

Supplementary Information

**DNA Origami: Thinking ‘Outside the Fold’ for Direct Integrity Testing
of Membranes for Virus Removal in Potable Reuse Applications**

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DNA nanostructure; quantitative polymerase chain reaction (qPCR)

Figure S1. Photographs of the single-element membrane system. **(Left)** Direction of flow is described and indicated by arrows. **(Right)** Due to the batch/recirculating configuration of the system, the feed water was constantly cooled to prevent temperature increases. This was accomplished by placing the feed-containing aluminum tank in a larger plastic tank filled with single-pass cooling water. The feed water to the system consisted of a full-scale tertiary effluent (primary clarification; activated sludge with full nitrification, partial denitrification, and biological phosphorus removal; secondary clarification; and ultrafiltration) that was pH-adjusted from 7.3 to 6.9 using sulfuric acid. Aliquots of each spiking stock (see main text) were added to the water, and the water was manually mixed. A 50-mL sample was collected into a conical tube to represent the combined feed water in the tank at time zero. The feed pump was then started, and the water was allowed to recirculate for 30 min prior to sample collection, at which point 50-mL feed and permeate samples were collected every 5-15 min.

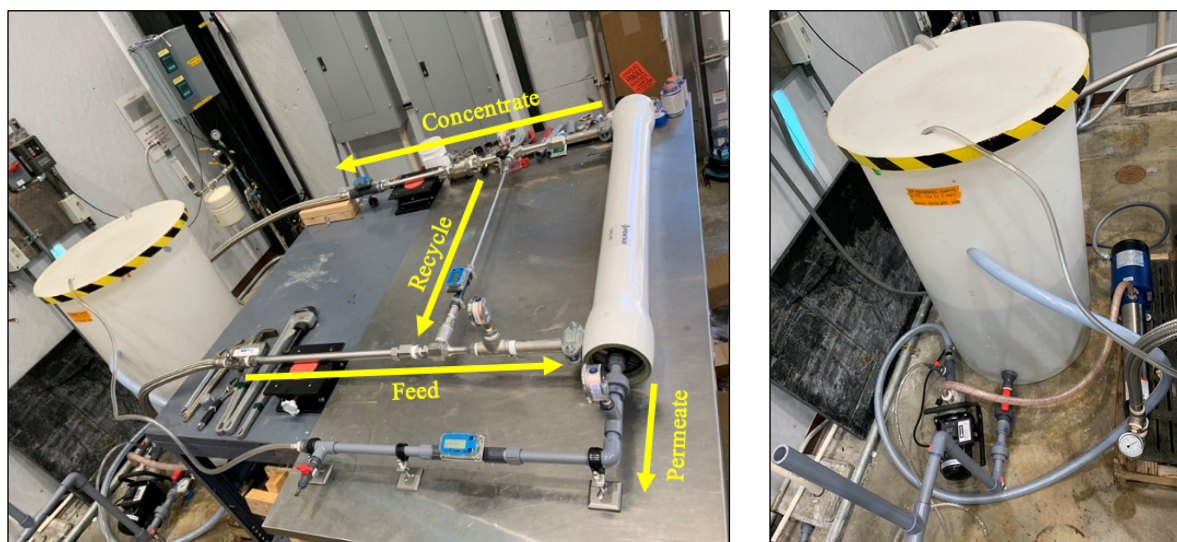


Table S1. Summary of general water quality parameters for the full-scale tertiary effluent (averages of two sample events). The treatment train consists of primary clarification, activated sludge (full nitrification, partial denitrification, and biological phosphorus removal), secondary clarification, and ultrafiltration.

Water Quality Parameter	Units	Value
pH		7.0
Total organic carbon (TOC)	mg-C/L	6.0
Electrical conductivity (EC)	$\mu\text{S}/\text{cm}$	1678
Alkalinity	mg/L as CaCO_3	94
Turbidity	NTU	<1
UV_{254}	cm^{-1}	0.12
Nitrate	mg-N/L	12
Nitrite	mg-N/L	<0.10
Ammonia	mg-N/L	0.65
Calcium	mg/L	92
Magnesium	mg/L	38
Hardness	mg/L as CaCO_3 (mM)	386 (3.86)

Table S2. Summary of operational conditions for each membrane.

