# Electronic Supplementary Information

## Stabilization of α-helix by the self-assembly of macrocyclic peptides on the surface of gold nanoparticles for molecular recognition

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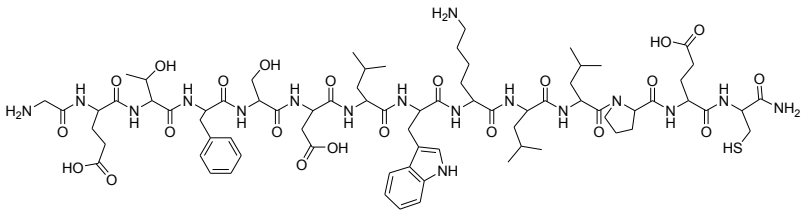
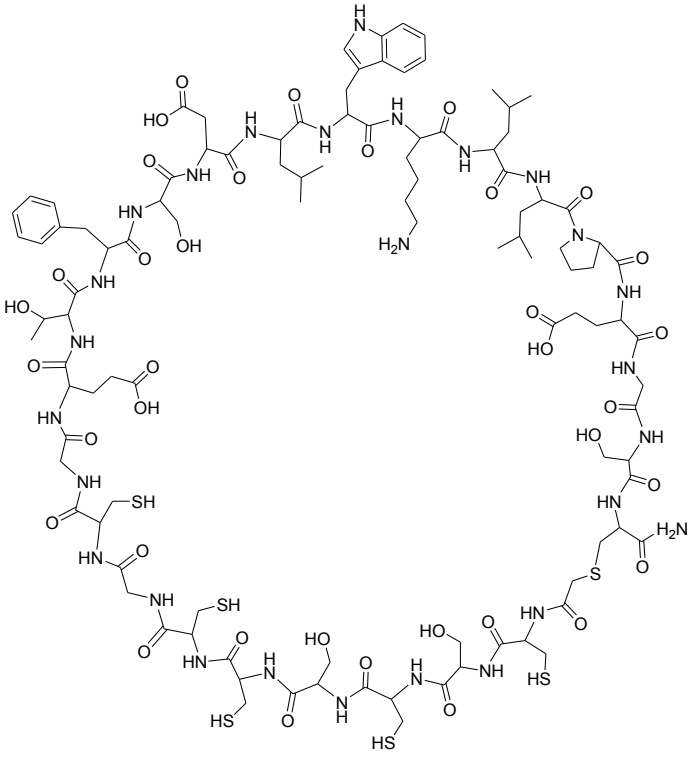
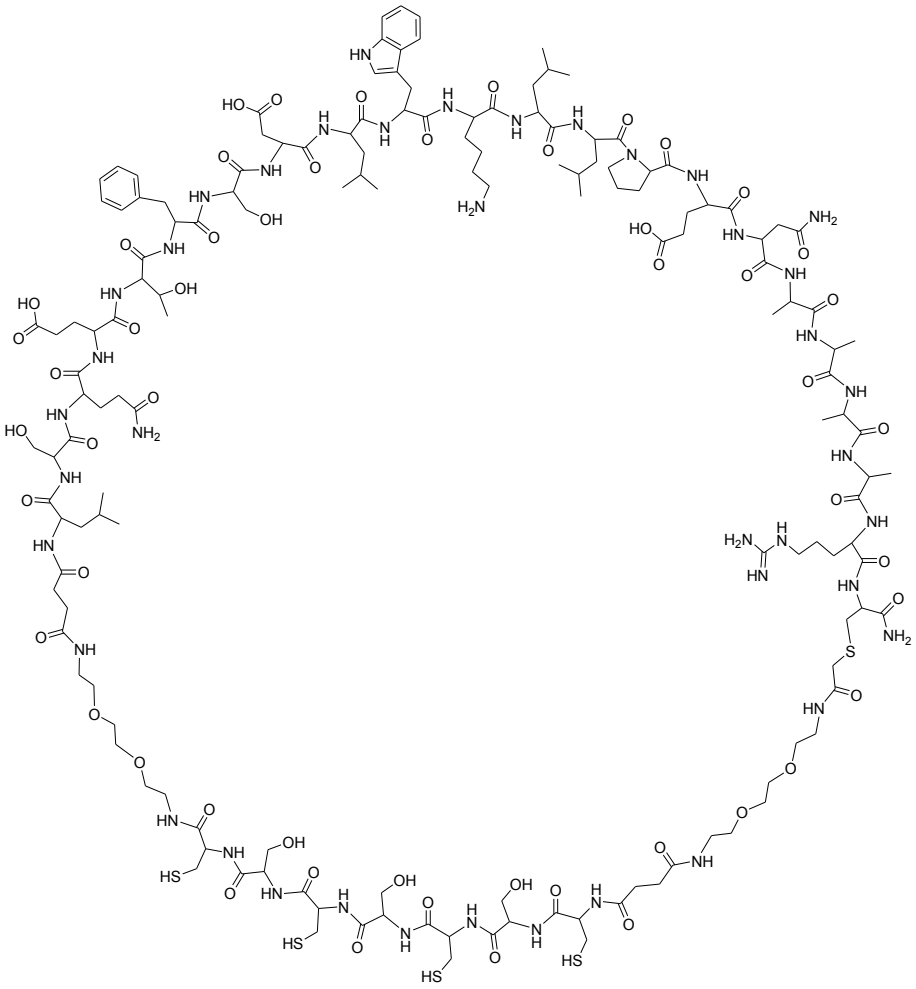


