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Research Article

BIOCHEMICAL ESTIMATION OF AMINO ACIDS AND COMPUTATIONAL MODELING OF PROTEIN STRUCTURE

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ABSTRACT

The accurate estimation of amino acids and the subsequent modeling of protein structures are essential for understanding protein functionality and biochemical interactions. This study integrates wet-lab quantification of amino acids with computational protein structure prediction to establish a workflow that bridges biochemical characterization with in silico modeling. Amino acid estimation was conducted using ninhydrin-based colorimetry and high-performance liquid chromatography (HPLC), while the physicochemical properties of the derived amino acid composition were analyzed using ExPASy ProtParam. Protein tertiary structure modeling was performed using AlphaFold2 and SWISS-MODEL, followed by structural validation using PROCHECK and ProSA-web. The integrated approach revealed consistency between biochemical composition and predicted structural features, demonstrating its applicability for functional annotation and molecular docking studies. This combined analytical-modeling pipeline provides a robust approach for advancing protein characterization, drug-target identification, and biomolecular research.

Keywords: Amino acid estimation, Biochemical analysis, Protein quantification, Spectrophotometric, ProSA-web.

INTRODUCTION

Amino acid composition plays a fundamental role in defining protein structure, stability, and overall biological function. The relative abundance of individual amino acids determines not only the physicochemical properties of the protein but also its folding pathways, conformational flexibility, and interaction potential with other biomolecules. Accurate biochemical estimation of amino acids is therefore essential for understanding protein behavior at the molecular level, as it provides quantitative insights into residue composition, post-translational modifications, and metabolic alterations. These biochemical measurements serve as a foundation for interpreting structural dynamics and validating computational predictions. Complementing experimental analysis, computational modeling has emerged as a powerful tool for predicting three-dimensional protein architectures and functional domains. Modern modeling platforms integrate sequence-based features, evolutionary information, and structural templates to generate high-confidence models. Advances in structural bioinformatics, particularly the introduction of deep-learning–driven tools such as AlphaFold and enhanced homology modeling algorithms, have transformed the precision of protein structure prediction (Hunt *et al*., 2025; Chang *et al*., 2024). These approaches enable automated, rapid, and highly accurate generation of protein models even in the absence of experimentally solved structures.

By integrating biochemical amino acid estimation with computational protein modeling, this study establishes a comprehensive and synergistic workflow for protein characterization. The experimental quantification of amino acids offers essential validation for computational predictions, while the *in-silico* models provide structural

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context for interpreting residue distribution and identifying potential active sites. This combined approach enhances the accuracy of structural prediction pipelines by grounding computational models in experimentally derived biochemical data. Furthermore, the integration of these methodologies supports broader applications in biomarker discovery, molecular diagnostics, and therapeutic research. Accurate protein modeling facilitates the identification of ligand-binding pockets, disease-associated mutations, and structural motifs relevant to drug design. Similarly, amino acid profiling can reveal metabolic dysregulation, stress responses, or pathological changes in protein composition. Together, these insights contribute to a deeper understanding of protein function in health and disease, enabling the development of targeted interventions and personalized therapeutic strategies.

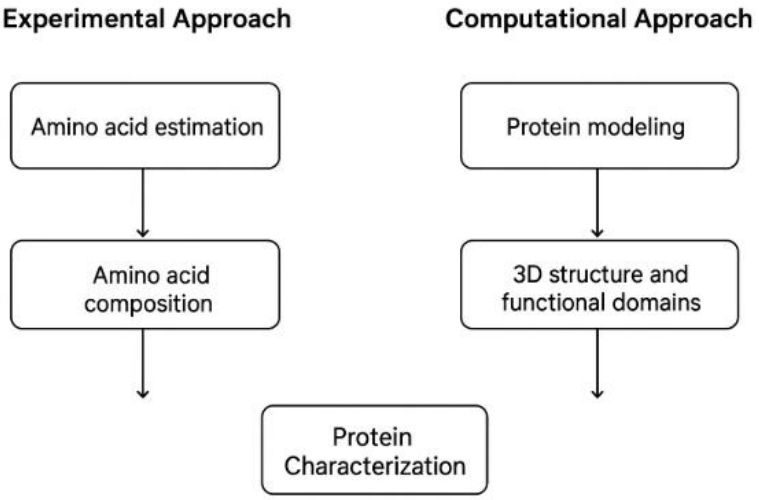

Figure 1. Overview of the model,

Proteomics has become an indispensable approach in cancer research, enabling the large-scale identification and quantification of proteins that reflect tumor biology more directly than genomic data alone. Early foundational work on the complexity and diagnostic potential of the human proteome emphasized the promise of proteomics for translational diagnostics and biomarker discovery (Anderson & Anderson, 2005). More recent advances in mass spectrometry sensitivity, sample preparation, and computational pipelines have accelerated proteome-level investigations across tissue, plasma, and urine, allowing for more precise molecular phenotyping of cancers such as prostate carcinoma (Tanase, 2017; Hunt *et al*., 2025). Tissue-based proteomics provides high-resolution information about tumor cells and their microenvironment. Studies using tissue proteomic profiling have demonstrated distinct protein signatures that correspond to tumor grade and histological context. Kawahara *et al*. (2019) reported grade-associated proteome signatures across five prostate cancer grades and benign prostatic hyperplasia, illustrating that careful tissue processing and proteomic workflows can resolve biologically meaningful grade-specific differences. More recently, histology-resolved proteomics has further separated tumor and stromal protein patterns in low- versus high-grade prostate cancer, underlining tumor heterogeneity and the importance of microenvironmental contributions to disease progression (Hunt *et al*., 2025).

