HD Physiology Newsletter

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Message from Project Leader

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Biological function (especially, high-level function) is the outcome of interactions between multi-level physiological systems, from the molecule level to the whole organism level. It is inherently complex, but is also highly organized. To unveil the logic of life, many scientists have been studying organisms and accumulating elemental knowledge by using the techniques of molecular and cellular biology. Now, we need to ask how close we are to the understanding the logic of life. I believe that one of the ways of understanding the logic of life is to establish the field of integrative multi-level systems biology, where physiological and pathological information can be described in high-definition (HD) across multiple scales of time and size. HD-Physiology has not yet been established; however, the progress towards the ultimate goal is valuable, since it helps us identify the aspects of life we have understood and those we have not. Furthermore, struggling against many obstacles on the road to the *in silico* human may help us to depict nature of life. For such a purpose, we have to utilize new mathematical models and information technologies to understand the basic concepts of life.

Research and development of open platform for multilevel modeling of physiological system

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Our mission is to promote the integrative multi-level systems biology. For this, integration of pieces of knowledge developed in each level is necessary and building mathematical models is the way. One of challenges that we have been tackling for these years is to establish fundamental technologies for modeling multi-level and multi-scale physiological systems, and for sharing models. We have been developing an open platform named “PhysioDesigner” as a next generation of *insilico*IDE on which users can create their own models and integrate data on the models (Fig. 1). They also can merge shared models easily. Models on PhysioDesigner are described in a format called ISML which we have also developed. A part of ISML specification is compatible with CellML, which is one of pioneering efforts in the same direction. There is a *de facto* standard language called SBML to describe wide range of subcellular biochemical phenomena. PhysioDesigner has a capability to create hybridized models by

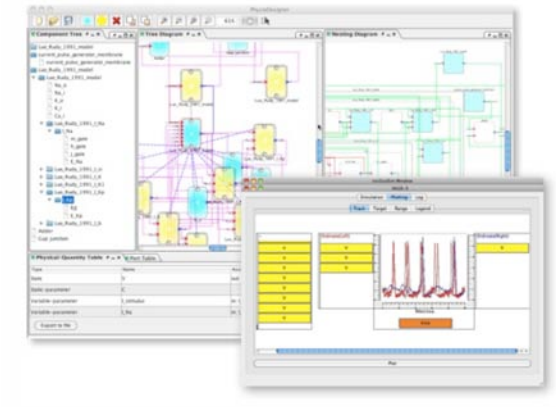

Fig. 1 Snapshot of PhysioDesigner

incorporating the biochemical models written in SBML at the same time for expressing a hierarchical network of biophysical phenomena. The most distinctive feature of PhysioDesigner is that morphometric data can be integrated in the models. In general, such data are used for defining a domain in which partial differential equations (PDEs) are solved, and several conditions, such as initial and boundary conditions, are defined. For this, we need to define segments on a morphometric object. PhysioDesigner equips a graphical interface to deal with meshed morphometric objects (Fig.2). In addition, we have also developed a simulator. In particular, the simulator supports a parallel computation technology, so that it can execute high performance simulations even in cases that

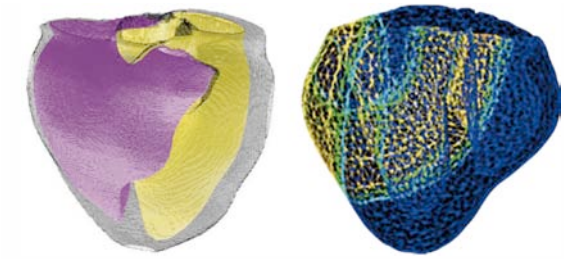

Fig. 2 An example of whole heart model

the size of a model is huge.

To make an appeal to researchers and to have an opportunity that takes user feedback to improve PhysioDesigner, we have held tutorials on PhysioDesigner. As the opportunity to the tutorial, we expect that research collaborations between the wet and dry researchers have been accelerated. Through these efforts, we are confident that PhysioDesigner can play one of key roles to promote the integrative multi-level systems physiology.

PhysioDesigner is available at http://physiodesigner.org

We also aim to develop mathematical models for cardiac conduction in collaboration with other groups of HD physiology project, and to analyze anti- and pro-arrhythmic mechanisms.

