Supplemental Figures for

Colorimetric Detection of *Staphylococcus aureus* Contaminated Solutions Without Purification

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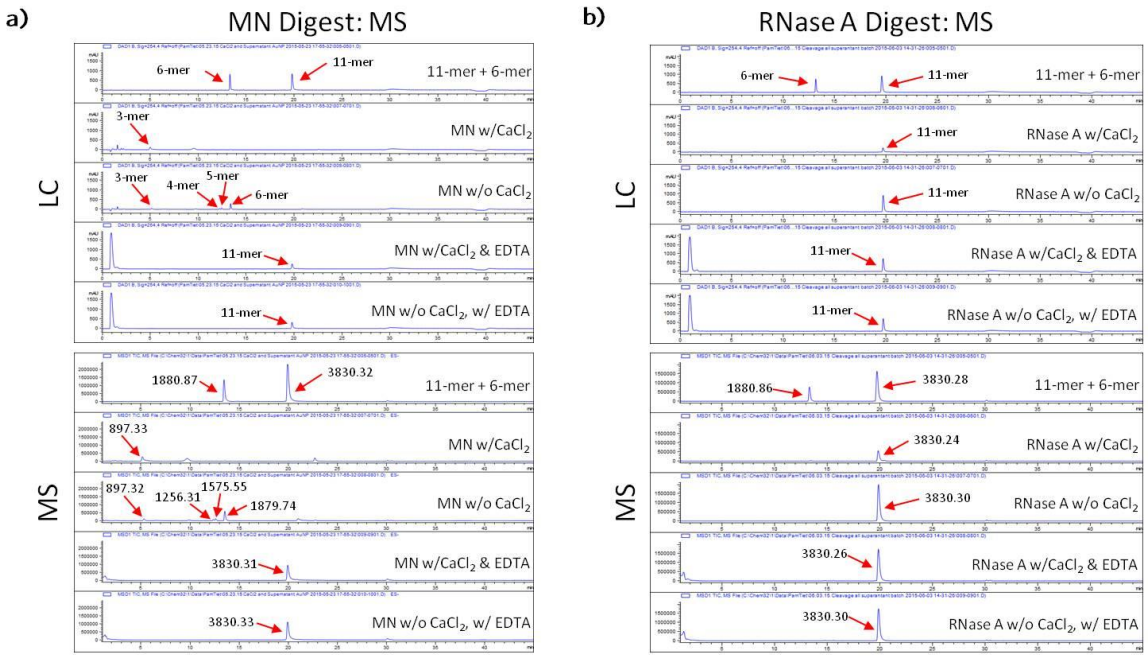


c)

Oligonucleotide	Sequence	Molecular Weight (g/mol)
11-mer	mCmUmCmG-T-T-mCmGmUmUmC	3830.7
HS-5-mer (remains on particle after cleavage)	mCmUmCmG-T	1888.5
6-mer (cleaved off sequence)	T-mCmGmUmUmC	1880.3
5-mer (cleaved off sequence)	mCmGmUmUmC	1576.1
4-mer (cleaved off sequence)	mGmUmUmC	1256.9
3-mer (cleaved off sequence	mUmUmC	897.6
2-mer (cleaved off sequence	mUmC	577.4

Figure S1. a) The 11-mer Oligonucleotide was digested with 9.917 µM MN and analyzed by LC-MS. MN w/Ca cleaved the 11-mer into even smaller oligonucleotides, whereas MN w/o Ca primarily cleaved the 11-mer between the unmodified deoxythymidines, which yielded 6-mers. The addition of EDTA suppressed cleavage from occuring. **b)** RNase A (0.126 µM) was used to digest the 11-mer oligonucleotide as a negative control. In all conditions, no cleavage peaks appeared and the 11-mer was left intact. **c)** The digested oligonucleotides and their sequences are listed along with their molecular weights for LC-MS analysis.

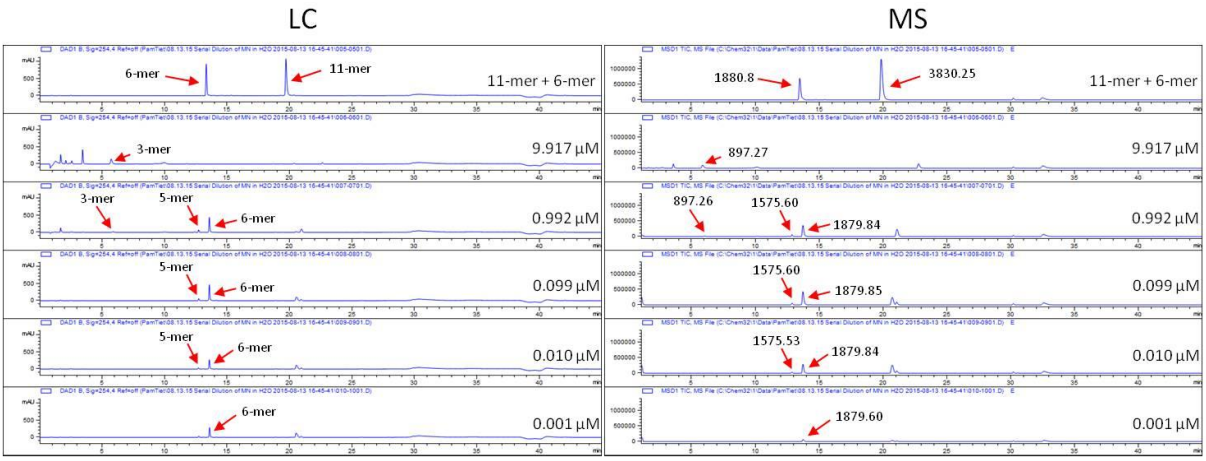


Figure S2. MN was serially diluted and used to digest the 11-mer oligonucleotide (400 pmoles). At the highest concentration used, MN cleaved multiple sites within the 11-mer. However, from 0.992 µM to 0.001 µM MN, the primary cleavage site was between the unmodified deoxythymidines.

