FIGURE S1. **Physical interaction between K153A mutant PfuUDG and PfuPCNA.**
Purified PfuPCNA was immobilized on a Biacore sensor chip, and various concentrations of the K153A mutant PfuUDG (0.2-1.0 ⌈M) were injected for 120 s. The equilibrium constant (K_D) was calculated from the obtained sensorgrams.

FIGURE S2. **Specific activity of the T154A/L155A/F156A mutant.**
The enzyme activities of wild-type (WT) and mutant (T154A/L155A/F156A) PfuUDG were compared. Various amounts of PfuUDG proteins, as indicated, were incubated with 100 fmol of uracil-containing DNA substrate (G:U*) in the reaction, as described in the “Experimental Procedures” The uracil excision efficiency is plotted as a function of the enzyme concentration.

FIGURE S3. **The molecular model of *Pfu*UDG-PCNA-DNA complex.**
The molecular model was constructed from the co-crystal structures of *T. thermophilus* UDG–dsDNA (a) and *P. furiosus* PCNA (b). The trimer of PfuPCNA (c) and the UDG-PCNA complex (d) was assembled by superposing the corresponding region in TthUDG on the newly identified PCNA binding site of PfuUDG. The homology model of PfuUDG was constructed from TthUDG (e), and then was used in place of the TthUDG in the complex-1 model. A B-type dsDNA model (f) was also assembled into complex-1, to build complex 2 (g). The assembled model was energy minimized with MOE to obtain the final PfuUDG-PfuPCNA-DNA complex, complex-3 (h).

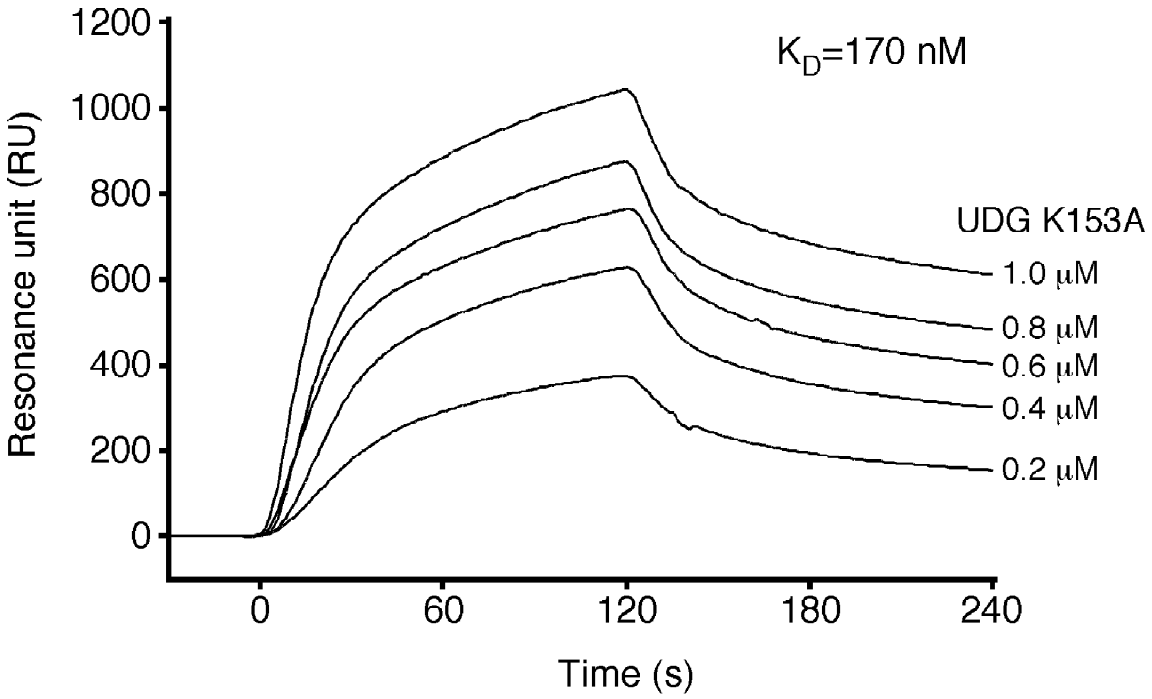

K_D=170 nM
UDG K153A
1.0 μM
0.8 μM
0.6 μM
0.4 μM
0.2 μM
Resonance unit (RU)
1200
1000
800
600
400
200
0
0
60
120
180
240
Time (s)

