Insights into the structural dynamics of Liver kinase B1 (LKB1) by the binding of STe20

Related Adapter α (STRAD α) and Mouse protein 25 α (MO25 α) co-activators

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Supplementary Information

1. Materials and Methods

1.1 Analysis of Correlation between residues

The Dynamic Cross Correlation Map (DCCM) between pairs of atoms i and j were obtained from the last 50ps of the trajectories of all systems. The covariance matrix (C_{ij}) was generated using the C α -atoms of residues (Hunenberger *et al.* 1995; Ichiye and Karplus 1991). The scaled version of covariance yields the correlation coefficients ranging between -1 and +1.

$$C_{ij} = \frac{\langle r_i r_j \rangle - \langle r_i \rangle \langle r_j \rangle}{\sqrt{\langle r_i^2 \rangle \langle r_j^2 \rangle}}$$

The negative value indicates the residues/domains moving in opposite direction (i.e. anti-correlated motion) and the positive value explains the concerted motions between residues. The value zero indicates the uncorrelated regions. The correlation analysis was performed using the CPPtraj module incorporated in AmberTools13 (Daniel R. Roe and Thomas E. Cheatham 2013).

1.2. Binding Free Energy Analysis

The binding free energy of LKB1 (as receptor) with ATP, STRAD α and MO25 α (as ligands) were calculated using g_mmpbsa tool and the equation used is given below (Kumari *et al.* 2014)

$$\Delta G_{\text{binding}} = \Delta G_{\text{complex}} - (\Delta G_{\text{receptor}} + \Delta G_{\text{ligand}})$$

Where,

$$G = \Delta E_{\text{mm}} + \Delta G_{\text{sol}}$$

$$\Delta E_{\text{mm}} = \Delta E_{\text{vdW}} + \Delta E_{\text{elec}}$$

$$\Delta G_{\text{sol}} = \Delta G_{\text{pol(PB)}} + \Delta G_{\text{nonpol(SASA-only)}}$$

$$\Delta G_{pol(PB)} = \nabla \cdot [\epsilon(r) \nabla \cdot \varphi(r)] - \epsilon(r) \kappa(r)^2 \text{Sinh}[\varphi(r)] + 4\pi \rho^f(r) / K_T$$

$$\Delta G_{nonpol} = \gamma \text{SASA} + \beta$$

The ΔE_{mm} is the molecular mechanics energy calculated from van der Waals and electrostatic energy. The $\Delta G_{pol(PB)}$ is the polar solvation energy calculated using Poisson Boltzmann method. The dielectric constants 8 and 80 were used for the solute and solvent, respectively and a grid space of 0.2 Å was used for polar solvation energy calculation. The non-polar solvation energy was calculated using SASA model with a probe radius of 1.4 Å.

1.3. Principal Component Analysis

Principal component analysis was used to extract the dominant modes of dynamics and the last 10 ns trajectories from the 30 ns simulation were used for all systems. The overall translational and rotational motions were removed by fitting into a reference structure and the results were presented based on the mass weighted analysis. A configurational space was constructed using Cartesian coordinates by generating the 3N-dimensional covariance matrix (C) of Ca atoms which is a symmetric matrix. The covariance matrix (C) was diagonalized by an orthogonal coordinate transformation to generate a set of eigenvectors and eigenvalues as explained below.

$$C = \langle (x - \langle x \rangle)(x - \langle x \rangle)^T \rangle$$

$$C = T \Lambda T^T$$

The eigenvectors represent the direction of atomic motions and the corresponding eigenvalues represent the magnitude of atomic fluctuations (Amadei *et al.* 1993; David and Jacobs 2014; Issack *et al.* 2012; Stepanova 2007).

1.4. Free energy landscape

The free energy landscape of LKB1 in 2D was generated using g_anaeig and g_sham

programs available in Gromacs package 4.5 (Walter R. P. Scott 1999). Both PC1 and PC2 modes were selected as the reaction coordinates for the 2D representation of free energy.

$$G(\text{pc1}, \text{pc2}) = -K_B T \ln P(\text{pc1}, \text{pc2})$$

Where, K_B is the Boltzmann constant, T is the temperature (300K) and $P(\text{pc1}, \text{pc2})$ is the probability distribution as a function of conformational sampling of reaction coordinates (Maisuradze *et al.* 2010; Tavernelli *et al.* 2003).

Figure S1

Superimposition of both crystal (PDB ID: 2WTK) and simulated (extracted at 30 ns) structures of LKB1(ATP)-STRAD α (ATP) is shown in panel A and the total number of H-bonds formed between LKB1 and STRAD α during simulation is plotted in panel B.

Figure S2

Superimposition of the crystal (colored in grey) and modeled (colored in pink and green) structures of LKB1(ATP)-STRAD α (ATP).

Figure S3

Superimposition of the crystal (colored in grey) and modeled (colored in cyan, pink and green) structures of LKB1(ATP)-STRAD α (ATP)-MO25 α .

Table S1

The list of H-bonds formed in LKB1:STRAD α and LKB1:MO25 α . Both donor and acceptor of the H-bond are superscripted as D and A, respectively.

LKB1:STRAD α (in the absence of MO25 α)	
ASN252 ^D	ARG331 ^A
GLN251 ^D	ARG74 ^A
LYS235 ^D	THR189 ^A
GLN225 ^D	GLU317 ^A
HIS223 ^D	HIS154 ^A
GLU110 ^D	ARG106 ^A
ASN109 ^D	ARG106 ^A
ARG331 ^D	ASN252 ^A
LYS191 ^D	GLU110 ^A
GLY188 ^D	ASP232 ^A
GLY187 ^D	ASN252 ^A
GLN123 ^D	GLU430 ^A
LYS122 ^D	PHE431 ^A
TYR118 ^D	ASP427 ^A
GLN112 ^D	GLU110 ^A
ARG106 ^D	GLU105 ^A
ARG106 ^D	CYS107 ^A
ARG103 ^D	ASP427 ^A
ARG74 ^D	GLN250 ^A
ARG74 ^D	GLN251 ^A
LKB1:STRAD α (in the presence of MO25 α)	
ALA284 ^D	SER69 ^A
GLN251 ^D	LEU72 ^A
ARG74 ^D	GLN251 ^A
LKB1:MO25 α	
HIS241 ^D	GLU165 ^A
ARG240 ^D	ALA205 ^A
ARG240 ^D	SER169 ^A
ARG240 ^D	LEU201 ^A
GLN109 ^D	LYS312 ^A
THR640 ^D	ALA316 ^A
THR64 ^D	PRO315 ^A
LYS312 ^D	ARG152 ^A
LYS312 ^D	GLU154 ^A
LYS312 ^D	GLN109 ^A
ARG301 ^D	HIS241 ^A
HIS202 ^D	ALA283 ^A
SER169 ^D	ASP239 ^A
TYR166 ^D	GLU235 ^A
ARG103 ^D	GLU315 ^A
GLN100 ^D	ASP316 ^A
LYS96 ^D	GLU317 ^A
LYS44 ^D	GLU315 ^A

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