receptor family. Odorant-bound ORs stimulate Gs-type G-proteins, Galpha-olf, which in turns activate cAMP-mediated signaling pathway in olfactory sensory neurons. To better understand the molecular basis for OR activation and G-protein coupling, we analyzed the effects of a series of site-directed mutations of a mouse OR, mOR-EG, on function. Thus, we identified amino acids involved in two steps of G protein activation by an OR, conformational changes within the OR and OR-G protein interactions. Our results provide insights into how ORs transition from an inactive to an active state, as well as where and how activated ORs interact with G-proteins. Recently, we have reported a rather surprising finding that insect ORs are not G protein-coupled receptors, but heteromeric ligand-gated ion channels. Despite the common function of the olfactory system as a chemosensor in various species, some differences between vertebrate and invertebrate appears to be found in properties of chemosignals, in receptor repertoire and their expression pattern, and in the mode of receptor signal transduction, which is likely a consequence of adaptation to the living environment during the processes of evolution.

2S10-2 嗅覚神経系の機能構築原理：嗅上皮から嗅球へ、さらには高次嗅覚中枢へ

Anatomical and functional architecture of the olfactory neural circuitry in zebrafish: from odor inputs to behavioral outputs

Yoshihiro Yoshihara (Lab for Neurobiology of Synapse, RIKEN Brain Science Institute)

Zebrafish is now becoming one of the most useful model organisms in neurobiology. In addition to its general advantageous properties (external fertilization, rapid development, transparency of embryos, etc.), the zebrafish is amenable to various genetic engineering technologies such as transgenesis, mutagenesis, gene knockdown, and transposon-mediated gene transfer. A transgenic approach unraveled two segregated neural circuits originating from ciliated and microvillous sensory neurons in the olfactory epithelium to distinct regions of the olfactory bulb, which likely convey different types of olfactory information (pheromones and odorants) to the higher olfactory centers. Furthermore, the two basic principles identified in mice, so-called "one neuron - one receptor rule" and "convergence of like axons to target glomeruli", are basically preserved also in the zebrafish, rendering this organism a suitable model vertebrate for studies of the olfactory system. In this talk, I will summarize recent advances in our knowledge on genetic, molecular, and cellular mechanisms underlying the development and functional architecture of the olfactory neural circuits in the zebrafish that lead to specific odor-induced behaviors.

2S10-3 ショウジョウバエの味受容細胞におけるモーダルシフト

Cross-modality sensing in gustatory receptor neurons of *Drosophila*

Teiichi Tanimura (Department of Biology, Graduate School of Sciences, Kyushu University)

Olfactory sensilla of *Drosophila* are located at the antennal segment and the maxillary palp. Gustatory sensilla are located at the labellum and the tarsal segment of legs. Both sensilla house sensory receptor neurons expressing seven transmembrane receptors, ORs and GRs. We expressed olfactory receptor genes in taste neurons to know if they gain ability to respond to odors. To this end we utilized a tungsten electrode recording method that enable us to record nerve responses to an odor. We found that taste neurons respond to odors if OR is expressed together with an ubiquitous Or83b. These results suggest the functional similarity between taste and olfactory neurons. Employing the Gal4-UAS system we expressed Or genes either in sugar cells or in bitter cells. Depending on the cell type a different feeding effect of an odor was observed. Sex pheromones are volatile or non-volatile in *Drosophila*. Volatile pheromones are sensed by olfactory neurons and we found that labellar Gr66a-expressing bitter-sensing taste neurons respond to a male specific non-volatile pheromone, 7-tricosene. 7-Tricosene is probably sensed by tarsal taste sensilla during courtship and we observed projection patterns from Gr66a-expressing neurons in tarsi. Projection patterns of Gr66a-expressing neurons from sensilla that might sense the sex-pheromone and those from other sensilla that are involved in feeding behavior are different. These new results demonstrate a cross-modality sensing in taste neurons of *Drosophila*.

