

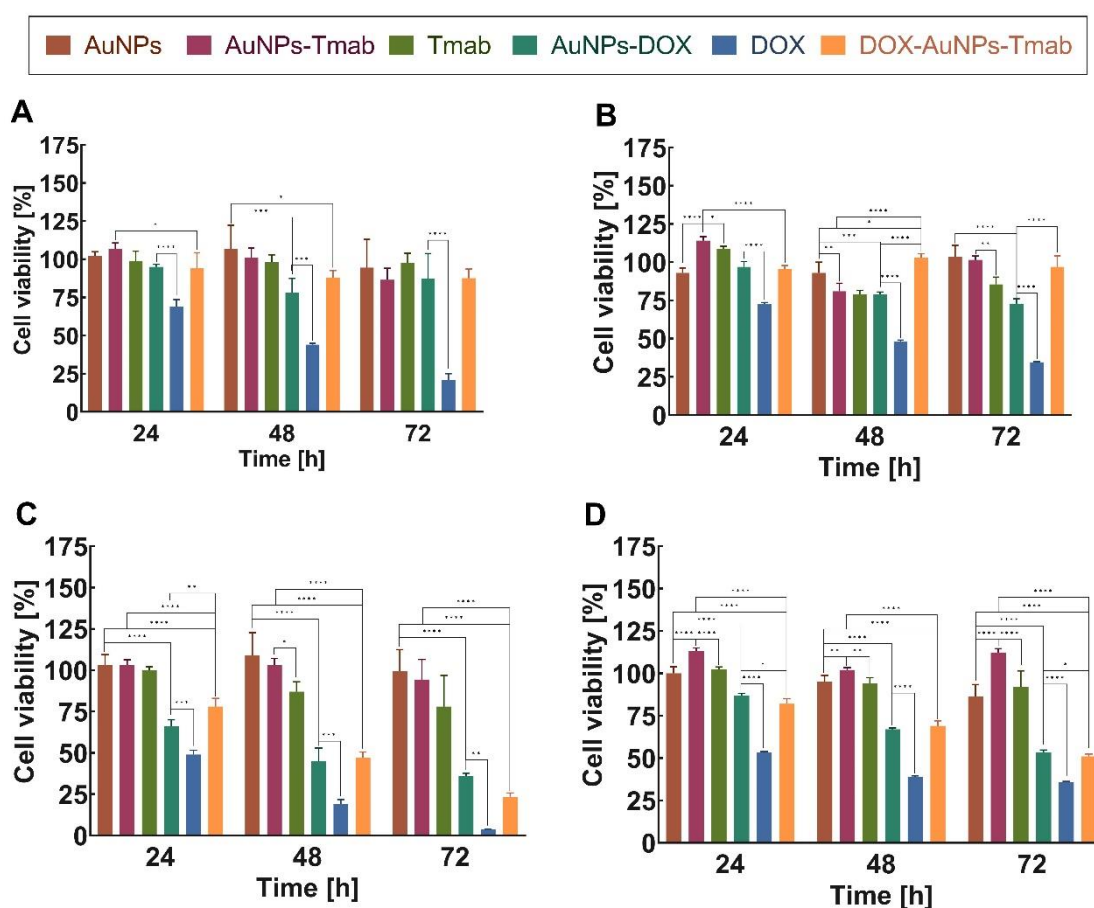
# Doxorubicin- and Trastuzumab-Modified Gold Nanoparticles as Potential Multimodal Agents for Targeted Therapy of HER2+ Cancers

