Supporting Information

NANODIAGNOSTICS TO MONITOR BIOFILM OXYGEN METABOLISM FOR ANTIBIOTIC SUSCEPTIBILITY TESTING

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Figure S1. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* PAO1 that has been challenged with serial 2-fold dilutions of tobramycin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* PAO1 from 1a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figures 2 and 3a.



Figure S2. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* BAA-2108 that has been challenged with serial 2-fold dilutions of tobramycin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* BAA-2108 from 2a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 3b.



Figure S3. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* BAA-2113 that has been challenged with serial 2-fold dilutions of tobramycin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* BAA-2113 from 3a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 3c.



Figure S4. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* BAA-2114 that has been challenged with serial 2-fold dilutions of tobramycin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* BAA-2114 from 4a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 3d.



Figure S5. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* PAO1 that has been challenged with serial 2-fold dilutions of colistin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* PAO1 from 5a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 4a.



Figure S6. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* BAA-2108 that has been challenged with serial 2-fold dilutions of colistin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* BAA-2108 from 6a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 4b.



Figure S7. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* BAA-2113 that has been challenged with serial 2-fold dilutions of colistin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* BAA-2113 from 7a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 4c.



Figure S8. Nanosensors embedded in active biofilms are able to report local oxygen concentration changes in response to antibiotics. a) Kinetic ratiometric intensity data for *P. aeruginosa* BAA-2114 that has been challenged with serial 2-fold dilutions of colistin in phosphate buffered saline (pH = 7.4). b) Normalized kinetic ratiometric intensity data for *P. aeruginosa* BAA-2114 from 8a. Data was normalized to an initial reading of each biofilm well submerged in PBS containing no antibiotic. (n = 4 biological replicates). This data was used to produce Figure 4d.



Figure S9. Data and attempted dose-response curve fits of *P. aeruginosa* BAA-2114 response to imipenem (IPM) and ampicillin (AMP). These fits do not provide useful data from which to determine the MBIC parameter, and are instead presented here to guide the eye.



Figure S10. Schematic for fabrication of oxygen-sensitive nanosensor and its incorporation into the bacterial biofilm for dynamic O_2 measurements in 96-well plate reader format.