Supplementary Information

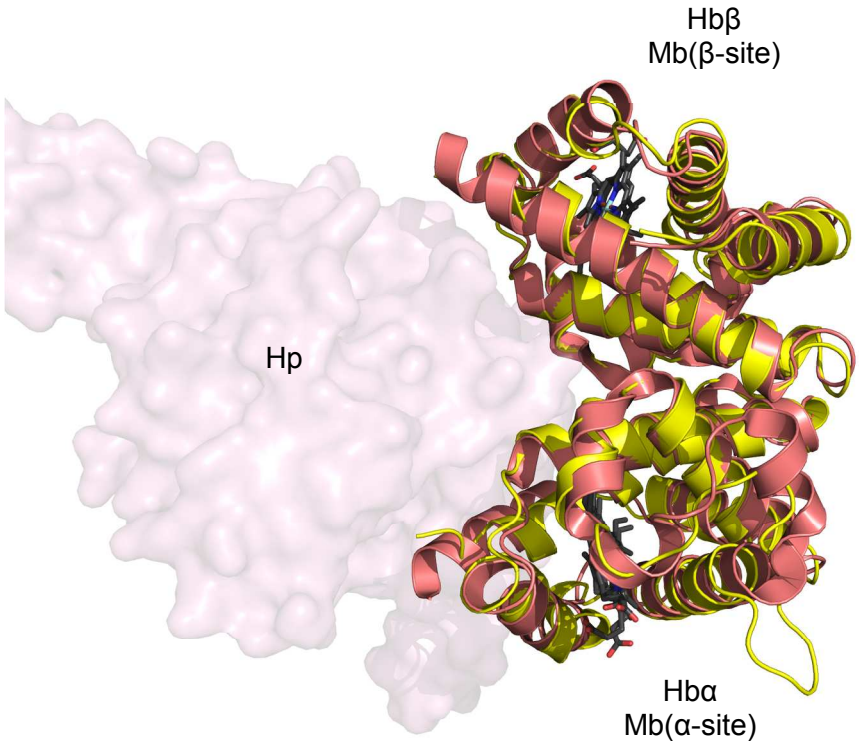


Figure S1. Structural alignment of free Mb and Mb bound to the Hp α-site and β. The 1.65 Å structure of free human Mb (PDB ID: 3RGK, red) superimposed on Mb from the homology model (yellow). The heme prosthetic group is shown as sticks and the total RMSD is 1.2 Å at both sites.

