SINGLE-WALLED CARBON NANOHORNS DECORATED WITH SEMICONDUCTOR QUANTUM DOTS TO EVALUATE INTRACELLULAR TRANPSORT

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SUPPLEMENTAL DOCUMENT

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SI.1. PEG-DSPE Toxicity

AlamarBlue was used to study the cytotoxicity of PEG-DSPE on MDA-MB-231 cells at various concentrations following manufacturer's protocol. Approximately 25,000 cells per well were seeded in TCPS 48-well dishes and incubated for 24 h (~70% confluent). Cells were then exposed to 0, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0, or 2.0 mg/mL PEG-DSPE in DMEM/F12 growth media for 24 h (*n=3*). PEG-DSPE solutions were removed and cells rinsed once with PBS. Next, 500 µL of 10% alamarBlue in growth media was added to each sample and incubated for 3 h, and 100 µL aliquots from each sample were taken in triplicate and placed into a 96-well dish, totaling 9 readings per experimental group. Absorbance was measured with a SpectraMAX M2^e microplate reader (Molecular Devices; Sunnyvale, CA) at 570 and 600 nm. Each experimental group (*n=3*) was tested and analyzed independently. The results were represented as the mean value ± sample standard deviation. Significance of results was verified with Student's t-test and a 95% confidence was used to determine statistical significance between groups. MDA-MB-231 cells were selected because they are a widely used cell line in our laboratory.

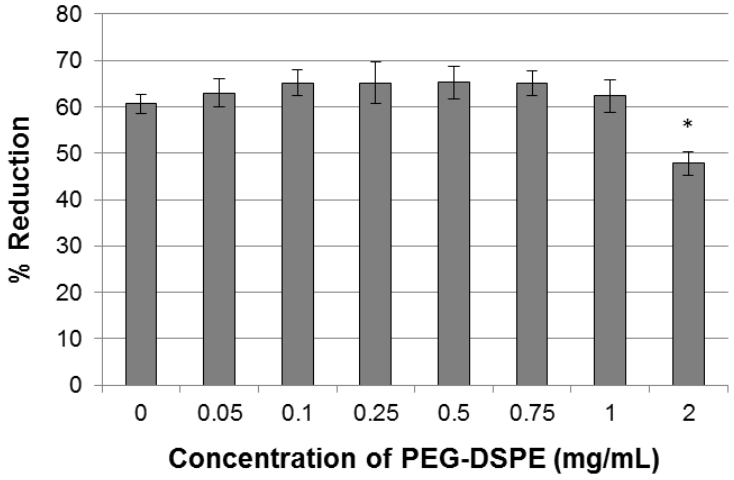


Figure S1. alamarBlue assay of MDA-MB-231 cells treated with PEG-DSPE at various concentrations shows minimal toxicity at concentrations ≤ 1 mg/mL. *Denotes statistical significance for *p<0.05*.

SI.2. Unfunctionalized Nanoparticle Control

Pristine single-walled carbon nanohorns (SWNHs) were bath sonicated for 15 min in chloroform (0.25 mg/mL) and CdSe/ZnS powder (as-purchased) was suspended in chloroform by hand mixing (1 mg/mL). Equal volumes of SWNH and QD suspensions were mixed without reagents and the resulting solution was dried on a TEM grid. QD self-assembly was mainly observed (Figure S2A). Minimal QD assembly on SWNHs was observed as shown in Figure S2B and may be attributed to hydrophobic interactions upon removal of the solvent.

