APAC DAE 2017

Continued Efforts to Discover New Drugs using Natural Resources

Charm of natural products and development of next-generation natural product chemistry

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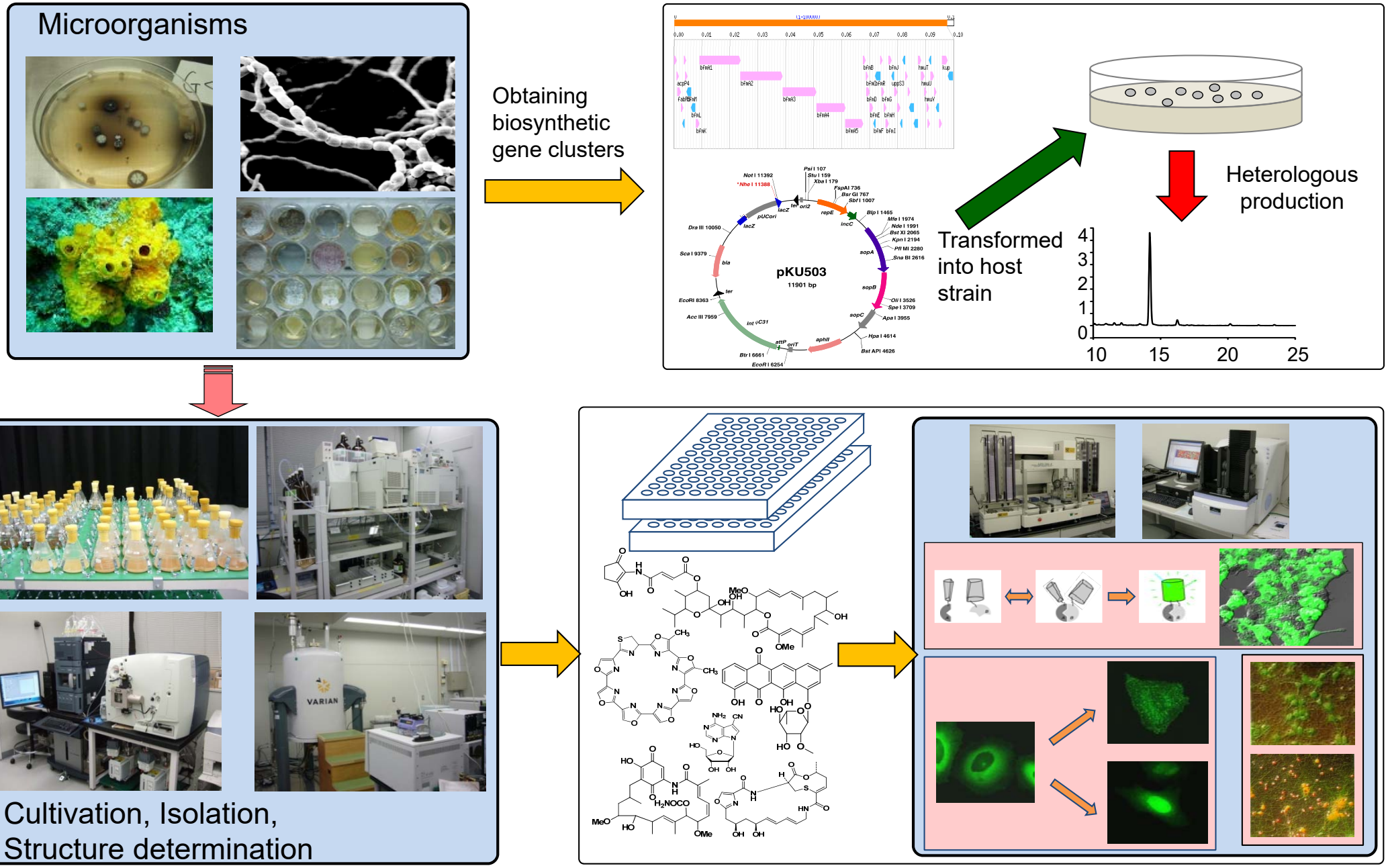
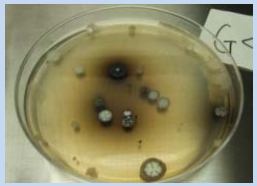
Purpose of Technology Research Association for Next generation natural products chemistry

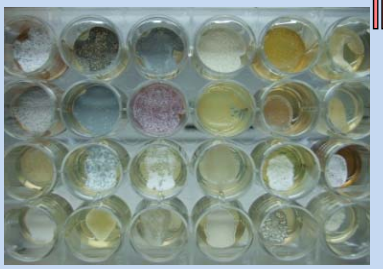
- Performing drug screenings with World-largest Natural Library (over 270,000)
- Deveropping Next-generation heterologous expression system



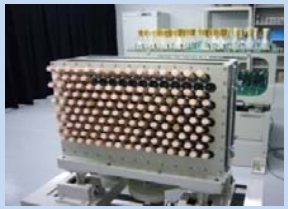
The 1st Topic: Drug discovery from natural product library

Sample collection



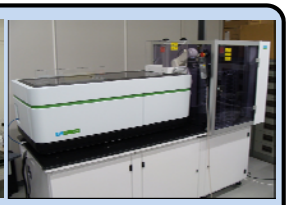
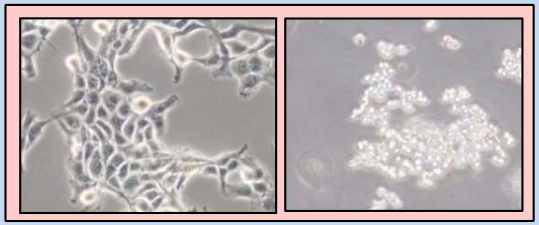
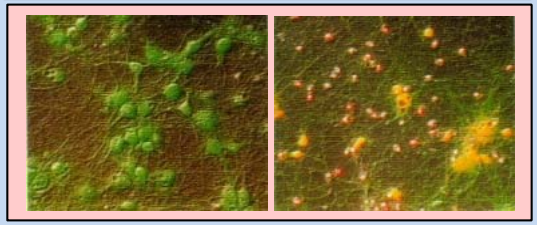
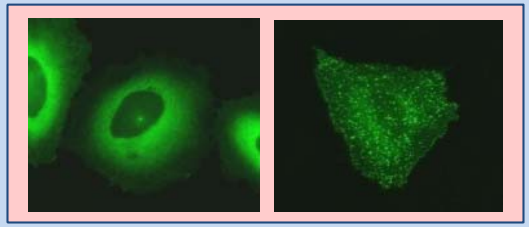



