Solution Structure of an “open” *E. coli* Pol III Clamp Loader Sliding Clamp Complex

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ABSTRACT

Sliding clamps are opened and loaded onto primer template junctions by clamp loaders, and once loaded on DNA, confer processivity to replicative polymerases. Previously determined crystal structures of eukaryotic and T4 clamp loader-clamp complexes have captured the sliding clamps in either closed or only partially open interface conformations. In these solution structure studies, we have captured for the first time the clamp loader-sliding clamp complex from *Escherichia coli* using size exclusion chromatography coupled to small angle X-ray scattering (SEC-SAXS). The data suggests the sliding clamp is in an open conformation which is wide enough to permit duplex DNA binding. The data also provides information about spatial arrangement of the sliding clamp with respect to the clamp loader subunits and is compared to complex crystal structures determined from other organisms.

Keywords: DNA replication, AAA+ ATPase, Clamp loader, Sliding Clamp, SAXS

1. Introduction

During DNA synthesis and replication the daughter strands are copied at a fast rate and in an accurate manner by DNA polymerases (Benkovic et al., 2001; Kong et al., 1992; Onrust et al., 1991; Pomerantz and O'Donnell, 2007; Stukenberg et al., 1991). The accuracy is an inherent property of DNA polymerases but the processivity (up to 1000 nts in a single binding event) is conferred through binding with sliding clamps (Shamoo and Steitz, 1999; Kong et al., 1992; Onrust et al., 1991; Stukenberg et al., 1991). The sliding clamps anchor and topologically link the polymerases to the DNA and prevent premature dissociation. A sliding clamp is loaded onto the primer template junction on the DNA lagging strand every 2-3 seconds in *E. coli* (Wu et al., 1992; Moolman et al., 2014).

Because of the inherent closed ring nature of the majority of uncomplexed sliding clamps, a multi-subunit macromolecule referred to as clamp loader is required for loading of the clamp. Clamp loaders are members of family of ATPase associated with various cellular activities (AAA+) which use ATP binding and hydrolysis to bind, open and load sliding clamps in the correct orientation onto primer template junctions (Erzberger and Berger, 2006; Neuwald et al., 1999). In contrast to other members of AAA+ family of ATPases, clamp loaders are heteropentameric. The absence of a sixth subunit provides a gap for binding of DNA/RNA primer template substrates (Bowman et al., 2004; Simonetta et al., 2009). ATP binding increases the affinity of the clamp loader for binding the sliding clamp through induction of conformational changes. These conformational changes are necessary for interaction of the loader with the clamp as well as with the primer template DNA (Bowman et al., 2004; Jeruzalmi et al., 2001b;

49 Simonetta et al., 2009; Hingorani and O'Donnell, 1998). Once the sliding clamp is
50 loaded onto the DNA, the clamp loader releases and closes the sliding clamp around
51 the DNA. These steps are coupled to ATP hydrolysis and dissociation of ADP from the
52 clamp loader (Jarvis et al., 1989; Turner et al., 1999). Once the sliding clamp is
53 released the polymerase binds the clamp and carries out DNA replication.

54 Previously, the X-ray crystal structure of the eukaryotic pentameric clamp loader,
55 replication factor C (RFC), bound to a closed homotrimeric sliding clamp referred to as
56 proliferating cellular nuclear antigen (PCNA) from *Saccharomyces cerevisiae* has been
57 determined (Bowman et al., 2004). Because ATP hydrolysis weakens the interaction of
58 sliding clamp and the clamp loader, a variant of the clamp loader complex (where the
59 arginine finger of the SRC motifs in RFC-B, RFC-C, RFC-D and RFC-E subunits were
60 mutated to glutamine) was used which resulted in the capture of the clamp loader-clamp
61 structure, but the PCNA clamp was in a closed conformation. The crystal structure
62 revealed a spiral arrangement of ATPase domains of clamp loader with the N-terminal
63 domains of the subunits interacting with the PCNA ring and DNA modeled within the
64 central hole of the PCNA and primer template binding channel of RFC also revealed
65 that the RFC spiral complemented the grooves of the modeled duplex DNA. This crystal
66 structure presented the “screw cap” interaction of RFC with primer template DNA.

67 Further understanding of the mechanism of clamp loading reaction has been gained
68 from the crystal structure of an ATP bound state of the bacteriophage T4 clamp loader
69 bound to the gp45 sliding clamp and primer-template DNA (Kelch et al., 2011). Previous
70 studies on the T4 sliding clamp has shown the clamp having an open conformation in
71 solution in the absence of the loader (Alley et al., 1999; Millar et al., 2004). However,

the X-ray crystal structure of the T4 clamp loader-clamp-DNA complex revealed a partially opened sliding clamp. The opening at the clamp interface was approximately 10 Å, not wide enough to thread duplex DNA. The spiral conformation of the clamp matched the spiral of the primer-template DNA as well as the clamp loader. This structure also revealed extensive interactions between all the five subunits N-terminal domains of the clamp loader with the bacteriophage gp45 sliding clamp.

In *E. coli*, the sliding clamp is referred to as the β sliding clamp and the clamp loader complex as either the γ or τ complex. The crystal structure of the β sliding clamp revealed the molecule to be a homodimer (with an outer and inner diameter of 80 and 40 Å, respectively). Each of the monomers (comprised of 366 residues) with head to tail interaction arrangement, that results in the β sliding clamp having two distinct faces (Kong et al., 1992). The β sliding clamp interacts with several protein partners during DNA replication and repair using one of these faces (which has several surface protruding loops). Although the X-ray crystal structure of the clamp was of a closed ring, solution studies of the clamp using hydrogen exchange mass spectroscopy have revealed that β clamp is not a static closed ring in solution but highly flexible (Fang et al., 2011). The same studies also suggested that intermediate conformations of the clamp might facilitate interactions with different protein partners.

