Supplementary Data

Structural basis of cooperative DNA recognition by the plasmid conjugation factor, TraM

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Table S1. Plasmids and oligonucleotides

	Description & references
pACYC184	Tc^r; (1)
pBAD24	Amp^r; (2)
pBluescript	Amp^r; (3)
pED208	(4)
pT7-5	Amp^r; (5)
pACM400	pACYC184 with *traM* from F; this work
pACM407	pACYC184 with a *traM* hybrid ; F $TraM^{1-55}$:pED208 $TraM^{56-127}$; this work
pJLD263	pJMTraD with a *traD* hybrid F $TraD^{1-709}$:pED208 $TraD^{729-736}$; this work
pJLD264	pJMTraD with a *traD* hybrid F $TraD^{1-709}$:pED208 $TraD^{729-736}$:R734D; this work
pJLEM200	pT7-5 with *traM* from pED208; this work
pJLM400	pBluescript KS (+) with *traM* from F expressed from the *lac* promoter (6)
pJLM401	pJLM400 with *traM* from R100; this work
pJLM402	pJLM400 with a R100 *traM* mutant R3K:I5N; this work
pJLM403	pJLM400 with a F *traM* mutant N5D; (6)
pJLM404	pJLM400 with *traM* from pED208; this work
pJLM407	pJLM400 with *traM* hybrid F $TraM^{1-55}$:pED208 $TraM^{56-127}$; this work
pJMTraD	pBAD24 with *traD* from F: (7)
pOX38-DM	Km^rCm^r, $TraD^-TraM^-$ derivative of pOX38-Km; (7)
pOX38-	Km^rCm^r, $TraM^-$ derivative of pOX38-Km ; (8)
pRFM200	pT7-5 with an F *Bst*BI-*Bgl*II fragment from *traM* to P_{finP}; (9)
pRF105	Amp^r; R100 *oriT* and *traM* gene cloned in pUC18 (10)
pRF911	Amp^r; F plasmid *sbmA* cloned in pBEND2 (11)
JLU3	CTA TAG GGA GAC CGG AAT TCG
JLU91	TTA GAA T TC TAA TAA GGT TTT TGA AAT GCC
JLU92	TTT CCC TAC CAC CAG AAC ATT CAA AGT G
JLU262	GCC ATC CGT TAC CTG CAG G
JLU263	ATA TAT AAG CTT TCA GTA TTC CCT TCC GTC ATC CAT ATC CTC CCC GCG CTC C
JLU264	ATA TAT AAG CTT TCA GTA TTC ATC TCC GTC ATC CAT ATC CTC CCC GCG CTC C
JLU601	TTG AAT TCG AAA GGT TTT ATC TTA TGG CCA GAG TAA TTT TGT ATA TCA G
JLU602	TTG GAT CCG TGG TTA ATT GTC ATC AAA TTG AAC CAG ATC AAA ATC CTG
JLU603	TTG AAT TCG AAA GGT TTT ATC TTA TGG CCA AAG TAA ATT TGT ATA TCA GTA ATG ATG TC
JLU608	ATG GAT CCA CCA GAA CAT TCA AAG TG
JLU611	GAA GGA GGC TTT AAT CAG ATG GAG TAC AAC AAG CTC ATG CTG GAA AAC G
JLU612	CTC CAT CTG ATT AAA GCC TCC TTC CTC CAT CTG AGC CTC ATG TAC AC
30BTA	GAT ACC GCT AGG GGC GCT GCT AGC GGT GCG
30BTB	CGC ACC GCT AGC AGC GCC CCT AGC GGT ATC
sbmAB-F	TTA TAT TAG GGG TGC TGC TAG CGG CGC GGT GTG TTT TTT TAT AGG ATA CCG CTA GGG GCG CTG CTA GCG GTG CGT
sbmAB-R	ACG CAC CGC TAG CAG CGC CCC TAG CGG TAT CCT ATA AAA AAA CAC ACC GCG CCG CTA GCA GCA CCC CTA ATA TAA
pED 4site	AGA TTC GAA TCT AGA TTC GAA TCT

