SUPPLEMENTARY MATERIALS

Figure S1 : Biodistribution of chemically modified siRNA formulated with chitosan A:

Polyplexes formulated with chitosan A and various chemically modified siRNAs (N/P=10), including 2′-deoxy, 2′-fluoro 2′-O-methyl –LNA (F/OMe-LNA), unlocked nucleic acid (UNA) and Hexitol nucleic acid (HNA) were injected into the tail vein of mice (n=2). The siRNA biodistribution in the kidney was examined by northern blot as described in Fig.1. Lane 1, 1 ng siRNA duplexes (Ctrl.); Lane 2, 1 ng siRNA/nanoparticle complex (chitosan/siR-F-Ome-LNA, UNA and HNA, respectively) (Ctrl.); Lane 3-4/5-6/7-8/9-10/11-12, liver (Li), kidney (Ki), spleen (Sp), lung (Lu), heart (He) from mouse 1 and 2 for each formulation, respectively.

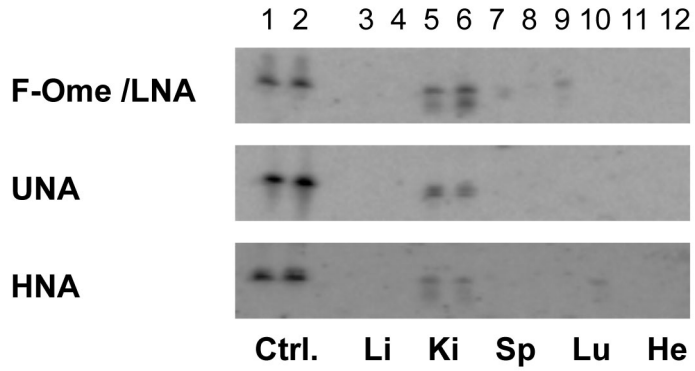


Figure S2. Biodistribution and blood clearance of unmodified siRNA formulated with chitosan A: Polyplexes formulated with chitosan A and unmodified siRNAs (N/P=10) were injected into the tail vein of mice (n=2). (a). The biodistribution was examined by northern blotting as described in Fig.1 (loading order was same as in Fig. S1). (b). siRNA blood clearance was evaluated by northern blotting for RNA purified from blood samples at various time course post-injection. Lane 1, 1 ng siRNA duplexes (Ctrl.); Lane 2, 1 ng siRNA/nanoparticle complex; 3-12, RNA isolated from blood 1, 5, 15, 30 min and 24 hrs post injection from mouse 1 and 2.

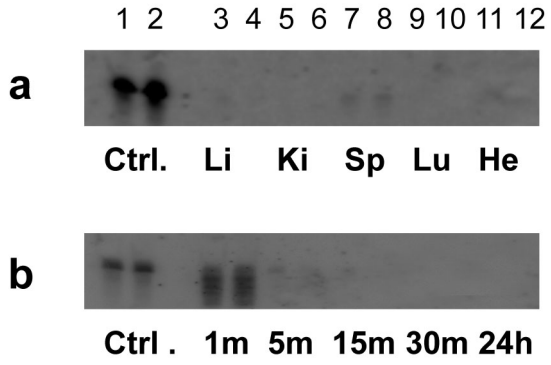
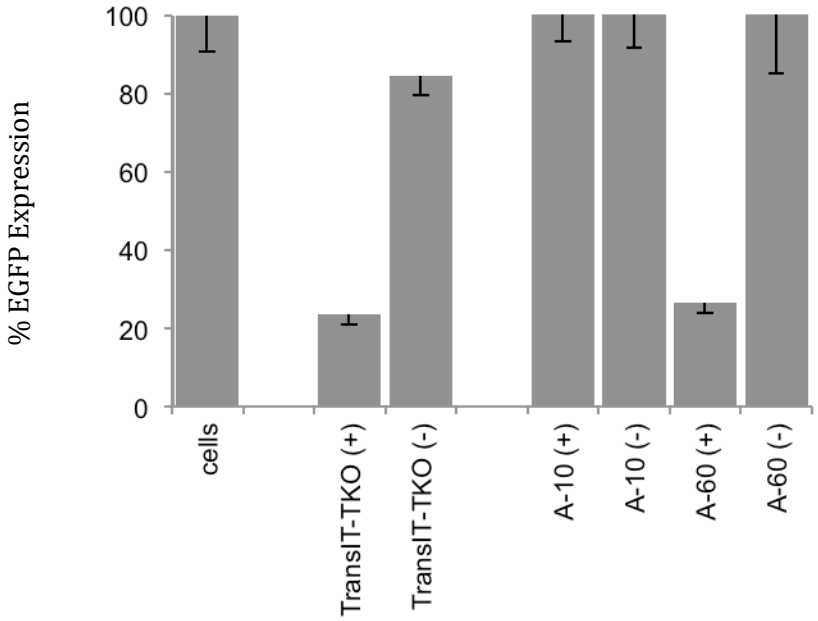


