BRAIN POWER

The infinitely complex human brain has recently given up more of its secrets. The Zhang Lab, a global leader in neuronal structural biology, has discovered the answer to a question that has been puzzling neuroscience for 60 years. Led by Prof Mingjie Zhang, the team has also shown that this finding has significant potential for greater understanding of neuropsychiatric disorders such as autism and schizophrenia, which afflict millions around the world.

The question involves one of the Zhang Lab's areas of focus over the past two decades: transmission of neuronal signals in synapses. In the human brain, the 1.5-liter powerhouse that determines everything about us, information is transmitted through around 10^{11} neurons. They send or receive signals from other cells, and from one neuron to the next, through synapses, which number around 10^{15}. Given the large numbers and nanometer scale of the molecular machinery, tracing the atomic-level underpinnings of this network is a daunting challenge.

But specializing in synapses, a fundamental unit for all kinds of neurons, has provided the Zhang Lab with a valuable way of cutting through such complexity. Synapses function as signal receiving, processing and transmitting apparatuses as well as memory storage and retrieval sites. Derived from the Greek word meaning conjunction, they encompass a jumping-off point for the signal, called the presynaptic terminal, a fluid-filled space or cleft, and the landing area or postsynaptic density.

While the postsynaptic density and its role as the signal receiving and processing unit was first observed six decades ago, how it forms and organizes itself remained a mystery. It is this enigma that the Zhang Lab has successfully solved.

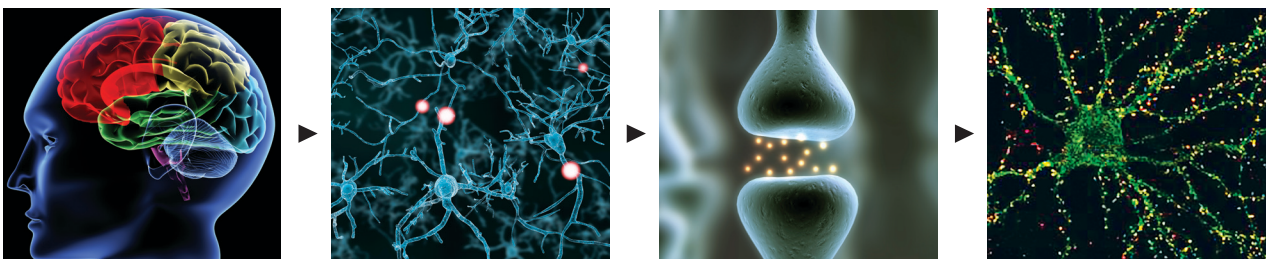
A focus on two abundant proteins in the postsynaptic density, SynGAP and PSD-95, led to the discovery. These proteins were already of interest to scientists as they were known to play a major role in learning and memory; and if mutated, were implicated in disorders such as autism and epilepsy. However, the breakthrough was accomplished by building on the Zhang Lab's years of leading studies on synaptic signaling complex assembly and regulation; and its systematic work to comprehend how the proteins critical for receiving and interpreting diverse brain signals are coordinated to form synaptic functional networks.

> "I chose to focus on the synapse, a tiny compartment just 0.5 micron or less in diameter, because it is a basic unit of communication for all kinds of neurons. The principles we learn from our studies will be applicable to essentially every aspect of brain cells"

PROF MINGJIE ZHANG
Kerry Holdings Professor of Science,
Academician, Chinese Academy of Sciences

Prof Mingjie Zhang (right) with Dr Menglong Zeng.

Transmission of neuronal signals in synapse



Network ▶ Circuit ▶ Synapse ▶ Neuron

Led by Prof Zhang, team leader Dr Menglong Zeng (then a PhD student and now a postdoctoral fellow) and other researchers leveraged such experience – and hundreds of lab hours spent in sample preparation to obtain the high-quality specimens necessary for rigorous characterization – to elucidate the structural basis for the interaction between SynGAP and PSD-95.

The study showed the two protein molecules could form an autonomously assembled network structure, with SynGAP forming a coiled-coil trimer that can bind to multiple copies of PSD-95. The researchers then deduced and demonstrated that binding of the two proteins induces the spontaneous formation of stable "oil-like" droplets through phase transition, a fundamental physical chemistry phenomenon.

