Magnesium confers stability to the PYL10-PP2C complex at high temperatures: *Insights from molecular dynamics simulations*

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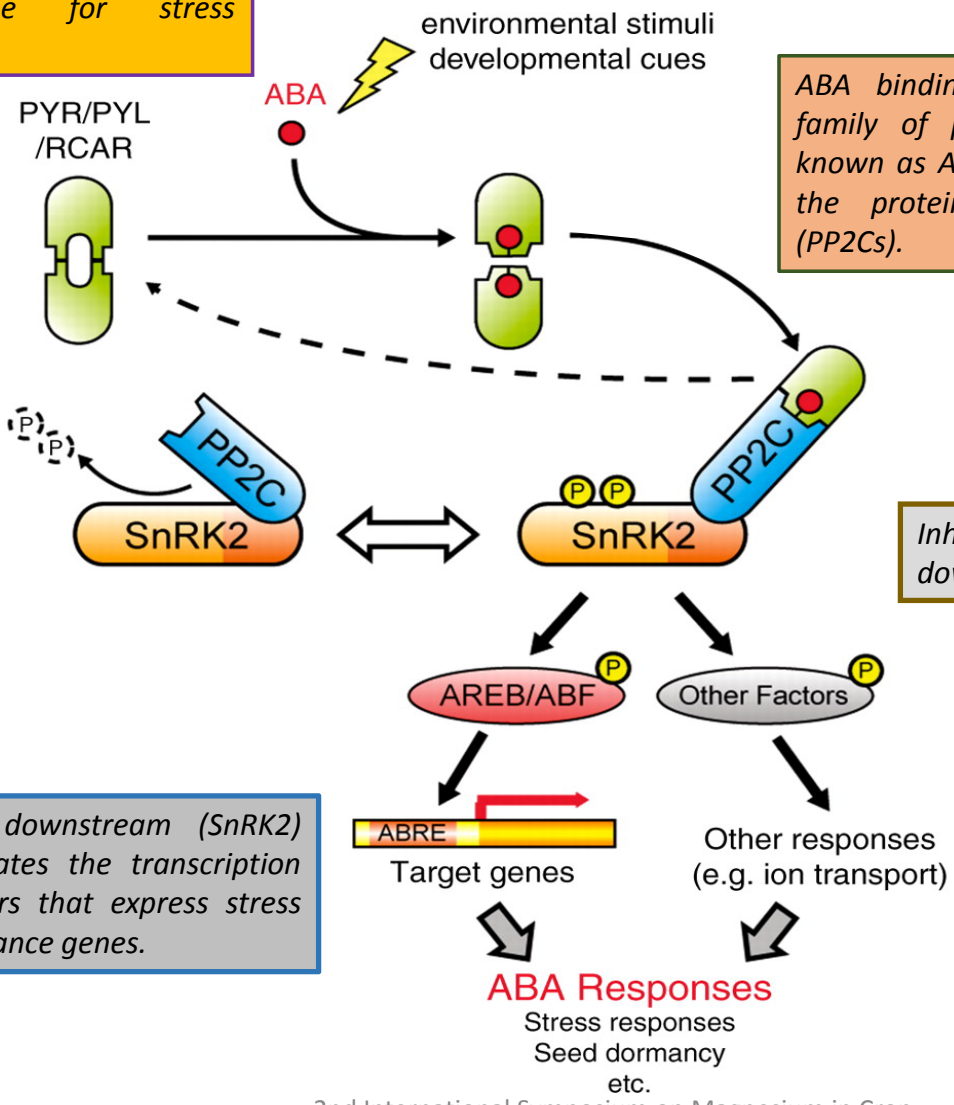
Outline

- Introduction
- Aim
- Method
- Results
- Discussion
- Conclusions and Future Remarks

INTRODUCTION

ABA-dependent pathway

Abscisic acid (ABA) is a pytohormone for stress protection.



ABA binding to PYR/PYL/RCAR family of proteins (PYLs), also known as ABA receptors, inhibits the protein phosphatase 2Cs (PP2Cs).

Inhibition of PP2C relieves the downstream kinases (SnRK2).

The downstream (SnRK2) activates the transcription factors that express stress tolerance genes.

INTRODUCTION

Structural changes during PYL and PP2C interaction

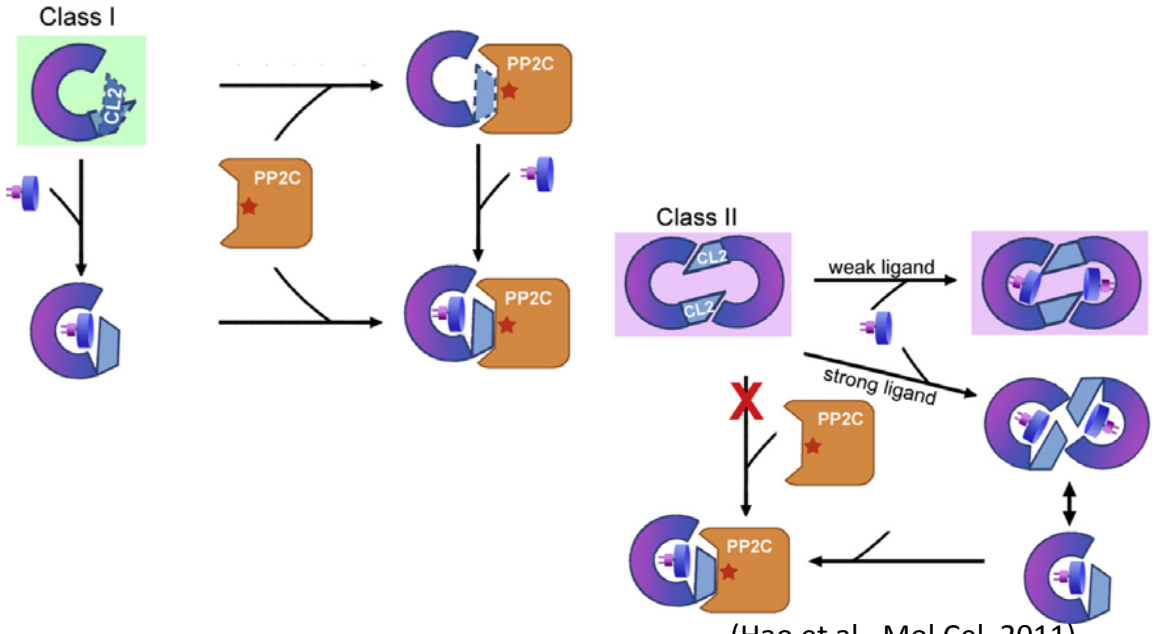
Along with ABA-dependent inhibition of PP2Cs by PYLs, a subclass of PYLs (Class I) was found to inhibit PP2C, even in the absence of ABA. The conformation of the C2 loop in the ABA-free form of PYL10 was similar to those attained in other members of PYLs when bound to ABA (Class II). Owing to the accessible C2 loop in Class I receptors, they can constitutively inhibit PP2Cs.

