Electronic Supplementary Material (ESI) for Molecular BioSystems

SUPPLEMENTAL INFORMATION

Differential Tus-*Ter* binding and lock formation: Implications for DNA replication termination in *Escherichia coli*

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SUPPLEMENTAL DATA

Table S1: Related to Figure 3. Kinetic parameters of Tus affinity for *Ter* sites and their *Ter*-lock variants at 250 and 150 mM KCl.

	10^{-5} k_a ± SEM (M^{-1} s^{-1})	10^{3} k_d ± SEM (s^{-1})	K_D (nM)	$t_{1/2}$ (s)
TerA	2.05 ± 0.17	2.2 ± 0.07	10.7	315.8
Ter-lockA	1.56 ± 0.29	0.21 ± 0.0.5	1.34	3309.9
TerB	1.57 ± 0.12	1.83 ± 0.08	11.6	379.8
Ter-lockB	1.52 ± 0.20	0.16 ± 0.01	1.05	4366.9
TerC	1.58 ± 0.12	2.86 ± 0.17	18	242.6
Ter-lockC	1.64 ± 0.16	0.20 ± 0.01	1.24	3408.9
TerD	1.88 ± 0.16	3.2 ± 0.15	17	217.4
Ter-lockD	1.34 ± 0.17	0.27 ± 0.016	1.99	2588.8
TerE	1.97 ± 0.1	5.1 ± 0.15	25.6	137.0
Ter-lockE	1.13 ± 0.07	0.23 ± 0.005	2.05	2979.3
TerF	2.47 ± 0.38	21.8 ± 2.20	88.4	31.8
Ter-lockF	NA	35.7 ± 4.25	NA	19.4
TerG	2.63 ± 0.31	4.4 ± 0.1	16.7	157.4
Ter-lockG	0.94 ± 0.06	0.3 ± 0.02	3.25	2276.5
TerH	NA	22 ± 1.2	NA	31.6
	4.10 ± 0.44	0.56 ± 0.04	1.36	1244.2
Ter-lockH	NA	NA	NA	59.1*
	5.04 ± 0.35	1.68 ± 0.09	3.34	411.7
TerI	NA	24.1 ± 0.4	31	28.7
	5.54 ± 1.01	0.73 ± 0.08	1.32	949.1
Ter-lockI	NA	3.53 ± 0.4	18.9	196.4
	3.17 ± 0.44	0.65 ± 0.06	2.05	1069
TerJ	NA	25.4 ± 0.4	NA	27.3
	3.20 ± 0.36	1.94 ± 0.10	6.07	356.8
Ter-lockJ	NA	2.1 ± 0.18	NA	332.1
	1.25 ± 0.2	0.52 ± 0.05	4.18	1322

Shaded rows represent low-salt data while clear rows correspond to high-salt data. NA: Data not available. * $t_{1/2}$ value was determined by direct visual analysis. See also Figure 4 for sensorgrams.

SUPPLEMENTAL FIGURE LEGEND

Figure S1: Related to Figure 2. Tus-GFP Aggregation rates in presence of *Ter, Ter-lock* or *oriC* (non-specific control) sequences obtained with GFP-Basta. **A**. Aggregation rates of Tus-GFP-*Ter* complexes in 150 mM KCl at 58 ̊C. **B**. Aggregation rates of Tus-GFP-*Ter-lock* complexes in 150 mM KCl at 58 ̊C. **C**. Aggregation rates of Tus-GFP-*Ter* complexes in 250 mM KCl at 52 ̊C. **D**. Aggregation rates of Tus-GFP-*Ter-lock* complexes in 250 mM KCl at 52 ̊C.

Figure S2: Multi-alignment of Tus with Tus from diverse bacteria. The alignment was generated with Tus from *E.coli* (1ECR_A) as the query sequence using the Conserved Domains Database (accessed from the NCBI website).[1] **A**. Top listed sequences (most conserved sequences excluding *E.coli* strains). Bacteria include: *Cronobacter sakazakii* ATCC BAA-894, *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578, *Enterobacter sp*. 638, *Shigella dysenteriae* Sd197, *Salmonella enterica* subsp. *enterica* serovar Paratyphi A str. ATCC 9150, *Sodalis glossinidius* str. 'morsitans', *Pectobacterium atrosepticum*, *Yersinia pseudotuberculosis* IP 31758, *Photorhabdus luminescens* subsp. *laumondii*. **B**. Most diverse sequences included: IncT plasmid R394, *Proteus vulgaris*, *E. coli* (putative terminator protein), *Pseudoalteromonas tunicata* D2, *Photobacterium damselae* subsp. *Piscicida*, *Moritella* sp. PE36, *Marinobacter sp*. ELB17, *Salmonella enterica* subsp. enterica serovar Typhi, *Pseudomonas fluorescens* SBW25. The shaded box highlights residues F140-G149 in the C(6) binding pocket domain. Residues with direct contacts to the C(6) are asterisked.

