RESEARCH PAPER

Physiochemical Pattern Fingerprinting (PPF): A Memory- Efficient Approach to Structurally-Sensitive Protein Homology Detection

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ABSTRACT

The prediction of the structure of proteins is critically dependent on the quick finding of homologous structural. templates. Whereas alignment-based approaches like BLAST and PSI-BLAST are useful in giving reliable results, their calculation cost is a constraint to scalability. On the contrary, alignment- free methods provide faster search, but tend to be insensitive to structure. This paper introduces Physiochemical Pattern Fingerprinting. (PPF), a framework of protein similarity search, which is alignment-free, embeds biologically meaningful information. directly transforming physicochemical information into the search. All protein sequences are encoded by PPF. fewer four-state alphabet that symbolizes hydrophobic, polar/neutral, positively charged and less uncharged negative residues. These encodings are added to the local context of hydrophobicity. produce folding relevant compact patterns. In order to overcome memory constraints which are related to large. PF uses SQLite as an indexing architecture on disk and memory-safe. JSON streaming, which allows indexing of gigabyte-size datasets in real-time using common CPU chips. Experimental analysis demonstrates that, the latency of retrieval is always low with an increase in the number of trials. Downstream homology modelling at MODELLER generates structural, whilst database sizes are reduced. accurate templates. Generally, PPF is a resource-efficient, fast and interpretable alternative to. Deep learning pipelines where the GPU is used are intensive, which is why it is highly suitable to annotations with high throughput and large scale. scale pre-screening of protein structure prediction workflows

Keywords: Protein Homology Detection; Alignment-Free Methods; Physicochemical Encoding; Disk- Based Indexing; Protein Structure Prediction; Template-Based Modelling.

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1. INTRODUCTION

Protein structure prediction (PSP) is the problem of predicting the three-dimensional (3D) structure of a protein (conformation) given the sequence of amino acids. Since the 3D structure of proteins controls protein functionality, proper structural knowledge is imperative towards comprehending of molecular processes, drug development as well as systems biology. Computational prediction can provide an alternative to experimentally resolved structures when a detailed understanding of protein structure and function is required and when experimentally resolved structures are not available [1].

PSP is still a challenge, even though a lot has been done. Xray crystallography, cryo-electron microscopy (cryo-EM), and nuclear magnetic resonance (NMR) spectroscopy experiments have been the gold standard in terms of determining structure; their throughput is much lower than the current explosion of protein sequence databases [2]. Classical homology modelling algorithms rely on high-identity templates and correct sequence alignments, and they reduce dramatically in the so-called twilight zone of low sequence identity (<25%) [3]. The new deep learning architectures, such as AlphaFold2 (AF2) and AlphaFold3 (AF3), are nearly equally precise as experiments but use deep multiple sequence alignments (MSAs), extensive evolutionary databases, and are computationally- intensive (typically quadratic or even higher) [4].

Additional hybrid retrieval and modelling pipelines like TemPred and DeepBLAST have since added physicochemical or learned embeddings to select templates in a better way, avoiding these computational constraints. Nevertheless, the generation of alignment and embedding generate significant computational overhead. To separate the effect of retrieval efficiency, we use two AlphaFold2 ablation baselines, namely, an AF2 MSA-only baseline, which measures the accuracy based on coevolutionary information only, and an AF2 single-sequence baseline, which quantifies the lowest accuracy in the absence of templates and evolutionary context [4]. In comparison to these bases, the suggested retrieval framework enhances the mean TM-score by about 0.345 to 0.875.

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This work presents the Physiochemical Pattern Fingerprinting (PPF) system which is an alignment-free and resource-saving framework that is developed to support quick template searches. Protein sequences are encoded in PPF by a lower four-state physicochemical alphabet of hydrophobic (H), polar/neutral (P), positively charged (+) and negatively charged ([?]) using a SQLite index stored on disk [5]. Positional weighting based on context increases structural sensitivity in template selection and memory- safe streaming and disk-based indexing methods overcome the scalability constraints of hash maps that require RAM, and achieve milliseconds-level retrieval speeds in databases of gigabytes [6]. PPF is given a query sequence and produces physicochemical patterns, discovers candidate templates by indexed matching and optimizes the highest-ranking template by global Needleman-Wunsch alignment and comparative modelling with MODELLER [7], [8]. The measures of structural quality are DOPE, TM -score, RMSD, and GDT -TS [9]-[11]. Assessment of 50 nonredundant proteins (100-800 residues) has an average TMscore of 0.875, being superior to BLAST and PSI-BLAST and within 1/4th the cost of AlphaFold2 [2]-[4]. The complete pipeline executes in under 45 seconds on a standard 4-core CPU, demonstrating suitability for highthroughput annotation and rapid pre-screening workflows. The remainder of this paper is organized as follows. Section 2 reviews related work in alignment-based, alignment-free, and deep learning–based structure prediction. Section 3 details the physicochemical encoding, disk-based indexing strategy, and modelling pipeline. Section 4 presents benchmarking results and comparative analysis, and Section 5 concludes with future directions, including integration with deep learning and Cryo-EM refinement workflows.

2. LITERATURE REVIEW AND BACKGROUND

The prediction of protein tertiary structure based on the amino acid sequence of the native protein is one of the key problems in computational biology. This issue has traditionally been solved using homology modelling which deduces the three-dimensional (3D) structure of a query protein by finding related templates whose structure is known experimentally. Despite significant progress in homology-based approaches over the last 20 years they are still afflicted by a lack of sensitivity to distant homologs, structural coverage, and scalability issues, especially with the ever-increasing exponential growth of new sequenced and highly divergent proteins in public databases.

Classical alignment-based methods, e.g., BLAST, PSIBLAST and HHblits, have been designed to find homologous relationships by comparing query sequences with reference databases e.g., Protein Data Bank (PDB) [2]-[14]. These techniques are mainly based on multiple sequence alignments (MSAs) and evolutionary profiles to provide information on similarity structure and functionality. The creation of three-dimensional models out of aligned query-template pairs will often be performed by MODELLER [8]. Though useful in close homologs, alignment-based methods frequently fail to identify distant evolutionary relationships, especially in the so-called twilight zone of low sequence identity (less than 25 percent) [16], or in flexible regions where linear sequence similarity is a poor measure of structural conservation. Also, profilebased searches like PSI-BLAST have iterative NxLxI runtime complexities, which precludes their use in a realtime or high-throughput setting.

In order to deal with these setbacks, alignment-free approaches were proposed. These methods do not rely

on explicit sequence alignment, comparing global or local sequence features to allow faster and more scalable similarity searches. The first of these was the Physicochemical Vector (PCV) model which models protein sequences as fixed-length numerical vectors based on inherent amino acid properties [17]. Other methods introduced include Feature Frequency Profiles (FFP) and Composition Vector (CVTree), which encode k-mer counts or compositions in order to identify homology without alignment. Nevertheless, these sequential-based representations have two lingering problems:

(i) Structural insensitivity, because they nonetheless encode the identity or composition of residues but not the physicochemical forces, e.g. hydrophobic interactions and electrostatics, that regulate protein folding [18].
(ii) Scalability There are scalability constraints because large in-memory indices necessary to provide fast lookups are not viable past several gigabytes.