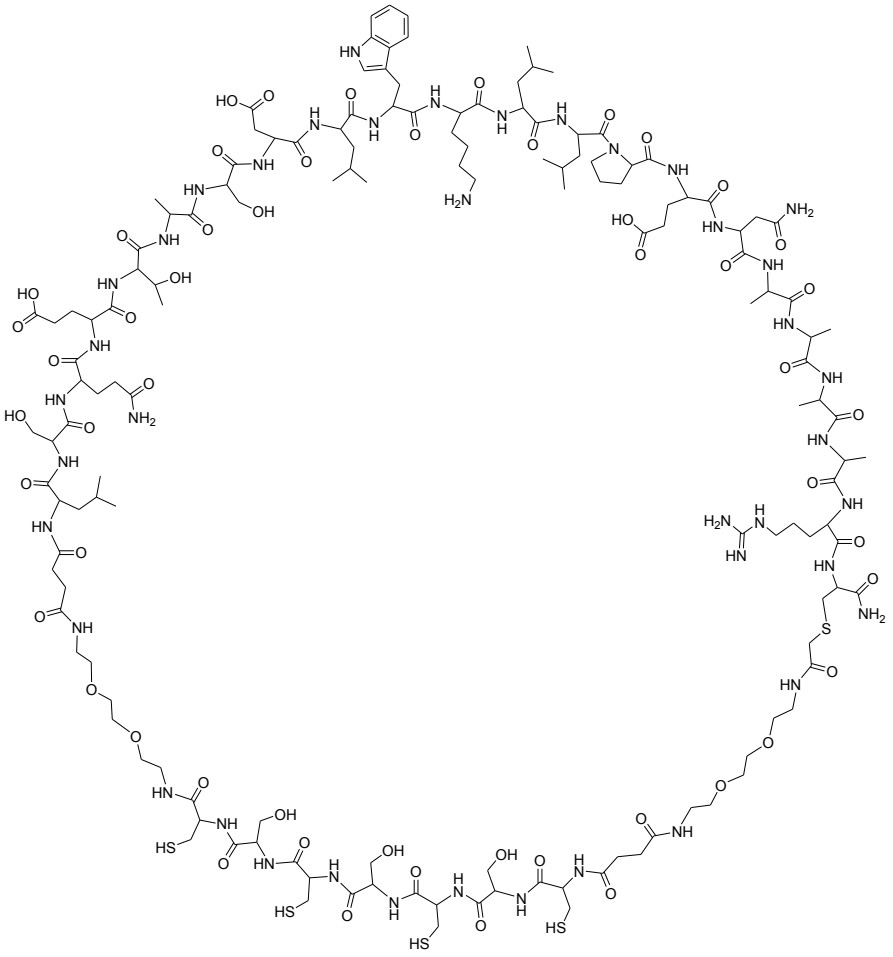
$p53_{17\text{-}28}$



Cyclo-$p53_{17\text{-}28}$



Cyclo-p53$_{14\text{-}29}$



Cyclo-p53$_{14\text{-}29(F19A)}$

**Figure S1.** Chemical structures of peptides.

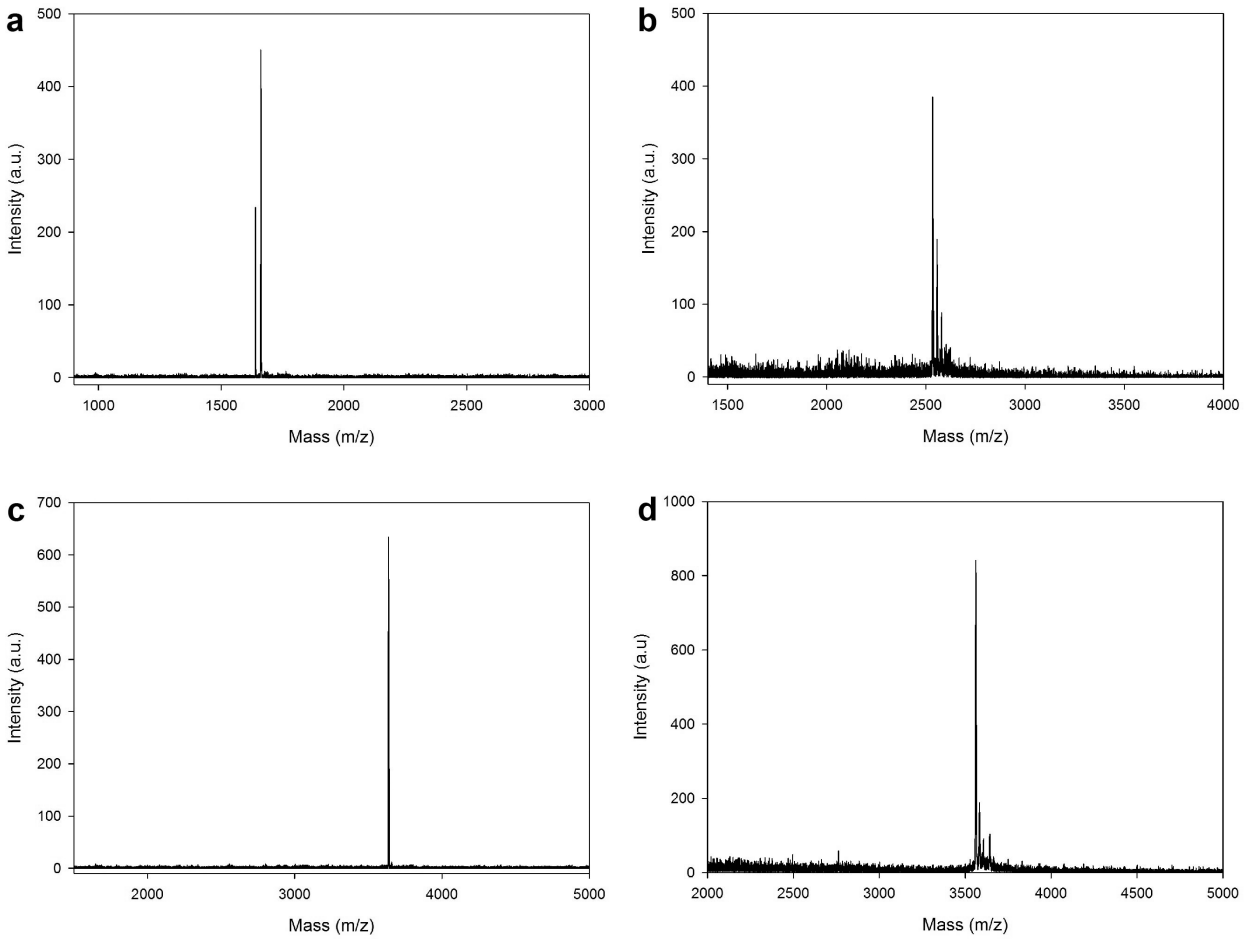



**Figure S2.** MALDI-TOF MS spectra of the peptides. (a) $p53_{17\text{-}28}$, (b) cyclo-$p53_{17\text{-}28}$, (c) cyclo-$p53_{14\text{-}29}$, and (d) cyclo-$p53_{14\text{-}29(F19A)}$.

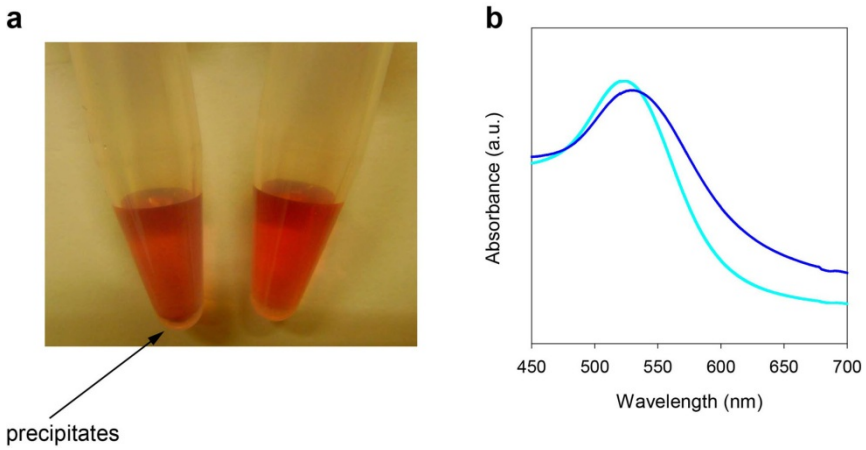



**Figure S3.** Recognition of MDM2-coated AuNPs by cyclic peptide/AuNP biohybrids. (a) Left: mixture of cyclo-p53$_{17\text{-}28}$ peptide/AuNP biohybrids and MDM2/Ni(II)-NTA-AuNP conjugates (mixture 3). Right: mixture of cyclo-p53$_{17\text{-}28}$ peptide/AuNP biohybrids and Ni(II)-NTA-AuNP conjugates (mixture 4). (b) UV/Vis absorption spectra. Blue: mixture 3, Light blue: mixture 4.

