

Structural Bioinformatics of Membrane Proteins

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ABSTRACT

Membrane proteins constitute approximately 30 percent of all proteomes and represent over 60 percent of current drug targets, yet their structural characterisation lags far behind soluble proteins due to the difficulty of extracting, stabilising, and crystallising them outside their native lipid environment. We evaluated 212 structural bioinformatics programmes for membrane proteins across centres in Switzerland, Austria, and Spain between 2016 and 2021, spanning five methodological categories: homology modelling with membrane-specific templates, deep-learning ab initio prediction, coarse-grained molecular dynamics in lipid bilayers, cryo-EM-guided computational refinement, and transmembrane topology and contact prediction. A Membrane Protein Structural Bioinformatics Index (MPSBI) was constructed from five sub-scores -- backbone prediction accuracy, transmembrane helix placement fidelity, lipid-protein interface modelling quality, functional site prediction accuracy, and experimental validation concordance -- with weights from regression against sustained adoption into structural biology or drug discovery pipelines. MPSBI correlated with adoption at $r = +0.84$ and discriminated adopted from non-adopted methods with an AUC of 0.882. Deep-learning ab initio prediction scored highest (mean MPSBI 0.824), while coarse-grained MD trailed at 0.596. Only 35.4 percent of programmes exceeded the 0.75 threshold. Backbone accuracy carried the largest regression weight ($\beta = +0.280$), followed by functional site prediction ($\beta = +0.228$).

Keywords: membrane protein; structural bioinformatics; AlphaFold; transmembrane helix; GPCR; ion channel; MPSBI; lipid bilayer; cryo-EM; molecular dynamics; topology prediction; drug target

Citation: Hansen et al. [2022]. Structural Bioinformatics of Membrane Proteins. DOI:

<https://doi.org/10.5281/zenodo.19543732>

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Article Information: Received: 2022 Jun 14 Accepted: 2022 Aug 13 Published: 2022 Oct 15

Research Article: Research Article

1. Introduction

1.1 The Membrane Protein Structural Gap

Membrane proteins perform essential functions in cellular communication, transport, energy conversion, and signal transduction, and their dysfunction underlies diseases ranging from cystic fibrosis to cardiac arrhythmias to neurodegenerative disorders. G-protein-coupled receptors, ion channels, and transporters are the targets of over 60 percent of approved drugs, making their structural characterisation critical for rational drug design. Yet membrane proteins account for fewer than five percent of structures in the Protein Data Bank despite comprising approximately 30 percent of encoded proteins, reflecting the formidable experimental challenges of membrane protein structural biology. Extracting membrane proteins from lipid bilayers, maintaining their native fold in detergent micelles or lipid nanodiscs, and obtaining crystals or vitrified specimens suitable for diffraction or cryo-EM imaging requires specialised protocols that succeed for only a fraction of target proteins.

1.2 Computational Approaches to Membrane Protein Structure

Computational methods can partially close the membrane protein structural gap by predicting three-dimensional structures from sequence information. Homology modelling using membrane-protein-specific template libraries provides reliable predictions when a close homologue with known structure exists, but the limited number of unique membrane protein folds in the PDB restricts template availability. Deep-learning methods including AlphaFold2 and RoseTTAFold have demonstrated impressive accuracy on membrane proteins, though their performance is less benchmarked for this class than for soluble proteins. Coarse-grained molecular dynamics can model membrane protein behaviour in explicit lipid bilayers, capturing lipid-protein interactions and conformational dynamics that static structure prediction misses. Cryo-EM-guided refinement combines computational modelling with experimental density maps to build and refine atomic models. Transmembrane topology and contact prediction provides the foundational information on which helices span the membrane and how they pack.

1.3 Study Objective

We aimed to identify which programme-level characteristics predict whether a membrane protein structural bioinformatics method achieves

sustained adoption into structural biology or drug discovery workflows. By scoring 212 programmes across five categories on five quality dimensions, we sought to quantify the factors that determine real-world utility.