C4
(110-116)

C3
(96-100)

C2
(80-86)

C1
(65-73)

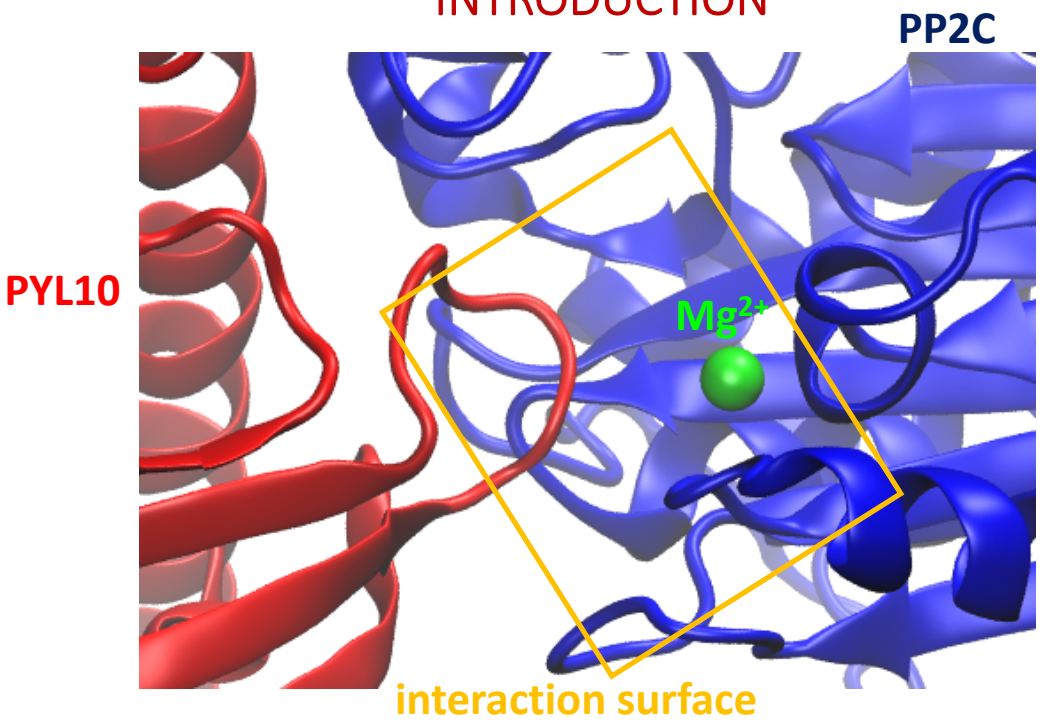


(Hao et al., Mol Cel, 2011).

PYLs have four conserved surface loops, among which the C2 loop is critical for inhibition of PP2C. *Upon ABA binding, the C2 loop in PYLs undergoes dramatic conformational rearrangement by which PYLs interact with PP2Cs.*

What does Mg do in all of this?

INTRODUCTION



- Investigation of the PYL10-PP2C complex showed that the C2 loop of PYL10 extended towards the PP2C surface where a magnesium (Mg) cation is coordinated. The close proximity (~5 Å) of the C2 loop of PYL10 to the Mg in PP2C implies that Mg might have a role in inhibition of PP2Cs by PYLs.
- Besides being an indispensable mineral for plant growth, recent studies showed that Mg levels altered plants' responses to stress (Hermans et al., New Phytol, 2010; Mengutay et al., Plant and Soil, 2013). Particularly, heat stress led reduction in growth and chlorosis in Mg deficient wheat and maize, whereas, when supplied with adequate Mg, the plants continued their normal growth at elevated temperatures (Mengutay et al., Plant and Soil, 2013)

AIM

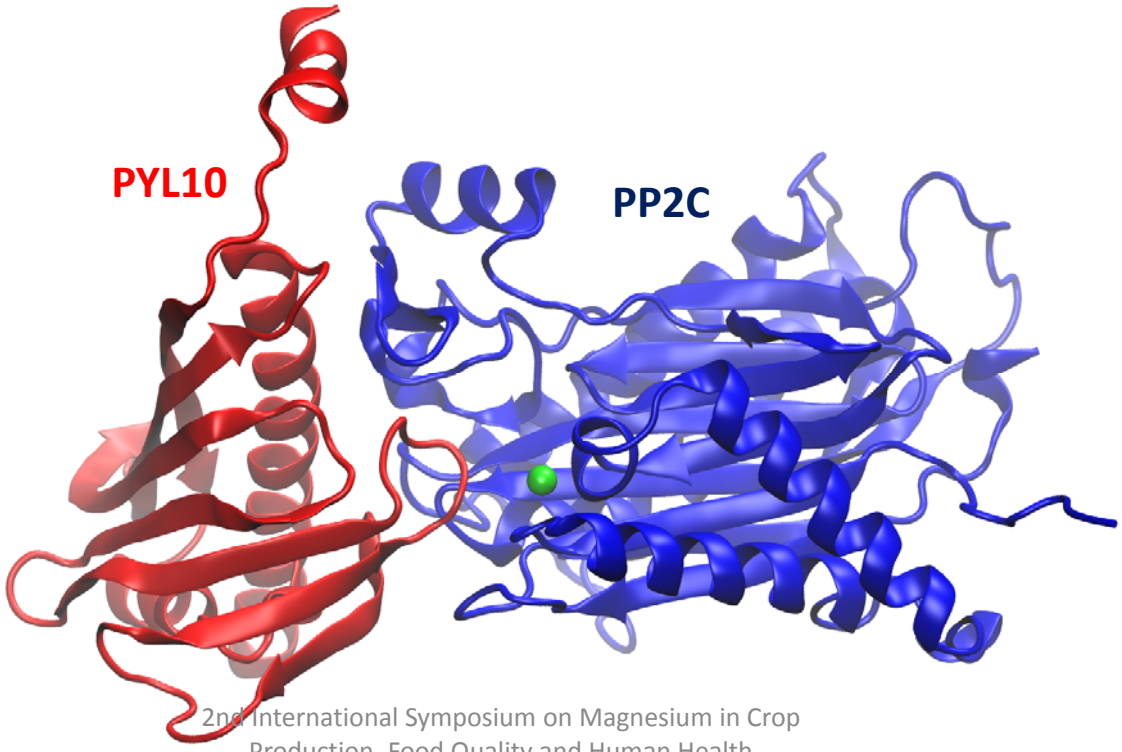
- Taken together with such experimental conclusions and the structural rationalizations made above, we surmise that depleted Mg might limit the interaction between PYLs and PP2Cs and thus reduce the expression of stress-related genes.
- This work investigates the impact of Mg^{2+} on the stability of the PYL10-PP2C complex using molecular dynamics (MD) simulations.

