SUPPORTING INFORMATION

Supplementary Data

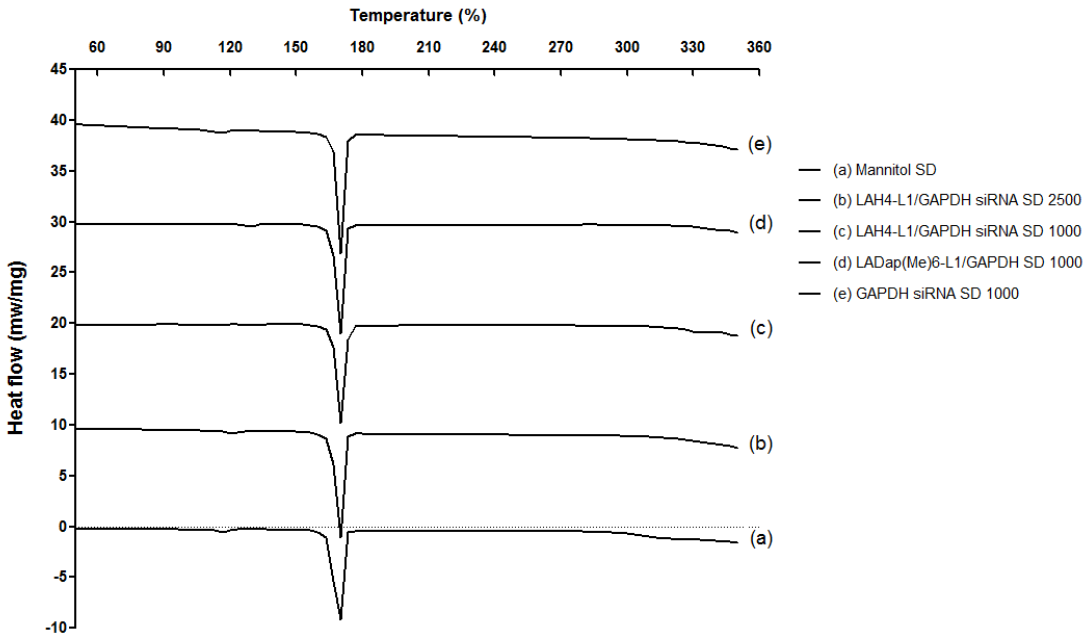


Figure S1. Differential scanning calorimetry (DSC) thermogram of spray-dried (SD) powders containing mannitol alone (a); LAH4-L1/GAPDH siRNA/mannitol (10/1/2500) (b); LAH4-L1/GAPDH siRNA/mannitol (10/1/1000) (c); LADap(Me)6-L1/GAPDH siRNA/mannitol (10/1/1000) (d); GAPDH siRNA/mannitol (1/1000) (e).

