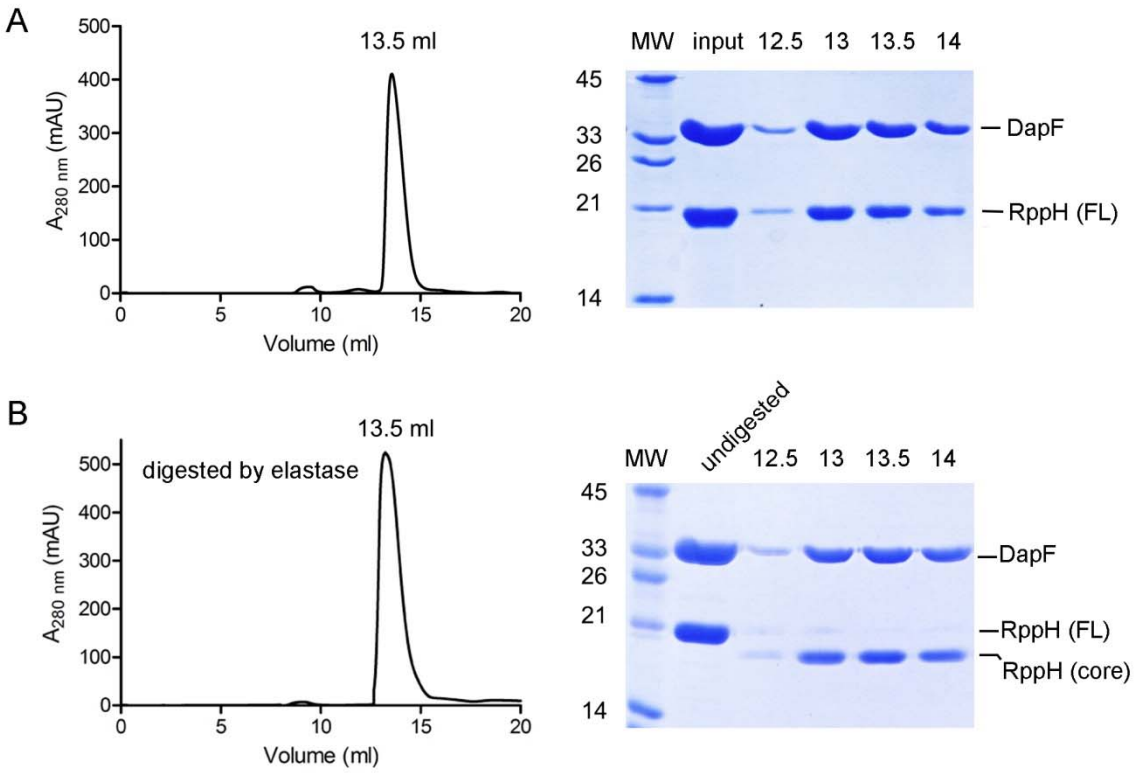
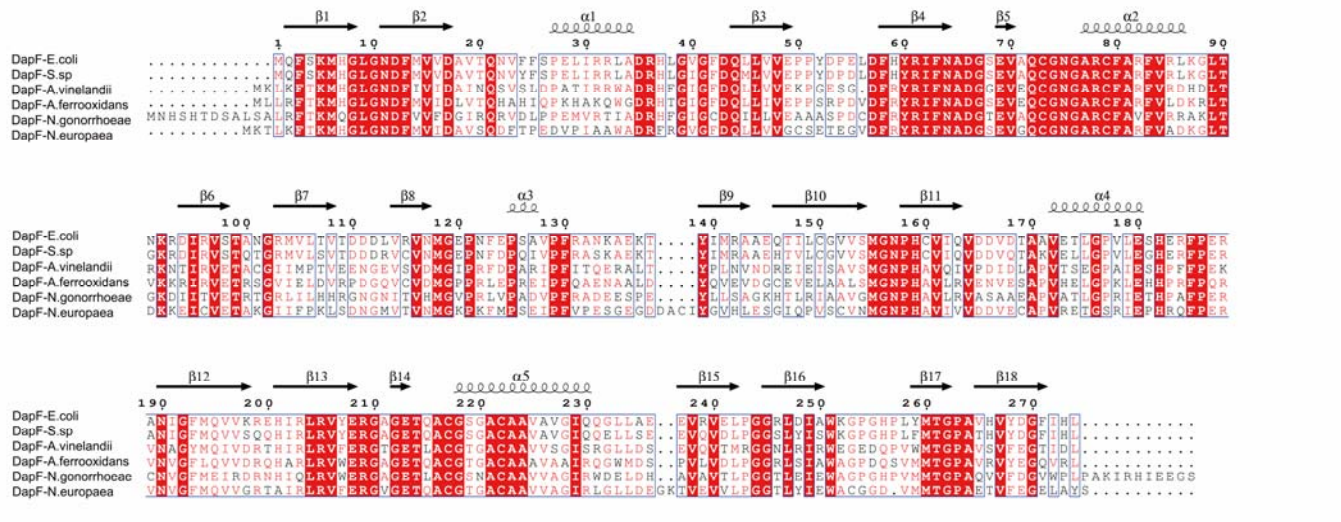
# Supplementary information

