Thermo-sensitive nanoparticles for triggered release of siRNA
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Efficient delivery of small interfering RNA (siRNA) is crucially required for cancer gene therapy. Herein, a thermo-sensitive copolymer with a simple structure, poly (ethylene glycol) methyl ether acrylate-b-poly (N-isopropylacrylamide) (mPEG-b-PNIPAM) was developed. A novel kind of thermo-sensitive nanoparticles (DENPs) was constructed for the cold-shock triggered release of siRNA by double emulsion–solvent evaporation method using mPEG-b-PNIPAM and a cationic lipid, 3β [N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol [DC-Chol]. DENPs were observed by transmission electron microscopy and dynamical light scattering before and after ‘cold shock’ treatment. The encapsulation efficiency (EE) of siRNA in DENPs, which was measured by fluorescence spectrophotometer was 96.8% while it was significantly reduced to be 23.2% when DC-Chol was absent. DENPs/siRNA NPs exhibited a thermo-sensitive siRNA release character that the cumulatively released amount of siRNA from cold shock was approximately 2.2 folds higher after 7 days. In vitro luciferase silencing experiments indicated that DENPs showed potent gene silencing efficacy in HeLa-Luc cells (HeLa cells steadily expressed luciferase), which was further enhanced by a cold shock. Furthermore, MTT assay showed that cell viability with DENPs/siRNA up to 200 nM remained above 80%. We also observed that most of siRNA was accumulated in kidney mediated by DENPs instead of liver and spleen in vivo experiments. Thus, DENPs as a cold shock responsive quick release model for siRNA or hydrophilic macromolecules delivery provide a new way to nanocarrier design and clinic therapy.

Keywords: thermo-sensitive nanoparticles; siRNA; PNIPAM; double emulsion–solvent evaporation method; cold shock

1. Introduction
The use of small interfering RNA (siRNA) in clinics is limited by its poor delivery into tumor cells \textit{in vitro} and \textit{in vivo} due to its high molecular weight, large size, and negative charge. To overcome this impediment, nanoparticles (NPs) have been employed to facilitate siRNA delivery.\textsuperscript{[1–3]} For example, hydrophilic siRNA was successfully loaded into PLGA NPs using double emulsion–solvent evaporation technique.\textsuperscript{[4,5]} The loaded siRNA is released through diffusion and/or polymer degradation.\textsuperscript{[6]} Therefore,
siRNA released from the mentioned carriers may take days to months. As a result, in some particular conditions, the concentration of siRNA released from these carriers may not be able to reach the therapeutic threshold promptly, especially for treatment of diseases which progresses rapidly. Therefore, it is very desirable to design a stimuli-responsively triggered on-demand release system.

Poly (N-isopropylacrylamide) (PNIPAM), a thermo-sensitive polymer, exhibits a lower critical solution temperature (LCST) in water around 32 °C.[7] Below LCST, PNIPAM is highly soluble in aqueous solution. Above 32 °C, PNIPAM becomes hydrophobic and water insoluble. Cooling can reverse solubility. This unique property of PNIPAM has been widely utilized for drug delivery, bioconjugation, and tissue engineering.[8–12] Herein, we attempted to encapsulate siRNA into DENPs in which a simple structure copolymer with excellent biocompatibility, PEGylated PNIPAM was used as the encapsulation matrix (as shown in Scheme 1). In order to improve the loading capacity of siRNA, cationic DC-Chol was selected to use. Additionally, to evade the RES system and prolong the circulation in the blood, PNIPAM was PEGylated. We hypothesized that the release of siRNA in the system can be accelerated by ‘cold shock’.

![Diagram showing the synthesis of mPEG-b-PNIPAM and the illustration of thermo-sensitive DENPs/siRNA NPs assembled from mPEG-PNIPAM copolymers for cold shock triggered release of siRNA.](image)

Scheme 1. (A) Synthesis of mPEG-b-PNIPAM by RAFT polymerization. (B) Illustration of thermo-sensitive DENPs/siRNA NPs assembled from mPEG-PNIPAM copolymers for cold shock triggered release of siRNA.
2. Materials and methods

