Supporting Information

Biologically Triggered Delivery of EGF from Polymer Fiber Patches

Si-Eun Kim, Parker W. Lee, and Jonathan K. Pokorski*

Department of Macromolecular Science & Engineering, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106, United States

jon.pokorski@case.edu

Experimental Section

Materials

Aminooxy-5(6)-TAMRA was purchased from Biotium, Inc. BL21(DE3) *E. coli* were purchased from Genlantis. Aniline, 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), N-hydroxyphthalimide, and α-cyano-4-hydroxycinnamic acid were purchased from Sigma-Aldrich. 1-Step TMB substrate, agarose, calcium chloride, isopropyl β-D-1-thiogalactopyranoside, kanamycin sulfate, potassium phosphate monobasic anhydrous, potassium phosphate dibasic anhydrous, pyridoxal 5'-phosphate, Miller LB broth, methanol, SeeBlue Plus2 protein ladder, sodium hydroxide, sulfuric acid, Tween-20, Tris-HCl, potassium carbonate, N,N-dimethylformamide, potassium iodine, sodium bicarbonate, chloroform-d_6, and urea were obtained from Fisher Scientific. Recombinant, human pro-MMP-9 expressed in CHO cells and MMP-2/MMP-9 Substrate I, Fluorogenic were purchased from EMD Millipore. Dimethyl sulfoxide (DMSO) was purchased from Amresco. Amino-phenyl mercuric acetate, imidazole, 4-hydroxyl benzophenone, 2-bromoethanol, dichloromethane, trimethylamine, and Triton X-114 were purchased from Acros Organics. Chemically competent NEB 5α *E. coli*, NcoI restriction enzyme, XhoI restriction enzyme, T4 polynucleotide kinase, and T4 ligase were purchased from New England Biolabs. Recombinant human epidermal growth factor was purchased from BD Bioscience. Mouse anti-human epidermal growth factor IgG and horse radish

peroxidase conjugated goat anti-mouse IgG secondary antibody was purchased from Life Technologies. HaCaT and A431 cells were generous gifts from the dermatology department at Case Western Reserve University.

Instrumentation

Proton nuclear magnetic resonance (^{1}H NMR) were recorded on a Varian Inova 600 MHz NMR spectrometer in deuterated solvents. Chemical shifts are reported in parts per million (ppm, δ) relative to residual solvent ($CDCl_3$, δ 7.26). Fast protein liquid chromatography (FPLC) was performed using a GE Healthcare AKTAFPLC 900 chromatography system equipped with a Superdex 75 10/ 300 GL size exclusion column. A Thermo Finnigan LCQ Advantage LC/MS (ESI) was used to confirm the molecular weight of synthesized aminooxy benzophenone. SDS polyacrylamide gel electrophoresis (PAGE) was performed on Novex NuPAGE 4-12% bis-tris protein gels (1.0 mm x 12 well) (35 minutes, 200 V, 1X NuPAGE MES SDS running buffer). Gels were stained with Coomassie SimplyBlue SafeStain (Life Technologies). Multilayer coextrusion was performed using the CLiPS two-component coextrusion system with 23 multipliers. ATR-FTIR imaging was conducted on a Digilab FTS 7000 spectrometer, a UMA 600 microscope. A high-intensity UV lamp (Bluepoint 4 Ecocure from Honle UV America Inc.) was used for surface modification of the PCL fibers with aminooxy benzophenone. The molecular weights of the synthesized proteins were measured on a Bruker Autoflex III MALDI-TOF/TOF mass spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix. UV-vis spectra were collected using a Shimadzu BioSpecnano UV-vis spectrophotometer.

