Supporting information

Delivery of siRNA using cationic rosette nanotubes for gene silencing

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Table S1: Characterization data of K1T-K15T

KnT	Characterization data
K1T	**^{1}H-NMR:** 9.17 (bs, 4H), 8.32-8.28 (m, 3H), 7.71-7.70 (m, 2H), 4.49-4.39 (m, 4H), 3.92 (t, *J* = 6.6 Hz, 1H), 3.55-3.48 (m, 4H), 3.38-3.33 (m, 2H), 2.86 (m, 6H), 1.92-1.85 (m, 2H), 1.72-1.71 (m, 2H), 1.50-1.43 (m, 2H). **^{13}C-NMR:** 172.7, 163.3, 161.1, 157.3, 157.0, 150.5, 83.8, 54.0, 51.8, 37.9, 30.5, 28.7, 28.7, 23.4, 22.7. **MALDI-TOF MS (THAP, positive mode):** *m/z*: Calcd for $C_{24}H_{34}N_{14}O_6$ 614.28; found 615.28 $[M + H]^+$, 637.27 $[M + Na]^+$ **Elemental analysis:** Calcd for $C_{24}H_{34}N_{14}O_6(CF_3CO_2H)_{2.5}(H_2O)(C_4H_{10}O)_{0.75}$, MW = 972.83: C 39.49, H 4.76, N 20.15. Found: C 39.55, H 4.59, N 20.22
K2T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.26-8.22 (m, 6H,), 7.70-7.64 (m, 2H), 4.45-4.35 (m, 4H), 3.90 (t, *J* = 6.6 Hz, 1H), 3.80 (t, *J* = 6.6 Hz, 1H), 3.58-3.51 (m, 4H), 3.36-3.32 (m, 2H), 3.12-3.09 (m, 2H), 2.85 (m, 6H), 1.86-1.67 (m, 6H), 1.46-1.26 (m, 6H). **^{13}C-NMR:** 172.8, 170.0, 163.1, 160.9, 157.0, 156.7, 150.2, 83.6, 54.3, 53.9, 53.7, 51.2, 40.1, 37.6, 31.3, 30.4, 28.6, 28.7, 23.2- 22.3. **Positive ESI–MS:** Calcd for $C_{30}H_{46}N_{16}O_7$ 742.4; found 743.3 $[M+H]^+$, 372.2 $[M+2H]^{2+}$ **High–resolution EI–MS** *m/z*: 372.1941 ($M+2H^+$ calcd 744.3881) **Elemental analysis:** Calcd for $C_{30}H_{46}N_{16}O_7(CF_3CO_2H)_3(H_2O)_2(C_4H_{10}O)$, MW = 1194.45: C 40.20, H 5.31, N 18.75. Found: C 40.21, H 5.24, N 18.84
K3T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.20-8.14 (m, 9H,), 7.70-7.69 (m, 2H), 4.45-4.35 (m, 4H), 3.90 (t, *J* = 6.6 Hz, 1H), 3.81-3.77 (m, 2H), 3.54 (m, 4H), 3.35-3.33 (m, 2H), 3.12-3.04 (m, 4H), 2.85 (s, 6H), 1.85-1.67 (m, 8H), 1.44-1.21 (m, 10H). **^{13}C-NMR:** 172.8, 170.3, 170.0, 163.1, 160.8, 156.9, 156.5, 150.1, 83.5, 54.4-53.7, 53.5, 51.0, 40.0, 37.5, 31.4, 31.2, 30.3, 28.5 , 28.6, 23.1-22.2. **Positive ESI-MS:** Calcd for $C_{36}H_{58}N_{18}O_8$ 870.5; found 436.2 $[M+2H]^{2+}$, 291.2 $[M+3H]^{3+}$ **High-resolution EI-MS** *m/z*: 436.2415 ($M+2H^+$ calcd 872.4830) **Elemental analysis:** Calcd for $C_{36}H_{58}N_{18}O_8(CF_3CO_2H)_{4.5}(H_2O)_2(C_4H_{10}O)$, MW = 1493.53: C 39.39, H 5.16, N 16.87. Found: C 39.51, H 5.21, N 16.53
K4T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.24-8.19 (m, 12H), 7.67-7.66 (m, 2H), 4.45-4.35 (m, 4H), 3.90 (t, *J* = 6.6 Hz, 1H), 3.80-3.76 (m, 3H), 3.55-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.12-3.03 (m, 6H), 2.85 (s, 6H), 1.85-1.71 (m, 10H), 1.45-1.21. **^{13}C-NMR:** 173.0, 170.6, 170.2, 163.3, 161.1, 157.3, 157.0, 150.5, 83.8, 54.8, 54.6, 54.2, 51.7, 40.4, 37.8, 31.8, 31.6, 30.6, 28.7, 28.9, 23.4-22.5. **Positive ESI-MS:** Calcd for $C_{42}H_{70}N_{20}O_9$ 998.6; found 500.3 $[M+2H]^{2+}$, 333.9 $[M +3H]^{3+}$ **High-resolution EI-MS** *m/z*: 500.2889 ($M+2H^+$ calcd 1000.5780) **Elemental analysis:** Calcd for $C_{42}H_{70}N_{20}O_9(CF_3CO_2H)_5(H_2O)_3(C_4H_{10}O)$, MW = 1696.63: C 39.62, H 5.40, N 16.50. Found: C 39.50, H 5.26, N 16.60

