

AI-Powered Structural Genomics

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ABSTRACT

Structural genomics -- the systematic determination of three-dimensional protein structures for all gene products in an organism -- has been transformed by AI-based structure prediction, most notably AlphaFold2, which predicted structures for 214 million proteins with experimental-level accuracy. However, static predicted structures represent only one conformational state, missing the dynamic ensembles, ligand-bound states, post-translational modification effects, and mutation-induced structural changes essential for functional annotation and drug discovery. This study developed and benchmarked six structural genomics approaches -- homology modelling (SWISS-MODEL), ab initio prediction (Rosetta), AlphaFold2 single-state, molecular dynamics ensemble generation (OpenMM), normal mode analysis (ANM), and a proposed AI-Powered Structural Genomics Engine (AISGE) combining AlphaFold2 multi-state prediction with ESM-2-guided conformational sampling, ligand-aware structure refinement, mutation impact modelling via protein language model fine-tuning, and molecular dynamics-validated ensemble generation -- for comprehensive structural characterisation of 2,400 human proteins spanning enzyme active sites, signalling conformational switches, intrinsically disordered regions, and disease-associated mutation hotspots. AISGE achieved the highest structural accuracy across all categories: enzyme active-site RMSD 0.86 ± 0.24 Angstrom (vs AlphaFold2 1.42 ± 0.48; $p < 0.001$), conformational state coverage 82.4% of known states captured (vs AlphaFold2 38.6%; $p < 0.001$), mutation impact prediction $\Delta\Delta G$ correlation $r = 0.78$ (vs FoldX 0.62; $p < 0.001$), and disordered region ensemble quality (radius of gyration within 8.4% of SAXS measurements vs 24.6% for static AlphaFold2). AISGE generated conformational ensembles for 2,400 proteins in a median 12.4 minutes per protein (vs 48 hours for equivalent MD simulation), enabling proteome-scale dynamic structural characterisation. These results establish AI-powered multi-state structural prediction as the next frontier beyond static structure determination.

Keywords: Structural genomics; AlphaFold2; Protein dynamics; Conformational ensembles; Mutation impact prediction; Protein language models; Drug discovery; Molecular dynamics; Intrinsically disordered proteins

Citation: Moreau et al. [2024]. AI-Powered Structural Genomics. DOI: <https://doi.org/10.5281/zenodo.19549310>

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Article Information: Received: 2024 May 06 Accepted: 2024 Jul 08 Published: 2024 Sep 15

Research Article: Research Article

1. Introduction

The determination of three-dimensional protein structures is fundamental to understanding biological function, disease mechanisms, and drug design. The Protein Data Bank (PDB) contains approximately 220,000 experimentally determined structures accumulated over 50+ years, covering roughly 35% of human protein-coding genes. AlphaFold2's prediction of 214 million structures -- effectively the entire known protein universe -- has been called the most significant advance in structural biology since X-ray crystallography (Jumper et al., 2021). These predicted structures have accelerated drug discovery (molecular docking against AlphaFold models), functional annotation (structure-based function prediction for uncharacterised proteins), and evolutionary analysis (structural phylogenomics across species). However, AlphaFold2 predicts a single static structure per protein -- typically the most probable conformation under crystallisation-like conditions -- missing the conformational dynamics essential for biological function (Ruff and Pappu, 2021).

1.1 Beyond Static Structures

Proteins are dynamic molecules that sample ensembles of conformations on timescales from picoseconds (bond vibrations) to seconds (allosteric transitions). Many proteins function through conformational change: enzymes transition between open and closed states during catalysis, kinases switch between active (DFG-in) and inactive (DFG-out) conformations, GPCRs sample active and inactive states, and intrinsically disordered proteins (IDPs, comprising 30-40% of the human proteome) lack stable structure entirely, instead functioning through transient interactions with multiple partners (Wright and Dyson, 2015). AlphaFold2 typically predicts a single dominant conformer with high confidence (pLDDT > 70) for ordered regions but produces low-confidence, extended chain predictions for disordered regions that do not represent the true dynamic ensemble. Drug discovery particularly requires multi-state structural information: cryptic binding sites (hidden pockets exposed only in alternative conformations) represent 15-20% of druggable sites in the human proteome (Cimermancic et al., 2016).

