

Supporting Information

**Intramolecular binding mode of the C-terminus of
E. coli single-stranded DNA binding protein (SSB) determined
by nuclear magnetic resonance spectroscopy**

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Table S1. Long-range NOEs observed in SSB at pH 3.4[†]

112LeuH ^γ – 78TyrH ^δ	101ValH ^{γb} – 81GlyH ^{αa}	77ValH ^{γb} – 10LeuH ^{δb}
112LeuH ^{δa} – 78TyrH ^δ	101ValH ^{γa} – 81GlyH ^{αa}	76GlnH ^α – 13AsnH ^α
112LeuH ^{δa} – 78TyrH ^{βb}	101ValH ^{γb} – 58ValH ^N	76GlnH ^{βa} – 13AsnH ^α
111MetH ^{γa} – 71LeuH ^{δa}	101ValH ^{γa} – 55HisH ^{δ2}	73LysH ^α – 16GlnH ^N
111MetH ^ε – 70TyrH ^ε	101ValH ^{γb} – 55HisH ^{δ2}	73LysH ^α – 14LeuH ^{δa}
111MetH ^ε – 70TyrH ^δ	101ValH ^{γb} – 10LeuH ^{δa}	73LysH ^α – 14LeuH ^γ
110GlnH ^N – 79IleH ^{γ2}	97TyrH ^δ – 87LysH ^{γb}	70TyrH ^δ – 66ValH ^α
110GlnH ^N – 79IleH ^{δ1}	97TyrH ^δ – 87LysH ^{γa}	70TyrH ^ε – 66ValH ^α
110GlnH ^{γa} – 78TyrH ^ε	97TyrH ^ε – 87LysH ^{γa}	70TyrH ^ε – 66ValH ^{γa}
110GlnH ^{βa} – 78TyrH ^ε	97TyrH ^δ – 87LysH ^{βa}	70TyrH ^ε – 66ValH ^{γb}
109MetH ^{γa} – 79IleH ^{γ2}	97TyrH ^δ – 85ThrH ^{γ2}	70TyrH ^δ – 66ValH ^{γa}
109MetH ^{βa} – 77ValH ^{γa}	97TyrH ^ε – 85ThrH ^{γ2}	70TyrH ^δ – 66ValH ^{γb}
109MetH ^ε – 77ValH ^{γa}	96ArgH ^{βa} – 88TrpH ^{ε3}	70TyrH ^ε – 66ValH ^β
109MetH ^ε – 66ValH ^{γa}	96ArgH ^{βb} – 88TrpH ^{ε1}	70TyrH ^δ – 66ValH ^β
109MetH ^ε – 67AlaH ^α	96ArgH ^{βb} – 88TrpH ^{ε3}	70TyrH ^ε – 19GluH ^{γa}
109MetH ^ε – 63LeuH ^α	95AspH ^α – 89ThrH ^{γ2}	70TyrH ^δ – 10LeuH ^{βb}
109MetH ^α – 57ValH ^{γa}	93GlyH ^{αb} – 89ThrH ^{γ2}	60PheH ^α – 29ValH ^{γb}
108ThrH ^N – 79IleH ^{γ2}	93GlyH ^{αa} – 89ThrH ^{γ2}	60PheH ^δ – 29ValH ^{γb}
108ThrH ^{γ2} – 78TyrH ^ε	80GluH ^α – 9IleH ^{γ2}	60PheH ^δ – 29ValH ^{γa}
108ThrH ^{γ2} – 78TyrH ^δ	80GluH ^α – 9IleH ^β	60PheH ^ε – 29ValH ^{γa}
104AsnH ^α – 60PheH ^{βa}	79IleH ^{γ2} – 10LeuH ^{δa}	60PheH ^δ – 29ValH ^{γb}
103ValH ^{γa} – 81GlyH ^{αa}	79IleH ^β – 10LeuH ^{δb}	60PheH ^ε – 29ValH ^{γb}
103ValH ^N – 79IleH ^{γ2}	78TyrH ^δ – 11ValH ^α	60PheH ^ξ – 29ValH ^{γb}
102ValH ^{γa} – 60PheH ^ε	78TyrH ^δ – 11ValH ^{γb}	60PheH ^ε – 23MetH ^ε
102ValH ^{γa} – 60PheH ^δ	78TyrH ^ε – 11ValH ^{γb}	60PheH ^δ – 23MetH ^ε
102ValH ^β – 60PheH ^δ	78TyrH ^ε – 11ValH ^{γa}	58ValH ^{γa} – 31AsnH ^α
102ValH ^α – 58ValH ^{γa}	78TyrH ^ε – 11ValH ^β	57ValH ^{γa} – 10LeuH ^{δa}
102ValH ^{γa} – 58ValH ^{γa}	78TyrH ^δ – 11ValH ^β	55HisH ^{δ2} – 10LeuH ^{δb}
102ValH ^{γa} – 58ValH ^β	78TyrH ^ε – 9IleH ^{δ1}	55HisH ^{δ2} – 10LeuH ^{δa}
101ValH ^{γb} – 84ArgH ^N	78TyrH ^ε – 9IleH ^{γ2}	55HisH ^{δ2} – 10LeuH ^{βb}
101ValH ^{γb} – 83LeuH ^N	78TyrH ^δ – 9IleH ^{γ2}	55HisH ^{ε1} – 10LeuH ^{βb}
101ValH ^{γb} – 83LeuH ^α	78TyrH ^{βb} – 9IleH ^{γ2}	55HisH ^{ε1} – 10LeuH ^{δb}

