**Figure S1: Biochemical characterization of Gp44 and its sequence alignment with σ54.**

**(A)** **ITC results for the affinity determinations of Gp44-β and Gp44-** **β', respectively. (B) Gp44(56-122) sequence aligned with σ54 (44-113) by NEEDLE.**

**A** 

**B**

**Gp44(56-122) 1 EEVVEEVVTEEE-DILEGVEVVEDEEEEEAAEDVEEPTSEED--SEDEWE 47**

 **..::|::.|.|| |..| .:|.|..:.|:.:|:....|: .:..|:**

 **σ54(44-113) 1 NPLLEQIDTHEEIDTRE----TQDSETLDTADALEQKEMPEELPLDASWD 46**

**Gp44(56-122) 48 ----EGYPVATEVEEDEDEEIEYP 67**

 **.|.|..|..:..:||...|.**

 **σ54(44-113) 47 TIYTAGTPSGTSGDYIDDELPVYQ 70**

**Figure S2: Growth attenuation assay: Graphs showing growth curves of *E. coli* cultures expressing different constructs of Gp44 and that of control cultures of empty pDE2 vector (see figure legend).**



**Table S1: DNA fragments used in NMR titration experiment.**

|  |  |  |  |
| --- | --- | --- | --- |
| Name  | Description | Sequence | Length |
| Pstrong(BBa\_K780003) | Strong constitutive promoter for Bacillus subtilis | aattgcagtaggcatgacaaaatggactca | 30 |
| T7A1(BBa\_I712074) | T7A1 promoter used by T7 polymerase in Escherichia coli  | agggaatacaagctacttgttctttttgca | 30 |

**Table S2: Structural statistics from the solution structure calculation for 6xHis Gp44 N-terminal region (1-55) (PDB ID: 6L6V, BMRB ID: 36290).**

|  |  |
| --- | --- |
| Gp5.7 | Value |
| NMR Distance and Dihedral Constraints |  |
| Distance constraints  |  |
|  Total NOE | 875 |
|  Intraresidue | 354 |
|  Interresidue | 521 |
|  Sequential (|*i-j*|)=1) | 181 |
|  Short range (2≤|*i-j*|)≤3) | 87 |
|  Medium range (4≤|*i-j*|)≤5) | 56 |
|  Long range (|*i-j*|)>5) | 197 |
| Total Dihedral angle Restraints  | 84 |
|  φ | 42 |
|  ψ | 42 |
|  Total RDCs  | 0 |
| Structural Statistics |  |
| Violations (mean and SD) |  |
|  Distance constraints (Å) | 0.024 ± 0.007 |
|  Dihedral angle constraints (°) | 0.30 ± 0.073 |
|  Maximum dihedral angle violation (°) | 0.64 |
|  Maximum distance constraint violation (Å) | 0.22 |
| Deviations from idealized geometry |  |
|  Bond length (Å) | 0.0015 ± 0.000 |
|  Bond angle (°) | 0.319 ± 0.006 |
|  Impropers (°) | 0.263 ± 0.011 |
| Average Pairwise rmsdα(Å) |  |
|  Heavy | 0.576 ± 0.0843 |
|  Backbone | 0.216 ± 0.0470 |

**Table S3: Parameters for molecular dynamics simulation of each fragments from Gp4456-122**

|  |
| --- |
| **MD simulation parameters** |
|  Integrator | md | Leap-frog integrator |
|  Nsteps | 250000000 | 2 \* 250000000 = 500 ns |
|  dt | 0.002 | fs |
| **Output control** |
|  nstxout | 0 |  |
|  nstvout | 0 |  |
|  nstfout | 0 |  |
|  nstenergy | 250000 |  |
|  nstlog | 250000 |  |
|  nstxout-compressed | 250000 |  |
|  compressed-x-grps | system |  |
| **Bond parameter** |
|  continuation | yes | Restarting after NPT |
|  constrain algorithm | LINCS | holonomic oncstrains |
|  constrains | H-bonds | Bonds involving H are constrained |
|  LINCS iteration | 1 |  |
|  LINCS order | 4 |  |
| **Neighbor searching** |
|  cutoff-scheme | Verlet |  |
|  ns type | grid |  |
|  nstlist | 10 |  |
|  rcoulomb | 1.0 nm |  |
|  rvdw | 1.0 nm |  |
| **Electrostatics** |
|  coulomb type | Particle Mesh Ewald for long-range electrostatics (PME) |
|  PME order | 4 | cubic interpolation |
|  fourier spacing | 0.16 |  |
| **Temperature Coupling** |
|  temperature coupling | V-rescale | modified Berendsen thermostat |
|  temperature coupling group | Protein | Non protein |
|  τ(t) time constant | 0.1 | 0.1 |
|  reference temperature (K) | 300 | 300 |
| **Pressure Coupling** |
|  pressure coupling | Parrinello-Rahman |  |
|  pressure coupling type | isotropic |  |
|  τ(t) time constant | 2.0 |  |
|  reference pressure (bar) | 1.0 |  |
|  compressibility (bar-1) | 4.5e-05 | isothermal compressibility of water |