Visualizing and Quantifying Antibody Flexibility from Simulations

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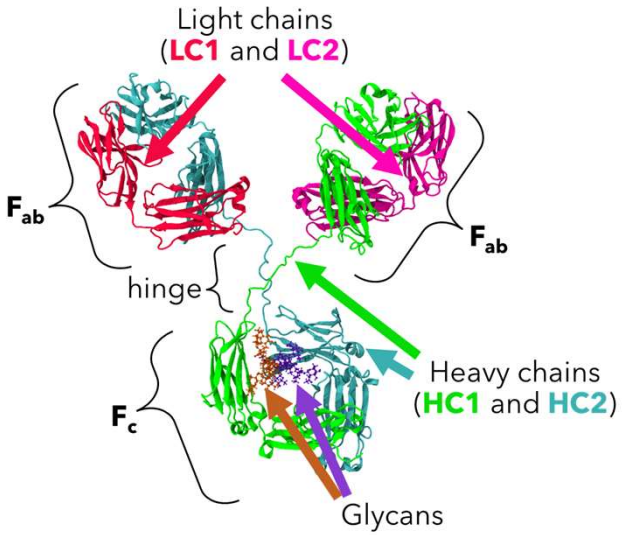
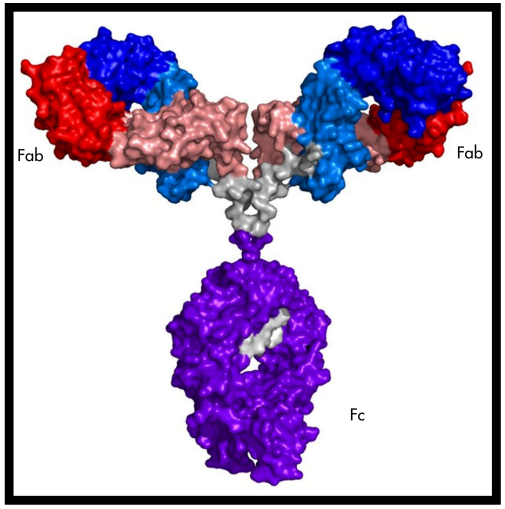
NIST Center for Neutron Research

NISTmAb Dynamics

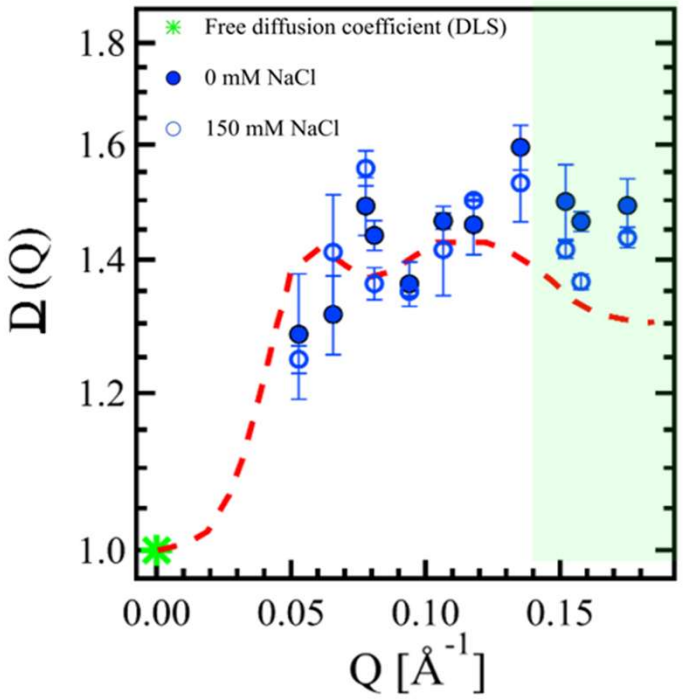
- NISTmAb (Reference Material 8671) is an standard monoclonal antibody critical for validating biopharmaceutical measurements
- Large Y shaped antibodies are highly flexible, which can influence shelf stability and target binding
- Experimentally, Neutron Spin Echo (NSE) quantifies the dynamics, but the results are complex and difficult to translate directly into 3D movement
- Molecular Dynamics (MD): Computational simulations used to study the physical movements of atoms and molecules





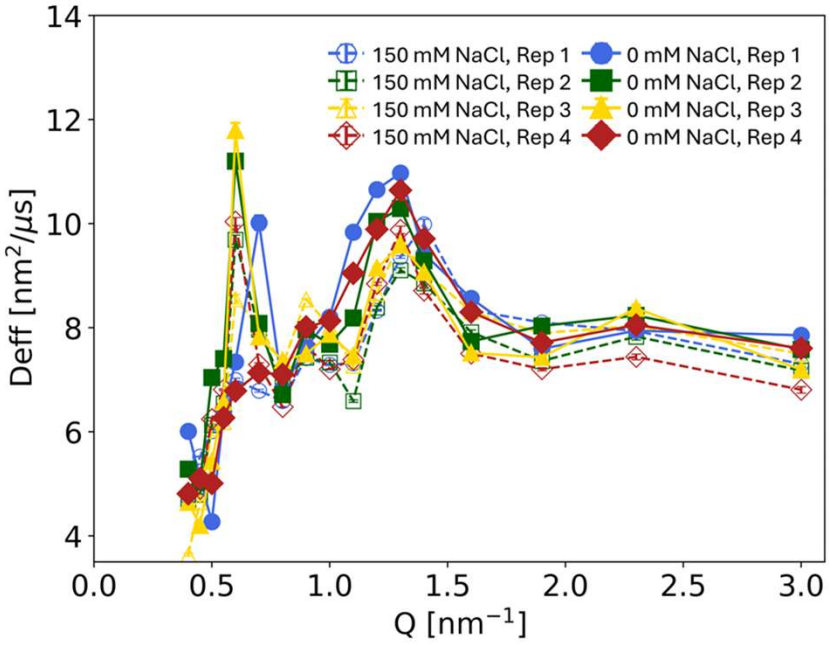
NIST (2015)

Length-Scale Dependent Dynamics



Neutron Spin Echo (NSE)

Nayem U. of Delaware (2020)

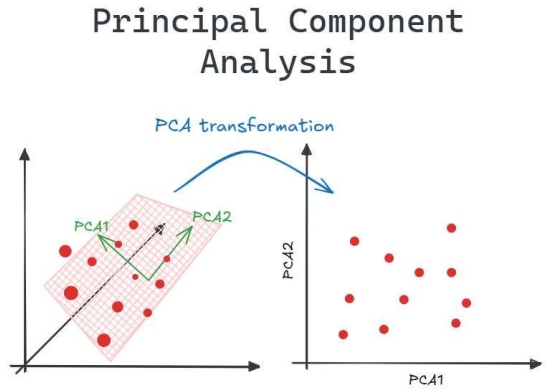


All-Atom Molecular Dynamics

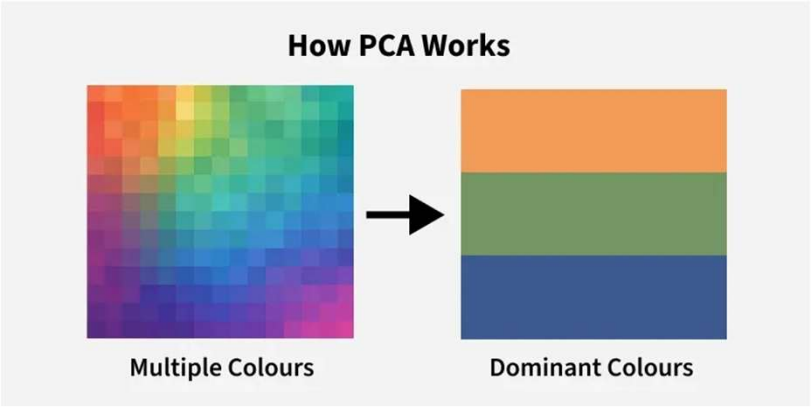
Hilburg et al. *In Preparation*

Principal Component Analysis

- Reduces the dimensionality of large datasets
- Retains maximum amount of original information
- Essential Space is a set of new coordinates that describe most of the total fluctuations



