Supporting Information

DNA Nanostructures for Targeted Antimicrobial Delivery
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Supporting information

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Section 1. Synthesis of DNA origami nanostructures

1.1 Synthesis of DNA origami nanostructures

We used M13mp18 single-stranded DNA as scaffold (New England Biolabs) and complementary oligonucleotides (Integrated DNA Technologies) to synthesise DNA origami nanostructures. 159 complementary oligos, as described by Yoshidome et al.\(^1\), fully occupy the whole length of the scaffold. The sequences of the oligonucleotides are listed in Table S-1, in a 5’ to 3’ orientation.

| Sequence Name | Sequence | Bases |
|---------------|----------|-------|
| Swf-001 1A-hp | TAA AAA TAC CGA ACG ACC TAA AAC TCT TCT TTT GAG GAA CAA GTT TTC TGG TAT CGC CAT TTT GCA GAT TC | 71 |
| Swf-002 1B-hp | ACC AGT CAT GGA TTA TTC CTC TTC TTT TGA GGA ACA AGT TTT CTT GTT TAC ATT GTT TTA TTA GTA A | 64 |
| Swf-003 1C-hp | TAA CAT CAT AGC AAT ATC TCT TCT TTT TGA GGA ACA AGT TTT CTT GTC TCT TTT TTT TTT CAA TGC GCC GAA T | 64 |
| Swf-004 1DE | CCT GAG AAT AGA CAG GAA CGG TAC TTT TTG CTT TGA CGA GCA CGG GGC GCC TAC TAT GGT TTT TGC GGG GC | 72 |
| Swf-005 1FG | TAG GGC GCC AAC AAA GGC AAA GGA TTT TAT CCG CAA AAT CCC TTT GTG GTT GCC GAA TTT TCC GCT TTT | 72 |
| Swf-006 1HI | CAG TCG GCC CTG CCT GCC CTC TTT TCG TCT GTC TGA TTA AAC GAC GGG TTT TGC CAG TCA CGA TTT TAG GGG ACG | 72 |
| Swf-007 1JK | AGC ACA GTT GCA TCT GCC AAG TTT TGT TTT TTA ACC CAA TTA AAA AAG CAG GGT GTT CAT GAG AAA ATT TTA GCC TTT TTT TAAC TGC TTT TAC TAC ATT TAA | 72 |
| Swf-008 2AB | GTC TGA AAC AGC ACC AGT ATT AAT AAA TGC TGG GAC AAC AGG CCC TTT | 64 |
| Swf-009 2CD | AGG GAT TTG TGT TTT TAT AAT CAG CGC AAA TTA ACC GTT GCT TGC ATG AGA AGG CTC AAT C | 64 |
| Swf-010 2EF | AAG GAA GTG GCC AAG TGT AGC GGT TTA ATG CGC CGC TAC ATA TAA CGT GCT TTT CTC CGA TTA A | 64 |
| Swf-011 2GH | CAT TAA TTA AAC CTC TCG TGG CAG ACG CGA AAA TCC TCT TAT AAA TCA AAA GAA TAT GGC GAG A | 64 |
| Swf-012 2IJ | CGT ACG CTA CGG GCC TCA GGA AGR GTT GAA TCC CGC AAG GGG TCC ACC TCT GCC TAA CTC A | 64 |
| Swf-013 2KL | GAG AGA TCT GGA GCA AAC AAG AGA TAA CAA TTA ACC GTC TTT TGA TAT CCA AAG GGT GTA TGA TAT CAC TAT ACC TCT TTT T | 64 |
| Swf-014 2MN | GCG GGA GAT AGA ACC CTC ATA TAT AAG ATT CAA AAG GTT GTA TGA TAT CAC TAT ACC TCT TTT T | 64 |
| Swf-015 3AB | GGT GAG GCC GCT ATT AGT CTT TAA TGG GCC CTA TAA TAC ACC TAA TAC ACC TAA TCT TTT T | 64 |
| Swf-016 3C | CTA TCG GCA AGA GTC TGT CCA TCA TGA GCC GAA CAA TTT TTT TCT TGA GAA CAA TAT TGA AAG ATT GCC ATC AAC TAA TAC ACC TAA TAC | 32 |
| Swf-017 3D | CCG AGT AAG GAG CTA AAG ACC AGG CGT TAG AA | 32 |
| Swf-018 3E | TCA GAG CGA CCA CAC CCA CCG GGC CGC AC GCT GC | 32 |
| Swf-019 3F | GCG TAA CCG GAA AGC CGG CGA AGC GGC CAA GA | 32 |
| Swf-020 3G | TAG GGT TGG CTT TGG CTC GGC AGC CTG CAT TA | 32 |
| Swf-021 3H | ATG AAT CGG TGC CTA ATG AGT GAG ATG CCT GC | 32 |
| Swf-022 3I | AGG TCG AGC CTG CAA GCC GAT TAA TCG CAC TC | 32 |
| Swf-023 3J | CAG CCA GCT CAC GTT GGT GTA GAT TTA AA | 32 |
