Designer mice for human disease
- A close view of Nobel Laureate: Oliver Smithies

Yau-Sheng Tsai, Institute of Clinical Medicine, National Cheng Kung University, Tainan, Taiwan
Pei-Jane Tsai, National Laboratory Animal Center, National Applied Research Laboratories, Taipei, Taiwan
Man-Jin Jiang, Cardiovascular Research Center, National Cheng Kung University, Tainan, Taiwan
Cherng-Shyang Chang, Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan

Two major innovations in the 1980s have changed all fields of biomedicine. Combination of gene targeting in mammalian cells with culture of embryonic stem cell enables scientists to study specific genes by creating "knockout mice". Through targeting and removing a specific gene, researchers can find out what happens once it is gone! Working on gene targeting in the Laboratory of Oliver Smithies and his wife, Nobuyo Maeda, in 2000~2006 made my American journey enlightening and fulfilling. Things I learned from Oliver and Nobuyo are not only research, but also the scientific temper and attitude these two distinguished scientists represent.

Designer mice for human disease

The 2007 Nobel Prize in Physiology or Medicine goes to a series of ground breaking discoveries regarding the gene targeting in mice, commonly called knockout technology. The principles for these discoveries are to introduce specific gene modification in mice by the use of embryonic stem (ES) cell (Figure 1). Drs. Mario R. Capecchi of the University of Utah's Howard Hughes Medical Institute, Martin J. Evans of the Cardiff University in United Kingdom, and Oliver Smithies of the University of North Carolina at Chapel Hill will share this year's Nobel Prize.

With their discoveries, the scientists can produce almost any type of DNA modification in the mouse genome, which importantly allows them to establish the roles of individual gene in human health and disease. To date, more than 10,000 different genes in mice, approximately half of the genes in the mammalian genome, have been studied with gene targeting. This technique is now being applied to study numerous human disorders, including cardiovascular and neuron-degenerative diseases, diabetes, and cancer. Ongoing international collaboration has set the roadmap to disrupt each of the ~20,000 genes in the mouse genome before 2010.

The Trio story in the 1980s

The genetic modification can be introduced through various ways. Homologous recombination ensures the newly introduced DNA into the correct location. Homologous recombination is a process of rearrangement occurring between any two highly similar regions of DNA. In 1958, Joshua Lederberg was recognized by a Nobel Prize with his studies in homologous recombination in bacteria. However, homologous recombination does not work as efficiently in mammalian cells. In the early 1980s, Smithies thought that genetic disorders could be treated by repairing mutated genes in human cells. In these attempts, Smithies discovered in 1985 that endogenous genes could be “marked” and “replaced” in a landmark paper in Nature (1), which demonstrated that successful integration by homologous recombination of a plasmid into the chromosomal β-globin gene of human erythroleukaemia cells.

In parallel with Smithies work, Capecchi independently observed the evidence of homologous recombination between introduced DNA and parental chromosome in 1982 (2). Capecchi in 1986 further demonstrated that the homologous recombination could occur at a relatively high frequency (3), which should be high enough to allow gene manipulation in the mammalian genome.

By 1986, all of these works by Smithies and Capecchi were carried out in the cell types, which could not be used to create genetically-modified animals. To make it possible, this technique requires another critical breakthrough: ES cell, which has the potential and capability to develop into an individual.

In 1981, Evans successfully isolated mouse ES cells from mouse embryo and grew them in cell culture (4). Evans and his colleagues took one step further and demonstrated that the isolated and cultured ES cell could be injected back to the blastocyst and created a chimeric mosaic mouse, which was published in Nature in 1984 (5). In 1986, Evans began to modify the genes in cultured ES cells by introducing a virus gene. He showed transfer of such virus DNA from ES cells, through mosaic mice, into the mouse germ line (6).