"Principal Component Analysis," mlpills.substack.com



"Principal Component Analysis," geeksforgeeks.com

- Variance is the magnitude of fluctuations along a specific PC
- Simplifies qualitative and quantitative analysis of complex systems

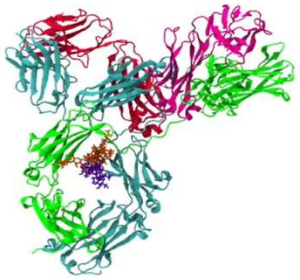
Principal Component Analysis for MD

Perform PCA on MD trajectories

- Trajectories are 3D coordinates of every atom over time, accounting for the physics of the molecule
- Resulting components are dynamic modes

Analysis:

- 4 ten μs independent trajectories
- Combined trajectory



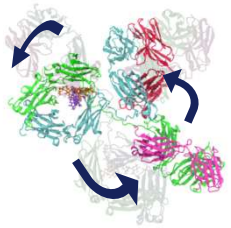
NISTmAb MD Trajectory

Tool and Atom Selection with Test Trajectory

Atom Selection	# of Atoms	MDAnalysis (HH:MM:SS)	AmberTools: CPPTRAJ (HH:MM:SS)
All atoms	20667	13:55:00	00:05:03
Heavy Atoms	10447	04:25:03	00:00:38
'Backbone' (CA, C, O, N atom-names)	5296	00:22:37	00:00:10
Atom-name 'CA' only	1326	00:00:48	<00:00:01

CPPTRAJ Implementation

01 Alignment



- Elimination of global translation and rotation (diffusion).
- Align to a reference to isolate internal motion

02 Correlation

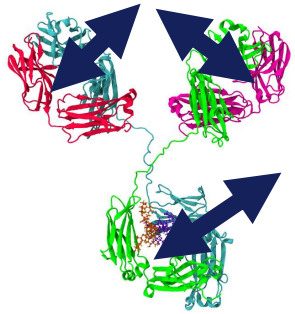
- Calculate the covariance matrix
- Captures the correlation between the displacement of all 'backbone' atoms

03 Diagonalization

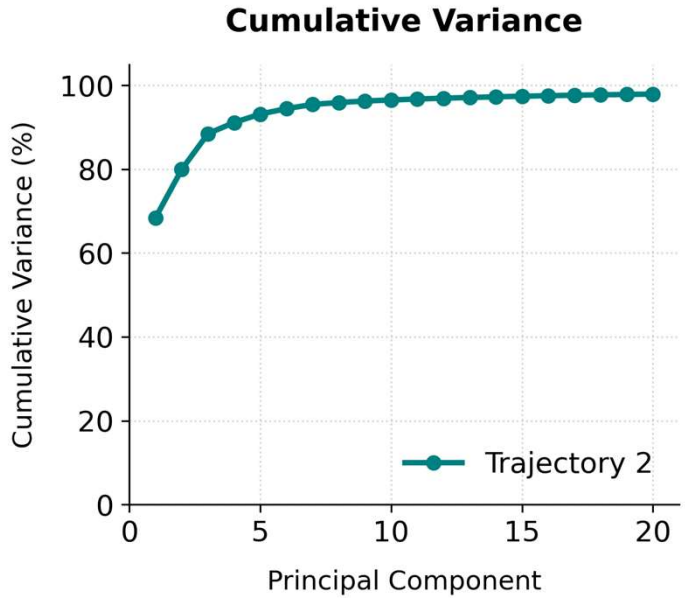
- Diagonalized the covariance matrix
- Principal components (eigenvectors)
- Associated variance (eigenvalues)

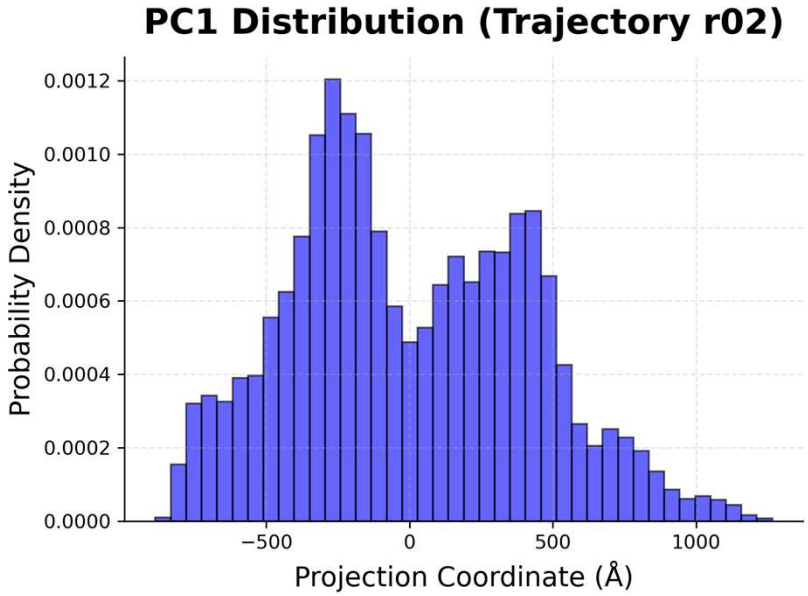
04 Projection

- Project the eigenvectors onto 3D coordinates of average structure
- Visualize PCs



