Vol: 02; Issue 01 (Jan – March 2026)
JOURNAL OF MEDICO INFORMATICS

Review

ISSN: 3108-2696 (Online)

MODELLER-Based Homology Modeling: A Critical Review of Protocols, Advances, and Computational Challenges

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Abstract

Homology modeling using MODELLER has been a cornerstone of computational structural biology for over three decades, yet its protocols, advances, and computational challenges have not been fully reviewed in the context of modern structural prediction methods. Despite breakthroughs from deep learning approaches like AlphaFold, MODELLER's satisfaction of spatial restraints methodology remains vital in template-based modeling, especially in low sequence identity cases, multi-template strategies, and specialized applications. This review critically examines current MODELLER protocols, recent methodological improvements, and persistent computational challenges. We analyzed literature on MODELLER applications, benchmarking, and algorithmic refinements, focusing on template selection, alignment optimization, model validation, and integration with modern computational methods. Our analysis shows that although MODELLER's core algorithm has remained largely unchanged since the 1990s, advances in template selection, multi-template modeling, and statistical potentials such as DOPE scoring have improved model accuracy. Recent work highlights the impact of optimized σ value estimation and incorporation of statistical potential terms, particularly in multi-template modeling. Nonetheless, challenges persist, including reduced accuracy in low sequence identity modeling, scalability issues, and poor handling of conformational flexibility. MODELLER retains unique strengths in template-based modeling, particularly when combined with modern validation strategies and machine learning–assisted template selection. Looking ahead, progress in hybrid modeling approaches, more accurate scoring functions, and greater computational efficiency will be essential for MODELLER to sustain its relevance within the rapidly evolving field of protein structure prediction.

1. Introduction

Over three decades, homology modelling or comparative modelling has been cornerstone of computational biology. The field play a key role in the understanding of protein structure that are more conserved than their sequences, based on the sequence similarity usually implying significant structural similarity. The basic principle of the homology modelling is used for predicting three-dimensional structures of protein by leveraging the growing repository of experimental determined structures in the Protein Data Bank. Computation tool, MODELLER, developed by Andrej Sali in 1989, which represents one of the most influential software in computational biology. This program implements as an automated approach to comparative modelling for predicting the protein structure by satisfaction of spatial restraint, a method inspired by NMR (Nuclear Magnetic Resonance) spectroscopy of proteins **(Pearce and Zhang 2021)**. The original model developed and maintained by Andrej Sali at the University of California, then the MODELLER has evolved through multiple versions, currently the version 10.7 has been used as of 2025 and maintained by Ben Webb. Owing to their strong statistical foundation, this software remains useful, taking spatial restraints from source protein structures data instead of depending only on the physical force fields. MODELLER is the most used software in this approach in spatial restraint-based modelling, with implement of ModBase as data source for generating the reliable model using this platform. Currently the integrate of artificial intelligence approach to this platform like AlphaFold2, still, MODELLER is used in the protein modelling, and it continues to serve critical roles in template-based modelling, especially for studying challenging conditions, like multi-templated approaches, low sequence similarity and specialized applications. The implementation of advanced computational approaches with MODELLER and its complementary relationships with AI-based tools for predicting the molecular structure has opened new avenues for hybrid modelling strategies. The present study aims to critically emphasize the current state of MODELLER-based homology modelling protocols, to evaluate the current advanced methods and identify insistent computational challenges those limits accuracy of modelling and efficiency in the contemporary structural biology landscape **(Webb and Sali 2016)**.

2. Theoretical Foundations of MODELLER

2.1. Satisfaction of Spatial Restraints Method

The incorporation of MODELLER in the comparative protein structure modelling via strong statistical, spatial restraints, a technique that generates a probability density function for location of each atom of the protein. The core step of the modelling process starts with alignment between target sequence and known related experimentally determined 3D structure (template). Based on the sequence alignment, and the statistical analysis of relationship between two homologous structures, the key features like many distance and dihedral angle of the target sequence can calculated. The method's foundation respites on a statistical analysis of relationships between pairs of homologous structures, formerly resultant from a database of 105 family alignments that included 416 proteins with known 3D structure. This analysis predicts the various correlations including relationships between equivalent Cα-Cα distances or equivalent mainchain dihedral angles from associated proteins **(Janson *et al.* 2019)**.