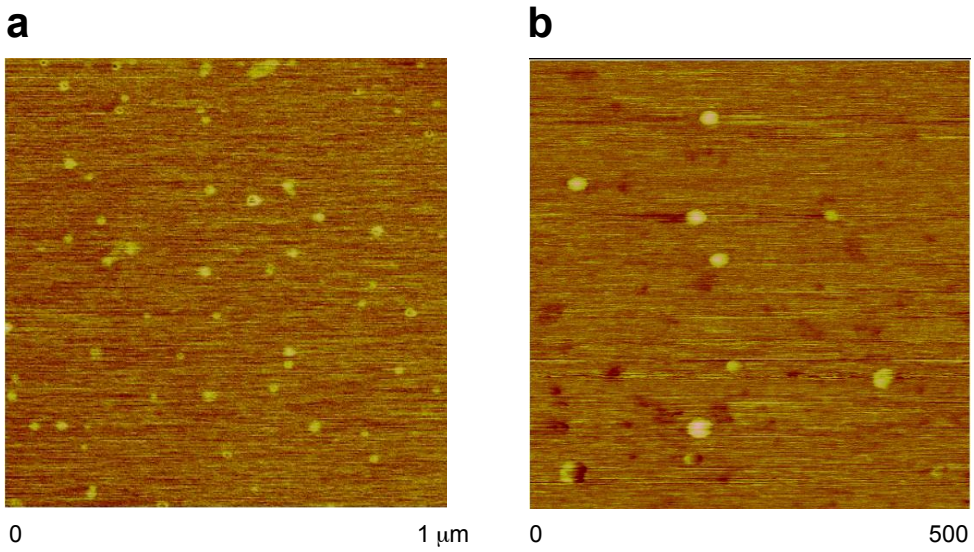



**Figure S4.** AFM images of (a) cyclo-p53$_{17\text{-}28}$ peptide/AuNP biohybrids and (b) cyclo-p53$_{14\text{-}29}$ peptide/AuNP biohybrids.

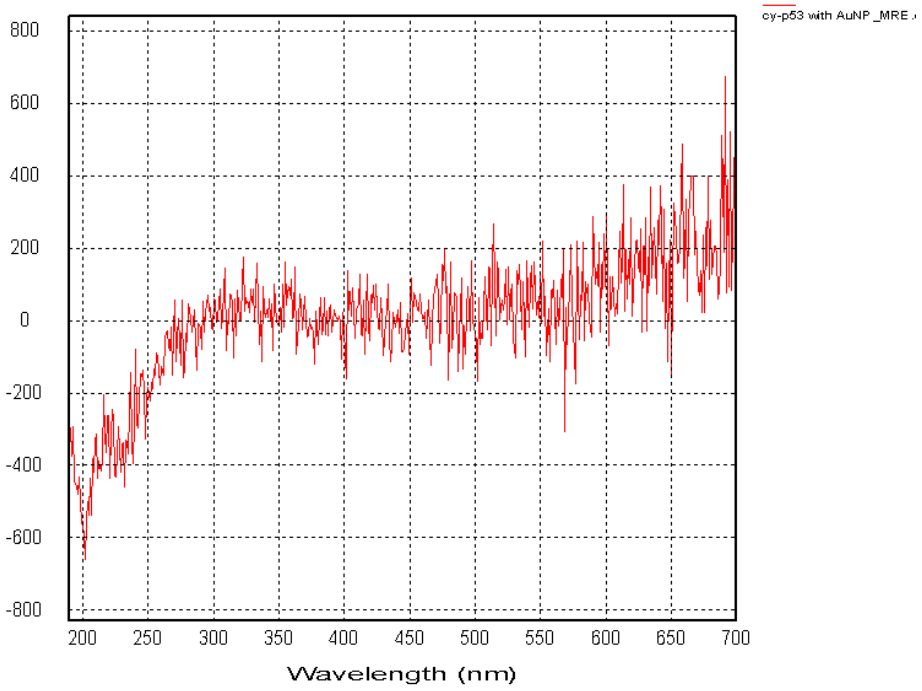



**Figure S5.** CD spectrum of cyclo-p53$_{14\text{-}29}$ peptide/AuNP biohybrids.

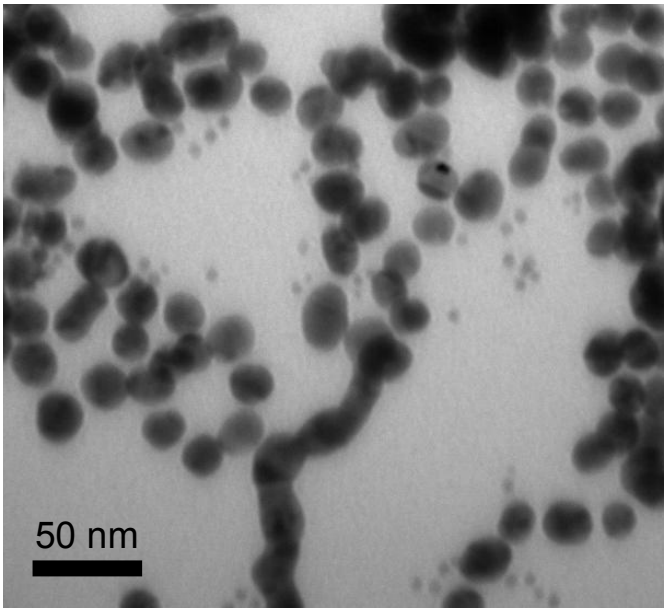



**Figure S6.** TEM image for the mixture of cyclo-p53$_{14\text{-}29}$ peptide/AuNP biohybrids (large nanoparticles) and MDM2/Ni(II)-NTA-AuNP conjugates (small nanoparticles).

