STRUCTURAL BIOLOGY

Volume 77 (2021)

Supporting information for article:

Structural studies of hemoglobin from two flightless birds, ostrich and turkey: insights into differing oxygen-binding properties

Pandian Ramesh, Selvarajan Sigamani Sundaresan, Nagaraj Shobana, Thangaraj Vinuchakkaravarthy, Kandasamy Sivakumar, Sayed Yasien and Mondikalipudur Nanjappa Gounder Ponnuswamy

Table S1 Percent identity matrix for α - and β - chains of ostrich, turkey and other avian species

	α-Chain										β-chain									
	Ostrich	Turkey	GLG	JQuail	Duck	Pigeon	BHG	Emu	Parrot	Cormorant	Ostrich	Turkey	GLG	JQuail	Duck	Pigeon	BHG	Emu	Parrot	Cormorant
Ostrich	**100**	87.94	90.07	87.23	89.36	80.14	89.36	87.23	84.4	84.4	**100**	96.58	97.26	96.58	97.26	90.41	96.58	95.21	90.41	91.78
Turkey	87.94	**100**	87.94	92.91	87.94	81.56	87.94	83.69	80.85	82.98	96.58	**100**	97.26	98.63	97.95	91.78	96.58	92.47	91.78	93.15
GLG	90.07	87.94	**100**	90.07	95.74	83.69	97.87	88.65	82.27	90.07	97.26	97.26	**100**	96.58	97.26	92.47	99.32	94.52	91.78	93.15
JQuail	87.23	92.91	90.07	**100**	88.65	83.69	88.65	86.52	84.4	85.82	96.58	98.63	96.58	**100**	97.95	91.1	95.89	93.15	92.47	93.84
Duck	89.36	87.94	95.74	88.65	**100**	82.98	93.62	85.82	80.85	89.36	97.26	97.95	97.26	97.95	**100**	91.78	96.58	92.47	91.78	94.52
Pigeon	80.14	81.56	83.69	83.69	82.98	**100**	81.56	82.27	80.14	82.98	90.41	91.78	92.47	91.1	91.78	**100**	91.78	90.41	89.73	89.73
BHG	89.36	87.94	97.87	88.65	93.62	81.56	**100**	86.52	80.14	87.94	96.58	96.58	99.32	95.89	96.58	91.78	**100**	93.84	92.47	92.47
Emu	87.23	83.69	88.65	86.52	85.82	82.27	86.52	**100**	82.27	87.94	95.21	92.47	94.52	93.15	92.47	90.41	93.84	**100**	91.78	91.78
Parrot	84.4	80.85	82.27	84.4	80.85	80.14	80.14	82.27	**100**	78.72	90.41	91.78	91.78	92.47	91.78	89.73	92.47	91.78	**100**	92.47
Cormorant	84.4	82.98	90.07	85.82	89.36	82.98	87.94	87.94	78.72	**100**	91.78	93.15	93.15	93.84	94.52	89.73	92.47	91.78	92.47	**100**

Table S2 Difference in the non-conversed residues of BHG, Ostrich, Turkey and GLG Hbs

S.No	Residue No	BHG	Ostrich	Turkey	GLG
			α-Chain		
1	4	Ala	Gly	Ala	Ala
2	5	Ala	Thr	Ala	Ala
3	8	Thr	Thr	Asn	Thr
4	13	Val	Ile	Ile	Val
5	15	Ser	Ser	Thr	Ser
6	18	Ser	Ser	Ala	Gly
7	19	Ser	Ser	Gly	Gly
8	34	Thr	Ile†	Ile†	Thr
9	35	Ala	Thr†	Thr†	Ala
10	38	Gln	Gln	Pro	Gln
11	49	Gln	His	Ser	Gln
12	57	Ala	Ala	Gly	Ala
13	63	Val	Ala	Val	Ala
14	64	Ala	Asn	Ala	Ala
15	67	Val	Ile	Ile	Val
16	70	Val	Val	Ala	Val
17	77	Ala	Ser	Ala	Ala
18	79	Ala	Ala	Thr	Ala
19	89	Gln	Gln	His	Gln
20	100	Phe	Leu†	Leu†	Phe
21	103	His	Gln†	Gln†	His
22	115	Ser	Ser	Ala	Ser
23	119	Ala	Pro†	Pro†	Pro
24	134	Thr	Ala	Thr	Thr
			β-Chain		
1	2	His	Gln	His	His
2	12	Thr	Ser	Thr	Thr
3	43	Ser	Ala	Ala	Ser
4	86	Ala	Ala	Ser	Ala
5	119	Ala	Thr†	Ser†	Ala
6	121	Glu	Glu	Asp	Glu
7	125	Asp	Glu†	Glu†	Glu

