Activesite_residues_R2.tif

**Fig.S1** Overlay of Pak1 structures (PDB ids: 4EQC - green, 4P90 - red, 4ZY6 - yellow and 5KBQ - dark blue). The active site residues are shown in ball and stick model.

Fig.S1.tif

**Fig.S2** Four Crystal structures along with their resolution selected for the study

**Fig.S1.tif**

**Fig. S3 (a-d)** Two-dimensional schematic ligand representations of Pak1and their respective co-crystal ligands.

1.tif2.tif3.tif4.tif

**Fig. S4 (a-d)** Computationally identified hydration sites overlapping with the Pak1co-crystal ligands a) 4EQC-FRAX597; b) 4P90-2K0; c) 4ZY6-4T6 and d) 5KBQ-IPV

**Table S1 Predicted occupancy and thermodynamic properties of selected hydration sites of 4EQC**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hydration Site** | **Occupancy** | **Overlap** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| 13 | 0.89 | 1.00 | 1.94 | 3.33 | 5.27 |
| 21 | 0.83 | 1.00 | -0.27 | 2.86 | 2.59 |
| 47 | 0.63 | 1.00 | 1.50 | 1.95 | 3.45 |
| 57 | 0.55 | 1.00 | 3.14 | 1.52 | 4.66 |
| 62 | 0.52 | 1.00 | 2.43 | 1.50 | 3.93 |
| 74 | 0.45 | 1.00 | -2.04 | 1.30 | -0.74 |
| 91 | 0.40 | 1.00 | 3.14 | 1.22 | 4.36 |
| 103 | 0.37 | 1.00 | -0.28 | 1.02 | 0.74 |
| 107 | 0.34 | 1.00 | -0.81 | 1.04 | 0.23 |
| 109 | 0.34 | 1.00 | 5.42 | 1.02 | 6.44 |

**Table S2 Predicted occupancy and thermodynamic properties of selected hydration sites of 4P90**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hydration Site** | **Occupancy** | **Overlap** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| 6 | 0.99 | 0.37 | -10.52 | 5.26 | -5.26 |
| 21 | 0.79 | 1.00 | 1.03 | 2.91 | 3.94 |
| 29 | 0.74 | 1.00 | 0.64 | 2.36 | 3.00 |
| 30 | 0.71 | 1.00 | -2.10 | 2.57 | 0.47 |
| 71 | 0.42 | 1.00 | 4.35 | 1.29 | 5.64 |
| 74 | 0.41 | 1.00 | 1.23 | 1.15 | 2.38 |
| 75 | 0.40 | 1.00 | 1.29 | 1.22 | 2.51 |
| 83 | 0.38 | 1.00 | 1.61 | 1.06 | 2.67 |
| 91 | 0.36 | 1.00 | 0.67 | 1.06 | 1.73 |
| 93 | 0.36 | 1.00 | 2.03 | 1.02 | 3.05 |
| 112 | 0.30 | 1.00 | 1.63 | 0.82 | 2.45 |
| 135 | 0.28 | 0.30 | 2.59 | 0.76 | 3.35 |

**Table S3 Predicted occupancy and thermodynamic properties of selected hydration sites of 4ZY6**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hydration Site** | **Occupancy** | **Overlap** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| 14 | 0.82 | 1.00 | 1.15 | 2.94 | 4.09 |
| 41 | 0.63 | 1.00 | 2.08 | 1.96 | 4.04 |
| 52 | 0.53 | 1.00 | 0.42 | 1.74 | 2.16 |
| 53 | 0.53 | 1.00 | 3.15 | 1.46 | 4.61 |
| 57 | 0.49 | 1.00 | -1.07 | 1.50 | 0.43 |
| 82 | 0.36 | 1.00 | 0.09 | 0.99 | 1.08 |
| 99 | 0.33 | 0.95 | 2.44 | 0.92 | 3.36 |

**Table S4 Predicted occupancy and thermodynamic properties of selected hydration sites of 5KBQ**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Hydration Site** | **Occupancy** | **Overlap** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| 14 | 0.84 | 1.00 | 0.50 | 3.01 | 3.51 |
| 39 | 0.65 | 1.00 | 3.37 | 2.43 | 5.80 |
| 48 | 0.58 | 1.00 | 0.29 | 1.91 | 2.20 |
| 54 | 0.55 | 1.00 | 1.67 | 1.61 | 3.28 |
| 67 | 0.48 | 1.00 | 0.92 | 1.59 | 2.51 |
| 84 | 0.40 | 1.00 | 1.39 | 1.18 | 2.57 |
| 88 | 0.38 | 1.00 | 1.33 | 1.03 | 2.36 |
| 89 | 0.38 | 1.00 | 2.89 | 1.08 | 3.97 |
| 108 | 0.33 | 0.96 | -0.10 | 1.00 | 0.90 |
| 114 | 0.32 | 1.00 | 2.29 | 0.87 | 3.16 |
| 119 | 0.31 | 1.00 | 2.68 | 0.89 | 3.57 |
| 134 | 0.29 | 1.00 | 1.21 | 0.81 | 2.02 |
| 137 | 0.29 | 0.46 | 0.49 | 0.78 | 1.27 |
| 144 | 0.28 | 1.00 | 0.77 | 0.86 | 1.63 |

