Protein & Peptide Letters, 2025, *32*, 519-524

RESEARCH ARTICLE

Purification, Crystallization, and Preliminary X-ray Diffraction Studies on Hemoglobin from the Angora Goat (*Capra Aegagrus Hircus*)

Farheen Pervez[1], Shobana Nagaraj[2], Mpho Setshedi[1], Arshad Mather[3], Ponnuswamy Mondikalipudur Nanjappa Gounder[4], Yasien Sayed[1] and Ramesh Pandian[1,*]

[1]*Protein Structure-Function Research Laboratory, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg 2050, South Africa;* [2]*Chromium Research Group, Department of Physics, University of Johannesburg, Auckland Park, Johannesburg 2006, South Africa;* [3]*Agricultural Research Council - Onderstepoort Veterinary Research, Private Bag X5, Onderstepoort, Pretoria 0110, South Africa;* [4]*Centre of Advanced Study in Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India*

Abstract: ***Introduction*:** Angora goats are a distinct breed that differs significantly from common goats and shares a similar appearance to sheep. In Angora goats, only the level of glutathione (GSH) is elevated during under-stimulated conditions, as well as after the period of hypoxic stress; however, no changes are found in 2,3-diphosphoglycerate (2,3-DPG) levels, which are commonly present in the red blood cells (RBCs) of most mammals. We chose the Angora goat for our investigation because no previous studies have been conducted on the structural and functional aspects of hemoglobin (Hb). In addition, no sequence or structural information is currently available in any database.

***Methods*:** Angora goat Hb was isolated and purified by anion-exchange chromatography, followed by crystallization using various methods. X-ray data collection for Angora goat Hb was performed under a liquid nitrogen cryo-stream using a *Bruker D8 Venture Bio Photon III 28-pixel array area detector* system.

***Results*:** Good diffracting crystals were obtained using the hanging-drop vapor-diffusion method with polyethylene glycol (PEG) 3350 as the precipitant in water, without the addition of any salt or buffer. The Angora goat Hb diffracted to a resolution of 1.85 Å, and the structure solution was obtained by the molecular replacement method, using the structure of domestic goat Hb as the starting model.

***Discussion*:** The solved structure of Angora goat crystallized in the monoclinic space group $P2_1$, consisting of one whole biological molecule in the asymmetric unit, with unit cell dimensions of a = 52.08 Å, b = 76.70 Å, c = 74.08 Å, and β = 91.77 °. The solvent content and Matthews coefficient (Vm) for the Angora goat Hb are 49.05% and 2.41 $Å^3$/Da, respectively, and are within the normal range for protein crystals.

***Conclusion*:** Purification, crystallization, and preliminary X-ray diffraction studies of Angora goat Hb were performed successfully. Structural refinement and biophysical characterization of Angora goat Hb are in progress in the absence and presence of GSH and 2,3-DPG.

ARTICLE HISTORY

Received: December 04, 2024
Revised: March 24, 2025
Accepted: April 21, 2025

DOI:
10.2174/0109298665370730250708065019

Keywords: Angora goat, hemoglobin, oxygen affinity, level of glutathione, hypoxic stress, protein crystals.

1. INTRODUCTION

Mammalian hemoglobins (Hbs) consist of four polypeptide chains (tetrameric form; α1β1 and α2β2); namely, two α- and two β-chains with 141 and 146 amino acids (AAs) in each chain, respectively, with the presence of an individual heme group in each chain [1-4]. Hemoglobin is an allosteric protein that maintains cooperativity during oxygen binding and release by transitioning between two alternative quaternary structures: the T state (tensed; deoxy form), which has low oxygen affinity, and the R state (relaxed; oxy form), which has high oxygen affinity [5-10]. Subsequently, structural analysis of hemoglobin from various species revealed the presence of several hemoglobin quaternary states, which include R2, RR2, R3, B, and Y [11-14]. In all mammals, 2,3-DPG is an important allosteric modulator of hemoglobin function and is available in red blood cells at concentrations between 4 and 13 mM/L [15-22].

*Address correspondence to this author at the Protein Structure-Function Research Laboratory, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg 2050, South Africa;
E-mail: ramesh.pandian@wits.ac.za

Previously, Moorthy *et al.* published work on the purification, crystallization, and preliminary X-ray diffraction studies on the (domestic) goat. The research in our current study differs from the domestic goat study by Moorthy *et al.* [23]. The unique features of our Angora goat are discussed below. Angora goats are a distinct breed that differs significantly from common goats, which are more closely related in appearance to sheep. Angora goats possess horns that are twisted in a spiraling pattern. They have long, woolly hair hanging in corkscrew ringlets, which are fine, white, and as shiny as silk. In addition, Angora goats produce fattier milk than common goats [24]. GSH and 2,3-DPG are available in significant quantities in the RBCs of mammals, including the Angora goat [25, 26]. Angora goats, under simulated conditions of altitude and hypoxic stress up to 5500 m, show an increased level (from 33% to 45%) of packed cell volume and concentration of hemoglobin (from 11.4 g/dL to 16.0 g/dL) in RBCs. In Angora goats, only the level of GSH is raised during, as well as after, the period of hypoxic stress, to approximately 20 mg/dL, but no changes are found in 2,3-DPG levels under any condition [26].

