Supplementary Materials

NMR Structure and Biophysical Characterization of Thermophilic Single-stranded DNA Binding Protein from *Sulfolobus solfataricus*

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Figure S1: Results of CD spectroscopy. (A) θ value at 190 to 250 nm from CD spectroscopy of $SsoSSB_{1-114}$ and $SsoSSB_{12-114}$ at 20°C. (B) Molar ellipticity at 228 nm of $SsoSSB_{1-114}$ and $SsoSSB_{12-114}$ from 20 to 80°C.

Figure S2: Number of distance constraints used for structure calculation. The number of Short-range (Intramolecular and sequential residue) constraints (white), mid-range constraints (cyan), and long-range constraints (red) are marked. The secondary structure from Uniprot showed the top of the graph.

Figure S3: Secondary structure comparison among the high-temperature NMR structure (PDB ID: 7WCG), the X-ray crystal structure (PDB ID: 1O7I), and the room temperature NMR structure in complex with ssDNA (PDB ID: 2MNA). Aromatic residues which are related to DNA binding are indicated in red.

Figure S4: (A) The lowest energy structure of $SsoSSB_{1-114}$ (PDB ID: 7WCG; The N-terminal part (residue number 1-11) is shown in magenta). (B) The crystal structure of TmaSSB (PDB ID: 1Z9F).,

Figure S5: T_m of hRPA70A measured by DSC.

Figure S6: (A) ^{1}H-^{15}N HSQC of $SsoSSB_{12-114}$. (B) Overlay of ^{1}H-^{15}N HSQC spectra of $SsoSSB_{1-114}$ (blue) and $SsoSSB_{12-114}$ (red). (C) Overlay of ^{1}H-^{15}N HSQC spectra of $SsoSSB_{12-114}$ with ssDNA dA(15) (green), $SsoSSB_{1-114}$ (blue), and $SsoSSB_{12-114}$ (red). Experiments were performed at 25°C.

Figure S7: Thermodynamic analysis by isothermal titration calorimetry. ssDNA was added to (A) $SsoSSB_{1-114}$ and (B) $SsoSSB_{12-114}$. The fitted curve represents a 1 to 1 binding model with the given K_d and n.

Figure S1

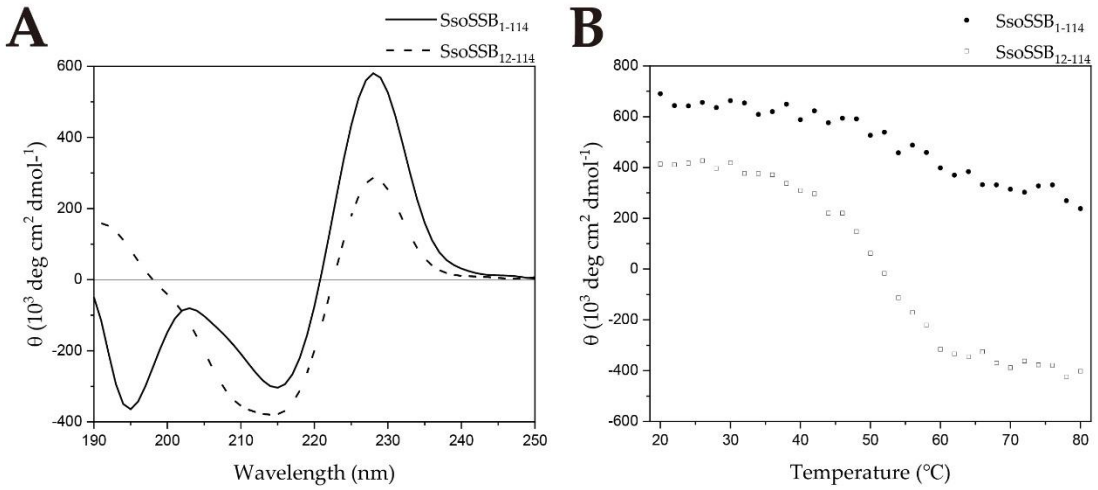

A
SsoSSB1-114
SsoSSB12-114
θ (10^3 deg cm^2 dmol^-1)
Wavelength (nm)
B
SsoSSB1-114
SsoSSB12-114
θ (10^3 deg cm^2 dmol^-1)
Temperature (°C)