KnT	Characterization data
K5T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.22-8.16 (m, 15H), 7.70-7.69 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.82-3.76 (m, 4H), 3.55-3.50 (, m, 4H), 3.36-3.33 (m, 2H), 3.12-3.01 (m, 8H), 2.85 (s, 6H), 1.87-1.69 (m, 12H), 1.46-1.18 (m, 18H). **^{13}C-NMR:** 172.9, 170.4, 170.0, 163.2, 160.9, 157.0, 156.7, 150.2, 83.6, 54.5, 54.3, 54.0, 51.2, 40.2, 37.6, 31.6, 30.4, 28.6, 28.7, 23.2-22.3. **Positive ESI-MS:** Calcd for $C_{48}H_{82}N_{22}O_{10}$ 1126.7; found 564.4 $[M+2H]^{2+}$ **High-resolution EI-MS** *m/z*: 564.3360 ($M+2H^{+}$ calcd 1128.6730) **Elemental analysis:** Calcd for $C_{48}H_{82}N_{22}O_{10}(CF_3CO_2H)_{6.5}(H_2O)_{1.5}(C_4H_{10}O)$, MW = 1968.70: C 39.64, H 5.19, N 15.65. Found: C 39.81, H 5.28, N 15.41
K6T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.25-8.22 (m, 18H), 7.66-7.65 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.76 (m, 5H), 3.56-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.11-3.02 (m, 10H), 2.85 (s, 6H), 1.87-1.69 (m, 14H), 1.45-1.09 (m, 22H). **^{13}C-NMR:** 172.9, 170.4, 170.1, 163.2, 160.9, 157.0, 156.7, 150.2, 83.6, 54.5, 54.3, 54.0, 51.2, 40.2, 37.7, 31.6, 30.4, 28.6, 28.8, 23.1-22.3. **Positive ESI-MS:** Calcd for $C_{54}H_{94}N_{24}O_{11}$ 1254.8; found 628.4 $[M+2H]^{2+}$, 419.3 $[M+3H]^{3+}$, 314.7 $[M+4H]^{4+}$ **High-resolution EI-MS** *m/z*: 419.2584 ($M+3H^{+}$ calcd 1257.7752) **Elemental analysis:** Calcd for $C_{54}H_{94}N_{24}O_{11}(CF_3CO_2H)_8(H_2O)_3(C_4H_{10}O)$, MW = 2294.80: C 38.71, H 5.18, N 14.64. Found: C 38.67, H 5.18, N 14.51
K7T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.24-8.21 (m, 21H), 7.67-7.66 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.76 (m, 6H), 3.56-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.11-3.02 (m, 12H), 2.85 (s, 6H), 1.87-1.70 (m, 16H), 1.40-1.09 (m, 26H). **^{13}C-NMR:** 173.0, 170.5, 170.1, 163.3, 161.0, 157.2, 156.9, 150.4, 83.7, 54.8, 54.7, 54.1, 51.5, 40.3, 37.7, 31.7, 30.5, 28.6, 28.9, 23.3-22.4. **Positive ESI-MS:** Calcd for $C_{60}H_{106}N_{26}O_{12}$ 1382.8; found 461.9 $[M+3H]^{3+}$, 346.7 $[M+4H]^{4+}$ **High-resolution EI-MS** *m/z*: 346.7190 ($M+4H^{+}$ calcd 1386.8775) **Elemental analysis:** Calcd for $C_{60}H_{106}N_{26}O_{12}(CF_3CO_2H)_9(H_2O)_3(C_4H_{10}O)$, MW = 2536.89: C 38.80, H 5.20, N 14.35. Found: C 38.81, H 5.35, N 14.25
K8T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.19-8.16 (m, 24H), 7.67-7.66 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.81-3.77 (m, 7H), 3.56-3.51 (m, 4H), 3.36-3.33 (m, 2H), 3.12-3.02 (m, 14H), 2.85 (s, 6H), 1.85-1.70 (m, 18H), 1.44-1.21 (m, 30H). **^{13}C-NMR:** 172.8, 170.4, 170.0, 163.2, 160.9, 157.0, 156.7, 150.2, 83.6, 54.5, 54.3, 53.9, 51.2, 40.1, 37.6, 31.5, 30.4, 28.6, 28.7, 23.2-22.3. **MALDI-TOF MS (THAP, positive mode):** *m*/z: Calcd for $C_{66}H_{118}N_{28}O_{13}$ 1510.94; found 1511.95 $[M + H]^{+}$, 1534.5 $[M + Na]^{+}$ **Elemental analysis:** Calculated for $C_{66}H_{118}N_{28}O_{13}(CF_3CO_2H)_{10.5}(H_2O)_{3.5}(C_4H_{10}O)$, MW = 2844.98: C 38.40, H 5.15, N 13.78. Found: C 38.56, H 5.07, N 13.53