Library preparation




In-house and companies' library

Screening




Anti-tumor

Neural protection

Signal transition

Purification


Structure determination


Large scale culture

Sample supply for animal models

Combination of large natural library and high-throughput random screening system

World-largest natural library

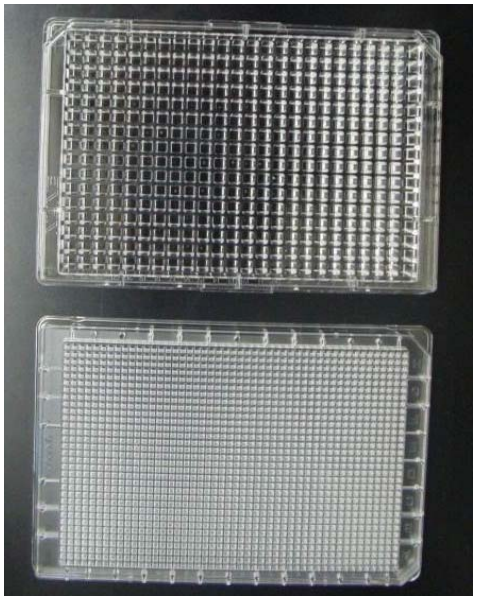
×

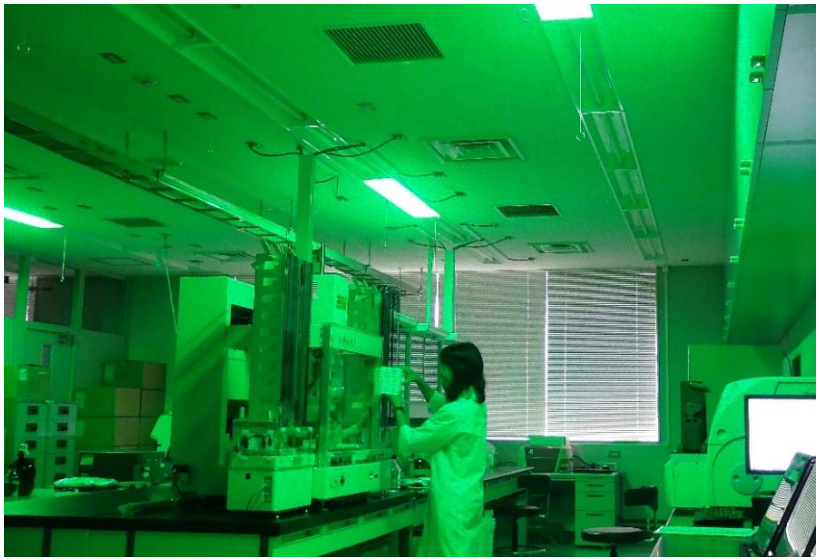
Total: ~ 270,000 sample

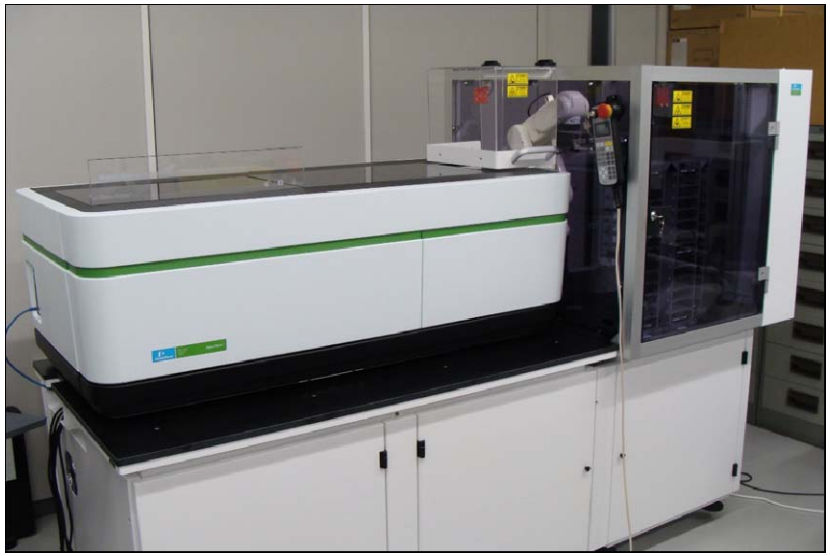
High-throughput screening system

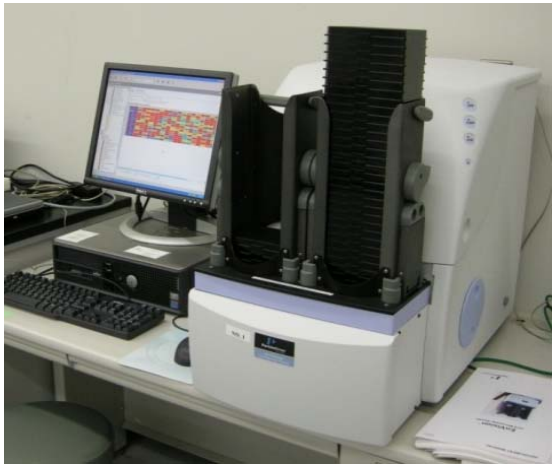
	Throughput
In vitro assay	> 100,000 assay / week
Cell based assay	~ 100,000 assay / week

High-performance natural product screening





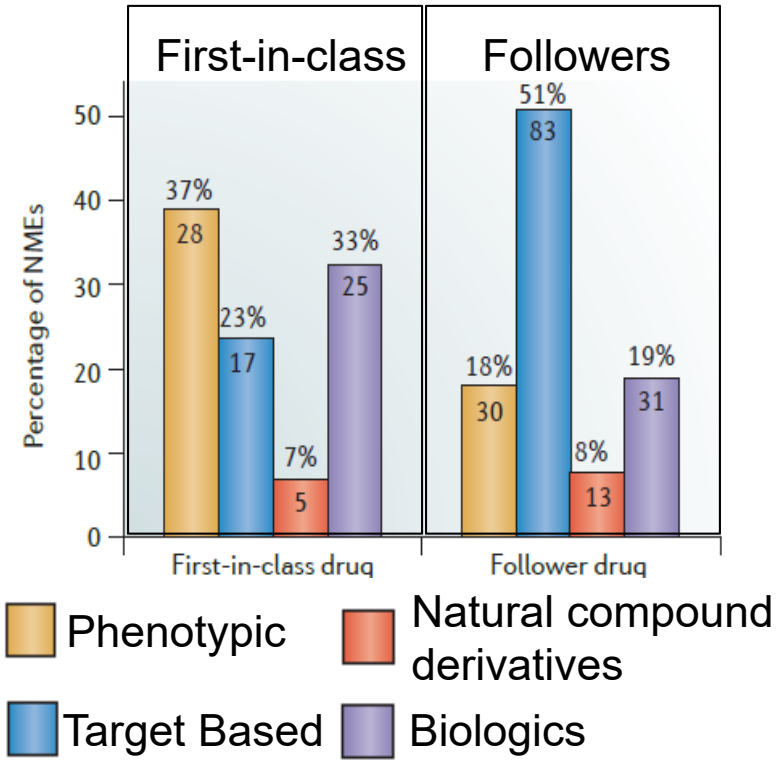


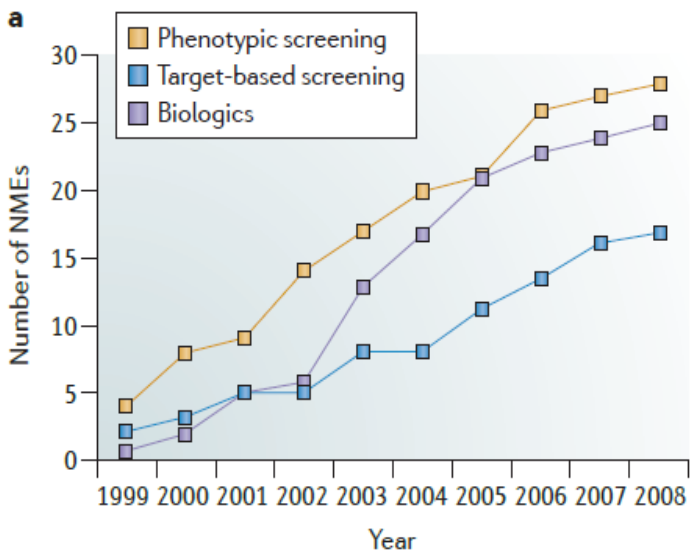


Trend of World: Return to Phenotypic screenings in Western countries

First in class drugs were discovered by phenotypic screenings

From 1998 to 2008, within approved clinical drugs, 37% of first-in-class drugs were discovered by phenotypic screenings (PS), while 23% ones were discovered by target based (TB) screenings.
To the contrary, 51% of followers were discovered by target based screenings.
From 2009～2015: 80 approved drugs, 21 were PS, 18 were TB, 21 were biologics





David C. Swinney & Jason Anthony,
How were new medicines discovered?
Nature Reviews Drug Discovery. **10**, 507-519 (2011)

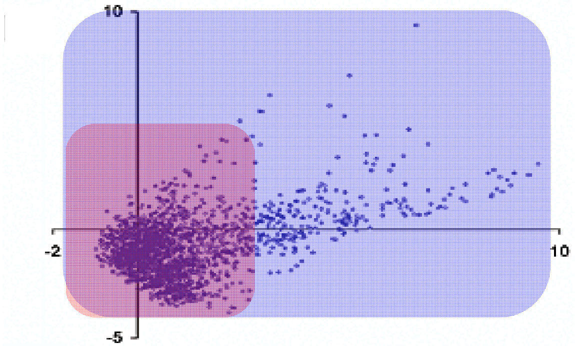
This paper encouraged mega-pharma to develop phenotypic screenings with modern technologies such as disease iPS and clinical isolated samples

