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# THE CAMELS, HUMANS AND BOVINES HAEMOGLOBIN: IN SILICO AND MOLECULAR DYNAMICS PERSPECTIVE AND BINDING POTENCY WITH HAEME

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## ABSTRACT

This study compared the molecular dynamics (MD) of haemoglobin (Hb) bound to haeme in humans, camels, and bovines. The camel haemoglobin alpha and beta subunits showed larger amino acid differences, compared with bovine and human Hb. The bovine Hb was more phylogenetically related to human Hb than camel Hb. The camel haemoglobin structure complexed with haeme showed the highest stability by showing the lowest root mean square deviations (RMSD) and root mean square fluctuations (RMSF), compared with bovine and human structures. The Molecular Mechanics/Poisson Boltzman Surface Area (MM/PBSA) method was used to estimate the binding potency of haeme with the studied Hbs. The binding free energy of haeme was calculated to be -688.062 for bovines, -897.019 for camels, and -585.291 for humans. As a consequence, camel Hb had the highest binding potency, followed by bovines, and then humans. The structure and binding features of camel Hb contributes to its role in adaptation to dehydration and the harsh environment by adopting of higher affinity for haeme.

**Key words:** Camel, haemoglobin, haeme, molecular dynamics

Erythrocytes, red blood cells (RBCs), contain a significant amount of the haemoglobin molecule. It is a type of oxygen-carrying protein that is responsible for giving blood its distinctive red colour. Haemoglobin in adult vertebrates is made up of four different protein chains, two of which are alpha chains and the other two being beta chains (Storz, 2018). In camel haemoglobin from Asian and African camels, the amino acid His2 binds to 2,3-diphosphoglycerate (DPG) and substantially decreases the oxygen affinity. However, in camel haemoglobin from Andean camelids, a replacement of the amino acid at second position of β-chain (His2-to-Asn) increases affinity for oxygen by suppressing binding of DPG. This aids in adaptation of those small camelids to high altitudes. The vicuna is the camelid species native to greatest elevational zone in the Andes (4,500–5,000 m), and it also has the highest blood oxygen affinity (P50 = 17.5) of all the Andean camelids. A mutation from *Ala* to *Thr* at position α-Ala130 and a substitution from *His* to *Asn* at position β-His2 are thought to be responsible for exceptionally high oxygen affinity of vicuna haemoglobin (Storz, 2007). This may be the reason why camels prefer to dwell in lowland areas.

*Camelus dromedarius* haemoglobin is an intriguing case study of adaptability to live in deserts at extremely high temperatures. Camelids that live at different altitudes survive in a variety of climates and environments. The structural analysis of camelid haemoglobin allows researchers to link oxygen affinity to adaptation to extremely high and dry settings (Balasubramanian *et al*, 2009).

Erythrocytes in animals have a spherical or concave form, which has been extensively proven. Camels, on the other hand, have very enucleated, ovaloid erythrocytes that are exceedingly tiny, flat, and in vast numbers. The RBC of a camel is a great example of an organism's ability to adapt to harsh environments. It is important to note that haemoglobin found in camels is very enormous for an animal of its size (camel haemoglobin is around the size of a pea). Camel RBCs are alls exceptionally resistant to osmotic haemolysis and that they may grow to 240% of their usual volume before rupturing (Adah *et al,* 2016; Al-Bassam *et al,* 2007; Oyewale *et al*, 2011).

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This study was conducted to compare the molecular dynamics of human, camel and bovine Hb bound with haeme. The aspects of comparison comprise the structure stability, Hb backbone fluctuations, structure compactness, the number of hydrogen bonds between Hb and haeme during simulations and strength of binding between Hb and haeme in different structures. The results of this study will shed new insights into the differences in interactions of Hb with haeme in humans, camel and bovine.

## Materials and Methods

### *Retrieval of haemoglobin chains sequences*

The sequences utilised in this work were obtained from the GenBank database and the protein database, which are found at (https://www.ncbi.nlm.nih.gov/). Human, dromedary camel, and bovine sequences were retrieved. CLC genomics software was used to import the alpha and beta chain sequences (Qiagen software, Denmark).