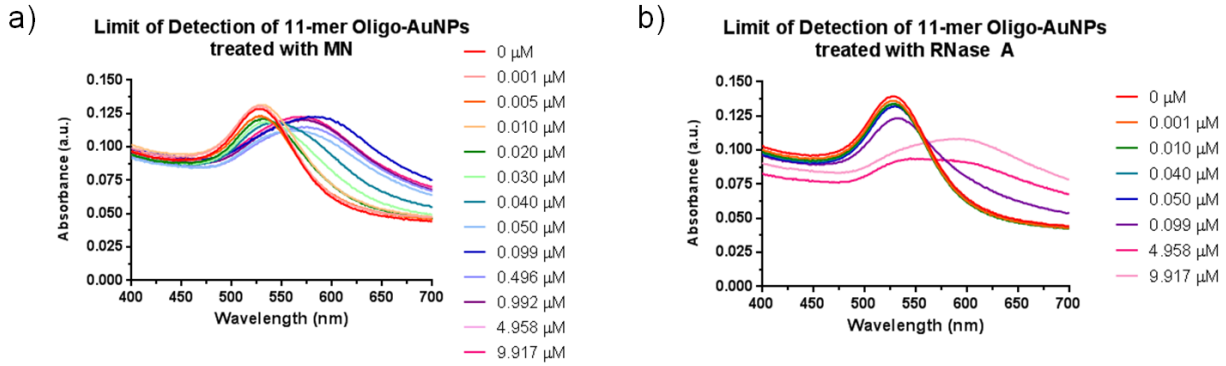


Figure S3. a) 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 0.040 µM to 9.917 µM MN. **b)** 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of Rnase A to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 4.958 µM to 9.917 µM RNase A.

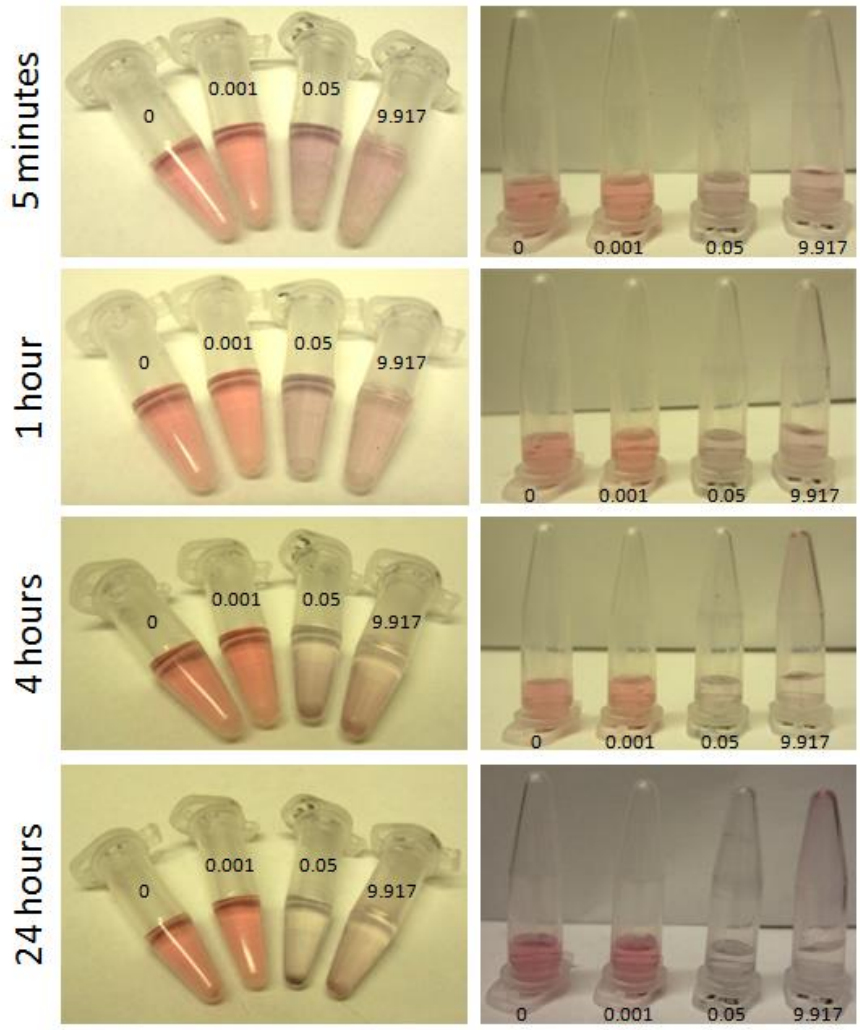


Figure S4 Oligo-AuNPs treated with 9.917 μM MN underwent a colorimetric change from pink to light purple in solution within 5 minutes. Such a high concentration of MN caused the Oligo-AuNPs to immediately aggregate and within 1 hour, we start to see Oligo-AuNPs precipitating and coating the eppendorf tube. After 4 and 24 hours, we see that the tube is coated in Oligo-AuNPs. When treated with an intermediate concentration, such as 0.05 μM MN, the Oligo-AuNP solution changed from pink to dark purple within 5 minutes, suggesting that precipitation due to aggregation is slower. A pellet is seen after 4 hours, however, even after 24 hour hours, Oligo-AuNPs still did not coat the eppendorf tube.

