Online Methods

Protein expression and purification

The catalytic domain of *Bacillus halodurans* RNase H1 (Bh-RNase HC, aa 59-196) was previously cloned into pET15b with an N-terminal His_6-tag (pWY2042), and E188A mutant (pWY2063) was also made previously [1]. Bh-RNase HC-K196A (pWY2780) was constructed for this study by site-directed mutagenesis. The enzymes were expressed and purified as previously described [1]. For simplicity RNase HC is referred to as RNase H1 below.

Preparation of RNA/DNA hybrid and co-crystallization with RNase H1

The RNA/DNA hybrid was made by annealing the oligo 5´- dArCrArUrCdG - 3´ and 3´- d(TGTAGC) - 5´ (IDT, IO USA) at 1.5 mM in TE buffer (10 mM Tris (pH 8.0) and 1 mM EDTA). The RNase H1 and RNA/DNA were mixed a molar ratio of 1:1.1 (300 μM RNase H1 and 330 μM RNA/DNA) and incubated for 30 min at 21°C. Crystals were grown by the hanging drop method at 21°C. Equal volumes of RNase H1-RNA/DNA complexes and the precipitant buffer (14% PEG3350, 20% glycerol, 200 mM KI, and 25 mM $CaCl_2$) were equilibrated against the well buffer (12% PEG3350, 20% glycerol, 200 mM KI, and 25 mM $CaCl_2$), and crystals of ~300 μm X 150 μm X 50 μm grew in 3-4 days.

Pre-reaction Ca^{2+} bound and EGTA/K^+ soaked structures

To obtain a Ca^{2+}-bound structure, mature crystals were transferred to a buffer containing 23% PEG3350, 20% glycerol, 0.2 M KI, 50 mM HEPES (pH 7.0), and 2 mM $CaCl_2$ for 45-60 min at 21°C before flash freezing and storing in LN_2 for data collection. To obtain EGTA soaked K^+ structures, crystals were soaked in 50 µl of stabilization buffer

containing 23% PEG3350, 20% glycerol, 0.2 M KI, 50 mM HEPES (pH 7.0), and 0.5 mM EGTA and incubated at 21°C for 45-60 min before stabilization in a 50 µl stabilization buffer with 30% glycerol for flash freezing in LN_2. To obtain the K^+ anomalous signal, data was collected at a home source X-ray generator (Rigaku RA-Micro7 HF mounted with a Rigaku Saturn A200 CCD detector, at λ=1.54 Å).

***In crystallo* reaction and time courses in $MgCl_2$ or $MnCl_2$**

Crystals of RNase H1-RNA/DNA complexes were transferred to 50 µl of stabilization buffer containing 23% PEG3350, 20% glycerol, 0.2 M KI, 50 mM HEPES pH 7.0, and 0.5 mM EGTA and incubated at 21°C for 45-60 min. Individual crystals were then transferred from the stabilization buffer to 50 µl of buffer containing 18% PEG3350, 0.2 M KI, 50 mM HEPES pH 7.0, and 2 mM $MgCl_2$ to allow *in crystallo* reactions to proceed for 40s, 80s, 120s…and 600s. At each desired end-point, a crystal was transferred to a cryo-protection buffer containing the reaction buffer and 30% glycerol for 10s before freezing in LN_2 to stop the reaction.

To initiate catalysis in the presence of rubidium ions, 0.2 M KI was substituted by 0.2 M RbCl in the stabilization, reaction and cryo-protection buffers and 5 mM $MgCl_2$ was used in the reaction and cryo-protection buffers. To initiate catalysis in the presence of lithium ions, 0.2 M KI was substituted by 0.2 M LiCl in the stabilization, reaction and cryo buffers and 2 mM or 5 mM $MgCl_2$ was used in the reaction and cryo-protection buffers.

For the E188A mutant, reactions were conducted in similar buffers as the WT enzyme. For K196A, reactions were performed in the presence of 0.2 M RbCl and 5-10 mM $MgCl_2$. For time-courses conducted in $MnCl_2$, crystals of RNase H1-RNA/DNA

containing WT, E188A or K196A enzyme were treated similarly using the same protocol described for $MgCl_2$, but $MgCl_2$ was substituted for 4 mM $MnCl_2$.

***In crystallo* titrations of K^+**

No more than 5 crystals of RNase H1-RNA/DNA complexes were transferred to 50 µl of stabilization buffer containing 23% PEG3350, 20% glycerol, 5 mM KI, 50 mM HEPES pH 7.0, and 0.5 mM EGTA, and incubated at 21°C for 45-60 min. A single crystal was then transferred to a fresh 50 µl drop of stabilization buffer for 5 min to further dilute any residual crystallization buffer. To initiate the reaction, the washed crystal was transferred to a reaction buffer containing 5, 25, 50, 100 to 300 mM KI for 120 s, and then dipped in cryo-protection buffer containing reaction buffer and 30% glycerol before freezing in LN_2 for data collection later.

***In crystallo* titration of Mn^{2+} and Mg^{2+}**

Concentration titrations in Mn^{2+} and 200 mM K^+ were done by following the *in crystallo* protocol and varying the concentration of Mn^{2+} in the reaction and cryo-protection buffers (from 2-100 mM). Crystals were soaked for 40 s in the reaction buffer before freezing and storing in LN_2 for data collection later. Concentration titrations in Mg^{2+} and 75 mM K^+ were done by following the *in crystallo* protocol and varying the concentration of Mg^{2+} in the reaction and cryo-protection buffers (from 1-80 mM). Crystals were soaked for 40 s in the reaction buffer before freezing and storing in LN_2 for data collection later.

X-ray data were collected at the 22-BM or 22-ID beam lines at the Advanced Photon Source (APS, Argonne, Illinois). Data for the RNase H1 soak in EGTA at 200mM

K^+ (Fig S1b, right panel and Supplementary Data Set 2) was collected at 1.54 λ using the home X-ray source (Rigaku MicroMax-007HF with Saturn A200 CCD detector, Rigaku). For native and Mn^{2+} anomalous datasets, 150° of data were collected at the wavelength of 1.0 Å. For Rb^+ anomalous datasets, 450° of data were collected at the wavelength of 0.8 Å. Diffraction data were indexed, processed and scaled using HKL2000 [2]. All crystals are in the $C2_1$ space group (Table 1, 2, Supplementary Data Set 2). The initial structure of RNase H1-RNA/DNA was solved by molecular replacement using MolRep (CCP4) [3] and the D192N mutant RNase H1 structure (PDB 1ZBL) as a search model, and the RNA/DNA molecule was built using COOT [4]. All structures were refined using PHENIX [5]. Geometric constraints were used to fix the atoms in the electron density. Occupancy was refined by fixing the occupancy and refining B factors until ligand and metal B factors were similar and the electron density in the Fo-Fc map was minimized [6]. Models and maps were viewed in COOT, and figures were generated using PyMol (www.pymol.com). ShelxC was used for preparing anomalous data and Anode was used for calculating the anomalous signal, which was converted into a map format using Shelx2map (CCP4). The anomalous maps were visulaized in COOT.

Calculation of unbiased |Fo|-|Fc| omit maps

Omit models were made by removing the scissile phosphate, the A and B metal ions, and if needed, the monovalent cations, water molecules, and active site residues from an appropriate ground state model [6]. Unit cell parameters in each sca file of a time course series were set to the same averaged values. The sca files were then truncated and converted to mtz format in AIMLESS (CCP4), combined in CAD (CCP4) and scaled

together to the appropriate ground state model in SCALEIT (CCP4). $||F_o|-|F_c||$ omit maps were calculated in SFALL (CCP4) for each dataset in a series using the F_o (SCALEIT output) and F_c of the omit reference model. FFT (CCP4) was then used to convert the SFALL outputs to map files that were visualized and analyzed in COOT. The python script "density_at_point" in COOT was used for measuring the peak heights of the 5′-phosphate product and metal ions [6]. The values were normalized using the RMSD of each $||F_o|-|F_c||$ map calculated in FFT.

Substrate preparation for in solution assays

The 5´-fluorescein (Fluor) labeled oligo 5´ Fluor-dT /AGT CAC CGT ArCrA rUrC G- 3´ (Integrated DNA Technologies, USA) was suspended in 10 mM TE at 100 μM concentration and purified by gel extraction methods. The RNA/DNA hybrid was annealed after mixing the above purified oligo with 3´- CGATGT ACG GTG ACT A – 5´ (Integrated DNA Technologies, USA) at a final concentration of 1-5 μM in TE, and annealed by heating to 75°C in a thermocycler and slowly cooled (1°C/min) to 4°C.

Activity assays of WT, E188A and K196A RNase H1 in solution

For each reaction, WT, E188A, or K196A RNase H1 was incubated with 100 nM of Fluor-labeled RNA/DNA substrate in 12.5 μl of 50 mM HEPES (pH 7.3), 1 mM DTT, 20 μg/ml BSA, 75 mM KCl, 2% glycerol for 10 min at 22°C. Reactions were initiated by adding 12.5 μl of $MgCl_2$ or $MnCl_2$ to a final concentration of 4 mM and incubated at 37°C for 30 min. The Mg^{2+}-dependent reactions were carried out with 12, 24, 48 and 96 nM of each enzyme, and the Mn^{2+}-dependent reactions were carried out with 0.03, 0.06, 0.12, 0.24, and 0.48 nM of each enzyme. The reactions were stopped by adding 25 μl 2X TBE buffer

(ThermoFisher) containing 100 mM EDTA and heated for 5 min at 95°C followed by incubation at 4°C for 2 min. Samples were run on 20% TBE urea gels and imaged with a Typhoon FLA 9500 biomolecular imager (GE Healthcare Life Sciences) using the emission filter: 520 BP 40 and laser: blue (488 nm) and RNA product was quantified with ImageJ.

Monovalent ion dependent RNase H1 activities

Activity assays were conducted as described above. 200 nM substrate and 100 nM WT RNase H1 were incubated in the buffer containing 200 mM KCl, LiCl, RbCl or NaCl in 50 mM HEPES (pH 7.0), 1 mM DTT, and 20 μg/ml BSA first. The reactions were initiated by the addition of 2 mM Mg^{2+} (final) and incubated at 22°C for 45 min.

Mn^{2+} titration in solution

The 5´-fluorescein (Fluor) labeled hybrid (500 nM) and 1 nM WT RNase HC was mixed and incubated in a crystallization-like buffer containing 50 mM HEPES (pH 7.0), 2.5% PEG3350, and 200 mM KCl for 10 min. The reactions were initiated by adding an equal volume of $MnCl_2$ to the final concentrations of 0.5, 1, 2, 4, 8, 16, 20, and 40 mM, and stopped after 5 min of incubation at 22°C.

