Supporting Information

Experiments

Materials: L-α-phosphatidylcholine (95%, Egg PC, Chicken) were purchased from Avanti Polar Lipids, Inc. PEGDA (Molecular weight: 575), Irgacure 2959, insulin (Human Recombinant, >27.5 units per mg, 91077C), ferric chloride hexahydrate, ammonium thiocyanate, chloroform (ethanol stabilized), poly(ethylenimine) (average molecular weight 25,000) were bought from Sigma-Aldrich. Extrusion polycarbonate membranes were purchased from Whatman GE. Micro BCA assay was purchased from Thermo Fisher.

Nanolipogel fabrication: A conventional procedure was used to form EggPC liposomes (18mM) encapsulating insulin and hydrogel constituents (Figure 1(B)). The Egg PC thin film was prepared using rotary evaporator(40℃, 1h) and then hydrated with a mixture of PBS pH 7.4 containing 2mg/mL insulin, PEGDA with various concentrations (10, 30 and 50% (w/v)) and 0.1% (w/v) photo-initiator (Irgacure 2959). After hydration of the lipid thin film, the dispersion was downsized by an extruder (T001, Northern lipids Inc.) through 0.2 and 0.1 μm membrane subsequently, to form large unilamellar vesicles (LUVs). LUVs suspension was dialyzed against with PBS pH 7.4 and 100 KDa cut off membrane to remove unencapsulated insulin, photo-initiator, and PEGDA monomers. The purified solution was finally exposed to UV-lamp of 365 nm (VL-8.L, Vilber, 1-2mW/cm^2) for 30 minutes forming NLGs. Bare liposomes were prepared by the same method except for the use of PEGDA solution during the hydration.

Encapsulation and Loading efficiency of Insulin Nanolipogels: Encapsulation and loading efficiency is determined from samples prior to crosslinking. To determine the drug amount, the lipid bilayer was dissolved using 1% Triton X100 (v/v). Micro BCA assay was used to determine the concentration of encapsulated insulin. The insulin standard curve samples were prepared with 1% Triton X-100. Encapsulation efficiency is calculated according to equation 1:

$$Encapsulation\ efficiency\ \%\ (EE\%) = \frac{Encapsulated\ insulin}{Total\ insulin} \times 100\% \quad (1)$$

Loading efficiency is calculated according to equation 2:

$$Loading\ efficiency\ \%(LE\%) = \frac{Encapsulated\ insulin\ mass}{Lyophlized\ Nanolipogel\ mass} \times 100\% \qquad (2)$$

Light scattering, Stewart assay, and Zeta potential: Dynamic light scattering (DLS) was done to measure the size of NLGs and confirm the formation of nanogels inside the core of NLGs by Malvern Zetasizer. For the measurement of the nanogel core, 1% v/v of Triton X-100 was added to the NLG to strip off the bilayer. To study the stability of NLGs and core change of NLGs, DLS of NLGs and nanogel cores were measured over time. To determine the lipid concentration in the NLGs, modified Stewart Assay was done according to a published paper[1]. Zeta potential of samples were measured by Malvern Zetasizer. The microstructures of the samples (nanogel cores and liposomes) were examined by a Brookhaven Laser Light Scattering system. Samples were kept at constant temperature (25 °C) for the duration of the measurements. The mean hydrodynamic radius (R_h) was obtained at a scattering angle of 90° based on the CONTIN method by DLS. Static light scattering (SLS) measurements were analyzed in a Berry plot to obtain the root-mean-square radius of gyration (R_g). Measurements were taken every 5° intervals between 50° and 140°. The ratio of R_g and R_h values is generally used to indicate the morphology of the microstructure of the nanoparticles.

