

Supporting Information

B/N-doped carbon nano-onions as nanocarriers for targeted breast cancer therapy

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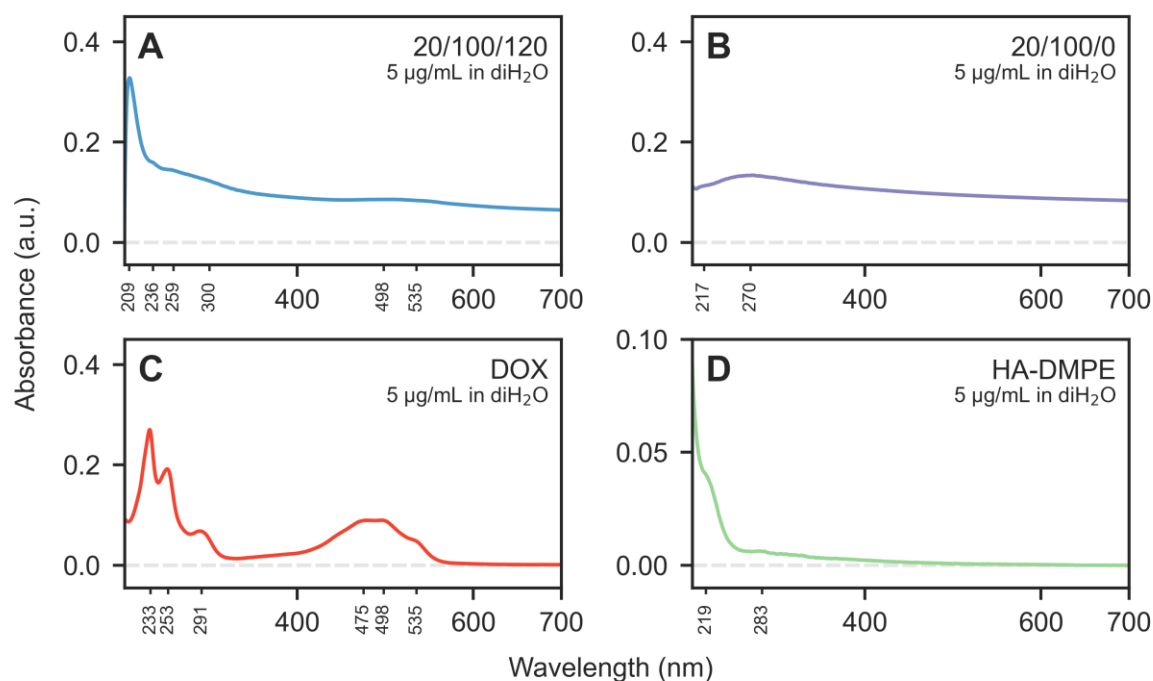


Fig. S1 UV-vis absorption spectra of 5 µg/mL deionised water dispersions of (A) DOX-loaded 20/100/120, (B) DOX-free 20/100/0 wt% HA-DMPE/BN-CNO/DOX, (C) DOX, and (D) HA-DMPE