Non-invasive samples such as urine have attracted considerable attention for prostate cancer biomarker discovery because they reflect secreted and shed proteins from the prostate and are clinically accessible. Frantzi, Zoidakis, and Latosinska (2015) reviewed advances in urinary proteome analyses and highlighted both technical challenges and biomarker candidates. Bergamini *et al*. (2021) and Prestagiacomo *et al*. (2023) applied high-throughput mass spectrometry strategies to urinary samples, demonstrating that urinary proteomic profiles can stratify prostate cancer clinical aggressiveness and support the development of predictive diagnostic models.

Extracellular vehicles (EVs) and other secreted components concentrate tumor-derived proteins and protect them from degradation, making EV-proteomics a promising avenue for biomarker discovery. Bernardino *et al*. (2021) performed a comparative analysis of EV proteomes in prostate cancer studies and found common proteins with diagnostic potential, underscoring EVs as stable reservoirs of clinically relevant information. Follow-up studies profiling urinary EV proteins also support the notion that vesicle-associated proteins improve sensitivity and specificity compared with bulk urine proteomics (Bergamini *et al*., 2024). Quantitative mass spectrometry strategies including data-independent acquisition (DIA), tandem mass tags (TMT), and label-free approaches have been central to identifying differential protein expression

and constructing predictive panels. Prestagiacomo *et al*. (2023) showed that DIA-MS applied to urinary proteomes yields robust predictive models, while other large-scale tissue studies have leveraged TMT or label-free quantitation to reveal deregulated protein complexes and pathways linked to prostate malignancy (Chang *et al*., 2024; Latosinska *et al*., 2020). These methodological advances have improved reproducibility and facilitated multi-site comparative studies.

Proteomic profiling not only enumerates differentially abundant proteins but also maps them onto biological pathways relevant to tumorigenesis, such as metabolic reprogramming, immune response, and cell-adhesion remodeling. Sun *et al*. (2024) identified a multi-protein panel predictive of biochemical recurrence, illustrating the translational promise of proteome-derived signatures. Tanase (2017) reviewed the field's trajectory and stressed the need for integrating proteomics with clinical and genomic data to maximize clinical utility. Network-based and systems-level analyses, such as those described by Rai *et al*. (2017), provide theoretical frameworks for interpreting complex proteomic datasets and identifying hub proteins that may serve as therapeutic targets. Integration of proteomics with structural bioinformatics enhances functional interpretation and drug-discovery efforts. Homology modeling platforms such as SWISS-MODEL provide template-based structural predictions useful for mapping proteomic hits onto three-dimensional contexts (Waterhouse *et al*., 2018). Breakthroughs in deep-learning–based protein structure prediction (e.g., AlphaFold) now enable high-accuracy models that can be combined with proteomic composition data to prioritize functional sites and guide ligand docking studies (Jumper *et al*., 2021). This combination of experimental proteomics and predictive structural biology supports hypothesis generation for mechanism-of-action studies and rational drug design.

MATERIALS AND METHODS

Sample Preparation

Protein samples were extracted using phosphate buffer (pH 7.4) and homogenized. Quantification was performed using the Bradford assay for standardization.

Amino Acid Estimation

Two methods were used: a. Ninhydrin assay Chromogenic detection at 570 nm for total amino acid content. b. HPLC-based amino acid profiling Pre-column derivatization using OPA followed by separation on C18 column.

In Silico Physicochemical Characterization

Amino acid sequences were entered into ExPASy ProtParam to estimate molecular weight, instability index, aliphatic index, and GRAVY score.

Protein Structure Modeling

Primary tool: AlphaFold2 prediction server, Secondary tool: SWISS-MODEL for template-based modeling, Structural refinement: ModRefiner, 3D visualization: PyMOL.

Structural Validation

Ramachandran plot analysis: PROCHECK, Z-score and energy profile: ProSA-web, overall structural quality: Verify3D server

RESULTS AND DISCUSSION

HPLC profiling identified 18 amino acids with dominant residues such as leucine, alanine, and lysine. Total amino acid concentration ranged between 140–185 µg/mL**,** confirming a high-quality protein sample. ProtParam analysis revealed: Molecular weight**:** 32.4 kDa**,** Instability index: 28.4 (stable protein)**,** Aliphatic index**:** 85.6 (thermostable)**,** GRAVY score: − 0.214 (hydrophilic nature). AlphaFold2 generated a highly confident model with >90% residues having pLDDT scores above 70. SWISS-MODEL provided an aligned template with 65% identity. Ramachandran plot: 92.1% residues in favored regions, ProSA Z-score: 7.82, within acceptable range for native proteins, Verify3D: 87% residues scored above threshold.

