Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Figures

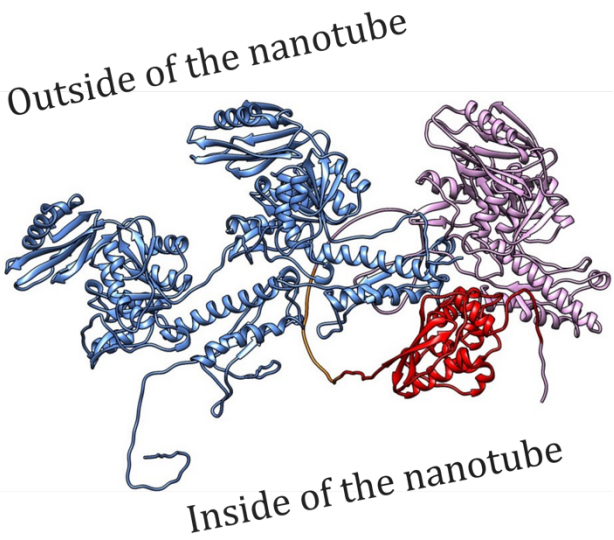


Figure S1. Representation of gp053 and 053SNAP assembly in the nanotube structure. Two native gp053 are shown in blue, chimeric 053SNAP is shown in magenta (gp053) and red (SNAP-tag). Inside and outside surfaces of the tube are indicated. Sheath protein structural modelling was carried out using AlphaFold 2. The models were visualised in Chimera 1.13.1.

