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CXDI and XANES combined for time-resolved study of biological complexes: simulations towards an XFEL experiment

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***Abstract.** Time-dependent CXDI patterns and Mg K-Edge XANES spectra for the human phosphoglycerate kinase were calculated for simulated enzyme conformations and analyzed in the frame of an XFEL experiment. The approach used can be applied to different biological complexes to obtain information about both the active site or the substrate and the state of the whole object.*

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Introduction

Experimental investigation of protein dynamics is a current problem which is becoming more and more solvable with the development of next generation synchrotron radiation sources – free electron lasers, providing extremely short pulses with ultrahigh brilliance sufficient for a single particle imaging. In contrast to the X-ray crystallography where only static states of a protein can be obtained, a single particle imaging provides information on the time evolution of systems under study. Highly coherent X-ray flashes scatter on the sample and diffraction patterns are registered by a detector. The structure of the sample can be then obtained by evaluating these patterns. A technique for obtaining and evaluating such kind of patterns is a Coherent X-ray Diffraction Imaging (CXDI).

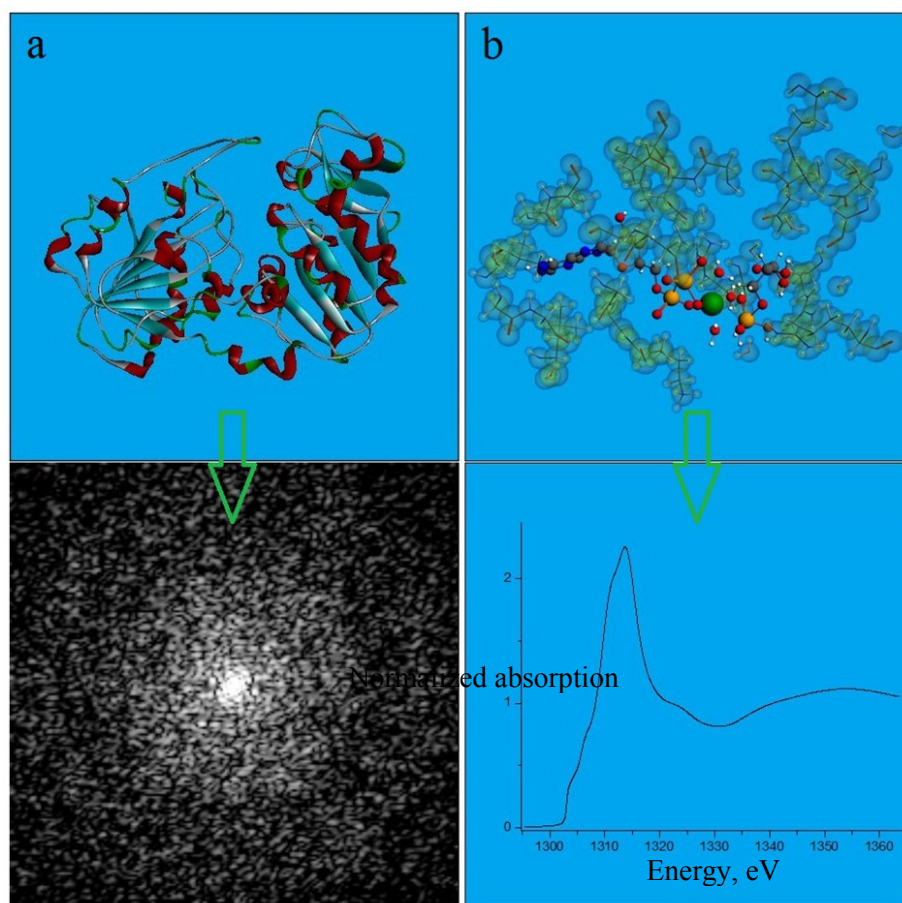


Figure 1. Illustration of a single step of the performed calculations. The enzyme structure obtained from molecular dynamics simulations is used for CXDI calculations (left). A structure of an active site is optimized by the molecular orbital package with fixed geometry of enzyme binding site (right top). The final structure is then used for the Mg K-edge XANES calculation (right bottom).

