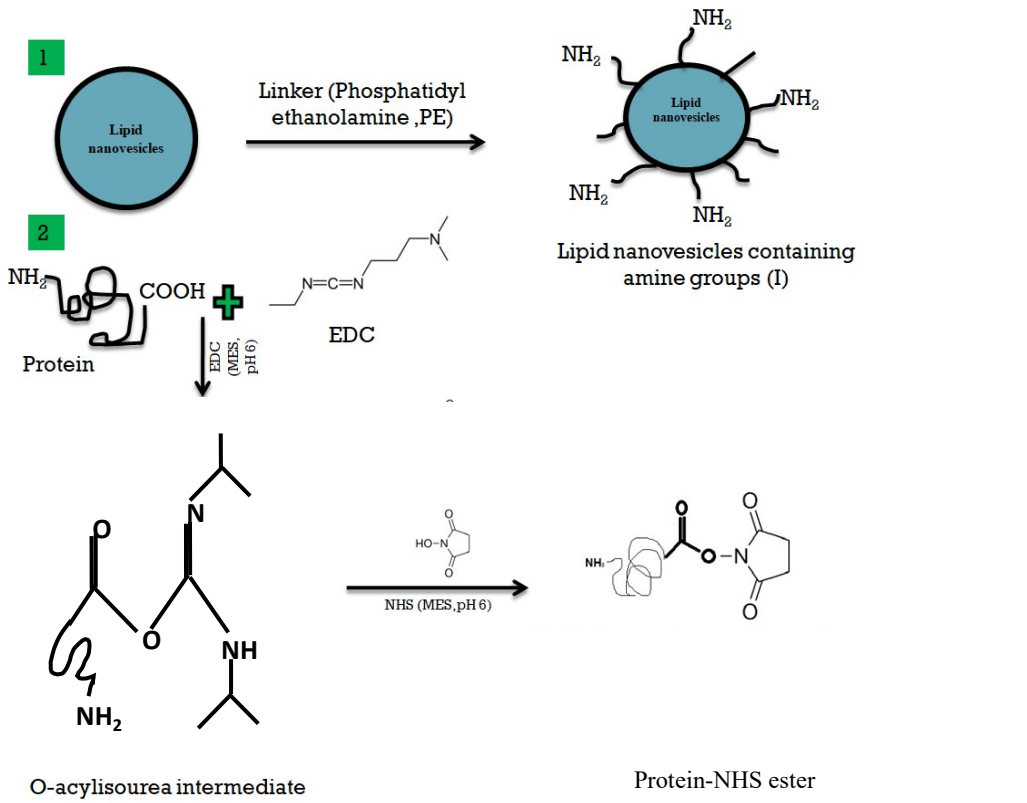
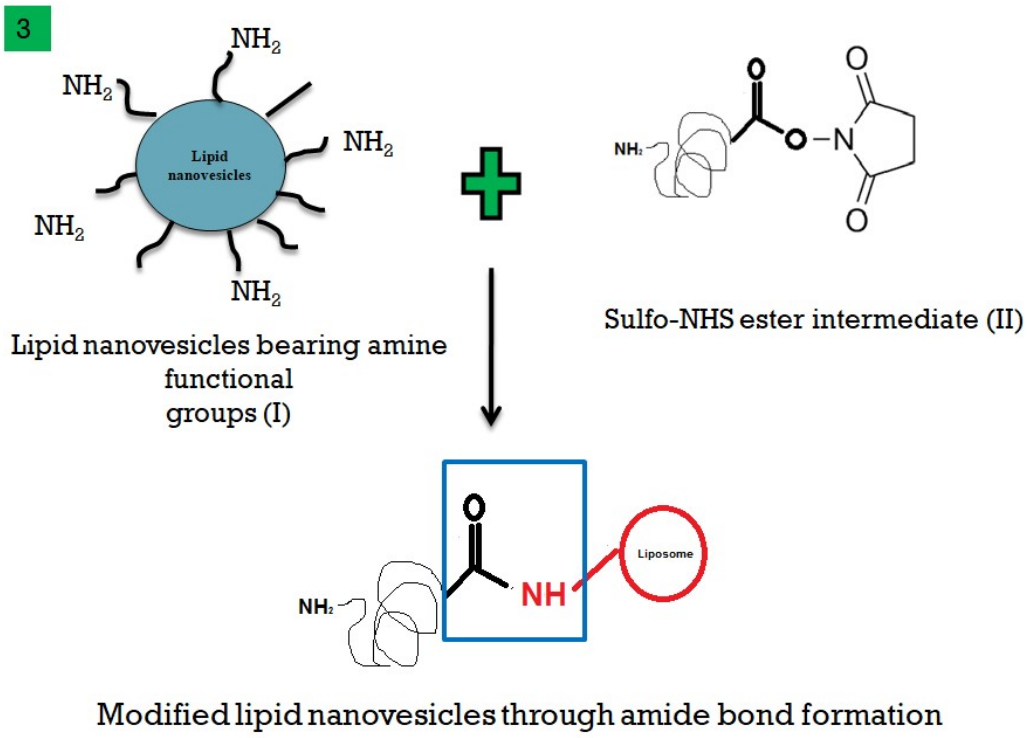
Supplementary Materials