Software Platform for Complex Tissue/Organ level simulation

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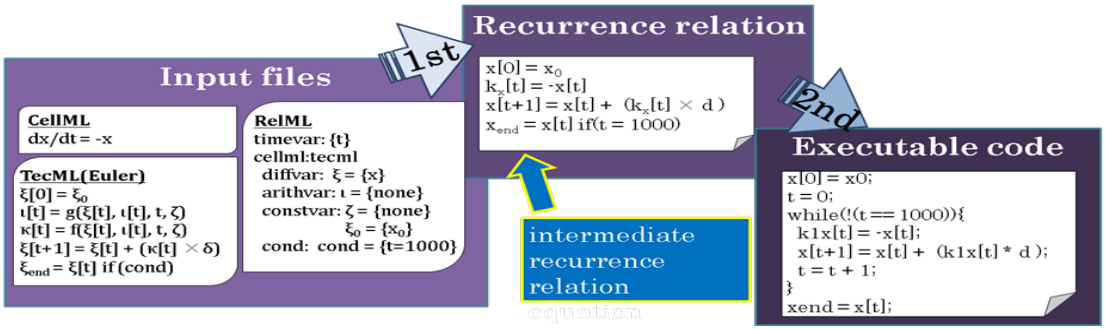
One important aspect of biological simulation model is to evaluate the role of certain small system in the large system or whole body quantitatively by combining a small detailed system into a large scale model. Due to the accumulation of the biological findings, the complexity of the large scale model is growing rapidly. The complexity of each model and the total system is already becoming incomprehensible for one researcher. This situation is similar with what happened in 1970s where software engineers faced of a problem called software crisis. As in those days, softwares which can support maintaining biological model simulation software correctness are becoming important. One solution of this problem is to use certain markup language to describe biological models. Since each model is written in mathematical equations, these models can be used in various situations without modifying the model descriptions. If each model file is created and maintained by the original researcher, the probability of bug appearance becomes negligible low. There are several description languages which can be used to describe multiscale multiphysics biological simulation models, such as in silico ML, SBML, CellML, FieldML. They are aimed to describe lumped parameter models using ordinary differential models or differential algebraic equations, and field models using partial differential equations or finite difference equations together with geometry descriptions. However, the multiscale multiphysics biological simulation program still requires a method how to combine these elementary model descriptions and also how to calculate this combination of equations provided by each elementary model. This calculation method is called coupling calculation schemes. The coupling calculation schemes include numerical calculation methods of temporal and spatial discretization, calculation order of equations provided by each elementary model and relations between each model. Since there was no description language suitable for this purpose, we are designing several languages. One is TecML (Time Evolution Calculation Markup Language) with which one can represent discrete time evolution calculation method for ODEs such as Euler method or Runge-Kutta method. We are also designing markup languages with which one can describe spatial discretization scheme and calculation order of the whole system. The former is essential for calculating the field model for example excitation propagation model of heart. The latter is essential for calculating pharmacokinetic models where several models of different time constants have to be calculated together, and also for calculating heart deformation model in which electrophysiological model and mechanical models are calculated together. By using our system, one can safely obtain a complex simulation program which is free from bugs.



Re-evaluation of the pathophysiological potential of TRPM4 channels for Ca^{2+}-dependent arrhythmia

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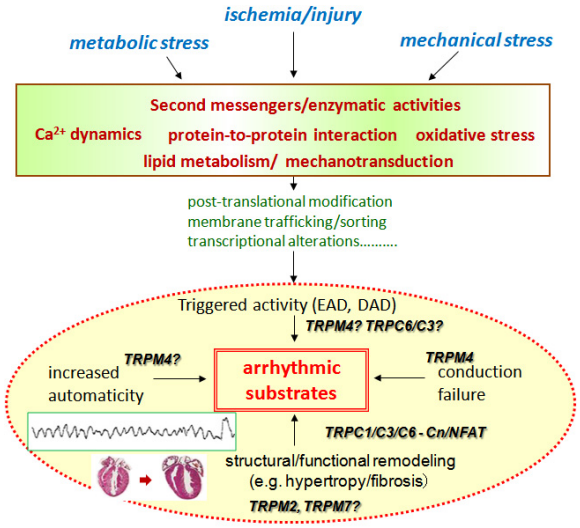
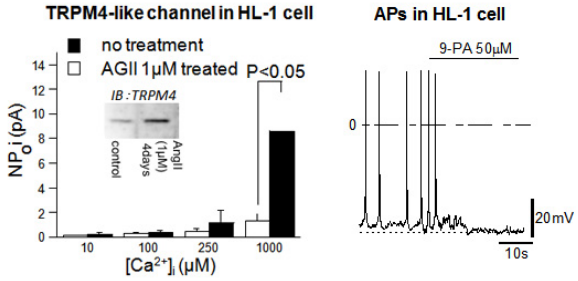
The purpose of the A02-2 research project is to explore the mechanisms underlying the acquired arrhythmogenicity associated with cardiac remodeling. In the second year of this project, we have focused on elucidating the role of TRPM4 channels therein. This channel has been implicated in familial conduction blocks and also shown to be upregulated in hypertrophied hearts under sustained mechanical and neurohormonal stresses (for review, refer to Inoue *et al.*, 2011)*. However, detailed investigations suggest that Ca^{2+}-dependent activation of this channel may require unphysiologically high concentrations of Ca^{2+} ($[Ca^{2+}]$) of ~mM. To reconcile this discrepancy, we re-investigated the Ca^{2+} sensitivity of this channel under as intact conditions as possible in both expression system and an immortalized atrial cardiomyocyte line HL-1.



