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Supporting Information
A nanoparticle-cell-nanoparticle cooperation network to enhance poly(I:C) induced apoptosis in cancer cells

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1. Chemicals

The chemicals tetraethylorthosilicate (TEOS), *n*-cetyltrimethylammonium bromide (CTAB), sodium hydroxide, sulforhodamine B, (3-aminopropyl)triethoxysilane (APTES), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), (3-isocyanatopropyl)triethoxisilane, 9-*cis*-retinoic acid, interferon-γ, D6046 DMEM medium, monoclonal anti-α-tubulin antibody, polyclonal anti-TLR3 antibody, anti-mouse IgG (whole molecule)-peroxidase antibody and the lysosome Isolation Kit (LYSISO1) were provided by Aldrich. Triethylamine was provided by Avantor Materials, Cell Proliferation Reagent (WST-1) by Roche, while polyinosinic:polycytidylic acid (poly(I:C)) was provided by American Custom Chemical Corporation. Western blotting detection reagents and analysis system kit and anti-rabbit IgG horseradish peroxidase linked antibody were purchased from GE Healthcare Life Sciences, while SK-BR-3 human breast carcinoma cells from American Type Culture Collection. All products were used as received.

2. General Techniques

The synthesised materials were characterized by powder XRD, TG analysis, elemental analysis, TEM, and N_2 adsorption-desorption techniques. Powder X-ray diffraction measurements were performed on a Seifert 3000TT diffractometer using CuK_α radiation. Thermogravimetric analysis were carried out on a TGA/SDTA 851e Mettler Toledo equipment, using an oxidant atmosphere (Air, 80 mL/min) with a heating program consisting on a heating ramp of 10°C per minute from 393 K to 1273 K and an isothermal heating step at this temperature for 30 minutes. Elemental analysis was performed in a CE Instrument EA-1110 CHN Elemental Analyzer. Transmission electron microscopy (TEM) images were performed in a Philips CM-10. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP2010 automated sorption analyser. The samples were degassed at 120°C in vacuum overnight. The specific surfaces areas were calculated from the adsorption data in the low pressures range using the BET model. Pore size was determined following the BJH method. UV-visible spectra were recorded with a JASCO V-650 spectrophotometer. Fluorescence measurements were carried out in a JASCO FP-8500 spectrophotometer. A VersaMax absorbance microplate reader by Molecular Devices was used to determine solubilized formazan concentration in viability assays and to quantify protein extracts in Bradford assay. Confocal microscopy imaging was performed employing a Leica TCS SPE (Leica Microsystems Heidelberg GmbH) inverted laser scanning confocal microscope using oil objective HC PL APO 40x. Flow cytometry assays were performed using a FC500 MPL Flow Cytometer (Beckman-Coulter, CA, USA) equipped with a 488nm laser. Ability to acquire samples in different formats (tubes or well plates.

3. Synthesis of Mesoporous Nanoparticles

Mesoporous silica nanoparticles (MSNs) were synthesised by the following procedure: *n*-cetyltrimethylammonium bromide (CTAB, 1.00 g, 2.74 mmol) was first dissolved in deionised water (480 mL). Then, NaOH (3.5 mL, 2.00 mol L^{-1}) in deionised water was added to the CTAB solution, followed by adjusting

the solution temperature to 80°C. TEOS (5.00 mL, 2.57 x 10^{-2} mol) was then added dropwise to the surfactant solution. The mixture was stirred for 2 h to give a white precipitate. Finally, the solid product was centrifuged, washed with deionised water and dried at 60°C (MSNs as-synthesised). To prepare the final porous nanoparticles (MSNs), the as-synthesised solid was calcined at 550°C using an oxidant atmosphere for 5 h in order to remove the template phase.

Synthesis of S(RA)$_{IFN}$

To obtain **S(RA)$_{IFN}$** material, 50 mg of calcined MSNs were suspended in a solution of 9-*cis*-retinoic acid (1 mg, 3.3 x 10^{-3} mmol) in 250 µL of acetonitrile and were stirred for 24 h at room temperature and safe from light. Then, (3-isocyanatopropyl)triethoxysilane (61.9 µL, 0.25 mmol) was added and the suspension was stirred for 5.5 h at room temperature and safe from light (**S(RA)$_{NCO}$**). Afterwards, the solid was centrifuged and dried under vacuum. Solid **S(RA)$_{NCO}$** (1 mg) was then suspended in a mixture of 700 µL of acetonitrile containing an excess of 9-*cis*-retinoic acid and 100 µL of a solution of interferon-γ (0.1 mg) in deionised water. Triethylamine (2 µL) were added to the mixture that was stirred during 2 h at room temperature and safe from light. Finally, this solid was isolated by centrifugation, washed with deionized water and dried under vacuum.

