Supporting information for: *Cyclodextrin Ion Channels*

Jonathan K.W. Chui and Thomas M. Fyles*

Voltage Clamp Data Acquisition

A model BC-525A bilayer clamp (Warner Instrument Corp.) was used for planar bilayer experiments. The analogue output was filtered with an 8-pole Bessel filter (Frequency Devices, model 902) and digitized with a 330 kHz digitizer (Axon Instruments, Digidata 1200A). Data acquisition was controlled by the pClamp8 software package (Axon Instruments). Data were collected at 10 Hz, analogue filtered at 1 Hz, and digitally filtered at 50 Hz. The headstage and the bilayer chamber (3 mL polystyrene cuvette with 250 µm diameter aperture held in a 5 mL PVC holder) were placed on a floating table and electrically shielded by a grounded aluminum Faraday cage. Agar salt bridges (2 M KNO3 in 1% Agar) were used to stabilize junction potentials and were employed between the electrolyte in each well of the cell and Ag/AgCl electrodes. Electrolyte solutions were prepared from high purity salts and nanopure water.

A stock solution of diphytanoyl phosphatidylcholine (diPhyPC) in chloroform (Avanti Polar Lipids; shipped on dry ice) was divided into sealed glass vials under an argon atmosphere and stored at -12 °C. For use in an experiment, a stream of dry nitrogen was passed through the vial for 1 hour. The dried lipid was diluted with decane to give a solution concentration of 25 mg/mL in lipid.

Bilayers were formed by either brushing or dipping: after lipid in decane had been introduced by brushing, a lipid/ decane film formed on the surface of the electrolyte, and bilayers could then be formed by withdrawal of 2-3 mL of electrolyte from the cell holder by syringe to expose one face of the aperture to the air-water interface held in the cell holder, followed by reintroduction of the electrolyte to oppose monolayers across the aperture in the cuvette. Bilayer quality was monitored via the capacitance and stability under applied potential, using the criteria previously described[1]. The measured voltage was applied with respect to the *trans* (cuvette) side of the bilayer, making the *trans* side the relative ground. Digitized data files were analyzed using the pClamp10 suite of programs.

The compounds are introduced to the membrane in two ways, depending on the solvent in which the compound can be dissolved:

Direct injection - all injection experiments utilized bilayers that were apparently stable at 100 mV for periods of 20 minutes or more. Aliquots (1-5 µL of transporter solutions in MeOH were injected with a microliter syringe as close as possible to the bilayer in the free well of the cuvette holder (*cis* side), and gently stirred with a stream of nitrogen for 5 minutes.

Pre-mixed into lipid - in this method, 1mol% of compound (in $CDCl_3$ or MeOH-d_4) was added to the diPhyPC/$CHCl_3$ solution, and solvent removed with a stream of N_2, and bilayer membrane prepared by brushing/dipping as described above. Most of the bilayers formed with this method gave bilayers with good quality.

Of the two methods, direct injection is preferable, as it allows monitoring of pristine bilayer prior to compound introduction. Following direct injection, channel behaviour typically appears within 20

minutes of compound introduction, and persists over period of hours. Once stabilized, continuous data acquisition of at least 30-60 minutes is required to provide sufficient statistical power for the power-law analysis; shorter acquisition periods (10 minutes) are sufficient to characterize other types of behaviors.

Power Law Fitting Procedure

Fitting experimental data to a power law requires two distinct steps. The first step transforms the irregular current trace into a list of opening times; this list is then fitted to a power law distribution.

Event List Generation Manipulation of the digitally filtered traces was carried out using Clampfit 10 of the pClamp suite. A customized threshold search was used to generate the list of events. The threshold was set across the fluctuating section of the trace to maximize the number of events. Within that segment, α is insensitive to the choice of threshold. A minimum duration was fixed at 50ms. The threshold search automatically logs *event start* and *event end* fromwhich the duration can be calculated. The resultant values were exported to the fitting program.

Power Law Fitting The list of opening durations, obtained above as a plain-text file, can then be fitted using the method of Clauset *et al*[2], implemented in python[3]. The code performs the Maximum Likelihood Estimate fit, and provides α, x_{min}, n, and p-value as outputs.

Summary of bilayer activity

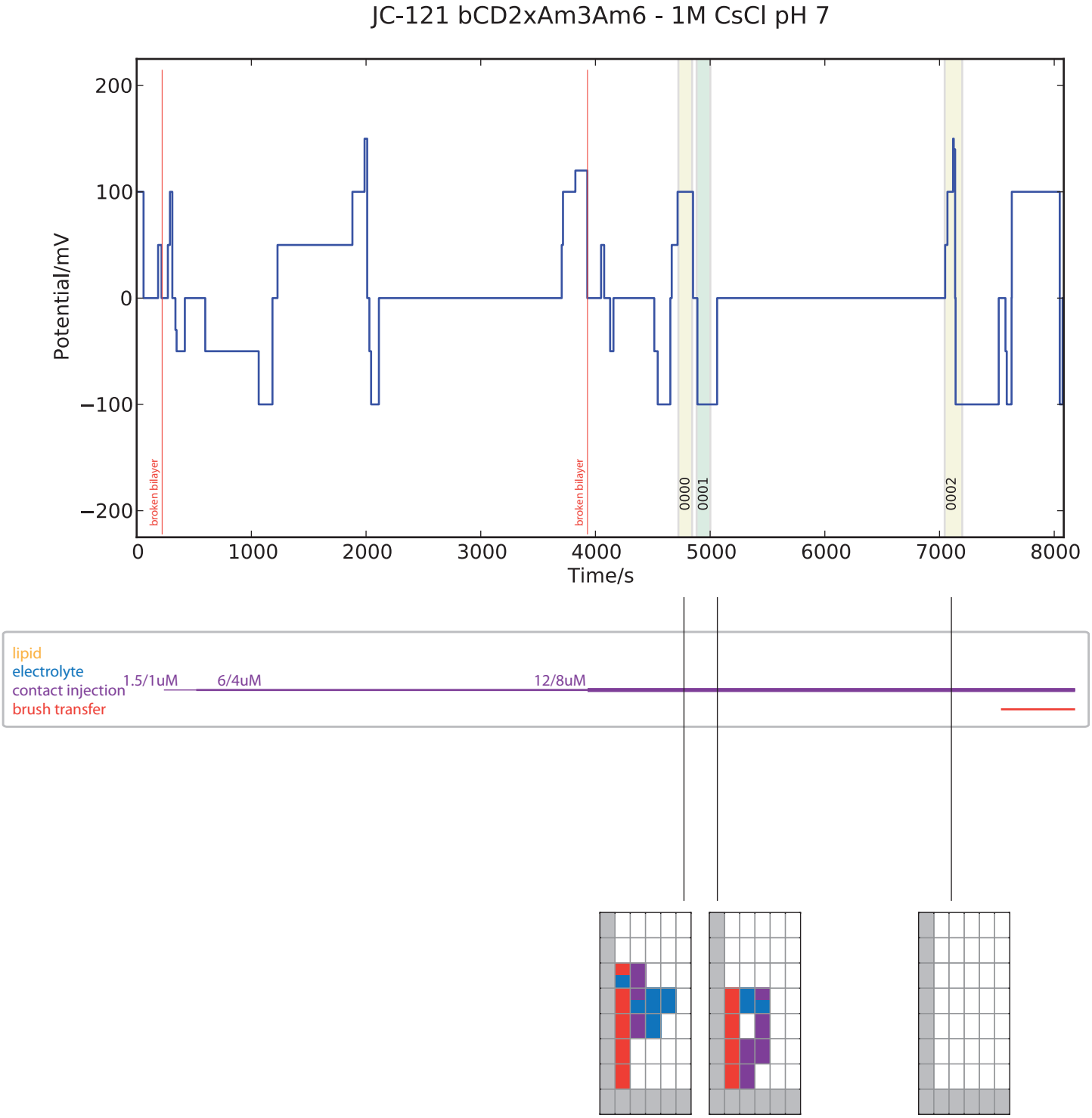
Annotated activity grids, as well as full conductance records (and expansions where appropriate), are provided below for every compound studied. The activity grids were prepared as previously described[4]. The summaries are arranged first by compound, then individual experiments. Within each experiment, the first page(s) summarizes the experimental conditions as well as activity grids charted; subsequent pages shows the full conductance record as the top panel, with expansions indicated by corresponding letters.

1. Fyles, T. M.; Knoy, R.; Müllen, K.; Sieffert, M., Membrane activity of isophthalic acid derivatives: ion channel formation by a low molecular weight compound. *Langmuir* **2001,** *17*, 6669-6674.

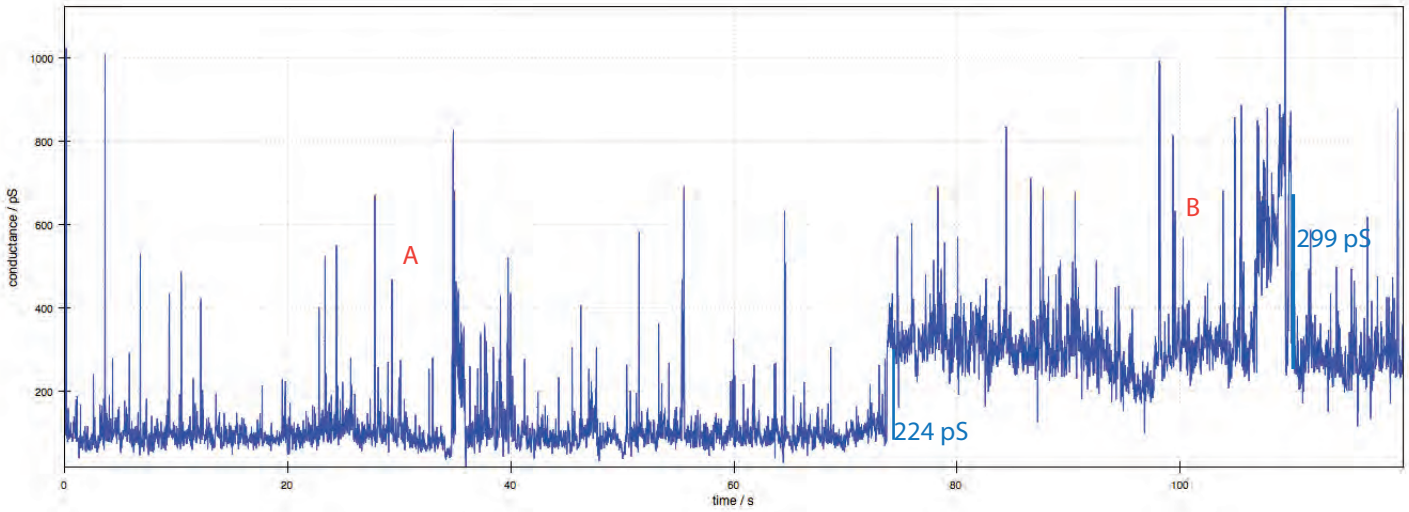
2. Clauset, A.; Shalizi, C. R.; Newman, M. E. J., Power-law distributions in empirical data. *SIAM Rev.* **2009,** *51*, 661-703.

3. Ginsberg, A. <http://code.google.com/p/agpy/wiki/PowerLaw> (accessed November 7).

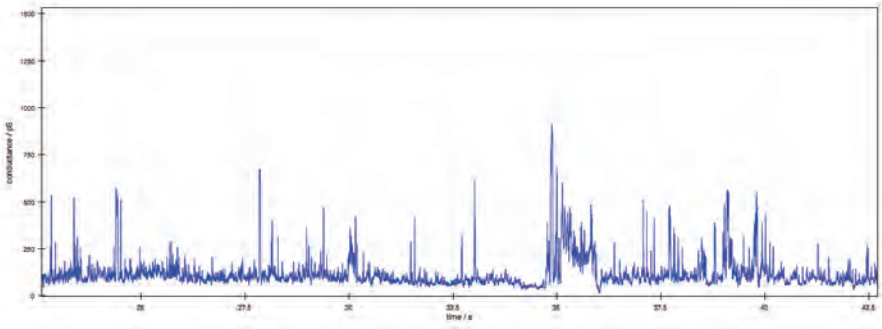
4. (a) Chui, J. K. W. A New Paradigm for Voltage-Clamp Studies of Synthetic Ion Channels. University of Victoria, Victoria, 2011; (b) Chui, J. K. W.; Fyles, T. M.; Luong, H., Planar bilayer activities of linear oligoester bolaamphiphiles. *Beilstein J. Org. Chem.* **2011,** *7*, 1562-1569.


JC-121 bCD2xAm3Am6 - 1M CsCl pH 7
Potential/mV
200
100
0
−100
−200
0
1000
2000
3000
4000
5000
6000
7000
8000
Time/s
broken bilayer
broken bilayer
0000
0001
0002
lipid
electrolyte
contact injection
brush transfer
1.5/1uM
6/4uM
12/8uM

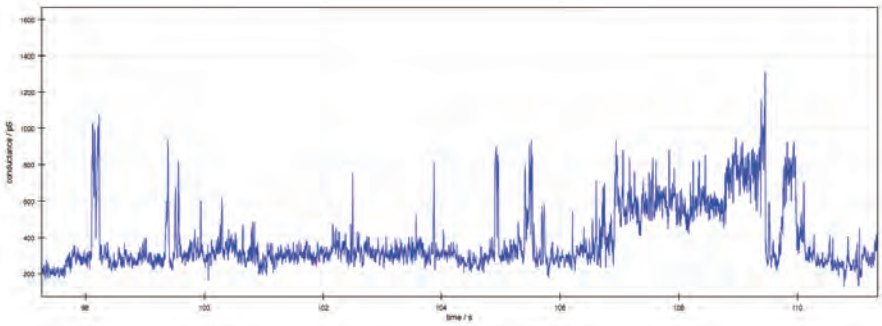
121-0000


conductance / pS
time / s
A
B
224 pS
299 pS

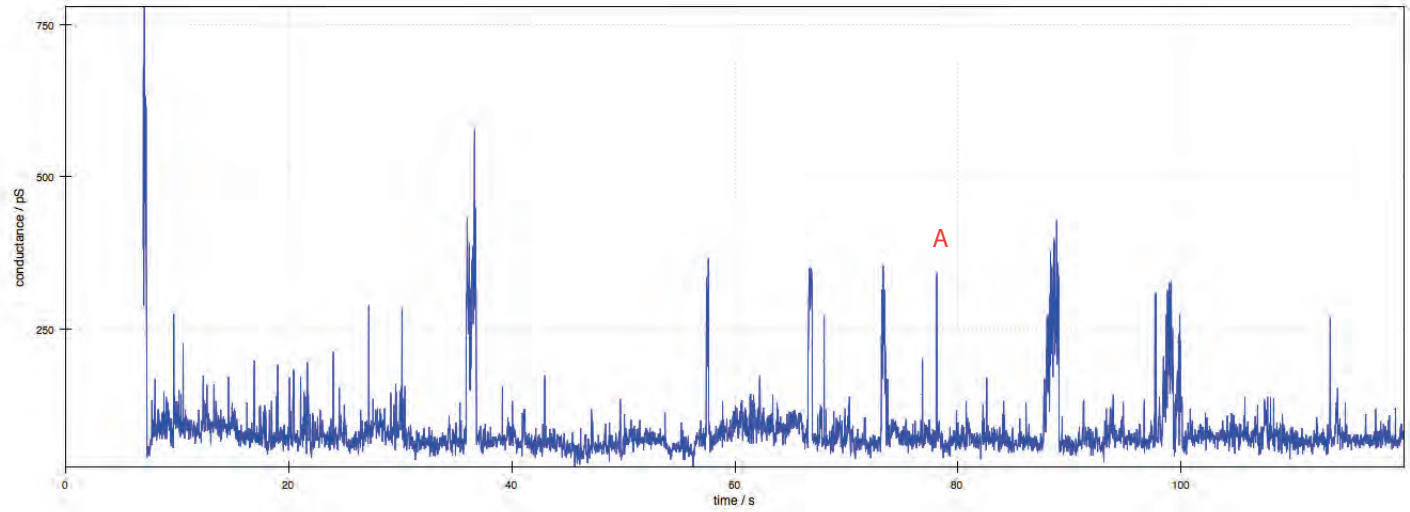
A


conductance / pS
time / s

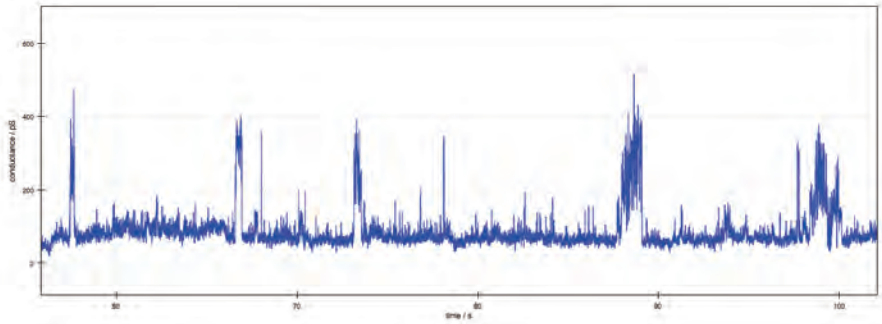
B


conductance / pS
time / s

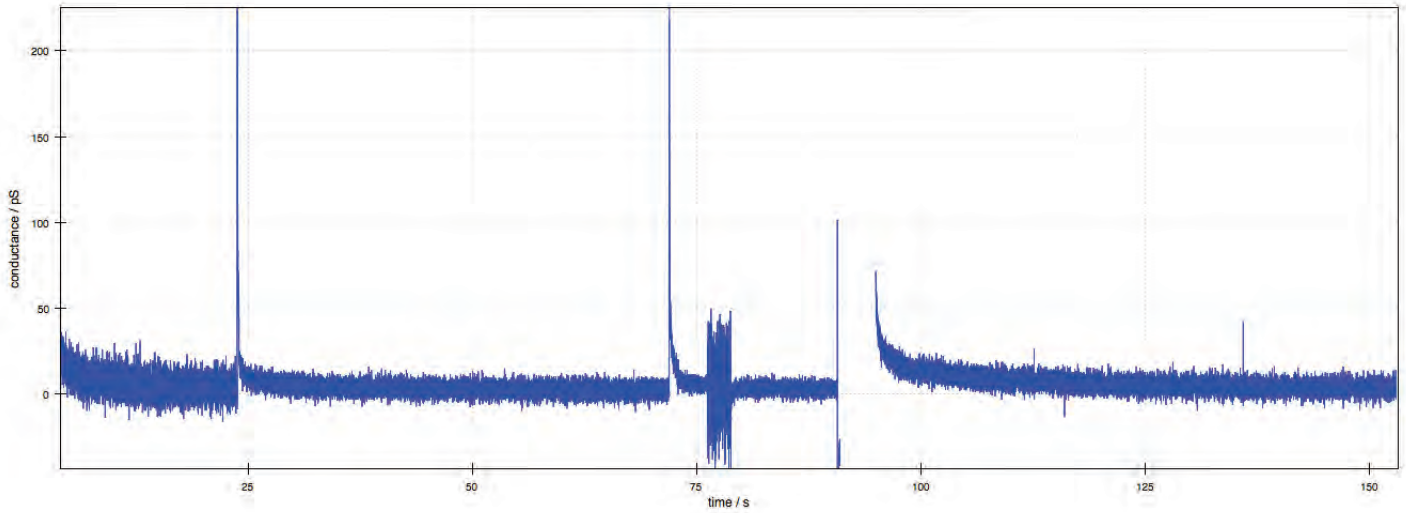
121-0001

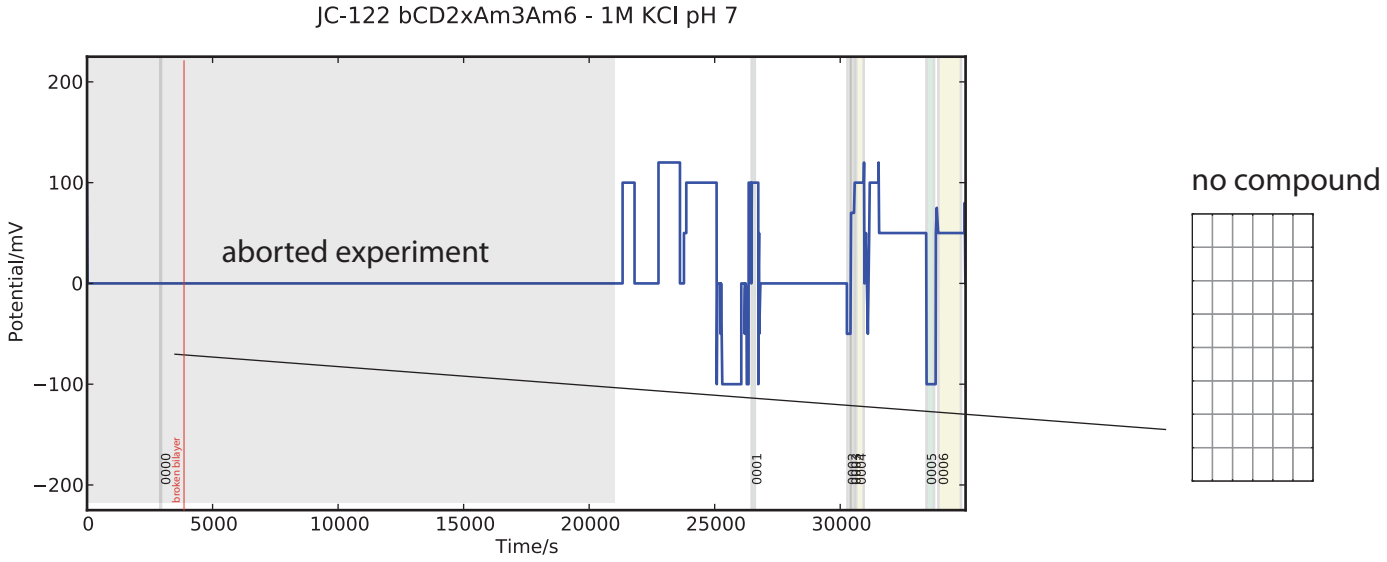


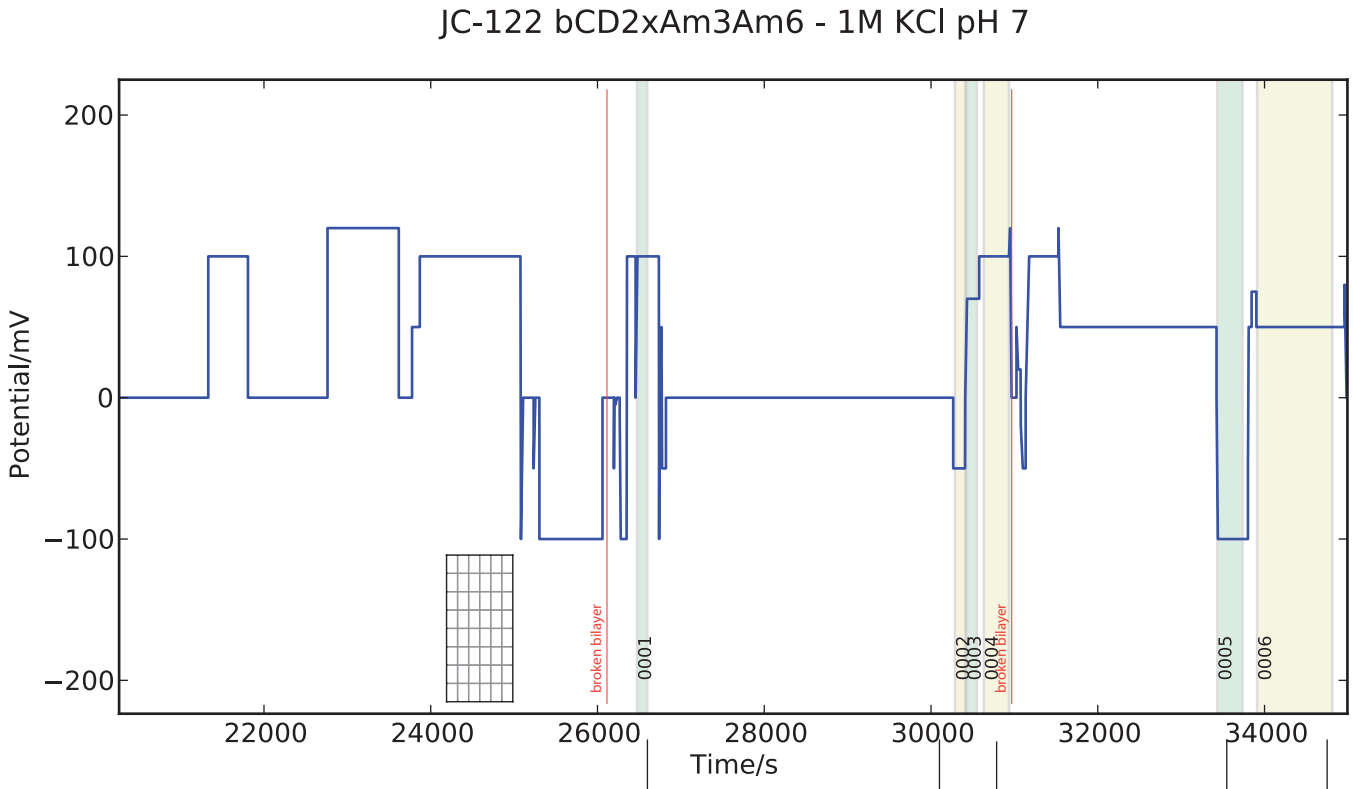
A

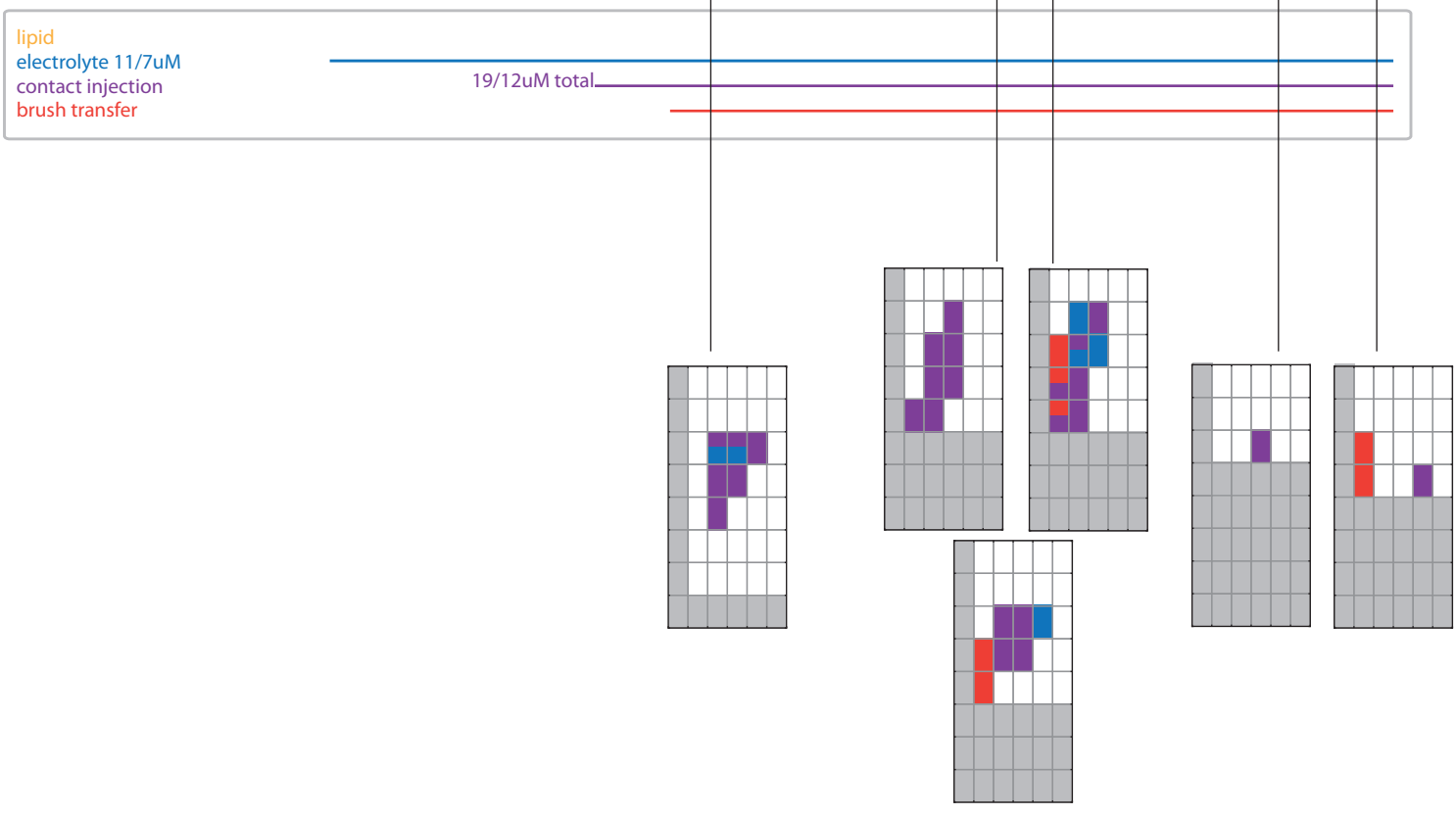


121-0002

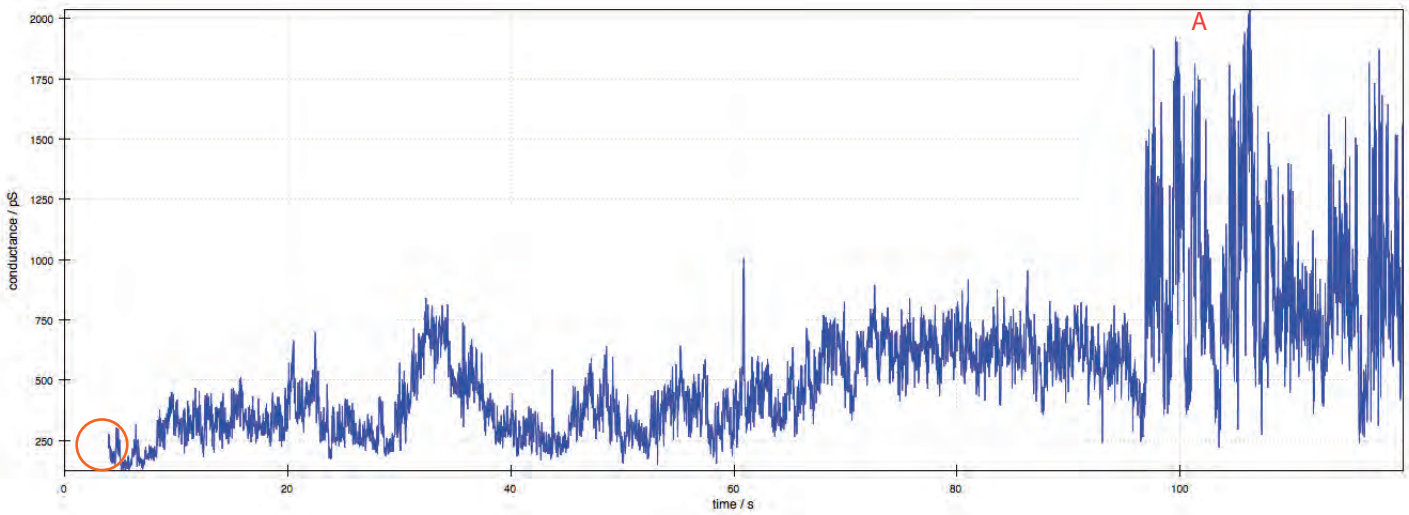



JC-122 bCD2xAm3Am6 - 1M KCl pH 7
aborted experiment
no compound
Potential/mV
Time/s
broken bilayer
0000
0001
0005
0006