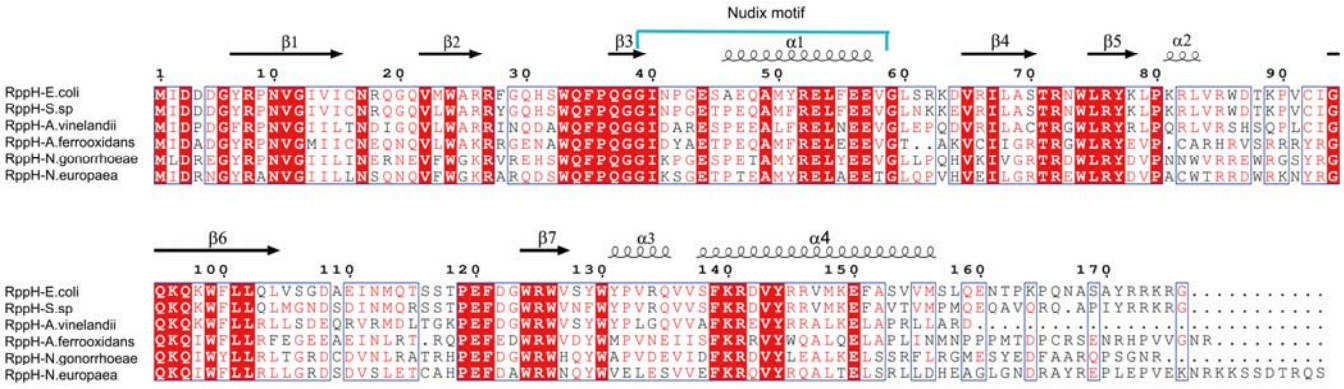
## DapF stabilizes the substrate-favoring conformation of RppH to stimulate its RNA-pyrophosphohydrolase activity in *Escherichia coli*



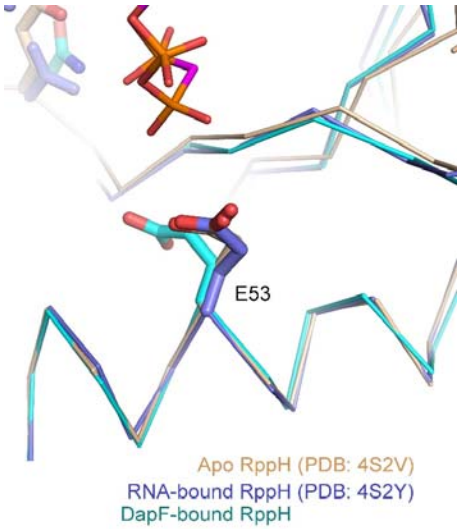

**Supplementary Figure S1.** Determination of the stable core of the DapF-RppH complex. (A) Full-length DapF co-eluted with full-length RppH in size exclusion chromatography and confirmed by SDS-PAGE. (B) Limited proteolysis of DapF-RppH by elastase. DapF co-eluted with RppH, with the former nearly undigested, while the latter became slightly smaller.