**Fig.S3_2.tif**

**Fig. S5 (a-g)** (a) The Root Mean Square Deviation (RMSD), (b) Root Mean Square Fluctuation (RMSF), (c) Secondary Structure Elements Assignment (SSE), (d) Ligand RMSF (e) Protein-Ligand Contacts (f) 2D Schematic Representation of Protein-Ligand Contacts and (g) Ligand properties such as RMSD, RoG, IntraHB, MolSA(Ǻ2), SASA (Ǻ2), and PSA (Ǻ2)

**Fig.S4.tif**

**Fig. S6 (a-g)** (a) The Root Mean Square Deviation (RMSD), (b) Root Mean Square Fluctuation (RMSF), (c) Secondary Structure Elements Assignment (SSE), (d) Ligand RMSF (e) Protein-Ligand Contacts (f) 2D Schematic Representation of Protein-Ligand Contacts and (g) Ligand properties such as RMSD, RoG, IntraHB, MolSA(Ǻ2), SASA (Ǻ2), and PSA (Ǻ2)

**Fig.S5.tif**

**Fig. S7 (a-g)** (a) The Root Mean Square Deviation (RMSD), (b) Root Mean Square Fluctuation (RMSF), (c) Secondary Structure Elements Assignment (SSE), (d) Ligand RMSF (e) Protein-Ligand Contacts (f) 2D Schematic Representation of Protein-Ligand Contacts and (g) Ligand properties such as RMSD, RoG, IntraHB, MolSA(Ǻ2), SASA (Ǻ2), and PSA (Ǻ2)

**Fig.S6.tif**

**Fig. S8 (a-g)** (a) The Root Mean Square Deviation (RMSD), (b) Root Mean Square Fluctuation (RMSF), (c) Secondary Structure Elements Assignment (SSE), (d) Ligand RMSF (e) Protein-Ligand Contacts (f) 2D Schematic Representation of Protein-Ligand Contacts and (g) Ligand properties such as RMSD, RoG, IntraHB, MolSA(Ǻ2), SASA (Ǻ2), and PSA (Ǻ2)

**Fig.S7.tif**

**Fig. S9 (a-g)** (a) The Root Mean Square Deviation (RMSD), (b) Root Mean Square Fluctuation (RMSF), (c) Secondary Structure Elements Assignment (SSE), (d) Ligand RMSF (e) Protein-Ligand Contacts (f) 2D Schematic Representation of Protein-Ligand Contacts and (g) Ligand properties such as RMSD, RoG, IntraHB, MolSA(Ǻ2), SASA (Ǻ2), and PSA (Ǻ2) **Supplementary Information**

**Molecular Docking of the best potent compounds to Pak2 and Pak3.**

Among the group-I Paks, Pak1 comprises of more than twenty crystal structures pertaining to catalytic domain available in Protein Data Bank (PDB). Hence, Pak2 and Pak3 (thiophosphorylated form available) crystals structures are modeled using Prime (Jacobson *et al*, 2004; M.P. Jacobson, R.A Friesner, Z. Xiang & B. Honig, 2002; Schrödinger, 2018) using existing structure of Pak1 (PDB id: 4EQC). The RMSD of the modeled structures Pak2 and Pak3 with respect to Pak1 is 0.136 Ǻ and 0.102 Ǻ respectively. The active site residues (Arg30 and Leu78) of Pak2 and Pak3 are predicted based on sequence alignment with the above mentioned structure of Pak1. The predicted compounds (4835785, 76038049, 32407813, 32198676 and 32945545) are docked into the active site pocket of Pak2 and Pak3. The enlisting of interacting residues along with scores are tabulated in **Table (S5 and S6)** along with the illustrations of the compounds interactions with Pak2 and Pak3 denote that these compounds even though interact with the active site residues comparatively these compounds have better binding affinity and docking score to Pak1 (**Fig. S10 and S11**). So from computational approaches, these compounds are specific to Pak1. Furthermore, experimental studies on these compounds will facilitate much deeper insights into selectivity among the group –I kinases.