† Residues involved in interface interactions

Table S3 Amino acid Composition of avian Hbs (α1β1)

Amino Acids	Ostrich (3FS4)			Turkey (3K8B)			GLG (1FAW)			JQuail (3JMP)			Duck (3EOK)			Pigeon (3MJU)			BHG (1A4F)			Emu (3WTG)			Parrot (2ZFB)			Cormorant (3WR1)		
	α	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**	**α**	**β**	**Total**
Ala (A)	15	17	32	18	16	34	19	17	36	21	16	37	21	17	38	17	17	34	19	17	36	19	18	37	16	17	33	21	21	42
Arg (R)	3	6	9	3	6	9	3	6	9	3	6	9	3	6	9	3	5	8	3	6	9	3	6	9	3	6	9	3	5	8
Asn (N)	4	7	11	4	7	11	3	7	10	3	7	10	3	7	10	4	7	11	3	7	10	4	7	11	4	8	12	3	6	9
Asp (D)	7	6	13	7	7	14	7	6	13	8	6	14	7	7	14	8	7	15	7	7	14	7	5	12	9	6	15	7	5	12
Cys (C)	2	3	5	2	3	5	2	3	5	2	3	5	2	3	5	1	3	4	2	3	5	2	3	5	2	3	5	2	3	5
Gln (Q)	4	6	10	2	5	7	4	5	9	4	5	9	3	5	8	4	5	9	4	5	9	4	6	10	5	5	10	3	5	8
Glu (E)	6	7	13	6	6	12	6	7	13	5	7	12	6	6	12	5	6	11	6	6	12	6	8	14	4	7	11	6	8	14
Gly (G)	8	8	16	9	8	17	9	8	17	9	8	17	9	8	17	11	9	20	8	8	16	5	7	12	10	10	20	8	9	17
His (H)	10	6	16	10	7	17	10	7	17	9	7	16	10	7	17	8	7	15	10	7	17	10	6	16	6	7	13	10	7	17
Ile (I)	8	7	15	8	7	15	5	7	12	6	7	13	5	7	12	5	8	13	5	7	12	4	8	12	5	6	11	4	7	11
Leu (L)	15	18	33	15	18	33	14	18	32	14	18	32	13	18	31	18	16	34	14	18	32	17	17	34	15	18	33	15	17	32
Lys (K)	12	10	22	12	10	22	12	10	22	12	10	22	12	10	22	12	11	23	12	10	22	12	10	22	12	10	22	13	10	23
Met (M)	1	1	2	1	1	2	1	1	2	1	1	2	2	1	3	0	0	0	1	1	2	1	1	1	1	0	1	2	1	3
Phe (F)	7	8	15	7	8	15	8	8	16	7	8	15	8	8	16	8	8	16	8	8	16	7	8	15	7	8	15	7	8	15
Pro (P)	5	5	10	6	5	11	5	5	10	5	5	10	5	5	10	5	4	9	4	5	9	6	4	10	6	4	10	5	4	9
Ser (S)	10	7	17	6	8	14	7	7	14	6	7	13	7	5	12	8	9	17	8	7	15	8	9	17	11	7	18	7	5	12
Thr (T)	8	6	14	9	6	15	8	6	14	9	7	16	6	8	14	6	6	12	8	6	14	10	5	15	8	6	14	8	8	16
Trp (W)	0	4	4	0	4	4	0	4	4	0	4	4	0	4	4	0	4	4	0	4	4	0	4	4	0	4	4	0	4	4
Tyr (Y)	4	2	6	4	2	6	4	2	6	4	2	6	4	2	6	3	2	5	4	2	6	4	2	6	4	2	6	4	2	6
Val (V)	12	12	24	12	12	24	14	12	26	13	12	25	15	12	27	15	12	27	15	12	27	13	12	25	13	12	25	13	11	24
Pyl (O)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sec (U)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**	-	-	**287**

