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Electronic Suplementary Information

for

Co-delivery of anti-PLK-1 siRNA and camptothecin by nanometric polydiacetylenic micelles results in a synergistic tumor cell killing

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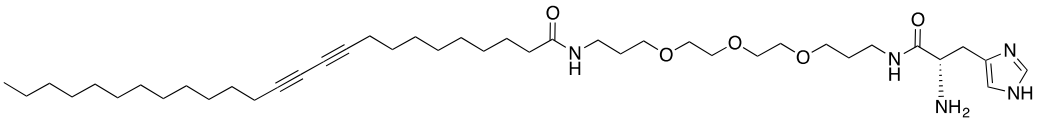
Surfactant synthesis



^{1}H NMR (400 MHz, MeOD, δ) 8.97 (s, 1H), 7.52 (s, 1H), 4.17 (t, *J*= 7 Hz, 1H), 3.65-3.55 (m, 8H), 3.53-3.44 (m, 4H), 3.35-3.22 (m, 6H), 2.24 (t, *J*=6.5 Hz, 4H), 2.18 (t, *J*=7.5 Hz, 2H), 1.77-1.69 (m, 4H), 1.64-1.57 (m, 2H), 1.54-1.47 (m, 4H), 1.43-1.28 (m, 26H, alkyl chain), 0.9 (t, *J*=7 Hz, 3H) ppm.

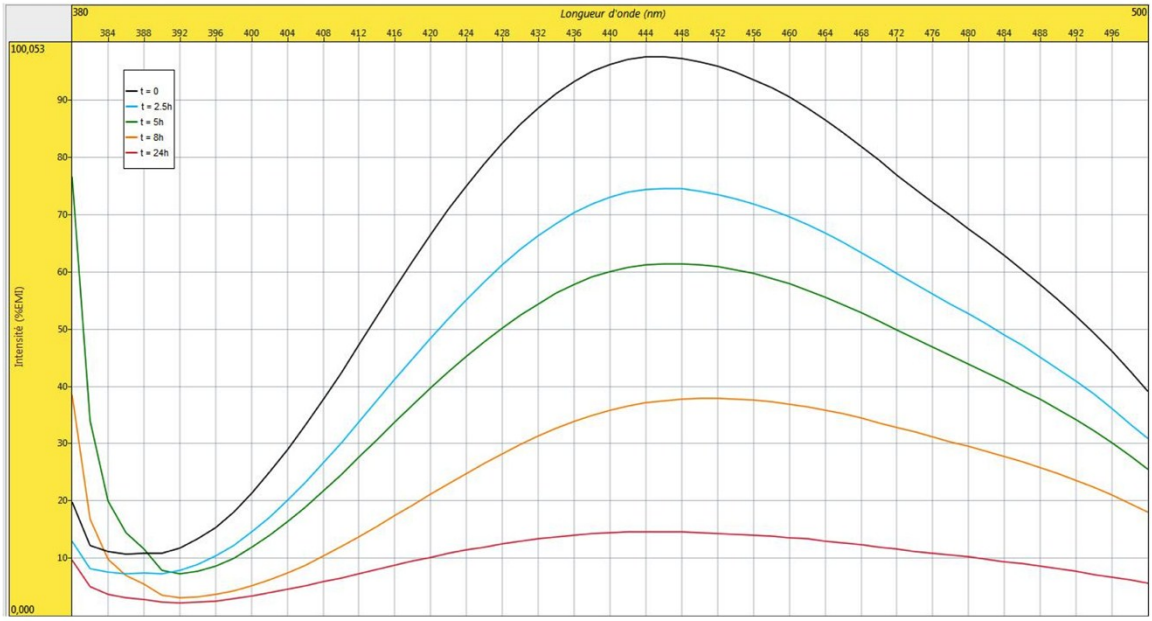
^{13}C NMR (75 MHz, MeOD, δ) 174.9, 161.6, 135.5, 132.6, 118.4, 115.9, 115.4, 76.6, 76.5, 70.1, 69.9, 69.8, 68.5, 68.3, 65.0, 53.5 38.7, 36.6 36.4, 35.8, 31.7, 29.4-28.1, 25.6, 22.3, 18.3, 13.1 ppm.

