Curli synthesis and biofilm formation in enteric bacteria are controlled by a dynamic small RNA module made up of a pseudoknot assisted by an RNA chaperone.

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Supplemental Material

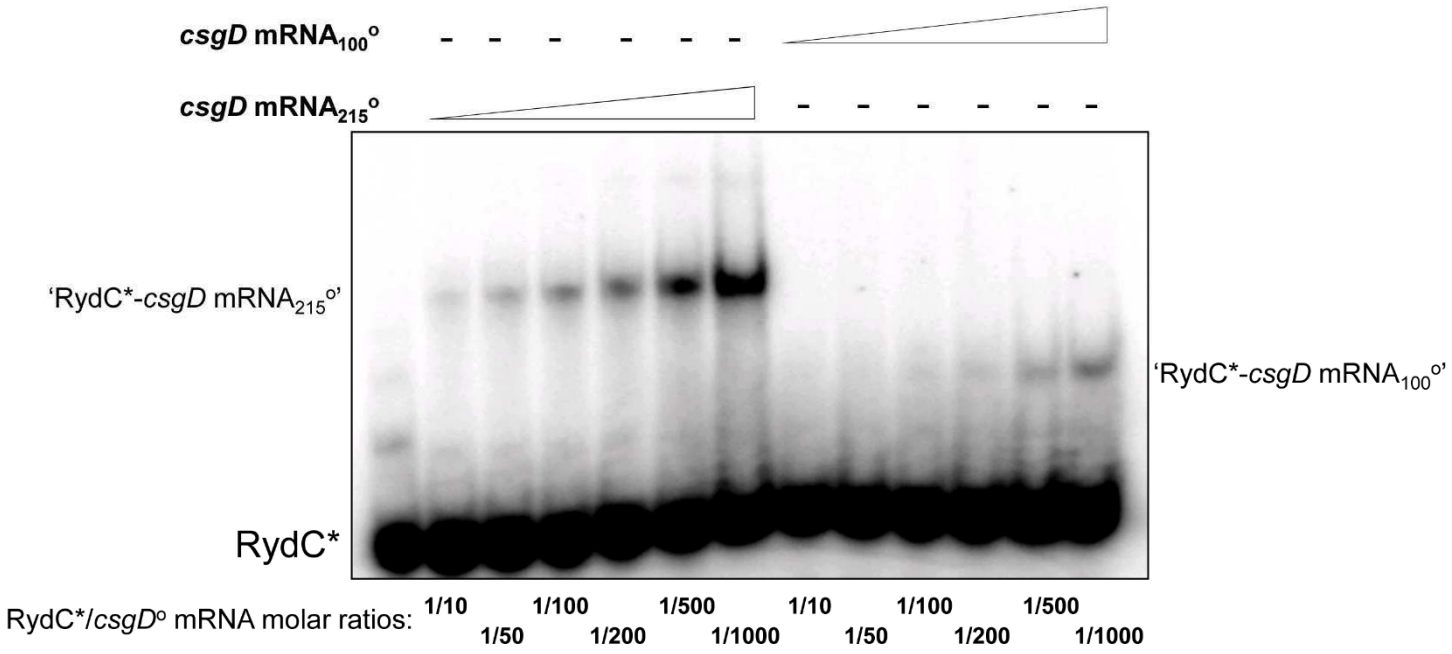

Figure S1. Complex formation between RydC and two *csgD* mRNA fragments of different lengths. *csgD* $mRNA_{100}$ and *csgD* $mRNA_{215}$ correspond to 100 and 215 nts from the *csgD* mRNA 5'-end, respectively. Native gel retardation assays of purified labelled RydC with increasing amounts of unlabelled *csgD* $mRNA_{215}$ and *csgD* $mRNA_{100}$ (10 to 1000-fold more than RydC) in the absence of the Hfq protein. This indicates that in the absence of the Hfq protein, the affinity between the two RNAs is weak.

