Binding-Induced Conformational Changes Involved in Sliding Clamp PCNA and DNA Polymerase DPO4

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HIGHLIGHTS
The mechanism of DPO4 binding to PCNA ring and PCNA dimer is investigated.

Two important intermediate states are found before reaching the final bound state.

Both PCNA3 and DPO4 can influence the PCNA12 planar conformation.
SUMMARY

Cooperation between DNA polymerases and DNA sliding clamp proteins is essential for DNA replication and repair. However, it is still challenging to clarify the binding mechanism and the movements of Y-family DNA polymerase IV (DPO4) on the proliferating cell nuclear antigen (PCNA) ring. Here we develop the simulation models of DPO4–PCNA123 and DPO4–PCNA12 complexes and uncover the underlying dynamics of DPO4 during binding and the binding order of the DPO4 domains. Two important intermediate states are found on the free energy surface before reaching the final bound state. Our results suggest that both PCNA3 and DPO4 can influence the PCNA12 planar conformation, whereas the impact of PCNA3 on PCNA12 is more significant than DPO4. These findings provide the crucial information of the conformational dynamics of DPO4 and PCNA, as well as the clue of the underlying mechanism of the cooperation between DPO4 and PCNA during DNA replication.

INTRODUCTION

DNA polymerases, which catalyze synthesis of poly-deoxyribonucleotides from mono-deoxyribonucleoside triphosphates (dNTPs), are crucial for DNA replication, repair, and, in some cases, cell differentiation (Steitz, 1999). In living cells, specialized DNA polymerases including mainly Y-family DNA polymerases can bypass various DNA lesions, although they can replicate undamaged DNA with low-fidelity and poor processivity (Ohmori et al., 2001; Friedberg et al., 2002). The Y-family DNA polymerases share little sequence identity with high-fidelity replicative DNA polymerases in the A- and B-families. However, the Y-family DNA polymerases have a conserved right-handed polymerase core of palm, thumb, and finger domains (Ling et al., 2001; Zhou and Elledge, 2000; Silvian et al., 2001; Trincao et al., 2001). There is an additional little finger (LF) domain located at the C terminus of the Y-family DNA polymerases, which has been shown to increase their overall binding affinity to DNA by contacting the DNA major groove (Ling et al., 2001; Silvian et al., 2001; Boudsocq et al., 2004).

Sulfolobus solfataricus DNA polymerase IV (DPO4) is a representative member of the Y-family DNA polymerases, and both its structure and functions have been investigated in great depth (Wong et al., 2008; Ling et al., 2001; Xing et al., 2009; Wang et al., 2012; Chu et al., 2014; Chu and Wang, 2018, 2020). In crystal structures, DPO4 exhibits large conformational changes upon binding to DNA (from apo state to DNA-bound state), in which the LF domain moves from close to the thumb domain (apo state) to close to the finger domain (DNA-bound state), suggesting the binding-induced “open to closed” mechanism. Similar conformational changes have also been inferred from the crystal structures of Y-family DNA polymerases in other species (Trincao et al., 2001; Uljon et al., 2004). Previous studies have shown that DPO4 is under a conformational equilibrium between multiple states during the DNA-binding process and the distributions of the conformations vary at different binding stages (Wang et al., 2012; Chu et al., 2014). However, a third structural state of DPO4 was reported in 2009, in which the LF domain exhibits a different configuration from that at the apo and DNA-bound states and is located on the surface of the proliferating cell nuclear antigen (PCNA) (Xing et al., 2009).

PCNA is a six-domain ring that clamps DNA and provides a platform for DNA-processing enzymes, such as DNA polymerases, DNA ligase I, and Flap endonuclease 1 (Fen1) in almost every DNA metabolic process, including replication, repair, recombination, modification, and cell cycle regulation (Kelman, 1997;
The PCNA ring surface facing the primer-extension direction forms a platform that tethers DNA-modifying enzymes (PCNA-interacting proteins, PIPs) on the DNA substrate as the clamps move along the DNA (Waga and Stillman, 1998; Warbrick, 2000). The PIPs interact with PCNA via the well-conserved PIP-box motif, Qxx(M/L)xxF(Y/W) (Warbrick, 2000; Dalrymple et al., 2001). Previous structural studies have shown that the PIP-box binds to the interdomain connecting loop (IDCL) of the PCNA ring and its proximal hydrophobic cavity (Gulbis et al., 1996). In addition, it has been found that the PCNA heterotrimeric ring (formed by three monomers PCNA1, PCNA2, and PCNA3) from *S. solfataricus* binds and stimulates Fen1, DNA polymerase B1 (Pol B1), DNA ligase 1, as well as DPO4 through their PIP-box regions (Dionne et al., 2003, 2008; Dore et al., 2006; Xing et al., 2009). The crystal structure of the full-length DPO4 (352 a.a.) and *S. solfataricus* PCNA (two monomers, PCNA12) complex (PCNA12–DPO4) was resolved in 2009 (Xing et al., 2009). Both native PAGE and DNA mobility shift assay results indicate that PCNA1 is a minimum and sufficient DPO4-binding partner (Xing et al., 2009). In this structure, the C-terminal PIP-box (residues 342–352) of DPO4 becomes ordered in the complexed structure with PCNA, whereas it is disordered in the apo and DNA-bound structures (Demarest et al., 2002). Thus, two intrinsically flexible hinge regions have been found in the DPO4. One is located on the flexible linker between the core and the LF domain, and the other is located at the C-terminal end of the LF domain. However, other DNA-processing enzymes do not have the first hinge region and the major conformational changes found in the Y-family polymerases (Silvian et al., 2001; Uljon et al., 2004; Wong et al., 2008; Ling et al., 2004). Moreover, comparison between the apo PCNA12 and the DPO4–PCNA12 complex structures has revealed a slightly off-planar movement of the PCNA1–PCNA2 plane in the DPO4-bound PCNA12 structure (Xing et al., 2009).

