Electronic Supplementary Information

Genetically engineered haemoglobin wrapped covalently with human serum albumins as an artificial O_2 carrier

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Results

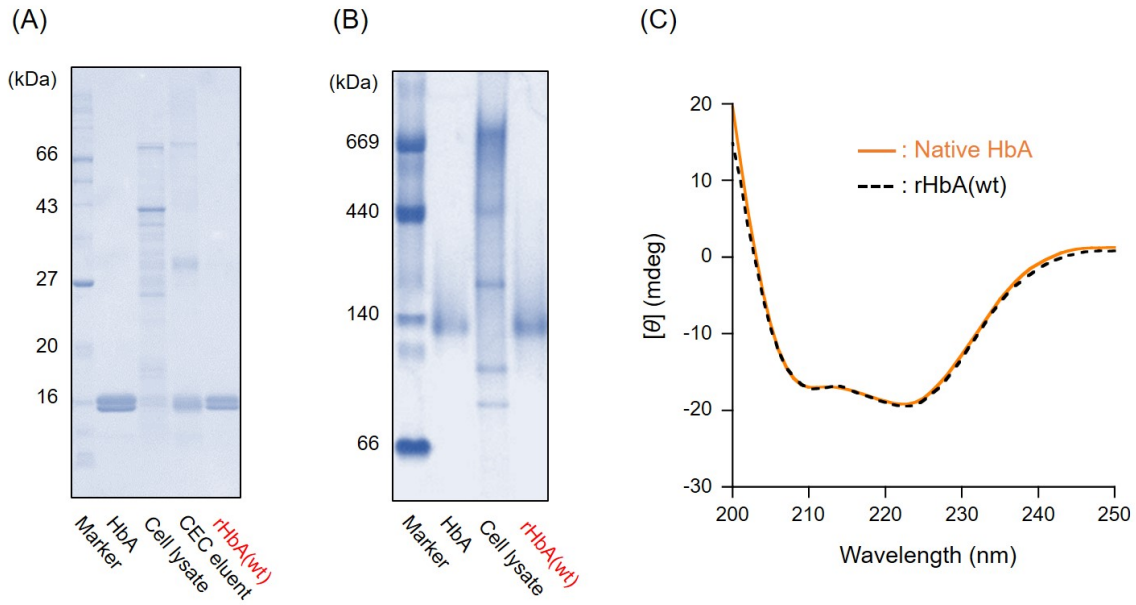

Fig. S1 (A) SDS-PAGE and (B) Native-PAGE patterns of cell lysate and purified rHbA(wt). (C) CD spectra of native HbA and rHbA(wt) (0.2 μM) in PBS solution (pH 7.4) at 25 °C .

Table S1 MALDI-TOF mass spectral data of rHbA(wt) and its mutants.

Haemoglobin	α chain [Da]		β chain [Da]	
	Found	Calcd.	Found	Calcd.
rHbA(wt)	15128	15126	15869	15867
rHbA(F)	15128	15126	15904	15901
rHbA(W)	15129	15126	15943	15940
rHbA(YQ)	15127	15126	15909	15908

Table S2 UV-vis. absorption spectral data of native HbA, rHbA(wt), its mutants, and these clusters at 25 ºC.

Haemoproteins	λ_{max} (nm)		
	oxy	deoxy	carbonyl
Native HbA	414, 541, 577	430, 555	420, 539, 569
rHbA(wt)	415, 541, 576	430, 555	419, 539, 569
rHbA(wt)-rHSA$_3$	414, 541, 576	430, 555	419, 539, 569
rHbA(F)	415, 541, 577	430, 555	419, 539, 569
rHbA(F)-rHSA$_3$	415, 541, 577	430, 556	420, 539, 569
rHbA(W)	415, 541, 577	430, 556	419, 538, 569
rHbA(W)-rHSA$_3$	414, 541, 576	430, 556	419, 538, 569
rHbA(YQ)	414, 540, 576	430, 555	420, 539, 569
rHbA(YQ)-rHSA$_3$	413, 540, 576	430, 556	420, 539, 569

Table S3 X-ray crystallography data collection and refinement statistics of rHbA(wt)

Wavelength (Å)	1.0000
Resolution range (Å)	48.42–2.28 (2.33–2.28)
Space group	$P\ 1\ 2_1\ 1$
Cell dimensions	a = 123.031 Å, b = 55.947 Å, c = 133.156 Å α = 90.00°, β = 98.27°, γ = 90.00°
Total reflections	233642
Unique reflections	80208 (12565)
Multiplicity	2.9 (2.9)
Completeness (%)	97.6 (93.6)
Mean I/sigma (I)	5.4 (0.7)
R_{meas}	0.17 (1.984)
R_{pim}	0.095 (1.116)
CC(1/2)	0.993 (0.338)
Refinement	
Resolution range (Å)	48.42–2.28
No. reflections	80196
R_{work} / R_{free}	0.226 / 0.268
No. atoms	
Amino acids	13085
Ligands	540
Waters	139
B-factors	
Amino acids	54.41
Ligands	49.46
Waters	43.19
RMS (bonds)	0.0039
RMS (angles)	1.337
Ramachandran favored (%)	95.98
Ramachandran outliers (%)	0.18
Clashscore	6.28

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

Table S4 List of primers used for construction of rHbA(X) (X= F, W, YQ).

Primer name	Sequence
βL28F forward	5’-GATGAAGTGGGTGGTGAAGCA**TTT**GGTCGTCTGCTGGTAGTG-3’
βL28F reverse	5’-CACTACCAGCAGACGACC**AAA**TGCTTCACCACCCACTTCATC-3’
βL28W forward	5’-GATGAAGTGGGTGGTGAAGCA**TGG**GGTCGTCTGCTGGTAGTG-3’
βL28W reverse	5’-CACTACCAGCAGACGACC**CCA**TGCTTCACCACCCACTTCATC-3’
βL28Y forward	5’-GATGAAGTGGGTGGTGAAGCA**TAC**GGTCGTCTGCTGGTAGTG-3’
βL28Y reverse	5’-CACTACCAGCAGACGACC**GTA**TGCTTCACCACCCACTTCATC-3’
βH63Q forward	5’-CCCAAGGTCAAAGCA**CAA**GGGAAGAAAGTCCTGGGCGCG-3’
βH63Q reverse	5’-CGCGCCCAGGACTTTCTTCCC**TTG**TGCTTTGACCTTGGG-3’