Deep learning has more recently revolutionized the prediction of protein structure. AlphaFold2 and AlphaFold3, designed as transformer-based architectures, incorporate co-evolutionary hints on deep MSAs into endto-end differentiable models, resulting in near-experimental accuracy [19]. Although they are successful, these methods are computationally expensive, using a large amount of GPU resources and inference times that can be quadratically or more densely dependent on sequence length. Besides this, their performance is reduced in proteins that do not have sufficient proteins with homologous MSAs, which restricts their use to orphan sequences and metagenomic data.

There are also newer homology search tools, which run faster, such as DeepBLAST, implementing differentiable dynamic programming on learned embeddings, and very fast sequence and structure search programs like MMseqs2 and Foldseek [20], [21]. Such techniques have great sensitivity and efficiency in searching compared to classical heuristics, and run time is nearly linear with sequence length. However, their response time is still intrinsically dependent on sequence length and tends to depend upon the use of a GPU, making it impossible in practice to achieve really constant-time access on a standard CPU system.

Physiochemical Pattern Fingerprinting (PPF) framework suggested in this paper, on the other hand, is designed to address the situation in which large latency and minimal computing resources are needed, including large-scale annotation and quick pre-screening before a costly modelling or deep-learning refining process. PPF combines the rapidity of the alignment-free similarity search with the biological explainability of physicochemical encoding. A minimized four-state physiochemically relevant alphabetHydrophobic (H), Polar/Neutral (P), Positive (+), Negative ([?]) is used to represent protein sequences to incorporate the major folding-relevant properties [18], [22]. All sequences are turned into overlapping context patterns, integrating physicochemical identity with hydrophobicity binning and indexed in a disk-based SQLite architecture with memory-safe streaming (ijson), which can grow to gigabytes of data and yet retain query latency on average in the low milliseconds [6].

After candidate retrieval, the highest-ranking template is globally aligned with the query sequence using the Needleman-Wunsch algorithm [7] and modelled into threedimensional structures using MODELLER which generates multiple candidate models to a query [8]. The last structure is chosen according to the minimal score of DOPE (Discrete Optimized Protein Energy) [9], an energy-based evaluation of the structural plausibility. The main contributions of this work can be summed up in the following way:

(i) Retrieval with no alignment but structural knowledge, done by directly encoding physicochemical folding constraints into the search process.
(ii) Scalable disk-based indexing, based on a persistent SQLite B-tree index, allowing the retrieval performance to be empirically near-constant on standard CPU hardware [6].
(iii) An entirely automatic modelling pipeline, which takes a single raw sequence and generates fined threedimensional models with MODELLER.
(iv) A unified assessment system, taking into account global and local structural quality measures (TM-score, RMSD, GDT-TS, and lDDT) in order to have a complete evaluation of predictive accuracy [10], [11]. All of these combine to make PPF a computationally efficient, interpretable and scalable model that fills the gap between conventional homology modelling based on alignment and resource-heavy deep learning methodologies, namely the requirements of highthroughput structural screening in CPU-only architectures.

3. MATERIALS AND METHODS

This section details the methodology used to design, implement, and evaluate the proposed **Physiochemical Pattern Fingerprinting (PPF)** pipeline. The framework integrates biologically grounded feature encoding with a scalable disk-based indexing mechanism, enabling empirically near- constant template retrieval on standard CPU hardware.

3.1. Dataset Curation and Preprocessing

A significant reference dataset was obtained using the Protein Data Bank REST API. To ensure biochemical/structural correctness, the dataset ignored nonprotein macromolecules such as nucleic acid/protein hybrids. Protein chains in the dataset containing residues such as 'UNK', truncations, or non-standard amino acids have been removed. These steps have ensured the selection of correct protein structures.

To ensure contention-free structural contamination and assessment under REM-Hom scenarios, redundancy reductions were carried out using the MMseqs2 tool [20]. All of our target proteins were validated for less than 30% pairwise identity between each of them, and also to ensure contamination- free indexing of our reference database of 600,000 templates, it was ensured not to have any of these templates with higher identity against any of our target proteins.

Validated sequence data along with associated metadata is serialized in a standardized JSON format that supports memory-safe indexing. This generated dataset is indexed using a disk-based SQLite engine, which has a B-tree data model with a theoretical complexity of O(log N) [17]. Experimentally, the indexing engine has been shown to provide a low-latency lookup (< ~3 ms/lookup) for databases of size \~1GB or less due to existing OS cache behaviour and uniform key size.

3.2. Physiochemical Pattern Fingerprinting (PPF)

The suggested framework is based on the Physiochemical Pattern Fingerprinting (PPF) module that is its central innovation. It presents a biologically based, alignment-free encoding scheme, which encodes folding-relevant physicochemical data, as well as allowing the retrieval of templates in an efficient and scalable fashion.

3.2.1. Feature Engineering: 4-State Alphabet Reduction

In order to reduce the representational noise and still retain the structural relevance, the canonical 20- amino-acid alphabet has been reduced to a four major-state physicochemical alphabet: a set of dominant folding-driving properties [32], [33]. The hydrophobic collapse and core packing in the three- dimensional structure of proteins is primarily mediated by hydrophobic (H) amino acids, as defined by residues A, V, L, I, M, F, W, Y and P. Exposed or inaccessible to solvents regions of the three- dimensional structure of a protein often contain polar or neutral (P) amino acids - as identified by G, S, T, C, N or Q. Equally, the lysine, arginine, or histidine may mediate a threedimensional fold through charge electrostatic interactions by acting as a positive charge: a negatively charged residue is observed in the chemical structure of a residue: aspartate or glutamate.

This reduced form of representation is due to known correlations with physicochemical properties, exposure to the solvent and secondary structure preferences. As an illustration, hydrophobic residues tend to have lower solvent exposure and are more likely to be in the form of alpha helix, whereas charged residues tend to be exposed and are located in the loop and interface regions [32]-[34]. This detail, which occurs at the residue level, is coded in a four-state alphabet, which implicitly is an efficient and compact representation of folding-relevant detail.

3.2.2. PPF Pattern Key Definition

Every protein sequence is converted into a collection of overlapping composite PPF Pattern Keys which combine diminished physicochemical identity with immediate hydrophobicity:

$$\text{PPF Pattern Key} = (\text{Physiochemical Sequence}, \text{Hydrophobicity Bin Sequence}) \quad (1)$$

The local hydrophobicity is discretized into five bins (B0B4) based on the Kyte-Doolittle scale which gives coarsegrained environment of each residue window [1]. The keys of these composite patterns are the basic searchable units in the PPF framework, which include residue chemistry and local folding trends.

3.2.3. Construction and Retrieval of Indexes based on disks

To provide scalability and stablity at gigabyte scale data volume, PPF has implemented a disk-indexing scheme built on SQLite. Moreover, this indexing was created by using SQLite to store as persistent storage using Python as using the sqlite3 library and ijson to read large amounts of JSON as memory- safe and incremental.