To evaluate the 'intact' Ca^{2+} sensitivity of TRPM4 channel heterologously expressed in HEK293 cells, we adopted two approaches; (1) β-escin-permeabilized cell-attached (C/A) recording with different concentrations of Ca^{2+} ($[Ca^{2+}]$); (2) ionomycin-permeabilized C/A recording combined with fura-2-based $[Ca^{2+}]$ measurement. Both methods gave far lower K_d values (<10μM) for $[Ca^{2+}]$ vs. TRPM4 open probability relationship than those obtained under cell-free conditions. Furthermore, in HEK cells coexpressing TRPM4 and voltage dependent Ca^{2+} channel ($\alpha_1C/\beta_2/\alpha_2\delta$), Ca^{2+} influx activated by a single depolarization sufficed to activate TRPM4-mediated inward current at the resting level of membrane potential, -80mV, and repetitive depolarizations at high frequencies further enhanced it. Finally, four-to-five day treatment of HL-1 cells with angiotensin II, a potent pro-hypertropic factor for the heart, greatly enhanced the expression level of TRPM4 with increased generation of spontaneous action potentials (APs) that could be abolished by a selective TRPM4 channel blocker, 9-phenanthrol via the inhibition of basal



membrane depolarization.
These results suggest that the Ca^{2+} sensitivity of TRPM4 channel would suffice to produce diastolic depolarizations linked with each AP cycle, and when TRPM4 channel expression is upregulated, this may lead to triggering arrhythmic excitations.
The quantification of this AP-linked activation of TRPM4 channels in a relevant AP simulation model will be the next challenge of our project.

*Inoue R, Duan Y, Hu Y, Ichikawa J. The pathophysiological implications of TRP channels in cardiac arrhythmia. *In: "Cardiac Arrhythmias"*, ***InTech Review***. ISBN 979-953-307-050-5, 2011

The regulatory mechanism of signal pathway of cardiomyocyte: integrative analysis of autonomic regulation

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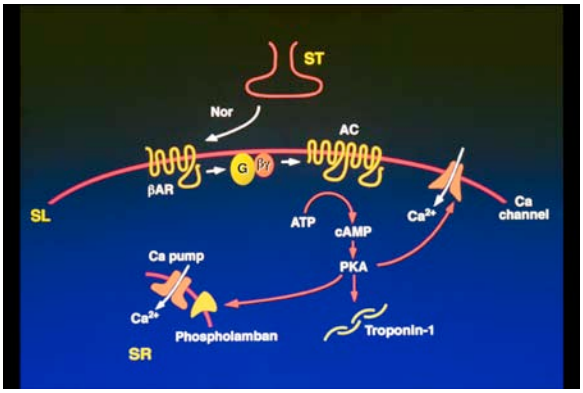
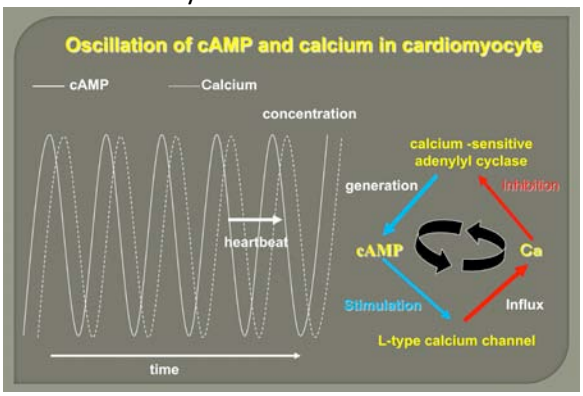
Sympathetic nerves regulate cardiac function via adrenergic receptors coupling adenylyl cyclase. Norepinephrine released from the synaptic terminal binds to β-adrenergic receptors on the cardiac sarcolemma and activates Gsα by exchanging guanosine diphosphate (GDP) for guanosine triphosphate (GTP). This reaction promotes the dissociation of the GTP-Gsα from Gβγ. The GTP-Gsα binds to and stimulates adenylyl cyclase (AC), which is a membrane-bound enzyme that catalyzes the conversion of ATP to cyclic AMP (cAMP). Cyclic AMP (cAMP) activates protein kinase A, and it phophorylates L-type calcium channel that enhances calcium entry into cardiomyocytes leading to increased contractility.