In this work a simulation of a possible experiment was performed for a phosphoglycerate kinase (PGK) [1], an enzyme which plays an important role in the glycolysis. It consists of two domains (N- and C-) which are associated with large-scale 'hinge-bending' conformational changes [2-4], which makes it an interesting object for the time-resolved investigation. PGK catalyzes the phosphate transfer from the 1,3-bisphosphoglycerate (bPG) bound to the N-domain to the adenosine diphosphate (ADP) bound to the C-domain to form an adenosine triphosphate (ATP) molecule. The phosphate transfer reaction requires domain closure to make the distance between a phosphoglycerate and a nucleotide (ADP) enough for chemical reaction. A divalent Mg atom usually binds to negatively charged nucleotides [5] which makes it possible to apply the X-ray Absorption Spectroscopy (XAS) to control the coordination of the absorbing Mg atom.

We applied XAS and CXDI techniques together to obtain complementary information on the sample structure. A process of domain closure was simulated by molecular dynamics (MD). Obtained structures were then used for a diffraction patterns' calculation (fig.1a). The structure of the active site (fig.1b) was optimized in the molecular orbital package (MOPAC) and Mg K-edge XANES spectra were calculated. The approach used can be applied to investigate dynamical properties of any metal containing biological sample.

Molecular dynamics simulations

The initial structures for the MD simulations were taken from the RSBS Protein Data Bank (PDB): a half-closed PGK of *Thermotoga maritima* (1VPE) [3] and an open human PGK (2XE7) [4]. Both structures presented in the RSBS PDB were obtained by X-ray diffraction on crystallized samples with a resolution of 2.00 Å for 1VPE and 2.20 Å for 2XE7. 1VPE has a 3-phosphoglyceric acid (3PG) and a phosphoaminophosphonic acid-anenylate ester (ANP) as a substrate. 2XE7 structure contains 3PG and ADP.

MD simulations were performed with the Fujitsu Scigress software using the MM3 force field [6]. The total function of potential energy of the system included the following terms: bond stretching, angle bending, torsion, stretch-bend interaction, torsion-stretch interaction, torsion-bend interaction, bend-bend interactions, Van der Waals' interactions, electrostatics (dipole approach), dihedral angle and hydrogen bonds. Van der Waals interaction between atoms separated by more than 9 Å was excluded.

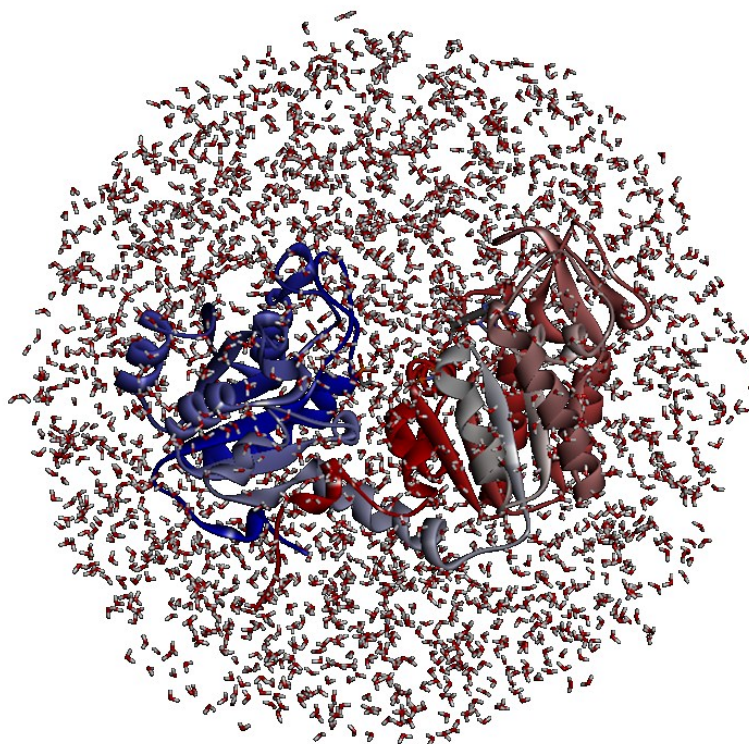


Figure 2. The structure of the phosphoglycerate kinase from *Thermotoga maritima* surrounded by water and optimized by Molecular Mechanics. This sample was used as the initial geometry for the Molecular Dynamics simulation of enzyme conformation from half-closed to closed state.

A half-closed structure of PGK (1VPE) was surrounded by a 9 nm sphere of water (fig.2). The initial geometry was optimized by molecular mechanics tools using the same MM3 force fields with the parameters described above. For the 1VPE a temperature was set to 300°K. For 2XE7 MD calculations were performed at different temperatures (200 – 450 °K).

At both closed and open conformations molecular mechanics geometry optimization was applied to obtain stationary point geometries (fig.3).

