**Supplementary Table S1.** Cryo-EM data collection and refinement statistics for Hfq/Crc/RNA structures

BS3 crosslinked Not crosslinked

PDB codes 6FUS,6FYN,6FXZ

EMDB codes 4320, 4326, 4325

**Data collection**

EM equipment Titan Krios G2, FEI Titan Krios G2, FEI

Voltage (kV) 300 300

Detector Falcon III Falcon III

Pixel size (Å) 1.09 1.09

Electron dose (e-/Å2/fraction) 0.37 0.40

Defocus range, step (µm) -1.25 to -3, δ=0.25 -1.25 to -3, δ=0.25

**Reconstruction**

Software RELION v 3b RELION v2.1

Complex (Hfq:Crc:RNA) 2:2:2 2:3:2 2:4:2 2:2:2 2:3:2 2:4:2

Molecular mass (kDa) 192 222 252 192 222 252

Number of particles used 73,187 45,311 35,376 25,408 18,898 14,900

Angular accuracies (°) 0.72 0.75 0.66 1.65 1.71 1.58

Offsets (pixels) 0.30 0.32 0.30 0.652 0.681 0.657

Symmetry C2 C1 C2 C2 C1 C2

Final resolution (Å) 3.1 3.4 3.2 4.4 4.5 4.4

Map-sharpening B factor (Å2) -97 -100 -82 -189 -172 -173

Non-hydrogen atoms 11466 13647 15770 11466 13647 15770

Protein residues 1292 1458 1820 1292 1558 1820

RNA bases 18 18 18 18 18 18

**Refinement**

Software PhenixRSRef

Model-to-Map Correlation Coef. 0.79 0.77 0.76

**Model Validation**

MolProbity score 1.64 1.77 1.69

EMRinger 3.8 3.4 3.2

All-atom clash score 5.07 5.44 5.26

***Ramachandran statistics*** (%)

Favored (overall) 94.57 92.64 93.81

Allowed (overall) 5.27 7.30 6.19

Outlier (overall) 0.16 0.06 0.0

***RMS deviations***

Bond length (Å ) 0.004 0.007 0.005

Bond angle (°) 0.84 0.88 0.908

**Validation (RNA)**

Correct sugar puckers (%) 89 89 89

Good backbone conformation (%) 39 39 39

**Supplementary Table S2.** Strains and plasmids used in this study

|  |  |  |
| --- | --- | --- |
| **Strain/plasmid** | **Genotype/relevant features** | **Source/reference** |
| ***P. aeruginosa*** |  |  |
| PAO1 |  | (Holloway et al., 1979) |
| PAO1Δ*crc* | PAO6673, in frame deletion of *crc* deletion | (Sonnleitner and Bläsi, 2014) |
| ***E. coli*** |  |  |
| XL1-Blue | *recA1 endA1 gyrA96 thi-1 hsdR17*(rK-, mK+) *supE44 relA1 lac* [F' *proAB* *lacIq lacZ*ΔM15::Tn*10*(Tcr)] | Stratagene |
| BL21(DE3) | *F-, ompT, hsdSB(rB-, mB-), dcm, gal, λ*(DE3) | Novagen |
| **Plasmids** |  |  |
| pETM14lic-His6Crc | Encodes Crc with a N-terminal cleavable His6-tag. Transcription of the *crc* gene is driven by a T7 promoter. KanR | (Milojevic et al., 2013) |
| pME9655 | Encoding the translational *amiE::lacZ* reporter gene. Tcr | (Sonnleitner and Bläsi, 2014) |
| pME4510*crc*Flag | pME4510 carrying PAO1 *crc* fused to a Flag-tag encoding sequence under control of its authentic promoter | (Sonnleitner et al., 2018) |
| pME4510*crc*(R140E)Flag | pME4510*crc*Flag derivative encoding the Crc(R140E) variant | This study |
| pME4510*crc*(E142R)Flag | pME4510*crc*Flag derivative encoding the Crc(E142R) variant | This study |
| pME4510*crc*(R229E)Flag | pME4510*crc*Flag derivative encoding the Crc(R229E) variant | This study |
| pME4510*crc*(R230E)Flag | pME4510*crc*Flag derivative encoding the Crc(R230E) variant | This study |
| pME4510*crc*(E142R, R229E)Flag | pME4510*crc*Flag derivative encoding the Crc(E142R, R229E) variant | This study |
| pME4510*crc*(E142R, R230E)Flag | pME4510*crc*Flag derivative encoding the Crc(E142R, R230E) variant | This study |
| pME4510*crc*(E142R, R229E, R230E)Flag | pME4510*crc*Flag derivative encoding the Crc(E142R, R229E, R230E) variant | This study |
| pME4510*crc*(E142A, R229E, R230E)Flag | pME4510*crc*Flag derivative encoding the Crc(E142A, R229E, R230E) variant | This study |
| pME4510*crc*(E193R)Flag | pME4510*crc*Flag derivative encoding the Crc(E193R) variant | This study |
| pME4510*crc*(E193R, R230E)Flag | pME4510*crc*Flag derivative encoding the Crc(E193R, R230E) variant | This study |
| pME4510*crc*(E193A, R230E)Flag | pME4510*crc*Flag derivative encoding the Crc(E193A, R230E) variant | This study |
| pETM14lic-His6CrcR140E | pETM14lic-His6Crc derivative encoding the Crc(R140E) variant | This study |
| pETM14lic-His6CrcE142R | pETM14lic-His6Crc derivative encoding the CrcE142R) variant | This study |
| pETM14lic-His6CrcR230E | pETM14lic-His6Crc derivative encoding the Crc(R230E) variant | This study |

**Supplementary Table S3. Oligonucleotides used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequencea** | **Mutation/orientation** |
| N142 | GCGTCGCAAGgaaCGCGAATACATC | CrcR140E/forward |
| O142 | GATGTATTCGCgttCCTTGCGACGC | CrcR140E/reverse |
| P142 | CAAGCGCCGCcgcTACATCTACTGC | CrcE142R/forward |
| Q142 | GCAGTAGATGTAgcgGCGGCGCTTG | CrcE142R/reverse |
| L142 | CGCCCTGCGCcgcGTCAGCCGC | CrcE193R/forward |
| M142 | GCGGCTGACgcgGCGCAGGGCG | CrcE193R/reverse |
| A145 | CCCCGGCCTAgaaCGCTTCGTGCGC | CrcR229E/forward |
| B145 | GCGCACGAAGCGttcTAGGCCGGGG | CrcR229E/reverse |
| R142 | CGGCCTACGCgaaTTCGTGCGCAAC | CrcR230E/forward |
| S142 | GTTGCGCACGAAttcGCGTAGGCCG | CrcR230E/reverse |
| I146 | CCCCGGCCTAgaagaaTTCGTGCGCAAC | CrcR299E,R230E/forward |
| J146 | GTTGCGCACGAAttcttcTAGGCCGGGG | CrcR299E,R230E/reverse |
| U173 | CAAGCGCCGCGccTACATCTACTGC | CrcE142A/forward |
| V173 | GCAGTAGATGTAggCGCGGCGCTTG | CrcE142A/reverse |
| W173 | CGCCCTGCGCGccGTCAGCCGC | CrcE193A/forward |
| X173 | GCGGCTGACggCGCGCAGGGCG | CrcE193A/reverse |

a mutated sequences are shown in small letters