Investigation of the underlying mechanisms and details of the binding process between PCNA and DPO4 is still challenging. Although molecular dynamics (MD) simulations provide a good way to study the important interactions of the protein systems and collect atomic structural information, it is rather time consuming for conventional MD simulations to deal with the protein systems with such large-scale conformational changes during binding. In the present study, structure-based models based on the energy landscape theory (Bryngelson and Wolynes, 1987; Shoemaker et al., 2000; Clementi et al., 2000; Turjanski et al., 2008; Ganguly et al., 2013; Lu and Wang, 2008) as well as the two-basin (and even multi-basin) models (Chu and Voth, 2007; Lu and Wang, 2008; Whitford et al., 2007; Okazaki et al., 2006; Chu et al., 2017; Chu and Wang, 2018; Liu et al., 2017) are developed for effectively sampling the PCNA–DPO4 binding process associated with the large conformational changes on DPO4. We develop a variety of different binding systems of DPO4 complexed with either PCNA12 or PCNA123, respectively. By performing the thermodynamic and kinetic binding MD simulations, we show the dynamic distributions of both DPO4 and PCNA conformations and identify the critical interactions and residues during binding. Our results uncover the underlying mechanisms and the details of different conformational changes during the DPO4 and PCNA binding process, thus making a significant contribution to the research of DNA replication.

**RESULTS AND DISCUSSION**

**Finger Domain Moves from Center to Side of PCNA**

DPO4 is a Y-family polymerase that has a polymerase core consisting of palm, finger, and thumb domains in addition to a fourth domain known as the little finger (LF) domain. In the structure of PCNA12–DPO4 (PDB: 3FDS), all the native contacts exist between three domains (finger, thumb, LF) as well as PIP-box of DPO4 and PCNA1 monomer (see Figure 1). Considering the native structure of PCNA12–DPO4, the PCNA1 monomer is crucial for the binding of DPO4. The different forms of DPO4 and its domain information are illustrated in Figure 1. DPO4 undergoes large-scale conformational changes upon DNA binding, with the movement of LF domain connected by the flexible linker loop (colored gray in Figures 1C–1E). In the DNA-bound state (Figure 1D, PDB: 2RDJ), the LF domain moves toward and interacts with the finger domain, binding with the DNA ligand strongly (“closed” form), whereas in the apo form (Figure 1E, PDB: 2RDJ), the LF domain changes to have strong interactions with the thumb domain (“open” form). When DPO4 binds with PCNA (Figure 1C, PDB: 3FDS), the LF domain moves away from the other domains to get interactions with PCNA1. In addition, the PIP-box of DPO4 is important for DPO4 binding to PCNA12. More than half (60.8%) of the native contacts between DPO4 and PCNA12 are located on this PIP-box region (Figure 1F). If we superimpose these different forms of DPO4 together, we can see that the rotating angle of LF domain continues to change throughout them, whereas the other domains (palm, finger, and thumb) almost remain unchanged.
After modeling the system of PCNA123 and DPO4, replica-exchange molecular dynamics (REMD) simulations were performed to obtain the free energy profiles. Room-temperature free energy profiles were extracted and projected onto several reaction coordinates. Here we define $Q_{\text{inter}}$, PCNA–DPO4 as the fraction of native contacts between PCNA and DPO4 (obtained from PDB: 3FDS, see Figure 1F); $Q_{\text{speci}}$, DPO4 as the fraction of the native contacts that are specific for DPO4 conformational changes between apo DPO4 and PCNA-bound forms (the intra-chain native contacts of DPO4 belong to only one form of DPO4 [apo DPO4 or PCNA12-bound DPO4]). As shown in Figure 2A, at room temperature, there is one basin with the lowest free energy (B state) as well as two basins with slightly higher free energy (I1 and I2 states). The B state (0.0 kT) is located at about $Q_{\text{inter}} = 0.86$, $Q_{\text{speci}} = 0.76$, where DPO4 is considered to fully bind with PCNA123. The other two intermediate states I1 and I2 are located at about $Q_{\text{inter}} = 0.62$, $Q_{\text{speci}} = 0.82$ and $Q_{\text{inter}} = 0.70$, $Q_{\text{speci}} = 0.72$, with free energy 0.7 and 0.4 kT higher than that of the B state, respectively. Although these three states have similar heights and locations on the free energy surface, PCNA123-bound DPO4 in these states exhibits different configurations and binding poses (see Figure 3A). In the B state, the finger, thumb, and LF domains of DPO4 have interactions with PCNA123 (Figure 3B), consistent with the PDB: 3FDS structure. In the I1 state, the DPO4 rotates on the axis of the PIP-box with the finger domain toward the center of the PCNA ring. The palm, thumb, and LF domains move away from their original binding sites (Figure 3C). In the I2 state, the finger and LF domains stay at their original binding sites while the thumb domain moves away from the PCNA ring (Figure 3D).
In all these three states, the C-terminal PIP-box region of DPO4 strongly and steadily binds to the surface of PCNA1 (see Figure 3A). The PIP-box (342–352, DPO4) is a well-conserved domain in PIPs. It has been observed experimentally that the C-terminal PIP-box of DPO4 is essential and sufficient for PCNA–DPO4 binding (Xing et al., 2009). However, this part is missing in the PDB: 2RDI (apo DPO4) and PDB: 2RDJ (DPO4 with DNA) structures. It suggests that the PIP-box of DPO4 acts as an anchor when DPO4 moves on the surface of PCNA123.

