Institute of Transformative Bio-Molecules

Change the world with molecules: Innovative interdisciplinary research between animal/plant biology and synthetic chemistry

The Institute of Transformative Bio-Molecules (ITbM) at Nagoya University is an international research center that creates a new research field through the integration of cutting-edge synthetic chemistry and animal/plant biology research. ITbM aims to develop molecules that change the way we live, i.e. "transformative bio-molecules", in order to "understand", "see", and "regulate" biological systems, and address urgent social issues on the environment, food production and medical technology.

ITbM
Nagoya University

Research Center's Information (FY 2015)

Center Director: Kenichiro Itami
Principal Investigators (PI): 11 (including 4 overseas researchers and 2 female researchers)
Other Researchers: 54 (including 18 overseas researchers and 15 female researchers)
Research Support Staff: 34
Administrative Division:
  Administrative Director: Tsuyoshi Matsumoto
  Administrative Staff: 12 (percentage of bilingual staff: 50%)
Satellites and Cooperative Organizations: ETH-Zürich, Switzerland; Queen's University, Canada; University of Washington, USA; University of Southern California, USA; NSF Center for Selective C-H Functionalization, USA; RIKEN Center for Sustainable Resource Science, Japan; and others
URL: http://www.itbm.nagoya-u.ac.jp

Major Research Achievements

1 Development of molecules that promote plant growth

Succeeded in discovering that plant growth is controlled by the opening/closure of stomata. Solutions to environmental and food production issues are expected to be derived by improvement in plant growth through the development of molecules that can control the number and opening/closure of stomata.

2 Development of molecules to combat the parasitic plant *Striga*

Achieved the development and commercialization of a fluorescent molecule "Yoshimulactone", which acts as a tool to elucidate the parasitic mechanism of the parasitic plant *Striga* that causes huge damage to the agricultural production in Africa.

3 Development of molecules to control plant reproduction

Accomplished for the first time in identifying the structure and function of long-sought key molecules, "LURE" and "AMOR", which are involved in the reproduction of plants. This outcome may eventually lead to full elucidation of the mechanism of plant reproduction.

4 Development of molecules to regulate the circadian clock of animals and plants

Gained access to molecules that can control the biological clock rhythm, and identified highly active molecules through structure activity relationship studies. Regulation of the circadian clock in animals and plants is expected to lead to the improvement of food production and development of drugs to treat sleep disorders in humans.

5 Development of molecules to visualize biological systems

Established synthetic routes for highly photostable and environmentally responsive fluorescent dye molecules, which are essential tools for live cell imaging that can be used to elucidate the various mechanisms occurring in biological systems.

Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8601, Japan
Phone: +81-52-789-3239
Email: office@itbm.nagoya-u.ac.jp

Background image: Photo of leaves. Leaves contain stomata, which are small holes located on the surface that allow gas exchange.

Aim of ITbM

ITbM aims to create a new interdisciplinary field of research through the integration of forefront synthetic chemistry and animal/plant biology, as well to deliver bio-functional molecules that will greatly change the way we live, i.e. "transformative bio-molecules". Humans have generated various transformative bio-molecules up to now. Representative examples in the pharmaceutical field are the well-known antibiotic, *Penicillin*, and the anti-influenza drug, *Tamiflu*. At ITbM, chemists and animal/plant biologists achieve full collaboration by breaking the walls between their fields, and work together to develop molecules that can solve various social issues, including the environment and food production, as well as contribute to advances in medical technology.

1 Development of molecules that promote plant growth

Toshinori Kinoshita (PI), Keiko Torii (PI), Kenichiro Itami (PI)

Plants have stomata, which are small pores located on the surface of leaves that control gas exchange with the external environment. Stomata are the primary inlet and outlet for the uptake of carbon dioxide (CO_2) and transpiration of water, respectively. During the day, plants open their stomata (Fig. 1a) and absorb CO_2 from the atmosphere to carry out photosynthesis and create nutrients necessary for their growth. In addition, stomata are responsible for adjusting the water content in leaves, i.e. stomata close when the water content is low (Fig. 1b) to protect the plant from water loss. Stomata are also involved in the global water regulation, with data suggesting that all water in the atmosphere pass through the stomata every half-year.

