Electronic Supplementary Material (ESI) for ChemComm.

Dual-Responsive Nanoparticles Release Cargo Upon Exposure to Matrix Metalloproteinase and Reactive Oxygen Species

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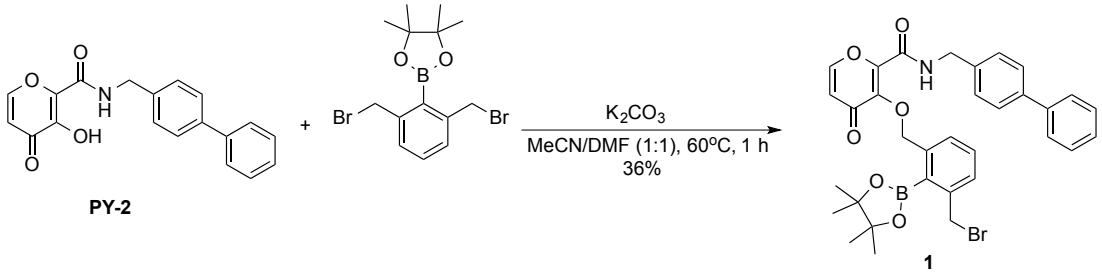
Supporting Information

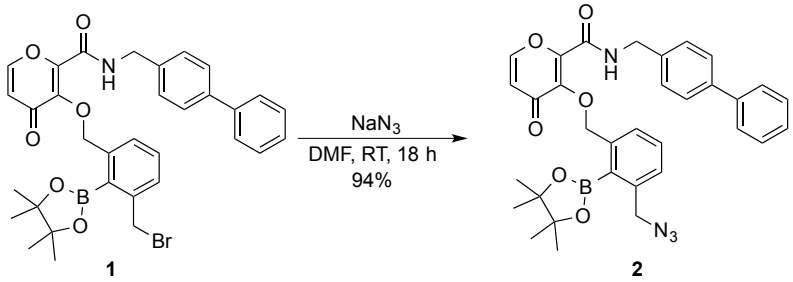
General Experimental Details

All chemicals were purchased from commercial suppliers (Sigma-Aldrich, Fisher Scientific) and were used without further purification. All reactions were carried out under N_2 in oven-dried glassware. Normal and reverse phase chromatography was performed using a CombiFlash *Rf* 200 automated system from TeledyneISCO (Lincoln, NE, USA). NMR spectra were recorded on a Varian FT 400 MHz NMR instrument. Mass spectrometry (MS) was performed at the Molecular Mass Spectrometry Facility (MMSF) in the Department of Chemistry and Biochemistry at the University of California, San Diego. Polymerizations were performed in a dry, N_2 atmosphere with anhydrous solvents. MMP-12 (catalytic domain, human, recombinant) was obtained from Enzo Life Sciences as a solution in 50 mM TRIS, pH 9.5, containing 5 mM calcium chloride, 500 mM sodium chloride, 20 μM zinc chloride, 0.5% Brij-35, and 30% glycerol. HPLC analyses of peptides were performed on a Jupiter 4u Proteo 90A Phenomenex column (150×4.60 mm) with a binary gradient, using a Hitachi-Elite LaChrom 2130 pump, equipped with a Hitachi-Elite LaChrom L-2420 UV-Vis detector at a flow rate of 1 mL min^{-1} and the following mobile phase: 0.1% trifluoroacetic acid in H_2O (A) and 0.1% trifluoroacetic acid in acetonitrile (B). Starting with 100% A, a linear gradient was run for 30 min to a final solvent mixture of 33% A and 67% B, which was held for 5 min before ramping up to 100% B over the course of 2 min. This level was held for an additional 4 min, before ramping back to 100% A, with constant holding at this level for 4 additional min. Polymer dispersities and molecular weights were determined by size-exclusion chromatography (Phenomenex Phenogel 5u 10, 1K-75K, 300×7.80 mm in series with a Phenomenex Phenogel 5u 10, 10K-100K, 300×7.80 mm) in 0.05 M LiBr in DMF, using a Shimadzu pump that was equipped with a multi-angle light scattering detector (DAWN-HELIOS, Wyatt Technology) and a refractive index detectors (Wyatt Optilab T-rEX) normalized to a 30,000 MW polystyrene standard. Hydrodynamic radius (R_h) was determined by DLS, through a Wyatt Dynapro NanoStar. Transmission Electron Microscopy was performed on an FEI Tecnai G2 Sphera at 200 KV. TEM grids were prepared with a 1% uranyl acetate stain on carbon grids from Ted Pella, Inc. In vitro fluorescence measurements were taken on a PTI QuantaMaster Spectrofluorometer.



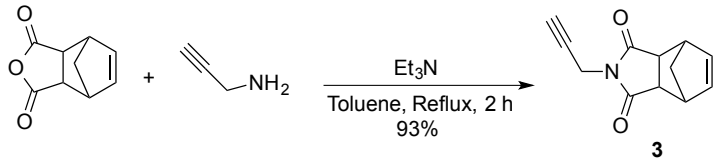
***N*-([1,1'-biphenyl]-4-ylmethyl)-3-((3-(bromomethyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-4-oxo-4*H*-pyran-2-carboxamide (1).**

2-(2,6-Bis(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane[1] (0.70 g, 1.8 mmol) was dissolved in MeCN (15 mL). To this was added K_2CO_3 (0.75 g, 5.4 mmol) and the reaction was heated to 60 ºC. After 30 min, a solution of **PY-2**[2] (0.29 g, 0.9 mmol) in DMF (15 mL) was added to the first mixture over the course of 10 min. Upon addition, the reaction was held at 60 ºC for 1 h. The reaction mixture was cooled to room temperature and the solvent was removed via rotary evaporation. The resulting crude product was purified by silica gel chromatography eluting 5-80% EtOAc in hexanes to afford the desired product in 36% yield (0.20 g, 0.3 mmol). ^{1}H NMR (400 MHz, Acetone-d_6): δ 8.30 (t, J = 6.0 Hz, 1H), 8.09 (d, J = 5.6 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.47-7.33 (m, 8H), 7.18 (d, J = 8.4 Hz, 2H), 6.50 (d, J = 5.6 Hz, 1H), 5.54 (s, 2H), 4.84 (s, 2H), 4.42 (d, J = 6.0 Hz, 2H), 1.37 (s, 12H). ESI-MS(+): *m/z* 652.20 $[M+Na]^+$.



