The C-terminal residue of phage Vp16 PDF, the smallest peptide deformylase, acts as an offset element locking the active conformation

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MATERIALS AND METHODS

Materials

All solvents and chemicals were purchased from SDS and Aldrich, respectively.

_V. parahaemolyticus_ phage PDF expression and purification

Rosetta2(DE3)pLysS were transformed with pET16b plasmid containing _Vp_16 PDF gene. Protein expression was induced with 0.5 mM IPTG for 3h at 37°C. For the crystal structure determination _Vp_16 PDF was purified as previously described ¹. For the enzymatic characterization, the cells were resuspended in buffer A (50 mM MES-KOH pH 4 and 80 mM NiCl₂). The supernatant was loaded to SP-Sepharose column equilibrated with buffer A. Bound proteins were eluted with buffer B (50 mM MES-KOH pH 4 and 80 mM NiCl₂, 1M KCl). Because a high purity, no second step of purification was necessary and purified protein was directly dialyzed overnight at 4°C against buffer A containing 55% glycerol and stored at -20°C.

Cloning, expression and purification of _E. coli_ PDF and chimeras

_E. coli_ PDF and _E. coli_ PDF(KLF)∆helices were cloned in pBAD and pET-22b(+) as previously reported ²;³. Site-directed mutagenesis to create the different chimeras was carried out in the wild type version of _E. coli_ PDF or _Vp_16 PDF present in both pBAD and pET-22b(+) plasmids by using the QuickChange Site-Directed Mutagenesis Kit (Stratagene). The C-terminal deleted versions of _E. coli_ PDF and corresponding mutants were generated from the corresponding full length versions in which a double stop was inserted after the codon corresponding to the 144th amino acid.
**Enzymology**

PDF enzymatic activity was measured by a spectrophotometric assay. The formate released by the deformylation reaction is used by FDH to convert NAD in NADH, the production of which being measured over time at 340 nm and controlled temperature (37°C). The 200µL reaction mixture contained 50 mM HEPES-NaOH pH 7.5, 12 mM NAD⁺, 4.5 U/mL FDH, 1 mM NiCl₂ and PDF enzyme (either 100 nM *Vp*₁₆ PDF or 10 nM *E. coli* PDF) previously diluted in 50 mM MES-KOH pH 4.0, 80 mM NiCl₂ for *Vp*₁₆ PDF or 50 mM HEPES-NaOH pH 7.5, 0.1 mg/mL BSA for *E. coli* PDF. The reaction was started by addition of 1-6 mM Fo-peptides as indicated in the legend of Figure. Kinetics parameters (*k*ₗₒₚ, *K*ₘ) were derived from iterative non-linear least square fits using the Michaelis-Menten equation based on the experimental data (Sigma-Plot, Kinetics module).

*Vp*₁₆ PDF inhibition was performed as previously described ⁴, ⁵.

**Immunological Methods**

Rabbit polyclonal antibodies raised against full length *E. coli* MetAP and *E. coli* PDF were used at 1:5000 dilution for Western blot analysis as previously described ⁶.
N-terminal proteomic analysis

The PAL421Tr was transformed at 30°C with pBAD plasmid expressing *Vp*16 PDF or *E. coli* PDF in the presence of ampicillin. Next day 10 mL cultures were inoculated with bacterial colony from the plate and grown for 24h in the presence of ampicillin and 0.2% arabinose at 37°C. After 24h, cultures were diluted to the OD600=0.05 and cultured at 30°C in the presence of 0.1% of arabinose for cells expressing *E. coli* PDF and 0.2% for *Vp*16 PDF to equalize the level of protein expression. Cells (4.6 x 10^10) were harvested and collected by centrifugation (4°C, 3300g, 30 min) at time points 2, 6, 8 and 24h. Cells were disrupted by grinding (MM300 grinder, Qiagen) in 50 mM HEPES-NaOH pH 8, 25 mM Ascorbic Acid, 25 mM L-Cystein and one tablet of antiprotease inhibitor cocktail (Roche). After centrifugation at 21,000 g for 30 min at 4°C, protein concentration in the supernatant was determined by Bradford protein assay.