a





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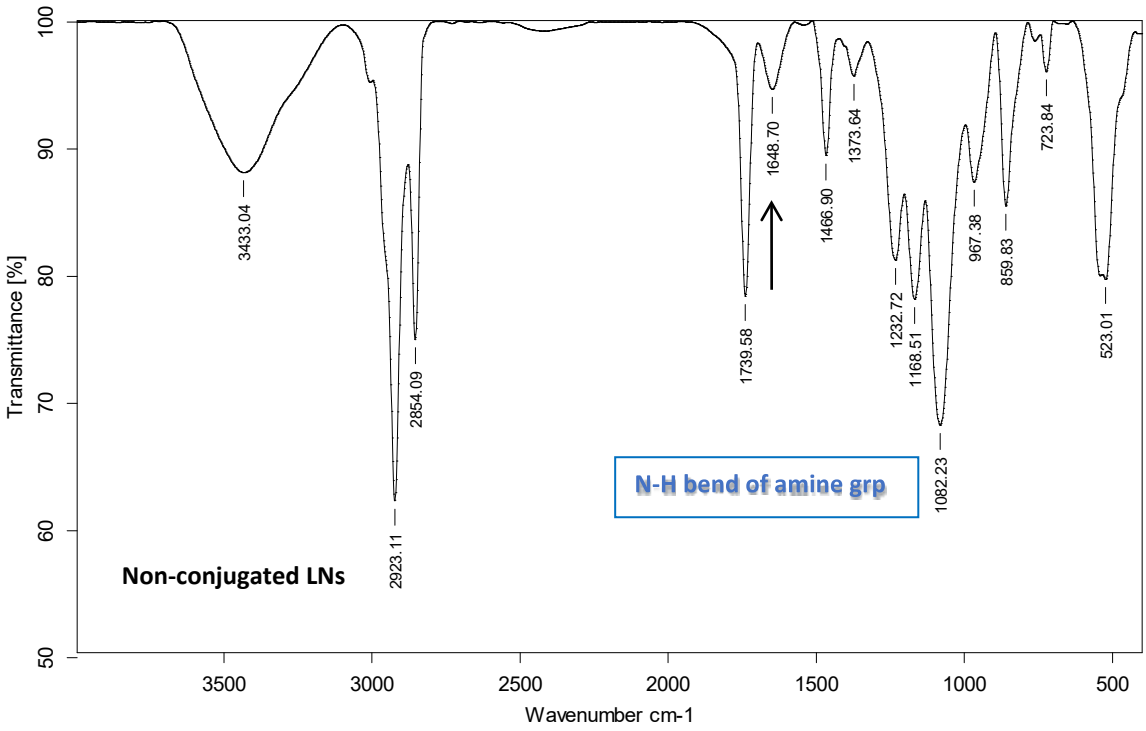
Transmittance [%]
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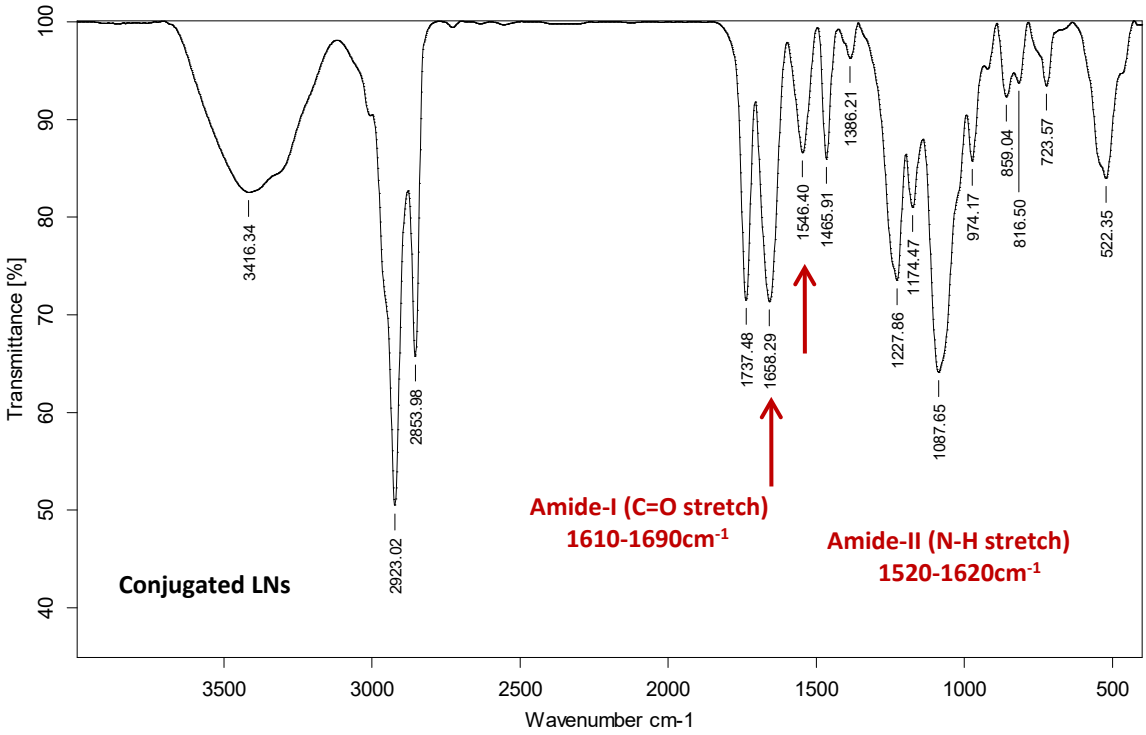
90
80
70
60
50
3433.04
2923.11
2854.09
1739.58
1648.70
1466.90
1373.64
1232.72
1168.51
1082.23
967.38
859.83
723.84
523.01
N-H bend of amine grp
Non-conjugated LNs
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1


