Supplemental Experimental Procedures

Secondary structure of TIP protein

Circular dichroism (CD) spectrum was collected on the expressed TIP protein that contained a His-tag (His-TIP) (1) and the TIP peptide (residues 28-64) using a Chirascan CD spectrometer (Applied Photophysics LTD, Surrey, UK). The His-TIP CD data was collected using a 1 mm quartz cell over a wavelength range of 200-280 nm. TIP peptide CD data were collected using a 0.1 mm quartz cell over a wavelength range of 180-280 nm. All CD data were collected at 25°C using 1 nm steps and 5 s averaging per data point. Data were converted to mean residue molar ellipticity (deg cm^2/dmol) after baseline subtraction of the buffer. The His-TIP protein was prepared for CD and NMR by dialyzing into phosphate buffer containing 50 mM $NaPO_4$ (pH 7.2), 0.3 M NaCl. Protein concentrations were determined using UV absorbance at 280 nm. The synthesized TIP peptide was dissolved in water. NMR spectra of uniformly ^{15}N-labeled TIP protein were collected on a Bruker 600 MHz spectrometer equipped with a Cryoprobe (Billerica, MA, USA). The CD spectra of His-TIP and TIP peptide were very similar (data not shown).

The TIP protein is largely unstructured in solution. Secondary structure analysis using circular dichroism showed His-TIP contained ~15% α-helix and ~48% random coil, while TIP peptide (residues 28-64) contains ~22% helix and ~33% random coil (Supplemental Figure S1A) [determined using CDNN (2)]. The lack of much spectral dispersion for the amide resonances in the HSQC spectrum (Supplemental Figure S1B) is consistent with the CD data. TIP does maintain a small amount of helicity by itself, which is apparently stabilized when binding to PCNA. TIP in the PCNA:TIP complex contains 33% α-helix.

Size exclusion chromatography of PCNA and PCNA complexes

Size exclusion chromatography was run prior to SAXS analysis as an additional purification step and to obtain a mono-disperse sample for accurate scattering measurements. Size exclusion chromatography results show that PCNA:TIP has an apparent molecular mass of 39 kDa, indicative of a monomer, whereas the free PCNA protein elutes as 109 kDa, consistent with the expected trimer (Supplemental Table S1). For PCNA:FEN-1 complex, the apparent molecular mass is 105 kDa, a result close to the 109 kDa for the free PCNA trimer (Supplemental Table S1). PCNA proteins and PCNA:peptide complexes were run on an ÄKTA purifier using a

Superdex-200 10/300 GL column (GE Healthcare Bio-Sciences, Pittsburgh, PA) as described in Material and Methods at 0.5 ml/min flow rate at 22°C. Half milliliter samples containing 20-200 μM protein were loaded onto the column and 0.5 ml fractions were collected. Molecular weight was determined using a standard curve and Gel Filtration standard (Bio-Rad, Hercules, CA).