Operational Parameter	Units	RO Hydranautics ESPA2-LD-4040	NF (1) Toray CSM NE4040-40	NF (2) Dupont Filmtec NF270
Feed Flow (FF)	gpm	8.20	9.42	9.29
Recycle Flow (RF)	gpm	0.92	1.00	0.92
Permeate Flow (PF)	gpm	0.89	1.03	1.03
Concentrate Flow (CF)	gpm	6.39	7.38	7.34
Recovery (PF/FF)	%	10.8%	10.9%	11.1%
Flux	gfd	15.9	17.5	18.1
Feed Pressure	psi	138	93	50
Molecular Weight Cutoff	Da	100–200 ^a	320–350 ^b	155–400 ^c

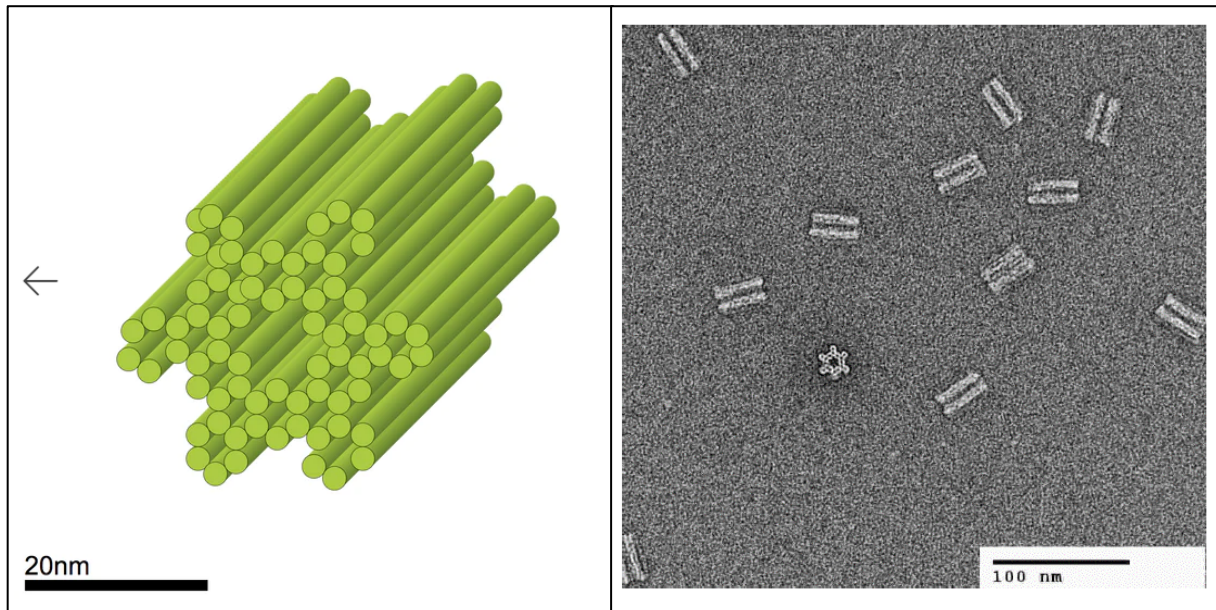
^aReferences: [1]–[3]. ^bReference: [4]. ^cReferences: [5]–[9].

Table S3. Summary of the experimental conditions for the three spiking experiments.