For N-termini enrichment samples were prepared as previously described (Bienvenut et al., 2012). 1 mg of protein was denatured and reduced followed by cysteine alkylation with iodoacetamide. After cold acetone precipitation, proteins were resuspended in 50 mM NH₄HCO₃ and digested by 1/100 (w/w) of TPCK treated porcine trypsin (Sigma-Aldrich) for 1.5h at 37°C, twice. Peptides were desalted with Sep-Pak columns and the retained material was eluted with 80% acetonitrile (ACN), 0.1% TFA and then evaporated to dryness. The collected material was resuspended in Strong Cation eXchange (SCX) LC buffer (5mM KH₂PO₄, 30% ACN and 0.05% formic acid) and injected into Alliance HPLC system using a fluorimeter detector (Waters) equipped with polysulfoethyl A column (200 x 2.1 mm, 5 µm 200 Å; PolyLC, Colombia, MD). Peptides were eluted with a KCl gradient (SCX-LC buffer B: 350 mM KCl in SCX-LC buffer A; 0-5 min, 0% B; 15-40 min, 5-26% B; 40-45 min, 26-35% B). Fractions were collected every 2 min for 40 min and the solvent was evaporated to
dryness before storage at -20°C. Fractions eluted from SCX columns with retention times of 3 to 22 min were analyzed as previously described \(^7\) with an Easy Nano-LC II (Thermo Scientific) coupled to a LTQ-Orbitrap™ Velos (Thermo Scientific).

**Crystallography: data collection and processing**

Diffraction data were collected on single crystals at 100 K on FIP-BM30A and PROXIMA1 beamlines at ESRF (Grenoble, France) and SOLEIL (Gif-sur-Yvette, France), respectively. Data were processed and scaled with XDS package \(^8\). Statistics are summarized in Table S2. The crystal structure of \(Vp\)16 PDF crystallized in form I was solved by molecular replacement with PHASER \(^9\) using a C-terminally truncated version of the *Pseudomonas aeruginosa* PDF (PDB code 1LRY \(^10\)) as the starting model. Structures of protein crystallized in form II was solved by rigid-body refinement in REFMAC \(^11\). Manual model building and refinement were done with TURBO-FRODO \(^12\) and REFMAC, \(^11\) respectively, yielded the final models that were validated by PROCHECK \(^13\). Refinement statistics are detailed in Table S2. Figures were generated using PyMOL (http://www.pymol.org).

Inspection of the density maps revealed the presence of several high electron density spots in both models, which could reasonably be interpreted as metal ions. In order to identify the nature of these ions, we performed X-ray fluorescence experiments on the crystal form II, which revealed the presence of two different metal ions, zinc and nickel. Anomalous difference maps calculated for each metal allowed us to locate zinc ions within the active site and nickel ions at the surface of the protein.

