Identification of Bacteria by Conjugated Oligoelectrolyte/ssDNA Electrostatic Complexes

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Supporting Information

Experimental Details

Bacterial strains were grown as follows. *Escherichia coli* strains K12 and FAD-1 were grown in LB Broth (10.0 g tryptone, 5.0 g yeast extract, and 10.0 g NaCl per liter dH_2O). *Sporomusa* strain DMG58 was grown in an anaerobic basal media as described by Möller[1] (containing 0.348 g K_2HPO_4, 0.227 g KH_2PO_4, 0.500 g NH_4Cl, 2.250 g NaCl, 0.002 g $FeSO_4 \cdot 7H_2O$, 0.000015 g $NaHSeO_3$, 2.000 g yeast extract, 2.000 g casitone, 0.001 g resazurin, 0.500 g $MgSO_4 \cdot 7H_2O$, 0.250 g $CaCl_2 \cdot 2H_2O$, 6.700 g betaine$\cdot H_2O$, 4.000 g $NaHCO_3$, 0.300 g cysteine-HCl$\cdot H_2O$, 0.300 g Na_2S, 3.0 mL Widdel Trace Elements, and 2.0 mL 10x Wolin Vitamins per liter dH_2O, prepared anoxic by flushing with 80/20 N_2/CO_2). *Rhodopseudomonas palustris* CGA009 was grown photosynthetically in as described in Pelletier[2] (containing 25.0 mL 0.5 M Na_2HPO_4, 25.0 mL 0.5 M KH_2PO_4, 10.0 mL 10% $(NH_4)_2SO_4$, 1.0 mL concentrated base, 1.0 mL 0.1 M Na-thiosulfate, 1.0 mL 2.0 mg mL^{-1} p-aminobenzoic acid; concentrated base containing 20.000 g nitrilotriacetic acid, 28.900 g $MgSO_4$•anhydrous, 6.670 g $CaCl_2 \cdot 2H_2O$, 0.0185 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.198 g $FeSO_4 \cdot 7H_2O$, and 100 mL Hunter's 'Metal 44' per liter dH_2O). *Streptococcus mutans* was grown in TS media (30.0 g tripticase-soy broth and 3.0 g yeast extract per liter dH_20). *Lactobacillus acidophilus* was grown in Lactobacilli MRS Broth (55.0 g Difco Lactobacilli MRS broth powder per liter dH_2O). All media preparations were autoclaved at 121ºC for 20 minutes per liter of liquid, unless otherwise specified in the provided references. *E. coli* K12 and FAD-1, *S. mutans,* and *L. acidophilus* were grown shaking at 100 rpm at 37 ºC. *Sporomusa*

and *R. palustris* were grown shaking at 100 rpm at 30 ºC. Cells were grown for 12-72 hours until the mid-exponential growth phase. Cell aliquots were stored in 10% glycerol at -20 ºC until use. Bacteria cells were separated from the media by centrifuging at 14000 rpm for 4 minutes in an Eppendorf Centrifuge 5415C. Cell aliquots were washed twice in 800 µL phosphate buffer (10 mM pH=7.5; PB) and resupsended in PB to yield a concentration of 1.0 OD at 600 nm. Bacteria cells were kept on ice until addition to the probe mixtures.

HPLC-purified ssDNA oligonucleotides were purchased from Integrated DNA Technologies, Inc (Skokie, IL) and resupended in TE buffer. Synthesis of FPF has previously been reported.[3] The probes were prepared by individually mixing the FPF, 100 µM FPF, aqueous solution and the $ssDNA_x$-FAM (x=1-5; 100 µM, 130 µM, 91 µM, 131 µM, 95 µM, respectively) solutions in phosphate buffer. The probe solutions were prepared to a final concentration of 4 µM FPF and 0.70 µM ssDNA, which yielded a $R_{+/-}$ = 1.2 charge ratio. The probes were vortexed (Fisher Scientific vortex mixer) on speed setting 9 for 60 minutes. Probe solutions of 190 µL were then aliquoted into a 96-well plate. The initial photoluminescence (PL) spectra were measured in a Varian Cary Eclipse fluorescence spectrometer equipped with an Eclipse microplate reader. Six bacteria solutions were aliquoted into each of the five probe wells to yield 0.05 OD. The well plate was placed on the vortex mixer for 15 minutes on the lowest setting before the final PL was recorded. Regularized discriminant analysis transformation of the patterns was generated using JMP (SAS Institute) statistical software.

