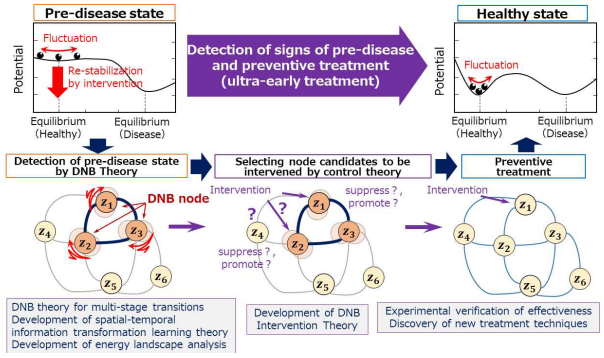
R&D item

1. Mathematical Approach to Complex Control System between Organs

Progress until FY2023

1. Outline of the project

The process to the onset of a disease is understood as state transition of the complex interaction network between organs, cells, and genes. A method called **Dynamical Network Biomarkers (DNB)** has been proposed to detect the pre-disease states by focusing on the "fluctuations" in gene mRNA expression levels, hormone concentrations and others. The effectiveness of DNB has been demonstrated for various diseases. On the other hand, there is no research on preventive treatment when such a pre-disease state is detected. Our goal is to expand DNB theory to **multi-stage transitions**, develop **its complementary theory** like spatiotemporal information transformation learning and energy landscape analysis, and then establish **preventive network treatment in the pre-disease states by** combining **DNB theory and control theory.**



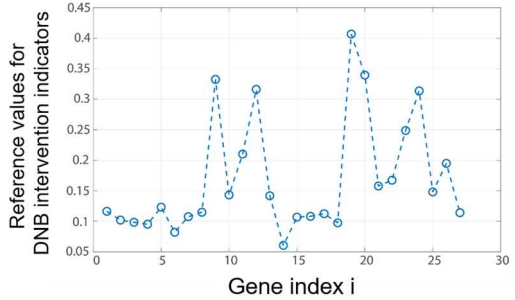
Development of the detection of pre-disease state and preventive treatment based on DNB theory

2. Outcome so far

In 2022, we developed the basic theory of DNB intervention based on a mathematical model of mRNA expression. In 2023, for more practical applications, we extended it to include the process from mRNA expression to protein production based on a hierarchical model of mRNAs and proteins. Specifically, we theoretically derived an index (the DNB intervention index) to identify candidate genes in the gene expression network that are highly effective for intervention from the mRNA or protein level to prevent diseases (re-stabilization). The relationship between mRNA intervention and the corresponding protein intervention was clarified and an approximation (a reference value) of the DNB intervention index was successfully derived even when only mRNA expression levels could be measured.

We have also proposed **mathematical methods to detect warning signals of multi-stage successive transitions** (Nat Comm, 2024) and **new theory** to complement the DNB theory so that we can detect **early warning signals for small deviation from healthy states** at ultra-early timing. Further, as an urgent important problem of our society, we studied **COVID-19** and found **a key gene DOCK2** for severe COVID-19 (Nature, 2022) and **optimal isolation guidelines** (Nat Comm, 2022) for COVID-19 patients.

We have collaborated **with the Saito Group at U. Toyama for experimental verification and the Fujiwara Group at U. Tokyo for the data management**. In addition to the **metabolic syndrome mouse model**, we have applied the developed theory to **the inflammatory bowel disease (IBD) mouse model** with these groups, and derived the reference values for the DNB intervention indicators shown in the figure below. Experiments conducted by the Saito group for protein intervention on the relevant mice confirmed the effectiveness of the method.



Collaborative research with Saito G and Fujiwara G: Application of gene data of IBD mouse model

Furthermore, we have successfully observed multi-stage state transitions in lung cancer mice. On the basis of the results from these mouse models, our experimental group is currently working on extending the analysis to human data.