Table S4 Physical Parameters for individual amino acids - Argos et al. 1982

Amino acids	HI	T_t	V_v	HP	υ
Ala	0.61	45	67	2.00	0.74
Arg	0.60	60	148	0.00	0.70
Asn	0.06	50	96	0.20	0.63
Asp	0.46	150	91	0.01	0.60
Cys	1.07	30	86	1.51	0.61
Gln	0.00	60	114	0.25	0.66
Glu	0.47	250	109	0.13	0.66
Gly	0.07	55	48	2.07	0.64
His	0.61	30	118	0.12	0.67
Ile	2.22	10	124	2.03	0.90
Leu	1.53	5	124	2.06	0.90
Lys	1.15	120	135	0.23	0.82
Met	1.18	20	124	1.47	0.75
Phe	2.02	-30	135	1.58	0.77
Pro	1.95	-8	90	0.93	0.76
Ser	0.05	50	73	0.92	0.63
Thr	0.05	50	93	0.94	0.70
Trp	2.65	-90	163	0.79	0.74
Tyr	1.88	-55	141	0.75	0.71
Val	1.32	24	105	2.00	0.86

Table S5 Hydrophobicity Index (HI) and Hydrophobicity (Hϕ)calculations

Amino acids	HI	Ostrich		Turkey		GLG		JQuail		Duck		Pigeon		BHG		Emu		Parrot		Cormorant	
		Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)	Total	HI (α1β1)
Ala	0.61	32	19.52	34	20.74	36	21.96	37	22.57	38	23.18	34	20.74	36	21.96	37	22.57	33	20.13	42	25.62
Arg	0.6	9	5.4	9	5.4	9	5.4	9	5.4	9	5.4	8	4.8	9	5.4	9	5.4	9	5.4	8	4.8
Asn	0.06	11	0.66	11	0.66	10	0.6	10	0.6	10	0.6	11	0.66	10	0.6	11	0.66	12	0.72	9	0.54
Asp	0.46	13	5.98	14	6.44	13	5.98	14	6.44	14	6.44	15	6.9	14	6.44	12	5.52	15	6.9	12	5.52
Cys	1.07	5	5.35	5	5.35	5	5.35	5	5.35	5	5.35	4	4.28	5	5.35	5	5.35	5	5.35	5	5.35
Gln	0	10	0	7	0	9	0	9	0	8	0	9	0	9	0	10	0	10	0	8	0
Glu	0.47	13	6.11	12	5.64	13	6.11	12	5.64	12	5.64	11	5.17	12	5.64	14	6.58	11	5.17	14	6.58
Gly	0.07	16	1.12	17	1.19	17	1.19	17	1.19	17	1.19	20	1.4	16	1.12	12	0.84	20	1.4	17	1.19
His	0.61	16	9.76	17	10.37	17	10.37	16	9.76	17	10.37	15	9.15	17	10.37	16	9.76	13	7.93	17	10.37
Ile	2.22	15	33.3	15	33.3	12	26.64	13	28.86	12	26.64	13	28.86	12	26.64	12	26.64	11	24.42	11	24.42
Leu	1.53	33	50.49	33	50.49	32	48.96	32	48.96	31	47.43	34	52.02	32	48.96	34	52.02	33	50.49	32	48.96
Lys	1.15	22	25.3	22	25.3	22	25.3	22	25.3	22	25.3	23	26.45	22	25.3	22	25.3	22	25.3	23	26.45
Met	1.18	2	2.36	2	2.36	2	2.36	2	2.36	3	3.54	0	0	2	2.36	1	1.18	1	1.18	3	3.54
Phe	2.02	15	30.3	15	30.3	16	32.32	15	30.3	16	32.32	16	32.32	16	32.32	15	30.3	15	30.3	15	30.3