Figure S2

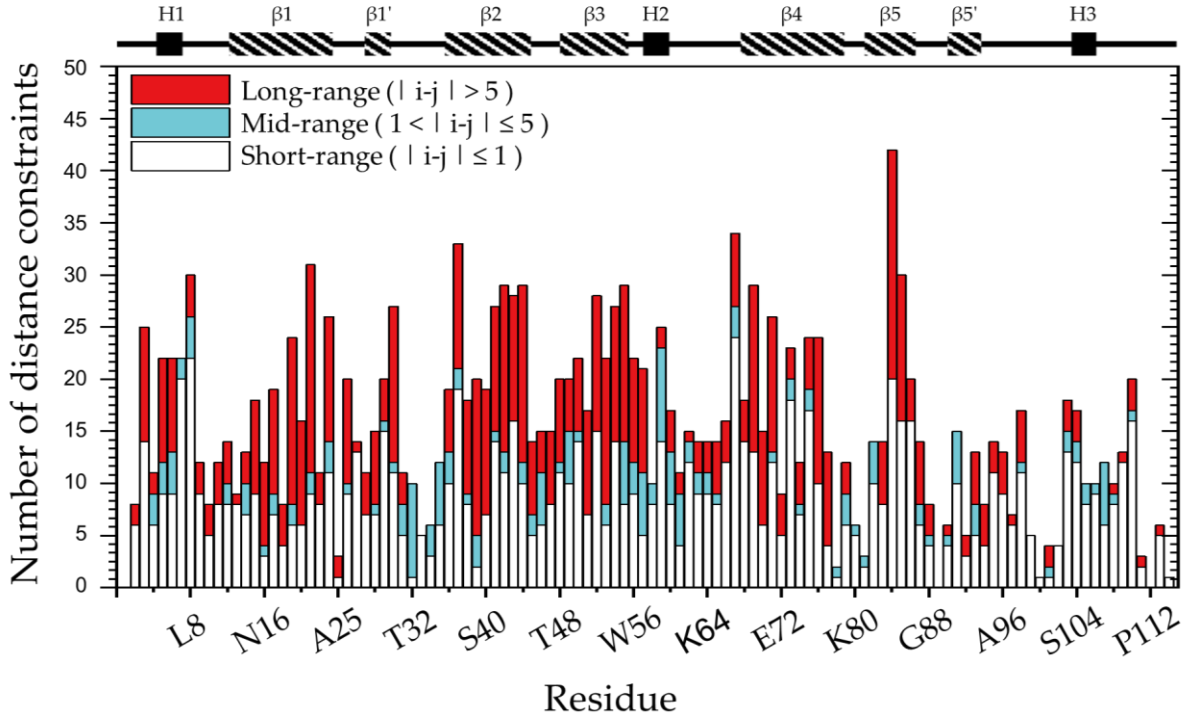

H1
β1
β1'
β2
β3
H2
β4
β5
β5'
H3
Long-range (| i-j | > 5)
Mid-range (1 < | i-j | ≤ 5)
Short-range (| i-j | ≤ 1)
Number of distance constraints
0
5
10
15
20
25
30
35
40
45
50
L8
N16
A25
T32
S40
T48
W56
K64
E72
K80
G88
A96
S104
P112
Residue

