181005@JSPS Alumni in Finland

Enzymes for biomass utilization

How do difficult biotechnologies contribute to Bioeconomy?

Graduate School of Agricultural and Life Sciences, The University of Tokyo

VTT Technical Research Centre of Finland

五十嵐 圭日子　Kiyohiko Igarashi

Advanced approaches for enzymatic biomass utilization and modification

Prof. Penttilä

Dr. Koivula

“BioAd”

Finland Distinguished Professor (FiDiPro) Program supported by TEKES => Business Finland

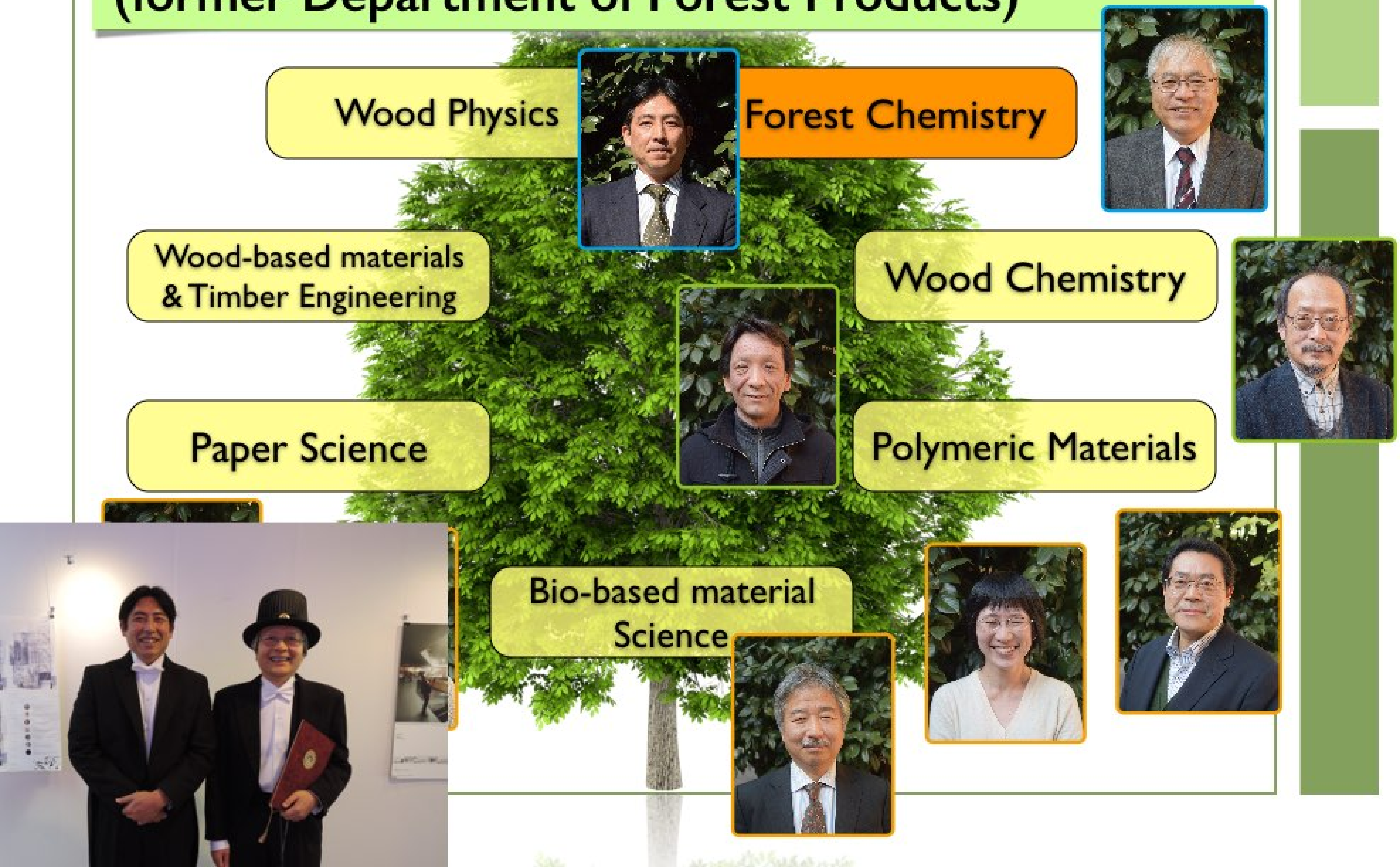
Biosynthesis

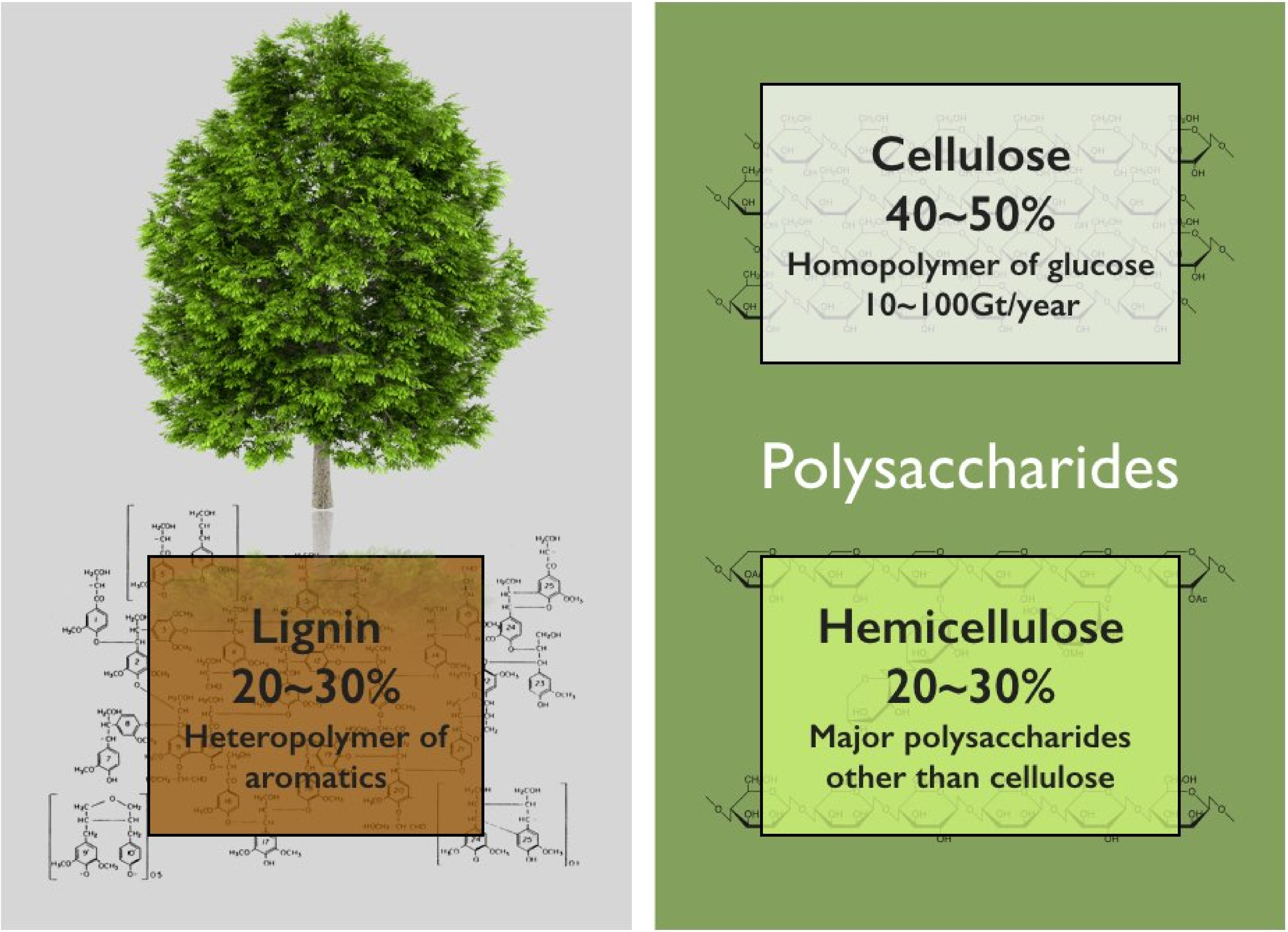
Biomodification

Biodegradation

@VTT

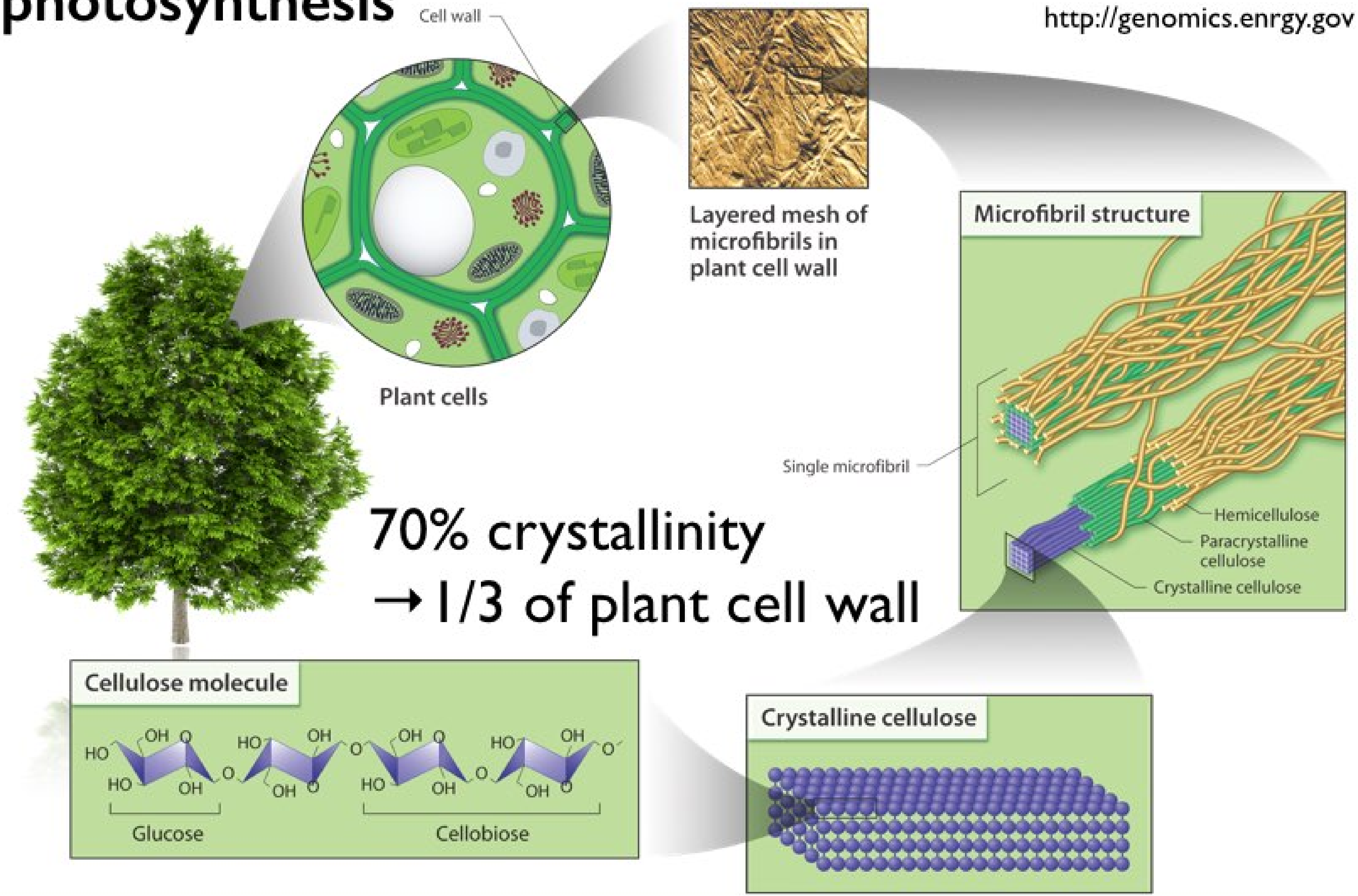
Department of Biomaterial Sciences
(former Department of Forest Products)