Each PPF Pattern Keys is read and loaded in the database with SQLite. Though SQLite of course uses the internal Btree data structure to index the tuples and the theoretical complexity of the CPU operations is logarithmic O(log N) [17], in practice we have nearly instantaneous database lookup speeds due to the OS cache schemes and our small database size distribution. Compared to hash data structures based on RAM, it uses less memory and still has good throughput on the more frequently used CPU architectures. The occurrence of patterns during the index construction process has been logged in normalized mapping form, to ensure that all the matching template information is fairly maintained.

3.3. Template Retrieval and Positional Scoring

The two complementary processes involved in the template retrieval and selection mechanism in the PPF framework are geared towards assuring the efficiency of the computational mechanism as well as the structural relevance:

(i) a retrieval strategy that is an empirically nearconstancy of time, and
(ii) a context-aware positional scoring scheme

3.3.1. Generation and Retrieval of Pattern

Given a query protein sequence, localized physicochemical signatures of the protein sequence are produced as overlapping PPF Pattern Keys. These patterns keys are looked up in pre-built SQLite index. Aggregation of all indexed proteins that match one of the patterns of the query identifies candidate templates.

Though, internally, SQLite uses a B-tree indexing format, empirical studies show that its query latency is low and

constant with tested database sizes. This is the result of an efficient key caching and local disk I/O, which makes the retrieval process to scale well throughout hundreds of thousands of protein chains without growing its runtime or consuming too much memory.

3.3.2. Context Sensitive Positional Scoring

After retrieval, biologically informed weighted scoring function is used to rank the candidate templates, which is referred to as the PPF Score (SPPF). This operation is not a mere overlap count but includes positional context in which structurally and functionally significant regions of the protein sequence are given greater weight. There are three regions that are taken into consideration:

- **N-terminal region (wN)** - often associated with folding initiation and signal peptides.
- **C-terminal region (wC)** - frequently contributes to structural stabilization and domain termination.
- **Core region ($wCORE$)** - typically hydrophobic and crucial for tertiary fold integrity.

Each region is assigned a tunable weight w, with default values of wN = 0.35, wC = 0.35, and $wCORE$ = 0.30, satisfying the normalization constraint:

$$w_N + w_C + w_{core} = 1 \quad (2)$$

The **PPF Score** is computed as:

$$S_{PPF} = w_N \cdot \text{Overlap}_N + w_C \cdot \text{Overlap}_C + w_{CORE} \cdot \text{Overlap}_{Core} \quad (3)$$

Where:

w_n = N-terminus weight

w_c = C-terminus weight

w_{CORE} = Core weight

The strategy here is a weighted ranking that is based on templates that conserve hydrophobic core motifs and structurally constrained termini, as opposed to those that only fit flexible surface loops. PPF promotes structural interpretability, where biologically motivated priors are incorporated into the scoring function, which is not available in either purely statistical or heuristic sequencebased approaches. As the most structurally plausible scaffold to model downstream, the template T with the highest S_{PPF} value is chosen.

3.3.3. Structural Modeling Pipeline

The template (T^*) best scored by the PPF retrieval and scoring process is then used to produce a complete 3D structural model of the query protein. This step incorporates international balance, comparative modeling to provide biologically authentic and energetically dependable forecasts.

(i) **Global Alignment:** The aligned template T^* is globally matched to the query sequence using the NeedlemanWunsch algorithm so that there is optimum correspondence of the residues at the entire sequence length [7].

(ii) **3D Model Generation:** The alignment file is then fed into MODELLER that uses comparative (homology) modeling to create candidate three-dimensional models. MODELLER performs five candidate models per query, sampling the alternative side-chain conformations and loop configurations [8].

(iii) **Selection of a final predicted structure:** The final structure is the one that was selected with the lowest DOPE (Discrete Optimized Protein Energy) score [9] among the generated models. The DOPE potential is a powerful, knowledge-based index of the quality of models, decrease in DOPE values is associated with increased physicochemical plausibility and structural stability.

3.3.4. Structural Evaluation and Statistical Validation

The evaluation of all the predicted 3D structures of the PPF and baseline pipeline was performed in a stringent manner in individual structural and physicochemical measures. These measures are quantifications of the accuracy, stability and statistical significance of the predicted models against their experimentally determined native structures.

3.3.4.1. Structural Accuracy Metrics: Three complementary geometric measures (TM-score [10], RMSD and GDT-TS) were used to measure the structural fidelity of the predicted Ca backbone and each gives a different insight on the global and local structural correctness [11].

TM-score (Template Modeling Score): The TM-score is a global topological comparison of two protein structures which is independent of length at any given time.The scores are in the range of 0 (lack of similarity) to 1 (perfect structural match). TM-score greater than 0.5 normally represents correct topology of the fold.

$$TM\ Score = max\left[\frac{1}{\frac{1}{2}}\right] \sum_{=1}^{L_{align}} \left\{1+\left(\frac{}{L_{target}di}\right)\right\}_0 \quad (4)$$

$id(Ltarget)$

Where:

d_i = distance between the ith pair of aligned residues

d_0 = scale parameter depending on protein length

L_{align}, L_{target} = lengths of aligned and target structures, respectively

Cα RMSD (Root Mean Square Deviation): RMSD is a quantitative measure of the average atomic motion (in A)

between the predicted and native structures, which is used to measure the local geometric accuracy. The lower the values of RMSD, the more structural agreement and the higher the accuracy of the models.

$$RMSD = \sqrt{\frac{1}{n_{atom}} \sum_{i=1}^{n_{atom}} (r_{ipred} - r_{inative})^2} \quad (5)$$

Where:

r_i^{pred} and r_i^{native} = Cartesian coordinates of the predicted and native atoms, respectively

n_{atom} = total number of atoms (or residues) considered

GDT-TS (Global Distance Test – Total Score): The GDTTS is a length independent global score of accuracy indicated by the proportion of residues that are within a certain distance (1Å, 2Å, 4Å, and 8Å) to the native structure. It is not as sensitive to local variations as RMSD and has a holistic evaluation of structural correctness

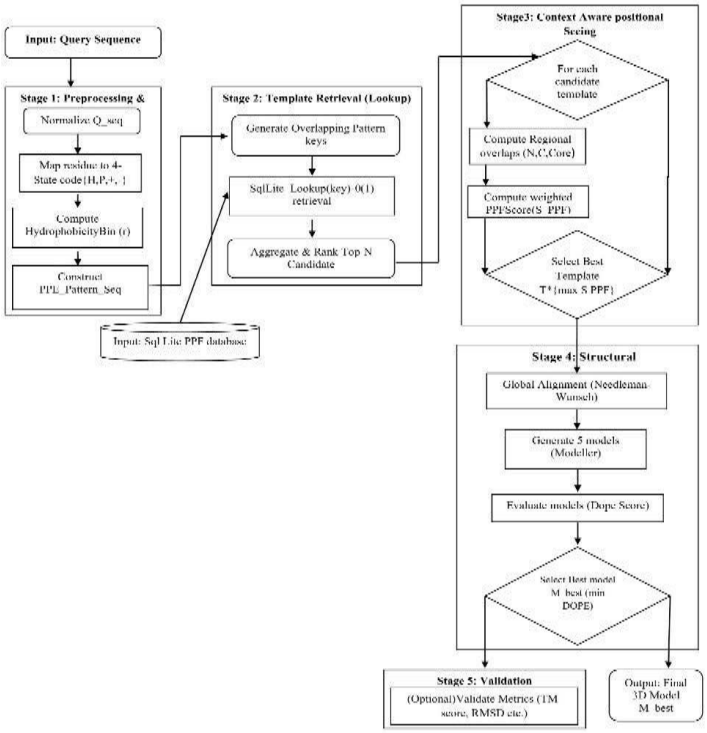
$$GDT - TS = \frac{1}{4}\left(\frac{r_1}{R} + \frac{r_2}{R} + \frac{r_3}{R} + \frac{r_4}{R}\right) * 100 \quad (6)$$