Pro	1.95	10	19.5	11	21.45	10	19.5	10	19.5	10	19.5	9	17.55	9	17.55	10	19.5	10	19.5	9	17.55
Ser	0.05	17	0.85	14	0.7	14	0.7	13	0.65	12	0.6	17	0.85	15	0.75	17	0.85	18	0.9	12	0.6
Thr	0.05	14	0.7	15	0.75	14	0.7	16	0.8	14	0.7	12	0.6	14	0.7	15	0.75	14	0.7	16	0.8
Trp	2.65	4	10.6	4	10.6	4	10.6	4	10.6	4	10.6	4	10.6	4	10.6	4	10.6	4	10.6	4	10.6
Tyr	1.88	6	11.28	6	11.28	6	11.28	6	11.28	6	11.28	5	9.4	6	11.28	6	11.28	6	11.28	6	11.28
Val	1.32	24	31.68	24	31.68	26	34.32	25	33	27	35.64	27	35.64	27	35.64	25	33	25	33	24	31.68
∑ (α1β1)	-	287	**270.26**	287	**274**	287	**269.64**	287	**268.56**	287	**271.72**	287	**267.39**	287	**268.98**	287	**268.1**	287	**260.67**	287	**266.15**
Hϕ* (α1β1α2β2)	**-**	-	**540.52**	-	**548**	-	**539.28**	-	**537.12**	-	**543.44**	-	**534.78**	-	**537.96**	-	**536.2**	-	**521.34**	-	**532.3**

* Hϕ= ∑HI × 2

Table S6 Comparison of the overall structure (tetramer) and dimer of the selected avian Hbs in terms of rmsd

		Ostrich	TMS	TOS	GLG	JQuail	Duck	Pigeon	BHG (Oxy)	BHG (AM)	Emu	Parrot	Cormorant
		Dimer (rmsd values in Å)											
Ostrich	Tetramer (rmsd values in Å)	100	0.369	0.358	0.380	0.667	0.425	0.347	0.424	0.471	0.461	0.575	0.481
TMS		0.424	100	0.109	0.333	0.565	0.336	0.384	0.281	0.295	0.402	0.517	0.489
TOS		0.477	0.180	100	0.351	0.568	0.361	0.381	0.293	0.311	0.385	0.555	0.493
GLG		0.467	0.731	0.750	100	0.610	0.376	0.424	0.304	0.299	0.379	0.485	0.398
JQuail		0.902	0.713	0.799	1.029	100	0.546	0.670	0.604	0.598	0.641	0.733	0.693
Duck		0.701	0.750	0.842	0.785	0.617	100	0.397	0.369	0.395	0.455	0.619	0.412
Pigeon		0.624	0.980	0.992	0.509	1.188	0.932	100	0.390	0.414	0.451	0.606	0.450
BHG Oxy		0.445	0.600	0.650	0.650	0.843	0.602	0.564	100	0.206	0.472	0.480	0.458
BHG AM		0.560	0.692	0.757	0.354	0.936	0.672	0.509	0.228	100	0.499	0.440	0.494
Emu		0.435	0.518	0.487	0.487	0.875	0.734	0.729	0.536	0.615	100	0.610	0.382
Parrot		0.739	0.956	0.982	0.526	1.191	0.972	0.681	0.657	0.575	0.765	100	0.660
Cormorant		0.581	0.588	0.596	0.662	0.751	0.557	0.837	0.518	0.576	0.573	0.954	100

