Supporting Information

Investigating peptide sequence variations for 'double-click' stapled p53 peptides

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Fmoc solid-phase peptide synthesis

Automated peptide synthesis was carried out on a CEM Liberty Automated Microwave Peptide Synthesiser using Merck Rink Amide MBHA resin LL (0.29-0.39 mmol/g loading). All peptide couplings were performed with 5 equivalents of Fmoc-protected amino acid in DMF, 5 equivalents of either HATU or HBTU in DMF, and 10 equivalents of DIPEA in NMP (2 M). Arginine was coupled using double couplings for 15 min each without microwave irradiation. All other amino acids were coupled as single couplings with 25 W power at 75 °C over 15 min. The side chain protecting groups used were *t*-Bu for aspartic acid, glutamic acid, serine, threonine, Boc for lysine and tryptophan, Pbf for arginine, and Trt for asparagine.

Fmoc deprotection was achieved with a solution of 20% piperidine in DMF, using 45 W power at 75 °C over 3 min. N-terminal capping, cleavage and HPLC purification of peptides were carried out as previously described.[1]

Peptide concentrations were determined by amino acid analysis at the Peptide Nucleic Acid Chemistry Facility at the Department of Biochemistry, University of Cambridge.

Double-click peptide stapling

Synthesis of linkers **A** and **B**, peptides stapling and purification was carried out as previously described.[1]

Peptide LCMS data

Data on wt $p53_{17-29}$, **1**, **1A** and **1B** was reported previously.[1]

The *m/z* ratios found represent the $[M+2H]^{2+}$ species in all cases except **6-8** and **6A-8A**, which are the $[M-2H]^{2-}$ species.

Peptide	Mass	*m/z* found	*m/z* calcd
2	1712.8	858.1	857.9
2A	1838.9	921.0	921.0
3	1695.9	849.4	849.5
3A	1821.9	913.1	912.5
4	1725.8	864.4	864.4
4A	1851.9	928.1	927.5
5	1654.8	829.0	828.9
5A	1780.9	892.1	891.9
6	1680.8	839.8	839.9
6A	1806.8	902.8	902.9

