Computational Modeling of Protein Kinase A and Comparison with Nuclear Magnetic Resonance Data

Lei Shi[1]
Advisor: Gianluigi Veglia[1,2]
Department of Chemistry[1], & Biochemistry, Molecular Biology and Biophysics[2]

ABSTRACT

Protein phosphorylation is fundamental in the modulation of myocardial contractility. Sarcoendoplasmic reticulum Ca^{2+} ATPase(SERCA) removes cytosolic Ca^{2+} to initiate relaxation, but the regulatory protein, phospholamban(PLN), decreases SERCA's affinity for free Ca^{2+}. Phosphorylation of PLN by Protein Kinase A (PKA) induces a relief of inhibition on SERCA and augments the rate of SERCA Ca^{2+} uptake. Here, we studied the interaction between PKA and PLN by nuclear magnetic resonance (NMR), computational docking and molecular dynamics (MD) simulations. Comparative simulations of PKA apo, binary and ternary states were performed, which provided molecular details to understand the mechanism of PKA substrate recognition.

Keyword

Protein kinase A, docking, molecular dynamics simulations, NMR

Physiology and Biochemistry of Cardiomyocytes

Our research focuses understanding the molecular mechanisms of the contraction and relaxation of cardiomyocytes, which are governed by the interactions of several proteins. We use biochemical, biophysical and theoretical approaches to study the structure, dynamics, and interactions of these proteins. The ultimate goal is to provide atomic detail models that can be used as a basis for rational drug design.

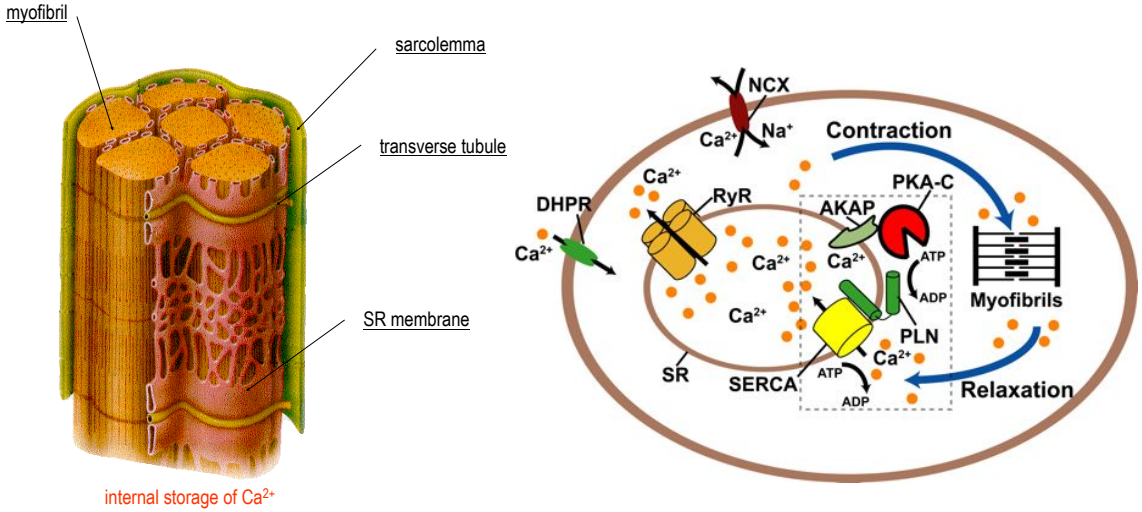


Figure 1. Diagram of the cardiac contractility mechanism. PLN (green) inhibits the calcium pump, SERCA (yellow), when unphosphorylated. Phosphorylation by PKA-C (red) relieves the inhibition.

MacLennan, DH and Kranias, EG, Nature Reviews (2003) 4, 566-577.