Spiking Experiment	Membrane	Type	DNA Nanostructure Formulation^a
1	Hydranautics ESPA2-LD-4040	RO	GP, NL, NS
	CSM NE4040-40	NF1	GP, NL, NS
	Filmtec NF270	NF2	GP, NL, NS
2	Hydranautics ESPA2-LD-4040	RO	GP, NL, NS
	CSM NE4040-40	NF1	GP, NL, NS
3	Hydranautics ESPA2-LD-4040	RO	GP, NL, OLS
	CSM NE4040-40	NF1	GP, NL, OLS

^aGP: gel-purified; NL: non-labeled; NS: non-stabilized; OLS: oligolysine-stabilized.

Figure S2. (Left) Illustration of the DNA nanostructure “Gear” shape and **(Right)** transmission electron microscope (TEM) photograph of the synthesized structures after gel purification. **Source:** tilibit nanosystems synthesis report.



Text S1. QA/QC for molecular analyses.

Standards and Equivalent Sample Volumes (ESVs). Standards were purchased from Integrated DNA Technologies (IDT, Coralville, IA) as gBlock Gene Fragments (**Table S4**) and processed according to manufacturer's instructions. The gBlock Gene Fragments were resuspended in 1X TE buffer to target a concentration of 10 ng/ μL . This concentration was further verified by quantification with a Qubit 4 Fluorometer and the dsDNA HS Assay Kit (Invitrogen). ThermoFisher's DNA Copy Number and Dilution Calculator ([DNA copy number calculator](#)) was then used to dilute each standard to a working stock of 10^8 gene copies (gc)/ μL based on the stock concentration and fragment size. The working stocks were serially diluted down to 10^1 gc/ μL for MS2 or 10^2 gc/ μL for the DNA nanostructures to generate assay-specific standard curves (see **Figure S3** for nanostructure standard curves). For the SYBR-based nanostructure assays, the intercalating dye requires dsDNA to fluoresce. Thus, to account for the difference in structure between the dsDNA gBlock Gene Fragments and the ssDNA nanostructures, observed starting quantities for the nanostructures (as determined from the qPCR instrument) were multiplied by two. For all qPCR assays, starting quantities were converted to reaction-specific concentrations (in gc/reaction) using standard curves, and sample-specific concentrations were calculated based on equivalent sample volumes (ESVs). **Equations S1-S3** define the ESVs for the (1) RT-qPCR analyses for MS2, (2) the direct extraction qPCR analyses for the nanostructures, and (3) the direct quantification qPCR analyses for the nanostructures, respectively.

Equation S1. Equivalent sample volume (ESV) calculation for direct extraction and RT-qPCR analysis of spiked MS2 bacteriophage.

$$\text{ESV (mL)} = \frac{1 \mu\text{L cDNA template}}{20 \mu\text{L total cDNA}} \times \frac{5 \mu\text{L reverse transcription template}}{60 \mu\text{L nucleic acid eluate}} \times 350 \mu\text{L sample} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} = 0.001458 \text{ mL}$$

Equation S2. Equivalent sample volume (ESV) calculation for direct extraction and qPCR analysis of spiked nanostructures.

$$\text{ESV (mL)} = \frac{1 \mu\text{L DNA template}}{60 \mu\text{L nucleic acid eluate}} \times 350 \mu\text{L sample} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} = 0.005833 \text{ mL}$$

Equation S3. Equivalent sample volume (ESV) calculation for direct qPCR analysis of spiked nanostructures.