2S10-4 衣擦れが苦痛に変わるとき

Touch sensation causes abnormal pain

Kazuhide Inoue (Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University)

Microglia are known as resident macrophages in the CNS. Microglia play a key role in neuropathic pain. Neuropathic pain is a severely disabling state that affects more than 15 millions of people in the world. This type of pain may be experienced after nerve injury. In the neuropathic pain state, touch stimulation frequently evokes strong pain sensation. We have shown that P2X4 receptors (P2X4Rs), which are upregulated in activated microglia in the spinal cord after nerve injury (Nature, 2003), and the stimulation of P2X4Rs causes release of brain-derived neurotrophic factor (BDNF) (Nature, 2005). BDNF causes a collapse of transmembrane anion gradient of lamina I neurons, resulting in the change of the inhibitory action of GABA to excitatory one. Thus, we postulate that BDNF released by the stimulation of P2X4Rs in microglia is a crucial signalling molecule for neuropathic pain. We also found that P2Y12 receptors and P2Y6 receptors are tightly related with not only chemotaxis or phagocytosis (Nature, 2007) but also the pain. These findings indicate that nucleotides play a key role in the communication between neuron and microglia.

3S1-1 はじめに：創薬の新局面と数理論の応用

New aspects of drug discovery and its representation by number theory

Kazuo Kuwata (Center for Emerging Infectious Diseases, Gifu Univ.)

Recent development in NMR techniques enabled us to observe the slow dynamics on the time scale of micro- to milliseconds of nuclei in a protein. However, the nature of such a slow motion is not well understood. For example, at room temperature the time constant of the refolding of prion protein is about 100 microseconds, but that of slow dynamics is between sub-millisecond to milliseconds. During such a slow motion, the native structure has a chance to unfold completely. In order to describe the protein dynamics in a large phase space, here we have constructed a novel representation theory, which is essentially based on the number theory. We could represent the conformational rearrangement with an extremely low probability. This formalism includes the Dirichlet series to represent the partition function, which consists of periodic trajectories with prime number steps.

3S1-2 蛋白質準安定状態の同定からプリオン病治療薬開発へ

The low-lying excited states: from identification to drug discovery

Yuji O. Kamatari (1). (1: Center for Emerging Infectious Diseases, Gifu Univ)

Proteins are intrinsically dynamic, which lead to excursions from the highly-populated ground state to less-populated excited states. The excited states, especially meta-stable states energetically close to the ground state, are thought to play important roles in protein function, stability, aggregation, lifetime etc. We have investigated the low-lying excited (N') states of lysozyme [1] and prion protein [2] using high pressure NMR spectroscopy and NMR relaxation. These data indicated that the conformational fluctuations are closely related to the cavity and hydration. In the case of prion protein, conformational transition to the scrapie form may occur through the N' states. Therefore, we have designed compounds that fit into a pocket created by residues undergoing the conformational rearrangements between the ground and N' states and found compounds that efficiently reduced formation of the scrapie form *in vitro* and *in vivo* [3]. Surface plasmon resonance and heteronuclear NMR showed that these compounds specifically binding to the flexible region around the pocket of the native protein. There results indicated that decrease of the N' population hampers the pathogenic conversion process of prion protein. [1] Y. O. Kamatari et al. (2001) Eur. J. Biochem. 268, 1782. [2] K. Kuwata et al. (2004) Biochemistry 43, 4439. [3] K. Kuwata et al. (2007) PNAS 104, 11921.

3S1-3 Understanding the Multiscale Dynamics of Complex Biological Systems from Single Molecule Experiments.

Understanding the Multiscale Dynamics of Complex Biological Systems from Single Molecule Experiments.

