

## Non-invasive micro-test technology and applications

Kai Sun<sup>1</sup>, Yunqi Liu<sup>2,3</sup>✉, Yanshu Pan<sup>4</sup>, Dongwei Di<sup>5</sup>, Jianfang Li<sup>6</sup>, Feiyun Xu<sup>7</sup>, Li Li<sup>8</sup>, Yoshiharu Mimata<sup>9</sup>, Yingying Chen<sup>10</sup>, Lixia Xie<sup>11</sup>, Siqi Wang<sup>12</sup>, Wenqian Qi<sup>13</sup>, Yan Tang<sup>14</sup>, Huachun Sheng<sup>15</sup>, Bing Wang<sup>16</sup>, Ruixue Sun<sup>17</sup>, Dingquan Tan<sup>18</sup>, Daohong Fu<sup>19</sup>, Ye Yin<sup>20</sup>, Ao Xue<sup>21</sup>, Yichao Shi<sup>22</sup>, Wenjing Shao<sup>23</sup>, Lei Gong<sup>24</sup>, Zhijian Jiang<sup>13</sup>, Wei Zhang<sup>14</sup>, Qiangsheng Wu<sup>25</sup>, Yaosheng Wang<sup>26</sup>, Minglin Lang<sup>23</sup>, Wenxiu Ye<sup>9</sup>, Weifeng Xu<sup>7</sup>, Shuhe Wei<sup>27</sup>, Weiming Shi<sup>5</sup>, Yue Jeff Xu<sup>2,3,28,29</sup>✉

<sup>1</sup> College of Life Sciences, Nanjing Normal University, Nanjing 210046, China

<sup>2</sup> Zhongguancun Xuyue NMT Industrial Alliance, Beijing 100080, China

<sup>3</sup> NMT International Alliance, Amherst, Massachusetts 01002, USA

<sup>4</sup> College of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 102488, China

<sup>5</sup> State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

<sup>6</sup> College of Biological Science, China Agricultural University, Beijing 100193, China

<sup>7</sup> Center for Plant Water-use and Nutrition Regulation and College of Resources and Environment, Joint International Research Laboratory of Water and Nutrient in Crop, Fujian Agriculture and Forestry University, Fuzhou 350002, China

<sup>8</sup> Institute of Agricultural Resources and Environment, Tianjin Academy of Agricultural Sciences, Tianjin 300380, China

<sup>9</sup> Peking University Institute of Advanced Agricultural Sciences, Shandong Laboratory of Advanced Agricultural Sciences at Weifang, Weifang 261000, Shandong, China

<sup>10</sup> Guangxi Forestry Research Institute, Nanning 530002, China

<sup>11</sup> Institute of Wetland Agriculture and Ecology, Shandong Academy of Agricultural Science, Jinan 250100, China

<sup>12</sup> Key Laboratory of Wastewater Treatment Technology of Liaoning Province, Academy of Environmental and Chemical Engineering, Shenyang Ligong University, Shenyang 110159, China

<sup>13</sup> Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

<sup>14</sup> Technical Institute of Physics and Chemistry, CAS, Beijing 100190, China

<sup>15</sup> Institute of Qinghai-Tibetan Plateau, Southwest Minzu University, Chengdu 610225, China

<sup>16</sup> Molecular Genetics Key Laboratory of China Tobacco, Guizhou Academy of Tobacco Science, Guiyang 550081, China

<sup>17</sup> College of Agriculture and Forestry, Hebei North University, Zhangjiakou 075000, Hebei, China

<sup>18</sup> Smart Health Institute, Chongqing Vocational College of Media, Chongqing 402560, China

<sup>19</sup> Institute of Biology, Humboldt University of Berlin, Berlin 10099, Germany

<sup>20</sup> College of Horticulture, Qingdao Agricultural University, Qingdao 266109, Shandong, China

<sup>21</sup> College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin 150040, China

<sup>22</sup> Department of Gastroenterology, Aerospace Center Hospital, Peking University Aerospace School of Clinical Medicine, Beijing 100049, China

**Electronic supplementary material** The online version of this article contains supplementary material, which is available to authorized users.

✉ Correspondence: [yunqi@nmtia.org.cn](mailto:yunqi@nmtia.org.cn) (Y. Liu),  
[jeffxu@youngerusa.com](mailto:jeffxu@youngerusa.com), [jeff@xuyue.net](mailto:jeff@xuyue.net) (Y. J. Xu)

- <sup>23</sup> CAS Center for Excellence in Biotic Interactions, College of Life Science, University of Chinese Academy of Sciences, Beijing 100049, China
- <sup>24</sup> State Key Laboratory of Grassland Agro-ecosystems, School of Life Sciences, Lanzhou 730000, China
- <sup>25</sup> College of Horticulture and Gardening, Yangtze University, Jingzhou 434025, Hubei, China
- <sup>26</sup> Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100081, China
- <sup>27</sup> Key Laboratory of Pollution Ecology and Environment Engineering, Institute of Applied Ecology, Shenyang 110016, China
- <sup>28</sup> Xuyue (Beijing) Sci. & Tech. Co., Ltd., Beijing 100080, China
- <sup>29</sup> YoungerUSA LLC, Amherst, Massachusetts 01002, USA

Received: 2 February 2024 / Accepted: 14 October 2024

**Abstract** Non-invasive micro-test technology (NMT) reveals dynamic ionic/molecular concentration gradients by measuring fluxes of ions and small molecules in liquid media in 1D, 2D or 3D fashions with sensitivity up to pico- ( $10^{-12}$ ) or femto- ( $10^{-15}$ ) moles per  $\text{cm}^2$  per second. NMT has been applied to study metabolism, signal transduction, genes and/or proteins physiological functions related to transmembrane ionic/molecular activities with live samples under normal conditions or stress. Data on ion and/or molecule homeostasis (IMH) by NMT in biomedical sciences, plant and crop sciences, environmental sciences, marine and space biology as well as traditional Chinese medicine are reviewed.

**Keywords** Non-invasive micro-test technology, Transmembrane transport, Ionic homeostasis,  $\text{H}^+$ -ATPase, Calcium signature, Physiology

## INTRODUCTION

Ion and/or molecule homeostasis (IMH) is a fundamental biological phenomenon essential for all living organisms (Ahmadi *et al.* 2023; Huang *et al.* 2022; Song *et al.* 2024; Sun *et al.* 2009a, 2009b, 2010; Xu 2023; Xu *et al.* 2022; Yang *et al.* 2010). Pathology of humans, animals, crops, insects and microbes is accompanied by abnormal IMHs. Excessive  $\text{H}^+$  accumulations, for instance, are the result of lactate formation even in the presence of  $\text{O}_2$  in tumor cells (Martinez-Outschoorn *et al.* 2011; Semenza 2007; Seyfried and Shelton 2010; Warburg 1956; Warburg *et al.* 1927). It is also well known that a plant will not grow properly if the supply of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{K}^+$  are out of balance (Guan *et al.* 2024; Martínez-Ballesta *et al.* 2020; Tyler 2017; Zhou *et al.* 2019).

However, it has been a challenge for biologists to fully comprehend IMHs due to: (1) ionic and molecular information related to IMH have to be collected with live samples; (2) multiple ions/molecules are preferably measured simultaneously in order to construct the network among relevant ion(s)/molecule(s); (3) ionic/molecular data in different levels, *i.e.* cell, tissue to organ, has to be collected; and (4) since biological materials are 3-dimensional per se, 1D or 2D form of data is

no longer accurate to reflect the 3D world (Kunkel *et al.* 2001, 2006; Wan *et al.* 2011; Xu *et al.* 2006; Yang *et al.* 2012).

Non-invasive micro-test technology (NMT) is a collection of techniques. Initial NMT hardware was developed based on the Vibration Probe (VP) Technique invented by Dr. Lionel Jaffe at Marine Biological Laboratory in the early 1990s (Degenhardt *et al.* 1998; Kochian *et al.* 1992; Kuhlreiber and Jaffe 1990; Smith 1995). VP was primarily focused on solving the problems of voltage signal drifts and instrumental background noise (Kunkel *et al.* 2006; McLamore and Porterfield 2011), while NMT went further emphasizing: (1) developing more ionic and molecular micro-sensors; (2) simultaneous multiple ion/ion and/or ion/molecule measurements; (3) 3D-flux data collection and visualization and (4) automation of NMT to be more efficient to collect flux data (Kunkel *et al.* 2006; Xu *et al.* 2006; Yin *et al.* 2006). The term Non-invasive Micro-test Technology, initially proposed by Yue Jeff Xu, is now widely accepted (Xu 2023).

This review will describe NMT about: (1) working principles based on the 2-point measurement scheme (2PMS); (2) spatial relationships among samples, NMT sensors and ionic/molecular gradients from physical

and chemical perspectives; and (3) ionic/molecular fluxes in relation to their gradients.

Comparisons will be made between NMT with other ion/molecule detection techniques, such as patch clamp and ion-selective microelectrodes.

NMT is now widely used in plant and crop sciences (Liu *et al.* 2023), animal science, microbiology, environmental science, and traditional Chinese medicine, as outlined below.

## NON-INVASIVE MICRO-TEST TECHNOLOGY (NMT)

### Principles

#### Two-points measurement scheme

Ionic/molecular fluxes are detectable when ions/molecules move from high concentrations to low concentrations. The concentration differences then result in ionic/molecular gradients (Fig. 1). In live biological samples, transmembrane activities of ions/molecules result in ionic/molecular gradients via their effluxes and/or influxes (Kunkel *et al.* 2001). In order to measure these ions/molecules selectively or specifically in a quantitative way, various sensors made from glass, optical fiber, and platinum-iridium alloy have been developed. These sensors' fabrications are based on different theories/mechanisms, such as ionophores, enzymatic reactions, electrochemistry, and fluorescent dyes

(McLamore and Porterfield 2011).

Due to the special demands of temporal and spatial resolutions, conventional ionic/molecular sensors usually have to be modified to fit NMT hardware configuration. Therefore, NMT ionic/molecular micro-sensors are designated to differentiate themselves from those traditional micro-sensors.

#### 1D, 2D, 3D and 6D measurements

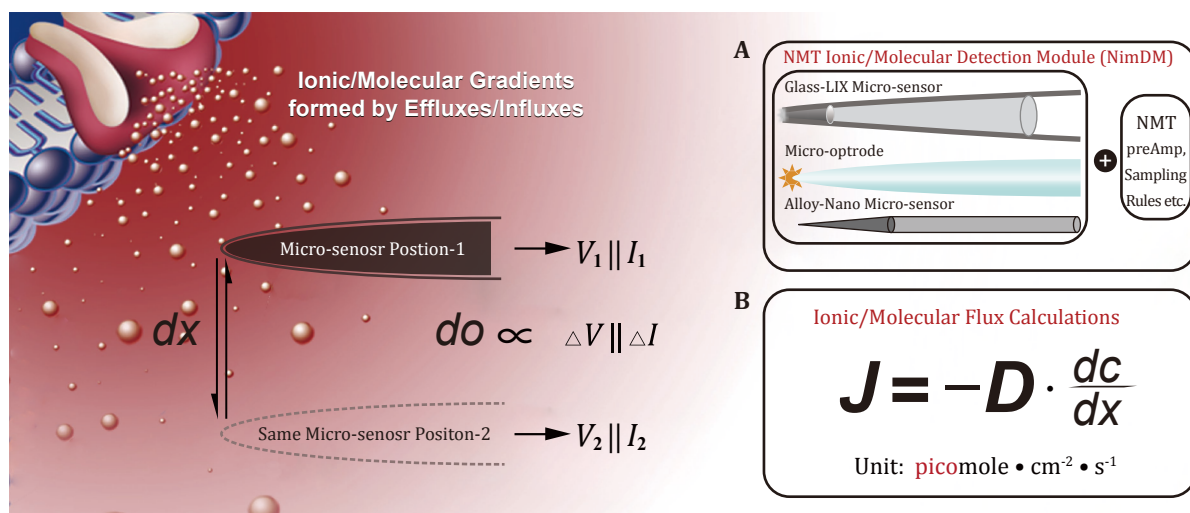
NMT flux data in 1D, 2D and 3D are essential to (1) validate results from molecular biology, biochemistry, microscopy, and omics experiments; (2) provide clues for research on new genes, and new mechanisms; (3) for network map connecting among genes, proteins, metabolites.