The magnitude and efficiency of these processes depend on the amount and subtypes of molecules exist in the signaling pathway. The one of integrative steps of neuronal stimuli to cardiomyocytes was activation of adenylyl cyclases following receptor-G protein activation in the sarcolemma.

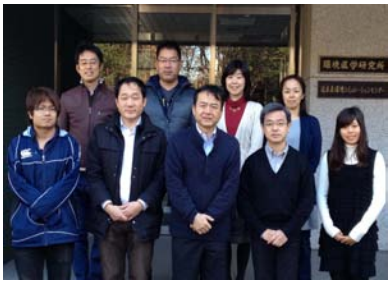


Ishikawa previously identified and characterized the major isoform of adenylyl cyclase expressed in the adult heart, type 5 adenylyl cyclase which was directly inhibited by calcium. It has been suggested that functional interaction between calcium and calcium-sensitive adenylyl cyclase influences the contraction properties of cardiomcyoytes. However this issue has not been extensively studied. In this project, we investigate influence of calcium on cAMP accumulation in the computational simulation models of cardiomyocyte. The results are compared with the data observed in cardiomyocytes or in vivo data obtained from genetically-modified mouse we have already established.



One of topics we are analyzing is the oscillation of cAMP and calcium. In the excitation-relaxation cycle of cardiomyocyte, intracellular cAMP level oscillates along with calcium ion. It is well known there is a delay of peak from cAMP to calcium. The cyclic oscillation of cAMP and calcium is demonstrated in the computational simulation model of cardiomyocyte, then an effect of calcium-sensitive adenylyl cyclase on cAMP oscillation is examined.

All these analysis contribute for the under standing of the roles of calcium-sensitive adenylyly cyclase in the physiological properties of cardiomyocyte.



Dynamics of excitation wave propagation and arrhythmias in the heart

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Ichiro Sakuma

The objective of the A2-4 project is to investigate the dynamics of excitation wave propagation and mechanisms of cardiac arrhythmias. Coordinated propagation of action potentials in the heart depends on intercellular current flow through gap junction (GJ) channels. Deranged expression and/or organization of GJ proteins (connexines) in the cardiac muscle have been demonstrated in a variety of pathological conditions, such as ischemia, inflammation and hypertrophy, and resulting alteration of GJ function is supposed to create arrhythmogenic substrates. We investigate the influence of GJ conductance on the dynamics of excitation wave propagation in isolated coronary-perfused rabbit hearts with the aid of high-resolution optical action potential mapping. Conduction velocity, CV) of cardiac excitation waves is affected by source-sink balance of intercellular current through GJs: when the excitation wave front is convex, CV is lower than the case of a flat wave front, because the local excitatory current supplied by the upstream excited cells distributes a large area downstream. This curvature effect is expressed by the following equation:

$$CV = CV_0 - D \cdot \kappa \quad [1]$$

where CV_0 is CV at a flat wave front ($\kappa = 0$) and D is the diffusion coefficient. We analyzed CV and κ during centrifugal propagation in a direction parallel to the fiber orientation and found that the relationship between two parameters follows the above equation and that pharmacological enhancement or inhibition of GJ coupling increases or decreases both D and CV_0 (Fig. 1). In addition, it is interesting that the regression lines between the two parameters before and after modification of GJ coupling crossed. An enhancement of GJ coupling increased CV at relatively low κ, whereas it paradoxically decreased CV at higher κ.

