Supplementary Materials

A two-way ANOVA test to investigate the effects of inhibitors on cellular uptake of fullerene

To assess the effects of inhibitors on cellular uptake of fullerene, a two-way ANOVA test was used from five separate experiments (two for addition of the fullerene to cells at the start of the incubation in deionized water (test 1, and test 4); one for addition of the inhibitors and the cells 7 d after the fullerene was dispersed into deionized water (test 2); and two for 14 d fullerene and water incubation prior to addition of the inhibitors and the cells (test 3, and test 5)). For each test, triplicate samples were prepared. Therefore, the total number of samples was 15. Table S1 shows the mass of fullerene particles taken up by the cells for each test.

Table S1. Mass of Cellular Uptake of Fullerene with Three Different Inhibitors for Fullerene Addition of 6 µg and 4 µg for test 1-3 and test 4-5, Respectively (results from tests1-3 are showed in Fig. 5)

mass (µg)	Control	sodium azide	2,4-dinitrophenol	nocodazole
Test 1	1.947	1.068	0.989	1.107
	1.243	1.971	1.112	1.220
	2.135	1.463	1.604	1.494
test 2	1.998	1.520	0.819	0.583
	2.075	1.535	1.660	0.644
	1.797	1.528	1.012	0.548
test 3	1.144	0.425	0.321	0.339
	0.512	0.661	0.358	0.332
	0.681	0.322	0.372	0.630
test 4	2.512	1.104	2.201	1.749
	1.305	1.343	2.108	1.900
	2.891	1.735	1.556	1.095
test 5	1.348	1.229	1.101	0.583
	1.966	1.540	1.474	1.531

	1.660	1.467	1.995	0.517
average	1.681	1.261	1.246	0.951

Statistical significance was defined as $p < 0.05$ using a two-way ANOVA test. When we compare amount of cellular uptake of control (without inhibitor) samples with cellular uptake with three inhibitors, the estimated p-value was 0.0008, indicating inhibitors significantly lowered the fullerene uptake by cells. From the comparison of cellular uptake of control samples with samples with each inhibitor, only nocodazole showed a significant difference ($p = 0.0009$). These results indicate that active transport are responsible to cellular transport of fullerene, and microtubule involved endocytosis can be one of the important transport mechanisms.