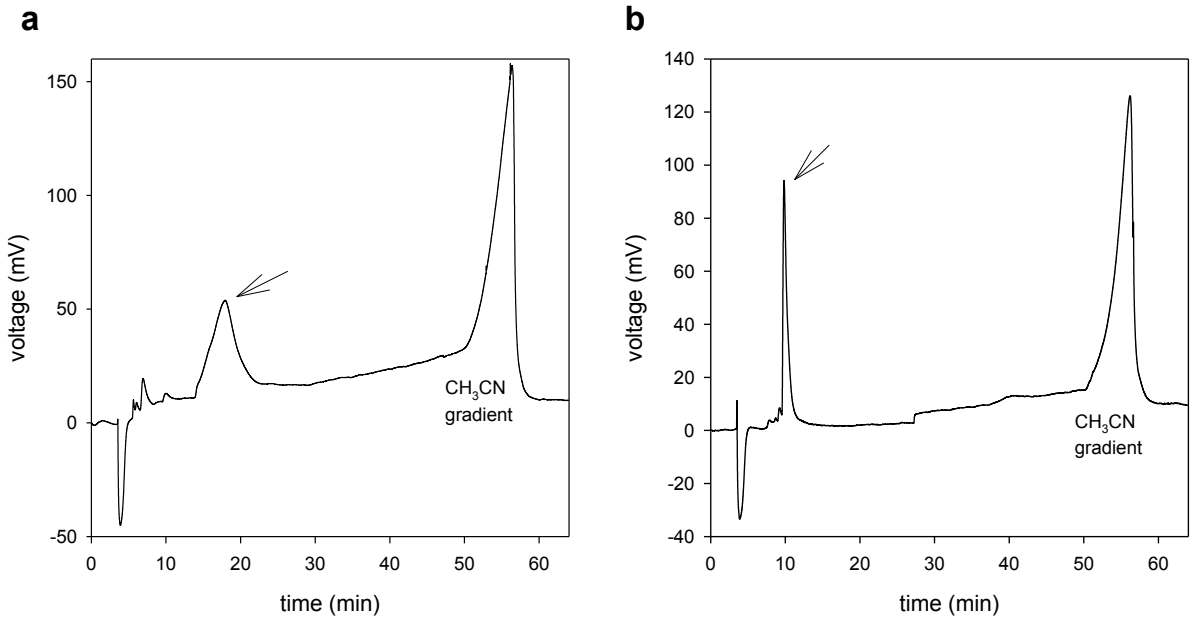



**Figure S7.** HPLC analyses of purified peptides. (a) cyclo-p53$_{17\text{-}28}$ peptide. (b) cyclo-p53$_{14\text{-}29}$ peptide.

