Supporting Information

A Nanometer Sized Enzyme Modulator for Precise Cancer Diagnosis and Treatment

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Table of Contents

ADDITIONAL EXPERIMENTAL SECTION

Characterization of CQD-KD1

CQD-KD1 was characterized by high-resolution transmission electron microscopy (TEM, Tecnai F20, Thermo Fisher Scientific, MA, USA), atomic force microscopy (AFM, Dimension Icon, Bruker Corporation, MA, USA), dynamic light scatter (DLS, ZETASIZER 3000, Malvearn Instruments, Ltd, Malvern, UK) analysis, and Fourier transform infrared spectroscopy (FTIR, Perkin Elmer, Waltham, MA,USA),. The fluorescence spectra of uncoupled CQD and CQD-KD1 (0.01 mg/ml) in phosphate buffer pH 7.4 were determined with a microplate reader (SynergyTM 4, BioTek Instruments).

Determination of the degree of labeling

2 mg CQD was activated by NHS in the presence of EDC in PB pH 6.0 buffer, followed by centrifugation with 16,000 rpm for 20 min. After washed with water, 0.4 mg/ml recombinant KD1 in PB pH 7.4 was added and incubated for 20 h. The nanodots were then centrifuged with 16,000 rpm for 20 min. The protein concentration in the supernatant was quantified with a BCA Protein Assay Kit (BioTeke Corporation, Beijing) using bovine serum albumin as the standard. Results were presented as nmol photosensitizers per mg cell protein. The labeled protein amount was calculated by subtracting the protein concentrations before and after the coupling reaction.

Photobleaching by household light

50 μg/ml CQD and CQD-KD1 were dispersed in PBS. 10 μg/ml fluorescein isothiocyanate (FITC) in PBS was set as the negative control. CQD, CQD-KD1, and FITC in PBS was exposed under the irradiation of 12.5 mW/cm^2 household light for 0-10 h. Samples were collected every hour and determined the fluorescence signal (ex488 and em520) with a microplate reader (SynergyTM 4, BioTek Instruments). The fluorescence signals were normalized by setting the fluorescence at 0 h as 100%.

Cell viability assay

Aliquots of exponentially growing MCF-7 or HELF cells (1×10^5 cells/ml) were placed in 96-multiwell plates a volume of 100 μl per well and incubated for 12-16 h at 37℃ with 5% CO_2. The cells were incubated with complete medium with various concentration of CQD and CQD-KD1 (0.01, 0.03, 0.1, 0.33, 1, 3.3, 10, 33, 100 μg/ml) for 2 h. One control column in the plate was filed with culture medium as a blank. The cells were washed with sterile PBS twice, then filled with 200 μl fresh culture medium. The cells were continuatively cultivated for another 12 h. cellular viability was then measured by adding 10 μl MTT solution (5 mg/ml in PBS) to each well followed by incubation for 4 h at 37℃. The supernatant was subsequently removed, and 100 μl dimethyl sulfoxide (DMSO) was refiled to solubilize the formazan crystals. Then samples were measured on

a microplate reader by recording the absorbance at 570 nm. The cell viability of treated samples was then obtained by comparison with the control columns.

Statistical analysis

GraphPad Prism 7 software (GraphPad Softwere, Inc., La Jolla, CA) was used for statistical analysis. Values are expressed as mean ± standard deviation of three samples obtained from three independent experiment. Statistical comparisons were made using one-way analysis of variance (ANOVA) and multiple comparisons between groups were performed using t test and $p<0.05$ was considered statistically significant.

Figure S1: Multiple domains of st14 and its cognate inhibitor HAI-1. The KD1 domain of HAI-1 binds to the catalytic domain of st14 and prevents the approach of substrates. CQD-KD1 was synthesized by immobilizing recombinant KD1 to the surface-exposed carboxyl groups of CQD.