Data availability

The coordinates and structure factors have been deposited in the Protein Data Bank under the accession codes: 6DMN, 6DMV, 6DO8, 6DO9, 6DOA, 6DOB, 6DOC, 6DOD, 6DOE, 6DOF, 6DOG, 6DOH, 6DOI, 6DOJ, 6DOK, 6DOL, 6DOM, 6DON, 6DOO, 6DOP, 6DOQ, 6DOR, 6DOS, 6DOT, 6DOU, 6DOV, 6DOW, 6DPP, 6DOX, 6DOY, 6DOZ, 6DP0, 6DP1, 6DP2, 6DP3, 6DP4, 6DP5, 6DP6, 6DP7, 6DP8, 6DP9, 6DPA,

6DPB, 6DPC, 6DPD, 6DPE, 6DPF, 6DPG, 6DPH, 6DPI, 6DPJ, 6DPK, 6DPL, 6DPM, 6DPN, 6DPO. Source data for Fig. 4a is available with the paper online. Other data and results are available from the corresponding author upon reasonable request.

References

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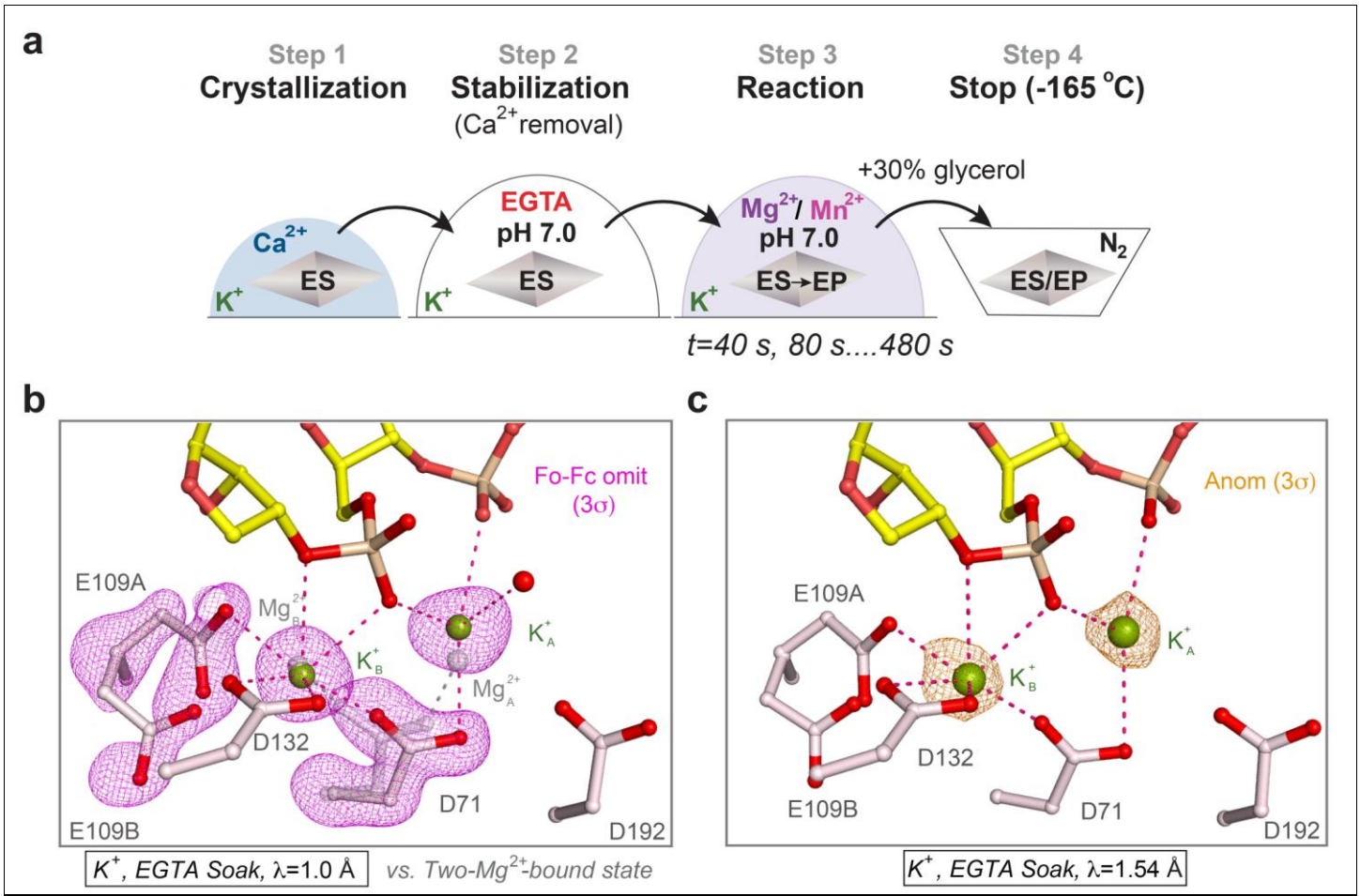
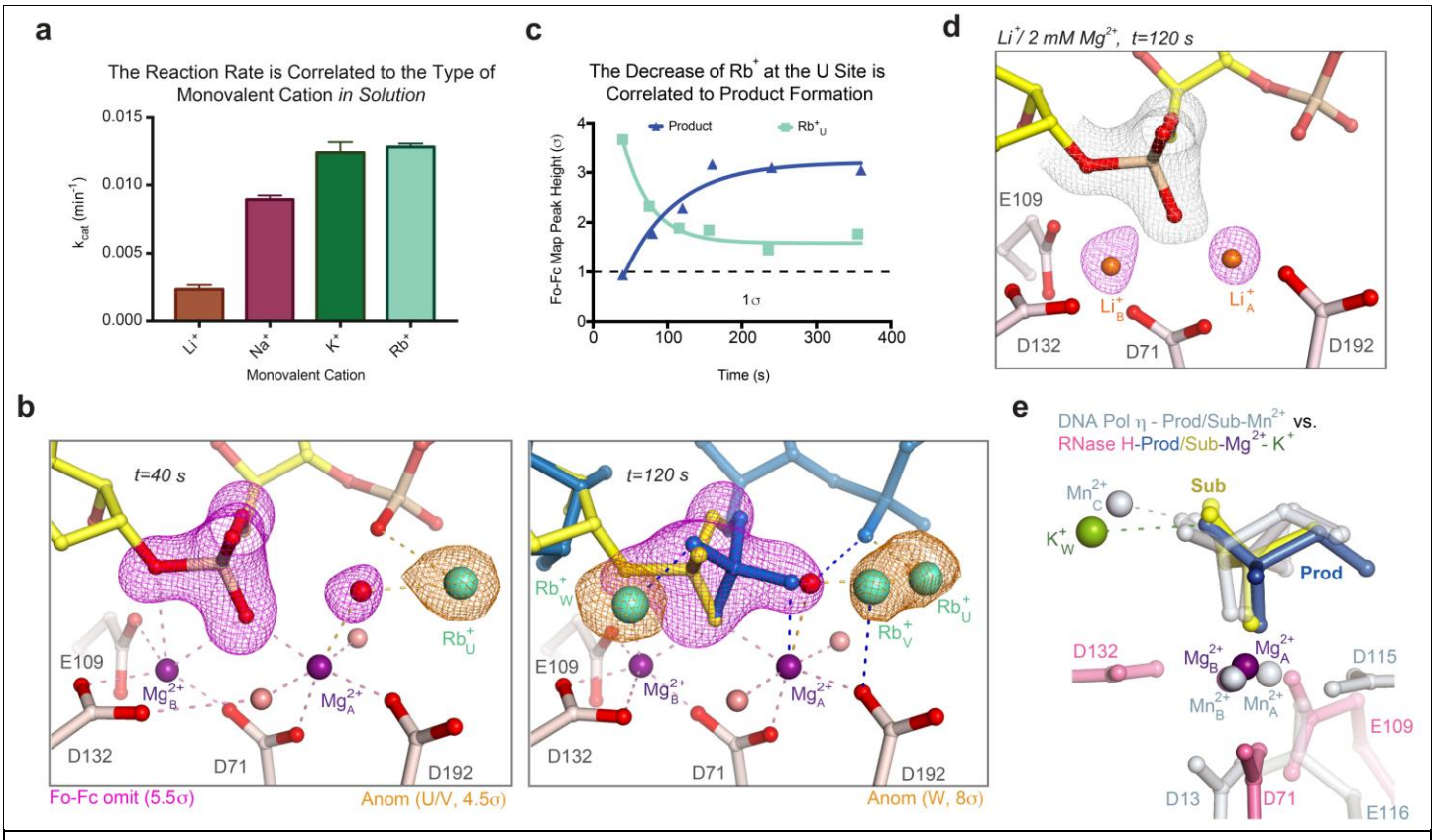


Supplementary Figure 1

RNA hydrolysis in crystallo.

