SUPPLEMENTARY INFORMATION

Solvent-driven, self-assembled acid-responsive poly(ketalized serine)/siRNA complexes for RNA interference

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Table S1. Molecular weight analysis of acid-labile polypeptides.

Sample	MW_n (Da)	MW_w (Da)	PDI	DP
Poly(kSer)'	23,900	27,500	1.15	127
Poly(kSer)	26,100	30,700	1.18	84

Acid-responsive peptides were polymerized using amino acid urethane derivative monomers and polymerized to high molecular weights. MALDI-TOF was used to measure the molecular weight of the peptides.

Table S2. Characterization of cross-linked poly(kSer)/siRNA complexes

Mole % of amines used for cross-linking	Size (nm)	Zeta potential (mV)	PDI
10	392.3	-17.6	0.555
50	150.2	-1.20	0.225

Using 50 mole % of amines on peptide for cross-linking, particles of sufficient size and charge necessary for therapeutic use were obtained. Using 10% equivalence of crosslinker to moles of amine did not produce stable, uniform particles as indicated by large size and PDI, therefore the zeta potential measurement cannot be accurately assessed and compared with that of stably crosslinked complexes in this case.

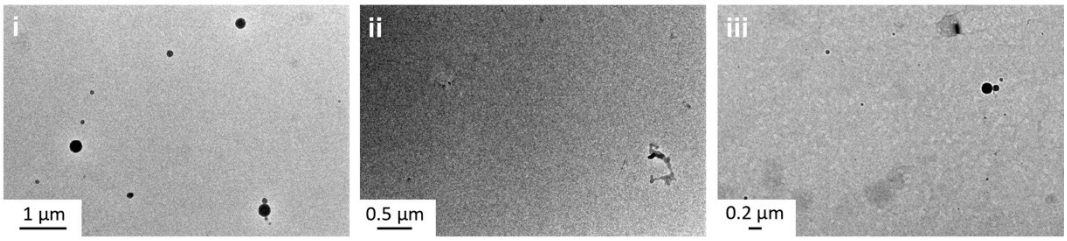

Figure S1. TEM images of i.) poly(kSerTFA)' and ii.) poly(kSerTFA) in water. iii.) TEM image of poly(kSer) in acetonitrile.

Both poly(kSerTFA)' and poly(kSerTFA) showed decreased self-assembly in water compared to acetonitrile, indicating that the solvent plays a major role in the self-assembly. Poly(kSerTFA) exhibited negligible self-assembly compared with poly(kSerTFA)' counterpart, indicating that increased hydrophilicity may inhibit self-assembly. Free amines exposed on the side chain of poly(kSer) also limited the self-assembly, further demonstrating the role of hydrophilicity in self-assembly.

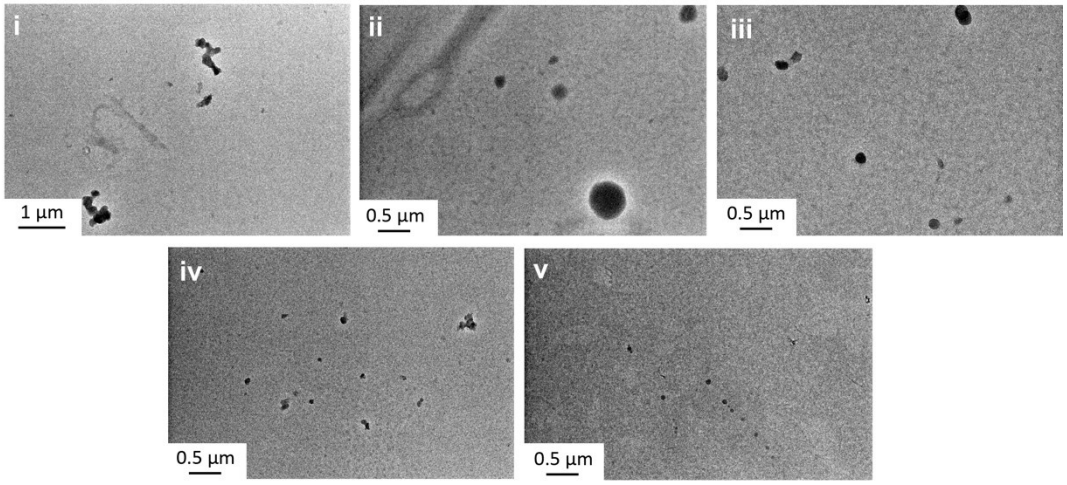

Figure S2. TEM images of poly(kSerTFA)' polymerized in different solvents. i) DMF, ii) THF, iii) DMAc, iv) DMSO, v) EtoAc.

