Supporting information

Enhanced Drug Loading in Polymerized Micellar Cargo

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Nanostructures derived from diacetylenic amphiphile **1**

At lower pH (~ 7), polymerized amphiphile **1** forms well defined ribbons (Figure 1a-b) which evolve to tubular nanostructures (Figure 1c-d) when heated above 70°C. At pH above 12, micelles are formed (Figure 1e-f-).

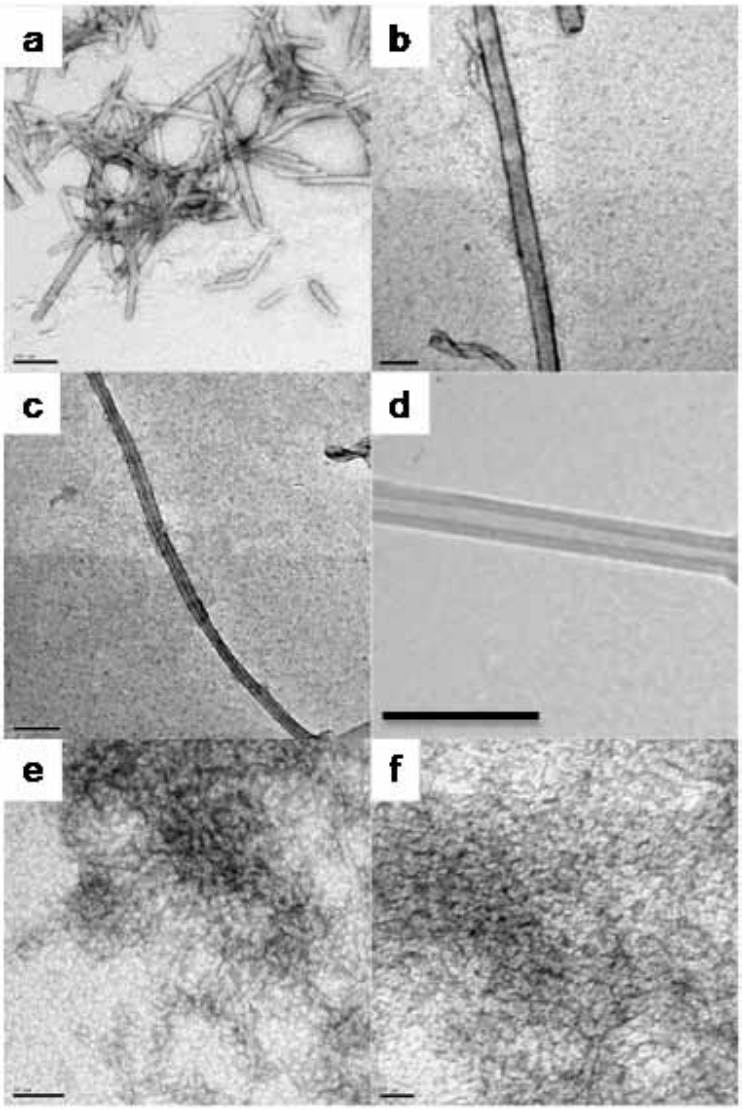


Figure S1 – Structure of polymerized amphiphile **1** in: (S1a-b) ribbon (pH 7) (S1c-d) nanotube (pH 7 and heated at 70°C) (S1e-f) micelles (pH=12)

Scale bars : (S1a) 200 nm (S1b) 50 nm (S1c) 100 nm (S1d) 50 nm (S1e) 50 nm (S1f) 20 nm.

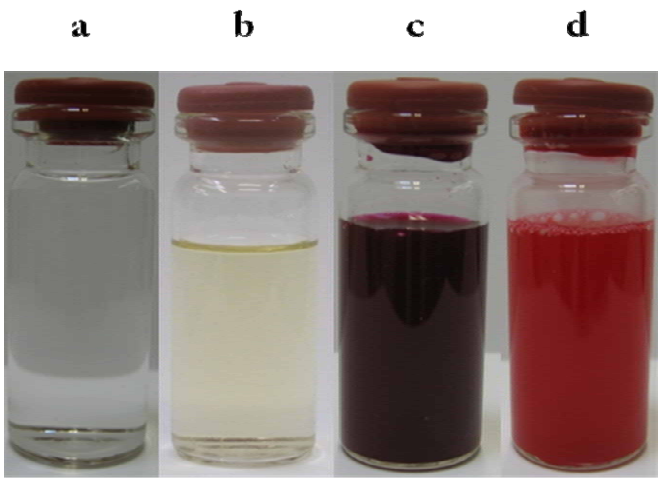


Figure S2. Pictures of solutions of (a) non-polymerized micelles, (b) polymerized micelles, (c) ribbons, and (d) coiled ribbons.

NMR spectra of photo-polymerizable amphiphile **1**

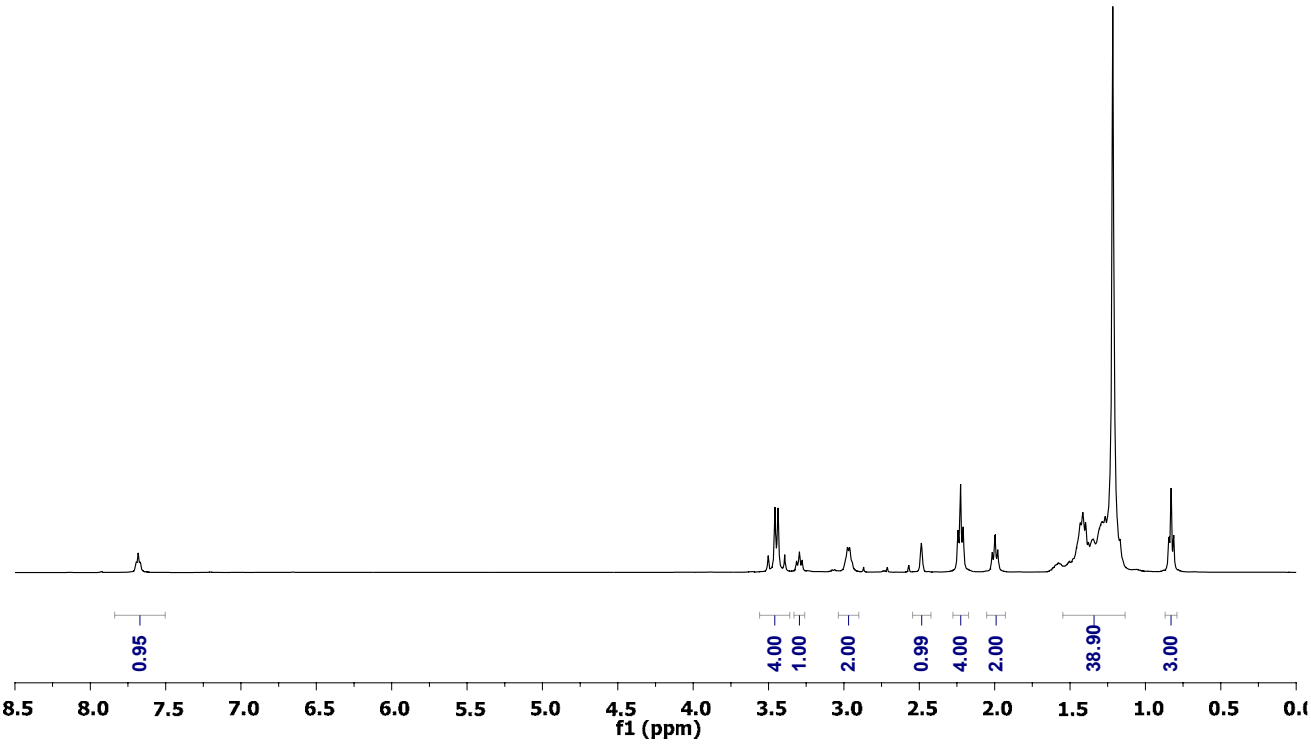


Figure S3. ^{1}H NMR of photo-polymerizable amphiphile **1** in DMSO-d_6

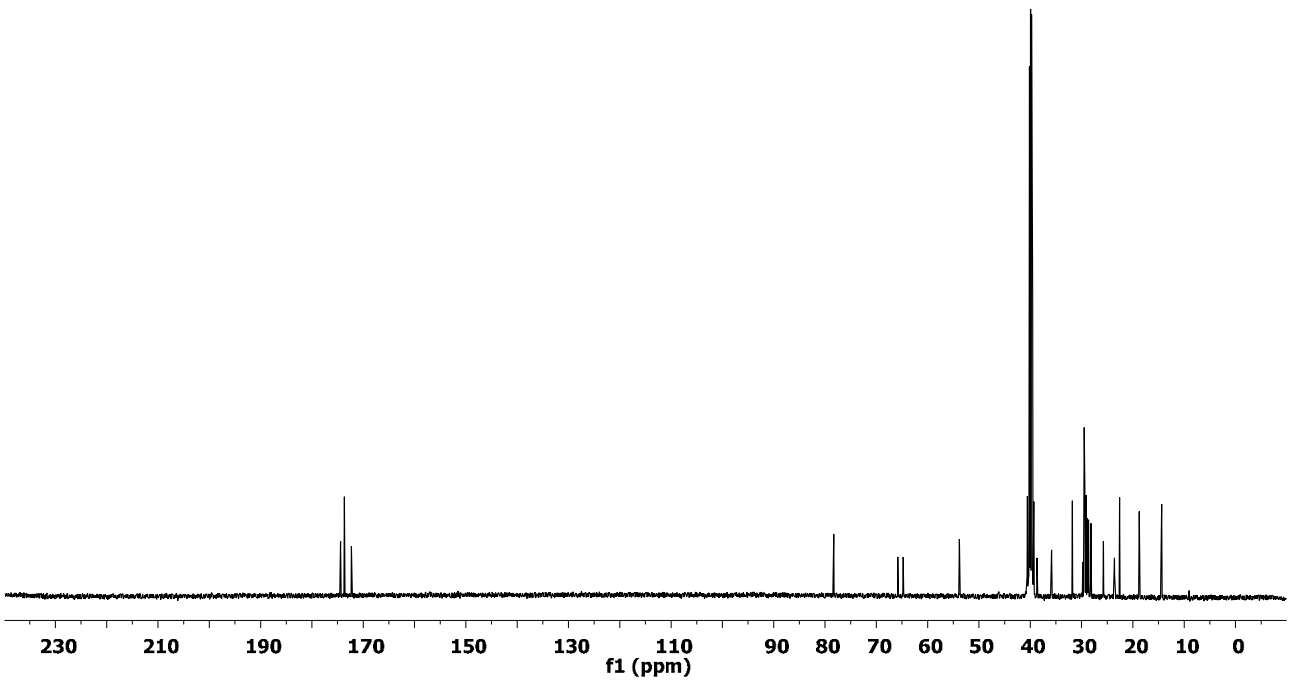


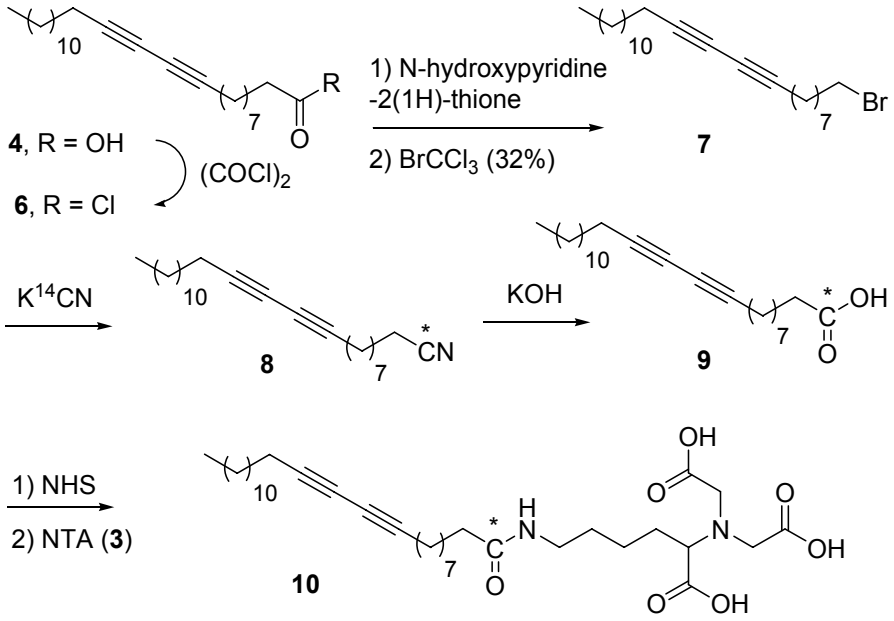
Figure S4. ^{13}C NMR of photo-polymerizable amphiphile **1** in DMSO-d_6

Synthesis of ^{14}C- amphiphile **10** (Scheme S1).

1-Bromotetracosa-9,11-diyne (7). 10,12-pentacosadiynoic acid **4** (1 g, 2.67 mmol, 1 equiv.) was solubilized in 10 mL of toluene and oxalyl chloride (1.1 mL, 5 equiv.) was added dropwise at 0 °C. The resulting solution was stirred at r.t. under N_2 for 12 hrs and concentrated under vacuum. The residue was taken back into toluene and evaporated to remove traces of oxalyl chloride (this operation was repeated 3 times). The resulting acid chloride **6** was used in the next step without further purification. DMAP (126 mg, 0.4 equiv.) and 2-mercaptopyridine *N*-oxide sodium salt (478 mg, 1.2 equiv.) were solubilized in 15 mL of $BrCCl_3$. The solution was heated to reflux and acid chloride **6**, in 5 mL of $BrCCl_3$, was added. The mixture was refluxed for an additional hour. The solution was diluted with 50 mL of Et_2O and washed 3 times with sat NaCl. The organic layer was dried over $MgSO_4$, filtered, and concentrated. Purification by column chromatography over SiO_2 (pentane) gave 349 mg of 1-bromotetracosa-9,11-diyne **7**. Yield: 32%. ^1H NMR ($CDCl_3$): 3.4 (t, J=6.8 Hz, 2H), 2.24 (t, J=6.8 Hz, 4H), 1.85 (m, 2H), 1.2-1.6 (m, 30H), 0.89 (t, J=6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$): 78.2 (2C), 65.2 (2C), 33.9 (1C), 32.7 (1C), 31.8 (1C), 26.7-29.6 (13C), 22.6 (1C), 19.1 (2C), 14.1 (1C).

*[1-^{14}C]-10,12-Pentacosadiynoic acid (**9**).* Bromo derivative **7** (82.1 mg, 201 µmol, 1 equiv.) and $K^{14}CN$ (36.2 mg, 2.7 equiv., 27 mCi, specific activity: 53 mCi/mmol) were solubilized in 10 mL of DMSO. The solution was stirred overnight at 80°C under Ar. The solution was then diluted with 20 mL of Et_2O and 30 mL of H_2O were added. The aqueous phase was extracted two times with Et_2O. The organic phases were collected, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography over SiO_2 (pentane/Et_2O: 90/10) gave ^{14}C-labeled nitrile **8** (160 µmol, 8.5 mCi). Compound **8** was solubilized in 10mL of EtOH and 10 mL of 40% aqueous KOH was added. The solution was heated to 80°C for 6 hrs. The solution was cooled to r.t. and 10% HCl was added until pH 1 is reached. The aqueous phase was extracted three times with Et_2O. The organic phases were collected, dried over $MgSO_4$, filtered and concentrated. Purification by column chromatography over SiO_2 (hexane/EtOAc/AcOH: 85/15/0.1) gave labeled carboxylic acid **9** (50 mg, 133 µmol, 7 mCi). Yield from **7**: 66%. Liquid scintillation counting: 140 µCi/mg. Radiochemical purity: 94.3%.

*N_2-(bis-carboxymethyl)-N_6-[1-^{14}C]-pentacosa-10,12-diynoyl-Lysine (**10**).* Under Ar, [1-^{14}C] carboxylic acid **9** (25 mg, 66 µmol, 3.5 mCi, 1 equiv.) and pentacosa-10,12-diynoic acid **4** (225 mg, 9 equiv.) were solubilized in 25 mL of anhydrous CH_2Cl_2. To this solution were added *N*-hydroxysuccinimide (116 mg, 1.5 equiv.) and EDC (195 mg, 1.5 equiv.). The solution was stirred at r.t. for 12 hrs. The organic phase was washed twice with H_2O, dried over $MgSO_4$, filtered and concentrated under vacuum to give the corresponding activated carboxylic acid (312 mg, 664 µmol) as a pale pink solid. The latter was diluted with 10 mL of DMF before being added to a mixture of 6-amino-2(bis-carboxymethyl-amino)-hexanoic acid **3** (230 mg, 1.3 equiv.) in 30 mL of DMF, 2 mL of H_2O, and 1 mL of NEt_3. The solution was stirred at room temperature for 12 hrs. The solution was concentrated, taken back into 30 mL of H_2O and slowly acidified to pH 1 with 1N HCl. The precipitate was centrifuged and washed 2 times with 1N HCl. The orange solid was then dried overnight under vacuum and over P_2O_5. Yield: quant. Liquid scintillation counting: 7.6 µCi/mg. Radiochemical purity: 91.0%. NMR ^{1}H (d_6-DMSO): 7.68 (t, J=5.6 Hz, 1H), 3.50 (AB, 4H, J_{AB}=17.6 Hz), 3.35 (t, J=7.3 Hz, 1H), 2.97 (m, 2H), 2.24 (t, J=6.8 Hz, 4H), 2.00 (t, J=7.2 Hz, 2H), 1.1-1.6 (m, 38H), 0.83 (t, J=6.8 Hz, 3H). MS (ESI/TOF) m/z: (ESI$^-$) 617 (100), 618 (26.3), 619 (5.2).



Scheme S1. Synthesis of ^{14}C- amphiphile **10**

Determination of the Critical Micelle Concentration of photo-polymerizable amphiphile **1**

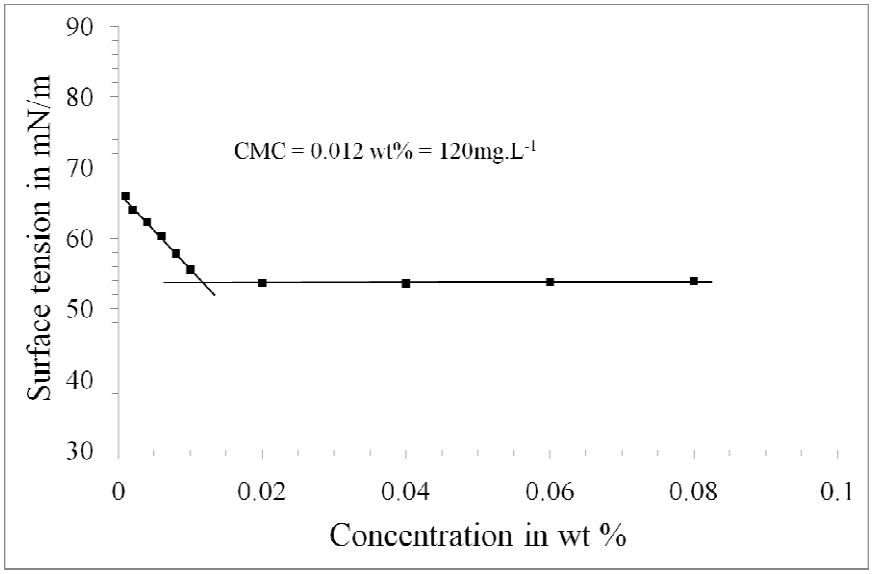


Figure S5- Measurement of the CMC of photo-polymerizable amphiphile **1** by the ring method

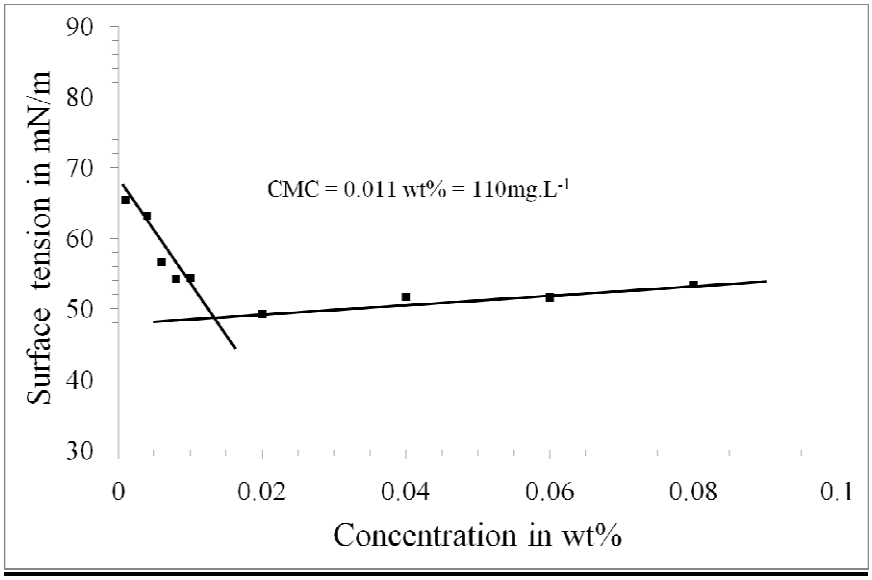


Figure S6- Measurement of the CMC photo-polymerizable amphiphile **1** by the plate method

Drug loading experiments

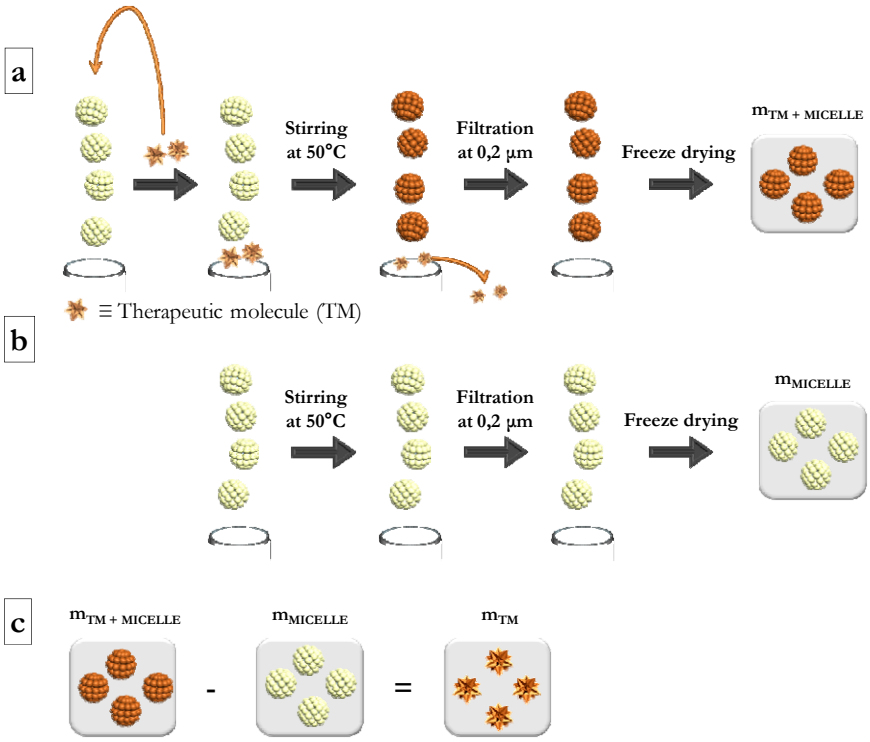


Figure S7 – Determination of the drug loading by mass balance: (a) inclusion of TM at 50°C (b) blank experiment (c) weight of solubilized TM determined by mass balance of the two experiments (a) and (b).

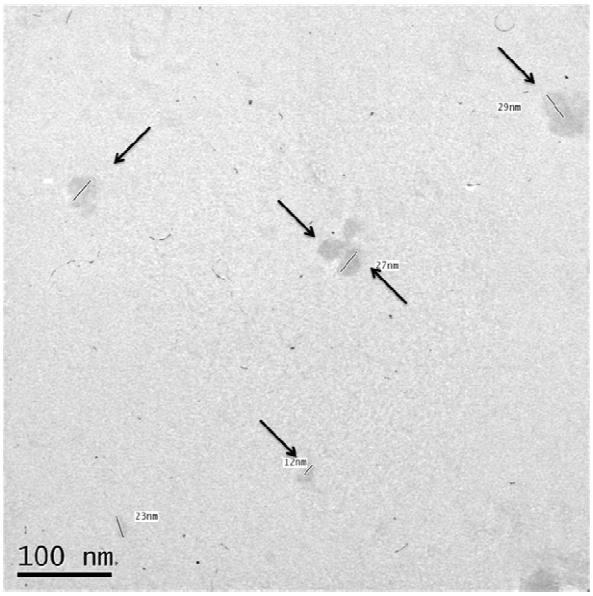


Figure S8 – TEM pictures of FLD loaded in polymerized micelles

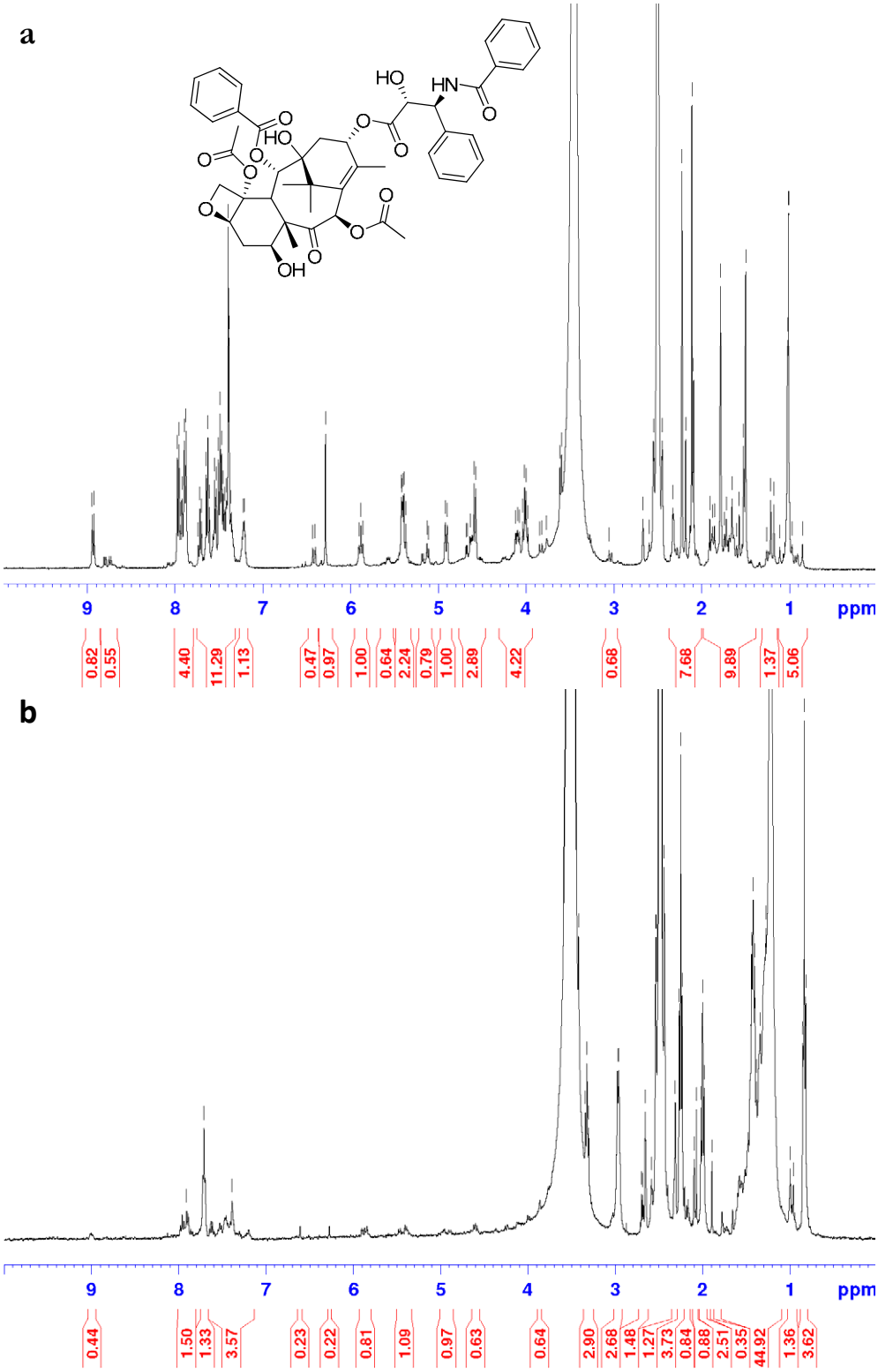


Figure S9 – ^{1}H NMR spectra of (a) Paclitaxel in DMSO-d_6/DCl, (b) Paclitaxel/micelle complex in DMSO-d_6/DCl

Tissue distribution of total radioactivity in the male wistar rat after single intravenous administration of [^{14}C]-polymerised micelles

Species/Strain/Gender: Rat/Wistar/Male
Method of administration/volume (mL/kg): intravenous bolus/ 2.5
Sampling times: 0.17 and 24
Radionuclide: ^{14}C
Compounds/batches: ^{14}C-polymerised micelles
Doses (MBq/kg – mg/kg): 4 – 100
Specific activity (MBq/ mg of total compound in formulation): .0365
Radiochemical purity (%): 100 in stock solution
Formulation: physiological saline (0.9 g/L NaCl)
Analyte: Total radioactivity ^{14}C
Samples: Saggital sections of each rat (at 10 min or 24h after dosing) and urine samples collected at 24h (rat 2) after dosing
Analytical methods: Radioluminography to quantify radioactive levels in tissues and liquid scintillation counting to measure radioactivity in urine.

TABLE S1: Tissue distribution of total radioactivity versus time after single intravenous administration of 4 MBq/kg [14C]-polymerised micelles and polymerised micelles (100 mg/kg) in male Wistar rats. Expressed in Bq/g

Time (h) Tissues/organs	0.17	24
ADRENAL GLANDS	35644	12224
BLOOD	50303	2275
BONE MARROW	22006	8605
BONE MINERAL	600	466
BRAIN	997	234
BROWN FAT	14783	2994
FAT	903	729
HEART	21137	2859
INTESTINE WALL	9052	14591
KIDNEY CORTEX	22465	11907
KIDNEY CORTICOMEDULLA	22789	4446
KIDNEY MEDULLA	37148	2358
LACHRYMAL GLAND	5282	4667
LIVER	36547	20639
LUNG	45005	5079
OESOPHAGUS WALL	1649	
PANCREAS	11422	4669
PITUITARY GLAND	9385	6271
PROSTATE		
SALIVARY GLANDS	8335	4387
SEMINAL VESICLES	128	1679
SKELETAL MUSCLE	2015	574
SKIN	2521	1491
SPINAL CORD	1212	480
SPLEEN	31053	14098
STOMACH WALL	2420	1285
TESTIS	1291	1504
THYMUS	3590	3533
THYROID GLAND	12428	2579
URINARY BLADDER WALL		
UVEAL TRACT	5264	1968

TABLE S2: Tissue distribution of total radioactivity versus time after single intravenous administration of 4 MBq/kg [^{14}C]-polymerised micelles and polymerised micelles (100 mg/kg) in male Wistar rats. Expressed in ng eq/g

Time (h) Tissues/organs	0.17	24
ADRENAL GLANDS	976662	335424
BLOOD	1378326	62415
BONE MARROW	602966	236122
BONE MINERAL	16440	12777
BRAIN	27309	6412
BROWN FAT	405061	82152
FAT	24752	20003
HEART	579155	78439
INTESTINE WALL	248029	400354
KIDNEY CORTEX	615561	326707
KIDNEY CORTICOMEDULLA	624420	121985
KIDNEY MEDULLA	1017863	64701
LACHRYMAL GLAND	144720	128067
LIVER	1001396	566305
LUNG	1233149	139354
OESOPHAGUS WALL	45183	
PANCREAS	312959	128113
PITUITARY GLAND	257163	172070
PROSTATE		
SALIVARY GLANDS		
SEMINAL VESICLES	3516	46070
SKELETAL MUSCLE	55221	15750
SKIN	69067	40912
SPINAL CORD	33209	13180
SPLEEN	850858	386845
STOMACH WALL	66309	35250
TESTIS	35365	41277
THYMUS	98359	96942
THYROID GLAND	340542	70756
URINARY BLADDER WALL		
UVEAL TRACT	144236	54000

TABLE S3: Tissue distribution of total radioactivity versus time after single intravenous administration of 4 MBq/kg [14C]-polymerised micelles and polymerised micelles (100 mg/kg) in male Wistar rats. Expressed in % dose/g

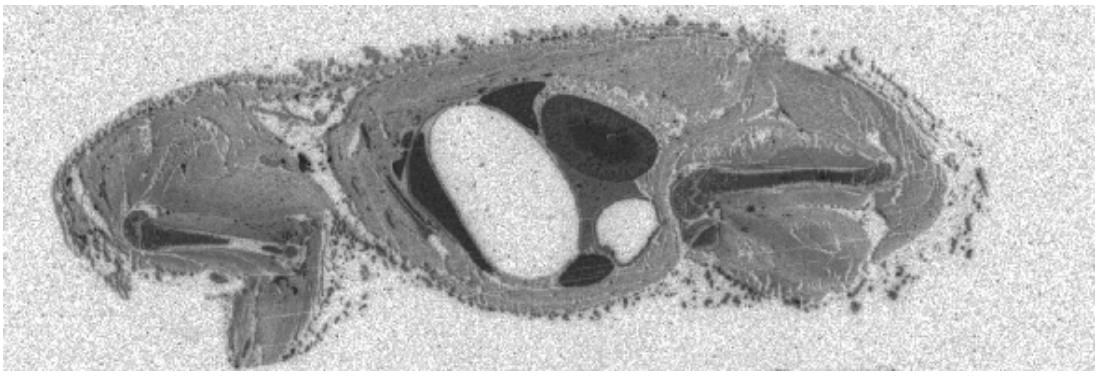
Time (h) Tissues/organs	0.17	24	$t_{1/2}$ (2 pts) (h)
ADRENAL GLANDS	4.17	1.44	15.5
BLOOD	5.89	0.268	5.35
BONE MARROW	2.58	1.01	17.7
BONE MINERAL	0.0703	0.0549	67.0
BRAIN	0.117	0.0276	11.5
BROWN FAT	1.73	0.353	10.4
FAT	0.106	0.0860	79.8
HEART	2.48	0.337	8.29
INTESTINE WALL	1.06	1.72	NC
KIDNEY CORTEX	2.63	1.40	26.3
KIDNEY CORTICOMEDULLA	2.67	0.524	10.2
KIDNEY MEDULLA	4.35	0.278	6.01
LACHRYMAL GLAND	0.618	0.550	142
LIVER	4.28	2.43	29.3
LUNG	5.27	0.599	7.60
OESOPHAGUS WALL	0.193		NC
PANCREAS	1.34	0.551	18.6
PITUITARY GLAND	1.10	0.740	41.7
PROSTATE			NC
SALIVARY GLANDS	0.976	0.517	26.0
SEMINAL VESICLES	0.0150	0.198	NC
SKELETAL MUSCLE	0.238	0.0677	13.2
SKIN	0.295	0.176	31.9
SPINAL CORD	0.142	0.0566	18.0
SPLEEN	3.64	1.66	21.1
STOMACH WALL	0.283	0.152	26.4
TESTIS	0.151	0.177	NC
THYMUS	0.420	0.417	1865
THYROID GLAND	1.46	0.304	10.6
URINARY BLADDER WALL			NC
UVEAL TRACT	0.616	0.232	16.9

NC: not calculated

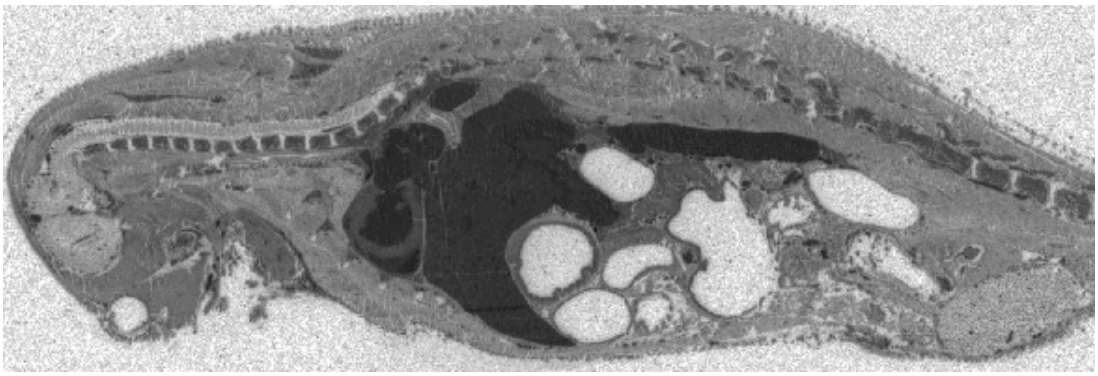
TABLE S4: Tissue distribution of total radioactivity versus time after single intravenous administration of 4 MBq/kg [14C]-polymerised micelles and polymerised micelles (100 mg/kg) in male Wistar rats. Tissue/blood ratios

Time (h) Tissues/organs	0.17	24
ADRENAL GLANDS	0.709	5.37
BLOOD	1	1
BONE MARROW	0.437	3.78
BONE MINERAL	0.0119	0.205
BRAIN	0.0200	0.103
BROWN FAT	0.294	1.32
FAT	0.0180	0.320
HEART	0.420	1.26
INTESTINE WALL	0.180	6.41
KIDNEY CORTEX	0.447	5.23
KIDNEY CORTICOMEDULLA	0.453	1.95
KIDNEY MEDULLA	0.738	1.04
LACHRYMAL GLAND	0.105	2.05
LIVER	0.727	9.07
LUNG	0.895	2.23
OESOPHAGUS WALL	0.0328	
PANCREAS	0.227	2.05
PITUITARY GLAND	0.187	2.76
PROSTATE		
SALIVARY GLANDS	0.166	1.93
SEMINAL VESICLES	0.00300	0.738
SKELETAL MUSCLE	0.0400	0.252
SKIN	0.0500	0.655
SPINAL CORD	0.0241	0.211
SPLEEN	0.617	6.20
STOMACH WALL	0.0481	0.565
TESTIS	0.0260	0.661
THYMUS	0.0710	1.55
THYROID GLAND	0.247	1.13
URINARY BLADDER WALL		
UVEAL TRACT	0.105	0.865

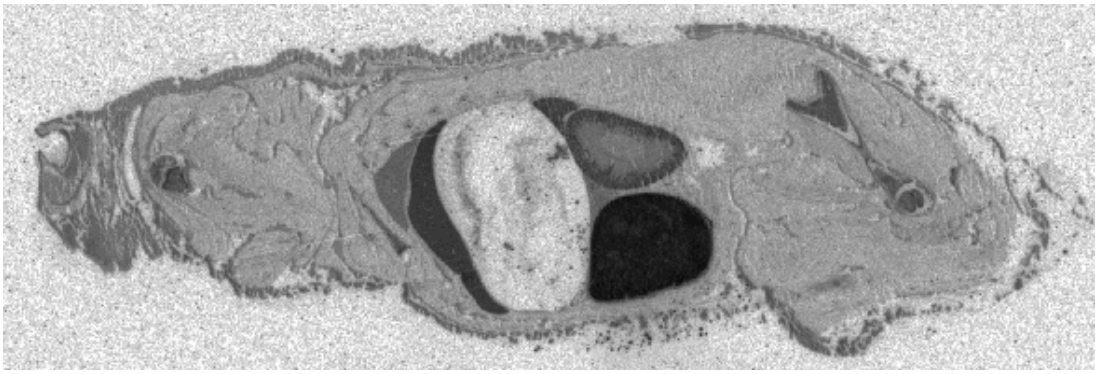
Quantitative tissue distribution of total radioactivity in the male C56 black mouse after single intravenous infusion of various formulations of ^{14}C-CPTD-1



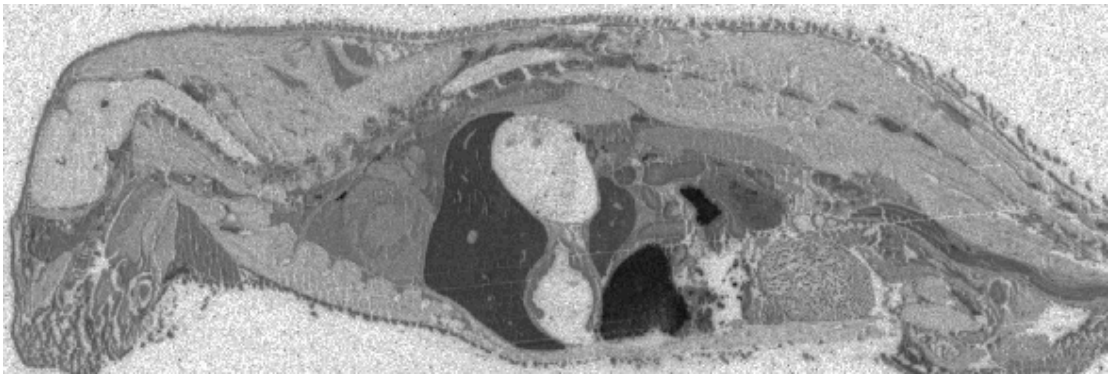
Rat 1 – Section 3 : distribution of radioactivity at 0.17 h (polymerised micelles at 100 mg/4MBq/kg/IV)



Rat 1 – Section 15 : distribution of radioactivity at 0.17 h (polymerised micelles at 100 mg/4MBq/kg/IV)



Rat 2 – Section 2 : distribution of radioactivity at 24 h (polymerised micelles at 100 mg/4MBq/kg/IV)



Rat 2 – Section 14 : distribution of radioactivity at 24 h (polymerised micelles at 100 mg/4MBq/kg/IV)

TABLE S5: Tissue distribution of total radioactivity versus time after single intravenous administration of 4 MBq/kg of ^{14}C-CPTD in non-labeled polymerized micelles and nanosuspension of the free ^{14}C-CPTD in C57 black male mice

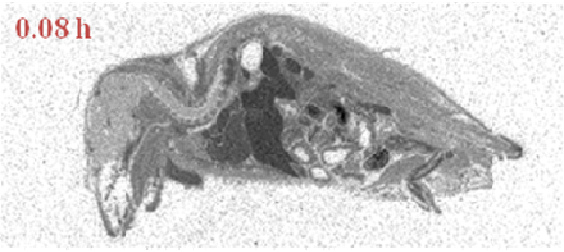
Tissue distribution (% dose/g)

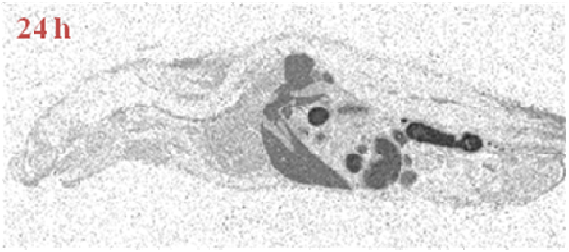
Time (h)	Tissues	Adrenal glands	Blood	Bone marrow	Brown fat	Heart	Kidney cortex	Lachrymal gland	Liver	Lungs	Pancreas	Salivary glands	Skeletal muscle	Spleen	Thymus
0.08	Nanosuspension	3.84	2.68	3.98	5.58	7.47	8.86	6.70	50.7	3.86	4.65	3.76	1.43	21.4	1.99
	Micelles	27.8	28.4	7.68	16.1	20.2	36.0	12.4	41.8	21.0	12.7	23.8	2.85	10.5	6.48
1	Nanosuspension	1.32	0.943	0.918	1.42	1.91	3.13	7.27	21.1	1.27	1.62	1.99	BQ(0.6)	3.43	1.32
	Micelles	3.89	7.73	2.95	2.50	3.42	5.68	4.21	9.65	4.68	1.76	3.21	BQ(0.4)	1.91	1.21
4	Nanosuspension	BQ(0.2)	BQ(0.3)	BQ(0.2)	BQ(0.2)	BQ(0.4)	0.665	1.06	2.87	BQ(0.4)	0.628	BQ(0.1)	BQ(0.0)	0.835	BQ(0.0)
	Micelles	3.06	3.46	1.61	1.69	1.98	6.09	1.56	8.05	1.91	BQ(0.8)	0.959	BQ(0.0)	1.94	BQ(0.7)
24	Nanosuspension	BQ(0.0)	BQ(0.1)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.2)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)
	Micelles	1.54	BQ(0.6)	1.04	BQ(0.5)	BQ(0.5)	2.53	BQ(0.3)	4.05	BQ(0.6)	BQ(0.0)	BQ(0.4)	BQ(0.0)	1.45	BQ(0.2)
48	Nanosuspension	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.4)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.0)	BQ(0.2)	BQ(0.0)
	Micelles	1.62	BQ(0.2)	1.54	BQ(0.6)	BQ(0.4)	2.68	BQ(0.6)	4.18	BQ(0.4)	BQ(0.4)	BQ(0.5)	BQ(0.0)	1.41	BQ(0.3)

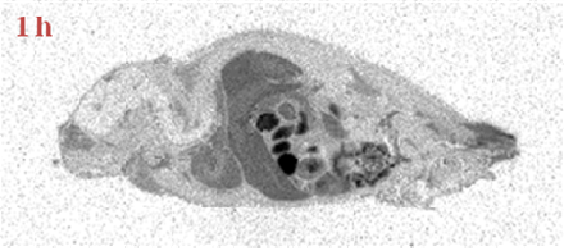
T1/2 (h)

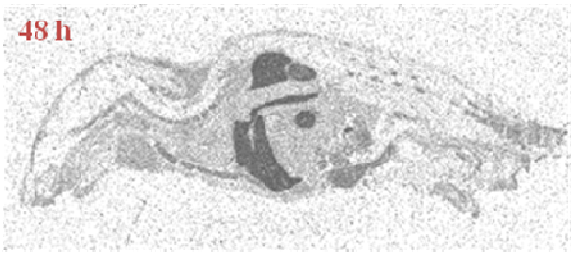
Tissues	Adrenal glands	Blood	Bone marrow	Brown fat	Heart	Kidney cortex	Lachrimal gland	Liver	Lungs	Pancreas	Salivary glands	Skeletal muscle	Spleen	Thymus
Nanosuspension	0.958	10.3	0.898	0.905	0.96	1.11	1.35	7.63	1.39	1.50	0.831	0.000	15.3	0.000
Micelles	37.7	11.4	31.3	23.8	16.5	36.9	12.9	38.6	19.5	25.5	23.1	0.000	95.1	28.3

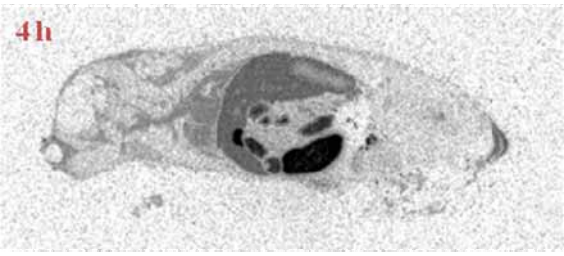
Polymerized micelles











Nanosuspension