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Supporting information for article:

The structure of class 3 nonsymbiotic plant haemoglobin from *Arabidopsis thaliana* reveals a novel N-terminal helical extension

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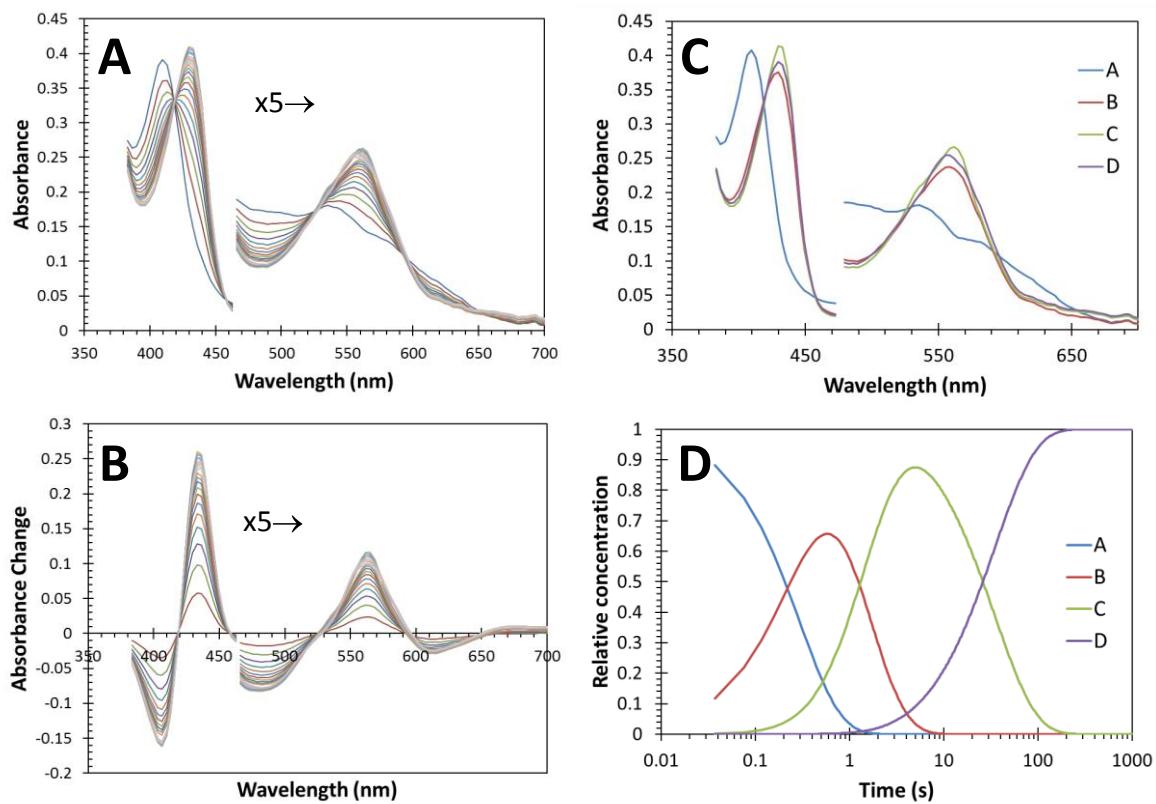


Figure S1 Dithionite reduction of ferric to ferrous AHb3. (A) Ferric AHb3 (5 μ M) was reduced by sodium dithionite (5 mM) in 0.1 M sodium phosphate buffer. For clarity, absorbance values above 460 nm have been multiplied by 5. (B) Difference spectra with ferric spectrum taken as baseline. (C) Fitted spectra to an A \rightarrow B \rightarrow C \rightarrow D serial mechanism with kinetics shown in panel (D).

