Purification, Crystallization and Preliminary Diffraction Studies of the Sulfolobus solfataricus PCNA Proteins in Different Oligomeric Forms†

ARTICLE *in* CRYSTAL GROWTH & DESIGN · OCTOBER 2007

Impact Factor: 4.89 · DOI: 10.1021/cg700961b

3 AUTHORS:

Guangxin Xing
The University of Western Ontario
7 PUBLICATIONS **177** CITATIONS
SEE PROFILE

Vladena Bauerová-Hlinková
Slovak Academy of Sciences
10 PUBLICATIONS **48** CITATIONS
SEE PROFILE

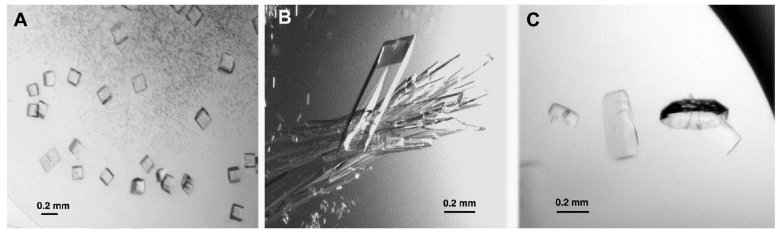
Hong Ling
The University of Western Ontario
31 PUBLICATIONS **2,588** CITATIONS
SEE PROFILE

Article

Purification, Crystallization and Preliminary Diffraction Studies of the *Sulfolobus solfataricus* PCNA Proteins in Different Oligomeric Forms

Guangxin Xing, Vladena Hlinkova, and Hong Ling

Cryst. Growth Des., **2007**, 7 (11), 2202-2205• DOI: 10.1021/cg700961b • Publication Date (Web): 23 October 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

CRYSTAL GROWTH & DESIGN
2007
VOL. 7, NO. 11
2202–2205

Purification, Crystallization and Preliminary Diffraction Studies of the *Sulfolobus solfataricus* PCNA Proteins in Different Oligomeric Forms[†]

Guangxin Xing, Vladena Hlinkova,[‡] and Hong Ling*

Department of Biochemistry, University of Western Ontario, London, Ontario, N6A 5C1, Canada

Received October 3, 2007

ABSTRACT: PCNA is a ring-shaped protein that encircles DNA and is essential for DNA metabolism, including DNA replication and repair. PCNA is either a homotrimer in eukaryotes and euryarchaeotes or a heterotrimer in some crenarchaeotes. The crenarchaeon *Sulfolobus solfataricus* encodes three PCNA homologues (PCNA1, PCNA2, and PCNA3). PCNA1 and PCNA2 form a stable dimer. The dimer then recruits PCNA3 to form the trimeric ring-shaped molecule that is typical for all PCNA proteins. We crystallized the PCNA3 monomer, the PCNA1–PCNA2 heterodimer, and the PCNA1–PCNA2–PCNA3 heterotrimer. The crystals diffract X-ray to 1.9, 2.6, and 2.5 Å resolutions, respectively. SAD phasing and molecular replacement solutions have confirmed that the crystals do contain the corresponding monomer, dimer, and trimer.

DNA sliding clamps play essential roles in DNA replication, DNA damage repair, and cell cycle control.[1–3] The prokaryotic clamps are referred to as β clamps, and the eukaryotic and archaeal sliding clamps are called the proliferation cell nuclear antigen (PCNA). Sliding clamps are ring-shaped molecules that encircle double-stranded DNA and tether their functional partners to the DNA substrates.[4] Although the sequence similarity is low among the homologues from different domains of life, structural studies showed that they all are composed of six topologically similar domains arranged into planar ring with pseudo-6-fold symmetry. In eukaryotes and euryarchaeotes, three identical PCNA monomers each consisted of two similar domains joined together in a head-to-tail fashion to form a homotrimer,[5,6] whereas crenarchaea mostly encode three distinct PCNA homologues.[7]