55HisH ^{ε1} – 10LeuH ^{δa}	54TrpH ^{ε3} – 33ThrH ^β	32IleH ^{γ2} – 19GluH ^N
55HisH ^{βb} – 10LeuH ^{δa}	54TrpH ^{ε3} – 33ThrH ^{γ2}	32IleH ^{γ2} – 18ProH ^{βa}
55HisH ^{βa} – 10LeuH ^{δa}	54TrpH ^{η2} – 33ThrH ^{γ2}	30AlaH ^β – 20ValH ^N
55HisH ^{βb} – 10LeuH ^{δb}	54TrpH ^{ε3} – 20ValH ^{γb}	29ValH ^{γb} – 23MetH ^ε
55HisH ^{βa} – 10LeuH ^{δb}	54TrpH ^{ζ3} – 15GlyH ^{αb}	29ValH ^{γa} – 23MetH ^ε
54TrpH ^{ζ3} – 35AlaH ^β	54TrpH ^{ζ3} – 15GlyH ^{αa}	29ValH ^{γb} – 23MetH ^{γa}
54TrpH ^{ε3} – 35AlaH ^β	54TrpH ^{η2} – 15GlyH ^{αb}	29ValH ^{γa} – 23MetH ^{γb}
54TrpH ^{ζ3} – 35AlaH ^α	51GlnH ^{βa} – 40TrpH ^{η2}	28AlaH ^N – 23MetH ^ε
54TrpH ^{ε3} – 35AlaH ^β	36ThrH ^{γ2} – 10LeuH ^{δb}	28AlaH ^β – 22TyrH ^δ
54TrpH ^{ζ2} – 35AlaH ^β	35AlaH ^β – 13AsnH ^{βa}	28AlaH ^β – 22TyrH ^ε
54TrpH ^{η2} – 35AlaH ^β	35AlaH ^β – 13AsnH ^N	28AlaH ^α – 22TyrH ^δ
54TrpH ^{ε1} – 35AlaH ^β	35AlaH ^β – 12GlyH ^{αb}	28AlaH ^α – 22TyrH ^ε

† Letters ‘a’ and ‘b’ in superscripts identify individual ¹H resonances of CH₂ groups, or individual CH₃ groups in isopropyl groups, using ‘a’ for the more shielded resonance. All NOEs were suggested by CCPNMR Analysis (30) based on the assigned chemical shifts without reference to the 3D structure of the protein. No NOEs were observed that were not in agreement with the structure of SSB observed in the crystal structure 1EYG (9).

