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Electronic Supplementary Information

Apoferritin protein cages: A novel drug nanocarrier for photodynamic therapy

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Materials & Supplies

Apoferritin from equine spleen 25 mg/mL in 50% glycerol and 0.075 M NaCl (A3660) and Methylene Blue were both obtained from Sigma. PD-10 column (17-0851-01) was purchased from GE Health, and Slide-A-Lyzer® Dialysis Cassettes, 100-500µl, 3500 MWCO (66335) from Thermo Fisher Scientific.

Preparation of Methylene Blue-Loaded Apoferritin Nanoparticles

Apoferritin with a concentration of 25 mg/mL was first diluted with 10 mM phosphate buffer saline (PBS) to make a 2.5 mL solution. The diluted apoferritin was loaded on a PD-10 column (GE Healthcare) and was washed with 4 mL of 10 mM PBS. An aliquot of 2 mL of the purified apoferritin was transferred into an 8 mL tube. The solution pH was first adjusted to 2 with 1 M HCl while magnetically stirring the mixture. Approximately 250 µl of 31.25 mM Methylene Blue (or 10 mg/mL) was slowly added into the apoferritin solution. The pH value was maintained for about 15 min. When the dissociation of apoferritin was completed, the pH value was adjusted to ~7.5 by adding 0.1 M NaOH dropwise. The mixture was continuously stirred for 2 h. The resulting solution was exhaustively dialyzed for 24 h to remove free MB against several changes of phosphate buffer using a Slide-A-Lyzer® Dialysis Cassette (Cat. #66333, Pierce) with a molecular weight cutoff (MWCO) of 3500. Finally, the MB-loaded apoferritin Nanoparticle solution was applied to a disposable PD-10 desalting column with an exclusion limit of 5000 for purification. The eluent was 50 mM phosphate buffer, pH 7.4.

BCA protein assay of MB-loaded apoferritin

The Bicinchoninic acid (BCA) method employs the reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium [Smith, P.K., et al, Anal. Biochem., Measurement of Protein Using Bicinchoninic Acid, 150, 76-85 (1985)]. The combination of BCA and Cu^{+1} creates a purple-colored product that absorbs at 562 nm. The amount of product formed is nearly linear with increasing protein concentrations over a broad working range (20-2000 µg/ml). A UV-Vis-NIR Spectrophotometer (Model: Shimadzu UV-3600) was used for absorption measurements, and a room temperature protocol with a 2 hour-incubation was chosen in our study.

Determination of the loading amount of methylene blue in apoferritin

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The MB content in apoferritin was determined by comparing fluorescence intensities of the MB-loaded apoferritin solution with those of a standard MB solution. A calibration curve of the standard MB was established by measuring fluorescence intensities of free MB dye at different concentrations. Fluorescence measurements were obtained from FluoroMax-3 Spectrofluorometer (Jobin Yvon/SPEX Division, Instruments S. A. Inc., Edison, NJ). All fluoroMax-3 functions are under total control of DataMax spectroscopy software. The measurements were taken at room temperature.

Cell Culture

MCF-7 cells were seeded in supplemented phenol-red-free minimum essential medium eagle (MEM, 100 Units/ml penicillin, 100 μg/ml streptomycin, 2 μg/ml insulin, 29.3 ml/L 7.5% sodium bicarbonate, 0.292 g/L L-glutamine, and 10% fetal bovine serum). All cell cultures were maintained at 37ºC, 5% CO2. Cell culture medium was changed three times weekly with supplemented MEM (MCF-7 cell lines).