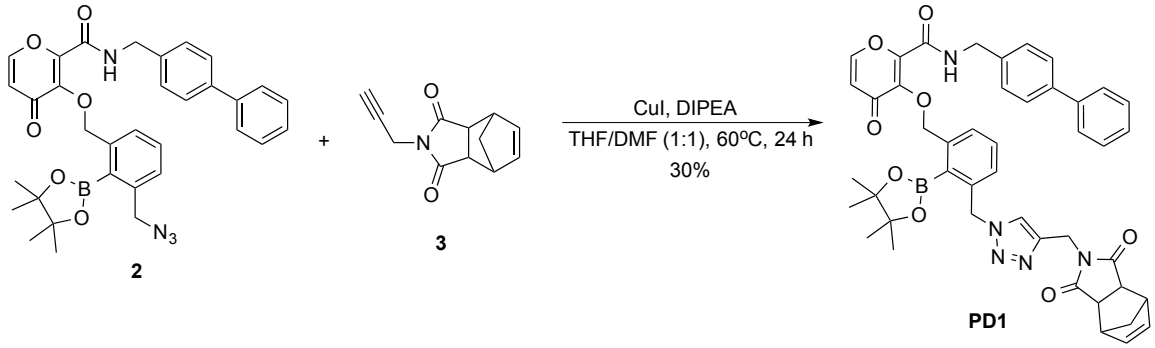
***N*-([1,1'-biphenyl]-4-ylmethyl)-3-((3-(azidomethyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-4-oxo-4*H*-pyran-2-carboxamide (2).**

Compound **1** (0.30 g, 0.5 mmol) was dissolved in DMF (15 mL). To this was added NaN_3 (0.09 g, 1.4 mmol) and the reaction was held at room temperature for 18 h. The solvent was then removed via rotary evaporation and the resulting crude product was purified by silica gel chromatography eluting 70% EtOAc in hexanes to afford the desired product in 94% yield (0.27 g, 0.4 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.28 (t, J = 6.0 Hz, 1H), 8.09 (d, J = 5.6 Hz, 1H), 7.63 (d, J = 7.2 Hz, 2H), 7.52-7.35 (m, 8H), 7.16 (d, J = 8.4 Hz, 2H), 6.50 (d, J = 5.6 Hz, 1H), 5.57 (s, 2H), 4.55 (s, 2H), 4.40 (d, J = 6.0 Hz, 2H), 1.36 (s, 12H). ESI-MS(+): *m/z* 593.21 $[M+H]^+$, 615.20 $[M+Na]^+$.



2-(prop-2-yn-1-yl)-3a,4,7,7a-tetrahydro-1*H*-4,7-methanoisoindole-1,3(2*H*)-dione (3).

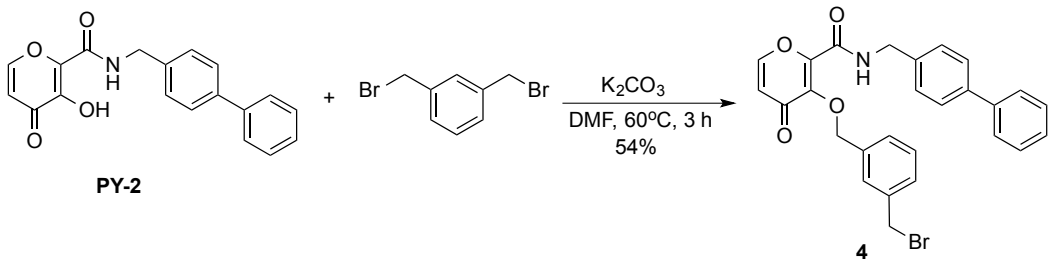
Cis-5-norbornene-*exo*-2,3,dicarboxylic anhydride (2.30 g, 14.0 mmol) was dissolved in toluene (120 mL). To this was added propargylamine (0.99 mL, 15.4 mmol) and triethylamine (Et_3N) (0.9 mL, 7.0 mmol) and the mixture was heated to reflux for 2 h. The mixture was cooled to RT and the solvent was removed via rotary evaporation. The resulting residue was brought up in CH_2Cl_2 (30 mL) and washed with 1 M HCl (3 × 30 mL). The organics were collected, dried over $MgSO_4$, and filtered rinsing with CH_2Cl_2 (50 mL). The solvent was removed via rotary evaporation to afford the purified product in 92% yield (2.61 g, 13.0 mmol). ^{1}H NMR (400 MHz, $CHCl_3$): δ 6.30 (t, J = 2.4 Hz, 2H), 4.24 (d, J = 2.0 Hz, 2H), 3.30 (t, J = 2.0 Hz, 2H), 2.73 (s, 2H), 2.19 (t, J = 2.4 Hz, 1H), 1.52 (m, 1H), 1.28 (m, 1H). APCI-MS(+): *m/z* 202.10 $[M+H]^+$.