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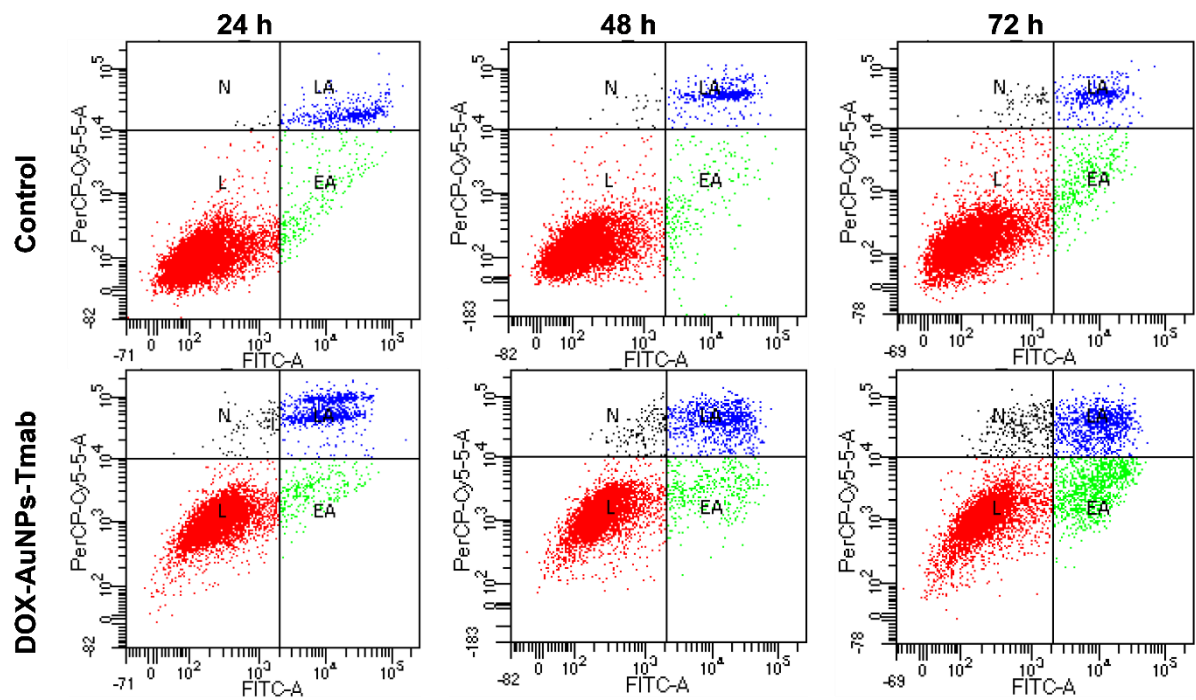
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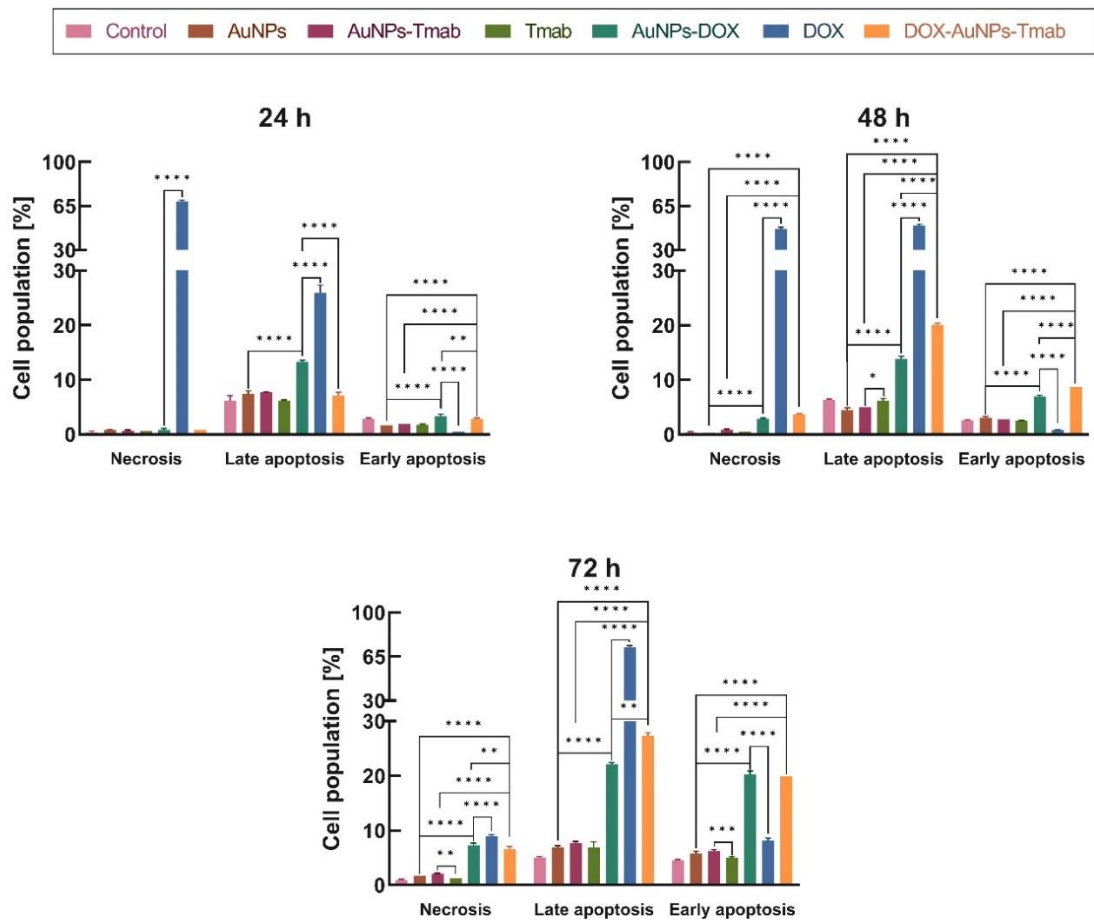
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**Figure S1.** Metabolic activity of SKOV-3 (A, C) and MDA-MB-231 (B, D) cells after treatment with AuNPs, AuNPs-Tmab, Tmab, AuNPs-DOX, DOX, DOX-AuNPs-Tmab. DOX concentrations are 2 µg/mL (A, B) and 15 µg/mL (C, D). Cells were incubated for 24 h, 48 h and 72 h and then the MTS assay was performed. Untreated cells were used as control (100% viability). Statistics were performed using one-way ANOVA test comparing AuNPs together with nanoparticles with doxorubicin and/or trastuzumab attached, and AuNPs-Tmab was compared with Tmab/DOX-AuNPs-Tmab and AuNPs-DOX vs. DOX/DOX-AuNPs-Tmab. Summarized data represented results from four replicates (n = 4; mean ± SD) and were considered significant if  $p \leq 0.01$  (\*\*),  $p \leq 0.001$  (\*\*\*),  $p \leq 0.0001$  (\*\*\*\*).