METHOD
Structure preparation

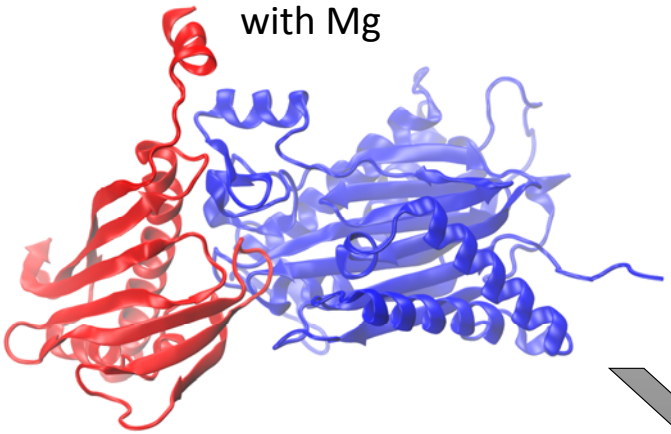
- PDB ID: 3RT0
  - PYL10 complexed with PP2C in the absence of ABA
  - Crystal structure (resolution: 2.11 Å)
  - Source: *Arabidopsis thaliana*
  - Asymetric unit composed of 2 heterodimers is shown right:
    - PYL chains: grey, orange
    - PP2C chains: blue, red
    - Crystal waters: ice blue balls
    - Magnesium ions: green
  - PYL: 171 residues (9-180)
  - PP2C: 339 residues (172-511)
  - Missing residues
    - PYL: 9-24, 128-129
    - PP2C: 172-179, 222-229, 273-277, 511

METHOD
Structure preparation

- Missing residues were modeled with I-Tasser (Zhang, BMC Bioinformatic, 2008).
  - RMSD = 0.0 → no change in the crystal structure
- 1 heterodimer is deleted.
- The protein complex PYL10-PP2C to be used in simulations is generated.
  - ~10,000 atoms



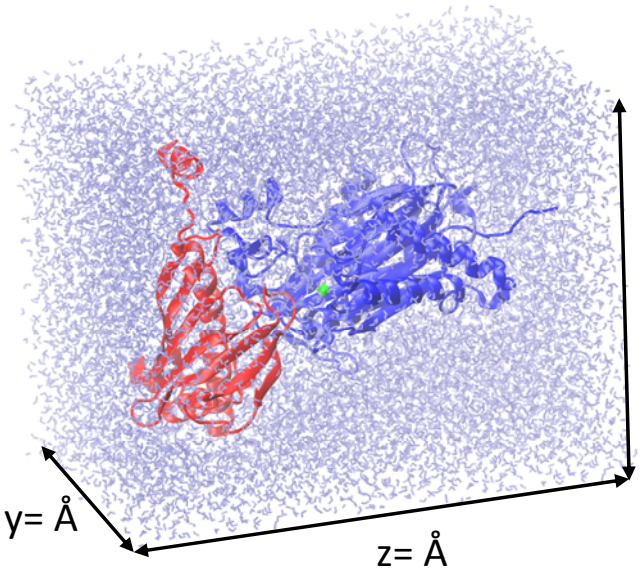
METHOD
Simulation systems



with Mg

without Mg

Placed in a water box



- Neuralization with NaCl using solute-protein distance cutoff at 5 Å.
- Overall charge should be close to zero to mimic the cellular media *e.g.* cytoplasm.

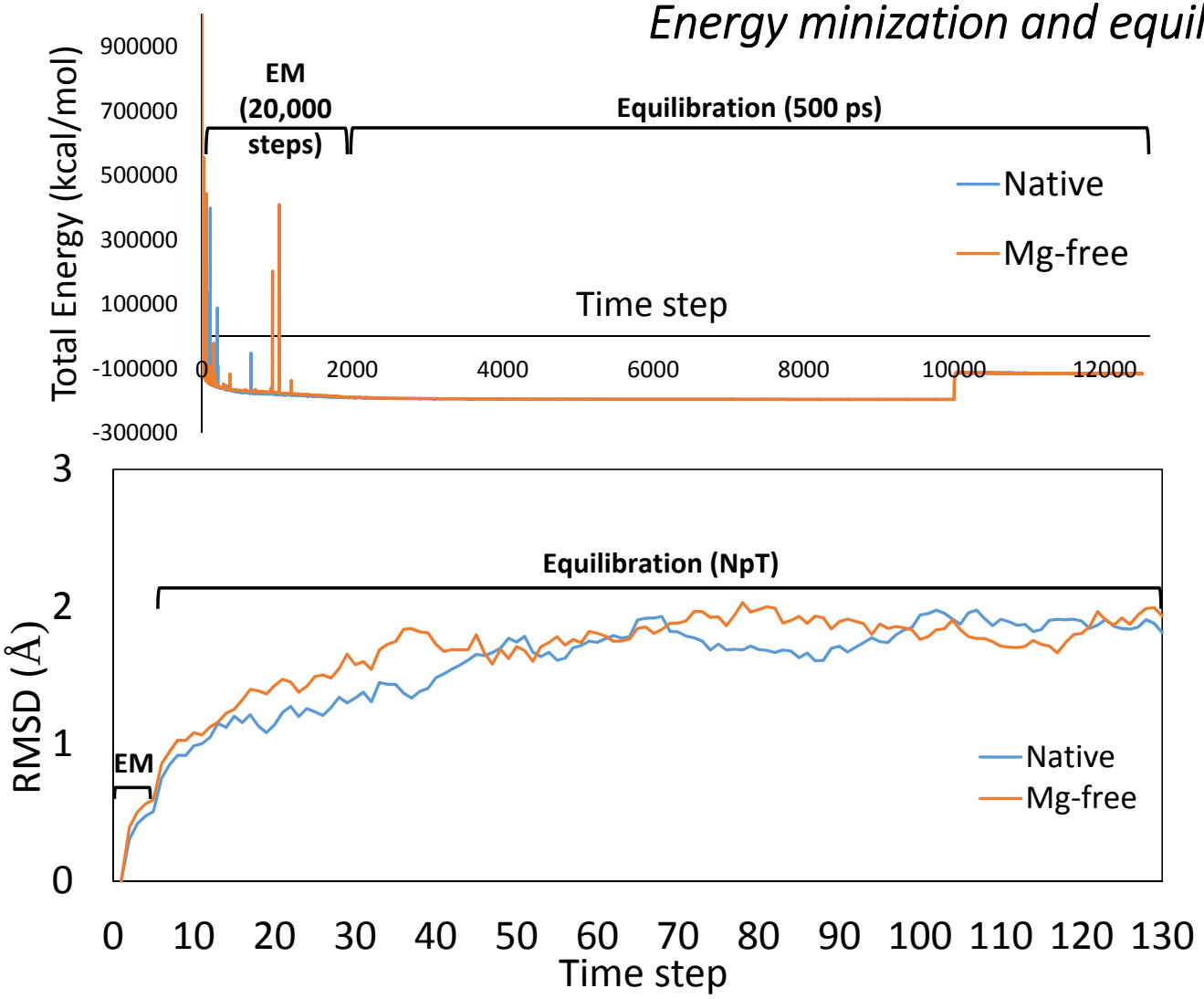
- 2 complex systems were generated.
  - **Native complex**
  - **Mg-free complex**
- Each composed of ~50,000 atoms.
- Ready for simulations!

