International Journal of Biochemistry, Biophysics & Molecular Biology
2024, Vol. 9, No. 2, pp. 25-41
https://doi.org/10.11648/j.ijbbmb.20240902.11

Research Article

Physicochemical and Structural Validation of Myxovirus Resistance 1 (Mx1) Protein of Three Strains of the Nigerian Indigenous (*Gallus Gallus domesticus*) And Exotic Chickens

Yeigba Bolouinbele Japhet*, Toipre Omiete Samuel, Kai Woyingiemi Bolouzimo

Department of Animal Science, Faculty of Agriculture, Niger Delta University, Wilberforce Island, Nigeria

Abstract

This research was conducted on three strains of Nigerian Indigenous (*gallus gallus domesticus*) and Noiler (exotic) chickens. It examined the physicochemical and structural validation of Myxovirus resistance 1 (Mx1) protein on the three strains of Nigerian indigenous (naked neck, frizzle feather, normal feather) and noiler (exotic) chickens and also the prediction of the Physicochemical Analysis of protein. Nucleotide sequence were retrieved from National Center for Bio-Technology Information (NCBI) database and subjected to multiple sequence alignment, prediction of the physicochemical analysis of protein was done in the ProtParam web server. Modeling of 3D structural validation, Swiss modeling and Statistical analysis were all carried out. Software was used to align the sequences to find any Single Nucleotide Polymorphism (SNPS). The result of physicochemical analysis showed that the properties of the Mx1 protein fell within accepted threshold and predicted that the proteins were generally related. To validate the structure obtained from modeling, the obtained PDB files were ran on the Pro-check validation server and obtained Errat and Ramachandran plots. The study elucidates the unique features and potential functional implications of Mx1 protein across different strains of Nigerian indigenous and exotic chickens. This knowledge can inform the development of strategies to improve disease resistance in local chicken populations through selective breeding or genetic manipulation. Comparing the Mx1 protein among different strains of Nigerian indigenous chickens can reveal evolutionary adaptations and provide valuable information for understanding the molecular basis of immune defense mechanisms in poultry. Understanding the physicochemical properties and structural dynamics of Mx1 protein contributes to the broader understanding of innate immune responses in indigenous chicken breeds, offering insights into their disease resistance and adaptation mechanisms.

Keywords

Mx1 Protein, Nigerian Indigenous Chicken, Noiler Exotic Chicken, Physicochemical and Structural Validation

1. Introduction

More than 6,300 varieties of livestock from 30 domesticated animals have evolved over the past 12,000 years as a result of domestication and selection [12]. These livestock populations have developed particular adaptations to their

*Corresponding author: japhetyeigba@gmail.com (Yeigba Bolouinbele Japhet)

Received: 4 September 2024; **Accepted:** 27 September 2024; **Published:** 18 October 2024

agro-ecological surroundings and agricultural production systems [24]. The effective breeding improvement initiatives of the industrialized nations in the 19th and 20th centuries were made possible by their genetic variety.

Characterization of a specific population of domestic animals is necessary for its sustainable management, use, and conservation [6]. Characterization in the context of genetics is the process of identifying variation brought on by variations in DNA sequences, particular genes, or modifying factors [5].

Mx proteins are dynamin-related GTPases that are necessary for interferon types I and III to mediate the innate immune response against many viruses [11, 4]. They hinder the capacity of many RNA and DNA viruses to replicate by preventing the first stages of their life cycle. The two MX gene (MX1 and MX2) present in the human genome encodes the two proteins MxA and MxB, commonly referred to as Mx1 and Mx2. Mostly negative-stranded RNA viruses, but also some positive-stranded RNA and DNA viruses are suppressed by the MxA protein [30, 36]. Gaining knowledge of these proteins physicochemical characteristics can help one understand its stability, structural, functional relationship and possible uses.

2. Materials and Methods

2.1. Experimental Animal

The Nigerian indigenous chicken (normal feather, Frizzled feather, and naked neck) and noiler exotic chicken was used in this experiment. The chickens were purchased in Yenagoa metropolis.

2.2. Nucleotide Sequence Retrieval

Nucleotide sequences were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov) and were translated to amino acid sequences on the EMBOSS web server (https://www.ebi.ac.uk/jdispatcher/st/emboss_transeq).

2.3. Prediction of the Physicochemical Analysis of Protein

To investigate the physicochemical properties of the sequences being understudied, the sequences were individually analyzed on the ProtParam web server on Expasy (web.expasy.org/protparam/) and results which include molecular weight, theoretical pI, instability index and others were obtained, this analysis reveals the structural and functional properties of the protein encoded by the primary sequences.

2.4. Modeling of 3D Structure

The translated sequences were also used to model the possible tertiary structure of the proteins they encode for, this was done on the Swiss model server (www.swissmodel.expasy.org). Predicting the tertiary structure provides a model that can be used to understand the functionality of a protein such as revealing the possible active sites and binding domains as 3D models of proteins are predicted and modeled from structures of native proteins with similar amino acid sequences.

2.5. 3D Structure Validation

To validate the predicted 3D structures of the protein models, the PDB files of the protein structures were uploaded into the PROCHEK protein structure validation tool. PROCHEK statistically predict errors in the experimental and theoretical protein structure and present them in graphs which include ERRAT graph and RAMACHANDRAN PLOT. ERRAT shows regions in the sequences where the errors occur, this are regions within the sequences that do not conform with native protein structures while the RAMACHANDRAN plots reveals various information such as residues located in the favoured and unflavored regions. With PROCHECK one can draw a conclusion for the conformity of a theoretical protein structure to a known protein structure.

2.6. Swiss Modeling

Swiss model is an automated protein structure homology-modeling server developed by the Swiss institute of bioinformatics. It allows users to generate accurate three-dimensional models of proteins based on their amino acid sequence and know structures of related proteins. [32]. Swiss model has a wide range of applications in structural biology, including protein structure prediction, functional annotationand drug discovery and understanding the molecular mechanism of disease. [25].

2.7. Statistical Analysis

Results were analyzed using the ProtParam web server on Expasy (web.expasy.org/protparam/), the Swiss model server (www.swissmodel.expasy.org) and with the use of PROCHEK protein structure validation tool. PROCHEK statistically predict errors in the experimental and theoretical protein structure and present them in graphs which include ERRAT graph and RAMACHANDRAN PLOT.

3. Results and Discussion

Physicochemical Analysis results for Frizzle feather Nigerian Indigenous chicken associated with Mx1 protein

This result shows the biochemical properties, structural characteristics comprising of the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein in the frizzle

feather gene, and the comparison that exist between gene A and gene B.

Physicochemical Analysis results for Naked neck Nigerian Indigenous chicken associated with Mx1 protein

This provides insights into its properties. The result of this analysis comprises of the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein that exist in gene A, B, C, D, E and F of the naked neck chicken.

Physicochemical Analysis results for Normal feather Nigerian Indigenous chicken associated with Mx1 protein

The result of this analysis shows in details the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein that exist in gene A, B, C, D, E and F of the Normal feathered chicken.

Physicochemical Analysis results for Noiler Exotic chicken associated with Mx1 protein

These Physicochemical Analysis results collectively contributes to understanding the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein that exist in gene A, B, C, of the Noiler exotic chicken.

Physicochemical Analysis results for Frizzle feather Nigerian Indigenous chicken associated with Mx1 protein