Transmittance [%]
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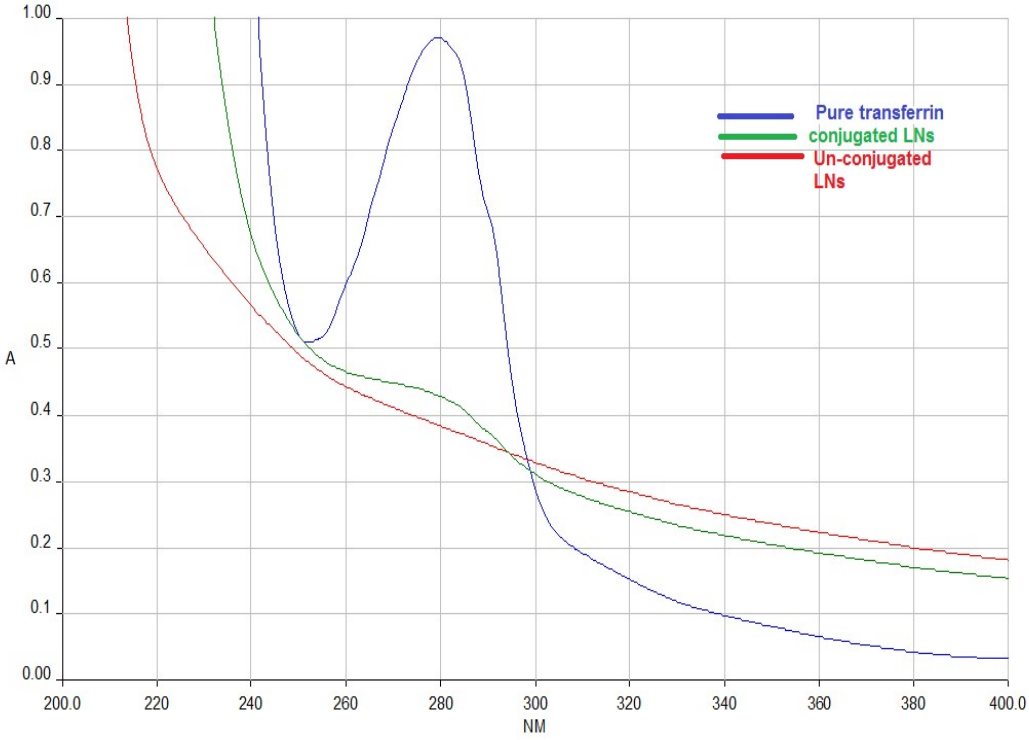
90
80
70
60
50
40
3416.34
2923.02
2853.98
1737.48
1658.29
1546.40
1465.91
1386.21
1227.86
1174.47
1087.65
974.17
859.04
816.50
723.57
522.35
Amide-I (C=O stretch)
1610-1690cm-1
Amide-II (N-H stretch)
1520-1620cm-1
Conjugated LNs
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

c


1.00
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.00
A
200.0
220
240
260
280
300
320
340
360
380
400.0
NM
Pure transferrin
conjugated LNs
Un-conjugated LNs

d

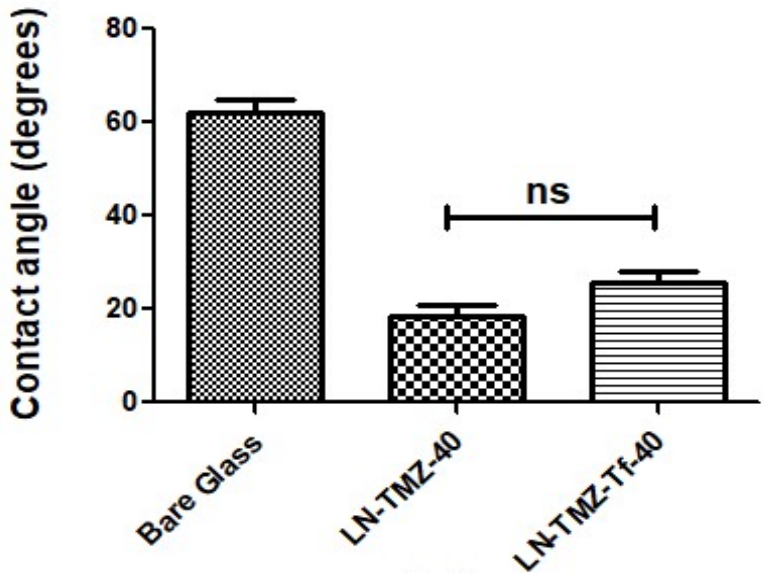
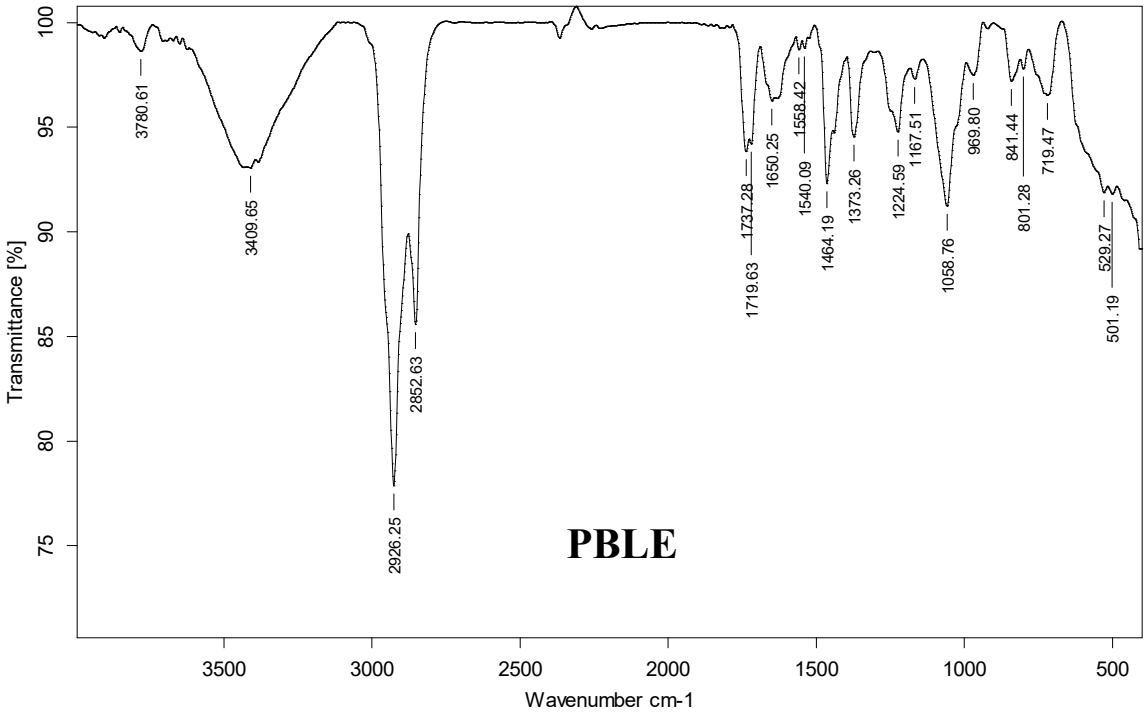