| Swf-024 3K | TTG TAA ACC GTA AAA CTA GCA TGT GAT GA | 32 |
| 5wf-028 3L | ACG GTA ATA TGC CGG AGA GGG TAG TCT AGC TG | 32 |
| 5wf-029 3M | ATA AAT TAG AGT AAT GTG TAG GTA TTT AAA TG | 32 |
| 5wf-030 3N | CAA TGC CTA CAT TAT GAC CCT GTA AAT CAT AC | 32 |
| 5wf-031 3OP | AGG CAA GGT TTA GCT ATA TTT TCA ATA ACA GTT GAT TCC CGC TCA ACA TGT TTT AAA TAT GCA A | 64 |
| 5wf-032 4A | TTT TGA ATG GTC AGT ATT AAT AAC ACC TGC ACC AGT GCC A | 40 |
| 5wf-033 4B | CTT GCT GGT TTA GCT TTA AAT TAG AGC TTG AAA CAG AGA TAG AAC CCT GAC AAT AT | 56 |
| 5wf-034 4G | CGG TCC ACA GTG TTG TTC CAG TTT ATT TAG AGC TTG AGC G | 40 |
| 5wf-035 4H | AGC CTG GGG CCA ACG CGG GGG GAG GCA GCA AG | 32 |
| 5wf-036 4I | GGG GAT GTT CTA GAG GAT CCC CGG AAG TGT AA | 32 |
| 5wf-037 4J | GTT AAT ATT TTG TTA AAC CGT AAT GGG ATA GGT TTC CGG CAC CGC TTC GGC GAA AG | 56 |
| 5wf-038 4O | ATA ACC TGC AAA GAA TTA GCA AAA ATC GTG TGT ACC AAA A | 40 |
| 5wf-039 4P | TTA AAT TTG TTA AAT AGT GAC CCA CTA CCT GAA AAT | 32 |
| 5wf-040 5-6A | AAG GAA TTG TCA GTT GGC AAA TCA AAG AAT ACG TGG CAC ATC TGA CCT GAA AGC GTA CAG TTG A | 64 |
| 5wf-041 5-6B | ATT ACC GCT AAT TTT AAA AGT TTG TTT GCC CGA AGC TTA TCA GCC ATT GCA ACA GGA GAA CAA T | 64 |
| 5wf-042 5F | TCG GAA CCC TAA AGG GAG CCC CCG GGA ACA AG | 32 |
| 5wf-043 5G | AGT CCA CTT GGC CCT GAG AGA GTT AGG CGG TT | 32 |
| 5wf-044 5H | TGC GTA TTA CGA GCC GGA AGC ATA GTA CCG AG | 32 |
| 5wf-045 5I | CTC GAA TTC GCT ATT ACG CCA GCT TGG TGC CG | 32 |
| 5wf-046 5J | GAA ACC AGA ACA AAC GGC GGA TTG AAT TCG CA | 32 |
| 5wf-047 5-6N | TAA AGC CTA ATC CCC CTC AAA TGC TCA TAA ATA TTC ATT GCA GAG CAT AAA GCT AAT TAA GCA A | 64 |
| 5wf-048 5-6O | TCA AAA AGT AGT CAG AAG CAA AGC TTG GAT ACA TTT CGC AAG ATT TAG TTG GAC CAG GAT TGC A | 64 |
| 5wf-049 6G | TCA CGG CCA TTA AAG AAC GTG GAC GCA CTA AA | 32 |
| 5wf-050 6H | CAC CGA CTC CAA AGA CAA AAG GGC GAT TAA GA | 32 |
| 5wf-051 6I | GGC CTC TTC GTA ATC ATG GTC ATA CAA TTC CA | 32 |
| 5wf-052 6J | TCC GTG GGG CAA AGC GCC ATT CGC TCG GTG CG | 32 |
| 5wf-053 6K | TTA AAT TTT TGT TAA ATC AGC TCA TCG GAT TC | 32 |
| 5wf-054 7A | CGC TGA GAG CCA GCA GCA AAT GAA AAA TCT AAC CTC AAT C | 40 |
| 5wf-055 7B | AAT ATC TGG AGG AAG GTT ATC AAT AAC TCG TAT TAA ATC CAG | 56 |
| 5wf-056 7G | GTC GAG GTG CCG TAA ATC CAA CGT CAA AGG GCA GAC GGG C | 40 |
| 5wf-057 7H | AAC AGC TGT TTC TTT TCA CCA GTG AAT TGT TA | 32 |
| 5wf-058 7I | TCC GCT CAG CTG TTT CCT GTG TGA CTG TTG GG | 32 |
| 5wf-059 7J | AAG GGC GAC ATT CAG GCT GGG CAA AGC GAG TAA CCA CCC GTT TTT TAA CCA ATA AA | 56 |
| 5wf-060 7O | CAA TAC TGC GGA ATC GTT TAA ACA GTT CAG AAT TTA CCC T | 40 |
| 5wf-061 7P | GAC TAT TAA TTA AGA GGA AGC CCG TCC TTT TGA TAA GAG GTC ATT TTT GGC GAT GG | 56 |
| 5wf-062 8-9A | AGA AAA CTA CCT CAA ATA TCA AAG AGC ATC ACC TTG CTG ATT TTC AAA TAT ATT TTA GAA CCG G | 64 |
| 5wf-063 8B | AAA GAA ACT TAC AAA CAA TTC GAC AAT ATC TTT AGG AGC ACT AAC AAG | 48 |
| 5wf-064 8C | CAT ATT CCC ACC AGA AGG AGC GGA TGC GGA AC | 32 |
| 5wf-065 8D | CAA AAT TAT GGA AGG GTT AGA ACC ATT ATC AT | 32 |