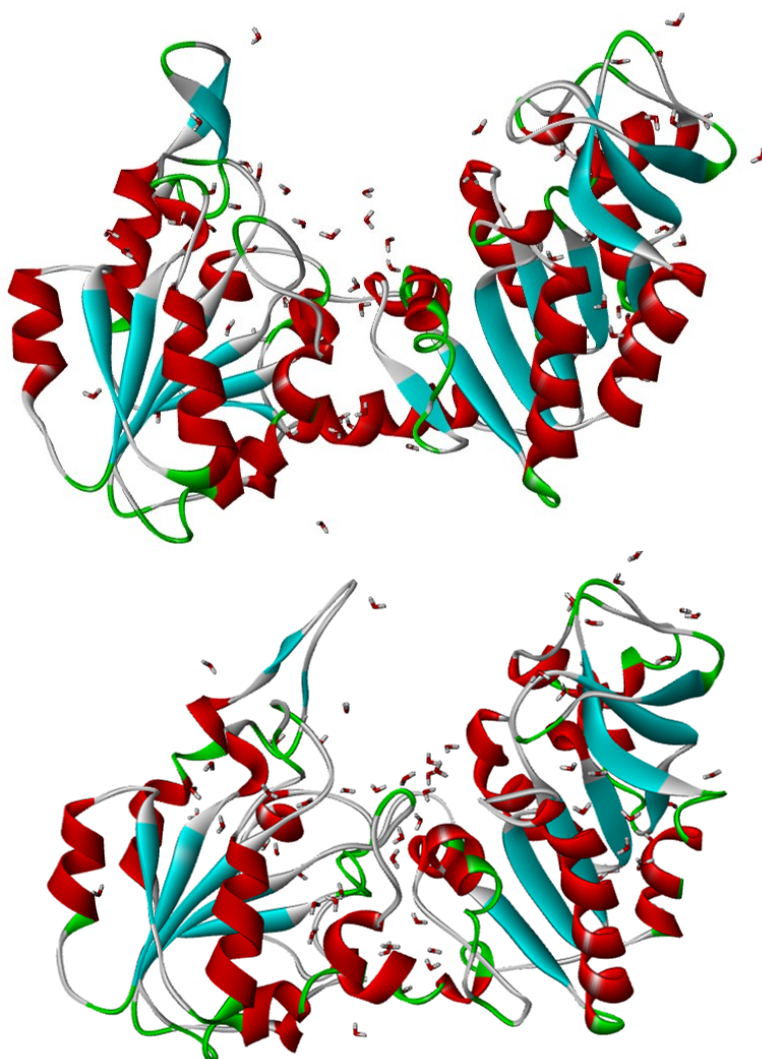


Figure 3. Structures corresponding to stationary geometries (Molecular Mechanics). Top: open conformation of human phosphoglycerate kinase taken as initial geometry for dynamics simulations. Bottom: optimized structure of closed state taken from molecular dynamics.

The 1VPE structure showed a tendency towards domain closure which supports the notion that the crystalline structure of PGK does not correspond to its stable state in free phase [4]. The timescale of domain closure is 10 picoseconds. This effect was independent of substrate presence. A closure takes place both with and without water. In further calculation only a few water close to the enzyme structure was taking into account.

The 2XE7 structure also showed a domain closure tendency on the time scale of 15 picoseconds without water. The distance between two carbon atoms of different domains was used to monitor the closure process (fig.4). For higher temperatures ($\sim 450^\circ\text{K}$) the enzyme stays in the open state.