Table S7 Comparison of the structure of α- and β-chains of the selected avian Hbs in terms of rmsd

		Ostrich	TMS	TOS	GLG	JQuail	Duck	Pigeon	BHG Oxy	BHG AM	Emu	Parrot	Cormorant
		β-Chain (rmsd values in Å)											
Ostrich	α-Chain (rmsd values in Å)	100	0.198	0.184	0.287	0.587	0.316	0.282	0.259	0.266	0.248	0.452	0.367
TMS		0.282	100	0.097	0.287	0.578	0.267	0.305	0.237	0.248	0.232	0.429	0.363
TOS		0.286	0.113	100	0.281	0.573	0.258	0.266	0.228	0.238	0.228	0.449	0.339
GLG		0.269	0.310	0.286	100	0.587	0.408	0.318	0.211	0.203	0.306	0.464	0.322
JQuail		0.603	0.543	0.515	0.614	100	0.514	0.598	0.596	0.595	0.620	0.685	0.651
Duck		0.327	0.289	0.237	0.283	0.531	100	0.316	0.348	0.339	0.356	0.518	0.392
Pigeon		0.501	0.423	0.422	0.460	0.763	0.429	100	0.309	0.298	0.300	0.496	0.335
BHG Oxy		0.425	0.257	0.279	0.381	0.577	0.291	0.475	100	0.186	0.278	0.432	0.331
BHG AM		0.416	0.252	0.281	0.348	0.566	0.289	0.503	0.190	100	0.266	0.414	0.325
Emu		0.455	0.451	0.378	0.365	0.586	0.437	0.491	0.492	0.501	100	0.451	0.367
Parrot		0.582	0.464	0.465	0.432	0.687	0.497	0.479	0.431	0.430	0.464	100	0.550
Cormorant		0.384	0.411	0.413	0.373	0.639	0.396	0.531	0.438	0.441	0.418	0.614	100

Table S8 Different types of interactions and their counts at the α1β1 and α1β2 interfaces of the selected avian Hbs

Interactions		**Ostrich**	**TMS**	**TOS**	**GLG**	**JQuail**	**Duck**	**Pigeon**	**BHG (oxy)**	**BHG (Aqua met)**	**Emu**	**Parrot**	**Cormorant**
H-bonds	**α1β1 interface**	7	8	9	7	6	6	6	7	4	7	5	6
Non-bonded contacts		84	96	100	70	91	82	79	79	66	98	63	85
Salt bridges		0	0	0	0	0	0	1	0	0	0	0	0
Disulphide bridges		0	0	0	0	0	0	0	0	0	0	0	0
Total number of interactions		91	104	109	77	97	88	85	86	70	105	68	91
Buried interface area (Å^2)		1966	1997	1952	1856	1908	1928	1909	1847	1816	1947	1826	1844
H-bonds	**α1β2 interface**	3	3	3	1	2	1	2	2	2	2	0	1
Non-bonded contacts		48	43	49	38	48	64	47	45	48	54	27	50
Salt bridges		0	0	0	0	0	0	0	0	0	0	0	0
Disulphide bridges		0	0	0	0	0	0	0	0	0	0	0	0
Total number of interactions		51	46	52	39	50	65	49	47	50	56	27	51
Buried interface area (Å^2)		873	828	737	904	895	1051	929	968	941	901	932	908

Table S9 H-bonding interactions of ostrich, TMS and TOS Hbs in α1β1 and α1β2 interfaces (Å)

