**Supplementary Table S1. Data collection and refinement statistics.**

| | AMPPNP | ADP | ADP-AlF3-ssDNA | apo |
|---|---|---|---|---|
| Wavelength | 0.97914 | 1.00001 | 0.97776 | 0.9753986 |
| Resolution range | 33.62 - 1.38 (1.43 - 1.38) | 57.21 - 2.269 (2.35 - 2.269) | 75.12 - 3.19 (3.304 - 3.19) | 48.56 - 1.695 (1.756 - 1.695) |
| Space group | P 21 21 21 | P 21 21 21 | P 31 21 1 | P 21 21 21 |
| Unit cell | 67.238 74.93 107.729 90 90 90 | 67.173 74.032 109.153 90 90 90 | 86.743 86.743 149.222 90 90 120 | 66.783 70.747 103.985 90 90 90 |
| Total reflections | 804634 (79664) | 327618 (31138) | 110624 (11373) | 295055 (8181) |
| Unique reflections | 110840 (10818) | 25850 (2511) | 11303 (1117) | 48257 (2538) |
| Multiplicity | 7.3 (7.4) | 12.7 (12.4) | 9.8 (10.2) | 6.1 (3.2) |
| Completeness (%) | 0.99 (0.98) | 1.00 (1.00) | 1.00 (1.00) | 0.87 (0.46) |
| Mean I/sigma(I) | 18.60 (2.39) | 27.56 (2.82) | 15.62 (2.59) | 24.99 (2.22) |
| Wilson B-factor | 15.34 | 50.21 | 107.94 | 21.69 |
| R-merge | 0.06131 (0.9668) | 0.05905 (1.057) | 0.1117 (1.061) | 0.04385 (0.4887) |
| R-meas | 0.06619 (1.039) | 0.06164 (1.102) | 0.118 (1.118) | 0.0477 (0.5862) |
| CC1/2 | 0.999 (0.739) | 1 (0.906) | 0.998 (0.932) | 0.999 (0.812) |
| CC* | 1 (0.922) | 1 (0.975) | 1 (0.982) | 1 (0.947) |
| Reflections used in refinement | 110825 (10818) | 25839 (2510) | 11299 (1117) | 48248 (2537) |
| Reflections used for R-free | 5444 (546) | 1343 (134) | 568 (46) | 2355 (128) |
| R-work | 0.1676 (0.2464) | 0.1945 (0.2832) | 0.2212 (0.3554) | 0.1693 (0.2241) |
| R-free | 0.1828 (0.2699) | 0.2490 (0.3519) | 0.2793 (0.4184) | 0.1940 (0.2538) |
| CC(work) | 0.961 (0.846) | 0.955 (0.878) | 0.919 (0.555) | 0.964 (0.885) |
| CC(free) | 0.958 (0.820) | 0.938 (0.783) | 0.926 (0.554) | 0.952 (0.853) |
| Number of non-hydrogen atoms | 4218 | 3585 | 3616 | 3908 |
| macromolecules | 3459 | 3481 | 3584 | 3461 |
| ligands | 41 | 29 | 32 | 5 |
| Protein residues | 429 | 431 | 429 | 429 |
| RMS(bonds) | 0.006 | 0.008 | 0.003 | 0.012 |
| RMS(angles) | 1.05 | 0.85 | 0.74 | 1.28 |
| Ramachandran favored (%) | 99 | 96 | 93 | 97 |
| Ramachandran allowed (%) | 0.94 | 3.3 | 6.1 | 2.3 |
| Ramachandran outliers (%) | 0.23 | 0.7 | 0.47 | 0.7 |
| Rotamer outliers (%) | 1.8 | 3.4 | 1.6 | 3.9 |
| Clashscore | 5.01 | 11.40 | 6.27 | 5.48 |
| Average B-factor | 24.24 | 67.57 | 118.06 | 30.71 |
| macromolecules | 21.61 | 67.82 | 118.13 | 29.67 |
| ligands | 24.35 | 65.05 | 110.98 | 29.24 |
| solvent | 36.90 | 56.57 | | 38.88 |
| Number of TLS groups | 7 | 5 | 6 | |

Statistics for the highest-resolution shell are shown in parentheses.