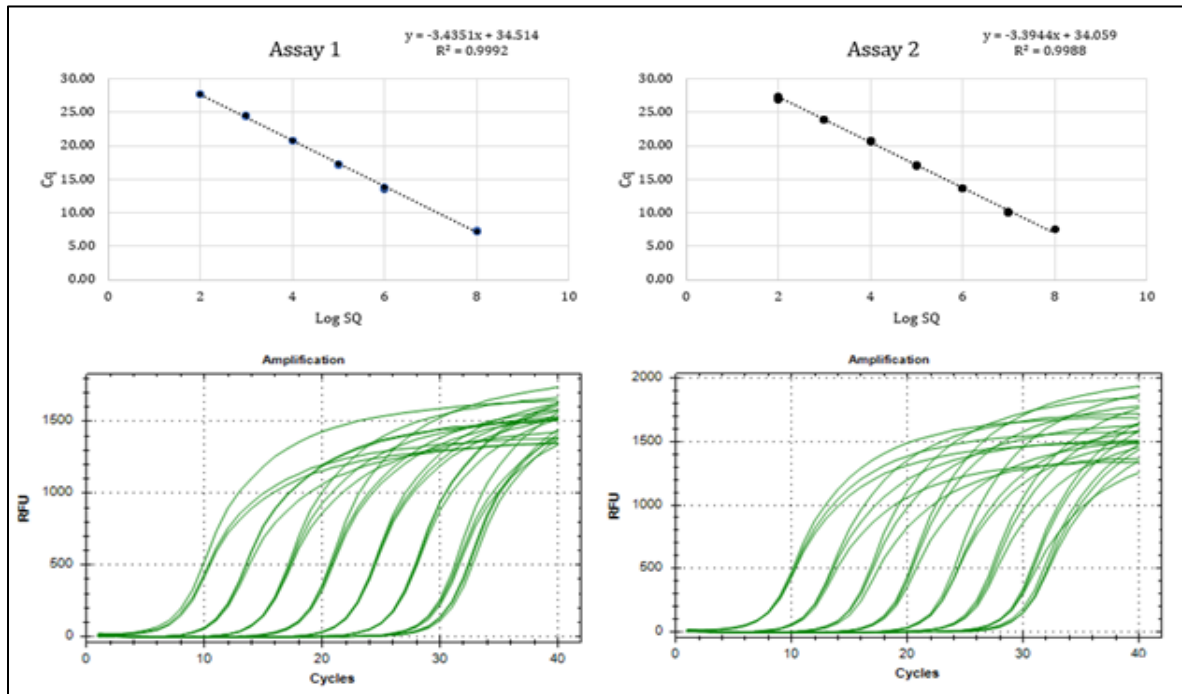
$$\text{ESV (mL)} = 1 \mu\text{L sample} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} = 0.001000 \text{ mL}$$

Table S4. Sequences used for gBlock Gene Fragment standards for the MS2 and DNA nanostructure assays.

Standard Name	Sequence	Length (bp)
MS2_STD	TAAAGTCTCCTTTCTCGATGGTCCATACCTTAGATGCGTTAGC ATTAATCAGGCAACGGCTCTCTAGATAGAGCCCTCAACCGGA GTTTGAAGCATGGCTTCTAACTTTACTCAGTTCGTTCTCGTCG ACAATGGCGGAACTGGCGACGTGACTGTCGCCCAAGCAAC TTCGCTAACGGGGTCGCTGAATGGATCAGCT	160
p7249-8064_STD1	AAACCATCTCAAGCCCAATTTACTACTCGTTCTGGTGTTTCTC GTCAGGGCAAGCCTTATTCCTGAATGAGCAGCTTTGTTACG TTGATTTGGGTAATGAATATCCGGTTCCTTGCAAGATTACTCT TGATGAAGGTCAGCCAGCCTATGCGCCTGGTCTGTACACCGT TCATCTGTCCTCTTTCAAAGTTGGTCAGTTCGGTTCC	163
p7249-8064_STD2	TCGGTACTTTATATTCTTATTACTGGCTCGAAAATGCCTCT GCCTAAATTACATGTTGGCGTTGTTAAATATGGCGATTCTCA ATTAAGCCCTACTGTTGAGCGTTGGCTTTACTGGTAAGAA TTTGTATAACGCATATGAT	98

*STD1 and STD2 were used for nanostructure assays 1 and 2, respectively (see Table 1 in main text for assays).

Figure S3. Regression and amplification curves for the two SYBR-based qPCR assays used for DNA nanostructure quantification. The curves illustrate the amplifications of the gBlock Gene Fragments used in assay development and to generate standard calibration curves for quantification.