Synthesis of S(sulf)$_{PIC}$

In a typical synthesis, 400 mg of template-free MSNs were suspended in a solution of sulforhodamine B (200 mg, 0.34 mmol) in 35 mL of acetonitrile in a round-bottomed flask and were stirred for 24 h at room temperature. Afterwards, (3-aminopropyl)triethoxysilane (480 µL, 2 mmol) was added to the suspension and the mixture was stirred for 24 h at room temperature (**S(sulf)$_{NH2}$**). Finally, the obtained solid was isolated by centrifugation and dried under vacuum. Then, 685 mg of this functionalised solid were dispersed in 122.3 mL of water containing sulforhodamine B (in order to inhibit the delivery of the dye from the pores to the bulk solution), EDC (2.34 g, 12.2 mmol) and poly(I:C) (171.25 mg, 3.8 µmol) and the resulting suspension was stirred for 6 h at room temperature. Finally, this solid was isolated by centrifugation, washed with abundant water and dried at 37°C for 24 h.

Synthesis of S(sulf)$_{IFN}$

100 mg of template-free MSNs were suspended in a solution of sulforhodamine B (50 mg, 0.085 mmol) in 9 mL of acetonitrile in a round-bottomed flask and were stirred for 24 h at room temperature. Then, (3-isocyanatopropyl)triethoxysilane (123.8 µL, 0.5 mmol) was added and the suspension was stirred for 5.5 h at room temperature, and successively centrifuged and dried under vacuum (**S(sulf)$_{NCO}$**). 4 mg of the dried solid were suspended in a mixture of 2.8 mL of acetonitrile containing an excess of sulforhodamine B, 400 µL of a solution of interferon-γ (0.1 mg) in deionised water and 2 µL of trimethylamine. The mixture was stirred during 2 h at room temperature and then centrifuged. The isolated solid was washed with deionized water and dried under vacuum.

4. Materials Characterization

The prepared solids were characterized using standard techniques. Figure SI-1a shows powder X-ray diffraction (PXRD) patterns of as-synthesized MSNs, calcined MSNs, **S(sulf)$_{NCO}$** and **S(sulf)$_{PIC}$**. The as-synthesized MSNs show the typical four low-angle reflections of a hexagonal-ordered matrix indexed at (100), (110), (200), and (210) Bragg peaks. The displacement of the (100) peak in the PXRD of the calcined MSNs and the broadening of the (110) and (200) peaks are related to the further condensation of silanol groups during the calcination step. Finally, for solids **S(sulf)$_{NCO}$** and **S(sulf)$_{PIC}$**, reflections (110) and (200) are less intense due to a reduction in contrast related to the functionalization process and to the filling of mesopores with sulforhodamine B. Nonetheless, the intensity of the (100) peak in these patterns strongly indicates that

the loading process with the dye and the additional functionalization with (3-isocyanatopropyl)triethoxysilane or with 3-(aminopropyl)trimethoxysilane and poly(I:C), respectively, did not modify the mesoporous MSNs scaffold.