An online interactive 3D animation application has been developed to not only visualize 3D flux data but also for additional features, such as zooming, spinning, panning, movie making and coloring (Fig. 2) (Wang *et al.* 2023b).

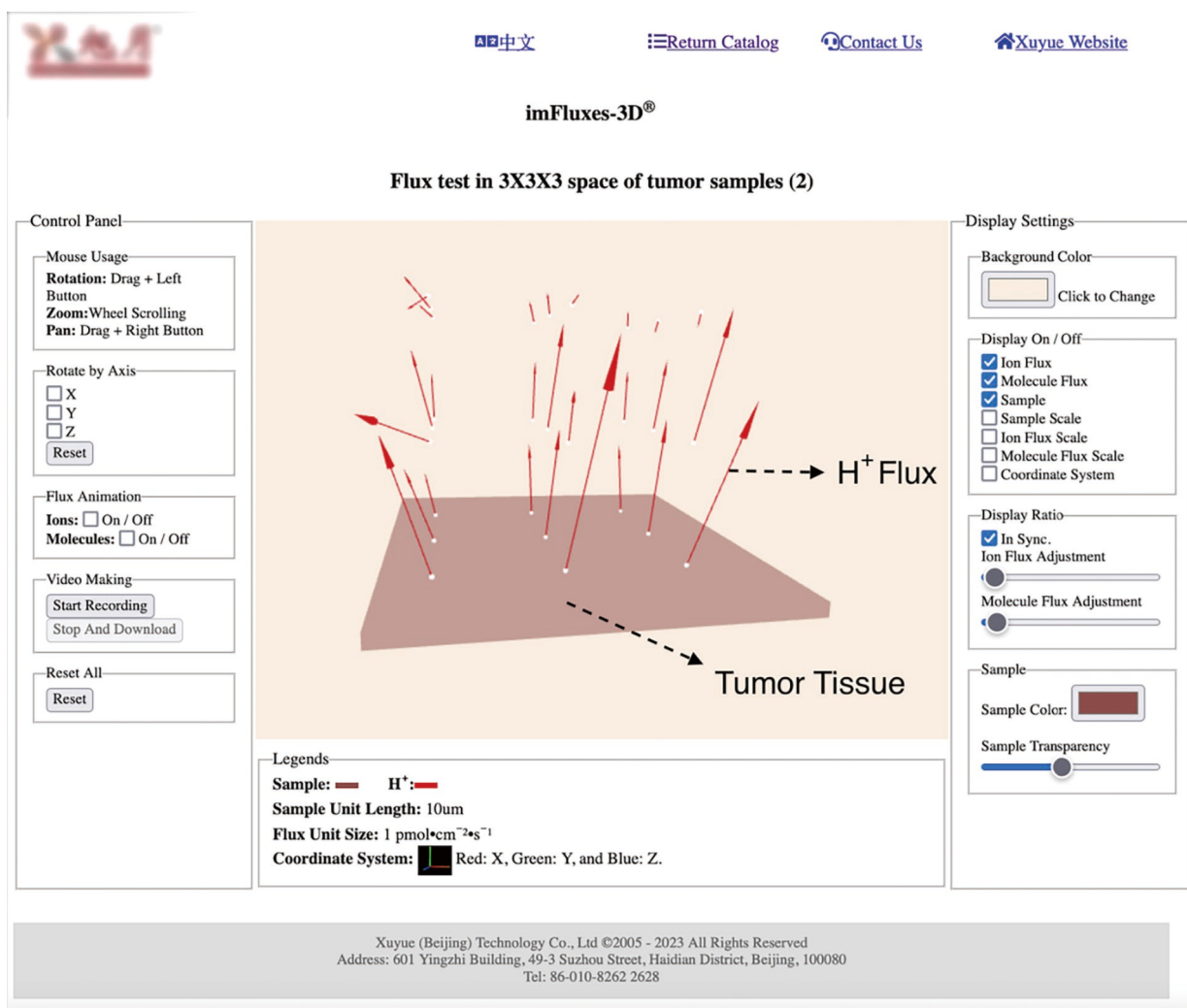
A recent NMT hardware design allows both NMT micro-sensor and the tested sample to move in their own 3D system independently, which is even more powerful with a versatile 6D NMT system (Xu 2023).

### Limitations of NMT

The types of NMT sensors and their temporal and spatial resolutions are limiting factors of NMT.



**Fig. 1** Working Principle of NMT. Diagram of NMT working theory. A NMT ionic/molecular micro-sensor undergoes two-point measurement at a known distance  $dx$  in the concentration gradient of the ions/molecules obtaining voltages  $V_1||I_1$  and  $V_2||I_2$  respectively. The concentration difference  $dc$  between the two points is proportional to  $\Delta V||\Delta I$  derived from  $V_1||I_1$  and  $V_2||I_2$ . **A** Fluorescent dyes/optical fibers, nano-carbon filaments, enzyme electrodes, fabricated metals/alloys, *etc.* can all be used to detect ions/molecules. **B** Fick's first law of diffusion is adopted to calculate ionic/molecular fluxes, where  $D$  is the diffusion coefficient of ions/molecules ( $\text{cm}^2/\text{s}$ )



**Fig. 2** 3D animation. Screenshot of imFluxes3D<sup>®</sup> online version. Top: navigation area and title of current 3D file displayed; Left: Control Panel; Middle: area for displaying the 3D animation of samples, ionic/molecular fluxes and legends. It will be updated in real time as the user adjusts the parameters in both “Control Panel” and “Display Settings”. Bottom: legends to the 3D animation above is displayed here; Right: Display Settings

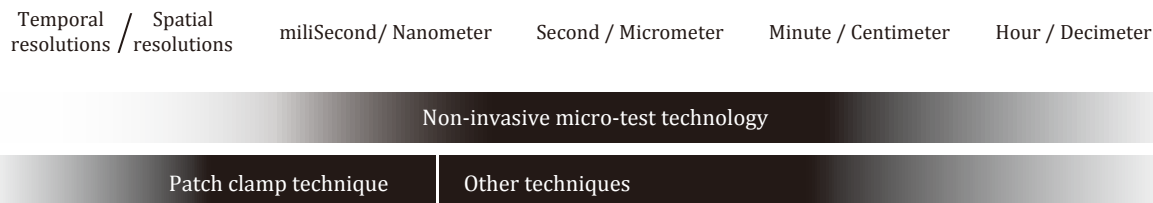
### NMT Micro-sensors

Knowing exactly which ions/molecules are measured is very straightforward, but adding new members to the current NMT sensor’s list is not an easy task. For example, some breakthroughs have been made to the most desired  $\text{Fe}^{2+}/^{3+}$  and  $\text{PO}_4^{3-}$  sensors recently after years of endeavors and efforts. The reasons that this progress has been slow are (1) only a few scientists are working in the field; (2) due to the inter-disciplinary nature of NMT sensor’s development; (3) unwillingness to develop new NMT sensors for the lack of commercial incentives.

### Temporal resolution

NMT’s temporal resolution is mainly determined by its responding time. When obtaining reliable and stable readings, the NMT sensor requires responding time in a few seconds range versus dozens of seconds or even minutes of conventional microsensors.

Although temporal resolution may be a limiting factor for NMT to go below seconds, biologists are taking advantage of its relative long-term detection capability of up to hours or even days (Fig. 3).



**Fig. 3** NMT temporal and spatial resolutions. Comparisons between NMT and other techniques. NMT resides in the range covering the gap between patch clamp and other techniques

**Comparative advantages over other techniques**

*NMT vs. Micro-electrodes*

NMT is different from traditional Ion Selective and/or molecular specific Micro-electrodes. On one hand, it is true to a certain extent because they both inform about the ions and/or molecules, on the other hand, much difference exists upon how much information each technique can reveal (Fig. 4).

*NMT vs. Patch Clamp*

E. Neher and B. Sakmann were awarded the Nobel Prize in 1991, after they invented the patch clamp technique to measure ionic currents in the plasma membrane of living cells (Xu and Qiu 1993).

One of the major breakthroughs with patch clamp is the successful GigaΩ seal between the polished glass micro-pipette and the plasma membrane. Yet, it also brings in significant mechanical impacts or even damages to the cells, which makes the mechanical stimulations unavoidable throughout every patch clamp experiment (Yang *et al.* 2012).

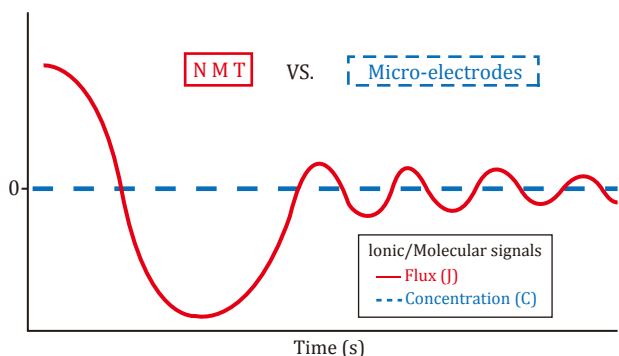
Therefore, a non-invasive way of detecting ions across cell membranes is very attractive. Especially for plant biologists to study salt or water processes, there is no need to enzymatically peel away the cell walls of plant cells which perturb the properties of cell membranes significantly (Xu 2023).

NMT can detect ion(s) movement(s) even under electrical neutral conditions (Fig. 5).

A valuable by-product of no-touching measurements of NMT is that it can measure a wide range of live samples in different sizes, from small intact organisms, isolated organs, tissues, cell layers, and single cells to aggregated organelles or microbes.

Detection of multi-ions/molecules simultaneously is another unique feature of NMT, which allows scientists to study the inter-relationships not only among ions but also among ions and molecules potentially paving the way to understand vital physiological functions of life (Kunkel *et al.* 2006; Xu *et al.* 2006).

However, patch clamps have good temporal and spatial resolutions (Fig. 3), which allow scientists to take advantage of both techniques to tackle tough scientific questions (Chen *et al.* 2007; Shabala *et al.* 2006; Smith *et al.* 1999).



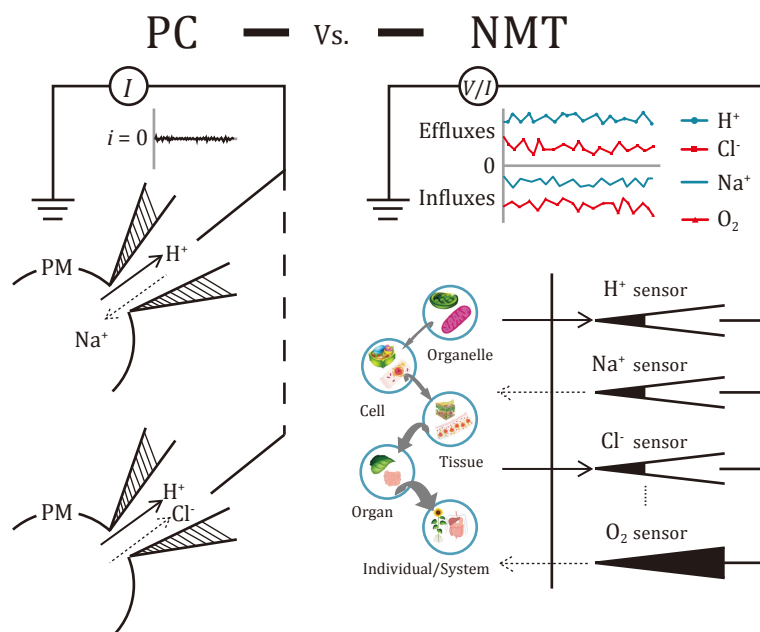
**Fig. 4** NMT versus micro-electrodes. Comparison between NMT and micro-electrodes. Under identical experimental conditions, NMT can pick up reliable and quantifiable ionic/molecular flux signals while micro-electrodes do not