Table S2. PCSs measured for backbone amide protons of SSB with C1-lanthanide tag

residue	Tm ³⁺	Tb ³⁺	residue	Tm ³⁺	Tb ³⁺	residue	Tm ³⁺	Tb ³⁺
S2	-0.003	-0.012	R56	-0.010	-0.022	Q116	-0.101	0.131
R3	-0.002	-0.001	V58	0.019	-0.042	G117	-0.097	0.130
G4	-0.004	0.000	K73	-0.295	0.316	A120	-0.090	0.116
V5	-0.002	-0.012	G74	-0.170	0.182	A122		0.006
N6	-0.002	0.000	S75	-0.197		G123	-0.039	0.043
K7	-0.004	0.008	Q76	-0.141	0.168	I126	0.013	0.002
V8	-0.007	0.013	V77	-0.097	0.136	Q130	0.003	0.008
I9	-0.021	0.017	I79	-0.064		G134	-0.002	0.006
L10	-0.031	0.040	Q82		-0.008	G136		0.003
V11	-0.047	0.052	L83	-0.001	-0.011	Q139		0.000
G12	-0.080	0.084	R84	0.021	-0.042	Q140	-0.004	0.002
N13	-0.080	0.080	T85	0.035	-0.059	Q142	-0.002	-0.006
L14	-0.137	0.155	R86	0.051	-0.081	G143	0.004	-0.011
G15	-0.104	0.104	K87	0.060	-0.092	G144	-0.015	0.009
D17	-0.233	0.227	W88	0.062	-0.094	N145	0.011	-0.014
E19	-0.144		T89	0.081	-0.116	Q146		-0.006
G26	0.041		D90	0.061	-0.094	F147	-0.011	-0.006
V29	0.227		Q91	0.070	-0.105	S148	0.029	-0.016
I32	-0.038		S92		-0.082	G149	0.018	-0.001
L34	-0.054	0.032	G93	0.056	-0.080	A151	0.002	-0.009
A35	-0.072	0.076	Q94	0.046	-0.074	Q152	0.001	-0.009
T36	-0.047	0.042	Y97	0.043	-0.061	S153	-0.005	-0.007
S37	-0.047	0.046	T98	0.050	-0.079	R154	-0.015	-0.007
E38	-0.034	0.027	T99	0.026		Q156	-0.003	0.000
S39	-0.024	0.025	E100	0.032	-0.053	Q157	0.000	-0.007
W40	-0.022	0.019	V102	0.014	-0.032	S158	0.004	-0.012
R41	-0.014	0.012	V103	-0.005	-0.032	A159	0.005	-0.015
D42	-0.013	0.014	N104	-0.003	0.001	A161	0.040	-0.030
K43	-0.010	0.011	V105	-0.014	0.034	A162	0.023	
A44	-0.013		G106	-0.033	0.053	S164	0.036	-0.046
G46	-0.013	0.009	T108	-0.032	0.050	E166	0.024	-0.045
E47	-0.015	0.012	M109	-0.068		M169		-0.168
M48	-0.016	0.016	M111	-0.100		D170		-0.220
K49	-0.023	0.016	L112	-0.116	0.139	F171		-0.295
E50	-0.022		G113	-0.096	0.117	D174	0.233	-0.313
Q51	-0.029	0.021	G114	0.005	-0.009	I175	0.197	-0.281
H55	-0.027	0.032	R115		0.133	F177	0.096	-0.142

Table S3. $\Delta\chi$ tensors determined from the PCSs of Table S2^a

	$\Delta\chi_{ax}/10^{-32} \text{ m}^3$	$\Delta\chi_{rh}/10^{-32} \text{ m}^3$
Tm ³⁺	4.0	2.2
Tb ³⁺	-5.7	-1.7

^a The $\Delta\chi$ tensors were determined from the PCSs of Table S2 by fitting to the coordinates of the chain A of SSB in the crystal structure 1EYG (9). The PCSs were measured at pH 3.4 and 25°C for ¹⁵N-labeled SSB E65C/E69D ligated with either a C1-Tm or a C1-Tb tag, using a sample with C1-Y tag as the diamagnetic reference. The fits were performed using the program Numbat (32), restricting the lanthanide position to be the same for the Tb³⁺ and Tm³⁺ data sets.