**Table S5: Compounds with Glide score, energy, Emodel score and Interacting residues from the Pak2.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound Code** | **Glide Score** | **Glide Energy**  **(kcal/mol)** | **Glide Emodel**  **(kJ/mol)** | **Interacting residues** |
| 4835785 | -8.547 | -56.251 | -70.997 | Asp138 |
| 76038049 | -8.421 | -44.597 | -69.113 | Leu78, Thr137 and Asp138 |
| 32407813 | -8.095 | -42.436 | -64.080 | Glu76, Tyr77 and Leu78 |
| 32198676 | -5.734 | -46.393 | -64.931 | Leu78 |
| 32945545 | -3.913 | -40.314 | -54.761 | Glu46 and Asp138 |

**Table S6: Compounds with Glide score, energy, Emodel score and Interacting residues from the Pak3.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compound Code** | **Glide Score** | **Glide Energy**  **(kcal/mol)** | **Glide Emodel**  **(kJ/mol)** | **Interacting residues** |
| 4835785 | -8.342 | -57.747 | -76.622 | Lys122, Asn125, Asp138 |
| 32407813 | -8.334 | -46.783 | -63.530 | Glu76, Tyr77 and Leu78 |
| 76038049 | -8.286 | -40.679 | -64.462 | Leu78, Thr137, Asp138, Phe139 |
| 32198676 | -3.039 | -48.478 | -71.354 | Cation-π interaction with Lys30 |
| 32945545 | -1.711 | -41.724 | -55.170 | Cation-π interaction with Lys30 |

**Pak2.tif**

**Fig.S10** Two-dimensional schematic ligand representations of protein-ligand interactions between Pak2 and top five hits

Pak3.tif

**Fig.S11** Two-dimensional schematic ligand representations of protein-ligand interactions between Pak3 and top five hits

**4EQC - WaterMap Analysis**

**Table S7a Interaction of water molecules – with two polar atoms of the main chain (Backbone…Backbone)**

Water Molecules: 140

Polar atoms: 592

No of interactions: 35

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| VAL328 | O | 3.184 | 8 | 3.398 | TYR330 | N | 92 | 1.95 | 3.52 | 5.47 |
| TYR346 | O | 2.653 | 59 | 3.472 | ALA348 | N | 53 | -1.44 | 1.92 | 0.48 |
| ALA348 | N | 3.546 | 17 | 3.196 | MET399 | N | 88 | 0.78 | 2.98 | 2.20 |
| HIS387 | O | 2.930 | 1 | 2.562 | ASP407 | O | 100 | 1.50 | 4.97 | 6.47 |
| ASP407 | N | 3.448 | 16 | 3.425 | PHE408 | N | 88 | 0.01 | 3.38 | 3.39 |
| O | 2.562 | 1 | 3.164 | PHE410 | N | 100 | 1.50 | 4.97 | 6.47 |
| O | 2.562 | 1 | 3.520 | CYS411 | N |

**Table S7b Interaction of water molecules – with two polar atoms of the side chain (Sidechain…Sidechain)**

Water Molecules: 140

Polar atoms: 231

No of interactions: 60

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| ARG299 | NH | 2.773 | 5 | 2.984 | GLU315 | OE | 96 | -6.27 | 4.01 | -2.26 |
| 2.773 | 2.610 |
| 3.373 | 42 | 2.710 | 67 | -1.46 | 2.15 | 0.69 |
| 3.269 | 80 | 2.787 | 44 | 0.03 | 1.40 | 1.43 |
| 3.174 | 33 | 3.374 | 74 | -3.23 | 2.60 | -0.63 |
| 3.174 | 3.435 | THR406 | OG |
| 3.174 | 2.701 | ASP407 | OD |
| 3.537 | 45 | 2.850 | 64 | -0.53 | 1.54 | 1.01 |
| GLU315 | OE | 2.984 | 5 | 2.610 | GLU315 | OE | 96 | -6.27 | 4.01 | -2.26 |
| 3.374 | 33 | 3.435 | THR406 | OG | 74 | -3.23 | 2.60 | -0.63 |
| 2.701 | ASP407 | OD |
| GLU345 | 2.934 | 2 | 2.727 | LYS404 | NZ | 97 | -3.72 | 4.68 | 0.96 |
| SER351 | OG | 3.346 | 10 | 3.258 | SER351 | OG | 91 | -4.12 | 3.50 | -0.62 |
| 3.141 | THR353 |
| 2.981 | ASP393 | OD |
| 2.932 |
| 3.258 | 3.141 | THR353 | OG |
| 2.981 | ASP393 | OD |
| 2.932 |
| 3.086 | 23 | 2.735 | ASP354 | 81 | -4.52 | 3.02 | -1.50 |
| ASP354 | OD | 2.729 | 22 | 3.064 | ASP354 | OD | 83 | -5.29 | 3.58 | -1.71 |
| 2.942 | THR541 | OG |
| 3.064 | THR541 |
| ASP389 | OD | 2.623 | 3 | 3.218 | ASN394 | ND | 97 | -2.74 | 4.40 | 1.66 |
| ASP393 | OD | 2.513 | 124 | 3.330 | ASP393 | OD | 30 | -3.13 | 2.08 | -0.66 |
| 3.069 |
| 3.330 |
| 3.559 | 58 | 2.674 | ASN394 | 53 | -5.69 | 2.17 | -3.52 |
| 2.358 | ASP407 |
| ASN394 | OD | 3.385 | 44 | 2.796 | ASP407 | OD | 65 | -3.35 | 2.25 | -1.10 |
| 2.674 | 58 | 2.358 | 53 | -5.69 | 2.17 | -3.52 |
| 2.723 | 133 | 3.083 | 29 | -3.48 | -0.92 | -2.56 |
| THR406 | OG | 3.194 | 20 | 3.522 | ASP407 | OD | 85 | -3.81 | 3.04 | -0.77 |
| 2.569 |
| 3.435 | 33 | 2.701 | 74 | -3.23 | 2.60 | -0.63 |
| ASP407 | OD | 3.522 | 20 | 2.569 | ASP407 | OD | 85 | -3.81 | 3.04 | -0.77 |