PCNA has been shown to interact with different proteins mainly through its interdomain-connecting loop (IDCL).[8,9] Most PCNA binding proteins interact with PCNA through a conserved motif with a consensus sequence of QXX(L/M/I)XX(F,Y-,H)(F,Y), in which X could be any amino acid. Different PCNA binding proteins may act sequentially on DNA substrate to complete a specific task in DNA replication and repair.[10] The crenarchaeon *Sulfolobus solfataricus* has three distinct PCNAs (PCNA1, PCNA2, and PCNA3) with low sequence identity (less than 25%). Biochemical studies have shown that PCNA1 and PCNA2 form a stable heterodimer. The dimer then recruits PCNA3 to form the functional heterotrimer that can specifically interact and stimulate the activity of DNA PolB1, Fen1, and DNA ligase I.[11] The three PCNAs have distinct IDCL and interact specifically with different proteins, sometimes simultaneously. Previous studies showed that PCNA1 interacts with Fen1; PCNA2 interacts with PolB1 and PCNA3 interacts with DNA ligase I and uracil DNA glycosylase.[11,12] The molecular basis of the specificity of PCNA in its interaction with different binding proteins remains elusive.

In attempts to understand the molecular mechanism of PCNA in DNA replication and repair, structural analyses on PCNAs from *S. solfataricus* have been carried out by several research laboratories. Two PCNA heterotrimer structures [13,14] and one PCNA12 dimer–Fen1 complex structure[15] have been reported. However, to the best of our knowledge, neither PCNA12 dimer nor PCNA3 monomer crystal structure have been available up to date. Here, we report the purification, crystallization, and preliminary X-ray diffraction analysis of the PCNA3 monomer, PCNA12 heterodimer, and PCNA123 heterotrimer. The trimeric crystal reported here is in a different crystal form from the previously reported structural analyses.[13,14] Three copies of the heterotrimers were identified in the asymmetric unit, which will provide three crystallographically independent structures of the trimer.

The plasmids (pET33-P1, pET30-P3) containing DNA fragments encoding PCNA1 (SSO0397) and PCNA3 (SSO0405) were kind gifts from Dr. S. Bell (University of Cambridge, U.K.). PCNA2 (SSO1047) was cloned into pET11a at the NdeI and *Bam*HI sites, which resulted in the plasmid pET11a-P2. Appropriate restriction nuclease sites were incorporated into the primers used in PCR. The plasmid pET33-P1 contains the full-length PCNA1 with the second amino acid residue changed from phenylalanine to valine because of the NcoI site used in the cloning procedure (249 amino acids, 27.5 kD). pET11a-P2 and pET30-P3 encode full-length PCNA2 (246 amino acids, 27.6 kD) and PCNA3 (244 amino acids, 27.5 kD), respectively. The plasmids were sequenced to confirm the correct sequences of the ORFs for all the three PCNA proteins.

We expressed all three PCNAs in *Escherichia coli (E. coli).* The *E. coli* strain BL21 (DE3) was used for recombinant PCNA overexpression. Same expression procedure was used to overexpress PCNA1 and PCNA3 at yield levels of 10–20 mg of targeted proteins per 1 L of cell culture. A single colony was picked from the plate of transformed BL21 (DE) cells harboring the recombinant plasmid into 25 mL of LB medium supplemented with 50 μg/mL kanamycin in 250 mL flasks. The cells were cultured at 37 °C with shaking in LB at 200 rpm overnight. The overnight cultures were then transferred into 1 L of fresh LB with 50 μg/mL kanamycin and grown at 37 °C till OD_{600nm} reached 0.8. The protein expression was then induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 4 h at

[†] Part of the special issue (Vol 7, issue 11) on the 11th International Conference on the Crystallization of Biological Macromolecules, Quebec, Canada, August 16–21, 2006 (preconference August 13–16, 2006).

* Corresponding author. Tel: (519) 661 3557. Fax: (519) 663 3175. E-mail: hling4@uwo.ca.

[‡] Present address: Institute of Molecular Biology, Slovak Academy of Sciences, Dubravska cesta 21, Bratislava, 84551, Slovakia.

10.1021/cg700961b CCC: $37.00
Published on Web 10/23/2007

37 °C before harvesting. The cell culture was centrifuged at 6500 g for 15 min at 4 °C. The Cell pellet was washed with cell lysis buffer (20 mM Tris HCl pH 7.5, 150 mM NaCl, 1 mM EDTA) and stored at −80 °C. PCNA2 was overexpressed by inoculating 1 L of LB medium containing 100 mg/mL ampicillin with a single colony and shaking at 200 rpm at 37 °C overnight without IPTG induction.

