Supporting Information

Structural and Oxygen Binding Properties of Dimeric Horse Myoglobin

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Table S1 Statistics of data collection and structure refinement.

Data collection		
X-ray source	SPring-8 (BL44XU)	
Wavelength (Å)	0.800	
Space group	$P2_12_12_1$	
Unit cell parameters		
a, *b*, *c* (Å)	57.3, 62.5, 83.4	
Resolution (Å)	20-1.05 (1.07-1.05)	
Total reflections	601280	
Number of unique reflections	139129 (6909)	
R_{merge} [a]	0.065 (0.450)	
Completeness (%)	100.0 (99.5)	
$\langle I/\sigma(I)\rangle$	13.2 (3.1)	
Redundancy	4.3 (3.5)	
Refinement		
Resolution (Å)	20-1.05	
Number of reflections	133007	
R_{cryst} [b] (%) (F > 0σ)	12.8	
R_{free} [b] (%)	16.8	
Number of observations per parameter	4.5	
Number of atoms in an asymmetric unit		
Protein	2398	
Water	639	
Heme	86	
Deviations from ideal geometry	RMSD	target σ
Bond distance (Å)	0.014	0.020
Angle distances (Å)	0.030	0.040
Chiral volumes (Å^3)		
C sp^3	0.078	0.100
C sp^2	0.088	0.100

Anisotropic displacement parameters	RMSD	target σ
DELU (Å^2)	0.005	0.010
SIMU (Å^2)	0.046	0.135
ISOR (Å^2)	0.098	0.100

Mean isotropic equivalent B-factor (Å^2)	
Main-chain atoms	11.5
Side-chain atoms	19.8
Heme atoms	9.8
Water atoms	28.8

Ramachandran plot (%)	
Favored	97.7
Allowed	2.3

Statistics for the highest-resolution shell are given in parentheses.

[a] $R_{merge}=\Sigma_{hkl} \mid I - \langle I \rangle \mid (\Sigma_{hkl} \mid I \mid)^{-1}$.

[b] $R_{cryst}=\Sigma_{hkl} \mid \mid F_{obs} \mid - k \mid F_{calc} \mid \mid (\Sigma_{hkl} \mid F_{obs} \mid)^{-1}$, k: scaling factor. R_{free} was computed identically, except where all reflections belong to a test set of 5% of randomly selected data.

Table S2 Root-mean-square deviation (rmsd) values of the 1–77 amino acid and the 86-153 amino acid regions between the structures of the monomer and each independent protomer.

	1–77 Amino acid residues	86–153 Amino acid residues
Between the protomers (Å)	0.68	0.38
Between the monomer and each independent protomer (Å)	0.56, 0.77	1.20, 1.22

