Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Table S1. Binding parameters for the formations of DHB_{DAXX}/SPEP complexes determined using computational methods[$]

Peptide	Ele	Vdw	Polar	Nonpolar	ΔG*
PEP_I	-836.9 (40)	-66.2 (1.4)	829.2 (33.1)	-12.7 (0.2)	-84.1 (7.1)
SPEP1	-929.4 (41)	-73.1 (1.4)	931.5 (42)	-13.5 (0.2)	-84.3 (2.3)
SPEP2	-850.5 (13)	-67.1 (0.6)	834.8 (8)	-12.5 (0.3)	-92.1 (1.8)
SPEP3	-853 (1.1)	-67.9 (2.3)	839.8 (7)	-12.7 (0.7)	-82.4 (3.3)
SPEP4	-564.4 (66)	-69.3 (1.6)	877 (31)	-11.9 (0.5)	-73.7 (3.3)
SPEP5	-892.6 (26)	-71.7 (7.2)	649 (67)	-11.3 (0.8)	-70.9 (5.5)
SPEP6	-641.1 (74)	-70.3 (3.6)	721 (25)	-12.3 (0.1)	-80.1 (4.0)
SPEP7	-726 (31)	-68.1 (1.1)	574 (64)	-11.2 (0.3)	-86.3 (1.4)

[$]All values are calculated in kcal mol^{-1} at 300K. Errors of 1 SD are shown in parentheses.

***Entropy changes are not calculated, therefore the binding energy calculated here corresponds to only the enthalpy contribution.**

Table S2: Binding parameters for the formation of DHB_{DAXX}/STPEP complexes determined using computational and experimental methods.

Peptide	Ele	Vdw	Polar	Nonpolar	ΔG*	Kd (nM)
STPEP1	-814.2 (52)	-63.7 (2.1)	799.5 (47)	-11.7 (0.5)	-90.1 (7)	n.d
STPEP2	-726.3 (15)	-62.1 (1.0)	712. 0 (14)	-11.8 (0.2)	-88.2 (1.5)	32.4 (2.6)
STPEP3	-630.7 (18)	-61.0 (3)	614.9 (17)	-11.5 (0.4)	-88.2 (4.3)	34.9 (2.4)
STPEP4	-447.7 (34)	-60.5 (1.8)	439.9 (31)	-11.2 (0.1)	-79.5 (1.0)	147.8 (12.4)
STPEP5	-772 (36)	-55.0 (3)	763 (32)	-10.7 (0.4)	-71.6 (7.5)	n.d
STPEP6	-694 (45)	-52.2 (6)	648.8 (44)	-10.4 (0.8)	-71.2 (9.1)	n.d
STPEP7	-662.7 (51)	-57.8 (0.7)	654.5 (45)	-10.8 (0.2)	-76.8 (5.5)	268.9 (17.3)

[$]Binding energies are calculated using MMPBSA method. All values are calculated in kcal mol^{-1} at 300K. Errors of 1 SD are shown in parentheses. *Entropy changes are not calculated, therefore the binding energy calculated here corresponds to only the enthalpy contribution. Kd values were determined from fluorescence anisotropy measurments.

Supplementary Figures:

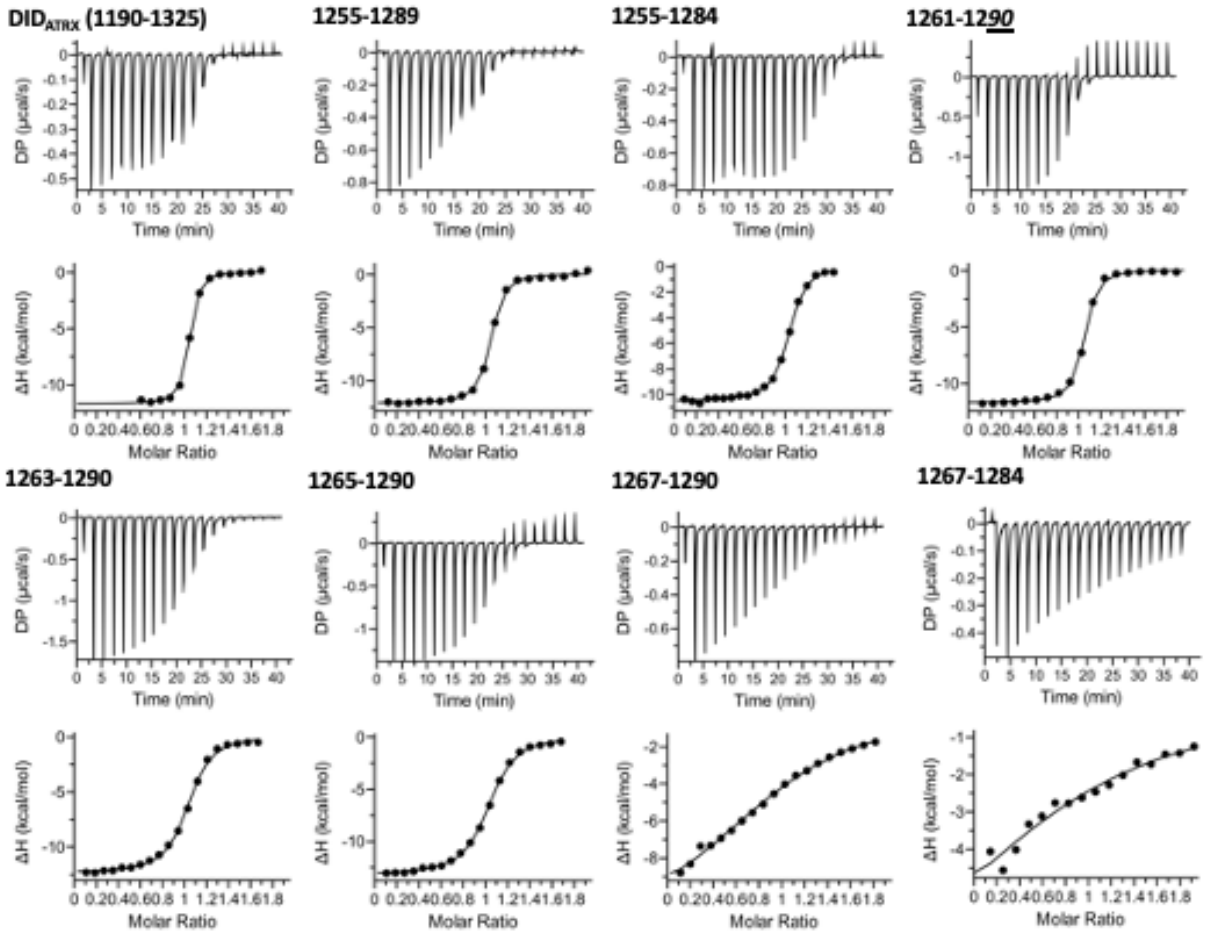

Figure S1. ITC isotherms for binding of DHB$_{DAXX}$ to peptide fragments of ATRX.

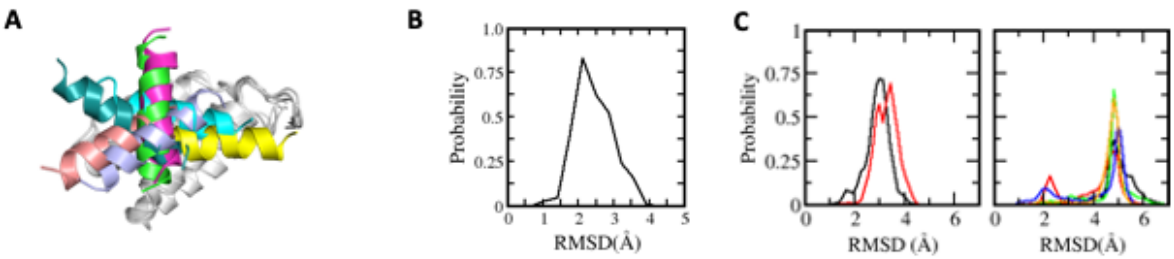

Figure S2. Stability of docked $DHB_{DAXX}/PEP_{RassF1C}$ and DHB_{DAXX}/PEP_{ATRX} complexes. A Cartoon representations showing 7 poses of the PEP_{ATRX} helix (multiple colours) docked with DHB_{DAXX} (grey). **B** RMSD of conformations of $PEP_{Rassf1C}$ sampled during MD simulations starting from the $DHB_{DAXX}/PEP_{Rassf1C}$ solution structure. **C** RMSD of conformations of PEP_{ATRX} peptides sampled during MD simulations starting from different docked poses of PEP_{ATRX}; (left) RMSD of the peptides with stable binding; (right) RMSD of peptides with unstable binding during MD simulations.

