Electronic Supplementary Information for:

Increased Ion Conductance Across Mammalian Membranes Modified with Conjugated Oligoelectrolytes

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Materials and Methods

Materials

All materials were used as received unless otherwise stated. **DSBN+** (1,4-bis(4'-(*N,N*-bis(6''-(*N,N,N*-trimethylammonium)hexyl)amino)-styryl)benzene tetraiodide), **DSSN+** (4,4'-bis(4'-(*N,N*-bis(6''-(*N,N,N*-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide), **COE1-5C**, and **DMI** (decane-1,10-bis(trimethylammonium iodide) or decamethonium iodide) were synthesized and characterized according to previously reported procedures.[1–4] Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum were purchased from Invitrogen Life Technologies (Grand Island, NY). **Tween 80**, poly-D-lysine, and HEPES buffer were purchased from Sigma-Aldrich (St. Louis, MO). Glass coverslips (12-545-101), NaCl, KCl, $MgCl_2$, NaOH, and KOH were purchased from Fisher Scientific (Houston, TX). Glass capillary tubes (7052 or 8520 glass) were purchased from Garner Glass Co. (Claremont, CA) or World Precision Instruments (Sarasota, FL). All solutions were prepared using ultrapure water obtained from a Millipore® Milli-Q water purification system (Billerica, MA).

Cell Culture

COS-1 cells (ATTC #CRL-1650) were cultured in DMEM with 10% fetal bovine serum on poly-D-lysine-coated glass coverslips to 20% confluence at 37 °C in an incubator with an atmosphere comprising 5% CO_2.

Electrophysiology

Ionic currents were recorded across excised patches in 'inside-out' configuration with patch clamp techniques using a Dagan 3900 patch clamp (Minneapolis, MN). Glass capillary tubes were pulled using a Sutter Instrument P-87 pipette puller (Novato, CA) and fire-polished to produce micropipettes with a final resistance of 1–3 MΩ in the test solutions. An agarose-based salt bridge was used as a reference electrode. An ITC-16 data acquisition interface with accompanying acquisition and analysis software (Pulse; HEKA Electronik, Lambrecht, Germany) were used to record currents which had been filtered at 2 kHz and digitized at 50 μs/point. Current was recorded using a pulse sequence that stepped from -100 mV to 80 mV in 20 mV increments from a holding potential of 0 mV. Each voltage pulse was applied over an interval of 100 ms with an interpulse interval of 1 s. The bath solution (4 mL) was composed of either 150 mM NaCl, 1.5 mM $MgCl_2$, and 10 mM HEPES (Solution A), or 150 mM KCl, 1.5 mM $MgCl_2$, and 10 mM HEPES (Solution B). The same solution as used for the bath was used for the pipette. In some cases, a concentration gradient was established across membrane patches using salt solutions of the following compositions: 27 mM NaCl, 123 mM KCl, 1.5 mM $MgCl_2$, and 10 mM HEPES (Solution C); or 123 mM NaCl, 27 mM KCl, 1.5 mM $MgCl_2$, and 10 mM HEPES (Solution D). If Solution C comprised the bath solution, Solution D was employed as the pipette solution, and vice-versa. All solutions were adjusted to pH 7.3 with NaOH or KOH, and all experiments were performed at room temperature (22–25 °C).

The stability of freshly excised membrane patches was evaluated by subjecting the patches to the above pulse sequence once every 0.5–2 minutes for a minimum of 15 minutes and monitoring changes in the magnitude of the average currents recorded at -100 mV and 80 mV. After patch stability was verified, the membrane modifying agent was then added to the bath solution and application of the pulse sequence was continued at the same frequency until the current reached a magnitude that was equivalent to the current across a patch-free pipette (usually ≥ +/- 20 nA), or if more than 60 minutes had elapsed since obtaining the membrane patch. Representative traces are shown in the corresponding figures (*vide infra*). For all patches that showed suitable stability upon patch excision, replication of the patch-clamping experiments was conducted with the results as summarized in Table S1.