Absorbance and Fluorescence of FPF and FAM-labeled ssDNA

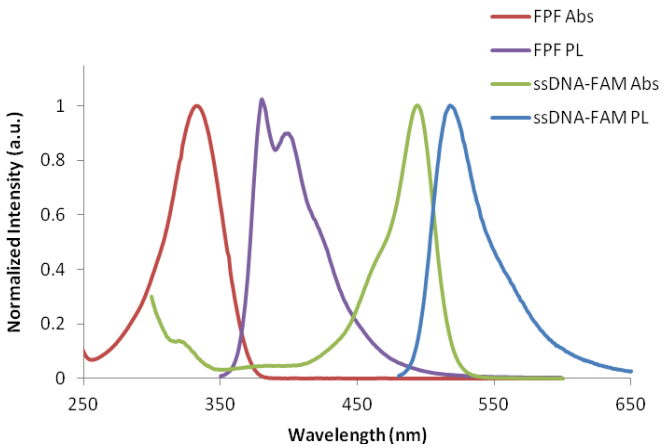

Figure S1. Normalized UV absorption and photoluminescence spectra of FPF and 5'-FAM labeled ssDNA.

Comparison of $R_{+/-}$ = 1.0 and $R_{+/-}$ = 1.2 response to *Escherichia coli* K12

The charge ratio of $R_{+/-}$ = 1.0 has previously been described to yield charge neutral complexes that are responsive to both cationic and anionic quenchers.[4] Charge ratios between the cationic oligomer and ssDNA$_1$-FAM of $R_{+/-}$ = 1.0 and $R_{+/-}$ = 1.2 were investigated for high bacterial response and good, reproducible signal stability, as shown in Figure S2. The charge ratio of $R_{+/-}$ = 1.0 yielded a higher δ response of 5.53 compared to $R_{+/-}$ = 1.2 of 4.88, but offered lower signal stability as reflected by the relative standard deviations of 6% and 2%, respectively. The charge ratio of $R_{+/-}$ = 1.2 was therefore chosen to carry out the investigation due to its higher signal stability.

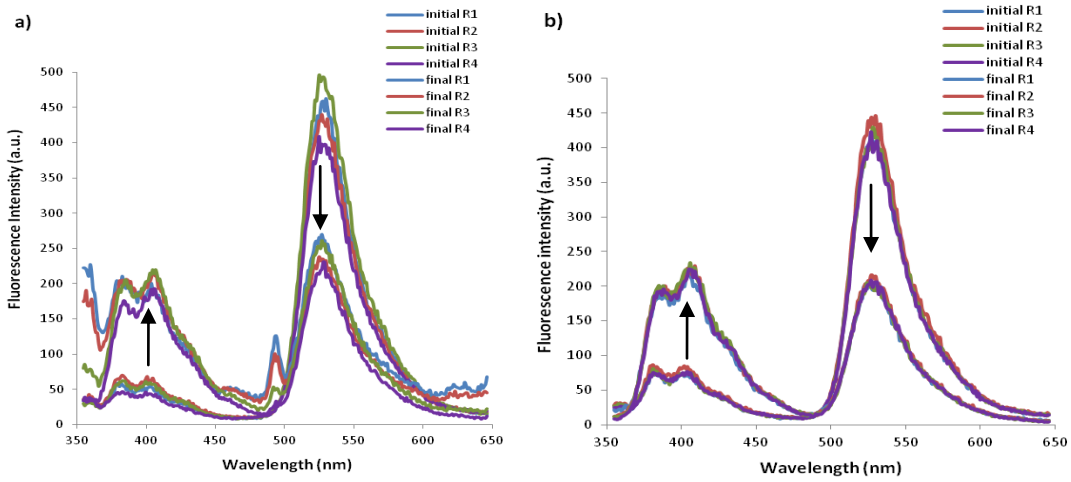

Figure S2. Four replicas (R1-R4) of the photoluminescence spectra of the initial FPF/ssDNA$_1$-FAM aggregate and the final spectrum after the addition of *Escherichia coli* K12, 0.05 OD a) charge ratio of $R_{+/-}$ = 1.0 and b) charge ratio $R_{+/-}$ = 1.2.

References

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