In 1985, Smithies made a phone call to Matins and discussed about the potential collaborations. Martins immediately broke his lab work and flied to U.S. with samples of his ES cell in his own pocket. Sooner after Matins returned to U.K., Capecchi took a visit with Martin to collect ES cells and learned the techniques in U.K.. Two year later, Smithies in 1987 first used homologous recombination to correct a mutant

HPRT gene, involved in a rare inherited human disease (Lesch-Nyhan syndrome), in cultured ES cells (7). At the same year, Capecchi also worked on HPRT gene and inactivated the functional HPRT gene by introducing a neomycin antibiotic resistance gene in ES cells (8). The strategies for introduction of antibiotic resistance gene, now referred the positive-negative selection (Figure 2), have been generally applied to today's knockout procedures.

After two landmark papers in 1987 published by Smithies and Capecchi groups for manipulating the genome in ES cells, 1989 saw the birth of HPRT-corrected mice by Smithies (9) and knockout mice by other groups. The birth of the knockout and gene-modified mice set the beginning of a new era in genetics.

Significance and impact on biomedical research

Before gene targeting, our understanding of the role of specific genes in human health and disease usually came from the knowledge of existing mutations in humans and animals. By the genetic linkage study or statistic association test, some specific gene can be "implied" in the certain disease. Although administration of gene products might add, to certain extent, understanding of gene function, it has been problematic to solve a "cause or consequence" puzzle. By mutating a gene to destroy its function, it becomes possible to distinguish between causation and correlation. This allows scientists to perform experimental testing of hypotheses regarding the function of specific genes. The discoveries made by Capecchi, Evans, and Smithies completely changed the style of contemporary biomedical science. More researches have been switched from rats to mice as model due to the resources and availability of gene-modified mouse models. The research activities applying this technology have been explored. Novel therapeutic strategies to cure human diseases will build on the experience of these gene-targeted mouse models. "Designer mice" have become indispensable in almost every aspect of biomedical research and been applied to all areas of biomedicine (Figure 3), and its impacts and benefits will continue to grow and increase in the coming future.

A close view of Nobel Laureate: Oliver Smithies

In 1996, I graduated from the Laboratory of Drs. Hua-Lin Wu and Guey-Yueh Shi in the Department of Biochemistry, National Cheng Kung University in Tainan, Taiwan. One big hurdle I had faced in the final part of my master thesis was how I could apply my genetically-engineered peptide to human patients, the ultimate goal of our research. We have tried very hard to set up an animal experimental model to take one step earlier before the real challenge for human patients. That was my first thought in

doing the animal experimental model.

After serious consideration, in 2000, I decided to join the research program of Pathology and Laboratory Medicine in the University of North Carolina at Chapel Hill, located in a relatively warmer region in U.S.. Because of the enthusiasm and passion for animal models, my first choice would be to join the Laboratory of Dr. Nobuyo Maeda, who generated apolipoprotein E knockout mice that have been used enormously in the research area of atherosclerosis. My life in the first few months in Chapel Hill was not that enjoyable due to the pressure from the lecture, language problem, and inexperience of animal experiments. I would not say our laboratory instruments and environments were “fantastic”, but they were really beyond my expectation of the so called “American dream”. “Give up” had come to my mind in one day. However, one thought had crossed my mind: why people in this laboratory love here? There must be something they enjoy but I did not find.

In the winter of 2001, when Dr. Pei-Jane Tsai, married to me after returning back to Taiwan, visited me in Chapel Hill. Pei-Jane and I had the first chance to talk with Oliver (Dr. Oliver Smithies is Dr. Nobuyo Maeda’s husband) in a dinner with Nobuyo. During the dinner, though with my poor English, I found Oliver very approachable and modest. Every story he mentioned was indeed like a lecture from the Biomedical textbook.