No Template Control (NTC) Amplification. Each standard curve included a no template control (NTC) and was run in triplicate alongside samples. All NTCs included in the MS2 qPCR assay exhibited no amplification, as expected. In contrast, NTCs for both nanostructure (NS) assays showed amplification

(C_q = 28.78 for NS assay 1 and C_q = 28.25 for NS assay 2), and this background signal was eventually linked to the SYBR-based mastermix. We hypothesized that this false-positive signal could be eliminated by pretreatment of the iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories) with DNase I. To test this hypothesis, 150 μL of the iTaq Universal SYBR Green Supermix was incubated with 17 μL of buffer containing MgCl₂ (a component of the DNase I kit) and 3 μL of DNase I at 37°C for 30 minutes, followed by 10 minutes at 65°C. In a separate control, 150 μL of the iTaq Universal SYBR Green Supermix was incubated with 17 μL of the buffer and 3 μL of water (instead of DNase I). This pretreatment step successfully eliminated NTC amplification but also reduced the overall performance of the NS assays, thereby negating its benefits. Instead of implementing this DNase I pretreatment, sample data were adjusted for the background signal originating from the mastermix (i.e., by subtracting the background gc quantity) to obtain nanostructure-specific concentrations.

Limits of Quantification (LoQs). Limits of quantification (LoQs) were determined for all assays by analyzing a set of test samples (i.e., standards) with known concentrations ranging from 1,000 to 1 gc/μL and comparing against NTCs and blanks in 9 replicates. The LoQ values (**Table S5**) were determined using a statistical t-test with 99% confidence, as per the U.S. EPA (Method Detection Limit Procedure – Revision 2 outlined in 40 CFR Part 136 Appendix B). For any sample that was determined to be non-detect or <LoQ during the membrane study, the LoQs in **Table S5** were imputed for those left-censored datapoints and used to calculate corresponding log reduction values (LRVs).

Table S5. Limits of quantification (LoQs) for the MS2 and nanostructure qPCR assays. The nanostructures were analyzed with and without direct extraction.

qPCR Assay	Reaction LoQ (gc/reaction)	ESV (mL)	Sample LoQ ^b (gc/mL)
Direct Extraction MS2 ^c	9	0.001458	6.17×10 ³
Direct Extraction Nanostructures	62 ^a	0.005833	1.06×10 ⁴
Direct Quantification (No Extraction) Nanostructures ^c	62 ^a	0.001000	6.20×10 ⁴

^aNanostructure LoQ accounts for background (NTC) amplification from mastermix for assay 1.

^bSample LoQ accounts for equivalent sample volume (ESV) in each qPCR reaction.

^cPrimary methods selected for sample analysis.

Table S6. MS2 concentrations for experiment samples as determined by **(top)** culturing, **(middle)** RT-qPCR without RNase treatment, and **(bottom)** RT-qPCR with RNase treatment prior to nucleic acid extraction. Red font indicates concentrations that were below the limit of quantification (LoQ).

MS2 CULTURE		RO (PFU/ml)				NF1 (PFU/ml)				NF2 (PFU/ml)			
Experiment	Sample	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV
1	0	6.33E+05	--	--	--	2.74E+06	--	--	--	1.54E+06	--	--	--
	1	--	1.63E+06	3.78E+00	5.64	--	1.73E+06	3.33E-01	6.71	--	7.47E+05	2.89E+00	5.41
	2	--	9.40E+05	6.67E+01	4.15	--	1.10E+06	1.44E+00	5.88	--	7.67E+05	1.29E+01	4.77
	3	--	1.39E+06	4.44E-01	6.50	--	1.03E+06	1.78E+00	5.76	--	7.00E+05	4.44E-01	6.20
	Avg.	--	1.32E+06	2.36E+01	5.43	--	1.28E+06	1.19E+00	6.12	--	7.38E+05	5.41E+00	5.46
2	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	1.16E+06	6.25E+01	4.27	--	1.23E+06	4.40E+03	2.45	--	--	--	--
	2	--	1.03E+06	2.55E+01	4.61	--	1.10E+06	7.17E+00	5.18	--	--	--	--
	3	--	1.02E+06	1.78E+01	4.76	--	1.10E+06	4.83E+00	5.36	--	--	--	--
	Avg.	--	1.07E+06	3.53E+01	4.55	--	1.14E+06	1.47E+03	4.33	--	--	--	--
3	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	1.05E+06	5.00E-01	6.32	--	1.03E+06	6.33E+00	5.21	--	--	--	--
	2	--	1.15E+06	9.50E+00	5.08	--	1.16E+06	1.50E+00	5.89	--	--	--	--
	3	--	1.06E+06	5.00E-01	6.33	--	1.07E+06	6.00E+00	5.25	--	--	--	--
	Avg.	--	1.09E+06	3.50E+00	5.91	--	1.09E+06	4.61E+00	5.45	--	--	--	--