**Table S7c Interaction of water molecules – with main and side chain polar atoms (Backbone…Sidechain)**

Water Molecules: 140

Polar atoms: 132

No of interactions: 23

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| ASP407 | N | 3.564 | 33 | 3.174 | ARG299 | NH | 74 | -3.23 | 2.60 | -0.63 |
| 3.448 | 16 | 2.547 | GLU315 | OE | 88 | 0.01 | 3.38 | 3.39 |
| PHE408 | N | 3.425 | 16 | 2.547 | GLU315 | 88 | 0.01 | 3.38 | 3.39 |
| ASN324 | O | 3.428 | 2 | 2.934 | GLU345 | 97 | -3.72 | 4.68 | 0.96 |
| ILE327 | O | 2.757 | 2.934 | GLU345 |
| TYR346 | O | 3.327 | 14 | 2.495 | GLU345 | 88 | -3.52 | 3.61 | 0.09 |
| SER351 | N | 3.283 | 19 | 2.587 | ASP354 | OD | 87 | -5.22 | 3.32 | -1.90 |
| ASP393 | O | 2.849 | 52 | 2.980 | ASP407 | 60 | -2.74 | 2.08 | -0.66 |
| 3.568 | 58 | 3.559 | ASP393 | 53 | -5.69 | 2.17 | -3.52 |
| 2.358 | ASP407 |
| 3.494 | 64 | 2.774 | ASP393 | 51 | -0.53 | 1.54 | 1.01 |
| ASP407 | N | 3.448 | 16 | 2.547 | GLU315 | OE | 88 | 0.01 | 3.38 | 3.39 |
| 3.564 | 33 | 3.174 | ARG299 | NH | 74 | -3.23 | 2.60 | -0.63 |
| 3.374 | GLU315 | OE |
| 2.701 | ASP407 | OD |
| O | 3.415 | 3 | 2.623 | ASP389 | OD | 97 | -2.74 | 4.40 | 1.66 |

**4P90 - Water Map Analysis**

**Table S8a Interaction of water molecules – with two polar atoms of the main chain (Backbone…Backbone)**

Water Molecules: 135

Polar atoms: 544

No of interactions: 14

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| VAL328 | O | 3.445 | 9 | 3.298 | TYR330 | N | 95 | 4.27 | 3.51 | 7.78 |
| TYR346 | O | 3.018 | 22 | 3.065 | ALA348 | N | 78 | -1.23 | 3.00 | 1.77 |
| LEU347 | N | 3.284 | 29 | 3.501 | LEU347 | O | 74 | 0.64 | 2.36 | 3.00 |
| ASP407 | N | 3.273 | 5 | 3.226 | PHE408 | N | 99 | -1.33 | 4.80 | 3.47 |