### *Multiple sequence alignment and phylogenetic tree*

The sequences of alpha and beta Hb chains were aligned by the sequence alignment tool and the tree was generated by CLC genomic software.

### *Molecular dynamics simulations*

Molecular dynamics simulations set up and conditions were performed as previously described with slight modifications (Kandeel *et al,* 2018; 2021). GROMACS simulation package (GROMACS 2020.4) was used to perform molecular dynamic dynamics simulations. MD simulation of alpha chain of Hb was carried out for 100 ns in water using CHARMM36 forcefield; trajectory and energy files were written every 10 ps. The system was solvated in a truncated octahedral box containing TIP3P Water molecules. The protein was centered in the simulation box within a minimum distance to the box edge of 1 nm to satisfy the minimum image convention efficiently. Potassium/chlorine ions were added to the complex to neutralise the overall system. Minimisation was carried out for 5000 steps using Steepest Descent Method, and the convergence was achieved within the maximum force < 1000 (KJ mol-1 nm-1) to remove any steric clashes. All systems were equilibrated at constant- volume and constant- temperature (NVT) and isobaric–isothermal (NPT) ensembles for 100ps (50,000 steps) and 1000ps (1,000,000 steps), using time steps 0.2 and 0.1 femtoseconds (fs), respectively, at a temperature of 300 °K to ensure a fully converged system for the production run which was carried out at a constant temperature of 300 °K and a pressure of 1 atm or bar (NPT) using a weak coupling velocity-rescaling (modified Berendsen thermostat) Parrinello-Rahman algorithms, respectively. Relaxation times were set to $\tau$ T = 0.1 ps and $\tau$ P = 2.0 ps. All bond lengths involving the hydrogen atom were kept rigid at ideal bond lengths using the Linear Constraint Solver (lincs) algorithm, allowing for a time step of 2 fs. The Verlet scheme was used for the calculation of non-bonded interactions. Periodic Boundary Conditions (PBC) were used in all x, y, z directions. Interactions within a short-range cutoff of 1.2 Nm were calculated in each time step. Particle Mesh Ewald (PME) was used to calculate electrostatic interactions and forces to account for a homogeneous medium outside the long-range cutoff. The production was run for 100ns for the complex.

### *Binding energy calculations*

Molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) was used in the study of biomolecular interactions and computational drug design. MM-PBSA binding energy of chosen molecules was calculated *via* g mmpbsa. This tool calculated the enthalpic components of MM-PBSA interaction using GROMACS and APBS packages.

## Results and discussion

### *Comparison of humans, dromedary camel and bovine Hb*

Multiple sequence alignment was generated for the Hb sequence from humans, camels and bovines (Figs 1 and 2). In the alpha subunit (Fig 1), the camel Hb beta subunit showed 21 and 23 amino acid differences with bovines and humans Hb, respectively. This accounts for 87.2 and 83.6 identity%. In the beta subunit (Fig 2), the camel Hb alpha subunit showed 25 and 24 amino acid differences with bovines and humans Hb, respectively. This accounts for 84.1 and 82.8 identity%. In both subunits, the camel proteins were more distant to humans, compared with the bovine proteins.

Several studies have found that the distal histidine residues α:His58 and β:His63 play an important role in haeme binding (Ali *et al*, 2022; Jameson *et al*, 1980). They are also important in controlling the rate and affinity of oxygen binding to human haemoglobin. These residues were conserved in human, camel and bovine Hb (Figs 1 and 2).

### *Root mean square deviations (RMSD)*

RMSD was calculated for the complex based on 'Backbone' atoms using GROMACS program.

RMSD graph (Fig 3, Column A) for protein complex showed that the structure remained stable throughout the simulation time with some fluctuation within the range of ~1 Å, which is a normal behaviour of globular protein. Ligand RMSD was calculated for the ligand-based on the ligand's atoms using GROMACS program and it is shown in Fig 3, column A. RMSD of ligand remained reasonably stable and throughout the simulation for all 3 complexes. The camel structure complex showed highest stability by showing the lowest RMSD, compared with bovine and human structures (Fig 3A). The RMSD profile of Haeme was lower than the protein during all simulation time, which was lower than the protein RMSD, indicating stable binding with the examined proteins.