Character of microbial secondary metabolites in clinical use

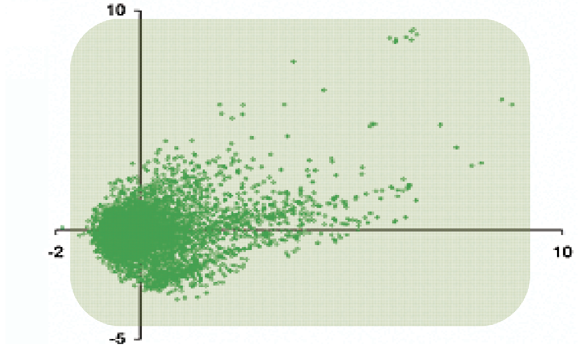
Natural products have large chemical spaces

Principal Component Analysis (PCA)

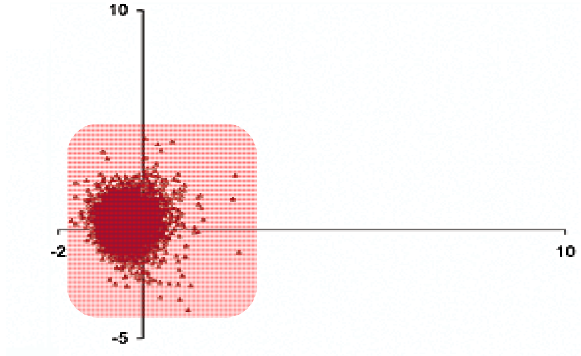
Natural products



Clinical drugs



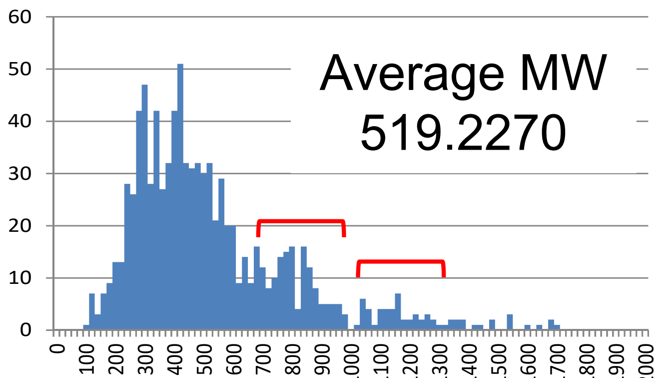
Synthetic compounds



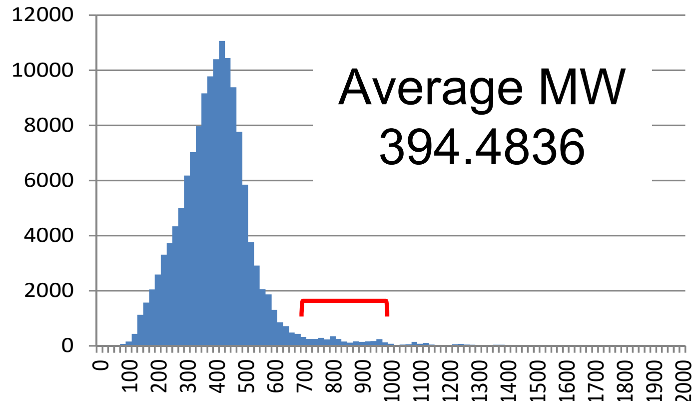
More than 60% of clinical drugs are natural product origin

J. Chem. Inf. Comput. Sci., **43**, 218 (2003)

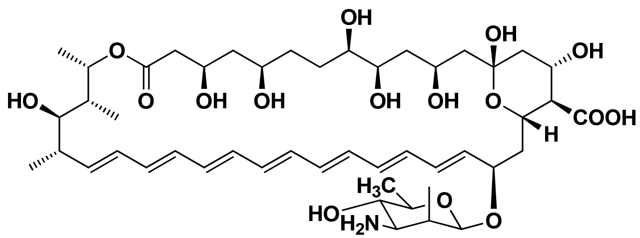
Natural products have large molecular weights (especially clinical drugs)



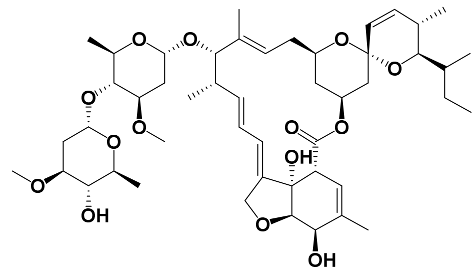
Isolated natural compounds (917)



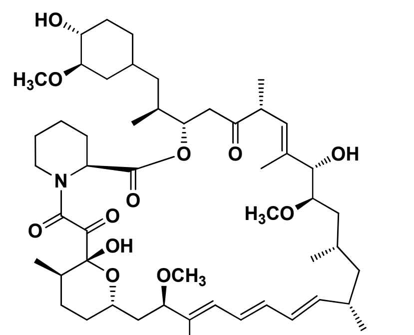
Commercially available library (10 companies,138050)



Amphotericin B (MW: 921)



Avermectin (MW: 860)



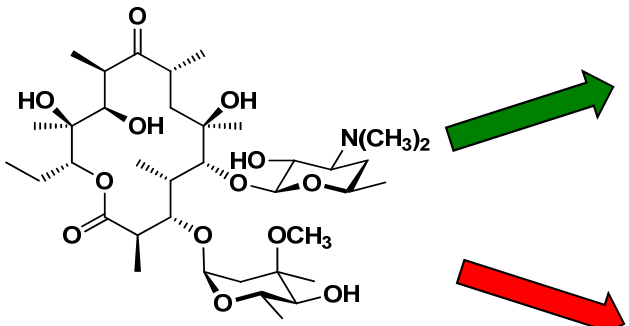
Rapamycin (MW: 899)

Middle molecular weight natural compounds are suitable for phenotypic and protein-protein interaction screenings

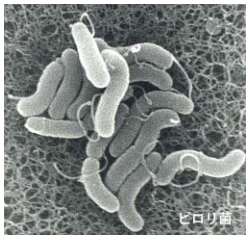
Natural compounds originally synthesized by protein so called “enzyme”, therefore show high affinity to proteins (catalytic domains resemble each other !)

Among natural compounds, circular compounds possess both rigidity and suitable flexibility to stabilize the binding between compounds and target proteins
(also reflect for activity expression and membrane permeability)

- Middle molecular weight natural compounds exhibit multiple activities against completely different targets
- Middle molecular weight natural compounds can cover total metabolic system, whole protein structures

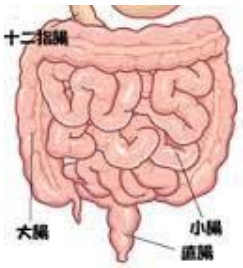


Erythromycin (MW 733)



Antibiotic activity through protein synthesis

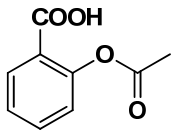
http://higashiosaka-naishikyo.com/treat/endoscope/pylori.html



Acts as non-peptide motilin agonists, which stimulates gastrointestinal motility.

⇨ Mimicking peptide structure acting on GPCR

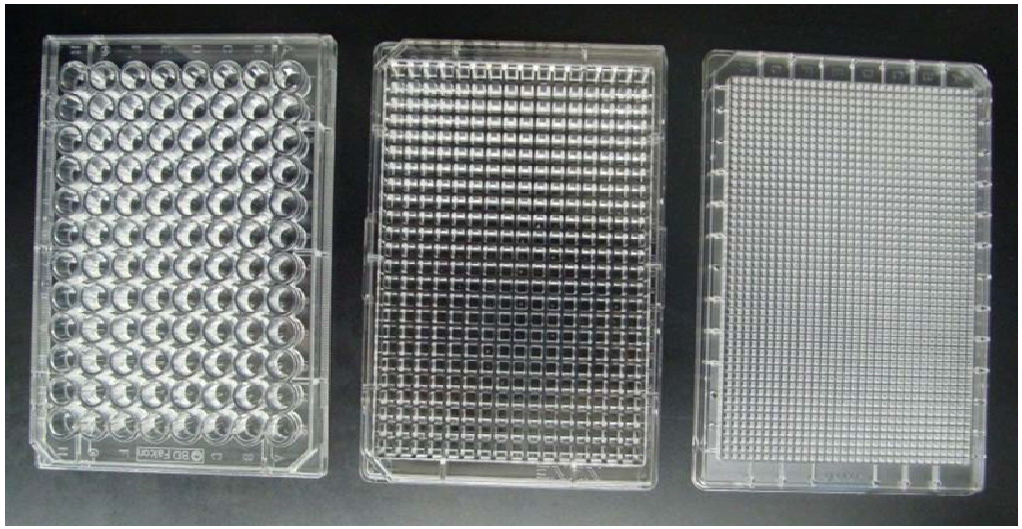
http://www.itawari.net/body/syoutyou.html



