Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Membrane Permeabilization by Conjugated Oligoelectrolytes Accelerates Whole-Cell Catalysis

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Experimental Methods

Cell culture

Escherichia coli K-12 (ATTC #10798, ATCC, VA) was cultured aerobically overnight from a frozen stock by growth in Luria Broth (10 g L^{-1} bacto tryptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl) at 37 ºC with shaking. For cells cultured for ß-galactosidase activity assays, Luria Broth was supplemented with 2% lactose for induction of *lacZ*. Cells were washed three times by centrifugation in M9 minimal media (6.8 g L^{-1} Na_2HPO_4, 3 g L^{-1} KH_2PO_4, 1 g L^{-1} NH_4Cl, 0.5 g L^{-1} NaCl) before use in assays.

Assay of outer membrane permeation

Cell suspensions were resuspended to a final OD_{600nm}= 1.0 in M9, stained with varying amounts of 1 mM DSSN+ in ultrapure water to achieve final concentrations of 0, 5, 10, 15 and 25 µM, and then incubated at room temperature for 1, 4 and 10 hours. Samples were removed at each time point and filtered using a 0.2 µm filter. Non-fluorescent 4-methylumbelliferyl phosphate disodium salt (MUP) from an alkaline phosphatase activity assay kit (Biovision, K422-500) was used to measure the activity of extracellular ALP. Supernatant samples were incubated with 80 µM MUP at 25ºC in a 96-well plate while monitoring fluorescence (Ex/Em = 360nm/440nm) over 90 min using a Spectra Max Plus 384 microplate spectrophotometer. Supernatant samples without MUP added were measured for background correction. All samples were measured in triplicate.

Assay of inner membrane permeation

Cell suspensions were resuspended to a final OD_{600nm}= 0.6 in M9, stained with varying concentrations of DSSN+ (0, 5, 10, 15 and 25 µM) and then incubated at room temperature for 5 hours. The permeability of the inner membrane of *E. coli* K-12 was determined by measuring the release of ß-galactosidase activity into the medium. Cell suspensions were filtered, and the resulting filtrates were incubated with 1.3 mM 2-nitrophenyl ß-D-galactopyranoside (ONPG, Sigma Aldrich) at 37ºC for 2 hours in a 96-well plate while monitoring the absorbance at 420 nm using a Spectra Max Absorbance plate reader. All samples were measured in triplicate.

Calibration against cell lysates

For both permeability assays, activities were compared against the activity of lysed cell suspensions. Cell suspensions of the same OD_{600nm} and same culture conditions used in the comparative assays were lysed via probe sonication in an ice bath. Complete lysis was confirmed by measuring OD_{600nm} to be equal to 0. Lysates were then serially diluted in M9 to produce dilutions of 50, 25, 12.5, 6.1, 3 and 1.5% cell lysate. These solutions were then tested following the respective assay procedures.

Substrate turnover assay

Cell suspensions were resuspended to an OD_{600nm}= 0.6 in M9, stained with varying concentrations of DSSN+ (0, 5, 10, 15 and 25 µM) for 30 minutes to allow for membrane intercalation, then centrifuged and resuspended in M9 to a final OD_{600nm}= 0.6. Cell turnover of ONPG, indicated by the linear rate of absorbance increase, was examined in triplicate for each staining concentration using a Spectra Max Absorbance plate reader to measure absorbance at 420nm over 2 hours while cells were incubated with 1.3 mM ONPG. Background corrections were made by subtracting the absorbance at 420 nm of DSSN+ stained cell suspensions that were not incubated with ONPG.

Calibration of ALP activity in cell lysates

E. coli cells were fully lysed via sonication and dilutions of the resulting lysate were measured for ALP activity. Lysates were serially diluted in M9 in ratios of 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. For each lysate dilution, we calculated the average rate of fluorescence change using the slopes of plots containing fluorescence intensity vs. time. The resulting rates were linearly related to the amount of cell lysate in order to generate a calibration curve to determine the relative degree of cell lysis of samples.

Alkaline Phosphatase Activity Fluorometric Assay Standards to control for DSSN+ interference

To determine whether DSSN+ has an effect on the hydrolysis of MUP or interferes with the activity of ALP, a standard curve was generated in accordance to the BioVision (#K422-500) Assay kit protocol. Using a 96-well plate, different amounts of MUP (0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well) were dispensed in the assay buffer. The same amount of ALP enzyme solution was added to each well. To test the effect of DSSN+, DSSN+ solution was added to each well to achieve a final concentration of 2 µM in order to mimic the maximum amount of DSSN+ left in solution after cell staining. Fluorescence was monitored in duplicates of the standard without DSSN+ and with DSSN+ at 440 nm.