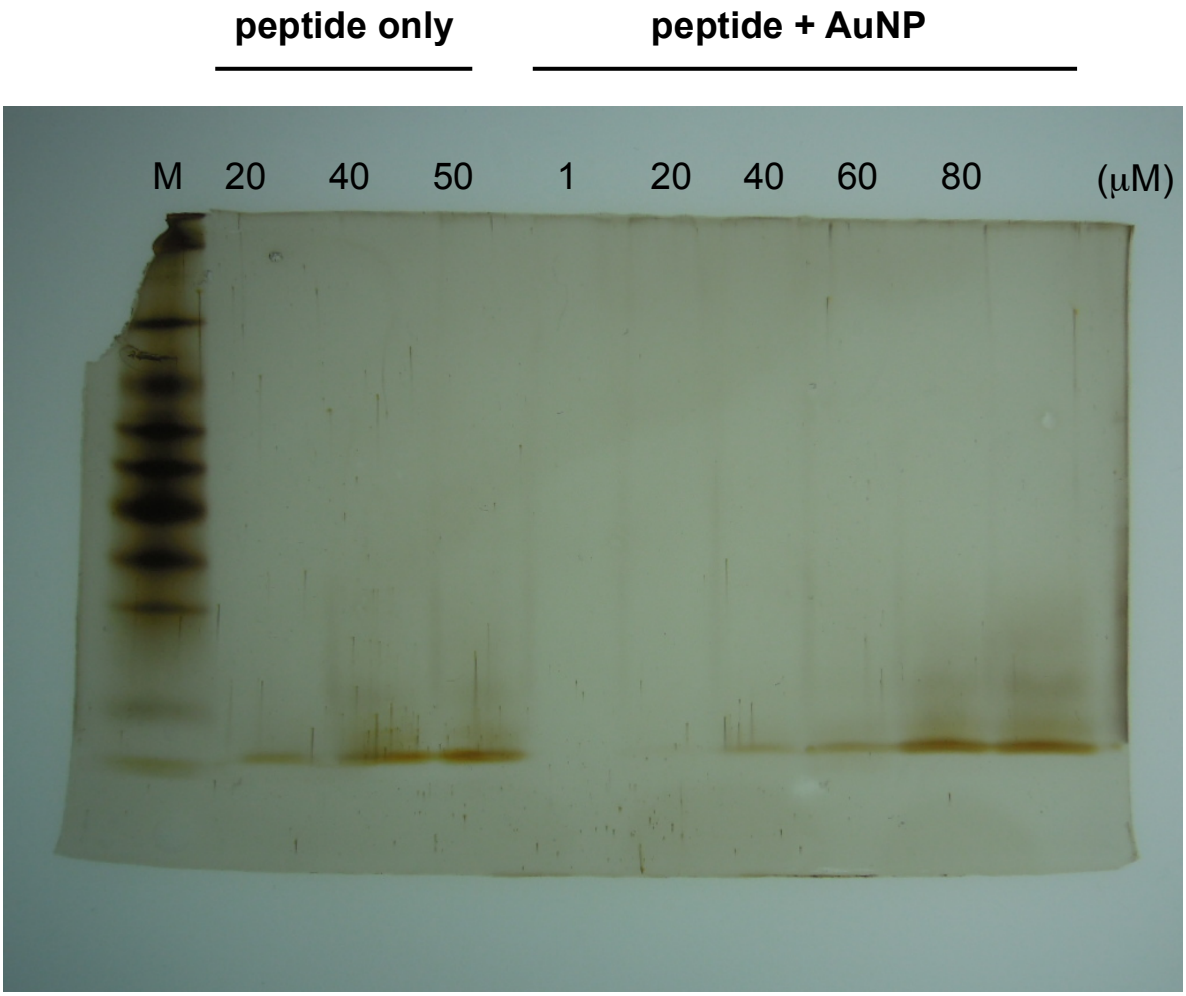



**Figure S8.** SDS-PAGE analysis to determine the amount of cyclo-p53$_{14\text{-}29}$ peptide bound to AuNP. The intensity of bands of the excess free peptide in the peptide and AuNP mixture was compared with that of separately loaded bands of free peptide by densitometric analysis. The analysis revealed that up to ~ 1700 peptide molecules can be bound to AuNPs.

