Supplementary materials

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Spatial attributes of the four-helix bundle group of bacteriocins - the high-resolution structure of BacSp222 in solution

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Table S1

Relevant parameters used for acquisition of NMR spectra.

Experiment (total experiment time)	Number of scans	Sparse, %	Nucleus	Maximum evolution time [ms]	Spectral width [Hz]
HNCO (4.86 h)	4	12.50	^{1}H	85.0	12020.0
			^{13}C	25.0	4000.0
			^{15}N	25.6	2500.0
(HACA)CONH (20.05 h)	4	51.60	^{1}H	85.0	12020.0
			^{13}C	25.0	4000.0
			^{15}N	25.6	2500.0
HN(CO)CA (6.3 h)	4	37.50	^{1}H	85.0	12020.0
			^{13}C	9.5	5282.0
			^{15}N	25.6	2500.0
HNCA (12.6 h)	4	75.00	^{1}H	85.0	12020.0
			^{13}C	9.5	5282.0
			^{15}N	25.6	2500.0
HNCACB (16.6 h)	8	31.25	^{1}H	85.0	12020.0
			^{13}C	6.4	12325.0
			^{15}N	25.6	2500.0
CBCA(CO)NH (8.3 h)	8	15.60	^{1}H	85.0	12020.0
			^{13}C	6.4	12325.0
			^{15}N	25.6	2500.0
HAHB](CO)NH (8.75 h)	8	19.50	^{1}H	85.0	12020.0
			^{1}H	5.3	12020.0
			^{15}N	25.6	2500.0
C(CO)NH (17.6 h)	8	9.70	^{1}H	85.0	12020.0
			^{13}C	9.1	14084.0
			^{15}N	25.6	2500.0
^{15}N-edited NOESYHSQC (47.4 h), mixing time (150 ms)	16	9.20	^{1}H	85.0	12020.0
			^{1}H	14.1	9101.4
			^{15}N	51.0	2500.0
^{13}C-edited NOESYHSQC (64.4 h), tuned to aromatic carbons mixing time (150 ms)	8	36.00	^{1}H	85.0	12020.0
			^{1}H	17.5	9101.4
			^{13}C	7.0	8500.0
^{13}C-edited NOESYHSQC (32 h), tuned to aliphatic carbons mixing time (150 ms)	8	12.50	^{1}H	85.0	12020.0
			^{1}H	14.1	9101.4
			^{13}C	9.0	14084.5
H(C)CH-TOCSY (15.5 h)	8	6.20	^{1}H	85.0	12020.0
			^{1}H	9.1	7306.5
			^{13}C	25.6	14084.0
^{1}H-^{13}C HSQC tuned to aromatic carbons (0.6 h)	16	100	^{1}H	85.0	12020.0
			^{13}C	9.0	7040.0
^{1}H-^{13}C HSQC tuned to aliphatic carbons (0.6 h)	4	100	^{1}H	85.0	12020.0
			^{13}C	16.0	16000.0
^{1}H-^{15}N HSQC (0.6 h)	8	100	^{1}H	85.0	12020.0
			^{15}N	102.4	2500.0
(HB)CB(CGCD)HD (10h)	256	100	^{1}H	85.0	12020.0
			^{13}C	7.9	5281.9
(HB)CB(CGCDCE)HE (10h)	256	100	^{1}H	85.0	12020.0
			^{13}C	7.9	5281.9

Table S2

The list of peptides and proteins possessing significant (Z-score equal or greater than 3.0) degree of structural similarity to BacSp222 fold (PDB entry 5lwc), generated by Dali server. Peptides are distinguished **bold**, procaryotic proteins are underlined, while eukaryotic proteins are written using regular characters.