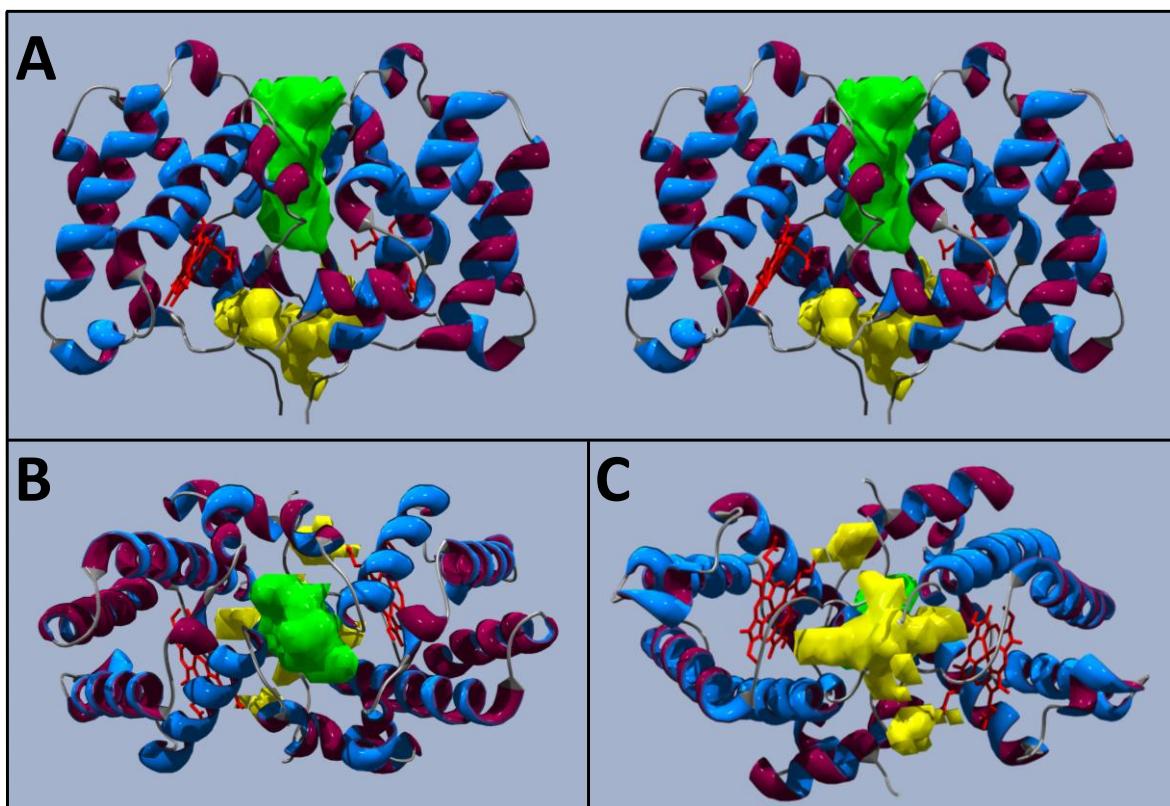


Figure S2 AHb3 dimer with solvent exposed cavities in green (689 \AA^3) and yellow (337 \AA^3). Cavity volumes calculated using SwissPdbViewer v4.1.0 (A) Stereo ‘front’ view (B) ‘top’ view and (C) ‘bottom’ view. Haems are modelled as red sticks.

