figure 1A and C.

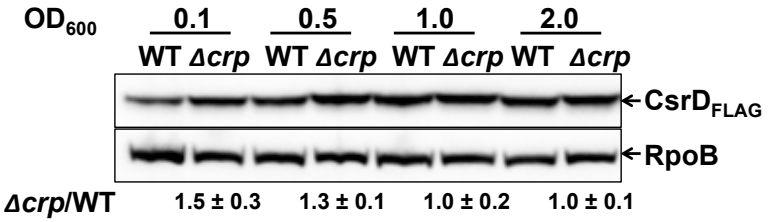


Figure S5. CRP has minimal or no effect on CsrD protein levels.

Western blots depicting effects of *crp* deletion on CsrD protein levels. RpoB was used as loading control. CsrD protein levels in *Δcrp* relative to those in the wild-type strain (WT) were given. Standard derivations from triplicate experiments are indicated.

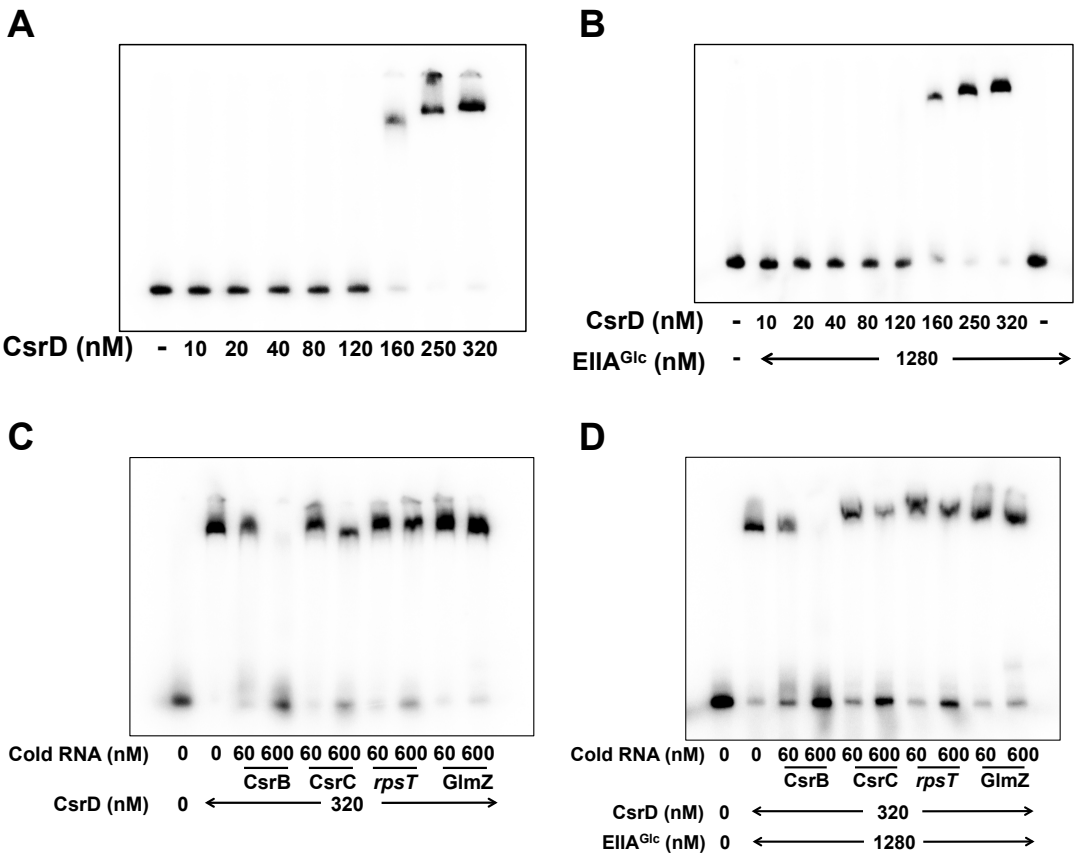

Figure S6. $EIIA^{Glc}$ has no effect on the binding of CsrD to CsrB RNA *in vitro*.

