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Supporting information for article:

Structure of *Chlamydomonas reinhardtii* THB1, a group 1 truncated hemoglobin with a rare histidine–lysine heme ligation

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Structure of *Chlamydomonas reinhardtii* THB1, a group 1 truncated hemoglobin with a rare histidine–lysine heme ligation

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Table S1 Top 10 structures selected by the program DALI (4XDI, chain B)

Protein- Chain	Z	r.m.s.d.	lali	nres	% id	Description	Reference
1S69-A	16.5	2.0	120	123	44	<i>Synechocystis</i> GlbN, ferric, cyanide bound	(Trent <i>et al.</i> , 2004)
4L2M-B	16.2	2.0	120	123	44	<i>Synechococcus</i> GlbN, ferric, cyanide bound	(Wenke <i>et al.</i> , 2014)
1DLY-A	15.7	2.0	116	121	42	<i>C. eugametos</i> TrHb1 (CtrHb), ferric, cyanide bound	(Pesce <i>et al.</i> , 2000)
3AQ5-A	15.3	2.1	112	117	28	<i>Tetrahymena pyriformis</i> , ferrous, oxygen bound	(Igarashi <i>et al.</i> , 2011)
1IDR-B	15.2	2.3	113	125	31	<i>Mycobacterium tuberculosis</i> , ferrous, oxygen bound	(Milani <i>et al.</i> , 2001)
1DLW-A	15.2	1.9	111	116	29	<i>P. caudatum</i> TrHb1, ferric, water bound	(Pesce <i>et al.</i> , 2000)
1RTX-A	14.7	2.3	117	123	43	<i>Synechocystis</i> GlbN, ferric, bis-histidine	(Hoy <i>et al.</i> , 2004)
4MAX-C	14.6	2.4	118	123	43	<i>Synechococcus</i> GlbN, ferric, bis-histidine	(Wenke <i>et al.</i> , 2014)
1UX8-A	12.2	2.6	110	118	18	<i>Bacillus subtilis</i> TrHb2, ferric, cyanide bound	(Giangiacomo <i>et al.</i> , 2005)
2IG3-B	12.1	2.7	110	124	16	<i>Campylobacter jejuni</i> TrHb3, ferric, cyanide bound	(Nardini <i>et al.</i> , 2006)

Z: Z-score as reported by DALI (Holm & Rosenström, 2010); lali: length of alignment; nres: number of residues; %id: percent sequence identity. Only one structure is listed when the search identified highly similar structures (e.g., the same protein with oxygen or cyanide bound). Highly similar structures with non-natural amino acid replacement(s) were removed from the table.

Table S2 Superposition of TrHb1 structures

	Structures	r.m.s.d. (Å)	# of C α pairs	% identity	Fig. S1
<u>With THB1</u>					
<i>Synechococcus</i> GlbN His–His	4XDI.B / 4MAX.C	1.09	69	46	<i>a</i>
<i>Synechococcus</i> GlbN His–CN	4XDI.B / 4L2M.B	0.97	82	46	<i>b</i>
<i>Synechocystis</i> GlbN His–His	4XDI.B / 1RTX	1.06	78	47	<i>d</i>
<i>Synechocystis</i> GlbN His–CN	4XDI.B / 1S69	0.97	82	47	<i>e</i>
<i>C. eugametos</i> CtrHb His–CN	4XDI.B / 1DLY	0.91	82	48	<i>f</i>
<i>M. tuberculosis</i> HbN His–O ₂	4XDI.B / 1IDR.A	1.02	78	39	<i>g</i>
<u>Other pairs</u>					
<i>Synechococcus</i> GlbN His–His / His–CN	4MAX.B / 4L2M.A	0.96	79	100	<i>c</i>
<i>Synechococcus</i> GlbN His–CN	4L2M.A / 1S69	0.67	118	60	
<i>Synechocystis</i> GlbN His–CN					
<i>Synechococcus</i> GlbN His–CN	4L2M.A / 1DLY	0.73	111	47	
<i>C. eugametos</i> CtrHb His–CN					
<i>Synechococcus</i> GlbN His–CN	4L2M.A / 1IDR.B	0.78	99	35	
<i>M. tuberculosis</i> HbN His–O ₂					
<i>Synechococcus</i> GlbN His–CN	4L2M.A / 1DLW	0.97	99	31	
<i>P. caudatum</i> HbN His–H ₂ O					