Synthesis of aminooxy-benzophenone

Aminooxy-benzophenone was synthesized based on a previously published procedure.[1]

2-(4-Benzoylphenoxy)ethanol (1). 4-Hydroxylbenzophenone (2 g, 10 mmol) and anhydrous potassium carbonate (2.76 g, 20 mmol) were stirred in a round bottom flask in 30 mL of *N,N*-dimethylformamide (DMF). 2-Bromoethanol (1.07 mL, 15 mmol) and potassium iodide (KI) (0.8 g, 4.8 mmol) were added, and

the reaction mixture was heated for 24 hours at 65 °C. After the reaction, the mixture was cooled to room temperature and then filtered. The yellow filtrate was slowly precipitated in 700 mL of deionized water in an ice bath. A white powder was collected by centrifuging at 10,000 rpm for 10 min (80% yield) ^{1}H NMR (600 MHz, chloroform-d) δ 7.82 (d, 2H), 7.76 (d, 2H), 7.57 (t, 1H), 7.47 (t, 2H), 6.99 (d, 2H) 4.17 (t, 2H), 4.01 (t, 2H). ESI-MS (*m/z, rel*%) 243.0 ($[M + H]^+$, 10%), 264.9 ($[M + Na]^+$, 90%) calculated for $C_{15}H_{14}O_3$.

Methyl sulfonyl benzophenone (2). 2-(4-Benzoylphenoxy)ethanol (2 g, 8 mmol) in DCM was cooled to 0 °C in an icebath. Triethylamine (TEA) (2 g, 2 mmol) was mixed and then methyl sulfonyl chloride (2.27 g, 1.2 mmol) was added by syringe into the same flask. The reaction was performed at room temperature and stirred overnight in a nitrogen atmosphere. Crude material was sequentially washed with saturated $NaHCO_3$ and brine solution. The organic layer was dried *in vacuo*. ^{1}H NMR (600 MHz, chloroform-d) δ 7.84 (d, 2H), 7.75 (d, 2H), 7.57 (t, 1H), 7.48 (t, 2H), 6.98 (d, 2H), 4.61 (t, 2H), 4.33 (t, 2H), 3.1 (s, 3H) ppm. ESI-MS (*m/z, rel*%) 321.0 ($[M + H]^+$, 100%), calculated for $C_{16}H_{16}O_5S$.

Aminooxy benzophenone (4). Methyl sulfonyl benzophenone (2 g, 6 mmol) was added to a round bottomed flask with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.93 mL, 7.5 mmol) and N- hydroxyphthalimide (1.3 g, 7.5 mmol) in DMF (50 mL). The reaction proceeded overnight at room temperature with stirring. The following day, DMF was removed by rotary evaporation. The crude reaction was re-suspended in dichloromethane (DCM) and DBU was filtered via flash silica column chromatography. The filtrate in DCM was washed with brine and sodium bicarbonate, dried over anhydrous sodium sulfate, and filtered. The dried *phthalimide benzophenone (3)* (1 g, 2.6 mmol) was re-dissolved in acetonitrile (50 mL). Hydrazine monohydrate (0.42 g, 13 mmol) was added and the mixture was stirred for two hours at room temperature. After concentrating the reaction by rotary evaporation, 20 mL of DCM was added and the mixture was filtered over a plug of celite under vacuum. The product was purified via silica flash chromatography (n-hexane:ethyl acetate, 1:2, v/v). ^{1}H NMR (600 MHz, chloroform-d) δ 7.88(d, 2H), 7.79 (d, 2H), 7.61 (t, 1H), 7.52 (t, 2H), 7.02 (d, 2H), 5.60 (s, 2H) 4.28 (t, 2H), 4.02 (t, 2H) ppm. ESI-MS (*m/z, rel*%) 280.0 ($[M + Na]^+$, 100%), calculated for $C_{15}H_{15}NO_3$

Protein sequences and plasmid generation

Epidermal growth factor used in this study was produced recombinantly in *E. coli* to incorporate a PLP reactive site and MMP cleavage site into the sequence. The protein sequence for the mutant, referred to as EGF-MMP, is as follows:

AKTHHHHHHVPLSLYSGNSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRD LKWWELR

The PLP reactive site is highlighted in red, the His tag for purification is highlighted in green, and the MMP cleavage site is highlighted in blue. The DNA sequence was generated from the protein sequence using GeneDesign 2.0 software and optimized for *E. coli* codon usage. The DNA sequence is given below with the added NcoI and XhoI cut sites underlined at the 5' and 3' ends respectively.