In vitro release: To evaluate the release profile, three groups of samples were prepared from the same batch of liposomes: a sample of uncrosslinked NLGs containing hydrogel monomers, photo-initiator and the drug (without UV-polymerization); a sample of NLGs (UV crosslinked), and bare liposomes. Triplicates of 1 ml of each sample were placed in 100kDa dialysis bags and suspended in 20 ml of PBS pH 7.4 (0.1% sodium azide) each. Release samples were placed in a 37 ℃ shaking incubator with a speed of 100 rpm. The concentration of released insulin was measured at regular time intervals using the micro-BCA assay.

Mesh size: The Flory-Rehner equation was used to calculate the mesh size of the nanogel-core inside NLG. In the case of NLG, nanogels are surrounded by lipid bilayers and in a constrained state. Therefore, we modified the calculation equation of mesh size to Equation (3). $V_{2,s1}$ was determined from the Equation (7) based on nanoparticle mass. $V_{2,s}$ was calculated from the hydrodynamic diameter in the equilibrium swollen state by DLS (Malvern Zetasizer Nano ZS). To achieve the equilibrium DLS size measurement, nanogels were incubated in PBS for three days

after the removal of lipid bilayer by 1% Triton X-100. The mesh size is calculated using the following sets of equations:

$$\xi = \left(V_{2,s1}\right)^{-\frac{1}{3}}\left(\bar{r}_0^{\,2}\right)^{\frac{1}{2}} \tag{3}$$

$$\left(\bar{r}_0^{\,2}\right)^{\frac{1}{2}} = l(2M_c/M_r)^{\frac{1}{2}} C_n^{\frac{1}{2}} \tag{4}$$

$$\frac{1}{M_c} = \frac{2}{M_n} - \frac{(\frac{v}{V_1})\left[Ln(1-V_{2,s}) + V_{2,s} + \chi_{12} V_{2,s}\right]}{\left[V_{2,s}^{1/3} - \frac{1}{2}V_{2,s}\right]}$$

(5)

$$V_{2,s} = \frac{V_p}{V_{gel}} = \left(\frac{r_{core}}{r_{eq}}\right)^3 \tag{6}$$

$$V_{2,s} = \frac{V_p}{V_{gel}} = \frac{1}{1 + Q\left(\frac{M_s}{M_d} - 1\right)/\rho}$$

ξ: mesh size, $V_{2,s1}$:polymer volume fraction inside NLG (in the constrained status), $V_{2,s}$:equilibrium polymer volume fraction (equilibrium nanogel after removal of the lipid bilayer of NLG), M_c: average molecular weight between two adjacent crosslinks, M_r: molecular weight of monomer (44), C_n: the Flory characteristic ratio (PEG: 4.0), l: bond length of polymer backbone(1.54×10^{-10}m), M_n: the number average molecular weight, χ_{12}: the solvent-polymer interaction parameter (PEG in water 0.426), V_p: polymer volume, V_{gel}: swollen gel volume, V_{dry}: dry mass of NLG, r_{eq}: the radius of equilibrium nanogel after stripping off the bilayer of NLG (measure it after swelling for 3 days by DLS), r_{core}: the radius of nanogel after stripping off the bilayer of NLG (measure it immediately by DLS), M_s: the swelling mass of NLGs by using ultra-high centrifuge (600,000 g, 1 hour), M_d: the dry mass of NLGs after lyophilization [2].

AFM: The elasticity of NLGs was determined by AFM force measurement according to a previous publication with modifications[3]. All the morphology and force measurements were done in distilled water. To promote NLG adsorption on mica surface, freshly cleaved mica discs (15mm, V1, Pelco, Ted Pella, USA) were immersed in poly(ethylenimine) (PEI) solution (1 g/L, 0.5M NaCl) for 15min, rinsed with distilled water, and dried in a dry box. For the AFM sample preparation, 10 µL NLGs solution was added to PEI coated mica surface and incubated in a covered petri dish at room temperature for 1 hour. Unbound NLG was removed by washing with 10µL distilled water three times. After washing, the NLG absorbed mica was put inside the open

liquid cell of AFM (Park NX10, Korea) and immersed in water immediately. PPP-CONTSCR (contact cantilever, k ~ 0.2 N/m, f ~ 25 kHz, tip radius <10nm) was used in this study. The AFM experiments were performed in contact mode, and the elasticity of NLGs was assessed by the measurement of Young's modulus. The Young's modulus was calculated from the force-distance curve with Hertz equation (Equation (8)) [4].

$$F = \frac{E}{1 - \upsilon^2} \frac{\tan \beta}{\sqrt{2}} \delta^2 \tag{8}$$

Where F is a quasi-static force, δ is the indentation depth, β is the face angle, ν is Poisson's ratio (0.5), and E is the Yung's modulus.