Selenomethionine (SeMet)-substituted PCNA1 (SeMet-PCNA1) and PCNA2 (SeMet-PCNA2) were prepared by a metabolic inhibition method.[16] Briefly, 25 mL of overnight starter culture was prepared as described above. The cells from the overnight culture were pelleted by centrifuging in 50 mL Falcon tubes at 3000 g for 15 min at room temperature. The cells were washed with 50 mL of M9 minimal medium, resuspended in 10 mL of M9 medium, and transferred into 1 L of M9 minimal medium with 100 mg/mL ampicillin (for expressing PCNA2) or 50 mg/mL kanamycin (for expressing PCNA1), supplemented with 1% thiamine and 0.4% glucose. Cells were incubated at 37 °C with shaking at 200 rpm till OD_{600nm} was about 0.45. Next, 50 mg of L-selenomethionine (Sigma) along with 100 mg of lysine, phenylalanine, threonine, isoleucine, leucine, and valine were added as solid chemicals to the culture. After 45 min, IPTG was added to a final concentration of 0.5 mM and the temperature was lowered to 22 °C for overnight induction of SeMet-PCNA1 or SeMet-PCNA2 protein expression. Cells were harvested and stored as described above.

We purified the proteins by heat treatment and liquid chromatography. Protein purification was done using a FPLC (GE Healthcare) at 4 °C. PCNA1, 2, and 3 are acidic proteins with predicted pI values of 5.17, 4.36, and 4.61, respectively. The cell paste from a half-liter culture was resuspended in buffer A (20 mM Tris HCl pH 7.5, 1 mM EDTA, 1 mM DTT, and 50 mM NaCl). One millimolar PMSF, 1 mM Benzamidine, 0.3 μM Leupeptin, and 5 μM Aprotinin were added before cells were lysed by three passes through a French pressure cell at 20000 psi. The PCNA1, PCNA2, and PCNA3 cell lysates were heated at 55, 70, and 75 °C, respectively, for 30 min to denature host proteins. Cell debris and denatured proteins were removed by centrifugation at 30000 g for 45 min. The supernatant was loaded onto a 5 mL HiTrapQ HP column (GE Healthcare) pre-equilibrated with buffer A. The column was then washed with 10 column volumes of buffer A. PCNA proteins were eluted from the column with a linear gradient of 50–500 mM NaCl over 12 column volumes. After heat treatment and the first ion-exchange step, significant contamination of DNA was observed when the $A_{280\ nm}/A_{260\ nm}$ ratio was measured. The Heparin affinity column was used to remove DNA contaminant from PCNA1. Fractions containing PCNA1 were pooled and applied to a 5 mL HiTrap Heparin column (GE Healthcare) pre-equilibrated with buffer B (50 mM NaCl, 20 mM HEPES pH 7.0, 0.1 mM EDTA, 1 mM DTT, 2.5% glycerol). After being washed with 10 column volumes, PCNA was eluted with a linear gradient of 50–500 mM NaCl. However, PCNA2 and PCNA3 do not bind the Heparin column. DNA contaminants with PCNA2 and PCNA3 were removed by a second pass through the HiTrap Q column. Pooled fractions containing PCNA2 or PCNA3 were diluted to a salt concentration similar to that of buffer A; a HiTrapQ ion-exchange chromatography step was repeated to remove the remaining DNA. The samples were concentrated to about 30 mg/mL using a Vivaspin 15R concentrator (Vivascience) with a 10 kD molecular weight cutoff (MWCO) and stored at 4 °C. PCNA1 and 2 form a very stable dimer, and PCNA3 binds to the PCNA12 dimer to form a heterotrimer.[11] A size exclusion column was used to further purify the proteins and protein–protein complexes. The PCNA12 dimer was purified by mixing concentrated PCNA1 and PCNA2 in an approximately equal molar ratio and incubated on ice for 30 min. The mixture was then passed through a Superdex 200 10/300GL gel filtration column (GE Healthcare) equilibrated in 10 mM Tris HCl pH 8.0, 150 mM NaCl, 1 mM EDTA. PCNA123 heterotrimer complex was purified in the same way as the PCNA12 dimer after mixing equal molars of PCNA12 dimer with PCNA3. Although the binding affinity between the PCNA3 and PCNA12 dimer is much lower than that between PCNA1 and PCNA2,[11] both the heterodimer and heterotrimer are stable enough to be purified by size exclusion chromatography (Figure 1). All three PCNAs have similar sizes of 244–249 amino acids and molecular weights of about 27.5 kD. A PCNA3 sizing column profile (green) is shown in Figure 1 as a reference point to the dimer and the trimer. The purified complexes were concentrated to 30 mg/mL using a Vivaspin 15R concentrator (30 kD MWCO) and stored at 4 °C. SeMet-PCNA12 dimer was purified in the same way as native complexes except that 5 mM DTT was added to all the buffers during purification to prevent oxidation of selenomethionine residues in the recombinant proteins. Selenomethionine incorporation was checked to be greater than 95% by MALDI-TOF analysis. The homogeneity and identity of the purified individual PCNAs, PCNA12 heterodimer, and PCNA123 heterotrimer were assessed by SDS-PAGE and judged to be greater than 95% pure.