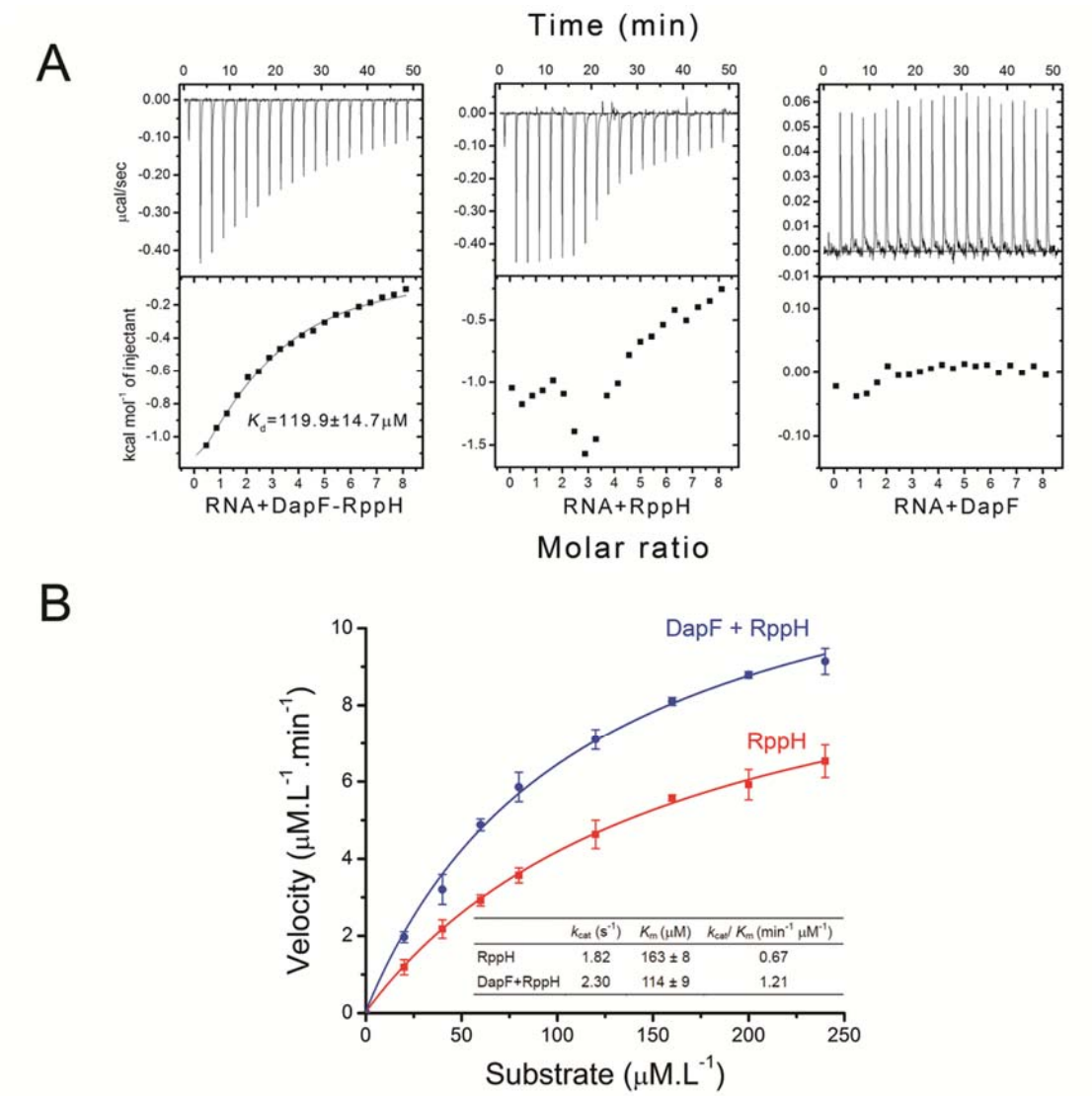

**Supplementary Figure S2.** Sequence alignment of DapF homologues. The sequence of *Escherichia coli* DapF (NCBI-GI: 447083398) was aligned with its homologues from *Acidithiobacillus ferrooxidans* (NCBI-GI: 497251752), *Neisseria gonorrhoeae* (NCBI-GI: 489783847), *Nitrosomonas europaea* (NCBI-GI: 499424712), *Serratia sp.* (NCBI-GI: 558558764) and *Azotobacter vinelandii* (NCBI-GI: 502037695). Secondary structural elements are indicated above the sequence alignment. Completely conserved amino acids are colored in red, while relatively conserved amino acids are enclosed in blue boxes. Square cyan brackets indicate the Nudix motif region. The alignment was generated using the MultAlin (1) and ENDscript (2) programs.



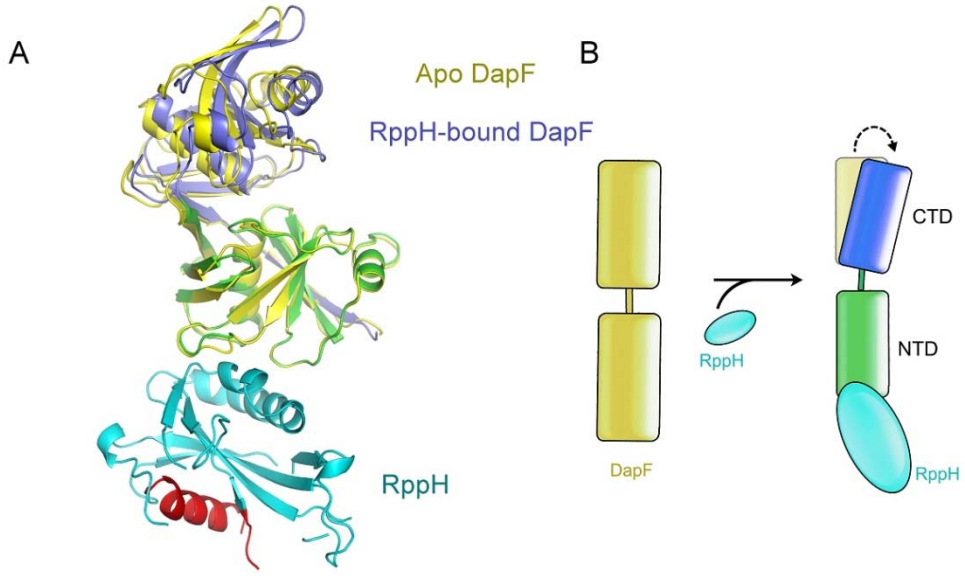

**Supplementary Figure S3.** Sequence alignment of RppH homologues. The sequence of *Escherichia coli* RppH (NCBI-GI: 446486635) was aligned with its homologues from *Acidithiobacillus ferrooxidans* (NCBI-GI: 501530552), *Neisseria gonorrhoeae* (NCBI-GI: 499253703), *Nitrosomonas europaea* (NCBI-GI: 499424430), *Serratia sp.* (NCBI-GI: 558558606) and *Azotobacter vinelandii* (NCBI-GI: 502037747). All labeling and methods used are the same as in Supplementary Figure S2.




**Supplementary Figure S4.** Structural comparison of the Nudix motif in various states of RppH. The side chains of key catalytic residue E53 exhibit similar orientation in various apo/bound forms of RppH.