From protein chemist to protein geneticist

As a child in England in the 1930s, Oliver’s dream was to be an inventor. His first ground breaking discovery was in 1950 when he had his first job in Toronto, Canada. His initial plan was to do something with insulin. He believed that insulin should result from a precursor. He thought he needs a method to differentiate insulin from its precursor. One day, he came up with an idea of using cooked potato starch, putting his insulin in the jelly, and running through it. This idea was later published in 1955 and became the most quoted paper in the biological science literature. The discovery of high-resolution gel electrophoresis led Oliver to win the prestigious Gairdner Foundation International Award.

With a useful tool, Oliver began looking at variations in the proteins caused by hereditary factors. By gel electrophoresis, he found that an allelic variation of a blood protein hepatoglobin had occurred though chromosomal recombination. He used his hand-made PCR machine (Figure 4) and cloned the second human gene, fetal globin gene. He found the evidence that allelic variation of fetal globin gene is caused

through homologous recombination. The pattern of migration led to his transition from being a protein chemist to protein geneticist, and he began to work on homologous recombination.

After that dinner, my impression on Oliver is not just my mentor's husband. I began to pay more attention on him. On one day of September 2001, the laboratory was in a big celebration. UNC Chancellor, Dean of School of Medicine, and Director of Pathology Department had come to the laboratory and talked with Oliver. Then I realized he won the Albert Lasker Medical Research Awards, which is often called "America's Nobel". Suddenly, I understand he is not just an ordinary person although the way he looked is like that.

His passion for science

His appearance at his bench every single day has been my greatest inspiration in research. He is still running his old fashion grease gel and using his 20-year-old hand-made thermocycler, which was developed even before introduction of the PCR machine (Figure 4). He simply enjoys working and craves understanding how things work. "It's not the achievements." Oliver explains "It's got more to do with curiosity, trying to solve problems, understand something." He said "If you do work and every day there's some enjoyment, then the science never gets boring because every day you have something new to look forward to".

Oliver has said he always enjoys the three things: he did some science; he took Nobuyo to the lunch; and he went flying. Although not many people, including Nobuyo, in the laboratory wanted to take the challenge with Oliver's flight, he still invited us to join him. He told us that flying in the clouds and finding out where you are just by the instruments are indeed like being in the darkroom and waiting for the development of critical gel film. He called this "runway moment" to describe his moment waiting for seeing the result and getting the real answer.

A real scientist

The way he taught me in paper writing is particularly impressive. In one Saturday, he spent a whole afternoon and went through every single word he corrected for me on my manuscript. He explained it by showing me the definition in the dictionary. Since then, I knew how to use certain words more precisely in English. His patience also can be seen during our regular laboratory meeting. We had listened to his description on glomerular filtration rate (GFR) for thousands of time. He helps everyone in the room understand what has been discussed, especially for those relatively new in the

laboratory.

In the celebration party for his Nobel Prize, the people in our laboratory concluded three keys for his success: hard work, love science, and good wife (Oliver immediately corrected to “nice wife”!). Oliver identified critical problems, he figures it out with his own hands, he comes up with innovative solutions, and he makes profound discoveries that launch a new era of science. He is the most gentlemanly, generous, non-self-promoting scientist I ever met. Without working with Oliver, my American journey would not be that rewarding. From Oliver, I realized that a Nobel Laureate can be that modest, approachable, and open-minded. Things I learned from Oliver and Nobuyo are not only research, but also the scientific temper and attitude these two distinguished scientists represent. I will always remind myself and apply this standard in my future career.

*We thank M. T. Lin for his reading and suggestions.
**Figures 5~8 are not described in the maintext, but attached in the end of this article.

