Supporting Information

Epigenetic DNA Modification N^6-Methyladenine Causes Site-Specific RNA Polymerase II Transcriptional Pausing

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Key words: *Transcription, Epigenetics, DNA methylation, N^6-methyladenine, RNA Polymerase II.*

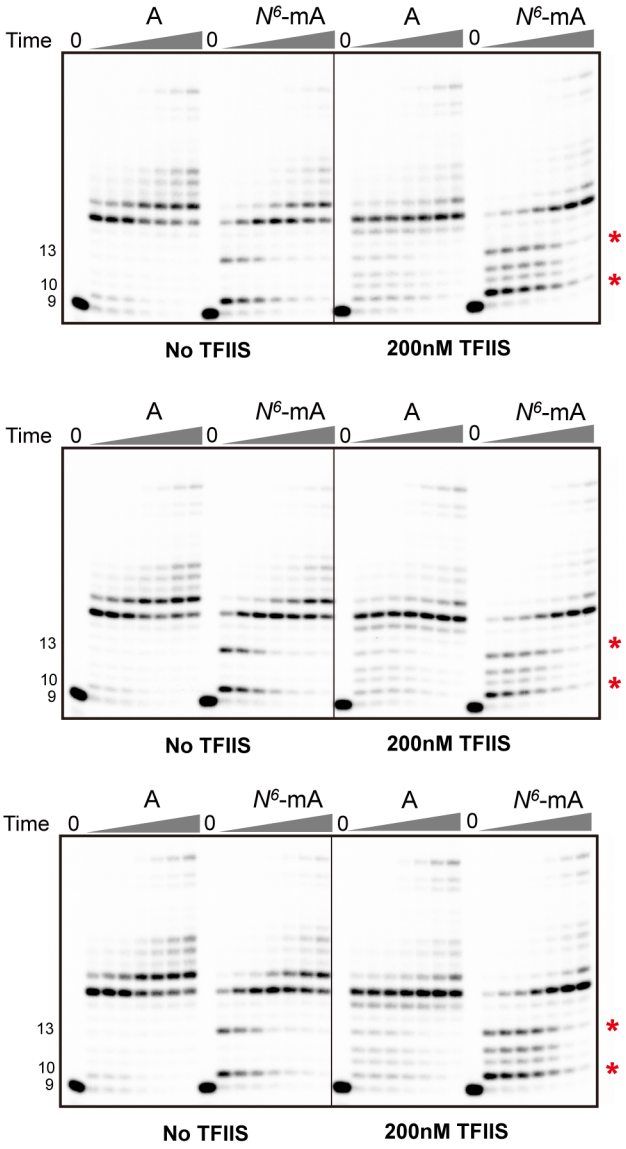

Figure S1. N^6-mA causes site-specific pol II pausing due to a slow incorporation step. Three additional repeats for Figure 1c and 1d.