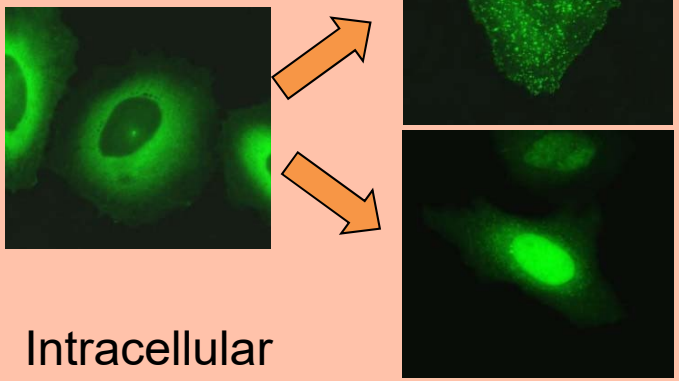
Example of low molecular compound：aspirin (MW 180)

Inhibitor of inflammation induction (antiinflammatory・Analgesic)

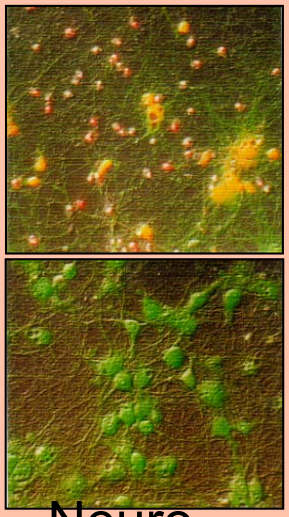
Phenotypic Screening (High Content Screening) by image analyzer



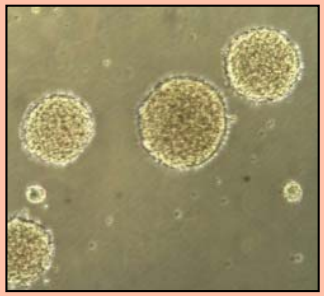
384-well / 8 min



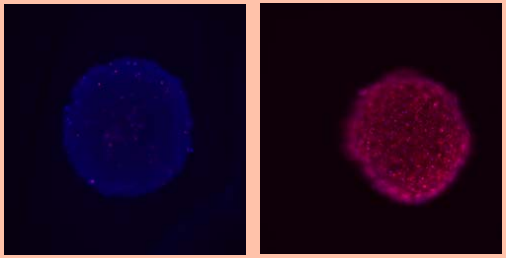
Intracellular
Protein Localization



Neuro-
protection



Sphere from
patients

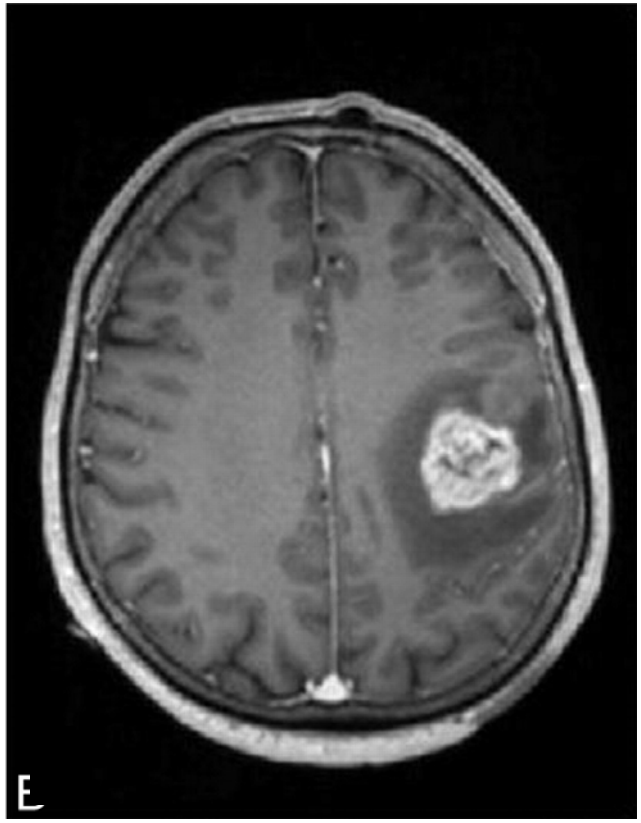


Anti-gliomer

Blue: Live Cell
Red: Dead cell

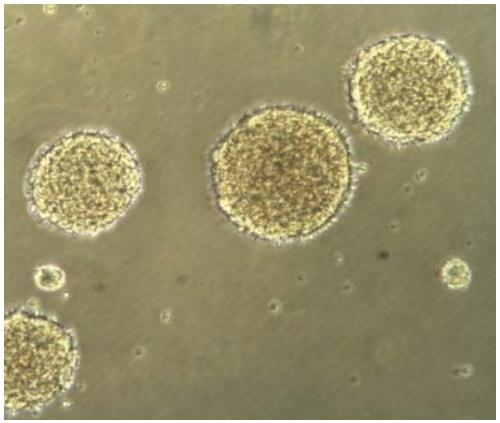
Glioblastoma multiforme (GBM)

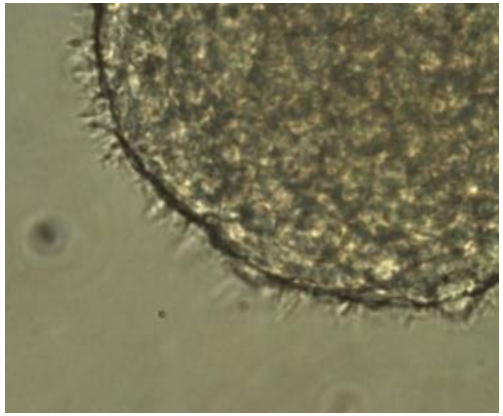
- Classified into Grade IV, which is the highest malignancy in glioma, and is bad prognosis after surgery
- Highly immature Mesenchymal type glioma cell grow fast and invasive
- No effective treatment including chemotherapy



MRI image of GBM

Am. J. Neuroradiol., **32**, 67-73 (2011)

