Acta Crystallographica Section D

Volume 70 (2014)

Supporting information for article:

Bacteriophage P22 tailspike: structure of the complete protein and function of the interdomain linker

Anaït Seul, Jürgen J. Müller, Dorothee Andres, Eva Stettner, Udo Heinemann and Robert Seckler

Supporting information

Table S1 Primers for cloning of the expression constructs.

Primer	*Sequence*
PBD-fw	CTAGATCATATGACAGACATCACTGCAAAC
PBD-rv	CCTGTCATTGATCAGCTTAAATTTTTTATCAGCTTC
Zip-fw	ATCATAGTGATCAAACAGATCGAAGACAAATC
Zip-rv	CTAATCACATATGTCATTCACCGATCAGTTTTTTG
Y108W-fw	GCTAACGTGTTGAAGTGGGATCCAGATCAATATTCAATAGAAGCTG
Del01	CGATCCAGATCAATATTCAATAGAAGCTGATAAAAAATTTAGATCAAACAGATCGAAGACAAA ATCG
Del02	CGATCCAGATCAATATTCAATAGAAGCTGATAAAAAATTTAAACAGATCGAAGACAAAATCG
Del03	TCAATAGAAGCTGATAAAAAATTTAAACAGATCGAAGACAAAATCG

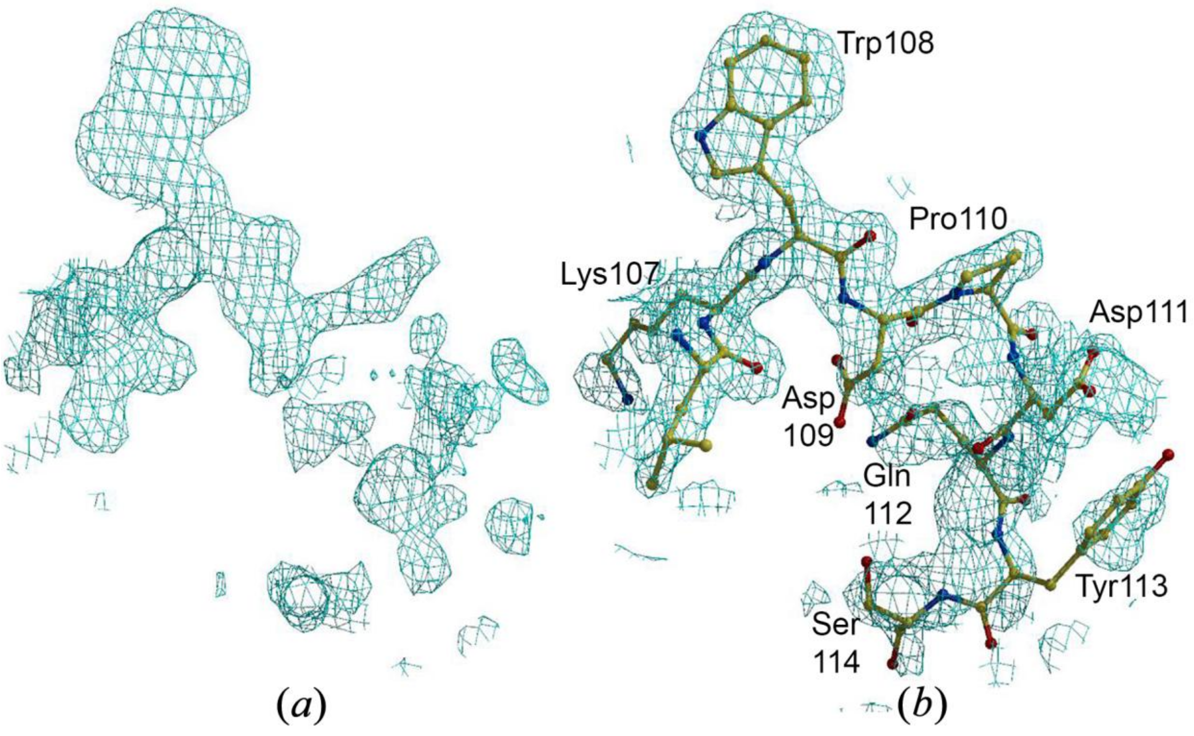

Figure S1 Electron density of PBDzip around residues K107-S114 before (*a*) and after (*b*) use of the BIAS REMOVAL SERVER (Reddy *et al.*, 2003). The contours following the 1σ level are displayed along with the fully refined structure.