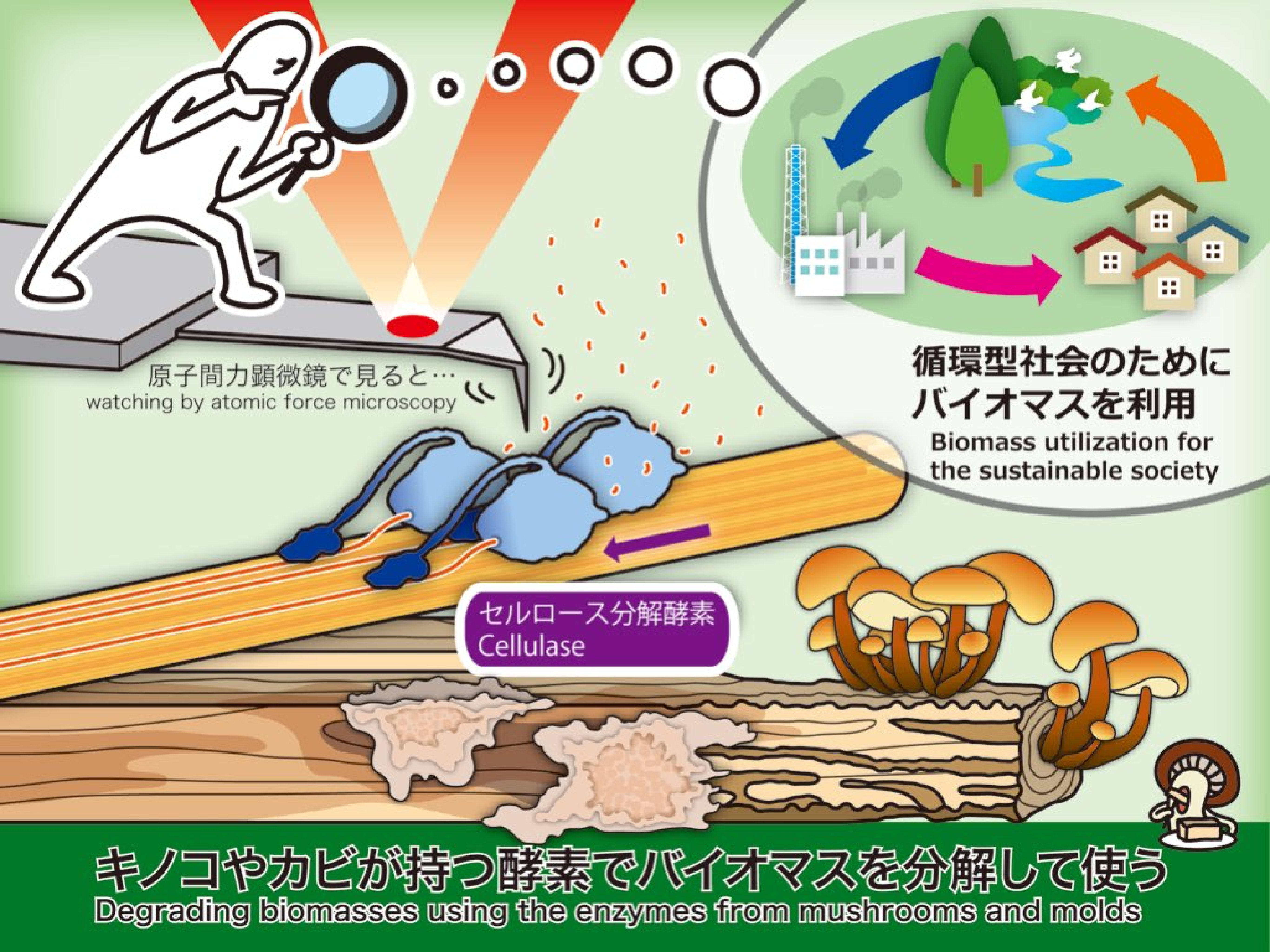

Cellulose
40~50%
Homopolymer of glucose
10~100Gt/year
Polysaccharides
Hemicellulose
20~30%
Major polysaccharides
other than cellulose
Lignin
20~30%
Heteropolymer of
aromatics

Cellulose is a major component of plant cell wall up to 50% and 10^{10}~10^{11} ton/year production by photosynthesis

http://genomics.enrgy.gov

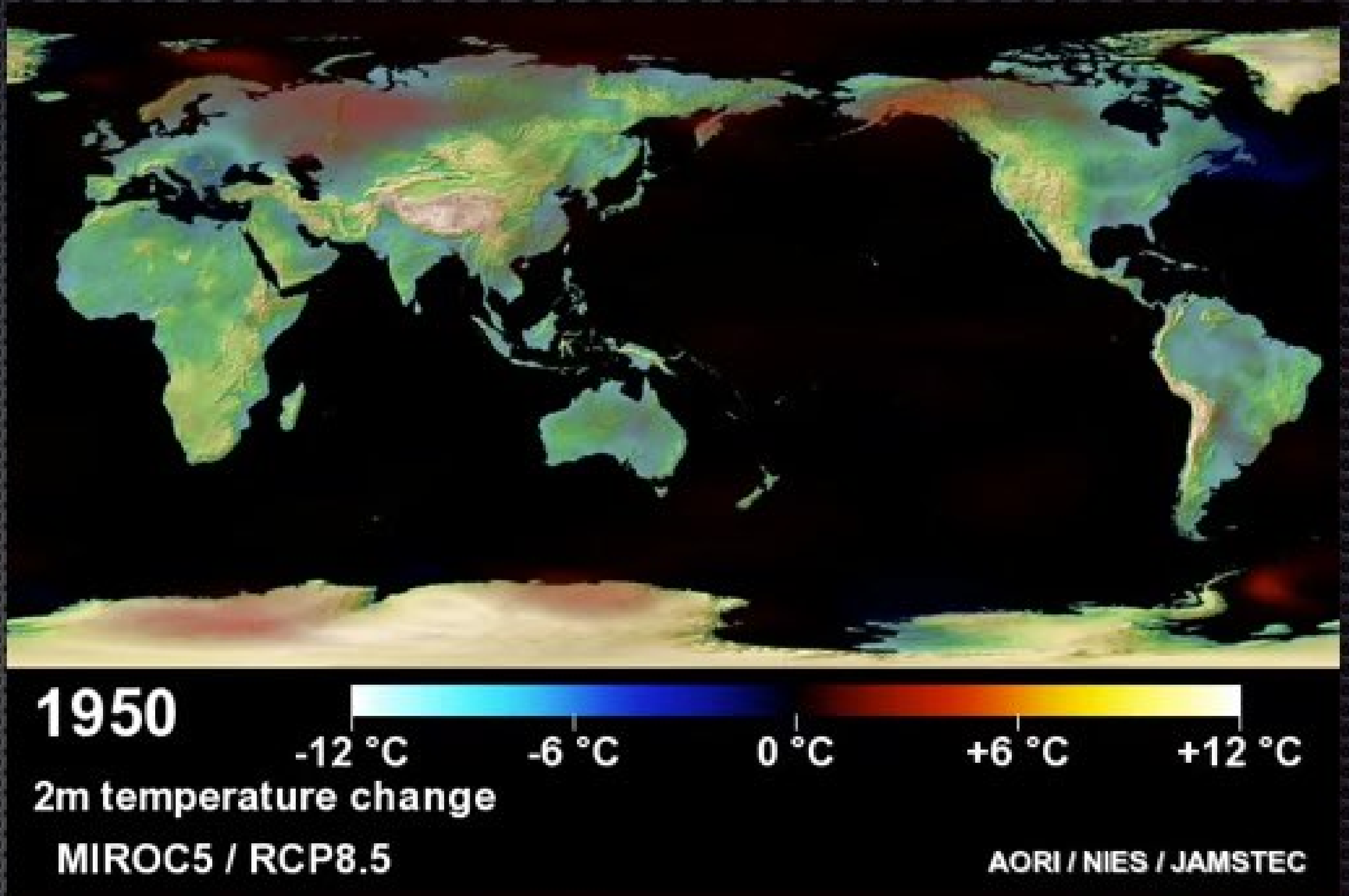


70% crystallinity
→ 1/3 of plant cell wall

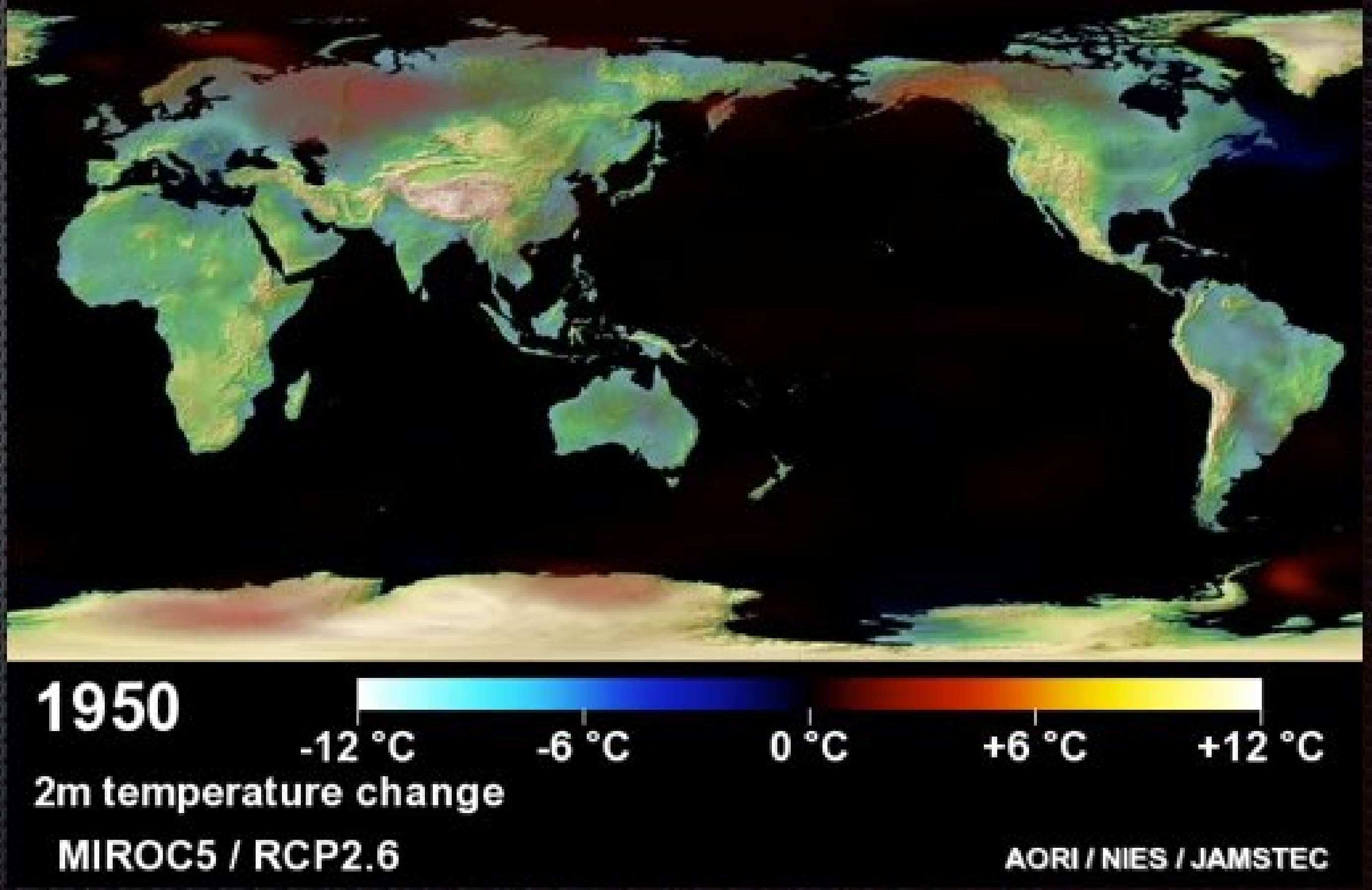

原子間力顕微鏡で見ると…
watching by atomic force microscopy
循環型社会のために
バイオマスを利用
Biomass utilization for
the sustainable society
セルロース分解酵素
Cellulase
キノコやカビが持つ酵素でバイオマスを分解して使う
Degrading biomasses using the enzymes from mushrooms and molds

Simulation of Temperature Change

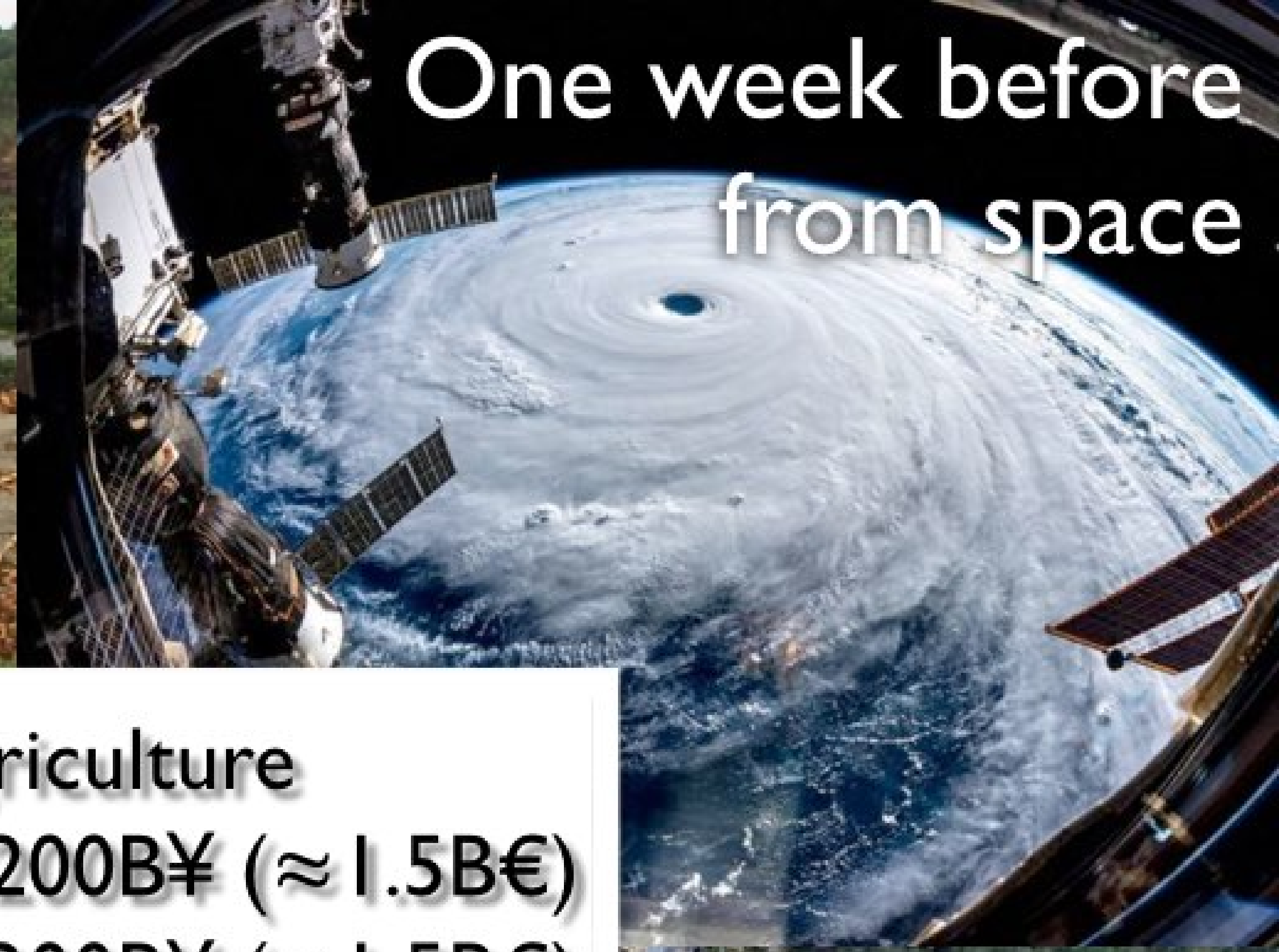
Scenario with
"no action"