	α1β1	Ostrich	TMS	TOS
1	Arg31(NH1) - (O)Phe122	3.13	3.11	3.14
2	Arg31(NH1) - (OE1)Gln127	3.04	2.92	2.83
3	Arg31(NH2) - (O)Phe122	2.81	2.73	2.66
4	Gln103(NE2) - (O)Asp108	3.29	3.00	3.09
5	Ile111(O) - (OG)Ser119 / (CG2)Thr119	3.26*	2.77	2.76
6	Leu117(O) - (NH1)Arg30	3.17	3.09	3.12
7	Leu117(O) - (NH2)Arg30	3.14	3.00	2.88
8	His122(ND1) - (NH1)Arg30	2.96	3.15	2.93
9	Asp126(OD1) - (OH)Tyr35	3.85*	3.63*	3.27
	α1β2			
1	Thr41(O) - (NH2)Arg40	3.00	2.97	4.39*
2	Thr41(OG1) - (NH2)Arg40	2.89	3.86*	3.34
3	Asp94(OD2) - (ND2)Asn102	2.86	2.85	2.8
4	Arg92(NH2) - (OE1)Gln39	3.94*	2.64	3.24

*Marked values are minimal distances of corresponding atoms

Table S10 Proα119 and Gluβ125 involved van der Waals contacts and minimal distances of corresponding atoms of ostrich, TMS and TOS, respectively (Å)

α1β1	Ostrich	TMS	TOS
Pro119 (N) - (CZ) Arg30	4.18*	3.88	3.84
Pro119 (N) - (NH1) Arg30	4.12*	3.89	3.78
Pro119 (N) - (NH2) Arg30	4.18*	3.80	3.71
Pro119 (CB) - (CD1) Ile33	3.80	3.63	3.72
Pro119 (CG) - (OE1) Glu26	7.74*	3.73	4.38*
Pro119 (CG) - (CD2) Leu55	3.83	3.79	3.99*
Pro119 (CD) - (NH2) Arg30	3.98*	3.76	3.65
Pro119 (CA) - (NH1) Arg30	4.17*	3.94*	3.78
Pro119 (O) - (CG2) Ile33	4.05*	3.96*	3.81
Ile34 (CD1) - (N) Glu125	4.00*	3.58	5.07*
Ile34 (CD1) - (CA) Glu125	3.92*	3.50	5.78*
Ile34 (CD1) - (CG) Glu125	3.93*	3.40	5.25*
Ile34 (CD1) - (CD) Glu125	4.10*	3.75	4.59*
Ile34 (CD1) - (OE2) Glu125	3.92*	3.78	3.87

*Marked values are minimal distances of corresponding atoms

Table S11 Geometry of heme groups and environment of avian oxy hemoglobins

	Ostrich		**GLG**		**JQuail**		**Pigeon**		**BHG Oxy**		**Emu**	
	α1	**β1**	**α1**	**β1**	**α1**	**β1**	**α1**	**β1**	**α1**	**β1**	**α1**	**β1**
Fe – His (F8) NE2 (Å)	2.12	2.17	2.19	2.14	2.40	1.89	2.11	2.09	2.10	2.07	1.96	2.08
Fe – His (E7) NE2 (Å)	4.27	4.14	4.49	4.45	4.93	4.85	4.28	4.27	4.52	4.51	4.44	4.34
Fe – O1 (Å)	1.66	1.73	1.74	1.75	1.93	2.53	2.19	2.04	1.78	1.81	1.62	1.90
O_2 – His (E7) NE2 (Å)	2.70	2.86	2.69	2.55	3.71	3.84	3.39	3.71	2.53	2.68	2.70	2.80
O_2 – Val (E11) CG2 (Å)	3.84	3.39	3.28	3.40	4.28	3.46	3.61	3.40	3.22	3.25	3.44	3.45
O_1 – Fe –His (F8)NE2 (°)	168.3	173.1	171.6	171.4	172.3	155.8	175.1	176.9	174.9	175.4	176.5	166.0
Fe – O_1 – O_2 (°)	126.7	127.7	145.8	130.3	85.8	104.6	129.5	123.3	170.2	158.7	137.1	104.8
Fe- Plane distance* (Å)	0.09	0.00	0.26	0.16	0.14	0.16	0.09	0.09	0.21	0.13	0.14	0.12

* The plane is defined as porphyrin ring atoms excluding the side chains (methyl, ethyl and acid group) and Fe atom.

