

Supplementary data for:

Crystal structure of Hfq from *Bacillus subtilis* in complex with SELEX-derived RNA aptamer: insight into RNA-binding properties of bacterial Hfq

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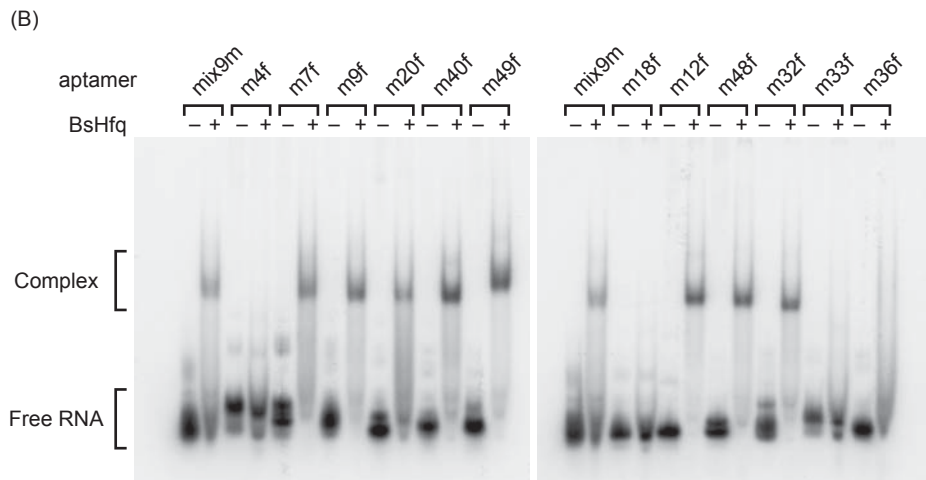
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Supplementary Table 1. List of primers.

Name	Sequence
<i>Hfq</i> -His expression plasmid	
ymaH-NcoI	5'- CGAACCATGGAACCGATTAATA -3'
ymaH-BglII	5'- TTTTAGATCTTTCGAGTTCAAGCTG -3'
Site-directed mutagenesis	
Y_F24A_s	5'- GAAAATACGTATGTCACGTGTTGCTTTGCTGAACGGCTTTC -3'
Y_F24A_a	5'- GAAAGCCGTTTCAGCAAAGCAACAGTGACATACGTATTTTC -3'
Y_F29A_s	5'- CTGTTTTTTTTGCTGAACGGCGCTCAGTTGCGGGGCCAG -3'
Y_F29A_a	5'- CTGGCCCCGCAACTGAGCGCCGTTTCAGCAAAAAAACAG -3'
Y_Q30A_s	5'- CTGTTTTTTTTGCTGAACGGCTTTGCGTTGCGGGGCCAG -3'
Y_Q30A_a	5'- CTGGCCCCGCAACGCAAAGCCGTTTCAGCAAAAAAACAG -3'
Y_R32A_s	5'- GAACGGCTTTCAGTTGGCGGGCCAGGTGAAAGG -3'
Y_R32A_a	5'- CCTTTCACCTGGCCCCGCAACTGAAAGCCGTTTC -3'
Y_N40A_s	5'- GTGAAAGGCTTTGATGCCTTTACCGTATTGTTGGAATCGG -3'
Y_N40A_a	5'- CCGATTCCAACAATACGGTAAAGGCATCAAAGCCTTTCAC -3'
Y_F41A_s	5'- GTGAAAGGCTTTGATAACGCTACCGTATTGTTGGAATCGG -3'
Y_F41A_a	5'- CCGATTCCAACAATACGGTAGCGTTATCAAAGCCTTTCAC -3'
Y_K56A_s	5'- CAGCAGCTTATATATGCACATGCGATCTCAACGTTTGCG -3'
Y_K56A_a	5'- CGCAAACGTTGAGATCGCATGTGCATATATAAGCTGCTG -3'
Y_H57A_s	5'- GCAGCAGCTTATATATAAAGCTGCGATCTCAACGTTTGCG -3'
Y_H57A_a	5'- CGCAAACGTTGAGATCGCAGCTTATATATAAAGCTGCTG -3'
<i>In vitro</i> transcription plasmid	
FM-49m30Nwt-F	5'- AATTCTAGAGAGAGATTAGATCCTGTCCGCGGCTAA -3'
FM-49m30Nwt-R	5'- AGCTTTAGCCGCGGACAGGATCTAATCTCTCTCTAG -3'
FM-49m30N1-F	5'- AATTCTAGAGAGACCTTAGATCCTGTCCGCGGCTAA -3'
FM-49m30N1-R	5'- AGCTTTAGCCGCGGACAGGATCTAAGGTCTCTCTAG -3'
FM-49m30N2-F	5'- AATTCTAGAGACCCCTTAGATCCTGTCCGCGGCTAA -3'
FM-49m30N2-R	5'- AGCTTTAGCCGCGGACAGGATCTAAGGGGTCTCTAG -3'
FM-49m30N3-F	5'- AATTCTAGACCCCTTAGATCCTGTCCGCGGCTAA -3'
FM-49m30N3-R	5'- AGCTTTAGCCGCGGACAGGATCTAAGGGGGTCTCTAG -3'
FM-49m30NwtTA-F	5'- AATTCTAGAGATAGATTAGATCCTGTCCGCGGCTAA -3'
FM-49m30NwtTA-R	5'- AGCTTTAGCCGCGGACAGGATCTAATCTATCTCTAG -3'
FM-49m30NwtAA-F	5'- AATTCTAGAGAAAGATTAGATCCTGTCCGCGGCTAA -3'
FM-49m30NwtAA-R	5'- AGCTTTAGCCGCGGACAGGATCTAATCTTTCTCTAG -3'
FM-49m30NwtCA-F	5'- AATTCTAGAGACAGATTAGATCCTGTCCGCGGCTAA -3'
FM-49m30NwtCA-R	5'- AGCTTTAGCCGCGGACAGGATCTAATCTGTCTCTAG -3'
FM-49m30NwtGGA-F	5'- AATTCTAGAGAGGAGATTAGATCCTGTCCGCGGCTAA -3'
FM-49m30NwtGGA-R	5'- AGCTTTAGCCGCGGACAGGATCTAATCTCTCTCTAG -3'

