

Supporting Information

Effect of distal histidines on hydrogen peroxide activation by manganese reconstituted myoglobin

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Table of Contents

1	Spectral changes of F43H Mn ^{III} Mb in the reaction with 100 equiv. H ₂ O ₂ in pH 7.4	2
2.	Kinetic data and spectral changes of Mn ^{III} Mb in the reaction with H ₂ O ₂	3
3.	Spectral changes of ABTS oxidation for Mn ^{III} Mb at pH 7.4	9
4.	ESI(+)-MS of different mutants	13

Spectral changes of F43H Mn^{III}Mb in the reaction with 100 equiv. H₂O₂ in pH 7.4

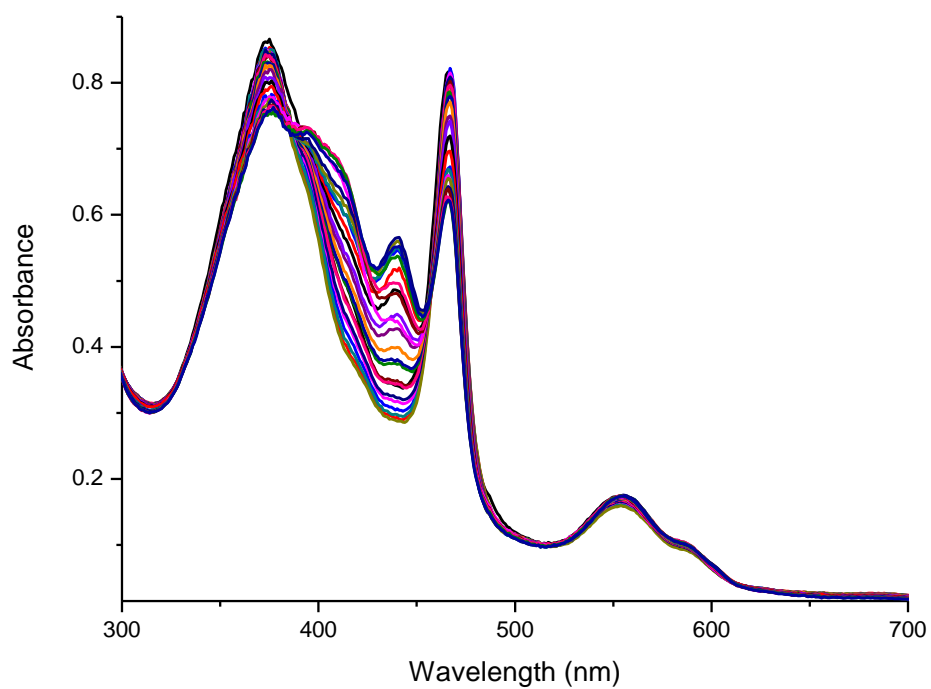
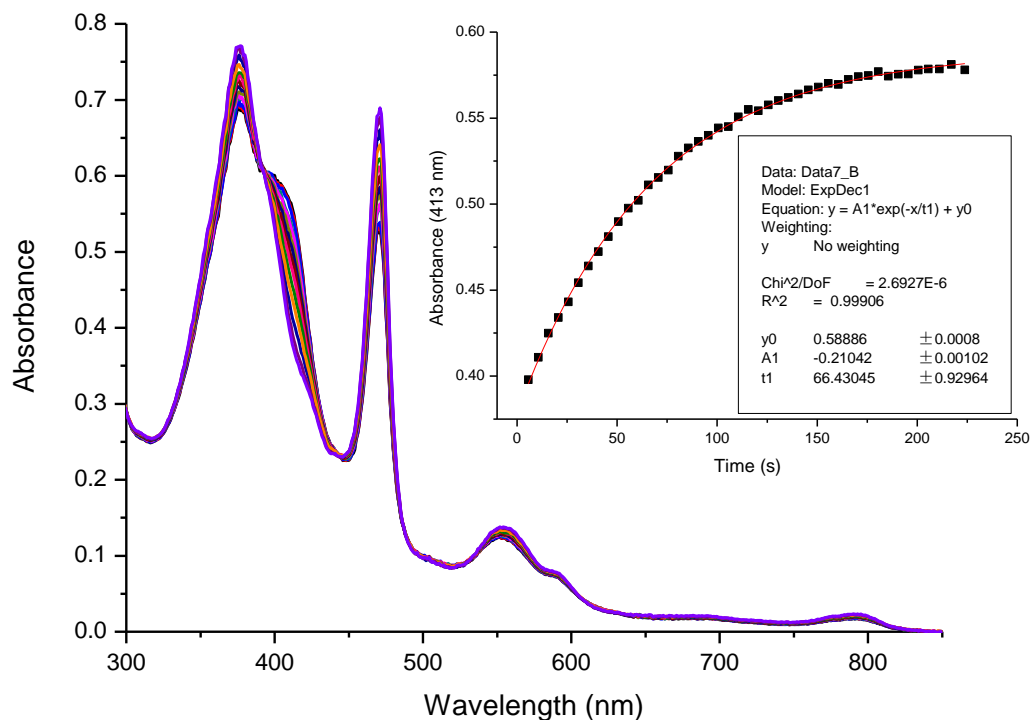


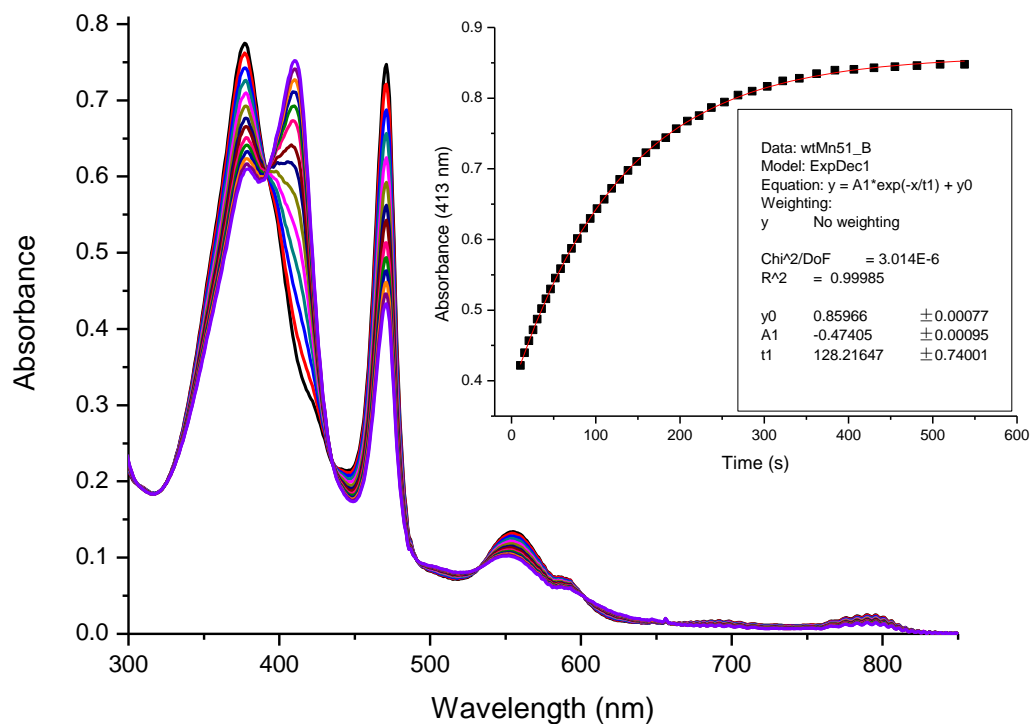
Figure S1. Spectral changes of F43H Mn^{III}Mb in the reaction with 100 equiv. H₂O₂ in pH 7.4

Kinetic data and spectral changes of Mn^{III}Mb in the reaction with hydrogen peroxide:

Conditions: the concentration was 29 μM for Mn^{III}Mb protein and 580 μM for H₂O₂, the reaction temperature was 20 °C.



