Title	Integrating carbon nanotubes and lipid bilayer for biosensing
Author(s)	Huang, Yinxi; Palkar, Preeti Vikas; Li, Lain-Jong; Zhang, Hua; Chen, Peng
Citation	Huang, Y., Palkar, P. V., Li, L. J., Zhang, H. & Chen, P. (2010). Integrating carbon nanotubes and lipid bilayer for biosensing. Biosensors and Bioelectronics, 25(7), 1834-1837.
Date	2009
URL	http://hdl.handle.net/10220/7432
Rights	© 2009 Elsevier. This is the author created version of a work that has been peer reviewed and accepted for publication by Biosensors and Bioelectronics, Elsevier. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: http://dx.doi.org/10.1016/j.bios.2009.12.011

Integrating carbon nanotubes and lipid bilayer for biosensing

Yinxi Huang[a], Preeti Vikas Palkar[a], Lain-Jong Li[b], Hua Zhang[b], Peng Chen[a],∗

[a] *School of Chemical and Biomedical Engineering, Bioengineering Division, Nanyang Technological University, 70 Nanyang Drive, Block N1.3, Singapore 637457, Singapore*
[b] *School of Materials Science and Engineering, Nanyang Technological University, Singapore 639798, Singapore*
∗ *Corresponding author.*
E-mail address: chenpeng@ntu.edu.sg (P. Chen).

ABSTRACT

Membrane proteins, which are the target of most drugs, are implicated in many critical cellular functions such as signal transduction, bioelectricity, exocytosis and endocytosis. Therefore, developing techniques to investigate the functions of membrane proteins is obviously important. Here, we have developed a novel system by integrating artificial lipid bilayer (biomimetic membrane) with single-walled carbon nanotube networks (SWNT-net) based field-effect transistor (FET), and demonstrated that such hybrid nanoelectronic biosensors can specifically and electronically detect the presence and dynamic activities of ionophores (specifically, gramicidin and calcimycin) in their native lipid environment. This technique can potentially be used to examine other membrane proteins (e.g. ligand-gated ion channels, receptors, membrane insertion toxins, and antibacterial peptides) for the purposes of biosensing, fundamental studies, or high throughput drug screening.

1. Introduction

Cell membrane mediates the complex communication and substance exchange between the cell and the environment or other cells *via* dynamic actions of molecular nanomachines associated with its lipid bilayer structure. Twenty–30% of the proteins encoded in the human genome are membrane proteins which are implicated in critical cellular functions such as signal transduction, bioelectricity, exocytosis and endocytosis (Wallin and von Heijne, 1998). On the other hand, cell membrane is an essential barrier to separate intracellular and extracellular space. Our immune system secretes a special class of proteins to attack intruding pathogenic bacteria by forming transmembrane channels (membrane attack complex) which allow water, ions, and other small molecules to move freely into and out of the bacteria, and quickly results in cell death. Similarly, bacteria secrete membrane attacking peptides (antibiotic or antibacterial ionophores) that are carriers of ions or can form transmembrane ion channels to kill the competing microorganisms nearby by eliminating the essential ionic gradients across the cell membrane. Not surprisingly, more than 60% of all drugs target on membrane proteins (Yildirim et al., 2007). Devising techniques to study the functions of membrane proteins is therefore of obvious significance for fundamental biology, diagnostics, and drug screening.

As proper functioning of membrane proteins requires association with lipid bilayer and cell membrane is too complex to isolate actions of a specific protein, systems based on artificial lipid bilayer (biomimetic membrane) have been employed (Chan and Boxer, 2007; Schuster and

Sleytr, 2006) to investigate the function of membrane-associated proteins, such as ionophores (Sato et al., 1998; Vallejo and Gervasi, 2007), pore-forming toxin (Bockenroth et al., 2008), receptors (Favero et al., 2005), voltage-dependent anion channel (Deniaud et al., 2007) and so on. Herein, we integrate artificial lipid bilayer with carbon nanotube devices and demonstrate that such hybrid nanoelectronic devices can be used to detect activities of membrane proteins in their native lipid environment.

