Supporting Information

Self-Assembly of Four Generations of RNA Dendrimers for Drug Shielding with Controllable Layer-by-Layer Release

Xin Li^{1,2}, Dr. Mario Vieweger^{1,2}, Dr. Peixuan Guo^{1,2,3,4,5}*



Figure S1. Sequence design and thermostability of new 3WJs. a) Sequence design and GC content of 3WJs, 3WJ-WT, 3WJ-H₀ and 3WJ-H₁. b) Thermostability evaluation of 3WJs including melting and annealing curve.



Figure S2. Paclitaxel release from RNA strands. a) Schematics of PTX release from RNA strands by esterase hydrolysis. b) Release profile of RNA-6 PTXs strands in 50% FBS for up to 24 h.



Figure S3. Characterizations of Ext-G₄-FA-PTX RNA dendrimers (a) Zeta potential measurement (mean \pm SD), (b) Thermal stability assay and (c) Enzymatic stability assay of Ext-G₄ and Ext-G₄-FA-PTX RNA dendrimers.



Figure S4. Shielding effect of RNA dendrimers using folate as model cargo demonstrated by flow cytometry.



Figure S5. Quantitative analysis of biodistribution in tumors and vital organs derived from organ images in Figure 6 (Average Radiant Efficiency, $[p/s/cm^2/sr] / [\mu W/cm^2]$).