Cryo-TEM: Cryo-TEM images of liposomes and NLGs were acquired under cryogenic conditions in order to avoid drying artifacts. The samples were prepared for cryo-TEM imaging via the plunge freezing method. A 3µl drop of the sample was placed onto a 300 mesh Lacey carbon grid (Ted Pella Inc., Redding, CA), blotted and then plunged into liquid ethane in order to vitrify NLG particles in a thin layer of ice. The samples thus prepared were stored in liquid nitrogen prior to image acquisition to maintain vitrification. Imaging was done using Carl Zeiss Libra 120 Plus system (Carl Zeiss, Jena, Germany) at 120kV with Gatan 626 cryo-holder (Gatan, Inc., Pleasanton, CA). A 2k x 2k CCD camera (Troendle (TRS), Sharpeye) was used to record the images. The sample temperature was maintained below -170 ℃ throughout the experiment.

Table S1 Insulin-nanolipogel particle characterization

	50% NLG	**30% NLG**	**10% NLG**	**Bare liposome**
Final drug concentration (µg/mL)	348.0 ± 48.4	545.9 ± 80.5	954.8 ± 97.3	702.4 ± 70.0
EE (%)	17.4 ± 2.4	27.3 ± 4.0	47.7 ± 4.9	35.4 ± 3.5
Lipid concentration (mg/mL) in final NLG solution	1.23 ± 0.07	4.23 ± 0.30	8.60 ± 0.42	10.42 ± 0.32
LE (%)	27.8 ± 3.1	27.3 ± 5.7	4.1 ± 2.8	4.3 ± 1.2
Size (nm)	190.3 ± 4.3	176.2 ± 13.7	184.4 ± 6.5	190.3 ± 20.5
PDI	0.10	0.10	0.12	0.05

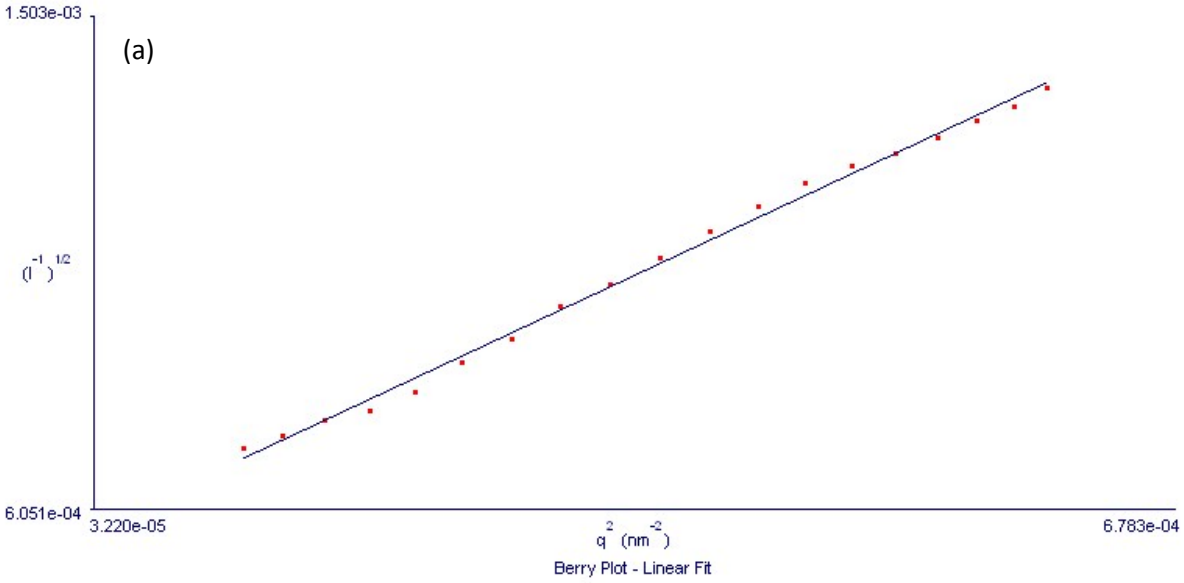
Zeta potential (mV)	1.2 ± 0.1	-1.3 ± 1.1	0.4 ± 0.7	-0.03 ± 0.02