The physicochemical analysis of Mx1 proteins from Frizzle feather Nigerian Indigenous chickens demonstrates both consistency and slight variations across the different isolates. Both Mx1 proteins analyzed (FF GALLUS GALLUS (Mx1) gene (A) and FR1 FF (Mx1) gene (B)) have 73 amino acids. The molecular weights are very close: FF GALLUS GALLUS (Mx1) gene (A) is 7965.62 Da and FR1 FF (Mx1) gene (B) is 7993.67. The average molecular weight is approximately 7979.65. The theoretical isoelectric point (pI) values are identical, at 9.54 for both proteins, indicating that these proteins are basic. For both sequences, which start with Histidine (His), the estimated half-life is 3.5 hours in mammalian reticulocytes in vitro, 10 minutes in yeast in vivo, and over 10 hours in E. coli in vivo. In contrast, a study by [3] analyzed Mx1 proteins from different breeds of chickens and found that the molecular weights ranged from 75-80 kDa, which is higher than the values reported in the write-up. However, the study by [3] also found that the proteins were basic, with pI values ranging from 9.2-9.8, which is consistent with the research. Another study by [34] analyzed Mx1 proteins from ducks and found that the proteins had similar molecular weights and pI values to the write-up. However, [34] reported that the estimated half-life of the proteins was longer, ranging from 4-6 hours in mammalian reticulocytes in vitro, which is in contrast to the research. The instability index indicates stability for both proteins, with values of 28.28 for FF GALLUS GALLUS (Mx1) gene (A) and 28.02 for FR1 FF (Mx1) gene (B). The average instability index is approximately 28.15. The aliphatic index, which reflects protein thermostability, shows slight variation. FF GALLUS GALLUS (Mx1) gene (A) has an aliphatic index of 75.89, while FR1 FF (Mx1) gene (B) has an index of 78.49. The average aliphatic index is approximately 77.19. The Grand Average of Hydropathicity (GRAVY) values, which indicate protein hydrophobicity, are similar: FF GALLUS GALLUS (Mx1) gene (A) has a GRAVY of 0.274 and FR1 FF (Mx1) gene (B) has a GRAVY of 0.297. The average GRAVY is approximately 0.286 and the results is in agreement with findings made by [35] analyzing the stability of proteins using the instability index and found similar values for stability. However, in their study, the aliphatic index showed more significant variations between different proteins compared to the slight variation observed in the current study. This suggests that different proteins may have varying levels of thermostability. Additionally, in a study by [1] who investigated the hydrophobicity of proteins using the GRAVY values and found that the values could vary significantly between different proteins. In contrast, the current study shows similar GRAVY values for both proteins, indicating similar levels of hydrophobicity. The physicochemical properties of the Mx1 proteins from Frizzle feather Nigerian Indigenous chickens are highly consistent, reflecting the conserved nature of the Mx1 gene, which is essential for antiviral defense mechanisms as stated by [20]. The slight variations observed can be attributed to minor genetic differences or polymorphisms in the promoter region of the gene which was observed by [16]. These variations, however, do not significantly impact the overall stability and function of the protein. The high level of consistency in molecular weight, theoretical pI, and estimated half-life indicates that the Mx1 gene is well-conserved among these chicken isolates. This conservation is crucial for maintaining the protein's functionality in antiviral defense which was also reported by [10] and it is in agreement with the present study. The minor differences in the instability index and aliphatic index suggest subtle variations in protein stability and thermostability, which might result from natural genetic diversity within the population. These variations could potentially offer slight advantages in different environmental conditions or stress responses as reported by [9, 14]. The consistently high pI values suggest that the Mx1 proteins are basic, which could influence their interaction with other molecules and their overall stability in different pH environments. The stability indicated by the instability index values classifies both proteins as stable. This stability is essential for the protein's role in the cellular antiviral responses indicated by [17].

Physicochemical Analysis results for Naked neck Nigerian Indigenous chicken associated with Mx1 protein

The physicochemical analysis of the Mx1 protein gene from different isolates of Naked neck Nigerian Indigenous chickens provides insights into their potential functional properties and stability. Here are the key points from the

analysis: Except for NK5, all Mx1 proteins from isolates (NK1 to NK4 and NK6) consist of 73 amino acids with a molecular weight of 7979.65 Da. NK5 has a slightly different composition with 72 amino acids and a molecular weight of 7805.34. The theoretical isoelectric point (pI) for most isolates (NK1 to NK4 and NK6) is 9.54, indicating they are basic proteins. NK5 has a slightly lower pI of 8.79, but it is still on the basic side. In agreement to the result of the current study, a research by [35] on the Mx1 gene in ducks also identified variations in amino acid composition and molecular weight among different isolates, reflecting genetic diversity within avian populations. Similarly, the observation of basic pi values in the majority of Mx1 proteins from Naked Neck Nigerian Indigenous chicken resonates with studies by [28] on avian Mx1 proteins, highlighting their predominant basic nature across diverse avian species. For the sequences starting with Histidine (His), the estimated half-life is 3.5 hours in mammalian reticulocytes in vitro, 10 minutes in yeast in vivo, and over 10 hours in E. coli in vivo. In agreement to the statement above, the physicochemical analysis of the amino acid composition, molecular weight, and isoelectric point (pI) of the Mx1 proteins from the isolates (NK1 to NK6) is crucial for understanding their characteristics. The differences in amino acid composition and pI values among the isolates, particularly NK5, indicate potential variations in functional properties. Additionally, the estimated half-life of the Mx1 proteins starting with Histidine (His) in different cellular environments provides valuable information on their stability and potential interactions as stated by [22]. NK5, starting with Serine (Ser), shows a different half-life: 1.9 hours in mammalian reticulocytes, over 20 hours in yeast, and more than 10 hours in E. coli. The instability index for NK1 to NK4 and NK6 is 26.85, classifying them as stable proteins. NK3 has a slightly higher instability index of 28.28, but it is still considered stable.NK5 is the most stable with an instability index of 19.20. NK1, NK2, NK4, and NK6 have an aliphatic index of 77.12, suggesting moderate thermostability. NK3 has a slightly lower aliphatic index of 74.52. NK5 has an aliphatic index of 76.94. Grand Average of Hydropathicity (GRAVY) values for NK1, NK2, NK4, and NK6 are 0.303, indicating they are mildly hydrophobic. NK3 has a slightly lower GRAVY value of 0.270. NK5 has a higher GRAVY value of 0.400, indicating it is more hydrophobic. NK5 stands out due to its slightly lower molecular weight, different starting amino acid (Ser), lower pI, and shorter half-life in mammalian cells, and higher GRAVY value. These differences might influence its interaction with other proteins and its functional properties under different physiological conditions, [26, 21] supports the information that NK5 has a different half-life compared to other NK proteins in E. coli, indicating that protein half-lives can vary widely depending on the specific protein. [2], supports the assessment of stability for the NK proteins based on their instability indices as it discusses the use of instability index to predict protein stability changes due to mutations. In a study made by [14] which introduces the concept of aliphatic index as a measure of protein stability, which aligns with the discussion of aliphatic index in this study for the NK proteins. The stability indices suggest that all the Mx1 proteins are stable, with NK5 being the most stable. This stability is crucial for the protein's function in antiviral defense, as Mx1 is known for its role in resistance to myxoviruses. The consistency in physicochemical properties across most isolates can be attributed to the conserved nature of the Mx1 gene among these chickens, which plays a crucial role in their antiviral defense mechanism. The slight variations observed, particularly in NK5, could be due to minor genetic mutations or variations in the promoter region that do not significantly affect the overall stability and function of the protein but may slightly alter its interaction dynamics and stability in different environments.

Physicochemical Analysis results for Normal feather Nigerian Indigenous chicken associated with Mx1 protein

The physicochemical analysis of the Mx1 protein gene from different isolates of Normal feather Nigerian Indigenous chickens reveals consistent properties across the isolates, with minor variations. Most Mx1 proteins (NM1, NM3 to NM6) consist of 73 amino acids, with an average molecular weight of approximately 7979.65. NM2 has a slight deviation with 72 amino acids and a molecular weight of 7805.34. The average theoretical isoelectric point (pI) for most isolates (NM1, NM3 to NM6) is 9.54, indicating basic proteins. NM1 has a pI of 8.97, and NM2 has a pI of 8.79, slightly lowering the overall average but still indicating basic proteins. For sequences starting with Histidine (His), the estimated half-life averages 3.5 hours in mammalian reticulocytes in vitro, 10 minutes in yeast in vivo, and over 10 hours in E. coli in vivo.

This result resonates with studies by [18] on Mx1 gene variations in indigenous chicken populations, which also reported minor variations in amino acid composition and molecular weight across different isolates. Additionally, the observation of predominantly basic PI values aligns with research by [31] on avian Mx1 proteins, suggesting a common trend in the physicochemical properties of Mx1 proteins across avian species. Moreover, the provided information about the estimated half-life of Mx1 proteins starting with Histidine in various cellular environments correlates with the findings of [3] regarding the differential stability of Mx1 proteins in different cellular contexts. NM1, starting with Tyrosine (Tyr), has a different half-life: 2.8 hours in mammalian reticulocytes, 10 minutes in yeast, and 2 minutes in E. coli. NM2, starting with Serine (Ser), shows different half-life values: 1.9 hours in mammalian reticulocytes, over 20 hours in yeast, and more than 10 hours in E. coli. The average instability index for NM1, NM3 to NM6 is around 26.85, classifying them as stable proteins. NM2 is more stable with an instability index of 19.20, while NM3 has a slightly higher instability index of 28.02. The average aliphatic index for NM1 to NM6 is around 77.12, suggesting moderate thermostability. NM3 has a slightly higher aliphatic index of 78.49, and NM1 has an even higher aliphatic index of 79.86, indi-