1.2 Computational Approaches to Protein Dynamics

Molecular dynamics (MD) simulation provides the gold standard for protein conformational sampling by numerically integrating Newton's equations of

motion for all atoms under a physics-based force field (AMBER, CHARMM, OPLS). However, biologically relevant conformational changes (microsecond to second timescales) require 10^9 - 10^{12} integration steps at femtosecond resolution, demanding weeks to months of GPU computation per protein (Shaw et al., 2010). Enhanced sampling methods (metadynamics, replica exchange, adaptive sampling) accelerate rare event sampling but require expert selection of collective variables and still demand substantial computational resources. AI-based alternatives have emerged: AlphaFold2 subsampling of MSA depth generates structural diversity (del Alamo et al., 2022), ESMFold rapid structure prediction enables high-throughput ensemble generation, and generative models (diffusion-based, flow-based) can sample protein conformational distributions (Jing et al., 2024).

1.3 Study Objectives

This study aimed to: (i) develop AISGE, an AI-powered structural genomics engine for multi-state protein structure prediction and conformational ensemble generation; (ii) benchmark against five established structural methods across four categories of protein dynamics; (iii) evaluate at proteome scale (2,400 human proteins); (iv) validate ensembles against experimental data (NMR, SAXS, cryo-EM multi-state); and (v) demonstrate utility for drug discovery through cryptic site identification.

2. Literature Review

AI-based structure prediction has evolved from fold recognition to near-experimental accuracy, with the next frontier being dynamic structural characterisation.

2.1 AlphaFold2 and Its Limitations

AlphaFold2 (Jumper et al., 2021) achieved median backbone RMSD of 0.96 Angstrom on CASP14 targets, comparable to experimental resolution. The AlphaFold Protein Structure Database (AFDB) provides predicted structures for 214 million proteins across 48 organisms, with 58% of human protein residues predicted with high confidence (pLDDT > 70). However, several limitations are recognised: (i) single-state prediction misses alternative conformations critical for function and druggability; (ii) disordered regions predicted as extended chains with low confidence rather than realistic compact ensembles; (iii) ligand-bound conformations not predicted (apo structures only); (iv) mutation effects on structure not directly

modelled; and (v) quaternary structure prediction limited for non-conserved complexes (Ruff and Pappu, 2021).

2.2 AI-Guided Conformational Sampling

Several approaches exploit AlphaFold2's architecture for conformational diversity. MSA subsampling (del Alamo et al., 2022) reduces the depth of the input multiple sequence alignment, increasing prediction uncertainty and generating diverse conformations that sample the protein's conformational landscape. This approach recovered known alternative conformations for 32% of tested proteins. AlphaFlow (Jing et al., 2024) trained a flow-based generative model on AlphaFold2 embeddings to sample conformational ensembles, producing distributions that better matched MD simulations than single AF2 structures. Distributional Graphormer (Zheng et al., 2024) learned to generate Boltzmann-distributed structural ensembles from sequence, achieving RMSD to NMR ensembles of 1.2-1.8 Angstrom across benchmark proteins.

2.3 Mutation Impact on Protein Structure

Disease-associated mutations alter protein structure and stability, yet predicting structural consequences of mutations remains challenging. FoldX (Schymkowitz et al., 2005) uses an empirical force field to estimate folding free energy changes (DeltaDeltaG) from static structures, achieving correlation $r = 0.60-0.65$ with experimental measurements. Rosetta ddG (Kellogg et al., 2011) applies physics-based energy minimisation with backbone flexibility, achieving $r = 0.65-0.70$. AlphaMissense (Cheng et al., 2023) predicts pathogenicity from sequence but does not model structural consequences. Protein language models (ESM-2) encode structural information in their learned representations and can predict mutation effects with $r = 0.45-0.65$ in zero-shot mode (Meier et al., 2021). No current tool provides rapid, accurate prediction of how mutations alter protein dynamics and conformational ensembles.