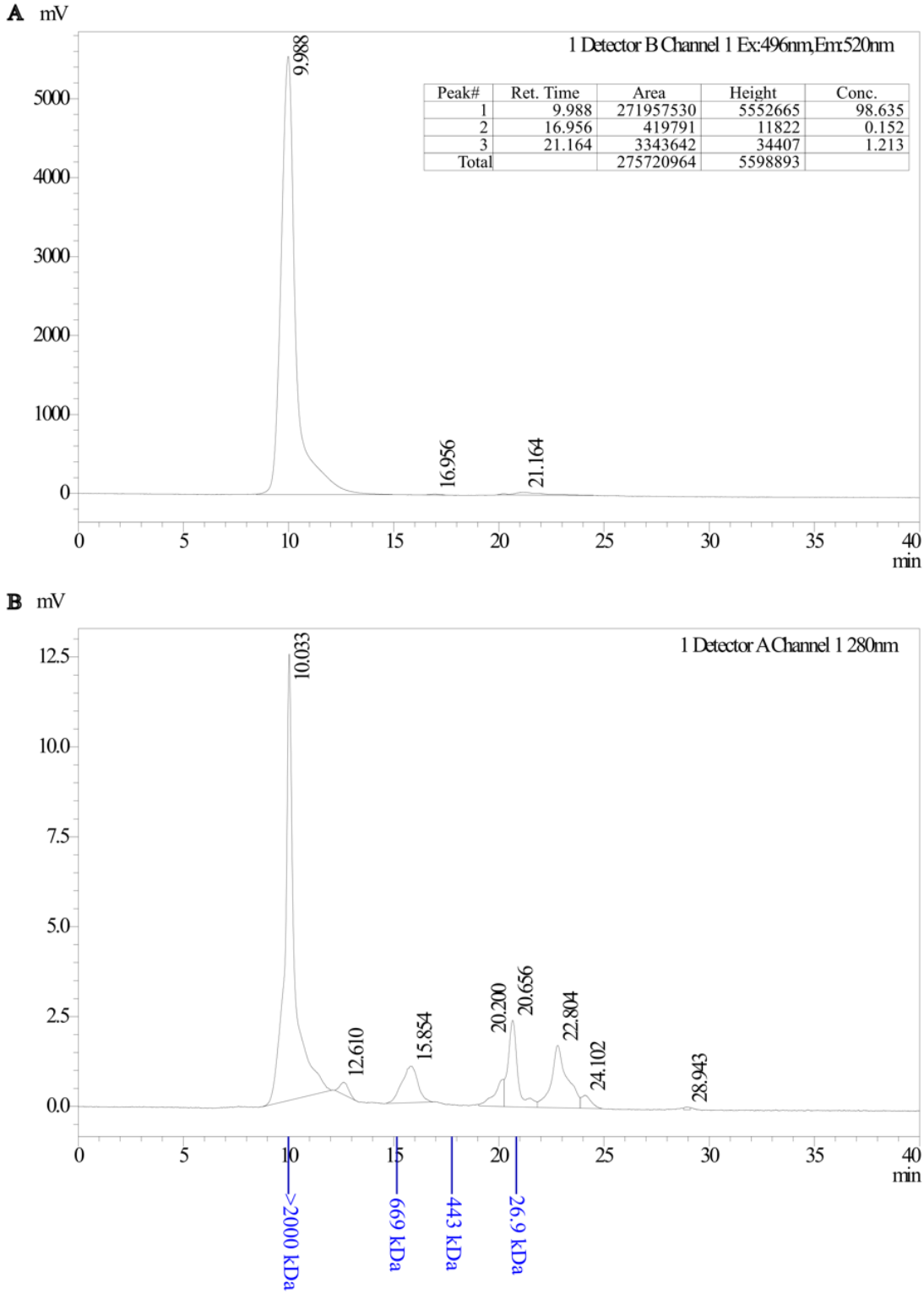


Figure S2. SEC-HPLC analysis of cell free extract of *E. coli* BL21(DE3)-053SNAP labelled with Alexa Fluor 488. The peak with the retention time of ~10 min of the fluorescence (A) and absorbance (280 nm, B) chromatograms represent the recombinant 053SNAP protein. The retention time of molecular mass standards are indicated in blue.

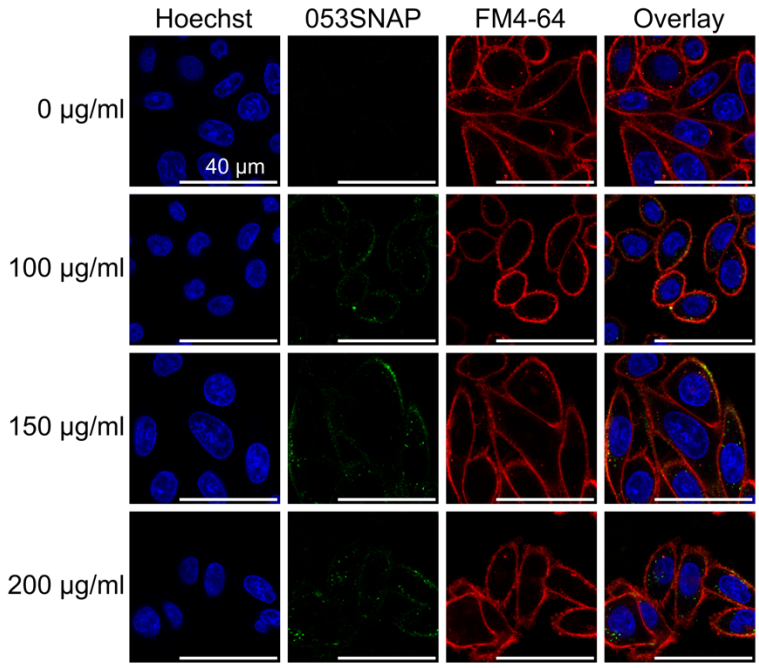


Figure S3. The internalisation of 053SNAP into SW480 cell line. SW480 cells were incubated for 4 hours with 100-200 µg/ml of non-PEGylated nanotubes composed of 053SNAP labelled with Alexa Fluor 488. Blue – Hoechst33342, red – membrane stain FM4-64, green – 053SNAP. Scale bar 40 µm.