Table S3 Geometry of coordinating lysines

Protein	PDB	2° str. ^a	Lys χ_{1-4}	C ε -N ζ -Fe angle	N ζ -Fe distance (Å)	N ζ H ₂ H-bond	Reference
THB1 His77-Lys53	4XDI	α -helix	tttp	118°	2.23	not detected	this work
M100K cyt <i>c</i> ₅₅₀ His19-Lys100	2BH5	loop	111°, t, -133°, t	129°	1.89	water	(Worrall <i>et al.</i> , 2005)
nitrite reductase NrFHA His140.C– Lys331.A	2J7A	β -strand	mttt	116 ± 5°	2.17 ± 0.06 ^b	Asn124.C	(Rodrigues <i>et al.</i> , 2006)
nitrite reductase NrFHA Lys151.A-H ₂ O	2J7A	turn	mmtm	123 ± 6°	2.20 ± 0.09	water	(Rodrigues <i>et al.</i> , 2006)

^aSecondary structure of the segment carrying the coordinating lysine. ^bAverage over all chains.

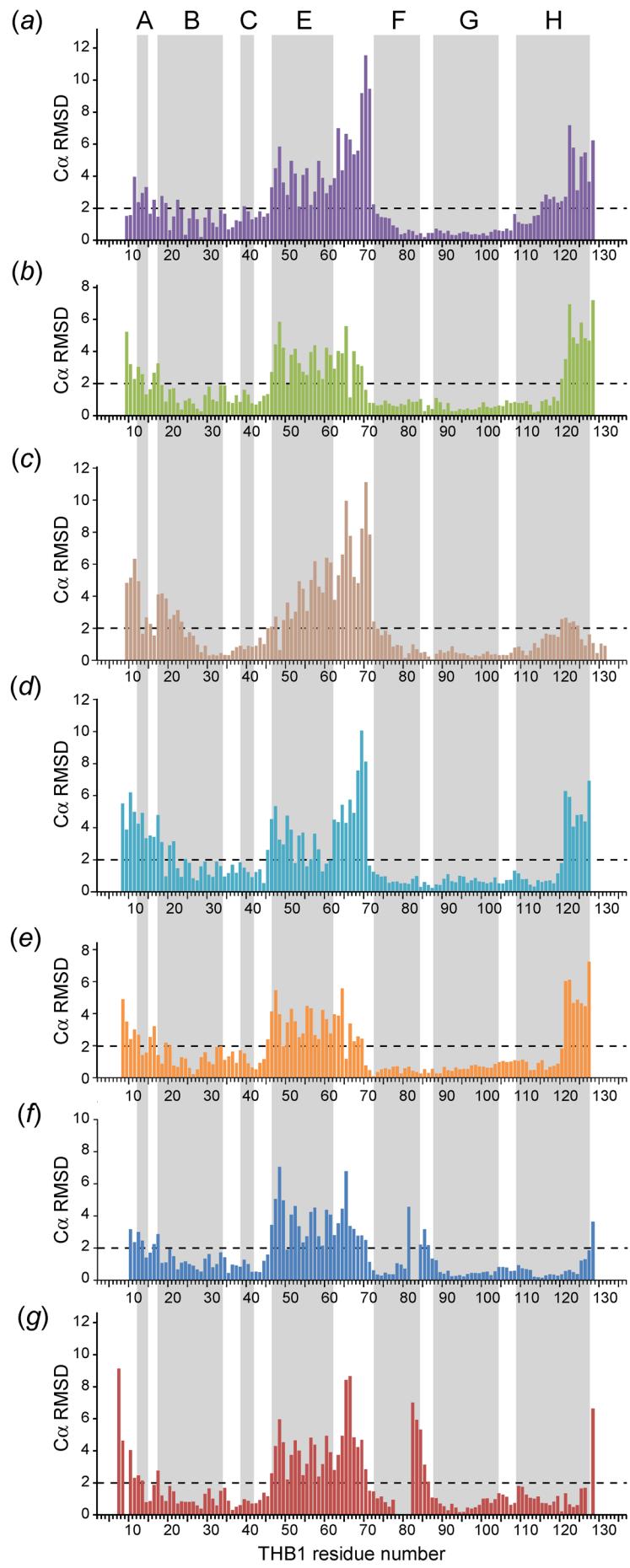


Figure S1 C α r.m.s.d. as a function of position for the overlay of (a) THB1 with *Synechococcus* GlbN in the *bis*-histidine state, (b) THB1 with *Synechococcus* GlbN