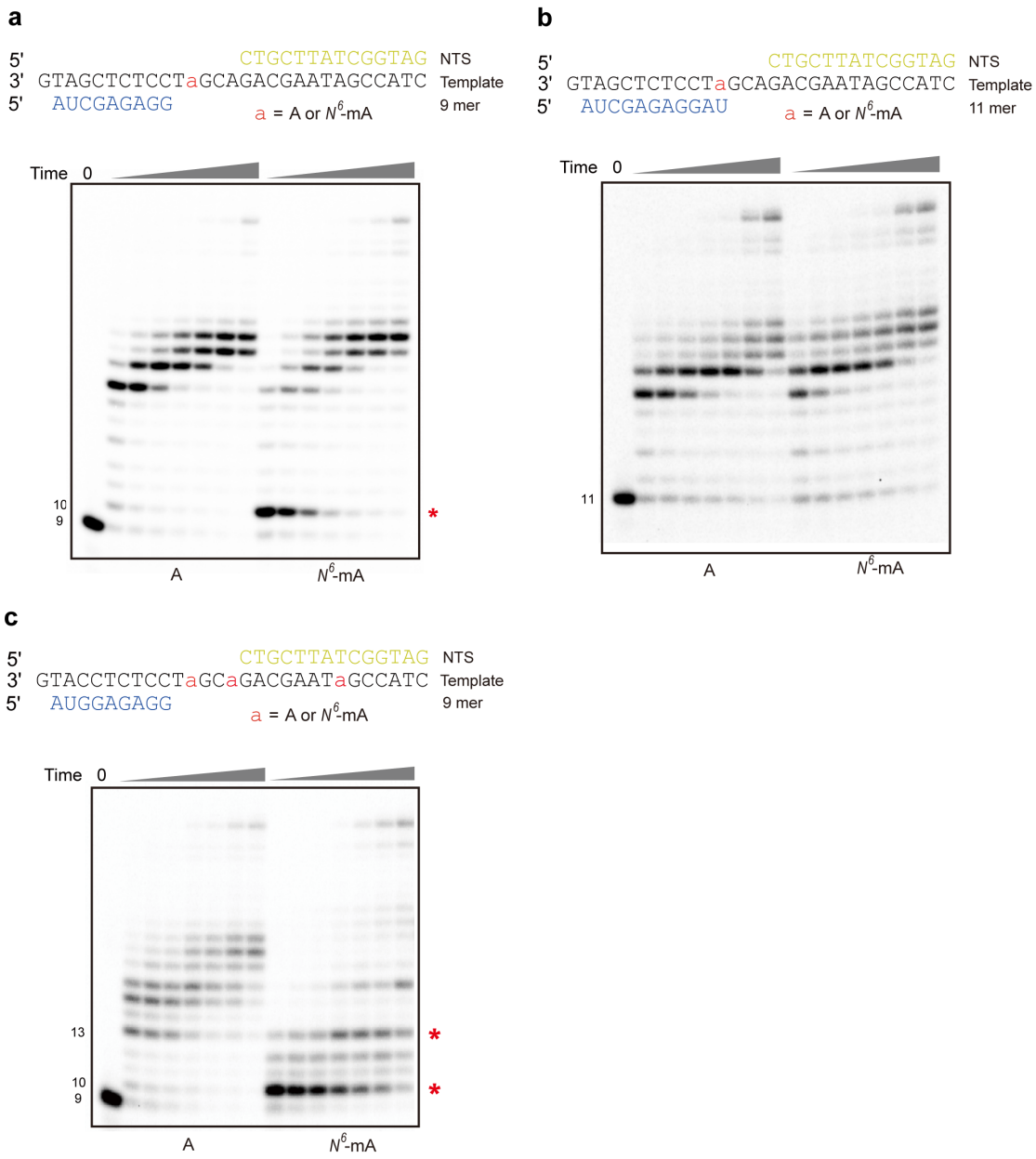

Figure S2. N^6-mA causes site-specific pol II pausing due to a slow incorporation step. (a) Gel analysis of nucleotide incorporation against N^6-mA, and (b) subsequent extension after N^6-mA in the presence of 25 μM NTP. Time points are 0s, 15 s, 30 s, 1 min, 2 min, 5 min, 20 min, and 1 hr, respectively. (c) N^6-mA cause site-specific pol II pausing (highlighted with *) in the presence of 1 μM TFIIS.

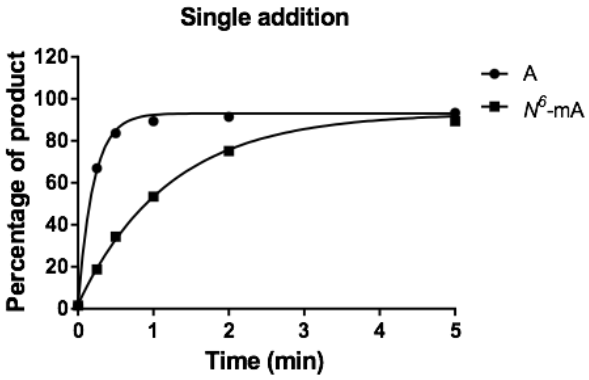

Figure S3. Quantitative analysis of observed kinetic rates for single UMP incorporation. For the unmodified template, $k_{obs,A}$=4.8 ± 0.3 min^{-1}; for the N^6-mA, $k_{obs,mA}$= 0.83 ± 0.04 min^{-1}.