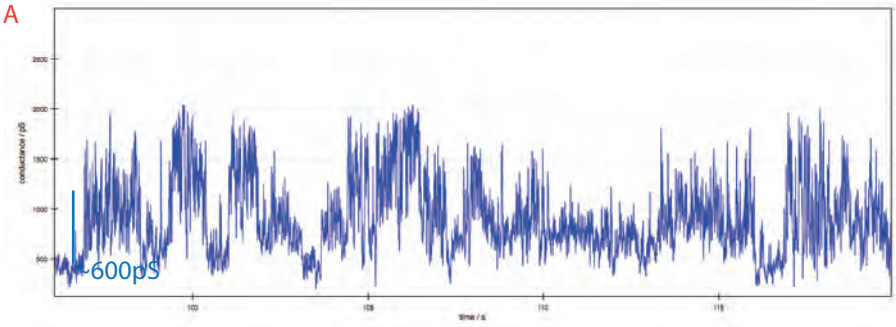

JC-122 bCD2xAm3Am6 - 1M KCl pH 7
Potential/mV
Time/s
broken bilayer
0001
0002
0003
0004
broken bilayer
0005
0006


lipid
electrolyte 11/7uM
contact injection
brush transfer
19/12uM total

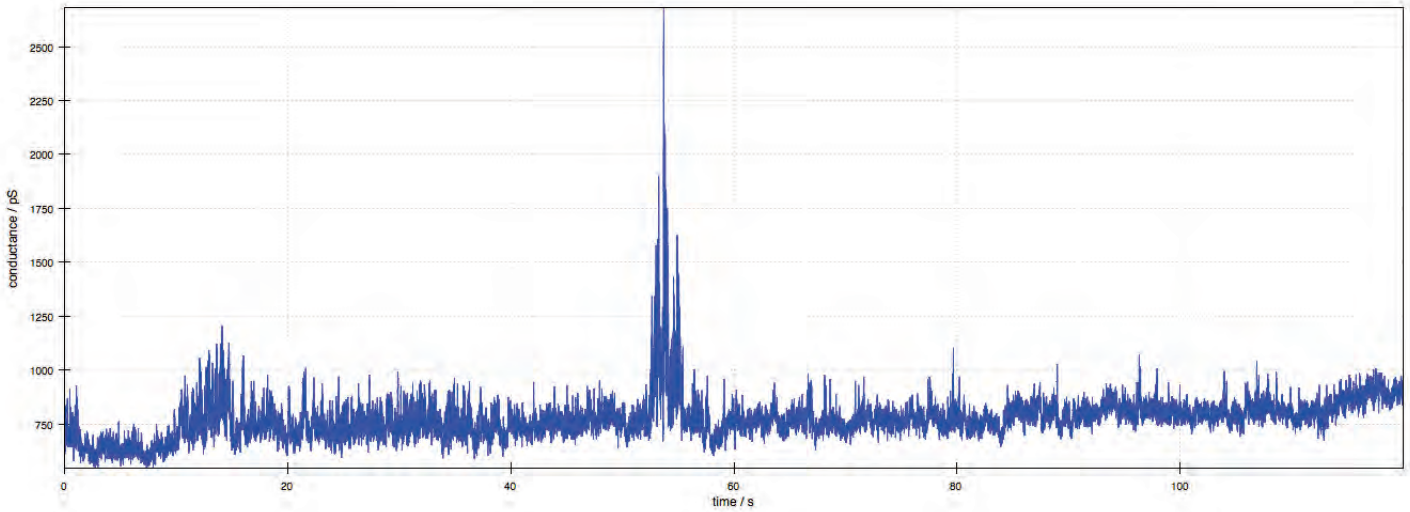
122-0001


A
conductance / pS
2000
1750
1500
1250
1000
750
500
250
0
20
40
60
80
100
time / s

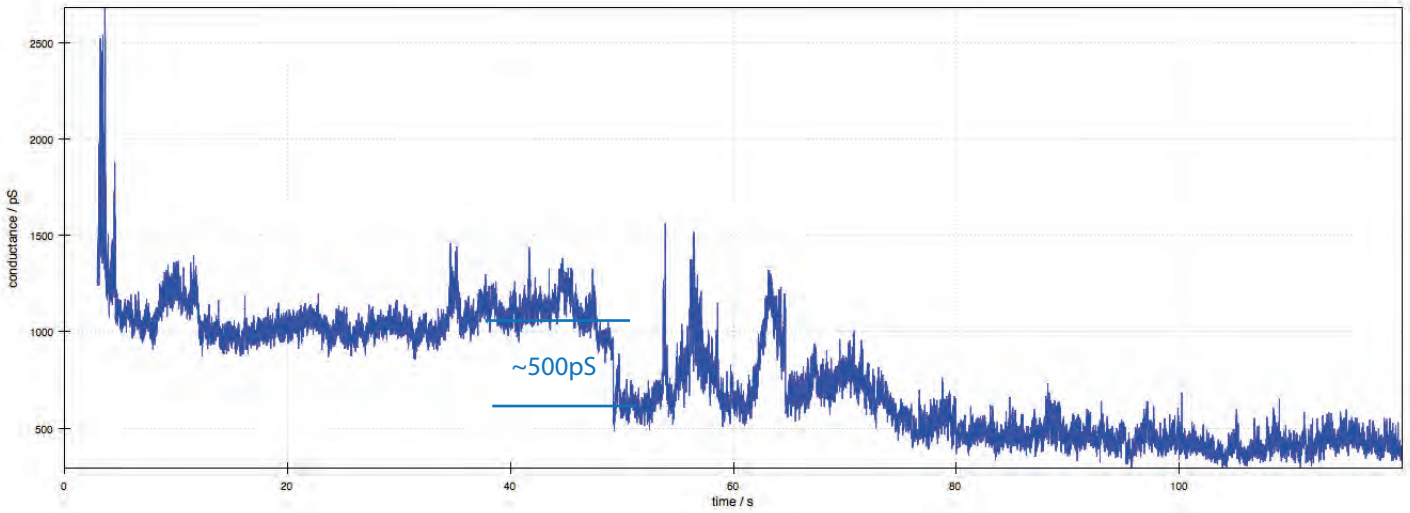
A


~600pS

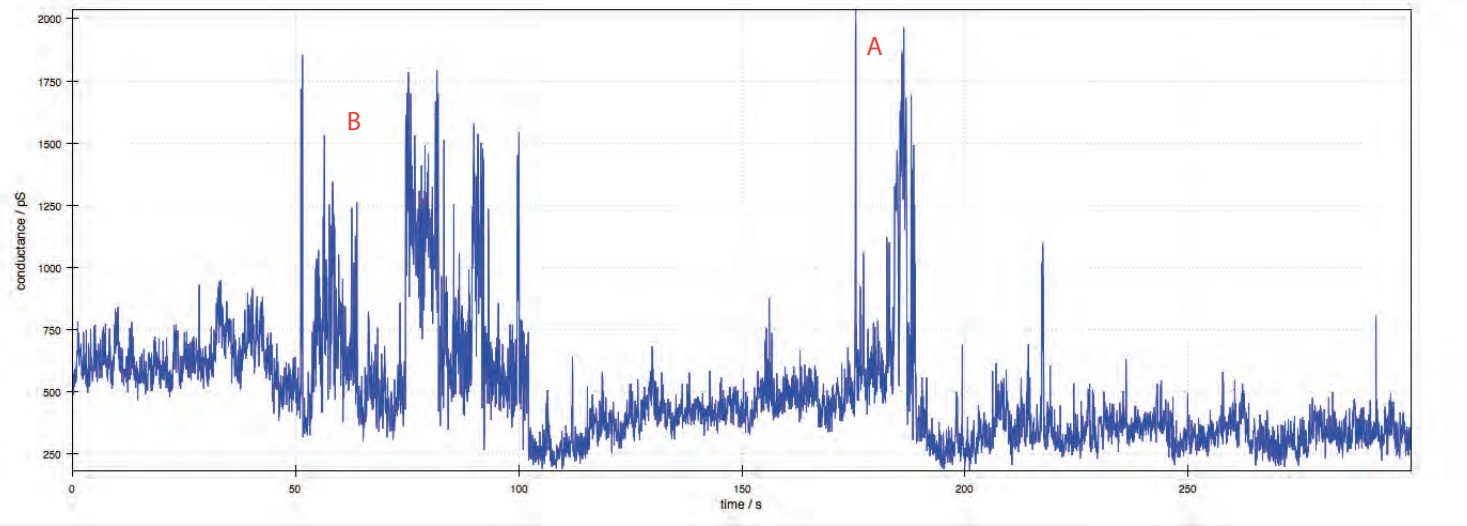
122-0002



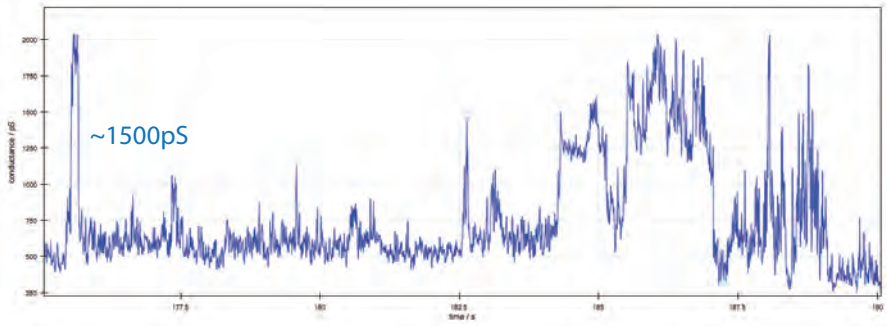
122-0003



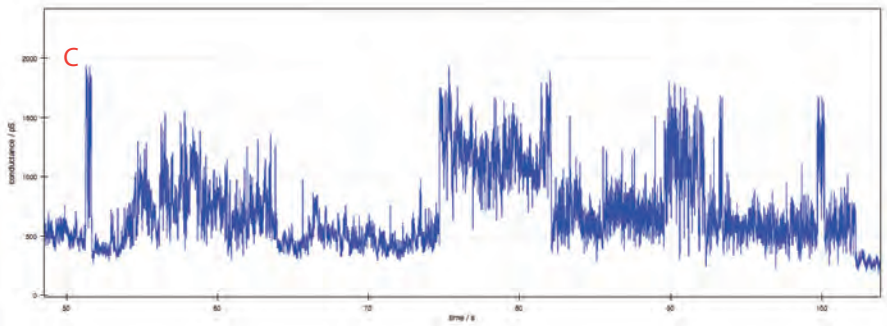
122-0004



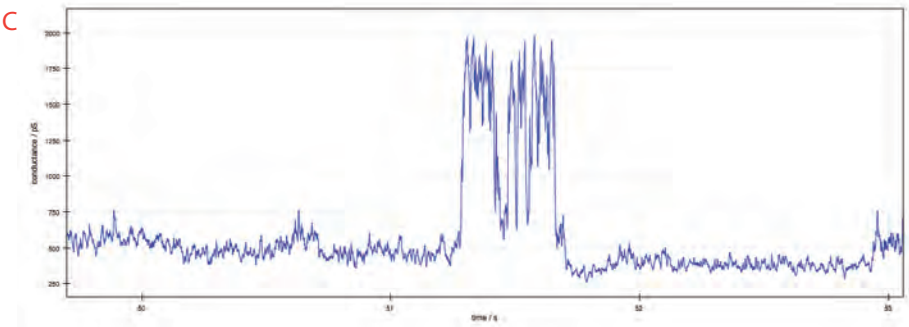
A



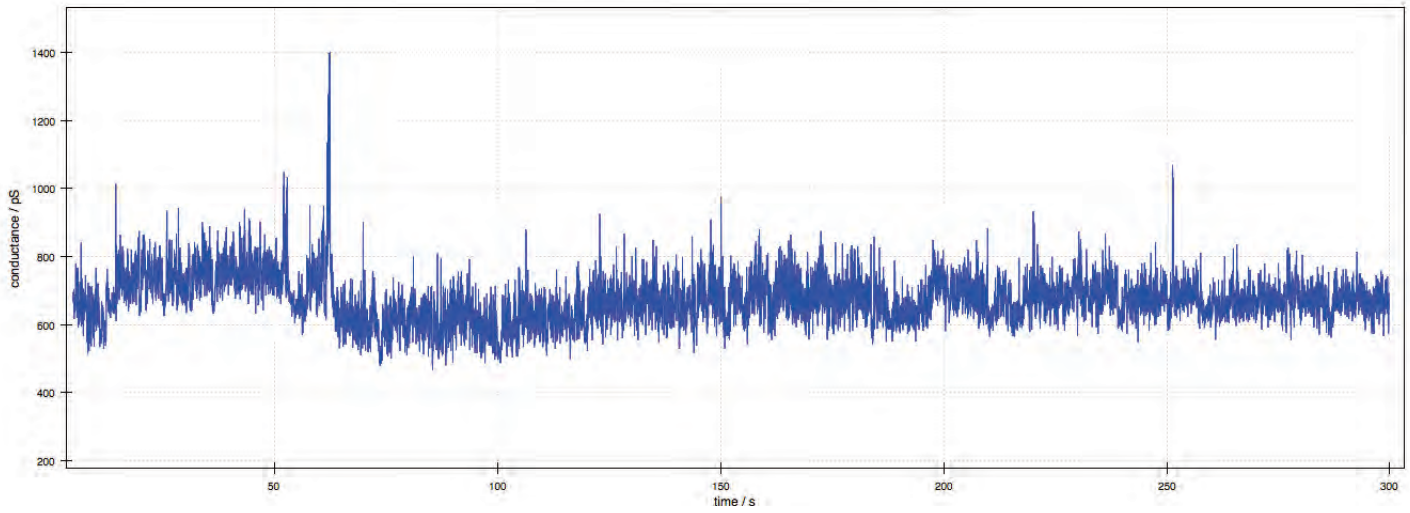
B



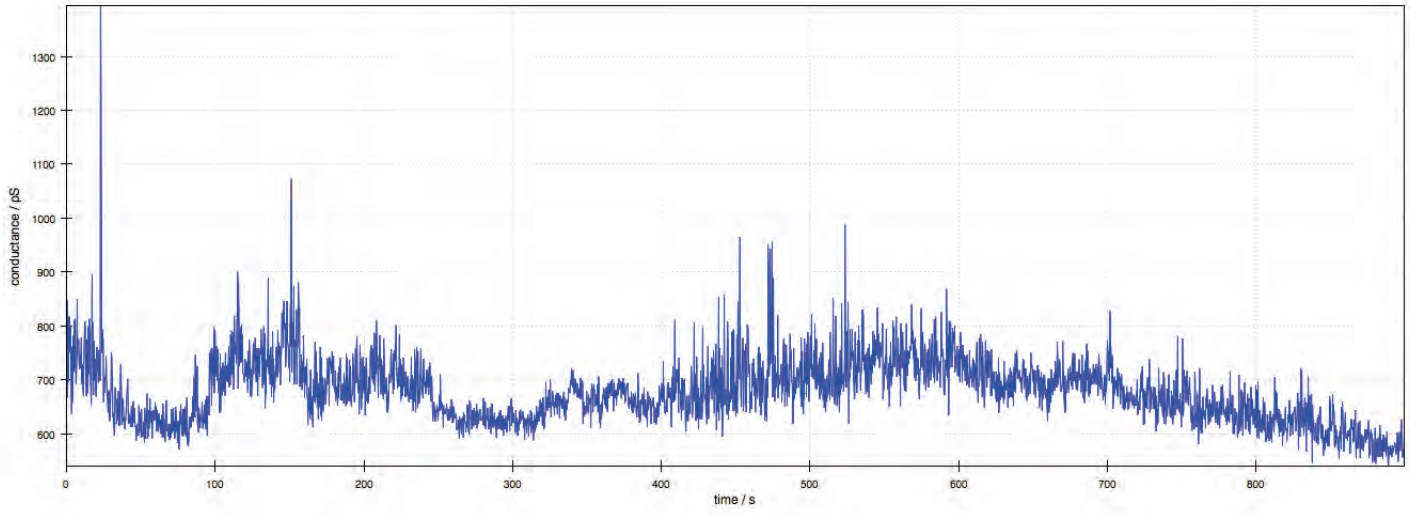
C

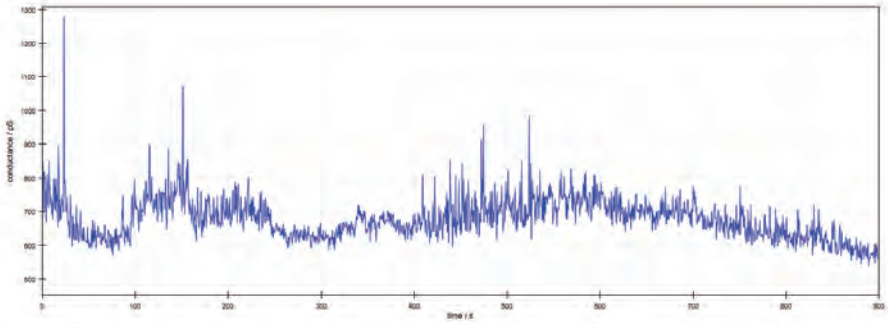


122-0005

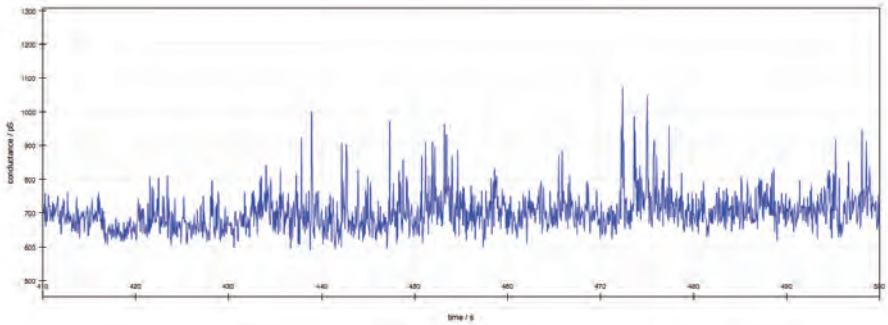


122-0006

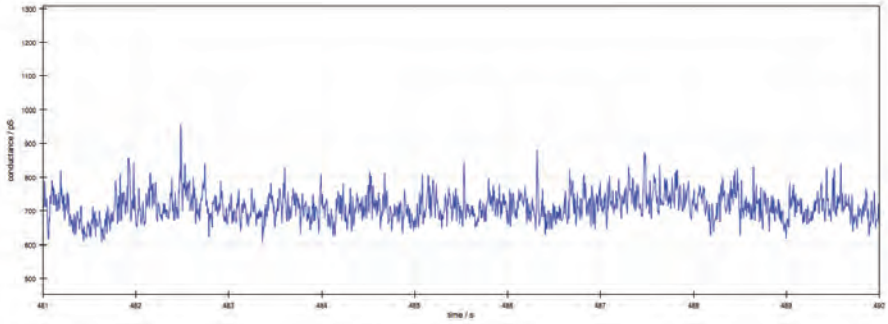




1000-fold reduction

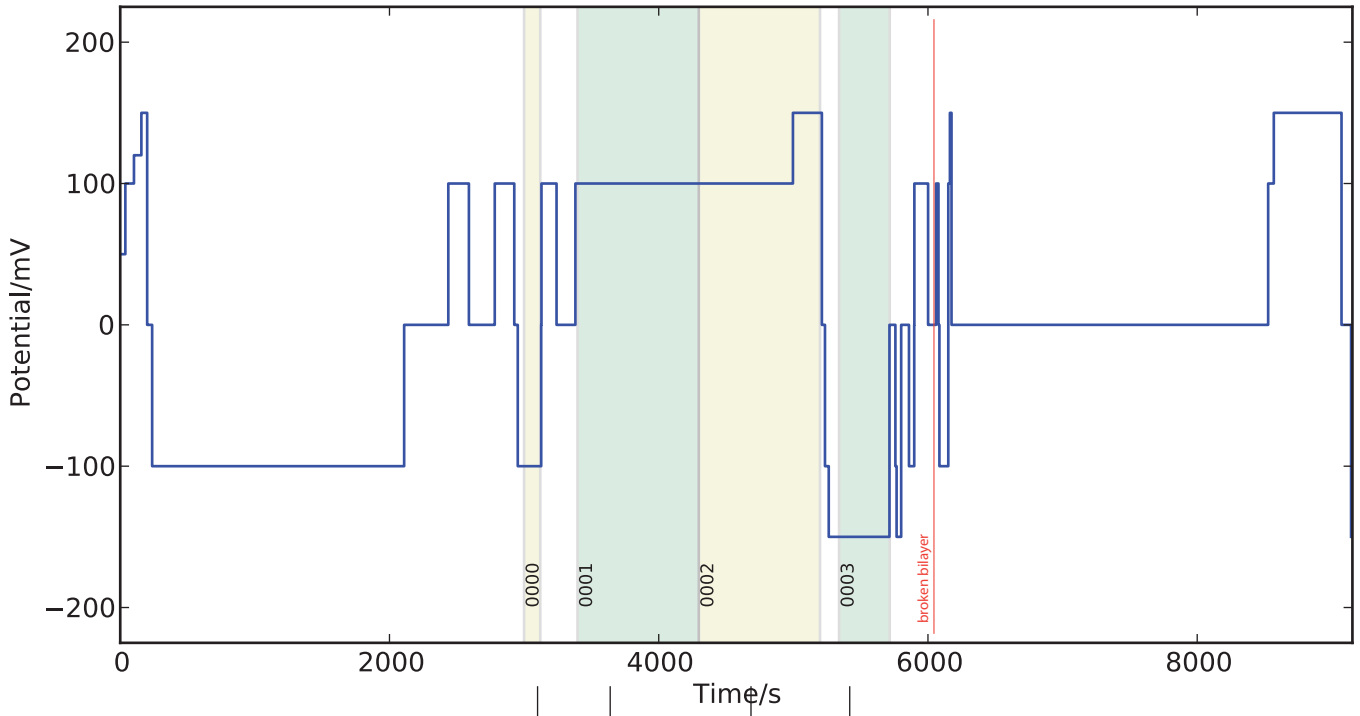


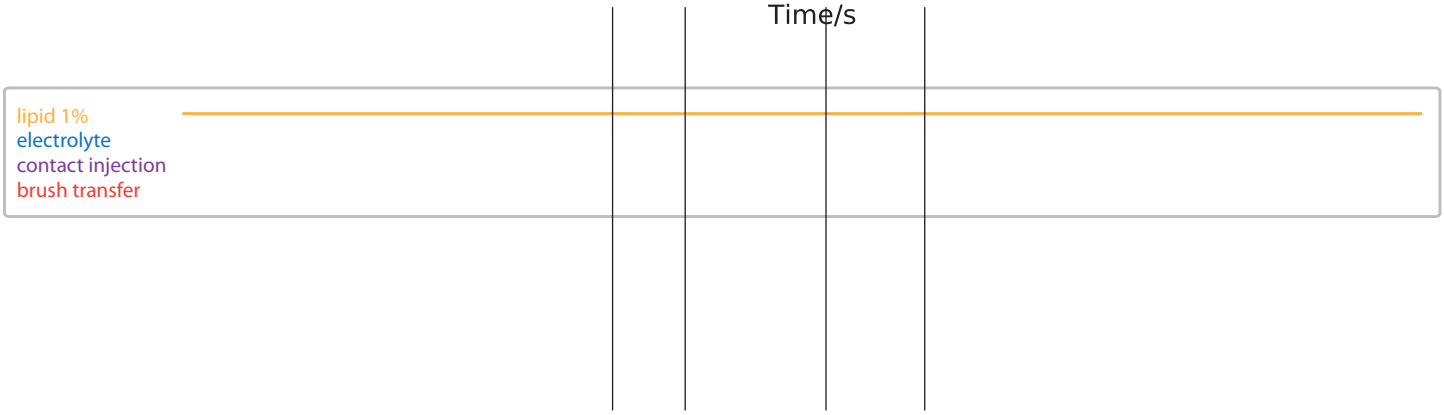
100-fold reduction

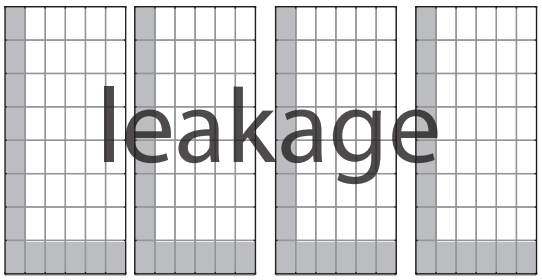


10-fold reduction

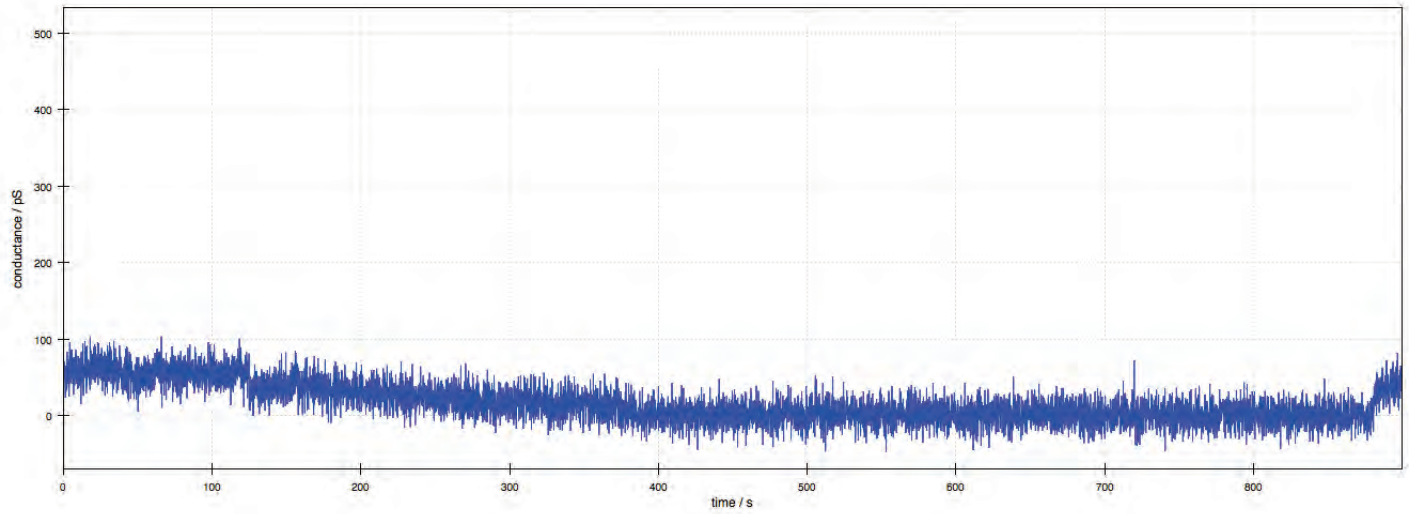
JC-123 bCD2xAm3Am6 - 1percent - 1M KCl pH 7



