Detecting metabolic activities of bacteria using a simple carbon nanotube device for high-throughput screening of anti-bacterial drugs

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Abstract

In this contribution, we demonstrate a simple carbon nanotube device to electrically detect the presence of bacteria (*E. coli*) with high sensitivity (<100 cfu/mL) and the glucose triggered metabolic activities of bacteria in real-time. As proof-of-concept demonstration, we also show that this nanoelectronic approach can be employed for high-throughput screening of anti-bacterial drugs.

Keywords: carbon nanotube, bacteria, nanoelectronic biosensors, drug screening

1. Introduction

One-dimensional single-walled carbon nanotubes (SWCNTs) configured as field-effect transistors (FETs) have been used to electrically, rapidly, and label-freely detect various biomolecules with high sensitivity (Allen et al. 2007; Byon et al. 2008; Gruner 2006; Huang et al. 2009; Kim et al. 2007; Sudibya et al. 2009). As the current flows solely on the surface, the conductance of SWCNT is highly sensitive to electrochemical disturbance imposed by the interacting biomolecules. Recently, nanoelectronic biosensors constructed by SWCNTs have also been used to detect the presence of bacteria (Garcia-Aljaro et al. 2010; So et al. 2008; Villamizar et al. 2008). So et al have demonstrated a FET device with single- or a few SWCNTs as the conducting (sensing) channel to detect *E. coli* with a detection limit of 10^3 cfu/mL (So et al. 2008). In the work of Vallamizar and co-workers, FETs based on a network of SWCNTs were developed to detect *Salmonella Infantis* with a detection limit of 100 cfu/mL (Villamizar et al. 2008). Comparing to the conventional culturing method and the state-of-the-art methods (namely, polymerase chain reaction -PCR or enzyme-linked immunosorbant assays – ELISA) which require specialized skills and are time consuming (hours to days), the nanoelectronic approach is simple, rapid and amenable for the development of portable lab-on-a-chip systems. However, these previously demonstrated SWCNT-FETs involve microfabrication of metal electrodes and in-situ chemical vapor deposition (CVD) growth of SWCNTs (Abe et al. 2007; Star et al. 2003; Star et al. 2006; Tang et al. 2006), which are non-trivial and not readily accessible to most labs.

Herein, we present a cheap SWCNT-network device readily made on bench-top and its ability to detect bacteria (*E. coli*) at low concentration (<100 cfu/mL). More importantly, we demonstrate, for the first time, that coupling the SWCNT-network FET with bacteria

enables real-time detection of the triggered metabolic activities of bacteria as an indicator of their susceptibility to antibacterial drugs. Such simple SWCNT nanoelectronic sensors would be instrumental to microbiologist for high-throughput functional studies and to pharmaceutical companies for high-throughput drug screening which is critical to fight the rapid evolving of bacterial drug-resistance.

2. Materials and Methods

2.1 Fabrication of SWCNT-network device

Carboxylated SWCNTs (Carbon Solutions) were dispersed in DI water by probe sonication for 30 min (0.02 mg mL^{-1}), followed by centrifugation (18000 rpm, 30 min) and filtration to remove large bundles and impurities. SWCNT-network was formed by drop-casting aqueous SWCNTs solution (3 µL) onto a glass coverslip and dried in 60°C oven. Two electrodes (source and drain) were subsequently prepared cross the SWCNT-network using silver conductive paint (RS Component). Finally, silicone rubber (Dow Corning) was used to insulate the electrodes and form the chamber for bacteria culturing and recording. Before culturing bacteria on it, SWCNT-network device was sterilized by immersion in 70% alcohol for 2 minutes, rinsed with DI water, and subsequently coated with poly-L-lysine (0.01 mg mL^{-1}).

2.2 Preparation of E. coli

E. coli K12 ER2925 (New England Biolab) was used in this study as the cell model. A stock culture of *E. coli* was prepared by growing the cells overnight in Luria Bertani (LB) medium at 37°C with continuous agitation. The harvested *E. coli* solution was stored at -80°C in 30% glycerol. Ten-fold serial dilutions of the stock culture were prepared in 0.85% sterile saline solution and 0.1 mL of each was plated on LB agar, and the number of colonies after overnight incubation at 37°C was counted for quantification. Further

dilutions in PBS solution (10 mM, pH 7.2) were made to produce the desired final concentration of *E. coli* for experiments.