2.1. Materials

The RAFT agent, 2-cyanoprop-2-yl N-carbazolylcarbodithioate (CYCBD), was synthesized as described previously.\(^{[13]}\) Poly (ethylene glycol) methyl ether acrylate (mPEG, \(M_n = 2055\)), azobisisobutyronitrile (AIBN), N-isopropylacrylamide (NIPAM), cholesteryl chloroformate, N,N-dimethylethylendiamine, ethanol, and acetone were purchased from Sigma–Aldrich. Dulbecco’s modified Eagle’s medium (DMEM), Lipofectamine 2000 and fetal bovine serum (FBS) were supplied by Invitrogen Corporation (Carlsbad, CA). Dichloromethane (DCM), triethylamine, anhydrous magnesium sulfate, sodium chloride (NaCl), hydrogen chloride (HCl), polyvinyl alcohol (PVA, \(M_n = 30,000\)), chloroform (CHCl\(_3\)), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich. Negative control siRNA (siN.C.), Cy5-labeled siRNA (Cy5-siRNA), siRNA targeting luciferase mRNA (siLuc) were purchased from Suzhou Ribo Life Science Co., Ltd (Suzhou, Jiangsu Province, China), and their sequences were as follows: siN.C.: sense: 5′-Cy5-CCUUGAGGCAUACUUCAAAdTdT-3′, antisense: 5′-UUUGAAGUAUGCCU-CAAGGdTdT-3′; Cy5-siRNA: sense strand: 5′-UUCUCC-GAACGUGUCAGudTdT-3′, antisense strand: 5′-ACGUGACACGUUCGGAGAAdTdT-3′, Cy5 fluorophore was labeled at 50 of the sense strand; siLuc: sense: 5′-CCCUAUUCUCCUUCUUCGCdTdT-3′, antisense: 5′-GCGAAGAAAAUGGAGdTdT-3′.

2.2. Synthesis of mPEG-b-PNIPAM

CYCBD (0.062 g, 0.2 mmol), AIBN (0.010 g, 0.05 mmol), NIPAM (2.26 g, 20 mmol), and THF (4 mL) were added to a dry schlenk flask which was degassed by three freeze-pump thaw cycles. The polymerization was carried out at 60 °C for 24 h. Then, poly (ethylene glycol) methyl ether acrylate (mPEG, \(M_n = 2055\), 0.411 g, 0.1 mmol), AIBN (0.005 g, 0.025 mmol), and THF (2 mL) were added to the above flask and purged by three repeated vacuum/nitrogen cycles. The polymerization was further carried out at 60 °C for 12 h. The product was purified by dialysis (MWCO 3500) in deionized water for 2 days and lyophilized. Yield: 94.2%.

2.3. Characterization of mPEG-b-PNIPAM

The \(^1\)H NMR spectra of the polymers were acquired on a Varian INOVA 500 MHz nuclear magnetic resonance instrument was used to study the molecular structure and composition of mPEG-b-PNIPAM. The solvent is CDCl\(_3\). \(^{13}\)C NMR spectrum of mPEG-b-PNIPAM in CDCl\(_3\) was also recorded. The gel permeation chromatography (GPC) system (Viscotek GPCmax, Malvern), equipped with a porous styrene divinylbenzene copolymer-based column (CLM3009, T6000 M, GeneralMixed, Org 300 × 7.8 mm), was used to measure the molecular weight distributions of mPEG-CYCBD and mPEG-b-PNIPAM. THF was used as an eluting solvent at the rate of 1 mL/min, and polystyrene was used as the molecular weight standard.

The thermal behaviors of dry mPEG-b-PNIPAM were characterized on the differential scanning calorimeter (NETSCZ 204, NETSCZ, Germany). The dry copolymer specimens were first heated from 0 to 60 °C under a nitrogen atmosphere at a heating rate of 5 °C/min, then cooled to 0 °C, and then reheated again to 60 °C at the same rate.
2.4. Synthesis of 3β [N-(N′, N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol)

Triethylamine (1.53 mL, 11 mmol) and cholesteryl chloroformate (4.5 g, 10 mmol) were dissolved in 40 mL anhydrous dichloromethane. N,N-Dimethylhexylenediamine (0.969 g, 11 mmol) dissolved in 10 mL anhydrous DCM was added dropwise to the solution in ice–water bath under stirring. Afterwards, the mixture was stirred at room temperature for 24 h. Then, DCM was evaporated and the raw product was washed with 1 N HCl in saturated NaCl (150 mL) by three times. The obtained solution was dried over anhydrous magnesium sulfate and evaporated to dryness. The crude product was recrystallized from ethanol and acetone, respectively, to obtain DC-Chol as a white powder. Yield: 72.8%.