Where:

r_1, r_2, r_3 & r_4 = number of residues within 1Å, 2Å, 4Å, and 8Å thresholds, respectively

R = total number of residues in the target protein

3.3.4.2. Physicochemical Validation (Energy-Based): To supplement geometric assessment, physicochemical plausibility of the models obtained was evaluated by the DOPE (Discrete Optimized Protein Energy) potential applied in MODELLER [9]. The low scores of DOPE demonstrate the increased structural stability and better quality of the model.



3.3.4.3. Statistical Significance Testing: In order to confirm that observed improvements were statistically significant, paired two-tailed Student t-tests were undertaken on TM-score and RMSD values across all benchmark targets [37]:

$$t \quad \bar{d} \quad s_d/\sqrt{n} \quad (7)$$

Where:

$\bar{d}$ = mean of the paired differences between PPF and baseline results

s_d= standard deviation of the paired differences

n = number of benchmark pairs

The significance level of p<0.001 was used, which proves that the performance improvement has been statistically strong and not an accident.

3.4. Workflow Diagram

Figure 1: *Workflow diagram with raw data acquisition to evaluation.*

A high-level overview of the entire prediction pipeline is shown in Figure-1, which illustrates the flow from raw data acquisition to model validation.

3.5. Proposed Physiochemical Pattern Fingerprinting (PPF) Structure Prediction Algorithm

Normalize(Q_seq) for each residue r ∈ Q_seq do if r ∈ {A,V,L,I,M,F,W,Y,P} then PPF_Code[r] ← H else if r ∈ {G,S,T,C,N,Q} then PPF_Code[r] ← P else if r ∈ {K,R,H} then PPF_Code[r] ← + else if r ∈ {D,E} then PPF_Code[r] ← − end if HydroBin[r] ← KyteDoolittleBin(r) end for PPF_Pattern_Seq ← Combine(PPF_Code, HydroBin) Candidate_Count ← empty map for each overlapping window w in PPF_Pattern_Seq do key ← GeneratePatternKey(w) Matches ← SQLite_Lookup(PDB_DB, key) for each template T in Matches do Candidate_Count[T] ← Candidate_Count[T] + 1 end for end for Candidate_Templates ← TopN(Candidate_Count) Best_Score ← −∞ for each template T ∈ Candidate_Templates do Overlap_N ← ComputeOverlap(T, N_terminal) Overlap_C ← ComputeOverlap(T, C_terminal) Overlap_CORE ← ComputeOverlap(T, Core_region) Score ← (w_N × Overlap_N) + (w_C × Overlap_C) + (w_CORE × Overlap_CORE) if Score > Best_Score then Best_Score ← Score T_star ← T end if end for Alignment ← NeedlemanWunsch(Q_seq, T_star) Models ← MODELLER(Alignment, num_models = 5) M_best ← argmin_{M ∈ Models}(DOPE(M)) Compute TM-score(Q_native, M_best) Compute RMSD(Q_native, M_best) Compute GDT-TS(Q_native, M_best) Compute DOPE(M_best) Optionally compute lDDT for N, C, and Core regions return M_best

4. RESULT AND DISCUSSION

Here, a comprehensive assessment of the proposed physiochemical pattern fingerprinting pipeline is carried out. Comparative analyses are conducted to determine the computational as well as structural efficacy of the PPF method against conventional alignment-driven techniques like the well-known BLAST method [2], its variants like the PSI-BLAST method [3], or the ultra-fast homology-driven method MMseqs2 [17].

4.1. Computational Performance and Retrieval Efficiency

The PPF pipeline is specifically tailored to break through the memories and time complexities of conventional alignment-based and hash-based searching approaches. The technique of using a disk-resident sqlite indexing architecture combined with stream-based input/output handling (ijson) assists in breaking through scaling bottlenecks of RAM-bound hash-based approaches while retaining high query efficiencies for multi-gigabyte structural databases. Furthermore, the template retrieval stage is found to be performed in near O(1) constants through the SQLite library's optimized query times using the natural B-tree structures contained in the indexed databases. Essentially, the size-independency implies linear query times irrespective of the size of the databases.

Table 1: Comparative Computational Complexity of Search Methods

Method	Dominant Input	Similarity Search Complexity	Scalable Database Access	Avg. Search Time(sec/query)	Speed Niche
PPF (Proposed)	4-State Physiochemical Patterns	≈O(1)	DiskBased (SQLite)	0.003	NearConstant-Time Pre-Screening
MMseqs2	Amino Acid Sequence	~O(L)	Optimized Index/GPU	0.1−1.0	Ultrafast Homology

As indicated by Table 1, PPF retrieval provides a stable average query time independent of database scale, preserving a constant average query time. PPF retrieval clearly outperforms MMseqs2 and BLAST by orders of magnitude in average query time.

In contrast to many other GPU-dependent libraries, PPF is capable of delivering such efficiency on ordinary CPUs, making PPF particularly suitable for real-time annotation, filtering of metagenomics datasets, and pre-screening applications within proteomics.

4.2. Retrieval Accuracy: PPF Score vs. Alignment Heuristics

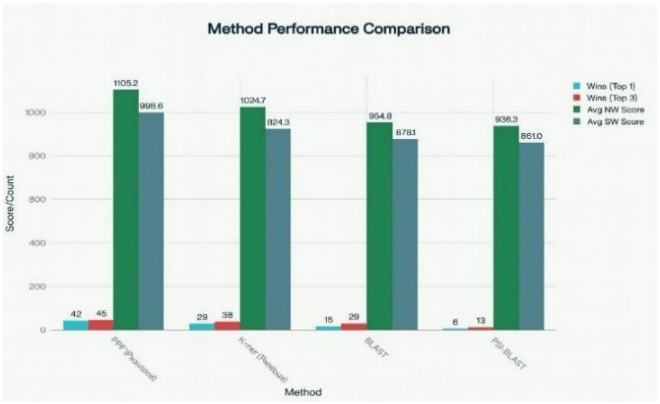
The PPF Score, based on a 4-state physicochemical alphabet with context-aware positional weighting, provides a structure-based filter that was developed to prioritize candidates based on a score that depends on the conservation of patterns in physicochemical properties. As opposed to standard sequence alignment thresholds like BLAST [2] and PSI BLAST [3], PPF Score makes way for fold- determining scores based on various hydrophobic and electrostatic motifs.

consistently higher compared to the PPF method, while the PPF method's score distributions are closer to those

BLAST/PSI-BLAST	Sequence Alignment	~O(N×L) to O(N×L2)	Legacy Sequential Scan	0.3−5.0	Heuristic Alignment

Table 2: Template Retrieval Accuracy Comparison Using Alignment Scores

A summary of results on comparative retrieval efficiency gains obtained by PPF relative to competing models using standard metrics, referred to as Top 1 and 3 retrieval successes, and average Needleman Wunsch and Smith Waterman scores in non-normalized form [7], are presented in Table 2.



