Electronic Supplementary Information

A cell permeable bimane-constrained PCNA-interacting peptide

Aimee J. Horsfall,[1-3] Beth A. Vandborg,[1,4] Zoya Kikhtyak,[5] Denis B. Scanlon,[1-2] Wayne D. Tilley,[5] Theresa E. Hickey,[5] John B. Bruning,[1,4]* Andrew D. Abell[1-3]*

Experimental

General information

Unless otherwise indicated, all starting materials were purchased from commercial sources and used without further purification. High-resolution mass spectra were collected using an Agilent 6230 ESI-TOF LCMS. RP-HPLC solvents were (A) H_2O with 0.1% TFA and (B) ACN with 0.1% TFA. Purity of all compounds was confirmed by analytical RP-HPLC on an Agilent 1260 HPLC equipped with a Phenomenex Luna C18(2) column (250 x 4.6 mm) over a gradient of 5-50% B (15 min). Purification was carried out by semi-preparative HPLC on a Gilson GX-Prep RP-HPLC system on a Phenomenex Aeris Peptide C18 (10 x 250 mm), over a gradient as specified in the individual compound sections. All graphs were generated using GraphPad Prism 8 software.

Synthesis & Characterisation

All peptides were synthesised by the Fmoc solid-phase peptide synthesis protocol detailed below, with all L-amino acids (unless otherwise specified), and then *N*-terminally acetylated before cyclisation on-resin. Peptides were subsequently cleaved from the resin (and simultaneously globally deprotected). See Scheme 1 and the following general procedures.

H-RQTSMTDFYHSK-NH_2

Solid-phase Peptide Synthesis of peptide 1: Rink Amide PL resin (0.2 mmol, 644 mg, 0.31 mmol/g, Agilent) was swollen in 1:1 DMF/DCM (15 mL) for 15 min. The Fmoc-protecting group was removed by treatment of the resin with a solution of 20% piperidine and 0.1 M HOBt in DMF (8 mL) for 15 min. The solution was drained and the resin washed with DMF (3 x 8 mL). Amino-acid couplings were achieved by addition of a solution of Fmoc-protected amino-acid (5 equiv), HATU (5 equiv) and DIPEA (10 equiv) in DMF (8 mL), to the resin and stirred intermittently for 1 h. The solution was drained and the resin washed with DMF (5 x 8 mL). The *N*-terminal Fmoc-protecting group was removed by treatment of the resin with a solution of 20% piperidine and 0.1 M HOBt in DMF (8 mL) for 10 min, the solution was drained and the resin washed with DMF (5 x 8 mL). A TNBS test* was used to verify each coupling (negative/colourless) and deprotection (positive/red) step, with steps repeated as necessary. Successive couplings and Fmoc-deprotections were repeated to achieve the desired sequence. The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 20-25% over 15 min. Pure fractions were combined and lyophilized to give the final purified product as a white fluffy powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{64}H_{98}N_{20}O_{20}S$: 500.5740, observed: $[M+3H]^{3+}$ 500.5741. RP-HPLC purity (C18, 215 nm) 95.1%.

**TNBS Test:*[1] A small spatula of swollen resin taken out and 1 drop each of TNBS (100 μL 5% w/v picrylsulfonic/trinitrobenzenesulfonic acid in H_2O added to 900 μL of DMF) and DIPEA solutions (100 μL in 900 μL of DMF) added and allowed to develop for 1 min. Clear/yellow beads indicated no free amine (negative), while red/orange beads showed free amine was present (positive).

General Procedure for Cleavage & Isolation:

The peptide was cleaved from the resin by addition of 92.5:2.5:2.5:2.5 TFA/TIPS/DODT/H_2O (10 mL) and rocked for 2 h. The TFA solution was pipetted from the resin and concentrated to 0.5-1 mL under a nitrogen stream, then peptide precipitated with diethyl ether (10 mL) and the mixture cooled to -20°C. The precipitate was pelleted by centrifugation (7600 rpm, 10 min), the supernatant decanted. The pellet was dried under a nitrogen stream, and then dissolved in 1:1 ACN/H_2O, before syringe filtering (0.2 μm) and lyophilised.

Solid-phase Peptide Synthesis of linear on-resin precursor peptide 2a*:*

[Ac-R(Pbf)Q(Trt)C(Mmt)S(tBu)MT(tBu)C(Mmt)FY(tBu)H(Trt)S(tBu)K(Boc)-RESIN]

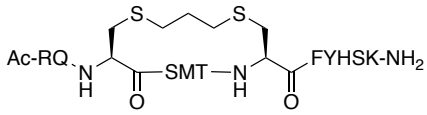
Rink Amide PL resin (0.2 mmol, 644 mg, 0.31 mmol/g, Agilent) was swollen in 1:1 DMF/DCM (15 mL) for 15 min. The Fmoc-protecting group was removed by treatment of the resin with a solution of 20% piperidine and 0.1 M HOBt in DMF (8 mL) for 15 min. The solution was drained and the resin washed with DMF (3 x 8 mL). Amino-acid couplings were achieved by addition of a solution of Fmoc-protected amino-acid (5 equiv), HATU (5 equiv) and DIPEA (10 equiv) in DMF (8 mL), to the resin and stirred intermittently for 1 h. The solution was drained and the resin washed with DMF (5 x 8 mL). The *N*-terminal Fmoc-

protecting group was removed by treatment of the resin with a solution of 20% piperidine and 0.1 M HOBt in DMF (8 mL) for 10 min, the solution was drained and the resin washed with DMF (5 x 8 mL). A TNBS test* was used to verify each coupling (negative/colourless) and deprotection (positive/red) step, with steps repeated as necessary. Successive couplings and Fmoc-deprotections were repeated to achieve the desired sequence. After the final Fmoc-deprotection, the *N*-terminus was protected with an acetyl functionality by reaction with acetic anhydride (470 μL) and DIPEA (870 μL) in DMF (10 mL) for 15 min. The resin was washed with DMF (3 x 8 mL), and DCM (5 x 8 mL).

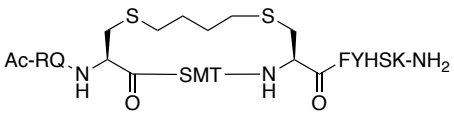
For peptides **3**-**7**, the cysteine side-chains were selectively deprotected: Mmt groups were removed by repetitive treatment of the resin with 2% TFA in DCM (8 mL) for 1 min, followed by washing with DCM (3 x 8 mL). Treatments were repeated until the solution no longer turned yellow on addition to the resin (~ 150-200 mL total). The resin was then further washed with DCM (5 x 8 mL) and DMF (5 x 8 mL).

Ac-RQTCMTCFYHSK-NH_2

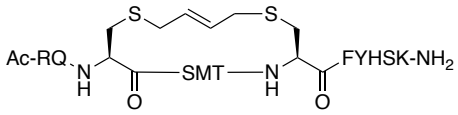
Peptide 2**:* Following peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to* ***2a' and *N*-terminal acetylation, the peptide was cleaved from the resin by *General Procedure for Cleavage and Isolation* to give peptide **2**. The crude peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **2** as a pale yellow fluffy powder. HRMS (ESI+) Expected $[M+4H]^{4+}$ for $C_{64}H_{98}N_{20}O_{18}S_3$: 383.6778, observed: $[M+4H]^{4+}$ 383.6702. [5]RP-HPLC purity (C18, 215 nm) 88.9%.



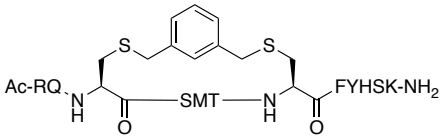
Peptide 3, propyl thioether cyclisation**:*[2] Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to* ***2a' the preswelled resin (0.1 mmol) with resin-bound *N*-terminally acetylated peptide and cysteines freshly deprotected, was transferred to a MW vessel with stirrer bar. NaI (17.5 equiv, 225 mg) in DMF (6 mL) was then added and the mixture stirred continuously while TCEP (0.5 equiv, 300 mg/ml, 18.9 μL) was then added and the vessel sealed and bubbled with N_2 for 15 min. Under a N_2 atmosphere, DIPEA (35 equiv, 274 μL) was added and the mixture stirred and bubbled with N_2 for a further 20 min. 1,3-Dibromopropane (3.5 equiv, 16 μL) was then added and the vessel sealed and reacted under MW for 2 min at 125°C. The vessel was then cooled, the resin removed and solution drained. The resin was washed with H_2O (5 x 5 mL), DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **3** as a white powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{67}H_{102}N_{20}O_{18}S_3$: 524.5692, observed: $[M+3H]^{3+}$ 524.5718. RP-HPLC purity (C18, 215 nm) 91.9%.



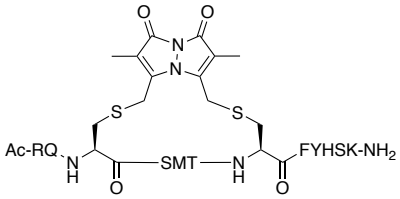
Peptide 4, butyl thioether cyclisation**:*[2] Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to* ***2a' the preswelled resin (0.1 mmol) with resin-bound *N*-terminally acetylated peptide and cysteines freshly deprotected, was transferred to a MW vessel with stirrer bar. NaI (17.5 equiv, 225 mg) in DMF (6 mL) was then added and the mixture stirred continuously while TCEP (0.5 equiv, 300 mg/ml, 18.9 μL) was then added and the vessel sealed and bubbled with N_2 for 15 min. Under a N_2 atmosphere, DIPEA (35 equiv, 274 μL) was added and the mixture stirred and bubbled with N_2 for a further 20 min. 1,4-Dibromobutane (3.5 equiv, 19 μL) was then added and the vessel sealed and reacted under MW for 2 min at 125°C. The vessel was then cooled, the resin removed and solution drained. The resin was washed with H_2O (5 x 5 mL), DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **4** as a white powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{68}H_{104}N_{20}O_{18}S_3$: 529.2416, observed: $[M+3H]^{3+}$ 529.2410. RP-HPLC purity (C18, 215 nm) 91.8%.



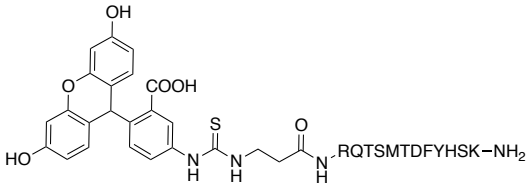
Peptide 5, trans-butene cyclisation: Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to* ***2a***' the preswelled resin (0.1 mmol) with resin-bound *N*-terminally acetylated peptide and cysteines freshly deprotected, was treated with a solution of trans-1,4-dibromo-2-butene (2 equiv, 42.8 mg) and DIPEA (4 equiv, 174 μL) in DMF (4 mL) was added to the resin, and reacted for 3 h with intermittent stirring. The solution was then removed and the resin washed with DMF (5 x 5 mL) and DCM (5 x 5 mL). In the case that a small cleave of the resin revealed incomplete reaction, the procedure was repeated as above and left to react overnight. The solution was then removed and the resin washed with DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **5** as a white and brown powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{68}H_{102}N_{20}O_{18}S_3$: 528.5692, observed: $[M+3H]^{3+}$ 528.5712. RP-HPLC purity (C18, 215 nm) 94.4%.



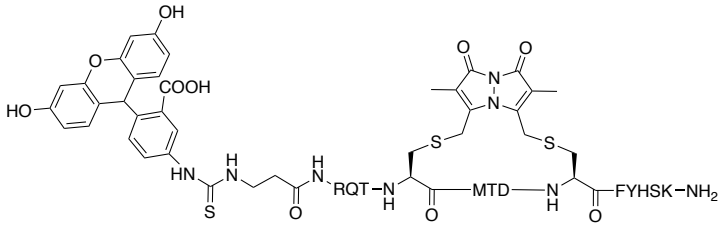
Peptide 6, xylene cyclisation: Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to* ***2a***' the preswelled resin (0.1 mmol) with resin-bound *N*-terminally acetylated peptide and cysteines freshly deprotected, was treated with a solution of dibromo-m-xylene (2 equiv, 47.4 mg) and DIPEA (4 equiv, 174 μL) in DMF (4 mL) was added to the resin, immediately following the cysteine Mmt group removal and washing procedure, and reacted for 3 h with intermittent stirring. The solution was then removed and the resin washed with DMF (5 x 5 mL) and DCM (5 x 5 mL). In the case that a small cleave of the resin revealed incomplete reaction, the procedure was repeated as above and left to react overnight. The solution was then removed and the resin washed with DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **6** as a white fluffy powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{72}H_{104}N_{20}O_{18}S_3$: 545.2411, observed: $[M+3H]^{3+}$ 545.2436. RP-HPLC purity (C18, 215 nm) 91.5%.