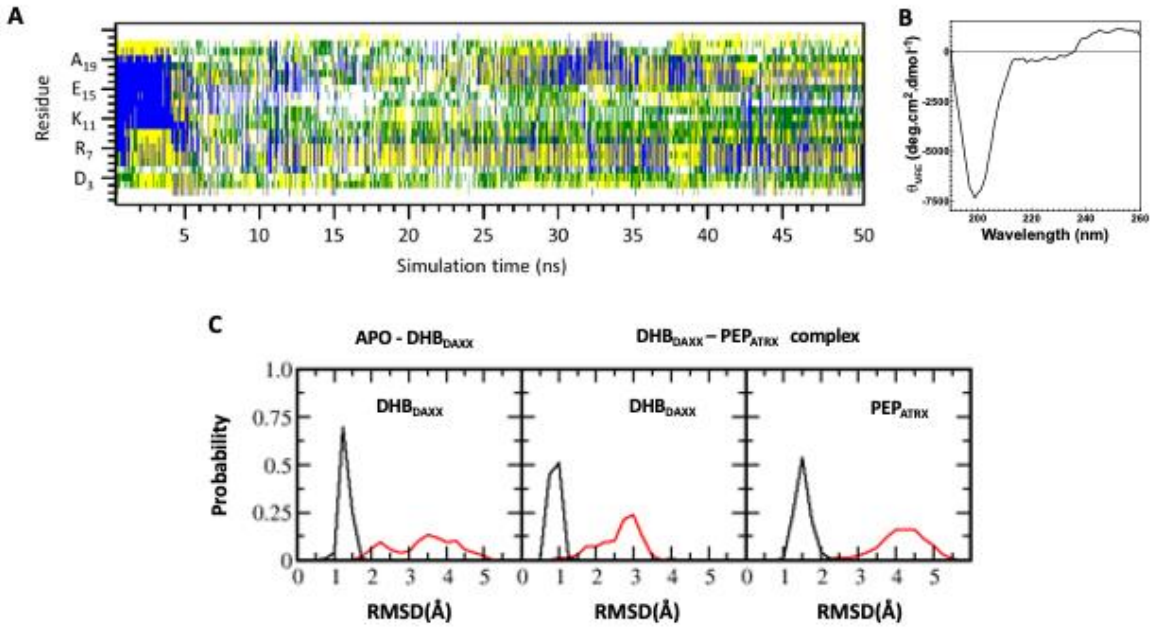

Figure S3. Structure and stability analysis of PEP_{ATRX} and DHB_{DAXX} in apo and bound forms.

A Time evolution of secondary structures in apo PEP_{ATRX} sampled during BP-REMD simulations (blue: α - helix, gray: 3_{10}- helix, yellow: turn, green: bend, white: coil). **B** CD spectrum of PEP_{ATRX} showing % helicity. **C** Probability distributions of RMSD of conformations sampled during simulations; (left) apo DHB_{DAXX}; (middle) bound DHB_{DAXX} from DHB_{DAXX}/PEP_{ATRX} complex, and (right) bound PEP_{ATRX} from DHB_{DAXX}/PEP_{ATRX} complex. Black and red corresponds to RMSD of conformations with or without the flexible N- and/or C- terminal residues described in the main text, respectively.