Strand(nt)(g/mol)1 H_0 -a/WT-b5'-3812099AUUCGCUGUGUGUGGUAGUGCCCACAUACUU UGUUGAUCC-3'40125662 H_0 -b/WT-b5'-4012566CACUACCACUUUGUCCUACGCCCACAUACU UUGUUGAUCC-3'014703 H_0 -c/WT-b5'-3611470CGUAGGACCAGCGAAUCCCACAUACUUUG UUGAUCC-3'012714 H_1 -a/ H_0 -b5'-38120745 H_1 -b/ H_0 -b5'-4012711CGGUAGCACUUUGCUGUGCGCACUACCACUUU UUGUCCUACG-3'0127116 H_1 -c/ H_0 -b5'-3611407CGCACAGCCAGCCAGCACGCCACUACCACUUUGU CCUACG-3'36114077Ext-H_1-a/ H_0 -b5'-6019108CCUAUUCAGGUGCGUGCUGUGUGUGCUACCG6019108
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
$ \begin{array}{cccc} & \operatorname{AUUCGCUGUGUGUGGUAGUGCCACAUACUU} \\ & \operatorname{UGUUGAUCC-3'} & & & & & & & & & & & & & & & & & & &$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
3 H_0 -c/WT-bCACUACCACUUUGUCCUACGCCCACAUACU UUGUUGAUCC-3'36114703 H_0 -c/WT-b5'-36114704 H_1 -a/H_0-b5'-38120746 H_1 -b/H_0-b5'-40127116 H_1 -c/H_0-b5'-40127117Ext-H_1-a/H_0-b5'-3611407
$ \begin{array}{ccccc} & & & & & & & & & & & & & & & & &$
$ \begin{array}{ccccc} 3 & H_0\mbox{-}c/WT\mbox{-}b & 5'\mbox{-} & 36 & 11470 \\ & CGUAGGACCAGCGAAUCCCACAUACUUUG \\ & UUGAUCC\mbox{-}3' & 38 & 12074 \\ & GCGUGCUGUGUGUGUGCUACCGCACUACCACUUU \\ & GCGUGCUGUGUGUGUGCUACCGCACUACCACUUU \\ & GUCCUACG\mbox{-}3' & 40 & 12711 \\ & CGGUAGCACUUUGCUGUGCGCACUACCACU \\ & UUGUCCUACG\mbox{-}3' & 40 & 12711 \\ & CGGCACAGCCAGCACQUUGCGCACUACCACU \\ & UUGUCCUACG\mbox{-}3' & 36 & 11407 \\ & CGCACAGCCAGCCAGCACGCCACUACCACUUUGU \\ & CCUACG\mbox{-}3' & 5'\mbox{-} & 36 & 11407 \\ & CGCACAGCCAGCCAGCACGCCACUACCACUUUGU \\ & CCUACG\mbox{-}3' & 60 & 19108 \\ & CCUAUUCAGGUGCGUGCUGUGUGCUACCG & 60 \\ \end{array} $
$ \begin{array}{cccc} CGUAGGACCAGCGAAUCCCACAUACUUUG \\ UUGAUCC-3' \\ 4 & H_1-a/H_0-b \\ 5'- & 38 \\ GCGUGCUGUGUGUGUGCUACCGCACUACCACUUU \\ GUCCUACG-3' \\ 5 & H_1-b/H_0-b \\ 5'- & 40 \\ UUGUCCUACG-3' \\ 6 & H_1-c/H_0-b \\ 5'- & 36 \\ UUGUCCUACG-3' \\ 6 & H_1-c/H_0-b \\ 5'- & 36 \\ UUGUCCUACG-3' \\ 7 & Ext-H_1-a/H_0-b \\ 5'- & 60 \\ CCUAUUCAGGUGCGUGCUGUGUGUGCUACCG \\ \end{array} $
$ \begin{array}{cccc} & & & & & & & & & & & & & & & & & $
$ \begin{array}{ccccc} 4 & H_1 \cdot a / H_0 \cdot b & 5' \cdot & 38 & 12074 \\ & & & & & & \\ & & & & & \\ & & & & & $
 GCGUGCUGUGUGUGCUACCGCACUACCACUUU GUCCUACG-3' H₁-b/H₀-b S'- CGGUAGCACUUUGCUGUGCGCACUACCACU UUGUCCUACG-3' H₁-c/H₀-b S'- CGCACAGCCAGCACGCACUACCACUUUGU CCUACG-3' Ext-H₁-a/H₀-b S'- CCUAUUCAGGUGCGUGCUGUGUGUGCUACCG
5 H ₁ -b/H ₀ -b GUCCUACG-3' 40 12711 5 H ₁ -b/H ₀ -b 5'- 40 12711 6 H ₁ -c/H ₀ -b 5'- 36 11407 6 H ₁ -c/H ₀ -b 5'- 60 19108 7 Ext-H ₁ -a/H ₀ -b 5'- 60 19108
5 H_1 -b/ H_0 -b5'-4012711CGGUAGCACUUUGCUGUGCGCACUACCACU UUGUCCUACG-3'UUGUCCUACG-3'36114076 H_1 -c/ H_0 -b5'-3611407CGCACAGCCAGCACGCCACUACCACUUUGU CCUACG-3'CCUACG-3'60191087Ext- H_1 -a/ H_0 -b5'-6019108
6 H1-c/H0-b 5'- 36 11407 7 Ext-H1-a/H0-b 5'- 60 19108 CCUAUUCAGGUGCGUGCUGUGUGUGUGUGCUACCG 60 19108
6 H ₁ -c/H ₀ -b 5'- 36 11407 CGCACAGCCAGCACGCCACUACCACUUUGU CCUACG-3' 60 19108 CCUAUUCAGGUGCGUGCUGUGUGUGCUACCG
6 H ₁ -c/H ₀ -b 5'- 36 11407 CGCACAGCCAGCACGCCACUACCACUUUGU CCUACG-3' 60 19108 7 Ext-H ₁ -a/H ₀ -b 5'- 60 19108
7 Ext-H1-a/H0-b 5'- 60 19108 CCUAUUCAGGUGCGUGCUGUGUGUGUGCUACCG 60 19108
7 Ext-H ₁ -a/H ₀ -b 5'- 60 19108 CCUAUUCAGGUGCGUGCUGUGUGCUACCG
7 Ext-H ₁ -a/H ₀ -b 5'- 60 19108 CCUAUUCAGGUGCGUGCUGUGUGCUACCG
CCUAUUCAGGUGCGUGCUGUGUGCUACCG
AUGUAAUUCAACACUACCACUUUGUCCUA
CG-3'
8 Ext-H ₁ -b/H ₀ -b 5'- 62 19841
UUGAAUUACAUCGGUAGCACUUUGCUGUG
CGAGGCUGAACAGCACUACCACUUUGUCCU
ACG-3'
9 Ext-H ₁ -c/H ₀ -b 5'- 58 18471
UGAAUAGGCACUACCACUUUGUCCUACG-3'
10 WT-b/H ₀ -a 5'- 38 12099
CCCACAUACUIUIGUUGAUCCAUUCGCUGU
GUGGUAGUG-3'
11 WT-b/H _{0-C} $5'_{-}$ 36 11470
CCCACAUACUIUIGUIGAUCCCGUAGGACCA
GCGAAU-3'
12 3WI-WT-a 5'-UUGCCAUGUGUAUGUGGG-3' 18 5784
13 $3WI-WT-b$ 5'-CCCACAUACUIUIGUIUGAUCC-3' 20 6253
14 3WJ-WT-c 5'-GGAUCAAUCAUGGCAA-3' 16 5140
$15 3WJ-H_{0-a} \qquad 5'-AUUCGCUGUGUGGUAGUG-3' \qquad 18 5784$
$16 3WJ-H_0-b \qquad 5'-CACUACCACUUUGUCCUACG-3' \qquad 20 6252$
$17 3WJ-H_0-c \qquad 5'-CGUAGGACCAGCGAAU-3' \qquad 16 5155$
18 $3WJ-H_1-a$ 5'-GCGUGCUGUGUGCUACCG-3' 18 5760