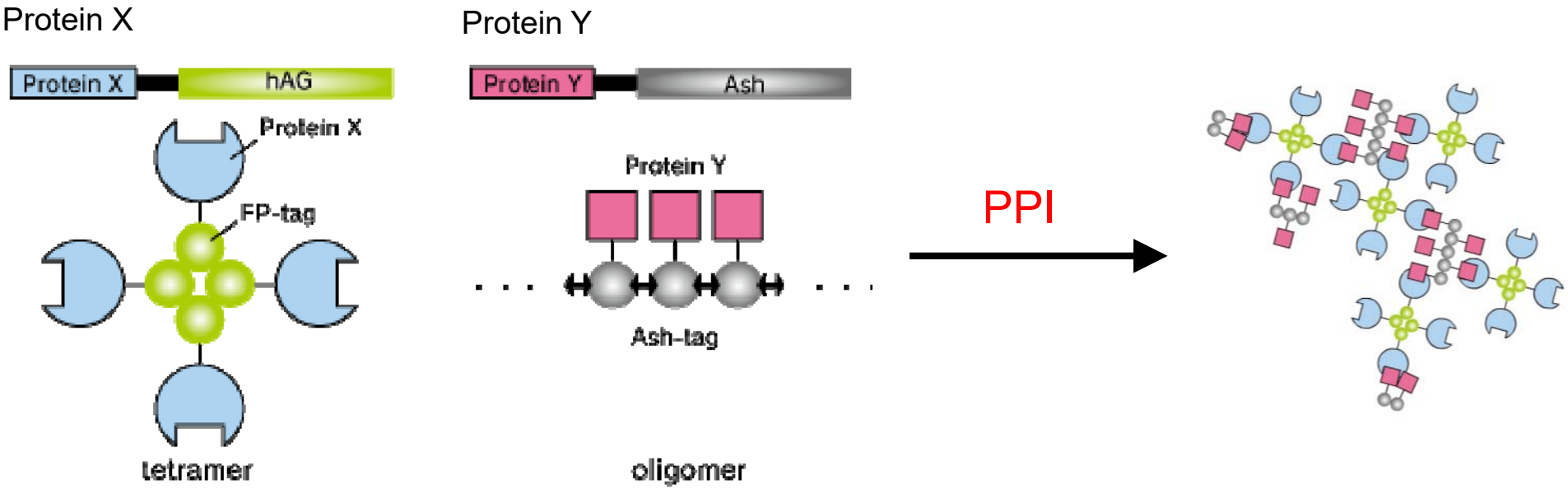


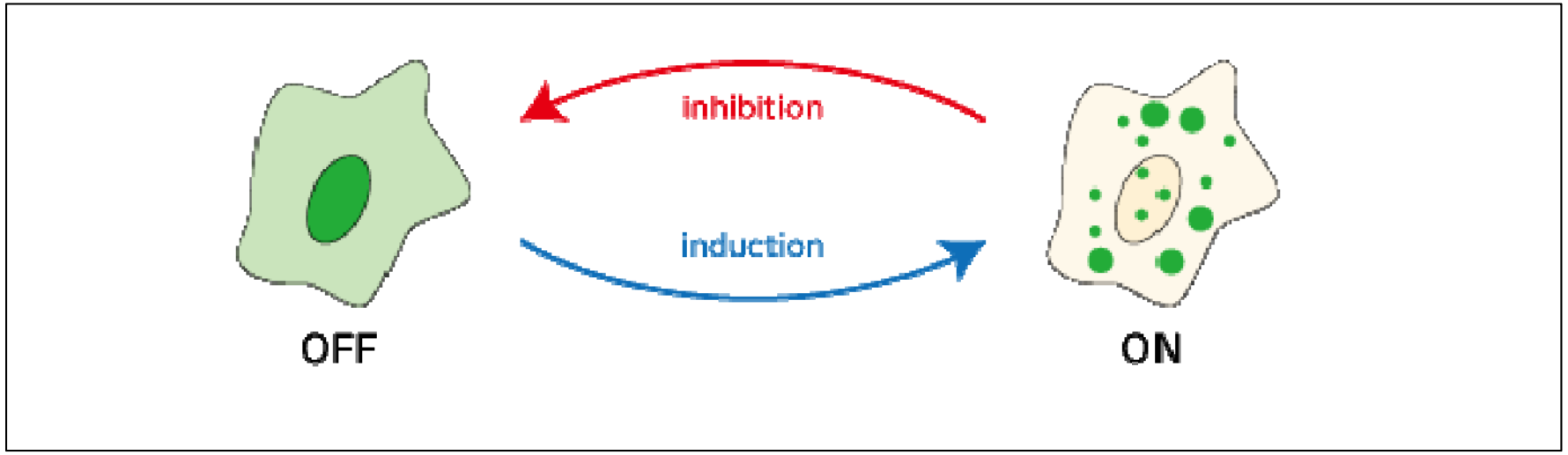


Sphere culture maintains cancer stem characters

Fluoppi (Fluorescent based technology detecting Protein-Protein Interactions) system

Fluoppi is efficient screening system for protein-protein interaction (PPI) regulators





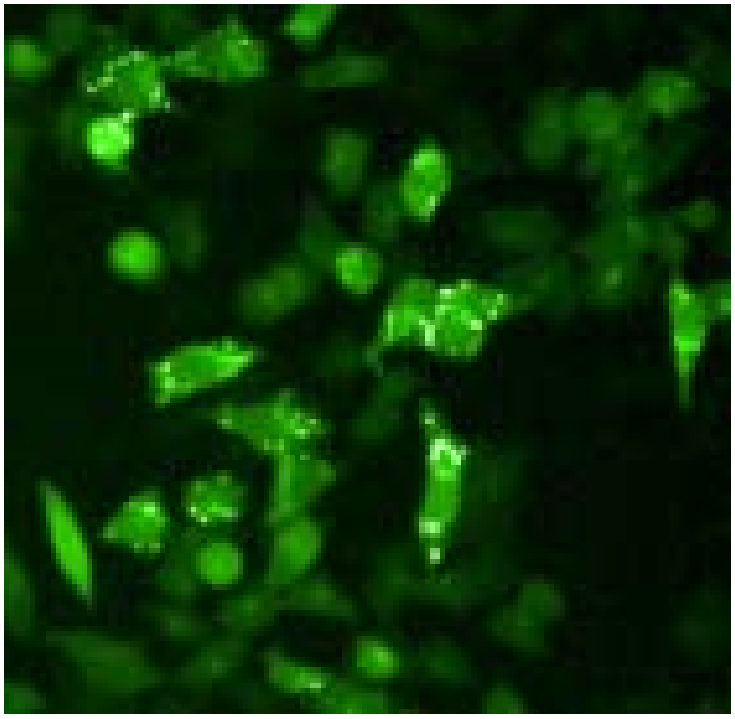
PPI induces the formation of foci in cells (reversible)

http://ruo.mbl.co.jp/bio/product/flprotein/fluoppi.html

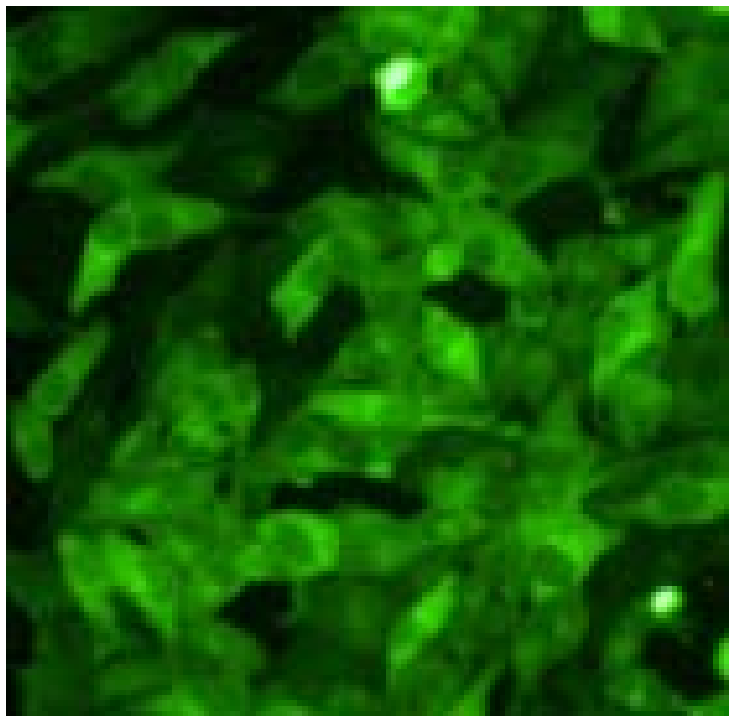
Screening for PPI inhibitors employing Fluoppi system

1st screening: isolated natural compounds (2,560)

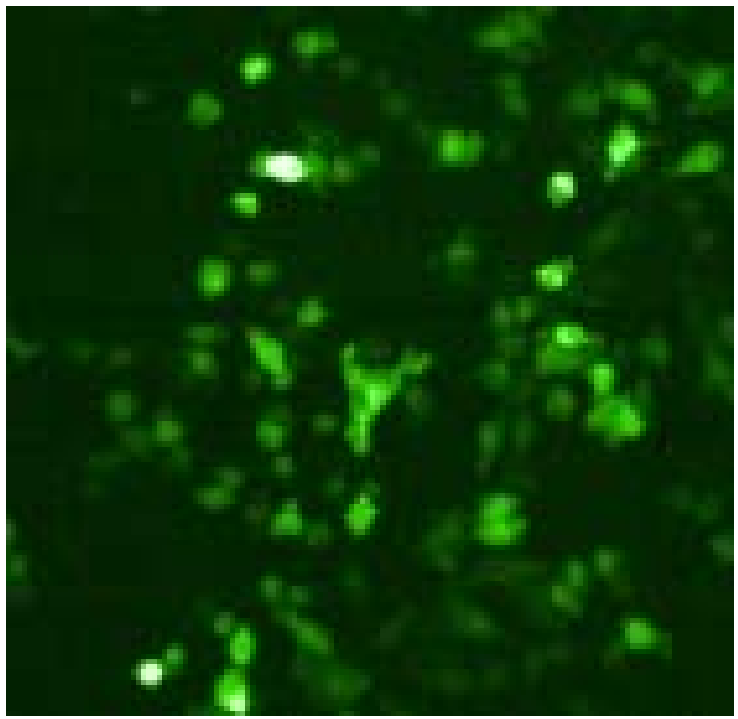
Control



True hit



False hit



Cytotoxic compound
(shrink cells were observed)

The 2nd Topic: Development of next generation natural product chemistry

Contrary to the charm and expectation of natural products, in fact, now the discovery rate of novel compounds significantly has decreased

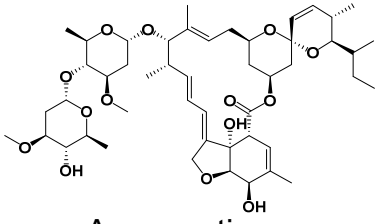
Indeed, we have hardly discovered novel unique compounds in the trial of chemical screening employing more than 1,000 strains cultured in 4 different suitable media.