2.5. Preparation of nanoparticles (DENPs) with siRNA encapsulation

siRNA-loaded nanoparticles were prepared by a double emulsion–solvent evaporation technique.[14] For example, an aqueous solution of siRNA (0.2 mg) in 25 μL of RNase free water was emulsified by sonication for 1 min at 37 °C in 0.5 mL of chloroform containing 1.0 mg of DC-Chol and 25 mg of mPEG-b-PNIPAM. The primary emulsion was further emulsified in 5 mL of PVA solution (1 mg/mL) by sonication (80 W for 2 min) at 37 °C to form a water-in-oil-in-water emulsion. The mixture was then added to 15 mL of RNase free water which was further stirred for 4 h to allow for the complete evaporation of chloroform.

2.6. Characterization of DENPs

The hydrodynamic size of DENPs/siRNA in water was studied by a dynamic light scattering (DLS) instrument (BrookhavenBI-200SM) at λ = 532 nm. Measurements of scattered light were made at an angle of 90° to the incident beam.

Morphology of DENPs/siRNA before and after cold shock was observed by the transmission electron microscopy (TEM) (JEM-100CX II). The sample before ‘cold shock’ was prepared by dipping the DENPs solution which was pre-equilibrated at 37 °C on the Formvar-coated copper TEM grid and dried in oven at 37 °C before measurement. The cold shock samples prepared were pre-equilibrated at 4 °C by adding a drop of polymeric nanoparticles solution on the grid and dried in the air. The colloidal stability of DENPs in PBS 7.4 with or without 10% FBS were investigated by DLS.

Encapsulation efficiency of siRNA in DENPs was determined by fluorescence spectrophotometer (Cary Eclipse-VARIAN). DENPs encapsulated with Cy5-siRNA were fetched and centrifuged (30,000 × g, 0.5 h) then the concentration of Cy5-siRNA in the supernatant was determined by fluorescence spectrophotometer at 664 nm using a standard curve method. The standard curve was constructed for concentrations in the range of 0.25–5 ng/μL using the peak areas by linear regression analysis. siRNA encapsulated into DENPs was calculated by subtracting the amount of siRNA in the supernatant from the total amount used for loading,[14] and the encapsulation efficiency (EE) was calculated according to Equation (1):

$$EE(\%) = \frac{\text{Amount of loaded siRNA}}{\text{Total amount of feeding siRNA}} \times 100\% \quad (1)$$
2.7. In vitro release of siRNA from DENPs
DENPs encapsulated with Cy5-siRNA were suspended in 2 mL of phosphate buffer (0.01 M, pH 7.4) in triplicate, and incubated at 37 °C with gentle shaking (70 rpm). Samples were taken at predetermined intervals and centrifuged (30,000 × g, 0.5 h) to monitor the release of siRNA. In the cold shock group, 15 min cold shocks at 4 °C were done before samples were taken at predetermined intervals. The concentration of Cy5-siRNA in the supernatant was determined by a Varian fluorescence spectrophotometer (Cary Eclipse-VARIAN) at room temperature, and the released amount of Cy5-siRNA was calculated accordingly.

2.8. In vitro cytotoxicity in HeLa-Luc cells
In vitro cytotoxicity of DENPs/siN.C. was determined by MTT assay. HeLa-Luc cells were seeded in 96-well plates at 1 × 10⁴ cells per well in 100 μL of complete DMEM medium supplemented with 10% FBS, and incubated at 37 °C in 5% CO₂ atmosphere for 24 h and subsequently treated with DENPs/siN.C. After 24 h incubation, 5 μL of MTT solution (10 mg/mL in PBS) was added to each well and incubated at 37 °C in 5% CO₂ for 4 h. The solution in the wells was aspirated gently and 50 μL of DMSO was added into each well to dissolve the formazan crystals and further incubated for 30 min at 37 °C. In the cold shock group, the cells were treated at 4 °C for 15 min cold shocks after 24 h incubation. Finally, the absorbance was read at 540 nm with a reference wavelength of 650 nm and the absolute absorbance (ODnet540) is OD540-OD650.