Moreover, we also compare the configuration of DPO4 at these states by projecting the free energy on the reaction coordinates of Q_{inter} PCNA–DPO4 and root-mean-square deviation (RMSD) of DPO4. We calculated the RMSD values of DPO4 with respect to the DPO4 in PDB: 3FDS (Figure 2B), PDB: 2RDJ (Figure 2C), and PDB: 2RDI (Figure 2D), respectively. It should be noted that the DPO4 at B state is close to the configuration of DPO4 in PDB: 3FDS (PCNA12-bound DPO4). The I1 state seems to be similar to the configuration of DPO4 in PDB: 2RDI (apo DPO4). In order to demonstrate the internal conformational changes of DPO4, we superimposed the representative frames of the three main states with the experimental structures of DPO4 (Figure 4A). The large conformational changes within DPO4 occur mainly on the LF domain, which is linked to the core part (palm, finger, and thumb domains) with a flexible loop region (colored gray in Figures 1C–1E). The LF domain of DPO4 in the I1 state is between that of DPO4 in PDB: 3FDS and PDB: 2RDJ and close to the DPO4 in PDB: 2RDI. The I2 state is difficult to be distinguished from the B state on the free energy surface of the Q_{inter} PCNA–DPO4 and RMSD of DPO4. It seems that, in the I2 state, the LF domain is different from that in other configurations of DPO4 but fairly close to the PDB: 3FDS structure (see Figure 4A).

In the previous works of PCNA and DPO4 complex (Xing et al., 2009), the researchers estimated the different conformations of DPO4 bound to PCNA when there is a section of dsDNA occupied in the center of PCNA ring. However, the structure of PCNA–DPO4–dsDNA complex (and that of the PCNA–dsDNA complex) has
yet to be resolved. All the structures in this paper were constructed using the published structures PDB: 2NTI/PDB: 2HI (apo PCNA123), PDB: 3FDS (DPO4–PCNA12), and PDB: 2RDI (apo DPO4). Our simulations here suggest that the I1 state may be an intermediate state between the extended form and the active form of DPO4 mentioned in this paper, close to the configuration of the apo form attaching to the PCNA ring, because the DPO4 starts to rotate and the core domain moves to the center hole of the PCNA ring.

DPO4 Binds to PCNA12 through Similar Intermediates

Previous experimental data have shown that PCNA1 and PCNA2 first form a stable heterodimer (PCNA12), which is then capable of recruiting PCNA3 to complete the ring structure (Xing et al., 2009; Vladena et al., 2008). In addition, only PCNA12 and DPO4 complex but no PCNA123 and DPO4 complex has been obtained. Here, in order to explore the function of PCNA3 on the PCNA ring, both PCNA12–DPO4 and PCNA123–DPO4 models were prepared for the REMD simulations. The PCNA12–DPO4 model has the same parameters as the PCNA123–DPO4 model, although the former does not include PCNA3.

After simulations, the free energy at room temperature was extracted and projected on the same reaction coordinates as above. Similar to PCNA123–DPO4, the PCNA12–DPO4 system also exhibits three states, one bound state as well as two intermediate states (B, I1, and I2 states), on the free energy surface of DPO4-binding path. As shown in Figure S2, these three states have the same location as in the PCNA123–DPO4 complex. However, the free energy values of these states are slightly different from before. The free energy of the I1 state (0.4 kT) is closer to that of the B state than to that of the I2 state (0.6 kT). This suggests that the PCNA without PCNA3 favors the movement of the finger domain of DPO4 into the center hole of PCNA.