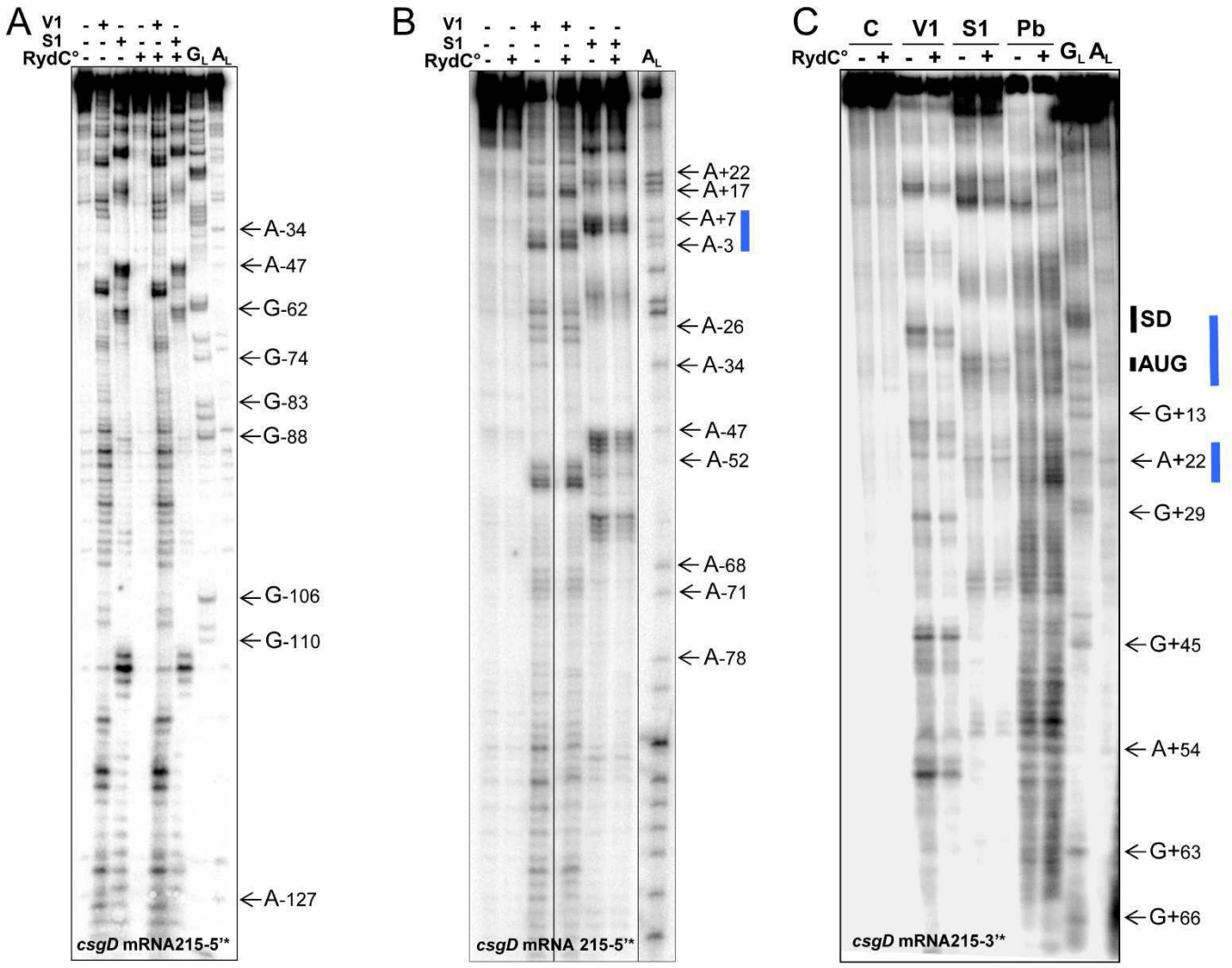

Figure S2. Structural analysis of the conformational changes in *csgD* mRNA$_{215}$ induced by complex formation with RydC. Autoradiograms of the cleavage products of 5'- (**A, B)** or 3'-labelled (**C**) *csgD* mRNA$_{215}$ by RNases V_1 (5.10^{-5} unit) and nuclease S_1 (2 units) in the presence or absence of unlabelled RydC at a 1:100 molar ratio. Lanes G_L, RNase T_1 hydrolysis ladder; lanes A_L, RNase U_2 hydrolysis ladder. The RNA sequences are indexed on the right sides of each panel. The conformational changes of the *csgD* mRNA upon complex formation with RydC are indicated with vertical blue bars. The SD sequence and AUG initiation codon of the mRNA are also shown.

