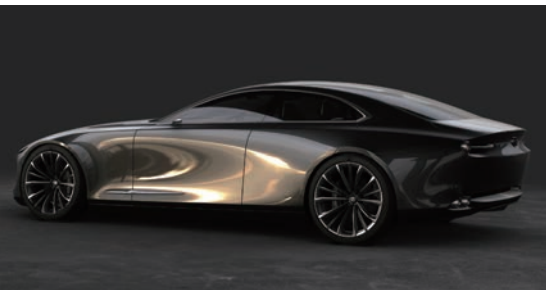
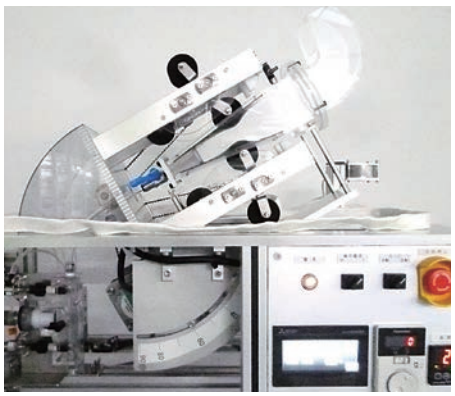
Volume
24

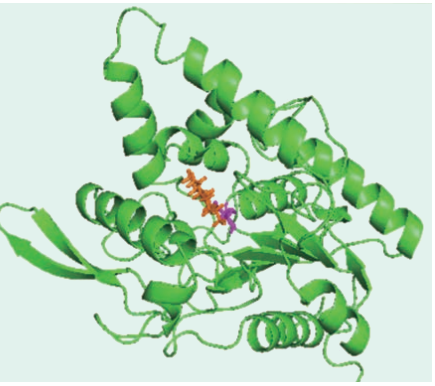
Enzyme Wave

2021

AMANO
Amano Enzyme







Seeing the invisible

Enzyme Wave vol.24

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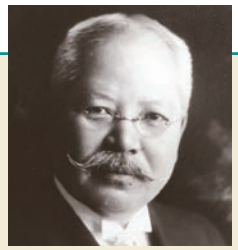
Information

Jokichi Takamine Study Group, NPO

Dr. Jokichi Takamine

Dr. Jokichi Takamine lived through the dramatic period of time from the end of the Tokugawa shogunate, Meiji era, and Taisho era. He left a great legacy as a scientist, entrepreneur, and an international goodwill ambassador. Dr. Takamine is called the father of modern biotechnology for his research and development of amylolytic enzymes derived from microorganisms, mainly Taka-Diastase.



Dr. Jokichi Takamine
(photo courtesy Great People of Kanazawa Memorial Museum)

Jokichi Takamine Study Group, NPO

The NPO, Dr. Jokichi Takamine Study Group, is engaged in educational activities, such as publishing journals and holding lectures, in order to make more people aware of Dr. Takamine, who made a great contribution to the development of science and technology in modern Japan, its commercialization, and goodwill between Japan and the United States.

Main activities

Although the COVID-19 pandemic that began in early 2020 has yet to subside, we are holding lectures almost as usual. In addition, our activities are being featured in books and the media on more occasions. We will continue to provide more accurate information, including the production of media materials, following the biographical manga.

Tomio Taki, vice chairperson, was honored as a person of special merit by Takaoka City for his efforts in honoring Dr. Takamine and his contribution to the project to preserve and recreate Shofuden Hall in Takaoka. (Left: Masaki Takahashi, mayor of Takaoka City)

Recreated exhibition in Shofuden Hall
Takaoka Chamber of Commerce, ground floor:
Takaoka Shoko Building, 1-40, Marunouchi, Takaoka City

Topic Shofuden Hall - development

In March 2020, Shofuden, which served as a social hall for Japan-U.S. friendship in the suburb of New York City, returned to Takaoka City in Toyama, the birthplace of Dr. Takamine, in the form of a partially relocated and recreated building using actual wall and ceiling paintings. You can feel the atmosphere of those days through the panels and furniture displays. Please come and visit Shofuden Hall!

Notification of the recruitment of new members

The Jokichi Takamine Study Group is accepting supporting members.

Supporting members will receive publications related to Dr. Takamine and regularly issued newsletters. They also receive information about lectures, events, and news.