in the cyanide-bound state, (c) *Synechococcus* GlbN in the *bis*-histidine state with the same in the cyanide bound state, (d) THB1 with *Synechocystis* GlbN in the *bis*-histidine state, (e) THB1 with *Synechocystis* GlbN in the cyanide-bound state, (f) THB1 with CtrHb in the cyanide bound state, and (g) THB1 with *M. tuberculosis* HbN in the oxy state. The dashed line indicates the 2 Å threshold for the matched pair count. Table S2 contains additional information.

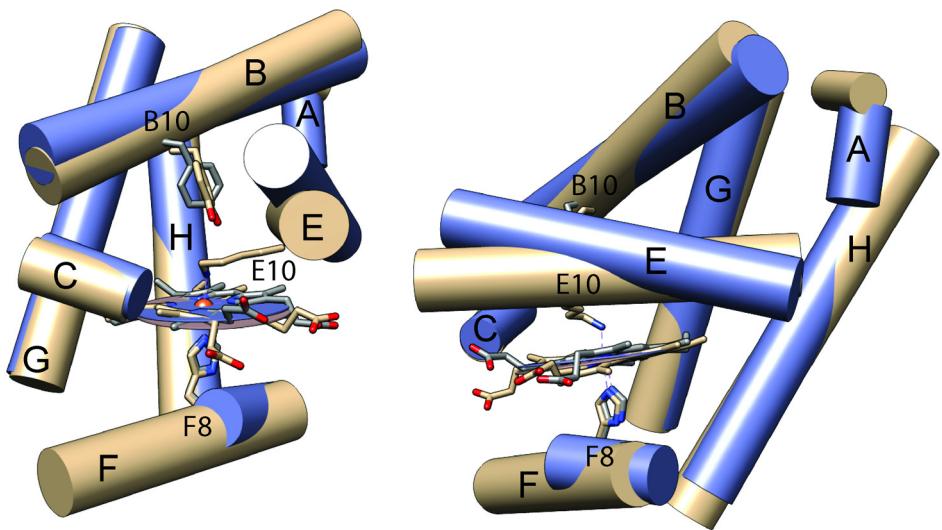


Figure S2 Superimposition of THB1 (PDB ID 4XDI, this work, sand colour) and CtrHb in the cyanide bound state (PDB ID 1DLY (Pesce *et al.*, 2000), purple colour). Note the position of Tyr B10. Unlike GlbN, CtrHb and THB1 do not attach the heme covalently through a histidine at the end of the H helix, and the structural overlap in this region is high.

