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Exploring Variation in Structure and Intrinsic Disorder of Lactoferrampin among Different Animal Species

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Abstract

Lactoferrampin (Lfampin) is second in order of importance among bioactive peptides derived from lactoferrin (Lf), a protein with multiple functions found chiefly in mammalian milk. This peptide has two shared features with other antimicrobial peptides; a net positive charge, and an amphipathic character. Lfampin linked to lactoferricin (another bioactive peptide derived from Lf) can be an efficient substitute to antibiotics in feed. Determining the variation in structure and intrinsic disorder of Lfampin among different animal species and human is vital to elucidate its antimicrobial activities. In this study, the amino acid sequences of parent protein of this peptide from 60 animal species and human were obtained from NCBI database and analyzed by multiple alignment, and phylogenetic tree of animal species was built. Lfampin was located in this alignment of parent protein of eighteen different animal species besides human, and then aligned individually. The variation in intrinsic disorder and disorder propensity was explored.

The hydrophobicity and charge characteristics of Lfampin primary structure in eighteen animal species and human were also determined. The region that includes the peptide displayed a range of 62.8% (*Sus scrofa*) to 97.1% (*Camelus ferus*) identity relative to *Camelus dromedarius* among 18 animal species and human. The variation in structure of the region that includes Lfampin is also correlated with diverse levels of intrinsic disorder as well as disorder propensity in its sequence among different animal species and human. Interestingly, no intrinsic disorder was detected in camel (*Camelus ferus*, and *Camelus dromedarius*) Lfampin or the 35-residues region that contains the peptide. *Chlorocebus sabaeus* Lfampin has zero net positive charge, while *Bos indicus*, *Bos grunniens*, and *Bos taurus* peptides have the highest net charge (=5). This high net charge can anticipate higher antimicrobial effects. Distribution of intrinsic disorder in bioactive proteins/peptides can identify their source such as animal milk source. Furthermore, intrinsic disorder can predict strength of peptide activity for its application in feed or food and as peptide drug.

Keywords: Disorder propensity; Intrinsic disorder; Lfampin; Lactoferrin; Multiple alignment; Phylogenetic tree

Introduction

Lactoferrampin is one of two antimicrobial peptides found in N-terminal region of Lf parent protein and comprises seventeen amino acids [1]. The parent protein of this peptide is involved in vast range of bioactivities from antimicrobial effects that are either based on its capacity to bind iron or not related to this capacity to immune system triggering [2-4]. These parent protein activities are mostly endorsed by its N-terminal region and high cationicity of this region [5].

The N-terminal sequence of Lf comprises two efficient bioactive peptides; lactoferricin (Lfcin) and Lfampin. Lfcin is released via pepsin hydrolysis of the parent protein [6]. Lfcin performs numerous activities comprising antimicrobial and immunological effects [7]. Lfampin is positioned in close proximity to Lfcin. The sequence of bovine Lfampin corresponds directly to residues 268–284 (WKLLSKAQEKFGKNKSR) in parent protein cationic N-terminal domain [1]. The structure of this peptide promotes its potential application as an antimicrobial peptide due to its net cationic charge and the existence of tryptophan in its sequence [8-10]. Bovine Lfampin could act against Gram-negative bacterial strains, Gram-positive bacterial strains, along with *Candida albicans* [1].

Lfampin linked to Lfcin can be applied as an efficient substitute to antibiotics (e.g., colistin sulfate) in feed of piglets weaned at age of three weeks to enhance their growth performance [11,12]. Both Lfampin and Lfcin possess superior antimicrobial effects than their parent protein [13]. Tanhaeian et al. (2020) could engineer food-grade *Lactococcus lactis* expressing a chimeric peptide of Lfampin linked to Lfcin that has antimicrobial effects and can be applied as bio-preservative in food industry [14].

Predicting intrinsic disorder in proteins is vital since it performs a wide range of functions in the cell. In spite of absence of stable 3D structure in these disordered proteins, they are responsible for countless functions in cell [15-17]. Research revealed that disordered region's primary structure varies considerably from that of ordered ones [18]. Thus, several prediction approaches were developed to predict disorder depending on the sequence of amino acids. RONN [19], GlobPlot [20], Predictor of Natural Disordered Regions (PONDR) [21], DISOPRED [22,23], DISEMBL [24], VL3 [25], and IUPred [26] can predict the likelihood that a definite amino acid residue is found in a disordered region via knowing the sequences of amino acids close to that residue. On the other hand, there are other methods that can predict disorder via amino acid sequences binary grouping into typically disordered sequences and typically ordered ones [27-29].

The aim of this work was to determine the variation in structure, hydrophobicity and charge characteristics of primary structure, intrinsic disorder, and disorder propensity of Lfampin among eighteen different animal species and human to elucidate its antimicrobial activities.

Materials and Methods

Protein sequences

The amino acid sequences of parent protein (Lf) of Lfampin from different animal species were searched in NCBI database (NCBI Blast: Protein Sequence, last accessed on 11/28/2023) via BLASTP program. The following sequence of camel parent protein (Accession: AHJ37525) was used as Query sequence in this search.

***Camelus dromedarius* Lf**

Length: 708 amino acids

<MKLFFPALLSLGALGLCLAASKKSVRWCTTSPAESSKCAQWQ
RRMKKVRGPSVTCVKKTSRFECIQAISTEKADAVTLDGGLVYDAGLD
PYKLRPIAAEVYGTENNPQTHYYAVAIAKKGTNFQLNQLQGLKSCH
TGLGRSAGWNIPMGLLRPFLDWTGPPEPLQKAVAKFFSASCVPCVD
GKEYPNLCQLCAGTGENKCACSSQEPYFGYSGAFKCLQDGAGDVAF
VKDSTVFESLPAKADRDQYELLCPNNTRKPVDAFQECHLARVPSHA
VVARSVNGKEDLIWKLLVKAQEKFGRGKPSAFQLFGSPAGQKDLLF
KDSALGLLRIPSKIDSGLYLGSNYITAIRGLRETAAEVELRRAQVVWC
AVGSDEQLKCQEWSRQSNQSVVCATASTTEDCIALVLKGEADALSL
DGGYIYIAGKCGLVPVLAESQQSPESSGLDCVHRPVKGYLAVAVVRK
ANDKITWNSLRGKKSCHTAVDRTAGWNIPMGLLFKNTDSCRFDEF
FSQSCAPGSDPRSKLCALCAGNEEGQNKCVPNSSERYYGYTGAFRCL
AENVGDVAFVKDVTVLDNTDGKNTEQWAKDLKLGDFELLCLNGTR
KPVTEAESCHLAVAPNHAVVSRIDKVAHLEQVLLRQQAHFGRNGQD
CPGKFCLFQSKTKNLLFNDNTECLAKLQGKTTYEEYLGPQYVTAIAK
LRRCSTSPLLEACAFLMR>

Adjustment of protein sequences and their alignment

After NCBI database search was accomplished, the top 113 Blast hits for Lf protein sequences from different mammals were generated. Repeated or similar sequences were removed and only 78 protein sequences were selected. These sequences were saved in FASTA format for additional exploration. Inspecting these 78 sequences, it could be concluded that they are derived from 60 different animal species and human Table 1. The sequences obtained from NCBI database were aligned using Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) [30]. The sequence identity of 78 lactoferrin sequences obtained from NCBI database relative to *Camelus dromedarius* (Accession: AHJ37525) was calculated. MEGA11 was also applied to build phylogenetic tree of parent protein among different animal species.

Table 1: Results of amino acid sequence search of parent protein (Lf) of Lfampin from 60 different animal species and human in NCBI database.

Protein description	Scientific name of animals	Organism	Query cover	Percent of Identity to query	Accession
Lactoferrin	*Camelus dromedarius*	Arabian camel (even-toed ungulates)	100%	99.29	AHJ37525
Lactotransferrin isoform X2	*Camelus ferus*	Wild Bactrian camel (even-toed ungulates)	100%	99.15	032314575
Lactotransferrin isoform X2	*Camelus bactrianus*	Bactrian camel (even-toed ungulates)	100%	99.15	010965654
Lactotransferrin precursor	*Camelus dromedarius*	Arabian camel (even-toed ungulates)	100%	98.87	001290496
Lactotransferrin isoform X2	*Vicugna pacos*	Alpaca (even-toed ungulates)	100%	97.60	006200784
Lactotransferrin isoform X2	*Balaenoptera acutorostrata*	Minke whale (whales & dolphins)	100%	81.64	057411496
Lactotransferrin isoform X1	*Balaenoptera acutorostrata*	Minke whale (whales & dolphins)	100%	81.52	057411495
PREDICTED: Lactotransferrin isoform X2	*Lipotes vexillifer*	Yangtze River dolphin (whales & dolphins)	100%	80.45	007470311
Lactotransferrin isoform X1	*Physeter catodon*	Sperm whale (whales & dolphins)	100%	80.93	007126155
Lactotransferrin isoform X2	*Balaenoptera musculus*	Blue whale (whales & dolphins)	100%	82.20	036725385
Lactotransferrin isoform X1	*Monodon monoceros*	Narwhal (whales & dolphins)	99%	81.02	029098394
Lactotransferrin isoform X1	*Lagenorhynchus albirostris*	White-beaked dolphin (whales & dolphins)	99%	80.88	060018141

