Electronic Supplementary Material (ESI) for Analyst

SUPPLEMENTAL INFORMATION

A Polyplex qPCR-based binding assay for protein-DNA interactions

Morgane J.J. Moreau and Patrick M. Schaeffer*

[a] *Comparative Genomics Centre, James Cook University, DB 21, James Cook Drive, Townsville, QLD 4811, Australia.*

[b] *School of Pharmacy and Molecular Sciences, James Cook University, DB 21, James Cook Drive, Townsville, QLD 4811, Australia.*

***Fax: +61 (0) 7 4781 6078; Tel: +61 (0) 7 4781 4448; E-mail:**

patrick.schaeffer@jcu.edu.au

MATERIAL AND METHODS

Protein expression and purification

The His_6-Tus-GFP[1, 2] 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 purity of proteins was assessed by SDS-PAGE (NEXT-GEL Amresco) and concentration was determined by standard Bradford assay.

Determination of Tus-Ter binding by qPCR DNA-binding assay

The method was developed to compare the affinity of Tus for each *Ter* site in polyplex. For this assay, genomic DNA regions (~150 bp) containing *Ter* sites or *oriC* sequences (non-specific binding control) were amplified from *E. coli* DH12S using a MyCycler (Bio-Rad) with Taq DNA polymerase (New England Biolabs). Oligonucleotides, used to amplify the individual genomic regions containing the *TerA-J* sites, were standardised for PCR and are described in Table S1. The protocol consisted of a denaturation step of 30 s at 95°C, followed by 40 cycles of 15 s at 95°C, 10 s at 60°C and 20 s at 68°C and a final extension step of 2 min at 68°C. DNA amplicons were purified and quantified after electrophoresis on an agarose gel using the image analysis software ImageJ (http://rsbweb.nih.gov/ij/). All DNA

amplicons were diluted to a final concentration of 6 nM in TBS (20 mM Tris pH 7.5, 150 mM NaCl and 0.005% Tween 20).

A solution containing one volume of Tus-GFP (6 nM in TBS) and one volume of each DNA amplicon (6 nM in TBS) was diluted in TBS buffer to obtain a final concentration of 0.4 nM of Tus-GFP and 4.4 nM of combined DNA amplicons. The reaction mix was left 10 min at room temperature to allow Tus binding to *Ter* sites. Streptavidin coated plates (Thermoscientific, Reacti-Bind^{TM} Streptavidin coated HBC black 96-well plates with SuperBlock blocking buffer) were coated overnight with 50 µl of 1 µg/ml biotinylated goat anti-GFP antibody (Ab 66858; Abcam) in TBS at 4°C. The Ab suspension was removed and the wells were washed with 200 µl of TBS. A volume of 50 µl of Tus-GFP-DNA reaction was bound to each well for 60 min at room temperature. The supernatant was removed and wells were washed 5-times with 200 µl of TBS. DNA amplicons were dissociated with 50 µl of TBS containing 0.5 M NaCl during 30 min at room temperature, transferred into a new tube and diluted 10-times with water to reduce the salt concentration (output). The salt concentration and dilution of the initial Tus-GFP-DNA reaction were adjusted (input) to match the output conditions. Background controls were obtained using the same protocol with the omission of the anti-GFP Ab binding step.

The IQ5 iCycler (Bio-Rad) was used for qPCR. Briefly, reactions contained 2 µl of input or output, 8 µl of primer pair (0.5 µM) and 10 µl of SensiMix SYBR & fluorescein mastermix (Bioline). The protocol used included 10 min activation at 95℃ followed by 40 cycles of 10 s at 95℃ and 15 s at 60℃. A melt-curve was carried out to verify that the correct regions were amplified. Standard curves were obtained for each primer set with a 10-fold serial dilution of input matching the output buffer conditions (10-, 100- and 1000-fold). ΔCt values were obtained by subtracting background Ct values (no Ab) from output Ct values (with Ab).

Slope values of the standard curves (for each primer set; see Figure S1) were used to obtain the enrichment factor using the relationship $10^{(\Delta Ct/slope)}$.

GFP-Basta

For GFP-Basta, we designed ten *Ter* oligonucleotides including the 23 bp *Ter* sequences followed by a stabilising 10-mer GC rich region (to elevate their T_m values above 70°C) and a non-specific control sequence from the *oriC* region (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 hybridised by heating at 80°C for 2 min followed by slow cooling to room temperature.