A significant component of the rural economy in South Africa's semi-arid regions is Angora goat farming. This activity produces more than half of the world's mohair, which is valued for its shine and sheen [27, 28]. Remarkably, South African Angora goats experience several poorly understood clinical diseases, including weaning shock, habitual abortion, and swelling diseases in their juvenile and adult years, but not in their kids [29-36]. It is worthwhile to note that the conditions mentioned above have been investigated using hematology and serum chemistry tests on blood. Considering the background and investigations mentioned above, we found that the amino acid sequences and three-dimensional structure of hemoglobin from Angora goats are not available in sequence (UniProt) and structural (Protein Data Bank; PDB) databases, respectively. In this regard, we were determined to solve the high-resolution structure of hemoglobin from the Angora goat. In this work, we report on the purification, crystallization, and preliminary X-ray diffraction analysis of Angora goat hemoglobin.

2. MATERIALS AND METHODS

2.1. Isolation and Purification

The procedures for purifying hemoglobin from different species were described by Sundaresan *et al.* and Ramesh *et al.* [36], respectively. Briefly, to avoid clotting, 5 mL of fresh whole blood (WB) from an Angora goat was collected in a prestored ethylenedia-minetetraacetic acid (EDTA) tube at the Agricultural Research Council - Onderstepoort Veterinary Research, Pretoria, South Africa. The RBCs were isolated from the WB by centrifugation at 1400 x g for 20 minutes at 4˚C. After centrifugation, the pellet was recovered and washed thrice with two volumes of 0.9% (*w/v*) saline solution. The recovered pellet was hemolyzed by the addition of three volumes of distilled/milliQ water with respect to the volume of the original blood sample. This solution was kept at 4˚C for 30 minutes for hemolysis to occur. After hemolysis, the mixture was centrifuged again at 5600 × g for 1 h at 4˚C, yielding a hemoglobin solution in the supernatant with no cell debris. Subsequently, the supernatant was loaded onto a 5 mL DEAE-cellulose anion-exchange column from Merck (Cytiva 17-5154-01; bed size: 16 mm × 25 mm; bed volume: 5 mL; matrix: 6% cross-linked agarose; particle size: 45-165 µm) pre-equilibrated with water [37]. Hemoglobin eluted at 0.1 M NaCl at a flow rate of 3 mL/min using a 0-1.0 M NaCl salt gradient (Figure **1**). The elution profile with UV absorbance and the Soret absorption band for Angora goat Hb near 420 nm is shown in Figure **1** [38, 39]. Analysis of the two selective fractions of purified Angora goat Hb was performed on a 12.5% native-PAGE, showing a single band in Lanes 1 and 2, respectively (Figure **2**) [40]. The purified Angora goat Hb was dialyzed against MilliQ water.