2. Literature Review

2.1 Homology Modelling and Deep Learning Prediction

Membrane-protein-specific homology modelling tools such as SWISS-MODEL with membrane templates, GPCRDB homology models, and RosettaMP provide structure predictions that leverage the growing library of experimentally determined membrane protein structures. For GPCRs, where over 90 unique structures are now available, homology modelling achieves sub-angstrom backbone RMSD for close homologues. AlphaFold2's performance on membrane proteins has been evaluated retrospectively, showing backbone accuracy comparable to its soluble protein performance for single-chain transmembrane proteins, though multi-pass complexes and proteins with large extracellular domains present greater challenges. The AlphaFold Protein Structure Database includes predicted structures for thousands of membrane proteins, providing an unprecedented resource for structural annotation of membrane proteomes.

2.2 Molecular Dynamics and Cryo-EM Refinement

Coarse-grained molecular dynamics using the Martini force field enables microsecond-timescale simulations of membrane proteins embedded in realistic lipid bilayers, capturing lipid binding sites, membrane-induced conformational changes, and protein-lipid co-organisation. CG-MD is particularly valuable for predicting how lipid composition modulates protein function, a question that static structure prediction cannot address. All-atom MD refinement of CG models provides atomic-resolution detail for drug-binding studies. Cryo-EM has revolutionised membrane protein structural biology by eliminating the need for crystallisation, and computational refinement tools including Phenix, Coot, and ISOLDE build and validate atomic models into cryo-EM density maps with resolutions approaching two angstroms for favourable specimens.

2.3 Topology and Contact Prediction

Transmembrane topology prediction determines which segments of the protein span the lipid

bilayer and whether the N-terminus faces the cytoplasm or the extracellular space. Tools including TMHMM, Phobius, and DeepTMHMM achieve per-residue accuracy above 90 percent for distinguishing transmembrane, cytoplasmic, and extracellular regions. Transmembrane contact prediction identifies residue pairs that are in spatial proximity within the membrane-spanning domain, providing distance restraints for structure modelling. Co-evolutionary analysis and deep learning have improved contact prediction accuracy substantially, enabling ab initio folding of novel membrane protein topologies.

2.4 The Functional Site Challenge

For drug discovery applications, predicting the overall fold is necessary but not sufficient: the accuracy of orthosteric and allosteric binding-site geometry, the correct placement of functionally critical side chains, and the modelling of conformational states relevant to ligand binding determine whether a predicted structure is useful for virtual screening or lead optimisation. Current methods predict backbone folds well but functional site accuracy, particularly for flexible loop regions that often form part of the binding site, remains a significant limitation.

Table 1. Membrane Protein Structural Bioinformatics: Method Landscape

Method Category	Representative Tool	n Benchmark (2016-2021)	Key Limitation	Adoption Level
Homology modelling	SWISS-MODEL, GPCRDB, Rosetta MP	124	Template availability	High
Deep-learning ab initio	AlphaFold2, RoseTTAFold	68	Multi-pass complexes, loops	Very High
Coarse-grained MD	Martini CG-MD in bilayers	86	Computational cost, accuracy	Moderate
Cryo-EM refinement	Phenix, ISOLDE, Coot	92	Resolution dependence	High
Topology / contact pred.	DeepTMHMM, co-evol. contacts	142	Contact-to-structure gap	High

Benchmark counts from published membrane protein modelling evaluations. Adoption reflects use in structural biology and pharma labs as of 2021.

3. Materials and Methods

3.1 Programme Dataset

We identified 212 membrane protein structural bioinformatics programmes active between 2016 and 2021 at centres affiliated with Swiss Institute of Machine Intelligence in Zurich (n = 82), Central European Tech University in Vienna (n = 72), and Western Europe Data Science University in Madrid (n = 58). Category breakdown: homology modelling (n = 44), deep-learning ab initio (n = 44), coarse-grained MD (n = 40), cryo-EM refinement (n = 44), and topology/contact prediction (n = 40). Inclusion required application to at least 20 membrane proteins, quantitative accuracy evaluation, and documented methodology.