MS2 MOLECULAR		RO (gc/ml)				NF1 (gc/ml)				NF2 (gc/ml)			
Experiment	Sample	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV
1	0	2.36E+07	--	--	--	1.36E+08	--	--	--	1.21E+08	--	--	--
	1	--	1.01E+08	6.17E+03	4.21	--	9.72E+07	6.17E+03	4.20	--	7.67E+07	6.17E+03	4.09
	2	--	1.23E+08	6.17E+03	4.30	--	7.48E+07	6.17E+03	4.08	--	4.68E+07	6.17E+03	3.88
	3	--	6.87E+07	6.17E+03	4.05	--	6.78E+07	6.17E+03	4.04	--	5.31E+07	6.17E+03	3.93
	Avg.	--	9.76E+07	6.17E+03	4.19	--	7.99E+07	6.17E+03	4.11	--	5.89E+07	6.17E+03	3.97
2	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	1.77E+08	6.17E+03	4.46	--	1.57E+08	7.24E+04	3.34	--	--	--	--
	2	--	1.29E+08	6.66E+03	4.29	--	1.50E+08	6.17E+03	4.39	--	--	--	--
	3	--	1.66E+08	6.17E+03	4.43	--	1.43E+08	6.17E+03	4.37	--	--	--	--
	Avg.	--	1.57E+08	6.33E+03	4.39	--	1.50E+08	2.82E+04	4.03	--	--	--	--
3	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	1.19E+08	6.17E+03	4.29	--	1.72E+08	6.17E+03	4.45	--	--	--	--
	2	--	9.80E+07	6.17E+03	4.20	--	1.34E+08	6.17E+03	4.34	--	--	--	--
	3	--	1.85E+08	6.17E+03	4.48	--	1.41E+08	6.17E+03	4.36	--	--	--	--
	Avg.	--	1.34E+08	6.17E+03	4.32	--	1.49E+08	6.17E+03	4.38	--	--	--	--

MS2 MOLECULAR (RNase)		RO (gc/ml)				NF1 (gc/ml)				NF2 (gc/ml)			
Experiment	Sample	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV
1	0	4.40E+07	--	--	--	4.01E+07	--	--	--	6.18E+07	--	--	--
	1	--	4.89E+07	6.17E+03	3.90	--	3.46E+07	6.17E+03	3.75	--	3.63E+07	6.17E+03	3.77
	2	--	6.73E+07	6.17E+03	4.04	--	3.55E+07	6.17E+03	3.76	--	2.73E+07	6.17E+03	3.65
	3	--	4.05E+07	6.17E+03	3.82	--	2.99E+07	6.17E+03	3.69	--	2.63E+07	6.17E+03	3.63
	Avg.	--	5.22E+07	6.17E+03	3.92	--	3.33E+07	6.17E+03	3.73	--	3.00E+07	6.17E+03	3.68
2	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	1.80E+08	1.46E+04	4.09	--	1.26E+08	9.41E+04	3.13	--	--	--	--
	2	--	1.11E+08	6.17E+03	4.26	--	1.15E+08	6.17E+03	4.27	--	--	--	--
	3	--	1.42E+08	6.17E+03	4.36	--	1.16E+08	6.54E+03	4.25	--	--	--	--
	Avg.	--	1.44E+08	8.98E+03	4.24	--	1.19E+08	3.56E+04	3.88	--	--	--	--
3	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	9.73E+07	6.17E+03	4.20	--	1.19E+08	6.17E+03	4.29	--	--	--	--
	2	--	1.15E+08	6.17E+03	4.27	--	1.13E+08	6.17E+03	4.26	--	--	--	--
	3	--	1.18E+08	6.17E+03	4.28	--	1.04E+08	6.17E+03	4.23	--	--	--	--
	Avg.	--	1.10E+08	6.17E+03	4.25	--	1.12E+08	6.17E+03	4.26	--	--	--	--

