Investigation into the Effect of Dimerization on PKR Activation with Enhanced Sampling Molecular Dynamics Methods

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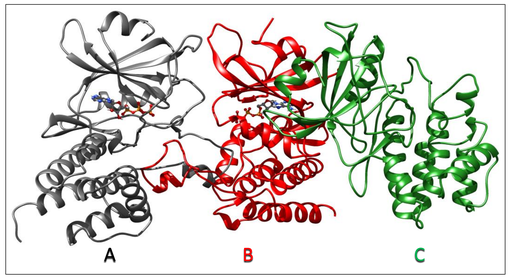
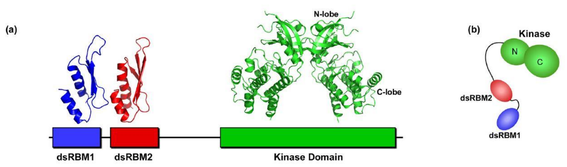
Abstract

Human protein kinase R (PKR) is a serine/threonine kinase which is centrally involved in the cell's innate immune response to viral infection. Upon binding viral dsRNA transcripts, PKR dimerizes and self-activates through autophosphorylation, enabling the downstream phosphorylation of eukaryotic translation initiation factor 2 subunit alpha (eIF2α). In its phosphorylated state eIF2α inhibits the translation of viral RNA products, arresting viral proliferation. The prevailing model of the self-activating dimerization event has PKR's protomers forming a back-to- back (BTB) homodimer, with their activation loops on opposite sides of the complex. In the B2B model, the protomers are autophosphorylated in a cis fashion when an intra subunit contact is formed between the activation loop and the ATP binding site. We propose that activation in fact occurs in a three-step process: back-to-back dimerization, which induces an active-like conformation of the protomers, followed by a second front-to-front trans event in which a third "substrate" protomer is phosphorylated by the active dimer. Here we focus on the first stage of activation, how back-to-back dimerization can induce an active conformation of the protomer. We use non-equilibrium molecular dynamics simulations to generate the activation pathways of PKR molecules in both monomer and back-to-back dimer states. A hallmark of activation in ser/thr kinases is the repositioning of their α-c-helix toward the enzyme's catalytic center, which is the coordinate we use to define the activation transition. From our pathways we perform extensive bias-exchange umbrella sampling simulations to sample and compute robust estimates of the free energy landscape for activation. From these free energy profiles we are able to compare barriers and overall free energy changes to evaluate how dimerization affects the energetics of activation.



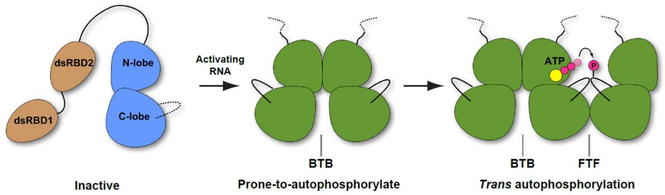
Protomers A, B, and C of the asymmetric unit of a new PKR crystal structure showing both loop-exchanged FTF and BTB dimer configurations.



a) Structure of PKR: Two double-stranded RNA binding domains connected to a C-terminal kinase domain via a linker region. **b) Schematic diagram of PKR** [1]

Background

- Human protein kinase R (PKR) is a serine/threonine kinase that works in the cell's innate immune response to viral infection, inhibiting dsRNA translation
- In the canonical model, PKR dimerizes in a back-to-back (BTB) orientation and self-activates through *cis* (intramolecular) autophosphorylation[1,4]
- A new structure by Dr. James Cole's (UConn) group indicates a novel, front-to-front (FTF) dimeric configuration of PKR, **suggesting the existence of an intermolecular *trans* mechanism of autophosphorylation**



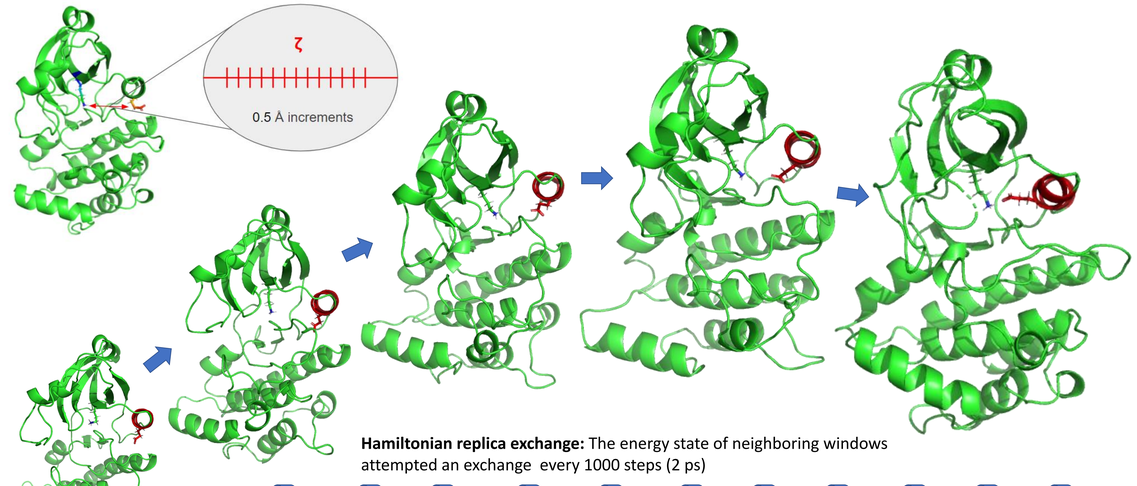
Three step activation mechanism: Our new model of PKR activation involves both BTB and FTF modes of dimerization. The BTB dimerization of inactive PKR monomers induces or stabilizes an active-like phosphorylation-competent conformation in one or both protomers. A third protomer adopts a loop-exchanged

