Electronic Supplementary Information

Core–shell protein clusters comprising haemoglobin and recombinant feline serum albumin as an artificial O_2 carrier for cats

Kyoko Yokomaku,[a] Motofusa Akiyama,[a] Yoshitsugu Morita,[a] Kiyohito Kihira[b] and Teruyuki Komatsu*[a]

[a] *Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan.*

[b] *JEM Utilization Center, Human Spaceflight Technology Directorate, Japan Aerospace Exploration Agency (JAXA), 2-1-1 Sengen, Tsukuba-shi, Ibaraki 305-8505, Japan.*

Corresponding Author: Prof. Dr. Teruyuki Komatsu

E-mail: komatsu@kc.chuo-u.ac.jp

Experimental

Purification of native FSA from feline plasma

Feline plasma was provided from Kyoritsu Seiyaku Corp. The frozen sample was thawed slowly in a refrigerator overnight at 4 °C and was centrifuged (10,000 × *g*, 30 min, 4 °C) to remove the cryoprecipitate. The supernatant was filtered through a track-etched polycarbonate membrane (Isopore membrane, 25 mm diameter, 50 nm pore; Millipore Corp.) to remove endogenous retroviruses. The filtrate was then brought to 50% saturation with ammonium sulfate. After leaving for 30 min at 4 °C, the solution was centrifuged (10,000 × *g*, 30 min, 2 °C) and the supernatant was filtered using a membrane filter (DISMIC-25CS, 0.2 μm pore; Toyo Roshi Kaisha Ltd.). The obtained solution was dialyzed against deionized water at 4 °C with subsequent addition of 11% volume of 500 mM sodium phosphate (pH 6.8). The resultant solution in 50 mM sodium phosphate (pH 7.0) was filtered using a membrane filter (C020A047A, 0.2 μm pore; Toyo Roshi Kaisha Ltd.).

The sample was applied to affinity chromatography (Toyopearl AF-Blue HC-650M; Tosoh Corp.). After washing with 50 mM sodium phosphate (pH 7.0), FSA was eluted with 50 mM sodium phosphate (pH 7.4) containing 3 M NaCl. The eluent was dialyzed against deionized water at 4 °C. Thereafter, 25% volume of 100 mM Tris-HCl (pH 8.0) was added, and the resulting 20 mM Tris-HCl solution (pH 8.0) of FSA was filtered using a membrane filter (C020A047A, 0.2 μm pore). Then the sample was subjected to anion exchange chromatography (Q Sepharose Fast Flow; GE Healthcare UK Ltd.) with 20 mM Tris-HCl (pH 8.0) as the running buffer. After washing with 20 mM Tris-HCl (pH 8.0) containing 100 mM NaCl, elution of FSA was performed with 20 mM Tris-HCl (pH 8.0) containing 300 mM NaCl. The eluent was dialyzed against deionized water at 4 °C, followed by addition of 11% volume of 10× phosphate-buffered saline (PBS, pH 7.4). At the last, the FSA solution was concentrated to 30 mL, and was sterilized with a membrane filter (DISMIC-25CS, 0.2 μm pore). All the purification processes were confirmed by SDS-PAGE analysis. The concentration of FSA was measured using a protein assay kit (Pierce 660 nm; Thermo Fisher Scientific K.K.). The cysteinyl thiol assay of CSA was performed by reaction with 4,4'-dithiopyridine (4,4'-DTP).[48]

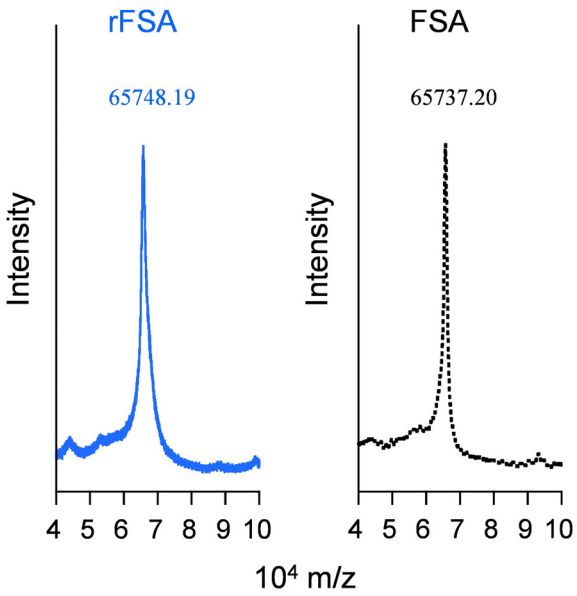

Fig. S1. MALDI-TOF MS spectra of rFSA and FSA.