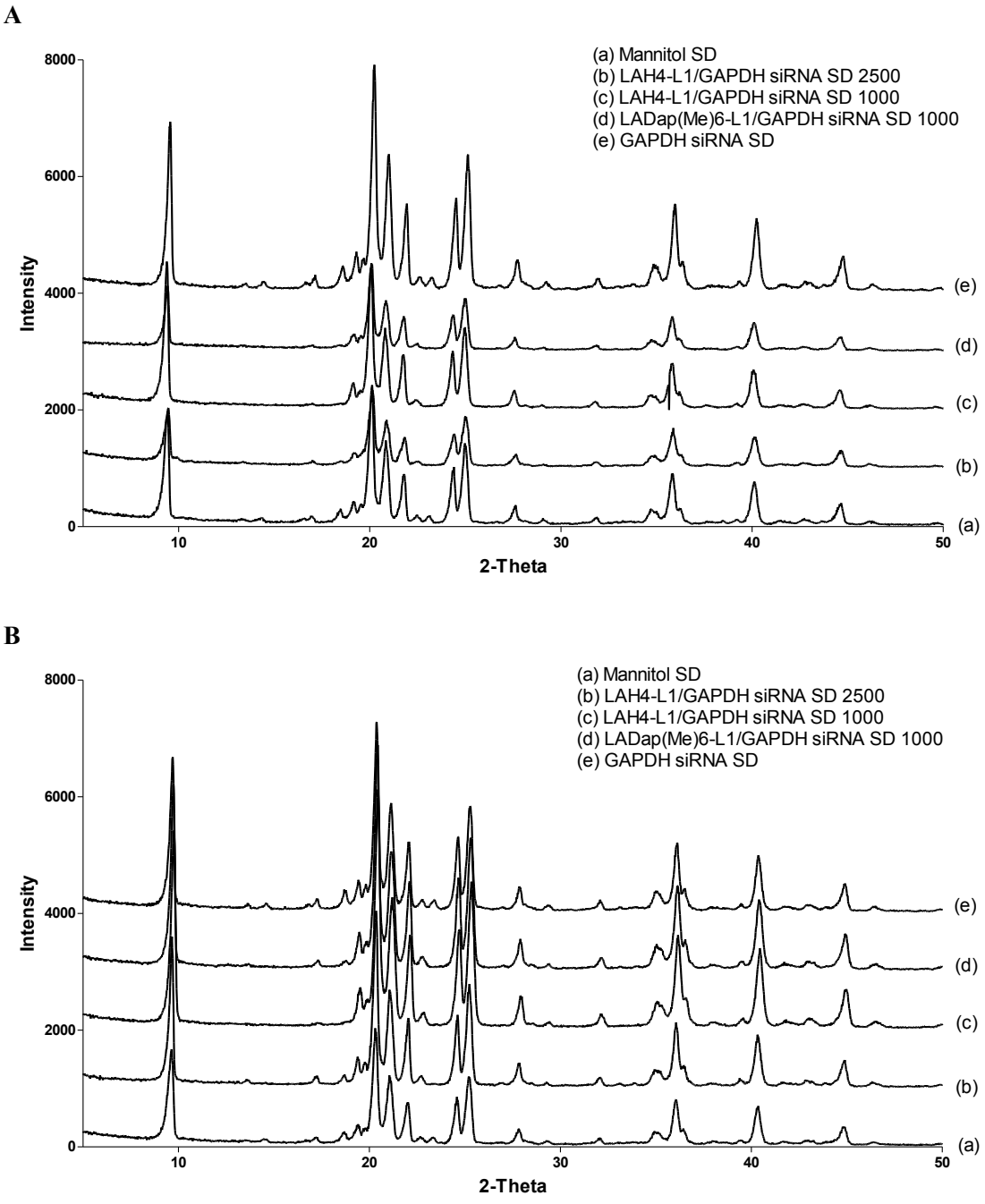


Figure S2. Powder X-ray diffraction (PXRD) pattern of spray-dried (SD) powders after preparation (A) and after five months of storage at 4 °C (B). The powders contain mannitol alone (a); LAH4-L1/GAPDH siRNA/mannitol (10/1/2500) (b); LAH4-L1/GAPDH siRNA/mannitol (10/1/1000) (c); LADap(Me)6-L1/GAPDH siRNA/mannitol (10/1/1000) (d); GAPDH siRNA/ mannitol (1/1000) (e).