 Table S1. Summary of sequences used in RNA dendrimer construction.

19	3WJ-H ₁ -b	5'-CGGUAGCACUUUGCUGUGCG-3'	20	6398					
20	3WJ-H ₁ -c	5'-CGCACAGCCAGCACGC-3'	16	5093					
21	Ext-H ₁ -a/H ₀ -b	<u>5'</u> -	60	19284					
	6 ALKs	CCUAU <u>U</u> CAGG <u>U</u> GCGUGCUGUGUGCUAC <u>C</u> G							
		AUG <u>U</u> AAUU <u>C</u> AACACUACCACUUUGUCCUA							
		CG-3'							
22	Ext-H ₁ -b/H ₀ -b_	<u>5'</u> -	62	20017					
	6 ALKs	UUGAA <u>U</u> UACA <u>U</u> CGGUAGCACUUUGCUGUG							
		<u>C</u> GAGG <u>C</u> UGAA <u>C</u> AGCACUACCACUUUGUCCU							
		ACG-3'							
23	Ext-H ₁ -c/H ₀ -b_	5'-	58	18585					
	6 ALKs	<u>CUGUUCAGCCU</u> CGCACAGCCAGCA <u>C</u> GCAC <u>C</u>							
		UGAA <u>U</u> AGGCACUACCACUUUGUCCUACG-3'							
All C and U are 2'-fluoro modified. All <u>5'</u> , <u>C</u> and <u>U</u> are 2'-O-propargyl modified.									

	RNA	Strand	Strand	Nucleotide	Terminal	Size	T_{m}	Ta	MW
	nanoparticle	Type	Amount	Amount	Ends	(nm)	(°C)	(°C)	(g/mol)
1	3WJ-WT	3	3	54	3	N/A	54.8	63.5	17177
2	$3WJ-H_0$	3	3	54	3	N/A	61.7	67.2	17191
3	$3WJ-H_1$	3	3	54	3	N/A	68.5	74.4	17251
4	G3-1	5	9	216	6	7.98	52.0	72.3	68908
5	G3-2	5	9	216	6	8.39	58.3	79.4	69010
6	Ext-G ₃	5	9	282	6	10.38	59.7	N/A	90238
7	G ₄	7	21	540	12	13.12	52.6	76.5	172444
8	Ext-G ₄	7	21	606	12	14.24	53.6	95.0	193672
9	Ext-G ₄ -FA-PTX	7	21	606	12	17.90	53.4	N/A	217780

 Table S2. Summary of RNA dendrimers characteristics.