Table S12 Geometry of heme groups and environment of aqua-met and met form of avian Hbs

	TMS		**TOS**		**Duck**		**BHG AM**		**Parrot**		**Cormorant**	
	α1	**β1**	**α1**	**β1**	**α1**	**β1**	**α1**	**β1**	**α1**	**β1**	**α1**	**β1**
Fe – His (F8) NE2 (Å)	2.32	2.30	2.18	2.18	2.20	2.20	2.24	2.04	2.16	2.02	2.47	2.15
Fe – His (E7) NE2 (Å)	4.38	4.33	4.21	4.25	4.29	4.17	4.35	4.21	4.44	4.43	4.26	4.57
Fe – O (Water) (Å)	2.65	2.37	2.31	2.32	-	-	-	-	-	-	-	-
O – His (E7) NE2 (Å)	2.44	2.71	2.38	2.75	-	-	-	-	-	-	-	-
O – Val (E11) CG2 (Å)	3.52	3.22	3.17	3.07	-	-	-	-	-	-	-	-
O – Fe –His (F8)NE2 (°)	172.7	173.6	178.3	176.0	-	-	-	-	-	-	-	-
Fe – O – His (E7) NE2 (°)	118.7	116.9	127.7	113.6	-	-	-	-	-	-	-	-
Fe- Plane distance* (Å)	0.15	0.14	0.15	0.13	0.10	0.09	0.15	0.13	0.13	0.18	0.02	0.10

* The plane is defined as porphyrin ring atoms excluding the side chains (methyl, ethyl and acid group) and Fe atom.

Table S13 RMSD between the heme groups of the ostrich and turkey Hbs with other avian Hbs

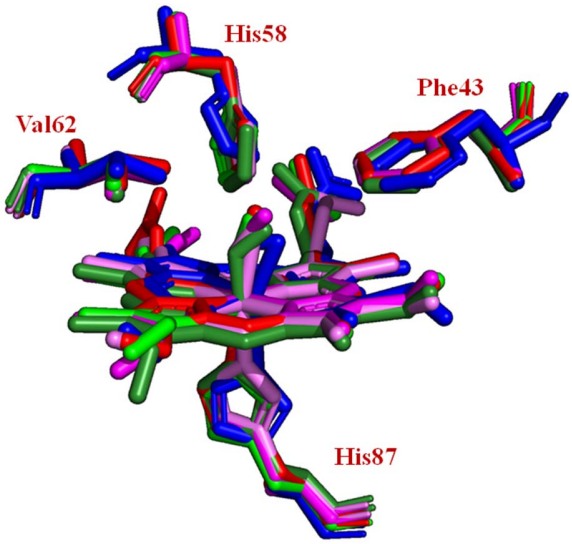
		Ostrich*	TMS	TOS	GLG*	JQuail*	Duck	Pigeon*	BHG Oxy*	BHG AM	Emu*	Parrot	Cormorant
		β – Heme (rmsd values in Å)											
Ostrich*	α – Heme (rmsd. values in Å)	100	0.081	0.052	0.158	0.387	0.127	0.147	0.090	0.127	0.110	0.106	0.182
TMS		0.055	100	0.074	0.176	0.437	0.131	0.180	0.141	0.153	0.157	0.170	0.180
TOS		0.106	0.061	100	0.156	0.401	0.092	0.141	0.096	0.125	0.122	0.118	0.186
GLG*		0.211	0.170	0.155	100	0.401	0.223	0.063	0.117	0.080	0.098	0.120	0.226
JQuail*		0.578	0.536	0.517	0.433	100	0.416	0.352	0.382	0.354	0.370	0.338	0.474
Duck		0.248	0.209	0.190	0.120	0.431	100	0.204	0.144	0.195	0.197	0.175	0.178
Pigeon*		0.155	0.134	0.139	0.141	0.529	0.129	100	0.112	0.057	0.084	0.092	0.247
BHG Oxy*		0.183	0.141	0.106	0.141	0.526	0.183	0.165	100	0.109	0.106	0.062	0.156
BHG AM		0.197	0.158	0.156	0.086	0.461	0.114	0.134	0.131	100	0.104	0.087	0.236
Emu*		0.063	0.036	0.084	0.162	0.524	0.199	0.120	0.162	0.153	100	0.096	0.234
Parrot		0.226	0.214	0.229	0.223	0.550	0.286	0.209	0.272	0.268	0.195	100	0.213
Cormorant		0.125	0.100	0.116	0.096	0.501	0.164	0.102	0.144	0.107	0.086	0.192	100

