

## Functional characterisation of two ferric-ion coordination modes of TtFbpA, the periplasmic subunit of an ABC-type iron transporter from *Thermus thermophilus* HB8

### Figure S1.

The not converged results of quantum calculation by Gaussian 16. Hydrogen atoms are represented as white balls. Tyrosine residues are represented as sticks.  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  ions are represented as ball and sticks. **A:** One water molecule and two  $\text{CO}_3^{2-}$  ions were input as the coordination ligands. **B:** Two  $\text{CO}_3^{2-}$  ions were input as the coordination ligands.

### Figure S2.

Purity verification of TtFbpA. **A:** Image of SDS-PAGE for the fractions after Ni-NTA resin affinity chromatography. The first lane on the left is the molecular marker. Lane FT is the flow-through after loading the Ni-NTA resin. Lane S1 is the fraction eluted by washing buffer 1. Lane S2 is the fraction eluted by washing buffer 2. Lane S3 is the fraction eluted by elution buffer 1. Lane S4 is the fraction eluted by elution buffer 2. Lane S5 is the fraction eluted by elution buffer 3. Lane S6 is the fraction eluted by elution buffer 4. Lane S7 is the Ni-NTA resin after elution. **B:** Image of SDS-PAGE for the verification of  $6 \times$  His-tag cleavage. The first lane on the left is the molecular marker. Lane 1 is TtFbpA that was incubated overnight. Lane 2 and Lane 3 are samples of the flow-through after loading on the Ni-NTA resin.

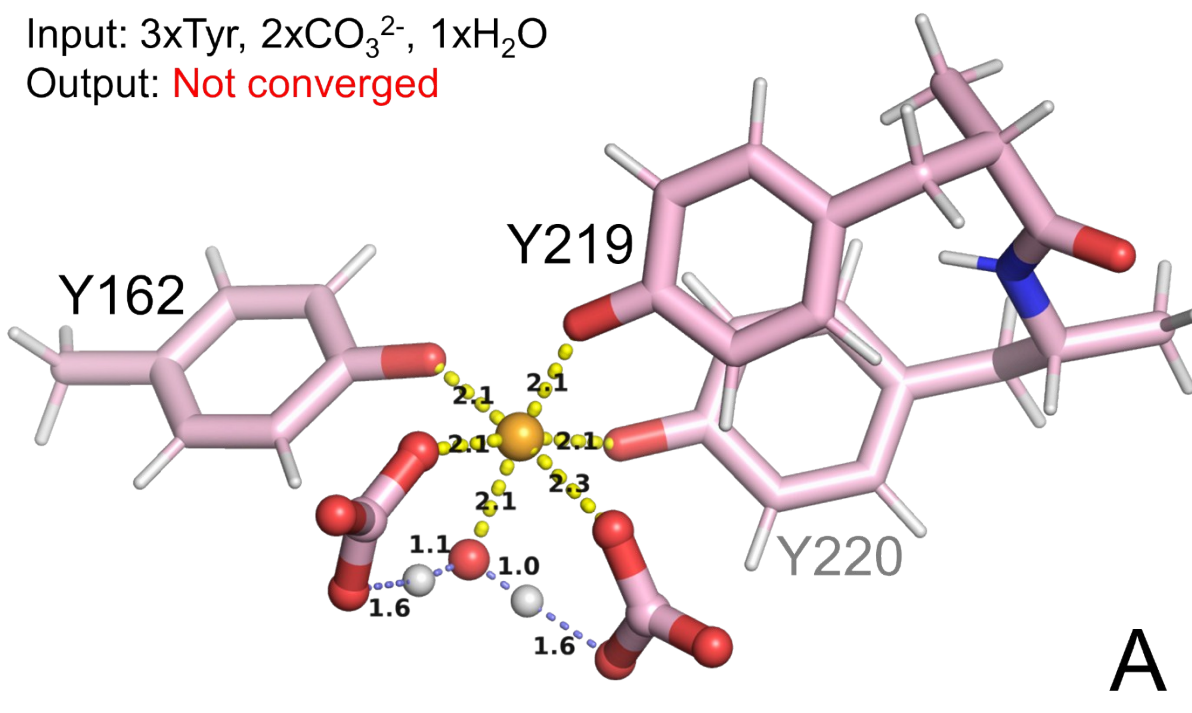
### Figure S3.