5'ATGCTACCATGGCTAAAACCCACCACCACCACCACCACGTTCCGCTGTCTCTGTACTCTGG TAACTCTGACTCTGAATGCCCGCTGTCTCACGACGGTTACTGCCTGCACGACGGTGTTTGCAT GTACATCGAAGCTCTGGACAAATACGCTTGCAACTGCGTTGTTGGTTACATCGGTGAACGTT GCCAGTACCGTGACCTGAAATGGTGGGAACTGCGTTAACTCGAGTGACTC 3'

The gene was produced via primer overlap PCR of primers purchased from Integrated DNA Technologies designed by GeneDesign 2.0 software. The PCR was run for 55 cycles and the product was purified using an agarose gel, the band excised, and the excised band was spin column purified. The purified product was digested with NcoI and XhoI and spin column purified. The product was then ligated via T7 DNA ligase into a pET28a(+) vector that had been digested with NcoI and XhoI. The ligation mixture was transformed into chemically competent NEB5α *E. coli* and plasmids were extracted from isolated colonies. The insertion of the sequence into the plasmid was verified via sequencing with T7 promoter primers and the pET28(EGF-MMP) plasmid was isolated. A mutant of the EGF-MMP protein was made to remove the MMP cutsite for control experiments (referred to as EGF), with the protein sequence given below:

AKTHHHHHHNSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRDLKWWELR

The PLP reactive site is highlighted in red and the His tag for purification is highlighted in green. Primers were designed for whole-plasmid PCR of the pET28(EGF-MMP) plasmid with the removal of the DNA sequence encoding the MMP cut-site amino acid sequence. PCR was run with the primers and pET28(EGF-MMP) plasmid and the resulting PCR mixture was treated with T7 polynucleotide kinase and then ligated with T7 DNA polymerase. The ligation mixture was transformed into chemically competent NEB5α *E. coli* cells and isolated colonies had plasmids sequenced as previously described. The pET28(EGF) plasmid was isolated from successfully transformed colonies.

Recombinant EGF protein expression and purification

Chemically competent BL21(DE3) *E. coli* cells were transformed with pET28(EGF-MMP) or pET28(EGF) and plated onto LB agar media containing 50 μg/mL kanamycin. After overnight incubation at 37 ºC, 100 mL of LB media containing 50 μg/mL kanamycin was inoculated with a well-isolated colony and incubated overnight at 37 °C with agitation at 250 rpm. The overnight culture was then diluted into 1000 mL of LB media containing 50 μg/mL kanamycin and incubated at 37 °C with agitation at 250 rpm. Cell culture growth was monitored by optical density at 600 nm (OD600) utilizing UV-vis spectroscopy. When the OD600 of the cultures reached approximately 0.8 (mid-log phase), protein expression was induced by addition of 10 mL of 100 mM IPTG resulting in a final concentration of 1 mM. Shaking was continued at 37 °C for an additional 6 hours, at which point cells were collected by centrifugation in an Eppendorf A-4−81 rotor at 4000 rpm (4 °C) for 30 min. The supernatant was decanted and the cell pellet was frozen at -80 °C overnight. Cells were resuspended in 50 mL of 50 mM phosphate buffer with 500 mM NaCl (pH 7.4) and this buffer was used for subsequent steps in the purification. Samples were lysed with a microtip sonicator (10 min total sonication time, cycles of 30 s on/30 s off, power output of 6) in an ice bath. The resulting cell debris was pelleted in an Eppendorf A-4−81 rotor at 4000 rpm (4 °C) for 30 min and the supernatant was decanted. The expressed protein was present in the pellet as an inclusion body. The inclusion bodies were denatured by incubating the re-suspended pellets in 50 mL of 4M urea phosphate buffer for 1 hour at 4°C. Solids were pulled out of the solution and the inclusion body solution was

