BEATING THE BRAIN DRAIN

In the past two decades, HKUST researchers have made many pace-setting contributions to molecular neuroscience, the area of life science focused on understanding the nervous system

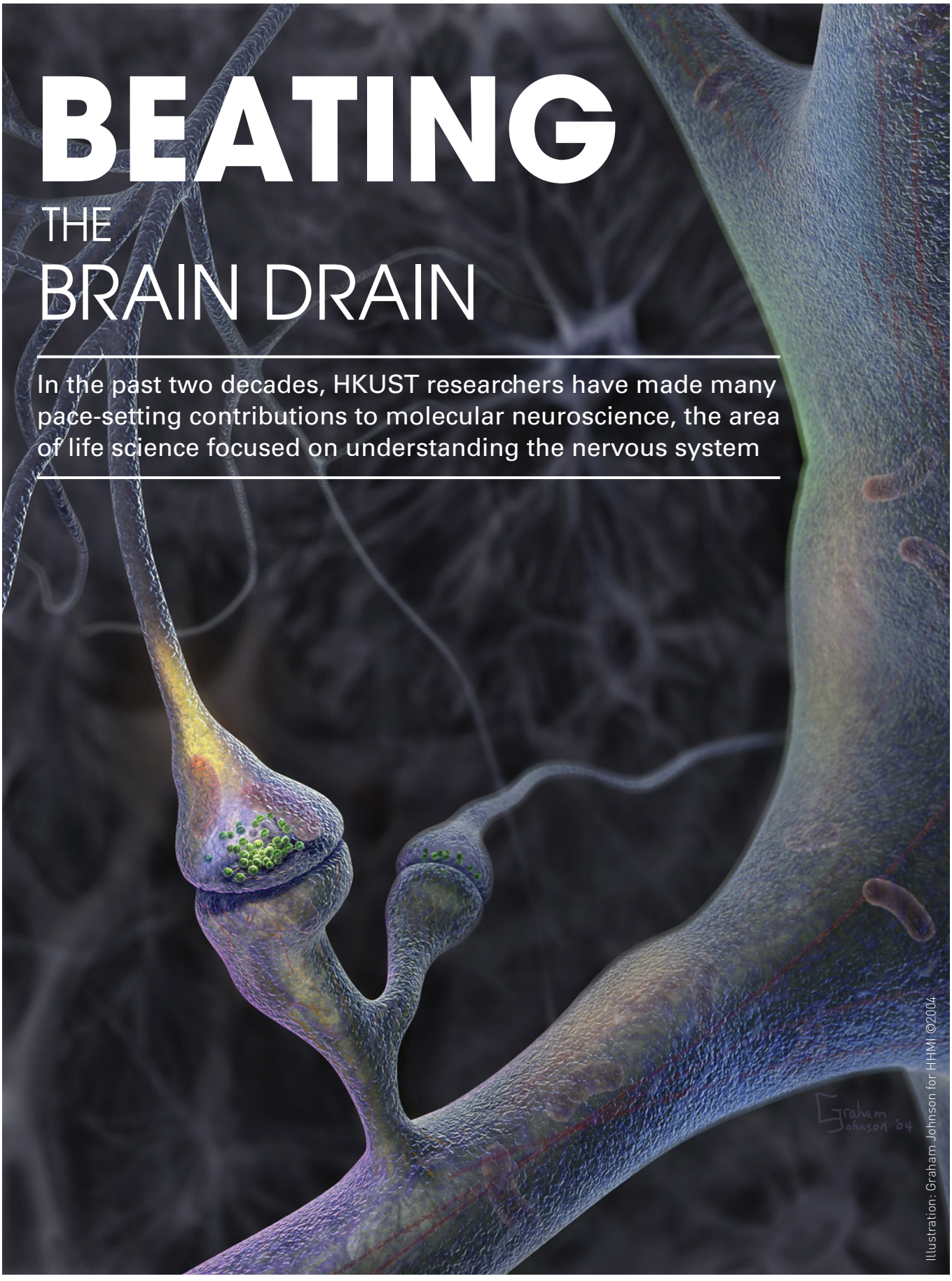


Illustration: Graham Johnson for HHMI ©2004

Neurodegenerative diseases emerge when synapse connections falter and neurons begin to fade at a faster rate than the normal aging process. How and why this happens, and what, if anything, can be done to slow or stop the process or even reverse the devastating effects, is one of the great challenges for life science and medicine.