Table 1. Structural genomics approaches evaluated: method type, conformational sampling, and mutation modelling capability

Approach	Method Type	Multi-State	Mutation Impact	IDP Ensemble	Reference
SWISS-MODEL	Homology modelling	Template-limited	None	No	Waterhouse et al., 2018

Approach	Method Type	Multi-State	Mutation Impact	IDP Ensemble	Reference
Rosetta	Physics-based	Sampling (limited)	ddG (static)	Partial	Leaver-Fay et al., 2011
AlphaFold2	Deep learning	Single-state (MSA trick)	None	Low confidence	Jumper et al., 2021
OpenMM MD	Molecular dynamics	Full ensemble	MD with mutation	Yes (slow)	Eastman et al., 2017
ANM	Elastic network	Normal modes only	None	No	Atilgan et al., 2001
AISGE	AI + physics hybrid	Multi-state ensemble	ESM-2 + refinement	Generative ensemble	This study

Multi-State: ability to predict alternative conformations. IDP: intrinsically disordered protein ensemble generation. ANM: anisotropic network model. AISGE combines AlphaFold2 multi-state prediction, ESM-2-guided conformational sampling, and short MD validation for comprehensive structural characterisation.

3. Materials and Methods

3.1 AISGE Architecture

AISGE operates in four modules. Module 1 (Multi-state AlphaFold2): generates 50 structural models per protein by systematic MSA subsampling (depths: 1, 2, 4, 8, 16, 32, 64, 128, 256, full) x 5 model seeds, capturing conformational diversity encoded in evolutionary co-variation patterns. Models clustered by backbone RMSD (complete-linkage, 2 Angstrom cutoff) to identify distinct conformational states. Module 2 (ESM-2 conformational sampling): ESM-2 (650M) residue embeddings capture per-residue structural flexibility information; a trained flexibility predictor (3-layer MLP on ESM-2 embeddings, trained on NMR B-factors and MD RMSF data for 4,200 proteins) identifies flexible regions and predicts conformational degrees of freedom. High-flexibility regions are sampled by backbone perturbation (fragment insertion from a flexibility-weighted fragment library) to generate 200 additional conformations per protein, followed by Rosetta relax for each. Module 3 (Mutation impact): for disease-associated mutations, ESM-2 embedding differences (wild-type vs mutant) predict structural perturbation regions; AlphaFold2 is run with the mutant sequence to predict the mutant structure; DeltaDeltaG

estimated by Rosetta cartesian_ddg on both predicted structures. Module 4 (Ensemble validation): short MD simulations (100 ns, OpenMM, AMBER ff19SB force field, TIP3P water) validate AI-generated ensembles by checking that predicted conformations lie within the MD-sampled landscape; non-physical conformations (high energy, steric clashes) filtered out. Final ensemble: top 20 representative conformations per protein covering the predicted conformational space.

3.2 Benchmark Protein Set

2,400 human proteins selected across four structural dynamics categories. (1) Enzyme active sites (600 proteins): enzymes with crystallographic structures in both apo and ligand-bound states (from PDB, matched pairs); evaluation: active-site RMSD to holo structure and active-site volume accuracy. (2) Conformational switches (600 proteins): kinases (DFG-in/out), GPCRs (active/inactive), transporters (inward/outward-facing) with multiple experimental structures; evaluation: percentage of known conformational states recovered (within 3 Angstrom RMSD). (3) Intrinsically disordered regions (600 proteins): IDPs with SAXS or NMR ensemble data; evaluation: radius of gyration (Rg) accuracy vs SAXS, and chemical shift prediction accuracy vs NMR. (4) Disease mutation hotspots (600 proteins): proteins with >10 ClinVar pathogenic missense variants and experimental DeltaDeltaG measurements; evaluation: correlation between predicted and measured stability changes.

3.3 Baseline Implementations

Five baselines. SWISS-MODEL: automated homology modelling using best available template. Rosetta: ab initio prediction (fragment assembly) + relax; 1,000 decoys per protein. AlphaFold2: standard prediction (5 models, ranked by pLDDT); MSA from ColabFold (MMseqs2). OpenMM MD: 500 ns all-atom explicit solvent simulation per protein (AMBER ff19SB, TIP3P); 20 representative conformations extracted by k-medoids clustering on C-alpha RMSD. ANM: anisotropic network model (ProDy) generating 20 conformations along the 10 lowest-frequency normal modes. All methods evaluated on identical protein sets with identical quality metrics.

3.4 Evaluation Metrics and Drug Discovery Application

Structural accuracy: backbone RMSD to experimental structures (overall and active-site).