2.2. Statistical Potentials and Scoring Functions

The implementation of advanced scoring functions known as Discrete

Received on : 2025-08-30
Revised on : 2025-09-13
Accepted on : 2025-09-24
Published Online : 2026-01-28
Review Model : Single-Blind Review
No. of Reviewers : Two
Edited by : Dr. Chandrabose Selvaraj
Vol and Issue : 02 (01)
Page No : 40-47
Plagiarism Level : 09% and 19% (AI)
Correspondence : Dr. T.M. Sundari
Contact Author : mownasundari[at]ldc.edu.in

Key Words:
Modeling
Algorithm
Alphafold
Modeller
Healthcare
Structure Biology
Energy

DOI: 10.64659/jomi/211255

Optimized Protein Energy (*DOPE*) is a critical advancement of -MODELLER's assessment capabilities. *DOPE* signifies an atomic distance-dependent statistical potential derived from a native protein structure of the sample, grounded completely in probability theory short of recourse to statistical mechanics. This approach significantly avoids the assumption of the Boltzmann distribution and permits for predict more accurate treatment of reference state complications. The score predicted by *DOPE* is extracted from a nonredundant set of 1427 experimentally predicted crystallographic structures and integrated into MODELLER to facilitate both structure refinement and model assessment. The normalized *DOPE* score signify by Z-score that provides a standardized measure where the negative values lower than -1 suggested native like structure, whereas the positive score indicated the poor models **(Shen and Sali 2006)**.

2.3. Comparative Modeling Principles

The theoretical foundation of MODELLER rests on several key principles derived from the analysis of protein structure relationships. The technique recognizes that spatial restraints should be gained empirically from databases of protein structure alignments rather than from theoretical considerations alone. This empirical approach allows the software to capture the complex relationships between sequence similarity and structural conservation that may not be fully captured by physical force fields. The restraints are articulated as conditional probability density functions that directly incorporate the uncertainty inherent in homology modeling. For example, possibilities for diverse values of mainchain dihedral angles are intended from the residue type, mainchain conformation of equivalent residues, and sequence similarity between related proteins **(Sali and Blundell 1993)**.

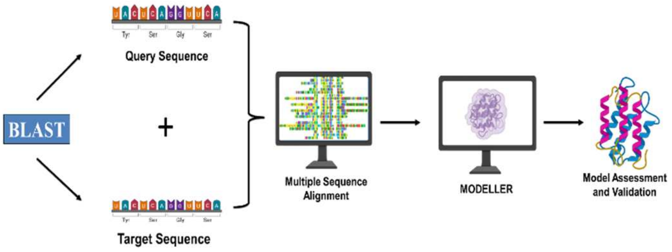

Figure.1. Workflow of Basic Homology Modeling

3. Basic Homology Modeling Workflow

3.1. Template Identification and Selection

Template identification represents the crucial first step in homology modeling, as the quality of selected templates directly impacts final model accuracy **(Figure.1).** The simplest template selection rule involves choosing structures with the highest sequence similarity to the target sequence, but several additional factors must be considered for optimal results. Database searching strategies typically employ BLAST, PSI-BLAST, or more sophisticated methods like HHsearch for remote homology detection. PSI-BLAST, utilizing position-specific scoring matrices, has proven particularly effective for identifying distantly related templates by iteratively updating scoring profiles to detect more remote homologs. Template quality assessment must consider multiple criteria beyond sequence similarity. The resolution and R-factor of crystallographic structures, or the number of restraints per residue for NMR structures, provide important indicators of structural accuracy. When multiple templates show similar sequence similarity, the one determined at the highest resolution should generally be preferred. The alignment coverage - the fraction of the query sequence that can be prophesied from the template - represents another crucial consideration. Templates with better coverage of the target sequence typically yield more complete and reliable models, even if sequence identity is slightly lower than alternatives with limited coverage **(Fiser 2010).**

3.2. Sequence Alignment Optimization

Accurate sequence-to-structure alignment represents the most critical factor affecting model quality, particularly when target-template sequence identity falls below 40%. The alteration of any single amino acid resides can result in an error of approximately 4 Å in the final model, as current modeling approaches mostly cannot improve from alignment errors. Multiple sequence alignment techniques provide enhanced sensitivity for detecting remote relationships and improving alignment accuracy. Profile-profile alignment methods, which generate sequence profiles for both target and template, offer improved performance by reducing noise familiarized by sequence drift in nonessential regions. When multiple templates are designated, an effective strategy involves superposing templates with each other first to find a multiple structure-based alignment, followed by alignment of the target sequence to this structural consensus. This approach leverages structural information to guide alignment decisions and can significantly improve model quality in challenging cases **(Fiser 2010).**