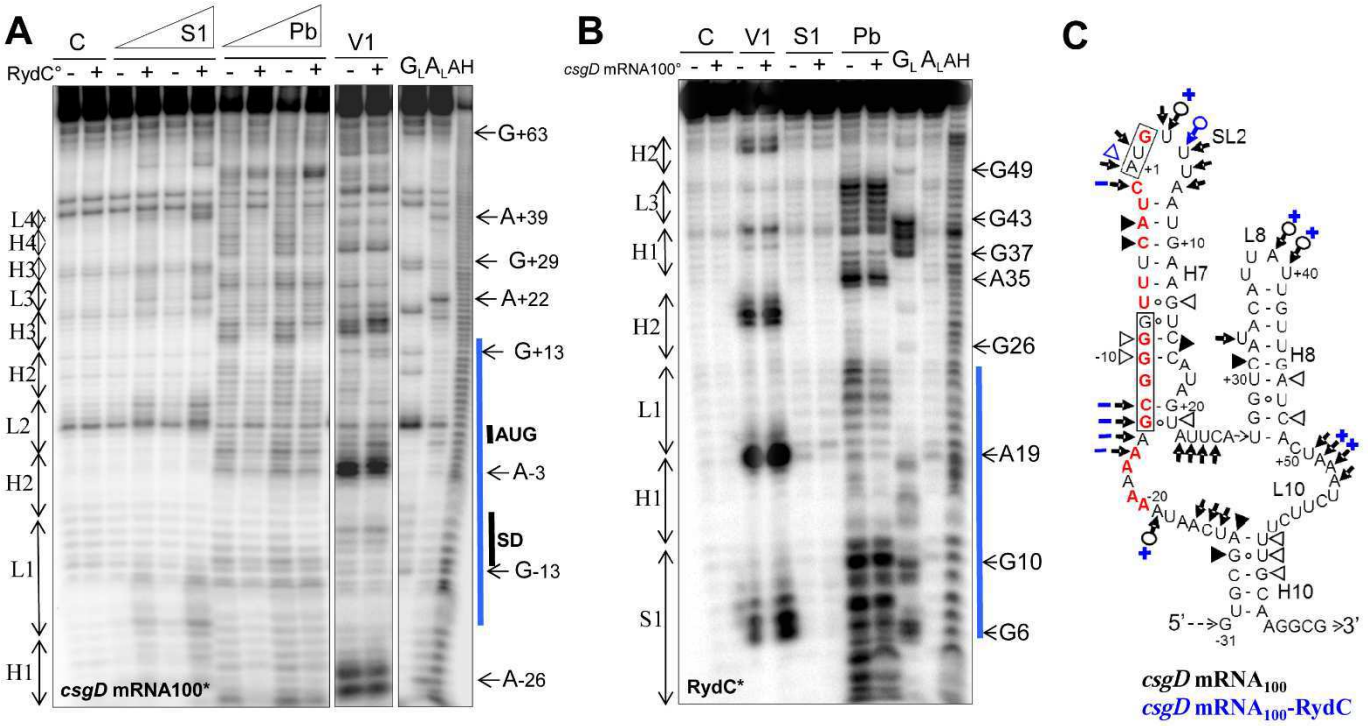

Figure S3.**Structural analysis of the conformational changes of RydC and *csgD* mRNA$_{100}$ induced by complex formation with *csgD* mRNA$_{100}$ and RydC, respectively**. **A, B.** Autoradiograms of cleavage products of 5'-labelled *csgD* mRNA$_{100}$ (100 nts-long) (A) or 5'-labelled RydC (B) by RNases V_1 (5.10^{-5} unit), nuclease S_1 (0.5 and 1 unit) and lead acetate (0.5 and 1 mM) in the presence or absence of either unlabelled RydC (A) or unlabelled *csgD* mRNA$_{100}$ (B) at 1:100 molar ratios. The *csgD* mRNA$_{100}$ or RydC structural domains are indicated on the left sides of each panel. Upon complex formation, the conformational changes of *csgD* mRNA$_{100}$ or RydC are highlighted by vertical blue bars. **C.** Secondary structure of the *csgD* mRNA$_{100}$ inferred from the probing results, which support the proposed model. Triangles are V_1 cuts; arrows capped by a circle are S_1 cuts; uncapped arrows are lead cuts. The cut and cleavage intensities are proportional to the darkness of the symbols. The structural domains are indicated and the AUG and SD sequences are outlined. The red nucleotides are those proposed to interact with RydC. Structural changes detected in the *csgD* mRNA$_{100}$ upon RydC complex formation are in blue.