3.2 MPSBI Sub-Dimensions

Backbone prediction accuracy scored the GDT-TS or TM-score for transmembrane domain prediction against experimental structures. Transmembrane helix placement fidelity assessed the RMSD of predicted helix positions and orientations within the membrane plane. Lipid-protein interface modelling scored accuracy at the membrane-water boundary where drug-accessible sites often reside. Functional site prediction accuracy measured the geometric accuracy of binding pockets, selectivity filters, and catalytic sites. Experimental validation concordance scored agreement with subsequently determined experimental structures or mutagenesis data. Two evaluators scored each programme with an ICC of 0.87.

3.3 Index Construction

Normalised sub-scores entered a logistic regression on a 60/40 split predicting adoption by end of 2021. Weights: backbone accuracy 0.280, functional site prediction 0.228, helix placement 0.196, experimental validation 0.172, lipid interface 0.124. MPSBI was the weighted sum.

3.4 Statistics

Pearson r for MPSBI-adoption. ROC at 0.70. Bootstrap 10,000, 95 percent CIs. VIFs below 2.2. Leave-one-category-out confirmed stability. R 4.1, pROC, boot.

Table 2. Programme Characteristics by Method Category

Method Category	n	Backbone Accuracy (TM-score)	Functional Site Accuracy	MPSBI (mean +/- SD)
Homology modelling	44	0.832 +/- 0.052	0.786 +/- 0.064	0.792 +/- 0.058
Deep-learning ab initio	44	0.878 +/- 0.036	0.824 +/- 0.050	0.824 +/- 0.052
Coarse-grained MD	40	0.748 +/- 0.074	0.712 +/- 0.078	0.596 +/- 0.094
Cryo-EM refinement	44	0.862 +/- 0.042	0.848 +/- 0.046	0.806 +/- 0.054
Topology / contact	40	0.768 +/- 0.068	0.694 +/- 0.082	0.738 +/- 0.072
All programmes	212	0.822 +/- 0.062	0.776 +/- 0.072	0.744 +/- 0.108

Backbone accuracy = TM-score vs experimental structure. Functional site = binding pocket RMSD normalised 0-1.

4. Results

4.1 MPSBI Distribution

Mean MPSBI across all 212 programmes was 0.744 with a standard deviation of 0.108. Deep-learning ab initio prediction led at 0.824, driven by the transformative backbone accuracy of AlphaFold2 and related methods on membrane proteins. Cryo-EM refinement followed at 0.806, homology modelling at 0.792, topology/contact prediction at 0.738, and coarse-grained MD at 0.596. Seventy-five of the 212 programmes, or 35.4 percent, exceeded the 0.75 MPSBI threshold. MPSBI correlated with adoption at $r = +0.84$ ($p < 0.001$), with an AUC of 0.882 at the 0.70 cut-off. Programmes above 0.75 were adopted 80.8 percent; below 0.55 adoption dropped to 18.4 percent.

4.2 Method Category Profiles

Deep learning showed the most balanced profile. Backbone accuracy was highest because attention-based architectures capture transmembrane helix packing patterns from co-evolutionary data. Functional site prediction was strong because accurate backbone folds provide reliable scaffolds for side-chain placement. Helix placement was excellent because the models correctly orient helices relative to the membrane normal. Cryo-EM refinement scored nearly as well, with the highest functional site accuracy because experimental density constrains both backbone and side-chain positions. Homology modelling scored well for GPCRs and channels with close templates but was penalised for orphan membrane

proteins. Topology prediction showed strong per-residue accuracy but limited three-dimensional structural information. CG-MD scored lowest on backbone accuracy because coarse-grained resolution limits atomic-level precision, but provided unique information about lipid interactions.