Contact angle (degrees)
80
60
40
20
0
ns
Bare Glass
LN-TMZ-40
LN-TMZ-Tf-40

Figure. S1. (a) Schematic representation of preparation of transferrin (Tf) modified lipid nanovesicles using N-hydroxysuccinimide (NHS) and 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) based chemistry in 2-(N-morpholino) ethanesulfonic acid (MES) buffer at pH 6.0. **(b)** Fourier transformed infra-red spectra (FTIR) of conjugated and non-conjugated lipid nanovesicles (LNs) and **(c)** UV-Visible spectra exhibiting characteristic absorbance peak of pure transferrin, transferrin conjugated and non-conjugated lipid nanovesicles (LNs) **(d)** Contact angle measurement of targeted (LNs-TMZ-Tf-40) and non-targeted (LNs-TMZ-40) nanovesicles with mucin (2%w/v) as the contacting fluid (n=3, mean±SEM, at no significant difference of $^{ns}p \geq 0.05$)

a



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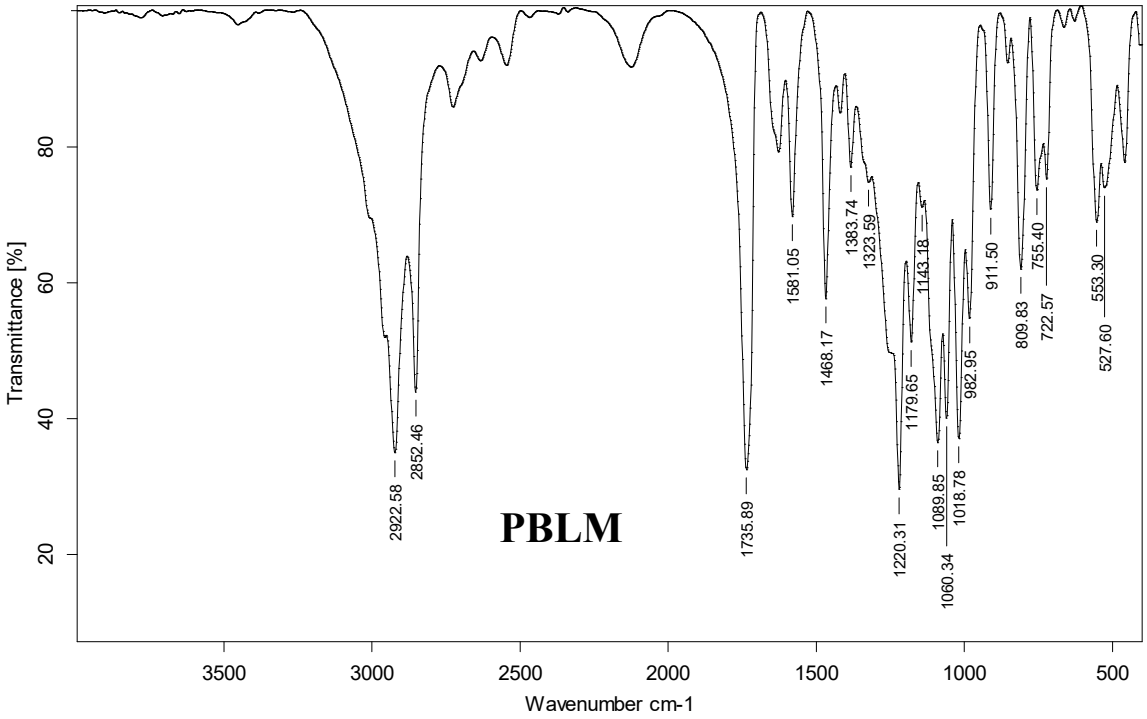

