

Supplementary Information

Acoustically-Mediated Intracellular Nanoparticle and Macromolecule Delivery

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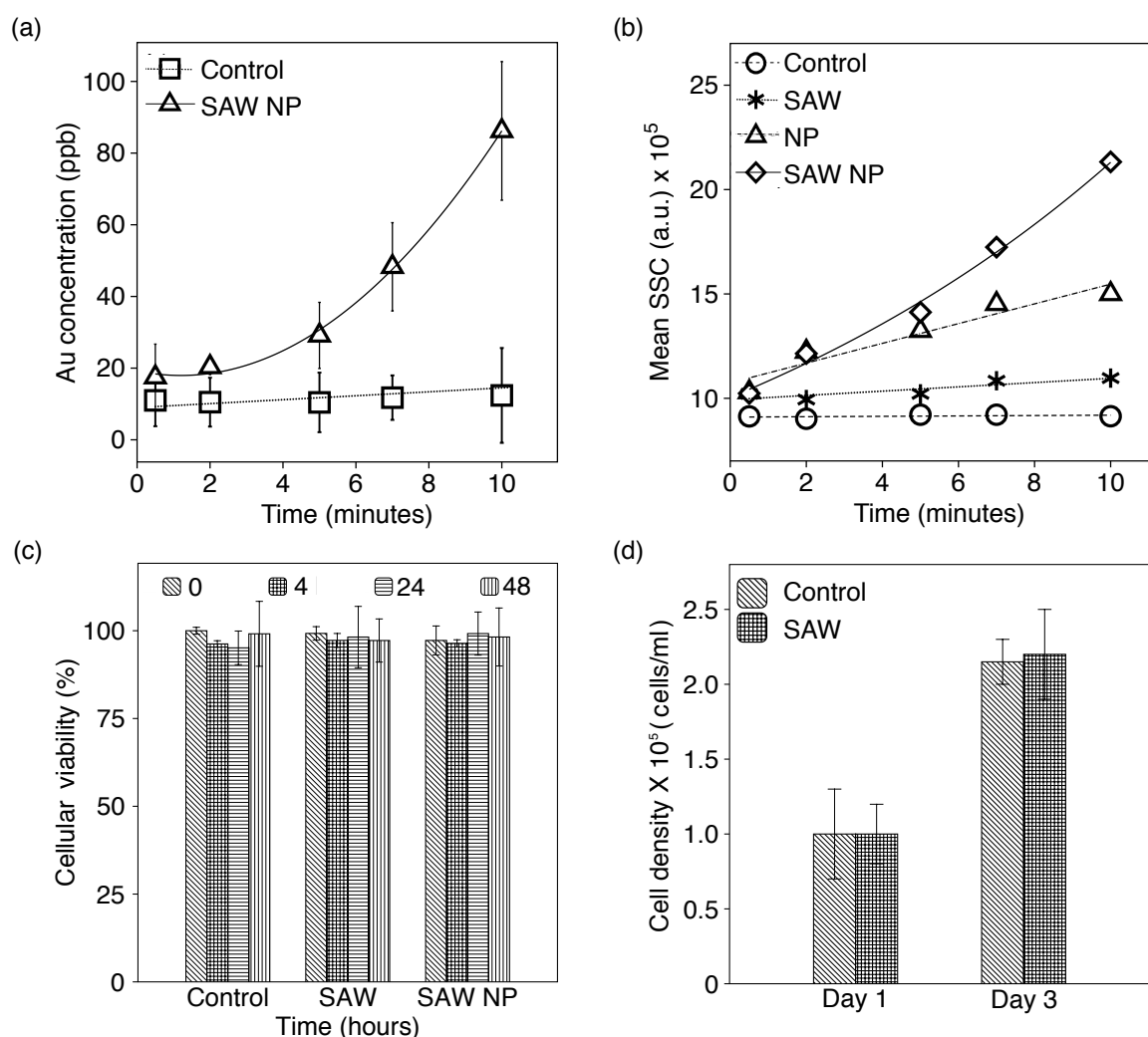


Figure S1: (a) Concentration of Au nanoparticles internalised in HeLa cells at different time points, quantified using ICP-MS. (b) Flow cytometry side scatter, showing an increase in the Au nanoparticles internalised within the HeLa cells in the presence of the acoustic radiation (SAW NP), as a function of time; also shown are the cases where the cells were only exposed to the SAW for the same duration, but in the absence of the Au nanoparticles (SAW), and, in the presence of the Au nanoparticles but in the absence of SAW irradiation (NP), in which case the nanoparticles are passively internalised within the cell. (c) Results from the MTT assay showing short- and long-term viability of the HeLa cells, immediately (0 min), 4 h, 24 h and 48 h after exposure to the SAW irradiation over a 10 min duration, both in the absence (SAW) and in the presence (SAW NP) of the Au nanoparticles. (d) HeLa cells exposed to the SAW, showing an increase in the number of cells after re-seeding post exposure to the SAW. The data are represented in terms of a mean value ($n = 3$) \pm the standard error. The asterisks *** indicate a statistically significant difference with $p < 0.001$.

