

## Simulation of catalytic water activation in mitochondrial F<sub>1</sub>-ATPase using a hybrid quantum mechanics/molecular mechanics approach: An alternative role for $\beta$ -Glu 188.

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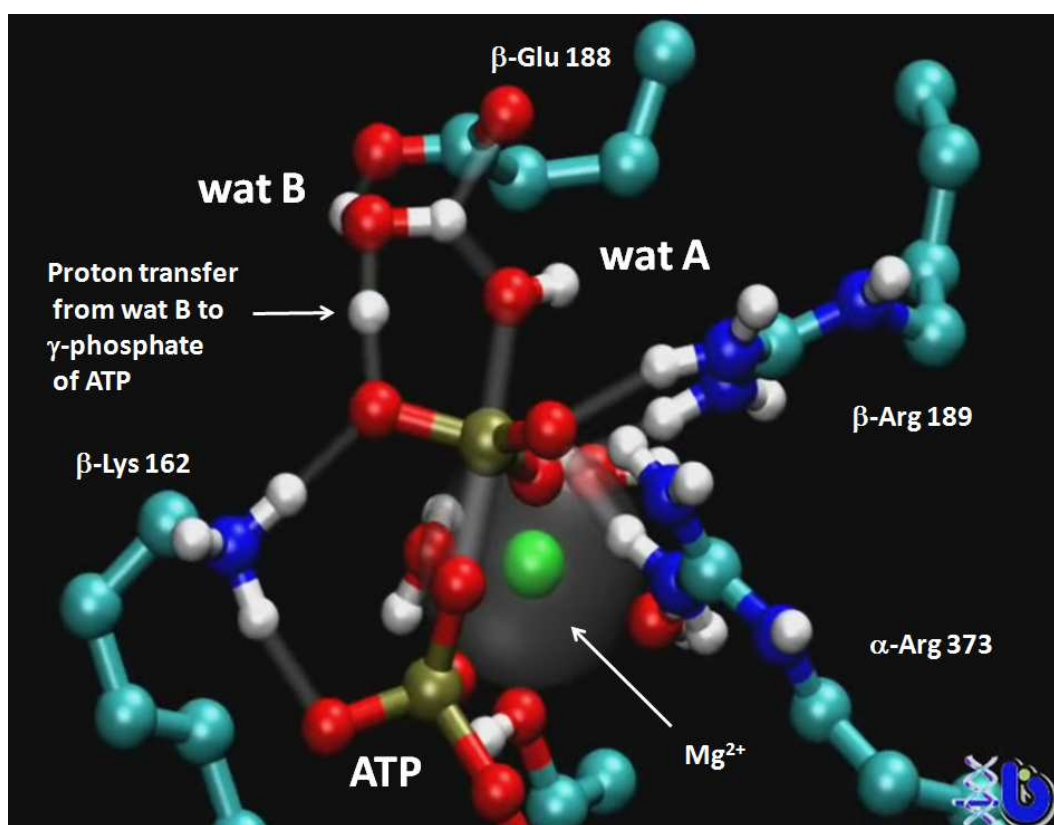
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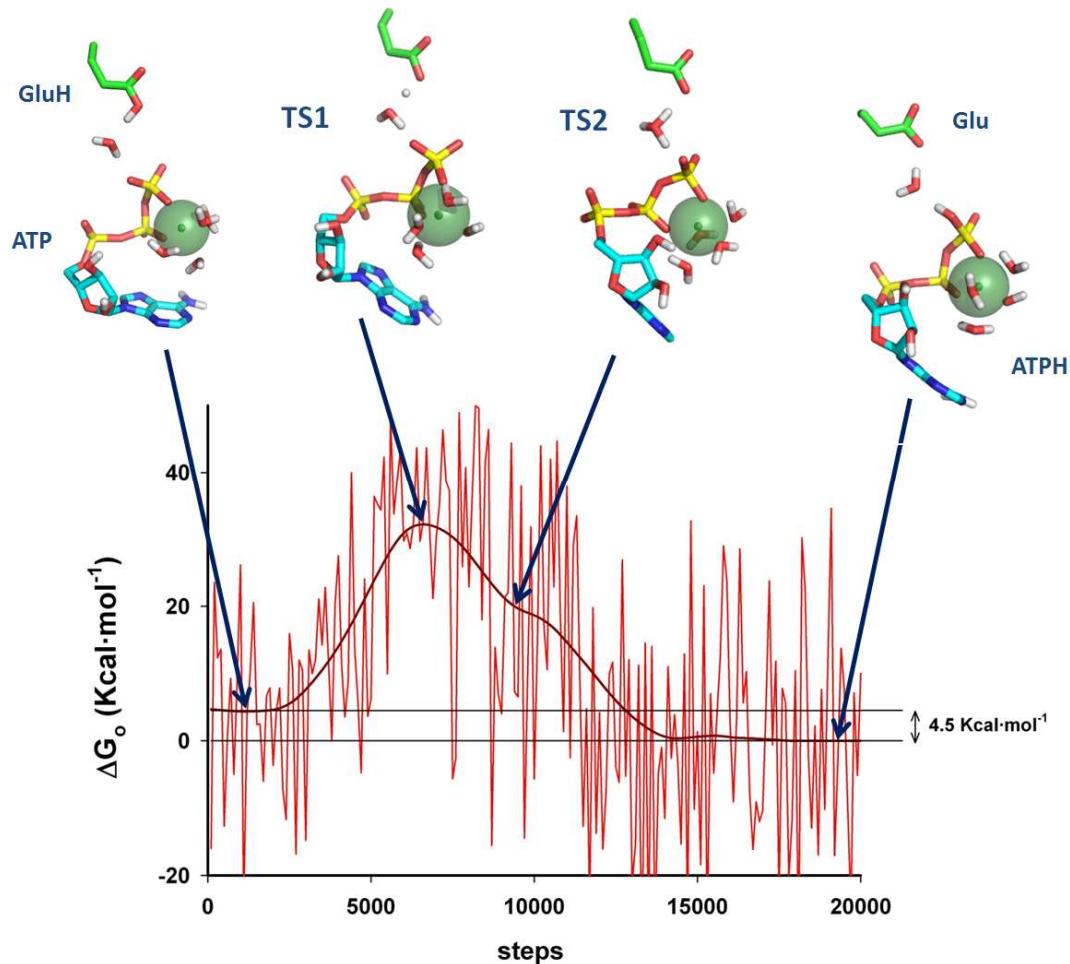
### SUPPORTING INFORMATION