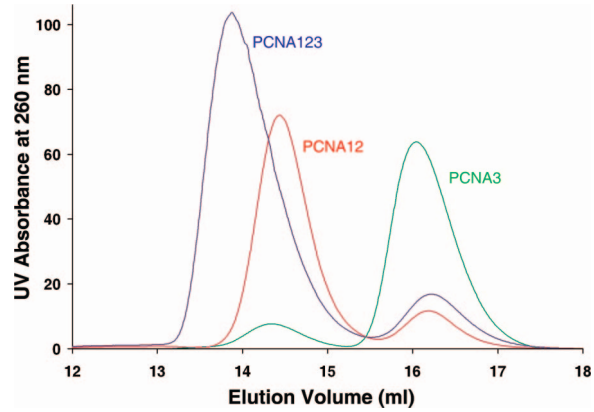


Figure 1. Elution profiles of the 28 kD monomeric PCNA3 (green), dimeric PCNA12 (red), and trimeric PCNA123 (blue) from a Superdex-200 size exclusion column in buffer of 10 mM Tris HCl pH 8.0, 150 mM NaCl, 1 mM EDTA.

All crystallization experiments were carried out using vapor diffusion techniques. Initial crystallization conditions were obtained by sparse-matrix screening with commercial crystal screening kits (Hampton Research, Emerald BioSystems) at 20 °C. Promising conditions were optimized by varying precipitant/protein concentration, buffering agents and pHs and additives with both hanging-drop and sitting-drop crystallization setups. The PCNA3 monomer and PCNA123 heterotrimer were crystallized in sitting-drops at 20 °C over 0.6 mL of well solution, and PCNA12 dimer was in hanging-drops. Initially, PCNA3 was crystallized with well solution containing 10% (w/v) PEG 3350 and 100 mM NH_4F. The condition was optimized using fine grid screening by varying the precipitant, salt, and protein concentrations. Curiously, the hanging-drop setup does not work for PCNA3 crystallization using the same well solution, attesting to the benefits of trying different setups when screening for protein crystallization conditions. One and a half microliters of PCNA3 at 10 mg/mL in 20 mM Tris-HCl pH 7.5, 100 mM