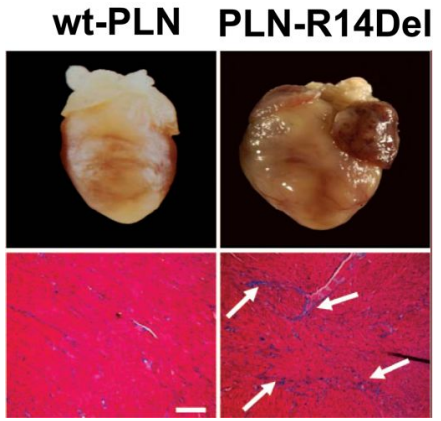


Figure 2. Mutations of PLN have been shown to lead to dilated cardiomyopathy, characterized by thickening of the heart wall muscle (top, right) and interstitial fibrosis (bottom, at arrows)

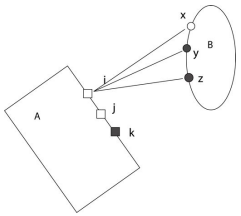
Haghighi, K, Kolokathis, F, et al, PNAS (2006)103, 1388-1393.

Molecular Docking of PKA/PLN using experimental NMR data

High Ambiguity Driven biomolecular DOCKing (HADDOCK)

Both biochemical and biophysical interaction data are imported as AIR restraints to dock protein complexes in the HADDOCK approach.

Definition of Ambiguous Interaction Restraints



Dominguez, C; et al J. AM. Chem. Soc. 125, 1731-1737

$$d_{iAB}^{eff} = \left(\sum_{m_A=1}^{Natoms} \sum_{k=1}^{NresB} \sum_{n_k=1}^{Natoms} \frac{1}{d_{mnk}^{6}} \right)^{-\frac{1}{6}}$$

Docking protocol

Position of Proteins 150 A away from each other and apply random rotations

↓

Rigid body energy minimization (rotation and translation) $Score_1 = 0.01xE_{vdw} + 1xE_{elec} + 0.01xE_{AIR} - 0.01xBSA + 1xE_{desolv}$

↓

Semi-flexible simulated annealing in torsion angle space

$Score_2 = 1.0xE_{vdw} + 1xE_{elec} + 0.1xE_{AIR} - 0.01xBSA + 1xE_{desolv}$

↓

Final refinement in explicit solvent

$Score_3 = 1.0xE_{vdw} + 0.2xE_{elec} + 0.01xE_{AIR} + 1xE_{desolv}$

↓

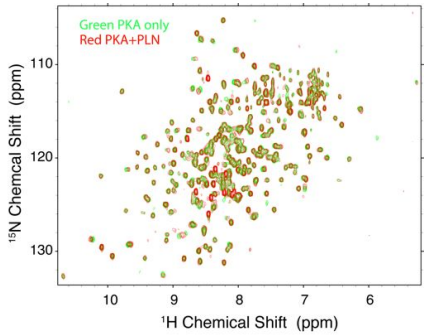
Cluster and analysis

Overall flowchart

Biochemistry Experiments

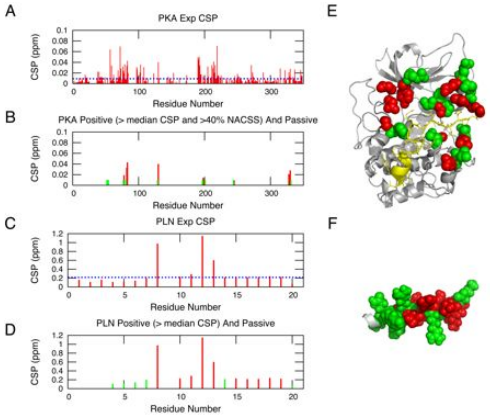
Biophysical Experiments

→



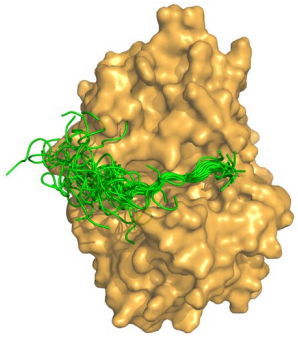
NMR Chemical Shift perturbation

↓



Defining Interacting Residues

←



NMR docked ensemble

Compare NMR Docking Model with X-ray Crystallography Structures

Our collaborator Susan S. Taylor from University of California, San Diego have recently obtained a 3 Å X-ray crystal structure of the PKA/PLN_{1-20} complex. We compared our model ensemble from NMR-driven docking with the crystal structure. There is a good overall agreement between model/structures solved by the two approaches.

