**Veyron et al. Supplementary data.**

**Analysis of the deAMPylation catalytic mechanism of EfFIC.**

We discuss below the mechanism of deAMPylation of EfFIC. First, it should be noted that FIC active sites do not ressemble the active site of Legionella de-AMPylase SidD, which has a canonical phosphatase fold (1) or the de-AMPylation domain of E. coli glutamine synthase adenylyl transferase (2), hence are not expected to share catalytic features with these enzymes. From a general enzymology perspective, two major mechanisms of (phospho)ester bond hydrolysis can be considered: anchimeric catalysis, which is assisted by the substrate (**Figure S2A)** or conventional acido-basic catalysis **(Figure S2B**). Both mechanisms involve four steps (see **Figure S2**) and share three chemical requirements: i) a proton attractor to increase the nucleophilic properties of the reactive oxygen (step 1) ii) a positively charged species, located in the neighborhood of the phosphate group to increase the susceptibility of the phosphorus to nucleophilic attack and stabilize the developing negative charge in the intermediate (steps 2 and 3), and iii) a proton donor, located close to the cleaved phosphor-ester bond to favor the production of the leaving group by giving up its proton (step 4). A major difference between the two scenarii is that nucleophilic attack is performed by the 2' hydroxyl of the ribose in the anchimeric mechanism, as described for a calcium-dependent phosphoinositide-specific phospholipase C from Pseudomonas (3), whereas it is achieved by the oxygen of an activated water molecule in the general acido-basic catalysis. The two mechanisms can be distinguished by at least two major features of the active site: i) anchimeric catalysis requires a proton attractor close to the ribose 2'OH, whereas the general acidic catalysis requires a proton attractor close to the nucleophilic water molecule, itself located close to the leaving phosphate group; ii) substrate-activated catalysis involves the formation of a cycle between the phosphorus and the 2'OH of the AMP moiety of the substrate.

Considering our EfFIC$^{WT}$-AMP-$Ca^{2+}$ structure as an acceptable mimic of the enzyme-AMPylated protein complex, EfFIC displays several important features: i) the AMP moiety is stabilized by multiple interactions with the active site, such that a large conformational change needed for cyclisation in the anchimeric reaction is unlikely ii) a water molecule coordinated by the conserved glutamate and $Ca^{2+}$ ion can be readily positionned for in line nucleophilic attack by completing the heptahedral coordination of $Ca^{2+}$ iii) no proton acceptor is situated closed to the 2'OH in the structures. Together, these observations impose a

mechanism based on acido-basic and electrostatic catalysis, as described in **Figures 5** and **S2B**.

**Supplementary Figure legends**

**Figure S1 (associated with Figures 1 and 2). Structural analysis of EfFIC.**

1A: Omit map showing the electron density of the inhibitory glutamate and the ADP moiety of ATPγS in $EfFIC^{WT}$-ATPγS contoured at 5.0 r.m.s.d.. The electron density of Glu190 is representative of all EfFIC structures determined in this study

1B: Omit map showing the electron density of $Ca^{2+}$ in $EfFIC^{WT}$-AMP-$Ca^{2+}$-bound structure contoured at 10.0 r.m.s.d.

**Figure S2 (associated with Figure 5). Comparison of anchimeric and acido-basic deAMPylation catalytic mechanisms.**

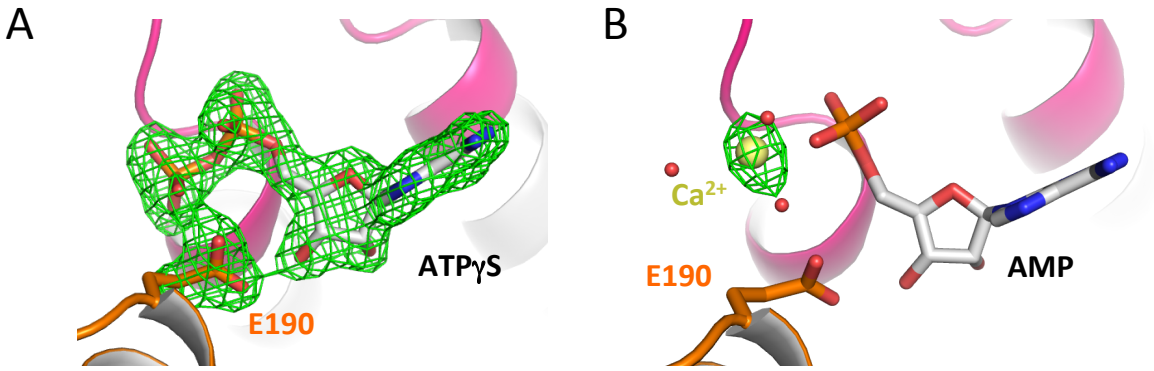
2A: Anchimeric catalysis.