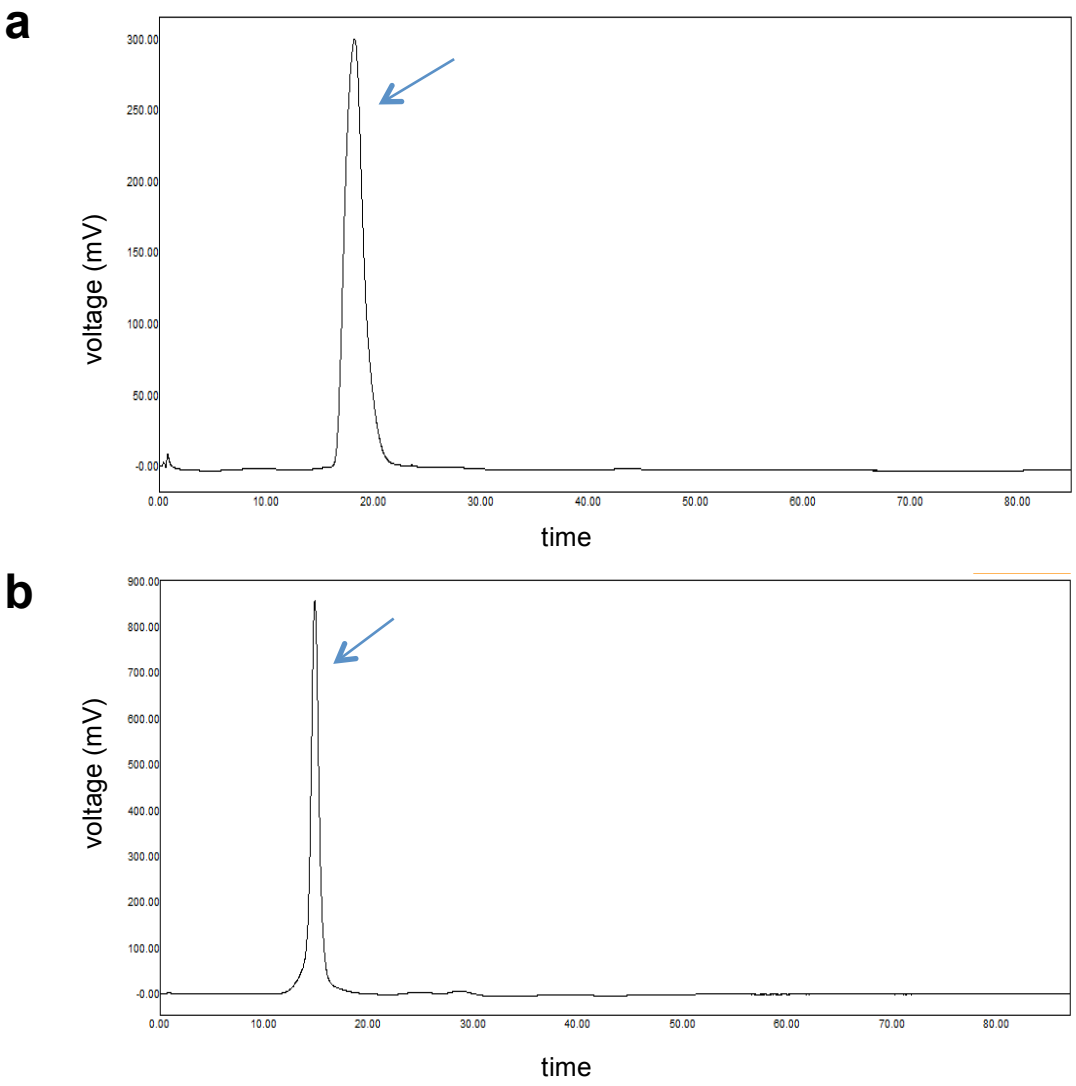



**Figure S9.** Gel filtration chromatograms of (a) cyclo-p53$_{17\text{-}28}$ peptide/AuNP biohybrids. (b) cyclo-p53$_{14\text{-}29}$ peptide/AuNP biohybrids.

# EXPERIMENTAL SECTION

## MATERIALS

Fmoc-amino acids were purchased from Novabiochem. The oligoethylene glycol-based linker N-(Fmoc-8-amino-3,6-dioxaoctyl) succinamic acid (Fmoc-PEG2-Suc-OH) was purchased from AnaSpec. Sodium citrate tribasic dihydrate and hydrogen tetrachloroaurate(III) ($HAuCl_4 \cdot H_2O$) were purchased from Sigma-Aldrich. Five-nanometer AuNPs derivatized with Ni(II)-NTA (Ni-NTA-Nanogold) were acquired from Nanoprobes.

## METHODS

### Peptide synthesis and the macrocyclization reaction

The peptide was synthesized on Rink Amide MBHA resin LL (Novabiochem) using standard Fmoc protocols in a Tribute peptide synthesizer (Protein Technologies, Inc). Standard amino acid protecting groups were employed except for cysteine, for which an acid-labile methoxytrityl (Mmt) group was used.

For cyclization, the peptide-attached resin (20 μmol of N-terminal amine groups) was swollen in N-methyl-2-pyrrolidone (NMP) for 30 min. Bromoacetic acid was then coupled to the N-terminal portion of the resin-bound peptide. Before addition to the resin, a mixture of bromoacetic acid (28 mg, 200 μmol) and N,N′-diisopropylcarbodiimide (15.5 μL, 100 μmol) in NMP was incubated for 10 min for carboxyl activation. Following the addition of this mixture to the resin, the reaction was allowed to continue for 1 h with shaking at room temperature in a 6 mL polypropylene tube with a frit (Restek). The resin was then washed successively with NMP

and dichloromethane (DCM). For orthogonal removal of the Mmt protecting group from the cysteine, the resin was treated with 1% trifluoroacetic acid (TFA) in DCM several times (1 min × ~7). The intramolecular cyclization reaction was performed in 3 mL of 1% diisopropylethylamine (DIPEA) in NMP overnight with shaking at room temperature. The resin was then successively washed with NMP, DMF, and THF and dried under reduced pressure. For cleavage and final deprotection, the resin was treated with a cleavage cocktail (TFA:1,2-ethanedithiol:thioanisole; 95:2.5:2.5) for 3 h and was triturated with *tert*-butyl methyl ether (TBME). The peptides were purified by reverse-phase HPLC (water-acetonitrile with 0.1% TFA). The molecular weight was confirmed by MALDI-TOF mass spectrometry. The purity of the peptides was >95%, as determined by analytical HPLC. The peptide concentration was determined spectrophotometrically in 8 M urea using the molar extinction coefficient of tryptophan (5,500 $M^{-1}cm^{-1}$) at 280 nm.