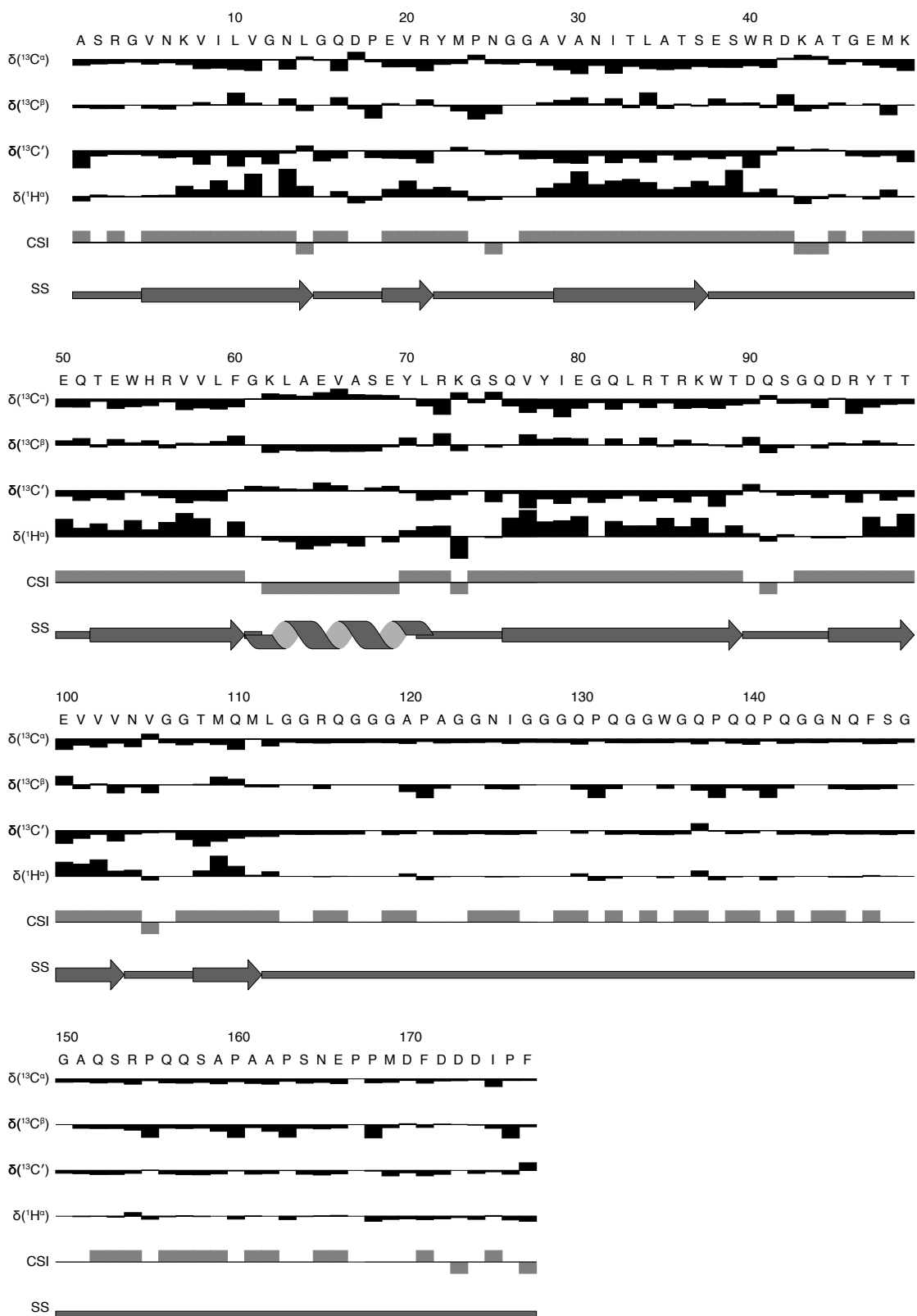


Figure S1. Secondary structure analysis of SSB. The figure was prepared using the program CCPNMR Analysis (30). The rows labeled $\delta(^{13}\text{C}^\alpha)$, $\delta(^{13}\text{C}^\beta)$, $\delta(^{13}\text{C}')$, and $\delta(^1\text{H}^\alpha)$ indicate the chemical shift differences observed for any given residue with respect to the random coil

values of C^α , C^β , C' , and H^α resonances, respectively. The row labeled CSI reports the chemical shift indices (38). The segments of regular secondary structure in the chain A of the crystal structure 1EYG (9) are depicted underneath.