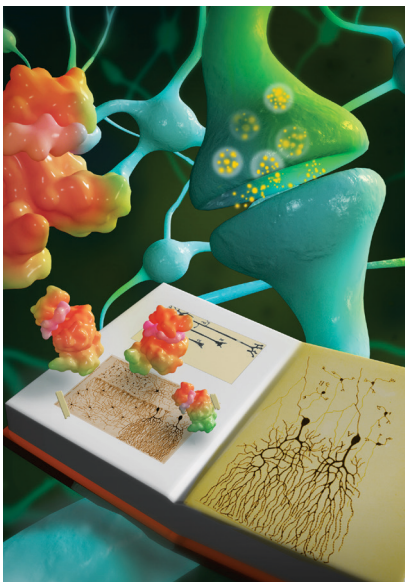
Excitingly, additional findings indicated that the SynGAP/PSD-95 protein complex is crucial for SynGAP stabilization in the postsynaptic density and for stopping neurons from overstimulation, or hyper-excitation. Experiments involving mutated SynGAP proteins, as found in autistic patients' brains, showed that such proteins altered the "oil-like" droplet formation, causing the synapse to overreact. This mechanism could explain why the genetic disorder occurs.

The project was carried out in collaboration with a research group from Johns Hopkins University and published in *Cell* in 2016.

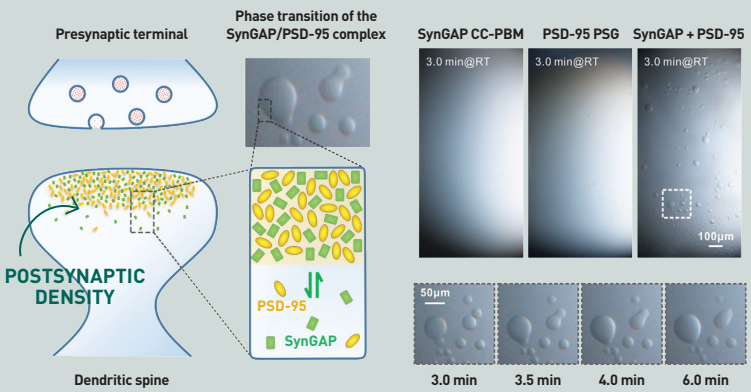
"The identification of phase transition as the underlying mechanism for synaptic protein assembly formation could lead to a paradigm shift in thinking in the biology field overall, as it represents a principle beyond those located in traditional textbooks," Prof Zhang said. "Phase transition can explain why in a test tube you can have the same protein existing in two phases, one extremely dense, the other diluted, and they can exchange. This is what we observed in living cells and systems but we didn't know how it happened. It may even be how the evolution of life got underway."

Over the years, research work by the Zhang Lab has been awarded numerous competitive grants, including the 2013 Areas of Excellence funding from the Hong Kong Research Grants Council, and led to a large number of publications in leading journals, including *Cell*, *Science*, *Developmental Cell*, and *Proceedings of the National Academy of Sciences* (*PNAS*).

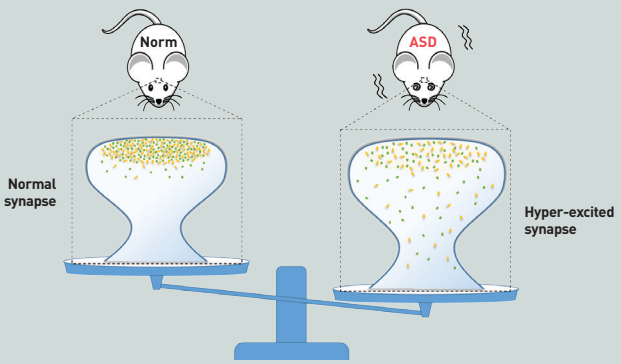
The Zhang Lab is currently hard at work to explore whether other synapses, such as neuron-muscle connections, use phase transition to build synaptic protein assemblies. They are also hoping that the findings may offer new strategies for developing therapeutics for treating neuropsychiatric disorders such as autism, which currently have no effective treatments. "When and whether we are able to translate our findings into real drugs lie a long way forward from our discoveries. The important thing is we now have a new direction," Prof Zhang said.



Neurons communicate with each other via synapses.



Prof Zhang suggested a model for phase transition-mediated formation of the postsynaptic density (PSD). The phase transition occurs and "oil-like" droplets form when PSD-95 and SynGAP bind together. The finding answers an important neuroscience question of how PSD can form and stably exist.



The mice with mutations of SynGAP have hyper-excited synapses and show autistic symptoms.