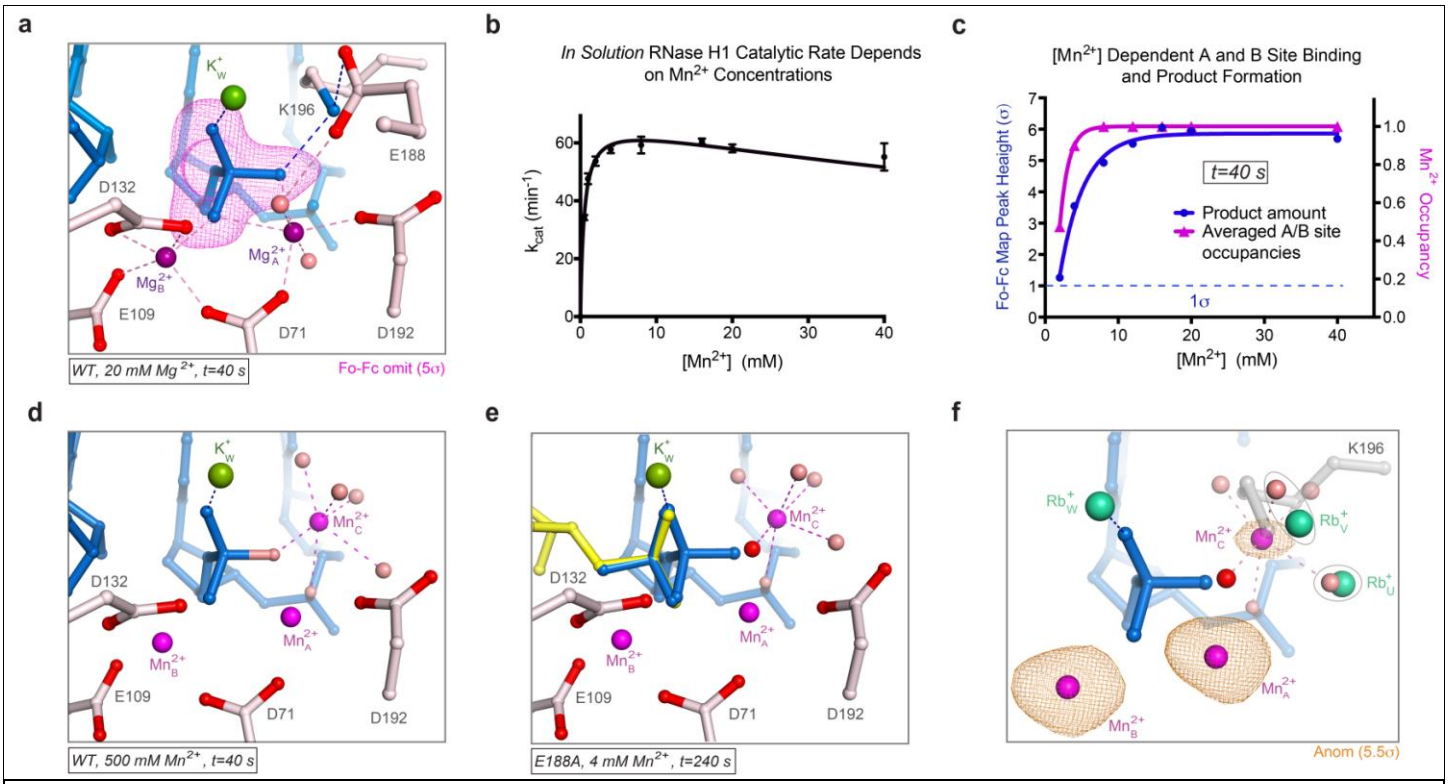
a, A diagram of co-crystallization of RNase H1 and RNA:DNA hybrid, procedure of in crystallo reaction and preparation for X-ray diffraction data acquisition. **b**,**c**, Structures of EGTA-soaked RNase H1–substrate co-crystals with data collected at λ = 1.0 Å and λ = 1.54 Å. Altered conformations of D71 and E109 in the absence of two canonical Me^{2+} ions are shown with the pink omit map (contoured at 3σ), and the reference structure in the presence of Mg^{2+} ions is shown as semitransparent gray sticks (D71) and spheres (Mg^{2+}). **c**, The presence of K^+ after EGTA soaking is confirmed by the anomalous signal (contoured at 3σ) at λ = 1.54 Å.



Supplementary Figure 2

Monovalent cations in RNase H1 catalysis.

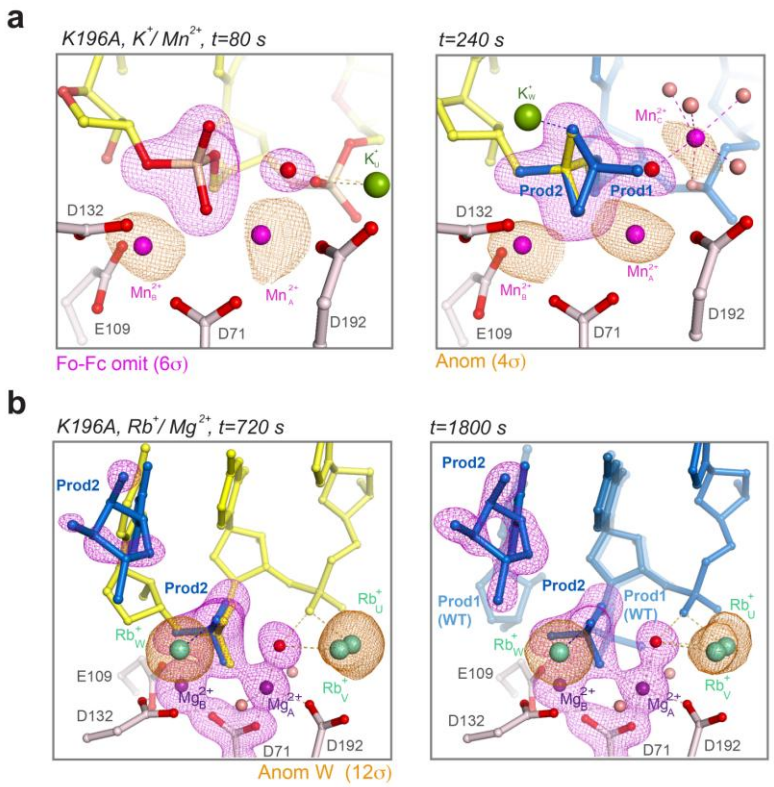
a, Solution analysis showing that Na^+, K^+ and Rb^+ support the RNase H1 reaction, but Li^+ causes much reduced catalysis. Here $n = 3$ independent experiments. The plotted values are the mean, and error bars represent 1 s.d. **b**, Identification of U, V and W monovalent cations. K^+ was substituted by Rb^+ in the in crystallo reaction, and the reaction process is shown at t = 40 s and 120 s after exposure to 5 mM Mg^{2+}. The substrate (yellow) and product (blue) structures are superimposed with the pink $F_o - F_c$ map (omitting the scissile-phosphate, contoured at 5.5σ) and Rb^+ anomalous map (golden mesh, contoured at 4.5σ for the U and V sites and 8σ for the W site). **c**, During the reaction time course, U-site occupancy decreased with increase in product formation. **d**, Li^+ persisted in occupying the A and B sites after a 120-s soak in 2 mM Mg^{2+}. This structure is superimposable on the structure before soaking in Mg^{2+}. Even though no $2F_o - F_c$ density (gray mesh) for Li^+ could be observed at 1 □, the omit $F_o - F_c$ map (magenta mesh) clearly shows the presence of Li^+ in the A and B sites. **e**, Comparison of the W-site K^+/Rb^+ in RNase H1 and the C-site Mn^{2+} in DNA Pol η in an orthogonal view of Fig. 2c.



Supplementary Figure 3

Evidence for a third Me^{2+} in wild-type RNase H1 catalysis.

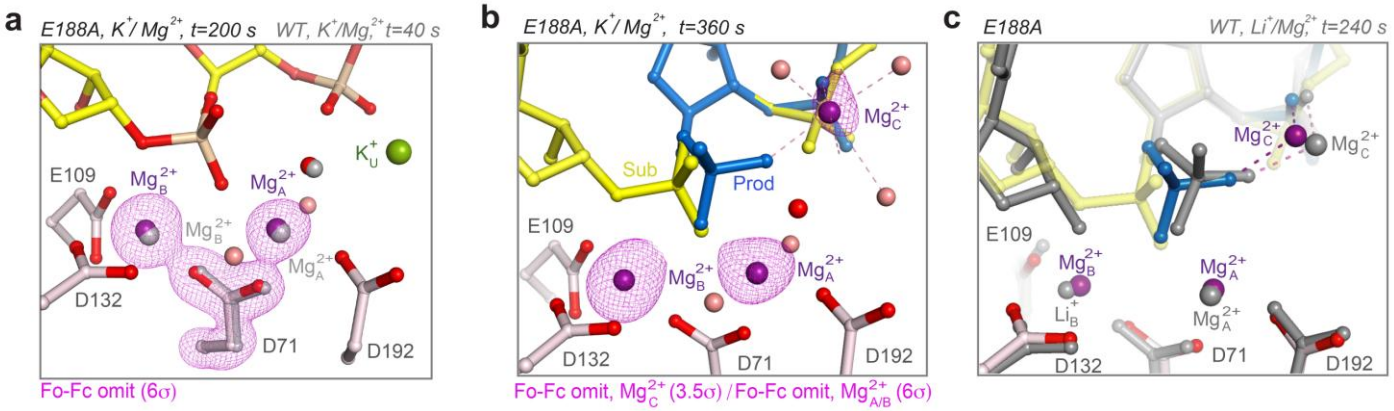
a, The in crystallo reaction catalyzed by WT RNase H1 was 80% complete with 20 mM Mg^{2+} at *t* = 40 s. E188 and K196 interact with each other. E188 also interacts with the A-site Mg^{2+} ion via a water molecule, and K196 interacts with the 5´-phosphate product. **b**, Dependence of RNase H1 catalytic rates on Mn^{2+} concentrations in solution. Initially increased concentration of Me^{2+} increases the reaction rate, probably owing to the low affinity of C-site Me^{2+}, but high concentrations of Me^{2+} eventually lead to prolonged binding of $Me^{2+}{}_C$ and prevent product release. Here *n* = 3 independent experiments. The plotted values are the mean, and error bars represent 1 s.d. **c**, In the in crystallo reaction, the A and B occupancies (magenta) and product formation (blue) required different Mn^{2+} concentrations. **d**, The in crystallo reaction catalyzed by WT RNase H1 was 100% complete with 500 mM Mn^{2+} at *t* = 40 s. K196 is disordered in this state, and $Mn^{2+}{}_C$ (magenta) occupies that space and similarly interacts with the 5´-phosphate product, as well as the downstream phosphate and four water ligands. **e**, $Mn^{2+}{}_C$ (magenta) also appears in the in crystallo reaction catalyzed by $RNaseH1^{E188A}$ at *t* = 240 s, where K196 is similarly disordered despite the presence of product. **f**, One of the water ligands of $Mn^{2+}{}_C$ overlaps with $K^+{}_U$, and another water ligand of $Mn^{2+}{}_C$ overlaps with $K^+{}_V$, both enclosed in an oval. If present, K196 (shown as semitransparent sticks) overlaps with $Mn^{2+}{}_C$.



Supplementary Figure 4

Defects of K196A mutant RNase H1.

a, In crystallo reactions with 4 mM Mn^{2+} rescued the slow reaction and most product inversion with the K196A mutation. The C-site Mn^{2+} is superimposable with that in the reaction catalyzed by $RNaseH1^{E188A}$ (Fig. 4b). The golden anomalous map (contoured at 3.5σ) and the pink $F_o - F_c$ omit map (contoured at 6σ) are also shown. **b**, In the in crystallo catalysis by $RNaseH1^{K196A}$, the 5′-phosphate product (blue) was inverted and adopted the same conformation as the substrate (yellow) by t = 720 s and most clearly at t = 1,800 s. The pink omit $F_o - F_c$ map (5σ) is superimposed onto the structural model. The three monovalent cations were confirmed by Rb^+ anomalous signals (golden mesh, contoured at 12σ for Rb^+_W and 3.5σ for Rb^+_U and Rb^+_V) as with WT RNase H1. **c**, Presence of a positively charged residue (K196 equivalent) adjacent to the C-terminal catalytic carboxylate is conserved in RNase H1 homologs.