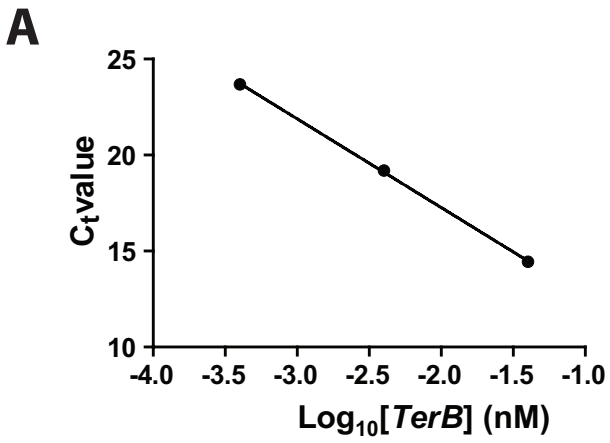
The aggregation rate constants of Tus-GFP alone or in complex with *Ter* or oriC sites were determined by the isothermal method of GFP-Basta. For these reactions, an equal volume of Tus-GFP (1.6 µM) in buffer B (buffer A with 150 mM final KCl concentration) was mixed with an equal volume of *Ter* DNA (2 µM) in buffer B (see Table S2 for oligonucleotide sequences). The reactions were left 10 min at room temperature to allow complex formation. Each reaction (70 µl) was heated at 58°C in a MyCycler (BioRad). After heating, samples were transferred to ice for 10 min to stop the reaction. Aggregates were then centrifuged at 18,000 rpm for 20 min at 4°C in a Beckman Coulter Microfuge 22R centrifuge using the rotor F12x8.2. The residual fluorescence in the supernatant after thermal denaturation was quantified by transferring 60 µl of the supernatant into a black 96-well plate (Nunclon) and the residual fluorescence was measured with a fluorescence plate reader (Victor V Wallace Perkin-Elmer). The excitation and emission filters were set at 460 nm and 535 nm respectively, with 40 nm bandwidth. The values obtained were normalised against the fluorescence of an untreated sample. Aggregation curves were fitted as described previously

to obtain aggregation rate constants (k_{agg})[1]. Aggregation half-lives ($t_{1/2\text{-}agg}$) were obtained as $\ln 2/k_{agg}$.

SPR

For SPR experiments, all *Ter* DNA were designed to include a single-stranded decamer overhang (velcro) after the base 23 to allow their hybridisation 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. Hybridisation was achieved by heating at 80°C for 2 min followed by slow cooling to room temperature.

Measurements were carried out at 20°C using a ProteON XPR36 (Bio-Rad) with freshly diluted His_6-Tus[1, 2] in buffer B. The biotinylated pCBio (5'-Biotin-CCCCGCCCCC-3') was used as a molecular "velcro" to capture the *Ter* oligonucleotides on the neutravidin NLC chip (Bio-Rad). The pCBio was immobilised onto the surface at 50 nM for 300 s at 25 µl/min. *Ter* DNA were hybridised through their complementary single stranded G_5CG_4 overhang to the pCBio at a concentration of 25 nM and flowrate of 25 µl/min during ~100 s. The kinetics of complex formation between Tus and *Ter* were measured in buffer B. Six Tus concentrations ranging from 30 nM to 0.9 nM in buffer B were injected at a flow rate of 25 µl/min, for 120 s, and dissociations were analysed over 900 s. When required, Tus was dissociated from *Ter* with 1 M NaCl injections (25 µl/min for 120 s). The surface was regenerated with 50 mM NaOH and 1 M NaCl (30 µl/min for 60 s), leaving the pCBio on the surface. Experiments were carried out at least in triplicate and fit to the Langmuir binding model with all the variables fitted locally.



B

Primer	Slope
TerA	-4.06
TerB	-4.62
TerC	-4.03
TerD	-4.71
TerE	-4.27
TerF	-3.89
TerG	-3.84
TerH	-4.24
TerI	-4.01
TerJ	-4.08
oriC	-4.09

Figure S1: A. qPCR standard curve for *TerB*. B. Slope values obtained for each qPCR standard curves.

Table S1: Oligonucleotides for PCR amplification of genomic *Ter* sites and *oriC* sequence in DH12S.