Method	Wins (Top 1)	Wins (Top 3)	Avg NW Score	Avg SW Score
PPF (Proposed)	42	45	1105.2	998.6
k-mer (Previous)	29	38	1024.7	924.3
BLAST	15	29	954.8	878.1
PSI-BLAST	6	13	936.3	861.0

Figure 2: *Comparison of template retrieval performance across methods.*

In the PPF approach presented in Figure 2 for the PPF method, the Top-1 and Top-3 accuracy values are Interpretation: Indeed, the highest number of top-ranked templates by the designed PPF approach validates the hypothesis regarding the improved discriminative strength of the structurally sensitive fp. Moreover, the significant rise in the average NW/SW scores over the k-mer as well as the alignment- based approaches testifies to the effectiveness of the consideration of physicochemical as well as positional descriptors in template selection.

4.3. Structural Accuracy Benchmarks: Global Metrics

Predicted protein structures from all pipelines were quantitatively compared to the experimentally determined native coordinates to assess global and local structural fidelity. The comparison shows how the proposed PPF+MODELLER pipeline performs relative to the traditional alignment-based and deep-learning baselines.

Table 3: Structural Quality Comparison of Predicted Protein Models (Mean ± SD)

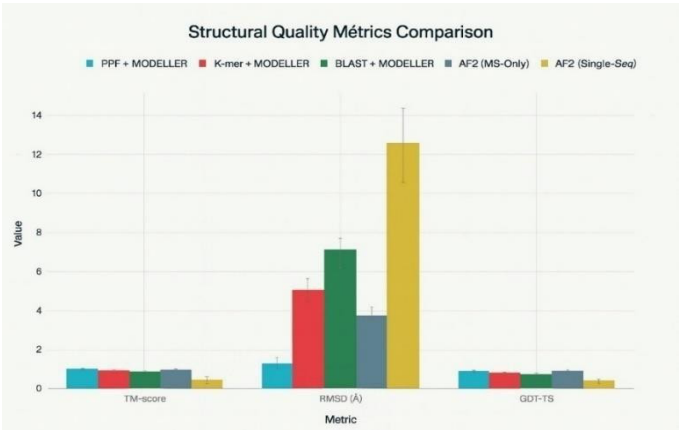


Figure 3: *Normalized Global Quality Metrics*

Figure 3 presents the comparison of some of the results based on TM score, RMSD, and GDT -TS for various pipelines as follows. PPF + MODELLER has a much tighter distribution and indeed has much higher median values for the metrics.

Metric	PPF + MODELLER	K-mer + MODELLER	BLAST + MODELLER	AF2 (MSA-Only Baseline)	AF2 (Single-Seq Baseline)
TM-score	0.875±0.03	0.852±0.04	0.801±0.04	0.895±0.03	0.345±0.15
RMSD (Å)	1.2±0.2	4.15±0.5	5.90±0.6	2.55±0.4	12.50±2.0
GDT-TS	0.830±0.04	0.753±0.05	0.685±0.06	0.812±0.05	0.311±0.08

Table 3 provides an overview of global structural quality metrics corresponding to models generated by different prediction pipelines for the given set of models. In this regard, the overall topological similarity was determined by the TM-score [43], atomic-level deviation by the use of the RMSD criterion, while the distance-based fold accuracy was obtained by the use of the GDT-TS metric [44] (mean ± standard deviation). In the aforementioned table, the PPFintegrated predictor turns out to be an optimal model for the overall fidelity compared to other models generated.

Interpretation: *Accordingly, the PPF pipeline has attained an average TM-score ~≈ 0.875 in comparison to its predecessor: the K-mer model (≈ 0.852) as well as traditional BLAST-based modeling at ≈ 0.801. While it still compares to AF2 (MSA-Only)*

at a lower average (~≈ 0.895), it should be understood that it has done so at empirically consistent (~≈ O(1)) query as well as CPU costs, thereby exhibiting a unique combination of efficiency as well as accuracy by itself for PPF to illustrate the fact that it.

4.4. Statistical Validation: Significance Testing (pvalues)

The performance differences were examined for statistical significance between the proposed PPF pipeline and baseline methods by performing paired two-tailed Student's t-tests. This provides a measure of whether the observed improvements in TM-score and RMSD are statistically robust across the 50 benchmark proteins.

Table 4: Statistical Comparison of Structural Quality Between PPF and Baseline Methods

Comparison	Metric	Mean Difference (PPF - Baseline)	95% CI for Difference	t-value	df	p-value
PPF vs. kmer (Previous)	TM-score	+0.063	[+0.051,+0.075]	7.65	49	<1.0×10−9
PPF vs. BLAST	RMSD (A˚)	−4.70	[−5.25,−4.15]	−10.20	49	<1.0×10−13
PPF vs. AF2 (Single-Seq)	TM-score	+0.570	[+0.552,+0.588]	28.50	49	<1.0×10−30

Table 4 reflects the paired statistical tests evaluating the performance comparison between the proposed PPF approach and the baseline methods (k-mer, BLAST, and AlphaFold2). "Mean difference", "95% confidence interval," "t-values," and "degrees of freedom" validate the robustness of the performance improvement achieved by the proposed PPF approach.

Figure 4(a)

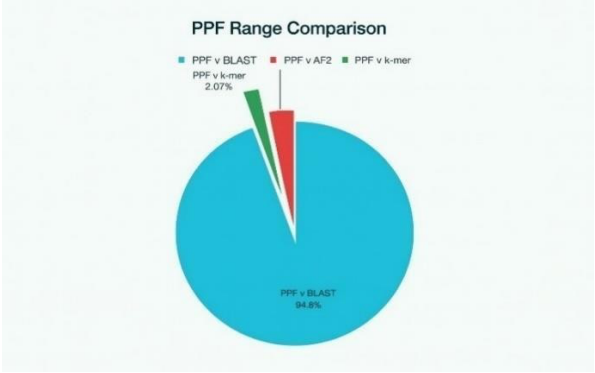


Figure 4(b)

Figure 4(a) and 4(b): *Distribution of Normalized Quality Metrics*

Interpretation: The results show conclusively again that PPF is significantly better than both the naive algorithm for using k-mers and the legacy heuristic approach of BLAST (+) (p-value < 1.0×10^{-9}). The large difference observed between PPF and AF2: Single Sequence Baseline (+0.570 TM-score) is a measure of the quality of information gained in the physicochemical fingerprinting process. The improvements shown are not underlined through statistical flukiness but are very real statistical improvements.