**Table S8b Interaction of water molecules – with two polar atoms of the main chain (Sidechain…Sidechain)**

Water Molecules: 135

Polar atoms: 205

No of interactions: 19

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| GLU315 | OE | 2.483 | 6 | 2.716 | ASP407 | OD | 99 | -10.52 | 5.26 | -5.26 |
| 2.993 | 32 | 3.373 | GLU315 | OE | 68 | 1.68 | 2.29 | 3.97 |
| GLU345 | OE | 2.631 | 2 | 2.666 | LYS404 | NZ | 100 | -6.97 | 5.42 | -1.55 |
| SER351 | OG | 3.271  3.369  3.350 | 12 | 2.320 | ASP354 | OD | 92 | -5.92 | 3.48 | -2.44 |
| 14 | 2.932 | THR353 | OG | 86 | -2.71 | 3.22 | 0.51 |
| 28 | 2.470 | ASP354 | OD | 74 | -4.98 | 2.53 | -2.45 |
| ASP354 | OD | 3.121 | 96 | 2.984 | 35 | -3.15 | 1.04 | -2.11 |
| 2.784 | 66 | 3.353 | THR541 | OG | 43 | -4.15 | 1.54 | 2.61 |
| 3.110 | 84 | 2.759 | 38 | -3.82 | 1.49 | -2.33 |
| ASP389 | OD | 2.607 | 8 | 3.434 | LYS391 | NZ | 96 | -6.21 | 4.67 | -1.54 |
|  | 2.714 | ASP407 | OD |  |  |  |  |
| ASN394 | OD | 2.809 | 25 | 2.899 | 76 | -6.14 | 3.03 | -3.11 |
| ASP407 | OD | 3.321 | 15 | 3.101 | 86 | -4.77 | 3.40 | -1.37 |

**Table S8c Interaction of water molecules – with main and side chain polar atoms (Backbone…Sidechain)**

Water Molecules: 135

Polar atoms: 122

No of interactions: 10

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| ASP407 | N | 3.273 | 5 | 2.482 | GLU315 | OE | 99 | -1.33 | 4.80 | 3.47 |
| PHE408 | 3.226 |
| ILE327 | O | 3.087 | 2 | 2.631 | GLU345 | OE | 100 | -6.97 | 5.42 | -1.55 |
| TYR346 | 3.423 | 17 | 2.480 | 84 | -3.82 | 1.49 | -2.33 |
| ALA348 | 3.443 | 95 | 3.523 | LYS538 | NZ | 35 | 1.08 | 1.04 | 2.12 |
| GLY349 | 3.433 | 16 | 3.233 | ASP354 | OD | 85 | -3.56 | 3.24 | -0.32 |
| SER351 | N | 3.084 | 12 | 2.320 | 92 | -5.92 | 3.48 | -2.44 |

**4ZY6 - Water Map Analysis**

**Table S9a Interaction of water molecules – with two polar atoms of the main chain (Backbone…Backbone)**

Water Molecules: 120

Polar atoms: 570

No of interactions: 24

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| TYR346 | O | 2.962 | 61 | 3.446 | ALA348 | N | 45 | -1.20 | 1.61 | 0.41 |
| LEU347 | N | 3.481 | 41 | 3.483 | LEU347 | O | 63 | 2.08 | 1.96 | 4.04 |
| ALA348 | 3.461 | 10 | 2.944 | MET399 | N | 92 | -1.72 | 3.23 | 1.51 |
| HIS387 | O | 2.802 | 1 | 2.666 | ASP407 | O | 100 | -0.17 | 5.05 | 4.88 |
| ASP407 | N | 3.046 | 7 | 3.390 | PHE408 | N | 96 | 0.77 | 3.81 | 4.58 |
| O | 2.666 | 1 | 3.232 | PHE410 | 100 | -0.17 | 5.05 | 4.88 |
| 3.449 | CYS411 |