1. Extraction Recovery of Fullerene

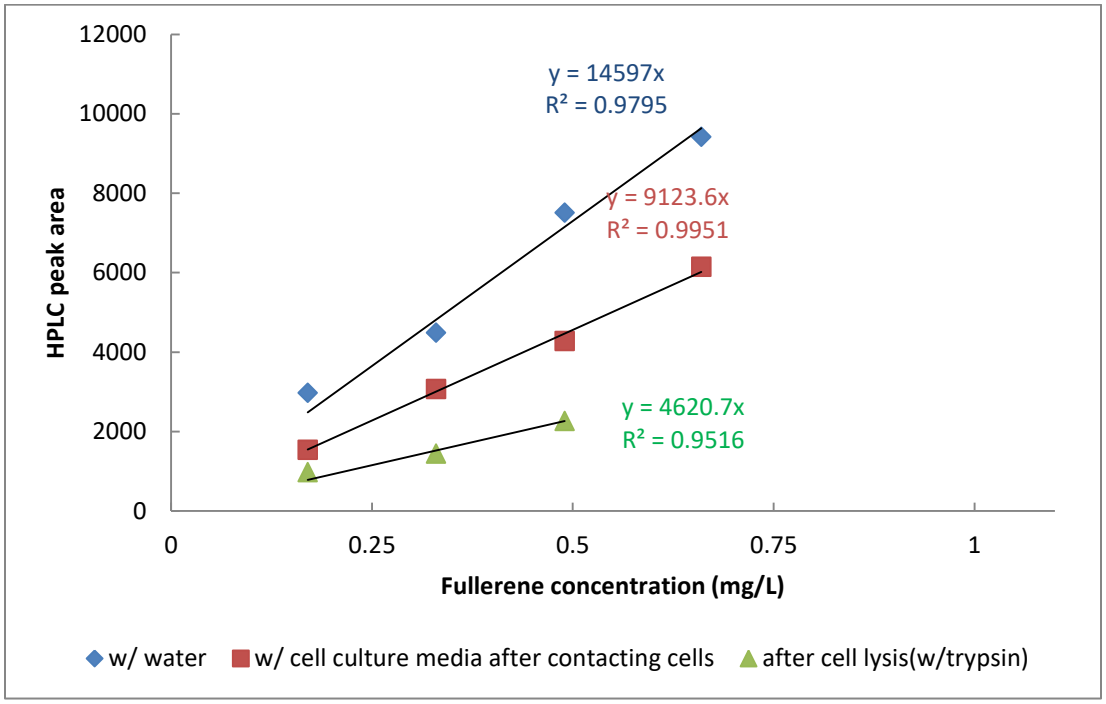

Fig. S1. Correlation between actual fullerene concentration and HPLC peak areas for fullerene extracted from water (blue diamond), cell culture media without FBS (red squares), and cell lysates (green triangles).

2. nC_{60} Mass Recovered from Reactors

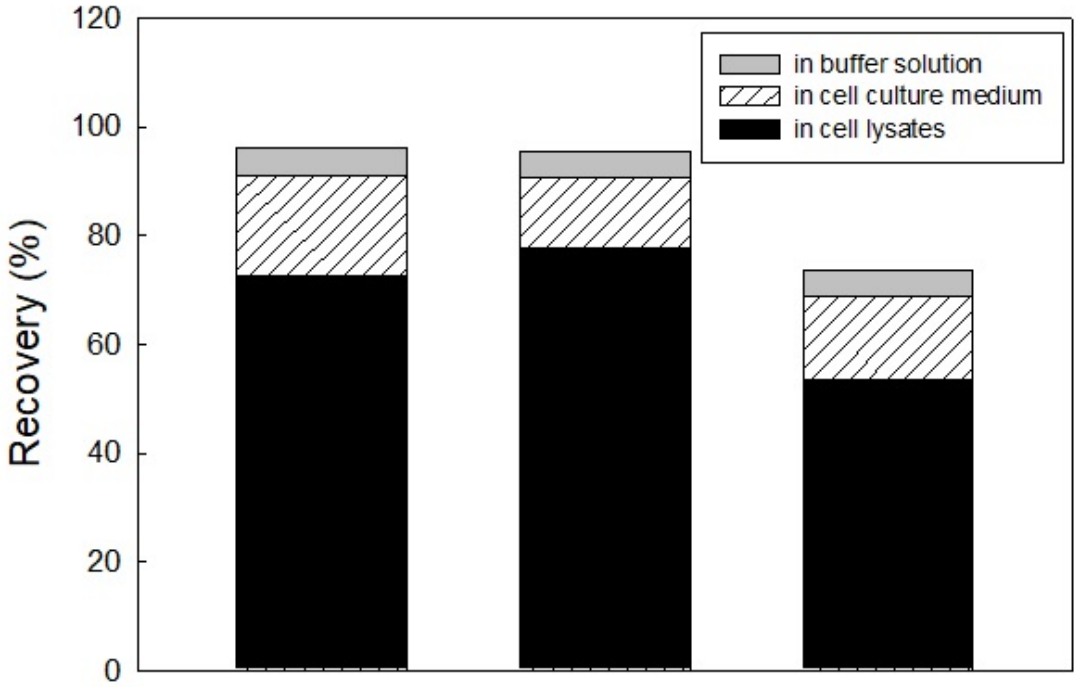

Fig. S2. Mass of fullerene nanoparticles recovered from the triplicate independent cellular uptake tests. Gray bars shows fullerene in phosphate-buffered saline, white bars indicate fullerene remaining in cell culture medium, and black bars indicate fullerene taken up by the Caco-2 cells. The initial fullerene concentrations added to each reactor was 7.8 mg/L. The three experiments were conducted using cells with the same passage number seeded at different wells, at the same day.