Supplementary Information

Development of CS-TPP-dsRNA nanoparticles to enhance RNAi efficiency in the yellow fever mosquito, *Aedes aegypti*

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Table S1 Formation of the chitosan and TPP nanoparticles

	TPP (mg/ml)					
CS (mg/ml)	0.25	0.5	0.75	1	1.25	1.5
1 mg	++	++	++	++	--	--
3 mg	××	××	++	++	++	++
5 mg	××	××	++	++	++	++
7 mg	××	××	××	++	++	++

++, opalescent suspension; ××, Clear solution; --, aggregates

Table S2 DLS analysis of CS-TPP nanoparticles

Ratio	**Size**	**Charge**	**PDI**
CS-TPP (1:1)	160±3.21	1.97±0.29	0.45±0.018
CS-TPP (3:1)	171±2.31	13.97±1.51	0.29±0.021
CS-TPP (5:1)	115±3.71	48.67±1.71	0.19±0.023
CS-TPP (7:1)	186.67±2.96	65.73±1.68	0.51±0.015

Table S3 DLS analysis of CS-TPP-dsRNA nanoparticles

Ratio	**Size (nm)**	**Charge (mv)**	**PDI**	**Percent dsRNA incorporated**
CS-TPP-dsRNA (1:1)	242.67±4.06	-18.37±1.97	0.31±0.04	32±2.31
CS-TPP-dsRNA (3:1)	263.33±5.61	11±0.93	0.39±0.02	61.67±2.03
CS-TPP-dsRNA (5:1)	181.67±3.48	34.37±0.94	0.26±0.02	81.33±2.03
CS-TPP-dsRNA (7:1)	3157±9.29	47.57±1.25	0.46±0.03	68±1.73

Table S4. List of primers used in this study. All primers except those labeled as qPCR were used for dsRNA synthesis.

S. No	Genes	Primer sequences (5'-3')	Amplicon Size (bp)	Accession No	References
1.	EGFP	FP: CGATGCCACCTACGGCAA RP: TGTCGCCCTCGAACTTCA	248		
2.	IAP1	FP: CTTCTGCCGAGTGGAAATCGG RP: ATATTCCGGTAGCTTCTGTTG	349	DQ993355.1	47
		qPCR-FP: GTGTTTGGCCAAGAAGGAAAG qPCR-RP: TGACTGAAGCGAGGATGTTG	118		
3.	SNF7	FP: ACGATGTCCACGAGATGATG RP: CAGGCAGATCGGTTGCT	222	XM_001659907.2	52
4.	SRC	FP: CGTCAAATGCAGCAGATCACCCAA RP: TGTTGGTTGTTCGAGGGAGAAGGT	431	XM_021845577.1	
5.	SSK	FP: TCATCTACCGAACGGGCTA RP: TTCTCTCCGAGGTGATGTG	303	XM_021848746.1	56
6.	MESH	FP: TGGAACGGTAGTCAACATCA RP: CCCACAGCGAGATCTGAAC	350	XM_021840549.1	
7.	HEL25E	FP: CAAGCTGTGCTAGGAATGGA RP: CGAGAATGAAATGCTTCAGGTG	355	XM_001658256.2	
8.	SAC1	FP: ATACGGTCGACAGGTTCTGG RP: CAGACGTTCTTGAACAGCCA	420	XM_001658506.2	57
9.	LRC	FP: GCCAGACTTGAGCAAACCTC RP: TTGTTGTTTGGCGTTAGCAG	327	XM_001658387.2	
10.	OTK	FP: GGTGCTGACCGAGGTACATT RP: AGGACTTTTCCGGTAGGCAT	338	XM_021853098.1	
11.	S7RP	qPCR-FP: ACCGCCGTCTACGATGCCA qPCR-RP: ATGGTGGTCTGCTGGTTCTT	131	CR938234.1	58