As stomata are essential for plant growth, ITbM's researchers figured that by being able to control the number and opening/closure of stomata, they would be able to produce plants with drought resistance, increase plant growth and reduce the amount of CO_2, a greenhouse gas, from the atmosphere.

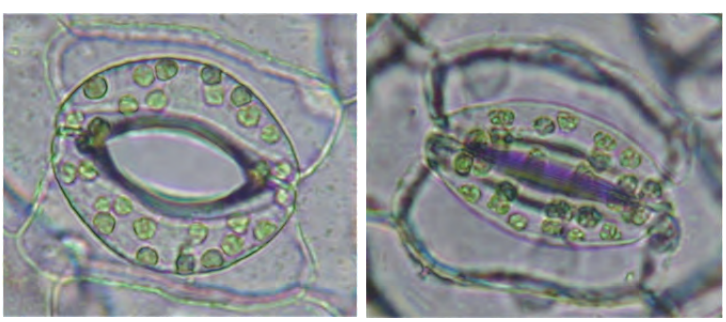


Fig. 1 Plant stomata in: (a) open state, (b) closed state

When the guard cells of the stomata are illuminated with sunlight, phototropins (photoreceptors) respond to the blue light and activate the plasma membrane proton pump (H^+-ATPase), eventually leading to stomatal opening. Using a model plant, *Arabidopsis thaliana*, plant biologists at ITbM have selectively increased the amount of H^+-ATPase in the guard cells of the stomata. In comparison to the wild type, the researchers found that this led to an approximately 25% and 15% increase in the amount of stomatal opening and CO_2 uptake (photosynthesis), respectively. As a result, a 1.4-1.6 times increase in plant weight was observed, thus indicating that the control of stomata was essential for plant growth.

ITbM aims to contribute towards solving food, energy and environmental issues by developing this technique to enable an increase in the production of food crops and plants for bio-fuels, as well as reduce the amount of CO_2 in the atmosphere with plants.

Y. Wang, et al: Proc. Nat. Acad. Sci. USA, 111, 533, 2014.

2 Development of molecules to combat the parasitic plant *Striga*

Toshinori Kinoshita (PI), Kenichiro Itami (PI), Takashi Ooi (PI)

Striga is a parasitic plant that causes drastic damage to agriculture in parts of Africa, Asia and Australia. This parasite is also known as witchweed from its beautiful purple-pink flowering (Fig. 2). *Striga* is a major threat for food crops, such as rice, corn and sugarcane as it infests the host crop plant through its roots by depriving them of their nutrients and water. The host plant eventually withers, leading to yield losses in approximately 40 million hectares of land, worth over 10 billion U.S. dollars, which affects over 100 million people. Therefore, an effective antidote for this situation is critical to ensure global food security.

Striga is known to detect host crop plants from a class of plant hormones called strigolactones, which are released by plants for their own growth. Yet, the full mechanism on how *Striga* detects strigolactones has been unclear up to now. Based on the hypothesis that *Striga* possesses a protein receptor to detect strigolactones, ITbM's chemists and biologists worked together to develop a molecule, "Yoshimulactone green (YLG)", which shows green fluorescence upon binding to a strigolactone receptor (Fig. 3). Using YLG, the research team was able to directly observe how the molecule interacts with the receptor during *Striga* germination by live cell imaging. The development of YLG was an interdisciplinary outcome that arose from 3 young researchers from ITbM, and the molecule was

Fig. 2 A crop field infested by *Striga*
(Photo by: Professor Abdel Gabar Babiker, Sudan University of Science and Technology)