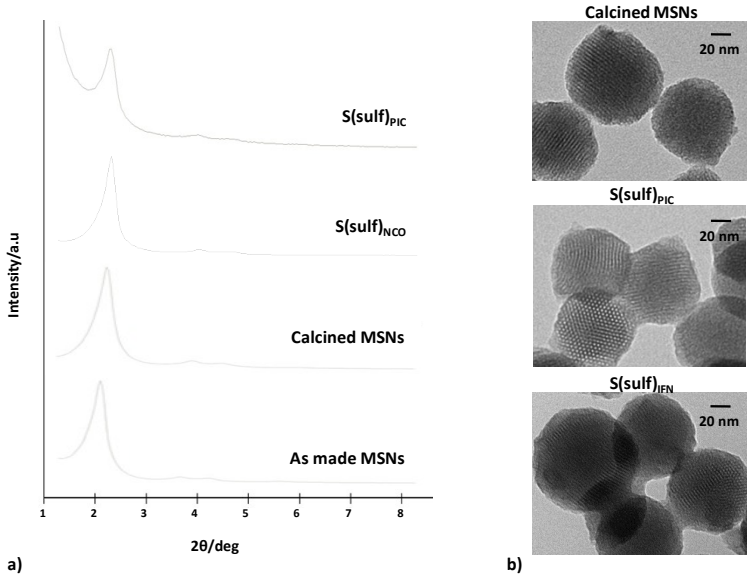

Figure SI-1. (a) Powder X-ray diffraction pattern of as made MSNs, calcined MSNs, solid **S(sulf)$_{NCO}$** and solid **S(sulf)$_{PIC}$** showing the typical reflections of the MSNs hexagonal array. (b) TEM representative images of calcined MSNs, solid **S(sulf)$_{PIC}$** and solid **S(sulf)$_{IFN}$** showing the typical porosity of the MSNs mesoporous matrix. Scale bars: 20 nm.

The N_2 adsorption-desorption isotherms of the calcined MSNs is shown in Figure SI-2a. A typical curve for mesoporous solids consisting of an adsorption step at intermediate P/P_0 values (0.25-0.4) is observed. This curve corresponds to a type IV isotherm, in which the observed step is due to nitrogen condensation inside the mesopores. The absence of a hysteresis loop in this interval and the narrow Barrett-Joyner-Halenda (BJH) pore distribution suggest there are uniform cylindrical mesopores (pore diameter of 2.68 nm and pore volume of 0.81 $cm^3\ g^{-1}$, calculated using the BJH model on the adsorption branch of the isotherm). The application of the Brunauer-Emmett-Teller (BET) model resulted in a value of 1034.8 $m^2\ g^{-1}$ for the total specific surface area. From the PXRD, porosimetry and TEM studies, the a_0 cell parameter (4.41 nm), pore diameter (2.68 nm), and value for the wall thickness (1.8 nm) were calculated. In addition to this adsorption step associated to the micelle-generated mesopores, a second feature appears in the isotherm at a high relative pressure ($P/P_0 > 0.8$). This adsorption corresponds to the filling of the large voids among the particles (pore diameter of 46.61 nm and pore volume of 0.25 $cm^3\ g^{-1}$, calculated by using the BJH model) and therefore must be considered as a textural-like porosity. On the other hand, the N_2 adsorption-desorption isotherm of **S(sulf)$_{PIC}$** is typical of mesoporous systems with partially filled mesopores (Figure SI-2b). In this case, and as expected, a lower N_2 adsorbed volume (BJH mesopore volume of 0.04 $cm^3 g^{-1}$) and surface area (127.3 $m^2\ g^{-1}$) were found when compared with the starting MSNs material.

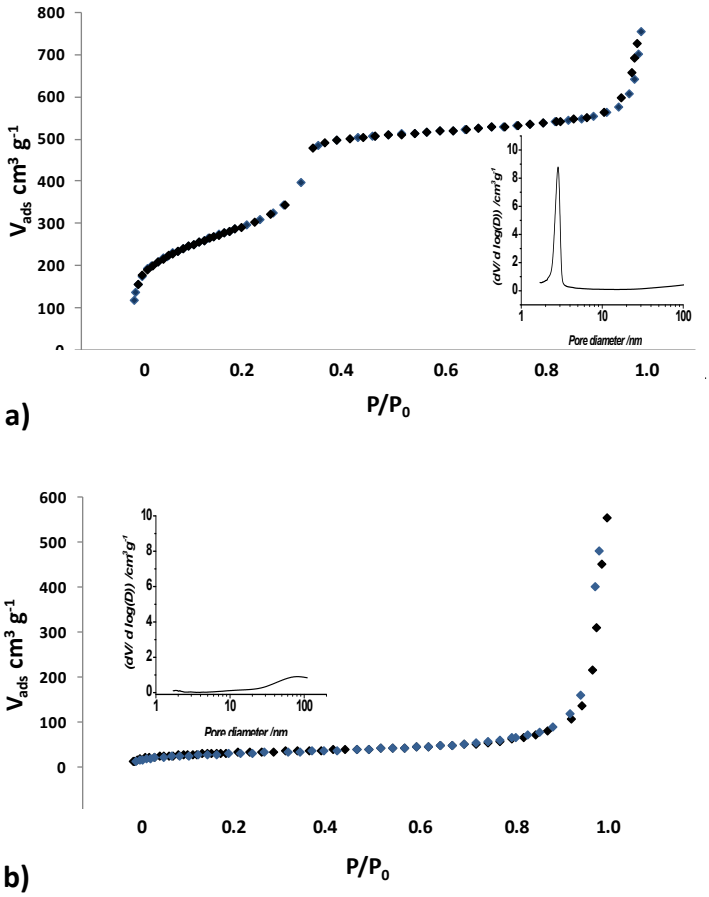

Figure SI-2. Adsorption-desorption isotherms for (a) calcined MSNs and (b) dye-loaded and functionalized **S(sulf)$_{PIC}$**. Inset: pore-size distribution of both solids.