*Marked species are belongs to oxy form

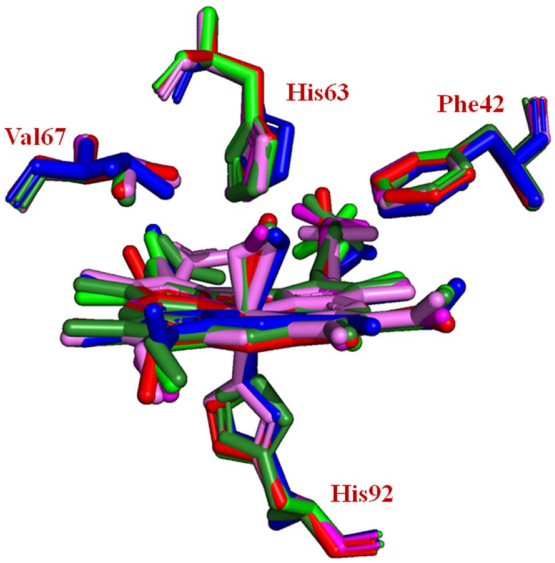
Table S14 The minimum distances (Å) between residues of IPP-binding site in all the avian Hbs

IPP Binding site residues	Ostrich	TMS	TOS	GLG	JQuail	Duck	Pigeon	BHG (Oxy)	BHG (AM)	Emu	Parrot	Cormorant
β1Val 1-β2Val 1	18.37	18.91	18.65	16.53	19.76	18.66	16.60	18.38	14.39	17.01	16.61	18.46
β1His2/Gln* - β2His2/Gln*	25.44*	26.02	25.79	23.59	26.70	25.62	24.11	25.02	23.61	24.10*	23.11	24.80
β1Lys 82-β2Lys 82	9.19	9.08	7.31	9.87	10.98	8.69	8.42	12.26	7.79	5.73	11.36	8.99
β1Arg 104-β2Arg 104	10.89	10.82	10.60	11.71	12.28	12.35	10.53	10.55	10.39	10.24	12.24	13.28
β1Arg135/Gly*-β2Arg135 /Gly*	15.94	15.96	15.75	15.26	15.89	16.22	13.39	15.52	14.65	15.31	15.39	15.59*
β1His 139-β2His 139	4.07	4.27	3.89	5.75	5.62	4.55	3.97	3.75	2.80	3.49	4.49	7.39
β1Arg 143-β2Arg 143	9.70	5.07	9.15	10.54	6.11	3.77	8.93	7.85	12.33	7.13	10.32	10.79
β1Lys 144-β2Lys 144	16.13	16.15	15.92	16.14	16.38	16.39	17.27	16.94	17.57	16.52	17.46	15.19
β1His 146-β2His 146	8.34	8.25	7.43	11.63	8.55	7.70	10.13	8.83	4.27	8.53	11.83	10.79

*marked values belong to the variant residues in respective species



(a)



(b)

Figure S1 Overlapped diagram of the heme environment of ostrich oxy Hb with other avian oxy Hbs. (a) α-chains and (b) β-chains with the colors forest green (ostrich), green (GLG), blue (JQuail), magenta (pigeon), red (BHG oxy form), and violet (emu), respectively.