SUPPORTING INFORMATION

A functional interplay between intein and extein sequence in protein splicing compensates for the essential block B histidine

Kristina Friedel[1], Monika Popp[2], Julian C. J. Matern[1], Emerich M. Gazdag[2], Ilka V. Thiel[1], Gerrit Volkmann[1], Wulf Blankenfeldt[2,3], Henning D. Mootz*

[1]Institute of Biochemistry
University of Muenster
Wilhelm-Klemm-Str. 2
48149 Münster, Germany

[2]Structure and Function of Proteins
Helmholtz Centre for Infection Research
Inhoffenstraße 7
38124 Braunschweig, Germany

[3]Institute for Biochemistry, Biotechnology and Bioinformatics,
Technische Universität Braunschweig
Spielmannstraße 7
38106 Braunschweig, Germany

* to whom correspondence should be addressed:
E-mail: Henning.Mootz@uni-muenster.de

Supporting Tables

Table S1. Rates of *trans*-splicing

	k_{SP} [$10^{-4}s^{-1}$]	
	M86 Int^C	**M86 Int^C(H73A)**
pep1	13.3 ± 0.9	-
pep2	4.1 ± 0.3	-
pep3	4.3 ± 0.3	-
pep4	5.7 ± 0.7	0.31 ± 0.06
pep5	6.5 ± 0.6	0.33 ± 0.04
pep6	14.8 ± 1.9	0.74 ± 0.11
pep7	22.8 ± 2.3	0.40 ± 0.04
pep8	2.0 ± 0.3	-
pep9	20.0 ± 5.3	0.25 ± 0.05

Table S2: Data collection, processing and refinement statistics of M86 mutants. Values in parentheses are for the highest resolution shell.

Dataset	**M86**	**M86 (G-1F)**	**M86 (G-1F, H73A)**
data collection and processing			
Beamline[¥]	X10SA, SLS	X06DA, SLS	X06DA, SLS
Detector	Pilatus 6M	Pilatus 2MF	Pilatus 2MF
Wavelength (Å)	0.9793	1	1
Resolution range (Å)	37.05-2.03 (2.08-2.03)	43.94-1.54 (1.56-1.54)	48.05-1.22 (1.24-1.22)
Space group	P1	$P4_222$	$P4_322$
Cell dimensions			
a, b, c (Å)	34.6, 40.9, 57.9	71.5, 71.5, 87,8	52.6, 52.6, 118.4
α, β, γ (°)	84.4, 83.5, 65.0	90, 90, 90	90, 90, 90
Mosaicity(°)[#]	0.28	0.11	0.07
Total reflections	62054 (4704)	547813 (27493)	2553000 (119585)
Unique reflections	18028 (1329)	34745 (1683)	49184 (2332)
Mean I/σ (I)	5.8 (1.9)	24.5 (2.0)	28.6 (2.0)
Multiplicity	3.4 (3.5)	15.8 (16.3)	51.9 (51.3)
Completeness (%)	96.8 (96.2)	100.0 (100.0)	98.1 (95.8)
R_{meas} (%)[§]	18.3 (89.7)	6.8 (170.1)	9.5 (321.2)
R_{pim} (%)[$]	9.8 (47.5)	2.3 (58.1)	1.8 (60.4)
$CC_{1/2}$[+]	98.2 (70.6)	100.0 (64.6)	100.0 (69.9)
Wilson B-factor (Å^2)	20.5	18.4	12.7
refinement			
Resolution range (Å)	31.95-2.03	43.94-1.53	48.05-1.22
R_{work} (%)	22.2	14.3	17.5
R_{free} (%)	24.2	17.0	18.8
No. of non-hydrogen atoms			
Protein	2394	1291	1317
Ligand	-	70	-
Water	177	221	248
R.m.s. deviations			
Bonds (Å)	0.002	0.018	0.006
Angles (°)	0.492	1.669	0.934
Average B-factors (Å^2)			
Protein	31.93	26	22
Ligand	-	63	-
Water	34.96	44	35
Ramachandran plot (%)			
Favored regions	98.0	97.6	98.0
Outliers	0	0	0
MolProbity score[‡]	0.98	1.61	1.12
molecules/ASU	2	1	1
PDB code	6FRH	6FRG	6FRE

[¥] SLS: Swiss Light Source (Paul Scherrer Institute, Villigen, Switzerland).

