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Supplemental Information

Size, Shape, and Sequence-Dependent Immunogenicity of RNA Nanoparticles

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SUPPLEMENTAL INFORMATION:

Supplemental Figures:

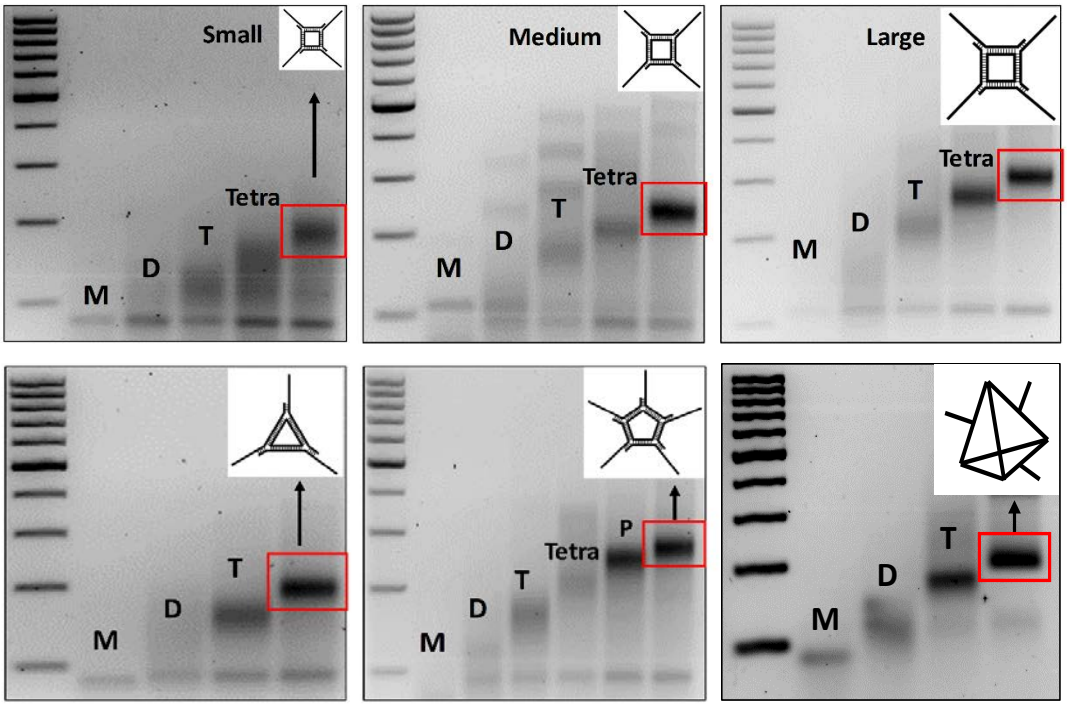


Figure. S1. Step-wise self-assembly of small, medium and large 2'F SQR-SEQ, 2'F TRI-SEQ, 2'F PENTA-SEQ and 2'F Tetrahedron-SEQ evaluated by 3% agarose gel (M=monomer, D=dimer, T=trimer, Tetra=tetramer, P=pentamer; ladder: 100 bp DNA).

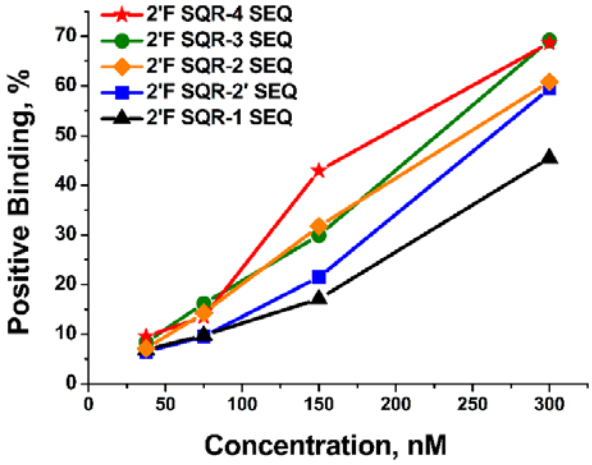


Figure. S2. Flow cytometry analysis showing the increased cellular binding of medium 2'F SQR-SEQ to RAW 264.7 cells as more copies of 2'F SEQ incorporated (2'F SQR-2 SEQ refers to 2'F Square with two SEQ extensions on the neighboring vertexes, and 2'F SQR-2' SEQ means 2'F Square with two SEQ extensions on the opposite vertexes).