For comparison of relative viability, all data are presented as the mean percentage ± SD in pentaplicate samples when compared with the absorbance value of mock-treated cells. Cell viability was calculated as:

\[
\text{Cell viability (\%)} = \left( \frac{\text{ODnet540 (sample)}}{\text{ODnet540 (mock)}} \right) \times 100\%
\]  

(2)

where ODnet540 (sample) is the absorbance at 540 nm of the transfected cells and ODnet540 (control) is the absorbance at 540 nm of the mock control (non-transfected cells).

2.9. In vitro luciferase silencing in HeLa-Luc cells
In vitro luciferase silencing in HeLa-Luc cells was performed as before with three wells per sample. In brief, the HeLa-Luc cells were seeded in 24-well plates with 5 × 10⁴ cells per well one day before transfection. Then the medium were removed and replaced by serum-free DMEM. Subsequently predetermined DENPs containing siRNA was added into each well. The siLuc was used to evaluate the silencing efficiency, and Lipofectamine were used as the positive controls. After 4 h post-transfection at 37 °C, the cells were incubated for 15 min at 4 °C for cold shock treatment. The cells without the cold shock treatment were incubated for additional 15 min at 37 °C. After additional 20 h incubation, the total protein was measured according to a BCA protein assay kit (Pierce, Rockford, IL).

2.10. In vivo experiments
Animals were maintained in the Center for Experimental Animals (an AAALAC accredited experimental animal facility) at Peking University. All procedures involving experimental animals were performed in accordance with protocols approved by the
Committee for Animal Research of Peking University, China, and conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985). For distribution test, male C57 mice, 5–6 weeks, and weighing 18–22 g, were supplied by the Academy of Military Medical Sciences of China. The DENPs/siRNA complexes (at the dose of 1 mg/kg) were administered via tail-vein injection. Three days later, mice were sacrificed by cervical dislocation, and the major tissues were isolated and examined using the Kodak in vivo imaging system. Normal saline was used as the negative control while naked siRNA was used as the positive control.

3. Results and discussion

3.1. Characterization of mPEG-b-PNIPAM

mPEG_{45}-b-PNIPAM_{110} was synthesized using reversible addition–fragmentation chain-transfer (RAFT) polymerization method as described. ^1H NMR and ^13C NMR spectra confirmed the chain structure as well as chemical composition of mPEG_{45}-b-PNIPAM_{110} (Figure 1(A) and Figure S1). The weight, molecular weight, and weight polydispersity of mPEG_{45}-CYCBD and mPEG_{45}-b-PNIPAM_{110} were determined by GPC as shown in Figure S2. The LCST of mPEG_{45}-b-PNIPAM_{110} was measured by DSC using the peak temperature of endotherm. As shown in Figure 1(B), the LCST of mPEG_{45}-b-PNIPAM_{110} was 28.7 °C, lower than that of PNIPAAm homopolymer as reported.\[^{15}\]

The thermodynamic basis for LCST depression has been elaborated.\[^{16,17}\] In brief, the addition of mPEG caused an ordering of water molecules, and then decreased the entropy of mixing. The more negative mixing entropy led to the depressed LCST. The temperature of mouse and human are 35–37 °C, so the lower LCST of mPEG_{45}-PNIPAM_{110} meant the PNIPAM segments remained hydrophobic in the systemic circulation, keeping the intact nanoparticle formation of DENPs with encapsulated siRNA. In addition, DC-Chol was made by the reaction of cholesteryl chloroformate and N,N-dimethylethylenediamine. The ^1H NMR spectrum is shown in Figure 2. As reported, the EE of siRNA was low if there is no cationic composition. So, cationic polymers as PEI were introduced to the system through a double emulsion method to enhance the

![Figure 1. (A) ^1H NMR spectra of mPEG_{45}-b-PNIPAM_{110}. (B) Endotherm of mPEG_{45}-b-PNIPAM_{110}.](image-url)
EE.[18,19] However, because of hydrophilic nature of PEI, which might lead to siRNA diffusion into the external aqueous phase during the preparation, the EE was about 79% as reported by Panyam and his co-workers which was also relatively lower.[20] Besides, the toxicity caused by non-degradable PEI may be another restriction for its application.[13] Therefore, in this work, we introduced DC-Chol to enhance the EE.