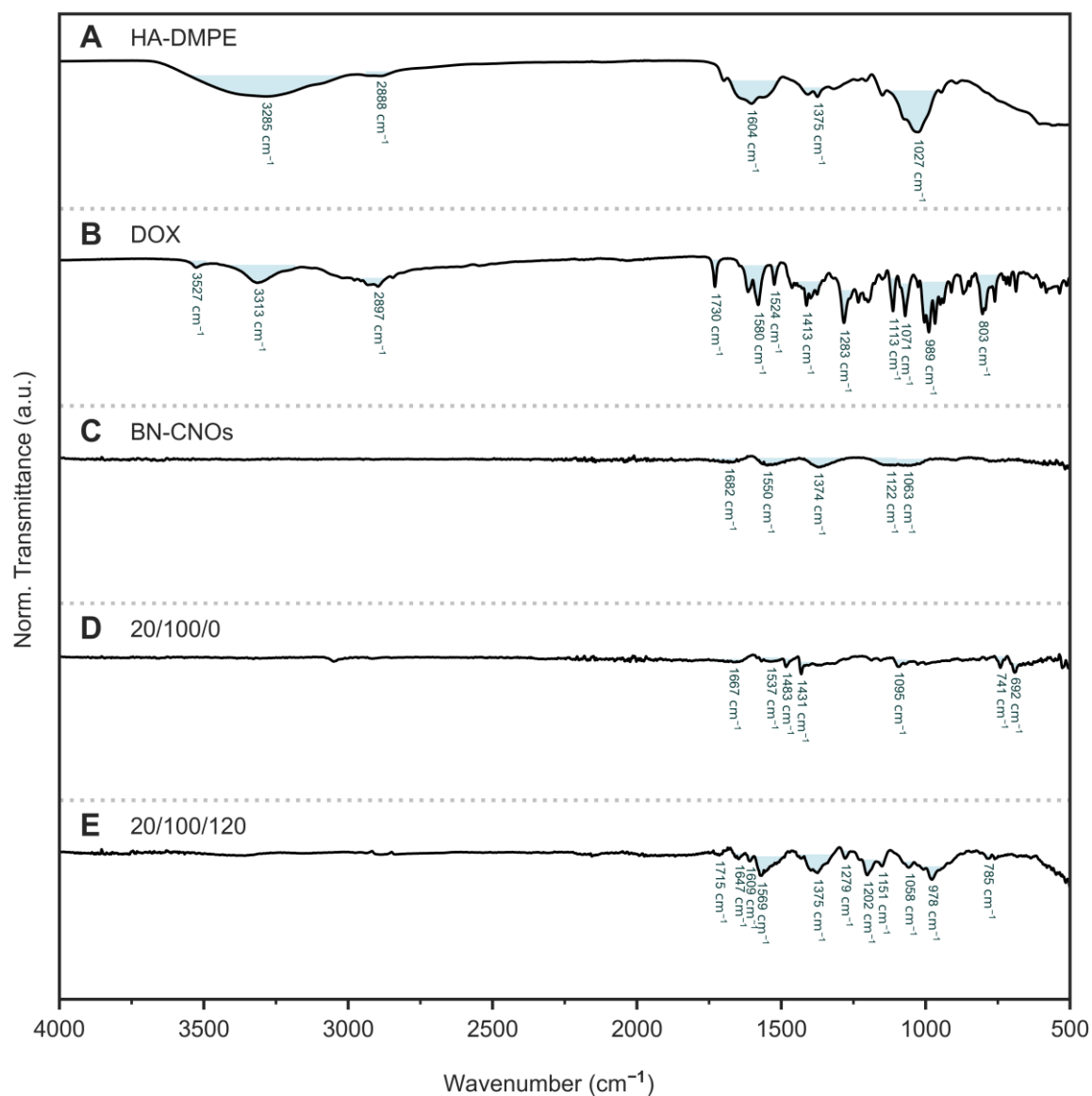


Fig. S2 ATR-FTIR spectra of (A) HA-DMPE, (B) DOX, (C) BN-CNOs, (D) the DOX-free 20/100/0 and (E) the DOX-loaded 20/100/120 wt% HA-DMPE/BN-CNO/DOX nanocarrier.

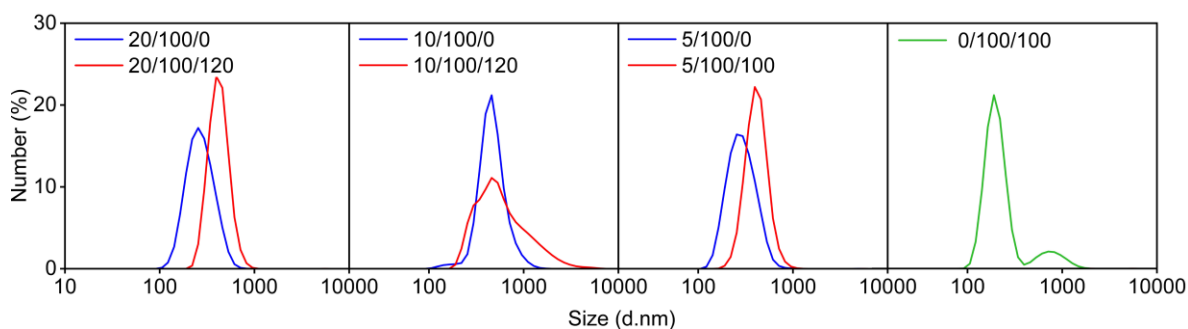


Fig. S3 DLS number (%) distributions (5 $\mu\text{g/mL}$ in deionized water) of the DOX-free HA-DMPE-loaded 20/100/0, 10/100/0 and 5/100/0 wt% formulations (red), the DOX-loaded and HA-DMPE-loaded 20/100/120, 10/100/120, 5/100/100 wt% formulations (blue) and the DOX-loaded HA-DMPE-free 0/100/100 wt% formulation (green) of the HA-DMPE/BN-CNO/DOX nanocarrier.

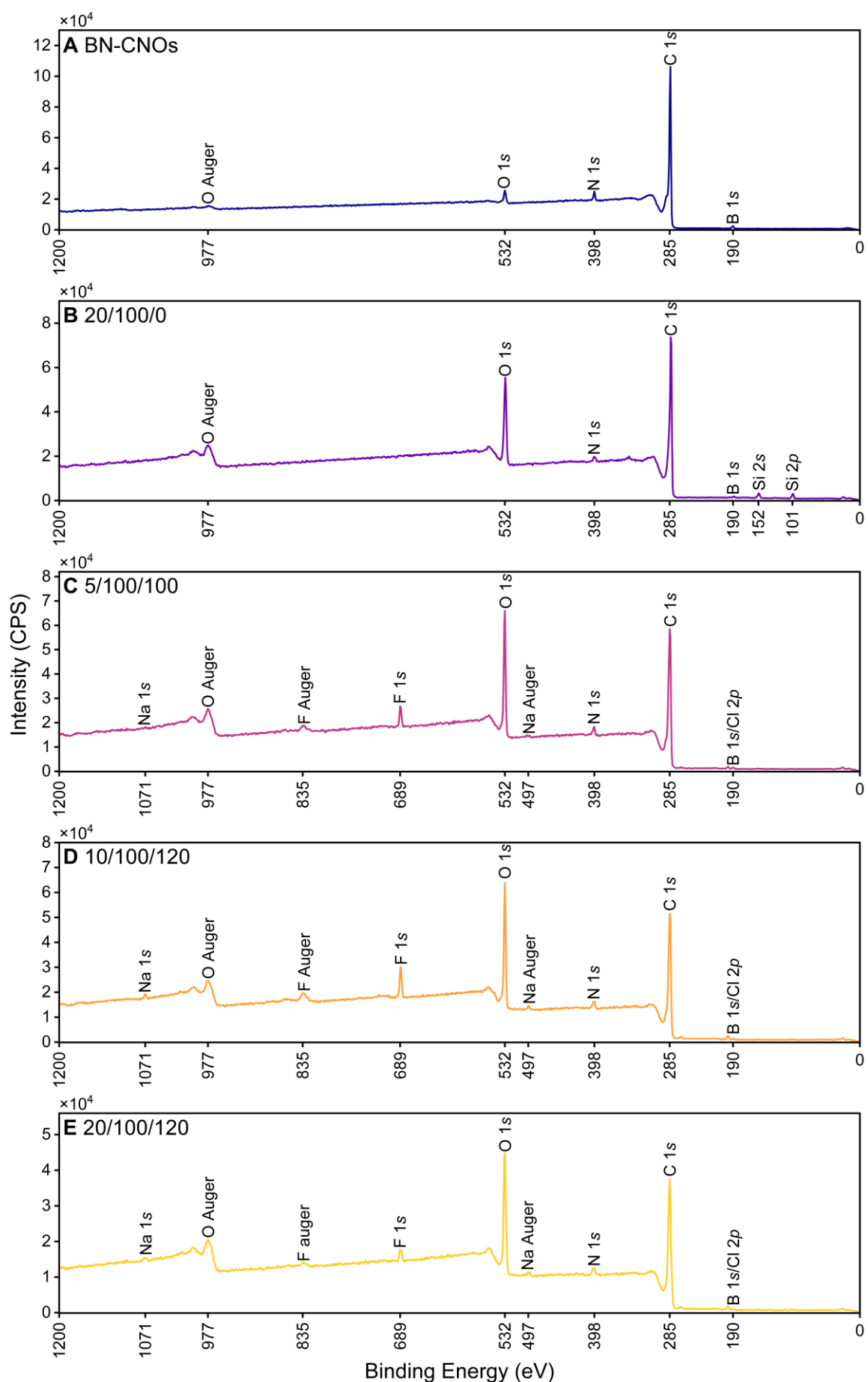


Fig. S4 XPS survey spectra of (A) BN-CNOs and (B) 20/100/0, (C) 5/100/100, (D) 10/100/120, and (E) 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulations

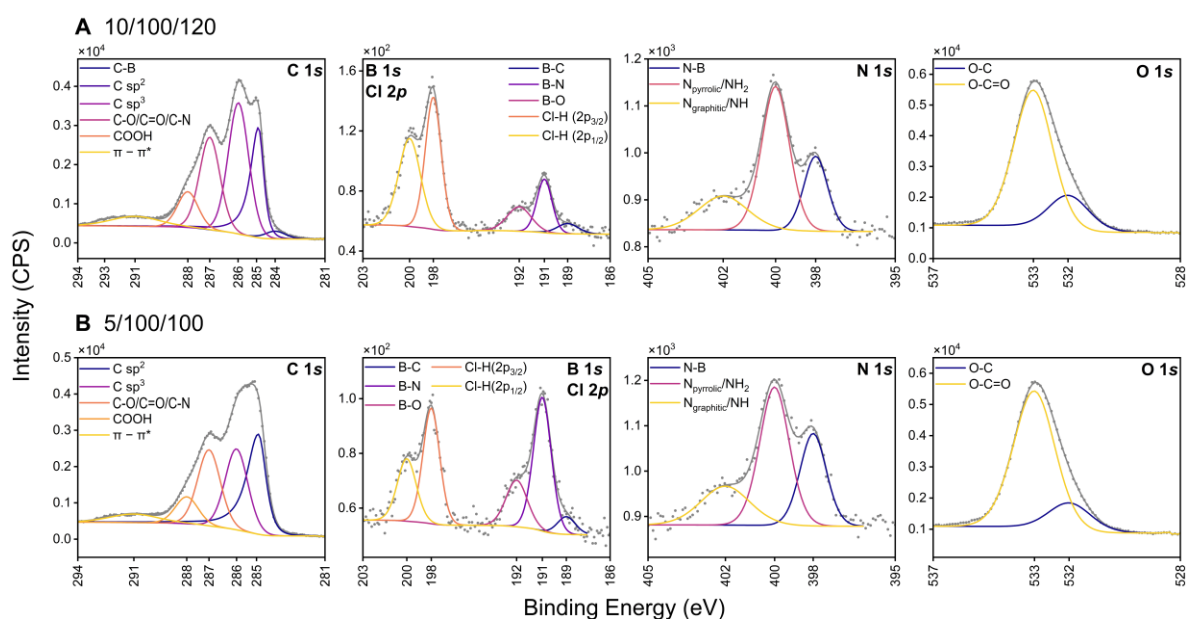


Fig. S5 High-resolution core level XPS spectra of (A) 10/100/120 (top row; C 1s, B 1s, N 1s, and O 1s); (B) 5/100/100 wt% HA-DMPE/BN-CNO/DOX formulation (bottom row; C 1s, B 1s, N 1s, and O 1s). Spectra include experimental data (grey, scatter) and the fitted peak deconvolution envelope (grey, line).

Table S1 XPS elemental composition in atomic percentage (at.%) of BN-CNOs, 20/100/0, 5/100/100, 10/100/120, and 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulations.

	C (at. %)	O (at. %)	N (at. %)	B (at. %)	Cl (at. %)
BN-CNOs	85.7	6.5	4.1	3.7	-
20/100/0	80.3	17.1	1.7	0.9	-
5/100/100	77.0	18.8	2.8	1.2	0.2
10/100/120	76.5	19.6	2.5	0.9	0.5
20/100/120	74.5	21.6	2.5	0.9	0.5

Table S2 Position (eV), area (relative %), and chemical state of deconvoluted C 1s peaks obtained from high-resolution XPS analysis of BN-CNOs, 20/100/0, 5/100/100, 10/100/120, and 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulations.

	C charging	C-B	C sp ²	C sp ³	C-O/ C=O/C=N	COOH	π-π*
BN-CNOs	-	-	284.5 (80.3 %)	286.5 (6.3 %)	288.0 (2.6 %)	289.0 (1.6 %)	291.0 (9.2 %)
20/100/0	-	-	284.8 (32.2 %)	285.3 (39.6 %)	287.2 (16.8 %)	288.8 (8.3 %)	290.7 (3.1 %)
5/100/100	-	-	284.5 (32.2 %)	285.7 (25.9 %)	287.1 (24.5 %)	288.3 (9.9 %)	291.1 (7.5 %)
10/100/120	-	283.6 (2.0 %)	284.5 (23.1 %)	285.6 (34.5 %)	287.1 (24.3 %)	288.2 (9.4 %)	291.0 (6.7 %)
20/100/120	282.9 (11.7 %)	284.0 (5.4 %)	284.5 (14.3 %)	285.6 (35.9 %)	287.1 (22.1 %)	288.4 (7.1 %)	291.2 (3.5 %)

Table S3 Position (eV), area (relative %), and chemical state of deconvoluted B 1s and Cl 2p peaks obtained from high-resolution XPS analysis of BN-CNOs, 20/100/0, 5/100/100, 10/100/120, and 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulations

	B-B	B-C	B-N	B-O	Cl-Na	Cl-H (2p _{3/2})	Cl-H (2p _{1/2})
BN-CNOs	-	189.5 (17.1 %)	190.5 (34.1 %)	191.8 (48.8 %)	-	-	-
20/100/0	-	-	190.6 (82.7 %)	192.6 (17.3 %)	-	-	-
5/100/100	-	189.0 (8.7 %)	190.7 (64.5 %)	192.4 (26.8 %)	-	198.3 (62.3 %)	199.9 (37.7 %)
10/100/120	-	188.9 (13.6 %)	190.5 (50.1 %)	192.3 (36.3 %)	-	198.2 (53.4 %)	199.8 (46.6 %)
20/100/120	187.6 (2.5 %)	189.1 (12.9 %)	190.6 (51.2 %)	192.3 (33.4 %)	196.5 (20.1 %)	198.3 (49.7 %)	199.9 (30.2 %)

Table S4 Position (eV), area (relative %), and chemical state of deconvoluted N 1s and O 1s peaks obtained from high-resolution XPS analysis of BN-CNOs, 20/100/0, 5/100/100, 10/100/120, and 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulations.

