Molecular dynamics shows how inhibitor-2 phosphorylation activates the protein phosphatase-1 • inhibitor-2 complex

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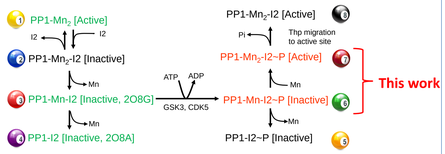
1 PP1 regulation by I2

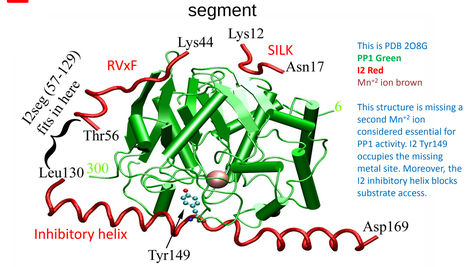


Inhibitor-2 (I2) squelches protein phosphatase-1 (PP1) enzyme activity by blocking access to the metal-containing active site. After I2 threonine-74 is phosphorylated, PP1 is activated without I2 dissociation.

The goal of this study was to see if molecular dynamics (MD) could give atomic resolution understanding about how this works.

Many similar structures of PP1 (green) are known. This work models PP1-Mn-I2 and examines phosphorylation-dependent events that could lead to PP1 activation (between steps 6 and 7 above).

2 I2 phosphorylation site is in an disordered segment



This is PDB 2O8G
PP1 Green
I2 Red
Mn^{+2} ion brown

This structure is missing a second Mn^{+2} ion considered essential for PP1 activity. I2 Tyr149 occupies the missing metal site. Moreover, the I2 inhibitory helix blocks substrate access.

This PP1-I2 structure from x-ray crystallography was missing 73 residues (I2seg) of I2 because they are "unstructured". **They have many potential structures.**

3 Molecular dynamics

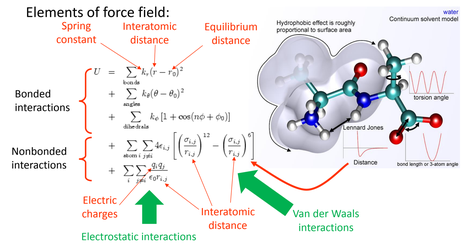
Molecular dynamics calculates the trajectory of atoms in the system by integration of Newton's equations of motion. The force field is parameterized using empirical data. Time steps are 1 femtosecond (10^{-15} sec).

$$\vec{r}(t+\Delta t) = \vec{r}(t) + \Delta t\vec{v}(t) + \frac{\Delta t^2 \vec{a}(t)}{2}$$

$$\vec{a}(t+\Delta t) = \frac{\vec{f}(t+\Delta t)}{m}$$

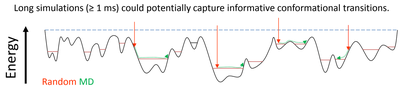
$$\vec{v}(t+\Delta t) = \vec{v}(t) + \frac{1}{2}\Delta t[\vec{a}(t) + \vec{a}(t+\Delta t)]$$

Elements of force field:

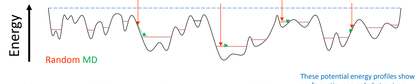


4 Many short simulations sample conformation space efficiently

Long simulations (≥ 1 ms) could potentially capture informative conformational transitions.

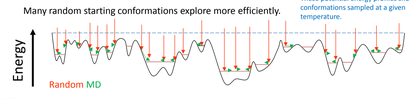


But in reality MD explores little conformational space (1.6 ns per day using 16 processors).



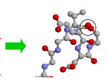
Many random starting conformations explore more efficiently.

These potential energy profiles show conformations sampled at a given temperature.

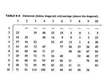


5 I2seg structures by Monte Carlo method

LLLEG…
GLEHH…
EEEGL…
LEEBG…
HBGLE…



1500 K for 50 ps
Cool to 300 K over 100 ps



Torsion angles of I2seg were made according to the frequencies of angles in known structured proteins.

Energy minimize to exclude those with steric clashes

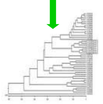
These models were fit to observed NMR data and end restraint (19 Å) by simulated annealing.