3.2. Characterization of DENPs

DENPs/siRNA NPs were prepared by a modified double emulsion method. In the first emulsion, small droplets of siRNA solution were dispersed in chloroform containing mPEG$_{45}$-b-PNIPAM$_{110}$ and cationic DC-Chol by probe sonication. The polar, cationic head group of amphiphilic DC-Chol will form complexes with siRNA at the water–oil interface. The hydrophobic part of DC-Chol will stay in the organic phase which is a key to stabilize the emulsion. In the second emulsion, PVA was used as a surfactant. To maintain the hydrophobicity of PNIPAM, the experiment was carried out at 37 °C.

The size distribution and zeta-potential of DENPs/siRNA were measured by DLS. The results were shown in Table 1. The morphology of DENPs/siRNA was observed by TEM. As shown in Figure 3(A), DLS size distribution of DENPs at 37 °C exhibited a sharp single peak at 220 nm while the zeta-potential was $-3.5 \pm 1.3$ mV. TEM images revealed that the size of DENPs at 37 °C was approximately 200 nm with a

| Formulation      | Diameters $^a$ (nm) | PDI $^a$     | Zeta-potential $^a$ (mV) |
|------------------|---------------------|--------------|--------------------------|
| Cold-shock NPs   | 550.2 ± 11.5        | 0.718 ± 0.09 | 3.9 ± 2.6                |
| Untreated NPs    | 198.7 ± 6.3         | 0.129 ± 0.02 | $-3.5 \pm 1.3$           |

$^a$Measured by DLS at a concentration of 1.0 mg/mL.
well-defined spherical shape (Figure 4(A)). To test the thermo-sensitivity of DENPs/siRNA, the size and morphology of DENPs/siRNA after ‘cold shock’ were also examined. Resultantly, DENPs exhibited two peaks at 120 and 611 nm as shown in Figure 3(B). When the temperature was below LCST of mPEG45-b-PNIPAM110, PNIPAM segments became hydrophilic. The outer layer thus began to dissociate, leading to the smaller peaks of DENPs; the PEG shielding caused particle aggregation and the peak at 611 nm. TEM showed the same result. From TEM, it was observed that the edge of DENPs appeared to be blurry after the cold shock. In Figure 4(B), obvious space between the shell and core could be observed (red arrows) while the hydrophilic outer layer seemed to be dissociated from the DENPs so that the particle distribution became more heterogeneous.

Figure 3. (A) Size distribution of DENPs/siRNA at 37 °C. (B) Size distribution of DENPs/siRNA after 15 min cold shock.

Figure 4. (A) TEM of DENPs/siRNA at 37 °C. (B) TEM of DENPs/siRNA after 15 min cold shock.
3.3. *In vitro release of siRNA from DENPs*

Firstly, the siRNA loading was characterized. The EE of siRNA in DENPs/Cy5-siRNA NPs, which was measured by fluorescence spectrophotometer for more than 4 times, was 96.8%. However, it was significantly reduced to be 23.2% when the DC-Chol was absent. The standard curve was shown in Figure S4. We further studied the *in vitro* release profile of siRNA from DENPs with or without cold shocks at pH 7.4. A 15 min cold shock at 4 °C was done at predetermined intervals before the samples were taken and measured. As shown in Figure 5(A), the cold shocks facilitated the siRNA release when compared to the control group. At the first sampling point at 12 h, promoted siRNA release was achieved abruptly after the shock. Compared with the control, the cumulatively released amount of siRNA from cold shock was approximately 2.2 folds higher after 7 days as schematically illustrated in Figure 5(B). We believed that the outer layer of the core consisted of PNIPAM segments, interacted with water, and became soluble after the cold shock, which started to dissociate from the NPs. After the dissociation, the layer next to outer layer was exposed to water, and the dissociation process occurred subsequently. During the dissociation, pore canal or space was formed in the core, which accelerated the siRNA release.

3.4. *In vitro cytotoxicity in HeLa-Luc cells*

We used HeLa-Luc cells, which stably expressed luciferase, to evaluate the gene silencing activity and to testify whether the gene silencing was thermo-sensitive. First of all, in order to detect the biocompatibility of DENPs, the cell viability determined by MTT was done and the *t*-test results showed that there was no significant difference in HeLa-Luc cells activities treated with DENPs with/without a 15 min cold shock (*p > 0.19). As shown in Figure 6(A), cell viability with DENPs/siN.C. up to 200 nM remained above 80% relative to untreated cells, suggesting its good biocompatibility.