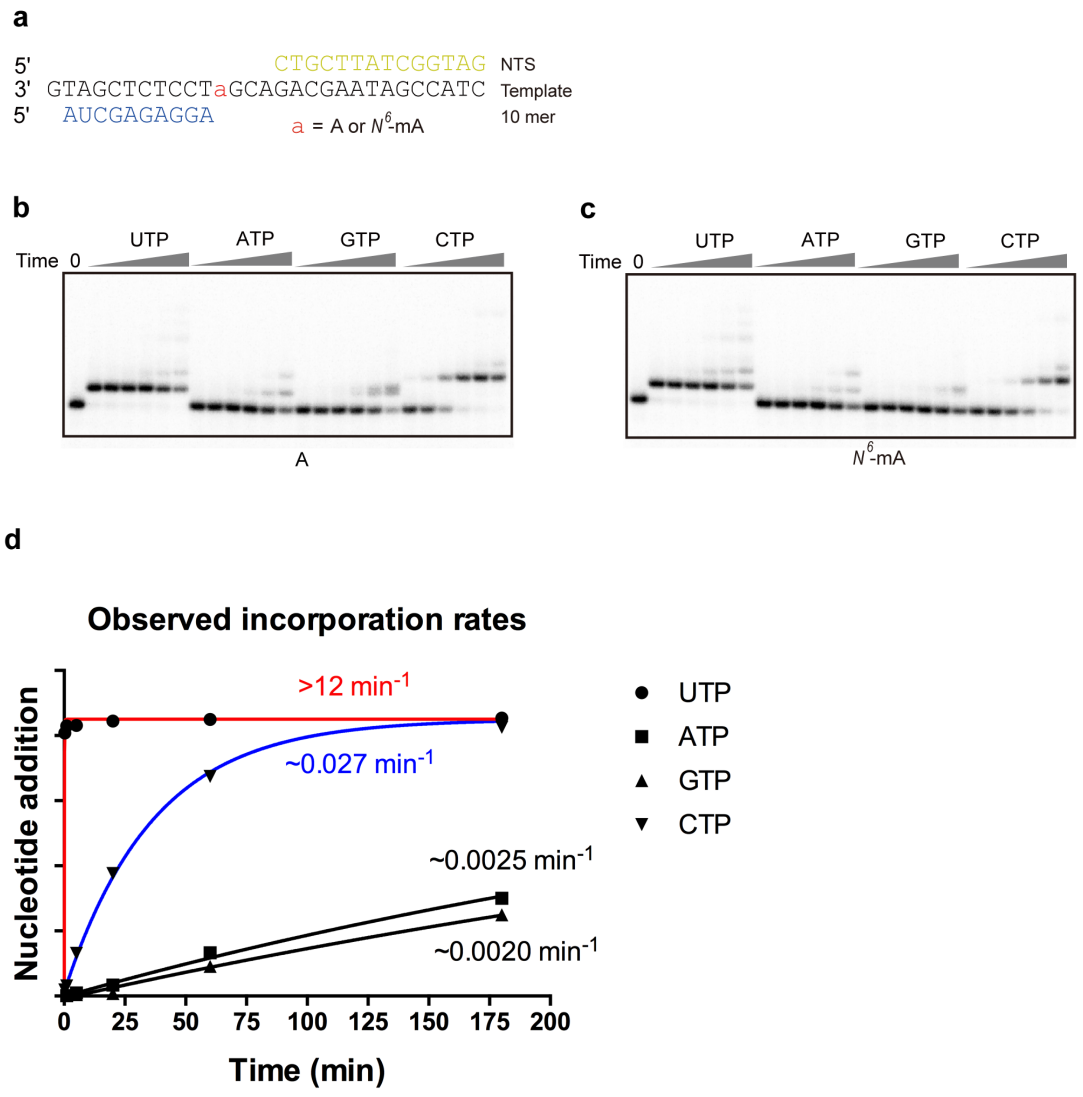

Figure S4. UMP is selectively incorporated opposite the N^6-mA site. (a) RNA/DNA scaffold used in this *in vitro* transcription assay. Single nucleotide addition for the unmodified template (b) and N^6-mA template (c). The nucleotide concentration is 1 mM. The first lane in the left refers to time zero. Other time points are 0, 15 s, 1 min, 5 min, 20 min, 1 hr, and 3 hr, respectively. (d) Quantitative analysis of nucleotide incorporation opposite of N^6-mA template.