[#] Value as reported by *AIMLESS*.[1]

[+] $CC_{1/2}$ Correlation coefficient between the intensities of two random half data sets.[2]

[§] $R_{meas} = \Sigma_{hkl} (N/(N-1))^{1/2} \Sigma_i | I_i(hkl) - \langle I(hkl)\rangle | / \Sigma_{hkl} \Sigma_i I_i(hkl)$, where N is the number of observations of the reflection with index hkl and I_i is the intensity of its i^th observation.

[$] $R_{pim} = \Sigma_{hkl} (1/(N-1))^{1/2} \Sigma_i | I_i(hkl) - \langle I(hkl)\rangle | / \Sigma_{hkl} \Sigma_i I_i(hkl)$ where N is the multiplicity.[3]

[‡] Value as reported by *MolProbity*.[4]

Table S3. Selected angles and sequences of all available intein crystal structures including flanking residue*

Intein	**Pdb[Ref]**	**native N-Extein (5aa)**	**N-Extein**	**Mutation within intein**	**C-Extein**	**native C-Extein (5aa)**	**τ ($C_{\alpha,-1}$-C‘-N)**	**φ(aa(-1))**	**ψ(aa(-1))**	**ω_1**	**φ(aa(1))**	**ψ(aa(1))**	**τ ($C_{\alpha,1}$-C‘-N)**
Mja KlbA	2JMZ[5]	TGHDG-	MNTGHDG-	N(G:7)A	-**S**SGTLHHHHHH	-CSGTL	112.9°	-93.0°	-169.0°	165.2°	-177.4°	170.8°	107.7°
Mxe GyrA	1AM2[6]	AAMRY-	A-	C(A:1)A	-	-TEAPL	104.9°	-	137.1°	0.7°	-136.8°	164.8°	109.2°
Npu DnaB	4O1R[7]	LRESG-	SGG-	C(A:1)A	-	-SIEQD	113.7° 112.6°	-88.5° 113.3°	171.2° 176.0°	172.1° -170.7°	-133.7° -138.1°	176.6°	110.7°
Npu DnaE	2KEQ[8]	KFAEY-	GG-	C(A:1)A	-	-CFNKS	113.8°	60.2°	-167.0°	172.6°	-158.3°	171.6°	112.2°
Npu DnaE	4KL5[9]	KFAEY-	SGG-	C(A:1)A	-**A**DNG	-CFNKS	113.3°	66.7°	-162.3	171.9°	-169.6°	171.1°	111.4°
Npu DnaE$_{split}$	4LX3[10]	KFAEY-	HHHHHH-	-	-	-CFNKS	102.9°	-137.6°	151.0°	-144.2°	-164.5°	169.7°	111.1°
Pho RadA	4E2U[11]	FGSGK-	SQHM-	C(A:1)A	-**A**Q	-TQLAH	110.3°	-117.5°	145.7°	-174.4°	-140.4°	-35.4°	116.5°
Sce VMAI	1EF0[12]	IIYVG-	MKAEEGKLEG-	C(A:1)A, N(G:7)A	-CGER	-CGERG	108.3° 99.1°	-94.2° -121.2°	139.5° 109.2°	-179.5° -177.2	-157.6° -109.0°	173.6° 124.7°	113.5° 115.0°
Sce VMAI	1JVA[13]	IIYVG-	MSNSDAIIYVG-	C(A:1)S, H(B:10)N, N(G:7)S	-**S**GERGNEMAE	-CGERG	112.3° 110.8°	-61.9° -62.1°	150.7° 144.1°	179.7° -179.1°	-166.3° -148.8°	-177.0° -173.7°	112.9° 111.8°
Sce VMAI	1GPP[14]	IIYVG-	MHHHHHHGSA-	-	-	-CGERG	109.8°	-95.2°	122.9°	-170.3°	-130.4	125.4	113.9°
Ssp DnaB	1MI8[15]	LRESG-	SG-	C(A:1)A, N(G:7)A	-SI	-SIEQD	110.7°	-82.0°	-153.6°	174.4°	173.0°	150.9°	109.5°
M86	6FRH	LRESG-	MLRESG-	C(A:1)A, N(G:7)A	-SIEQDKLGG	-SIEQD	112.6° 112.6°	-102.0° -101.3	-176.1° -176.7°	178.9° 178.5°	-157.0° -157.9°	158.7° 158.8°	108.5° 108.5°
M86(G-1F)	6FRG	LRESG-	MLRESG-	C(A:1)A, N(G:7)A, G(-1)F	-SIEQDKLGG	-SIEQD	106.7°	-125.0°	168.1°	-173.3°	-167.0°	171.6°	111.6°
M86(G-1F, H73A)	6FRE	LRESG-	MLRESG-	C(A:1)A, N(G:7)A, G(-1)F, H(B:10)A	-SIEQDKLGG	-SIEQD	107.0°	-124.0°	52.2°	-158.6°	-76.5°	153.9°	114.1°
Ssp DnaE	1ZDE[16]	KFAEY-	IIAMEKFAEY-	C(A:1)A, N(G:7)A,	-CFNISTGP	-CFNKS	111.2°	-64.3°	165.8°	-179.2°	-158.0°	159.1°	112.2°
Ssp DnaE$_{redox-trapped}$	3NZM[17]	KFAEY-	KSPDPFCPG-	-	-	-CFNKS	114.5°	-123.5°	68.0°	179,1	-127.3°	159.9°	110.4°
Ssp DnaE(T69A)	4GIG[18]	KFAEY-	KSPDPFCPG-	T(B:7)A	-	-CFNKS	115.6°	-91.9°	33.7°	179.2°	-101.1°	154.4°	112.0°
Tvo VMA	4O1S[7]	FGSGK-	SGGK-	C(A:1)A	-**A**	-TVIQH	110.8° 108.9°	-75.6° -69.0°	126.6° 116.5°	179.6° -179.6°	-159.4° -155.1°	170.3° 171.5°	112.0° 110.0°