Peptide 7, bimane cyclisation:[3] Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to* ***2a***' the preswelled resin (0.1 mmol) with resin-bound *N*-terminally acetylated peptide and cysteines freshly deprotected, was treated with a solution of dibromobimane (2 equiv, 70 mg) and DIPEA (4 equiv, 174 μL) in DMF (6 mL), and reacted for 3 h with intermittent stirring. The solution was then removed and the resin washed with DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **7** as a pale yellow fluffy powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{74}H_{106}N_{22}O_{20}S_3$: 573.9119, observed: $[M+3H]^{3+}$ 573.9140. RP-HPLC purity (C18, 215 nm) 97.3%.



Peptide 8, fluorescein attachment: Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to **1***' the preswelled resin (0.1 mmol) with resin-bound peptide with *N*-terminal free amine, was coupled to Fmoc-β-alanine (5 equiv) with HATU (5 equiv) and DIPEA (10 equiv) in DMF for 1 h with intermittent stirring. The solution was then drained from the resin, and the Fmoc group removed by treatment of the resin with 20% piperidine in DMF (5 mL) for 10 min with intermittent stirring, and the resin then washed with DMF (3 x 5 mL), and subsequently treated with fluorescein isothiocyanate (2 equiv, 78 mg) and DIPEA (4 equiv, 70 μL) in DMF (4 mL) and stirred intermittently for 20 min. The solution was then drained and the resin thoroughly washed with DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Luna C18(2) Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **8** as a yellow fluffy powder. HRMS (ESI+) Expected $[M+4H]^{4+}$ for $C_{88}H_{114}N_{22}O_{26}S_2$: 490.7007, observed: $[M+3H]^{3+}$ 490.6728. RP-HPLC purity (C18, 215 nm) 93.6%.



Peptide 9, bimane cyclisation and fluorescein attachment:[3] Following linear peptide assembly as described in '*Solid-phase Peptide Synthesis of linear on-resin precursor peptide to **2a***' the preswelled resin (0.1 mmol) with the exception that the N-terminal Fmoc was not removed prior to the cysteine Mmt deprotection. The resin-bound peptide, with N-terminal Fmoc and cysteines freshly deprotected, was treated with a solution of dibromobimane (2 equiv, 70 mg) and DIPEA (4 equiv, 174 μL) in DMF (6 mL), and reacted for 3 h with intermittent stirring. The solution was then removed and the resin washed with DMF (5 x 5 mL) and then treated with 20% piperidine in DMF (5 mL) for 10 min with intermittent stirring. The solution was then drained and the resin washed with DMF (3 x 5 mL) and then a solution of Fmoc-β-alanine (5 equiv), HATU (5 equiv) and DIPEA (10 equiv) in DMF added and stirred intermittently for 1 h. The solution was then drained from the resin, and the Fmoc group removed by treatment of the resin with 20% piperidine in DMF (5 mL) for 10 min with intermittent stirring, and the resin then washed with DMF (3 x 5 mL), and subsequently treated with fluorescein isothiocyanate (2 equiv, 78 mg) and DIPEA (4 equiv, 70 μL) in DMF (4 mL) and stirred intermittently for 20 min. The solution was then drained and the resin thoroughly washed with DMF (5 x 5 mL) and DCM (5 x 5 mL), then dried with diethyl ether (3 x 5 mL). The peptide was then cleaved from the resin as described by *General Procedure for Cleavage and Isolation*. The cyclised peptide was purified by semi-preparative RP-HPLC and Phenomenex Aeris Peptide C18 Column (10 x 250 mm) over a linear gradient of 25-50% B (15 min). Pure fractions were combined and lyophilized to give the final purified peptide **7** as a yellow powder. HRMS (ESI+) Expected $[M+3H]^{3+}$ for $C_{97}H_{122}N_{24}O_{24}S_4$: 712.6059, observed: $[M+3H]^{3+}$ 712.5945. RP-HPLC purity (C18, 215 nm) 93.4%.

SPR Protocol:

The running buffer used for ligand attachment and analyte binding experiments was 10 mM HEPES buffer with 150 mM NaCl, 3 mM EDTA and 0.05% Tween20, adjusted to pH 7.4 with 2 M NaOH. Experiments were performed on a GE S200 Biacore System at 25°C. A GE CM5 sensor chip was primed with running buffer and preconditioned per the manufacturer's recommendation with successive injections (2 x 50 s, 30 μL/min) of each 50 mM NaOH, 10 mM HCl, 0.1% SDS, 0.85% H_3PO_4 and glycine pH 9.5. The surface was then activated with a solution of 0.2 M EDC and 50 mM NHS (600 s, 10 μL/min).

hPCNA (5 μL, 12 mg/mL) was diluted into running buffer (245 μL). Only once the preactivation was complete was the protein further diluted to a final concentration of 25 μg/mL in 10 mM NaAc (~pH 4.6) by addition of hPCNA/HEPES (50 μL) to a solution of 100 mM NaAc (50 μL) and water (400 μL). This solution was immediately injected over only the second flow cell (10 μL/min) until the ~1500 RU was reached at stabilisation. Both flow cells were then blocked with 1.0 M ethanolamine pH 8.5 (600 s,

10 µL/min). The chip was left to stabilise for two hours before sample injections commenced. After stabilisation a final protein level of 1380 RU was achieved.

Peptides (approx. 2 mg by weight) were dissolved in milliQ H_2O (50 µL) and centrifuged (7800 rpm, 10 min) to remove any particulate. The peptide stock concentration was determined by UV absorbance (A_λ), where 2 µL of the stock was further diluted in water (20-40 fold) and a measurement taken in triplicate with a Nanodrop2000 and baselined to 750 nm absorbance. The peptide stock solution concentration was then calculated per $c = (A_\lambda/\varepsilon_\lambda.l).DF$ where concentration is in mol/L, A_λ is absorbance at λ nm calculated as an average of three readings, λ is the appropriate wavelength (here, 205 or 380 nm), l is the pathlength in cm (1 mm for Nanodrop), and ε_λ is the molar absorptivity at λ nm and DF is the dilution factor. The ε_{205} was estimated for peptides **1**-**6** using Anthis 2013,[4] and ε_{380} used for bimane-containing peptide **7** as reported in Shen 2011,[5] and are recorded in Table S2. The peptides were then diluted into running buffer before further dilution as necessary.

The steady state affinity experiments were all run at 30 µL/min, a contact time of 30 s and dissociation time of 40 s, followed by regeneration of 2 M NaCl (2 x 30 s). Each peptide was serially diluted (1 in 2) eight times, and run from least to most concentrated following a blank injection. After the concentration range was optimised, the experiment was repeated to ensure reproducibility. The top concentration of the final concentration range for each peptide sample is tabulated in Table S1. The data was analysed using the provided Biacore S200 Evaluation software to give a steady state K_D value and associated standard error, as shown in Table S1.

NMR Analysis

^1H and ^{13}C NMR 1D and 2D spectra were recorded on an a Varian Inova 600 MHz instrument in 10% aq. D_2O at 298K, pH ~5, and referenced to DSS at 0 ppm. ES suppression sequences were used for all ^1H 1D and 2D homonuclear spectra. ROESYAD and zTOCSY were obtained for all compounds. gHMBCAD and gHSQCAD experiments were collected with 256 scans. Chemical shifts are reported in ppm (δ). Full ^1H assignments for peptides **2** and **7** are included in Tables S4 and S5. Secondary shifts for **2** and **7** were calculated relative to the random coil shifts reported by Wishart 2011[6] and nearest neighbour corrections applied (Tables S4 and S5, Fig. S7, green and blue). The secondary shift for **7** were also calculated by subtraction from the linear precursor peptide **2** (Table S6, Fig. S7, purple).

Protein Expression and Purification

A glycerol stock of *E. coli* BL21 (λDE3) cells carrying a hPCNA-pMCSG19 plasmid (with no purification tag) were grown in a 50 mL overnight culture. Eight 1 L baffled flasks of LB with 100 µg/mL of ampicillin were inoculated with 6.3 mL of the overnight culture. Cultures were incubated at 37°C until OD600 of 0.5 and induced with 0.5 mM IPTG. Cultures were grown overnight at 16°C with shaking at 200 rpm. Cultures were pelleted at 5000xg for 20 min. After removing the supernatant, pellets were resuspended in 30 mL of Buffer A (20 mM Tris pH 7.5, 20 mM NaCl, 2 mM DTT), then lysed by 5 rounds of cell disruption by a microfluidics cell disrupter. Lysate was pelleted at 45,000xg for 60 min and the supernatant was collected for purification. hPCNA was purified at 4°C by fast protein liquid chromatography (FPLC), using an anion exchange DEAE column (HiTrap DEAE FF 5 mL column), equilibrated in Buffer A (20 mM Tris pH 7.5, 20 mM NaCl, 2 mM DTT), and protein was eluted using Buffer B (20 mM Tris pH 7.5, 0.7 M NaCl, 2 mM DTT). Fractions were analysed by SDS-PAGE and those of interest indicating containing protein at ~28 kDa were selected and pooled and treated with ammonium sulphate to bring the concentration to 1.5 M. Protein was purified again by a hydrophobic column (HiTrap Phenyl FF [high sub] 5 mL column) equilibrated in Buffer C (20 mM Tris pH 7.5, 20 mM NaCl, 2 mM DTT, 0.5 mM EDTA, 1.5 M Ammonium Sulphate), and protein was eluted using Buffer D (20 mM Tris pH 7.5, 0.5 mM EDTA, 2 mM DTT). Fractions were analysed by SDS-PAGE and those of interest were dialysed overnight in Buffer E (20 mM Tris pH 7.5, 20 mM NaCl, 2 mM DTT). Equilibrated protein was purified using an anion exchange Q Sepharose column (ENrich Q 10 × 100 mm 8 mL column), equilibrated in Buffer F (20 mM Tris pH 7.5, 20 mM NaCl, 2 mM DTT), protein was eluted using Buffer G (20 mM Tris pH 7.5, 0.7 M NaCl, 2 mM DTT). Protein pool was concentrated using a centrifugal filter unit (30 kDa molecular mass cut off) to a volume of less than 10 mL and purified using the size exclusion column (HiPrep 26/60 Sephacryl S-200 HR 300 mL column), equilibrated in Buffer H (20 mM Tris pH 7.5, 50 mM NaCl, 2 mM DTT, 0.5 mM EDTA), and protein was eluted using the same buffer. Fractions were analysed by SDS-PAGE and those of interest were pooled. Fractions were analysed by SDS-PAGE and those of interest were pooled and dialyzed overnight against storage Buffer I (20 mM Tris pH 7.5, 10% glycerol, 2 mM DTT, 0.5 mM EDTA). Protein for crystallography was concentrated to ~10 mg/mL using a centrifugal filter unit (50 kDa molecular mass cut off) and stored at -80°C.

Protein-peptide co-crystallisation experiments

hPCNA was mixed with peptide of interest at 1:1.2 molar ratio, and after incubation on ice for 30 minutes, the sample was pelleted at 16,000×g for 10 min to remove aggregates. The supernatant containing peptide bound protein was stored at -80°C.

