**Chrono\_\_\_: Temporal Trajectory Analysis of Protein Motion from Static Structure Predictions**

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**Abstract**

Brief overview of discovering shared dynamic signatures between MD simulations and AlphaFold3 predictions, validation across protein designs, and proteome-wide application.

**1. Introduction**

* **Problem Setup**: Static structure prediction vs. dynamic protein function
* **Gap**: AlphaFold provides snapshots, but proteins are dynamic machines
* **Hypothesis**: AF3 confidence patterns encode dynamic information accessible via PCA
  + **Contrast this with the belief that this is not broadly true**!
* **Contributions**: (1) MD-AF3 signal correlation, (2) design validation, (3) software package, (4) proteome application

**3. Results**

**3.1 Core Discovery: MD-AF3 Signal Correspondence from ensemble RMSF:**



Figure S4. AlphaFold3 and molecular dynamics rmsf comparison. *Structural dynamics comparison across 20 protein variants. Mean root-mean-square fluctuation (RMSF) values from molecular dynamics (MD) simulations are shown for all variants (solid blue line) and compared with AlphaFold 3 (AF3) predictions. The AF3 RMSF (solid black line) was computed as the root mean square deviation of each Cα atom from its mean predicted position. Additionally, mean per-residue pLDDT confidence scores from AF3 (dashed line) are included. While pLDDT values show only weak correspondence to experimental dynamics, AF3 RMSF closely mirrors the MD-derived RMSF trends across all variants.*

**Motivation to time sorting**

* **Correlation analysis**: Quantitative overlap between AF3 principal components and MD normal modes
* **Physical interpretation**: Which structural features drive shared signals
* **Statistical validation**: Significance testing across multiple systems
* **Mechanistic insight**: Why static predictions capture dynamic information

-> NanoLuc SI picture – comp against own MD results

**3.2 Validation on Simulated Protein Systems**

**- LipA – against own MD and known function**

**- AD Kinase – against external MD**

**- HIV-1 Protease – against external MD**

**- Onconase – against external MD**

* **RMSD plots 2x2 panel, MD from crystal, and chronosort from first frame**
* **RMSF for four designs**
* **PCA – @Will make 2x2 pymol figs (nice quality)**
* **Table of cosine sim between MD and AF3 eigenvecs, compare against random ones.**
* **How do both types of vecs give us the same info?**
* **Four protein designs**: Diverse fold families/functional classes
* **Ground truth comparison**: Experimental/simulation data where available
* **Prediction accuracy**: Motion prediction performance metrics
* **Failure mode analysis**: Where/when the method breaks down

**3.3 AlphaDynamics Software Package**

* **Implementation**: Python package architecture and key functions
* **Usability**: Input/output formats, computational requirements
* **Reproducibility**: Benchmarking and example workflows
* **Integration**: Compatibility with existing structural biology tools
* **Implications**: What this means for structure-function relationships
* **Limitations**: Current method boundaries and assumptions
* **Future directions**: Extensions to protein-protein interactions, drug design
* **Broader impact**: Democratizing protein dynamics prediction

**2. Methods**

**A diagram of a computer program

AI-generated content may be incorrect.**

**2.1 AF3 Run**

**2.2 ChronoSort**

**2.3 Principal Component Extraction Framework**

* PCA methodology for AF3 confidence/coordinate data
* Feature engineering from structural predictions
* Dimensionality reduction and component interpretation

**2.4 Molecular Dynamics Comparison Pipeline**

* MD simulation protocols
* Dynamic mode extraction from trajectories
* Cross-correlation analysis between AF3-PCA and MD modes

2.5 PCA visualization

2.6 Random proteins? Really big ones?

**References**