Single Trajectory Analysis

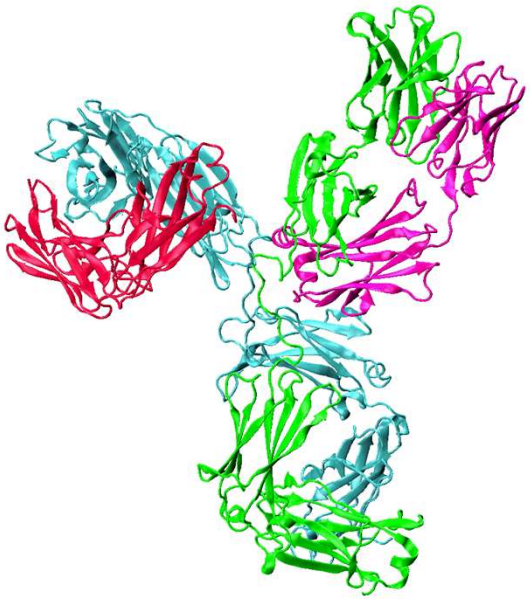




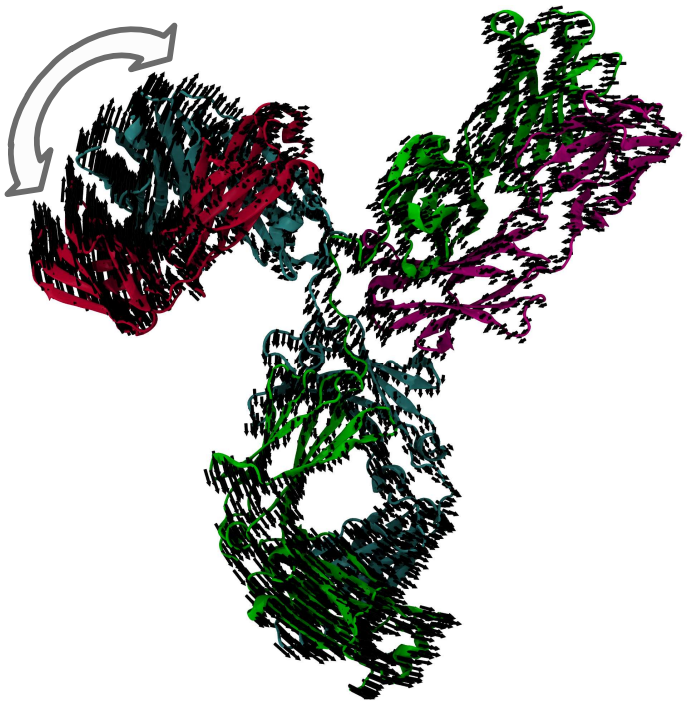
PC1 accounts for 68% of the total variance

Probability density of this motion and how often the protein adopts each position

PC1 of a Single Trajectory

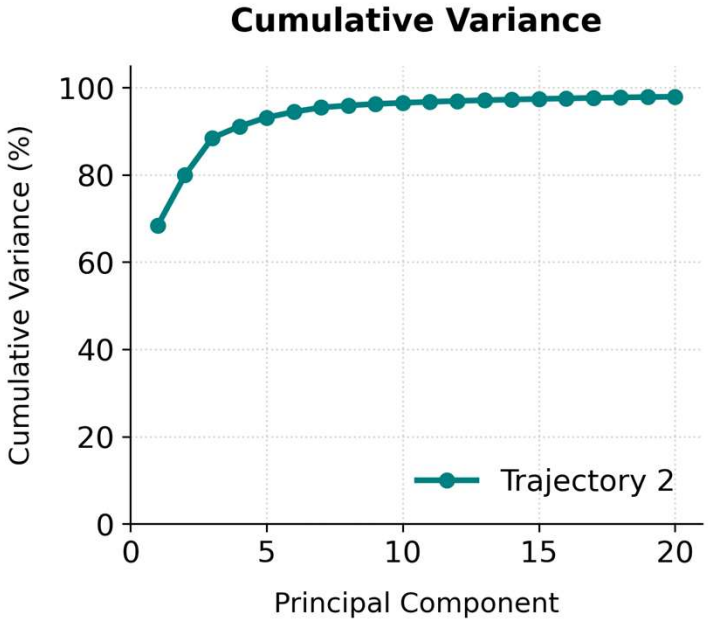


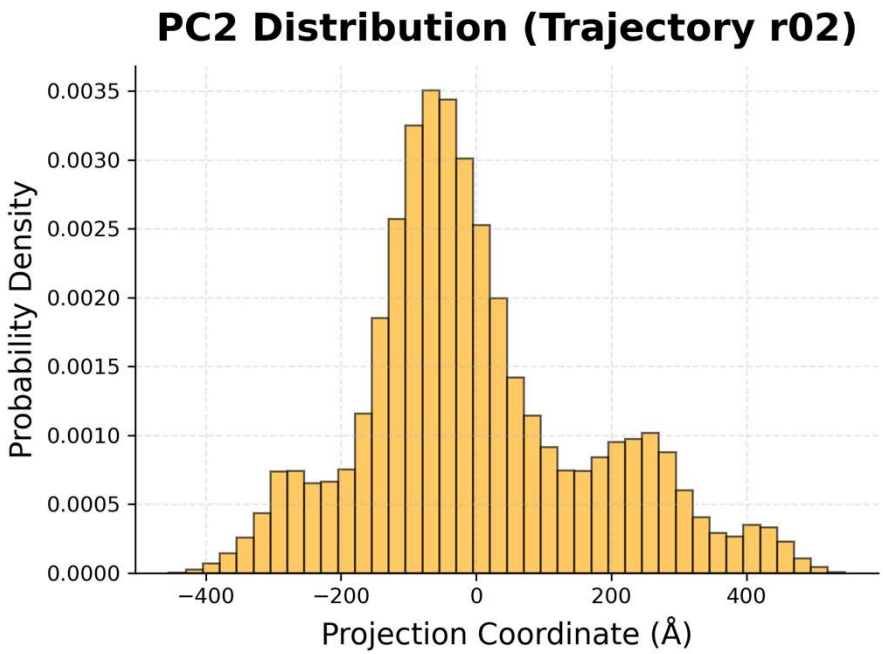
The dominant mode of flexibility (PC1) exhibits