Poly(kSerTFA)' was polymerized in organic solvents of varying polarity. The previously observed uniformity of self-assembled particles in acetonitrile was not achieved in the selected solvents.

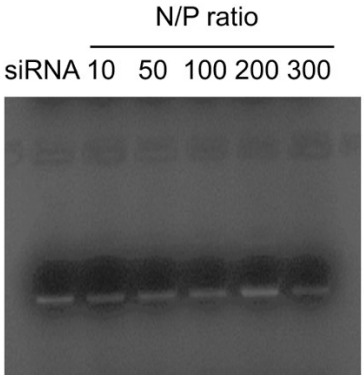

Figure S3. Gel electrophoresis of non-cross-linked poly(kSer)'/siRNA at different N/P ratios.

Without cross-linking, poly(kSer)' showed poor complexation with siRNA even at high N/P ratios, possibly due to increased hydrophobicity and steric hinderance of methyl ketal groups.

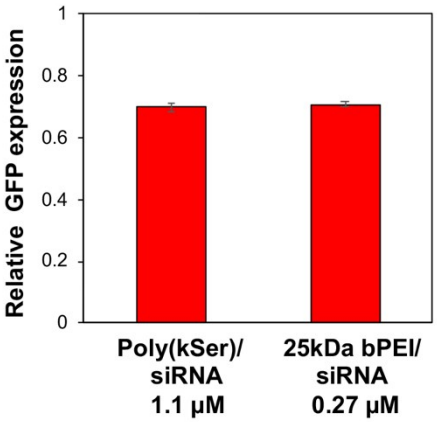

Figure S4. Gene silencing of EL4-GFP suspension cells treated with cross-linked poly(kSer)/GFP siRNA complexes in comparison with bPEI/GFP siRNA polyplexes.

Poly(kSer)/siRNA added to cells at 1.1 µM siRNA was able to silence gene expression with nearly identical efficacy as 25 kDa bPEI.

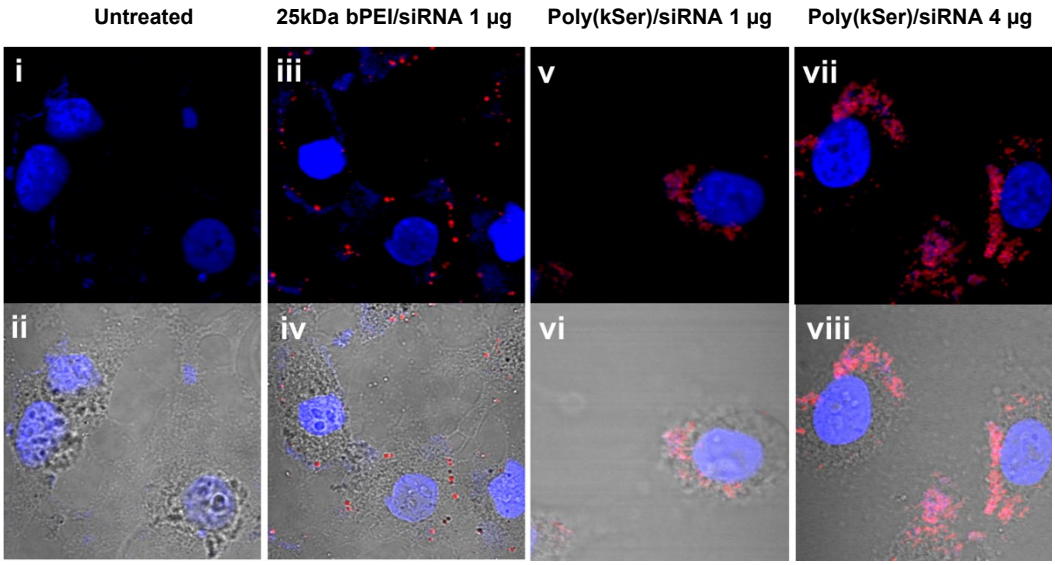

Figure S5. Confocal laser scanning microscopy showing cellular uptake of Cy3-labeled siRNA in HeLa cells treated with i-ii.) blank, , iii-iv.) 25 kDa bPEI/siRNA at 1 µg, v-vi.) cross-linked poly(kSer)/siRNA at 1 µg, vii-viii.) cross-linked poly(kSer)/siRNA at 4 µg.

Cellular uptake of nanoparticles was confirmed using cross-linked poly(kSer)/siRNA delivering 1 µg and 4 µg siRNA. Dispersed red seen in samples using cross-linked poly(kSer) indicates acid-responsive properties that allow siRNA to get out of the endosome within a 4 h timeframe in contrast to 25kDa bPEI, which showed punctate red localization.