**Table S9b Interaction of water molecules – with two polar atoms of the main chain (Sidechain…Sidechain)**

Water Molecules: 120

Polar atoms: 213

No of interactions: 44

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| LYS299 | NZ | 2.597 | 13 | 3.593 | GLU315 | OE | 87 | -2.37 | 2.95 | 0.58 |
| 3.044 | THR406 | OG |
| 3.373 | 27 | 2.559 | GLU315 | OE | 74 | -7.01 | 3.25 | -3.76 |
| 3.211 | ASP407 | OD |
| 2.833 | 35 | 2.480 | GLU315 | OE | 66 | 0.44 | 2.46 | 2.90 |
| 3.036 | 47 | 3.450 | 59 | -2.61 | 1.86 | -0.75 |
| 3.495 |
| GLU315 | OE | 3.593 | 13 | 3.044 | THR406 | OG | 87 | -2.37 | 2.95 | 0.58 |
| 2.559 | 27 | 3.211 | ASP407 | OD | 74 | -7.01 | 3.25 | -3.76 |
| 3.466 | 33 | 2.269 | 71 | -4.15 | 2.96 | -1.19 |
| 3.450 | 47 | 3.495 | GLU315 | OE | 59 | -2.61 | 1.86 | -0.75 |
| GLU345 | OE | 2.684 | 6 | 2.445 | LYS404 | NZ | 96 | -6.63 | 5.26 | -1.37 |
| SER351 | OG | 3.206 | 4 | 2.438 | ASP354 | OD | 98 | -6.21 | 4.23 | -1.98 |
| 3.264 | 8 | 3.020 | THR353 | OG | 93 | -2.64 | 3.67 | 1.03 |
| 3.346 | 17 | 2.520 | ASP354 | OD | 80 | -4.10 | 2.72 | -1.38 |
| THR353 | 3.453 | 3 | 2.492 | ASP393 | OD | 99 | -7.15 | 4.53 | -2.62 |
| ASP354 | OD | 2.818 | 45 | 3.386 | ASP354 | OD | 60 | -2.47 | 1.89 | -0.58 |
| 2.731 | 5 | 3.200 | LYS538 | NZ | 98 | -5.72 | 4.72 | -1.00 |
| 2.672 | 22 | 3.049 | THR541 | OG | 76 | -5.76 | 3.30 | -2.46 |
| ASP389 | ND | 3.045 | 25 | 3.285 | LYS391 | NZ | 74 | -0.95 | 2.41 | 1.46 |
| 3.516 | 24 | 3.431 | ASN394 | OD | -5.63 | 2.52 | -3.11 |
| 2.463 | ASP407 |
| LYS391 | NZ | 2.853 | 12 | 2.589 | ASP393 | OD | 88 | -3.76 | 3.25 | -0.51 |
| 3.302 |
| 3.285 | 25 | 3.553 | THR427 | OG | 74 | -0.95 | 2.41 | 1.46 |
| 3.115 | 50 | 2.877 | ASP393 | OD | 54 | -3.19 | 1.75 | -1.44 |
| SER392 | NZ | 3.023 | 3 | 2.492 | 99 | -7.15 | 4.53 | -2.62 |
| ASP393 | OD | 2.589 | 12 | 3.302 | 88 | -3.76 | 3.25 | -0.51 |
| ASN394 | 3.431 | 24 | 2.463 | ASP407 | 74 | -5.63 | 2.52 | -3.11 |
| ASP407 | 3.146 | 32 | 3.288 | 72 | -3.01 | 2.29 | -0.72 |

**Table S9c Interaction of water molecules – with main and side chain polar atoms (Backbone…Sidechain)**

Water Molecules: 120

Polar atoms: 125

No of interactions: 17

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| ASP407 | N | 3.046 | 7 | 2.446 | GLU315 | OE | 96 | 0.77 | 3.81 | 4.58 |
| PHE408 | 3.390 |
| GLY409 | 3.248 | 33 | 3.446 | 71 | -4.15 | 2.96 | -1.19 |
| ASN324 | O | 3.485 | 6 | 2.684 | GLU345 | 96 | -6.63 | 5.26 | -1.37 |
| PRO325 | 3.308 |
| TYR346 | 3.192 | 9 | 2.577 | 92 | -4.03 | 4.01 | -0.02 |
| ALA348 | 3.458 | 31 | 2.870 | LYS538 | NZ | 72 | -1.75 | 2.34 | 0.59 |
| SER351 | N | 3.149 | 4 | 2.438 | ASP354 | OD | 98 | -6.21 | 4.23 | -1.98 |
| GLY349 | O | 3.473 | 5 | 2.731 | 98 | -5.72 | 4.72 | -1.00 |
| ASP407 | N | 3.046 | 7 | 2.446 | GLU315 | OE | 96 | 0.77 | 3.81 | 4.58 |
| GLY409 | 3.248 | 33 | 2.269 | ASP407 | OD | 71 | -4.15 | 2.96 | -1.19 |

**5KBQ - Water Map Analysis**

**Table S10a Interaction of water molecules – with two polar atoms of the main chain (Backbone…Backbone)**

Water Molecules: 146

Polar atoms: 578

No of interactions: 33

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| ILE327 | O | 2.770 | 66 | 3.512 | VAL328 | O | 48 | 1.96 | 2.26 | 4.22 |
| VAL328 | 3.243 | 5 | 3.075 | TYR330 | N | 98 | 3.20 | 3.88 | 7.08 |
| TYR346 | 3.015 | 90 | 3.117 | ALA348 | 37 | -1.51 | 1.33 | -0.18 |
| LEU347 | N | 3.556 | 54 | 3.147 | LEU347 | O | 55 | 1.67 | 1.61 | 3.28 |
| ALA348 | 3.580 | 20 | 2.853 | MET399 | N | 81 | -2.47 | 2.97 | 0.50 |
| HIS387 | O | 2.529 | 1 | 2.716 | ASP407 | O | 100 | -0.45 | 4.85 | 4.40 |
| ASP407 | 2.716 | 3.538 | PHE410 | N |
| 3.409 | CYS411 |
| 3.312 | 8 | 3.063 | PHE410 | 94 | -1.74 | 4.04 | 2.30 |