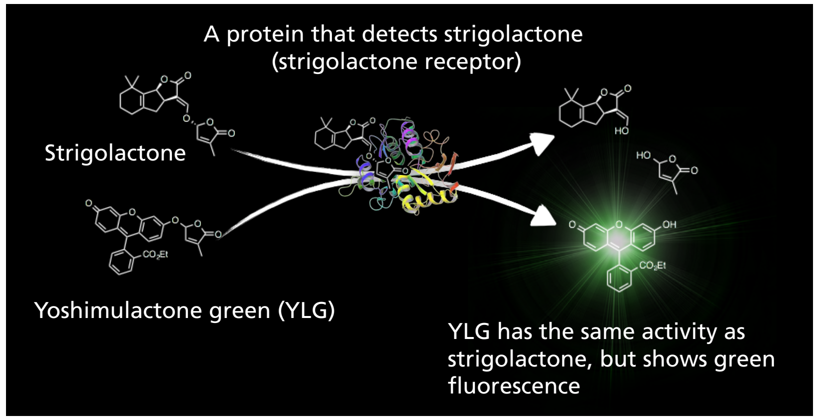


Fig. 3 Reaction mechanism of Yoshimulactone green (YLG): YLG exhibits a similar activity to strigolactone. Upon binding to a strigolactone receptor, the molecule is decomposed to release a molecule that shows green fluorescence.

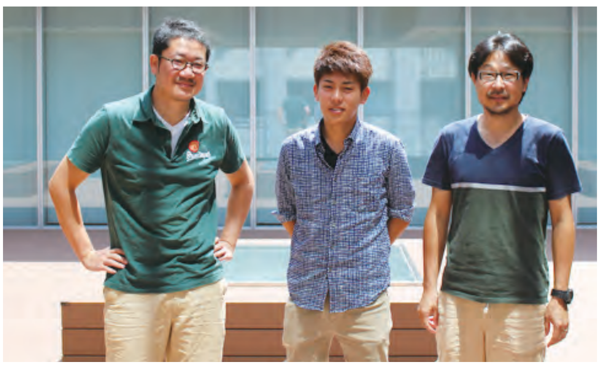


Fig. 4 YLG development team. From the left: Dr. Shinya Hagihara, Mr. Masahiko Yoshimura, Dr. Yuichiro Tsuchiya

named after the graduate student Masahiko Yoshimura, who designed and synthesized the molecule (Fig. 4).

The outcome of this research is an important step forward for elucidating the parasitic mechanism of *Striga*. YLG is now commercially available (from TCI Co., Ltd.) and can now be used by researchers around the world, which is expected to accelerate *Striga* research.

Y. Tsuchiya, et al: Science, 349, 864, 2015.

3 Development of molecules to control plant reproduction

Tetsuya Higashiyama (PI), Kenichiro Itami (PI), Jeffrey Bode (PI)

Rapeseed plants are known as the main ingredient for rapeseed oil and are essential for our daily life. Rapeseed originally occurred from the natural crossing of different species, and is a successful example of crossbreeding. However, most organisms have a barrier known as reproductive isolation in order to maintain their own species, which therefore makes fertilization between different species a rather difficult event. Clarification of the mechanism for reproductive isolation is considered as the key to bring about successful crossbreeding. In 2012, ITbM's plant biologists have shed light on this unresolved mystery of 140 years by identifying an attractant molecule, a "LURE" peptide, which is secreted from within the plant's ovule to attract the pollen tube. When pollen pollinates on the pistil, a pollen tube grows towards the ovule. The synergid cells located next to the egg cells within the ovule secrete LURE and guide the pollen tube towards it (Fig. 5). The pollen tube that reaches the synergid cell releases its sperm cells, which fertilizes the egg cells. The structure of LURE depends on the plant species and is known to have an important role in the conservation of species.

Moreover, ITbM's plant biologists have also found that ovules secrete a molecule that activates the pollen tubes for fertilization. The researchers named the molecule "AMOR", taken from the Latin word meaning "love" and "cupid", which brings together the female and male organs together. AMOR is a large glycoprotein, which contains a sugar chain characteristic for plants. Through the collaboration between biologists and chemists, ITbM's team chemically synthesized a disaccharide molecule containing two sugar units, which is similar to the two units located at the terminus

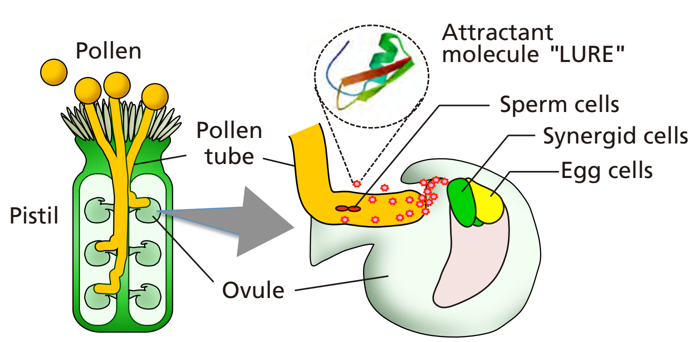


Fig. 5 Pollen tube guidance by the attractant molecule "LURE" in *Arabidopsis thaliana.*

of AMOR. Studies showed that this disaccharide exhibits the same properties as AMOR, and this key molecule is expected to be released soon on the market.