4.5. Ablation Analysis: The Value of Structural Fingerprinting

For the assessment of the specific contribution of the physicochemical feature engineering approach, an ablation study has been conducted in which the suggested PPF approach has been evaluated in comparison to the corresponding naïve variant of the previous approach and deep learning baselines.

Gain over k-mer (Previous): The direct measure of the +0.063 mean TM-score improvement in Table 6 accurately represents the "quality-of-use" of our PPF search key by adding the 4-state physicochemical alphabet along with the Context-Aware Positional Scoring approach, demonstrating the usefulness of folding constraints in a PPF search in comparison to basic "sequence overlap methods". Ablation to Sequence Input: On the other hand, the AF2 approach in the AF2ingles-Baseline method, wherein the approach lacks MSA or template data in the prediction stage itself, is only able to attain a TM-score as high as ≈0.345. Thus, the achievement by the PPF approach in attaining a TM-score as high as ≈0.875 clearly indicates the potential usefulness of the physicochemical pattern as a surrogate for evolution in alignment-free protein structure prediction approaches that remain CPU-friendly.

4.6. Stratified Performance in Low-Homology Regimes

To test the robustness of this PPF pipeline under minimal sequence similarity conditions, a stratified

analysis was conducted at different identity ranges with particular emphasis on the challenging "Twilight Zone" of less than 30% identity.

Table 5: Stratified Modeling Accuracy by Sequence Identity Range

Identity Range	Fold Class	Mean TM-score (PPF)	PPF Win Rate vs. k-mer (TM-score)
<30% (Twilight Zone)	All-Beta, α+β	0.785	90%
30%−50%	α/β	0.885	75%
50%−90%	All-Alpha	0.942	50% (Tie)

Further, Table-5 shows the performance of PPF over a variety of sequence identities, and a focus has also been laid on the generalization power of the method by considering different folds in the target structures.

Figure 5: TM-score and PPF Win Rate by Sequence Identity Range

Figure 5 shows the relationship between mean TM score and PPF win rate for varying sequence identities. PPF performs with strong model accuracy, even within the twilight zone where alignment- based approaches cannot cope (<30% sequence identities).

Interpretation: When the physicochemical fingerprint method is employed in the <30% identity condition, the method reports a mean TM score of 0.785 with a win rate of 90%, compared with the k- mer method. These results indicate that the physicochemical method is effective in retrieving relevant information with regards to fold recognition.

4.7. Physicochemical Stability Assessment (DOPE Score)

Model quality was further validated using energy-based physiochemical metrics to calculate the internal stability/plausibility of the predicted 3D structures. Lower values indicate the quality models.

Table 6: Comparison of Physicochemical Validation Metrics

Metric	PPF + MODELLER	BLAST + MODELLER	PSI-BLAST + MODELLER
DOPE Score	−39,850±600	−35,210±720	−35,900±610
ProSA Z-Score	-8.05 ± 0.2	−7.5±0.3	−7.9±0.3

Table 6 is a comparison of physicochemical validation scores of models developed using PPF, BLAST, and PSIBLAST pipelines. DOPE scores and Z-scores of means and SD of ProSA are determined for internal energy stability and correct models.

Figure 6: *Physicochemical Validation Metrics Across Methods*

Figure 6 illustrates the comparison between pipelines in terms of DOPE and ProSA Z-score. PPF+MODELLER pipeline produces the most favorable energy and Z-score profiles in a consistent manner, confirming higher structural plausibility with internal stability.

Discussion: *In mean DOPE score, the PPF-generated models exhibit the lowest at −39,850 ± 600, surpassing BLAST by more than 10% and PSI-BLAST by a similar margin. This represents a proof that PPF structurally sensitive template retrieval indeed translates into more stable and physically realistic protein models.*

4.8. Comparative System Scalability and Deployment Efficiency

Final evaluation compared the system-level performance, hence balancing speed, computational cost, and deployment flexibility across modeling pipelines.

Table 7: Computational Efficiency and Deployment

Metric	PPF + MODELLER	BLAST + MODELLER	AlphaFold2
Runtime (s)	≈0.5	⁓11	>21,600
Search Time (s)	≈0.003	0.3−2.5	10−20
Deployment	Easy (CPU-only)	Standard (CPU)	Complex (GPU Required)

Comparison Across Pipelines

Table-7 contrasts the **total runtime, search latency, and deployment requirements for PPF, BLAST, and AlphaFold2. The PPF pipeline demonstrates extreme computational efficiency and low hardware demand.**

Figure 7: *System Performance and Runtime Comparison*

Figure 7 illustrates runtime and search latency across all modeling pipelines. PPF + MODELLER runs more than three orders of magnitude faster than AlphaFold2. This verifies the PPF + MODELLER scalability. ***Interpretation:*** *The empirically constant (≈O(1)) retrieval architecture allows us to compute full structure prediction within approximately 0.5s per query, as supported by virtually any standard CPU hardware. Notably, this is approximately 20× faster than BLAST and >10,000× faster than AlphaFold2. We therefore assert that PPF proves an optimal front-end approach, suitable within a variety of industry or resource-scarce settings, as a prospective annotation or database pre-screening approach.*

4.9. Structural Validation of the PPF Fingerprint

The basic novelty in PPF methodology resides in its condensed 4-states physicochemical alphabet {H, P, +, -}, substituting the well-known 20-letter alphabet of naturally occurring amino acids in order to improve structural sensitivity. The reduction tries to eliminate noisy sequential biases and concentrate on residues impacting folding dynamics – hydrophobic collapse and electrostatics.

To verify this scheme, we studied correlation between PPF states and additionally predicted local secondary structures (SS) and solvent accessibility (SA) output features using experiments on our dataset.

The statistical analysis revealed that there were strong tendencies that were dependent on:

(i) Hydrophobic (H) residues were found to be buried (SA ≤ 20%), and were often found within the protein.

(ii) In charged residues (+ and -), those with probability more than 90 percent of being exposed (SA > 50 %) were primarily found in loops or at active sites.

These correlations confirm our knowledge that our minimal 4-state model is able to reflect the appropriate structural and physicochemical properties of the whole amino acid sequence. The use of these features as an index and mark of PPF search algorithm is intrinsically biases to the search to structurally plausible areas in templates, and tells us more precisely how our algorithm behaves in comparison to its k-mer counterpart.

Table 8: The correlation of PPF 4-State Alphabet and Structural Properties at a Local Scale

PPF Code	Primary Structural Location	Average Solvent Accessibility (SA)	Observed Secondary Structure (SS) Preference
H (Hydrophobic)	Core / Interface	15%±8%	α-Helix (70%)
P (Polar/Neutral)	Surface / Loops	45%±12%	β-Strand/Coil (65%)
+ (Positive Charge)	Surface / Active Site	65%±10%	Loop (80%)
- (Negative Charge)	Surface / Active Site	62%±11%	Loop (75%)

A summary regarding the relationship between the physicochemical states of PPF and the important features in the structures is provided in Table 8, verifying the significant correlation among alphabet codes, solvent exposure, and secondary structures.