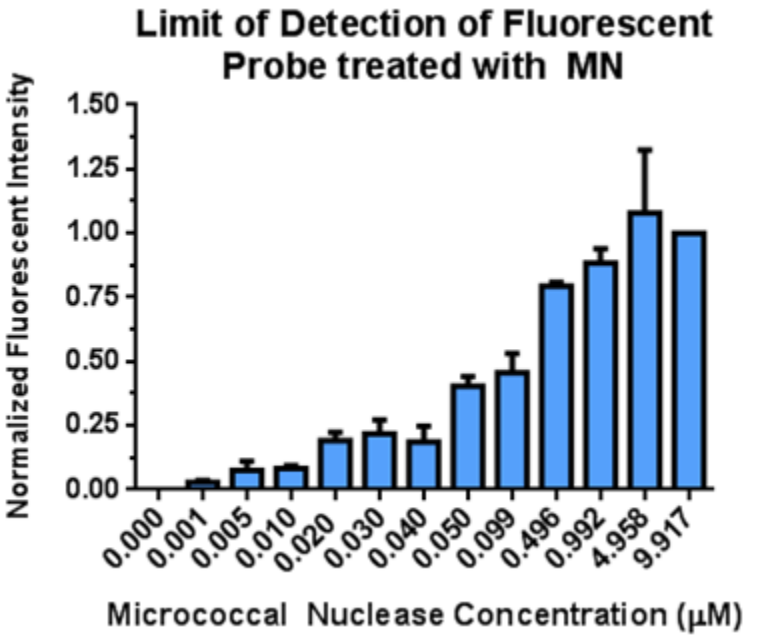
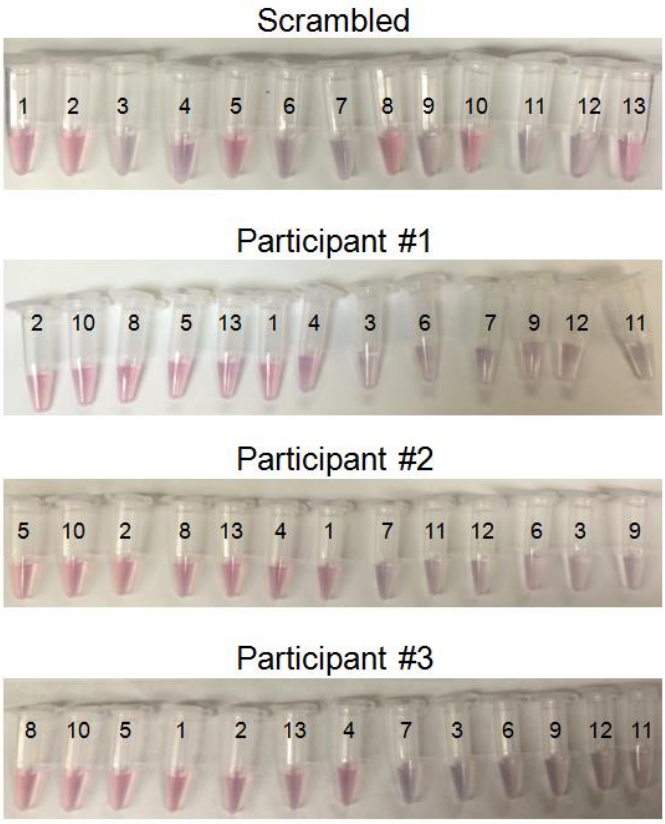


Figure S5. The limit of detection of the fluorescent probe was determined to be 0.496 μM MN.



Tube #	Concentration (μM)
1	0.040
2	0.005
3	4.958
4	0.030
5	0.001
6	0.992
7	0.050
8	0
9	9.917
10	0.010
11	0.099
12	0.496
13	0.020

Figure S6. 11-mer Oligo-AuNPs were treated with varying concentrations of MN and the tubes were scrambled. Participants were able to sort and differentiate between aggregated and non-aggregated samples.