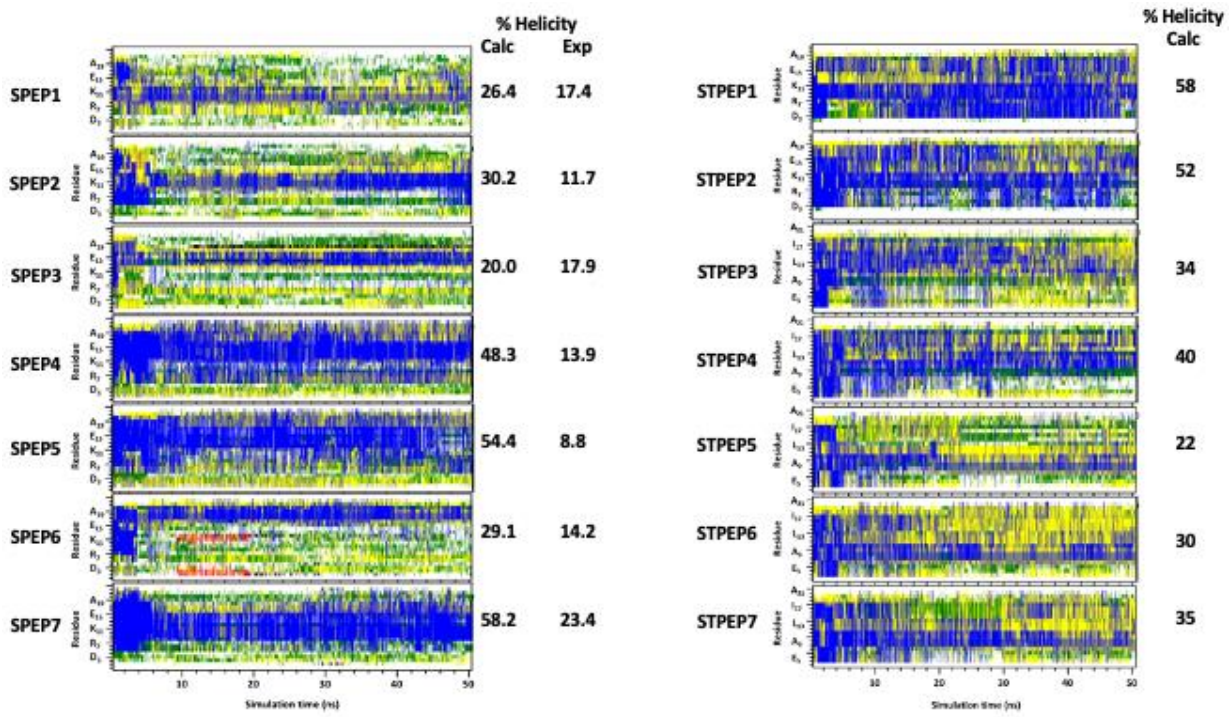

Figure S4. Conformational analysis of stapled and stitched peptides. Time evolution of the secondary structure of SPEP 1-7 and STPEP 1-7 during BP-REMD simulations (blue: α - helix, grey: 3_{10}- helix, yellow: turn, green: bend, white: coil, red: beta strand). The overall peptide conformations sampled were used to calculate percentage helicity ('Calc'). For the stapled peptides, SPEP 1-7, percentage helicity obtained by fitting CD spectra ('Exp') is plotted alongside.

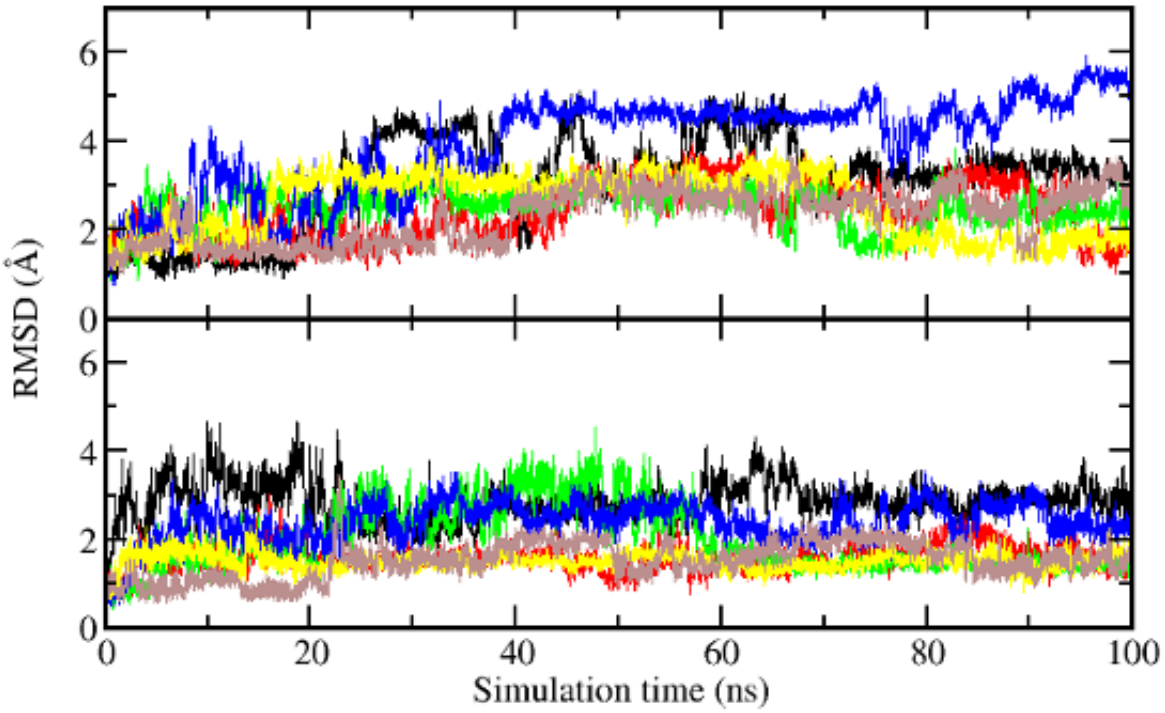

Figure S5. Computational analysis of the stability of DHB_{DAXX}/SPEP complexes. RMSD of conformations sampled during MD simulations of DHB_{DAXX}/SPEP complexes; (top) DHB_{DAXX} and (bottom) stapled peptides (SPEP). The different colors, black, red, green, blue, yellow, brown and grey corresponds to different DHB_{DAXX} /SPEP 1 to 7 complexes in numerical order.