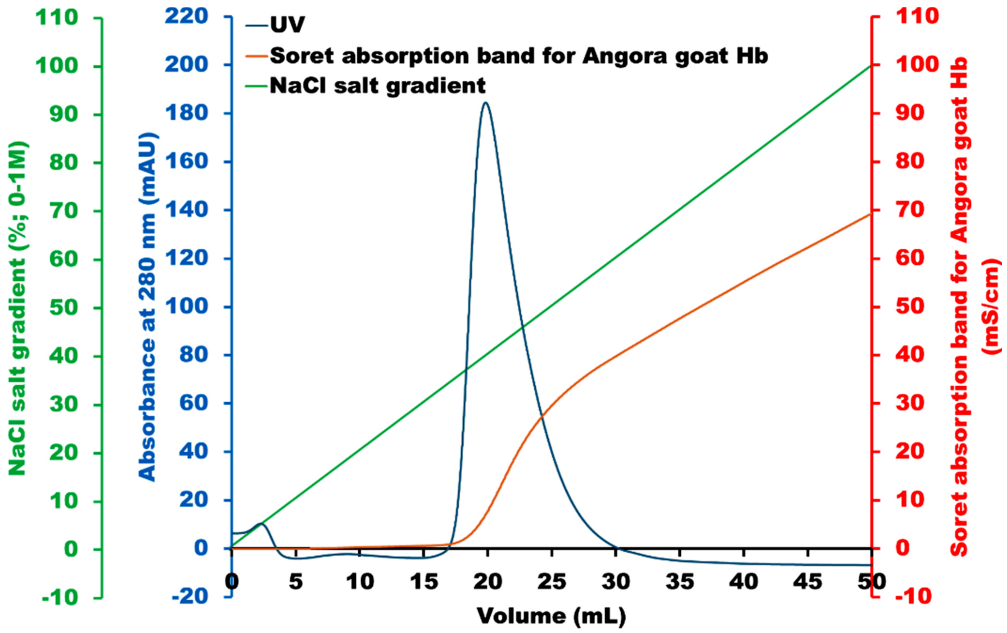

Figure 1. Elution profile of Angora goat Hb. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

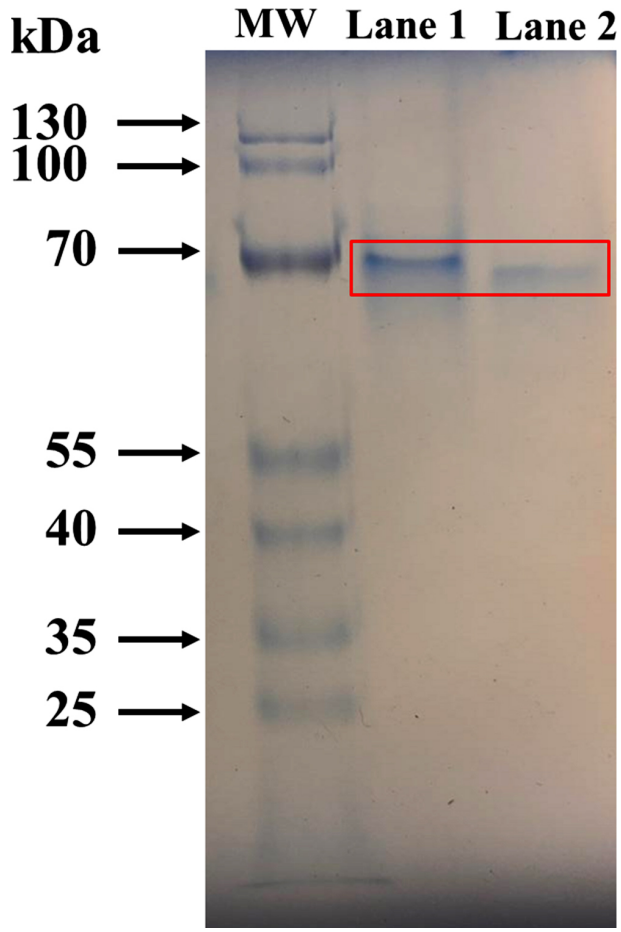

Figure 2. 12.5% Native-PAGE of Angora goat Hb (MW: Molecular weight marker; Lane 1 and Lane 2: Purified fractions 1 and 2 of Angora goat Hb, respectively). *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

2.2. Crystallization

Initially, crystallization conditions for Angora goat hemoglobin (Hb) were screened using sitting-drop vapor-diffusion and micro-batch methods with a PEG/Ion screening kit (Hampton Research, USA). The kit uses polyethylene glycol 3350 (PEG 3350) as a precipitant under varying protein concentrations. We obtained good diffracting crystals of Angora goat Hb by the hanging-drop vapor-diffusion method.

2.3. Data Collection and Processing

A high-quality crystal of Angora goat Hb (Figure **3**) was mounted in a nylon cryo-loop and soaked briefly with an oil-based cryoprotectant, Parabar 10312 (Hampton Research, USA), previously called Paratone, to reduce temperature damage to the crystal. Data collection was performed at 100 K under a constant liquid nitrogen cryo-stream using a *Bruker D8 Venture Bio Photon III 28-pixel array area detector* with dual source available at our in-house X-ray facility at the University of the Witwatersrand, Johannesburg, South Africa. Preliminary data collection for calculating the unit cell parameters and full data collection belonging to the Angora goat Hb was done using a *Cu Kα IμS DIAMOND* source (50 kV, 1.2 mA) controlled by the *PROTEUM4* software package [41]. One of the diffraction images belonging to the Angora goat Hb is shown in Figure **4**. The diffraction images were processed using *SAINT* for data integration, *SADABS* for empirical absorption correction and data scaling, and *SCALEPACK* and *AIMLESS/ POINTLESS* for generating an MTZ file, all of which are built into the *PROTEUM4* software suite [41-46]. Data collection and processing statistics are listed in Table **1**.