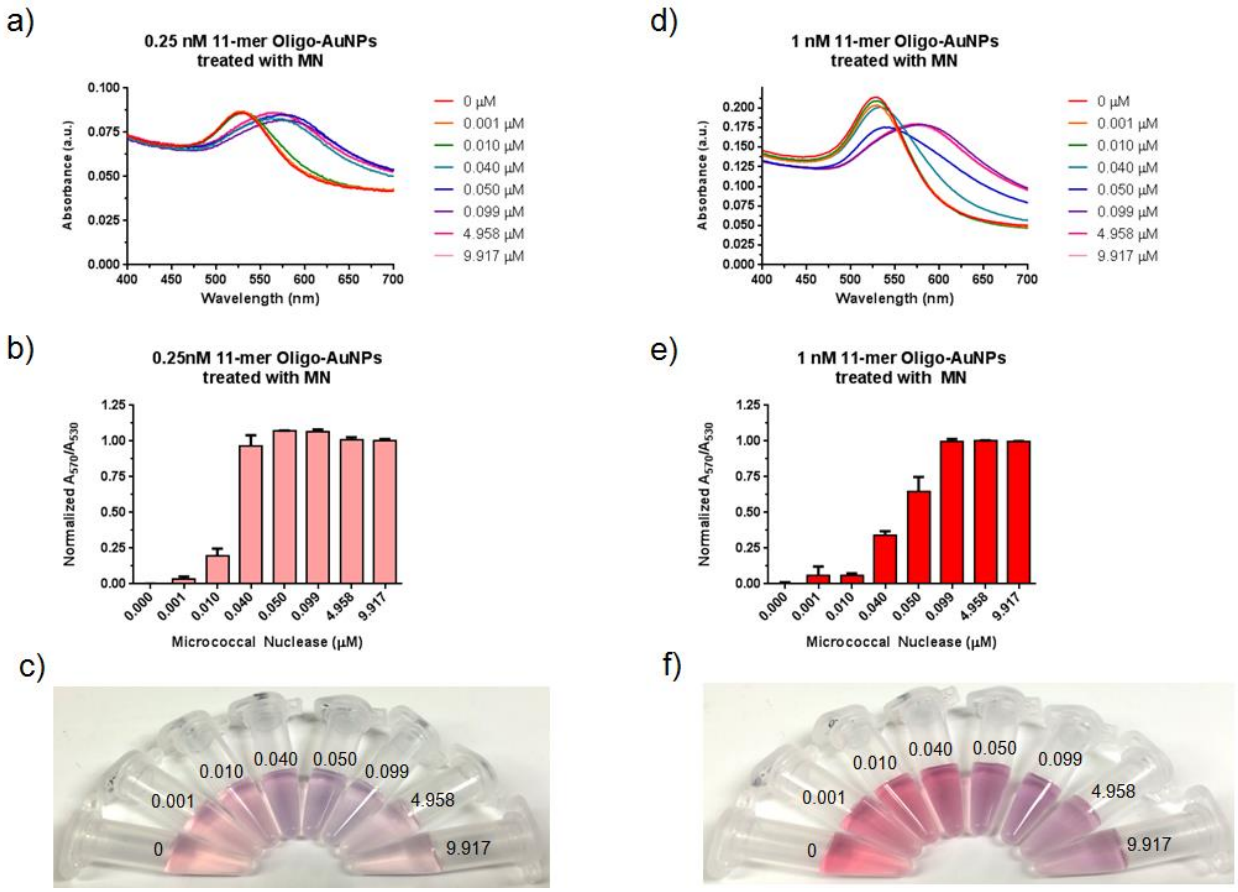


Figure S7. a-c) 11-mer Oligo-AuNPs (0.25 nM) were treated with varying concentrations of MN to determine the limit of detection. There was a shift in the UV-absorbance and a color change in solution when Oligo-AuNPs were treated with 0.040 µM to 9.917 µM MN. **d-f)** 11-mer Oligo-AuNPs (1 nM) were treated with varying concentrations of MN to determine the limit of detection. There was a shift in the UV-absorbance and a color change in solution when Oligo-AuNPs were treated with 0.050 µM to 9.917 µM MN

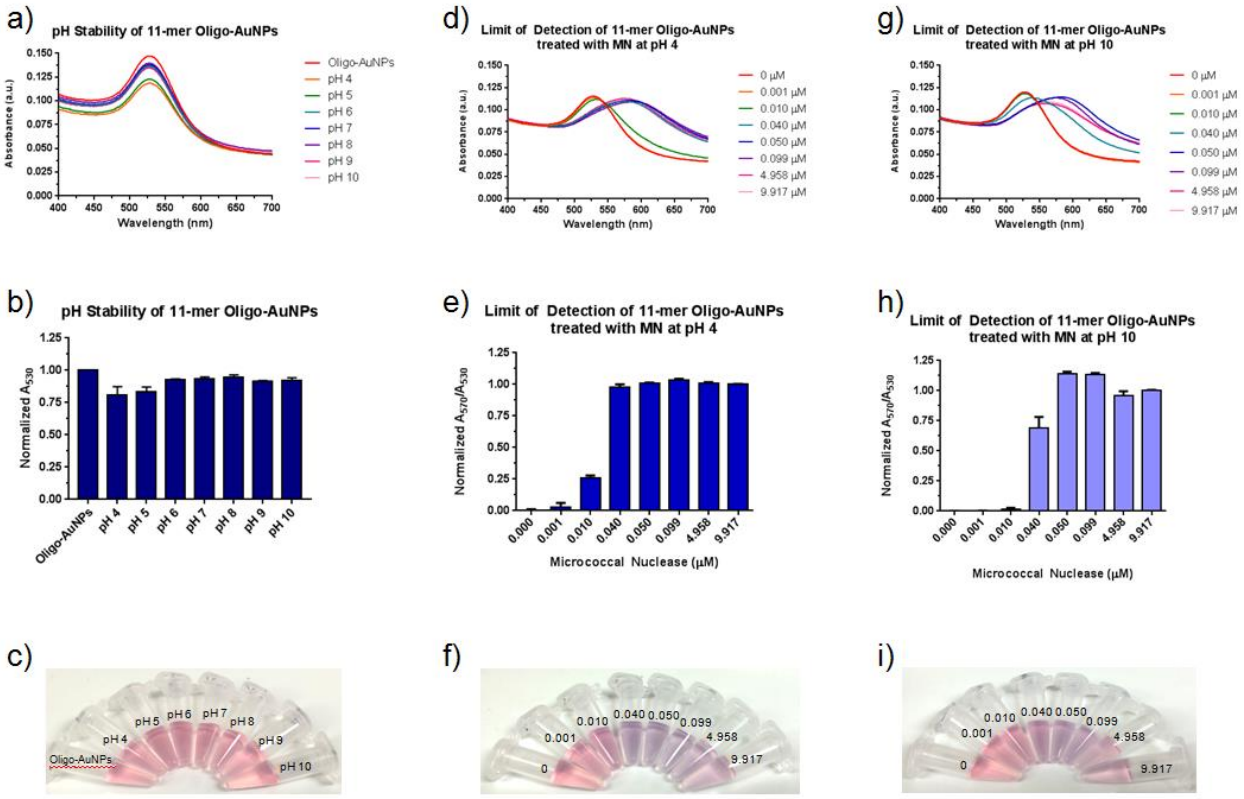


Figure S8. a-c) 11-mer Oligo-AuNPs (0.5 nM) were stable in solution from pH 4 -10 and the λ_{max} remained at 530 nm. **d-f)** 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN at pH 4 to determine the limit of detection. There was a shift in the UV-absorbance and a color change in solution when Oligo-AuNPs were treated with 0.040 µM to 9.917 µM MN. **g-i)** 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN at pH 10 to determine the limit of detection. There was a shift in the UV-absorbance and a color change in solution when Oligo-AuNPs were treated with 0.040 µM to 9.917 µM MN.