Chun Biu Li(1), Haw Yang(2) and Tamiki Komatsuzaki(1). (1: Molecule & Life Nonlinear Sciences Laboratory Research Institute for Electronic Science (RIES) Hokkaido University; 2:)

The rapid developments in the single molecule spectroscopy enable us to reveal detailed conformational dynamics of protein in the molecular level that would otherwise be masked in ensemble measurements. Recently we have established a novel data-driven methodology [1] to extract the underlying biological modeling directly from the single molecule experimental time series. The outcomes are the multiscale state-space networks describing the protein conformational dynamics at different timescales with high predictive power. Based on the current innovations of network theory [2], I will present in this talk the potentiality of using the multiscale state-space network as a mathematical platform for the study of diverse aspects of biological phenomena, such as the response from external stimulus, robustness and adaptability of protein functions, etc. We expect that our methodology can lead to new insights into biomedical applications, such as drug discovery, when prediction and controlling tasks are performed based on time series data. References: [1] "Multiscale Complex Network of Protein Conformational Fluctuation in Single-molecule Time Series", C.B. Li, H. Yang, T. Komatsuzaki, Proceedings of National Academy of Sciences USA, Vol. 15, pp536 (2008). [2] "Network Biology: Understanding the Cell's Functional Organization", A.-L. Barabasi, Z.N. Oltvai, Nature Reviews, Vol. 5, pp101 (2004).

3S1-4 低分子化合物の抗プリオン活性予測法構築に向けて：アッセイ結果を説明する評価関数の探索

Toward the construction of anti-prion activity prediction method for small compounds: modeling of evaluation scheme from assay results

Hironori K. Nakamura. (Division of Prion Research, Center for Emerging Infectious Diseases, Gifu University)

Prion diseases such as Creutzfeldt-Jakob disease (CJD) in human and bovine spongiform encephalopathy (BSE) in cow are infectious fatal neurodegenerative diseases, and there is no efficient treatment at this stage. These are caused by transformation of prion protein from normal cellular form to abnormal scrapie form. It is the important issue how we can find drug-candidate compounds for these diseases efficiently. Our group previously found a new anti-prion compound, GN8 with IC₅₀=1μM, by *in silico* screening [1]. After the discovery, we further conducted *in silico* screening to obtain anti-prion drug candidates, using AutoDock and ZINC compound database. We evaluated the anti-prion activity of selected compounds using the cell line model. Consequently, we obtained lots of compounds with a variety of anti-prion activity, and four compounds had particularly high anti-prion activity among two hundreds compounds [2].

Our aim here is to suggest better model/theory to describe our assay results. First, we exhaustively searched better protocol to recognize hit compounds more efficiently by focusing solely the energy/score of the best docking mode. It was not successful. Instead one statically calculated value using many docking mode information showed a better result for predicting anti-prion activity. A statistical view of docking for prion protein may be more important than the case of enzyme.

[1] Kuwata *et al.* (2007) *PNAS* 104, 11921-26.

[2] Hosokawa-Muto, Kamatari, Nakamura, Kuwata (in preparation)

3S1-5 レア・イベントとしてのタンパク質ダイナミクスと複合体形成 Protein dynamics as rare event, in connection with complex formation

Akio Kitao (1,2). (1: *Inst Cell Biol Sci, Univ of Tokyo*; 2: *JST, CREST*)

If protein dynamics is examined energetically in close-up view, protein native state should be found to be highly frustrated as commonly seen in other condensed systems. This frustration also appears as multiple conformational substates on protein energy landscape. At room temperature, a protein molecule tends to be trapped around a single substate within Terahertz time range or so but conformational transitions from one substate to another are more likely to occur in slower time range.

In highly frustrated systems, it is expected that relatively weak perturbation can induce conformational change among substates. Also in the process of complex formation of proteins, inter-molecular interaction can play a role as a sort of perturbation and may induce conformational change.

Conformational change between substates separated by relatively high energy barrier should occur as "rare" events, which is expected to be essential to various protein functions including complex formation processes. In this talk, I am going to consider the connection between rare protein dynamics and complex formation.