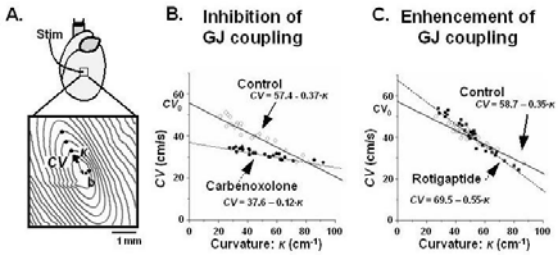

Fig. 1. Relationship between CV and κ.

Spiral wave reentry (rotor) is a principle mechanism of fibrillation/tachycardia (Fig. 2). Spiral wave reentry induced in the setting of GJ uncoupling was more stationary with a shorter functional block line and lasted longer than controls. In contrast, SW reentry induced in the setting of GJ enhancement was characterized by decremental conduction and block near the rotation center, resulting in a prominent drift and self-termination of the rotor by collision with the anatomical boundaries. The decremental conduction could be explained by the GJ enhancement-induced paradoxical inhibition of propagation of excitation waves with an extreme convex curvature (Fig. 1). These results indicate that SW reentry in the heart is regulated by intercellular electrical coupling.

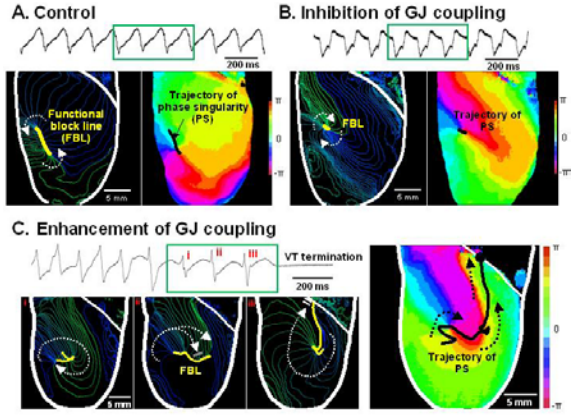

Fig. 2. Dynamics of SW reentry after inhibition or enhancement of intercellular coupling.

Predicting disposition dynamics of small molecules through chemoinformatic analysis of molecular interactions

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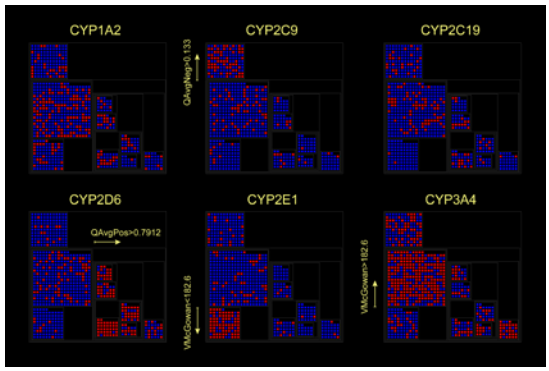
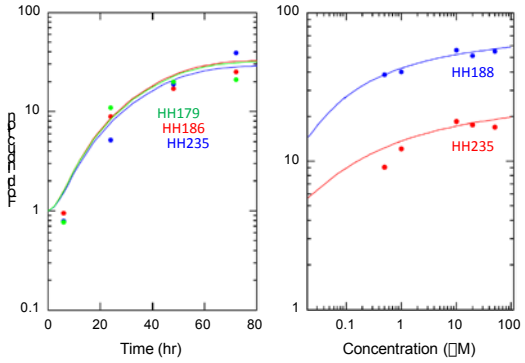
The A3-1 project study group aims to develop a mathematical model that represents disposition dynamics of small molecules through chemoinformatic analysis of their interaction with biological molecules. Homeostasis of small molecules is being maintained by functional proteins such as transporters and metabolizing enzymes and their relevant biological networks. Some small molecules influence the regulatory networks and alter disposition of other molecules. If it is the case for highly bioactive molecules, unwanted biological response might occur. In drug discovery settings, therefore, it is eager to delineate their structure-activity relationships at the level of biological networks. Our present study comprises two parts: that is, one is chemoinformatic analysis of molecular interactions for ligand-regulated functional proteins, and the other is system dynamics analysis of regulatory networks. Seamless integration of these two aspects allows us to predict disposition of small molecules from their chemical structures. Prior to the analyses of structure-activity relationships, we have been developing a natural language processing-based text mining system to systematically collect information on interactions between chemicals and proteins. For cytochrome P450s (CYPs), a superfamily of drug metabolizing enzymes, the system could successfully detect chemical names from the texts and classify them into substrates, inhibitors, or inducers. The system gave a fairly good performance with recall and precision of 85% and 92%. When the entire PubMed database was analyzed by this system, about 700 CYP substrates were obtained. Furthermore, analysis of structure-activity relationships for CYP-mediated metabolism using the data revealed: CYP2C9 substrates are mostly anionizable compounds; in contrast, CYP2D6 substrates are cationizable compounds;