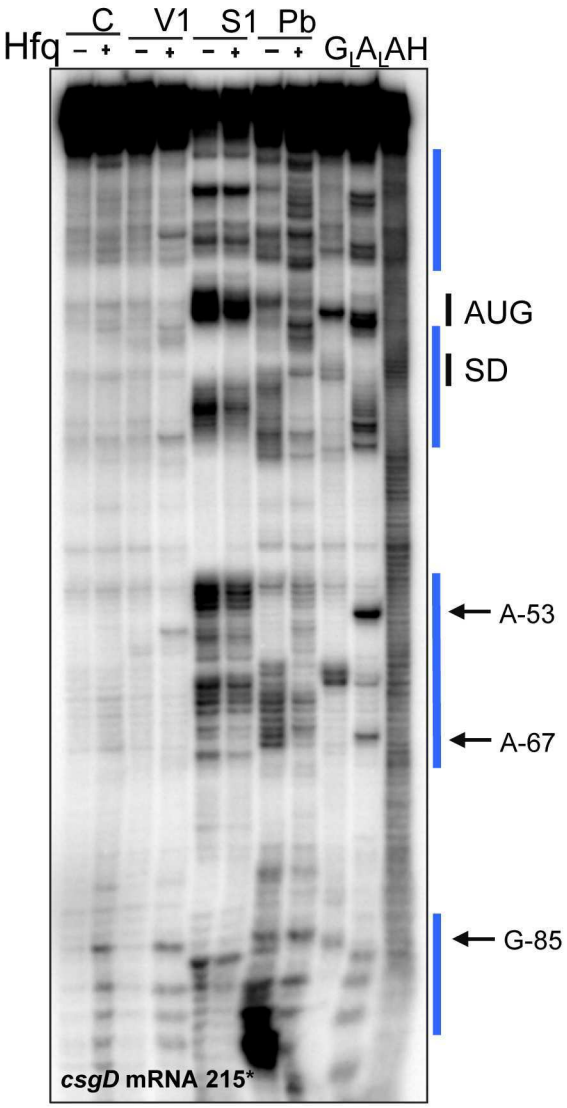

Figure S4. Structural analysis of the conformational changes of *csgD* mRNA$_{215}$ induced by complex formation with Hfq. Autoradiograms of the cleavage products of 5'*csgD*mRNA$_{215}$ by RNases V_1 (15.10^{-5} unit), nuclease S_1 (0.5 units), and lead acetate (1 mM) in the presence or absence of Hfq at a 1:20 molar ratio. Lanes G_L, RNase T_1 hydrolysis ladder; lanes A_L, RNase U_2 hydrolysis ladder. The RNA sequences are indexed on the right sides of the panels. Upon complex formation with Hfq, the conformational changes in *csgD* mRNA are emphasized by the vertical blue bars. The SD sequence and AUG initiation codon of the mRNA are indicated

Table S1. Strains used and constructed in this study.

Strain	Description	Source,Reference
E. coli MG1655Z1	Z1(lacR tetR SpR)	(4)
E. coli MG1655Z1 Δ*rydC*	Z1(lacR tetR SpR) ΔRNA1114::Cm	(4)
E. coli MG1655Z1 pUC18	MG1655Z1 + pUC18	(4)
E. coli MG1655Z1 pUC18-*rydC*	MG1655Z1 + pUC18-RNA1114	(4)
S enterica subsp. bongori+pUC18	*Salmonella enterica subsp. bongori* + pUC18	This study
S. enterica subsp. bongori+pUC18-*rydC*	*Salmonella enterica subsp. bongori* + pUC18-RNA1114	This study
S. sonnei+pUC18	*Shigella sonnei* + pUC18	This study
S. sonnei+pUC18-*rydC*	*Shigella sonnei* + pUC18-RNA1114	This study

Table S2. DNA oligodeoxyribonucleotides used in this study.