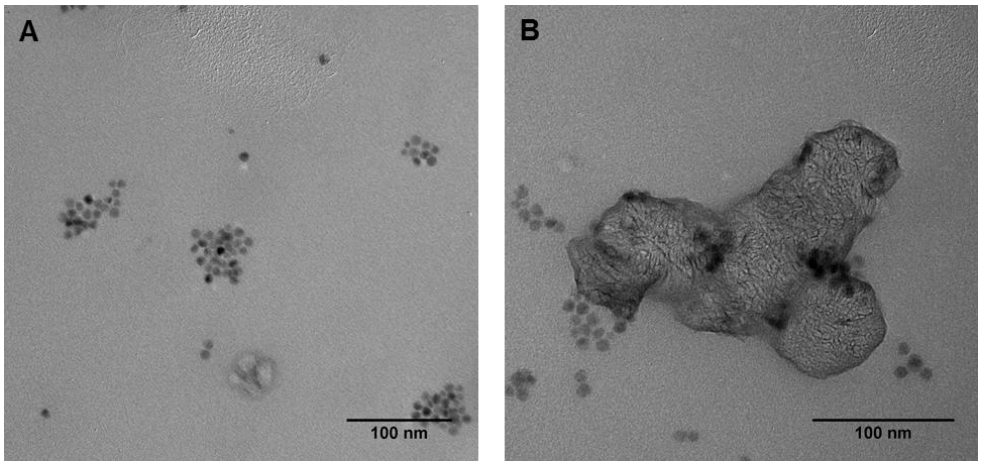


Figure S2. Pristine SWNH and QDs (1:4 weight ratio) were physically mixed without reaction products (EDC, NHS, or AET) and analyzed with TEM to confirm minimal physioadsorption of QDs to exohedral SWNH surface. (A) Self-assembled regions of QDs were observed throughout the sample, which were not observed after SWNH-QD conjugation described in the manuscript. (B) SWNH aggregates had minimal QD physioadsorption upon physical mixing of nanomaterials.

SI.3. SWNH-QD Conjugation Stability Experiment

Single-walled carbon nanohorns (SWNHs) were also conjugated to quantum dots (QDs) using a more conventional method, similar to those used for single-walled carbon nanotubes (SWNTs) [46] and SWNHs [18] in previously published work (Figure S3). SWNHs were acid oxidized using the same procedures in the manuscript. Surface ligand exchange was then performed on as-purchased QDs. During this process, the QDs undergo surface ligand exchange at the phase interface, replacing the octadecylamine capping ligand with 2-aminoethanethiol (AET), creating amine-terminated QDs that are water dispersible (QD-AET). QDs were suspended in chloroform to a concentration of 1.0 mg/mL. Equal volume of 2.0 M AET was added to the solution and stirred vigorously for 24 h. The thiol containing end of AET forms a much stronger bond to the ZnS shell than the Zn-amine bond of the octadecylamine, causing it to be replaced. Subsequently, equal volume sterile dH_2O was added to the mixture and stirred vigorously for 24h to move QDs from the organic phase to the aqueous phase. Acid oxidized (SWNHox) were sonicated in sterile dH_2O for 45-60 min to make a final concentration of 0.1 mg/mL. Then, 0.1 M EDC was added to the SWNHox suspension and stirred for 5 min, followed by the addition of 5.0 mM NHS. QD-NH_2 were then added to the reaction vessel (4:1 QD to SWNH weight ratio), pH was altered between 6.0 and 7.0 with 1.0 M HCl and stirred vigorously for 14-24 h at room temperature. The product (SWNH-QD(2)) was filtered with a 0.22 μm-pore nylon filter membrane and rinsed four times with dH_2O. The product was sonicated off the membrane filter

for 1 min in sterile dH_2O. SWNH-QD(2) was then analyzed by TEM and compared to the SWNH-QD produced using the method in the main manuscript (SWNH-QD(1), Figure 5). Additionally, the conjugates were analyzed for stability over time. Conjugates were left in solution for 5 days and subsequently imaged with TEM.

SWNH-QD complexes, where modified QDs (QD-AET) and SWNHox were conjugated with EDC were not stable over 5 days (Figure S4B) as evidenced by the separation of QDs from SWNHs when dropped on the TEM grid. However, SWNH-QD complexes synthesized by thiol-functionalization remained conjugated after 5 days (Figure S4A).