Nature still possesses many possibilities of microbial resources

- Only 0.1 ~ 1.0 % of microorganisms in nature can be cultured
- Natural compounds from marine biology are produced by symbiotic microbes
- Even for culturable microbes, only 1/10 ~ 1/3 of biosynthesis genes can be utilized

Ex.: Obtained compounds and biosynthesis genes in actinomycetes

Streptomyces avermitilis: 12/37
Streptomyces griseus : 10/32
Streptomyces coelicolor : 10/25
Saccharopolyspora erythraea: 3/25



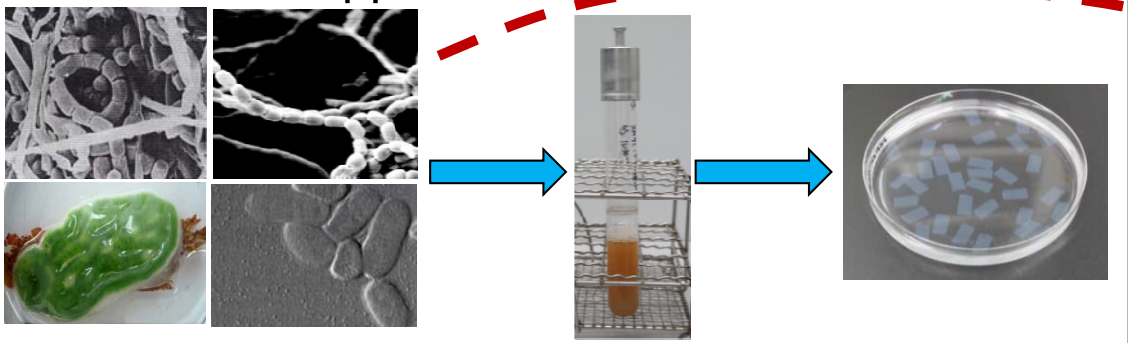
Avermectin

https://www.kitasato-u.ac.jp/lisci/international/OmuraSatoshi.html

Heterologous expression system could be one of the final (ultimate) technique to draw the potency of microorganisms

Heterologous expression of microbial secondary metabolites

Conventional approach



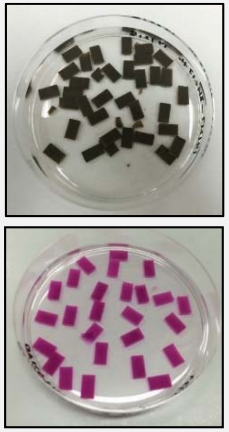
Genome sequence

Clone sequence

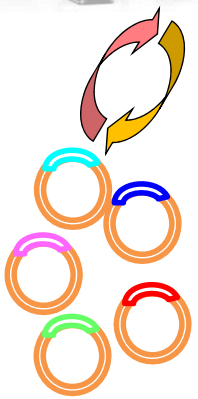
Metagenomic approach

Soil

Marine biology

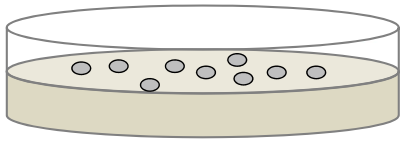


Preparing BAC library



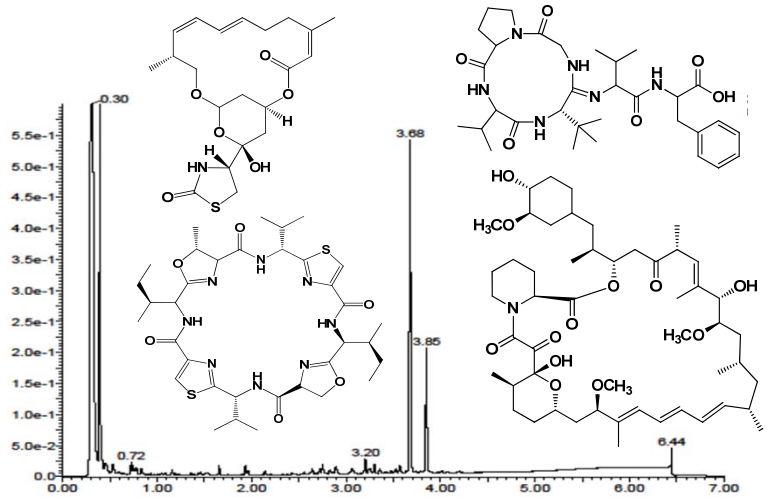
Cloning biosynthesis clusters

Transforming into host strains



Clean host

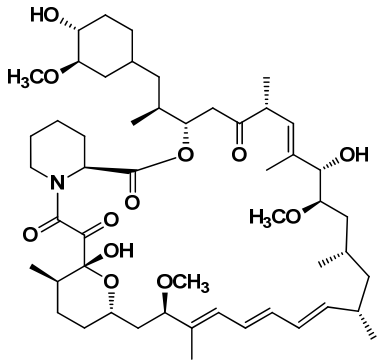
Heterologous expression



Targets of heterologous expression system

- Macrolides (Type-I PKS) are representative microbial secondary metabolites approved as clinical drugs
- Organic synthesis of these compounds is quite difficult

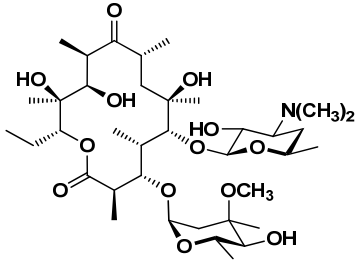
Rapamycin
(107 kbp)



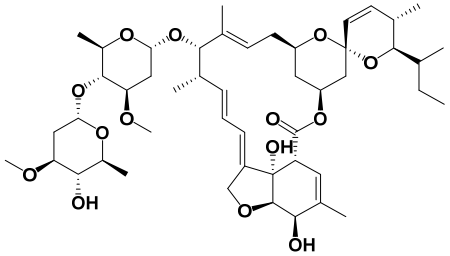
Organic synthesis
Reaction steps: 34 (～5 years), yield 2%

Biosynthesis
Enzyme reaction: 60 (1 weeks)
Enzymatic genes: 75 genes

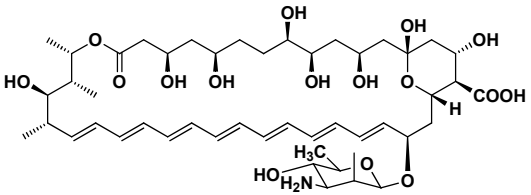
- Heterologous expression is also difficult since their biosynthesis gene clusters are extremely large



Erythromycin (55 kbp)



Avermectin (81 kbp)