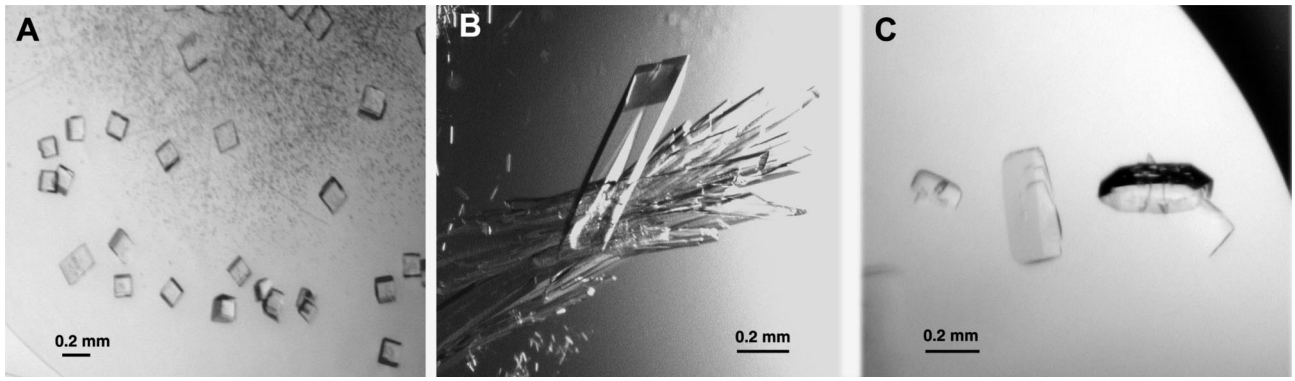


Figure 2. Crystals of (A) PCNA3, (B) PCNA12 dimer, and (C) PCNA123 heterotrimer.

Table 1. Data Collection Statistics and Preliminary Structure Solutions[a]

		PCNA12 dimer		
crystal	PCNA3 monomer	native	SeMet	PCNA123 trimer
space group	$P4_12_12$	$P2_12_12$	$P2_12_12$	$P2_12_12$
unit cell (Å) (*a*, *b*, *c*)	85.8, 85.8, 264.2	105.0,112.7, 101.9	105.0, 112.7, 101.8	148.1, 222.3, 80.2
wavelength (Å)	1.1000	0.9731	0.9791 (peak)	0.9195
beamline	NSLS- X12B	APS-8BM	APS-8BM	APS-8BM
resolution (Å)	30–1.9 (1.92–1.9)	30–2.6 (2.66–2.6)	30–3.3 (3.42–3.3)	30–2.5 (2.59–2.5)
R_{merge}[b]	0.110 (0.490)	0.076 (0.555)	0.093 (0.545)	0.126 (0.700)
no. of unique reflns	75802	37787	34749 (I^+/I^-)	88416
completeness (%) [b]	96.3 (65.6)	99.9 (99.8)	99.6 (100)	95.4 (93.6)
$I/\sigma I$	28.9 (2.2)	22.1 (1.8)	25.2 (1.8)mo)	18.5 (1.9)
redundancy	23.3 (5.8)	5.1 (4.2)	6.0 (4.0)	6.4 (6.3)
mosaicity (deg)	0.32	0.41	0.66	0.53
molecules per AU[c]	4	2	2	3
phasing methods	MR		SAD	MR

[a] Data in the highest-resolution shell are shown in parentheses. [b] $R_{merge} = \Sigma_h\Sigma_i |I_{hi} - \langle I_h \rangle|/\Sigma\langle I_h \rangle$, where I_{hi} is the intensity of the *i*th observation of reflection h, and $\langle I_h \rangle$ is the average intensity of redundant measurements of the h reflections. [c] AU stands for asymmetric unit.

NaCl was mixed with 1.5 μL of well solution (12.5% (w/v) PEG 3350, 0.15 M NH_4Cl). Crystals appeared after two days (Figure 2A). PCNA12 dimer was exchanged to a buffer containing 50 mM HEPES pH 7.0, 30 mM NaCl before crystallization drops were set up. PCNA12 dimer was crystallized with well solution of 30% (v/v) 2-methyl-2,4-pentanediol (MPD), 20 mM $CaCl_2$, and 100 mM sodium acetate, pH 4.6 first. Crystal quality was improved by changing pH and streak seeding. Native PCNA12 dimer crystals were obtained by mixing 2 μL of PCNA12 dimer at 10 mg/mL with 2 μL of well solution (25% (v/v) MPD, 20 mM $CaCl_2$, and 100 mM MES, pH 6.5). The crystals appeared in 1–2 days (Figure 2B). SeMet-PCNA12 dimer crystals were obtained under the same crystallization conditions by streak seeding with crushed native PCNA12 dimer crystals. Ten milligrams per milliliter PCNA123 heterotrimer in 10 mM Tris HCl pH 8.0, 150 mM NaCl, and 1 mM EDTA was used in the initial crystallization screening, and crystals were found with well solution containing 2.0 M ammonium sulfate, 100 mM HEPES pH 7.5, and 2% (v/v) PEG 400. Crystal grew to a bigger size when ammonium sulfate concentration was increased to 2.5 M, but crystals were thin layered and disintegrated after a few days. Glycerol (1.6%) was added into the heterotrimer complex buffer, and 1.5 μL of PCNA123 was mixed with 1.5 μL of the same well buffer in a sitting drop. Crystals appeared in 1–2 weeks (Figure 2C). The resultant heterotrimer crystals appeared to be a single crystal and were more stable and diffracted with better resolutions. The heterotrimer crystal diffraction was further improved by annealing once by blocking the liquid nitrogen stream about 15 s. The resolution was increased from 2.8 to 2.5 Å after annealing.