Furthermore, the B state conformations of both PCNA123–DPO4 and PCNA12–DPO4 are very similar to the conformation in PDB: 3FDS, with RMSD values of about 3.72 and 3.07 Å, respectively (as shown in Figure S3).
In the previous study of the PCNA12–DPO4 structure, the authors identified two intrinsically flexible hinge regions in DPO4 based on the multiple conformations (Ling et al., 2001; Wong et al., 2008; Xing et al., 2009). The main one of these hinges is located on the flexible linker between the core and the LF domain. Consequently, we analyzed the movement of the LF domain with respect to the core part by calculating the dihedral \( u \) of the four domains (finger, palm, thumb, and LF). \( u \) is defined with the center of mass (COM) sites of the finger (COM1), palm (COM2), thumb (COM3), and LF (COM4); see Figure 4B. As shown in Figure 4C, from the beginning of the binding process to the I1 state, the relative positions of the four domains are similar to those in the \( \text{apo} \) DPO4 structure (PDB: 2RDI); from I1 state to B state, the peak of the \( u \) distribution moves from the position of PDB: 2RDI to PDB: 3FDS; whereas at the bound state, the relative positions of the four domains are close to those in the PCNA12-bound DPO4 structure (PDB: 3FDS). Moreover, with the additional PCNA3, the \( u \) of PCNA123-bound DPO4 has similar changes to that of the PCNA12-bound DPO4. Therefore, when DPO4 binds to PCNA123 or PCNA12, the conformational changes of DPO4 almost keep the same.

**Thumb and LF Domains Touch PCNA First**

In order to investigate the binding order, we divided the native inter-molecular contacts into several parts (between three domains and PIP-box of DPO4 and PCNA; here the palm domain does not have native inter-molecular contacts with PCNA, see the analysis in the first section of Results and Discussion) and then analyzed their Q values at different stages of the binding path. As shown in Figure 5, here the situations of these native interactions along the binding path of DPO4 to PCNA123 and PCNA12 are similar. At the first stage of binding (\( Q_{\text{inter}} < 0.1 \)), the thumb and LF domains of DPO4 touch PCNA123 (or PCNA12) first, whereas the other domains have much lower proportion of interactions with PCNA in this period. At the middle stage of binding (\( Q_{\text{inter}} \sim 0.1–0.4 \)), PIP-box begins to bind with PCNA, with mean Q increased from about 0.1 to 0.6. The main inter-molecular interactions belong to the LF domain and PIP-box of DPO4. LF domain undergoes two main shifts, one before \( Q_{\text{inter}} \sim 0.2 \) and the other after \( Q_{\text{inter}} \sim 0.2 \). After the second shift (\( Q_{\text{inter}} \sim 0.38 \)), the mean Q between the LF domain and PCNA decreases to about 0.1. Then the LF domain and PIP-box continue binding to PCNA. At I1 state (\( Q_{\text{inter}} \sim 0.6 \)), PIP-box binds almost fully to PCNA, whereas LF binds to PCNA with about half native contacts. The finger and thumb domains have few contacts with PCNA. At I2 state (\( Q_{\text{inter}} \sim 0.7 \)), both LF domain and PIP-box bind strongly with PCNA, whereas the finger and thumb domains remain out of the final binding site. After the B state (\( Q_{\text{inter}} > 0.8 \)), all the parts of DPO4 find their binding places. These results are consistent with the representative configurations extracted from the trajectory above (Figures 3B–3D).
Furthermore, the contact map between PCNA and DPO4 can clearly reveal the changes of individual inter-molecular interacting pairs during the binding, including the native and non-native contact pairs. At the beginning of the binding process ($Q_{\text{inter}} < 0.1$), there are several non-native contacts between LF and PIP-box of DPO4 and PCNA2 monomer (Figures S4A and S5A). At this stage, these non-native contacts in the PCNA12–DPO4 complex are more and stronger than that in the PCNA123–DPO4 complex, which may be related to the flexibility of the PCNA12 dimer (see the next section). For DPO4 to PCNA123, the inter-molecular contacts with probability higher than 0.2 locate between residues 196, 302, and 304 of DPO4 (thumb and LF) and PCNA1. For DPO4 to PCNA12, contacts with similar strength change to locate between residues 301, 302, 304, 339, and 349 of DPO4 (LF and PIP-box) and PCNA1. It suggests that the initial complex formed at the beginning of DPO4 binding to PCNA123 is a bit different from that of DPO4 binding to PCNA12. The simulation results show that the mean binding time of DPO4 to PCNA123 ($5.36 \pm 1$) is a bit higher than that of DPO4 to PCNA12 ($4.02 \pm 1$). The interactions between PCNA12 and DPO4 at the beginning may favor the binding process. In addition, there are a few non-native contacts between LF of DPO4 and PCNA2 monomer at the I2 state of PCNA12–DPO4 binding, whereas these non-native contacts do not exist at the same stage of PCNA123–DPO4 binding (see Figures S4D and S5D). Besides, at the I2 state of both PCNA123–DPO4 and PCNA12–DPO4 binding processes, the palm domain of DPO4 has non-native contacts with the C terminus of PCNA1, which do not appear at the other stages of binding. At the B state of both PCNA123–DPO4 and PCNA12–DPO4 binding processes, DPO4 forms inter-molecular interactions with PCNA1 only, the same as that in PDB: 3FDS (see Figure 1F). At every stage of DPO4 binding to PCNA123 or PCNA12, contacts between DPO4 and PCNA mainly locate on the PCNA1 monomer. This is consistent with previous experimental results that PCNA1 is the minimum and sufficient DPO4-binding partner Xing et al. (2009).

