Supplemental Material

Structure and mechanism of the polynucleotide kinase component of the bacterial Pnkp-Hen1 RNA repair system

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Supplemental Table S1
Supplemental Figures S1, S2, S3 and S4

Table S1

Crystallographic data and refinement statistics

	Native Pnkp-(1-170)	SeMet Pnkp-(1-170)
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions (Å) @ 130K	a = 43.69	a = 45.48
	b = 71.50	b = 67.08
	c = 118.59	c = 119.62
Diffraction data quality		
Resolution, Å	45.6 - 2.1 (2.21 - 2.10)	50.0 - 2.0 (2.03 - 2.0)
Radiation source / wavelength	NSLS X25 / 1.10 Å	NSLS X25 / 0.97 Å
Processing software	Mosflm/Scala	Scalepack
R_{sym}*, %	11.9 (48.9)	10.5 (39.2)
Unique reflections[a]	22214 (2970)	47289 (2380)
Mean redundancy	5.7 (4.2)	9.6 (2.8)
Completeness, %	98.9 (92.9)	99.8 (99.2)
Mean $I/\sigma I$	10.8 (3.3)	29.31 (2.8)
Phasing statistics		
Phasing method	Phenix AutoMR	Se-SAD (8 Se sites)
Resolution, Å	45.6 - 2.1	44.5 - 2.0
Signal to noise†	RFZ = 19.5, TFZ = 29.4	Ano = 0.13
Figures of merit§		0.37/0.70
Refinement and model statistics ($F>\sigma F$)		
Resolution (Å)	35.8 – 2.1 (2.16 – 2.10)	44.5 – 2.0 (2.05 – 2.00)
Completeness, %	98.8 (89.0)	98.8 (93.0)
R_{free}¶/R_{work}, %	22.8 /17.7 (27.5/20.9)	23.0/17.5 (35.2/23.5)
RMS deviation: bonds/angles	0.007 Å/ 1.10°	0.008 Å/ 1.22°
Protomers / ASU	2	2
NCS RMS Deviation	0.36 Å over 170 C_α	0.57 Å over 169 C_α
NCS restraints	none	none
Ramachandran plot: favorable/outliers	97.4% / none	99.4% / none
B-factors, Å^2		
Overall / Wilson	26.1/21.4	22.9/14.4
Protein (by chain)	26.2/27.0	22.2/23.2
Mean TLS anisotropy	0.25 (1 TLS group)	NA
PDB ID	4GP6	4GP7
Model contents		
Protein residues	341	342
Heteroatoms	2 ADP, 2 Mg^{2+}	2 ATP, 2 Mg^{2+}, 1Na^+, 1 citrate
Water	237	189

Standard definitions are used for all parameters. Figures in parentheses refer to data in the highest resolution bin. The refinement and geometric statistics come from PHENIX.

* R_{sym} output as R_{meas} by SCALA and R_{lin} by SCALEPACK.

† Signal to noise for anomalous phasing (Ano) of the SeMet-kinase is the fraction of data for which $D_{ano} \geq 3\sigma D_{ano}$, as calculated in XTRIAGE. Signal to noise values for the native kinase structure are described by the rotation function Z-score (RFZ) and translation function Z-score (TFZ) from molecular replacement, as calculated in PHENIX.

§ FOM's are from PHASER/RESOLVE for heavy atom phasing.

¶ R_{free} sets consisted of 8% of data chosen at random against which structures were not refined.

[a] F^+ and F^- were treated as equivalent for the native and independent for SeMet dataset.