2. Materials and methods

2.1. Fabrication of SWNT-net device

Carboxylated-SWNTs (Carbon Solution) were sonicated for 30 min and dispersed in DIH_2O, followed by centrifugation and filtering for removal of the large bundles and impurities. Subsequently, pentane was added on top of 0.05 mg/ml SWNT solution to form an organic layer above the SWNT suspension. Following injection of ethanol, SWNTs precipitated out and formed a visible thin layer sandwiched between the organic and the aqueous phases. Evaporation of pentane overnight left the SWNT film floating on the top of the water. The SWNT thin film (~ 8 mm^2) was then transfer-printed onto a cleaned glass coverslip, followed by rinsing and heating at $120°C$ for 2h in a vacuum oven. Source and drain electrodes were then prepared across the SWNT-net using silver conductive paint (RS Component). Finally, silicone rubber (Dow Corning) was used to insulate the electrodes and create the chamber (2–3 cm^2) for bilayer deposition and recording.

2.2. Formation of lipid bilayers on SWNT-net device

Giant unilamellar vesicles (GUVs) were prepared by the simple hydration method according to the reported protocol with modification (Kim et al., 2006). 1,2-Dioleoyl-sn-glycero3-phosphocholine (DOPC), 1,2-Dioleoyl-sn-glycero-3-phosphoric acid (DOPA) and cholesterol (Sigma) were mixed at 9:1:3 by weight in chloroform:methanol (2:1 v/v) to make a lipid solution at 10 mg/ml. 100 µl lipid mixture in a 1 ml glass test tube was dried by N_2 gas blowing to form a lipid film and then placed in vacuum oven for >6 h to thoroughly remove the organic solvent. The completely dried lipid film was then prehydrated at $50°C$ with N_2 saturated 0.1 M sucrose solution for 15–25 min. Then 1 ml of an aqueous solution containing 0.1 M glucose and 10mM KCl, which had been N_2 purged, was added gently to the test tube. The tube was sealed under N_2 and incubated at $37°C$ overnight to harvest GUVs. SWNT-net device was incubated with 0.01 mg/ml poly-l-lysine (PLL) at $4°C$ overnight, and then rinsed with DI H_2O. The solution with GUVs was incubated with SWNT-net device at $50°C$ for 1 h to allow fusion of GUVs onto the SWNT-net and subsequent formation of lipid bilayer, followed by DI H_2O rinsing.

2.3. Incorporation of GramD and calcimycin

GramD or calcimycin was first dissolved in ethanol and diluted by a factor of 100 with DI water. As the ionophores can self-assemble into the lipid bilayer, the ionophore solution was added to the recording chamber to reach desired final concentration and incubated with lipid bilayer coated SWNT-net device for 30 min followed by DI water rinsing.

2.4. Electrical measurements

The SWNT-net current was continuously monitored at ambient condition using a semiconductor device analyzer (Agilent, B1500A), while the source and drain electrodes were

biased at 400 mV and an Ag/AgCl reference electrode (0 V) was immersed in the bath solution. In the experiment to characterize the SWNT-net, the gating voltage was applied *via* an Ag/AgCl electrode in a solution containing 0.1 M glucose and 10mM KCl. All added solutions containing Na^+, K^+, Ca^{2+}, ATP, or BSA were of the same ionic strength (0.12 M) adjusted with glucose.

3. Results and discussion

Owing to its unique electrical, physiochemical, and one-dimensional structural properties, single-walled carbon nanotubes have been successfully used as biosensors to detect the presence of biomolecules (Gruner, 2006) and more recently dynamic release of biomolecules from live cells (Huang et al., 2009; Sudibya et al., 2009). Two-dimensional SWNT networks (SWNT-net) made of SWNT bundles of ~20 nm were fabricated using phase-separation facilitated self-assembly as previously reported (Huang et al., 2009; Zhang et al., 2009) (Fig. 1a) in order to interface with two-dimensional lipid bilayer (~5 nm thick). As shown in Fig. 1b, the conductance of SWNT-net is highly sensitive to the electrical potential applied to the bath solution, suggesting that our SWNT-net is ideal to detect biomolecules or biological processes that cause electrochemical perturbations in the vicinity of nanotubes. We chose the SWNT-net devices with similar conductance (~5 μS) and on/off ratio (~50) for all the experiments. All the devices showed very small leakage conductance (~1 nS).