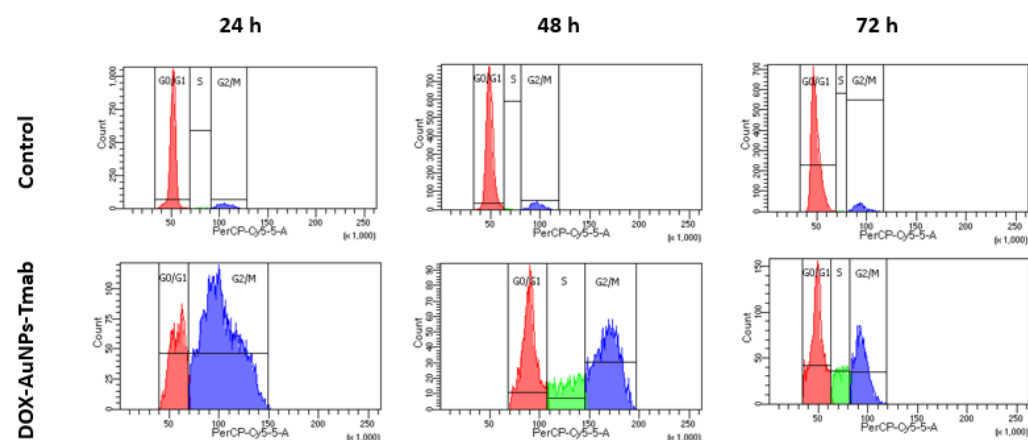


**Figure S2.** Representative graphs of apoptosis analysis results for the control group and for cells treated with DOX-AuNPs-Tmab after 24 h, 48 h and 72 h. DOX concentration is 7  $\mu$ g/mL.