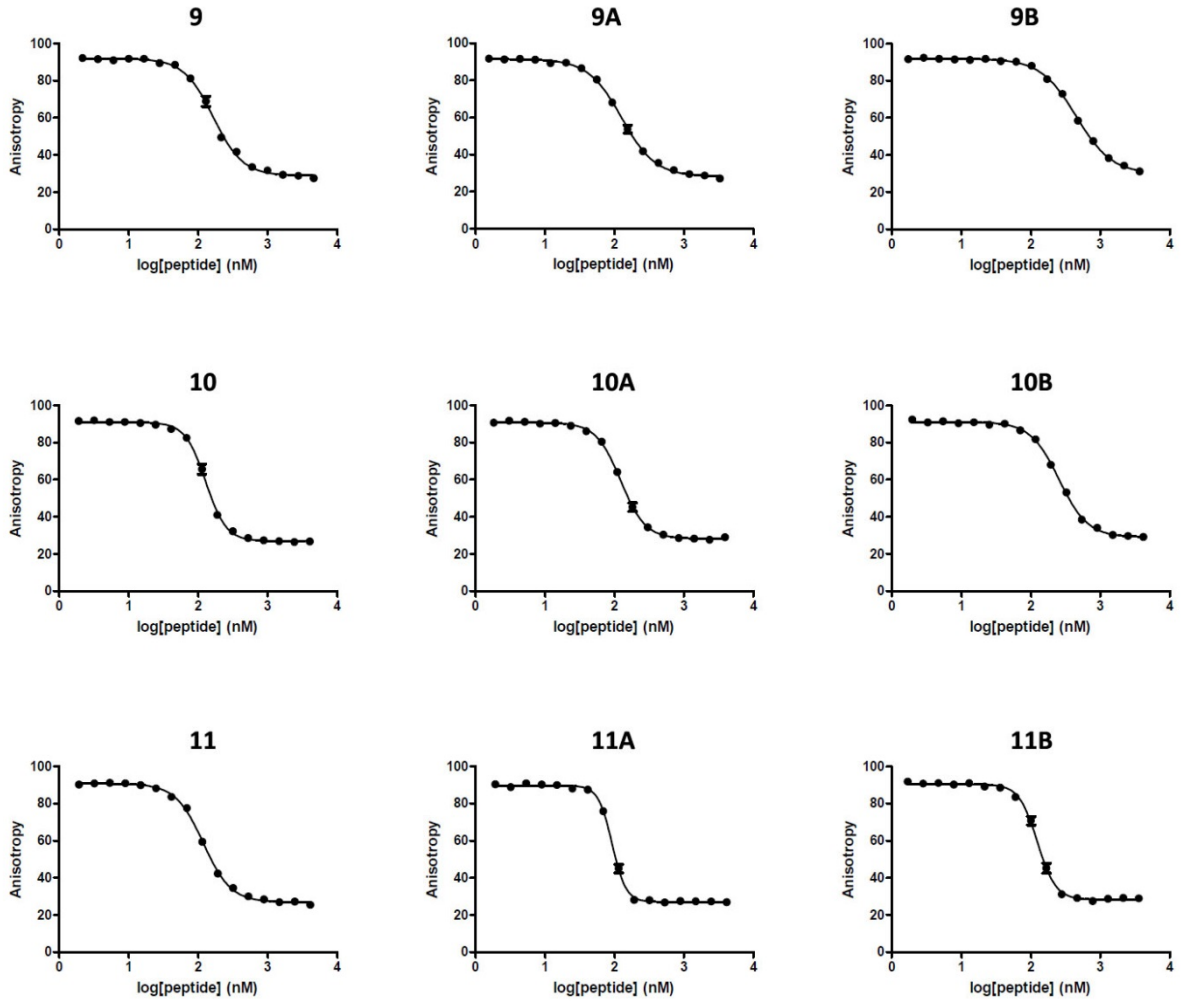
7	1652.7	825.8	825.9
7A	1778.8	888.7	888.9
8	1708.8	853.9	853.9
8A	1834.8	917.1	916.9
9	1727.9	865.9	865.9
9A	1853.9	929.2	928.0
9B	2365.2	1184.5	1184.1
10	1783.9	891.9	893.0
10A	1910.0	956.6	956.5
10B	2421.3	1212.5	1212.1
11	1727.9	865.5	865.4
11A	1853.9	928.7	928.5
11B	2365.2	1184.3	1184.1

Circular dichroism

Circular dichroism spectra were obtained on a Chirascan CD spectrometer at 20 ^{o}C using a 1 mm path length, scanning between 260 and 190 nm at 0.2 nm/s with a bandwidth of 1.0 nm and response time of 0.5 s. Each spectrum is the average of three scans. p53 peptides were dissolved in 85:15 water/acetonitrile, whilst model peptides were dissolved in 20 mM sodium phosphate buffer at pH 7.4, with accurate concentrations determined by amino acid analysis. Helicity was calculated based on mean residue ellipticity at 222 nm as previously reported.[2]

Competitive fluorescence polarisation assay

Fluorescence polarisation assay and binding affinity calculations were carried out as we have previously described.[1] Experiments were conducted twice independently, each in triplicate. Data on wt $p53_{17\text{-}29}$, **1**, **1A** and **1B** was reported in our previous work.[1]



Gene reporter assay

Assay was carried out as previously described,[3] with measurements read on a Tecan Infinite 200 Pro plate reader.

References

1. Y. H. Lau, P. D. Andrade, S.-T. Quah, M. Rossmann, L. Laraia, N. Sköld, T. J. Sum, P. J. E. Rowling, T. L. Joseph, C. Verma, M. Hyvönen, L. S. Itzhaki, A. R. Venkitaraman, C. J. Brown, D. P. Lane, D. R. Spring, *Chem. Sci.*, 2014, doi: 10.1039/c4sc00045e
2. Bird, G. H., Bernal, F., Pitter, K. & Walensky, L. D. Chapter 22: Synthesis and Biophysical Characterization of Stabilized α-Helices of BCL-2 Domains. *Methods Enzymol.*, 2008, **446**, 369–386.
3. C. J. Brown, S. T. Quah, J. Jong, A. M. Goh, P. C. Chiam, K. H. Khoo, M. L. Choong, M. A. Lee, L. Yurlova, K. Zolghadr, T. L. Joseph, C. S. Verma, D. P. Lane, *ACS Chem. Biol.*, 2012, **8**, 506–512.