3.3. Model Construction and Generation

The automated model building process in MODELLER utilizes the AutoModel class, which implements the satisfaction of spatial restraints algorithm. The spatial restraint satisfaction procedure combines distance and dihedral angle restraints derived from template structures with CHARMM energy terms to generate the final model. Multiple model generation strategies are typically employed to sample conformational space and identify the most reliable structural prediction. Based on the different random seeds, this software can predict multiple models and allowing the assessment of model consistency and find the well-defined versus poorly defined regions. Further optimization protocols involved in this approach include iterative refinement with conjugate gradient methods a molecular dynamics simulation satisfy spatial restraints while maintaining appropriate stereochemistry. This optimization process includes several cycles of variable target function which effectively optimize and ensure convergence to a stable structural solution.

3.4. Model Assessment and Validation

The *DOPE* scoring system plays a critical role in model assessment in MODELLER, which provides both absolute and normalized Z-score of a model which signify the quality of the generated model. The normalized Z-score provided the standardized measure the enhances the comparative analysis between different models and assessment of model reliability. Stereochemical validation tools like Verify3D and ProSA were integrated in MODELLER based on *DOPE* score that evaluate the model quality based on several perspectives. These tools also assess the characteristics consistent of native protein structures in terms of amino acids environment and overall fold quality. Comparative evaluation metrics provides the ranking of different model predicted for a single target and facilitate selection of the most reliable structures. This multiple assessment criteria offers more robust model evaluation than reliance on single scoring functions **(Figure.1)** **(Eswar *et al.* 2006)**.

4. From Advanced Modeling Protocols

4.1. Multi-Template Modeling Approaches

Multi-template modeling has developed as a powerful strategy for improving model accuracy, particularly in cases where no single template provides complete coverage or optimal quality for all regions of the target protein. Template combination strategies involve using different templates for different regions of the target, leveraging the strengths of multiple structural templates. Automated template superposition algorithms in MODELLER facilitate the alignment of multiple template structures, creating a structural framework that can incorporate information from diverse sources. Region-specific template selection allows optimization of template choice for different parts of the target protein, potentially using high-resolution templates for conserved regions and more distantly related templates for variable regions. Quality improvement through template diversity has been demonstrated in numerous studies, with multi-template approaches often yielding superior results compared to single-template modeling. The ability to combine complementary structural information from multiple templates helps compensate for limitations in individual template structures **(Figure.2) (Larsson *et al.* 2008).**

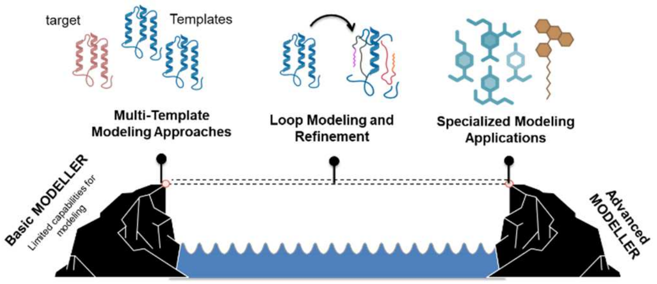

Figure.2: Represents the Advanced Modeling Protocols

4.2. Loop Modeling and Refinement

Loop regions represent challenges in homology modeling due to their high variability even among closely related proteins. MODELLER incorporates specialized algorithms for ab initio loop modeling, which attempts to predict loop conformations without relying on template structures for these regions. The LoopModel class in MODELLER automatically identifies insertion regions in alignments where template information is unavailable and applies specialized refinement procedures. Users can also manually define specific loop regions for targeted refinement using the select_loop_atoms() routine. Statistical potential-based refinement methods, particularly those incorporating *DOPE* scoring, have improved loop modeling accuracy significantly. The integration of cyro-EM data in molecular modeling, especially comparative or de nova modelling, demonstrates that the translation of density maps into restraint information. This conformational change guides the sampling and density itself and does not propose a novel structure, instead it is served as a probabilistic distribution of atomic positions that biases the search towards models consistent with the map. The incorporation of cryo-EM data as supplementary restraints in loop modeling signifies an evolving application that can further improve accuracy in appropriate cases **(Haddad *et al.* 2020)**. Several studies have been conducted by incorporating the GNNs into cryo-EM workflows for evaluating the atomic position and spatial relationships. For instance, graph neural networks (GNNs) have been used for automated model building directly from cryo-EM density maps. In this integrated approach, the density map is served as a graph where nodes correspond to candidate atomic positions and edges are represented the spatial relationships. The incorporation of cryo-EM density as external spatial restraints in the MODELLER also have been studied exclusively. For instance, Sali et al used em_density and Gaussian mixture model–based restraint functions in the MODELLEER, where they significantly demonstrate the density maps for bias structure refinement **(Webb and Sali 2016).**