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Protein expression and purification

The His_6-Tus-GFP proteins (Tus-GFP) were expressed in *E. coli* BL21(*DE3*)RIPL in Overnight Express Instant TB Medium (Novagen) at 16°C. Cells were lysed by French press in lysis buffer (50 mM phosphate, pH 7.8, 10% glycerol v/v, 2 mM β-mercaptoethanol, 300 mM NaCl) and the lysate was cleared by centrifugation at 40,000 *g* for 35 min. The lysate was then incubated with Profinity IMAC Ni-charged resin (Bio-Rad) to purify Tus-GFP through its N-terminal His_6 tag. Proteins were eluted in lysis buffer supplemented with 200 mM imidazole and were ammonium sulphate precipitated (0.5 g/ml). The pellet was resuspended in Buffer A (50 mM Tris, pH 7.6, 250 mM KCl, 0.1 mM EDTA and 0.2 mM β-mercaptoethanol) and dialysed (SnakeSkin pleated dialysis tubing 10,000 MWCO; Pierce) twice against 200 ml of Buffer A at 4°C. The His_6-Tus protein (Tus) was expressed, lysed and purified as for Tus-GFP. After ammonium sulphate precipitation, Tus was resuspended in Buffer A. The purity of proteins was assessed by SDS-PAGE (NEXT-GEL Amresco) and concentration was determined by standard Bradford assay.

GFP-Basta

Oligonucleotides for GFP-basta were designed to include the 23 bp *Ter* or *Ter-lock* sequences or a 23 bp sequence from the *oriC* (non-specific DNA control) followed by a stabilizing 10-mer GC rich region in order to elevate their T_m values above 70°C (Table S2). All complementary oligonucleotide pairs (100 µM each in 10 mM Tris-HCl pH 7.6, 1 mM EDTA (TE) supplemented with 50 mM KCl) were hybridized by heating at 80°C for 2 min followed by slow cooling to room temperature.

Table S2: Oligonucleotide sequences used for GFP-Basta

	Ter	***Ter-lock***
TerA	AATTAGTATGTTGTAACTAAAGTGGGGGCGGGG TTAATCATACAACATTGATTTCACCCCCGCCCC	TATGTTGTAACTAAAGTGGGGGCGGGG TTAATCATACAACATTGATTTCACCCCCGCCCC
TerB	AATAAGTATGTTGTAACTAAAGTGGGGGCGGGG TTATTCATACAACATTGATTTCACCCCCGCCCC	TATGTTGTAACTAAAGTGGGGGCGGGG TTATTCATACAACATTGATTTCACCCCCGCCCC
TerC	ATATAGGATGTTGTAACTAATATGGGGGCGGGG TATATCCTACAACATTGATTATACCCCCGCCCC	GATGTTGTAACTAATATGGGGGCGGGG TATATCCTACAACATTGATTATACCCCCGCCCC
TerD	CATTAGTATGTTGTAACTAAATGGGGGGCGGGG GTAATCATACAACATTGATTTACCCCCCGCCCC	TATGTTGTAACTAAATGGGGGGCGGGG GTAATCATACAACATTGATTTACCCCCCGCCCC
TerE	TTAAAGTATGTTGTAACTAAGCAGGGGGCGGGG AATTTCATACAACATTGATTCGTCCCCCGCCCC	TATGTTGTAACTAAGCAGGGGGCGGGG AATTTCATACAACATTGATTCGTCCCCCGCCCC
TerF	CCTTCGTATGTTGTAACGACGATGGGGGCGGGG GGAAGCATACAACATTGCTGCTACCCCCGCCCC	TATGTTGTAACGACGATGGGGGCGGGG GGAAGCATACAACATTGCTGCTACCCCCGCCCC
TerG	GTCAAGGATGTTGTAACTAACCAGGGGGCGGGG CAGTTCCTACAACATTGATTGGTCCCCCGCCCC	GATGTTGTAACTAACCAGGGGGCGGGG CAGTTCCTACAACATTGATTGGTCCCCCGCCCC
TerH	CGATCGTATGTTGTAACTATCTCGGGGGCGGGG GCTAGCATACAACATTGATAGAGCCCCCGCCCC	TATGTTGTAACTATCTCGGGGGCGGGG GCTAGCATACAACATTGATAGAGCCCCCGCCCC
TerI	AACATGGAAGTTGTAACTAACCGGGGGGCGGGG TTGTACCTTCAACATTGATTGGCCCCCCGCCCC	GAAGTTGTAACTAACCGGGGGGCGGGG TTGTACCTTCAACATTGATTGGCCCCCCGCCCC
TerJ	ACGCAGTAAGTTGTAACTAATGCGGGGGCGGGG TGCGTCATTCAACATTGATTACGCCCCCGCCCC	TAAGTTGTAACTAATGCGGGGGCGGGG TGCGTCATTCAACATTGATTACGCCCCCGCCCC
oriC	CCGGCTTTTAAGATCAACAACCTGGAAAGGATCA GGCCGAAAATTCTAGTTGTTGGACCTTTCCTAGT	TTTAAGATCAACAACCTGGAAAGGATCA GGCCGAAAATTCTAGTTGTTGGACCTTTCCTAGT

