EF-X IN SILICO: MODELING PROTEIN DYNAMICS IN AN ELECTRIC FIELD

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ABSTRACT: Protein functions result from local and collective atomic motions that span a wide range of time scales. An integrated analysis of experimental and simulation data can shed light on the detailed mechanism of these motions. Applying a high electric field to protein crystals enables conformational changes that can be captured by time-resolved X-ray crystallography. Such an experiment (referred to as EF-X) carried out on a human PDZ domain obtained a series of atomistic "snapshots" of ensemble averages protein dynamics at 50 to 100 ns time intervals. Here, we present a molecular dynamics (MD) study of the same system and provide a detailed picture of the protein dynamics in between the experimental "snapshots". We replicated the experimental conditions and system geometry in the presence and absence of an electric field. By constructing a model of the protein crystal as a 3x3x3 supercell with a total of 108 individual proteins, we achieved extensive sampling of the protein conformational ensemble at the sub-millisecond time scale. In the study, we quantify the effects of the electric field on the protein structure and crystal symmetry. This work demonstrates how MD simulations can complement information obtained in EF-X experiments by providing the higher spatial and temporal resolution of underlying dynamical processes.

Experiment[1]

Electric Field-stimulated X-ray Crystallography (EF-X)

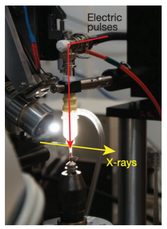


Fig. 1: A view of the assembled experimental EF-X apparatus[1].

- An experiment to determine time-dependent protein dynamics at the atomic level.
- A protein crystal is subjected to an electric field along one of the crystallographic axes.
- X-ray pulses are perpendicular to the electric field, which allows collect diffraction data from different angles.

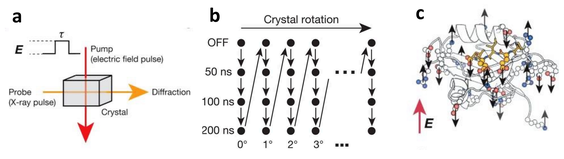


Fig. 2: Experimental protocol and principles of EF-X[1]. (a) E-field is applied along the crystal axis perpendicular to the X-ray pulses. **(b)** The protocol is repeated for a series of crystal rotations to collect diffraction data. **(c)** A sampling of charged residues in LNX2^{PDZ2}, exemplifying potential actuators for applied electric fields.

Physical Concept

- A strong electric field induces conformational changes in a protein crystal: atomic motions due to the charges of the protein atoms and solvent molecules.
- Conformational changes are captured by time-resolved X-ray crystallography: structural "snapshots" are taken 4 times: 0, 50, 100, 200 ns after application of E-field.

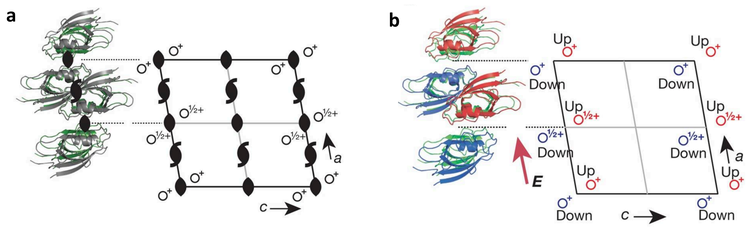


Fig. 3: Crystal symmetry breaking in the EF-X experiment. (a) PDZ domain crystallizes in the *C*2 space group – four molecules per unit cell and one molecule per asymmetric unit. **(b)** E-field ***E*** (applied along the *a* dimension), all rotational symmetry is broken. This results in a new unit cell with two molecules per asymmetric unit (red and blue)—one experiencing +*E*, and one experiencing −*E*.

Results

- Tested on human PDZ domain (LNX2^{PDZ2}): small, well-characterized globular protein.
- Rotational symmetry of the crystal is broken due to the electric field.
- Crystal contains protein chains of two orientations ("up" and "down") relative to the direction of the electric field.

Model

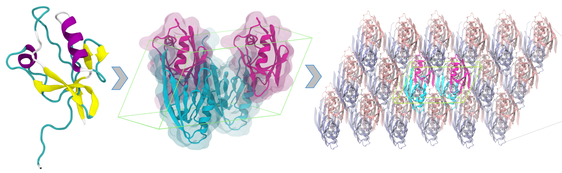
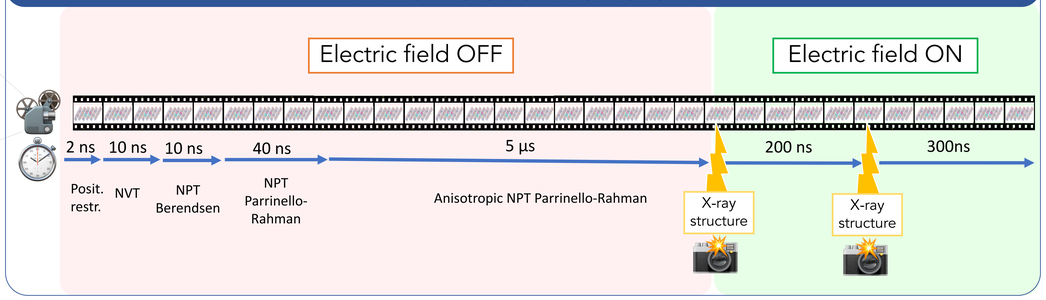