Supplemental Figures and Tables

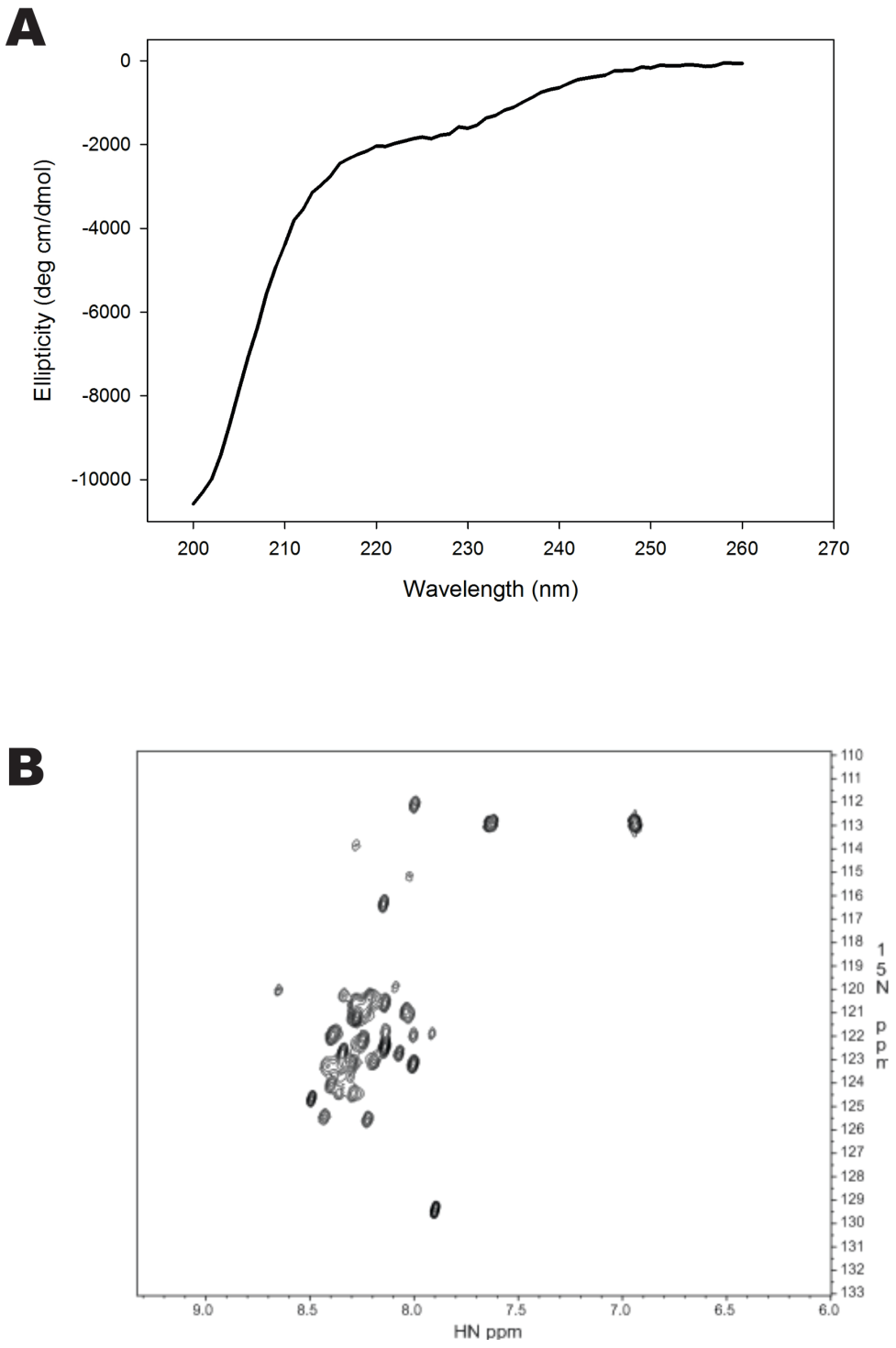

Figure S1: The TIP protein is partially disordered. A. The observed circular dichroism spectrum of His-TIP shows very little secondary structure. B. The ^{1}H-^{15}N HSQC 2D NMR spectrum of the TIP protein in the same buffer. The lack of proton chemical shift dispersion for a 64-residue protein indicates the TIP protein is not well folded.