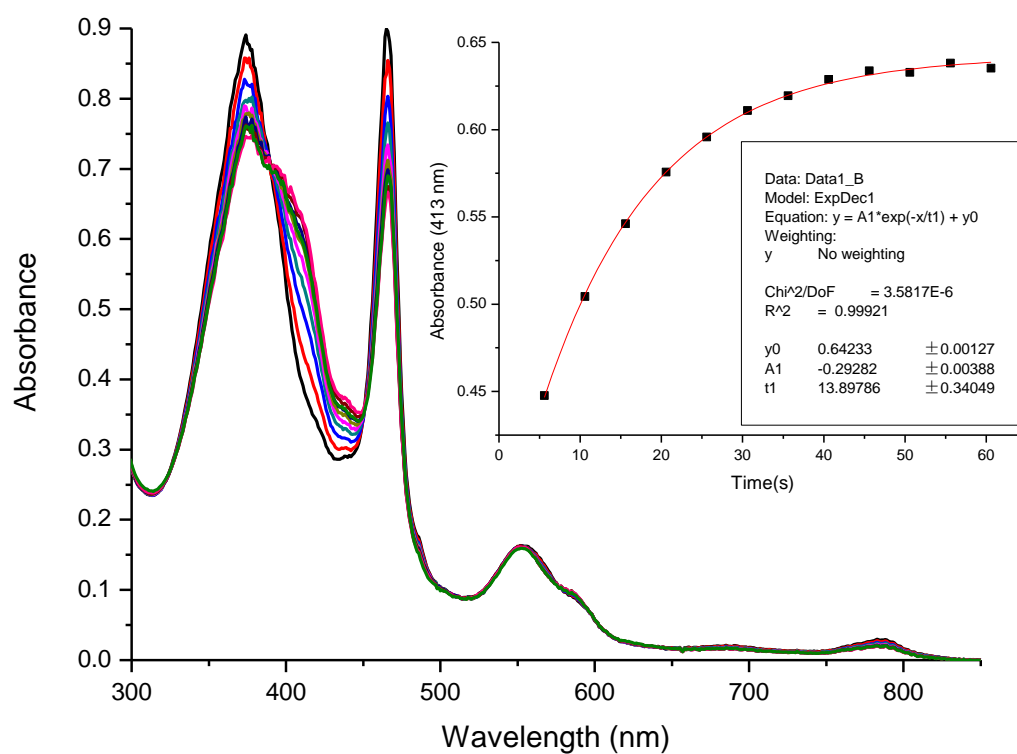
(a)



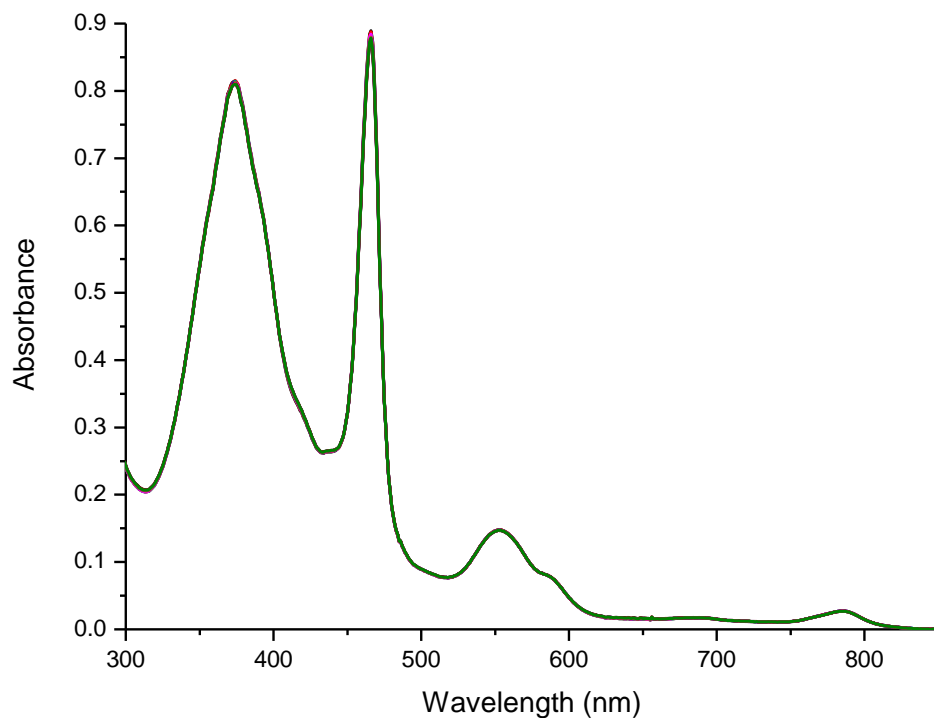
(b)

Figure S2 Spectral change of wild-type Mn^{III}Mb in the reaction with H₂O₂ at a) pH 7.4 and b) pH

5.1. Insets: trace for the formation of $\text{Mn}^{\text{IV}}=\text{O}$ Por and the exponential fitting function, $k_{\text{obs}}=1/t_1$

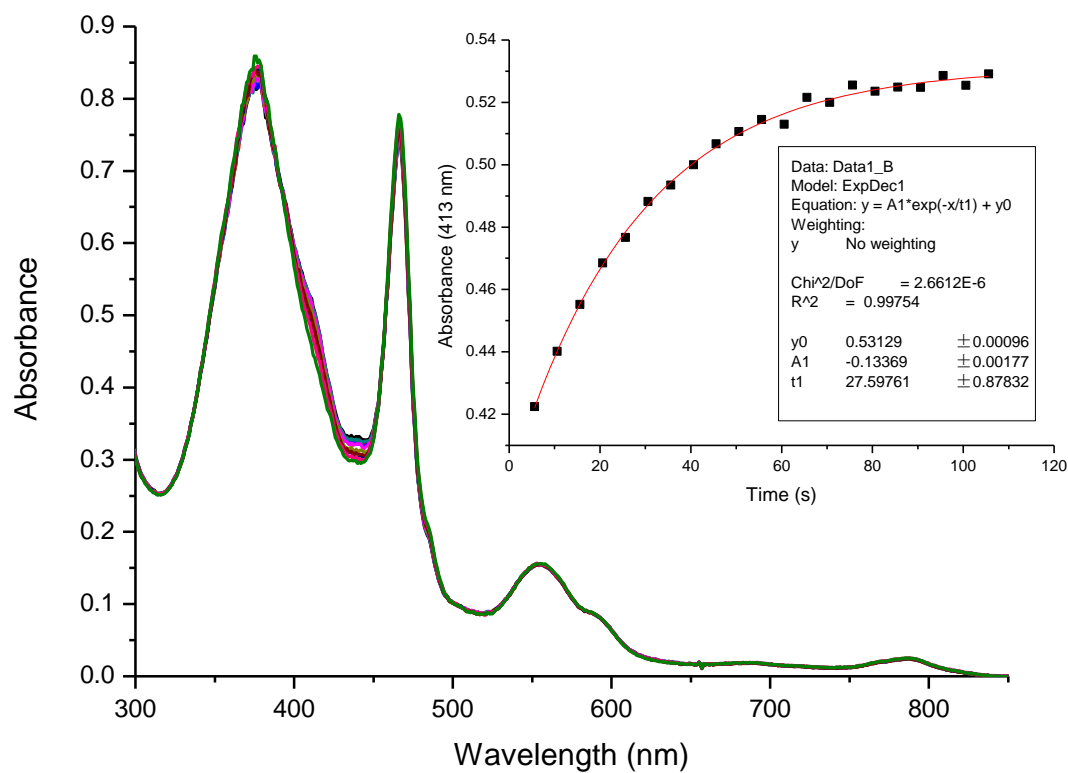


(a)

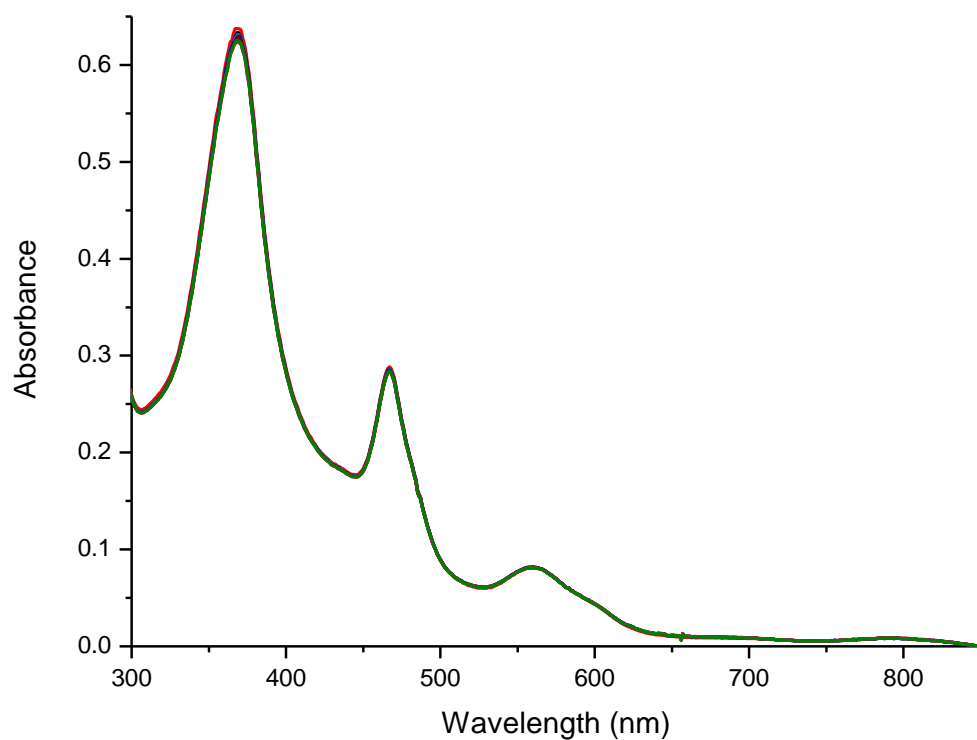


(b)

