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Supporting Information

Modulating the Folding and Binding of Peptides Using a Stimuli-Responsive Molecular Tweezer

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KEYWORDS: β-hairpin-based molecular tweezer, Stimulus-responsive peptide folding, α-helix stabilization, Controlled capture-and-release, Multivalent binding

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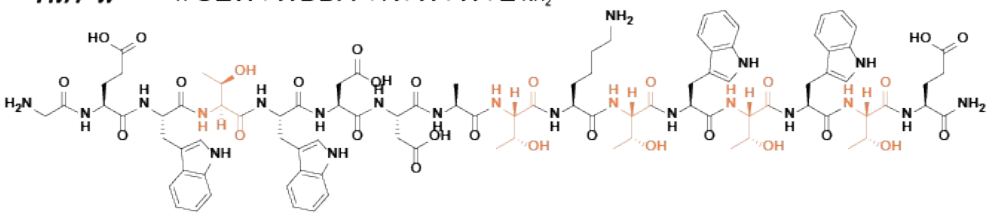
TMT-w H-GEWTWDDA-TKTWTWTE-NH_2



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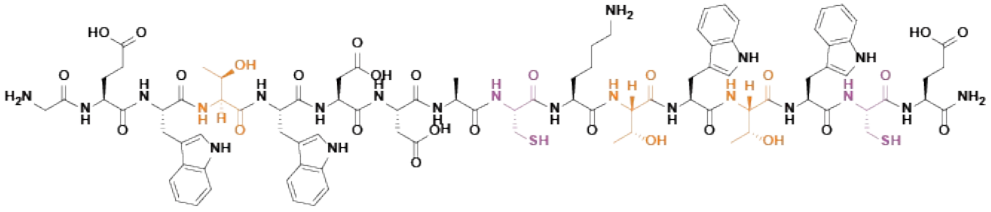

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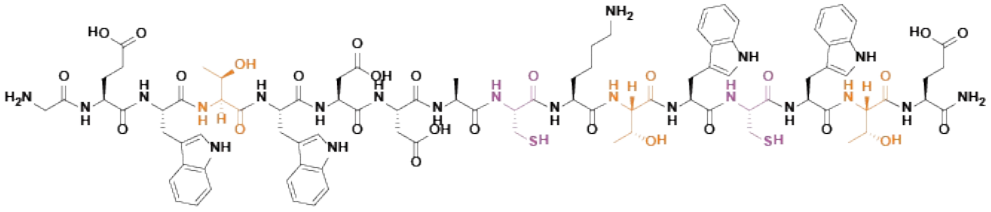

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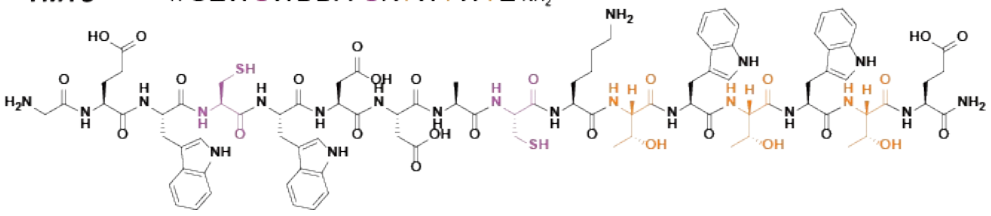
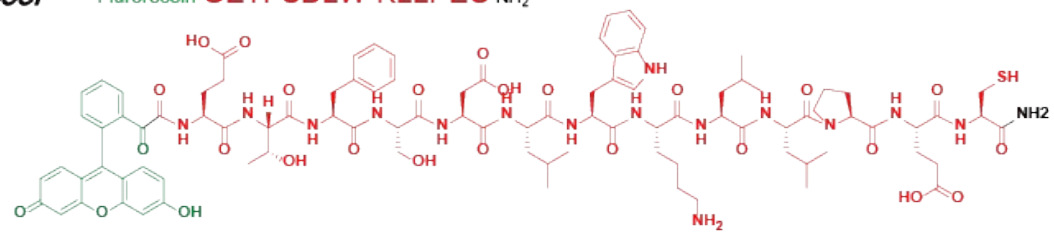
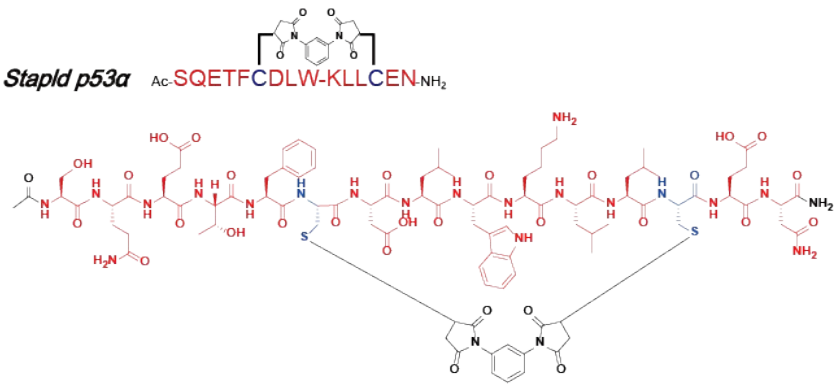