Supplementary Figure 5

Defects of E188A mutant RNase H1.

a, In crystallo catalysis by E188A RNase H1 reveals delayed binding of the A-site Mg^{2+}, which took 200 s to displace K^+ ions instead of 40 s as in WT RNase H1 (shown in gray). **b**, By t = 360 s, the C-site Mg^{2+} along with its coordination ligands and the shifted 5′-phosphate product were observed as WT enzyme in the presence of Li^+/Mg^{2+}. Structures in **a** and **b** are superimposed with pink $F_o - F_c$ omit maps contoured at 6σ for 200 s and 3.5σ for 360 s. **c**, Comparison of the third Mg^{2+} in the reactions catalyzed by WT (Li^+) (shown in gray) and E188A mutant RNase H1. For the WT structure, Li^+ was modeled in the B site because it had much lower $2F_o - F_c$ signal than the A site while the five coordination ligands were the same as when the B site was fully occupied.

Table S1 Data collection and refinement statistics

(3) RNase H1^{WT} reaction with 2 mM Mg^{2+} and 200 mM K^{+} (Fig. 2a)

	200 s PDB 6DOB	240 s PDB 6DOC	360 s PDB 6DOD
Data collection			
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions			
a, b, c (Å)	81.4, 38.1, 62.1	81.4, 37.9, 61.9	81.6, 38.0, 62.4
a, b, γ (º)	90, 96.2, 90	90, 96.3, 90	90, 96.3, 90
Resolution (Å) [1]	50.0 - 1.34 (1.37 - 1.34)	50.0 - 1.50 (1.53 - 1.50)	50.0 - 1.54 (1.57 - 1.54)
R_{pim} [1]	0.038 (0.856)	0.042 (0.683)	NA
R_{sym} or R_{merge} [1]	0.051 (>1.0)	0.061 (0.871)	0.079 (0.822)
$I/\sigma(I)$ [1]	15.6 (0.84)	14.5 (1.52)	10.0 (1.27)
$CC_{1/2}$ [1]	(0.161)	(0.568)	NA
Completeness (%) [1]	97.1 (91.2)	95.1 (95.2)	99.0 (93.6)
Redundancy [1]	2.7 (2.0)	2.9 (2.7)	3.0 (2.6)
Refinement			
Resolution (Å)[1]	23.4 - 1.34	23.5 - 1.50	31.0 - 1.54
No. reflections[1]	40941	28741	28053
R_{work} / R_{free}	16.9 / 19.6	18.8 / 22.1	16.0 / 19.5
No. atoms			
Protein / RNA/ DNA	2096 / 135 / 121	2096 / 135 / 121	2096 / 135 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	3 K^{+}	3 K^{+}	3 K^{+}
Halides[4]	4 I^{-}	4 I^{-}	4 I^{-}
Water/ Solvent	196 / 56	193 / 56	196 / 56
Occupancy			
RS: PS	0.45 : 0.55	0.35 : 0.65	0.35 : 0.65
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.10 K^{+}	0.10 K^{+}	0.10 K^{+}
W site	0.30 K^{+}	0.35 K^{+}	0.35 K^{+}
B-factors			
Macromolecules	23.2	25.2	25.8
Me_A / Lig_A[2]	18.5 / 21.6	21.0 / 24.6	18.8 / 20.5
Me_B / Lig_B[2]	18.3 / 20.0	18.7 / 17.5	17.3 / 19.5
Me_U / Lig_U[2]	27.2 / 25.5	29.9 / 28.5	27.8 / 24.7
Me_W / Lig_W[2]	22.4 / 18.1	22.3 / 22.8	17.8 / 19.6
Solvent[3]	39.0	39.1	40.2
R.m.s deviations			
Bond lengths (Å)	0.009	0.010	0.009
Bond angles (º)	1.12	1.19	1.15

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(4) RNase H1^{WT} reaction with 2 mM Mg^{2+} and 200 mM K^{+} (Fig. 2a)-Cont'd

	420 s PDB 6DOE	540 s PDB 6DOF	600 s PDB 6DOG
Data collection			
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions			
a, b, c (Å)	81.6, 38.0, 62.4	81.8, 37.9, 62.5	81.6, 37.9, 62.2
a, b, γ (º)	90, 96.3, 90	90, 96.2, 90	90, 96.2, 90
Resolution (Å) [1]	50.0 - 1.45 (1.48 - 1.45)	50.0 - 1.43 (1.486 - 1.43)	50.0 - 1.28 (1.31 - 1.28)
R_{pim} [1]	NA	NA	0.035 (0.518)
R_{sym} or R_{merge} [1]	0.072 (0.568)	0.074 (0.613)	0.051 (0.691)
$I/\sigma I$ [1]	12.1 (1.24)	10.6 (1.57)	14.0 (1.17)
$CC_{1/2}$ [1]	NA	NA	(0.572)
Completeness (%) [1]	98.0 (91.1)	99.0 (99.5)	98.9 (96.2)
Redundancy [1]	2.9 (2.3)	3.0 (2.6)	2.7 (2.2)
Refinement			
Resolution (Å)[1]	31.0 - 1.45	31.1 - 1.43	20.3 - 1.28
No. reflections[1]	33083	34641	47259
R_{work} / R_{free}	17.0 / 19.8	16.1 / 18.7	16.1 / 18.3
No. atoms			
Protein / RNA/ DNA	2096 / 135 / 121	2096 / 135 / 121	2096 / 135 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	3 K^{+}	3 K^{+}	3 K^{+}
Halides[4]	4 I^{-}	4 I^{-}	4 I^{-}
Water/ Solvent	196 / 56	196 / 56	196 / 56
Occupancy			
RS: PS	0.45 : 0.55	0.45 : 0.55	0.35 : 0.65
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.10 K^{+}	0.10 K^{+}	0.10 K^{+}
W site	0.30 K^{+}	0.30 K^{+}	0.35 K^{+}
B-factors			
Macromolecules	22.6	23.6	22.8
Me_A / Lig_A [2]	18.8 / 21.7	20.2 / 25.2	18.2 / 22.2
Me_B / Lig_B [2]	17.7 / 19.6	16.9 / 21.1	17.1 / 18.8
Me_U / Lig_U [2]	31.3 / 26.6	27.7 / 29.9	21.2 / 26.3
Me_W / Lig_W [2]	19.4 / 19.1	19.1 / 21.9	22.8 / 21.1
Solvent[3]	37.5	38.4	38.5
R.m.s deviations			
Bond lengths (Å)	0.009	0.008	0.008
Bond angles (º)	1.15	1.13	1.10

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(5) RNase H1^{WT} Substrate Complexes - EGTA Soaks (Fig.S1b)

	EGTA Soak/200 mM K^+ PDB **6DOH**	EGTA Soak/200 mM K^+ [5] PDB **6DOI**
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	81.2, 37.8, 61.8	81.4, 38.0, 62.1
a, b, γ (º)	90, 96.1, 90	90, 96.2, 90
Resolution (Å) [1]	19.1 - 1.40 (1.45 - 1.40)	22.0 - 1.95 (2.02 - 1.95)
R_{pim} [1]	0.065 (0.707)	NA
R_{sym} or R_{merge} [1]	0.096 (0.947)	0.070 (0.215)
$I/\sigma(I)$ [1]	8.7 (0.78)	14.1 (3.6)
$CC_{1/2}$ [1]	NA	NA
Completeness (%) [1]	97.4 (83.2)	92.3 (78.1)
Redundancy [1]	2.8 (2.2)	3.1 (2.3)
Refinement		
Resolution (Å)[1]	19.1 - 1.40	22.0 - 1.95
No. reflections[1]	35608	12946
R_{work} / R_{free}	16.8 / 20.0	14.7 / 19.5
No. atoms		
Protein / RNA / DNA	2034 / 122 / 121	2016 /122 / 121
Me^{2+}	1 Ca^{2+}	1 Ca^{2+}
Me^{+} [4]	3 K^+	2 K^+
Halides[4]	4 I^-	4 I^-
Water/ Solvent	175/ 36	166 / 36
Occupancy		
RS: PS	1.0 : 0	1.0 : 0
A site	0.30 Ca^{2+}	0.30 Ca^{2+}
B site	NA	NA
A′ site	0.70 K^+	0.70 K^+
B′ site	0.70 K^+	0.70 K^+
B-factors		
Macromolecules	27.3	30.6
Me_I / $Lig_{A'}$ [2]	27.2 / 27.2	22.3 / 25.0
Me_{II} / $Lig_{B'}$ [2]	23.2 / 22.0	24.5 / 22.8
Solvent[3]	41.9	39.2
R.m.s deviations		
Bond lengths (Å)	0.008	0.009
Bond angles (º)	1.01	1.02

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

[5] Collected at λ = 1.54 Å

(6) RNase H1^{WT} 120 s reaction with 2 mM Mg^{2+} and 5-300 mM K^+ (Fig. 2b)

	5 mM PDB 6DOJ	25 mM (1) PDB 6DOK	25 mM (2) PDB 6DOL	50 mM PDB 6DOM
Data collection				
Space group	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions				
a, b, c (Å)	81.9, 37.4, 62.3	81.6, 37.4, 62.2	81.6, 37.1, 62.1	81.6, 37.3, 62.2
a, b, γ (º)	90, 96.7, 90	90, 96.6, 90	90, 96.4, 90	90, 96.4, 90
Resolution (Å) [1]	20.0 - 1.40 (1.42 - 1.40)	20.0 - 1.38 (1.41 - 1.38)	20.0 - 1.43 (1.45 - 1.43)	20.0 - 1.42 (1.45 - 1.42)
R_{pim} [1]	0.048 (0.643)	0.049 (0.686)	0.044 (0.941)	0.053 (0.844)
R_{sym} or R_{merge} [1]	0.071 (0.933)	0.073 (0.867)	0.063 (>1.0)	0.078 (>1.0)
$I/\sigma(I)$ [1]	11.4 (1.12)	10.3 (0.98)	12.0 (0.91)	11.8 (1.04)
$CC_{1/2}$ [1]	(0.502)	(0.376)	(0.349)	(0.361)
Completeness (%) [1]	99.7 (97.9)	96.9 (79.3)	98.9 (97.3)	99.6 (98.8)
Redundancy [1]	3.0 (2.7)	2.9 (2.0)	3.0 (2.9)	2.9 (2.7)
Refinement				
Resolution (Å)[1]	19.3 - 1.40	18.7 - 1.38	20.1 - 1.43	19.9 - 1.42
No. reflections[1]	36467	36837	33646	34702
R_{work} / R_{free}	14.5 / 18.5	14.9 / 17.0	15.1 / 18.5	14.8 / 18.4
No. atoms				
Protein / RNA/ DNA	1990 / 122/ 121	1997 / 122 / 121	1997 / 122 / 121	2047 / 147 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	1 K^+	1 K^+	1 K^+	3 K^+
Halides[4]	1 I^-	2 I^-	2 I^-	3 I^-
Water/ Solvent	203 / 44	201 / 32	201 / 32	193 / 38
Occupancy				
RS: PS	1.0 : 0	0.8 : 0	0.8 : 0	0.65 : 0.35
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.10 K^+	0.15 K^+	0.15 K^+	0.25 K^+
W site	0 K^+	0 K^+	0 K^+	0.25 K^+
B-factors				
Macromolecules	30.0	31.4	31.0	28.0
Me_A / Lig_A [2]	23.5 / 28.1	26.7 / 30.9	27.0 / 31.1	24.1 / 26.8
Me_B / Lig_B [2]	22.6 / 23.2	24.8 / 27.3	26.3 / 27.6	22.4 / 22.9
Me_U / Lig_U [2]	NA	28.6 / 35.8	29.5 / 34.6	29.9 / 28.9
Me_W / Lig_W [2]	NA	NA	NA	32.2 / 31.9
Solvent[3]	46.2	47.5	46.2	44.0
R.m.s deviations				
Bond lengths (Å)	0.007	0.008	0.007	0.009
Bond angles (º)	1.07	1.00	1.00	1.22