KnT	Characterization data
K9T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.23-8.20 (m, 27H), 7.68-7.67 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.81-3.76 (m, 8H), 3.55-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.11-3.06 (m, 16H), 2.85 (s, 6H), 1.85-1.71 (m, 20H), 1.45-1.21 (m, 34H). **^{13}C-NMR:** 172.7, 170.3, 169.9, 163.0, 160.7, 156.7, 156.3, 150.0, 83.4, 54.2, 54.1, 53.6, 50.7, 39.9, 37.5, 31.3, 30.1, 28.5, 28.5, 23.2-22.1. **Positive ESI-MS:** Calcd for $C_{72}H_{130}N_{30}O_{14}$ 1639.04; found 547.7 $[M+3H]^{3+}$, 411.0 $[M+4H]^{4+}$ **High-resolution EI-MS** *m/z*: 410.7670 ($M+4H^{+}$ calcd 1643.0674) **Elemental analysis:** Calcd for $C_{72}H_{130}N_{30}O_{14}(CF_3CO_2H)_{11}(H_2O)_4(C_4H_{10}O)$, MW = 3039.08: C 38.71, H 5.27, N 13.82. Found: C 38.79, H 5.34, N 13.74
K10T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.20-8.17 (m, 30H), 7.69-7.68 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.77 (m, 9H), 3.55-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.12-3.03 (m, 18H), 2.85 (s, 6H), 1.86-1.71 (m, 22H), 1.45-1.21 (m, 38H). **^{13}C-NMR:** 172.7, 170.3, 169.9, 163.0, 160.7, 156.7, 156.3, 150.0, 83.4, 54.2, 54.1, 53.6, 50.7, 39.9, 37.5, 31.4, 30.2, 28.5, 28.8, 23.2-22.1. **Positive ESI-MS:** Calcd for $C_{78}H_{142}N_{32}O_{15}$ 1767.1; found 590.4 $[M+3H]^{3+}$, 442.8 $[M+4H]^{4+}$, 354.4 $[M+5H]^{5+}$ **High-resolution EI-MS** *m/z*: 590.0502 ($M+3H^{+}$ calcd 1770.1551) **Elemental analysis:** Calcd for $C_{78}H_{142}N_{32}O_{15}(CF_3CO_2H)_{13}(H_2O)_4(C_4H_{10}O)$, MW = 3395.16: C 38.19, H 5.13, N 13.20. Found: C 38.24, H 5.35, N 13.13
K11T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.18-8.15 (m, 33H), 7.70-7.69 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.77 (m, 10H), 3.55-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.12-3.03 (m, 20H), 2.85 (s, 6H), 1.85-1.70 (m, 24H), 1.45-1.21 (m, 42H). **^{13}C-NMR:** 172.7, 170.3, 169.9, 163.1, 160.7, 156.8, 156.5, 150.1, 83.5, 54.3, 54.2, 53.7, 50.9, 39.9, 37.5, 31.4, 30.1, 28.6, 28.6, 23.1-22.1. **Positive ESI-MS:** Calcd for $C_{84}H_{154}N_{34}O_{16}$ 1895.2; found 633.1 $[M+3H]^{3+}$, 475.1 $[M+4H]^{4+}$, 380.1 $[M+5H]^{5+}$ **High-resolution EI-MS** *m/z*: 474.8149 ($M+4H^{+}$ calcd 1899.2573) **Elemental analysis:** Calcd for $C_{84}H_{154}N_{34}O_{16}(CF_3CO_2H)_{14}(H_2O)_6(C_4H_{10}O)$, MW = 3673.26: C 37.91, H 5.21, N 12.96. Found: C 37.89, H 5.02, N 13.00
K12T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.21-8.18 (m, 36H), 7.69-7.68 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.77 (m, 11H), 3.55-3.51 (m, 4H), 3.35-3.32 (m, 2H), 3.12-3.07 (m, 22H), 2.85 (s, 6H), 1.85-1.70 (m, 26H), 1.45-1.21 (m, 46H). **^{13}C-NMR:** 172.7, 170.3, 169.9, 163.1, 160.7, 156.8, 156.5, 150.1, 83.5, 54.3, 54.2, 53.7, 50.9, 39.9, 37.5, 31.4, 30.2, 28.6, 28.6, 23.0-22.1. **Positive ESI-MS:** Calcd for $C_{90}H_{166}N_{36}O_{17}$ 2023.3; found 675.8 $[M+3H]^{3+}$, 507.1 $[M+4H]^{4+}$, 405.9 $[M+5H]^{5+}$ **High-resolution EI-MS** *m/z*: 405.6722 ($M+5H^{+}$ calcd 2028.3596) **Elemental analysis:** Calcd for $C_{90}H_{166}N_{36}O_{17}(CF_3CO_2H)_{13}(H_2O)_3(C_4H_{10}O)$, MW = 3633.34: C 39.65, H 5.41, N 13.87. Found: C 38.24, H 5.35, N 13.13