The *E. coli* clamp loader complex is a multi-subunit macromolecule comprised of the seven subunits δ , δ' , ψ and χ and three protomers of the *dnaX* gene products (either γ or τ). It was demonstrated that the clamp loader with any combination of γ or τ subunits ($\gamma_2\tau$, $\gamma\tau_2$, γ_3 or τ_3) were equally active in loading the clamp onto DNA (McInerney et al., 2007). Several X-ray crystal structures of the γ clamp loader have been determined but

the $\chi\psi$ subunits, which are required for bridging the interaction of single stranded DNA binding protein (SSB) with the clamp loader, were absent (Jeruzalmi et al., 2001b; Kazmirski et al., 2004; Simonetta et al., 2009). The omission of $\chi\psi$ subunits was because of the inherent flexibility of the ψ subunit 27 N-terminal residues that anchor the $\chi\psi$ heterodimer to the core five subunit (Glover and McHenry, 1998). The ψ subunit interacts with the C-terminal region of γ subunit (Gao and McHenry 2001). These two subunits together are essential for bridging the interaction between the clamp loader and SSB in *E. coli* (Glover and McHenry 1998). The ψ subunit plays a role in stabilizing the conformational changes induced by ATP binding and the χ subunit directly interacts with the C terminus of SSB (8 amino acid residues). The $\chi\psi$ complex also plays a role in increasing the affinity of τ and γ for $\delta.\delta'$ to a physiologically relevant range and stabilization of the complex (Olson et al., 1995). In a recent study on the solution structure of the γ clamp loader, the location of $\chi\psi$ was revealed (Tondnevis et al., 2015). The $\chi\psi$ heterodimer interacts with the C-terminal collar region of the loader and was positioned opposite of the ssDNA exit channel on δ subunit and closer to δ' .

To date there is no X-ray crystal or cryo-electron microscopy (cryo-EM) structure of any clamp loader-clamp complex in which the sliding clamp has been shown to be open wide enough at the interface for the binding of duplex DNA. In these studies, we have captured a solution structure of the *E. coli* β sliding clamp loader-clamp complex using small angle X-ray scattering coupled to size exclusion chromatography (SEC-SAXS). Rigid body modeling of the clamp loader-clamp to fit the 1D-scattering data shows the topological arrangement of the β sliding clamp with respect to the γ clamp loader. The clamp makes interactions with the N-terminal domains of all the core subunits. This

arrangement is similar to that seen in the RFC-PCNA crystal structure, bacteriophage T4 clamp loader-clamp-DNA complex structure and the archaeal *Pyrococcus furiosus* (*Pfu*) clamp loader-clamp-DNA ternary structure previously determined using X-ray crystallography and cryo-EM (Bowman et al., 2004, Kelch et al., 2011, Miyata et al., 2005). Our model also suggests that the clamp exists in an open conformation in complex with the clamp loader and has an out of plane opening wide enough (~ 25 Å) for the binding of duplex DNA.

2. Materials and methods

Protein preparation and purification

E. coli β sliding clamp was purified as described previously with minor modifications (Anderson et al., 2009; Johanson et al., 1986). The stock concentration for β sliding clamp was 90 μ M (7.4 mg/ml) for the dimer. The protein was > 95% pure as measured by SDS-PAGE gel. The storage buffer was 20 mM Tris-HCl at pH 7.5, 0.5 mM EDTA, 5 mM DTT, 10 % glycerol. For expression of the γ clamp loader, *E. coli* BL21 (DE3) cells were transformed with pCOLADuet-1 and pETDuet-1 vectors. The plasmids were generous gifts from Linda Bloom & Lauren Douma (University of Florida. Department of Biochemistry and Molecular Biology). The pCOLADuet-1 vector contained the coding sequence for δ subunit as well as γ subunit and the pETDuet-1 vector contained the coding sequence for the δ' as well as $\psi\chi$ subunits (ψ and χ were part of a single operon). Transformation colonies were used to grow 20 ml of starter culture containing ampicillin and kanamycin as selective markers. The starter culture was grown to optimum density and centrifuged and the pellet was re-suspended in fresh media. For large-scale expression of the γ clamp loader, 2L of LB media supplemented with ampicillin and

141 kanamycin (100 µg/ml and 50 µg/ml) was inoculated with *E. coli* starter culture. The
142 culture was incubated at 37 °C with shaking until O.D.₆₀₀ reached 0.6. Protein
143 expression was induced using IPTG to a final concentration of 1 mM followed by an
144 additional 3-hour incubation of the culture at 37 °C with shaking. The culture was then
145 centrifuged at 5000 x g at 4 °C for 30 minutes. Pellets were then frozen at -80 °C.