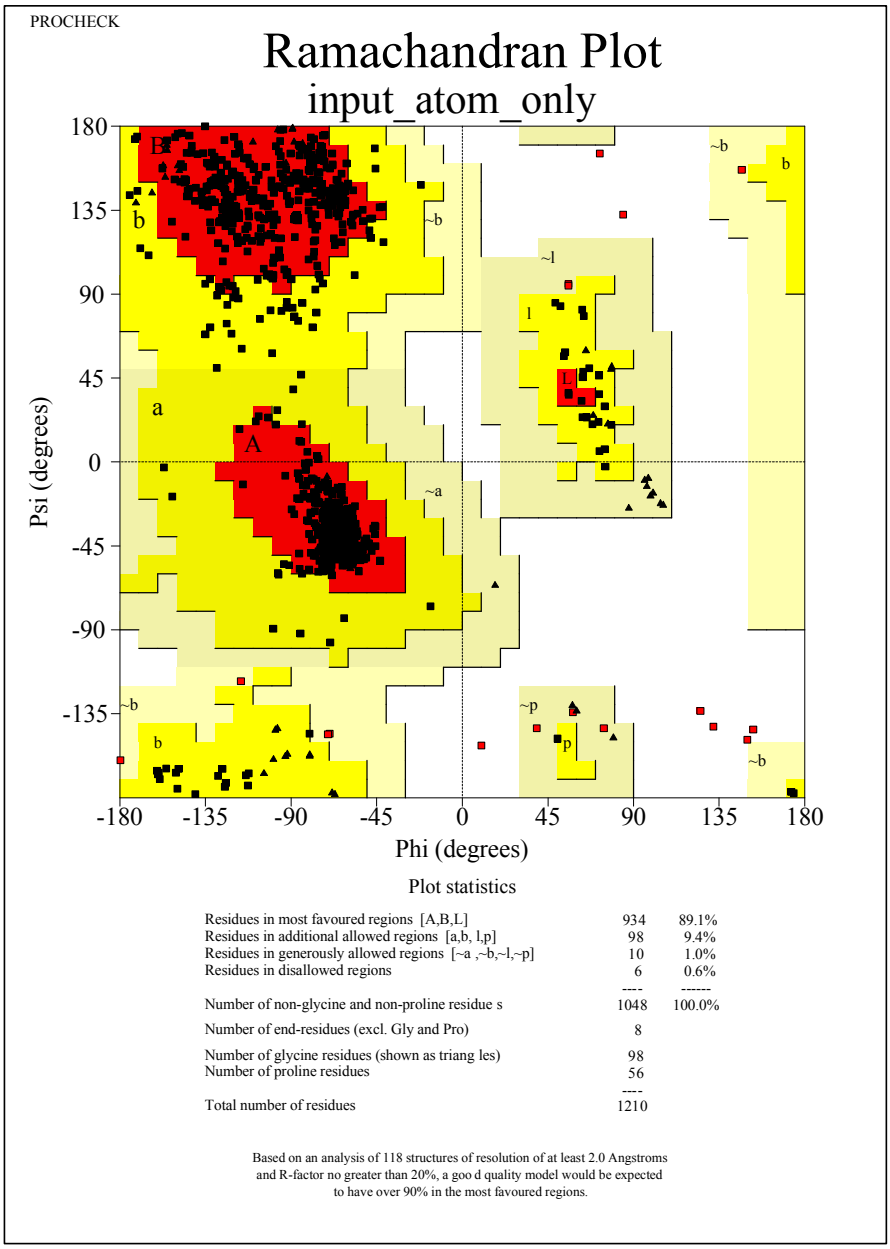


Figure S2. Ramachandran plot prepared by PROCHECK[36] for the Mb_2/Hp homology model shown in Figure 2A. Glycine and proline residues are represented by triangles and squares are used for all other residues. The most favorable regions of the Ramachandran plot are shown in red and contain 89.1% of the Mb/Hp residues. Additionally allowed regions (9.4%) are shown in dark yellow, generously allowed (1.0%) regions are in light yellow, and disallowed regions are in white. These regions contain 9.4%, 1.0% and 0.6% of the Mb/Hp residues.