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Lactotransferrin isoform X1	*Tursiops truncatus*	Common bottlenose dolphin (whales & dolphins)	99%	81.02	019802369
Lactotransferrin isoform X1	*Balaenoptera musculus*	Blue whale (whales & dolphins)	100%	82.09	036725384
Lactotransferrin isoform X2	*Physeter catodon*	Sperm whale (whales & dolphins)	100%	80.65	028334746
Hypothetical protein DBR06_SOUSAS21910028	*Sousa chinensis*	Indo-pacific humpbacked dolphin (whales & dolphins)	99%	79.60	TEA23950
Lactotransferrin isoform X2	*Balaenoptera ricei*	Rice's whale (whales & dolphins)	100%	81.78	059795411
Lactotransferrin	*Delphinus delphis*	Saddleback dolphin (whales & dolphins)	99%	80.88	059877399
Lactotransferrin	*Globicephala melas*	Long-finned pilot whale (whales & dolphins)	99%	80.59	030701198
Lactotransferrin isoform X1	*Orcinus orca*	Killer whale (whales & dolphins)	99%	80.74	004283903
Lactotransferrin isoform X1	*Mesoplodon densirostris*	Blainville's beaked whale (whales & dolphins)	100%	80.87	059966870
Lactotransferrin isoform X2	*Monodon monoceros*	Narwhal (whales & dolphins)	99%	80.74	029098395
Lactotransferrin	*Delphinapterus leucas*	Beluga whale (whales & dolphins)	99%	81.02	022429072
Lactotransferrin isoform X1	*Balaenoptera ricei*	Rice's whale (whales & dolphins)	100%	81.66	059795410
PREDICTED: Lactotransferrin	*Rhinolophus sinicus*	Chinese rufous horseshoe bat (bats)	100%	77.26	019579600
PREDICTED: Lactotransferrin isoform X1	*Lipotes vexillifer*	Yangtze River dolphin (whales & dolphins)	100%	80.79	007470310
Lactotransferrin	*Equus asinus*	Ass (odd-toed ungulates)	99%	77.90	044610851
Lactotransferrin precursor	*Equus caballus*	Horse (odd-toed ungulates)	99%	77.62	001157446
Lactotransferrin	*Equus quagga*	(odd-toed ungulates)	99%	77.62	046528116
PREDICTED: Lactotransferrin	*Equus przewalskii*	Przewalski's horse (odd-toed ungulates)	99%	77.48	008542061
Lactotransferrin isoform X2	*Mesoplodon densirostris*	Blainville's beaked whale (whales & dolphins)	100%	81.21	059966871
Lactotransferrin	*Rhinolophus ferrumequinum*	Greater horseshoe bat (bats)	100%	76.41	032988762
Lactotransferrin	*Diceros bicornis minor*	(odd-toed ungulates)	99%	79.18	058412731
Lactotransferrin	*Phocoena sinus*	Vaquita (whales & dolphins)	99%	80.06	032504925
PREDICTED: Lactotransferrin	*Hipposideros armiger*	Great roundleaf bat (bats)	100%	75.42	019485988
PREDICTED: Lactotransferrin	*Myotis brandtii*	Brandt's bat (bats)	100%	79.66	005877141
Lactotransferrin isoform X3	*Mesoplodon densirostris*	Blainville's beaked whale (whales & dolphins)	100%	80.93	059966873
lactotransferrin isoform X2	*Myotis lucifugus*	Little brown bat (bats)	100%	79.52	006097173
Lactotransferrin isoform X1	*Lagenorhynchus obliquidens*	Pacific white-sided dolphin (whales & dolphins)	100%	80.37	026952807
Lactotransferrin isoform X1	*Neophocaena asiaeorientalis asiaeorientalis*	Yangtze finless porpoise (whales & dolphin)	99%	79.77	024607171
Lactotransferrin isoform X1	*Myotis myotis*	Bats	100%	79.38	036188933
Lactotransferrin	*Myotis daubentonii*	Daubenton's bat (bats)	100%	79.38	059520279
Lactotransferrin	*Manis pentadactyla*	Chinese pangolin (placentals)	100%	76.27	KAI5207914
Lactotransferrin	*Eptesicus fuscus*	Big brown bat (bats)	100%	79.38	008152421
Lactotransferrin	*Manis pentadactyla*	Chinese pangolin (placentals)	100%	76.13	036739216
Lactotransferrin	*Manis javanica*	Malayan pangolin (placentals)	100%	75.71	036861506
PREDICTED: Lactotransferrin isoform X1	*Miniopterus natalensis*	bats	100%	77.97	016058233
Lactotransferrin	*Piliocolobus tephrosceles*	Ugandan red Colobus (primates)	100%	75.07	023087181

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Hypothetical protein JEQ12_008530	*Ovis aries*	Sheep (even-toed ungulates)	100%	76.69	KAG5197801
Lactotransferrin isoform X2	*Myotis myotis*	Bats	100%	78.95	036188934
Lactoferrin	*Bos grunniens*	Domestic yak (even-toed ungulates)	100%	75.14	ALE66318
Lactoferrin precursor	*Ovis aries*	Sheep (even-toed ungulates)	100%	76.55	AGF69235
Lactoferrin	*Bos indicus*	Zebu cattle (even-toed ungulates)	100%	75.00	AEK29439
Lactotransferrin	*Trachypithecus francoisi*	Francois's langur (primates)	100%	74.93	033060828
Lactotransferrin	*Rhinopithecus roxellana*	Golden snub-nosed monkey (primates)	100%	74.79	010373088
Lactoferrin	*Ovis aries*	Sheep (even-toed ungulates)	100%	76.41	ACT76166
Lactotransferrin	*Gorilla gorilla gorilla*	Western lowland gorilla (primates)	100%	74.68	004034071
Lactotransferrin	*Budorcas taxicolor*	Takin (even-toed ungulates)	100%	76.55	052495122
PREDICTED: Lactotransferrin isoform X2	*Miniopterus natalensis*	Bats	100%	77.68	016058234
Lactotransferrin	*Cervus canadensis*	Even-toed ungulates	100%	75.85	043299278
Lactotransferrin precursor	*Ovis aries*	Sheep (even-toed ungulates)	100%	76.27	001020033
Lactotransferrin	*Moschus berezovskii*	Chinese forest musk deer (even-toed ungulates)	100%	76.69	055269623
Lactotransferrin isoform X1	*Sus scrofa*	Pig (even-toed ungulates)	100%	74.86	020924222
Lactotransferrin isoform X1	*Bubalus carabanensis*	Carabao (even-toed ungulates)	100%	76.69	055413340
Lactoferrin	*Sus scrofa*	Pig (even-toed ungulates)	100%	74.86	AAL40161
Lactotransferrin	*Oryx dammah*	Scimitar-horned oryx (even-toed ungulates)	100%	76.30	040102748
Lactotransferrin	*Callithrix jacchus*	White-tufted-ear marmoset (primates)	100%	74.08	002758329
Lactotransferrin	*Phacochoerus africanus*	Common warthog (even-toed ungulates)	100%	74.86	047628102
Lactotransferrin	*Odocoileus virginianus texanus*	Even-toed ungulates	100%	75.85	020771788
Lactotransferrin; Precursor	*Sus scrofa*	Pig (even-toed ungulates)	100%	74.72	P14632
Lactotransferrin	*Panthera tigris*	Tiger (carnivores)	100%	77.26	042834703
Lactotransferrin	*Neofelis nebulosa*	Clouded leopard (carnivores)	100%	77.26	058583187
Lactoferrin	*Capra hircus*	Goat (even-toed ungulates)	100%	75.85	ACT53713
Lactoferrin	*Capra hircus*	Goat (even-toed ungulates)	100%	75.85	ABD49106
Lactotransferrin isoform X2	*Chlorocebus sabaeus*	Green monkey (primates)	100%	74.08	007982150
Lactotransferrin	*Bos taurus*	Cattle, Artiodactyla	100%	75.5	BAB03470
Lactotransferrin	*Homo sapiens*	Human	100%	73.9	NP-002334

Peptide sequences and their alignment

Lfampin was located in the alignment of parent protein of different animal species. The 35-residues region of Lfampin (the sequence of amino acids that corresponds directly to formerly identified Lfampin derived from bovine lactoferrin (17 amino acids) with added 18 residues near C-terminal end of peptide from the parent protein sequence) was aligned individually among 18 animal species and human using BioEdit version 7 [31]. The selected 18 animal species include *Camelus dromedarius* (Arabian camel, even-toed ungulates), *Camelus ferus* (wild Bactrian camel, even-toed ungulates), *Physeter catodon* (Sperm whale, whales & dolphins), Lagenorhynchus albirostris (White-beaked dolphin, whales & dolphins), *Equus asinus* (Ass, odd-toed ungulates), *Phocoena sinus* (Vaquita, whales & dolphins), Myotis brandtii (Brandt's bat, bats), Miniopterus natalensis (bats), *Bos grunniens* (Domestic yak, even-toed ungulates), *Bos indicus* (Zebu cattle, even-toed ungulates), *Trachypithecus francoisi* (Francois's langur, primates), Gorilla gorilla gorilla (Western lowland gorilla, primates), Budorcas taxicolor (Takin, even-toed ungulates), *Sus scrofa* (Pig, even-toed ungulates), *Capra hircus* (Goat, even-toed ungulates), *Chlorocebus sabaeus* (Green monkey, primates), *Bos taurus* (Cattle, Artiodactyla), and Oryx dammah (Scimitar-horned oryx, even-toed ungulates). Sequence identity of 20 sequences of Lfampin region relative to *Camelus dromedarius* Lfampin region was determined.