3. Future plans

We have expanded the DNB intervention theory to include protein interventions, in addition to mRNA. Future challenges include extending this theory to hierarchical networks of genes and cells and adapting it for single-cell sequencer data. It is also our important future problem to **combine the DNB theory with its complementary theory toward a comprehensive system for ultra-early medicine of many diseases.**

R&D item

2. Experimental Approach to Complex Control System between Organs

Progress until FY2023

1. Outline of the project

Our R&D item has the following three objectives:

1. To Promote the project by applying mathematical methods to our health sciences research with respect to the pre-disease states in collaboration with the Mathematical Approaches Team within the project.
2. To lead the realization of ultra-early precision medicine through medical intervention in the pre-disease states.
3. To build a pre-disease database by collecting temporal and comprehensive biological information on multiple organs in animal models and humans.

Specifically, we are conducting validation of mathematical methods such as DNB analysis applied for health sciences using animal models and clinical samples. We are also providing pre-disease datasets to the GakuNin RDM through the project's Mathematical Collaboration Team.

2. Outcome so far

Functional analysis of DNB genes selected through collaborative research within the project

We performed functional analysis of 15 DNB genes selected from the adipose tissue dataset of spontaneously developing metabolic syndrome (TSOD) mice by the control theory in collaboration with Prof. Jun-ichi Imura's group (Tokyo Institute of Technology) using the fruit fly model system in metabolism. We knocked down each DNB gene in the adipose tissue of flies and identified two genes, which ameliorates (gene X) and female-specifically mimic (gene Y) the effects of high fat diet feeding. We reported the effectiveness of the *Drosophila* model system in combination with DNB theory to identify the functional genes on Mebyo (Akagi *et al.*, *Cells*, 2023).

② Inter-organ network of TSOD mice and pre-disease dataset of HFD-fed C57BL/6 mice

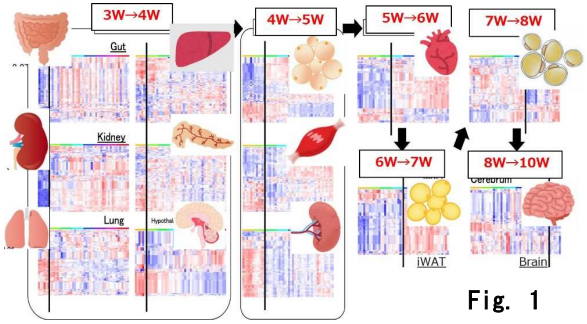


Fig. 1

To reveal the inter-organ network, we investigated how changes in gene expression propagate among organs in TSOD mice (Fig. 1). We also obtained comprehensive gene expression data and registered them to the pre-disease database from 13 organs of diet-induced obese mice (C57BL/6) at 16 time points.

③ Detection of precancerous state (pre-disease state) of hematopoietic tumors using Raman microscopy

We applied DNB theory and energy landscape analysis (ELA) in collaboration with Prof. Naoki Masuda's group (State University of New York) to Raman spectra obtained from plasma cells of the bone marrow of patients with hematopoietic tumors. The DNB analysis identified MGUS as a pre-disease state in the progression of the multiple myeloma stage (Fig. 2a).

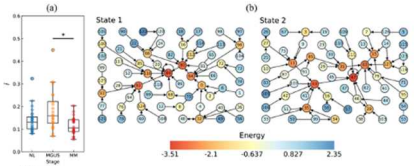


Fig. 2

The ELA revealed two stable states: the normal stage and multiple myeloma in the progression (Fig. 2b). We published two papers on validating the detection of pre-disease states using Raman spectroscopy, DNB theory, and ELA for human samples. (Oshima *et al.*, *Int. J. Mol. Sci.* 2023, Yonezawa *et al.*, *Int. J. Mol. Sci.* 2024).

④ Biology of DNB genes detected in IBD model

To investigate the biological significance of the 27 DNB genes in inflammatory bowel disease (IBD), we calculated control theory-based DNB intervention scores in collaboration with Prof. Jun-ichi Imura's group and investigated the expression of DNB genes in patients with IBD using GEO database. Then, we performed the intervention in Wars and found that Wars may play a protective role against IBD. A patent application has been filed with JST on these results.

3. Future plans

We are working on more detailed single cell analysis and intercellular network analysis using visceral adipose tissues from high-fat diet-induced metabolic syndrome mice and humans. In addition, by improving the Raman microscope, we are also developing a clinical examination system that can be applied to detect the pre-disease state of human hematopoietic tumors. Moreover, the detailed biology of Wars as a candidate intervening gene will be further investigated in mice and humans.