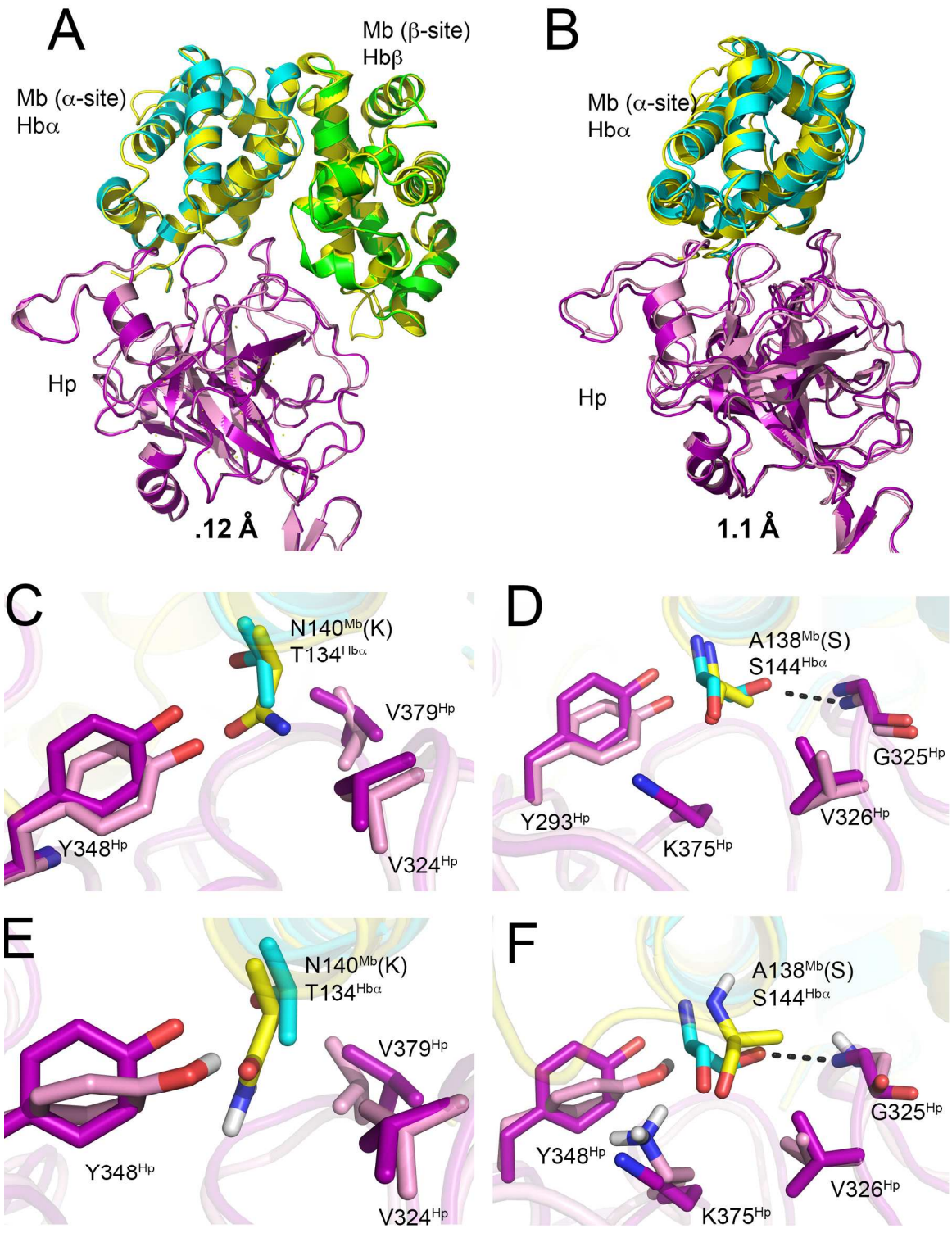


Figure S3. Structure of human Hb/Hp complex superimposed on the equine Mb/ human Hp (A) homology model and (B) docked models. (C-F) Closeups of the equine Mbα-site /Hp interface showing the equine Mb interface residues that are not identical to human Mb as yellow sticks.

The corresponding human residue is in parentheses. Structures: Hbα cyan, Hbβ green, Hp purple and models: Mb yellow, Hp pink, heme as sticks.