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(7) RNase H1^{WT} 120 s reaction with 2 mM Mg^{2+} and 5-300 mM K^+ (Fig. 2b)-Cont'd

	100 mM (1) PDB 6DON	100 mM (2) PDB 6DOO	200 mM (1) PDB 6DOP
Data collection			
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions			
a, b, c (Å)	81.9, 37.6, 62.1	81.5, 37.7, 62.4	81.5, 37.6, 61.8
a, b, γ (º)	90, 96.4, 90	90, 96.2, 90	90, 95.8, 90
Resolution (Å) [1]	20.0 - 1.42 (1.44 - 1.42)	20.0 - 1.44 (1.47 - 1.44)	20.0 - 1.25 (1.28 - 1.25)
R_{pim} [1]	0.055 (0.955)	0.042 (0.850)	0.057 (0.651)
R_{sym} or R_{merge} [1]	0.077 (>1.0)	0.061 (>1.0)	0.085 (0.902)
$I/\sigma(I)$ [1]	10.5 (1.14)	13.5 (0.81)	8.27 (1.17)
$CC_{1/2}$ [1]	(0.316)	(0.350)	(0.465)
Completeness (%) [1]	99.1 (99.6)	99.7 (99.0)	95.8 (86.0)
Redundancy [1]	3.0 (2.7)	3.0 (2.9)	3.1 (2.5)
Refinement			
Resolution (Å)[1]	18.8 - 1.42	18.9 - 1.44	19.9 - 1.25
No. reflections[1]	34701	34009	48949
R_{work} / R_{free}	15.6 / 19.5	14.5 / 17.0	16.2 / 17.8
No. atoms			
Protein / RNA/ DNA	2047 / 147 / 121	2047 / 147 / 121	2047 / 147 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	3 K^+	3 K^+	2 K^+
Halides[4]	4 I^-	4 I^-	4 I^-
Water/ Solvent	184 / 44	184 / 44	180 / 44
Occupancy			
RS: PS	0.50 : 0.50	0.65 : 0.35	0.70 : 0.30
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.25 K^+	0.25 K^+	0.25 K^+
W site	0.25 K^+	0.25 K^+	0 K^+
B-factors			
Macromolecules	30.0	28.5	25.9
Me_A / Lig_A [2]	25.2 / 31.7	22.1 / 26.2	24.8 / 29.3
Me_B / Lig_B [2]	26.3 / 27.9	21.1 / 26.1	22.8 / 24.4
Me_U / Lig_U [2]	32.5 / 32.0	27.5 / 27.4	30.9 / 32.8
Me_W / Lig_W [2]	28.8 / 42.5	24.7 / 44.7	NA
Solvent[3]	46.8	44.4	40.9
R.m.s deviations			
Bond lengths (Å)	0.008	0.009	0.007
Bond angles (º)	1.15	1.21	1.15

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(8) RNase H1^{WT} 120 s reaction with 2 mM Mg^{2+} and 5-300 mM K^{+} (Fig. 2b)-Cont'd

	200 mM (2) PDB 6DOQ	300 mM (1) PDB 6DOR	300 mM (2) PDB 6DOS
Data collection			
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions			
a, b, c (Å)	81.4, 37.9, 61.8	81.5, 37.7, 62.8	81.2, 37.4, 62.0
a, b, γ (º)	90, 96.0, 90	90, 96.6, 90	90, 96.0, 90
Resolution (Å) [1]	20.0 - 1.42 (1.45 - 1.42)	20.0 - 1.50 (1.53 - 1.50)	20.0 - 1.32 (1.34 - 1.32)
R_{pim} [1]	0.049 (0.786)	0.037 (0.745)	0.036 (0.782)
R_{sym} or R_{merge} [1]	0.072 (>1.0)	0.054 (>1.0)	0.051 (>1.0)
$I/\sigma(I)$ [1]	12.7 (0.99)	16.6 (1.04)	16.0 (0.95)
$CC_{1/2}$ [1]	(0.291)	(0.368)	(0.316)
Completeness (%) [1]	99.6 (99.7)	99.7 (96.7)	99.7 (99.3)
Redundancy [1]	3.0 (2.8)	3.0 (2.5)	3.0 (2.9)
Refinement			
Resolution (Å)[1]	19.8 - 1.42	19.4 - 1.50	19.8 - 1.32
No. reflections[1]	35101	30500	43775
R_{work} / R_{free}	14.9 / 17.7	15.5 / 19.4	15.5 / 17.1
No. atoms			
Protein / RNA/ DNA	2047 / 147 / 121	2047 / 147 / 121	2047 / 147 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	3 K^{+}	3 K^{+}	2 K^{+}
Halides[4]	4 I^{-}	4 I^{-}	4 I^{-}
Water/ Solvent	180 / 44	177 / 44	177 / 44
Occupancy			
RS: PS	0.65 : 0.35	0.50 : 0.50	0.70 : 0.30
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.25 K^{+}	0.25 K^{+}	0.25 K^{+}
W site	0.15 K^{+}	0.25 K^{+}	0 K^{+}
B-factors			
Macromolecules	28.1	28.9	26.0
Me_A / Lig_A[2]	24.7 / 26.2	25.7 / 27.3	25.2 / 26.8
Me_B / Lig_B[2]	22.7 / 25.6	24.8 / 25.8	23.2 / 23.1
Me_U / Lig_U[2]	30.0 / 31.1	32.0 / 31.2	31.8 / 29.9
Me_W / Lig_W[2]	24.6 / 42.4	32.1 / 31.2	NA
Solvent[3]	42.8	44.0	40.7
R.m.s deviations			
Bond lengths (Å)	0.008	0.009	0.008
Bond angles (º)	1.14	1.17	1.20

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(9) RNase H1^{WT} reaction with 5 mM Mg^{2+} and 200 mM Rb^{+} (Fig. S2b)

	40 s PDB 6DOT	120 s PDB 6DOU
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	81.6, 37.2, 62.1	81.4, 37.7, 61.9
a, b, γ (º)	90, 96.6, 90	90, 96.0. 90
Resolution (Å) [1]	30.0 - 1.42 (1.45 - 1.43)	20.0 - 1.48 (1.52 - 1.49)
R_{pim} [1]	0.042 (0.460)	0.035 (0.605)
R_{sym} or R_{merge} [1]	0.120 (>1.0)	0.105 (>1.0)
$I/\sigma(I)$ [1]	15.5 (2.00)	17.2 (1.00)
$CC_{1/2}$ [1]	(0.365)	(0.824)
Completeness (%) [1]	100.0 (99.9)	96.6 (87.7)
Redundancy [1]	8.5 (5.6)	9.3 (5.4)
Refinement		
Resolution (Å)[1]	23.4 - 1.42	19.1 - 1.48
No. reflections[1]	34770	29597
R_{work} / R_{free}	14.2/ 16.8	15.2 /18.4
No. atoms		
Protein / RNA/ DNA	1996 / 122 / 121	1999 / 135 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	3 Rb^{+}	5 Rb^{+}
Halides[4]	3 Cl^{-}	3 Cl^{-}
Water/ Solvent	214 / 34	188 / 40
Occupancy		
RS: PS	1.0 : 0	0.50 : 0.50
A site	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.35 Rb^{+}	0.10 Rb^{+}
V site	0	0.10 Rb^{+}
W site	0	0.25 Rb^{+}
B-factors		
Macromolecules	25.4	31.3
Me_A / Lig_A [2]	20.1 / 29.0	25.6 / 32.2
Me_B / Lig_B [2]	20.2 / 21.8	25.3 / 28.7
Me_U / Lig_U [2]	40.0 / 36.0	29.2 / 34.5
Me_U / Lig_V [2]	NA	37.0 / 37.0
Me_W / Lig_W [2]	NA	32.0 / 28.4
Solvent[3]	41.6	45.5
R.m.s deviations		
Bond lengths (Å)	0.007	0.008
Bond angles (º)	1.01	1.08

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(10) RNase H1^{WT} reaction with 5 mM Mg^{2+} and 200 mM Rb^{+} (Fig. S2c)

	80 s PDB 6DOV	160 s PDB 6DOW	240 s PDB 6DPP	360 s PDB 6DOX
Data collection				
Space group	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions				
a, b, c (Å)	81.6, 37.4, 61.7	82.0, 37.5, 62.5	81.5, 38.0 62.5	81.3, 37.9, 62.1
a, b, γ (º)	90, 96.6, 90	90, 96.8, 90	90, 96.8, 90	90, 96.7, 90
Resolution (Å) [1]	20.0 - 1.52 (1.55 - 1.52)	20.0 - 1.50 (1.53 - 1.50)	20.0 - 1.45 (1.47 - 1.45)	20.0 - 1.45 (1.47 - 1.45)
R_{pim} [1]	0.032 (0.666)	0.040 (>1.0)	0.037 (0.750)	0.029 (0.778)
R_{sym} or R_{merge} [1]	0.098 (>1.0)	0.114 (>1.0)	0.106 (>1.0)	0.053 (>1.0)
$I/\sigma(I)$ [1]	22.0 (0.90)	14.9 (0.90)	17.5 (0.96)	23.9 (0.97)
$CC_{1/2}$ [1]	(0.456)	(0.868)	(0.293)	(0.495)
Completeness (%) [1]	99.2 (92.1)	99.8 (97.1)	98.5 (83.3)	99.7 (99.5)
Redundancy [1]	10.9 (5.6)	9.3 (4.9)	8.9 (3.7)	8.8 (8.1)
Refinement				
Resolution (Å)[1]	20.0 - 1.52	19.4 - 1.50	19.4 - 1.45	19.3 - 1.45
No. reflections[1]	28106	30353	33303	33565
R_{work} / R_{free}	15.3 /19.0	16.4 /19.8	15.1/ 17.1	15.4 /17.5
No. atoms				
Protein / RNA/ DNA	1996 / 135 / 121	1987 / 135 / 121	1963 / 135 /121	1997 / 135 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	5 Rb^{+}	5 Rb^{+}	5 Rb^{+}	5 Rb^{+}
Halides[4]	3 Cl^{-}	3 Cl^{-}	3 Cl^{-}	3 Cl^{-}
Water/ Solvent	207 / 28	179 / 34	180 / 38	184 / 61
Occupancy				
RS: PS	0.75 : 0.25	0.35 : 0.65	0.30 : 0.70	0.30 : 0.70
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.15 Rb^{+}	0.10 Rb^{+}	0.10 Rb^{+}	0.10 Rb^{+}
V site	0.10 Rb^{+}	0.10 Rb^{+}	0.10 Rb^{+}	0.10 Rb^{+}
W site	0.15 Rb^{+}	0.30 Rb^{+}	0.30 Rb^{+}	0.30 Rb^{+}
B-factors				
Macromolecules	29.0	34.5	26.9	26.3
Me_A / Lig_A [2]	23.8 / 33.4	26.9 / 34.1	20.9 / 23.4	19.6 / 23.9
Me_B / Lig_B [2]	19.8 / 26.3	28.0 / 31.2	20.0 / 21.5	18.4 / 21.4
Me_U / Lig_U [2]	37.6 / 34.3	35.9 / 36.2	31.8 / 26.4	32.1 / 26.9
Me_V/ Lig_V [2]	36.5 / 43.2	37.7 / 37.1	32.6 / 23.0	34.4 / 22.3
Me_W / Lig_W [2]	37.0 / 36.3	30.7 / 27.8	25.3 / 20.8	22.7 / 18.7
Solvent[3]	40.5	46.3	41.3	40.0
R.m.s deviations				
Bond lengths (Å)	0.008	0.008	0.008	0.008
Bond angles (º)	1.16	1.03	1.02	1.07