3S1-6 量子化学計算に基づく創薬研究へのアプローチ - プログラム開発と生体分子への応用 -

Drug discovery based on quantum mechanical calculation: program development and application to a biomolecular system

Takeshi Ishikawa (1). (1: *Division of Prion Research, Center for Emerging Infectious Disease, Gifu University*)

In recent years, ab initio quantum mechanical calculations have been applied to large molecules including biomolecular systems. The fragment molecular orbital (FMO) method, which was developed by Kitaura *et al.*, is one of the most efficient approaches for the quantum mechanical investigation of such molecules. In the FMO method, a target molecule is divided into small fragments and only calculations of the fragments and pairs of fragments are required for evaluating the total energy or the total density. This feature reduces the computational effort significantly and enables us to carry out quantum mechanical calculations of large molecules. Additionally, the inter fragment interaction energy (IFIE) can be obtained in the FMO calculations. The IFIE is known to be very useful for analyzing the interaction in biomolecular systems such as ligand-protein interaction. In this speech, we will show the potential of the FMO method in the field of drug discovery. At the beginning of my speech, the theoretical aspects of the FMO method will be explained. Second, the results of calculations for the prion protein (PrP) complexed with a small molecule will be shown as an illustrative example of application to a real biomolecular system. Finally, we will introduce the drug discovery software developed in our laboratory.

3S2-1 Mg²⁺輸送体 MgtE による Mg²⁺ホメオスタシス機構の構造・機能解析 Structure and Mechanism of the MgtE Mg²⁺ transporter

Motoyuki Hattori (1). (1: *Graduate of School of Bioscience and Biotechnology, Tokyo Institute of Technology*)

The magnesium ion Mg²⁺ is a vital element involved in numerous physiological processes. It remains obscure how Mg²⁺ transporters selectively recognize and dehydrate the large, fully hydrated Mg²⁺ cation for transport. The MgtE family of Mg²⁺ transporters is ubiquitously distributed in all phylogenetic domains, and human homologues have been functionally characterized and suggested to be involved in magnesium homeostasis. Recently we reported the crystal structures of the full-length *Thermus thermophilus* MgtE at 3.5 Å resolution, and of the cytosolic domain in the presence and absence of Mg²⁺ at 2.3 Å and 3.9 Å resolutions, respectively (Hattori, *et al.*, 2007 *Nature*). Based on these structures, we proposed a Mg²⁺ homeostasis mechanism. However, the present resolution of the full-length structure does not allow a detailed discussion on its coordination chemistry and ion its ion selectivity. Moreover, functional analyses are necessary to provide evidence to support the proposed homeostasis mechanism. Here we solved the crystal structure of the full-length MgtE at 2.9 Å resolution. In this structure, we identified new putative Mg²⁺ binding sites. Moreover, a combination of genetic and biochemical techniques demonstrates that the proposed Mg²⁺ homeostasis mechanism, in which the cytosolic domain of MgtE function as a Mg²⁺ sensor regulating the gating of the channel by sensing the intracellular Mg²⁺ concentration.

3S2-2 神経伝達物質トランスポーターホモログに見る膜を介した物質輸送の構造基盤

Structural basis for substrate transport across a lipid bilayer by a neurotransmitter transporter homolog

Atsuko Yamashita (RIKEN Spring-8 Center)

Secondary transporters are integral membrane proteins which catalyze translocation of a wide array of chemical substances across a membrane bilayer utilizing pre-existing ionic electrochemical gradients. LeuT, a sodium-dependent leucine transporter from *Aquifex aeolicus*, is one amenable to high-resolution crystallographic structure analyses as well as detailed biochemical analyses.

LeuT is a stable homolog protein of Na⁺/Cl⁻-dependent neurotransmitter transporters (neurotransmitter sodium symporters; NSS). NSS plays significant roles for termination of synaptic transmission in the central nervous system of vertebrates by uptake of neurotransmitters, including the biogenic amines (serotonin, dopamine, etc.) and amino acids (γ-aminobutyric acid, glycine, etc.), from the synapse to the cytoplasm of neurons and glia. Dysfunction of these transporters causes various psychiatric disorders, such as depression. NSS members are the targets of therapeutic compounds for these diseases, such as antidepressants.

The first crystal structure of LeuT was solved at 1.65 Å under a state with the substrates, L-leucine and sodium ions, binding to the molecule. We recently found that tricyclic antidepressants (TCA), which target NSS, non-competitively inhibit the LeuT transport, and solved the structure of LeuT under an "inhibited" state in complex with TCA. Structural bases for the LeuT transport will be discussed based on these crystal structures under different functional states, and results from accompanying functional analyses.