Figure S3

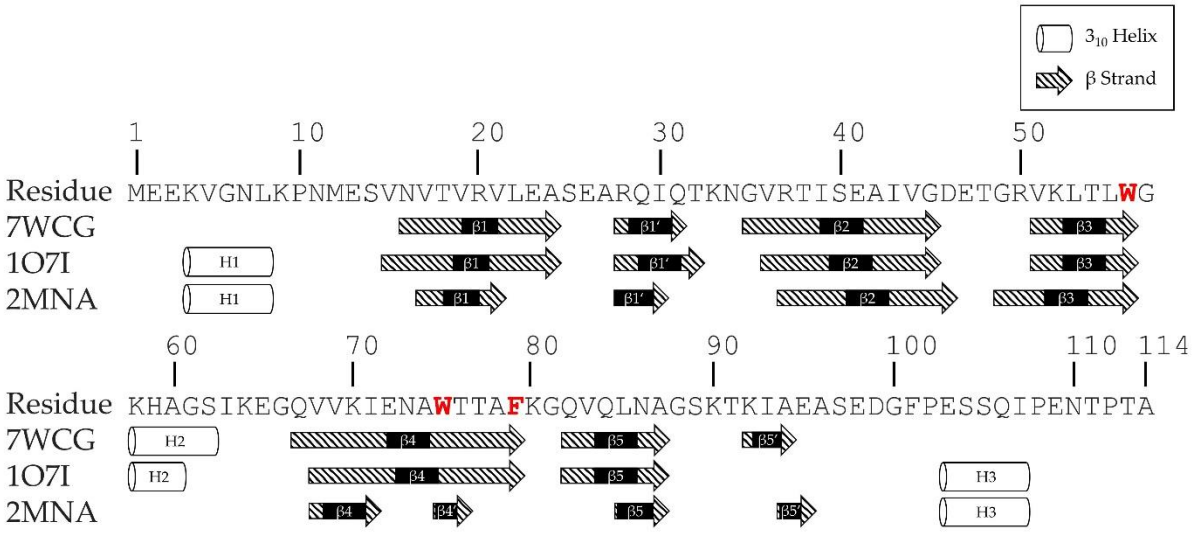

3₁₀ Helix
β Strand
1
10
20
30
40
50
Residue MEEKVGNLKPNMESVNVTVRVLEASEARQIQTKNGVRTISEAIVGDETGRVKLTLWG
7WCG
1O7I
2MNA
H1
β1
β1′
β2
β3
60
70
80
90
100
110
114
Residue KHAGSIKEGQVVKIENAWTTAFKGQVQLNAGSKTKIAEASEDGFPESSQIPENTPTA
H2
β4
β5
H3