	N-B	N _{pyridinic}	N _{pyrrolic} / NH ₂	N _{graphitic} / NH	O=C	O-C	O-C=O
BN-CNOs	398.4 (30.7 %)	398.9 (62.4 %)	400.4 (5.0 %)	401.5 (1.9 %)	-	532.5 (100 %)	-
20/100/0	398.4 (43.9 %)	-	-	400.6 (56.1 %)	530.9 (1.9 %)	532.3 (11.2 %)	533.5 (86.9 %)
5/100/100	398.3 (28.9 %)	-	399.9 (48.6 %)	401.9 (22.5 %)	-	532.1 (18.2 %)	533.3 (81.8 %)
10/100/120	398.2 (24.0 %)	-	399.8 (53.7 %)	401.9 (22.3 %)	-	532.1 (21.6 %)	533.4 (78.4 %)
20/100/120	397.1 (7.6 %)	398.2 (18.1 %)	399.9 (54.5 %)	402.3 (19.8 %)	530.8 (17.9 %)	532.1 (19.6 %)	533.4 (62.5 %)

Analysis of 20/100/0 wt% HA-DMPE/BN-CNO/DOX XPS spectra:

Attaching HA-DMPE to the BN-CNOs increases the O content to 17.1%, diluting the N and B contributions to 1.7% and 0.9% respectively, as seen in the survey spectrum (Fig. S4B) and elemental analysis (Tab. S1) of the DOX-free 20/100/0 wt% HA-DMPE/BN-CNO sample. The 10.6% increase in O can be attributed to the multiple oxygen-containing groups in the HA-DMPE, this serves to increase the solubility of the nanocarrier. The high-resolution core level C 1s spectrum of this 20/100/0 wt% material (Fig. 1B) reveals a large decrease in sp² C (32%), with sp³ C increasing to 40% due to the HA-DMPE, which contains mostly sp³ carbon. The π - π^* contribution was also significantly diluted to 3% due to the lack of delocalised electrons in the polymer. The amount of carbon bound to O/N increased to 25%

due to the HA-DMPE. The B 1s spectrum of HA-DMPE functionalised BN-CNOs shows the boron is split between B-N (83%) and B-O (17%). A 54% increase in the N-graphitic/NH contribution to the N 1s spectrum is noted upon polymer functionalisation, resulting from the primary amine groups in the conjugate polymer. The N-B contribution increased by over 10% to 43%. The additional O containing groups from the HA-DMPE resulted in a more complex O 1s spectrum, with 2%, 11%, and 87% contributions arising from O=C, O-C, and O-C=O groups, all of which are present in the HA-DMPE polymer and contribute to the negative ZP and enhanced water solubility of the HA-DMPE/BN-CNOs.

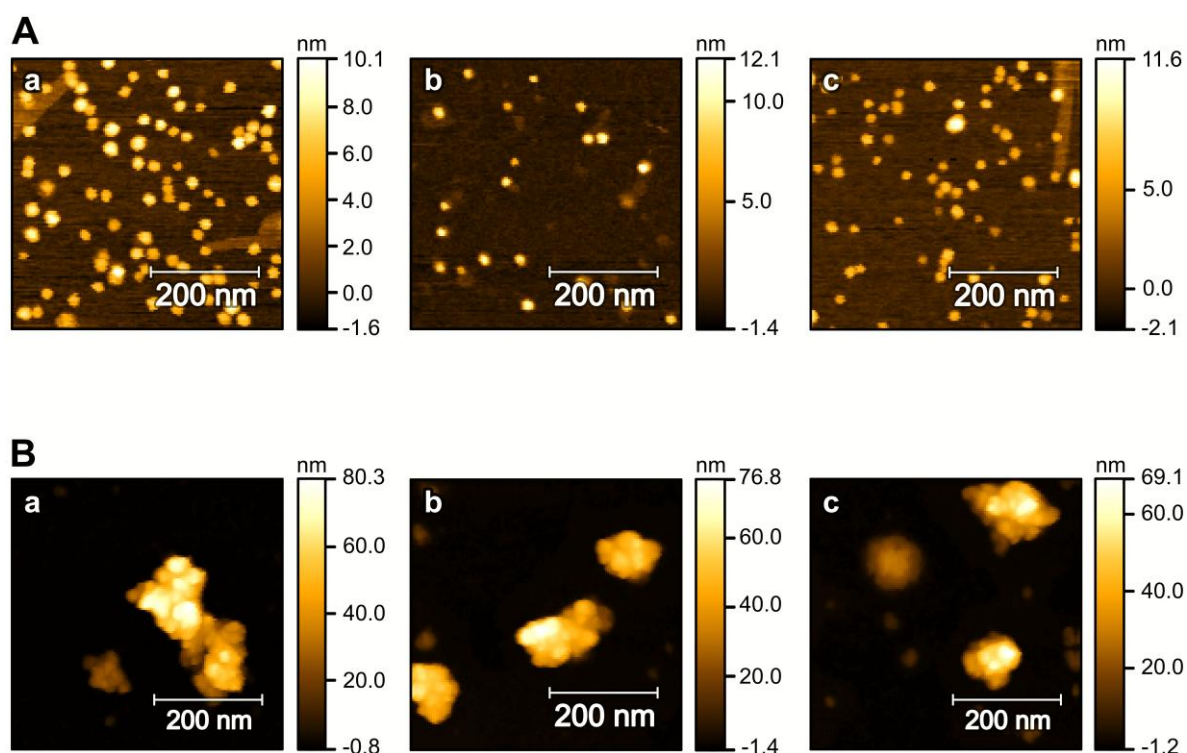


Fig. S6 AFM micrographs of (A) BN-CNOs, and (B) the 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulation. All micrographs represent different $0.5 \times 0.5 \mu\text{m}$ regions of the batch material (scalebars = 200 nm).