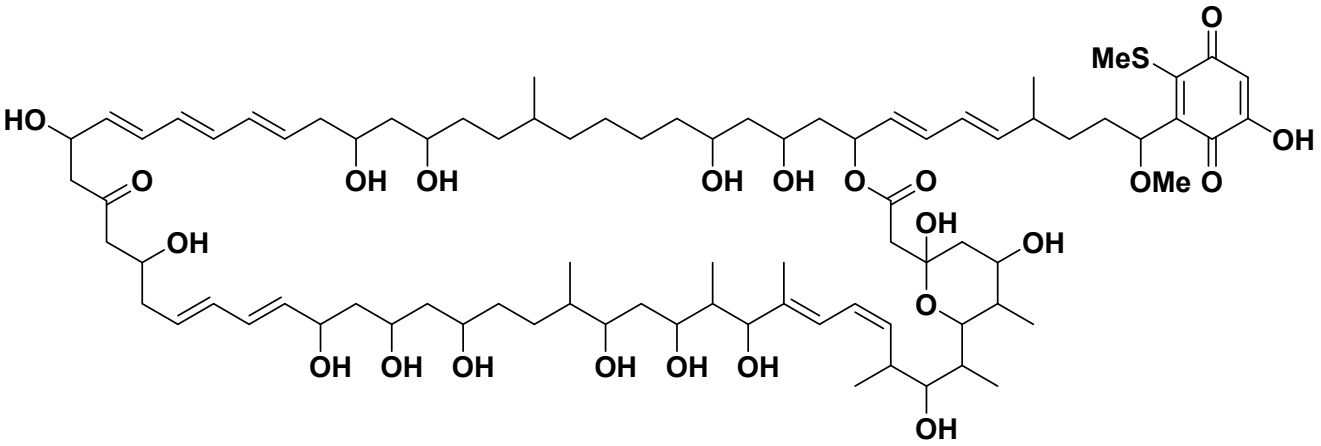
Amphotericin B (143.5 kbp)

To clone these large gene cluster, BAC technology is necessary

	BAC	Cosmid	PCR
Gene library size	～ 250 kbp	~ 45 kbp	1 ~ 2 kbp

BAC: Bacterial Artificial Chromosome

This technique allows us to isolate cryptic biosynthetic gene clusters from unculturable microorganisms and circumstance DNA (metagenomes)



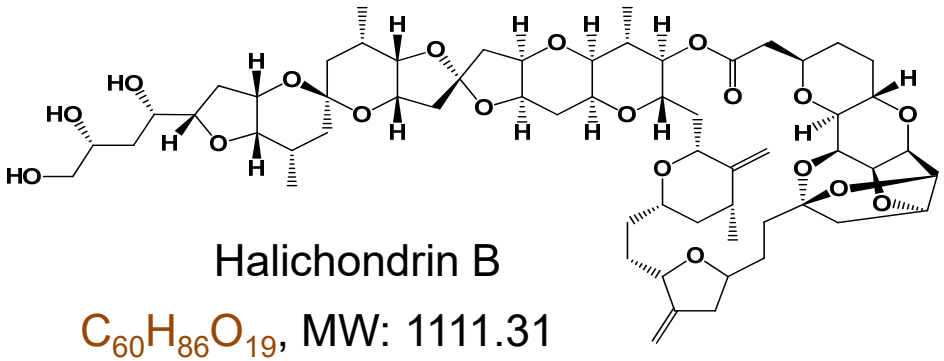
Quinolidomicin

$C_{83}H_{132}O_{23}S$, MW: 1529.99

Cluster size: 223 kbp,
Insert size: 232 kbp

Largest macrolide produced by terrestrial microorganism

Technically, it is enough to clone halichondrin biosynthesis gene cluster



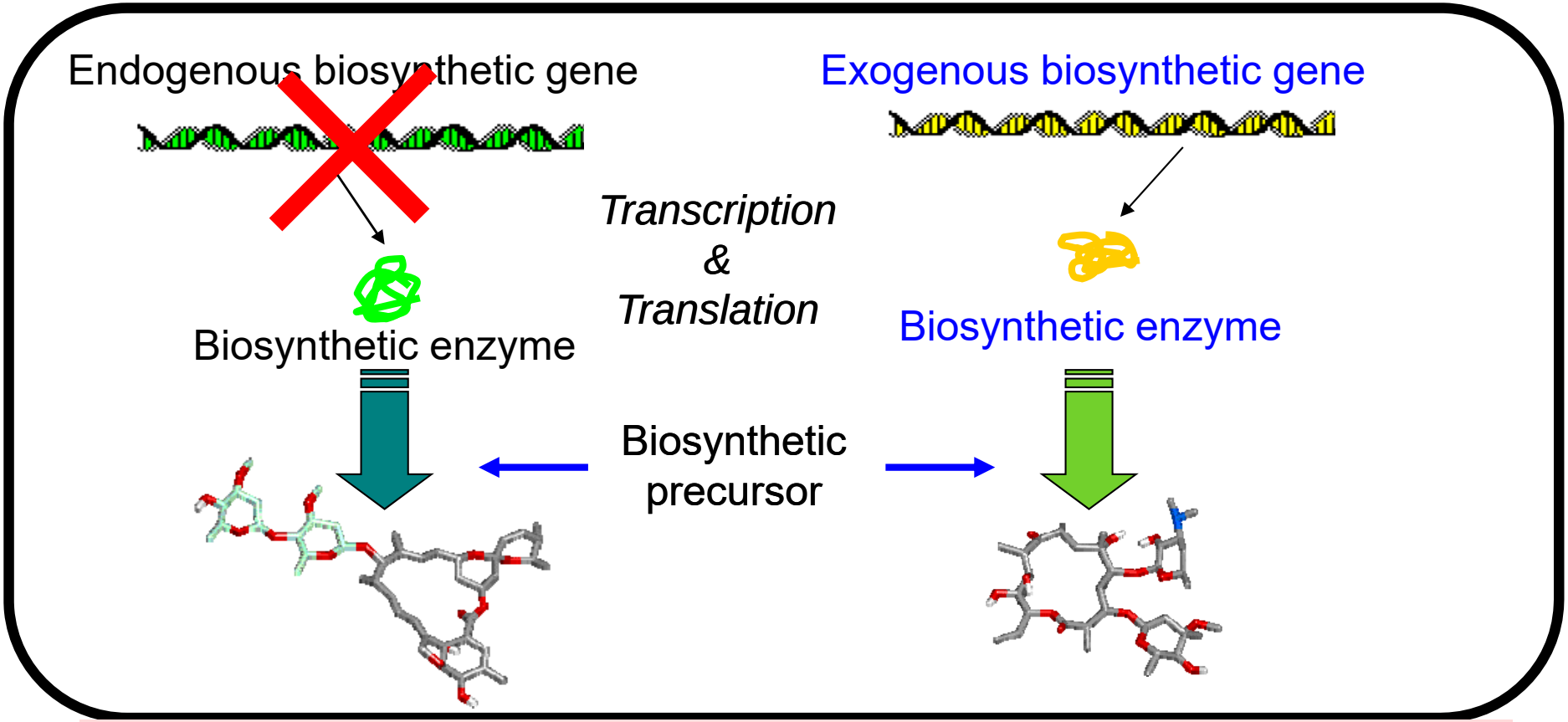
Halichondrin B

$C_{60}H_{86}O_{19}$, MW: 1111.31

Isolate from *Halichondria* sp. (1986)

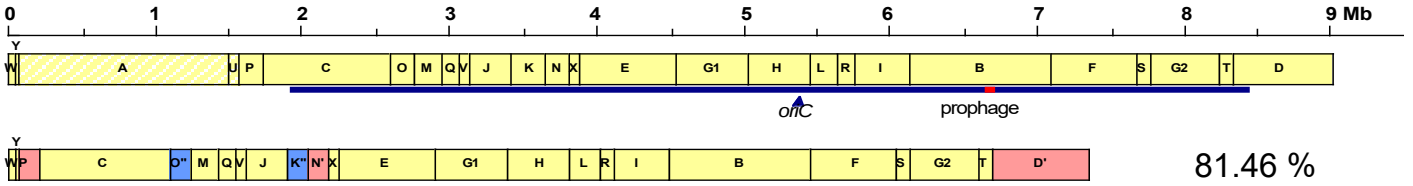
Produced by symbiotic microorganism (unidentified)