**PCNA12 Becomes Open and Twisted without PCNA3**

The PCNA123 ring structure is closed and remains almost planar when it is isolated (see PDB: 2HII). In the previous experimental studies, it was found that the PCNA12 heterodimer becomes somewhat open and twisted when compared with PCNA123 (Xing et al., 2009). The DPO4 binding does not significantly affect the open conformation of the PCNA12 but marginally stabilizes the off-plane spiral conformation. Here we aim to quantify the different conformations of PCNA12 by calculating the angle and dihedral of the dimer. As illustrated in Figure 6A, the interface between PCNA1 and PCNA2 (yellow cartoons) acts as the hinge of PCNA12 during this kind of conformational change. The angle of PCNA1(COM1)–hinge(COM2)–PCNA2(COM4) is defined as the opening angle of PCNA12 ($\theta_{12}$); the dihedral of PCNA1(COM1)–hinge1(COM2)–hinge2(COM3)–PCNA2(COM4) is defined as the off-plane angle of PCNA12 ($\varphi_{12}$). For apo PCNA123 (PDB: 2HII), $\theta_{12}$ and $\varphi_{12}$ are 102.678° and $-0.592°$. Without PCNA3, apo PCNA12 (PDB: 2I04) has slightly higher $\theta_{12}$ (110.130°) and lower $\varphi_{12}$ ($-32.145°$) than apo PCNA123. When PCNA12 is bound with DPO4 (PDB: 3FDS), $\theta_{12}$ changes slightly (109.888°), whereas $\varphi_{12}$ becomes a little higher ($-24.704°$) compared with apo PCNA12.
In our simulation, we collected all the $q_{12}$ and $\phi_{12}$ data during the binding process. As shown in Figure 6B, it is clear that the removal of PCNA3 will lead to an increase in the mean $q_{12}$ of about 2°/C14 and a decrease in the mean $\phi_{12}$ by about 10°/C14. Whether DPO4 is bound or not, $q_{12}$ does not change significantly. Similar results can be obtained for PCNA123. However, the middle stage ($Q_{inter} \approx 0.1–0.4$) of DPO4 binding to PCNA12 exhibits much larger fluctuations in $\theta_{12}$ and $\phi_{12}$ than that of DPO4 binding to PCNA123. In addition, the $\theta_{12}$ of PCNA123 (with or without DPO4) is much more concentrated than that of PCNA12 (with or without DPO4, see Figure 6C). These results suggest that PCNA3 may have a role in stabilizing the PCNA12 conformation. Moreover, $\phi_{12}$ of DPO4-bound PCNA123 is similar to that of unbound PCNA123. However, $\phi_{12}$ of DPO4-bound PCNA12 is slightly higher (by about 1°) than that of unbound PCNA12, which is consistent with the experimental structures. This also implies that PCNA3 may help maintain the PCNA planar conformation.

Limitations of the Study

Both the Y-family DNA polymerase IV (DPO4) and proliferating cell nuclear antigen (PCNA) are essential for DNA replication and translesion synthesis. However, the cooperation between DPO4 and PCNA and the movement of DPO4 on PCNA are still unclear. Here, based on a series structures of apo DPO4, apo PCNA, and the DPO4–PCNA complex, we have developed the structure-based coarse-grained model for both trimeric PCNA–DPO4 complex (PCNA123–DPO4) and dimeric PCNA–DPO4 complex (PCNA12–DPO4) and found three main states during DPO4 reaching PCNA ring. Here in this study, we did not incorporate the ligand DNA in our simulation model since there is no structure of DPO4-PCNA complexed with the ligand DNA, which is located in the center of the PCNA ring. Further molecular dynamics investigations based on the structure of DPO4–PCNA complexed with the ligand DNA will be in urgent need in the future.

Resource Availability

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Jin Wang (jin.wang.1@stonybrook.edu).
Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
All relevant data are available from the authors upon reasonable request.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101117.

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AUTHOR CONTRIBUTIONS
W.-T.C., Z.S., and J.W. designed research; W.-T.C. performed research; W.-T.C., Z.S., and J.W. analyzed data; and W.-T.C., Z.S., and J.W. wrote the paper.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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Supplemental Information

Binding-Induced Conformational Changes Involved in Sliding Clamp PCNA and DNA Polymerase DPO4

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Supporting information

Binding-induced conformational changes involved in sliding clamp PCNA and DNA polymerase DPO4