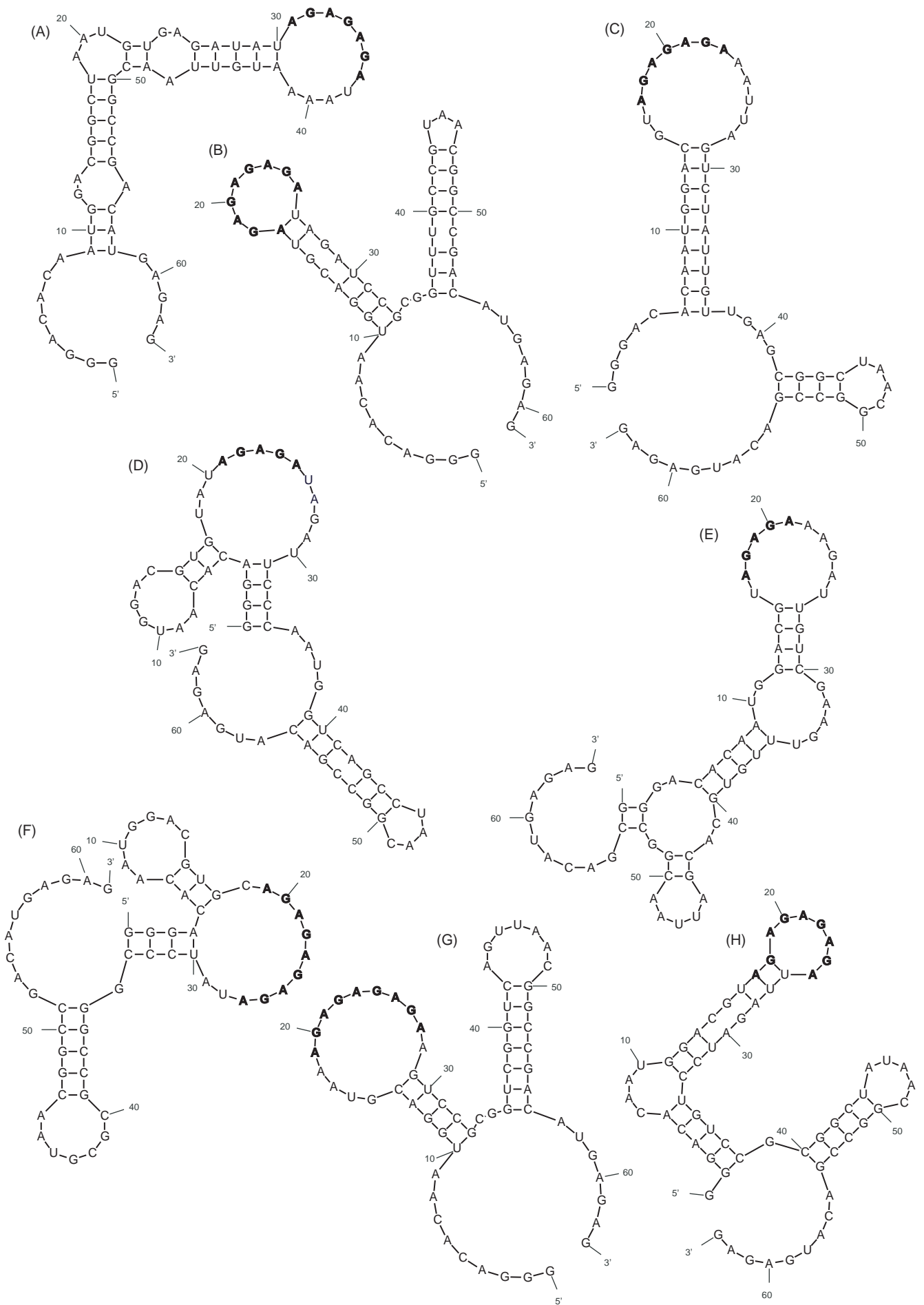
(A)