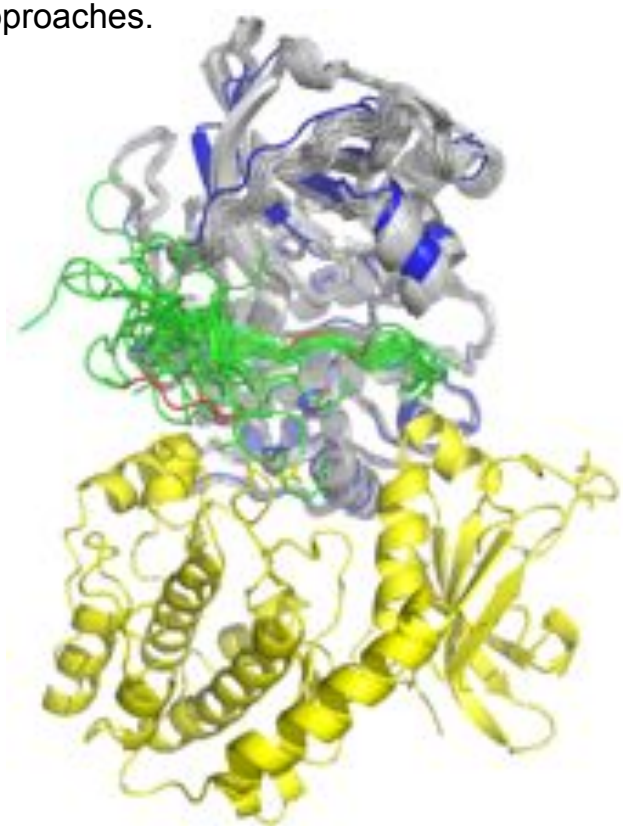


Figure 3. Overlay of the NMR docking ensemble with the x-ray crystal structure. The crystal structure contains two PKA enzyme in one asymmetric unit (yellow and blue). PLN_{1-20} in the crystal structure is colored in red. The model of PKA from NMR structures is colored in grey, while PLN_{1-20} is shown in green. The figure was produced by overlaying backbone atoms of PKA using Pymol.

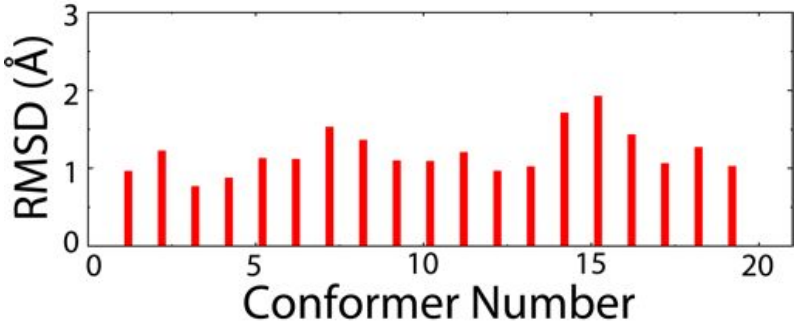


Figure 4. Structure analysis of the 20 conformers of PLN in the NMR ensemble. The rmsd was calculated by overlaying with structure number 20, which agrees best with the PLN in the x-ray crystal structure.

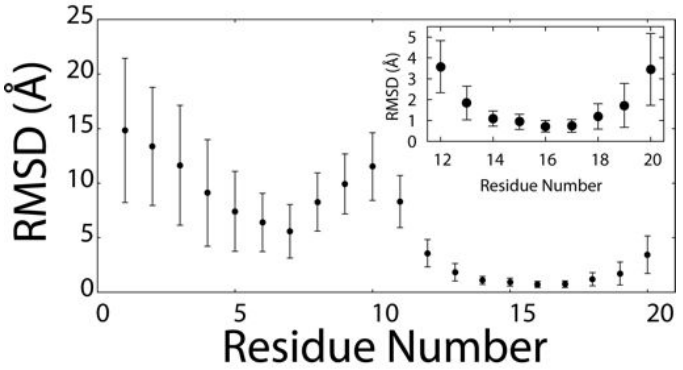


Figure 5. Average and standard deviation for the RMSD of the NMR modelwith respect to PLN in the crystal structure. The PLN RMSD for each conformer was calculated by overlaying the backbone of the PKA.