KnT	Characterization data
K13T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.23-8.19 (m, 39H), 7.69-7.68 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.75 (m, 12H), 3.55-3.50 (m, 4H), 3.35-3.32 (m, 2H), 3.13-3.03 (m, 24H), 2.85 (s, 6H), 1.86-1.66 (m, 28H), 1.45-1.17 (m, 50H). **^{13}C-NMR:** 172.7, 170.3, 169.9, 163.1, 160.7, 156.7, 156.4, 150.0, 83.5, 54.3, 54.2, 53.6, 50.8, 39.9, 37.5, 31.4, 30.2, 28.6, 28.6, 23.0-22.1. **MALDI-TOF MS (THAP, positive mode):** *m/z*: Calcd for $C_{96}H_{178}N_{38}O_{18}$ 2151.42; found 2152.4 $[M + H]^+$, 2175.7 $[M + Na]^+$ **Elemental analysis:** Calcd for $C_{96}H_{178}N_{38}O_{18}(CF_3CO_2H)_{15.5}(H_2O)_{2.5}(C_4H_{10}O)$, MW = 4037.41: C 38.95, H 5.20, N 13.18. Found: C 38.95, H 5.26, N 12.96
K14T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.14-8.06 (m, 42H,), 7.72-7.71 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.75 (m, 13H), 3.55-3.50 (m, 4H), 3.35-3.32 (m, 2H), 3.10-3.03 (m, 26H), 2.85 (s, 6H), 1.86-1.71 (m, 30H), 1.45-1.21 (m, 54H) **^{13}C-NMR:** 172.9, 170.4, 170.0, 163.2, 160.9, 157.0, 156.7, 150.2, 83.6, 54.5, 54.3, 53.9, 51.2, 40.1, 37.6, 31.6, 30.4, 28.8, 28.8, 23.2-22.3. **MALDI-TOF MS (THAP, positive mode):** *m/z*: Calcd for $C_{102}H_{190}N_{40}O_{19}$ 2279.51; found 2280.5 $[M + H]^+$, 2304.3 $[M + Na]^+$ **Elemental analysis:** Calcd for $C_{102}H_{190}N_{40}O_{19}(CF_3CO_2H)_{16}(H_2O)_3(C_4H_{10}O)$, MW = 4231.50: C 39.15, H 5.29, N 13.23. Found: C 39.15, H 5.46, N 13.04
K15T	**^{1}H-NMR:** 9.16 (bs, 4H), 8.14-8.06 (m, 45H), 7.72-7.71 (m, 2H), 4.45-4.35 (m, 4H), 3.91 (t, *J* = 6.6 Hz, 1H), 3.80-3.75 (m, 14H), 3.55-3.50 (m, 4H), 3.35-3.32 (m, 2H), 3.10-3.03 (m, 28H), 2.85 (s, 6H), 1.86-1.70 (m, 32H), 1.45-1.21 (m, 58H). **^{13}C-NMR:** 172.9, 170.4, 170.0, 163.2, 160.9, 157.0, 156.7, 150.2, 83.6, 54.5, 54.3, 53.9, 51.2, 40.1, 37.6, 31.6, 30.4, 28.8, 28.8, 23.1-22.3. **MALDI-TOF MS (THAP, positive mode):** *m/z*: Calcd for $C_{108}H_{202}N_{42}O_{20}$ 2407.61; found 2408.6 $[M + H]^+$, 2431.2 $[M + Na]^+$ **Elemental analysis:** Calcd for $C_{108}H_{202}N_{42}O_{20}(CF_3CO_2H)_{16}(H_2O)_4(C_4H_{10}O)$, MW = 4037.41: C 38.49, H 5.43, N 13.43. Found: C 39.51, H 5.44, N 13.29

Table S2: Molecular weight (MW) of K1T to K15T, molar concentration (mM) and net charge per molecule of the RNTs solutions (1 mg/mL)

Compounds	MW (g/mol)	Concentration (mM)	Net charge
K1T	972.83	1.03	+1
K2T	1194.45	0.84	+2
K3T	1493.53	0.67	+3
K4T	1696.63	0.59	+4
K5T	1968.70	0.51	+5
K6T	2294.80	0.44	+6
K7T	2536.89	0.39	+7
K8T	2844.98	0.35	+8
K9T	3039.08	0.33	+9
K10T	3395.16	0.29	+10
K11T	3673.26	0.27	+11
K12T	3633.34	0.28	+12
K13T	4037.41	0.25	+13
K14T	4231.50	0.24	+14
K15T	4037.41	0.25	+15

Sample preparation for characterization by microscopy

KnT RNTs and siRNA complexation

RNTs samples were diluted with deionized water (0.012 μg/μL). K5T, K10T and K15T RNTs/siRNA complexes (molar ratio of 20:1, 0.1 nmol siRNA) were prepared in 50 μL SFM, followed by 30 min incubation and dilution in deionized water (0.012 μg/μL RNTs).

Scanning electron microscopy (SEM)

The SEM samples were prepared by depositing the diluted solutions on carbon-coated 400-mesh copper grids and blotting after 20 s. All samples were air-dried and heated on a hotplate for 5 min prior to imaging. The SEM images were obtained at 30 kV accelerating voltage, 20 μA and a working distance of 5 to 8 mm on a high-resolution Hitachi S-4800 cold field emission SEM and ultra high-resolution Hitachi S-5500 cold field emission SEM.

Atomic Force Microscopy (AFM)

The solutions were deposited on clean HOPG substrates (1 x 1 cm^2) and spin-coated at 2500 rpm for 30 s. The surface morphology was visualized using a Digital Instruments/Veeco Instruments MultiMode Nanoscope IV AFM equipped with an E scanner. Silicon cantilevers (MikroMasch USA, Inc.) with low spring constants of 4.5 N/m in tapping mode (TM-AFM), a scan rate of 0.5-1 Hz and amplitude setpoint of 1 V were used.

Transmission electron microscopy (TEM)

Samples for TEM imaging were prepared by depositing a drop of prepared solution on a carbon-coated 400-mesh copper grid, then, blotted after 10 s. The sample was stained using uranyl acetate (2% aqueous solution) for 2 min. The grid was then blotted, air-dried and heated on the hotplate prior to imaging. The samples were visualized on JEOL 2200 FS TEM – 200kV Schottky field emission instrument equipped with an in-column omega filter. Bright field TEM images were acquired using energy filtered zero loss beams (slit width 10 eV).