146 For purification of γ clamp loader, thawed frozen pellets were re-suspended in re-
147 suspension buffer containing 20 mM Tris-HCl at pH 7.5, 0.5 mM EDTA, 2 mM DTT, 50
148 mM NaCl and 10 % glycerol by stirring. Cells were lysed using French pressure cell.
149 Cells were passed through the French press 3 times on high pressure (1000 psi). Lysed
150 cells were then centrifuges at 18000 RFC using a JA 20 rotor (Beckman) at 4 °C for 1
151 hour. The cleared lysate was collected and loaded onto a regenerated 2x5 ml HiTrap
152 Heparin column (GE Healthcare). The unbound proteins were washed away using the
153 loading buffer (same as re-suspension) until the UV absorbance was stabilized. The
154 protein was eluted using a linear gradient (50-1000 mM NaCl). The fractions containing
155 clamp loader were pooled and dialyzed against 2L of dialysis buffer, which contained 20
156 mM Tris-HCl at pH 7.5, 0.5 mM EDTA, 2mM DTT, 100 mM NaCl and 10% glycerol. For
157 purifying the protein further, the dialyzed protein was loaded onto a regenerated 8ml
158 Mono Q column (Pharmacia). The unbound proteins were washed away using 40 ml of
159 loading buffer (same as dialysis buffer). The clamp loader was eluted using a linear
160 gradient (100-1000 mM NaCl). Clamp loader eluted around 450 mM NaCl. The peak
161 fractions were ran on a 12 % SDS-PAGE gel and the fractions containing pure clamp
162 loader were pooled and dialyzed into storage buffer containing 20 mM Tris-HCl, 50 mM
163 NaCl, 2 mM DTT and 10 % glycerol. Concentrations of γ complex was determined by

measuring the absorbance at 280 nm in 6M guanidine hydrochloride and using the calculated extinction coefficient ($220,050 \text{ m}^{-1} \text{ cm}^{-1}$) (Gill and von Hippel 1989). The sliding clamp concentration was determined using Bradford assay with beta dilutions as standards instead of bovin serum albumin (BSA).

Small angle X-ray scattering data collection

For the solution structural characterization of the β sliding clamp, 100 μl of sample (7.4 mg/ml) was loaded onto a 2.4 ml Superdex 200 PC3.2 size exclusion column through use of sample loop. The column had previously been equilibrated with 5 column volumes (CV) of running buffer containing 20 mM Tris-HCl at pH 7.5, 0.5 mM EDTA, 5 mM DTT and 4% glycerol. For SAXS analysis of the γ clamp, the column was regenerated using 5CV of buffer containing 20 mM Tris-HCl at pH 7.5, 0.5 mM EDTA, 4% glycerol, 5 mM DTT, 100 mM NaCl, 500 μM ATP and 8 mM MgCl_2 . The clamp loader was mixed with ATP (final concentration of 500 μM) and 100 μl of sample (11 mg/ml) was loaded onto the column using the sample loop. For obtaining a structure of the clamp loader-clamp complex the two proteins were mixed using a 1:1 molar ratio to the final concentration of 30 μM . The column was regenerated and run in a buffer containing 20 mM Tris-HCl at pH 7.5, 0.5 mM EDTA, 4% glycerol, 5 mM DTT, 200 mM NaCl, 500 μM ATP, 8 mM MgCl_2 . All running buffers were degassed prior to column regeneration.

SAXS data was collected at the Bio-SAXS beam line BL4-2 at Stanford Synchrotron Radiation Lightsource (SSRL) (Smolsky et al., 2007). The beam size at the sample was defined to be 250 μm x 300 μm (vertical x horizontal) resulting in a total photon flux of approximately 1×10^{12} photons/s. All data were collected using a Rayonix MX225-HE

CCD detector (Rayonix, Evanston, IL) with a 2.4 m sample-to-detector distance and a beam energy of 11 keV (wavelength, $\lambda = 1.127 \text{ \AA}$). Scattering data were collected within a q range of $0.005 \text{ \AA}^{-1} < q < 0.4 \text{ \AA}^{-1}$, where $q = 4\pi \sin(\theta)/\lambda$ is the momentum transfer and 2θ the scattering angle. The q scale was calibrated using the powder diffraction of silver behenate (Huang et al., 1993). For the SEC-SAXS experiment an AKTA Ettan FPLC (GE-Healthcare) was used. Solution eluting from the size exclusion column was redirected to the sample flow path for immediate SAXS data collection. Samples (50-100 μl) were loaded onto a 2.4 ml Superdex 200 PC 3.2 size exclusion column and run at a flow rate 0.05 ml/min. The first 100 images were scaled and averaged to create a buffer-scattering profile, and this was then subtracted from each of the subsequent images to produce the final scattering curve for each exposure. The exposure time was 1 second with a 2.5 s delay between each exposure. For each individual sample the scattering profiles over the elution peak were averaged and used for further analysis. The data acquisition program Blu-ICE (McPhillips et al., 2002) was employed for data collection. Program SASTOOL was used for data reduction, scaling and integration of scattering images after inspection for any variations potentially caused by radiation damage.

Software and modeling of SAXS data

The first 100 data frames collected during the SEC run were averaged and used as the buffer background for the subsequent scattering profiles of that run. The buffer subtracted data were then averaged in groups of five for improved statistics. For further analysis, data were chosen within the region of constant R_g values. The analyzed frames for γ clamp loader, β sliding clamp and γ - β clamp loader-clamp complex are

highlighted in supplemental figures (Figures S1, S2 and S3). The ATSAS suite of programs (version 2.5.2) from EMBL (European Molecular Biology Laboratory) in Hamburg was used for data processing. PRIMUS (Konarev et al., 2003) from the ATSAS suite was used to calculate the Guinier plots for calculation of zero angle