Gel filtration chromatography of TtFbpA (**A**) and image of SDS-PAGE for the fractions from the elution peak collected from 13 mL to 17 mL (**B**). The first lane on the left is the molecular marker.

### Figure S4.

**A:** SDS-PAGE results for each apo and  $\text{Fe}^{3+}$ -bound TtFbpA under different pH conditions after adjusting the protein concentration to 0.1 mM. The first lane on the left is the molecular marker. **B:** Absorbance spectra (300–700 nm) of peak 2 from the gel filtration chromatography of  $\text{Fe}^{3+}$ -bound TtFbpAs at pH 6.0 and 5.0. The absorbance spectrum of 0.2 mM  $\text{FeCl}_3$  (300–700 nm) is shown as a reference.

Input: 3xTyr, 2xCO<sub>3</sub><sup>2-</sup>, 1xH<sub>2</sub>O  
Output: **Not converged**



Input: 3xTyr, 2xCO<sub>3</sub><sup>2-</sup>  
Output: **Not converged**

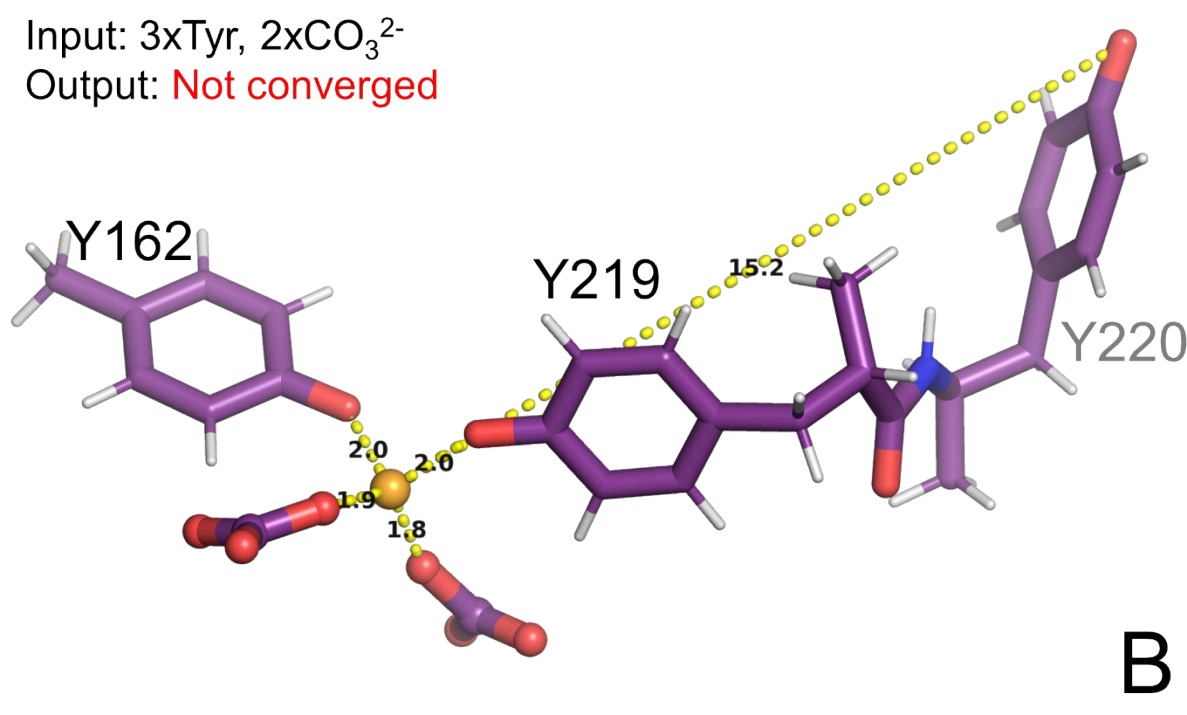
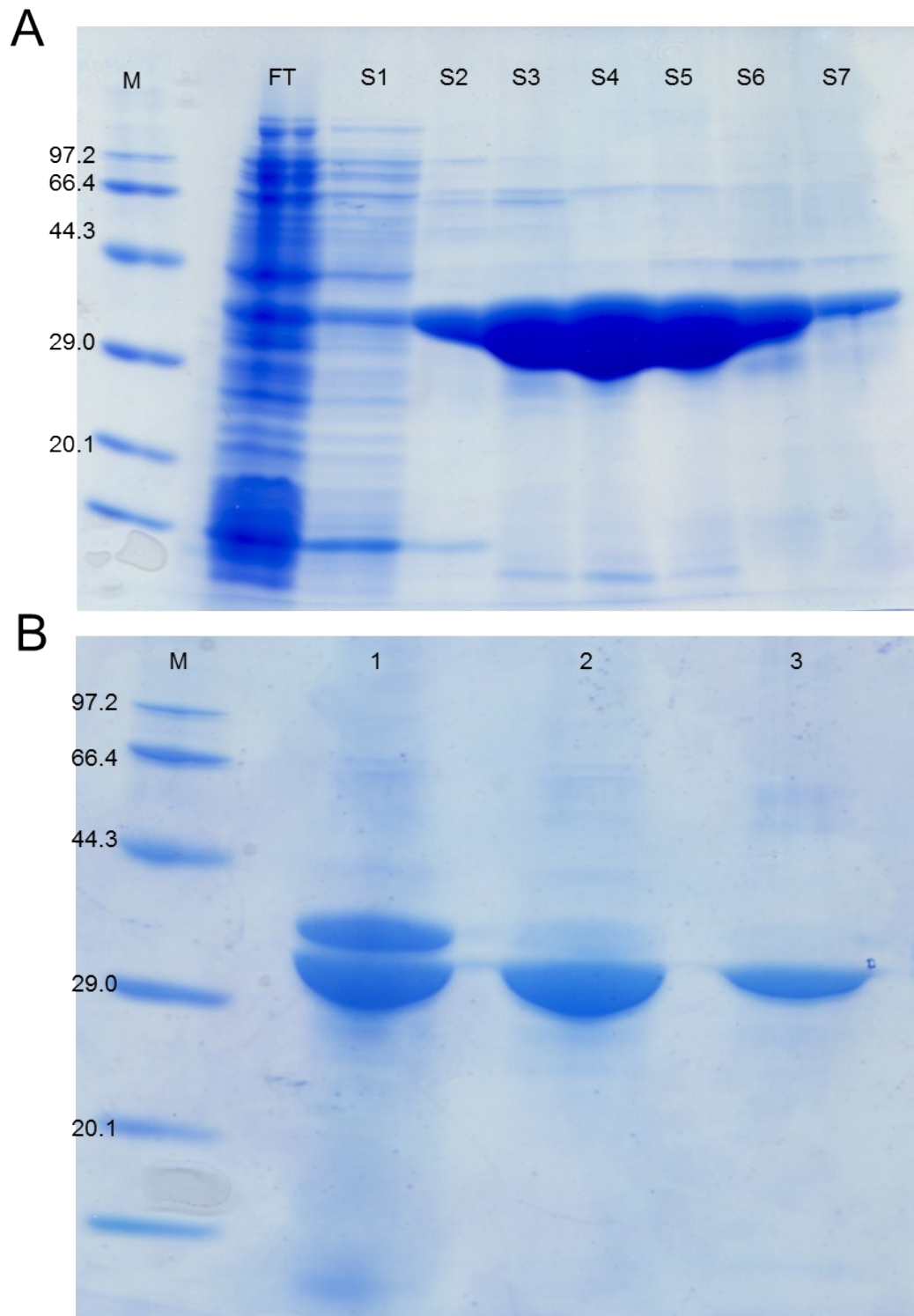
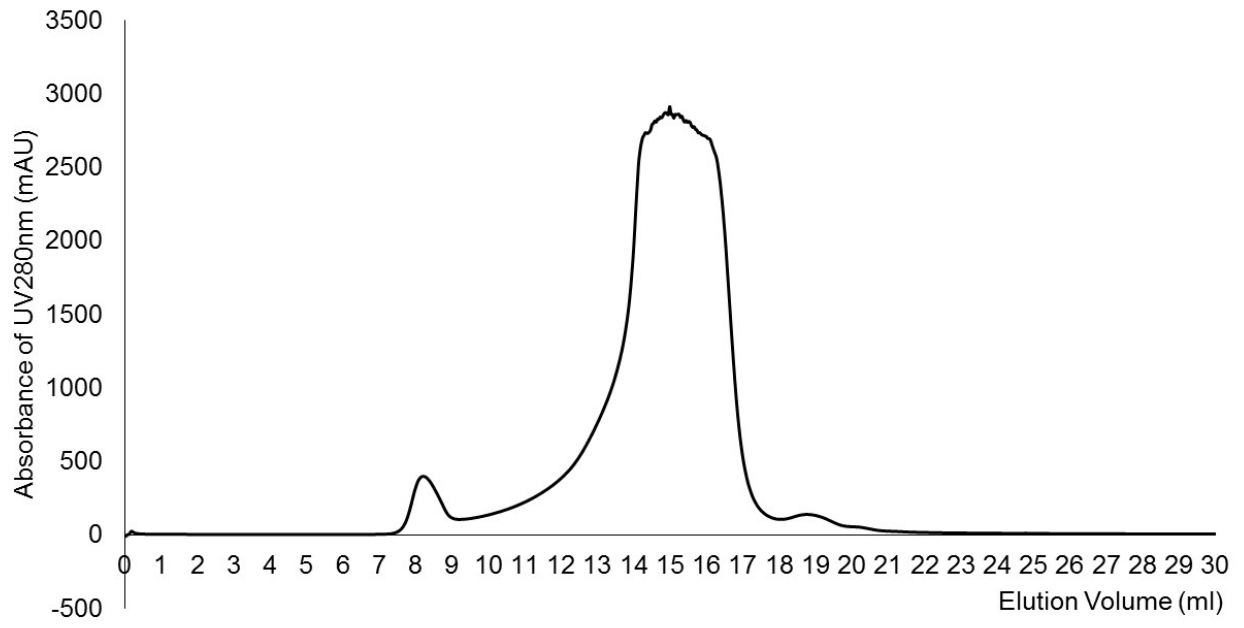