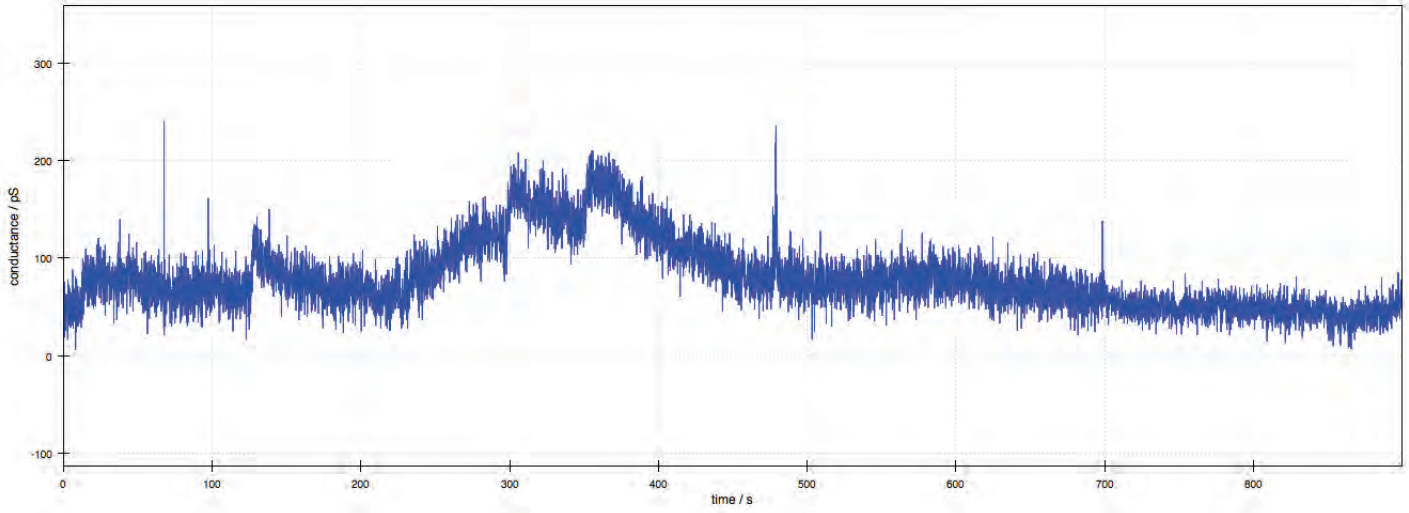




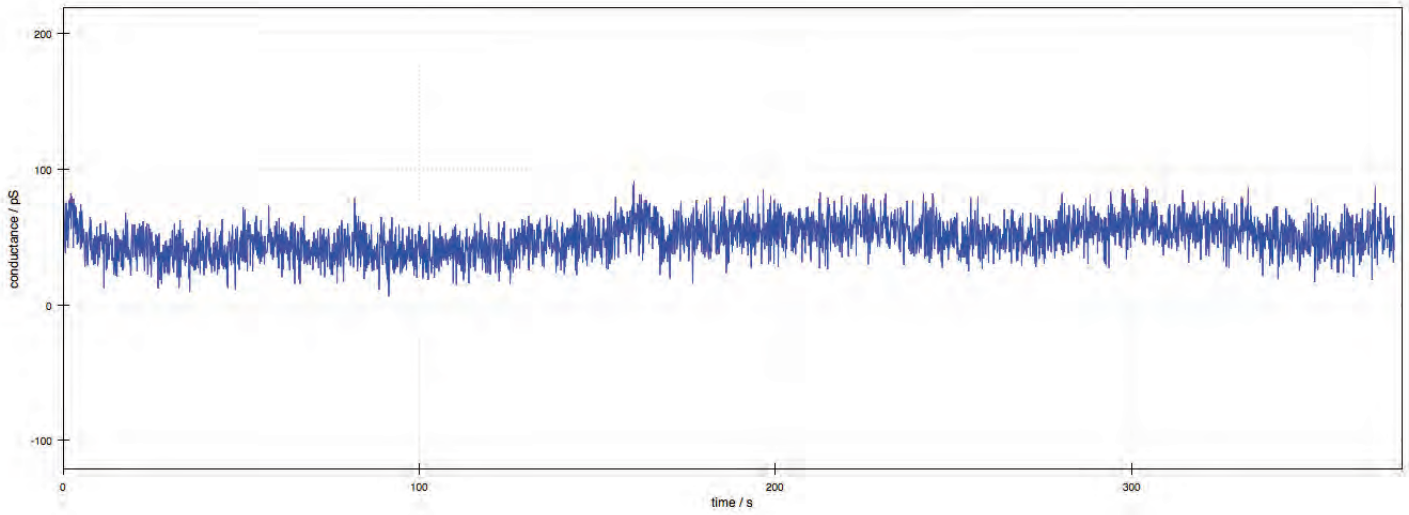
123-0001



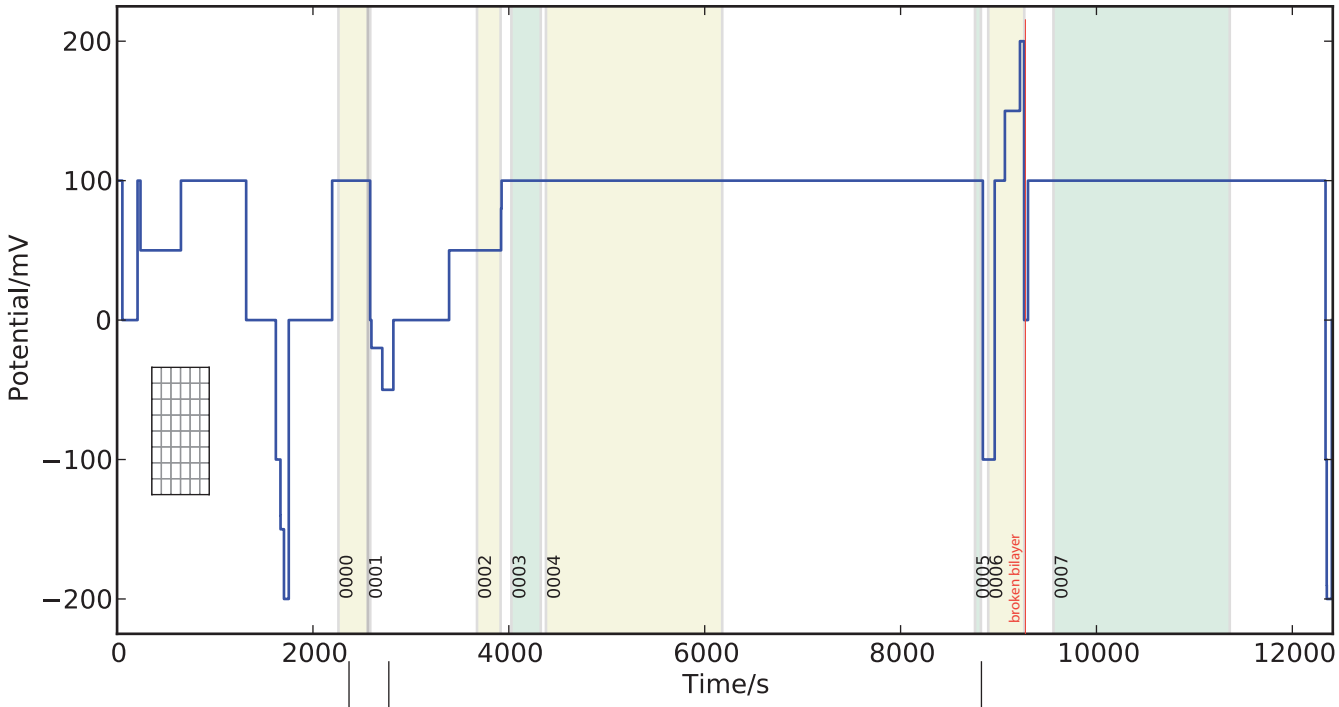
123-0002

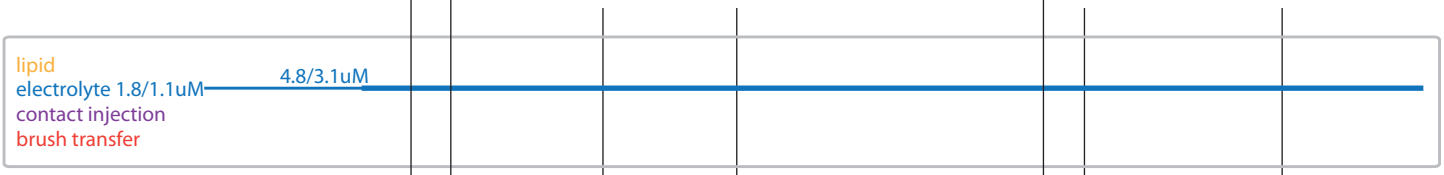


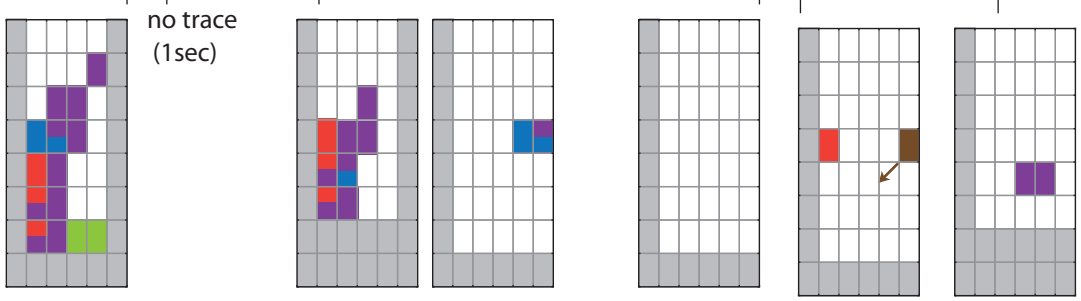
123-0003

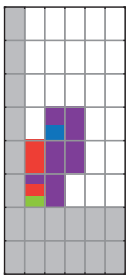


JC-118 bCD2xAm3OMe - impure - 1M CsCl



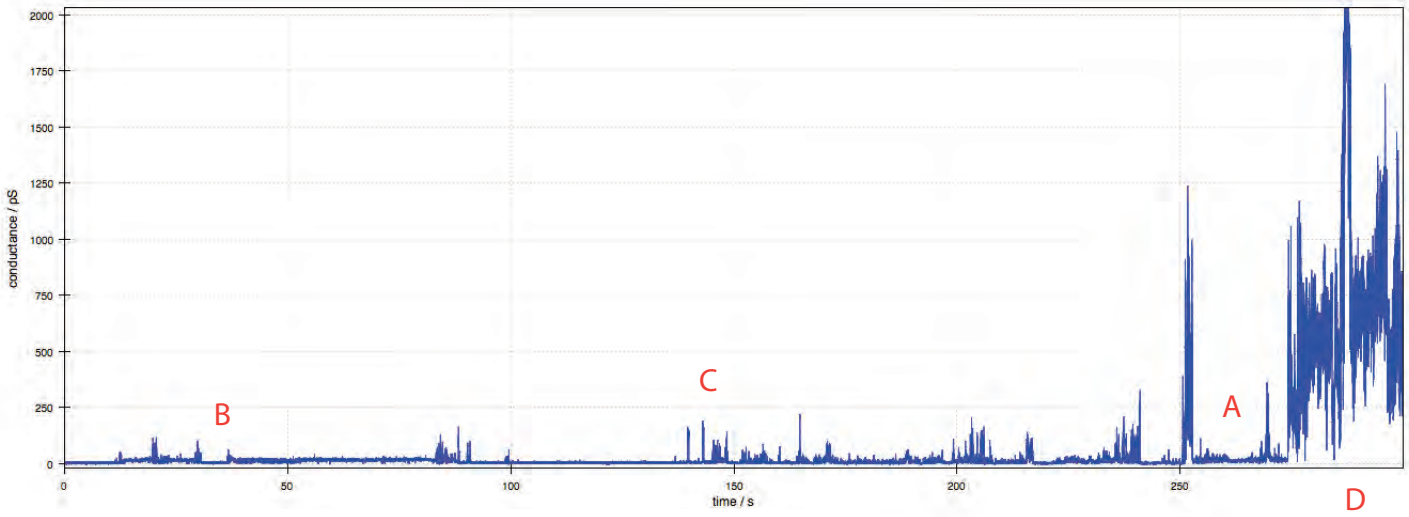




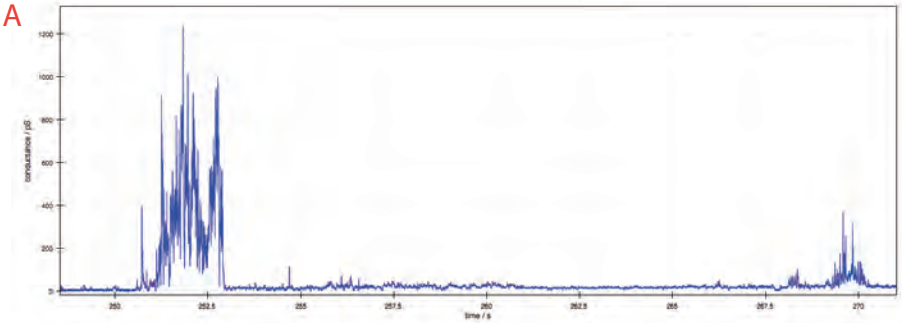


sample chromatographed
but not entirely clean by NMR

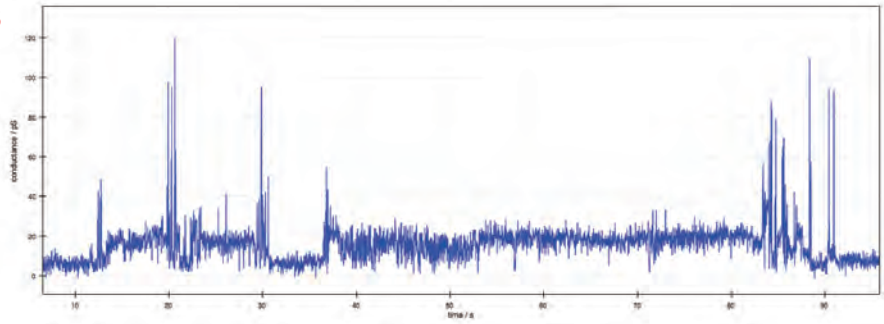
118-0000



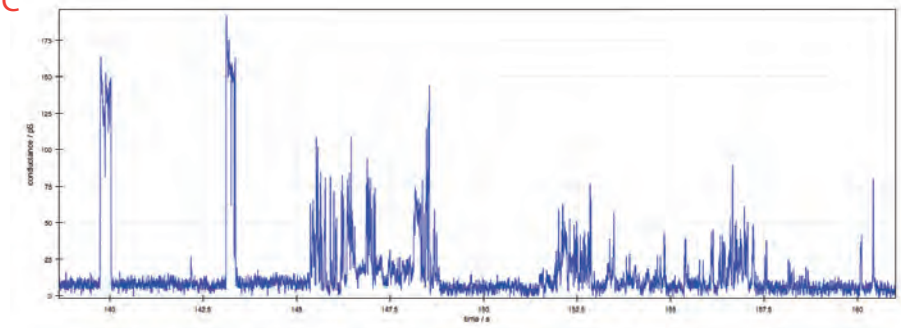
A



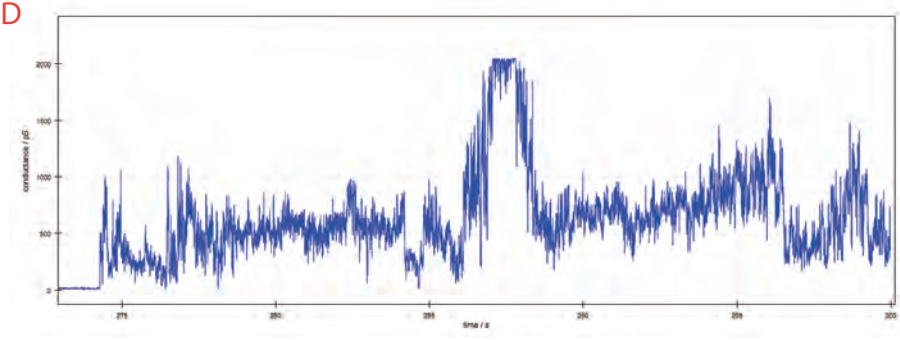
B



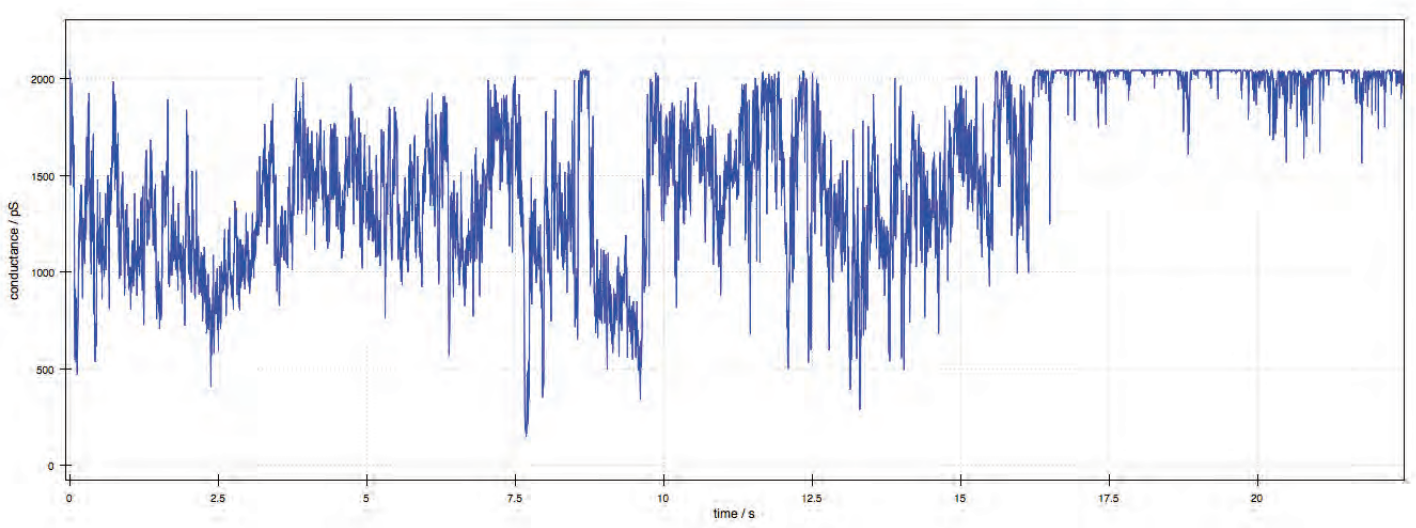
C



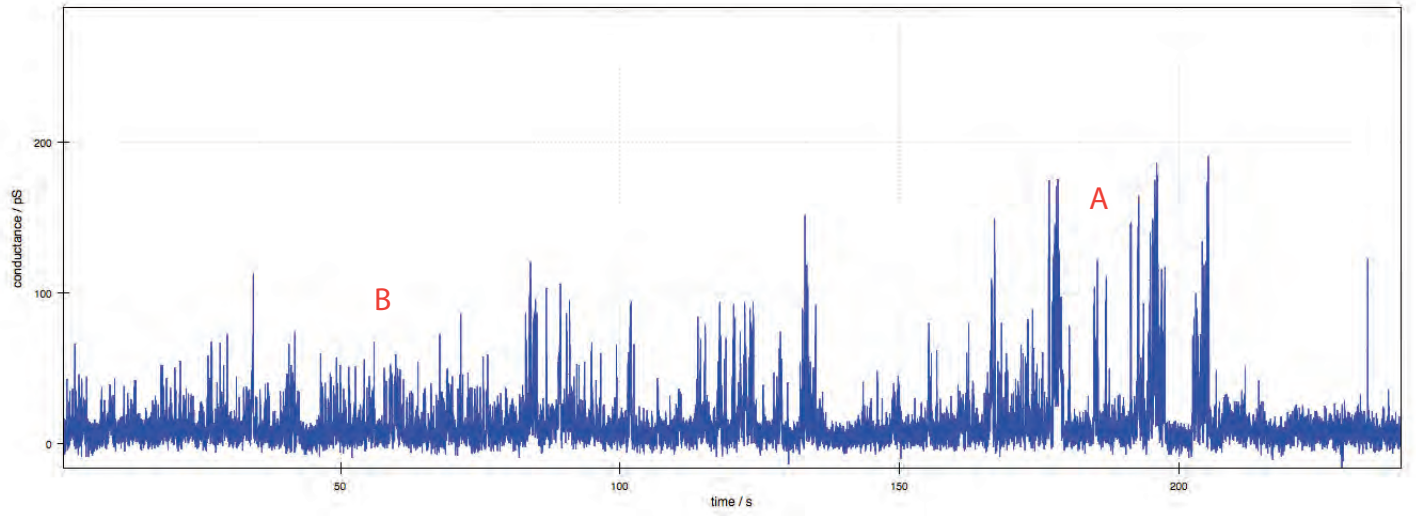
D



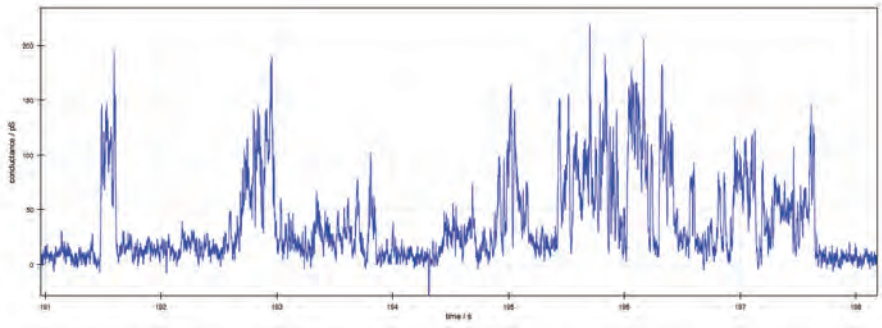
118-0001



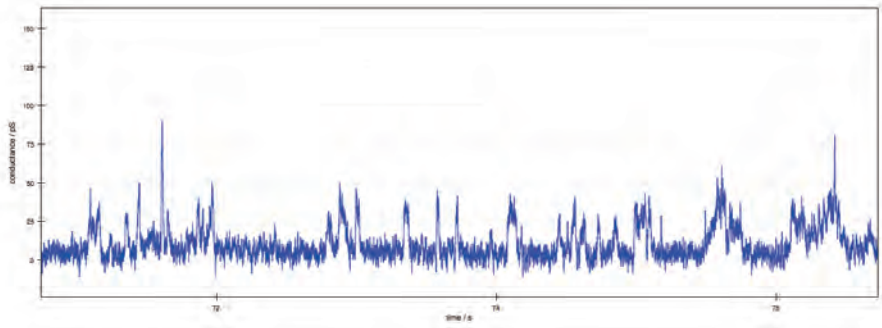
118-0002



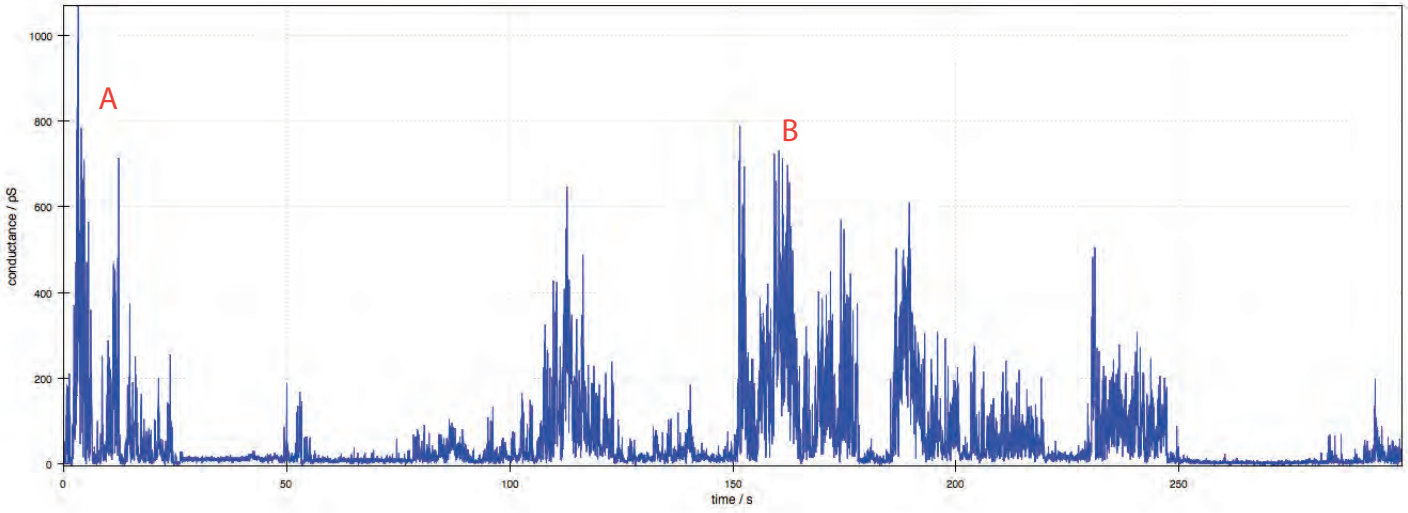
A



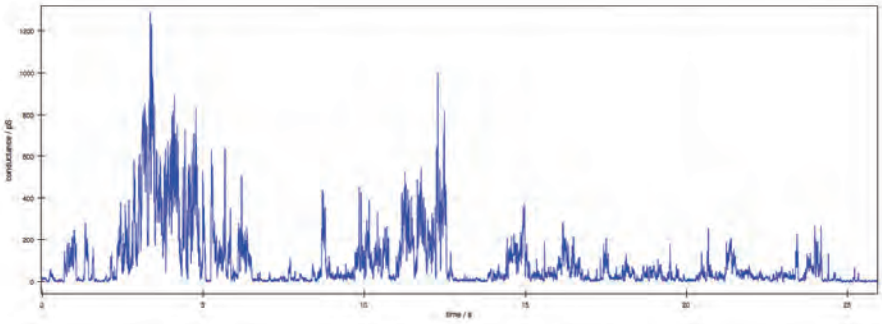
B



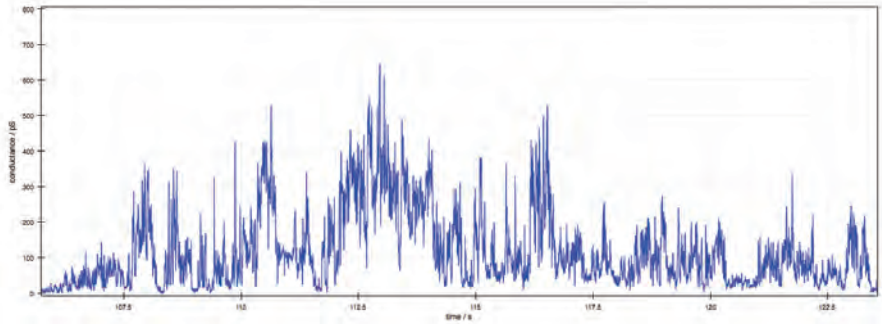
118-0003



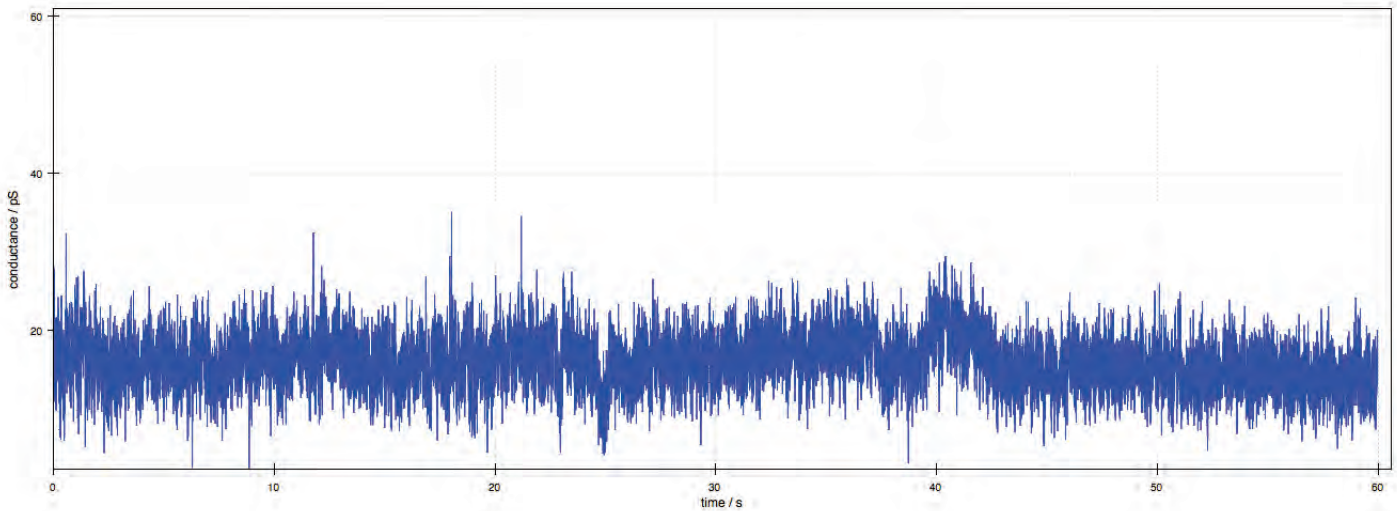
A



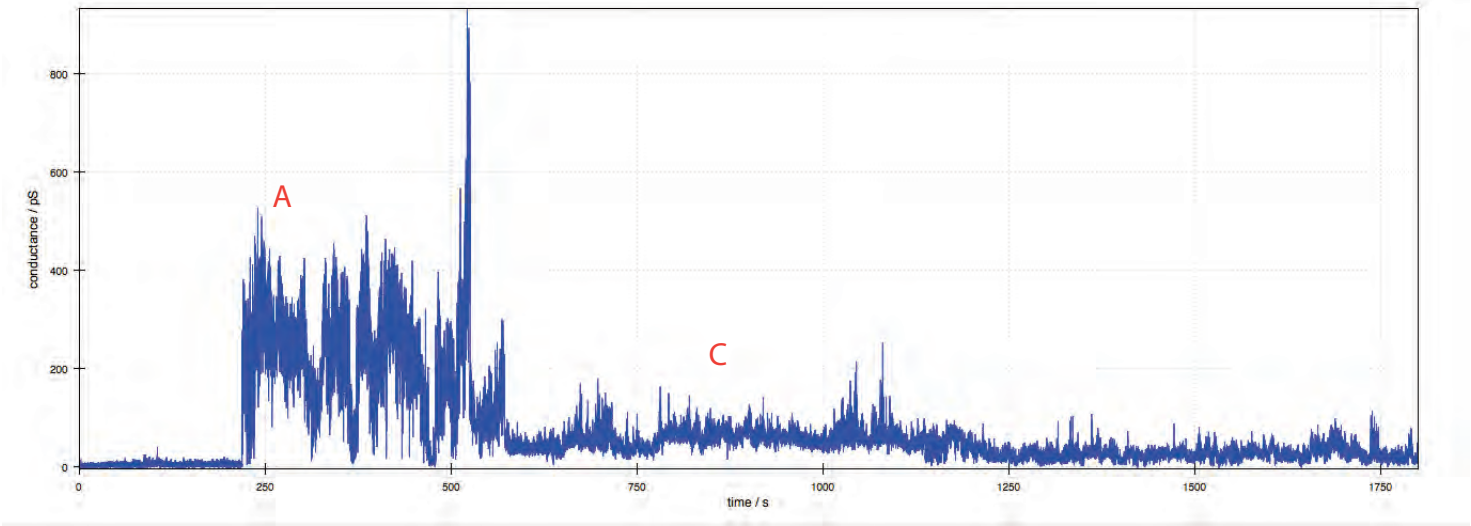
B



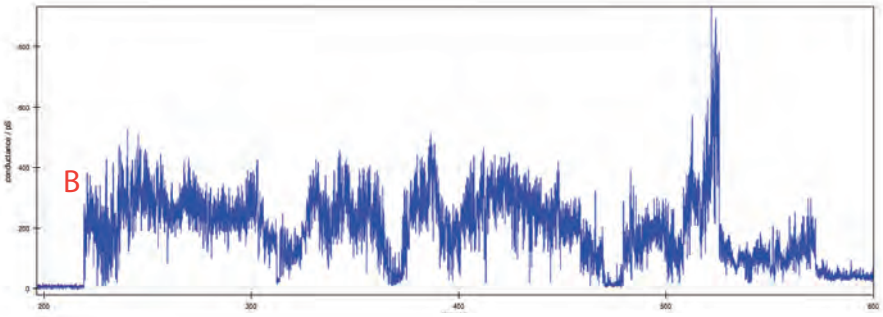
118-0005



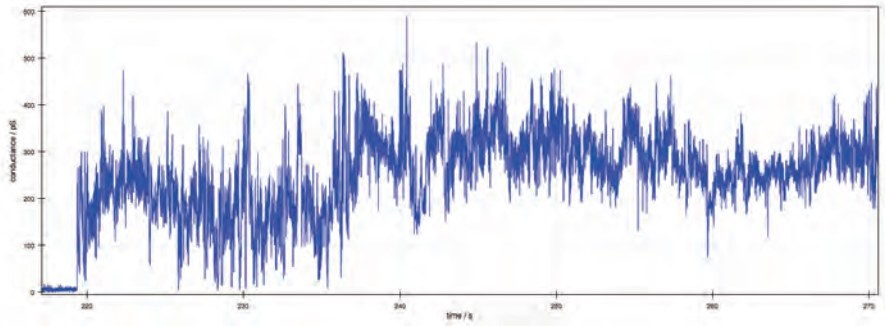
118-0004



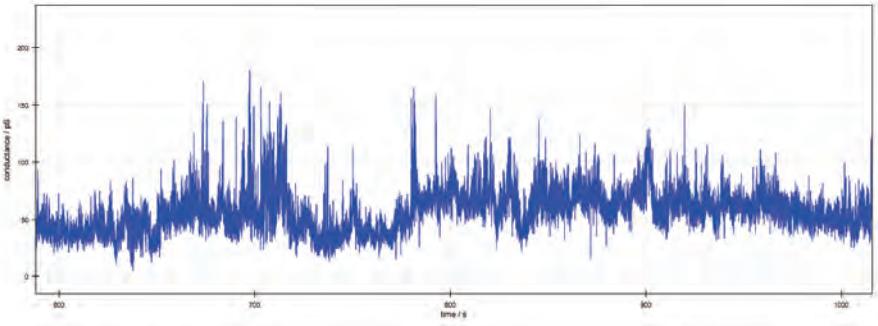
A