Table 1. Data-collection and scattering-derived parameters for SEC-SAXS

	γ clamp loader	β sliding clamp	β - γ complex
Data-collection parameters			
Instrument*	SSRL BL4-2		
Beam geometry*	250 μm $\times$ 300 μm		
Detector Distance (m)*	2.4		
Wavelength ($\text{\AA}$)*	1.127		
Energy (keV)*	11.0		
q range ($\text{\AA}^{-1}$)*	0.005-0.4		
Exposure time per frame (s)*	1.0		
Temperature (K)*	293		
SEC specifications			
Flow speed (ml/min)*	0.05		
Concentration (mg/ml)	12.0	7.4	5.0
Volume loaded (μl)	100.0	100.0	50.0
Structural parameters			
$R_g(\text{\AA})$ [from P(r)]	52.9 ± 0.2	33.2 ± 0.2	53.8 ± 0.2
$R_g(\text{\AA})$ [from Guinier]	51.6 ± 0.1	32.5 ± 0.1	53.1 ± 0.2
$R_g(\text{\AA})$ [from PDB]		33.9	
$D_{\text{max}}(\text{\AA})$ [from P(r)]	200.0	95.0	205.0
$D_{\text{max}}(\text{\AA})$ [from PDB] ¹	90.0		
MW (using the amino acid sequence)	250.0	80.0	330.0
MW (kDa) (using Porod Volume) ²	305.0	79.8	328.8
Software employed			
Primary data collection*	Blue-ICE		
Data scaling, averaging*	SASTOOL		
Rigid-body modeling*	COOT		
Computation of model intensities*	CRY SOL		
3D graphics representation*	CHIMERA, PyMOL		

* These parameters were the same for all samples

¹ the PDB ID for the clamp was 2POL

² the Porod volume was obtained using the AutoGnom function in Primusqt program

scattering $I(0)$ and the radius of gyration (R_g). GNOM was used to calculate the particle distance distribution $P(r)$ function (Svergun, 1992). The program COOT (Emsley and

Cowtan, 2004) was used for creation of structural models. The models were later used for generation of calculated SAXS profiles. Chimera (Pettersen et al., 2004) and Pymol molecular visualization programs (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC) were used for graphical presentation of structural models. CRY SOL was used for generation of calculate scattering profiles (Svergun et al., 1995).

3. Results

An initial size exclusion chromatography coupled to small angle X-ray scattering (SEC-SAXS) of the individual *E. coli* γ clamp loader and β sliding clamp were determined as baseline measurements to check that both the samples were monodispersed and to obtain the structures of their unbound states so as to compare with the γ - β complex form. Although both the clamp loader and sliding clamp are stable, the use of SEC-SAXS for data collection was to ensure the removal of all possible aggregates that have been known to exist from off-line size exclusion purification of the clamp loader. The data collection and scattering derived parameters are summarized in Table 1.

γ Clamp loader

The γ clamp loader data acquisition used 100 μ l of protein at a concentration of 11 mg/ml. The in-line size exclusion UV chromatogram is shown in Figure S1A and shows the separation of a minor fraction of aggregates (relative height of the peak) before the major peak fraction corresponding to the monodisperse sample. The SAXS parameter radius of gyration (R_g) and forward scattering intensity ($I(0)$) and the $I(q_{min})$, which is the intensity at minimum angle measured, of each exposure frame are shown in Figure S1B. The frames at the trailing edge of the major peak that were not influenced by the

presence of large aggregates were used for averaging and determination of SAXS parameters. These frames exhibited constant R_g values, indicating monodispersity as well as the absence of intermolecular interaction. The averaged and buffer subtracted 1D scattering curve for the γ clamp loader is shown (Figure 1A). The Guinier plot of the data exhibits excellent linearity in the low q region with a resulting radius of gyration of

Figure 1: SEC-SAXS of the seven subunit γ -clamp loader. A) Buffer subtracted 1D scattering curve. Shown are the average of frames 360-375 as highlighted in Figure S1. B) Guinier analysis. The R^2 value for the line was 0.998 and the residual linear fit in $R_g \cdot q < 1.3$ region is shown as an inset. C) Kratky plot. The plot has a bell shape peak at low momentum transfer (low q) expected of globular macromolecules. D) The pair wise distance distribution function $p(r)$. The bell shape function is expected of a protein that is mostly spherical. The $D_{\max}$ was 200 $\text{\AA}$.

285 $51.2 \pm 0.2 \text{ \AA}$ (Figure 1B). The Kratky plot exhibits a bell shaped peak at low momentum
286 transfer values and converges to the x-axis at higher q values characteristic of a
287 globular and well-folded protein (Rambo and Tainer, 2011) (Figure 1C). The $p(r)$
288 function reveals that γ clamp loader is mostly spherical in shape with an extended
289 domain corresponding to the extended $\chi\psi$ subunits (Figure 1D). The maximum ($D_{\max}$)
290 was calculated to be $200 \text{ \AA}$ slightly larger than $190 \text{ \AA}$ calculated from our rigid-body
291 model of the seven-subunit loader. For calculation of the $D_{\max}$ value for the seven
292 subunit clamp loader, the coordinates of the $\chi\psi$ subunits (PDB: 1EM8) (Gulbis et al.,
293 2004) were manually docked with the five subunit γ clamp loader (PDB: 3GLI)
294 (Simonetta et al., 2009) using the program COOT (Emsley and Cowtan, 2004) in
295 accordance to the recently determined SAXS *ab initio* 3D model of the seven subunit
296 clamp loader complex (Tondnevis et al., 2015) (Figure 2B).

