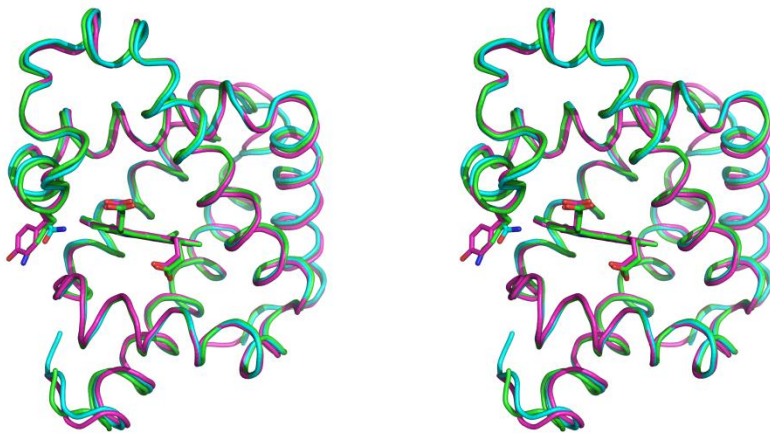


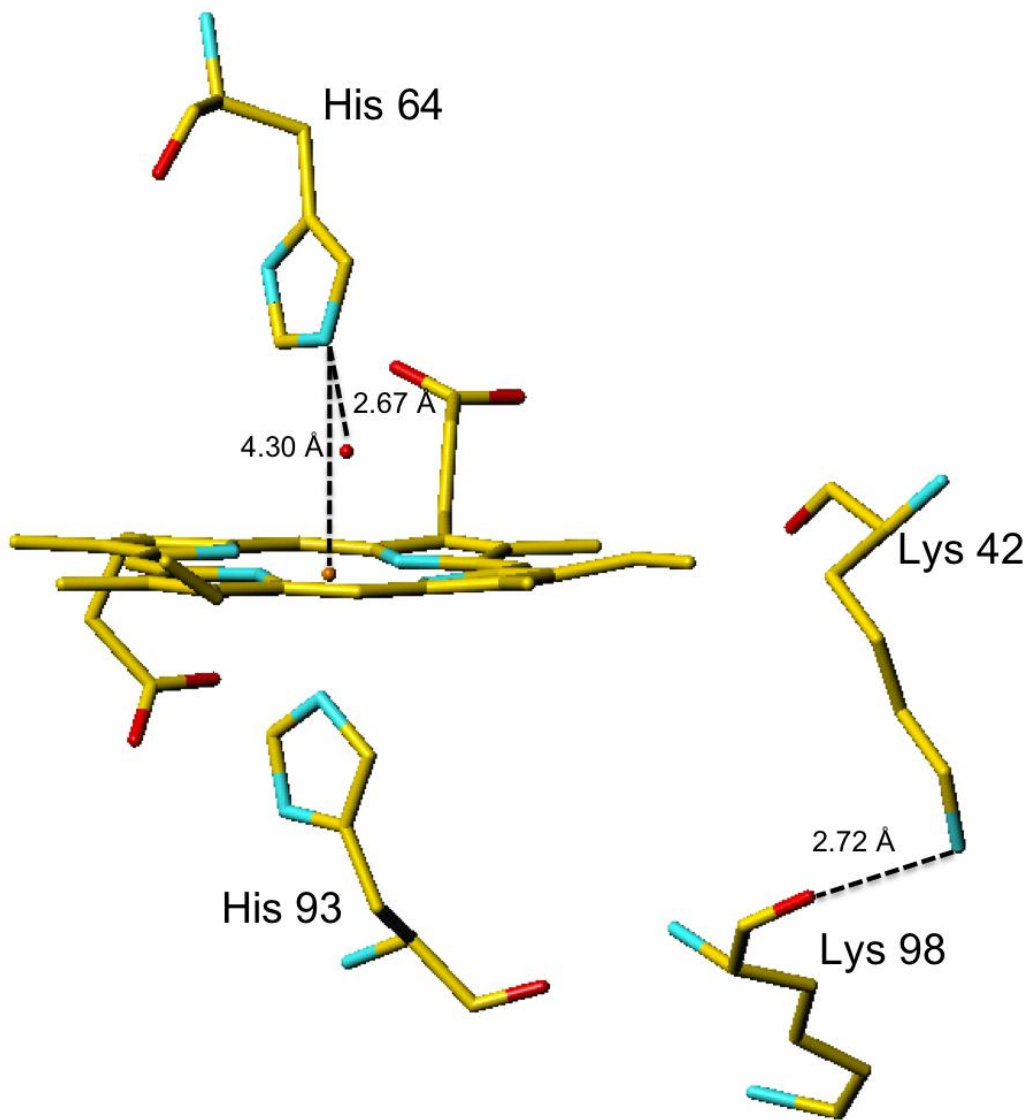
Supplementary material for:

Structures of K42N and K42Y sperm whale myoglobins point to inhibitory role of distal water in peroxidase activity

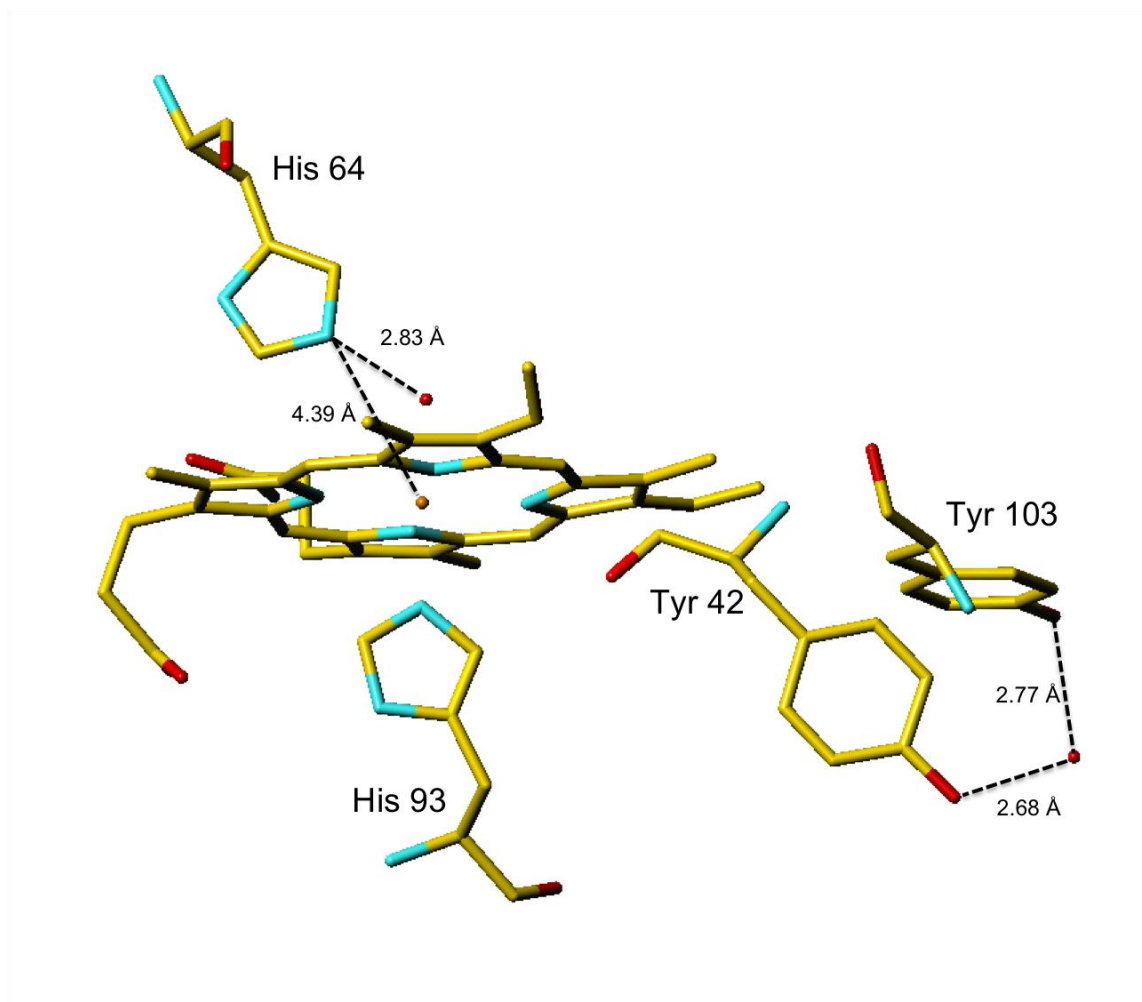
Chunxue Wang, Leslie L. Lovelace, Shenfang Sun, John H. Dawson and Lukasz Lebioda.