2.3 Electrical measurements.

A 400 mV source-to-drain voltage was applied across SWCNT-network while the current was continuously monitored using a semiconductor device analyzer (B1500A, Agilent). All measurements were carried out in 10 mM PBS (pH 7.2) with an Ag/AgCl reference electrode (V_g=0 V) immersed in solution. The PLL-coated SWCNT device was exposed to certain concentrations of *E. coli* in the absence or presence of the testing antibiotics: Ampicillin (Sigma), Chloramphenicol (Sigma), Kanamycin (Calbiochem) or Penicillin-Streptomycin mixture (Gibco).

3. Results and discussion

The carboxylated SWCNTs well dispersed in DI water were drop-casted onto a coverslip (as illustrated in Figure 1A). At a mildly elevated temperature (60°C), the SWCNTs swam to the edge of the droplet during water evaporation due to the interstitial capillary force and formed a ring of thin-film SWCNT-network with a diameter of ~ 3 mm and edge width of ~140 μm (Figure 1B-E). After being washed and dried, the source and drain electrodes were prepared across the SWCNT device using conductive silver paste, followed by coating of silicone rubber to insulate the electrodes and to define the recording chamber. Finally, positively charged poly-L-lysine was coated on the SWCNT-FET device to promote the attachment of bacteria whose surface is populated with negatively charged lipopolysaccharides.

(Fig. 1)

During the experiments, PBS solution (300 μL) was added in the recording chamber and an Ag/AgCl electrode was immersed to apply gate voltage (V_g). At V_g = 0 mV, the

average conductance of our SWCNT device is about 15.4 +/- 1.4 μS (n=15). While biasing the device at V_{ds} = 400 mV and the liquid-gate electrode at V_g = 0 mV, introduction of *E. coli* to the final concentration of 100 cfu/mL caused obvious decrease of the device current over time (Figure 2A). The current (or conductance) decrease reached a plateau in ~2 hours as the bacteria gradually settled down onto the SWCNT network. Appreciable response could be noticed at 20th minute with a signal-to-noise ratio of ~10 (~32 nA current decrease vs. ~3 nA current noise). In contrast, the SWCNT conductance remained stable in the absence of bacteria. Figure 2B depicts a SEM image of an *E. coli* on SWCNT network. The rate of bacteria attachment at the concentration of 10^5 cfu/mL was shown in Figure 2C as quantified under the phase-contrast optical microscope. As shown in Figure 2D, the electrical characteristics (conductance versus V_g) were significantly altered by the attachment of bacteria in an incubation-time (bacteria-density) dependent manner.

(Fig. 2)

Our experiments corroborated the ability of SWCNT sensors to detect bacteria. The achieved detection limit (< 100 cfu/mL) is comparable to the state-of-the-art ELISA analysis (Sunwoo et al. 2006). The observed conductance decrease induced by bacteria is consistent with the previously reported SWCNT sensors (So et al. 2008). But this is not the field-effect due to the negatively charged bacterial surface because the *p*-type characteristics of SWCNT-FET (Figure 2D) would predict an increase in conductance if it is the case. It is conceivable that the conductance decrease may be attributable to doping effects imposed by the interacting biomolecules on the bacterial surface or scattering effects imposed by the charged interacting molecules on bacteria. The underlying mechanisms deserve further investigations.

It is known that glucose metabolism in bacteria leads to release of organic acids (e.g., pyruvic, citric, and lactic acids), and consequently, extracellular acidification (Solé et al. 2000). We hypothesized that discharge of organic acids into the nano-gap between the SWCNTs and the interfacing bacterial surface would significantly alter the local pH (i.e., the local electrochemical environment) at the vicinity of SWCNTs, thus possibly change the SWCNT conductance (Figure 3A). We first verified that the conductance of our carboxylated SWCNTs is actually highly responsive to pH change as shown in Figure S1 in the supplementary material.