**List of the 262 sequences selected to represent PDF sequence diversity and to construct the new phylogenetic tree**

Type 1B
Halococcus-hamelinensis (archaea); Roseobacter-sp-MED193; Rhodobacterales-bacterium-HTCC2083; Roseobacter-denitrificansRoseovarius-217; Magnetospirillum-magnetcum-AMB1; Magnetospirillum-magnetotacticum-MS1; Phaeobacter-gallaeciensis-BS107; Sinorhizobium-meliloti1/2; Agrobacterium-tumefaciens1/2; Caulobacter-phageCcr; Colossus Sinorhizobium-meliloti2/2; Agrobacterium-tumefaciens2/2; Vibrio-cholerae1/2; Vibrio-parahaemolyticus1/2; Enterobacter-aerogenes; Escherichia-coli; Haemophilus-influenzae; Neisseria-gonorhoeae; Chromobacterium-violaceum; Physcomitrella-patens1/3; Pseudomonas-aeruginosa1/2; Methylcoccus-capsulatus2/2; Xanthomonas-oryzae2/2; Vibrio-cholerae2/2; Vibrio-parahaemolyticus2/2; Rickettsiae-prowazekii; Rickettsia-conorii2/3; Ehrlichia-canis1/1; Rickettsia-conorii3/3; Clostridium-sordellii1/2; Peptoclostridium-difficile2/2; Clostridium-tetani; Clostridium-acetobutylicum1/2; Clostridium-botulinum2/3; Clostridium-clostridioforme2/2; Clostridium-perfringens2/2; Candidatus-arathomitus; Clostridium-botulinum1/3; Clostridium-beijerinckii1/2; Clostridium-acetobutylicum2/2; Peptoclostridium-difficile1/2; Bacillus-oceanisediminis1/3; Geobacillus-stearothermophilus1/2; Bacillus-cereus1/2; Bacillus-subtilis2/2; Enterococcus-faecalis1/2; Listeria-monoctogenes1/2; Helicobacter-pylori; Myxococcus-xanthus3/3; Mycobacterium-tuberculosis; Mycobacterium-leprae; Mycobacterium-smegmatis; Nocardia-vulneris3/4; Rhodococcus-wratislaviensis1/2; Corynebacterium-diphtheriae1/2; Corynebacterium-doosanense2/2; Corynebacterium-amycolatum1/2; Nocardia-vulneris1/4; Rhodococcus-wratislaviensis2/2; Streptomyces-avermitilis2/3; Streptomyces-avermitilis1/3; Gardnerella-vaginalis1/2; Bifidobacterium-longum1/3; Thermobifida-fusca; Nocardia-vulneris4/4; Corynebacterium-amycolatum2/2; Corynebacterium-diphtheriae2/2; Corynebacterium-doosanense1/2; Capronia-semi-immersa Cladophialophora-carriionii; Cladophialophora-psammophila; Propionibacterium-humerusii; Propionibacterium-acnes; Tropheryma-whipplei-strTwist; Aquifex-aelicus; Hydrogenobacter-thermophilus-TK6; Ricinus-
communis1B; Populus-trichocarpa1B; Vitis-vinifera1B; Glycine-max1B; Medicago-
truncatula1B; Lycopersicon-esculentum1B; Arabidopsis-thaliana1B; Hordeum-vulgare1B;
Oryza-sativa1B; Micromonas-pusilla; Micromonas-sp.RCC299-1B; Ostreococcus-
lucimarinus1B; Chlamydomonas-reinhardtii1B; Galdieria-sulphuraria; Thalassiodra-
pseudonana2/2-diatom; Cyanidioschyzon-merolae-rhodophyte; Thalassiosira-
pseudonana1/2-diatom; Thermotoga-spRQ2; Thermotoga-naphthophila; Thermotoga-maritima-MSB8;
Thermotoga-petrophila-RKU1; Thermotoga-neapolitana-DSM4359; Petrotoga-mobilis;
Bigelowiella-natans1B; Plasmodium-vivax; Plasmodium-knowlesi; Plasmodium-falciparum;
Plasmodium-yoelii; Acanthamoeba-castellanii1/2 amoebozoa; Psychroflexus-torquis-
ATCC700755; unidentified-eubacterium-SCB49; Polaribacter-irgensii-23P; Flavobacterium-
seoulense; Sphingobacterium-spiritivorum-ATCC33861; Algoriphagus-sp-PR1; Microcella-
marinaBacteroides-sp.D1; Bacteroides-ovatusATCC8483; Bacteroides-thetaiotaomicron-VPI-
5482; Pseudomonas-gingivalis1/2; Chlorobium-tepidum; Cyanothece-sp.PCC7424;
Crocosphaera-watsoniiWH8501; Synechocystis-sp-PCC8821; Acaryochloris-marina-
MBIC11017; Gloeobacter-violaceus; Prochlorococcus-marinus-strMIT9215; Prochlorococcus-
marinus; Synechococcus-spRS9917; Borrelia-burgdorferi; Treponema-pallidum;
Myxococcus-xanthus2/3.

**Type 1C**

Streptobacillus-moniliformis-DSM1211; Leptotrichia-buccalis; Seballdella-termitidis-
ATCC33386; Methanocorpusculum-bavaricum1/2; Methanocorpusculum-labreanum-Z2/2;
Methanocorpusculum-bavaricum2/2; Methanocorpusculum-labreanum-Z1/2;
Methanomicrobium-mobile; Deinococcus-radiodurans.