Protein Dynamics Probed by NMR Relaxation Measurements

In addition to the correct folding (structure), flexibility (dynamics) is another essential factor for a protein to function properly. Nuclear magnetic resonance (NMR) is an important technique that can be used to study protein dynamics. By using NMR relaxation measurements, we monitored the fast (ps-ns) and slow (ms) dynamics of PKA different states, including apo, binary and ternary. Dynamics of PKA were enhanced after binding the nucleotide AMP-PNP (binary) and substrate PLN (ternary). This implies that PKA does not function through an induced fit mechanism, rather through conformational selections.

NMR relaxation data

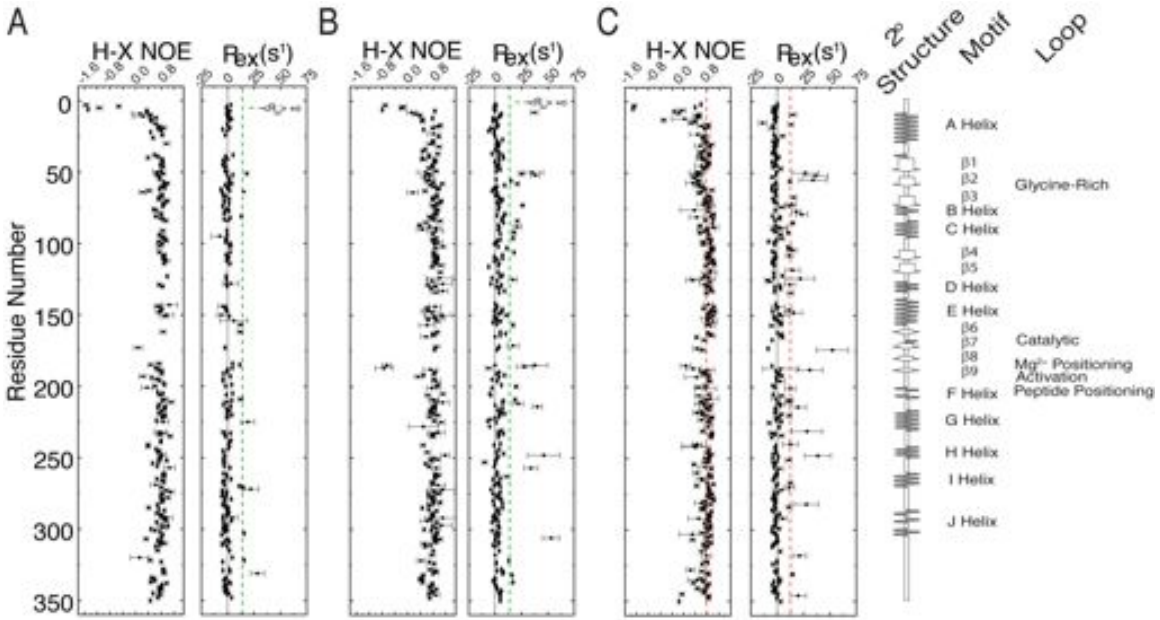


Figure 6. The H-X NOE and R_{ex} for apo form, binary form (PKA+AMPPNP) and ternary form (PKA+AMPPNP+PLN) from NMR from relaxation measurements.