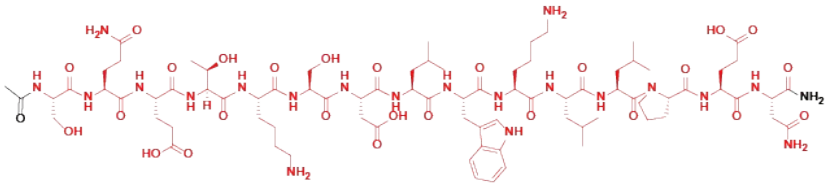
p53f Flurorecein-GETFSDLW-KLLPEC-NH_2

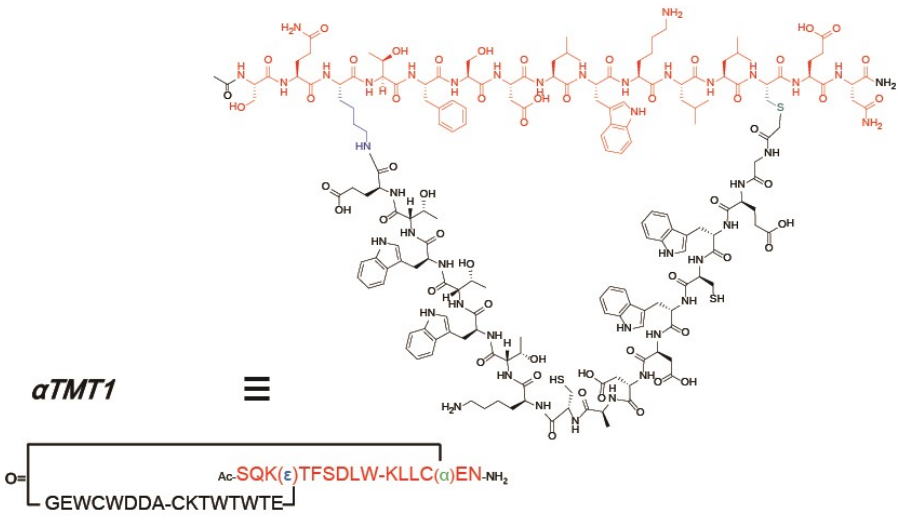


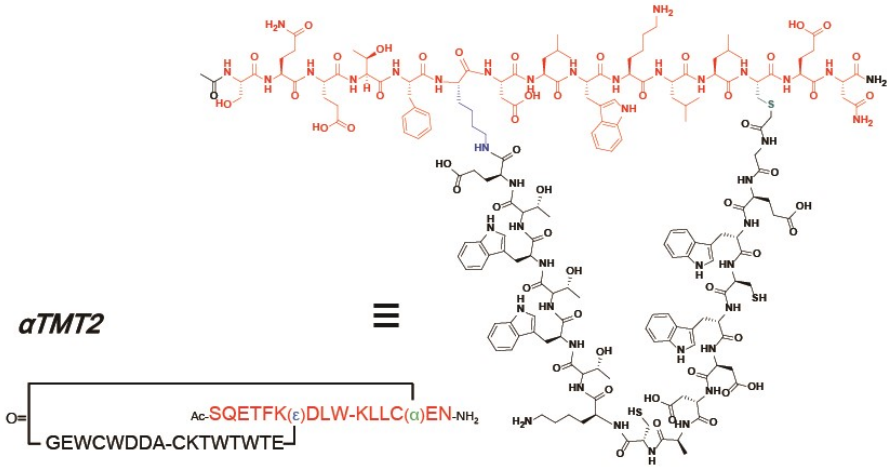
Stapld p53α Ac-SQETFCDLW-KLLCEN-NH_2



Native unstapled p53α Ac-SQETFSDLW-KLLPEN-NH_2




αTMT1
≡
Ac-SQK(ε)TFSDLW-KLLC(α)EN-NH2
GEWCWDDA-CKTWTWTE


αTMT2
≡
Ac-SQETFK(ε)DLW-KLLC(α)EN-NH2
GEWCWDDA-CKTWTWTE

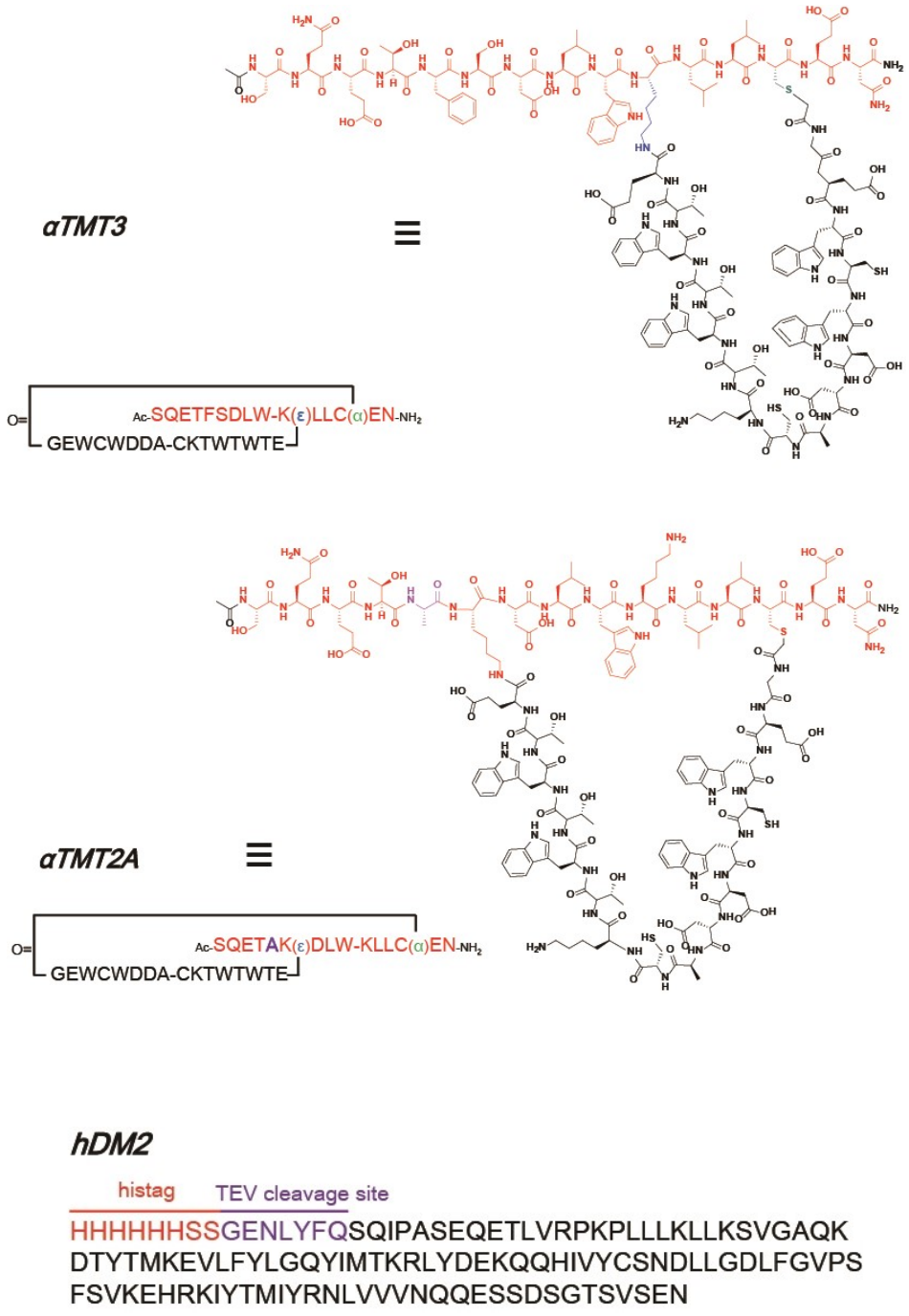


Fig. S1 Chemical structures of peptides and sequence of the expressed hDM2 proteins.