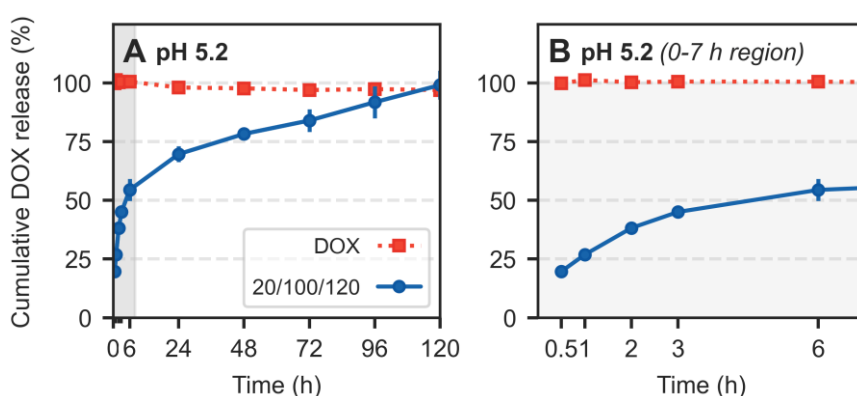


Fig. S7 (A) Extended cumulative DOX release profile up to 120 h for the 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulation in 0.01 M sodium acetate buffer (pH 5.2). The region marked in grey has been zoomed in and presented in (B). DOX is shown as a control.

Table S5 Percentage cumulative DOX release and standard deviations from the triplicate drug release analysis of the HA-DMPE/BN-CNO/DOX nanocarrier formulations with varying HA-DMPE and DOX content (20/100/120, 10/100/120, 5/100/100, and 0/100/100 wt%) in 0.01 M sodium acetate buffer (pH 5.2) and 0.01 M PBS buffer (pH 7.4) at all measured time points up to 72 h. The extended-release studies up to 120 h for the 20/100/120 wt% formulation in pH 5.2 buffer are also shown.

Time (h)	20/100/120 DOX release (% \pm σ)		10/100/120 DOX release (% \pm σ)		5/100/100 DOX release (% \pm σ)		0/100/100 DOX release (% \pm σ)	
	pH 7.4	pH 5.2	pH 7.4	pH 5.2	pH 7.4	pH 5.2	pH 7.4	pH 5.2
0.5	3.2 \pm 0.2	19.7 \pm 1.3	3.8 \pm 1.0	17.0 \pm 1.8	1.7 \pm 0.3	9.7 \pm 3.1	1.5 \pm 0.5	3.3 \pm 1.2
1	3.7 \pm 0.4	26.8 \pm 1.2	4.2 \pm 0.2	17.4 \pm 0.4	2.1 \pm 0.8	15.7 \pm 6.4	2.1 \pm 0.2	3.3 \pm 0.6
2	4.5 \pm 0.3	38.2 \pm 2.1	5.2 \pm 0.1	22.8 \pm 2.7	2.1 \pm 0.7	21.3 \pm 3.1	2.4 \pm 0.5	5.3 \pm 0.6
3	4.8 \pm 0.3	45.1 \pm 0.8	5.5 \pm 0.2	24.2 \pm 2.9	2.5 \pm 0.5	25.7 \pm 5.7	2.9 \pm 0.1	5.3 \pm 0.6
6	4.7 \pm 0.6	54.4 \pm 4.7	6.0 \pm 0.2	27.5 \pm 5.0	3.7 \pm 1.2	31.7 \pm 3.1	3.9 \pm 0.1	6.0 \pm 1.0
24	5.0 \pm 0.3	69.7 \pm 3.4	5.8 \pm 0.4	33.4 \pm 1.4	3.3 \pm 0.6	36.3 \pm 7.2	3.6 \pm 0.5	13.0 \pm 1.1
48	5.7 \pm 0.3	78.3 \pm 2.8	6.6 \pm 0.0	34.3 \pm 1.0	3.9 \pm 0.9	41.7 \pm 6.8	5.0 \pm 0.0	13.0 \pm 1.1
72	6.9 \pm 0.6	84.0 \pm 4.8	7.8 \pm 0.3	37.5 \pm 1.7	4.8 \pm 1.0	46.7 \pm 8.4	5.5 \pm 0.5	14.0 \pm 0.8
96	-	91.8 \pm 6.8	-	-	-	-	-	-
120	-	99.1 \pm 6.2	-	-	-	-	-	-

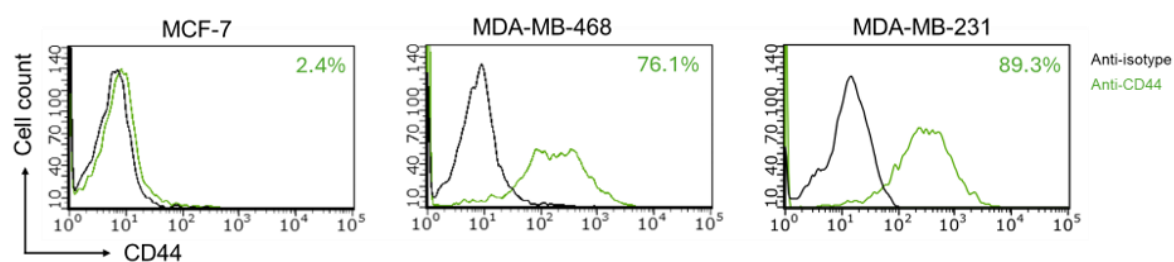


Fig. S8 Flow cytometry results of CD44 receptor expression in MCF-7, MDA-MB-468, and MDA-MB-231 cell lines (% positive cells)