These findings agree with the previously reported camel Hb structure stability. At different conditions of temperature and salt, camel Hb was more stable than the human structure (Ali *et al*, 2022). The camel Hb showed higher stability over bovine and human structures.

***Root mean square fluctuations (RMSF)***

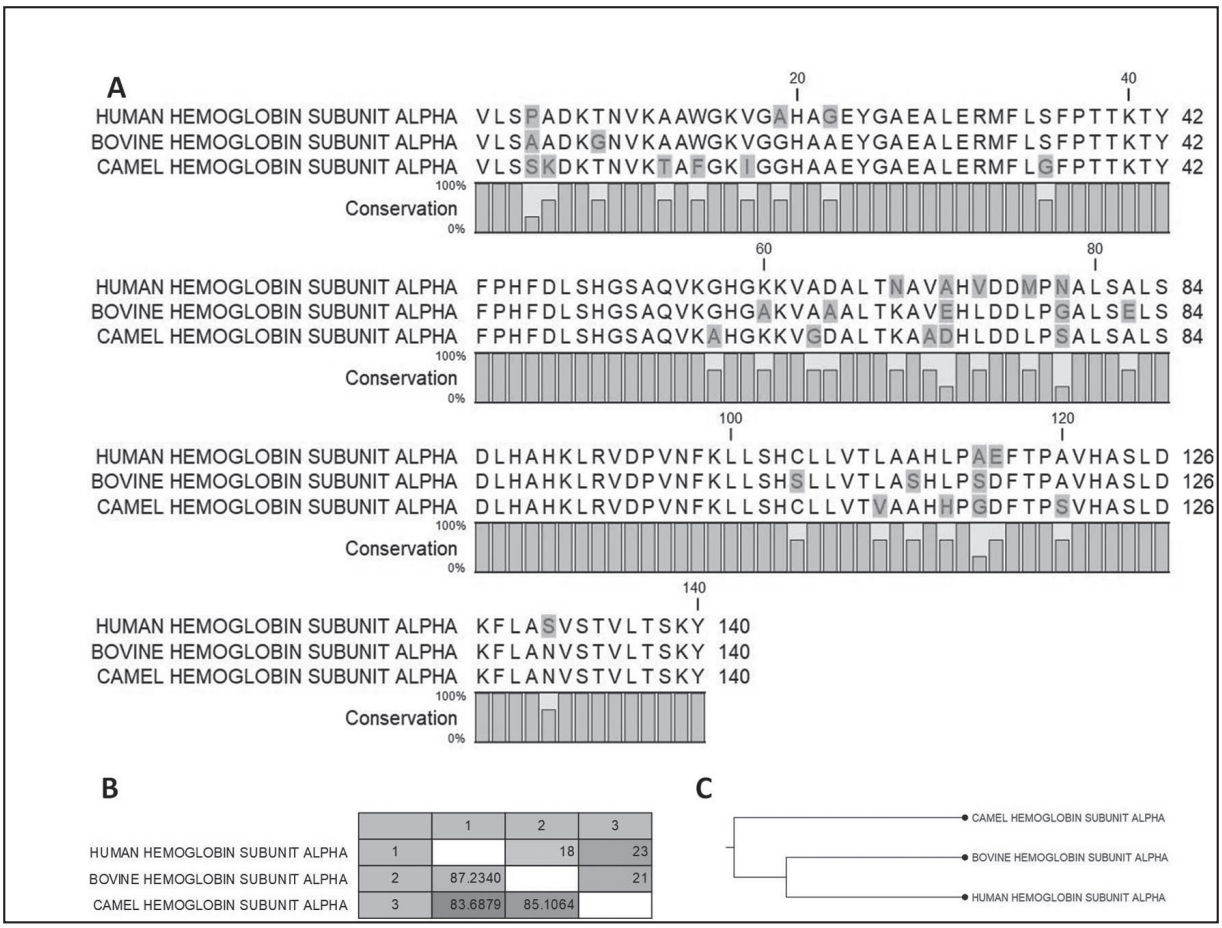
RMSF was calculated for protein complex based on 'C-alpha' atoms using GROMACS program. Overall, the fluctuation intensity remains below 2.5 Å except for some residues which represent a loop or turn in the protein (Fig 3, Column B). In this context, the camel protein residues showed the lowest RMSF value indicating the stability of complexes with Haeme.