Figure S4

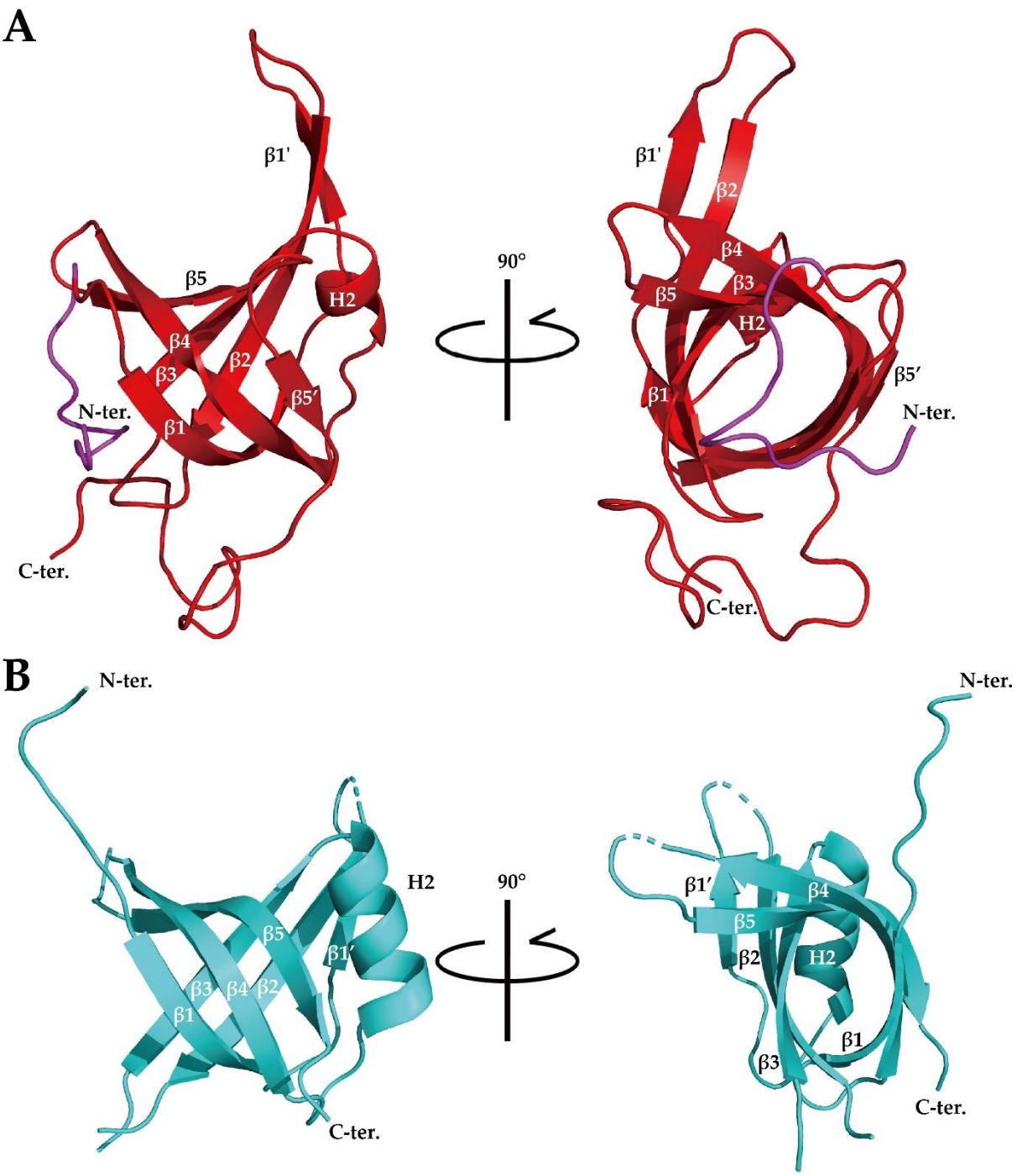

A
β1'
β5
H2
β4
β2
β3
β5′
N-ter.
β1
C-ter.
90°
β1'
β2
β4
β3
β5
H2
β5′
β1
N-ter.
C-ter.
B
N-ter.
H2
β5
β1′
β3
β4
β2
β1
C-ter.
90°
N-ter.
β1′
β4
β5
β2
H2
β1
β3
C-ter.

Figure S5

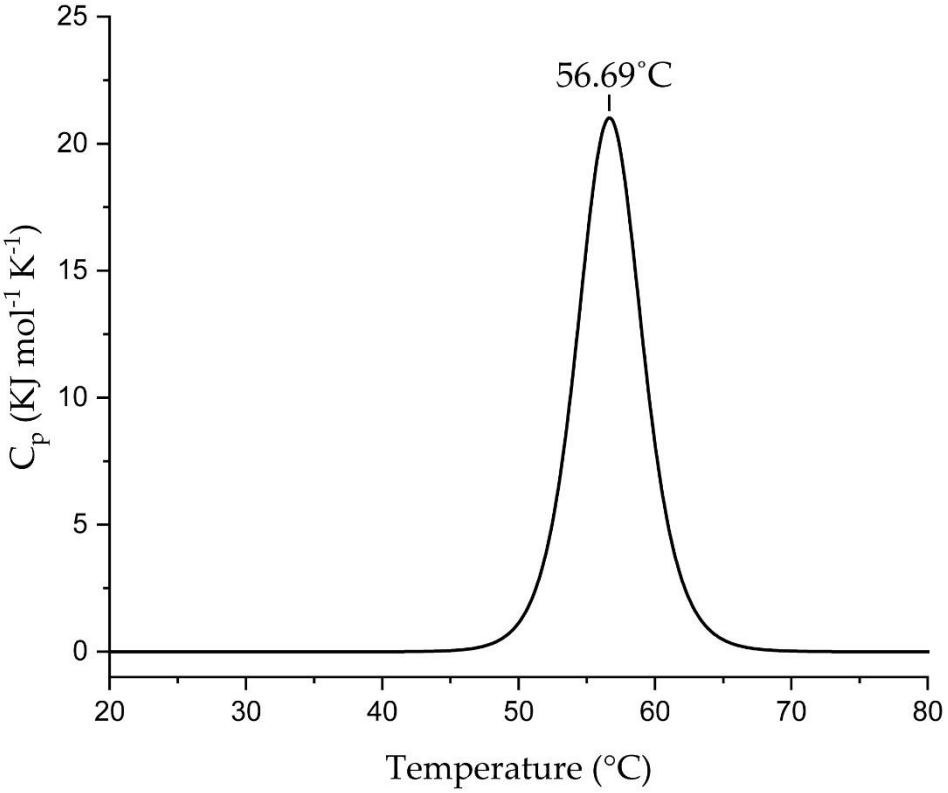

56.69°C
25
20
15
10
5
0
C_p (KJ mol^{-1} K^{-1})
20
30
40
50
60
70
80
Temperature (°C)