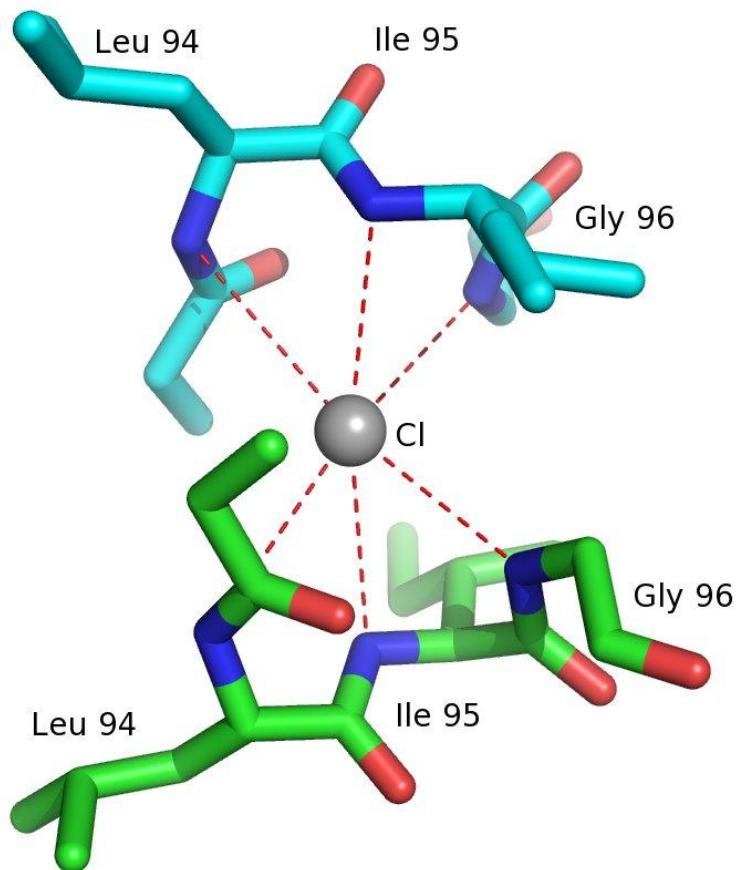


Figure S3 Inter-subunit binding of chloride. Chloride binds in a pocket between monomers, with weak (3.2-3.5 Å) interactions with 6 backbone amides.

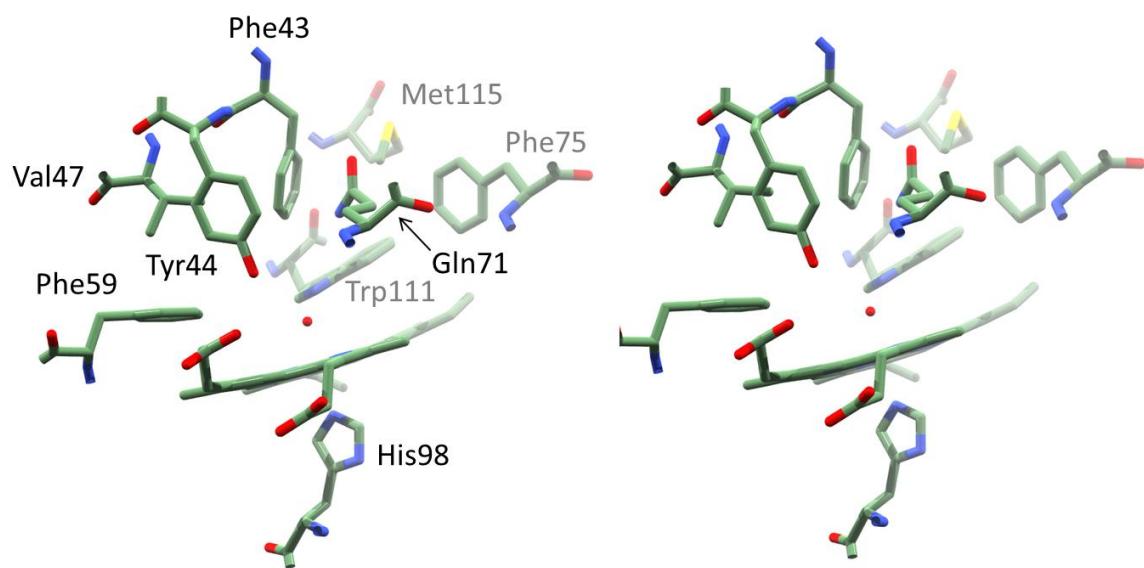


Figure S4 Stereoview of distal side of haem pocket. The distal residues include tyrosine 44 and tryptophan 111 that can potentially form hydrogen bonds with haem-bound ligands.