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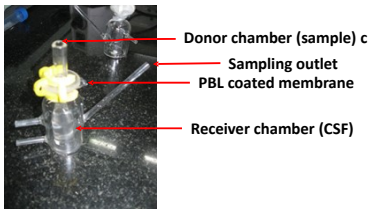
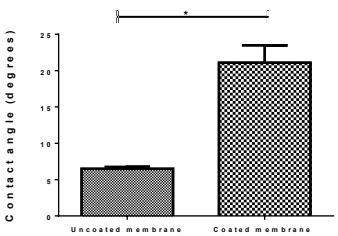
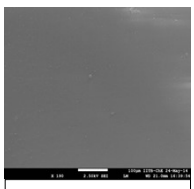
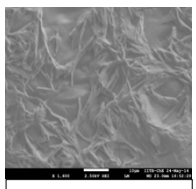


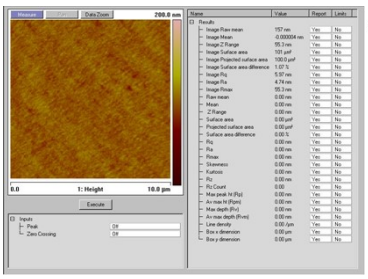




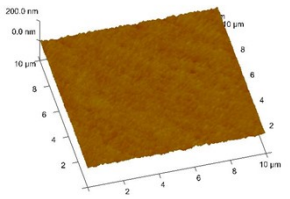
Uncoated membrane



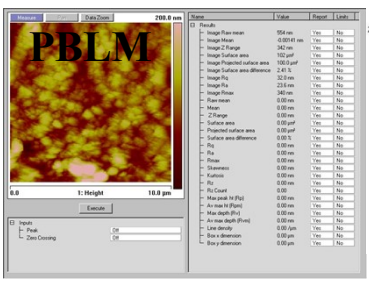
PBL Coated membrane



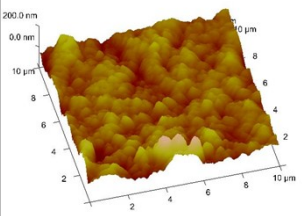
2-D AFM surface of uncoated membrane



3-D AFM surface of uncoated membrane



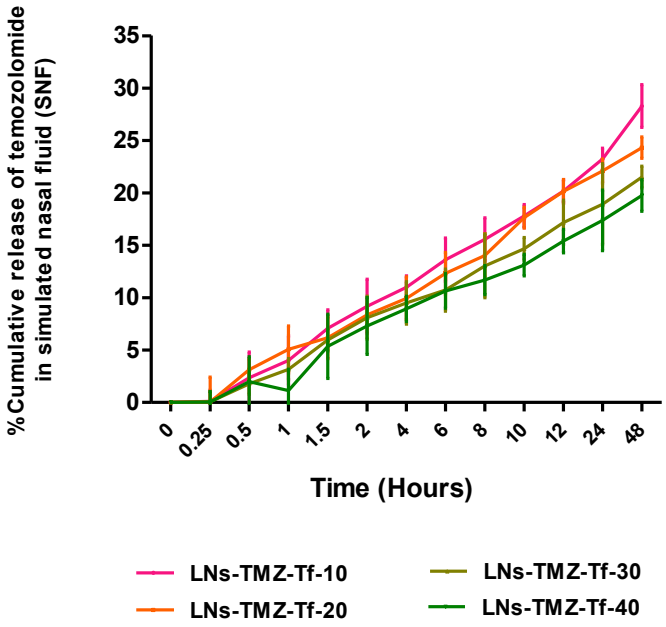
2-D AFM surface of PBL coated membrane



3-D AFM surface of PBL coated membrane

Figure. S2. *Ex-vivo* Parallel Artificial Membrane Permeability Assay (PAMPA). (a,b) Characterization of porcine brain lipid extract (PBLE) by fourier transformed infra-red spectroscopy (FTIR) **(a)** IR spectra of porcine brain lipid extract (PBLE) and **(b)** Polar brain lipids mixture (made by mixing the constituents lipids) to be impregnated on the porous filter for PAMPA study **(c)** Schematic of *ex-vivo* Parallel artificial membrane permeability assay (PAMPA) using Franz's diffusion cells showing contact angle of the porcine brain lipid extract (PBLE) coated membrane with water as the contacting fluid and uncoated membrane as control (*$p \leq 0.05$, $n=3$) with characterizations of membranes by Cryo-FEG-SEM (scale bar: 10µm for PBL coated and 100µm for uncoated membrane) and Atomic Force Microscopy (scale bar: 10µm for PBL coated and uncoated membrane).