4.2. Specialized Modeling Applications

Protein-ligand complex modelling is another key approach in MODELLER can incorporate the cofactors and heteroatoms into predicated structures. The template selections of structures that contains similar ligands should prioritize in these applications; the mode of protein-ligand interaction mode often conserved among related protein structures. MODELLER allows the users to add their own restraints, like data from experiments, ensures the tools flexible for several research needs. However, these flexibility makes its adaptability for diverse research that need for supportive experimental scenarios. Multi-domain protein modelling offers the specific challenges associated with domain orientation and interaction of inter-domain. MODELLER can even take up the large protein system and makes it suitable for these applications via careful selection of template and validation become key factor for multi-domain targets. **(Figure.2)(Xu *et al.* 2023).**

5. Theoretical Framework & Computational Architecture

5.1. Machine Learning Integration

The integration of machine learning approaches with MODELLER has opened new possibilities for enhanced template selection and alignment optimization. Deep learning-enhanced template selection algorithms can identify more sophisticated patterns in sequence-structure relationships, potentially improving template choice for challenging modeling cases. ML-assisted alignment optimization represents another promising area, where machine learning models can predict optimal alignment parameters or identify problematic alignment regions. These hybrid approaches combine the statistical rigor of MODELLER's spatial restraints method with the pattern recognition capabilities of modern machine learning. Hybrid modeling approaches that combine MODELLER with neural networks have shown particular promise in recent studies. The AlphaMod pipeline, which integrates AlphaFold2 predictions with MODELLER refinement, demonstrated improvements in prediction accuracy for certain protein classes, showing rising accuracy of approximately 34% over AlphaFold2 alone in unsupervised setups **(Chen *et al.* 2024)**. Recently the integration of two different potential approaches has been employed as effective methods to enhance the effectiveness of the approaches. The incorporation of comparative modeling and machine learning approaches in the structure prediction such as AlphaMod, where the AlphaFold2 derived structural model incorporates with MODELLER's comparative modeling and refinement capabilities. In this approach, AlphaFold2 predicts the high-quality templates, while MODELLER describes conformational variability, imposes experimental restraints and enables ensemble generation **(Hiranuma et al., 2023)**.

5.2. Enhanced Validation Methods

Advanced scoring functions beyond *DOPE* have been developed to provide more comprehensive model assessment. These include physics-based validation approaches that incorporate experimental data and ensemble-based model assessment methods that evaluate model consistency across multiple predictions. Cross-validation strategies for model reliability have gained importance as the field recognizes the need for more robust quality assessment. These approaches help identify cases where models may appear high-quality according to computational metrics but lack experimental validation. The development of confidence estimation methods has become increasingly important for practical applications of homology models. These methods help users understand the reliability of different model regions and make informed decisions about which parts of a model are suitable for specific applications **(Guedes *et al.* 2018)**.

5.3. High-Throughput Modeling Capabilities

Automated pipeline development has made MODELLER more accessible for large-scale applications. These pipelines can process hundreds or thousands of sequences automatically, making comparative modeling feasible for genome-scale studies and systematic structural biology projects. Batch processing optimization has improved computational efficiency for large datasets, enabling more effective use of computational resources. Performance scaling for large datasets has become increasingly important as structural genomics projects generate ever-larger numbers of modeling targets. Cloud computing integration has made high-performance MODELLER applications more accessible to researchers without access to dedicated computational infrastructure. This democratization of computational resources has expanded the user base and enabled more ambitious modeling projects **(Figure 3)**.