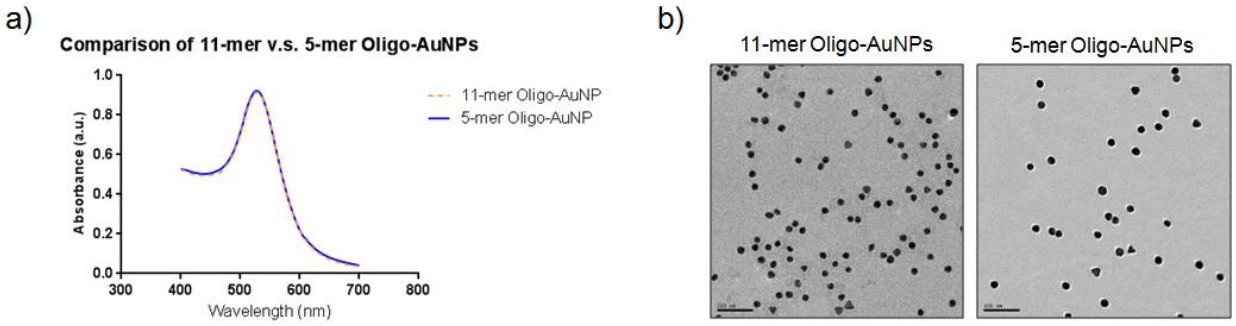


Figure S9. a) The 11-mer and 5-mer Oligo-AuNPs had an equivalent λ_{max} at 530nm. **b)** TEM images were taken of the 11-mer Oligo-AuNPs and 5-mer Oligo-AuNPs (30,000x magnification).

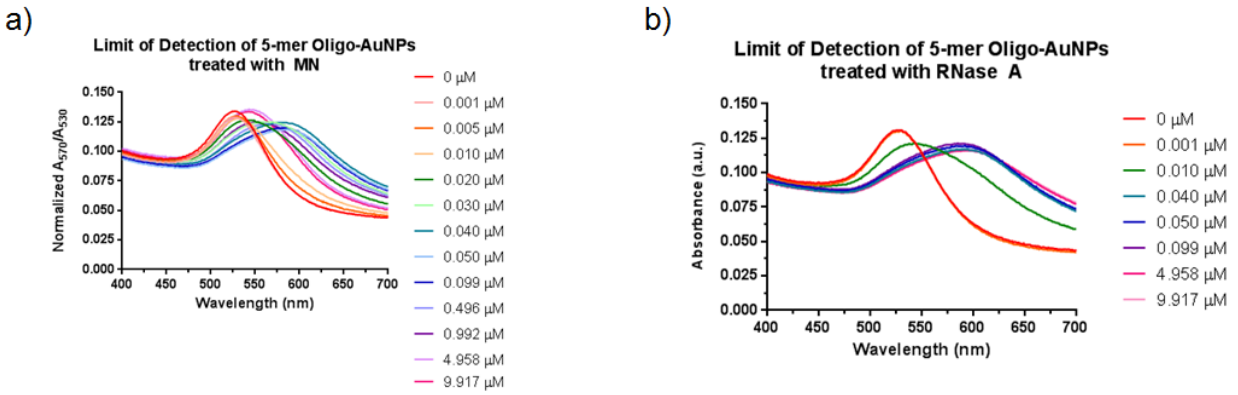


Figure S10. a) 5-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 0.020 µM to 9.917 µM MN. **b)** 5-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of RNase A to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 0.010 µM to 9.917 µM RNase A.

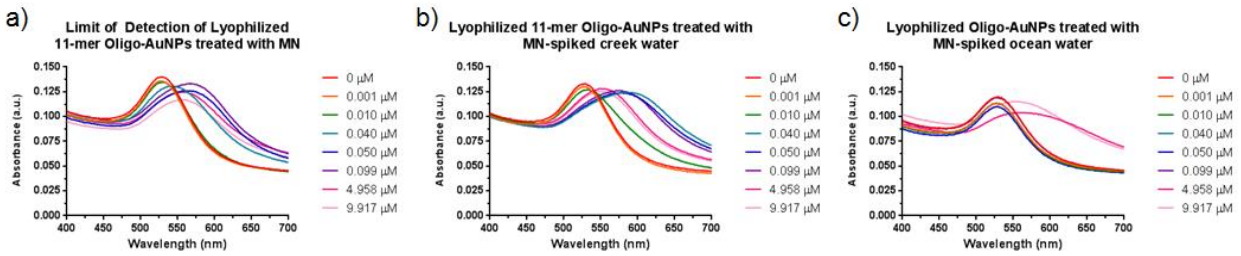


Figure S11. **a)** Lyophilized 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 0.040 µM to 9.917 µM MN. **b)** Lyophilized 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN spiked into creek water to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 0.040 µM to 9.917 µM MN. **c)** Lyophilized 11-mer Oligo-AuNPs (0.5 nM) were treated with varying concentrations of MN spiked into ocean water to determine the limit of detection. There was a shift in the UV-absorbance when Oligo-AuNPs were treated with 4.958 µM to 9.917 µM MN.