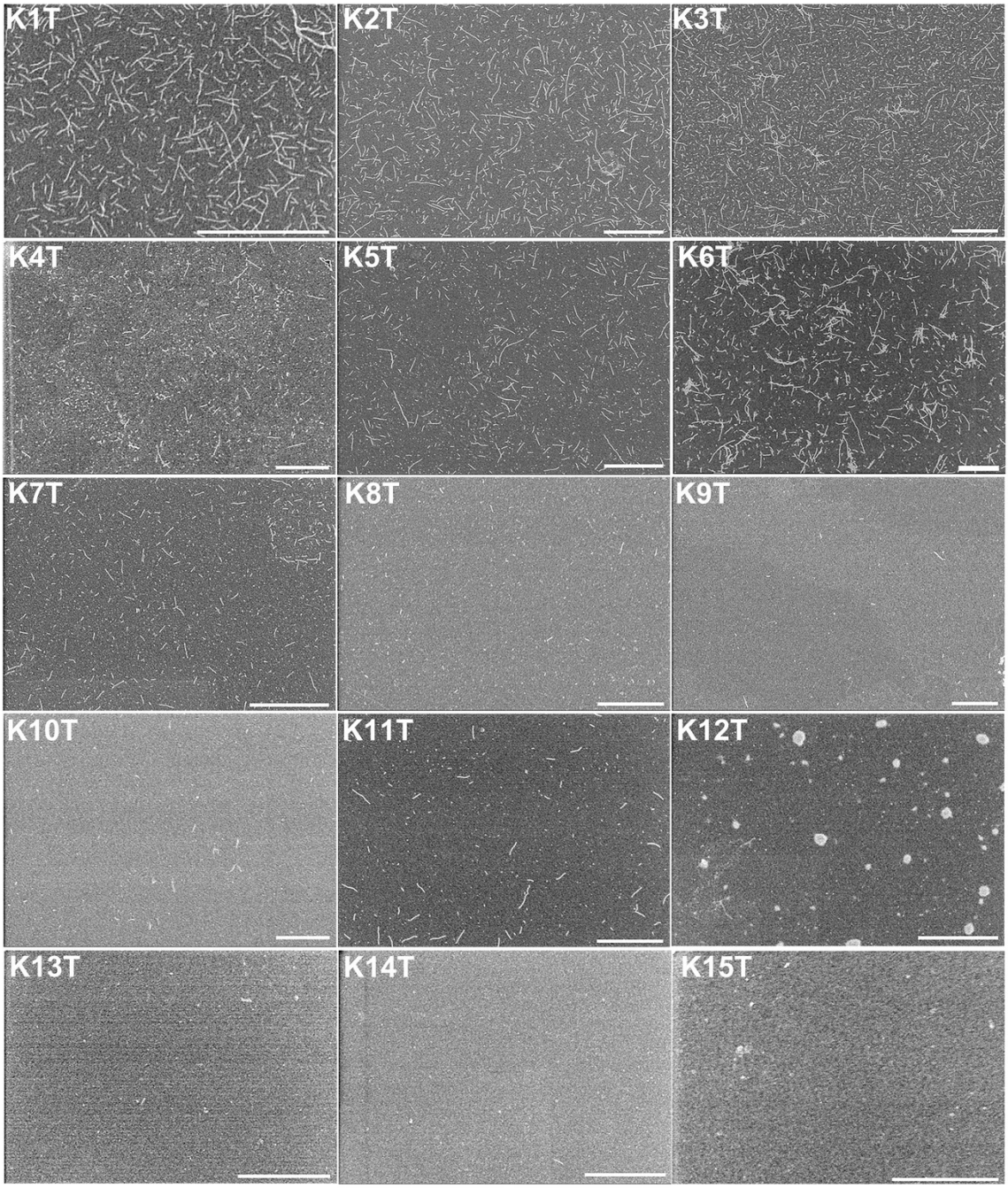

Fig. S1. SEM images of K1T to K15T RNTs in water (0.012 mg/mL). Scale bar = 500 nm

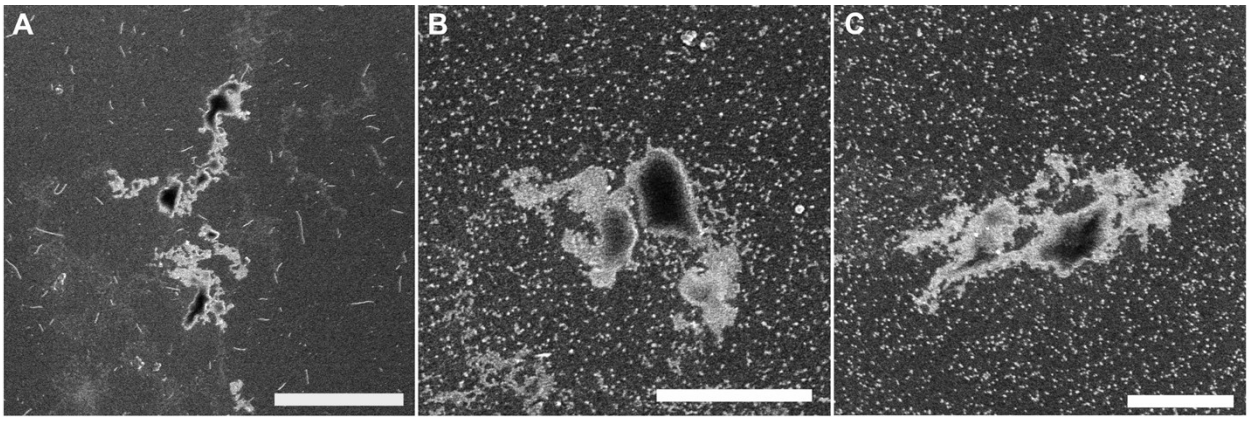

Fig. S2: SEM images of A) K5T, B) K10T and C) K15T RNTs and siRNA at molar ratio of 20:1 respectively. Scale bar = 500 nm

