Differential toxicity of gold-doxorubicin in cancer cells vs. cardiomyocytes as measured by real-time growth assays and fluorescence lifetime imaging microscopy (FLIM)

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Supplementary Data



**Figure S1.** Schematic of nanoparticle constructs. Au-tiopronin is covered with approximately 200 ligands per particle. Binding to doxorubicin is via the primary amine of the molecule, and approximately 1/10 of the tiopronin ligands are conjugated to Dox during the reaction.



**Figure S2**. Phase-contrast images of cultured B16 Cells on a 96 well plate (a) without analysis, (b) with a customized Discrete Object Analysis and (c) with a customized Field Analysis from SoftMax Pro software. The white areas indicate the cell regions that were delineated after analysis. Note the improved detection Panel (c) compared with Panel (b). Dead cells (black) were omitted from the count.



**Figure S3**. Real-time plot of B16 cell confluence, under standard growth conditions, measured by Field Analysis (a) or by Discrete Object Analysis (b). The points are experimental measurements and the curves are fits to a sigmoidal function. Each data point is the mean  $\pm$  standard error of the mean (SEM) of six replicates and has been normalized to the highest mean value and to zero.



**Figure S4**. Dose-response effect of Dox and Au-Dox on B16 cell viability determined by SRB assay vs. phase-contrast microscopy. The curves are fits to the Hill equation. Each data point is the mean of six replicates  $\pm$  the standard error of the mean (SEM) and has been normalized to the highest and lowest mean value.



**Figure S5**. Growth curves of cardiomyocytes after 40 min exposure to Dox or Au-Dox with subsequent washing.

	τ <sub>1</sub> (B16)	τ <sub>2</sub> (B16)	τ <sub>1</sub> (CM)	τ <sub>2</sub> (CM)
Au-Dox 1 hr				
cytoplasm	$3.6 \pm 0.3$	1.2±0.1	4.46±0.04	1.2±0.1
Au-Dox 4 hr				
cytoplasm	$3.91 \pm 0.06$	1.0±0.3	4.73±0.05	1.38±0.04
Au-Dox 1 hr				
nucleus	$2.5 \pm 0.2$	$1.00\pm0.03$	2.4±0.2	1.11±0.05
Au-Dox 4 hr				
nucleus	3.9±0.6	1.19±0.06	2.81±0.06	1.08±0.06
Free Dox				
cytoplasm	2.40±0.05	0	2.14±0.05	0.000
Free Dox nucleus	1.32±0.05	0	1.44±0.05	0.000

**Table S1.** Fits to decay curves for averages of 7-10 spots in the nucleus and cytoplasm of B16 cells and cardiomyocytes (CM). All fits showed  $\chi^2 \sim 1$  and random distributions of residuals. The curve for free Dox did not change between 1 hr and 4 hr.