Cloning of TusGFP expression vector pPMS1259

The vector pPMS1259 was engineered to afford a His_6-Tus fusion gene cassette for the expression of N-terminal His_6-Tus tagged proteins using pETMCSIII[1] as backbone vector and contains the following features. The original *BamHI* site was deleted by endfilling, resulting in the sequence GGATCGATCC. The original *NdeI* site was deleted by oligonucleotide insertion of a TCT codon resulting in the sequence CATTCTATG followed by the Tus coding sequence. The *Tus* gene is followed by a linker sequence (see Fig. S1) and is fused in frame with the NdeI/EcoRI *GFP* containing fragment obtained from pMH1200[2]. The linker region contains *BamHI*, *NdeI* and *SpeI* sites for subcloning of potential genes of interest. Partial sequence and details of pPMS1259 are provided in Fig. S1.

TCCCGCGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGTAATAATTTTGTTTAATC**GGATCGAT**
CCTAAGGAGGTTAATATT***ATG***CACCATCACCATCAC**CATTCTATG**GCGCGTTACGATCTCGTAGACCGACTCAACACTAC
CTTTCGCCAGATGGAACAAGAGCTGGCTATATTTGCCGCTCATCTTGAGCAACACAAGCTATTGGTTGCCCGCGTGTTCT
CTTTGCCGGAGGTAAAAAAAGAGGATGAGCATAATCCGCTTAATCGTATTGAGGTAAAACAACATCTCGGCAACGACGCG
CAGTCGCTGGCGTTGCGTCATTTCCGCCATTTATTTATTCAACAACAGTCCGAAAATCGCAGCAGCAAGGCCGCTGTCCG
TCTGCCTGGCGTGTTGTGTTACCAGGTCGATAACCTTTCGCAAGCAGCGTTGGTCAGTCATATTCAGCACATCAATAAAC
TCAAGACCACGTTCGAGCATATCGTCACGGTTGAATCAGAACTCCCCACCGCGGCACGTTTTGAATGGGTGCATCGTCAT
TTGCCGGGGCTGATCACCCTTAATGCTTACCGCACGCTCACCGTTCTGCACGACCCCGCCACTTTACGCTTTGGTTGGGC
TAATAAACATATCATTAAGAATTTACATCGTGATGAAGTCCTGGCACAGCTGGAAAAAAGCCTGAAATCACCACGCAGTG
TCGCACCGTGGACGCGCGAGGAGTGGCAAAGAAAACTGGAGCGAGAGTATCAGGATATCGCTGCCCTGCCACAGAACGCG
AAGTTAAAAATCAAACGTCCGGTGAAGGTGCAGCCGATTGCCCGCGTCTGGTACAAAGGAGATCAAAAACAAGTCCAACA
CGCCTGCCCTACACCACTGATTGCACTGATTAATCGGGATAATGGCGCGGGCGTGCCGGACGTTGGTGAGTTGTTAAATT
ACGATGCCGACAATGTGCAGCACCGTTATAAACCTCAGGCGCAGCCGCTTCGTTTGATCATTCCACGGCTGCACCTGTAT
GTTGCGAATTTG**GGATCC**GGCGGT**CATATG**ACTAGT-pMH1200(GFP)-AGATCC*TAA***GAATTC**AGCATCTGCTTCAGC
GAGTGCCTCCGCATCAGCGAGTGCATCCGCCAGCGCGAGTGCCAGCGCTTCAGCATCTGCCTCAGCGTCCG**CCATGG**

Figure S1 Sequence and details of pPMS1259 from the original endfilled *BamHI* to the *NcoI* site. The start and stop codons are italicized. Sequences in bold represent deleted restriction sites. Sequences in bold and underlined represent restriction sites. The grey shaded sequence represents the linker between Tus and GFP and the black sequence corresponds to the insertion site and flanking sequences of pMH1200 (GFP) segment.

Cloning of the Tus expression vector pMM001

For the construction of the plasmid pMM001 encoding the Tus protein followed by the linker sequence, a stop codon (TGA) was introduced directly downstream of the linker in pPMS1259 by the following DNA manipulation. The plasmid pPMS1259 was digested with *NdeI* resulting in the linearization of the plasmid and the loss of a fragment of *GFP*. The 5'-overhangs were end-filled with Phusion DNA polymerase (Finnzymes, Espoo, Finland), resulting in the deletion of

NdeI sites. Owing to the presence of an adenosine nucleotide following both *NdeI* sites, a TGA stop codon was created upon recircularization of the end-filled plasmid by T4 DNA ligase to generate pMM001.