**NMT APPLICATIONS**

**Medicine and healthy**

*Disease treatments*

Glioma is a common brain tumor with high morbidity and mortality. Recent studies have shown that photodynamic therapy (PDT) can be used to kill the glioma cells due to increased extracellular glutamate concentrations in C6 gliomas and upregulated glutamate receptor AMPA expression. However, how PDT affects the related transportations of Ca<sup>2+</sup> and K<sup>+</sup> across the cell membranes remains elusive. Researchers have demonstrated with NMT that significant Ca<sup>2+</sup> uptake and K<sup>+</sup>



**Fig. 5** NMT versus Patch Clamp. Comparison between NMT and Patch Clamp technique. Left panel: there are no currents when two identical electrically charged ions move in the opposite direction, as well as two opposite electrically charged ions moving in the same direction. Right panel: regardless of the sizes of the samples and their electrical charges, as long as there are specific ionic/molecular sensors available, NMT can pick up all the ionic and molecular signals.  $H^+$ ,  $Na^+$ ,  $Cl^-$  and  $O_2$  sensors are used to illustrate not only the different types of NMT sensors used simultaneously with different samples, but also effluxes and/or influxes of multiple ions/molecules detected at the same time in one NMT experiment. In the following, we will outline some recent progress made in different research areas in biology and medicine with the assistance of the NMT technology

loss occurred after PDT treatment resulting in the death of C6 glioma cells (Hu *et al.* 2014). Antagonist CNQX eliminated the changes of  $Ca^{2+}$  and  $K^+$  therefore direct evidence was provided for the involvement of changes in  $Ca^{2+}$  and  $K^+$  transportations in PDT interventions (Hu *et al.* 2014). To date, NMT has been successfully used to correlate both  $O_2$  and  $Ca^{2+}$  fluxes to the early signals of PDT-induced apoptosis as well as the intracellular ROS (Song *et al.* 2015). Intracellular pH (pHi) and extracellular pH (pHe) are important parameters for maintaining various physiological functions. The drug resistance study method (DRSM) was established using non-invasive micro-test technology (NMT), which can be used to study the correlations among organs, tissues, extracellular ions, molecular activities and anti-cancer drug resistance (Ma *et al.* 2012). The study suggested the correlation between extracellular  $H^+$  activity and anti-cancer drug resistance by using ADR (doxorubicin) with both sensitive and resistant strains of MCF-7 cells (Song *et al.* 2008).

$Ca^{2+}$  has been demonstrated to be a key player in pancreatic (Ren *et al.* 2022) functions (Cui and Kanno 1997). Abnormal insulin secretion of pancreas  $\beta$  cells is a major defect in type 2 diabetes. Impaired mitochondrial function and abnormal  $Ca^{2+}$  absorption have been

previously found in studies in human and animal diabetic models. NMT experiments found that the change in  $Ca^{2+}$  influx caused by the abnormal mitochondria of pancreatic beta cells rather than  $Ca^{2+}$  channel activity is the main reason for abnormal insulin secretion in diabetic mice (Li *et al.* 2012a). Islet  $\beta$  cell transplantation is an ideal treatment for type-I diabetes and generating  $\beta$  cells from induced pluripotent stem cells (iPSCs) from patients is a promising strategy. Increased glucose levels lead to membrane depolarization resulting in an influx of  $Ca^{2+}$  which triggers insulin exocytosis. Therefore, researchers used  $Ca^{2+}$  influxes as one of the benchmarks to evaluate whether the pancreas  $\beta$  cells derived from iPSCs are healthy (Bai *et al.* 2021).

NMT also was widely applied in pharmacological research. Bumetanide is a potent loop diuretic increasing the urine flow by blocking NKCC2, which may relate to its side effects in the digestive system, such as loss of appetite, nausea or/and vomiting due to abnormal  $H^+$  secretion.  $H^+$  fluxes in the gastric mucosa of mice revealed by NMT demonstrate that bumetanide (10  $\mu\text{mo/L}$ ) significantly inhibited histamine-induced  $H^+$  fluxes, but had no effect on basal  $H^+$  fluxes, suggesting NKCC plays a role in acid secretion (Zheng *et al.*

2020). Entacapone, an inhibitor of Catechol-O-methyltransferase (COMT), is widely used in the treatment of Parkinson's disease (PD). However, about 10% of PD patients with entacapone treatment clinically experience diarrhea. NMT results indicate that the cause of the gastrointestinal side effects of entacapone is primarily the profound secretion of  $\text{Cl}^-$  (Li *et al.* 2015). Emodin (1,3,8-trihydroxy-6-methylantraquinone) is a well-known ingredient of herb-based laxatives, but the working mechanism is still unclear.  $\text{Cl}^-$  secretion was found to be the main player in the emodin-induced colonic physiological process accompanied by mast cell degranulation and activation of cholinergic and non-cholinergic submucosal neurons (Xu *et al.* 2012).

NMT has also been used to detect apoptosis, which often occurs in inflammatory responses. Similar to  $\text{Ca}^{2+}$ , which has been demonstrated to be involved in ROS production and mitochondria depolarization (Hu *et al.* 2008),  $\text{K}^+$  has been proven to be a player in apoptosis. Programmed cell death (apoptosis) occurs in almost all cells, including oocytes and embryos. Understanding the mechanism of apoptosis can provide cues for preterm birth, cancer and aging. When cells initiate programmed death by  $\text{H}_2\text{O}_2$ , cells shrink, potassium channels are activated, and  $\text{K}^+$  effluxes are detected outside the embryos (Trimarchi *et al.* 2000a, b).

Alzheimer's disease (AD) is a common, irreversible central nervous system degenerative disorder characterized by progressive cognitive dysfunction, memory loss, and psychobehavioral changes. Its pathology is characterized by massive extracellular deposition of amyloid- $\beta$  ( $\text{A}\beta$ ) that forms senile plaques and the intracellular accumulation of abnormally modified tau protein that forms neurofibrillary tangles (NFTs). The main pathogenesis hypotheses include:  $\text{A}\beta$  tangles, Tau hyperphosphorylation, energy metabolism disorders, oxidative stress, excitatory neurotoxicity, metal ion homeostasis, *etc.* (Chung *et al.* 2010; Ray *et al.* 2011; Shabala *et al.* 2010). Among them, the changes of  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{H}^+$ ,  $\text{O}_2$ , and  $\text{H}_2\text{O}_2$  plasma dynamic balance are closely related to the development of AD, which could be detected using NMT. Evidence suggest that transmembrane  $\text{Ca}^{2+}$  transportation regulation is crucial to understand the mechanism of many human diseases (Li *et al.* 2011). Especially, the intracellular  $\text{Ca}^{2+}$  overload is an important mechanism for the cytotoxic effects of  $\text{A}\beta$ . Therefore, rapid and accurate detection of dynamic changes in neuronal transmembrane  $\text{Ca}^{2+}$  flux can not only help to understand the mechanism of cell maintenance of  $\text{Ca}^{2+}$  homeostasis and normal functional activities, but also help to reveal the pathogenesis of AD and other diseases related to  $\text{Ca}^{2+}$  signaling. Researchers used NMT to study the effect of a novel anti-T2DM drug

GLP-1/GIP/Gcg tri-receptor agonist (Triagonist) on the transmembrane fluxes of  $\text{Ca}^{2+}$  in neurons in *ex vivo* brain slices. The results showed that Triagonist maintained the  $\text{Ca}^{2+}$  homeostasis of neurons and avoided intracellular  $\text{Ca}^{2+}$  overload by regulating the transmembrane  $\text{Ca}^{2+}$  fluxes of neurons in the CA1 region of the hippocampus of 3xTg-AD mice (Li *et al.* 2020). Moreover, both  $\text{K}^+$  and  $\text{Ca}^{2+}$  have been found to be key players in the immune system and the brain (Shu *et al.* 2007). It is imperative to correlate the deposition of amyloid- $\beta$  ( $\text{A}\beta$ ) that forms senile plaques and the homeostasis of  $\text{K}^+$  and  $\text{Ca}^{2+}$  to tackle the mechanism of how AD develops.  $\text{K}^+/\text{Ca}^{2+}$  fluxes data also suggest that long term exposure to  $\text{A}\beta$  is detrimental since it diminishes the ability of cortical neurons to maintain  $\text{K}^+/\text{Ca}^{2+}$  homeostasis, which in turn may provide a new insight into the early indicators of AD (Ray *et al.* 2011; Shabala *et al.* 2010). One well-known feature of AD is a relatively higher level of reactive oxygen species (ROS) that overcomes the anti-oxidative stress mechanism of the brain. Meanwhile, the Cu (II)- $\text{A}\beta$  complex has been demonstrated to be highly toxic to neurons, and Zn7MT-2A can alleviate the toxicity. In a previous study, a significant  $\text{K}^+$  efflux was detected with the presence of Cu(II)- $\text{A}\beta$ 1-40 suggesting that Cu(II)- $\text{A}\beta$  can lead to disruption of  $\text{K}^+$  homeostasis in cultured cortical neurons, while the addition of Zn7MT-2A can inhibit the disruption (Chung *et al.* 2010; Howells *et al.* 2012).

### Toxicology

Zebrafish embryos were used as a model to investigate the toxic effects of CuNPs. The results show that CuNP exposure can impair two subtypes of ionocytes and their associated functions, including  $\text{Na}^+/\text{Ca}^{2+}$  uptake and  $\text{H}^+/\text{NH}_4^+$  excretion in zebrafish embryos (Lee *et al.* 2020; Shabala *et al.* 2012).

### Clinical medicine

Cell volume regulation is the basis of various cellular functions, such as cell division, cell proliferation, apoptosis, cell migration, and cell regulation, all of which require changes in cell volume and the involvement of transmembrane ions and molecules (Chen *et al.* 2011). Previously,  $\text{K}^+$  and  $\text{Cl}^-$  were found to be tightly coupled in regulatory volume reduction (RVD), it has been proved, however, not the case by using NMT. Not only for the first time that the transport of  $\text{K}^+$  and  $\text{Cl}^-$  is not coupled during hypotonic-induced RVD, but also  $\text{H}^+$  efflux has been found to play an important role in cell volume regulation, which may be a target for the

treatment of nasopharyngeal carcinoma (Yang *et al.* 2012).

Myopia has been a public health issue because of its increasingly high prevalence worldwide. The occurrence of myopia correlates with both the disturbance of ionic homeostasis and the change in the local microenvironment of the ciliary muscle.  $K^+$  fluxes in ciliary muscles from guinea pigs demonstrated myopia-triggered  $K^+$  influx, which leads to the disturbance of the microenvironment within ciliary muscles, providing a possible approach to treating myopia in clinical practice (Wu *et al.* 2020). Using live catfish retinal level cells,  $H^+$  fluxes under the stimulation of a variety of substances suggested that the neurotransmitter Glu induced alkalization at the outer surface of the cell membrane, and the size of the effect of Glu was related to the buffer, especially extracellular  $Ca^{2+}$  (Kreitzer *et al.* 2007).

Understanding the ionic homeostasis of wound healing at the single cell level is crucial to decipher the tissue, organ and or disease physiology (Zhang *et al.* 2022a).

$Ca^{2+}$  influxes are required for single cell wound healing while tested with *Xenopus laevis* oocytes (Luxardi *et al.* 2014).

Furthermore, NMT has also been widely used in the mechanism study of traditional Chinese Medicine. Traditional Chinese medicine and leucous, astragalus sugar complex have been proposed to facilitate the rapid repair process of intestinal mucosa via activation signaling pathway of potassium channel mediated by polyamine.  $K^+$  effluxes were observed with leucous and astragalus sugar complex treatments promoting the repair of gastrointestinal mucosal injury (Wang *et al.* 2018).

NMT was adopted to study the acupuncture mechanism. The results indicated that dynamic changes of  $Ca^{2+}$ ,  $Na^+$ , and  $H_2O_2$  fluxes had been observed. (1) In the early phase of skeletal muscle regeneration,  $Ca^{2+}$  efflux decreased, while  $Na^+$  influx increased, accompanied by increased  $H_2O_2$  efflux; (2) Acupuncture intervention increased  $Ca^{2+}$  efflux in the early phase of skeletal muscle regeneration and advanced the  $Na^+$  influx phase, with the decrease of  $H_2O_2$  efflux, and the effect was related to the interaction of TRP with NOX2 (Liu *et al.* 2018).