Arrows showing the atomic displacement

Single Trajectory Analysis

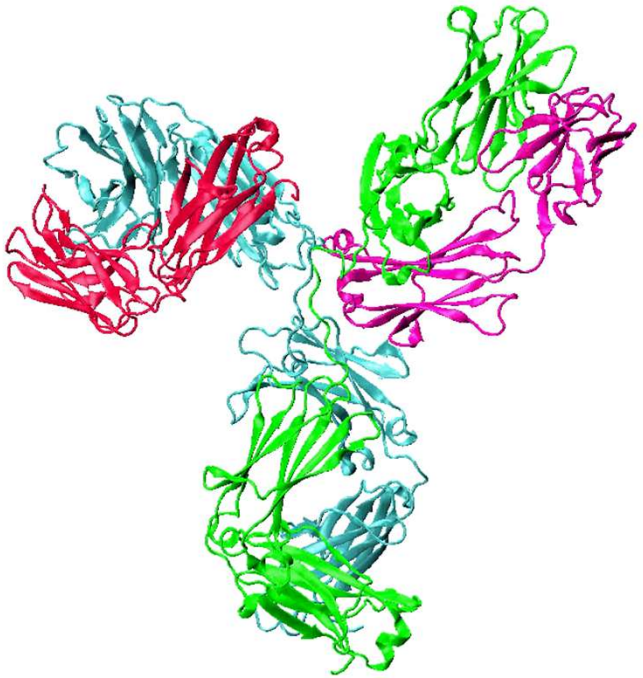




PC2 accounts for an additional 12 % of the total variance

Probability density of PC2 and how often the protein adopts each position

PC2 of a Single Trajectory



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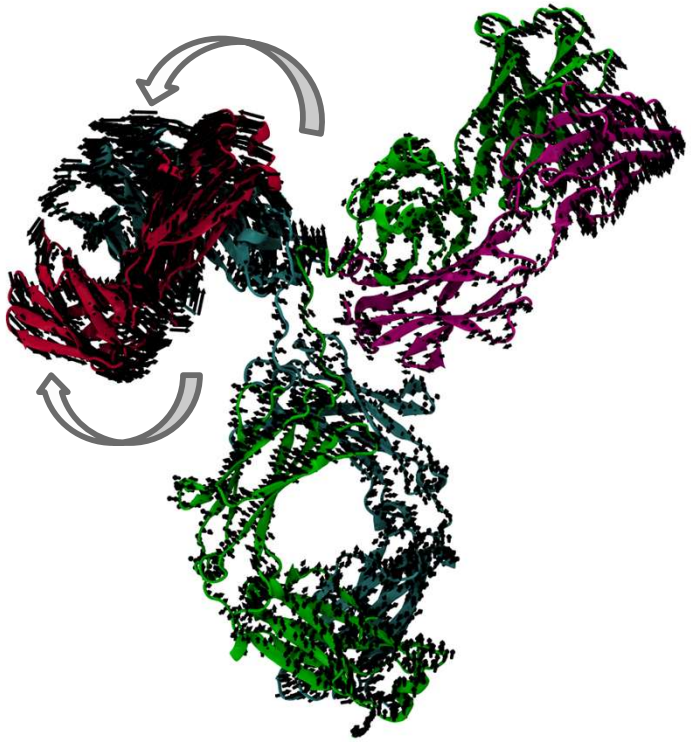
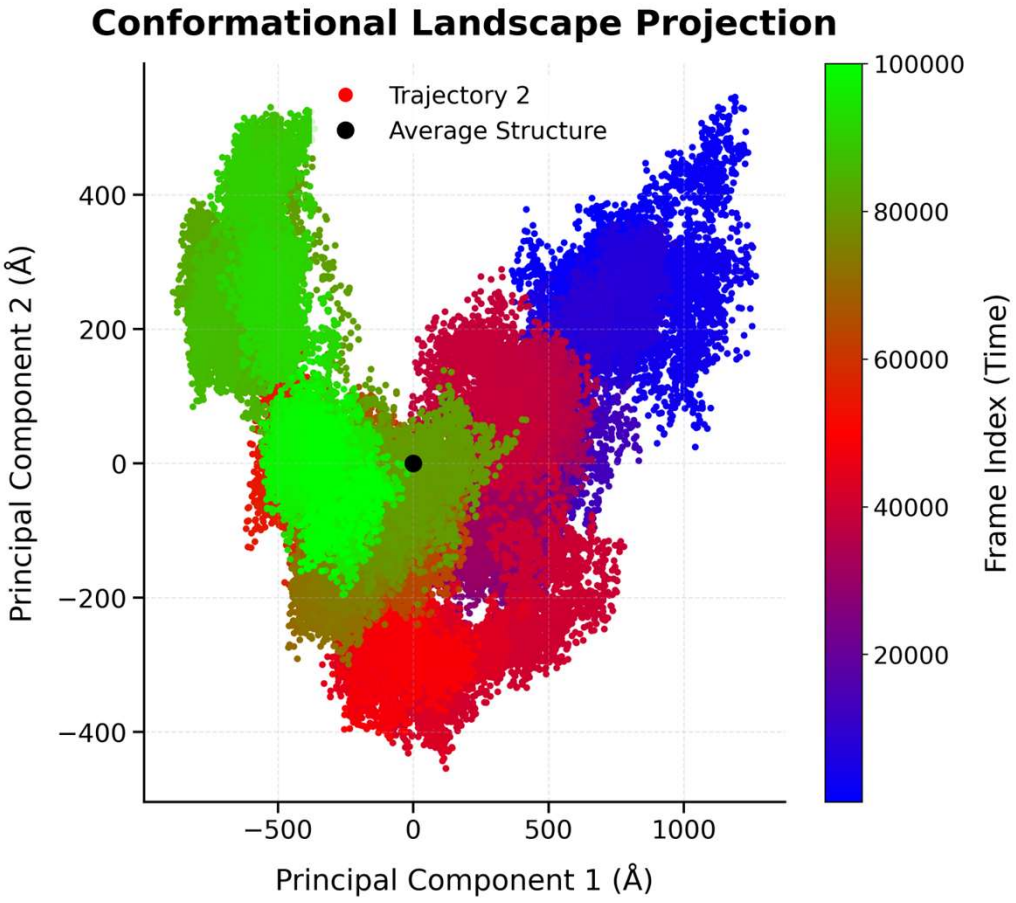
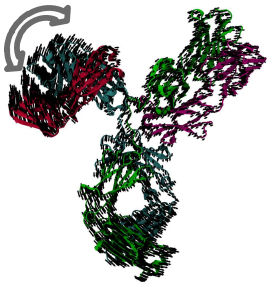


Arrows showing the atomic displacement

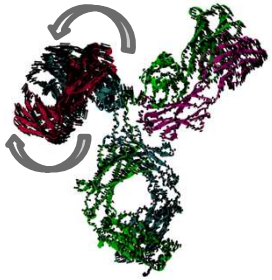
PC1 vs. PC2 for a Single Trajectory



- Trajectory in 2-dimensional space
- Accounts for 80% of the variance in the trajectory
- The average structure is used as a reference for the alignment and projection
- Trajectory migrates across configurational landscape



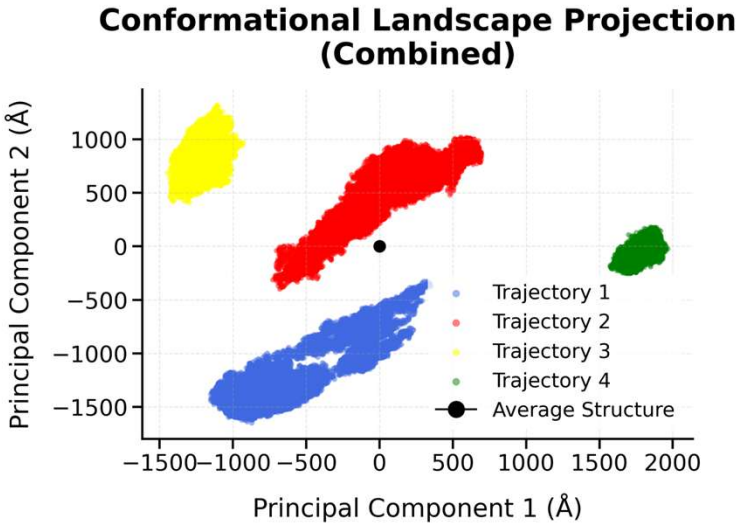
PC1

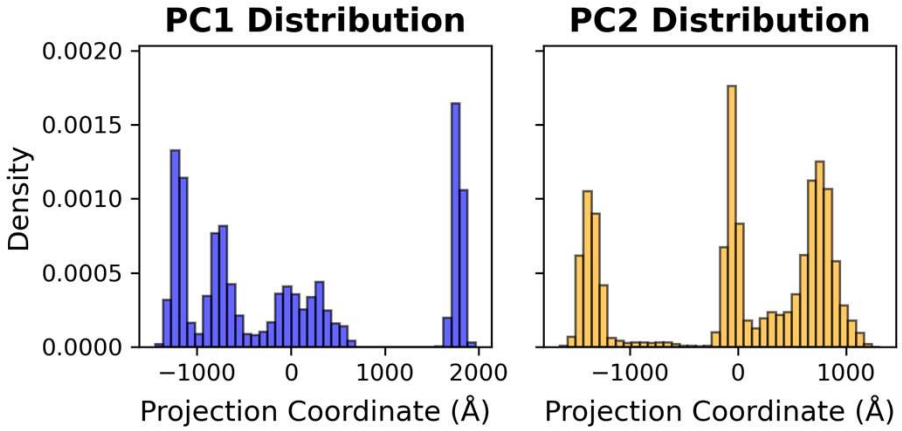


PC2

4 Replicates Combined Trajectory

Combined trajectories to evaluate whether the same dynamic modes are happening in all replicates