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(11) RNase H1^{WT} reaction with 2 mM Mg^{2+} and 200 mM Li^{+} (Fig. S2d)

	120 s PDB 6DOY
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	81.9, 37.4, 62.0
a, b, γ (º)	90, 96.8, 90
Resolution (Å) [1]	20.0 - 1.45 (1.47 - 1.45)
R_{pim} [1]	0.049 (0.776)
R_{sym} or R_{merge} [1]	0.083 (>1.0)
$I/\sigma(I)$ [1]	13.1 (0.97)
$CC_{1/2}$ [1]	(0.433)
Completeness (%) [1]	96.0 (92.9)
Redundancy [1]	3.8 (3.4)
Refinement	
Resolution (Å)[1]	19.3 - 1.45
No. reflections[1]	31921
R_{work} / R_{free}	14.8 /18.7
No. atoms	
Protein / RNA/ DNA	2019 / 122 / 121
Me^{+} [4]	2 Li^{+}
Halides[4]	4 I^{-}
Water/ Solvent	188 / 44
Occupancy	
RS: PS	1.0 : 0
A site	1.0 Li^{+}
B site	0.7 Li^{+}
B-factors	
Macromolecules	31.4
Me_A / Lig_A[2]	20.7 / 25.6
Me_B / Lig_B[2]	23.6 / 24.5
Solvent[3]	47.1
R.m.s deviations	
Bond lengths (Å)	0.007
Bond angles (º)	1.22

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(12) RNase H1^{WT} 40 s reaction with 1-80 mM Mg^{2+} and 75 mM K^{+} (Fig. 3a)

	1 mM PDB 6DOZ	2.5 mM PDB 6DP0	5 mM PDB 6DP1	7.5 mM PDB 6DP2
Data collection				
Space group	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions				
a, b, c (Å)	81.6, 37.5, 62.5	81.5, 37.8, 61.8	81.8, 37.4, 61.8	81.4, 37.3, 62.3
a, b, γ (º)	90, 90.7, 90	90, 96.1, 90	90, 96.5, 90	90, 96.6, 90
Resolution (Å) [1]	20.0 - 1.57 (1.63 - 1.57)	20.0 - 1.45 (1.47 - 1.45)	20.0 - 1.42 (1.44 - 1.42)	19.9 - 1.66 (1.72 - 1.66)
R_{pim} [1]	0.027 (0.682)	0.057 (0.702)	0.042 (0.870)	0.045 (0.779)
R_{sym} or R_{merge} [1]	0.040 (0.916)	0.083 (0.977)	0.061 (1.231)	0.063 (1.075)
$I/\sigma(I)$ [1]	21.7 (0.90)	11.3 (1.09)	14.6 (0.95)	13.8 (0.86)
$CC_{1/2}$ [1]	0.999 (0.438)	(0.206)	(0.373)	0.995 (0.385)
Completeness (%) [1]	98.9 (87.8)	88.6 (93.4)	98.0 (94.2)	96.7 (93.0)
Redundancy [1]	3.0 (2.4)	3.0 (2.8)	3.0 (2.8)	3.0 (2.7)
Refinement				
Resolution (Å)[1]	19.4 - 1.57	19.1 - 1.45	19.2 - 1.42	19.9 - 1.66
No. reflections[1]	25891	29457	34456	21230
R_{work} / R_{free}	15.2 / 18.5	19.0 / 23.6	15.3 / 19.0	15.7 / 19.6
No. atoms				
Protein / RNA/ DNA	2048 / 122 / 121	2054 / 122 / 121	2054 / 122 / 121	2048 / 122 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	2 K^{+}	2 K^{+}	2 K^{+}	2 K^{+}
Halides[4]	3 I^{-}	4 I^{-}	4 I^{-}	4 I^{-}
Water/ Solvent	169 / 26	182 / 32	199 / 32	159 / 28
Occupancy				
RS: PS	0.95 : 0.15	0.90 : 0.10	0.80 : 0.20	0.85 : 0.15
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.2 K^{+}	0.35 K^{+}	0.3 K^{+}	0.35 K^{+}
W site	0	0	0	0
B-factors				
Macromolecules	37.3	28.6	28.6	36.8
Me_A / Lig_A [2]	32.8 / 37.2	26.3 / 29.3	26.5 / 27.4	31.0 / 31.3
Me_B / Lig_B [2]	33.4 / 29.0	27.9 / 22.0	24.6 / 22.3	28.4 / 29.0
Me_u / Lig_u [2]	54.1 / 40.9	47.5 / 34.0	45.2 / 33.53	54.5 / 33.9
Me_W / Lig_W [2]	NA	NA		
Solvent[3]	47.0	41.8	42.4	45.3
R.m.s deviations				
Bond lengths (Å)	0.008	0.008	0.007	0.008
Bond angles (º)	1.06	0.98	1.00	1.04

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(13) RNase H1^{WT} 40 s reaction with 1-80 mM Mg^{2+} and 75 mM K^+ (Fig. 3a and S3a)

	10 mM PDB 6DP3	20 mM PDB 6DP4	40 mM PDB 6DP5	80 mM PDB 6DP6
Data collection				
Space group	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions				
a, b, c (Å)	81.5, 38.1, 61.7	81.5, 37.7, 62.1	81.9, 37.5, 62.2	81.8, 37.3, 62.6
a, b, γ (º)	90, 96.0, 90	90, 96.5, 90	90, 96.5, 90	90, 96.7, 90
Resolution (Å) [1]	20.0 - 1.46 (1.49 - 1.46)	20.0 - 1.38 (1.40 - 1.38)	20.0 - 1.43 (1.45 - 1.43)	20.0 - 1.40 (1.42 - 1.40)
R_{pim} [1]	0.046 (0.692)	0.033 (0.708)	0.046 (0.735)	0.063 (0.706)
R_{sym} or R_{merge} [1]	0.067 (0.969)	0.048 (0.964)	0.065 (0.928)	0.085 (0.814)
$I/\sigma(I)$ [1]	15.1 (1.07)	17.0 (1.07)	11.3 (1.20)	7.8 (1.01)
$CC_{1/2}$ [1]	(0.434)	(0.371)	0.990 (0.278)	0.986 (0.409)
Completeness (%) [1]	99.5 (98.8)	97.3 (99.0)	94.0 (86.6)	90.4 (78.5)
Redundancy [1]	2.9 (2.7)	2.8 (2.7)	2.6 (2.1)	2.3 (1.7)
Refinement				
Resolution (Å)[1]	19.1 - 1.46	19.2 - 1.38	20.0 - 1.43	19.4 - 1.40
No. reflections[1]	32665	37958	32560	33058
R_{work} / R_{free}	17.1 / 19.8	15.7 / 17.6	15.3 / 17.6	16.3 / 18.6
No. atoms				
Protein / RNA/ DNA	2103 / 135 / 121	2073 / 135 / 121	2076 / 135 / 121	2099 / 135 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	3 K^+	3 K^+	3 K^+	3 K^+
Halides[4]	3 I^-	3 I^-	4 I^-	4 I^-
Water/ Solvent	134 / 54	182 / 42	140 / 8	147 / 28
Occupancy				
RS: PS	0.30 : 0.70	0.20 : 0.80	0.35 : 0.65	0.15 : 0.85
A site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.10 K^+	0.10 K^+	0.1 K^+	0.10 K^+
W site	0.35 K^+	0.40 K^+	0.35 K^+	0.40 K^+
B-factors				
Macromolecules	28.9	26.2	27.2	26.1
Me_A / Lig_A [2]	20.7 / 25.7	21.4 / 25.5	22.4 / 26.9	19.0 / 22.0
Me_B / Lig_B [2]	19.4 / 24.2	20.6 / 23.3	21.5 / 24.3	18.8 / 22.0
Me_u/ Lig_u [2]	33.8 / 31.8	27.2 / 27.7	30.3 / 31.5	16.6 / 23.9
Me_W / Lig_W [2]	33.2/ 24.8	28.5 / 26.3	37.8 / 24.3	28.1 /24.6
Solvent[3]	42	39.9	37.8	35.5
R.m.s deviations				
Bond lengths (Å)	0.008	0.008	0.008	0.008
Bond angles (º)	1.08	1.07	1.20	1.03

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(14) RNase H1^{WT} reaction with 500 mM Mn^{2+} and 200 mM K^{+} (Fig. 3b)

	40 s PDB **6DP7**
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	82.0, 37.6, 61.9
a, b, γ (º)	90, 96.8, 90
Resolution (Å) [1]	20.0 - 1.40 (1.43 - 1.40)
R_{pim} [1]	0.105 (>1.0)
R_{sym} or R_{merge} [1]	0.249 (>1.0)
$I/\sigma(I)$ [1]	6.8 (3.8)
$CC_{1/2}$ [1]	(0.179)
Completeness (%) [1]	99.5 (100)
Redundancy [1]	7.7 (7.4)
Refinement	
Resolution (Å)[1]	17.98 - 1.38
No. reflections[1]	38088
R_{work} / R_{free}	17.7 / 20.1
No. atoms	
Protein / RNA/ DNA	1873 / 118 / 121
Me^{2+}	4 Mn^{2+}
Me^{+} [4]	1 K^{+}
Halides[4]	3 I^{-}
Water/ Solvent	169 / 8
Occupancy	
RS: PS	0 : 1.0
A site	1.0 Mn^{2+}
B site	1.0 Mn^{2+}
W site	0.40 K^{+}
B-factors	
Macromolecules	28.7
Me_A / Lig_A[2]	18.3 / 23.6
Me_B / Lig_B[2]	18.0 / 19.3
Me_W / Lig_W[2]	32.7 / 20.0
Solvent[3]	39.2
R.m.s deviations	
Bond lengths (Å)	0.006
Bond angles (º)	0.94