6. Comparative Analysis with Contemporary Methods

6.1. MODELLER vs. AlphaFold Integration

The relationship between MODELLER and AlphaFold2 represents complementary rather than competitive applications. Template-based modeling using MODELLER remains superior when high-quality templates are available, particularly for applications requiring specific conformational states or ligand-bound structures. Quality assessment comparative studies have shown that MODELLER models often outperform AlphaFold2 predictions when appropriate templates are available. In a study of G-protein-coupled receptors, MODELLER achieved an average RMSD of 2.17 Å compared to 5.53 Å for AlphaFold2, largely due to the availability of high-quality templates with near-identical sequences. Hybrid approaches leveraging both methods show particular promise for challenging modeling scenarios. The integration of AlphaFold2 predictions as starting points for MODELLER refinement, or vice versa, can combine the strengths of both approaches while mitigating their individual limitations **(Gil Zuluaga *et al.* 2023).**

6.2. Benchmarking Against Modern Predictors

CASP competition performance analysis has provided valuable insights into MODELLER's capabilities relative to contemporary methods. While newer deep learning methods have excelled in template-free modeling, MODELLER continues to perform competitively in template-based categories, particularly for targets with good template availability. Accuracy comparisons with deep learning methods reveal that the choice of optimal method depends heavily on target characteristics and template availability. For targets with high-quality templates (>70% sequence identity), MODELLER often outperforms neural network-based methods, while the reverse is true for targets lacking good templates. Structural prediction benchmarking studies have emphasized the continued importance of template-based methods in the overall landscape of protein structure prediction. The complementary nature of different approaches suggests that hybrid strategies may represent the future direction of the field **(Kryshtafovych *et al.* 2019)**.

7. Computational Challenges and Limitations

7.1. Sequence-Structure Relationship Limitations

Low sequence identity modeling remains one of the most significant challenges in MODELLER-based homology modeling. When sequence identity falls below 30%, alignment accuracy becomes the primary limiting factor, and even sophisticated alignment methods may produce errors that propagate through the modeling process. Twilight zone homology detection, referring to cases where sequence identity falls between 20-30%, presents difficulties. In this range, statistical significance of sequence similarity becomes questionable, and structural similarity may not be reliably predicted from sequence relationships alone. Alignment errors in distant homology modeling can have catastrophic effects on final model quality. Since MODELLER cannot recover from fundamental alignment errors, the accuracy of the initial sequence-structure alignment sets an upper bound on achievable model quality **(Bender *et al.* 2020).**

7.2. Computational Efficiency Issues

Optimization algorithm scalability presents challenges for large protein systems, where the number of spatial restraints can become computationally prohibitive. The iterative optimization process may require substantial computational time for complex targets, limiting throughput in high-volume applications. Memory usage in large protein modeling can become problematic, particularly for multi-domain proteins or protein complexes. The storage requirements for restraint matrices and coordinate sets can exceed available memory on standard computational systems. Processing time considerations for complex systems often require trade-offs between model accuracy and computational feasibility. While more extensive optimization generally improves model quality, the computational cost may be prohibitive for routine applications or high-throughput studies **(Heo and Feig 2024).**

7.3. Model Quality Assessment Challenges

Reliability of scoring functions remains a fundamental challenge in computational structural biology. While *DOPE* and other statistical potentials provide useful metrics, they may not accurately reflect model quality in all cases, particularly for unusual protein families or non-globular proteins. False positive identification in model validation can lead to overconfidence in poor models. The correlation between computational scores and actual structural accuracy, while generally positive, shows significant variation that can mislead users about model reliability. The correlation between scores and actual structural accuracy depends heavily on the specific protein family and modeling scenario. Statistical potentials derived from globular proteins may not accurately assess models of membrane proteins or other specialized protein classes **(Eramian *et al.* 2006).**

7.4. Conformational Flexibility Modeling

Dynamic region prediction represents a fundamental limitation of static homology modeling approaches. Protein regions that undergo significant conformational changes during function cannot be accurately modeled without incorporating information about multiple conformational states. Allosteric site modeling presents particular challenges, as these regions may adopt different conformations contingent on the functional state of the protein. Template structures may not represent the conformational state relevant to the intended application of the model. Conformational state selection becomes critical when multiple template structures are available in different functional states. The choice of appropriate conformational state for modeling varies on the proposed use of the model and may not be obvious from sequence analysis alone **(Varadi *et al.* 2025).**