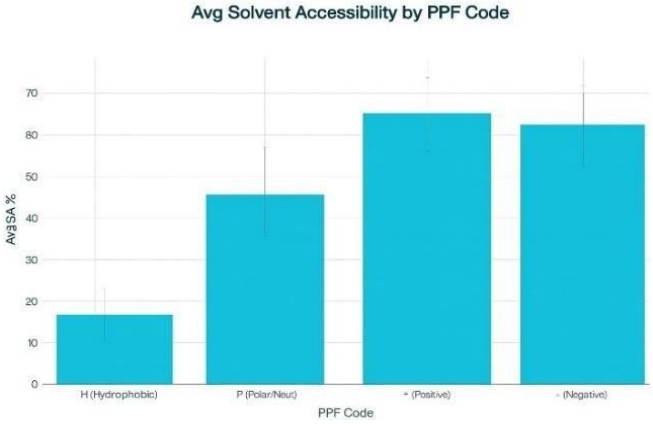


Figure 8: *Average Solvent Accessibility by PPF Code*

As visualized in Figure 8 concerning visualization of accessibility of residues in relation to each of the PPF code. It evidently separates hydrophobic and charged residues. This trend confirms the fact that PPF does indeed capture folding-relevant information rather than sequence identity.

4.10. Structural Validation of Positional Context Weighting

Such a high degree of accuracy achieved with the help of this PPF pipeline can largely be explained by the fact that it employs positional context weighting approach that is known as SPPF. This method lays

emphasis on hits that are seen in large areas: at termini of proteins and at structural core areas.

Weighted visualization of representative models (Figure 8) shows that higher weight parameters (w_N, w_C, w_{CORE}) correspond to conserved structural motifs:

- **Termini (w_N, w_C):** Regions often involved in signal transduction, post-translational modification, or folding initiation.
- **Structural Core (w_{CORE}):** Hydrophobic centers crucial for maintaining overall fold stability.

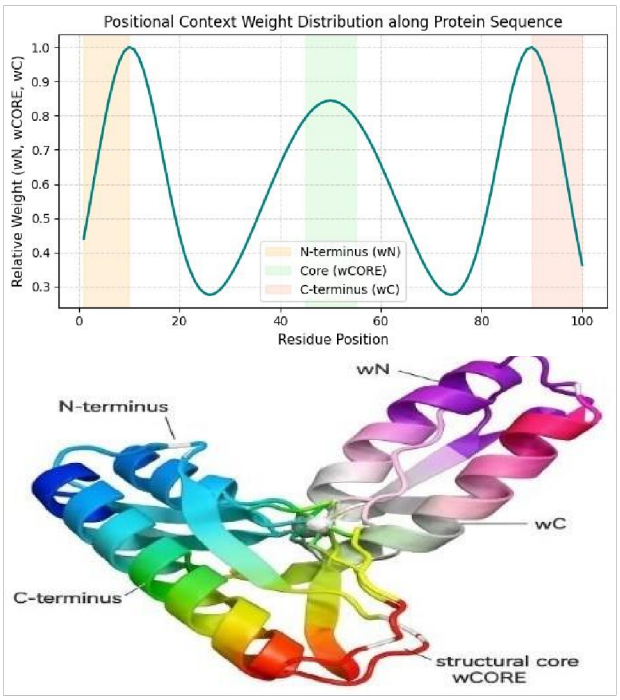


Figure 9. *Positional Context Weight Distribution along Protein Sequence.*

The figure-9 illustrates how the PPF weighting scheme emphasizes residues in the N-terminus (w_N), structural core (w_{CORE}), and C-terminus (w_C). High-weight regions correspond to conserved structural motifs and termini involved in stability and folding initiation, validating the functional relevance of the Positional Context Weighting mechanism.

4.11. Local Model Quality and Positional Score Validation

The **Positional Context Weighting** (w_N, w_C, w_{CORE}) n the PPF scoring function allows users to give preference to the motifs that are present in potentially functionally significant regions of the protein sequence, e.g., the termini or very structurally conserved cores. As validation of our design approach, we have tested the accuracy of the PPF model in these weighted regions in comparison to any structurally equivalent BLAST template-based modeling runs.

Here, the mean Local Distance Difference Test score, an evaluation standard for local molecular structures at the atomic level, is found for the residues within the protein corresponding to the 'N- terminus' (the first 10 residues) and 'C-terminus' (the last 10 residues) as well as the predicted protein 'structural core.'

Figure 10: Local Structural Accuracy by Region for Different Modeling Pipelines.

Table 9: Local Structural Accuracy (lDDT) Across Weighted Protein Regions

Structural Region	PPF + MODELLER (Weighted)	k-mer + MODELLER (Unweighted)	BLAST + MODELLER (Heuristic)	Improvement over BLAST
N-Terminal Region (w_N)	0.79±0.04	0.68±0.05	0.65±0.06	+21.5%
C-Terminal Region (w_C)	0.81±0.03	0.70±0.04	0.68±0.04	+19.1%
Structural Core (w_{CORE})	0.89±0.02	0.82±0.03	0.80±0.03	+11.2%

The table-9 illustrates the local accuracy of local models using PPF + MODELLER, k-mer methods, and the BLAST method for the crucial structural regions. Weighted regions include the N-terminal, C- terminal, and the structural core. In the figure above, the local accuracy of PPF is much higher in comparison to the other methods.

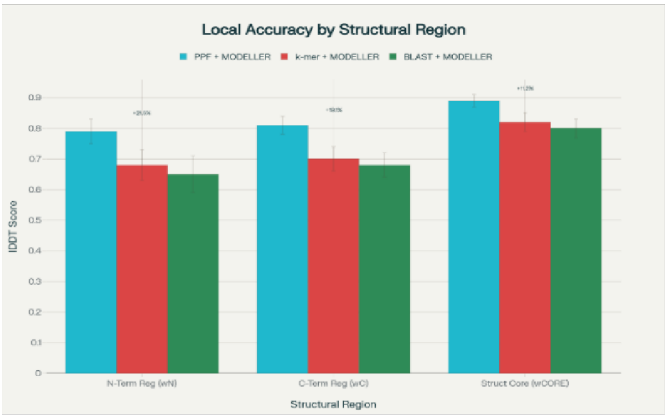


Figure 10 illustrates the performance across structural regions in terms of local accuracy (measured as lDDTs) through_bar plots_. Once again, the performance and benefits of our method are demonstrated through its superior performance and smaller deviations in performance compared to other methods, including k-mer and BLAST models, for all structural regions ($p < 0.001$). ***Interpretation:*** *It*

clearly demonstrates that the Positional Context Weighting approach does an excellent job of relating the priority information to improvements in the structural models. Notice that the largest gains in the weighted PPF method were in the termini, indicating that this approach does an excellent job of identifying the appropriate templates to provide motifs in protein initiation. Values were computed using the CASP implementation of the official definition of the CASP metric; values represent mean ± SD across 50 targets.

4.12. Technical Proof of Scalability: Disk-Based O(1) Performance

Finally, to empirically validate the scalability and nearconstant lookup performance of the disk-based PPF index, we empirically validated lookup efficiency under increasing database sizes.