Figures

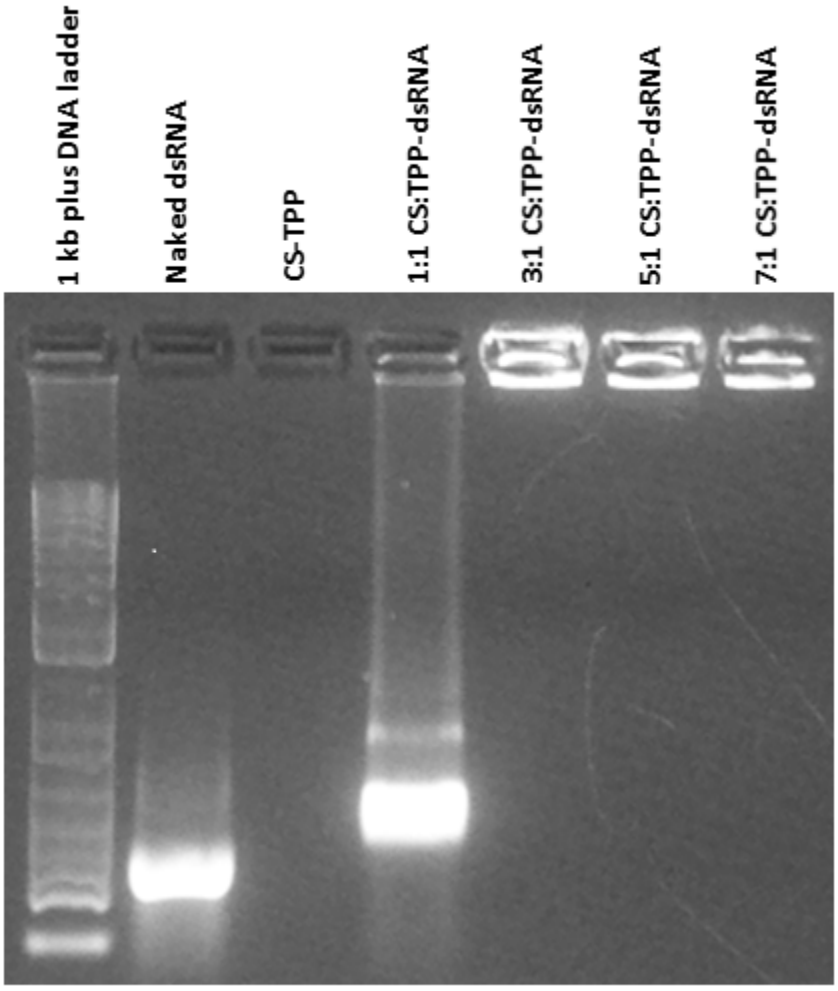

Fig S1 Gel retardation assay of nanoparticle complexes formed with different ratios of chitosan (CS). TPP and dsRNA were conjugated with different ratios of chitosan and analyzed by gel electrophoresis.

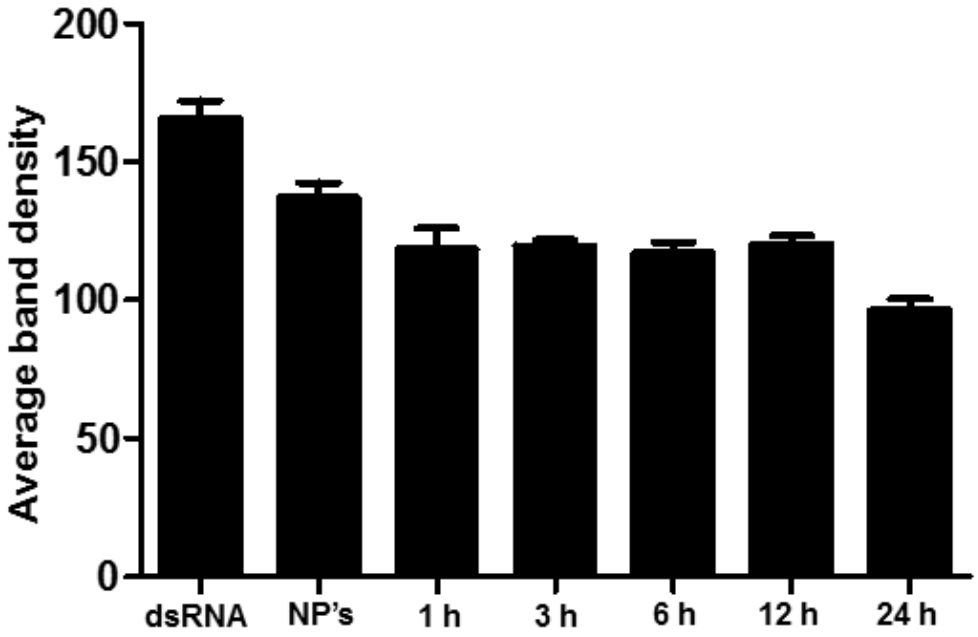

Fig S2 Stability of CS-TPP-dsRNA nanoparticle complexes exposed to lumen contents of mosquito larvae. CS-TPP-dsRNA nanoparticles were exposed to lumen contents collected from *Aedes aegypti* larvae. At 1, 3, 6, 12 and 24 h after mixing dsRNA and lumen contents, the samples were collected and analyzed in agarose gels. The gels were stained with GelRed® and photographed under UV light. The bands from scanned gel images were quantified and Mean+SE (n=3) are shown.