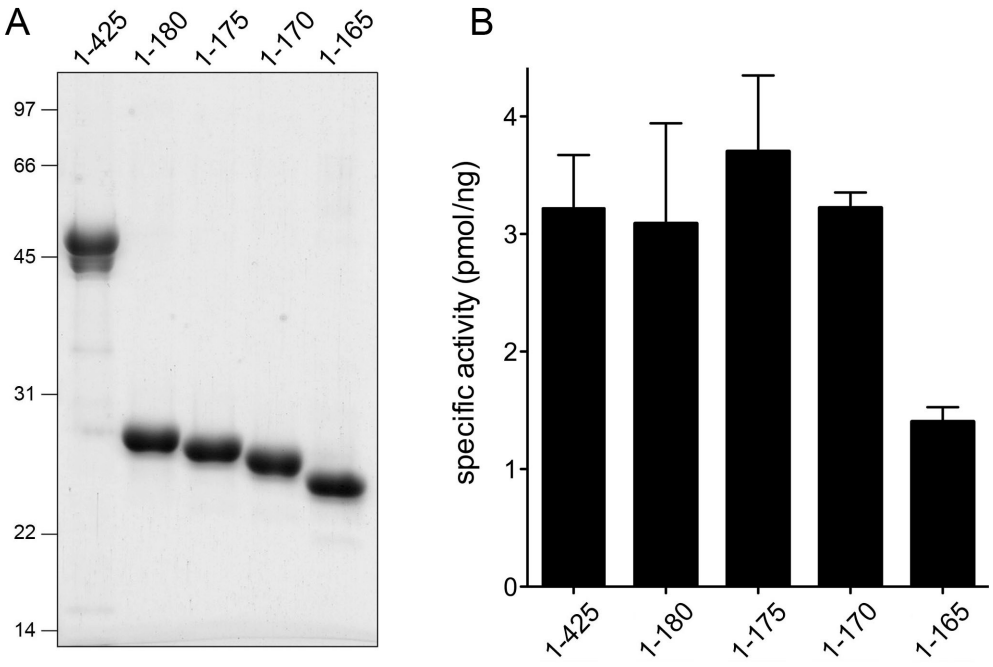

Figure S1. **Deletion analysis of *Cth*Pnkp demarcates an N-terminal kinase domain.**
(A) Aliquots (8 μg) of the Ni-agarose preparations of the indicated His_{10}*Cth*Pnkp proteins were analyzed by SDS-PAGE. The polypeptides were visualized by staining with Coomassie blue dye. The positions and sizes (kDa) of marker polypeptides are indicated on the left. (B) Kinase reaction mixtures contained 180 pmol of 36-mer 5'-OH DNA oligonucleotide and serial 2-fold dilutions of the indicated *Cth*Pnkp proteins (from 6.25 to 200 ng). Specific activity (pmol of label transfer from [γ^{32}P]ATP to the 36-mer DNA per ng of input protein) was determined from the slope of the titration curve in the linear response range. Each datum in the bar graph is the average of three separate enzyme titration experiments ± SEM.

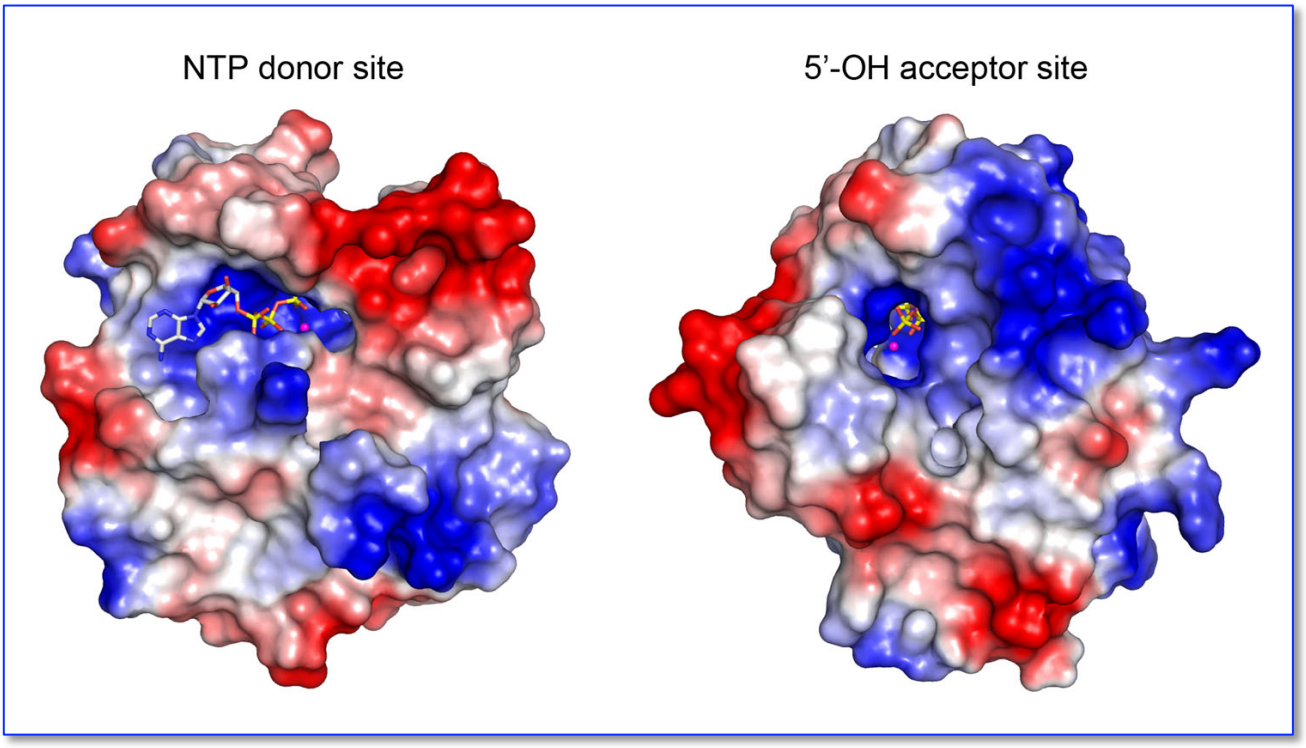

Figure S2. **Surface views of the NTP phosphate donor site and the polynucleotide 5’-OH phosphate acceptors site**. The surface models and vacuum electrostatics were generated in Pymol. The ATP is shown as a stick model and Mg^{2+} is depicted as a magenta sphere.