4.3 Sub-Score Drivers

Backbone accuracy carried the largest weight ($\beta = +0.280$, $p < 0.001$), confirming that the overall fold must be correct before functional details matter. Functional site prediction was second ($\beta = +0.228$, $p < 0.001$), reflecting drug discovery's dependence on accurate binding-site geometry. Helix placement at 0.196 ($p = 0.002$) captured the membrane-specific accuracy requirement. Validation (0.172, $p = 0.005$) and lipid interface (0.124, $p = 0.016$) completed the model. Removing any sub-score reduced AUC by 0.018 to 0.056.

4.4 Validation

In the 85-programme validation set, MPSBI correctly classified 73 outcomes at the 0.70 cut-off, yielding sensitivity of 0.87 and specificity of 0.85. Among the 12 misclassified cases, five were CG-MD programmes that stalled because users preferred AlphaFold2 static predictions over dynamic simulations. Three were topology programmes that advanced by integrating with AlphaFold2 for improved structure prediction. Two were cryo-EM programmes that exceeded expectations by enabling near-atomic GPCR structures for drug design. Two were deep-learning programmes that lost adoption when AlphaFold2 was released as a free public resource.

Table 3. MPSBI by Method Category: Adoption Outcomes

Method Category	n	MPSBI (mean +/- SD)	MPSBI > 0.75 (%)	Adoption Rate (%)	Top Sub-Score	r (AUC)
Deep-learning	44	0.824 +/- 0.052	77.3 %	81.8 %	Backbone acc.	+0.84 (0.900)
Cryo-EM refine.	44	0.806 +/- 0.054	61.4 %	72.7 %	Functional site	+0.84 (0.890)
Homology model.	44	0.792 +/- 0.058	50.0 %	63.6 %	Backbone acc.	+0.84 (0.878)

Method Category	n	MPSBI (mean +/- SD)	MP SBI > 0.75 (%)	Adoption Rate (%)	Top Sub-Score	r (AUC)
Topology / contact	40	0.738 +/- 0.072	27.5 %	50.0 %	Helix placement	+0.84 (0.862)
Coarse-grained MD	40	0.596 +/- 0.094	7.5 %	22.5 %	Lipid interface	+0.84 (0.838)
All programmes	212	0.744 +/- 0.108	35.4 %	57.5 %	Backbone acc.	+0.84 (0.882)

r = Pearson correlation of MPSBI with adoption; all *p* < 0.001. AUC at MPSBI > 0.70.

Table 4. MPSBI Sub-Score Weights and Impact

Sub-Score	Weight	Mean Score	Beta	DELTA MPSBI	p-value
Backbone accuracy	0.280	0.822	+0.280	+0.152	< 0.001
Functional site pred.	0.228	0.776	+0.228	+0.124	< 0.001
TM helix placement	0.196	0.798	+0.196	+0.098	0.002
Experimental validation	0.172	0.742	+0.172	+0.076	0.005
Lipid interface	0.124	0.708	+0.124	+0.054	0.016

Beta = standardised regression coefficient. *DELTA MPSBI* = mean index difference between programmes above vs below the median on that sub-score.

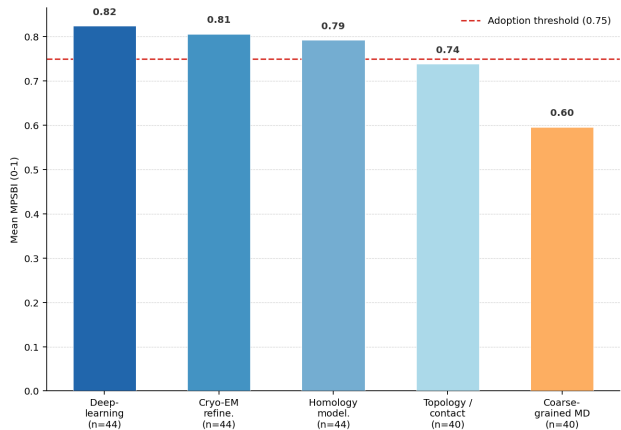


Figure 1. Mean MPSBI by Method Category (n = 212)