If you would like to join us, please write your name (if you are a corporate body, company/organization name and department), postal code, address, phone number (no cell phone number accepted), occupation, age, and sex on a letter or a postcard and mail it to us. We will return a bank transfer form to pay the initiation fee and the annual membership fee.

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*For more details, please visit our website.
http://www.npo-takamine.org/ask.html

Column

Brand style of Japan

Introduction of the author

Ikuo Maeda

Managing executive officer in charge of Design and Brand Style, Mazda Motor Corporation

[Background]

Graduated from the Department of Design and Crafts, Kyoto Institute of Technology, visiting professor at Hiroshima City University

Mr. Maeda has led Mazda Design since 2009, advocating "Kodo Design - the design that moves spirits," and has won many design awards around the world, including the WCOTY Design Award and the European Most Beautiful Car of the Year twice.

Member of the Study Group on Industrial Competitiveness and Design organized by the Ministry of Economy, Trade and Industry. Holds an international Class C license and is active as a racecar driver.

Amano Enzyme celebrated its 120th anniversary two years ago, and since the 121st year, we have been providing a series of contributions on Japanese tradition and culture.

In this second edition, Mr. Maeda, Managing Executive Officer of Mazda Motor Corporation, a Japanese automobile manufacturer with a strong presence in the world, writes about Japanese aesthetics and brand style.

I have always believed that there is power in beauty.

Although there is no precise definition, I believe that powerful beauty can only be expressed when the philosophy of the creator comes to fruition in tangible form.

In Japan, there is a spirit of creation that takes time to sophisticate, and there is a culture that connects seemingly useless efforts to value. The delicate methods of expression, such as lacquerware, metalwork, and ceramics, are recognized as embodying Japan's unique sense of beauty, and are highly regarded, especially in the West. These powerful beauties are not easy to create.

On the other hand, I think it is rare to come into contact with Japan's unique sense of beauty in our daily lives. In addition, I rarely find unified style. Meanwhile, Europe, and Italy in particular, is a country with beautiful scenery of the same hue everywhere you go. Part of the reason is that they continue to use old buildings, but the buildings, signs, and everything else in the city are unified within a certain color range. I feel that the beauty of the style is not only because of the strict rules but also because all the people take pride in the Italian style and continue to protect it themselves.

As a result of the continuous chaos of many different styles, the Japanese have forgotten the importance of a unified aesthetic style. What kind of unified message does the brand of Japan have? Should we have one to begin with? I think it is about time that we find the answer to that question.

Japanese car manufacturing has a history that is a fair match to its European predecessors. Mazda also celebrated its 100th anniversary last year. All the while, has the Japanese automobile industry contributed to the establishment of Japanese aesthetics and style? The answer is probably "no." Business efficiency has been the top priority, and there has been a history of repeated overproduction of products with poor quality. This efficiency-oriented industry may have created the tendency of Japanese customers who get bored of things easily and quickly.