Here begins our new MIRAI
MOONSHOT RESEARCH & DEVELOPMENT PROGRAM

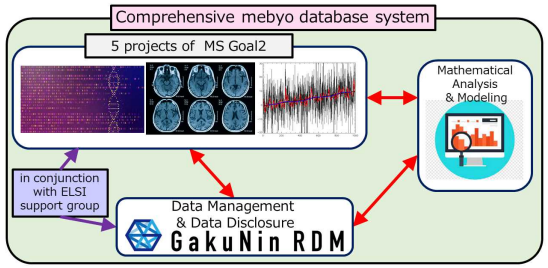
R&D item

3. Mathematical Collaboration with Other Moonshot Projects, Development of Data Base, and ELSI Supporting System

Progress until FY2023

1. Outline of the project

This R&D item can be divided into two sub-themes: One is **"Mathematical Collaboration with other Moonshot Projects"** and the other is **"Database Construction"**. Mathematical collaboration involves the construction of **a mathematical analysis infrastructure** that can be used across the entire Moonshot Goal 2, as well as mathematical collaboration on disease data obtained in Goal 2. In particular, we will construct **a comprehensive mathematical analysis method** for detecting the pre-disease state (Mebyo) just before the transition from a healthy state to a diseased state.



Conceptual diagram of the construction of a comprehensive Mebyo database system

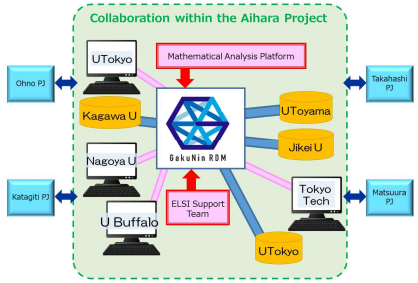
In addition, the database construction aims to compile the results of the entire project, construct **a comprehensive Mebyo database of complex organ regulatory systems**, and make it widely available to society. **In collaboration with all the projects in Moonshot Goal 2**, we will construct this comprehensive Mebyo database based on experimental data, clinical data, and cross-sectional mathematical analysis data related to various diseases.

Through these efforts, we will contribute not only to the project's goals of comprehensive mathematical understanding of complex organ regulatory systems and early precision medicine, but also to the realization of **a society capable of predicting and preventing diseases in the ultra-early stages**, which is **the goal of Moonshot Goal 2 as a whole**.

2. Outcome so far

In the mathematical collaboration, the construction of mathematical analysis methods and the development of mathematical analysis software have been conducted in order to start mathematical analysis using artificial data generated by mathematical models and existing open data etc., in anticipation of data analysis of experimental and clinical data that will be obtained in **all the 5 projects in MS Goal 2**. For example, software has been developed and released for each of the Aihara Project's original mathematical analysis methods, such as **DNB analysis and ASURAT**. The released software has been tested on **the GakuNin RDM**, and it has been verified that the software can be used for the entire Moonshot Goal 2. Thus, the construction of the mathematical analysis platform is steadily progressing.

In addition, in database construction, we have completed the design of a **Moonshot Goal 2 database** and have begun collecting and sharing experimental and clinical data from all the five projects. The database working group has been organized to concretely work toward the use of the database for the entire Goal 2 project. In addition, we are working closely with **the ELSI supporting team** to consider appropriate responses to ethical, legal, and social issues (ELSI) that may arise in comprehensive Mebyo database construction.



MS Goal 2 Database by GakuNin RDM

3. Future plans

In mathematical collaborative research, we will continue to build **a data sharing system to continue to promote mathematical collaboration within each project and across all the projects under MS Goal 2**. In addition, we will also continue to construct a mathematical analysis platform that can be easily used **across the entire Goal 2**. By releasing various mathematical analysis methods as software that can be used by everyone in Goal 2, it is expected that mathematical collaboration will be promoted.

As for the database construction, data sharing within MS Goal 2 will be promoted as soon as possible. To this end, we will resolve issues related to data sharing and promote data storage by taking **a leading role within MS Goal 2**. In addition, we will work with **the ELSI supporting team in our project** to create a database guideline in order to establish common usage methods and rules for the comprehensive Mebyo database construction.

JST Japan Science and Technology Agency