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1 Transparent Methods

1.1 Initial model of simulation

The initial coarse-grained $C_\alpha$ structure-based models (SBM) were constructed for PCNA123–DPO4 and PCNA12–DPO4 complexes, by using SMOG on-line toolkit (Noel et al., 2010; Clementi et al., 2000; Noel et al., 2012; Lammert et al., 2009). This $C_\alpha$ model includes one bead on the $C_\alpha$ atom of each residue of the system. The model of heterotrimeric ring PCNA123 from *S. solfataricus* was generated from two PDB structures, 2HII (*apo* *S. sol* PCNA123) (Pascal et al., 2006) and 3FDS (*S. sol* PCNA12 with DPO4 bound) (Xing et al., 2009). Trimeric ring PCNA123 (2HII) contains PCNA1 (Met1-Leu249), PCNA2 (Met1-Ala245), and PCNA3 (Met1-Lys243). In addition, the model of the binding partner DPO4 was built from the 3FDS (PCNA-bound DPO4) and 2RDI (*apo* DPO4) (Wong et al., 2008). DPO4 is a 352 a.a. protein. Two-basin SBM was prepared for both PCNA123 trimer and DPO4, respectively, with the similar method used before (Chu and Voth, 2007; Chu et al., 2014; Wang et al., 2012b; Okazaki et al., 2006; Chu and Wang, 2018; Liu et al., 2017; Chu et al., 2017b). The key to construct a model with multiple basins is to build a mixed contact map which integrates multiple structural information together. In this work, all the intra-chain native contacts within PCNA (or within DPO4) can be collected into core (native contacts involved in both structures) and specific (native contacts involved in only one structure). All the inter-monomer native contacts within PCNA123 are obtained from 2HII. And the inter-chain native contacts between PCNA and DPO4 come from 3FDS.

The modeled two-basin Hamiltonian can be given as:

$$U = U_{\text{bond}} + U_{\text{angles}} + U_{\text{dihedrals}} + U_{\text{LJ}} + U_{\text{charged}}$$

For the local terms of the Hamiltonian, the bond term is expressed as $U_{\text{bond}} = K_r (r - r_0)^2$. Here, $K_r$ is the strength of the bond term, which is set to 100$\varepsilon$. For the large-scale conformational changes between native structures, the pseudo angle and dihedral terms are divided into non-hinge and hinge regions, by comparing the differences of angle and dihedral degrees in the native structures:

$$U_{\text{angles}} + U_{\text{dihedrals}} = U_{\text{non-hinge}} + K_{\text{hinge}} U_{\text{hinge}}$$

where $U_{\text{non-hinge}}$ and $U_{\text{hinge}}$ have the same functional form (angles: $K_\theta (\theta - \theta_0)^2$; dihedrals: $K^{(1)}_\phi (1 - \cos(\phi - \phi_0)) + K^{(3)}_\phi (1 - \cos(3(\phi - \phi_0)))$). Here, $\theta_0$ and $\phi_0$ are the angle and dihedral values in the closed state. For non-hinge regions, $K_\theta = 20 \varepsilon$, $K^{(1)}_\phi = \varepsilon$, $K^{(3)}_\phi = 0.5 \varepsilon$. Hinge term, which is defined as before (Chu and Wang, 2018; Chu et al., 2017b, 2014), is set to control the flexibility of the model. In hinge regions, $K_\theta$ and $K_\phi$ are rescaled by 0.01, mimicking the flexibility.
The non-local term is in the form of Lennard-Jones (LJ) interactions, which can be divided into the term of native contacts (attractive, $U_{\text{attractive}}$) and the term of non-native contacts (repulsive, $U_{\text{repulsive}}$) within the protein ($U_{\text{LJ}} = U_{\text{attractive}} + U_{\text{repulsive}}$). All the native contacts were identified by the Shadow Algorithm (Noel et al., 2012). The expression of $U_{\text{attractive}}$ can be given as:

$$U_{\text{attractive}} = K_{\text{intra}} \ast U_{\text{LJ}}(\gamma_{\text{intra}}) + K_{\text{inter}} \ast U_{\text{LJ}}(\gamma_{\text{inter}})$$

$$= K_{\text{core}} \ast U_{\text{LJ}}(\gamma_{\text{core}}) + K_{\text{speci}} \ast U_{\text{LJ}}(\gamma_{\text{speci}}) + K_{\text{interP}} \ast U_{\text{LJ}}(\gamma_{\text{interP}}) + K_{\text{interD}} \ast U_{\text{LJ}}(\gamma_{\text{interD}})$$

(S3)

, where the LJ attractive expression is $5 \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - 6 \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{10}$. In this model, the $U_{\text{attractive}}$ term can be divided into intra-chain interaction terms and inter-chain interaction terms. The former includes the core part and the specific part. And the latter contains the potentials between PCNA monomers (interP) and between DPO4 and PCNA (interD). The parameter $K_{\text{core}}$ values for PCNA123 and DPO4 are set to 2.0 and 3.0 ($K_{\text{speci}}$ values for both PCNA123 and DPO4 are 1.5), respectively, in order to let PCNA123 and DPO4 have similar thermo-stability (see the next subsection). The repulsive interactions have the potential function as $K_{NC} \left( \frac{\sigma_{NC}}{r_{ij}} \right)^{12}$.

