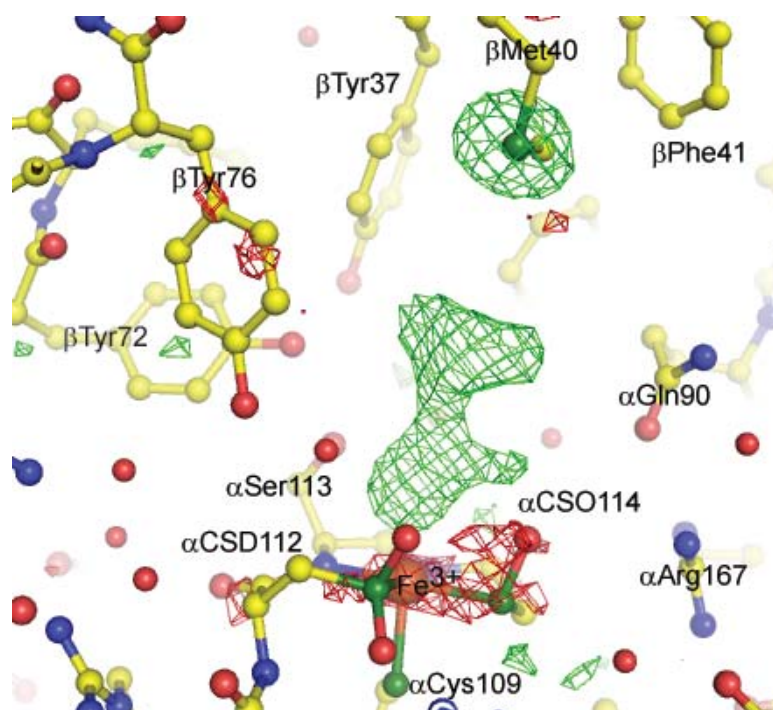


**Supplemental Figure for**

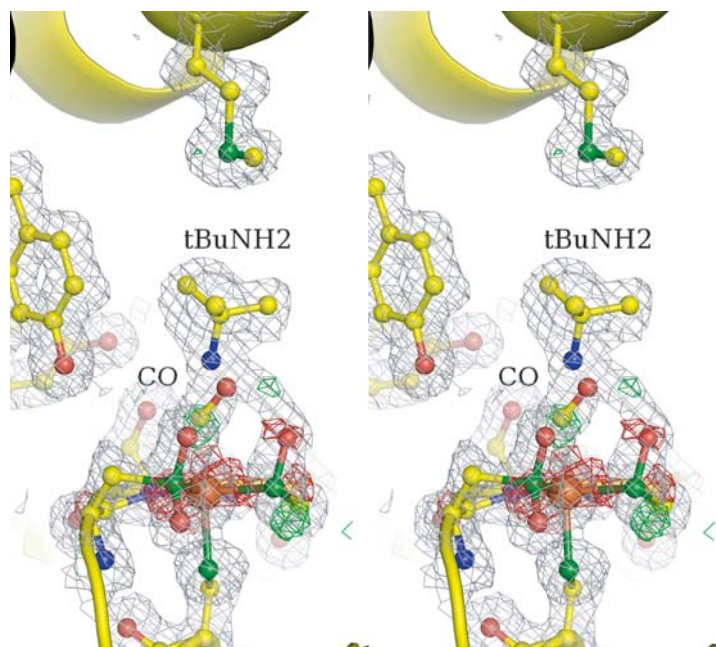
**“Catalytic mechanism of nitrile hydratase proposed by time-resolved  
X-ray crystallography using a novel substrate, *tert*-butylisonitrile”**

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Supplementary Fig. S1 **Structure around the non-heme Fe<sup>3+</sup> center of *ReNHase* with *t*BuNC after light illumination for 340 min.**  $F_o - F_c$  electron density (3.0  $\sigma$  contour as green and -3.0  $\sigma$  contour as red) superimposed on the refined structure. NO and *t*BuNC molecules and S $\gamma$  of  $\beta$ Met40 were excluded from the calculation.  $\alpha$ CSD112 and  $\alpha$ CSO114 indicate  $\alpha$ Cys112-SO<sub>2</sub><sup>-</sup> and  $\alpha$ Cys114-SO<sup>-</sup>, respectively. Yellow, blue, red, green and brown spheres represent carbon, nitrogen, oxygen, sulfur and iron atoms, respectively.



Supplementary Figure 2: **Structure around the non-heme Fe<sup>3+</sup> center of *ReNHase* with *tBuNC* after light illumination for 440 min.**  $F_o-F_c$  electron density (3.0  $\sigma$  contour as green and -3.0  $\sigma$  contour as red) and  $2F_o-F_c$  electron density (1.0  $\sigma$  contour as gray) superimposed on the refined structure at 440 min. *tBuNH<sub>2</sub>* and CO were included in the calculation. Yellow, blue, red, green and brown spheres represent carbon, nitrogen, oxygen, sulfur and iron atoms, respectively.