Figure S3 Spectral change of F43H Mn^{III} Mb mutant in the reaction with H_2O_2 at a) pH 7.4 and b) pH 5.1. Insets: trace for the formation of $\text{Mn}^{\text{IV}}=\text{O}$ Por and the exponential fitting function, $k_{\text{obs}}=1/t_1$



(a)



(b)

Figure S4. Spectral change of L29H/F43H Mn^{III}Mb mutant in the reaction with H₂O₂ at a) pH 7.4 and b) pH 5.1. Insets: trace for the formation of Mn^{IV}=O Por and the exponential fitting function, $k_{\text{obs}}=1/t_1$

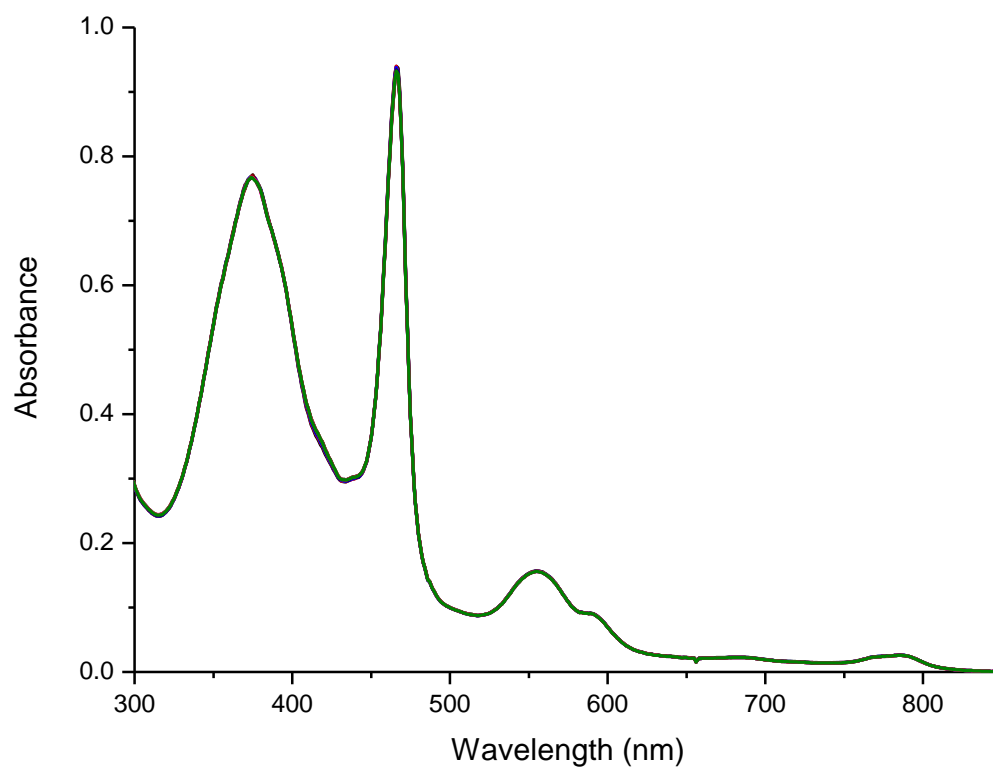


Figure S5 Spectral change of L29H Mn^{III}Mb mutant in the reaction with H₂O₂ at pH 7.4.

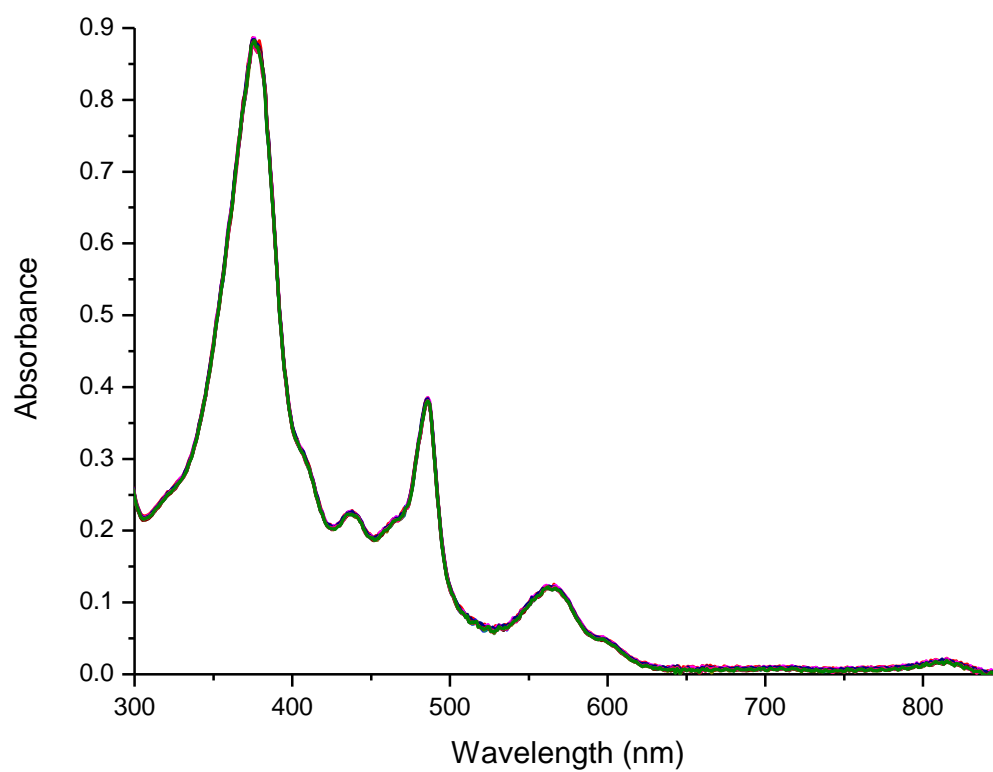


Figure S6. Spectral change of H64F Mn^{III}Mb mutant in the reaction with H₂O₂ at pH 7.4.

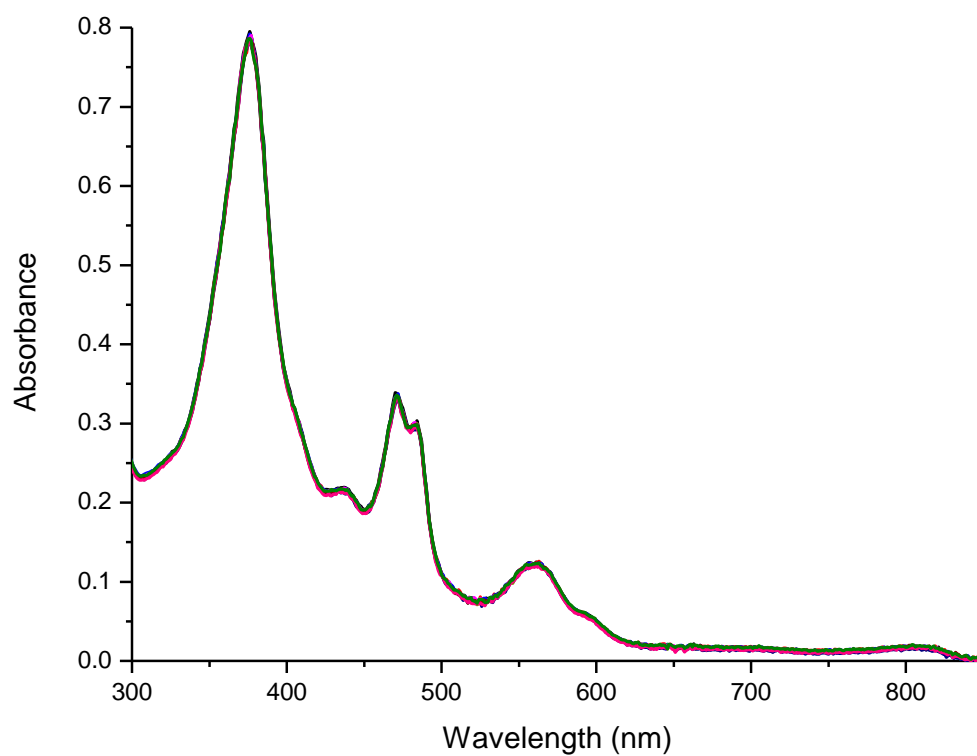


Figure S7. Spectral change of F43H/H64F Mn^{III}Mb mutant in the reaction with H₂O₂ at pH 7.4.