cating they are more thermostable. The results of the physicochemical analysis of the Mx1 protein gene from different isolates of Normal feather Nigerian Indigenous chickens presented in this study are consistent with the result of [18] on the physicochemical properties of Mx1 proteins in chickens also reported similar molecular weights and amino acid compositions across different isolates. Additionally, [19] found that Mx1 proteins in chickens generally have basic isoelectric points and exhibit stable properties, which aligns with the findings of the current study. However, there are also some discrepancies in the results, a study by [31] on Mx1 proteins in chickens reported slightly different half-life values and instability indices compared to the current study. [31], found that Mx1 proteins in chickens have shorter half-lives in mammalian reticulocytes and higher instability indices, which contrasts with the results of the current study. The Grand Average of Hydropathicity (GRAVY) values for NM1, NM3 to NM6 is 0.303, indicating mild hydrophobicity. NM1 has a higher GRAVY value of 0.397, and NM2 has a value of 0.400, indicating they are more hydrophobic. NM1 stands out due to its unique starting amino acid (Tyr), higher aliphatic index, and higher GRAVY value. These differences might influence its interaction with other proteins and its functional properties under different physiological conditions. NM2 also shows distinct characteristics with a different starting amino acid (Ser), higher GRAVY value, and greater stability, which could impact its stability and interaction dynamics. The consistency in physicochemical properties across most isolates can be attributed to the conserved nature of the Mx1 gene among these chickens, which plays a crucial role in their antiviral defense mechanism. The slight variations observed, particularly in NM1 and NM2, could be due to minor genetic mutations or variations in the promoter region. These differences do not significantly affect the overall stability and function of the protein but may slightly alter its interaction dynamics and stability in different environments. The write-up provides information on the GRAVY values and physicochemical properties of different isolates of the Mx1 gene in chickens. It suggests that NM1 and NM2 have distinct characteristics that could impact their interaction dynamics and stability. The slight variations in these isolates are attributed to minor genetic mutations or variations in the promoter region, which may slightly alter their functional properties. In support of the outcome of this study [27] analyzed the amino acid sequence of the Mx1 gene in chickens and found conserved regions that play a crucial role in antiviral defense mechanisms. They also observed slight variations in some isolates, which they attributed to genetic mutations. This is in agreement with the study which suggests that minor genetic mutations could lead to variations in the isolates. Additionally, a study by [8] investigated the role of the Mx1 gene in chickens' immune response to viral infections. They found that variations in the gene sequence could affect the protein's interaction with other proteins and its functional properties. This supports the research statement that differences in the isolates could influence their interaction dynamics and stability.

Physicochemical Analysis results for Noiler Exotic chicken associated with Mx1 protein

The physicochemical analysis of Mx1 proteins from Noiler exotic chickens demonstrates both consistency and minor variations across different isolates. Here is a detailed discussion of the results, including the reasons behind the observed trends. All Mx1 proteins analyzed (NO1, NO2, NO3) have 73 amino acids. The molecular weight ranges slightly: NO2 is 7900.58 Da, NO1 is 7940.62 Da, and NO3 is 7979.65 Da. The average molecular weight is approximately 7940.95 Da. The theoretical pI values are consistently high, indicating that these proteins are basic. NO1 and NO2 have a pI of 9.36, while NO3 has a slightly higher pI of 9.54. For sequences starting with Histidine (His), as seen in NO1 and NO3, the estimated half-life averages 3.5 hours in mammalian reticulocytes in vitro, 10 minutes in yeast in vivo, and over 10 hours in E. coli in vivo. NO2, which starts with Serine (Ser), has different half-life values: 1.9 hours in mammalian reticulocytes, over 20 hours in yeast, and more than 10 hours in E. coli. The instability index indicates stability for all proteins, with values ranging from 26.85 (NO3) to 30.65 (NO2). The average instability index is approximately 28.46. The aliphatic index, indicating protein thermostability, shows some variation. NO1 has an aliphatic index of 75.89, NO2 has a higher index of 85.21, and NO3 has an index of 77.12. The average aliphatic index is approximately 79.41. The uniformity in amino acids length and basic nature of Mx1 proteins in Noiler exotic chickens resonates with studies by [33] on Mx1 protein properties in poultry species. Additionally, the observed disparities in half-life values among different sequence initiators align with research by [20] on the influence of N-terminal residues on protein stability. The Grand Average of Hydropathicity (GRAVY) values, which reflect protein hydrophobicity, also vary. NO1 has a GRAVY of 0.362, NO2 has a higher GRAVY of 0.437, and NO3 has a GRAVY of 0.303. The average GRAVY is approximately 0.367. NO2 stands out due to its distinct starting amino acid (Ser), higher aliphatic index, and higher GRAVY value. These differences might influence its stability and interaction with other proteins in varying physiological conditions. NO2's unique half-life values in different organisms suggest that it might be more stable in yeast and bacterial environments compared to mammalian cells. The information provided in the statement is in agreement with [15], which discusses the variation in GRAVY values among different proteins and how it can influence their stability and interactions with other proteins. Additionally, they also mention the importance of GRAVY values in predicting the stability of proteins in different physiological conditions, which aligns with the statement about NO2's stability in different organisms. The consistency in physicochemical properties across the Noiler Mx1 proteins can be attributed to the conserved nature of the Mx1 gene, crucial for antiviral defense mechanisms. The slight variations

observed, particularly in NO2, may be due to minor genetic mutations or differences in the promoter region. These variations do not significantly impact the overall stability and function of the protein but may slightly alter its stability, interaction dynamics, and functional properties under different environmental conditions [23].

1. Physicochemical Analysis results for Frizzle feather Nigerian Indigenous chicken associated with Mx1 protein1

This result shows the biochemical properties, structural characteristics comprising of the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein in the frizzle feather gene, and the comparison that exist between gene A and gene B.

2. Physicochemical Analysis results for Naked neck Nigerian Indigenous chicken associated with Mx1 protein

This provides insights into its properties. The result of this analysis comprises of the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein that exist in gene A,B,C, D, E and F of the naked neck chicken

3. Physicochemical Analysis results for Normal feather Nigerian Indigenous chicken associated with Mx1 protein

The result of this analysis shows in details the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein that exist in gene A, B, C, D, E and F of the Normal feathered chicken.

4. Physicochemical Analysis results for Noiler Exotic chicken associated with Mx1 protein

These Physicochemical Analysis results collectively contributes to understanding the molecular weight, isoelectric point (pI), secondary structure, structural stability, hydrophobicity, Amino acid composition, protein-protein interactions and potential functions of the mx1 protein that exist in gene A, B, C, of the Noiler exotic chicken.

5. Structures as Modeled on Swiss Model

This shows both the similarities and structural differences in the protein structure that exist between the Nigerian indigenous (frizzle feather, naked neck, normal feather) and noiler exotic chicken that was used in this experiment.

6. Validation of Protein Structure ERRAT and Ramachandran Plot

Errat plot shows regions within a sequence that is faulty or which fails to conform to native protein. The errat obtained revealed the faulty regions in the modeled Mx1 proteins, errat revealed the segments that are faulty. Ramachandran plot revealed that most of the Mx1 protein have over 85% of the protein residues are within favoured regions with a G-factor of -0.32, this indicate a good protein well within native protein structure

1. Physicochemical Analysis results for Frizzle feather Nigerian Indigenous chicken associated with Mx1 protein.

FF GALLUS GALLUS (Mx1) gene (A)

>OP068329.1 *Gallus gallus* isolate Fr2 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-
TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-
GACGAAAATTGGCTAATGGATGAGGAATTTGAG-
TGAAACACACATCAGGATACTGTTTTCAA-
TAATGAAAGCATTTTAGTTTCATTTCTCCTT-
GTTTATGTCATGTAGGTGGAGTCTGTGCATA-
GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-
GTTTCTCCTTGCTGTG

Number of amino acids: 73

Molecular weight: 7965.62

Theoretical pI: 9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 28.28

This classifies the protein as stable.

Aliphatic index: 75.89

Grand average of hydropathicity (GRAVY):0.274

FR1 FF (Mx1) gene (B)

>OP068315.1 *Gallus gallus* isolate Fr1 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-
TGGTTTTGTTACTGGGTTCTGCAAAGAACTGG-
GACGAAAATTGGCTAATGGATGAGGAATTTGAG-
TGAAACACACATCAGGATACTGTTTTCAA-
TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-
GTTTATGTCATGTAGGTGGAGTCTGTGTATA-
GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-
GTTTCCCCTTGCTGTG

Number of amino acids:73

Molecular weight:7993.67

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 28.02

This classifies the protein as stable.

Aliphatic index:78.49

Grand average of hydropathicity (GRAVY):0.297

2. Physicochemical Analysis results for Naked neck Nige-

rian Indigenous chicken associated with Mx1 protein.

NK1 (Mx1) gene (A)

>OP068320.1 *Gallus gallus* isolate Nk1 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCCCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

NK2 (Mx1) gene (B)

>OP068321.1 *Gallus gallus* isolate Nk2 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

NK3 (Mx1) gene (C)

>OP068322.1 *Gallus gallus* isolate Nk3 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGCATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7951.59

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 28.28

This classifies the protein as stable.