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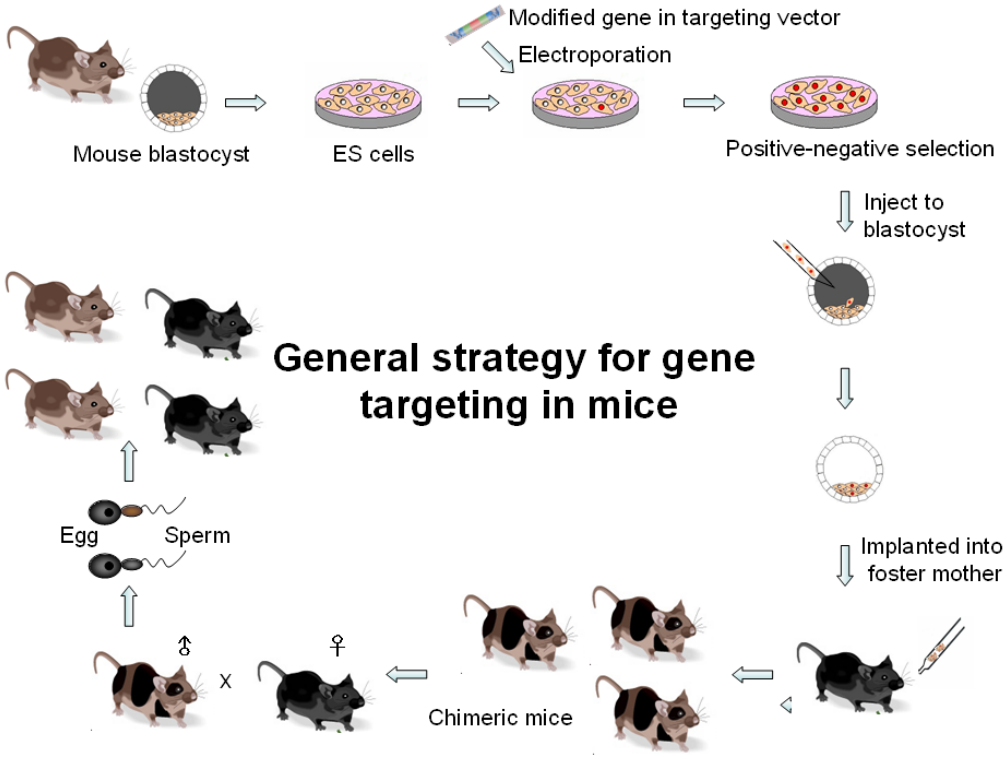

Figure 1. General strategy for gene targeting in mice. To use this technique, scientists introduce a genetic change into the mouse ES cells. ES cells containing desired modification are selected and enriched (Figure 2), and then microinjected into mouse blastocysts, which are injected into foster mothers. The mice born from these embryos are chimeric (mosaic) and then bred to transmit the modified gene to their progeny.

