# Specificity of carbon nanotube accumulation and distribution in cancer cells revealed by K-means clustering and principal component analysis of Raman spectra†

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**Abstract**

Single-walled carbon nanotubes (SWCNTs) show great potential for their application as cancer therapeutic nanodrugs, but the efficiency and mechanism of their accumulation in the cell, the modulation of cell activity, and the strong dependence of the results on the type of capping molecule still hinder the transfer of SWCNTs to the clinic. In the present study, we determined the mechanism and sequence of accumulation, distribution and type discrimination of SWCNTs in glioma cells by applying K-means clustering and principal component analysis (PCA) of Raman spectra of cells exposed to SWCNTs capped with either DNA or oligonucleotides (ON). Based on the specific biochemical information uncovered by PCA and further applied to K-means, we show that the accumulation of SWCNT–DNA occurs in two phases. The first phase involves the transport of SWCNT–DNA through vesicles and its redistribution in the cytoplasm, which is reflected in two SWCNT-related clusters. The second phase begins after 18 hours of interaction between cells and SWCNT–DNA. PCA shows the appearance of two SWCNT-associated PC loadings, reflected by the addition of a new cluster of SWCNTs with a narrowed and shifted G-peak in the spectra. It is caused by the loss of DNA capping and clumping of SWCNTs and triggered by the acidic conditions in autolysosomes resulting from the fusion of transport vesicles with lysosomes. SWCNTs penetrate all cellular compartments after 42–66 hours and lead to cell death. The clumped SWCNTs are released to the outside. In contrast, SWCNT–ON is hardly accumulated in glioma cells and after 72 hours of exposure to SWCNT–ON, the accumulation of SWCNTs corresponds to the first stage without reaching the second. PCA made it possible to separate the characteristics of cellular components against the high-intensity Raman signal from nanotubes and, thus, to propose the mechanism of accumulation and metabolism of nanomaterials in living cells without the use of additional research approaches. Our results elucidate the time dependence of the accumulation of SWCNTs on the capping molecule. We expect that our results can make an important contribution to the use of these nanomaterials in the clinic.