**Supplementary Table S2. SAD data collection and phasing statistics**

| **Data collection (Se-Met)** | |
|---|---|
| Wavelength (Å) | 0.9792 |
| Resolution range | 49.64 - 2.438 (2.525 - 2.438) |
| Space group | P 21 21 21 |
| Unit cell | 66.948 73.969 107.31 90 90 90 |
| Total reflections | 300540 (29886) |
| Unique reflections | 20489 (2007) |
| Multiplicity | 14.7 (14.9) |
| Completeness (%) | 1.00 (1.00) |
| Mean I/sigma(I) | 39.76 (7.59) |
| Wilson B-factor | 39.80 |
| R-merge | 0.07222 (0.4397) |
| R-meas | 0.0748 (0.4551) |
| CC1/2 | 1 (0.968) |
| CC* | 1 (0.992) |
| **Anomalous signal and phasing** | |
| Anomalous completeness | 99.8 (91) |
| Anomalous multiplicity | 7.8 (7.6) |
| CC ano | 0.847 (0.178) |
| R-ano | 0.059 (0.428) |
| Mean I/sigma(I) ano | 2.149 (1.026) |
| FOM | 0.43 |
| FOM after density modification | 0.69 |
| **Refinement** | |
| Reflections used in refinement | 20479 (2007) |
| Reflections used for R-free | 1006 (102) |
| R-work | 0.2139 (0.2723) |
| R-free | 0.2364 (0.2549) |
| CC(work) | 0.944 (0.843) |
| CC(free) | 0.927 (0.813) |
| Number of non-hydrogen atoms | 3609 |
| macromolecules | 3447 |
| ligands | 32 |
| Protein residues | 427 |
| RMS(bonds) | 0.020 |
| RMS(angles) | 1.88 |
| Ramachandran favored (%) | 92 |
| Ramachandran allowed (%) | 6.4 |
| Ramachandran outliers (%) | 1.2 |
| Rotamer outliers (%) | 9.4 |
| Clashscore | 22.42 |
| Average B-factor | 53.27 |
| macromolecules | 53.34 |
| ligands | 83.42 |
| solvent | 44.20 |
| Number of TLS groups | 0 |

Statistics for the highest-resolution shell are shown in parentheses.

**Supplementary Table S3. SAXS data collection and analysis**

| **Data-collection parameters (BsPif1)** | |
|---|---|
| Instrument | BL19U2 |
| Beam geometry (mm) | 0.40×0.15(H×V) |
| Wavelength (Å) | 1.033 Å |
| q range ($Å^{-1}$) | 0.01-0.5 |
| Exposure time (s) / nb frames | 1 / 10 |
| Concentration range ($mg.ml^{-1}$) | 1-7 |
| Temperature | 288 |
| I(0) ($cm^{-1}$) [from P(r)] | 4350 |
| Rg (Å) [from P(r)] | 28.0 |
| I(0) ($cm^{-1}$) [from Guinier] | 4350 |
| Rg (Å) [from Guinier] | 28.0 |
| $D_{max}$ (Å) | 95.6 |
| Porod estimate ($Å^3$) | 95744 |
| **Molecular-mass determination** | |
| Partial specific volume ($cm^3.g^{-1}$) | 0.738 |
| Contrast ($\times 10^{10}$ $cm^{-2}$) | 2.872 |
| Molecular mass $M_r$ [from I(0)] | 59917 |
| Calculated monomeric $M_r$ from sequence | 46673 |
| **Data processing** | |
| Primary data reduction | xx |
| Data processing | PRIMUS |
| *Ab initio* analysis | DAMMIF |
| Number of models | 50 |
| Model [2] | 1.200 ± 0.031 |
| Validation and averaging | DAMAVER |
| NSD | 0.559 ± 0.084 |
| Rigid-body modeling | DADIMODO |
| Computation of model intensities | CRYSOL |
| Model [2] | 2.2 |

SUPPLEMENTARY FIGURES LEGENDS

**Supplementary Fig. S1**. Schematic diagram of BsPif1 DNA helicase. (a) Coloring denotes the 1A (blue), 1B (orange), 2A (red), 2B (cyan), CTD (pink) domain. (b) Structural alignment of BsPif1 with human Pif1, ScPif1, RecD2 and Dda. Secondary structure alignment is for BsPif1. Conserved helicase motifs (I to VI) and specific Pif1 motifs (A, B, C and PFSS) are indicated.