| 5wf-066 8E | AAA TTG CGT TTG CAC GTA AAA CAG TAC CAT AT | 32 |
| 5wf-067 8F | GAA AAA CCG TCT ATC AAA TCA AGT TTT TTG GGA AAT AAA G  | 40 |
| 5wf-068 8K | CTA ACG GAA ACG CCA TCA AAA ATA ATC AAC ATT AAA TGT G  | 40 |
| 5wf-069 8L | TAC GAG GCA CAA CAT TAT TAC AGG AAA ACG AA  | 32 |
| 5wf-070 8M | CCC TCG TTA TAG TAA GAG CAA CAC AAA GGA AT  | 32 |
| 5wf-071 8N | ATA GCG TCT ACC AGA CGA CGA TAA TAT CAT AA  | 32 |
| 5wf-072 8O | CAA ATA TCA ATC AAA AAT CAG GTC AAC GAG AAT GAC CAT ATA GAC TGG  | 48 |
| 5wf-073 8P | ATT CGA GCA CAG GTC AGG ATT AGA GAG TAC TTT TAA TTG CAA AGA CT  | 48 |
| 5wf-074 8Q | TAA TAG ATT TAG AAG TAT TAG ACT GAA ACA GTA CAT AAA TAT TAC CT  | 48 |
| 5wf-075 8R | TTT TAA TGT GAT TAT CAG ATG TTA TAC TTT GTA ATA AAG TTA CAA  | 48 |
| 5wf-076 8S | AAT CGC GCA TTG CTT TGA ATA CCA TAG ATT TTC AGG TTT AAC GTC AGA  | 48 |
| 5wf-077 9-10F | ATC ACC CAG GGC GAT GGC CCA CTA GAG AGA TAA CCC ACA ATT GAG CGC TAA TAT CAC GTG AAC C  | 64 |
| 5wf-078 9-10J | CTA TTT CGG TAT AAA CAG TTA ATG CCT GTA GCC AGC TTT CAT TCG CGT CTG GCC TTC CCC CTG C  | 64 |
| 5wf-079 9KL | ACG TTA ATT AGA AAG ATT CAT CAG CAG ATA CAT CAC GCC AAG AAC GAG  | 48 |
| 5wf-080 9MN | TAG TAA ATC CTG ACG AGA AAC ACC AAA CCA AAA TAG CGA GTT AAT AGT  | 48 |
| 5wf-081 9O | AAA ATG TTC AGA CGG TCA ATC ATC TAG CCG GAA CGA GGC GGC GTT TTA  | 48 |
| 5wf-082 10-11A | CAA GAC AAA GTT AAT TTC ATC ATC TTC TGA CCT AAA TTT AAT GTA AAT GCT  | 48 |
| 5wf-083 10BC | GTG AGT GAT AAT ACA TTT GAG TAG TAG AGC CGT CAA TAG ATC CAA TCG  | 48 |
| 5wf-084 10D | TGT TTG GAG CAA TTC ATC AAT TAA CAA TTT CAT TTG ACA ATA TAT  | 48 |
| 5wf-085 10E | TGA ATA TAA TAA CGG ATT CGC CTG AGA GGC GAA TTA TTC AAT CCT GAT  | 48 |
| 5wf-086 10KL | AAC TAA TGT TGA GAT TTA GGA ATA TCA GGA CGT TGG GAA GAA AAA TCT  | 48 |
| 5wf-087 10MN | GCA AAA GAA GTG AAT AAG GCT TGC TGG GCT TGA GAT GGT TCC ACA TTC  | 48 |
| 5wf-088 10O | ATG TTA CTA GGG AAC CGA ACT GAC GAT TTT GCC AGA GGG GAG GCT TTT  | 48 |
| 5wf-089 10-11P | GAA CCA GAA CAC TCA TCT TTG ACC AAA AGA ATA CAC TAA ACC GGA AGC AAA CTC CAT TCA AAG C  | 64 |
| 5wf-090 11B | GAT GCA AAA ATA GTG AAT TTA TCA GAC GCT GAG AAG AGT CAT AAC CTT  | 48 |
| 5wf-091 11C | GCT TCT GTG AAA ATT AAT TAC ATT ACA AAC AT  | 32 |
| 5wf-092 11D | CAA GAA AAA AAA GAA GAT GAT GAA TTT CAA TT  | 32 |
| 5wf-093 11E | ACC TGA GCT ACA TCG GGA GAA ACA CAG TAA CA  | 32 |
| 5wf-094 11F | GTA CCT TTC AAA GTC AGA GGG TAA GAA TTG AGT TAA GCC C  | 40 |
| 5wf-095 11K | TTG AGT AAC AGT GCC CGA ACC TAT TAT TCT GAT GGC TCA T  | 40 |
| 5wf-096 11L | TAT ACC AGA TGC GAT TTT AAG AAC CAT TGT GA  | 32 |
| 5wf-097 11M | ATT ACC TTT AAT TTC AAC TTT AAT ACA AAG CT  | 32 |
| 5wf-098 11N | GCT CAT TCT ACC CAA ATC AAC GTA AGA ACC GG  | 32 |
| 5wf-099 11O | ATA TTC ATC AAC TTT GAA AGA GGA GTG TCG AAA TCC GCG ACC TGC TCC  | 48 |
| 5wf-100 12A | ACT ATA TGG TTT GAA ATA CCG ACC GTG TGA TAA ATA AGG C  | 40 |
| 5wf-101 12B | AAA TCG TCG CTA TTA ACG ATA GCT TAG ATT AAA AAT CAT AGG TCT GAG GTT ATA TA  | 56 |
| Sequence  | Sequence | Length |
|-----------|----------|--------|
| Swf-102 12G | AGA AAC GCA ATA ATA AGA GCA AGA AAC TGA ACA ACC TGA A | 40 |