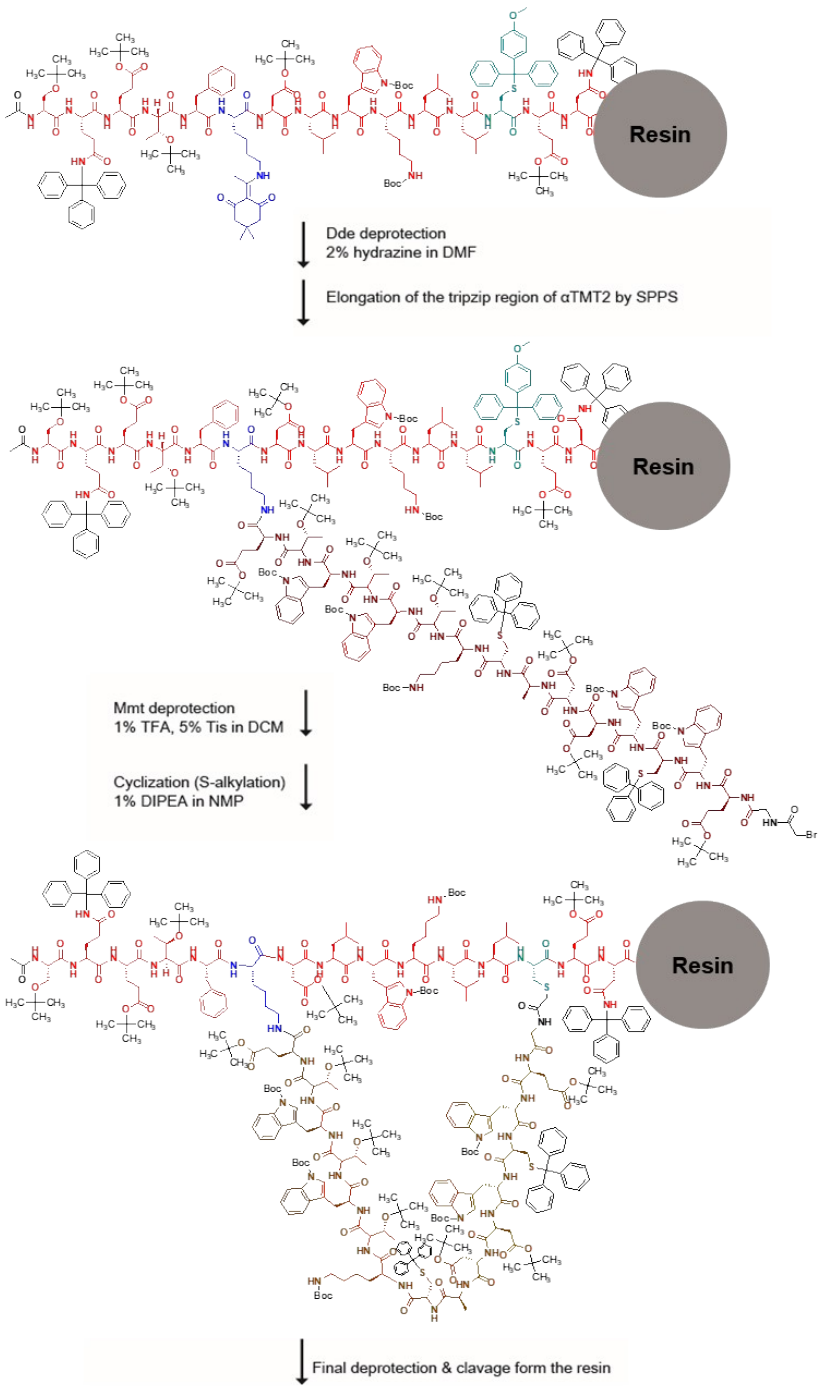


Fig. S2 Reaction scheme of the αTMT2 synthesis.

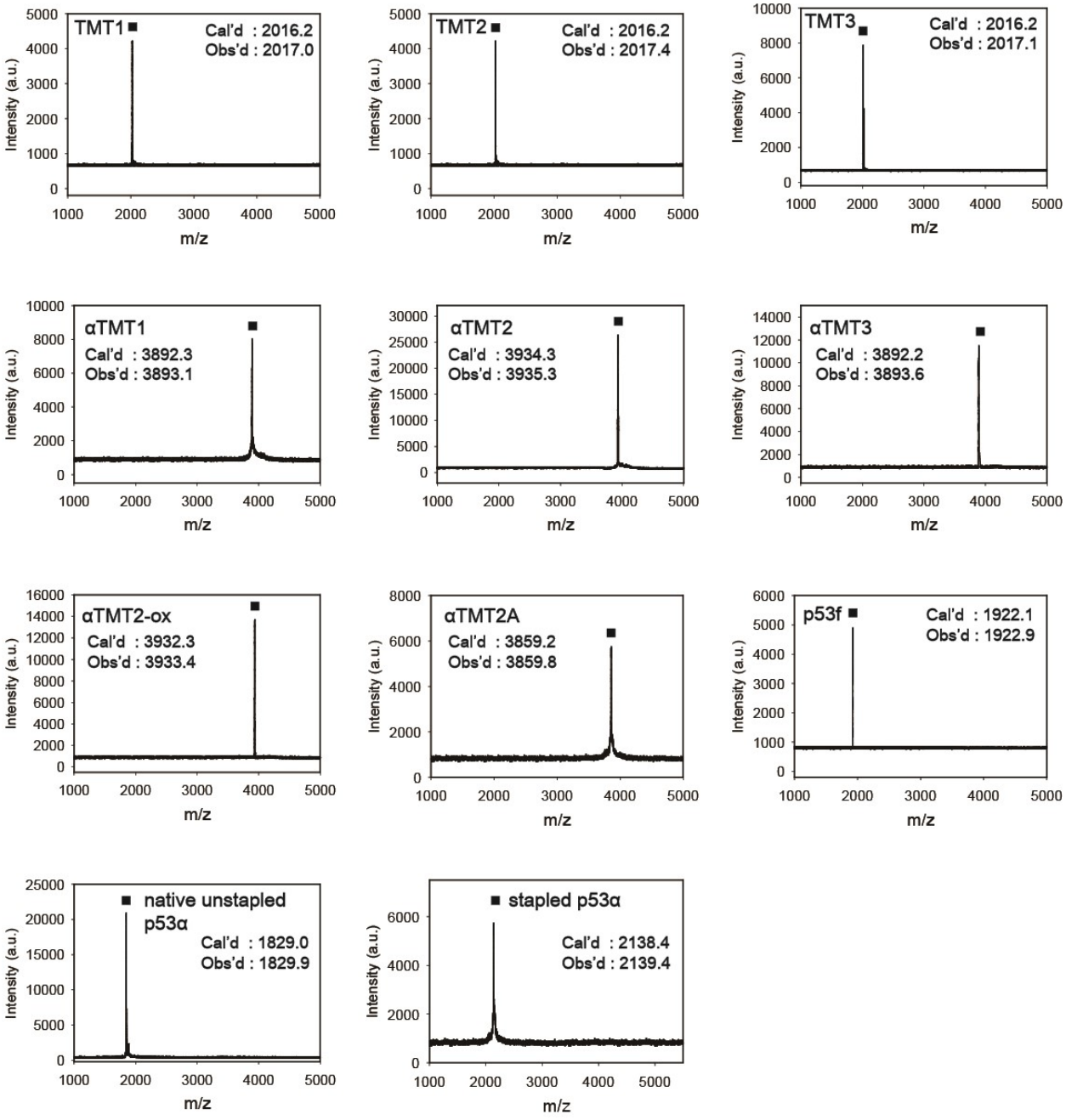


Fig. S3 MALDI-TOF MS spectra of peptides.