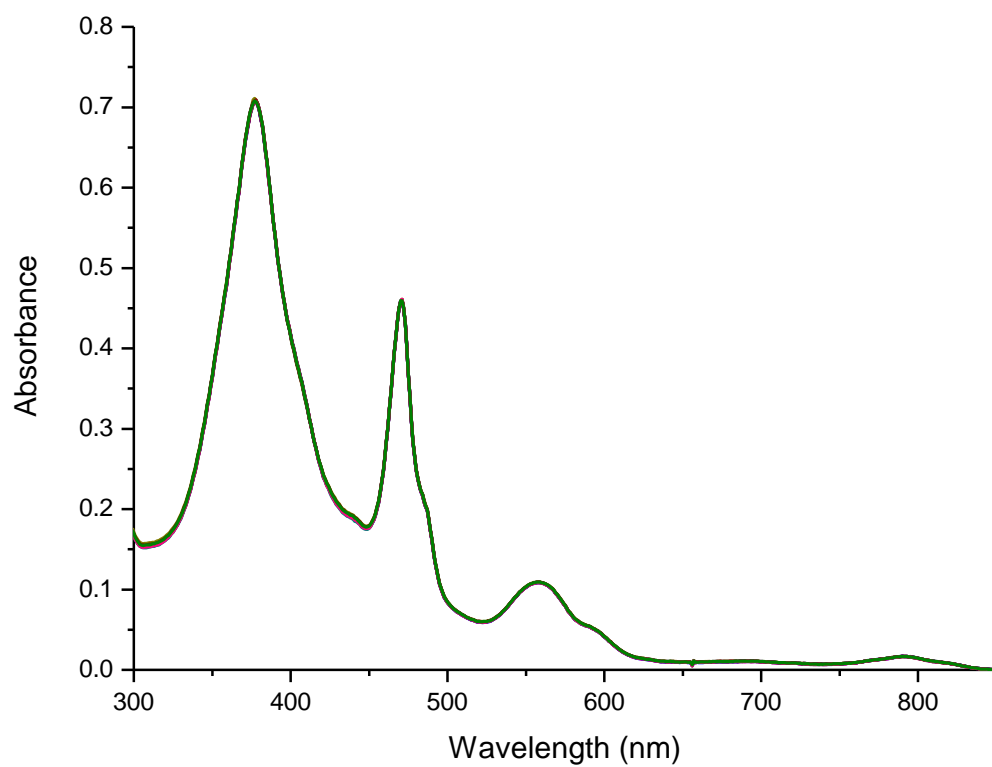


Figure S8. Spectral change of L29H/H64F Mn^{III}Mb mutant in the reaction with H₂O₂ at pH 7.4.

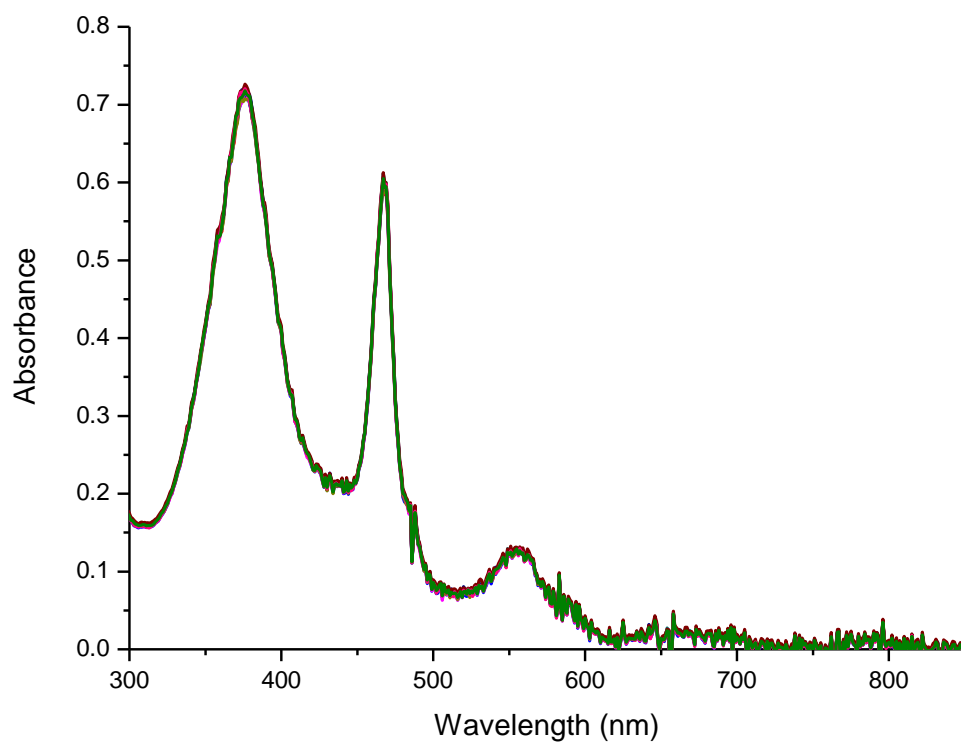


Figure S9. Spectral change of L29H/F43H/H64F Mn^{III}Mb mutant in the reaction with H₂O₂ at pH 7.4.

Spectral changes of ABTS oxidation for Mn^{III}Mb at pH 7.4:

Conditions: the concentration was 29 μ M for protein and 580 μ M for ABTS and H₂O₂, the reaction temperature was 20 °C.

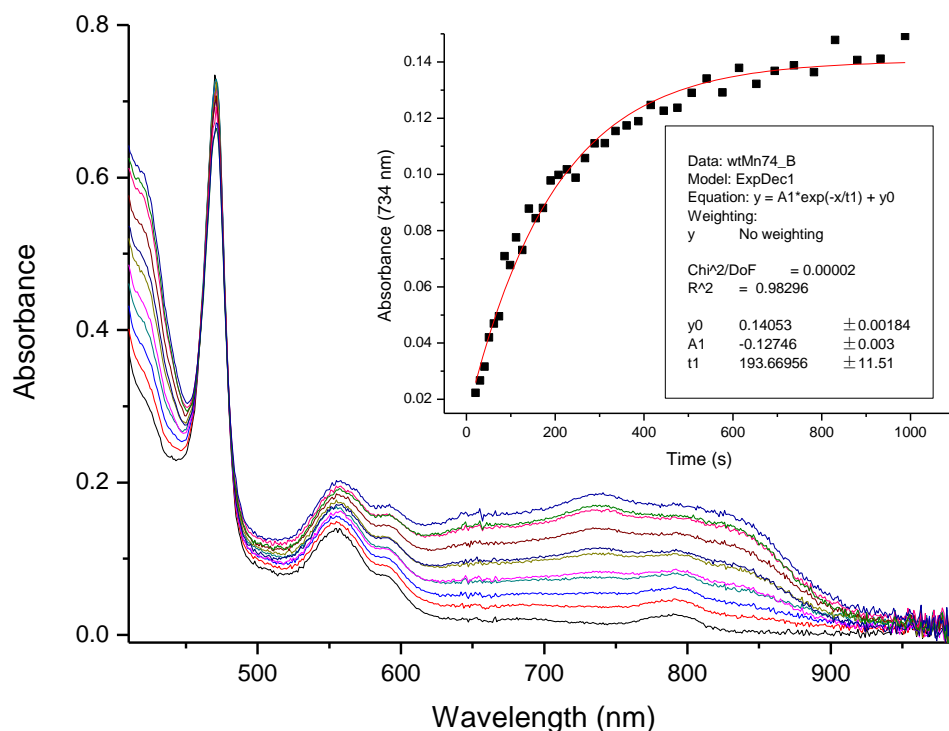


Figure S10. Spectral change of ABTS oxidation at pH 7.4 for wild-type Mn^{III}Mb.