| Swf-103 12H | AAG GCC GGA AAG ACA CCA CGG AAT CAT ATA AA | 32 |
| Swf-104 12I | CAG AGCCAA AAC GTC ACC AAT GAA CCA TTA GC | 32 |
| Swf-105 12J | AAC ATG AAA GTA TTT ATA ATG ACG GGG TCA GTG CCC CAC CTT ACG AGC GCC GCC ACC CT | 56 |
| Swf-106 12O | GAT AAA TTC AGA TGA AGC GTG TAC AAG AGT AAT CTT GAC A | 40 |
| Swf-107 12P | AGA CGG ACC CAA CCT AAA ACG AAA GAG GCC CCA GCG ATT ATA CCA CTG GCC CT | 56 |
| Swf-108 13-14A | GAG CCA GTT GTA ATT TAG GCA GAG CTC CGG CTT AGG TTG GAG ACT ACC TTT TTA ACG CAT TTT C | 64 |
| Swf-109 13-14B | CCC TTA GAT TTA CGA GCA TGT AGA TAA TAT CCC ATC CTA AAT CCT TGA AAA CAT AGT TAA TTT T | 64 |
| Swf-110 13F | GGA AGC GCA TTA GAC GGG AGA ATT AA CAAT GA | 32 |
| Swf-111 13G | AAT AGC AAT ACA TAA AGG TGG CAA AAG TTT AT | 32 |
| Swf-112 13H | TTT GTC ACA CCA GTA GCA CCA TTA ACC ATC GA | 32 |
| Swf-113 13I | TAG CAG CAC GCC ACC CTC AGA ACC CAG AA | 32 |
| Swf-114 13J | CCA CCA CCT ACT GGT AAT AAG TTT GAG GCT GA | 32 |
| Swf-115 13-14N | AAA AGG AGG AAA ATC TCC AAA AAA CTG GCT GAC CTT CAT CAG ACC AGG CGC ATA GGA AGG CTC C | 64 |
| Swf-116 13-14O | ACA AAG TAG CGG CTT TGT CGG GAT TTT CAG GGA GTT AAA GCA ACG GAG ATT TGT ATC GGA CGA A | 64 |
| Swf-117 14G | AAA ATA CAT AGC TAT CTT ACC GAA AAA AAC AG | 32 |
| Swf-118 14H | GCA AAA TCA ATC AAT AGA AAA AAA TAC CGT AG | 32 |
| Swf-119 14I | CTC AGA GCC CGT AAT CAG TAG CGA TAG AGC CA | 32 |
| Swf-120 14J | CAG GAG TGA GAG CCG CCG CCA GCA CCG CCT CC | 32 |
| Swf-121 14K | GAC TCC TCA AGA GAA GGA TTA GGA TGA TGA TA | 32 |
| Swf-122 15A | GTT AAA TAA GAA TCA ACC CCG GAA TCA TAA TTT TTA ACA A | 40 |
| Swf-123 15B | CGC CAA CAA ATA AGA GAA TAT AAA TCC TGA ACA AGA AAA AAA CCA ATC AAT AAT CG | 56 |
| Swf-124 15G | ACA GAG AGA ATA ACA TGC CCT TTT TTA GAA AAT ACG CAG T | 40 |
| Swf-125 15H | ATG TTA GCA TAT GGT TTA CCA GCG TGA GCC AT | 32 |
| Swf-126 15I | TTG GGA ATC AGA ATC AAG TTT GCC AGA GCC AC | 32 |
| Swf-127 15J | CAC CGG AAT TGA CAG GAG GTT GAG CGT CAT ACA TGG CTT TTT AGC GGG GTT TTG CT | 56 |
| Swf-128 15O | ATA ATT TTT TCA GTG TCC TTT TTA AAT TGT ATC GGA TTC GGT C | 40 |
| Swf-129 15P | GCT GAG GCC GTC ACC CTC AGC ACG TTTCCA TTA AAC AGG TAA AAT ACG TAA TGC CA | 56 |
| Swf-130 16AB | TAG ATA AGG TAC CGA CAA AAG GAA GTT ATT GAG AAT CGC CAT AAC TAG AAA AAG CCT GTT TAG TAT C | 64 |
| Swf-131 16C | GGA AATC ATG CTG TCT TTC TCT ATC ATC AAC AA | 32 |
| Swf-132 16D | CGG GAG GTT ACC GCG CCC AAT AGC TCA TCG TA | 32 |
| Swf-133 16E | AGA GCC TAT TTG AAG CCT TAA ATC CCG ACT TG | 32 |
| Swf-134 16F | CAG CCT TTA TTT GCC AGT TAC AAA GTC TTC TT | 32 |
| Swf-135 16G | CTC CTT ATG TAA GCA GAT AGC CGA GAA AAT AG | 32 |
| Swf-136 16H | CAC AAG ATG GCC GCC AGG GTG GTT ATT GCC CT | 32 |
| Swf-137 16I | CGG GAA CCT TTA GGC TCA GCT ATC ACC GT | 32 |
| Swf-138 16J | CCA GTA AGG CAG GTC AGA CGA TTG CAA AAT CA | 32 |
| Swf-139 16K | GCC ACC CTC AGT ACC AGG CGG ATA TTA CCG TT | 32 |
| Swf-140 16L | ACA CTG AGC AGA ACC GCC ACC CTC TTA GTA CC | 32 |
| Swf-141 16M | CCA GAC GTT TTC GTC ACC AGT ACA GTA CCG TA | 32 |
Table S-1. Sequences of the 159 complementary oligos.