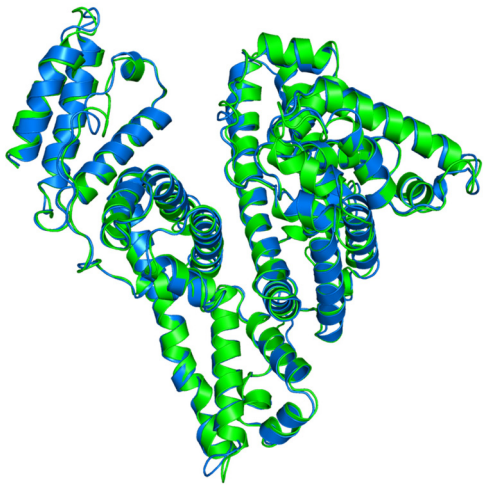

Fig. S2. Superposition of crystal structures of rFSA (light blue) and rHSA (light green, PDB ID: 1E78).[36]

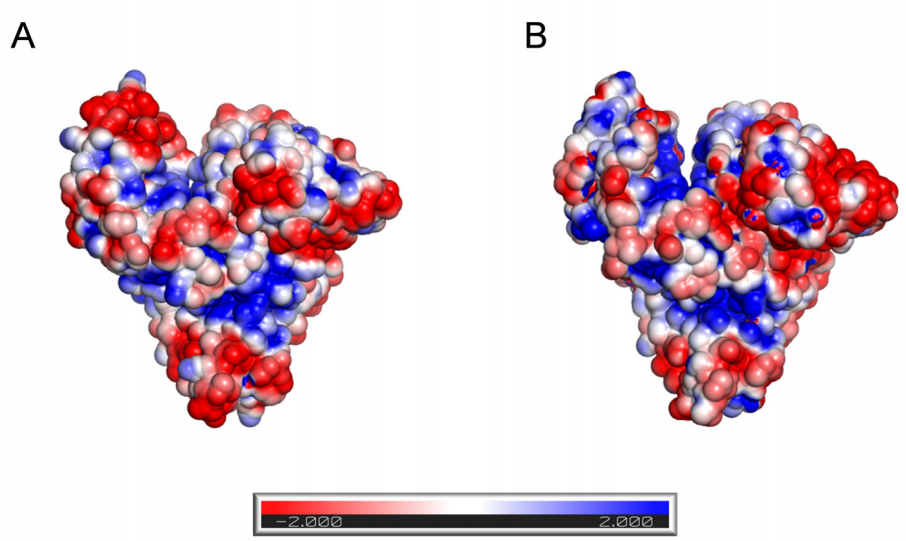

Fig. S3. Surface electrostatic potential representations of (A) rFSA and (B) rHSA. Blue and red respectively represent positive charge and negative charge density. Calculations were carried out using Adaptive Poisson-Boltzmann Solver (APBS) and PyMOL. The pdb files were converted to pqr files for APBS electrostatics calculations by PDB2PQR service.[S1] (PDB ID of rHSA: 1E78)[36]

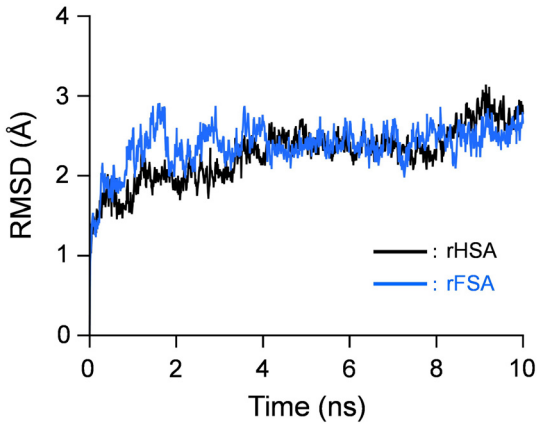

Fig. S4. Time evolution of the RMSD of Cα atoms from the starting structure during 10 ns MD simulations of rFSA and rHSA. RMSD values reached plateau at about 4 ns. (PDB ID of rHSA: 1AO6)[37]