2B: Acido-basic catalysis.

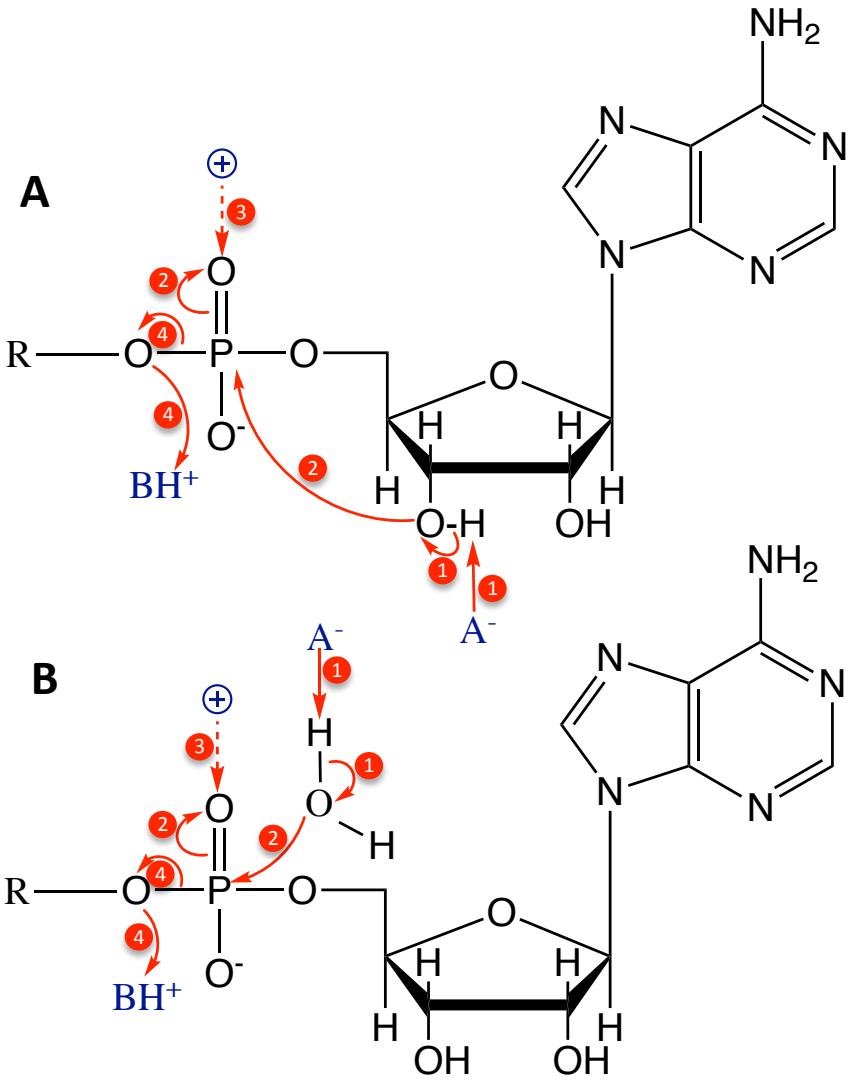
Both mechanisms involve four steps, as indicated: (1) activation of the reactive oxygen through proton attraction (2); nucleophilic attack on the positively charged phosphorus triggering P=O π electrons rearrangement and production of a pentavalent intermediate harboring an additional negative charge; (3) stabilization of the intermediate by a positive charge in the catalytic site, which also contribute to elicit the electrophily of the phosphorus and (4) facilitation of phosphor-ester bond cleavage through protonation of the leaving hydroxylate group. R: AMPylated protein. A- : basic form of an acidic catalyst (proton attractor). BH+ : acidic form of a basic catalyst (proton donor).

**References**


1. Chen Y*, et al.* (2013) Structural basis for Rab1 de-AMPylation by the Legionella pneumophila effector SidD. *PLoS pathogens* 9(5):e1003382.
2. Xu Y, Carr PD, Vasudevan SG, & Ollis DL (2010) Structure of the adenylylation domain of E. coli glutamine synthetase adenylyl transferase: evidence for gene duplication and evolution of a new active site. *J Mol Biol* 396(3):773-784.
3. Moroz OV*, et al.* (2017) The structure of a calcium-dependent phosphoinositide-specific phospholipase C from Pseudomonas sp. 62186, the first from a Gram-negative bacterium. *Acta Crystallogr D Struct Biol* 73(Pt 1):32-44.




Supplementary Figure S1




Supplementary Figure S2