Professor Yimon Aye

Shining Light on Reactive Molecules' Signalling Codes

Q: How can chemistry research help us develop better treatments for psychiatric conditions in the future?

A: Although this is not particularly our area of research, tackling a disease or a health condition is usually achieved by understanding the disease mechanism. Most psychiatric conditions can be related to brain chemistry in some way. Chemistry research can help us better understand how the changes in the brain chemistry or chemical biological processes in the brain can accommodate, or even exacerbate, the condition. Let's say a certain neurotransmitter level is significantly lower in people who are diagnosed with schizophrenia. There are many questions that can be asked here: Is this localized to brain or nervous system? Is it because cells cannot uptake this specific chemical neurotransmitter – such as a problem with receptor or cells not producing this neurotransmitter enough? What happens when we administer people with this neurotransmitter? Does it alleviate the symptoms? All of these questions need answers and in this particular case, we would need both biology and chemistry research to understand the situation. Chemistry side help us to design molecules to study the disease as well as design drugs with well understood chemical-biological mechanisms to treat the disease.

Q: When designing pharmacological probes to investigate mechanistic signalling processes, how would you ensure that the mechanistic perturbation establishes a causal link and screen for cross interactions?

A: This is why you would need many experiments and most importantly many negative and positive controls in your experiments. There are many ways to understand if a certain correlation found in an experiment is also a causation. Roughly, you can check gain of function, loss of function, rescue experiments and check the downstream pathways. Perhaps the most informative way, though, is to design a mutant of the target protein (such as mutant missing the functional cysteine that you heard in the lecture) such that your chemical probe is no longer able to interact and affect the mutant protein, and show that the probe-induced signalling changes otherwise seen in the cells expressing the wild-type proteins are ablated in mutant protein expressing cells.

Q: What do you believe would be the best way to administer drugs related to treating neurological disorders?

A: One big challenge in treating neurological disorders is blood brain barrier. This barrier is very selective and will allow only certain chemicals to pass based on their physicochemical features. Yet, most neurological drugs are still administered orally or via IV or IM, as it is easier, and much more comfortable for the patients, and also constitute most convenient ways to administer drugs.

Q: Are the worms Asexual or Sexual?

A: The worms reproduce sexually. But, under normal conditions, they are mainly hermaphrodites (male percentage is lower 1%). This means that a single organism can both produce eggs and sperm. C. elegans hermaphrodites are self-fertilizers meaning that the sperm and eggs come from the same hermaphrodite (earthworms for instance are cross fertilizers). Under stress conditions or in some mutants as we showed, the male percentage will go up and worms mainly reproduce via mating if there are males available.

Q: You said that if multiple worms have different and desired mutations, you join them together. How do you achieve this?

A: This is standard practice in biology research called 'genetic crossing'. It is done via letting a male worm with mutation A and female worm with mutation B mate and checking their progeny to find a worm that has both of the mutations. This is called setting up a cross and then screening the progeny. This can be done with all organisms that can reproduce sexually: mice, fruit flies, worms, humans! It is how you inherit traits from both your mother and your father essentially.

With the worms, you just need to pick around 15 males and 5 hermaphrodites (there are no female C. elegans, they are either male or hermaphrodites that can both produce eggs and sperm but when there are males available, they will mainly mate rather than self-fertilize) and wait for a few days. Then, you can screen the progeny. Screening is much easier if your worms have phenotypes. Let's say you are crossing dumpy worms with fluorescent worms. You pick 5 fluorescent hermaphrodites (it's usually not easy to get mutant-unhealthy worms to give mating males) and 15 dumpy males then check for fluorescent and dumpy worms in the progeny. If there is no particular phenotype to look at, you have to check the genes. And the easiest way to do this is PCR.

Q: As someone looking to work in drug engineering what would be your top tips?

A: Drug discovery is a multidisciplinary field that requires you to be able to communicate and understand ideas in several different fields. Therefore practicing talking and explaining science to your friends is certainly one great way to start. In terms of learning objectives, keep an open mind. Obviously, you need to learn chemistry, but keep an eye out for courses on biology, signalling, and biological engineering all of which will be helpful to you.

Q: What exactly would you need to treat Huntington's disease, and would it be possible to cure it with the research that is going on right now?

A: We approach drug design by trying to understand what chemical signals at specific biological locales and disease contexts are causing harm in that disease and what signals that provide beneficial outcomes in healthy people have been "turned off" in disease state. By identifying the good signals that have been turned off, and learning how to turn them back on using designer chemicals we create in the lab, we hope we can help suppress disease symptoms. By understanding what signals are bad, we can develop diagnostics, or we can try to prevent them from happening. As Huntington's disease is a genetic disease, these methods would not "cure" the disease in the sense of making the mutation go away, rather they would prevent the downstream damage caused by the disease and help to restore healthy brain function.

Q: What is the current study the team is doing and the drug being tested. And for the organism getting tested, how are they getting exposed to the drugs? I am curious about the practical process.

A: Worms can be exposed to drugs in liquid media (effectively where the worms swim in the presence of specific bacteria species (their food). We also screen cell lines and also zebrafish. In these cases, we usually administer the drug via the media as well. As we work with reactive chemical signals that impact numerous oxidative stress-associated diseases, spanning cardiovascular, diabetes, neurodegenerative, and autoimmune disorders and cancer, we actually work with a lot of disease pathways and protein targets, and seek to modulate them all with precision context and control.

Q: How might understanding those codes help us distinguish between their normal roles in healthy cells and their harmful effects in healthy cells and their harmful effects in disease?