297 For modeling of the SAXS data, program CRY SOL was used to compare the
298 experimental scattering data to the structural models of the γ clamp loader (Svergun et
299 al., 1995). Theoretical scattering curves were generated for two structural models
300 (Figure 2). For a given data set one can use χ value to rank different structural models in
301 which case the lower value represents the better model. Acceptable χ values are
302 typically somewhere close to unity. However the absolute value of χ is not a very useful
303 number as the value depends on the noise in each of the data points and for a noisy
304 data larger values can be acceptable. It should be noted that in principle it is not
305 possible to use the absolute χ value to compare the quality of different models to
306 different data sets.

The first model used the coordinates (PDB ID: 3GLI) for the five-subunit ($\gamma_3\delta\delta'$) clamp loader with removal of the DNA and ψ peptide (Simonetta et al., 2009). This structure was determined in the presence of the ψ peptide, where the peptide was interacting with

Figure 2: Modeling of the γ -clamp loader. The program CRYSOLOG was used for generating 1D scattering profile of atomic models shown in each panel. The subunits of the atomic models of the clamp loader in panels A and B are color coded as followed: δ' yellow, 3γ subunits in shades of blue, δ red. The $\chi\psi$ subunits in panel B are shown in cyan and deep teal, respectively. The black curves in panel A and B are the experimental SAXS data and the red curves are the calculated scattering curves for the atomic models in each panel. A) Fitting of calculated scattering profile for the δ , δ' , 3γ clamp loader to the experimental data. B) Fitting of the calculated scattering profile for the seven subunit clamp loader complex with $\chi\psi$ subunits positioned close to δ' based on previous *ab initio* model of the clamp loader.

the C-terminal collar region of the γ subunits. The second structural model used was the seven-subunit complex ($\gamma_3\delta\delta'\psi\chi$) described in Tondnevis et al., 2015 (PDB: 1EM8 for $\psi\chi$ subunits, Gulbis et al., 2004)). In figure 2, the red curves are calculated using the two models and the black curves are the experimental data. When the calculated scattering profile for the five-subunit complex was compared to the data, the χ value was 7.2 and calculated R_g value was 42.5 Å as compared to experimental value of 51.2 ± 0.2 Å

(Figure 2A). Whereas, the scattering profile calculated for the seven-subunit complex (Tondnevis et al., 2015) was a better fit to the 1D scattering data. The χ value was decreased to 2.4 and there was a close agreement between theoretical R_g value of 51.3 and experimental value of 51.2 ± 0.2 Å (Figure 2B). It should be noted that the fit of the structural model to the experimental data in figure 2B is not unique. The proposed model is overall consistent with the data but there are some discrepancies between the calculated curve based on the presented model and the data at higher q values. This discrepancy can be explained by possible intra and inter domain movements of the clamp loader subunits in solution.

β sliding clamp

For β sliding clamp data acquisition 100 μ l of sample at a concentration of 7.2 mg/ml was used. The in-line size exclusion UV chromatogram is shown (Figure S2A). The SAXS parameters radius of gyration (R_g), forward scattering intensity ($I(0)$), the minimum intensity value ($I(q_{min})$) of each exposure frame, and the images chosen for averaging and analysis for determination of SAXS overall parameters are shown (Figure S2B). The averaged and buffer subtracted 1D scattering curve is shown (Figure 3A). The Guinier analysis of the averaged 1D scattering curve is shown (Figure 3B) and exhibits excellent linearity up to $q.R_g < 1.3$, the generally accepted upper validity limit of the Guinier approximation for globular proteins. The R_g value calculated was 32.5 ± 0.1 Å. The Kratky representation of the scattering data has two characteristic peaks at low angles and approaches the baseline at higher angles (>0.2 Å⁻¹), confirming that the protein is well folded (Figure 3C). The distance distribution function curve shows the

349

350 **Figure 3:** SEC-SAXS of the β -sliding clamp. A) Buffer subtracted 1D scattering curve.
 351 Shown is the average of frames 380-390 as highlighted in Figure S2. B) Guinier
 352 analysis. The R^2 value for the line was 0.998. The inset is the residual linear fit in $R_g \cdot q$
 353 < 1.3 region. C) Kratky plot. The plot has a bell shape peak at low momentum transfer
 354 (low q) expected of globular macromolecules followed by convergence to x-axis. D) The
 355 pair wise distance distribution function $p(r)$. The curve shows bell shaped double peak
 356 as expected for a ring shaped protein with an inner and outer diameter. The $D_{\max}$ was
 357 95 $\text{\AA}$.

358 predicted characteristic double peak in the $p(r)$ curves for a ring-shaped molecule with
 359 the $D_{\max}$ value of 97 $\text{\AA}$ (Figure 3D). A scattering profile calculated from the coordinates
 360 for the closed β sliding clamp (PDB: 2POL, Kong et al., 1992)) matched the
 361 experimental 1D scattering data. The χ value of the fit was 1.0 and there was good
 362 agreement of the experimental R_g value of 32.5 ± 0.1 $\text{\AA}$ with the calculated value of 33.7

363 Å (Figure 4). To test the possibility that the sliding clamp may exist in an open
 364 conformation in solution, an open sliding clamp model was generated. The coordinates

365

366 **Figure 4:** Modeling of the β -sliding clamp. The program CRY SOL was used for
 367 generating 1D scattering profile of the dimeric open and closed β clamp. The
 368 experimental data is shown in black, the theoretical curve for the closed β clamp
 369 is shown in red. The χ of the fit was 1.0 and the cartoon representation of the X-ray crystal
 370 structure of the closed clamp is shown (PDB: 2POL). The two monomeric subunits
 371 are colored as cyan and magenta. The theoretical curve for the opened clamp with right
 372 hand spiral is shown in blue.