* a recent paper reporting *Ssp* DnaE structures was not included in the analysis because the extein residues were arranged for cyclization and are therefore likely to be in a highly strained conformation.[19]

Table S4. Expression plasmids and amino acid sequences

Protein	Reference	Expression plasmid	Vector backbone	Sequence (intein sequence underlined)
WT Int^C-Trx-His$_6$	Ref[20]	pCL20	pSU38	MGTSSTGKRVSIKDLLDEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYILKTRLGRTIKATANHRFLTIDGWKRLDELSLKEHIALPRKLESSSLQLSPEIEKLSQSDIYWDSIVSITETGVEEVFDLTVPGPHNFVANDIIVHNSIEGSGGGSDKIIHLTDDSFDTDVLKADGAILVDFWAHWCGPCKMIAPILDEIADEYQGKLTVAKLNIDHNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGSGSRSHHHHHH
WT Int^C(H73A)-Trx-His$_6$	Ref[21]	pJB04	pSU38	WT Int^C with H73A mutation
WT Int^C(H73A, N154A, S+1A))-Trx-His$_6$	This work	pPJ03	pSU38	WT Int^C with H73A, N154A, S+1A mutation
M86 Int^C-Trx-His$_6$	Ref[22]	pIT21	pET16b	MGTSSTGKRVPIKDLLGEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYTLKTRLGRTIKATANHRFLTIDGWKRLDELSLKEHIALPRKLESSSLQLAPEIEKLPQSDIYWDPIVSITETGVEEVFDLTVPGLRNFVANDIIVHNSIEGSGGGSDKIIHLTDDSFDTDVLKADGAILVDFWAHWCGPCKMIAPILDEIADEYQGKLTVAKLNIDHNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLAGSVDRSHHHHHH
M86Int^C(H73A)-Trx-His$_6$	This work	pKF14	pET16b	M86 Int^C with H73A mutation
M86 Int^C(H73A, N154A, S+1A))-Trx-His$_6$	Ref[22]	pIT28	pET16b	M86 Int^C with H73A, N154A, S+1A mutation
MBP-WT-Trx	This work	pKF18	pMST[23]	MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRGTLESGCISGDSLISLASTGKRVSIKDLLDEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYILKTRLGRTIKATANHRFLTIDGWKRLDELSLKEHIALPRKLESSSLQLSPEIEKLSQSDIYWDSIVSITETGVEEVFDLTVPGPHNFVANDIIVHNSIEGSGGTGMSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLA
MBP-WT(H73A)-Trx	This work	pKF28	pMST	WT intein with H73A mutation
MBP-WT(H73A, N154A, S+1A)-Trx	This work	pKF23	pMST	WT intein with H73A, N154A, S+1A mutation
MBP-M86-Trx	Ref[24]	pAba12	pMST	MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIEGRGTLESGCISGDSLISLASTGKRVPIKDLLGEKDFEIWAINEQTMKLESAKVSRVFCTGKKLVYTLKTRLGRTIKATANHRFLTIDGWKRLDELSLKEHIALPRKLESSSLQLAPEIEKLPQSDIYWDPIVSITETGVEEVFDLTVPGLRNFVANDIIVHNSIEGSGGTGMSDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLA
MBP-M86(H73A)-Trx	This work	pKF21	pMST	M86 intein with H73A mutation
MBP-M86(H73A, N154A, S+1A)-Trx	This work	pKF27	pMST	M86 intein with H73A, N154A, S+1A mutation
MBP-WT(G-1A)-Trx	This work	pKF190	pMST	WT intein with G-1A mutation
MBP-WT(G-1A, H73A)-Trx	This work	pKF193	pMST	WT intein with G-1A, H73A mutation
MBP-WT(G-1A, H73A, N154A, S+1A)-Trx	This work	pKF205	pMST	WT intein with G-1A, H73A, N154A, S+1A mutation