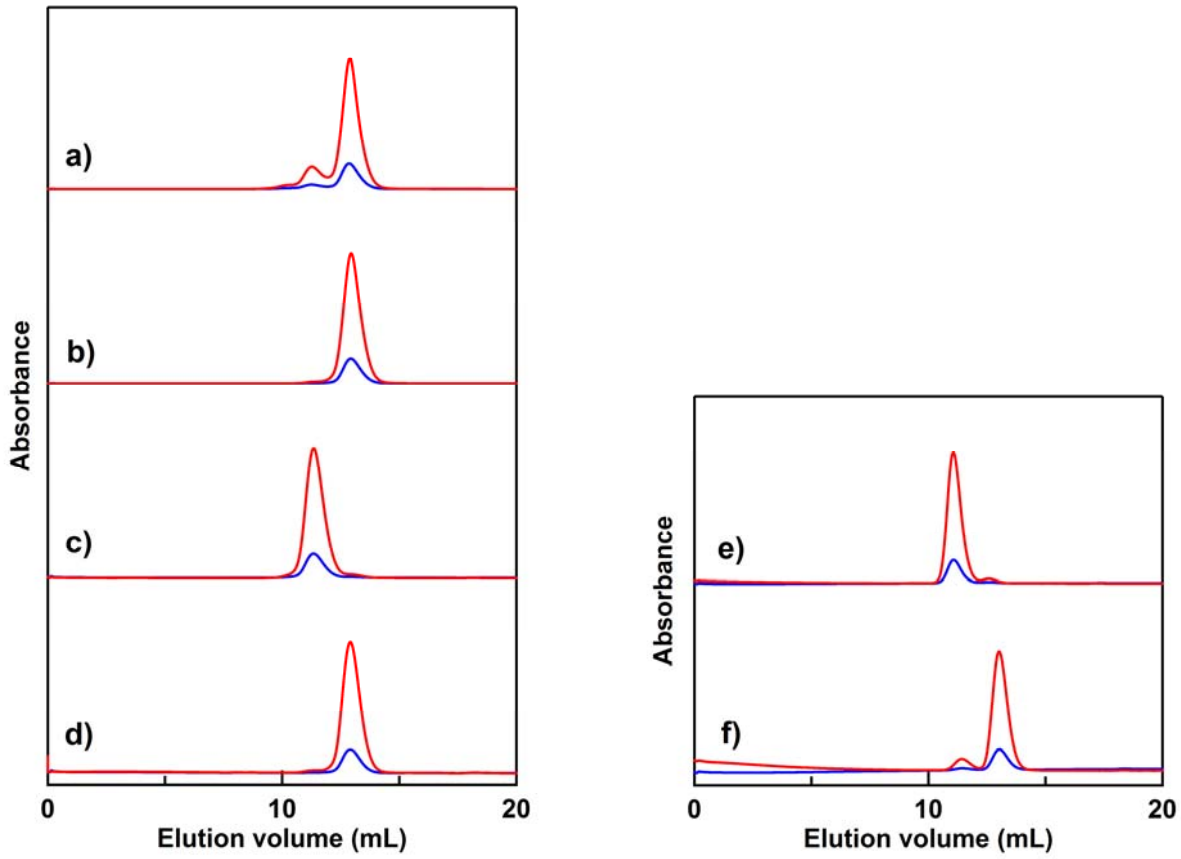

Figure S1 Elution curves of horse metMb. a) Elution curve after incubation under 5% (v/v) ethanol, subsequent lyophilization, and dissolution to buffer; b) elution curve of purified monomeric metMb; c) elution curve of purified dimeric metMb; d) elution curve of dimeric metMb after incubation at pH 7.0 for 5 min at 70ºC; e) elution curve of dimeric metMb after incubation at pH 7.0 for 3 days at 4ºC; f) elution curve of dimeric metMb after incubation at pH 5.0 for 15 min at 4ºC. Measurement conditions: column: Superdex 75 10⁄300 GL; flow rate: 0.2 mL⁄ min; monitoring wavelength: 409 (red) and 280 nm (blue); solvent: 50 mM potassium phosphate buffer; pH: 7.0; temperature: 5 °C.

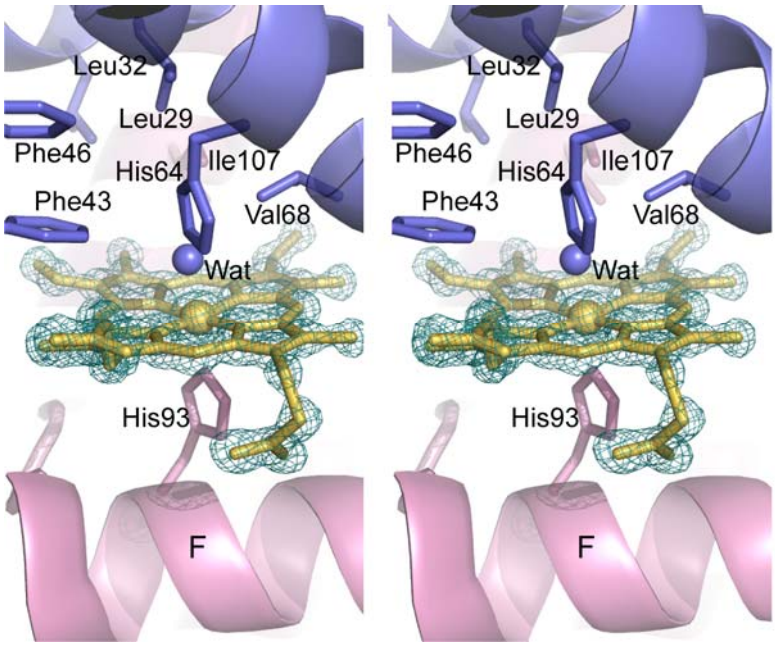

Figure S2 Crystal structure of dimeric horse Mb with a 2*F*o-*F*c omit electron density map of the heme. The Heme is shown as a yellow stick model. The 2*F*o-*F*c electron density map is contoured at 2σ and shown as a green mesh. Side-chain atoms of His64 and His93 (labeled as H64 and H93) and amino acids in the heme pocket are shown as stick models with labels.

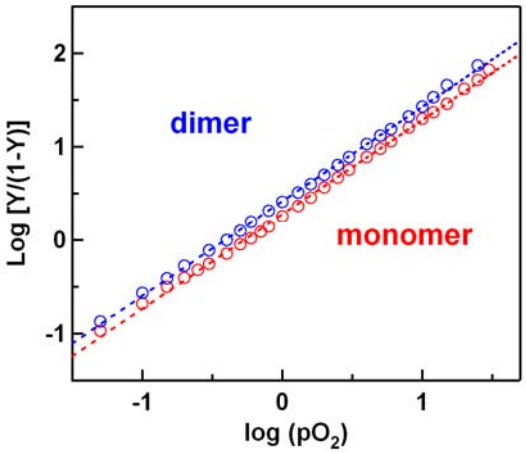

Figure S3 Hill plots of monomeric and dimeric horse Mbs for oxygen binding. pO_2 and Y represent the partial oxygen pressure (mmHg) and the fractional oxygen saturation of Mb, respectively. Best-fitted linear lines are indicated by broken lines for monomeric (red) and dimeric (blue) Mbs. Measurement conditions are the same as those in Fig. 6.