A: We believe that nature has evolved a tool kit of reactive chemical molecules that modulate protein function. These molecules are upregulated during normal physiological stress but healthy cells have regulatory mechanisms to defend such routine stress, but these mechanisms are overloaded or deregulated during disease. These chemical molecules work to defend cell stress and damage by reacting with specific proteins and changing the protein's function, influencing cellular communication and decision-making outcomes. The interaction of a reactive molecule with a specific protein is essentially similar to the way many drugs behave. So if we understand these natural switches, we can develop drugs that target those "organic" switches and mimic natural cell signalling outcomes.

Q: Are there any diseases where changes in reactive-molecule signalling are already known to play an important role?

A: There are very strong correlations between reactive molecule signalling and many diseases, including neurodegenerative diseases, immune disorders, and cancers. However, the precise protein targets, and how those individual reactive molecule targeting events suppress, support, or promote disease are unknown. Our ongoing studies fill in this large piece of a puzzle that is very important for understanding and eventually curing disease.

Q: How do you regulate temperature in the incubators to establish optimum growth of the worms?

A: The worms are very resilient creatures, so they are not that sensitive to temperature changes. Generally, they are okay between 17 degrees to 25 degrees. The main difference is that they grow faster at increased temperatures. What we have in the lab are simple incubators that mainly functions as a thermostat. So, we don't have to do anything, we just put them in the incubator and set a certain temperature! How you decide on a certain temperature can be trickier. If you are going away for a long time, you would like to keep them around 15 degrees or you can keep plate stocks at 15 degrees for months. For diseases worms, we keep them at lower temperatures to keep metabolic activity lower. If you want to grow them faster, you would keep them at 22-23 degrees. There are some very interesting worms that express a certain phenotype only when they are kept at higher temperatures, 25 degrees.

Q: What are the worms for?

A: We use them as model organisms. A model organism is used widely in research to understand biology better where human research is not possible, feasible or very expensive. There are many model organisms: fruit flies, mice, worms, zebrafish, yeast and sometimes even plants. Worms in particular are very good model organisms because they are easy, economic to maintain, give a lot of progeny in a short time, they have short life cycle and still they share around 50-60% disease causing proteins with humans.

Q: Are there any other organisms like C. elegans that also help in this sort of research?

A: Yes! We call them "model organisms". A model organism is used widely in research to understand biology better where human research is not possible, feasible or very expensive. There are many model organisms: fruit flies, mice, worms, zebrafish, yeast and sometimes even plants. Many Nobel prize winning discoveries in chemistry, and physiology & medicine have been enabled by research leveraging these model organisms. In our lab, we use C. elegans and zebrafish. One can also use mice as they are closer relatives to humans but there are also numerous limitations in using mice than working with worms or zebrafish. For instance, it is challenging to maintain thousands or hundreds of mice as we can do for worms

and zebrafish, respectively. Having a healthy sample size (no. of animals) in biological experiments is generally important for scientific rigor and reproducibility.

Q: How might artificial intelligence and chemistry work together to discover new medicines faster?

A: Artificial intelligence can help design new inhibitors or predict new drug targets (or both) for instance. In our laboratory we are developing AI-based tools to predict reactive chemical molecules and their cognate protein targets based on our own data.

Q: Did most of them (the worms) mutate on their own or did you insert genes by 'cutting and sticking'?

A: They can also mutate on their own but the strains we have in the lab are made by labs around the world. We ordered some of the mutant worms from a research centre called CGC and borrowed some of them from another lab. C. elegans community is very open and people are very kind, sharing strains, materials with each other all the time. You can also buy strains from CGC for very cheap and do your own research! The fluorescent worms were made by us and a company that we are working with. Coming back to your question, the fluorescent and Huntington's model are mainly produced by injecting a plasmid (a small, circular DNA molecule which is used as a vector to carry a certain gene of interest) that has the gene that you want to insert to the gonad of the worms. Then, you irradiate the progeny with gamma rays, mainly to create breaks in the DNA. When the worms try to repair those breaks, they will sometimes also integrate your gene of interest that you injected into their genome. It is also possible to create mutant worms with just irradiation and screening after that for certain phenotypes. People also use CRIPSR to create mutant strains nowadays.

Q: What has the research into precision medicines so far yielded? Are there any types of diseases we've managed to tackle via targeting certain molecules in cells instead of the whole protein?

A: Precision medicine has been hugely successful. Many inhibitors that have revolutionized the cancer field target a specific mutant protein that is known to exist in cancers and cause a disease, like the specific example shown in the lecture where a drug appending a covalently reactive fragment was approved that targets a single mutation on a key signalling protein. People had looked for such a molecule for more than 40 years. It is helping to save many lives. Our own research helps mine such reactive fragments and their target protein pairs that uniquely stem from Nature's chemical signalling mechanisms, and in the process we are also uncovering basic biological processes of life beyond establishing nature-inspired drug development and design principles.

Q: How do you use AI in this process, when deciding the drugs to cure neuron related disorders?

A: We have not yet used AI to design molecules to cure neuron-related disorders. Many other groups and companies are doing this but the methods to predict and de novo design chemical binders are still significantly limited compared to predictive tools to design proteins de novo. We are currently using AI to help us identify proteins that functionally interact with reactive molecules.

Q: How can furthering our information and knowledge on decoding natural chemicals help in drug discovery?

A: Proteins sensitive to specific native reactive molecules are prone to drug interaction. Once we have identified a specific sensitive protein, we have a robust workflow in place to design molecules that target this protein, hijacking the natural signalling pathways.

Q: How do you regulate other essentials for life other than Temperature for the worms?

A: We grow them on a media that has some essential nutrient for the worms, especially cholesterol and some salts. Other than that, we grow some bacteria on the same media as food source for the worms. This is almost everything they need! They don't require a lot to grow, they are simple organisms.