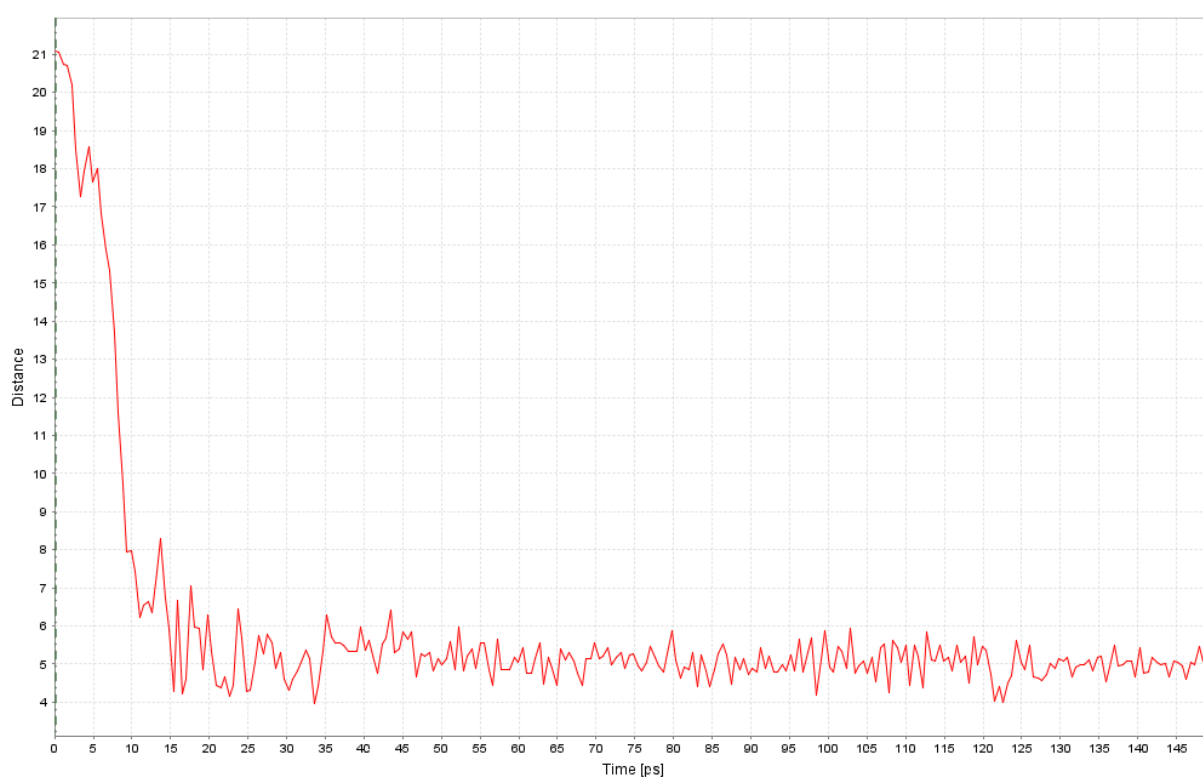


Figure 4. Time dependence of the inter-domain distance at room temperature. A distance in Å between the two selected carbons corresponding to the two different domains is plotted.

CXDI calculation

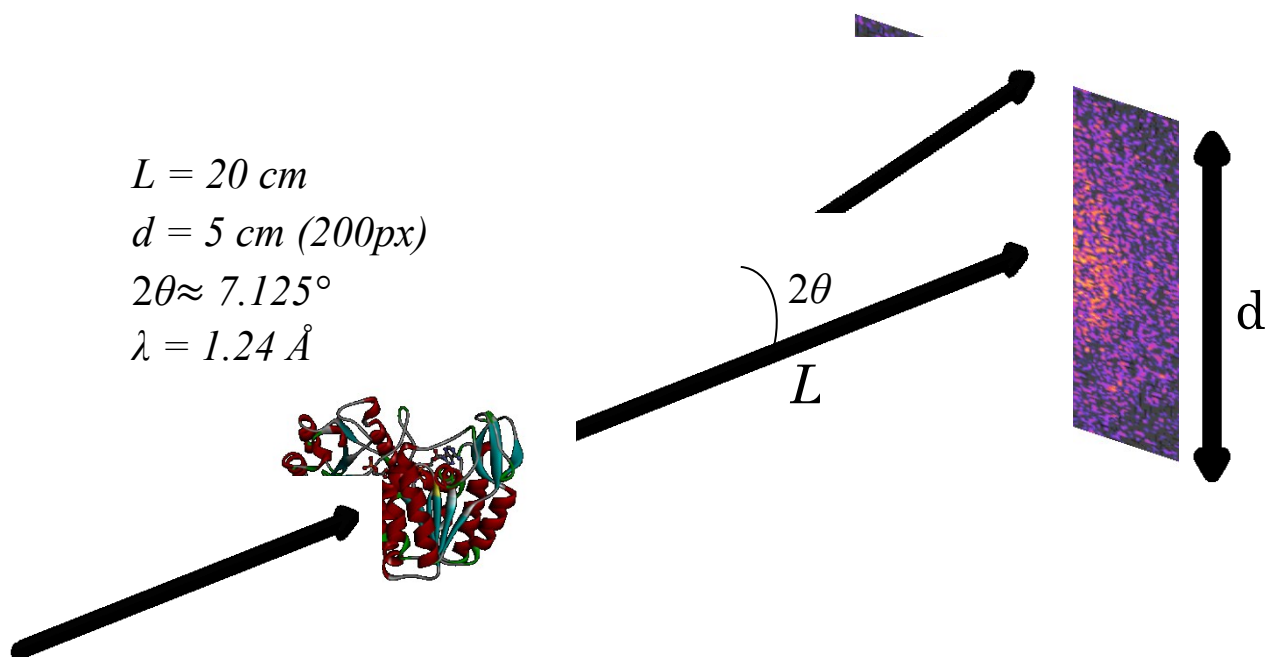


Figure 5. Typical geometry of a CXDI experiment and parameters used in calculations.