Membrane depolarization has been found to be accompanied by the transmural pressure, the typical myogenic response of renal and cerebral arteries of the rat. A  $Cl^-$  efflux from rat cerebral arteries with a temperature dependence correlated with myogenic contraction suggesting that  $Cl^-$  efflux through  $Cl^-$  channels contributes to the depolarization associated with myogenic contraction (Doughty and Langton 2001).

Oseltamivir phosphate (OP) is an antiviral drug that is used in the treatment and prophylaxis of both influenza A and influenza B. It is effective against all known influenza viruses that can infect humans, including pandemic influenza viruses and may be the most appropriate antiviral option against avian influenza caused by H5N1. An OP sensor based on ion association complexes and electrochemical reactions using a polyvinyl chloride (PVC) membrane has been successfully developed. The sensor can be used to determine (OP) in tablets. Compared to the reported HPLC method, the developed method is simple, accurate and precise (Hamza *et al.* 2017).

Acupuncture has a good therapeutic effect on skeletal muscle injury, and according to recent research trends, the mechanism is related to the TRP channel-mediated reactive oxygen species signaling pathway to restore early cytoplasmic  $Ca^{2+}$  homeostasis. The changes of  $Ca^{2+}$ ,  $Na^+$ , and  $H_2O_2$  dynamic flow rates in the early phase after skeletal muscle injury were detected by acupuncture intervention, aiming to explore the role of the interaction between TRP channel and NADPH oxidase 2 in the mechanism of acupuncture effect (Liu and Zhang 2018).

Uncoupling protein (UCP) is an inner mitochondrial membrane protein that eliminates transmembrane proton gradients on both sides of the inner mitochondrial membrane, slowing down the oxidative phosphorylation process driven by the proton gradient and hindering the normal production of ATP. Reduced UCP3 in prediabetes and diabetes is associated with insulin resistance, but the function of UCP3 is unclear. The study of the  $O_2$  fluxes caused by UCP3 demonstrates no significant change in oxygen consumption in the UCP3 super expression group, and the chemical decoupling agent significantly increased oxygen consumption (MacLellan *et al.* 2005).

## Animal study

### Entomology

In insects, heterodimeric glycoprotein hormones play an important role in myelination and cuticle hardening during development. With the recent discovery of two novel subunits forming an additional heterodimeric glycoprotein hormone in mammals, similar glycoprotein hormone forming subunits have been identified in insects. Research done by O'Donnell *et al.* suggests that AedaeGAP2/GPB5 can be used to regulate the physiological phenomenon of low  $Na^+$  and high  $K^+$  in the process of digestion and absorption of red blood cells by

female mosquitoes, and direct evidence of physiological function has been obtained (Nguyen and Donini 2010; Paluzzi *et al.* 2014).

#### *Animal physiology and behavior*

E-C coupling contraction of the muscle relies on the movement of  $\text{Ca}^{2+}$  from different sources, such as the intracellular stores and/or from the extracellular media. The  $\text{Ca}^{2+}$  flux data suggested that the acetylcholine-induced  $\text{Ca}^{2+}$  efflux was the result of, first,  $\text{Ca}^{2+}$  influx through voltage-sensitive L-type  $\text{Ca}^{2+}$  channels, then the rapid extrusion of  $\text{Ca}^{2+}$  by an outwardly directed carrier such as the Na-Ca exchanger as demonstrated by  $\text{Li}^+$  substitution experiments. NMT has provided new insights into the active and complex role the sarcolemma plays in  $\text{Ca}^{2+}$  homeostasis and regulating  $\text{Ca}^{2+}$  redistribution during excitation-contraction coupling (Devlin and Smith 1996).

### Plants biology

#### *Abiotic stress*

Quantitative  $\text{K}^+/\text{H}^+$  fluxes under salt stress detected by NMT suggested a  $\text{K}^+$  preservation anti-salt stress mechanism via repolarization of the plasma membrane and silencing of Guard cell outward-rectifying  $\text{K}^+$  (GORK) channel (Gong *et al.* 2023; Wu *et al.* 2024; Zeng *et al.* 2024). The other way to control  $\text{K}^+$  fluxes is to go through the key signal ROS that activates the Non-selective cation channel (NSCC) and the pathways generated extracellular ROS (Pang *et al.* 2006; Shabala and Hariadi 2005; Sun *et al.* 2009c; Tang *et al.* 2018). Taking advantage of direct physiological evidences from NMT, a number of attempts have been made to find out a way to maintain IMH,  $\text{Na}^+/\text{K}^+$  homeostasis specifically, to combat with plant salt stress (Chinnusamy *et al.* 2004; Guo *et al.* 2009; Lou *et al.* 2020; Qu *et al.* 2021; Shabala *et al.* 2005; Shahzad *et al.* 2022; Zhang *et al.* 2022b).

Extreme temperature is a key factor limiting global crop plant distribution and yield. NMT was able to demonstrate that there was a significant difference in  $\text{Ca}^{2+}$  influxes after cold treatment between COLD1/transgenic lines and corresponding wild types (Ma *et al.* 2015). Supported by  $\text{Ca}^{2+}$ ,  $\text{H}^+$ ,  $\text{K}^+$ , and  $\text{Na}^+$  fluxes data, Lsi1 and OsCNGC9 genes have been found to enhance the cold tolerance of crops by regulating low-temperature-induced calcium influx and cytosolic calcium elevation (Liu *et al.* 2024; Wang *et al.* 2021).

$\text{Cd}^{2+}/\text{Cu}^{2+}/\text{Pb}^{2+}$  influxes have been detected in live samples, such as roots, leaves, algae, biofilms, bacteria,

and fungi, as well as with different live parts/organs, such as root xylem, stem, xylem duct, mesophyll cells, intact vacuoles, *etc.* (Cheng *et al.* 2023; Cuin and Shabala 2007; Jiang *et al.* 2022; Li *et al.* 2016, 2017a, 2024; Lv *et al.* 2018; Papoyan *et al.* 2007; Yang *et al.* 2022b; Zhang *et al.* 2020). The changes in the absorption of  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  were studied with plant tissues and cells in the presence of heavy metals (Li *et al.* 2017a, 2017b, 2012b; Lin *et al.* 2013; Ma *et al.* 2016; Wang *et al.* 2022, 2023a). NMT data of  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$  fluxes help to identify OsHIPP9/BcHIPP16 membrane proteins to absorb nutrient metal Cu and heavy metal Cd in plants (Niu *et al.* 2021; Xiong *et al.* 2023).

Drought is a great threat to crop yields and quality worldwide. lows of  $\text{H}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{H}_2\text{O}_2$  have been monitored with live plant samples to correlate energy, nutrient metabolisms,  $\text{K}^+$  homeostasis, signal transduction, ROS response to water control in plants (Chen *et al.* 2005; Li *et al.* 2019; Mak *et al.* 2014; Sun *et al.* 2024). NMT study provides important evidence for the dual role of plasma membrane  $\text{H}^+$ -ATPases in light-induced stomatal opening and ABA-induced stomatal closure, highlighting the importance of  $\text{H}^+$  in plasma membrane cation and anion influx and efflux (Pei *et al.* 2022).

#### *Plant immunity*

NMT results found that herbivory pretreatment altered  $\text{Ca}^{2+}$  flux via different  $\text{Ca}^{2+}$  channels or transporter which help *Ammopiptanthus nanus* to prevent  $\text{K}^+$  leakage (Chen *et al.* 2020).  $\text{Ca}^{2+}$  flux data also indicated that the wheat susceptibility factor TaBln1, which interacts with TaCaM3 to impair  $\text{Ca}^{2+}$  influx could inhibit plant defenses (Guo *et al.* 2022).  $\text{Ca}^{2+}$  influx and  $\text{H}^+$  efflux data indicated that eATP-promoted stomatal opening possibly involves the heterotrimeric G protein, ROS, cytosolic  $\text{Ca}^{2+}$ , and plasma membrane  $\text{H}^+$ -ATPase (Gong *et al.* 2023; Hao *et al.* 2012; Sun *et al.* 2012; Zhao *et al.* 2016). NMT results indicated that melatonin,  $\text{H}_2\text{O}_2$  and  $\text{Ca}^{2+}$  attenuated ABA-induced  $\text{K}^+$  efflux and subsequent cell death (Guo *et al.* 2023).

#### *Plant hormones signaling*

After Indole acetic acid (IAA) treatment, the  $\text{H}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  fluxes of *Arabidopsis thaliana* roots were detected (Yang *et al.* 2022a), which provided key evidences for IAA-induced  $\text{H}^+$  alkalinization and then  $\text{H}^+$ -induced exosomes in roots (Li *et al.* 2021). NMT experiments have indicated that the  $\text{Ca}^{2+}$  uptake of the mutant is impaired resulting in the pollen tube tip not moving towards the ovule. Meanwhile, the fluxes of  $\text{Cl}^-$  and  $\text{K}^+$  did

not differ significantly between mutant and wild type (Meng *et al.* 2020).

### Plant nutrition

Sa *et al.* (2019) found that root-associated ectomycorrhizal fungi (EMF) are involved in promoting root  $\text{NO}_3^-$  uptake of poplar by detecting  $\text{NO}_3^-$  fluxes around the root surface. Similarly, Peng *et al.* (2021) used the NMT to monitor and compare  $\text{K}^+$  flux profiles across the meristematic zone, root elongation and mature zones of *Liquidambar styraciflua* and observed that more  $\text{K}^+$  is absorbed by the roots colonized by a common soil saprotroph, *Clitopilus hobsonii*. Sun *et al.* (2022) demonstrated that fungus-regulated  $\text{NO}_3^-/\text{NH}_4^+$  dynamics alters plant response to  $\text{NO}_3^-/\text{NH}_4^+$  nutrition during symbiotic fungus *Phomopsis liquidambaris*-*Arabidopsis* interaction by examining N fluxes at the plant-fungal interface (Peng *et al.* 2021; Sa *et al.* 2019; Sun *et al.* 2022).

The acidity and alkalinity of the surrounding environment also affect ion exchange. In soybeans and coniferous trees, proton efflux at lower pH is beneficial for maintaining sustained absorption of  $\text{NH}_4^+$ , for plants to adapt to acidic soil (Hawkins and Robbins 2010). *Glomus mosseae* (GM, fungus) and *Bradyrhizobium japonicum* (BJ, fungus) promoted the outflow of  $\text{H}^+$  in soybean roots, and the increase of phosphorus content was positively correlated with the outflow of  $\text{H}^+$  (Ding *et al.* 2012).

### Environmental science

Bacteria biofilms use potassium channel-mediated electrical signals for cell-to-cell communication although it is unknown whether these signals play a role in *Geobacter* sp. when surrounded by an intense electric field.

Steady and reliable  $\text{K}^+$  fluxes detected by NMT provide preliminary evidence to reveal the role of potassium channels in electroactive biofilms in *Agrobacterium* reducing ensembles (Jing *et al.* 2020).

Seagrass is the only higher angiosperm that submerges in seawater. The seagrass ecosystem is, nevertheless, subject to numerous stressors brought on by human activity, including hypoxia, hyper salinity, eutrophication, temperature, and others. The findings from  $\text{NH}_4^+$  and  $\text{H}^+$  flux data demonstrated that an increase in  $\text{NH}_4^+$  content in seawater encouraged absorption, which might reprogram the nitrogen metabolism (Chen *et al.* 2019; Fang *et al.* 2020).

The safety of freshwater ecosystems is critical to humans, so real-time monitoring of levels of harmful

chemicals and biological agents in freshwater environments is essential. Using  $\text{O}_2$  fluxes as a Biosafety Indicator, a real-time effect of atrazine, cadmium chloride, pentachlorophenol, malathion, and potassium cyanide on respiratory oxygen consumption in fathead minnow embryos two days after fertilization was detected (Sanchez *et al.* 2008).