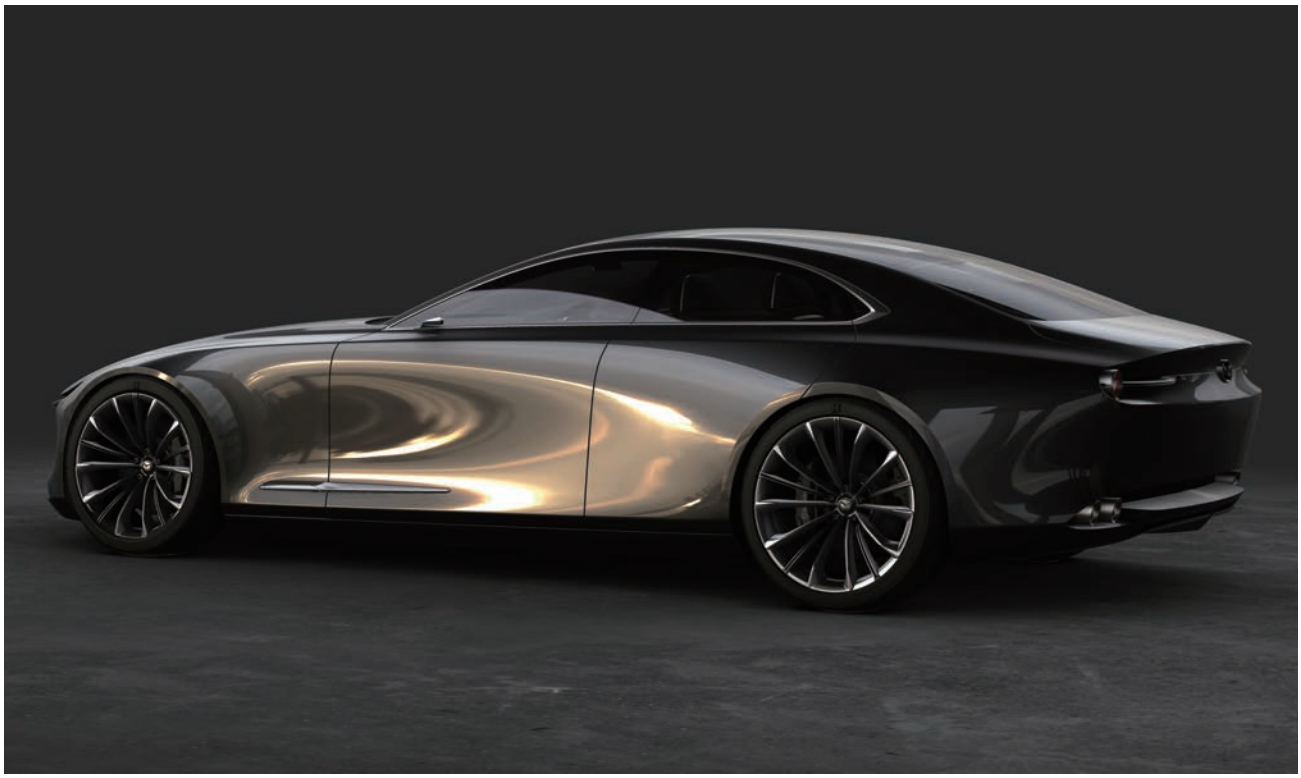
It is said that artists are in the business of presenting their own sense of problems to the world, while designers are in the business of solving the problems of their customers. Yet, I believe that the role of designers will be much broader in the future. In the ten years since I took over the leadership of Mazda design, I have been driven by the aforementioned desire to create a beautiful Japanese style. I have faced questions like what I could do with car design to achieve this. What should be the design of a car that embodies the Japanese sense of beauty? We have been exploring these questions by setting a theme of pursuing the “Kodo Heartbeat Design” that moves the spirit.

The underlying philosophy is the "aesthetics of subtraction.”

I aimed to express the sense of life and tension that is created when elements are stripped down to the bare minimum. This is the same concept as Japan's unique sense of beauty of believing that spirits exist in tools. This approach is the exact opposite of today's efficiency-centered manufacturing trend, but we have come to believe that the source of Japan's manufacturing and style creation can be found in the careful and painstaking search for answers through co-creation with the master form-makers who are our assets.

It is rare to find a landscape where cars are not in sight, except in the real wilderness. In other words, the car is an element of the landscape, or more specifically, a part of the style. It is no exaggeration to say that the design of a car creates the style of a country. Be aware of this and do not opt for quick and easy designs. I believe that we designers have a responsibility to do so.

We have put behind the days when convenience and mass consumption were symbols of affluence. Today, surrounded by beautiful things and living in a neat and uniform style seems to be the true wealth. I would like to establish a Japanese brand style that embodies such a lifestyle.



[Vision Coupe]

Trend

Smart fermentation industry through IT and AI

Introduction of the author

Jun Ogawa

Professor of Applied Life Sciences, Graduate School of Agriculture, Kyoto University

[Background]

After working as a research assistant at the Graduate School of Agriculture, Kyoto University, a visiting researcher at the French National Research Institute for Agriculture, and a special professor at the Research Division of Microbial Sciences, Kyoto University, he assumed his current position in 2009. Ogawa specializes in fermentation physiology and brewing science. Since FY 2020, he has been in charge of research and development for the NEDO project: Development of Production Technology for Bio-based Products to Accelerate Carbon Recycling / Research and Development of Data-driven Integrated Bio-manufacturing Management System (Data-driven iBMS).

IT and AI technologies have made remarkable progress in recent years, and these technologies are beginning to be introduced into the fermentation industry. This article is about the current status of IT and AI technologies and fermentation technology, and the technological elements necessary for further progress.

