Supporting information for

Single-stranded DNA uptake during gonococcal

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Supporting Figures

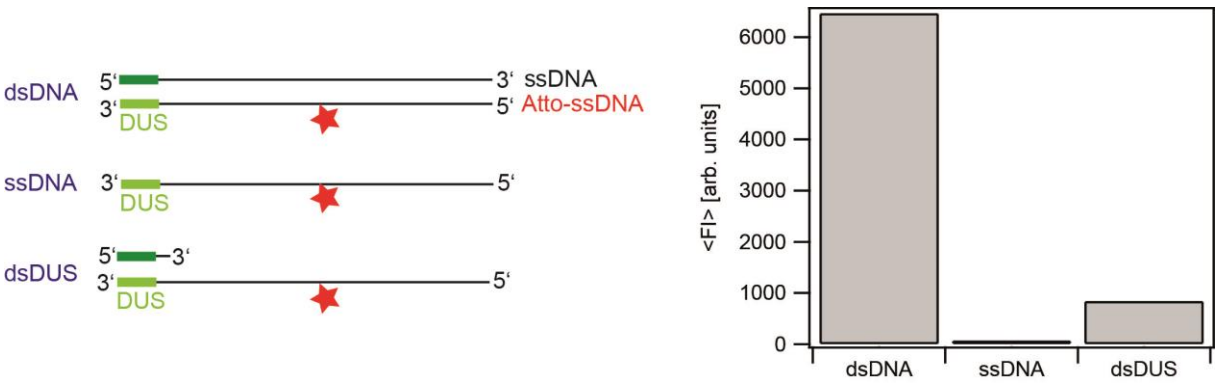

Fig. S1. Mid-labeling confirms that the import efficiency of ssDNA is increased by dsDUS. Gonococci (*ΔpilV*, Ng005) were incubated for 1 h with DNA fragments containing a single dye molecule at the center of the fragment. Subsequently they were treated with DNase I. The fragments consisted of dsDNA, ssDNA, or ssDNA with 16 b complementary oligonucleotide containing DUS. Average fluorescence intensity of individual cells. (N > 500 for each condition)

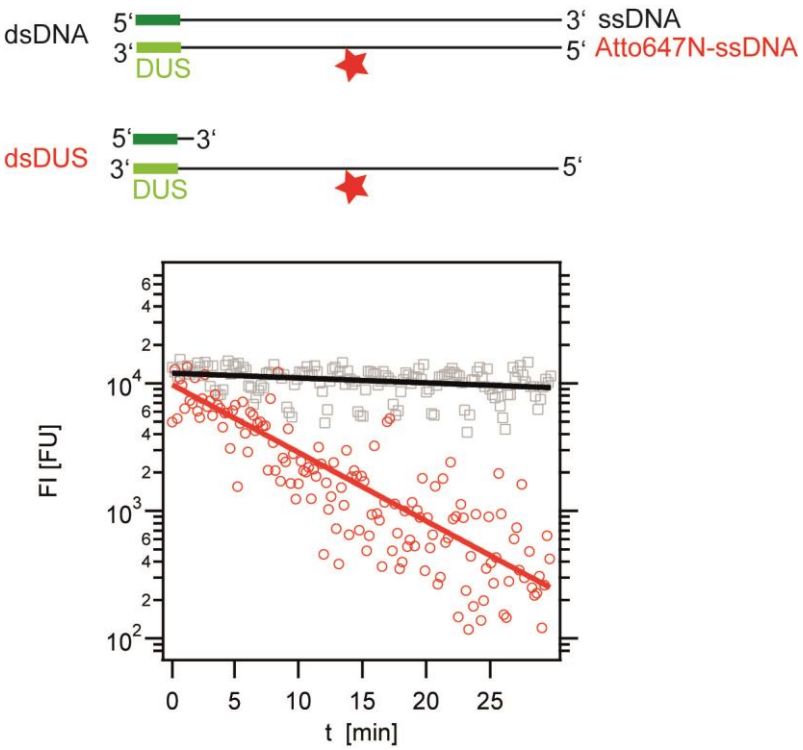

Fig. S2 Decay of imported dsDNA and dsDUS DNA. Gonococci (*ΔpilV*, Ng005) were incubated for 1 h with DNA fragments containing a single dye molecule at the center of the fragment. Single cell fluorescence intensity was monitored starting immediately after DNA removal. Cells were not treated with DNase. Grey: dsDNA, red: dsDUS. Full lines are exponential fits to the data.

