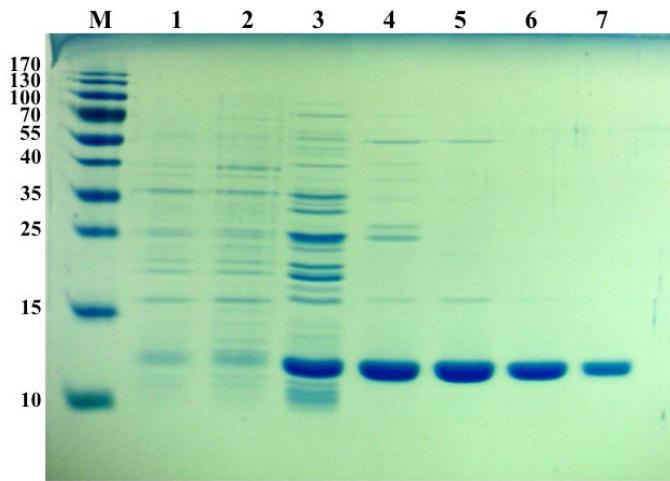
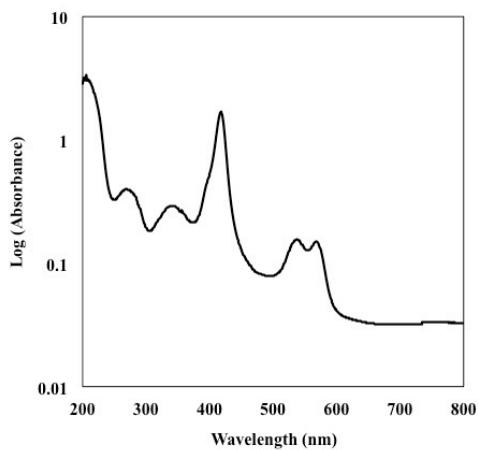


Supplementary Material



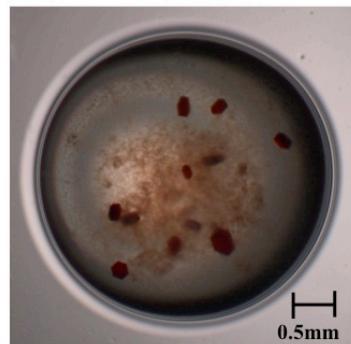
Supplementary Fig. S1.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) image showing the progress of purification. The quality of the sample was analyzed by SDS-PAGE. The band between 10 kD and 15 kD protein markers was used as an indicator of purity. The post-dialysis lysate fraction from the previous experiment was loaded to ensure the expression of hemoglobin. The sample fractions were as follows; M, the protein ladder (Thermo Fisher Scientific, Waltham, USA); 1, post-dialysis crude lysate from previous experiment; 2, post-dialysis crude lysate; 3, post Q-chromatography fraction; 4, post SP-chromatography 1st fraction; 5, post SP-chromatography 8th fraction; 6, post SP-chromatography 16th fraction; 7, post SP-chromatography 22nd fraction.



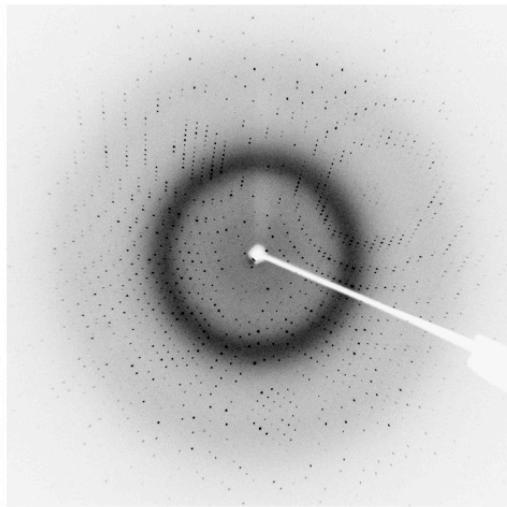
Supplementary Fig. S2.

UV-Visible spectrum of purified deer mouse hemoglobin. The ratio of the absorbance at 418 nm (Soret) and 280 nm (protein) was calculated for the quality analysis. Since oxygenated heme protein gave two peaks at 541 nm and 577 nm, the absorbance values at these two peaks were used for the estimation of hemoglobin concentration.