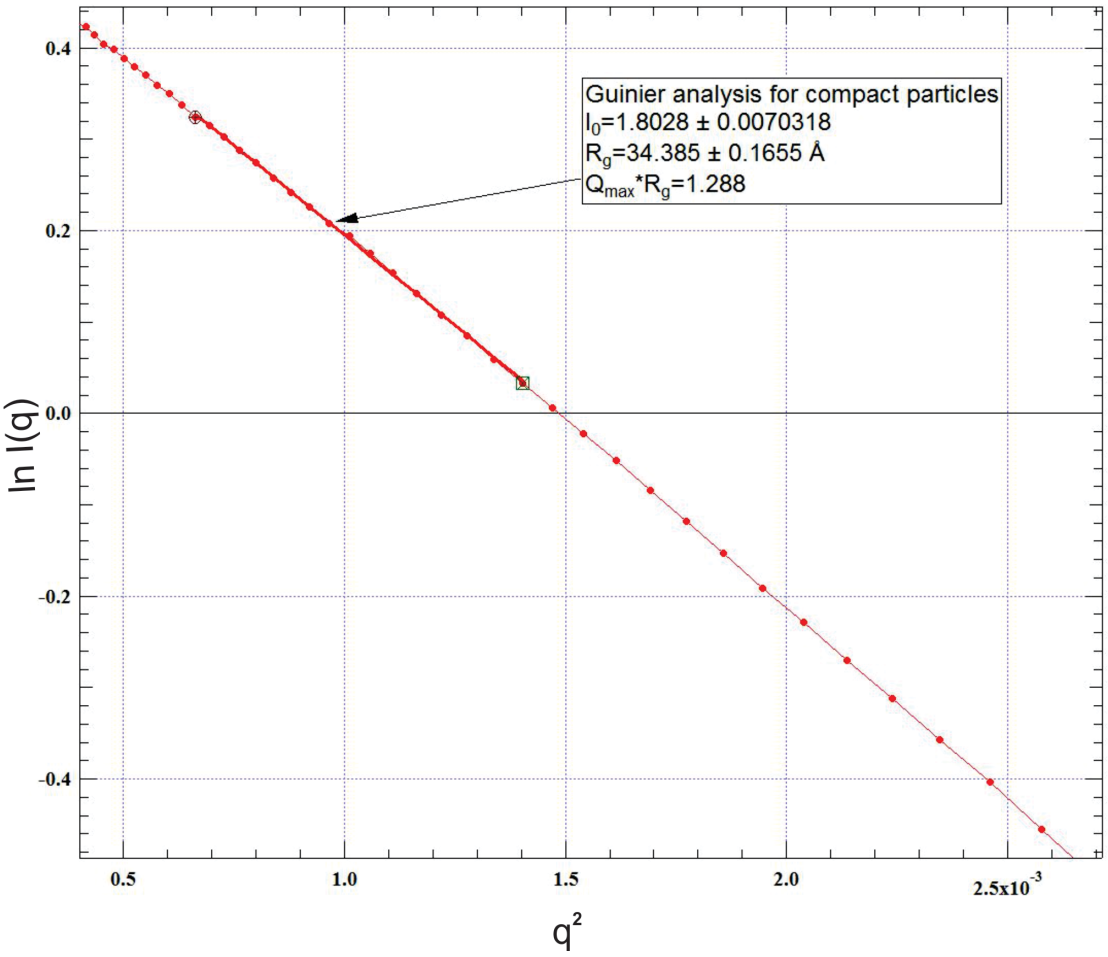

Figure S2: Example of a Guinier fit of the SAXS data to obtain I_o and R_g for PCNA:FEN-1. Guinier fits were obtained by taking the natural logarithm of $I(q)=I_o\exp(-q^2Rg^2/3)$, an approximation that is valid for values of $Q_{max}*R_g < 1.3$.