METHOD
Molecular dynamics simulations

- NAMD v2.9 program with CHARMM27 all-atom FF for proteins.
- Water is treated explicitly using the TIP3P model.
- The systems are fully energy minimized and carefully equilibrated under constant temperature and volume for 0.5 ns before production runs.
- Then they are heated slowly from 10 K to 298 K in 30 ps followed by 5 ns of equilibration under constant temperature (300 K) and pressure (1 atm) (NpT ensemble).
- The high-temperature simulations are carried out at 450 and 550 K for 10 ns under constant volume and temperature.
- The simulations were repeated to test for reproducibility.

RESULTS

Energy minization and equilibration



- The total energy of both systems reduced to low levels
- RMSD graph shows the rms displacements of the backbone atoms (C, N, Cα) of the complex.
- Equilibration simulations showed that the protein complex is stable.
- The productions simulations were performed from the last time point of the equilibration simulation.

RESULTS

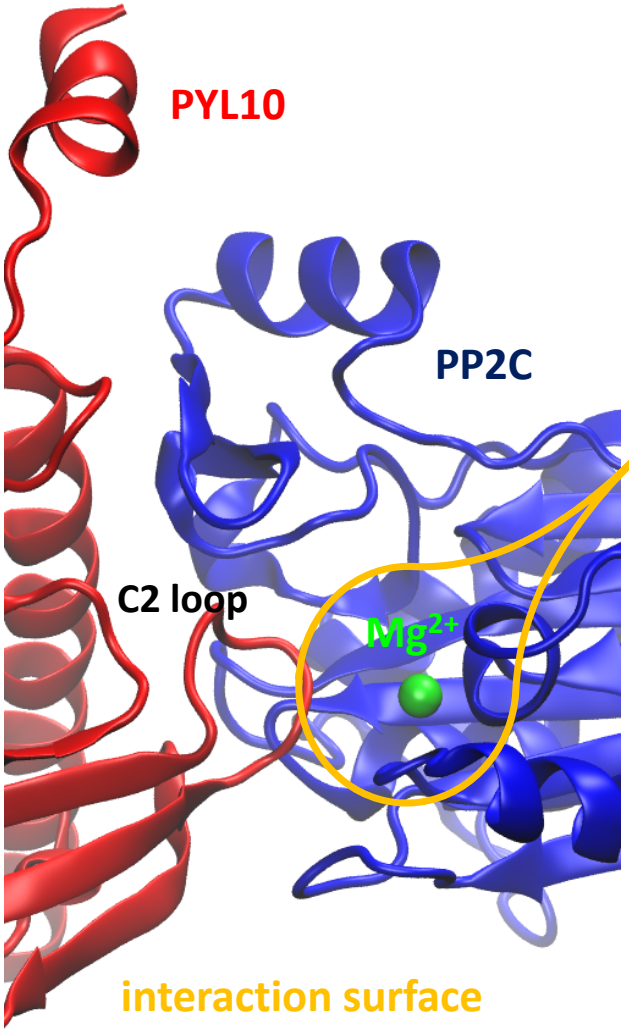
Molecular dynamics – What to search?

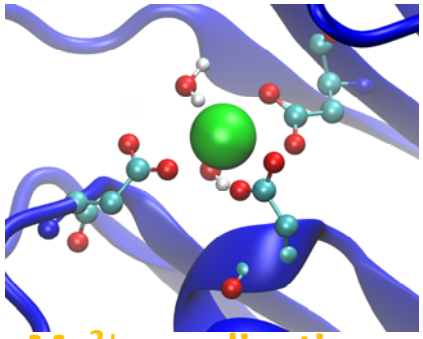
System	Production	Post-production
Native (3RT0) **Mg-free**	5 ns (300 K)	10 ns (450 & 550 K)

- Root mean square displacements (RMSD) of the backbone atoms (C, N, Cα) would give an idea about the quality of the simulations i.e. Whether they are acceptable for assesments of the dimer stability.
- Two criteria for asssesing the impact of Mg on the stability of the PYL10-PP2C complex were used.
  - Cα distance of D261 of PYL10 and S81 of PP2C to probe the compactness of the heterodimer, which was 11 Å in the crystal structure.
  - The root mean square fluctuations (RMSF) of the C2 loop (residues from 79 to 86) of PYL10 for analyzing the interaction state of the C2 loop.
- Reduced compactness and increased fluctuations would refer to less stable dimer, while higher compactness and lower fluctuations would reflect a bound state.

RESULTS

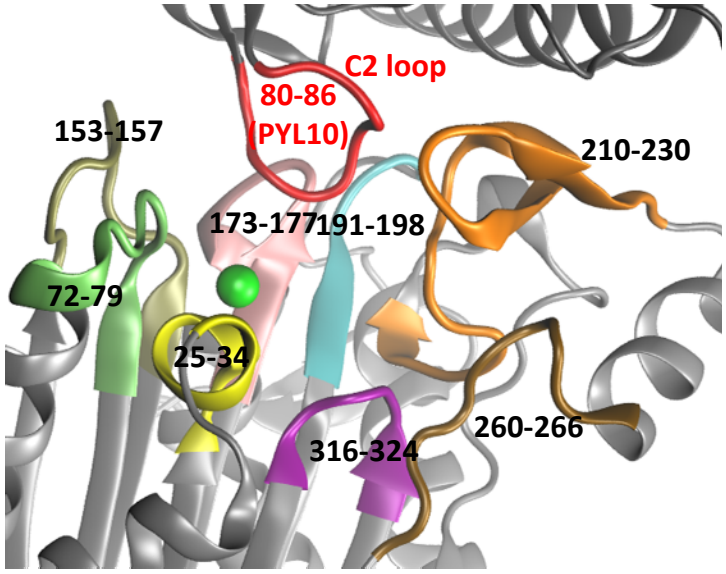
Molecular dynamics – Where to look?

