
STRUCTURAL
BIOLOGY

**Volume 73 (2017)**

**Supporting information for article:**

# The structure of the Pfp1 protease from a hyperthermophilic archaeon, *Thermococcus thioreducens*, in two crystal forms

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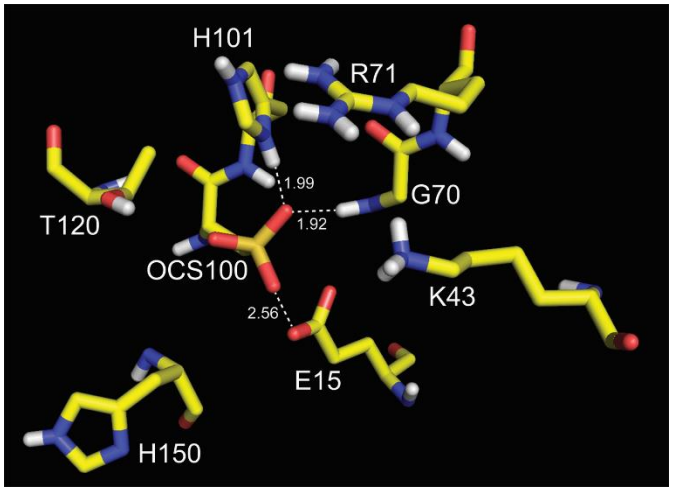


**Figure S1** The environment around the active site OCS100 of the dodecameric protein. In the hexameric form, Arg71 has a different conformation and His150 has two conformations. Water molecules and the hydrogen atom on the cysteine-sulfonic acid, which is presumed to be directed toward Glu15, have been omitted for clarity. Hydrogen bonds and the inter-oxygen distance involving the Cys-$SO_3H$ are labelled.