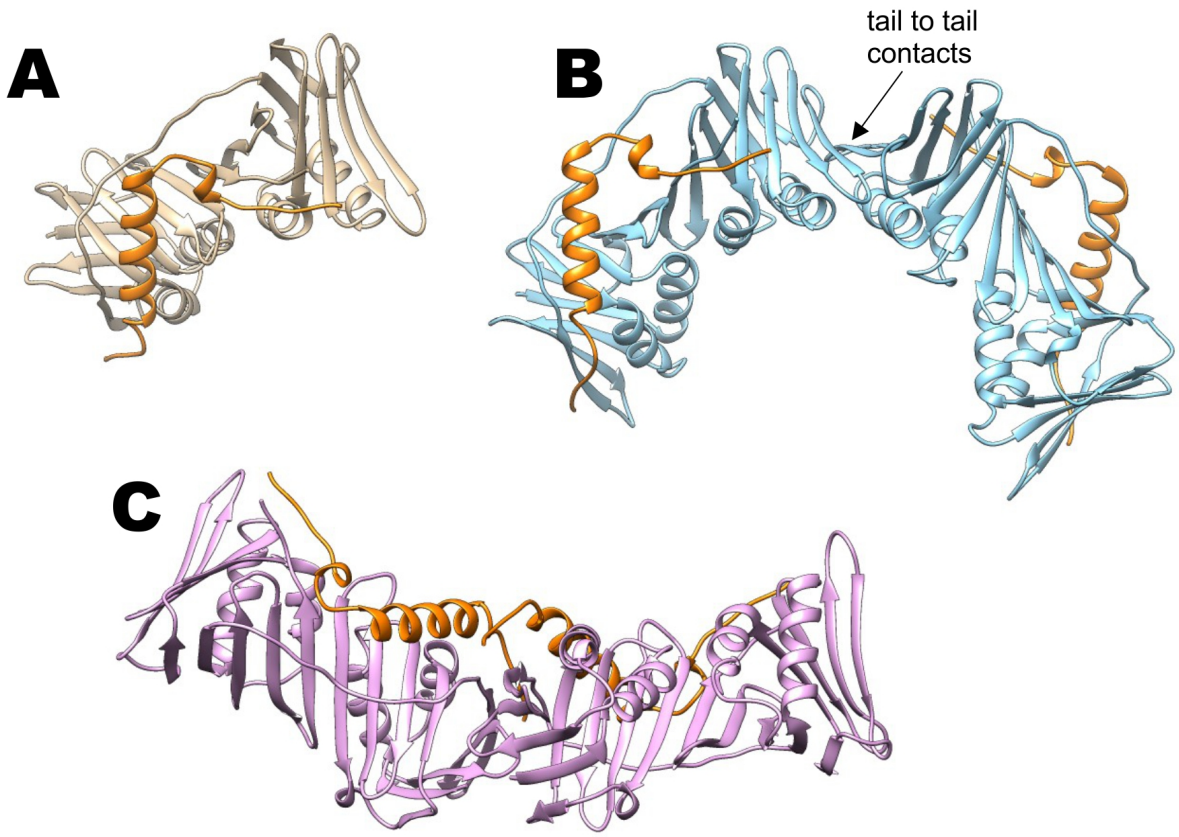

Figure S3. Ribbon figure of the models used in fitting the solution SAXS data for PCNA:TIP. Model A, PCNA:TIP (1:1) complex; Model B, two PCNA:TIP molecules that make crystallographic contacts between neighboring unit cells; Model C, two copies of TIP in the asymmetric unit. Although the protein-protein contacts in Model C do not make stable intermolecular interactions, this model was used as an approximation to a more linear 2:2 complex.