m4 f	UGGAAAGGGUAAUAUGUAUGACAUGGCC
m7 f	GCUAAUGUGAGAUAAU <u>AGAGAGAG</u> AUAAAUGU
m9 f	<u>UAGAGAGAG</u> AUAGAUCGCGGUUUGCCG
m20 f	UGUA <u>UAGAG</u> AUAGAUUCCAAUGGUCAGCC
m40 f	UGCAGAGAGAGAU <u>AUCCCGGGCCGCGC</u> G
m49 f	<u>UAGAGAGAG</u> AUUAGAUCUGUCCGCGGCCUA
m18 f	GAGGUGGGAGUGUCAAAUUUCGAUUGGCCA
m12 f	<u>UAGAGAG</u> AAAUAGUCUAUUGUUGAGCGGC
m48 f	UAA <u>AGAGAG</u> AGAAGUCCGCGGUCGGUCAGU
m32 f	<u>UAGAG</u> AAAGAUUGUCGAAGUUUGUGCACGAU
m33 f	GAGGGUGGGUAGUUUUCUAGAAGUGGUCGG
m36 f	AUAAAA <u>AGAG</u> AAUCCGGUCAGUCUGGCCA

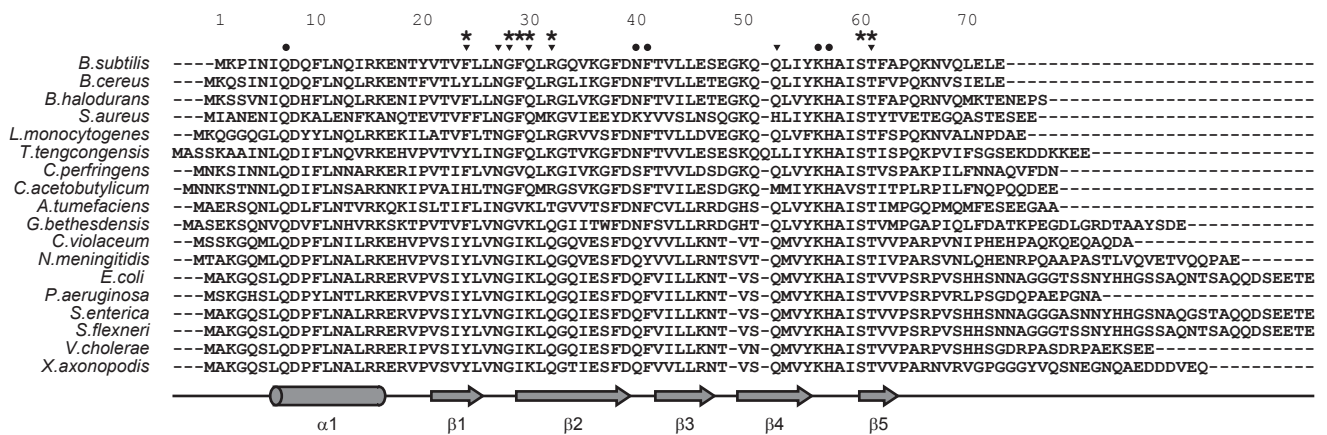


Supplementary Figure 1. *In vitro* selection of RNA aptamers that bind to BsHfq.

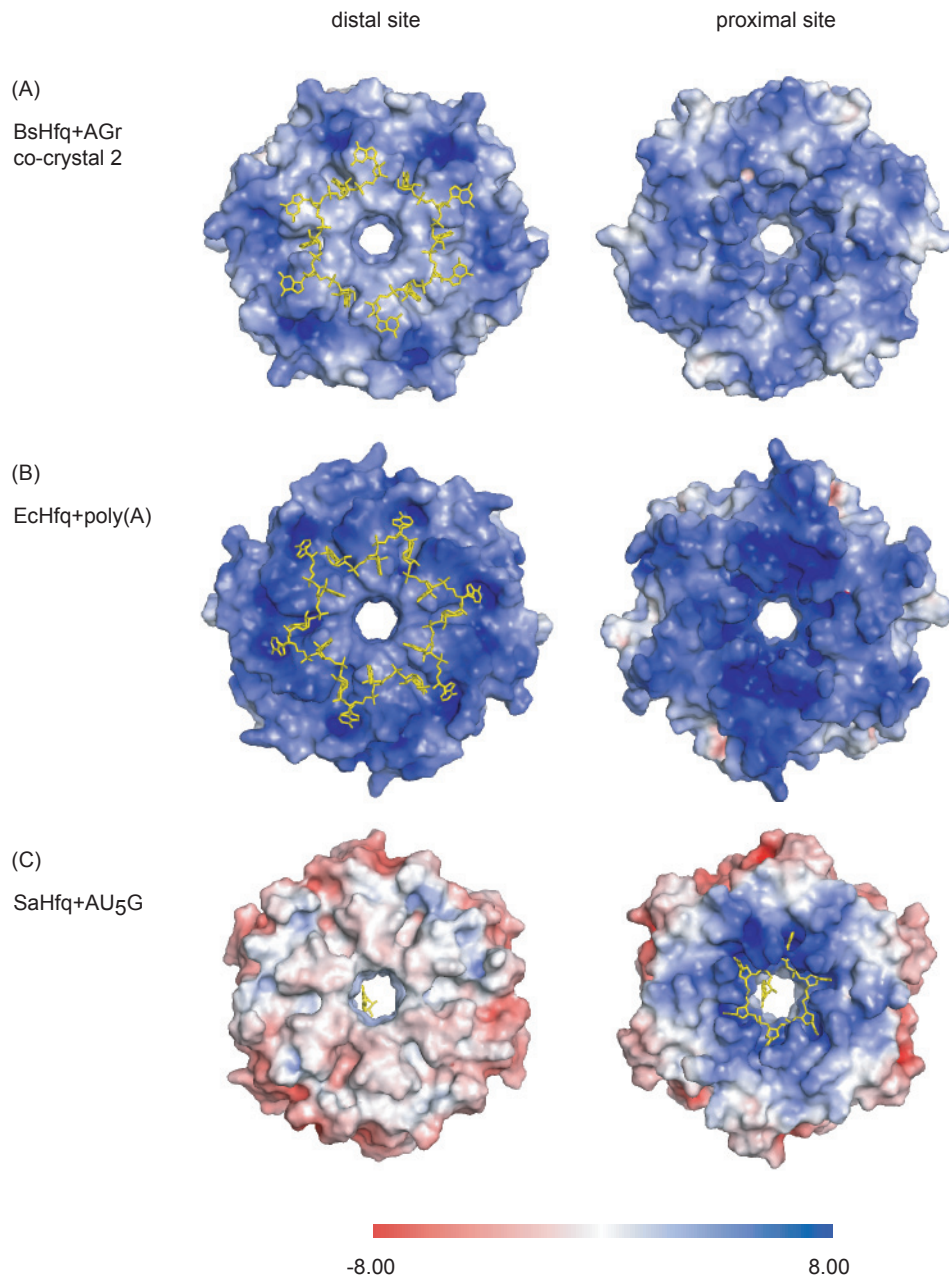
(A) Random sequence regions of clones isolated from SELEX round 9 pool. Individual clones are named left of sequences. Sequences of AG repeats are indicated by underline. (B) EMSA of RNA aptamers binding with BsHfq. ³²P-RNA (60,000 cpm) was incubated in the absence (-) or presence (+) of 12 pmol BsHfq-His. Mixture of SELEX sequences after round 9 indicated as mix9m.