HKUST scientists have been immersed in clarifying these mysteries over the past 20 years. Leveraging on multidisciplinary expertise, they are working at the cutting-edge of discovery and offer fresh hope that the battle against dementia and other such devastating conditions can be won. Their significant research findings have resulted in a stream of innovative avenues in molecular neuroscience.

World-class Discoveries

Breakthroughs have been derived from undertaking focused research at the molecular level. By unraveling signaling mechanisms underlying normal brain functions, and those that specifically exhibit aberrant behavior in diseased conditions, HKUST scientists are shedding light on the molecular basis of neurodegenerative diseases.

Exciting work is on-going, with teams of HKUST scientists engaged in critical research on:

- Identification and delineation of neuronal signal transduction pathways in synapse development and plasticity, and the regulation of neuronal survival;
- Cellular and molecular mechanisms of synaptic dysfunction, neuronal death, and age-related triggers in Alzheimer's disease;
- Identification of biomarkers for Alzheimer's disease;
- Aberrant proteins and signaling mechanisms in Huntington's and Parkinson's diseases;
- Understanding mechanisms for differentiation and integration of neural stem cells;
- Development of state-of-the-art imaging technology for brain research;
- Developing potential therapeutic approaches to slow the progression of neurodegenerative diseases.

> "Research into neurodegenerative diseases is vital because there are no cures and clear understanding is still lacking. People suffering from Alzheimer's and Parkinson's, among others, are rapidly increasing due to aging populations worldwide. This is a challenge we must address"

PROF NANCY IP

The Morningside Professor of Life Science,
Director, State Key Laboratory of
Molecular Neuroscience,
Academician, Chinese Academy of Sciences,
Foreign Associate, US National Academy
of Sciences

Such research was given added impetus following the establishment of the Molecular Neuroscience Center at HKUST in 1999, which brought together a core team of talented researchers to push exploratory frontiers. This was initiated by Prof Nancy Ip who has been an instrumental figure in achieving the University's research strengths in this area. In 2001, the Center was awarded major support of HK$26.8 million for five years under the Areas of Excellence (AoE) Scheme initiated by the Hong Kong University Grants Committee. This was later extended by HK$27.5 million for another three years.

Potential Drug Leads

HKUST researchers have broken new ground in understanding neurodegenerative diseases. Over the years, a number of proteins (for example, cyclin-dependent kinase 5, α2-chimaerin, EphA4 and PICK1), and their signal transduction pathways have been investigated to reveal the pivotal roles they play in brain functions, such as neuronal survival, differentiation and synaptic plasticity. Most recently, they have identified the protein interleukin (IL)-33 as a potential Alzheimer's treatment. These novel findings have been recognized internationally and published in prestigious academic journals.

The University's scientists have also identified potential therapeutic interventions to slow neurodegeneration. Leveraging their strong expertise in traditional Chinese medicinal herbs, the team has identified small molecules derived from such herbs as potential drug leads. This research has been supported by innovative use of the latest *in vitro* and *in vivo* drug development technologies. The University is also in the process of licensing several patents to biopharmaceutical companies, which will invest in further development of these drug leads into viable treatments.

Prof Nancy Ip reviewing experimental data with her students.

Proper brain functions depend on the intricate interplay of well-coordinated signaling pathways in brain cells that are induced by extracellular stimuli. Cell surface receptors serve as links to transduce signals to the intracellular targets, and dysregulation of these signaling events can result in neurodegenerative diseases. The research undertaken by Prof Ip, is focused on unraveling molecular mechanisms essential for modulation of different brain functions, in particular neuronal communication as well as wiring of neural circuit, both critical for learning and memory. Through research, Prof Ip and her team are opening doors to new understanding of the brain structure and its function to help combat brain diseases and disorders, many of which are currently incurable.

EphA4 Breakthrough

Prof Ip and her team had an exciting breakthrough with their discovery that cell surface receptor protein EphA4 is a key player in Alzheimer's disease pathology. EphA4 regulates the signaling between neurons, and hence brain plasticity that is vital for normal cognitive functions including memory. It dampens neuronal communication through two mechanisms. The first is morphological, in reducing the number of communication points, known as synapses, between brain cells. The second is biochemical, by degrading or reducing the number of neurotransmitter receptors that are responsible for communication between excitatory neurons.