Diffraction data for PCNA3 were collected at Beamline X12B at the National Synchrotron Light Source (Brookhaven, NY). Diffraction data for native and selenomethionine substituted PCNA12 dimer, and PCNA123 trimer were collected at Beamline 8-BM of the Advanced Photon Source (Argonne, IL). PCNA3 crystals were picked up using a cryoloop (Hampton Research) and soaked briefly in the cryo-buffer (15% PEG 3350, 0.2 M NH_4Cl, 25% (v/v) ethylene glycol). Crystals were flash-cooled in liquid nitrogen prior to X-ray diffraction experiments. PCNA123 crystallization drops contain a high concentration of ammonium sulfate that forms crystals quickly when exposed to air. Malonate was reported to be a universal cryoprotectant and stabilizing solution for salt-grown macromolecular crytals[17] and was used as cryoprotectant for PCNA123 crystals. Twenty microliters of cryo-buffer (2.5 M sodium malonate, 0.5 M KBr) was added directly to the PCNA123 heterotrimer crystal drops. Potassium bromide was included as an attempt to collect data for SAD phasing. Crystals were then pulled out immediately using cryoloops and flash-cooled in liquid nitrogen. PCNA12 crystals were fished from the drop and directly flash-cooled in the stream of liquid nitrogen for the data collection because of the high concentration of MPD in crystallization solution. Diffraction data were collected at 100 K and images were processed with HKL2000 suite and DENZO/SCALEPACK.[18] The crystallographic parameters and data statistics are listed in Table 1. The structures have been solved by selenium SAD phasing and molecular replacement. We solved the PCNA3 structure by molecular replacement using the structure of PCNA from *Sulfolobus tokodai* (PDB ID 1UD9) as a search model, and refined to 1.9 Å resolution with R and R_{free} of 20.3 and 25.0%, respectively, using CNS.[19] The structure of the PCNA12 dimer was solved by the selenium single anomalous dispersion (SAD) phasing method using SHELXD.[20] The dimer and PCNA3 were constructed into a heterotrimer that was used as

a search model for PCNA123 trimer structure determination. The PCNA123 trimer structure was solved by molecular replacement using PHASER.[21] Two PCNA12 dimers and three PCNA123 trimers were identified in the asymmetric units (Table 1). The dimer and trimer structures are in final refinement with R_{free} values currently below 30% at 2.6 and 2.5 Å resolution, respectively. The structural details will be reported in another paper.

Acknowledgment. We thank S. Bell for the plasmids pET33-P1 and pET30-P3, Y. Zhang for diffraction tests on PCNA3 crystals, A. Soares for data collection of PCNA3, and I. Kourinov for synchrotron beamline support.