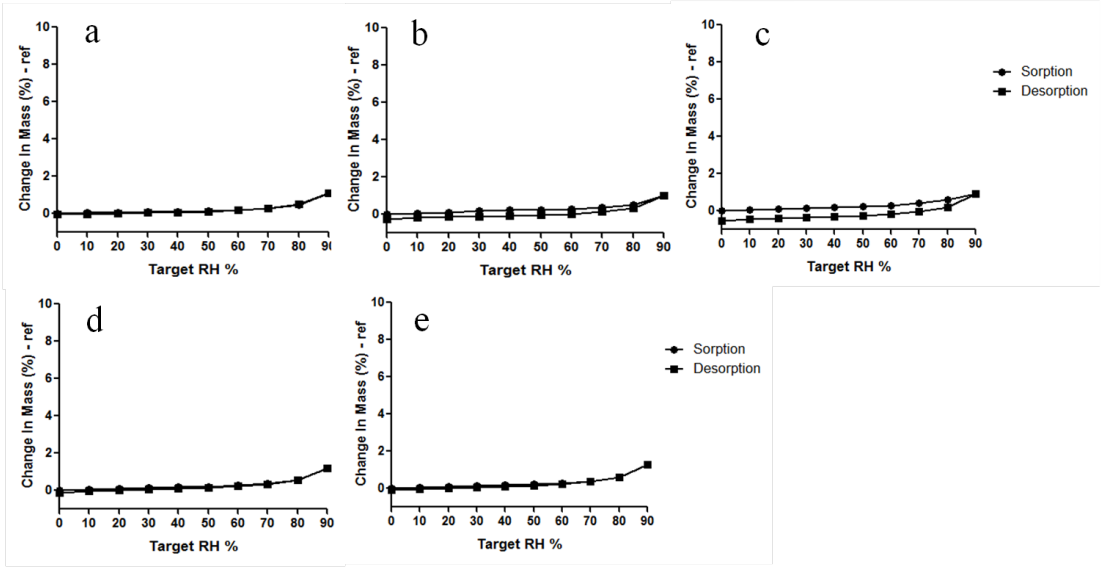
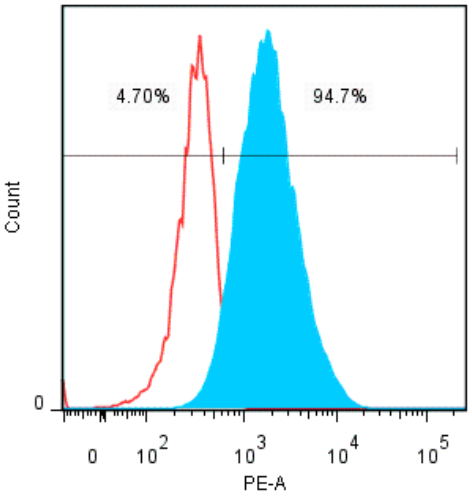


Figure S3. Moisture sorption isotherms of sorption (●) and desorption (■) of spray-dried (SD) powders containing mannitol alone (a); GAPDH siRNA/mannitol (1/1000) (b); LAH4-L1/GAPDH siRNA/mannitol (10/1/2500) (c); LAH4-L1/GAPDH siRNA/mannitol (10/1/1000) (d); LADap(Me)6-L1/GAPDH siRNA/mannitol (10/1/1000) (e).

A



B

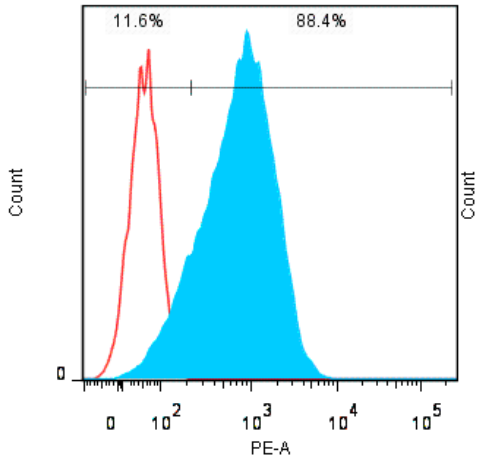


Figure S4. Flow cytometry study the cellular uptake of LAH4-L1/siGLO cyclophilin B siRNA complexes in A549 cells (A) and MDCK cells (B). siRNA (siGLO cyclophilin B) was labeled with red fluorescent signal and the flow cytometry measurement was carried out at 3 h post-transfection.