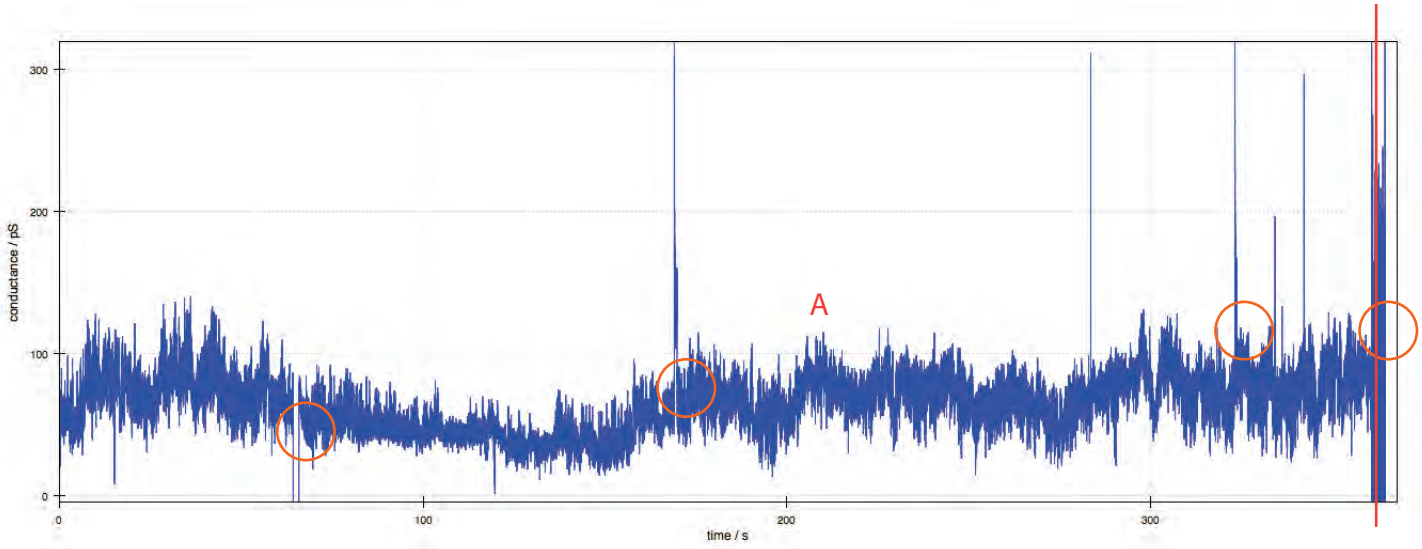
B



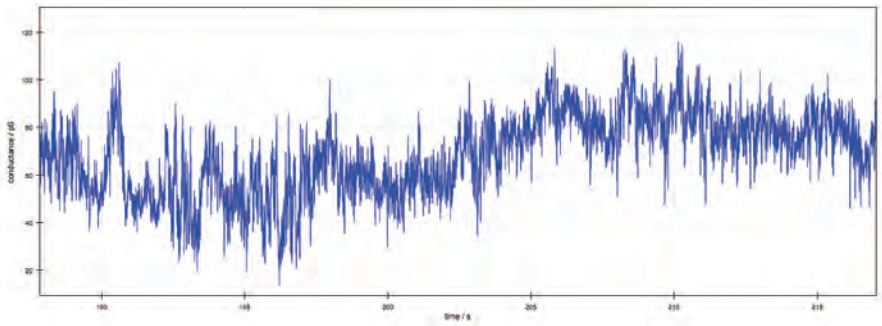
C



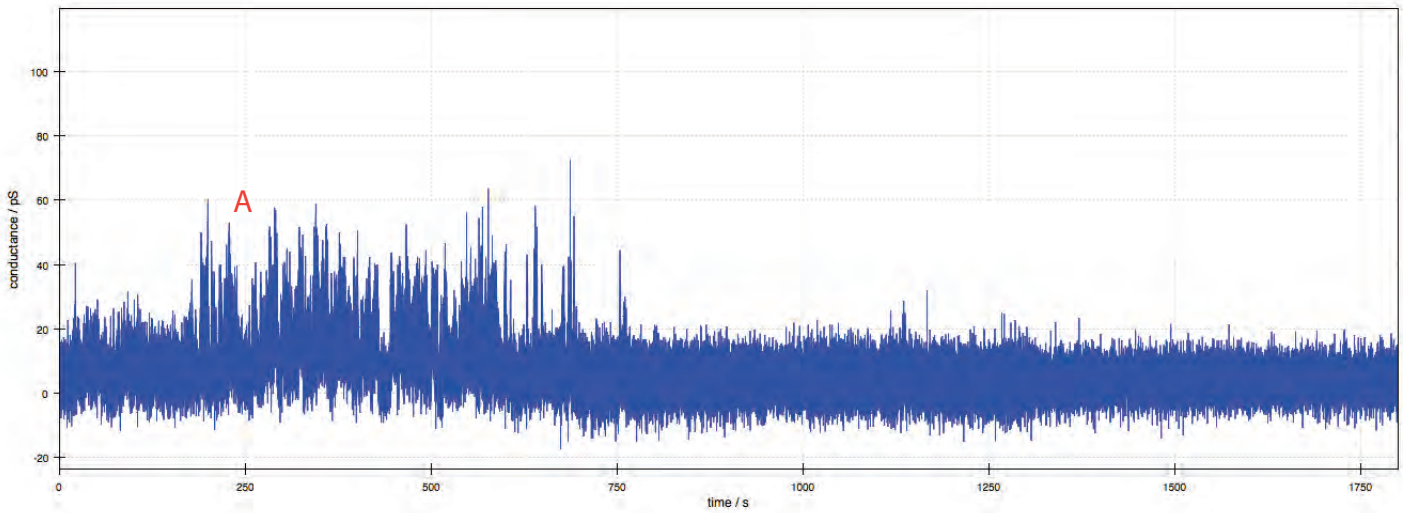
118-0006



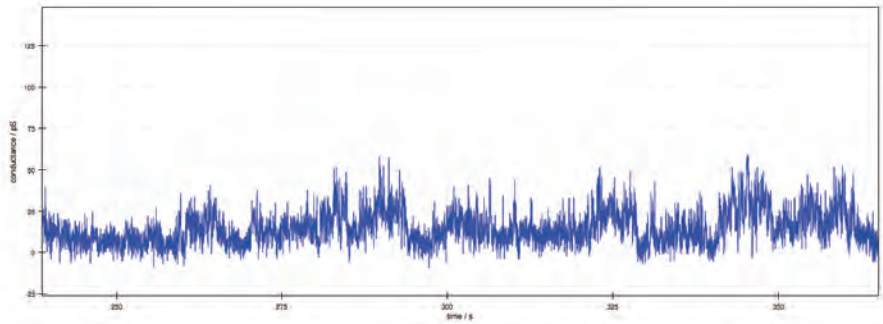
A

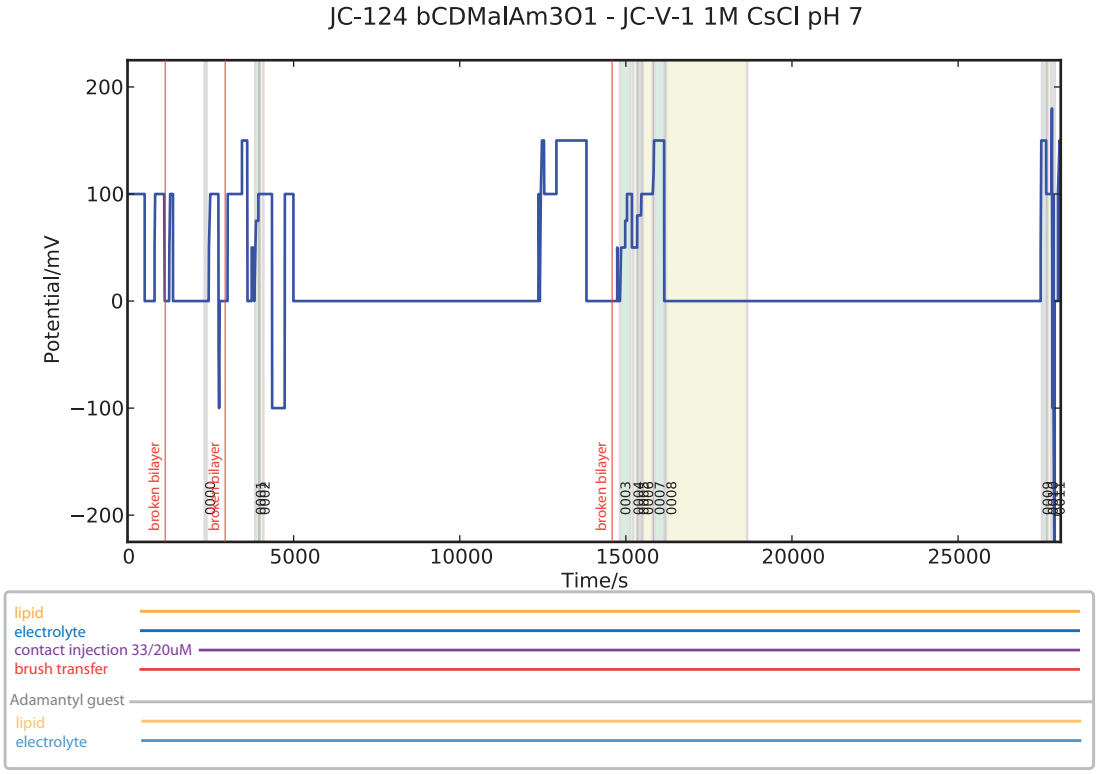


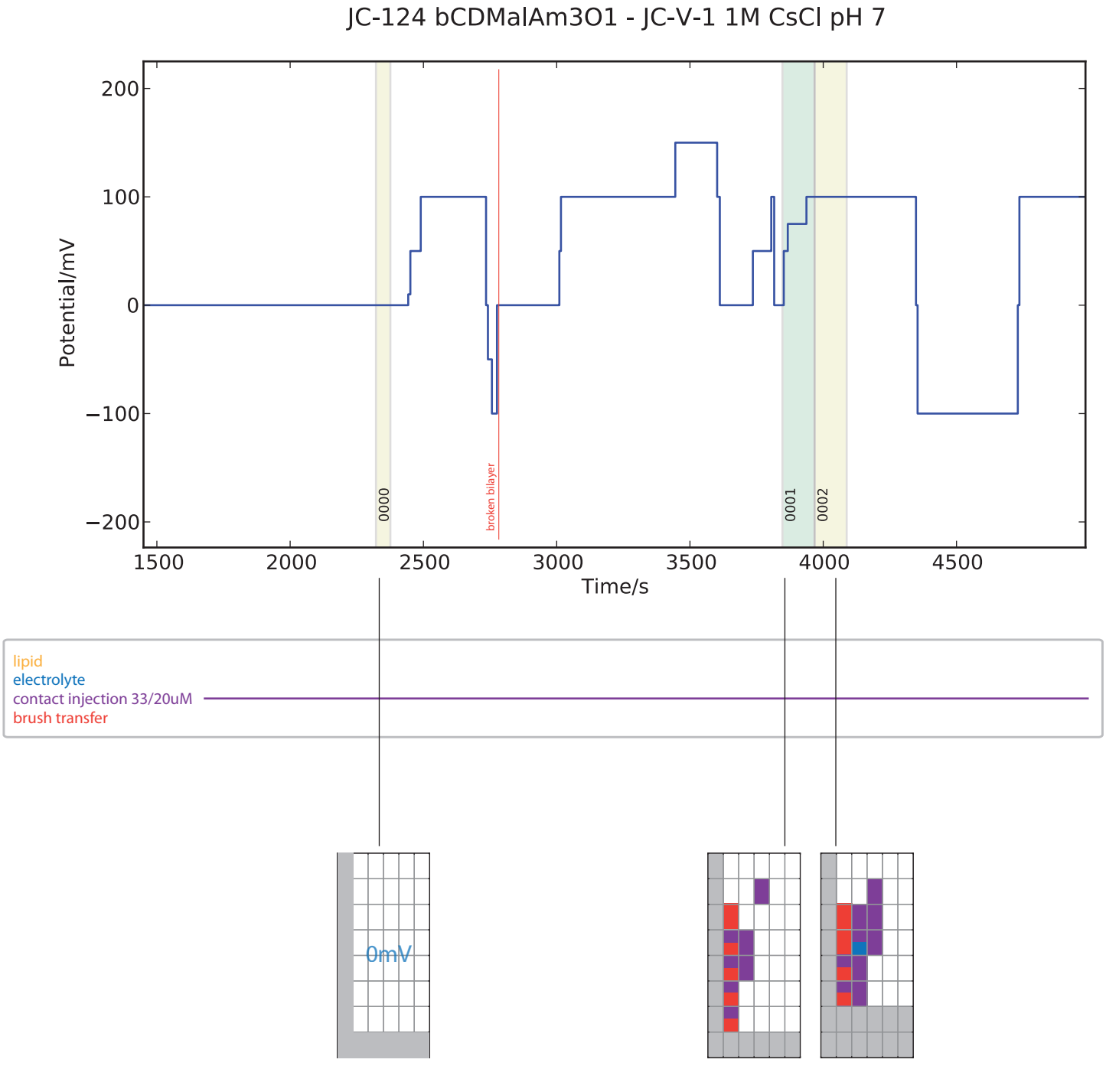
118-0007

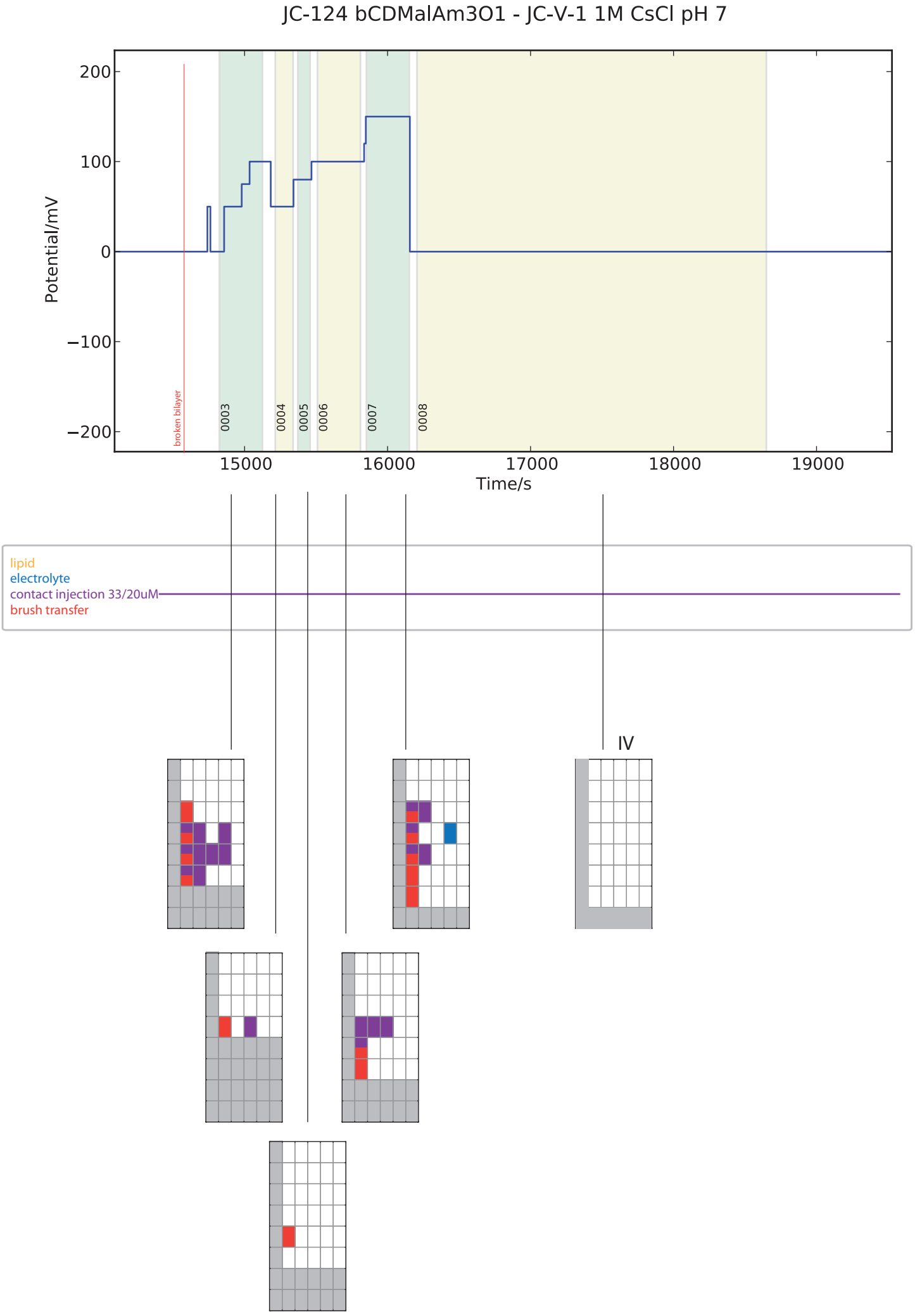


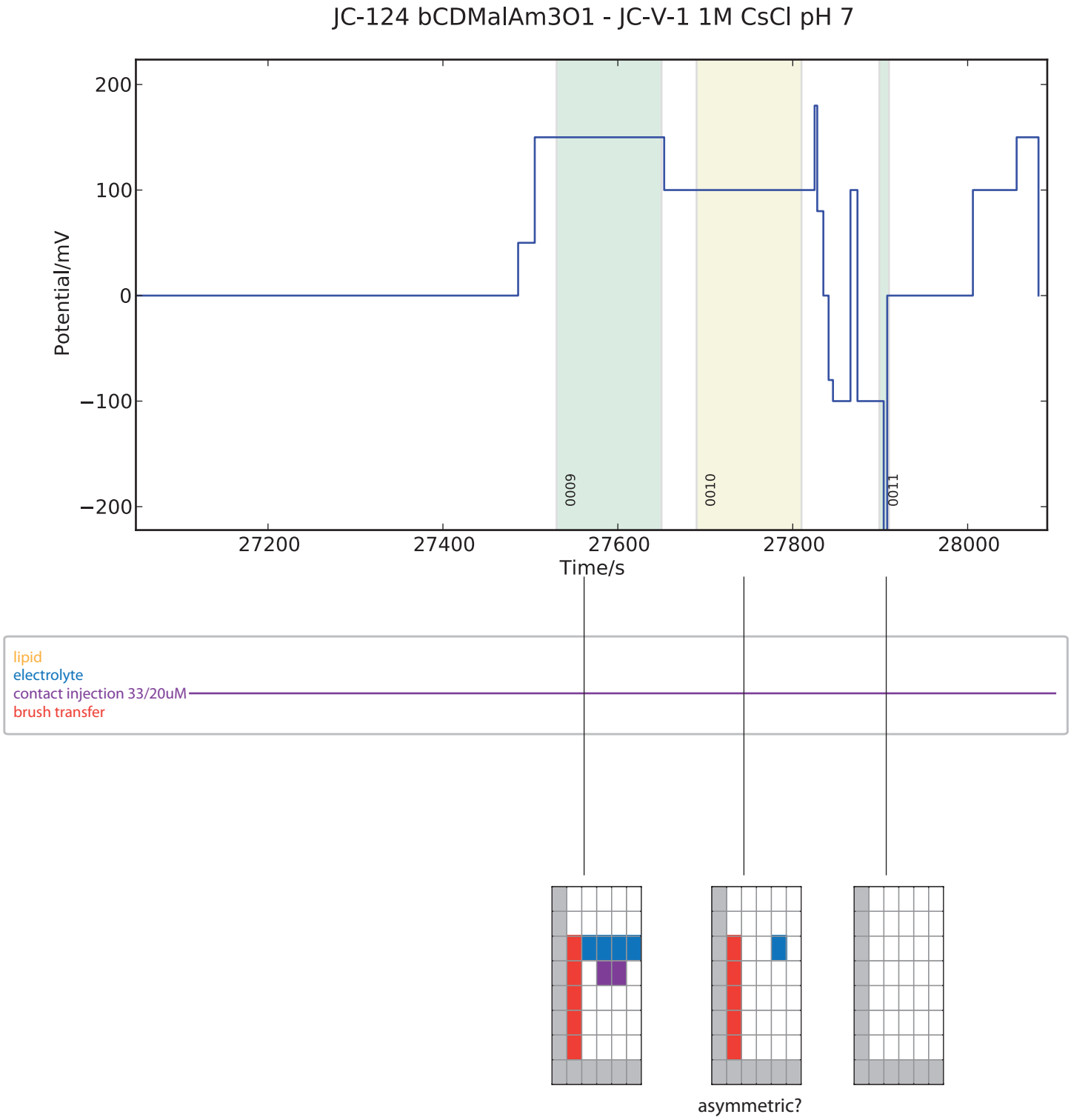
A



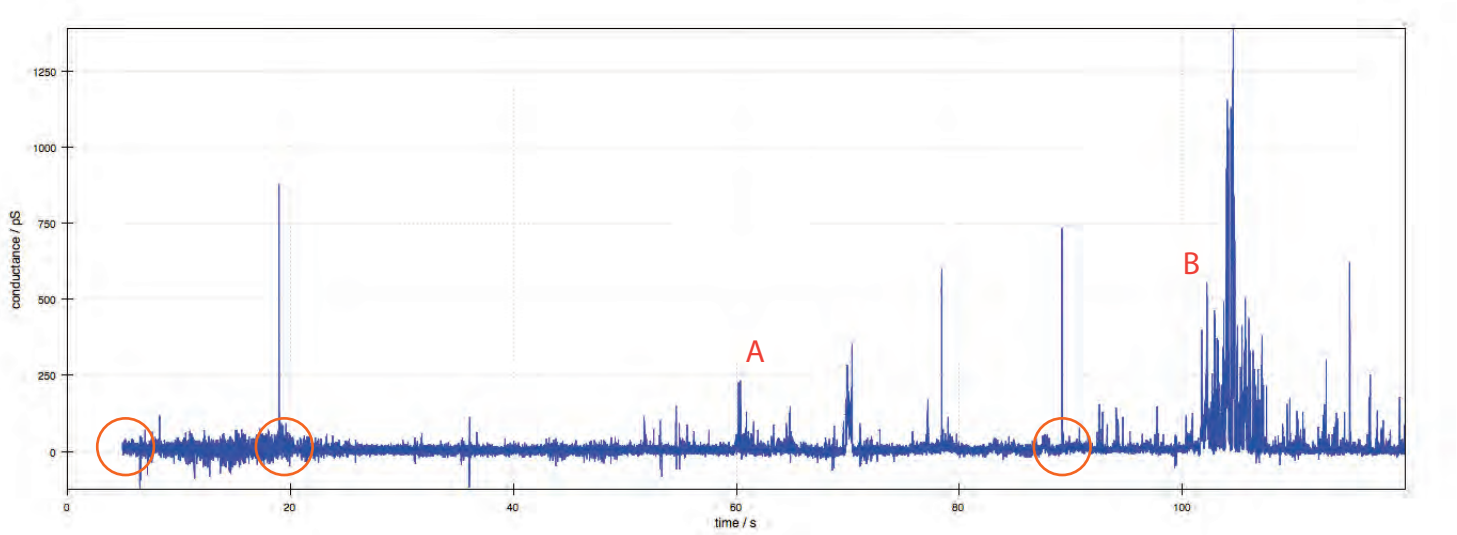

JC-124 bCDMalAm3O1 - JC-V-1 1M CsCl pH 7
Potential/mV
Time/s
broken bilayer
broken bilayer
broken bilayer
lipid
electrolyte
contact injection 33/20uM
brush transfer
Adamantyl guest
lipid
electrolyte


JC-124 bCDMalAm3O1 - JC-V-1 1M CsCl pH 7
Potential/mV
Time/s
0000
broken bilayer
0001
0002
lipid
electrolyte
contact injection 33/20uM
brush transfer
0mV

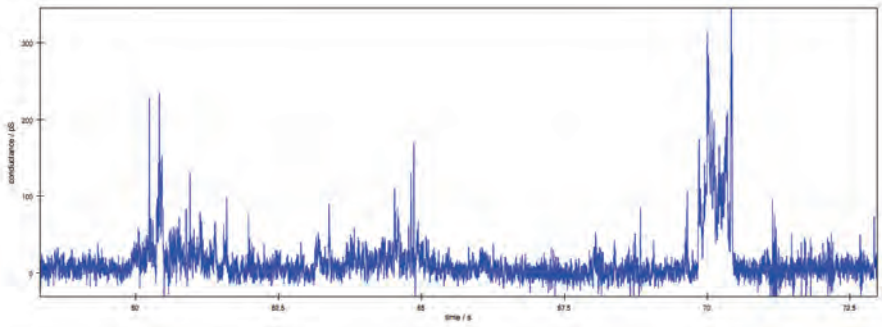

JC-124 bCDMalAm3O1 - JC-V-1 1M CsCl pH 7
Potential/mV
200
100
0
−100
−200
broken bilayer
0003
0004
0005
0006
0007
0008
15000
16000
17000
18000
19000
Time/s
lipid
electrolyte
contact injection 33/20uM
brush transfer
IV


JC-124 bCDMalAm3O1 - JC-V-1 1M CsCl pH 7
Potential/mV
200
100
0
−100
−200
27200
27400
27600
27800
28000
Time/s
0009
0010
0011
lipid
electrolyte
contact injection 33/20uM
brush transfer
asymmetric?