Crystals were grown by hanging drop vapor diffusion method in 24-well limbro plates containing 500 µL well solution, by mixing 1 µL protein and peptide with equal volume of well solution.[7-9] Diffracting crystals of hPCNA bound to **5** were formed in 0.18 M magnesium acetate + 19.5% (polyethylene glycol) PEG 3350 at a temperature of 16°C after one and a half weeks. Diffracting crystals of hPCNA bound to **3** were formed in 0.18 M magnesium acetate 19.2% PEG 3350 at a temperature of 16°C after one and a half weeks. Diffracting crystals of hPCNA bound to **6** were formed in 0.18 M magnesium acetate + 19.3% PEG 3350 at a temperature of 16°C after one and a half weeks. Crystals were mounted on cryoloops, cryoprotected using paratone-N, and flash cooled in liquid nitrogen.[7-9] Data was collected at 100 K using the MX2[10] (peptide **3**) and MX1[11] (peptides **5** and **6**) beamline at the Australian Synchrotron (Clayton, Vic). Diffraction data was indexed and integrated using XDS (X-ray Detector Software).[12] Pointless (CCP4i)[13] was used to create a mtz file for scaling. Data was scaled using Aimless (CCP4i) to a resolution of 3.30 Å for **5**, 3.30 Å for **3** and 2.80 Å for **6**. Phasing was solved by molecular replacement using Phaser MR (CCP4i)[14] using a search model (PDB: 1AXC, human).[15] Solutions were refined in phenix.refine[16] in iterative rounds with manual rebuilding in Coot.[17, 18] Data collection and refinement statistics for hPCNA in complex with **3**, **5**, or **6** are summarised in Table S2. The final structures are deposited on the RCSB database under accession numbers 7M5L, 7M5M and 7M5N, respectively.

Computational modelling

The model of peptide **1** bound to hPCNA was constructed using the solved structure of hPCNA bound with $p21_{141-155}$ peptide as a starting template (PDB ID: 7KQ1), and the necessary residues deleted and unresolved side-chains of residues were modelled into the computational structure.

Models of hPCNA and peptide structures **4** and **7** were constructed by drawing the peptide structure in ChemDraw 18.0. Using SMILES, the peptide structure was made into a pdb through phenix eLBOW.[19] The .pdb and .cif files were used to dock the peptide **4** or **7**, in place of peptide **3** in the .mtz map of the hPCNA-bound **3** (PDB code: 7M5L), replacing the propyl-linker peptide **3** with the computational peptide. Manual refinement of the computational linker into the .mtz map was done in Coot. Energy minimisation/annealing (n=30) for refinement was carried out in ICM-Pro Molsoft.[20, 21] Refined models were analysed using PyMOL[22] to validate the model by comparing against the $p21_{141-155}$ structure (7KQ1), and assess side-chain interactions. The resulting structures were visualised in PyMOL and are depicted in Figures S1-S6. Additional analysis was carried out using the RING server[23] and PoseView[24].

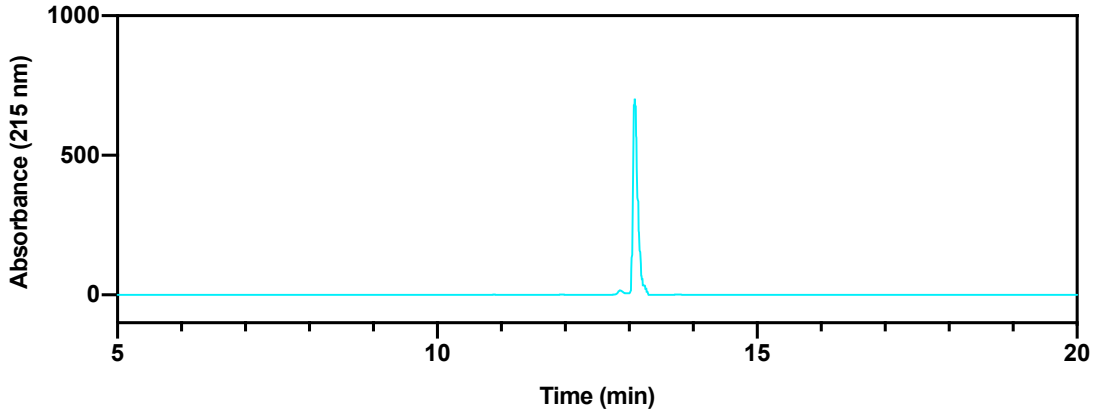
Cell imaging

MDA-MB-468 mKate is a breast cancer cell line that was lenti-virally modified to stably express nuclear fluorescent (ex 588nm; em 635nm) mKate protein. Cells were maintained in DMEM (Sigma, D5671) base media supplemented with 2 mM L-Glutamine (Sigma, G7513) and 1 mM Sodium Pyruvate (Sigma, S8636) at 37°C and 5% CO_2. For experiments, cells were seeded at ~70% confluency onto glass coverslips inside wells of a 6-well culture plate containing 2 mL of media. The cells were cultured for 48 h to allow for attachment, then treated 24 h with 10 µM peptide **7** or **8** while being protected from light to sustain the florescent signal. After treatment, cells were washed with ice cold PBS (Gibco, 14190144) two times for 5 min to remove residual media and peptide. Cells were fixed with 4% PFA (10% Neutral Buffered Formalin, ChemSupply, #1258) for 10 min at rt, followed by two 5 min wash steps with PBS at rt. Coverslips were then mounted onto microscope slides using DAKO fluorescent mounting medium (S302380-2) and sealed with clear nail polish (Sally Hansen). Slides were allowed to dry overnight. The following day, samples were imaged using a Confocal Olympus FV3000 microscope (Adelaide Microscopy).

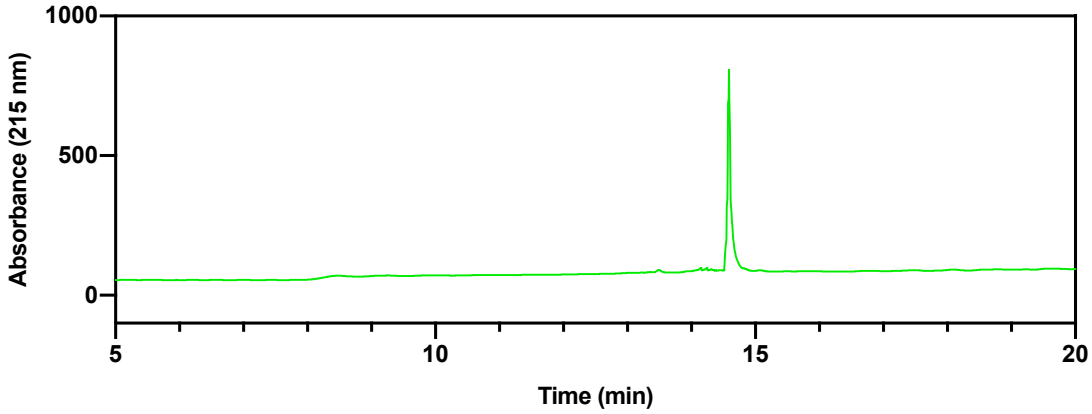
Cell nuclei were visualised using a 594 nm laser with a detection range of 600-700 nm. A 488 nm laser with a detection range of 490-534 nm was used for peptide **8**, which contains a FITC fluorophore. A 405 nm laser with a detection range of 410-485 nm was used for peptide **7**, which contains the Bimane fluorophore. Images were taken with a 30x silicon oil objective, with a 2x zoom setting with imaging software (Olympus, Cell Sens), bringing the total image magnification to 60x.

Supplementary Information

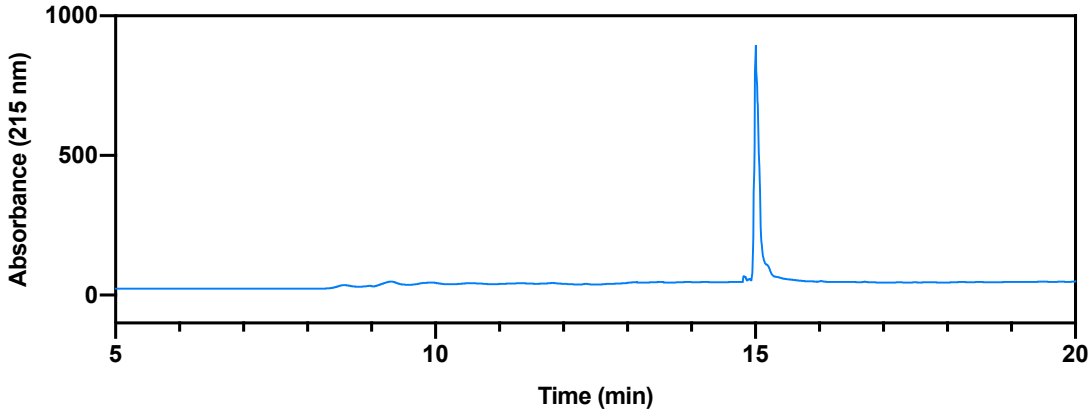
HPLC spectra



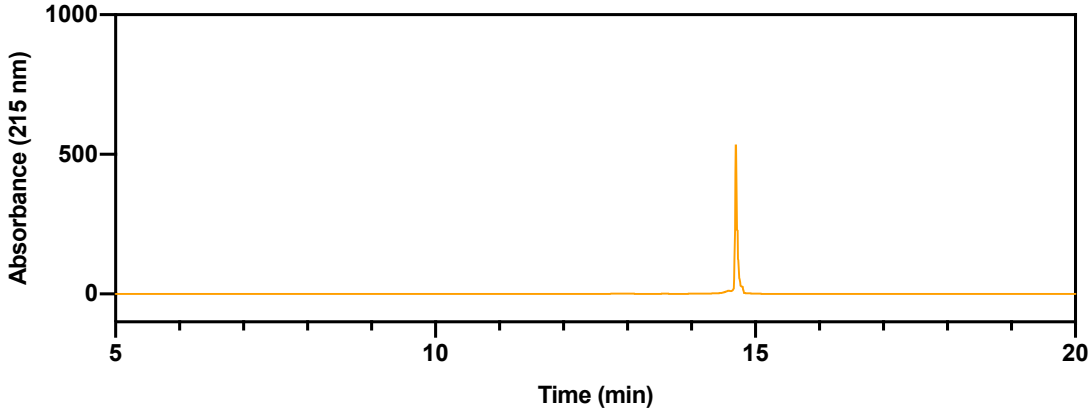
Analytical HPLC spectrum of peptide 1 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.



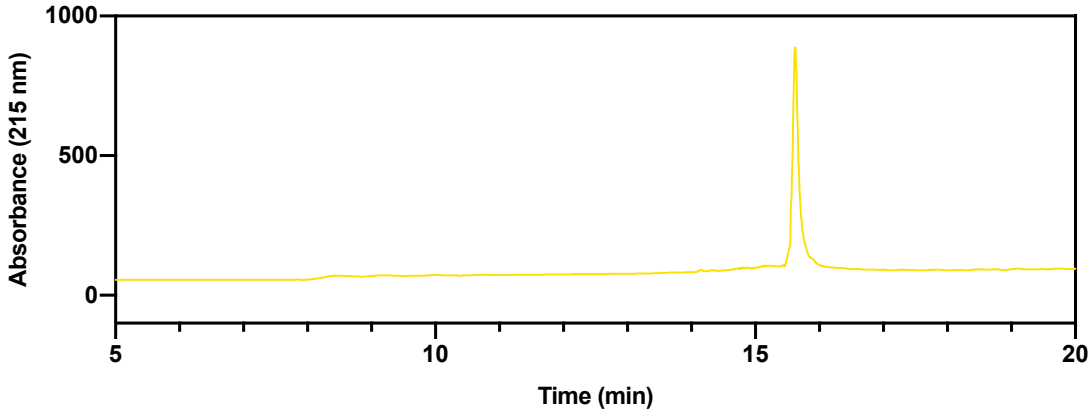
Analytical HPLC spectrum of peptide 3 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.



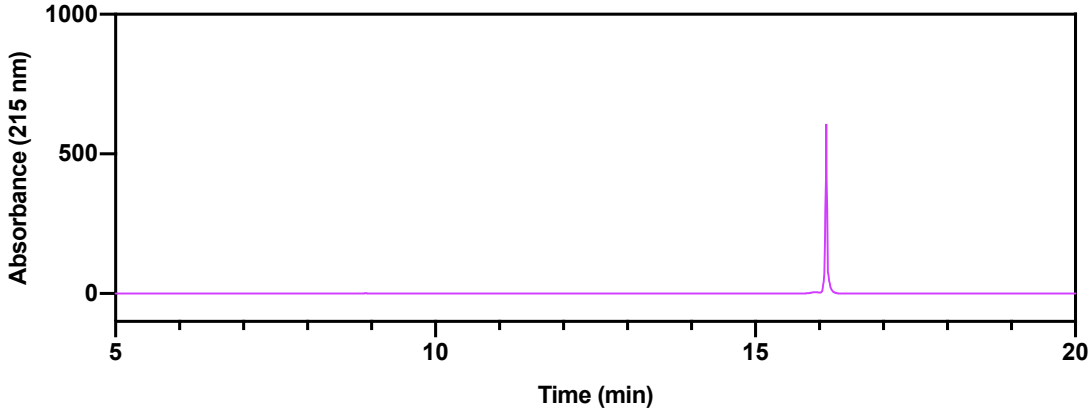
Analytical HPLC spectrum of peptide 3 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.