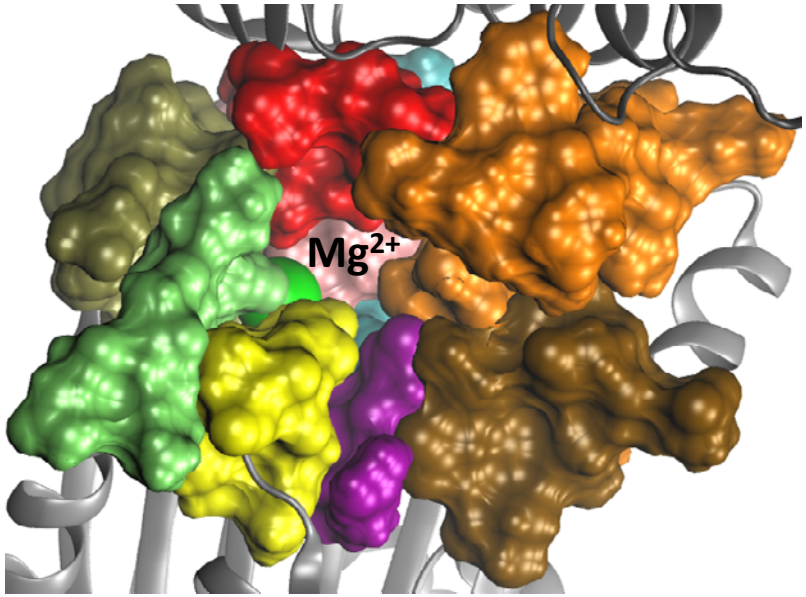




Mg2+ coordination

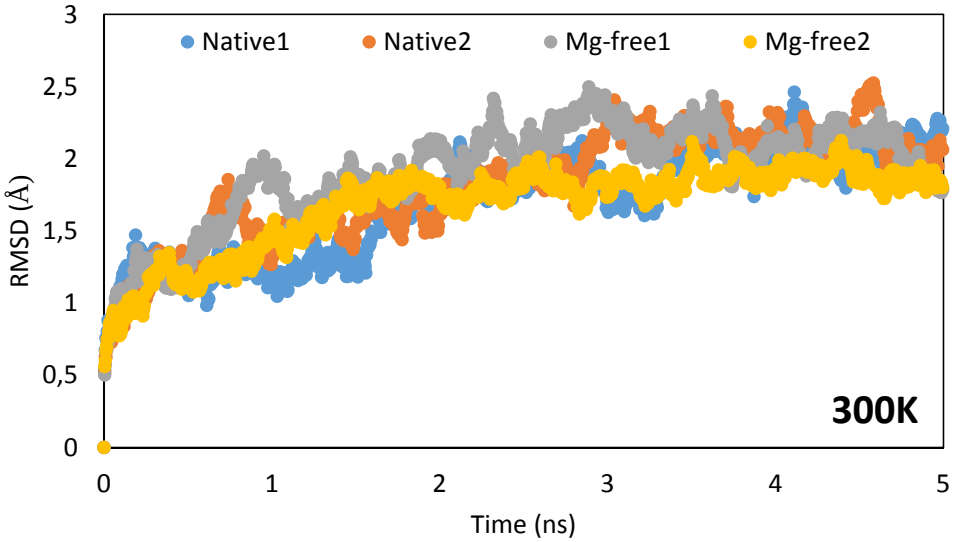
- Mg2+ coordination by 3 Aspartates and 2 crystal waters.
- Mg coordination domain in PP2C is located on the protein surface where the C2 loop of PYL10 is extended towards.
- The surface render of the Mg coordination domain together with the neigbouring regions shows that Mg coordination generates a tight interaction surface with the C2 loop.