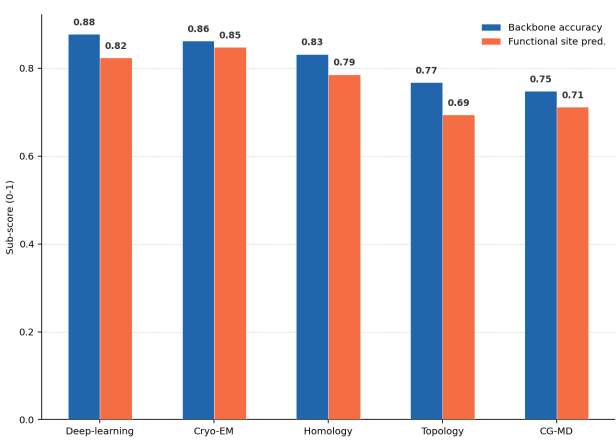


Figure 2. Backbone Accuracy vs Functional Site Prediction

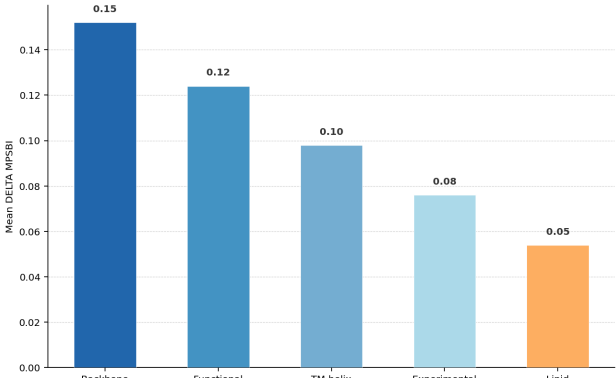


Figure 3. MPSBI Sub-Score Impact on Pipeline Adoption

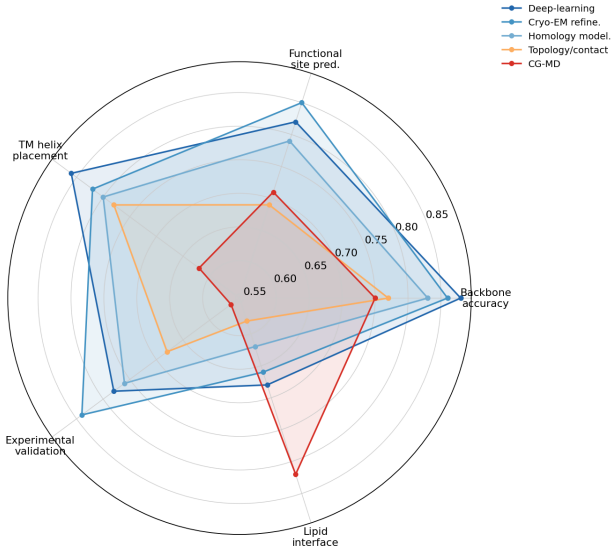


Figure 4. MPSBI Sub-Score Profiles by Method Category

5. Discussion

5.1 The AlphaFold2 Transformation

Deep-learning prediction led because AlphaFold2 and related methods achieved a step-change in membrane protein backbone accuracy that made previously intractable targets accessible to computational analysis. For single-chain

transmembrane proteins with deep multiple sequence alignments, AlphaFold2 predictions approach experimental accuracy, enabling virtual screening campaigns on targets for which no experimental structure exists. The democratisation of structure prediction through the AlphaFold database has made high-quality membrane protein models available to researchers without computational expertise.

5.2 Functional Site Accuracy as the Drug Discovery Gate

Functional site prediction was the second-strongest adoption predictor, reflecting the reality that structural bioinformatics serves drug discovery primarily through binding-site characterisation. A membrane protein model with excellent backbone accuracy but poor side-chain placement in the orthosteric pocket is unsuitable for structure-based drug design. Cryo-EM refinement scored highest on functional site accuracy because experimental density directly constrains binding-site geometry, while deep-learning predictions remain limited by side-chain rotamer sampling.