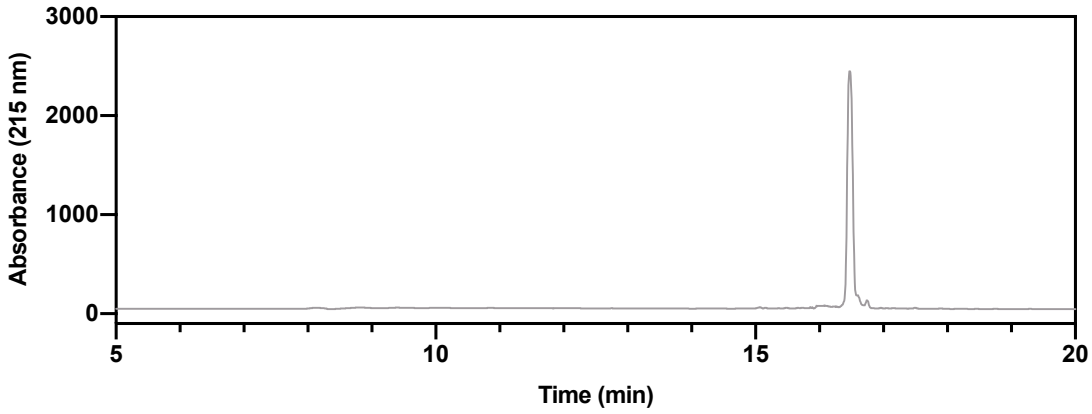
Analytical HPLC spectrum of peptide 5 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.



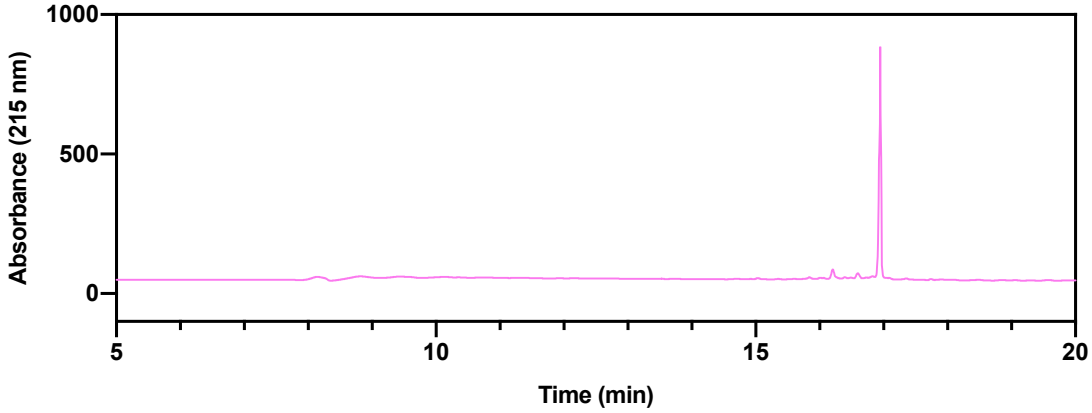
Analytical HPLC spectrum of peptide 6 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.



Analytical HPLC spectrum of peptide 7 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.

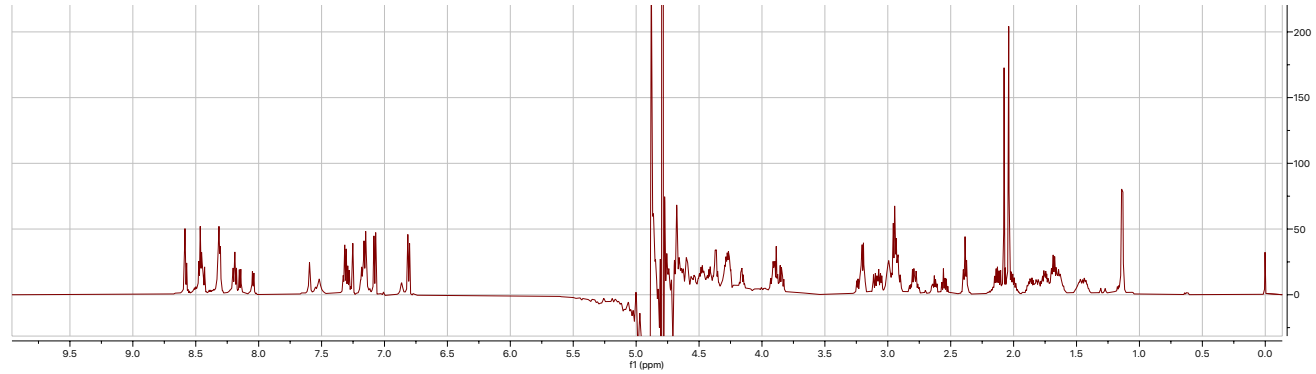


Analytical HPLC spectrum of peptide 8 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.

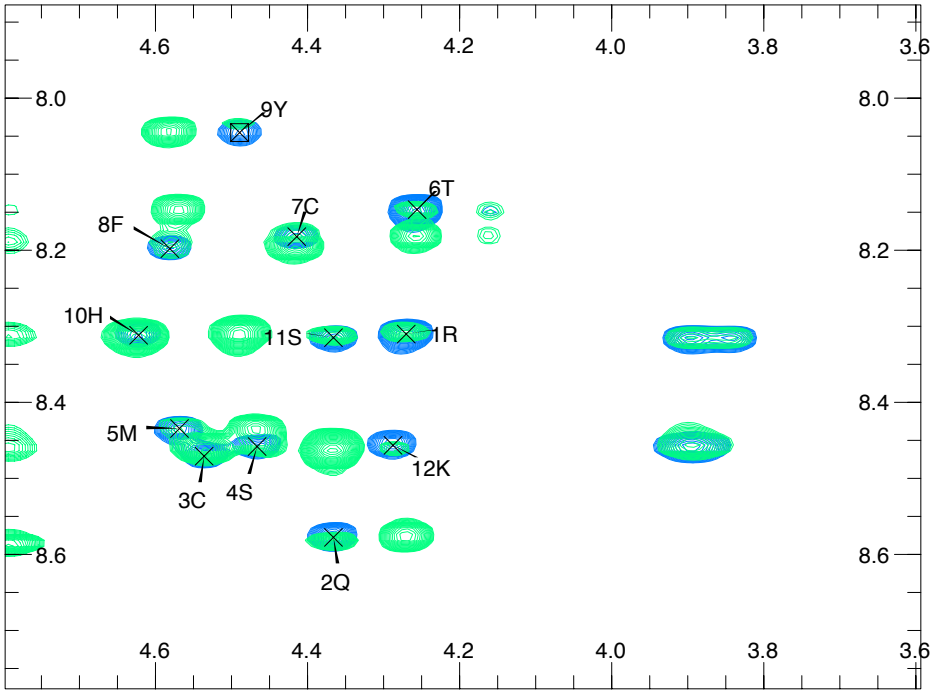


Analytical HPLC spectrum of peptide 9 at 215 nm. Collected over a gradient of 5-50% ACN with 1% TFA, in water with 1% TFA, over 15 minutes (between 5-20 min) at 1.5 mL/min.

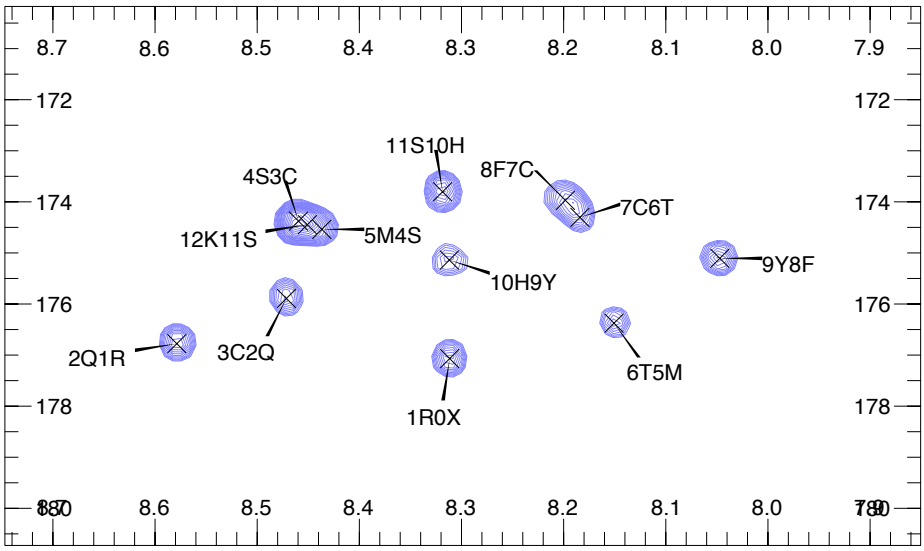
NMR Characterisation



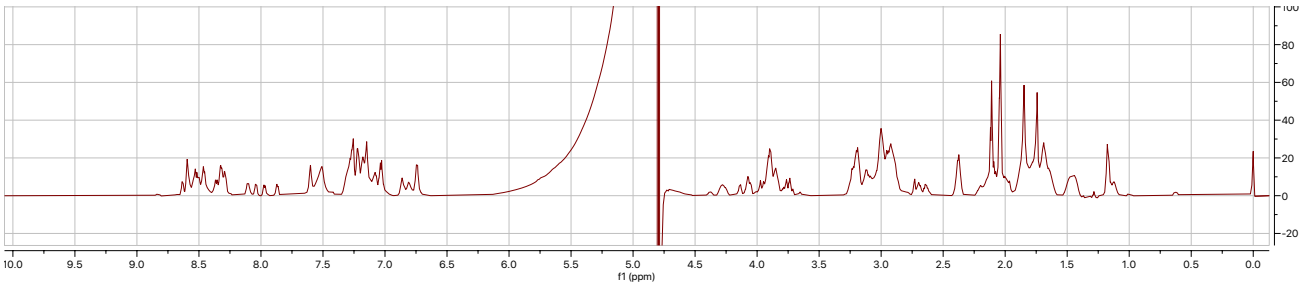
1D ^{1}H waterES spectrum for peptide 2, referenced to DSS at 0 ppm



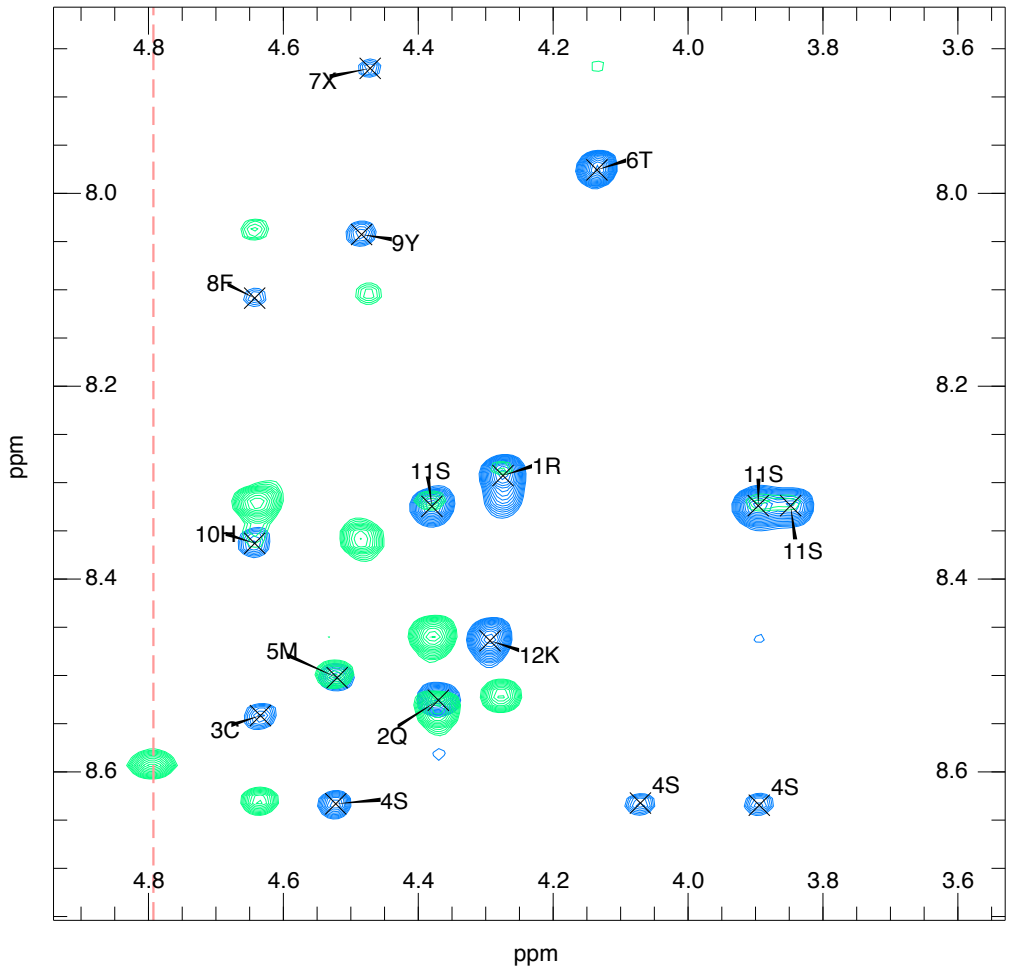
2D ^{1}H TOCSY (blue) and ROESY (green) for peptide 2 in the mainchain region. Assignments of TOCSY spectrum shown. Green cross peaks at 4.5 ppm (y-axis, F2) correspond to the residual HDO peak.