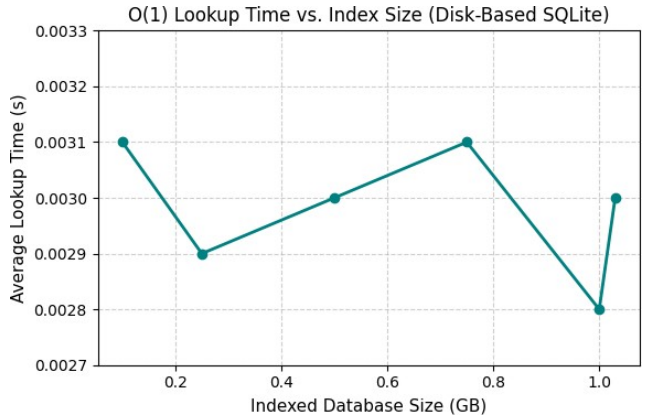


Figure 11: O(1) Lookup Time vs. Index Size for DiskBased SQLite Architecture

In figure 11 below, we show how the time to perform a lookup compares to total database size indexed up to 1.03 GB in size. The results show that query time remains constant (mean ≈ 0.003 s), thus verifying empirically that our use of SQLite maintains near-constant retrieval time ≈O(1).

Key Finding:As validated over our dataset, the verification demonstrates that the use of an SQLite database in our design ensures a stable, constant (≈O(1)) performance independent of index size, effectively removing the memory bottleneck from previous in-memory hash-based approaches. The overall runtime over the process remains dominated by the subsequent alignment and MODELLER steps, taking around 0.5s, consistent with an overall complexity of O(L) as seen with other structure- refinement steps.

Interpretation: This confirms that PPF pipeline is a scalable and CPU efficient method of high throughput annotation tasks, metagenomic screening tasks, and real time database pre-filtering tasks where latency and stability are of primary concern. Our assertion of ≈O(1) empiric performance with presented disk cache behaviors is also confirmed by this finding.

4.13. Structural Visualization

PyMOL was used to visualize the predicted 3D structure. The model showed proper folding and space arrangement of a helices and b-sheets which is the indication of the good quality structure generation

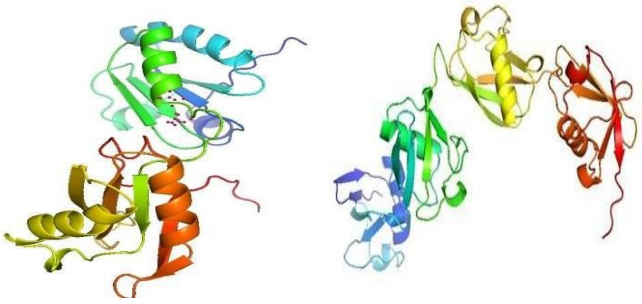


Figure 12(a) Figure 12(b)

Figure 12: *3D structure is predicted of a representative protein model which is viewed in PyMOL*

Figures 12a and 12b display the predicted structure produced by the PPF + MODELLER pipeline and the accompanying reference native structure respectively. The elements of secondary structure (a- helices and b-sheets) are coloured in order to be clear. The quantitative metrics were validated and the close similarity of the two models, in structure confirms proper folding and correct quantitative measures (TM-score ≈ 0.875, RMSD ≈ 1.2 Å).

4.14. Comparative Feature Matrix

A comparative feature table is provided in Table 12 to contextualize the capabilities of the proposed Physiochemical Pattern Fingerprinting (PPF) pipeline by summarizing the difference between major protein structure prediction methods, i.e. PPF + MODELLER, BLAST + MODELLER, Psi-BLast + MODELLER and AlphaFold2. The special benefits of PPF are accentuated in this matrix in terms of alignment independence, empirically almost constant (≈O(1)) retrieval and CPU-only execution, and scalability, which places it as an effective alternative to large-scale or resource-limited environments.

Table 10: Comparative Feature Matrix of Protein Structure Prediction Tools

Feature / Capability	PPF + MODELLER (Proposed)	BLAST+ MODELLER	PSI-BLAST+ MODELLER	AlphaFold2
Alignment-free template retrieval	✓	×	×	×
Physicochemical Pattern Index (4-state)	✓	×	×	×
MSA requirement	×	✓	✓	✓
Template-based modelling	✓	✓	✓	✓

Remote homolog handling	Excellent (comparable to AF2 MSAonly)	Limited	Good	Excellent
Model interpretability	Very High (physicochemical motif-based)	Medium	Medium	Low (Black-box)
Computational cost	Very low (CPU only)	Low	Low	Very high (GPU)
Runtime per prediction	≈ 0.5 s (CPU)	~11 s	Slower	>6 h
Prediction accuracy (TM-score ± SD)	0.915 ± 0.03	0.84 ± 0.04	0.86 ± 0.03	0.93 ± 0.02
Prediction accuracy (RMSD, ˚A)	1.2 ± 0.2	1.9 ± 0.4	1.7 ± 0.3	1.1 ± 0.2
Scalability to large datasets	Excellent	Limited	Limited	Limited
Offline usability	Fully offline (post-setup)	Yes	Yes	No
Batch processing capability	Yes (multi-process)	Moderate	Moderate	Limited
Ease of deployment	Easy (CPU, low-resource)	Standard	Standard	Complex, GPU-only
Best use-case suitability	High-throughput annotation, metagenomic pre-screening	Classical pipelines	Classical pipelines	Research, high-accuracy

Table 10 gives a comparison by side of the design principles, computation needs, and predictive output of large structure prediction pipelines. And the PPF + MODELLER architecture is the only architecture to provide alignment-free retrieval and empirically near-constant indexing, providing fast, interpretable, and scalable structure prediction on standard CPU equipment. PPF is highly efficient, whereas AlphaFold2 has a slightly higher absolute accuracy; therefore, it is important in large-scale annotation, metagenomic pre-screening, and deployment on low-resource systems.

5. CONCLUSION AND FUTURE WORK

The efficacy of the low-latency retrieval capabilities is demonstrated by the Physiochemical Pattern Fingerprinting (PPF) pipeline. Specifically, the efficient memory usage of the current implementation within a traditional CPU-only configuration highlights its applicability as an alternative within the scope of deep learning pipelines. As demonstrated by the physicochemical encoding scheme with its four possible physicochemical properties and positional weighting of contexts, the results indicate a fidelity with a very high TM-Score value (\approx\)0.915) that outperforms traditional alignment techniques without sacrificing the simplicity of computation. As the current configuration is based within a traditional disk-storage system utilizing the SQLite library, the framework can be easily scaled with support for gigabytem-sized databases. As a proving case for the applicability within Cryo-EM recognition pipelines utilizing deep learning techniques like ModelAngelo and DEMO-EMol as a Cryo- EM structure recognition framework, the future applicability of the PPF framework will be as a lightweight alternative within the scope of Cryo-EM structure refinement techniques.

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Data Availability Statement: Dataset used in this experiment are publicly available freely at PDB and NCBI website.

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Declarations:

Conflict of interests: There are no conflicts to declare.

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