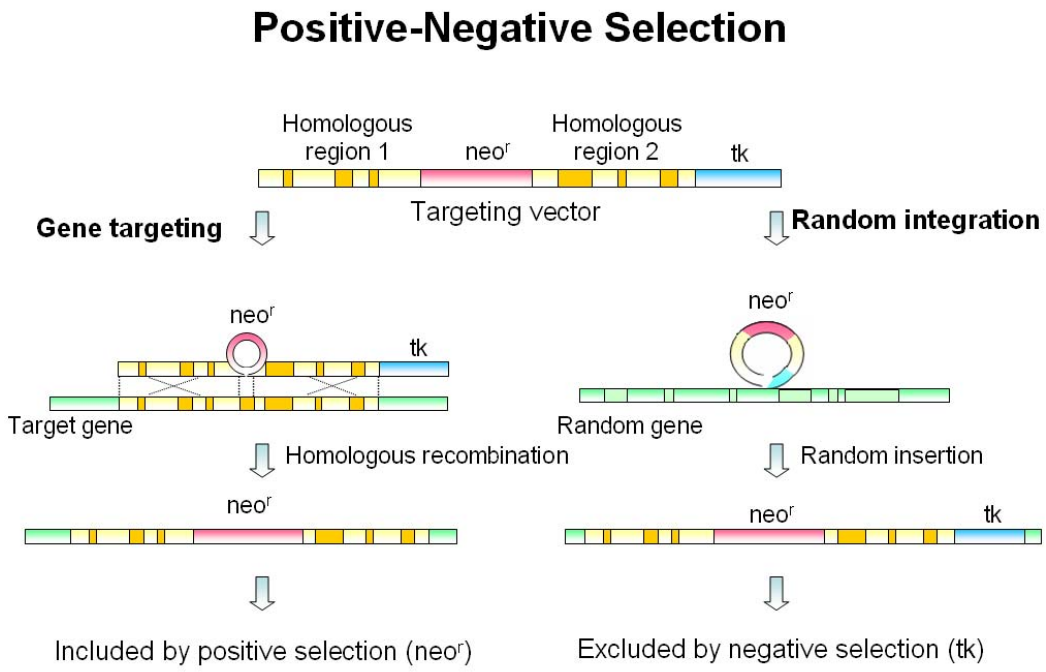

Figure 2. Positive-negative selection. Capecchi in 1988 used "positive-negative selection" to enrich and select ES cells containing desired genetic modification. An antibiotic neomycin resistance (neo^r) element is introduced to replace and disrupt the exon of targeted gene, followed by a thymidine kinase (tk) element at its end. Homologous recombination with the target gene introduces only neo^r; whereas random integration with random gene inserts both neo^r and tk.

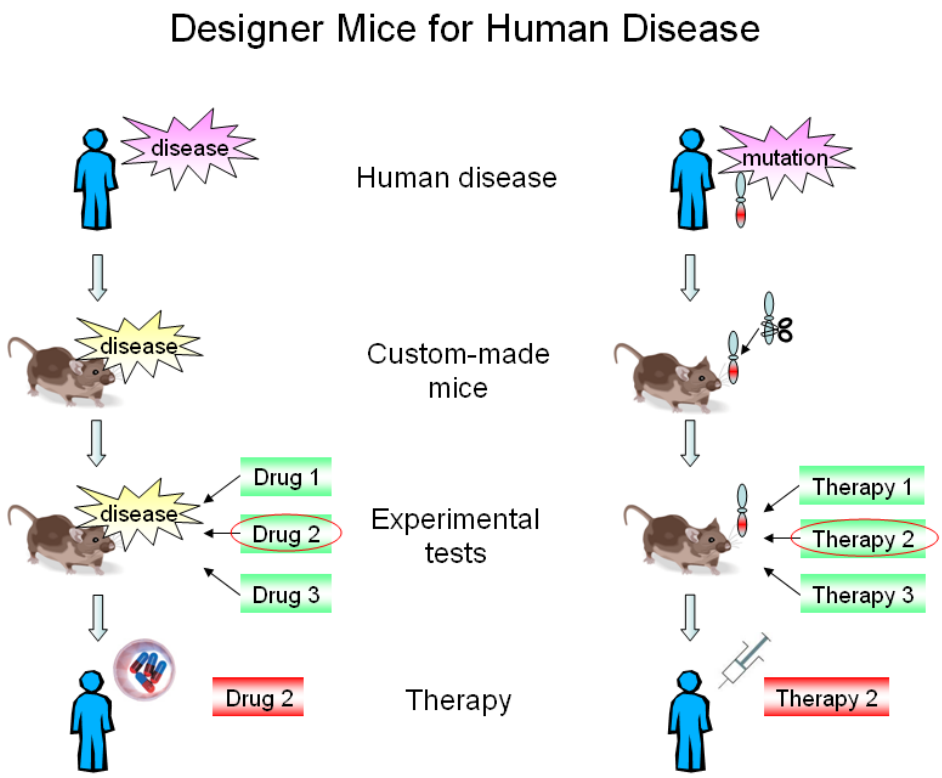

Figure 3. Designer mice for human disease. If scientists can "order" a custom-made experimental model to do the pre-test and find out the feasible therapies, it will greatly advance medical treatment for many major diseases. Furthermore, specific mutations carried by patients with specific diseases can be introduced into the mouse genome and tested for different therapeutic methods. The discoveries in the 1980s made all this possible for today's biomedical research.