With the advancement of information technology (IT), it has become possible to analyze vast amounts of information in a short period of time and easily extract meaningful information from it. The act of Googling is symbolic of these technologies. In addition, the advances in artificial intelligence (AI) technology is making it possible to design the direction of gene modification for function up-grading based on the results of an analysis. We are now in an age where the next move made by AI can make even the greatest Go and chess masters groan.

This trend is also entering the fermentation industry. Starting from the fact that organisms can now be described in terms of universal information, such as gene sequences, it has become possible to extract functions through gene analysis using IT. Also, coupled with genetic manipulation techniques, artificial control of biological functions has become realized. In the development of enzymes that play an important role in material production, mutation and selection technologies have been greatly streamlined by IT, making it possible to produce highly functional enzymes in a short period of time. In addition, AI is now able to design the direction of genetic modifications for higher functionality by surveying a vast number of successful and failed examples. Furthermore, artificial metabolic pathways incorporating the resulting highly functional enzymes have been designed and are about to be installed in hosts with ample genetic engineering technology, such as *E. coli* and yeast, and applied to fermentation production. This is a technical field known as synthetic biology.

In addition, in microbial cultivation, various environmental changes associated with growth are monitored, analyzed by IT, and AI predicts the outcome and proposes a response, creating a situation in which operations can be carried out like automated car driving. A symbolic example of this is the introduction of IT and AI, along with various monitoring devices, into the brewing process, which was once regarded as the craftsmanship of the *toji* (master sake brewer), to ensure the consistent supply of high-quality sake.

These efforts will lead to the accumulation of new experiences, and IT and AI technologies seem to be evolving in a chain reaction beyond our imagination. However, the author, who deals with a variety of microorganisms on a daily basis, believes that in order for these to truly contribute to biotechnology, especially fermentation

technology in which organisms play a leading role, a fusion with the characteristics of organisms must be achieved. The characteristics of organisms are individuality and diversity.

I'm going to change the subject a little here, so please forgive me. My hobby is music. In a sense, it is easy to construct music digitally because the components of music are things that can be expressed in mathematical equations, such as harmony and rhythm. However, what is needed for music to stir people's hearts is a strong individuality like that of Mozart or Beethoven, as well as a leap forward to create diversity that goes beyond the existing boundaries. This is no different from the characteristics of living things.

So, what are the technological elements necessary for IT and AI technologies to confront the individuality and diversity of living things? I believe that three factors are important. The first is the analysis of genes with unknown functions. Even in *E. coli,* 20% to 30% of its genome is said to have unknown functions. Under these circumstances, it is impossible to see the individuality of expression that goes into each wrinkle and the rich diversity that results from it. The other two are technologies that create individuality and diversity. The second factor is related to breeding. This is the development of a transformation technology that can add additional characteristics to cells with inherent individuality. So far, there are only a limited number of microorganisms (hosts) to which genetic engineering technology can be easily applied. The diversity of individuality at the host level is not sufficient yet. The third is a method to create individuality as a group. By interweaving unique cells, it might become possible to make manifest the characteristics of a group, such as national characters and traits. In terms of technology, the control technology for complex culture systems may fall into this category.

I believe that if we can freely handle the individuality and diversity of living things and create new individuality through IT and AI technologies in collaboration with above mentionedtechnologies, we will be able to create biotechnology and fermentation technologies that will realize a truly sustainable recycling society.

Report

Human gastric digestion simulator (Usefulness of digestive enzymes)

Digestive enzymes are effective in relieving heaviness in the stomach and indigestion and have been used for centuries. The forerunner was Takadiastase, which was developed and commercialized by Dr. Jokichi Takamine, and was launched in Japan by Sankyo Shoten (now Daiichi Sankyo Healthcare) in 1899. In 1900, the use of animal-derived pancreatin also started in medical digestive enzymes. Amano Enzyme (then known as Amano Pharmaceutical) began manufacturing and selling malt-derived diastase in 1948, koji mold-derived biodiastase in 1950, and pancreatin in 1953. Subsequently, amylase to degrade starch, protease to degrade protein, lipase to degrade fat, and cellulase to degrade fiber came to be used as microbial-derived enzymes in complex digestive enzyme agents.