7.5. Complex Biological System Modeling

Membrane protein modeling limitations stem from the underrepresentation of membrane proteins in structural databases and the challenges associated with modeling lipid-protein interactions. The unique environment of membrane proteins often requires specialized modeling approaches that go beyond standard homology modeling protocols. Multi-subunit complex assembly challenges arise when modeling protein complexes, where quaternary structure prediction becomes as important as tertiary structure modeling. The interfaces between subunits may not be accurately predicted from individual subunit models. Protein-protein interaction interface modeling requires careful consideration of the biological context and may benefit from experimental constraints such as cross-linking data or evolutionary covariance information **(Koehler Leman *et al.* 2015).**

7.6. User Interface and Accessibility

The learning curve for new users represents a significant barrier to MODELLER adoption. The command-line interface and Python scripting requirements can be intimidating for users without computational backgrounds, limiting accessibility for experimental biologists. Documentation and tutorial comprehensiveness, while extensive, can be overwhelming for new users who need guidance on basic applications. The balance between comprehensive coverage and accessible introduction remains challenging. Error handling and troubleshooting support could be improved to help users diagnose and resolve common problems. Cryptic error messages and limited diagnostic information can frustrate users and impede successful model generation.

7.7. Integration with Modern Workflows

Compatibility with current computational infrastructure varies depending on system configuration and dependencies. Installation and configuration challenges can prevent users from effectively utilizing the software. Pipeline integration challenges arise when attempting to incorporate MODELLER into automated workflows or web-based services. Standardization of input/output formats and programmatic interfaces could facilitate better integration. Version control and reproducibility issues can complicate collaborative research and method development. Ensuring consistent results across different versions and computational environments remains challenging.

8. Methodology Practice for Modeling

8.1. Template Selection Best Practices

Multi-criteria template evaluation should consider sequence similarity, structural quality, resolution, and functional relevance. The optimal template may not always be the one with highest sequence identity, particularly when functional considerations are important. Structural quality assessment using resources like the PDBREPORT database can identify templates with known structural problems or unusual features. Avoiding templates with significant structural errors or missing regions can improve model quality significantly. Evolutionary relationship consideration through phylogenetic analysis can help identify the most appropriate templates for specific modeling applications. Templates from the same evolutionary lineage may provide better structural frameworks than more distant homologs with higher sequence identity **(Binbay *et al.* 2023).**

8.2. Model Validation Strategies

Multi-metric validation approaches that combine *DOPE* scores with stereochemical validation tools provide more robust model assessment. No single validation method is perfect, so combining multiple approaches increases confidence in model quality evaluation. Integration of tools like Verify3D and ProsaII can identify models with incorrect fold topology or unusual structural features that may not be detected by *DOPE* scoring alone. These complementary approaches assess different aspects of model quality. MolProbity clash score analysis can identify steric conflicts and other stereochemical problems that may not be adequately addressed during the modeling process. Resolving these issues through local refinement can improve model quality for downstream applications **(Wlodawer 2017)**.

8.3. Quality Control Measures

Systematic error identification through analysis of model quality as a function of sequence identity and template characteristics can help users understand the limitations of specific models. This analysis enables informed decisions about model reliability for different applications. Region-specific accuracy assessment recognizes that different parts of a model may have different reliability levels. Loop regions and areas with poor template coverage typically show lower accuracy than well-conserved regions. Confidence scoring interpretation requires understanding of the statistical basis of scoring functions and their limitations. Users should be trained to interpret normalized *DOPE* scores and other metrics in the context of their specific applications **(Miller *et al.* 2022).**

8.4. Optimization Strategies

Parameter tuning for specific applications can significantly improve modeling efficiency without sacrificing accuracy. Understanding the trade-offs between optimization thoroughness and computational cost enables more effective resource utilization. Computational resource allocation should consider the complexity of the modeling target and available computational infrastructure. Simple targets may not require extensive optimization, while challenging cases may benefit from additional computational investment. Parallel processing implementation can reduce wall-clock time for model generation, particularly when generating multiple models or performing extensive optimization. Modern multi-core systems can significantly accelerate MODELLER calculations **(Joo *et al.* 2009).**

8.5. Workflow Automation

Scripting and pipeline development can improve reproducibility and enable high-throughput applications. Well-designed scripts can standardize modeling protocols and reduce user error. Batch processing optimization allows efficient processing of multiple targets and can take advantage of computational resources more effectively. Queue-based systems can manage computational resources and prioritize modeling tasks. Result analysis automation can standardize model assessment and facilitate comparison between different modeling approaches. Automated reporting can help users quickly identify successful models and problematic cases **(Cieslik and Mura 2011).**