Expression and purification of his_6-tagged proteins

Escherichia coli BL21-(DE3)-RIPL cells were transformed with pMM001 (Tus) or pPMS1259 (TusGFP) for protein production. Single colonies were first isolated and grown overnight in LB supplemented with ampicillin (100 μg/mls) and chloramphenicol (50 μg/mls). Flasks of 100 mls of OEIM medium (Overnight Express Instant TB medium, Merck) were inoculated and incubated at 37ºC and 200 rpm until OD reached 1.5. Cells were then incubated at least another 24 hours at 16°C and 200 rpm. Cells were harvested when OD was steady for a minimum of 6 h and centrifuged at 7741 g during 10 minutes at 4 ºC and re-suspended in ice-cold lysis buffer (buffer A: 45 mM Na_2HPO_4, 5mM NaH_2PO_4, 10% glycerol (v/v), 300 mM NaCl, 2mM β-mercaptoethanol) at 7ml/g of cell pellet. Cells were lysed twice at 12000 p.s.i in a cooled French press followed by centrifugation at 39191 g during 40 minutes at 4 ºC. The clarified lysate (≈ 15 mls) was mixed with 2 mls of buffer A-equilibrated Ni-charged beads (IMAC, Bio-Rad) and gently rocked during one hour at 4°C. The beads were transferred into a column and after elution of the lysate, the flow-through was passed again through the column. Ni-charged beads were then washed with 15 ml of buffer A + 10 mM imidazole. The proteins were eluted with buffer A + 200 mM imidazole. The fractions containing the his_6-proteins were pooled, $(NH_4)_2SO_4$ precipitated (at 0.5 g/ml) at 4˚C and centrifuged at 18000 g during 50 minutes at 4˚C. The pellets were resuspended with 500 μl of buffer B (45 mM Na_2HPO_4, 5mM NaH_2PO_4, 10% glycerol (v/v), 2mM β-mercaptoethanol) and stored at -80 ºC. Protein concentrations were determined by standard Bradford assay (SIGMA®) and purity (see Fig. S2) was assessed by SDS-PAGE (ProPure Next Gel 10%, Amresco)

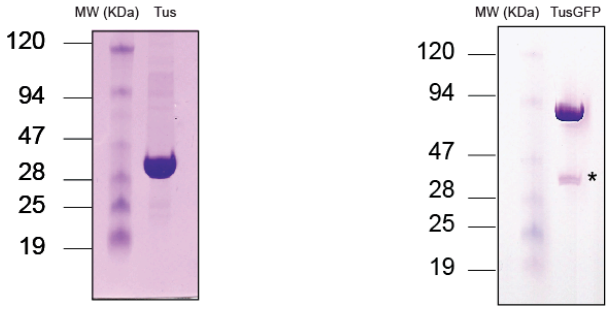

Figure S2. Coomassie gels of purified His_6-Tus (Tus) and His_6-TusGFP (TusGFP). Sizes of molecular weight marker are as indicated on the left. The star indicates Tus protein contamination resulting from minor proteolysis of TusGFP.