SPR

For SPR experiments, all *Ter* and *Ter-lock* were designed to include a single-stranded decamer overhang (velcro) after the base 23 to allow their hybridization to a biotinylated complementary oligonucleotide (Table S3). Individual oligonucleotides were resuspended in 10 mM Tris-HCl pH 7.6, 1 mM EDTA (TE) supplemented with 50 mM KCl to a final concentration of 100 µM. They were combined by mixing 25 µl of the oligonucleotide containing the 10-mer overhang with 50 µl of the complementary oligonucleotides, and 175 µl of Buffer A. Hybridization was achieved by heating at 80°C for 2 min followed by slow cooling to room temperature.

Table S3: *Ter* and *Ter-lock* DNA sequences used for SPR

	Ter	***Ter-lock***
TerA	AATTAGTATGTTGTAACTAAAGTGGGGGCGGGG TTAATCATACAACATTGATTTCA	TATGTTGTAACTAAAGTGGGGGCGGGG TTAATCATACAACATTGATTTCA
TerB	AATAAGTATGTTGTAACTAAAGTGGGGGCGGGG TTATTCATACAACATTGATTTCA	TATGTTGTAACTAAAGTGGGGGCGGGG TTATTCATACAACATTGATTTCA
TerC	ATATAGGATGTTGTAACTAATATGGGGGCGGGG TATATCCTACAACATTGATTATA	GATGTTGTAACTAATATGGGGGCGGGG TATATCCTACAACATTGATTATA
TerD	CATTAGTATGTTGTAACTAAATGGGGGGCGGGG GTAATCATACAACATTGATTTAC	TATGTTGTAACTAAATGGGGGGCGGGG GTAATCATACAACATTGATTTAC
TerE	TTAAAGTATGTTGTAACTAAGCAGGGGGCGGGG AATTTCATACAACATTGATTCGT	TATGTTGTAACTAAGCAGGGGGCGGGG AATTTCATACAACATTGATTCGT
TerF	CCTTCGTATGTTGTAACGACGATGGGGGCGGGG GGAAGCATACAACATTGCTGCTA	TATGTTGTAACGACGATGGGGGCGGGG GGAAGCATACAACATTGCTGCTA
TerG	GTCAAGGATGTTGTAACTAACCAGGGGGCGGGG CAGTTCCTACAACATTGATTGGT	GATGTTGTAACTAACCAGGGGGCGGGG CAGTTCCTACAACATTGATTGGT
TerH	CGATCGTATGTTGTAACTATCTCGGGGGCGGGG GCTAGCATACAACATTGATAGAG	TATGTTGTAACTATCTCGGGGGCGGGG GCTAGCATACAACATTGATAGAG
TerI	AACATGGAAGTTGTAACTAACCGGGGGGCGGGG TTGTACCTTCAACATTGATTGGC	GAAGTTGTAACTAACCGGGGGGCGGGG TTGTACCTTCAACATTGATTGGC
TerJ	ACGCAGTAAGTTGTAACTAATGCGGGGGCGGGG TGCGTCATTCAACATTGATTACG	TAAGTTGTAACTAATGCGGGGGCGGGG TGCGTCATTCAACATTGATTACG
Velcro pCBio	Biotin-CCCCGCCCCC	

All oligonucleotides were obtained from Sigma.

1. A. Marchler-Bauer, S. Lu, J. B. Anderson, F. Chitsaz, M. K. Derbyshire, C. DeWeese-Scott, J. H. Fong, L. Y. Geer, R. C. Geer, N. R. Gonzales, M. Gwadz, D. I. Hurwitz, J. D. Jackson, Z. Ke, C. J. Lanczycki, F. Lu, G. H. Marchler, M. Mullokandov, M. V. Omelchenko, C. L. Robertson, J. S. Song, N. Thanki, R. A. Yamashita, D. Zhang, N. Zhang, C. Zheng and S. H. Bryant, *Nucleic Acids Res.*, 2011, **39**, D225-229.