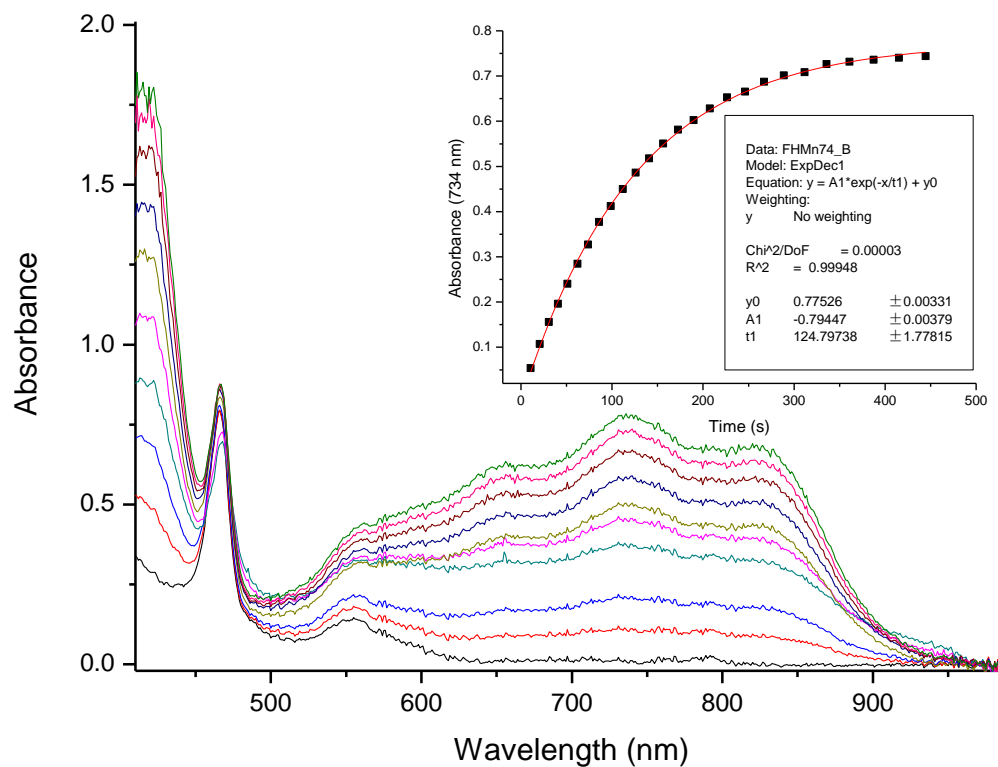


Figure S11. Spectral change of ABTS oxidation at pH 7.4 for F43H Mn^{III}Mb mutant.

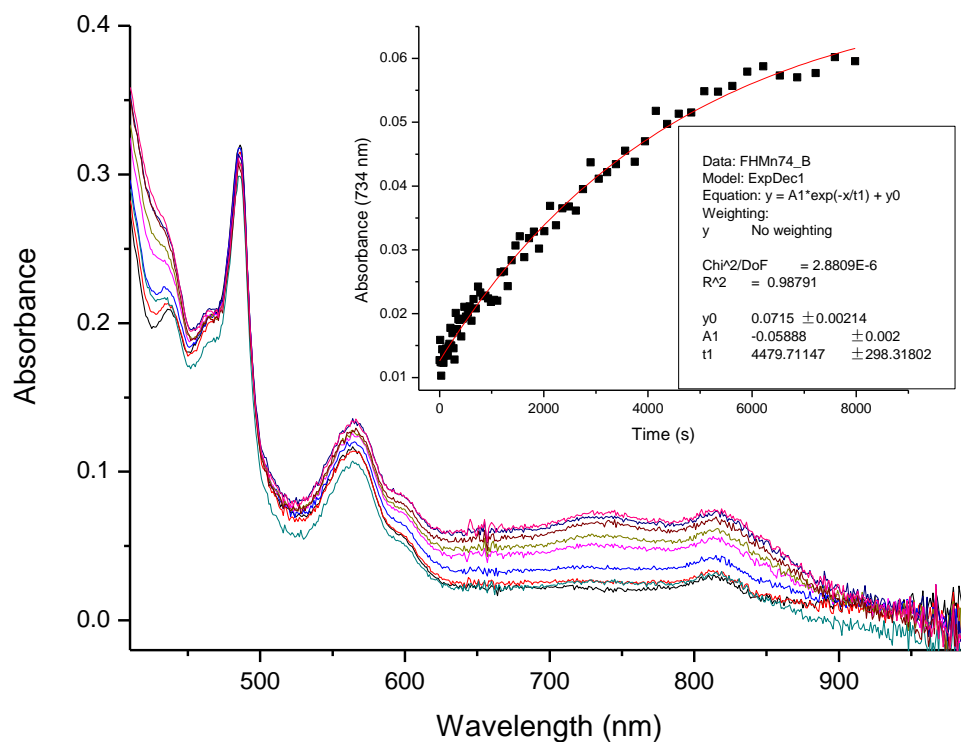


Figure S12. Spectral change of ABTS oxidation at pH 7.4 for H64F Mn^{III}Mb mutant.

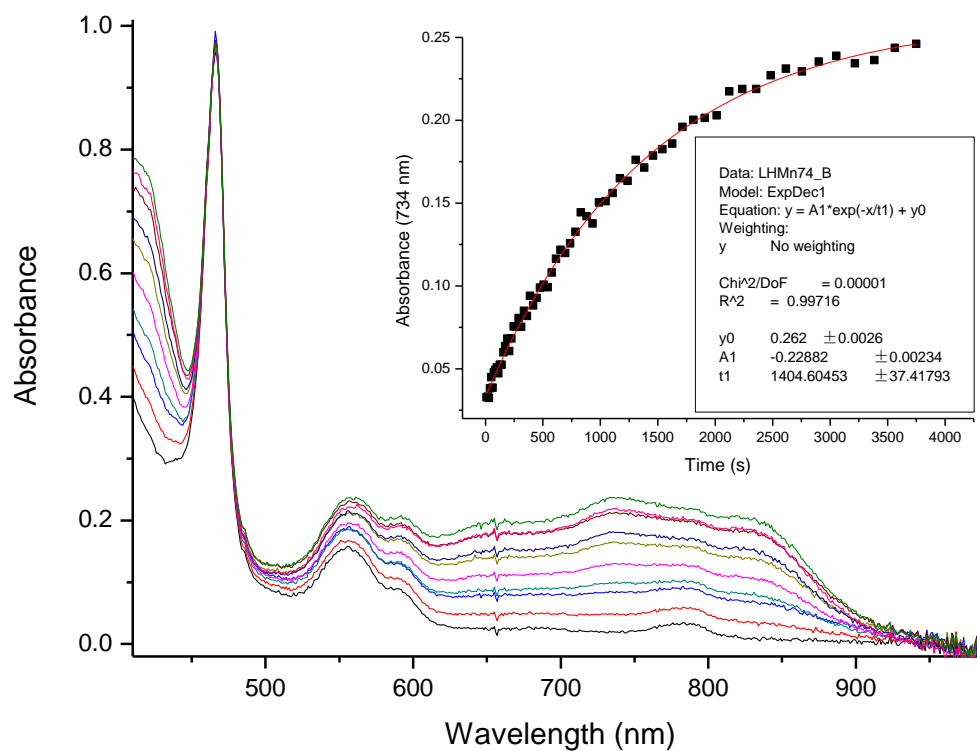


Figure S13. Spectral change of ABTS oxidation at pH 7.4 for L29H Mn^{III}Mb mutant.