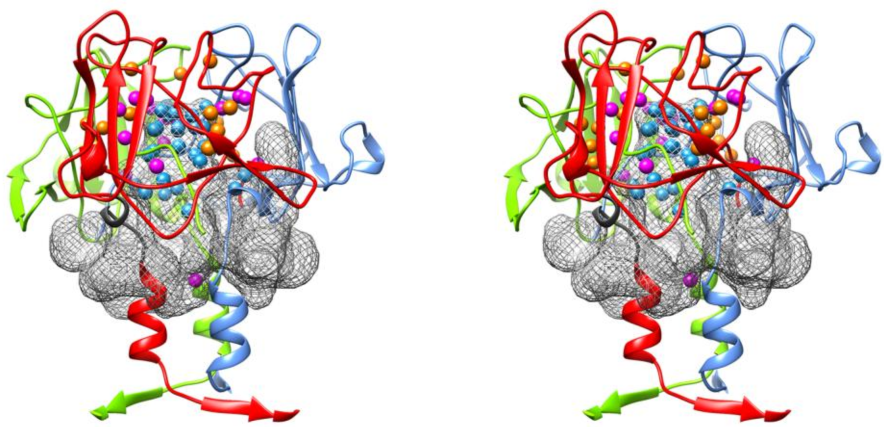

Figure S2 Inner solvation of the trimeric PBD of TSP-Y108W (stereo view). Chains are colored as in Fig. 3. Solvent tunnels pass through the triple helix, and a large cavity (bordered by a mesh (generated with SURFNET (Laskowski, 1995))) with channels toward the bulk solvent is covered by the protein chains. 15 water molecules are buried within the trimeric PBD, and 15 more waters are located in deep clefts between the chains. Water molecules are represented by spheres, colored blue when located in the cavity, orange when buried, and magenta when positioned in deep clefts between protein chains. The peptide stretch V105-N111 (black in chain A) resides at the surface of the PBD. Because the interior of the PBD is solvent-accessible, the linker peptide is almost completely hydrated.

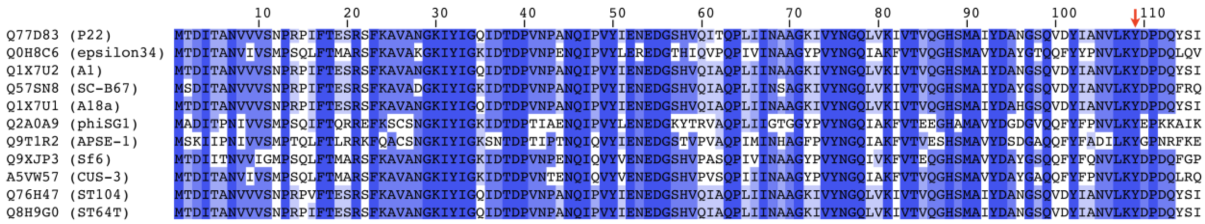

Figure S3 Alignment of tailspike PBDs. The first 116 residues of nine (putative) tailspike proteins from enterobacterial phages and two phages of endosymbionts were aligned with ClustalW (Larkin *et al.*, 2007). The source organisms are given in parentheses. Residues are blue-shaded according to percentage of identity. The highly conserved tyrosine 108 (here residue 109, as the N-terminal methionine is included) is marked by a red arrow. The four residues following Y108 (linker residues) are fully conserved except for the sequences from the endosymbiont phages, phiSG1 (phage of tsetse fly endosymbiont *Sodalis glossinidius*) and APSE-1 (phage of aphid endosymbiont *Acyrthosiphon pisum*).

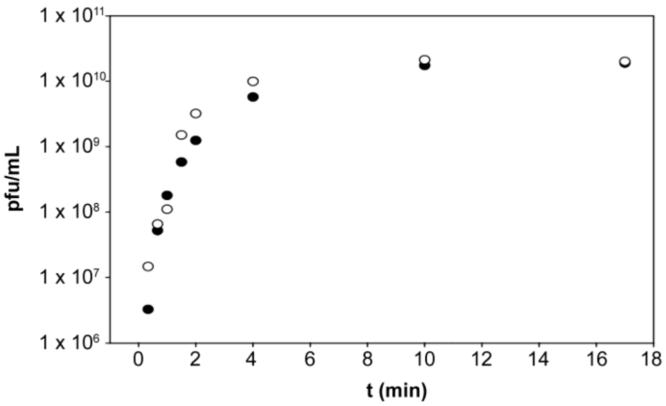

Figure S4 Tailing kinetics of TSP wild-type and TSP-Y108W. The experiment was performed at 4 °C with an excess of tailspike molecules over phage heads of 195:1. Both wild-type (empty circles) and mutant (filled circles) tailspike bind to phage heads and produce infectious particles.