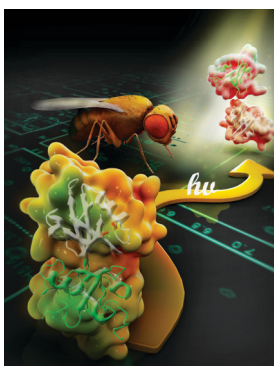
Deciphering Proteins

The mysteries of the vastly complex world of proteins are being unraveled through many different structural biology advances at the Zhang Lab. The following highlights several key discoveries:

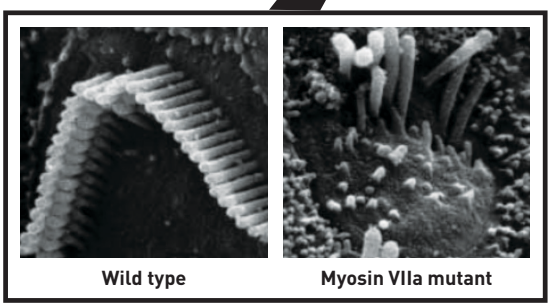
Hereditary Deafness and Blindness

Researchers have systematically elucidated proteins (for example, myosin I, myosin III, myosin VI, myosin VII, myosin X, and myosin XV) and protein interaction networks (for example, the Usher complex) that are required for the proper development and function of sound-detecting hair cells in human ears and light-receiving photoreceptors in human eyes. This work led to significant findings that mutation of myosin VIIa can affect the proper development and normal functioning of hair cells in human ears and eyes which can lead to severe deaf-and-blindness (Usher syndrome) in new-borns and young children. (*Cell*, 2009; *Science*, 2011; *Nature Structural and Molecular Biology*, 2015; *eLife*, 2016; among others)

Using fruit fly photoreceptors as a model, Prof Zhang and his team found that the INAD scaffold protein in microvilli of photoreceptors in animal eyes can undergo a light-dependent architectural or molecular shape change, regulating light signal detection speed and signal output amplitude, as a result of a rapid oxidation/reduction cycle. The finding expanded understanding of how animal photoreceptors can detect such a broad intensity of light signals at a very rapid speed and how human eyes in the same way regulate their response to different light conditions; and helped research into disorders such as night blindness and retinitis pigmentosa. (*Cell*, 2011)



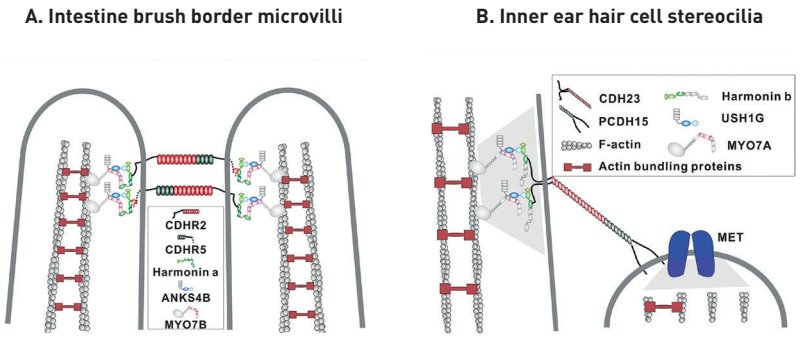
Research into the mechanisms underlying the light-detecting capabilities of fruit flies (drosophila) led to insights on human visual disorders.



Myosin VIIa is a motor protein responsible for transporting various cargos in living cells and maintaining stereocilia structures in hair cells. Genetic mutations in the cargo-binding tail of myosin VIIa, in complex with adaptor protein Sans, can cause Usher syndrome, a genetic disorder resulting in hearing and vision loss or impairment.

Digestive Aid

The Zhang Lab has assisted in the identification of gene mutations that may cause digestive diseases. The team recognized the striking similarities, both in appearance and mechanistic features, between two structures in separate parts of the human body that perform vastly different functions. Stereocilia are organelles of hair cells that respond to fluid motion for different functions in the human body, including hearing and balance. Brush border microvilli, located on the surface of epithelial cells in areas such as the small intestines and proximal tubules of kidneys, are cellular membrane protrusions that increase cells' surface area and help with absorption, secretion, and cellular adhesion. Findings showed that both systems use very similar sets of proteins to organize their multi-protein complex, building tip-links that can sustain mechanical strains. Current knowledge of stereocilia tip-link complex is more advanced than that of microvilli tip-link complex, thus by identifying the similarity between these two systems, existing understanding of the former may provide insight into understanding of the latter. They anticipate that this characterization will assist understanding of certain gut or kidney diseases. (*Developmental Cell*, 2016; and *PNAS*, 2017)



Protein interaction networks in brush border microvilli (Figure A) and inner ear stereocilia (Figure B). Prof Zhang's studies characterized interactions biochemically and structurally in microvilli and discovered that microvilli and stereocilia tip-link complexes are strikingly similar.