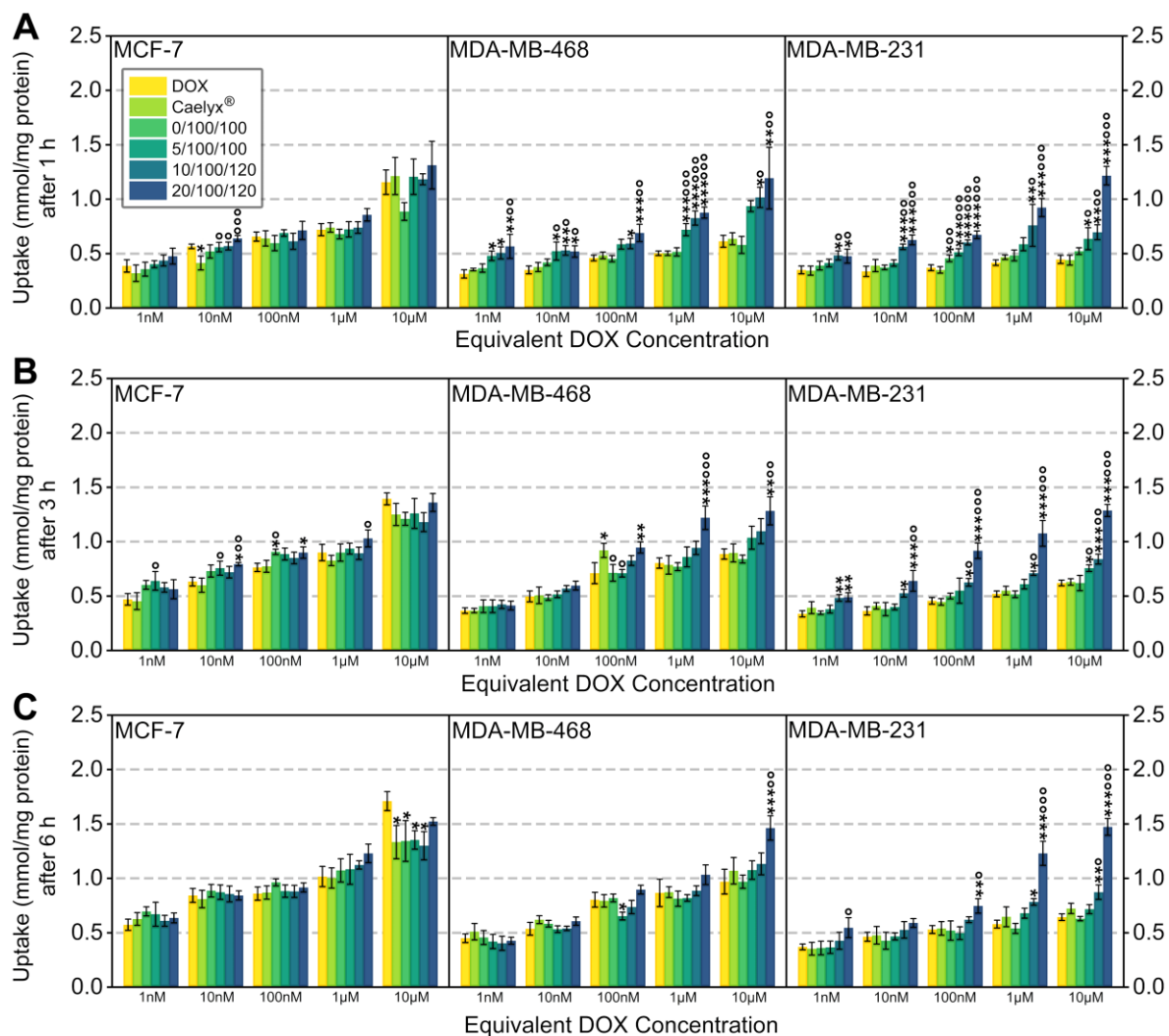


Fig. S9 1 h (A), 3 h (B), and 6 h (C) uptake of DOX, Caelyx®, and various HA-DMPE/BN-CNO/DOX formulations in MCF-7, MDA-MB-468, and MDA-MB-231 cells. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX, and ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$ vs. Caelyx®.