Figure S1

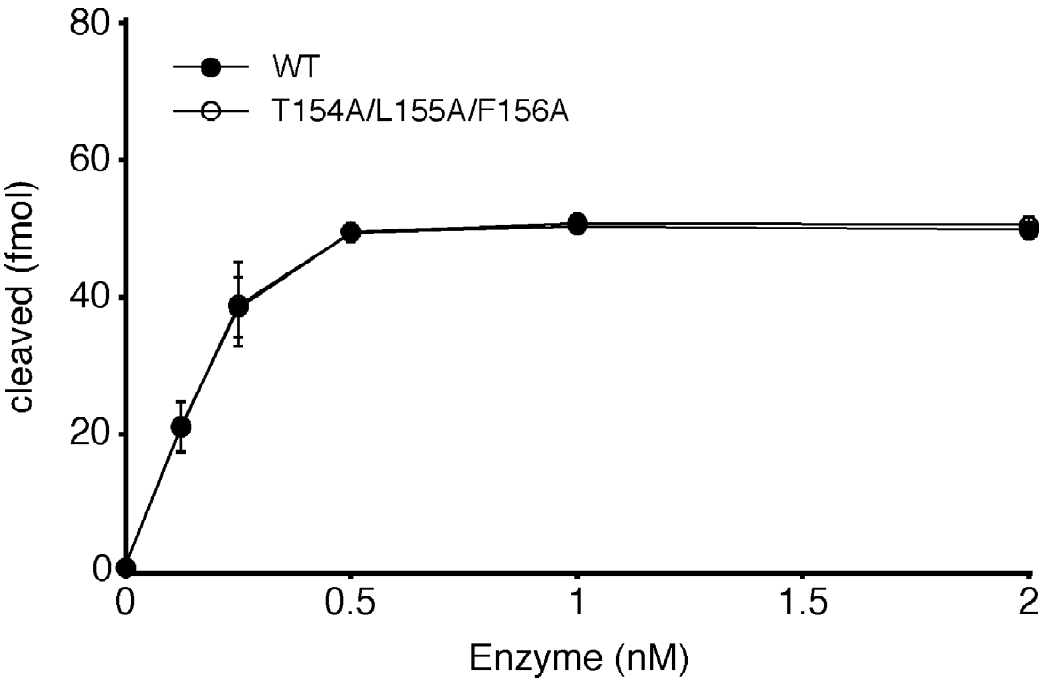
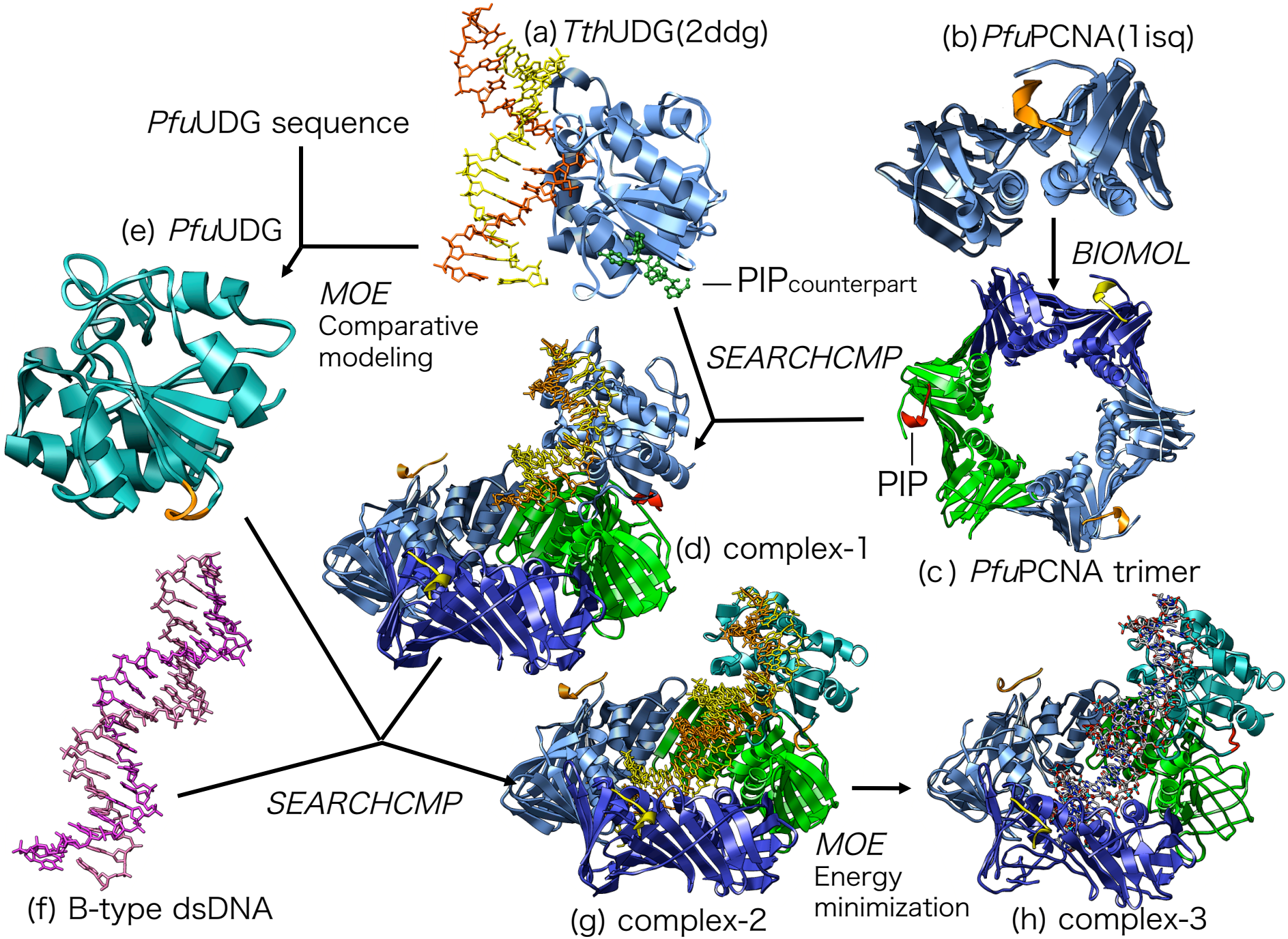

80
60
40
20
0
cleaved (fmol)
WT
T154A/L155A/F156A
0
0.5
1
1.5
2
Enzyme (nM)

Figure S2


(a) *Tth*UDG(2ddg)
(b) *Pfu*PCNA(1isq)
*Pfu*UDG sequence
(e) *Pfu*UDG
MOE
Comparative
modeling
PIP$_{counterpart}$
BIOMOL
SEARCHCMP
PIP
(d) complex-1
(c) *Pfu*PCNA trimer
SEARCHCMP
(f) B-type dsDNA
(g) complex-2
MOE
Energy
minimization
(h) complex-3