A and B. EMSA experiments were carried out with 0.6 nM labeled CsrB and increasing concentrations of CsrD (0-320 nM) in the absence or presence of $EIIA^{Glc}$.

C and D. Competition assays were performed using 0.6 nM labeled CsrB with indicated concentration of CsrD and $EIIA^{Glc}$ in the absence or presence of 100-fold and 1,000-fold molar excess of unlabeled competitor sRNAs, CsrB, CsrC, and GlmZ and mRNA, *rpsT*.

**Supplementary tables**

Table S1. Bacterial Strains

| Strain | Description | Reference |
|---|---|---|
| MG1655 | Prototrophic E. coli K12 | Michael Cashel |
| MG1655 *Δcrr* | MG1655 with unmarked *crr* deletion | This study |
| MG1655 *ΔcsrD* | MG1655 with unmarked *csrD* deletion | This study |
| MG1655 *Δcrp* | MG1655 with marked *crp* disruption-$Cam^r$ | This study |
| MG1655 *ΔcyaA* | MG1655 with marked *cyaA* deletion-$Kan^r$ | This study |
| MG1655 *Δcrr ΔcsrD* | MG1655 with unmarked *crr* deletion and marked *csrD* deletion-$Kan^r$ | This study |

| | | |
|---|---|---|
| MG1655 *Δcrr Δcrp* | MG1655 with unmarked *crr* deletion and marked crp disruption-Cam$^r$ | This study |
| MG1655 *Δcrr ΔcyaA* | MG1655 with unmarked *crr* deletion and marked *cyaA* deletion-Kan$^r$ | This study |
| MG1655 *csrD*$_{FLAG}$ | MG1655 with in-frame, CTD 3X-FLAG tag at native *csrD* locus | This study |
| MG1655*Δcrr csrD*$_{FLAG}$ | *csrD*$_{FLAG}$ allele introduced by transduction using P1vir - Kan$^r$ | This study |
| MG1655 *Δcrp csrD*$_{FLAG}$ | *csrD*$_{FLAG}$ allele introduced by transduction using P1vir - Kan$^r$ | This study |
| MG1655 *crr*$_{FLAG}$ | MG1655 with in-frame, CTD 3X-FLAG tag at native *crr* locus | This study |
| MG1655 H91A | MG1655 with a His91Ala exchange (CAC to GCC) at native *crr* locus | This study |
| MG1655 H91A *crr*$_{FLAG}$ | *crr*$_{FLAG}$ allele introduced by transduction using P1vir | This study |
| BL21(DE3) | Host for expression | (Studier & Moffatt, 1986) |
| DE5α | Host for plasmid amplification | (Woodcock *et al.*, 1989) |
| PW1096 | *Vibrio cholerae* C6706str2 | (Thelin & Taylor, 1996) |
| PW1197 | PW1096 with in frame *mshH* deletion | This study |
| PW1198 | PW1096 with in frame *crr* deletion | This study |
| PW1207 | PW1096 with in fram *mshH* | This study |

| | |
|---|---|
| | and *crr* deletions |

Table S2. Plasmids and related primers

| Name | Description | Relevant Primers | Reference |
|---|---|---|---|
| pBR322 | Cloning vector - $Amp^r$ $Tet^r$ | N/A | (Bolivar *et al.*, 1977) |
| pCRR | pBR322 derivative carrying *crr* gene between EcoRI and HindIII sites- $Amp^r$ | P1/P2 | This study |
| pCRRH91A | pCRR derivative carrying *crr* with a His91Ala exchange (CAC to GCC) | P1/P17/P2 | This study |
| pCRRH91D | pCRR derivative carrying *crr* with a His91Asp exchange (CAC to GAC) | P1/P19/P2 | This study |
| pBYH4 | pBR322 derivative carrying *csrD* gene in EcoRI site- $Amp^r$ | N/A | (Suzuki et al., 2006) |
| pET24CRR | pET24a derivative carrying *crr* gene between NdeI and XhoI sites | P3 /P4 | This study |
| pΔTM | pMAL-c5x derivative carrying DNA encoding 156-646 aa of CsrD between NcoI and EcoRI sites | P5/P10 | This study |
| pΔEAL | pMAL-c5x derivative carrying DNA encoding 156-385 aa of CsrD between NcoI and EcoRI | P6/P7 | This study |