3S2-3 細菌型 Sec トランスロコン複合体の結晶構造解析 Crystal structure of bacterial Sec translocon

Tomoya Tsukazaki (1), Hiroyuki Mori (2), Shuya Fukai (3), Ryuichiro Ishitani (4), Anna Perederina (5), Dmitry G. Vassilyev(5), Koreaki Ito (2), Osamu Nureki (1,4). (1 : *Graduate School of Bioscience & Biotechnology, Tokyo Institute of Technology*; 2 : *Institute for Virus Research, Kyoto Univ.*; 3 : *SRRO, Univ. of Tokyo*; 4 : *Institute of Medical Science, Univ. of Tokyo*; 5 : *Dep. of Biochemistry & Molecular Genetics, Univ. of Alabama*)

The Sec translocation machinery facilitates the movement of newly-synthesized secretory and membrane proteins across and into membranes. The prokaryotic SecYE complex, embedded in the cytoplasmic membrane, functions as an evolutionarily conserved core component of the protein-conducting channel, the Sec translocon. Here, we present the first crystal structure, at 3.2 angstrom resolution, of the SecYE complex from a SecA (bacterial translocation motor)-containing organism, *Thermus thermophilus*, in complex with a specific Fab fragment. Comparisons with the previously reported structures of the Sec translocon revealed a significant conformational change in the transmembrane helices, which creates a hydrophobic crevasse that is open to the cytoplasm and is probably used for signal peptide entry. Interestingly, the Fab fragment interactions with SecY involve the SecA-binding interface at the tip of the cytoplasmic protrusion, possibly mimicking the SecY-SecA contacts and stabilizing this "pre-open" state of SecY. Intramolecular disulfide cross-linking experiments revealed that the pre-open state also exists in physiological membranes, and it probably represents the first conformational transition of the Sec translocon during protein translocation.

3S2-4 Bacterial multidrug efflux transporter, AcrB - The pumping mechanism

Bacterial multidrug efflux transporter, AcrB - The pumping mechanism

Satoshi MURAKAMI (1) (1: *Dept. Life Science, Tokyo Institute of Technology*)

Resistance nodulation cell-division (RND) transporters are one of the main cause of the bacterial multidrug resistance. They pump a wide range of antibiotics out of the cell by proton motive force. AcrB and its homologues are the major RND transporter in *Escherichia coli* and other Gram-negative bacteria. Recently, the crystal structures of AcrB have been determined by different space groups. All these structures are consistent with asymmetric trimer. Each monomer has different conformation corresponding to one of the three functional states of the transport cycle. Transporting hydrophobic drug was bound in the periplasmic domain on one of the three monomers. The transport pathway with alternating access mechanism is located at the hydrophilic domain protruded into the periplasmic space while this mechanism of other transporter families like ABC and MFS transporter is located in the membrane embedded region. For the RND, protonation might take place also asymmetrically at the functionally important charged residues in the transmembrane region. The structures indicate that drugs are transported by a three-step functional rotation in which substrates undergo ordered binding change.

[1] Murakami, S. *et al.*, *Nature*, 1997, **419**, 587.[2] Murakami, S. *et al.*, *Nature*, 2006, **443**, 173.

3S2-5 古細菌型ロドプシンと無脊椎動物のロドプシンとの構造比較 Structural comparison of archaeal and invertebrate rhodopsins

Tsutomu Kouyama (1) (1: *Dept Physics, Graduate School of Science, Nagoya University*)

For structural investigation of oligomeric complexes of archaeal rhodopsins, we have previously developed the membrane fusion method, by which the trimeric bacteriorhodopsin-lipid complex was crystallized into the hexagonal P622 crystal. Recently, we applied this method to prepare the P321 and P63 crystals of archaeorhodopsin-2 without destroying the trimeric structure [1]. These crystals provided information as to the physiological roles of native lipids including bacterioruberine. Structural comparison of archaeal rhodopsins showed that the structure of the retinal-binding pocket was well conserved, while the structure of the protein-lipid interface was more variable during the evolution. For structural investigations of invertebrate rhodopsins, we also tried to develop a