Figure 4. 20-year-old hand-made thermocycler (PCR machine) developed by Oliver in our laboratory.

Figure 5. A news conference at UNC on Monday afternoon after the announcement of Nobel Prize.

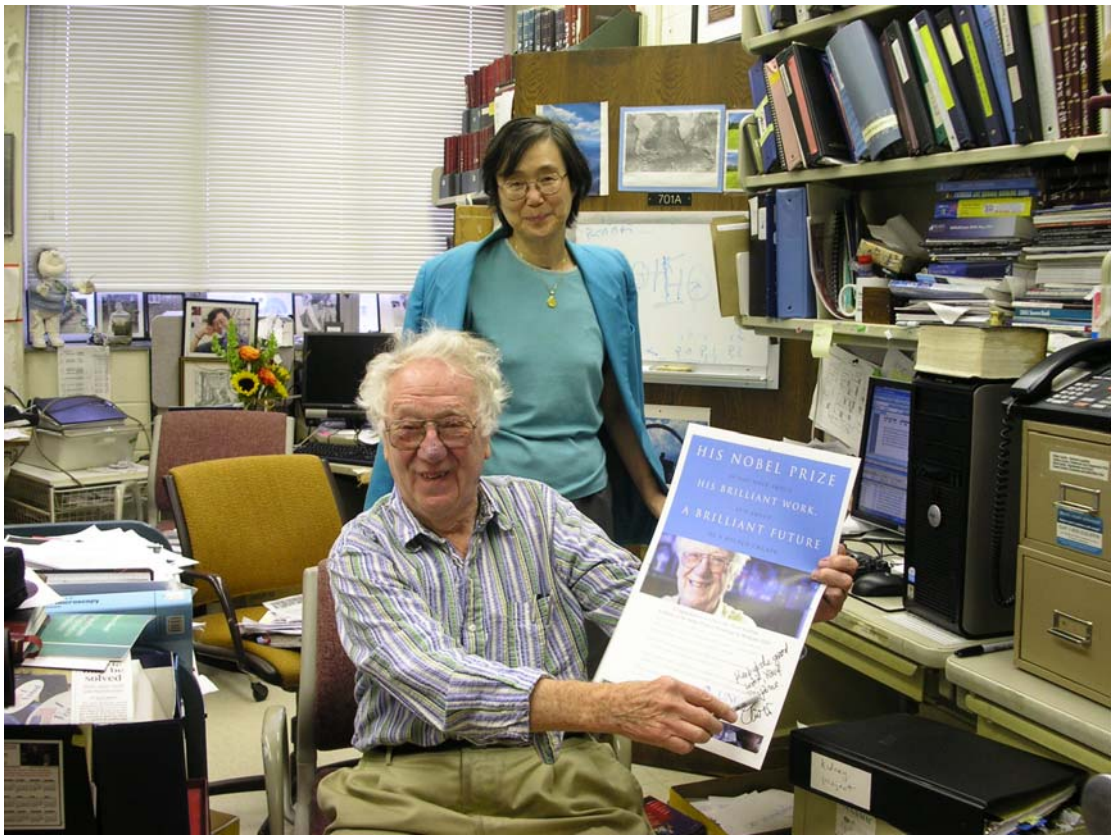

Figure 6. Nobuyo and Oliver with his celebration poster in 2007.