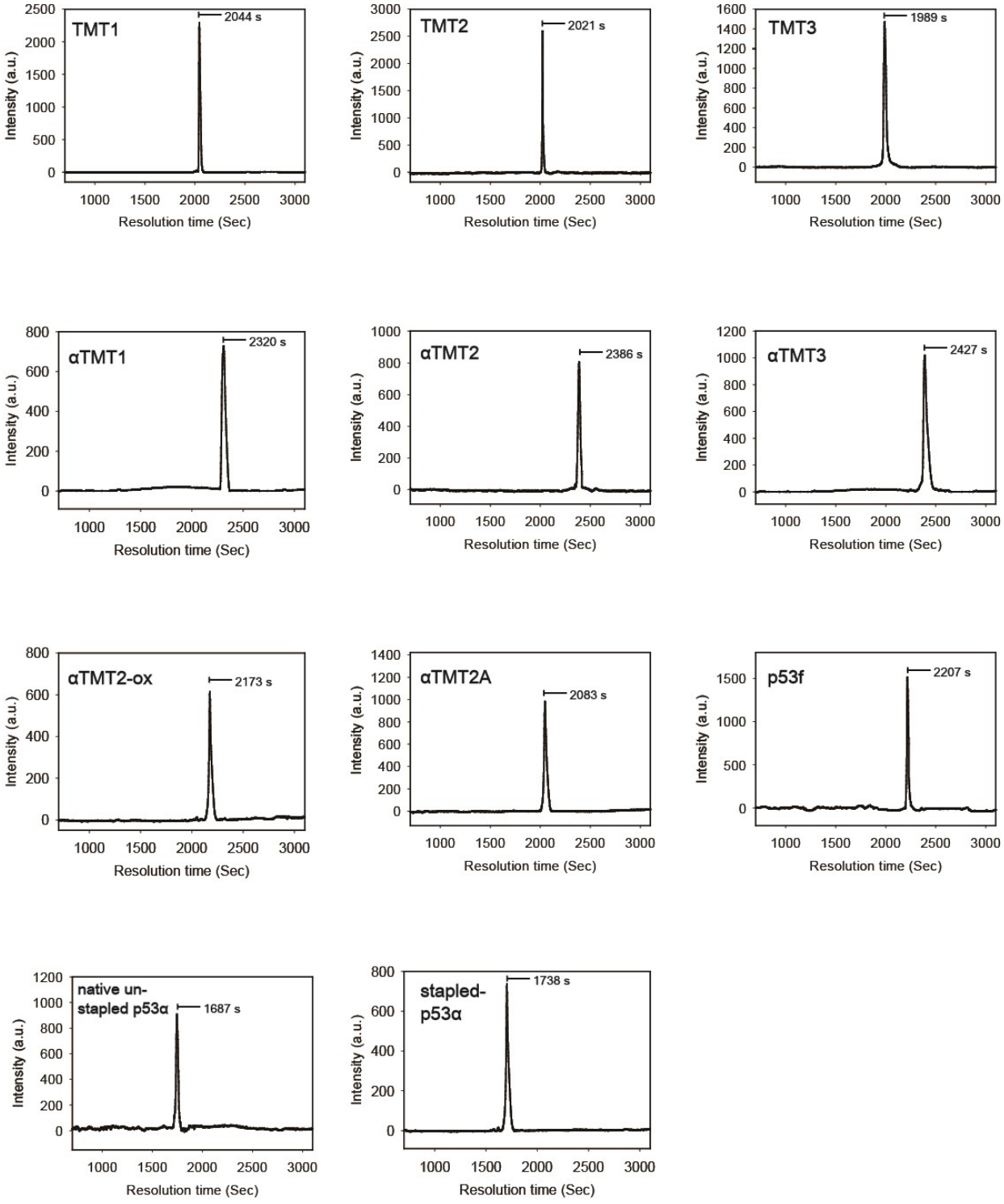


Fig. S4 HPLC chromatograms of peptides. Reverse-phase (RP) HPLC was performed using a Vydac C18 column (10 × 250 mm, 300 Å) with a gradient of 30% to 50% MeCN with 0.1% TFA.

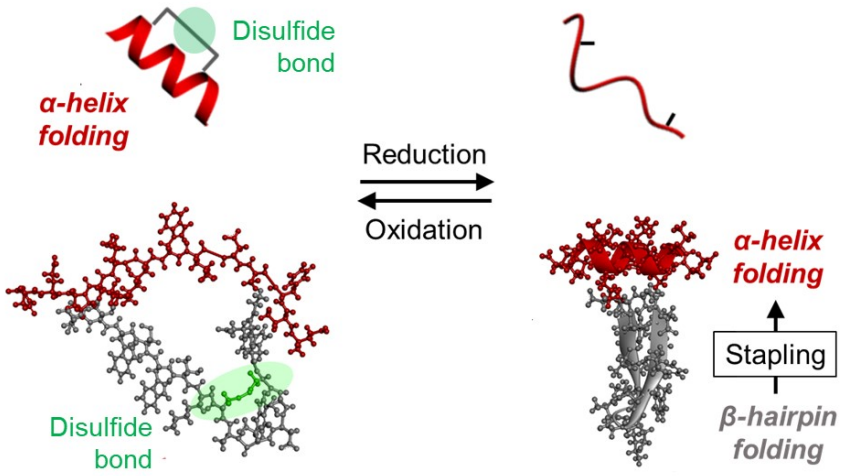


Fig. S5 Schematic illustration of redox-responsive α-helix stabilization behaviors of the typical disulfide stapling (top) and αTMT (bottom) strategies.