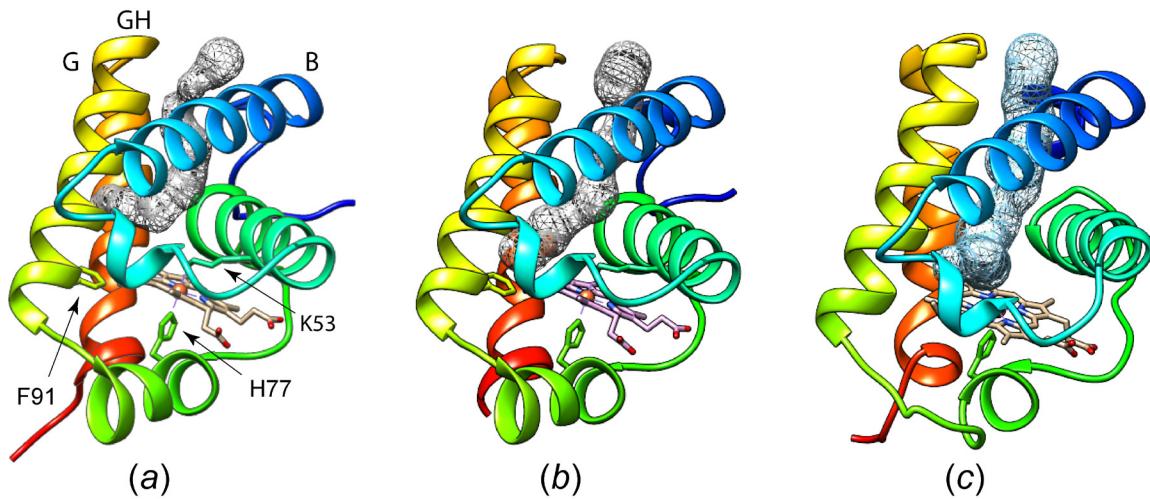
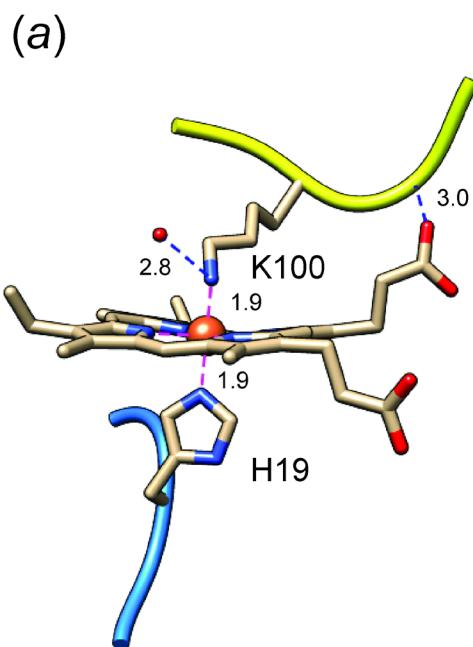
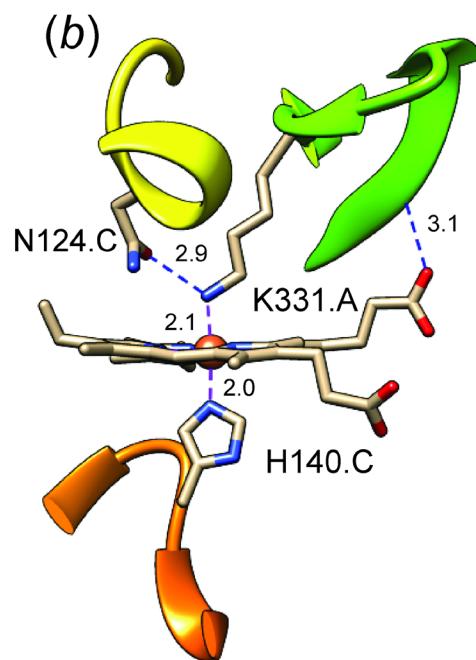


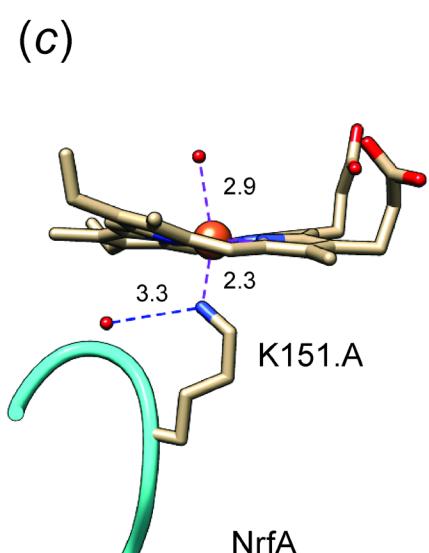
Figure S3 Pores in the structure of THB1 chain A (a) and chain B (b). The pores extend from the B/GH interface toward the heme. Also shown are Lys53 (E10), His77 (F8), and Phe91 (G5). (c) Corresponding tunnel in the structure of CtrHb (PDB ID 1DLY (Pesce *et al.*, 2000)) (Milani *et al.*, 2001). The pores and tunnel were calculated with *MOLEonline 2.0* (Berka *et al.*, 2012). The interior threshold was 1.25 Å for CtrHb (default value), 1.10 Å for 4XDI chain A and 1.15 Å for chain B. The volume of the pores (~180 Å³ for 4XDI chain A, ~230 Å³ for 4XDI chain B, and ~370 Å³ for CtrHb) was estimated with *USCF Chimera* (Pettersen *et al.*, 2004) (surface calculation using the variable radius of the pore, followed by calculation of the volume defined by the surface).



