**Minireview Article**

**Optimization in Protein structure and function prediction of silk of Tasar silkworm, *Antheraea mylitta* Drury**

**Abstract**

Protein structure and function prediction is an arduous task in computational biology. Understanding the structure of protein facilitates in understanding its function. The Tasar silkworm, *Antheraea mylitta* Drury,is a vital species in sericulture, producing high-quality silk fibres with unique properties. Beyond its economic significance, the Tasar silkworm is a model organism for studying insect biology, development, and evolution. Despite recent advances in genomics and proteomics, which have enabled the exploration of the Tasar silkworm proteome, there still exist hitches in the atomic accuracy of its protein structure prediction in the era of deep learning. Here, we endeavour to bridge the existing knowledge gap of algorithmic modelling techniques, especially deep neural networks in protein prediction methods of this valuable lepidopteran, leading to a better understanding of its biological processes and potential applications.

**Keywords:** Optimization, Protein structure and function, algorithmic modelling, deep neural network

**Introduction**

Tasar cocoons are reported to be the largest among all the silk producing insects in the world [1]. Tasar silk producing silkworm *Antheraea mylitta* Drury is highly heterogenic with several ecoraces in varied topographical diverse areas exhibiting diversity in phenotypic characters and forms an integral part of forest ecosystem [2] However, it is on the verge of extinction due to its weakness in voltinism, emergence, hatching, low yield etc. [3],[4],[5]. The extinction process can be mitigated to some extent by deciphering the proteome enabling the identification of functional proteins, their interactions and regulation. The current paper discusses the optimization of machine learning and deep learning methods to be used for protein structure and function prediction of silk of Tasar silkworm *Antheraea mylitta* Drury.

The advancements in computational biology have made molecular modelling of biological compounds a reality [6] For instance, proteins—composed of polypeptide chains-undergo intricate folding processes at multiple structural levels to become functional. After synthesis by ribosomes, these chains first fold into secondary structures such as alpha helices and beta sheets, often connected by turns [7].This is followed by tertiary [8] and quaternary folding [9], which are essential for achieving the final three-dimensional conformation necessary for proper protein function. In nature, although many varieties of Protein folding are seen, but SCOP (Structural Classification of Proteins) and CATH are two most common ones [10]. The amino acids that make up the polypeptide determine the folding, aka the interaction between the structures, determines the physio-chemical nature of the Proteins. Hence understanding the constitutive aminoacids of a protein is crucial to understand the the mechanism of function of proteins [11]

Although understanding the constituents of the proteins in the language of Amino Acids sounds easy, but physically understanding the inter-aminoacid interactions in secondary structures and further folding is tricky. Hence, for this purpose use of traditional prediction methods along with AI powered one have proven quite helpful.

**PROTEIN PREDICTION METHODS**

Various methods are been employed for protein prediction, which broadly categorized into several approaches. Homology-based methods include tools such as BLAST (Basic Local Alignment Search Tool) and PSI-BLAST (Position-Specific Iterative BLAST), along with HMMER, which utilizes Hidden Markov Models (HMM) for sequence alignment and comparison. Machine learning-based approaches involve algorithms like Support Vector Machines (SVM), Random Forests, Neural Networks, and Gradient Boosting, which can identify patterns from large datasets. Deep learning-based techniques offer advanced analysis through architectures such as Convolutional Neural Networks (CNNs), Recurrent Neural Networks (RNNs), and Long Short-Term Memory (LSTM) networks. Additionally, physicochemical property-based methods predict proteins based on features like hydrophobicity, charge, and secondary structure. Ab initio methods, which predict protein structures from scratch without relying on homologous templates, include tools such as Rosetta, trRosetta (transform-restrained Rosetta), I-TASSER (Iterative Threading Assembly Refinement), and QUARK. Hybrid methods combine multiple strategies from the aforementioned categories to enhance prediction accuracy. Furthermore, template-based modeling compares the target protein with known structures as templates or by aligning smaller fragments from existing proteins.