HRMS (ESI) m/z: 714.554 $[M+H]^+$ calculated for $C_{41}H_{72}N_5O_5$, 714.554; found 714.554

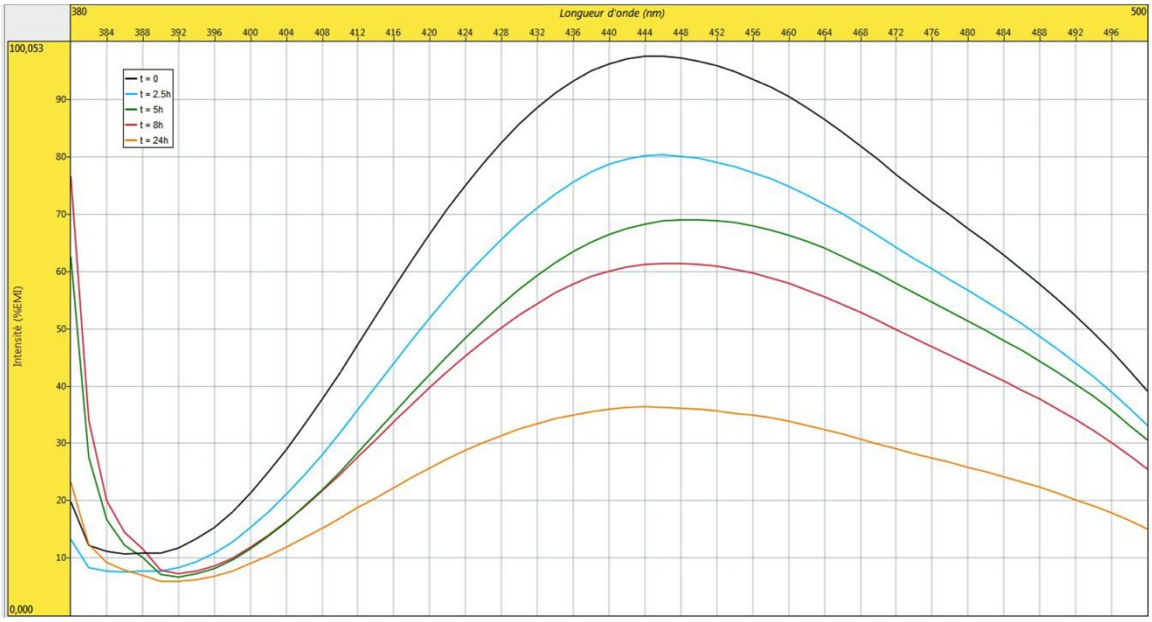
In vitro CPT release

The *in vitro* drug release profiles were performed according to the literature (Defan Yao et al. *chemical communications*, 2017,**53**, 1233-1236). CPT was encapsulated into the micelles (10 w%) and the profiles for the *in vitro* drug release were obtained by dialyzing the CPT-micelles in TBS (Tris-buffered saline, 50 mM) at different pH (4.5, 6 and 7.4) for 24h at 25°C. At several time intervals (2h, 5h, 8h and 24h), the fluorescence of the CPT encapsulated into the micelles was measured by fluorescence spectroscopy at 440 nm. The fluorescence of the empty micelle was taking as a blank. The CPT release was indicated by the reduction of the fluorescence signal by taking into reference the fluorescence of the CPT-micelle observed in t = 0.