Giant unilamellar vesicles (GUVs) were prepared with DOPC (1,2-Dioleoyl-sn-glycero-3-phosphocholine), DOPA (1,2-Dioleoylsn-glycero-3-phosphoric acid), and cholesterol (Fig. S1 in Supplementary Material). Positively charged poly-L-lysine (PLL) was coated on the SWNT-net to facilitate fusion of negatively charged GUVs with SWNT-net and subsequent formation of lipid bilayer. Nanorough meshworks of SWNT-net and polymeric cushion of PLL prevent direct contact between membrane proteins and the solid supporting surface which may compromise the functions of membrane proteins. Using lipid-bilayer inserting fluorescent probe (DiOC18), we show that lipid bilayer was successfully deposited on SWNT-net with good coverage (Fig. S2 in Supplementary Material).

As a proof-of-principle demonstration, bilayer-SWNT hybrid sensors were utilized here to detect two membrane inserting ionophores secreted by bacteria: gramicidin and calcimycin. Gramicidin from soil bacterial species *Bacillus brevis* is one of the best characterized pore-forming antibiotic ionophore. It dimmerizes in a head-to-head fashion to form a transmembrane channel that selectively allows the electrogenic flux of monovalent cations (e.g. K^+, Na^+) (Andersen et al., 1999) (Fig. 1c). Calcimycin produced by bacteria *Streptomyces chartreusensis* is a mobile ion carrier, which is able to complex with divalent metal ions, in particular, with Ca^{2+} (Boeckman et al., 1991). Operating by a shuttle mechanism, it constantly transports ions across the lipid bilayer from high concentration to low concentration (Pohl et al., 1990) (Fig. 1c). The hybrid sensor of lipid bilayer on SWNT-net is illustrated in Fig. 1c. We speculate that ionic flux produced by the ionophores will cause accumulation of ions at the narrow interface between the lipid bilayer and SWNT-net, and consequently change the electrical potential in the vicinity of nanotubes whereby the conductance of the voltage-biased SWNT-net is modulated, leading to measurable current signal.

In a typical experiment demonstrated in Fig. 2, gramicidin was incorporated into the lipid-bilayer on SWNT-net by incubation of 0.1 μM gramicidin for 30 min followed by DIH_2O

washing and subsequent bathing in 0.12 M glucose solution. As shown in Fig. 2a, when bias the SWNT-net at V_{ds} = 400 mV, and pulses of bath solution containing 1 mM K^+, or 10mM K^+, or 10mM Na^+ were delivered locally onto fluorescently labeled lipid bilayer on SWNT-net using a glass micropipette with a tip diameter of ~2 μm positioned ~1 μm above the SWNT-net, obvious current decreases were observed. As expected, higher concentration of K^+ caused larger current reduction. The decrease in SWNT-net current is due to flux of K^+ or Na^+ ions through gramicidin channels into the narrow gap between the SWNT-net and the bilayer. And the accumulation of these cations increases the electrical potential at the bilayer-SWNT gap, thus decreases the SWNT-net conductance due to the p-type electrical characteristics of SWNT-net (Fig. 1). It is noted that the SWNT-net responses reached equilibrium after some time, presumably because, at the equilibrium, the increase in electrical potential due to ion accumulation counteracted the diffusion flow of the cations due to concentration gradient.

In contrast to K^+, delivery of Ca^{2+} was not able to produce any appreciable nanotube response because Ca^{2+} ions are not permeable to gramicidin channels (Fig. 2b). In addition, both ATP and BSA molecules, which can interact with bare SWNTs and consequently alter their conductance (Huang et al., 2009), did not cause nanotube response (Fig. 2b). These control experiments indicate that the presence and activities of ionophores can be specifically detected without interference from impermeable ions or biomolecules present in the biological solutions.

