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Supporting information for article:

Structure of the double-stranded DNA-binding type IV secretion protein TraN from *Enterococcus*

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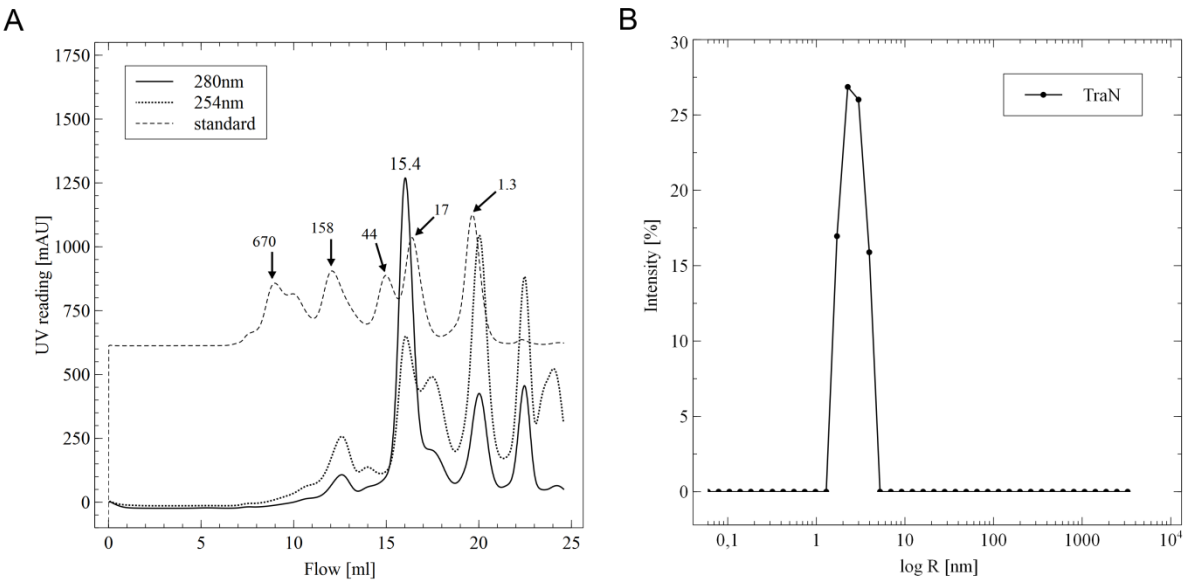
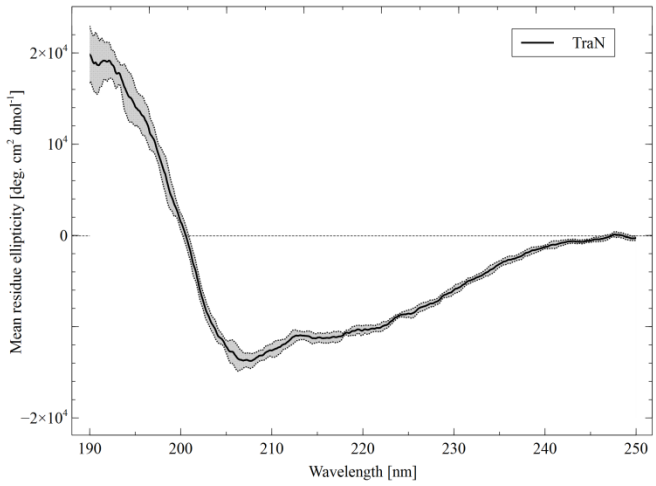


