**Supporting Information**

**Polyphosphate Kinase from *Burkholderia cenocepacia*, One Enzyme Catalyzing a Two Step Cascade Reaction to Synthesize ATP from AMP**

Dianelis T. Monterrey1, †,\* Leire Azcona,1 Julia Revuelta,1 Israel Sánchez-Moreno,1 and Eduardo García-Junceda1,\*

1 Departamento de Química Bioorgánica, Instituto de Química Orgánica General, CSIC (IQOG-CSIC). Juan de la Cierva 3, 28006 Madrid, (Spain); [leireazcona.sa@gmail.com](mailto:leireazcona.sa@gmail.com) (L.A.); [julia.revuelta@iqog.csic.es](mailto:julia.revuelta@iqog.csic.es) (J.R.) [isra.sanchez@iqog.csic.es](mailto:isra.sanchez@iqog.csic.es) (I.S-M.)

† Current address: Department of Biocatalysis, Institute of Catalysis and Petrochemistry, CSIC (ICP-CSIC). Marie Curie 2, 28049 Madrid (Spain)

**\*** Correspondence: [d.monterrey@csic.es](mailto:d.monterrey@csic.es) (D.T.M.)

[eduardo.junceda@csic.es](mailto:eduardo.junceda@csic.es) (E.G.-J-)



**Figure S1**. Sequence alignment of PPK2-III proteins from *B. cenocepacia* and *M. ruber* (Mrub\_2488). Walker A and B motifs highlighted in red and blue, respectively and the cap module highlighted in purple; the conserved residues, glutamic acid (E137) is highlighted in green and glutamine (Q74) in yellow.



**Figure S2.** SDS-PAGE analysis of expression and IMAC purification of *Bc*PPK2-III. MWM: molecular weight marker; Lane 1: cell-free extract; Lane 2: pass-through fraction; Lane 3: elution of proteins nonspecifically bound to resin; Lane 4: elution of recombinant protein; Lane 5: resin with proteins not removed after washing and elution.



**Figure S3.** Peptide mass fingerprint and major secondary structural motifs of *Bc*PPK2-III. The identified peptides are highlighted in bold and underlined with their corresponding molecular mass in Dalton (Da). Sequences corresponding to -helix are shown in pink and those corresponding to -sheets in orange. Segments corresponding to "coil-coil" structures are highlighted in gray.



**Figure S4.** (**a**) AlphaFold prediction colored by model confidence band; (**b**) Sequence coverage. Number of homologous sequences identified per position

Imagen en blanco y negro

Descripción generada automáticamente

**Figure S5.** Transmission electron microscopy images (sTEM) of the nanoflower structure formed between Mn2+ and the shorter chains of phosphate donor groups.



**Figure S6.** Agarose gel of restriction analysis of plasmid pET-28b(+)-*ppk-III*, purified from three different colonies. MWM = molecular weight marker.



**Figure S7.** (**a**) Chromatogram of the standard mixture of polyP, ATP, ADP and AMP. (**b**) Chromatogram of *Bc*PPK2-III reaction after 3.5h, with AMP as the starting substrate. (**c**) Chromatogram of *Bc*PPK2-III reaction after 3.5h, with ADP as the starting substrate.