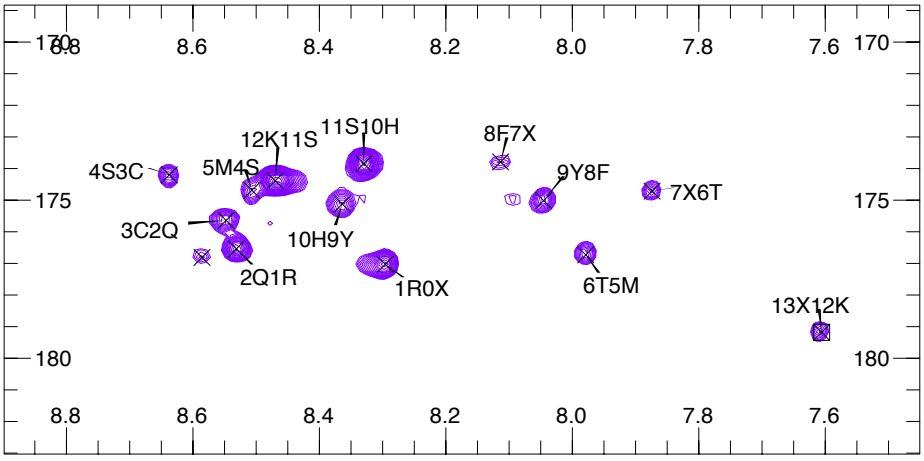
2D heteronuclear HMBC for peptide 2 in the mainchain region. Assignments are NH/CO (f2/f1).



1D ^{1}H wet1D spectrum for peptide 7, referenced to DSS at 0 ppm



2D ^{1}H TOCSY (blue) and ROESY (green) for peptide 7 in the mainchain region. Red dotted line indicates HDO crosspeaks**.** Assignments of TOCSY spectrum shown.



2D heteronuclear HMBC for peptide 7 in the mainchain region. Assignments are NH/CO (f2/f1).X represents the bimane modified cysteine residue.

SPR information

Table S1: SPR data. ε_λ is the extinction coefficient for the peptide at wavelength λ nm. A best estimate is used for modified peptides **3**-**6**. ε_{205} was calculated with the online calculator detailed in Anthis 2013.[4] Top Conc is the highest concentration of 8x 1 in 2 dilutions, run sequentially from lowest to highest concentration. K_D is the affinity constant, K_D SE is the standard error of the K_D, and χ^2 is a measure of the goodness of fit, all calculated using the inbuilt Biacore S200 Evaluation Software. Ass/Diss is the contact and dissociation times, respectively, in seconds for each injection cycle.

Name	ε_λ	λ (nm)	Top Conc (nM)	Affinity K_D (nM)	K_D SE (nM)	χ^2	Ass/Diss (s)
Peptide 1	56820	205	500	102.3	5.3	0.0701	40/60
Peptide 2	58200	205	20000	NS	-	-	40/60
Peptide 3	58200*	205	2000	769.1	78	0.250	40/60
Peptide 4	58200*	205	5000	1994	140	0.160	40/60
Peptide 5	58200*	205	5000	2818	80	0.106	40/60
Peptide 6	58200*	205	5000	3855	350	0.527	40/60
Peptide 7	4694[5]	380	2000	570.5	30	0.118	40/60
Peptide 9	4694[5]	380	30000	25190	1900	0.0547	40/60
*The $\varepsilon_{\lambda\ of}$ **2** was used as an approximation of the peptide concentration							

Crystallographic Data Statistics

Table S2: Data collection & Refinement statistics. Data collection and refinement statistics of hPCNA bound with peptide **3** (RCSB PDB ID: 7M5L), hPCNA bound with peptide **5** (RCSB PDB ID: 7M5M), and hPCNA bound with peptide **6** (RCSB PDB ID: 7M5N). Statistics for the highest-resolution shell are shown in parentheses.

	Peptide 3	**Peptide 5**	**Peptide 6**
PDB ID	7M5L	7M5M	7M5N
Wavelength	0.9537	0.9537	0.9537
Resolution range	48.76 - 3.0 (3.107 - 3.0)	38.17 - 3.001 (3.108 - 3.001)	38.37 - 3.11 (3.221 - 3.11)
Space group	P 21 21 21	P 31 2 1	P 31 2 1
Unit cell	70.96 84.468 134.21 90 90 90	83.579 83.579 187.494 90 90 120	83.365 83.365 181.155 90 90 120
Total reflections	33470 (3284)	312221 (29680)	27339 (2646)
Unique reflections	16736 (1643)	15794 (1532)	13672 (1195)
Multiplicity	2.0 (2.0)	19.8 (19.4)	2.0 (2.0)
Completeness (%)	99.62 (98.48)	99.58 (98.52)	94.21 (88.85)
Mean I/sigma(I)	10.20 (0.68)	13.79 (2.43)	11.31 (3.06)
R-merge	0.03674 (0.9641)	0.2337 (1.466)	0.1039 (0.585)
R-meas	0.05195 (1.363)	0.2399 (1.505)	0.1469 (0.8273)
R-pim	0.03674 (0.9641)	0.05348 (0.337)	0.1039 (0.585)
CC1/2	1 (0.496)	0.999 (0.827)	0.992 (0.557)
CC*	1 (0.814)	1 (0.952)	0.998 (0.846)
Reflections used in refinement	16684 (1619)	15761 (1530)	12911 (1195)
Reflections used for R-free	830 (72)	787 (75)	624 (56)
R-work	0.2599 (0.3938)	0.2364 (0.3278)	0.2699 (0.3649)
R-free	0.2638 (0.4255)	0.2749 (0.3665)	0.3088 (0.4653)
CC(work)	0.950 (0.577)	0.945 (0.848)	0.880 (0.659)
CC(free)	0.954 (0.715)	0.949 (0.748)	0.964 (0.674)
Number of non-hydrogen atoms	5636	5611	5324
macromolecules	5395	5443	5173
ligands	241	168	151
Protein residues	769	763	755
RMS(bonds)	0.003	0.004	0.004
RMS(angles)	0.81	0.75	0.84
Ramachandran favored (%)	95.02	92.82	94.32
Ramachandran allowed (%)	4.59	6.91	5.01
Ramachandran outliers (%)	0.39	0.27	0.68
Rotamer outliers (%)	0.00	0.19	0.22
Clashscore	12.02	27.90	13.92
Average B-factor	106.51	64.71	60.98
macromolecules	105.29	63.97	60.73
ligands	133.71	88.78	69.52
Number of TLS groups	1	1	1
Twin Law	Not applicable	Not applicable	-h, -k, l

[a] $Rmerge = \Sigma\ |I - < I >|/\Sigma I$.

[b] $Rpim = \Sigma h\ [1/(^n/_h - 1)]^{1/2}\ \Sigma i|<I_h> - I_{h,i}|/\Sigma_h\ \Sigma_i\ I_{h,i}$ (2)

[c] $Rwork = \Sigma|F_o - F_c|/\Sigma|F_o|$ for all data excluding data used to calculate Rfree.

[d] $Rfree = \Sigma|F_o - F_c|/\Sigma|F_o|$ for all data.

hPCNA-peptide structures supplementary data

Table S3: Percentage buried surface area (BSA, A^2 | %) of PIP-box residues (excluding the covalently linked residues Cys145* and Cys149*) and the total BSA of the PIP-box residues including the covalent linker.

Peptide bound to hPCNA	**p21$_{141-155}$**	**Peptide 3**	**Peptide 3**	**Peptide 4**	**Peptide 5**	**Peptide 6**	**Peptide 7**
Structure type	Cocrystal PDB: 7KQ1	Cocrystal PDB: 7M5L	Computational (confidence) model	Computational model	Cocrystal PDB: 7M5M	Cocrystal PDB: 7M5N	Computational model
Glu$_{144}$	98.61 | 70	141.15 | 50	112.31 | 80	133.02 | 80	117.83 | 60	92.99 | 40	143.85 | 80
Ser$_{146}$	31.97 | 60	47.30 | 50	40.34 | 50	35.30 | 40	38.11 | 40	35.78 | 40	72.12 | 40
Met$_{147}$	140.69 |100	147.53 | 70	113.37 | 100	124.28 | 100	142.54 | 100	130.03 | 100	138.45 | 100
Thr$_{148}$	34.23|40	58.75 | 40	17.31 | 20	23.89 | 30	20.03 | 30	29.80 | 30	27.08 | 30
Phe$_{150}$	66.67 |50	77.50 | 70	82.30 | 60	83.20 | 50	75.24 | 50	70.27 | 40	87.12 | 60
Tyr$_{151}$	132.92 | 90	133.53 | 90	129.25 | 90	140.41 | 70	142.60 | 100	165.87 | 90	145.93 | 90
Glu$_{144}$ - Tyr$_{151}$ (including linker)	252.53 | 90	568.39 | 60	533.24 | 60	561.39 | 60	589.45 | 60	552.08 | 50	652.59 | 60