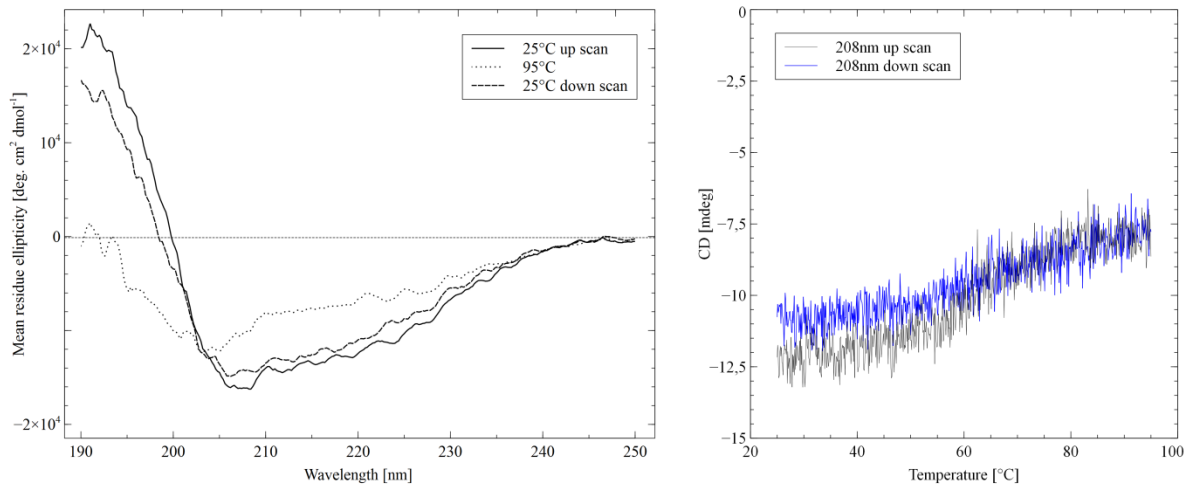
Figure S1 Biophysical characterization of TraN. **A:** TraN elutes as a single peak from the Superdex 200 size exclusion column; 280 nm (black) and 254 nm (dotted) readings are shown; a standard is shown with its molecular weight (discontinued). **B:** In the mono-dispersity analysis via DLS, TraN appears as a narrow peak.



(a)

Secondary structure	Amount [%]
Alpha helices	34
Beta strands	19
Turns	19
Unordered	27

(b)



(c)

Figure S2 CD analysis of TraN. **A:** TraN is folded in solution; the black curve represents the average of 10 individual wavelength scans; the standard deviation is displayed as a grey area. **B:** Secondary structure content of TraN; the NRMSD is 0.018. **C:** TraN unfolding and refolding characteristics. The CD spectra are shown at 25 and 95 °C and after cooling to 25 °C (left panel). The temperature scan (up- and down-scan) is shown in the right panel.