Once these mechanisms were understood, mouse models were used to confirm the link with Alzheimer's disease. The groundbreaking findings were published in 2007 and 2011 in *Nature Neuroscience*, a leading journal in the field.

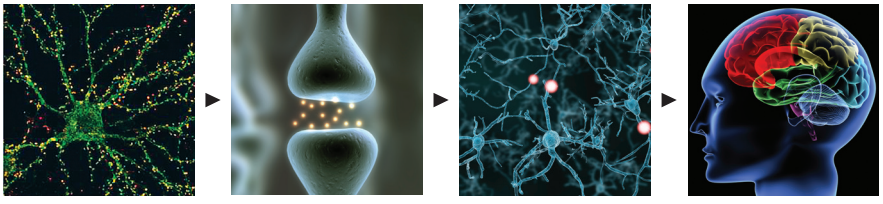
Chinese Medicine 'Blockade'

This discovery, the culmination of more than a decade of research, was closely followed by a second major breakthrough – identification of a small molecule derived from a traditional Chinese medicine herb to block EphA4 activity and rescue neuronal communication impairment observed in Alzheimer's disease. The team explored ways to block the negative EphA4 regulatory pathway, turning to a traditional Chinese medicine library for potential "blockades". This translational stage of the research was funded by the Hong Kong government's Innovation and Technology Commission and the S. H. Ho Foundation. After testing dozens of potential compounds, a hit was identified from the herb *gou teng* (*Uncaria rhynchophylla)* in collaboration with computer scientists.

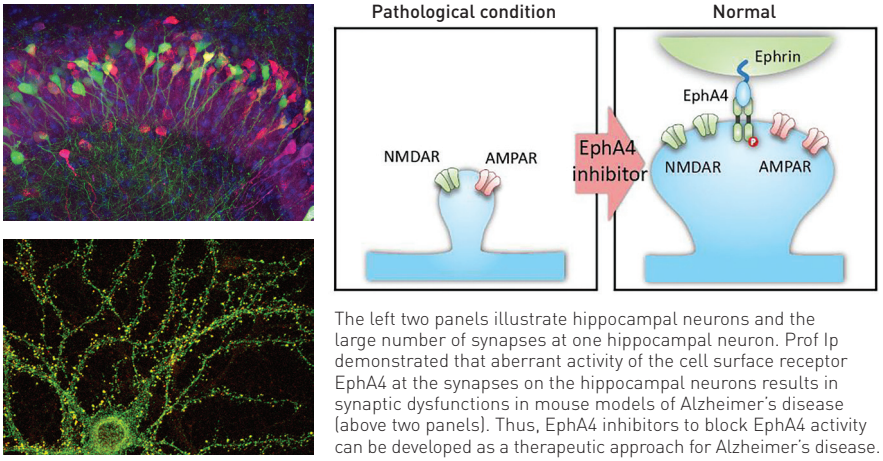
The team prepared the active molecule and orally administered it to transgenic model mice exhibiting Alzheimer's disease-like pathologies (AD model mice). The resulting effect was that neuronal communication was restored to normal and synaptic impairment was rescued. The findings were published in 2014 in *Proceedings of the National Academy of Sciences* (*PNAS*), and received worldwide attention and strong media interest since they represented a potential new strategy for developing treatments for Alzheimer's disease.

Fresh Light on IL-33

Another recent significant discovery by Prof Ip and her team may be a potential game-changer in the approach to developing therapies for Alzheimer's disease. The team demonstrated the importance of the immune function in the disease



Proper neuronal architecture and communication is integral for normal circuit activity and brain function.



The left two panels illustrate hippocampal neurons and the large number of synapses at one hippocampal neuron. Prof Ip demonstrated that aberrant activity of the cell surface receptor EphA4 at the synapses on the hippocampal neurons results in synaptic dysfunctions in mouse models of Alzheimer's disease (above two panels). Thus, EphA4 inhibitors to block EphA4 activity can be developed as a therapeutic approach for Alzheimer's disease.

pathology and identified the IL-33 protein as a potential treatment for Alzheimer's.