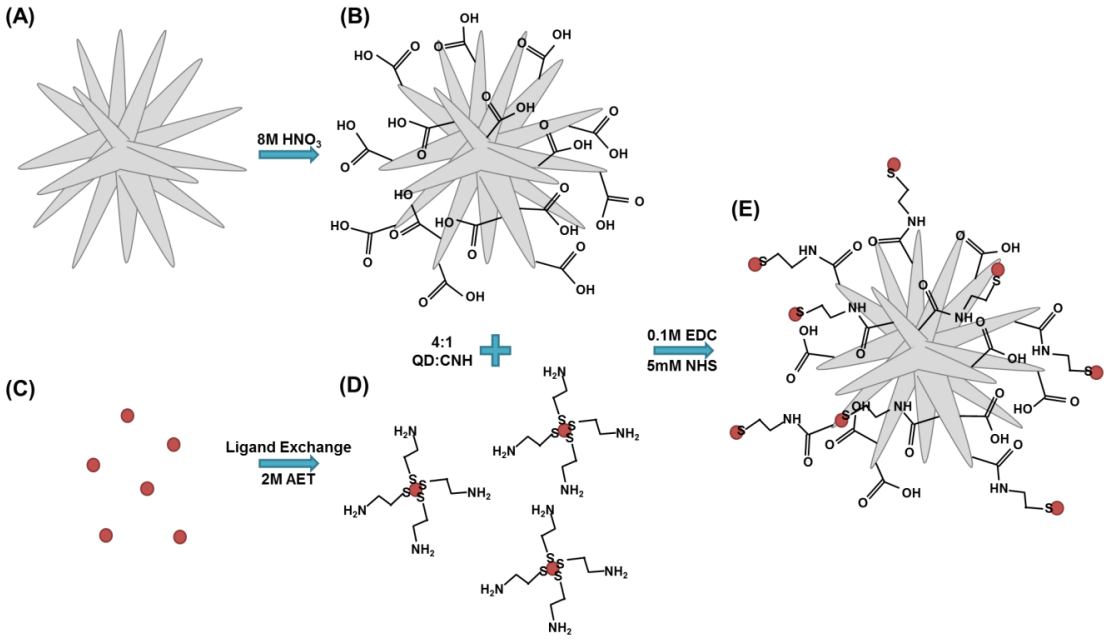


Figure S3. Illustration of QD conjugation to SWNH exohedral surface using conventional method (Scheme 1). Pristine SWNHs (A) were acid oxidized to produce SWNHox (B). QDs (C) underwent surface ligand exchange to produce amine-functionalized water-dispersible QD-NH2 (D). Carbodiimide coupling was used to conjugate SWNHox and QD-NH_2 (E).

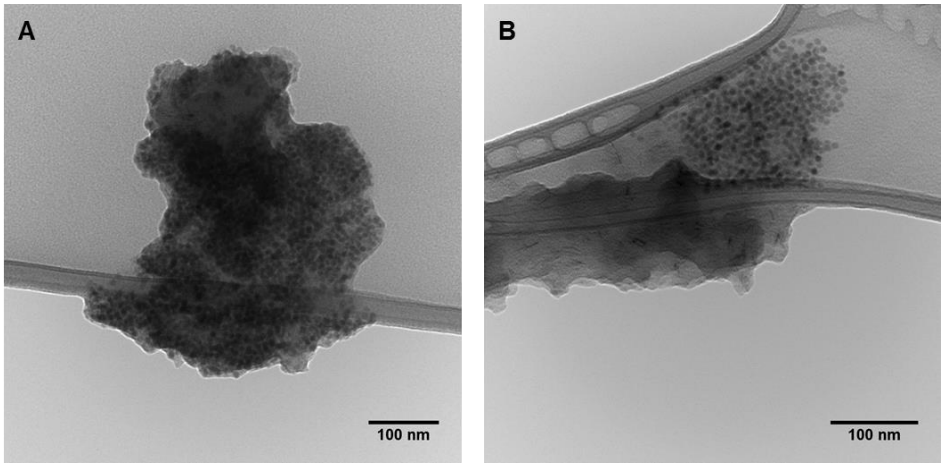


Figure S4. TEM images of SWNH-QD conjugation products after 5 days incubation in solution using (A) the multi-dentate approach discussed in the manuscript (Figure 1 in manuscript, SWNH-QD(1)) and (B) the traditional coupling approach discussed in this supplementary document (Figure S3, SWNH-QD(2)).