***Radius of gyration (ROG)***

The radius of gyration was calculated for the complex based on 'C-alpha' atoms using GROMACS program. The slight fluctuation within the 1 Å Rog value during the MD simulation time indicates a slight opening and closing of the N and C terminal domains (Fig 3). This indicates the general compactness of the examined systems.

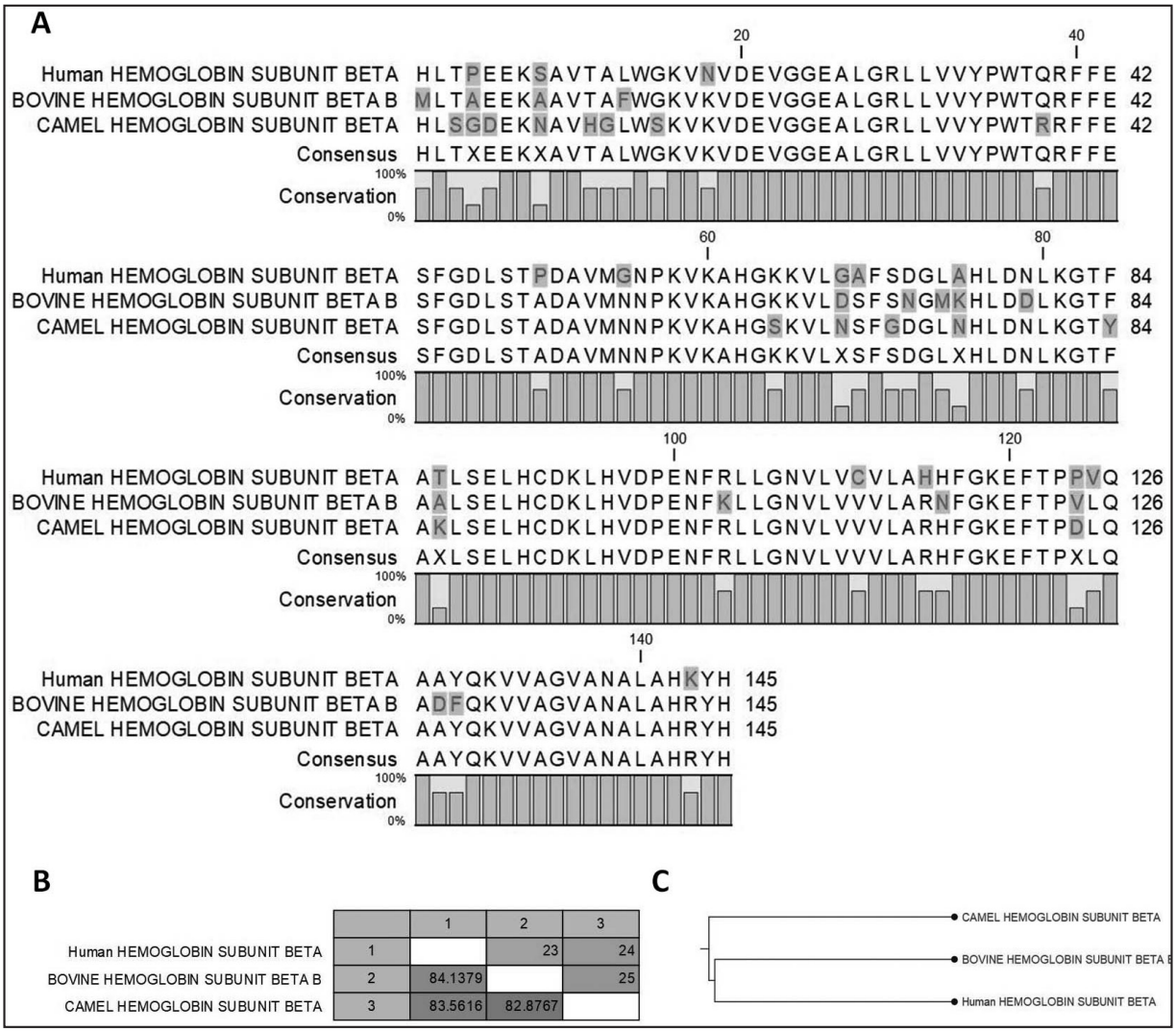
***Hydrogen bonds (Hb-haeme)***

The total number of hydrogen bonds formed between ligand and protein during 100 ns of the