Depending on their origin, digestive enzymes have different pH ranges in which they work effectively. Digestive enzymes derived from microorganisms, such as koji mold, work well in the acidic range. Animal-derived pancreatin works well in the neutral to alkaline range, but stops working when it becomes acidic. As these characteristics indicate, digestive enzyme preparations have been devised to suit the characteristics of each enzyme, such as blending ingredients in acidic and neutral dissolvable granules.

Digestive enzymes have been used for such a long time, and Amano Enzyme is working to discover new functions of digestive enzymes. In the process, the effect of digestive enzymes derived from koji mold on improving the intestinal microflora has been discovered. The photo shows a digestion experiment using a continuous human gastric digestion simulator, which reproduces gastric digestion, including gastric peristalsis, evacuation from the stomach to the intestines, and secretion of gastric acid and pepsin (digestive enzymes of the stomach) for the observation of the effect of digestive enzymes on food digestion and the analysis of products (Fig.1). This demonstrated, among other things, the enhancement of amino acid release from food by administration of digestive enzymes.

Through these efforts, Amano Enzyme will continue to search for the new functions of digestive enzymes and provide useful information related to health and food.

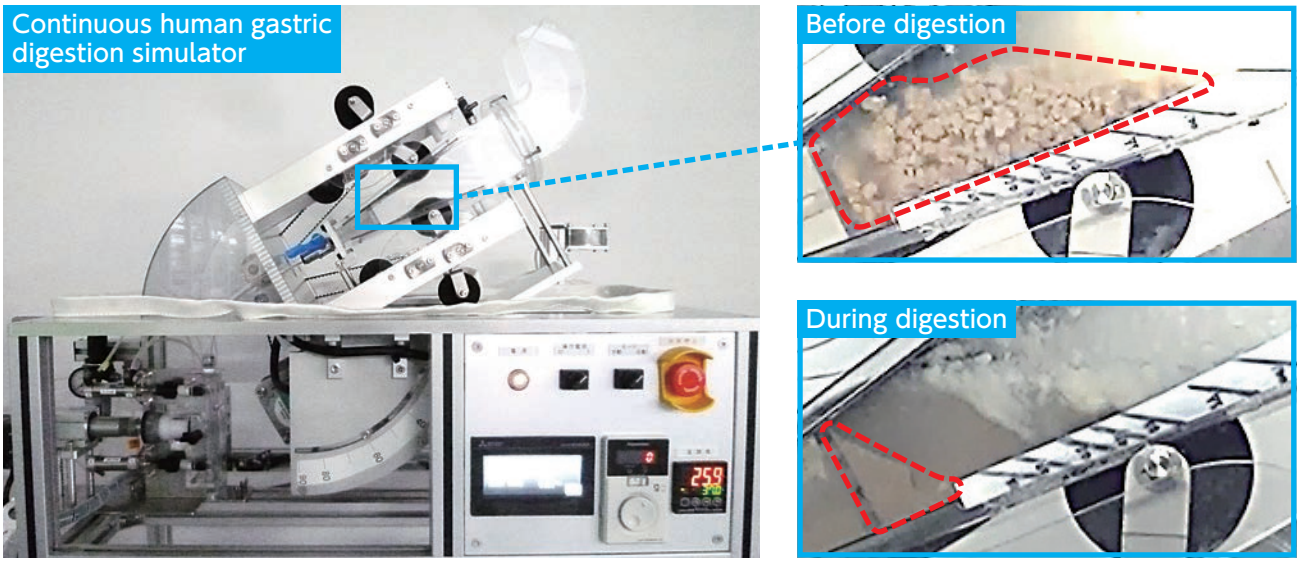


Fig.1 An experiment in a continuous human gastric digestion simulator
The photo shows how minced beef is digested in a container that mimics the stomach.
The dashed line indicates digestion residue.

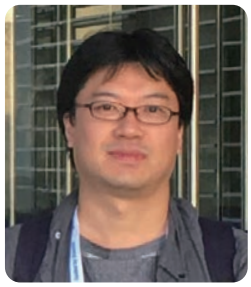
Report

Lipase design by MD

Introduction of the author

Tomoshi Kameda

Senior researcher, Artificial Intelligence Research Center, National Institute of Advanced Industrial Science and Technology and visiting associate professor, Graduate School of Life Science, Hokkaido University.



[Background]

Graduated from the Faculty of Science at Kyoto University and the doctoral program at Kobe University, Graduate School, Doctoral Program, PhD (Science). Kameda has consistently studied biopolymers, such as proteins and DNA, using theoretical methods. He mainly uses molecular dynamics (MD) simulations but also uses bioinformatics and machine learning.