We similarly incorporate calcimycin into lipid bilayer on SWNT devices. In a typical experiment shown in Fig. 3a, local application of Ca^{2+} resulted in significant reduction in SWNT-net current in a concentration dependent manner. Furthermore, as expected, local delivery of K^+ or ATP or BSA did not trigger any SWNT-net response (Fig. 3b). In another experiment, calcimycin (0.1 μM) together with 10mM Ca^{2+} was administrated, and reduction in SWNT-net was also observed with ~100s delay presumably due to the time required for calcimycin insertion (Fig. 3c). In some experiments, the bilayer-SWNT devices were incubated with gramicidin or calcimycin at a lower concentration (1 nM) for 30 min pre-insertion. Local delivery of K^+ or Ca^{2+} ions could still cause obvious response of SWNT-net (e.g., 10mM K^+ or Ca^{2+} caused current decrease of ~500 pA). In addition, all experiments were repeated independently for 3–4 times, and all the SWNT-net current responses were significantly larger than the noise level (~50 pA) with device-to-device variation within ±25%.

4. Conclusions

In summary, we have developed a novel system integrating artificial lipid bilayer with SWNT-net field-effect transistor, and demonstrated that such hybrid nanoelectronic biosensors can selectively detect the presence and activities of ionophores in their native lipid environment. This technique can potentially also be used to examine other membrane proteins (e.g. ligand-gated ion channels as illustrated in Fig. 1c, receptors, membrane insertion toxins, and antibacterial peptides) for the purposes of biosensing, fundamental studies, or high throughput drug screening.

Acknowledgment

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.12.011.

References

Andersen, O.S., Apell, H.J., Bamberg, E., Busath, D.D., Koeppe, R.E., Sigworth, F.J., Szabo, G., Urry, D.W., Woolley, A., 1999. Nature Structural Biology 6 (7), 609–1609.

Bockenroth, I.K., Atanasova, P.P., Toby, A., Jenkins, A., Koper, I., 2008. Langmuir 24 (6), 2954.

Boeckman, R.K., Charette, A.B., Asberom, T., Johnston, B.H., 1991. Journal of the American Chemical Society 113 (14), 5337–5353.

Chan, Y.H.M., Boxer, S.G., 2007. Current Opinion in Chemical Biology 11(6), 581–587.

Deniaud, A., Rossi, C., Berquand, A., Homand, J., Campagna, S., Knoll, W., Brenner, C., Chopineau, J., 2007. Langmuir 23 (7), 3898–3905.

Favero, G., Campanella, L., Cavallo, S., D'Annibale, A., Perrella, M., Mattei, E., Ferri, T., 2005. Journal of the American Chemical Society 127 (22), 8103–8111.

Gruner, G., 2006. Analytical and Bioanalytical Chemistry 384 (2), 322–335.

Huang, Y.X., Sudibya, H.G., Fu, D.L., Xue, R.H., Dong, X.C., Li, L.J., Chen, P., 2009. Biosensors & Bioelectronics 24 (8), 2716–2720.

Kim, Y.H., Rahman, M.M., Zhang, Z.L., Misawa, N., Tero, R., Urisu, T., 2006. Chemical Physics Letters 420 (4–6), 569–573.

Pohl, P., Antonenko, Y.N., Yaguzhinsky, L.S., 1990. Biochimica et Biophysica Acta 1027 (3), 295–300.

Sato, H., Hakamada, H., Yamazaki, Y., Uto, M., Sugawara, M., Umezawa, Y., 1998. Biosensors & Bioelectronics 13 (9), 1035–1046.

Schuster, B., Sleytr, U.B., 2006. Current Nanoscience 2 (2), 143–152.

Sudibya, H.G., Ma, J.M., Dong, X.C., Ng, S., Li, L.J., Liu, X.W., Chen, P., 2009. Angewandte Chemie-International Edition 48 (15), 2723–2726.

Vallejo, A.E., Gervasi, C.A., 2007. Journal of Electroanalytical Chemistry 603 (1), 51–58.

Wallin, E., von Heijne, G., 1998. Protein Science 7 (4), 1029–1038.

Yildirim, M.A., Goh, K.I., Cusick, M.E., Barabasi, A.L., Vidal, M., 2007. Nature Biotechnology 25 (10), 1119–1126.

Zhang, J., Fu, D.L., Chan-Park, M.B., Li, L.J., Chen, P., 2009. Advanced Materials 21 (7), 790–793.