606 member ensemble

Minimize fit and calculate Cα distance matrix

~1500 random structures

Use cluster analysis to find a small number of models that adequately represent the ensemble.



14 reference models statistically represent this ensemble.

6 Results

The 14 reference I2seg models were used for three types of MD simulations in an unphosphorylated and phosphorylated state.

1. Free I2seg fragments (73 residues, 1086 atoms, 28 simulations, 20 ns each)
2. PP1-Mn-I2 complexes (427 residues, 6835 atoms, 28 simulations, 10-20 ns each)
3. PP1-Mn_2-I2 complexes (427 residues, 6836 atoms, 28 simulations, 5-10 ns each)

Free I2seg fragments

• Essentially no secondary structure except for a ten residue α-helix, which formed ~30% of the time. The helix was previously detected by NMR.

• Time averaging MD structures fit NMR chemical shifts better than the initial static structures.

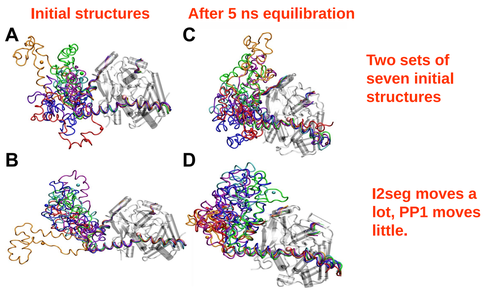
• Phosphorylation increased the equilibrium end distance during 20 ns molecular dynamics.

Models were built with 18.9 Å end restraint so that they would fit the PP1-I2 structure in PDB 2O8G.

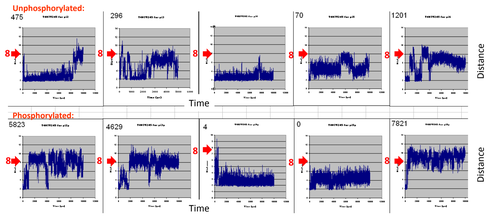
Unphosphorylated models without restraint.

Phosphorylated models without restraint.

7 PP1-Mn-I2 dynamics



8 I2 Tyr149 pops out when I2 is phosphorylated

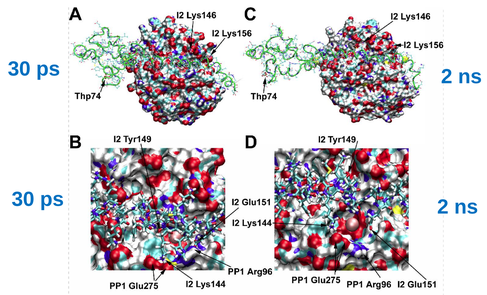


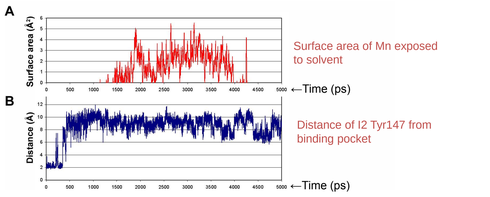
These plots show the I2 Tyr149 OH to PP1 Val245 amide H distance for 5 unphosphorylated and 5 phosphorylated PP-I2 10 ns trajectories. In total 14 trajectories of each were simulated (>480 ns total).

This distance is greater than 8 Å **4%** of the time when I2 is unphosphorylated in which it prevents a second metal from occupying a PP1 binding site.

When I2 is phosphorylated, the distance is greater than 8 Å **37%** of the time, leaving the metal binding site open.

9 I2 phosphorylation promotes PP1 active site exposure





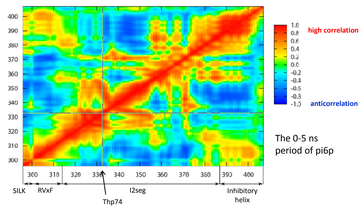
In the PP1-Mn-I2 simulations, I2 phosphorylation promoted greater motion of the I2 inhibitory helix around the PP1 active site. This favored displacement of I2 Tyr147 and allowed PP1 to acquire a second metal for activity.