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Prediction of natural disordered regions and disorder propensity in Lfampin

Prediction of natural disordered regions in 20 amino acid sequences of 35-residues region of Lfampin from 18 animal species and human was done by PONDR (www.pondr.com, last accessed on 11/29/2023). Furthermore, prediction of disorder propensity and binary disorder in 20 sequences of Lfampin region from 18 animal species and human was performed via flDPnn Server (biomine.cs.vcu.edu/webresults/flDPnn/20231129035754/results.html, last accessed on 11/29/2023). The flDPnn Server generated the following data: binary disorder (value of 1 represents disordered residue, and 0 represents ordered residue), disorder propensity (greater value signifies higher possibility that a certain residue is disordered), binary protein-binding (value of 1 represents disordered protein-binding residue, 0 represents other disordered residue, and X represents ordered residue), protein-binding propensity, binary DNA-binding, DNA-binding propensity, binary RNA-binding, RNA-binding propensity, binary linker, and linker propensity.

Hydrophobicity and charge characteristics of Lfampin primary structure

The primary structure of 20 amino acid sequences of Lfampin from 18 animal species and human was analyzed by HeliQuest CompuParam version3 (https://heliquest.ipmc.cnrs.fr/cgi-bin/ComputParams.py, last accessed on 12/2/2023). This analysis generated different characteristics of the peptide including physico-chemical properties (hydrophobicity, hydrophobic moment, and net positive charge), polar and nonpolar residues, uncharged and charged residues, aromatic residues, and special residues.

Results and Discussion

Despite the fact that Lfampin is neighboring Lfcin in cationic N-terminal domain of their parent protein and have comparable cationic and amphipathic features, their ability to kill bacterial pathogens differs from each other. The reason for this difference in their antibacterial activity is the significant variation in their amino acid composition and chain length, and consequently great variation in their structures [5]. Lfampin has a crucial contribution to Lf effects on bacterial membrane [1]. Lfampin can kill various Gram-negative bacterial strains, Gram-positive bacterial strains, parasites, and yeasts as reported in MilkAMP database (a comprehensive database of antimicrobial peptides of dairy origin) [1,32].

The comparison of sequence and primary structure of Lfampin from different animal species and human is important to elucidate the peptide antimicrobial action and predict which one is a candidate for developing therapeutic peptide. The present study provides the first comprehensive description of sequence, hydrophobicity, and charge characteristics of primary structure of Lfampin from 18 different animal species and human.

MEGA11 alignment of 78 sequences obtained from NCBI database of parent protein (Lf) from 60 animal species and human is demonstrated in Figure 1. The amino acid sequences of lactoferrin in 60 different animal species and human showed a range of identity of 73.9% (Homo sapiens) to 99.1% (*Camelus ferus*, and Camelus bactrianus) relative to lactoferrin of *Camelus dromedarius* Table 2. Figure 2 illustrates phylogenetic tree built by MEGA11 of parent protein (Lf) among different animal species.

Table 2: Sequence identity list of 78 parent protein sequences obtained from NCBI database relative to *Camelus dromedarius* (Accession: AHJ37525).

Sequence Accession	Organism	Percent identity
AHJ37525	*Camelus dromedarius*	100%
032314575	*Camelus ferus*	99.1%
010965654	*Camelus bactrianus*	99.1%
001290496	*Camelus dromedarius*	98.8%
006200784	*Vicugna pacos*	97.5%
057411496	*Balaenoptera acutorostrata*	81.6%
057411495	*Balaenoptera acutorostrata*	81.5%
007470311	*Lipotes vexillifer*	80.4%
007126155	*Physeter catodon*	80.9%
036725385	*Balaenoptera musculus*	82.2%
029098394	*Monodon monoceros*	80.7%
060018141	*Lagenorhynchus albirostris*	80.6%
019802369	*Tursiops truncates*	80.7%
036725384	*Balaenoptera musculus*	82.0%
028334746	*Physeter catodon*	80.6%

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TEA23950	*Sousa chinensis*	78.8%
059795411	*Balaenoptera ricei*	81.7%
059877399	*Delphinus delphis*	80.6%
030701198	*Globicephala melas*	80.3%
004283903	*Orcinus orca*	80.5%
059966870	*Mesoplodon densirostris*	80.8%
029098395	*Monodon monoceros*	80.5%
X022429072	*Delphinapterus leucas*	80.7%
059795410	*Balaenoptera ricei*	81.6%
019579600	*Rhinolophus sinicus*	77.2%
007470310	*Lipotes vexillifer*	80.7%
044610851	*Equus asinus*	77.6%
001157446	*Equus caballus*	77.4%
046528116	*Equus quagga*	77.4%
008542061	*Equus przewalskii*	77.2%
059966871	*Mesoplodon densirostris*	81.2%
032988762	*Rhinolophus ferrumequinum*	76.4%
058412731	*Diceros bicornis minor*	78.9%
032504925	*Phocoena sinus*	79.8%
019485988	*Hipposideros armiger*	75.4%
005877141	*Myotis brandtii*	79.6%
059966873	*Mesoplodon densirostris*	80.9%
006097173	*Myotis lucifugus*	79.5%
026952807	*Lagenorhynchus obliquidens*	80.3%
024607171	*Neophocaena asiaeorientalis asiaeorientalis*	79.5%
036188933	*Myotis myotis*	79.3%
059520279	*Myotis daubentonii*	79.3%
KAI5207914	*Manis pentadactyla*	76.2%
008152421	*Eptesicus fuscus*	79.3%
036739216	*Manis pentadactyla*	76.1%
047705068	*Prionailurus viverrinus*	76.5%
036861506	*Manis javanica*	75.7%
016058233	*Miniopterus natalensis*	77.9%
023087181	*Piliocolobus tephrosceles*	75.0%
KAG5197801	*Ovis aries*	76.6%
036188934	*Myotis myotis*	78.9%
ALE66318	*Bos grunniens*	75.1%
AGF69235	*Ovis aries*	76.5%
AEK29439	*Bos indicus*	75.0%
033060828	*Trachypithecus francoisi*	74.9%
010373088	*Rhinopithecus roxellana*	74.7%
ACT76166	*Ovis aries*	76.4%
004034071	*Gorilla gorilla gorilla*	74.6%
052495122	*Budorcas taxicolor*	76.5%
016058234	*Miniopterus natalensis*	77.6%
043299278	*Cervus Canadensis*	75.8%

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001020033	*Ovis aries*	76.2%
055269623	*Moschus berezovskii*	76.6%
020924222	*Sus scrofa*	74.7%
055413340	*Bubalus carabanensis*	76.6%
AAL40161	*Sus scrofa*	74.7%
040102748	*Oryx dammah*	76.3%
002758329	*Callithrix jacchus*	74.0%
47628102	*Phacochoerus africanus*	74.7%
020771788	*Odocoileus virginianus texanus*	75.8%
P14632	Precursor *Sus scrofa*	74.5%
042834703	*Panthera tigris*	77.2%
058583187	*Neofelis nebulosa*	77.2%
ACT53713	*Capra hircus*	75.8%
ABD49106	*Capra hircus*	75.8%
007982150	*Chlorocebus sabaeus*	74.0%
BAB03470	*Bos taurus*	75.5%
NP-002334	*Homo sapiens*	73.9%

BioEdit alignment of amino acid sequences of the 35-residues Lfampin region from 18 animal species and human is shown in Figure 3. The identity results of the 35-residues region that contains Lfampin differ from those of its parent protein or lactoferrin. The 35-residues region that includes the peptide displayed a range of 62.8% (*Sus scrofa*) to 97.1% (*Camelus ferus*) identity relative to *Camelus dromedarius* among 18 animal species and human Table 3. Data in Figure 3 and Table 3 support the presence of significant variance in sequence of the 35-residues region that includes Lfampin among 18 different animal species and human, which can lead to differences in its structure, hydrophobicity, charge characteristics, intrinsic disorder, disorder propensity, and thus its biological activities.

Disordered proteins or peptides are receiving raising attention since a lot of of them are functionally essential. However, disorder information for a given protein or peptide is mostly extracted from X-ray analysis of its crystal. This means that most proteins that are difficult to crystallize or those not crystallized yet even if they are disordered will not be reported. Therefore, intrinsic disorder prediction from protein sequences that have been experimentally determined based on difference from disorder among structure-unknown protein data will benefit reporting more disordered proteins [17]. PONDR algorithms are among the methods that predict disorder depending on structure-known records from already crystallized proteins [21].

