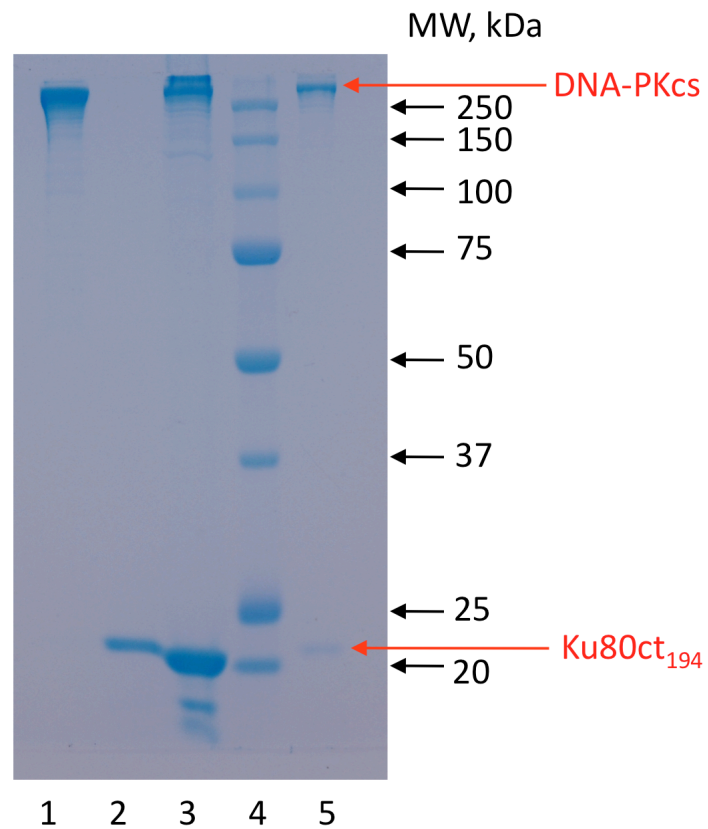


Supplementary Information

Supplementary Table 1. Data collection, phasing and refinement statistics for DNA-PKcs and Ku80ct₁₉₄ complex crystal structure.

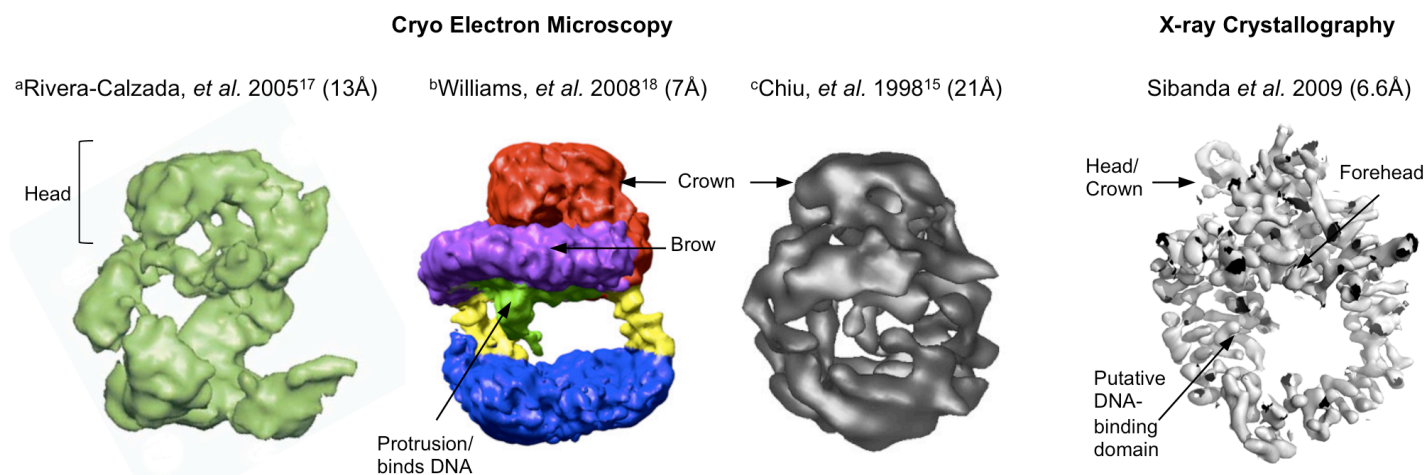
Ta ₆ Br ₁₂ ²⁺ derivative		
Data collection		
Space group	P2 ₁	P2 ₁
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	164.91, 132.91, 291.29	165.05, 133.26, 292.34
<i>α</i> , <i>β</i> , <i>γ</i> (°)	90.0, 103.75, 90.0	90.0 103.86, 90.0
	<i>Peak</i>	<i>Inflection</i>
Wavelength	1.2549	1.2552
Resolution (Å)	100 - 6.6 (6.75 – 6.60)	100 - 7.1 (7.27 – 7.10)
<i>R</i> _{merge} (%)	7.7 (55.0)	7.9 (63.1)
< <i>I</i> /σ <i>I</i> >	10.1	9.8
Completeness (%)	88.6 (74.9)	87.3 (70.7)
Redundancy	3.2	3.3
Refinement		
Resolution (Å)	100 – 6.6	
No. reflections	22,653	
<i>R</i> _{work} / <i>R</i> _{free} (%)	44.2/44.1	
No. Ala residues	4064	
modelled in asymmetric unit		