Table SI-1. BET-specific surface values, pore volumes and pore sizes calculated from the N_2 adsorption–desorption isotherms for calcined MSNs and **S(sulf)$_{PIC}$** material.

Sample	S_{BET} [m^2g^{-1}]	Pore Volume [cm^3g^{-1}]	Pore size [nm]
Calcined MSNs	1034.8	0.81	2.68
S(sulf)$_{IC}$	127.3	0.04	2.02

The contents of loaded sulforhodamine B and grafted (3-isocyanatopropyl)triethoxysilane in **S(sulf)$_{NCO}$**, loaded 9-*cis*-retinoic acid in **S(RA)$_{IFN}$** and grafted poly(I:C) and loaded sulforhodamine B in **S(sulf)$_{PIC}$** were determined by elemental and thermogravimetric analyses (Table SI-2). Moreover, the content of 9-*cis*-retinoic acid in solid **S(RA)$_{IFN}$** was also determined by UV-visible studies. For this purpose, the content of 9-*cis*-retinoic acid present in the washing solutions was quantified and subtracted to the quantity used in the loading process of the solid. In addition from thermogravimetric studies the amount of interferon-γ was

determined in **S(RA)$_{IFN}$**. The contents calculated through this method compared well with those obtained from elemental analyses.

Table SI-2. Content (α, mg/g of solid) of the different loading molecules, of (3-isocyanatopropyl) triethoxysilane, interferon-γ and of poly(I:C) in **S(sulf)$_{NCO}$**, **S(RA)$_{IFN}$** and **S(sulf)$_{PIC}$**.

Sample	$\alpha_{9\text{-cis-retinoic acid}}$	α_{dye}	$\alpha_{isocyanate}$	$\alpha_{poly(I:C)}$	α_{IFN}
S(sulf)$_{NCO}$	-	200	123.9	-	-
S(RA)$_{IFN}$	45.8	-	-	-	2.29
S(sulf)$_{PIC}$	-	16.2	-	179.6	-

5. Delivery studies

Assays of *in vitro* cargo release of **S(sulf)$_{IFN}$** and **S(sulf)$_{PIC}$** solids were performed using purified lysosomal extract as trigger. In a typical experiment, 1 mg of **S(sulf)$_{IFN}$** or **S(sulf)$_{PIC}$** was suspended in 500 µL of deionised water. Then, each suspension was divided into two aliquots of 250 µL and 1 mL of 10 mM PBS or 1 mL of purified lysosomal extract was added. To obtain the purified lysosomal extract the Lysosome Isolation Kit (LYSISO1) was used, following supplier's instructions for extractions from animal tissues and purification. Dye delivery from **S(sulf)$_{IFN}$** and **S(sulf)$_{PIC}$** was monitored via the emission band of the dye centred at 575 nm (λ_{ex}= 554 nm). In both cases, in absence of purified lysosomal extract, negligible cargo release was observed, indicating that the nanoparticles were tightly capped. In contrast, in presence of purified lysosomal extract the uncapping of the pores was clearly observed (see Figure 2 in the manuscript). These facts demonstrated that cargo delivery is triggered by the presence of lysosomal enzymes that hydrolysed interferon-γ or poly(I:C) caps in **S(sulf)$_{IFN}$** and **S(sulf)$_{PIC}$** nanoparticles.

Besides, cargo release from **S(sulf)$_{IFN}$** and **S(sulf)$_{PIC}$** in the presence of selected interferents (i.e. Na^+, K^+, Ca^{2+}, Fe^{3+}, Cys, GSH, H_2O_2) and at different pH (4, 7 and 9) was studied (Figure SI-3). For these purposes, each solid (0.5 mg) was suspended in water at different pH values (1250 µL at pH 4, 7 and 9), in PBS 10 mM (blank) and in water at pH 7 containing the selected interferents (10 mM). Delivery in the presence of purified lysosomal extract were also carried out. After 10 h the emission of sulforhodamine at 575 nm (λ_{ex}= 554 nm) was measured. As could be seen, none of the inteferents tested nor the pH of the media induced sulforhodamine release from **S(sulf)$_{IFN}$** and **S(sulf)$_{PIC}$** nanoparticles. A remarkable release of the entrapped fluorophore was only observed in the presence of lysosomal extract.