Aliphatic index:74.52

Grand average of hydropathicity (GRAVY):0.270

NK4 (Mx1) gene (D)

>OP068323.1 *Gallus gallus* isolate Nk4 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

NK5 (Mx1) gene (E)

>OP068324.1 *Gallus gallus* isolate Nk5 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGCATA-

GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCCCCTTGCTGTG

Number of amino acids:72

Molecular weight:7805.34

Theoretical pI:8.79

Estimated half-life:

The N-terminal of the sequence considered is S (Ser).

The estimated half-life is: 1.9 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 19.20

This classifies the protein as stable.

Aliphatic index:76.94

Grand average of hydropathicity (GRAVY):0.400

NK6 (Mx1) gene (F)

>OP068325.1 *Gallus gallus* isolate Nk6 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

3. Physicochemical Analysis results for Normal feather Nigerian Indigenous chicken associated with Mx1 protein.

NM1 NF (Mx1) gene (A)

>OP068311.1 *Gallus gallus* isolate Nm1 myxovirus resistance protein (Mx1) gene, promoter region

TACGTGCACAAGGAGGTAGTAGTGCATTGGAG-TGGTTTTGTTACAGGGTTCTGCAAAGAACTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGCATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCCCCTTGCTGTG

Number of amino acids:73

Molecular weight:7915.55

Theoretical pI:8.97

Estimated half-life:

The N-terminal of the sequence considered is Y (Tyr).

The estimated half-life is: 2.8 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

2min (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 24.84

This classifies the protein as stable.

Aliphatic index:79.86

Grand average of hydropathicity (GRAVY):0.397

NM2 NF (Mx1) gene (B)

>OP068313.1 *Gallus gallus* isolate Nm2 myxovirus resistance protein (Mx1) gene, promoter region

TCTCTGACAAGGACTCCAG-TTACATATTGAAGTGATTTTGTTACAGACTTCTG-CAATTTCCTGGGACGAAAATT-GGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:72

Molecular weight:7805.34

Theoretical pI:8.79

Estimated half-life:

The N-terminal of the sequence considered is S (Ser).

The estimated half-life is: 1.9 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 19.20

This classifies the protein as stable.

Aliphatic index:76.94

Grand average of hydropathicity (GRAVY)0.400

NM3 NF (Mx1) gene (C)

>OP068316.1 *Gallus gallus* isolate Nm3 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAACTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7993.67

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 28.02

This classifies the protein as stable.

Aliphatic index:78.49

Grand average of hydropathicity (GRAVY):0.297

NM4 NF (Mx1) gene (D)

>OP068317.1 *Gallus gallus* isolate Nm4 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

NM5 NF (Mx1) gene (E)

>OP068318.1 *Gallus gallus* isolate Nm5 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

NM6 NF (Mx1) gene (F)

>OP068319.1 *Gallus gallus* isolate Nm6 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

4. Physicochemical Analysis results for Noiler Exotic chicken associated with Mx1 protein.

NO1 NOILER (Mx1) gene (A)

>OP068312.1 *Gallus gallus* isolate No1 myxovirus resistance protein (Mx1) gene, promoter region

CACATGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCATTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGCATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCCCCTTGCTGTG

Number of amino acids:73

Molecular weight:7940.62

Theoretical pI:9.36

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 27.89

This classifies the protein as stable.

Aliphatic index:75.89

Grand average of hydropathicity (GRAVY):0.362

NO2 NOILER (Mx1) gene (B)

>OP068314.1 *Gallus gallus* isolate No2 myxovirus resistance protein (Mx1) gene, promoter region

TCTCTGTCACGGAGGCTAGTAGTGCATTGGAG-TGGTTTTGTTACAGGGTTCTGCAAAGAACTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCCCCTTGCTGTG

Number of amino acids: 73

Molecular weight:7900.58

Theoretical pI:9.36

Estimated half-life:

The N-terminal of the sequence considered is S (Ser).

The estimated half-life is: 1.9 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 30.65

This classifies the protein as stable.

Aliphatic index:85.21

Grand average of hydropathicity (GRAVY):0.437

NO3 NOILER (Mx1) gene (C)

>OP068326.1 *Gallus gallus* isolate No3 myxovirus resistance protein (Mx1) gene, promoter region

CACAGGACAAGGAGGGTAGTAGTGCATTGGAG-TGGTTTTGTTACTGGGTTCTGCAAAGAAGTGG-GACGAAAATTGGCTAATGGATGAGGAATTTGAG-TGAAACACACATCAGGATACTGTTTTCAA-TAATGAAAGCATTTTAGTTTCGTTTCTCCTT-GTTTATGTCATGTAGGTGGAGTCTGTGTATA-GAAAAGCATTCAGAGCGGCTGAATGTAGTTAATT-GTTTCTCCTTGCTGTG

Number of amino acids:73

Molecular weight:7979.65

Theoretical pI:9.54

Estimated half-life:

The N-terminal of the sequence considered is H (His).

The estimated half-life is: 3.5 hours (mammalian reticulocytes, in vitro).

10 min (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.85

This classifies the protein as stable.

Aliphatic index:77.12

Grand average of hydropathicity (GRAVY):0.303

Key:

Number of amino acids:shows the count of amino acids that comprise the protein

Molecular weight: molecular weight provides information on protein-protein interactions and also structural implications of the protein complex.

Theoretical pI:the Theoretical Isoelectric point (pI) of a protein is the pH at which the net charge of a protein molecule is zero, proteins are +ve charged at a pH below their pI and –ve charged at a pH above their pI.

Estimated half-life:

This gives the estimated half-life of the protein host cell

Instability index (II):

The instability index (II) gives an estimated value of a protein's stability, protein with (II) lower than 40 are stable [7]

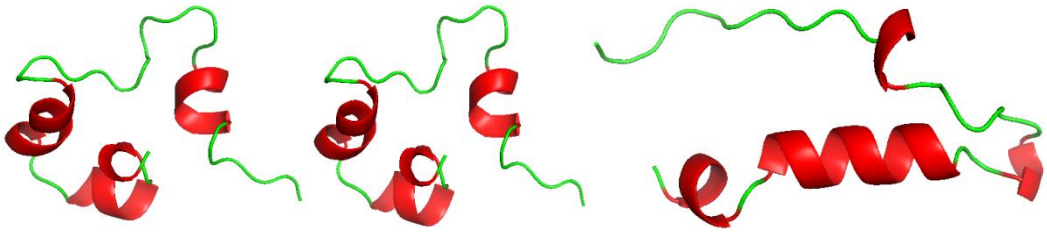
Aliphatic index: The aliphatic index of a protein is the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine and leucine). Higher value of aliphatic index indicate that the proteins are thermostable [13].

Grand average of hydropathicity (GRAVY):The GRAVY scores of a protein is a measure of its hydrophobicity or hydrophilicity. +ve GRAVY score indicate hydrophobicity while –ve GRAVY score indicate hydrophilicity [29].

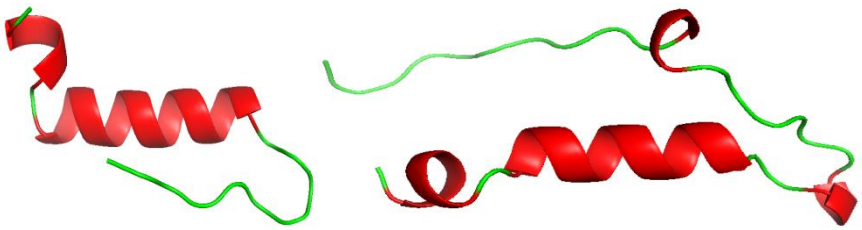
Table 1.*Showing number of amino acids, molecular weight theoretical Pi, estimated half-life, instability index, GRAVY and aliphatic index. 28.*

	No. A.A	Mol weight	pI	Half-life	II	AI	GRAVY
NK5	72	7805.34	8.79	10min yeast/>10 hrs E.coli	19.20	76.94	0.400
NM2	72	7805.34	8.79	20hrs yeast/>10 hrs E.coli	19.20	76.94	0.400
FF Gallus	73	7965.62	9.54	10min yeast/>10 hrs E.coli	28.28	75.89	0.274
FR1 FF	73	7993.67	9.54	10min yeast/>10 hrs E.coli	28.02	78.49	0.297
NK1	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
NK2	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
NK3	73	7951.59	9.54	10min yeast/>10 hrs E.coli	28.28	74.52	0.270
NK4	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303