incubated with 8 mL of HisPur cobalt resin that had been washed and equilibrated to buffer. The inclusion body solution was allowed to incubate for 1 hour and then loaded into a fritted syringe. The resin bed was allowed to settle under gravity flow and washed with 4 M urea buffer containing 5 mM imidazole for 16 column volumes. The resin bed was then eluted with an imidazole gradient all made in 4 M urea buffer of 15, 25, 35, 45, 55, 65, 75, 85, 95, and 105 mM imidazole. 6 mL was used for each concentration and fractions were collected under gravity flow. The column was fully eluted using 6 mL of 150 mM imidazole and the fractions were analyzed using SDS-PAGE. Fractions containing pure recombinant protein were pooled together and refolded through sequential dialysis against buffer containing 3, 2, and 1 M urea using 3.5K MWCO dialysis tubing for a minimum of 4 hours each. The refolding was then completed via dialysis against phosphate buffer 3 times for a minimum of 4 hours each. The refolded protein solution was concentrated using 3.5K MWCO of centrifugal spin filtration (8,000 rpm) and quantified via 280 nm absorbance (ε_{280} = 20,315 and 18,825 M^{-1} cm^{-1} for EGF-MMP and EGF respectively). The purified proteins were analyzed via SDS-PAGE, FPLC, MALDI-TOF MS.

EGF-MMP cleavage test by MMP-9

100 mg/mL of EGF-MMP was prepared (n = 3) in 2 mL of PBS buffer. In order to activate MMP-9 enzyme, amino-phenyl mercuric acetate (APMA) (3.5 mg, 10 mmol) was dissolved in 1 ml of DMSO and the solution was diluted with reaction buffer (50 mM Tris-HCL pH 7.4, 1 mM $CaCl_2$, 0.05% Triton X-100) to a final concentration of 2 mM APMA. 100 µL of the APMA solution was added to 900 µL of phosphate buffer (50 mM phosphate buffer with 500 mM NaCl, pH 7.4), then 200 nM of MMP-9 (1 µL) was added and reacted for 16 hours at 37 °C. 10 µL of activated MMP-9 was added to the protein sample, after 60 and 120 minutes respectively, the samples were collected. MALDI-TOF was utilized to measure the molecular weight of EGF-MMP.

Melt coextrusion of PCL fibers

The melt coextrusion process began with PCL (CAPA 6800 pellets, MW = 80 kg/mol) and PEO. In order to match the rheology of PCL and PEO for the melt extrusion processing, two different molecular weights

of PEO (Dow Chemical, POLYOX N80 (MW = 200 kg/mol) and POLYOX N10 (MW = 100 kg/mol) were used with a ratio of 30:70 (200 kg/mol :100 kg/mol, N80:N10). The mixture of two molecular weights of PEO was used to ensure a viscosity match between PEO and PCL, critical to maintaining fiber uniformity. The two grades of PEO were pre-mixed using a Haake Rheodrive 5000 twin screw extruder and pelletized. The viscosities of the obtained PEO blend and PCL melt matched at 180 °C, which was chosen as the extrusion temperature. PEO and PCL were completely dried at 40 °C under high vacuum for 48 h in advance of co-extrusion to prevent void volume formation from residual moisture. PCL fiber domains embedded in a PEO matrix were fabricated via multilayer co-extrusion at 180 °C. 18 vertical multipliers and 5 horizontal multipliers were utilized in this process. Finally, this structure went through a 3″ exit die, and the extruded tape contained 8192 by 32 fiber domains. The extruded tape was collected on a chill roll at room temperature with a speed of 15 rpm.

Preparation of non-woven PCL fiber mats

To remove the separating PEO domains, the composite tape was cut (10 cm length) and placed in a water bath while stirring (12 hours). After prolonged immersion the majority of the PEO was removed from the PCL/PEO composite tape yielding PCL fiber bundles. Two strips of PCL fiber bundles were stacked in a cross-ply (90 °) on a metal plate and covered by an aluminum grid of mesh size (250 μm). A high pressure waterjet, through a 0.010" diameter nozzle, was swept across the fibers parallel to the directions of the fibers on the top and bottom of the fiber mat for 5 minutes each with a 500 psi rotator pressure. This process removed the remaining residual PEO and produced a non-woven fiber mat. The pore size of the fiber mats was determined by a porometer. The specific surface area of the fiber mat was analyzed via multipoint Brunner-Emmett−Teller (BET) analyzer (Micrometrics, TriStar III) after degassing at 40 °C under nitrogen for 24 h.