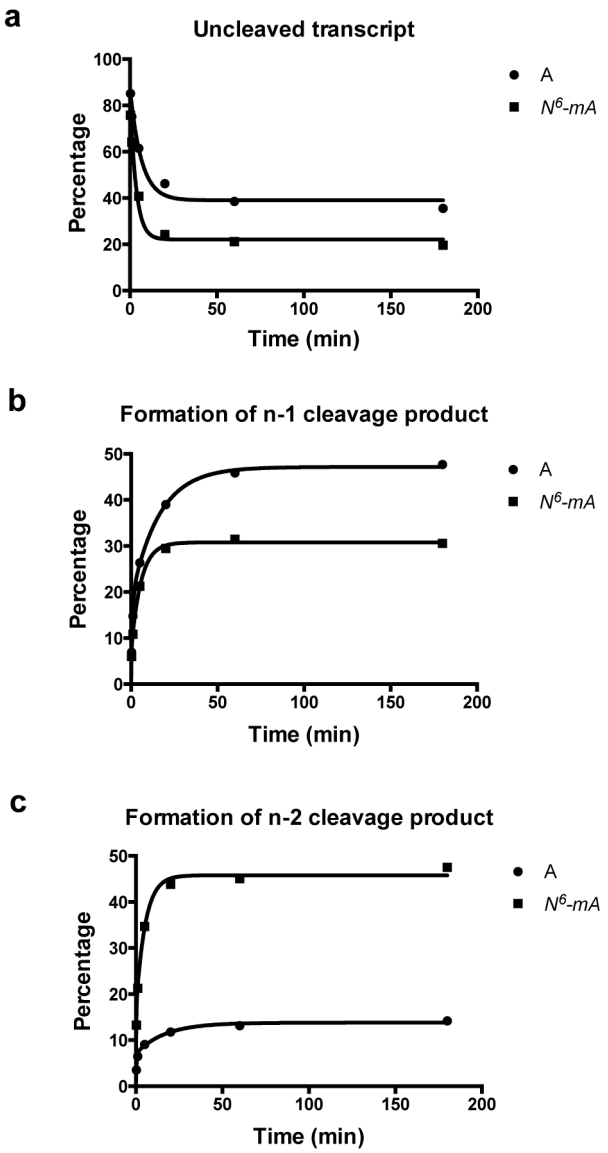

Figure S5. Analysis of TFIIS-stimulated transcript cleavage rates and patterns in the unmodified A template and N^6-mA template. (a) Quantitative analysis of overall observed transcript cleavage rates derived by the disappearance of starting material using two-phase decay fitting model. $k_{obs,6mA} = 4.9 \pm 1.9$ min^{-1} for N^6-mA template and $k_{obs,A} = 1.2 \pm 0.3$ min^{-1} for unmodified template. (b) Formation of n-1 cleavage product (pre-translocation state derived cleavage) using two-phase decay fitting model. $k_{obs,6mA} = 4.8 \pm 2.0$ min^{-1} for N^6-mA template and $k_{obs,A} = 1.3 \pm 0.3$ min^{-1} for unmodified template. Population of pre-translocation derived by curve plateau are 31 ± 1% for the N^6-mA template and 47 ± 1% for the unmodified A template. (c) Formation of n-2 cleavage product (backtracked state derived cleavage) using

two-phase decay fitting model. $k_{obs,6mA}$ = 5 ± 2 min^{-1} for N^6-mA template and $k_{obs,A}$= 2.4 ± 0.8 min^{-1} for unmodified template. Population of backtracked state derived by curve plateau are 46 ± 1% for the N^6-mA template and 14 ± 0.4% for the unmodified A template.