Methods

- Since there is no extant structure of an inactive PKR molecule, a homology model was built using the I-TASSER[7] software and used as the basis for the activation simulations
- Using GROMACS[6] COM pulling, a steered MD (SMD) trajectory was generated in which the E308 residue of the α-C-helix was displaced toward K296 near the catalytic center, with which it must form a salt bridge for PKR to be active -- a distance of ~14 Å
- Umbrella sampling windows were selected at 0.5 Å intervals along this SMD pathway
- 24 windows were run in a Hamiltonian Replica Exchange system for ~200 ns. These replicas were run with a harmonic restraint keeping them near their origin, each having a probability of exchanging its Hamiltonian with a neighbor every 1000 steps
- The resultant data was analyzed using the GROMACS implementation of the Weighted Histogram Analysis Method (WHAM)

Is active-like PKR less energetically favorable as a monomer?

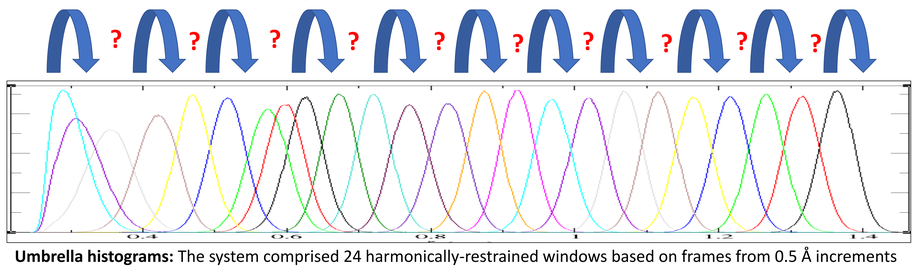
To investigate the plausibility of the three-step activation hypothesis, the free energy profile of activation for a PKR monomer was compared to that of a dimer in computer simulations. First an activation pathway – here, the repositioning of the α-C-helix in an initially inactive monomer – was generated using steered molecular dynamics simulations. Bias exchanged umbrella sampling along this pathway allowed us to maximally sample the transition's free energy surface and accurately calculate the free energy of the reaction coordinate.



Hamiltonian replica exchange: The energy state of neighboring windows attempted an exchange every 1000 steps (2 ps)

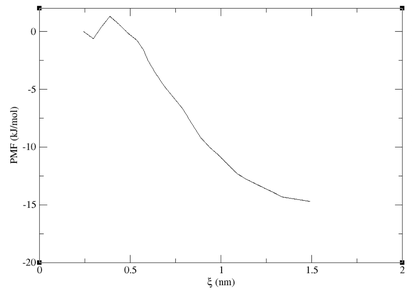
$$w(x_i \rightarrow x_j) = \min[1, \exp(-\Delta)]$$
$$\Delta = (\beta)\left[(E^i(r_j) - E^i(r_i)) - (E^j(r_j) - E^j(r_i))\right]$$

Exchange criterion:
β = 1/RT
E^i, E^j = Hamiltonian for given replica
r_j r_i = Replica 1, 2

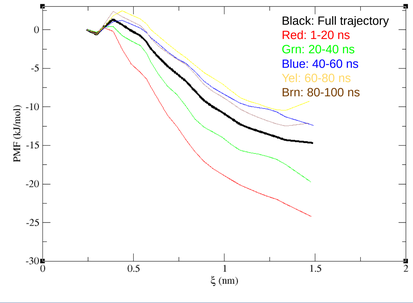


Umbrella histograms: The system comprised 24 harmonically-restrained windows based on frames from 0.5 Å increments along the reaction coordinate, **ζ** (shown here in nm)

Results



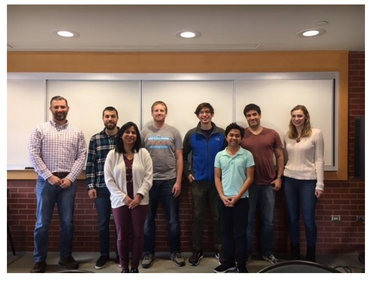
Potential of Mean Force (PMF) curve: The free energy of the α-C-helix displacement can be calculated based on the PMF derived from WHAM analysis. Here the ΔG of the reaction coordinate is **-13.387 kJ/mol**



Block averaging: The PMF was plotted for 5 running 20 ns blocks over the first 100 ns of the HREX simulation. The fluctuating plots suggest good convergence of the full simulation on a stable value

Current and Future Work

We are currently modeling an *inactive* back-to-back dimer, which will be the subject of a similar HREX simulation, in which one or both α-C-helices of the dimer are displaced toward the catalytic center of the molecule. This will enable a comparison of free energy between both systems. A lower energy barrier for the activation transition in the dimer would suggest the BTB dimeric configuration plays a role in facilitating or stabilizing an autophosphorylation-competent state in PKR, capable of fully activating the kinase



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References: [1] Cole, Trends Biochemical Sciences 2007. 32(2), 57–62. [2] Dey et al, Journal of Biol. Chem. 2014. 289(9), 5747-5757 [3] Mieczkowski et al, The EMBO Journal 2008 27, 3186–3197 [4] Vijay et al, NIH Methods 2010. 52(1): 99–105. [5] Beenstock, Trends in Biochemical Sciences 2016. 41(11), 938-951 [6] Uchida et al 2014. Nature Sci Rep.4:7395. [6] Hess, B., C. Kutzner, ., E. Lindahl. 2008. GROMACS 4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. J. Chem. Theory Comput. 4:435–447. [7] J Yang, R Yan, A Roy, D Xu, J Poisson, Y Zhang. The I-TASSER Suite: Protein structure and function prediction. Nature Methods, 12: 7-8 (2015)