**Supplementary Fig. S2**. Representative regions of the electron density. Electron density results from a map calculated using $2F_0$-Fc coefficients contoured at 2 sigmas in the nucleotide binding site. (a) AMPPNP; (b) ADP•AlF3; (c) ADP; (d) apo structure.

**Supplementary Fig. S3**. The putative water molecule could act as the nucleophile for ATP hydrolysis. The yellow broken line connects the presumptive attacking water and the γ-phosphate atom.

**Supplementary Fig. S4**. Localization of mutated residues at BsPif1-ssDNA-ADP•AlF3 ternary complex (a) and in BsPif1-apo (b).

**Supplementary Fig. S5**. Comparison of BsPif1 structures. Structures are superimposed on 1A domain. (a) Comparison of AMPPNP structure (blue) on ADP-AlF3-ssDNA structure (red). Cα r.m.s.d is 3.0 Å. (b). Comparison of ADP structure (green) on ADP-AlF3-ssDNA structure (red). Cα r.m.s.d is 3.29Å. Structural changes are limited in BsPif1 belonging to space group P212121 Cα r.m.s.d between AMPPNP and ADP is 1.23Å.

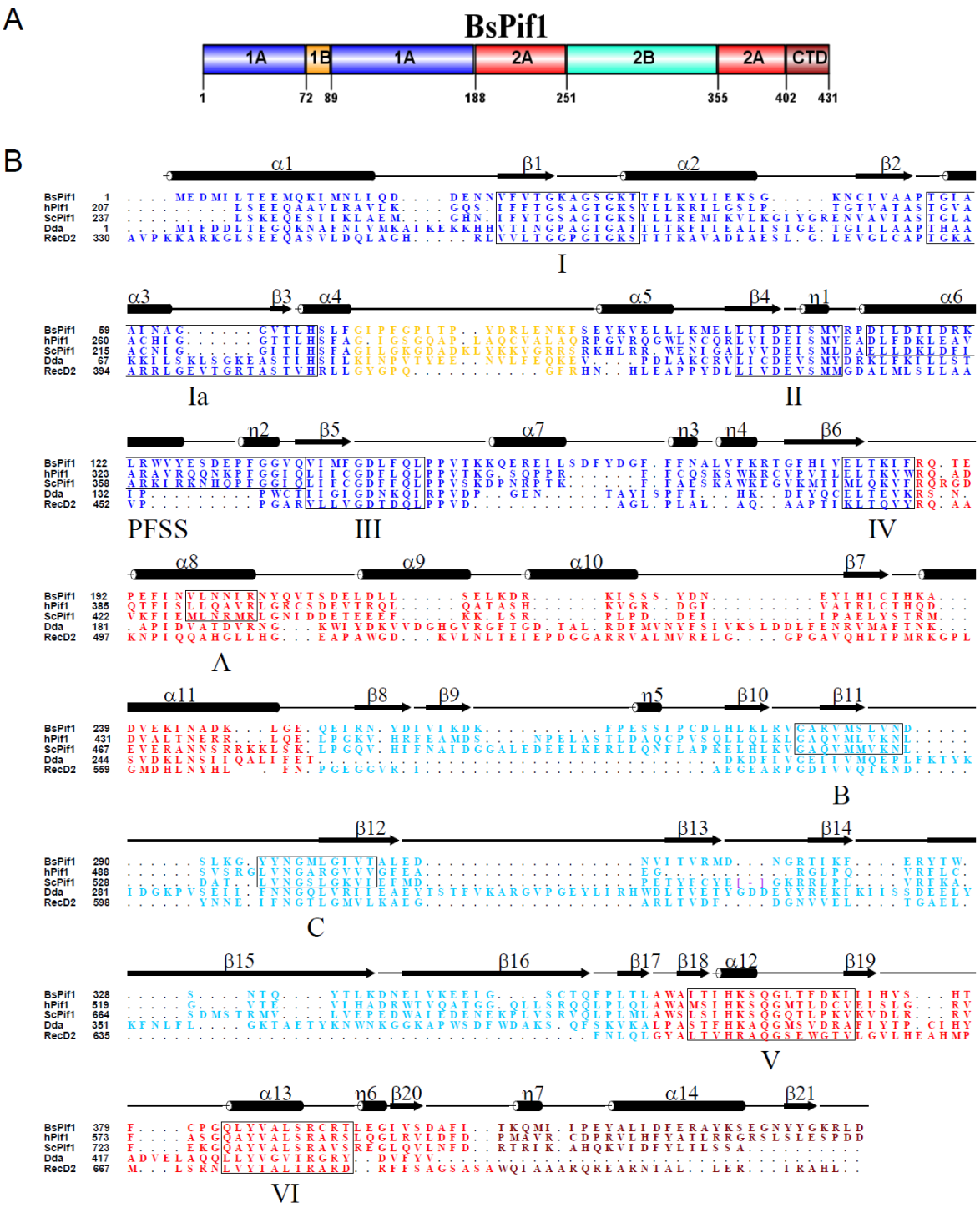
**Supplementary Fig. S6**. Disulphide bridge in the L95C/E339C mutant. (a) BsPif1-ssDNA-ADP•AlF3 ternary complex structure shows that the domain 2B is far away from domain 1B. (b) introducing of cysteine residues at positions of I339 and L95 establish a disulphide bridge between domains 1B and 2B. (c) The $2F_0$-Fc electron density map contoured at 2σ level shows clearly the disulphide bridge between the two mutated residues.