Protein structure prediction can be approached using two broad categories: **Experimental Methods** and **Computational Methods**. Experimental techniques such as X-ray crystallography, NMR spectroscopy, and cryo-electron microscopy (Cryo-EM) are widely employed for determining the three-dimensional (3D) structure of proteins, each with its own strengths and limitations [12]. In X-ray crystallography, the protein must be purified and crystallized under optimal conditions before being exposed to an X-ray beam to obtain diffraction patterns [13] In NMR spectroscopy, it analyzes proteins in solution, especially if the protein structure is flexible[14]. Cryo-EM, involving electron beams and lenses, provides direct imaging of biomolecules, and in certain cases, electron diffraction from 2D/3D crystals is employed as well, to determine the structural information [15],[16]. As for the Computational methods, Homology Modelling, Fold Recognition (also known as threading), and AB Initio Modelling are widely used. Homology modelling is considered as one of the most straightforward and reliable techniques when the target protein shares >30% sequence identity with a known template structure [17],[18]. The process involves multiple steps such as template identification (using BLAST: [19] or FASTA: [20] sequence alignment (e.g., CLUSTAL W: [21] structural modelling, loop modelling, side chain optimization, energy minimization, and model validation ([22]sequentially. Loop regions, which often contribute to protein flexibility and functional specificity, are modelled using knowledge-based methods supported by software such as MODELLER [23], Swiss-Model [24] Insight ([25], and 3D-Jigsaw [26]. Energy functions using Monte Carlo or Molecular Dynamics Simulations can be applied to optimize loop conformations ([27], However, a low energy state does not always indicate correct folding, as some misfolded structures can also exhibit energetically favourable conformations [28] Hence, model validation should include verification for Torsion Angles, Bond Lengths, Bond Angles, and Hydrophobic Core Packing [29] In cases where sequence similarity is below 30%, Threading or Fold Recognition both are preferred [30],[31]. These techniques specialize in detecting distant homologs by matching the target sequence to known folds using structural features. 3D profiles, which can assess the compatibility of amino acids between multiple proteins with structural environments, are also preferred in some, like Tools of Profile-3D and PROSPECT [32],[33]. Ab Initio Modelling, such as the ROSETTA platform (developed by: David Baker lab; [34],[35] can construct protein models without a template, using fragment assembly techniques based on structures from the Protein Data Bank (PDB). Despite its innovative strategy, the accuracy of Ab Initio Models is comparatively lower compared to template-based methods [36]

Protein structure prediction is continuously benchmarked through community-wide experiments like the Critical Assessment of protein Structure Prediction (CASP), which classifies prediction targets into Template-Based Modelling (TBM) and Free Modelling (FM) categories [37] Furthermore, machine learning techniques (ML Techniques), including Support Vector Machines (SVM) and kernel methods, are increasingly being integrated into structural bioinformatics recently. These approaches leverage both homogeneous and heterogeneous datasets to recognize structural and functional patterns ,[.38]

**UTILISING PROTEOMICS AND PROTEIN PREDICTION METHODS IN TASAR SILKWORM**

Proteomic techniques can also be effectively used to study protein alterations throughout embryonic development, larval stages, and pupation of the tropical tasar silkworm *Antheraea mylitta* Drury. By integrating proteomics with genomics, we can gain a deeper understanding of gene expression and protein function in various silkworm species, including both closely and distantly related species of *Antheraea mylitta*. This integrative approach may be proven as a very useful approach towards enhancing tasar silkworm breeding, sericulture practices, and biotechnological innovations across the country. The identification of vitellogenin, a female-specific protein present in the hemolymph, marked a significant milestone in insect biochemistry, first reported in Hyalophora cecropia by [39] This discovery paved a smoother pathway for deeper molecular insect studies. Subsequently, the mapping of the silkworm genome[40][41] enabled the application of advanced proteomic technologies to silkworm research. In this context, two-dimensional electrophoresis (2D-PAGE) combined with mass spectrometry has proven particularly effective for analyzing this protein’s expression in various tissues such as silk glands, fat body, skeletal muscle, and haemolymph [42][43] The development of the silk gland, which undergoes significant growth during the fourth and fifth larval instars, is largely affected by environmental factors such as light, diet, temperature, and humidity .In a key contribution to silk protein characterization, [44] successfully isolated, purified, and analysed proteins from the cocoon peduncle of Antheraea mylitta. Further advancements were made in this field )[45], who identified 169 distinct proteins in *Bombyx mori* cocoon silk using shotgun LC-MS/MS techniques. In addition to the primary silk proteins, fibroin and sericin, studies have revealed the presence of enzymes, protease inhibitors, and proteins of yet-unknown function. The resulting mass spectrometry data were analysed using MaxQuant software (version 1.3.0.1) [46], while the database searches were conducted against the integrated silkworm proteome resource containing 35,379 protein sequences from NCBI and SilkDB [47]. In parallel, amino acid sequence analysis of tasar silkworm proteins has provided critical insights into their structure, function, and biochemical properties. Structural predictions can be achieved through homology modelling when similar known protein structures are available, or via ab initio methods in the absence of such templates. These computational approaches aid in understanding biological functions such as enzymatic activity, ligand-binding affinity, subcellular localization, post-translational modifications (e.g., phosphorylation, glycosylation), and interactions with other proteins or small molecules like hormones and drugs. To support these predictions, several robust bioinformatics tools are employed. BLAST and HMMER facilitate sequence alignment and motif detection, while SWISS-MODEL and I-TASSER assist in 3D structural modelling. Functional annotations can be predicted using Functome, subcellular localization through SubLoc, and post-translational modifications via NetPhos. This review highlights a comparative analysis of these protein prediction methods, emphasizing both their advantages and limitations in advancing silkworm proteomics.