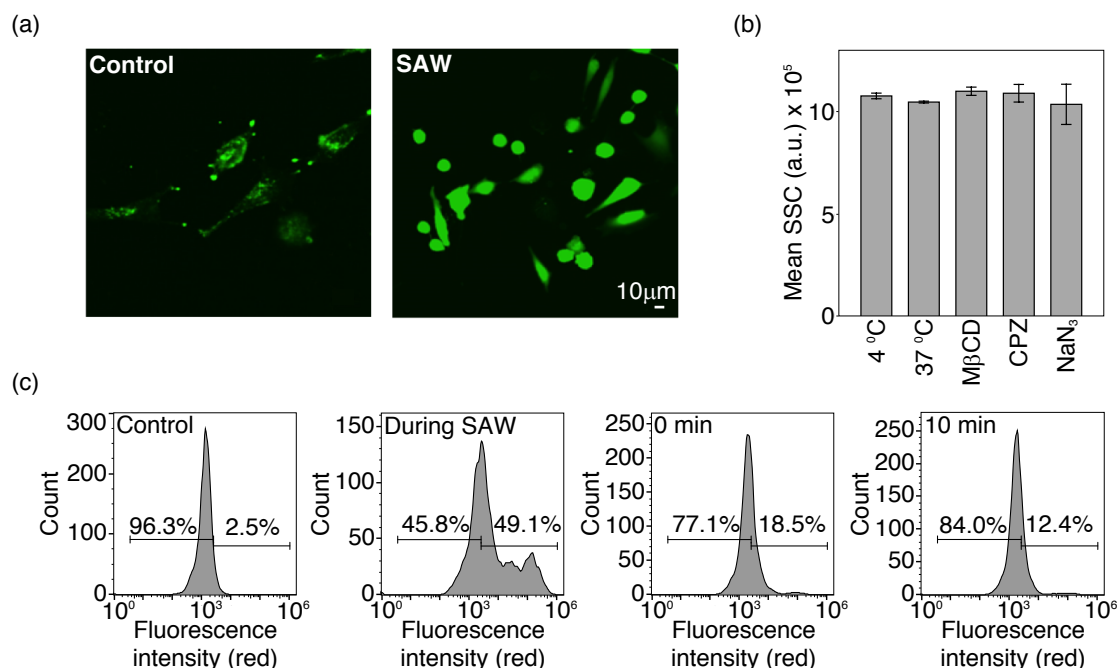


Figure S2: (a) Fluorescence microscopy images showing the distribution of calcein, a membrane-impermeant fluorophore, in HeLa cells in the presence (SAW) and absence (control) of the SAW irradiation. (b) Results of the flow cytometry mean side scatter (SSC), showing negligible reduction in the uptake of AuNPs upon SAW irradiation over a duration of 5 mins at 4 °C and in the presence of various endocytosis inhibitors (methyl- β -cyclodextrin (M β CD), chlorpromazine (CPZ) and sodium azide (NaN₃)) compared to that when the SAW irradiation experiment is carried out under ambient conditions (37 °C). The error bars denote mean values ($n = 4$) \pm the standard error. (c) Results from flow cytometry experiments showing an increase in the propidium iodide (PI) uptake into HeLa cells when they are exposed to the SAW irradiation; this is in contrast to the control experiments in the absence of the SAW, where no PI is internalised by the cells, at least over a comparable 10 min duration for the SAW exposure. It can however be seen that removal of the SAW excitation immediately (0 min) arrests the PI intake. No further PI internalisation is observed, even after 10 mins following relaxation of the SAW irradiation.