**Type 1A**

Methylcoccus-capsulatus1/2; Myxococcus-xanthus1/3; Bos-taurus; Muntiacus-muntjak-
vaginalis-deerCanis-familiaris; Homo-sapiens; Pan-troglodytes-chimpanzee; Mus-
musculusRattus-norvegicus; Gallus-gallus; Meleagris-gallopavo turkey; Tetraodon-nigroviridis; Fugu-rubripes; Gasterosteus-aculeatus; Oryzias-latipes-Japanese-medaka; Danio-rerio; Oncorhynchus-mykiss; Xenopus-tropicalis; Ixodes-scapularis-tick-arachnide; Boophilus-microplus-tick-arachnide; Strongylocentrotus-purpuratus-echynoderm; Drosophila-melanogaster2/2; Drosophila-melanogaster1/2; Anopheles-gambiae; Aedes-aegypti yellow; Oncometopia-nigricans;Apis-mellifera; Hydra-magnipapillata2/2; Hydra-magnipapillata1/2; Batrachochytrium-dendrobatidis; Spizellomyces-punctatus; Physcomitrella-patens2/3; Physcomitrella-patens3/3; Lycopersicon-esculentum1A; Arabidopsis-thaliana1A; Oryza-sativa1A; Triticum-aestivum1A; Chlamydomonas-reinhardtii1A; Ostreococcus-lucimarinus1A; Streptomyces-coelicolor1; Streptomyces-avermitilis3/3; Nocardia-vulneris2/4; Gardnerella-vaginalis2/2; Bifidobacterium-longum2/3; Xanthomonas-oryzae1/2; Pseudomonas-aeruginosa2/2.

**Type 4**

Cyano-phage-GOS-JCVI-1502.1906; Cyano-phageGOS-JCVI-1149-1577; Cyano-phagePRSM6; Cyano-phageGOS-JCVI-1906; Stenotrophomonas-phage-vB; SmaS-DLP 6; Caulobacter-phageCr30; Synechococcus-phageACG-2014f; Synechococcus-phage S-SSM7; Ehrlichia-canis2/2; Hammodia-hammondi; Toxoplasma-gondii; Neospora-caninum.

**Type 3**

Vibrio-phage-henriette-12B8; Methanoculleus-marisnigri; Candidatus-Methanoperedens-nitroreducens; Methanosarcina-soligelidi; Methanobacterium-formicicum; Methanobacterium-thermoautotrophicum; Methanobrevibacter-filiformis; Trypanosoma-cruzi2/2; Trypanosoma-brucei2/2; Leishmania-major2/2; Trypanosoma-cruzi1/2; Trypanosoma-brucei1/2; Leishmania-major1/2; Thermoplasmales-archaeon; Streptococcus-dysgalactiae2/2; Streptococcus-pyogenes2/2; Streptococcus-uberis2/2; Streptococcus-thermophilus2/2; Streptococcus-agalactiae2/2; Streptococcus-suis2/2; Streptococcus-
pneumoniae2/2; Clostridium-botulinum3/3; Clostridium-beijerinckii2/2; Clostridium-sordelli2/2; Erysipelotrichaceae-bacterium2/2; Bifidobacterium-longum3/3; Lactobacillus-helvetica2/2; Rickettsia-conorii1/3; Staphylococcus-simiae2/2; Staphylococcus-aureus2/2; Clostridium-perfringens1/2; Paramecium-tetraurelia2/2; Nostoc-punctiforme; Malawimonas-jakobiformis-mitochondrial-cristae; Blastocystis-homini-diatom; Clostridium-clostridioforme1/2; Candidatus-thorarchaeota archaea; Lokiarchaeum-sp archaea; Sawyeria-marylandensis; Polysphondylium-pallidum; Acytostelium-subglobosum; Dictyostelium-discoideum; Acanthamoeba-castellanii2/2; Paramecium-tetraurelia1/2.