| | 1 | 2 | 3 |
|---|---|---|---|
| HUMAN HEMOGLOBIN SUBUNIT ALPHA | 1 | | 18 | 23 |
| BOVINE HEMOGLOBIN SUBUNIT ALPHA | 2 | 87.2340 | | 21 |
| CAMEL HEMOGLOBIN SUBUNIT ALPHA | 3 | 83.6879 | 85.1064 | |

**Fig 1.** Sequence comparisons of Bovine, Camel and Human HB Alpha subunit. A) Pairwise sequence alignment. The different residues are highlighted. B) Pairwise sequence comparison. The upper right panel is the number of differences. The lower left panel is the percent identity. C) Phylogenetic relations of Bovine, Camel and Human HB Alpha subunit.




| | | 1 | 2 | 3 |
|---|---|---|---|---|
| Human HEMOGLOBIN SUBUNIT BETA | 1 | | 23 | 24 |
| BOVINE HEMOGLOBIN SUBUNIT BETA B | 2 | 84.1379 | | 25 |
| CAMEL HEMOGLOBIN SUBUNIT BETA | 3 | 83.5616 | 82.8767 | |

**Fig 2.** Sequence comparisons of bovine, camel and human HB Beta subunit. A) Pairwise sequence alignment. The different residues are highlighted. B) Pairwise sequence comparison. The upper right panel is the number of differences. The lower left panel is the percent identity. C) Phylogenetic relations of Bovine, Camel and Human HB Alpha subunit.

simulation time is shown in (Fig 4). A conserved average of one Hbond was observed in all examined structures. Therefore, there are almost conserved features of the binding space around haeme in the studied structures.

### *Average center-of-mass distance*

The average center-of-mass distance between ligand and protein during 100 ns of the simulation time is shown in (Fig 4). There was no observable difference in human, camel and bovine structures.

### *Contact frequency (CF) analysis*

To further evaluate the binding between the protein and the ligands, a contact frequency (CF) analysis was performed using contact free.tcl module in Visual Molecular Dynamics (VMD) with a cutoff of 4 Å. The residues with higher CF% are shown in (Fig 5). The camel protein showed higher contact frequency with PHE98, HIS45, ASN97 and LEU129. There was almost conserved contact time with important residue α:His58, implying almost conserved binding with this residue. The residues α:Phe43 and α:Phe98 showed higher contact frequency with haeme in camels than in human or bovine Hb (Fig 5). This agrees with the previously described role of these residues in sustained interaction with haeme at extreme salinity and temperatures (Ali *et al*, 2022).

### *Potential energy, pressure and temperature*

The potential energy, pressure, and temperature of the system during 100 ns of MD simulation as obtained from GROMACS edr file are shown in (Fig 6). The graph shows converged potential energy,