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(15) RNase H1^{WT} reaction with 5 mM Mg^{2+} and 200 mM Li^{+} (Fig.3c)

	240 s PDB **6DP8**
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	81.4, 37.7, 61.8
a, b, γ (º)	90, 95.4, 90
Resolution (Å) [1]	50.0 - 1.32 (1.37 - 1.32)
R_{pim} [1]	0.032 (0.671)
R_{sym} or R_{merge} [1]	0.047 (0.891)
$I/\sigma(I)$ [1]	21.9 (1.07)
$CC_{1/2}$ [1]	(0.413)
Completeness (%) [1]	94.7 (71.4)
Redundancy [1]	3.0 (2.4)
Refinement	
Resolution (Å)[1]	23.5 - 1.32
No. reflections[1]	41833
R_{work} / R_{free}	14.7 / 17.0
No. atoms	
Protein / RNA/ DNA	2037 / 122 / 121
Me^{2+}	2 Mg^{2+}
Me^{+} [4]	1 Li^{+}
Halides[4]	3 Cl^{-}
Water/ Solvent	208 / 56
Occupancy	
RS: PS	0:1.0
A site	1.0 Mg^{2+}
B site	1.0 Li^{+}
C site	1.0 Mg^{2+}
B-factors	
Macromolecules	22.2
Me_A / Lig_A[2]	17.8 / 18.1
Me_B / Lig_B[2]	17.0 / 15.5
Me_C / Lig_C[2]	19.5 / 20.2
Solvent[3]	38.5
R.m.s deviations	
Bond lengths (Å)	0.007
Bond angles (º)	1.06

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(16) RNase H1^{WT} 40 s reaction with 2-40 mM Mn^{2+} and 200 mM K^+ (Fig. S3c)

	2 mM PDB 6DP9	4 mM PDB 6DPA	8 mM PDB 6DPB	12 mM PDB 6DPC
Data collection				
Space group	C 1 2 1	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions				
a, b, c (Å)	81.1, 38.0, 61.4	81.2, 37.7, 62.1	81.5, 37.3, 62.2	81.8, 37.4, 61.9
a, b, γ (º)	90, 96.0, 90	90, 96.2, 90	90, 96.8, 90	90, 96.8, 90
Resolution (Å) [1]	20.0-1.40 (1.42-1.40)	20.0-1.49 (1.52-1.49)	50.0-1.33 (1.35-1.33)	20.0-1.34 (1.36-1.34)
R_{pim} [1]	0.031 (0.636)	0.042 (0.618)	0.032 (0.81)	0.041 (0.699)
R_{sym} or R_{merge} [1]	0.046 (0.910)	0.062 (0.868)	0.046 (>1.0)	0.061 (0.966)
$I/\sigma(I)$ [1]	20.0 (1.20)	13.0 (1.06)	20.4 (0.88)	14.2 (1.04)
$CC_{1/2}$ [1]	(0.570)	(0.282)	(0.380)	(0.462)
Completeness (%) [1]	91.8 (90.2)	96.0 (98.3)	94.9 (86.8)	92.6 (90.0)
Redundancy [1]	3.0 (2.8)	2.8 (2.6)	3.1 (2.5)	2.9 (2.5)
Refinement				
Resolution (Å)[1]	19.0-1.40	19.2-1.49	32.1-1.32	19.2-1.34
No. reflections[1]	33786	34029	41029	38831
R_{work} / R_{free}	15.1 / 18.4	15.4 / 18.8	14.8 / 17.2	14.9 / 18.0
No. atoms				
Protein / RNA/ DNA	2013 / 122 / 121	1955 / 135 / 121	1919 / 135 / 121	1919 / 135 / 121
Me^{2+}	2 Mn^{2+}	2 Mn^{2+}	4 Mn^{2+}	5 Mn^{2+}
Me^{+} [4]	3 K^+	3 K^+	3 K^+	3 K^+
Halides[4]	4 I^-	4 I^-	4 I^-	4 I^-
Water/ Solvent	181 / 32	169 / 30	212 / 36	213 / 36
Occupancy				
RS: PS	1.0 : 0	0.70 : 0.30	0.40 : 0.60	0.30 : 0.70
A site	0.65 Mn^{2+} / 0.35 K^+	0.80 Mn^{2+}	1.0 Mn^{2+}	1.0 Mn^{2+}
B site	0.30 Mn^{2+} / 0.35 K^+	0.90 Mn^{2+}	1.0 Mn^{2+}	1.0 Mn^{2+}
C site	0	0	0	0
U site	0	0.20 K^+	0.20 K^+	0.15 K^+
W site	0	0.20 K^+	0.30 K^+	0.40 K^+
B-factors				
Macromolecules	27.5	27.8	25.5	23.7
Me_A / Lig_A[2]	28.7 / 40.8	24.6 / 28.4	19.4 / 22.2	17.5 / 19.4
Me_B / Lig_B[2]	23.8 / 28.3	25.3 / 25.7	18.0 / 21.9	16.2 / 18.9
Me_C / Lig_C[2]	NA	NA	NA	NA
Me_U / Lig_U[2]	NA	28.2 / 25.6	24.2 / 23.8	19.9 / 21.0
Me_W / Lig_W[2]	NA	25.4 / 15.8	23.0 / 16.0	26.7 / 18.0
Solvent[3]	43.6	42.4	40.1	37.6
R.m.s deviations				
Bond lengths (Å)	0.007	0.008	0.007	0.007
Bond angles (º)	0.99	1.14	1.06	1.06

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(17) RNase H1^{WT} 40 s reaction with 2-40 mM Mn^{2+} and 200 mM K^+ (Fig. S3c)-Cont'd

	16 mM PDB **6DPD**	20 mM PDB **6DPE**	40 mM PDB **6DPF**
Data collection			
Space group	C 1 2 1	C 1 2 1	C 1 2 1
Cell dimensions			
a, b, c (Å)	81.3, 38.0, 61.3	81.0, 38.5, 60.6	81.7, 37.6, 61.2
a, b, γ (º)	90, 96.3, 90	90, 96.0, 90	90, 96.2, 90
Resolution (Å) [1]	20.0-1.46 (1.49-1.46)	20.0-1.58 (1.61-1.58)	19.920.0
R_{pim} [1]	0.044 (0.686)	0.098 (0.682)	0.069 (0.744)
R_{sym} or R_{merge} [1]	0.068 (0.973)	0.141 (0.944)	0.102 (>1.0)
$I/\sigma(I)$ [1]	14.8 (1.12)	6.7 (1.05)	7.6 (0.83)
$CC_{1/2}$ [1]	(0.530)	(0.339)	(0.383)
Completeness (%) [1]	99.4 (98.4)	95.2 (95.8)	98.0 (95.0)
Redundancy [1]	3.2 (2.8)	2.9 (2.7)	2.7 (2.5)
Refinement			
Resolution (Å)[1]	19.8-1.46	18.8-1.56	19.9-1.56
No. reflections[1]	32500	24895	25955
R_{work} / R_{free}	15.0 / 17.1	17.2 / 21.6	16.2 / 20.7
No. atoms			
Protein / RNA/ DNA	1900 / 123 / 121	1900 / 123 / 121	1900 / 123 /121
Me^{2+}	6 Mn^{2+}	5 Mn^{2+}	5 Mn^{2+}
Me^{+} [4]	1 K^+	1 K^+	1 K^+
Halides[4]	5 I^-	5 I^-	5 I^-
Water/ Solvent	186 / 47	167 / 44	167 / 44
Occupancy			
RS: PS	0 : 1.0	0 : 1.0	0 : 1.0
A site	1.0 Mn^{2+}	1.0 Mn^{2+}	1.0 Mn^{2+}
B site	1.0 Mn^{2+}	1.0 Mn^{2+}	1.0 Mn^{2+}
C site	0.15 Mn^{2+}	0	0
U site	0	0	0
W site	0.60 K^+	0.60 K^+	0.60 K^+
B-factors			
Macromolecules	28.4	29.2	32.1
Me_A / Lig_A [2]	20.4 / 20.5	19.1 / 19.2	22.2 / 21.5
Me_B / Lig_B [2]	19.3 / 20.3	17.7 / 19.5	21.1 / 21.8
Me_C / Lig_C [2]	26.1 / 23.9	NA	NA
Me_U / Lig_U [2]	NA	NA	NA
Me_W / Lig_W [2]	33.3 / 21.1	30.9 / 23.4	34.2 / 22.4
Solvent[3]	43.0	38.5	41.6
R.m.s deviations			
Bond lengths (Å)	0.007	0.008	0.008
Bond angles (º)	0.97	0.98	1.05

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(18) RNase H1^{E188A} reaction with 4 mM Mn^{2+} and 200 mM K^{+} (Fig. 4b)

	240 s PDB **6DPG**
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	81.6, 37.6, 62.0
a, b, γ (º)	90, 96.6, 90
Resolution (Å) [1]	20.0 - 1.38 (1.40 - 1.38)
R_{pim} [1]	0.047 (0.782)
R_{sym} or R_{merge} [1]	0.070 (>1.0)
$I/\sigma(I)$ [1]	13.1 (1.03)
$CC_{1/2}$ [1]	(0.373)
Completeness (%) [1]	96.4 (93.8)
Redundancy [1]	3.1 (2.8)
Refinement	
Resolution (Å)[1]	20.0 - 1.38
No. reflections[1]	37154
R_{work} / R_{free}	15.9 / 17.5
No. atoms	
Protein / RNA/ DNA	2225 / 173 / 121
Me^{2+}	4 Mn^{2+}
Me^{+} [4]	3 K^{+}
Halides[4]	3 I^{-}
Water/ Solvent	177 / 44
Occupancy	
RS: PS	0.20 : 0.80
A site	0.90 Mn^{2+}
B site	1.0 Mn^{2+}
C site	0.40 Mn^{2+}
W site	0.40 K^{+}
B-factors	
Macromolecules	31.0
Me_A / Lig_A[2]	20.7 / 23.9
Me_B / Lig_B[2]	21.0 / 22.7
Me_C / Lig_C[2]	29.2 / 28.4
Me_W / Lig_W[2]	31.8 / 25.3
Solvent[3]	45.9
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (º)	1.26

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(19) RNase H1^{E188A} reaction with 2 mM Mg^{2+} and 200 mM K^{+} (Fig. 4c)