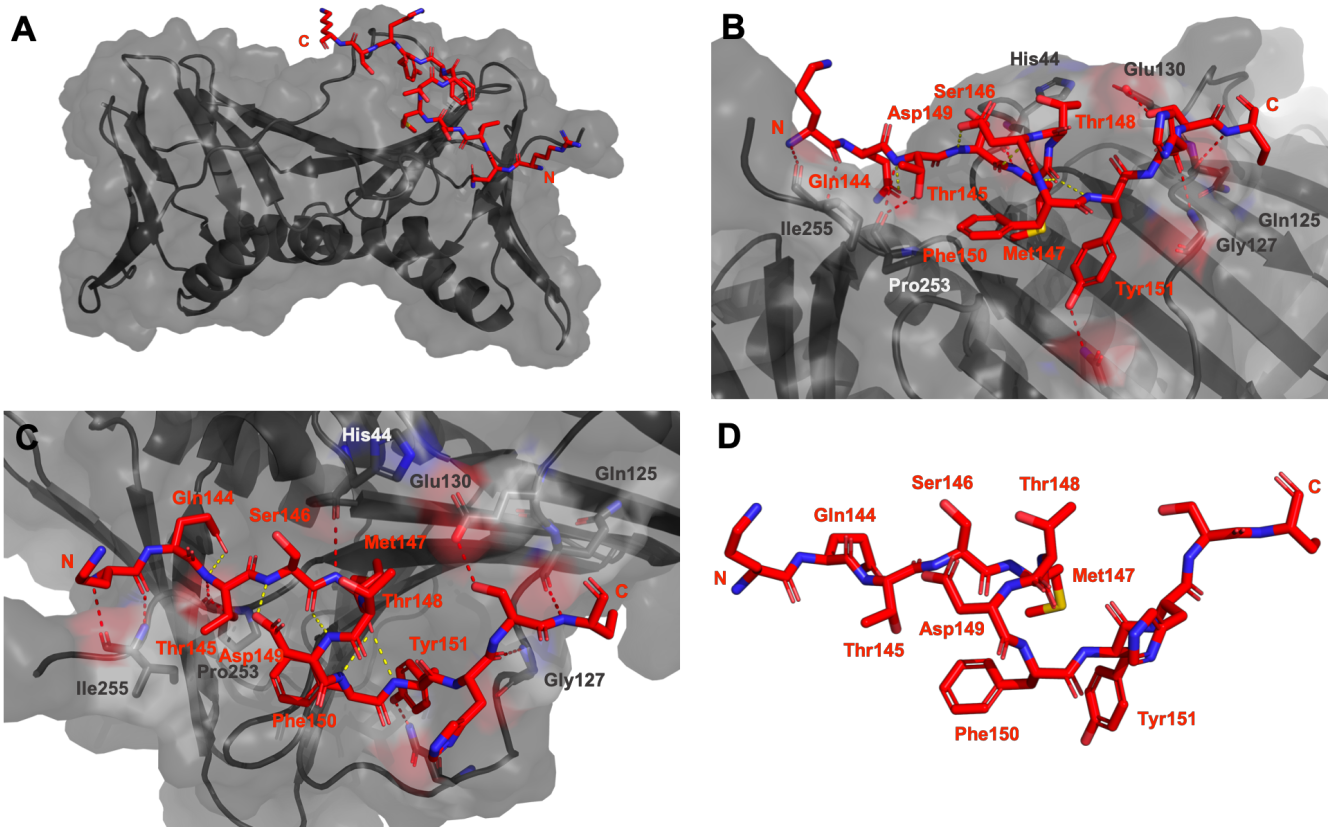


Figure S1: Computational model of peptide **1** bound to hPCNA. Peptide **1** is shown in red as sticks, and hPCNA in grey with cartoon and transparent surface representation. The PIP-box residues of peptide **1** are labelled in red, and PCNA residues in grey. Intermolecular interactions are shown as red dashes, and intramolecular interactions as yellow dashes.

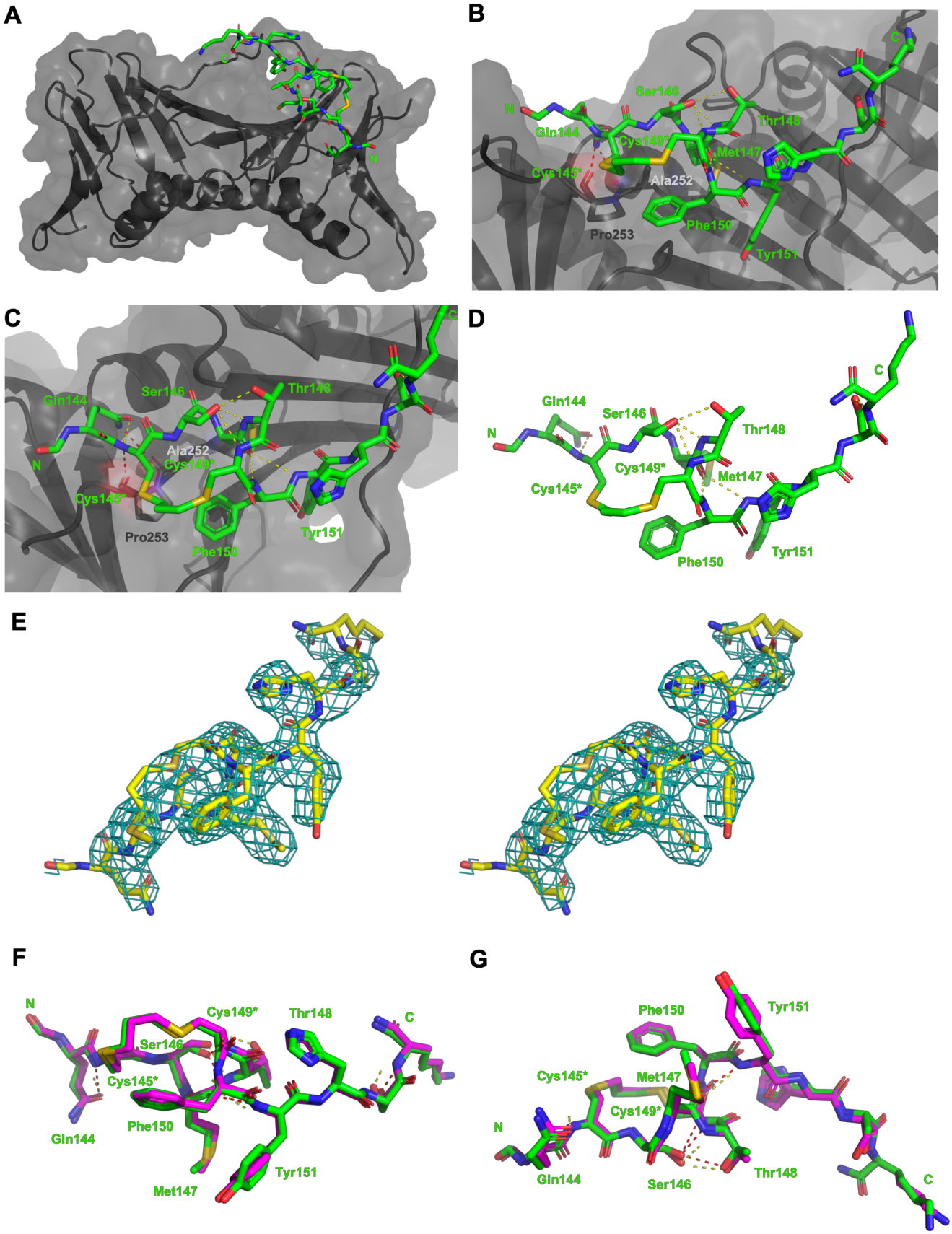


Figure S2: Co-crystal structure of peptide **3** bound to hPCNA (PDB: 7M5L). Peptide **3** is shown in green as sticks, and hPCNA in grey with cartoon and transparent surface representation (**A**-**C**). **B**-**D** The PIP-box residues of peptide **3** are labelled in green, and PCNA residues in grey. Intermolecular interactions are shown as red dashes, and intramolecular interactions as yellow dashes. **E** Representative electron density of peptide **3** (yellow, sticks) shown as a wall-eye stereo image of reduced model bias feature-enhanced map,[25] view contoured at 1.0σ. **F** & **G** Overlay of co-crystal structure of peptide **3** from cocrystal structure (green) and the computational validation model of **3** (pink), where the intramolecular interactions of the crystal structure are in yellow, and in red for the model.

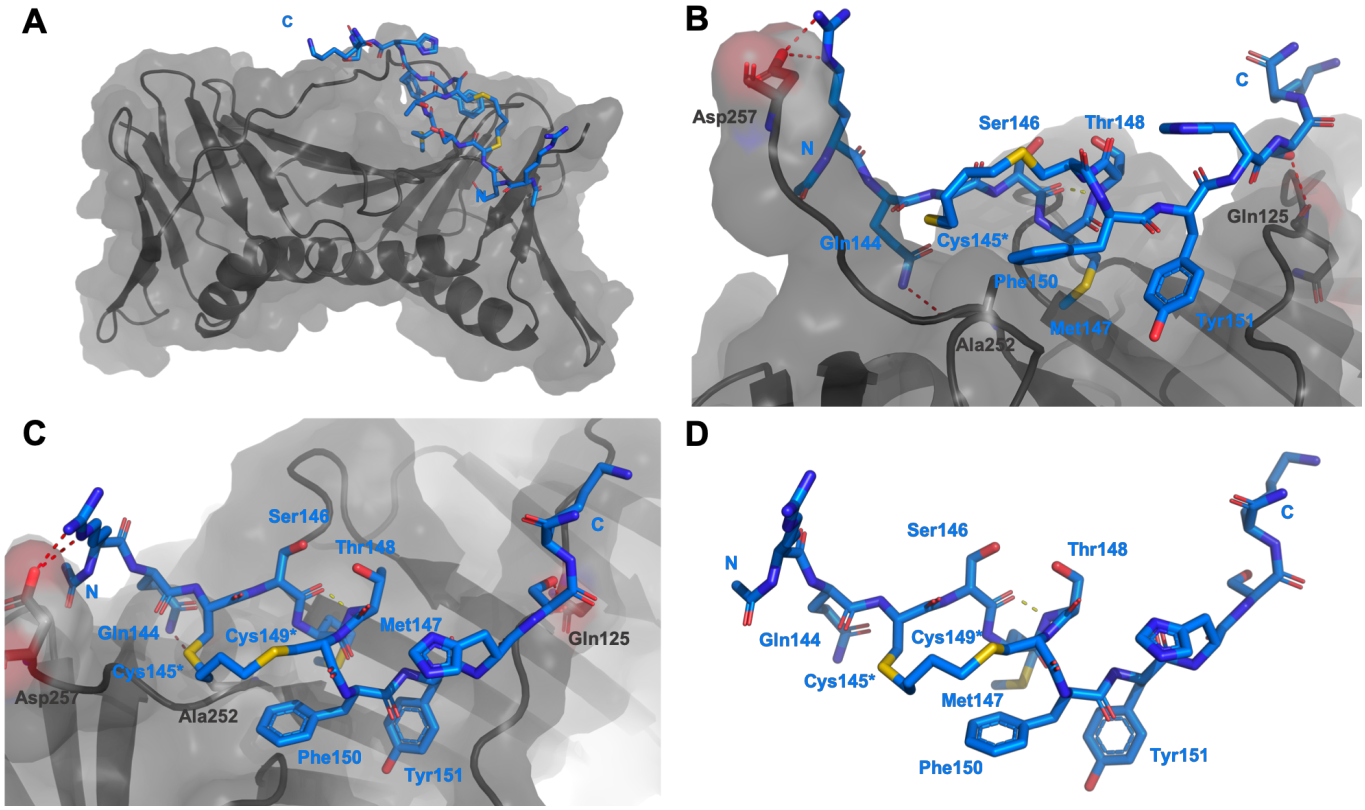


Figure S3: Computational model of peptide **4** bound to hPCNA. Peptide **4** is shown in blue as sticks (**A**-**D**), and hPCNA in grey with cartoon and transparent surface representation (**A**-**C**). The PIP-box residues of peptide **4** are labelled in blue, and PCNA residues in grey. Intermolecular interactions are shown as red dashes, and intramolecular interactions as yellow dashes.