a



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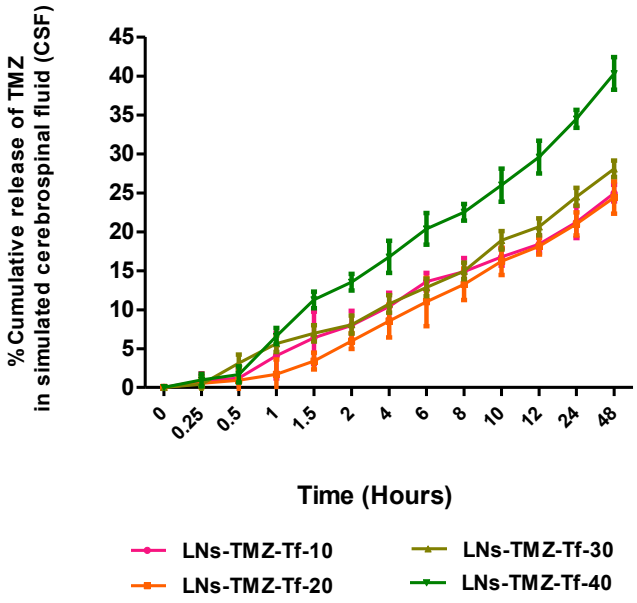
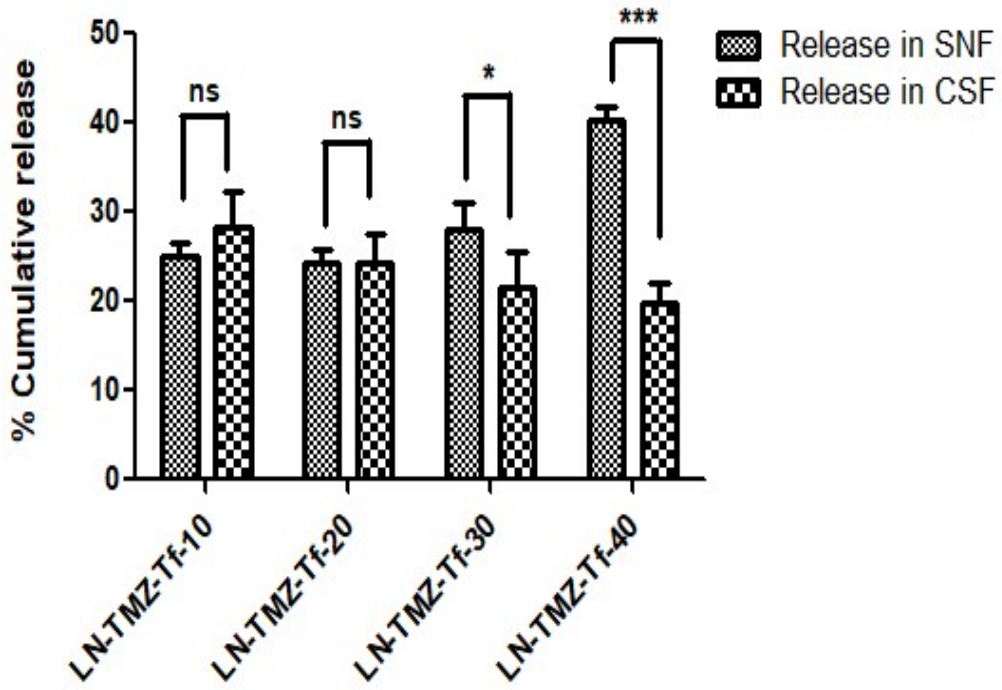
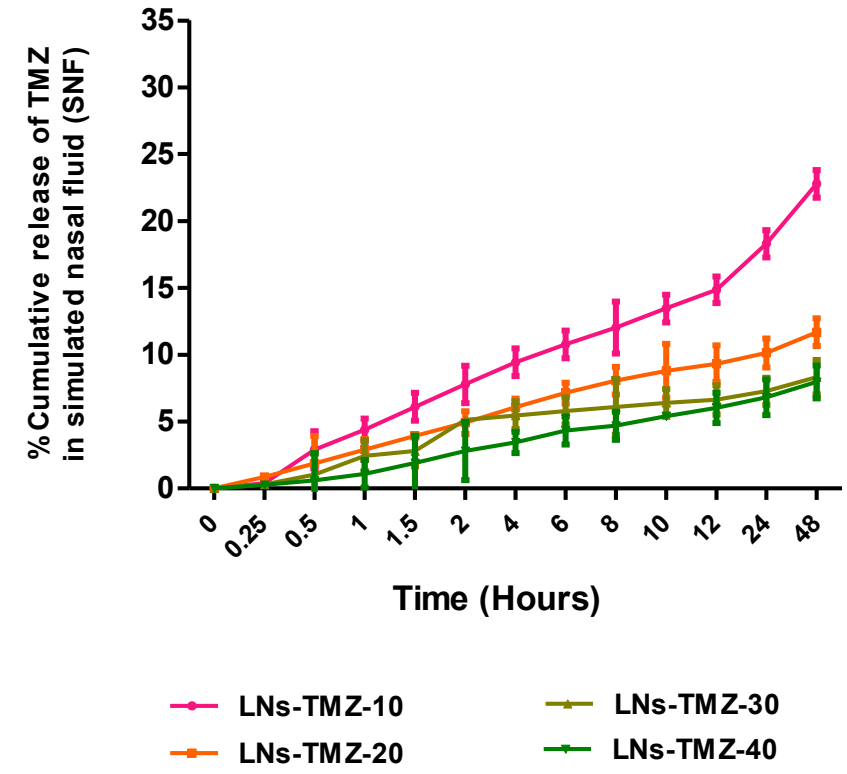

% Cumulative release of TMZ in simulated cerebrospinal fluid (CSF)
45
40
35
30
25
20
15
10
5
0
0
0.25
0.5
1
1.5
2
4
6
8
10
12
24
48
Time (Hours)
LNs-TMZ-Tf-10
LNs-TMZ-Tf-20
LNs-TMZ-Tf-30
LNs-TMZ-Tf-40