MBP-M86(G-1A)-Trx	This work	pKF191	pMST	M86 intein with G-1A mutation
MBP-M86(G-1A, H73A)-Trx	This work	pKF192	pMST	M86 intein with G-1A, H73A mutation
MBP-M86(G-1A, H73A, N154A, S+1A)-Trx	This work	pKF207	pMST	M86 intein with G-1A, H73A, N154A, S+1A mutation
MBP-WT(G-1T)-Trx	This work	pKF213	pMST	WT intein with G-1T mutation
MBP-WT(G-1T, H73A)-Trx	This work	pKF215	pMST	WT intein with G-1T, H73A mutation
MBP-WT(G-1T, H73A, N154A, S+1A)-Trx	This work	pKF194	pMST	WT intein with G-1T, H73A, N154A, S+1A mutation
MBP-M86(G-1T)-Trx	This work	pKF195	pMST	M86 intein with G-1T mutation
MBP-M86(G-1T, H73A)-Trx	This work	pKF208	pMST	M86 intein with G-1T, H73A mutation
MBP-M86(G-1T, H73A, N154A, S+1A)-Trx	This work	pKF196	pMST	M86 intein with G-1T, H73A, N154A, S+1A mutation
MBP-WT(G-1L)-Trx	This work	pKF197	pMST	WT intein with G-1L mutation
MBP-WT(G-1L, H73A)-Trx	This work	pKF198	pMST	WT intein with G-1L, H73A mutation
MBP-WT(G-1L, H73A, N154A, S+1A)-Trx	This work	pKF199	pMST	WT intein with G-1L, H73A, N154A, S+1A mutation
MBP-M86(G-1L)-Trx	This work	pKF200	pMST	M86 intein with G-1L mutation
MBP-M86(G-1L, H73A)-Trx	This work	pKF201	pMST	M86 intein with G-1L, H73A mutation
MBP-M86(G-1L, H73A, N154A, S+1A)-Trx	This work	pKF203	pMST	M86 intein with G-1L, H73A, N154A, S+1A mutation
MBP-WT(G-1H)-Trx	This work	pKF55	pMST	WT intein with G-1H mutation
MBP-WT(G-1H, H73A)-Trx	This work	pKF56	pMST	WT intein with G-1H, H73A mutation
MBP-WT(G-1H, H73A, N154A, S+1A)-Trx	This work	pKF58	pMST	WT intein with G-1H, H73A, N154A, S+1A mutation
MBP-M86(G-1H)-Trx	This work	pKF63	pMST	M86 intein with G-1H mutation
MBP-M86(G-1H, H73A)-Trx	This work	pKF64	pMST	M86 intein with G-1H, H73A mutation
MBP-M86(G-1H, H73A, N154A, S+1A)-Trx	This work	pKF66	pMST	M86 intein with G-1H, H73A, N154A, S+1A mutation
MBP-WT(G-1F)-Trx	This work	pKF59	pMST	WT intein with G-1F mutation
MBP-WT(G-1F, H73A)-Trx	This work	pKF60	pMST	WT intein with G-1F, H73A mutation
MBP-WT(G-1F, H73A, N154A, S+1A)-Trx	This work	pKF62	pMST	WT intein with G-1F, H73A, N154A, S+1A mutation
MBP-M86(G-1F)-Trx	This work	pKF67	pMST	M86 intein with G-1F mutation
MBP-M86(G-1F, H73A)-Trx	This work	pKF68	pMST	M86 intein with G-1F, H73A mutation
MBP-M86(G-1F, H73A, N154A, S+1A)-Trx	This work	pKF70	pMST	M86 intein with G-1F, H73A, N154A, S+1A mutation