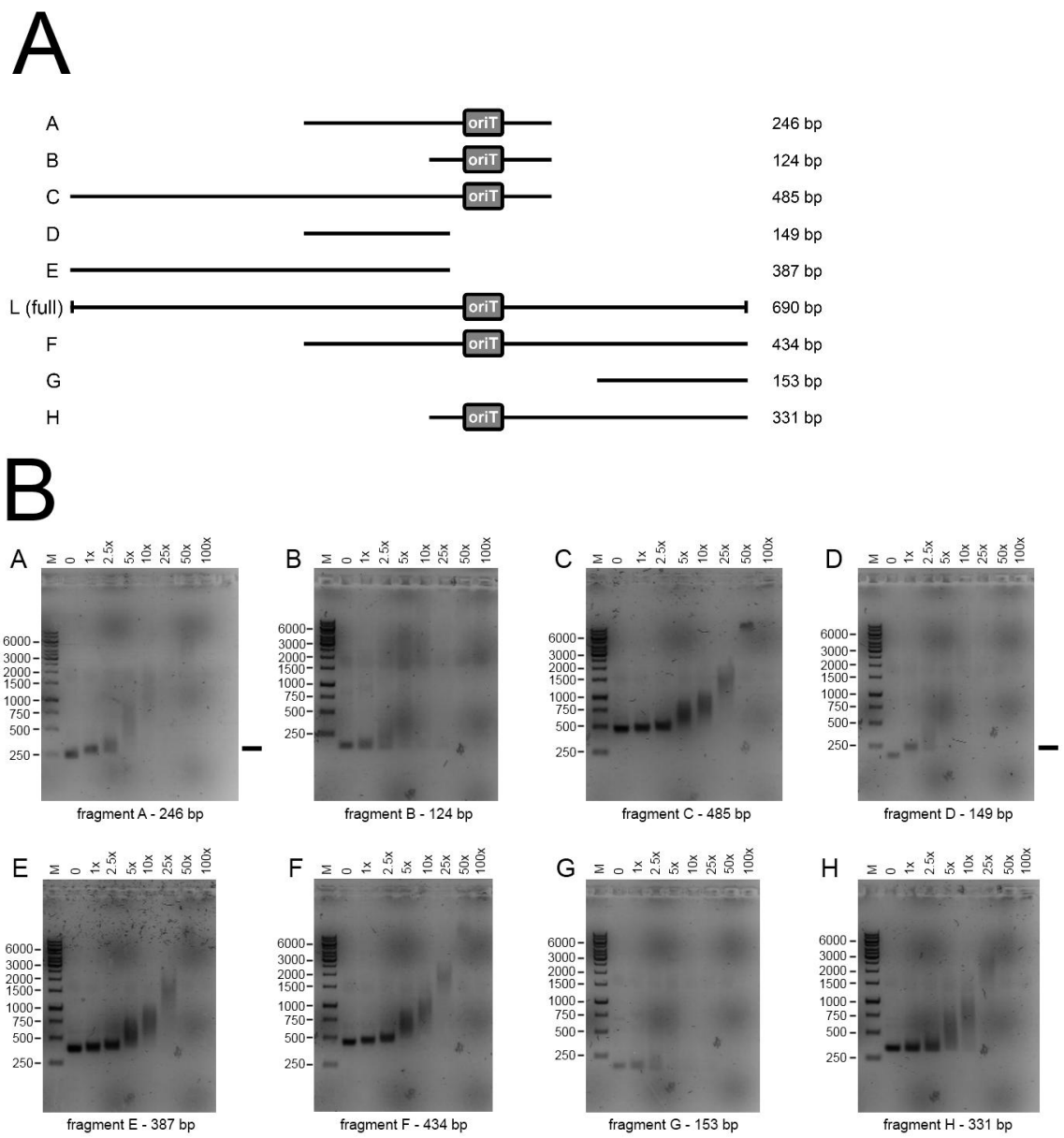


Figure S3 The putative TraN binding region. **A:** The *oriT* upstream/downstream fragments used in EMSA experiments. The pIP501 *oriT* sequence is indicated; the length of the fragments is given. **B:** TraN specifically shifts fragments A and D already at an equimolar protein :: DNA ratio; all fragments are co-operatively shifted at higher TraN concentrations; lane M: molecular weight standard (GeneRuler 1 kb DNA Ladder, ThermoFisher Scientific, Waltham, Massachusetts, USA).

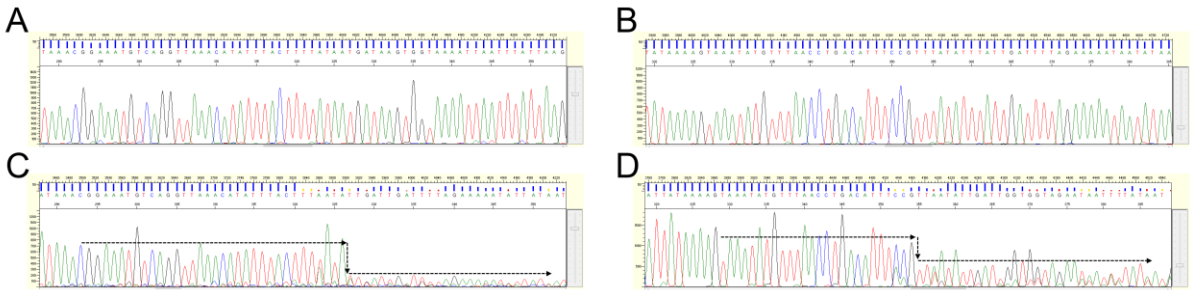


Figure S4 Exemplary data set for identification of the TraN preferred binding site. Sequencing chromatograms of fragment L forward strand (**A, C**) and reverse strand (**B, D**) with no (**A, B**), respectively 15 minutes (**C, D**) T4 exonuclease digestion. Dashed lines were added to highlight the decrease of the signal intesity. The sequencing data were analyzed with free Sequence Scanner v1.0 software from Applied Biosystems.