Plants use environmental signals to guide root growth and response so that they can adapt to the environment. For instance, roots can direct their growth and response in relation to gravity, light, gradients of temperature, humidity, ions, chemicals and oxygen. These environmental signals will be translated into physiological responses via ionic/molecular transportation. In root gravitropism,  $\text{H}^+$  efflux or  $\text{Ca}^{2+}$  influx in root apexes was weaker in IPG-grown roots than those in TPG-grown roots (Xu *et al.* 2013).

A list of live samples tested by NMT is provided in Table 1, and a list of categorized references by NMT applications fields is also made available as a quick reference with NMT (supplementary Table S1).

### CONCLUSION

Abnormal IMHs can lead to pathology, while the activities of ions and small molecules are relatively difficult to study.

NMT, an integration of multi-techniques and multi-disciplinary knowledge, is evolving. It is constantly being improved functions to better fit their specific studies (Chi *et al.* 2021; Li *et al.* 2014; Lv *et al.* 2013; Song *et al.* 2017; Zhu *et al.* 2007).

The involvements of  $\text{H}^+$  and  $\text{O}_2$  in tumor physiology have been studied (Hsu and Sabatini 2008; Parks *et al.* 2013; Seyfried and Shelton 2010), as well as  $\text{H}^+$  and/or  $\text{O}_2$  manipulations in tumor micro-environments (Bailey *et al.* 2012; Parks *et al.* 2013; Yang and Li 2019; Zhang *et al.* 2021).

A few decades have passed since the first  $\text{Ca}^{2+}$  flux data was collected by biologists from a single cell (Jaffe and Nuccitelli 1974; Kuhlreiber and Jaffe 1990). NMT-based ions and molecules Omics (imOmics) is the study of IMH pertinent to a specific physiological process via collections and integral analysis of ions and/or molecules activities (Xu *et al.* 2023).

In this multi-dimensional-omics age, scientists are trying to make a functional network map, it has been demonstrated that the study of IMHs by NMT could play a significant role in not only connecting but verifying data from other techniques for a better understanding of a specific physiological function, more importantly from live samples.

**Table 1** Summary of samples tested by NMT

Plant samples	Number of publications
<i>Arabidopsis thaliana</i>	107
Rice	79
Poplar	38
Wheat	36
Tobacco	24
Apple	14
Cotton	12
Tea plant	11
Corn	10
Alfalfa, rape	9
Soybean	8
Sweet potato	7
Tomato, cucumber, southeast <i>Sedum</i> , mining accompanying <i>Sedum</i>	6
Trifoliate orange, Spruce	5
<i>Solanum nigrum</i> , <i>Limonium bicolor</i> , duckweed, mangrove, <i>Leymus chinensis</i>	4
Grape, <i>Porphyra haitanensis</i> , Chinese cabbage, <i>Suaeda salsa</i> , pumpkin, white thorn, cassava, <i>Phyllostachys edulis</i>	3
Potato, autumn eggplant, Peas, pear, star grass, ryegrass, <i>Polyporus umbellatus</i> , mung bean, maple fragrance, sweet sorghum, willow	2
Candlestick fruit, tall fescue, white stem, green cottage, <i>Phellodendron amurense</i> , licorice, lily, Bidong eggplant ( <i>Petunia</i> ), peanut, Chinese fir, Chinese cabbage, <i>Spartina alterniflora</i> , Jiangli, child ginseng, reed, Chinese chestnut, watermelon, <i>Zizania latifolia</i> , Minjiang fir, red thick shell (begonia fruit), <i>canna</i> plantain, Xiaojin Haitang, Heguo taro, mulberry, sand holly, water caltrop, black fruit goji berries, <i>Halophila beccarii</i> , sugarbeet, banana, <i>Malus hupehensis</i> , Mi Zi, <i>Dendrobium officinale</i> , strawberry, Huanghua Hongsha, white feather fan bean, cucumber grass, bitter grass, <i>Microsorium pteropus</i> , goldfish grass, <i>Sorghum</i> , millet, blueberries, lettuce, Ningxia goji berries, <i>Spiraea japonica</i> , sour jujube, oats, <i>Bidens bipinnata</i> , <i>Iris</i> , <i>Citrus</i> , wolf tail grass, <i>Celosia argentea</i>	1
Other samples	
Zebrafish	9
Green guppy	6
Clawed toad	4
Yeast cell	4
Metallic material	3
Fungi	3
Biological materials, rat colon, <i>Phanerochaete chrysosporium</i> , mice	2
Rat, guinea pig, sludge, mouse pancreatic islet tissue, nasopharyngeal cancer cells, human oral epidermoid carcinoma cells, C6 glioma cells, oral squamous cell carcinoma cells, pearl oyster, liver cells, acellular cortical bone slices, I- $\beta$ cell, <i>Escherichia coli</i> , <i>Aspergillus niger</i> , <i>Geobacter</i> , microalgae	1

**Acknowledgements** Many thanks to Prof. Tingyun Kuang (Institute of Botany, Chinese Academy of Sciences), Prof. Lionel Jaffe (Marine Biological Laboratory, The University of Chicago), Prof. Fuyu Yang (Institute of Biophysics, Chinese Academy of Sciences), Prof. Kechun Lin (Peking University), Prof. Weiping Zhao (Capital Normal University), Prof. Zesheng Qiu (Capital Normal University), Prof. Weihua Wu (China Agricultural University), Prof. Kang Chong (Institute of Botany, the Chinese Academy of Sciences), Prof. Weicai Yang (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences), Prof.

Yan Guo (China Agricultural University), Prof. Tao Xu (Institute of Biophysics, Chinese Academy of Sciences), Prof. Shaoliang Chen (Beijing Forestry University), Prof. Zhibin Luo (Institute of Forestry, Chinese Academy of Forestry Sciences), Prof. Liwei Wang (Jinan University), Prof. Lixin Chen (Jinan University), Prof. Xingjie Liang (National Center for Nanoscience and Technology), Prof. Jian Sun (Jiangsu Normal University), Prof. Jinxia Zhu (Capital Medical University), Prof. Jinshun Qi (Shanxi Medical University), Prof. Marshall Porterfield (Purdue University), Prof. Sergey Shabala (The University of Western

Australia), Prof. Lana Shabala (University of Tasmania). We thank Prof. Li Zhang (Peking Union Medical College Hospital), Prof. Ning Yu (Chinese People's Liberation Army (PLA) General Hospital), Prof. Jingqian Feng (Medical School, Quzhou College of Technology), Prof. Lihong Wang (School of Environment and Civil Engineering, Jiangnan University), Dr. Heping Wan (School of Life Sciences, Jiangnan University), Ms. Wen Zhang (School of Life Sciences, Shandong University) and Mr. Jianfei Song (College of Horticulture Science and Engineering, Shandong Agricultural University) for critical reading of the manuscript.

### Compliance with Ethical Standards

**Conflict of Interest** Kai Sun, Yunqi Liu, Yanshu Pan, Dongwei Di, Jianfang Li, Feiyun Xu, Li Li, Yoshiharu Mimata, Yingying Chen, Lixia Xie, Siqi Wang, Wenqian Qi, Yan Tang, Huachun Sheng, Bing Wang, Ruixue Sun, Dingquan Tan, Daohong Fu, Ye Yin, Ao Xue, Yichao Shi, Wenjing Shao, Lei Gong, Zhijian Jiang, Wei Zhang, Qiangsheng Wu, Yaosheng Wang, Minglin Lang, Wenxiu Ye, Weifeng Xu, Shuhe Wei, Weiming Shi, and Yue Jeff Xu declare that they have no conflict of interest.

**Human and animal rights and informed consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

### References

- Ahmadi F, Samadi A, Sepehr E, Rahimi A, Shabala S (2023) Potassium homeostasis and signaling as a determinant of *Echinacea* species tolerance to salinity stress. *Environ Exp Bot* 206: 105148. <https://doi.org/10.1016/j.envexpbot.2022.105148>
- Bai C, Ren Q, Liu H, Li X, Guan W, Gao Y (2021) miR-212/132-enriched extracellular vesicles promote differentiation of induced pluripotent stem cells into pancreatic beta cells. *Front Cell Dev Biol* 9: 1–15
- Bailey KM, Wojtkowiak JW, Hashim AI, Gillies RJ (2012) Targeting the metabolic microenvironment of tumors. *Adv Pharmacol* 65: 63–107
- Chen S, Ye S, Cui S, Yu N (2011) The transport mechanism of  $Ca^{2+}$  in the isolated OHCs of guinea pig. Master's Thesis of Fujian Medical University
- Chen T, Wang W, Xu K, Xu Y, Ji D, Chen C, Xie C (2019)  $K^+$  and  $Na^+$  transport contribute to  $K^+/Na^+$  homeostasis in *Pyropia haitanensis* under hypersaline stress. *Algal Res* 40: 101526. <https://doi.org/10.1016/j.algal.2019.101526>
- Chen Y, Cao C, Guo Z, Zhang Q, Li S, Zhang X, Gong J, Shen Y (2020) Herbivore exposure alters ion fluxes and improves salt tolerance in a desert shrub. *Plant Cell Environ* 43: 400–419
- Chen Z, New I, Zhou M, Mendham N, Zhang G, Shabala S (2005) Screening plants for salt tolerance by measuring  $K^+$  flux: a case study for barley. *Plant Cell Environ* 28: 1230–1246
- Chen Z, Pottosin II, Cuin TA, Fuglsang AT, Tester M, Jha D, Zepeda-Jazo I, Zhou M, Palmgren MG, Newman IA, Shabala S (2007) Root plasma membrane transporters controlling  $K^+/Na^+$  homeostasis in salt-stressed barley. *Plant Physiol* 145: 1714–1725
- Cheng Y, Chen X, Liu W, Yang L, Wu J, Wang Y, Yu W, Zhou J, Fayyaz P, Luo ZB, Deng S, Shi W (2023) Homolog of human placenta-specific gene 8, PcPLAC8-10, enhances cadmium uptake by *Populus* roots. *J Hazard Mater* 460: 132349. <https://doi.org/10.1016/j.jhazmat.2023.132349>
- Chi S, Yang H, Jiang P, Chen H (2021) Application of non-invasive micro-test technology (NMT) in environmental protection research. *Metall Manag* 1: 141–153
- Chinnusamy V, Schumaker K, Zhu JK (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signalling in plants. *J Exp Bot* 55: 225–236
- Chung RS, Howells C, Eaton ED, Shabala L, Zovo K, Palumaa P, Sillard R, Woodhouse A, Bennett WR, Ray S, Vickers JC, West AK (2010) The native copper- and zinc-binding protein metallothionein blocks copper-mediated  $A\beta$  aggregation and toxicity in rat cortical neurons. *PLoS One* 5: e12030. <https://doi.org/10.1371/journal.pone.0012030>
- Cui ZJ, Kanno T (1997) Photodynamic triggering of calcium oscillation in the isolated rat pancreatic acini. *J Physiol* 504: 47–55
- Cuin TA, Shabala S (2007) Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. *Plant Cell Environ* 30: 875–885
- Degenhardt J, Larsen PB, Howell SH, Kochian LV (1998) Aluminum resistance in the *Arabidopsis* mutant alr-104 is caused by an aluminum-induced increase in rhizosphere pH. *Plant Physiol* 117: 19–27
- Devlin CL, Smith PJ (1996) A non-invasive vibrating calcium-selective electrode measures acetylcholine-induced calcium flux across the sarcolemma of a smooth muscle. *J Comp Physiol* 166: 270–277
- Ding X, Sui X, Wang F, Gao J, He X, Zhang F, Yang J, Feng G (2012) Synergistic interactions between *Glomus mosseae* and *Bradyrhizobium japonicum* in enhancing proton release from nodules and hyphae. *Mycorrhiza* 22: 51–58
- Doughty JM, Langton PD (2001) Measurement of chloride flux associated with the myogenic response in rat cerebral arteries. *J Physiol* 534: 753–761
- Fang Y, Jiang Z, Zhao C, Li L, Ranvilage CIPM, Liu S, Wu Y, Huang X (2020) Efficient heat dissipation and cyclic electron flow confer daily air exposure tolerance in the intertidal seagrass *Halophila beccarii* Asch. *Front Plant Sci* 11: 1–14
- Gong J, Wang Z, Guo Z, Yao L, Zhao C, Lin S, Ma S, Shen Y (2023) DORN1 and GORK regulate stomatal closure in *Arabidopsis* mediated by volatile organic compound ethyl vinyl ketone. *Int J Biol Macromol* 231: 123503. <https://doi.org/10.1016/j.jbiomac.2023.123503>
- Guan L, Yin L, Liu Y, Yan J, Wang B, Luan M, Lan W (2024) A plasma membrane-localized transporter remobilizes aleurone layer magnesium for seed germination in rice. *Plant J* 119: 1449–1464
- Guo KM, Babourina O, Rengel Z (2009)  $Na^+/H^+$  antiporter activity of the SOS1 gene: lifetime imaging analysis and electrophysiological studies on *Arabidopsis* seedlings. *Physiol Plant*