**Supplementary Fig. S7**. (a) Presentation of the spatial configurations of the Motif A-C and PFSS motif. (b) Interactions of PFSS with the pin-loop. The residues in PFSS helix (green) and in pin-loop (orange) are shown.

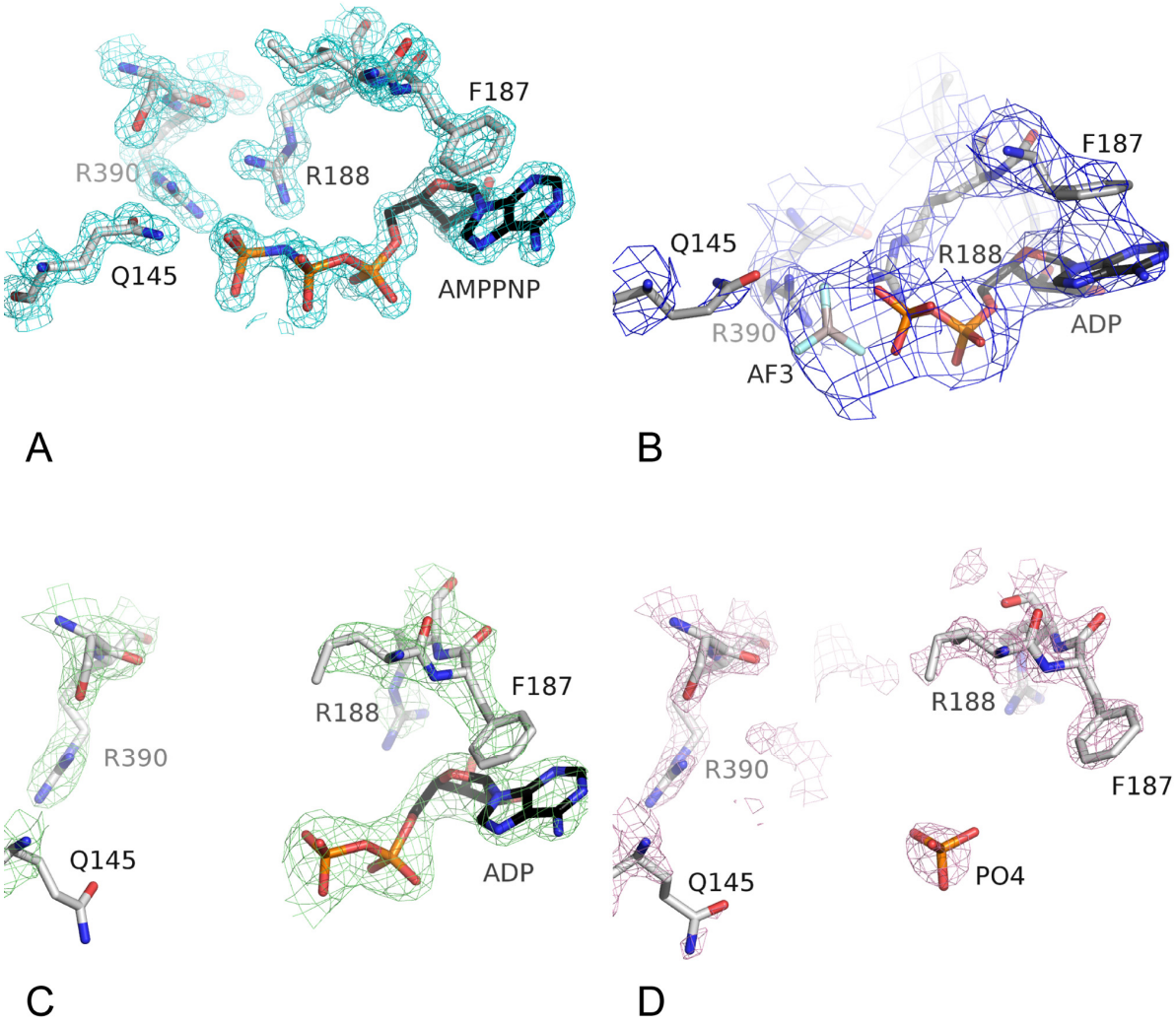
**Supplementary Fig. S8**. Solution conformations of BsPif1. Fit of the experimental data obtained with BsPif1 protein alone to the calculated intensities from the model by CRYSOL with BsPif1 "apo" P212121 crystal form (a) and BsPif1 "complex" in P3121 crystal form (b). Initial $\chi^2$ is 5.5 and 7.5, respectively. The residual $(I_{exp}(q)-I_{calc}(q))/s_{exp}$ is indicated below. (c) The final $\chi^2$ after flexible fitting is 2.2. (d) Superposition of SAXS envelope with "apo" structure (cyan) and model after flexible fitting (green) The 1B domain is in blue (X-ray structure) and orange (model).

**Supplementary Fig. S9**. (a-c) Comparison of the conformation of 1B domain in BsPif1, RecD2 and Dda. The structures were superimposed on 1A domain. (a) BsPif1 structure. Colors are the same as in Figure 1. (b). Comparison with RecD2 (3GPL, magenta). (c) Comparison with Dda (3UPU, yellow). (d-f) Comparison of Pin/wedge organization SF1B and SF1A helicases. (d) Structure of BsPif (this work), (e) PcrA (PDB code 3PJR) and (f) UvrD (PDB code 3LFU) have been superimposed on 1A domain and are shown in the same orientation. The wedge and the helix which may have the same function as PFSS are colored in orange and green respectively.