References

(1) Moldovan, G. L.; Pfander, B.; Jentsch, S. PCNA, the maestro of the replication fork. *Cell* **2007**, *129* (4), 665–79.
(2) Maga, G.; Hubscher, U. Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *J Cell Sci.* **2003**, *116* (Pt 15), 3051–60.
(3) Tsurimoto, T. PCNA, a multifunctional ring on DNA. *Biochim. Biophys. Acta* **1998**, *1443* (1–2), 23–39.
(4) Hingorani, M. M.; O'Donnell, M. Sliding clamps: a (tail)ored fit. *Curr. Biol.* **2000**, *10* (1), R25–9.
(5) Krishna, T. S.; Kong, X. P.; Gary, S.; Burgers, P. M.; Kuriyan, J. Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell* **1994**, *79* (7), 1233–43.
(6) Matsumiya, S.; Ishino, Y.; Morikawa, K. Crystal structure of an archaeal DNA sliding clamp: proliferating cell nuclear antigen from Pyrococcus furiosus. *Protein Sci.* **2001**, *10* (1), 17–23.
(7) Tsurimoto, T. PCNA binding proteins. *Front. Biosci.* **1999**, *4*, D849–58.
(8) Gomes, X. V.; Burgers, P. M. Two modes of FEN1 binding to PCNA regulated by DNA. *EMBO J.* **2000**, *19* (14), 3811–21.
(9) Gulbis, J. M.; Kelman, Z.; Hurwitz, J.; O'Donnell, M.; Kuriyan, J. Structure of the C-terminal region of p21(WAF1/CIP1) complexed with human PCNA. *Cell* **1996**, *87* (2), 297–306.
(10) Warbrick, E. The puzzle of PCNA's many partners. *Bioessays* **2000**, *22* (11), 997–1006.
(11) Dionne, I.; Nookala, R. K.; Jackson, S. P.; Doherty, A. J.; Bell, S. D. A heterotrimeric PCNA in the hyperthermophilic archaeon Sulfolobus solfataricus. *Mol. Cell* **2003**, *11* (1), 275–82.
(12) Dionne, I.; Bell, S. D. Characterization of an archaeal family 4 uracil DNA glycosylase and its interaction with PCNA and chromatin proteins. *Biochem. J.* **2005**, *387* (Pt 3), 859–63.
(13) Williams, G. J.; Johnson, K.; Rudolf, J.; McMahon, S. A.; Carter, L.; Oke, M.; Liu, H.; Taylor, G. L.; White, M. F.; Naismith, J. H. Structure of the heterotrimeric PCNA from Sulfolobus solfataricus. *Acta Crystallogr., Sect. F* **2006**, *62* (Pt 10), 944–8.
(14) Pascal, J. M.; Tsodikov, O. V.; Hura, G. L.; Song, W.; Cotner, E. A.; Classen, S.; Tomkinson, A. E.; Tainer, J. A.; Ellenberger, T. A flexible interface between DNA ligase and PCNA supports conformational switching and efficient ligation of DNA. *Mol. Cell* **2006**, *24* (2), 279–91.
(15) Dore, A. S.; Kilkenny, M. L.; Jones, S. A.; Oliver, A. W.; Roe, S. M.; Bell, S. D.; Pearl, L. H., Structure of an archaeal PCNA1-PCNA2-FEN1 complex: elucidating PCNA subunit and client enzyme specificity. *Nucleic Acids Res.* **2006**.
(16) Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin. *J. Mol. Biol.* **1993**, *229* (1), 105–24.
(17) Holyoak, T.; Fenn, T. D.; Wilson, M. A.; Moulin, A. G.; Ringe, D.; Petsko, G. A. Malonate: a versatile cryoprotectant and stabilizing solution for salt-grown macromolecular crystals. *Acta Crystallogr., Sect. D* **2003**, *59* (Pt 12), 2356–8.
(18) Otwinowski, Z., Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. In *Methods in Enzymology*; Carter, C. W., Jr., Sweet, R. M., Eds.; Academic Press: New York, 1997; Vol *276*, pp 307–326.
(19) Brünger, A. T.; Adams, P. D.; Clore, G. M.; Delane, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuszewski, J.; Nilges, M.; Pannu, N. S. Crystallography and NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr., Sect. D* **1998**, *54*, 905–921.
(20) Schneider, T. R.; Sheldrick, G. M. Substructure solution with SHELXD. *Acta Crystallogr., Sect. D* **2002**, *58* (Pt 10 Pt 2), 1772–9.
(21) McCoy, A. J.; Grosse-Kunstleve, R. W.; Storoni, L. C.; Read, R. J. Likelihood-enhanced fast translation functions. *Acta Crystallogr., Sect. D* **2005**, *61* (Pt 4), 458–64.

CG700961B