Supplementary Figure 2. Secondary structure models of aptamers with binding affinity for BsHfq. (A) m7f ($\Delta G = -8.6$ kcal/mol, calculated by MFOLD), (B) m9f (-9.9 kcal/mol), (C) m12f (-15.5 kcal/mol), (D) m20f (-15.3 kcal/mol), (E) m32f (-3.4 kcal/mol), (F) m40f (-14.3 kcal/mol), (G) m48f (-16.5 kcal/mol) and (H) m49f (-15.1 kcal/mol). Structures were derived using MFOLD program (41). Only most stable predicted structures are shown. Nucleotides comprising AG repeats are highlighted in bold type.

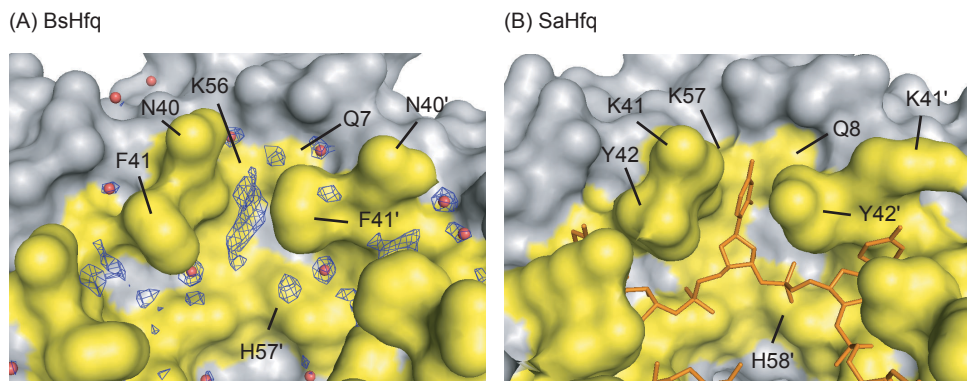


Supplementary Figure 3. Multiple sequence alignment of bacterial Hfqs. Numbering at top corresponds to BsHfq sequence and secondary structural elements are shown below these sequences. Stars, circles and triangles indicate amino acid residues involved in RNA binding in BsHfq-AGr, SaHfq-AU₅G, and EcHfq-poly(A), respectively. Sequences were aligned using ClustalW program and manually edited.



Supplementary Figure 4. Electrostatic surface potential of (A) BsHfq (co-crystal 2), (B) EcHfq (PDB ID: 3GIB), and (C) SaHfq (PDB ID: 1KQ2).

Left and right panels show views of distal and proximal sites of Hfq, respectively. Potential scales range from -8 kT/e (red) to 8 kT/e (blue). Surface electrostatic potentials were calculated by PyMOL APBS (<http://apbs.sourceforge.net>) tools and figure was generated using PyMOL software.



Supplementary Figure 5. Proximal sites of hexameric BsHfq and SaHfq.

(A) Surface representation of pocket of BsHfq surrounded by F41 and its neighboring residues. Part of $2F_o - F_c$ electron density map (blue) is shown at 1.5σ . Residues involved in AU₅G binding in SaHfq are shown in yellow and labeled.

(B) Pocket of SaHfq with AU₅G (orange). Residues in direct contact with AU₅G are shown in yellow and labeled.