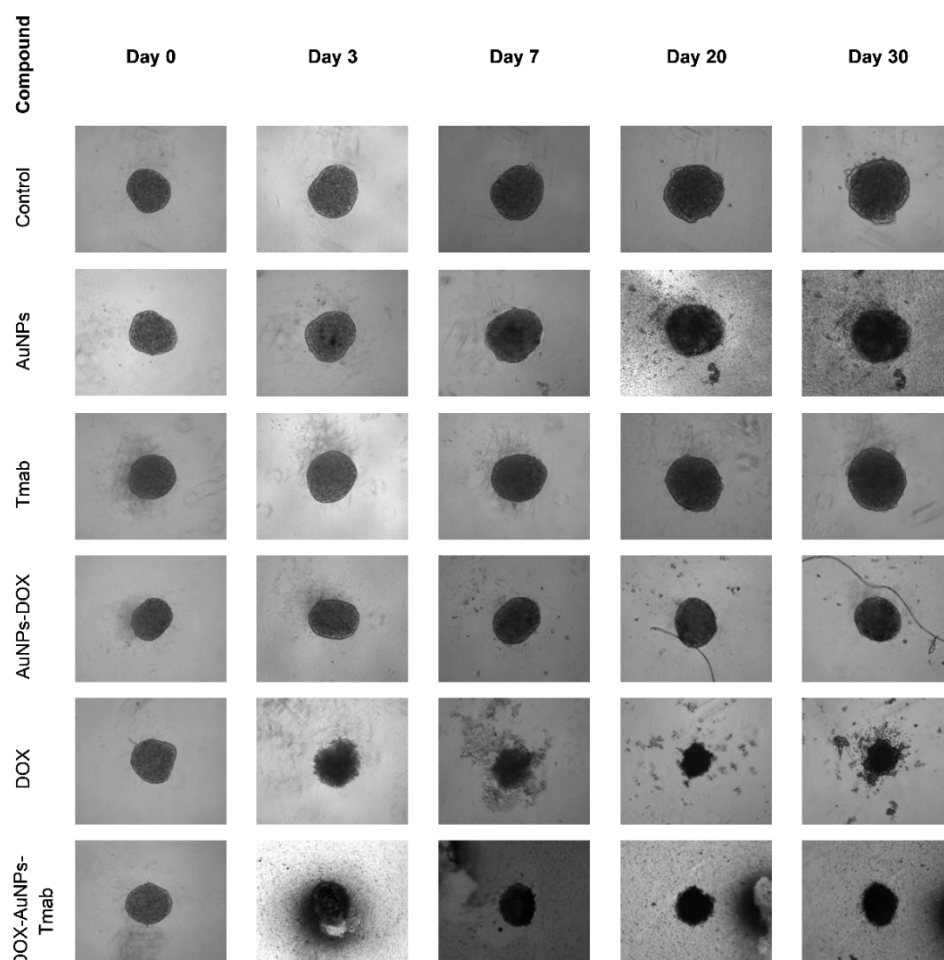


**Figure S3.** Distribution of SKOV-3 cell populations (necrosis, late and early apoptosis) treated with AuNPs, AuNPs-Tmab, Tmab, AuNPs-DOX, DOX, and DOX-AuNPs-Tmab after 24 h, 48 h and 72 h.

Data are presented from four replicates ( $n = 4$ , mean  $\pm$  SD). DOX concentration is 15  $\mu\text{g/mL}$ . Untreated cells were used as control, while statistics (one-way ANOVA test) were performed similarly as in the case of cytotoxicity studies with the use of MTS assay (Figure S1). Statistical significance was considered significant if  $p \leq 0.05$  (\*),  $p \leq 0.01$  (\*\*),  $p \leq 0.001$  (\*\*\*),  $p \leq 0.0001$  (\*\*\*\*).



**Figure S4.** Representative graphs of cell cycle analysis results for the control group and DOX-AuNPs-Tmab cell treatment after 24 h, 48 h and 72 h. DOX concentration is 7  $\mu\text{g/mL}$ .



**Figure S5.** Microscopic images showing the morphology of control and compound-treated SKOV-3 spheroids on several selected measurement days.