***N*-([1,1'-biphenyl]-4-ylmethyl)-3-((3-((4-((1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2*H*-4,7-methanoisoindol-2-yl)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)-4-oxo-4*H*-pyran-2-carboxamide (PD1).**

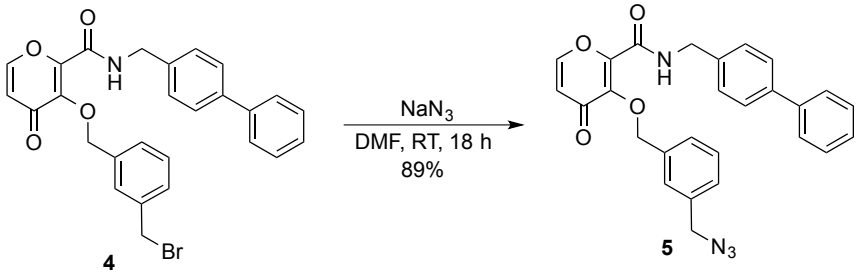
Compound **2** (0.26 g, 0.4 mmol) was dissolved in dry THF (15 mL) and DMF (15 mL). To this was added **3** (0.12 g, 0.6 mmol) and *N,N*-diisopropylethylamine (DIPEA) (0.12 mL, 0.7 mmol) and the mixture was stirred at room temperature for 15 min. To this was added CuI (17 mg, 0.09 mmol) and the reaction was held at 60 ºC for 24 h. The solvent was then removed via rotary evaporation and the resulting crude product was purified by silica gel chromatography eluting CH_2Cl_2 followed by 10% MeOH in CH_2Cl_2. The product was further purified by reverse phase prep HPLC eluting a gradient of 5-100% acetonitrile in water (both contain 0.1% formic acid) to obtain the purified

product in 30% yield (0.11 g, 0.1 mmol). ^{1}H NMR (400 MHz, Acetone-d_6): δ 8.24 (t, J = 6.0 Hz, 1H), 8.10 (d, J = 5.6 Hz, 1H), 7.76 (s, 1H), 7.64 (d, J = 7.2 Hz, 2H), 7.52-7.33 (m, 7H), 7.22 (d, J = 7.2 Hz, 1H), 7.14 (d, J = 8.0 Hz, 2H), 6.50 (d, J = 5.6 Hz, 1H), 6.29 (t, J = 2.0 Hz, 2H), 5.68 (s, 2H), 5.56 (s, 2H), 4.61 (s, 2H), 4.36 (d, J = 6.0 Hz, 2H), 3.10 (t, J = 2.0 Hz, 2H), 2.66 (s, 2H), 1.36 (s, 12H) 1.31 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 176.9, 175.4, 158.8, 155.3, 147.8, 147.2, 142.6, 141.9, 140.8, 139.9, 138.0, 137.8, 130.8, 129.9, 129.5, 129.1, 128.1, 127.5, 127.1, 127.0, 123.1, 117.4, 85.0, 74.9, 53.4, 47.8, 45.3, 42.7, 42.6, 33.6, 24.7. HRMS (ESI) calcd for $[C_{45}H_{44}BN_5O_8Na]^+$: 815.3211; Found: 815.3209.



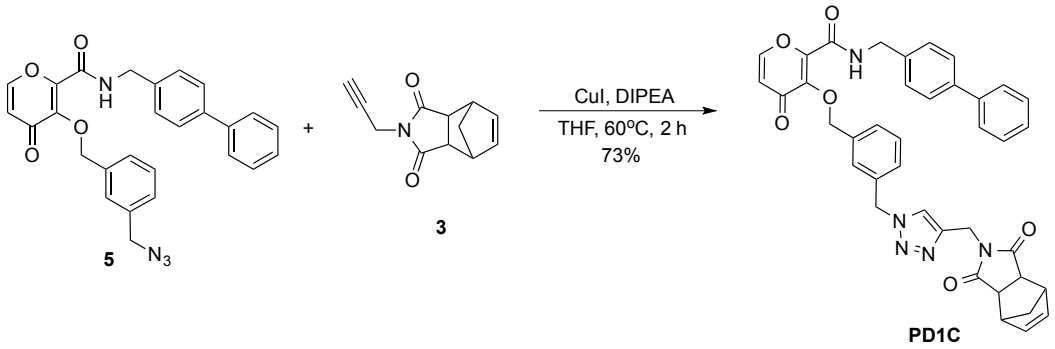
***N*-([1,1'-biphenyl]-4-ylmethyl)-3-((3-(bromomethyl)benzyl)oxy)-4-oxo-4*H*-pyran-2-carboxamide (4).**

1,3-Bis(bromomethyl)benzene (0.74 g, 2.8 mmol) was dissolved in dry THF (15 mL). To this was added K_2CO_3 (0.26 g, 1.9 mmol) and the reaction was heated to 60 ºC. After 30 min, a solution of **PY-2** (0.30 g, 0.9 mmol) in DMF (15 mL) was added to the first mixture over the course of 2 h. Upon addition, the reaction was held at 60 ºC for 3 h. The reaction mixture was cooled to room temperature and the solvent was removed via rotary evaporation. The resulting crude product was purified by silica gel chromatography eluting 5-80% EtOAc in hexanes to afford the desired product in 54% yield (0.26 g, 0.5 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.19 (t, J = 6.0 Hz, 1H), 8.21 (d, J = 5.6 Hz, 1H), 7.63 (d, J = 7.2 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 7.45 (t, J = 7.6 Hz, 3H), 7.49-7.33 (m, 4H), 7.30 (d, J = 5.2 Hz, 2H), 6.54 (d, J = 5.6 Hz, 1H), 5.14 (s, 2H), 4.63 (s, 2H), 4.46 (d, J = 6.0 Hz, 2H). ESI-MS(+): *m/z* 526.19 $[M+Na]^+$.