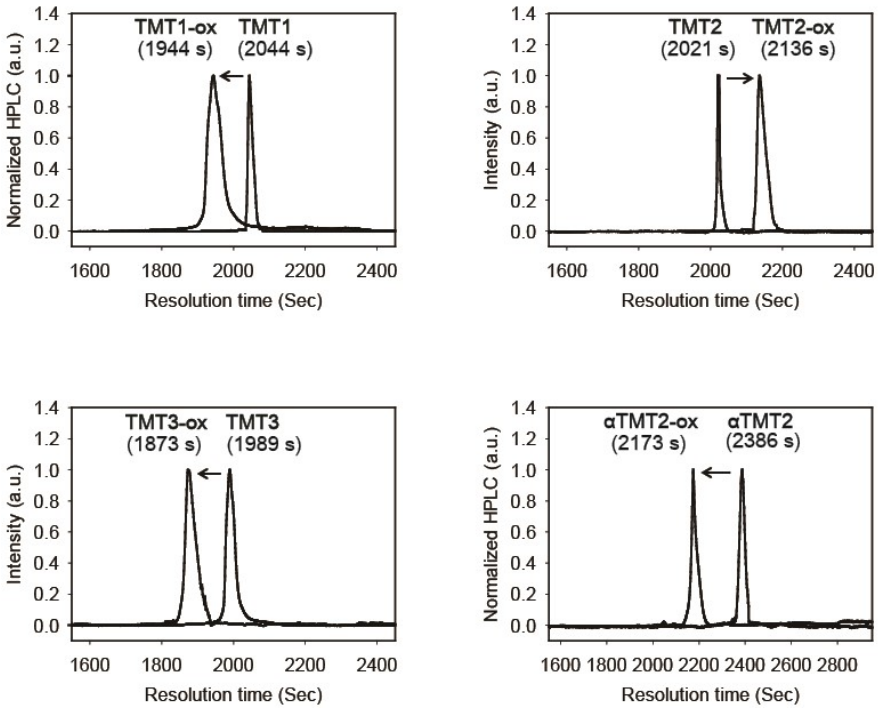


Fig. S6 HPLC peak shift after oxidation (disulfide bond formation) of peptides.

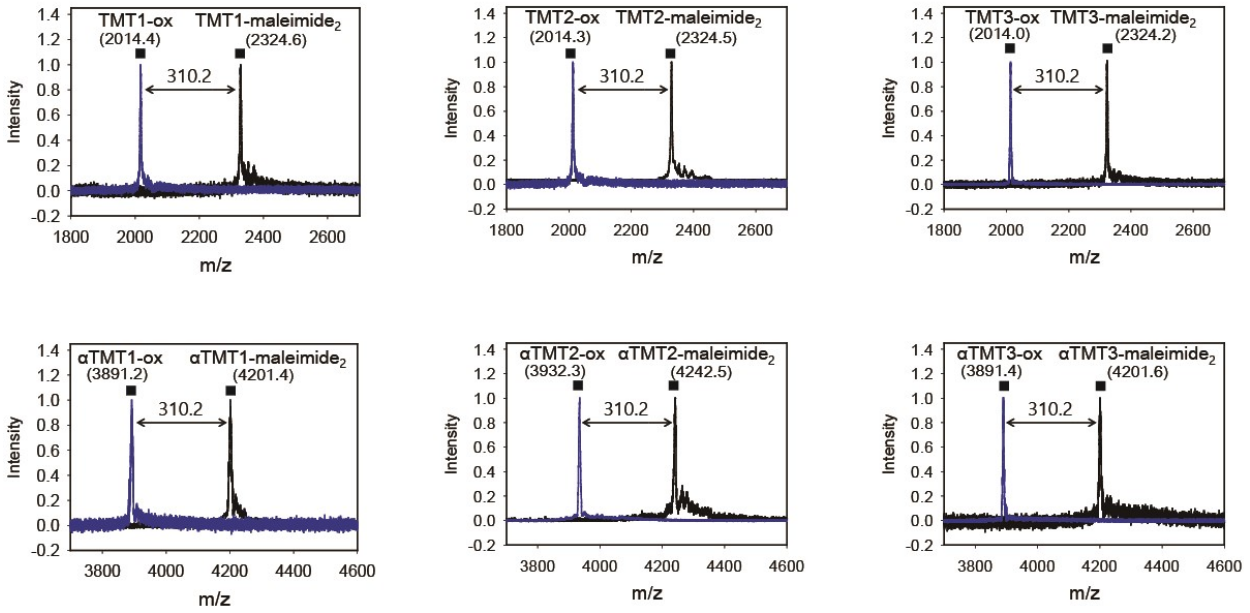


Fig. S7 MALDI-TOF MS spectra of peptides and their conjugates with n-methoxycarbonyl maleimide (MW: 155.11 Da).

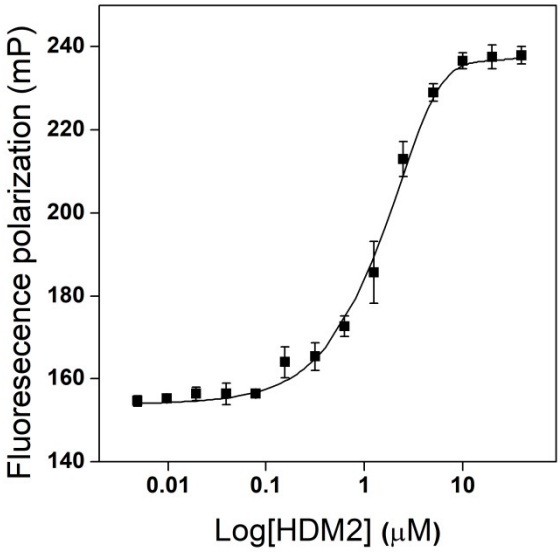


Fig. S8 Changes in the FP intensity of p53f (100 nM) upon titration with hDM2 in Tris-Cl buffer solution (pH 8.0, 20 mM, 10 mM imidazole, 150 mM NaCl, 10% glycerol) at room temperature. The filter sets were 485/15 excitation and 535/25 emission.

Peptides	α-Helix Sequence	EC50 (μM)	K_d (μM)
Nutlin-3a	-	0.154	0.145
αTMT1	$_{Ac-}$SQK(ε)TFSDLWKLLC(α)EN$_{-NH2}$	0.547	0.515
αTMT2	$_{Ac-}$SQETFK(ε)DLWKLLC(α)EN$_{-NH2}$	0.149	0.140
αTMT3	$_{Ac-}$SQETFSDLWK(ε)LLC(α)EN$_{-NH2}$	0.655	0.616
αTMT2A	$_{Ac-}$SQETAK(ε)DLWKLLC(α)EN-$_{-NH2}$	4.584	4.314
αTMT2-ox	$_{Ac-}$SQETFK(ε)DLWKLLC(α)EN$_{-NH2}$	0.569	0.536
p53α-stapled	$_{Ac-}$SQETFCDLWKLLCEN$_{-NH2}$	0.343	0.323

Fig. S9 Calculated EC50 and dissociation constant (K_d) values for the p53f/hDM2 complexes with the investigated peptides.