*Highest resolution shell is shown in parenthesis.



Supplementary Figure 1. SDS-PAGE of DNA-PKcs.

A sodium dodecyl sulphate (SDS) poly-acrylamide gel showing in lanes: 1) purified full length DNA-PKcs; 2) expressed and purified Ku80ct₁₉₄ domain; 3) DNA-PKcs complexed with Ku80ct₁₉₄ domain; 4) Precision Plus protein standards from Bio-RAD; 5) washed and dissolved crystals showing the presence of DNA-PKcs and Ku80ct₁₉₄ designated by red arrows.



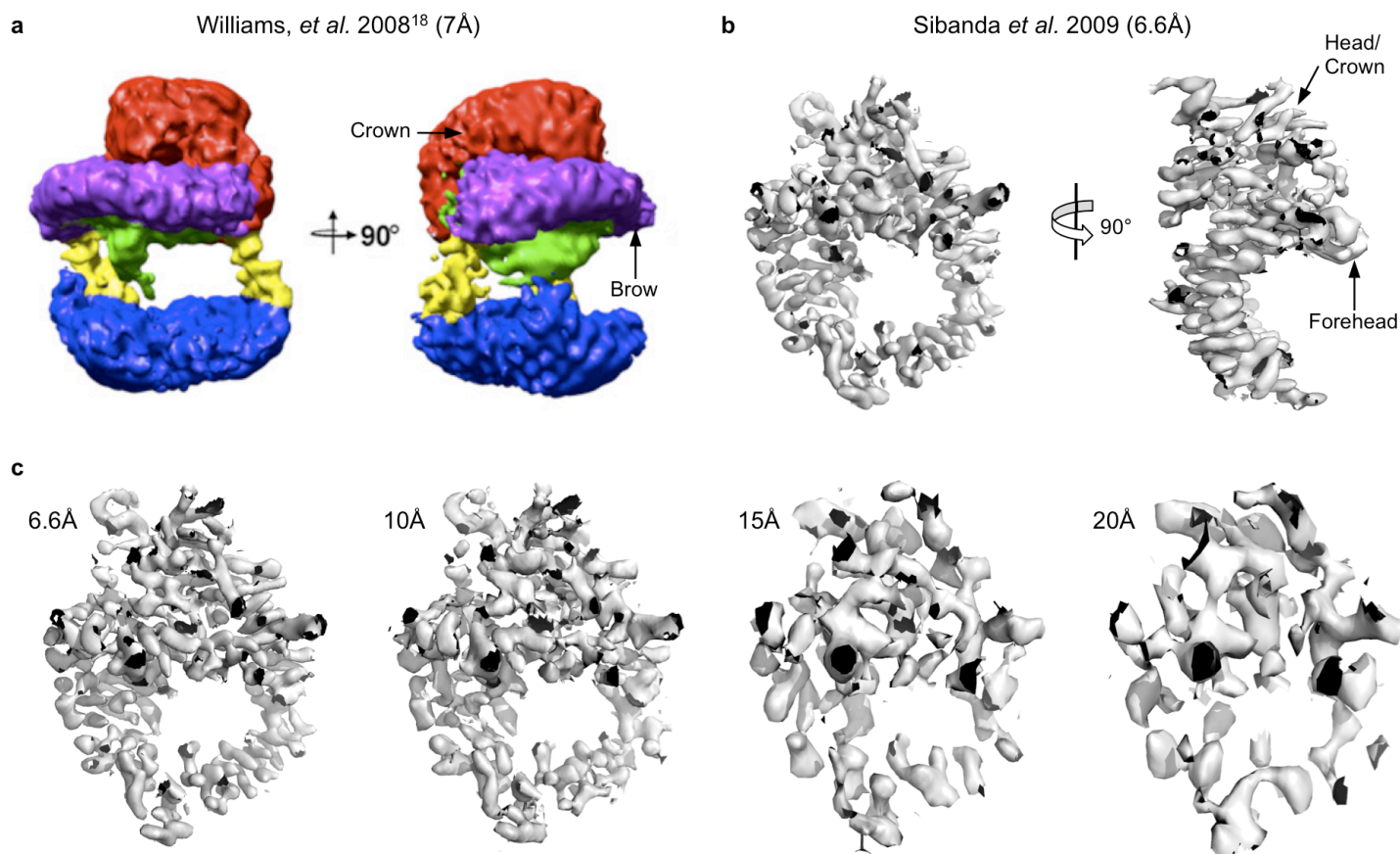
Supplementary Figure 2. Equivalent views of DNA-PKcs as defined by cryo-electron microscopy and X-ray crystallography.

