Why Commercial Banks Held Excess Reserves: The Japanese Experience of the Late 1990s

We investigated, empirically, why Japanese banks held excess reserves in the late 1990s. Specifically, we pin down two factors explaining the demand for excess reserves: a low short-term interest rate, or call rate, and the fragile financial health of banks. The virtually zero call rate increased the demand for excess reserves substantially, and a high bad loans ratio largely contributed to the increase in excess reserve holdings. We found that the holdings of excess reserves would fall by two-thirds if the call rate were to be raised to its level prior to the adoption of the zero-interest-rate policy, and the bad loans ratio were to fall by 50%.

**JEL codes:** E42, E51, E52, G21

**Keywords:** excess reserves, bad loans, zero-interest-rate policy.

**Japanese banks** have chronically held excess reserves since the late 1990s. Figure 1 illustrates the ratio of actual reserves to required reserves for commercial banks as a whole. The increasing trend in the excess reserve ratio has been conspicuous since the summer of 2001, and this ratio reached a high of 5.88 in October 2003.\(^1\) The excess reserve ratio typically parallels the supply of reserves. In fact, the reserve supply began to increase when the Bank of Japan announced that it would provide ample funds to push down the uncollateralized overnight call rate, or short-term inter-bank money market rate, as low as possible, in February

\(^1\) There are two spikes in the figures, each of which corresponds to Year 2000 and Fiscal Year 2002 problems. On those occasions, the policy authority provided ample liquidity to meet a surge in demand and secure stability in the financial markets.

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Why Commercial Banks Held Excess Reserves: The Japanese Experience of the Late 1990s

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Introduction

In the midst of severe depression during the 1990s, the Bank of Japan (BOJ) adopted the "zero-interest-rate policy" to set the call rate or the short-term inter-bank interest rate as low as possible in February 1999. Furthermore, the BOJ switched its operating target for money market operations from the call rate to the outstanding balances of the reserves held at the BOJ in March 2001. The BOJ committed itself to fulfill its policy goal and supplied tremendous amount of reserves to commercial banks. Commercial banks are required to hold certain amount of reserves, depending on the amount and type of deposits, at their current accounts at the BOJ. The reserves in excess of required reserves are called excess reserves. It should be noted that even if the banks keep excess reserves, there is no interest earned on it. In spite of no interest income the banks still held reserves in far excess of required ones. Figure 1 shows the ratio of actual reserves to required reserves for commercial banks as a whole. The increasing trend in the excess reserve ratio has been conspicuous since the summer of 2001, and this ratio reached a high of 5.88 in October 2003. Figure 2 illustrates the frequency distribution of the excess reserve ratios of 145 commercial banks of our panel data set in 1994, 1999 and 2001. The frequency distribution was more dispersed and less skewed to the right in later years.

The purpose of this paper is twofold. First, we construct a theoretical model to explain this phenomenon. Second, we test the implications derived from the theoretical model using the panel data of Japanese commercial banks. We find that our theoretical model explains the banks’ behavior to hold excess reserves reasonably well. Empirical investigation reveals that virtually-zero short-term interest rate and banks’ balance sheet deterioration by bad loans are responsible for banks’ holding excess reserves.

A Theoretical Model of Bank Demand for Reserves

We assume that the bank optimally allocates its resources between the interest-bearing asset and reserves with no interest, to maximize its expected interest income, while simultaneously taking into account the cost incurred in the case of reserve shortages (Freixas and Rochet(1997)). It is possible for the bank to put all the resources into interest-bearing asset but then the bank runs the risk of being unable to meet large, unanticipated withdrawals of deposits. Such a situation may be costly for the bank, as it gives rise to apprehension among depositors regarding the fragility of the bank’s balance sheet, and depositors may thus be prompted to further withdraw their deposits.