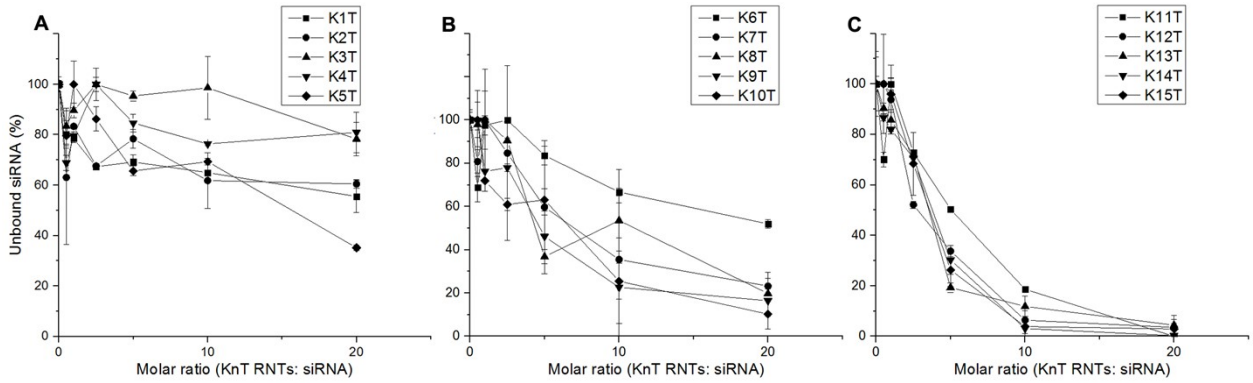

Fig. S3. Binding study of KnT RNTs (n = 1 – 15) and siRNA in PBS buffer (molar ratio of 0.5, 1, 2.5, 5, 10, 20:1) using agarose gel retardation assay.

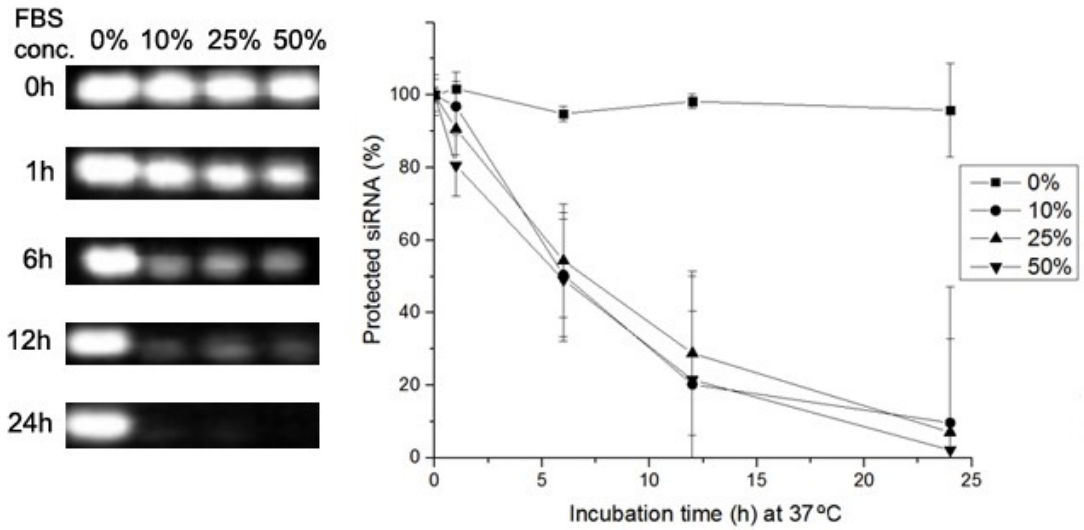

Fig. S4: Study of siRNA degradation in 0%, 10%, 25% and 50% of FBS for 0, 1, 6, 12 and 24 hours at 37 °C

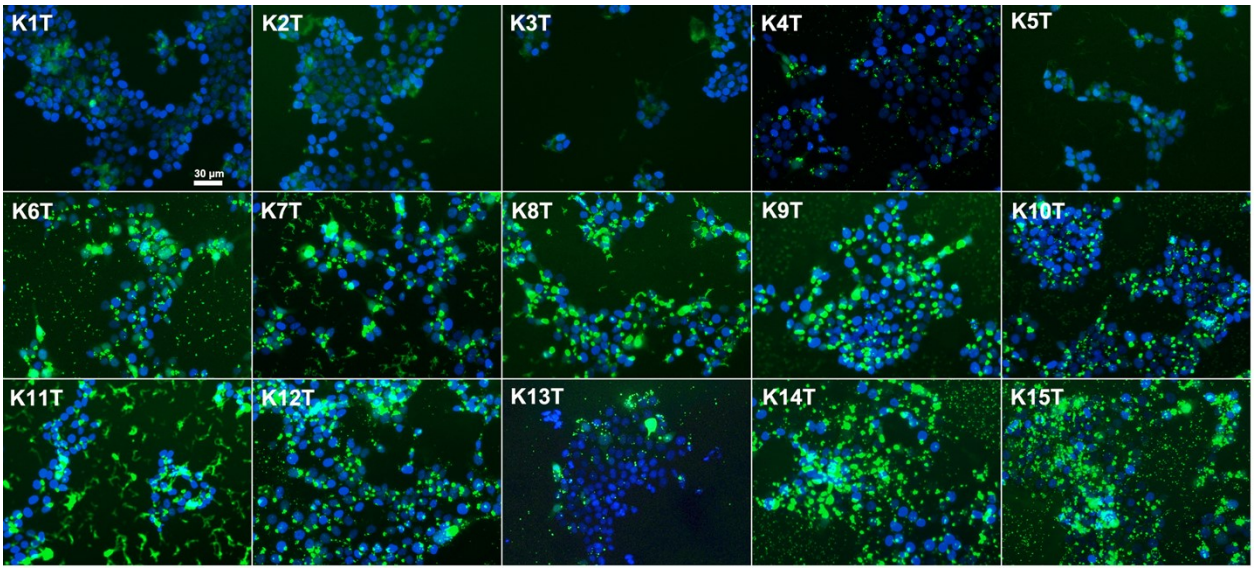

Fig. S5. Fluorescence imaging of HCT116 cells transfected with K1T - K15T RNTs complexes with siRNA (20:1 molar ratio) at 24 h.