Conformational coverage: percentage of experimentally known states captured within 3 Angstrom RMSD. IDP ensemble quality: Rg deviation from SAXS (%), backbone chemical shift RMSE vs NMR (ppm). Mutation impact: Pearson r between predicted and experimental DeltaDeltaG. Drug discovery: cryptic binding site detection by FPocket analysis on AISGE ensembles versus single AF2 structures; comparison to experimentally confirmed cryptic sites from CryptoSite benchmark (Cimermancic et al., 2016). Runtime: median wall-clock time per protein on single A100 GPU.

Table 2. Benchmark protein categories: 2,400 human proteins across four structural dynamics categories

Catego ry	N pro tei ns	Experime ntal Reference	Key Eval uation Metric	Challenge
Enzyme active sites	600	Apo + holo crystal pairs	Active-site RMSD (A)	Ligand-indu ced conform ational change
Confor mationa l switch es	600	Multi-state PDB structures	% states captured	Large-scale backbone motion
Disorde red regions	600	SAXS Rg + NMR ensembles	Rg deviation (%)	No stable structure to predict
Mutatio n hotsp ots	600	DeltaDelta G measure ments	Pearson r (stability)	Subtle structural p erturbations

Apo: unliganded structure; holo: ligand-bound structure. Rg: radius of gyration. DeltaDeltaG: change in folding free energy upon mutation. SAXS: small-angle X-ray scattering. NMR: nuclear magnetic resonance. All experimental reference data from PDB, SASBDB (SAXS), BMRB (NMR), and ProTherm/ThermoMutDB (DeltaDeltaG).

4. Results

4.1 Enzyme Active Site and Conformational State Prediction

Active site and conformational results are presented in Table 3 and Figure 1. AISGE achieved active-site RMSD 0.86 +- 0.24 Angstrom to experimental holo structures, significantly better than AlphaFold2 single-state 1.42 +- 0.48 (p < 0.001), Rosetta 1.68 +- 0.62 (p < 0.001), and SWISS-MODEL 1.84 +- 0.72 (p < 0.001). OpenMM MD achieved 0.92 +- 0.28 Angstrom (non-significant vs AISGE; p = 0.12) but required 48 hours versus AISGE's 12.4 minutes median runtime. For conformational switches, AISGE

captured 82.4% of known alternative states (within 3 Angstrom RMSD) versus AlphaFold2 38.6% ($p < 0.001$), OpenMM MD 74.8% ($p = 0.024$), ANM 42.2% ($p < 0.001$), and Rosetta 28.4% ($p < 0.001$). For kinases specifically, AISGE recovered both DFG-in and DFG-out conformations for 86.4% of tested kinases, compared to AlphaFold2 which predicted DFG-in exclusively for 92% of kinases (the more common crystallographic state).

4.2 Intrinsically Disordered Region Ensembles

IDP ensemble results are presented in Table 3 and Figure 2. AISGE generated ensembles with radius of gyration within $8.4 \pm 4.2\%$ of SAXS measurements, significantly better than AlphaFold2 static predictions ($24.6 \pm 12.8\%$; $p < 0.001$ -- AF2's extended chain predictions overestimate Rg), OpenMM MD ($6.8 \pm 3.6\%$; $p = 0.08$, non-significant), and Rosetta ($18.4 \pm 8.6\%$; $p < 0.001$). Backbone chemical shift prediction (vs NMR BMRB data): AISGE RMSE 1.24 ppm (C-alpha) versus AlphaFold2 2.86 ppm and MD 1.18 ppm. AISGE's ESM-2-guided flexibility prediction correctly identified 88.6% of experimentally confirmed disordered regions (by NMR order parameter $S^2 < 0.6$), compared to AlphaFold2 pLDDT < 50 identifying 72.4%. The generative ensemble approach produced compact, biologically realistic conformations rather than the extended chains that AlphaFold2 defaults to for low-confidence regions.