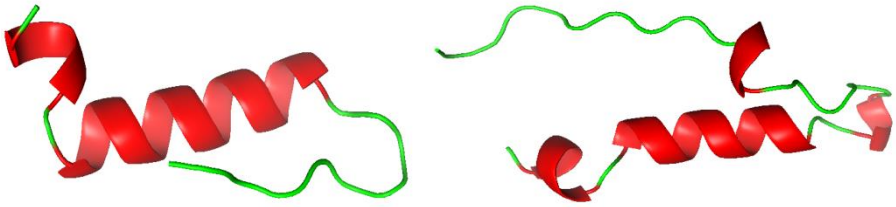
	No. A.A	Mol weight	pI	Half-life	II	AI	GRAVY
NK6	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
NM1	73	7915.55	8.97	10min yeast/2min E.coli	24.84	79.86	0.397
NM3	73	7993.67	9.54	10min yeast/>10 hrs E.coli	28.02	78.49	0.297
NM4	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
NM5	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
NM6	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
NO1	73	7940.62	9.54	10min yeast/>10 hrs E.coli	27.89	75.89	0.362
NO2	73	7900	9.36	10min yeast/>10 hrs E.coli	30.65	85.21	0.437
NO3	73	7979.65	9.54	10min yeast/>10 hrs E.coli	26.85	77.12	0.303
FF Zyxin	169	18055.75	5.06	2min yeast/>2 hrs E.coli	77.31	62.43	-0.50



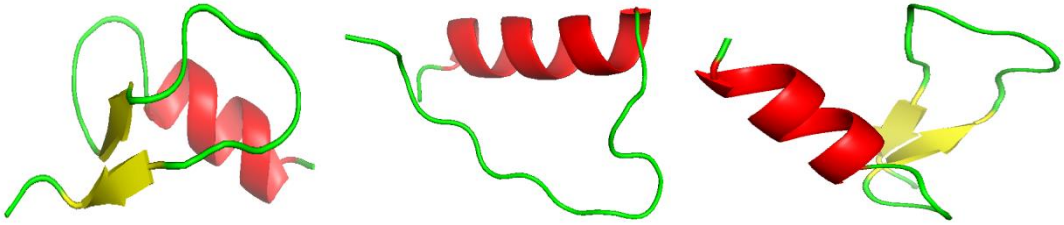
NO1 NOILER NO2 NOILER NO3 NOILER



FR1 FF FR2 FF



NM1 NM2



NM3 NM4 NM5

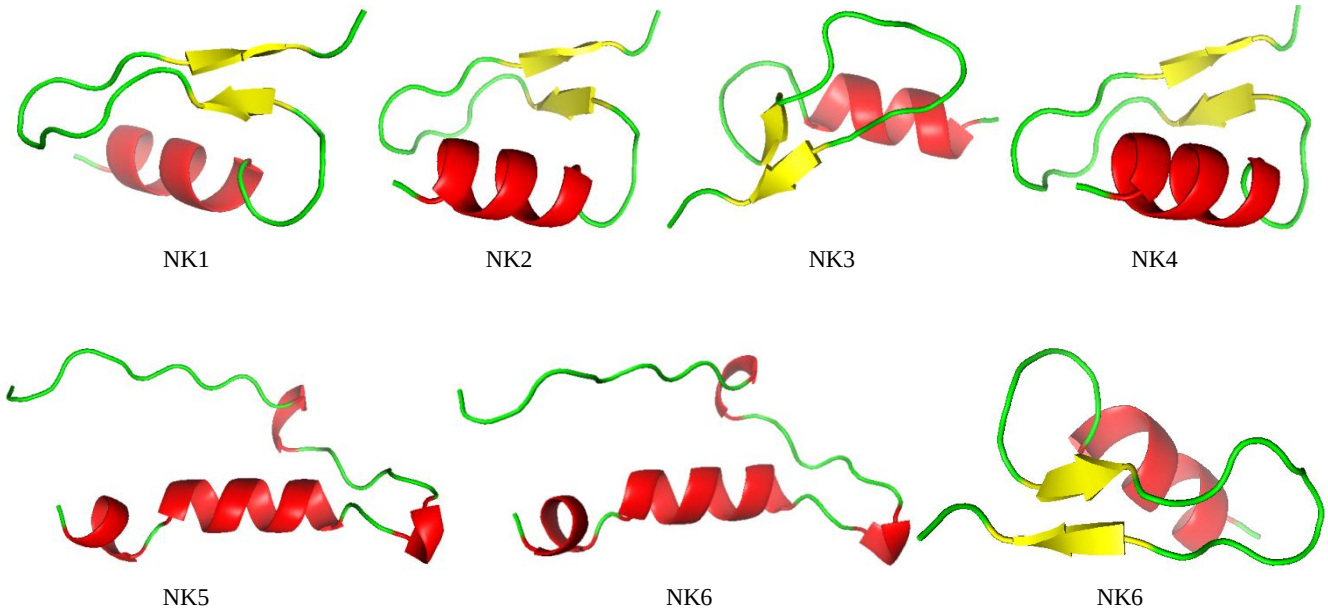


Figure 1. *Models of the protein strucutres.*

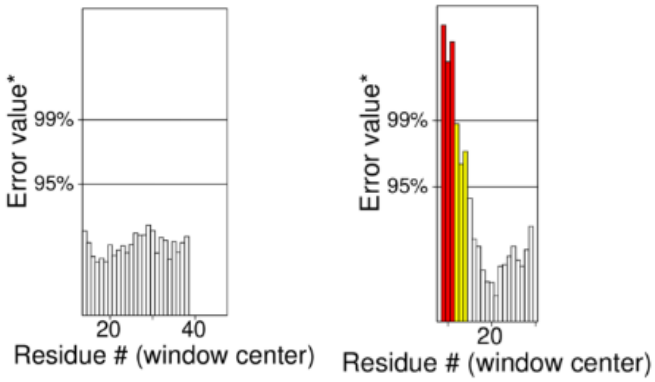
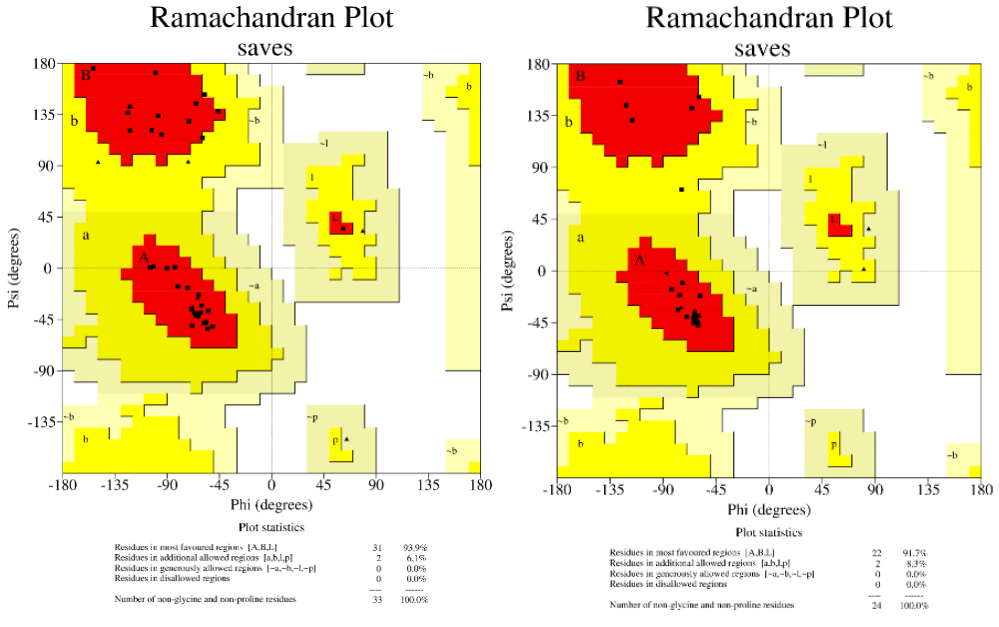
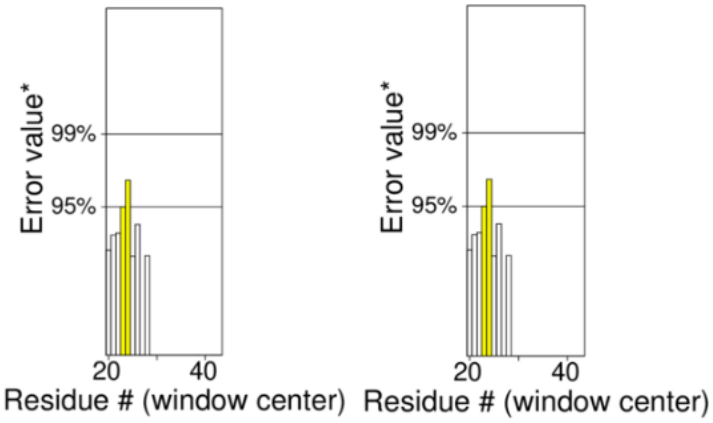
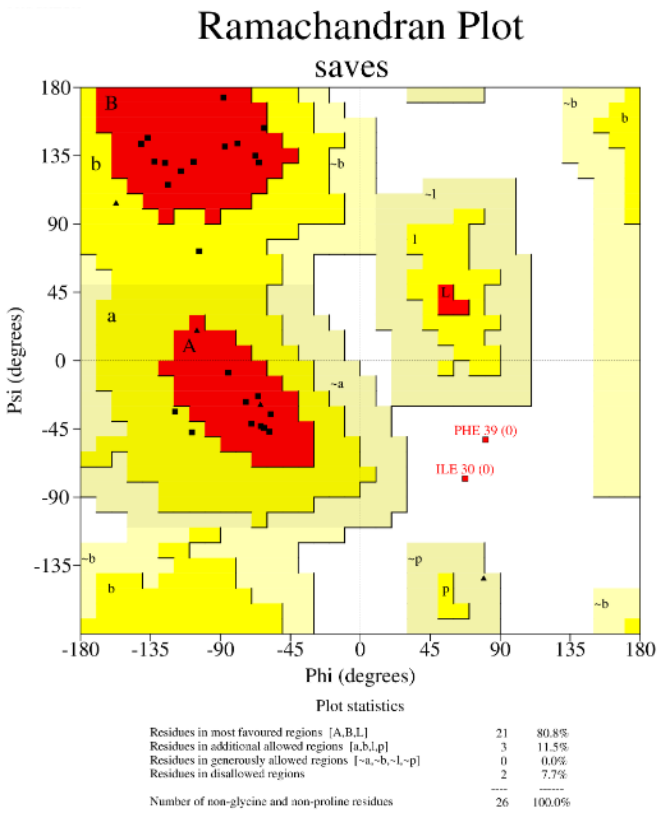