The DNA nanostructures were made by mixing together 2 μL M13mp18 DNA (10 nM), 5μL oligonucleotide mix (each oligo 200 nM), 2 μL origami buffer 10x (20 mM MgCl2, 100 mM Tris-HCl, pH=7.6), and 11 μL deionised MilliQ water. EDTA was omitted from the origami buffer for the whole of this study (normally included at 1mM), so as not to interfere with the bacterial populations. The mixture was annealed from 85 to 25 °C at a rate of –1.0 °C/min. After annealing, excess oligonucleotides were removed using a Micro Biospin column (Bio-Rad) packed with Sephacryl S-300 (GE Healthcare).

1.2 Modifications to the 5-well frames

The following oligonucleotides were modified to carry aptamers that can bind *E.coli* and *B.subtilis* [2]. The aptamers sequence is CAT ATC CGC GTC GCT GCG CTC AGA CCC ACC ACC ACG CAC C (in red in the table below).
| Sequence Name | Sequence                                                                 | Bases |
|---------------|--------------------------------------------------------------------------|-------|
| Swf-009 1NO-apt | AAA ATT TTA GCC TTT ATT TCA ACG TTT TTC TAC TAA TAG TAG TAA AAG GGT GGC ATC AAT TTT TT C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 105   |
| Swf-015 2KL-apt | GAG AGA TCT GGA GCA AAC AAG AGA CAA TCA TAT GTA CCC CAG ATT GTA TAA GCA AAG GCC GCA TTT TTT CAT ATC CGC GTC GCT GCG CTC AGA CCC ACC ACC ACG CAC C | 109   |
| Swf-159 18NO-apt | TAG TGG CGC CGA CAA TAA CAG CTT GAT ACC GAT TTT TTT CAG CGG AGT GAG AAA ACA ACT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 105   |
| Swf-001 1A-apt | TAA AAA TAC CGA ACG ACC TAA AAT GCC ATG CAG ATT CTT TTT CAT ATC CGC GTC GCT GCG CTT TTA AGT GAG AAA ACA ACT TTT TT C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 85    |
| Swf-023 3G-apt | TAG GGT TGG CTG GTT TGC CCC AGC CTG CAT TAT GTA CCC CAG ATT GTA TAA GCA AAG GCC GCA TTT TTT CAT ATC CGC GTC GCT GCG CTC AGA CCC ACC ACC ACG CAC C | 77    |
| Swf-043 5G-apt | AGT CCA CTT GGC CCT GAG AGA GTT AGG CGG TTT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 77    |
| Swf-045 5I-apt | CTC GAA TTC GCT ATT ACG CCA GCT TGG TGC CGT TTT TTT CAT ATC CGC GTC GCT GCG CTT TTA AGT GAG AAA ACA ACT TTT TT C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 93    |
| Swf-075 9D-apt | TTT TAA TGT GAT TAT CAG ATG ATG TTA TAC TTC TGA ATA AAG TTA CAA | 93    |
| Swf-111 13G-apt | AAT AGC AAT ACA TAA AGG TGG CAA AAG TTT ATT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 77    |
| Swf-113 13I-apt | TAG CAG CAC GCC ACC CTC AGA ACC CAC CAG AAT TTT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 77    |
| Swf-136 16H-apt | CAC AAC ATG GCC GCC AGG GTG GTT ATT GCC CTT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 77    |
| Swf-138 16I-apt | CCA GTA AGG CAG GTC AGA CGA TTG CAA AAT CAA AAT ATT CTT TTA CTT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 77    |
| Swf-152 18A-apt | AAT AAA CAT TTT AGT ATA AAG CCA ACG CTA TAC ABA TTA CCT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 89    |
| Swf-079 9KL-apt | AGC TTA ATT AGA AAG ATT CAT CAG ATG ATA CAT AAC GCC AAG AAC GAG TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 93    |

**Table S-2.** Sequences of the 14 aptamer-modified oligos.

The following oligonucleotides were functionalised with Alexa 647 molecules.

| Sequence Name | Sequence                                                                 | Bases |
|---------------|--------------------------------------------------------------------------|-------|
| Swf-025 3I 647 | /5Alex647N/TTT TTA GGT CGA CGC TGC AAG ACC ATT AAT CGC ACT C | 37    |
| Swf-126 15I 647 | /5Alex647N/TTT TGG GAA TCA GAA TCA AGT TTG CCA GAG CCA C | 37    |
| Swf-083 10BC 647 | /5Alex647N/TTT TTA TGT CTA ATA AAG CCA GCA TAA TCA GAT TTT T T C ATA TCC CGG TCG CTG CGC TCA GAC CCA CCA CGC ACC | 53    |
| Swf-081 90 647 | /5Alex647N/TTT TTA AAA TGT TCA GAC GGT CTA TAC TAT AGC CGG AAC GAG GCG GCC TTT TA | 53    |