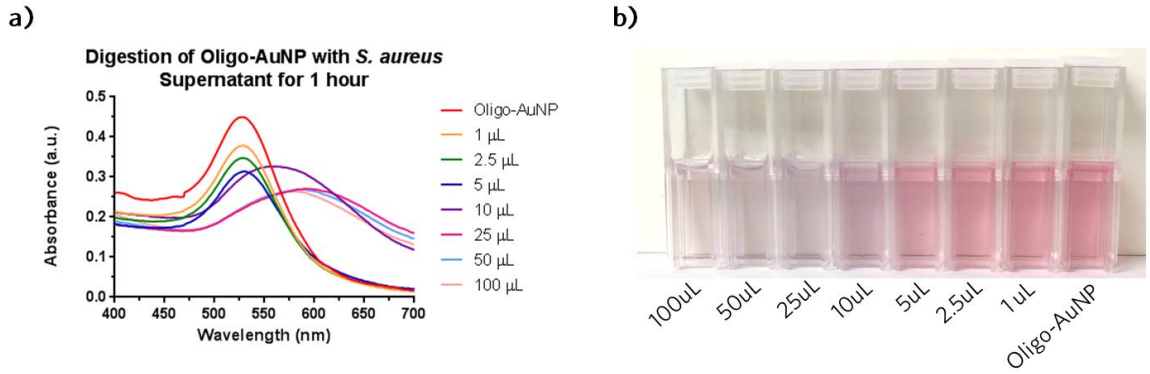
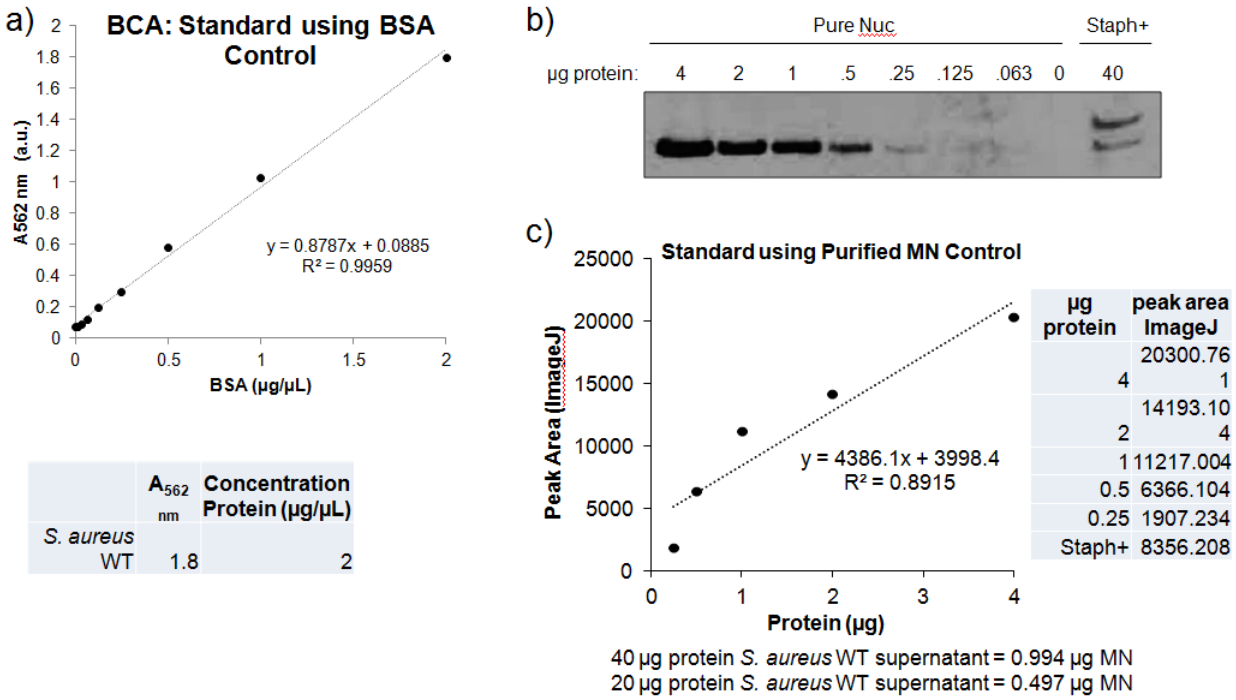


Figure S12. **a)** The minimum volume of *S. aureus* WT supernatant needed was 10 µL. Using 10 µL, the λ_{max} shifted to 560 nm. The study was drawn out to 1 hour in order to allow the lower volumes (1 µL-5 µL) of *S. aureus* WT supernatant used more time to digest since the λ_{max} for these conditions did not shift after 5 minutes. **b)** A colorimetric change in solution from red to purple occurred within 5 minutes when 10µL to 100 µL of *S. aureus* supernatant was used. After extending the incubation time to 1 hour, the lower volumes (1 µL-5 µL) of *S. aureus* WT supernatant used to treat Oligo-AuNPs did not undergo a color change.



	$A_{562\ nm}$	Concentration Protein (µg/µL)
S. aureus WT	1.8	2

µg protein	peak area ImageJ
4	20300.761
2	14193.104
1	11217.004
0.5	6366.104
0.25	1907.234
Staph+	8356.208

Figure S13. A western blot was performed in order to determine how much MN protein is in the *S. aureus* WT supernatant. **a)** BSA control was serially diluted to create a standard curve then assayed along with the *S. aureus* WT supernatant using Pierce BCA Protein Assay kit. **b)** A western blot was performed using serially diluted purified MN along with *S. aureus* WT supernatant (40 µg protein). **c)** A standard curve was generated from the western blot. It was determined that from the 40 µg protein (*S. aureus* WT supernatant) loaded, only 0.994 µg is attributed to MN.