Type 2

Streptococcus-uberis1/2; Streptococcus-thermophilus1/2; Streptococcus-agalactiae1/2; treptococcus-pneumoniae1/2; Streptococcus-dysgalactiae1/2; Streptococcus-suis1/2; Enterococcus-faecalis2/2; Listeria-monocytogenes2/2; Bacillus-subtilis1/2; Geobacillus-stearothermophilus2/2; Bacillus-oceanisediminis3/3; Bacillus-cereus2/2; Staphylococcus-simiae1/2; Staphylococcus-aureus1/2; Bacillus-oceanisediminis2/3; Lactobacillus-helvetica1/2; Erysipelotrichaceae-bacterium1/2; Mycoplasma-pneumoniae; Phialophora-attae

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**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1 Complementation of strain PAL421Tr by different PDFs.** pBAD plasmids encoding different PDFs (*E. coli, Vibrio parahaemolyticus* phage and marine viral PDFs named: 1906, 1906QR deleted version, the last two amino acids, of 1906, and 2750) were used to transform strain PAL421Tr at 30°C. Strains were streaked out in parallel at 42°C on
LB Petri dishes containing PDF expression inducer arabinose (0.1%). pBAD corresponds to the empty cloning vector used as control.

**Figure S2 Complementation of strain PAL421Tr by different *E. coli* PDF chimeras.** pBAD plasmids encoding different PDF chimeras were used to transform strain PAL421Tr at 30°C. Strains were serially diluted and spotted in parallel at 42°C on LB Petri dishes containing PDF expression inducer arabinose. a) Outline of used chimeras. b) Image of the Petri dishes incubated at 30°C and 42°C at different concentration of arabinose.

**Figure S3 Full-length gel and blots of Figure 3a are presented.**

**SUPPLEMENTAL TABLES**

**Table S1. Comparative enzymatic constants of *Vp*16 PDF and *E. coli* PDF for different formylated tripeptides.**

| Peptide          | *Vp*16 PDF |              |              |              | *E. coli* PDF |              |              |              |
|------------------|------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
|                  |            | $K_m$ (mM)   | $k_{cat}$ (sec$^{-1}$) | $k_{cat} / K_m$ (M$^{-1}$ s$^{-1}$) | $k_{cat} / K_m$ (%) | $K_m$ (mM)   | $k_{cat}$ (sec$^{-1}$) | $k_{cat} / K_m$ (M$^{-1}$ s$^{-1}$) | $k_{cat} / K_m$ (%) |
| Fo-Met-Ala-Ser   | 2.3        | 20           | 8,478        | 100          | 2.59         | 57           | 22,800       | 100          |
| Fo-Met-Lys-Leu   | 2.9        | 27.4         | 9,448        | 111          | 0.29         | 344          | 86,000       | 377          |
| Fo-Met-Pro-Ala   | 1.4        | 5.3          | 3,711        | 44           | 5.1          | 82           | 16,080       | 70           |
| Fo-Met-Ser-Asn   | 12         | 27.4         | 2,286        | 27           | 5.8          | 89           | 15,355       | 67           |
| Fo-Met-Thr-Thr   | 1.0        | 9.0          | 8,950        | 105          | 1.4          | 70           | 49,366       | 216          |

The catalytic efficiency for the Fo-Met-Ala-Ser was taken as 100% activity.
**Table S2. Crystallographic data and refinement statistics**

|                | Form I                      | Form II                  |
|----------------|-----------------------------|--------------------------|
| **Ligand**     | PEG                         | PEG-1,000                |
| **Data collection** |                             |                          |
| Wavelength (Å) | 0.97978                     | 0.98011                  |
| Resolution range (Å) | 50.0-1.7 (1.80-1.70)         | 50.0-1.5 (1.54-1.50)     |
| Space group    | P 32 2 1                    | P 32 2 1                 |
| Unit cell      |                             |                          |
| a, b, c (Å)    | 64.73, 64.73, 128.53        | 64.26, 64.26, 125.69    |
| α, β, γ (°)    | 90, 90, 120                 | 90, 90, 120              |
| Unique reflections | 35064                      | 48793                    |
| Redundancy     | 9.0 (8.9)                   | 4.3 (4.4)                |
| Completeness (%)| 99.8 (99.8)                 | 99.7 (99.5)              |
| I/σ            | 20.33 (3.28)                | 17.78 (2.45)             |
| R_sym (%)      | 7.6 (75.1)                  | 4.0 (50.2)               |
| CC_1/2         | 99.9 (89.9)                 | 99.9 (82.6)              |
| **Refinement** |                             |                          |
| R_work         | 0.1707                      | 0.1867                   |
| R_free         | 0.2013                      | 0.2096                   |
| Number of atoms|                             |                          |
| Protein        | 2036                        | 5051                     |
| Ligand / Ions  | 0 / 4                       | 20 / 5                   |
| Water          | 113                         | 329                      |
| R.m.s. deviations |                             |                          |
| Bond lengths (Å)| 0.010                      | 0.009                    |
| Bond angles (°)| 1.30                       | 1.31                     |