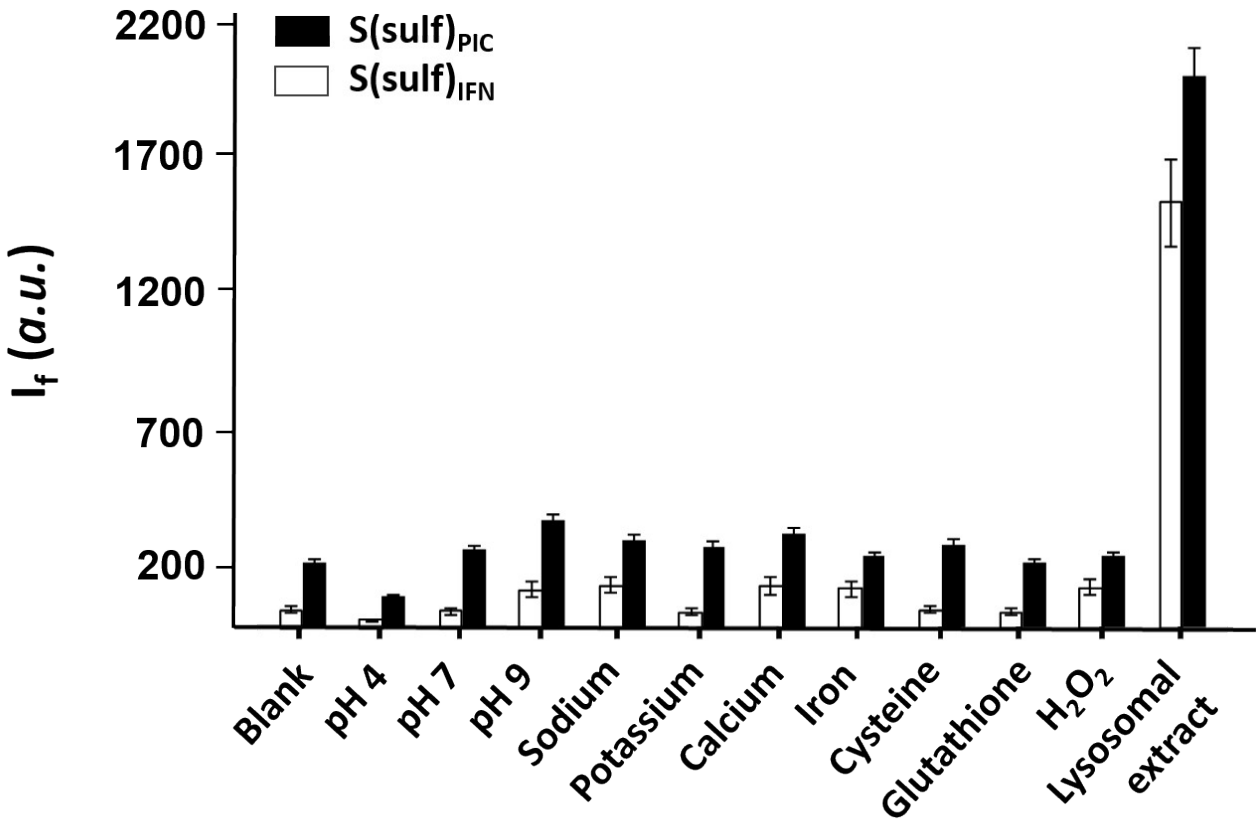

Figure SI-3. Sulforhodamine release (emission at 575 nm after excitation at 554 nm) from **S(sulf)$_{IFN}$** (white bars) and **S(sulf)$_{PIC}$** (black bars) nanoparticles alone (blank), at different pH values, in the presence of selected interferents (at 10 mM) and in the presence of lysosomal extract. Emission of sulforhodamine at 575 nm (λ_{ex}= 554 nm) was measured after 10 h.