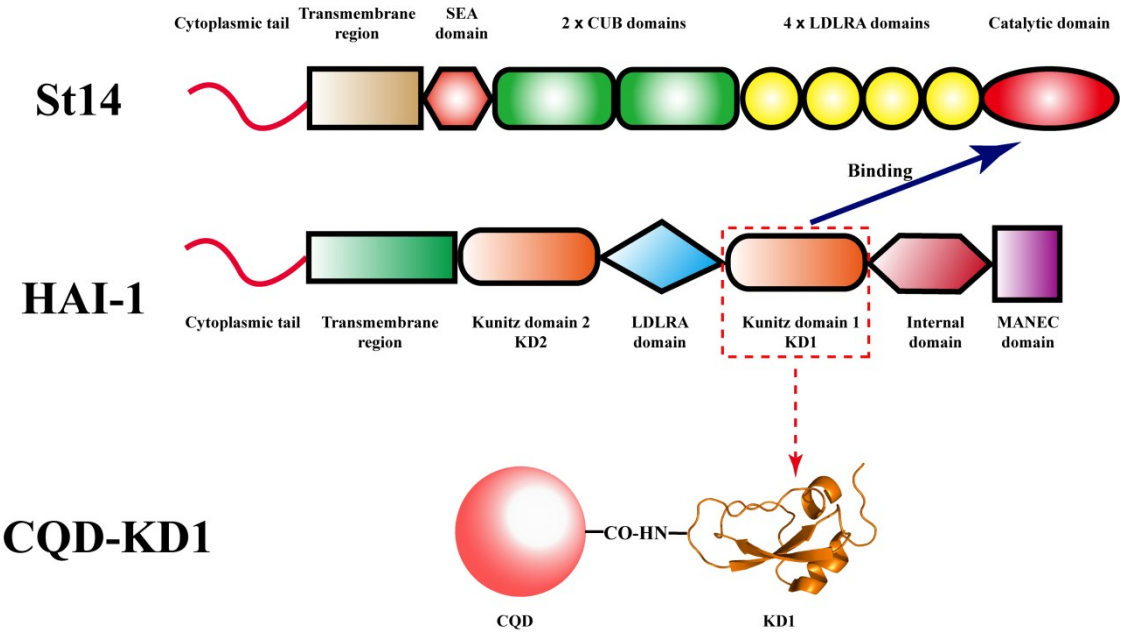

Figure S2: SDS-PAGE electrophoretogram demonstrates the high purity of the recombinant KD1. The theoretical molecular size of the recombinant KD1 is 7.7 kDa.

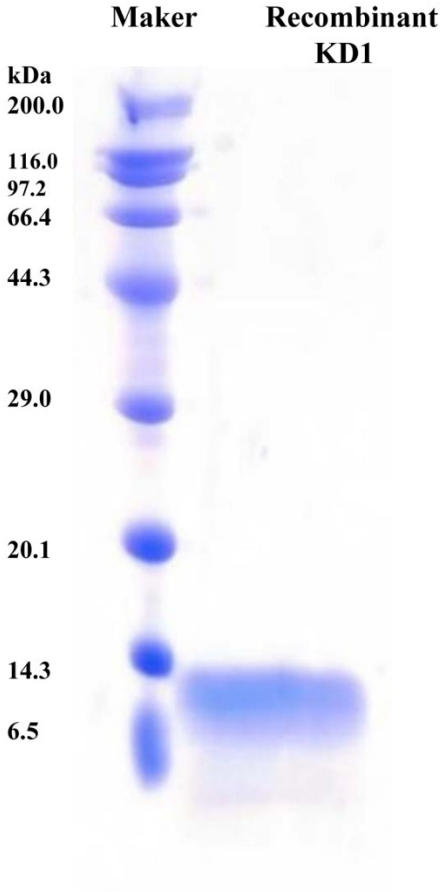

Figure S3: Transmission electron microscopy (TEM) graphics of unmodified CQD (A) and CQD-KD1 (B). Left column: 5 nm scale, middle column: 10 nm scale, and right column: 20 nm scale. TEM data showed that CQD-KD1 was of diameters ranged 5-8 nm slightly higher than the diameters of the uncoupled CQD (1-5 nm) likely due to the conjugation of the protein-based moiety