No.	PDB entry	Z-score	RMSD	Number of residues	Percent of identical residues	Description
1	**2n8o-A**	**5.4**	**2.1**	**51**	**27**	**BACTERIOCIN AUREOCIN A53**
2	1i2t-A	5.0	3.1	61	6	HYD PROTEIN
3	**2n8p-A**	**4.9**	**2.2**	**53**	**25**	**LACTICIN Q**
4	3ntw-C	4.6	3.3	56	8	E3 UBIQUITIN-PROTEIN LIGASE UBR5
5	3kus-B	4.5	3.2	70	8	POLYADENYLATE-BINDING PROTEIN 1
6	**2m5z-A**	**4.2**	**2.2**	**44**	**18**	**ENTEROCIN 7A**
7	3f6t-B	4.1	2.3	517	2	ASPARTATE AMINOTRANSFERASE
8	**2m60-A**	**3.8**	**2.4**	**43**	**21**	**ENTEROCIN 7B**
9	2zy5-D	3.7	2.4	511	8	L-ASPARTATE BETA-DECARBOXYLASE
10	2lkl-A	3.6	2.1	81	9	ERYTHROCYTE MEMBRANE PROTEIN 1 (PFEMP1)
11	4yxy-B	3.5	3.0	91	19	DTOR_9X31L
12	2mj5-B	3.5	2.4	52	14	Ubiquitin-associated (UBA)
13	2zy2-A	3.4	2.4	521	8	L-ASPARTATE 4-CARBOXYLYASE
14	3fri-A	3.4	2.9	242	13	16S RRNA METHYLASE
15	1h3l-A	3.3	2.3	75	11	RNA POLYMERASE SIGMA FACTOR
16	5he9-E	3.2	2.9	56	12	HELICASE LOADER
17	4ae4-B	3.1	2.3	115	13	UBIQUITIN-ASSOCIATED PROTEIN 1
18	3b74-A	3.1	2.5	307	15	UNCHARACTERIZED PROTEIN YKL091C
19	3q8g-A	3.1	2.5	306	15	CRAL-TRIO DOMAIN-CONTAINING PROTEIN
20	2o7g-B	3.1	2.3	88	4	PROBABLE RNA POLYMERASE SIGMA-C FACTOR
21	2n5l-A	3.0	2.5	57	12	RIBONUCLEASE ZC3H12A