Scenario to achieve
"below +2°C"
Paris Agreement

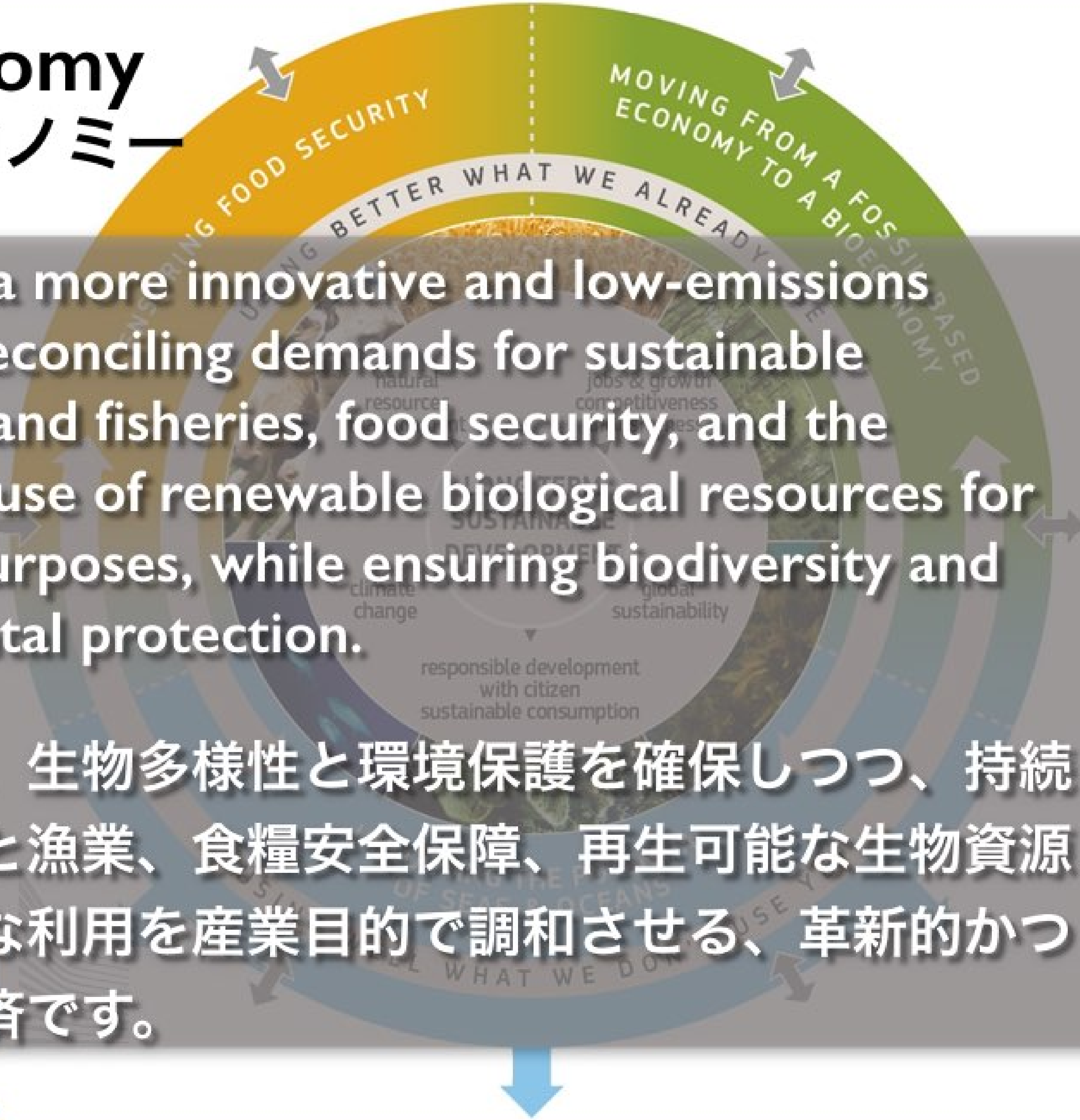


By MIROC5 Climate Model
(AORI/NIES/JAMSTEC/MEXT)



Damage for Agriculture
2017: 200B¥ (≈1.5B€)
pred. 2018: 200B¥ (≈1.5B€)
>1T¥ (≈7.7B€) for all damages

Bioeconomy
バイオエコノミー



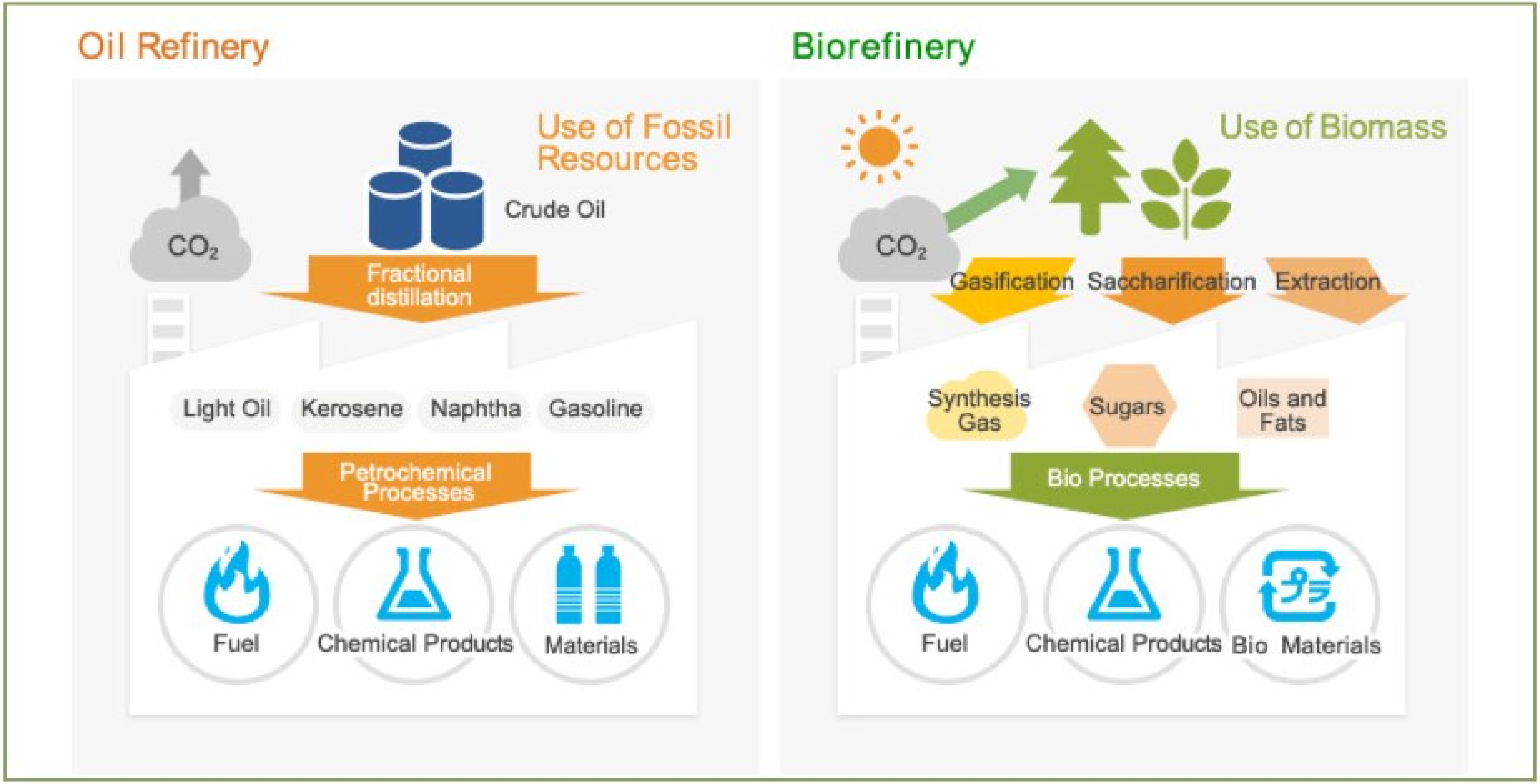
The goal is a more innovative and low-emissions economy, reconciling demands for sustainable agriculture and fisheries, food security, and the sustainable use of renewable biological resources for industrial purposes, while ensuring biodiversity and environmental protection.

その目的は、生物多様性と環境保護を確保しつつ、持続可能な農業と漁業、食糧安全保障、再生可能な生物資源の持続可能な利用を産業目的で調和させる、革新的かつ低排出の経済です。

European
Commission

BIOECONOMY STARTS here

Oil refinery vs Biorefinery



http://www.viaspace.com/biochemicals_bio_plastics.php

Bioeconomy
in everyday life

Catalogue
Bioeconomy Apartment
Exhibition, 9-10 November
Brussels, 2015

Bike

Sector:
Consumer goods

Producer:
Lignotube Technologies

Sources: abet/fotolia.com (left), Lignotubes technologies (right)

Raw material

Unlike materials such as aluminium, iron or carbon, **wood** is a renewable resource, for which you only need sunlight and CO_2 for photosynthesis. Meanwhile, engineered wood has caught up in terms of strength and processability. The German company Lignotube Technologies uses real wood veneer as the basis for lightweight tubes for bicycles.

Procedure

Inventors at Lignotube Technologies have developed a resource-saving procedure for **lightweight hollow tubes** called Lignotubes, which are made from a multi-layer composite material of wood veneers. The thin-walled tubes are lightweight and robust and their production uses a minimal amount of real wood. The individual layers of veneer are crosswise glued. The first product is a designer bicycle built using a Lignotubes frame.

Contribution to the bioeconomy

7

Toilet brush

Sources: Philipp Graf/BIOCOM AG (left), Tecnaro (right)

Sector:
Consumer goods

Producers:
Tecnaro | Bio.k

Raw material

Plastics are for the most part, petroleum based. But there are now procedures that use the renewable raw material **wood** as a raw material source. A large proportion is made up from lignin. Lignin is a waste product during paper production and is usually burnt afterwards. But the German company Tecnaro uses it as a key component for biobased plastics, which can be used to produce a wide range of household products.

Procedure

Tecnaro produces a **bio-composite** material from a mix of biopolymers derived from renewable raw materials. The result is a granular material, which like plastic, can be processed in injection moulding machines, extruders or presses in many different ways. Furthermore, the products are completely biodegradable and compostable. They can, for example, be used for the production of toilet brushes.

Contribution to the bioeconomy

10

Dishes

Sources: oly5/fotolia.com (left), Andrea Izzotti/fotolia.com (right)

Sector:
Consumer goods

Producers:
Magu | Capventure

Raw material

Fast-growing plants such as bamboo are easily cultivated and are therefore increasingly used by tableware manufacturers as a renewable resource. Companies like German Magu or Dutch company Capventure, offer, for example, bamboo tableware, consisting of up to 60% shredded **bamboo fibres**. The plants come from plantations which are regularly cut and replanted.

Procedure

So that colourful cups, plates and bowls can be made from renewable raw materials, the bamboo fibres are first ground and mixed with dyes and other raw materials, such as corn. For durability, a synthetic resin is often added to the bamboo, which makes the products food safe, odour and taste neutral, durable, dishwasher safe and can be cleaned hygienically. Some companies use natural **resin** as a binding agent.

Contribution to the bioeconomy

24

Trainers

Sources: rutchapon/fotolia.com (left), Puma (right)

Sector:
Consumer goods

Producer:
Puma

Raw material

The waste which accumulates during food is usually thrown away. This is also true for **rice husks**. German sportswear manufacturer Puma uses this waste material for its eco-friendly trainers "Re-suede". The rice husks replace a portion of the rubber content used for the outsoles. Therefore less petroleum-based rubber is used. This reduces energy consumption and increases the environmental balance.

Procedure

The remake of Puma's classic trainer "Suede" was designed as an eco-product based mainly on **recycling**. Compared to conventional products, it reduces CO_2 emissions by 80%. But it's not just the outsole that's made from waste materials. The synthetic Ultrasuede upper material is also comprised of recycled polyester fibres. And what's more – the shoe comes in sustainable packaging – Puma's "Clever Little Bag".

Contribution to the bioeconomy

16

Bioethanol

Sources: Clariant/Rötzer (left), Kathryn Cross/TGAC

Sector:
Chemical industry

Producer:
Clariant

Raw material

Biofuels such as bioethanol are derived from renewable raw materials. Until now, sugars from arable crops have been used. To avoid competition with food production, residual materials such as **straw** have come to the attention of several biofuel manufacturers. This is because straw or wood is largely composed of lignocellulose fibres, which has a high potential for energy conversion.