(Fig. 3)

To test our hypothesis, the SWCNT-network FETs were first incubated with *E. coli* suspension in PBS (10^5 cfu/mL) for 1 or 3 h, followed by gentle rinse and replacement with fresh PBS. While the SWCNT current was continuously monitored, glucose was added into the recording chamber to reach different final concentrations. Indeed, as expected, when glucose (1 mM) was applied to a SWCNT device pre-incubated with *E. coli* for 1 h, immediate increase of SWCNT current was observed (Figure 3B, middle trace), and the current further increased upon addition of new doses of glucose. Similar recording from a SWCNT device pre-incubated with *E. coli* for a longer time (3 h) was shown in parallel (Figure 3B, top trace). With higher density of deposited bacteria, this device produced a larger response at a given glucose concentration. In comparison, glucose was not able to trigger any response from a device without bacteria (Figure 3B, bottom trace). The statistics of these experiments is summarized in Figure 3C. As seen, the magnitude of SWCNT response is positively correlated with the concentration of glucose and the bacteria density (the pre-incubation time). These observations clearly

suggest that the SWCNT responses were resulted from the glucose-induced bacterial metabolism.

Current detection methods, such as PCR, are not able to distinguish dead cells from live ones. Our detection of glucose metabolism can provide unambiguous evidence of the existence of live bacteria, with a signal strength reflecting their number. The metabolic activities of bacteria can serve as an indicator of their susceptibility to drugs (Deland 1972). Antibacterial drugs jeopardize bacterial survival by inhibiting protein or DNA synthesis, attacking the outer bacterial wall, inhibiting synthesis of outer wall or inner membrane, or combination of the above. These actions directly or indirectly impair the metabolic activities of such simple life forms.

As the proof-of-concept demonstration, we examined the effects of several common antibacterial drugs (ampicillin, kanamycin, chloramphenicol, mixture of penicillin and streptomycin) on glucose metabolism using our SWCNT-network FETs. The devices were pre-incubated with 10^5 cfu/mL *E. coli* for 3 h in the presence of the testing antibacterial drug (at a dose of 10 μg/mL), followed by replacement with fresh PBS. The drugs did not affect the bacteria attachment onto SWCNTs. The inhibitory effects of these drugs on the induced metabolic activities of *E. coli* (indicated by increase of SWCNT conductance) at different concentrations of glucose are shown in Figure 3D.

Ampicillin, which inhibits a critical enzyme (transpeptidase) for peptidoglycan synthesis of bacterial cell wall, significantly reduced the SWCNT response to the application of 55 mM glucose by ~33.8 % as compared to the response without the drug. Kanamycin and chloramphenicol, which inhibit protein synthesis, gave more profound inhibition. The cocktail of penicillin and streptomycin was commonly used to prevent infection in animal cell culture, because these two antibiotics can synergistically, thus

more effectively, kill bacteria. Inhibition of peptidoglycan synthesis by penicillin facilitates penetration and disruption of bacterial wall by streptomycin. Therefore, not surprisingly, the mixture of penicillin and streptomycin (10 μg/mL altogether) produced most potent inhibition on bacterial metabolism. And as anticipated, reducing the dose of a drug reduces its inhibitory effect (data not shown). Bacteria can grow confluently on our devices to form a biofilm. It is known that bacterial biofilm, whose formation involves in a variety of infectious diseases (e.g., the fatal cystic fibrosis), is more resistant to antibacterial drugs. In agreement with this notion, we found that the inhibitory effects of all the tested drugs were less potent on *E. coli* biofilms (Figure S2 in the supplementary material).

4. Conclusions

In summary, we demonstrate a simple SWCNT device for high-throughput detection, functional studies, and drug screening of bacteria. In the future work, the recognition elements, such as antibodies (Garcia-Aljaro et al. 2010) or aptamers (So et al. 2008), could be functionalized onto SWCNTs for detection of specific bacterial strains. With the rapid advance of SWCNT based nanoelectronics (Luo et al. 2008; Wei et al. 2009; Zhao et al. 2009), we envision that their sensitivity and capability would be greatly extended. And nanoelectronic biosensors based on SWCNT and other nanostructured materials (e.g., nanowire (Eschermann et al. 2009; Lin et al. 2008; Patolsky et al. 2006), graphene (Dong et al. 2010; Huang et al. 2010; Lu et al. 2009; Mohanty and Berry 2008) will continue to make significant impacts in various aspects of biomedicine. Nanoelectronic biosensors have been coupled with animal cells to detect their dynamic activities for high-throughput functional studies (He et al. 2010; Huang and Chen 2010; Pui et al. 2009a; Pui et al. 2011; Pui et al. 2009b) and high-throughput drug screening (Pui et al. 2010). The

herein demonstrated nanoelectronics-microoragnism interface adds a new dimension to this emerging technology.