Figure S3: Impact of N/P ratio on knockdown efficacy *in vitro* and biodistribution *in vivo*:

(a). EGFP expressing H1299 cell line was transfected with Chitosan A/siRNA nanoparticles at N/P=60 and N/P=10 ratio, each containing 50 nM siNRA; (+) and (-) denoted the presence of siRNA against EGFP and scrambled siRNA, respectively; TransIT-TKO (Mirus Corp) reagent was used for comparison.



(b). The biodistribution was examined by northern blotting as described in Fig.1 for chitosanA/siRNA particle with N/P ration of either 10 or 60 as denoted. Loading order: Lane 1, 1 ng siRNA/nanoparticle complex; 2-9, RNA isolated from liver (Li1 & Li2), kidney (Ki1 & Ki2), spleen (Sl1 & Sp2) and lung (Lu1 & Lu2) harvested at 24 hrs post injection from mouse 1 and 2.

The N/P=10 particle formulation tended to form agglomerates that deposited in the lungs in contrast to N/P=60 that exclusively accumulated in the kidneys.

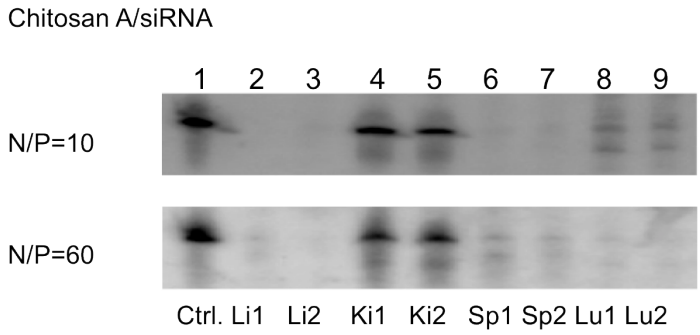


Figure S4. Stability of chitosan-A/siRNA nanoparticles: DLS measurement of Chitosan-A/siRNA particles with N/P ratio 60 was performed in water (a) (refractive index of 1.330 and a viscosity of 0.8872 cP), and compared to the measurement of FBS (b), as well as that incubated in 95% FBS for 0 hr (c) and 24 hr (d) (b-d: refractive index of 1.330 and a viscosity of 1.5 cP [1]). The particles remain fully dispersed after the incubation, but the peak of the size distribution histogram of Chitosan-A/siRNA particles shifted.