Table S7. Comparison of qPCR assays for quantification of nanostructure spiking stock.

Experiment	Nanostructure Processing		Assay 1 ^a	Assay 2
	Purification	Stabilization	Mean ± SD (gc/mL)	Mean ± SD (gc/mL)
1	Gel-purified	Non-stabilized	(2.1±0.3)×10 ¹³	(1.7±0.2)×10 ¹³
2	Gel-purified	Non-stabilized	(8.5±0.8)×10 ¹³	(8.9±0.9)×10 ¹³
3	Gel-purified	Oligolysine	(5.8±0.8)×10 ¹³	(5.9±0.8)×10 ¹³

^aNanostructure assay 1 was ultimately selected for quantifying DNA nanostructures in experimental samples.

Table S8. Nanostructure concentrations as determined by qPCR for experiment samples with **(top)** direct quantification (i.e., no nucleic acid extraction) without DNase treatment, **(middle)** direct extraction without DNase treatment, and **(bottom)** direct quantification (i.e., no nucleic acid extraction) with DNase treatment. Red font indicates concentrations that were below the limit of quantification (LoQ).

NS MOLECULAR (No Extraction)		RO (gc/mL)				NF1 (gc/mL)				NF2 (gc/mL)			
Experiment	Sample	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV
1 (Old, Gel-Purified, Non-Stabilized)	0	1.05E+05	--	--	--	6.56E+06	--	--	--	2.77E+05	--	--	--
	1	--	5.39E+06	6.20E+04	1.94	--	4.59E+05	6.20E+04	0.87	--	8.46E+04	6.20E+04	0.13
	2	--	4.60E+06	6.20E+04	1.87	--	2.24E+05	6.20E+04	0.56	--	8.59E+04	6.20E+04	0.14
	3	--	1.13E+06	6.20E+04	1.26	--	9.87E+04	6.20E+04	0.20	--	7.00E+04	6.20E+04	0.05
	Avg.	--	3.71E+06	6.20E+04	1.69	--	2.61E+05	6.20E+04	0.54	--	8.02E+04	6.20E+04	0.11
2 (New, Gel-Purified, Non-Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	2.27E+08	6.20E+04	3.56	--	7.70E+05	8.85E+04	0.94	--	--	--	--
	2	--	1.55E+08	6.20E+04	3.40	--	2.15E+05	1.01E+05	0.33	--	--	--	--
	3	--	7.00E+07	6.20E+04	3.05	--	1.73E+05	7.64E+04	0.35	--	--	--	--
	Avg.	--	1.51E+08	6.20E+04	3.34	--	3.86E+05	8.86E+04	0.54	--	--	--	--
3 (New, Gel-Purified, Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	4.14E+07	1.10E+05	2.58	--	1.27E+06	6.20E+04	1.31	--	--	--	--
	2	--	4.82E+07	6.20E+04	2.89	--	3.12E+05	7.06E+04	0.65	--	--	--	--
	3	--	2.16E+07	6.20E+04	2.54	--	1.35E+05	6.70E+04	0.30	--	--	--	--
	Avg.	--	3.71E+07	7.80E+04	2.67	--	5.72E+05	6.65E+04	0.75	--	--	--	--