Table S2. Complementation of TraM and TraD knockout

Complementing Proteins		Mating Efficiency (T/D)[a]
No TraM	TraD [F^{1-709}:$pED^{729-736}$]	$<1 \times 10^{-6}$
No TraM	TraD [F^{1-709}:$pED^{729-736}$:R734D]	$<1 \times 10^{-6}$
F TraM	TraD [F^{1-709}:$pED^{729-736}$]	1×10^{-6}
F TraM	TraD [F^{1-709}:$pED^{729-736}$:R734D]	3×10^{-6}
F TraM	F TraD	1×10^{-1}
TraM [F^{1-55}:pED^{56-127}]	TraD [F^{1-709}:$pED^{729-736}$]	1×10^{-1}
TraM [F^{1-55}:pED^{56-127}]	TraD [F^{1-709}:$pED^{729-736}$:R734D]	$<1 \times 10^{-6}$
TraM [F^{1-55}:pED^{56-127}]	F TraD	$<1 \times 10^{-6}$

[a] Determined by assaying donor ability of cells containing pOX38-MK3 or pOX38-DM and the complementing plasmid(s). T/D, transconjugants per donor. "$<1 \times 10^{-6}$" refers to no detectable donor ability.

Supplementary Figures

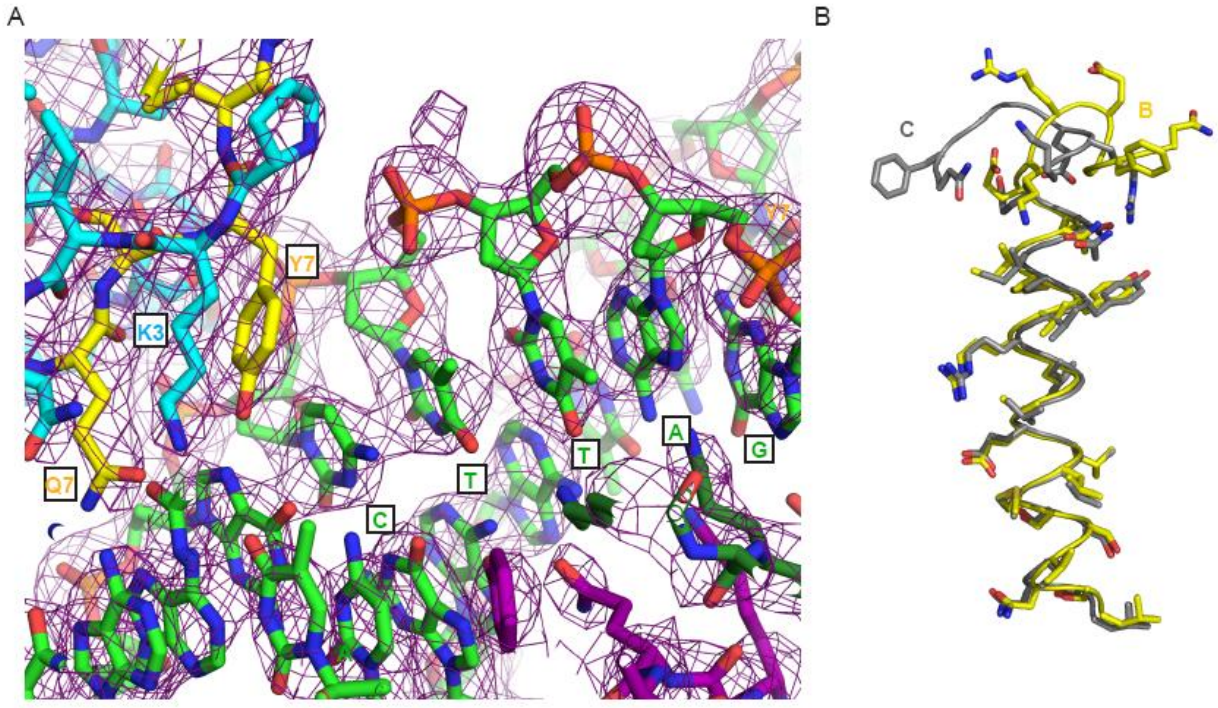

Figure S1

(A) Prime-and-switch map showing electron density at the protein-DNA interface contoured at 1.9σ.

(B) Superimposition of Cαs of two chains from the same N-terminal domain bound to *sbmA*, residues 33-60 of Chain B and Chain C, showing that α2 of Chain C becomes unwound relative to ChainB.