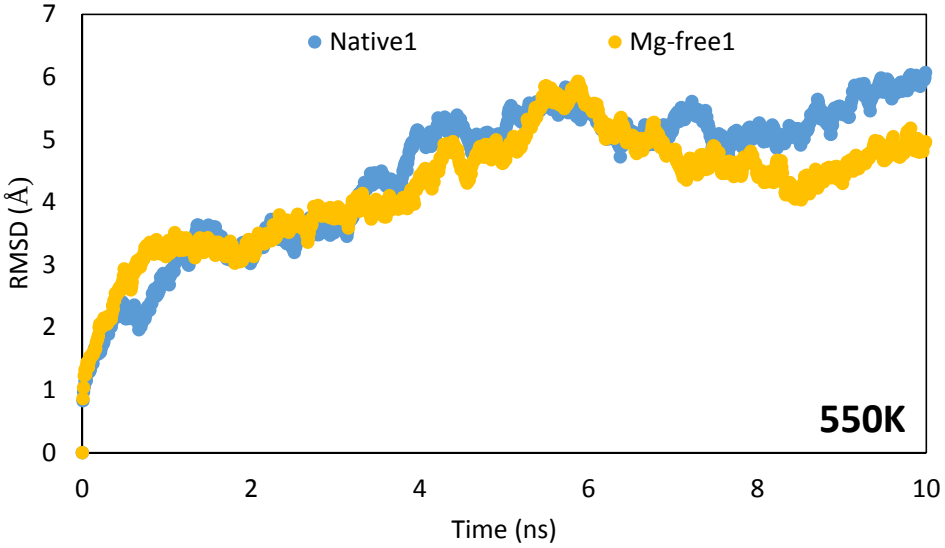


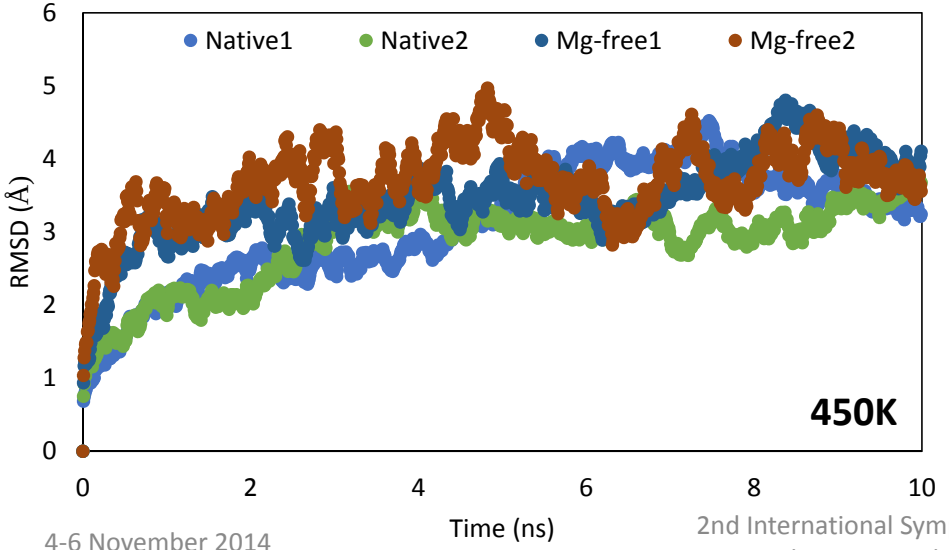


RESULTS

Molecular dynamics - RMSD



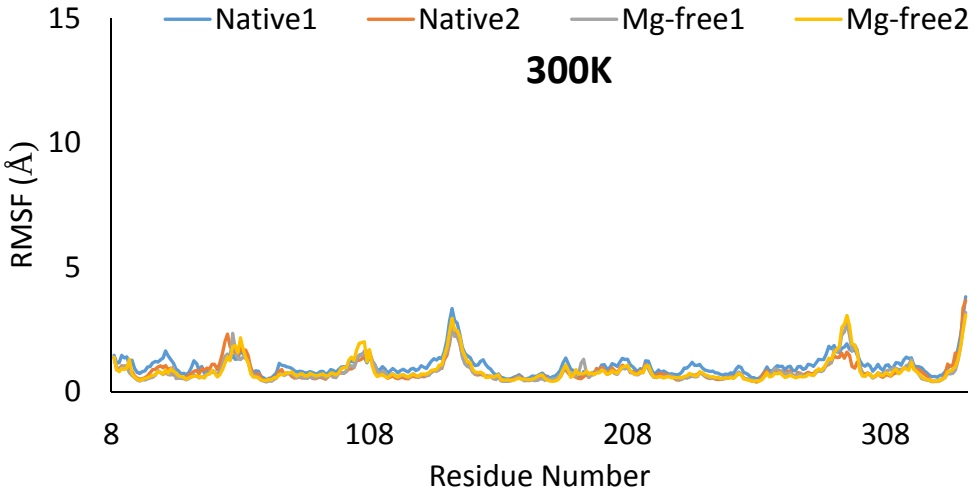


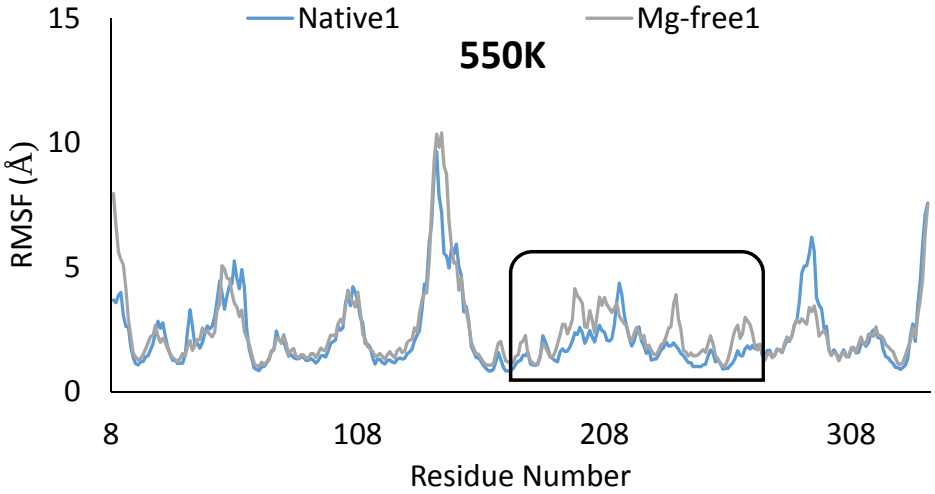


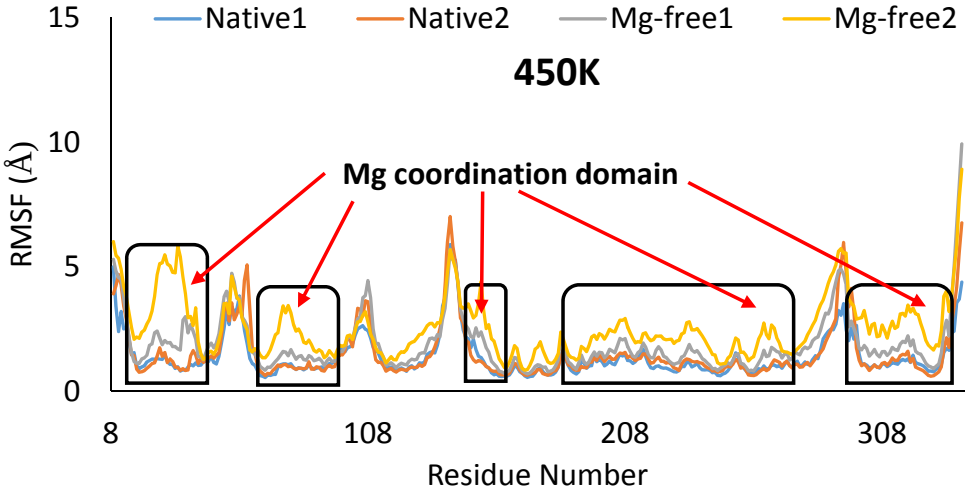
- Root mean square displacements (RMSD) of the backbone atoms (C, N, Cα) for these simulations indicated a stabilized pattern at all temperatures.
- Hence these simulations were acceptable for analyzing the stability of the PYL10-PP2C complex.

