Review of: "Revealing a hidden intermediate of rotatory catalysis with X-ray crystallography and Molecular simulations"

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This work seems impressive in combining structural determination and molecular dynamics (MD) simulation together to present key structural intermediates and dominant reaction mechanisms of rotary catalysis of V-1 ATPase. I have particular interest to learn it from a perspective of comparing the V-1 system with F1-ATPase, which we have studied its intrinsic inter-subunit coordination around the ATPase ring for sequential hydrolysis using MD and kinetic analyses (http://dx.doi.org/10.1016/j.bpj.2017.08.015)

In current V1-ATPase work, the authors suggest that Pi release proceeds prior to ADP release by analyzing structural and energetic characteristics. While I'm impressed by comprehensive free energy calculations and enhanced sampling approaches to characterize the Pi release process, I found little being addressed directly or comparatively on ADP release yet to V1. In F1-ATPase, evidence had shown on ADP release prior to Pi release instead. Then a key question is, what are the essential structural dynamics characters that distinguish V1 from F1? Although it is noted in current study that electrostatic interaction of ADP is stronger than Pi with the binding pocket, it may be commonly true (due to more negative charges on ADP) for both V1 and F1 or similar systems. Likely, additional features are needed to be examined, comparatively.

In Discussion, I hope that the authors can address a bit more on the 'unique asymmetry of the A3B3 ring' that facilities Pi release. Besides, the free energy barrier of Pi release appears to be quite substantial indeed (i.e., reaching ~ 6 kcal/mol; what would that be for ADP?). In F1, we noted that Pi release can indeed be facilitated by either ATP binding (from 'upstream' neighboring pocket) or ATP hydrolysis ('downstream' neighboring pocket). Hence, the claim made currently on "the Pi release mechanism presented here is generalizable' in Discussion may not be well supported.

In addition, from our previous learning on F1-ATPase ring, ATP binding likely opens up the neighboring pocket to facilitate ADP release. Hence, I'm also wondering if that is likely true as well for V1 system. Although ADP release can be rate limiting, with assistance from ATP binding to neighboring site, such a slow process can be possibly accelerated. Furthermore, can the authors point out more clearly wether the

coupling between neighboring sites (e.g. Pi release modulates ATP binding site) is more through the A/B intersubunit interface, or more via the coordination from the central stalk?

Last, for simulation implementations, although being conducted systematically, I found them sometime lack of details. For example, how the replica numbers were determined (in REST2 simulations), how the collective coordinates or CVs were defined (in funnel metadynamics), etc.. The network allostery and mutual information analyses, although interesting, seem to ignore contributions from amino acids side chains. In the kinetic estimation for Pi release (using mean first passage time), it is a bit risky to use diffusional coefficients from F1 system determined previously, due to the exact reason I raised above: in F1 Pi likely releases more reluctantly, after ADP release; while in V1 here, Pi release seems to happen first, before ADP release; hence, the ‘friction’ impacts or diffusional coefficients may be quite different in two systems.