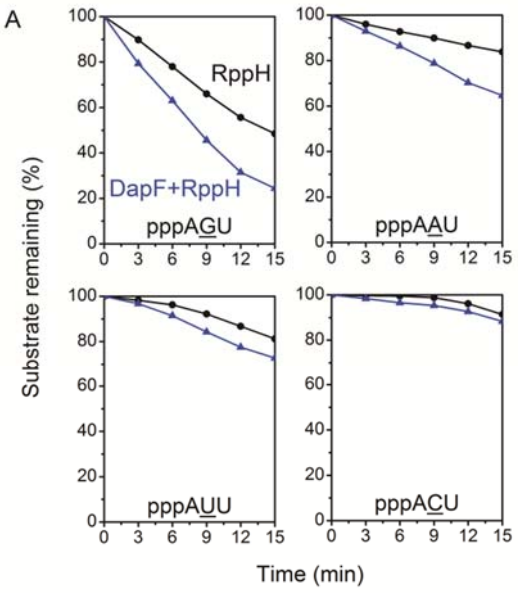



**Supplementary Figure S5.** The binding of RppH and substrate RNA. (A) Interactions of RNA and RppH are examined by ITC at 37 ºC in 50 mM HEPES, pH7.5, 150 mM NaCl. Left panels are original signals (up) and the deltaH value (down) of 2 mM RNA titrated into 50 µM DapF-RppH complex. Black line in the panel below represents the fitting curve of deltaH of the reaction and the calculated $K_d$ value was shown in the box. Middle and right panels represent 2 mM RNA titrated into 50 µM RppH and 50 µM DapF, respectively. (B) Determination of the enzymatic properties of RppH for RNA hydrolysis. Velocities of RppH at different concentration of RNA, in the absence (red squares) or presence (blue circles) of DapF, were fitted to Michaelis-Menten equation equipped in Origin 8.0 to get the kinetic parameters of RppH for RNA hydrolysis. The error bar of the velocity represents standard deviation of two independent measurements. Kinetic parameters of the reactions were list in the inlet table.




**Supplementary Figure S6.** The C-terminal domain of DapF undergoes a moderate conformational change upon binding to RppH. (A) Structural alignment of DapF in apo state and in RppH-bound state. The structure of apo DapF is labeled in yellow and RppH-bound DapF in blue; the green portion represents the superimposed moiety of DapF in different states. The RppH molecule is labeled in cyan and the Nudix motif in red. (B) Illustrated model of RppH binding to DapF. RppH binding induces a conformational change in DapF. The color of proteins is the same as in diagram (A).

A




B

| | Substrate remaining (%) | | | |
|---|---|---|---|---|
| | pppAGU | pppAAU | pppAUU | pppACU |
| RppH | 48.5 | 81.3 | 83.8 | 91.4 |
| DapF+RppH | 24.4 | 64.6 | 72.8 | 88.2 |

**Supplementary Figure S7.** The effect of DapF on hydrolysis of distinct substrates by RppH. (A) Substrate remained in the reaction was measured by MonoQ ion exchange chromatography during 15 min, with an interval of 3 min. Four short triphosphorylated RNAs were examined. The back filled circles and the blue filled triangles represent substrate remaining without or with DapF in reaction, respectively. (B) Summary of four substrates remaining at 15 min, with the absence or presence of DapF in the reaction.

**Supplementary Table S1.** Interatomic distances for the hydrogen bonds and hydrophobic interactions on interface of DapF-RppH complex.

| Molecule | Residue | Atom1 | Distance (Å) | Atom2 | Residue | Molecule |
|---|---|---|---|---|---|---|
| DapF | P54 | O | 3.01 | OG | S128 | RppH |
| | L56 | O | 2.60 | | | |
| | F58 | CE1 | 3.61 | CD1 | W130 | |
| | L89 | O | 2.86 | NE1 | | |
| | V19 | CG1 | 3.24 | CE3 | | |
| | T20 | O | 2.76 | NE | R145 | |
| | | | 2.90 | NH2 | | |
| | N22 | ND2 | 2.52 | OD1 | D142 | |
| RppH | R145 | NH1 | 2.90 | OD1 | D142 | RppH |

*The interatomic distances were generated using the COCOMAPS program (3).

**REFERENCES**


1. Corpet, F. (1988) Multiple sequence alignment with hierarchical clustering. *Nucleic acids research*, **16**, 10881-10890.
2. Gouet, P., Robert, X. and Courcelle, E. (2003) ESPript/ENDscript: Extracting and rendering sequence and 3D information from atomic structures of proteins. *Nucleic acids research*, **31**, 3320-3323.
3. Vangone, A., Spinelli, R., Scarano, V., Cavallo, L. and Oliva, R. (2011) COCOMAPS: a web application to analyze and visualize contacts at the interface of biomolecular complexes. *Bioinformatics (Oxford, England)*, **27**, 2915-2916.