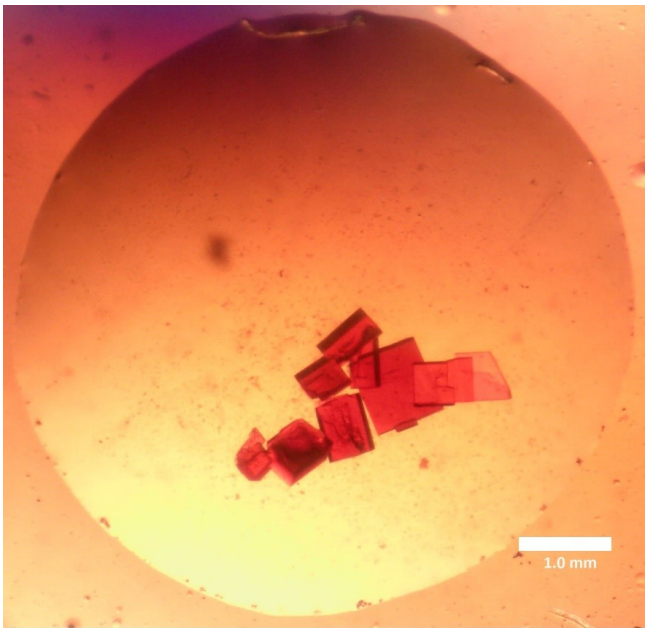

Figure 3. Crystals of Angora goat Hb. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

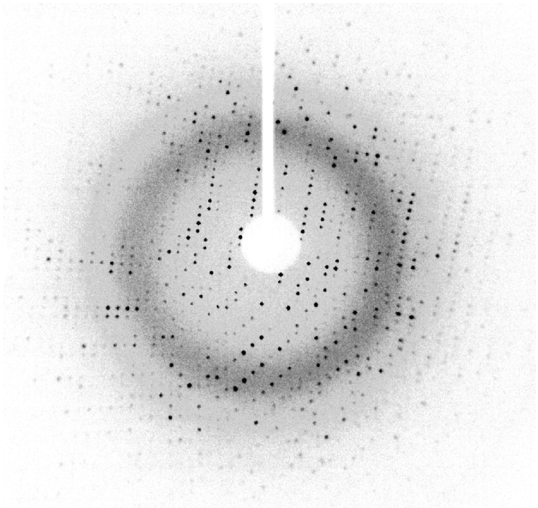

Figure 4. Diffraction pattern of Angora goat Hb crystal. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3. RESULTS AND DISCUSSION

The native form of hemoglobin was purified from the Angora goat to a final concentration of 22.5 mg/mL in MilliQ water in the absence of salt. Good-quality crystals

Table 1. Data collection and processing statistics of Angora goat Hb.

Light Source	Cu Kα
Wavelength (Å)	1.5418
Detector	*Bruker D8 Venture Bio PHOTON III 28-pixel area detector*
Temperature (K)	100
Crystal to detector distance (cm)	10.1
Exposure time (seconds)	40
Oscillation angle (°)	1.0
Space group	$P2_1$
Unit cell parameters (Å, °)	-
a, b, c, β,	52.08, 76.70, 74.08, 91.77
Resolution range (Å)	24.79 -1.85 (1.89-1.85)*
No. of observed reflections	470340 (11214)*
No. of unique reflections	49759 (3042)*
Completeness (%)	99.9 (99.4)*
I/σ(I)	8.4 (0.8)*
Rmerge¥	0.188 (1.662)*
Multiplicity	9.5 (3.7)*
Matthews coefficient V_M(Å3/Da)	2.41
Solvent content (%)	49.05

Note: *Values in parentheses are for the highest-resolution shell.
¥$R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the observed reflection and $\langle I(hkl)\rangle$ is the mean intensity of the reflection.

suitable for diffraction were obtained within a week using the hanging-drop vapor-diffusion method. The reservoir buffer consisted of 1 mL 12.5% PEG 3350 in water. A single drop consisted of 3 µL protein solution and 3 µL of reservoir solution. The crystals belonged to the monoclinic space group $P2_1$, as indicated by systematic absences, with unit cell parameters of a = 52.08 Å, b = 76.70 Å, c = 74.08 Å, and β = 91.77°. The data were processed to a resolution of 1.85 Å with a completeness of 99.9%. The data collection statistics are given in Table **1**. Evaluation of the crystal packing parameters revealed that the lattice accommodates one full biological molecule in a tetrameric form of Angora goat Hb, consisting of two α-chains and two β-chains in the asymmetric unit. The Matthews coefficient (Vm) and the solvent content for the collected data set for Angora goat Hb are 2.41 $Å^3$/Da and 49.05%, respectively, which are within the range observed commonly for protein crystals [47]. Structure solution of the Angora goat Hb was carried out by the molecular replacement method with a domestic goat Hb as the starting model (*PDB id: 3D1A*; Sathya Moorthy, Neelagandan, Balasubramanian & Ponnuswamy; unpublished work) using the program *PHASER* built in the *PHENIX* software [48-50]. The α- and β-subunit sequences from the domestic goat and sheep share 100% and 95.17% identity, respectively [51]. However, in the case of Angora goat Hb, there are differences in both subunits. This means that it is completely novel and unique when compared to the domestic goat. Since it is a high-resolution structure, several mutations were observed on both the α- and β-subunits of Angora goat Hb (data not shown) during refinement and after structure solution. The mutations observed in the Angora goat Hb may introduce structural and functional changes; however, these will be dealt with in a forthcoming manuscript. Structure refinement of Angora goat Hb and functional studies of ligand binding to GSH and 2,3-DPG are currently in progress.