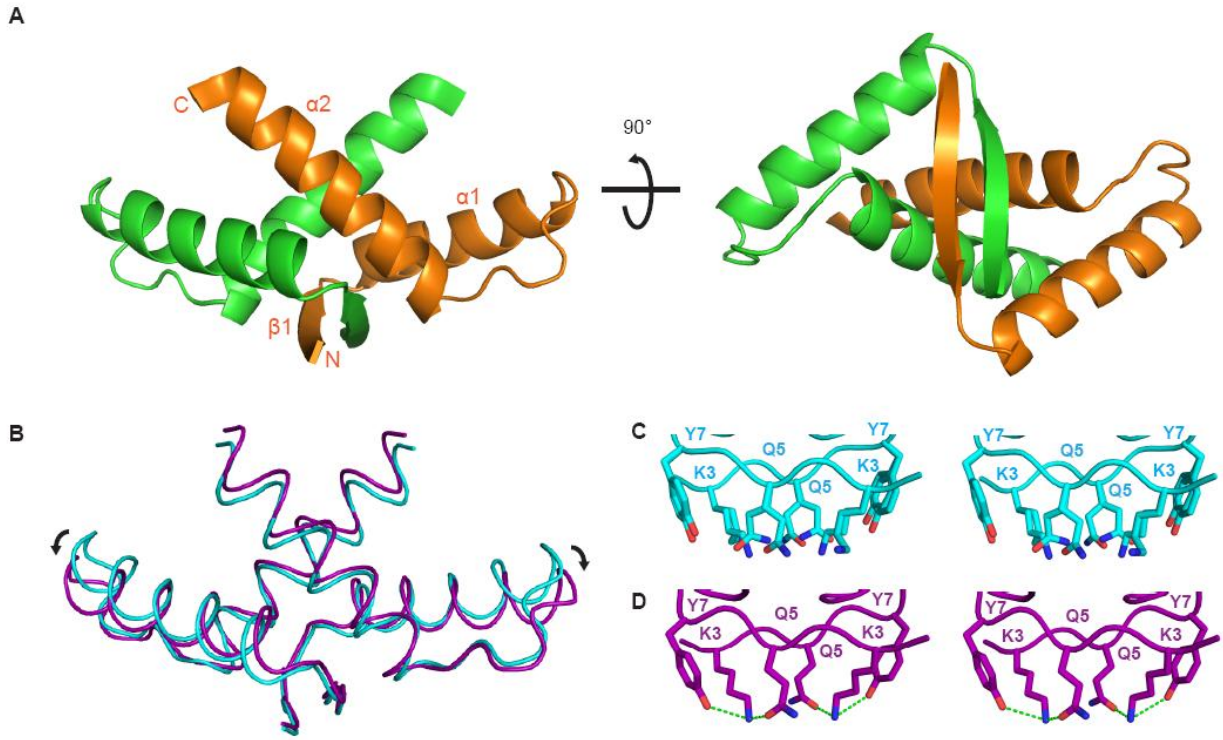

Figure S2

(A) Orthogonal views of the N-terminal domain of pED208 TraM, solved to 1.3Å resolution

(B) Superimposition of Cαs of the apo 1.30 Å N-terminal domain pED208 TraM structure (cyan) with the 2.90Å N-terminal domain TraM structure bound to *sbmA* (purple). Shift in α1-α2 loop upon binding to DNA is indicated with arrows.

(C) Stereo view of the β-sheet of pED208 TraM when unbound, showing alternate conformers of Lys3, Glu5, and Tyr7

(D) Stereo view of the β-sheet DNA-binding residues of pED208 TraM when bound to *sbmA*, showing hydrogen bond interactions between Lys3, Glu5, and Tyr7 in their DNA-bound conformation