Mapping of dynamics onto structures

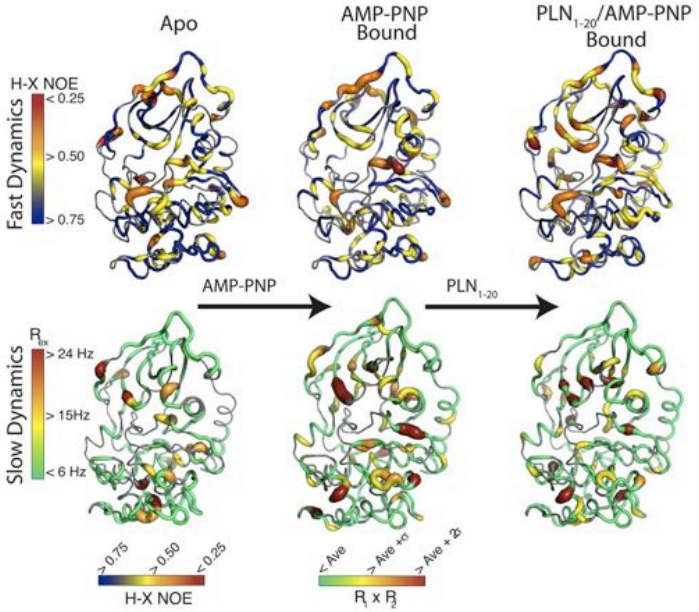
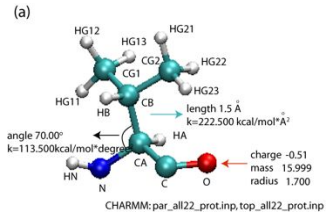


Figure 7. Mapping of H-X NOE and R_{ex} (Figure 6) onto the crystal structure of PKA. Thicker ribbons and red color indicates more dynamic regions.

Masterson, LR; et al in preparation

Molecular Dynamics Simulations

CHARMM force field



$$V = \sum_{bonds} k_b(b-b_0)^2 + \sum_{angles} k_\theta(\theta-\theta_0)^2 + \sum_{torsion\ angle} k_\varphi[1+\cos(n\phi-\delta)] + \sum_{impropers} k_w(w-w_0)^2 + \sum_{Urey-Bradley} k_u(u-u_0)^2 + \sum_{nonbonded} 4\varepsilon[(\frac{R_{min}}{r_{ij}})^{12}-(\frac{R_{min}}{r_{ij}})^{6}] + \frac{q_i q_j}{\varepsilon_0 r_{ij}}$$

Brooks, BR; J. Comp. Chem. 4, 187-217 (1983)

Newton equations of motions

$$f_i = -\frac{\partial V}{\partial r_i}, m_i\ddot{r}_i = f_i = -\frac{\partial V}{\partial r_i}$$

Simulation details

- NAMD with CHARMM27 force field
- Langevin dynamics (with a damping constant of 1.0 ps^{-1}) at constant temperature (310 K) and constant pressure (1 atm) with Nosé-Hoover Langevin piston pressure control.
- Electrostatic interactions particle-mesh Ewald (PME) summation and non-bond interactions with cutoff of 9 Å.
- Shake algorithms were applied to all bonds involving hydrogen atoms and time step of 2 fs.
- Each system was solvated in a cubic box of 80 x 80 x 80 $Å^3$.
- Counter ions were added to reach neutrality and ionic strength of ~150 M.
- Systems are heated gradually from 30K to 310K with harmonic restraints on backbone atoms
- In the first 5 ns, the harmonic restraints were kept on and released thereafter. In total, 35 ns simulations were performed. The last 10 ns trajectory was used for analysis.
- Trajectories are saved every 1 ps.