- 137: 155–165
- Guo S, Zhang Y, Li M, Zeng P, Zhang Q, Li X, Xu Q, Li T, Wang X, Kang Z, Zhang X (2022) TaBln1, a member of the Blufensin family, negatively regulates wheat resistance to stripe rust by reducing  $\text{Ca}^{2+}$  influx. *Plant Physiol* 189: 1380–1396
- Guo Y, Zhu J, Liu J, Xue Y, Chang J, Zhang Y, Ahammed GJ, Wei C, Ma J, Li P, Zhang X, Li H (2023) Melatonin delays ABA-induced leaf senescence via  $\text{H}_2\text{O}_2$ -dependent calcium signalling. *Plant Cell Environ* 46: 171–184
- Hamza SM, Rizk NMH, Matter HAB (2017) A new ion selective electrode method for determination of oseltamivir phosphate (Tamiflu) and its pharmaceutical applications. *Arab J Chem* 10: S236–S243
- Hao L-H, Wang W-X, Chen C, Wang Y-F, Liu T, Li X, Shang Z-L (2012) Extracellular ATP promotes stomatal opening of *Arabidopsis thaliana* through heterotrimeric G protein  $\alpha$  subunit and reactive oxygen species. *Mol Plant* 5: 852–864
- Hawkins BJ, Robbins S (2010) PH affects ammonium, nitrate and proton fluxes in the apical region of conifer and soybean roots. *Physiol Plant* 138: 238–247
- Howells C, Saar K, Eaton E, Ray S, Palumaa P, Shabala L, Adlard PA, Bennett W, West AK, Guillemin GJ, Chung RS (2012) Redox-active Cu(II)- $\text{A}\beta$  causes substantial changes in axonal integrity in cultured cortical neurons in an oxidative-stress dependent manner. *Exp Neurol* 237: 499–506
- Hsu PP, Sabatini DM (2008). Cancer cell metabolism: Warburg and beyond. *Cell* 134: 703–707
- Hu F, Sun WW, Zhao XT, Cui, ZJ, Yang WX (2008) TRPV1 mediates cell death in rat synovial fibroblasts through calcium entry-dependent ROS production and mitochondrial depolarization. *Biochem Biophys Res Commun* 369: 989–993
- Hu SL, Du P, Hu R, Li F, Feng H (2014) Imbalance of  $\text{Ca}^{2+}$  and  $\text{K}^+$  fluxes in C6 glioma cells after PDT measured with scanning ion-selective electrode technique. *Lasers Med Sci* 29: 1261–1267
- Huang X, Guo X, Yan G, Zhang Y, Yao Y, Qiao Y, Wang D, Chen G, Zhang W, Tang C, Cao F (2022) Dapagliflozin attenuates contrast-induced acute kidney injury by regulating the HIF-1 $\alpha$ /HE4/NF- $\kappa$ B pathway. *J Cardiovasc Pharmacol* 79: 904–913
- Jaffe LF, Nuccitelli R (1974) An ultrasensitive vibrating probe for measuring steady extracellular currents. *J Cell Biol* 63: 614–628
- Jiang P, Zheng Y, Liu J, Yu G, Lin F (2022) Pathways of cadmium fluxes in the root of the hyperaccumulator *Celosia argentea* Linn. *Environ Sci Pollut Res* 29: 44413–44421
- Jing X, Yang Y, Ai Z, Chen S, Zhou S (2020) Potassium channel blocker inhibits the formation and electroactivity of *Geobacter* biofilm. *Sci Total Environ* 705: 135796. <https://doi.org/10.1016/j.scitotenv.2019.135796>
- Kochian L, Shaff J, Kühtreiber W, Jaffe L, Lucas W (1992) Use of an extracellular, ion-selective, vibrating microelectrode system for the quantification of  $\text{K}^+$ ,  $\text{H}^+$ , and  $\text{Ca}^{2+}$  fluxes in maize roots and maize suspension cells. *Planta* 188: 601–610
- Kreitzer MA, Collis LP, Molina AJA, Smith PJS, Malchow RP (2007) Modulation of extracellular proton fluxes from retinal horizontal cells of the catfish by depolarization and glutamate. *J Gen Physiol* 130: 169–182
- Kühtreiber WM, Jaffe LF (1990) Detection of extracellular calcium gradients with a calcium-specific vibrating electrode. *J Cell Biol* 110: 1565–1573
- Kunkel J, Li-Yih L, Yue X, Prado A, Feijó J, Hepler P (2001) The strategic use of Good buffers to measure proton gradients around growing pollen tubes. *Cell Biol Plant Fungal Tip Growth* 328: 81–94
- Kunkel JG, Cordeiro S, Xu Y (Jeff), Shipley AM, Feijó JA (2006) Use of non-invasive ion-selective microelectrode techniques for the study of plant development. In: *Plant Electrophysiology*, Volkov AG (eds). Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 109–137
- Lee CY, Horng JL, Liu ST, Lin LY (2020) Exposure to copper nanoparticles impairs ion uptake, and acid and ammonia excretion by ionocytes in zebrafish embryos. *Chemosphere* 261: 128051. <https://doi.org/10.1016/j.chemosphere.2020.128051>
- Li F, Marshall Porterfield D, Zheng XY, Wang WJ, Xu Y, Zhang ZM (2012a) Abnormal mitochondrial function impairs calcium influx in diabetic mouse pancreatic beta cells. *Chin Med J (Engl)* 125: 502–510
- Li J, Han Q, Duan L, Wang P, Li H, Wang S, Zhang J (2014) Applications and advances of non-invasive micro-test technique in plant physiology researches. *Plant Physiol J* 50: 1445–1452
- Li L, Verstraeten I, Roosjen M, Takahashi K, Rodrigue, L, Merrin J, Chen J, Shabala L, Smet W, Ren H (2021) Cell surface and intracellular auxin signalling for  $\text{H}^+$  fluxes in root growth. *Nature* 599: 273–277
- Li LS, Liu CZ, Xu JD, Zheng LF, Feng XY, Zhang Y, Zhu JX (2015) Effect of entacapone on colon motility and ion transport in a rat model of Parkinson's disease. *World J Gastroenterol* 21: 3509–3518
- Li LZ, Yu SY, Peijnenburg WJGM, Luo YM (2016) Determining the fluxes of ions ( $\text{Pb}^{2+}$ ,  $\text{Cu}_2^+$  and  $\text{Cd}^{2+}$ ) at the root surface of wetland plants using the scanning ion-selective electrode technique. *Plant Soil* 414: 1–12
- Li LZ, Tu C, Peijnenburg WJGM, Luo YM (2017a) Characteristics of cadmium uptake and membrane transport in roots of intact wheat (*Triticum aestivum* L.) seedlings. *Environ Pollut* 221: 351–358
- Li LZ, Tu C, Wu LH, Peijnenburg WJGM, Ebbs S, Luo YM (2017b) Pathways of root uptake and membrane transport of  $\text{Cd}^{2+}$  in the zinc/cadmium hyperaccumulating plant *Sedum plumbizincicola*. *Environ Toxicol Chem* 36: 1038–1046
- Li S, Yu J, Zhu M, Zhao F, Luan S (2012b) Cadmium impairs ion homeostasis by altering  $\text{K}^+$  and  $\text{Ca}^{2+}$  channel activities in rice root hair cells. *Plant Cell Environ* 35: 1998–2013
- Li S, Liu J, An Y, Cao Y, Liu Y, Zhang J, Geng J, Hu T, Yang P (2019) MsPIP2;2, a novel aquaporin gene from *Medicago sativa*, confers salt tolerance in transgenic *Arabidopsis*. *Environ Exp Bot* 165: 39–52
- Li S, He Z, Qiu W, Yu M, Wu L, Han X, Zhuo R (2024) SpCTP3 from the hyperaccumulator *Sedum plumbizincicola* positively regulates cadmium tolerance by interacting with SpMDH1. *J Hazard Mater* 472: 134517. <https://doi.org/10.1016/j.jhazmat.2024.134517>
- Li T, Jiao J-J, Su Q, Hölscher C, Zhang J, Yan X-D, Zhao H-M, Cai H-Y, Qi J-S (2020) A GLP-1/GIP/Gcg receptor triagonist improves memory behavior, as well as synaptic transmission, neuronal excitability and  $\text{Ca}^{2+}$  homeostasis in 3xTg-AD mice. *Neuropharmacology* 170: 108042. <https://doi.org/10.1016/j.neuropharm.2020.108042>
- Li Z, Liu L, Deng Y, Ji W, Du W, Xu P, Chen L, Xu T (2011) Graded activation of CRAC channel by binding of different numbers of STIM1 to Orai1 subunits. *Cell Res* 21: 305–315
- Lin L-Y, Pang W, Chuang W-M, Hung G-Y, Lin Y-H, Horng J-L (2013) Extracellular  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  modulate aminoglycoside blockade of mechanotransducer channel-mediated  $\text{Ca}^{2+}$  entry in zebrafish hair cells: an *in vivo* study with the SIET. *Am J Physiol Physiol* 305: C1060–C1068
- Liu A, Zhang X (2018) A study on the effect of acupuncture intervention on the changes of early phase  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ , and  $\text{H}_2\text{O}_2$  flow rates in rat skeletons induced by centrifugal exercise.