9. Methodological Evolution and Emerging Trends

9.1. Hybrid Modeling Approaches

Integration with deep learning methods represents a promising direction for improving MODELLER's capabilities. Hybrid approaches that combine the statistical rigor of spatial restraints with the pattern recognition capabilities of neural networks show particular promise. Combination with experimental data constraints, including NMR restraints, cross-linking data, and cryo-EM information, can significantly improve model accuracy. The integration of sparse experimental data with computational modeling has shown dramatic improvements in model quality. Multi-scale modeling implementations that combine atomistic modeling with coarse-grained approaches may enable modeling of larger systems while maintaining accuracy in critical regions. These approaches could expand the applicability of MODELLER to complex biological assemblies **(Seffernick and Lindert 2020).**

9.2. Enhanced Validation Methods

Physics-based validation integration could provide more accurate assessment of model quality, particularly in cases where statistical potentials may be inadequate. The combination of knowledge-based and physics-based approaches may provide more robust validation. Experimental validation correlation studies help calibrate computational metrics against experimental data, improving the interpretation of model quality scores. These studies are essential for understanding the practical utility of different validation approaches. Confidence estimation improvements through machine learning and ensemble methods could provide more accurate estimates of model reliability. Better confidence estimates would enable more informed use of homology models in downstream applications **(Wang *et al.* 2023)**.

10. Technological Integration

10.1. High-Performance Computing Applications

GPU acceleration implementation could significantly reduce computational time for MODELLER calculations, particularly for optimization-intensive steps. Modern graphics processing units are well-suited to the parallel calculations required for spatial restraint satisfaction.

Cloud computing integration has made high-performance modeling more accessible to researchers without dedicated computational resources. Cloud-based MODELLER services could democratize access to advanced modeling capabilities. Distributed processing capabilities could enable modeling of very large systems or high-throughput applications that exceed the capabilities of single-node systems. Distributed computing frameworks could facilitate collaborative modeling efforts(**Grosdidier *et al.* 2009**).

10.2. Integration with Structural Biology Ecosystem

Compatibility with structure visualization tools like PyMOL and ChimeraX could improve the user experience and facilitate model analysis. Seamless integration with visualization software would reduce barriers to model interpretation. Integration with experimental structure databases could provide automated access to templates and validation data. Direct connection to databases like the Protein Data Bank could streamline the modeling process. Workflow integration with other computational tools could create comprehensive structural biology pipelines that combine modeling with other computational approaches. Standardized interfaces could facilitate tool interoperability (**Meng *et al.* 2023**).

10.3. Accuracy Improvement Strategies

Template quality enhancement methods, including error correction and structure refinement, could improve the starting point for homology modeling. Better templates naturally lead to better models, making template improvement a high-priority area. Alignment accuracy optimization through machine learning and structural information could address one of the primary limitations of current methods. Improved alignment methods could significantly extend the range of targets accessible to homology modeling. Scoring function refinement based on larger structural databases and improved statistical methods could provide better model assessment. As structural databases grow, statistical potentials can be improved and specialized for different protein classes (**Szilagyi and Zhang 2014**).

10.4. Usability Enhancement

User interface modernization could make MODELLER more accessible to non-computational users. Graphical interfaces and web-based services could expand the user base significantly. Educational resource development, including tutorials and training materials, could improve user competency and reduce barriers to adoption. Better educational resources would enable more effective use of the software's capabilities. Community support infrastructure improvement could provide better assistance to users and facilitate collaborative development. Strong community support is essential for maintaining and improving complex scientific software.

11. Case Studies and Applications

11.1. High-Impact Biological Applications

Drug discovery represents one of the most important applications of MODELLER-based homology modeling. The ability to generate structural models of target proteins has accelerated the structure-based drug design process, particularly for targets where experimental structures are unavailable. Homology models have been successfully used in virtual screening campaigns, lead optimization, and understanding drug-target interactions. Protein function prediction has benefited significantly from MODELLER-generated structural models. The three-dimensional context provided by homology models enables more accurate prediction of functional sites, protein-protein interaction interfaces, and allosteric regulation mechanisms. These applications have been particularly valuable in functional genomics projects where large numbers of proteins require functional annotation. Evolutionary relationship elucidation through structural modeling has provided insights into protein family evolution and functional diversification. Comparative modeling enables researchers to understand how structural changes relate to functional evolution and to identify key evolutionary innovations in protein families (**Vyas *et al.* 2012**).