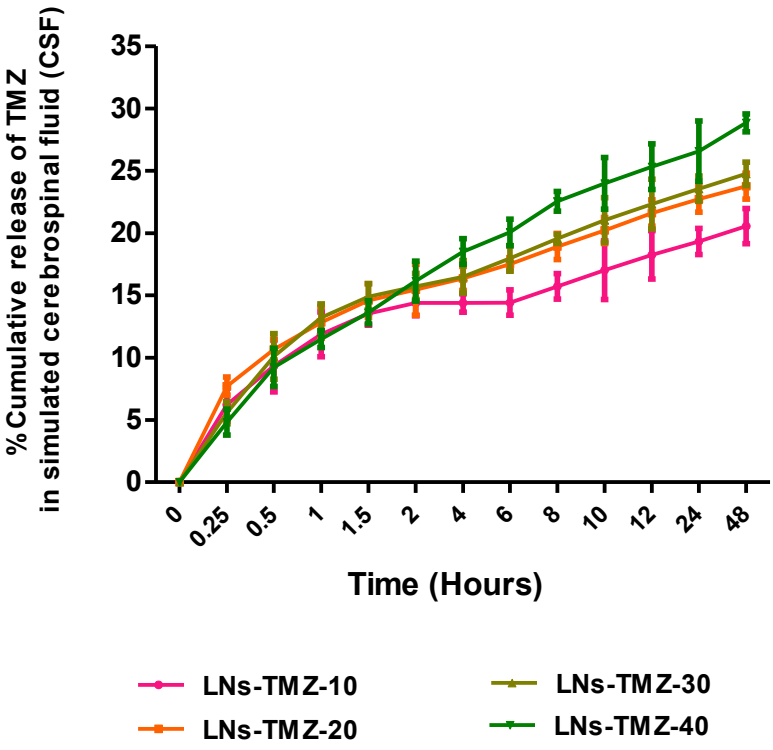
c


% Cumulative release
50
40
30
20
10
0
ns
ns
*
***
Release in SNF
Release in CSF
LN-TMZ-Tf-10
LN-TMZ-Tf-20
LN-TMZ-Tf-30
LN-TMZ-Tf-40

d


% Cumulative release of TMZ in simulated nasal fluid (SNF)
35
30
25
20
15
10
5
0
0
0.25
0.5
1
1.5
2
4
6
8
10
12
24
48
Time (Hours)
LNs-TMZ-10
LNs-TMZ-20
LNs-TMZ-30
LNs-TMZ-40

e


% Cumulative release of TMZ in simulated cerebrospinal fluid (CSF)
35
30
25
20
15
10
5
0
0
0.25
0.5
1
1.5
2
4
6
8
10
12
24
48
Time (Hours)
LNs-TMZ-10
LNs-TMZ-20
LNs-TMZ-30
LNs-TMZ-40

f

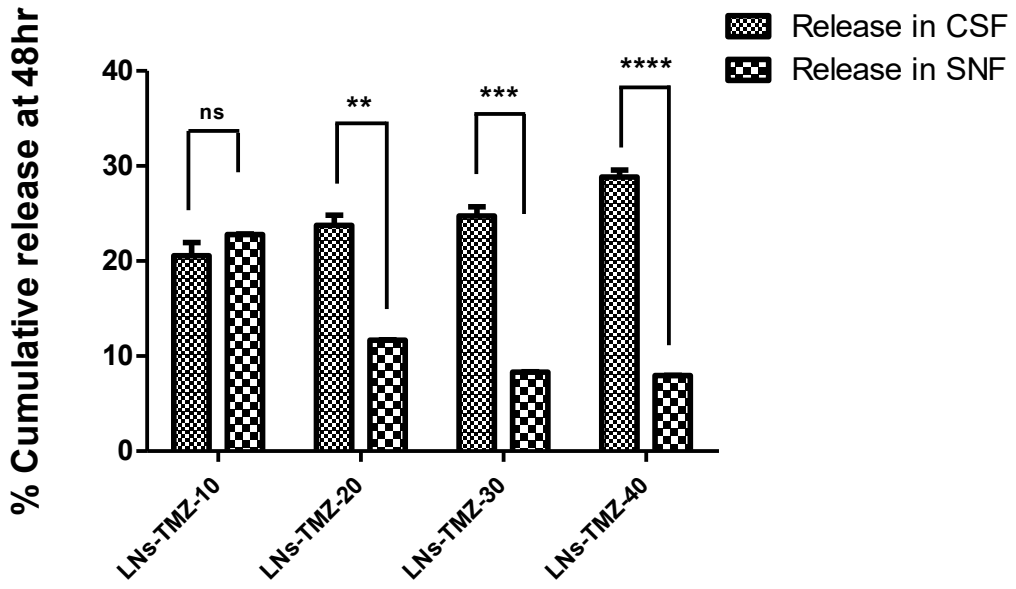


Figure S3. *In-vitro* release kinetics of nanovesicles (**a-c**) Percent cumulative release of TMZ encapsulated targeted nanovesicles in simulated nasal fluid (SNF) and simulated cerebrospinal fluid (CSF) at 37^0C for 48hr and their comparative evaluation in both the fluids at 48hr (**d-f**) Percent cumulative release of TMZ encapsulated non-targeted nanovesicles in simulated nasal fluid (SNF) and simulated cerebrospinal fluid (CSF) at 37^0C for 48hr and their comparative evaluation in both the fluids at 48hr. Values were presented as mean ± SEM, *(n=3)*. One-way ANOVA showing significant difference at $***p \leq 0.001$, $*p \leq 0.05$ and $^{ns}p \geq 0.05$ as non-significant difference*, (n=3*).