Table S5. ESI-MS-analysis of Int^N-peptides

	$[M+H]^+$ [Da]	**$[M+2H]^{2+}$ [Da]**		**$[M+3H]^{3+}$ [Da]**	
	calc.	**calc.**	**obs.**	**calc.**	**obs.**
pep1	1967.1	984.1	983.6	656.4	656.2
pep2	1981.2	991.1	990.6	661.1	660.8
pep3	1995.2	998.1	997.7	665.7	665.5
pep4	2011.2	1006.1	1005.6	671.1	670.8
pep5	2023.2	1012.1	1011.6	675.1	674.9
pep6	2047.2	1024.1	1023.6	683.1	682.8
pep7	2057.3	1029.1	1028.7	686.4	686.2
pep8	2023.2	1012.1	1011.6	675.1	674.8
pep9	2057.3	1029.1	1028.7	686.4	686.2

Supporting Figures

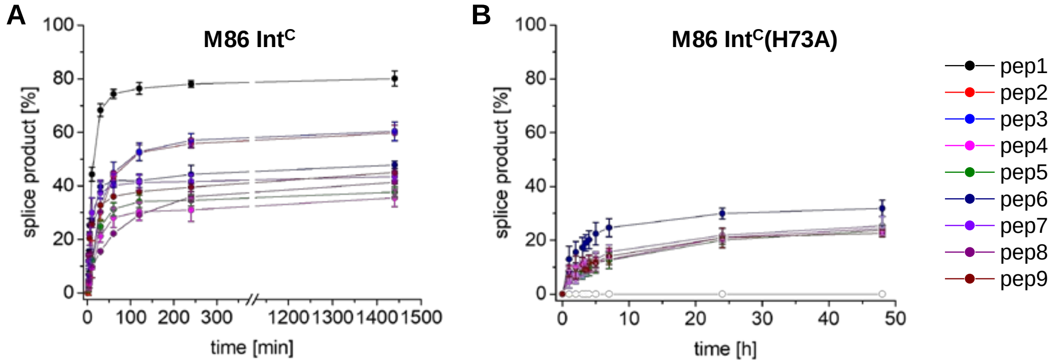

Figure S1. Time-courses of semi-synthetic protein *trans*-splicing. Reaction schemes are as shown in Figure 1A. Shown are the time-dependent analyses of the reactions as described in Figures 1 and 4. Aliquots were removed from the reaction mixtures at the indicated time points and analyzed. Yields of protein *trans*-splicing were determined by densitometric analysis of Coomassie-stained SDS-PAGE gels. Error-bars indicate standard deviations.