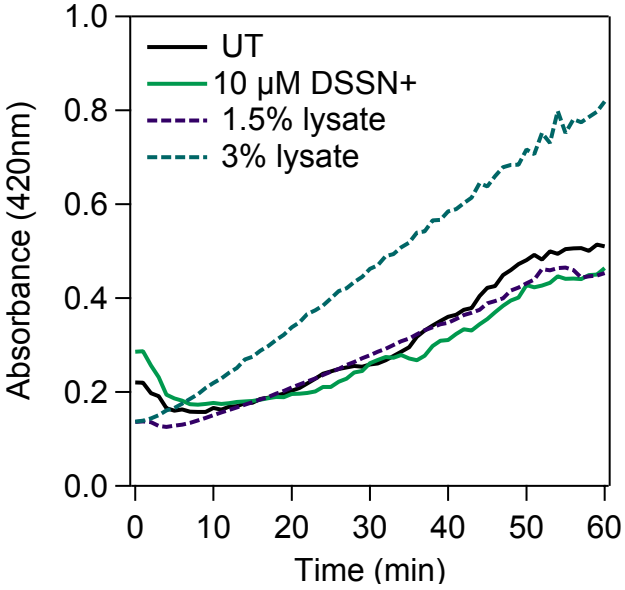

Figure S1. Permeation of *E.coli* IM by DSSN+ determined optically by measuring absorbance of ONP at 420 nm. ß-galactosidase activity in the supernatant from UT (solid black) and 10 µM DSSN+ stained *E. coli* (solid green) shown over incubation time with ONPG and compared with absorbance of 1.5% (dashed purple) and 3% (dashed blue) cell lysate solutions. All measurements are an average of triplicates.

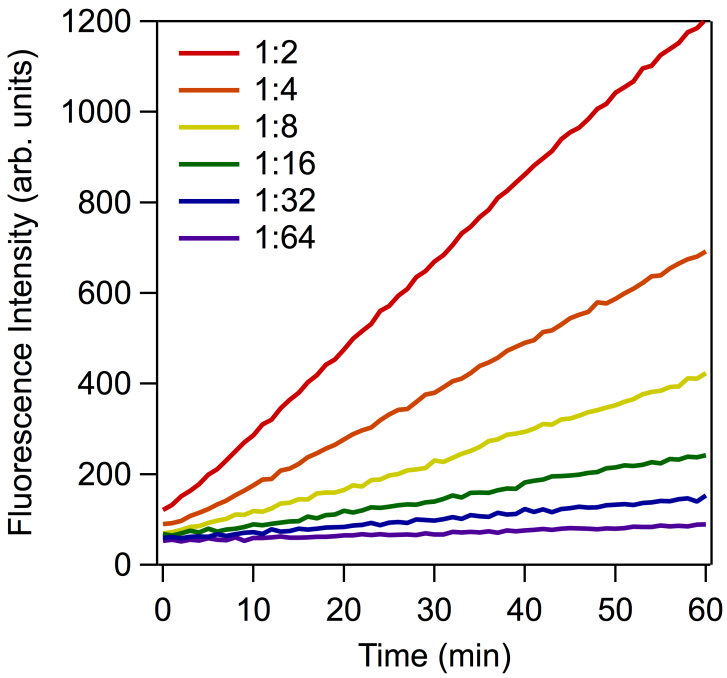

Figure S1. ALP activity of cell lysates dilutions (1:2, 1:4, 1:8, 1:16, 1:32, 1:64) determined by fluorescence intensity of 4-MU measured at 440 nm over time. All measurements are an average of triplicates.