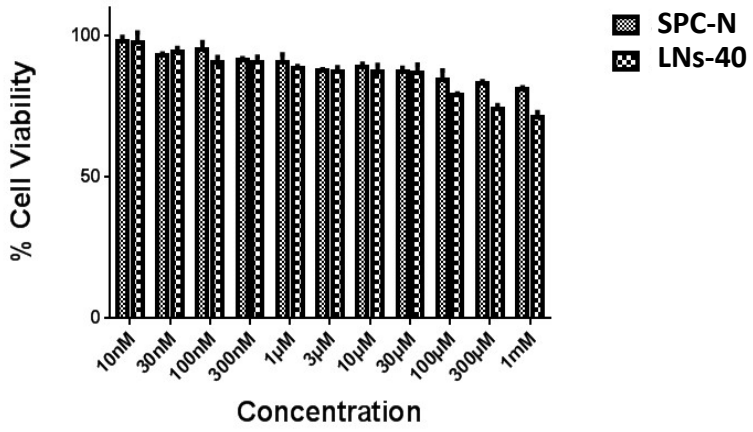


Figure S4. *In-vitro* biocompatibility of SPC nanovesicles (SPC-N) and blank nanovesicles (LNs-40) on fibroblast cells.

Table S1. Band assignments for IR spectra of porcine brain lipid extract

Wave number (cm^{-1})	Vibrations	Assignment
2960	υ_{as} ($C\text{-}H_3$)	Saturated fatty acids
2924	υ_{as} ($C\text{-}H_2$)	Saturated fatty acids
2852	υ_{sym} ($C\text{-}H_2$)	Saturated fatty acids
1737-1720	υ (C=O)	Phospholipids
1368	∂ (CH_3)	Fatty acids
1222-1224	υ_{as} (PO_2)	Phospholipids
1071	υ_s (SO_3)	Sulfatides
970	N^+-(CH_3)	Phosphatidylcholine

Table S2. Stability profile of non-targeted TMZ encapsulated nanovesicles as per ICH guidelines.

Parameters	Time intervals				
	Day 0	1 month		6 month	
		4^0C	25^0C±60RH (%)	4^0C	25^0C±60RH (%)
Particle size (nm)	121±8.2	142.2±3.5	162.8±2.1	169.2±10.9	192.2±3.2
PDI	0.2±0.003	0.3±0.002	0.3±0.002	0.2±0.002	0.2±0.001
Zeta potential (mV)	-29.2±1.8	-30.8±3.9	-30.2±4.4	-30.9±3.1	-31.1±13.2
% EE	87.4±2.6	85.3± 4.2	84.8± 3.9	84.8±12.9	84.5± 13.2

PDI: Polydispersity index,
% EE: Percent encapsulation efficiency
RH: Relative humidity

Table S3. Brain pharmacokinetics profile of targeted and non-targeted TMZ nanovesicles and free drug in tumor free animals.

Time intervals (hrs)		Free drug (p.o)			Free drug (i.n)			LNs-TMZ-40 (i.n)			LNs-TMZ-Tf-40 (i.n)		
		Conc. (µg/ml)	SEM	N	Conc. (µg/ml)	SEM	N	Conc. (µg/ml)	SEM	N	Conc. (µg/ml)	SEM	N
0.0	0.0	0.0000	0.0000	6	0.000	0.000	6	0.000	0.00	6	0.000	0.000	6
0.5	0.5	228.0900	123.7700	6	388.830	78.904	6	554.893	105.09	6	1265.892	56.980	6
1.0	1.0	493.7210	68.8230	6	1089.997	114.890	6	1512.904	92.67	6	1888.977	84.890	6
2.0	2.0	590.0360	74.8300	6	712.083	144.780	6	1178.883	100.42	6	1440.042	62.043	6
4.0	4.0	301.0470	32.0900	6	576.119	32.990	6	1032.760	128.67	6	1205.993	78.883	6
8.0	8.0	182.0978	21.7800	6	357.783	68.620	6	717.877	23.78	6	893.505	30.402	6
24.0	24.0	62.8020	18.9800	6	106.892	57.890	6	401.920	28.67	6	576.922	69.542	6

Table S4. Plasma pharmacokinetics profile of targeted and non-targeted TMZ nanovesicles and free drug in tumor free animals.

Time intervals (hrs)	Free drug (p.o)			Free drug (i.n)			LNs-TMZ-40 (i.n)			LNs-TMZ-Tf-40 (i.n)		
	Conc. (µg/ml)	SEM	N	Conc. (µg/ml)	SEM	N	Conc. (µg/ml)	SEM	N	Conc. (µg/ml)	SEM	N
0.0000	0.0000	0.00	6	0.000	0.000	6	0.000	0.00	6	0.000	0.00	6
0.5000	533.9210	21.03	6	583.908	18.560	6	417.044	18.97	6	378.743	20.92	6
1.0000	958.9930	49.32	6	820.774	39.320	6	685.403	23.78	6	439.919	33.45	6
2.0000	1262.0320	102.10	6	938.566	27.530	6	572.099	31.09	6	344.795	39.77	6
4.0000	523.4180	45.32	6	428.972	40.090	6	318.674	20.98	6	198.932	12.98	6
8.0000	234.9120	19.09	6	209.689	18.998	6	153.554	8.99	6	102.977	18.78	6
24.0000	97.4480	8.47	6	86.058	10.980	6	62.997	12.45	6	39.046	10.34	6