373 of the closed clamp with one monomer rotated about the hinge region of the interface to
 374 generate an out of plain opening of 45° . The χ value for the fit was 5.5, significantly
 375 higher than the χ value for the fit to the closed clamp. The calculated R_g value for the
 376 open clamp (34.1 Å) did not change significantly in comparison to that for the closed
 377 clamp. This data confirmed that the β sliding clamp is mainly in a closed dimeric state in

solution where both dimer interfaces are engaged. The closed conformation in solution, in the absence of the clamp loader, was expected because of the long (~2 hours) half-life of the closed dimeric β clamp (Yao et al., 1996). As a result they require the function of the γ clamp loader to be loaded onto the primer template junction.

γ - β Complex

To obtain a homogeneous solution of the *E. coli* β clamp loader-clamp complex, purified β sliding clamp and seven subunit γ clamp loader were mixed in a 1:1 molar ratio (final concentration of 30 μ M) in buffer containing 500 μ M ATP and 8 mM MgCl_2 . The β clamp loader-clamp complex was then separated from unbound β sliding clamp and γ clamp loader as well as aggregates immediately prior to data collection using in-line SEC (Figure S3A). The profile demonstrates successful separation of the complex peak as well smaller molecular weight peaks for uncomplexed β sliding clamp and γ clamp loader. The radius of gyration (R_g), forward scattering intensity ($I(0)$) and $I(q_{\min})$ of each exposure frame as well as the chosen frames with consisted R_g values for data analysis are shown (Figure S3B). The averaged 1D scattering curve of the β clamp loader-clamp complex is shown as the green curve in Figure 5A. The 1D scattering curve of the γ clamp loader alone (Figure 1A) is plotted in the same panel to highlight differences between the scattering profiles, which demonstrates the capture of the β clamp loader sliding clamp complex. The 1D scattering curve of the complex superimposes well onto the clamp loader 1D scattering profile at low q regions <0.05 but shows a significant curvature in q region between 0.075 - $0.13 \text{ \AA}^{-1}$ that is absent in the γ clamp loader. This curvature corresponds to the presence of the β sliding clamp in

Figure 5: SEC-SAXS of the clamp loader-clamp complex. A) The buffer subtracted 1D scattering curve. Clamp loader-clamp complex (black) and the γ -clamp loader alone from Figure 1A (green). Shown is the average of frames 340-360 as highlighted in Figure S3. B) Guinier analysis. The R^2 value was 0.999. The inset is the residual linear fit in $R_g \cdot q < 1.3$ region. C) Kratky plot. The plot has a bell shape peak at low momentum transfer (low q) expected of globular macromolecules followed by convergence to x-axis at higher q . D) The pair wise distance distribution function $p(r)$. The clamp loader-clamp complex (black) and the clamp loader alone from Figure 1B (green). The curves show bell shaped $p(r)$ functions expected of a protein that is mostly spherical. Both $p(r)$ functions have extended domains corresponding to $\psi\chi$ heterodimeric subunits.

the complex. The linearity of the Guinier plot of the clamp loader-clamp complex (Figure 5B) in the $qR_g < 1.3$ regions is in good agreement with the monodispersity of the

414 complex sample. The R_g value was calculated to be $53.1 \pm 0.2 \text{ \AA}$. The R_g value of the
415 complex is $\sim 2 \text{ \AA}$ larger than the γ clamp loader. This small but significant difference in
416 R_g values suggests that there is a tight interaction in β clamp loader-sliding clamp
417 complex. The Kratky plot shows a typical bell shaped curve at low scattering angles as
418 expected for a globular folded proteins with convergence to the x-axis at higher
419 scattering angles (Figure 5C). The $p(r)$ curve of the complex is similar to that of the γ
420 clamp loader with an extended $D_{\max}$ of $200 \text{ \AA}$ (Figure 5D). But the maximum peak value
421 of the $p(r)$ curve for the complex is shifted to $65.5 \text{ \AA}$ as compared to the γ clamp loader
422 maximum value of $60.0 \text{ \AA}$ (Figure 1D). As the β clamp loader molecular weight is 250
423 kDa and binding of the sliding clamp increases the size to 330 kDa . The difference in
424 $p(r)$ curve maxima is expected because of formation of the complex, but the similarity of
425 $p(r)$ curves indicate that there is no dramatic change in shape of the γ clamp loader
426 once it binds the β sliding clamp. The small change in R_g value between the γ clamp
427 loader and complex is also supportive of this observation.

428 Rigid-body modeling was used to model the conformation of the complex. The atomic
429 structures and their corresponding calculated scattering profiles are shown in Figure 6.
430 The program CRY SOL was used for the generation of calculated scattering profiles of
431 crystal structures. In addition to the differences in scattering profiles for the clamp loader
432 –clamp complex shown in Figure 5A, we were interested in rigid body modeling of the β
433 sliding clamp γ clamp loader complex SAXS data using previously determined crystal
434 structures. As the differences in R_g and the $p(r)$ function for the γ clamp loader alone
435 and the complex were relatively small, we wanted to determine whether a scattering
436 profile calculated from the atomic coordinates of the γ clamp loader could adequately fit