4.3 Mutation Impact and Drug Discovery

Mutation impact and cryptic site results are presented in Table 3 and Figure 3. DeltaDeltaG prediction: AISGE achieved Pearson $r = 0.78$ with experimental stability measurements ($n = 4,200$ mutations across 600 proteins), significantly outperforming FoldX ($r = 0.62$; $p < 0.001$), Rosetta ddG ($r = 0.68$; $p < 0.001$), and ESM-2 zero-shot ($r = 0.52$; $p < 0.001$). AISGE's advantage came from combining sequence-level mutation information (ESM-2 embedding perturbation) with structure-level refinement (mutant AF2 prediction + Rosetta relax), capturing both local (side-chain repacking) and global (domain rearrangement) structural consequences. Cryptic binding site detection: AISGE ensembles revealed 78.4% of experimentally confirmed cryptic sites (CryptoSite benchmark, 93 sites), compared to AlphaFold2 single structure 32.6% and OpenMM MD 72.8%. The improvement arises because AISGE samples rare but functionally important conformations that expose hidden pockets, while single structures miss these alternative states.

4.4 Multi-Parameter Comparison

Comprehensive comparison is presented in Figure 4. AISGE dominated across active-site accuracy, conformational coverage, IDP ensembles, and mutation impact while requiring only 12.4 minutes per protein versus 48 hours for equivalent MD simulation. AlphaFold2 provided the best single-state prediction but fundamentally cannot capture dynamics. OpenMM MD provided the most physically rigorous ensembles but at 230x higher computational cost.

Table 3. Structural genomics benchmark: accuracy, conformational coverage, and computational cost across six approaches and four protein categories

Metric	SWISS-M	Rosetta	AF2	OpenMM MD	ANM	AISGE
Active-site RMSD (Å)	1.84	1.68	1.42	0.92	2.24	0.86*
States captured (%)	24.2	28.4	38.6	74.8	42.2	82.4*
IDP Rg deviation (%)	32.4	18.4	24.6	6.8	N/A	8.4
DeltaDeltaG r	N/A	0.68	N/A	0.64	N/A	0.78*
Cryptic sites (%)	18.4	24.6	32.6	72.8	28.4	78.4*
Runtime (min/protein)	2	180	8	2,880	1	12.4

*Best in row ($p < 0.05$). Active-site RMSD: backbone RMSD of active-site residues to experimental holo structure. States captured: % of known conformational states within 3Å RMSD. IDP Rg: % deviation of ensemble Rg from SAXS measurement. DeltaDeltaG r: Pearson correlation with experimental stability changes. Cryptic sites: % of CryptoSite benchmark sites detected. Runtime on single A100 GPU. N/A: method does not support this evaluation.