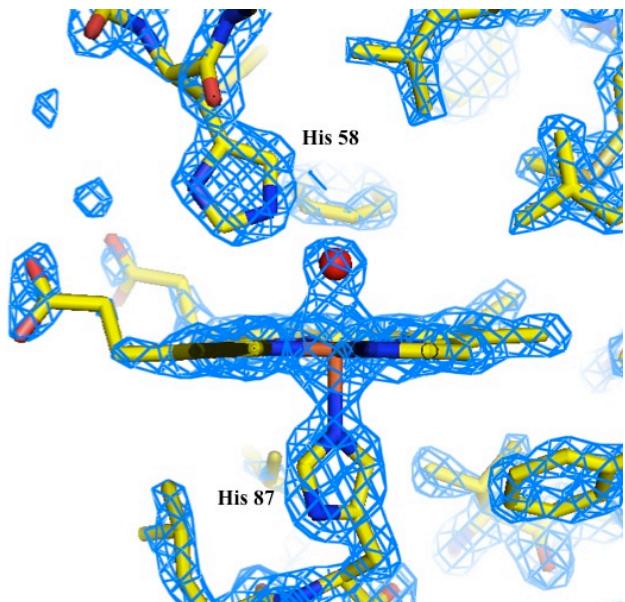
Supplementary Fig. S3.

Deer mouse hemoglobin crystals. A solution of deer mouse hemoglobin with an initial concentration of 0.2 mM heme was equilibrated against 1.0 ml 28% (w/v) PEG 3350 in 50 mM sodium-potassium phosphate buffer (pH 8.0).



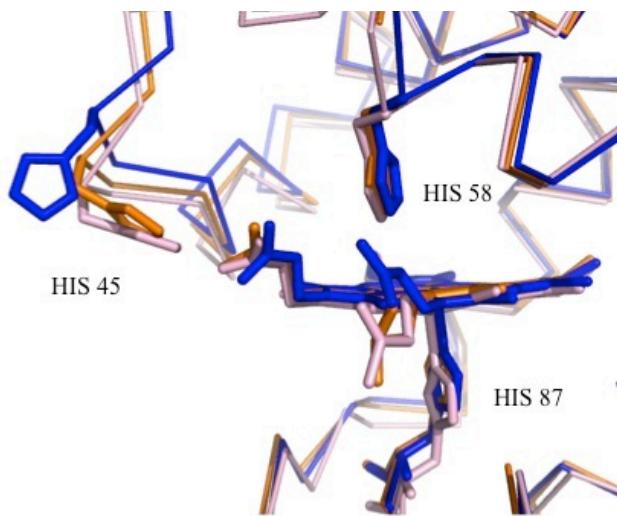
Supplementary Fig. S4.

X-ray diffraction image from the deer mouse hemoglobin crystal. The size of the detector was 300 mm × 300 mm.



Supplementary Fig. S5.

Sigma-A-weighted $2Fo - Fc$ electron density map of heme of α subunit in deer mouse hemoglobin. The contour level is 2.0σ , and a red sphere indicates the oxygen atom in the bound water molecule.



Supplementary Fig. S6.

Superimposed coordinates of $\alpha 1\text{HIS}45$ between deer mouse and human hemoglobin. Superimposed structures of deer mouse hemoglobin (blue), and of human hemoglobin in the deoxy (light pink) and oxy (orange) states, centered on $\alpha 1\text{HIS}45$.