STUDY'S LIMITATIONS

One of the main challenges in the current study is that we lack basic molecular information regarding Angora goat Hb. There is no amino acid sequence available for Angora goat Hb in common databases such as UniProt. Additionally, the full three-dimensional structure has not been reported in structural databases, *e.g.,* the Protein Data Bank (PDB). The above missing information makes it difficult to perform detailed bioinformatics studies, create accurate structural models, or compare its functions with other Hbs. Without these key details, our understanding of Angora goat Hb is limited.

CONCLUSION

The purification of Angora goat Hb from whole blood was done successfully, followed by crystallization at pH 7 in a water solution in the presence of PEG 3350. Angora goat Hb crystals diffracted up to 1.85 Å resolution within the monoclinic space group $P2_1$, with unit cell parameters of a = 52.08 Å, b = 76.70 Å, c = 74.08 Å, and β = 91.77°. We successfully obtained the structure solution of Angora goat Hb using molecular replacement with the domestic goat Hb as a starting model. Structure refinement and biophysical characterization studies of Angora goat Hb with and without GSH and 2,3-DPG are in progress.

AUTHORS' CONTRIBUTIONS

FP was responsible for study design, isolation, purification, crystallization, data collection, data analysis, and writing of the original draft. SN and MS contributed to the study design, data collection, data analysis, and writing through review and editing. AM participated in study design, sample collection, and manuscript review and editing. MNP was involved in study design, data analysis, and writing through review and editing. YS contributed to study design, supervision, data analysis, and manuscript review and editing. RP oversaw the study design, funding acquisition, supervision, project administration, data collection, data analysis, and manuscript review and editing.

LIST OF ABBREVIATIONS

AA = Amino Acid

DEAE = Diethylaminoethyl

2,3-DPG	=	2,3-diphospoglycerate
EDTA	=	Ethylenediaminetetraacetic Acid
GSH	=	Glutathione
Hb	=	Hemoglobin
NaCl	=	Sodium Chloride
Native-PAGE	=	Native-polyacrylamide Gel Electrophoresis
PDB	=	Protein Data Bank
PEG	=	Polyethylene Glycol
RBC	=	Red Blood Cell
WB	=	Whole Blood

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

An animal research ethical clearance waiver was obtained from the University of the Witwatersrand Animal Research Ethics Committee with the reference "**Waiver 19-04-2023-O**". No drugs or medicines were tested using live animals. The project utilized blood samples that were pre-stored from dead or live animals on the Agricultural Research Council farms for various purposes.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

This work was financially supported by C3-rated scientist money awarded by the National Research Foundation of South Africa (Grant number: RPND023 & RPAN023). The authors thank Wits University and the National Research Foundation of South Africa for their generous funding to purchase a *Bruker D8 Venture Bio PHOTON III 28-pixel array area detector* diffractometer with a dual source, under the National Equipment Program, with Grant Number 129920.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

RP thanks the Animal Research Ethics Committee at the University of the Witwatersrand for approving the handling of Angora blood samples for this research project. RP expresses gratitude to the Agricultural Research Council - Onderstepoort Veterinary Research for providing blood samples from the Angora goat for this study. The authors thank Dr. Olamide Jeje for producing the Native-PAGE of Angora goat Hb.

REFERENCES

[1] Perutz, M.F. The Croonian Lecture, 1968. The haemoglobin molecule. *Proc. R Soc. Lond. B. Biol. Sci.,* **1969**, *173*(1031), 113-140. http://dx.doi.org/10.1098/rspb.1969.0043 PMID: 4389425

[2] Weissbluth, M.; Weissbluth, M. *Hemoglobin: Cooperativity and Electronic Properties*; Springer, **1974**. http://dx.doi.org/10.1007/978-3-642-80801-2

[3] Poyart, C.; Wajcman, H.; Kister, J. Molecular adaptation of hemoglobin function in mammals. *Respir. Physiol.,* **1992**, *90*(1), 3-17. http://dx.doi.org/10.1016/0034-5687(92)90130-O PMID: 1455096

[4] Jensen, F.B.; Fago, A.; Weber, R.E. Hemoglobin Structure and Function. In: *Fish. physiology*; Elsevier, **1998**; pp. 1-40.

