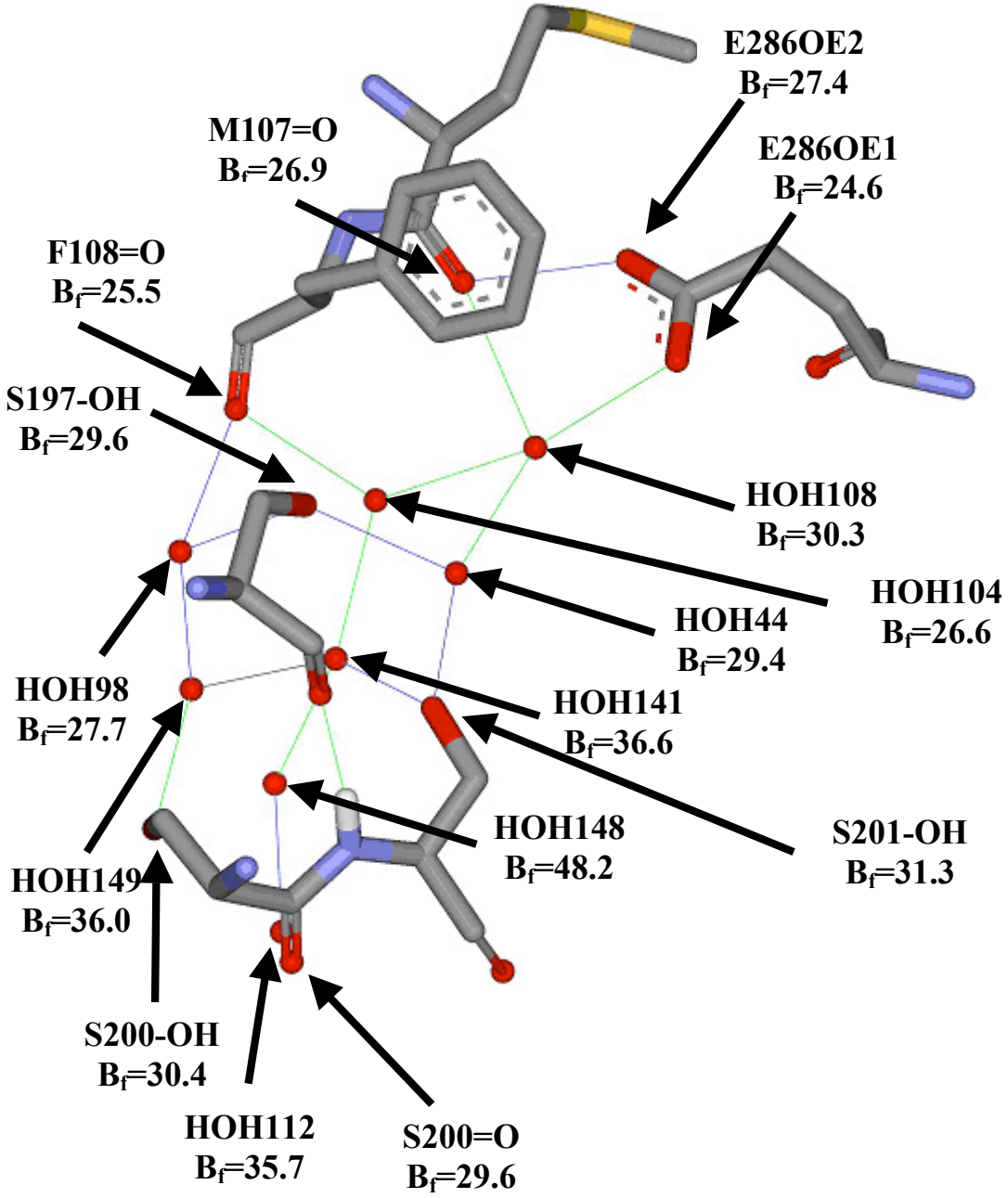
**Supporting Information for "Storage of an Excess Proton in the Hydrogen-bonded Network of the D-pathway of Cytochrome c Oxidase: Identification of a Protonated Water Cluster", by Jiancong Xu, Martyn A. Sharpe, Ling Qin, Shelagh Ferguson-Miller and Gregory A. Voth**




**Average of all atoms in amino acid $B_f$= 27.3**
**Average amino acid O-atoms with H-bonds $B_f$= 28.6**
**Average O-atoms of water in cluster $B_f$=33.8**
**Average $B_f$ of the entire oxidase crystal = 36.5**

The figure on the previous page shows the top of the D pathway in the crystal structure of the I-II subunit of Rs CcO (2GSM). The temperature factors (B factor) of the atoms around this region are listed. The B factor is used to describe thermal disorder of atoms in the molecular structure, as well as the static disorder of the crystal. This area in the structure is well resolved, which can be seen by the relatively low B factors, as well as the electron density map (cf. Figure 5 in PNAS (103), 16117-22).

The B factors for water 141 and water 149 are relatively low (in the mid 30 $Å^2$), almost equal to the overall B factor of the entire structure, and comparable to other water molecules resolved in the nearby region. To further confirm the validity of the assignments of these two water molecules, Qin et al. (unpublished data) did another refinement run during which the occupancy of waters 141 and 149 was set to be 0.5 each. The final B factor after refinement was 21.5 and 20.8, respectively. These B factor values are too low to be realistic for a fractionally occupied site. Therefore, it is concluded that the occupancies of these water molecules are close to 1, and that waters 141 and 149 are in fact two separate water molecules with a short distance rather than one water molecule with partial occupancy at each position.

The overall positional error of this PDB structure is 0.10 Å based on maximum likelihood, 0.14 Å based on R value, and 0.13 Å based on free R value.