***N*-([1,1'-biphenyl]-4-ylmethyl)-3-((3-(azidomethyl)benzyl)oxy)-4-oxo-4*H*-pyran-2-carboxamide (5).**

Compound **4** (0.26 g, 0.5 mmol) was dissolved in DMF (30 mL). To this was added NaN_3 (0.07 g, 1.0 mmol) and the reaction was held at room temperature for 18 h. The solvent was then removed via rotary evaporation and the resulting crude product was purified by silica gel chromatography eluting 70% EtOAc in hexanes to afford the desired product in 89% yield (0.21 g, 0.5 mmol). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.19 (t, J = 6.0 Hz, 1H), 8.21 (d, J = 5.6 Hz, 1H), 7.62 (d, J = 7.2 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 7.2 Hz, 2H), 7.37-7.30 (m, 7H), 6.54 (d, J = 5.6 Hz, 1H), 5.17 (s, 2H), 4.46 (d, J = 6.0 Hz, 2H) 4.39 (s, 2H). ESI-MS(+): *m/z* 489.15 $[M+Na]^+$.



***N*-([1,1'-biphenyl]-4-ylmethyl)-3-((3-((4-((1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2*H*-4,7-methanoisoindol-2-yl)methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzyl)oxy)-4-oxo-4*H*-pyran-2-carboxamide (PD1C).**

Compound **5** (0.30 g, 0.6 mmol) was dissolved in dry THF (15 mL). To this was added **3** (0.17 g, 0.8 mmol) and DIPEA (0.17 mL, 1.0 mmol) and the mixture was stirred at room temperature for 15 min. To this was added CuI (24 mg, 0.13 mmol) and the reaction was held at 60 ºC for 2 h. The solvent was then removed via rotary evaporation and the resulting crude product was purified by silica gel chromatography eluting CH_2Cl_2 followed by 10% MeOH in CH_2Cl_2. The product was further purified by reverse phase prep HPLC eluting a gradient of 5-100% acetonitrile in water (both contain 0.1% formic acid) to obtain the purified product in 73% yield (0.31 g, 0.5 mmol). ^{1}H

NMR (400 MHz, Acetone-d_6): δ 8.41 (t, J = 6.0 Hz, 1H), 8.02 (d, J = 5.6 Hz, 1H), 7.79 (s, 1H), 7.64 (d, J = 6.8 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.44 (t, J = 7.2 Hz, 2H), 7.39 (d, J =8.8 Hz, 3H), 7.37-7.32 (m, 2H), 7.28 (t, J = 7.2 Hz, 1H), 7.23 (dt, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 6.44 (d, J = 5.6 Hz, 1H), 6.26 (t, J = 2.0 Hz, 2H), 5.48 (s, 2H), 5.30 (s, 2H), 4.66 (s, 2H), 4.54 (d, J = 6.0 Hz, 2H), 3.10 (t, J = 2.0 Hz, 2H), 2.67 (s, 2H), 1.31 (m, 2H). ^{13}C NMR (100 MHz, Acetone-d_6): δ 177.1, 175.5, 158.9, 155.4, 148.9, 146.2, 142.8, 140.8, 140.1, 138.0, 137.9, 137.1, 136.4, 129.3, 129.2, 129.1, 128.9, 128.6, 128.5, 127.6, 127.2, 127.0, 123.1, 117.4, 73.9, 53.3, 47.8, 45.3, 42.9, 42.7, 33.7. HRMS (ESI) calcd for $[C_{39}H_{33}N_5O_6Na]^+$: 690.2323; Found: 690.2322.