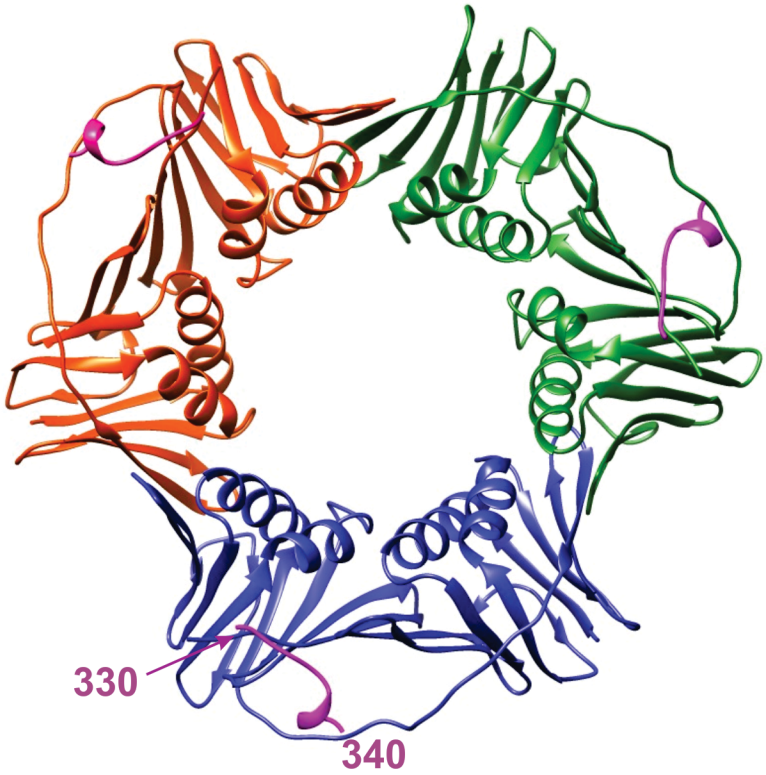

Figure S4. X-ray crystal structure of PCNA bound to the FEN-1 derived peptide. A peptide consisted of residues 330-340 of the *T. kodakarensis* FEN-1 protein (encoded by the TK1281 gene) was crystallized with PCNA (PDBid: 5DAI). The FEN-1 peptides are shown in magenta.