Procedure

The Swiss chemical company Clariant has established a biorefinery demonstration plant, in which wheat straw bioethanol is produced. With the help of enzymes, the lignocellulose is decomposed and recovered from the plant fibre into its individual components. The resulting sugar molecules serve as food for **yeast** and the fungi ferment them into alcohol. This can then be added to premium petrol for petrol engines.

Contribution to the bioeconomy

6

EuropaBioAgnes Borg氏Twitterより

Global Bioeconomy Summit 2018@Berlin

source: Biobased industry Consortium

Enzyme (from Wikipedia)

Enzymes are macromolecular biological catalysts. Enzymes accelerate chemical reactions. The molecules upon which enzymes may act are called substrates and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life...

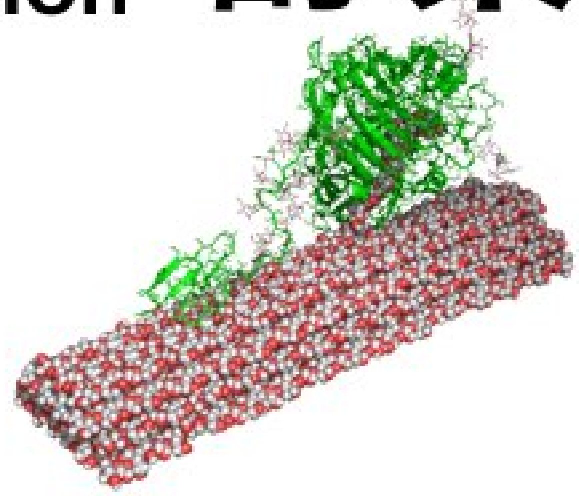
Enzymes are known to catalyze more than 5,000 biochemical reaction types. Most enzymes are proteins...

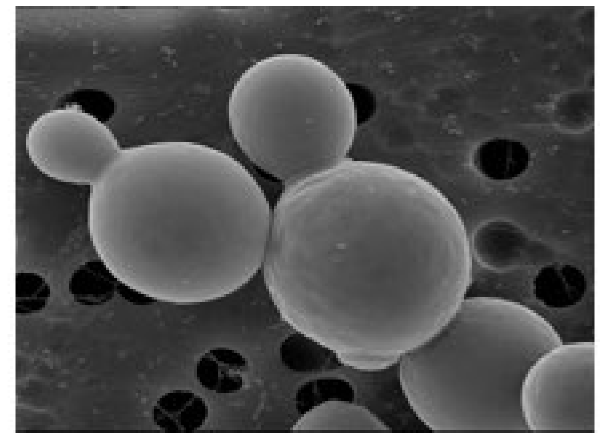
Enzymes' specificity comes from their unique three-dimensional structures.

kouso　　koubo

Essence of fermentation **酵素≠酵母** Mother of fermentation

10nm





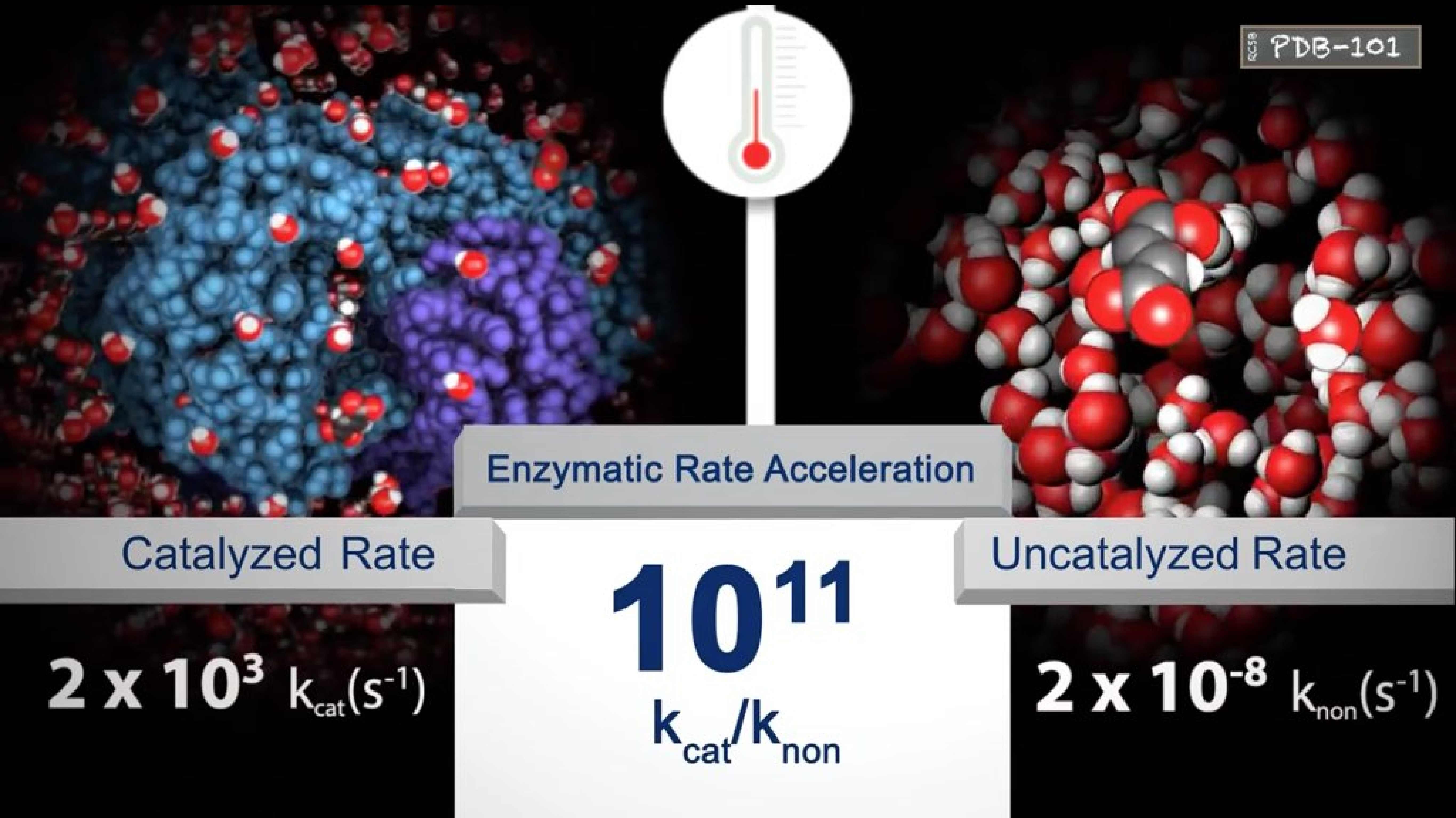
3μm

In 1877, German physiologist Wilhelm Kühne (1837–1900) first used the term *enzyme*, which comes from Greek ἔνζυμον, "leavened" or "in yeast", to describe this process.
The word *enzyme* was used later to refer to nonliving substances such as pepsin, and the word *ferment* was used to refer to chemical activity produced by living organisms.