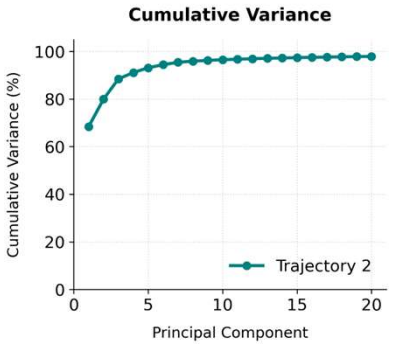


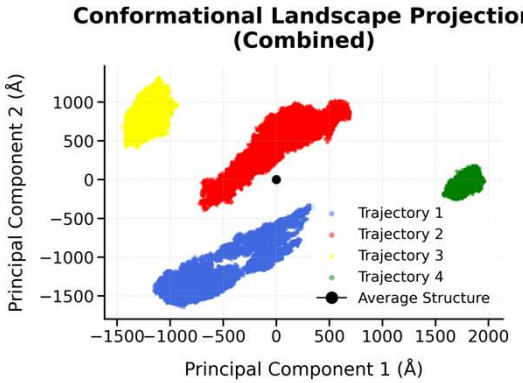


- Reveals that different trajectories sample distinct regions of the configurational space
- Mostly tells us about difference between trajectories instead of motion/dynamic mode

Summary

The essential dynamics of NISTmAb trajectories are each dominated by a few collective modes

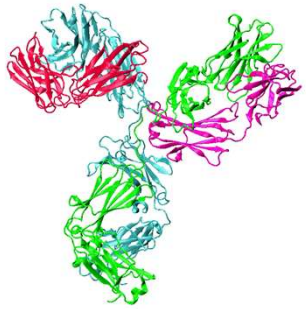




Sampling Limitations: Lack of overlap between replicates suggests that 10 μs is insufficient to fully sample the conformational landscape

Next Steps: Longer simulations or larger ensembles for more complete mapping

Visualizations enable the integration of MD simulations with Neutron Spin Echo (NSE) for improved characterization of flexible biomacromolecules



Acknowledgements

Dr. Shayna Hilburg

Dr. Yun Liu

NIST Center for Neutron Research

NIST RCHPC (Research Computing & High Performance Computing)

Prof. Norman Wagner and group (University of Delaware)

SURF Organizers: Cara O'Malley Dr. Julie Borchers, Dr. Kelsi Rehmann, and Dr. Susana Marujo

Support for SURF students was provided by the Center for High Resolution Neutron Scattering, a partnership between the National Institute of Standards and Technology and the National Science Foundation under Agreement No. DMR-2010792.

CHRNS NSF CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING NIST

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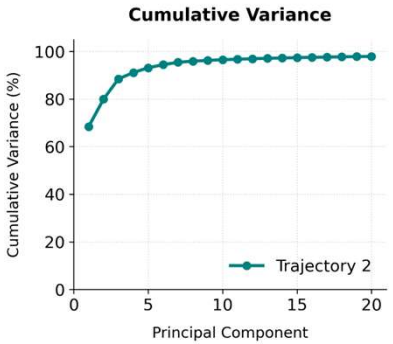

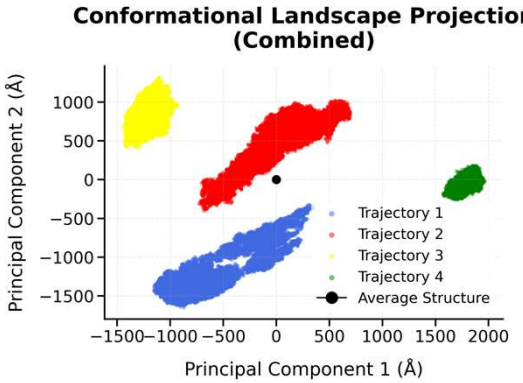

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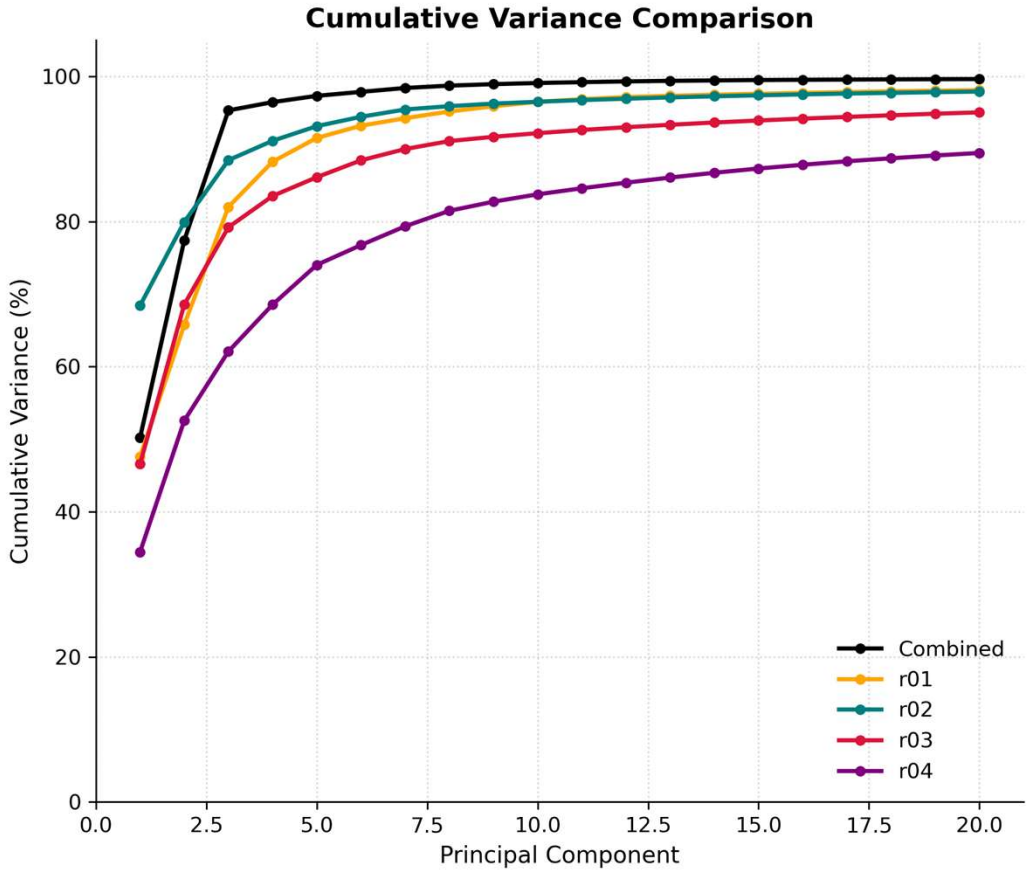
Cumulative Variance of Combined Trajectory