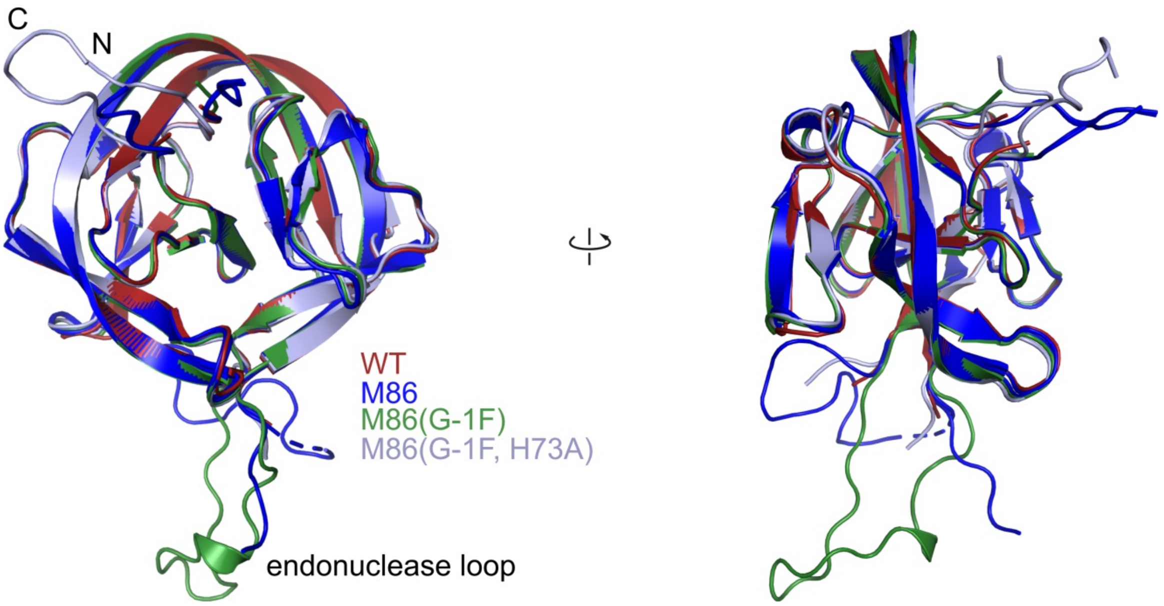

Figure S**2: Illustration of endonuclease loop and extein sequences.** Shown are two perpendicular views of an overlay of WT *Ssp* DnaB (PDB entry 1MI8; red), the two copies of M86 contained in the asymmetric unit of the crystal form investigated in this study (blue), M86(G-1F) (green) and M86(G-1F, H73) (light blue). Note the different orientations of the endonuclease loop, which is a consequence of different crystal environments.

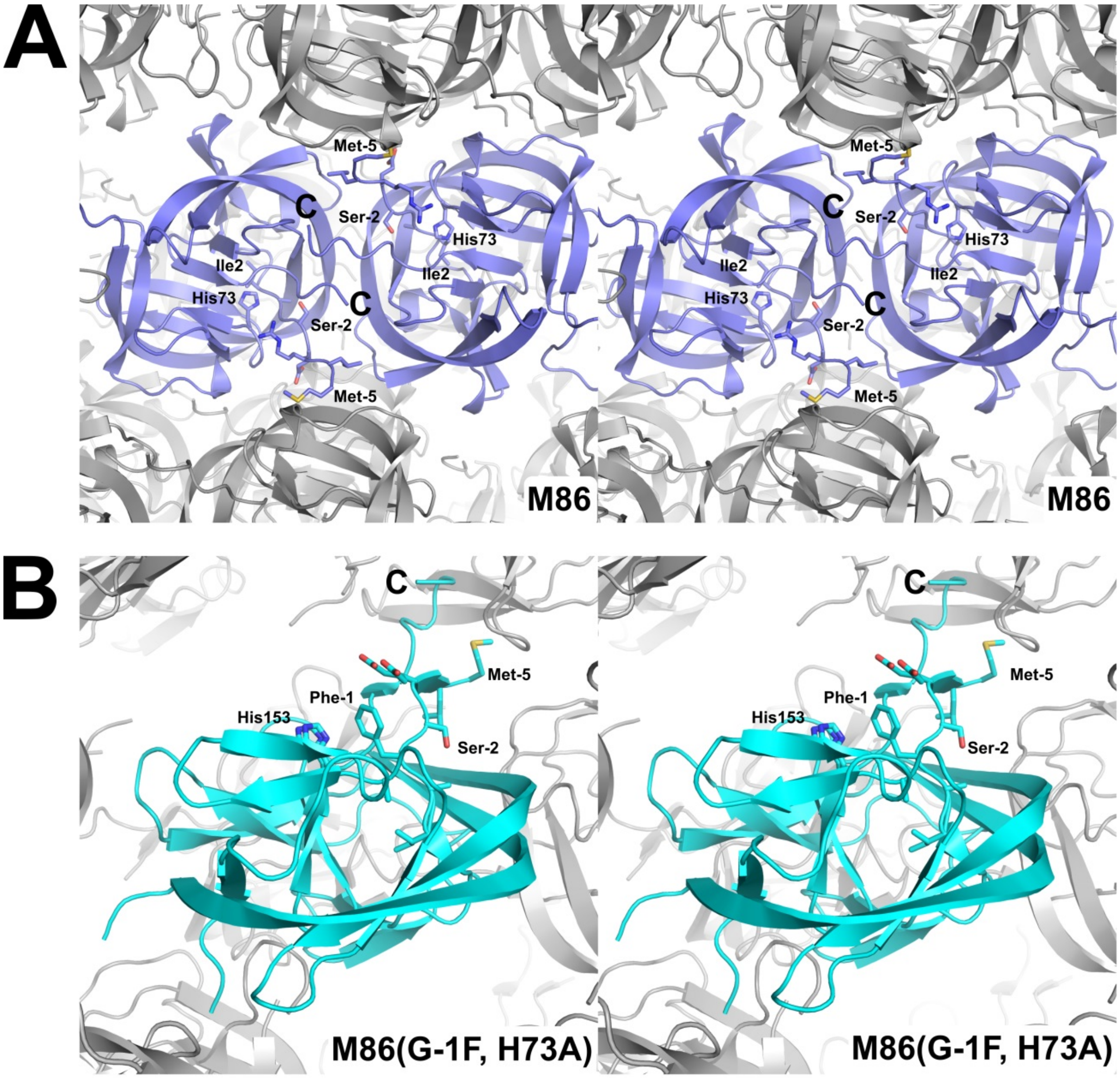

Figure S3. Cross-eyed stereo plots of the crystal packing environments of the N- and C-terminal regions. (A) M86 (blue) and (B) M86(G-1F, H73A) (cyan). The termini of WT *Ssp* DnaB (PDB entry 1MI8) and of M86(G-1F) are not involved in crystal contacts (not shown).