We are using computational methods, such as molecular dynamics (MD) simulation and machine learning, to enhance the functionality of enzymes. In this article, we use MD to represent proteins, water, and compounds as a collection of atomic spheres on a computer, and investigate their shapes and movements to enhance the functionality of lipase.

Currently, the optical isomer purity of L-menthol by extraction from plant materials and chemical methods is more than 99.0%, which meets pharmaceutical grade standards, while that by enzymatic synthesis is only 97.0%. If we can develop an enzyme that achieves a purity of 99.0% or higher, it will be possible for companies without advanced chemical processes to produce high-purity menthol. Therefore, we aimed to improve the optical isomeric purity of menthol when synthesized by lipase.

Lipase has an open structure when not bound to a substrate (apo) and a closed structure when bound (holo), and its three-dimensional structure changes significantly. In the lipase, the subject of this study, the only three-dimensional structure obtained was an open structure at the apo. We therefore placed L-menthyl acetate, the precursor of L-menthol, at the active site of the open structure and performed holo-state MD [1], which successfully reproduced the closed structure on the computer. Based on this closed structure, MD [1] was performed with the menthyl acetate of the L and D bodies in place, respectively. From the results of the two computations, the amino acid residues that contacted the D body well and did not contact the L body were identified. We assumed that by mutating this amino acid, we could reduce the reactivity to the D-body while maintaining the reactivity to the L-body, resulting in improved purity. When experiments were conducted on the mutant, we succeeded in obtaining a purity of up to 99.5%.

The docking simulation, which predicts the complex structure by changing only the relative configuration without changing the structure of the protein/substrate itself, cannot be applied to enzymes with large structural changes such as lipase. This study shows the usefulness of MD, which can take into account structural changes in the protein itself.

[1] In this study, a special MD called the ALSD method was used to promote the structural change
J. Ikebe et al. (2014) *J Comput Chem* 35:39-50

Enzyme: Lipase, Substrate: Menthyl acetate, Active site residue: S87 (PDB code: 1OIL)

Symposium

The 3rd Sino-Japan Symposium on Biocatalysis and Biotransformation

In recent years, environmental pollution caused by the rapid development of chemical industries especially in China and other Asian countries has become a social problem. Therefore, it is important to changes the industrial processes to that of minimize the effects on humans, ecosystems, and the environment. This trend has been spurred on by global efforts to realize a sustainable society as exemplified by the recent SDGs movements. Industrial processes using enzymes, which are a sustainable method of practicing green chemistry, are attracting more attention as a promising solution.

Amano Enzyme holds the Japan-Sino Symposium on Biocatalysis and Biotransformation every other year in collaboration with Zhejiang University in China. The purpose of this symposium is to connect Chinese companies interested in green chemistry using enzymes and professors and researchers from Japanese and Chinese universities and public research institutions who are conducting the latest research in this field and to disseminate the latest research findings to Chinese companies.

On December 12, 2020, the third symposium was held in Hangzhou, China. Because of the impact of COVID-19, we had to participate remotely from Japan. Also, there was a limit on the number of participants at the Hangzhou venue, but on the day of the conference, we had about 120 participants from companies and universities in China, which was just under the limit. A total of 13 lectures were given, including remote lectures by Professors Yasuhisa Asano (Toyama Prefectural University) and Kosuke Honda (Osaka University), who were invited from Japan, followed by lively Q&As and discussions.

In spite of the difficult and unusual situation, many participants participated in the conference, and we realized that enzymes are still attracting a lot of attention in China. Amano Enzyme is going to continue organizing this symposium to help people with green chemistry using enzymes.