Philips, JC; et al Journal of Computational Chemistry, 26:1781-1802, 2005

Snapshots of initial structures

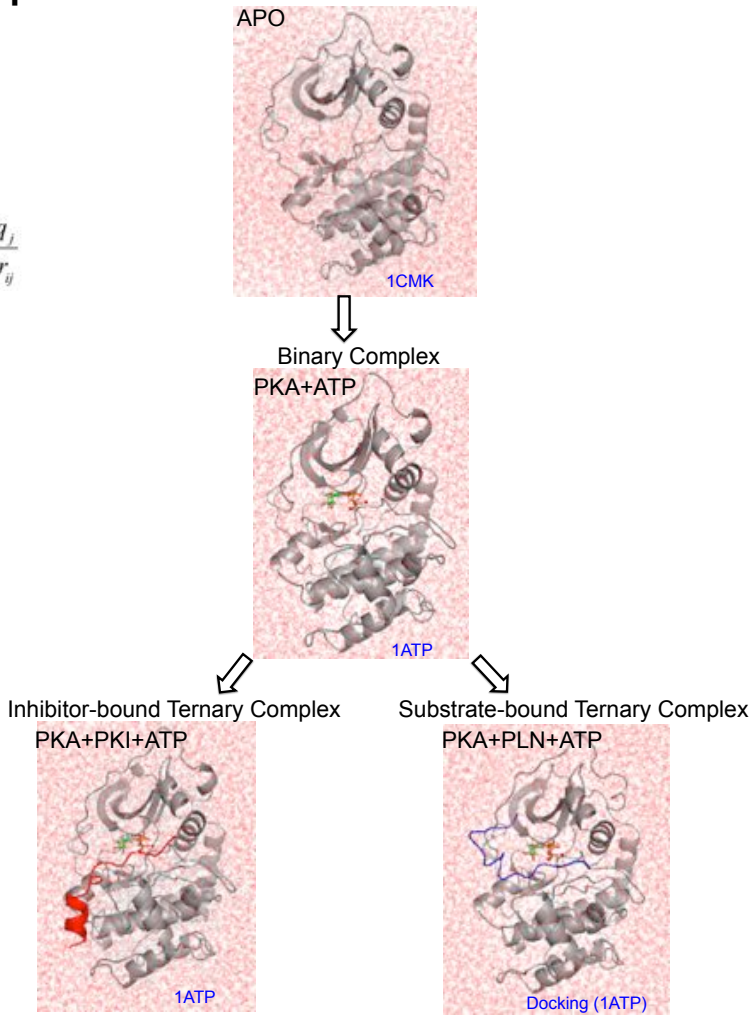


Figure 8. Snapshots of thesimulations. Four different simulations were performed including apo form (starting from crystal structure 1CMK), binary form (starting from crystal structure 1ATP after removing PKI), inhibitor PKI bound ternary form (from 1ATP) and substrate PLN bound form (starting from the NMR docking model).

Analysis of Molecular Dynamics Simulations

After initial equilibration with harmonic restraints on the protein backbone atoms, each of the four systems was simulated for 30 ns. The last 10 ns in each trajectory were used for structural analysis. We analyzed the root mean square deviation (RMSD) of different components of the systems. The RMSD is an indicator of the overall fluctuations of an selected group of atoms. In order to probe the fluctuations along the sequence, we used root mean square fluctuations (RMSF), which is the average RMSD of each residue in the simulation. Our analysis was consistent with NMR observations in that the apo form of PKA increased its dynamics after binding to nucleotide (binary) and substrate PLN (ternary). Surprisingly, the dynamics were quenched in the ternary complex with the inhibitor PKI.