CYP2E1 preferentially metabolizes smaller compounds; and there is a positive correlation between metabolic susceptibility toward CYP3A4 and molecular volume. The text-mining analysis was applied to the CYP3A4 network and detected 15 regulatory proteins. Since pregnane X receptor (PXR) was detected most frequently in the text mining analysis, we further intended to investigate structure-activity relationship of PXR activation. When results of text mining were merged with data reported in publications and registered in PubChem Bioassay database, 270 human PXR agonists and 248 non-agonists were obtained. The analysis suggested that electronic properties, particularly, existence of functional groups enabling π-π interaction, are important in the interaction with PXR. We are also challenging to construct a kinetic-dynamic model for CYP3A4 induction, specifically aiming to explain in vivo metabolic autoinduction of rifampicin quantitatively.



Development of Novel Chemical Modulators of Pleiotropic Actions of Nuclear Receptors

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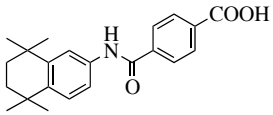
Tomoya Hirano, Shinya Fujii

Nuclear receptors are ligand-inducible transcription factors, and control various biological phenomena, including growth, development, metabolism, and homeostasis. Endogenous ligands for nuclear receptors are hydrophobic hormones, such as steroid hormones, thyroid hormones, activated vitamin A and D, and some metabolic signals. Nuclear receptors have become one of the most significant molecular targets for drug discovery in the fields of cancer, metabolic syndrome, autoimmune diseases, and so on. Am80 is synthetic retinoid, that is retinoic acid receptor (RAR) agonist, and was approved as drug for acute promyelocytic leukemia (APL) in Japan.

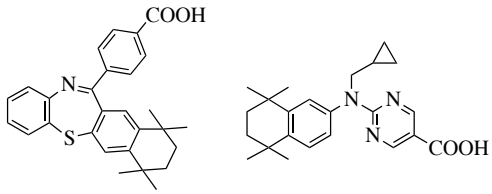
RAR agonists (Retinoids)



Am80
(Tamibarotene)

In A3 project, we focused on the nuclear receptors related to metabolisms, such as RARs, retinoid X receptors (RXRs), vitamin D receptor (VDR), liver X receptors (LXRs), and steroid and xenobiotic receptor (SXR). In order to clarify their functions and separate pleiotropic biological activities of each nuclear receptor, we developed novel ligands of nuclear receptors with different structure, compared to the endogenous or conventional synthetic ligands. For example, we have developed several types of specific ligands for RXRs whose endogenous ligand is 9-*cis*-retinoic acid. RXRs form heterodimers with various nuclear receptors, and are silent partner of RAR, VDR, and thyroid hormone receptors (TRs), while RXR agonists can activate heterodimers of RXRs with LXRs, farnesoid X receptors (FXRs), and peroxisome proliferator-activated receptors (PPARs). Among synthesized compounds, both HX630 and PA042 can activate PPAR-RXR heterodimers, while only PA024 can activate LXR-RXR heterodimers. Selective functions of RXR ligands would be useful tools for elucidation of nuclear receptors. Besides activated hydrophobic vitamin A and D, vitamin K was reported to act as SXR ligand. SXR has significant roles in the detoxification and clearance of foreign toxic substances including drugs. We tried to develop novel vitamin K derivatives with unique biological activities.

RXR agonists



HX630 PA024

We also developed fluorescent ligands for nuclear receptors. Fluorescent ligands could be utilized for the elucidation of localization of the receptor under fluorescent microscopy, or for construction of screening assay system for novel ligand molecules. For example, 7-diethylamino-6-phenylcoumarin is specific antagonist for progesterone receptor (PR), and its fluorescence increased by binding to PR, which suggested that it might be a fluorescent sensor for PR.

PR antagonist (fluorescent)