Figure S1. Lu *et al.*

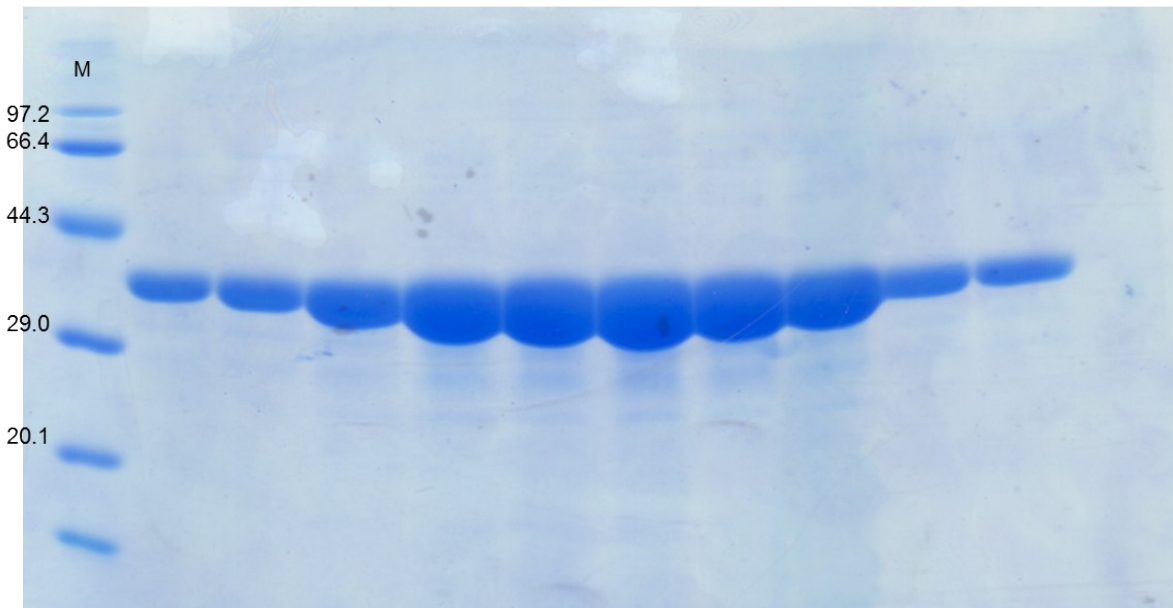


**Figure S2.** Lu *et al.*

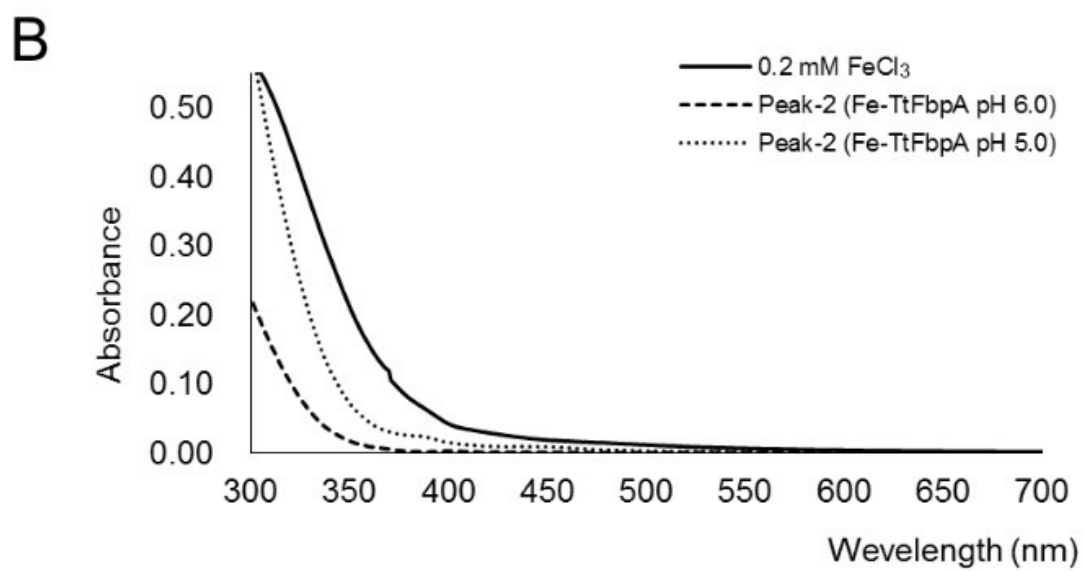