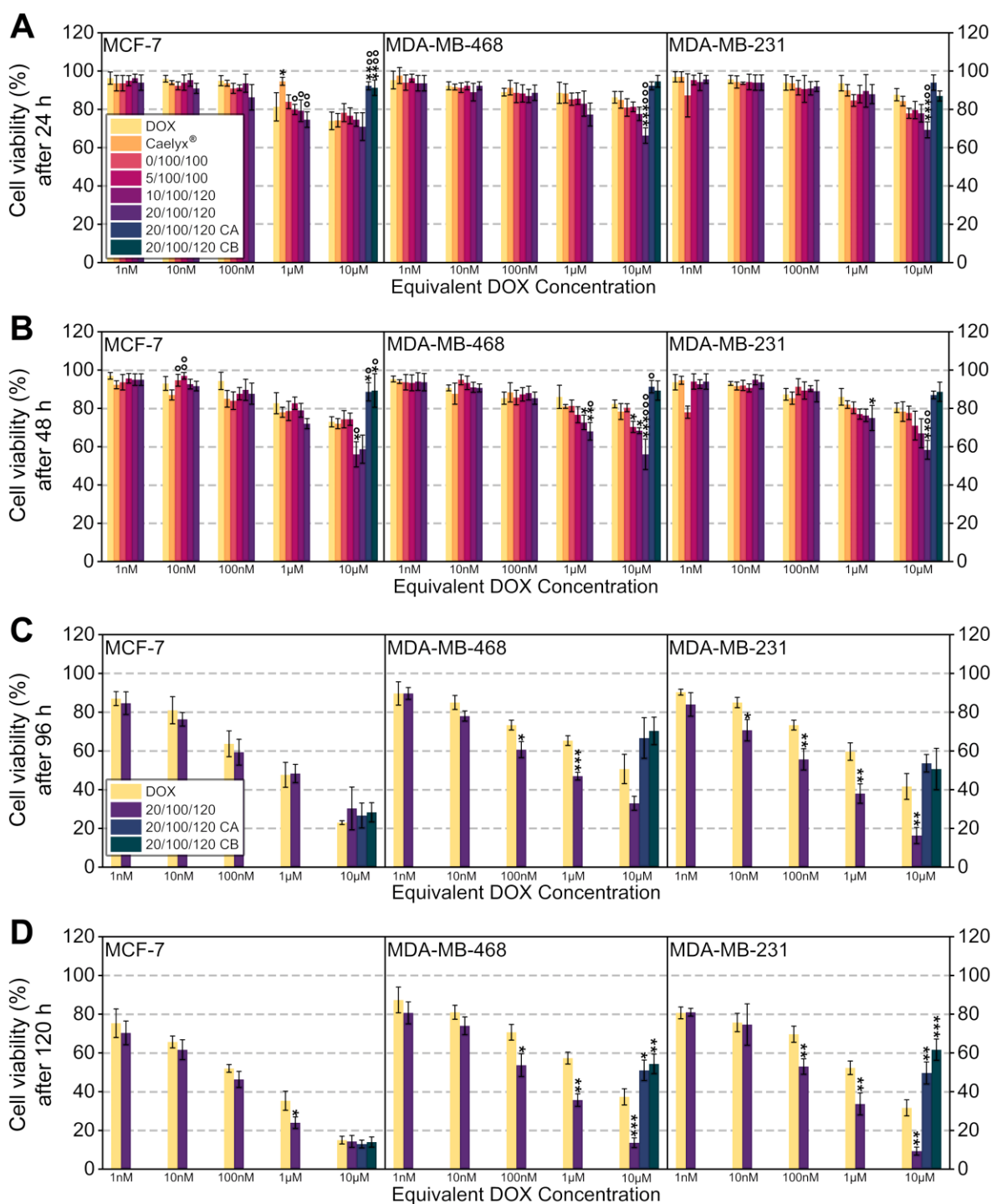


Fig. S10 24 h (A) and 48 h (B) MCF-7, MDA-MB-468, and MDA-MB-231 cell viability treated with various concentrations of DOX, Caelyx®, and different HA-DMPE/BN-CNO/DOX formulations; (C) and (D) represent the extended studies carried out at 96 and 120 h for the 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulation. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX, and ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$ vs. Caelyx®. Competitive co-incubations with excess HA or anti-CD44 blocking antibodies are labelled as CA and CB, respectively.