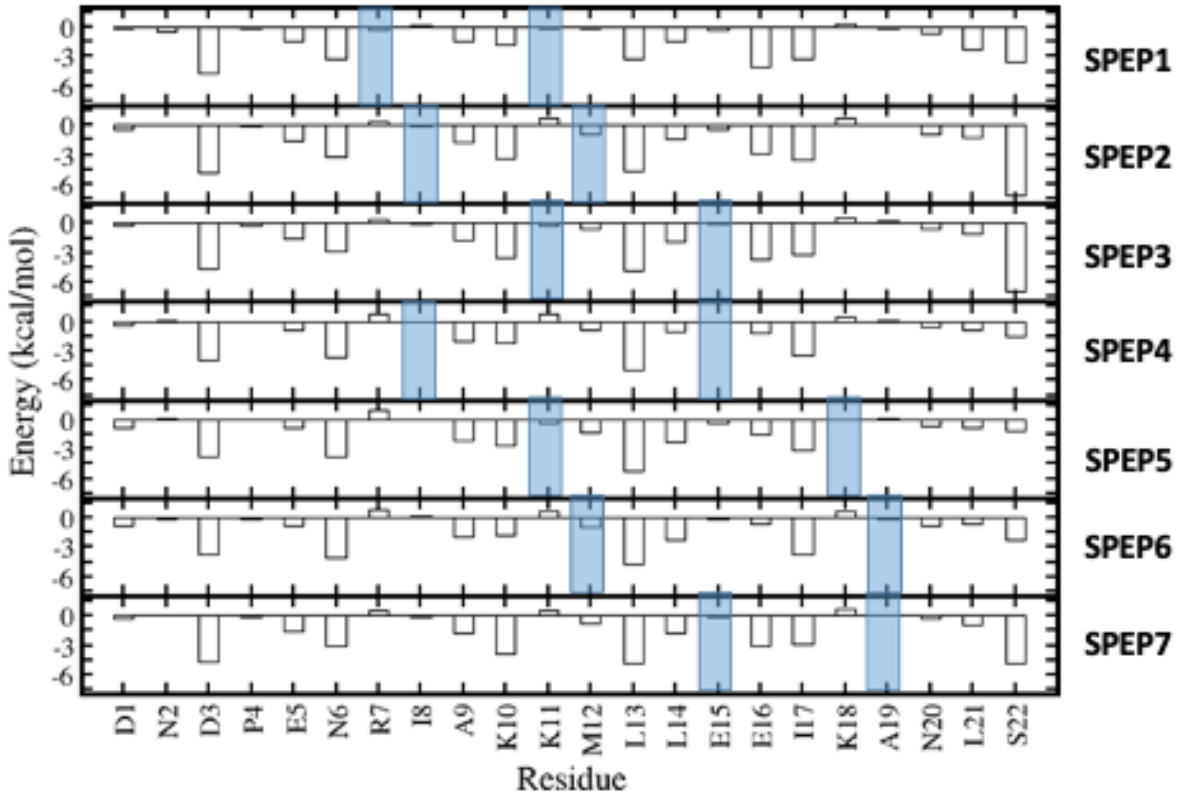

Figure S6: Per-residue contributions to binding of DHB_{DAXX} by SPEP1-7 calculated from MD simulations. Binding free energies of individual SPEP residues to DHB_{DAXX} were calculated using the MMPBSA approach (see Methods). The staple linker positions are highlighted in blue.