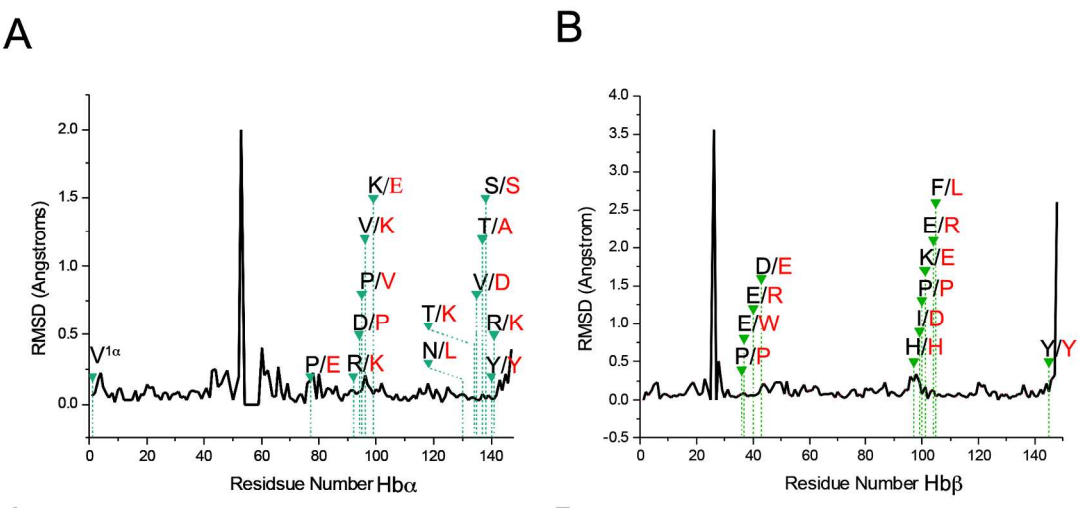
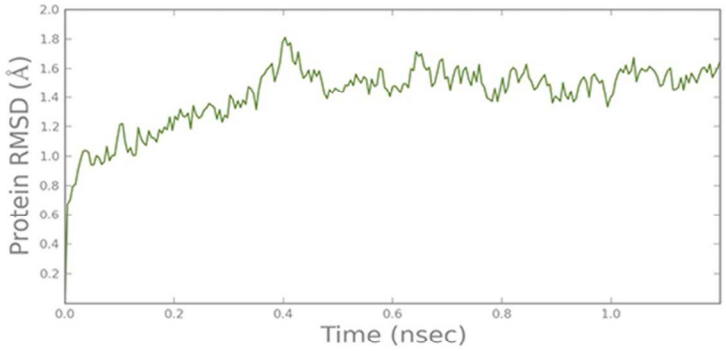
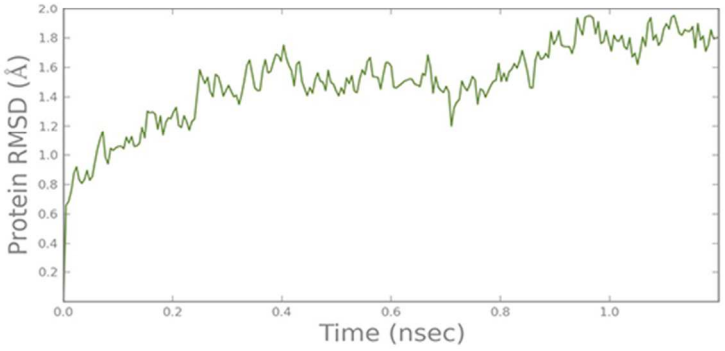


Figure S4. RMSD per residue in angstroms for structural alignments of Hb/Hp structures and Mb/Hp models as calculated by VMD[38]. (A) Hbα/Mb(α-site) RMSD. (B) Hbβ to Mb(β-site) RMSD. Hb residues, black and corresponding Mb residues, red.

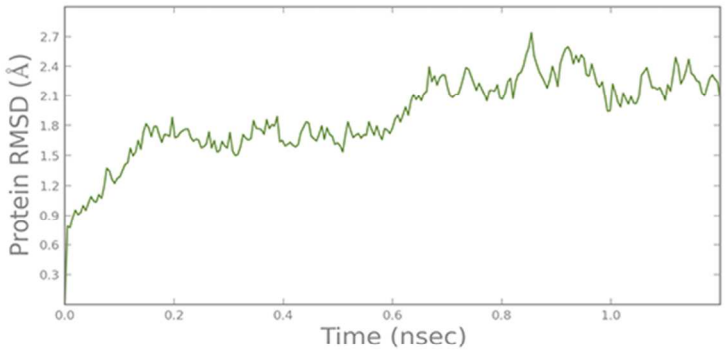
A.


Protein RMSD (Å)
Time (nsec)

B.


Protein RMSD (Å)
Time (nsec)

C.


Protein RMSD (Å)
Time (nsec)

D.