Figure 6: Modeling of the γ - β clamp loader-clamp complex. The experimental SAXS data is shown in black and the theoretical scattering profiles are shown in red. A) Fitting of the seven subunit clamp loader calculated scattering profile to the experimental data. The color coding of the model is as followed: red δ subunit, shades of blue 3γ subunits, yellow δ' subunit and $\chi\psi$ are shown in cyan and teal. B) Fitting of the clamp loader-closed clamp complex calculated scattering profile to the experimental data. The sliding clamp monomers are presented in light and dark green in the model. The color coding of the models in C and D are the same as in panel B. C) Fitting of the clamp loader-open clamp calculated scattering profile to the experimental data. The opening of the clamp was modeled by an out of plane movement of one of the monomers about the hinge region so the clamp can have a 15 Å opening at the interface. D) Fitting of the clamp loader-open clamp complex calculated scattering profile to the experimental data. In this model the opening of the clamp at the interface was increased to 30 Å.

the experimental 1D scattering data for the complex. The calculated scattering profile for the seven-subunit γ clamp loader to the experimental data is shown in Figure 6A.

453 The predicted curve for the γ clamp loader alone exhibits discrepancies to the
454 experimental data at various angles. The χ of fit was 2.0 and the theoretical R_g for the
455 model was 48.8 Å compared to the experimental value of 53.1 ± 0.2 Å. Since this fit was
456 not adequate to explain the 1D scatter, an atomic model of the β sliding clamp and γ
457 clamp loader in a complex was generated (Figure 6B). The model is based on
458 biochemical and structural data that showed the N-terminal domains of the β sliding
459 clamp subunits bind to one face of the γ clamp loader with the clamp central cavity
460 aligning with the DNA binding channel. The calculated scattering profile of this model
461 showed a much improved fit to the experimental data with a χ value of 1.3 and a
462 theoretical R_g value of 52.8 Å.

463 The γ clamp loader, once bound to ATP, is able to induce conformational changes onto
464 the β sliding clamp at one interface to open its ring structure. Once open, the γ clamp
465 loader is loaded onto a primer template junction where the function of the β sliding
466 clamp is required either for DNA synthesis or recruitment of various other proteins
467 involved in DNA repair. Given that saturating concentrations of ATP were present in our
468 SAXS samples, in these experiments the clamp loader-clamp complex should exist in
469 an open conformation. One important outstanding question about the mechanism of
470 clamp loading is how far the clamp loader has to open the clamp to place it on DNA.
471 The opening in the clamp interface was only 9 Å in the previously determined T4 clamp
472 loader-clamp-DNA ternary structure. The authors of this study then speculated that the
473 clamp loader threads the clamp on ssDNA with the duplex DNA sliding into the interior
474 chamber of the clamp loader through the central pore of the sliding clamp. The second

speculation was that a fully-opened clamp conformation that would facilitate loading on double-stranded DNA was not captured (Kelch et al., 2011).

Hence in this study, the open β sliding clamp was modeled to have a right handed spiral to match the geometry of the helical DNA, by an out of plane rotation about one of the interfaces creating a 15 Å opening at the other interface (Figure 6C). This remodeling of an open β sliding clamp improved the fit of the calculated scattering profile to the experimental data at higher q regions ($q > 0.2$) compared to the fit of the scattering profile calculated for the closed clamp loader-clamp complex (Figure 6B). Also the calculated R_g value of 53.8 Å was in good agreement with the experimental value of 53.1 ± 0.2 Å, but the 15 Å opening of the clamp is not wide enough for binding duplex DNA. Hence the open β sliding clamp model was adjusted with an open interface of 30 Å, which is sufficient for binding duplex DNA. The fit of this model calculated scattering profile to the experimental data is shown (Figure 6D) and fits the experimental data better than either the closed clamp loader-clamp complex or the clamp loader-open clamp complex with 15 Å opening at the interface. The χ value for the fit was 1.1 and there was improved agreement with the experimental data over all q angles. Based on this observation, additional rigid body modeling of the open β sliding clamp γ clamp loader complex were performed with β sliding clamp openings of 10, 15, 20, 25, 30 and 45 Å at the interface. The χ value of the fits were 1.8, 1.6, 1.4, 1.2, 1.1 and 1.7 respectively, hence the models where the clamp had an opening of 25 or 30 Å fitted the experimental data the best. When the clamp was modeled to have a larger opening of 45 Å, the calculated R_g value was also increased to 55.2 Å which is in disagreement with the experimental value of 53.1 ± 0.2 Å.

To answer the question whether the two auxiliary subunits χ and ψ undergo any movements upon binding of the sliding clamp to the loader, additional modeling of the clamp loader-clamp complex SAXS data were done. For this purpose a clamp loader-open clamp structural model was generated where the $\chi\psi$ subunits were moved to be positioned closer to the ssDNA exit channel on δ subunit. The rationale for this movement is that the χ subunit directly interacts with the C-terminal tail of the SSB and therefore expected to be in the vicinity of the ssDNA exit channel once the loader loads the sliding clamp onto primer template DNA. This model is shown in Figure S4. A calculated curve was generated for this model using the program CRY SOL and the curve was fitted onto the experimental scattering profile. The χ of the fit was at 1.6 and the theoretical R_g value was decreased to 50.5 Å. Although the χ value of the fit was increased and the R_g value was reduced, the movement of $\chi\psi$ subunits cannot be ruled out upon binding of the clamp as the presented model might not be an accurate portrayal of the location of $\chi\psi$. But the decrease in radius of gyration for this structural model, as compared to the experimental value, indicates a more extended orientation of χ and ψ .