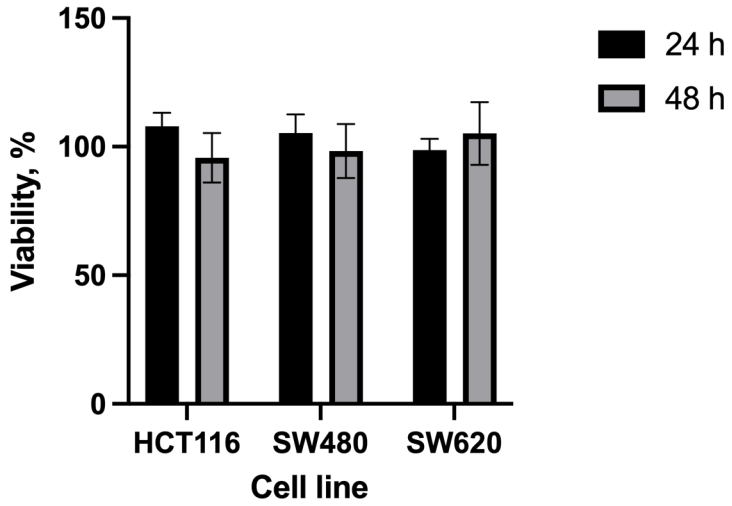


Figure S4. The viability of HCT116, SW480, and SW620 cells. The cells were incubated with 053SNAP (50 µg/ml) for 24 h and 48 h. Data are represented as means ± SD; n=3. None of the differences were statistically significant.

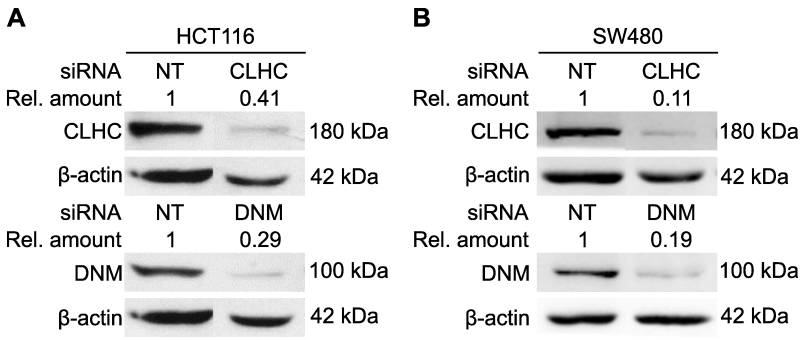


Figure S5. The efficiency of siRNA silencing. The efficiency of silencing of clathrin heavy chain (CLHC) and dynamin-2 (DNM) was defined by protein level in HCT116 (A) and SW480 (B) cells. Data are represented as relative amount of protein using β-actin as loading control.