Fig. 4: Stages of model building. A crystallographic unit cell was reconstructed using CHARMM-GUI for the human PDZ domain (PDB ID: 5E11). The unit cell was then replicated 3 times along each crystallographic axis to obtain a 3x3x3 supercell layout

MD simulation flowchart



Simulation: Electric Field OFF

Simulation setup

- Two force fields: AMBER14sb and CHARMM36m (not shown) with TIP3P and CHARMM-modified TIP3P water models, respectively.
- The supercell with 108 individual chains is simulated in GROMACS 2019.1.

Objectives

- Equilibrate the supercell to reach a structural convergence before the application of an electric field.
- Use EF-X structure at 289K (E-field OFF) to evaluate accuracy of MD simulations.

Results

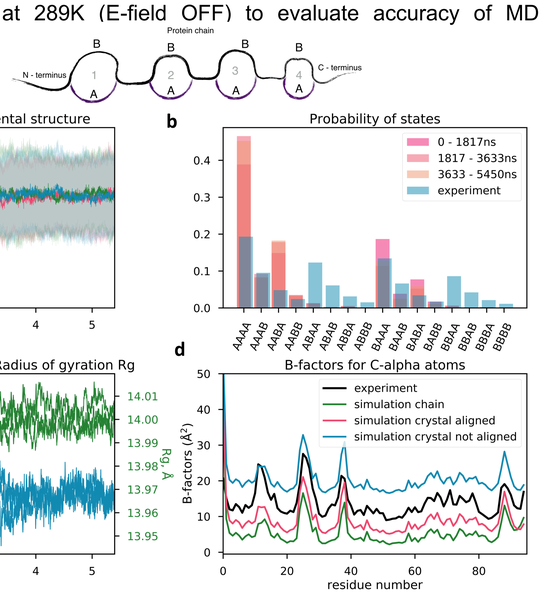


Fig. 5: Results of E-field OFF MD simulations. (a) RMSD from the experimental structure (PDB ID: 5E11) for 3 independent trajectories averaged over all chains in the crystal. Shaded area shows st. dev. (N = 108)**. (b)** Distribution over 16 alternate conformations for a specific simulation interval. **(c)** Fraction of native contacts Q and radius of gyration as a metric of convergence for 3 independent simulations. **(d)** Cα B-factors estimated from MD simulation using a number of approaches[2] in comparison to the experimental values.

Simulation: Electric Field ON

Simulation setup

- Setup is equivalent to E-field OFF MD simulation.
- Constant electric field = 1 MV/cm is applied along ***a*** crystallographic axis.

Objectives

- Simulate the equilibrated crystal for 500 ns in the presence of E Field.
- Use EF-X structure (E-Field ON) to evaluate accuracy of MD simulations.

Results

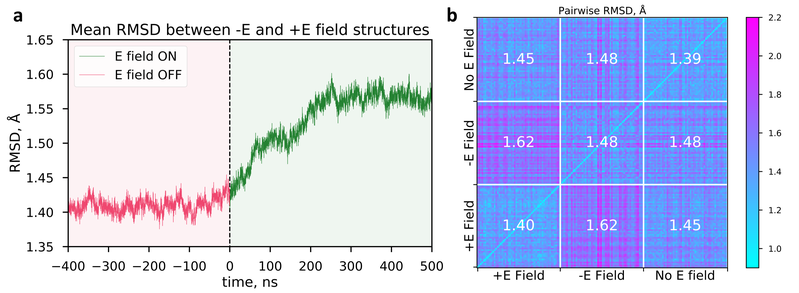


Fig. 6: Results of E-field ON MD simulations. (a) Average pairwise RMSD between all +E and –E chains in a crystal as a function of simulation time. At time = 0, the electric field of 1 MV/cm is turned on and the curve is monotonically increasing. **(b)** Heat map of all to all RMSD, consisting of three blocks of 54 structures each: +E and –E chains at t = 800 ns, and the third block shows structures with no E field at t = 0.

Conclusion

- Equilibration of a protein crystal requires more than 5 µs of MD simulation, which is substantially longer than for solvated proteins.
- E-field ON MD simulation of the PDZ domain supercell shows a crystal symmetry breaking – the effect observed in EF-X experiment due to the electric field.

References

[1] Hekstra DR, White KI, Socolich MA, Henning RW, Šrajer V, Ranganathan R. (2016) Electric-field-stimulated protein mechanics. Nature. 540: 400-405.

[2] Cerutti, DS, Case, DA. (2019) Molecular dynamics simulations of macromolecular crystals. *WIREs Comput Mol Sci*.; 9:e1402.

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