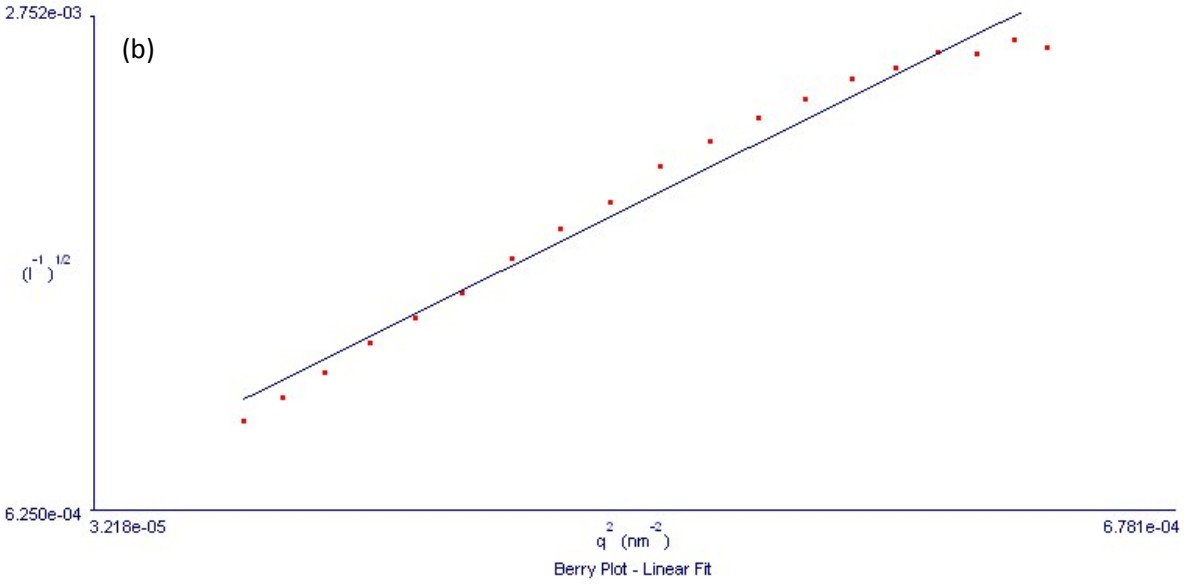
Table S2 Light scattering characterization of nanoparticle structures