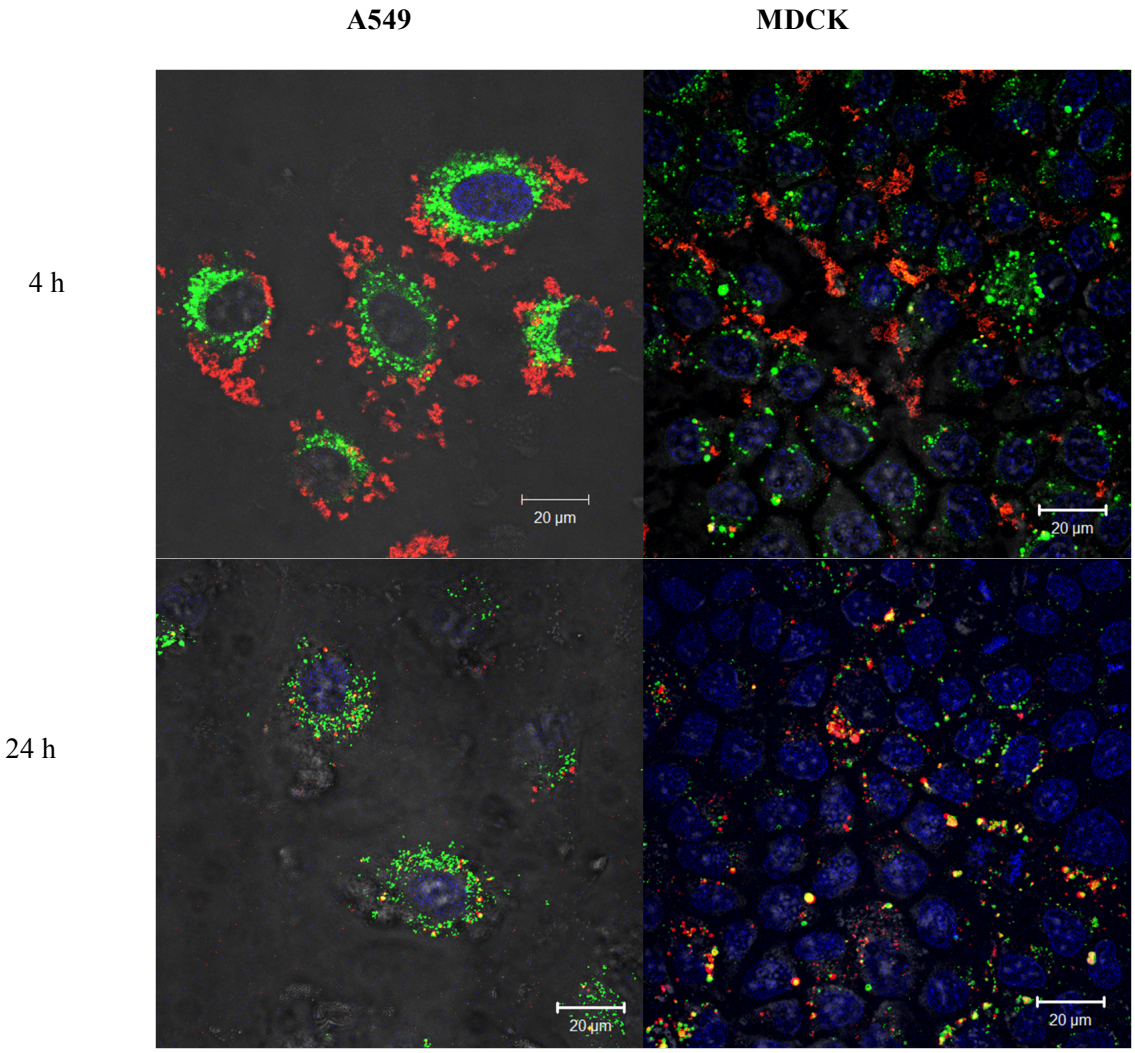


Figure S5. Live cell confocal imaging of LAH4-L1/ siGLO cyclophilin B siRNA complexes (10/1 weight ratio) in A549 and MDCK cells. siRNA were fluorescently labeled with rhodamine (red), lysosomes were labeled with Lysotracker™ (green) and the nucleus were stained with Hoechst (blue). Images were taken at 4 h and 24 h post-transfection. Scale bar = 20 µm.