**Table S10b Interaction of water molecules – with two polar atoms of the main chain (Sidechain…Sidechain)**

Water Molecules: 146

Polar atoms: 219

No of interactions: 48

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| SER281 | OG | 3.084 | 72 | 3.154 | LYS299 | NZ | 45 | -3.17 | 1.47 | -1.70 |
| LYS299 | NZ | 3.458 | 24 | 3.486 | GLU315 | OE | 79 | -1.32 | 2.73 | 1.41 |
| 2.700 |
| 2.753 | 36 | 2.327 | 66 | -5.00 | 2.33 | -2.67 |
| GLU315 | OE | 3.486 | 24 | 2.700 | 79 | -1.32 | 2.73 | 1.41 |
| 3.061 | 29 | 3.355 | 74 | -0.23 | 2.42 | 2.19 |
| 3.204 | 31 | 3.157 | 72 | -6.92 | 2.76 | -4.16 |
| GLU345 | OE | 3.124 | 22 | 2.502 | LYS404 | NZ | 80 | -4.47 | 3.82 | -0.65 |
| 2.981 | 122 | 3.142 | 31 | -2.03 | 0.92 | -1.11 |
| SER351 | OG | 3.151 | 3 | 2.835 | ASP354 | OD | 98 | -5.12 | 4.12 | -1.00 |
| 3.272 | 11 | 3.004 | THR353 | OG | 89 | -2.91 | 3.49 | 0.58 |
| 3.449 | 23 | 2.459 | ASP354 | OD | 80 | -4.62 | 2.68 | -1.94 |
| ASP354 | OD | 2.586 | 38 | 3.595 | THR357 | OG | 66 | -2.99 | 2.45 | -0.54 |
| 2.981 | 59 | 3.290 | ASP354 | OD | 52 | -2.64 | 1.65 | -0.99 |
| 2.441 | 41 | 3.228 | THR541 | OG | 64 | -5.14 | 2.32 | -2.82 |
| ASN389 | ND | 3.558 | 32 | 3.494 | LYS391 | NZ | 72 | -3.67 | 2.34 | -1.33 |
| 2.910 | 33 | 3.409 | LYS391 | 69 | -0.48 | 2.17 | 1.69 |
| 3.558 | 32 | 3.305 | ASN394 | OD | 72 | -3.67 | 2.34 | -1.33 |
| 3.585 | ASN394 | ND |
| 3.306 | 8 | 2.878 | ASP407 | OD | 94 | -1.74 | 4.04 | 2.30 |
| 2.899 | ASP407 |
| 3.558 | 32 | 2.605 | ASP407 | 72 | -3.67 | 2.34 | -1.33 |
| 3.484 | 42 | 3.332 | ASP407 | 64 | -2.81 | 2.22 | -0.59 |
| 2.815 | ASP407 |
| LYS391 | NZ | 2.780 | 19 | 2.617 | ASP393 | OD | 82 | -3.73 | 3.06 | -0.67 |
| 2.953 | ASP393 |
| 3.494 | 32 | 3.305 | ASN394 | 72 | -3.67 | 2.34 | -1.33 |
| 3.585 | ASN394 | ND |
| 2.605 | ASP407 | OD |
| 3.409 | 33 | 3.508 | THR427 | OG | 69 | -0.48 | 2.17 | 1.69 |
| 3.454 | 57 | 2.977 | ASP393 | OD | 54 | -3.11 | 1.61 | -1.50 |
| THR353 | OG | 2.936 | 99 | 2.909 | 35 | -6.10 | 1.69 | -4.41 |
| ASP393 | OD | 2.617 | 19 | 2.953 | 82 | -3.73 | 3.06 | -0.67 |
| ASN394 | 3.505 | 32 | 3.585 | ASN394 | ND | 72 | -3.67 | 2.34 | -1.33 |
| 2.605 | ASP407 | OD |
| ND | 3.585 |
| ASP407 | OD | 2.878 | 8 | 2.899 | 94 | -1.74 | 4.04 | 2.30 |
| 3.332 | 42 | 2.815 | 64 | -2.81 | 2.22 | -0.59 |