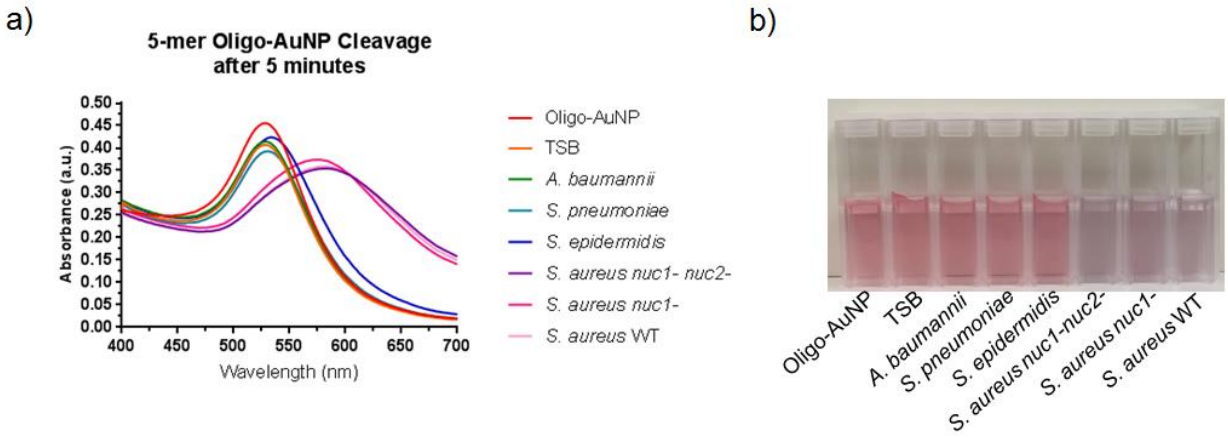


Figure S14. 5-mer Oligo-AuNPs (0.5 nM) were treated with various bacterial supernatants. **a)** The various *S. aureus* supernatants caused aggregation to occur and the λ_{max} to shift from 530 nm

to 570 nm. **b)** Treatment with various *S. aureus* supernatants resulted in a color change from red to purple in solution.

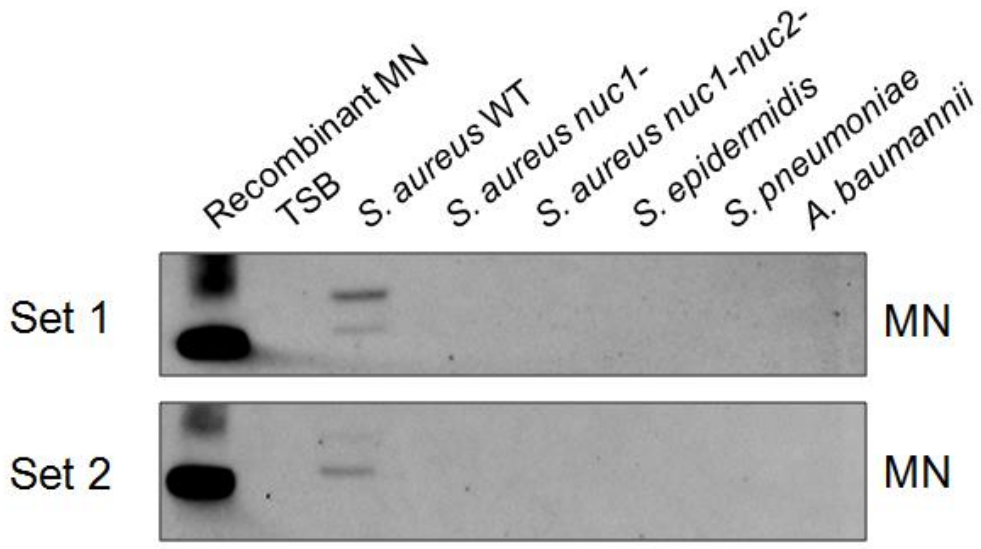


Figure S15. Two different sets of supernatants from *S. aureus* WT, *S. aureus nuc1-*, *S. aureus nuc1-nuc2-*, *S. epidermidis*, *S. pneumoniae*, and *A. baumannii* were analyzed for MN expression by Western blot. Supernatant from *S. aureus WT* is the only condition that expressed MN, meaning that complete knock-out of MN was successful in *S. aureus nuc1-* and *S. aureus nuc1-nuc2-*.

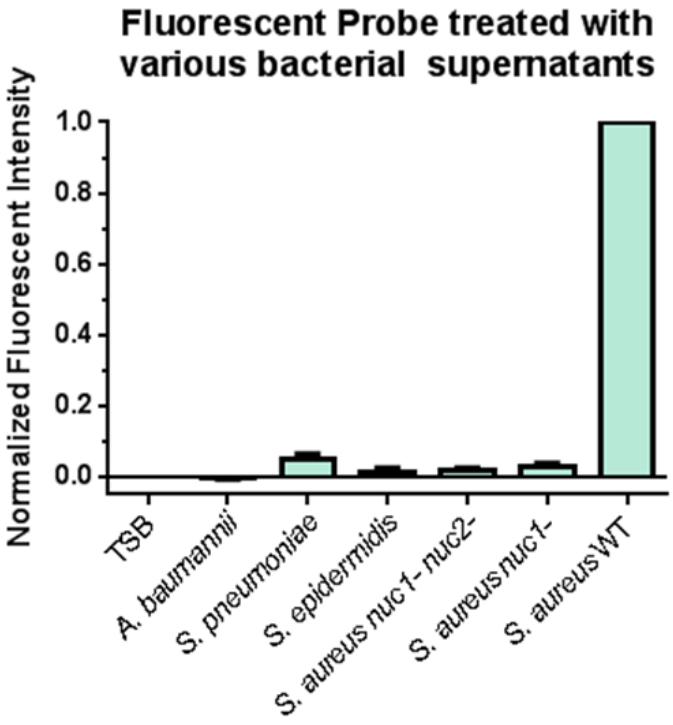


Figure S16. The fluorescent probe was treated with various bacterial supernatants. The fluorescent probe was specific to only the *S. aureus* WT supernatant.