Figure S6

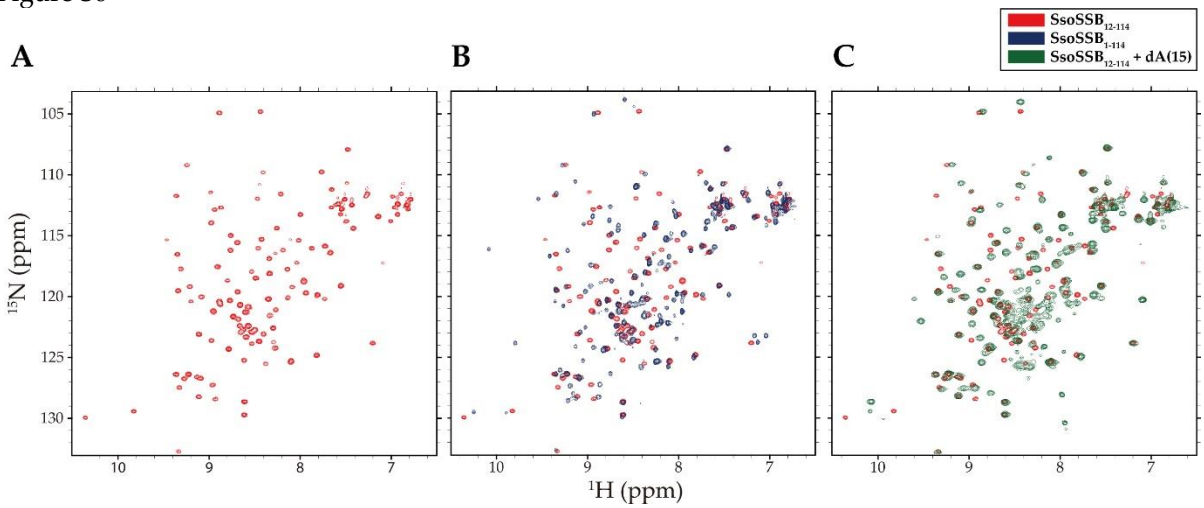
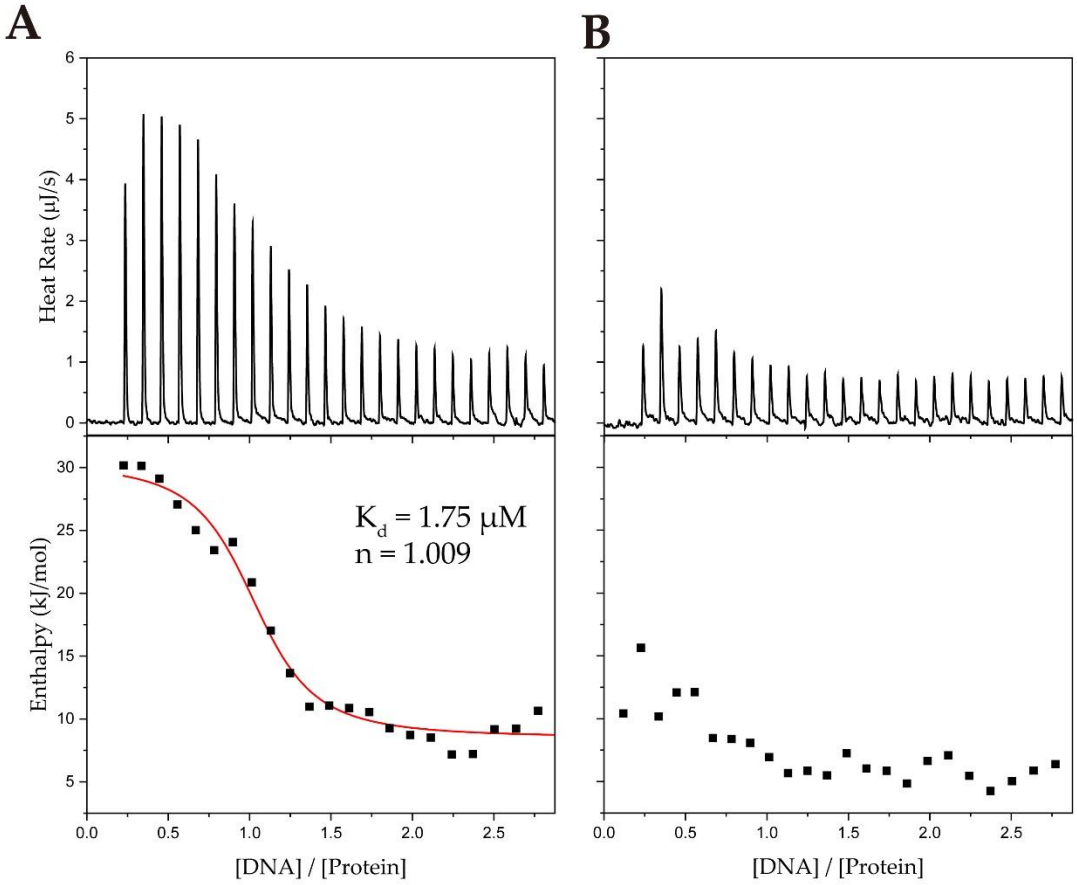

A
B
C
SsoSSB12-114
SsoSSB1-114
SsoSSB12-114 + dA(15)
15N (ppm)
1H (ppm)
105
110
115
120
125
130
10
9
8
7

Figure S7


A
B
Heat Rate (µJ/s)
Enthalpy (kJ/mol)
K_d = 1.75 µM
n = 1.009
[DNA] / [Protein]
[DNA] / [Protein]