SI.4. Calculation of alamarBlue Percent Difference

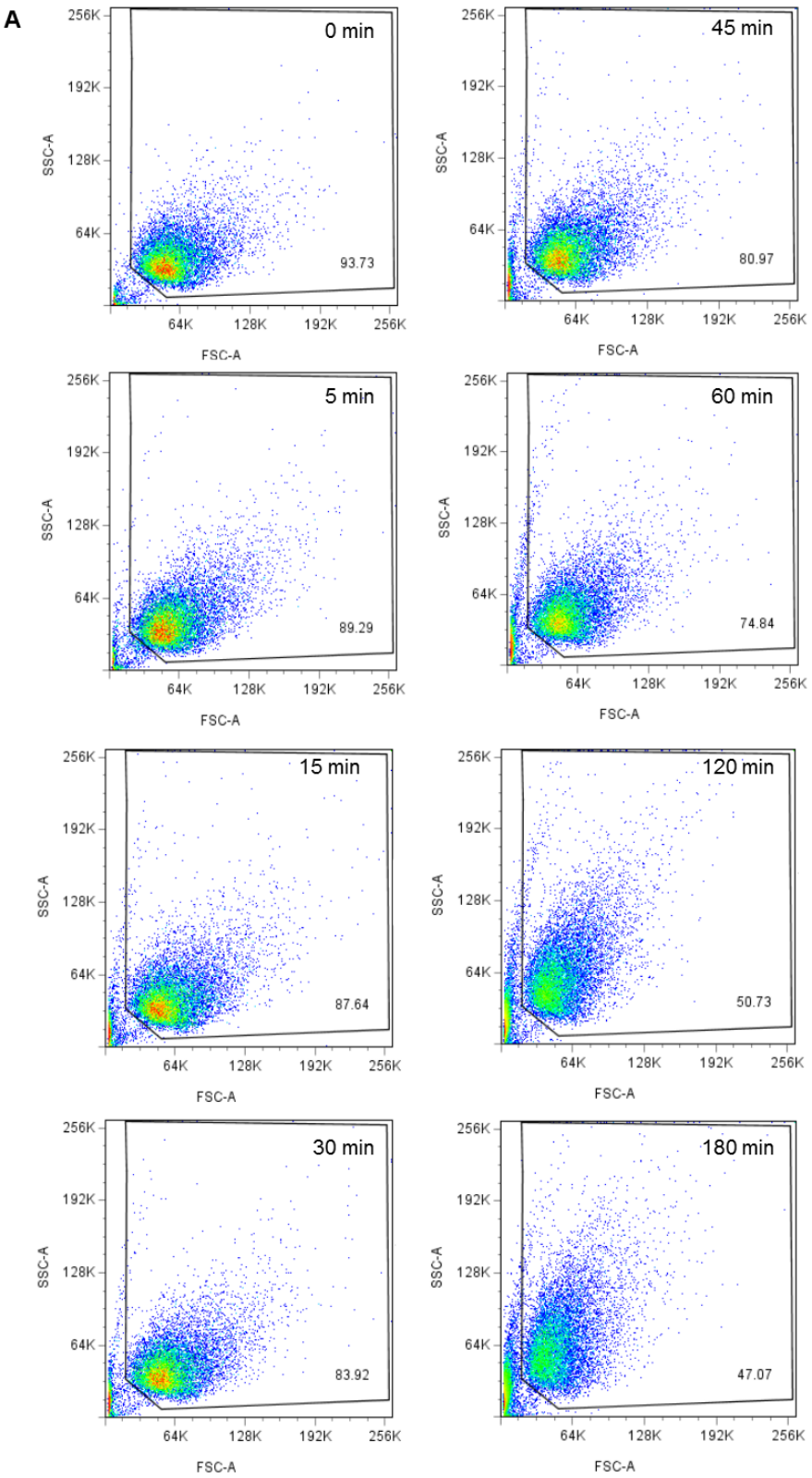
Percent difference of alamarBlue reduction compared to the non-treated control (*i.e.*, 100% difference is equal to 100% of that in the non-treated control groups) was quantified using the following equation:

$$\%\, Difference = \frac{(O_{600} \times A_{570}) - (O_{570} \times A_{600})}{(O_{600} \times P_{570}) - (O_{570} \times P_{600})} \qquad \text{(E.S1)}$$

Where O_{570}= molar extinction coefficient of oxidized alamarBlue at 570 nm, O_{600}= molar extinction coefficient of oxidized alamarBlue at 600 nm, A_{570}= absorbance of test wells at 570 nm, A_{600}= absorbance of test wells at 600 nm, P_{570}= average absorbance of control wells at 570 nm, P_{600}= average absorbance of control wells at 600 nm.

SI.5. Flow Cytometry Side Scattering

Side scattering information from flow cytometry provides information about the cellular internal complexity (Figure S5). The population of all cell lines shift from forward scattering to side scattering as the number of SWNH-QDs are internalized, *i.e.*, as median fluorescence intensity increases with time.