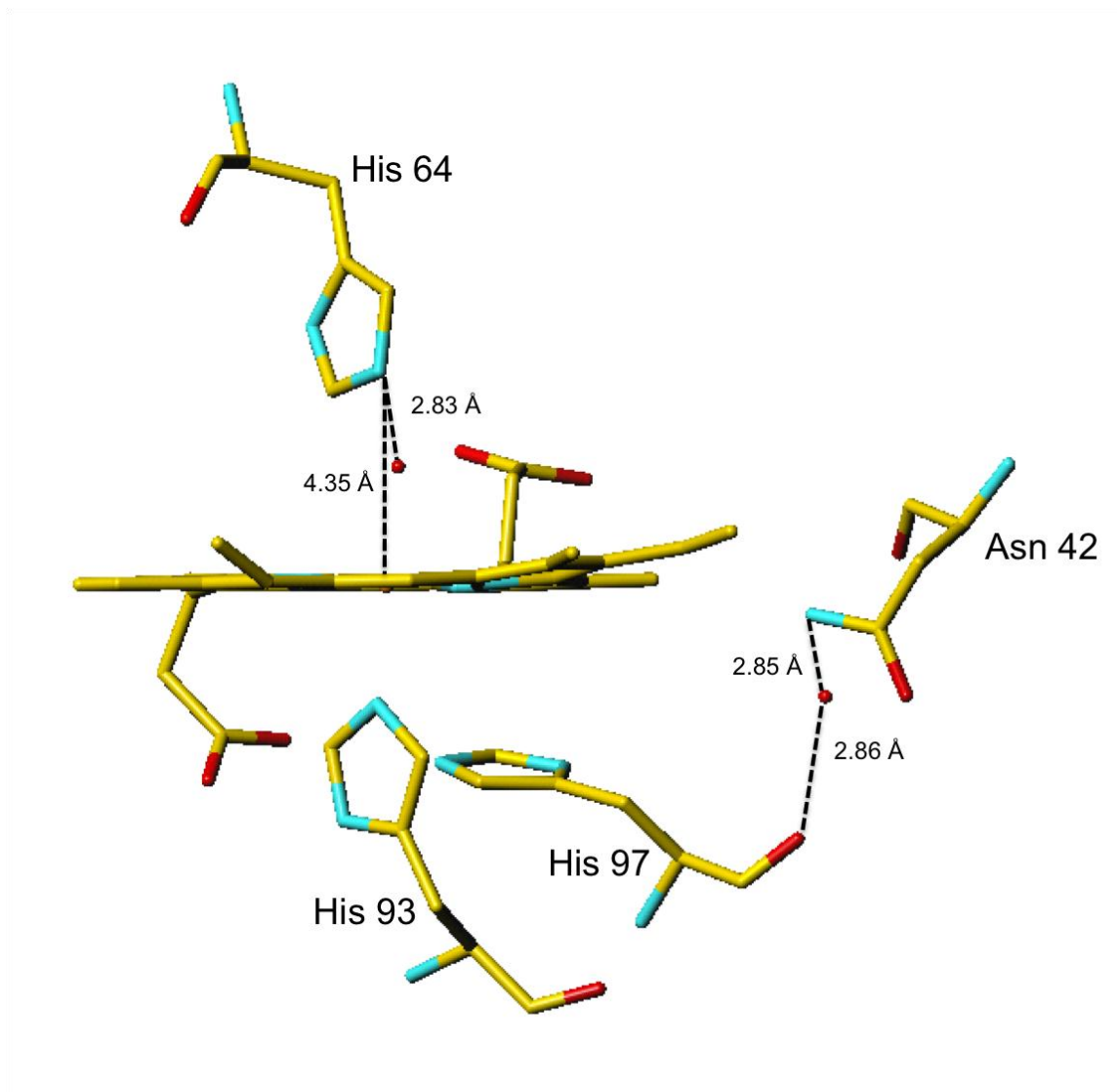
Supplementary Figure S1. Stereoview ribbon diagram of superposition of wild type Mb (green; PDB code: 1A6K), K42Y Mb (pink) and K42N Mb (turquoise). The heme and the mutation site in position 42 are shown in stick.