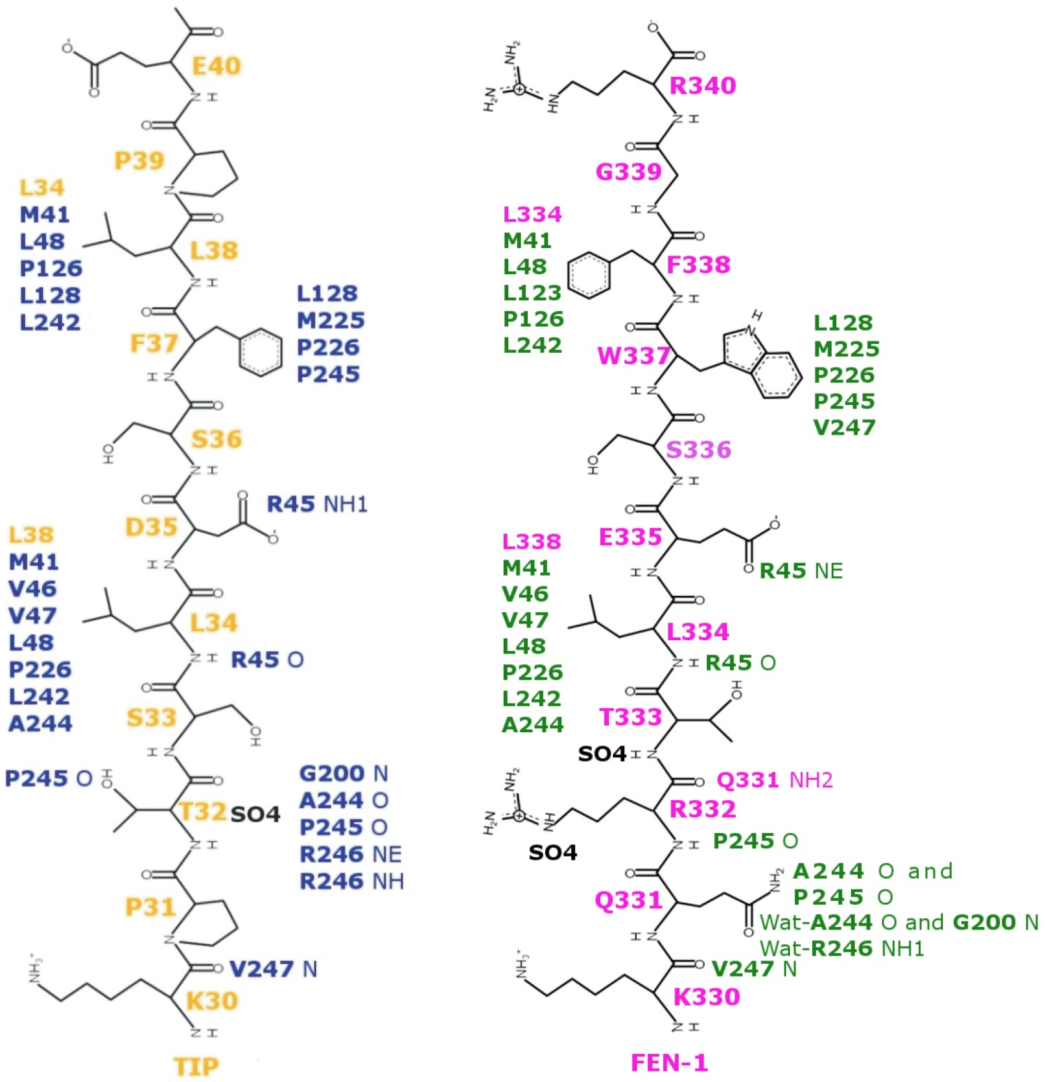

Figure S5. Schematic representation of the canonical (FEN-1) and non-canonical (TIP) PIP motifs interactions in the PIP binding site of PCNA. The central backbone in the figure is the PIP motif. PCNA:TIP is on the left and PCNA:FEN-1 is on the right. Residues labled in gold are TIP residues at the PIP binding site. PCNA residues that interact with the PIP site are labeled in blue for the PCNA:TIP complex. FEN-1 residues are labeled in pink, while PCNA residues interacting with FEN-1 are shown in green.