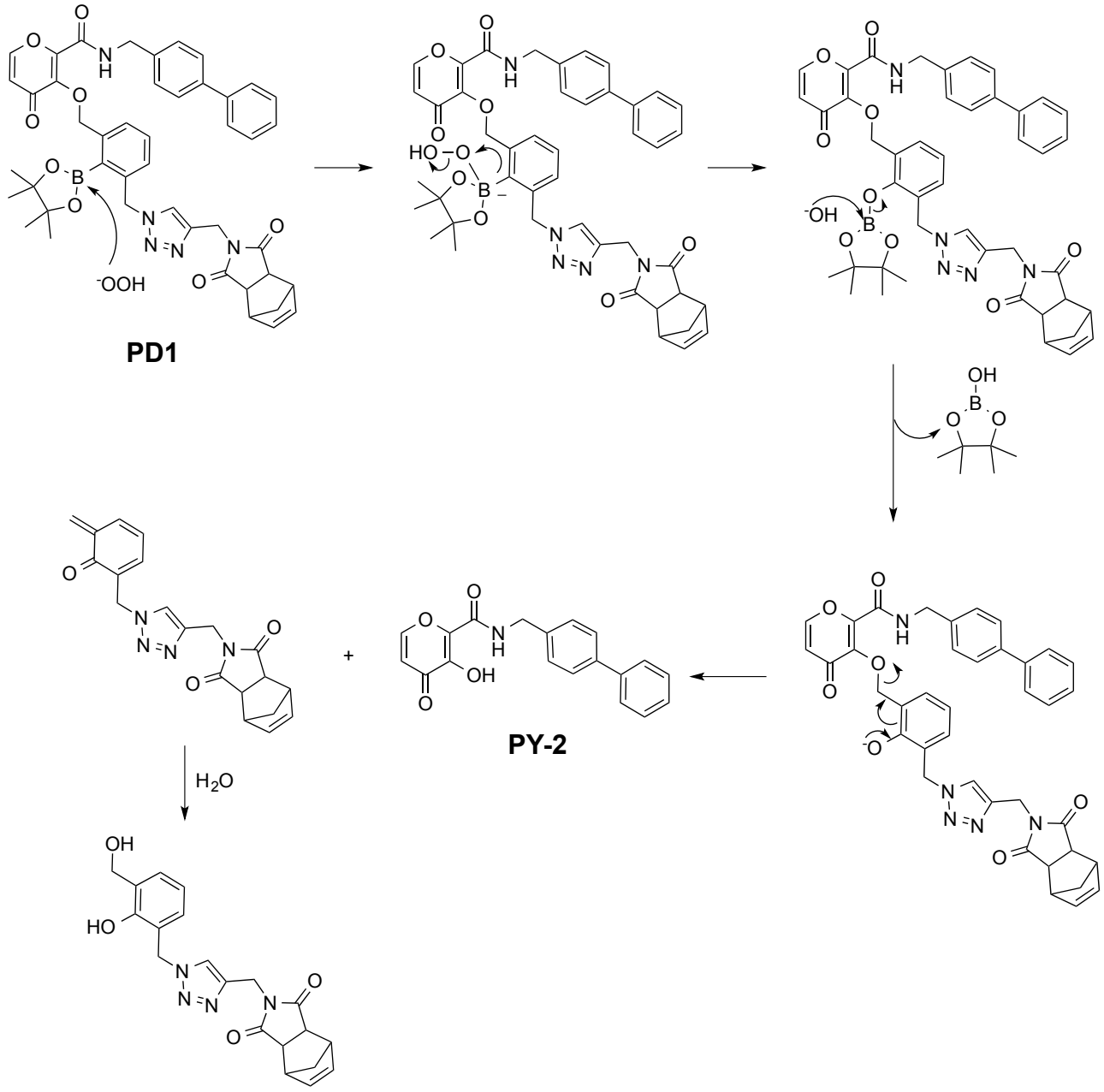


Figure S1. Disassembly mechanism of monomer **PD1** in the presence of H_2O_2 resulting in the release of an active MMP inhibitor (**PY-2)**.

HPLC Analysis of Monomers

Analytical HPLC was performed on a HP Series 1050 System equipped with a Poroshell 120 reverse-phase column (EC-C18, 4.6×100mm, 2.7µm). Separation was achieved with a flow rate of 1 mL min^{-1} and the following mobile phase: 2.5% ACN + 0.1% formic acid in H_2O (A) and 0.1% formic acid in ACN (B). Starting with 95% A and 5% B, a linear gradient was run for 15 min to a final solvent mixture of 5% A and 95% B, which was held for 5 min before ramping back down to 95% A and 5% B over the course of 2 min, with constant holding at this level for an additional 4 min. All compounds were prepared in HEPES buffer (50 mM, pH 7.4) at a concentration of 200 µM. An authentic sample of **PY-2** was prepared to compare with reaction traces. To determine the efficiency of cleavage of the monomers, a 1 mM solution of test compound (1.0 mL) in HEPES buffer (50 mM, pH 7.4) was prepared and treated with H_2O_2 (20 equiv, 20 mM). The sample was incubated at 37 ºC for 2 h prior to analysis.

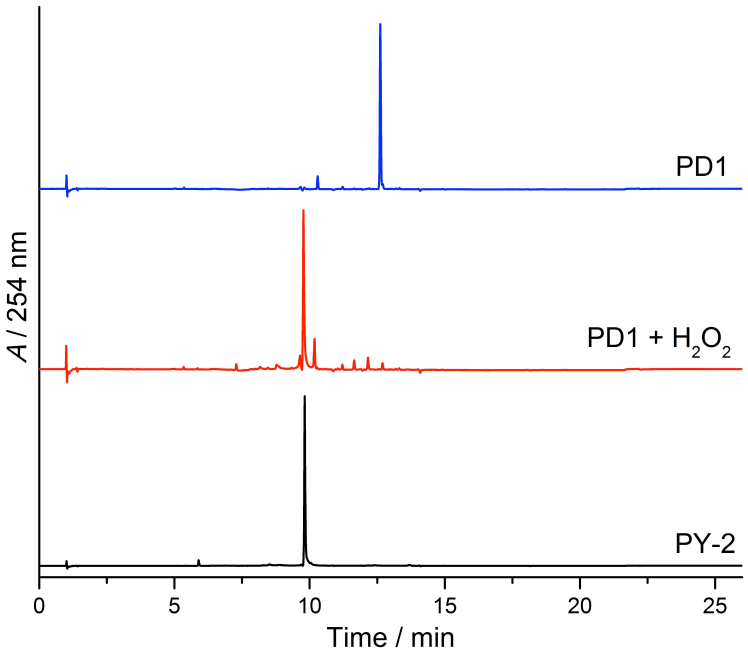


Figure S2. HPLC traces of **PD1** (top, blue), **PD1** after treatment with H_2O_2 (20 equiv) for 2 h at 37 °C (middle, red), and **PY-2** (bottom). Retention times are 12.61 min for **PD1** and 9.82 min for **PY-2**.

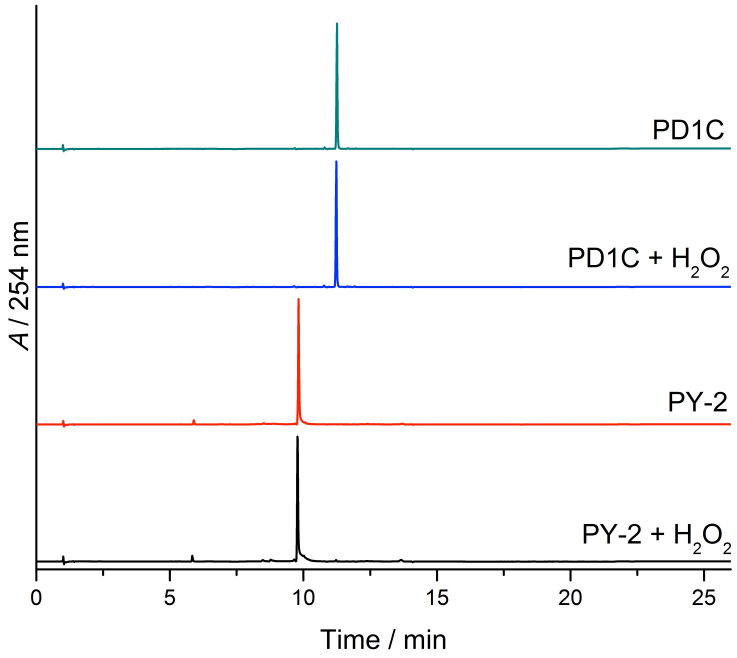


Figure S3. HPLC traces of **PD1C** (green), **PD1C** after treatment with H_2O_2 (20 equiv) for 2 h at 37 °C (blue), and **PY-2** (red), and **PY-2** after treatment with H_2O_2 (20 equiv) for 2 h at 37 °C (black). Retention times are 11.25 min for **PD1C** and 9.82 min for **PY-2**.