3.5. *In vitro luciferase silencing in HeLa-Luc cells*

In the gene silencing experiments, cells were treated with DENPs encapsulating siLuc at different doses from 50 to 250 nM, and the luciferase expression was analyzed after 24 h. As shown in Figure 6(B), free siLuc at 300 nM did not exhibit silencing

![Figure 5](image-url)
efficiency, while DENPs with increasing siLuc dose showed significantly improved luciferase expression knockdown efficacy. Compared to positive control, which was transfected with Lipofectamine 2000 at 50 nM siLuc, DENPs/siLuc at dose of 150–250 nM showed improved gene silencing efficacy. It is notable that a cold shock at 150 and 200 nM siLuc significantly enhanced luciferase silencing efficiency. t-test assay showed significant differences between untreated and cold shock groups at the dose of 150 and 200 nM.

3.6. In vivo experiments

Biodistribution of DENPs/siRNA was investigated. DENPs/Cy5-siRNA NPs were intra-venously injected into C57 mice at a dose of 1 mg/kg siRNA. Three days post IV injection, the organs of mice were collected and examined using ex vivo imaging system. As shown in Figure 7(A) and (B), the higher amount accumulations of siRNA delivered by DENPs in all the examined organs than the naked siRNA indicate that DENPs can prolong the circulation of siRNA. Especially, siRNA was dominately located in kidney in the case of DENPs with 4 times of naked siRNA, which means DENPs may provide a potential way in kidney therapeutics. Naked siRNA has been reported to accumulate in kidney and selectively suppresses gene function in renal proximal tubules.[21] Nevertheless, previous works showed siRNA delivered by polymer [22] or inorganic nanoparticles [23] were mainly accumulated in liver, spleen, and lung. On one hand, the PEGylation in DENPs might reduce the opsonization and aggregation of vectors and minimize the clearance by RES [24] and different from the most positive-charged siRNA carriers, DENPs with zeta-potential of $-3.5 \pm 1.3$ mV might show quite different distribution behavior such as the dominated accumulation in kidney. Most of the siRNA was delivered into kidney instead of liver, lung, and spleen as shown in Figure 7(A) and (B). On the other hand, DENPs might prolong the circulation half-life of siRNA in kidney which enhanced the siRNA enrichment in kidney. Further detailed works such as the biodistribution and the antitumor efficiency in tumor-bearing mice with or without a ‘cold shock’ will be done.

Figure 6. (A) Cell viabilities. (B) Relative luciferase expression in HeLa-luc cells after treatment with DENPs/siLuc with or without cold shock. Each bar represents the mean S.E. of three experiments. *$p < 0.05$. 

[Image of Figure 6]
The characteristic of the DENPs that exhibited a ‘cold shock’ responsive siRNA release makes them a high potential in therapies for cancers or other diseases combined with hypothermic patches or local cryosurgery probes, since the cooling and heating steps of cold shock are a usual part of a cryotherapy procedure [25] which has been extensively studied and shown a high potential when compared to the radical surgical intervention.[26,27]

4. Conclusion
In summary, a thermo-responsively triggered on-demand siRNA release system was designed and engineered by double emulsion–solvent evaporation technique using a simple structure copolymer, mPEG-b-PNIPAM which exhibited good biocompatibility. The cold shock changed the morphology and size of DENPs, contributing to the facilitated siRNA release from DENPs in vitro. DENPs showed potent gene silencing efficacy in HeLa-luc cells, which was further enhanced by a cold shock. In vivo distribution showed that most of the siRNA was accumulated in kidney instead of liver and spleen. This work provided a cold shock responsive quick release model for siRNA or hydrophilic macromolecules release.

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Figure 7. (A) In vivo Cy5-siRNA distribution in isolated organs at 3 days after intravenous injection of 1 mg/kg Cy5-siRNA to C57 mice with DENPs. (B) Mean fluorescence intensities of DENPs/Cy5-siRNA in isolated organs. Each group contained three mice.
Supplemental data

Supplemental data for this article can be accessed http://dx.doi.org/10.1080/09205063.2014.997559.

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