28 **Tables**

Strain	Relevant genotype	Source/Reference
MS11 Ng001	*wild type*	
GV1 (*ΔpilV*) Ng005	*recA6ind(tetM) pilVfs (G-1)*	(31)
ΔpilQΔpilV Ng055	*pilQ::m-Tn3cm* *recA6ind(tetM) pilVfs*	(21) (24)
ΔpilTΔpilV Ng056	*pilT::m-Tn3cm* *recA6ind(tetM) pilVfs*	(21) (43)
MS11 Δnuc Ng164	*nuc::kan*	This study
ΔnucΔpilV Ng058	*nuc::kan* *recA6ind(tetM) pilVfs*	This study
ΔpilQΔnucΔpilV Ng163	*pilQ::m-Tn3cm* *nuc::kan* *recA6ind(tetM) pilVfs*	This study
ΔpilTΔnucΔpilV Ng162	*pilT::m-Tn3cm* *nuc::kan* *recA6ind(tetM) pilVfs*	This study

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30 **Table 1 Bacterial strains used in this study**

31

	Sequence
Cy5-ssDNA-end	5’-Cy5-CTCCTGACTGTTCGCGCCCAGAATAAAATCCATCGCTGACTGC GTATCCAGCTCACTCTCAATGGTGGCGGCATACATCGCCTTCACATTC AGACGGCAT-3’
Atto647-ssDNA-mid	5’-CTCCTGACTGTTCGCGCCCAGAATAAAATCCATCGC[T-Atto647N]GACTGCGTATCCAGCTCACTCTCAATGGTGGCGGCATACATC GCCTTCACATTCAGACGGCAT-3’
oligo Watson-DUS	5’-ATGCCGTCTGAATGTG-3’
oligo control	5’-TGTGAAGGCGATGTATG-3’
HG1	5’ATGGCTAAAATGAGAATATCACCGGAATTGAAAAAACTG3'
HG2	5’CTAAAACAATTCATCCAGTAAAATATAATATTTTATTTTCTCCCAAT CAG 3'
HG3	5’ATGCCGTCTGAAGTCCCTGAACGAAGTGTCCGGTTTG 3'
HG4	5’CAGTTTTTTCAATTCCGGTGATATTCTCATTTTAGCCATCTCTGAACC GGATTTCAGACGGCATC 3'
HG5a	5’CTGATTGGGAGAAAATAAAATATTATATTTTACTGGATGAATTGTTT TAGAGACACCGCACGGCCTTGAACG 3'
HG6	5’ATGCCGTCTGAACCCGAGTTGGCGATCAGTGCC 3'
HG11	5’ACTGCCCGTGGAAGCCGTCG 3'
HG12	5’GGACGAAAAACGGAAACCACACATACG 3'
ngch_L01	5’CGCGGTAATCAGGGTGGCGACCTCTTGGCTGGCGAGCATTTTTTCAA AACGTGCTTTTTCGACGTTCAAAATTTTACCTTTGAGCGGCAAAATCG CTTGGAATTTGCGGTCGCGGCCCTGCATGGCGGAACCGCCTGCGGAGT TGCCCTCGACGAGGTAGAGTTCAGACAGGGCAGGGTCTTTTTCTTGGC AGTCGGCGAGTTTGCCGGGCAGTCCCAAGCCGTCCATCACGCCTTTGC GGCGGGTGATTTCGCGGGCTTTGCGGGCGGCTTCGCGTGCGCGGGCGG CTTCAGACGGCAT
kh33	5’ATGCCGTCTGAAGCCGCCCGCGCACGC
kh34	5’CGCGGTAATCAGGGTGGCGAC

32 **Table 2 Oligonucleotides used in this study**