| | | | |
|---|---|---|---|
| | sites | | |
| pΔGGDEF | pMAL-c5x derivative carrying DNA encoding 156-223 and 393-646 aa of CsrD between NcoI and EcoRI sites | P6/P8 and P9/P10 | This study |
| pΔHAMP | pMAL-c5x derivative carrying DNA encoding 192-646 aa of CsrD between NcoI and EcoRI sites | P11/P10 | This study |
| pΔcoil | pMAL-c5x derivative carrying DNA encoding 156-199 and 220-646 aa of CsrD between NcoI and EcoRI sites | P6/P12 and P13/P10 | This study |
| pEAL | pMAL-c5x derivative carrying DNA encoding 393-646 aa of CsrD between NcoI and EcoRI sites | P14/P10 | This study |
| pKOV | Vector for homologous recombination | N/A | (Link *et al.*, 1997) |
| pKOVH91A | pKOV derivative carrying *crr* with a His91Ala exchange (CAC to GCC) between NotI and BamHI sites | P15/P17and P18/ P16 | This study |

Table S3. Primers

| Primer # | Sequence (5'-3') | Function |
|---|---|---|

| | | |
|---|---|---|
| P1 | CAGTACCAGGAATTCTTTACACTTTATGCTTCCGGCTCGTATATTGTGTGGAAGAAATAATTTTGTTTAACTTTAAG | pCRR and pH91D construction |
| P2 | CAGGACCATAAGCTTTTACTTCTTGATGCGGATAACCGGGGT | pCRR construction |
| P3 | ACATGATCTCATATGGGTTTGTTCGATAAACTGAAATCTC | pET24CRR construction |
| P4 | ACATGATCTCTCGAGCTTCTTGATGCGGATAACCGGGGTT | pET24CRR construction |
| P5 | CCGATTCCATGGGCCGCTGGTTACAACGGCAACTTGCCG | pDTM construction |
| P6 | CATGCCATGGGCCGCTGGTTACAACGGCAACTTGC | pDEAL, pDGGDEF and pDcoil construction |
| P7 | ACCGGAATTCTTAGTAAATAGCCCAGCTATTGCCGC | pDEAL construction |
| P8 | AACATTACCGCGTCCATAAGAGCGGATCAG | pDGGDEF construction |
| P9 | CTGATCCGCTCTTATGGACGCGGTAATGTT | pDGGDEF construction |
| P10 | ACCGGAATTCTTAAACCGAGTATCTTTGTGAATAT | pDTM, pDGGDEF, pDHAMP, pDcoil and pEAL construction |
| P11 | CATGCCATGGGCCCGCCCAGAACCAGCAGTGC | pDHAMP construction |
| P12 | GGCGGCATAAGAGCGGATCAGCGCACTGCTGGTTCT | pDcoil construction |
| P13 | AGAACCAGCAGTGCGCTGATCCGCTCTTATGCCGCC | pDcoil construction |
| P14 | CATGCCATGGGCGGACGCGGTAATGT | pEAL construction |