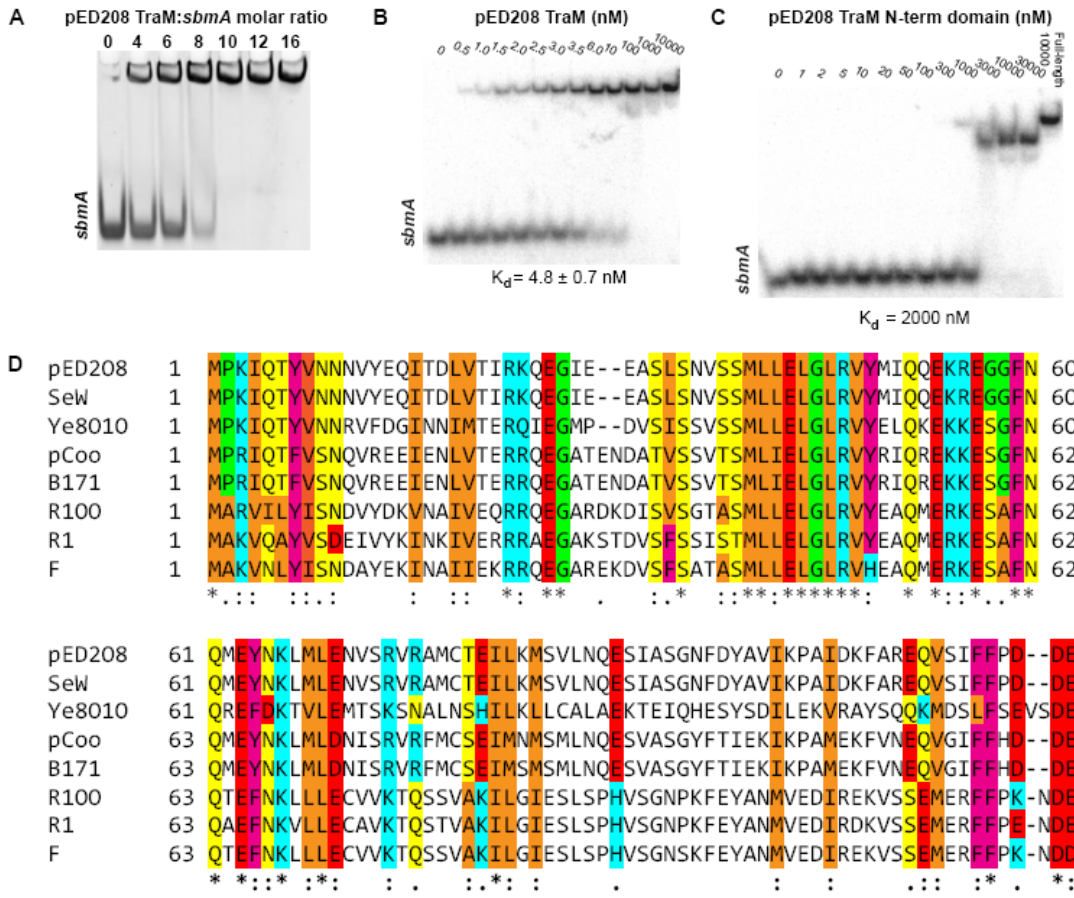

Figure S3

(A) Binding stoichiometry of pED208 TraM to 24bp *sbmA* analyzed by electrophoretic mobility shift assay.

(B) Binding affinity of pED208 TraM for 24 bp *sbmA*

(C) Binding affinity of pED208 TraM N-terminal domain for 24bp *sbmA*

(D) Sequence alignment of TraM homologues. SeW: *Salmonella enterica* subsp. enterica serovar Weltevreden str. HI_N05-537. Ye8010; *Yersinia enterocolitica* str. 8010. Basic residues are highlighted in cyan, acidic residues in red, hydrophobic residues in orange, aromatic residues in magenta, polar aliphatic residues in yellow, and Gly and Pro in green. ClustalW consensus symbols for each residue are shown ("*": perfect identity; ":": conserved substitutions; ".": semi-conserved substitutions)