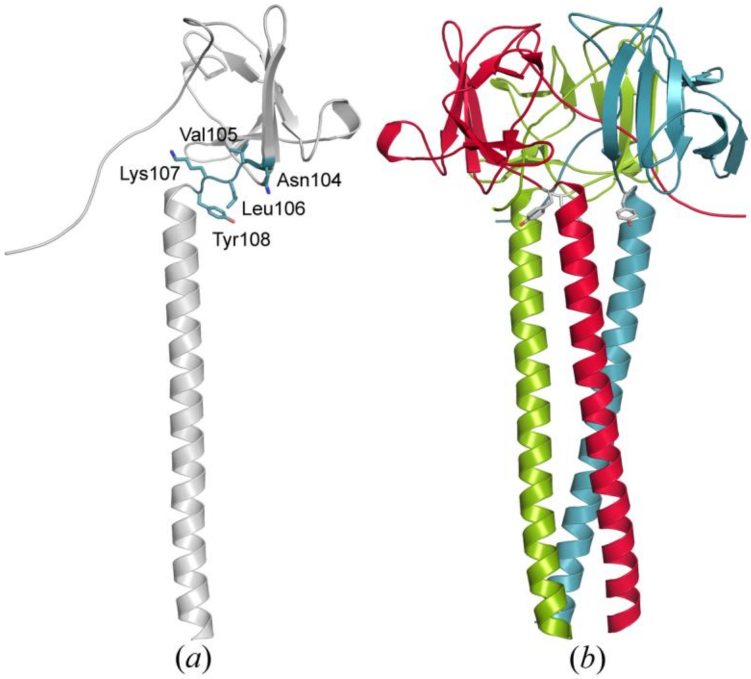

Figure S5 Structure of pre-PBDzip. (*a*) The mechanical strain in pre-PBDzip leads to a divergence of the coiled-coil structure towards the N-terminus, as well as to an elongation of the coiled coil, and thus to the unwinding of the small helix present in the native PBD. Side chains of residues from this small helix are shown in blue and labeled. The construct of PBD and coiled-coil zipper was used as a template to fit the distorted tailspike monomer chain A into the cryo-EM map. (*b*) In the trimer, chains A, B, and C are colored differently, and the side chain of Y108 is shown in gray.

```
pre-PBDzip    1 TDITANVVV SNPRPIFTES RSFKAVANGK IYIGQIDTDP VNPANQIPVY
PBDzip        1 TDITANVVV SNPRPIFTES RSFKAVANGK IYIGQIDTDP VNPANQIPVY
PBD           1 TDITANVVV SNPRPIFTES RSFKAVANGK IYIGQIDTDP VNPANQIPVY

pre-PBDzip   50 IENEDGSHVQ ITQPLIINAA GKIVYNGQLV KIVTVQGHSM AIYDANGSQV
PBDzip       50 IENEDGSHVQ ITQPLIINAA GKIVYNGQLV KIVTVQGHSM AIYDANGSQV
PBD          50 IENEDGSHVQ ITQPLIINAA GKIVYNGQLV KIVTVQGHSM AIYDANGSQV

pre-PBDzip  100 DYIANVLKYD PDQYSIEADK KFKLIKQIED KIEEILSKIY HIENEIARIK
PBDzip      100 DYIANVLKWD PDQYSIEADK KFK---QIED KIEEILSKIY HIENEIARIK
PBD         100 DYIANVLKYD PDQYSIEADK KFKYSLEHHH HHH

pre-PBDzip  150 KLIGE
PBDzip      147 KLIGE
```

Figure S6 Sequence alignment of pre-PBDzip, PBDzip, and the truncated head-binding domain (PBD). The underlined sequences correspond to the residues visible in the electron density of the isolated PBD (PDB ID: 1LKT) and RBD (PDB ID: 1TYX), the sequence of the linker region is highlighted in blue with the mutated Trp108 shown in red, the sequence of the isoleucine zipper (residues 3-31 of the Ebola virus membrane fusion subunit GP2) is highlighted in gray.