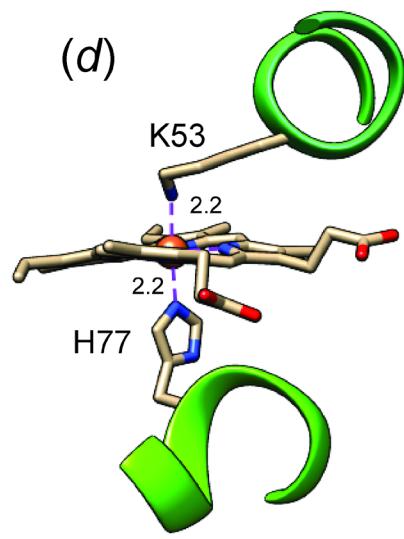
M100K cytochrome c-550
Paracoccus versutus
 2BH5



NrfHA
Desulfovibrio vulgaris
 2J7A



NrfA
Desulfovibrio vulgaris
 2J7A



THB1
Chlamydomonas reinhardtii
 4XDI

Figure S4 Available examples of lysine coordination: PDB ID 2BH5 (Worrall *et al.*, 2005), 2J7A (Rodrigues *et al.*, 2006), and 4XDI (this work). Distances are in Å.

S1. NMR data collection

NMR data were collected at a proton frequency of 600 MHz on a Bruker Avance or Avance II spectrometer equipped with a cryoprobe. Probe temperature was 298 K. Samples were (1) ¹⁵N-labeled THB1 (~1.4 mM) in 25 mM borax buffer pH 9.5, reduced with 7.5 mM dithionite, and (2) ¹⁵N-labeled CtrHb (~1.4 mM) in 100 mM borax buffer pH 10, reduced with 6 mM dithionite, both under argon. Following reduction, THB1 and CtrHb solutions (~300 μ L) were transferred into NMR Shigemi tubes and sealed with Parafilm; samples prepared in this manner remained completely reduced over the course of data acquisition. The solvent was 90% H₂O, 10% D₂O. ¹H spectra in Fig. S5 were acquired with ¹⁵N-decoupling (¹⁵N frequency centered in the amide region, 118–120 ppm). Under these conditions, incomplete decoupling of the Lys E10 N ζ H₂ protons is observed for THB1 (Fig. S5a). The flip-back WATERGATE NOESY spectrum in Fig. S5c was collected on the same CtrHb sample with a mixing time of 80 ms and ¹⁵N decoupling.