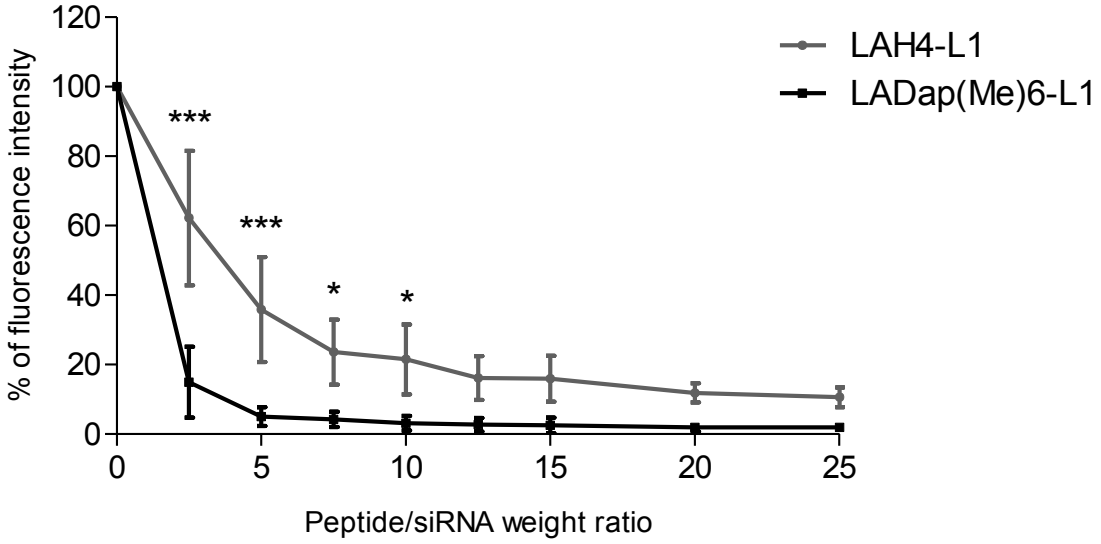


Figure S6. siRNA binding affinity was performed on LAH4-L1 and LADap(Me)6-L1 peptides by fluorescent displacement assay. SYBR® gold was used as nucleic acid dye. Peptides were titrated to siRNA/dye complexes. The dye was displaced from the complexes, leading to the decrease of fluorescence intensity. Percentage change of fluorescence intensity was plotted against peptide/siRNA ratios (up to 25:1) upon titration (n=3). Significant difference of fluorescence signal between LAH4-L1 and LADap(Me)6-L1 peptide/siRNA complexes at the same weight ratio was determined using unpaired t-test. *$p<0.05$, ***$p<0.001$.

Supplementary Experimental Section

Thermal analysis

Differential scanning calorimetry (DSC) was employed to study the thermal response of each powder sample. Approximately 5 mg of each sample was placed in a sealed 40 µl aluminum crucible and scanned using a Mettler Toledo DSC (Mettler Toledo, Greifensee, Switzerland) between 30 °C and 350 °C under 250 cm^3/min nitrogen purge at a heating rate of 10 °C /min. Exothermic and endothermic peak temperatures, onset temperature, and heat of enthalpy (DH) were determined using a STARe software.

Powder X-ray diffraction (PXRD)

The crystalline structure of SD powders was studied by a powder X-ray powder diffractometer (PXRD; Shimadzu XRD-6000, Shimadzu Corporation, Kyoto, Japan). Cu-Kα radiation at current of 30 mA and voltage of 40 kV was applied. The experiments were performed in the 2θ mode with a scanning range 5-50° at the speed of 2° per minute. To confirm the stability of the crystalline form, XRD was performed again for all samples after five-month storage at 4 °C.