Statistics for the highest-resolution shell are shown in parentheses.
Table S3. Summary of the primers used in the study. The sequences of the primers (ThermoFisher Scientific), in the directions "forward" and then "reverse", are presented from 5' to 3'. The nucleotides corresponding to the mutated amino acids are shown in red.

| Constructions | Sequence (5'-3') |
|---------------|-----------------|
| *Vp*16 PDF(VTI)helices | CTGAACGGCGTAACGATAATGGATTATCTGAGTCCGCTG CAGCGGACTCAGATAATCCATTTATCGTTACGCGGTCAG |
| *Vp*16 PDF(VTF)helices | CTGAACGGCGTAACGATTATGGATTATCTGAGTCCGCTG CAGCGGACTCAGATAATCCATTATCGTTACGCGGTCAG |
| *E. coli* PDF(KLF)Δhelices | CCTGGTGACGAAAAGTTTATGGATTATCTGTCACCGCTG CAGCGGTCAGATAATCCATTTATCGTTACGCGGTCAG |
| *E. coli* PDF(VTI)helices | CACCTGGTGACGACTACATGTTTATGGATTATCTGTCACCGCTG CAGCGGTCAGATAATCCATTTATCGTTACGCGGTCAG |
| *E. coli* PDF(VTI)Δhelices | CAGCATGAGATGGATCACCTGGTGACGACTACATGTTTATGGATTATCTGTCACCGCTG CAGCGGTCAGATAATCCATTTATCGTTACGCGGTCAG |
| *E. coli* PDF(KLI)helices | CACCTGGTGACGACTACATGTTTATGGATTATCTGTCACCGCTG CAGCGGTCAGATAATCCATTTATCGTTACGCGGTCAG |
Table S4. Protein sequence of wild type PDFs and chimeras used in this study. The mutated amino acids with respect to the wild version are shown in red. The protein sequence corresponding to the wild type sequence of *E. coli* PDF is shown in green whereas that of *Vp*16 PDF is shown in black.