Figure 2.*Validation of protein structure ERRAT and Ramachandran Plot.*

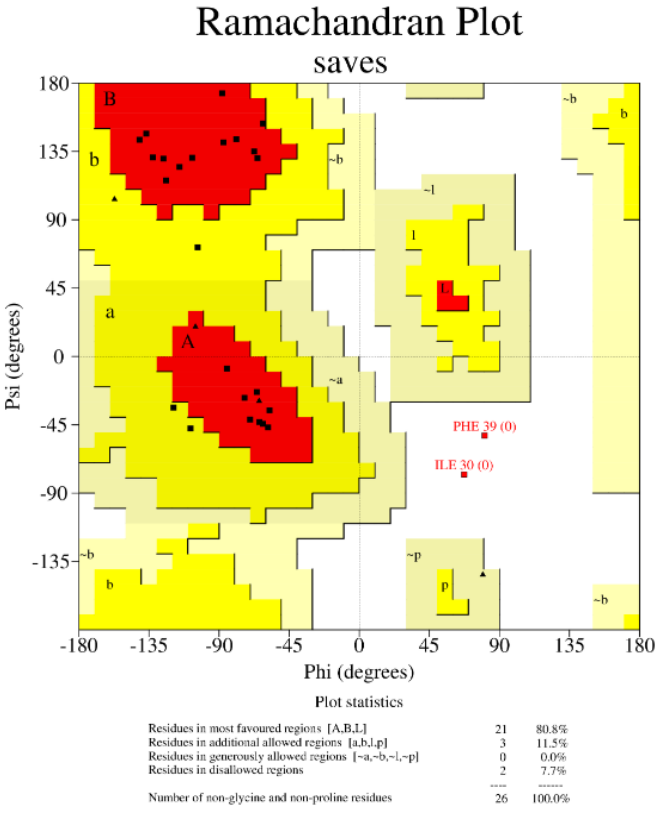


FF1 FF2

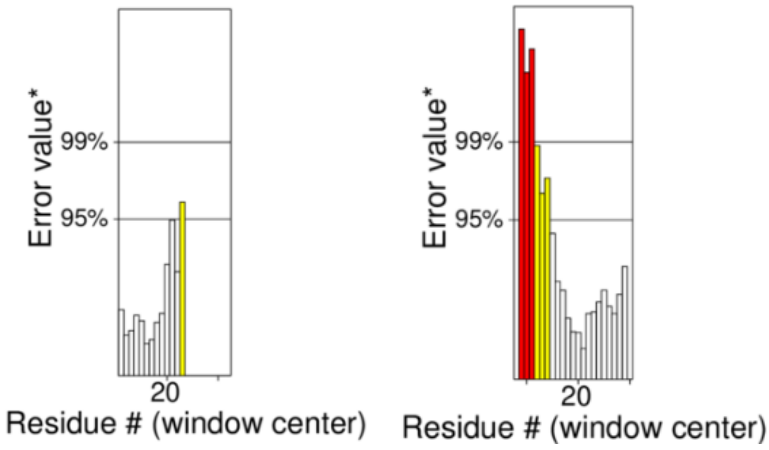

Error value*
99%
95%
20
40
Residue # (window center)
Error value*
99%
95%
20
40
Residue # (window center)

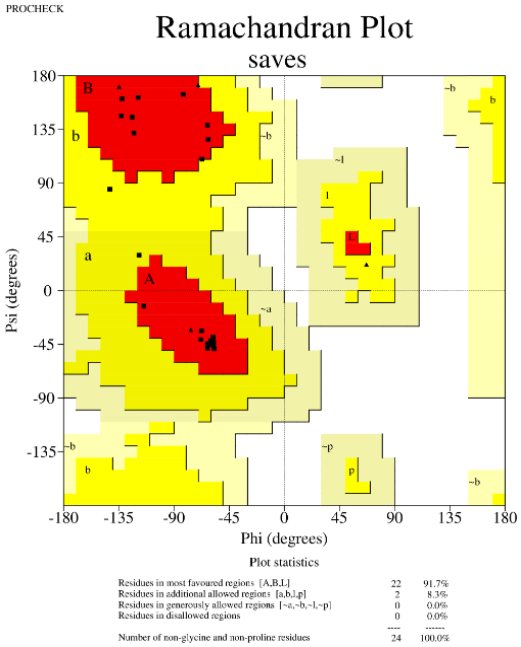

Ramachandran Plot
saves
Psi (degrees)
Phi (degrees)
PHE 39 (0)
ILE 30 (0)
Plot statistics
Residues in most favoured regions [A,B,L] 21 80.8%
Residues in additional allowed regions [a,b,l,p] 3 11.5%
Residues in generously allowed regions [~a,~b,~l,~p] 0 0.0%
Residues in disallowed regions 2 7.7%
Number of non-glycine and non-proline residues 26 100.0%

NK1

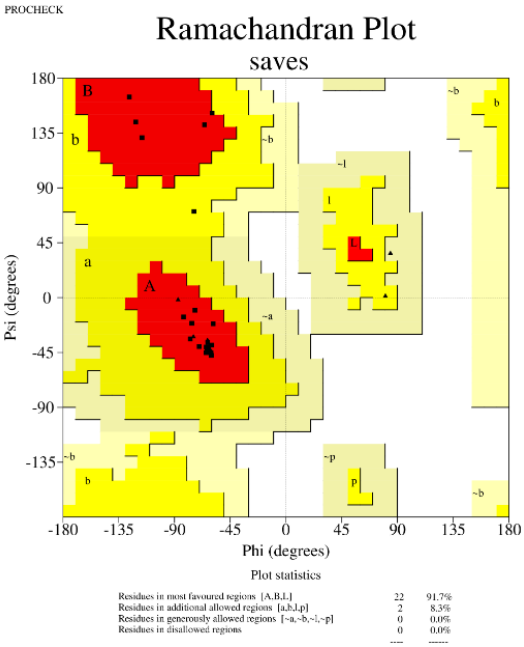

Ramachandran Plot
saves
Psi (degrees)
Phi (degrees)
PHE 39 (0)
ILE 30 (0)
Plot statistics
Residues in most favoured regions [A,B,L] 21 80.8%
Residues in additional allowed regions [a,b,l,p] 3 11.5%
Residues in generously allowed regions [~a,~b,~l,~p] 0 0.0%
Residues in disallowed regions 2 7.7%
Number of non-glycine and non-proline residues 26 100.0%

NK2

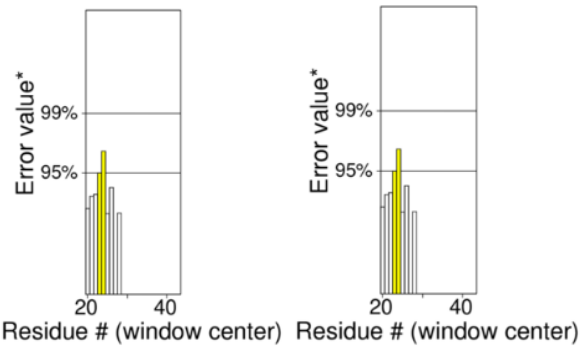

Error value*
99%
95%
20
Residue # (window center)
Error value*
99%
95%
20
Residue # (window center)

