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Supplementary Information for

Photodynamic activity of viral nanoparticles conjugated with C_{60}

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Experimental Section

Production of Qβ. A pET28 plasmid containing the Qβ coat protein-encoding gene was used to transform *E. coli* cells. Intact Qβ virus-like particles were produced and purified using established procedures.[1] Virus concentration was determined using the modified Lowry assay (Pierce), and particle integrity was determined by size exclusion chromatography. Yields obtained were 25 mg Qβ per L cell culture.

Bioconjugation procedures. Qβ was modified with Oregon Green 488 (O488, Invitrogen) and C_{60} at solvent-exposed surface Lys side chains. *N*-hydroxysuccinimide (NHS) ester chemistry was used to conjugate O488 and an azide ligation handle (azido $(PEO)_4$ propionic acid succinimidyl ester, Invitrogen) to Qβ (Scheme 1 in the manuscript). The reactions were carried out at a final concentration of 2 mg/mL Qβ in 0.1 M potassium phosphate buffer (pH 7.0) with 10% DMSO overnight at room temperature. A molar excess of 2000 and 1000 was used of O488 and azide, respectively. Particles were purified using 10 kDa molecular weight cut-off centrifugal filter units (Millipore), the product analyzed using UV/vis spectroscopy, native and denaturing gel electrophoresis, and size exclusion chromatography (Figure 1 in the manuscript). The concentration of Qβ particles was determined using the modified Lowry assay. Recovery was approximately 50% from the starting material. Next, copper-catalyzed azide-alkyne cycloaddition (CuAAC) was used to conjugate a propargyl-O-PEG-C_{60} derivative[2] (Scheme 1 in the manuscript). The reaction was carried out at a concentration of 1 mg/mL Qβ in 0.1 M potassium phosphate buffer (pH 7.0) using a molar excess of 250 propargyl-O-PEG-C_{60} and aminoguanidine (at 5 mM in final solution), $CuSO_4$ (0.5 mM) with tris-(benzyltriazolylmethyl)amine (THTPA) (2 mM), and L-ascorbic acid (5 mM).[3] The reaction was carried out for 2 hours at room temperature, then purified by size exclusion chromatography (SEC) using a Superose6 column on the ÄKTA Explorer chromatography system (GE Healthcare) at a flow rate of 0.4 mL/min using 0.1 M potassium phosphate buffer (pH 7.0).

Gel electrophoresis. Native gel electrophoresis was performed using 1.2% agarose gels in 1x TBE buffer (45 mM Tris, 45 mM boric acid, 1.25 mM EDTA in MilliQ water) with 10 μg of sample. Protein subunits were analyzed on denaturing 4–12% NuPAGE gels (Invitrogen) using 1x MOPS running buffer (Invitrogen) and 10 μg of sample with LDS sample buffer and 4 μL of β-mercaptoethanol to reduce disulfide bonds. The sample was then heated to 100°C for 5 minutes. After separation, the gel was photographed using AlphaImager (Biosciences) imaging system before and after staining with Coomassie Blue.

Cell assays. PC-3 cells (ATCC) were grown and maintained in F12 medium (Invitrogen) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin at 37°C in a 5% CO_2 humidified atmosphere (all reagents were obtained from Gibco).

First, cell interactions were evaluated using confocal microscopy to assess whether conjugation of C_{60} affected cellular uptake. PC-3 cells (15,000 cells/750 μL F12/well) were grown for 24 h on glass coverslips placed in an untreated 24-well plate. The media was then replaced with 250 □L of fresh F12 containing 5 μg of Qβ-O488 and Qβ-O488-C_{60} and incubated at 37°C, 5% CO_2 for 3 h. Post incubation, cells were washed thoroughly with sterile saline and incubated for another 3 h in 250 □L of fresh medium. The cells were then fixed using 4% (v/v)

paraformaldehyde and 0.3% (v/v) glutaraldehyde in DPBS for 5 min. Cell membranes were stained using wheat germ agglutinin (WGA) conjugated with Alexa Fluor 555 (WGA-A555) (Invitrogen) at 1 μg/mL in 5% goat serum (GS) (Invitrogen) in DPBS for 1 h. Cell nuclei were stained using 4',6-diamidino-2-phenylindole (DAPI) (MP Biomedicals) diluted 1:9500 in DPBS for 10 min. All steps were carried out in the dark at room temperature; in between each step the coverslips were washed 3x with DPBS. The coverslips were then mounted using Permount (Fisher) on glass slides and sealed using nail polish. Confocal images were obtained using Olympus FluoView^{TM} FV1000 LSCM and data processed using ImageJ software.

Next, drug efficacy was tested using white light therapy. PC-3 cells (15,000 cells/100 μL F12/well) were seeded in a treated 96 well plate for 24 h at 37°C and 5% CO_2. Triplicates of Qβ, C_{60}, and Qβ-C_{60} in 100 μL of medium were added to live cells in increasing amounts, matching the concentrations of Qβ and C_{60} to Qβ-C_{60}, respectively. Following incubation for 3 h, the medium was removed, the cells were washed with saline, and 100 μL of fresh medium was added. The cells were returned to the incubator for 24 h to allow bound particles to be internalized. A mirror was then used to reflect white light from a Proxima DP1000x projector onto the cells in a 6 by 8 in. rectangle at a dose of 2 mW/cm^2 (Figure 2B). The cells were irradiated for 1 h (6.4 J/cm^2). The cell plate was then incubated at 37°C for 96 h. Cell viability was measured using an XTT cell proliferation assay kit (ATCC). Cell viability data are shown in Figure 2C.

An XTT cell proliferation assay kit (ATCC) was used to assess cellular viability after light therapy. 50 μL of the activated XTT solution was added to each well then the plate was incubated at 37°C and 5% CO_2 for 2 hours. A Tecan Infinite 200 plate reader was used to measure absorbance at 450 and 630 nm. The specific absorbance was calculated as: $A_{450\ nm} - A_{630\ nm} - A_{450\ nm}$ (media only blank). The % cell viability was then determined by normalizing to the dark cells only control.

Supplementary Figures

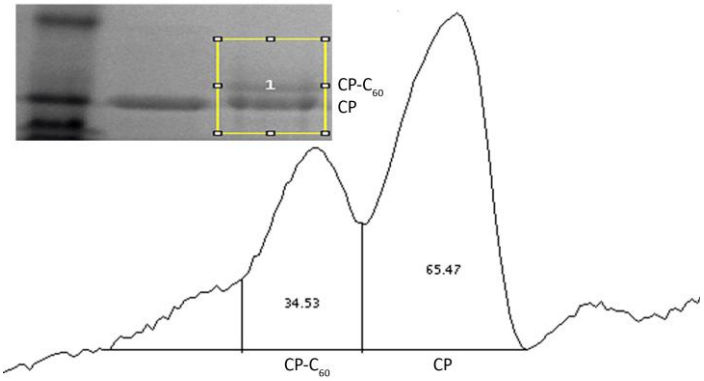

Figure S1. Protein band density analysis for quantification of C_{60} attached per Qβ particle using ImageJ band analysis tool (http://imagej.nih.gov/ij). Inset shows lane being analyzed. The lower mobility band indicates covalent attachment of C_{60}. Approximately a third of the possible 720 coat proteins of Qβ are labeled, resulting in an estimate of 60±10% C_{60} drugs attached. The error was approximated from the uncertainty in determining band boundaries as well as from analyzing multiple gels.

References

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