It is shown that the optimal demand for reserves is determined so that marginal decrease in the liquidity shortage cost brought about by increasing reserves, or the marginal benefit of increasing reserves may be equal to the marginal cost of increasing reserves. Figure 3 illustrates the way in which the optimal level of excess reserves is determined. The vertical axis measures the probability that the deposit withdrawal exceeds reserves and the horizontal axis measures reserves. The downward-sloping curve shows the bank’s subjective probability that the deposit withdrawal exceeds reserves, which decreases as reserves increase. When the downward-sloping curve intersects with the ratio of interest rate (rL) to the penalty rate (rp,i) or marginal cost of holding reserves, the optimal reserve holdings (R) is determined. The penalty rate is the loss the bank will incur in case of reserve shortage. The ratio of interest rate to the penalty rate measures the opportunity cost of holding reserves.

The demand for reserves increases as the interest rate falls and the penalty rate rises. This model, while simple, can explain the surge in excess reserve holdings by Japanese banks that took place in the late 1990s. The call rate was virtually zero under the zero-interest-rate policy, and the BOJ maintained the call rate to a level as low as possible under the new monetary policy regime.

When the balance sheet of a bank deteriorates by non-performing loans, the bank may anticipate a large amount of...
deposit withdrawals. In other words, the downward-sloping curve of the bank's subjective probability of deposit withdrawal might shift upward. It implies that the bank maintains higher level of reserves for precautionary purpose.

Estimation of Bank Demand Equations for Reserves

As was shown above, the optimal demand for reserves crucially depends on the bank's perception of deposit withdrawal. By assuming a specific distribution function for stochastic deposit withdrawal, we can derive the optimal demand equation for reserves to be estimated. In particular we assume a Pareto distribution with its parameters affected by the bank's balance sheet or the ratio of bad loans to total loans as well as deposits.

For estimation purposes, we transform the demand equation for reserves into the deviation from required reserves. In one case, banks do not hold any excess reserves, so the deviation of optimal reserves from the required level is zero. In the other case where the optimal demand for reserves exceeds the required level, the demand for excess reserves is expressed as a function of deposit, bank's health and opportunity cost of holding reserves (the ratio of call rate to the penalty rate). Since we can identify from the data which banks held excess reserves, we may estimate the system of reserve demand equations, using censored regression models.

Using the panel data set of 145 individual banks (9 city banks, 3 long-term credit banks, 7 trust banks, and 126 regional banks) during 1991-2002, we estimate the optimal demand equation for reserves. Note that the bad loans ratio is only available after 1997, so that we also use the rate of change in the share price of individual banks. Use of share price change has the merit that we can make full use of the whole sample period from 1991 in estimation.

The estimation is conducted for six specifications, where three variables are used to represent the banks' financial health (two measures of bad loans ratio and rate of change in the bank's share price), and where two account for the penalty rate (constant case and the ratio of operating profits to total equity as the variable penalty rate). The estimation results are quite satisfactory and stable irrespective of specifications. The coefficient estimates of the three key variables determining the demand for reserves are statistically significant. Deposits exert a significantly positive effect on reserve holdings. The call rate has a negative effect on the demand for reserves. The two bad loans ratios also affect the demand for reserves positively, which implies that banks with higher bad loans ratios increased their reserves. Furthermore, increases in the rate of change in share prices decreased the demand for reserves significantly.

Based on the parameter estimates of the demand equation for reserves, and using simulation analysis, we evaluated quantitatively the extent to which changes in the short-term interest rate and/or banks' bad loans ratios affected the demand for excess reserves. Specifically, we took the following steps. First, we calculated the theoretical values of excess reserves by substituting the actual exogenous variables for 2002 into the estimated demand equation for reserves, and subtracted the required reserves. Next, we calculated the predicted value of excess reserves under different scenarios of the call rate and the bad loans ratio. We assumed that the call rate was raised to its level prior to the adoption of the zero-interest-rate policy. In other words, the call rate was set to its level in March 1998, or to 0.25%. With regard to the banks' financial health, we considered a case in which the bad loans ratio was halved. This scenario is consistent with the Koizumi Structural Reform Plan, introduced in October 2002, which specified that non-performing loans should be reduced by 50% within one year. The predicted excess reserves thus calculated were summed up across banks, and the aggregated excess reserves were compared with their baseline value. Figure 4 shows the extent to which banks' excess reserves are reduced under the different scenarios for the call rate and the bad loans ratio under the specification where the penalty rate is constant and the ratio of non-performing loans under the Financial Reconstruction Law is used. When the call rate is raised, excess reserves are reduced by as much as 70%. When the banks' financial health is improved, excess reserves are reduced by 13%. When increases in both the call rate and banks' financial health are implemented simultaneously, the holdings of excess reserves are reduced almost by 75%.