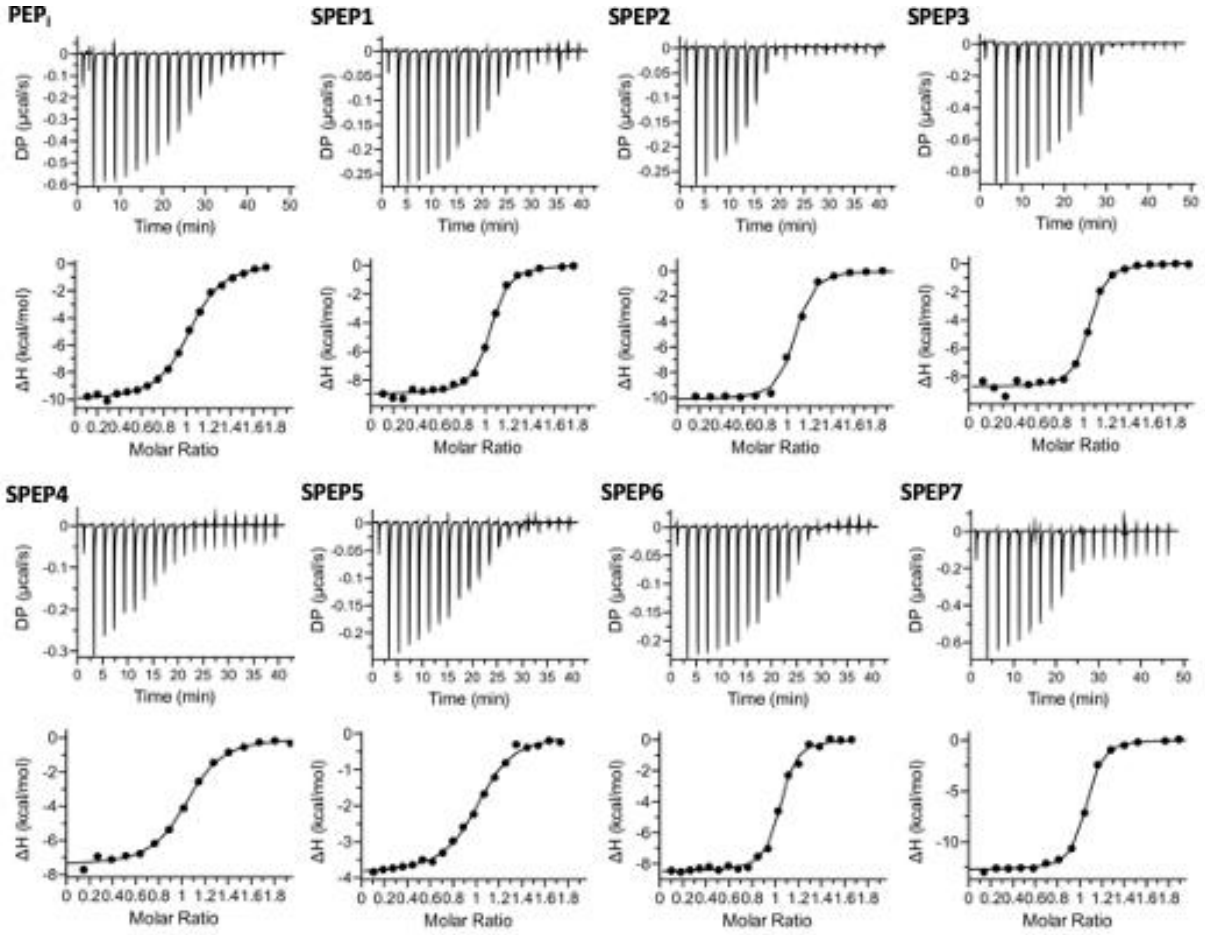

Figure S7. ITC isotherms for binding of DHB_{DAXX} to SPEP1 to 7.

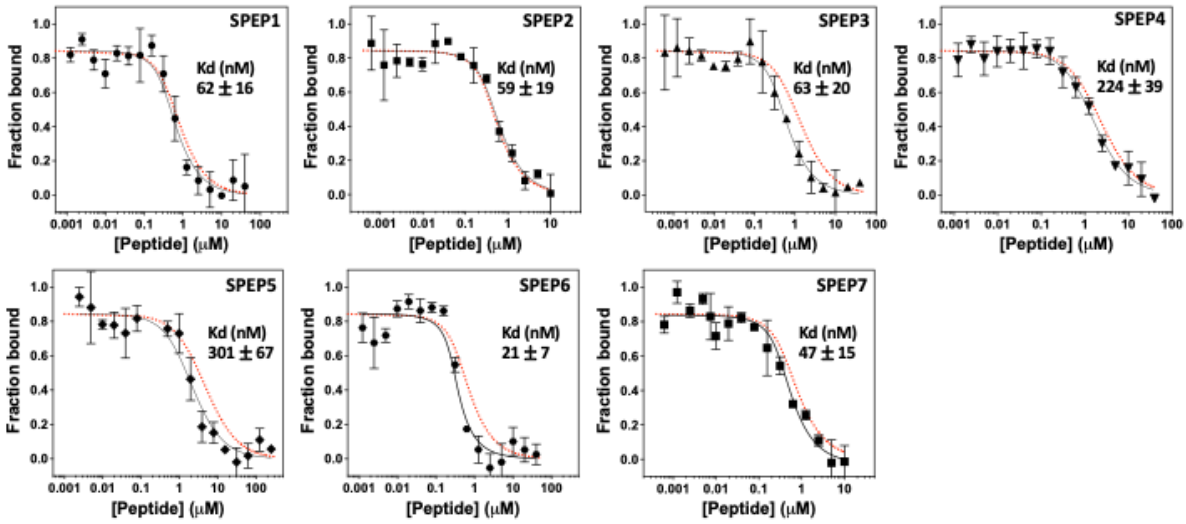

Figure S8. Competitive displacement of FAM-SPEP7 from DHB_{DAXX} by non-fluorescent SPEP peptides 1-7. Binding data with standard error bars derived from three independent measurements are shown for each peptide (closed symbols). Curves obtained from fitting the data to a competitive binding model (solid black line) are shown with simulated curves using Kd values obtained from ITC data (dashed red line) included for comparative purposes.