Prediction of natural disordered regions in 20 sequences of Lfampin region from 18 animal species and human performed by PONDR is illustrated in Figure 4 revealed that the highest PONDR score was for *Capra hircus* (goat Lfampin region), followed by 3 entirely overlapped curves for PONDR score representing *Bos grunniens* (domestic yak Lfampin region), *Bos indicus* (Zebu cattle Lfampin region), and *Bos taurus* (cattle Lfampin region). PONDR VLXT NNP statistics of 20 sequences of Lfampin region in Table 4 demonstrated an interesting and surprising fact: both *Camelus dromedarius* and *Camelus ferus* have zero number of disordered regions, zero overall percent of disorder, and that 17-residues Lfampin along with 35-residues Lfampin region are ordered. *Physeter catodon*, *Sus scrofa*, *Phocoena sinus*, and *Lagenorhynchus albirostris* have 1 disordered region within 35-residues Lfampin region not in 17-residues Lfampin Table 4. Although *Equus asinus* has 2 disordered regions but neither of them is within 17-residues Lfampin and both are found in 35-residues Lfampin region Table 4. Another unexpected data was those of *Bos indicus*, *Bos grunniens*, and *Bos taurus*, which have identical sequences for 17-residues Lfampin and 35-residues Lfampin region, and consequently the same number of disordered regions within 17-residues Lfampin and 35-residues Lfampin region as well as overall percent of disorder of 2 and 51.43%, respectively Table 4. The highest overall percent of disorder was for *Capra hircus* Lfampin (62.86%), whereas the lowest overall percent of disorder was for Homo sapiens (5.71%) Table 4.

The intrinsic disorder data of PONDR for camel Lfampin obtained in this study totally disagree with our previous report for camel Lf as well as Lfcin, which revealed that two long regions of camel lactoferrin including Lfcin sequence have greater disorder than the corresponding regions in both human and bovine proteins and peptides leading to superior antibacterial effects of camel protein and Lfcin as confirmed experimentally [3].

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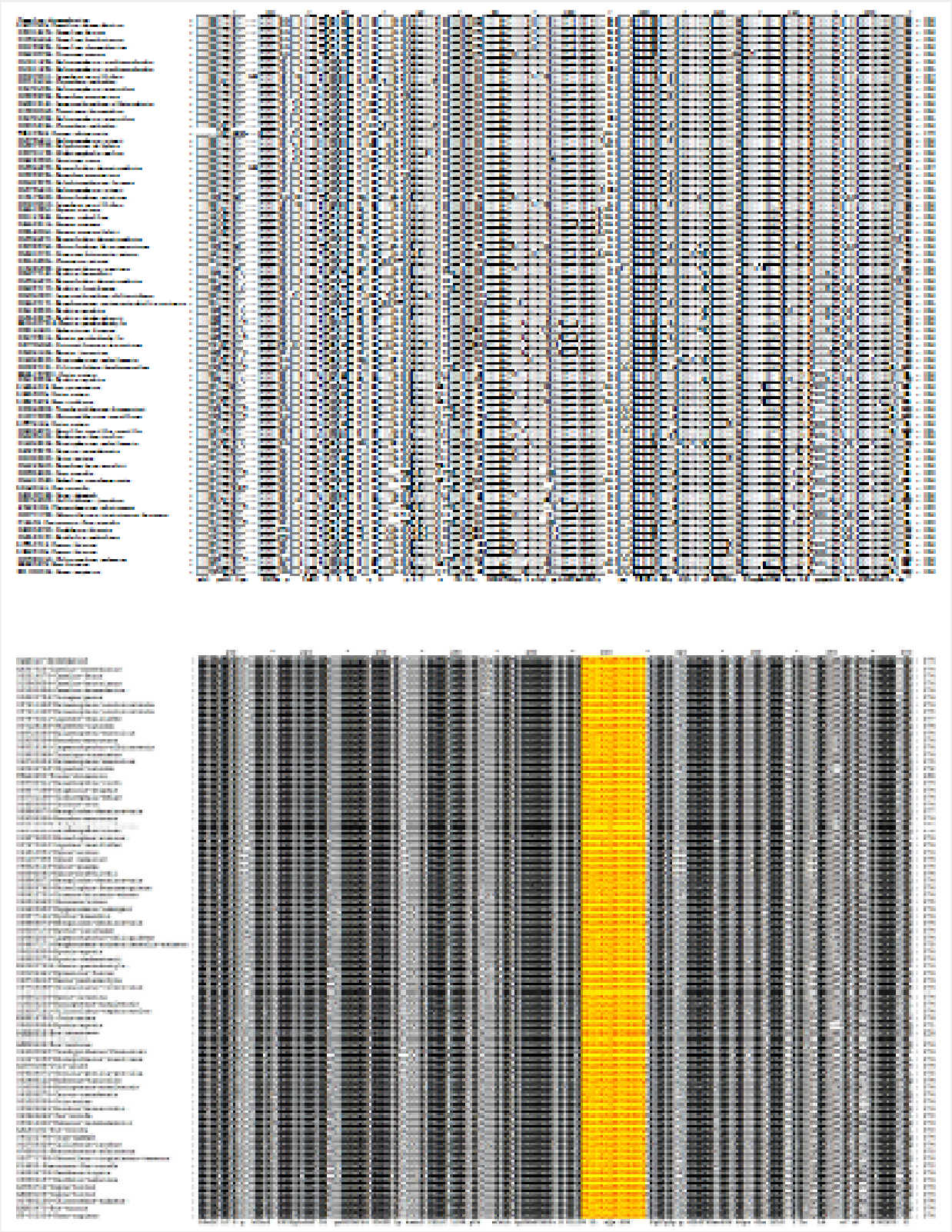

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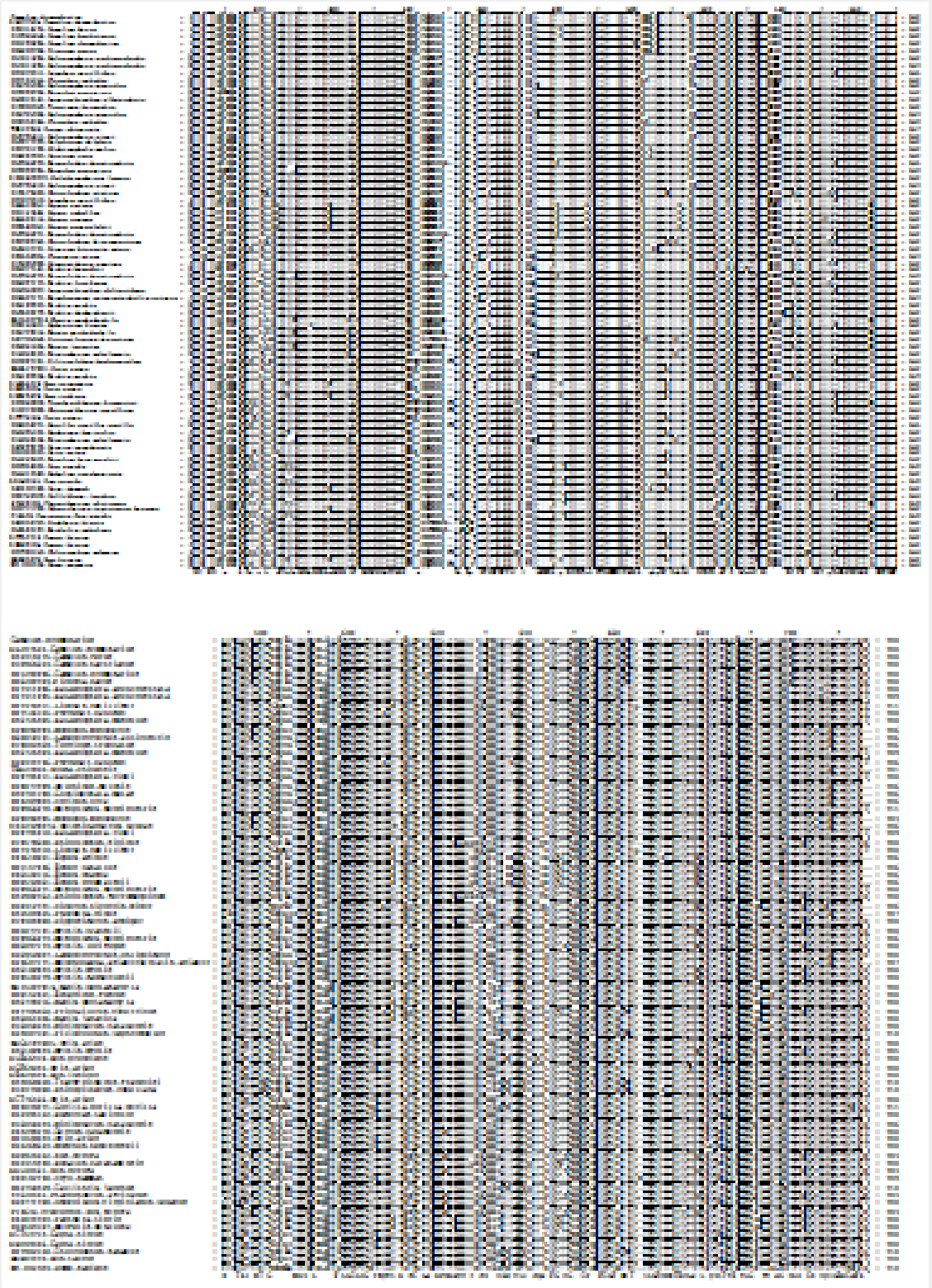

Figure 1: MEGA11 alignment of 78 sequences obtained from NCBI database of parent protein (Lf) from 60 animal species and human. Yellow highlighted sequences represent Lfampin located in this alignment of parent protein of different animal species.