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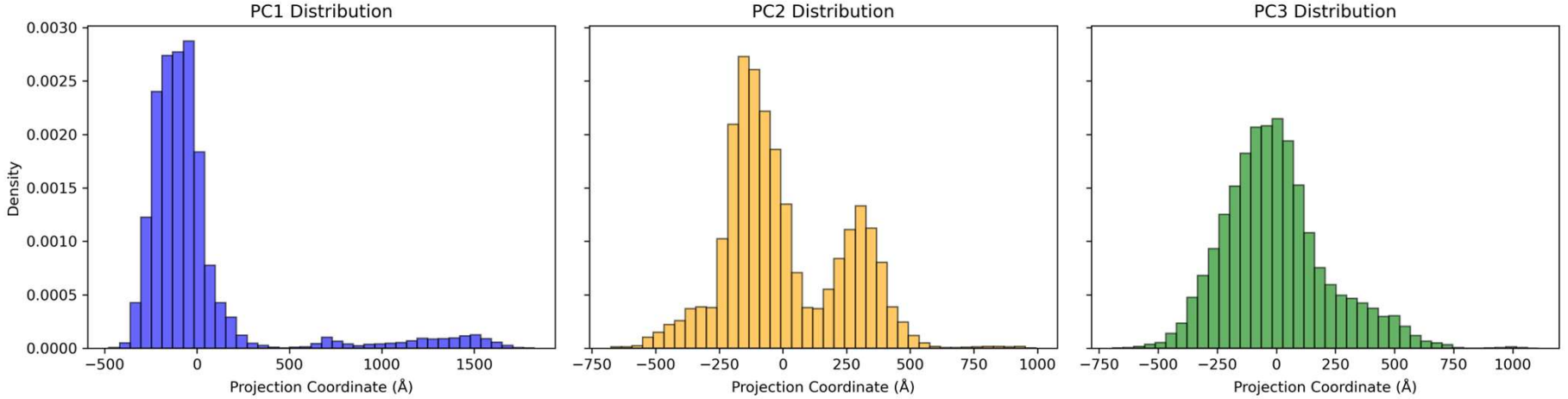
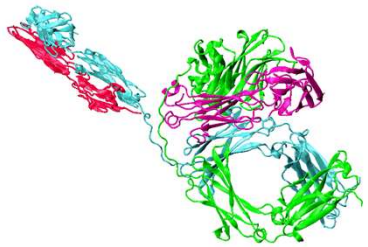
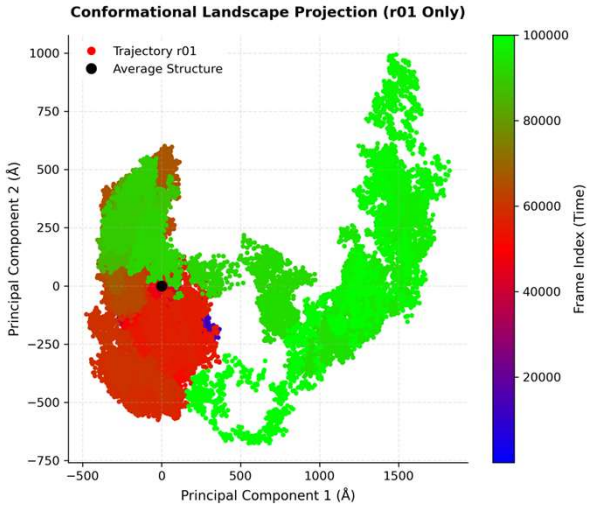
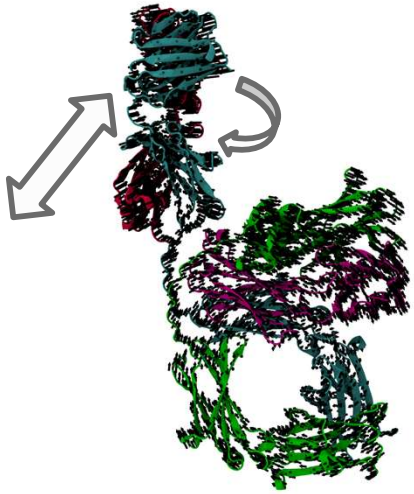
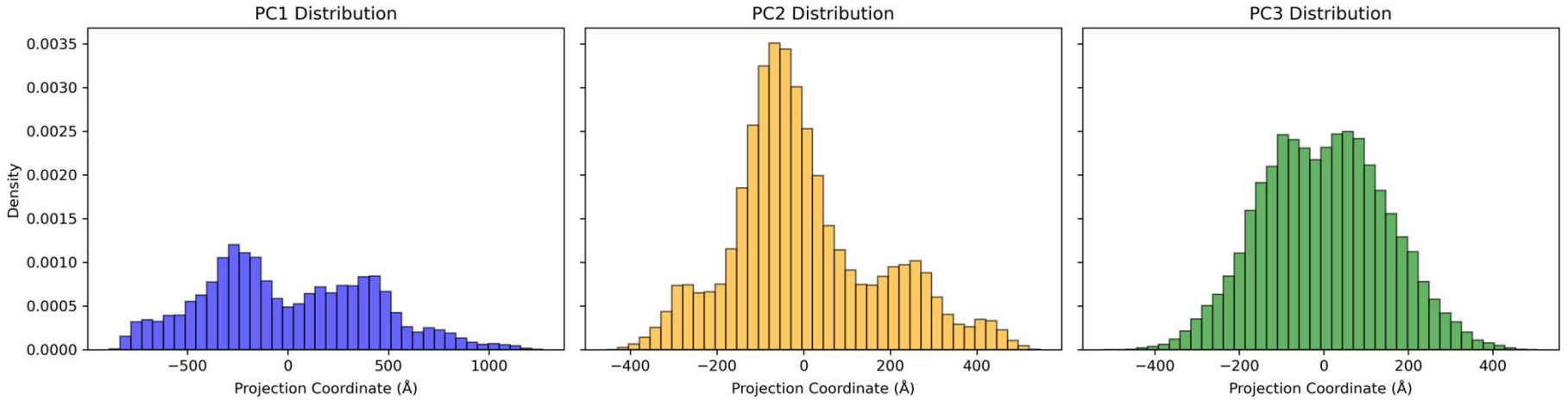


