

**Supplementary Information for
Regulation of Reversible Conformational Change, Size Switching and
Immunomodulation of RNA Nanocubes**

Peter Blanco Carcache^{a,b,c,d}, Sijin Guo^{a,b,c,d†}, Hui Li^{a,b,c,d†}, Kaiming Zhang^{e†}, Congcong Xu^{a,b,c,d}, Wah Chiu^f and Peixuan Guo^{a,b,c,d*}

#Address correspondence to: Peixuan Guo, PhD
Sylvan G. Frank Endowed Chair in Pharmaceuticals and Drug Delivery
The Ohio State University
Email: guo.1091@osu.edu

This PDF file includes:

Supplementary Information Text

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Materials and methods SI

The design, synthesis, and self-assembly of RNA nanocubes SI

Briefly, DNA templates for generating the RNA strands were made by polymerase chain reactions (PCR). DNA oligo's for PCR were directly ordered from Integrated DNA Technologies, Inc. (Coralville, IA). For a 50 μ l transcription reaction, 10 μ l of DNA template with 10 μ l of 5 $\times$ transcription buffer, 10 μ l of 25 mM NTPs solution, 5 μ l of 100 mM DTT, 10 μ l of T7 RNA polymerase, and 5 μ l 0.05% (vol/vol) DEPC water were mixed together and incubated at 37 $^{\circ}$ C for 4 hrs. The transcription reaction was stopped by adding 1 μ l of DNase I and incubating it at 37 $^{\circ}$ C for another 30 min to digest the DNA template. For generating 2'-F modified RNA tetrahedrons, Y693F mutant T7 polymerase and 2'-F modified cytosine (C) and uracil (U) nucleotides were used to synthesize the corresponding RNA strands. For a 50 μ l 2'-F transcription reaction, 2.5 μ g of DNA template diluted in 15 μ l 0.05% (vol/vol) DEPC water, 5 μ l of 10 $\times$ 2'-F transcription buffer, 5 μ l of 100 mM DTT, 5 μ l of 50 mM 2'-F CTP, 5 μ l of 50 mM 2'-F UTP, 5 μ l of 50 mM ATP, 5 μ l of 50 mM GTP, and 5 μ l of Y639F mutant T7 RNA polymerase were mixed together and incubated at 37 $^{\circ}$ C overnight. After transcription, the RNA strands were purified by 8 M urea, 8% denaturing PAGE, respectively.

The detailed sequences for assembling the designed RNA cubes are listed below:

Strand 1:

5'- GGUGCUACACGAUGUGUAGCCAGACUUAGCGGAAUGUUCGAUGUGUAC
GGGAAGAGCCUAUGCCCAUCCAUGUGUACAUGCGAGGCCGUCCAAUACCGAU
GUGUACGAUUACAGCU -3'

Strand 2:

5'- GGCUCUCCCCGUACUUUGUUCUGUGUCGUCCAAGACUGUCGAAAUCAU
GUUACAGUGCCCAUACUCGACAUACUAAACUCAAACUGCCCAUGGUGAUACGUU
GUUUCAUGGAUGGGCAUA -3'

Strand 3:

5'- GGGCAGUUGAGUUGUUUCAUGAGUAUACCACGAUCUGCUCGAUACUUU
GUUCGUACUCCGUCUCCUCAACCUAUUAUCGGUAUUGGACGGCCUCGCAU
GUACUAAACGUAUCACCAU -3'

Strand 4:

5'- GGAUCAGCUCAUACUAAGACUACGUGUACACUUGCAACUUUGUUUCAU
CGUGUAGCACCAGCUGUAAUCGUACUUUGUUAGGUUGAGGAAGACGGAGUA
CGAAUCAUCAGUAGCAGGC -3'

Strand 5:

5'- GGACGACACAGAAUCAUCGAACAUUCCGCUAAGUCUGGCUACUAAAGU
UGCAAGUGUACACGUAGUCUUGUUUCAUUAACCGACGUAUGCGUGAUGCUAU
ACUUUGUUUCGACAGUCUU -3'

Strand 6:

5'- GGCACUGUAACAUGUGUAUAGCAUCACGCAUACGUCGGUAAUGUGUAU
GAGCUGAUCCGCCUGCUACUGAUGUGUAUCGAGCAGAUCGUGGUAUACUCA
UGUGUAUGUCGAGUAUG -3'