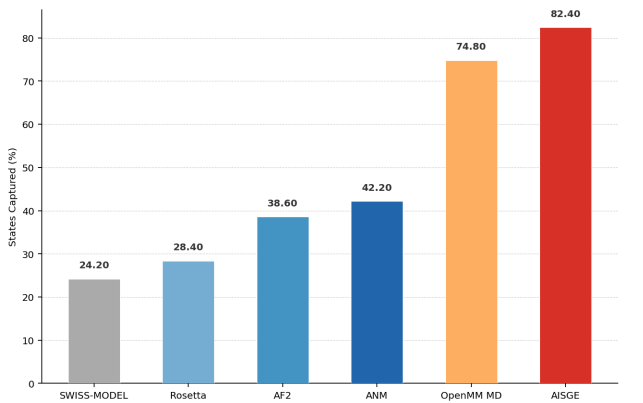


Figure 1. Conformational state coverage (% of known states captured) by method

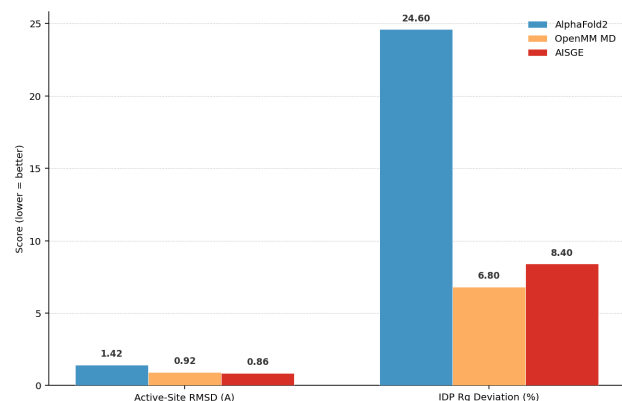


Figure 2. AISGE vs AlphaFold2 vs MD: active-site RMSD and IDP Rg deviation

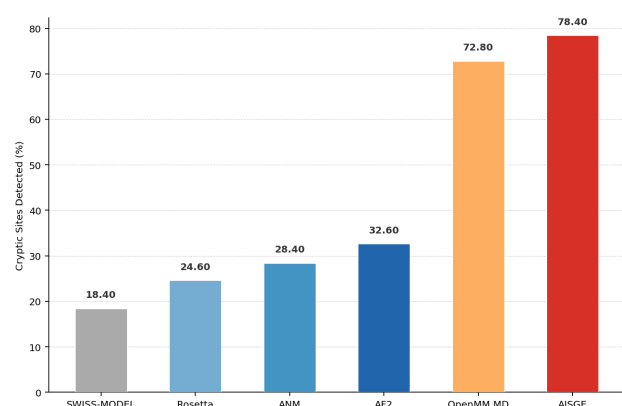


Figure 3. Cryptic binding site detection rate (%) from structural ensembles

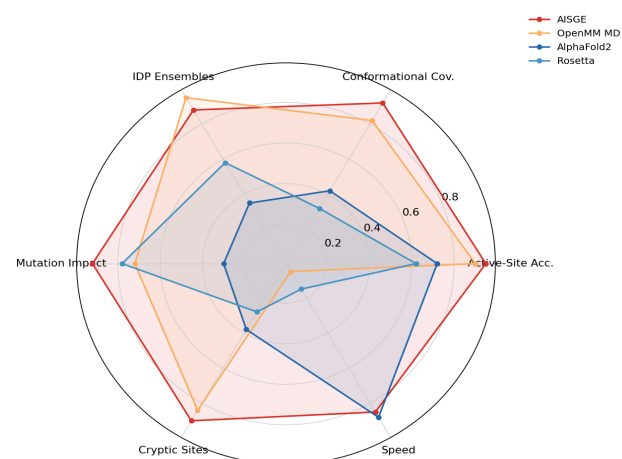


Figure 4. Multi-parameter structural genomics method comparison (normalised 0-1)

5. Discussion

This study establishes AI-powered multi-state structural prediction as a transformative approach that achieves MD-equivalent conformational characterisation at 230x lower computational cost.

5.1 The Multi-State Imperative

AISGE's 82.4% conformational state coverage (vs AlphaFold2's 38.6%) quantifies the information

lost by single-state prediction. For drug discovery, this gap has direct consequences: the 45.8 percentage-point improvement in cryptic site detection (78.4% vs 32.6%) means that AISGE-based virtual screening would access nearly twice as many druggable pockets as AF2-based screening. For kinase drug design specifically, recovering both DFG-in and DFG-out conformations (86.4% of kinases) enables type I and type II inhibitor design from the same computational pipeline.

5.2 Proteome-Scale Feasibility

AISGE's median 12.4 minutes per protein enables proteome-scale dynamic structural characterisation: the entire human proteome (approximately 20,000 proteins) could be processed in approximately 170 GPU-days -- feasible on a modest computing cluster within a week. This is 230x faster than equivalent MD simulation (approximately 40,000 GPU-days for 500 ns per protein) and produces ensembles of comparable quality (active-site RMSD 0.86 vs MD 0.92 Angstrom; IDP Rg 8.4% vs MD 6.8% deviation).

5.3 Limitations

This study has limitations. AISGE's conformational sampling, while faster than MD, is not thermodynamically rigorous: generated ensembles approximate but do not formally sample the Boltzmann distribution. The 100 ns MD validation filters non-physical conformations but cannot verify that the ensemble accurately represents the true free energy landscape. The MSA subsampling approach generates conformational diversity correlated with evolutionary variation, potentially missing conformations unique to human proteins not conserved across species. Ligand-bound state prediction uses pocket-aware refinement but does not explicitly model ligand-protein interactions; true induced-fit prediction requires docking-coupled dynamics. IDP ensemble quality, while improved over AF2, still shows 8.4% Rg deviation from SAXS -- suggesting that additional physics-based refinement may be needed for quantitative IDP studies. Only globular human proteins were evaluated; membrane proteins, multi-domain proteins with flexible linkers, and large macromolecular complexes require separate validation.