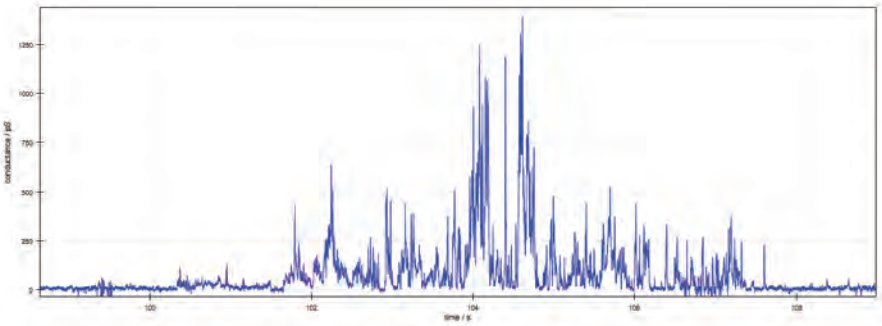
124-0001


conductance / pS
1250
1000
750
500
250
0
A
B
0
20
40
60
80
100
time / s

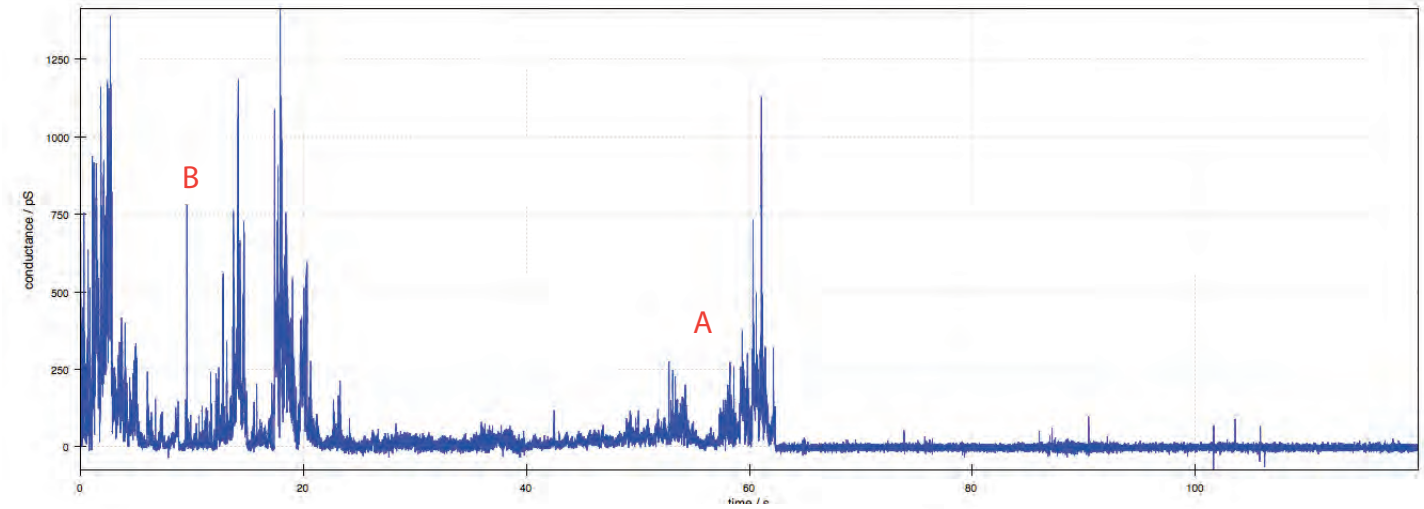
A



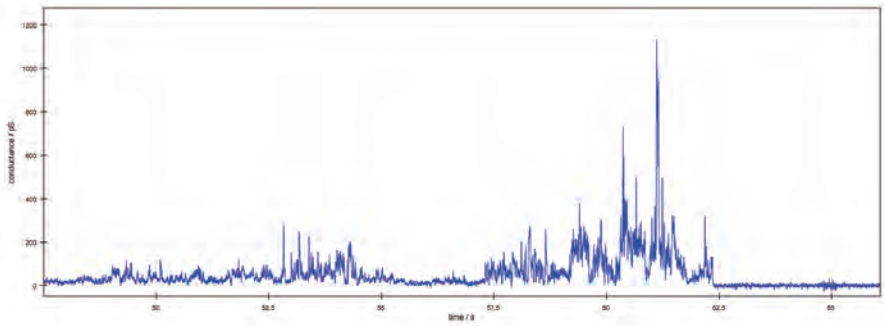
B



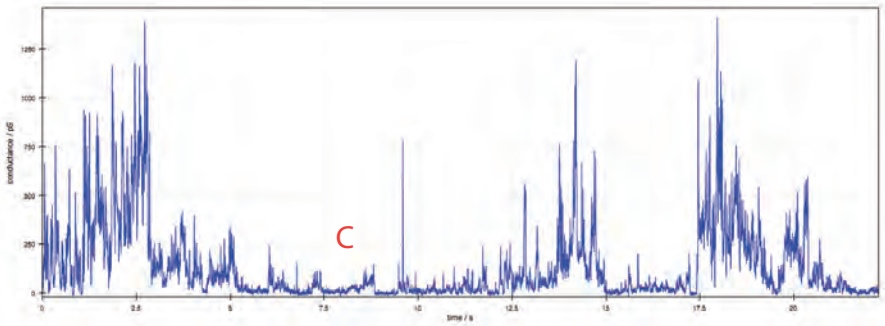
124-0002



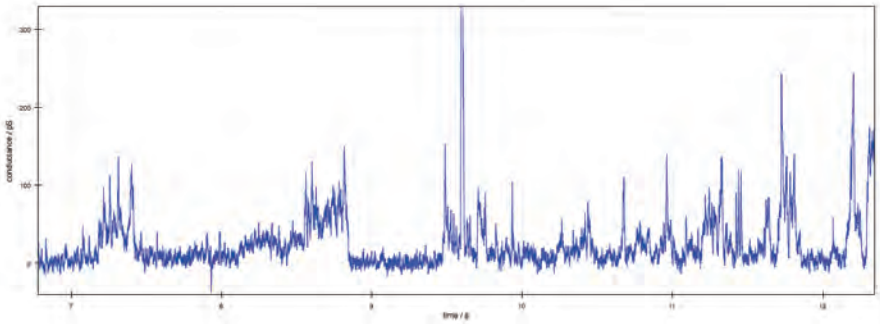
A



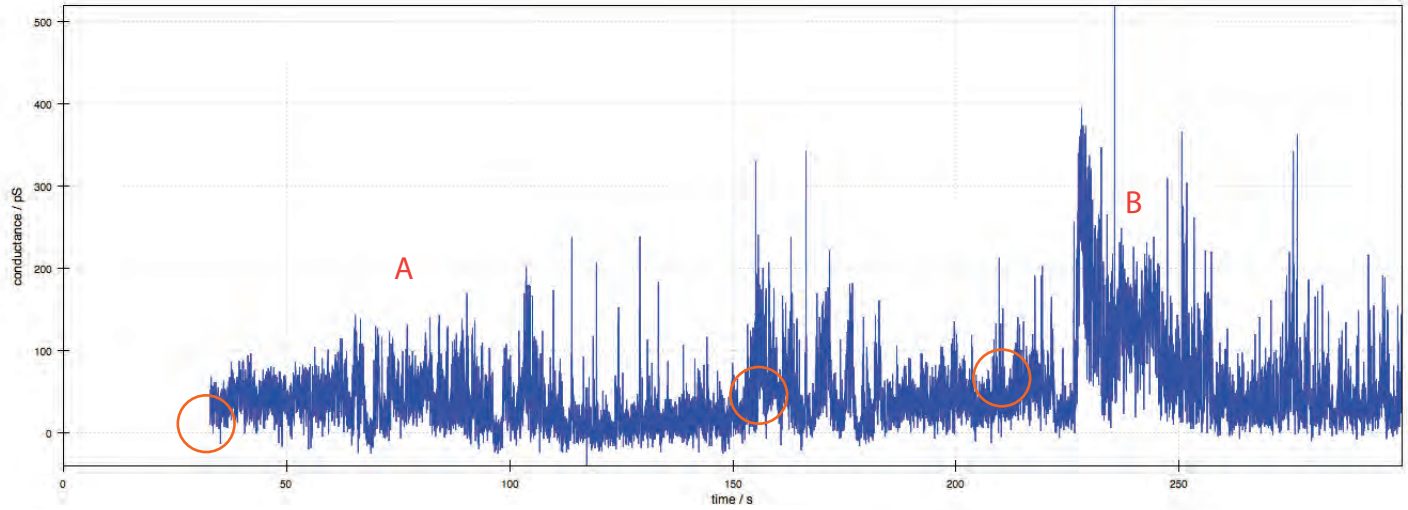
B



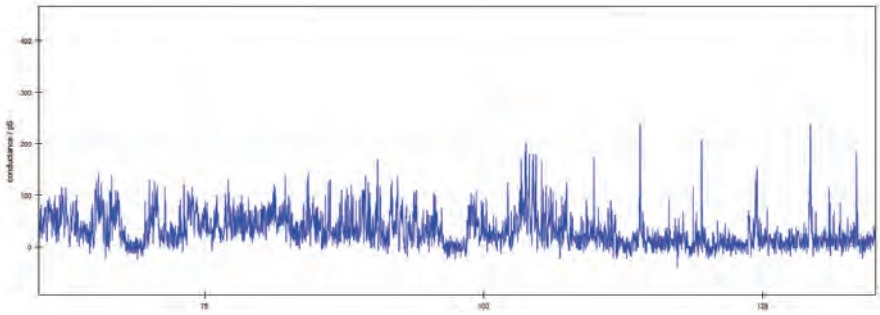
C



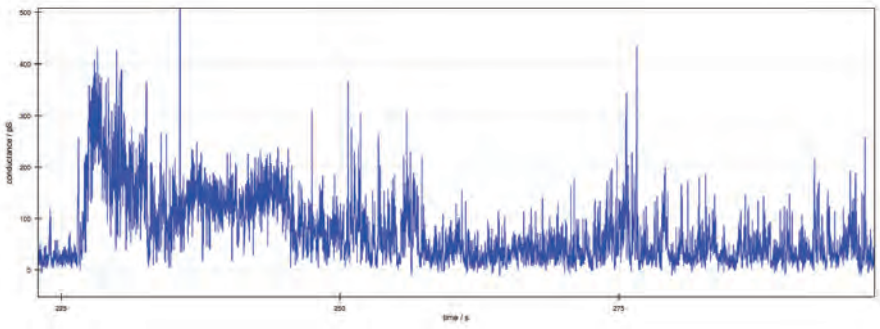
124-0003



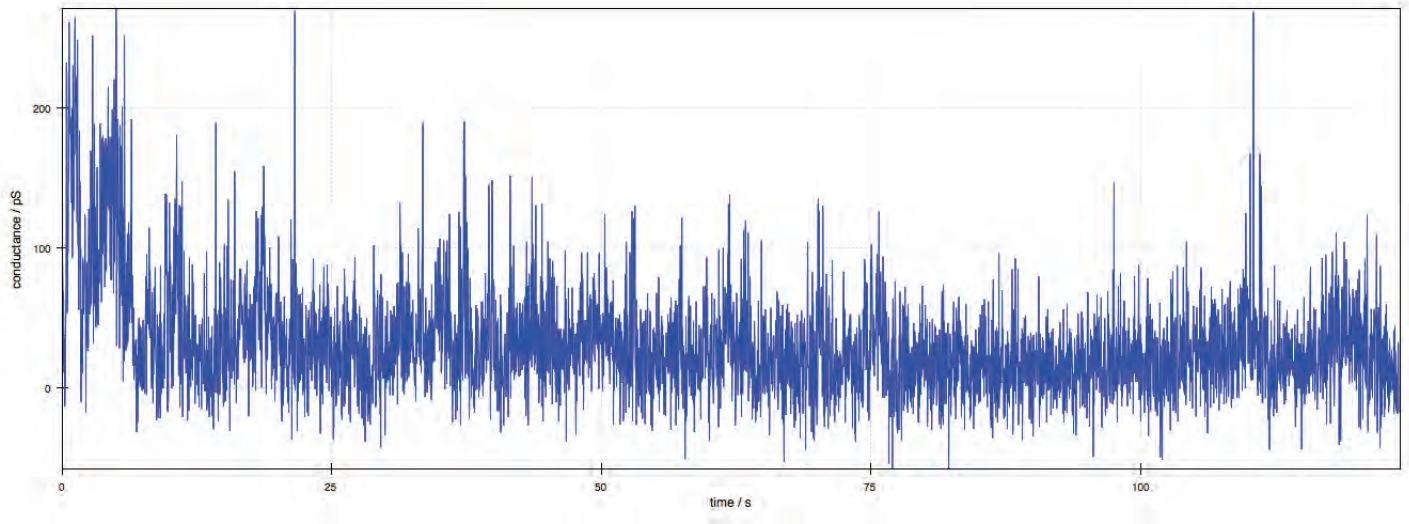
A



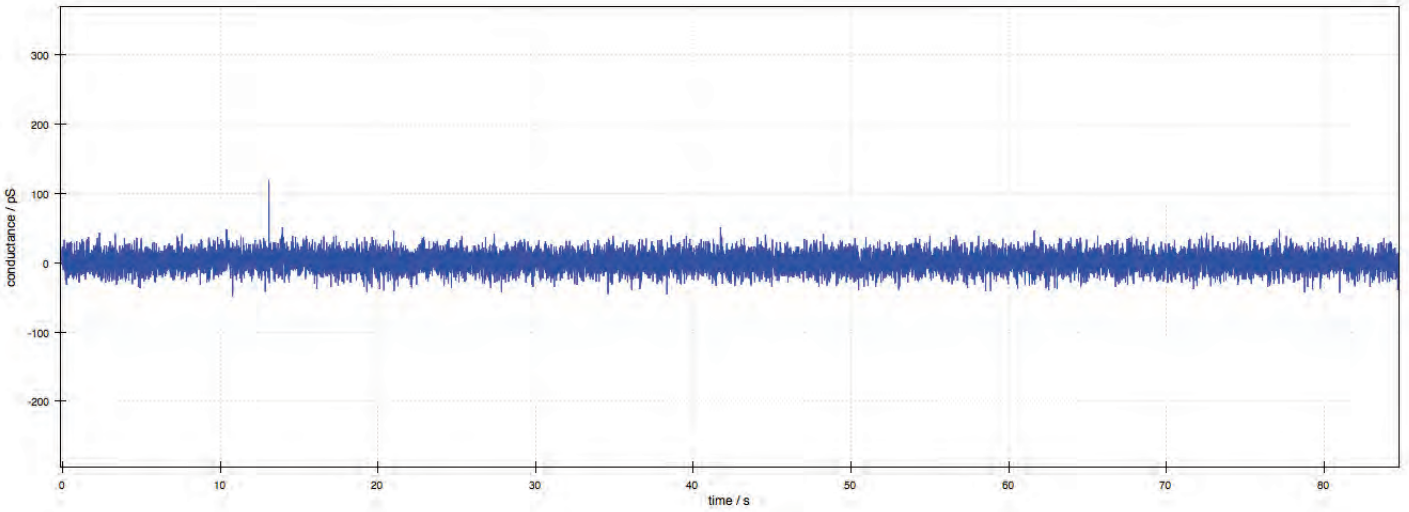
B



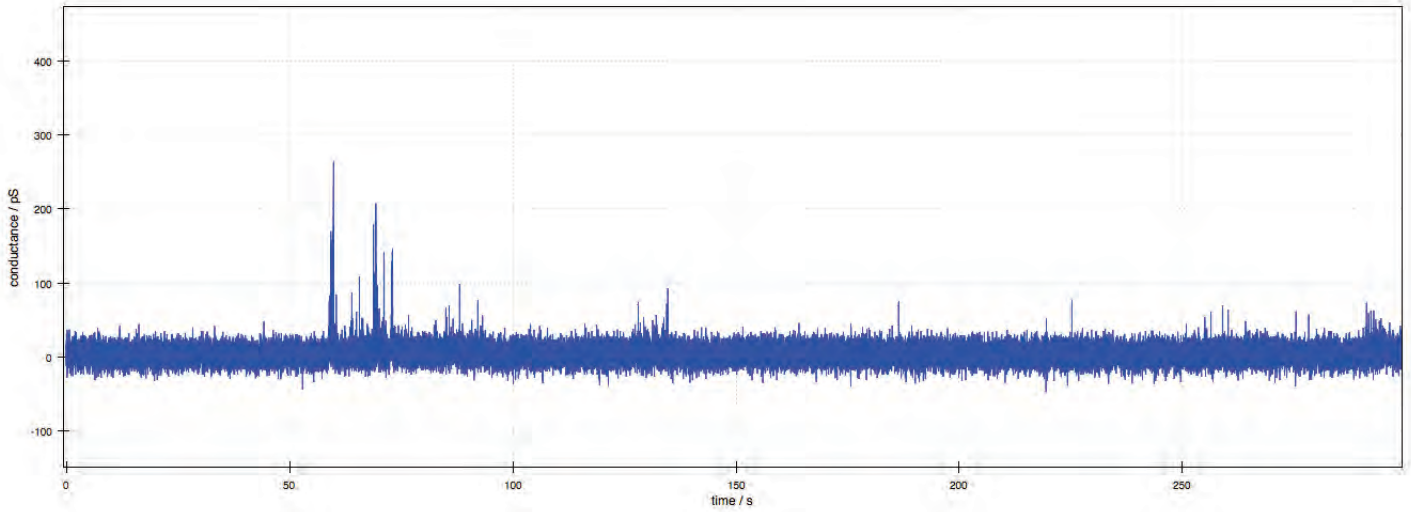
124-0004



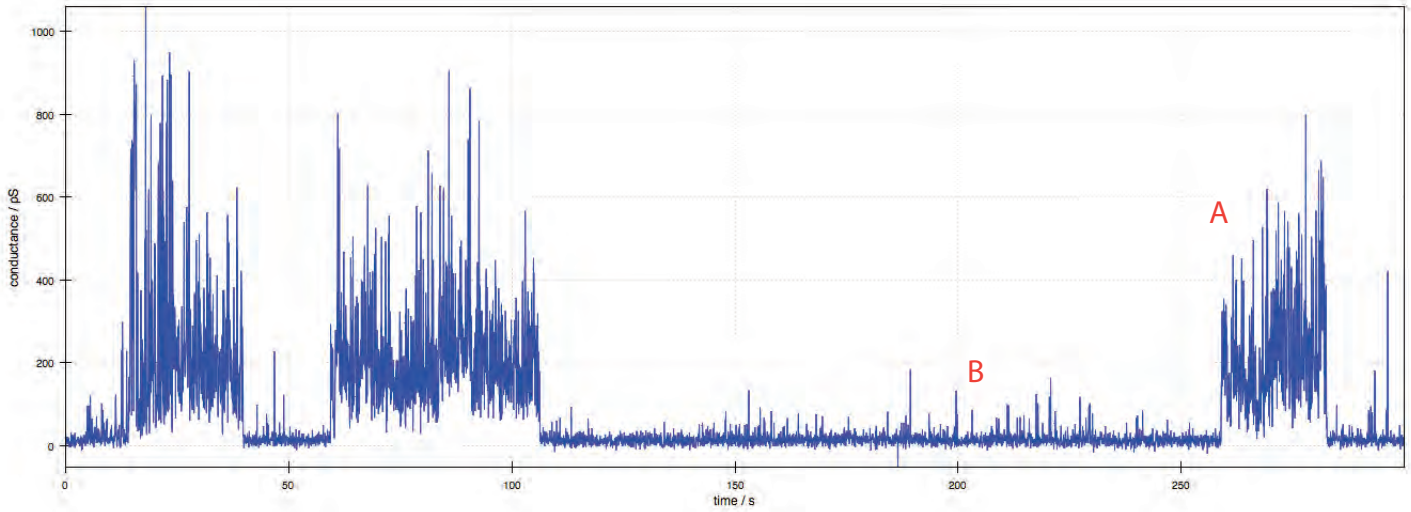
124-0005



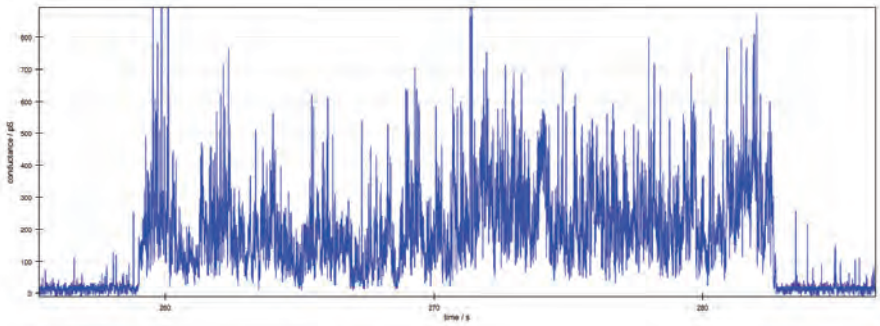
124-0006



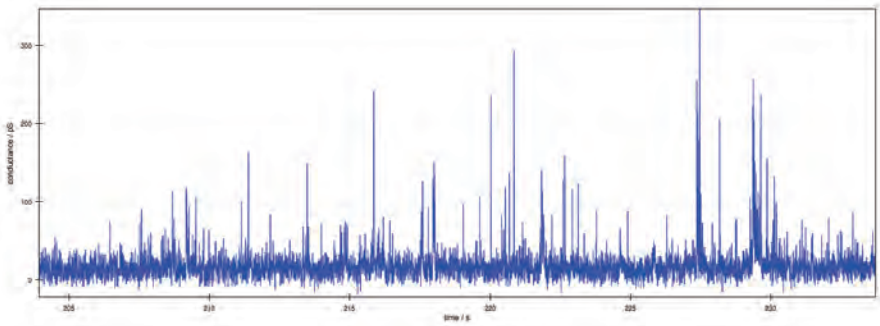
124-0007



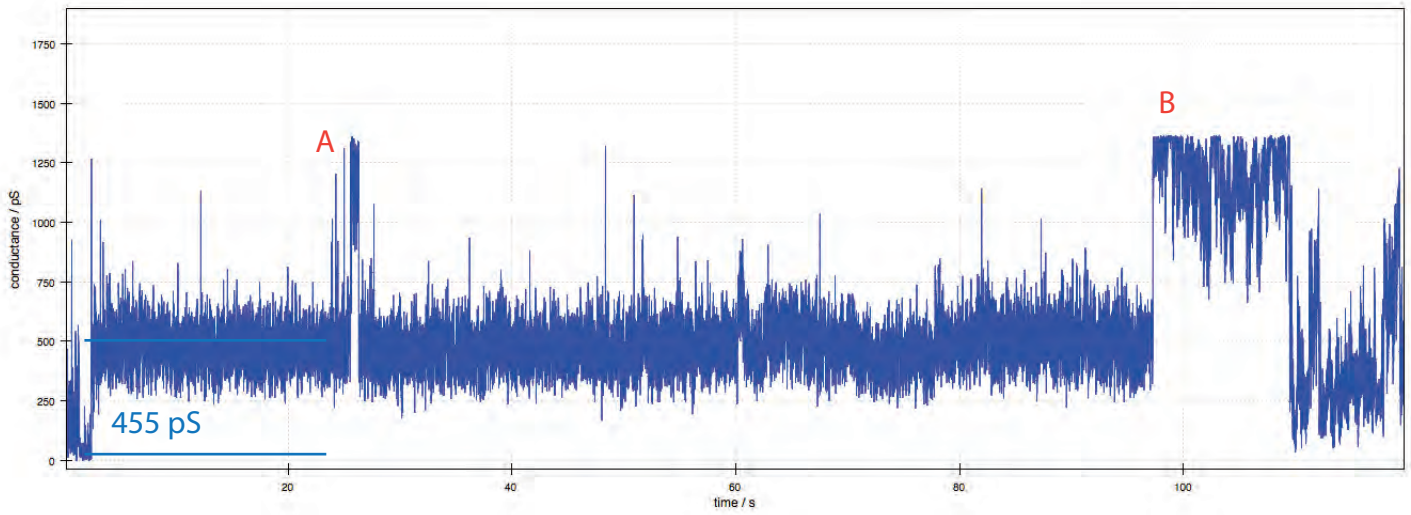
A



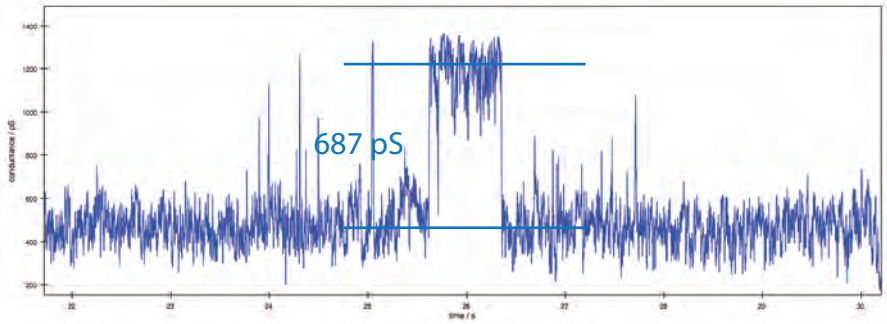
B



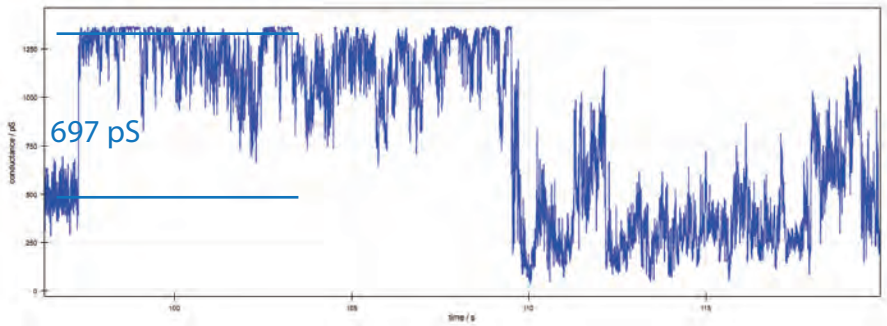
124-0009



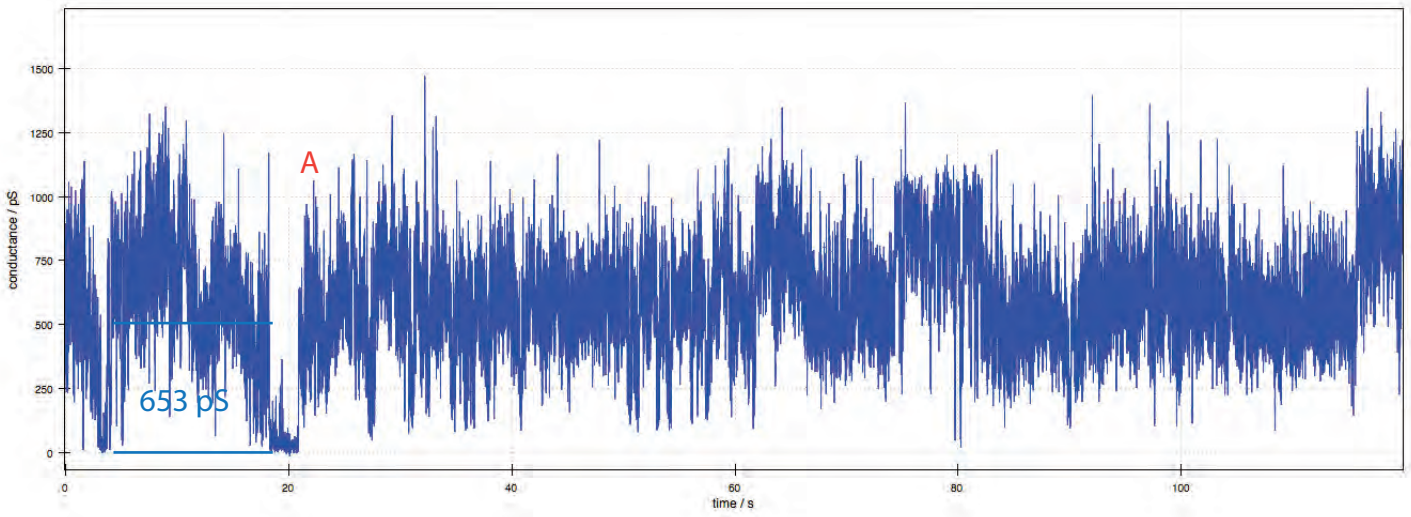
A



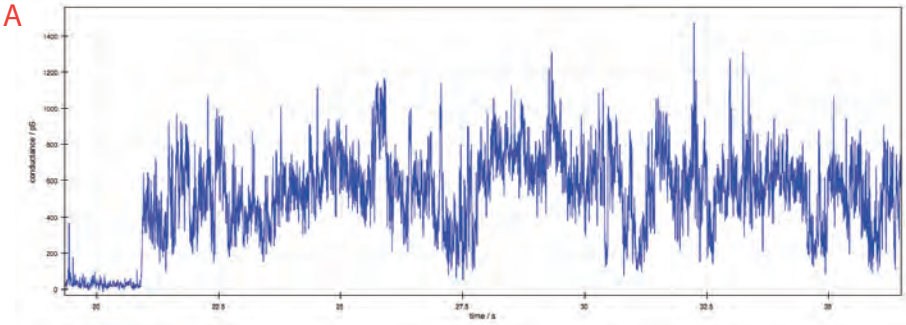
B



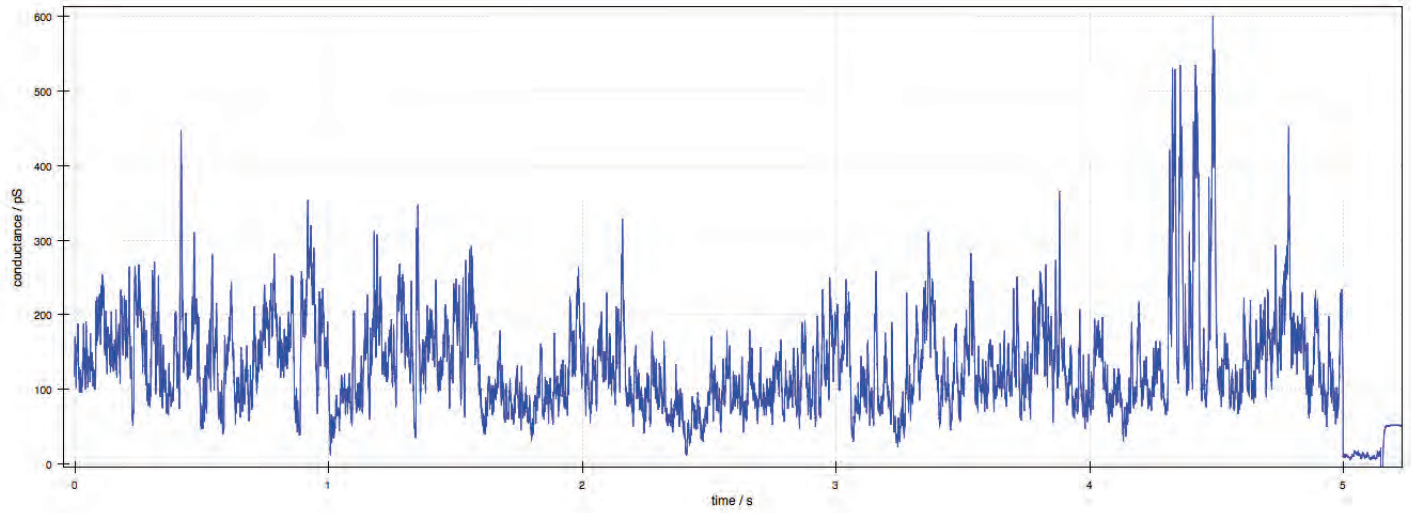
124-0010

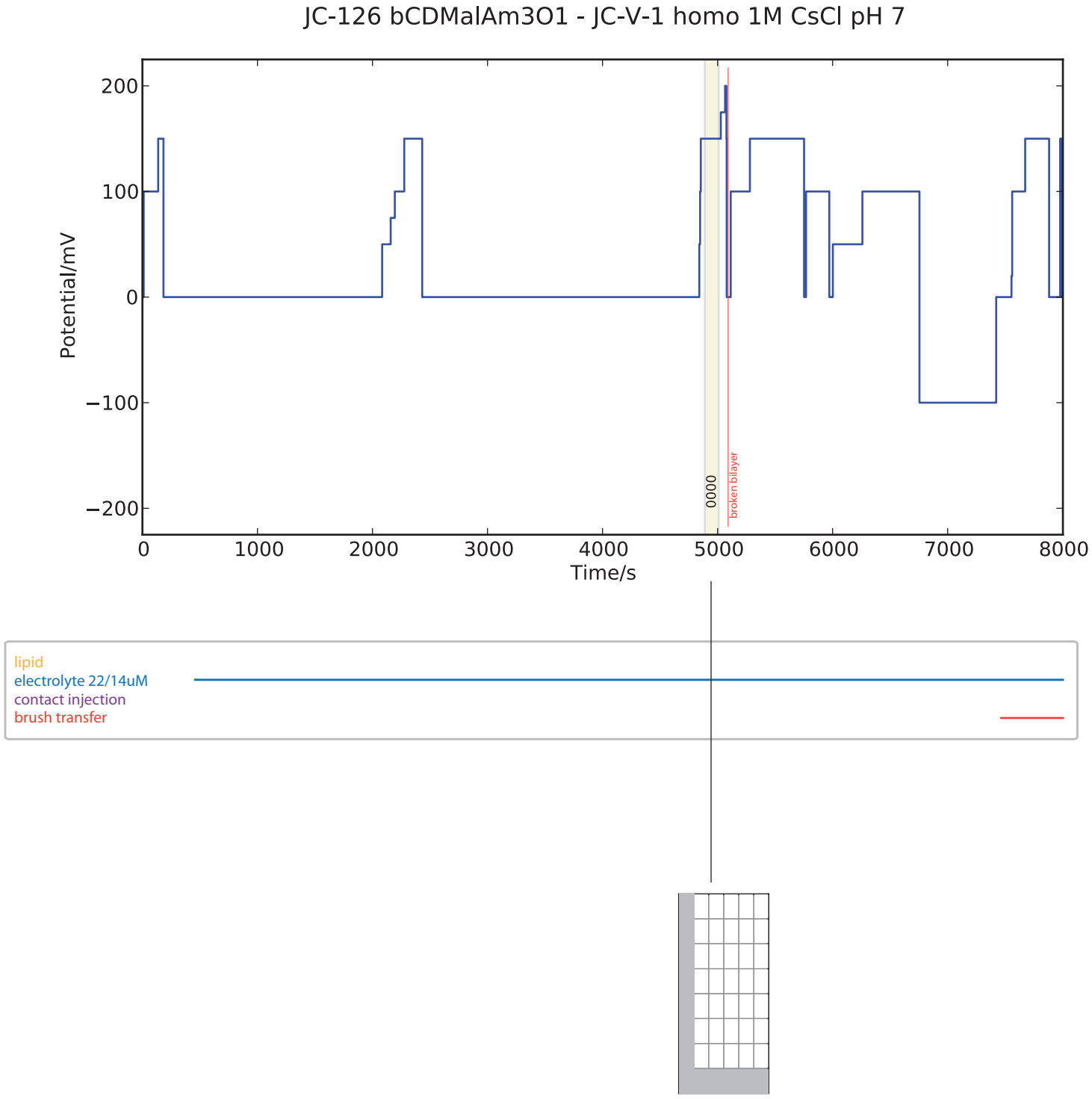


A

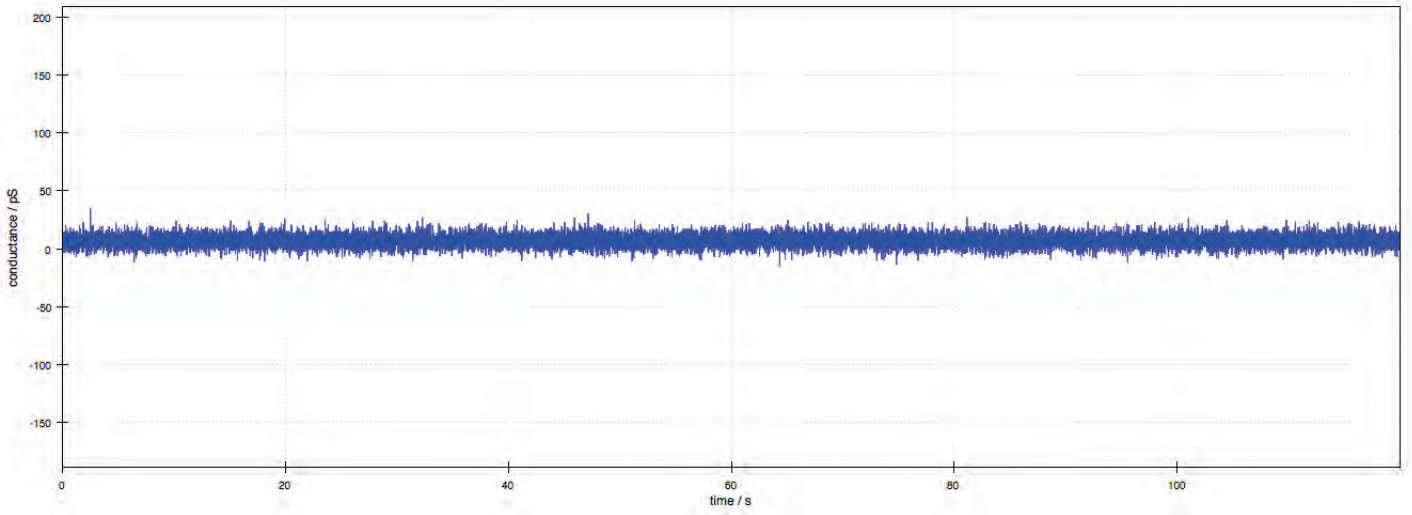


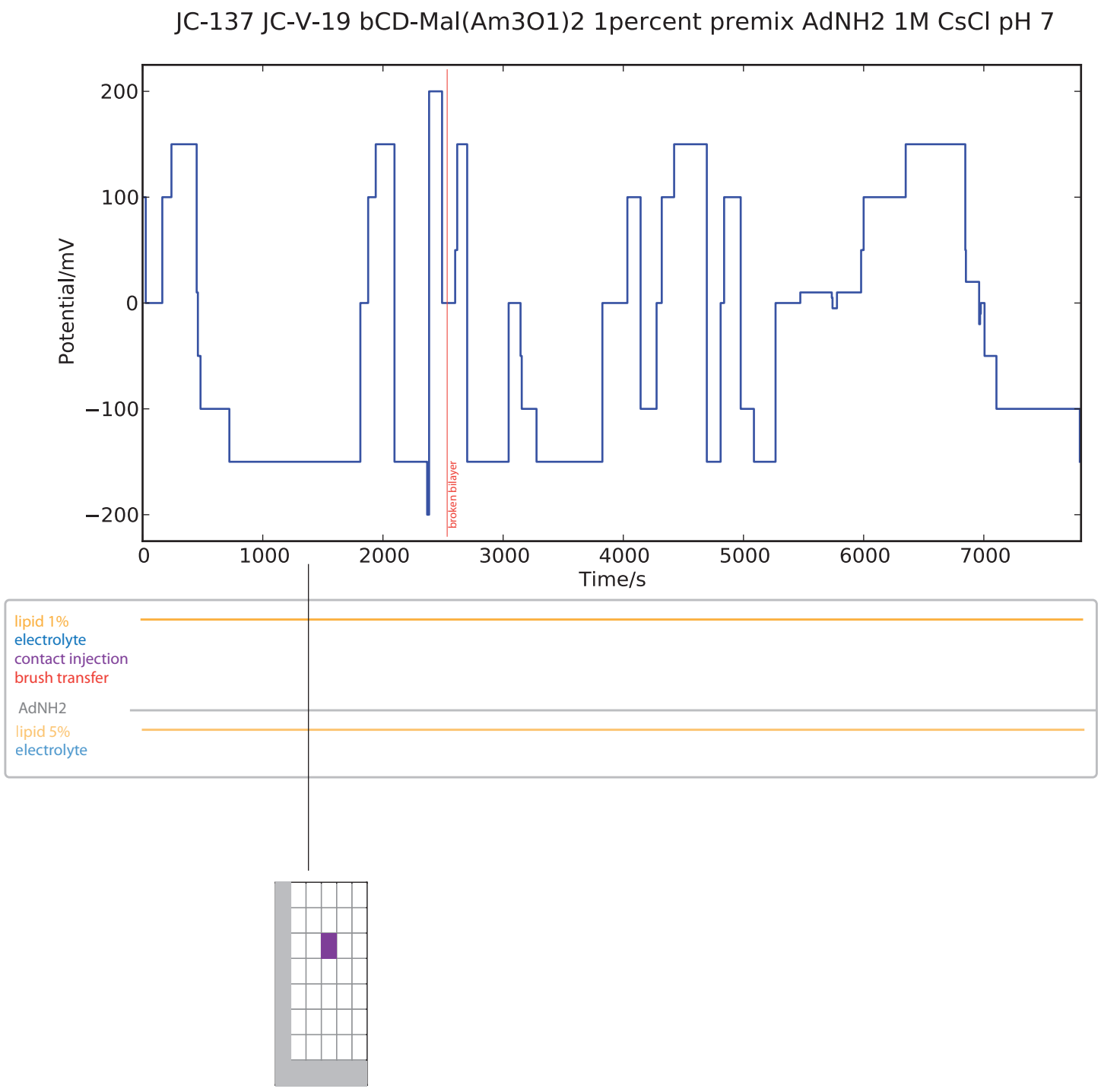
124-0011

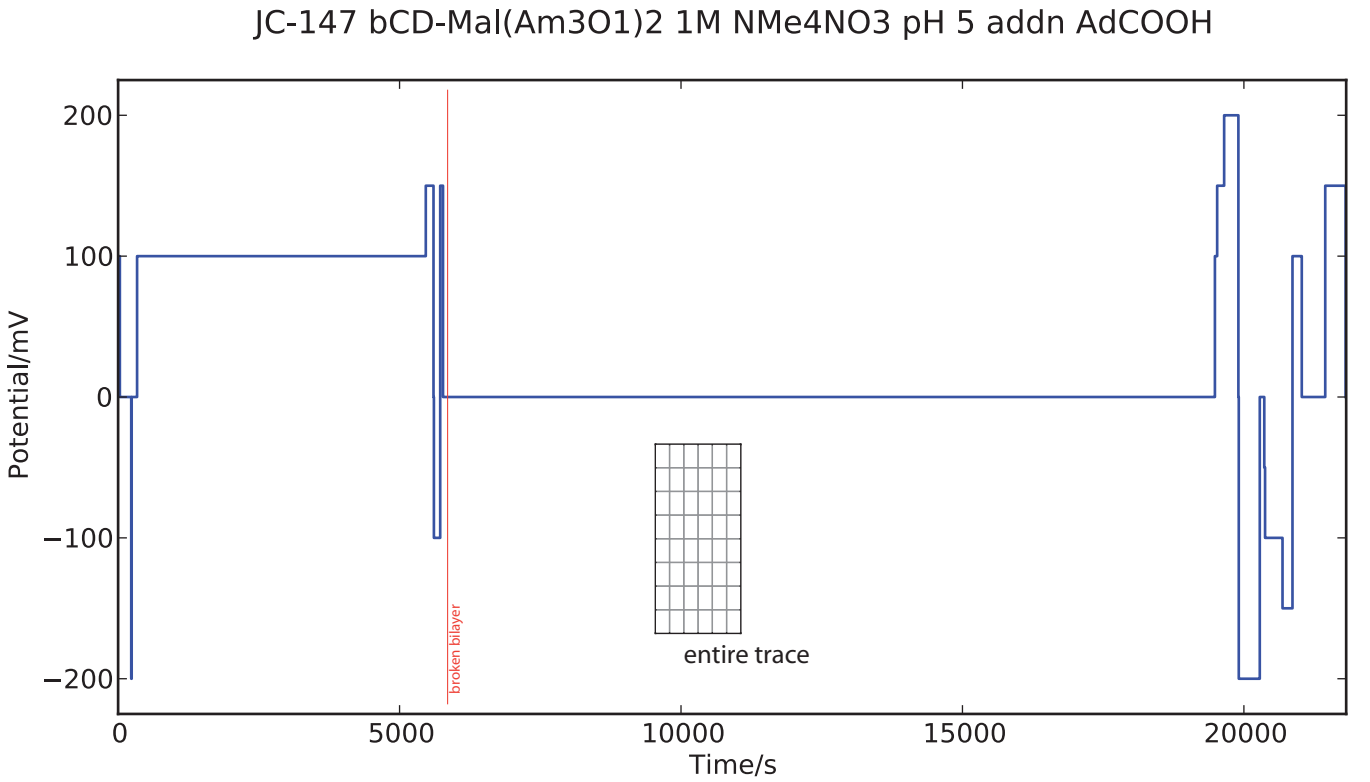