	120 s PDB **6DPH**
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	80.9, 37.1, 61.8
a, b, γ (º)	90, 96.3, 90
Resolution (Å) [1]	50.0 - 1.35 (1.37 - 1.35)
R_{pim} [1]	0.029 (0.547)
R_{sym} or R_{merge} [1]	0.043 (0.775)
$I/\sigma(I)$ [1]	19.1 (1.09)
$CC_{1/2}$ [1]	(0.524)
Completeness (%) [1]	98.5 (86.0)
Redundancy [1]	2.9 (2.5)
Refinement	
Resolution (Å)[1]	23.2 - 1.34
No. reflections[1]	40257
R_{work} / R_{free}	16.0 / 19.2
No. atoms	
Protein / RNA/ DNA	2105 / 122 / 121
Me^{2+}	2 Mg^{2+}
Me^{+} [4]	3 K^{+}
Halides[4]	4 I^{-}
Water/ Solvent	202 / 46
Occupancy	
RS: PS	1.0 : 0
A site	0.60 Mg^{2+} / 0.40 K^{+}
B site	0.60 Mg^{2+} / 0.40 K^{+}
C site	0
B-factors	
Macromolecules	26.6
Me_A / Lig_A[2]	24.6 / 21.4 (Mg^{2+}) , 16.3 / 20.5 (K^{+})
Me_B / Lig_B[2]	16.7 / 27.6 (Mg^{2+}) , 17.9 / 20.5 (K^{+})
Me_C / Lig_C[2]	NA
Solvent[3]	41.2
R.m.s deviations	
Bond lengths (Å)	0.009
Bond angles (º)	1.06

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(20) RNase H1^{K196A} reaction with 10 mM Mg^{2+} and 200 mM Rb^{+} (Fig. 4d)

	40 s PDB **6DPI**
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	81.6, 37.3, 62.4
a, b, γ (º)	90, 97.0, 90
Resolution (Å) [1]	20.0 - 1.35 (1.37 - 1.35)
R_{sym} or R_{merge} [1]	0.098 (>1.0)
R_{pim} [1]	0.036 (0.702)
$I/\sigma(I)$ [1]	18.1 (0.99)
$CC_{1/2}$ [1]	(0.380)
Completeness (%) [1]	97.0 (91.7)
Redundancy [1]	8.2 (3.8)
Refinement	
Resolution (Å)[1]	20.0 - 1.35
No. reflections[1]	40008
R_{work} / R_{free}	14.5 / 17.4
No. atoms	
Protein / RNA/ DNA	1981 / 122 / 121
Me^{2+}	2 Mg^{2+}
Me^{+} [4]	3 Rb^{+}
Halides[4]	3 Cl^{-}
Water/ Solvent	203 / 36
Occupancy	
RS: PS	1.0 : 0
A site	1.0 Mg^{2+}
B site	1.0 Mg^{2+}
U site	0.30 Rb^{+}
V site	0
W site	0
B-factors	
Macromolecules	24.1
Me_A / Lig_A[2]	16.7 / 21.4
Me_B / Lig_B[2]	14.6 / 17.0
Me_U / Lig_U[2]	27.2 / 22.2
Me_V/ Lig_V[2]	NA
Me_W / Lig_W[2]	NA
Solvent[3]	40.4
R.m.s deviations	
Bond lengths (Å)	0.008
Bond angles (º)	1.28

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(21) RNase H1^{K196A} reaction with 4 mM Mn^{2+} and 200 mM K^{+} (Fig. S4a)

	80 s PDB **6DPJ**	240 s PDB **6DPK**
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	81.9, 37.5, 62.4	81.5, 37.6, 61.7
a, b, γ (º)	90, 97.2, 90	90, 96.8, 90
Resolution (Å) [1]	20.0 - 1.55 (1.60 - 1.57)	20.0 - 1.39 (1.41 - 1.39)
R_{pim} [1]	0.077 (0.646)	0.051 (0.435)
R_{sym} or R_{merge} [1]	0.115 (0.882)	0.087 (0.718)
$I/\sigma(I)$ [1]	7.4 (0.97)	12.2 (1.46)
$CC_{1/2}$ [1]	(0.438)	(0.641)
Completeness (%) [1]	96.6 (77.2)	99.5 (96.0)
Redundancy [1]	2.9 (2.1)	3.6 (3.2)
Refinement		
Resolution (Å)[1]	18.8 - 1.55	19.9 - 1.39
No. reflections[1]	26181	37272
R_{work} / R_{free}	17.4 / 21.4	14.3 / 16.8
No. atoms		
Protein / RNA/ DNA	2104 / 122 / 121	2224 / 172 / 121
Me^{2+}	2 Mn^{2+}	4 Mn^{2+}
Me^{+} [4]	2 K^{+}	2 K^{+}
Halides[4]	4 I^{-}	3 I^{-}
Water/ Solvent	130 / 14	189 / 44
Occupancy		
RS: PS	1.0 : 0	0.20 : 0.80
A site	1.0 Mn^{2+}	1.0 Mn^{2+}
B site	1.0 Mn^{2+}	1.0 Mn^{2+}
C site	NA	0.40 Mn^{2+}
U site	0.30 K^{+}	NA
W site	NA	0.40 K^{+}
B-factors		
Macromolecules	37.4	24.3
Me_A / Lig_A[2]	28.9 / 31.5	18.0 / 23.2
Me_B / Lig_B[2]	27.6 / 29.9	17.0 / 17.5
Me_C / Lig_C[2]	NA	26.2 / 25.5
Me_U / Lig_U[2]	26.8 / 33.6	NA
Me_W / Lig_W[2]	NA	18.7 / 20.9
Solvent[3]	42.5	40.7
R.m.s deviations		
Bond lengths (Å)	0.008	0.008
Bond angles (º)	1.03	1.09

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(22) RNase H1^{K196A} reaction with 10 mM Mg^{2+} and 200 mM Rb^{+} (Fig. S4b)

	720 s PDB **6DPL**	1800 s PDB **6DPM**
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	81.8, 37.5, 62.7	81.8, 37.4, 62.7
a, b, γ (º)	90, 97.3, 90	90, 97.7, 90
Resolution (Å) [1]	20.0 - 1.45 (1.47 - 1.45)	20.0 - 1.68 (1.71 - 1.68)
R_{pim} [1]	0.038 (0.923)	0.053 (0.700)
R_{sym} or R_{merge} [1]	0.091 (>1.0)	0.123 (>1.0)
$I/\sigma(I)$ [1]	19.2 (1.0)	14.7 (1.15)
$CC_{1/2}$ [1]	(0.276)	(0.437)
Completeness (%) [1]	99.0 (91.7)	100 (100)
Redundancy [1]	8.0 (3.2)	8.2 (6.7)
Refinement		
Resolution (Å)[1]	19.5 - 1.45	20.1 - 1.68
No. reflections[1]	33423	21641
R_{work} / R_{free}	14.8 / 17.9	16.0 / 19.9
No. atoms		
Protein / RNA/ DNA	1989 / 145 / 121	1944 / 123 / 121
Me^{2+}	2 Mg^{2+}	2 Mg^{2+}
Me^{+} [4]	6 Rb^{+}	6 Rb^{+}
Halides[4]	3 Cl^{-}	3 Cl^{-}
Water/ Solvent	169 / 56	181 / 40
Occupancy		
RS: PS	0.55 : 0.45	0 : 1.0
A site	1.0 Mg^{2+}	1.0 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}
U site	0.25 Rb^{+}	0.20 Rb^{+}
V site	0.20 Rb^{+}	0.20 Rb^{+}
W site	0.45 Rb^{+}	0.70 Rb^{+}
B-factors		
Macromolecules	26.5	24.8
Me_A / Lig_A[2]	19.7 / 24.8	20.5 / 19.4
Me_B / Lig_B[2]	18.2 / 26.0	16.7 / 19.0
Me_U / Lig_U[2]	28.5 / 24.0	25.9 / 20.7
Me_V/ Lig_V[2]	40.6	37.04
Me_W / Lig_W[2]	22.4 / 17.6	19.3 / 20.5
Solvent[3]	42.3	36.3
R.m.s deviations		
Bond lengths (Å)	0.007	0.008
Bond angles (º)	1.23	1.22

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides

(23) RNase H1^{E188A} reaction with 2 mM Mg^{2+} and 200 mM K^{+} (Fig. S5a and S5b)

	200 s PDB **6DPN**	360 s PDB **6DPO**
Data collection		
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	81.5, 37.6, 62.0	81.3, 38.0, 61.9
a, b, γ (º)	90, 96.2, 90	90, 96.1, 90
Resolution (Å) [1]	20.0 - 1.49 (1.53 - 1.50)	20.0 - 1.45 (1.47- 1.45)
R_{sym} or R_{merge} [1]	0.063 (0.858)	0.042 (0.988)
R_{pim} [1]	0.044 (0.633)	0.028 (0.690)
$I/\sigma(I)$ [1]	14.5 (0.99)	24.2 (0.95)
$CC_{1/2}$ [1]	(0.561)	(0.526)
Completeness (%) [1]	98.4 (92.8)	98.4 (97.1)
Redundancy [1]	2.7 (2.2)	3.0 (2.8)
Refinement		
Resolution (Å)[1]	19.9 - 1.50	19.8 - 1.45
No. reflections[1]	29802	33161
R_{work} / R_{free}	16.2 / 18.3	16.2 / 19.0
No. atoms		
Protein / RNA/ DNA	2005 / 122 / 121	2115 / 151 / 121
Me^{2+}	2 Mg^{2+}	3 Mg^{2+}
Me^{+} [4]	2 K^{+}	1 K^{+}
Halides[4]	4 I^{-}	4 I^{-}
Water/ Solvent	185 / 36	171 / 42
Occupancy		
RS: PS	1.0 : 0	0.60 : 0.40
A site	1.0 Mg^{2+}	0.80 Mg^{2+}
B site	1.0 Mg^{2+}	1.0 Mg^{2+}
C site	0	0.40 Mg^{2+}
U site	0.35	NA
B-factors		
Macromolecules	30.3	30.5
Me_A / Lig_A[2]	25.1 / 28.1	25.9 / 33.3
Me_B / Lig_B[2]	23.8 / 24.6	26.9 / 25.6
Me_C / Lig_C[2]	NA	39.9 / 46.4
Me_U / Lig_U[2]	35.2 / 29.4	NA
Solvent[3]	41.3	42.3
R.m.s deviations		
Bond lengths (Å)	0.008	0.008
Bond angles (º)	1.02	1.08

[1] Data in the highest resolution shell is shown in the parenthesis.

[2] B-factors of metal ions and their protein, solvent, and nucleotide ligands.

[3] Water, ethylene glycol and glycerol

[4] B factors and occupancies are not listed for non-catalytic monovalent cations and halides