References to the publications and resolutions of the models are given above. Colour-coding of these EM structures are as given in their respective publications. The X-ray crystallographic experimental F_o electron density map defined by the multi-wavelength anomalous dispersion method, is from the present work.

^aReprinted from *Structure*, vol. 13, A. Rivera-Calzada, J. P. Maman, L. Spagnolo, L. H. Pearl and O. Llorca, Three-Dimensional Structure and Regulation of the DNA-Dependent Protein Kinase Catalytic Subunit (DNA- PKcs), pp. 243-255, ©2005, with permission from Elsevier.

^bReprinted from *Structure*, vol. 16, D. R. Williams, K.-J. Lee, J. Shi, D. J. Chen and P. L. Stewart, Cryo-EM Structure of the DNA-Dependent Protein Kinase Catalytic Subunit at Subnanometer Resolution Reveals α Helices and Insight into DNA Binding, pp. 468-477, ©2008, with permission from Elsevier.

^cReprinted from *Journal of Molecular Biology*, vol. 284, C. Y. Chiu, R. B. Cary, D. J. Chen, S. R. Peterson and P. L. Stewart, Cryo-EM Imaging of the Catalytic Subunit of the DNA-Dependent Protein Kinase, pp. 1075-1081, ©1998, with permission from Elsevier.



Supplementary Figure 3. Equivalent orthogonal views of DNA-PKcs as defined.

a) By cryo-electron microscopy¹⁸ at 7Å resolution (but at 6.7 Å resolution when estimated by the Fourier shell correlation at the 0.5 threshold; colour-coding of the EM structure is as given in Williams, *et al.*¹⁸) and b) X-ray crystallographic experimental F_o density at 6.6Å resolution defined by the multi-wavelength anomalous dispersion method from the present work. c) X-ray crystallographic experimental F_o electron density map recalculated with various resolution cut-offs (all are contoured at 1.0 sigma level) for comparison with the electron microscopy models in Supplementary Figure 2.

a) Reprinted from Structure, vol. 16, D. R. Williams, K.-J. Lee, J. Shi, D. J. Chen and P. L. Stewart, Cryo-EM Structure of the DNA-Dependent Protein Kinase Catalytic Subunit at Subnanometer Resolution Reveals α Helices and Insight into DNA Binding, pp. 468-477, ©2008, with permission from Elsevier.

Supplementary Movie 1.

This movie shows the 360 degrees view of a single DNA-PKcs molecule displayed as molecular surface. The colour-coding of the molecule is as follows: green – the ring structure; light green – the forehead that is part of the ring structure; cyan – the putative DNA-binding domain; magenta – the larger C-terminal region that carries the FAT and FATC domains; yellow – the kinase domain (QuickTime; 7.6 MB).