The electrostatic potential ($U_{\text{charged}}$) is computed by the Debye–Hückel model (Azia and Levy, 2009; Givaty and Levy, 2009; Chu et al., 2012; Wang et al., 2012a), which can be used to quantify the strength of the charge-charge attraction and repulsion in varying ion strengths:

$$U_{\text{charged}} = \Gamma_{DH} \times K_{\text{coulomb}} B(\kappa) \sum_{i,j} q_i q_j \exp \left( -\kappa r_{ij} \right)$$

(S4)

In Eq. S4, $K_{\text{coulomb}} = 4\pi \varepsilon_0 = 138.94 kJ/\text{mol} \cdot \text{nm}^{-1} \cdot \text{e}^{-2}$ is the electric conversion factor; $B(\kappa)$ is the salt-dependent coefficient; $\kappa^{-1}$ is the Debye screening length, which is directly influenced by the solvent ion strength (IS)/salt concentration $C_{\text{salt}}$ ($\kappa \approx 3.2 \sqrt{C_{\text{salt}}}$); $\varepsilon_r$ is the dielectric constant, which was set to 80 during the simulations. $\Gamma_{DH}$ is the energy scaled coefficient for balancing the total energy. In our model, Lys and Arg have a positive point charge (+e), Asp and Glu have a negative point charge (-e). All the charges are placed on the $C_{\alpha}$ atoms. In the physiological salt concentration ($C_{\text{salt}} = 0.15M$), $\kappa$ is $1.24 \text{ nm}^{-1}$. As a result, we set $\Gamma_{DH} = 0.535$ in our simulations, in order to have $V_{DH}$ for two opposite charged atoms located at a distance of 0.5 nm equal to the native contact energy. Here in our simulations, physiological salt concentration ($C_{\text{salt}} = 0.15M$) was set as the simulated ionic strength. When a native contact is an ionic pair (salt bridge), we rescaled its interaction strength by 0.1 so that its energetic contribution will be comparable to other native contacts (Levy et al., 2007).
1.2 Parameter calibration

In this study, we use coarse-grained structure-based model to investigate the conformational changes during the binding process of DPO4 to PCNA123/PCNA12. As a result, the simulation temperature is not the same as the real temperature (Clementi et al., 2000; Lammert et al., 2009). In our previous studies, we compared the simulated folding temperature and the experimental melting temperature of a protein. And then the simulated room temperature was determined according to the ratio of melting temperature and room temperature (Chu et al., 2017b,a). However, there are no experimental results about the melting temperature of PCNA (PCNA123 or PCNA12) or DPO4 available. Therefore, we calculated the folding temperature values of PCNA1, PCNA2, PCNA3, and DPO4, as well as the binding temperature between PCNA1 and PCNA2, between PCNA2 and PCNA3, between PCNA1 and PCNA3. First of all, the $K_{\text{core}}$ of PCNA123 was set to 2.0 and the $K_{\text{core}}$ of DPO4 was tuned to make sure that both PCNA123 and DPO4 are stable at room temperature. After a series of REMD simulations, we found that when $K_{\text{core}}$ of DPO4 is 3.0, DPO4 has similar thermo-stability as PCNA123 (see Fig. S1). The folding temperature ($T_f$) of PCNA123 is about 2.29 (in reduced unit), and the $T_f$ of DPO4 is about 2.19. While within PCNA123, the $T_f$ of PCNA1 (2.28) is similar as that of PCNA2 (2.27), but higher than that of PCNA3 (2.14).

Then we set $K_{\text{interP}}$ to 1.0 (the parameters between PCNA1 and PCNA2, PCNA1 and PCNA3, PCNA2 and PCNA3 are the same). We can also recognize the binding temperature $T_b$ between the two monomers of PCNA123 (as shown in Fig. S1B). The $T_b$ values between PCNA1 and PCNA2, between PCNA1 and PCNA3, between PCNA2 and PCNA3 are 1.73, 1.69 and 1.70, respectively. Therefore, the interactions between PCNA1 and PCNA2 are slightly stronger than that between PCNA1 and PCNA3, between PCNA2 and PCNA3. According to the heat capacity curves of PCNA123 and DPO4, we set the simulation temperature 1.24 (about 149 K in Gromacs) corresponds to the room temperature (about 0.7 $T_b$), in order to ensure the stability of PCNA ring at room temperature. In our model, the binding energy between PCNA1 and PCNA2 is higher than that between PCNA3 and PCNA1 or between PCNA3 and PCNA2, which is consistent with the experimental findings that the interactions between PCNA1 and PCNA2 is stronger than others (Dionne et al., 2003).

We next tuned the parameter $K_{\text{interD}}$ between PCNA and DPO4 to 2.0, after building the PCNA–DPO4 complex system. With this setting, the system is occupied with DPO4 bound state at room temperature (Fig. S1).

1.3 MD simulation

All simulations were performed with Gromacs 4.5.5 (Hess et al., 2008). The coarse grained molecular dynamics simulations (CGMD) used Langevin equation with constant friction coefficient $\gamma = 1.0$. The cutoff for non-bonded interactions was set to 3.0 nm. The MD time step was set to 0.5 fs and the trajectories were saved every 10 ps. To enhance the sampling of binding events, a strong harmonic potential was added if the distance between the center of mass of PCNA
and DPO4 is greater than 8.0 nm (Tribello et al., 2014).