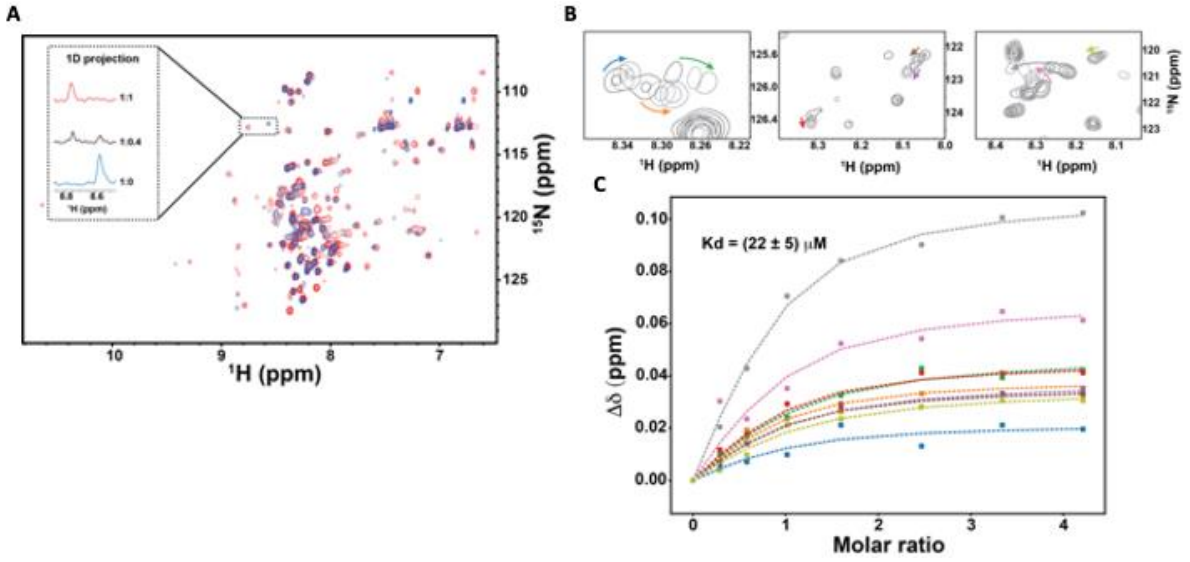

Figure S9. ^{1}H-^{15}N HSQC spectra showing the formation of the ^{15}N NSIM-DHB$_{DAXX}$/FAM-SPEP7 pre-complex and subsequent binding of SUMO-1 in sequential titrations. A. Overlaid ^{1}H-^{15}N HSQC spectra of unbound ^{15}N-NSIM-DHB$_{DAXX}$ (blue) and a saturated complex formed with FAM-SPEP7 (red) are shown. The transition of a single resonance projected in 1D (inset) with an additional mid-titration point is included (black), indicating slow exchange. **B.** (Top) Three panels showing overlaid sections of the ^{1}H-^{15}N HSQC spectra of ^{15}N-NSIM-DHB$_{DAXX}$/FAM-SPEP7 in the absence (black) and presence (grey) of increasing quantities of SUMO-1. Multi-coloured arrows indicate 9 individual resonances whose discrete chemical shift changes, in fast exchange, were measured during the titration. (Bottom) Titration curves of the 9 resonances, coloured as indicated above. The reported Kd value is the mean ± SD of the 9 individually fitted curves. Combined amide chemical shift changes, Dd (ppm), were calculated as $((Dd_{1H})^2+(0.2Dd_{15N})^2)^{1/2}$.