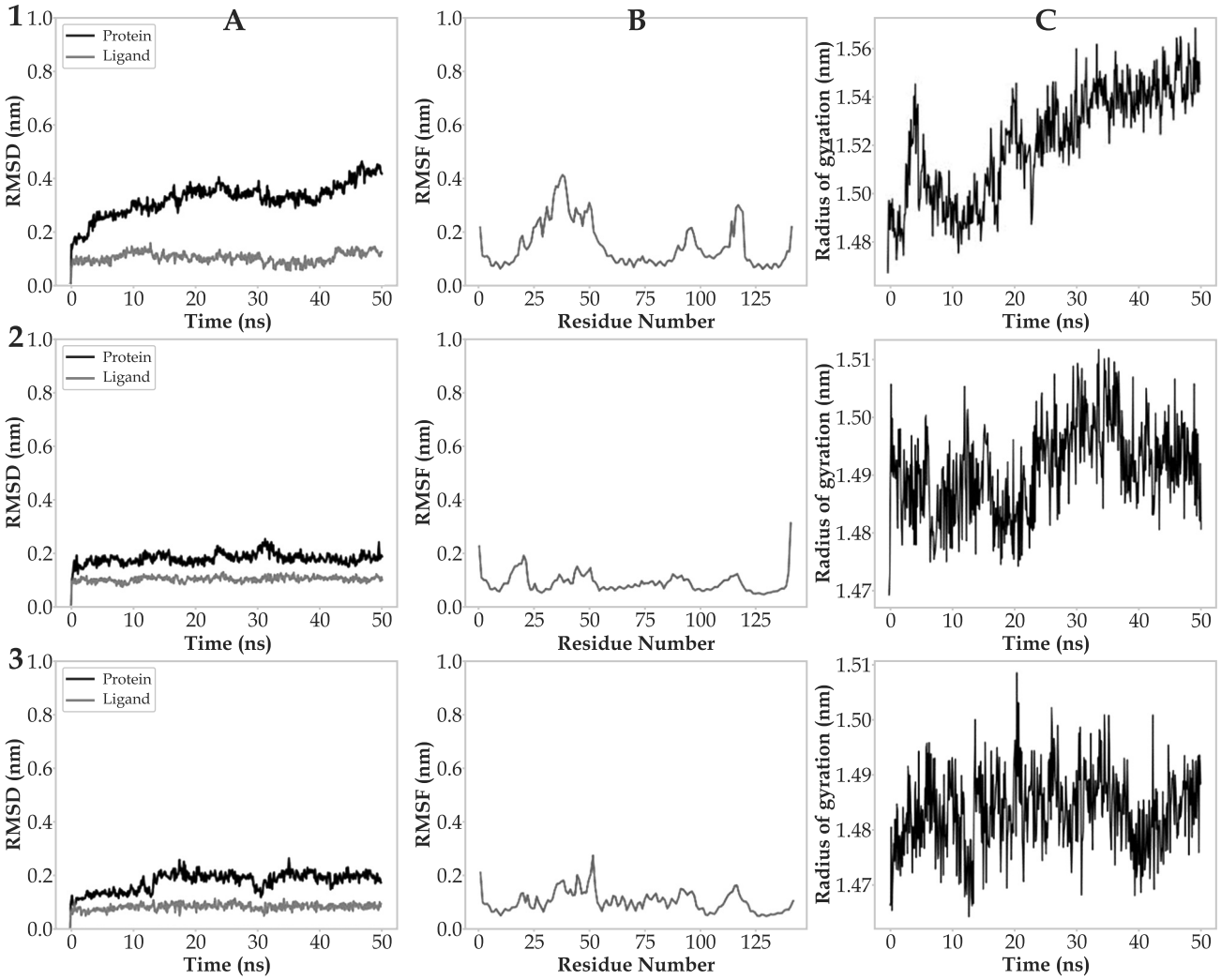



**Fig 3.** RMSD of the bovine, camel and human HB bound with Haeme. From left to right: (A) RMSD, (B) RMSF and (C) Radius of gyration of the complexes during 100ns MD simulation. Bovine (top), Camel (middle) and Human (bottom).

pressure, and temperature throughout the 100ns simulations.

### *MM/PBSA binding energy*

MM/PBSA method was selected for rescoring complexes because it is the fastest force field-based method that computes the free energy of binding, as compared to the other computational free energy methods, such as free energy perturbation (FEP) or thermodynamic integration (TI) methods. The MM/PBSA calculation was performed using g-mmpbsa software. The calculated binding free energies are shown in table 3.

The binding capacity of the camel protein with haeme was found to be the highest. It was estimated that the binding free energy of haeme was -688.062 ± 58.805 for bovine, -897.019 ± 42.708 for camel, and -585.291 ± 70.592 for humans, respectively. As a result, the binding potency was recurod highest in camel Hb, followed by bovines, and then humans.

In conclusion, after 100 ns simulation, the camel Hb showed lower backbone RMSD, RMSF and higher binding potency with haeme. The stronger haeme binding with Hb indicates stronger oxygen binding power, higher oxygen transport and storage capacity and higher tissue oxygen delivery. These features support the higher structural competency of camel Hb to adapt to adverse conditions.