Sequences of *Ter* variants

Oligonucleotides used in this study were obtained from SIGMA-ALDRICH :

```
Br:  5′—CTTTAGTTACAACAT(BrdU)CTTAT-3′
P1:  5′-ATAAGAATGTTGTAACTAAAG-3'
P2:       5′-TATGTTGTAACTAAAG-3'
P3:       5′-AATGTTGTAACTAAAG-3'
P4:  5′-ATAAGTATGTTGTAACTAAAG-3'
P5:  5′-GGGGAAATGTTGTAACTAAAG-3'
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EMSA

Oligonucleotide pairs were prepared by mixing equal volumes of each oligonucleotide (50 μM final concentration in buffer C: 10 mM Tris pH 8.0, 1 mM EDTA and 125 mM KCl) followed by 2 minutes incubation at 72˚C and a slow cooling step in order to obtain optimal annealing. His_6-proteins were diluted to a concentration of 50 μM in buffer B. Equal volumes of His_6-proteins (2 μl) and oligonucleotide pairs (2 μl) were mixed and incubated for 20 minutes in the dark at room temperature to allow the formation of protein-DNA complexes. The complexes were subjected to agarose gel electrophoresis (1%). Gels were stained with Coomassie Brilliant Blue for 1 hour and destained overnight in a solution of 40% isopropanol/10% acetic acid.

Photocrosslinking kinetics

The protein-DNA complexes were prepared as described in the EMSA section. Drops of the complex were spotted under the cover of a Nunclon 96 well plate with a precooled block placed on top to avoid overheating, then irradiated using a UV-transilluminator set at 312 nm (Vilber Lourmat). Drops were hanging at ~7 mm above the surface of the transilluminator. Crosslinking yields for reaction times ranging from 30 s to 9 min were assessed by SDS-PAGE followed by Coomassie blue staining. Reaction samples were denaturated during 2 minutes at 90°C in loading buffer (Amresco) and loaded in a 10% acrylamide gel (ProPure Next Gel 10%, Amresco). Gels were run for 1 hour at 40 mA, stained with Coomassie Brilliant Blue and destained in a solution of 40% isopropanol/10% acetic acid. Gels were scanned and analysed using ImageJ software.

KCl-dependence of photocrosslinking

Oligonucleotide pairs were prepared as described above. The protein-DNA complexes were prepared as above except that they were supplemented with KCl to obtain final concentrations of 62.5, 150, 250, 350, 450, 550, 650, 750, 850 and 950 mM. Drops were irradiated during 6 min at 312 nm and photocrosslinking yields were assessed as described above.

Evaluation of UV_{312}-induced DNA damage

We evaluated the extent of damage that UV irradiation could cause to DNA and proteins. We treated TusGFP with each of the *Ter* variants, followed by 6 min of UV irradiation and an EMSA (lanes 2-11, Fig. S3). UV irradiation induced a partial quenching of TusGFP fluorescence but the presence of one single protein band confirmed the integrity of the TusGFP-DNA complexes. When TusGFP and Br/P2 were separately UV-irradiated for 6 minutes and then incubated 20 minutes in the dark to allow binding, the presence of one single band confirmed that Tus and Br/P2 were still able to bind, suggesting that there is no substantial degradation of Tus or DNA after UV irradiation (lane 12, Fig S3).

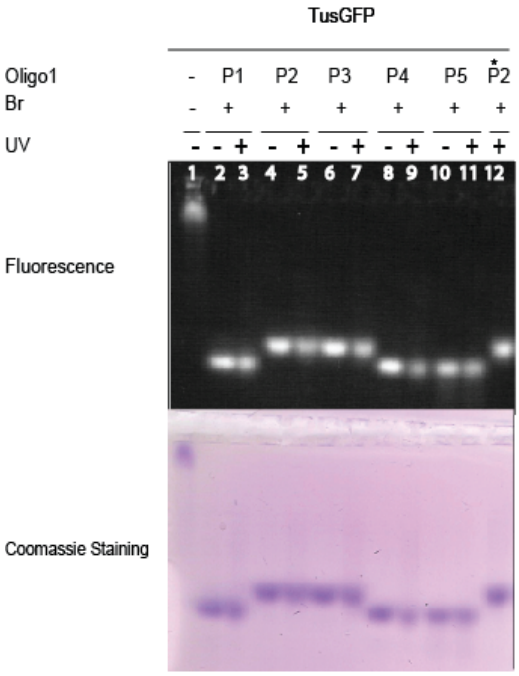

Figure S3. EMSA of the TusGFP-*Ter* complex. TusGFP proteins were incubated with *Ter* variants (lane 2,3 : Br/P1; lane 4,5 : Br/P2; lane 6,7 : Br/P3; lane 8,9 : Br/P4; lane 10,11 : Br/P5). Half of the mixture was subjected to UV-irradiation for 6 minutes, the other half was kept in the dark. As a control, lane 1 shows the migration of free TusGFP. For lane 12 (*), TusGFP and Br/P2 *Ter* sequences were separately UV-irradiated for 6 minutes and then incubated 20 minutes in the dark to allow binding. The agarose gel was first exposed to UV (top panel) and then

stained with Coomassie Brilliant Blue and destained to visualize free and DNA-bound proteins (bottom panel).

1. C. Neylon, S. E. Brown, A. V. Kralicek, C. S. Miles, C. A. Love and N. E. Dixon, *Biochemistry*, 2000, **39**, 11989-11999.

2. K. Ozawa, M. J. Headlam, D. Mouradov, S. J. Watt, J. L. Beck, K. J. Rodgers, R. T. Dean, T. Huber, G. Otting and N. E. Dixon, *FEBS J*, 2005, **272**, 3162-3171.