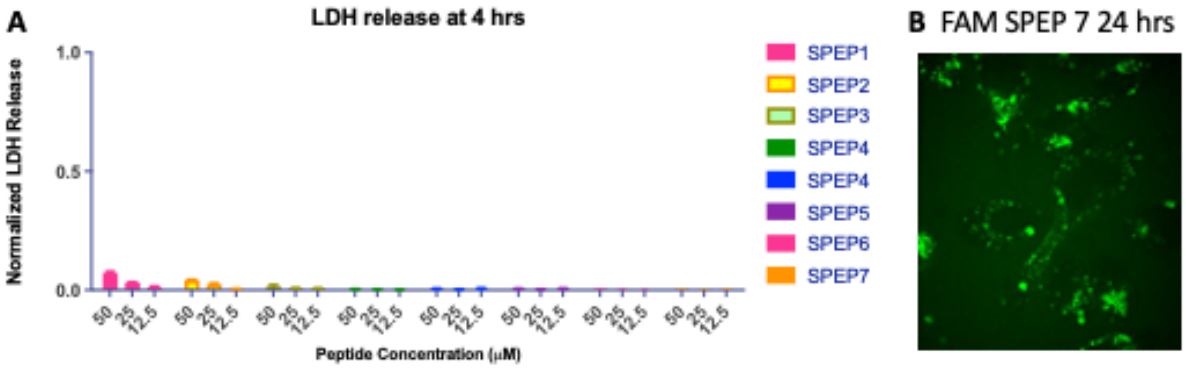

Figure S10. Cellular toxicity and localization of stapled peptides. A HCT116 cells were titrated with peptides in the presence of 2 % serum and LDH release was assessed after 4 hrs incubation. **B** Cellular uptake was assessed by live cell imaging after treating HCT116 cells for 4 hrs with 25 uM of FAM-SPEP7 in the same conditions.

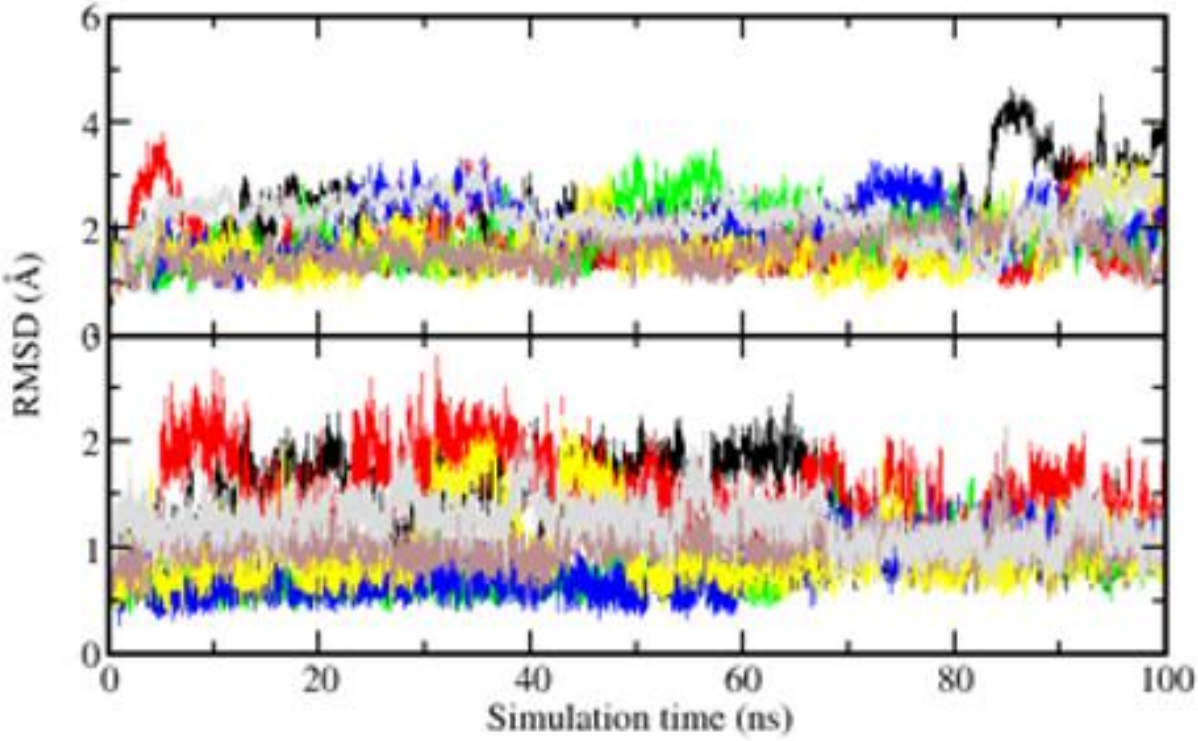

Figure S11. Computational analysis of the stability of DHB_{DAXX}/STPEP complexes. RMSD of conformations sampled during MD simulations of DHB_{DAXX}/STPEP complexes; (top) DHB_{DAXX} and (bottom) stapled peptides (SPEP). The different colors; black, red, green, blue, yellow, brown and grey, correspond to different DHB_{DAXX} /STPEP1 to 7 complexes in numerical order.