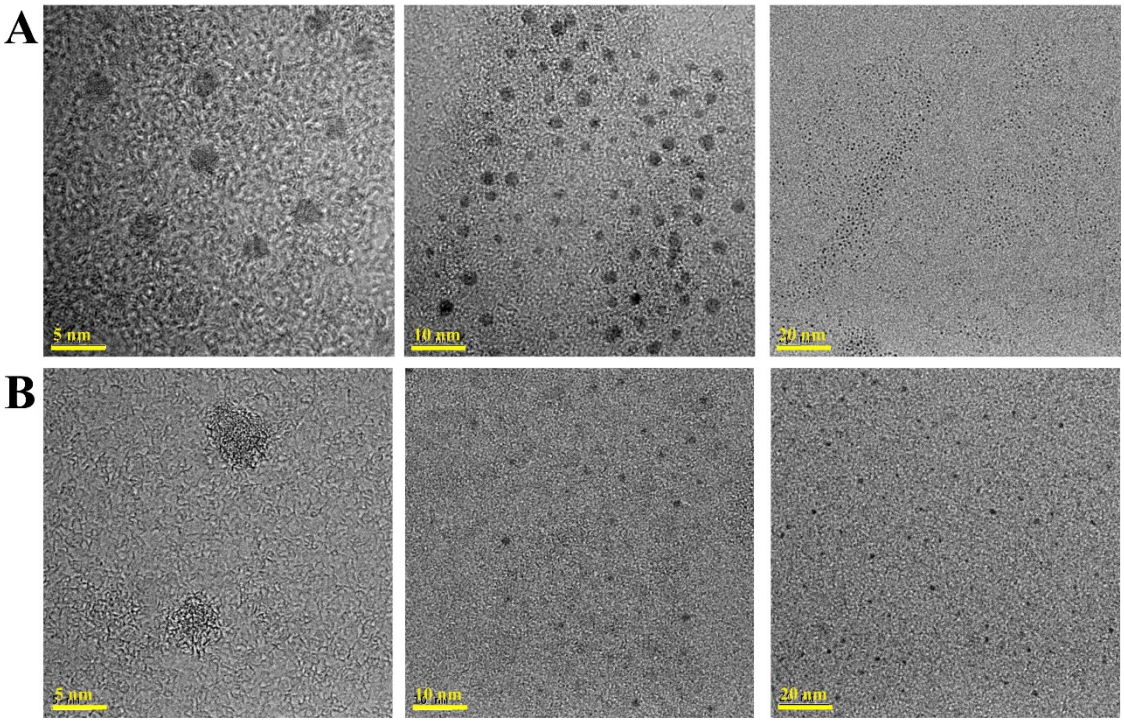

Figure S4: The atomic force microscopy (AFM) images of CQD and CQD-KD1. Left column: the images of the tip forces. Middle column: the images of the heights. Right column: height profile along the white line in the AFM image.

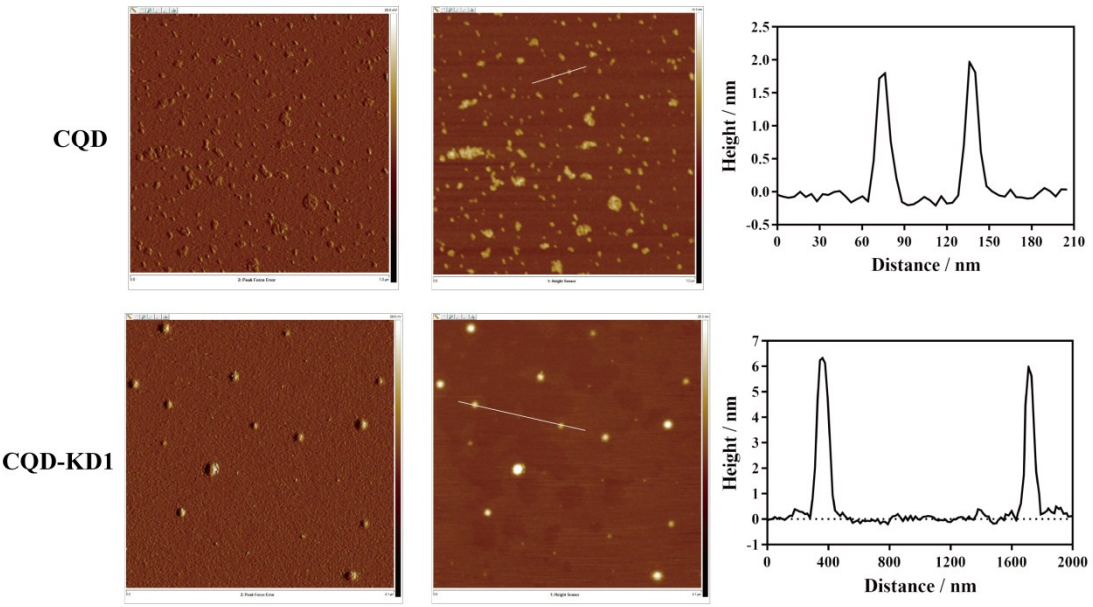

Figure S5: The infra-red spectra of unmodified CQD (blue), recombinant KD1 (green) and CQD-KD1 (red). The FTIR spectra showed a drastically increased absorbance at 3000-3500 cm^{-1} corresponding to the various hydrogens of the conjugated protein compared to the carboxyl hydrogens of the uncoupled CQD.