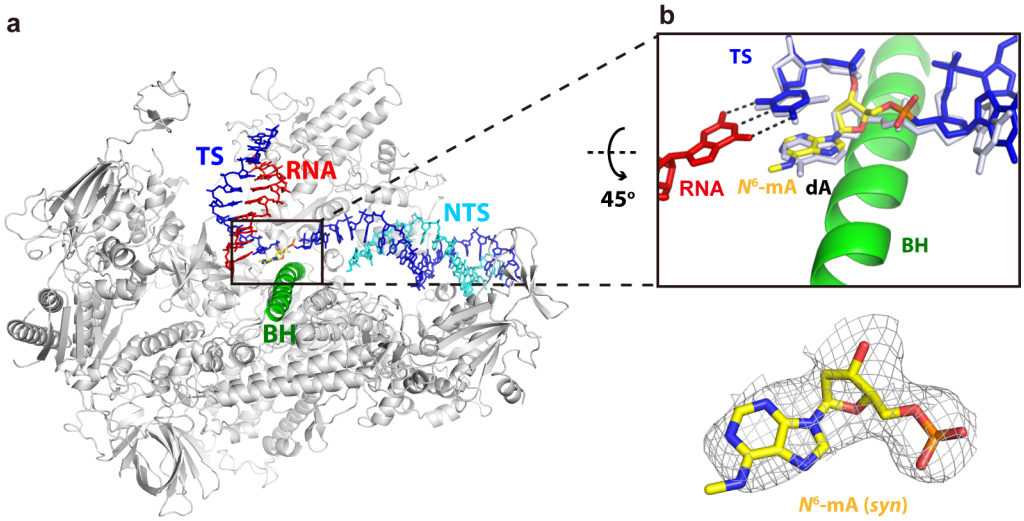

Figure S6. Crystal structure of RNA pol II elongation complex with an N^6-mA at i+1 position (EC-I). (a) Overall structure of pol II EC. All representations and colors are the same as pol II EC-II in Figure 3. (b) Comparison of N^6-mA with the corresponding canonical template nucleotide (silver colored, PDB code 2NVZ). Bottom panel: 2*F*o-*F*c map (grey) of N^6-mA is contoured at 1.0σ.