Names	Sequences (5'→ 3')	Purposes
csgD215rev	CGCCTGCAAAGAAGATTTAGT	*csgD* $mRNA_{215}$ transcription toeprint *csgD* $mRNA_{100}$ transcription, *csgD* $mRNA_{115}$ transcription
csgD215for	TAATACGACTCACTATAGGATGTAATCCATTAGTTTTATATTTTACCC	*csgD* $mRNA_{215}$ transcription
csgD100for	TAATACGACTCACTATAGGGTGCGATCAATAAAAAAAGCGGGGTTTCAT	*csgD* $mRNA_{100}$ transcription
csgD503	TTGCAACCCTTAATTGACAACACGTTCTTGAT	*csgD* $mRNA_{503}$ transcription
csgDΔ5'UTRfor	TAATACGACTCACTATAGGTTTAATGAAGTCCATAGTA	*csgD* $mRNA_{\Delta 5'UTR}$
csgD115rev	ACCTGACAGCTGCCTCTAAAA	*csgD* $mRNA_{115}$ transcription
csgDnorth	CAATGTCGCGGTACGGGTAATCTTCAGGCGTATTTAGCAA	*csgD* mRNA northern
RydCfor	CGGGATCCTAATACGACTCACTATAGGGCTTCCGATGTAGACCCGTT	RydC transcription
RydCrev	AAGAAAACGCCTGTACTAAAAC	RydC transcription
RydCnorth	ACCGACCCGTGGTACAGGCG	RydC northern
$RydC_{\Delta 5'}$for	TAATACGACTCACTATAGGCCTGTACCACGGGTCGGTTTTAGTACAGGCGTTTTCTT	RydC transcription
$RydC_{\Delta 5'}$rev	AAGAAAACGCCTGTACTAAAACCGACCCGTGGTACAGGCCTATAGTGAGTCGTATTA	$RydC_{\Delta 5'}$ transcription
tmRNAnorth	GTTTTAACGCTTCAACCCCA	tmRNA northern
5Snorth	CTTCTGAGTTCGGCATGGGC	5S rRNA northern
csgBAnorth	AACTGCAGCACCGTTGCCACCACCGAACTGTTTAACCGTCATTTAAT	*csgBA* mRNA northern

$RydC_{H1}$for	GAAATTAATACGACTCACTATAGGCTTCCGATGTAGACCCGTATTCTT CGCCTGTACCTGCCCAGGGTTTTAGTACAGGCGTTTTCTT	$RydC_{H1}$ transcription
$RydC_{H1}$rev	AAGAAAACGCCTGTACTAAAACCCTGGGCAGGTACAGGCGAAGAAT ACGGGTCTACATCGGAAGCCTATAGTGAGTCGTATTAATTTC	$RydC_{H1}$ transcription
$RydC_{H2}$for	GAAATTAATACGACTCACTATAGGCTTCCGATGTACTGGGCAATTCTT CGCCTGTACCACGGGTCGGTTTTAGTACAGGCGTTTTCTT	$RydC_{H2}$ transcription
$RydC_{H2}$rev	AAGAAAACGCCTGTACTAAAACCGACCCGTGGTACAGGCGAAGAATT GCCCAGTACATCGGAAGCCTATAGTGAGTCGTATTAATTTC	$RydC_{H2}$ transcription
$RydC_{H3}$for	GAAATTAATACGACTCACTATAGGCTTCCGATGTACTGGGCAATTCTT CGCCTGTACCTGCCCAGGGTTTTAGTACAGGCGTTTTCTT	$RydC_{H3}$ transcription
$RydC_{H3}$rev	AAGAAAACGCCTGTACCAAAACCCTGGGCAGGTACAGGCGAAGAATT GCCCAGTACATCGGAAGCCTATAGTGAGTCGTATTAATTTC	$RydC_{H3}$ transcription
RydCPCRQ1	AAGAAAACGCCTGTACTAA	Real-Time PCR
RydCPCRQ2	CTTCCGATGTAGACCCGTA	Real-Time PCR
tmRNAPCRQ1	GGCAAGCGAATGTAAAGACTGA	Real-Time PCR
tmRNAPCRQ2	CCGCGTCCGAAATTCCTA	Real-Time PCR
csgDPCRQ1	CACGGAATCAGCCCTCCTTA	Real-Time PCR
csgDPCRQ2	GCCGATACGCAGCTTATTCAG	Real-Time PCR