Fig.S4a: Fresh Chitosan-A/siRNA in water

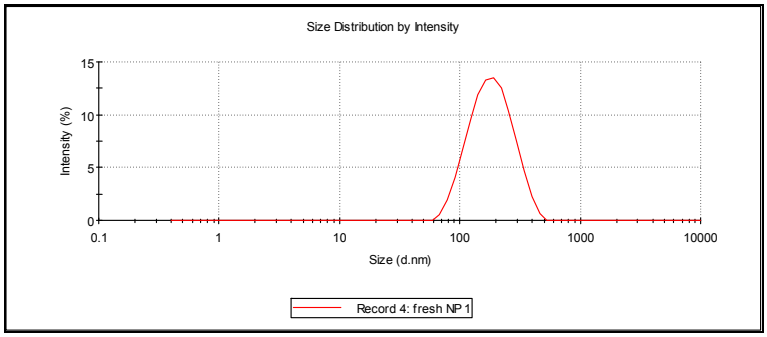


Fig.S4b: FBS

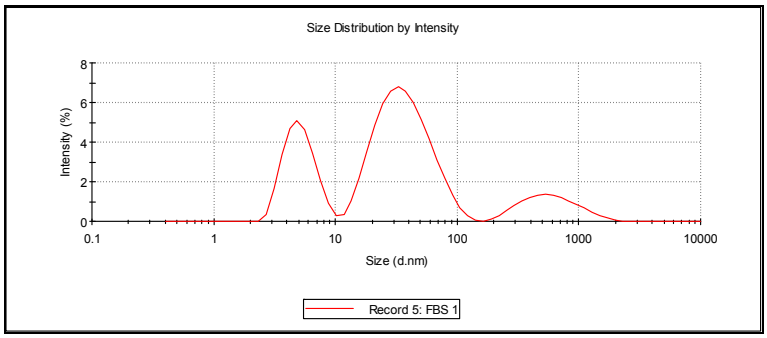


Fig.S4c: Fresh Chitosan-A/siRNA in 95% FBS

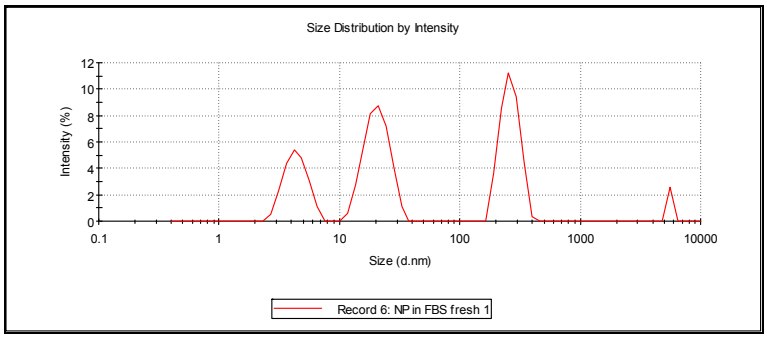


Fig. S4d: Chitosan-A/siRNA incubated in 95% FBS for 24 hrs

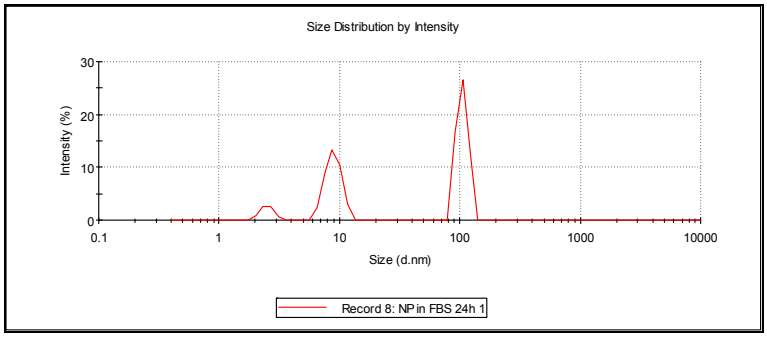


Table S1: Characteristics of four types of chitosan used in the present study

Chitosan	Degree of Deacetylation[a] (%DD)	Molecular Weight[b] (kDa)
A	98.2 ± 0.5	40.4 ± 0.7
B	98.5 ± 1.2	137.2 ± 2.7
C	97.7 ± 0.4	251.3 ± 5.9
D	97.6 ± 0.8	270.9 ± 3.4

Note:

[a] Degree of deacetylation was measured by UV spectrophotometry as in [2]. The data (average ± SD) is the representative of the measurement of 3 independent replicates.

[b] Molecular weight was measured by SEC-MALLS [3]. The data (average ± SD) is the representative of the measurement of independent duplicates.

[1] Westerhof N, Stergiopulos N and Nobel MIM, Chapter 1. Viscosity. In: Snapshots of Hemodynamics: An aid for clinical research and graduate education 2nd Edition. Springer. 2004: 3-6

[2] Hein S, Ng CH, Stevens WF, Wang K (2008) Selection of a practical assay for the determination of the entire range of acetyl content in chitin and chitosan: UV spectrophotometry with phosphoric acid as solvent, J Biomed. Mater. Res. 86B: 558-568.

[3] Chrtistensen BE, Vold IMN, Vårum KM (2008) Chain stiffness and extension of chitosans and periodate oxidised chitosans studied by size-exclusion chromatography combined with light scattering and viscosity detectors, Carbohydr. Polym. 74: 559-565.

Table S2: Mean particles size and polydisperisity for five formulations of chitosan/siRNA nanoparticles

Sample	N/P ratio	z-average diameter	Number mean diameter (nm)	Polydispersity
A	10	1512 ± 531	249 ± 32	1.00 ± 0.00
A	60	419 ± 38	269 ± 6	0.50 ± 0.01
B	10	550 ± 7	203 ± 7	0.80 ± 0.04
C	10	296 ± 3	200 ± 1	0.39 ± 0.01
D	10	422 ± 12	196 ± 3	0.73 ± 0.02

The data (average ± SD) are the representative of the measurements of 4 independent replicates.