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Figure 2: Phylogenetic tree built by MEGA11 of parent protein (Lf) among different animal species.

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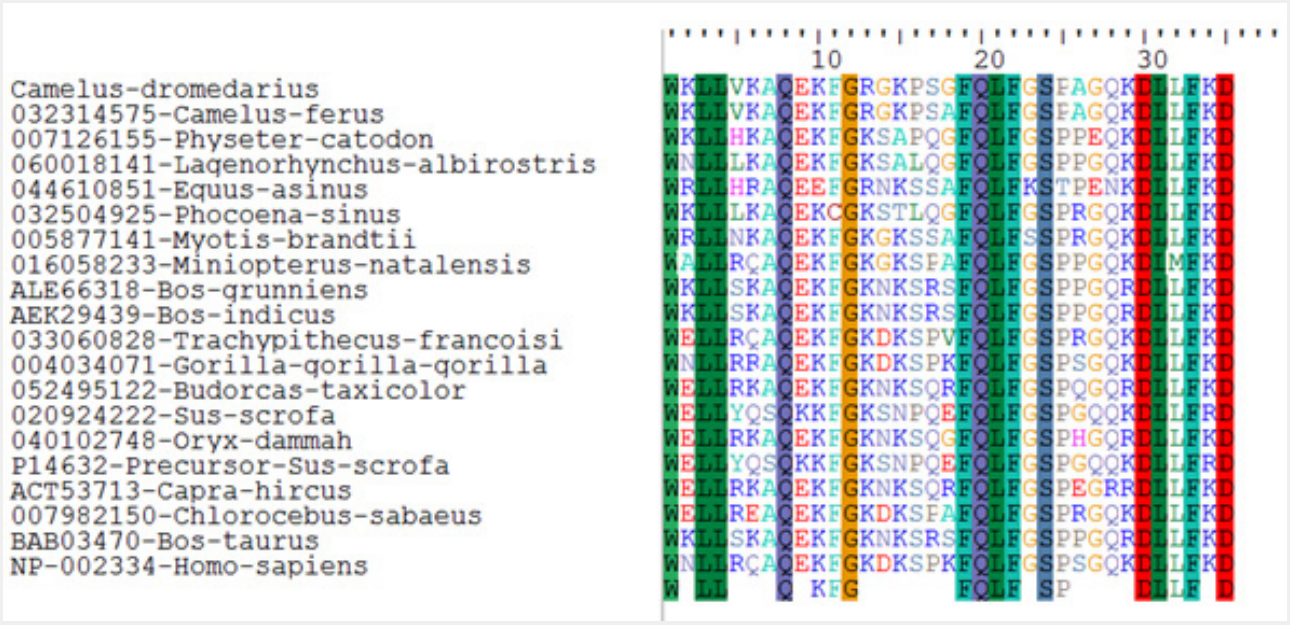

Figure 3: BioEdit alignment of sequences of Lfampin region from 18 animal species and human.

Table 3: Sequence identity list of 20 sequences of Lfampin region relative to *Camelus dromedarius* Lfampin region.

Sequence of Lfampin region	Percent identity
AHJ37525-*Camelus dromedarius*	100%
032314575-*Camelus ferus*	97.1%
007126155-*Physeter catodon*	80.0%
060018141-*Lagenorhynchus albirostris*	77.1%
044610851-*Equus asinus*	65.7%
032504925-*Phocoena sinus*	77.1%
005877141-*Myotis brandtii*	80.0%
016058233-*Miniopterus natalensis*	74.2%
ALE66318-*Bos grunniens*	77.1%
AEK29439-*Bos indicus*	77.1%
033060828-*Trachypithecus francoisi*	74.2%
004034071-*Gorilla gorilla gorilla*	74.2%
052495122-*Budorcas taxicolor*	74.2%
020924222-*Sus scrofa*	62.8%
040102748-*Oryx dammah*	77.1%
P14632-Precursor *Sus scrofa*	62.8%
ACT53713-*Capra hircus*	71.4%
007982150-*Chlorocebus sabaeus*	74.2%
BAB03470-*Bos taurus*	77.1%
NP-002334-Homo sapiens	74.2%

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Table 4: VLXT NNP statistics of 20 sequences of Lfampin region generated by PONDR.

Animal species	Number of residues disordered	Number Disordered Regions	Overall percent disordered	Predicted disorder segment	Longest Disordered Region	Average Prediction Score	Average Strength
Camelus dromedarius	0	0	0.00	0	0	0.3101	0
Gorilla gorilla gorilla	6	2	17.14	[8]-[12] [29]-[29]	5	0.3580	0.5446 0.5003
Myotis brandtii	10	2	28.57	[7]-[10] [26]-[31]	6	0.3719	0.5222 0.5985
Physeter catodon	7	1	20.00	[25]-[31]	7	0.3943	0.6198
Chlorocebus sabaeus	14	2	40.00	[4]-[11] [26]-[31]	8	0.4120	0.5616 0.5795
Miniopterus natalensis	7	2	20.00	[8]-[10] [26]-[29]	4	0.3413	0.5075 0.5167
Sus scrofa	8	1	22.86	[28]-[35]	8	0.3588	0.6224
Camelus ferus	0	0	0.00	0	0	0.3090	0
Phocoena sinus	6	1	17.14	[26]-[31]	6	0.3398	0.5785
Trachypithecus francoisi	10	2	28.57	[6]-[9] [26]-[31]	6	0.3754	0.5225 0.5781
Oryx dammah	13	2	37.14	[4]-[10] [29]-[34]	7	0.3837	0.5378 0.6088
Equus asinus	3	2	8.57	[29]-[30] [32]-[32]	2	0.4026	0.5170 0.5649
Budorcas taxicolor	17	2	48.57	[4]-[11] [26]-[34]	9	0.4311	0.5476 0.6249
Lagenorhynchus albirostris	6	1	17.14	[26]-[31]	6	0.3259	0.5575
Bos indicus	18	2	51.43	[4]-[11] [25]-[34]	10	0.4569	0.5555 0.6789
Bos grunniens	18	2	51.43	[4]-[11] [25]-[34]	10	0.4569	0.5555 0.6789
Bos taurus	18	2	51.43	[4]-[11] [25]-[34]	10	0.4569	0.5555 0.6789
Capra hircus	22	2	62.86	[4]-[11] [21]-[34]	14	0.5866	0.5476 0.7910
Homo sapiens	2	2	5.71	[9]-[9] [29]-[29]	1	0.3353	0.5027 0.5003
Precursor *Sus scrofa*	8	1	22.86	[28]-[35]	8	0.3588	0.6224

Figure 5 displayed results of prediction of disorder propensity and binary disorder in 20 sequences of Lfampin region from 18 animal species and human by flDPnn Server. These results of flDPnn disorder propensity vary significantly among analyzed sequences of Lfampin region. Distribution of intrinsic disorder in bioactive proteins/peptides as well as disorder propensity can identify their source such as animal milk source in this case. Yacouba et al. (2017) could identify animal meat source depending on the intrinsic disorder distribution peculiarities in mitochondrial cytochrome b amino acid sequences [33]. They showed that discrimination between meat of avian and animal species could be predicted from the proportions of Ser-Pro-Ala, and Leu-Ile in mitochondrial cytochrome b amino acid sequences [33].

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Table 5: HeliQuest CompuParam results of Lfampin of 18 animal species and human.

