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Supporting Information

Pendant Ionic Groups of Conjugated Oligoelectrolytes Govern the Ability to Intercalate into Microbial Membranes

Alexander W. Thomas, Chelsea Catania, Logan E. Garner, and Guillermo C. Bazan*

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1. Reagents and syntheses

Reagents

Unless otherwise noted, all chemicals and materials were purchased from Fisher or Sigma Aldrich and were used as received. *E. coli* lipid extract was purchased from Avanti Polar Lipids.

Syntheses of 1 and **DSSN+** has previously been reported.[1]

Synthesis of 2

A 250 mL round bottom flask was charged with **1** (1 eq., 1 g, 0.8 mmol), potassium cyanide (20 eq., 1.04 g, 15.9 mmol), 18-crown-6 (1 eq., 0.21 g, 0.8 mmol), and 200 mL of acetonitrile and equipped with a reflux condenser. The resulting solution was stirred under reflux for 24 hours. The solution was then allowed to cool and the solvent was removed by rotary evaporation. The residue was dissolved in CH_2Cl_2 and washed with water (2X) and brine. The combined organic phases were dried over $MgSO_4$ and the solvent removed to yield crude **2**. NMR analysis indicated the crude consisted almost entirely of a mixture of 18-crown-6 and the target compound. Further purification was achieved via silica gel chromatography. 0.380 g (60% yield) of an orange solid was collected. ^{1}H NMR (500 MHz, CD_2Cl_2): δ 7.50 (d, *J* = 8.5 Hz, 4H), 7.47 (d, *J* = 8.5 Hz, 4H), 7.38 (d, d, *J* = 8.8 Hz, 4H), 7.12 (s, 2H), 7.06 (d, *J* = 16.2 Hz, 2H), 6.89 (d, *J* = 16.2 Hz, 2H), 6.63 (d, *J* = 8.8 Hz, 4H), 3.31 (t, 8H), 2.35 (t, 8H), 1.65 (m, 16H), 1.50 (m, 8H), 1.38 (m, 8H). FD-MS: 850 (M^+), 823 ($(M\text{-}CN)^+$), 425 (M^{2+}). Elemental analysis (CHN) calculated: C, 81.84; H, 8.29; N, 9.87. Found: C, 81.10; H, 8.26; N, 8.67.

Synthesis of DSSNcarb

A 25 mL microwave reaction tube was charged with **2** (1 eq., 0.1 g, 0.116 mmol), 8 M aq. potassium hydroxide (100 eq., 1.5 mL, 11.7 mmol), water (3.5 mL) and methanol (15 mL), sealed, and placed into the reaction chamber of a Biotage Initiator microwave reactor. Under rigorous stirring at (900 RPM) the temperature of the reaction solution was ramped from ~ 30 °C (temperature upon sealing) to 100 °C over the course of 2 min and then further increased to 150 °C and stirred at this temperature for 2 hours. The reaction solution, now homogenous following the microwave and still warm was allowed to cool. The solvent was removed and the crude product dissolved in methanol. The target compound was then precipitated and washed with diethyl ether. 41 mg (30% yield) of an orange solid was collected. ^{1}H NMR (500 MHz, CD_3OD): δ 7.51 (d, *J* = 8.3 Hz, 4H), 7.47 (d, *J* = 8.3 Hz, 4H), 7.37 (d, *J* = 8.65 Hz, 4H), 7.14 (s, 2H), 7.07 (d, *J* = 16.2 Hz, 2H), 6.90 (d, *J* = 16.2 Hz, 2H), 6.65 (d, *J* = 8.65 Hz, 4H), 3.31 (m, 8H), 2.17 (t, 8H), 1.62 (m, 16H), 1.39 (m, 16H). ^{13}C NMR (800 MHz, CD_3OD): δ 182.98, 149.26, 139.21, 137.20, 130.04, 128.80, 128.68, 127.73, 127.16, 125.99, 124.01, 112.93, 66.90, 39.28, 30.84, 28.42, 28.14, 27.90, 15.44. IR (cm^{-1}): 1655, 1560 (peaks indicative of carboxylate functional groups, other IR absorbance peaks omitted).

Synthesis of DSSNpyr

Compound **1** (0.25 g, 0.141 mmol) was stirred in ~100 mL of pyridine at room temperature for 4 days. After 2 days, ~100 mL methanol was added to aid dissolution. Volatiles were removed and the resulting solid was slurried in hexanes and filtered to collect 0.291 g (93% yield) of an orange solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.11 (d, *J* = 5.6 Hz, 8H), 8.63 (t, *J* = 7.8 Hz, 4H), 8.19 (t, *J* = 7.5 Hz 8H), 7.58 (d, J = 8.3Hz, 4H), 7.53 (d, J = 8.3Hz, 4H), 7.40 (d, *J* = 8.6 Hz, 4H), 7.25 (s, 2H), 7.15 (d, *J* = 16.2 Hz, 2H), 6.94 (d, *J* = 16.2 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 4H), 4.62 (t, *J* = 7.4 Hz, 8H), 3.28 (m, 8H), 1.94 (m, 8H), 1.52 (m, 8H), 1.33 (m, 16H). ESI/TOF-MS: 265 $(M\text{-}4I)^{4+}$, 396 $((M\text{-}3I)^{3+}$), 658 $(M\text{-}2I)^{2+}$. Elemental analysis (CHN) calculated: C, 56.57; H, 5.77; N, 5.35. Found: C, 57.6; H, 6.67; N, 4.71.