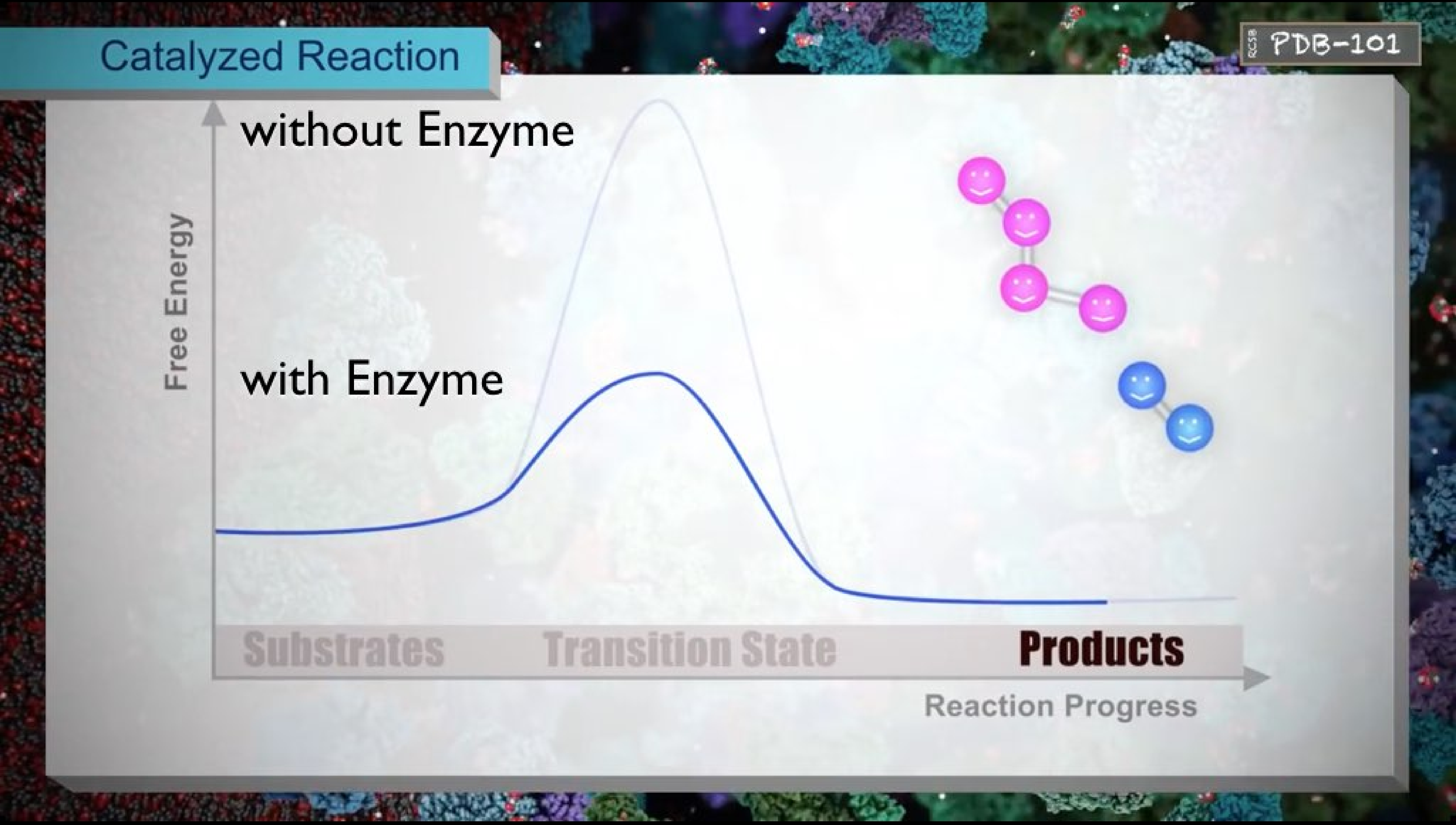
Wikipedia continued

ENZYMES

extremely higher velocity

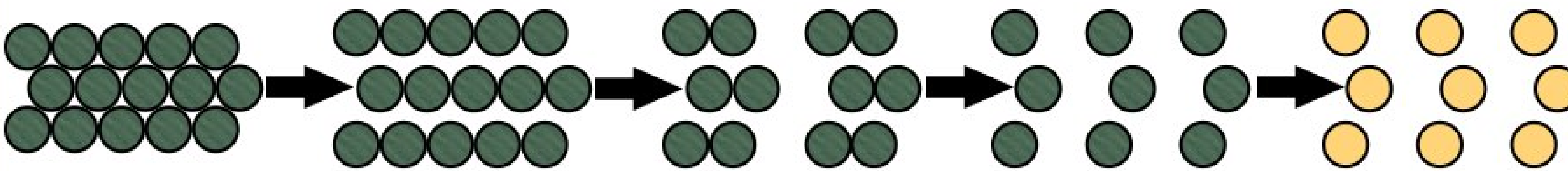


with less energy

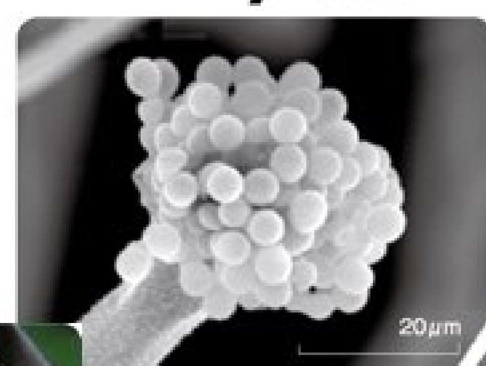


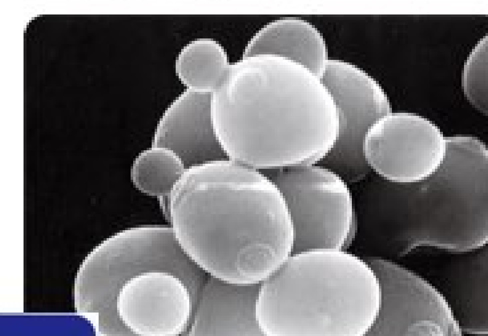
Process of Japanese Sake brewery

Pretreatment Saccharification Fermentation



Mill & Heat **Amylase** **α-glucosidase** **Yeast**



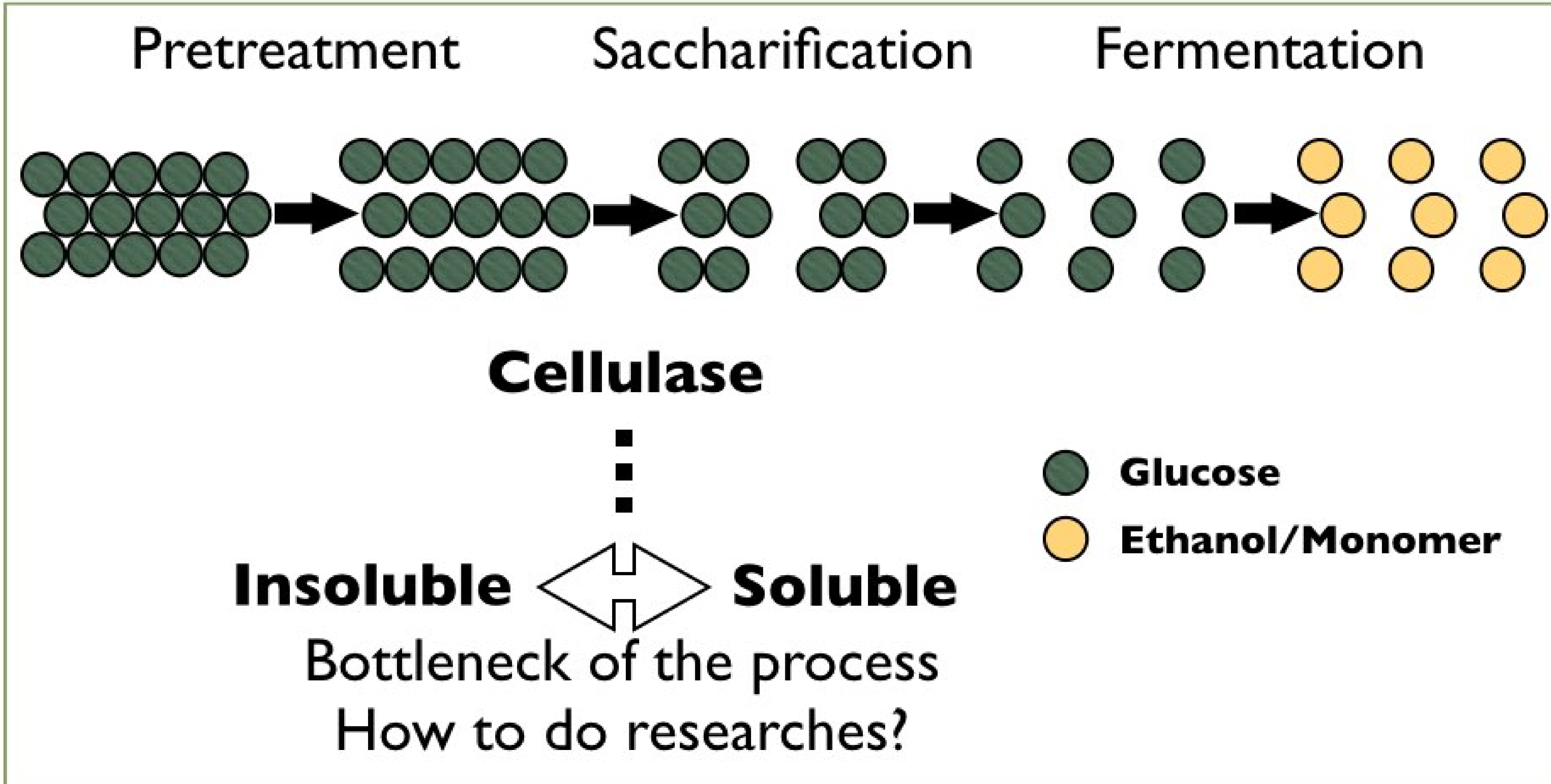


Glucose

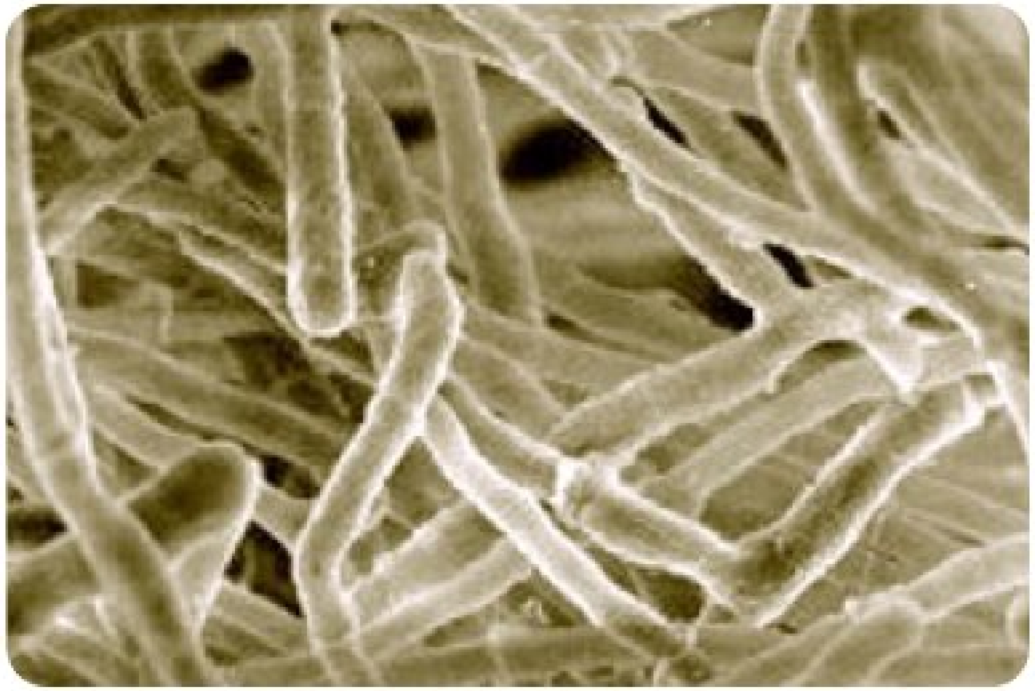
Ethanol

from National Research Institute of Brewing

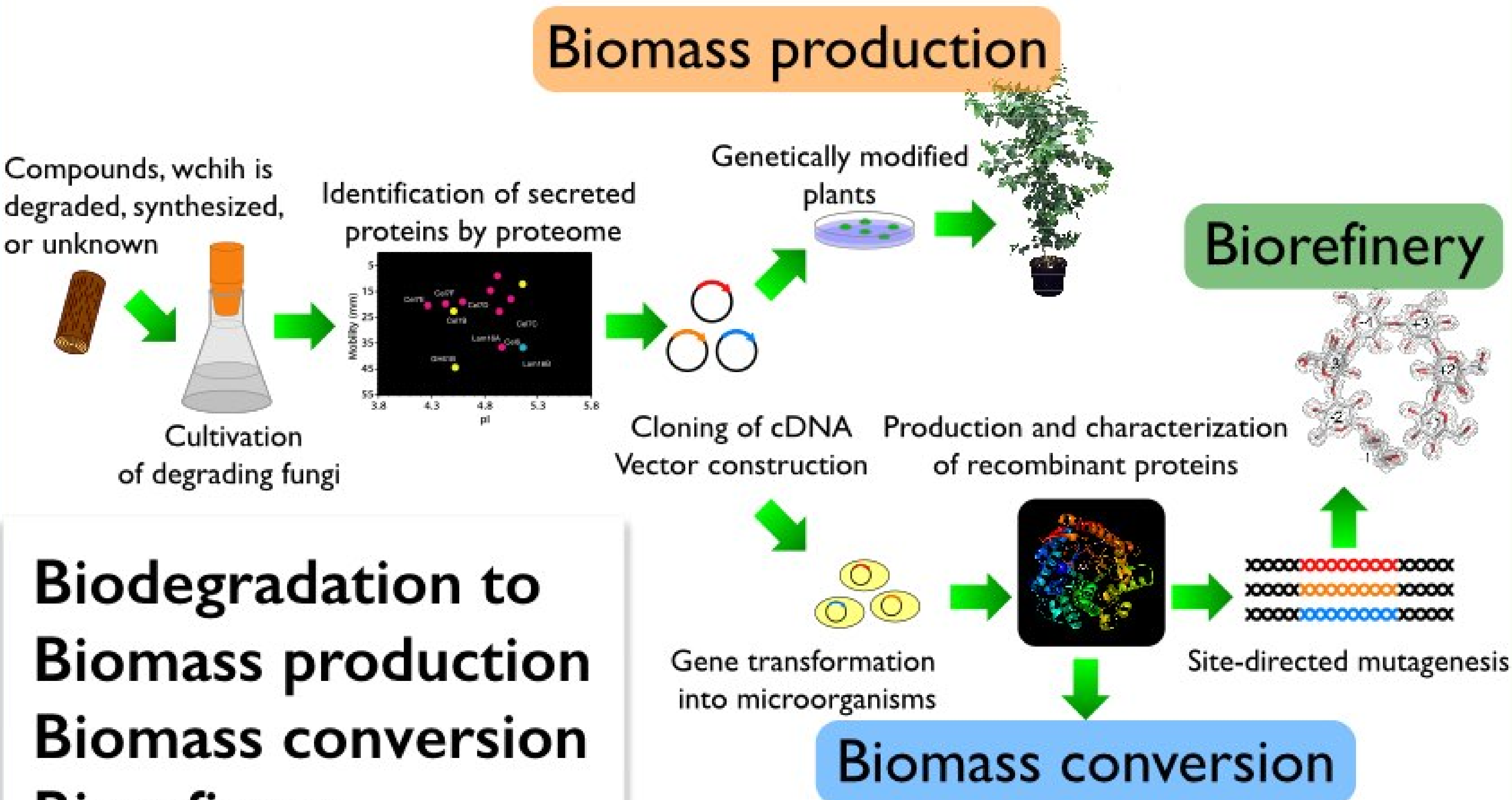
What is the difference between starch and cellulose?



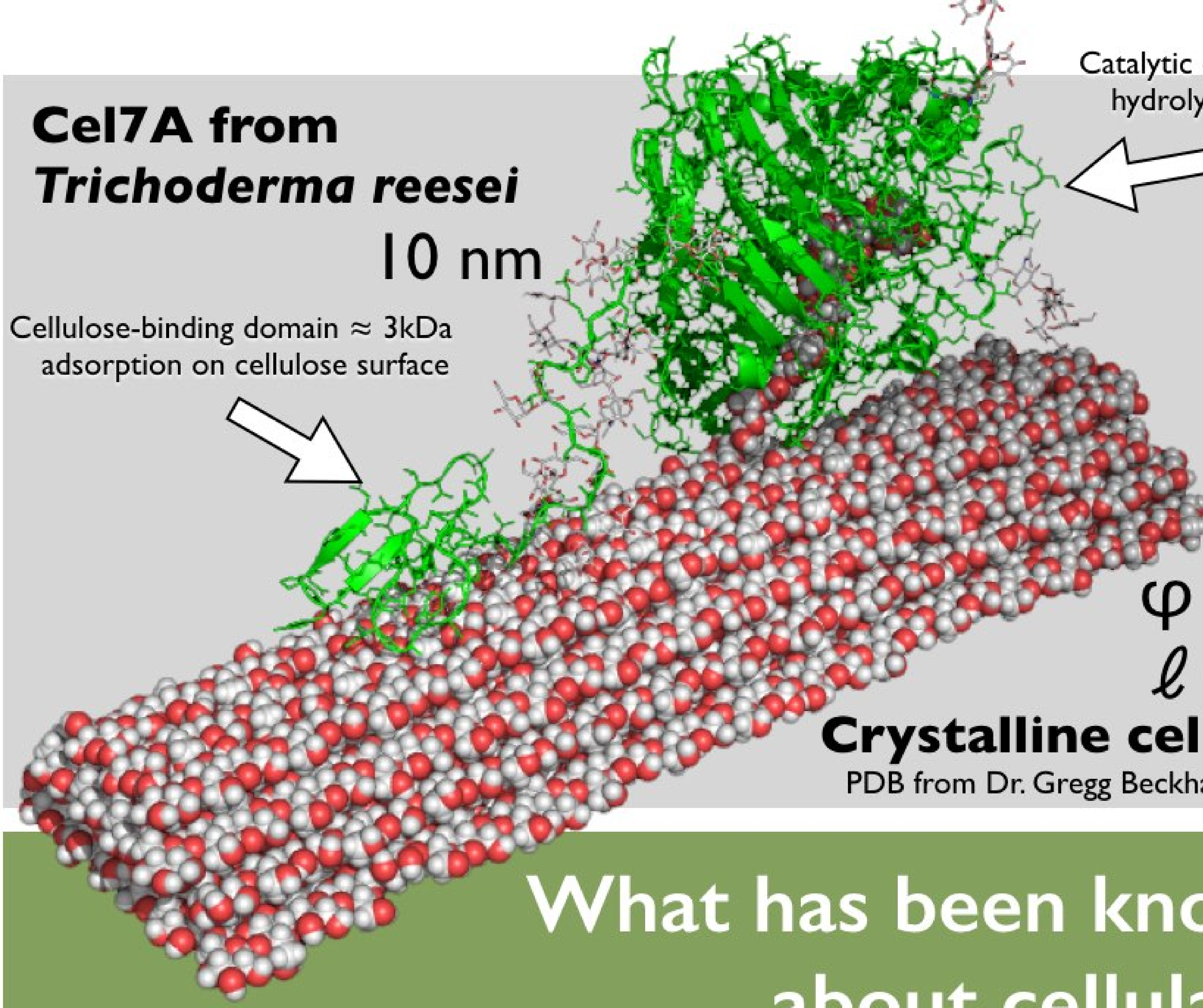
Wood-rotting fungi consist with "mushroom" and "mold"