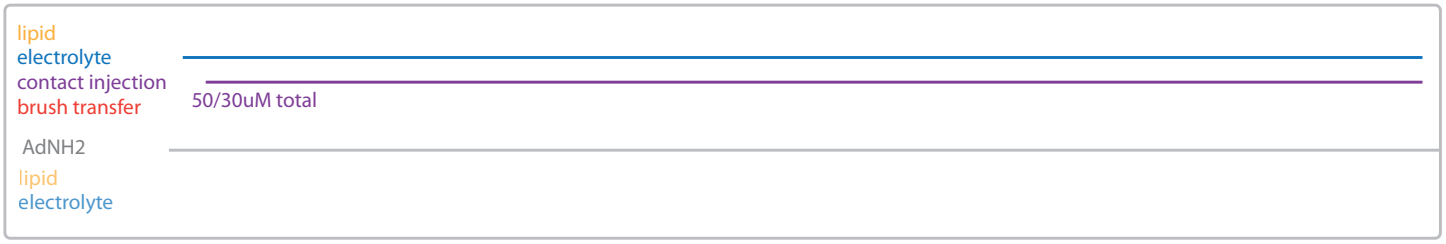

JC-126 bCDMalAm3O1 - JC-V-1 homo 1M CsCl pH 7
Potential/mV
200
100
0
−100
−200
0
1000
2000
3000
4000
5000
6000
7000
8000
Time/s
0000
broken bilayer
lipid
electrolyte 22/14uM
contact injection
brush transfer

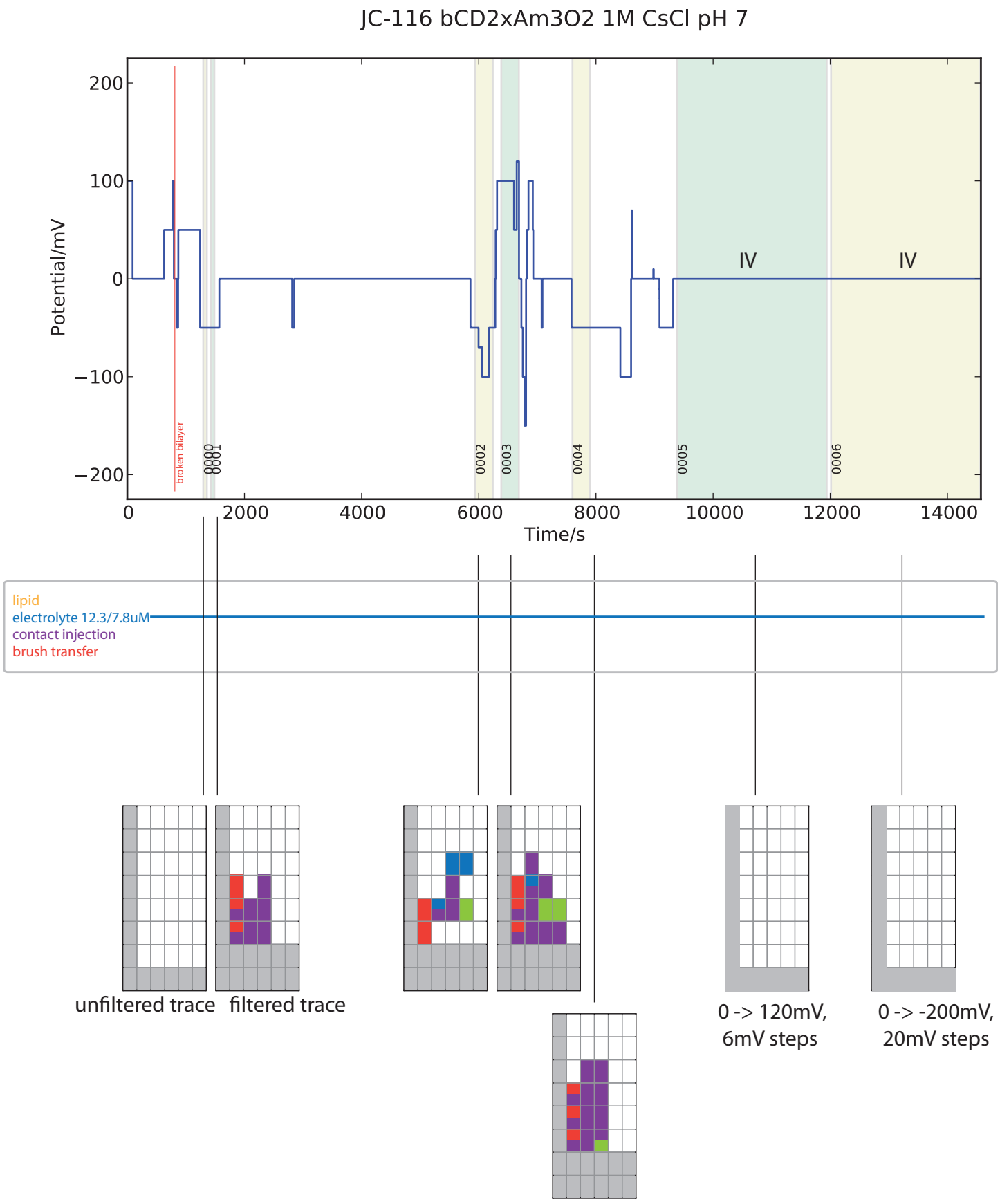
126-0000


200
150
100
50
0
-50
-100
-150
conductance / pS
0
20
40
60
80
100
time / s

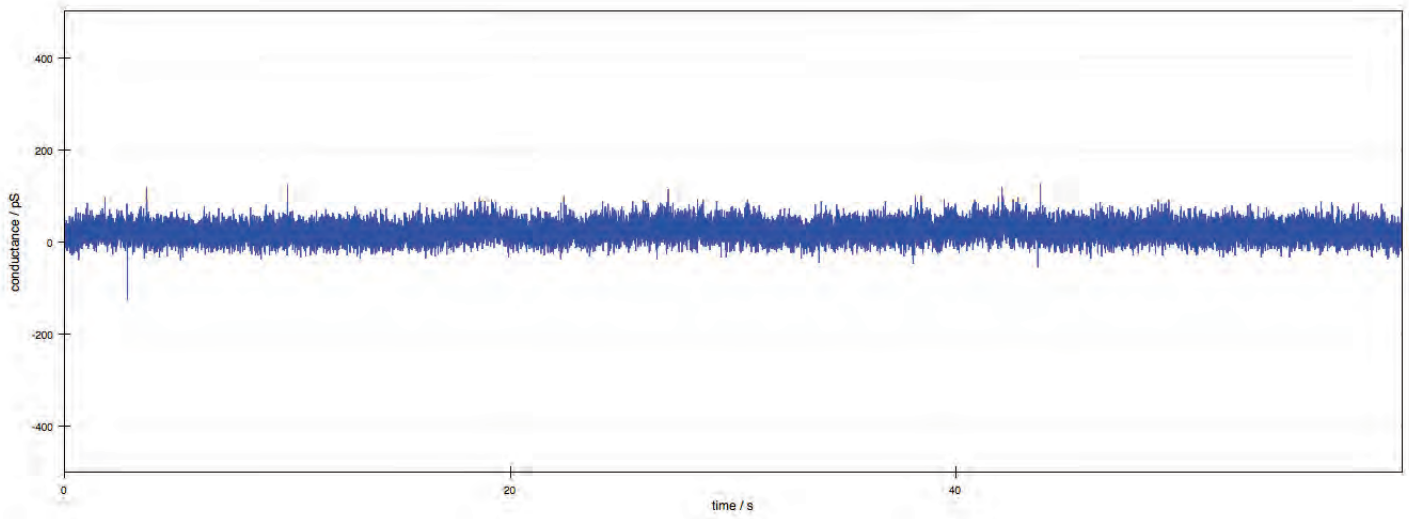

JC-137 JC-V-19 bCD-Mal(Am3O1)2 1percent premix AdNH2 1M CsCl pH 7
Potential/mV
200
100
0
−100
−200
0
1000
2000
3000
4000
5000
6000
7000
Time/s
broken bilayer
lipid 1%
electrolyte
contact injection
brush transfer
AdNH2
lipid 5%
electrolyte


JC-147 bCD-Mal(Am3O1)2 1M NMe4NO3 pH 5 addn AdCOOH
Potential/mV
200
100
0
−100
−200
0
5000
10000
15000
20000
Time/s
broken bilayer
entire trace

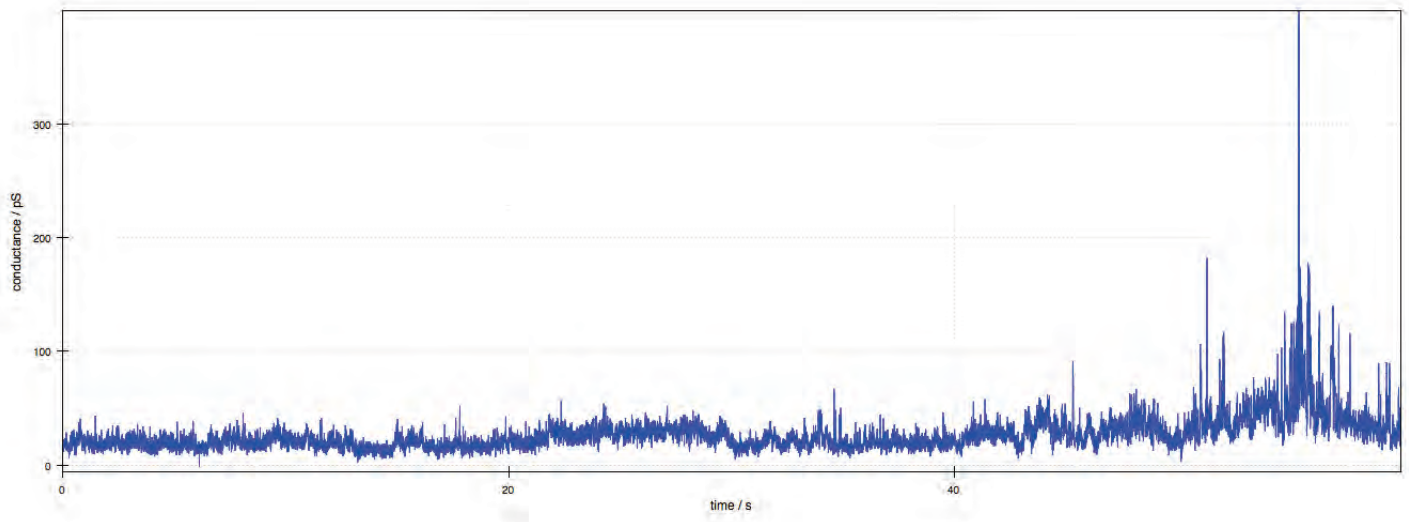

lipid
electrolyte
contact injection
brush transfer
50/30uM total
AdNH2
lipid
electrolyte


JC-116 bCD2xAm3O2 1M CsCl pH 7
Potential/mV
200
100
0
−100
−200
0
2000
4000
6000
8000
10000
12000
14000
Time/s
IV
IV
broken bilayer
0000
0001
0002
0003
0004
0005
0006
lipid
electrolyte 12.3/7.8uM
contact injection
brush transfer
unfiltered trace
filtered trace
0 -> 120mV,
6mV steps
0 -> -200mV,
20mV steps