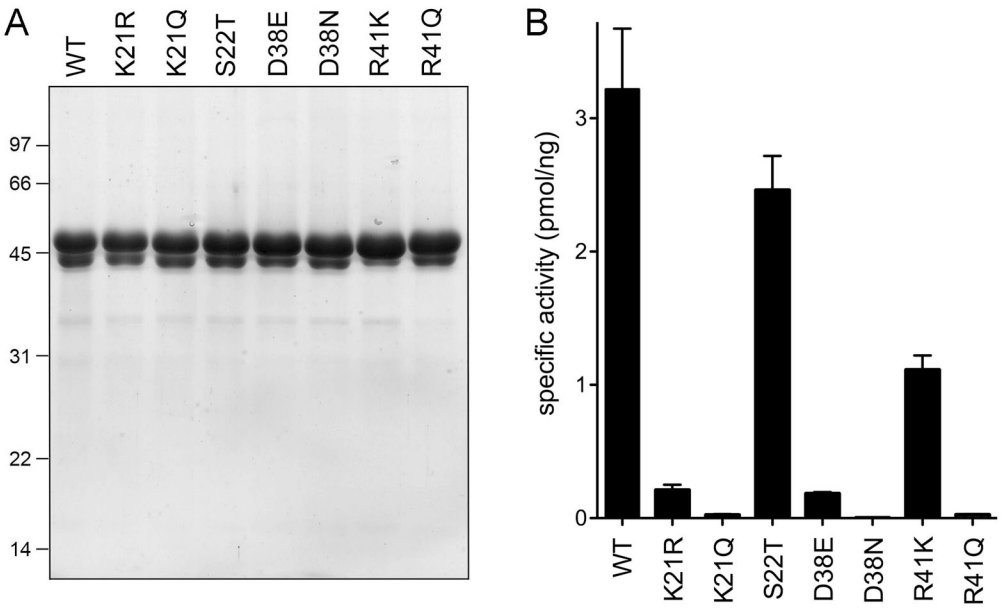

Figure S3. **Effects of conservative mutations on kinase activity**. (A) Aliquots (8 μg) of the Ni-agarose preparations of the indicated His_{10}*Cth*Pnkp-(1-425) proteins were analyzed by SDS-PAGE. The polypeptides were visualized by staining with Coomassie blue dye. The positions and sizes (kDa) of marker polypeptides are indicated on the left. (B) Kinase reaction mixtures contained 180 pmol of 36-mer 5'-OH DNA oligonucleotide and serial 2-fold dilutions of the indicated *Cth*Pnkp proteins (from 6.25 to 200 ng). Specific activity was determined from the slope of the titration curve in the linear response range. Each datum in the bar graph is the average of three separate enzyme titration experiments ± SEM.

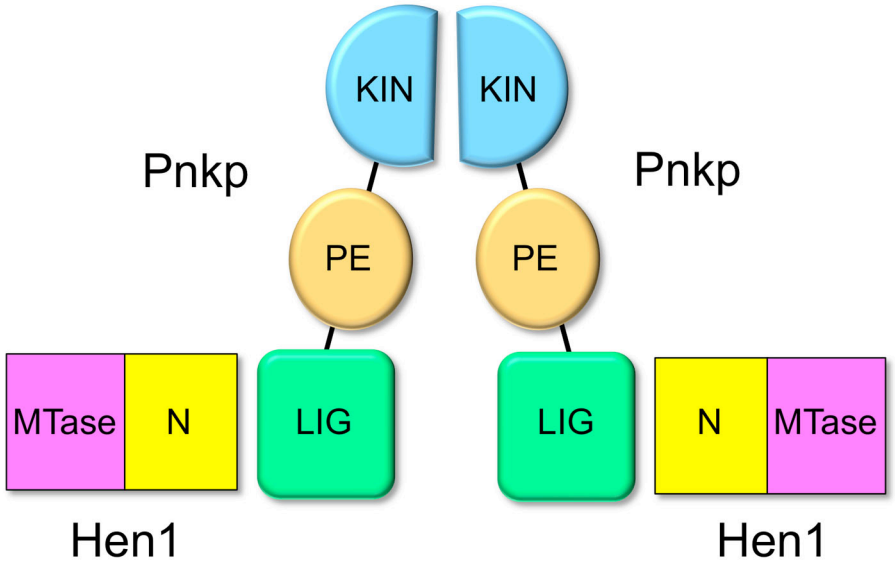

Figure S4. **Model for domain organization of the Pnkp-Hen1 RNA repair complex**.