Supplementary Figure S2. The heme and the environment of Lys42 in Mb.

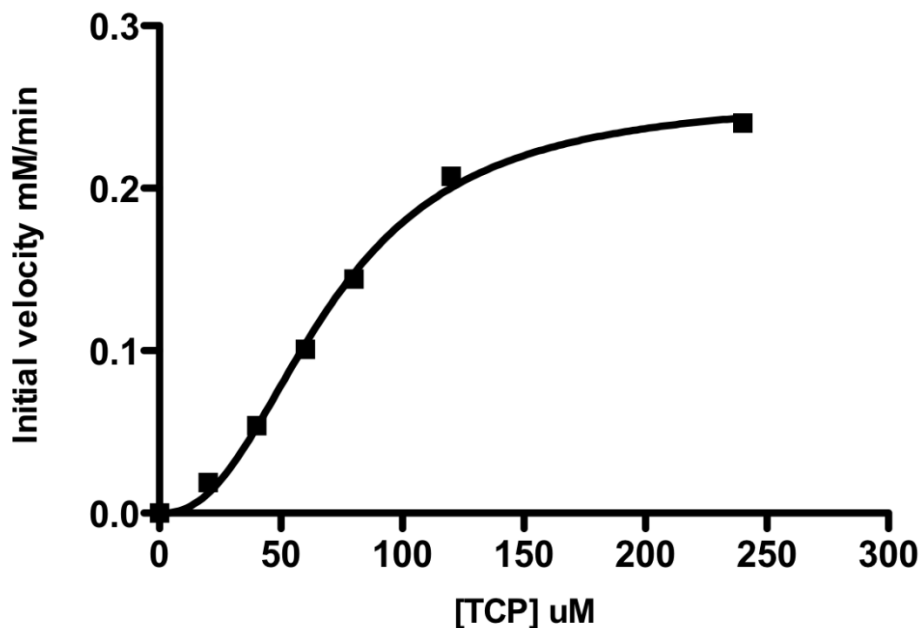


Supplementary Figure S3. The heme and the environment of Tyr42 in K42Y.