11.2. Challenging Modeling Scenarios

MODELLER can succeed even in the low sequence identity (below 30%) via careful selection of template choice and alignment optimization; however, the successful model still offers the valuable structural insights during appropriate handling. Modelling of multi-domain complex offers another important area in MODELLER which has achieved notable successes. The ability of the software to handle the large quantity proteins with multiple domains has qualified modelling of complex regulatory proteins and large enzyme complexes. Modelling of membrane proteins are quite challenging, but MODELLER can generate useful model of membrane proteins when appropriate templates are available. The implementation specialized protocols and validation application are needed for specialized requirements of membrane protein modelling (**Bender *et al.* 2020**).

11.3. Common Pitfalls and Solutions

Template selection errors is one of the most common failures in modelling, these errors are often results from overemphasis on sequence similarities without reflecting quality of the structure and functional relevance or experimental conditions. Multi-criteria template selection and evaluation, careful consideration are the important solution to evaluate the modelling objectives. Alignment quality issues as identified as the primary challenge in many failed modelling attempts, specifically in low sequence identity cases, poor alignments can render even the best modelling algorithms ineffective. The enhanced alignment methos and manual intervention are the critical aspects that can address number of problems. Hence, the validation interpretation mistakes occurs when users misinterpret the quality metrics or fail to recognize the challenges of the predicted models. Better understanding about the scoring function and its limitations with experimental validation can reduce the inappropriate use of low-quality models.

11.4. Improvement Strategies Based on Failures

For addressing the failures of modelling, incorporating error analysis approaches have been implemented to systematically identify the source and development strategies for improvements. These applications ensure the understanding of particular models those failed and avoid similar issues in future applications. The finding learned from failed models; quality control applications has been incorporated to predict mode robust modelling protocols. These protocols further incorporate with multiple validation steps and decision points that help identify the problematic model before they have used in downstream applications. The development of best practices via analysis of both successful and failure modelling has provide more reliable protocols. The cumulative information of MODELLER experience has led to generate and standardized approaches to enhance the probability of successful modelling outcomes.

12. Conclusion

MODELLER remains one of the unique approaches in the modern computational landscape despite the emergence of potential deep learning approaches like AlphaFold2. The main strength of the MODELLER is its ability to leverage high-quality templates structures and integrated the experimental constraints, makes it specific valuable for requiring specific conformational states or ligand-protein complex structures. The complementary role of other deep learning approaches has been used as emerging approaches which recognizes the different approaches excel in several scenarios. MODELLER remains superior methods for template-based modelling when high quality templates are available while deep learning methods are excelled in template-free scenarios. This unique advantage in specific applications including the ability to predict protein-ligand complex, incorporating the experimental restraints leads the prediction of multiple conformational states. These capabilities make MODELLER exceptional for certain types of structural biology applications.

13. Disclosure Statements

13.1. Author Contribution

PS: Writing – Original Draft Preparation; Writing – Review and Editing; Validation; **TMS:** Conceptualization; Correction and Supervision. The author has read and approved the final manuscript.

13.2. Declaration of Generative AI

The authors declare that no generative AI tools were used in the drafting, writing, or editing of the manuscript. All scientific interpretations and conclusions are the author's own. AI-based tools were used only for language grammar refinement and formatting purposes, and the final content was verified and approved by the authors.

13.3. Ethics approval (for clinical/animal studies)

This study did not involve the participation of human subjects, the use of identifiable human data or tissue, or any experiments on live animals. Consequently, the requirement for ethical approval or informed consent did not apply.

13.4. Informed Consent Statement

Not applicable.

13.5. Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

13.6. Acknowledgment

The authors thankfully acknowledge the Lady Doak College, Madurai, Tamil Nadu, India for providing necessary facilities for performing this review work.

13.7. Funding Statement

None

13.8. Conflicts of Interest

The authors declare that they have no known financial, personal, academic, or other relationships that could inappropriately influence, or be perceived to influence, the work reported in this manuscript. All authors confirm that there are no competing interests to declare.

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How to Cite: Suguna, P., and Sundari, TM. (2026). MODELLER-Based Homology Modeling: A Critical Review of Protocols, Advances, and Computational Challenges. *Journal of Medico Informatics*, 02(01), 40-47. **doi:** http://doi.org/10.64659/jomi/211255