IL-33 is a protein found in a wide variety of cell types in humans, and modulates immune functions. The team discovered that IL-33 function is compromised in individuals with mild cognitive impairment, those who are at high risk of developing Alzheimer's disease. To elucidate its role in disease pathology, the protein was injected into AD model mice, with astonishing results. The mice rapidly recovered their neuronal communication and memory. Additionally, IL-33 injection for only two consecutive days was sufficient to reduce the levels of beta-amyloid (Aβ) protein and, in turn, decrease the deposits of amyloid plaque, a major pathological hallmark of the disease, in the mice brains.

The team further demonstrated that IL-33 mobilized microglia, the immune cells of the brain, to the amyloid plaques to promote the clearance of the Aβ protein. It has been hypothesized that defects in the mechanism underlying Aβ clearance is one of the leading causes of Alzheimer's disease. With additional investigation, IL-33 was found to trigger changes in the microglia, which in turn reduced overall inflammation in the brain. This is a critical finding since inflammation contributes to and drives the pathology of the disease.

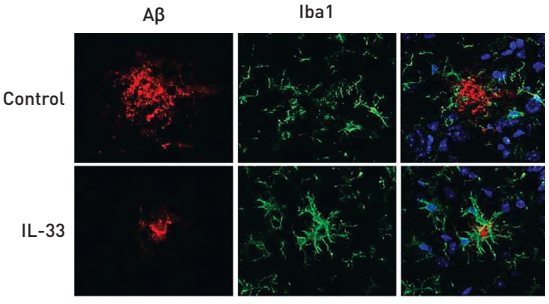
The study was published in *PNAS* in April 2016. The team aims to build upon its findings to further understand the mechanisms underlying Alzheimer's disease as well as evaluate the viability of using IL-33 as a clinical treatment in human. The research has been supported by the Hong Kong Research Grants Council's Collaborative Research Fund.

Search for Early Diagnosis

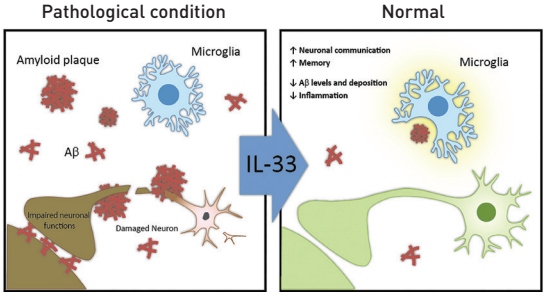
While the interdisciplinary team continues to investigate the complex signaling mechanisms between brain cells and other potential triggers for malfunction, the hunt is now on for biomarkers for early Alzheimer's disease and mild cognitive impairment.

Toward this goal, Prof Ip has initiated a critical project with clinicians in Hong Kong, Mainland China, and globally to identify biomarkers associated with early Alzheimer's and mild cognitive impairment. Success in this area could potentially lead to diagnostic tools and tests that simply and effectively identify individuals with these conditions, prior to manifestation of symptoms.

Furthermore, the question of why some patients with mild cognitive impairment develop Alzheimer's disease, while others do not is also being investigated. The genetic make-up of an individual, combined with inflammatory and environmental factors, are among the potential factors. To unravel these mysteries, Prof Ip is investigating inflammation in the brain to understand the interplay between the immune and nervous systems.



The immune system contributes to the pathogenesis of Alzheimer's disease, but the basis is unclear. Prof Ip demonstrated that injection with IL-33 recruits immune cells to the amyloid plaques, which then triggers amyloid clearance.



Overall, IL-33 restores neuronal communication and memory in the mouse model of Alzheimer's disease and alleviates Alzheimer's disease pathology by reducing inflammation and amyloid toxicity.

Partner State Key Laboratory: **Focus on Molecular Neuroscience Research**

Opening ceremony for Partner State Key Laboratory of Molecular Neuroscience.

The Partner State Key Laboratory (PSKL) of Molecular Neuroscience, established in 2010 by the Ministry of Science and Technology of China, is the first laboratory focused on molecular neuroscience research in Hong Kong. The core focus of the PSKL is to develop frontier research in neuroscience and address fundamental questions related to function of the nervous system and neurological diseases.

The PSKL has forged a long-term partnership with the State Key Laboratory of Neuroscience in Shanghai, and established a research team at the HKUST Shenzhen Research Institute to broaden the platform for innovative basic research as well as drive initiatives in translational research through collaborations with industrial partners.