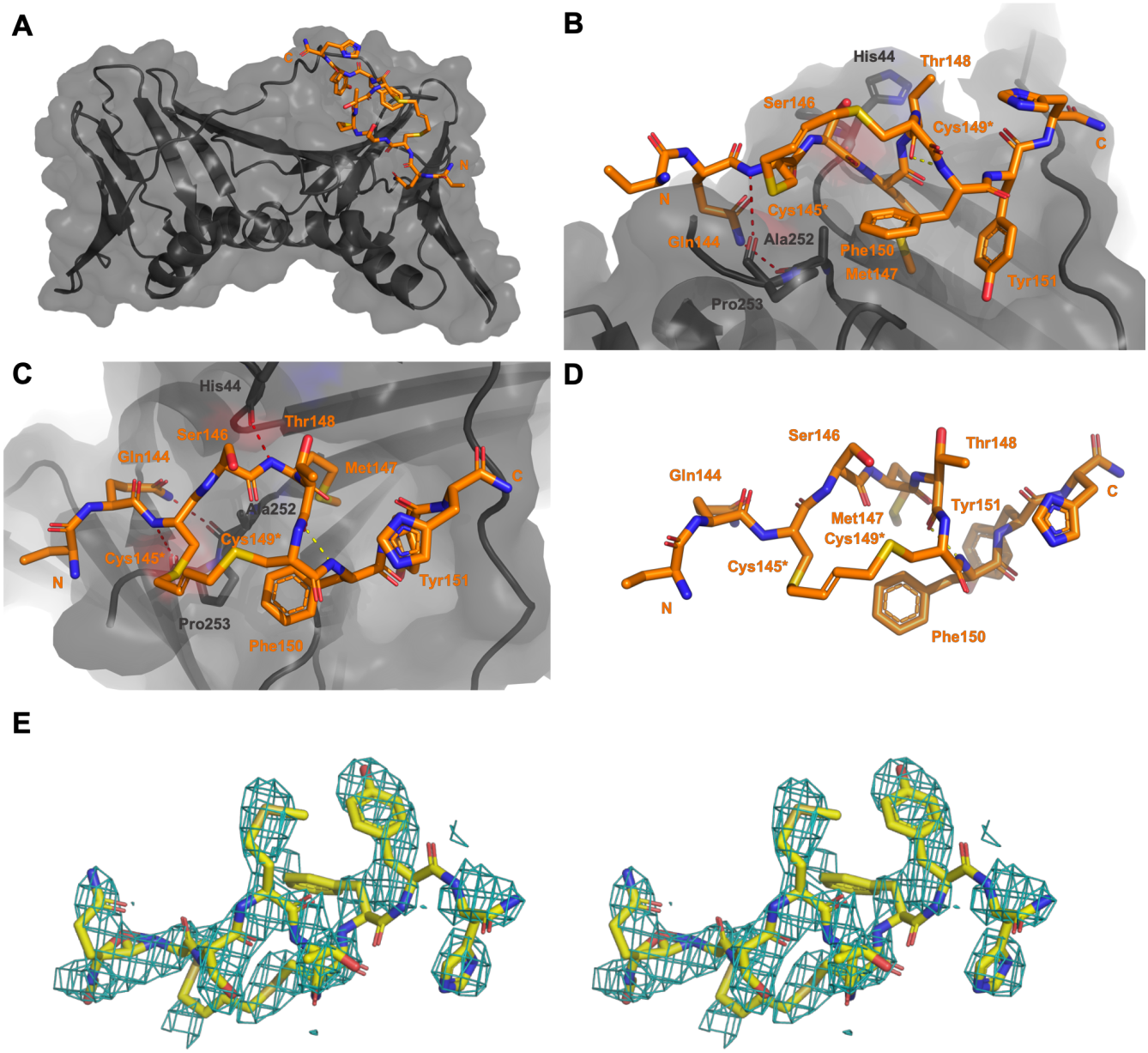


Figure S4: Co-crystal structure of peptide **5** bound to hPCNA (PDB: 7M5M). **A**-**D** Peptide **5** is shown in orange as sticks, and hPCNA in grey with cartoon and transparent surface representation (**A**-**C**). The PIP-box residues of peptide **3** are labelled in orange, and PCNA residues in grey. Intermolecular interactions are shown as red dashes, and intramolecular interactions as yellow dashes. **E** Representative electron density of peptide **5** (yellow, sticks) shown as a wall-eye stereo image of reduced model bias feature-enhanced map,[25] view contoured at 1.0σ.

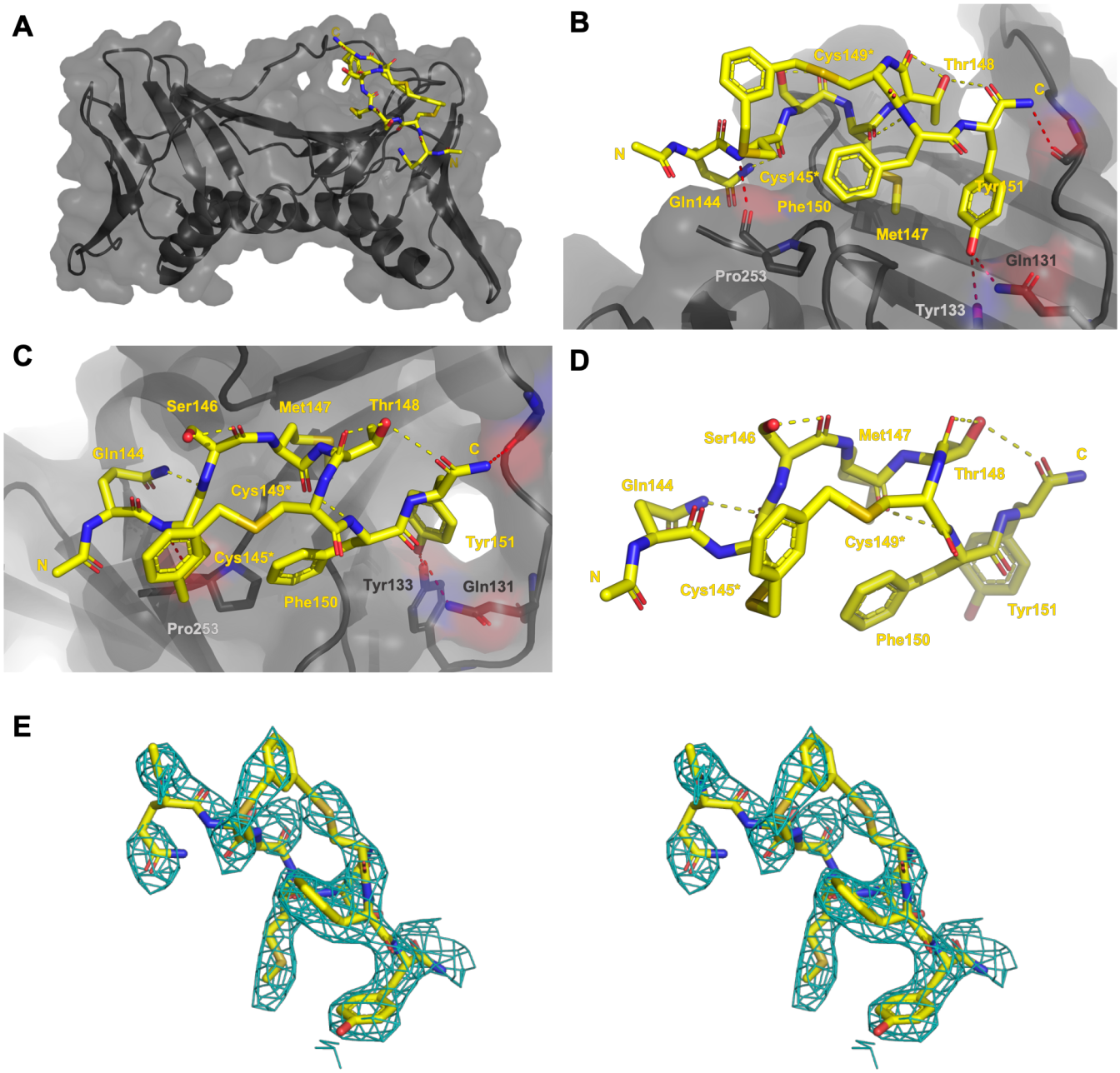


Figure S5: Co-crystal structure of peptide **6** bound to hPCNA (PDB: 7M5N). Peptide **6** is shown in yellow as sticks (**A**-**E**), and hPCNA in grey with cartoon and transparent surface representation (**A**-**C**). The PIP-box residues of peptide **6** are labelled in yellow, and PCNA residues in grey. Intermolecular interactions are shown as red dashes, and intramolecular interactions as yellow dashes. **E** Representative electron density of peptide **6** (yellow, sticks) shown as a wall-eye stereo image of reduced model bias feature-enhanced map,[25] view contoured at 1.0σ.

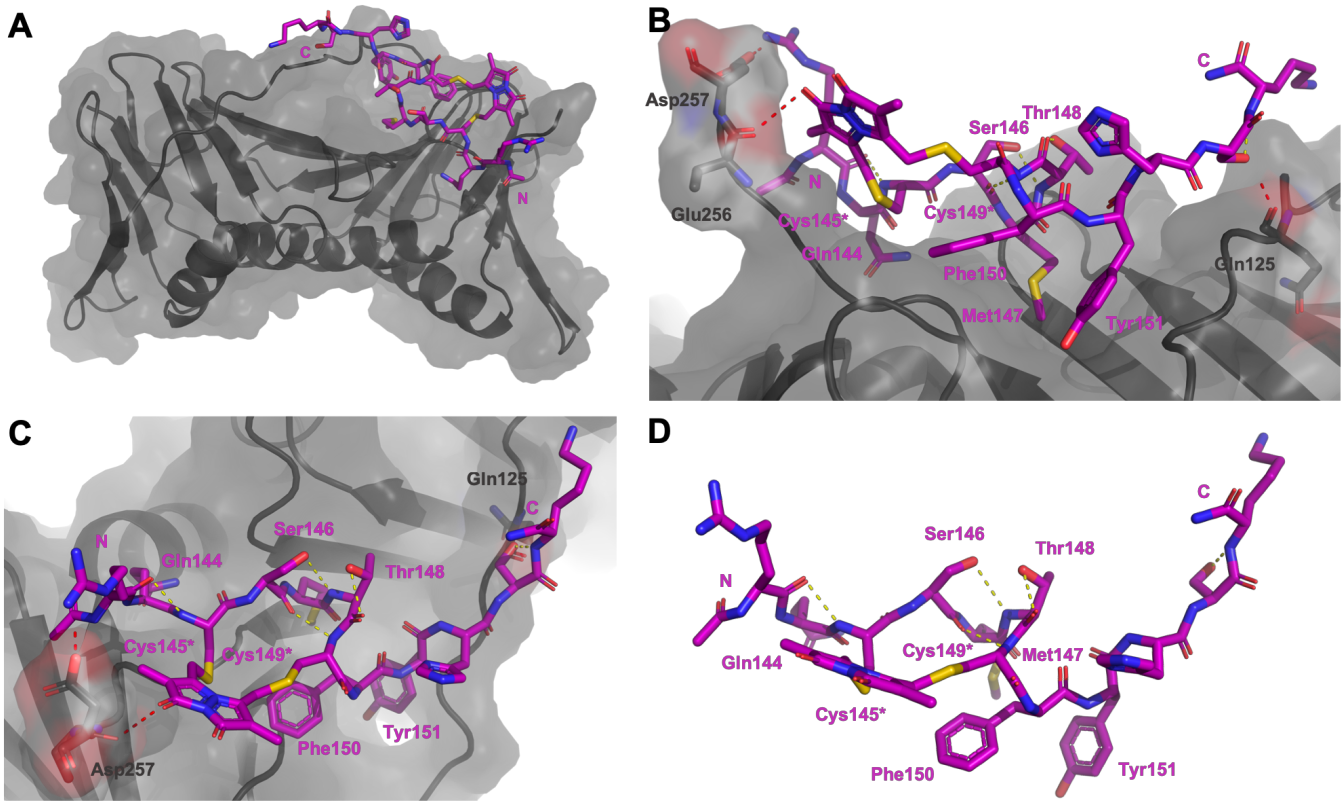


Figure S6: Computational model of peptide **7** bound to hPCNA. Peptide **7** is shown in purple as sticks (**A**-**D**), and hPCNA in grey with cartoon and transparent surface representation (**A**-**C**). The PIP-box residues of peptide **7** are labelled in purple, and PCNA residues in grey. Intermolecular interactions are shown as red dashes, and intramolecular interactions as yellow dashes.

Main-chain assignments and secondary shift analysis

Table S4: NMR resonance assignments for main-chain of peptide 2, and secondary shift calculations. All values in ppm. RC – random coil value from Wishart 2011[6]. NN – nearest neighbour corrections applied to random coil value; Hα did not require NN corrections due to the sequence used here. Δδ – Secondary shift values calculated as the difference between the observed resonance (Hα, Cα, CO or NH) and the respective NN value (obs-NN), shown graphically in green in Figure S7.