Peptide (PS, PSC) Synthesis

Peptides were synthesized using an AAPPTEC Focus XC automated synthesizer. Both L- and D-amino acids were purchased from AAPPTEC and NovaBiochem. *N*-(hexanoic acid)-cis-5-norbornene-exo-dicarboximide was prepared according to published protocols.[3] Peptide monomers (PS, PSC) were synthesized via standard FMOC-based peptide synthesis using Rink Amide MBHA resin (AAPTEC) in a standardized fashion. FMOC was deprotected using a solution of 20% 4-methylpiperidine in DMF. Amino acid couplings were carried out using *N,N,N′,N′*-Tetramethyl-O-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) and DIPEA (resin/amino acid/HBTU/DIPEA 1:3.5:3.4:4). The final peptide monomers were cleaved from the resin using a mixture of Trifluoroacetic acid , H_2O, and triisopropylsilane (95:2.5:2.5) for 40 min, where TFA is trifluoroacetic acid and TIPS is triisopropylsilane. The peptides were precipitated and washed with cold ether. For purification and analysis, the peptides were dissolved in a solution of 0.1% TFA in water and purified via preparative HPLC. Peptide identities and purities were confirmed using ESI-MS and HPLC monitoring at 214 nm.

Polymer Synthesis

Monomers were polymerized via ROMP, using Grubbs' modified second generation catalyst [$(H_2IMES)(pyr)_2(Cl)_2Ru$=CHPh].[4] The catalyst (1 equiv) was dissolved in DMF-d_7 and added to **PD1 or PDC1** (depending on which polymer was desired, 10 equiv) in DMF-d_7 to a final volume of 500 μL in a 1.5 mL Eppendorf tube under N_2 and stirred for 2 h. 30 μL was removed for analysis via SEC-MALS. To generate two diblock copolymers whose hydrophobic blocks were identical, 235 μL of polymer solution was transferred to a new Eppendorf tube, and **PSC** (4 equiv) was added to the new aliquot, while **PS** (4 equiv) was added to the original polymer solution, and stirred for an additional three hours. The resulting polymers were terminated with ethyl vinyl ether (2 equiv) and characterized via SEC-MALs.

Nanoparticle Formulation

Copolymers were dissolved separately in DMSO at concentrations of 1.0 mg/mL with respect to polymer, followed by addition of equivalent volume of Dulbecco's phosphate buffered saline (DPBS, 1×, no calcium, no magnesium, pH 7.4). This solution was then transferred to Slide-A-Lyzer mini dialysis cups (500 μL capacity, 3.5K MWCO) and dialyzed to DPBS overnight, switching out the dialysis buffer for fresh DPBS after 12 h. Nanoparticle formulation was confirmed by DLS using a Wyatt Dynapro NanoStar.

Table S1. Polymeric properties of all systems, where *m* and *n* represent the number of repeat units, M_n is the number average molecular weight, M_n/M_w is polymer dispersity, and *R* is hydrodynamic radius of nanoparticle systems made from the polymers, as determined by DLS.

	m	**n**	**M_n**	**M_n/M_w**	**R(nm)**
PD1-PS	6	2	7444	1.076	53.1
PD1-PSC	6	2	7399	1.091	52.3
PD1C-PS	5	2	11690	1.035	51.1

Transmission Electron Microscopy (TEM)

Small (3.5 μL) aliquots of nanoparticle sample were utilized via a standard procedure. Briefly, the sample was loaded onto carbon grids (Ted Pella Inc.) that had previously been glow-discharged, using an Emitech K350 glow discharge unit, and plasma-cleaned for 90 s in an E.A. Fischione 1020 unit. The sample grid was then transferred to a grid holder, and analyzed via an FEI Sphera microscope operating at 200 keV. Micrographs were recorded on a 2K x 2K Gatan CCD camera. Note: the diameters observed by TEM are smaller than those observed by DLS, which we attribute to the drying of the nanoparticles during TEM sample preparation.

Nanoparticle Stability (pH, Temperature)

To confirm that our nanoparticle system is reactive only to MMP and H_2O_2, a series of DLS experiments were performed to probe for morphology change in response to changes in pH and temperature. For these studies, we utilized **PD1C-PS**, as the Cohen lab has previously demonstrated the boronic ester linkages are selectively sensitive to H_2O_2 only.[5,6] As expected, the average hydrodynamic radius of the nanoparticle system remained unchanged when the pH was adjusted from 7-4, nor when adjusted from 7-10 (Figure **S5**). Additionally, the neutral nanoparticle system was incubated at 25 °C and 37 °C for 24 h to probe for temperature sensitivity. As with the pH variation studies, there was no change in the hydrodynamic radius with elevated temperature (Figure **S6**).