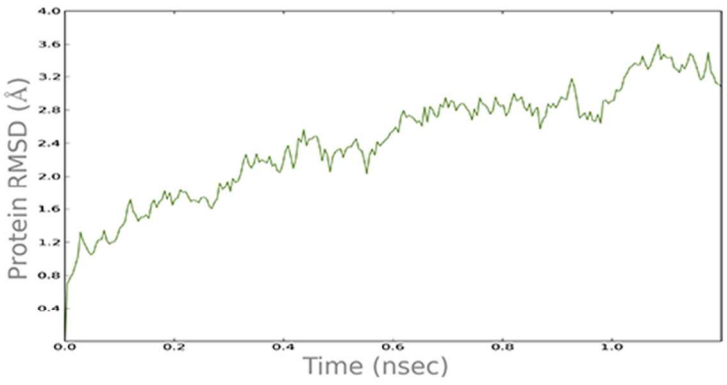

Protein RMSD (Å)
Time (nsec)

Figure S5. Results from 1.2 ns molecular dynamics (MD) simulations for (A) Hb/Hp (PDBID: 4F4O[2]), (B) Hbα/Hp model derived from Hb/Hp structure, (C) human Mb/Hp model and (D) equine Mb/ human Hp model. The root mean square deviations (RMSD) at time x, $RMSD_x$, was calculated using the Schrödinger interactive protein structure quality analysis tool as:

$$RMSD_x = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(r_i'\left(t_x\right) - r_i\left(t_0\right)\right)}$$

where N is the number of atoms in the structure, $r(t_0)$ and $r'(t_x)$ are the positions of the selected atom at time 0 and in the frame recorded at time t_x, respectively. Atom positions were determined after superimposing the structure at t_x on the reference structure at t_0. For the Hb/Hp simulation, the reference structure at t_0 is the X-ray crystal structure. For all other simulations, the reference structure is the model.