Photochemistry

20 mg of aminooxy benzophenone, was dissolved in methanol (MeOH) (10 mg ml^{-1}). Non-woven PCL fiber mats were cut to 1 cm × 1 cm, width and length. Each sample was soaked in the aminooxy benzophenone solution for 5 min and then air-dried at room temperature. The dried samples were placed on a glass slide and irradiated using a UV source with a 320-390 nm filter for 10 minutes and the fiber mat was flipped and irradiated again (Intensity = 33.5 mW cm^{-2}, $n = 3$). Unreacted aminoxy benzophenone was removed by washing the fiber mats in MeOH overnight for a total of 3 washes and then the samples were vacuum dried.

Transamination of EGF by PLP

200 mM of pyridoxal 5'-phosphate (PLP) was dissolved in PBS buffer and titrated with 6N NaOH to pH 6.5. A 1:1 (protein:PLP, v/v) solution (the final concentrations of PLP and the protein were 100 mM and 5 mM, respectively) was made with a final pH of 6.5 and incubated at 37 °C for 1 hour. After incubation, the solution was filtered using 3.5K MWCO centrifuge filter. The purification proceeded until no residual PLP was observed in the flow-through by UV-Vis spectroscopy at 414 nm. In order to determine the success of the PLP reaction, the presence of the ketone on the protein EGF-MMP (or control EGF) was probed by MALDI which was used to investigate the molecular weight of the unmodified and ketone-modified protein. In order to probe ketone formation, ketone functionalized protein was conjugated to aminooxy-5(6)-TAMRA dye in the presence of 10 mM of aniline, incubating at 37 °C. After 2 hours, extra TAMRA dye was filtered with 3.5 kDa cut off spin filter. Protein conjugated with aminooxy-5(6)-TAMRA was analyzed with fast protein liquid chromatography (FPLC) monitoring at 555 nm, which corresponds to TAMRA dye emission.

Bioconjugation of EGF protein to polymer fiber mat

Oxime chemistry was used to conjugate ketone-EGF (either control EGF or EGF-MMP) to the aminooxy amine-modified-PCL fiber mat. First, the fiber mats were placed in 20 mL scintillation vials and a solution of EGF (60 µg/mL) was added to the vial. 1%v/v of aniline was added as a catalyst and the reaction

proceeded for 2 hours at 37 °C. After 2 hours, the fiber mats were removed from the EGF solution and rigorously washed with PBS buffer to completely remove free EGF and catalyst. Complete removal of excess protein was monitored by measuring the waste PBS at 280 nm via UV-Vis spectroscopy. For all FPLC experiments, 2 column volumes of mobile phase (50 mM phosphate buffer, 150 mM NaCl, pH 7.4) was passed at a flow rate of 0.4 mL/min.

ATR-FTIR measurement for affinity of EGF-MMP on the alkoxyamine PCL fiber mat

1 cm x 1 cm of PCL fiber mat was prepared and soaked in 20 mg of aminooxy benzophenone in MeOH in the same manner described previously. Only one side of the sample was exposed to the UV source and then washed in pure MeOH to remove unreacted aminooxy benzophenone. To confirm the specific attachment of ketone functionalized EGF-MMP to the aminooxy benzophenone-PCL fiber mat, oxime click chemistry was performed following the same bioconjugation procedure previously described. After washing and drying of the fiber mat, ATR-FTIR was utilized to investigate the presence of EGF-MMP on both sides of the PCL fiber mat.