- Master's Thesis of Qufu Normal University
- Liu A, Zhang X, Duan M, Ding J (2018) OR-018. The study of acupuncture intervention on the dynamic changes of  $\text{Ca}^{2+}$ ,  $\text{Na}^+$ ,  $\text{H}_2\text{O}_2$  flux at earlier time points of rat skeletal muscle regeneration following eccentric exercise. *Exerc Biochem Rev* 1: OR-018. <https://doi.org/10.14428/eb.v1i2.9193>
- Liu B, Zhang J, Ye N (2023) Noninvasive micro-test technology: monitoring ion and molecular flow in plants. *Trends Plant Sci* 28: 123–124
- Liu S, Zheng Y, Zhao L, Gulam M, Ullah A, Xie G (2024) CALMODULIN-LIKE16 and PIN-LIKES7a cooperatively regulate rice seedling primary root elongation under chilling. *Plant Physio*. 195: 1660–1680
- Lou L, Yu F, Tian M, Liu G, Wu Y, Wu Y, Xia R, Pardo JM, Guo Y, Xie Q (2020) ESCRT-I component VPS23A sustains salt tolerance by strengthening the SOS module in *Arabidopsis*. *Mol Plant* 13: 1134–1148
- Luxardi G, Reid B, Maillard P, Zhao M (2014) Single cell wound generates electric current circuit and cell membrane potential variations that requires calcium influx. *Integr Biol* 6: 662–672
- Lv H, Teng Z, Wang S, Feng K, Wang X, Wang C, Wang G (2018) Voltammetric simultaneous ion flux measurements platform for  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Hg}^{2+}$  near rice root surface: utilizing carbon nitride heterojunction film modified carbon fiber micro-electrode. *Sensors Actuators B Chem* 256: 98–106
- Lv J, Miao L, Cai R, Wu H, Xu H, Zhou X (2013) Application of non-invasive micro-test technique in study of plant roots growth and development. *Biotechnology* 23: 89–93
- Ma X, Zhang LH, Wang LR, Xue X, Sun JH, Wu Y, Zou G, Wu X, Wang P., Wamer WG, Yin JJ, Zheng K, Liang XJ (2012) Single-walled carbon nanotubes alter cytochrome c electron transfer and modulate mitochondrial function. *ACS Nano* 6: 10486–10496
- Ma Y, Dai X, Xu Y, Luo W, Zheng X, Zeng D, Pan Y, Lin X, Liu H, Zhang D, Xiao J, Guo X, Xu S, Niu Y, Jin J, Zhang H, Xu X, Li L, Wang W, Qian Q, Ge S, Chong K (2015) COLD1 confers chilling tolerance in rice. *Cell* 160: 1209–1221
- Ma Z, Huang B, Xu S, Chen Y, Cao G, Ding G, Lin S (2016) Ion flux in roots of Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) under aluminum stress. *PLoS One* 11(6): e0156832. <https://doi.org/10.1371/journal.pone.0156832>
- MacLellan JD, Gerrits MF, Gowing A, Smith PJS, Wheeler MB, Harper M (2005) Physiological increases in uncoupling protein 3 augment fatty acid oxidation and decrease reactive oxygen species production without uncoupling respiration in muscle cells. *Diabetes* 54: 2343–2350
- Mak M, Babla M, Xu S-C, O'Carrigan A, Liu X-H, Gong Y-M, Holford P, Chen Z-H (2014) Leaf mesophyll  $\text{K}^+$ ,  $\text{H}^+$  and  $\text{Ca}^{2+}$  fluxes are involved in drought-induced decrease in photosynthesis and stomatal closure in soybean. *Environ Exp Bot* 98: 1–12
- Martínez-Ballesta M del C, Egea-Gilabert C, Conesa E, Ochoa J, Vicente MJ, Franco JA, Bañon S, Martínez JJ, Fernández JA (2020) The importance of ion homeostasis and nutrient status in seed development and germination. *Agronomy* 10: 504. <https://doi.org/10.3390/agronomy10040504>
- Martinez-Outschoorn UE, Lin, Z, Trimmer C, Flomenberg N, Wang C, Pavlides S, Pestell RG, Howell A, Sotgia F, Lisanti MP (2011) Cancer cells metabolically “fertilize” the tumor micro-environment with hydrogen peroxide, driving the Warburg effect: implications for PET imaging of human tumors. *Cell Cycle* 10: 2504–2520
- McLamore ES, Porterfield DM (2011) Non-invasive tools for measuring metabolism and biophysical analyte transport: self-referencing physiological sensing. *Chem Soc Rev* 40: 5308. <https://doi.org/10.1039/c0cs00173b>
- Meng J, Liang L, Jia P, Wang Y, Li H, Yang W (2020) Integration of ovular signals and exocytosis of a  $\text{Ca}^{2+}$  channel by MLOs in pollen tube guidance. *Nat Plants* 6: 143–153
- Nguyen H, Donini A (2010) Larvae of the midge *Chironomus riparius* possess two distinct mechanisms for ionoregulation in response to ion-poor conditions. *Am J Physiol Integr Comp Physiol* 299: R762–R773
- Niu M, Bao C, Zhan J, Yue X, Zou J, Su N, Cui J (2021) Plasma membrane-localized protein BcHIPP16 promotes the uptake of copper and cadmium in planta. *Ecotoxicol Environ Saf* 227: 112920 <https://doi.org/10.1016/j.ecoenv.2021.112920>
- Paluzzi J-P, Vanderveken M, O'Donnell MJ (2014) The heterodimeric glycoprotein hormone, GPA2/GPB5, Regulates ion transport across the hindgut of the adult mosquito, *Aedes aegypti*. *PLoS One* 9(1): e86386. <https://doi.org/10.1371/journal.pone.0086386>
- Pang JY, Newman I, Mendham N, Zhou M, Shabal AS (2006) Microelectrode ion and  $\text{O}_2$  fluxes measurements reveal differential sensitivity of barley root tissues to hypoxia. *Plant Cell Environ* 29: 1107–1121
- Papoyan A, Piñeros M, Kochian LV (2007) Plant  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  status effects on root and shoot heavy metal accumulation in *Thlaspi caerulescens*. *New Phytol* 175: 51–58
- Parks SK, Chiche J, Pouysségur J (2013) Disrupting proton dynamics and energy metabolism for cancer therapy. *Nat Rev Cancer* 13: 611–623
- Pei D, Hua D, Deng J, Wang Z, Song C, Wang Y, Wang Y, Qi J, Kollist H, Yang S, Guo Y, Gong Z (2022) Phosphorylation of the plasma membrane  $\text{H}^+$ -ATPase AHA2 by BAK1 is required for ABA-induced stomatal closure in *Arabidopsis*. *Plant Cell* 34: 2708–2729
- Peng L, Shan X, Yang Y, Wang Y, Druzhinina IS, Pan X, Jin W, He X, Wang X, Zhang X, Martin FM, Yuan Z (2021) Facultative symbiosis with a saprotrophic soil fungus promotes potassium uptake in American sweetgum trees. *Plant Cell Environ* 44: 2793–2809
- Qu Y, Guan R, Bose J, Henderson SW, Wege S, Qiu L, Gilliam M (2021) Soybean CHX-type ion transport protein GmSALT3 confers leaf  $\text{Na}^+$  exclusion via a root derived mechanism, and  $\text{Cl}^-$  exclusion via a shoot derived process. *Plant Cell Environ* 44: 856–869
- Ray S, Howells C, Eaton ED, Butler CW, Shabala L, Adlard PA, West AK, Bennett WR, Guillemain GJ, Chung RS (2011) Tg2576 cortical neurons that express human Ab are susceptible to extracellular  $\text{A}\beta$ -induced,  $\text{K}^+$  efflux dependent neurodegeneration. *PLoS One* 6(4): e19026. <https://doi.org/10.1371/journal.pone.0019026>
- Ren H, Li Y, Han C, Yu Y, Shi B, Peng X, Zhang T, Wu S, Yang X, Kim S, Chen L, Tang C (2022) Pancreatic  $\alpha$  and  $\beta$  cells are globally phase-locked. *Nat Commun* 13: 3721. <https://doi.org/10.1038/s41467-022-31373-6>
- Sa G, Yao J, Deng C, Liu J, Zhang Y, Zhu Z, Zhang Y, Ma X, Zhao R, Lin S, Lu C, Polle A, Chen S (2019) Amelioration of nitrate uptake under salt stress by ectomycorrhiza with and without a Hartig net. *New Phytol* 222: 1951–1964
- Sanchez BC, Ochoa-Acuña H, Porterfield DM, Sepúlveda MS (2008) Oxygen flux as an indicator of physiological stress in fathead minnow (*Pimephales promelas*) embryos: a real-time biomonitoring system of water quality. *Environ Sci Technol* 42: 7010–7017
- Semenza GL (2007) HIF-1 mediates the Warburg effect in clear cell renal carcinoma. *J Bioenerg Biomembr* 39: 231–234
- Seyfried TN, Shelton LM (2010) Cancer as a metabolic disease. *Nutr*

- Metab (Lond)* 7: 7. <https://doi.org/10.1186/1743-7075-7-7>
- Shabala S, Hariadi Y (2005) Effects of magnesium availability on the activity of plasma membrane ion transporters and light-induced responses from broad bean leaf mesophyll. *Planta* 221: 56–65
- Shabala L, Cuin TA, Newman IA, Shabala S (2005) Salinity-induced ion flux patterns from the excised roots of *Arabidopsis* sos mutants. *Planta* 222: 1041–1050
- Shabala L, Howells C, West AK, Chung RS (2010) Prolonged A $\beta$  treatment leads to impairment in the ability of primary cortical neurons to maintain K<sup>+</sup> and Ca<sup>2+</sup> homeostasis. *Mol Neurodegener* 5: 30. <https://doi.org/10.1186/1750-1326-5-30>
- Shabala L, Walker EJ, Eklund A, Randall-Demillo S, Shabala S, Guven N, Cook AL, Eri RD (2012) Exposure of colonic epithelial cells to oxidative and endoplasmic reticulum stress causes rapid potassium efflux and calcium influx. *Cell Biochem Funct* 31: 603–611
- Shabala S, Demidchik V, Shabala L, Cuin TA, Smith SJ, Miller AJ, Davies JM, Newman IA (2006) Extracellular Ca<sup>2+</sup> Ameliorates NaCl-induced K<sup>+</sup> loss from *Arabidopsis* root and leaf cells by controlling plasma membrane K<sup>+</sup>-permeable channels. *Plant Physiol* 141: 1653–1665
- Shahzad B, Shabala L, Zhou M, Venkataraman G, Solis CA, Page D, Chen Z, Shabala S (2022) Comparing essentiality of SOS1-mediated Na<sup>+</sup> exclusion in salinity tolerance between cultivated and wild rice species. *Int J Mol Sci* 23: 9900. <https://doi.org/10.3390/ijms23179900>
- Shu H-F, Wang B-R, Wang S-R, Yao W, Huang H-P, Zhou Z, Wang X, Fan J, Wang T, Ju G (2007) IL-1 $\beta$  inhibits I-K and increases [Ca<sup>2+</sup>]<sub>i</sub> in the carotid body glomus cells and increases carotid sinus nerve firings in the rat. *Eur J Neurosci* 25: 3638–3647
- Smith PJ (1995) Non-invasive ion probes--tools for measuring transmembrane ion flux. *Nature* 378: 645–646
- Smith PJS, Hammar K, Porterfield DM, Sanger RH, Trimarchi JR (1999) Self-referencing, non-invasive, ion selective electrode for single cell detection of trans-plasma membrane calcium flux. *Microsc Res Tech* 46: 398–417
- Song J, Tang Y, Zhang P, Xu Y (2008) Direct evidence for the correlation between extracellular H<sup>+</sup> activities and drug-resistance of tumor cells by non-invasive micro-test technique. *ACTA Biophys Sin* 24: 191–197
- Song L, Li C, Zou Y, Dai F, Luo X, Wang B, Ni J, Liu Q (2015) O<sub>2</sub> and Ca<sup>2+</sup> fluxes as indicators of apoptosis induced by rose bengal-mediated photodynamic therapy in human oral squamous carcinoma cells. *Photomed Laser Surg* 33: 258–265
- Song L, Xu C, Zhang L, Li J, Jiang L, Ma D, Guo Z, Wang Q, Wang X, Zheng H (2024) Trehalose along with ABA promotes the salt tolerance of *Avicennia marina* by regulating Na<sup>+</sup> transport. *Plant J* 119: 2349–2362
- Song X, Li Y, Zhang C (2017) Non-invasive micro-test technique and its application. *Anal Instrum* 2: 78–83
- Sun J, Dai S, Wang R, Chen S, Li N, Zhou X, Lu C, Shen X, Zheng X, Hu Z, Zhang Z, Song J, Xu Y (2009a) Calcium mediates root K<sup>+</sup>/Na<sup>+</sup> homeostasis in poplar species differing in salt tolerance. *Tree Physiol* 29: 1175–1186
- Sun J, Chen S-L, Dai S-X, Wang R-G, Li N-Y, Shen X, Zhou X-Y, Lu C-F, Zheng X-J, Hu Z-M, Zhang ZK, Song J, Xu Y (2009b) Ion flux profiles and plant ion homeostasis control under salt stress. *Plant Signal Behav* 4: 261–264
- Sun J, Chen S, Dai S, Wang R, Li N, Shen X, Zhou X, Lu C, Zheng X, Hu Z, Zhang Z, Song J, Xu Y (2009c) NaCl-induced alternations of cellular and tissue ion fluxes in roots of salt-resistant and salt-sensitive poplar species. *Plant Physiol* 149: 1141–1153
- Sun J, Wang MJ, Ding MQ, Deng SR, Liu MQ, Lu CF, Zhou XY, Shen X, Zheng XJ, Zhang ZK, Song J, Hu ZM, Xu Y, Chen SL (2010) H<sub>2</sub>O<sub>2</sub> and cytosolic Ca<sup>2+</sup> signals triggered by the PM H<sup>+</sup>-coupled transport system mediate K<sup>+</sup>/Na<sup>+</sup> homeostasis in NaCl-stressed *Populus euphratica* cells. *Plant Cell Environ* 33: 943–958
- Sun J, Zhang X, Deng S, Zhang C, Wang M, Ding M, Zhao R, Shen X, Zhou X, Lu C (2012) Extracellular ATP signaling is mediated by H<sub>2</sub>O<sub>2</sub> and cytosolic Ca<sup>2+</sup> in the salt response of populus euphratica cells. *PLoS One* 7: e53136. <https://doi.org/10.1371/journal.pone.0053136>
- Sun K, Lu F, Huang PW, Tang MJ, Xu FJ, Zhang W, Zhou JY, Zhao P, Jia Y, Dai CC (2022) Root endophyte differentially regulates plant response to NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> nutrition by modulating N fluxes at the plant–fungal interface. *Plant Cell Environ* 45: 1813–1828
- Sun W, Xia L, Deng J, Sun S, Yue D, You J, Wang M, Jin S, Zhu L, Lindsey K (2024) Evolution and subfunctionalization of CIPK6 homologous genes in regulating cotton drought resistance. *Nat Commun* 15: 5733. <https://doi.org/10.1038/s41467-024-50097-3>
- Tang X, Yang X, Li H, Zhang H (2018) Maintenance of K<sup>+</sup>/Na<sup>+</sup> balance in the roots of *Nitraria sibirica* Pall. In response to NaCl stress. *Forests* 9(10): 601. <https://doi.org/10.3390/f9100601>
- Trimarchi JR, Liu L, Porterfield DM, Smith PJS, Keefe DL (2000a) A non-invasive method for measuring preimplantation embryo physiology. *Zygote* 8: 15–24
- Trimarchi JR, Liu L, Smith PJ, Keefe DL (2000b) Noninvasive measurement of potassium efflux as an early indicator of cell death in mouse embryos. *Biol Reprod* 63: 851–857
- Tyler SEB (2017) Nature's electric potential: a systematic review of the role of bioelectricity in wound healing and regenerative processes in animals, humans, and plants. *Front Physiol* 8: 627. <https://doi.org/10.3389/fphys.2017.00627>
- Wan Y, Wan Y, McLamore E, Fan L, Hao H, Porterfield DM, Zhang Z, Wang W, Xu Y (Jeff), Lin J (2011) Non-invasive measurement of real-time oxygen flux in plant systems with a self-referencing optrode [EB/OL]. PROTOCOL (Version 1) available at Protocol Exchange. <https://doi.org/10.1038/protex.2011.266>
- Wang D, Li R, Hu C, Shi Y, Hu L, Chen W (2018) Research of glycoconjugates from *Atractylodes macrocephala* and *Astragalus membranaceus* on the influence of potassium signaling pathway during IEC-6 cell migration. *Pharmacol Clin Chinese Mater Medica* 34: 108–114
- Wang J, Ren Y, Liu X, Luo S, Zhang X, Liu X, Lin Q, Zhu S, Wan H, Yang Y, Zhang Y, Lei B, Zhou C, Pan T, Wang Y, Wu M, Jing R, Xu Y, Han M, Wu F, Lei C, Guo X, Cheng Z, Zheng X, Wang Y, Zhao Z, Jiang L, Zhang X, Wang YF, Wang H, Wan J (2021) Transcriptional activation and phosphorylation of OScNGC9 confer enhanced chilling tolerance in rice. *Mol Plant* 14: 315–329
- Wang S, Dai H, Ji D, Skuza L, Li L, Grzebelus D, Wei S (2022) Transport characteristics and mechanisms of Cd by *Bidens Pilosa* L. roots under different exogenous inorganic ions and pH conditions. SSRN. <http://dx.doi.org/10.2139/ssrn.4042479>
- Wang S, Dai H, Ji D, Cui S, Jiang C, Skuza L, Li L, Grzebelus D, Wei S (2023a) Influencing factors of *Bidens pilosa* L. hyper-accumulating cadmium explored by the real-time uptake of Cd<sup>2+</sup> influx around root apices under different exogenous nutrient ion levels. *Toxics* 11: 227. <https://doi.org/10.3390/toxics11030227>
- Wang Y, Lu X, Yang X, Li X, Xu Y (2023b) ImFluxes-3D<sup>®</sup>--Revealing the dynamic three-dimensional appearance of ion molecular flux in living organisms. *NMT Commun* 1: 63–65
- Warburg O (1956) On the origin of cancer cells. *Science* 123(3191): 309–314
- Warburg O, Wind F, Negelein E (1927) THE metabolism of tumors in the body. *J Gen Physiol* 8: 519–530
- Wu S, Guo D, Wei H, Yin X, Zhang L, Guo B, Xu F, Hao Y, Jiang W, Bi H

