# Supplementary data

**Table S1**. Primers used in this study. *Eco*RI sites are italicized, *Nde*I sites are highlighted in bold, *Xho*I sites are underlined in bold and *Bam*HI sites are underlined.

| Primer | Sequence |
|---|---|
| *PEB4-Nde*I-F | 5’ CCGG*GAATTC***CATATG**AAAAAATTTTCTTTAGTC 3’ |
| *PEB4-Xho*I-R | 5’ AATA*GAATTC***CTCGAG**TTTATATTCCACTTTAGC 3’ |
| *PEB4*VN-*Nde*I-F | 5’ AATA**CATATG**TTTTTAATTTTAGTTAAT 3’ |
| *PEB4*VN-*Bam*HI-R | 5’ AATAGGATCCATCATAAAAAGCTTTAACTTTAGC 3’ |
| *PEB4*VC-*Bam*HI-F | 5’ AATAGGATCCAAAGGTCAAATCAAATTTG 3’ |
| *PEB4*VC-*Xho*I-R | 5’ AATA**CTCGAG**CAGCATGATCAGTGTGTA 3’ |
| *cj1289-Nde*I-F | 5’ AATA**CATATG**AATACCATTAATGCTATAGC 3’ |
| *cj1289-Xho*I-R | 5’ AATA**CTCGAG**TCTTAAATATTCTATATTGA 3’ |
| *cj0694-Nde*I-F | 5’ AATA**CATATG**CTTACTTGGATGCAACAT 3’ |
| *cj0694-Xho*I-R | 5’ AATA**CTCGAG**ATTCCCTTTATAATAAAT 3’ |

**Table S2**. Full structural statistical data for PEB4

| | **Native** | **Se-Met derivative (Peak)** | **Se-Met derivative (Inflection)** | **Se-Met derivative (Remote)** |
|---|---|---|---|---|
| **Data collection** | | | | |
| Beam line (DLS) | I0-2 | I0-2/I0-4[#] | I0-2/I0-4[#] | I0-2 |
| Wavelength (Å) | 0.9780 | 0.9801 | 0.9803 | 0.9745 |
| Space group | C2 | C2 | C2 | C2 |
| Unit Cell (Å) | a=81.4 Å, b=91.6 Å, c=61.6 Å, α= γ=90°, β=102.3° | a=81.2 Å, b=91.7 Å, c=61.3 Å, α= γ=90°, β=102.7° | a=81.2 Å, b=91.7 Å, c=61.3 Å, α= γ=90°, β=102.7° | a=81.2 Å, b=91.7 Å, c=61.3 Å, α= γ=90°, β=102.7° |
| Resolution range (Å) | 39.8 – 2.2 | 46.4 – 2.6 | 39.3-2.5 | 19.9-2.6 |
| No of measured reflections | 251133 | 277591 | 102951 | 46409 |
| No of unique reflections | 22272 | 13585 | 15296 | 13954 |
| Completeness (%) * | 99.5 (100.0) | 100.0 (100.0) | 99.9 (100.0) | 98.2 (90.9) |
| $R_{pim}$ *,[1] | 0.023 (0.138) | 0.027 (0.199) | 0.034 (0.418) | 0.033 (0.194) |
| Mn<I/sd> * | 20.3 (5.7) | 30.0 (6.3) | 19.5 (2.2) | 12.5 (3.3) |
| **Refinement** | | | | |
| R / $R_{free}$ [2,3] | 0.234/0.276 | | | |
| Overall B-factor (Å$^2$) | 48.2 | | | |
| Composition of AU | 1 polypeptide chain (residues 22-273),144 waters | | | |
| **Geometry** | | | | |
| RMSD in bond distances (Å) | 0.011 | | | |
| RMSD in bond angles (°) | 1.1 | | | |
| **Ramachandran** | | | | |
| % most favored | 97.2 | | | |
| % additionally allowed | 2.8 | | | |

# Data were collected from the same crystal at identical wavelengths on both stations as checked by fluorescence scans

* Data in parentheses correspond to the highest resolution shell

1) $R_{pim} = \sum_{hkl}[1/(N-1)]^{1/2} \sum_i |I_i(hkl) - I(hkl)| / \sum_{hkl} \sum_i I_i(hkl)$

2) R-factor = $\Sigma \mid F_{obs} - F_{calc} \mid / \Sigma F_{obs}$

3) $R_{free}$ calculated as as in 2) but using 5% of experimental data excluded from refinement for validation.