**Table S-3.** Sequences of the 4 Alexa 647-modified oligos.

The following oligonucleotides were functionalised to carry biotin:

| Sequence Name | Sequence                                                                 | Bases |
|---------------|--------------------------------------------------------------------------|-------|
| Swf-057 7H-bio | AAC AGC TGT TTT TTA TCA CCA GTG TTT TT/3Bio/ | 29    |
| Swf-066 8E-bio | AAA TTT CGT TGG CAC GCA ATT AAT CTT CTA AAA CAG TTT TT/3Bio/ | 29    |
| Swf-096 11L-bio | TAT ACC AGA TGC GAT TTT AAG AAC TTT TT/3Bio/ | 29    |
| Swf-020 3D-bio | /5Biosg/TTT TTA GCT CTA AAG AGG CTG TAG AA | 29    |
| Swf-028 3L-bio | /5Biosg/TTT TTA TGC CGG AGA GGG TAG TCT AGC TG | 29    |
Table S-4. Sequences of the 10 biotin-modified oligos.

| Sequence   | Sequence                  | Length |
|------------|---------------------------|--------|
| Swf-070 8M-bio | /SBiosg/TTT TTA TAG TAA GAG CAA CAC AAA GGA AT | 29     |
| Swf-092 11D-bio | /SBiosg/TTT TTA AAA GAA GAT GAT GAA TTT CAA TT | 29     |
| Swf-104 12I-bio | /SBiosg/TTT TTA AAC GTC ACC AAT GAA CCA TTA GC | 29     |
| Swf-133 16E-bio | /SBiosg/TTT TTT TTG AAG CCT TAA ATC CCG ACT TG | 29     |
| Swf-141 16M-bio | /SBiosg/TTT TTT TTC GTC ACC AGT ACA GTA CCG TA | 29     |

Section 2. Imaging of the nanostructures

2.1 Atomic Force Microscopy (AFM)

Origami tiles were diluted ten times in origami buffer and 25 µl of the sample were deposited on freshly cleaved mica and incubated at room temperature for 10 minutes. The samples were then washed 5 times with 1 ml of origami buffer. The nanostructures were imaged in origami buffer, using a Dimension FastScan AFM microscope (Bruker). The probes used were FastScan-D probes (Bruker), with a resonant frequency of 90 kHz, a spring constant of 0.21 Nm⁻¹ and a nominal tip radius of 8 nm.

DNA origami tiles were incubated with 0.17 μM Streptavidin (Sigma Aldrich) for 5 minutes at room temperature, after which they were filtered using a Millipore filter (Millipore, MA, USA) unit with molecular weight cut-off (MWCO) of 100 KDa to remove free protein. The tiles were then prepared for AFM imaging as described above.

To load the streptavidin functionalised origami tiles with the antimicrobial enzyme, the tiles were incubated with 1mg/ml biotinylated lysozyme (Chicken Lysozyme protein, Egg whites, GeneTex) for 10 minutes at room temperature after which they were filtered and imaged as above.
2.2 AFM data analysis

Images of all datasets were plane-fitted using the speed-optimised plane correction function of the SPIP software (Image Metrology A/S, Hørsholm, Denmark), which fits each line in the horizontal axis to a polynomial equation. SPIP was also used for calculation of the volumes of proteins attached to the DNA origami tiles. The “inspection window” feature of SPIP was used to zoom into individual tiles and then the “circular area of interest” tool was used to allow the software to calculate only the volume of the protein rather than that of the whole tile, according to the following equation:

$$ Z_{\text{net volume}} = Z_{\text{material volume}} - Z_{\text{void volume}} $$

where $Z_{\text{material volume}}$ is the volume of all pixels inside the shape’s contour with a $Z$ value greater than zero:

$$ Z_{\text{material volume}} = \sum_{(x,y) \in \text{shape}[Z \geq 0]} Z(x,y) \, dx \, dy $$
where $dx$ and $dy$ are the point spacings in the X and Y directions of the image, respectively. $Z_{\text{void volume}}$ is the volume of all pixels inside the shape’s contour with a Z value lower than or equal to zero:

$$Z_{\text{void volume}} = \sum_{\{Z(x,y) \leq 0\}} Z(x,y) \, dx \, dy$$

where $dx$ and $dy$ are the point spacing in the X and Y directions of the image, respectively.

For cross-sections of sample features, tile dimensions measurements, as well as for the 3D rendering of the images, Nanoscope 1.9 software (Bruker) was used. Volume histograms were drawn with bin widths chosen according to Scott’s equation[3], using GraphPad Prism.

“Theoretical” molecular volumes of proteins based on molecular mass were calculated using the equation by Schneider et al[4]:

$$V = (M_0 / N_0)(V_1 + dV_2)$$

where $M_0$ is the molecular mass, $N_0$ is Avogadro’s number, $V_1$ and $V_2$ are the partial specific volumes of protein and water, respectively, and $d$ is the extent of protein hydration. The partial specific volume of a typical protein ($V_1$) is considered to be 0.74 cm$^3$g$^{-1}$, and the extent of protein hydration ($d$) has been estimated to be 0.4 g of water/g of protein. The partial specific volume of water ($V_2$) is 1 cm$^3$g$^{-1}$.