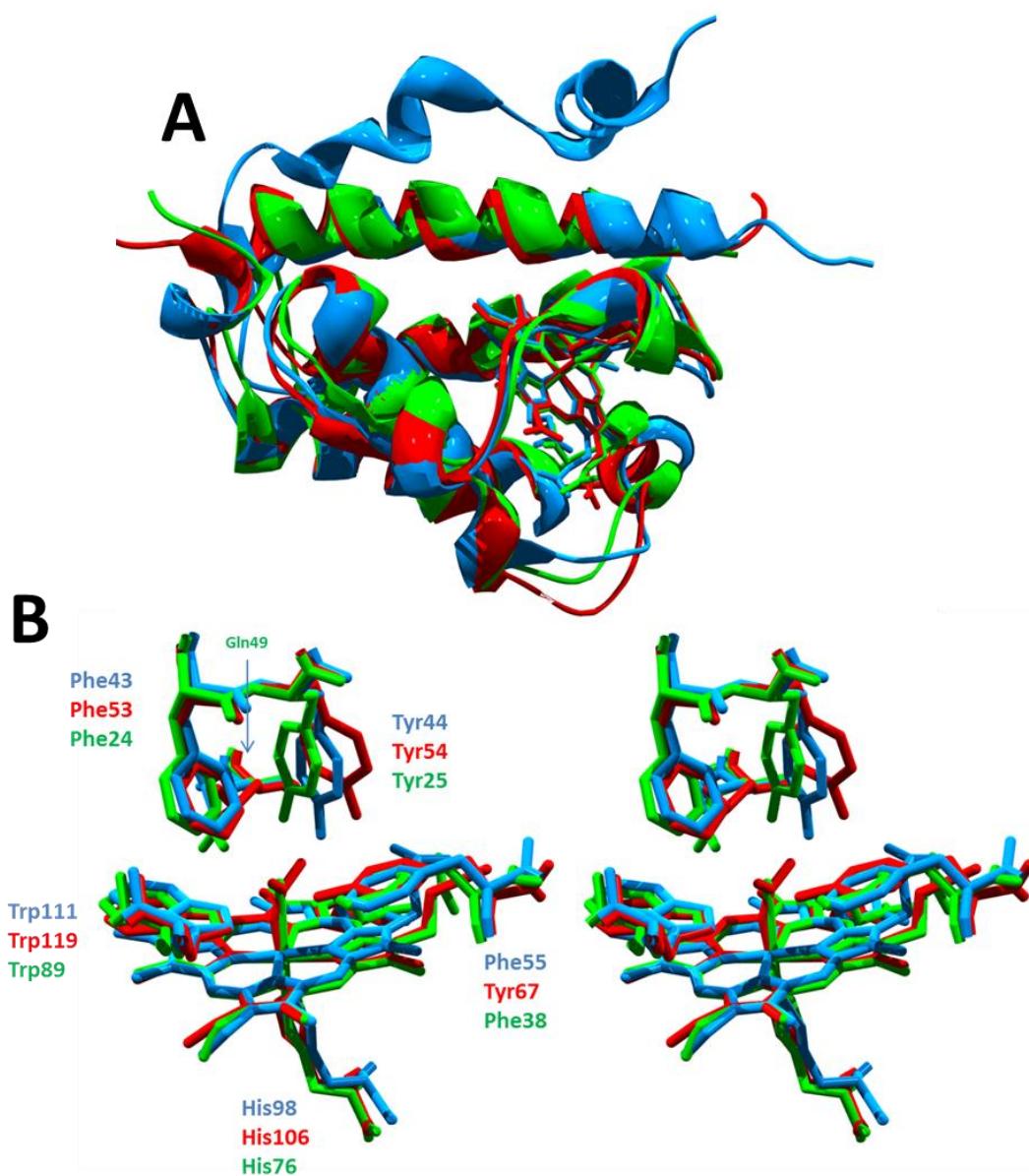


Figure S5 Superposition of the AHb3 crystal structure (blue) with the closest homology matches identified using PDBEfold, the haemoglobin from *T. fusca* (red, PDB code 2bmm) and *B. subtilis* truncated haemoglobin (green, PDB code 1ux8). Note that neither structure of bacterial globins contains the N-terminal helical domain present in AHb3 (A) Comparison of alpha helices cartoon representation and (B) comparison of distal hem pocket composition (stereo view) and overall structure of AHb3 (blue), *T. fusca* truncated haemoglobin (red) and *B. subtilis* truncated haemoglobin (green). Note the variability in the orientation of the tyrosine residue, likely correlated with the exogenous haem ligand present in each structure. SwissPdbViewer v4.1.0 was used to calculate superpositions.