	Forward Primer	Reverse Primer	Amplicon (bp)
TerA	CAACCATTAAACCGATTCGCGGTC	ATTTCCACGGGTAGAATGCTCG	145
TerB	TTACCTCTGCCTGACACTACGC	CGATCTCGTAGACCGACTCAACA	123
TerC	GCATGTGGCACCTGTTAATG	TAGCACAACGGACGTACAGC	123
TerD	CCTTTGCGCGGCATGATG	CGCCGGATTTCATTAAGAGCCAT	125
TerE	GAAGTCGCCGTCTGGTTTAT	TACGGCGGAAGTTAATGGTC	172
TerF	CACATCTTCGGGAGTCGGTTC	CAGCAGCGTTTACCACTCAACC	131
TerG	GGCAAGCCGCCATCATCG	TGGGAGATCAACATACAACCGTG	171
TerH	TGAAGGACAAACTGGAAACGCTGA	ATTGTGGTGGCGGTAGTCTG	148
TerI	ATTGCTGGAACGGTTGATTGCG	TGCTACGTAAAGACGGCGAG	168
TerJ	CCACCATGTTGACGATACGACG	TGTTCGGCATCACCAGAATATTGG	122
oriC	CGCACTGCCCTGTGGATAACAA	CCCTCATTCTGATCCCAGCTTA	114

Table S2: Oligonucleotide sequences used for GFP-Basta

	Ter
TerA	AATTAGTATGTTGTAACTAAAGTGGGGGCGGGG TTAATCATACAACATTGATTTCACCCCCGCCCC
TerB	AATAAGTATGTTGTAACTAAAGTGGGGGCGGGG TTATTCATACAACATTGATTTCACCCCCGCCCC

TerC	ATATAGGATGTTGTAACTAATATGGGGGCGGGG TATATCCTACAACATTGATTATACCCCCGCCCC
TerD	CATTAGTATGTTGTAACTAAATGGGGGGCGGGG GTAATCATACAACATTGATTTACCCCCCGCCCC
TerE	TTAAAGTATGTTGTAACTAAGCAGGGGGCGGGG AATTTCATACAACATTGATTCGTCCCCCGCCCC
TerF	CCTTCGTATGTTGTAACGACGATGGGGGCGGGG GGAAGCATACAACATTGCTGCTACCCCCGCCCC
TerG	GTCAAGGATGTTGTAACTAACCAGGGGGCGGGG CAGTTCCTACAACATTGATTGGTCCCCCGCCCC
TerH	CGATCGTATGTTGTAACTATCTCGGGGGCGGGG GCTAGCATACAACATTGATAGAGCCCCCGCCCC
TerI	AACATGGAAGTTGTAACTAACCGGGGGGCGGGG TTGTACCTTCAACATTGATTGGCCCCCCGCCCC
TerJ	ACGCAGTAAGTTGTAACTAATGCGGGGGCGGGG TGCGTCATTCAACATTGATTACGCCCCCGCCCC
oriC	CCGGCTTTTAAGATCAACAACCTGGAAAGGATCA GGCCGAAAATTCTAGTTGTTGGACCTTTCCTAGT

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

	Ter
TerA	AATTAGTATGTTGTAACTAAAGTGGGGGCGGGG TTAATCATACAACATTGATTTCA
TerB	AATAAGTATGTTGTAACTAAAGTGGGGGCGGGG TTATTCATACAACATTGATTTCA
TerC	ATATAGGATGTTGTAACTAATATGGGGGCGGGG TATATCCTACAACATTGATTATA
TerD	CATTAGTATGTTGTAACTAAATGGGGGGCGGGG GTAATCATACAACATTGATTTAC
TerE	TTAAAGTATGTTGTAACTAAGCAGGGGGCGGGG AATTTCATACAACATTGATTCGT
TerF	CCTTCGTATGTTGTAACGACGATGGGGGCGGGG GGAAGCATACAACATTGCTGCTA
TerG	GTCAAGGATGTTGTAACTAACCAGGGGGCGGGG CAGTTCCTACAACATTGATTGGT
TerH	CGATCGTATGTTGTAACTATCTCGGGGGCGGGG GCTAGCATACAACATTGATAGAG
TerI	AACATGGAAGTTGTAACTAACCGGGGGGCGGGG TTGTACCTTCAACATTGATTGGC
TerJ	ACGCAGTAAGTTGTAACTAATGCGGGGGCGGGG TGCGTCATTCAACATTGATTACG
Velcro pCBio	Biotin-CCCCGCCCCC

All oligonucleotides were obtained from Sigma.

REFERENCES

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