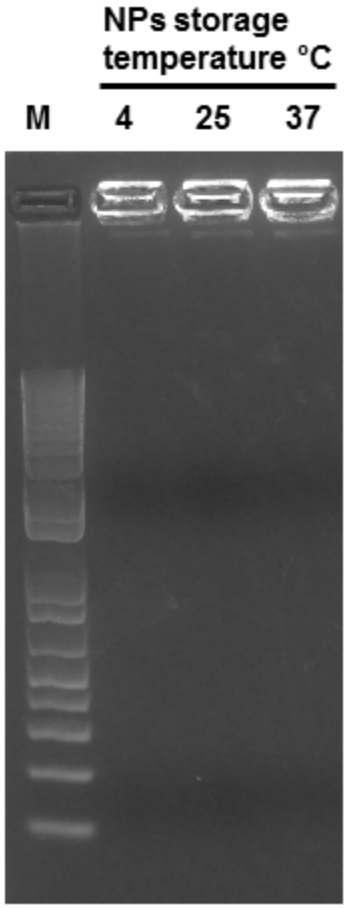

Fig S3 Storage stability of CS-TPP-dsRNA nanoparticles. NPs were prepared and stored at 4, 25 and 37°C for 10 days and analyzed by gel electrophoresis. M, 1 kb plus DNA ladder.

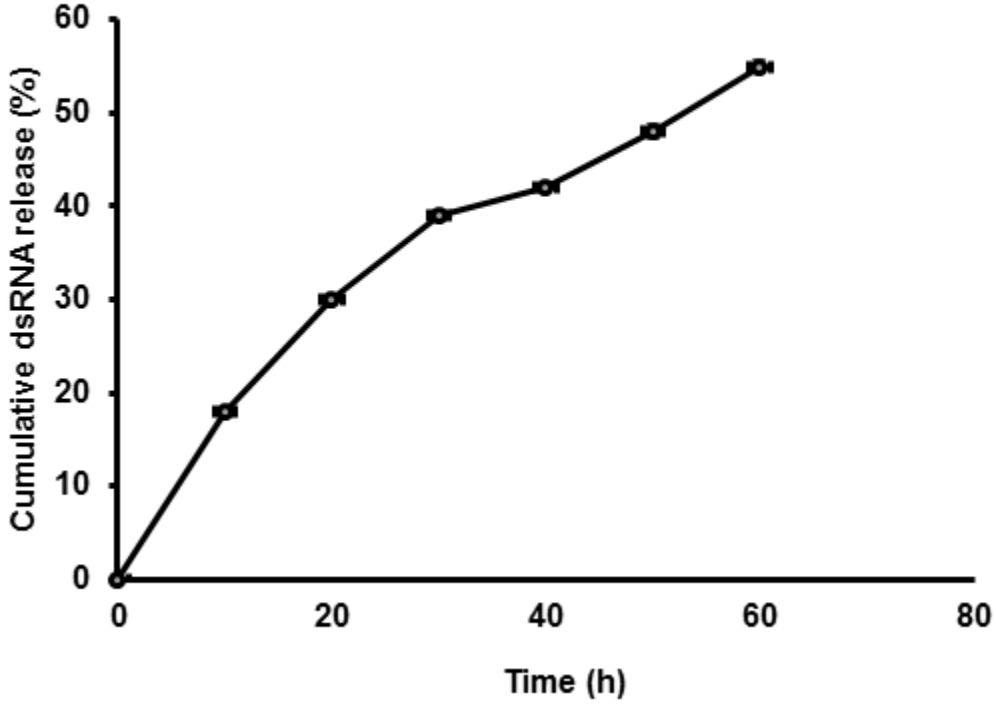

Fig S4 The profile of cumulative dsRNA release. CS-TPP-dsRNA NPs were incubated in 1X PBS buffer, pH 7.4, for different time intervals. The dsRNA released was quantified. The data shown are Mean ± SE (n=3).