Aging vs Amyloid

Prof Karl Herrup has championed an alternative hypothesis that emphasizes aging rather than amyloid as the key contributor to the disease. His work focuses on the biology underlying the process of cell death that occurs during the course of Alzheimer's disease, searching for the molecular triggers that start the cell death, and the strategies we can use to try to prevent it.

The genesis of human neurons stops almost completely by one year of age; after that, mature adult neurons are incapable of cell division. Yet in the regions of the Alzheimer's disease brain where cell death occurs, Prof Herrup and his team have shown that neurons are trying to do the impossible. They are trying to divide. Prof Herrup has been responsible for identifying the molecular details of why this happens. His search for the signals that fool the cells into making what is essentially a lethal move has led to some remarkable findings. He sees the initiation of cell division as a good, but ultimately fatal instinct. "Neurons sense damage and like skin cells trying to heal a cut, their instinct is to increase their numbers by trying to divide," he said. "But this is not possible in an adult neuron and the instinct goes sour on them. I call it divide and die."

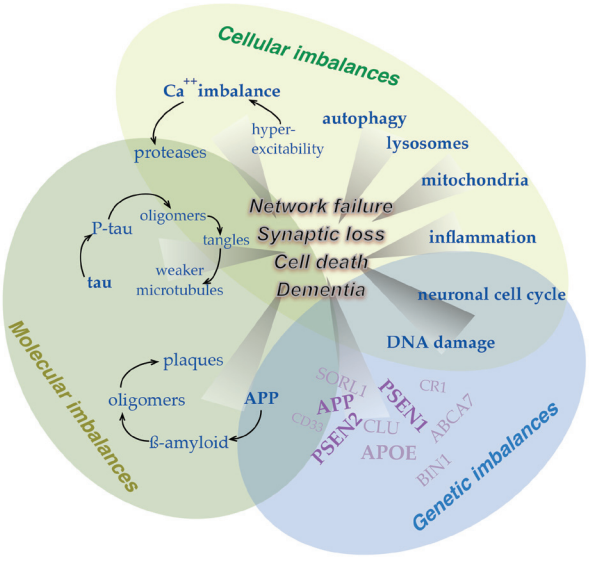
> "We can conceive of Alzheimer's disease as aging plus an injury that triggers a decline and a cascade of events. It is not normal aging, but you don't need the amyloid peptide to create it"

PROF KARL HERRUP

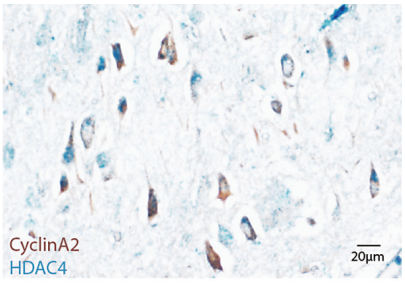
Chair Professor and Head, Division of Life Science, Co-director, HKUST Super-Resolution Imaging Center

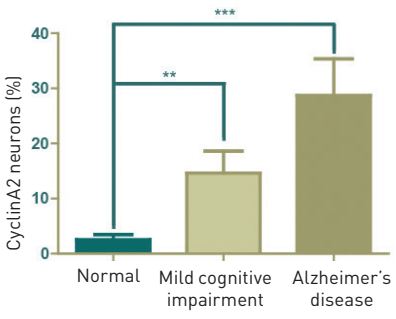
What forces an adult neuron into this situation? Prof Herrup and his team have pioneered work implicating an abnormal immune response. The brain of the person with Alzheimer's has long been recognized as being in a state of chronic inflammation. Both genetic and epidemiological studies point to the importance of this process and the roles that the immune system and neural inflammation can play in modulating neurodegenerative disease.

Over the years, this broad view of the origins of the cells death has led Prof Herrup to raise questions about and ultimately challenge the most prevalent disease model of Alzheimer's – the so-called amyloid cascade hypothesis, which regards the accumulation of beta-amyloid peptides as the root cause of Alzheimer's disease. His views on the topic were recently published in *Nature Neuroscience*. This distinction has important implications for future research – both basic biological science as well as clinical trials.