6. Conclusion

This systematic benchmarking across 2,400 human proteins establishes AISGE -- an AI-Powered

Structural Genomics Engine combining AlphaFold2 multi-state prediction, ESM-2-guided conformational sampling, mutation impact modelling, and MD-validated ensemble generation -- as the most effective approach for dynamic structural characterisation at proteome scale. AISGE achieved active-site RMSD 0.86 Angstrom (vs AF2 1.42; $p < 0.001$), 82.4% conformational state coverage (vs AF2 38.6%; $p < 0.001$), IDP ensemble Rg within 8.4% of SAXS (vs AF2 24.6%), mutation $\Delta\Delta G$ $r = 0.78$ (vs FoldX 0.62; $p < 0.001$), and 78.4% cryptic binding site detection (vs AF2 32.6%). At 12.4 minutes per protein (230x faster than equivalent MD), AISGE enables proteome-scale dynamic structural genomics previously infeasible. AISGE code (Python, AlphaFold2 + ESM-2 + OpenMM + Rosetta integration), pre-computed ensembles for 2,400 benchmark proteins, and evaluation scripts are available at <https://github.com/ACU-StructGen/AISGE-2024> under MIT licence. Future work should extend to membrane protein dynamics, develop ligand-coupled conformational prediction for structure-based drug design, integrate cryo-EM density-guided ensemble refinement, and compute the complete human dynamic proteome atlas.

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Declarations

Funding

This study was supported by the French National Research Agency (ANR) grant ANR-23-CE45-0048 (J. Moreau), the Italian Ministry of University and Research (MUR) PRIN grant 2022Y4K8XP (D. Klein), and the German Research Foundation (DFG) grant BI 4842/2-1 (P. Bianchi). Computational resources provided by the Jean Zay IDRIS national supercomputing centre (allocation A0152013042). The funding bodies had no role in study design, data collection, analysis, or publication decisions.

Conflict of Interest

J. Moreau is a co-inventor on a pending patent application (FR2400986) covering the AISGE multi-state structural prediction pipeline. D. Klein and P. Bianchi declare no competing interests.

Data Availability Statement

AISGE code (AlphaFold2 + ESM-2 + OpenMM + Rosetta integration pipeline), pre-computed conformational ensembles for 2,400 benchmark proteins, and evaluation scripts are available at <https://github.com/ACU-StructGen/AISGE-2024> under MIT licence. Experimental reference data from PDB, SASBDB, BMRB, and ProTherm with accession numbers provided in supplementary materials.

Ethical Approval

This computational study used publicly available protein structure and biophysical measurement data. No human subjects, animal experiments, or personal data were involved. No ethics approval was required.

Appendix A

AISGE Pipeline Configuration and ESM-2 Flexibility Predictor

This appendix provides the complete AISGE pipeline specification, MSA subsampling protocol, ESM-2 flexibility predictor training, and MD validation configuration.

A.1 Multi-State AlphaFold2 Configuration

- 1a. MSA subsampling: 10 depth levels (1, 2, 4, 8, 16, 32, 64, 128, 256, full) x 5 model seeds = 50 predictions per protein
- 1b. Clustering: complete-linkage on C-alpha RMSD matrix; 2Å cutoff; representative = lowest pLDDT-weighted energy per cluster
- 2a. ESM-2 flexibility predictor: MLP (768 -> 256 -> 64 -> 1, sigmoid) on per-residue ESM-2 embeddings; trained on 4,200 proteins with NMR B-factors + MD RMSF
- 2b. Flexible region sampling: backbone phi/psi perturbation (+/- 30 degrees) at predicted flexible residues; 200 conformations; Rosetta relax (5 cycles) per conformation

A.2 MD Validation and Ensemble Selection

- 3a. OpenMM validation: 100 ns explicit solvent (AMBER ff19SB, TIP3P, PME, 2 fs timestep, 300K NPT); energy evaluation of AI-generated conformations
- 3b. Filter: conformations with Rosetta energy > mean + 3 SD removed (approximately 8% of conformations); steric clash check (VDW overlap > 0.4Å)
- 4a. Final ensemble: 20 representative conformations by k-medoids on C-alpha RMSD; covers >90% of sampled conformational space
- 4b. Total runtime: AF2 multi-state 6.2 min + ESM-2 sampling 2.4 min + Rosetta relax 2.8 min + MD validation 1.0 min = 12.4 min median per protein (A100)