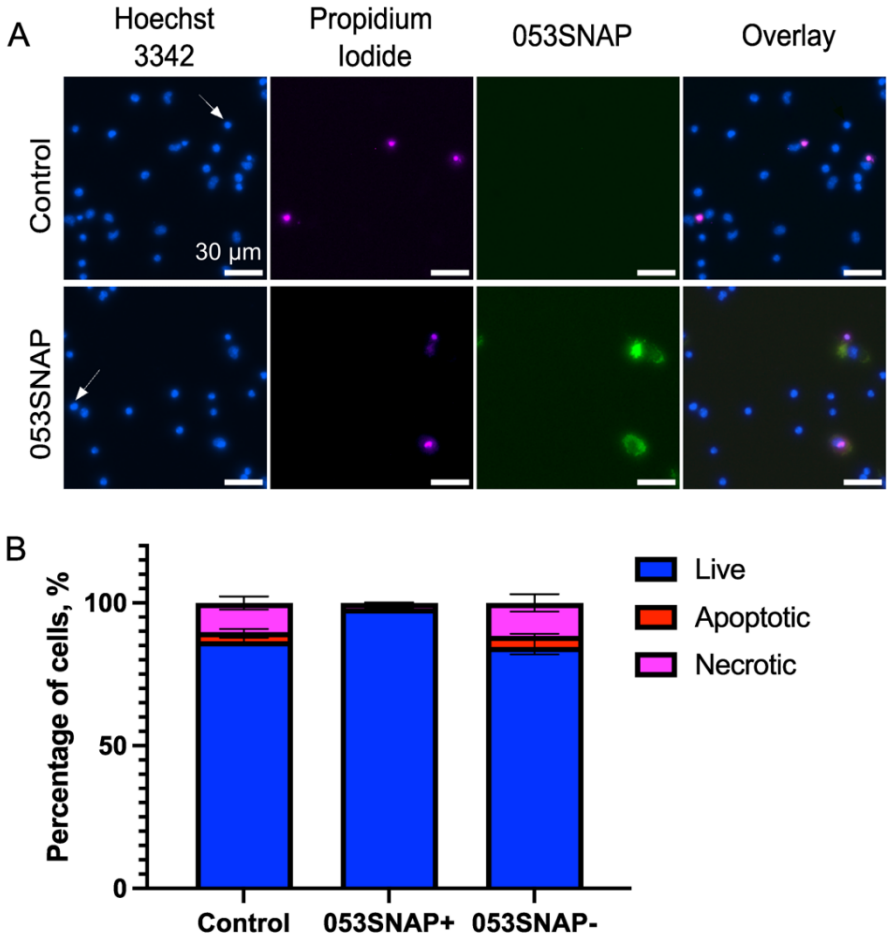
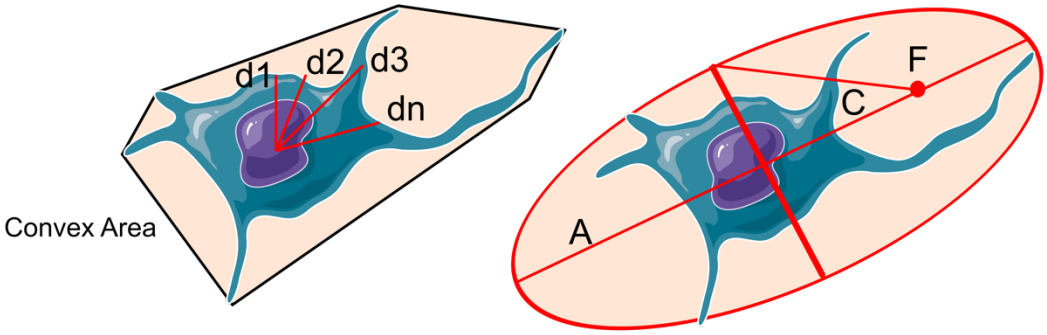


Figure S6. The evaluation of peritoneal macrophage viability after internalizing 053SNAP. (A) Live peritoneal macrophages after incubation with 053SNAP (green) stained with Hoechst33342 (blue) and propidium iodide (magenta), arrowheads shows apoptotic cells; scale bar 30 μm. (B) The percentage of live cells (negative for propidium iodide, negative for apoptotic nuclei), apoptotic cells (negative for propidium iodide, positive for apoptotic nuclei) and necrotic cells (positive for propidium iodide, negative for apoptotic nuclei) that have or have not internalized 053SNAP nanotubes (053SNAP+ and 053SNAP–, respectively) compared to control samples that were not treated with nanotubes. Data are represented as means ± s.e.m. None of the differences were statistically significant.



Circularity (Compactness) = μ^2_d/Area

Eccentricity = C/A

Solidity = Area/Convex Area

Figure S7. Schematic representation of peritoneal macrophage morphological analysis performed on segmented cell masks. d_n- distance between the centre and the edge of the cell; μ^2_d- mean squared distance between the centre and the edge of the cell; A- semi-major axis of an ellipse; F- focus of an ellipse; C- half of the distance between the foci of an ellipse. Cell drawing from https://smart.servier.com/.