Basidiomycetes *Phanerochaete chrysosporium*	Ascomycetes *Trichoderma reesei*
	
White-rot Brown-rot	Soft-rot

We can convert biomass if fungi can "eat" it

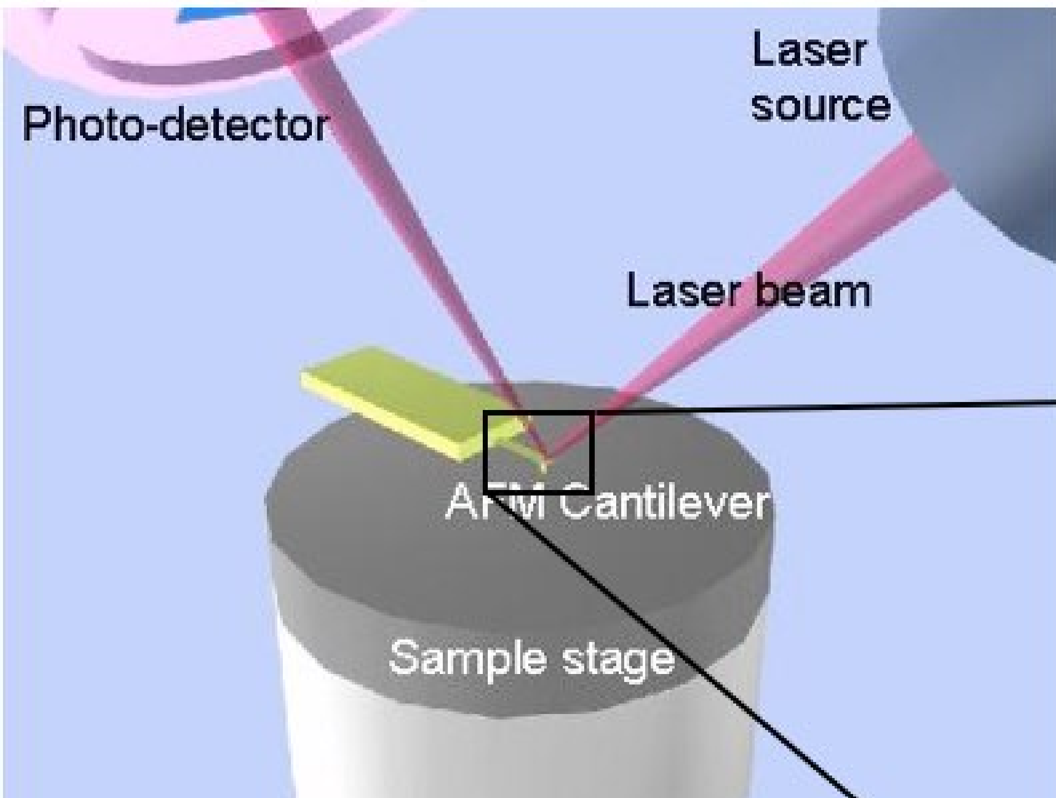


Biodegradation to
Biomass production
Biomass conversion
Biorefinery

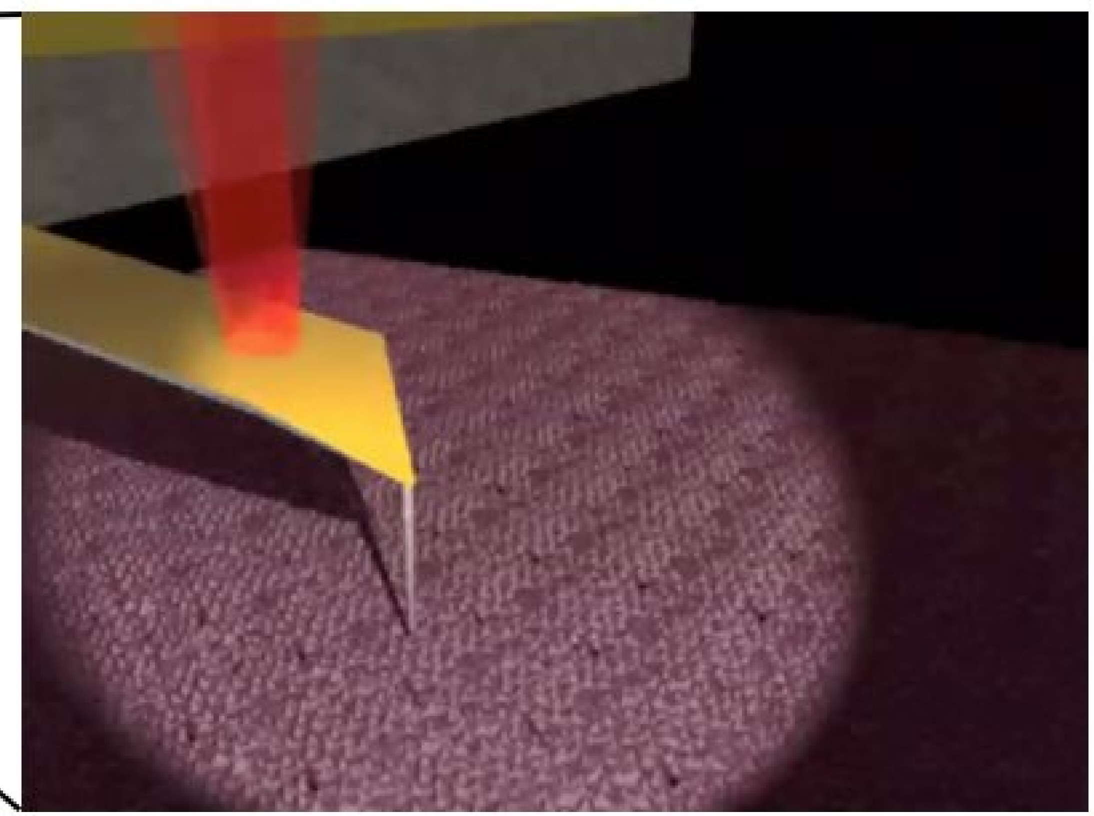


What has been known about cellulase?

Atomic force microscopy (AFM) to observe molecules



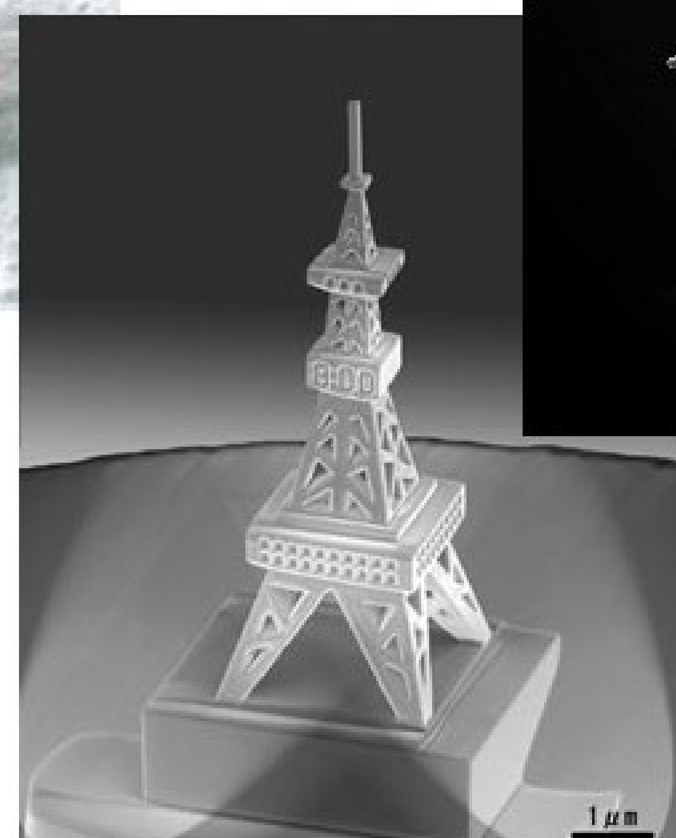
Scanning the surface of objects by the small needle (tip) on cantilever



Advantages

- space resolution less than nm
- not affected by the feature of sample
- do not choose the condition (liquid, air etc.)

milli=>micro=>nano technology
Japanese likes small (tiny?) things

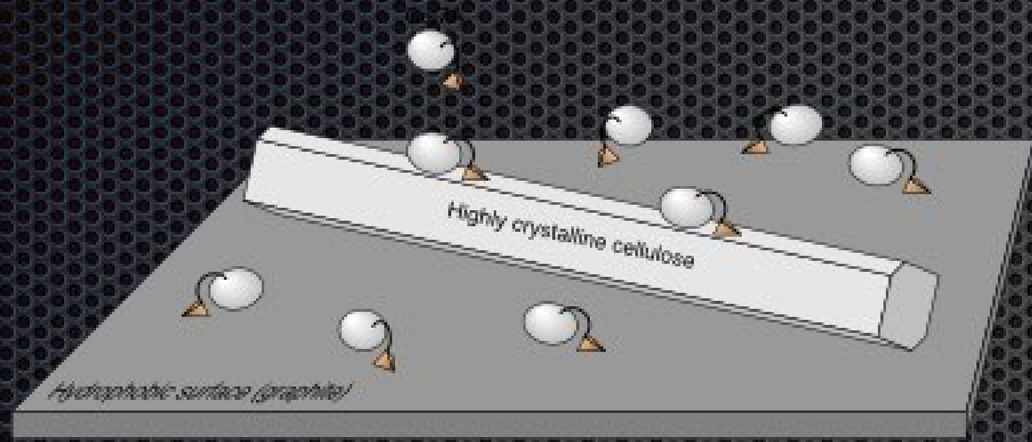


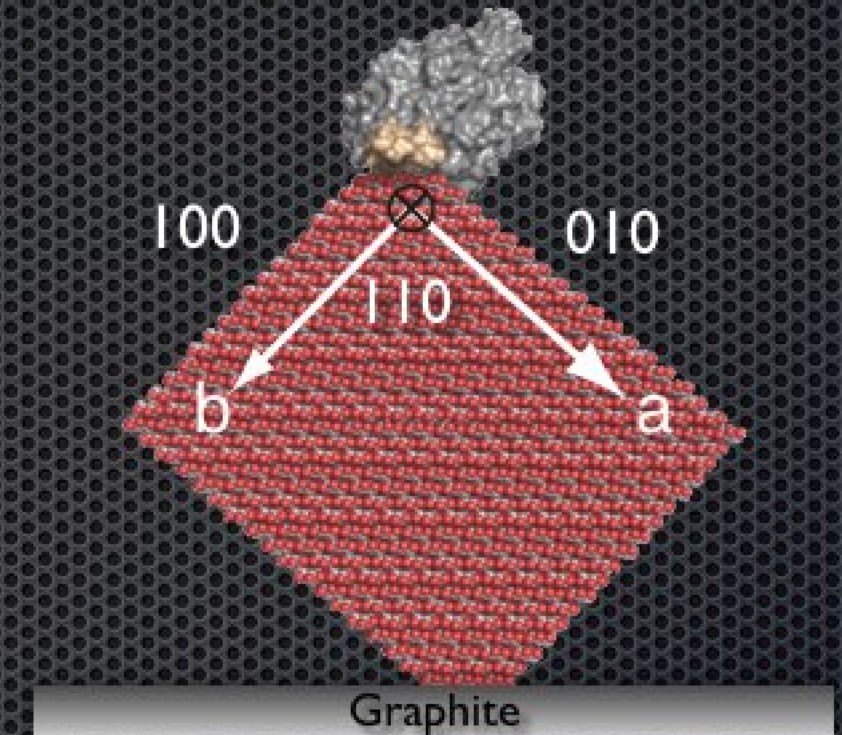
Hitachi High-Technologies nanoart

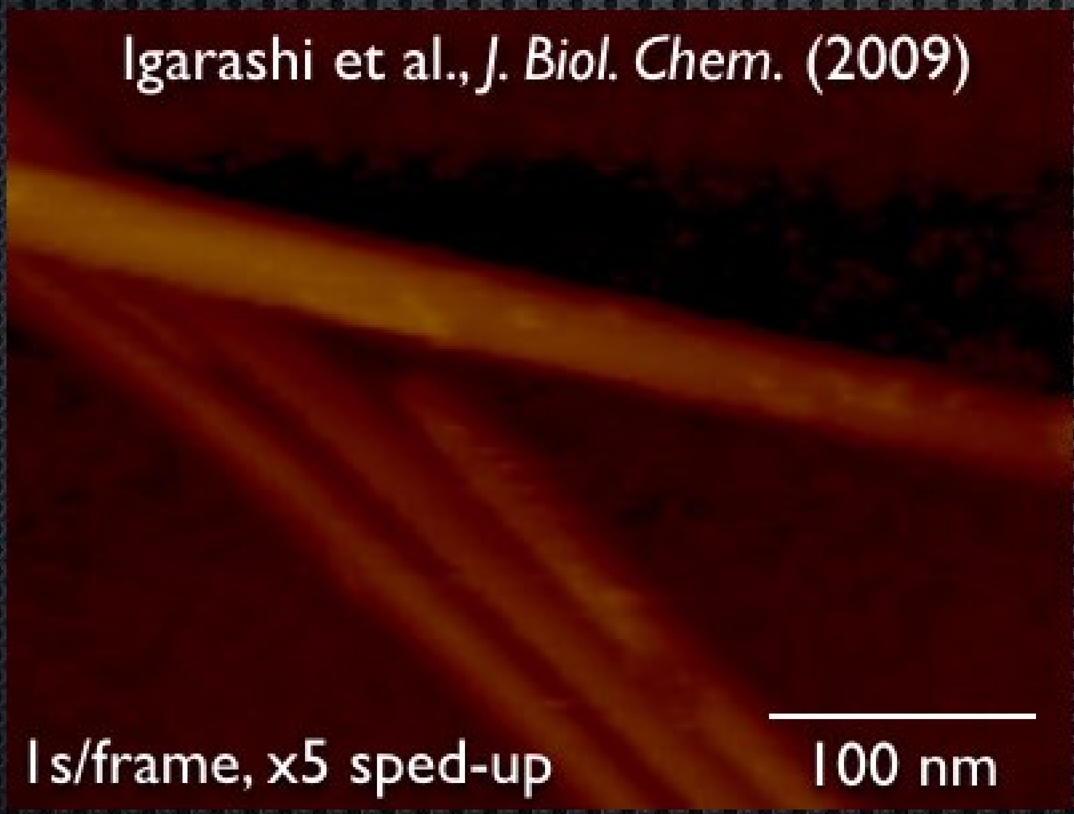
mm μm nm!