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**Table 3.** Calculated binding free energies of Haeme binding with Bovine, Camel and Human HB [kJ/mol].

| Complex | ΔG | van der Waal energy | Electrostatic energy | Polar solvation energy | Solvent-accessible surface area (SASA) energy |
|---|---|---|---|---|---|
| Bovine | -688.062 ± 58.805 | -246.185 ± 14.942 | -857.242 ± 171.840 | 443.664 ± 127.511 | -28.298 ± 1.935 |
| Camel | -897.019 ± 42.708 | -264.881 ± 4.544 | -862.527 ± 82.713 | 257.009 ± 78.213 | -26.619 ± 0.844 |
| Human | -585.291 ± 70.592 | -257.899 ± 10.052 | -552.638 ± 174.680 | 251.729 ± 106.498 | -26.482 ± 1.453 |

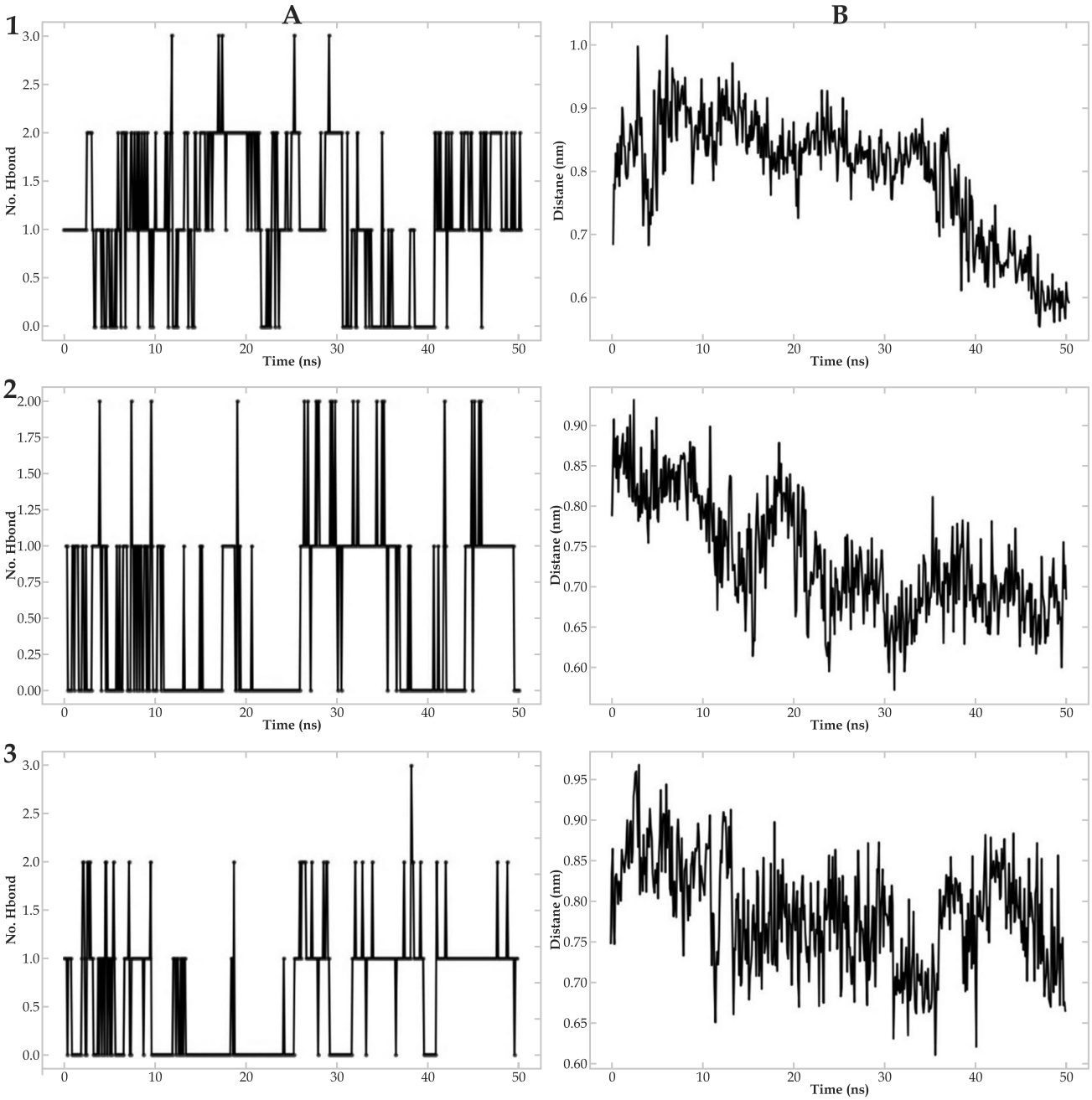