NS MOLECULAR (Extraction)		RO (gc/mL)				NF1 (gc/mL)				NF2 (gc/mL)			
Experiment	Sample	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV
1 (Old, Gel-Purified, Non-Stabilized)	0	3.84E+03	--	--	--	3.08E+06	--	--	--	3.44E+04	--	--	--
	1	--	6.60E+05	1.06E+04	1.79	--	1.43E+05	1.06E+04	1.13	--	1.06E+04	1.06E+04	0.00
	2	--	7.67E+05	1.31E+04	1.77	--	4.65E+04	1.06E+04	0.64	--	1.06E+04	1.06E+04	0.00
	3	--	4.73E+05	1.06E+04	1.65	--	1.06E+04	1.06E+04	0.00	--	1.06E+04	1.06E+04	0.00
	Avg.	--	6.33E+05	1.14E+04	1.74	--	6.67E+04	1.06E+04	0.59	--	1.06E+04	1.06E+04	0.00
2 (New, Gel-Purified, Non-Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	5.87E+07	1.06E+04	3.74	--	4.38E+05	1.66E+04	1.42	--	--	--	--
	2	--	3.64E+07	1.15E+04	3.50	--	4.64E+04	1.06E+04	0.64	--	--	--	--
	3	--	2.31E+07	1.32E+04	3.24	--	1.28E+04	1.06E+04	0.08	--	--	--	--
	Avg.	--	3.94E+07	1.18E+04	3.50	--	1.66E+05	1.26E+04	0.71	--	--	--	--
3 (New, Gel-Purified, Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	9.22E+06	1.61E+04	2.76	--	1.99E+05	1.13E+04	1.25	--	--	--	--
	2	--	1.07E+07	1.53E+04	2.84	--	6.80E+04	1.06E+04	0.81	--	--	--	--
	3	--	2.39E+06	1.17E+04	2.31	--	1.41E+04	1.06E+04	0.12	--	--	--	--
	Avg.	--	7.44E+06	1.44E+04	2.64	--	9.37E+04	1.08E+04	0.73	--	--	--	--

NS MOLECULAR (DNase)		RO (gc/mL)				NF1 (gc/mL)				NF2 (gc/mL)			
Experiment	Sample	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV	Tank	Feed	Permeate	LRV
1 (Old, Gel-Purified, Non-Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	--	--	--	--	--	--	--	--	--	--	--
	2	--	--	--	--	--	--	--	--	--	--	--	--
	3	--	--	--	--	--	--	--	--	--	--	--	--
	Avg.	--	--	--	--	--	--	--	--	--	--	--	--
2 (New, Gel-Purified, Non-Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	4.62E+05	6.20E+04	0.87	--	2.02E+05	6.20E+04	0.51	--	--	--	--
	2	--	5.06E+05	6.20E+04	0.91	--	1.55E+05	9.87E+04	0.20	--	--	--	--
	3	--	5.33E+05	6.20E+04	0.93	--	1.77E+05	1.03E+05	0.24	--	--	--	--
	Avg.	--	5.00E+05	6.20E+04	0.91	--	1.78E+05	8.79E+04	0.31	--	--	--	--
3 (New, Gel-Purified, Stabilized)	0	--	--	--	--	--	--	--	--	--	--	--	--
	1	--	6.40E+05	6.20E+04	1.01	--	2.56E+05	8.87E+04	0.46	--	--	--	--
	2	--	4.34E+05	6.20E+04	0.85	--	1.73E+05	7.49E+04	0.36	--	--	--	--
	3	--	4.68E+05	6.20E+04	0.88	--	1.32E+05	6.20E+04	0.33	--	--	--	--
	Avg.	--	5.14E+05	6.20E+04	0.91	--	1.87E+05	7.52E+04	0.38	--	--	--	--

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