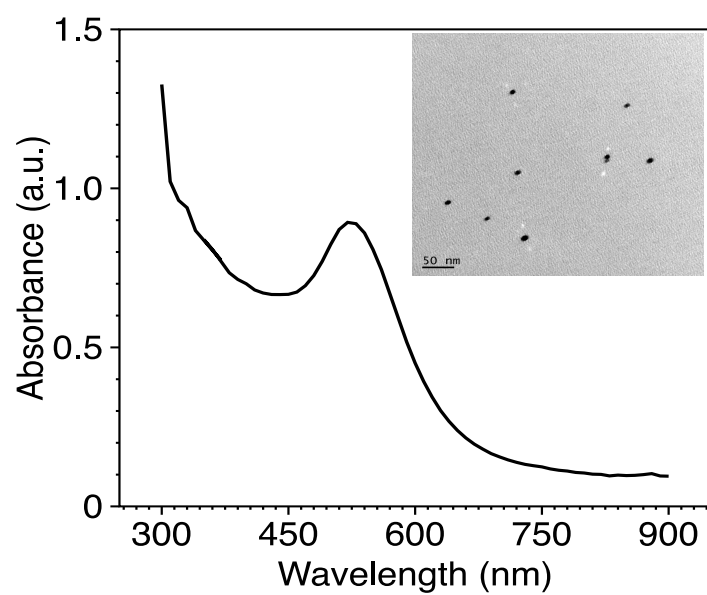


Figure S3: Characteristic UV peak at 519 nm, typical of Au NPs. The inset shows a representative TEM image of the Au NPs, indicating their shape and dimension (10 ± 5 nm), and that they are free from aggregation.

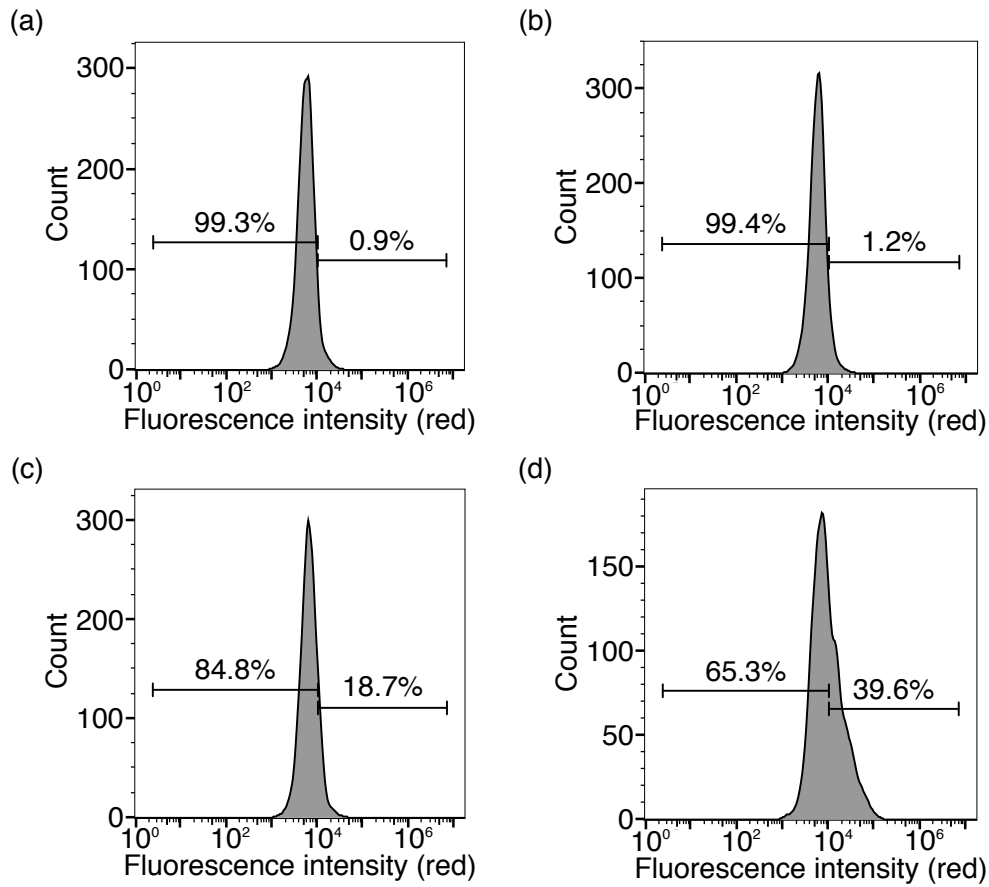


Figure S4: Internalisation efficiency of Cy3-labelled GAPDH siRNA in HeLa cells from measurements of the fluorescence intensity using flow cytometry for unexposed cells (a) without siRNA, (b) with naked siRNA in the absence of the transfection agent Lipofectamine®, and, (c) in the presence of both siRNA and Lipofectamine®, and, (d) cells that were exposed to the SAW irradiation for 5 mins in the presence of Lipofectamine® and siRNA.