Supplementary Table S1

Interface analysis of deer mouse hemoglobin and three forms of human hemoglobin

		Deer mouse	Human		
		4H2L	Deoxy 2DN2	Oxy 2DN1	CO 2DN3
$\alpha 1 - \beta 2$ (Å)					
Hydrogen bonds					
$\alpha 1$ LYS40 NZ	$\beta 2$ HIS146OXT	-	2.83	-	-
$\alpha 1$ THR41 OG1	$\beta 2$ ARG40 NH2	3.34	-	2.49	2.7
$\alpha 1$ TYR42 OH	$\beta 2$ ASP 99 OD1	-	2.51	-	-
$\alpha 1$ LEU91 O	$\beta 2$ ARG40 NE	-	2.91	-	-
$\alpha 1$ LEU91 O	$\beta 2$ ARG40 NH2	-	2.97	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ PRO36 O	-	-	2.83	3.15
$\alpha 1$ ARG92 NH1	$\beta 2$ GLN39 O	3.60	-	-	-
$\alpha 1$ ARG92 NH1	$\beta 2$ ASP 43 OD1	3.05	-	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ ASP 43 OD1	3.16	-	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ ASP 43 OD2	3.03	-	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ GLU43 OE2	-	2.49	-	-
$\alpha 1$ ASP 94 OD1	$\beta 2$ TRP 37 NE1	-	2.85	-	-
$\alpha 1$ ASP 94 OD2	$\beta 2$ ASN102ND2	2.76	-	2.83	2.74
$\alpha 1$ ASN97 ND2	$\beta 2$ ASP 99 OD1	-	2.85	-	-
$\alpha 1$ ARG141NH1	$\beta 2$ VAL34 O	-	2.97	-	-
$\alpha 1$ ARG141NH2	$\beta 2$ VAL34 O	3.27	-	-	-
Salt bridges					
$\alpha 1$ ARG92 NH1	$\beta 2$ ASP 43 OD1	3.05	-	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ ASP 43 OD1	3.16	-	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ ASP 43 OD2	3.03	-	-	-
$\alpha 1$ ARG92 NH1	$\beta 2$ GLU43 OE2	-	3.37	-	-
$\alpha 1$ ARG92 NH2	$\beta 2$ GLU43 OE2	-	2.49	-	-
$\alpha 1 - \alpha 2$ (Å)					
Hydrogen bonds					
$\alpha 1$ VAL 1 N	$\alpha 2$ SER 138 O	3.57	-	-	-
$\alpha 1$ VAL 1 N	$\alpha 2$ ARG 141 O	3.22	-	-	-
$\alpha 1$ VAL 1 N	$\alpha 2$ ARG 141OXT	2.99	-	-	-
$\alpha 1$ ASP 6 OD1	$\alpha 2$ ARG141 NH1	3.61	-	-	-
$\alpha 1$ ALA123 O	$\alpha 2$ ARG141 NH2	2.64	-	-	-
$\alpha 1$ ASP 126 OD2	$\alpha 2$ ARG141 NH1	-	2.66	-	-
$\alpha 1$ LYS 127 NZ	$\alpha 2$ ARG141OXT	-	2.77	-	3.05*
$\alpha 1$ SER 138 O	$\alpha 2$ VAL1 N	3.57	-	-	2.58
$\alpha 1$ ARG141 O	$\alpha 2$ VAL1 N	3.22	-	-	-
$\alpha 1$ ARG141 OXT	$\alpha 2$ VAL1 N	2.99	-	-	-

α1 ARG141 NH1	α2 ASP 6 OD1	3.61	-	-	-
α1 ARG141 NH2	α2 ALA123 O	2.64	-	-	-
α1 ARG141 NH1	α2 ASP 126 OD2	-	2.78	-	-
α1 ARG141 NH2	α2 ASP 126 OD2	-	2.95	-	-
α1 ARG141OXT	α2 LYS 127 NZ	-	2.81	-	3.05*
α1 ARG141 O	α2 LYS 127 NZ	-	-	-	-

Salt bridges

α1 ASP 6 OD1	α2 ARG141 NH1	3.61	-	-	-
α1 ASP126 OD2	α2 ARG141 NH1	-	2.66	-	-
α1 ASP126 OD2	α2 ARG141 NH2	-	3.05	-	-
α1ARG141 NH1	α2 ASP 6 OD1	3.61	-	-	-
α1ARG141 NH1	α2 ASP 126 OD2	-	2.78	-	-
α1ARG141 NH2	α2 ASP 126 OD2	-	2.95	-	-

α1 - β1 (Å)**Hydrogen bonds**

α1 ARG31 NH1	β1 PHE122 O	2.98	3.01	2.82	3.10
α1 ARG31 NH2	β1 PHE122 O	2.81	2.76	3.05	2.80
α1 ARG31 NH1	β1 GLN127 OE1	2.99	2.94	-	2.90
α1 ARG31 NH2	β1 GLN127 OE1	-	-	2.92	-
α1 CYS34 O	β1 SER 128 OG	3.31	-	-	-
α1 HIS 103 NE2	β1 GLN131 OE1	2.79	2.76	2.75	2.75
α1 CYS104 SG	β1 GLN127 NE2	3.75	3.89	-	3.85
α1 PRO114 O	β1 HIS 116 NE2	2.81	2.78	2.69	2.78
α1 PHE117 O	β1 ARG30 NH1	-	-	-	3.24
α1 PHE117 O	β1 ARG30 NH2	2.74	2.79	2.85	2.92
α1 THR 118 O	β1 ARG 30 NH1	3.36	-	-	-
α1 ASP126 OD1	β1 TYR35 OH	3.85	-	-	-
α1 ASP126 OD2	β1 TYR35 OH	-	3.22	3.69	3.43

β1 - β2 (Å)**Hydrogen bonds**

β1 VAL1 N	β2 HIS 146 OXT	-	-	-	2.82
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Subunit interface analyses of deer mouse hemoglobin and three forms of human hemoglobin (deoxy, oxy, and carbon monoxy) were estimated by PISA.

Asterisk (*) indicates that the α1 ARG141OXT atom used for the distance calculation was missing connections to any neighboring atoms.