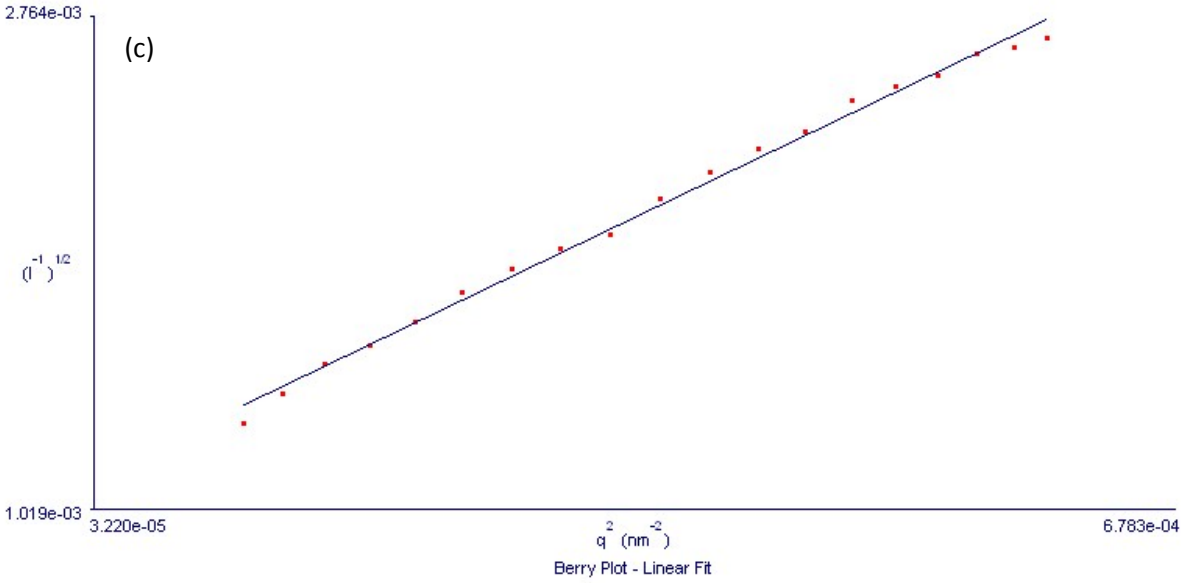
Sample	R_g (nm)	R_h (nm)	R_g/R_h	Structure
Bare liposome	89.1	89.7	0.99	vesicle
30% NLG	165.8	158.0	1.05	vesicle
10% NG	124.6	136.9	0.91	sphere
30% NG	110.0	139.1	0.79	sphere
50% NG	74.7	120.8	0.62	sphere

Table S3 Stability data

	Nanolipogels		Nanogels	
	Mean Size, nm	PDI	Mean Size, nm	PDI
Day 0	171.1	0.12	199.5	0.71
Day 1	178.7	0.1	253.7	0.64
Day 7	170.1	0.18	232.5	0.59
Day 14	172.5	0.12	169.6	0.74
Day 21	177.8	0.13	88.0	0.7
Day 28	168.7	0.22	65.0	1


1.503e-03
(a)
$(I^{-1})^{1/2}$
6.051e-04
3.220e-05
q^2 (nm^{-2})
6.783e-04
Berry Plot - Linear Fit


2.752e-03
(b)
$(I^{-1})^{1/2}$
6.250e-04
3.218e-05
q^2 (nm^{-2})
6.781e-04
Berry Plot - Linear Fit


(c)
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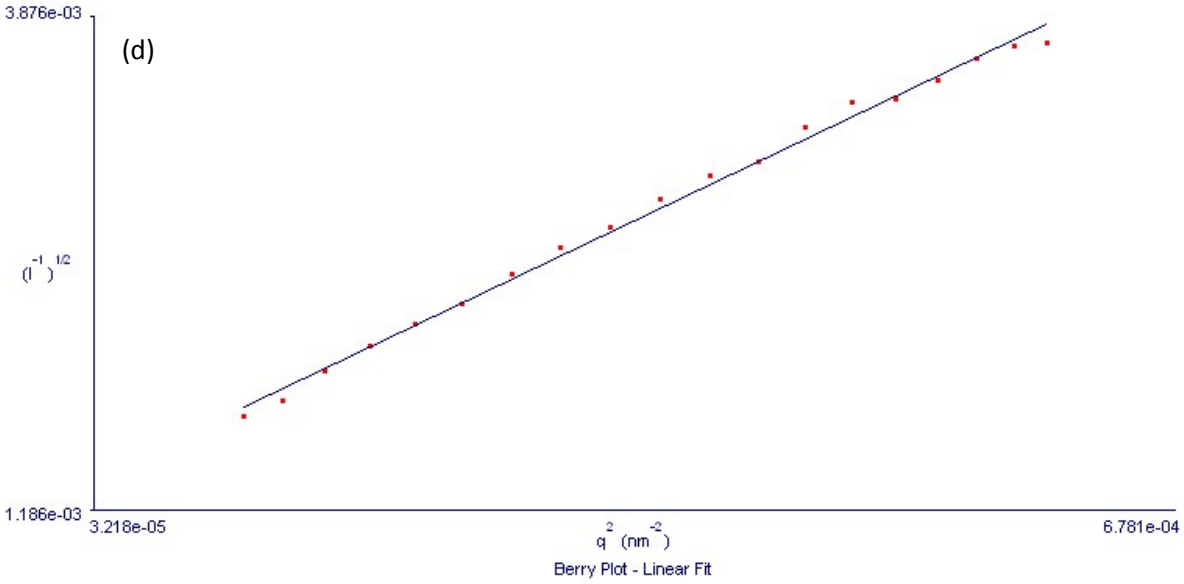
3.220e-05
q^2 (nm^{-2})
6.783e-04
Berry Plot - Linear Fit