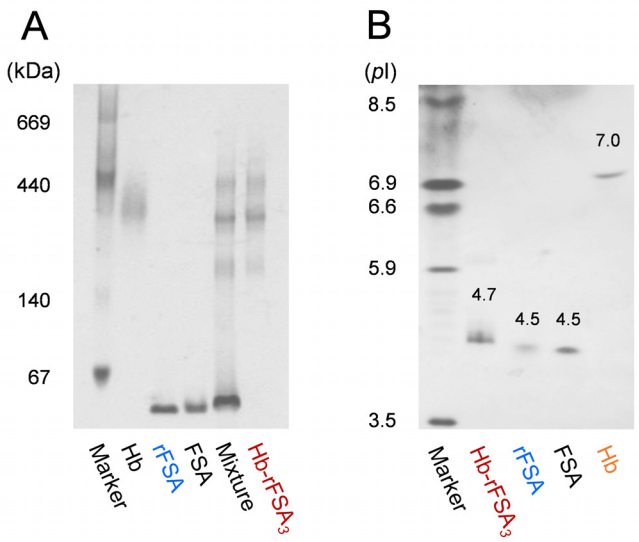

Fig. S5. (A) Native-PAGE and (B) IEF of Hb-rFSA$_3$ cluster.

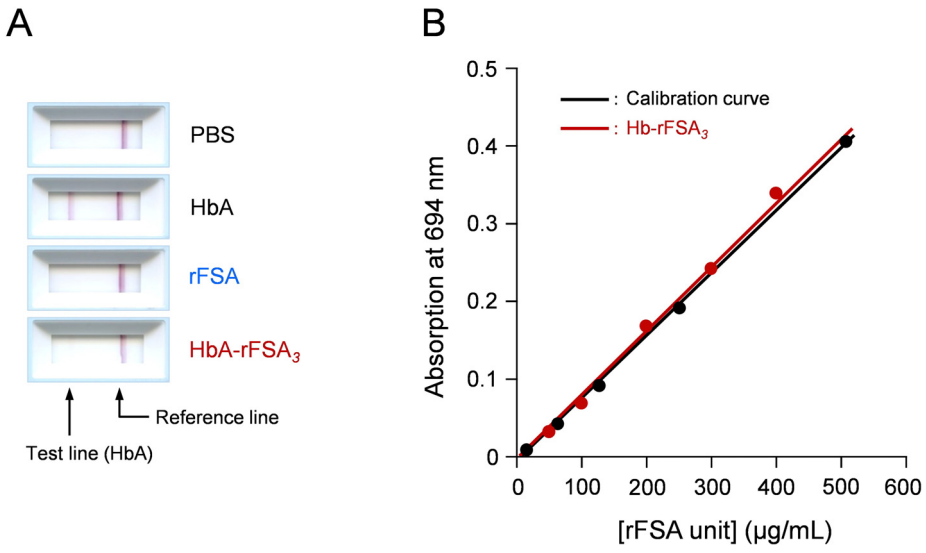

Fig. S6. (A) Immunological reactivity of Hb-rFSA$_3$ cluster against anti-HbA antibody. The result of quick chaser occult blood test kit. (B) Immunological reactivity of Hb-rFSA$_3$ cluster against anti-FSA antibody. The relation between FSA unit concentration of the sample and absorption intensity of the reactant solution at 694 nm.

Table S1 Comparison of amino acid sequences of FSA and HSA. The first row represents FSA sequence (colors correspond to the subdomain colors in Figure 2) and the second row represents HSA sequence (black). The yellow-marked amino acids are different kind pairs between FSA and HSA. The homology of these proteins is 81.9%.