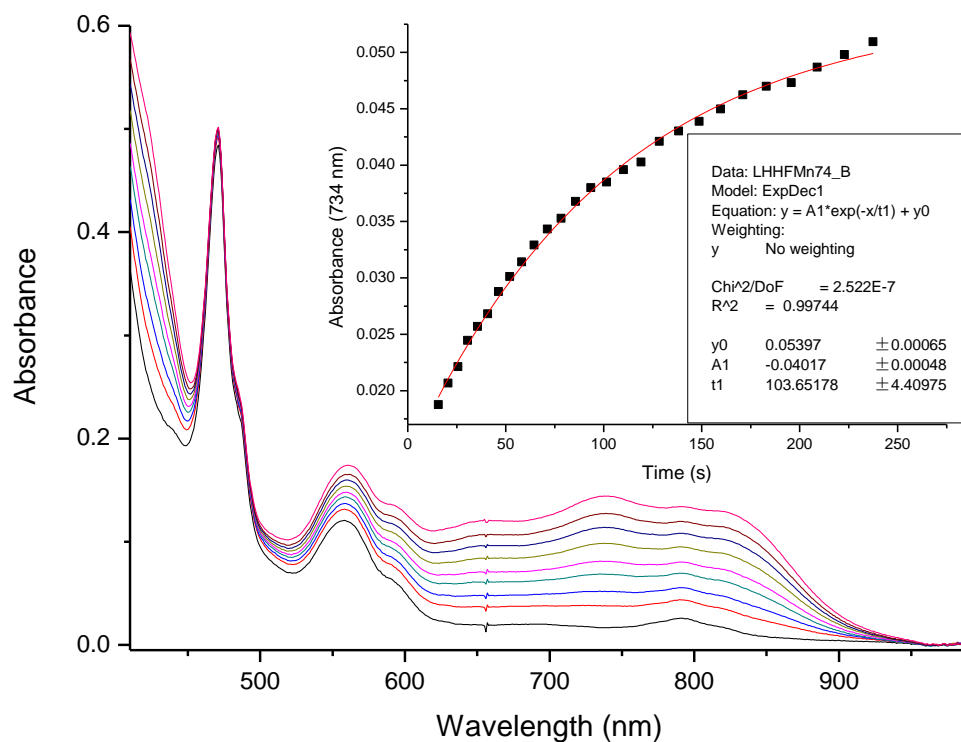


Figure S14. Spectral change of ABTS oxidation at pH 7.4 for L29H/H64F Mn^{III}Mb mutant.

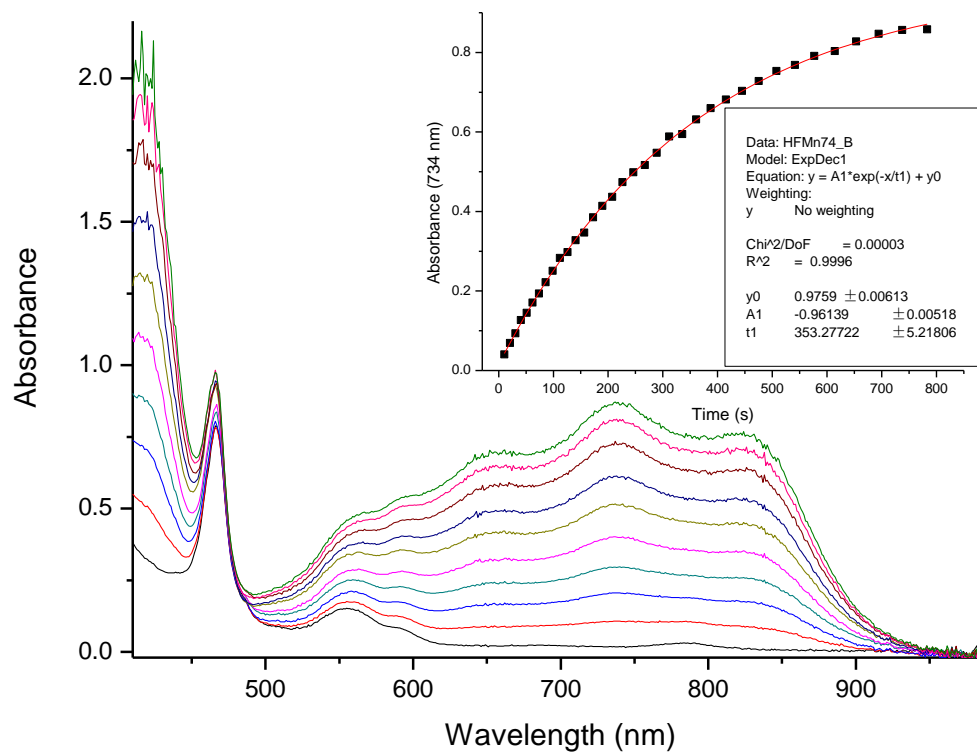


Figure S15. Spectral change of ABTS oxidation at pH 7.4 for L29H/F43H Mn^{III}Mb mutant.

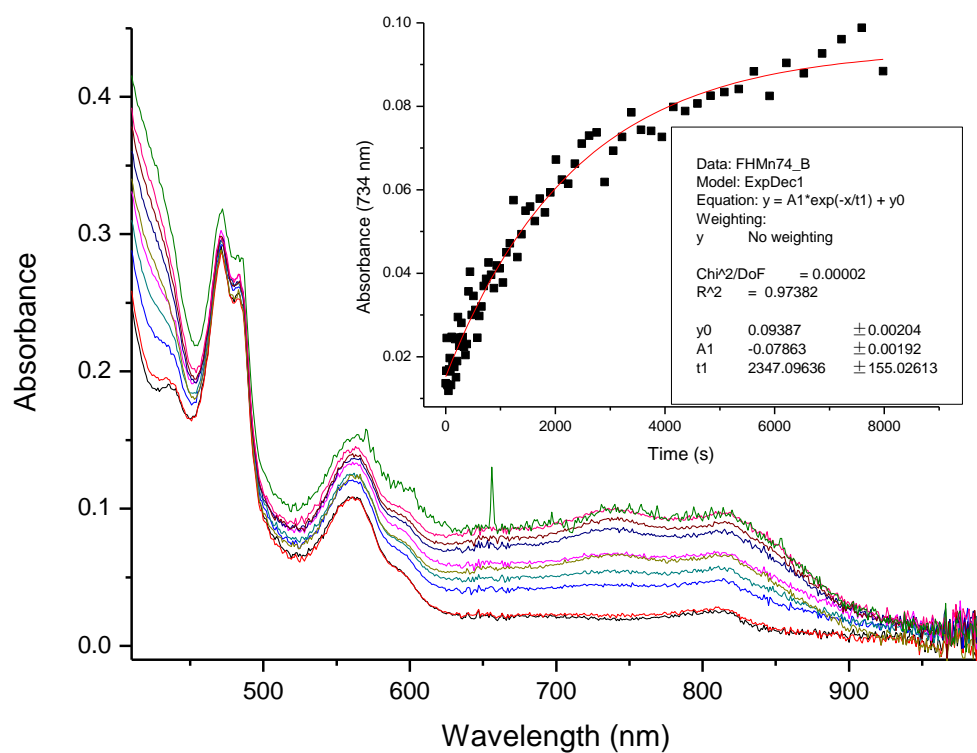


Figure S16. Spectral change of ABTS oxidation at pH 7.4 for F43H/H64F Mn^{III}Mb mutant.