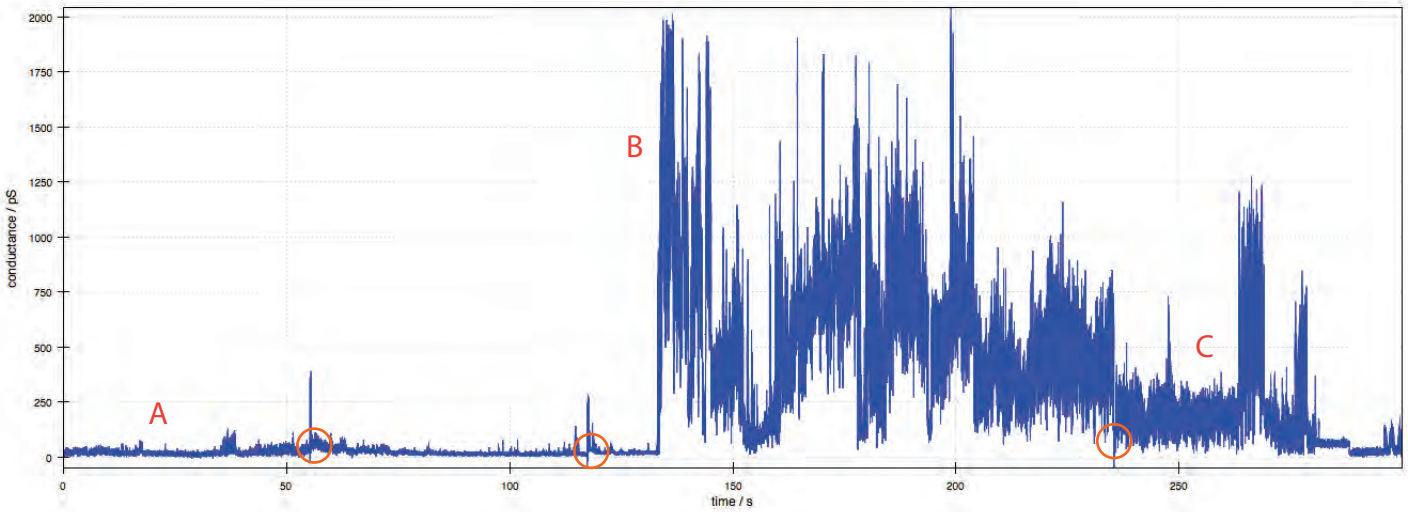
116-0000



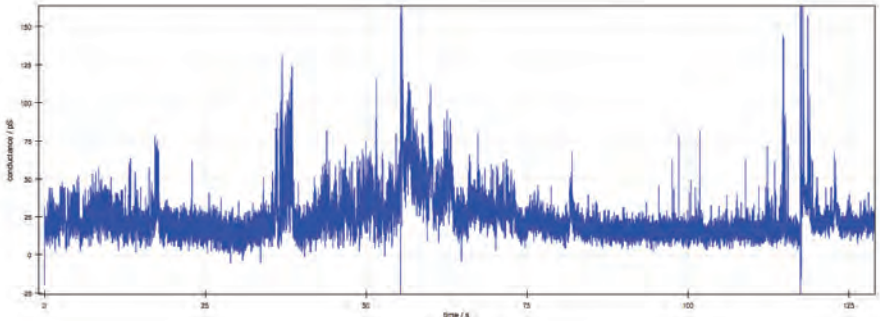
116-0001



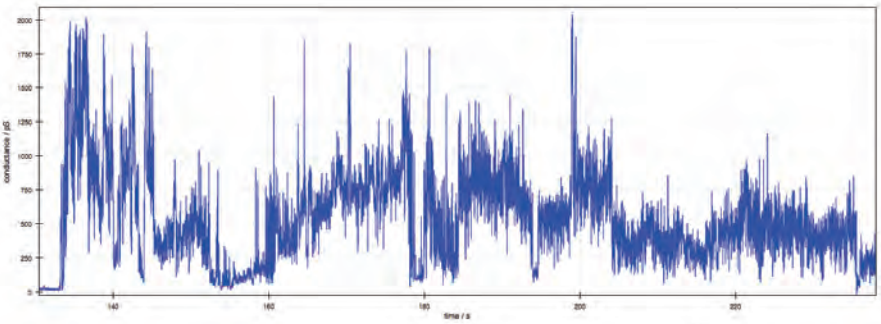
116-0002



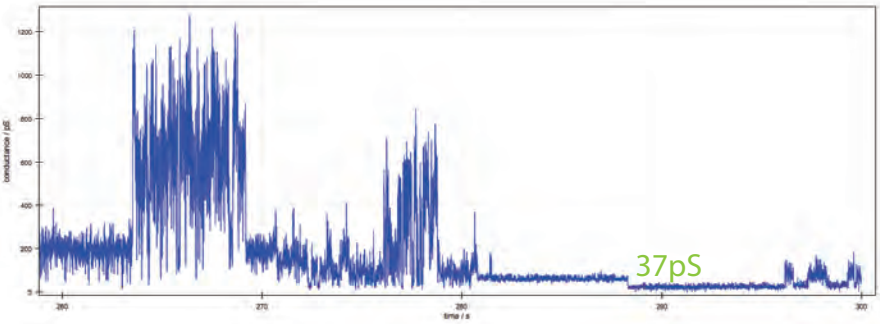
A



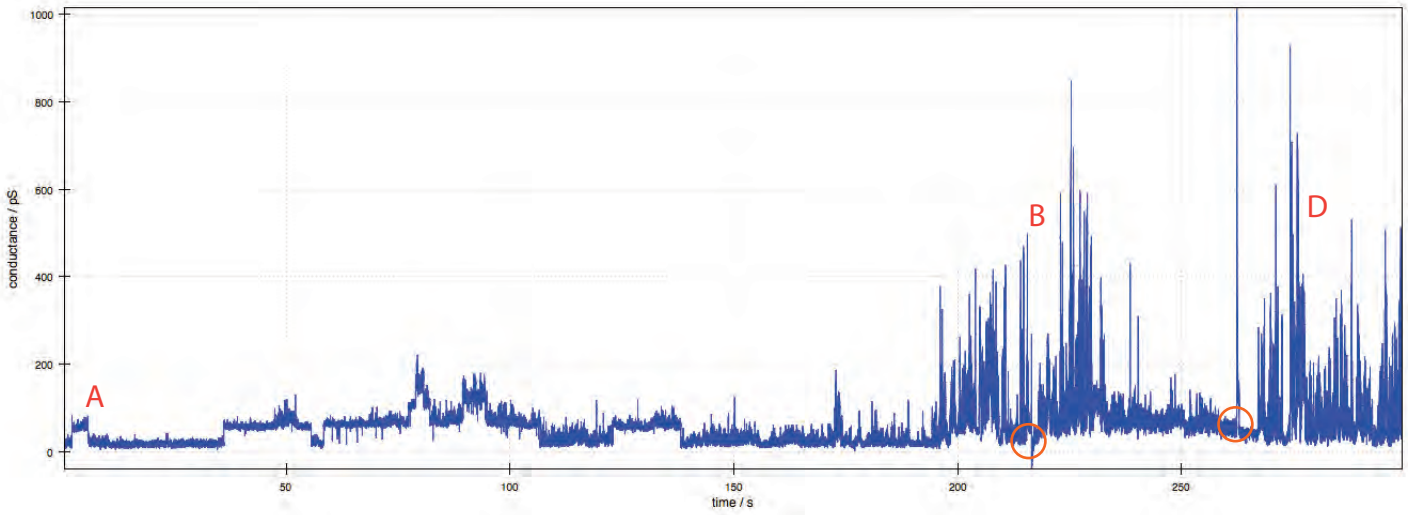
B



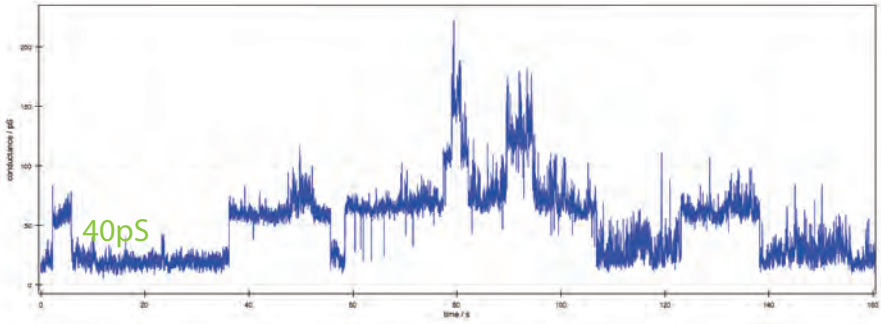
C



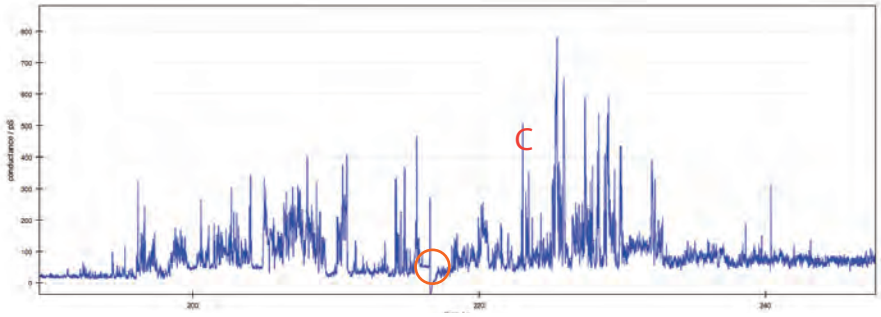
116-0003



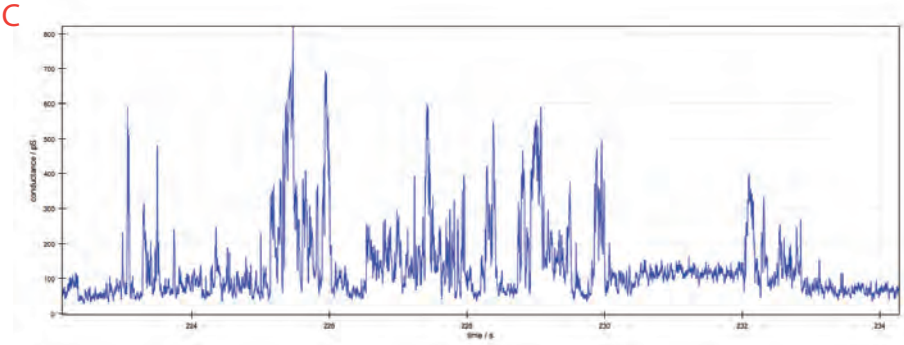
A



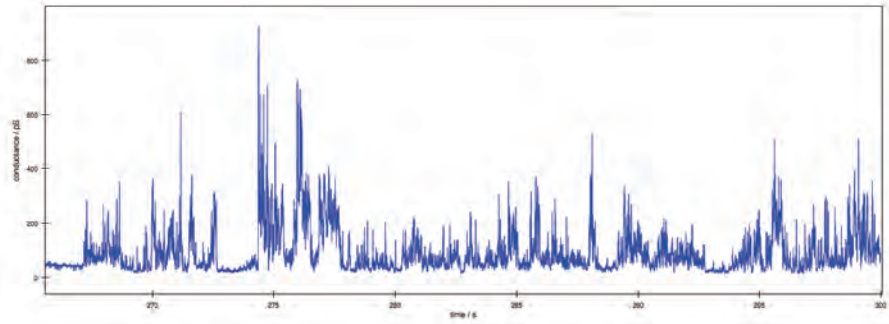
B



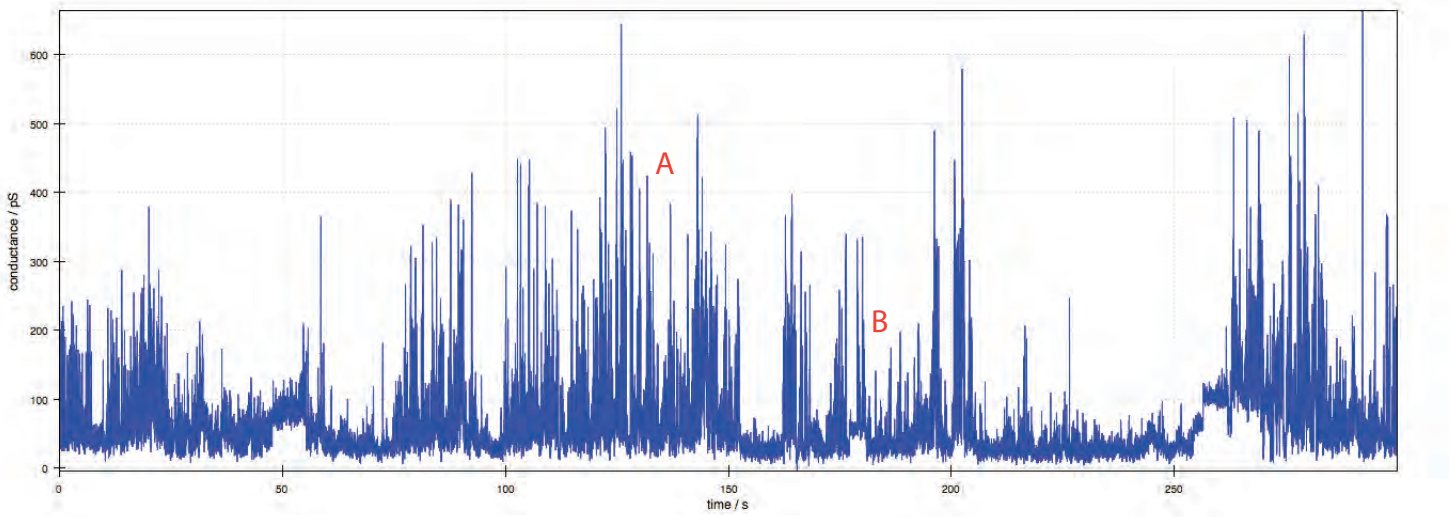
C



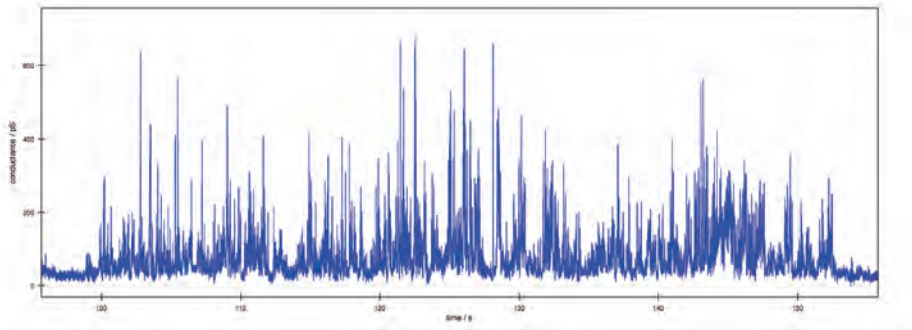
D



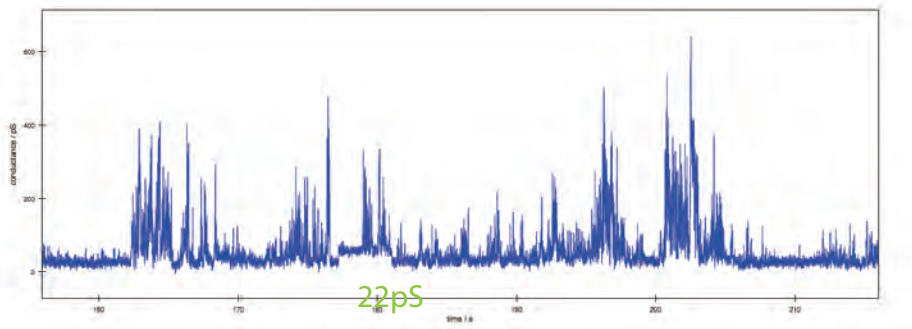
116-0004

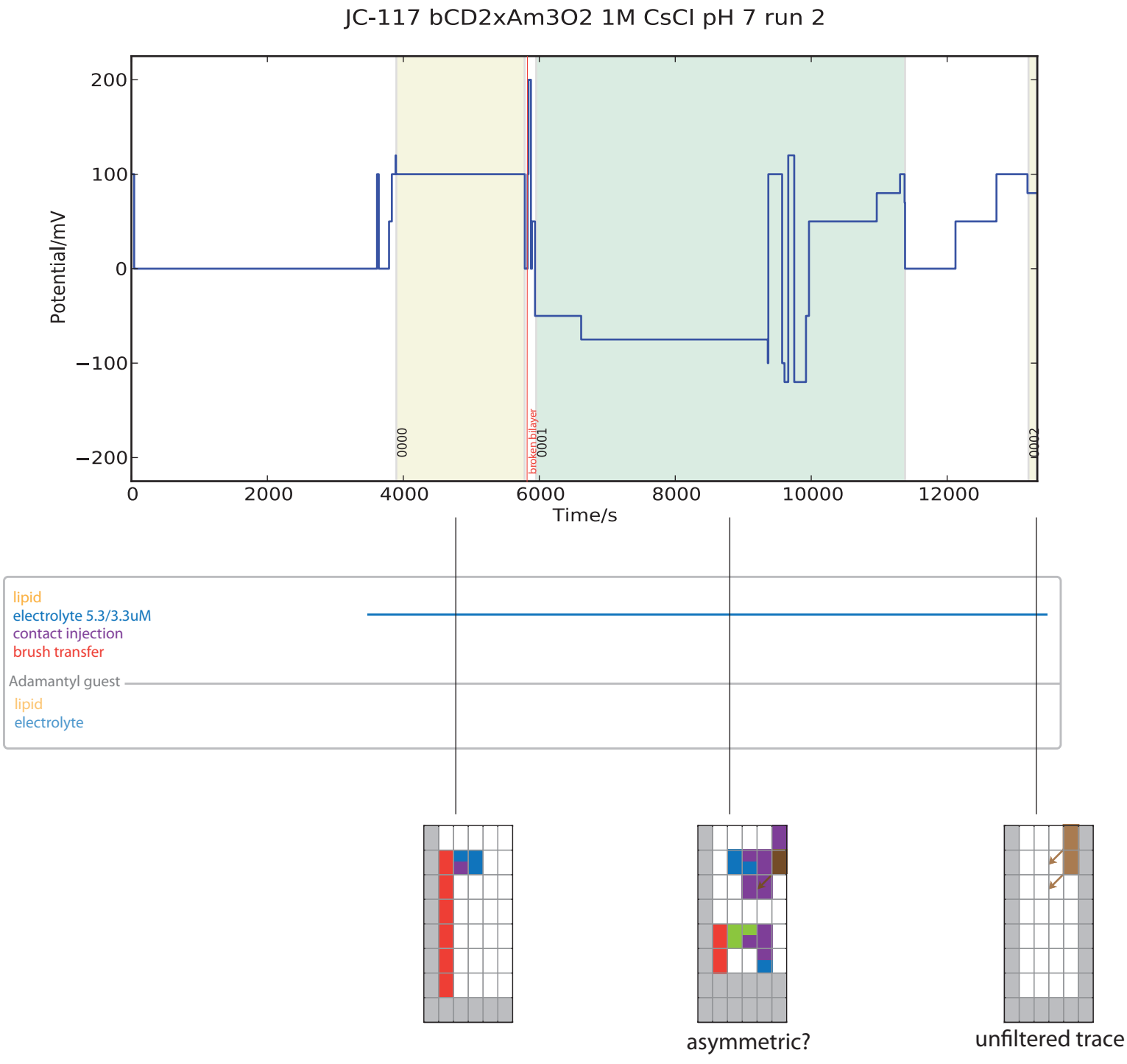


A

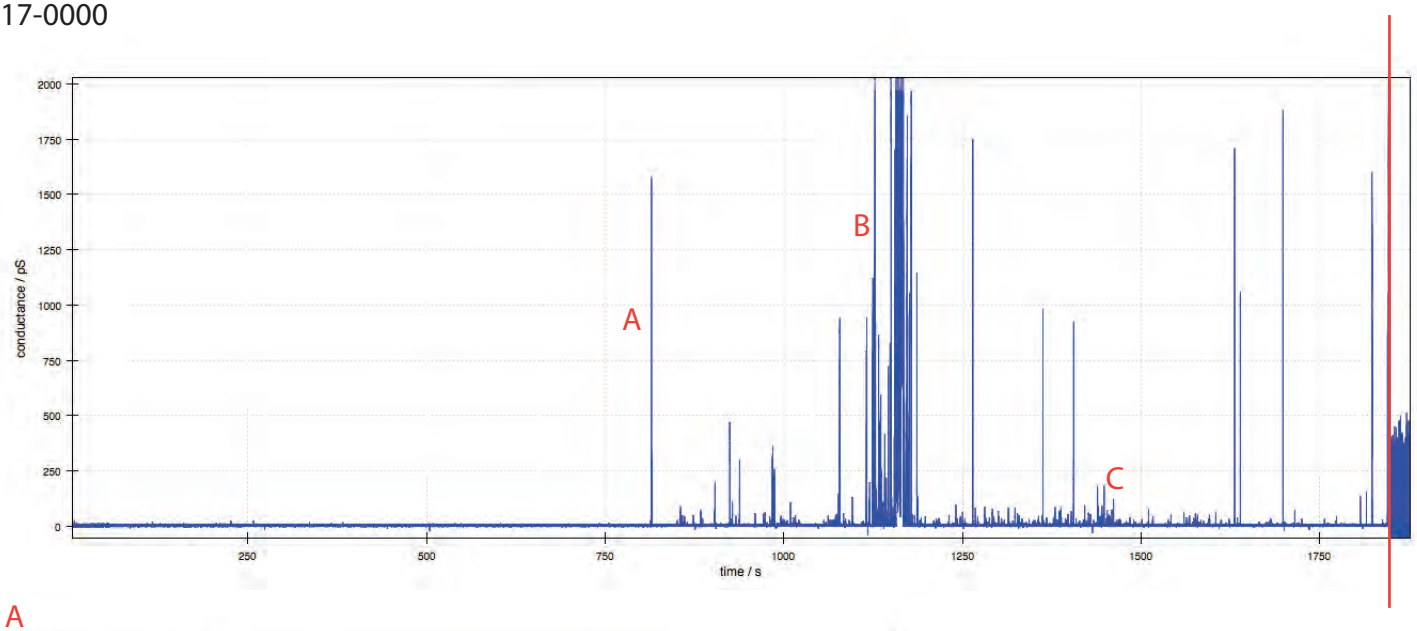


B

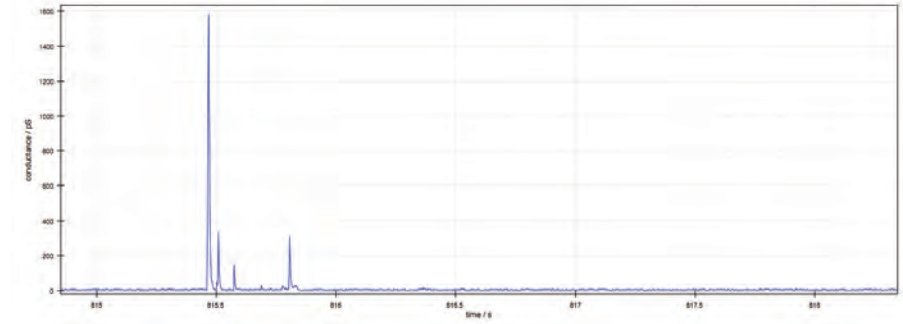



JC-117 bCD2xAm3O2 1M CsCl pH 7 run 2
Potential/mV
200
100
0
−100
−200
0
2000
4000
6000
8000
10000
12000
Time/s
0000
broken bilayer
1000
0002
lipid
electrolyte 5.3/3.3uM
contact injection
brush transfer
Adamantyl guest
lipid
electrolyte
asymmetric?
unfiltered trace