2. Confocal microscopy

All images were obtained via laser scanning confocal microscopy using an Olympus FluoView 1000 spectral scanning microscope equipped with a 60 x 1.30 silicon oil immersion lens. All images were processed using ImageJ.[2]

3. Cell culture
Escherichia coli K-12 (ATCC 10798) was grown aerobically in Luria Broth (10 g L^{-1} bacto tryptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl) overnight at 37°C and used for both imaging and inoculum of microbial fuel cells (MFCs).

4. Cell staining
Before staining, *E. coli* was rinsed twice from the growth medium with phosphate buffered saline (PBS) containing the following: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 1.8 mM KH_2PO_4 at pH 7.4. *E. coli* cells were centrifuged for 4 minutes at 6000 rcf. 0.5 mL of OD_{600} 1 cells were stained with 10 μM COE and allowed to sit in the dark for 20 minutes at room temperature before rinsing twice. Samples to be imaged were then resuspended in 100 μL of PBS and 5 μL were dropped onto a clean glass slide and a cover slip placed on top. Cover slips were sealed with clear nail polish and all samples were imaged within 2 hours.

5. MFC construction and operation
U-tube MFCs were constructed as previously described.[1,3,4] In short, devices were assembled from two L-shaped glass tubes separated by a Nafion® N117 membrane and sealed using an O-ring and a 28/15 stainless steel pinch clamp. Nafion® membranes were treated at 80 ºC in 3% hydrogen peroxide solution, followed by ultrapure water, then 0.5 M sulfuric acid solution, and ultrapure water again for 1 hour each. Anode and cathode electrodes were constructed out of carbon felt (2 cm x 5 cm), strung with titanium wire and placed in their respective chambers. Assembled devices were filled with ultrapure water and sterilized by autoclaving. After sterilization, the water was removed and the anode and cathode chambers were filled with sterile LB medium. Concentrated aqueous COE (DSSN+, DSSNcarb-, or DSSNpyr+) solutions were added to the anode chambers to a final concentration of 10 μM. Both chambers were then inoculated with overnight *E. coli* culture to a starting OD_{600} of 0.1. The final volume in all chambers was 20 mL. Anode chambers were sealed with a sterile silicon stopper and cathode chambers were loosely capped by a sterile glass scintillation vial, with the cathode only partially submerged to promote “air–wicking”. The assembled devices were then connected to an external resistor of 10 kΩ, a multiplexer and a digital multimeter (PXI-2575, PXI-4065, National Instruments, Austin, TX) controlled by a LabView program for automatic data acquision. Power densities were characterized by switching through a series of external resistors (1, 10, 51, 100, 200, 500 and 1000 kΩ) after 46 hours of operation. Densities were estimated by normalizing by the area of the felt electrodes (10 cm^2) as previously calculated.[4] Each condition was run in triplicate.

6. MFC electrode imaging
An approximately 1 cm x 1 cm square was cut from the carbon felt anode electrodes and dipped twice in PBS to dislodge loosely attached cells. For DAPI staining, the square was submerged in a solution of 5 μM DAPI in PBS for ~30 minutes. One edge of the square was then touched to a paper towel to wick away most of the moisture. A large drop of silicone immersion oil was placed directly onto the square and this was placed face down onto a piece of cover glass. A piece of tape was used to secure the square to the glass before imaging.

7. Liposome preparation
25 mg *E. coli* lipid extract was dissolved in 5 mL chloroform in a 25 mL round bottom flask. The solvent was removed via rotary evaporation to yield a film coating the bottom of the flask. The flask was placed under vacuum overnight. The lipid film was dissolved in 5 mL PBS by rotating the flask in a 30 °C water bath for 1 hour. This solution was then sonicated to near clarity while in an ice bath. The resulting liposome solution was used in aliquots for confocal characterization. COEs were added extraneously from concentrated stock solutions in PBS to ~1 mol% of lipid, roughly estimated assuming an average lipid molecular weight of 800 g mol^{-1}.