Biosynthesis cluster: ~ 200 kbp



"Versatile host" for secondary metabolism

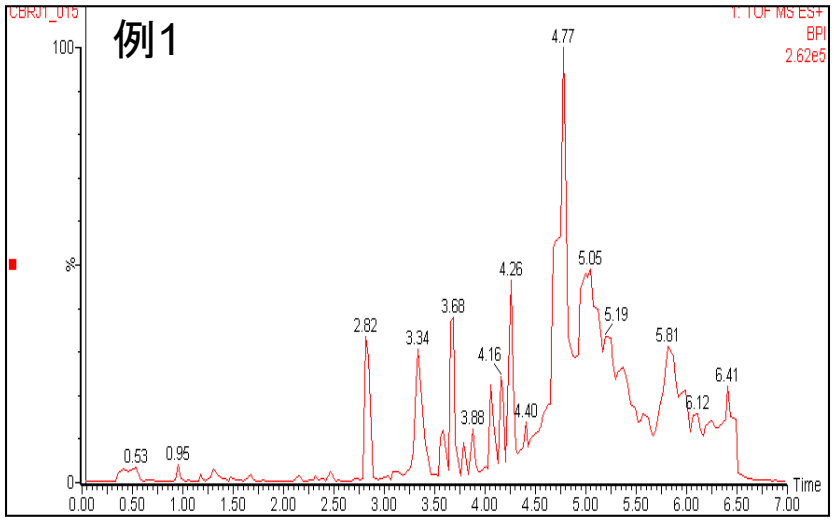
Strain	Genome size bp (ORFs)	Genotype
wild type	9,025,608 (7,582)	—
SUKA17	7,352,064 (6,310)	SUKA17 *DSAV2161-2167*

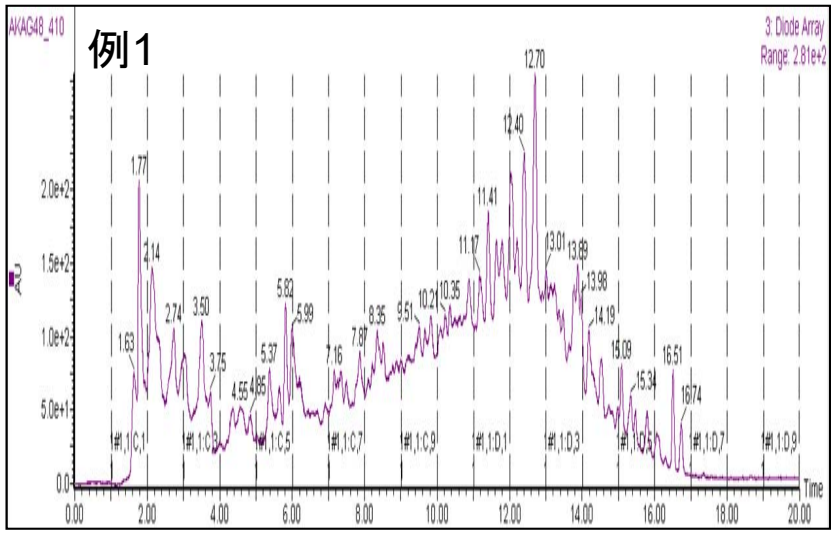


Properties of Mega-deletion mutants of *S. avermitilis* (*Special Use of Kitasato Actinobacteria*)

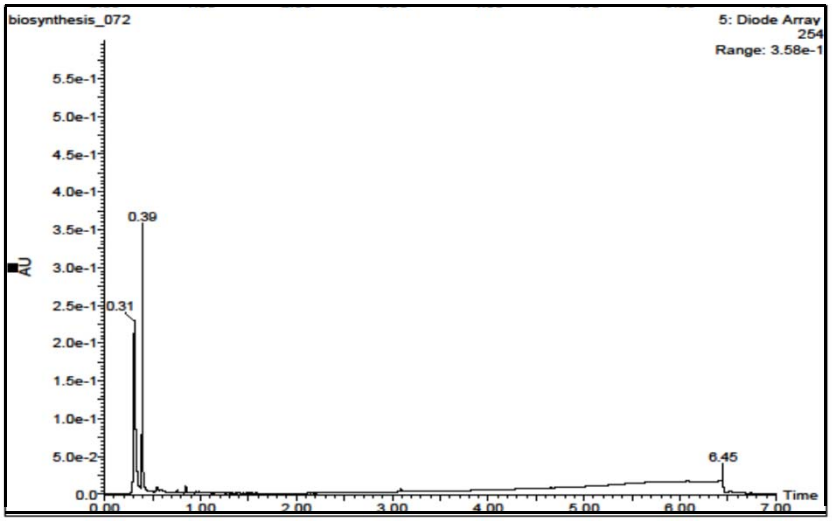
Advantage of production of heterologous expression by SUKA (high purity)

Wild strains produce many compounds: Isolation is bottleneck

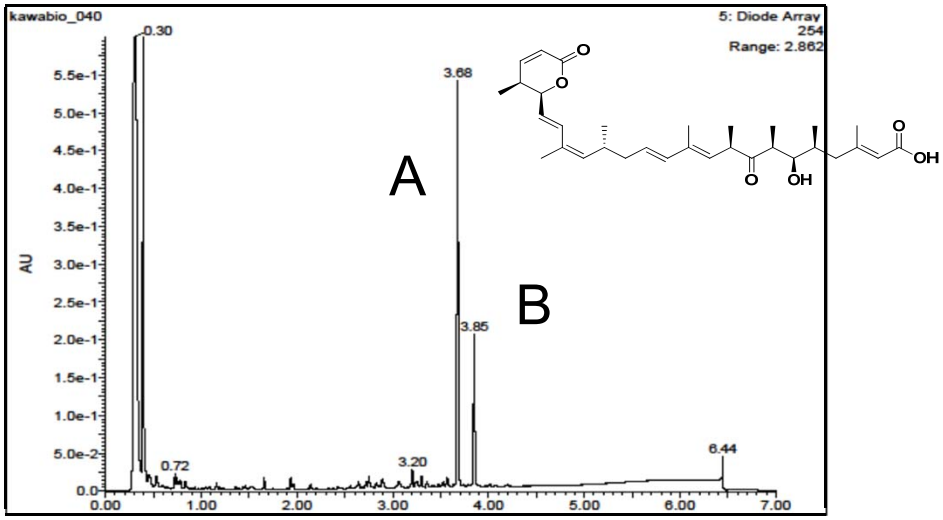




Control (− gene): No products



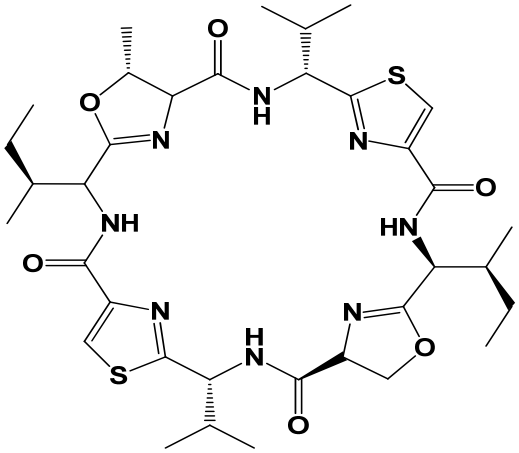
Transformant (+ gene)



Only introduced compounds can be produced

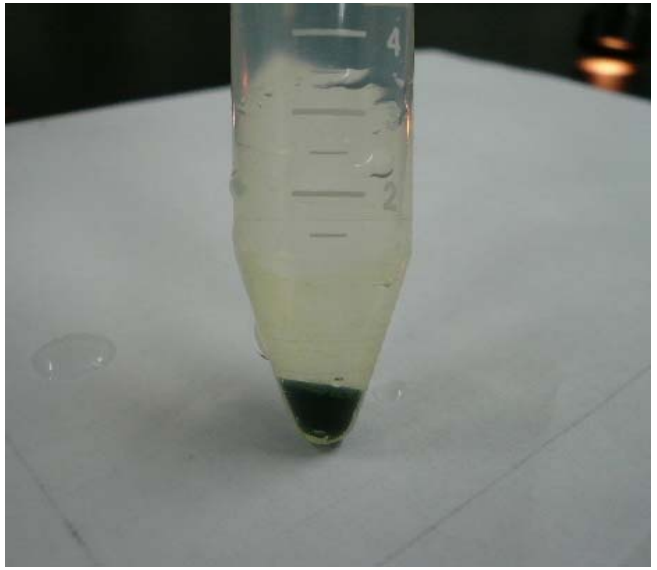
Cloning of biosynthesis gene cluster from unculturable marine microorganism

Lissoclinum patella



Patellamaide A

Ishigaki island, Okinawa



Prochloron (unculturable microorganism)