EGF release kinetic by MMP-9

Control EGF or EGF-MMP conjugated fiber mats were prepared (n = 3) stored in 2mL of PBS buffer. In order to activate MMP-9 enzyme, amino-phenyl mercuric acetate (APMA) (3.5 mg, 10 mmol) was dissolved in 1 ml of DMSO and then the solution was diluted with reaction buffer (50 mM Tris-HCL pH 7.4, 1 mM $CaCl_2$, 0.05% Triton X-100) to a final concentration of 2 mM APMA in solution. 100 µL of APMA solution was added to 900 µL of phosphate buffer (50 mM phosphate buffer with 500 mM NaCl, pH 7.4) and then, 200 nM of MMP-9 (1µL) was added and reacted for 16 hours at 37°C. The protein conjugated PCL fiber mat was soaked in 1 mL of phosphate buffer in an Eppendorf tube (n = 3). 10 µL of activated MMP-9 was added to each sample, after 10 minutes the buffer was collected and 1 mL of fresh phosphate buffer with 10 µL of activated MMP-9 solution was added to fiber mats. This process was repeated at time points of 10, 30, 60 and 120 minutes.

The released EGF samples from the fiber mat conjugated with either control EGF or EGF-MMP was evaluated via indirect ELISA. An EGF standard curve was generated using concentrations from 100 to 1 ng/mL of recombinant human EGF to determine the concentration of the released protein and released samples were plated at 1X or 10X dilutions. Nunc Maxisorp 96-well plates were coated with 50 µL of EGF at 4 ºC overnight. The wells were then blocked with 200 µL of blocking buffer (5% dry milk in PBS, pH 7.4) at room temperature for 2 hours. The wells were then incubated with mouse anti-EGF IgG at 5 µg/mL in 100 µL blocking buffer for 2 hours at room temperature. The wells were then incubated with 100 µL of 100 ng/mL of horseradish peroxidase labeled goat anti-mouse IgG in blocking buffer for 2 hours at room temperature. The wells were washed between each incubation step using 4X 300 µL of 0.1% w/v Tween-20 in PBS, pH 7.4. The wells were developed using 100 µL of 1-step TMB substrate at 4 ºC for 10 minutes. The reaction was stopped with 50 µL of 2 M H_2SO_4 and the absorbance was read at 450 and 540 nm in triplicate for each sample. The absorbance at 450 nm was subtracted from the absorbance at 540 nm and the EGF concentration in the released sample was determined via comparison to the standard curve and correction via the dilution factor if necessary.

Cell viability test

HaCaT and A431 cells were grown and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% newborn calf serum (NCS) (Omega Scientific), 1 mM sodium pyruvate, 1% of penicillin/streptomycin, and 2 mM GlutaMax. Cells were grown at 37 °C in a humidified 5% CO_2 and 95% air atmosphere. Cells used for *in-vitro* experiments were used at passage numbers less than 15. A431 and HaCaT cells were plated in 96-well plates in triplicate (10^4 cells/well) in 100 µL of complete DMEM. After 18 hours, the media was replaced with serum free media and incubated for 6 hours. Following serum starvation, EGF prepared in serum-free DMEM (100 µL/well) was added at the indicated concentrations to the cells and allowed to incubate for 72 hours at 37 °C. Cells were then assayed for viability using the MTT assay. MTT (5 mg/mL in DPBS) was combined with complete DMEM (85:15 DMEM: MTT, 25 µL/well) and added to each individual well and incubated at 37 °C for approximately 2 hours (the assay was stopped

when significant accumulation of purple formazan crystals was visibly observed in control wells). Media was carefully aspirated and DMSO was added (200 µL/well) to dissolve the purple MTT-formazan crystals. Absorbance of the dissolved formazan was quantified at 570 nm using a UV-Vis plate reader and cell viability was determined as a fraction of absorbance relative to untreated control wells. The average values are presented with standard deviation.