6. Cell culture and treatment

Poly(I:C), 9-*cis*-retinoic acid, interferon-γ and materials **S(RA)$_{IFN}$** and **S(sulf)$_{PIC}$** were tested *in vivo*, using SK-BR-3 human breast carcinoma cells. Cells were cultured at 37°C in DMEM medium containing 1000 mg L^{-1} glucose, phenol red, 10% foetal calf serum and 2 mM glutamine. All the tested compounds and materials were dissolved in full-serum media just before treatments.

7. Viability Assays

Quantitation of cell viability rates was determined using cell proliferation reagent WST-1 following supplier's instructions. Cells were seeded in 96-well plates (25,000 cells/well) in triplicate. In the poly(I:C) or **S(sulf)$_{PIC}$** material alone assay, the compound at concentrations of 0.001, 0.01, 0.1, 1, 10, 25, 50, 100 and 500 μg mL^{-1} or the nanoparticles at equivalent Poly(I:C) concentrations were added 48 h after seeding, during additional 48 h. In the viability assay with poly(I:C) combined with 9-*cis*-retinoic acid and interferon-γ or with both **S(RA)$_{IFN}$** and **S(sulf)$_{PIC}$** nanoparticles, 0.3 μg mL^{-1} of 9-*cis*-retinoic acid and 15 ng mL^{-1} of interferon-γ or **S(RA)$_{IFN}$** solid at a concentration of 0.3 μg mL^{-1} of equivalent 9-*cis*-retinoic acid was added to the plates during 4 h, and then the medium was removed and replaced with poly(I:C) or **S(sulf)$_{PIC}$** nanomaterial, respectively, in DMEM at the same concentrations of the previous assay.

After 48h of treatment, medium was removed and replaced with PBS with 1000 mg L^{-1} glucose to avoid

phenol red interference with the absorbance reading, WST-1 was added and the plates were incubated during 1 h at 37°C. Finally, the absorbance at 490 nm was measured. The obtained results are shown in Figure 3 in the manuscript.

Flow cytometry was also employed as a complementary technique for measure the cell viability. Cellular viability was determined by measuring the capacity of cells to exclude the dye 4',6-diamidino-2-phenylindole (DAPI). Cells were seeded in 12-well plates ($2 \cdot 10^5$ cells/well) in triplicate. In the poly(I:C) or **S(sulf)$_{PIC}$** assay, poly(I:C) at concentration of 500 μg mL^{-1} or the nanoparticles at an equivalent poly(I:C) concentration were added 48 h after seeding, during additional 48 h. In the viability assay with poly(I:C) combined with 9-*cis*-retinoic acid and interferon-γ or using both **S(RA)$_{IFN}$** and **S(sulf)$_{PIC}$** nanoparticles, 0.3 μg mL^{-1} of 9-*cis*-retinoic acid and 15 ng mL^{-1} of interferon-γ or **S(RA)$_{IFN}$** at a concentration of 0.3 μg mL^{-1} were added and then the medium was removed and replaced with poly(I:C) or **S(sulf)$_{PIC}$** nanomaterial in DMEM for 48h. In an additional experiment, after 48 h seeding, cell were treated with 3.5 μM of TLR3/dsRNA complex inhibitor for 4 h, and then the medium was removed and replaced with free media with or not **S(sulf)$_{PIC}$** nanoparticles for 48h. Then, cells were detached using trypsin and were resuspended in Dulbecco's Phosphate Buffered Saline (DPBS) containing 0.1 μg/mL DAPI. Cells were incubated during 5 minutes at room temperature and then were proceeded to analysis by flow cytometry. The obtained results are shown in Figure 4 in the manuscript.

8. Western blotting

TLR3 and α-tubulin antibodies were used to probe blots of protein extracts prepared using triton lysis buffer (10 mM Tris, pH 7.4, 137 mM NaCl, 2 mM EDTA, 1% Triton X-100, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 2 mM sodium pyrophosphate, 10% glycerol, 1 mM PMSF, and protease inhibitors cocktail). Immune complexes were detected by chemiluminescence. Densitometric analysis was carried out with the ImageJ program.

9. Confocal Microscopy Imaging

Internalization of **S(sulf)$_{PIC}$** nanoparticles and dye release in SK-BR-3 cells was observed *in vitro* by confocal fluorescence microscopy (Figure S3). About 8×10^5 SK-BR-3 cells per well were cultured on 6-well plates, and 48 h later **S(sulf)$_{PIC}$** nanoparticles at 50 μg mL^{-1} of equivalent poly(I:C) were added for additional 48 h.