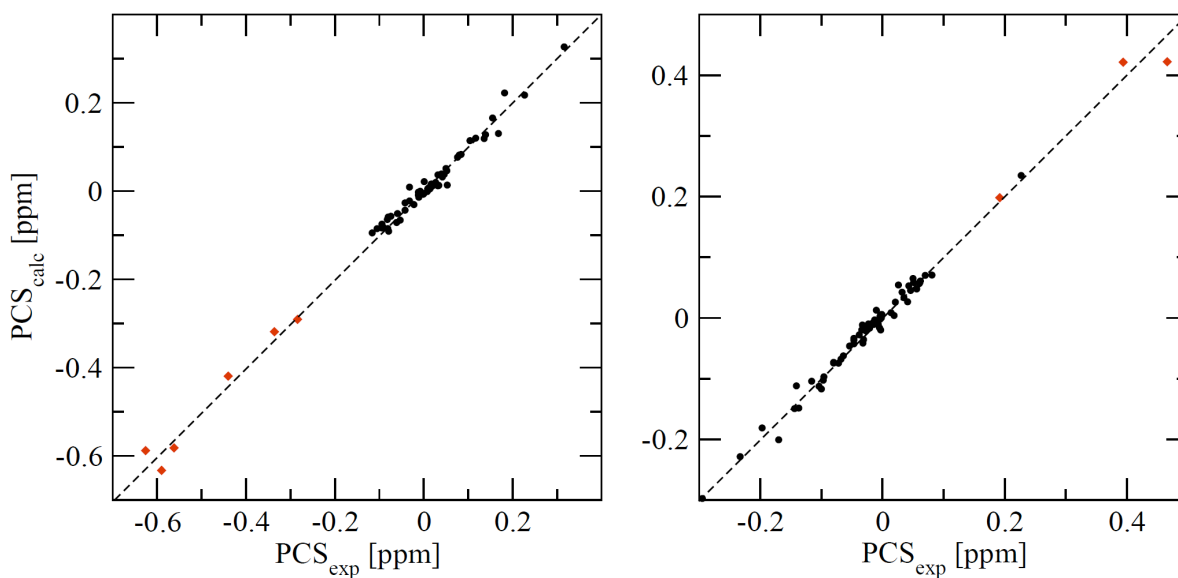


Figure S2. Calculated versus experimental PCSs for the PCS-Rosetta structure that best fulfills the NMR data. The PCS data from residues in the OB-domain are shown in black while PCSs from residues in the C-peptide are shown in red. The left and right panels show the data for Tb^{3+} and Tm^{3+} , respectively.

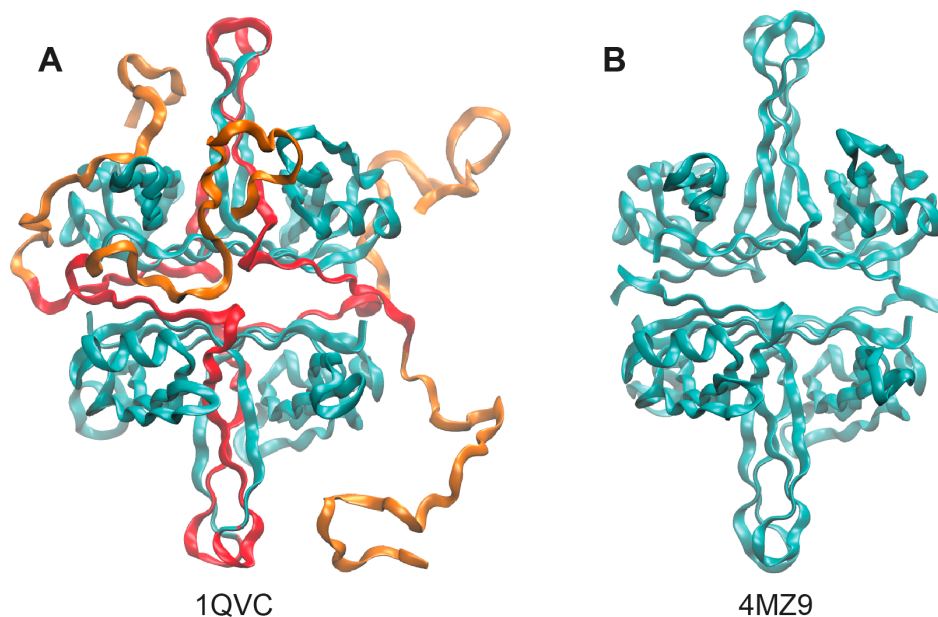


Figure S3. Revision of the 2.2 Å X-ray crystal structure of presumably proteolysed full length SSB (12). (A) Original structure, PDB code 1QVC; $R = 0.247$, $R_{\text{free}} = 0.317$. The OB-fold is shown in cyan, except that regions where the amino acid sequence is out of register with the electron density (from residue 90) are shown in red. The orange segments of the C-domain show weak and discontinuous electron density, with few clear protein–protein or protein–solvent interactions. (B) Revised structure, based on the deposited structure factors (PDB code 4MZ9), showing only the OB-fold (residues 1–114); $R = 0.210$, $R_{\text{free}} = 0.262$.

Supplementary References:

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