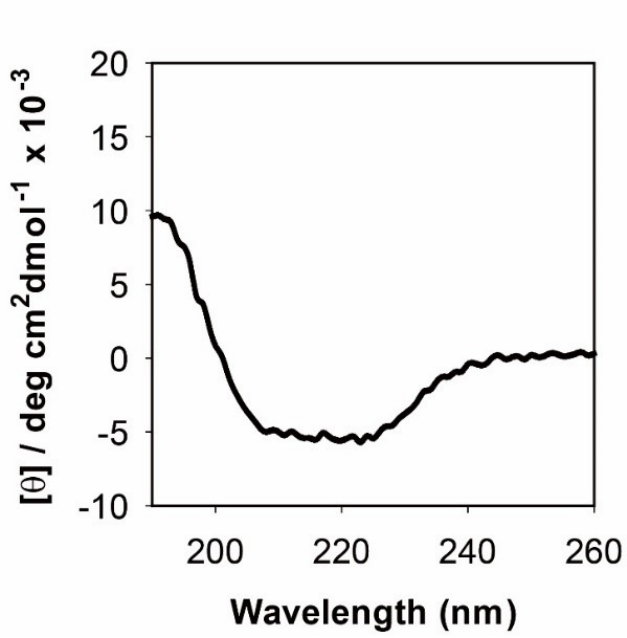


Fig. S10 CD spectrum of the stapled p53α peptides (20 μM) in PBS (pH 7.0, 20 mM, 150 mM NaCl).

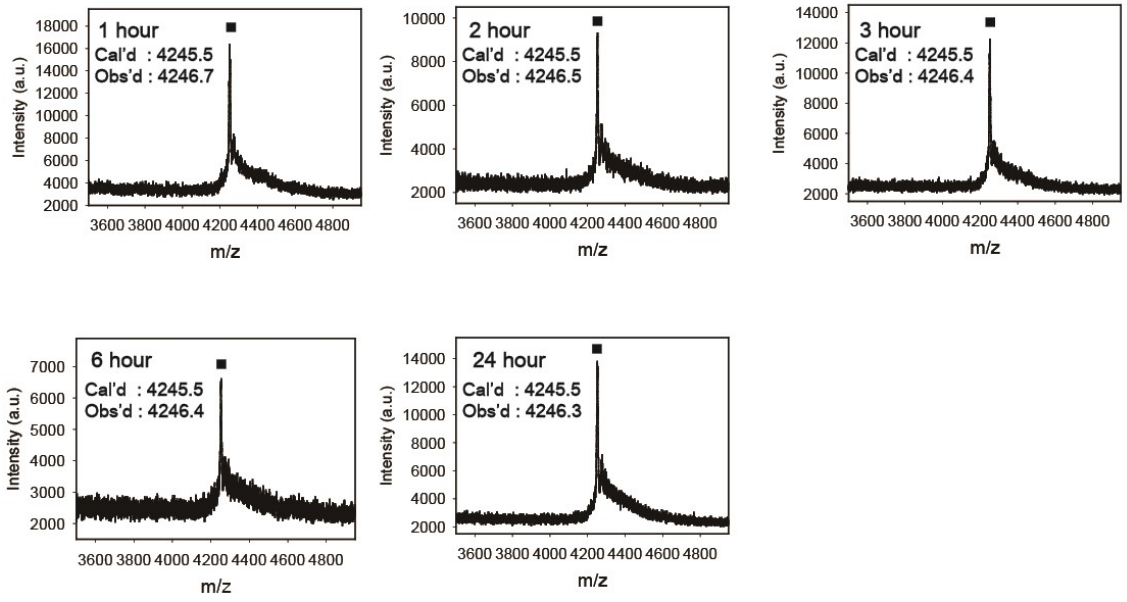


Fig. S11 MALDI-TOF analysis of the αTMT2 peptides after incubation in PBS (pH 7.0, 20 mM, 150 mM NaCl) for different times at room temperature and n-methoxycarbonyl maleimide (2 mM) coupling.

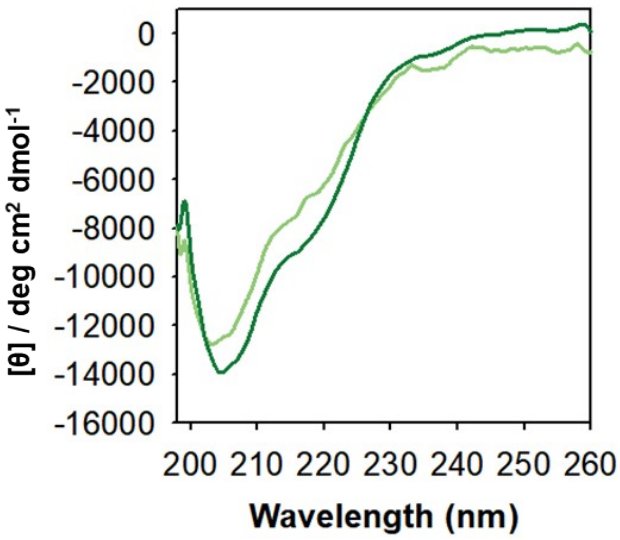


Fig. S12 CD spectra of αTMT2 (green) and αTMT2-ox (light green) in PBS (pH 7.0, 20 mM, 150 mM NaCl) at room temperature. [peptide] = 20 μM.