Animal species	Hydrophobicity	Hydrophobic moment (μH)	Polar residues + GLY (n/%)	Nonpolar residues (n/%)	Uncharged residues + GLY	Charged residues	Aromatic residues
Camelus dromedarius	0.212	0.372	11 / 61.11	7 / 38.89	GLN 1, SER 1, GLY 3	LYS 4, ARG 1, GLU 1	TRP 1, PHE 1
Gorilla gorilla gorilla	0.012	0.308	12 / 66.67	6 / 33.33	GLN 1, SER 1, ASN 1, GLY 1	LYS 4, ARG 2, GLU 1, ASP 1	TRP 1, PHE 1,
Myotis brandtii	0.086	0.425	12 / 66.67	6 / 33.33	GLN 1, SER 2, ASN 1, GLY 2	LYS 4, ARG 1, GLU 1	TRP 1, PHE 1
Physeter catodon	0.213	0.452	11 / 61.11	7 / 38.89	GLN 2, HIS 1, SER 1, GLY 2	LYS 4, GLU 1	TRP 1, PHE 1
Chlorocebus sabaeus	0.103	0.355	11 / 61.11	7 / 38.89	GLN 1, SER 1, GLY 1	LYS 3, ARG 1, GLU 3, ASP 1	TRP 1, PHE 1
Miniopterus natalensis	0.222	0.304	10 / 55.56	8 / 44.44	GLN 2, SER 1, GLY 2	LYS 3, ARG 1, GLU 1	TRP 1, PHE 1
Sus scrofa	0.196	0.326	12 / 66.67	6 / 33.33	GLN 3, SER 2, ASN 1, GLY 1	LYS 3, GLU 2	TYR 1, TRP 1, PHE 1
Camelus ferus	0.229	0.383	10 / 55.56	8 / 44.44	GLN 1, SER 1, GLY 2	LYS 4, ARG 1, GLU 1	TRP 1, PHE 1
Phocoena sinus	0.338	0.431	11 / 61.11	7 / 38.89	GLN 2, SER 1, THR 1, GLY 2	LYS 4, GLU 1	TRP 1
Trachypithecus francoisi	0.177	0.383	11 / 61.11	7 / 38.89	GLN 2, SER 1, GLY 1	LYS 3, ARG 1, GLU 2, ASP 1	TRP 1, PHE 1
Oryx dammah	0.023	0.393	13 / 72.22	5 / 27.78	GLN 2, SER 1, ASN 1, GLY 2	LYS 4, ARG 1, GLU 2	TRP 1, PHE 1
Equus asinus	0.111	0.398	12 / 66.67	6 / 33.33	GLN 1, HIS 1, SER 2, ASN 1, GLY 1	LYS 1, ARG 3, GLU 2	TRP 1, PHE 1
Budorcas taxicolor	-0.033	0.348	13 / 72.22	5 / 27.78	GLN 2, SER 1, ASN 1, GLY 1	LYS 4, ARG 2, GLU 2	TRP 1, PHE 1
Lagenorhynchus albirostris	0.376	0.428	10 / 55.56	8 / 44.44	GLN 2, SER 1, ASN 1, GLY 2	LYS 3, GLU 1	TRP 1, PHE 1
Bos indicus	0.012	0.433	13 / 72.22	5 / 27.78	GLN 1, SER 3, ASN 1, GLY 1	LYS 5, ARG 1, GLU 1	TRP 1, PHE 1
Bos grunniens	0.012	0.433	13 / 72.22	5 / 27.78	GLN 1, SER 3, ASN 1, GLY 1	LYS 5, ARG 1, GLU 1	TRP 1, PHE 1
Bos taurus	0.012	0.433	13 / 72.22	5 / 27.78	GLN 1, SER 3, ASN 1, GLY 1	LYS 5, ARG 1, GLU 1	TRP 1, PHE 1
Capra hircus	-0.033	0.348	13 / 72.22	5 / 27.78	GLN 2, SER 1, ASN 1, GLY 1	LYS 4, ARG 2, GLU 2	TRP 1, PHE 1
Homo sapiens	0.056	0.269	12 / 66.67	6 / 33.33	GLN 2, SER 1, ASN 1, GLY 1	LYS 4, ARG 1, GLU 1, ASP 1	TRP 1, PHE 1
Precursor *Sus scrofa*	0.196	0.326	12 / 66.67	6 / 33.33	GLN 3, SER 2, ASN 1, GLY 1	LYS 3, GLU 2	TYR 1, TRP 1, PHE 1

f5

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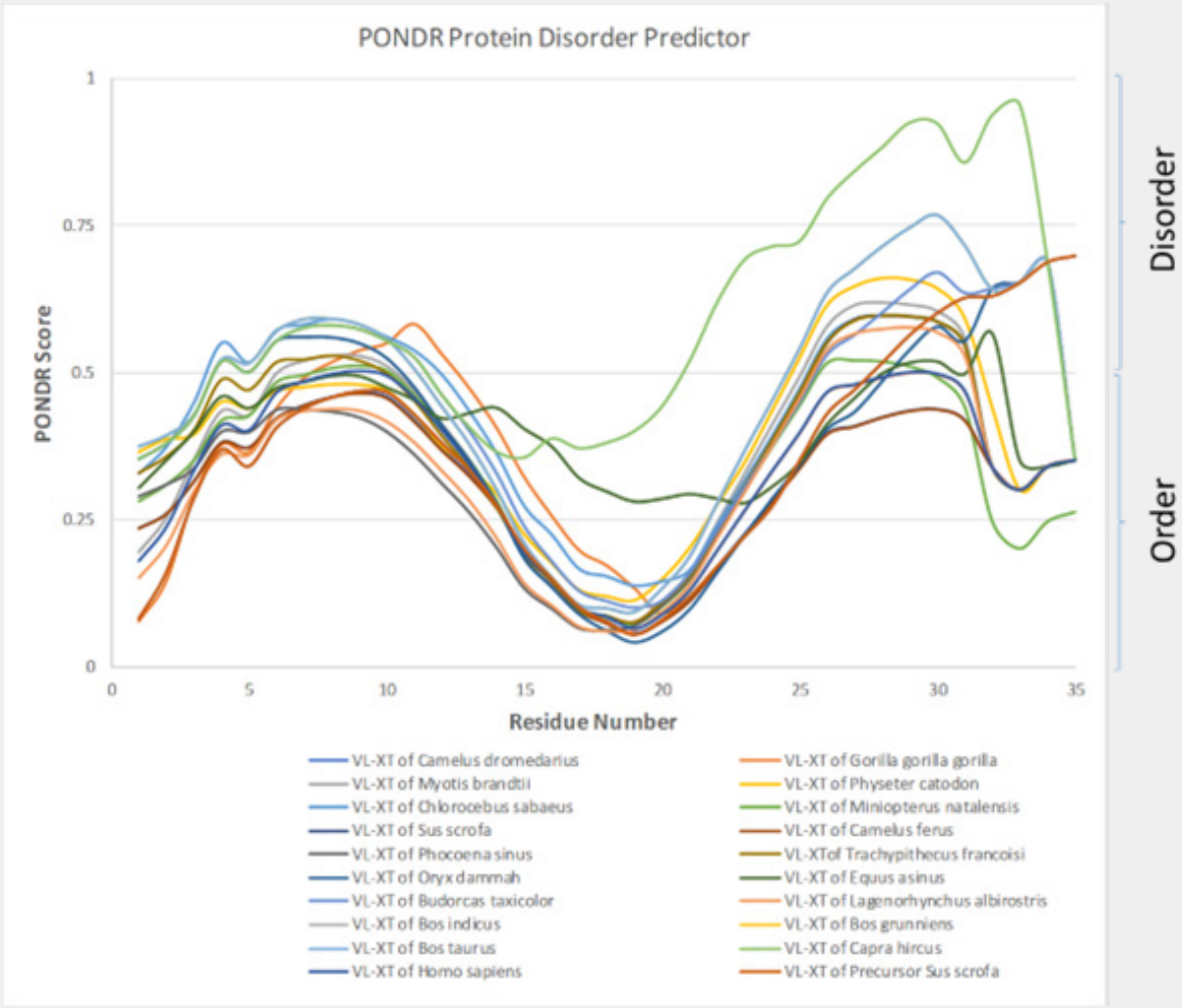

Figure 4: Prediction of natural disordered regions in 20 sequences of Lfampin region from 18 animal species and human by PONDR.

Enhancing the net positive charge of antimicrobial peptides such as Lfampin, i.e., improving cationicity of the peptide will significantly enhance its antibacterial effects or its antimicrobial effects in general. Additionally, predicting net positive charge of peptide can anticipate its antimicrobial effect and its potential application in feed or food, and as a peptide drug. HeliQuest CompuParam results of Lfampin of 18 animal species and human in Figure 6 and Table 5 demonastrated that *Chlorocebus sabaeus* Lfampin has zero net positive charge, while *Bos indicus*, *Bos grunniens*, and *Bos taurus* peptides have the highest net charge (=5). These net charge results of *Bos taurus* Lfampin in this work disagree with corresponding results of *Bos taurus* Lfcin we previously reported [34]. In our previous study, camel Lfcin has superior net charge over *Bos taurus* Lfcin and displayed greater antibacterial effects as confirmed experimentally [34].

Furthermore, Sus scrofa and *Trachypithecus francoisi* Lfampin have a net positive charge of only 1 Figure 6. Human Lfampin has a lower net positive charge (=3) relative to that of *Bos taurus* (=5), a result that agrees with that reported who increased the net positive charge of human Lfampin to enhance its Candidacidal and antibacterial effects [10].

Conclusion

Analysis of amino acid sequences, hydrophobicity, and charge characteristics of primary structure, number of disordered regions, overall percent of disorder, and disorder propensity of Lfampin from 18 different animal species and human recommends *Bos grunniens* (domestic yak Lfampin), *Bos indicus* (Zebu cattle Lfampin), and *Bos taurus* (cattle Lfampin) as candidates for developing therapeutic peptides either alone or linked to Lfcin in a chimeric peptide. These data should be experimentally validated. The absence of intrinsic disorder in camel Lfampin can be applied in identification of milk of this animal. *Capra hircus* (Goat Lfampin) with a net positive charge of 4 and 62.86% overall percent of disorder needs further investigation as a novel therapeutic peptide.