RESULTS

Molecular dynamics – RMSF (PP2C)



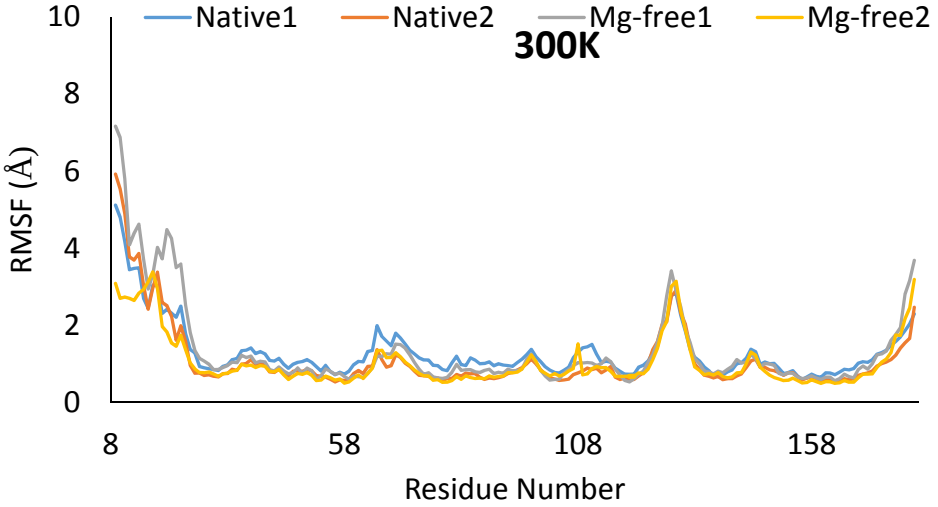


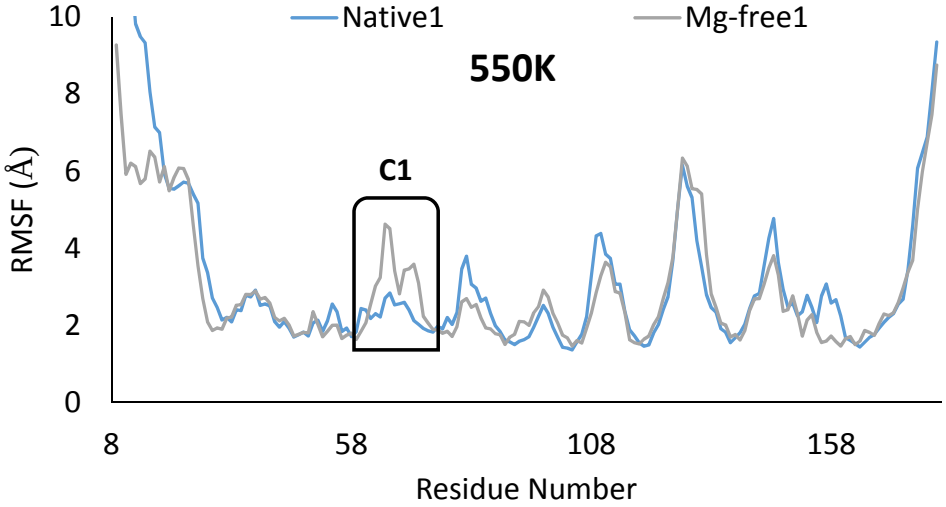


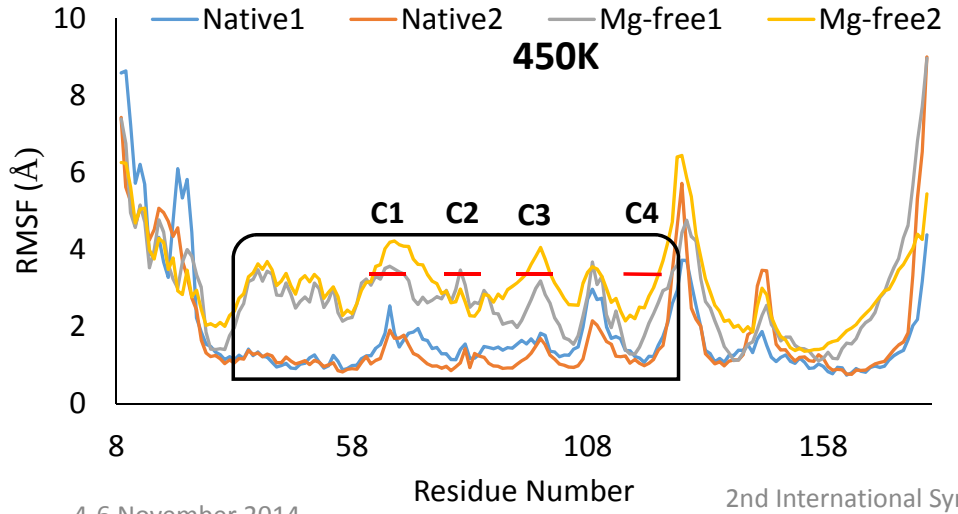
- At 300K → Mg presence does not have any effect on the fluctuations.
- At 450K → Mg-free complex shows increased fluctuations at certain regions (Mg coordination domains).
- At 550K → The proteins in the complex started to unfold, yet the fluctuations in the region between 150-250 in PP2C fluctuated more in the absence of Mg cation.

RESULTS

Molecular dynamics – RMSF (PYL10)