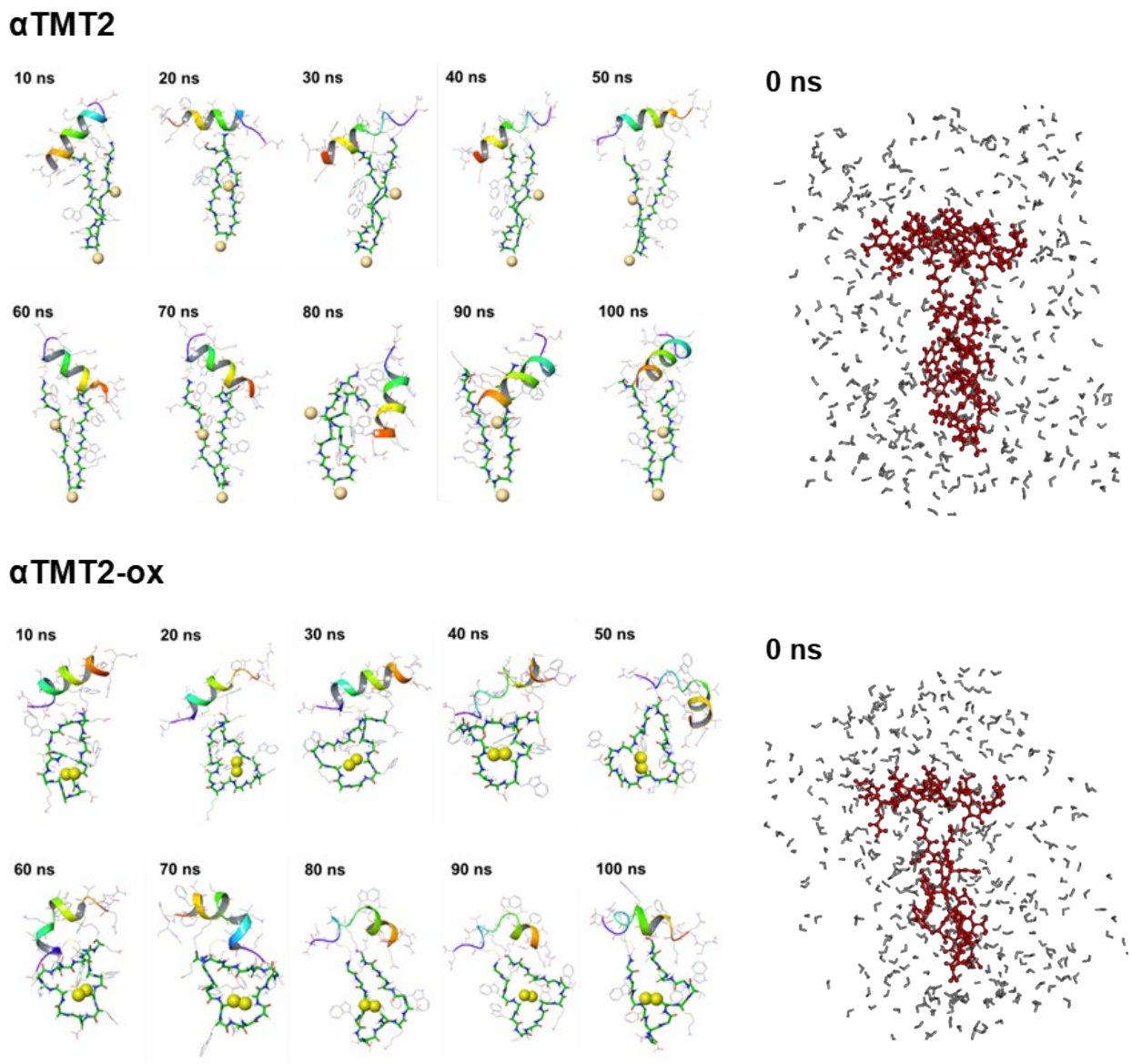


Fig. S13 MD simulation results of folding behaviors of αTMT2 and αTMT2-ox and snapshots of the input models with their geometry.

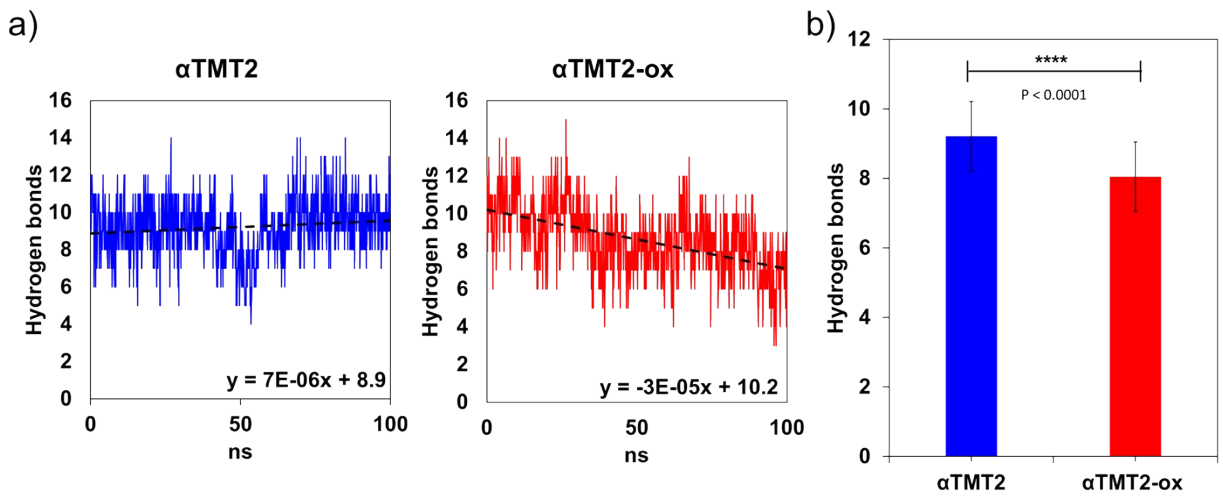


Fig. S14 (a) The numbers of hydrogen bonds in the helical backbone of αTMT2 and αTMT2-ox and (b) the average numbers of these hydrogen bonds over the simulation time (100 ns).