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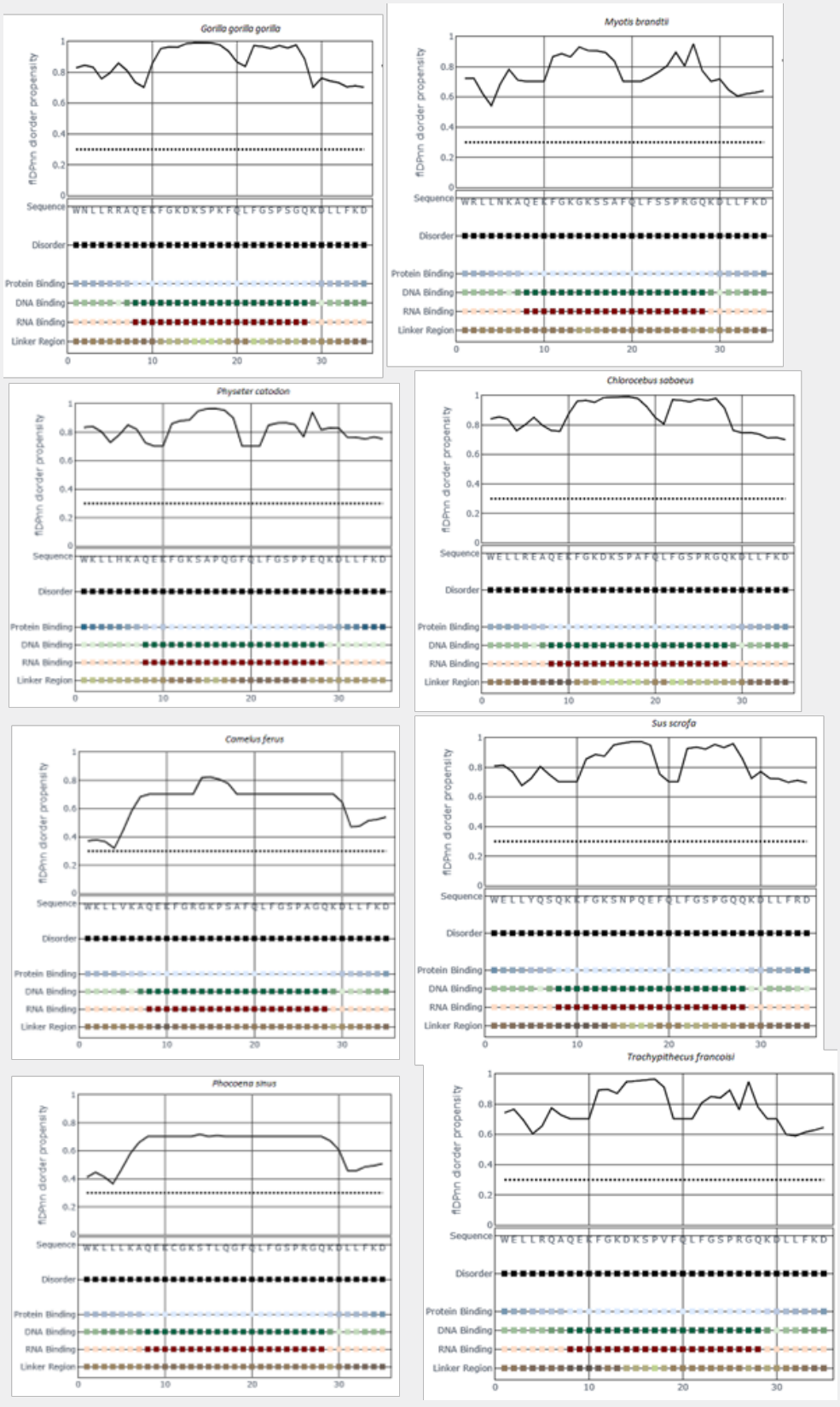

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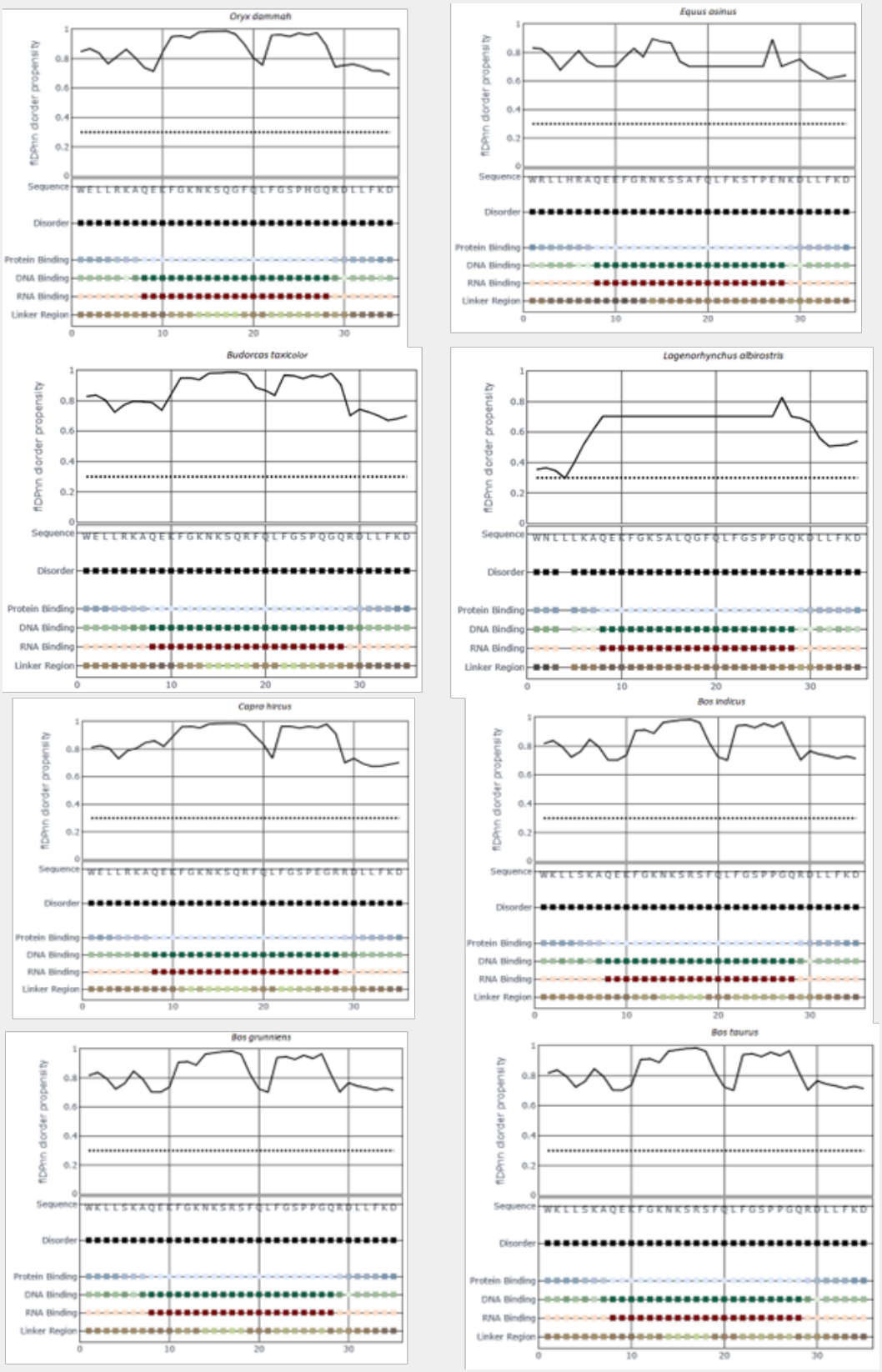

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Figure 5: Prediction of disorder propensity and binary disorder in 20 sequences of Lfampin region from 18 animal species and human by flDPnn Server.

