**Supplementary File 1**

**Structure and mechanistic features of the prokaryotic minimal RNase P**

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**Supplementary File 1a. Cryo-EM data collection, refinement and validation statistics.**

|  |  |
| --- | --- |
|  | Hhal2243  (EMD-12878)  (PDB: 7OG5) |
| **Data collection and processing** |  |
| Magnification | 105,000 |
| Voltage (kV) | 300 |
| Electron exposure (e-/Å2) | 40 |
| Defocus range (µm) | 1.5 – 2.4 |
| Pixel size (Å) | 0.833 |
| Symmetry imposed | C1 |
| Initial particle images (no.) | 2,749,587 |
| Final particle images (no.) | 1,736,597 |
| Map Resolution (Å) | 3.37 |
| FSC threshold | 0.143 |
| Map resolution range | 3.37 – 5 Å |
|  |  |
| **Refinement** |  |
| Map sharpening *B* factor (Å2) | 181 |
| Model composition |  |
| Non-hydrogen atoms | 15444 |
| Protein residues | 1892 |
| Ligands | 0 |
| *B* factors (Å2) |  |
| Protein | 106.70 |
| Ligand | 0 |
| R.m.s. deviations |  |
| Bond Lengths (Å) | 0.01 |
| Bond angles (°) | 0.933 |
| Validation |  |
| MolProbity score | 2.33 |
| Clashscore | 19.16 |
| Poor rotamers (%)  Ramachandran plot | 0 |
| Favored (%)  Allowed (%)  Outliers (%) | 89.91  10.09  0.00 |

**Supplementary File 1b**: Dynamic mass distribution determined by mass photometry

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Peak** | **Molecular weight (kDa)** | **Amount (%)** |
| **Hhal2243 wt** | 1 | 295 | 88 |
| **Aq880 wt** | 1 | 42 | 7 |
|  | 2 | 78 | 13 |
|  | 3 | 129 | 9 |
|  | 4 | 182 | 13 |
|  | 5 | 234 | 11 |
|  | 6 | 277 | 47 |
| **Aq880\_184-191** | 1  2  3  4  5 | 42  83  133  183  235 | 32  50  12  4  2 |
| **Aq880\_181-191** | 1 | 42 | 55 |
|  | 2 | 81 | 41 |
|  | 3 | 134 | 4 |
| **Aq880\_179-191** | 1 | 44 | 90 |
| **Aq880\_177-191** | 1 | 52 | 92 |
| **Aq880\_R125A** | 1  2  3  4  5 | 85  134  181  246  284 | 3  5  6  15  59 |
| **Aq880\_R129A** | 1  2  3  4  5 | 71  135  185  247  286 | 3  6  7  17  58 |
| **Aq880\_ K119A/R123A/ R125A/K127A/R129A** | 1  2  3  4  5 | 94  143  193  243  278 | 3  4  9  19  58 |

**Supplementary File 1c**: **Primers & Plasmids used in this study**

|  |  |
| --- | --- |
| **constructs in pET-28a(+)** | **sequence (5‘🡪 3‘)** |
| aq880 | AAG CCA TGG ATG TGT TCG TTC TCG ACA C &  CTC TCG AGA AAC CTG TGT CTT ACC AAG C |
| aq880\_∆184-191 | TTT CTC GAG CAC CAC CAC CAC CAC C &  AATGTTTTTGAAATTCTTAGGGTCTATG |
| aq880\_∆181-191 | TTT CTC GAG CAC CAC CAC CAC CAC C &  GAA ATT CTT AGG GTC TAT GAG TTT TAT ACC |
| aq880\_∆179-191 | TTT CTC GAG CAC CAC CAC CAC CAC C &  CTT AGG GTC TAT GAG TTT TAT ACC TAT C |
| aq880\_∆177-191 | TTT CTC GAG CAC CAC CAC CAC CAC C &  GTC TAT GAG TTT TAT ACC TAT CTT GTC CG |
| aq880\_∆172-191 | TTT CTC GAG CAC CAC CAC CAC CAC C &  TAT CTT GTC CGC CCA TGT TCT GAG GCC |
| aq880\_K119A/R123A/ R125A/K127A/R129A | GCG CAG CTA TTA ATG CCC CGA CGT CTT CAC &  TCG CGG AGG CGT ACG CGG AAG CCC TCA GG |
| aq880\_R125A/R129A | GCC TAG CTA TTA ATT TCC CGA CGT CTT CAC &  TCG CGG AGA AGT ACG CGG AAG CCC TCA GG |
| aq880\_R125A | GCC TAG CTA TTA ATT TCC CGA CGT CTT CAC &  TCG CGG AGA AGT ACA GGG AAG CCC TCA GG |
| aq880\_R129A | GCC TAG CTA TTA ATT TCC CGA CGT CTT CAC &  TCA GGG AGA AGT ACG CGG AAG CC |
| hhal2243 | TTT GCT AGC CGC CGA TTC GTG CTC G &  TTT GCT CAG CCT ACC CGG CGG GCT G |

**Supplementary References**

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