[5] Monod, J.; Wyman, J.; Changeux, J.P. On the nature of allosteric transitions: A plausible model. *J. Mol. Biol.,* **1965**, *12*(1), 88-118. http://dx.doi.org/10.1016/S0022-2836(65)80285-6 PMID: 14343300

[6] Koshland, D.E.; Némethy, G.; Filmer, D. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry,* **1966**, *5*(1), 365-385. http://dx.doi.org/10.1021/bi00865a047 PMID: 5938952

[7] Perutz, M.F. Stereochemistry of cooperative effects in haemoglobin. *Nature,* **1970**, *228*(5273), 726-734. http://dx.doi.org/10.1038/228726a0 PMID: 5528785

[8] Perutz, M.F. Mechanisms of cooperativity and allosteric regulation in proteins. *Q Rev. Biophys.,* **1989**, *22*(2), 139-237. http://dx.doi.org/10.1017/S0033583500003826 PMID: 2675171

[9] Wyman, J.; Gill, S.J. *Binding and Linkage: Functional Chemistry of Biological Macromolecules*; University Science Books, **1990**.

[10] Mihailescu, M.R.; Russu, I.M. A signature of the T → R transition in human hemoglobin. *Proc. Natl. Acad. Sci. USA,* **2001**, *98*(7), 3773-3777. http://dx.doi.org/10.1073/pnas.071493598 PMID: 11259676

[11] Silva, M.M.; Rogers, P.H.; Arnone, A. A third quaternary structure of human hemoglobin A at 1.7-A resolution. *J. Biol. Chem.,* **1992**, *267*(24), 17248-17256. http://dx.doi.org/10.1016/S0021-9258(18)41919-9 PMID: 1512262

[12] Mueser, T.C.; Rogers, P.H.; Arnone, A. Interface sliding as illustrated by the multiple quaternary structures of liganded hemoglobin. *Biochemistry,* **2000**, *39*(50), 15353-15364. http://dx.doi.org/10.1021/bi0012944 PMID: 11112521

[13] Safo, M.K.; Abraham, D.J. The X-ray structure determination of bovine carbonmonoxy hemoglobin at 2.1 Å resoultion and its relationship to the quaternary structures of other hemoglobin crystal forms. *Protein Sci.,* **2001**, *10*(6), 1091-1099. http://dx.doi.org/10.1110/ps.48301 PMID: 11369847

[14] Safo, M.K.; Abraham, D.J. The enigma of the liganded hemoglobin end state: a novel quaternary structure of human carbonmonoxy hemoglobin. *Biochemistry,* **2005**, *44*(23), 8347-8359. http://dx.doi.org/10.1021/bi050412q PMID: 15938624

[15] Rapoport, S.; Guest, G.M. Distribution of acid-soluble phosphorus in the blood cells of various vertebrates. *J. Biol. Chem.,* **1941**, *138*(1), 269-282. http://dx.doi.org/10.1016/S0021-9258(18)51432-0

[16] Bunn, H.F. Differences in the Interaction of 2,3-diphosphoglycerate with certain mammalian hemoglobins. *Science,* **1979**, *172*(3987), 1049. http://dx.doi.org/10.1126/science.172.3987.1049.

[17] Bunn, H.F.; Seal, U.S.; Scott, A.F. The role of 2,3-diphosphoglycerate in mediating hemoglobin function of mammalian red cells. *Ann. N.Y. Acad. Sci.,* **1974**, *241*(1), 498-512. http://dx.doi.org/10.1111/j.1749-6632.1974.tb21906.x PMID: 4530676

[18] Bartlett, G.R. Phosphate compounds in red cells of reptiles, amphibians and fish. *Comp. Biochem. Physiol. A Physiol.,* **1976**, *55*(3), 211. http://dx.doi.org/10.1016/0300-9629(76)90133-X

[19] MacDonald, R. Red cell 2,3-diphosphoglycerate and oxygen affinity. *Anaesthesia,* **1977**, *32*(6), 544-553. http://dx.doi.org/10.1111/j.1365-2044.1977.tb10002.x PMID: 327846

[20] Scott, A.F.; Bunn, H.F.; Brush, A.H. The phylogenetic distribution of red cell 2,3 diphosphoglycerate and its interaction with mammalian hemoglobins. *J. Exp. Zool.,* **1977**, *201*(2), 269-288. http://dx.doi.org/10.1002/jez.1402010211 PMID: 894234

