those observed in previous studies conducted in laboratory rats, and this difference may suggest that cetaceans metabolize Ag differently. Therefore, a presumptive metabolic pathway of Ag in cetaceans is proposed (Figure 2). It is presumed that Ag/Ag compounds can enter the cetacean body via dietary intake and then be delivered to the liver through the gastrointestinal tract and portal circulation. Ag/Ag compounds are considered to be conjugated to proteins during portal circulation. Most of the protein-conjugated Ag/Ag compounds are taken up by hepatocytes, degraded in lysosomes, released into the cytoplasm of hepatocytes, conjugated to proteins, and stored in the lysosomes of hepatocytes. Some Ag/Ag compounds with protein conjugation may remain in blood circulation or may be released from hepatocytes into blood circulation during the renewal of hepatocytes. The Ag/Ag compounds in blood circulation can be subsequently delivered to multiple organs (such as the kidneys), penetrate the glomeruli, and then be reabsorbed by proximal renal tubular epithelium.

Our study demonstrated that there were no lesions with marked intralesional Ag deposition and no statistically significant correlation between the lesions and Ag concentrations. However, stranded cetaceans are not laboratory animals and are not well controlled in that they are not exposed to only a single contaminant. Therefore, further investigations are warranted to study the systemic Ag distribution, the cause of death/stranding, and the infectious diseases in stranded cetaceans with different Ag concentrations to comprehensively evaluate the negative health effects caused by Ag in cetaceans. Most importantly, this study demonstrated that Ag contamination in cetaceans living in the North Pacific Ocean is more severe than that in other marine regions of the world. The level of Ag deposition in cetaceans living in the former area may have had negative impacts on their health conditions. Furthermore, these 7 cetacean species have different habitats and prey, but the Ag concentration did not significantly differ among the different species. This finding suggests that Ag contamination may exist in all compartments of the marine ecosystem.

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Dr. Wen-Ta Li
Graduate Institute of Molecular and Comparative Pathobiology
heerolee1104@gmail.com

Chian-Ren Jeng
Professor, Graduate Institute of Molecular and Comparative Pathobiology
crjeng@ntu.edu.tw

Application of the silicon nanowire field-effect transistor to monitor neuronal activities

The zinc ion (Zn^{2+}) is a common ingredient of many over-the-counter multivitamin and mineral dietary supplements. Many enzymes incorporate Zn^{2+} as a co-factor and participate in various physiological activities, such as apoptosis, DNA synthesis, gene expression, immunity, and neurotransmission. The free intracellular Zn^{2+} concentration ($[Zn^{2+}]_i$) is maintained at a sub-nM level by Zn^{2+} transporters at the membranes of organelles and binding proteins. Our previous reports have shown that treating neurons with dopamine or Zn^{2+} elevates $[Zn^{2+}]_i$ and causes cell death. Dopamine elevates $[Zn^{2+}]_i$ with a change of hundreds of pM and activates the autophagic pathway via the D1 receptor-protein kinase A (PKA)-nitric oxide (NO) cascade in cultured cortical neurons. In addition, blocking this elevation in $[Zn^{2+}]_i$ suppresses dopamine-in-

duced autophagosome formation (*Hung et al.*, 2017). These findings reveal that intracellular Zn^{2+} homeostasis is important for the activation of various signaling pathways responsible for neuronal survival.

In synaptic vesicles of glutamatergic neurons, a high concentration of Zn^{2+}, 100-300 μM, is coreleased with glutamate upon stimulation, thereby elevating the local extracellular Zn^{2+} concentration ($[Zn^{2+}]_e$). Abundant extracellular Zn^{2+}-chelating proteins are suggested to maintain $[Zn^{2+}]_e$ at a low sub-nM level. Extracellularly, Zn^{2+} can bind to the *N*-methyl-D-aspartate (NMDA) receptor and regulate the kinetics of the conjugated ion channels; Zn^{2+} can also facilitate the aggregation of amyloid β (Aβ) and enhance neurodegeneration. Some Zn^{2+}-related metal ionophores, such as PBT2, are under clinical trials to verify the importance of Zn^{2+} homeostasis in neurodegenerative diseases. However, most studies adopt a high concentration of Zn^{2+} (> 100 μM), and whether physiological levels of $[Zn^{2+}]_e$ are sufficient to activate these physiological responses is not clear, as traditional biological approaches cannot accurately monitor $[Zn^{2+}]_e$ in real time.

Over the past several years, with the collaboration of Dr. Yit-Tsong Chen (Department of Chemistry, NTU) and Chii-Dong Chen (Institute of Physics, Academia Sinica), we have focused on exploring the capabilities of silicon nanowire field-effect transistors (SiNW-FETs) in detecting molecules released from neurons (Figure 1). The SiNW-FET-based biosensor is a reliable, sensitive, label-free, and real-time tool that has been widely applied to analyze the presence of biological molecules. Through modification of the surface with appropriate receptors, the device is able to recognize specific target molecules with a sensitivity at the pM level. Compared to the production and manipulation required for other similar techniques, such as Biacore, which requires an expensive optical instrument, the low-cost production and ease of manipulation of this device makes this technique ideal for biological research. Furthermore, the SiNW-FET could be applied to detect cell activities in real time. We have obtained a patent for applying this design to study biomolecular interactions (*Reusable Nanowire Field-Effect Transistor System for Detecting Biomolecular Interactions, US Patent number 8420328; 2013/4/16*).

To detect various molecules released from cultured cells, we modified the surface of SiNW-FET with oligonucleotide aptamers. Aptamers can form a structure to bind target molecules specifically. We have verified that dopamine and neuropeptide Y are released differentially from pheochromocytoma (PC12) cells stimulated with different concentrations of ATP (*Banerjee et al.*, 2016); in addition, we have shown that the efflux of K^+ from excited neurons elevates the local extracellular K^+ concentration (*Anand et al.*, 2017).