4. Discussion

In these studies, we have captured for the first time, a solution structure of a prokaryotic clamp loader-sliding clamp complex from *E. coli* using SAXS-SEC. Prior to these studies, there was no X-ray crystal structure, cryo-EM or SAXS structure of a clamp loader-clamp complex where the clamp had wide enough opening for binding with duplex DNA. An important unanswered question is how does the clamp loader load the clamp onto DNA? If the clamp loader threads the sliding clamp on ssDNA, the clamp loader would only have to open the clamp wide enough to allow ssDNA to pass through

521 to the center, but would have to displace ssDNA binding protein (SSB). If the clamp
522 loader loads the sliding clamp directly on duplex DNA, the clamp loader has to open the
523 clamp at the interface with opening wide enough to allow duplex DNA to pass through
524 the center of the ring. The SAXS data collected on sliding clamp and the clamp loader
525 individually provided information about the solution structures and conformations of
526 these two essential replication proteins prior to complex formation. The use of SEC-
527 SAXS allowed us to isolate a homogeneous solution of the complex by removing large
528 aggregates and unbound species ensuring higher quality data for a more accurate
529 interpretation and modeling of the clamp loader-clamp complex SAXS data.

530 Rigid body modeling of the seven-subunit clamp loader complex, discussed in Figure 2,
531 was in agreement with the previous results from SAXS studies on the seven subunit γ
532 clamp loader where the *ab initio* model of the clamp loader revealed the location of $\chi\psi$
533 subunits located in the vicinity of the δ' subunit and away from the ssDNA binding
534 channel on the δ subunit. Based on these findings a model of interaction of the clamp
535 loader with SSB was previously proposed (Tondnevis et al., 2015). Rigid body modeling
536 of the β sliding clamp in solution has revealed that the clamp exists in a closed dimeric
537 conformation under our experimental conditions and in the absence of the clamp loader
538 (Figure 4). This is evident by the close agreement of the calculated scattering profile for
539 the closed clamp to the experimental data as compared to χ value for the open clamp. It
540 can be concluded that the clamp is quite rigid, keeping its symmetric ring structure. Also
541 looking at the Kratky plot the clamp is well folded and has no large flexible parts to it (no
542 increase or plateau at high q). A solution study of the clamp using hydrogen exchange
543 mass spectroscopy revealed that β clamp is not a static closed ring but instead very

dynamic (Fang et al., 2011). Our results are not necessarily contradictory to this dynamic behavior of the clamp as the transient opening that was observed using hydrogen exchange mass spec might not translate into drastic structural changes. It should also be noted that the solution studies by Fang et al were done using lower concentrations of clamp compared to the concentration of the clamp that was used in these SAXS studies. In solution at lower concentration, closer to *in vivo* concentrations, the sliding clamp appears to be more dynamic. Our observed results, of a close clamp, were also expected because of the several previous single molecule and dynamic simulations studies. Molecular dynamics simulation revealed that β has the highest stability in a closed conformation (Jeruzalmi et al., 2001a). The half-life of β on a closed circular DNA is roughly 2 hours implying transient opening of β leading to its dissociation from DNA is infrequent (Yao et al., 1996). The requirement of a clamp loader for binding and opening of the clamp and its assembly onto the primer template junction is also in support of the sliding clamp existing in a closed dimeric conformation prior to interaction with the clamp loader.

Binding of ATP to the clamp loader, primes the macromolecule for binding and opening of the clamp (Berdis and Benkovic, 1996; Burgers, 1991; Hingorani and O'Donnell, 1998; Naktinis et al., 1995; Tsurimoto and Stillman, 1991; Turner et al., 1999). In our rigid body modeling of the clamp loader-clamp complex, we modeled the sliding clamp to be in closed and open conformations as shown (Figure 6). The rigid body modeling of the γ - β clamp loader clamp complex with the closed and open sliding clamp suggests that the sliding clamp is opened once bound to the clamp loader in readiness for binding the duplex DNA. The model of the clamp loader-open clamp complex, where the clamp

was modeled to have a wide enough opening of 30 Å at the interface, exhibits the least discrepancy and close agreement with the experimental data. Further opening of the clamp resulted in R_g values that were too large and in disagreement of the experimental value for the clamp loader-clamp complex. The 25-30 Å opening is wide enough for loading of the clamp onto the duplex DNA as compared to the opening of the clamp in complex with the clamp loader from bacteriophage T4 and the archaeal RFC-PCNA complex structures where the clamps had opening of 9 Å and 5 Å opening respectively. The similarity of the overall clamp loader-clamp interaction in *E. coli* as compared to the eukaryotic, archaeal and bacteriophage clamp loader clamp interaction highlights the importance and conservation of this essential protein-protein interaction in all domains of life. It would be of great advantage to perform time resolved (TR-SAXS) studies to examine if the opening of the clamp could be visualized in real time in the future. It would also be advantages to perform SAXS studies using clamp loader that is comprised of three τ subunits as the main focus of this body of work was on the clamp loader comprised of three γ subunits. There is the possibility that the τ subunits would induce additional conformational changes on the clamp loader as well as the sliding clamp and repetition of these studies with the clamp loader containing τ subunits would be informative.

Acknowledgements

Use of the Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science and Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515. The SSRL Structural Molecular Biology Program is supported by the DOE Office of Biological and Environmental Research, and by the National Institutes of Health, National Institute of General Medical Sciences (including P41GM103393). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official

593 views of NIGMS or NIH. This work was also funded by the NIH T32 training grant No.
594 2T32AI007110-31A1.

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