[21] Bunn, H.F. Regulation of hemoglobin function in mammals. *Am. Zool.,* **1980**, *20*(1), 199-211. http://dx.doi.org/10.1093/icb/20.1.199

[22] Bunn, H.F. Evolution of mammalian hemoglobin function. *Blood,* **1981**, *58*(2), 189. PMID: 7018619

[23] Moorthy, P.; Neelagandan, K.; Balasubramanian, M.; Ponnuswamy, M. Purification, crystallization and preliminary X-ray diffraction studies on goat *(Capra hircus)* hemoglobin: A low oxygen affinity species. *Protein Pept. Lett.,* **2009**, *16*(4), 454-456. http://dx.doi.org/10.2174/092986609787847992 PMID: 19356147

[24] Hayes, J.L. *The Angora Goat: Its Origin, Culture and Products*; Press of AA Kingman: Boston, **1868**. http://dx.doi.org/10.5962/bhl.title.42758

[25] Jaffé, E.R. Hereditary hemolytic disorders and enzymatic deficiencies of human erythrocytes. *Blood,* **1970**, *35*(1), 116-134. http://dx.doi.org/10.1182/blood.V35.1.116.116 PMID: 4244328

[26] O'Dea, J.D.; Agar, N.S. Glutathione and 2,3-diphosphoglycerate in the blood of hypoxic ruminants. *Res. Vet. Sci.,* **1980**, *29*(2), 153-156. http://dx.doi.org/10.1016/S0034-5288(18)32656-0 PMID: 7465990

[27] Jordaan, D.; Kirsten, J. Investigating alternative governance systems for the South African mohair supply chain. *Agrekon,* **2008**, *47*(2), 258-284. http://dx.doi.org/10.1080/03031853.2008.9523800

[28] Pienaar, L.; Partridge, A.; Morokong, T. *The Mohair Industry: Economic Impact of Possible Market Closure*; Western Cape Department of Agriculture, **2018**.

[29] van Rensburg, S.J. Reproductive physiology and endocrinology of normal and habitually aborting angora goats. *Onderstepoort J. Vet. Res.,* **1971**, *38*(1), 1-62. PMID: 5170806

[30] Wentzel, D.; Viljoen, K.S.; Botha, L.J. Physiological and endocrinological reactions to cold stress in the Angora Goat. *Agroanimalia,* **1979**, *11*(2), 19-22.

[31] Snyman, M.A.; Snyman, A.E. The possible role of *Ostertagia circumcincta,* coccidiosis and dietary protein level in the development of swelling disease in Angora goat kids. *J. S Afr. Vet. Assoc.,* **2005**, *76*(2), 63-68. http://dx.doi.org/10.4102/jsava.v76i2.399 PMID: 16108523

[32] Snyman, M.A. Body weight and growth rate of South African Angora goat kids under different pre- and post-weaning management systems. *S Afr J. Anim. Sci.,* **2007**, *37*(2), 132-141. http://dx.doi.org/10.4314/sajas.v37i2.4037

[33] Sundaresan, S.S.; Ramesh, P.; Sivakumar, K.; Ponnuswamy, M.N. Purification, crystallization and preliminary X-ray analysis of haemoglobin from ostrich (*Struthio camelus*). *Acta. Crystallogr Sect F Struct. Biol. Cryst Commun,* **2009**, *65*(7), 681-683. http://dx.doi.org/10.1107/S1744309109019009 PMID: 19574638

[34] Sundaresan, S.S.; Ramesh, P.; Shobana, N.; Vinuchakkaravarthy, T.; Yasien, S.; Ponnuswamy, M.N.G. Crystal structure of hemoglobin from mouse (*Mus musculus*) compared with those from other small animals and humans. *Acta. Crystallogr F Struct. Biol. Commun,* **2021**, *77*(4), 113-120. http://dx.doi.org/10.1107/S2053230X2100306X PMID: 33830076

[35] Ramesh, P.; Sundaresan, S.S.; Sathya Moorthy, P.; Balasubramanian, M.; Ponnuswamy, M.N. Structural studies of haemoglobin from pisces species shortfin mako shark (*Isurus oxyrinchus)* at 1.9 Å resolution. *J. Synchrotron Radiat.,* **2013**, *20*(6), 843-847. http://dx.doi.org/10.1107/S0909049513021572 PMID: 24121325

[36] Ramesh, P.; Sundaresan, S.S.; Shobana, N.; Vinuchakkaravarthy, T.; Sivakumar, K.; Yasien, S.; Ponnuswamy, M.N.G. Structural studies of hemoglobin from two flightless birds, ostrich and turkey: Insights into their differing oxygen-binding properties. *Acta. Crystallogr D Struct. Biol.,* **2021**, *77*(5), 690-702. http://dx.doi.org/10.1107/S2059798321003417 PMID: 33950023