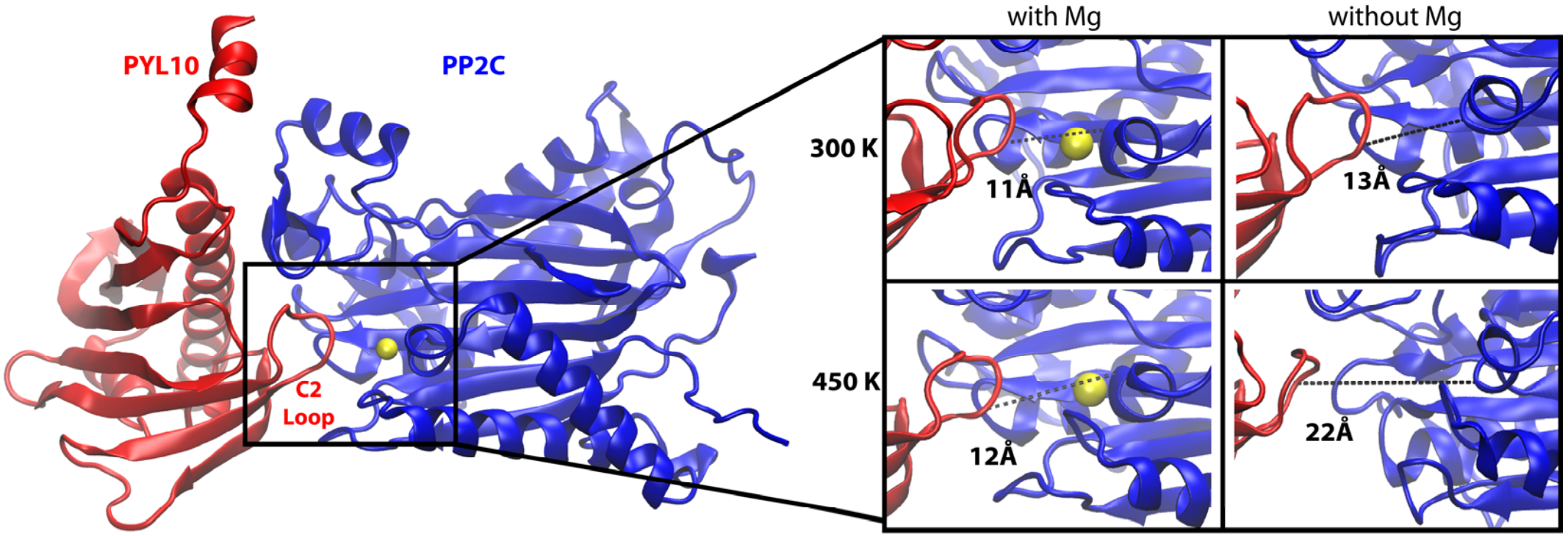




- At 300K → Mg presence does not have any effect.
- At 450K → Mg-free complex shows increased fluctuations at certain regions (in black rectangle).
- At 550K → The proteins in the complex started to unfold, yet the C1 loop in PYL10 fluctuated more in the absence of Mg cation.

RESULTS

Molecular dynamics – Visual inspections

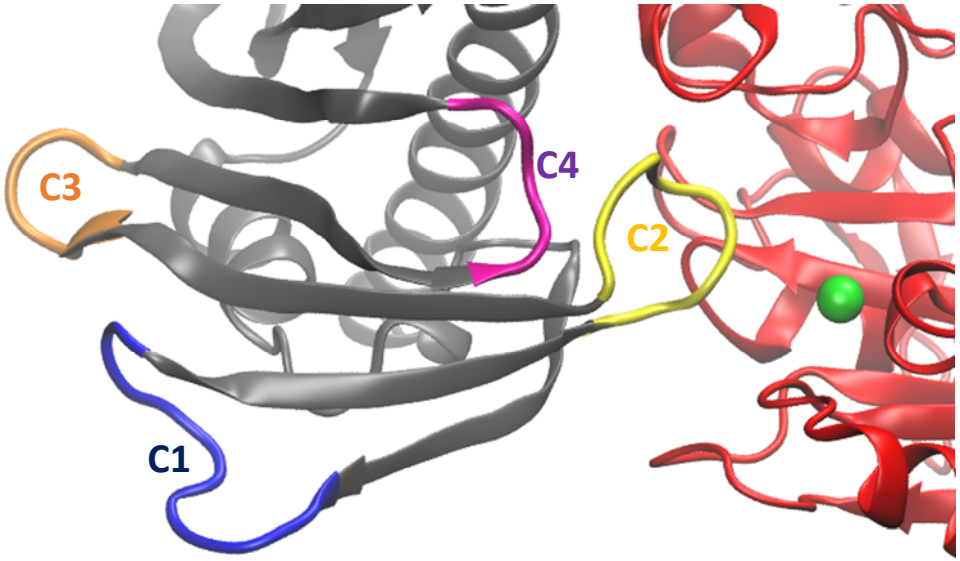


- At the end of the simulations, the snapshots of the subunit interface and the distance between the C2 loop of PYL10 and the Mg coordination domain of PP2C were illustrated.
- Mg absence did not have any effect on the compactness of the subunit interface at 300 K. On the other hand, at 450 K Mg absence led to a less compact interaction surface with an increased C2 loop and Mg coordination distance.
- The snapshots of 550 K simulations were not shown, because proteins became largely unfolded at the end.

RESULTS

The structural impact of Mg

RMSF (Å)	300 K		450 K	
	Native	Mg-free	Native	Mg-free
C1 (65-73)	1.2±0.2	1.2±0.1	1.7±0.2	**3.6±0.6**
C2 (80-86)	0.7±0.1	0.7±0.1	1.2±0.1	**2.7±0.2**
C3 (96-100)	1.0±0.0	1.0±0.0	1.6±0.1	**3.3±0.6**
C4 (110-116)	0.9±0.1	0.9±0.1	1.8±0.3	**2.6±0.3**



- Four loops that are critical to the interaction with PP2C fluctuated less than 1.5 Å regardless of Mg presence at 300 K. This suggests an almost zero mobility in those loops. Especially, the C2 loop fluctuated the least, which indicated that this loop is involved in a tight interaction.
- All of the four loops fluctuated significantly higher at 450 K in the absence of Mg than in the presence. In the native complex the fluctuations is slightly higher but still lower than 2 Å. But in the absence of Mg high temperature simulations led higher flexibility in all of the loops. This will imply that these loops cannot involve in any interactions with such kind of high fluctuations.

DISCUSSION

Mg has a direct impact on the stability of PYL10-PP2C complex at high temperatures

- In the presence of Mg;
  - After 10 ns simulations at 450 K, the distance between monomers were similar (12 Å) with the crystal structure (11 Å).
  - Also, at 450 K the fluctuations in the C1-to-C4 loops were comparable with those at 300 K.

- In the absence of Mg;
  - The distance between PYL10 and PP2C rose to 22 Å, which was profoundly higher than the crystal structure (11 Å).
  - Also the fluctuations in the C2 loop was elevated in the absence of Mg when compared with the condition at 300 K.
  - These data suggest that the interaction between the C2 loop of PYL10 and PP2C surface was compromised in the absence of Mg at high temperatures.

CONCLUSIONS
& Future remarks

- This study attempted to reveal the molecular mechanism leading Mg-deficiency related to heat-stress that was established in (Mengutay et al., Plant and Soil, 2013).
- Our results showed that at 450 K the removal of Mg negatively affected the stability of PYL10-PP2C by blocking the interaction of the C2 loop of PYL10 with PP2C.
- In this sense, our findings elaborated the experimental results showing the importance of Mg for tuning stress related genes and also for optimizing tolerance.
- The ABA-dependent or the constitutive inhibition of PP2Cs is realized through the conserved C2 loop, suggesting a unique inhibition mechanism of PP2Cs by PYLs and also reinforcing the importance of C2 loop in the inhibition mechanism.
- Considering the close proximity of the Mg domain to the C2 loop proposes that together with the C2 loop, Mg would also impart in this unique inhibition of PP2Cs, regardless of ABA presence.

THANKS TO

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- Thanx to you