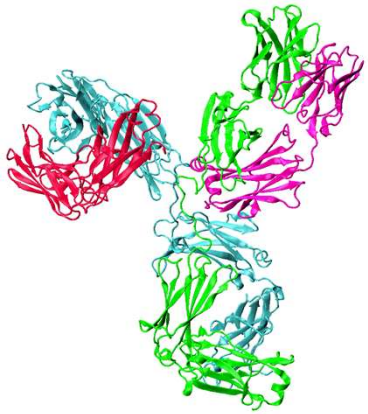


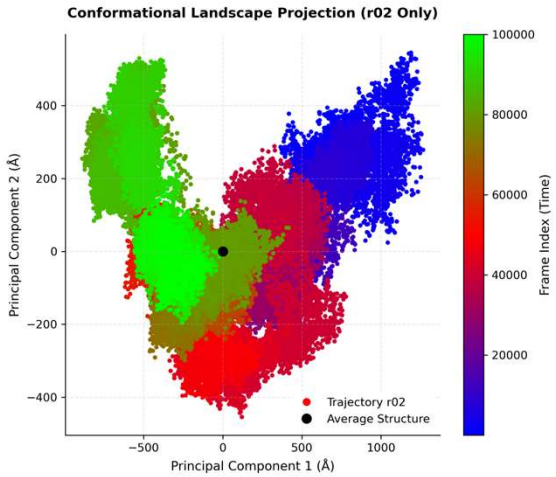


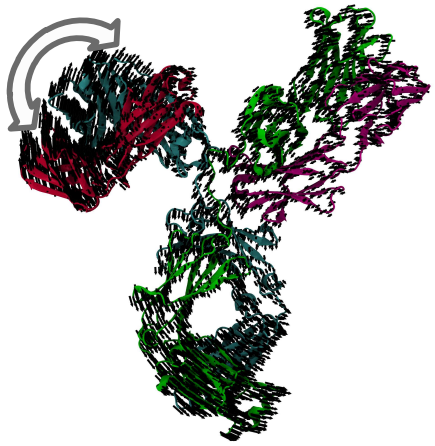


Second Trajectory Data

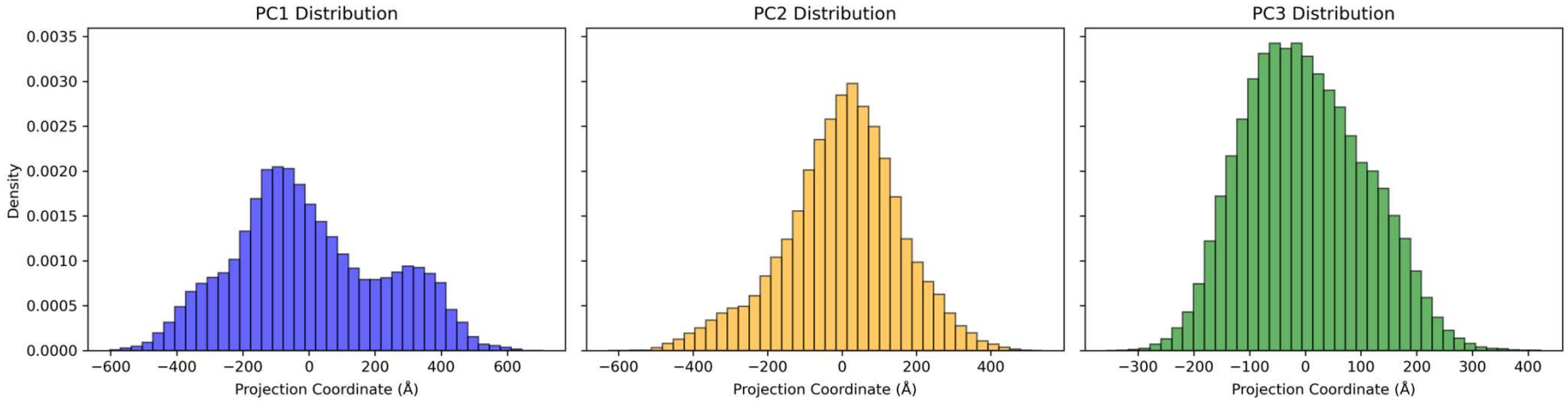


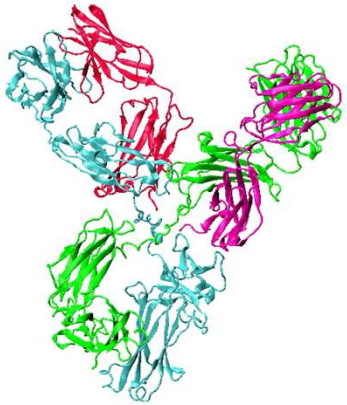


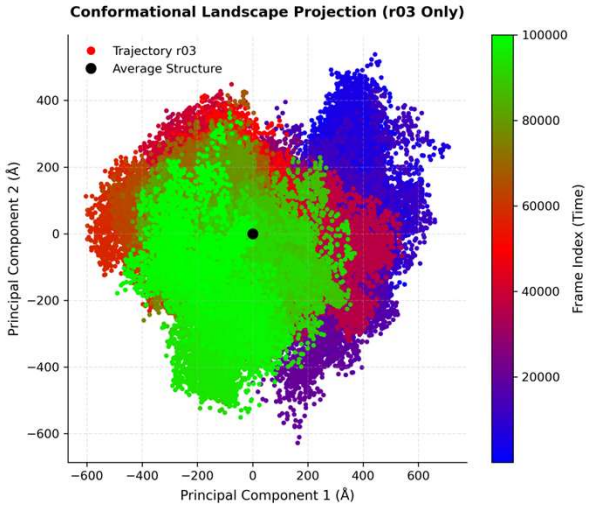


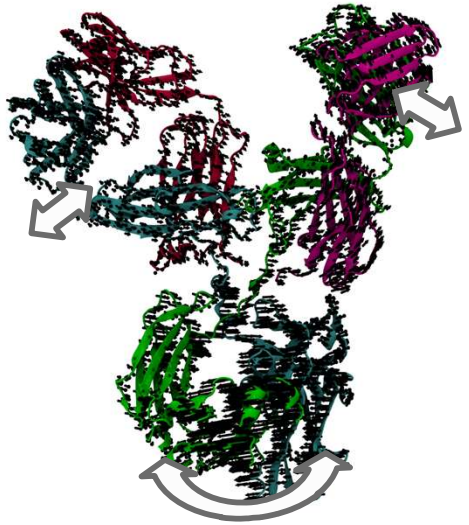


Third Trajectory Data

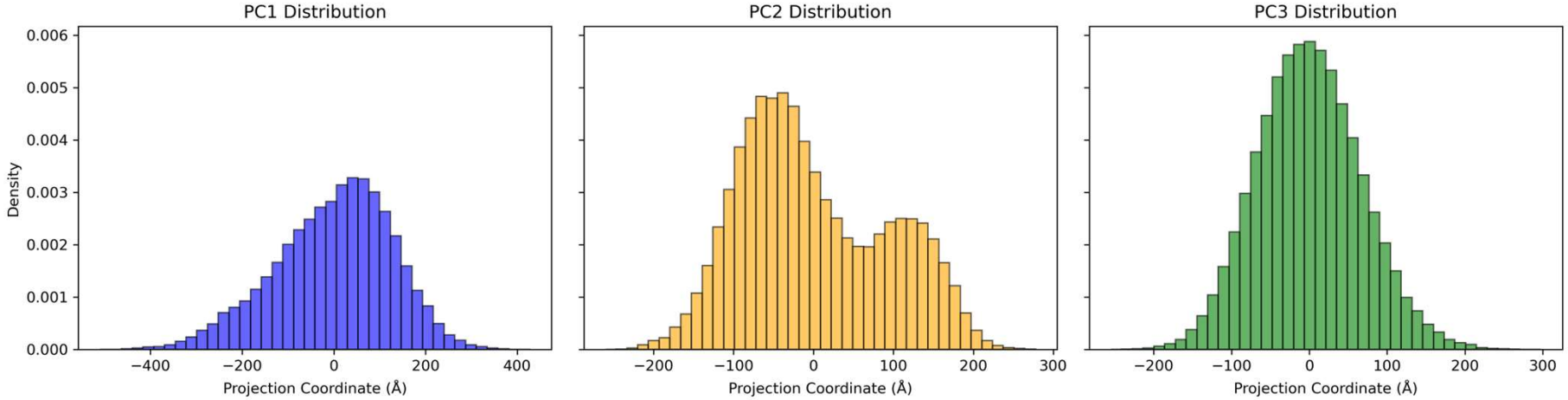