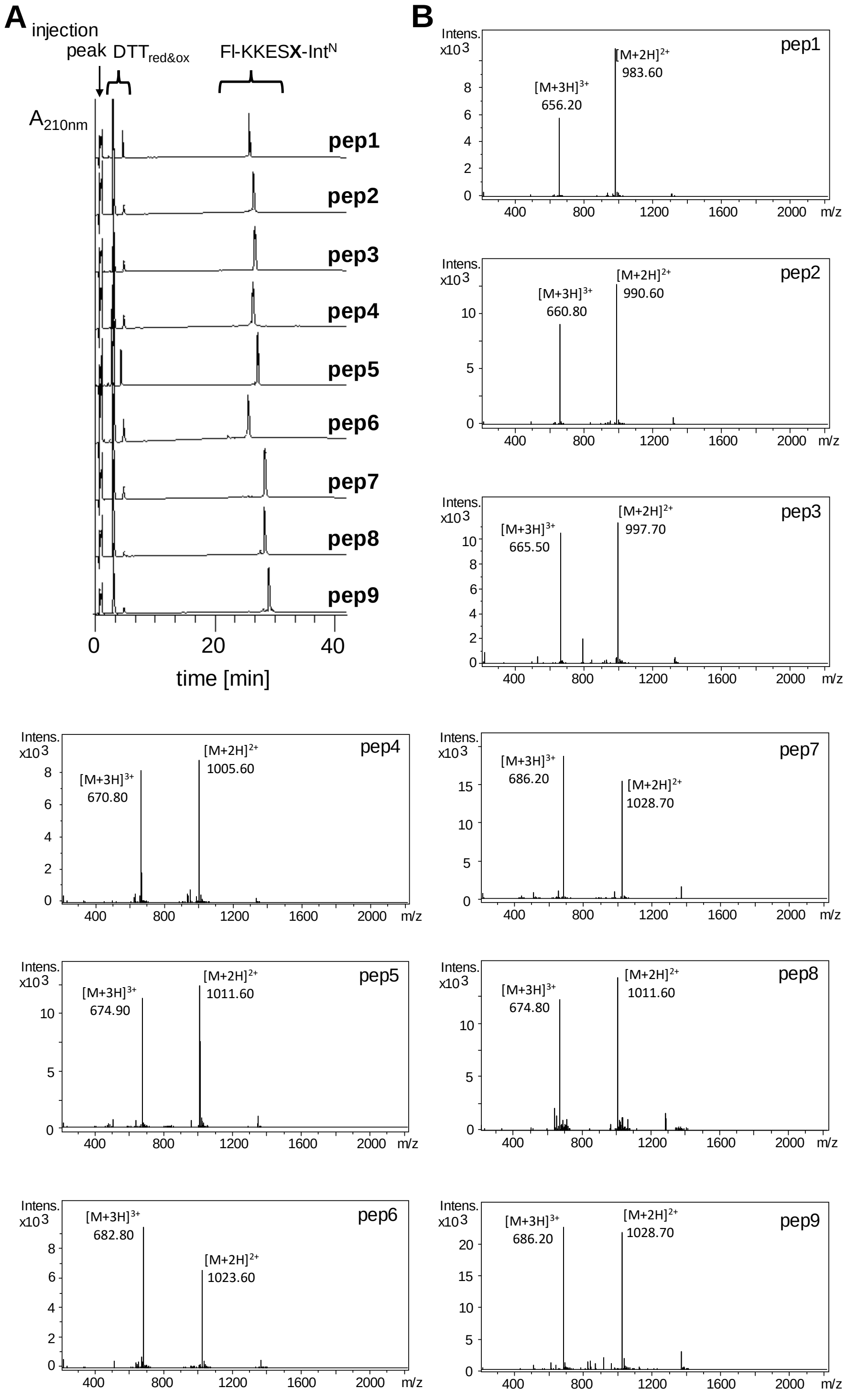

Figure S4. Purity of Int^{N}-peptides. (**A**) Shown are analytical HPLC traces. Note that the peptides appear as a double peak due to the 5,6-isomers of the carboxyfluoresceine moiety. (**B**) ESI-MS analysis. See Table S5 for an overview of observed and calculated masses. The contamination in **pep3** has a mass of 1589 Da (observed).