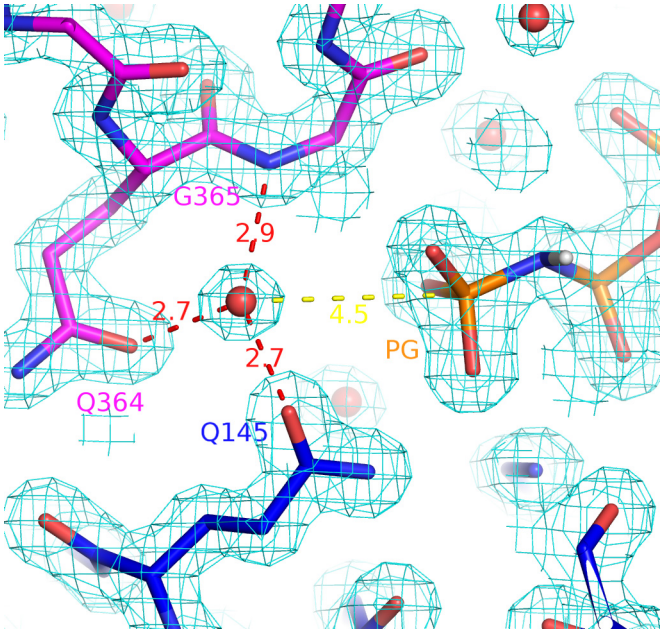
Supplementary Figure S1



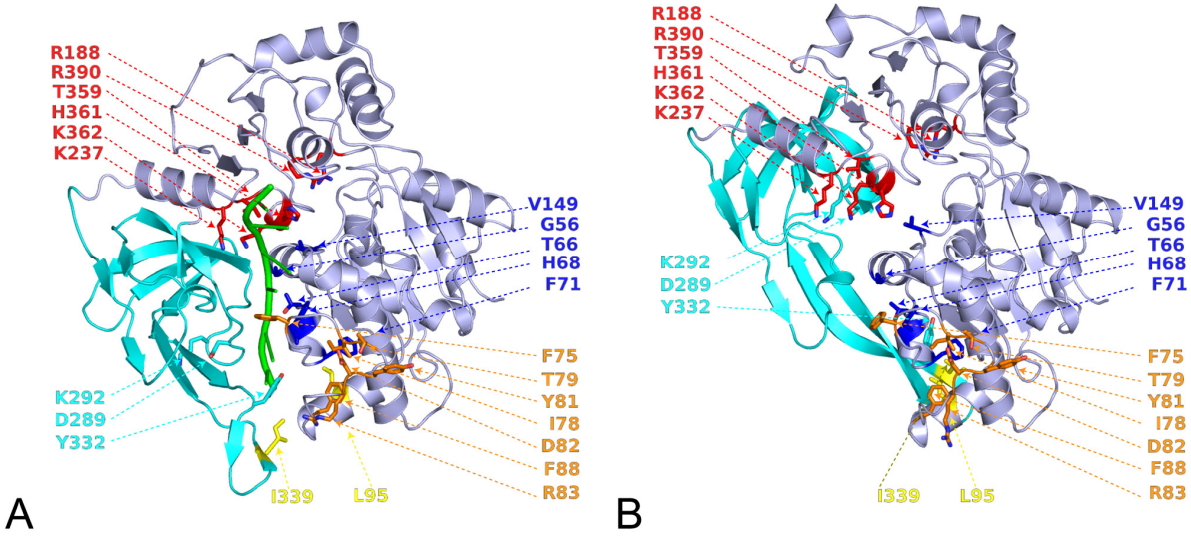
Supplementary Figure S2



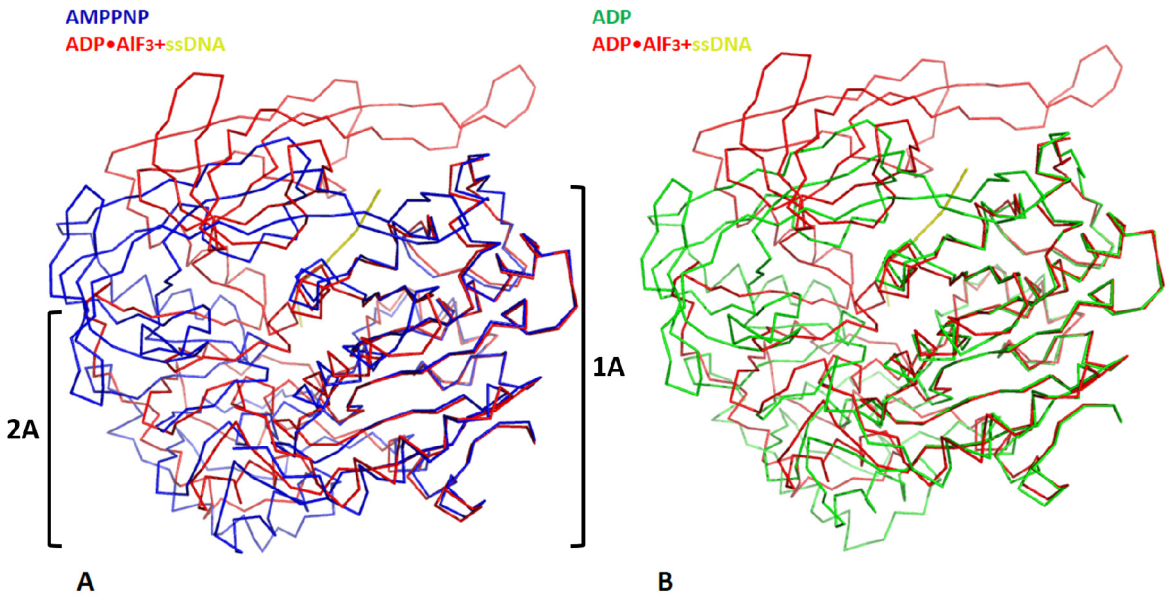