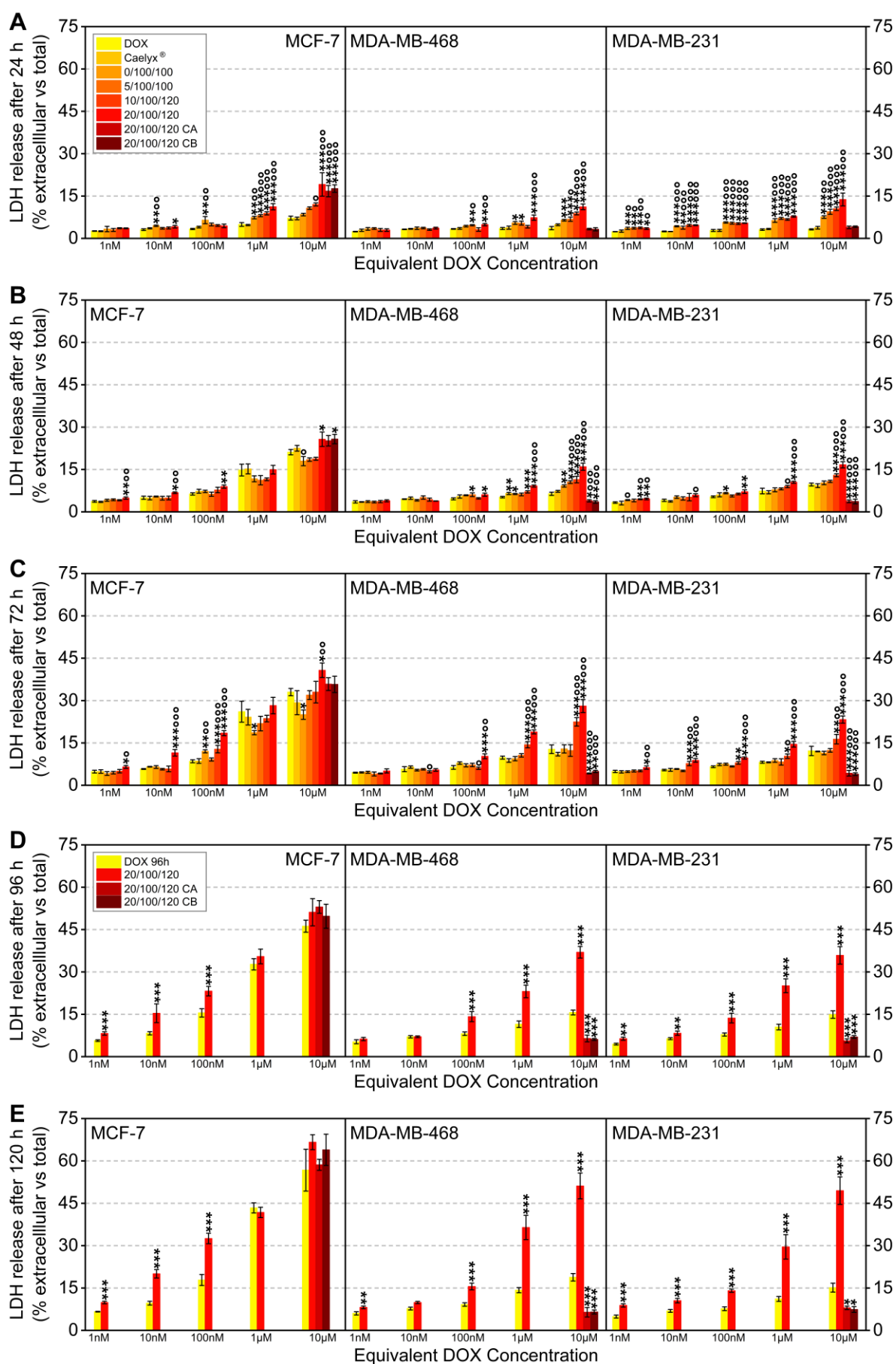


Fig. S11 24 h (A), 48 h (B), and 72 h (C) MCF-7, MDA-MB-468, and MDA-MB-231 extracellular LDH treated with various concentrations of DOX, Caelyx®, and different HA-DMPE/BN-CNO/DOX formulations; (D) and (E) represent the extended studies carried out at 96 and 120 h for the 20/100/120 wt% HA-DMPE/BN-CNO/DOX formulation. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX, and ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$ vs. Caelyx®. Competitive co-incubations with excess HA or anti-CD44 blocking antibodies are labelled as CA and CB, respectively.

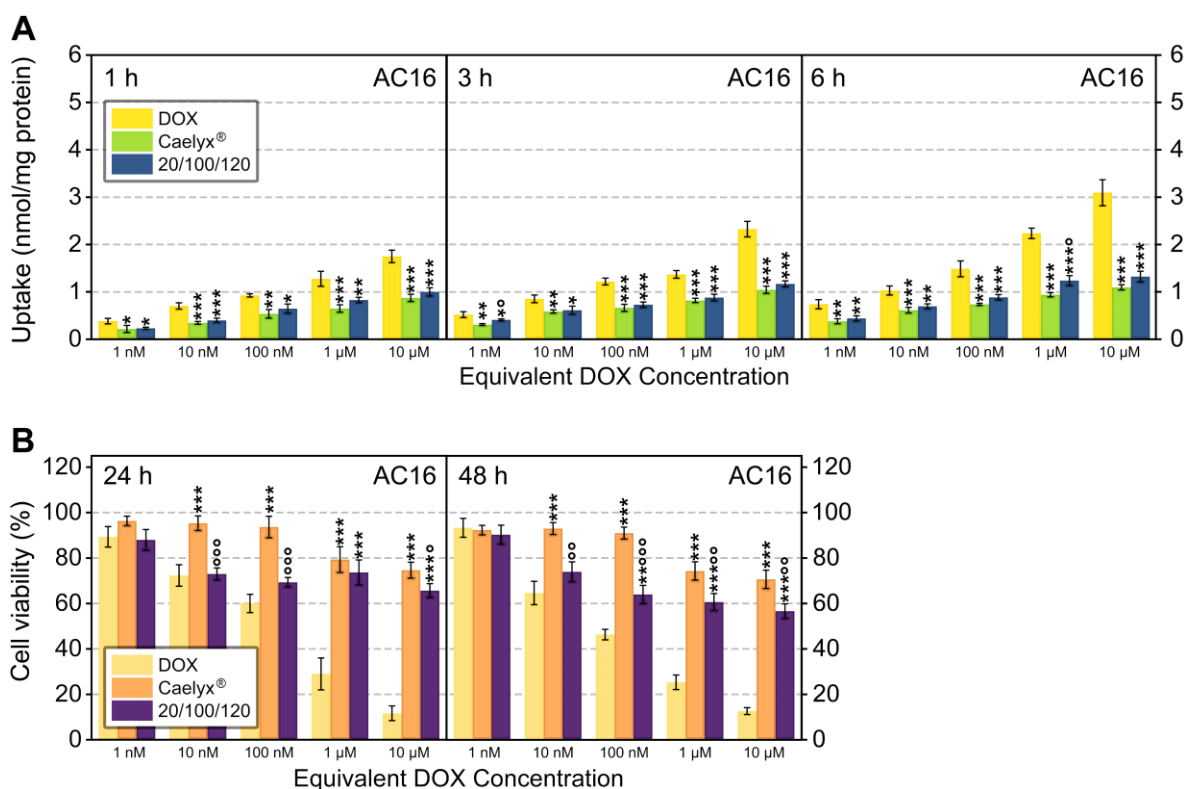


Fig. S12 AC16 1, 3, and 6 h cell uptake (A) and 24, and 48 h cell viability (B) with varying concentrations of DOX, Caelyx®, and 20/100/120 wt% HA-DMPE/BN-CNO/DOX. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX, and ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$ vs. Caelyx®.

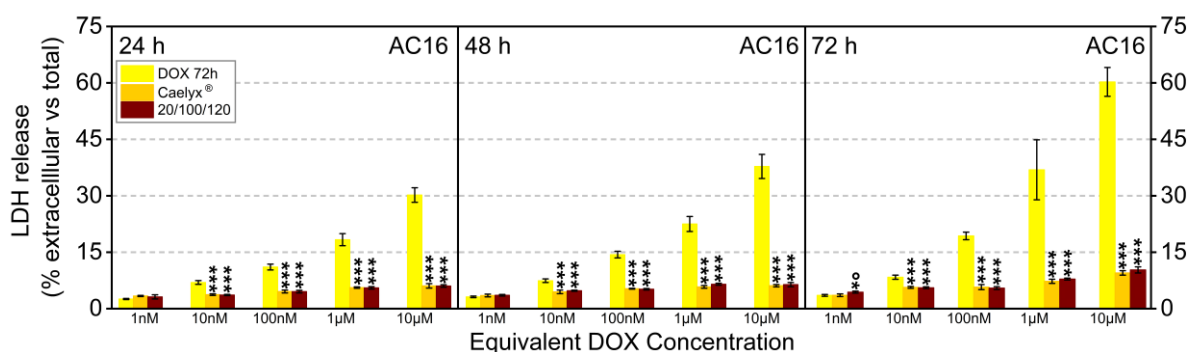


Fig. S13 Extracellular LDH, measured at 24 h (A), 48 h (B), and 72 h (C) measured in AC16 cells with varying concentrations of DOX, Caelyx®, and 20/100/120 wt% HA-DMPE/BN-CNO/DOX. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. DOX, and ° $p < 0.05$, °° $p < 0.01$, °°° $p < 0.001$ vs. Caelyx®.