**Table S3**. Full structural statistical data for Cj1289

| | **Se-Met derivative (Final refinement)** | **Se-Met derivative (Peak)** | **Se-Met derivative (Inflection)** | **Se-Met derivative (High-Remote)** |
|---|---|---|---|---|
| Beam line (DLS) | I0-3 | I0-3 | I0-3 | I0-3 |
| Wavelength (Å) | 0.9840 | 0.9801 | 0.9803 | 0.9686 |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| Unit Cell (Å) | a=49.6Å, b=94.6Å, c=124.6Å, α=β=γ=90° | a=49.6Å, b=94.6Å, c=124.4Å, α=β=γ=90° | a=49.6Å, b=94.6Å, c=124.5Å, α=β=γ=90° | a=49.6Å, b=94.5Å, c=124.3Å, α=β=γ=90° |
| Resolution range (Å) | 62.3-2.3 | 75.3-2.8 | 75.3-2.8 | 75.2-2.8 |
| No of measured reflections | 189232 | 263217 | 158243 | 52696 |
| No of unique reflections | 26871 | 15074 | 15070 | 14626 |
| Completeness (%) * | 100 (100.0) | 100 (100.0) | 100 (100.0) | 98.3 (99.6) |
| $R_{pim}$ *,[1] | 0.052 (0.379) | 0.035 (0.169) | 0.051 (0.291) | 0.063 (0.271) |
| Mn<I/sd> * | 14.3 (3.2) | 22.3 (6.3) | 15.5 (4.0) | 9.0 (2.9) |
| **Refinement** | | | | |
| R / $R_{free}$ [2,3] | 0.225/0.280 | | | |
| Overall B-factor ($Å^2$) | 56.5 | | | |
| Composition of AU | 2 polypeptide chains, 99 waters | | | |
| **Geometry** | | | | |
| RMSD in bond distances (Å) | 0.008 | | | |
| RMSD in bond angles (°) | 1.1 | | | |
| **Ramachandran** | | | | |
| % most favored | 96.0 | | | |
| % additionally allowed | 4.0 | | | |

* Data in parentheses correspond to the highest resolution shell

1) $R_{pim} = \Sigma_{hkl}[1/(N-1)]^{1/2} \Sigma_i |I_i(hkl) - I(hkl)| / \Sigma_{hkl} \Sigma_i I_i(hkl)$

2) R-factor = $\Sigma \mid F_{obs} - F_{calc} \mid / \Sigma F_{obs.}$

3) $R_{free}$ calculated as in 2) but using 5% of experimental data excluded from refinement for validation.

**FIGURE S1**

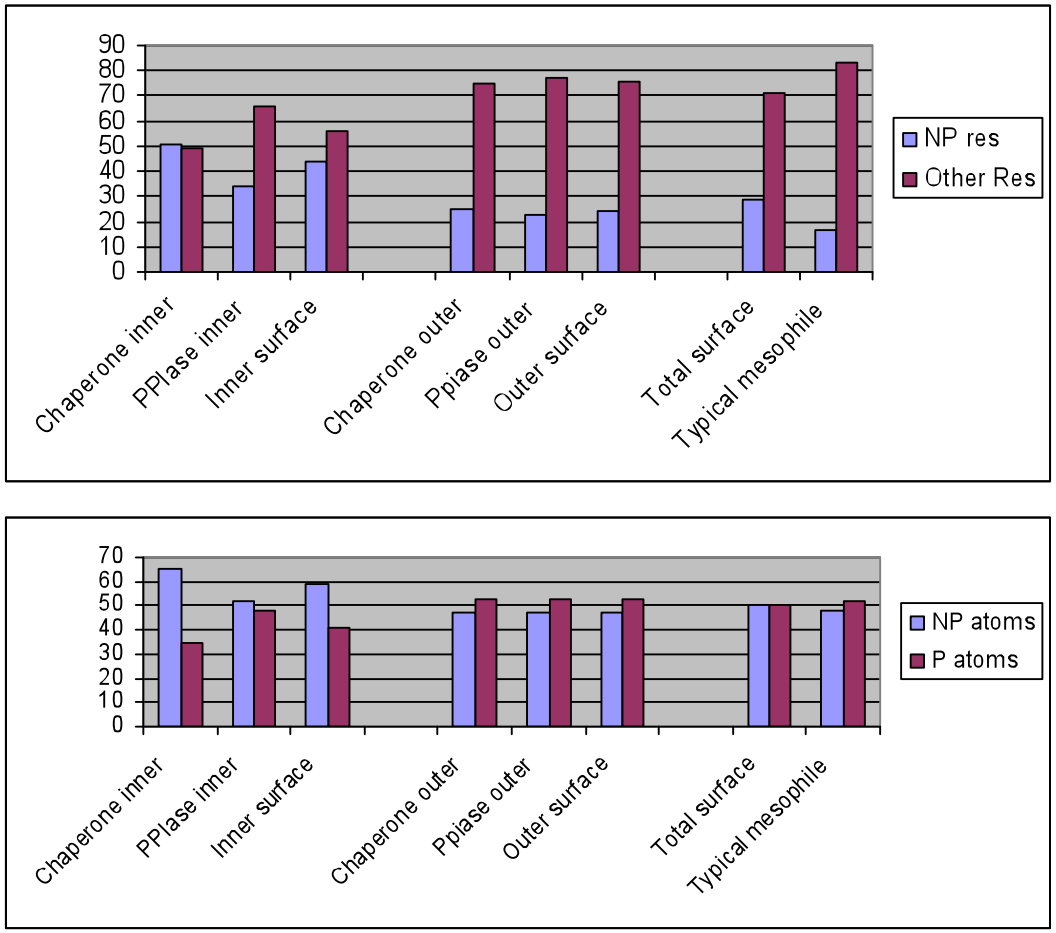


**Figure S1. The inner surfaces of the central cavity of PEB4 are significantly more hydrophobic than the outer surfaces, and than the surfaces of typical mesophiles.** Upper Panel: Proportions (%) of non-polar (blue) surface residues compared to proportion (%) of other (purple) surface residues. Lower Panel: Proportions (%) of non-polar surface atoms (blue) compared to proportion (%) of polar atoms (purple). In both panels from left to right the data are for the chaperone domain inner surface; the PPIase domain inner surface; total inner surface; chaperone domain outer surface; PPIase domain outer surface; total outer surface; total of inner and outer surfaces; values for typical mesophile from Fukuchi and Nishigawa (47) (upper panel) and Britton *et al*. (56) (lower panel).