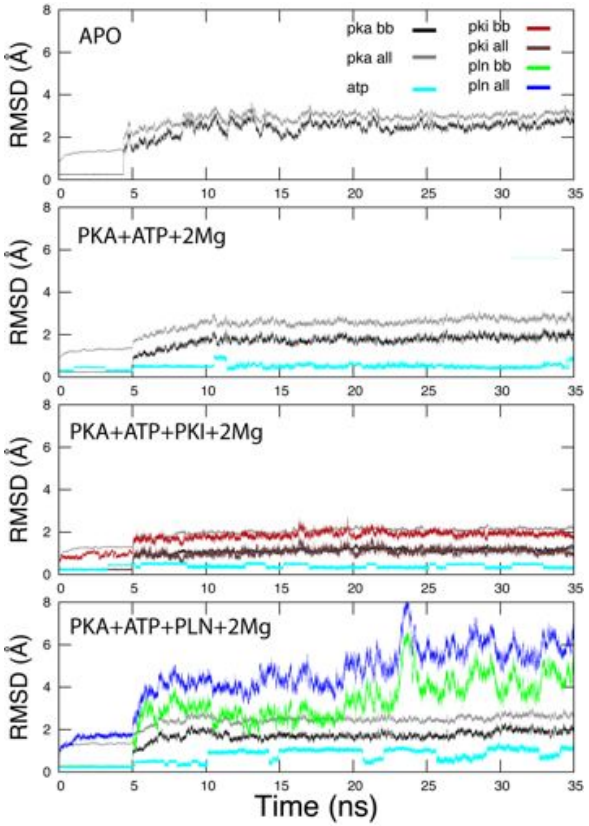


Figure 9. RMSD of the backbone and sidechain of PKA, ATP, PKI and PLN in four different simulations.

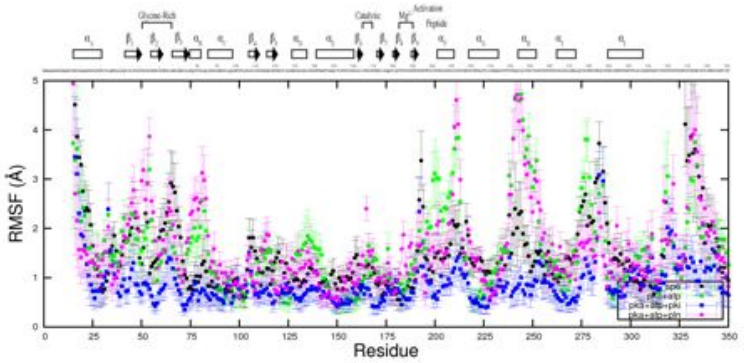


Figure 10. Average and standard deviations of the RMSF of each residues in four different simulations.

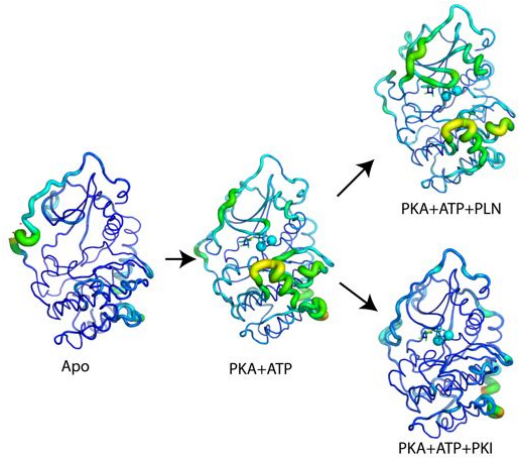


Figure 11. Mapping of the RMSF data to the crystal structure after normalizing all RMSF values by a common factor. Thicker ribbon and green color indicates larger fluctuations.

Model of PKA Recognition & Catalytic Function: Dynamic Energy Landscape

Reaction Scheme

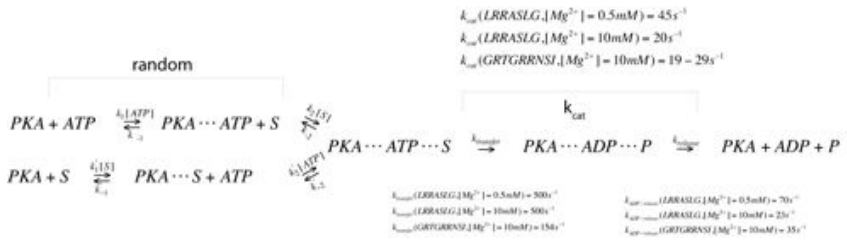


Figure 12. Enzymatic mechanism of PKA phosphorylation of PLN. PKA binds the ligand and substrates in a random order before the chemical steps. Product release is the rate-limiting.

Conclusions

PKA is an important enzyme that regulates many cellular functions. Based on NMR data, we determined the complex model of PKA/ PLN_{1-20} complex, which agrees well with x-ray crystal structure.

By performing comparative MD simulations, we also indentified the change in dynamics of different forms of PKA. PKA/substrate complex needs to maintain a certain degree of freedom for enzyme turnover. The inhibitor of PKA works by locking the enzyme in a stable, inactive state.