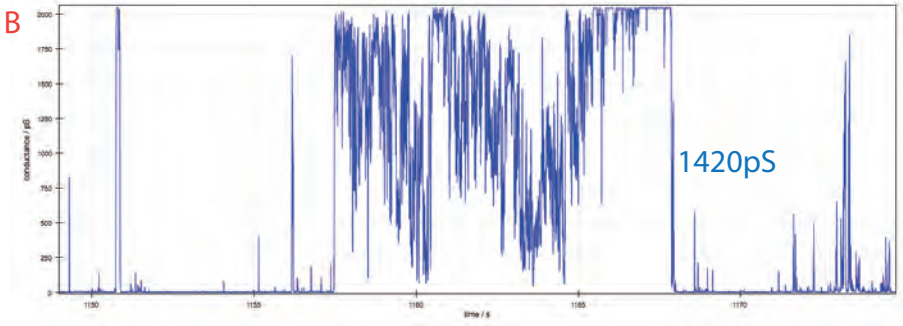
117-0000



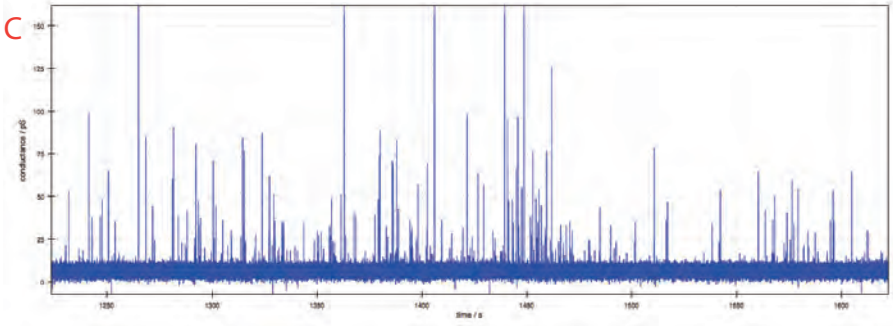
A



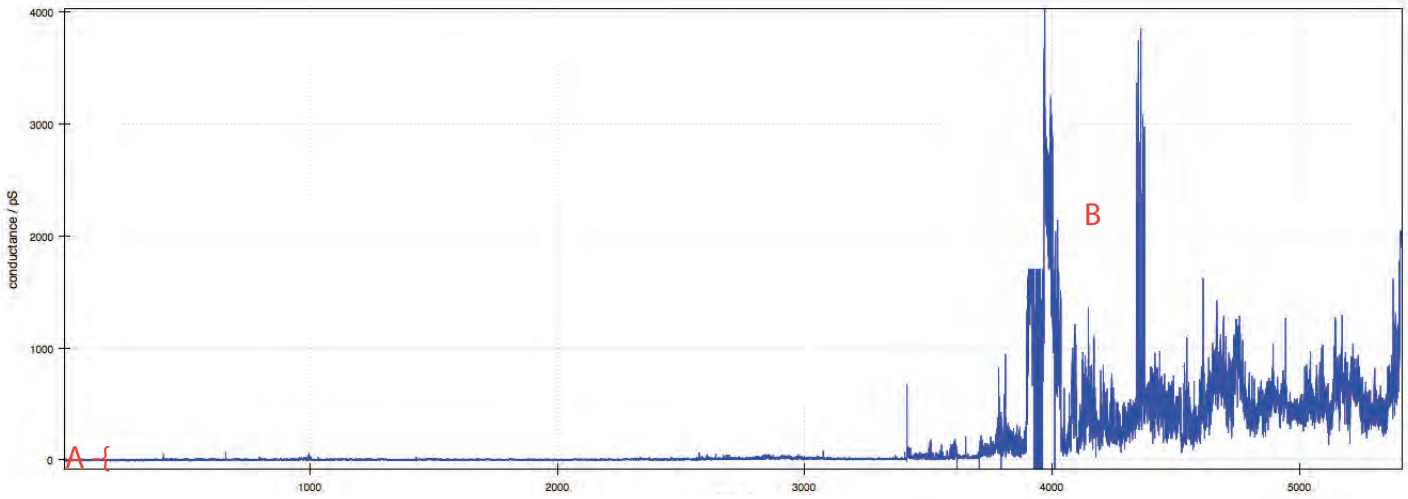
B

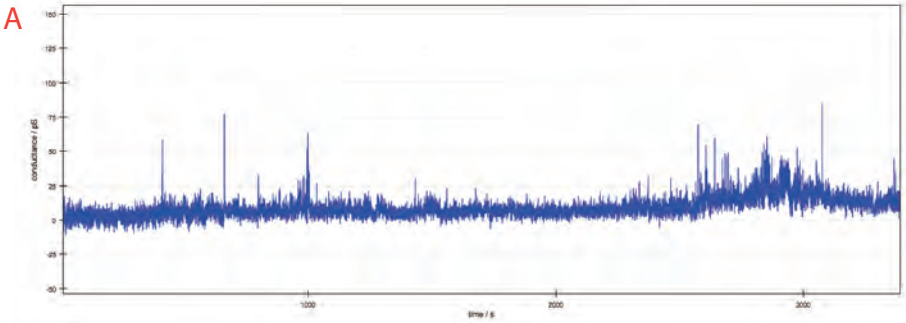


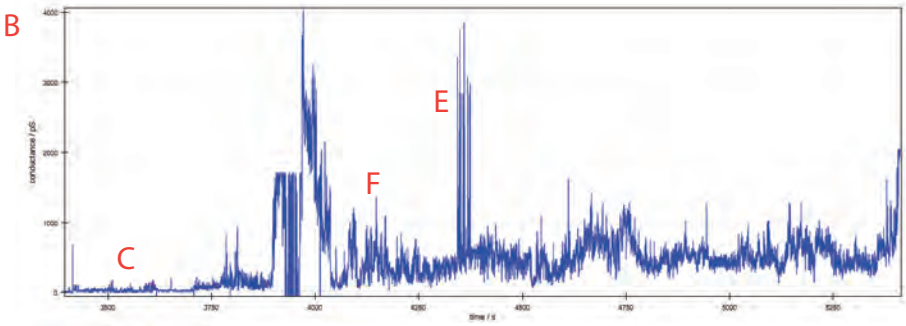
C

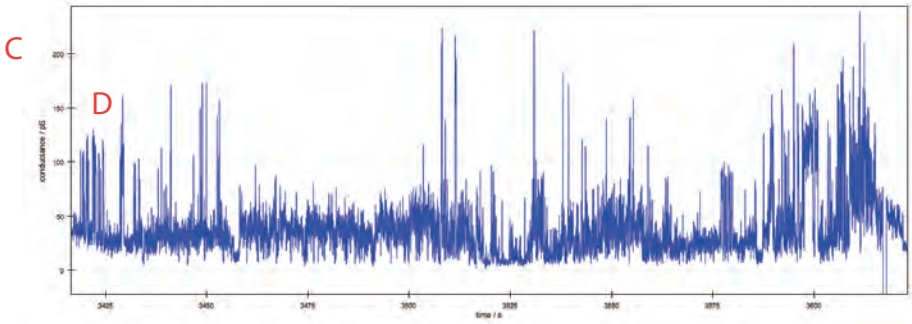


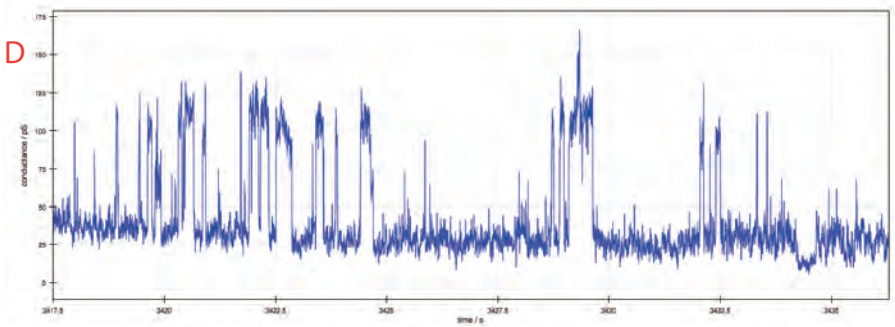
117-0001


conductance / pS
4000
3000
2000
1000
0
A
B
1000
2000
3000
4000
5000

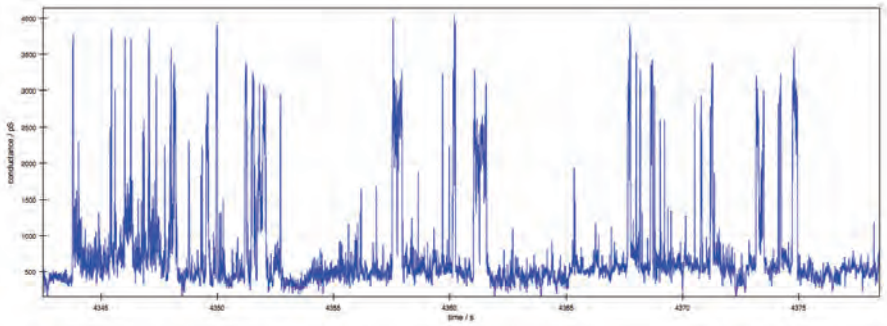

A


B
C
E
F

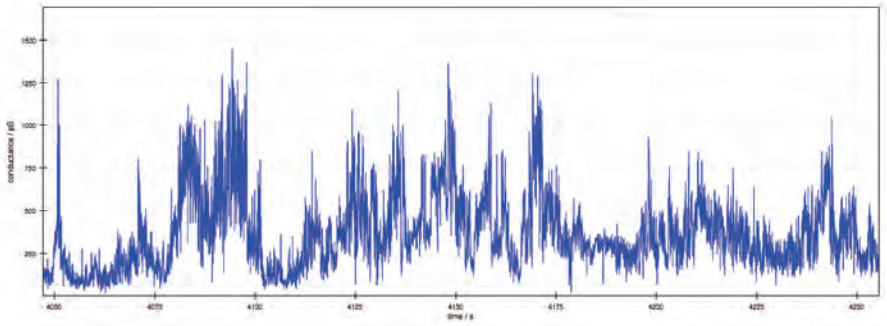

C
D


D

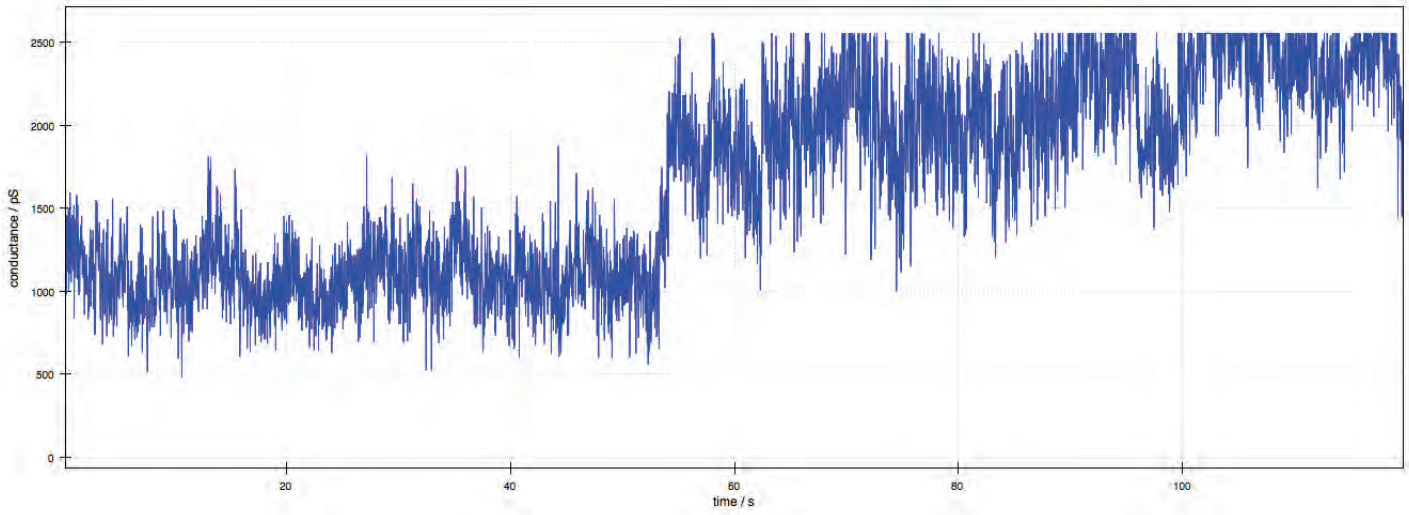
E

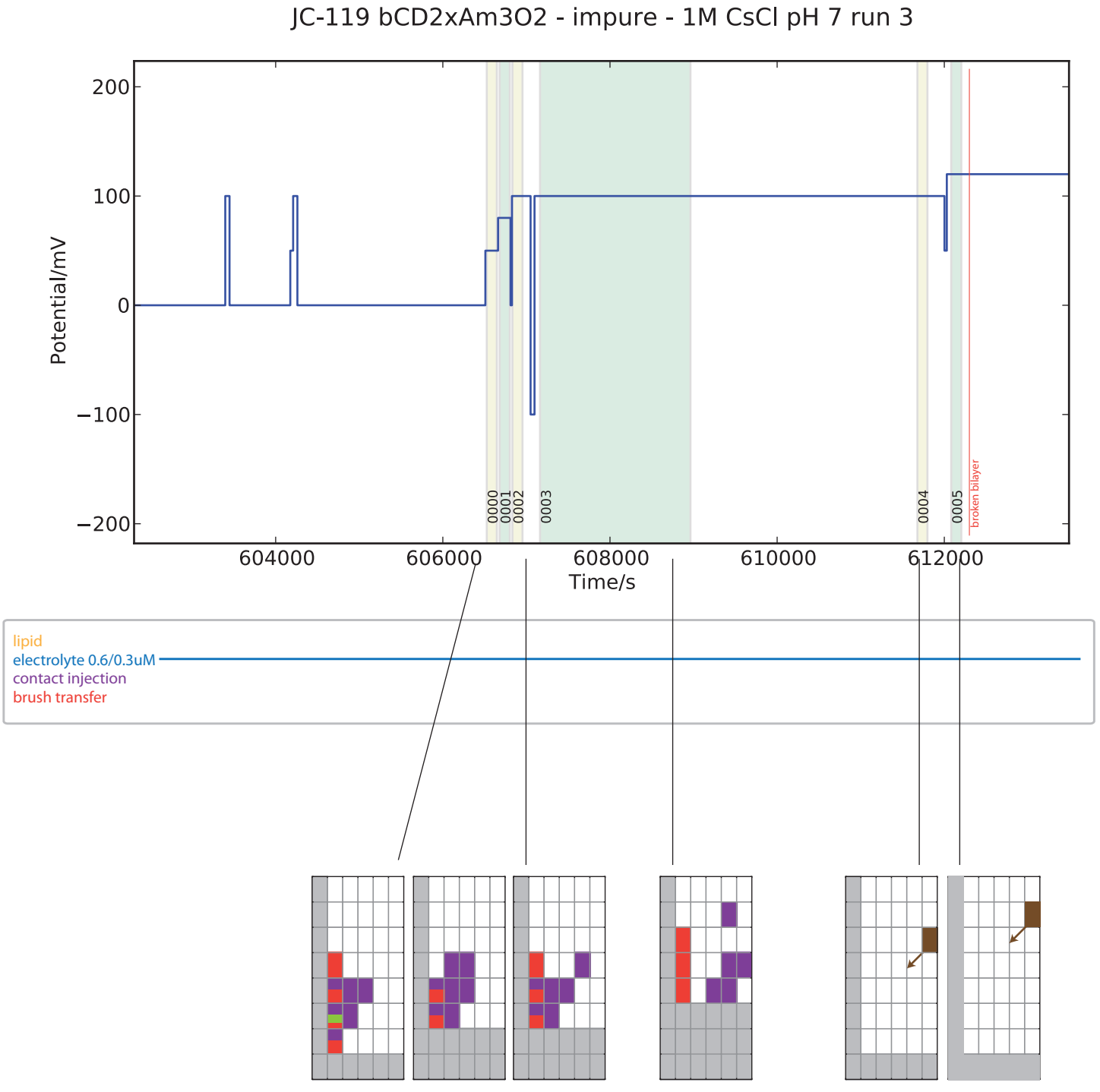


F

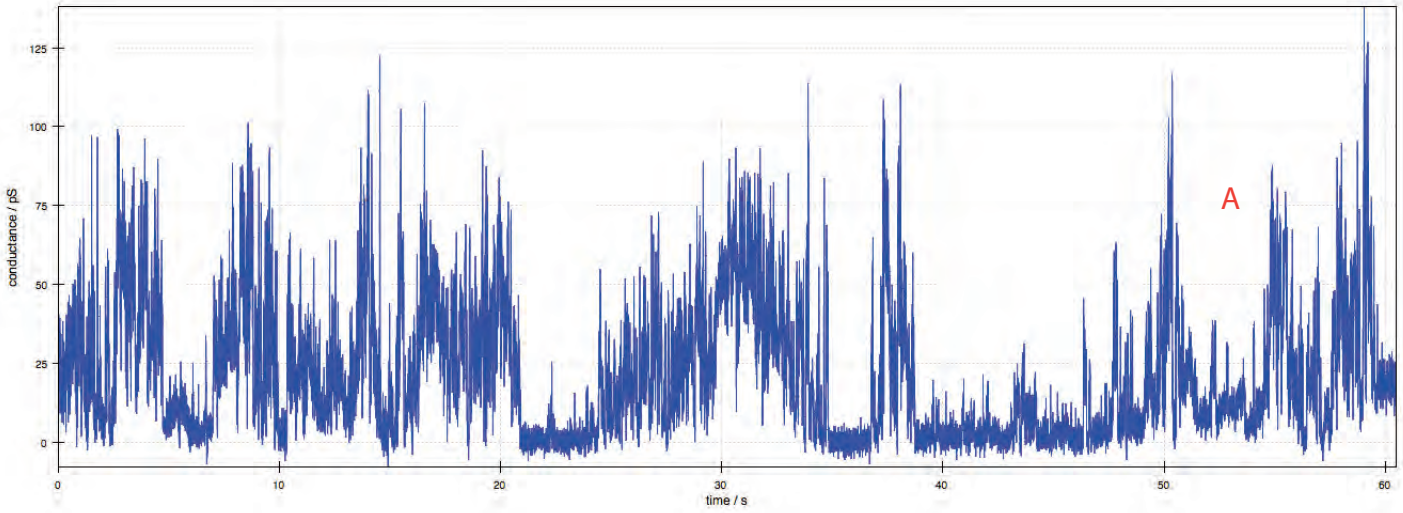


117-0002

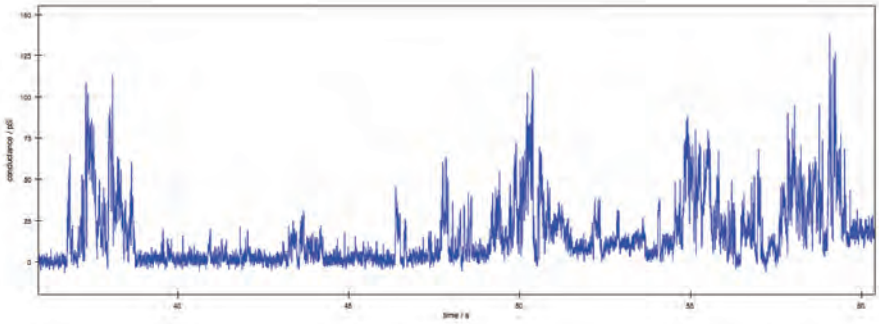



JC-119 bCD2xAm3O2 - impure - 1M CsCl pH 7 run 3
Potential/mV
200
100
0
−100
−200
604000
606000
608000
610000
612000
Time/s
0000
0001
0002
0003
0004
0005
broken bilayer
lipid
electrolyte 0.6/0.3uM
contact injection
brush transfer

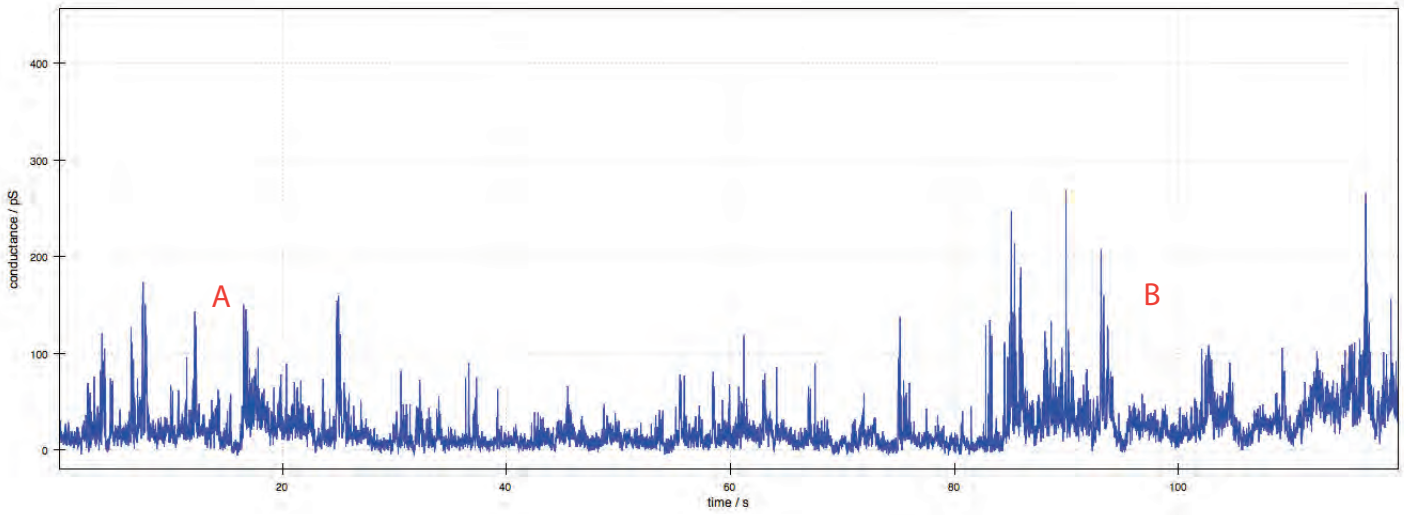
119-0000



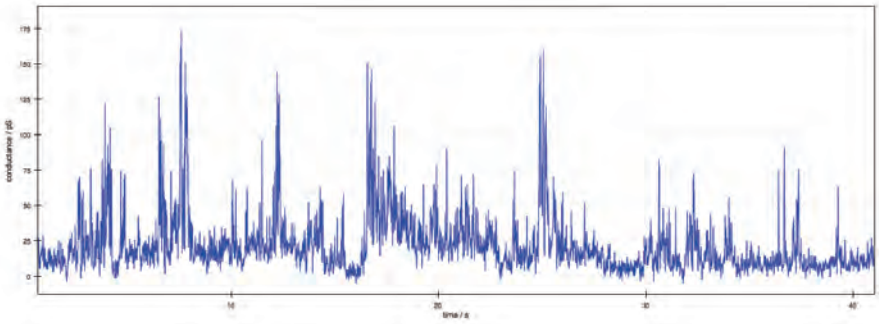
A



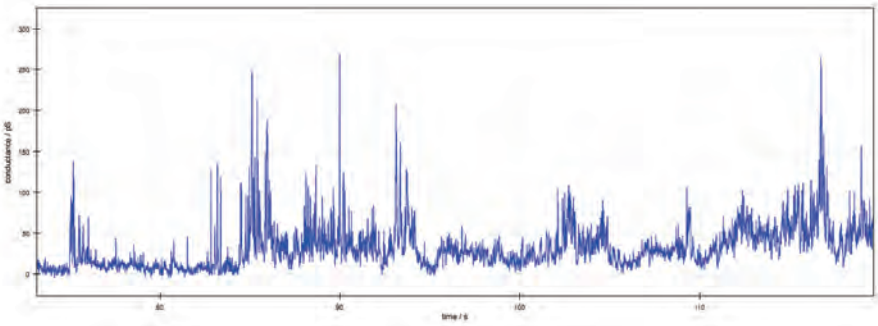
119-0001



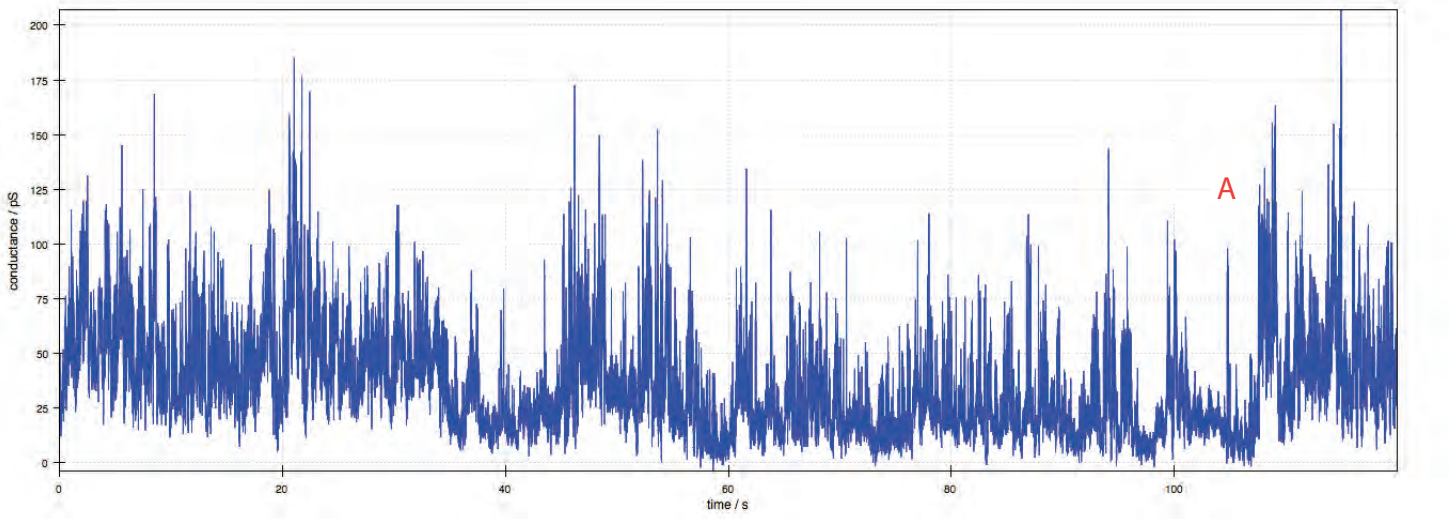
A



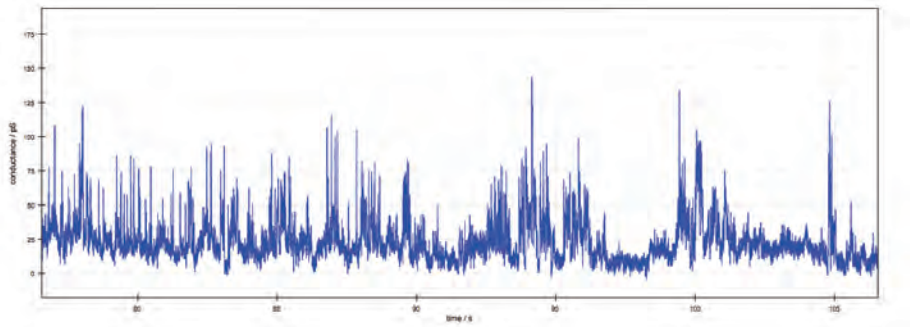
B



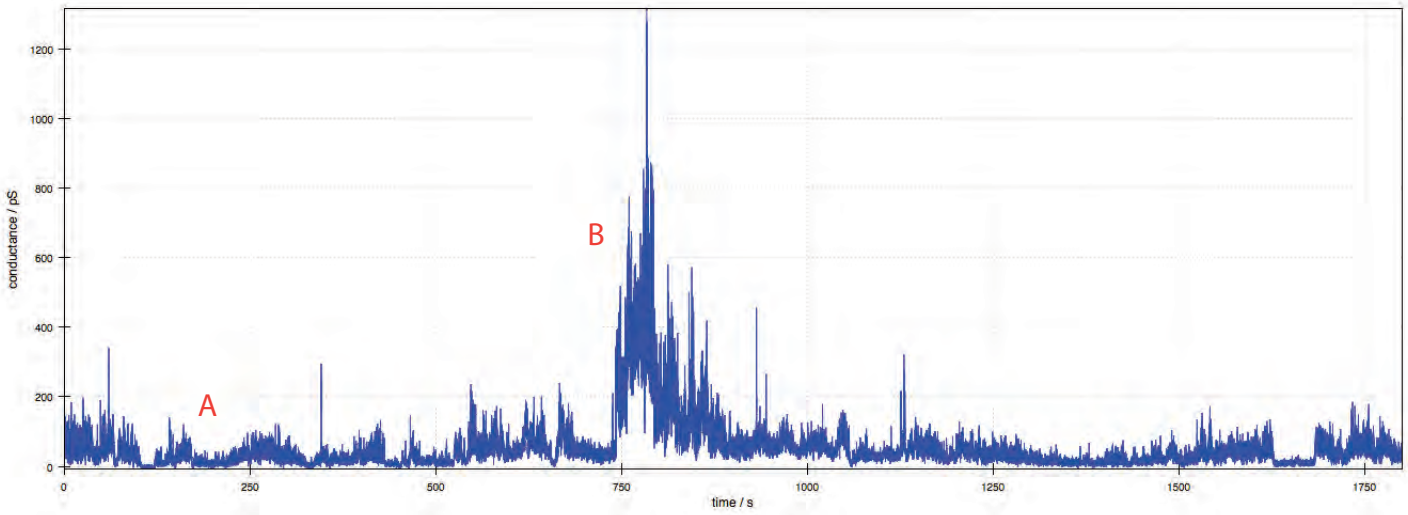
119-0002



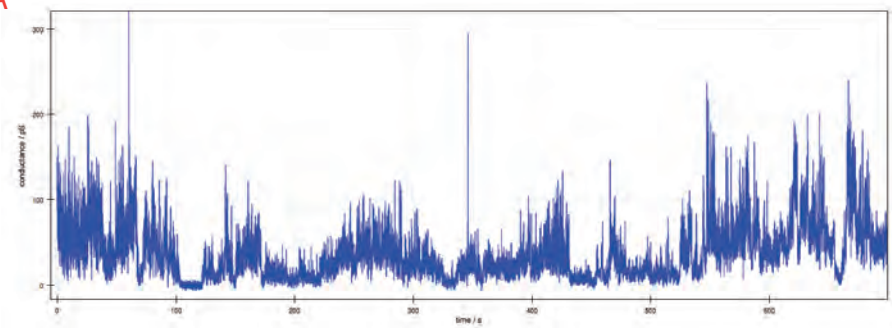
A



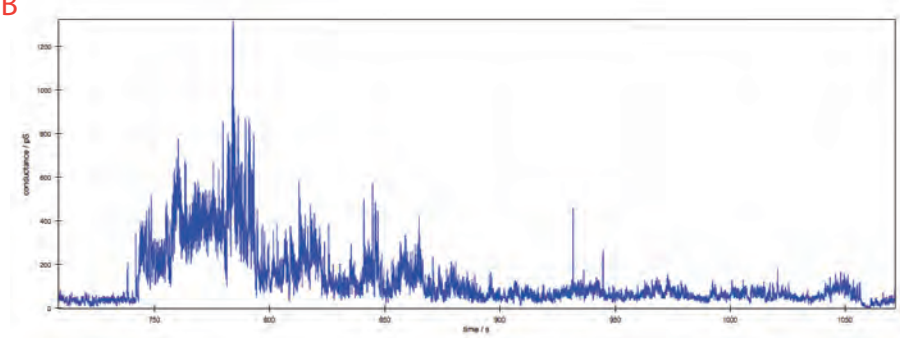
119-0003



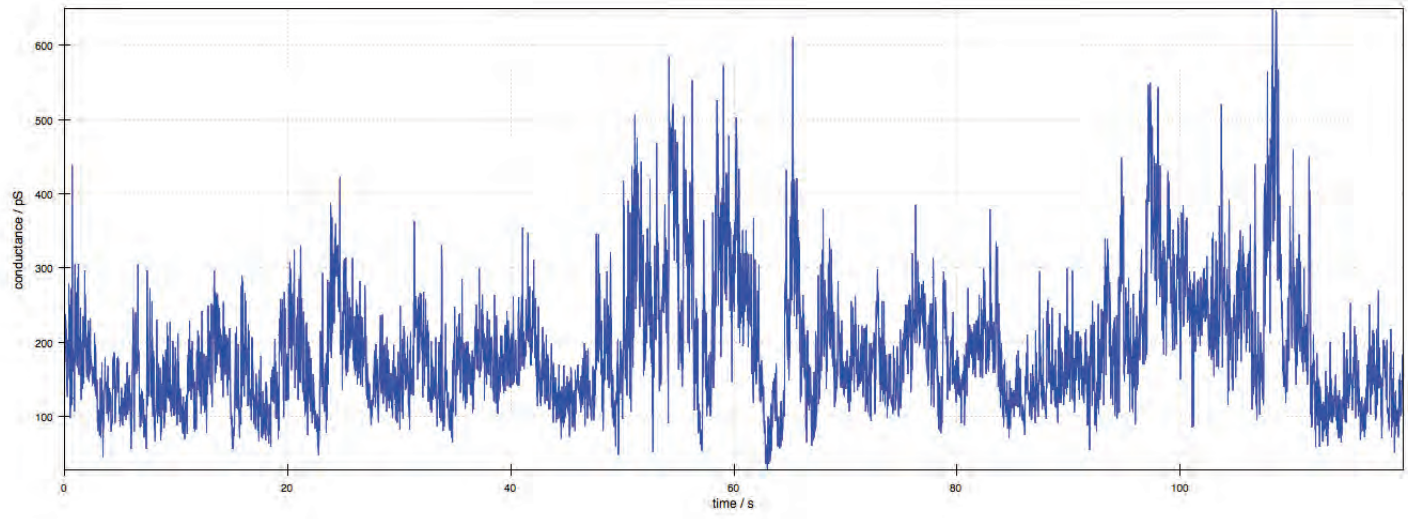
A



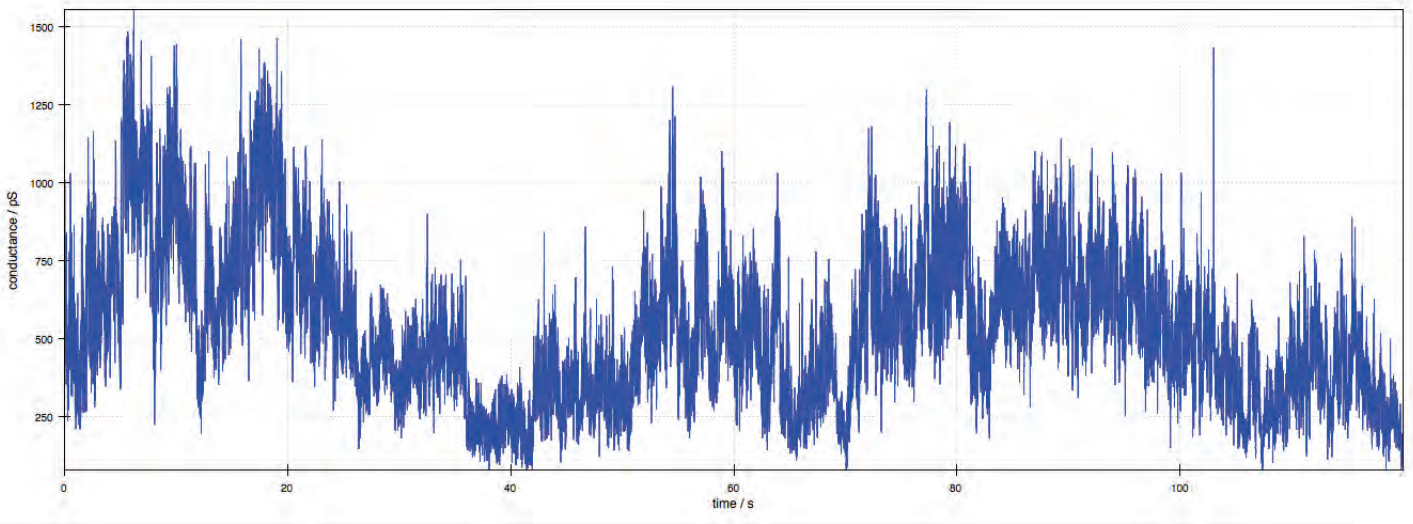
B

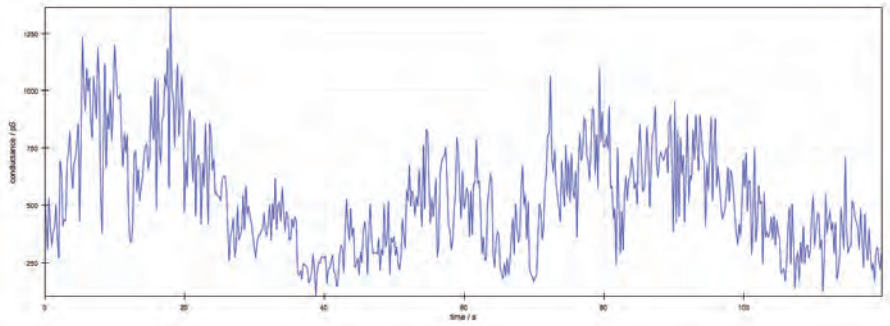


119-0004

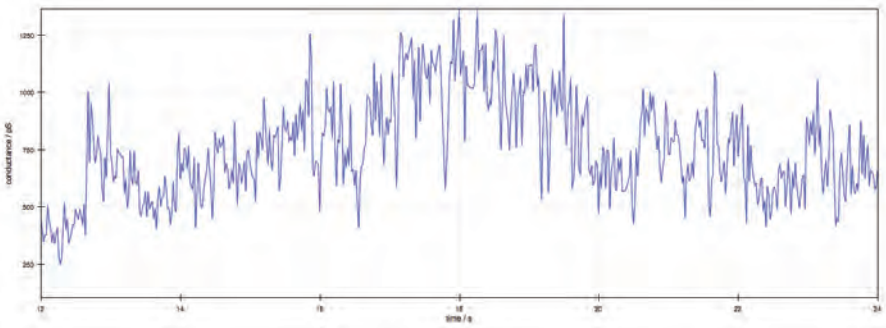


119-0005

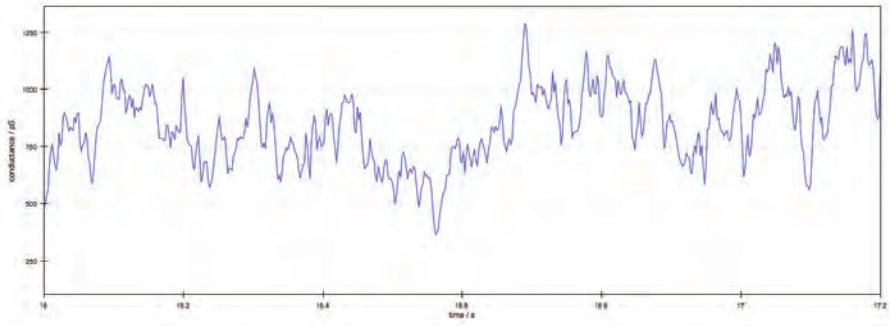




1000x decimation



100x decimation



10x decimation