

The present document provides the supporting information for the article titled

**Structural and functional significance of the N- and C-terminal appendages
in *Arabidopsis* truncated hemoglobin**

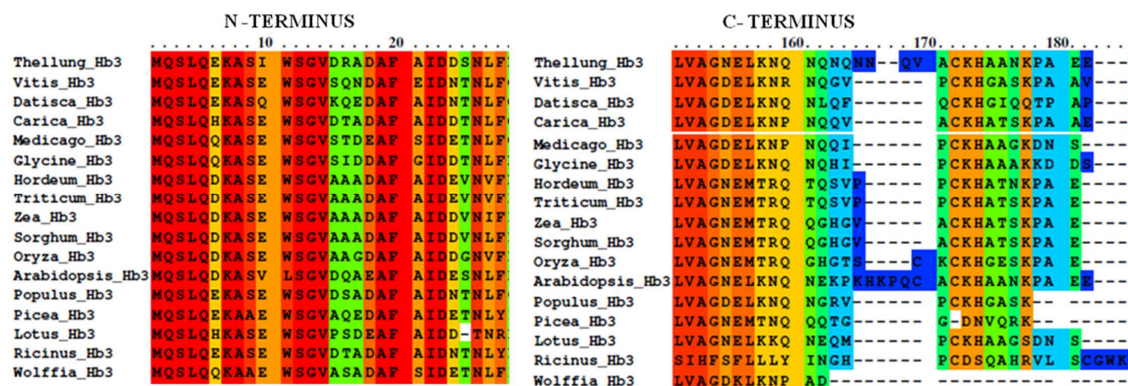
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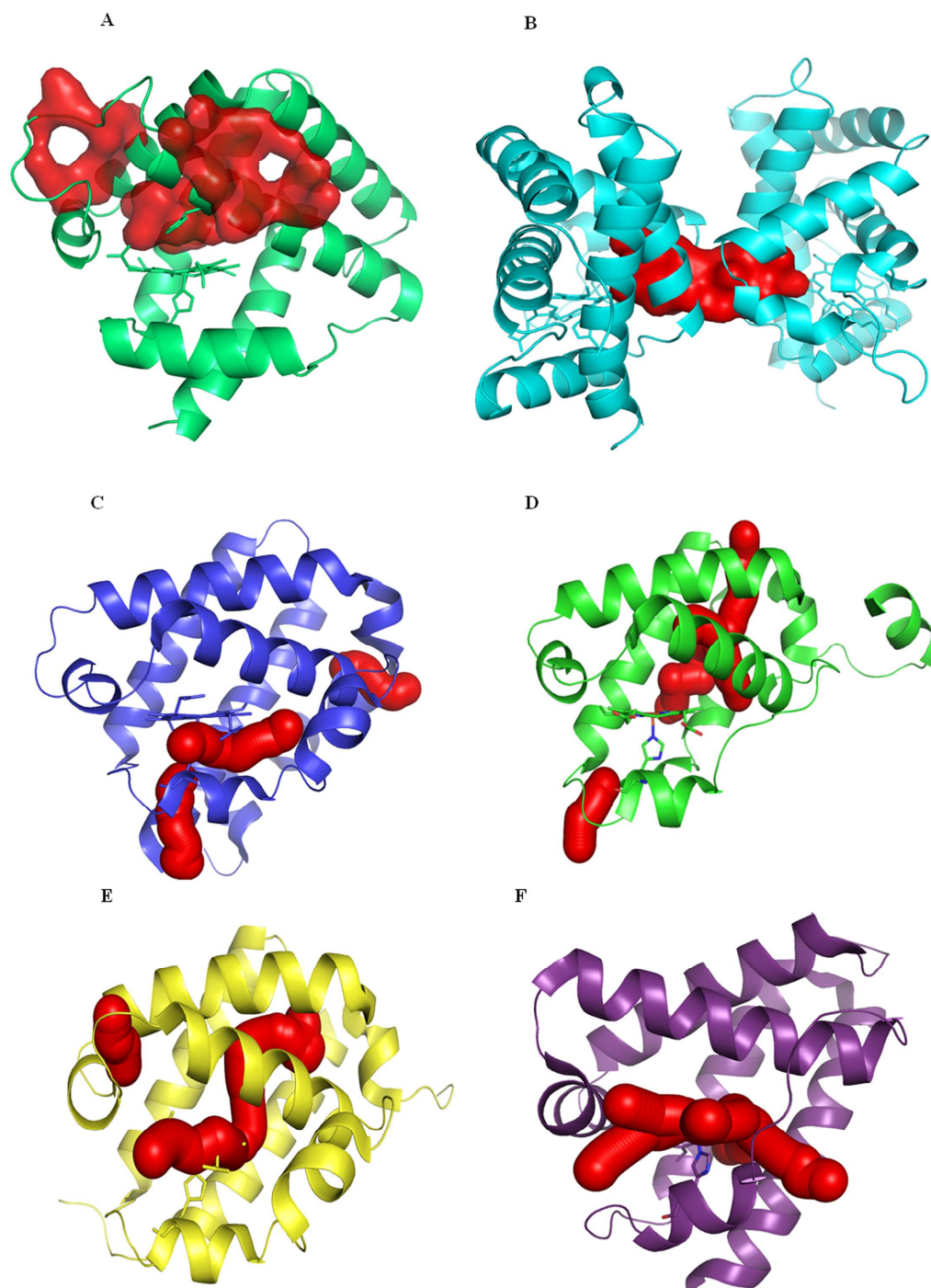
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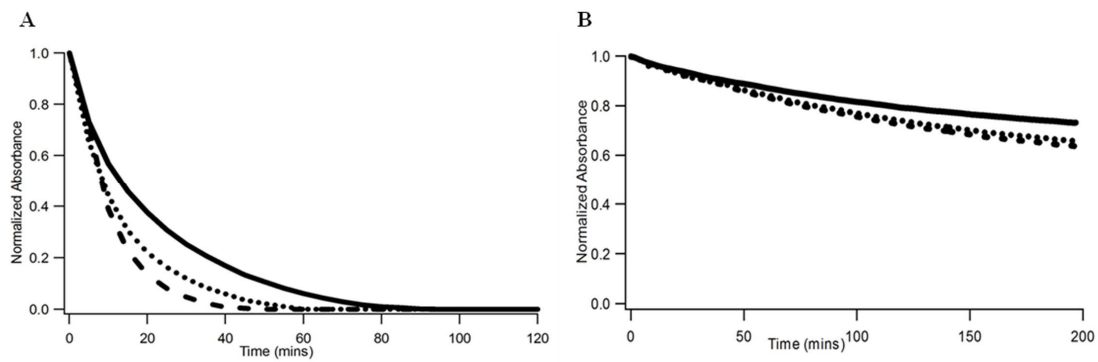
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Supplementary Fig. 1 Multiple sequence alignment of the N- and C-terminal extensions
among plant truncated hemoglobins display highly conserved N –terminus.