	1	11	21	31	41
FSA	EAHQSEIAHR	FNDLGEEHFR	GLVLVAFSQY	LQQCPFEDHV	KLVNEVTEFA
HSA	DAHKSEVAHR	FKDLGEENFK	ALVLIAFAQY	LQQCPFEDHV	KLVNEVTEFA
	51	61	71	81	91
	KGCVADQSAA	NCEKSLHELL	GDKLCTVASL	RDKYGEMADC	CEKKEPERNE
	KTCVADESAE	NCDKSLHTLF	GDKLCTVATL	RETYGEMADC	CAKQEPERNE
	101	111	121	131	141
	CFLQHKDDNP	GFGQLVTPEA	DAMCTAFHEN	EQRFLGKYLY	EIARRHPYFY
	CFLQHKDDNP	NLPRLVRPEV	DVMCTAFHDN	EETFLKKYLY	EIARRHPYFY
	151	161	171	181	191
	APELLYYAEE	YKGVFTECCE	AADKAACLTP	KVDALREKVL	ASSAKERLKC
	APELLFFAKR	YKAAFTECCQ	AADKAACLLP	KLDELRDEGK	ASSAKQRLKC
	201	211	221	231	241
	ASLQKFGERA	FKAWSVARLS	QKFPKAEFAE	ISKLVTDLAK	IHKECCHGDL
	ASLQKFGERA	FKAWAVARLS	QRFPKAEFAE	VSKLVTDLTK	VHTECCHGDL
	251	261	271	281	291
	LECADDRADL	AKYICENQDS	ISTKLKECCG	KPVLEKSHCI	SEVERDELPA
	LECADDRADL	AKYICENQDS	ISSKLKECCE	KPLLEKSHCI	AEVENDEMPA
	301	311	321	331	341
	DLPPLAVDFV	EDKEVCKNYQ	EAKDVFLGTF	LYEYSRRHPE	YSVSLLLRLA
	DLPSLAADFV	ESKDVCKNYA	EAKDVFLGMF	LYEYARRHPD	YSVVLLLRLA
	351	361	371	381	391
	KEYEATLEKC	CATDDPPACY	AHVFDEFKPL	VEEPHNLVKT	NCELFEKLGE
	KTYETTLEKC	CAAADPHECY	AKVFDEFKPL	VEEPQNLIKQ	NCELFEQLGE
	401	411	421	431	441
	YGFQNALLVR	YTKKVPQVST	PTLVEVSRSL	GKVGSKCCTH	PEAERLSCAE
	YKFQNALLVR	YTKKVPQVST	PTLVEVSRNL	GKVGSKCCKH	PEAKRMPCAE
	451	461	471	481	491
	DYLSVVLNRL	CVLHEKTPVS	ERVTKCCTES	LVNRRPCFSA	LQVDETYVPK
	DYLSVVLNQL	CVLHEKTPVS	DRVTKCCTES	LVNRRPCFSA	LEVDETYVPK
	501	511	521	531	541
	EFSAETFTFH	ADLCTLPEAE	KQIKKQSALV	ELLKHKPKAT	EEQLKTVMGD
	EFNAETFTFH	ADICTLSEKE	RQIKKQTALV	ELVKHKPKAT	KEQLKAVMDD
	551	561	571	581	
	FGSFVDKCCA	AEDKEACFAE	EGPKLVAAAQ	AALA	
	FAAFVEKCCK	ADDKETCFAE	EGKKLVAASQ	AALGL	

Table S2 X-ray crystallography data collection and refinement statistics of rFSA

Wavelength (Å)	1.0000
Resolution range (Å)	46.8–3.402 (3.524–3.402)
Space group	C 1 2 1
Cell dimensions	a = 106.908 Å, b = 49.145 Å, c = 123.869 Å α = 90°, β = 110.171°, γ = 90°
Total reflections	53018
Unique reflections	15776 (2644)
Multiplicity	3.4 (3.1)
Completeness (%)	96.8 (94.0)
Mean I/sigma (I)	3.72 (0.82)
B-factor (Å^2)	114.12
R-merge	0.252 (1.434)
Refinement	
Resolution range (Å)	46.8–3.2 (3.524–3.402)
No. reflections	8299 (788)
R-work	0.2859 (0.5042)
R-free	0.2967 (0.4432)
No. non-hydrogen atoms	4391
Macromolecules	4391
RMS (bonds)	0.002
RMS (angles)	0.49
Ramachandran favored (%)	93.46
Ramachandran outliers (%)	1.24
Clashscore	4.91
Average B-factor	115.07

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

Table S3 UV-visible absorption spectral data of Hb-rFSA$_3$ and Hb-FSA$_3$ clusters in PBS solution (pH 7.4) at 25 °C

	λ_{max} (nm)		
Hemoproteins	oxy	deoxy	carbonyl
Hb-rFSA$_3$	414, 541, 577	429, 556	419, 538, 569
Hb-FSA$_3$	414, 541, 577	429, 556	419, 538, 569
Hb-HSA$_3$ [a]	413, 541, 577	430, 556	420, 538, 569
Hb [a]	414, 541, 577	430, 555	420, 538, 569
HbA [b]	415, 541, 577	430, 555	419, 540, 569

[a] From ref. 25. [b] Ref. 42.

Reference

S1. T. K. Dolinsky, J. E. Nielsen, J. A. McCammon and N. A. Baker, *Nucleic Acids Res.*, 2004, **32**, W665–W667.