## **Description of Additional Supplementary Files**

File Name: Supplementary Movie 1

**Description**: A morph movie demonstrating the Cy substrate tunnel switch (ArCP entry side) controlled by the key residue F372.

File Name: Supplementary Movie 2

**Description**: The trajectory of PchE (highlighted with one chain) describing the changes between the catalytic steps by a series of 120 cryo-EM maps reconstructed by cryoDRGN software (left, PCA projection; middle, cryo-EM maps; right, schematics).