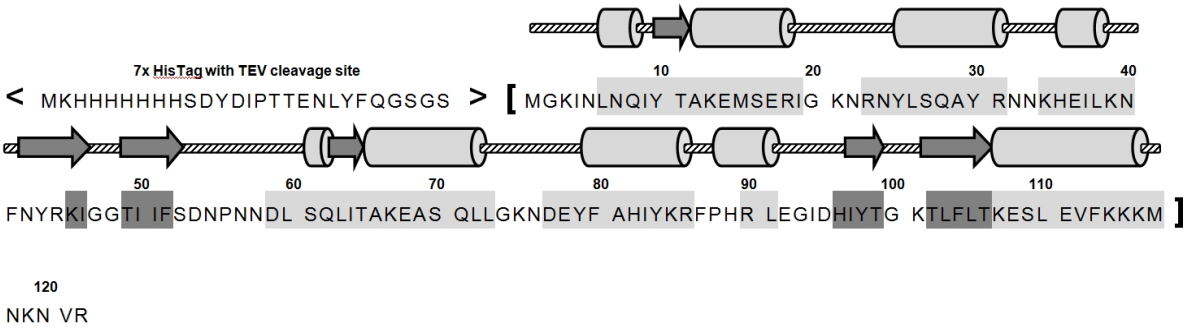


Figure S5 The TraN aa sequence and the TraN construct. Secondary structure features predicted by PsiPred are marked in light grey (helices) and dark grey (beta sheets); the 7xHis-tag was lost during crystallization; 5 residues on the C-terminus are not visible in the crystal structure; square brackets highlight the sequence present in the X-ray structure; secondary structure elements found in the X-ray model are shown as light grey tubes (helices) and dark grey arrows (beta sheets).