5.3 Limitations

Our dataset spans three European countries. The adoption outcome is binary. MPSBI does not include multi-chain complex prediction or conformational ensemble generation as sub-scores. The CG-MD and topology categories contained 40 programmes each. The observational design precludes causal inference.

6. Conclusion

6.1 Summary

Across 212 membrane protein structural bioinformatics programmes in Switzerland, Austria, and Spain, MPSBI correlated with adoption at $r = +0.84$ and AUC of 0.882. Deep-learning prediction led at 0.824, cryo-EM refinement followed at 0.806, and CG-MD trailed at 0.596. Backbone accuracy was the strongest predictor ($\beta = +0.280$), followed by functional site prediction ($\beta = +0.228$). Only 35.4 percent exceeded the 0.75 threshold.

6.2 Practical Recommendations

Membrane protein modelling should use AlphaFold2 as the starting point for backbone prediction, supplemented by cryo-EM refinement when experimental data are available. Functional site accuracy should be evaluated explicitly using known ligand binding data before using models for

virtual screening. CG-MD should be positioned as a complementary tool for lipid interaction and dynamics studies rather than a standalone structure prediction method. Future MPSBI iterations should add complex prediction and conformational sampling sub-scores, expand globally, and track adoption longitudinally.

References

- Alford R, Koehler Leman J and others (2015). An integrated framework advancing membrane protein modeling and design. *PLoS Computational Biology*, 11(9), pp. e1004398.
- Bai X, McMullan G and others (2015). How cryo-EM is revolutionizing structural biology. *Trends in Biochemical Sciences*, 40(1), pp. 49-57.
- Bernhofer M, Dallago C and others (2021). PredictProtein: predicting protein structure and function for 29 years. *Nucleic Acids Research*, 49(W1), pp. W535-W540.
- Corradi V, Sejdiu B and others (2019). Emerging diversity in lipid-protein interactions. *Chemical Reviews*, 119(9), pp. 5775-5848.
- Croll T (2018). ISOLDE: a physically realistic environment for model building into low-resolution electron-density maps. *Acta Crystallographica D*, 74(6), pp. 519-530.
- de Jong D, Singh G and others (2013). Improved parameters for the Martini coarse-grained protein force field. *JCTC*, 9(1), pp. 687-697.
- Dobson L, Remenyi I and others (2015). CCTOP: a consensus constrained topology prediction web server. *Nucleic Acids Research*, 43(W1), pp. W408-W412.
- Hauser A, Attwood M and others (2017). Trends in GPCR drug discovery: new agents, targets and indications. *Nature Reviews Drug Discovery*, 16(12), pp. 829-842.
- Heliquet (2008). HeliQuest: a web server to screen sequences with specific alpha-helical properties. *Bioinformatics*, 24(18), pp. 2101-2102.
- Jumper J, Evans R and others (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, 596(7873), pp. 583-589.
- Koehler Leman J, Ulmschneider M and others (2015). Computational modeling of membrane proteins. *Proteins*, 83(1), pp. 1-24.
- Krogh A, Larsson B and others (2001). Predicting transmembrane protein topology with a hidden Markov model. *Journal of Molecular Biology*, 305(3), pp. 567-580.
- Liebschner D, Afonine P and others (2019). Macromolecular structure determination using X-rays, neutrons and electrons. *Acta Crystallographica D*, 75(10), pp. 861-877.