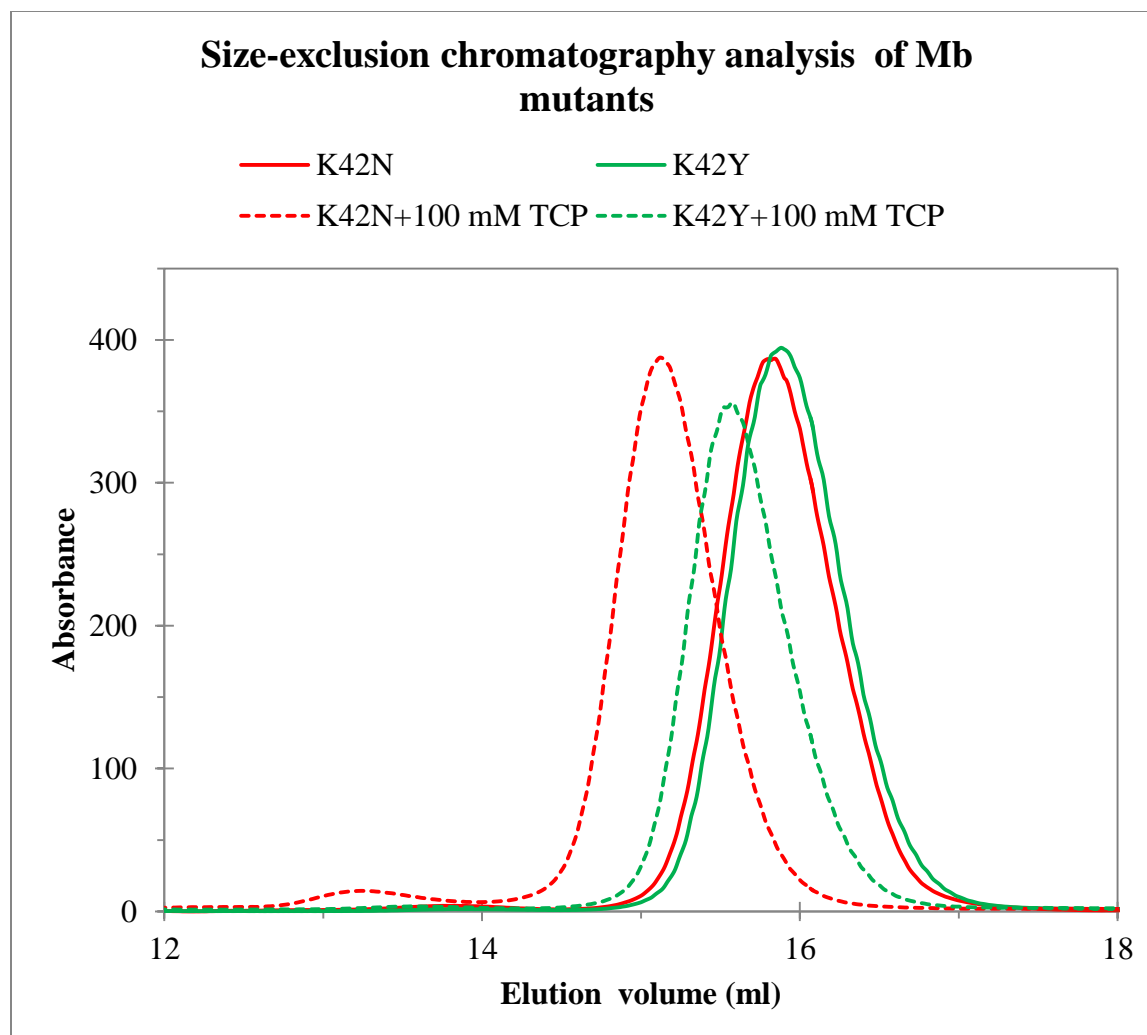


Supplementary Figure S4. The heme and the environment of Asn42 in K42N.