The venue

Professor Li-rong Yang (Zhejiang University)

Professor Kosuke Honda (Osaka University)

In the screen : Professor Yasuhisa Asano (Toyama Prefectural University)

Prof. Yasuhisa Asano (Toyama Prefectural University)	Structural, functional analyses and application of animal and plant-derived hydroxynitrile lyases
Prof. Jianping Wu (Zhejiang University)	Molecular engineering of glutamate dehydrogenases
Prof. Kohsuke Honda (Osaka University)	*In vitro* synthetic metabolic pathways consisting of thermophilic enzymes
Prof. Gaowei Zheng (East China University of Science and Technology)	Development of imine reductases and amine dehydrogenases for the synthesis of chiral amines
Nobuyuki Urano (Amano Enzyme Inc.)	Protein engineering for industrial application
Prof. Kequan Chen (Nanjing Tech University)	Enzyme-catalyzed amino acid derivatization
Prof. Jun Ge (Tsinghua University)	Enzyme catalyst engineering
Prof. Qiaqing Wu (Institute of Industrial Biotechnology, Chinese Academy of Sciences)	Selectivity control and synthetic applications of nitrilase-catalyzed hydrolysis of nitriles
Prof. Jianbo Wang (Hunan Normal University)	Mechanistic investigation of the P450-catalyzed asymmetric sulfoxidation
Prof. Yunzi Luo (Tianjin University)	Multi-modular engineering of Saccharomyces cerevisiae for high-titre production of tyrosol and salidroside
Prof. Haoran Yu (Zhejiang University)	Development of rational design methods for enzyme engineering
Kexin Liu Zhuxian Zhou (Zhejiang University)	Tailor-made polymer-cisplatin nanovesicles for acidresponsive drug delivery
Ying Zhao, Lin Zhang (Zhejiang University)	Preparation of positively charged nanofiltration membrane based on Donnan equilibrium effect

Introduction of the website, Seeing the invisible

In addition to the company's website, Amano Enzyme created a new special website, Seeing the invisible, which was launched in January 2021.

Fermentation has been used in human activities since as far back as B.C. and is widely used around the world in the fields of food, beverages, and clothing. However, it has been a short time since it was known that fermentation was caused by the action of enzymes, and even now, not many people know about enzymes.

This website was created to help non-enzymatic experts understand that enzymes are used in many areas of our daily lives and are an essential part of our lives. In addition to introducing enzymes, this website features interviews with people involved in fermentation, making it an enjoyable read for visitors to the site.

We hope that many people will become interested in enzymes and become familiar with them, as well as learn about their potential in a sustainable society through this website. The URL for the website is listed below. We hope that you will take a look at it and introduce it to your family and friends.

https://mienaimono.jp/en/

Conference presentation

In 2020 Amano Enzyme has decided academic presentations such as the following.
Please look forward to the activities of the future of Amano Enzyme.

Conference/Meeting	Date	Title	Speaker
The 106th General Meeting of the Japanese Society of Gastroenterology	Aug. 11th-30th, 2020 (Web)	Evaluation of total digestive activity of medical digestive enzymes using digestion flask model (Second report).	Takahashi, A., Ko, S.
		Evaluation of digestive enzymes in the digestion tract using gastric digestion simulator	Takahashi, A., Kobayashi, I., Ko, S.
The 72th Congress of the Vitamin Society of Japan	Sep. 4th-13th, 2020 (Web)	Glucose Dehydrogenase for glucose measurement - the change of the characteristics due to the difference of coenzyme -	Nishio, K.
BioJapan 2020 World Business Forum	Oct. 14th-16th, 2020 (Yokohama, Japan)	Development of Industrial Enzymes Using Molecular Dynamics Simulations. - Production method for valuable chemical products using enzyme design technology. -	Ishihara, S.
The 51th General Meeting of the Japanese Society of Digestion and Absorption	Nov. 21th, 2020 (Web)	Evaluation of digestive enzymes in the digestion tract using continuous-type gastric digestion simulator	Takahashi, A., Ko, S.
		Actual digestive activity of medical digestive enzymes at low pH in the duodenum.	Takahashi, A., Ko, S.

2021 Exhibitions

Date	Exhibition	Location
March 16-18	Food Ingredients China 2021	Shanghai (China)
May 2-5	2021 AOCS Annual Meeting & Expo	Portland (USA)
May 12-14	ifia Japan 2021	Yokohama (Japan)
May 19-20	Chemspec Europe 2021	Frankfurt (Germany)
June (TBD)	CPhI China 2021	Shanghai (China)
June 3-July 2	FBIF 2021	Hangzhou (China)
July 18-21	IFT21	Chicago (USA)
July 21-23	15th Food Proteins Course North America 2021	Chicago (USA)
September 15-17	Food Ingredients Asia 2021	Bangkok (Thailand)
September 22-23	Vitafoods Asia 2021	Singapore
September 28-29	2021 Protein Trends & Technologies Seminar	Itasca (USA)
October 1-3	2021Specialty Coffee Expo	New Orleans (USA)
October 5-7	Vitafoods Europe 2021	Geneva (Switzerland)
October 25-28	Supply Side West 2021	Las Vegas (USA)

For details and the latest information, please refer to our website or each exhibition website.