Acknowledgement

Financial support was kindly provided by a Doctoral Dissertation Fellowship (DDF) from Graduate School, University of Minnesota and National Institute of Health (NIH) grant to to GV (NIH GM64742, K02HL080081). The computational resources were provided by the Minnesota Supercomputing Institute (MSI). Veglia group members have provided a lot of helpful discussions.

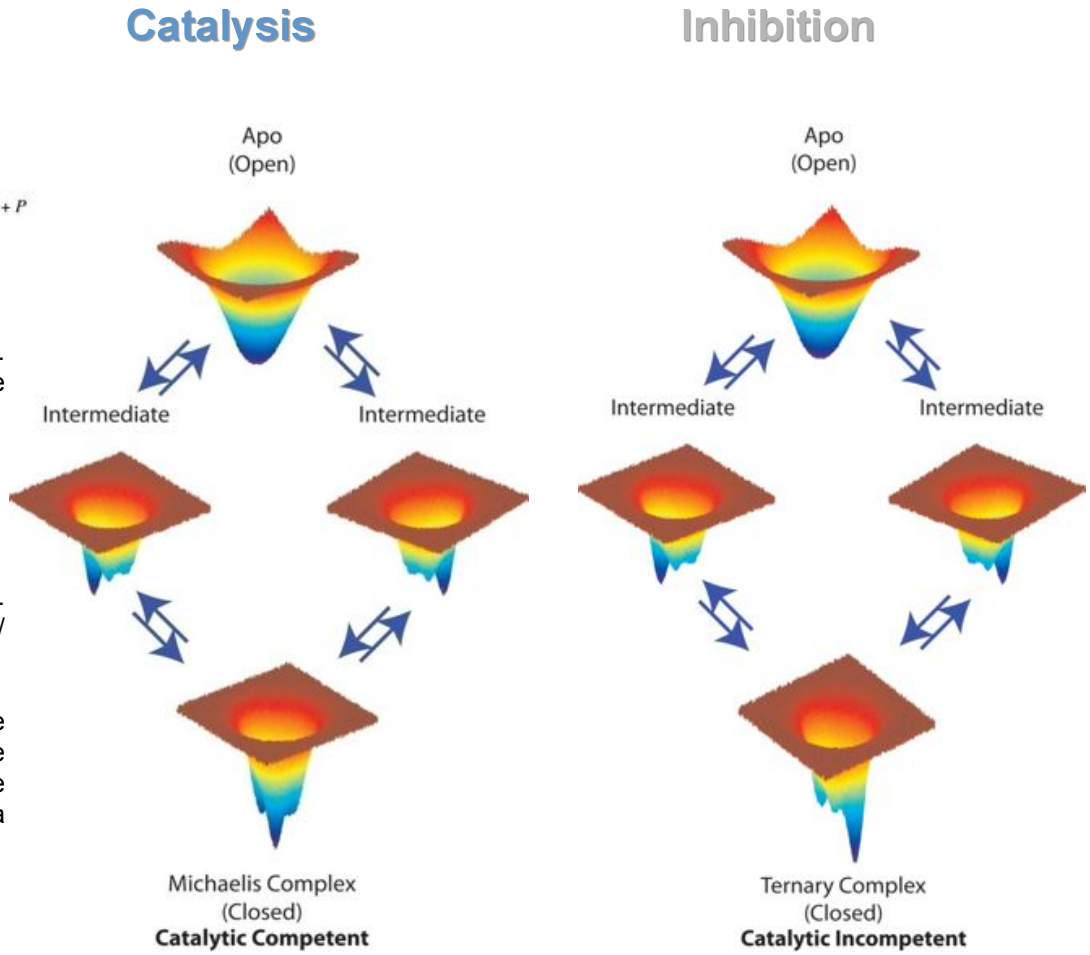


Figure 13. Change of potential energy surface (PES) of PKA upon binding substrate and inhibitor. Substrate-bound PKA complex retains a certain degree of dynamics which can facilitate product release. The inhibitor can trap the enzyme in a lower-energy state, in which turnover cannot occur.