2.3 direct Stochastic Optical Reconstruction Microscopy (dSTORM) 

Fluorescence microscopy experiments

The fluorescence microscopy experiments performed on the origami structures were conducted on a custom-built microscope based on an Olympus (Center Valley, PA) IX-73 frame with a 100x 1.49 NA oil objective lens (Olympus UAPON100XOTIRF) and a 647-nm laser (MPB Communications Inc. VFL-P-300-647-OEM1-B1). The samples were imaged in total internal reflection fluorescence (TIRF) mode and dSTORM images collected on an Andor iXon Ultra 897 camera as described previously[5].

dSTORM on origami structures

16000 frames were acquired for the dSTORM reconstructions, each recorded at 20 ms exposure time using an EM gain of 200 over a 256x256 pixel region. The pixel size in the image plane was measured to be 118 nm. The raw single molecule data sets were reconstructed using ThunderSTORM[6], and visualized as averaged shifted histograms with a magnification factor of 10. The peak-to-peak distance between the fluorophores tethered to the origami structures was measured by taking a cross-sectional profile in Fiji/ImageJ[7] between two bright spots in different regions of interest in the reconstructed image, and using a custom MATLAB (Natick, MA) script to measure the average distance between two peaks. Representative large fields of view can be seen in Figure S.2.
**Figure S.2.** dSTORM reconstruction of origami structures labelled with AlexaFluor647 molecules attached to four anchor points. (a) Large FOV image of the dSTORM reconstruction, with insets to highlight regions of interest containing origami structures. (b) shows a sub-panel from the large FOV image in (a) with 6 regions selected arbitrarily to highlight origami structures. (c) Zoomed-in insets of the origami structures highlighted in (b), with two (2), three (1,6), and four (3,4,5) detected fluorophore locations.
Section 3. Imaging of bacterial populations

3.1 Sample preparation for SIM
Each one of the bacterial strains was grown overnight (OD$_{600}$ of ~1 in the case of *E. coli* and ~0.6 in the case of *B. subtilis*). Prior to SIM imaging the cultures were spun down and washed three times in origami buffer. The bacteria were then resuspended in 100 μl origami buffer. In the case of *B. subtilis*, the bacterial pellet was resuspended in 100 μl origami buffer containing 1 μg/ml Nile red dye (Sigma-Aldrich, 72485) to enable visualisation of the bacteria. This step was not required for *E. coli*, as the BL21(DE3) *E.coli* cells used in this experiment had been transformed with pUC19GFP plasmid, and express GFP, meaning no additional staining is required for this strain.

Subsequently, 10 μl of bacterial suspension were mixed with 10μl of DNA origami (final concentration ~10 nM) and incubated with shaking at room temperature for 15 mins. The bacteria were gently centrifuged and resuspended in origami buffer to remove excess or unbound origami tiles. 2μl of the sample were deposited on a glass coverslip and an agarose pad was positioned over the sample to prevent the bacteria from moving during imaging. Another coverslip was positioned on top to minimise drying of the agarose pads.

3.2 Structured Illumination Microscopy (SIM)
Images of the sample were collected using 3-color SIM for optical sectioning\cite{8}. A ×60/1.2 NA water immersion lens (UPLSAPO 60XW, Olympus) focused the structured illumination pattern onto the sample, and the same lens was also used to capture the fluorescence emission light before imaging onto an sCMOS camera (C11440, Hamamatsu). The wavelengths used for excitation were 488 nm (iBEAM-SMART-488, Toptica), 561 nm (OBIS 561, Coherent), and 640 nm (MLD 640, Cobolt). Images were acquired using custom SIM software described previously\cite{9}.

An automated analysis routine for processing SIM images was written in MatLab. In order to quantify the overlap of the DNA origami (in magenta) with the bacterium body (in green), the code defines the percentage of the bacterium surface covered by DNA origami as the ratio between the number of overlapping pixels (pixels where both colours have non-zero intensity values) and the number of all pixels corresponding to single bacterium.

SIM imaging experiments were repeated three times, each time five fields of view were analysed to determine the DNA origami coverage of each of the two bacterial strains, with ~ 825 single bacteria for *E. coli* and ~750 single bacteria for *B. subtilis* analysed in total.

Representative large fields of view from where statistical analyses were obtained can be seen in Figures S.3 and S.4. The images in Figure 3 of the manuscript are subsets of those.
Figure S.3. Large field of view (42x42 μm) of E. coli (in green) decorated with DNA origami (in magenta). Overlap of the two colours is shown in white.
Figure S.4 Large field of view (42x42 µm) of B. subtilis (in green) decorated with DNA origami (in magenta). Overlap of the two colours is shown in white.
**Section 4. Bacterial growth curves**

Bacterial cell culture studies were conducted using *E. coli* BL21(DE3), expressing GFP and *B. subtilis* (BS168). All experiments were conducted in LB medium, supplemented with carbenicillin (100 μg/ml) for *E. coli* and chloramphenicol (25 μg/ml) for *B. subtilis*. Bacterial starter cultures were grown overnight, and the bacteria were then diluted 1:100 into 150µl LB, and grown over 16 hours in a shaking plate reader, at 37°C, with measurements taken every 5 minutes, in the following conditions:

| Sample  | Condition                                      |
|---------|------------------------------------------------|
| 1       | LB                                             |
| 2       | LB + 10 nM DNA origami                        |
| 3       | LB + 0.3 µM free lysozyme                     |
| 4       | LB + 10 nM DNA origami carrying ~0.3µM lysozyme |
| Control | LB + 10 nM DNA origami w/o aptamers           |
| 2       | LB + Origami Buffer (10mM Tris, 2mM MgCl₂)    |