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- Video V1. Video demonstrating proton movements in the QM region of the QM/MM system.
- Figure S1. Simulation of proton transference between a Glu residue and an ATP molecule through a hydronium intermediate in solution.



**Video V1. Video demonstrating proton movements in the QM region of the QM/MM system.** First: a proton is transferred from the attacking water molecule (wat A) to the second water molecule (wat B). Second, a different proton is transferred from wat B to ATP. The role of  $\beta$ -Glu 188 in stabilizing proton movements and transient hydronium ion during the water activation is highlighted.



**Figure S1. Simulation of proton transference between a Glu residue and an ATP molecule through a hydronium intermediate in solution.** A minimal system was simulated in a water box containing a protonated Glu residue, the catalytic water and an ATP molecule. From the initial state (left), proton was forced to move away from the initial GluH state until an energy maximum was reached (transition state TS1). From that position, the system moved spontaneously to a structure where the gamma phosphate of ATP is protonated (ATPH). The difference of  $\Delta G_0$  values between the initial and the final states is 4.5 kcal·mol<sup>-1</sup>, which corresponds to 3.3 pKa units, in the range of the experimentally measured difference of 3.0 - 3.3 pKa units between the two groups in solution. In the same trajectory, the position of the system when the hydronium group is completely formed (TS2 in the figure) suggested that, from this structure, the system will move spontaneously (downhill) to the protonated state of ATP. On the contrary, from this same position, it is predicted that the system would not move easily to the protonated state of Glu residue due to the presence of the  $\Delta G_0$  barrier of TS1.