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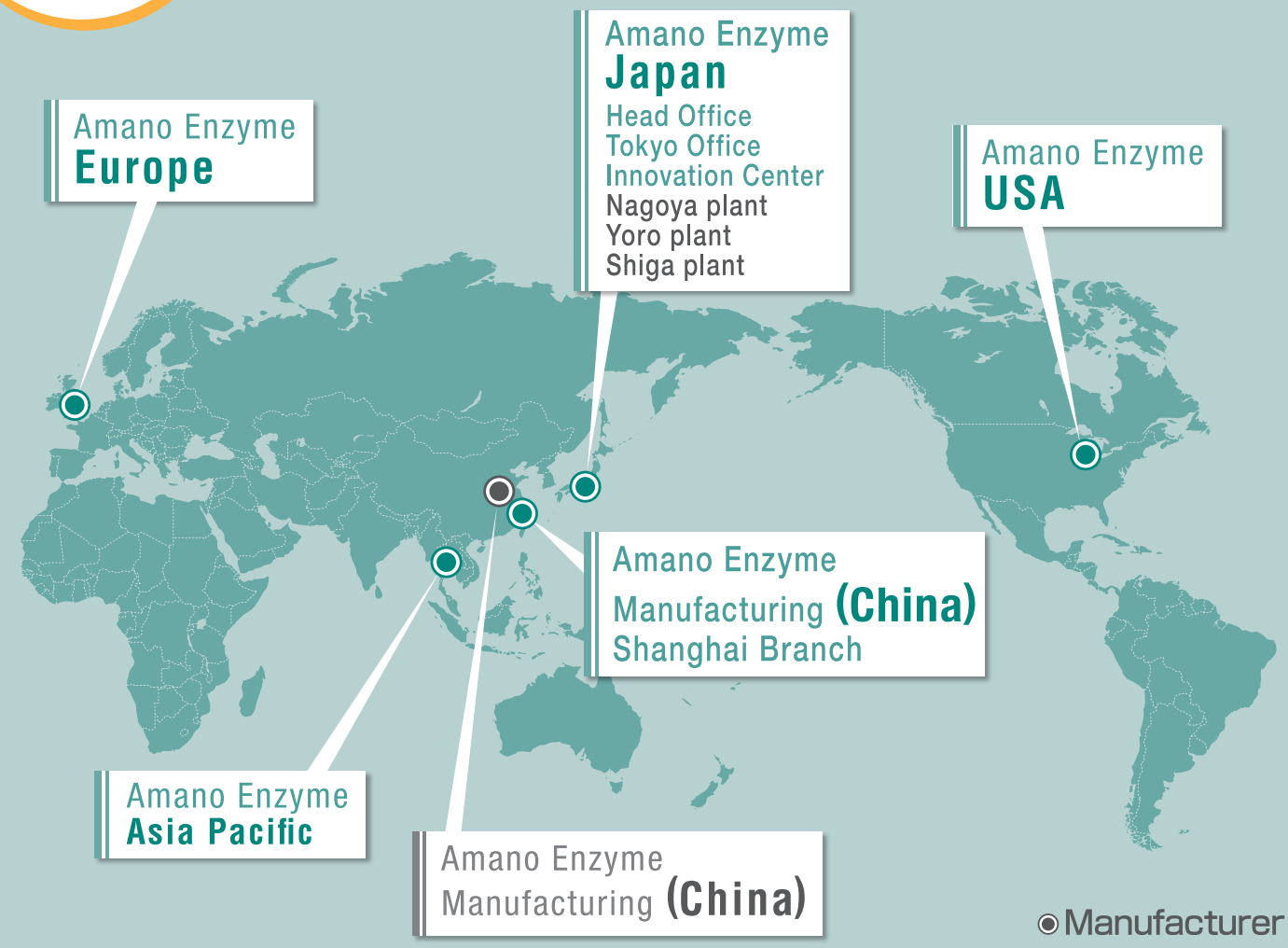
Amano Enzyme

World Network



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April.2021