| | | |
|---|---|---|
| | TCGCTGGCGTA | |
| P15 | ATAAGAATGCGGCCGCAAAGATCTGC CAGCTATTACGCTGG | pKOVH91A construction |
| P16 | CGCGGATCCTGATAGCCGATTTGACT GCCAGAAT | pKOVH91A construction |
| P17 | GGTGTCGATACCGAAGGCGACGAACA GTTCAAC | pCRRH91A and pKOVH91A construction |
| P18 | GTTGAACTGTTCGTCGCCTTCGGTAT CGACACC | pKOVH91A construction |
| P19 | GGTGTCGATACCGAAGTCGACGAACA GTTCAAC | pCRRH91D construction |
| S1 | CGTAACCGTGGGTGAAACCCCGGTTA TCCGCATCAAGAAGGACTACAAAGAC CATGACGG | MG1655 $crr_{FLAG}$ construction |
| S2 | AAATGGCGCCGATGGGCGCCATTTTT CACTGCGGCAAGAACATATGAATATC CTCCTTAG | MG1655 $crr_{FLAG}$ construction |
| S3 | TGATACTAACGTGAAAAAATATTCACA AAGATACTCGGTTGACTACAAAGACC ATGACGG | MG1655 $csrD_{FLAG}$ construction |
| S4 | AGCGCGCATTATTCTACGTGAAAACG GATTAAACGGCAGGCATATGAATATC CTCCTTAG | MG1655 $CsrD_{FLAG}$ construction |
| R1 | GTAATACGACTCACTATAGTCGACAG GGAGTCAGACAAC | RNA CsrB synthesis |
| R2 | AAAAAAAGGGAGCACTGTATTCACAG CGCTCCCGGTTCGTTTCGCAG | RNA CsrB synthesis |
| R3 | GTAATACGACTCACTATAGGATAGAG CGAGGACGCTAACAGGAAC | RNA CsrC synthesis |
| R4 | AAGAAAAAAGGCGACAGATTACTCTG | RNA CsrC synthesis |

| | | |
|---|---|---|
| | TCGCCTTTTTTCCTGACTC | |
| R5 | GTAATACGACTCACTATAGGCCTTTGA ATTGTCCATATAGAACAC | RNA *rpsT* synthesis |
| R6 | AAAAAAACCCGCTTGCGCGGGCTTTT TCACAAAGCTTCAGC | RNA *rpsT* synthesis |
| R7 | TAATACGACTCACTATAGGGTAGATGC TCATTCCATCTC | RNA GlmZ synthesis |
| R8 | AAAAAAACGCCTGCTCTTATTACGGAG CAGGCGTTAAAAC | RNA GlmZ synthesis |
| RP1 | TAATACGACTCACTATAGGGTAAAAGG TGCTCCCTGCATCTAATC | *V. cholerae* CsrB probe synthesis |
| RP2 | TGGTGATCTTCAGGAAGAAGAATCG | *V. cholerae* CsrB probe synthesis |
| RP3 | TAATACGACTCACTATAGGGATCCTTT CAGCGAACTCCGAGCATC | *V. cholerae* CsrC probe synthesis |
| RP4 | CAGGATGAGAAGTGGTGAGGATGAC | *V. cholerae* CsrC probe synthesis |
| RP5 | TAATACGACTCACTATAGGGCAATCCC GCTACTAATAGGTGCTCC | *V. cholerae* CsrD probe synthesis |
| RP6 | CAAGGATTGGTCATCTTCAGGACGA | *V. cholerae* CsrD probe synthesis |
| RP7 | GTAATACGACTCACTATAGGTTCGTTT CGCAGCATTCCAG | *E. coli* CsrB probe synthesis |
| RP8 | GCGTTAAAGGACACCTCCAGG | *E. coli* CsrB probe synthesis |
| RP9 | GTAATACGACTCACTATAGGTCTTACA ATCCTTGCAGGC | *E. coli* CsrC probe synthesis |
| RP10 | GAGGACGCTAACAGGAACAATG | *E. coli* CsrC probe synthesis |

Table S4. Molecular weight of CsrD in solution

| Protein | Molecular Mass (kDa) | | Quaternary structure[c] |
|---|---|---|---|
| | Calculated[a] | Experimental[b] | |
| $CsrD^{\Delta TM}$ | 99.3 | 359 | Tetramer |
| $CsrD^{\Delta HAMP}$ | 95.1 | 107 | Monomer |
| $CsrD^{\Delta Coil}$ | 97.0 | 107 | Monomer |
| $CsrD^{\Delta GGDEF}$ | 80.3 | 344 | Tetramer |
| $CsrD^{\Delta EAL}$ | 69.4 | 296 | Tetramer |

a. Molecular mass of the protein calculated from the primary sequence.

b. Molecular mass determined using size exclusion chromatography.

c. Deduced quaternary structure.

## References


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