**RESULTS AND DISCUSSION**

One of the major challenges in protein structure and function prediction for the Tasar silkworm lies in the limited availability and quality of genomic and proteomic data. This scarcity hinders the training and validation of computational prediction models. As of March 2021, while the Protein Data Bank (PDB) housed around 180,000 protein structures [48] databases like UniProt and TrEMBL had catalogued approximately 207 million protein sequences by the end of 2020 (UniProt Consortium, 2021). The vast disparity between available sequence and structure data creates a bottleneck for accurate model training.

Proteins from the Tasar silkworm are often complex, which adds to the difficulty of structural prediction. The lack of closely related species with well-characterized proteins further limits the accuracy of homology-based models. In particular, predicting reliable 3D structures for large and intricate silk proteins such as fibroin and sericin remains a substantial challenge. Additionally, assigning specific functions to predicted protein sequences is often inconclusive, and post-translational modifications (PTMs) like phosphorylation and glycosylation—crucial to protein function—are difficult to predict with current tools. Another layer of complexity arises in the prediction of protein–protein interactions, which are essential to understanding biological processes. The sheer volume of protein sequences demands considerable computational resources, both for storage and analysis. Moreover, existing algorithms may fail to capture the full spectrum of protein behaviour, and no universally accepted protocols currently exist for validating predictions. Variations in input parameters and methodology further affect the consistency and reproducibility of outcomes. Function prediction is further complicated by moonlighting proteins, which perform multiple unrelated biochemical or biophysical roles [49][50] Some enzymes, for instance, have dual roles—acting catalytically and also regulating transcription or translation through interactions with DNA, RNA, or transcription factors [51]

To address some of these challenges, machine learning approaches have been integrated into protein prediction frameworks. These models consider factors such as the frequency of Gene Ontology (GO) terms, amino acid sequence patterns, domain and motif presence, and various biophysical attributes [52]. A recent review by [53] offers a comprehensive overview of machine learning methodologies in protein function prediction, including feature selection techniques, algorithm types, model implementations, and evaluation strategies. The complexity increases with multi-domain proteins and longer sequences, which tend to have higher radii of gyration, making structural modeling more difficult [54][55]. In cases where no structural homologs with significant sequence similarity are found in PDB, alternative strategies like threading or fold recognition are employed. These methods attempt to align target sequences with known structural folds, exploiting the evolutionary conservation of protein structures [56] [57][58][59][60]There is also growing recognition of the functional importance of microproteins—those with fewer than 100 amino acids—which may serve regulatory, structural, or other roles [61] Community initiatives like the Critical Assessment of Functional Annotation (CAFA) have played a pivotal role in benchmarking algorithm performance and have reported steady improvements over the last decade [62][63][64]

**CONCLUSION**

Protein prediction in the Tasar silkworm is an emerging and rapidly evolving field that holds great promise for advancing our understanding of this economically and ecologically significant insect. Through the application of cutting-edge computational techniques and integrative strategies, researchers are now able to predict protein structures, functions, and properties with increasing accuracy. Potential applications of this research span improved silk production, novel biomaterials, and future biopharmaceutical developments. To realize these benefits, future research should prioritize: such as Generation of high-quality genomic and proteomic datasets; Development of advanced AI and machine learning-based prediction models; Integration of multi-omics data (genomics, transcriptomics, proteomics); Experimental validation of computational predictions; Exploration of industrial and biotechnological applications.