8. Supplemental figures

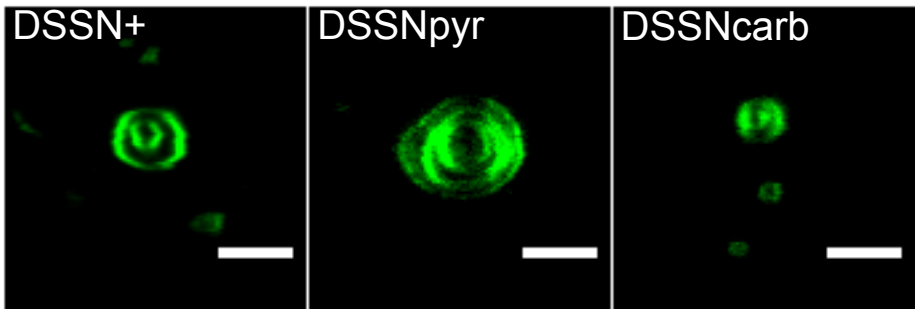

Figure S1. Confocal micrographs of *E. coli* lipid extract multilamellar liposomes stained with 1 mol% COE based on lipid concentration. These images are taken from the same samples as Figure 1 in the main text but are intended to highlight the COE ability to penetrate inner layers of multilamellar liposomes. Jaggedness in some images results from liposome movement during laser scanning image collection. Laser excitation was at 405 nm with emission collected 480 – 580 nm. Scale bars are 3 μm.

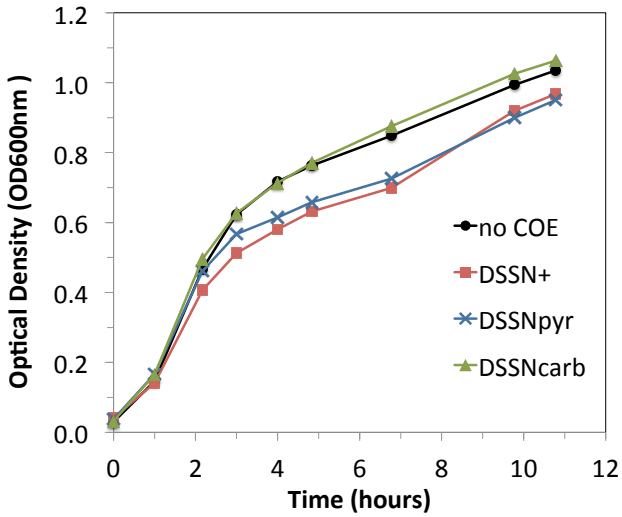

Figure S2. Growth of *E. coli* in LB medium supplemented with 10 μM COE compared to a no-COE control. 35 μL of an overnight culture was used to inoculate 3.5 mL of LB in tubes at 37 ºC shaking at 200 rpm. Results shown are an average of 4 cultures.

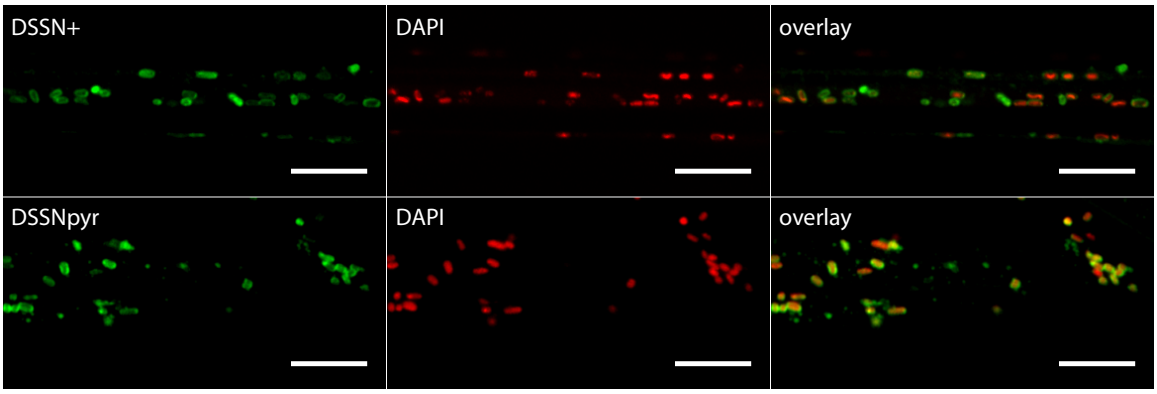

Figure S3. Confocal micrographs of graphite felt electrode fibers after MFC operation with COEs then subsequently stained with nucleic acid stain DAPI. Excitation for DAPI was 405 nm with emission collected 415 – 450 nm as to not collect COE emission, which starts after 450 nm.[1] Excitation for COEs was 488 nm in order to avoid exciting DAPI, with emission collected 500 – 600 nm. Scale bars are 10 μm.

9. References

[1] L. E. Garner, J. Park, S. M. Dyar, A. Chworos, J. J. Sumner, G. C. Bazan, *J. Am. Chem. Soc.* **2010**, *132*, 10042–10052.
[2] W. S. Rasaband, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012.
[3] L. E. Garner, A. W. Thomas, J. J. Sumner, S. P. Harvey, G. C. Bazan, *Energ. Environ. Sci.* **2012**, *5*, 9449–9452.
[4] V. B. Wang, J. Du, X. Chen, A. W. Thomas, N. D. Kirchhofer, L. E. Garner, M. T. Maw, W. H. Poh, J. Hinks, S. Wuertz, et al., *Phys Chem Chem Phys* **2013**, *15*, 5867–5872.