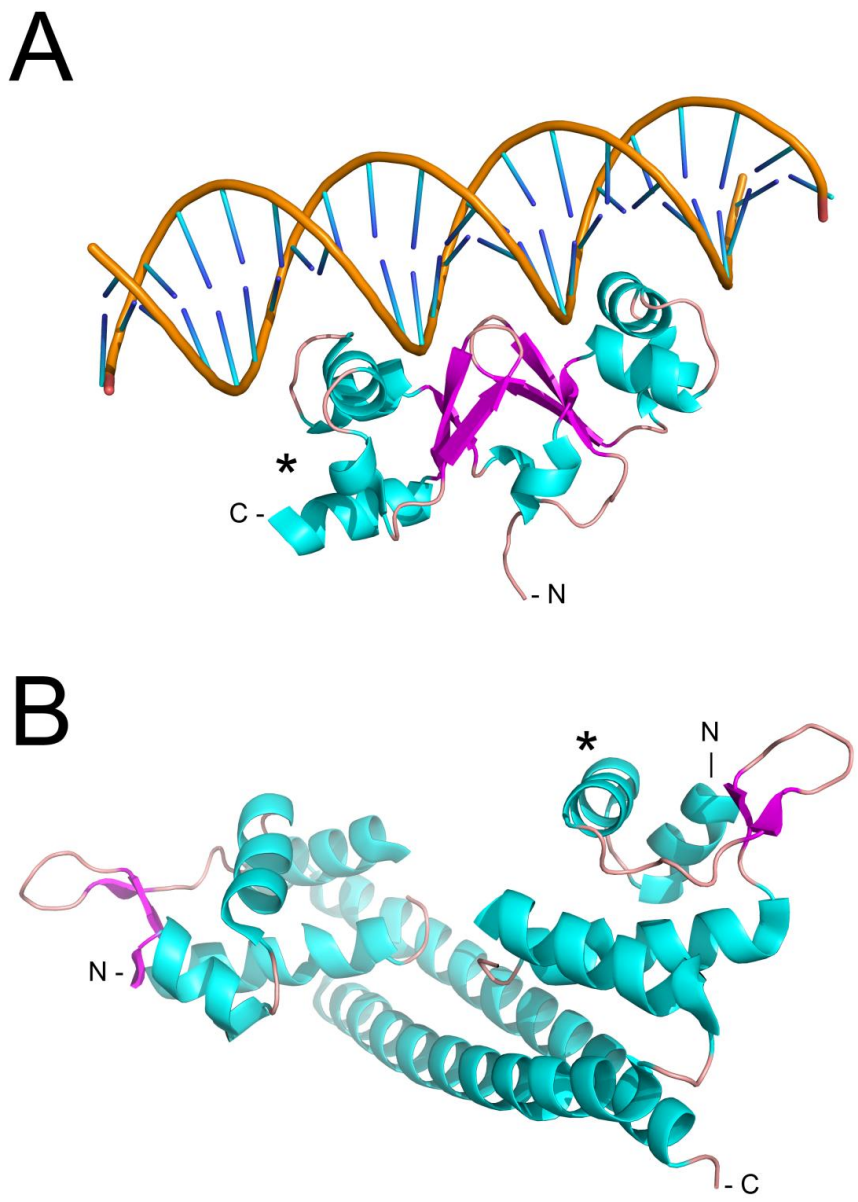


Figure S6 Proposed binding mode of TraN to dsDNA and comparison to a MerR dimer. **A:** Proposed binding mode of TraN to random ds B-DNA. Both molecules are shown in cartoon representation with secondary structure elements highlighted (helices in cyan, strands in purple). The 3D model of the DNA was generated with the DNA structure modelling server 3D-DART (van Dijk and Bonvin, 2009) and docked manually to the TraN model in PyMOL. **B:** Cartoon representation of the MerR dimer structure (PDB: 3GPV) with secondary structure elements highlighted (helices in cyan, strands in purple). The two MerR molecules form the quaternary structure via a two-helix coiled-coil motif. The two DNA interaction motifs (the TraN-like half sites) are placed on the opposite sides of the dimer structure with the primary DNA interaction helices in parallel, as in the TraN structure. An asterisk (*) marks the two structurally corresponding HTH domains of TraN (**A**) and MerR (**B**), respectively.

Type	Gram	Species	System	Protein
α	+	*Enterococcus faecalis*	pIP501	TraN
	+			crystal
	+	*Enterococcus faecalis*	pRE25	Orf38
	+	*Enterococcus faecalis*	pAM-beta1	OrfB
	+	*Enterococcus faecium*	pVEF3	P52
	+	*Enterococcus italicus*	DSM15952 (genomic)	HMPREF9088_2233
	+	*Enterococcus faecalis*	7L76 (genomic)	ENT03500

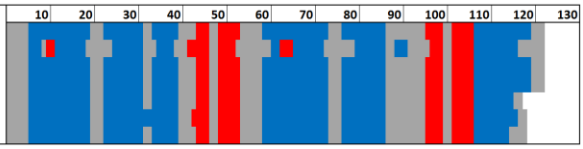


Figure S7 Results of the secondary structure based search for TraN-like proteins. Secondary structure (Psipred) and trans-membrane motif (HMMTOP, TMPRED) prediction for G+ TraN-like proteins from conjugative plasmids, transposons, ICEs and GIs; alpha helices (blue) and beta strands (red) are highlighted.

Appendix A. Supplemental Table

Table S1 Validating the structural similarity of TraN to itself and related structures.

A

TraN - CTD	PDB code	Residues aligned	Sequence identity [%]	Secondary structure similarity [%]	Superfamily reliability [%]	Fold reliability [%]
TraN **N-terminal domain**		4 - 56	28.8	80.8	97.0	99.1
Excisionase (Xis) **Tn*916* - *Enterococcus faecalis***	1Y6U	9 - 64	12.5	87.5	89.3	98.5
Excisionase ***Klebsiella pneumoniae***	2KVV	5 - 70	10.3	91.4	63.3	93.5
Excisionase (Xis) **bacteriophage Lambda**	1RH6	1 - 50	12.5	75.0	84.3	98.0
Excisionase (Xis) **bacteriophage HK022**	1PM6	1 - 53	11.8	70.6	58.8	91.5
Transcriptional regulator **MerR family - *Bacillus thuringiensis***	3GPV	20 - 72	5.7	81.1	35.3	68.4

B

TraN - CTD	PDB code	Number of aligned residues	Number of gaps	Sequence identity [%]	Core RMSD achieved [Å]
TraN **N-terminal domain**		51	3	31.4	1.78
Excisionase (Xis) **Tn*916* - *Enterococcus faecalis***	1Y6U	53	3	13.2	2.78
Excisionase ***Klebsiella pneumoniae***	2KVV	53	2	7.6	2.84
Excisionase (Xis) **bacteriophage Lambda**	1RH6	44	2	11.4	1.75
Excisionase (Xis) **bacteriophage HK022**	1PM6	46	3	13.0	1.98
Transcriptional regulator **MerR family - *Bacillus thuringiensis***	3GPV	50	3	8.0	2.42

CTD - C-terminal domain; Alignments were performed with MATRAS (**A**) and SUPERPOSE in Coot (**B**)