A
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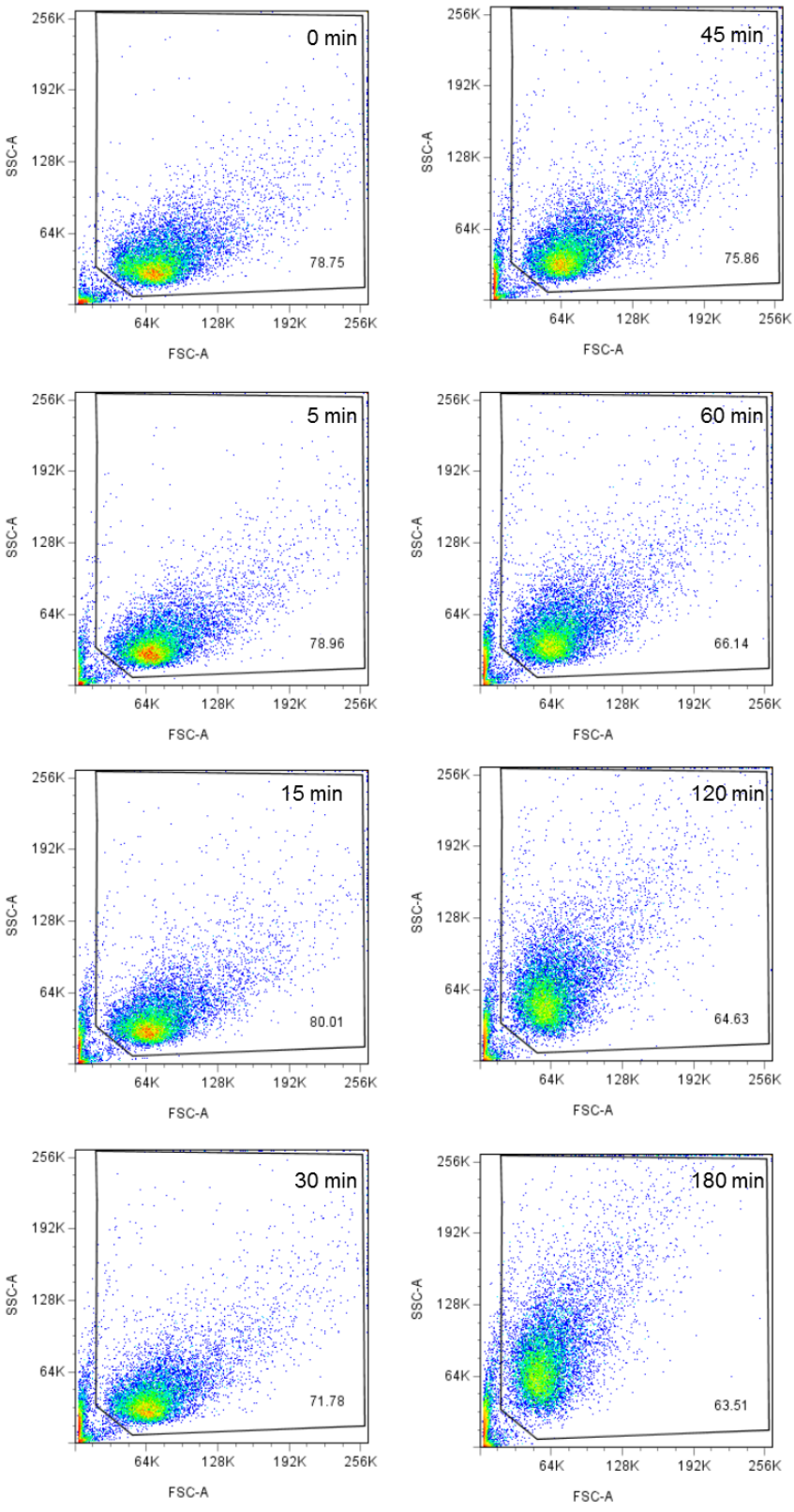
93.73
45 min
80.97
5 min
89.29
60 min
74.84
15 min
87.64
120 min
50.73
30 min
83.92
180 min
47.07
SSC-A
FSC-A
64K
128K
192K
256K