Acknowledgment

We acknowledge the support from an A*Star SERC grant (#072 101 0020).

Supplementary material

Additional information as noted in text. This material is available free of charge via the Internet at http://www.sciencedirect.com.

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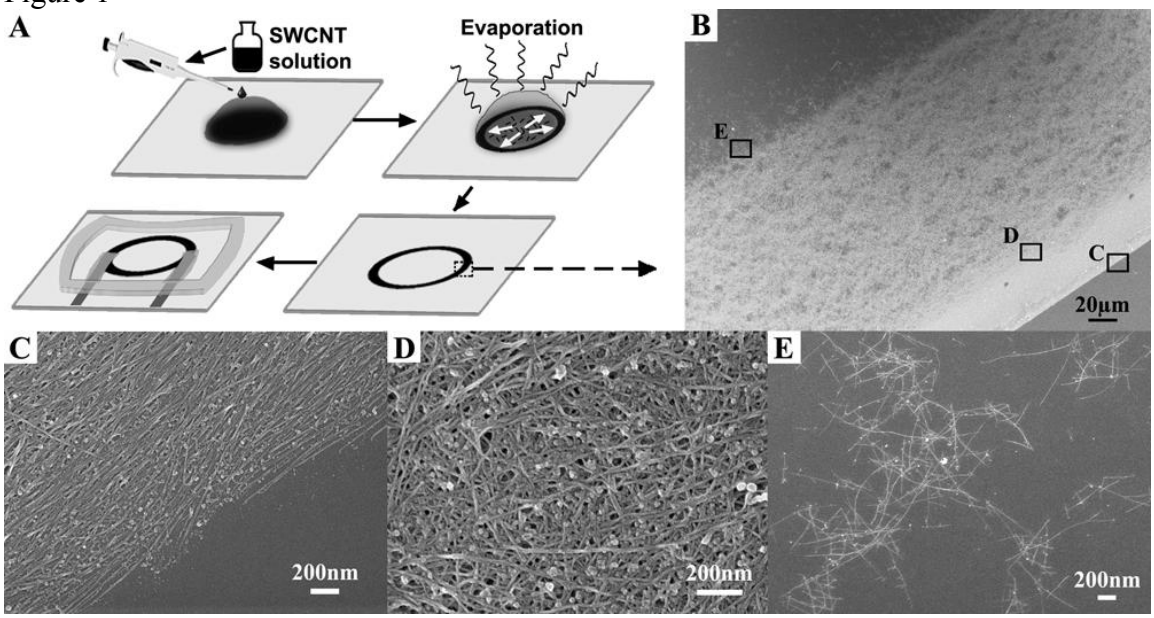
Figure legends:

Figure 1. A) Schematic illustration of SWCNT-network device fabrication. B) Scanning electron microscopy (SEM) image of the resulting SWCNT-network ring. C), D) and E) Zoom-in SEM images of different regions on SWCNT-network as indicated by the squares in B.

Figure 2. A) Percentage change of SWCNT-network conductance over time without (squares) or with (circles) addition of *E. coli* (100 cfu/mL). Each data point is the average from 3 devices. The error bars indicate the standard errors. B) SEM image of an E. coli on poly-L-lysine (PLL) coated SWCNT-network. C) Number of *E. coli* per mm^2 attached on SWCNT-network (counted under phase-contrast optical microscope), when SWCNT-network was incubated with *E. coli* (10^5 cfu/mL) for 15 min or 1 h or 3 h. D) Conductance vs. gate voltage of bare PLL-coated SWCNT devices (circles), devices pre-incubated with *E. coli* (10^5 cfu/mL) for 1 h (squares) or 3 h (triangles). The conductance is normalized to the maximum conductance of the bare devices. Each data point is the average from 6 devices. The error bars indicate the standard errors.