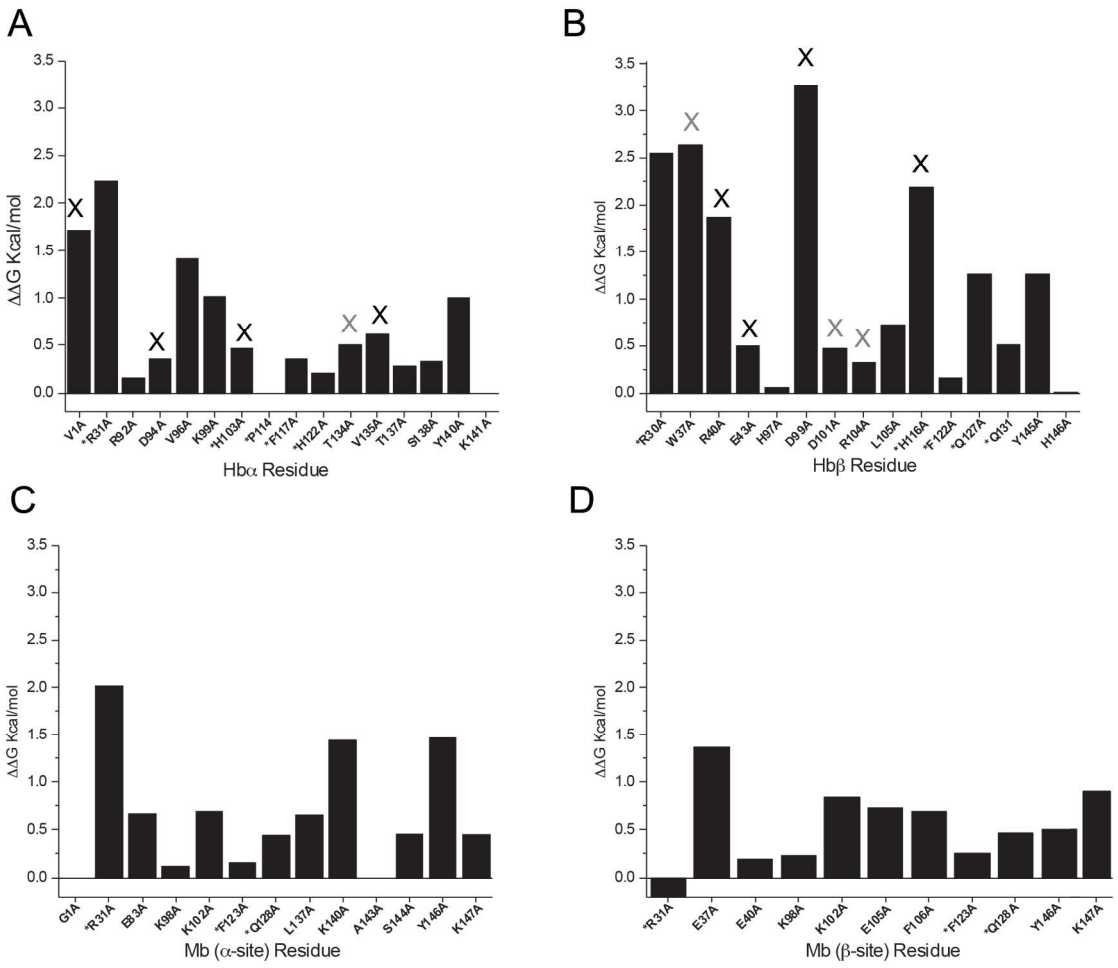


Figure S6. Predicted effects of alanine substitutions on the binding energy of the (A,B) Hb/Hp structures and (C,D) human Mb/Hp models calculated by the DrugScorePPI Web server[42] in kcal/mole. A black "X" denotes a non-conserved residue in Mb and a gray "X" denotes a partially conserved residue. The results were categorized into three sets: hot spots (ΔΔG ≥ 1.5 kcal/mol), warm residues (0.5 - 1.5 kcal/mol), and unimportant residues (< 0.5 kcal/mol).[50, 51] Residues on the horizontal axis marked with * are in the Hbα/β heterodimer interface.

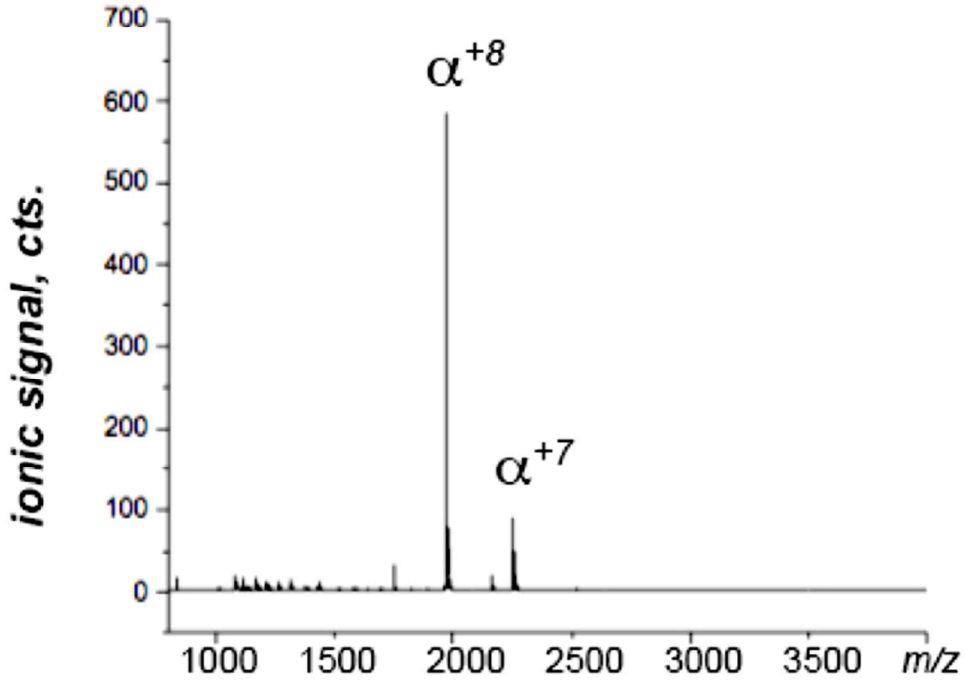


Figure S7. Native ESI mass spectra of isolated Hbα chains reconstituted with heme. The mass spectrum shows that isolated Hbα is monomeric.