B


0 min
78.75
45 min
75.86
5 min
78.96
60 min
66.14
15 min
80.01
120 min
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FSC-A
64K
128K
192K
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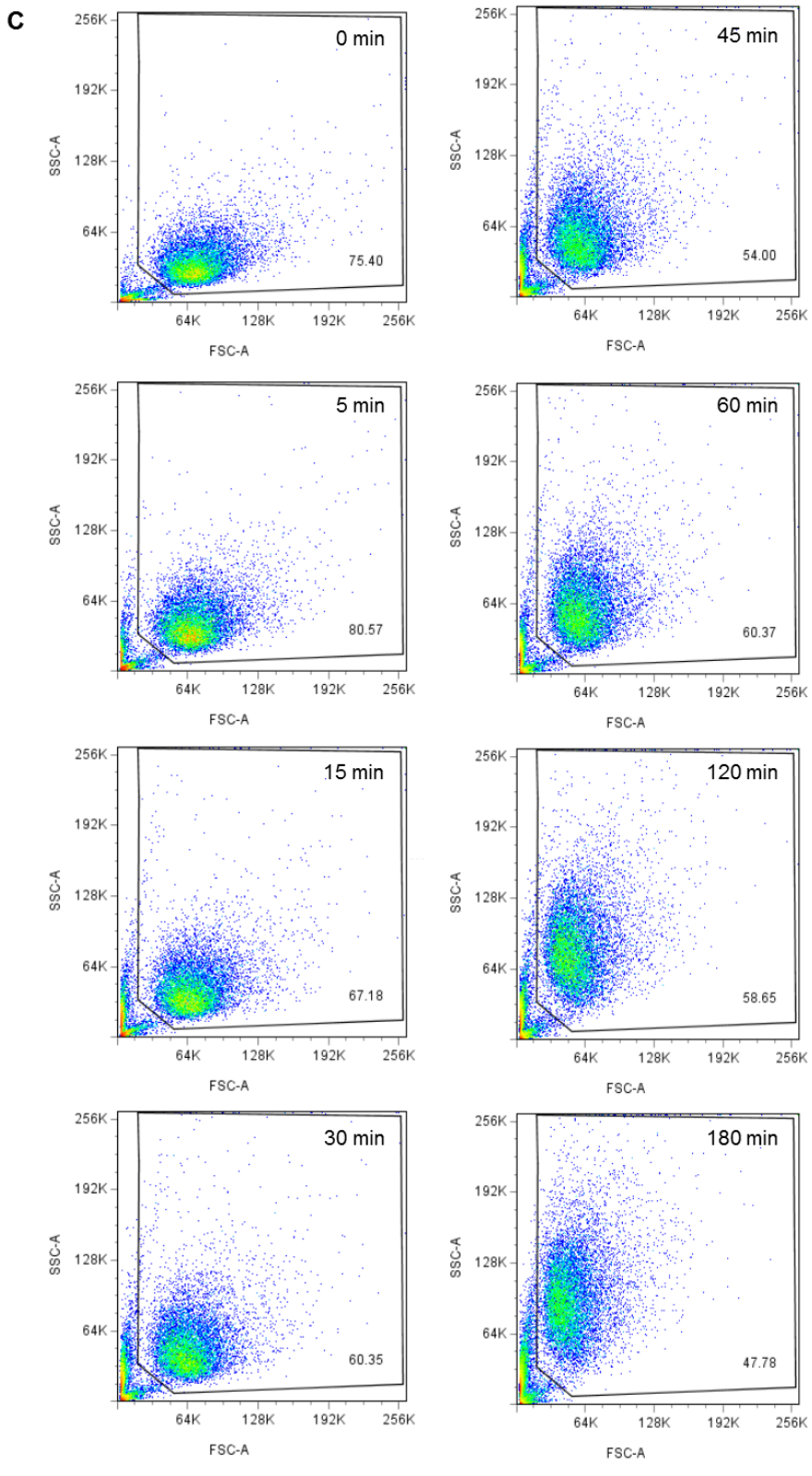

Figure S5. Flow cytometry raw data side scatter vs. forward over 3 h for (A) AY-27, (B) MDA-MB-231, and (C) U-87 MG cell lines.

SI.6. Calculation of Uptake Kinetics and Efficiency

Table S1. Slopes of cellular uptake kinetics and uptake efficiencies determined by linear regression of the linear regions of the SWNH-QD uptake curves obtained by FACS. [a,b]Corresponding uptake curves are shown in Figures 6A and 6B in the main manuscript.

Cell Line	Cell Type	Uptake Kinetics (% positive)[a]	Uptake Efficiency (median FL)[b]
AY-27	Rat bladder transitional cell carcinoma	0.70	5.52
MDA-MB-231	Human mammary gland adenocarcinoma	1.54	21.9
U-87 MG	Human glioblastoma	2.73	36.5