Mb K42N substrate-velocity curve

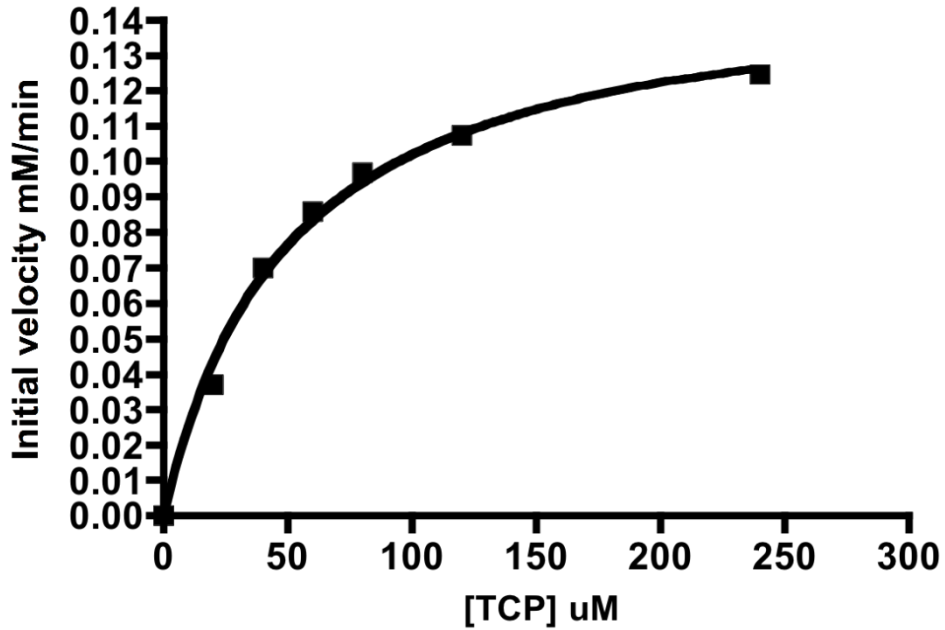


Supplementary Figure S5. The same data as presented in Figure 4 but fitted to the allosteric sigmoidal model with variable Hill coefficient, h . The model, $V=V_{\max}[\text{TCP}]^h/((K_{\text{half}})^h + [\text{TCP}]^h)$, yielded the following values with stds, referring to the last digit, in parentheses: $V_{\max}=0.259$ (9) mM/min, $h=2.4$ (2) $K_{\text{prime}}=(K_{\text{half}})^h = 26$ (20) μM . The considerations limiting the value of h to 2 for a dimer led to the model shown in Figure 4, which has a much lower std. for K_{prime} .

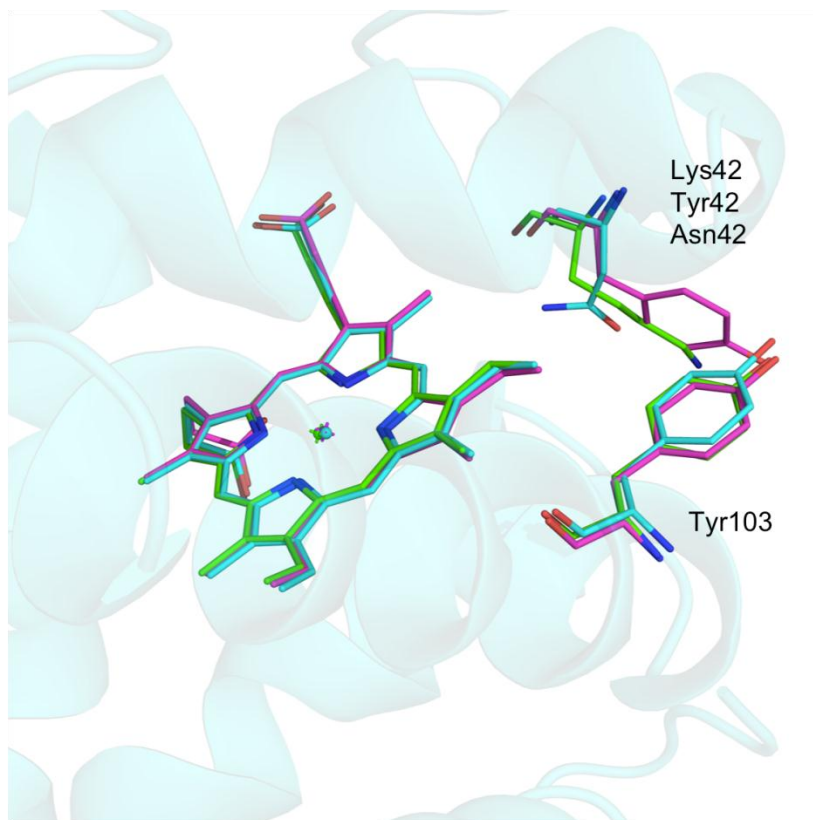


Supplementary Figure S6. Size exclusion chromatography analysis of K42Y and K42N Mb variants in the presence or absence of 100 mM TCP. The analysis was conducted using Superdex 75 and proteins were eluted with 20 mM Tris pH 8.0.

Mb K42Y substrate-velocity curve



Supplementary Figure 7. The dehaloperoxidase kinetics of K42Y Mb as a function of TCP concentration in 50 mM citrate buffer (pH 5.4) at 4 °C. The curve represents the best-fit to the Michaelis - Menten model with parameters $V_{\text{max}} = 0.153 (6) \text{ mM/min}$, $K_{\text{m}} = 50 (6) \mu\text{M}$.



Supplementary Figure S8. Spatial position relationship of Tyr 103 and the amino acid at position 42. Both of them are located at the surface of the protein.

Supplementary Table S1. DHP site-directed mutagenesis primers

Name	Sequence (5'-3')
K42N Mb	CCG GAA ACT CTG GAA AAT TTC GAT CGT TTC AAA
K42Y Mb	CCG GAA ACT CTG GAA TAT TTC GAT CGT TTC AAA