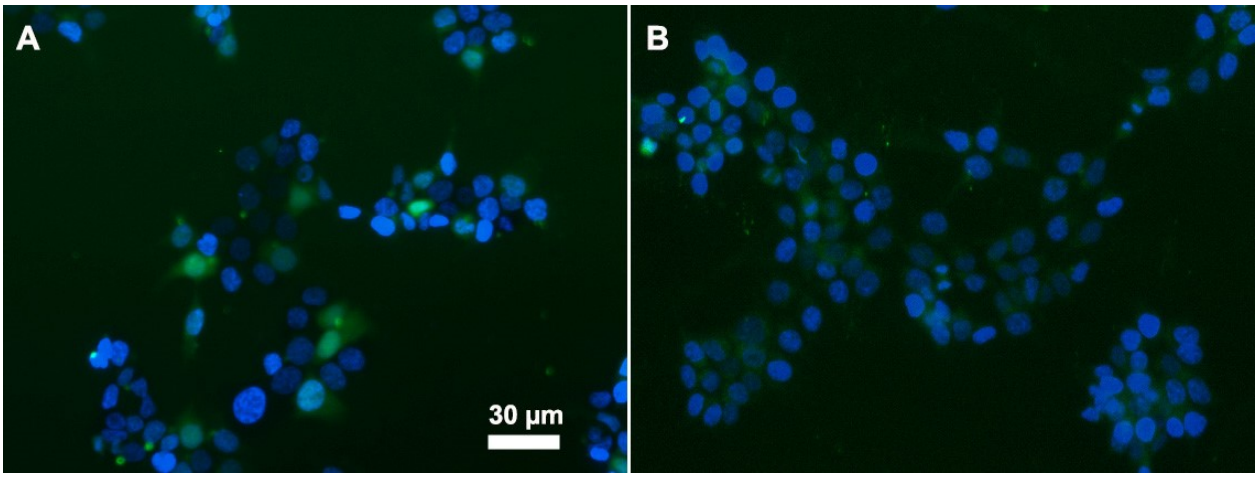

Fig. S6. Fluorescence imaging of HCT116 cells transfected with (A) positive control INTERFERin/FAM-siRNA and (B) negative control of FAM-siRNA alone. Blue: nuclei (DAPI), green: FAM-siRNA

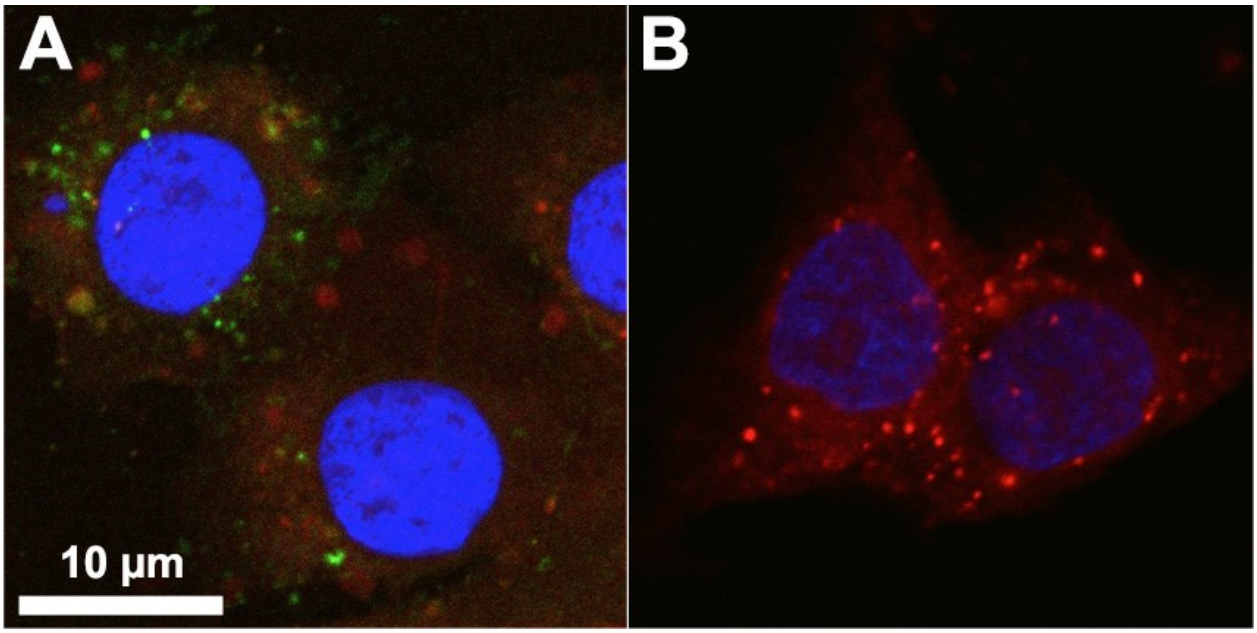

Fig. S7. Confocal imaging of HCT116 cells transfected with (A) positive control INTERFERin/FAM-siRNA and (B) negative control of FAM-siRNA alone. Blue: nuclei (DAPI), green: FAM-siRNA, red: LysotrackerRed DND-99.