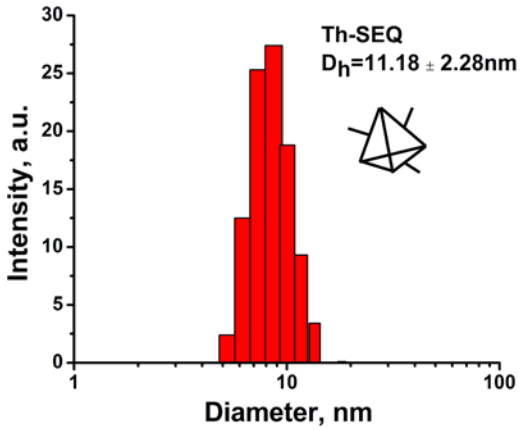
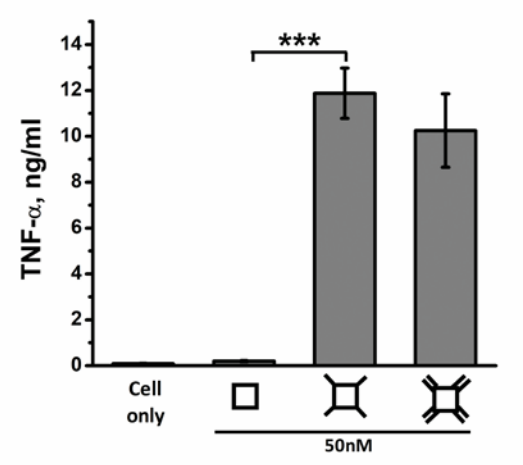


Figure. S3. Size distribution histogram of 2'F Tetrahedron (Th)-SEQ measured by DLS (n=3).

Comparison of 2'F SQR-SEQ and 2'F SQR-double-stranded SEQ

a. Cytokine TNF-α induction



b. Cytokine IL-6 induction

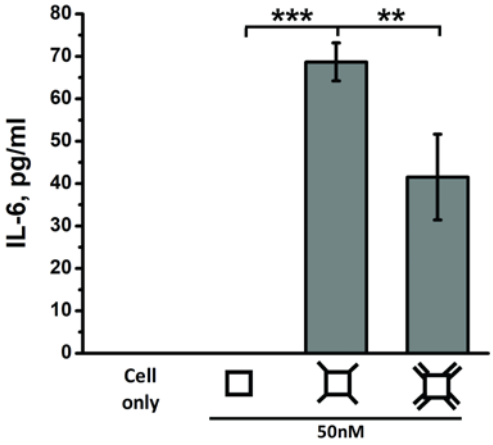


Figure. S4. Cytokines **a.** TNF-α and **b.** IL-6 induction by medium 2'F SQR-SEQ, 2'F SQR-double-stranded SEQ and control groups (concentrations refer to nanoparticles; results were presented as mean±standard deviation, n=3, $^{**}P<0.01$, $^{***}P<0.001$, analyzed by student's t test).

Table S1. Sequences for primary RNA nanoparticles (5'→3')

SEQ (20nt)	UCCAUGACGUUCCUGACGUU
Mutated SEQ I (20nt)	UCCAUGAGCUUCCUGACGUU
Mutated SEQ II (20nt)	UCCAUGAGCUUCCUGAGCUU
Scramble sequence(20nt)	GCAGCUUUGGCUGAGCGUAU
Complementary SEQ (20nt)	AACGUCAGGA ACGUCAUGGA
Small SQR A-SEQ (63nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCCGUCAAUCAUGACCGU ACUUUGUUGCACGCCC
Small SQR B-SEQ (63nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCGACCCAAUCAUGUCUCU ACUUUGUUGGCUGGCC
Small SQR C-SEQ (63nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCCAGCCAAUCAUGCACAU ACUUUGUUGACGGCCC
Small SQR D-SEQ (63nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCGUGCAAUCAUGUAGUU ACUUUGUUGGGUCGCC
Small SQR E (48nt)	GGUCAUGUGUAUGUGCAUGUGUAGAGACAUGUGUAACUACAUGUGU AC
Medium SQR A-SEQ (73nt) TRI A-SEQ (73nt) PENTA A-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCCGUCAAUCAUGGCAAG UGUCCGCCAUACUUUGUUGCACGCCC
Medium SQR B-SEQ (73nt) PENTA B-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCGACCCAAUCAUGGCAAC GAUAGAGCAUACUUUGUUGGCUGGCC
Medium SQR C-SEQ (73nt) TRI C-SEQ (73nt) PENTA C-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCCAGCCAAUCAUGGCAAU AUACACGCAUACUUUGUUGACGGCCC
Medium SQR D-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCGUGCAAUCAUGACAAG CGCAUCGCAUACUUUGUUGGGUCGCC
Medium SQR E (88nt)	GGACACUUGUCAUGUGUAUGCGUGUAUAUUGUCAUGUGUAUGCUCUA UCGUUGUCAUGUGUAUGCGAUGCGCUUGUCAUGUGUAUGGC
TRI B-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCGUGCAAUCAUGGCAAC GAUAGAGCAUACUUUGUUGGCUGGCC
TRI D (66nt)	GGACACUUGUCAUGUGUAUGCGUGUAUAUUGUCAUGUGUAUGCUCUA UCGUUGUCAUGUGUAUGGC
PENTA D-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCCCUACAAUCAUGGCAAG CGCAUCGCAUACUUUGUUGGGUCGCC