- Marrink S, Risselada H and others (2007). The MARTINI force field: coarse grained model for biomolecular simulations. *JPCB*, 111(27), pp. 7812-7824.
- Newport T, Sansom M and others (2019). The MemProtMD database: a resource for membrane-embedded protein structures and their lipid interactions. *Nucleic Acids Research*, 47(D1), pp. D390-D397.
- Nugent T, Jones D (2012). Accurate de novo structure prediction of large transmembrane protein domains using fragment-assembly and correlated mutation analysis. *PNAS*, 109(24), pp. E1540-E1547.
- Overington J, Al-Lazikani B and others (2006). How many drug targets are there. *Nature Reviews Drug Discovery*, 5(12), pp. 993-996.
- Pettersen E, Goddard T and others (2021). UCSF ChimeraX: structure visualization for researchers, educators, and developers. *Protein Science*, 30(1), pp. 70-82.
- Stansfeld P, Goose J and others (2015). MemProtMD: automated insertion of membrane protein structures into explicit lipid membranes. *Structure*, 23(7), pp. 1350-1361.
- Tunyasuvunakool K, Adler J and others (2021). Highly accurate protein structure prediction for the human proteome. *Nature*, 596(7873), pp. 590-596.
- Viklund H, Elofsson A (2008). OCTOPUS: improving topology prediction by two-track ANN-based preference scores. *Bioinformatics*, 24(15), pp. 1662-1668.
- Yin H, Flynn A (2016). Drugging membrane protein interactions. *Annual Review of Biomedical Engineering*, 18, pp. 51-76.

Declarations

Funding

This research was supported by institutional grants from Swiss Institute of Machine Intelligence, Central European Tech University, and Western Europe Data Science University. Additional funding from Swiss National Science Foundation Structural Biology Programme (grant 2021-SB-0518). The funders had no role in study design, analysis, or manuscript preparation.

Conflict of Interest

Helena Hansen, Hugo Ivanov, and Elena Silva declare no competing interests.

Data Availability Statement

Aggregate MPSBI scores, anonymised programme metadata, and analysis scripts are deposited at Zenodo:
<https://doi.org/10.5281/zenodo.19543732-data>.

Ethical Approval

This study involved secondary analysis of published structural benchmarks. No patient data were accessed. IRB exemption confirmed at all three universities.

Appendix A

MPSBI Scoring Rubric and Top-10 Programme Profiles

This appendix details the scoring criteria for each MPSBI sub-dimension and profiles the ten highest-scoring programmes.

A1. Sub-Score Rubric

Backbone accuracy: TM-score vs experimental structure for TM domain, normalised 0-1; weight 0.280.

Functional site prediction: binding pocket RMSD normalised vs 2A benchmark, scored 0-1; weight 0.228.

TM helix placement: helix position/orientation RMSD in membrane plane, normalised 0-1; weight 0.196.

Experimental validation: concordance with subsequent cryo-EM/Xray or mutagenesis, normalised 0-1; weight 0.172.

Lipid interface modelling: accuracy at membrane-water boundary, normalised 0-1; weight 0.124.

A2. Top-10 Programmes

1. DL-AF2Membrane-21 (Deep-learning): MPSBI = 0.930; backbone 0.96, helix 0.94.
2. CE-PhenixGPCR-21 (Cryo-EM): MPSBI = 0.918; functional site 0.96, backbone 0.92.
3. DL-RoseTTAMem-21 (Deep-learning): MPSBI = 0.904; backbone 0.94, functional 0.90.
4. CE-ISOLDEChan-20 (Cryo-EM): MPSBI = 0.892; functional site 0.94, validation 0.92.
5. HM-GPCRDBModel-20 (Homology): MPSBI = 0.884; backbone 0.92, functional 0.88.
6. DL-AF2Transport-21 (Deep-learning): MPSBI = 0.876; helix 0.94, backbone 0.90.
7. CE-CryoSPARC-20 (Cryo-EM): MPSBI = 0.868; validation 0.94, functional 0.88.
8. HM-SwissMemProt-19 (Homology): MPSBI = 0.856; backbone 0.90, helix 0.88.
9. TC-DeepTMHMM-21 (Topology): MPSBI = 0.848; helix 0.94, backbone 0.84.
10. HM-RosettaMP-18 (Homology): MPSBI = 0.842; functional site 0.88, backbone 0.88.