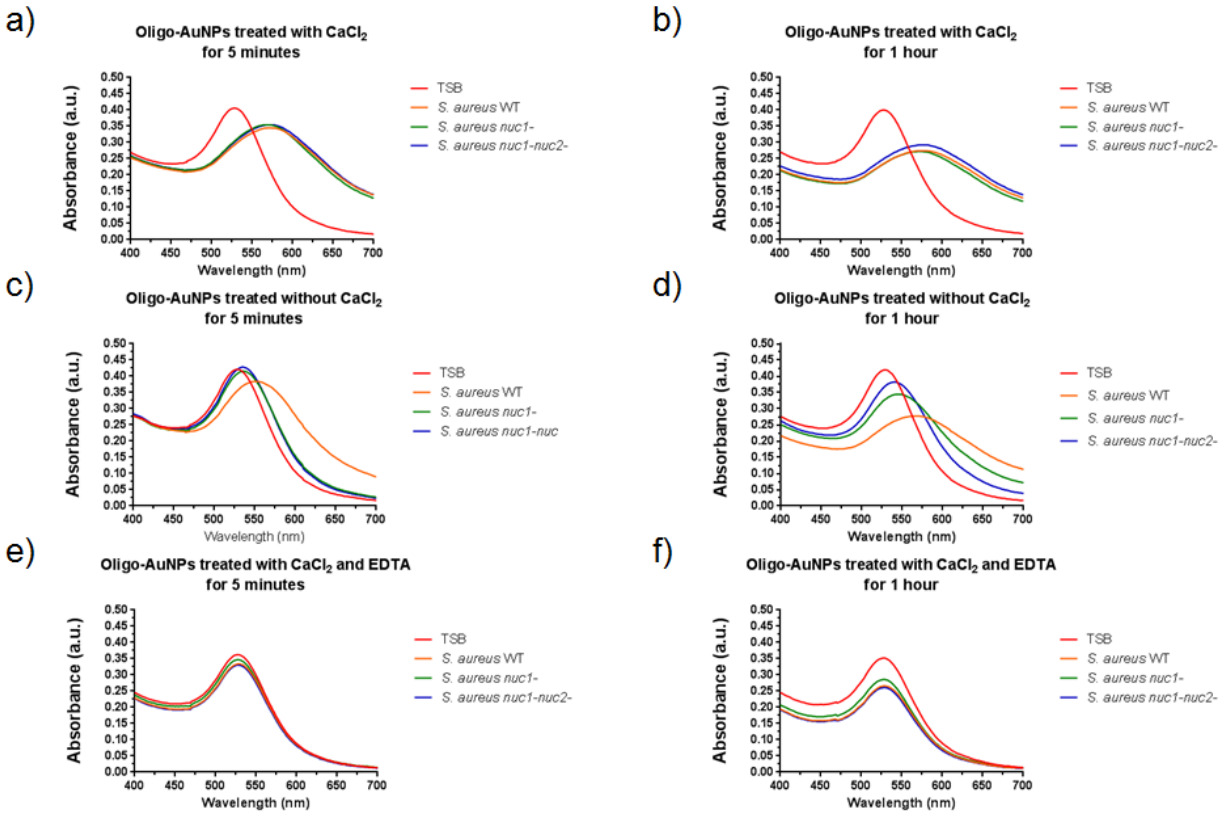


Figure S17. a) 11-mer Oligo-AuNPs (0.5 nM) were treated with TSB or supernatant from *S. aureus* WT, *S. aureus nuc1-*, and *S. aureus nuc1-nuc2-* in the presence of 2 mM $CaCl_2$ for 5 minutes. There was a shift in λ_{max} from 530 nm to 570 nm for the *S. aureus* conditions. **b)** 11-mer Oligo-AuNPs (0.5 nM) were treated with TSB or supernatant from *S. aureus* WT, *S. aureus nuc1-*, and *S. aureus nuc1-nuc2-* in the presence of 2 mM $CaCl_2$ for 1 hour. There was a shift in λ_{max} from 530 nm to 570 nm for the *S. aureus* conditions and a decrease in absorbance due to aggregation of the Oligo-AuNPs. **c)** 11-mer Oligo-AuNPs (0.5 nM) were treated with TSB or supernatant from *S. aureus* WT, *S. aureus nuc1-*, and *S. aureus nuc1-nuc2-* without $CaCl_2$ for 5 minutes. Supernatant from *S. aureus* caused a shift in λ_{max} from 530 nm to 560 nm. *S. aureus nuc1-* and *S. aureus nuc1-nuc2-* did not cause a shift in UV absorbance. **d)** 11-mer Oligo-AuNPs (0.5 nM) were treated with TSB or supernatant from *S. aureus* WT, *S. aureus nuc1-*, and *S. aureus nuc1-nuc2-* without $CaCl_2$ for 1 hour. Supernatant from *S. aureus* caused a shift in λ_{max} from 530 nm to 570 nm. *S. aureus nuc1-* caused a shift in in λ_{max} from 530 nm to 545 nm and *S. aureus nuc1-nuc2-* caused a shift in in λ_{max} from 530 nm to 540 nm. **e, f)** 11-mer Oligo-AuNPs (0.5 nM) were treated with TSB or supernatant from *S. aureus* WT, *S. aureus nuc1-*, and *S. aureus nuc1-nuc2-* with $CaCl_2$ and EDTA for both 5 minutes and 1 hour. After 5 minutes or 1 hour, no shift in the UV absorbance was seen.