Fluorescence Microscopy

Fluorescence images were acquired using an automated Olympus IX81 using an Olympus LUCPlanFLN 40x/0.6NA objective and a Sedat 89000 Quad Fluorescence Filter Set (Chroma Technology Corp, Bellows Falls, VT, USA) applying a FITC filter for excitation (490/20x) and emission (525/36nm) (#8900, Semrock, Lake Forest, Il, USA). Images were recorded using a Hamamatsu ImagEM CCD Camera (#C9100-13, Hamamatsu Corporation, Bridgewater, NJ) controlled by MetaMorph Software (Molecular Devices, Sunnyvale, CA, USA), and processed using Fiji open source software.[5] Time-lapse images were taken at 4 s intervals for the first 6 minutes of acquisition and at 60 s intervals thereafter.

Tables and Figures

Table S1. Replication of Patch-Clamp Recordings

Membrane Modifier	Concentration [μM]	Comments on Replication
none (control patch)	N/A	7 out of 7 patches maintained low conductance (< |300| pA) for at least 60 minutes from the time at which the patch was first excised from the cell
DSSN+	5	7 out of 8 patches showed sustained nA-range current
	2.5	2 out of 3 patches showed sustained nA-range current
	12.5	2 out of 2 patches showed rapid patch rupture after COE addition
DSBN+	5	3 out of 4 patches showed increasing patch current and rupture shortly after COE addition
COE1-5C	5	6 out of 6 patches showed increasing patch currents shortly after COE addition
DMI	5	2 out of 2 patches remained unchanged for ≥ 15 minutes after addition of the modifier, followed by sudden patch rupture
Tween 80	5	2 out of 2 patches remained unchanged for ≥ 20 minutes after addition of the modifier, followed by sudden patch rupture

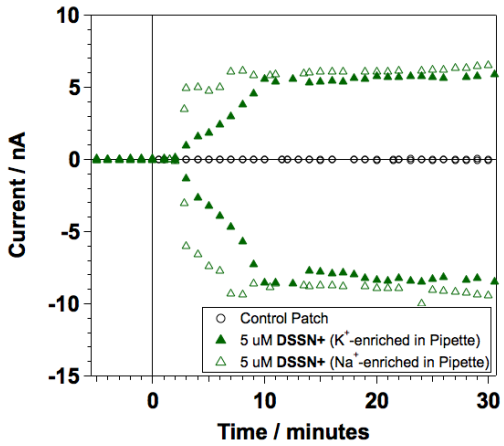

Fig. S1 Comparison of currents measured as a function of time across inside-out membrane patches at -100 mV (negative currents) and +80 mV (positive currents) of applied potential after addition of 5 µM **DSSN+** (addition occurred at t = 0 minutes) to the mixed NaCl- and KCl-containing bath solutions.

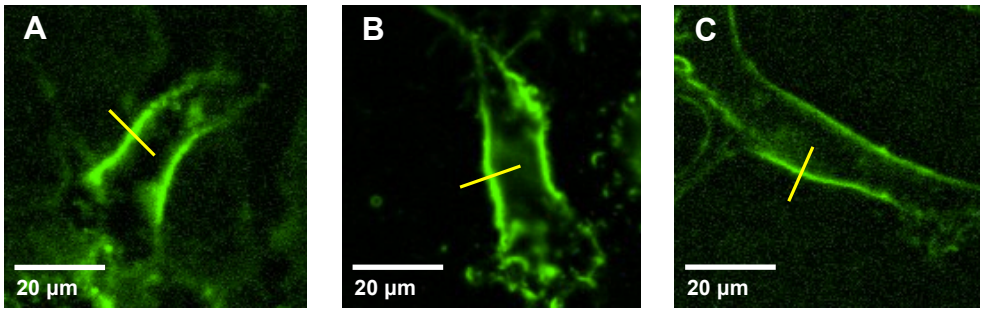

Fig. S2 Fluorescence micrographs of COS-1 cells stained with 5 µM of each of: (A) **DSBN+**; (B) **DSSN+**; and (C) **COE1-5C**, captured at the end of time-lapse experiments. The yellow line in each image indicates the location from which emission intensity data were extracted to generate the traces depicted in Fig. 5 of the manuscript.

References

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