(d)
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$(I^{-1})^{1/2}$
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3.218e-05
q^2 (nm^{-2})
6.781e-04
Berry Plot - Linear Fit

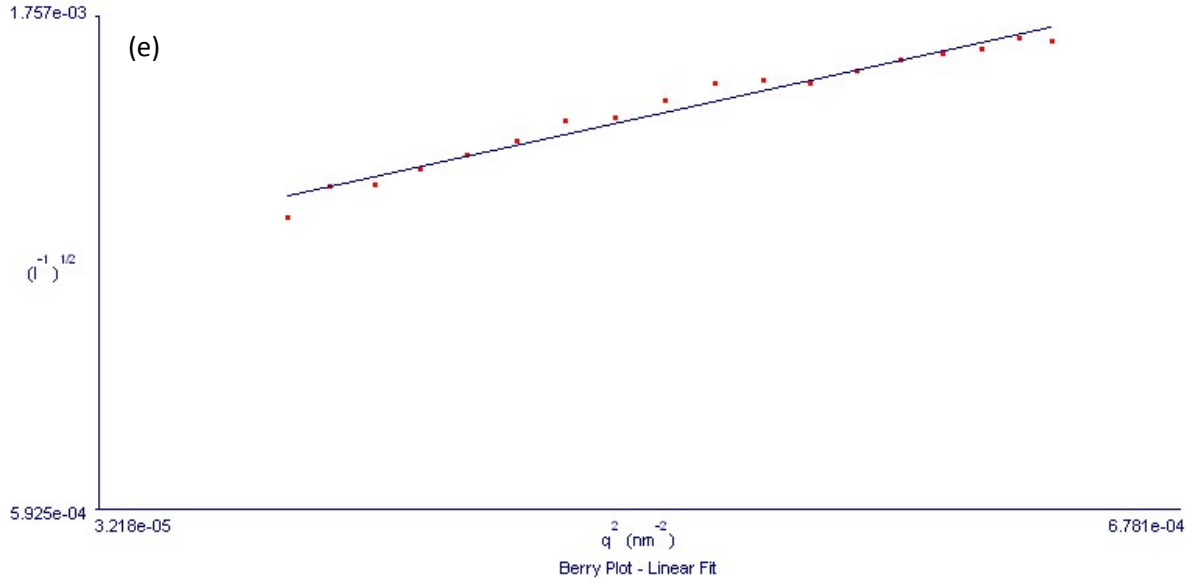

Figure S1: Partial Zimm diagram for (a) bare EggPC liposome, (b) 30% NLG, (c) 10% NG, (d) 30% NG and (e) 50% NG at 25 °C.

References:

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3. A. Pfau, W. Schrepp and D. Horn, *Langmuir*, 1999, **15**, 3219-3225.
4. J. J Roa, G. Oncins, J. Diaz, F. Sanz and M. Segarra, *Recent Patents on Nanotechnology*, 2011, **5**, 27-36.