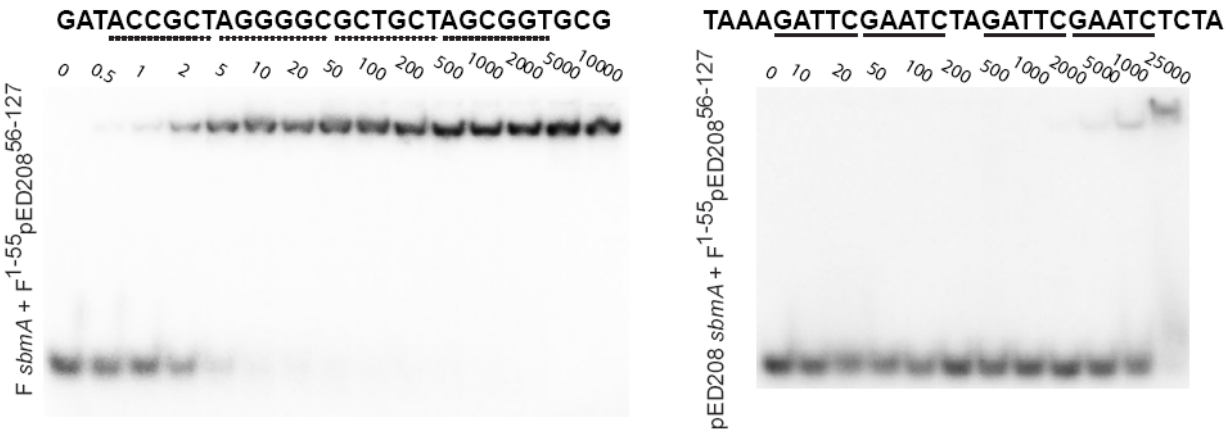

Figure S4

Binding of a chimeric TraM composed of the the F N-terminal domain and the pED208 C-terminal to F *sbmA* DNA (left panel) or pED208 *sbmA* DNA (right panel), monitored by electrophoretic mobility shift assay.

Supplementary Movies

Movie S1, related to Figure 5. Morph of B-DNA conformation *sbmA* to the conformation observed in the crystal structure, illustrating unwinding and kinking of DNA. Morph was created with CNS (12) using the script from the Morph Server (13). Movie was created with Pymol (http://www.pymol.org).

Movie S2, related to Figure 5. Morph of B-DNA conformation *sbmA* to the conformation observed in the crystal structure, orthogonal view to better illustrate DNA kinking. Morph was created with CNS (12) using the script from the Morph Server (13). Movie was created with Pymol (http://www.pymol.org).

Supplementary Experimental Procedures

Growth media and bacterial strains

Cells were grown in LB (Luria-Bertani**)** broth or on LB solid medium containing appropriate antibiotics or other supplements. Antibiotics were used at the following final concentrations: ampicillin (Amp), 50 µg/mL; kanamycin (Km), 25 µg/mL; spectinomycin (Spc), 100 µg/mL; nalidixic acid (Nal), 40 µg/mL, and tetracycline (Tet), 10 µg/ml. IPTG (isopropylthio-β-D-galactoside) was used at a final concentration of 1 mM. The following *Escherichia coli* strains were used: XK1200 [F^- Δ*lacU124* Δ*(nadA gal attλ bio*) *gyrA* (Nal^r)] (14), ED24 (F^- Lac^-Spc^r) (15), DH5α [Δ*lacU169 (Φ80 lacZΔM15) supE44 hsdR17 recA1 endA1 gyrA96 (*Nal^r*) thi-1 relA1*] (16) and BL21-DE3 [F^- *ompT hsdS* ($r_B^-m_B^-$) *galλ*(DE3)] (Stratagene) .

Plasmids and plasmid construction

Plasmids pJLM400 and pJLM403 (6), pOX38-MK3 (8), pRF105 (10), pRF911 (11), pED208 (4), pRFM200 (9), pACYC184 (1), pOX38-DM (7), pJMtraD (7), pBluescript KS (+) (3), pBAD 24 (2), and pT7-5 (5) have been described previously. Plasmids and primers used in this work are described in Table S2. The 0.5-kb *Eco*RI-*Bam*HI fragment of the PCR products amplified from pED208 using primer pair JLU91 was cloned into the *Eco*RI-*Bam*HI sites of pT7-5 or pBluescript KS (+), resulting in pJLEM200 or pJLM404, respectively. The 0.5-kb *Eco*RI-*Bam*HI fragment of the PCR products amplified from pRF105 using primer pair JLU601 and JLU602, or JLU603 and JLU602, was cloned into the *Eco*RI-*Bam*HI sites of pJLM400, resulting in pJLM401 or pJLM402, respectively.

Overlap extension (Ho *et al*., 1989) was used to construct pJLM407 expressing a hybrid TraM (F $TraM^{1-55}$:pED208 $TraM^{56-127}$). PCR primer pair JLU3 and JLU612 were used to amplify a fragment containing F *traM*$^{1-55}$ from pRFM200. Primer pair JLU611 and JLU608 were used to amplify a fragment containing pED208 *traM*$^{56-127}$ from pJLEM200. Primer pair JLU3 and JLU608 were used to amplify the full-length hybrid *traM* (F $TraM^{1-55}$:pED208 $TraM^{56-127}$) fragment, which was further digested by *Eco*RI and *Bam*HI and cloned into the *Eco*RI-*Bam*HI sites of pBluescript KS (+), resulting pJLM407.