These discoveries are expected to increase the plant's fertilization efficiency and seed production, which may eventually lead to increased success in the crossbreeding of plants.

H. Takeuchi et al: Nature, 531, 245, 2016.
A. G. Mizukami et al: Curr. Biol., 26, 1091, 2016.

4 Development of molecules to regulate the circadian clock of animals and plants

Takashi Yoshimura (PI), Steve Kay (PI), Kenichiro Itami (PI), Stephan Irle (PI), Florence Tama (PI)

Our bodies contain a circadian clock, which regulates various daily rhythms, such as sleep/wake rhythm, hormone secretion, and metabolism. Disruption of the circadian rhythm may lead to sleep disorders, obesity, and other lifestyle diseases as well as mental disorders. Therefore, studies are being carried out to develop molecules that can control the circadian clock and relieve related disorders.

Based on the discovery of a period-lengthening molecule, KL001, by Kay's group in 2012, ITbM has carried out research to synthesize related molecules by synthetic chemistry and theoretical calculations. Through structure-activity relationship studies, ITbM's team succeeded in designing new molecules that show strong period-lengthening activity. These molecules are considered to act by targeting cryptochrome, which is a clock protein responsible for regulating the circadian clock.

This research is expected to lead to further understanding of disorders related to the circadian clock along with the development of potential treatments. In addition, the circadian clock has been reported to be the key factor for seasonal reproduction in animals, and application of this research towards the control of animal reproduction may lead to a potential increase in food production.

T. Oshima, et al: Angew. Chem. Int. Ed., 54, 7193, 2015.
J. W. Lee, et al: ChemMedChem, 10, 1489, 2015.

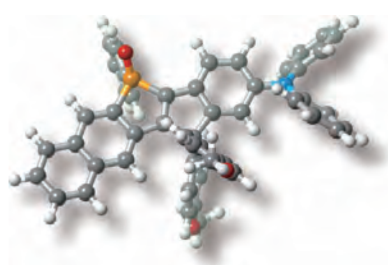
5 Development of molecules to visualize biological systems

Shigehiro Yamaguchi (PI), Tetsuya Higashiyama (PI), Cathleen Crudden (PI)

Bioimaging is an essential technique to study the localization and movement of molecules in living cells. Advances in the development of multipotent fluorescent dyes and super resolution microscopy have largely opened up the field of bioimaging. In particular, **st**imulated **e**mission **d**epletion (STED) microscopy (2014 Nobel Prize in Chemistry) has up to ten times higher resolution in living cells with respect to conventional microscopy. In addition, this technique enables nanoscale visualization of biological systems, such as organelles and proteins within cells, which has been difficult by previous techniques. Yet, the gradual degradation of fluorescent dyes bound to proteins, when exposed to the high intensity light necessary for super resolution microscopy has been a major obstacle for long-term observations.

To overcome this issue of reduced resolution in STED imaging due to photodegradation, ITbM's chemists and biologists developed a new fluorescent dye, "C-Naphox" that has enhanced photostability relative to conventional dyes (Fig. 6a). C-Naphox has demonstrated to be extremely photoresistant with almost no degradation of fluorescence even after prolonged STED imaging in live cells. Conventional fluorescent probes usually decompose after a few hours of irradiation, but the fluorescence of C-Naphox in HeLa cells persisted and remained unchanged even after 50 recordings under STED conditions.

The research team has also succeeded in developing a fluorescent molecule with a similar structure to C-Naphox that can change its color by detecting the polarity of the surrounding environment in biological systems. The dye molecule shows a color spectrum from red to green depending on the polarity of the organic solvent, in which it is dissolved in (Fig. 6b). Upon treatment of the cell with the dye, selective uptake of the dye by a lipid droplet is observed, and green emission is observed.

