Table 1
Crystal data and refinement statistics.

Values in parentheses refer to the highest resolution shell.

	Oxy	Oxy 93% r.h.	Oxy 90% r.h.	Oxy 88% r.h.	Oxy 84% r.h.	Oxy 25% methanol	Deoxy	Deoxy 90% r.h.
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P2_1$	$P2_1$
Unit-cell parameters (Å, °)	$a = b = 53.7$, $c = 193.8$	$a = b = 54.2$, $c = 195.7$	$a = b = 54.2$, $c = 194.9$	$a = b = 54.2$, $c = 194.2$	$a = b = 53.6$, $c = 193.3$	$a = b = 52.8$, $c = 192.1$	$a = 63.3$, $b = 83.3$, $c = 53.8$, $\beta = 99.4$	$a = 89.3$, $b = 82.9$, $c = 76.3$, $\beta = 99.3$
Unit-cell volume (Å^3)	558859.1	574896.2	572546.1	570489.7	555343.2	535544.1	279872.2	556435.7
Z	4	4	4	4	4	4	2	4
Solvent content (%)	43.3	44.8	44.6	44.3	42.8	40.6	43.4	43.0
Resolution range (Å)	20–2.5	20–2.6	20–2.6	20–2.8	20–3.3	20–3.1	20–2.1	30–2.7
Last resolution shell (Å)	2.6–2.5	2.7–2.6	2.7–2.6	2.9–2.8	3.4–3.3	3.2–3.1	2.17–2.1	2.8–2.7
No. of observations	66131	65312	50116	32801	16345	20857	145765	85616
No. of unique reflections	10041	9707	9245	7436	4467	5010	30095	30721
Completeness of data (%)	95.4 (92.8)	99.9 (100.0)	96.3 (99.4)	97.4 (99.3)	94.6 (95.4)	98.4 (98.2)	93.5 (93.2)	98.0 (99.4)
Merging R for all reflections (%)	12.1 (35.3)	12.9 (37.0)	12.8 (30.0)	11.7 (41.2)	10.4 (40.3)	11.4 (42.4)	11.0 (32.5)	11.5 (48.1)
Average $I/\sigma(I)$	5.4	5.8	7.9	8.0	4.1	4.2	7.6	6.4
R factor (%)	20.3	19.7	20.1	21.0	20.8	20.0	20.1	19.0
R_{free}	25.6	25.8	26.5	27.0	26.1	26.8	25.2	26.3
No. of protein atoms	2192	2192	2192	2192	2192	2192	4384	8768
No. of water O atoms	58	45	32	24	15	29	180	170
No. of haem atoms	86	86	86	86	86	86	172	344
R.m.s. deviation from ideality								
Bond distances (Å)	0.003	0.011	0.009	0.008	0.012	0.009	0.008	0.021
Bond angles (°)	1.4	1.3	1.2	1.3	1.4	1.4	1.2	1.2
Dihedral angles (°)	19.3	20.2	19.5	20.6	19.7	19.2	19.1	19.2
Improper angles (°)	1.3	1.5	1.2	1.3	1.4	1.5	1.0	1.2
Ramachandran plot, % non-glycine or non-proline residues in								
Most favored regions	88.1	85.1	88.0	84.3	82.3	85.1	93.2	88.8
Additional allowed regions	11.9	13.7	10.8	14.1	15.7	12.5	6.8	10.8
Generously allowed region	0.0	1.2	1.2	1.6	2.0	2.4	0.0	0.4
Disallowed regions	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Expected coordinate error from Luzzati plot (Å)	0.34	0.30	0.31	0.35	0.38	0.45	0.30	0.38

collected from oxyhaemoglobin crystals at r.h. values of 98 and 95% and from deoxyhaemoglobin crystals at r.h. values of 95 and 93%, as the unit-cell parameters at these r.h. values were nearly the same as those of the respective native crystals. Crystal data, data-collection statistics and refinement statistics pertaining to the data collected and used in the analysis are given in Table 1.

3.2. Haem stereochemistry

The tertiary structure of the protein is remarkably similar in all the crystal forms. However, the position of the ferrous iron with respect to the porphyrin ring in the low-humidity deoxy crystal presents an interesting case. The relevant distances in the native high-salt (Fermi *et al.*, 1984) and low-salt (Kavanaugh *et al.*, 1992) deoxy crystals, in the native oxy crystals and in the two molecules of the 90% r.h. form are listed in Table 2 and illustrated in Fig. 4. As is well known, the ferrous iron shifts from the haem plane towards the proximal histidine by about 0.6 Å (by about 0.4 Å from the plane of the porphyrin N atoms) in the deoxy form, while it lies in the plane in the oxy form in both the subunits. Consequently, the distance between the ferrous iron and the coordinating N atoms are a little longer in the former. Surprisingly, the displacement of the ferrous iron from the haem plane and the Fe–N distances in

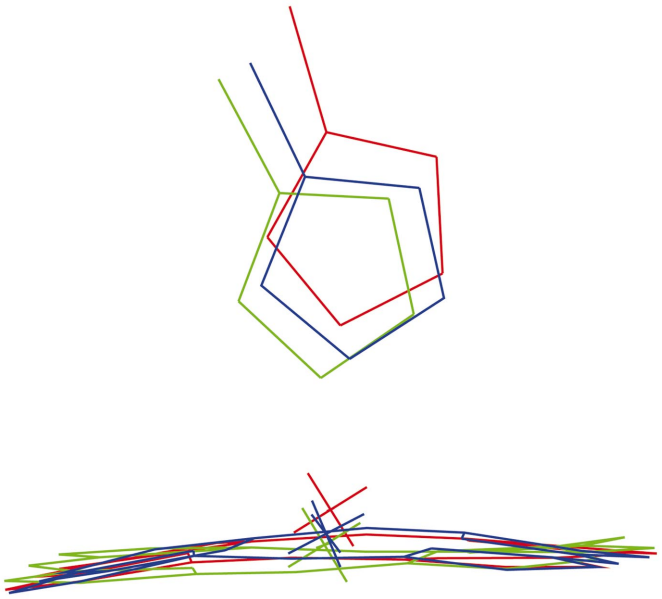

Figure 4
Haem environment of the α-subunit of native deoxy (red), molecule 1 of deoxy 90% r.h. (blue) and oxy (green) structures.