PENTA E-SEQ (73nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCGUGCAAUCAUGGCAAAUAUGCGCCAUACUUUGUUGUAGGGCC
PENTA F (110nt)	GGACACUUGUCAUGUGUAUGCGUGUAUAUUGUCAUGUGUAUGCUCUAUCGUUGUCAUGUGUAUGCGAUGCGCUUGUCAUGUGUAUGGCGCAUAUUUGUCAUGUGUAUGGC
Large SQR A-SEQ (93nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCCGUCAAUCAUGGCAAGUGUCCGCAAGCAUAGCUCGGAUAGCCUCAUACUUUGUUGCACGCCC
Large SQR B-SEQ (93nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCGACCCAAUCAUGGCAACGAUAGAGGCAUAGUCGACCUAUGCAUCCAUACUUUGUUGGCUGGCC
Large SQR C-SEQ (93nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGCCAGCCAAUCAUGGCAAUAUACACGCGAGUUGCCACGAGGACGCUCAUACUUUGUUGACGGCCC
Large SQR D-SEQ (93nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCGUGCAAUCAUGACAAGCAUCGCAUUCCGUGUCGUAGUCCUUCGCAUACUUUGUUGGGUCGCC
Large SQR E (168nt)	GGACACUUGUCAUGUGUAUGAGCGUCCUCGUGGCAACUCGCGUGUAUAUUGUCAUGUGUAUGGAUGCAUAGGUCGACUAUGCCUCUAUCGUUGUCAUGUGUAUGCGAAGGACUACGACACGGAAUGCGAUGCUUGUCAUGUGUAUGAGGCUAUCCGAGCUAUGCUUGC
Th A-SEQ (115nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGACUGAUACGAAUCAUCGUGUAGCACCAGCUGUAAUCGAUGUGUACGGGAAGAGCCUAUGCCCAUCCUACUUUGUUCUACUAUGGCG
Th B-SEQ (115nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGUGCUACACGAUGUGUAGCCAGACUUAGCGGAAUGUUCGUACUUUGUUCAUGCGAGGCCGUCCAAUACCGAAUCAUCGAUUACAGCU
Th C-SEQ (115nt)	GGUCCAUGACGUUCCUGACGUUUUUUUGGGCAGUUGAGAUGUGUACGAACAUUCCGCUAAGUCUGGCUACUUUGUUCGUAUCAGUCCCGCCAUAGUAGAAUCAUCGUAUCACCAU
Th D (88nt)	GGCCUCGCAUGAAUCAUCUCAACUGCCCAUGGUGAUACGAUGUGUAGGAUGGGCAUAGGCUCUUCCCGUACUUUGUUCGGUAUUGGAC
RNA SQR (small, medium & large)	Reference (54)
RNA TRI, SQR & PENTA	Reference (59)
RNA Th	Reference (43)

(SEQ: specific sequence, TRI: triangle, SQR: square, PENTA: pentagon, Th: tetrahedron)

Supplemental Methods:

Flow Cytometry Assay

5×10^5 RAW 264.7 cells were suspended in Opti-MEM medium in 1.5mL eppendorf tubes. Cy3-labled RNA nanoparticles were diluted in Opti-MEM medium at 100nM and incubated with cells at 37 °C for 1.5 hours. After washing with PBS buffer (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) to remove unbound nanoparticles, cells were re-suspended in PBS buffer and the cell binding efficacy was determined by FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).