Structures from MD simulation were used for CXDI calculations. For each picosecond a diffraction pattern was calculated using the MOLTRANS program [7]. An X-ray wavelength of 1.24 \AA was set. According to the values described in fig.5 the resolution is about 10 \AA . The plane of the enzyme closure angle was oriented perpendicularly to the incoming beam. Diffraction patterns were collected for 30 ps with a step of 1 ps. Diffraction patterns of the 1VPE model at selected time steps are plotted in fig.6a. The corresponding projections of the enzyme are shown (fig.6b).

Small Angle X-ray Scattering (SAXS) signals for open and closed conformations were evaluated in water solution by CRY SOL [8]. The calculated radial intensity distributions are shown in fig.7.

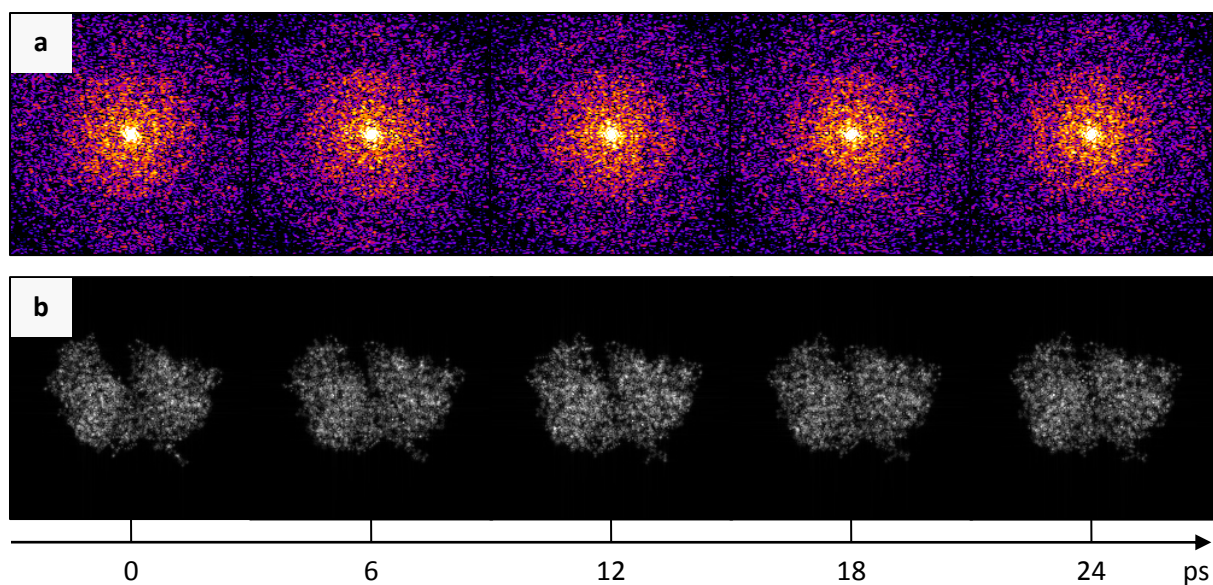


Figure 6. Diffraction patterns and corresponding projections of PGK during its transition from the half-closed to the closed states.