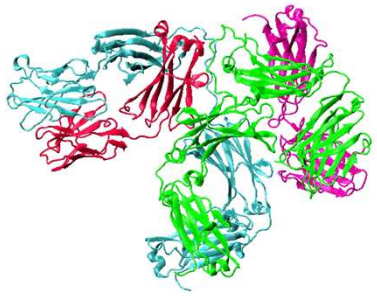


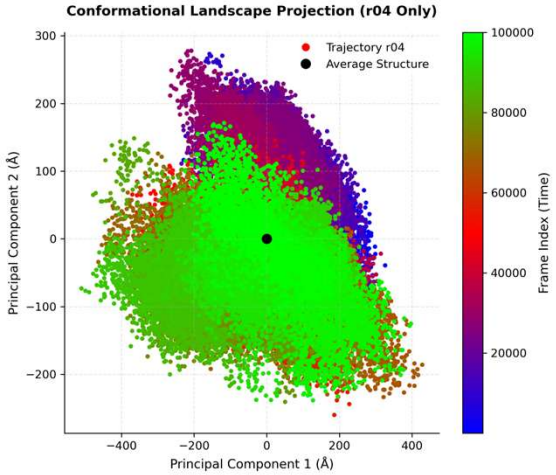


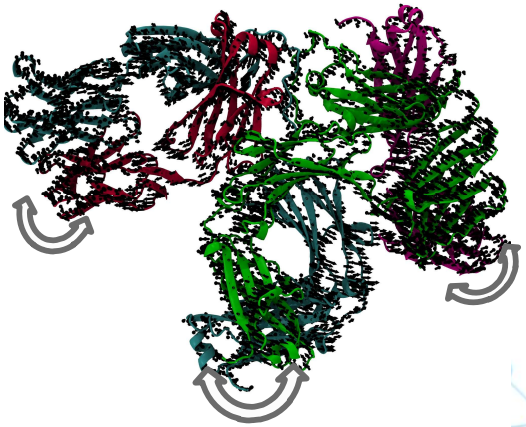


Fourth Trajectory Data

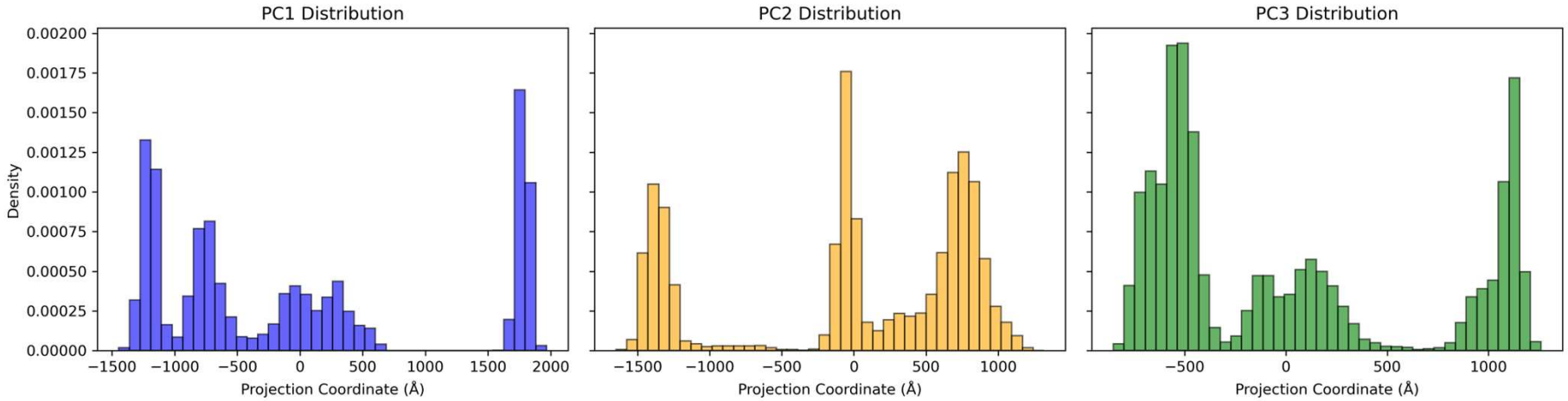


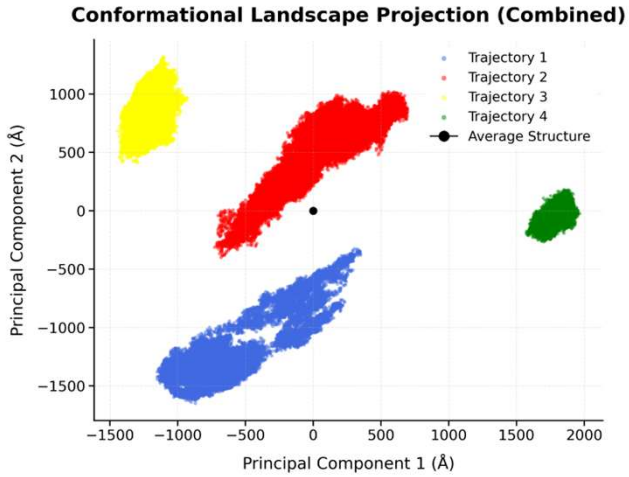




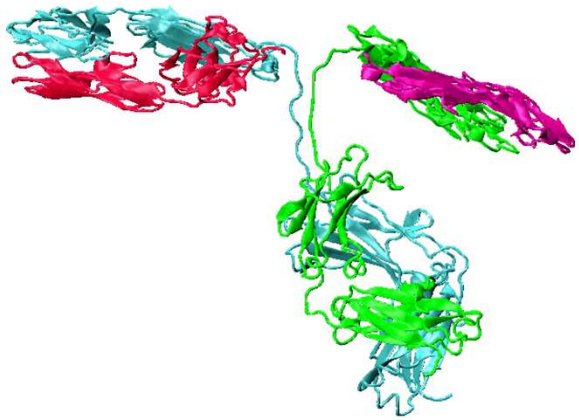


Combined Trajectory Data





Molecular Visualizations Combined Trajectory



PC1 of Combined