In this report, (*Anand et al.*, 2018) (Figure 2), we first chemically modified the Zn^{2+}-sensitive fluorophore, FluoZin-3, so it can anchor onto the SiNW-FET (FZ-3/SiNW-FET). This FZ-3/SiNW-FET has good specificity against Zn^{2+} (dissociation constant, K_d, ~12 nM) over other divalent cations. To quantify the Zn^{2+} released from excited neurons in real time, we placed a coverslip seeded with cultured embryonic cortical neurons atop an FZ-3/SiNW-FET, and the $[Zn^{2+}]_e$ increased to ~110 nM upon stimulation with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). Blockers against AMPA receptors (AMPARs) and exocytosis greatly suppressed the AMPA-induced elevation in $[Zn^{2+}]_e$. In addition, a SiNW-FET modified with Aβ (Aβ/SiNW-FET) bound Zn^{2+} with a K_d of ~633 nM. Placing neurons atop the Aβ/SiNW-FET and stimulation with AMPA induced a significant change in conductance. These results reveal that neurons release Zn^{2+} from synaptic vesicles upon stimulation and that the free $[Zn^{2+}]_e$ surrounding the excited neurons is high enough to bind Aβ.

$[Zn^{2+}]_e$ homeostasis is important for the health of the brain. Understanding the $[Zn^{2+}]_e$ surrounding neurons is critical in evaluating the physiological functions of Zn^{2+}. Our findings show that the $[Zn^{2+}]_e$ surrounding overexcited neurons is ~115 nM, which is already sufficient to support the formation of the Zn^{2+}-Aβ complex. Considering that Aβ fibrillary aggregates are a pathogenic factor for Alzheimer's disease, the Zn^{2+} coreleased with glutamate at the axonal terminals must be under strict regulation to avoid the formation of Aβ-Zn^{2+} complexes. In addition, this $[Zn^{2+}]_e$ level is able to bind to NMDA receptors, which have a high affinity (~ nM level) for Zn^{2+}, to regulate neurotransmission. In conclusion, our SiNW-FET system is sufficiently sensitive to characterize physiological $[Zn^{2+}]_e$ in real time and examine how Zn^{2+} homeostasis modulates neuronal activities.

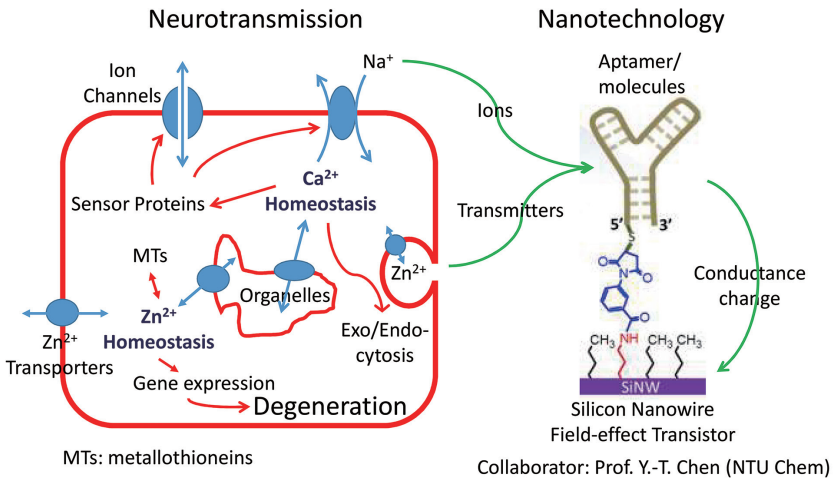

Figure 1. Applying the SiNW-FET technique to study molecules released from cells. Biological approaches reveal that both intracellular Ca^{2+} and Zn^{2+} signaling pathways share similar mechanisms to modulate cell activities. Various transporters or channels regulate the fluxes of ions across those membrane systems; ion-binding proteins in the cytosol activate signaling pathways and regulate gene expression. To detect the few molecules released from neurons via the transporters/channels or synaptic vesicles, we modified the SiNW-FET with different aptamers or molecules as the receptors. The binding of molecules to the receptors changes the field potential surrounding the nanowire, resulting in changes in conductance.

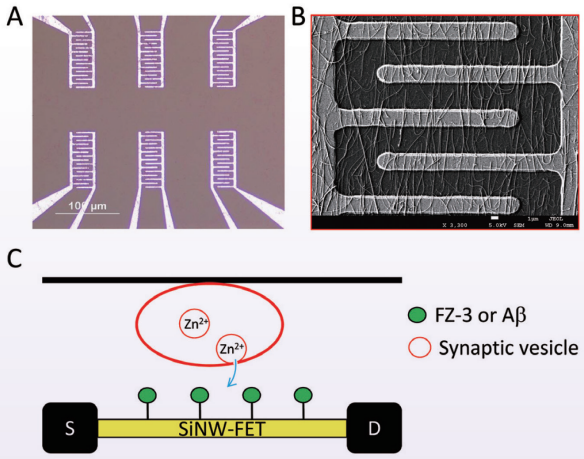

Figure 2. The SiNW-FET device. A. An image of the device that contains 6 pairs of comb-like circuits. B. A scanning electron microscopy images shows part of the circuit covered with silicon nanowires. C. Illustration of the experimental design. Cells grown on coverslips are placed atop the device with cells facing the circuit.

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Chien-Yuan Pan
Professor, Department of Life Science
cypan@ntu.edu.tw