[37] Knapp, J.E.; Oliveira, M.A.; Xie, Q.; Ernst, S.R.; Riggs, A.F.; Hackert, M.L. The structural and functional analysis of the hemoglobin D component from chicken. *J. Biol. Chem.,* **1999**, *274*(10), 6411-6420. http://dx.doi.org/10.1074/jbc.274.10.6411 PMID: 10037733

[38] Lavrik, N.L. On the nature of the spectral shift of the soret band of erythrocyte oxyhemoglobin when organic molecules are added to an erythrocyte suspension. *Biophysics (Oxf),* **2022**, *67*(1), 72-77. http://dx.doi.org/10.1134/S0006350922010079

[39] Sekyonda, Z.; An, R.; Goreke, U.; Man, Y.; Monchamp, K.; Bode, A.; Zhang, Q.; El-Gammal, Y.; Kityo, C.; Kalfa, T.A.; Akkus, O.; Gurkan, U.A. Rapid measurement of hemoglobin-oxygen dissociation by leveraging Bohr effect and Soret band bathochromic shift. *Analyst (Lond),* **2024**, *149*(9), 2561-2572. http://dx.doi.org/10.1039/D3AN02071A PMID: 38501195

[40] Davis, B.J. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.,* **1964**, *121*(2), 404-427. http://dx.doi.org/10.1111/j.1749-6632.1964.tb14213.x PMID: 14240539

[41] *PROTEUM4 Version 20214-1 Data Collection Software Which Includes SAINT Version 840B, SADABS-2016/2 and XPREP Version 2014/2*; Bruker AXS Inc.,: Madison, Wisconsin, **2021**.

[42] Sheldrick, G.M. *Program. for Empirical Absorption Correction of Area Detector Data*; Sadabs, **1996**.

[43] Evans, P. Scaling and assessment of data quality. *Acta. Crystallogr D Biol. Crystallogr,* **2006**, *62*(1), 72-82. http://dx.doi.org/10.1107/S0907444905036693 PMID: 16369096

[44] Otwinowski, Z.; Minor, W. Denzo and Scalepack. In: *International Tables for Crystallography*; Wiley, **2006**. http://dx.doi.org/10.1107/97809553602060000677

[45] Evans, P.R. An introduction to data reduction: space-group determination, scaling and intensity statistics. *Acta. Crystallogr D Biol. Crystallogr,* **2011**, *67*(4), 282-292. http://dx.doi.org/10.1107/S090744491003982X PMID: 21460446

[46] Otwinowski, Z. Denzo and Scalepack. In: *International Tables for Crystallography*; , **2012**; pp. 282-289. http://dx.doi.org/10.1107/97809553602060000833

[47] Matthews, B.W. Solvent content of protein crystals. *J. Mol. Biol.,* **1968**, *33*(2), 491-497. http://dx.doi.org/10.1016/0022-2836(68)90205-2 PMID: 5700707

[48] Rossmann, M.G. The molecular replacement method. *Acta. Crystallogr A,* **1990**, *46*(2), 73-82. http://dx.doi.org/10.1107/S0108767389009815 PMID: 2180438

[49] McCoy, A.J.; Grosse-Kunstleve, R.W.; Adams, P.D.; Winn, M.D.; Storoni, L.C.; Read, R.J. *Phaser* crystallographic software. *J. Appl. Cryst,* **2007**, *40*(4), 658-674. http://dx.doi.org/10.1107/S0021889807021206 PMID: 19461840

[50] Adams, P.D.; Afonine, P.V.; Bunkóczi, G.; Chen, V.B.; Davis, I.W.; Echols, N.; Headd, J.J.; Hung, L.W.; Kapral, G.J.; Grosse-Kunstleve, R.W.; McCoy, A.J.; Moriarty, N.W.; Oeffner, R.; Read, R.J.; Richardson, D.C.; Richardson, J.S.; Terwilliger, T.C.; Zwart, P.H. *PHENIX* : A comprehensive Python-based system for macromolecular structure solution. *Acta. Crystallogr. D. Biol. Crystallogr,* **2010**, *66*(2), 213-221. http://dx.doi.org/10.1107/S0907444909052925 PMID: 20124702

[51] Kamariah, N.; Ponnuraj, S.M.; Moovarkumudalvan, B.; Ponnuswamy, M.N.G. Structural studies on a low oxygen affinity hemoglobin from mammalian species: Sheep *(Ovis aries). Biochem. Biophys. Res. Commun,* **2014**, *450*(1), 36-41. http://dx.doi.org/10.1016/j.bbrc.2014.05.062 PMID: 24858681