Figure 3. A) Illustration of SWCNT-FET and bacteria interface for detection of bacterial metabolic activities as an indicator of susceptibility to antibacterial drugs. B) Real-time current recordings (V_{ds} = 400 mV and V_g = 0 mV) of a SWCNT device pre-incubated with *E. coli* (10^5 cfu/mL) for 3 h, a device pre-incubated with *E. coli* for 1h, and a bare SWCNT device, with application of glucose to the PBS recording buffer at indicated time points to reach the concentrations of 1, 3, 6, 10, and 15 mM. C) Percentage change in conductance of bare SWCNT devices (circle), devices pre-incubated with *E. coli* for 1h (squares), and devices pre-incubated with *E. coli* for 3h (triangles), upon application of different concentrations of glucose. Each data point is the average from 6 devices. The error bars indicate the standard errors. D) Percentage change in conductance of SWCNT devices pre-incubated with *E. coli* for 3h without (squares) or with antibacterial drugs (10 μg/mL), upon application of different concentrations of glucose. Each data point is the average from 6 devices. The error bars indicate the standard errors.

Figure 1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solution
Evaporation
B
E
D
C
20μm
C
200nm
D
200nm
E
200nm

Figure 2

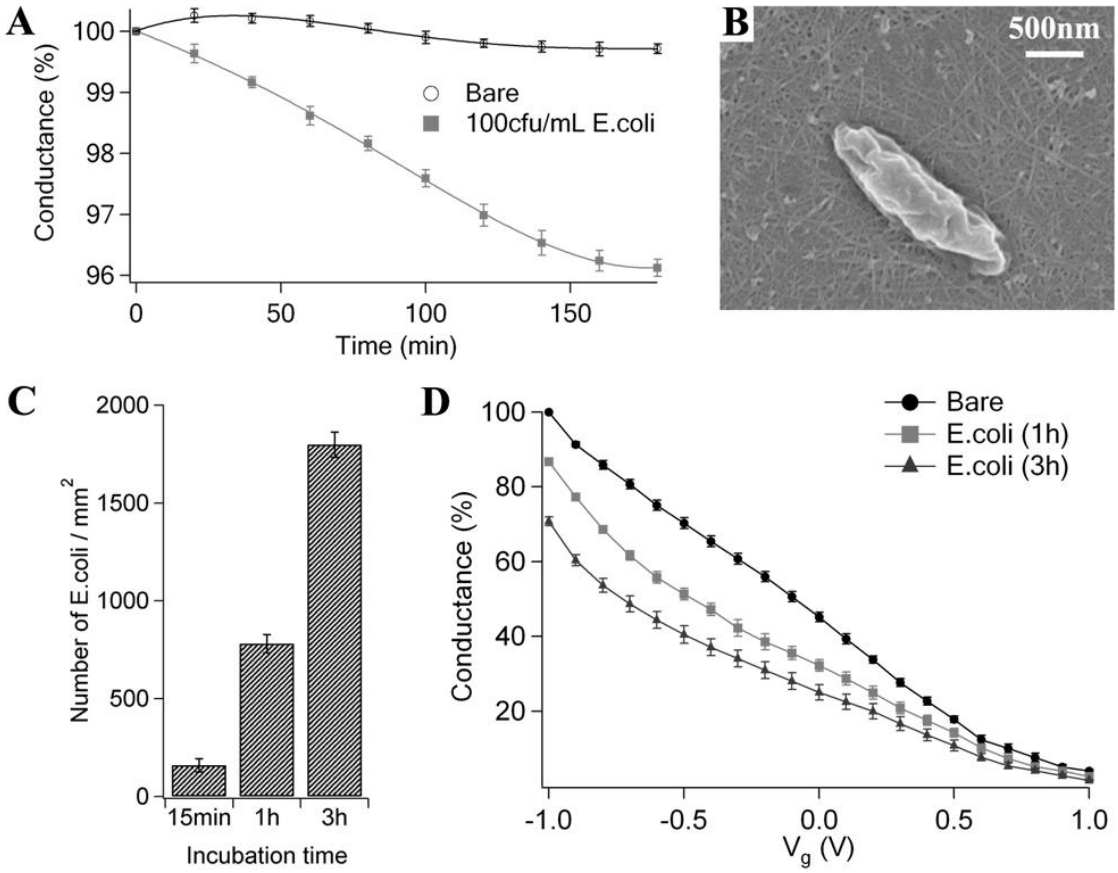
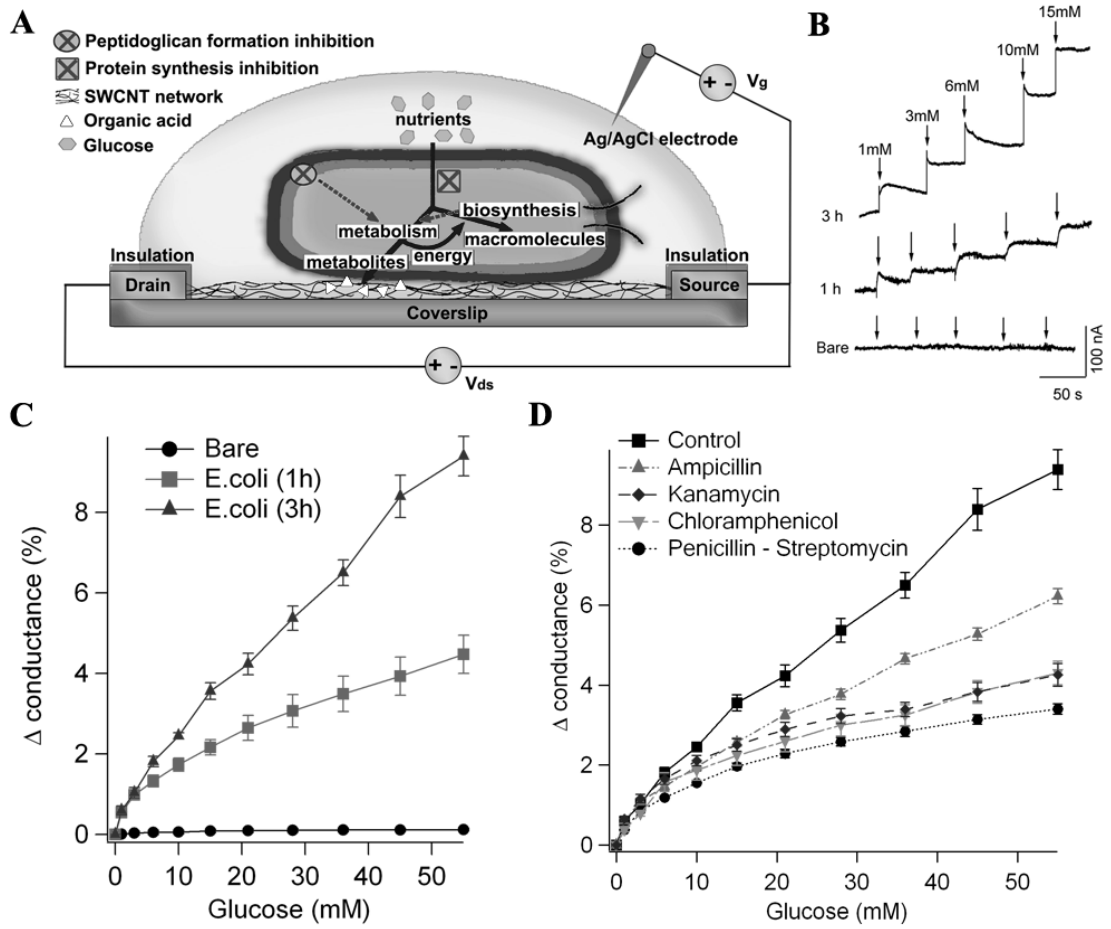

A
Conductance (%)
100
99
98
97
96
0
50
100
150
Time (min)
Bare
100cfu/mL E.coli
B
500nm
C
Number of E.coli / mm^2
2000
1500
1000
500
0
15min
1h
3h
Incubation time
D
Conductance (%)
100
80
60
40
20
-1.0
-0.5
0.0
0.5
1.0
V_g (V)
Bare
E.coli (1h)
E.coli (3h)

Figure 3


A
Peptidoglican formation inhibition
Protein synthesis inhibition
SWCNT network
Organic acid
Glucose
nutrients
Ag/AgCl electrode
Vg
biosynthesis
metabolism
macromolecules
metabolites
energy
Insulation
Insulation
Drain
Source
Coverslip
Vds
B
15mM
10mM
6mM
3mM
1mM
3 h
1 h
Bare
100 nA
50 s
C
Bare
E.coli (1h)
E.coli (3h)
Δ conductance (%)
Glucose (mM)
D
Control
Ampicillin
Kanamycin
Chloramphenicol
Penicillin - Streptomycin
Δ conductance (%)
Glucose (mM)