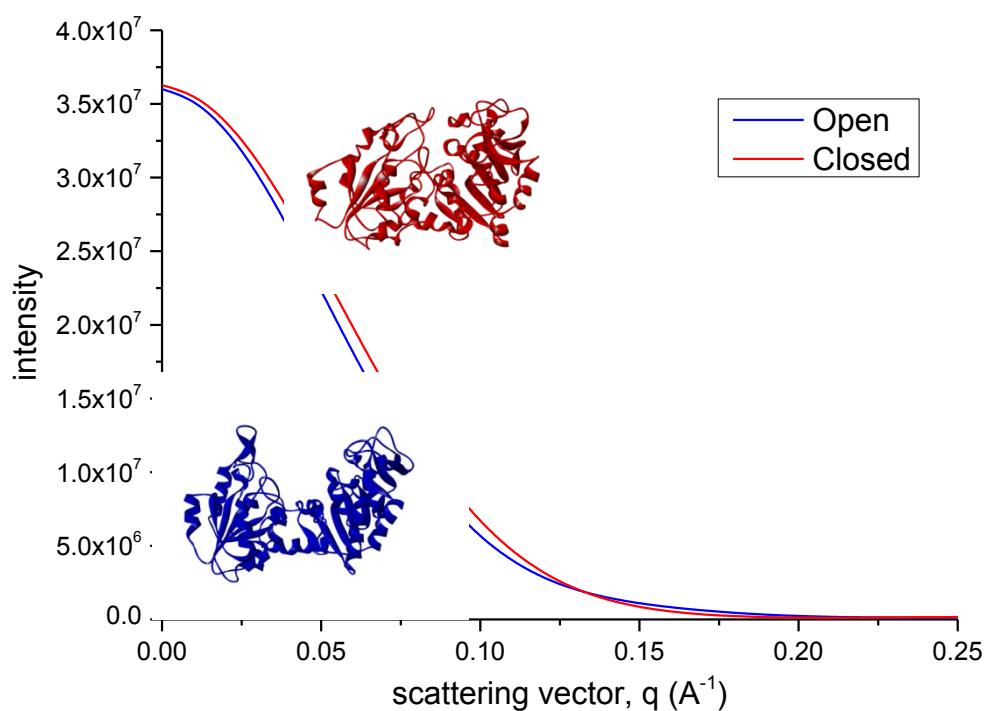


Figure 7. Results of SAXS calculation for the open (blue) and the closed (red) states of PGK in water. The structures used are described in fig.3.

Substrate optimization and XANES study

While CXDI measurements are useful to get information of the overall structure of the enzyme, no detailed information about substrate (ADP and 3PG) is obtained. If the sample contains metal atom (or other atom which is unique in the sample), so-called absorbing atom, we can use XANES to investigate the atomic and electronic structure of the local environment around the absorbing atom.

XANES spectra are sensitive to even small changes in the local geometry, so precise structures are required to fit calculated spectra to experimental data. To obtain a more precise geometry of the substrate than that achieved by classical molecular dynamics, we carried out semi-empirical calculations in the molecular orbital package MOPAC2012 [9]. The PM7 semi-empirical Hamiltonian was used to obtain molecular orbitals.