Supplementary Figure S3



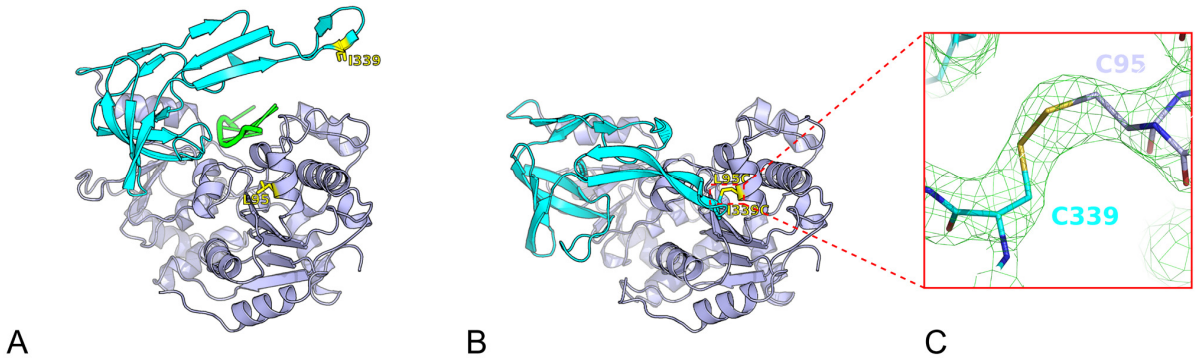

Supplementary Figure S4



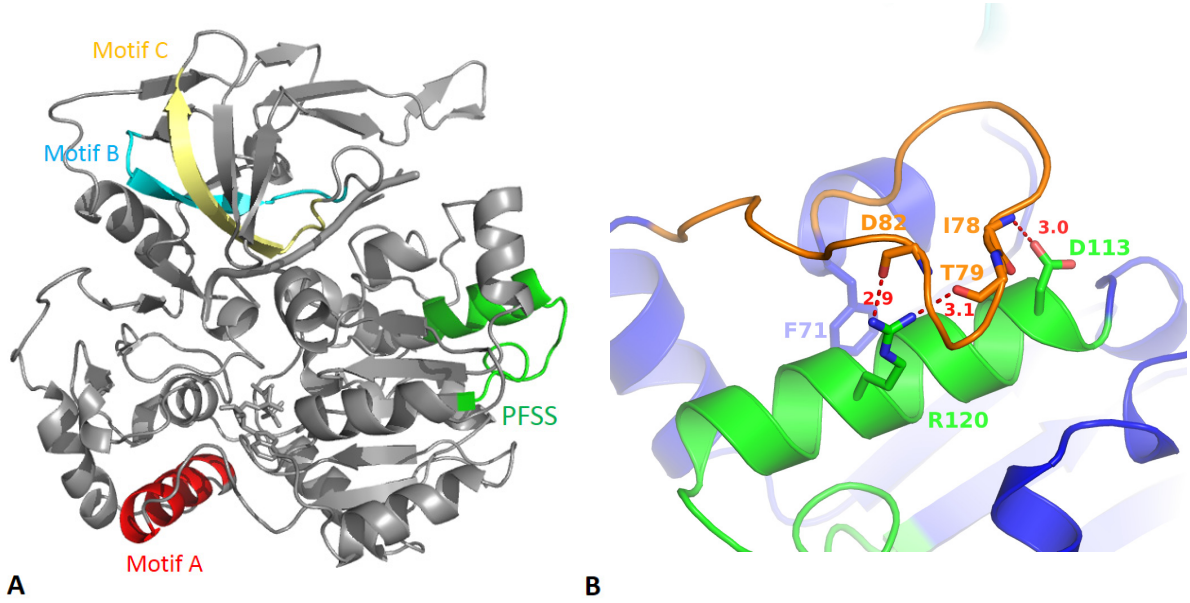