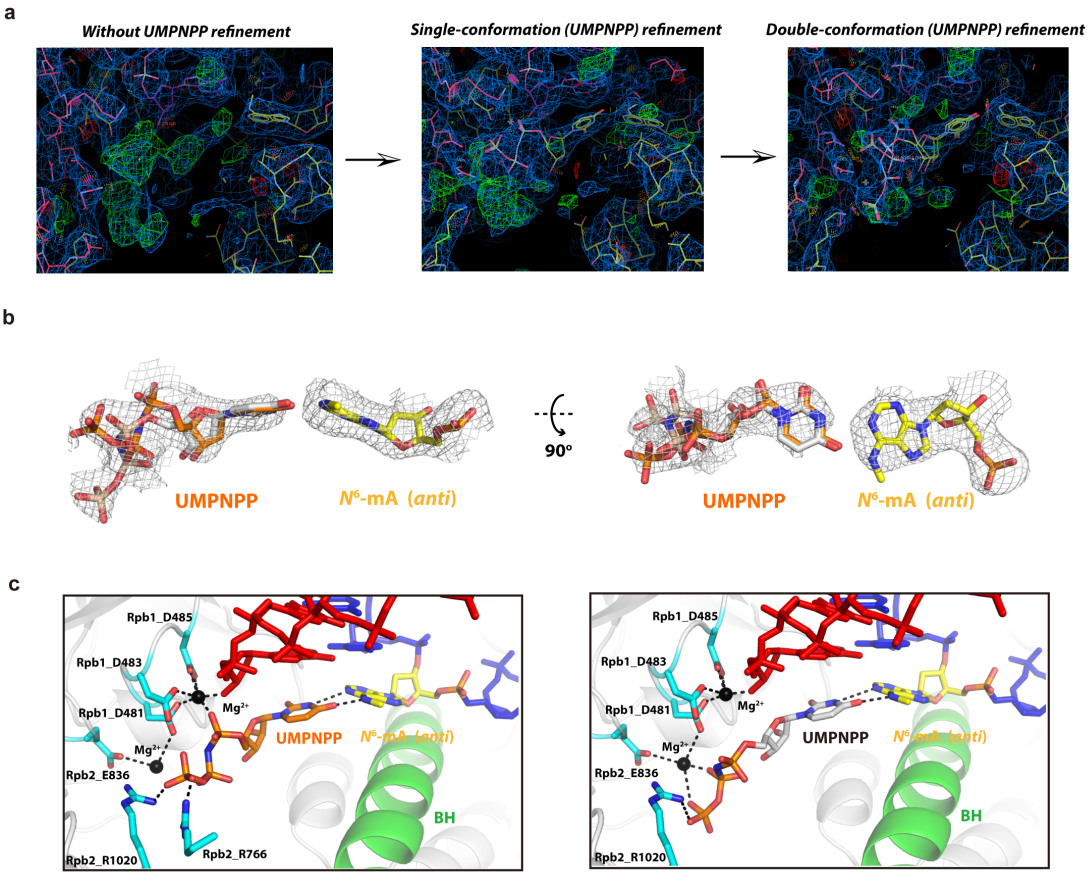

Figure S7. Electron density maps of pol II EC-II and two conformations of UMPNPP. (a) The original refinement process of UMPNPP is shown in software of COOT. 2*F*o-*F*c map is colored as blue (1.0σ), and *F*o-*F*c map is colored as green (3.0σ). (b) 2*F*o-*F*c maps (grey) of UMPNPP and N^6-mA are contoured at 1.0σ from two views. Two conformations of UMPNPP are colored as white and orange sticks, respectively. (c) Interaction network of hydrogen bonds between two different conformations of UMPNPP and N^6-mA.

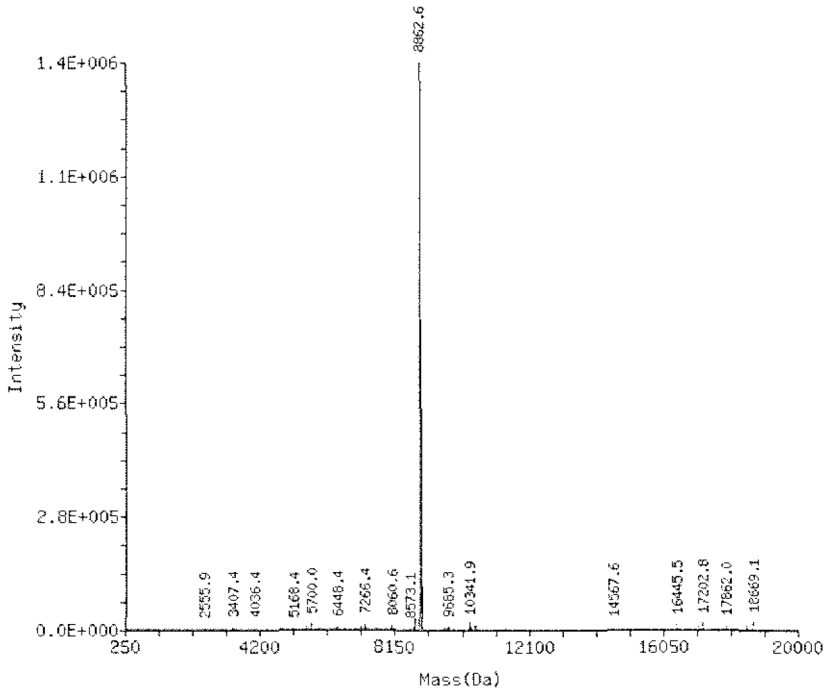

Figure S8. Representative deconvoluted ESI mass spectrum characterization of synthetic N^6-mA containing DNA oligo. The sequence is 5' CTACCGATAAGCAGACG(N^6-mA)TCCTCTCGATG 3'. The theoretical molecular weight is 8860.8 Da.

Table S1. X-ray Diffraction Data and Refinement Statistics.

	EC-I	EC-II
Data collection		
Space group	C2	C2
Cell dimensions		
a, b, c (Å)	167.2, 222.0, 192.7	168.7, 224.0,193.1
α, β, γ (°)	90, 100.6, 90	90, 101.0,90
Resolution (Å)	95.8-3.6 (3.67-3.6)	96.4-3.4 (3.58-3.4)
Mean I/σ	5.0 (1.3)	5.5 (1.3)
CC 1/2	0.98 (0.53)	0.98 (0.47)
Completeness (%)	100 (100)	99.6 (99.8)
Redundancy	5.4 (5.4)	3.7 (3.7)
Refinement		
Resolution (Å)	81.0-3.6	82.8-3.4
Diffraction Anisotropy a, b, c (Å)	4.0, 3.6, 3.6	3.7, 3.4, 3.4
No. reflections	71390	83721
R_{work}/R_{free}	0.227/0.259	0.227/0.254
RMSD bands	0.002	0.002
RMSD angles	0.514	0.536
Ramachandran favored (%)	92.5	92.6