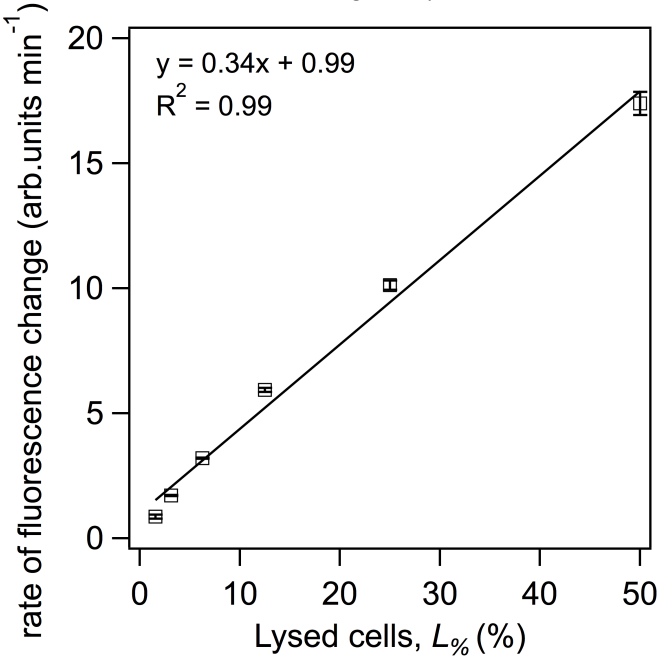

Figure S2. ALP calibration curve determined by measuring the rate of fluorescence change in different concentrations of cell lysate solutions, where $L_{\%}$ is equivalent to the ratio of cell lysate to buffer as a percentage.

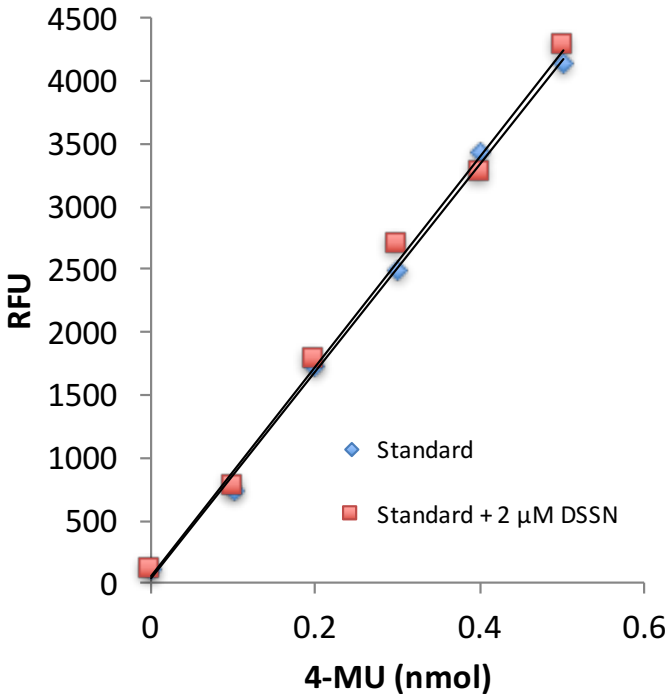

Figure S3. ALP hydrolysis of MUP measured by fluorescence intensity at 440 nm in standards prepared without (blue diamond) and with (red square) DSSN+. All measurements performed in duplicate.

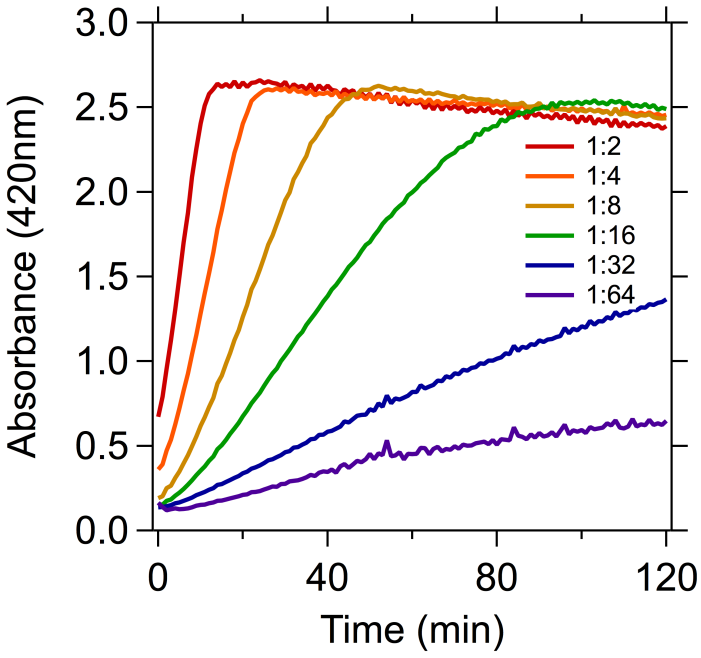

Figure S4. ß-galactosidase activity of cell lysates dilutions (1:2, 1:4, 1:8, 1:16, 1:32, 1:64) determined by absorbance measured at 420 nm over time. All measurements are an average of triplicates.