**Table S10c Interaction of water molecules – with main and side chain polar atoms (Backbone…Sidechain)**

Water Molecules: 146

Polar atoms: 123

No of interactions: 20

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Positively Charged atoms** | | **Distance** (Ǻ) | **Water Molecule/ Hydration Site** | **Distance** (Ǻ) | **Positively Charged atoms** | | **Occupancy (%)** | **ΔH (kcal/mol)** | **–TΔS (kcal/mol)** | **ΔG (kcal/mol)** |
| Res (A) | Atom |  |  |  | Res (A) | Atom |  |  |  |  |
| ASP407 | N | 3.141 | 6 | 2.457 | GLU315 | OE | 96 | 1.22 | 3.98 | 5.20 |
| ASN324 | O | 3.323 | 22 | 3.124 | GLU345 | OE | 80 | -4.47 | 3.82 | -0.65 |
| ILE327 | 3.047 |
| TYR346 | 3.579 | 21 | 2.459 | 81 | -3.25 | 3.02 | -0.23 |
| SER351 | N | 3.194 | 3 | 2.835 | ASP354 | OD | 98 | -5.12 | 4.12 | -1.00 |
| ASP407 | O | 2.716 | 1 | 3.385 | HIS387 | ND | 100 | -0.45 | 4.85 | 4.40 |
| 3.312 | 8 | 2.878 | ASP407 | OD | 94 | -1.74 | 4.04 | 2.30 |
| 3.312 | 2.899 |
| PHE410 | N | 3.063 | 8 | 2.878 |
| 2.899 |

**Table S11a Interaction of water molecules – with two polar atoms of the main chain (Backbone…Backbone) along with its crystallographic B-factor and Occupancy**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **PDB id** | **Water Res.No** | **B-factor** | **Interactions** | **Hydration site** | **Occupancy (%)** |
| 1 | 4EQC | 742 | 22.13 | Val328-Tyr330 | 8 | 92 |
| 2 | 713 | 38.57 | Tyr346-Tyr346 | - | - |
| 3 | 714 | 53.30 | Ala348-Gly350 | - | - |
| 4 | 757 | 35.76 | Gly349-Ser351 | 25 | 81 |
| 5 | 778 | 20.23 | His387-Asp407  Asp407-Phe410  Asp407-Cys411 | 1 | 100 |
| 6 | 727 | 30.95 | Asp407-Phe408 | 16 | 88 |
| 7 | 4P90 | 704 | 77.76 | Val328-Tyr330 | 9 | 95 |
| 8 | 703 | 46.42 | His387-Asp407  Asp407-Phe410  Asp407-Cys411 | - | - |
| 10 | 4ZY6 | 778 | 50.93 | Ala348-Met399 | 10 | 92 |
| 11 | 717 | 38.03 | Gly349-Ser351 | - | - |
| 12 | 710 | 39.80 | His387-Asp407  Asp407-Phe410  Asp407-Cys411 | 1 | 100 |
| 13 | 5KBQ | 703 | 43.74 | His387-Asp407  Asp407-Phe410  Asp407-Cys411 | 1 | 100 |

**Table S11b Interaction of water molecules – with two polar atoms of the side chain (Sidechain…Sidechain) along with its crystallographic B-factor and Occupancy**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **PDB id** | **Water Res.No** | **B-factor** | **Interactions** | **Hydration site** | **Occupancy (%)** |
| 1 | 4EQC | 733 | 28.97 | Arg299 -Glu315  -Asp407  Glu315-Asp407 | 5 42 80 33 45 | 96  67  44  74  64 |
| 2 | 908 | 44.12 | Asp354-Asp354 | 22 | 83 |
| 3 | 717 | 30.12 | Lys391-Asp393 | - | - |
| 4 | 906 | 47.77 | Asp393-Asp393 | 124 | 30 |
| 5 | 730 | 33.91 | Thr406-Asp407  Asp407-Asp407 | 20  33 | 85  74 |
| 6 | 4ZY6 | 727 | 48.75 | Lys299-Thr406 | 13 | 87 |
| 7 | 721 | 55.83 | Asp354-Thr357 | - | - |
| 8 | 5KBQ | 704 | 65.15 | Asn389-Asp407  Asp407-Asp407 | 42 32 | 64  72 |
| 9 | 701 | 64.60 | Lys391-Asn394  Glu345-Asp407  Asn394-Asn394  Asn394-Asp407 | 32 | 72 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **PDB id** | **Water Res.No** | **B-factor** | **Interactions** | **Hydration site** | **Occupancy (%)** |
| 1 | 4EQC | 706 | 36.00 | Ser281-Arg299 | - | - |
| 2 | 727 | 30.95 | Asp407-Glu315  Phe408-Glu315 | 33  16 | 74  88 |
| 3 | 731 | 46.89 | Asp393-Asp407 | 52 | 60 |
| 4 | 906 | 47.77 | Asp393-Asp393  Asp393-Asp393 | 58  64 | 53  51 |
| 5 | 778 | 20.23 | Asp407-His387 | - | - |
| 6 | 849 | 38.60 | Asp407-Asp389 | 3 | 97 |
| 11 | 5KBQ | 703 | 43.74 | Asp407-His387 | 1 | 100 |
| 12 | 704 | 65.15 | Phe410-Asp407 | 8 | 94 |

**Table S11c Interaction of water molecules – with two polar atoms of the main and side chain (Mainchain…Mainchain) along with its crystallographic B-factor and Occupancy**