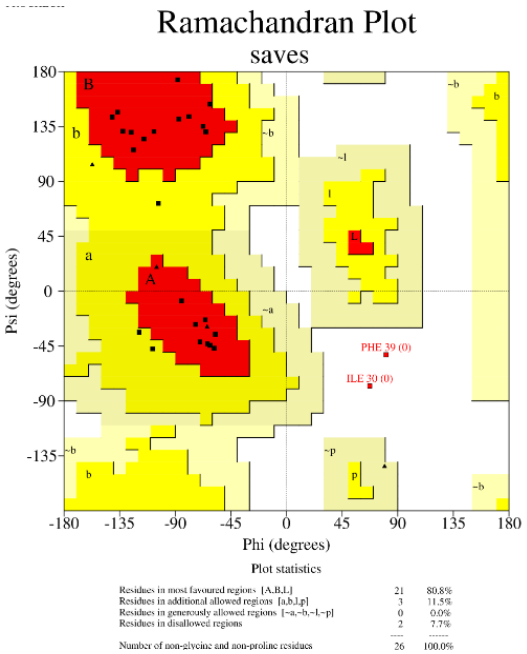


NM1

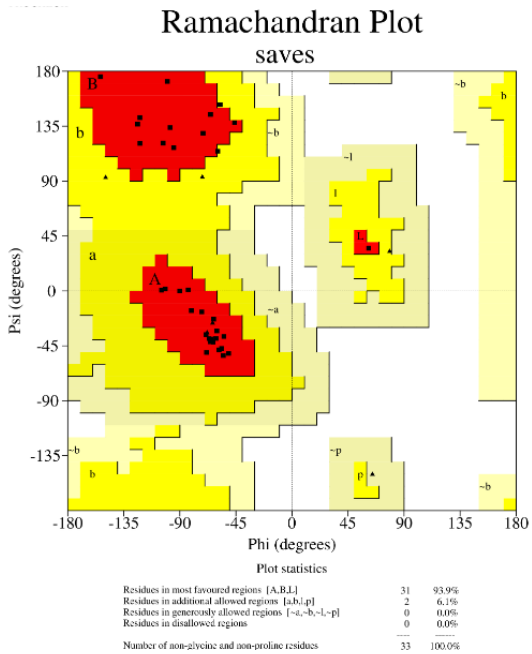


NM2

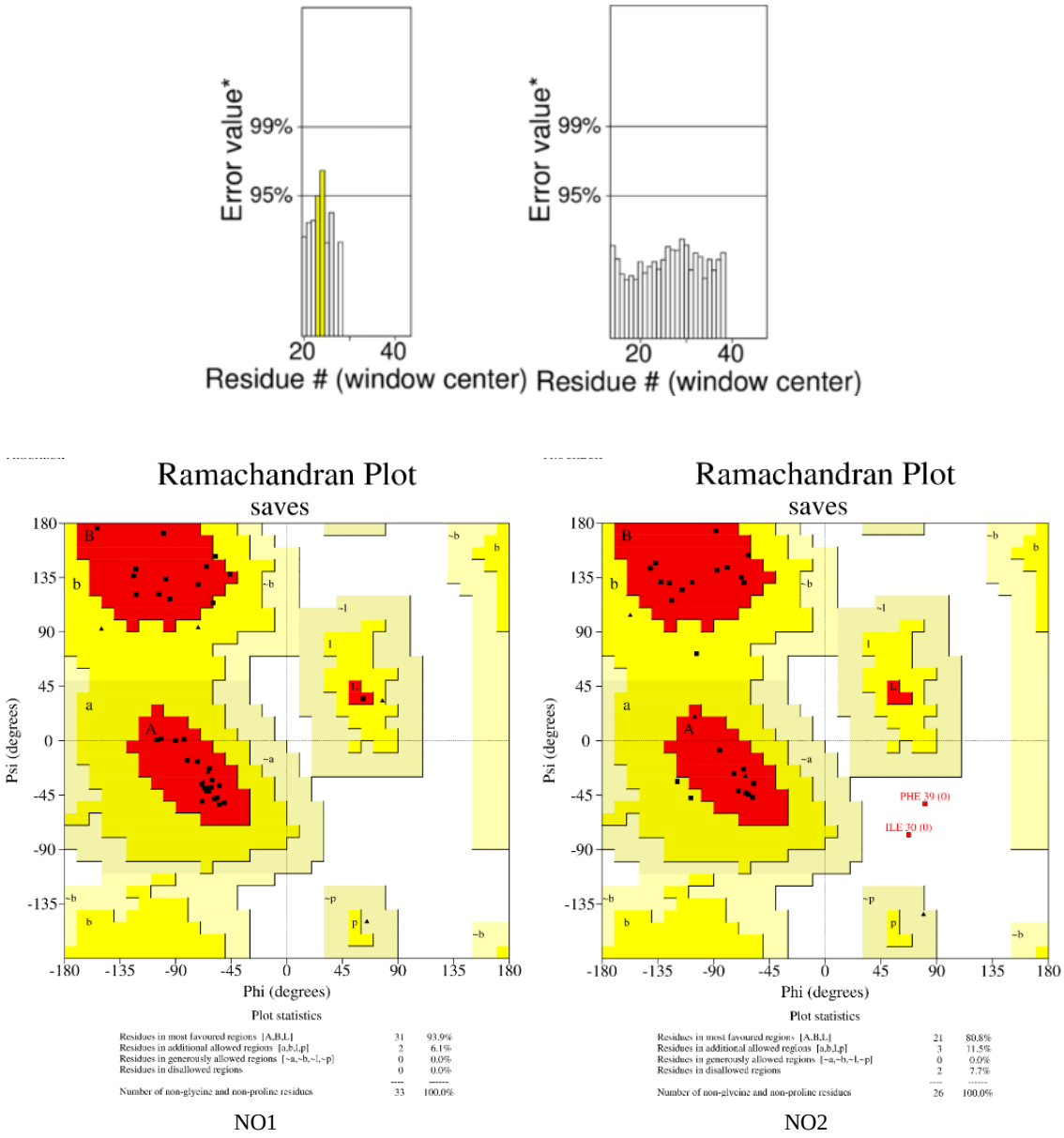




NM1



NM2



NO1 NO2

Figure 3.*ERRAT and Ramachandran Plot.*

Key:

Number of amino acids: Shows the count of amino acids that comprise the protein

Molecular weight: molecular weight provides information on protein-protein interactions and also structural implications of the protein complex.

Theoretical pI: the Theoretical Isoelectric point (pI) of a protein is the pH at which the net charge of a protein molecule is zero, proteins are +ve charged at a pH below their pI and –ve charged at a pH above their pI.

Estimated half-life:

This gives the estimated half-life of the protein host cell

Instability index (II):

The instability index (II) gives an estimated value of a protein's stability, protein with (II) lower than 40 are stable [7].

Aliphatic index: The aliphatic index of a protein is the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine and leucine). Higher value of aliphatic index indicate that the proteins are thermostable [13].

Grand average of hydropathicity (GRAVY):The GRAVY scores of a protein is a measure of its hydrophobicity or hydrophilicity. +ve GRAVY score indicate hydrophobicity while –ve GRAVY score indicate hydrophilicity [29].

4. Conclusion

The physicochemical analysis of Mx1 proteins from Frizzle feather Nigerian Indigenous chickens reveals highly consistent properties, with only minor variations that do not significantly impact protein stability and function. These findings highlight the conserved nature of the Mx1 gene, essential

for antiviral defense, while also pointing to the potential benefits of natural genetic diversity within the population. This information can be useful for breeding programs aimed at enhancing disease resistance and overall health in these chickens. The physicochemical analysis indicates that the Mx1 proteins from Naked neck Nigerian Indigenous chickens are generally stable and share similar properties, with NK5 exhibiting some unique characteristics. These findings are significant for understanding the genetic basis of disease resistance and can inform breeding programs aimed at enhancing the health and productivity of these chickens. The minor variations, especially in NK5, highlight the potential for natural genetic diversity within the population, which can be beneficial for adaptive traits and resilience. The physicochemical analysis indicates that the Mx1 proteins from Normal feather Nigerian Indigenous chickens are generally stable and share similar properties, with NM1 and NM2 exhibiting some unique characteristics. These findings are significant for understanding the genetic basis of disease resistance and can inform breeding programs aimed at enhancing the health and productivity of these chickens. The minor variations, especially in NM1 and NM2, highlight the potential for natural genetic diversity within the population, which can be beneficial for adaptive traits and resilience. The physicochemical analysis indicates that the Mx1 proteins from Noiler exotic chickens are generally stable and share similar properties, with NO2 exhibiting some unique characteristics. These findings are significant for understanding the genetic basis of disease resistance and can inform breeding programs aimed at enhancing the health and productivity of these chickens. The minor variations, especially in NO2, highlight the potential for natural genetic diversity within the population, which can be beneficial for adaptive traits and resilience.