(a)


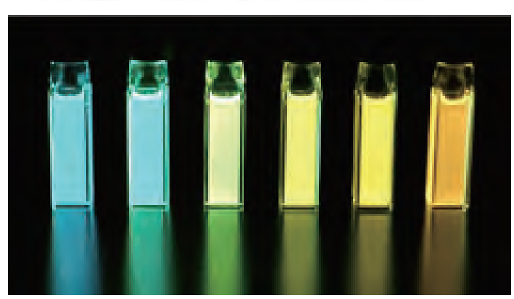
(b)


Fig. 6 (a) Structure of a new photostable fluorescent dye "C-Naphox"
(b) A fluorescent molecule that changes its color depending on solvent polarity

ITbM's chemists and biologists are working together to develop probe molecules and techniques applicable for STED microscopy. Some of the fluorescent probes developed at ITbM are commercially available as LipiDye, and their use in bioimaging is expected to accelerate biological research.

C. Wang et al: Angew. Chem. Int. Ed., 54, 15213, 2015.
E. Yamaguchi et al: Angew. Chem. Int. Ed., 54, 4539, 2015.

As a WPI center

Within 4 years since its establishment, ITbM has been generating a number of significant results from its interdisciplinary research. This is a result of an intensive collaboration between synthetic chemistry and animal/plant biology on top of the high caliber of each researcher. ITbM started under the call of the center director, where ITbM's PIs, who are rising stars in the fields of chemistry and biology, gathered in the same spirit. Driven by the need to integrate different disciplines, ITbM's researchers have come together to start new research projects and are sharing the excitement of integrative studies, which would have been difficult in a single research group or field alone.

ITbM's interdisciplinary research is greatly accelerated by ITbM's unique research environment called "Mix Labs" and "Mix Offices". These "Mix" spaces have been created to remove the walls between research groups and integrate researchers from different fields so that they can carry out research in the same space. ITbM's research center is built under this "Mix" concept. The effect of mixing has generated more results than initially expected, with the faculty, postdoctoral researchers, and graduate students forming their own research teams, proposing their original research projects and generating interdisciplinary research outcomes (Fig. 7).

ITbM has set up 4 sub-centers that promote ITbM's research: Molecular Structure Center, Chemical Library Center, Live Imaging Center and Peptide Protein Center. Each sub-center has a coordinator, who not only assists analytical measurements, but also actively participates in research projects to promote interdisciplinary research.

ITbM involves many researchers from overseas. Active exchange of researchers, especially graduate students, with overseas cooperative organizations, including USA, Canada, Switzerland and Germany, is being carried out. In addition, ITbM organizes many international meetings on a regular basis and hosts 3 international awards, 2 in organic chemistry (Nagoya Medal and Hirata Award) and 1 in biology (Tsuneko and Reiji Okazaki Award), which helps in building international networks.

Fig. 7 A fume hood in the Mix Lab for conducting chemistry experiments.

ITbM's Administrative Division also plays a role in promoting interdisciplinary research and international collaborations. ITbM provides support in English and help in the daily lives of overseas researchers. What makes ITbM different from the usual Japanese university administrative system is the presence of the Research Promotion Division and the Strategic Planning Division within the center. These 2 divisions consist of researchers with a background in ITbM's related fields (chemistry and biology), and provide seamless support for ITbM, through managing intellectual properties, help in presenting/exchanging research results, public relations and outreach, as well as social implementation of scientific outcomes.

ITbM's female scientists are receiving high recognition for their active performance, which is reflected in the number of awards and honors received by the faculty as well as graduate students. Nagoya University has been supportive for promoting female leaders, and provides nurseries and after-school care for children to create an environment for female researchers to be able to continue their research. These endeavors have been recognized by the international community, in which Nagoya University was selected as one of the ten leading global universities that lay out concrete commitments and begin charting their progress toward achieving gender parity (HeForShe IMPACT 10×10×10 University Parity Report). ITbM benefits from this environment and provides support for female scientists.

The outcomes and measures taken by ITbM are strongly supported by the WPI program and by Nagoya University. ITbM will continue its challenge in cutting edge research under this support and by the strong leadership of the center director.

Tsuyoshi Matsumoto (ITbM), Ayako Miyazaki (ITbM)