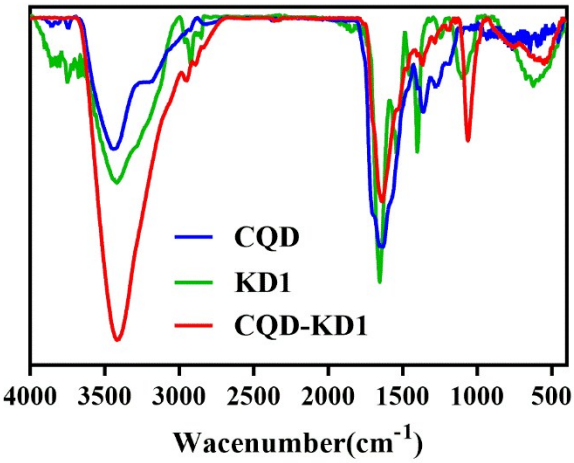

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Figure S6: Cytotoxicity of CQD and CQD-KD1 in the murine breast cancer cell line 4T1. The values were represented as Mean ± SEM

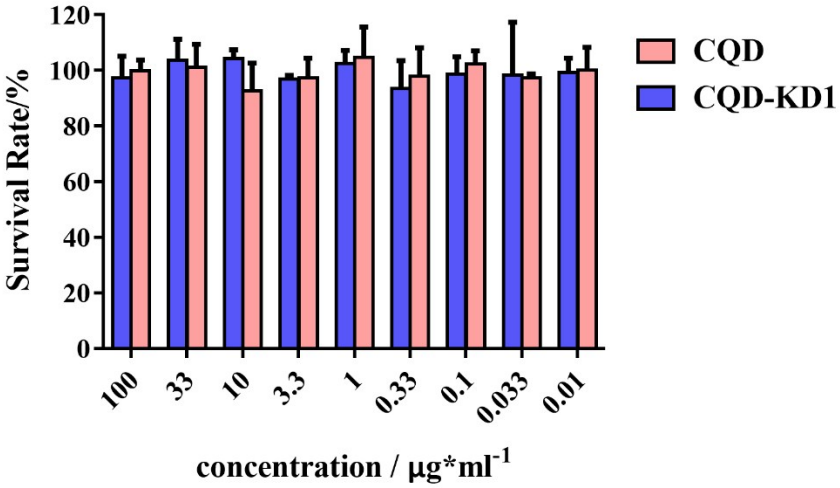

Figure S7: Body weights of balb/c mice implanted with MCF-7 (A) and 4T1 (B) with the treatments of saline, 0.077 mg/kg KD1, 6.4 mg/kg CQD, 0.64 mg/kg, and 6.4 mg/kg CQD-KD1. The values were represented as Mean ± SEM.

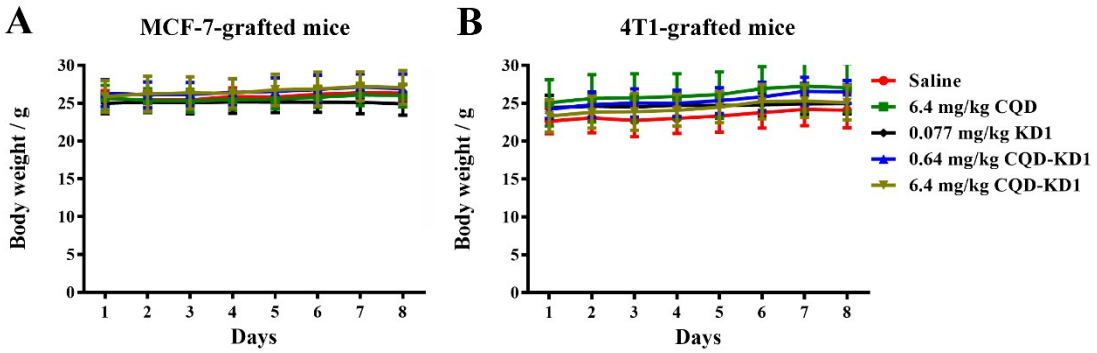

Figure S8: The graphic images of the resected lungs from normal mice, mice with implanation administrated with saline, recombinant KD1, CQD-KD1 in the lung metastatic mice model.