The causes of Alzheimer's disease can be roughly grouped into three categories (shaded ovals): cellular events (light green), genetic events (blue) and molecular events (dark green), and the various elements interact with each other. Thus, for example, inflammation can enhance the deposition of beta-amyloid peptides, which in turn can influence the deposition of tau and impair synaptic function.





The brown stains show proteins that are normally only found in dividing cells. Their presence in these neurons is a sure sign that something is not right. The blue stain, a separate protein HDAC4, moves to the nucleus when a cell tries to divide. Normal neurons cannot divide, but in Alzheimer's diseased patient the neurons try. This attempt will probably kill them. The histogram shows that as Alzheimer's progresses, the fraction of cells that are trying to divide increases.

PROF KENNY K CHUNG
Associate Professor of Life Science

Comprehending Parkinson's

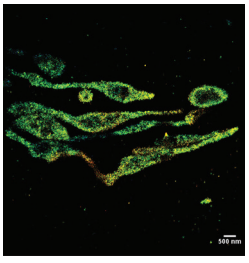
Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease and the major risk factor for the disease is aging. People with family history of this disease have an increased risk of getting the disorder.

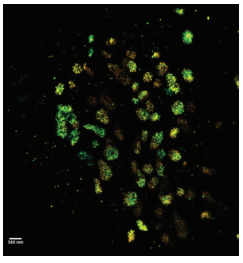
Prof Kenny K Chung has identified important molecular mechanisms of neurodegeneration, focused on familial Parkinson's disease – the form that is caused by genetic mutation(s). He has been working on genes involved in familial cases focusing on the gene known as parkin, its functions in relation to mitochondria, and the neural pathways affected during the disease. Prof Chung and his team have found one of the redox signaling pathways that can affect the function of parkin, and later on other protective genes. This is through nitric oxide, an important signaling molecule in the brain. Once this pathway is understood, one might be able to find ways of pathway modulation for potential therapy for the disease.

PROF HYOKEUN PARK
Assistant Professor of Life Science

Hunting Huntington's

Prof Hyokeun Park is investigating the roles of brain-derived neurotrophic factor (BDNF). Produced in cell bodies in neurons and transported to synapses, BDNF is important for maintaining synapses in neurons. Research has found that there is a lack of BDNF in the striatum in Huntington's disease patients. Prof Park has observed that there are differences in transport and their release of BDNF-containing vesicles in Huntington's disease mice compared with normal mice. "BDNF is very important for neuron survival. If we can increase the BDNF level in the striatum in Huntington's disease system, it may help slow down the progression of Huntington's disease," he said.





Mitochondrial dysfunction is one of the major contributors to Parkinson's disease.

Left: Dysfunctional mitochondria in a model of Parkinson's disease.

Far left: Healthy mitochondria.

The Power of Proteins

HKUST neuroscientists have made significant advances in understanding synapse and protein trafficking involving kinases C (PICK1), thrombospondin and neuroligin, as well as G-protein-coupled receptors (GPCRs), among others. These are some of the many proteins responsible for changes in neuron communication and information processing that can lead to neurodegeneration.

PICK1 Regulation

Prof Jun Xia, Professor of Life Science, and his team have looked into proteins that are important for synapse formation and function, with advances on thrombospondin and neuroligin published in *Nature Neuroscience*. "Each cell has about 25,000 proteins. Several hundreds are thought to be implicated in neurodegeneration. But we know very little how these proteins act individually or collectively in contributing to neurodegenerative diseases," said Prof Xia.

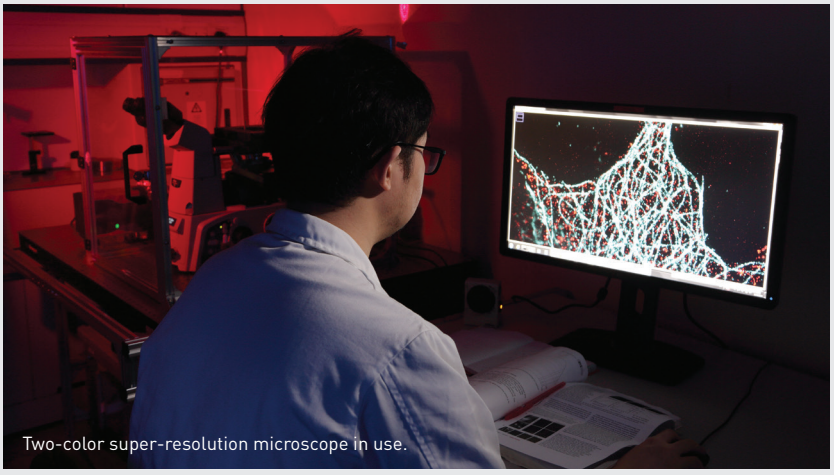
Their studies found that thrombospondin can bind to neuroligin to promote synapse formation. More notably they investigated the regulation of protein trafficking by the PICK1 protein, and how abnormal protein trafficking can contribute to neuron degeneration. Without PICK1 synapse strength cannot be changed, affecting brain plasticity that is vital for normal cognitive functions, including learning and memory.