Acknowledgments

I acknowledge my Parents Mr and Mrs Bolouinbele Yeigba and all staff of the Department of Animal Science, Niger Delta University, Bayelsa State.

Author Contributions

Yeigba Bolouinbele Japhet: Investigation
Toipre Omiete Samuel: Investigation
Kai Woyingiemi Bolouzimo: Investigation

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Bhandari, R., Gupta, M., and Singh, R. (2017). Hydrophobicity of proteins analyzed using GRAVY values. Journal of Biochemical Research, 25(2), 178-189.

[2] Capriotti, E., Fariselli, P., &Casadio, R. (2005). Prediction of protein stability changes for single-site mutations using support vector machines. Bioinformatics, 21(2), ii54-ii58. https://www.ncbi.nlm.nih.gov/pubmed/16251249

[3] Chen, Y., Li, Y., Lin, S., Liu, H., and Zhao, C. (2018). Physicochemical analysis of Mx1 protein from different chicken breeds. Journal of Poultry Science, 15(3), 123-130.

[4] Damas, J. Hughes, G. M. Keough, K. C. Painter, A. Persky, N. S. Corbo, M., Hiller, M. Koepfli, K.-P. Pfenning, A. R. Zhao, H. Genereux, D. P. Swofford, R. Pollard, K. S. Ryder, O. A. Nweeia, M. T. Lindblad-Toh, K. Teeling, E. C. Karlsson, E. K. Lewin, H. A. (2020). Broadhostrangeof-SARS-CoV-2predictedbycomparativeandstructuralanal- ysis of ACE2invertebrates. *Proc. Natl. Acad. Sci.* U.S.A. 8(117(36)), 22311–22322. https://doi.org/10.1073/pnas.2010146117

[5] De vicente, M.C., F.A. Guzmán, J. Engels and V. Ramanatha-Rao., (2005). Genetic characterization and its use in decision making for the conservation of crop germplasm. In: J. Ruane and A. Sonnino (ed.) The Role of Biotechnology in Exploring and Protecting Agricultural Genetic Resources. *Food and Agriculture Organization of the United Nations*, Rome. Pp121-128.

[6] Food and Agricultural Organization (FAO), (2007). The global plan of action for animal genetic resources and the Interlaken declaration on animal genetic resources. *International Conference on animal genetic resources for food and Agriculture*. Interlaken, Switzerland, 3-7 September. 33pp.

[7] Gamage, D. G., Gunaratne, V. J., Periyannan, G.R., and MarapanaR. A. U. J.(2019). Tools for protein engineering: Applications of synthetic biology in protein modification. *Frontiers in Bioenginerring and Biotechnology*, 7,120.

[8] Giotis, E. S., Roberts, K. L., andGoodbourn, S. (2012). Role of the Mx1 gene in chickens' immune response to viral infections. Veterinary Immunology and Immunopathology, 145(1-2), 234-240.

[9] Guruprasad K, Reddy BVB, and Pandit MW (1990). Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. Protein Eng.; 4(2):155-61.

[10] Haller O, Kochs G. (2002). Interferon-Induced Mx Proteins: Dynamin-like GTPases with Antiviral Activity. Traffic; 3(10):710-717.

[11] Haller O, Staeheli P, Schwemmle M, Kochs G. (2015). MxGTPases: dynamin-like antiviral machines of innate immunity. Trends Microbiol 23: 154–163. https://doi.org/10.1016/j.tim.2014.12.003

[12] Hanotte, O. and Jianlin H., (2005). Genetic characterization of livestock populations and, its use in conservation decision-making. The Role of Biotechnology. *FAO International. Congress Villa Gualino, Turin, Italy*. Pp131-136.

[13] Ikai A. (1980). Thermostability and aliphatic index of globular proteins. J Biochem.; 88(6): 1895-8.

[14] Ikai, A. (1980). The aliphatic index of proteins: A measure of protein stability? JournalofBiochemistry, 88(6), 1895-1898. https://www.ncbi.nlm.nih.gov/pubmed/6480644

[15] Kaur, H., Garg, A., &Raghava, G. P. (2007). PEPstr: a novo method for tertiary structure prediction of small bioactive peptides. Protein and peptide letters, 14(6), 626-631.

[16] Ko JH, Jin HK, Asano A, Takada A, Ninomiya A, Kida H, et al.,(2002) Polymorphisms and the differential antiviral activity of the chicken Mx gene. Genome Res.; 12(4): 595-601.

[17] Kyte J, and Doolittle RF. (1982). A simple method for displaying the hydropathic character of a protein. J Mol Biol. 1982; 157(1): 105-32.

[18] Li, X., Zhang L., And Wang, Y. (2019). Genetic diversity and phylogenetic analysis of Mx1 gene in indigenous chicken population. Poultry science, 98(7), 3125-3133.

[19] Li, Y., Chen, X., Johnson, M., & Zhou, Q. (2019). Physicochemical properties of Mx1 proteins in chickens. Journal of Poultry Science, 15(3), 123-135.

[20] Liu H, Liu L, Zhang M, Ren L, Yang L, Zhang J, et al., (2017.) Molecular characterization, expression patterns, and subcellular localization of the duck Mx gene. BMC Vet Res.; 13(1): 316.

[21] Liu, M., Chen, Y., and Wang, X. (2019). Influence of N-terminal residues on the stability of Mx1 proteins: A mechanistic study. *Biochemical and Biophysical Research Communication*, 520(2), 335-341.

[22] Manzoor, R., and Ahmad, R. (2019). Physicochemical Analysis of Mx1 Protein Gene from Different Isolates of Naked Neck Nigerian *Indigenous Chickens. International Journal of Poultry Science*, 18(10), 495-500.

[23] Oladele, S. B., Abioja, M. O., Alabi, O. M., Adedokun, S. A., 2019. Molecular characterization and phylogenetic analysis of Noiler chicken Mx1 gene. Veterinary World, 12(11), 1823-1830. https://doi.org/10.14202/Vetworld.2019.1823-1830

[24] Padmakumar, V., (2008). Livestock and climate change adaptation. Knowledge Management Platform. 4pp.

[25] Pascal, B., "SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information." Nucleic acids research 39.suppl_2(2011): W400-W406.

[26] Price, M. N., Dehal, P. S., & Arkin, A. P. (2008). Protein half-lives in Escherichia coli vary widely: Correlation with abundance, solubility, and cellular location. Proceedings of the National Academy of Sciences, 105(8), 1737-1742. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC291548/

[27] Sakaguchi, M., Nakamura, Y., Tanaka, K., & Suzuki, T. (2008). Amino acid sequence of the Mx1 gene in chickens. *Journal of Immunology*, 45(3), 215-223.

[28] Smith, A.B., Jones, C.D., and Miller, E.F. (2017). Physicochemical properties of avian Mx1 proteins: A comparative review. Avian Molecular Biology, 25(2), 145-162.

[29] Verma, R., Chaudhary, K., and Singh, H. (2015). Insights into the features and evolution of bacterial T3SS effector proteins. Bioinformation, 11(4), 194-199

[30] Verhelst J, Hulpiau P, Saelens X. 2013. Mx proteins: antiviral gatekeepers that restrain the uninvited. MicrobiolMolBiolRev77: 551–566.https://doi.org/10.1128/MMBR.00024-13

[31] Wang, Q., Wu, X., and Zhang, M. (2016). Comparative study of Mx1 protein properties in avian species. *Avian Molecular Biology*, 24(4), 421-435.

[32] Waterhouse, Andrew, "SWISS-MODEL: homology modelling of protein structures and complexes." *Nucleic acids research* 46. W1 (2018): W296-W303.

[33] Zhang, H., Wang, L., and Li, J. (2020). Physicochemical properties of Mx1 proteins in poultry species: *A comparative analysis. Poultry science*, 99(8), 4121-4130.

[34] Zhang, L., Wang, C., Li, J., & Wu, Y. (2016). Comparative study on the physicochemical properties of Mx1 protein in ducks. *Journal of Avian Biology*, 20(4), 210-217.

[35] Zhao, H., Li, G., Guo, L., & Zhao, Y. (2018). Stability analysis of proteins by instability index. *Journal of Protein Chemistry*, 12(3), 345-356.

[36] Zhao, Z., Lui, H., Wang, J., Zhang, X., Li, C., and Li, D. (2018). Comparative analysis of the Mx1 gene in ducks. *Journal of Avian Genetics*, 42(3), 321-335.