Scratch cell migration test

Fiber mats were sterilized with 70 % ethanol and dried, then a fiber mat was fixed on the side wall of each well in a 12 well culture plate with nail polish. HaCaT cells grown in DMEM supplemented with 10% FBS and 1% penicillin/ streptomycin were seeded onto each well of 12 well tissue culture plate (1 x 10^6 cells per well). Cell confluence reached around 80% as a monolayer after 48 hours. The monolayer was gently and slowly scratched with a sterilized 10 µL pipette tip across the center of the well in a straight line in one direction. The resulting scratch was imaged via optical microscopy. Each well was washed twice with PBS to remove detached cells and the wells were replenished with serum free medium. 10 µL of activated MMP-9 was added to each well. After 24 hours, cells were imaged again and *ImageJ* was utilized to measure scratch distance.[2]

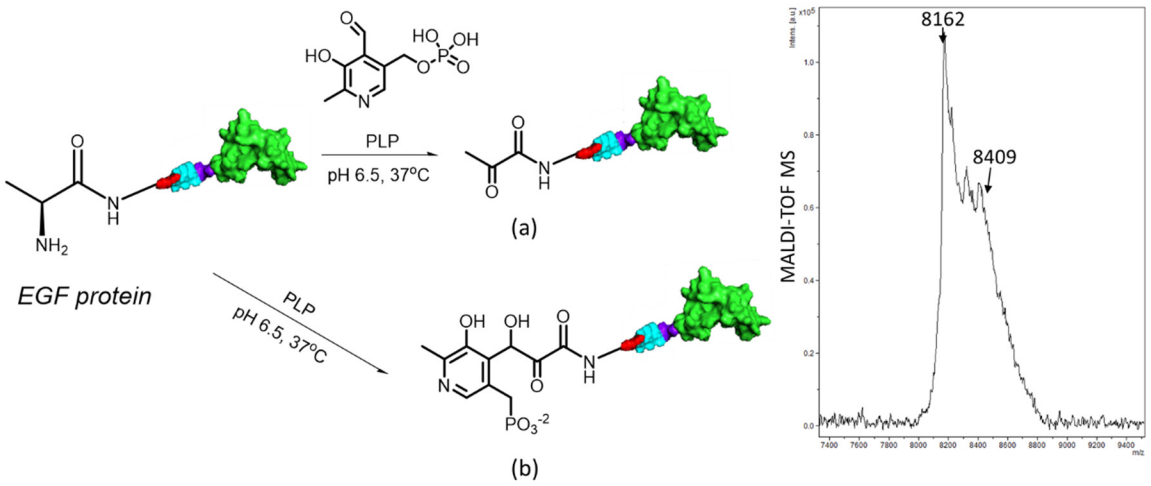

Figure S1. Proposed mechanism for site-specific modification of the protein in the presence of PLP. MALDI-TOF mass spectrum indicates protein (a) at 8162 m/z and (b) at 8409 m/z, matching model molecular weights, indicating modified EGF-MMP.