List of Figures

Fig. 1   (a) SEM image of a SWNT network. (b) Conductance of SWNT-net device is sensitive to the gate voltage (V_g) applied to the bath solution. V_{ds} =400 mV. (c) Schematic illustration of the hybrid device of SWNT-net and lipid bilayer with embedded membrane proteins.

Fig. 2   (a) SWNT current recorded when the solutions of K^+ (1 or 10mM) and Na^+ (10 mM) were locally delivered onto the lipid bilayer pre-incorporated with gramicidin. (b) SWNT responses to local delivery of Ca^{2+} (10mM), ATP (10 mM) and BSA (10 µM).

Fig. 3   SWNT-net current recorded when (a) Ca^{2+} (1 or 10 mM) or (b) Na^+ (10mM), ATP (10mM) and BSA (10 µM) was locally delivered to the calcimycin incorporated hybrid device of lipid bilayer and SWNT-net. (c) SWNT-net response when 0.1 µM calcimycin together with 10mM Ca^{2+} was delivered locally onto the pure lipid bilayer.
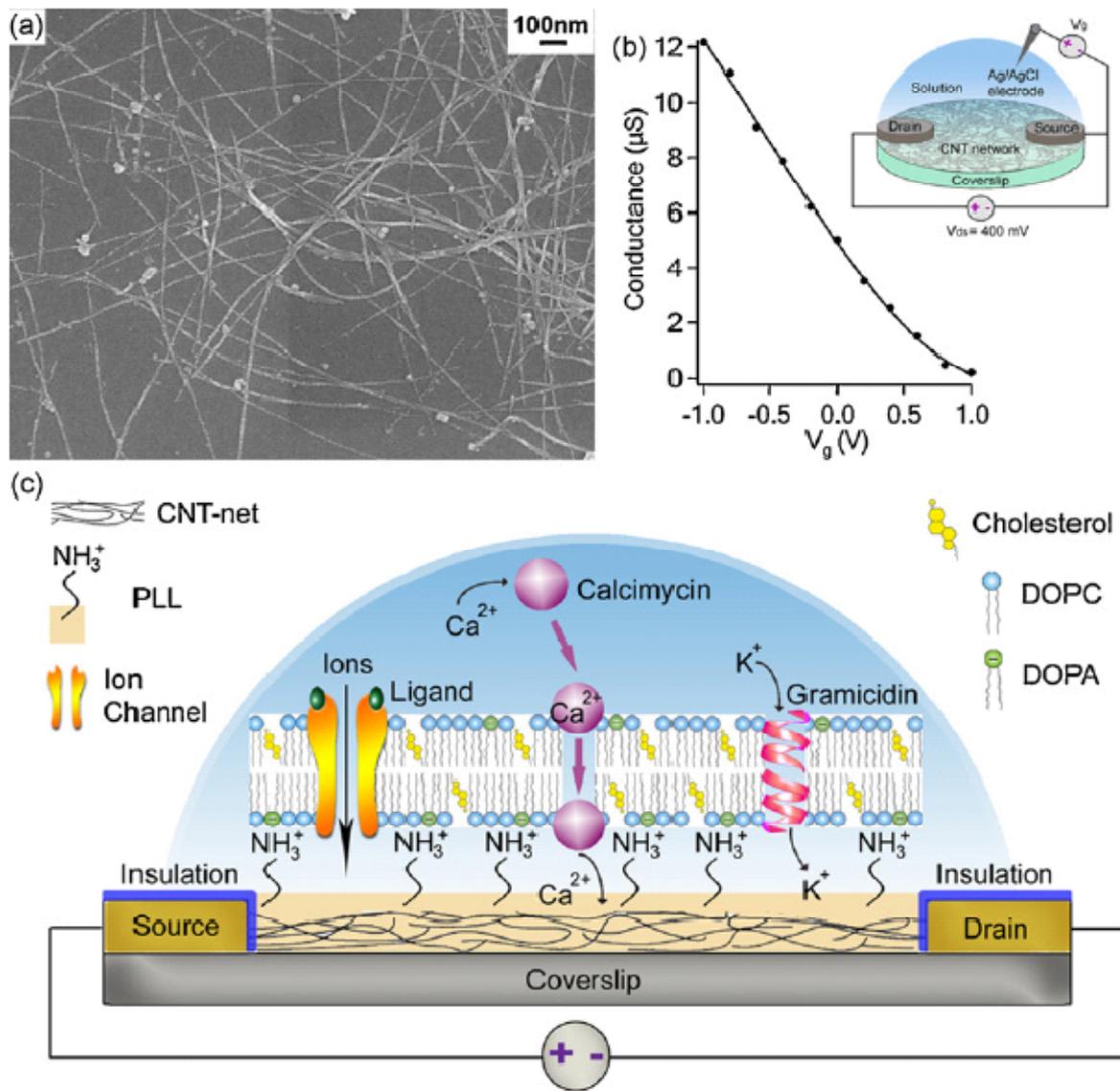
Fig. 1