- (2020) Disrupted potassium ion homeostasis in ciliary muscle in negative lens-induced myopia in *Guinea* pigs. *Arch Biochem Biophys* 688: 108403. <https://doi.org/10.1016/j.abb.2020.108403>
- Wu X, Li J, Song L, Zeng L, Guo Z, Ma D, Wei M, Zhang L, Wang X, Zheng H (2024) NADPH oxidase-dependent H<sub>2</sub>O<sub>2</sub> production mediates salicylic acid-induced salt tolerance in mangrove plant *Kandelia obovata* by regulating Na<sup>+</sup>/K<sup>+</sup> and redox homeostasis. *Plant J* 118: 1119–1135
- Xiong S, Kong X, Chen G, Tian L, Qian D, Zhu Z, Qu LQ (2023) Metallochaperone OsHIPP9 is involved in the retention of cadmium and copper in rice. *Plant Cell Environ* 46: 1946–1961
- Xu Y (2023) Non-invasive micro-test technology—2022. *NMT Commun* 1: 3–9
- Xu Y, Qiu Z (1993) Patch-clamp technique and its applications to and prospects for the studies of higher plant cells. *Plant Physiol Commun* 29: 169–174
- Xu J-D, Liu S, Wang W, Li L-S, Li X-F, Li Y, Guo H, Ji T, Feng X-Y, Hou X-L, Zhang Y, Zhu JX (2012) Emodin induces chloride secretion in rat distal colon through activation of mast cells and enteric neurons. *Br J Pharmacol* 165: 197–207
- Xu W, Ding G, Yokawa K, Baluška F, Li QF, Liu Y, Shi W, Liang J, Zhang J (2013) An improved agar-plate method for studying root growth and response of *Arabidopsis thaliana*. *Sci Rep* 3: 1273. <https://doi.org/10.1038/srep01273>
- Xu W, Wang Y, Xie J, Tan S, Wang H, Zhao Y, Liu Q, El-Kassaby YA, Zhang D (2022) Growth-regulating factor 15-mediated gene regulatory network enhances salt tolerance in poplar. *Plant Physiol* 191: 2367–2384
- Xu Y, Sun T, Yin L-P (2006) Application of non-invasive microsensing system to simultaneously measure both H<sup>+</sup> and O<sub>2</sub> fluxes around the pollen tube. *J Integr Plant Biol* 48: 823–831
- Xu Y, Zhang Z, Gao H, Liu Y, Li L, Ye B, Ju X, Ma Y, Zhang Q, Guo W (2023) Bio-imOmics project. *NMT Commun* 1: 44–50
- Yang C, Li B (2019) Maintaining proton homeostasis is an essential role of the Warburg effect in proliferating cells. *Cancer Biol Med* 16: 391–395
- Yang B, Wang J, Yu M, Zhang M, Zhong Y, Wang T, Liu P, Song W, Zhao H, Fastner A, Suter M, Rentsch D, Ludewig U, Jin W, Geiger D, Hedrich R, Braun DM, Koch KE, McCarty DR, Wu WH, Li X, Wang Y, Lai J (2022a) The sugar transporter ZmSUGAR1 of the nitrate transporter 1/peptide transporter family is critical for maize grain filling. *Plant Cell* 34: 4232–4254
- Yang G, Ma F, Wang Y, Feng CG, Li P, Xu Y, Zhao WZ, Yin LP (2010) Vesicle-related OsSEC27P enhances H<sup>+</sup> secretion in the iron deficient transgenic tobacco root. *Chinese Sci Bull* 55: 3298–3304
- Yang L, Zhu L, Xu Y, Zhang H, Ye W, Mao J, Chen L, Wang L (2012) Uncoupling of K<sup>+</sup> and Cl<sup>-</sup> transport across the cell membrane in the process of regulatory volume decrease. *Biochem Pharmacol* 84: 292–302
- Yang L, Ren Q, Ma X, Wang M, Sun J, Wang S, Wu X, Chen X, Wang C, Li Q, Sun J (2022b) New insight into the effect of riluzole on cadmium tolerance and accumulation in duckweed (*Lemna turionifera*). *Ecotoxicol Environ Saf* 241: 113783. <https://doi.org/10.1016/j.ecoenv.2022.113783>
- Yin Li, Shangguan Y, Xu Y (2006) Non invasive scanning ion selective electrode technology and its application in higher plant research. *Prog Nat Sci* 16: 262–266
- Zeng P, Xie T, Shen J, Liang TYin L, Liu K, He Y, Chen M, Tang H, Chen S, Shabala S, Zhang H, Cheng J (2024) Potassium transporter OsHAK9 regulates seed germination under salt stress by preventing gibberellin degradation through mediating OsGA2ox7 in rice. *J Integr Plant Biol* 66: 731–748
- Zhang H, Cui Y, Zhuo X, Kim J, Li H, Li S, Yang H, Su K, Liu C, Tian P, Li X, Li L, Wang D, Zhao L, Wang J, Cui X, Li B, Pan H (2022a) Biological fixation of bioactive bone cement in vertebraloplasty: the first clinical investigation of borosilicate glass (BSG) reinforced PMMA bone cement. *ACS Appl Mater Interfaces* 14: 51711–51727
- Zhang L, Ke J, Min S, Wu N, Liu F, Qu Z, Li W, Wang H, Qian Z, Wang X (2021) Hyperbaric oxygen therapy represses the warburg effect and epithelial–mesenchymal transition in hypoxic NSCLC cells via the HIF-1 $\alpha$ /PFKP axis. *Front Oncol* 11: 691762. <https://doi.org/10.3389/fonc.2021.691762>
- Zhang Y, Sun R, Wang L, Zhu Y, Tuyiringire D, Yang Y, Li K, Han W, Wang Y, Yan L (2020) Physiological responses of *Arthrobacter sp.* JQ-1 cell interfaces to co-existed di-(2-ethylhexyl) phthalate (DEHP) and copper. *Ecotoxicol Environ Saf* 205: 111163. <https://doi.org/10.1016/j.ecoenv.2020.111163>
- Zhang Y, Yao J, Yin K, Liu Z, Zhang Y, Deng C, Liu J, Zhang Y, Hou S, Zhang H, Yu D, Zhao N, Zhao R, Chen S (2022b) Populus euphratica phospholipase D $\delta$  increases salt tolerance by regulating K<sup>+</sup>/Na<sup>+</sup> and ROS homeostasis in *Arabidopsis*. *Int J Mol Sci* 23(9): 4911. <https://doi.org/10.3390/ijms23094911>
- Zhao N, Wang S, Ma X, Zhu H, Sa G, Sun J, Li N, Zhao C, Zhao R, Chen S (2016) Extracellular ATP mediates cellular K<sup>+</sup>/Na<sup>+</sup> homeostasis in two contrasting poplar species under NaCl stress. *Trees Struct Funct* 30: 825–837
- Zheng LF, Ji T, Guo ZH, Wang T, Xiu XL, Liu XY, Li SC, Sun L, Xue H, Zhang Y (2020) Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter 2 located in the human and murine gastric mucosa is involved in secretagogue-induced gastric acid secretion and is downregulated in lipopolysaccharide-treated mice. *Eur J Pharmacol* 880: 173162. <https://doi.org/10.1016/j.ejphar.2020.173162>
- Zhou Z, Zhang B, Liu H, Liang X, Ma W, Shi Z, Yang S (2019) Zinc effects on cadmium toxicity in two wheat varieties (*Triticum aestivum* L.) differing in grain cadmium accumulation. *Ecotoxicol Environ Saf* 183: 109562. <https://doi.org/10.1016/j.ecoenv.2019.109562>
- Zhu J-Y, Gao R-F, Xu Y (2007) Application of selective micro-electrode in plant physiological research. *J Plant Physiol Mol Biol* 33: 101–108