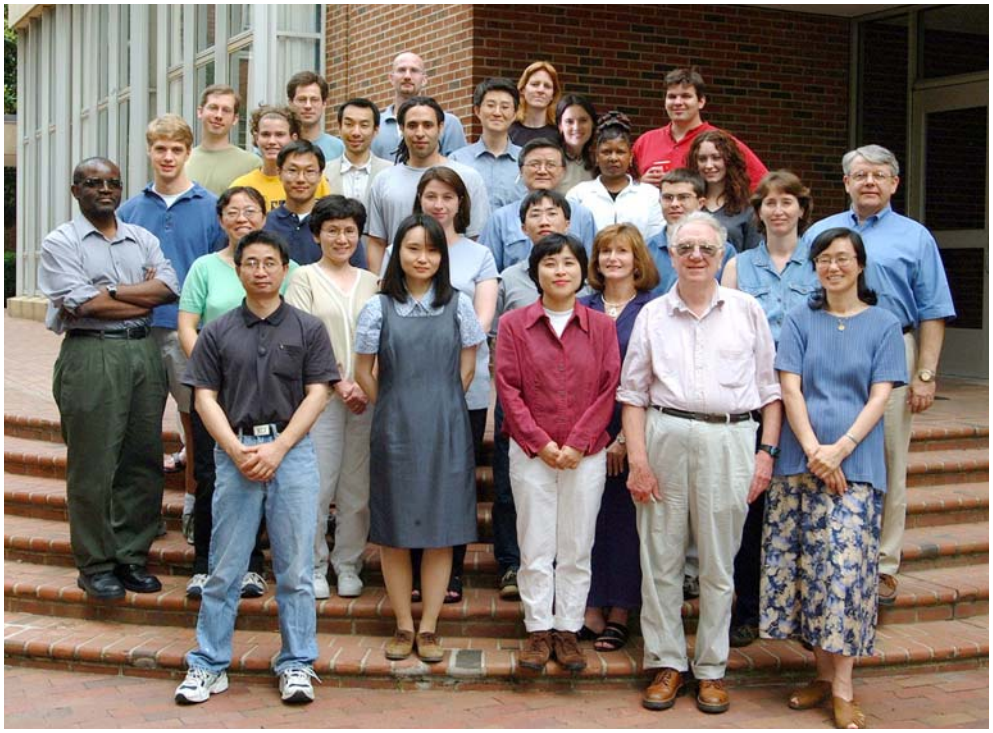

Figure 7. The Oliver/Nobuyo family in 2000.

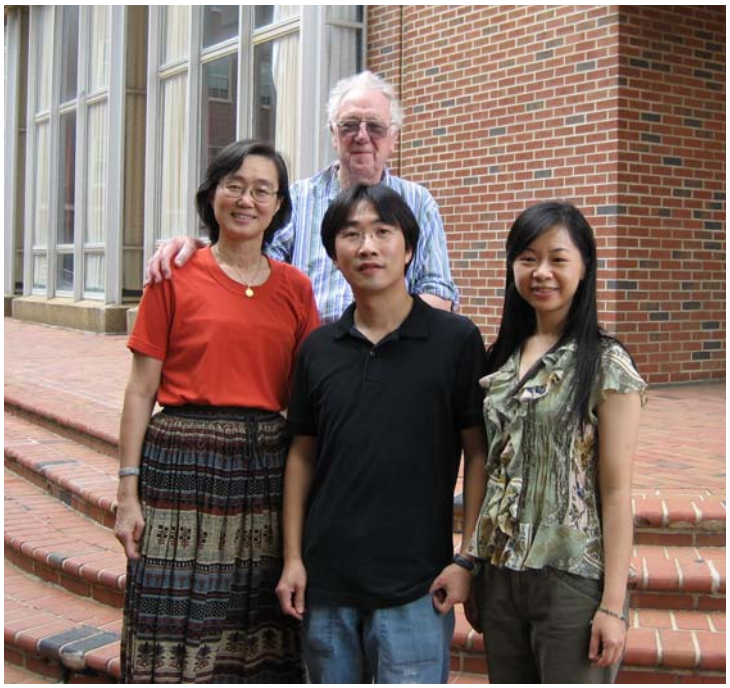

Figure 8. Nobuyo, Oliver, Pei-Jane, and I after my farewell party in 2006.