

SUPPLEMENTARY MATERIAL

Table 1. Interactions of buried waters (networked as well as isolated) with secondary-structural elements in the protein.

| Secondary structures involved in the water-mediated interactions | Number of waters involved | |
|--|------------------------------|----------------------------|
| | Networked buried waters (12) | Isolated buried waters (8) |
| α -helix - β -strand | - | 1 |
| β -strand - β -strand | 2 | 1 |
| $\beta\alpha$ loop - α -helix | 1 | 1 |
| $\beta\alpha$ loop - β -strand | 6* | 2 |
| $\beta\alpha$ loop - $\beta\alpha$ loop | 1 | 1 |
| $\alpha\beta$ loop - α -helix | 1 | 1 |
| $\alpha\beta$ loop - β -strand | 1 | - |
| $\beta\alpha$ loop, α -helix and β -strand | - | 1 |

* It may be seen that more networked buried waters are involved in bridging $\beta\alpha$ loops and β -strands.

Table 2. Buried water interactions in RTUX and CTUX**(a). Isolated buried waters**

| Water residue number (as in CTUX) | Protein atoms | |
|--------------------------------------|--|---|
| | Main Chain | Side Chain |
| Wat501 | Ile235 O (β 7) | Tyr17 OH (β 1) Thr265 OG1 (β 8) |
| Wat525 | Pro45 O (β 2) Asn47 O ($\beta_2\alpha_2$) Met49 N ($\beta_2\alpha_2$) His83 O (β 3) | - |
| Wat504 | Met49 O ($\beta_2\alpha_2$) Thr84 O (β 3) | His111 NE2 (α 3) |
| Wat538 | Met116 O (α 3) | Trp126 NE1 (β 4) Asp164 OD2 (α 4) |
| Wat509 | Ala132 O ($\beta_4\alpha_4$) Arg140 O ($\beta_4\alpha_4$) | Tyr152 OH (α 4) |
| Wat530 | Asp164 O (α 4) Ala167 O ($\alpha_4\beta_5$) | Arg161 NH1 (α 4) |
| Wat506 | Ala132 N ($\beta_4\alpha_4$) Asn172 O (β 5) | Asp173 OD2 (β 5) |
| Wat502 | Gly268 O ($\beta_8\alpha_8$) Val269 O ($\beta_8\alpha_8$) Leu283 N ($\beta_8\alpha_8$) | - |

The location in the secondary-structural element of the given residue is indicated in parentheses. Note that isolated buried waters are involved in more interactions (17) with main-chain atoms than with side-chain atoms (8). Also, among the interactions with the main-chain atoms, carbonyl oxygen is involved in 14 interactions and amide nitrogen in only three interactions.

(b). Buried waters which are networked with other waters

| Water residue number (as in CTUX) | Protein atoms | | Heteroatoms |
|--------------------------------------|--|---|-------------|
| | Main chain | Side chain | Water |
| Wat511 | Thr236 O ($\beta 7$) | Thr44 OG1 ($\beta 2$) Asp81 NH1 ($\beta 3$) | Wat533 |
| Wat533 | - | Asn127 OD2 ($\beta 4$) Glu237 OE2 ($\beta 7$) | Wat511 |
| Wat518 | Trp51 N ($\beta 2\alpha 2$) Thr84 O ($\beta 3$) | - | Wat545 |
| Wat545 | Trp87 O ($\beta 3\alpha 3$) Ser89 O ($\beta 3\alpha 3$) | - | Wat518 |
| Wat529 | - | Thr142 OG1 ($\beta 4\alpha 4$) Tyr152 OH ($\alpha 4$) | Wat514 |
| Wat514 | His88 N ($\beta 3\alpha 3$) Asn130 O ($\beta 4$) Glu131 O ($\beta 4$) | His88 ND1 ($\beta 3\alpha 3$) | Wat529 |
| Wat515 | - | Asp177 OD1 ($\beta 5\alpha 5$) Ser206 OG ($\beta 6$) Thr208 OG1 ($\beta 6\alpha 6$) | Wat535 |
| Wat535 | Leu176 O ($\beta 5\alpha 5$) | - | Wat515 |
| Wat519 | - | Tyr17 OH ($\beta 1$) Arg81 NE ($\beta 3$) Tyr170 OH ($\beta 5$) | Wat563 |
| Wat503 | Leu176 N ($\beta 5\alpha 5$) Thr208 N ($\beta 6\alpha 6$) | Asp177 OD1 ($\beta 5\alpha 5$) Ser206 OG ($\beta 6$) | Wat505 |
| Wat558 | Leu225 O ($\alpha 6$) Thr230 N ($\alpha 6\beta 7$) Ala226 O ($\alpha 6$) | Thr230 OG1 ($\alpha 6\beta 7$) | Wat579 |
| Wat524 | Trp267 O ($\beta 8\alpha 8$) | Asp239 OD2 ($\beta 7$) Ser274 OG ($\beta 8\alpha 8$) | Wat561 |

The location in the secondary-structural element of the given residue is indicated in parentheses. The first eight waters in the table define four water dimers. Note that networked buried waters are involved in more interactions (18) with side-chain atoms than with the main-chain atoms (15). Also among the interactions with the main-chain atoms, carbonyl oxygen is involved in ten interactions and amide nitrogen in five interactions.