Q67XG0	<i>A. thaliana</i>	QS <u>LQDKASVLS</u> GVD <u>QAEAF</u> AIDESENLFDKLG--LQTFINLSTNFY
R0GWW9	<i>C. rubella</i>	QSLQEKA <u>SVL</u> SGVDQAEAF <u>AIDESEN</u> LFDFKLG--LQTFINLSTNFY
K4CM33	<i>S. lycopersicum</i>	ASLEQKASEW <u>SGV</u> DPKDAVAVDE <u>EIN</u> LFQKLG--LQTFINLSTNFY
G7I4F4	<i>M. truncatula</i>	QSLQQKASEW <u>SGV</u> STDEAFS <u>IDETN</u> LFQKLG--LQTFINLSTNFY
I1M9X1	<i>G. max</i>	QSLQQKASEW <u>SGV</u> SIDDA <u>FGIDDT</u> NLFQKLG--LQTFINLSTNFY
D7SUH5	<i>V. vinifera</i>	QSLQEKA <u>SEW</u> SGVSQND <u>DAFEID</u> NTLFQKLG--LQTFINLSTNFY
Q7Y079	<i>D. glomerata</i>	QSLQEKA <u>SQW</u> SGVKQ <u>EDAFAID</u> NTLFQKLG--LQTFVNLSNFY
M5XFQ7	<i>P. persica</i>	QSLQAKAA <u>EW</u> SGVDSADA <u>FAIDES</u> NLFDFKLG--LQAFVNLSTNFY
Q94FG6	<i>H. vulgare</i>	QSLQDKASEW <u>SGV</u> AAAD <u>AF</u> AIDE <u>DEVN</u> FEALGGTPQPFVDSLSTNFY
O31607	<i>B. subtilis</i>	-----GQSFNAPYEAI <u>G</u> --EELLSQLVDTFY
	<i>T. fusca</i>	-----TF--YEAVGG-EETFTRLARRFY
Q67XG0	<i>A. thaliana</i>	FFRHTAFFLVAGNELK-NQNEK-PKHKP <u>QCA</u> CKHAANKPAEE----
R0GWW9	<i>C. rubella</i>	FFRHTAYFLVAGNDLK-NQDEK-PKH <u>QCA</u> CKHAAKPAEE----
K4CM33	<i>S. lycopersicum</i>	FFRHTAYFLVAGVELK-NQNKG-----TG <u>CKN</u> -CSHHH-----
G7I4F4	<i>M. truncatula</i>	FFRHTAYFLVAGDELK-NPNQQ-----IP <u>CK</u> HAAGK-DNS----
I1M9X1	<i>G. max</i>	FFRHTAFFLVAGDELK-NQNQH-----IP <u>CK</u> HAAAKKDDS----
D7SUH5	<i>V. vinifera</i>	FFRHTAFFLVAGDELK-NRNQG-----VP <u>CK</u> HGASKPAAV----
Q7Y079	<i>D. glomerata</i>	FFRHTAYFLVAGDELK-NQNLQ-----FO <u>CK</u> HGIQQTPAP----
M5XFQ7	<i>P. persica</i>	RLELTARHMVAGDELKKNQTQAQ---QPPPSKHGT <u>SNNH</u> A <u>CK</u> KKI
Q94FG6	<i>H. vulgare</i>	YLVYAA <u>YAMVAGNEMT</u> -RQTQS-----VPP <u>CK</u> HATNKPAE----
O31607	<i>B. subtilis</i>	FFRHTAFFLVNQTEAEDRSS-----
	<i>T. fusca</i>	FFRHTAFFLVNVPE-----

Figure S6 (Upper panel) Sequence alignments of the N terminal domain of nonsymbiotic plant haemoglobin class 3 (*A. thaliana* through to *H. vulgare*) and truncated bacterial haemoglobins (*B. subtilis* and *T. fusca*). Identified alpha helical segments forming the dimeric interface of AHb3 are shown in underlined bold. In all other class 3 plant proteins the 2 alpha helical sections are largely conserved, but absent in bacterial truncated proteins. (Lower panel) Sequence alignments of C-terminal domain of non-symbiotic plant haemoglobin class 3 (*A. thaliana* through to *H. vulgare*) and truncated bacterial haemoglobins (*B. subtilis* and *T. fusca*). One of the two cysteine residues of the disordered C terminal domain from *A. thaliana* is largely conserved in other class 3 plant globins (bold, underlined) with the possible exception of *P. persica*.