10 Dynamic cross correlation

Cross-correlations calculate how frequently two atoms move together.
Cross correlations, C_{ij} are defined by

$$C_{ij} = \frac{\langle \Delta r_i(t) \bullet \Delta r_j(t) \rangle}{(\langle \Delta r_i(t)^2 \rangle \langle \Delta r_j(t)^2 \rangle)^{1/2}} \quad \text{where} \quad \Delta r_i(t) = r_i - \langle r_i(t) \rangle$$

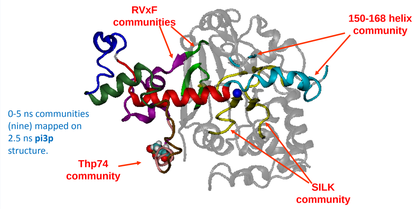
$r_i(t)$ = position of i atom at time, t.
< > means average, • dot product



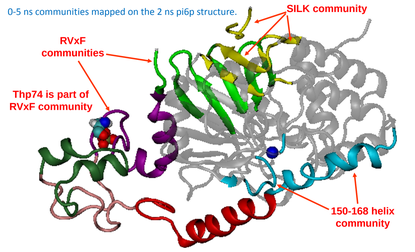
The 0-5 ns period of pi6p

11 Dynamic network communities

Each residue can be treated like a node in a network. Edges connect nonadjacent nodes which spend much time together. Communities of residue nodes that move together can be identified.



I2 residues are in dynamic communities with PP1 residues. These include the SILK, RVxF, and 150-168 helix domains of I2. Their motion is correlated with PP1 residues including those near the active site.



In this trajectory, there were only 7 I2 communities, 9 or more communities was more common. This trajectory had more vigorous I2 displacements at the PP1 active site. Perhaps the fewer communities enhanced phosphorylation –induced motion in I2seg to the PP1 active site.

12 Dynamical Community Distributions

PP1-I2 community distribution was calculated for two 5 ns intervals for the 28 unphosphorylated and phosphorylated trajectories.

I2 communities with three or more adjacent nodes (residues) were color coded. Each row is one trajectory.

• There are four constant I2 communities: SILK, RVxF, I2seg helix, and 130-148 helix.

• The SILK, RVxF, and 150-168 helix include PP1 residues. This provides a mechanism for I2 to influence PP1 structure.

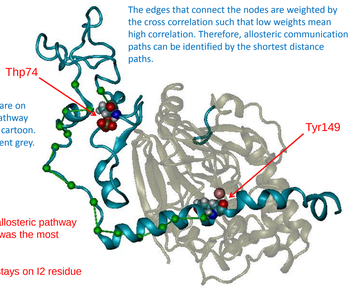
• Community distribution is different between models and varies with time. This is like a feature common to inherently disordered proteins like I2.

• I2 phosphorylation increased the time-dependent variation in community distribution.

• Thr74 was not in a consistent community and was frequently at the edge of a community. This could aid in its phosphorylation-dependent influence of structure at a distance.

13 Allosteric communication paths

The edges that connect the nodes are weighted by the cross correlation such that low weights mean high correlation. Therefore, allosteric communication paths can be identified by the shortest distance paths.

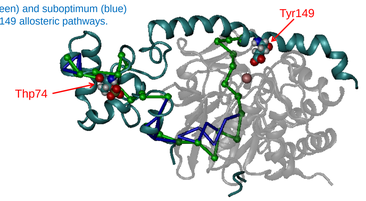


Green nodes on Cα that are on the I2 Thr72 to Tyr149 pathway are mapped on a blue I2 cartoon. PP1 is shown in transparent grey.

This optimal 17 node allosteric pathway from Thp74 to Tyr149 was the most common (21 /31).

This shorter pathway stays on I2 residue nodes.

Optimum (green) and suboptimum (blue) Thp74 to Tyr149 allosteric pathways.

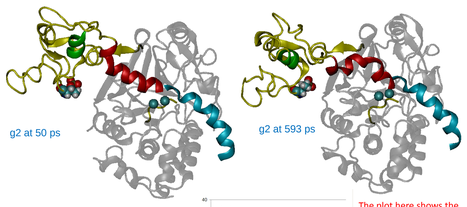


In this example, the 30 node pathway travels through PP1 residues.
This type of pathway was used in one-third of the pathways.
The allosteric communication pathway here uses the RVxF I2 domain connection to PP1.
These results inspire potential mutants that could impair the communication path to test these predicted allosteric paths.