The 1.5-kb *Eco*RI-*Sca*I fragment from pJLM400 or pJLM407 was cloned into the *Eco*RI-*Sca*I sites of pACYC184, resulting in pACM400 or pACM407, respectively. The 1.1-kb *Pst*I-*Hind*III fragment of PCR products amplified from pJMtraD using primer pair JLU262 and JLU263, or JLU262 and JLU264, was cloned into the *Pst*I-*Hind*III sites of pJMtraD, resulting in pJLD263 or pJLD264, respectively.

Overexpression and purification of F TraM, R100 TraM, and R100 R3K:I5N TraM

DH5α cells containing pJLM400, pJLM401 or pJLM402, were grown in 100 mL of LB broth containing ampicillin and 0.4% glucose at 37°C. After 12 hours, the 100 mL culture was added into 1 L of LB with ampicillin and grown under the same conditions for 3 hours. IPTG was added to a final concentration of 1 mM and the culture was grown for another 3 hours before harvesting. All the following steps were performed at 4°C or on ice. The cell pellet was suspended in 75 mL of buffer containing 50mM Tris-HCl, pH7.5 and one tablet of Complete protease inhibitor cocktail (Roche), and sonicated for 30 seconds, 6 times. Cell debris was removed by centrifugation at 39,000 x *g* for 1 hour. Ammonium sulfate (20 grams) was dissolved in the extracted supernatant. After centrifugation at 39,000 x *g* for 1 hour, the supernatant was loaded onto a 25 mL HIC column (Phenyl Sepharose™ 6 Fast Flow, GE healthcare Life Sciences), eluted with Tris-HCl (50 mM, pH 7.5) and a 1 to 0 M ammonium sulfate gradient. The fractions containing TraM (~40 mL) was brought to 135 mL with malonic acid (50 mM, pH 5.5), loaded onto a 25 mL cation-exchange column (SP Sepharose™ Fast Flow, GE healthcare Life Sciences), and was eluted with malonic acid (50 mM, pH 5.5) and a 0 to 1 M NaCl gradient. The pooled TraM fractions were concentrated to 5 mL using an Amicon® ultracentrifuge filter (Millipore), loaded onto a size exclusion column (Hiload® 26/60 Superdex 75 prep grade, GE Healthcare Life Sciences), and was eluted with 50mM sodium phosphate, 150 mM NaCl, pH 7.5. Fractions containing purified TraM were concentrated and the buffer was exchanged for 0.5 M ammonium acetate using an Amicon® ultracentrifuge filter (Millipore) to a final volume of 1 mL. Protein concentration was determined using BCA protein assays (Pierce) following the manufacturer's instructions.

Expression and purification of pED208 TraM

BL21-DE3 cells containing pJLEM200 were grown in LB media containing 100ug/ml ampicillin at 37°C until the OD 600 reached ~0.7, followed by induction with 0.5 mM IPTG for 5 hours at 27°C. Harvested cells from 1 L of media were suspended in 100 ml of 50 mM Tris pH 7.5, 150 mM NaCl, 1 mM DTT containing 1 Complete, EDTA-free protease inhibitor tablet (Roche). The cells were lysed by sonication for 20 seconds 6 times, followed by centrifugation at 39,000 x g for 45 minutes to remove cell debris. 35 grams of ammonium sulfate was added to the clarified cell lysate, and dissolved by stirring for 15 minutes on ice. Precipitated proteins were removed by centrifugation at 39,000 x g for 30 minutes. 10 grams of ammonium sulfate was dissolved in the resulting supernatant, followed by centrifugation as in the previous step. The resulting pellet containing TraM was dissolved in 100 ml of 50 mM Tris, pH 7.5, 1 M ammonium sulfate. The dissolved pellet was loaded onto a 25 ml HIC column *(*Phenyl Sepharose 6 Fast Flow™, GE Healthcare Life Sciences) and eluted by a decreasing gradient of 50 mM Tris pH 7.5, 1 M ammonium sulfate. Ammonium sulfate was added to fractions containing TraM to 45grams per 100 ml and dissolved by stirring on ice for 15 minutes, followed by centrifugation at 39,000 x g for 30 minutes to precipitate TraM. The resulting pellet was dissolved in 100 ml of 50 mM L-Histidine, pH 5.5, and loaded onto a 25 ml anion exchange column (Q Sepharose Fast Flow™, GE Healthcare Life Sciences) and eluted by increasing gradient of 50 mM L-Histidine, pH 5.5, 1 M NaCl. TraM-containing fractions were concentrated and exchanged into 50 mM MES pH 6.8, 300 mM NaCl by centrifugation at 2,400 x g in 15 ml concentrators (Amicon Ultracel 10K, Millipore). The concentrated fraction was loaded onto a 300 ml size exclusion column (HiLoad 26/60 Superdex75™ prep grade, GE Healthcare Life Sciences) and eluted with the same buffer. TraM-containing fractions were concentrated in 15 ml concentrators (Amicon Ultracel 10K, Millipore) and exchanged into 0.5 M ammonium acetate. Protein concentration was determined using BCA protein assays (Pierce) following the manufacturer's instructions.