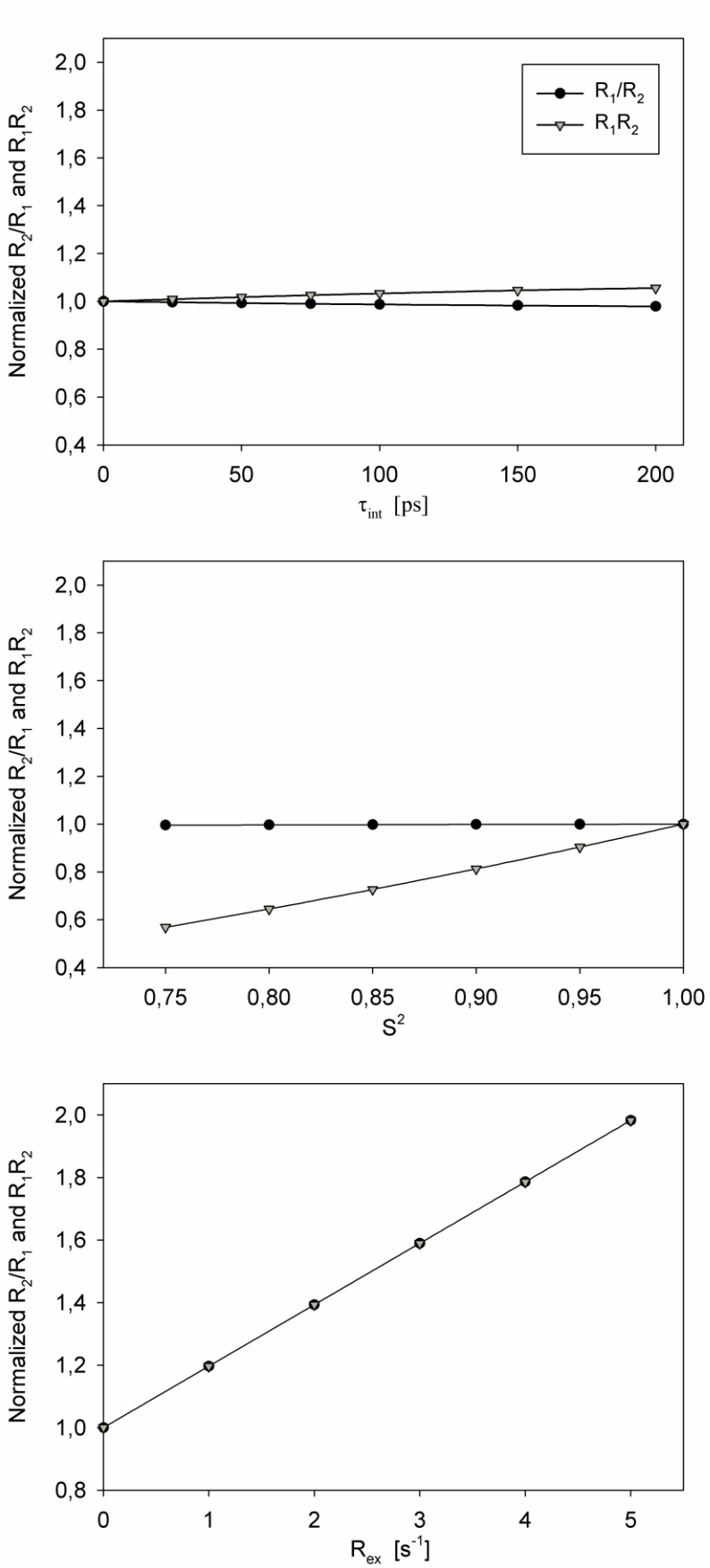
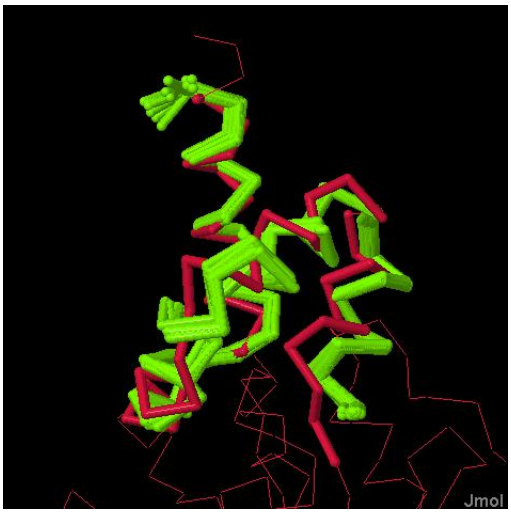
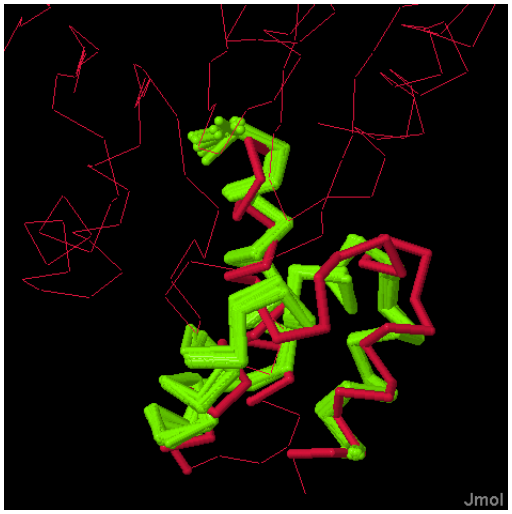


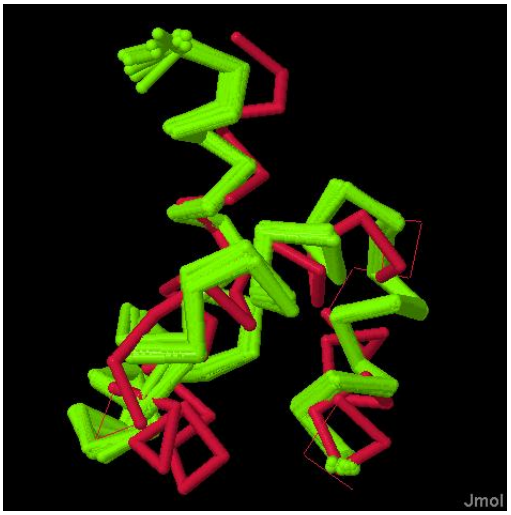
Fig. S1. Calculated relationships between normalized R_2/R_1 and R_1R_2 and local parameters of MFA. $R_2/R_1(\tau_{int})$ and $R_1R_2(\tau_{int})$ functions – upper panel, $R_2/R_1(S^2)$ and $R_1R_2(S^2)$ functions – central panel, $R_2/R_1(R_{ex})$ and $R_1R_2(R_{ex})$ – lower panel. Input data: τ_R=3.1 ns and B_0=16.4 T were used in calculations. Upper panel S^2=0.90, R_{ex}=0; central panel τ_{int}=10 ps, R_{ex}=0; lower panel: τ_{int}=10 ps, S^2=0.90