14 PP1-Mn_2-I2 simulations

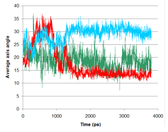
The PP1-Mn-I2 trajectories offered several opportunities to insert a second Mn^{+2} ion when I2 Tyr149 vacated the second metal binding site. Twelve trajectories from unphosphorylated and phosphorylated had a second metal inserted and then simulated for up to 10 ns with and without I2 phosphorylation.

Addition of the second metal affected the structure very little.
However, there were significant differences seen when I2 was phosphorylated.



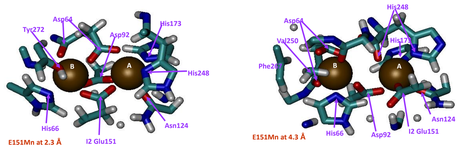
The inhibitory helix became very distorted in some phosphorylated trajectories, but in no unphosphorylated trajectories.

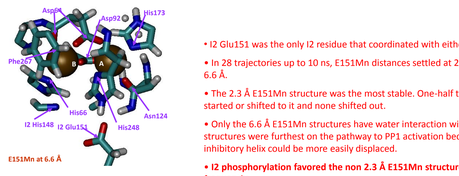
The stress released upon helix reformation could displace I2 residues near the PP1 active site.



The plot here shows the average angle of the helix backbone hydrogen bonds versus time. Three helices are color-coded: green I2seg helix, red 130-148 helix (high in NMR) and cyan 150-168 helix.

15 Metal Coordination in PP1-Mn_2-I2 simulations





• I2 Glu151 was the only I2 residue that coordinated with either Mn ion.

• In 28 trajectories up to 10 ns, E151Mn distances settled at 2.3, 4.3, 5.7, or 6.6 Å.

• The 2.3 Å E151Mn structure was the most stable. One-half trajectories started or shifted to it and none shifted out.

• Only the 6.6 Å E151Mn structures have water interaction with Mn. These structures were furthest on the pathway to PP1 activation because I2 inhibitory helix could be more easily displaced.

• I2 phosphorylation favored the non 2.3 Å E151Mn structures <u>twice</u> as frequently.

Conclusions

1. Molecular dynamics (MD) faithfully captures features of the unstructured I2seg 73 residue segment of I2. These include time-averaged NMR chemical shifts, low frequency ten residue helix formation, and highly variable structure.

2. In the context of PP1-Mn-I2 MD, I2seg residues form a sharp boundary between PP1 and I2 atoms visible crystallographically with low positional fluctuation and those of I2seg with high fluctuation (**7**).

3. Phosphorylation-dependent differences observed in MD trajectories predict how I2 phosphorylation might lead to PP1-I2 activation.

4. I2seg phosphorylation increased the favored end distance (**6**). This might provide force to pry I2 residues 130-145 away from the PP1 active site.

5. I2 phosphorylation in PP1-I2 increased the frequency that I2 Tyr149 exited the second metal binding site. I2 migrated from the PP1 active site in one phosphorylated trajectory sufficiently to allow substrates and a second metal to easily bind (**8, 9**).

6. I2 phosphorylation adds ~200 kcal/mol potential energy to I2seg, which increases occurrences of rapid transitions. Some of these can be ascribed to electrostatic attractions involving the phosphate. With limited observations at this point, the rapid transitions are all unique; favored transitions have not been documented.

7. Several communities of I2 residues that move together are common across models and time. Three I2 communities include many PP1 residues including those near the active site (**11, 12**). This data hints at how I2 could possibly modulate PP1 structure as predicted biochemically.

8. Allosteric paths connect movements near I2 Thr74, which is phosphorylated, to I2 residues, which block the PP1 active site (**13**). Most paths travel only through I2 although one-third travel through a common path including PP1 residues.

9. Addition of a second metal to PP1-Mn-I2 perturbs the structure little in structures where I2 Tyr149 left the metal binding site.

10. In PP1-Mn2-I2 simulations, I2 phosphorylation favored moving I2 Glu151 away from the metals to allow further displacement from the active site. Phosphorylation also distorted the I2 130-145 helix sometimes, which could enhance dislocation (**14, 15**).

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