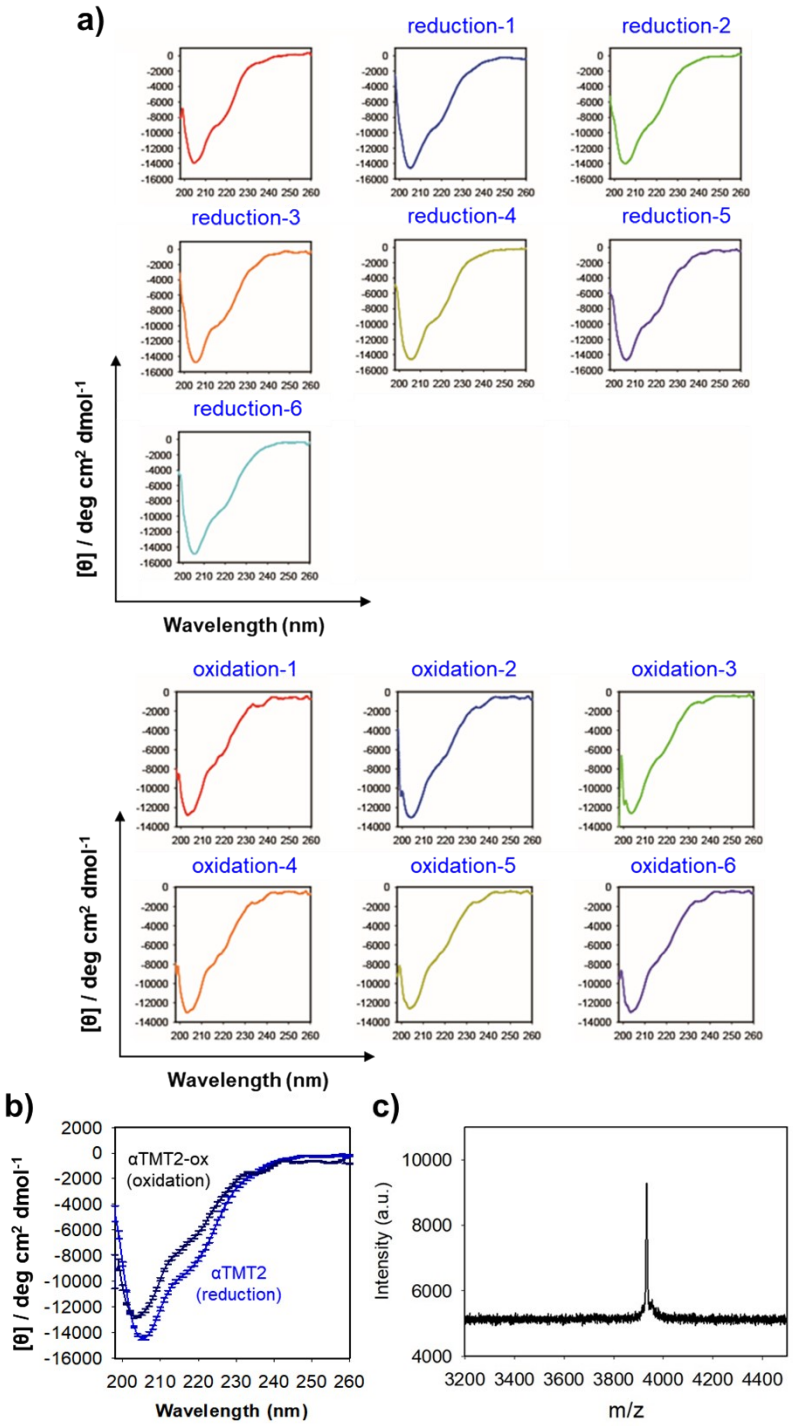


Fig. S15 (a) CD spectra of αTMT2 and αTMT2-ox during the six cycles of oxidation-reduction processes. (b) Averaged CD spectra of the oxidized and reduced αTMT2 peptides from the repeated oxidation-reduction experiment. (c) MALDI-TOF spectrum of αTMT2 after the repeated oxidation-reduction experiment (calculated MW: 3934.3, observed MW: 3935.6).

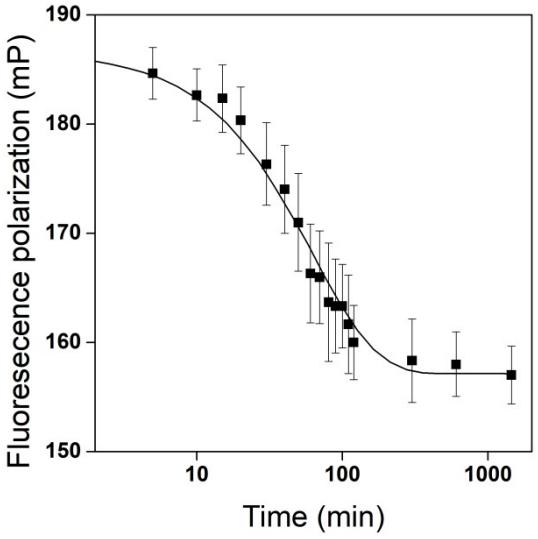


Fig. S16 Time-dependent FP competition assay on the αTMT2-ox peptides against p53f/hDM2 (100 nM/4 μM) complexes in Tris-Cl buffer solution (pH 8.0, 20 mM, 10 mM imidazole, 150 mM NaCl, 10% glycerol) at room temperature, with the reducing agents (1 mM TCEP). The filter sets were 485/15 excitation and 535/25 emission. The experiment was performed in triplicate.

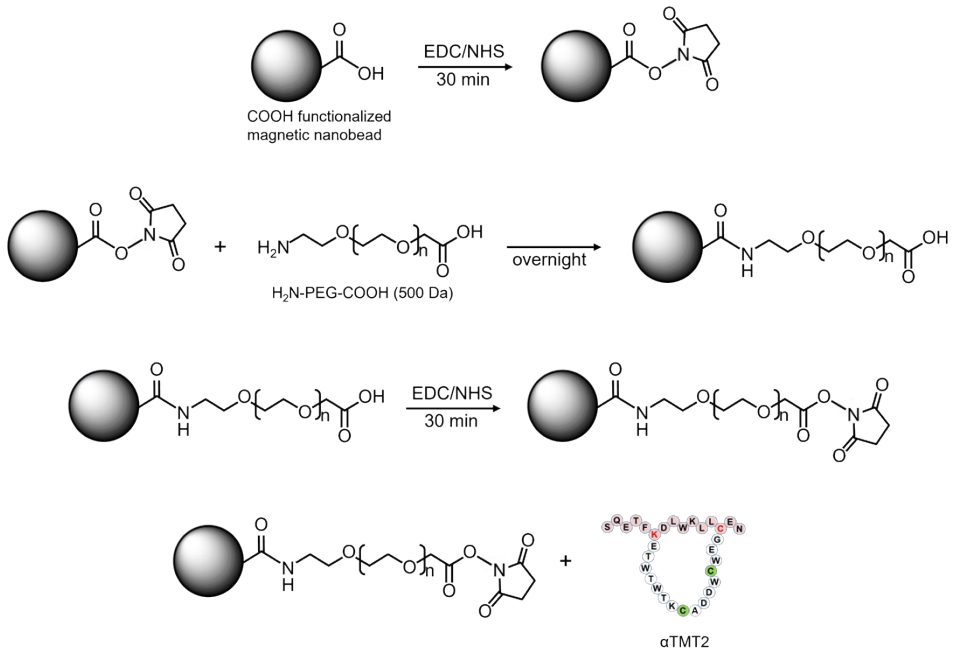


Fig. S17 Synthetic scheme of αTMT2-bead.

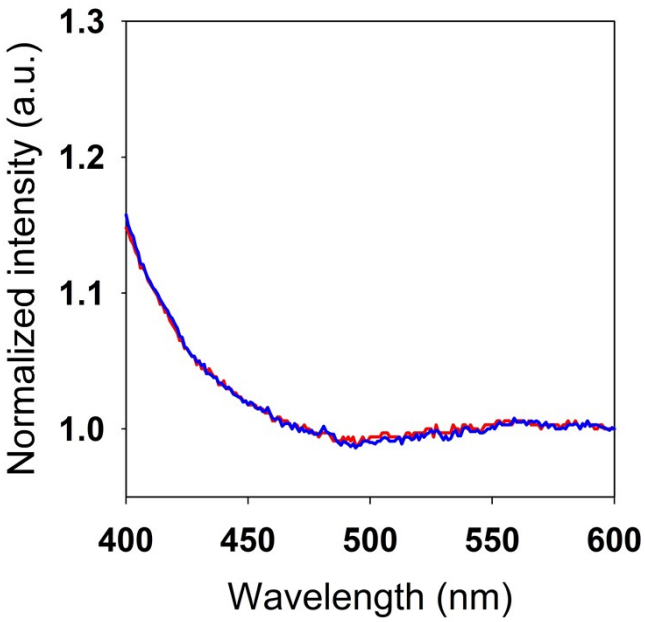


Fig. S18 The capture-and-release (CAR) experiment using PEG-magnetic bead conjugates without peptides. UV-Vis absorption spectra of released hGCs before (red) and after (blue) 5 h incubation in PBS (pH 7.0, 20 mM, 150 mM NaCl) containing the oxidizing agents (35 μM) at room temperature.