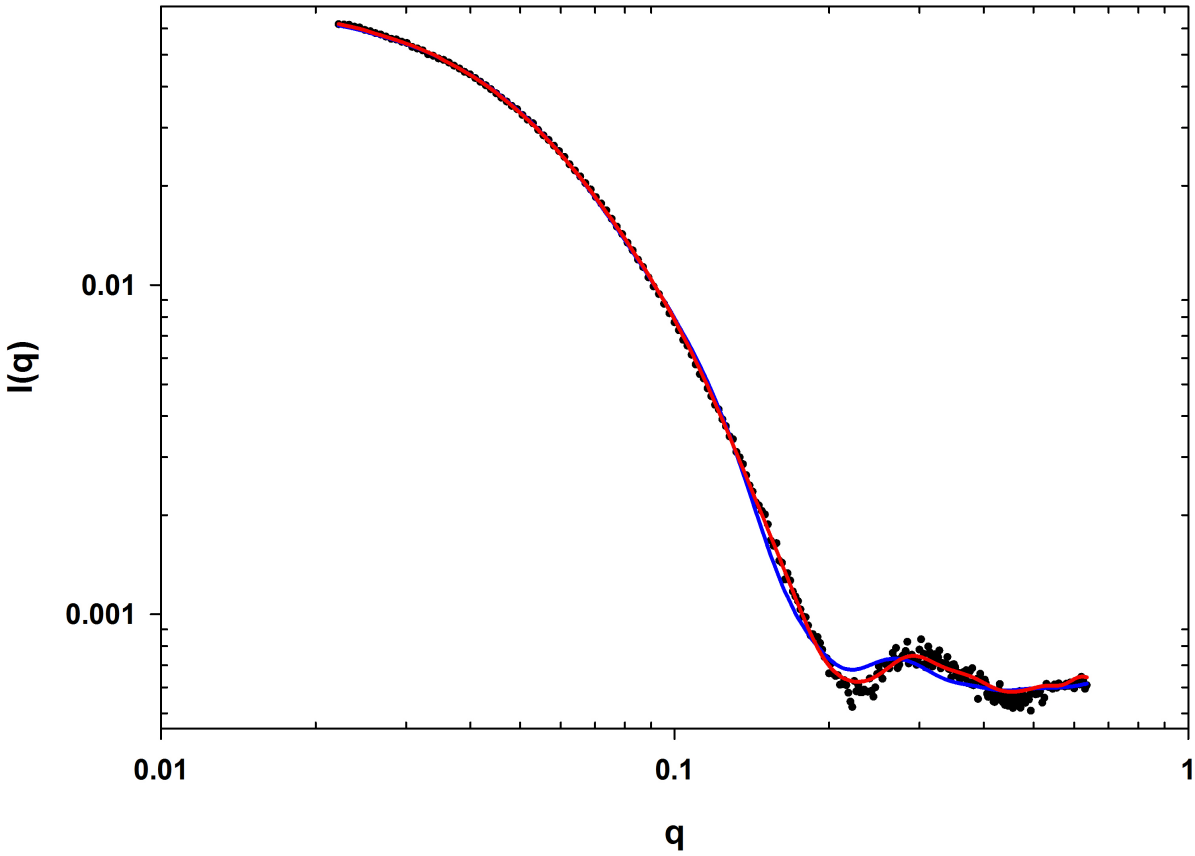

Figure S6. Comparison of modeled SAXS data for an equilibrium fit between Models A and B (blue line, $\chi \sim 0.41$) and Models A and C (red line, $\chi \sim 0.23$) (Supplemental Figure S3). The experimental SAXS data shown here (data points) was collected at 4.1 mg/ml, where the signal to noise is sufficient to distinguish between the models.

Table S1. Size determination of PCNA proteins and complexes.

	Retention time (ml)	MW measured (kDa)	MW calculated (kDa)
PCNA	15.5	109	30
PCNA:TIP (28-64)	17.0	39	34
PCNA:FEN-1 peptide	15.6	105	31
mPCNA	17.4	29	30

Table S2. Invariants from Guinier analysis of experimental SAXS data[1]

	Concentration[2] (mg/ml)	Rg[3] (Å)	Io[3]	Io/c
Monomeric PCNA	1.8	25.1±2.6	0.131±0.002	0.073
PCNA[4]	2.7	35.4±0.2	0.079±0.005	0.029
	2.0	35.4±0.9	0.058±0.001	0.029
PCNA:TIP peptide	6.4	30.4±0.1	0.475±0.004	0.074
	4.8	29.3±2.8	0.326±0.005	0.068
	4.1[4]	32.0±0.3	0.074±0.001	0.018
	2.1[4]	27.7±0.3	0.028±0.001	0.013
PCNA:FEN-1 peptide	7.3	33.4±1.1	1.770±0.007	0.024
	5.5	35.1±0.1	1.340±0.008	0.024
	4.6[4]	37.9±0.5	0.242±0.004	0.053
	2.3[4]	37.2±0.1	0.116±0.003	0.050

[1] The Qmax*Rg was ≤ 1.3 for all Guinier analyses.

[2]The errors in protein concentration are estimated to be 10-20%.

[3]Reported ± error in Rg and Io is the standard deviation from AutoRg in Primus.

[4]SAXS data for PCNA, PCNA:TIP at 4.1 and 2.1 mg/ml and PCNA:FEN-1 at 4.6 and 2.3 mg/ml PCNA:FEN-1 were collected on a different date using a similar experimental setup.

Table S3. Interface areas between PCNA and PCNA complexes in $Å^2$.

Interface between PCNA domains I and II	
PCNA alone	814
With TIP protein	738
With FEN-1 peptide	839
Interface of complexes	
PCNA:TIP protein	1486
PCNA:FEN-1 peptide	581

Supplemental References

1. Ladner, J.E., Pan, M., Hurwitz, J. and Kelman, Z. (2011) Crystal structures of two active proliferating cell nuclear antigens (PCNAs) encoded by *Thermococcus kodakaraensis*. *Proc Natl Acad Sci U S A*, **108**, 2711-2716.

2. Böhm, G., Muhr, R. and Jaenicke, R. (1992) Quantitative analysis of protein far UV circular dichroism spectra by neural networks. *Protein Eng*, ***5***, 191-195.