Supporting Information

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Filamentous Artificial Virus from Self-Assembled Discrete Nanoribbon

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Figure S1. Chemical structures of supramolecular building blocks. Glu: d-glucose, Man: d-mannose, FAM: 5-carboxyfluorescein.
**Figure S2.** a) CD spectra of GFP siRNA (green) and Glu-KW β-ribbon/GFP siRNA complexes (blue). b) CD spectra of dsDNA (green) and Glu-KW β-ribbon/dsDNA complexes (blue). Sequences of dsDNA: 5’-GCAGCAGACTTCTTCAAGTT-3’ and 5’-CTTGAAGAAGTCGTGCTGCTT-3’.
Figure S3. a) Measurements of $R_H$ distributions of Glu-KW $\beta$-ribbon (bright blue) and Glu-KW $\beta$-ribbon/GFP siRNA complexes (dark blue, $+/−$: 2) by dynamic light scattering. b) $R_H$ distributions of NH$_2$-KW $\beta$-ribbon (sky blue) and NH$_2$-KW $\beta$-ribbon/GFP siRNA complexes (dark blue, $+/−$: 2).
**Figure S4.** CD spectra of a) Man-RKW (15 μM in PBS) and b) Glu-RKW (15 μM in PBS).

**Figure S5.** a) $R_H$ distributions of Man-RKW β-ribbon (bright blue) and Man-RKW β-ribbon/GFP siRNA complexes (dark blue, $+/−$: 2) by dynamic light scattering. b) $R_H$ distributions of Glu-RKW β-ribbon (bright blue) and Glu-RKW β-ribbon/GFP siRNA complexes (dark blue, $+/−$: 2) by dynamic light scattering.
Figure S6. HPLC traces of the building blocks. Linear gradient of 5-40% acetonitrile over 30 min on C$_{18}$ column.
Figure S7. MALDI-TOF mass spectra of the building blocks.
Figure S8. Silencing (knockdown) of endogenous gene expression. a) Knockdown of GAPDH mRNA. b) Knockdown of Lamin A/C mRNA. GAPDH and Lamin A/C mRNA expression was normalized to levels of β-actin mRNA. Mean ± SD (n = 3). The results indicate that Glu-KW β-ribbon can efficiently transfect siRNA and knockdown the expression of endogenous mRNA.
METHODS

Synthesis of supramolecular building blocks

For loading first amino acid, Fmoc-Gly-OH (1.63 g, 5.5 mmol) and \( N,N \)-diisopropylethylamine (DIPEA, 22 mmol) was dissolved in dichloromethane (DCM) and added to 2-chlorotrityl chloride resin (2 g, Substitution on the resin: 1.4 mmol/g). After 2 h of reaction, the resin was washed thoroughly with DCM and DMF. The peptide portion of the supramolecular building block was then synthesized on the above Fmoc-Gly-2-chlorotrityl resin using standard Fmoc protocols on Applied Biosystems model 433A peptide synthesizer. The resin was then washed with DMF, treated with 20% piperidine in DMF for 30 min, and washed with DMF and DCM. For coupling D-glucose to the peptide, \( N,N' \)-diisopropylcarbodiimide (DIC, 19 \( \mu \)L, 123 \( \mu \)mol) was added to a solution of carboxymethyl 2,3,4,6-tetra-O-acetyl-\( \beta \)-D-glucopyranoside\(^{[1]} \) (50 mg, 123 \( \mu \)mol) and \( N \)-hydroxysuccinimide (29 mg, 250 \( \mu \)mol) in DCM (1 mL). For coupling D-mannose to the peptide, carboxymethyl 2,3,4,6-tetra-O-acetyl-\( \alpha \)-D-mannopyranoside was used instead. Following overnight reaction at RT, the solvent was evaporated under reduced pressure. The activated acid was dissolved in \( N \)-methyl-2-pyrrolidone (NMP, 1 mL), mixed with DIPEA (250 \( \mu \)mol), and added to the resin-bound peptide (50 \( \mu \)mol based on the peptide substitution on the resin). The reaction continued overnight with shaking at room temperature. Following washing the resin, acetyl protecting groups from the glucose were deblocked by the treatment with 10% hydrazine/DMF for 5 h. The resin was washed and dried. The dried resin was treated with cleavage cocktail (TFA: 1,2-ethanedithiol: thioanisole; 95: 2.5: 2.5) for 3 h, and was triturated with tert-butyl methyl ether to yield Glu-KW. FAM-KW was synthesized similarly using 5-carboxyfluorescein, succinimidyl ester (Anaspec Inc). The supramolecular building blocks were purified by reverse-phase HPLC (water/acetonitrile with 0.1% TFA). The molecular weight was confirmed by MALDI-TOF mass spectrometry. The purity of the building blocks was >95% as determined by analytical HPLC. Concentration was determined spectrophotometrically in water/acetonitrile (1:1) using a molar extinction coefficient of tryptophan (5500 M\(^{-1}\) cm\(^{-1}\)) at 280 nm.