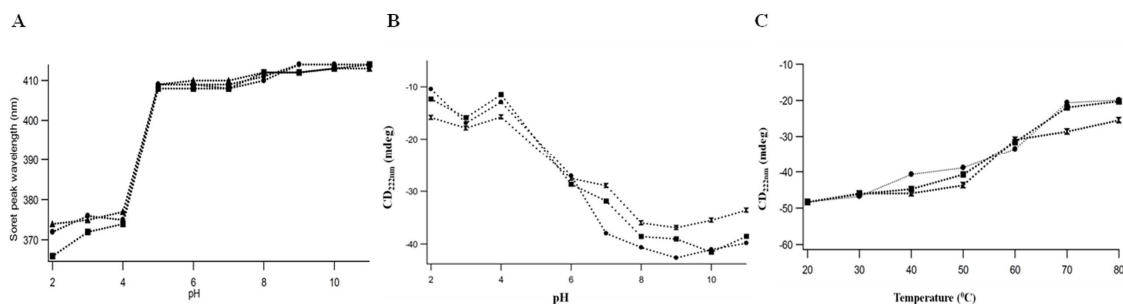


Supplementary Fig. 2 Comparative tunnel topologies of crystal structures of 3-on-3 and 2-on-2 hemoglobins (A) Symbiotic plant hemoglobin (Lba); Pdb id : 1MBN (B) *Arabidopsis* nonsymbiotic hemoglobin 1; Pdb id : 3ZHW (C) *Arabidopsis* truncated haemoglobin (trHb) 3; Pdb id : 4C44 (D) *Mycobacterium tuberculosis* trHbN-Group I trHb; Pdb id : 1IDR (E) *Geobacillus stearothermophilus* trHb- Group II trHb; Pdb id : 2BKM (F) *Campylobacter jejuni* trHb - Group III trHb; Pdb id : 2IG3. The tunnels are indicated in red.



Supplementary Fig. 3 Effect of the N- and C-terminal deletions on autooxidation and heme dissociation rates of AHb3.

(A) Time course displaying normalized change of ratio of $A_{581\text{nm}}/A_{630\text{nm}}$ for AHb3 (solid line), AHb3- $\Delta 25\text{C}$ (dotted line) and AHb3- $\Delta 25\text{N}$ (dash) indicating rates of autooxidation. (B) Time course displaying heme dissociation rates at pH 7.0 for AHb3 and its mutants. Normalized changes of the ratio, $A_{409\text{ nm}}/A_{630\text{ nm}}$, for the globins were plotted, indicating similar rates of heme dissociation.



Supplementary Fig. 4 Stability profiles of AHb3 and its mutants against denaturants.

(A) Comparative pH titration profile of AHb3 (circle), AHb3-Δ25C (square) and AHb3-Δ25N (triangle) probed by absorbance measurements display similar stability. Effect of (B) pH and (C) temperature on the integrity of secondary structure of AHb3 (circle), AHb3-Δ25C (square) and AHb3-Δ25N (triangle) were investigated using far-UV CD spectroscopy. CD_{222nm} was plotted as a function of the corresponding denaturing conditions. All the globins exhibited similar stability