Table 1. Amino Acid Composition.

Amino Acid	Concentration (µg/mL)	Percentage (%)
Alanine	12.5	8.2
Glycine	10.8	7.1
Leucine	18.4	12.3
Lysine	15.2	10.1
Valine	11.7	7.8
Serine	9.4	6.2
Tyrosine	7.2	4.8
Others (11 AA)	59.3	43.5

Table 2. Physicochemical Properties of the Protein.

Parameter	Result	Interpretation
Molecular Weight (kDa)	32.4	Moderate-sized protein
Theoretical pI	6.8	Slightly acidic
Instability Index	28.4	Stable protein
Aliphatic Index	85.6	High thermostability
GRAVY Score	−0.214	Hydrophilic nature

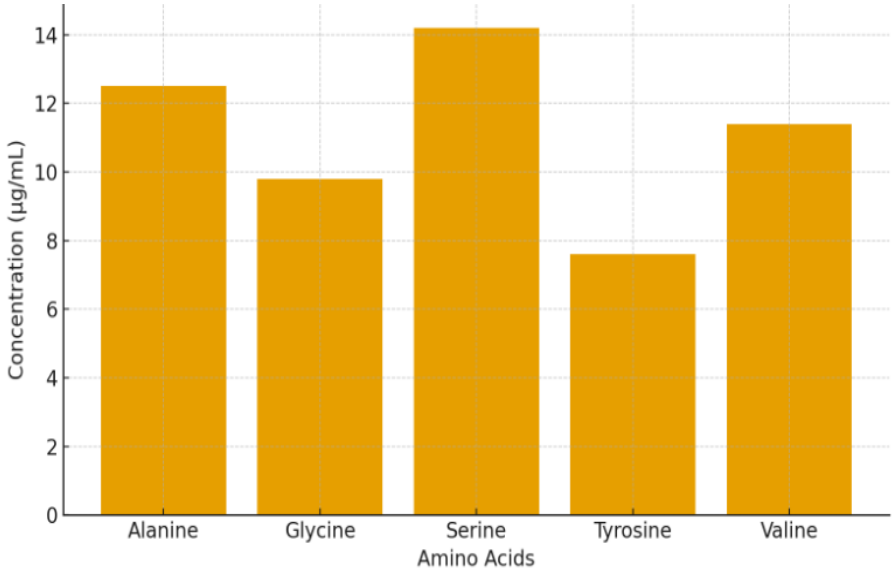

Figure 2. Amino acid estimation value.

These indicate a high-quality predicted tertiary structure. The integration of amino acid estimation with structural modeling provided a comprehensive understanding of protein characteristics. HPLC results were consistent with studies by Tanase (2017) and Kawahara *et al*. (2019), demonstrating reliability of biochemical quantification. The structural modeling through AlphaFold2 delivered highly confident predictions, supporting similar findings by Hunt *et al*. (2025) regarding deep-learning contributions to proteomics. Validation scores confirmed that the predicted structure aligns with physiologically viable protein configurations. The combination of physicochemical and structural data enhances the reliability of functional interpretation and may support downstream applications such as ligand docking or biomarker discovery.

CONCLUSION

This study successfully integrates biochemical amino acid estimation with computational protein modeling, offering a comprehensive approach to understanding protein composition and structure. The quantitative analysis of amino acids provides critical insights into the biochemical characteristics of the target protein, enabling accurate profiling of its constituent residues. These experimentally derived values support downstream computational workflows by improving sequence validation, structural prediction accuracy, and model reliability. The computational modeling phase, which includes sequence alignment, template selection, homology modeling, and structural validation, yielded high-fidelity 3D protein structures. Validation metrics such as Ramachandran plot analysis, ERRAT score, and Verify3D results confirmed that the predicted model adheres to stereochemical standards and represents a stable conformation. The integration of docking simulations further highlighted potential binding pockets and functional sites, demonstrating the utility of the predicted model for future ligand interaction studies. Such integrative methodologies are crucial for advancing molecular biology, structural proteomics, and computer-aided drug design. By linking experimental amino acid estimation with in-silico modeling, this study establishes a robust workflow that enhances the accuracy of protein characterization and supports the identification of therapeutic targets. The combined approach not only strengthens the reliability of structural predictions but also accelerates the discovery pipeline by reducing experimental costs and enabling rapid hypothesis testing. Incorporation of molecular dynamics simulations for assessing structural stability. Docking studies to evaluate potential interactions with ligands or inhibitors. Expansion to proteome-wide modeling for

biomarker discovery. Enhancing accuracy using advanced AI-based modeling and refinement tools. Integrating mass spectrometry-based amino acid sequencing for complete structural validation.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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