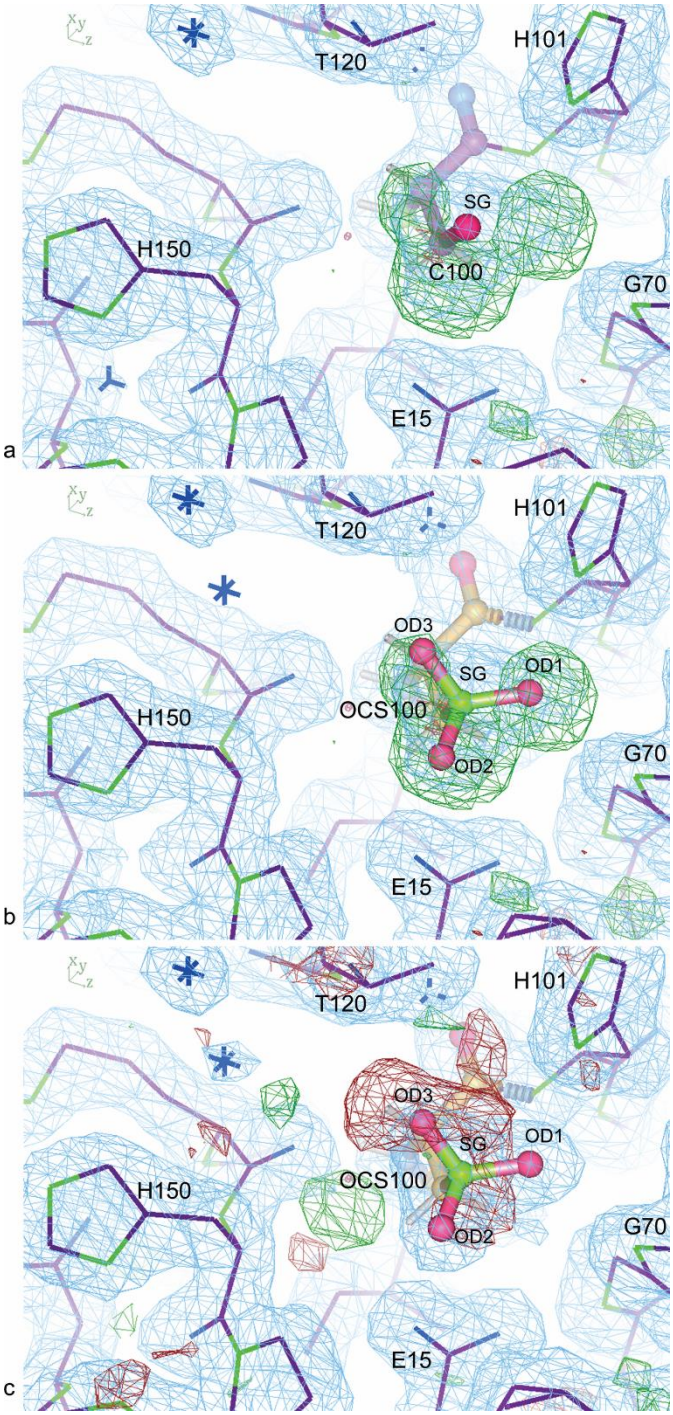


**Figure S2** In (a)-(c), the models are chain A of the large cell dodecamer structure and is representative of all chains in both the large and small cell structures. In (a), an early model before mutating Cys100 to OCS100 is displayed with the 2Fo-Fc and Fo-Fc maps calculated from the model superposed. It is quite clear from the triangularly situated lobes of difference density that the S atom of Cys100 is oxidized. In (b), the refined model in which the Cys100 is mutated to the fully oxidized OCS100 residue is shown with the same maps found in (a). The final model is shown in (c) with the final 2Fo-Fc and Fo-Fc maps superposed. Although we modelled Cys100 as fully oxidized, the negative density seen here and the smaller lobe of positive difference density at OD3 seen in (b)

suggest that Cys100 is probably in at least two oxidation states. The B factors of the O atoms increase with OD1 < OD2 < OD3 for all molecules in both structures. By comparison, in the study of a similar protein from *Staphylococcus aureus* by Kim *et al.* (2016), the authors found the native to be in the Cys-$SO_2H$ oxidation state. They intentionally oxidized it further to the Cys-$SO_3H$ oxidation state. In their final model (PDB ID 4Y1R), only the A chain was modelled in the Cys-$SO_3H$ oxidation state and the B factors of the O atoms refined to the same increasing order of magnitude as seen in *T. thioreducens*, with the non-hydrogen bonded O atom having a magnitude about twice that of the other two O atoms. The B chain was modelled as Cys-$SO_2H$ and there was no difference density at the OD3 position. So, it is unclear if the Cys residues were indeed fully oxidized in their structure.

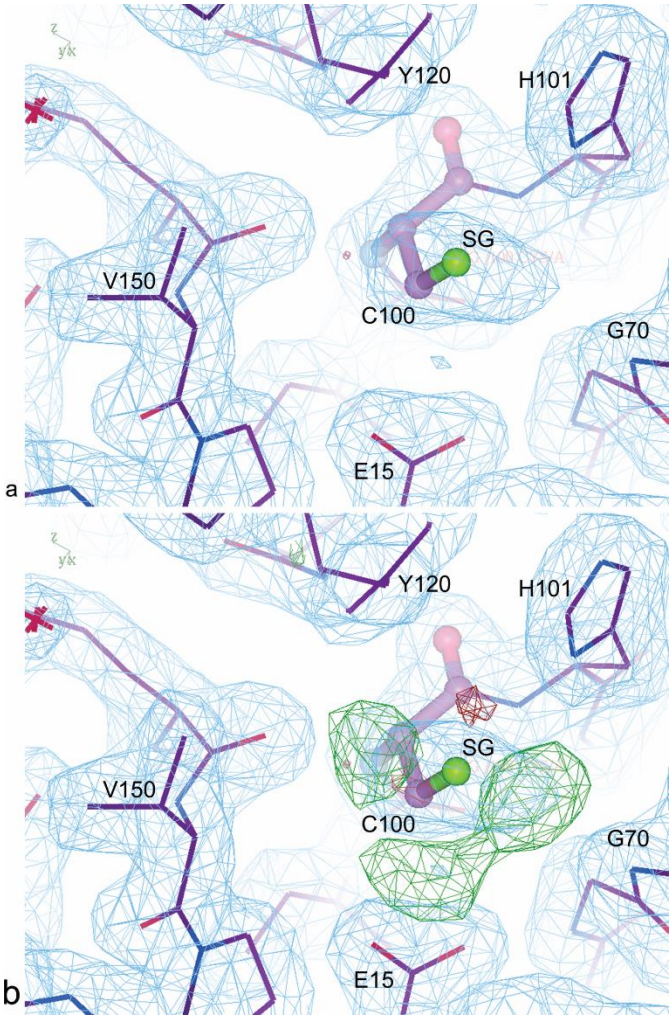


**Figure S3** The displayed model is the deposited model for the protein from *P. horikoshii* (PDB ID 1G2I; Du *et al.*, 2000). In (a), is the final 2Fo-Fc map superposed on the model. In (b), the final Fo-Fc map is added to the image in (a). It seems apparent that Cys100 in the *P. horikoshii* protein is also oxidized in a similar manner as that of the *T. thioreducens* protein with the O atom in the amide pocket between His101 and Gly70 showing up most prominently in the difference map.