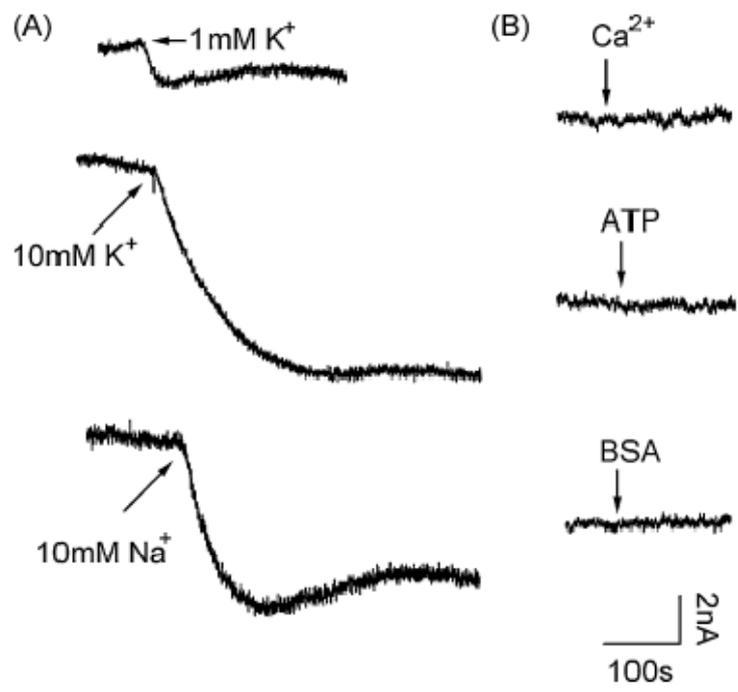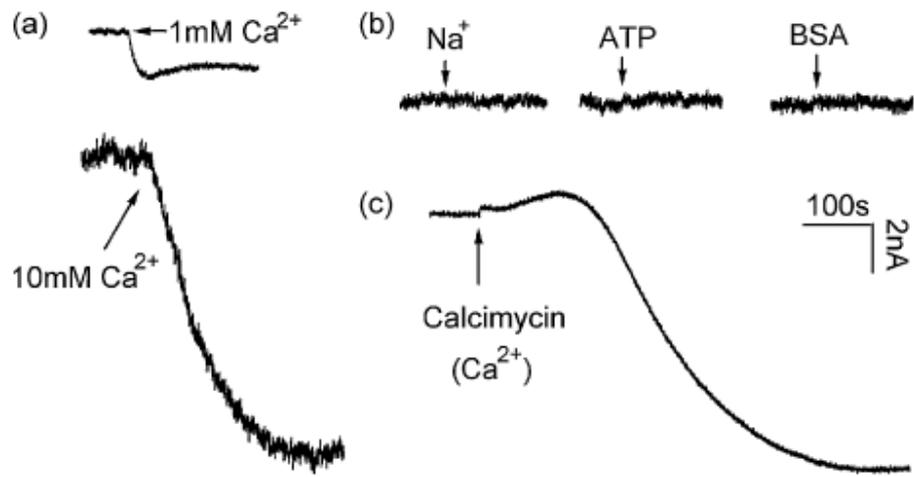Fig. 2

Fig. 3