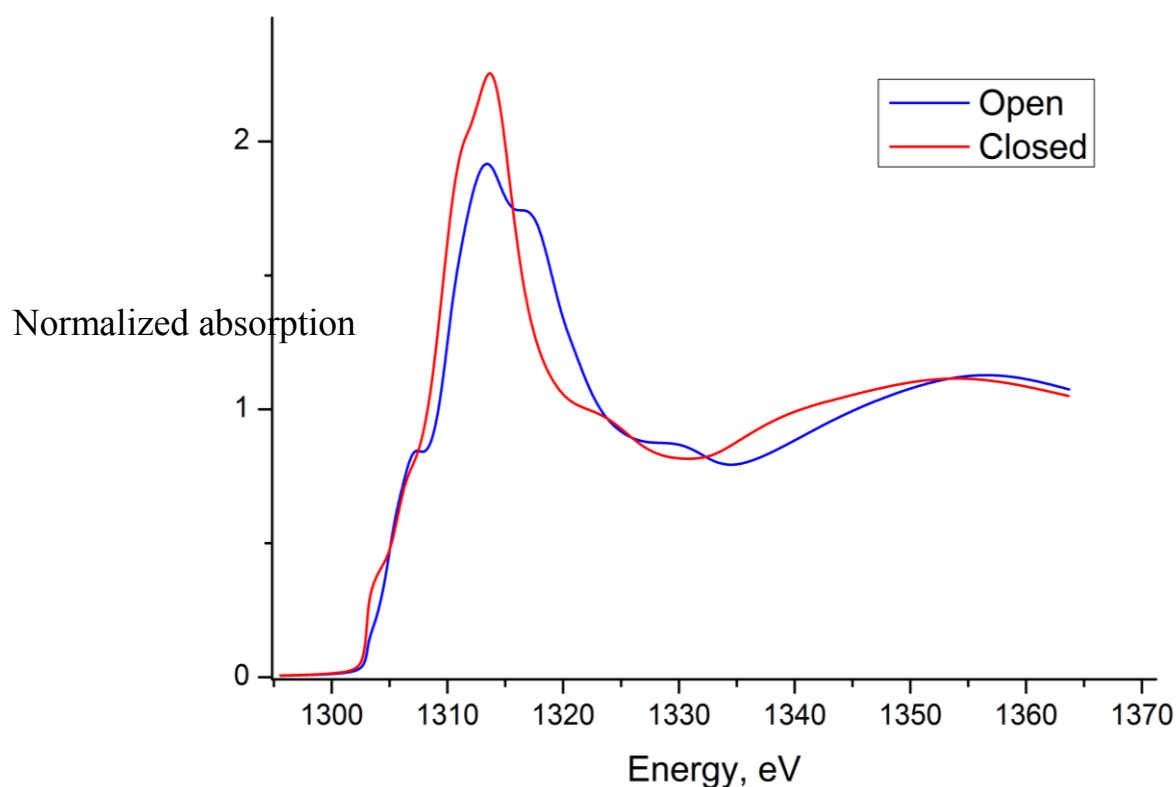


Figure 8. Mg K-edge XANES for MgADP in open (blue) and closed (red) conformations.

The geometry optimization of MgADP and 3PG was done at each picosecond time step; structures from MD were used as initial geometries. The binding sites of the enzyme with the molecules of the substrate and water molecules were involved in MOPAC optimization. Coordinates of binding site atoms were considered to be fixed as they refer to some intermediate steps of the conformational path and should not be optimized. We assume molecules of the substrate (MgADP and 3PG) to be flexible enough to fit changes of the binding site structure so they are at every time in the optimal position for the given binding site geometry. Magnesium atom bound to the oxygen atoms of ADP was also surrounded by water molecules to take into account an effect of non-covalent Mg-O bonding between Mg and water molecules.

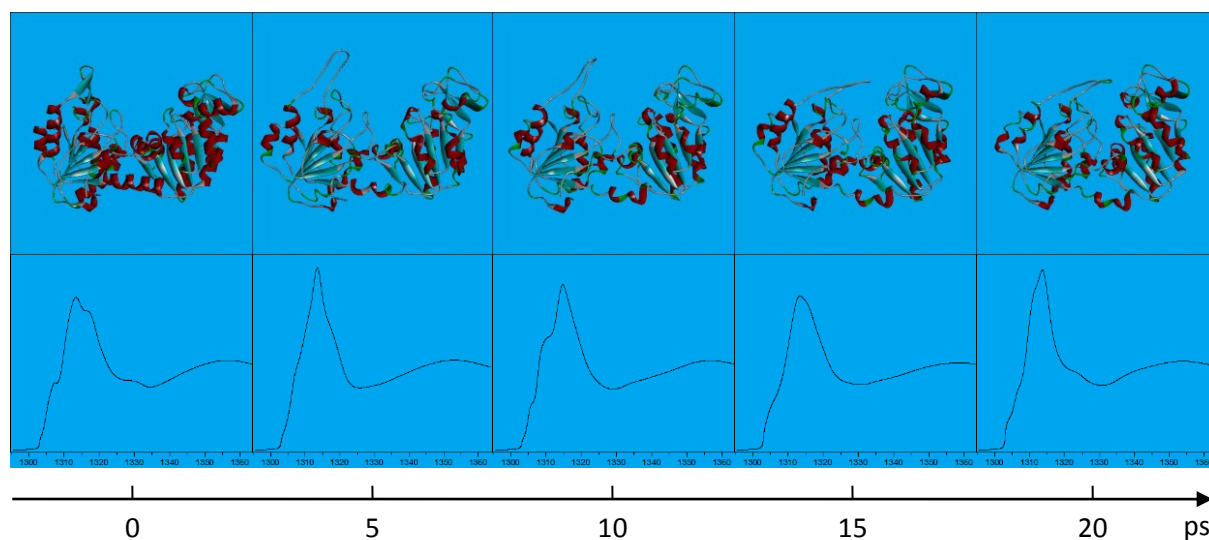


Figure 9. Enzyme structure and corresponding diffraction patterns and Mg K-edge XANES of human phosphoglycerate kinase during its transition from open to closed state.

Mg K-edge XANES spectra were calculated with FEFF9 code [10-11]. The structures involved in FEFF calculations represent spherical clusters with a radius of 13 Å around the absorbing Mg atom. Full multiple scattering calculations were performed within a sphere of 8 Å radius, and the radius of self-consistent field calculation was set as 4 Å. XANES spectra for the open and closed states are shown in fig.8. In the fig.9 corresponding CXDI patterns are presented together with Mg XANES for the total closure process.