**Fig 4.** Hydrogen bonds and distance of the bovine, camel and human HB bound with haeme. (A) Hydrogen Bonds (Protein-ligand) and (B) Average distance between ligand and the protein for of the complexes during 100ns MD simulation. Bovine (top), Camel (middle) and Human (bottom).

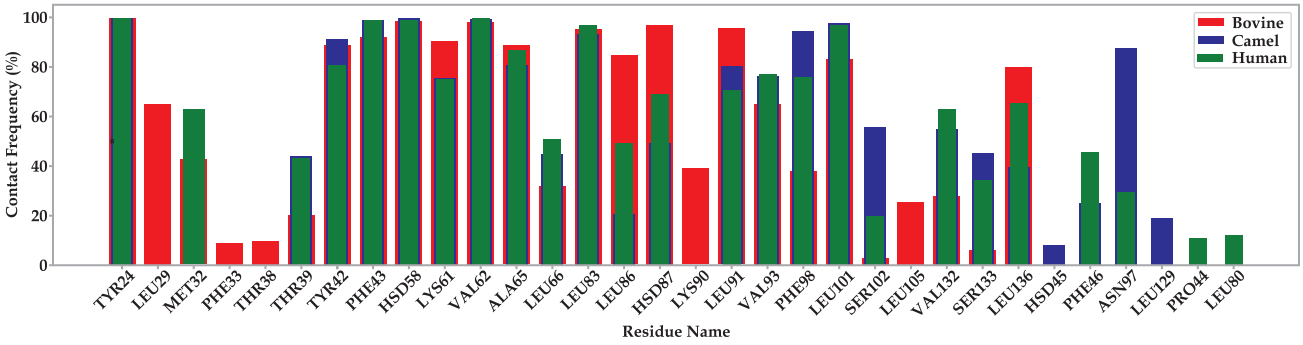


**Fig 5.** Contact frequency (CF) analysis of Bovine, Camel and Human HB with Haeme.

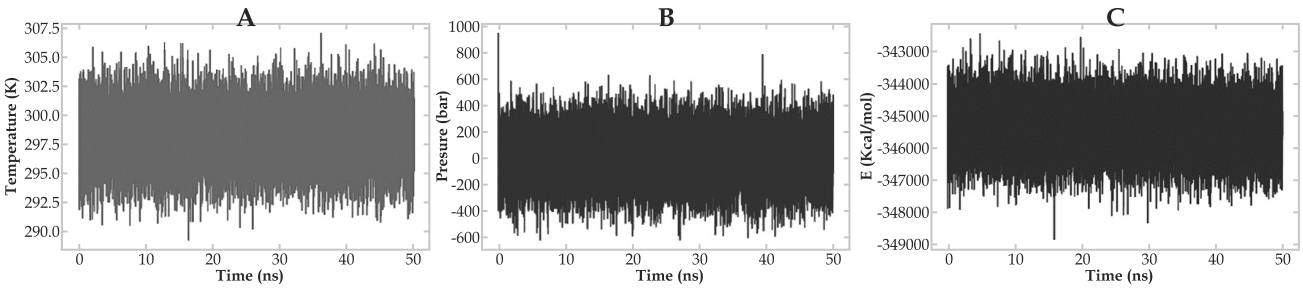


**Fig 6.** Potential energy, pressure and temperature during MD simulation. (A) temperature, (B) pressure and (C) potential energy during the 100ns MD simulations.

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