Supplementary Figure S5



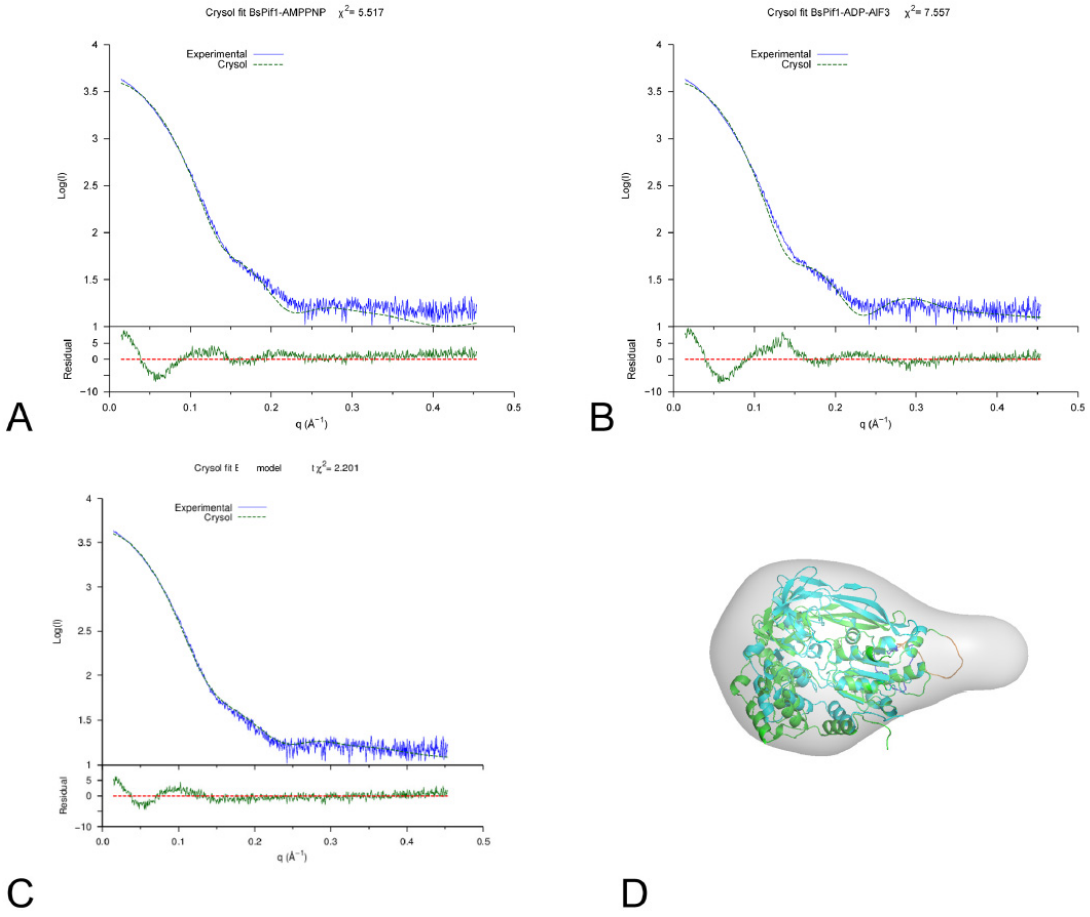

Supplementary Figure S6



Supplementary Figure S7




Supplementary Figure S8



Supplementary Figure S9