According to the optimization results the Mg atom was octa-coordinated, having water and phosphate oxygen atoms as nearest neighbors. An effect of water

movement on Mg K-edge XANES is shown in fig.10. As in the closed conformation one of the nearest to Mg water molecules is replaced by a phosphate the spectra should be less smeared in closed conformation.

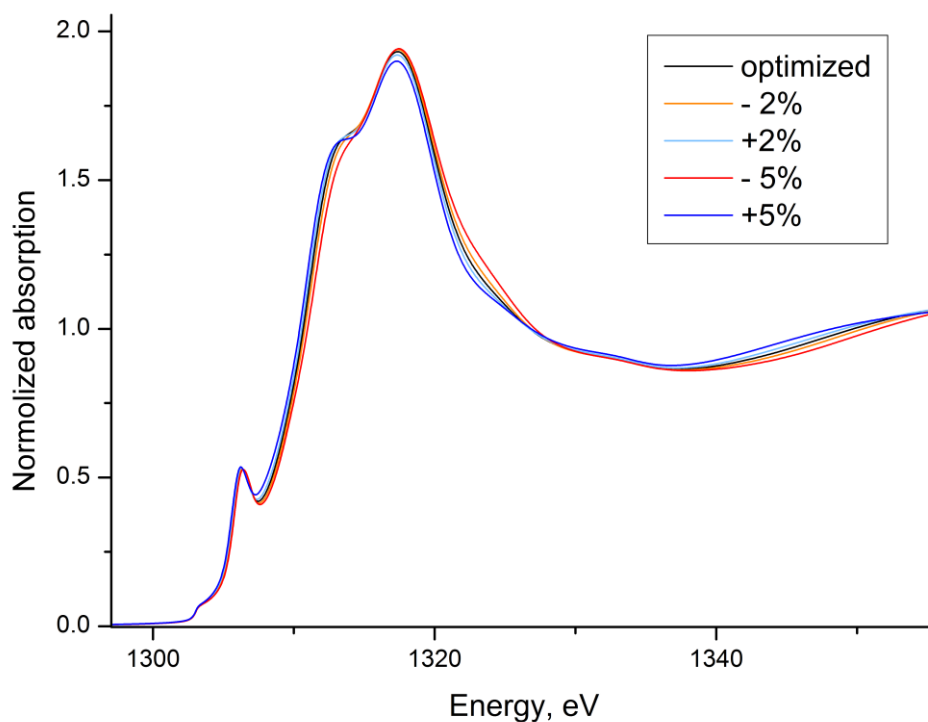


Figure 10. Effect of water movements on Mg K-edge XANES. The black curve corresponds to the optimized geometry (MOPAC). Colored curves reflect the dependence of XANES on movements of one water molecule in direction towards and out of the Mg atom by 2% and 5% of the optimal Mg-O distance.

Results and discussion

The PGK conformation change from the half-open and half-closed to the closed state were simulated by molecular dynamics methods. We find that enzyme structures obtained by X-ray crystallography do not refer to stable positions in the free phase. Both 1VPE and 2XE7 showed a closure tendency which is physically reasonable for making ATP formation possible. Potential energy calculations also showed that the closed structure is more preferable, mainly due to electrostatic interaction and hydrogen bonding. For 1VPE we observed no substrate dependence. Water presence insignificantly affected the total closure time. The closure time for 2XE7 was about 15 picoseconds (fig.4).

CXDI patterns calculated using parameters mentioned above have enough resolution to distinguish enzyme conformations even for transition from half-closed to closed (fig.6).

But one can obtain such kind of patterns only if it is possible to put enzymes in a known order. Otherwise all possible orientations will contribute to one pattern and only radial intensity distribution will yield information, mainly on the size and the shape of the sample (fig.7).

The closure process appeared to be temperature dependent. For high temperatures (400 – 450°K) PGK stayed in the open conformation while for lower temperatures domains were closing. Such dependence might give an experimental possibility to control PGK conformation.

XANES calculations showed high sensitivity to local changes of the substrate molecules. Water molecules close to Mg atom also affect XANES spectra. In experimental conditions smearing of the obtained XANES spectrum caused by water molecules presented (fig.10) as the Mg nearest neighbors should be reduced in closed state of enzyme.

In summary, we suggest an approach to measure CXDI and XANES simultaneously, to follow both the overall structure of the sample and changes in the active site. This approach might be applied to various biological samples, like metal containing proteins, which are associated with conformational changes. Simulations of conformational processes may give additional knowledge possible structural variations, which should help in experimental data evaluation.

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