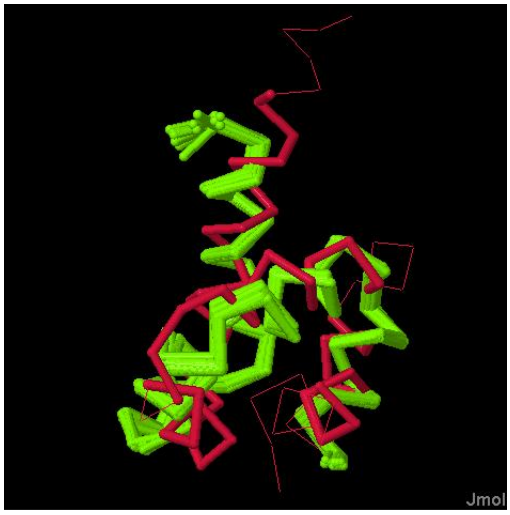
L-aspartate beta-decarboxylase (PDB entry 2zy5)



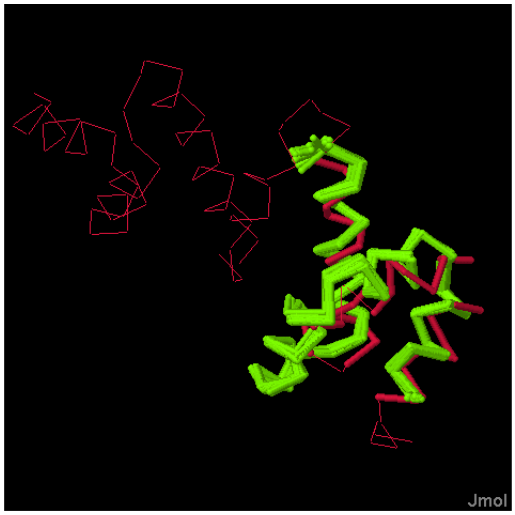
16s rRNA methylase (PDB entry 3fri)



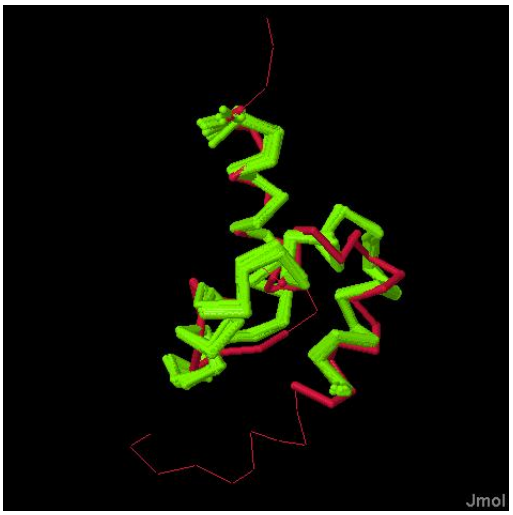
E3 ubiquitin-protein ligase (PDB entry 3ntw)



Polyadenylate-binding protein 1 (PDB entry 3kus)



Ubiquitin-associated protein 1 (PDB entry 4ae4)



Regnase (MCPIP-1) (PDB entry 2n5l)

Fig. S2. Images showing the similarity of the BacSp222 fold (green) and selected particular domains of prokaryotic and eukaryotic proteins (red), which are listed in Supplementary Materials Table S2 and described in the main text of the manuscript. All the overlaid stick models were generated using the Dali server and presented from the same perspective, looking from the N-terminal alpha-helix of the BacSp222 molecule, located in the right bottom corner (identically as presented in the left panel of Fig. 2A). The end of the C-terminal helix is located underneath the structures, in the left upper corner of each image.