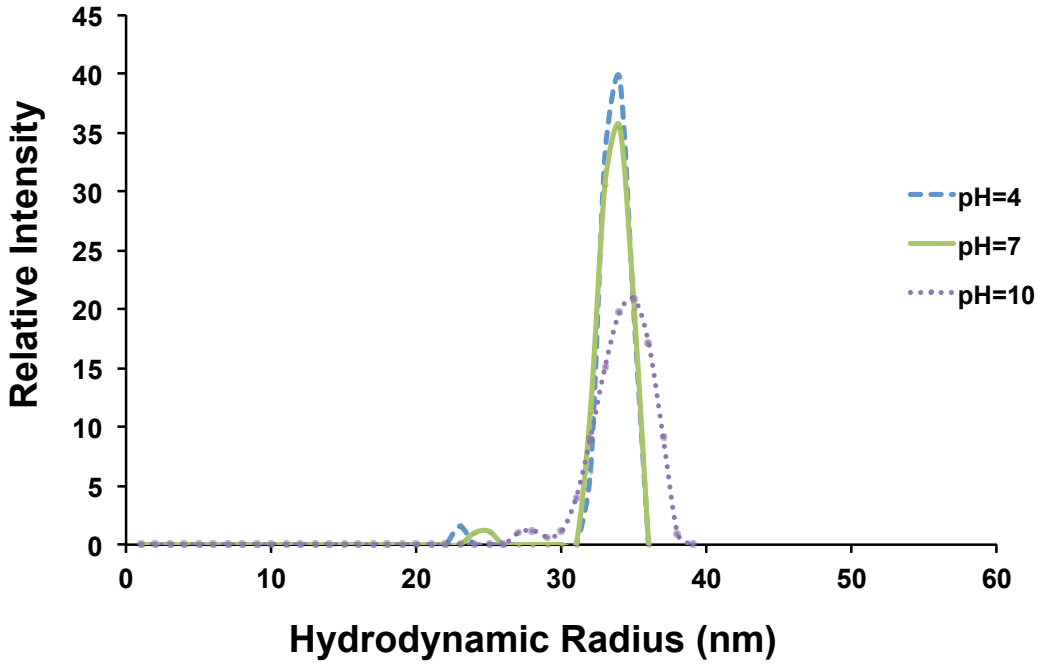


Figure S5. DLS traces of **PD1-PS** at pH 7 (green line), pH 4 (blue dashed line) and pH 10 (purple dotted line). The average hydrodynamic radius under all three conditions is ~35 nm.

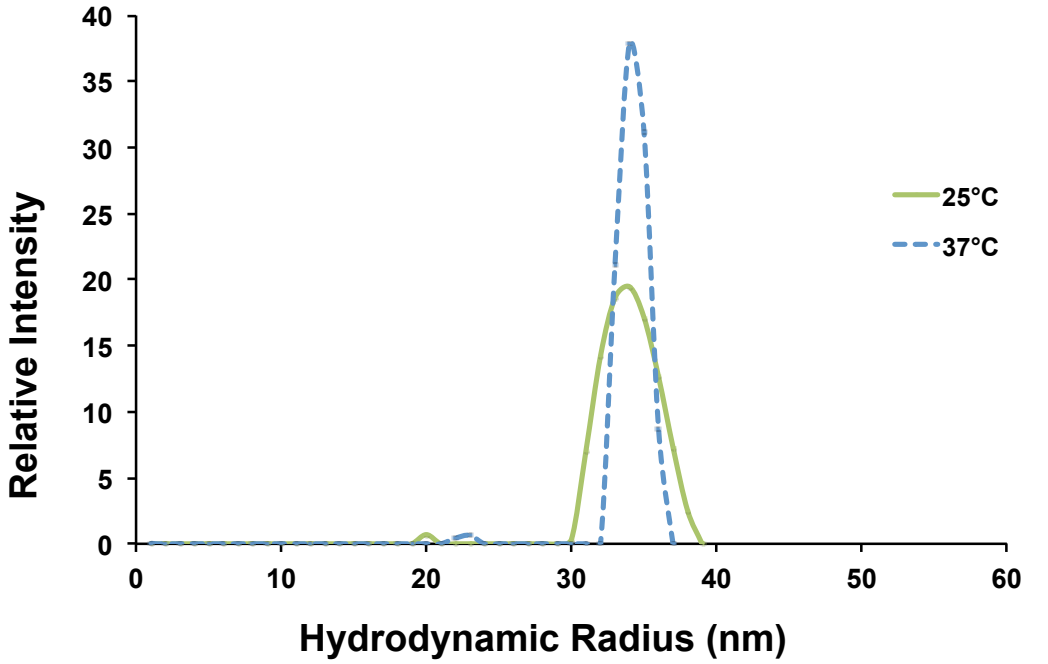


Figure S6. DLS traces of **PD1-PS** after 24 hours incubation at 25 °C (green line) and 37 °C (blue dashed line). The average hydrodynamic radius for both temperature conditions is ~35 nm.

Peptide Cleavage by MMP-12

300 uL (100 uM with respect to peptide concentration) of **PS** and **PSC** were each incubated with MMP-12 (100 nM) at 37 °C. At given time points, small aliquots (30 μL) were removed from each reaction vessel and analyzed by HPLC (absorbance = 214 nm) to monitor for the presence of peptide cleavage fragment, whose resulting sequence is LAGGERDG. ESI-MS was utilized for the peak that eluted at 11.45 min to determine fragment MW. As there is no observable increase in peak area between 4.5 and 5.5 h of incubation, it was determined that maximum peptide cleavage under these conditions occurs after 4.5 h. As a control, **PSC** was incubated with MMP-12 under the same conditions. No peak was observed, indicating **PSC** is not susceptible to enzymatic degradation.