CD, TEM, AFM, and DLS studies
CD spectra were measured using a JASCO model J-810 spectropolarimeter. Spectra were recorded from 250 nm to 190 nm using a 0.1 cm path-length cuvette, and scans were repeated three times and averaged. Molar ellipticity was calculated per amino acid residue. For TEM experiment, 3 μL of an aqueous solution of sample (15 μM in PBS) was placed onto a holey carbon-coated copper grid, and 3 μL of 2 % (w/w) uranyl acetate solution was added for negative staining. The sample was deposited for 1 min, and excess solution was wicked off by filter paper. The dried specimen was observed with a JEOL-JEM 2010 instrument operating at 120 kV. The data were analyzed with DigitalMicrograph software. For AFM, 1 μL of the sample in PBS was deposited onto a freshly cleaved mica surface for 1 min, washed with water several times, and dried in air. The images were obtained in tapping mode with a Nanoscope IIIa instrument (Digital Instruments). AFM scans were taken at setpoint of 0.8-1 V and scanning speed was 1-2 Hz. Dynamic light scattering experiment was performed at room temperature with ALV/CGS-3 Compact Goniometer System equipped with He-Ne laser operating at 632.8 nm. The scattering angle was 90°. Before measurement, the sample was centrifuged at 16,110 × g for 20 min to sediment any dust particles. The size distribution was determined by using a constrained regularization method.

**Exogenous gene (GFP) knockdown**

For RNAi experiment, HeLa cells (1 × 10⁴) were seeded on 96-well plate in DMEM with 10% FBS. Next day, the cells were transfected with GFP siRNA complexes. GFP siRNA AS: 5’-CUUGAAGAAGUCGUGCUCU-3’, GFP siRNA SS: 5’-GCAGCAGCACUUCUUAAGU-3’. At 4 h postincubation, the cells were washed with PBS. The cells were then transfected with 150 ng of pEGFP-C1 (Clontech) using LF2000 for 3 h. At 72 h posttransfection, the cells were washed with PBS and treated with trypsin (0.05% with EDTA, Gibco) for 10 min. The cells were then transferred to FACS tube containing 3 mL PBS and spun down by centrifugation. The GFP fluorescence from the cells was analyzed by FACSCalibur flow cytometer (Becton Dickinson) using argon laser to excite cells at 488 nm. Typically 5 × 10³ cells were sorted, and data were analyzed with CELLQUEST software.

**Endogenous gene (human GAPDH and Lamin A/C) knockdown**

HeLa cells were seeded in 24-well culture plates. After 24 h incubation in DMEM
containing 10% FBS, the cells were rinsed once and replaced with 1 mL of DMEM. Then, 500 μL DMEM containing 100 pmol of the siRNA (GAPDH targeting, Lamin A/C targeting, or GFP targeting) complex was added on the culture for 4 h. After removing the transfection mixture, 2 mL of DMEM containing 10% FBS was added. After incubation of 24 h, the total RNA was prepared by using 500 μL of Trizol reagent (Invitrogen) according to the manufacturer’s recommendation. The gene expression was measured using quantitative RT-PCR. Six hundred nanograms of total RNA was reverse-transcribed in a volume of 20 μL for 2 h at 42 °C using SuperScript RT II kit following the manufacturer’s recommendation (Invitrogen). After diluting the RT mixture 6 fold in dH₂O, 10 ng of cDNA was used for quantifying the mRNA level of each gene using the LightCycler 480 (Roche): After initial denaturation of the template for 1 min at 95 °C, 42 thermal cycles of 15 sec at 95 °C and 25 sec at 60 °C and 30 sec at 72 °C were run in a final volume of 20 μL with 8 pmol of each primer using SYBR Green I dye (Invitrogen) for PCR product detection according to the manufacturer’s recommendation. All mRNA expression was normalized to level of human β-actin mRNA which is also determined by the quantitative RT-PCR from the same total RNA sample.

**siRNA sequences:**

Lamin A/C: Sense, CUGGACUCCAGAAGAACAdTdT  
Antisense, UGUUCUUCUGGAAGUCCAGdTdT  
GAPDH: Sense, GUGUGAACCAUGAGAAGUA(dTdT)  
Antisense: UACUUCUCAUGGUUCACAC(dTdT)

**PCR primer sequences:**

Human GAPDH; forward primer (CCAGCAAGAGCACAAGAGGAAGAG) and reverse primer (AGGAGGGAGATTCATGCTGTGGTG).  
Human Lamin A/C; forward primer (GTACGGCTCTCATCAACTCCACTG) and reverse primer (TCCTCATCCTCGGTCTCTCAAC).  
Human β-actin; forward primer (GCACCTTCCAGCCTTCTTCC) and reverse primer (GCGGATGTCCACGTCACACTTC).
**Microscopy studies**

For microscopic observation of intracellular delivery, HeLa cells ($1 \times 10^4$) were seeded on 8-well Lab-tek II chambered coverglass system (Nunc) in DMEM with 10% FBS and cultured overnight. The cells were treated with coassembled Glu-KW/FAM-KW β-ribbon or Glu β-ribbon/nile red/siRNA ternary complex for 3 h. The cells were then washed three times with PBS and DMEM (without phenol red) was added. For staining acidic organelles, LysoTracker Red DND-99 (Invitrogen) was added at 50 nM for 5 min. Live cell images were observed with Nikon Eclipse TE2000-U inverted fluorescence microscope. For confocal images, Nikon Eclipse TE2000-U inverted microscope equipped with PerkinElmer UltraVIEW RS confocal scanner was used.

**References**