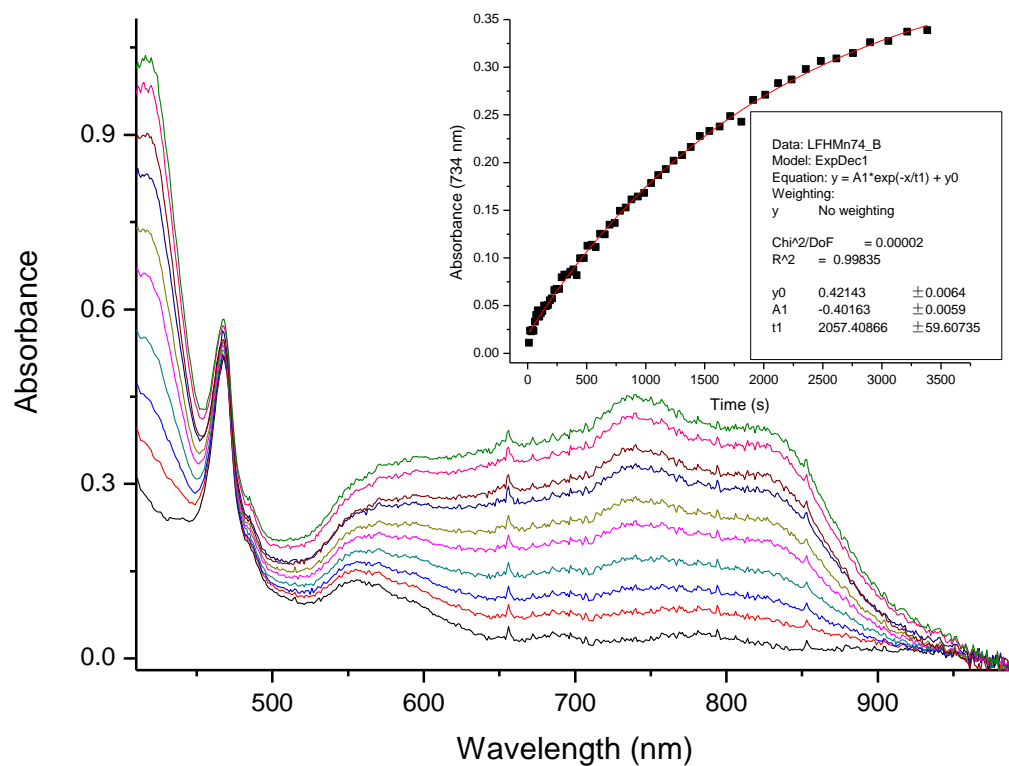
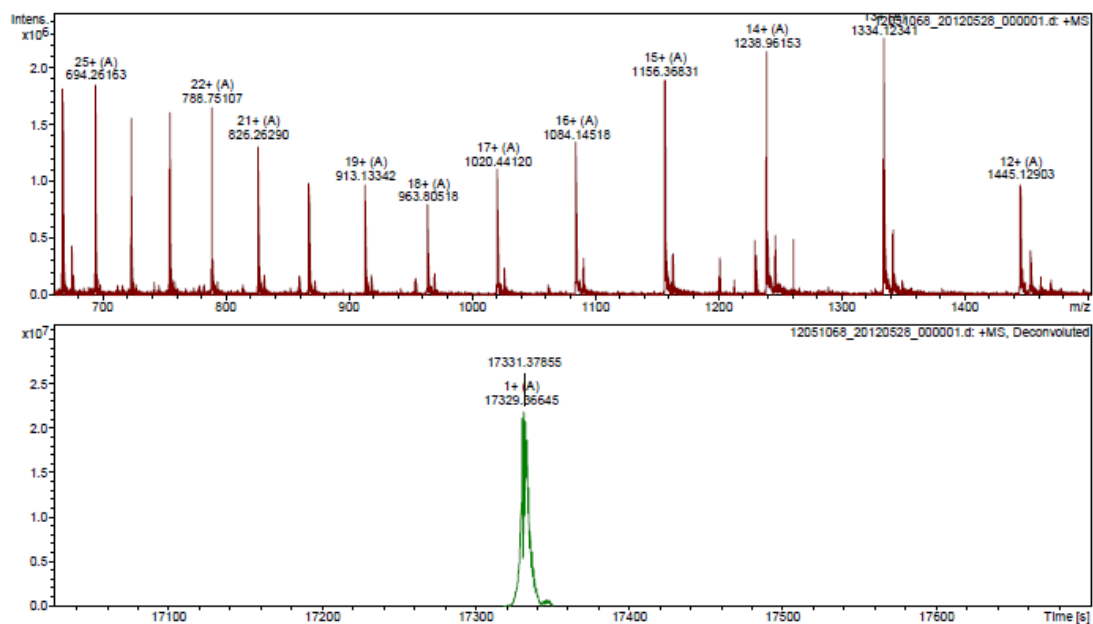


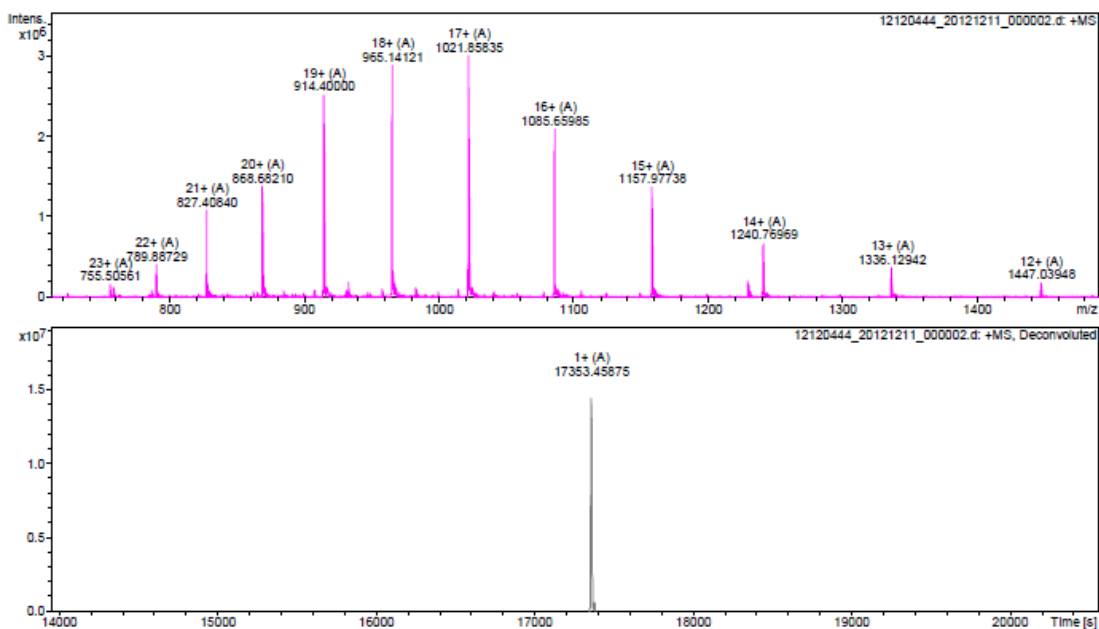
Figure S17. Spectral change of ABTS oxidation at pH 7.4 for L29H/F43H/H64F Mn^{III}Mb mutant.

ESI(+)-MS of different mutants:

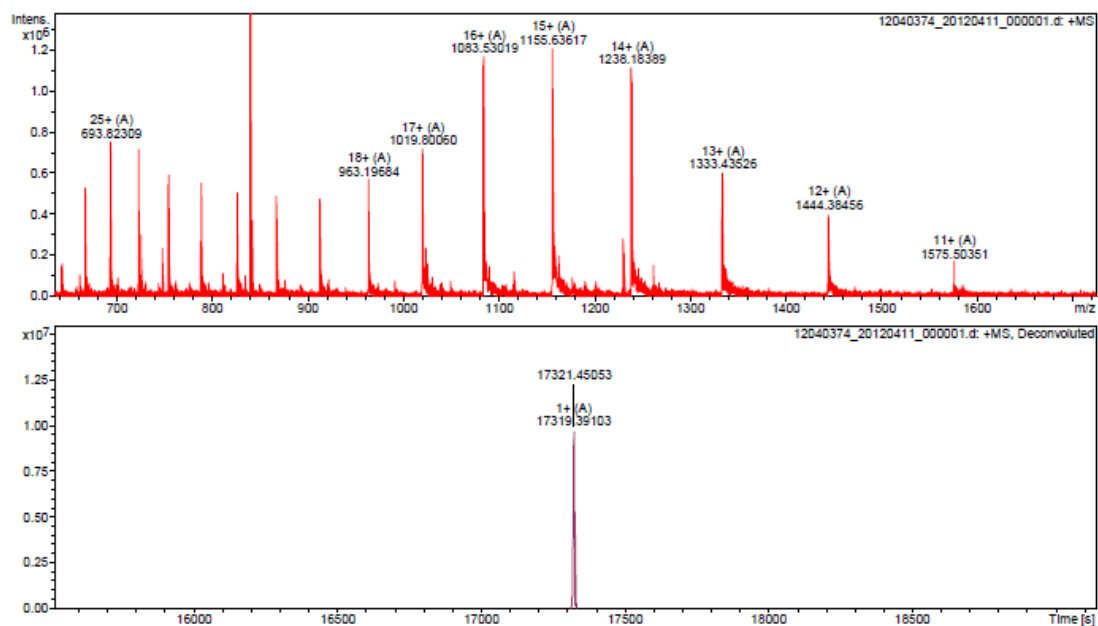
Wild-type Mb:



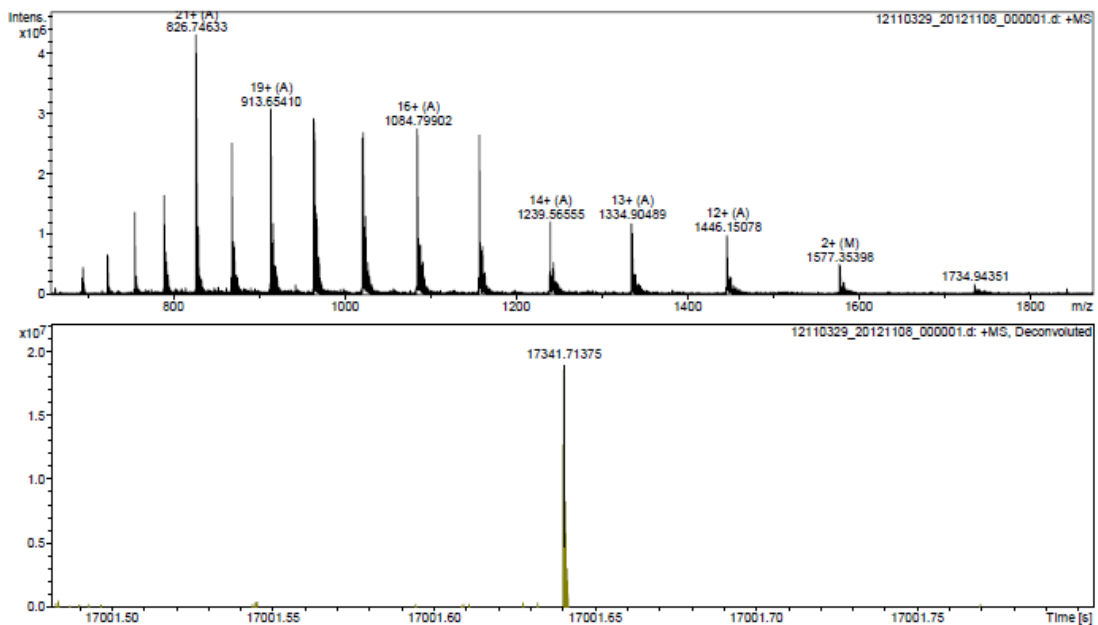
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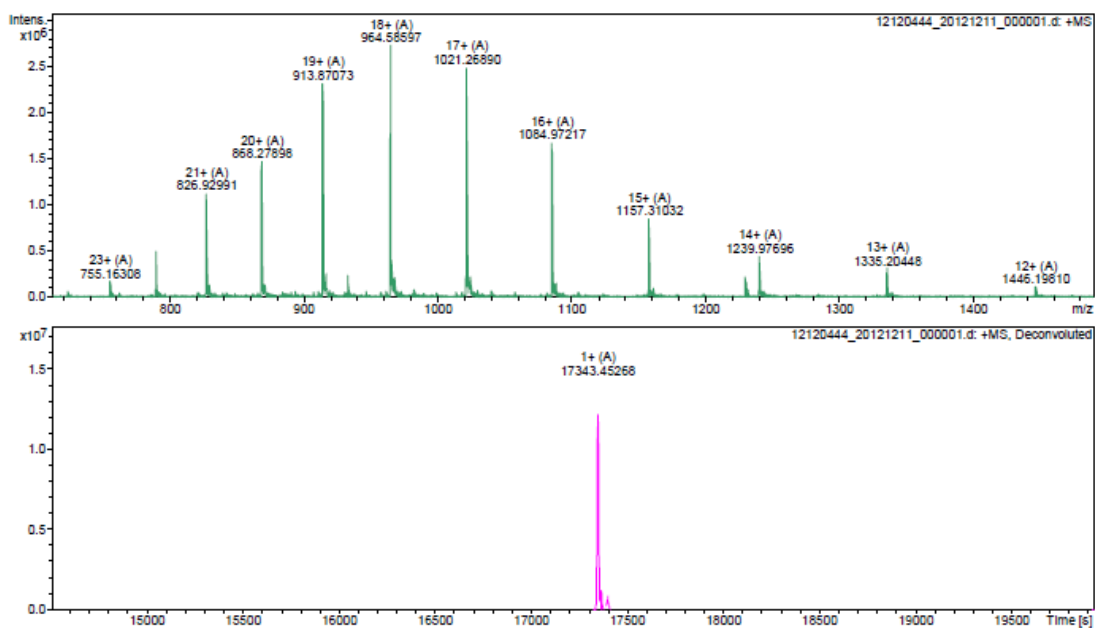
F43H Mb:



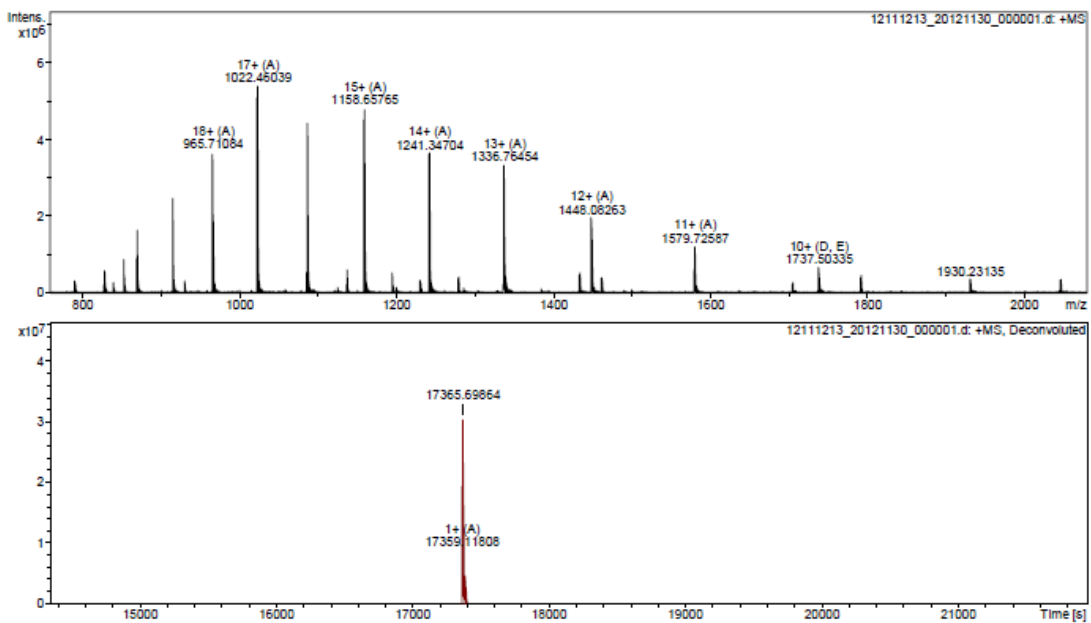
H64F Mb:



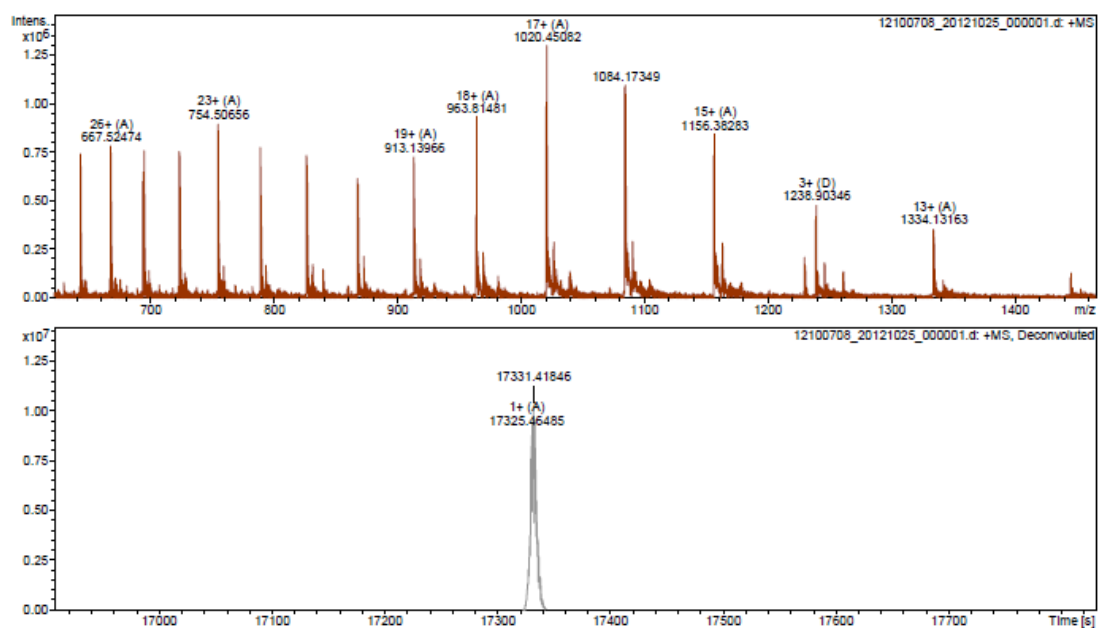
L29H/F43H Mb:



L29H/H64F Mb:



F43H/H64F Mb:



L29H/F43H/H64F Mb:

