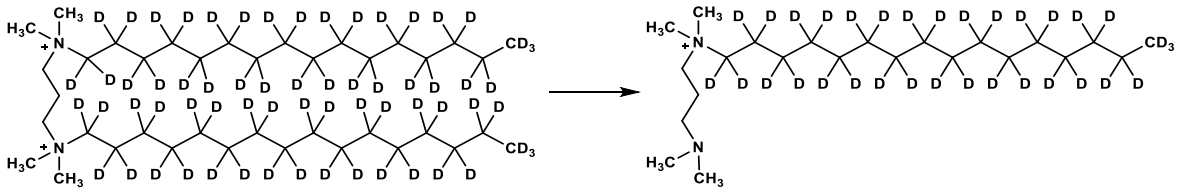
Supplement to the published article: Jin, W., Al-Dulaymi, M., Badea, I. *et al.* Cellular Uptake and Distribution of Gemini Surfactant Nanoparticles Used as Gene Delivery Agents. *AAPS J* **21**, 98 (2019). https://doi.org/10.1208/s12248-019-0367-1

Appendix A- Supplementary Figure and Table

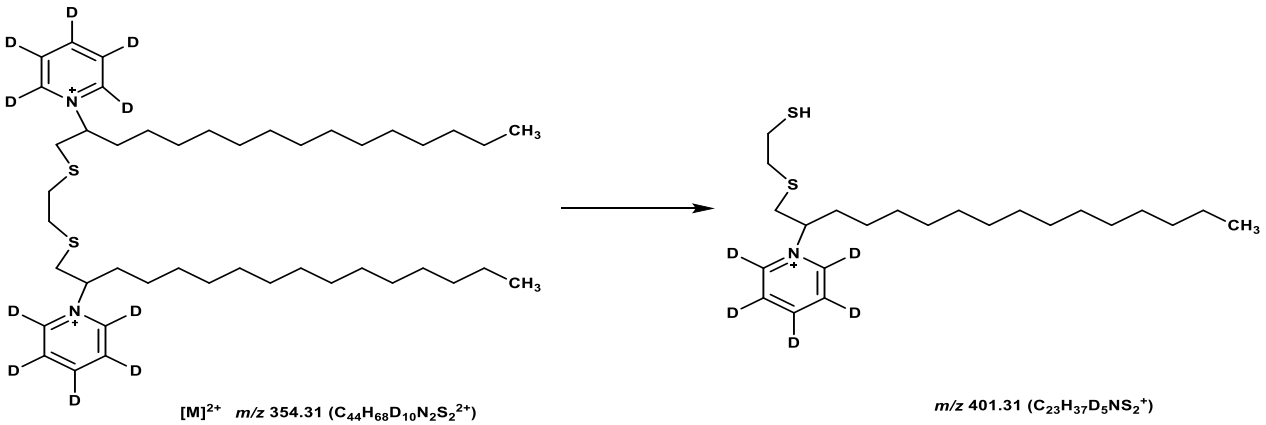
A 16-3-16-D_{66}



$[M]^{2+}$ *m/z* 323.51 ($C_{39}H_{18}D_{66}N_2^{2+}$)

m/z 388.61 ($C_{23}H_{18}D_{33}N_2^{+}$)

B 16(Py)-S-2-S-(Py)16-D_{10}



$[M]^{2+}$ *m/z* 354.31 ($C_{44}H_{68}D_{10}N_2S_2^{2+}$)

m/z 401.31 ($C_{23}H_{37}D_5NS_2^{+}$)

C 16-7N(GK)-16-D_4

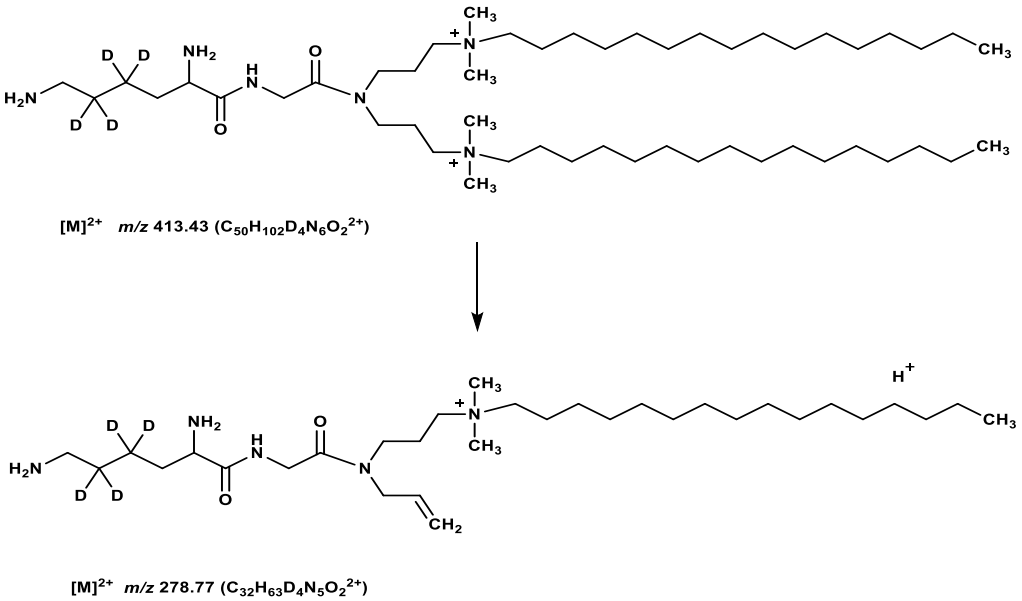


Figure S1. Structures of (A) 16-3-16-D_{66} and the monitored product ion, (B) 16(Py)-S-2-S-16(Py)-D_{10} and the monitored product ion, and (C) 16-7N(GK)-16-D_4 and the monitored product ion.

Table 1. Cellular uptake and distribution of the three gemini surfactant in PAM 212 cells at various time points, expressed as an absolute amount.

Subcellular fraction	2hr			5hr			8hr		
	16-3-16	16(Py)-S-2-S-16(Py)	16-7N(GK)-16	16-3-16	16(Py)-S-2-S-16(Py)	16-7N(GK)-16	16-3-16	16(Py)-S-2-S-16(Py)	16-7N(GK)-16
Cyto (nmoL)	0.15	0.20	0.20	0.13	0.26	0.42	0.10	0.25	0.40
Mito (nmoL)	0.23	0.18	0.29	0.29	0.62	0.63	0.16	0.49	0.56
Nuc (nmoL)	0.30	0.27	0.32	0.34	1.04	0.81	0.33	0.92	0.47
PM (nmoL)	0.03	0.02	0.22	0.06	0.15	0.57	0.03	0.12	0.42
Total uptake (nmoL)	0.72	0.67	1.04	0.82	2.06	2.42	0.63	1.77	1.86
Uptake (%)	1.3%	1.2%	7.3%	1.4%	3.6%	17.0%	1.1%	3.1%	13.0%

(Dose amount: 57nmoL for 16-3-16 and 16(Py)-S-2-S-16(Py), and 14.25nmoL for16-7N(GK)-16. Cyto-cytosol, Mito-mitochondria, Nuc-nucleus and PM-plasma membrane)

Appendix B- Supplementary Methods and Results

Western blotting procedure

To solubilize membrane proteins, the freshly collected nuclear, mitochondrial and plasma membrane fractions were resuspended in 150µL of ice-cold RIPA buffer (10mM Tris-HCl [pH 8], 150mM NaCl, 5mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS and 1x protease inhibitor cocktail) for 30min on ice. The clarified extracts were collected after centrifugation (15,000 *x g*, 20min, 4˚C). No protein recovery was needed for the cytosolic fraction.

An appropriate volume of 4x Laemmli sample buffer containing 600mM dithiothreitol was added to each fraction, which was then incubated for 10min in a 37˚C water bath. Approximately 20µg of proteins from each fraction was loaded on 4-15% SDS-PAGE gradient gel and electrophoresed for 1.5h at 120V. Proteins were then transferred to nitrocellulose membrane at 400mA for 1.75h. After a 2h room temperature incubation in TBST buffer (20mM Tris [pH 7.5], 150mM NaCl and 0.05% Tween-20) containing 5% non-fat dried milk, the membrane was incubated overnight at 4˚C with a primary antibody specific to each subcellular fraction. The following day, the membrane was washed 6 times with TBST and incubated in secondary antibody for 2h at room temperature. Finally, the membrane was washed 6 times with TBST and developed with an enhanced chemiluminescence assay kit (Bio-Rad, Canada). Immunoreactive bands were visualized using a VersaDoc™ Image System (Bio-Rad Laboratory, Hercules, CA, USA).

Western blotting analysis of subcellular fractions

Western blotting was conducted with organelle- or compartment-specific markers to verify the relative purify of each subcellular fraction. As showed in Figure S2, each marker was most abundant in the expected fraction. The modest amount of SDH70 in fraction 1 likely reflects a small number of residual, unbroken cells in the preparation. The results indicated that differential centrifugation of homogenates allowed for the successful isolation of enriched nuclear, mitochondrial, plasma membrane and cytosolic fractions from PAM 212 cells.

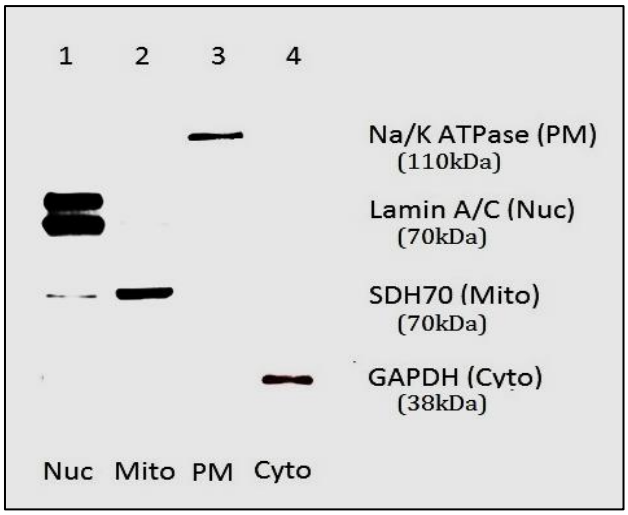


Figure S2. Western blot analysis of the subcellular fractions. Lane 1: nucleus (Nuc); lane 2: mitochondria (Mito); lane 3: plasma membrane (PM); lane 4: cytosol (Cyto). Samples were probed with antibodies for organelle-specific markers. The protein band sizes are: Na^+/K^+ ATPase (110kDa) for plasma membrane, Lamin A/C (70kDa) for nucleus. DH70 (70kDa) for mitochondria, and GAPDH (38kDa) for cytosol.