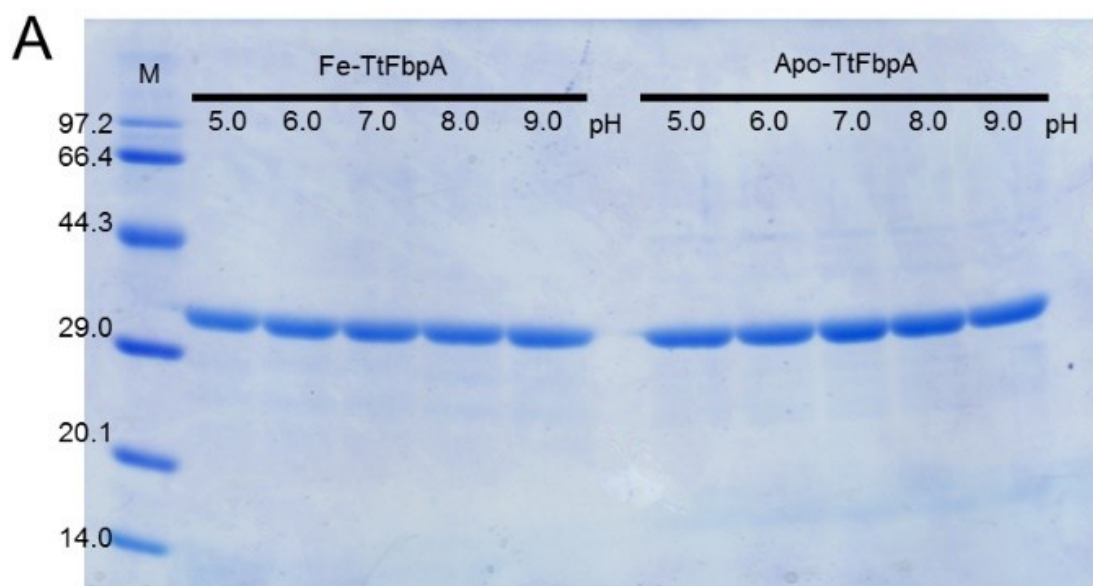
**A**



**B**



**Figure S3.** Lu *et al.*



**Figure S4.** Lu *et al.*