G-protein

Meanwhile, Prof Yung Hou Wong, Chair Professor of Life Science, and Director of HKUST Biotechnology Research Institute, is studying the molecular mechanisms of how a cell responds to extracellular signals, such as neurotransmitters and hormones. This often involves G-protein-coupled receptors (GPCRs) that use G-proteins to regulate cellular communications in the brain and other organs. They are fundamental to cell communication and malfunctioning of either G-proteins or their receptors may lead to diseases. For example, a defective dopamine receptor system causes Parkinson's disease.

Prof Wong and his team have also screened and identified molecules from traditional Chinese medicinal herbs that work on melatonin receptors, GPCRs that regulate our biological clock. The new drug candidates promise to be more useful than current drugs in treating sleep disorders with fewer side effects, and may also have implications for neurodegenerative diseases, since clinical research shows that patients with these diseases who sleep better have a better prognosis. Melatonin receptors have also been linked with mechanisms that can protect neurons.

Insights into an Infinitesimal World



Two-color super-resolution microscope in use.

Subcellular structures can now be visualized by super-resolution optical microscopes, to the nanometer level.

At HKUST Super-Resolution Imaging Center, physicists Prof Michael Loy and Prof Shengwang Du have taken the lead to work with life scientists, chemists, computer scientists and mathematicians to build state-of-the-art super-resolution localization fluorescence microscopes that are able to resolve tiny structures in cells or tissues that cannot be visualized with traditional optical microscopes. The successful implementation of this technology now underpins the University's strengths in many frontier research areas, including the study of neurodegenerative diseases.

In an on-going study to reveal the molecular organization of subcellular organelles, the central research platform developed focuses on two state-of-the-art microscopes: one stochastic optical reconstruction microscopy (STORM) machine, capable of spatial resolutions of 20 nanometers; and a light sheet microscope with improved sample preservation and fast acquisition times. These advanced tools are helping scientists explore the dynamics of synaptic vesicles in nerve cells and lipid droplets in intestinal cells, and the response of mitochondria to various biological stressors, among others. The project has received almost HK$8 million in funding from Hong Kong Research Grants Council.

The super-resolution microscope allows HKUST neuroscientists to work within a spatial scale previously inconceivable in the probing of the nervous system. The customized microscope is capable of capturing multiple channels at the same time, discerning the relationships between proteins or structures being investigated with high accuracy.

In addition, the team is working on constructing an advanced two-photon light sheet microscope for the observation of living specimens at ultra-high speed, assisted by equipment project funding from the Hong Kong Research Grants Council. Subcellular processes in single cells or embryos can now be recorded in multicolor at speeds of up to 500 frames per second. This provides a new angle to address many biological questions.

PROF MICHAEL LOY

Chair Professor of Physics,
Co-director, HKUST Super-Resolution Imaging Center

> "The whole system of super-resolution microscopy is quite complex. If you are a biologist, you might not know what to do with it. If you are a physicist, you might not know what to look at. We recognized this and the two disciplines got together"

Right: Super-resolved structure of the Ephrin receptor (EphA4, red) at peripheral of post synaptic density (PSD95, green) at synaptic region (Prof Nancy Ip Lab).

Far right: Super-resolution microscope can clearly resolve the synaptic structure in mouse brain. (a)(c) and (e) show the EPI-fluorescent image which fails to tell the details of structure. (b) (d) and (f) are super-resolution images acquired by a custom-designed two-color localization microscope. The pre- and post-synaptic structures are clearly resolved. Scale bars: 1μm in (a) and (b); 200nm in (c) and (d).