2	Hα	RC	NN	Δδ		Cα	RC	NN	Δδ		CO	RC	NN	Δδ		NH	RC	NN	Δδ
1R	4.27	4.34	4.34	-0.07		56.29	56.0	56.0	0.29		176.8	176.3	175.82	0.98		8.31	8.23	8.23	0.08
2Q	4.37	4.34	4.34	0.03		55.89	55.7	55.7	0.19		175.9	176.0	175.49	0.41		8.58	8.32	8.47	0.11
3C	4.54	4.55	4.55	-0.01		58.25	58.2	58.2	0.05		174.39	174.6	174.20	0.19		8.47	8.32	8.47	0.00
4S	4.47	4.47	4.47	0.00		58.46	58.3	58.3	0.16		174.51	174.6	173.91	0.60		8.46	8.31	8.51	-0.05
5M	4.57	4.48	4.48	0.09		55.70	55.4	55.4	0.30		176.38	176.3	176.30	0.08		8.43	8.28	8.44	-0.01
6T	4.26	4.35	4.35	-0.09		62.11	61.8	61.8	0.31		174.3	174.7	174.19	0.11		8.15	8.15	8.30	-0.15
7C	4.41	4.55	4.45	-0.04		58.28	58.2	57.97	0.31		173.98	174.6	173.77	0.21		8.18	8.32	8.34	-0.16
8F	4.58	4.62	4.52	0.06		57.65	57.7	57.48	0.17		175.1	175.8	174.67	0.43		8.2	8.3	8.39	-0.19
9Y	4.49	4.55	4.55	-0.06		57.74	57.9	57.9	-0.16		175.14	175.9	175.00	0.14		8.05	8.12	8.22	-0.17
10H	4.62	4.73	4.73	-0.11		54.78	55.0	55.0	-0.22		173.8	174.1	173.46	0.34		8.31	8.42	8.42	-0.11
11S	4.37	4.47	4.47	-0.1		58.19	58.3	58.3	-0.11		174.41	174.6	173.88	0.53		8.31	8.31	8.51	-0.20
12K	4.29	4.32	4.32	-0.03		56.22	56.2	56.2	0.02		179.1	176.6	176.60	2.50		8.46	8.29	8.45	0.01

Table S5: NMR resonance assignments for main-chain of peptide 7, and secondary shift calculations. All values in ppm. RC – random coil value from Wishart 2011[6]. NN – nearest neighbour corrections applied to random coil value; Hα did not require NN corrections due to the sequence used here. Δδ – Secondary shift values calculated as the difference between the observed resonance (Hα, Cα, CO or NH) and the respective NN value, shown graphically in blue in Figure S7.

7	Hα	RC	NN	Δδ		Cα	RC	NN	Δδ		CO	RC	NN	Δδ		NH	RC	NN	Δδ
1R	4.27	4.34	4.34	-0.07		56.15	56.0	56.0	0.15		176.54	176.3	175.82	0.72		8.29	8.23	8.23	0.06
2Q	4.37	4.34	4.34	0.03		55.87	55.7	55.7	0.17		175.64	176.0	175.49	-0.36		8.53	8.32	8.47	0.06
3C*	4.63	4.55	4.55	0.08		55.53	58.2	58.2	-2.67		174.20	174.6	174.20	-0.40		8.54	8.32	8.47	0.07
4S	4.52	4.47	4.47	0.05		58.34	58.3	58.3	0.04		174.69	174.6	173.91	0.09		8.63	8.31	8.51	0.12
5M	4.52	4.48	4.48	0.04		56.55	55.4	55.4	1.15		176.73	176.3	176.30	0.43		8.50	8.28	8.44	0.06
6T	4.14	4.35	4.35	-0.21		62.60	61.8	61.8	0.8		174.71	174.7	174.19	0.01		7.98	8.15	8.30	-0.32
7C*	4.47	4.55	4.45	0.02		-	58.2	57.97	-		173.79	174.6	173.77	-0.81		7.87	8.32	8.34	-0.47
8F	4.64	4.62	4.52	0.12		-	57.7	57.48	-		175.01	175.8	174.67	-0.79		8.11	8.30	8.39	-0.28
9Y	4.48	4.55	4.55	-0.07		57.73	57.9	57.9	-0.17		175.12	175.9	175.00	-0.78		8.04	8.12	8.22	-0.18
10H	4.64	4.73	4.73	-0.09		54.81	55.0	55.0	-0.19		173.84	174.1	173.46	-0.26		8.36	8.42	8.42	-0.06
11S	4.38	4.47	4.47	-0.09		58.25	58.3	58.3	-0.05		174.39	174.6	173.88	-0.21		8.32	8.31	8.51	-0.19
12K	4.29	4.32	4.32	-0.03		-	56.2	56.2	-		179.13	176.6	176.60	2.53		8.46	8.29	8.45	0.01

Table S6: NMR resonance assignments for main-chain of peptide 2 and 7, and secondary shift calculation for 7, relative to 2. All values in ppm. Δδ – Secondary shift values calculated as the difference between the observed resonance (Hα, Cα, CO or NH) of **7**, and the respective value for **2** (7-2), shown graphically in purple in Figure S7.

7-2	7Hα	2Hα	Δδ		7Cα	2Cα	Δδ		7CO	2CO	Δδ		7NH	2NH	Δδ
1R	4.27	4.27	0		56.15	56.29	-0.14		176.54	176.80	-0.26		8.29	8.31	0.02
2Q	4.37	4.37	0		55.87	55.89	-0.02		175.64	175.90	-0.26		8.53	8.58	0.05
3C*	4.63	4.54	0.09		55.53	58.25	-2.72		174.20	174.39	-0.19		8.54	8.47	-0.07
4S	4.52	4.47	0.05		58.34	58.46	-0.12		174.69	174.51	0.18		8.63	8.46	-0.17
5M	4.52	4.57	-0.05		56.55	55.70	0.85		176.73	176.38	0.35		8.50	8.43	-0.07
6T	4.14	4.26	-0.12		62.60	62.11	0.49		174.71	174.30	0.41		7.98	8.15	0.17
7C*	4.47	4.41	0.06		-	58.28	-		173.79	173.98	-0.19		7.87	8.18	0.31
8F	4.64	4.58	0.06		-	57.65	-		175.01	175.10	-0.09		8.11	8.20	0.09
9Y	4.48	4.49	-0.01		57.73	57.74	-0.01		175.12	175.14	-0.02		8.04	8.05	0.01
10H	4.64	4.62	0.02		54.81	54.78	0.03		173.84	173.8	0.04		8.36	8.31	-0.05
11S	4.38	4.37	0.01		58.25	58.19	0.06		174.39	174.41	-0.02		8.32	8.31	-0.01
12K	4.29	4.29	0		-	56.22	-		179.13	179.10	0.03		8.46	8.46	0.00

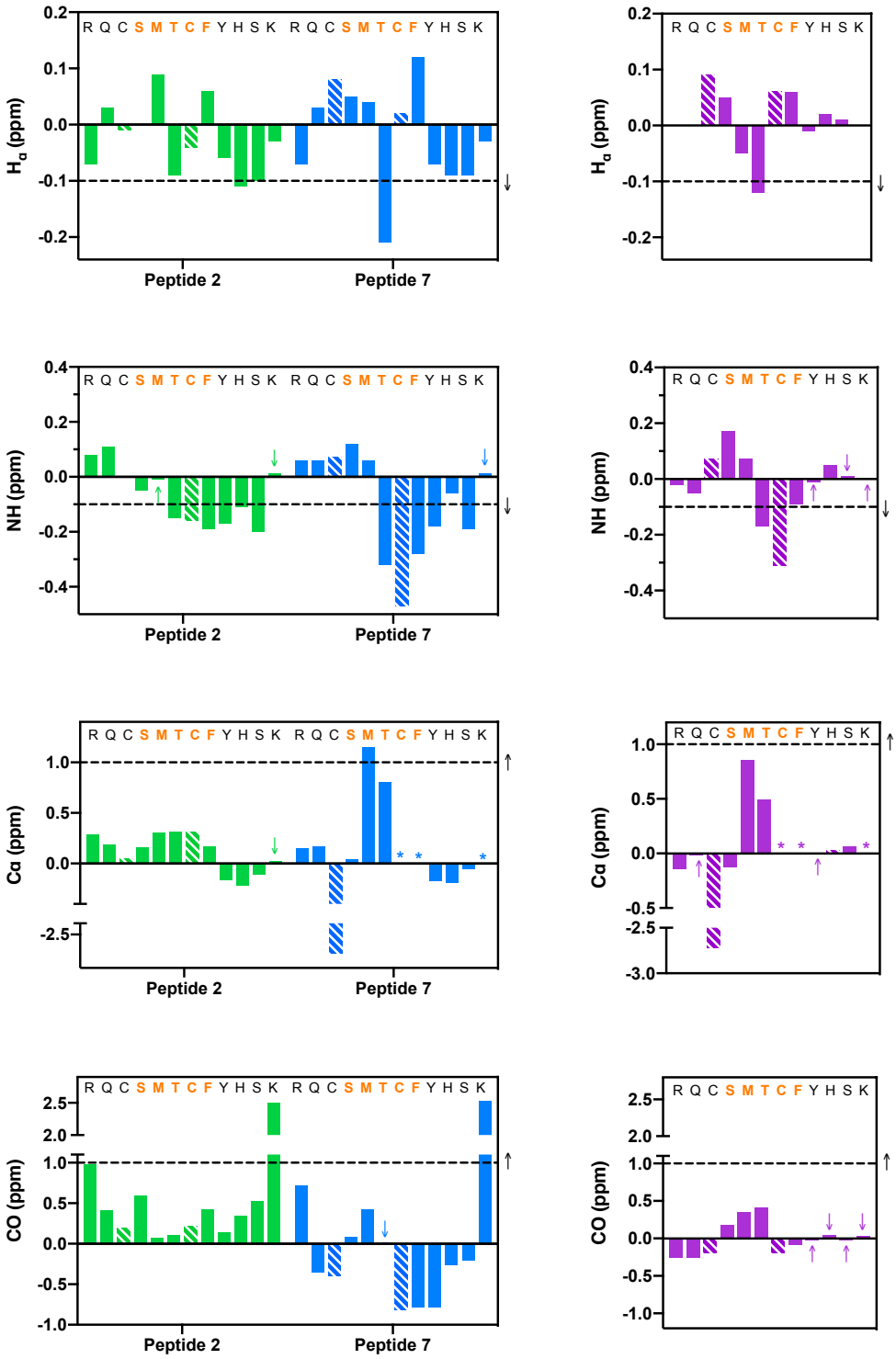


Figure S7: NMR secondary shift of mainchain resonances of peptide **2** (green) and **7** (blue) calculated relative to literature random coil values, with nearest neighbour corrections applied; and for peptide **7** relative to linear precursor peptide **2** (purple). Each bar represents the resonance for each consecutive amino-acid in the sequence, where the sequence is annotated that the top of each panel. The segment of peptide where helical structure is anticipated is indicated in orange writing. Arrows indicate values which are too close to zero to be easily observed, stars indicate values which could not be reliably extracted from the NMR spectrum. The black dashed line and black arrow, on each panel marks the threshold that should be surpassed by three consecutive residues to indicate the presence of a helical motif.

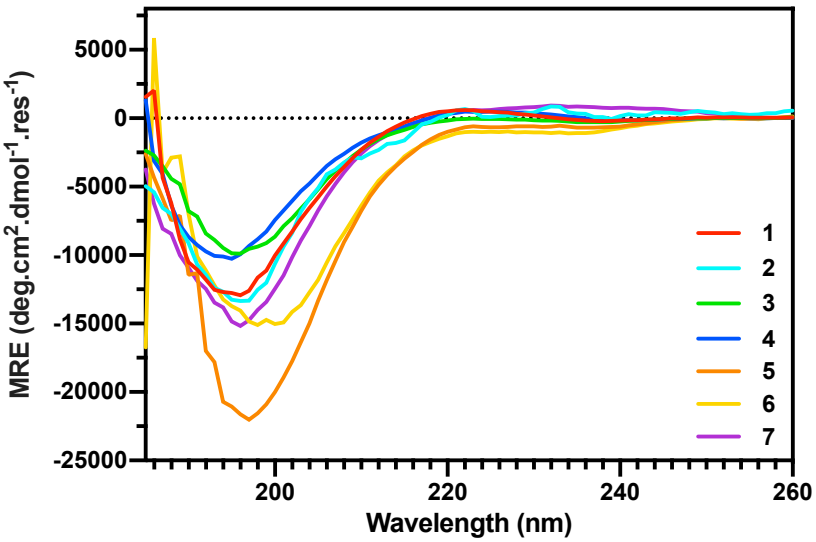


Figure S8: Circular dichroism spectra collected for peptides **1**-**7** in 10 mM phosphate buffer at pH 7.2. The spectra shown are the average of 8 scans, collected at 50 nm/min with a pitch of 1 mm in a 1 mm cuvette, using a Jasco J-810 (UniSA BioPhysical Characterisation facility); then smoothed using the in-built software with a Savitsky-Golay function and a convolution width of 7.

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