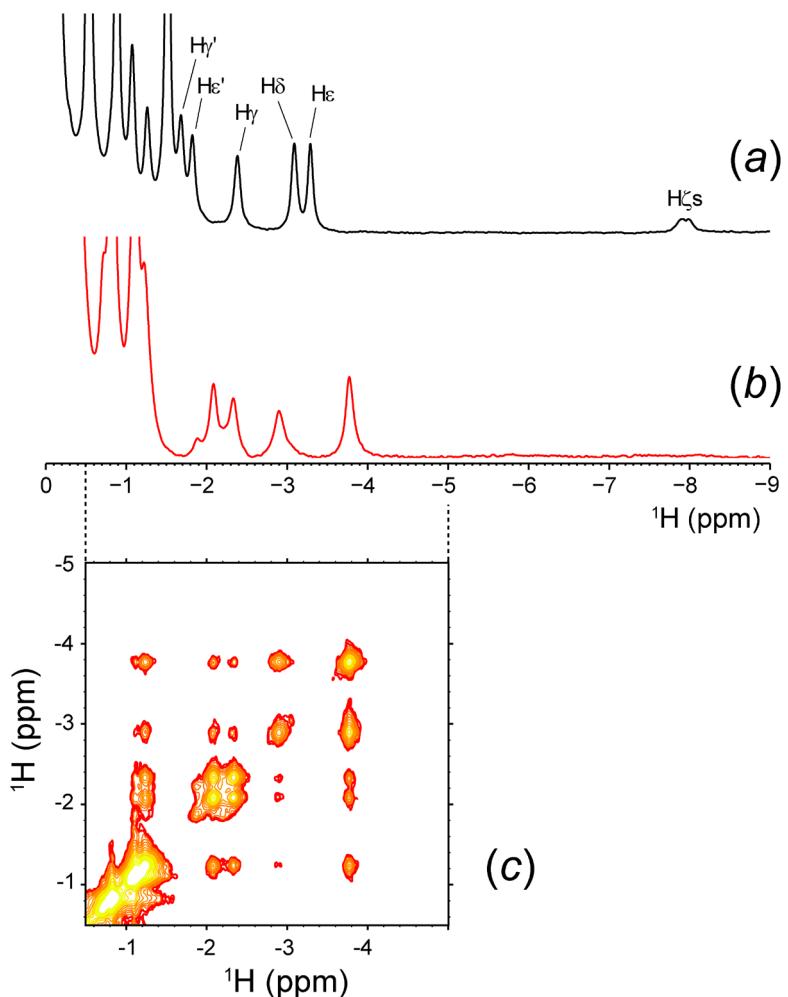


Figure S5 One-dimensional ¹H NMR spectra of alkaline ferrous (a) THB1 (pH 9.5) (Johnson *et al.*, 2014) and (b) CtrHb (pH 10) showing highly upfield-shifted resonances assigned to an axial Lys E10. The spectrum of CtrHb has broad lines at this pH. The Lys E10 amino head group detectable in THB1 at -8 ppm exchanges too rapidly for detection in CtrHb. The NOESY data shown in (c) were collected on the same CtrHb sample. The cross peaks demonstrate that the shifted protons attributed to Lys E10 are spatially close to each other. These signals are inconsistent with Tyr B10 ligation.

S2. References

- Berka, K., Hanák, O., Sehnal, D., Banáš, P., Navrátilová, V., Jaiswal, D., Ionescu, C.-M., Svobodová Vařeková, R., Koča, J. & Otyepka, M. (2012). *Nucleic Acids Res.* **40**, W222-W227.
- Giangiacomo, L., Ilari, A., Boffi, A., Morea, V. & Chiancone, E. (2005). *J. Biol. Chem.* **280**, 9192-9202.
- Holm, L. & Rosenström, P. (2010). *Nucleic Acids Res.* **38**, W545-W549.
- Hoy, J. A., Kundu, S., Trent, J. T., 3rd, Ramaswamy, S. & Hargrove, M. S. (2004). *J. Biol. Chem.* **279**, 16535-16542.
- Igarashi, J., Kobayashi, K. & Matsuoka, A. (2011). *J. Biol. Inorg. Chem.* **16**, 599-609.
- Johnson, E. A., Rice, S. L., Preimesberger, M. R., Nye, D. B., Gilevicius, L., Wenke, B. B., Brown, J. M., Witman, G. B. & Lecomte, J. T. J. (2014). *Biochemistry* **53**, 4573-4589.
- Milani, M., Pesce, A., Ouellet, Y., Ascenzi, P., Guertin, M. & Bolognesi, M. (2001). *EMBO J.* **20**, 3902-3909.
- Nardini, M., Pesce, A., Labarre, M., Richard, C., Bolli, A., Ascenzi, P., Guertin, M. & Bolognesi, M. (2006). *J. Biol. Chem.* **281**, 37803-37812.
- Pesce, A., Couture, M., Dewilde, S., Guertin, M., Yamauchi, K., Ascenzi, P., Moens, L. & Bolognesi, M. (2000). *EMBO J.* **19**, 2424-2434.
- Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C. & Ferrin, T. E. (2004). *J. Comput. Chem.* **25**, 1605-1612.
- Rodrigues, M. L., Oliveira, T. F., Pereira, I. A. & Archer, M. (2006). *EMBO J.* **25**, 5951-5960.
- Trent, J. T., 3rd, Kundu, S., Hoy, J. A. & Hargrove, M. S. (2004). *J. Mol. Biol.* **341**, 1097-1108.
- Wenke, B. B., Lecomte, J. T. J., Heroux, A. & Schlessman, J. L. (2014). *Proteins* **82**, 528-534.
- Worrall, J. A., van Roon, A. M., Ubbink, M. & Canters, G. W. (2005). *FEBS J.* **272**, 2441-2455.