**Gold nanoparticle synthesis**

Gold colloids were prepared by the sodium citrate reduction of $HAuCl_4$ as reported previously.[1] A 50 mL sample of 0.01% aqueous $HAuCl_4$ (0.243 mM) was vigorously boiled with stirring in a round-bottomed flask equipped a reflux condenser. Next, 7.5 mL of 0.1% aqueous sodium citrate (3.4 mM) was quickly added to the boiling $HAuCl_4$ solution. The color of the solution changed from yellow to dark purple and then to wine red within 3 min. The mixture boiled for another 10 min. The solution was allowed to cool slowly to room temperature with continuous stirring overnight. The concentration of gold colloids was determined spectrophotometrically as previously reported.[2]

**Conjugation of peptides to AuNPs**

Typically, 1 mL of a 1.1 nM citrate gold nanoparticle solution was centrifuged at the maximum speed (RCF: 16110 × g) for 20 min at 4 °C, and the supernatant was removed. The AuNP pellet was resuspended in a 0.5 mL solution of peptides dissolved in 30% 2,2,2-trifluoroethanol (TFE), and the solution was incubated for 1 h. Next, 0.5 mL of triethylene glycol mono-11-mercaptoundecyl ether (TGMUE; 592 μM) in 70% TFE was added to the mixture, which was then incubated for 2 h. TFE was evaporated from the aqueous solution using a centrifugal evaporator system (Labconco) until ~0.3 mL of solution remained. The peptide/AuNP biohybrids were purified by gel filtration chromatography on a Superdex 200 column (GE Healthcare Life Science). The concentration of gold colloids was determined spectrophotometrically as previously reported.[2]

**MDM2 protein expression and purification**

Six-His (His6)-tagged recombinant human MDM2 (hDM2) (N-terminal domain spanning amino acids 17-125) was overexpressed in E. coli. The plasmid pET28a-hMDM2(17-125) [kanamycin-resistance (KanR), kindly provided by Dr. Gregory Verdine, Harvard], which encodes a truncated human hDM2 protein (a.a. 17-125) with an N-terminal six-His (His6)-tag and a thrombin cleavage site, was introduced into the *E. coli* strain BL-21-Codon Plus (DE3)-RIPL [chloramphenicol-resistance (CmR), Agilent Technologies] using a standard chemical transformation method. A single transformed colony was cultured in LB media with Cm (25 μg/mL) and Kan (20 μg/mL) at 30 °C until the optical density (OD) at 600 nm was ~0.5. The culture was then treated with isopropyl β-d-thiogalactoside (IPTG; 0.1 mM) at 16 °C for 18 h to

induce the expression of the recombinant protein. The *E. coli* were collected by centrifuging the culture at 6000 rpm (Sorvall) for 10 min at 4 °C.

The purification steps were performed at 4 °C, unless otherwise specified. The *E. coli* cell pellet was resuspended in lysis buffer [20 mM Tris·HCl pH 8.0, 500 mM NaCl, 10% glycerol, 20 mM imidazole, 0.1% (v/v) NP-40, and 5 mM β-mercaptoethanol] containing a protease inhibitor cocktail (1 mM PMSF, 10 μg/ml aprotinin, 5 μg/ml pepstatin and 5 μg/ml leupeptin) and was treated with lysozyme (1 mg/ml) for 45 min to lyse the bacterial cell wall. The resuspended cells were then sonicated to break open the cells and to shear the genomic DNA until the cell lysate was no longer viscous. After removing the cell debris by centrifugation at 14000 × g for 10 min, the recombinant protein was purified from the cell lysate in lysis buffer using Ni-NTA agarose beads following the standard purification protocol provided by the vendor (Qiagen), except for the elution, which was performed in lysis buffer containing 250 mM imidazole. The sample purity and identity were confirmed using SDS-PAGE and MALDI-TOF mass spectrometry. The concentration of the purified MDM2 protein was determined using the Micro BCA protein assay (Pierce).

**CD spectroscopy (CD)**

CD spectra were measured using a Chirascan Circular Dichroism spectrometer equipped with a Peltier temperature controller (Applied Photophysics., Ltd). The CD spectra for peptides (1-5 μM) were recorded form 260 to 190 nm using a 2 mm path-length cuvette. CD spectra for the peptide/AuNP biohybrids (concentrations of ~ 1 μM and ~1 nM for the peptide and AuNPs, respectively).

**Transmission electron microscopy (TEM)**

One microliter of sample was placed on a carbon-coated copper grid and completely dried. The specimen was observed with a JOEL-JEM 2010 instrument operating at 120 kV. The data were analyzed using DigitalMicrograph™ software.

**Atomic force microscopy (AFM)**

One microliter of sample in water was placed on a freshly cleaved mica surface and completely dried. The images were acquired in tapping mode using a Nanoscope IV instrument (Digital Instruments). The AFM scans were performed at a setpoint of 0.8-1 V, and the scanning speed was 1-2 Hz.

1. Nath, N.; Chilkoti, A. *Anal. Chem.* **2002,** *74*, 504-509.
2. Haiss, W.; Thanh, N. T.; Aveyard, J.; Fernig, D. G. *Anal. Chem.* **2007,** *79*, 4215-4221.