**Table S-5. Experimental conditions for *E. coli***

| Sample  | Condition                                      |
|---------|------------------------------------------------|
| 1       | LB                                             |
| 2       | LB + 10 nM DNA origami                        |
| 3       | LB + 0.3 µM free lysozyme                     |
| 4       | LB + 10 nM DNA origami carrying ~0.3µM lysozyme |
| Control | LB + 10 nM DNA origami w/o aptamers           |
| 2       | LB + Origami Buffer (10mM Tris, 2mM MgCl₂)    |

**Table S-6. Experimental conditions for *B. subtilis***

The OD values at 600nm were collected and used for the creation of growth curves. For each condition, 9 individual growth curves were analysed and averaged. Individual growth curves were fitted in MATLAB using the curve fitting toolbox, to a re-parameterised Gompertz growth model[10], to extract growth rates.

DNA origami carrying lysozyme were prepared as described in Section 2.1 and added to the samples where appropriate.
The growth rates for *E. coli* and *B. subtilis* grown in the presence of DNA origami without aptamers are presented in Figure S.5:

**Figure S.5:** Growth rates of *E. coli* and *B. subtilis* in the presence of DNA origami with and without aptamers.

To further assess the bacterial growth beyond the first eight hours, we plotted the OD values for the bacterial cultures between eight and sixteen hours of growth. No changes were observed, apart from the expected population decline due to the depletion of nutrients in the growth medium and the accumulation of toxic metabolic by-products.

**Figure S.6:** Averaged growth curves for *B. subtilis* (n=9, left) and *E. coli* (n=9, right) show that the growth plateaus after eight hours of culture and no significant changes are observed beyond that point.
Section 5. Binding affinity of DNA nanostructures
We estimated the apparent dissociation constant $K_d$ of the aptamer-functionalised nanostructures, to better understand their affinity for the bacterial targets and the impact of having many of them locally concentrated into a multivalent complex.

The aptamers used have a dissociation constant $K_d$ of 27.2 nM for *E. coli* and 9.97 nM for *B. subtilis* according to Song et al.\(^2\). Recently, Csizmar et al.\(^{11}\) have used multivalent scaffolds to target tumour cells and have proposed the following equation to quantify the effect of the multiple valency, $N$, in a molecular scaffold, on its apparent affinity:

$$K = \frac{K_{d,1}}{N^2}$$

where $K_{d,1}$ is the affinity of a single-target ligand, and $K_{d,N}$ is the apparent affinity of the multiple-target ligand.

Our DNA nanostructure carries 14 aptamers; we thus obtain an apparent $K_{d,N}$ for *E. coli* and *B. subtilis* of 141 pM and 50 pM respectively.

Section 6. Cell viability assay
In order to explore the future potential of the DNA origami nanostructures to be used *in vivo* for selective bacterial targeting, we performed a mammalian cell viability assay. We used the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega), to assess the effects of the DNA origami on mammalian cells. COS-7 cells were plated in a 96-well plate at concentration of 10,000 cells/well in 100µl of media (DMEM+10%FBS). 20µl of CellTiter 96® AQueous One Solution Reagent were added per well and the cells were incubated at 37°C for 2 hours in a humidified, 5% CO2 atmosphere. After 2 h, the absorbance at 490nm was measured, using a 96-well plate reader. The measurements were performed in triplicates.
Section 7. Enzyme activity in the context of targeted delivery through DNA nanostructures

One question that is of great interest in the context of enzyme delivery through DNA nanostructures is the way in which the enzymatic activity is affected by binding of the enzyme to the DNA scaffold and how the high local enzyme concentrations afforded by the delivery vehicle affect the activity of the enzyme against its target.

In the case of lysozyme, it is possible that the loading of multiple enzymes on the same delivery platform leads to synergistic action that enhances its antimicrobial activity. Previous work has indeed indicated that the targeted delivery of several lysozyme molecules does increase antimicrobial activity locally. For example, it has been reported that all of Dextran-conjugated lysozyme\textsuperscript{[12]} and chitosan-lysozyme\textsuperscript{[13]} and selenium-lysozyme\textsuperscript{[14]} nanoparticles loaded with increasing amounts of lysozyme increase activity of the enzyme. The activity of lysozyme has also been shown to increase through delivery via "Engineered Water Nanostructures"\textsuperscript{[15]}. So overall there is plausible evidence that multiple loading sites provide cumulative benefits for antimicrobial applications, which will be explored in future work.

It is also possible that charge effects mediated by the DNA origami platform affect enzyme function. Although there is no available literature on lysozyme / DNA origami effects of this nature, an increased enzymatic activity was observed when other enzymes (i.e. not lysozyme) were coupled to DNA origami. For example, T. Morii’s group used DNA origami to assemble ribulose biphosphate carboxylase/oxygenase (RuBisCO). They show that the enzymatic activity is retained upon binding and possibly enhanced\textsuperscript{[16]}. Similarly, Zhao et al. showed that GOx/HRP enzyme pairs exhibit enhanced catalytic activity when bound to DNA nanocages\textsuperscript{[17]}, while Ora et al. report intact
activity of enzymes bound on DNA origami for delivery to mammalian cells\[^{18}\]. Potentially these effects are indeed mediated by the charge of the DNA scaffold.

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