Brilliant high-speed AFM apparatus in Kanazawa University

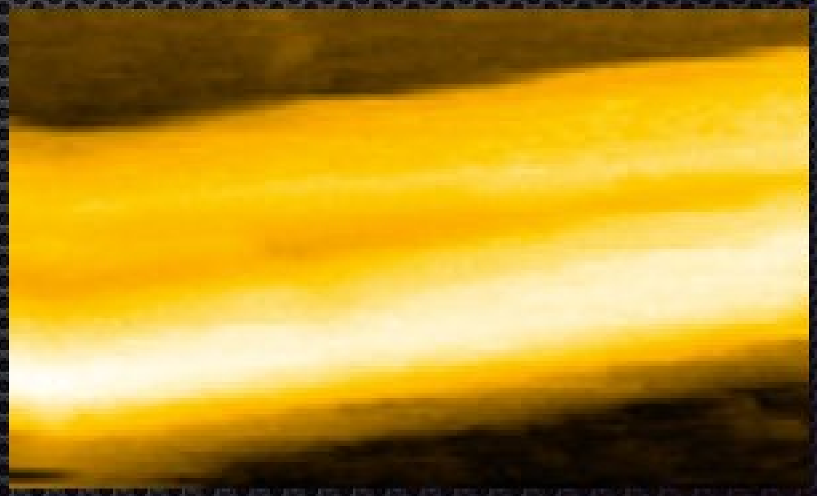
Enhanced time/space resolution of HS-AFM shows detailed movement of *Trichoderma* Cel7A







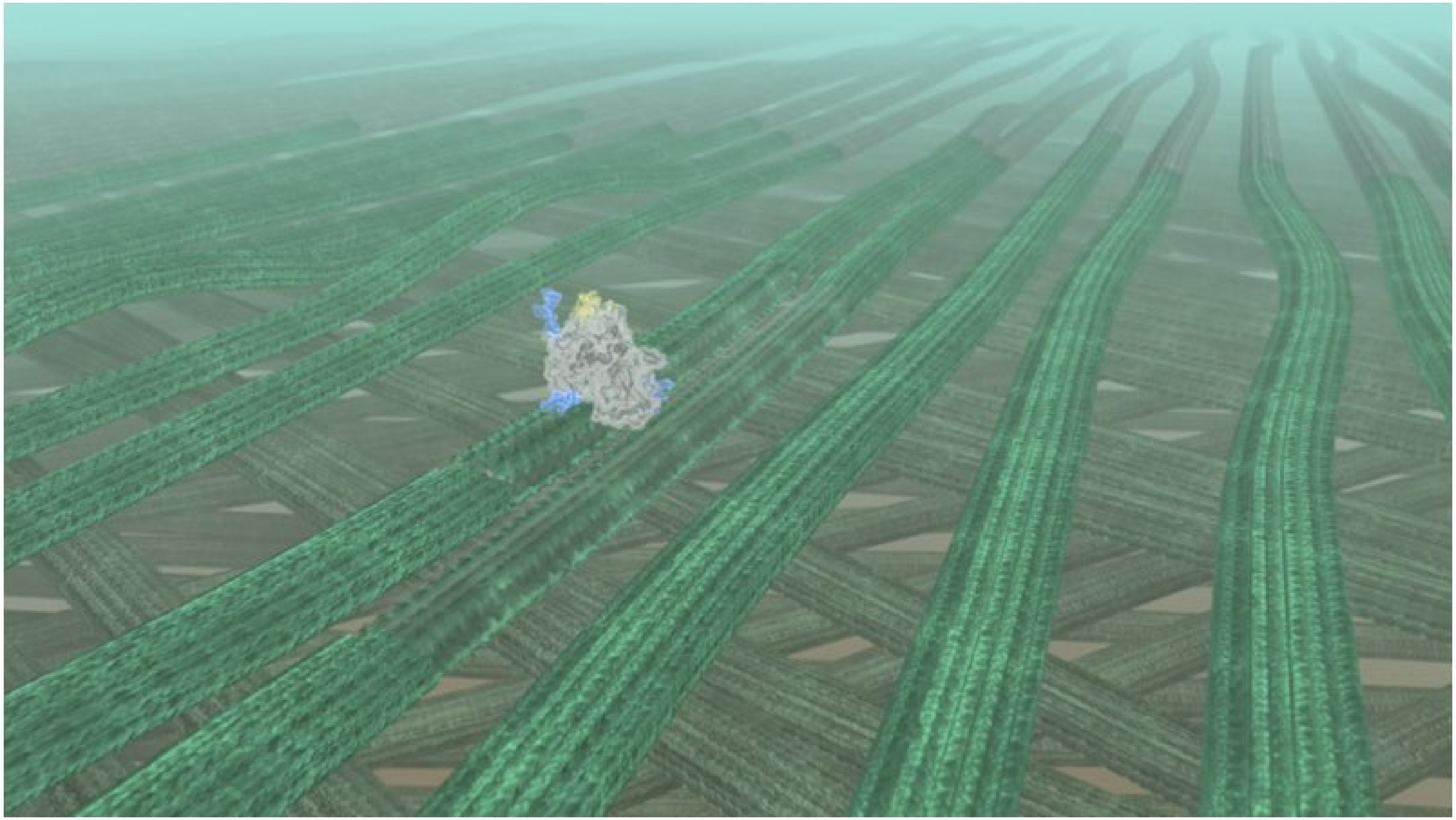
Igarashi, Uchihashi et al., *Science* (2011)



0.3s/frame
x10 sped-up

50 nm

Reaction of cellobiohydrolase from *Trichoderma reesei* "Processivity"



High Quality Protein Crystallization Project

このプロジェクトは、地上における結晶化条件検討の効率化をはかるとともに、結晶生成の場を宇宙に移すことで、より大型で高品質な結晶を作製し、タンパク質の構造研究をステップアップさせることを目的としています。

高品質タンパク質結晶を宇宙でつくる

ISS実験棟「きぼう」

プログレス補給船

打ち上げ

バイコヌール宇宙基地

ズ宇宙船

回収

タンパク質の調整 → 結晶化条件の検討

今後の結晶研究

従来の結晶研究

地上で結晶生成

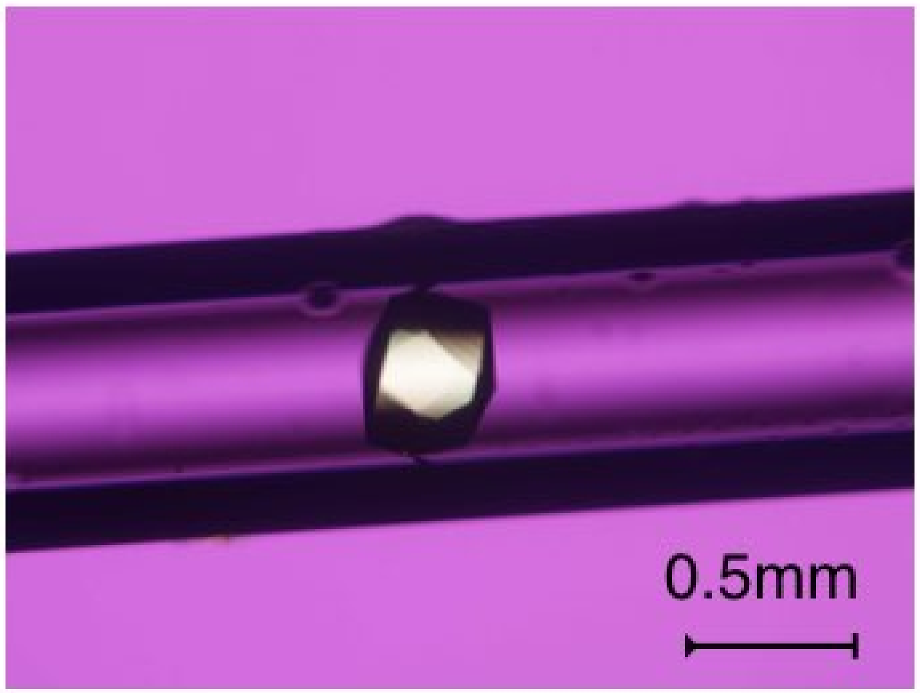
重力影響下
結晶は小型で均一になりにくい

結晶の解析

X線結晶構造解析

中性子結晶構造解析

Crystallization of *Pc*Cel45A at microgravity



沈殿材：60% MPD

結晶化法：PCG

結晶化温度：20 ˚C

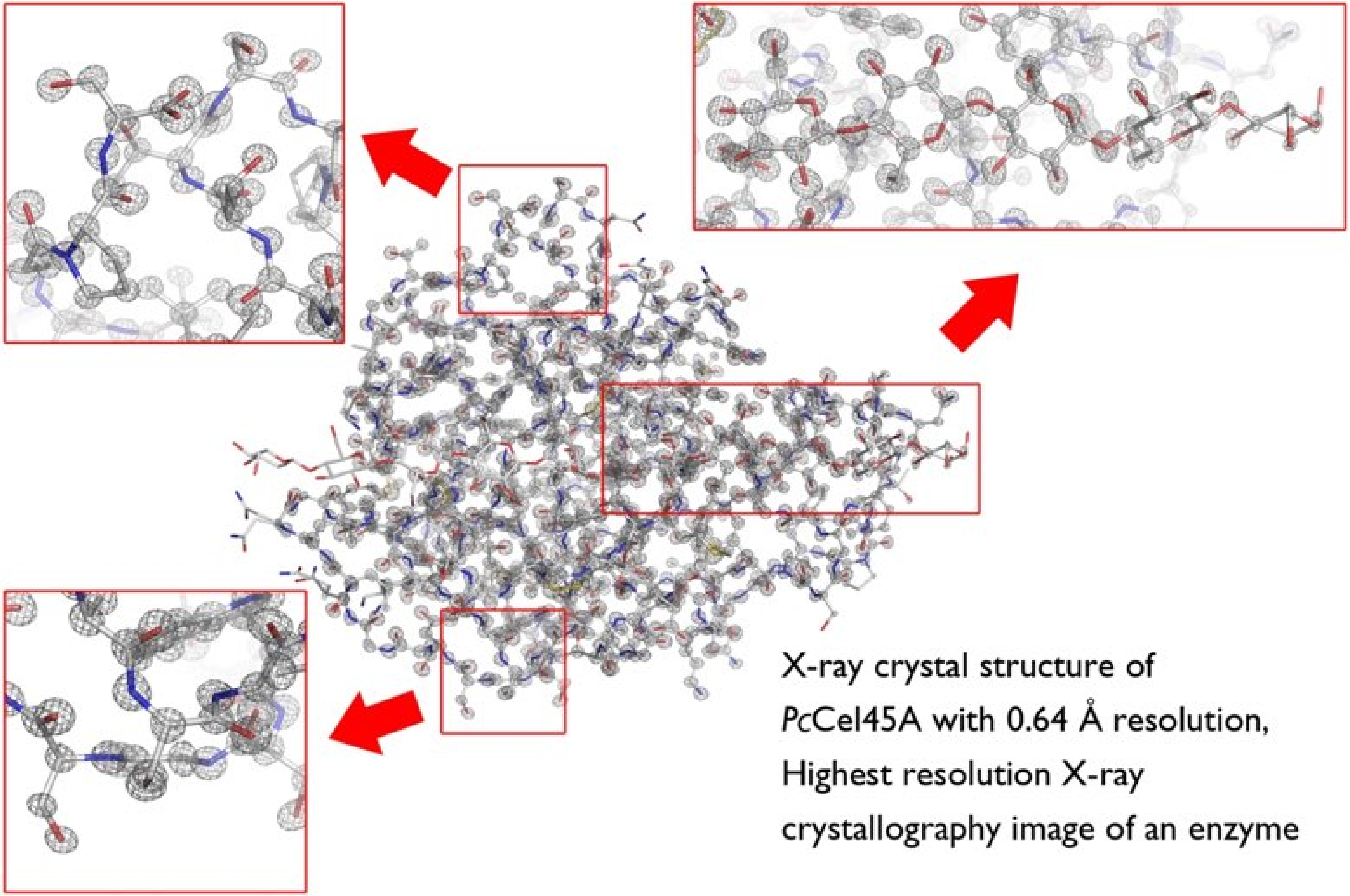
結晶化化期間：２週間+α

再現性：◎

（地上ではVD法CD法共に再現性高）

Space Group	$P2_12_12_1$
Cell dimensions	a=45.5, b=58.1, c=62.9 (Å)
Beam Line	SPring-8 BL44XU
Wavelength	0.720
Resolution Range (Å)	40 - 0.83
Total Number of Reflections	766,867
Number of Unique Reflections	156,957
Linear R-factor in shell <40.00 - 2.84>	0.064
Completeness (%)	98.3
Completeness in shell <0.84 - 0.83>	94.7
Mean I/sigma	47.8
Mean I/sigma in shell <0.84 - 0.83>	4.2
Total Linear R-Merge	0.071
Linear R-factor in shell <0.84 - 0.83>	0.260
Mosaicity Range	0.16 - 0.29