Oligonucleotide DNA purification and annealing for crystallization, titration analysis, and MALLS

Synthetic DNA oligonucleotides were purified on a 7 mL anion exchange column (Source 15Q) under denaturing conditions (10 mM NaOH) and eluted by increasing gradient of 10mM NaOH, 1M NaCl. DNA-containing fractions were desalted with a Sep-PAK® (C18) cartridge in a volatile buffer (30%

acetonitrile in 0.1 M triethylammonium bicarbonate), lyophilized and resuspended in water. Oligonucleotide DNA solutions were quantified by absorbance at 260 nm. The 30-base pair F *sbmA* DNA was generated by annealing of 30BTA and 30BTB, and F *sbmAB* by annealing F *sbmAB*–F and F *sbmAB*-R. The 24-base pair pED208 *sbmA* DNA was generated by annealing the perfectly palindromic sequence pED 4site. Mixed oligos were heated in a water bath to 100°C and slow cooled to room temperature in 10 mM Tris pH 7.5 and 100 mM NaCl at a final concentration of 0.5 mM double stranded DNA.

Titration analysis of pED208 TraM binding to *sbmA*

Each binding mixture contained 1.5 µM of 24 base-pair *sbmA*, the indicated molar ratio of TraM, 50mM Tris-HCl (pH 7.5), and 10% glycerol in a 15 µl volume. Samples were incubated for 10 minutes at room temperature. Mixtures were run on TBE-buffered 12% polyacrylamide gel in 1X TBE at 4°C and 200 Volts for 45 minutes. DNA and DNA-protein complexes were visualized by ethidium bromide staining.

Competition of Wild-type pED208 *sbmA* by pED208 *sbmA* mutants

0.1 nM of P^{32}-labelled pED208 24 bp wild-type *sbmA* was mixed with 50 nM of TraM (enough to effectively bind all unbound DNA) in 50 mM Tris pH 7.5, 10% glycerol, and 30 ng/µl bovine serum albumin (Pierce). TraM-*sbmA* complexes were incubated for 10 minutes, followed by addition of unlabelled competitor *sbmA* oligos in the amount of times the concentration of labeled oligo indicated. Mixtures with competitor DNA were incubated for 48 hours. All incubations were done at room temperature. Mixtures were run at 200 V on a 1x TBE-buffered 12% acrylamide gel for 45 minutes at 4°C. Gel bands were visualized by phosphor screen. Proportion of bound radioligand was fitted to a 3-parameter logistic curve for obtaining the IC_{50}.

Multi-angle laser light scattering

All following steps were performed at 25 °C. Purified TraM (200 µg) or purified TraM (120 µg) plus 200 µg of 30-base pair *sbmA* DNA was applied to a Superose™ 12 HR 10/30 column eluted with the same buffer. The effluent from this column was run directly through in-line DAWN EOS™ multi-angle laser

light scattering (MALLS) and Optilab rEX^{TM} differential refractive index detectors (Wyatt Technologies, Santa Barbara, CA). ASTRA v.4.90 software was used to process the data.

Titration analysis of F TraM-*sbmA* binding

Each binding mixture contained 1 μM of 30 bp *sbmA*, 50 mM Tris-HCl (pH 7.5), and 10% glycerol. Increasing amount of purified F plasmid TraM was added with different molar ratios to the 30 bp *sbmA*. The final volume of each sample is 10 μl. The resulting mixture was loaded onto a TBE-buffered 12% polyacrylamide gel and was run in 1x TBE (90 mM Tris-borate, 1 mM EDTA) at 4°C and 40 Volts for 4 hours. DNA and DNA-protein complexes were visualized by ethidium bromide staining.

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