Interestingly, polypeptide studies suggest that shorter sequences can introduce more complexity and lower confidence in structural prediction [65][66]. This is due to the surface elements responsible for physicochemical interactions and binding often not being directly involved in the folding process, which means they may not significantly affect pharmacological or toxicological behavior [67] In conclusion, this comprehensive review of protein prediction in *Antheraea mylitta* provides critical insights and lays a robust foundation for future investigations. Continued advancements in this field are essential for unlocking the full biological and industrial potential of Tasar silk proteins.

**Table-1: Highlights of different approaches to protein structure prediction in a tabular form**

|  |  |  |
| --- | --- | --- |
| Year | Discoverer/Author | Type of Approach |
| 1969 | Braune et al. | Comparative modelling (Summers and Karplus, 1989)[68] |
| 1974 | Richard Corey and Irving Kuntz | First protein folding algorithm (DREIDING) (Niazi et al., 2024) [69] |
| 1991 | Bowie et al.[32] | Threading (Xu et al., 2007) [70] |
| 1994 | Moult et al.[37] | CASP(Critical Assessment of Structure Prediction) (Moult et al., 1995) |
| 1995 | Sali et al.[71] | MODELLER |
| 1997 | Altschul et al.[19] | PSI-BLAST(Position Specific Iterative Basic Local Alignment Search Tool) |
| 1997 | Baker et al. | Rosetta (Simons et al. 1999)[[72] |
| 1999 | Jones[58] | PSIPRED(Position Specific Iterative Blast based secondary structure prediction) |
| 2004 | Zhou and Zhou[73] | SPARKS |
| 2005 | Zhang et al.[74] | I-TASSER(Iterative Threading ASSEmbly Refinement) (Zhang, 2008) |
| 2005 | XU et al.[75] | QUARK |
| 2018 | Senior et al.[76] | Alphafold |
| 2019 | Ingraham et al.[77] | NEMO(NF-Kappa-B Essential Modulator) |
| 2019 | AlQuraishi et al.[78] | RGN(Recurrent Geometric Network) |
| 2019 | Mao et al.[79] | GDFold(Gradient Decent Folding) |
| 2020 | Yang et al.[80] | trRosetta(transform restrained Rosetta) |
| 2021 | Ju et al.[81] | CopulaNet |
| 2021 | Google Deep mind | Alphafold 2 (Jumper et al, 2021) [82] |
| 2021 | Baek et al.[83] | RoseTTA fold |
| 2022 | Brandes et al.[84] | Protein BERT |
| 2022 | Chowdhury et al.[85] | RGN2(Recurrent Geometric Network 2) |
| 2022 | Yu et al.[86] | Profold single(Protein fold single) |
| 2022 | Wu et al.[87] | Omegafold |
| 2022 | Kandthil et al. [88] | DMP fold 2 |
| 2022 | Lin et al. [89] | ESM fold (Evolutionary Scale Modeling) |
| 2024 | Google DeepMind and Isomorphic Labs | Alphafold 3 (Abramson et al., 2024)[90] |

**Table 2: Current Prediction Methods: (A)Prokaryotic localization predictors**

**(B) Eukaryotic localization predictors**

|  |  |  |
| --- | --- | --- |
| **(A)Prokaryotic localization predictors** | | |
| Prediction Method | Proposer or Discoverer | Year of Discovery |
| PSORT I | Nakai K. And Kanehisa[91] | 1991 |
| PSORT B | Nakai K. and Kanehisa [92] | 2001 (Gardy et al, 2003)[93] |

|  |  |  |
| --- | --- | --- |
| **(B) Eukaryotic localization predictors** | | |
| Prediction Method | Proposer or Discoverer | Year of Discovery |
| PSORT II | Nakai K. and Horton P.[94] | 1999 |
| i PSORT | Bannai et al.[95] | 2001 (Bannai et al., 2002) |
| Target P | Emanulsson et al.[96] | 2000 |
| ESLPred | Bhasin and Raghava[97] | 2004 |

**Table-3: Prokaryotic and Eukaryotic localization predictors**

|  |  |  |
| --- | --- | --- |
| Prediction Method | Proposer or Discoverer | Year of discovery |
| NNPSL | Reinhardt and Hubbard[98] | 1998 |
| Subloc(Subcellular Localization Prediction) | Hua and Sun [99] | 2001 |

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Disclaimer (Artificial intelligence): Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.**References**

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