Supporting References

1. P. R. Evans and G. N. Murshudov, *Acta Crystallogr D Biol Crystallogr*, 2013, **69**, 1204-1214.
2. P. A. Karplus and K. Diederichs, *Science*, 2012, **336**, 1030-1033.
3. M. S. Weiss, *J. Appl. Cryst.*, 2001, **34**, 130-135.
4. V. B. Chen, W. B. Arendall, 3rd, J. J. Headd, D. A. Keedy, R. M. Immormino, G. J. Kapral, L. W. Murray, J. S. Richardson and D. C. Richardson, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 12-21.
5. M. A. Johnson, M. W. Southworth, T. Herrmann, L. Brace, F. B. Perler and K. Wuthrich, *Protein Sci*, 2007, **16**, 1316-1328.
6. T. Klabunde, S. Sharma, A. Telenti, W. R. Jacobs, Jr. and J. C. Sacchettini, *Nat Struct Biol*, 1998, **5**, 31-36.
7. A. S. Aranko, J. S. Oeemig, D. Zhou, T. Kajander, A. Wlodawer and H. Iwai, *Mol Biosyst*, 2014, **10**, 1023-1034.
8. J. S. Oeemig, A. S. Aranko, J. Djupsjobacka, K. Heinamaki and H. Iwai, *FEBS Lett*, 2009, **583**, 1451-1456.
9. A. S. Aranko, J. S. Oeemig, T. Kajander and H. Iwai, *Nat Chem Biol*, 2013, **9**, 616-622.
10. Q. Wu, Z. Gao, Y. Wei, G. Ma, Y. Zheng, Y. Dong and Y. Liu, *Biochem J*, 2014, **461**, 247-255.
11. J. S. Oeemig, D. Zhou, T. Kajander, A. Wlodawer and H. Iwai, *J Mol Biol*, 2012, **421**, 85-99.
12. B. W. Poland, M. Q. Xu and F. A. Quiocho, *J Biol Chem*, 2000, **275**, 16408-16413.
13. R. Mizutani, S. Nogami, M. Kawasaki, Y. Ohya, Y. Anraku and Y. Satow, *J Mol Biol*, 2002, **316**, 919-929.
14. E. Werner, W. Wende, A. Pingoud and U. Heinemann, *Nucleic Acids Res*, 2002, **30**, 3962-3971.
15. Y. Ding, M. Q. Xu, I. Ghosh, X. Chen, S. Ferrandon, G. Lesage and Z. Rao, *J Biol Chem*, 2003, **278**, 39133-39142.
16. P. Sun, S. Ye, S. Ferrandon, T. C. Evans, M. Q. Xu and Z. Rao, *J Mol Biol*, 2005, **353**, 1093-1105.
17. B. P. Callahan, N. I. Topilina, M. J. Stanger, P. Van Roey and M. Belfort, *Nat Struct Mol Biol*, 2011, **18**, 630-633.
18. A. K. Dearden, B. Callahan, P. V. Roey, Z. Li, U. Kumar, M. Belfort and S. K. Nayak, *Protein Sci*, 2013, **22**, 557-563.
19. L. M. Kick, S. Harteis, M. F. Koch and S. Schneider, *Chembiochem*, 2017, **18**, 2242-2246.
20. C. Ludwig, M. Pfeiff, U. Linne and H. D. Mootz, *Angew Chem Int Ed Engl*, 2006, **45**, 5218-5221.
21. J. Binschik and H. D. Mootz, *Angew Chem Int Ed Engl*, 2013, **52**, 4260-4264.
22. J. H. Appleby-Tagoe, I. V. Thiel, Y. Wang, Y. Wang, H. D. Mootz and X. Q. Liu, *J Biol Chem*, 2011, **286**, 34440-34447.
23. H. Wu, M. Q. Xu and X. Q. Liu, *Biochim Biophys Acta*, 1998, **1387**, 422-432.
24. J. K. Bocker, K. Friedel, J. C. Matern, A. L. Bachmann and H. D. Mootz, *Angew Chem Int Ed Engl*, 2015, **54**, 2116-2120.