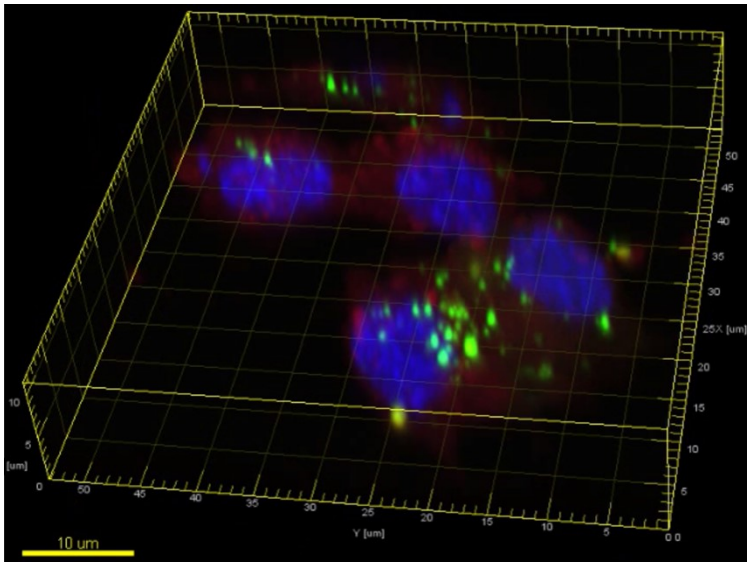

Fig. S8. 3D confocal image of HCT116 cells transfected with K10T RNTs/siRNA for 2h. Scale bar = 10 µm

Cytotoxicity

MTT assay was used to assess the toxicity effects of K1T to K5T RNTs to HCT116 cells at increasing dosage from 0.75 μM to 12 μM RNTs for 24h (Fig. S11) and K10T, K15T RNTs for various time points. Due to the low-charged nature of the five RNTs, result showed no significant cell death caused by the increase in RNTs concentrations, with the lowest cell viability observed to be 80%. This is important for future *in vivo* application of the RNTs as nanocarriers.

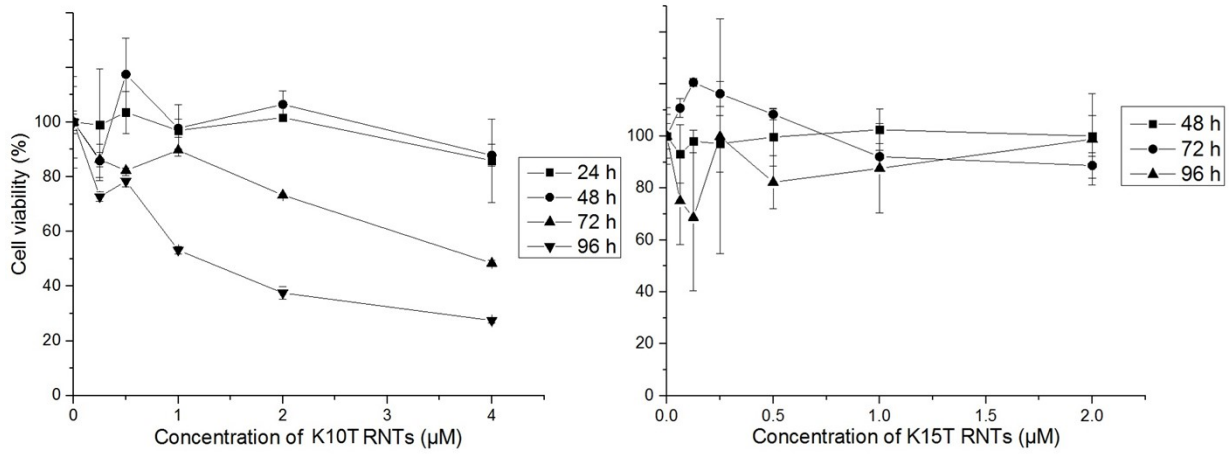

Fig. S9. MTT assay measuring cell viability (%) of HCT116 transfected with (A) K10T RNTs and (B) K15T RNTs at various concentrations and incubation time.

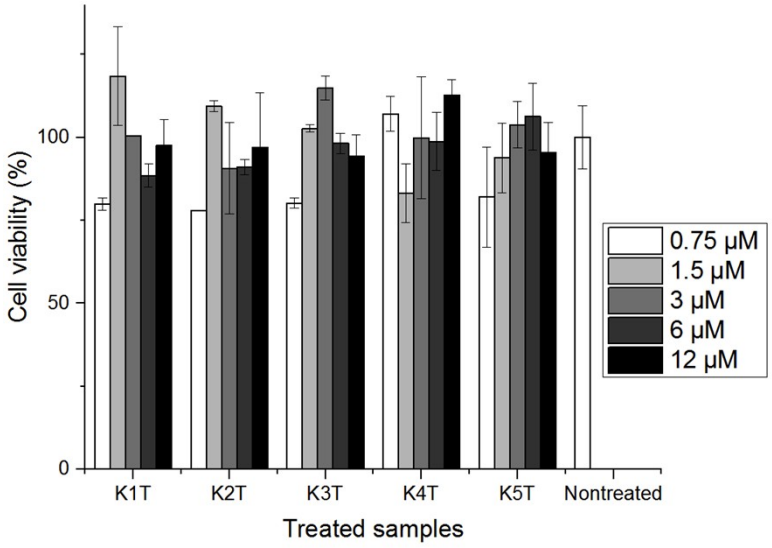

Fig. S10. MTT assay measuring cell viability (%) of HCT116 cells transfected with K1T to K5T RNTs