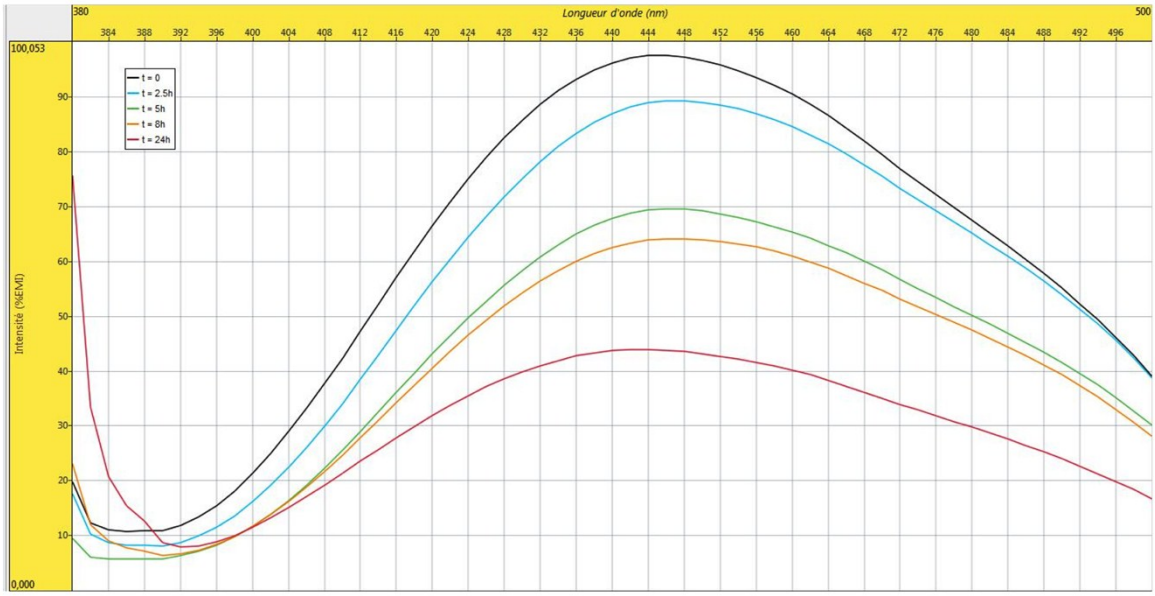
A. pH = 7.5


380
Longueur d'onde (nm)
500
384 388 392 396 400 404 408 412 416 420 424 428 432 436 440 444 448 452 456 460 464 468 472 476 480 484 488 492 496
100,053
Intensité (%EMI)
90
80
70
60
50
40
30
20
10
0,000
t = 0
t = 2.5h
t = 5h
t = 8h
t = 24h

B. pH = 6


380
Longueur d'onde (nm)
500
384 388 392 396 400 404 408 412 416 420 424 428 432 436 440 444 448 452 456 460 464 468 472 476 480 484 488 492 496
100,053
Intensité (%EMI)
90
80
70
60
50
40
30
20
10
0,000
t = 0
t = 2.5h
t = 5h
t = 8h
t = 24h

C. pH = 4.5



D.

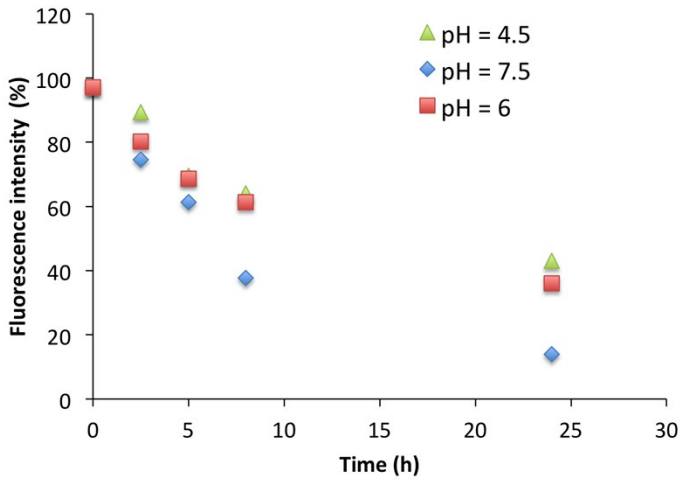


Fig S1. A-C Fluorescence intensity of CPT encapsulated in micelles at several time intervals (0, 2.5, 5, 8 and 24 hours) at pH 7.5 **(A)**, 6 **(B)** and 4.5 **(C)**. **D**. Release profiles of CPT from CPT-micelles dialyzed in TBS (50 mM, pH = 7.5, 6 and 4.5).

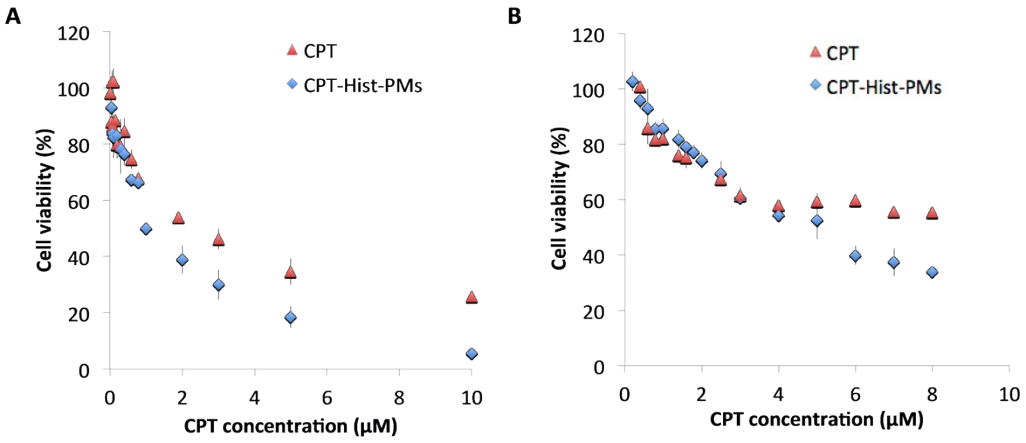


Fig S2. **A-B.** MTT cytotoxicity assay performed 48h after addition of CPT +/- micelles on HeLa (**A**) and MDA-MB-231 cells (**B**). Cell viability is given for free-CPT (red triangle) and CPT-Hist-PMs (blue diamond).

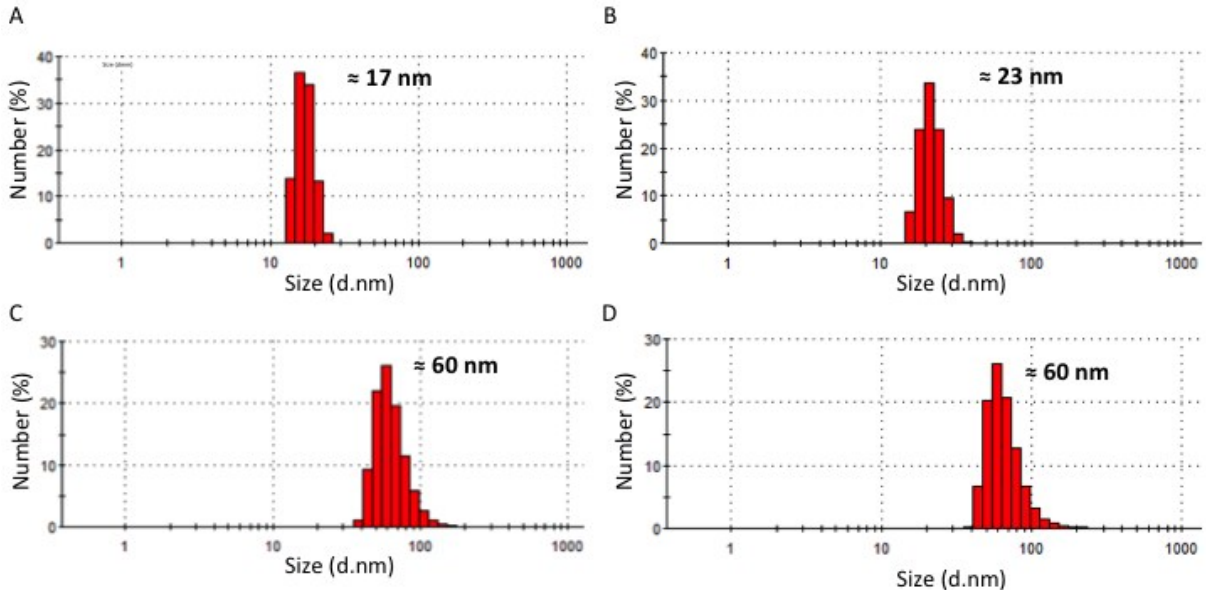


Fig S3. A. Size distribution of Hist-PMs in HBG (PdI = 0.452). **B.** Size distribution of CPT-Hist-PMs (1/10 w/w) in HBG (PdI = 0.434). **C.** Size distribution of siPLK-1-Hist-PMs (N/P = 5.6) in HBG (PdI = 0.215). **D.** Size distribution of siPLK-1/CPT-Hist-PMs (N/P = 5.6, 1/10 w/w ratio CPT/Micelle) in HBG (PdI = 0.213).

Figure S4A and S5A: “Dose M+CPT” represents the concentration of CPT encapsulated into Hist-PMs, which is added on cells.
“Dose M+plk1” represents the concentration of siPLK-1 complexed with Hist-PMs added on cells.
The effect is defined by x% inhibition induced by the combination of the two drugs.
“CI” is the combination index (T.-C. Chou *et al.*, *Pharmacol. Rev.*, 2006, **58**, 621–681.).

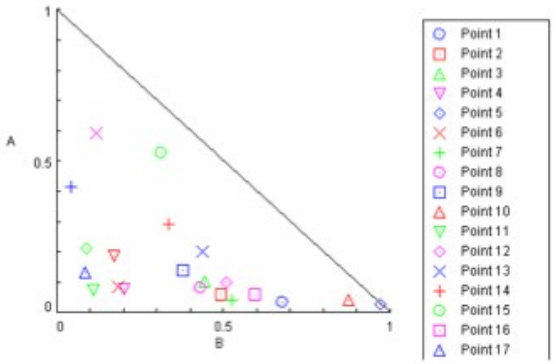
Figure S4B and S5B represent dose-normalized isobologram for CPT-Hist-PMs and siPLK1-Hist-PMs with normalization of dose with ED_{50} to unity on both *x* and *y*-axes. The *line* on the graph corresponds to the additive interaction. If the combination data points fall on the hypotenuse or lower left or upper right is indicated an additive effect or synergism or antagonism, respectively.
The two isobolograms evidence a synergistic relationship for all points.

Figure S4C and S5C represent the IC_{50} of M+CPT (CPT-Hist-PMs), M+plk1 (siPLK-1-Hist-PMs) and CPT (free camptothecin).

A

Dose M+CPT	Dose m+plk1	Effect	CI
80.0	150.0	0.67	0.71141
200.0	150.0	0.74	0.55099
400.0	150.0	0.76	0.54894
800.0	150.0	0.88	0.27900
40.0	150.0	0.58	0.99982
1000.0	150.0	0.89	0.26988
80.0	100.0	0.632	0.57061
200.0	100.0	0.68	0.51512
400.0	100.0	0.71	0.51880
40.0	100.0	0.5	0.91876
1000.0	100.0	0.9	0.18671
80.0	50.0	0.46	0.60856
200.0	50.0	0.5	0.64162
400.0	50.0	0.57	0.62717
800.0	50.0	0.59	0.83887
40.0	50.0	0.42	0.65436
1000.0	50.0	0.85	0.21652
80.0	10.0	0.33	0.35788
200.0	10.0	0.49	0.30347
400.0	10.0	0.42	0.71170
1000.0	10.0	0.68	0.45919

B



C

Drug/Combo	
M+CPT	987.737
m+plk1	113.861
CPT	1972.09

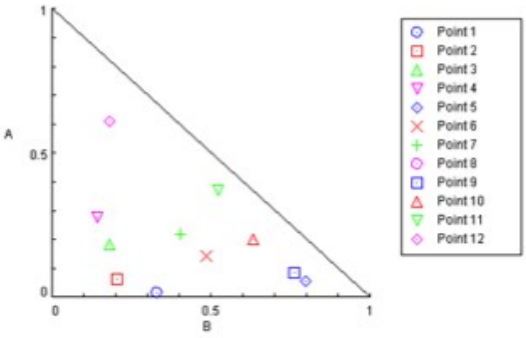
Fig S4. **A**. Summary table of inhibition induced by the co-delivery of the drugs with their combination index in HeLa cells after 48h of transfection. **B.** Associated isobologram of the co-delivery. **C.** IC_{50} in nM of the different systems in HeLa cells.

A

CI Data for Non-Constant Combo: syn (M+CPT+M+plk1)

Dose M+CPT	Dose M+plk1	Effect	CI
40.0	10.0	0.32	0.34410
200.0	10.0	0.39	0.26972
600.0	10.0	0.41	0.36367
1000.0	10.0	0.45	0.41646
200.0	50.0	0.43	0.85625
600.0	50.0	0.51	0.63060
1000.0	50.0	0.54	0.62678
40.0	100.0	0.48	1.18289
400.0	100.0	0.55	0.84949
1000.0	100.0	0.58	0.83322
2000.0	100.0	0.61	0.89400
2000.0	10.0	0.41	0.78979

B



C

Drug/Combo	
M+CPT	4104.88
cpt	4861.17
M+plk1	96.3833

Fig S5. **A**. Summary table of inhibition induced by the co-delivery of the drugs with their combination index in MDA-MB-231 cells after 48h of transfection. **B.** Associated isobologram of the co-delivery. **C**. IC_{50} of the different systems in MDA-MB-231 cells.

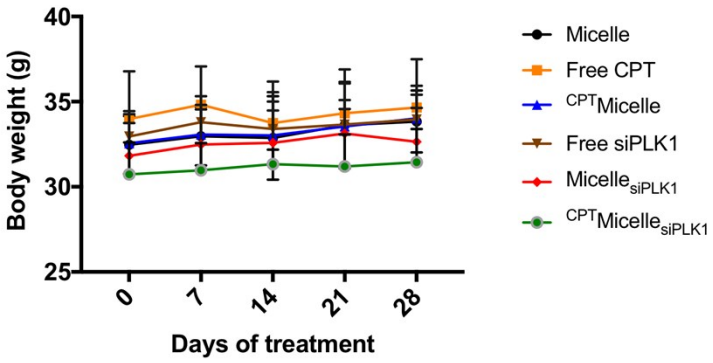


Fig. S6: Changes of body weights of mice after peritumoral injection of Micelle, free CPT, ^{CPT}Micelle, free siPLK1, $Micelle_{siPLK1}$ and $^{CPT}Micelle_{siPLK1}$ in MDA-MB-231 tumor-bearing mice.