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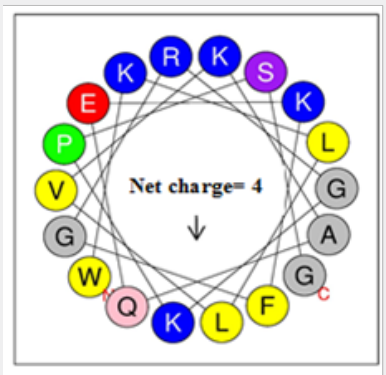
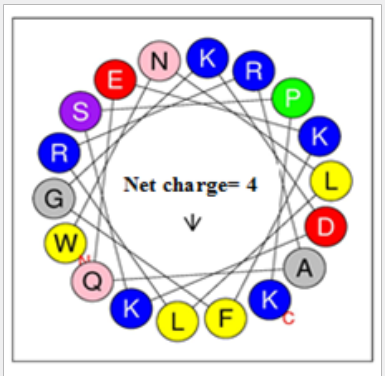
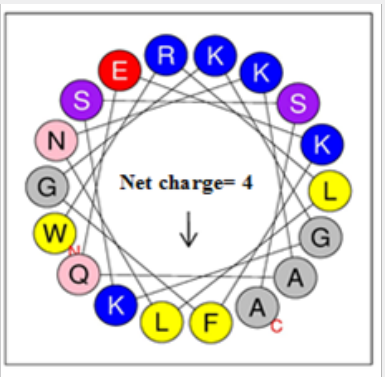


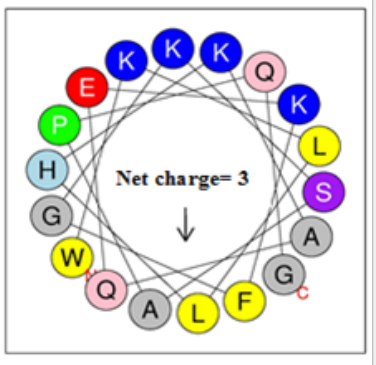
Camelus dromedarius



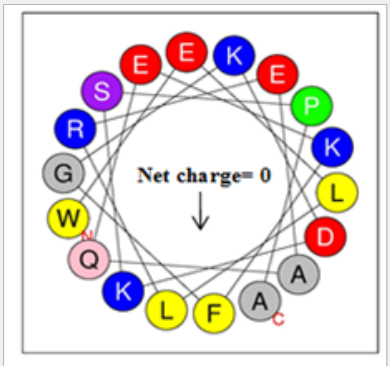
Gorilla gorilla gorilla



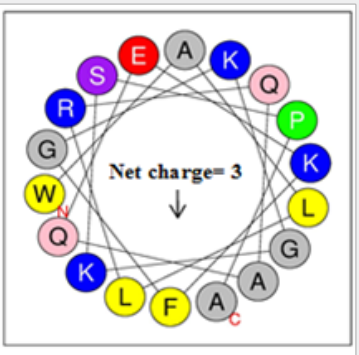
Myotis brandtii



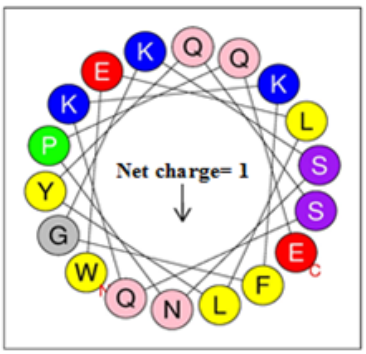
Physeter catodon



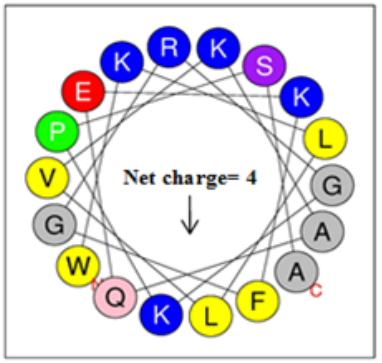
Chlorocebus sabaeus



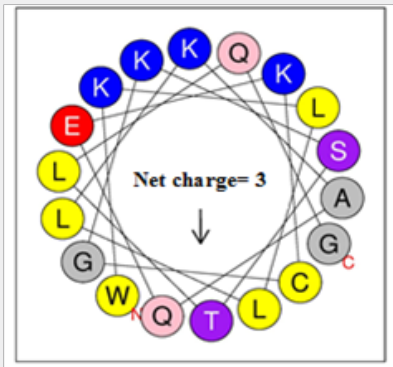
Miniopterus natalensis



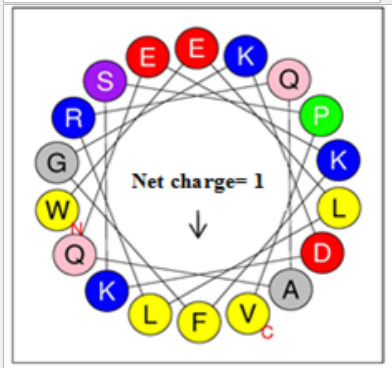
Sus scrofa
Precursor *Sus scrofa*



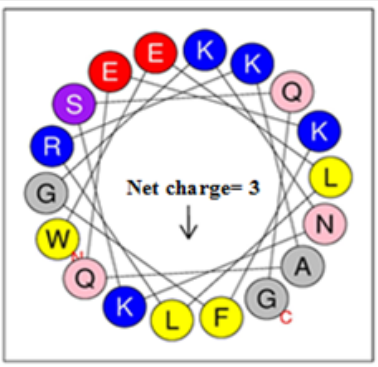
Camelus ferus



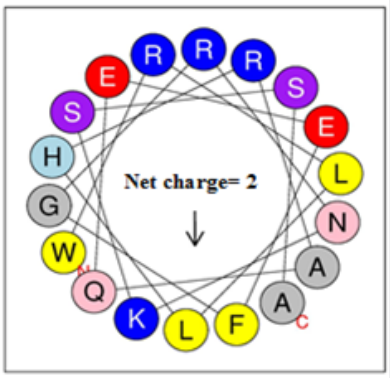
Phocoena sinus



Trachypithecus francoisi



Oryx dammah



Equus asinus

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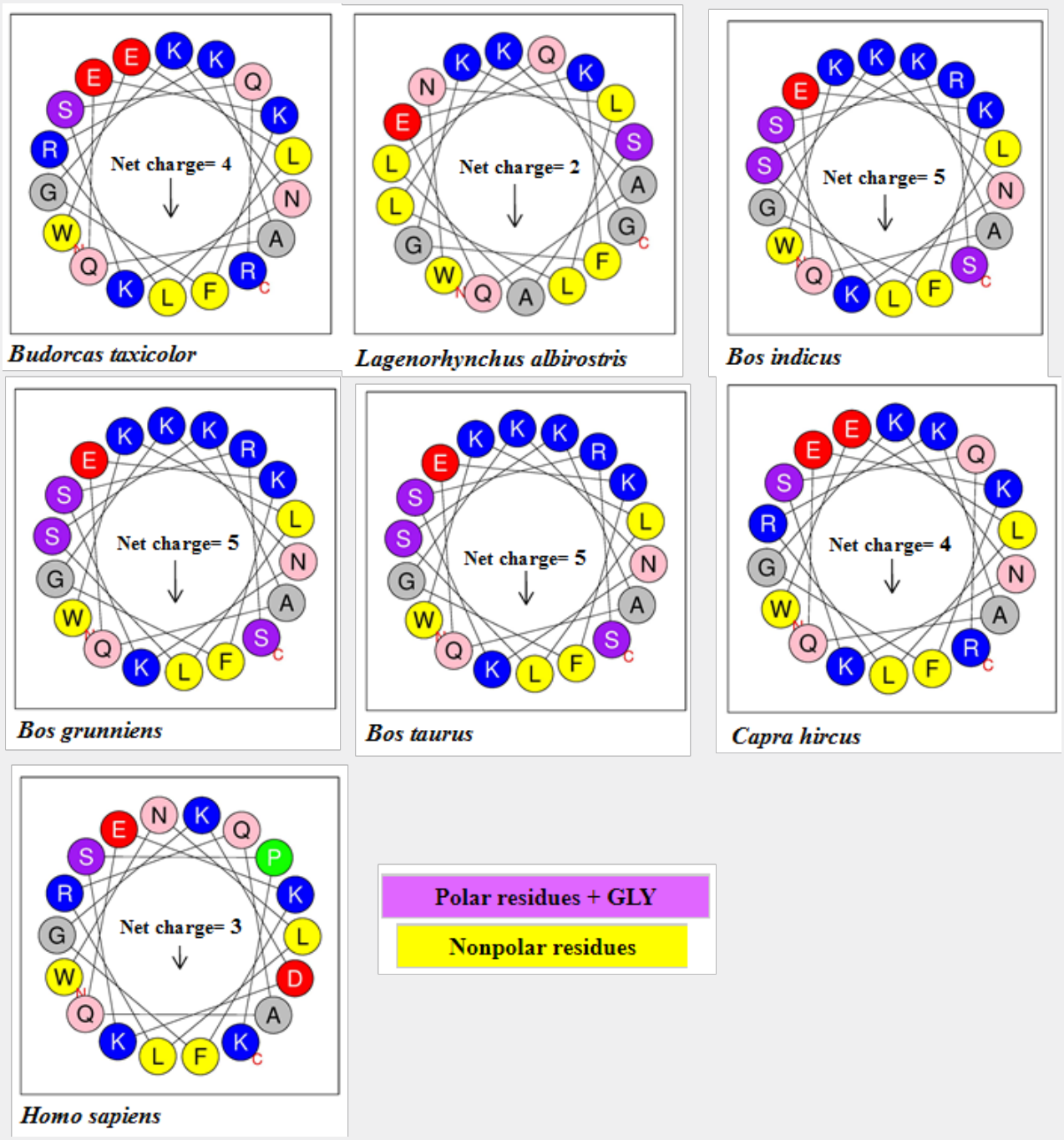

Figure 6: HeliQuest CompuParam diagrams of Lfampin of 18 animal species and human.

Conflict of Interest

None

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