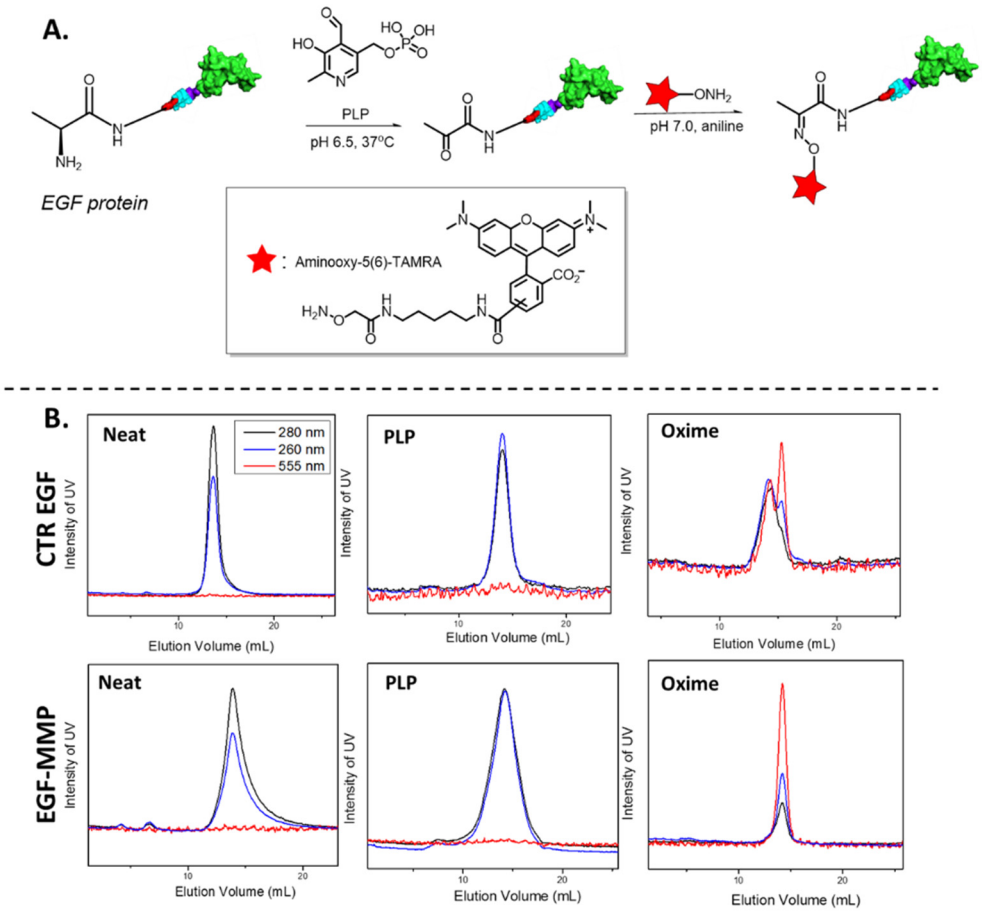

Figure S2. (A) Scheme of oxime ligation of the TAMRA dye with the ketone-modified EGF protein, (B) FPLC trace of control EGF and EGF-MMP (left); FPLC trace of ketone modified control EGF and EGF-MMP (middle); FPLC trace of TAMRA conjugated control EGF and EGF-MMP(right). The maximum absorbance of aminooxyl-5(6)-TAMRA is shown at 555 nm (red). The other wavelengths monitored were 280 nm (black) and 260 nm (blue).

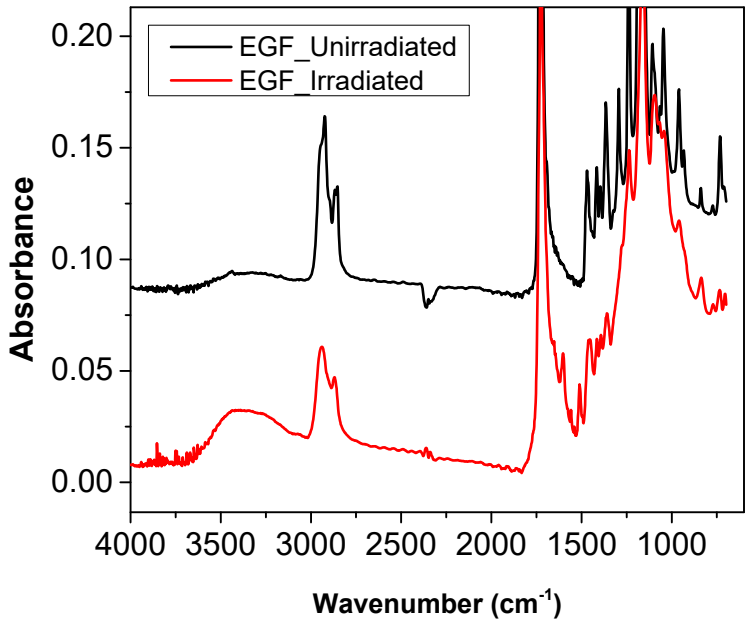

Figure S3. ATR-FTIR spectra of EGF-MMP conjugation to PCL fiber mat. The spectrum from the back of the fiber mat is shown in black (no UV irradiation) and the spectrum from the front is shown in red (UV irradiation).

References

(1) Kim, S.-E.; Wallat, J. D.; Harker, E. C.; Advincula, A. A.; Pokorski, J. K. *Polym. Chem.* **2015**, *6* (31), 5683–5692.

(2) Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. *Nat. Methods* **2012**, *9* (7), 671–675.