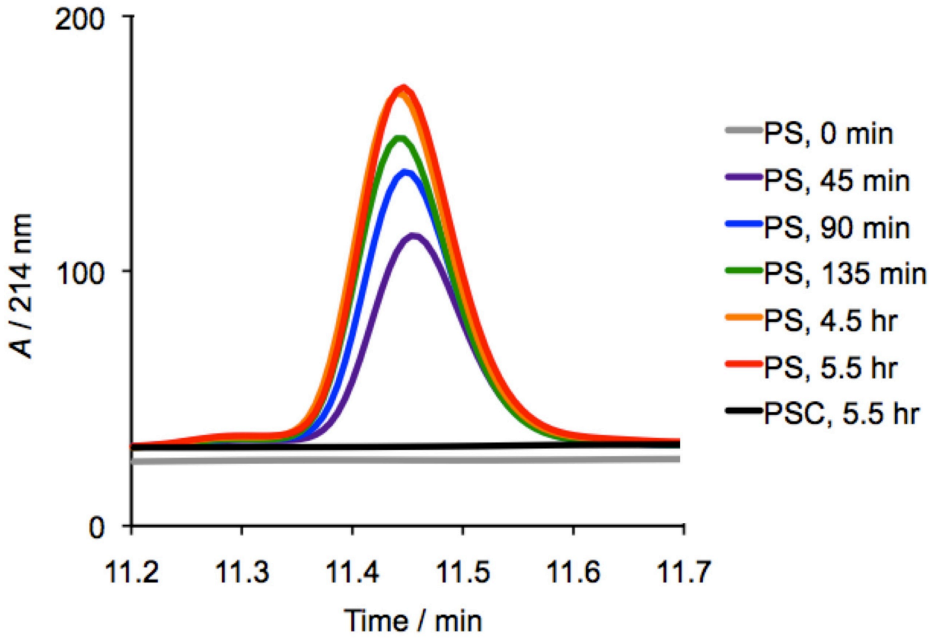


Figure S7. HPLC traces of **PS** (monomer) after treatment with MMP-12 at time 0 min (gray), 45 min (purple), 90 min (blue), 135 min (green), 4.5 h (orange), and 5.5 h (red) at RT. HPLC trace of **PSC** after treatment with MMP-12 for 5.5 h (black) at room temperature. The retention time for the cleaved product is 11.45 min.

Nanoparticle Degradation Assays

To evaluate the capability of nanoparticles to respond to MMP and change shape, **PD1-PS** and **PD1-PSC** (100 uM with respect to peptide concentration in each sample) were treated with MMP-12 (100 nM) in a total reaction volume of 100 µL at 37 °C overnight. Dynamic light scattering (DLS) and TEM (dry state, negative uranyl acetate stain) were used to evaluate nanoparticle size before and after enzyme exposure, as shown in Figure 2 (main text) and Figure S6.

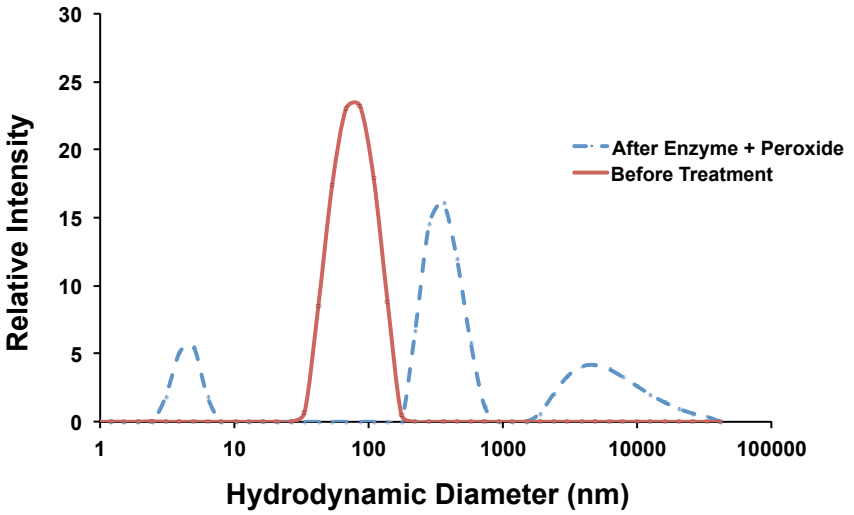


Figure S8. DLS traces of **PD1-PS** before treatment with MMP-12 and H_2O_2 (red) and after treatment with MMP-12 and H_2O_2 (blue dash). The hydrodynamic diameter of **PD1-PS** before treatment is 85 nm by DLS, whereas a range of diameters is detected (5 nm, 256 nm, and aggregates >1000 nm) after treatment.

MMP-Inhibition Assays

MMP-12 (catalytic domain, human, recombinant) was purchased from Enzo Life Sciences. The assays were carried out in 96-well plates using a PerkinElmer HTS 7000 Plus Bio Assay plate reader. **PD1-PS**, **PD1-PSC**, and **PD1C-PS** (100 µM with respect to peptide, 300 µM with respect to MMP inhibitor) were incubated at 37 °C with MMP-12 (100 nM) in DPBS at a total volume of 50 µL for 4 h. To measure MMP activity, a fluorogenic substrate was introduced at 4 h (1 µL, 200 µM, Enzo Life Sciences, Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 where Mca = (7 methoxycoumarin-4-yl)-acetyl and Dpa = N-3-(2,4- dinitrophenyl)-L-diaminopropionyl)) and fluorescence intensity was measured every minute for 60 min with excitation and emission wavelengths at 328 nm and 393 nm, respectively. Measurements were performed in triplicate. Fluorescence intensity was

plotted as a function of time, with the slope of the line being indicative of enzyme activity. As a negative control, an authentic sample of **PY-2** (300 μM) was incubated with MMP-12 and H_2O_2 under the same conditions.

MMP activity in the presence of H_2O_2 was evaluated with MMP-12 and **PD1-PS**, **PD1-PSC**, and **PD1C-PS** with a 4 h incubation of enzyme with nanoparticle and H_2O_2 at the same concentrations as the aforementioned experiment (100 μM with respect to peptide, 300 μM with respect to MMP inhibitor, 100 nM with respect to enzyme, 6 mM with respect to H_2O_2). A control sample containing 10 uL H_2O_2 (9 mM in DPBS) in DPBS was also prepared to confirm that H_2O_2 did not inhibit MMP-12. Enzyme activity with inhibitor was calculated with respect to the control experiment (no H_2O_2 added). Measurements were performed in triplicate in two independent experiments.

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