For thermodynamic simulations, REMD simulations with 28 replicas were performed on both PCNA123–DPO4 and PCNA12–DPO4 systems to overcome the energy barriers between unbound and bound states. The initial conformations of the two systems are at unbound state. That is, we put DPO4 (from 3FDS) and PCNA123/PCNA12 (from 2HII) at distance of about 6.0 nm to ensure that they do not have interactions at the beginning of REMD simulations. Then the profiles of free energy curve or surface can be obtained by using WHAM algorithm (Kumar et al., 1992, 1995).

For kinetic simulations, 200 individual MD runs started with varying unbound configurations and velocities were performed on PCNA123–DPO4 and PCNA12–DPO4 systems, respectively. Each kinetic run starts from the unbound state ends to the bound state. Likewise, at the beginning, DPO4 was put far away from PCNA123/PCNA12 at distance of about 6.0 nm to 8 nm. These procedures make sure that DPO4 binds to PCNA123/PCNA12 in different directions.

We define that a native contact is formed if the Cα-Cα distance between any given native atom pair is within 1.2 times of its native distance. And a non-native contact is considered to be formed if the distance between the two beads of a contact pair is within 10 Å. For each kinetic run, the binding time (the first passage time of binding, $FPT_{on}$) equals to the simulation time of reaching the bound state firstly. Since the simulated binding time is different from the experimental binding time, we collected the $FPT_{on}$ values in the unit of $\tau$ ($\tau = 1$ ns).
Fig. S1  Related to Figure 2. (A) The heat capacity curves of apo PCNA123 (red) and apo DPO4 (gold) as a function of temperature, as well as the heat capacity curves of PCNA1 (green), PCNA2 (blue), and PCNA3 (magenta) in apo PCNA123. (B) The heat capacity curves of the interactions between PCNA1 and PCNA2 (red), PCNA1 and PCNA3 (green), PCNA2 and PCNA3 (blue) as a function of temperature. Here the temperature in these coarse-grained SBM simulations is different from the real temperature.
Fig. S2  Related to Figure 2. The free energy profile as a function of (A) the fraction of native contacts between PCNA and DPO4 ($Q_{\text{inter}, \text{PCNA-DPO4}}$) and the fraction of specific native contacts of DPO4 ($Q_{\text{speci}, \text{DPO4}}$), (B) $Q_{\text{inter}, \text{PCNA-DPO4}}$ and RMSD of DPO4 with respect to the DPO4 in 3FDS ($\text{RMSD}_{\text{DPO4}}(3\text{FDS})$), (C) $Q_{\text{inter}, \text{PCNA-DPO4}}$ and RMSD of DPO4 with respect to the DPO4 in 2RDJ ($\text{RMSD}_{\text{DPO4}}(2\text{RDJ})$), (D) $Q_{\text{inter}, \text{PCNA-DPO4}}$ and RMSD of DPO4 with respect to the DPO4 in 2RDI ($\text{RMSD}_{\text{DPO4}}(2\text{RDI})$). These free energy data were extracted at $T = 1.24$ (in reduced unit, mimicking the room temperature).

Fig. S3  Related to Figure 2. The free energy profile as a function of specific native contacts of DPO4 ($Q_{\text{speci}, \text{DPO4}}$) and RMSD of PCNA12–DPO4 with respect to 3FDS ($\text{RMSD}_{\text{com}}(3\text{FDS})$) in the system PCNA123–DPO4 (A) and PCNA12–DPO4 (B). These free energy data were extracted at $T = 1.24$ (in reduced unit, mimicking the room temperature).
**Fig. S4**  Related to Figure 5. The evolution of the contact map between PCNA123 and DPO4 during the binding path. Each native and non-native contacts are illustrated with a triangle and a circle. The probability of each contact is shown with gradient color. The PCNA1 (1–249, gray), PCNA2 (250–494, cyan), and PCNA3 (495–737, magenta) are labeled along the x axis. The domains of DPO4 are labeled along the y axis with the same color as in Fig. 1. Here a non-native contact is considered to be formed if the distance between the two beads of a contact pair is within 10 Å.
Fig. S5  Related to Figure 5. The evolution of the contact map between PCNA12 and DPO4 during the binding path. Each native and non-native contacts are illustrated with a triangle and a circle. The probability of each contact is shown with gradient color. The PCNA1 (1–249, gray), PCNA2 (250–494, cyan), and PCNA3 (495–737, magenta) are labeled along the x axis. The domains of DPO4 are labeled along the y axis with the same color as in Fig. 1. Here a non-native contact is considered to be formed if the distance between the two beads of a contact pair is within 10 Å.
Fig. S6 Related to Figure 6. Distribution of both $\theta_{12}$ (horizontal axis) and $\varphi_{12}$ (vertical axis) at unbound state of PCNA12 (A) and PCNA123 (B), and at bound state of PCNA12 (C) and PCNA123 (D). Note that the numbers of data points in the four panels are not the same.
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