| PDF                | Amino acid sequence                                                                 |
|--------------------|-------------------------------------------------------------------------------------|
| *Vp*16T            | MKILDDAPELHAIAAEVPHGEDVKDLVLMATAAAGGIGLANQVGVLKRIIIVLR CPTFKGCVINPIITRHTDGHVSYSCPGLSYPGKTAVKRRNKVVVEGMDWQPIITA AKGLTACFLQHEIDHLNGVTI |
| 1906               | MILQLPNTHPIHLERVKCSYDLDRAEISKILYENMVHNGVGLSANQIGINERVFI MVKDLCENEILTCFNPKIVKQSSKTVMEEGCLSYPDEIFIEIERTVIVKVEDNKV NHKIKLEGFAARVFLHEFDHMQQINFTQ |
| 1577               | MILQLPNTHPIHLERMNKCSYDLDNEIISKILHENMIIHEGEGVGLSANQIGMSERVFI MMDIDETEETITCFNPRIVKVYDEEVEFEEGCLSYPEIIINIQPRRNIVKVYEDNKV NHKVKLEGFAARVFLHEYDGMEIGNFTQ |
| 2750               | MILELPNTPIHLKVKPCYNLDRNLSKTLIENMLHNYGIGISANQIGIWARAFA MVRDIEHNEIMVCFNPRIKITYAEEEVEMEEGCLSYPKLFLIKRPDRIVKVEYEDVKK THKKVKLGASVRQFQGEYNGEMGI |
| *Vp*16 PDF(VTI)helices | MKILDDAPELHAIAAEVPHGEDVKDLVLMATAAAGGIGLANQVGVLKRIIIVLR CPTFKGCVINPIITRHTDGHVSYSCPGLSYPGKTAVKRRNKVVVEGMDWQPIITA AKGLTACFLQHEIDHLNGVTMDYSLPLKQQRIRQKEVKLRLKARA |
| *Vp*16 PDF(VTF)helices | MKILDDAPELHAIAAEVPHGEDVKDLVLMATAAAGGIGLANQVGVLKRIIIVLR CPTFKGCVINPIITRHTDGHVSYSCPGLSYPGKTAVKRRNKVVVEGMDWQPIITA AKGLTACFLQHEIDHLNGVTMDYSLPLKQQRIRQKEVKLRLKARA |
| *E. coli* PDF(KLF)Δhelices | MAVLQVLHIPERLKVAKPVEEVNAEIQRIVDMMETMAYEAGIGLAAQTQV DIHRQRIIIVDSERDNERLVLINPELLEKSGTIEEVEGCLSIPEQRALVFRA EKVIRKALDRDGKPFELEADGLLAICIQHEMDHLVGKLF |
| *E. coli* PDF(VTI)helices | MAVLQVLHIPERLKVAKPVEEVNAEIQRIVDMMETMAYEAGIGLAAQTQV DIHRQRIIIVDSERDNERLVLINPELLEKSGTIEEVEGCLSIPEQRALVFRA EKVIRKALDRDGKPFELEADGLLAICIQHEMDHLVGTVI MDYSLPLKQQRIR QKEVKLRLKARA |
| *E. coli* PDF(VTI)Δhelices | MAVLQVLHIPERLKVAKPVEEVNAEIQRIVDMMETMAYEAGIGLAAQTQV DIHRQRIIIVDSERDNERLVLINPELLEKSGTIEEVEGCLSIPEQRALVFRA EKVIRKALDRDGKPFELEADGLLAICIQHEMDHLVGTVI |
| *E. coli* PDF(KLI)helices | MAVLQVLHIPERLKVAKPVEEVNAEIQRIVDMMETMAYEAGIGLAAQTQV DIHRQRIIIVDSERDNERLVLINPELLEKSGTIEEVEGCLSIPEQRALVFRA EKVIRKALDRDGKPFELEADGLLAICIQHEMDHLVGKLI MDYSLPLKQQRIR QKEVKLRLKARA |
Figure S2

A

| Strain                | Temperature | Glucose  | Arabinose |
|-----------------------|-------------|----------|-----------|
| **E. coli PDF**       | 30 °C       | 0.5 %    | 0.2 %     |
| **Vp16 PDF**          | 30 °C       | 0.5 %    | 0.5 %     |
| **E. coli PDF (KLF)Δhelices** | 30 °C       | 0.5 %    | 1 %       |
| **E. coli PDF (VTI)helices** | 42 °C       | 0.5 %    |           |
| **E. coli PDF (KLI)helices** | 42 °C       | 0.5 %    |           |
| **E. coli PDF (VTI)Δhelices** | 42 °C       | 0.2 %    |           |

B

- **pBAD**
- **pBAD E. coli PDF**
- **pBAD Vp16 PDF**
- **pBAD E. coli PDF (KLF)Δhelices**
- **pBAD E. coli PDF (VTI)helices**
- **pBAD E. coli PDF (KLI)helices**
Figure S3

Western blot

|       | 24h | 2h  | 6h  | 8h  |
|-------|-----|-----|-----|-----|
| Ec    | Vp  | Ec  | Vp  | Ec  | Vp  |

anti-MetAP

anti-PDF

Comassie brilliant blue gel

|       | 24h | 2h  | 6h  | 8h  |
|-------|-----|-----|-----|-----|
| Ec    | Vp  | Ec  | Vp  | Ec  | Vp  |

E.coli PDF

phage Vp16 PDF