X-ray crystal structure of *Pc*Cel45A with super high resolution

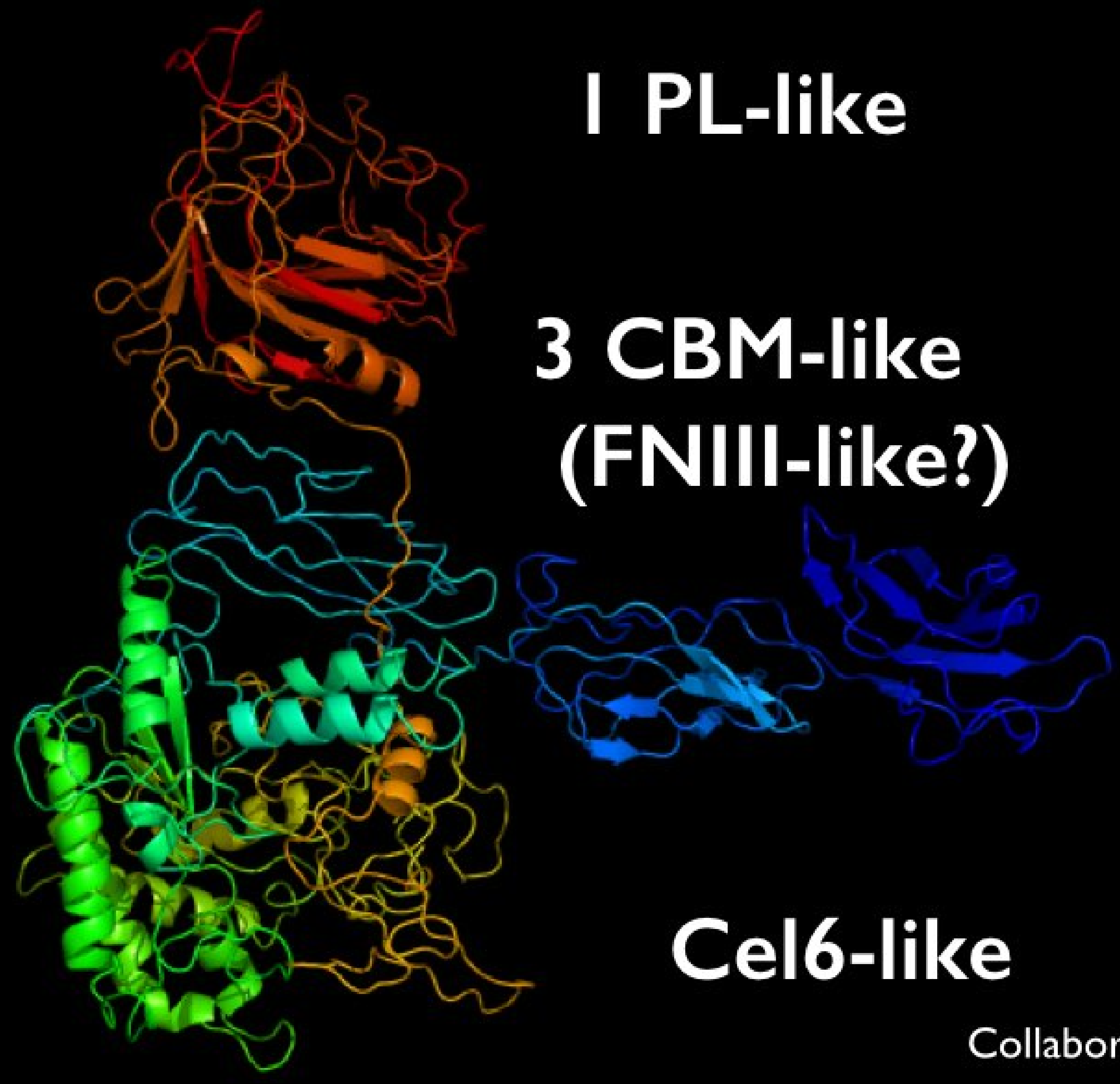


X-ray crystal structure of *Pc*Cel45A with 0.64 Å resolution, Highest resolution X-ray crystallography image of an enzyme

Giant Larvacean Houses: Rapid Carbon Transport to the Deep Sea Floor

Robinsson, *et al.*, *Science* (2005)

Unusual multi-domain cellulase from deep sea bacteria



Collaboration with Dr. Deguchi@JAMSTEC

in single molecular study

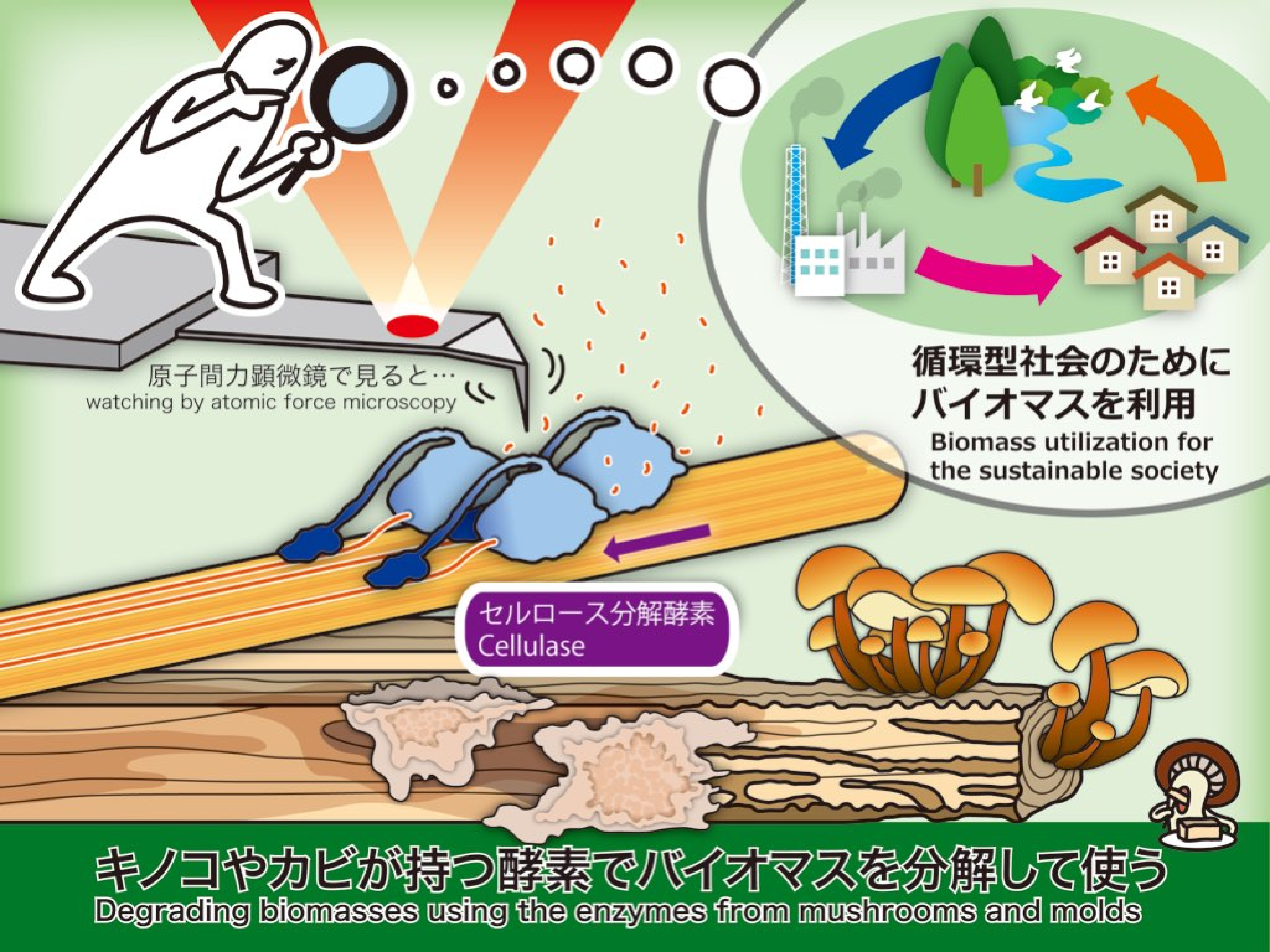
$\approx$ **10^3**

in biochemical experiments

1 μM x 1 mL x 6×10^{23} $\approx$ **10^{15}**

in bioethanol plant

5 kg cellulase x 6×10^{23} $\approx$ **10^{23}**


原子間力顕微鏡で見ると…
watching by atomic force microscopy
循環型社会のために
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セルロース分解酵素
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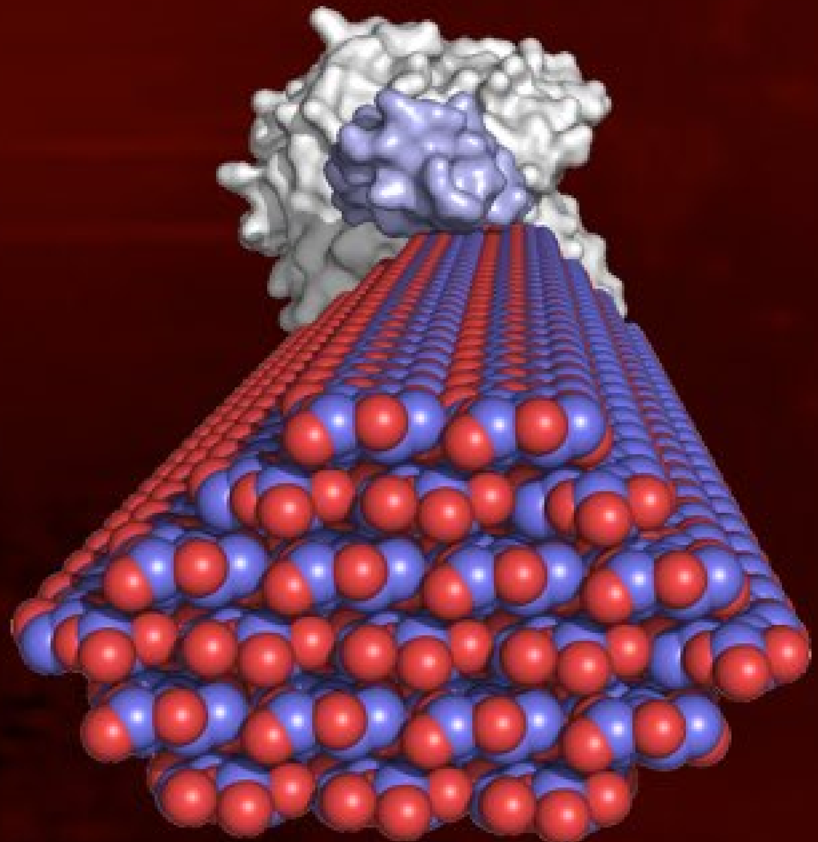

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Mr. Shingo Fukuda

University of Tokyo
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Prof. Masahisa Wada
Prof. Ryota Iino
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Dr. Takuya Ishida
Dr. Akihiko Nakamura
Mr. Yusuke Shibafuji

National Food Reserach Institute
Dr. Satoshi Kaneko

VTT Biotechnology
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Dr. Anu Koivula

Niigata University
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Prof. Kazushi Suzuki
Dr. Hayuki Sugimoto

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Prof. Hiroyuki Ohno
Dr. Hirotoshi Matsumura
Dr. Kouta Takeda

Taisei Corporation
Dr. Kouki Yoshida

科研費
KAKENHI

革新的研究開発推進プログラム
ImPACT
Impulsing Paradigm Change through Disruptive Technologies Program

x20 sped-up

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Ibaraki biological crystal diffractometer (iBIX) BL03
Spring-8 BL44XU (Prof. Atsushi Nakagawa in Osaka Univ.)
Photon Factory BL5A, BL17A, NW12A, NE3A

謝辞

THE PLANT CELL WALL
THE PLANT CELL WALL AS INFORMATION-PROCESSING SYSTEM

科学研究費補助金 新学術領域研究

植物細胞壁の情報処理システム「 植物細胞壁成分の合成酵素および分解酵素を用いた細胞外情報処理空間の動的可視化」

文部科学省 MINISTRY OF EDUCATION, CULTURE, SPORTS, SCIENCE AND TECHNOLOGY-JAPAN

高品質タンパク質結晶化プロジェクト
High Quality Protein Crystallization Project

地球観測技術等調査研究委託事業

高品質蛋白質結晶化技術の宇宙科学研究拠点形成

JAXA

JAXAオープンラボ公募

「水」から考えるエコフレンドリーな洗剤用酵素の開発

科研費 KAKENHI

科学研究費補助金 基盤研究（B）

未利用バイオマスの完全酵素糖化を目指したβ-グリカナーゼ新規アッセイ法の開発

茨城県

iBIX プロジェクト課題

バイオマス高度利用に向けた立体反転型糖質加水分解酵素の活性に関与する水分子の機能解析

Elucidation of the strategies of mechanical optimization in plants toward the establishment of the bases for sustainable structure system

Prof. Taku Demura in NAIST & 11 PIs
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