Powder moisture sorption

Dynamic vapour sorption (DVS) was employed to study the moisture sorption of powder formulations with respect to humidity exposure. Around 5 mg of sample was weighted into a metal sample pan and then placed inside the sample chamber of DVS (Surface Measurement Systems, Alperton, UK). The samples were exposed to the relative humidity (RH) of 0 % to 90 % at 10 % RH increments (at 25 °C). The environmental RH was increased from 0 % to 90 % for the sorption cycle and decreased from 90 % to 0% for the desorption cycle. Equilibrium moisture content at each humidity step was determined by a mass rate (dm/dt) of 0.002% per min.

Cellular uptake

A549 and MDCK cells were seeded on a 6-well plate (2 x 10^5 cells per well) and incubated for 24 h. LAH4-L1 peptides/siRNA complexes containing 2 µg of siGLO

(Cy3-siRNA) prepared in 1ml of Opti-MEM-1 were added to the cells which were incubated for 3 h. The cells were then washed with warm PBS and collected after trypsinization. To quench extracellular fluorescence signal, trypan blue solution in PBS (0.04% v/v) was added to the cells for 2 min. The cells were centrifuged and washed with cold PBS solution thrice. Finally, the cells were re-suspended in 1 ml cold PBS solution and sieved with a cell strainer (BD Bioscience, USA). 1 x 10^4 cells were monitored and evaluated by FACSCantoII Analyzer (BD Bioscience, USA) to determine the cellular uptake of LAH4-L1 peptide/cy3-siRNA complexes. Cellular uptake (%) was defined as the percentage of cells with positive fluorescence signal compared to untreated control.

Confocal live cell imaging

A549 and MDCK cells were seeded on a 35mm Mattek glass bottom culture dish (Mattek Corp. Ashland, MA) (1 x 10^5 cells per dish) and incubated for overnight. LAH4-L1/siRNA complexes containing 2 μg of siGLO (Cy3-siRNA) prepared in 1ml of Opti-MEM-1 were added to the cells which were incubated for 3 h. The cells were then washed with warm PBS twice and replaced with completed cell culture medium. The cells were imaged at 4 h and 24 h post-transfection. To stain cell nuclei, 1 μg/ml Hoechst 33258 (Invitrogen, CA, USA) was added and incubated with the cells for 30 min. At 5 min prior to imaging, the Hoechst stain solution was removed and replaced with Opti-MEM-1. For lysosomes labeling, 50 nM Lysotracker Green DND-26 (Invitrogen, CA, USA) was added to the cells 2 min before imaging. During live cell imaging, the cells were incubated at 37°C and 5% CO_2 in a heated Perspex box. Confocal laser scanning microscopy (Carl Zeiss LSM 510 Meta/Axicam system, Germany) with argon laser (488nm) and HeNe laser (543nm) was used to visualize the cells. The microscope was equipped with Plan-Apochromat 64x/1.4 oil DIC objective lens. The images were analysed with Zeiss LSM Image Browser (Version 4.2.0.121).

siRNA binding assay

To investigate the binding affinity of peptides to siRNA, fluorescent displacement assay was performed. 0.1 μg siRNA was added into 3 ml of 1 x SYBR® Gold solution (pH 7.4). The

fluorescence intensity of the intercalated dye was quantified by fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies, Inc., Santa Clara, CA, USA) at 495 nm excitation and 537 nm emission wavelengths. LAH4-L1 or LADap(Me)6-L1 peptide was then titrated to siRNA/dye mixtures, leading to the decrease of fluorescence intensity upon binding with siRNA. Intercalation of the dye with naked siRNA represents 100% fluorescence intensity. SYBR® Gold solution in TAE buffer was employed as control. The background fluorescence was subtracted from all measured values before analysis. Percentage change of fluorescence intensity was plotted against the weight ratio of peptides to siRNA upon titration. Experiments were repeated three times independently.