Conclusions

We empirically investigated why Japanese banks held excess reserves in the late 1990s. We were able to pin down two factors that explain the demand for excess reserves: a low short-term interest rate, or call rate, and banks' fragile financial health. The nearly zero call rate substantially increased the demand for reserves, and the high bad loans ratio also contributed to the observed increase in reserve holdings. A quantitative evaluation of these factors was also conducted. It turns out that our simulation exercise is pretty accurate in predicting the amount of excess reserves thereafter. After the bad loans ratio peaked in 2002, it has exhibited a declining trend and now it is more than halved than its peak value for the banking sector as a whole. The call rate also started to rise after the BOJ ceased quantity-easing policy in 2006 March. Then the actual total excess reserves in 2006 were reduced by 71% compared to 2004 figures. It is exactly what our model predicted, hinting that our model of the bank's demand for reserves in the late 1990s is correctly specified.

References

Three-Dimensional Bulk Fermiology of $\text{CeRu}_2\text{Ge}_2$ in the Paramagnetic Phase by Soft X-Ray $h\nu$-Dependent (700–860 eV) ARPES

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By virtue of the soft x-ray angle-resolved photoelectron spectroscopy, the three-dimensional bulk fermiology has been successfully performed for a strongly correlated Ce compound, ferromagnet CeRu$_2$Ge$_2$ in the paramagnetic phase. A clear difference of the Fermi surface topology from either band calculation or the Haas–van Alphen results in the ferromagnetic phase is observed and interpreted by considering the difference of the 4f contribution to the Fermi surfaces in the paramagnetic phase.

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Fermi surface (FS) topology dominates the macroscopic properties of solids such as resistivity, specific heat, and magnetic susceptibility. Therefore, the study of FSs is essential. The quantum oscillation measurement using the de Haas–van Alphen (dHvA) effect is known [1] as a powerful technique to evaluate the cross sections of the FSs. The dHvA measurement has so far been applied to many strongly correlated rare-earth materials [2−4]. The consistency between this experimentally observed FS and the band-structure calculation for CeSe$_2$ [5,6] is understood as the dHvA measurement, a conclusive tool to qualitatively judge whether the 4f electrons are “itinerant” or “localized” in the ground state of strongly correlated Ce compounds. However, a FS which is composed by high effective mass electrons could not be observed by the dHvA measurement [7] because the observation of such FSs requires a high-magnetic field and an ultralow temperature. In addition, the FSs change their shapes in accord with possible phase transitions at higher temperatures above several tens of K, where the dHvA measurement is inapplicable.

The low-$h\nu$ angle-resolved photoelectron spectroscopy (ARPES) is known to be a useful technique to reveal the characters of the two-dimensional and/or surface FSs as seen in many cases of high-$T_c$ cuprates [8]. When the surface electronic structures are not much different from the bulk electronic structures, even surface-sensitive low-$h\nu$ ARPES with changing $h\nu$ in the normal emission could reveal the bulklike band dispersions along the surface normal direction [9]. As for correlated electron systems, however, the low-$h\nu$ three-dimensional (3D) ARPES band mapping has been rarely applied for fermiology except for the cases of simple transition metals [10] because the surface vertical dispersion ($k_z$) is very different from the bulk $k_z$ dispersion. For rare-earth compounds, ARPES measurements have been performed for $\text{XRu}_2\text{Si}_2$ ($\text{X} = \text{La, Ce, Th, U}$) by using $h\nu$ within a range of 14–220 eV [11]. Recently, high-energy ($h\nu > 360$ eV) photoemission became feasible with high energy resolution and is found to be very effective for probing bulk electronic structures [12−15]. In this Letter, we demonstrate the potential of soft x-ray $h\nu$-dependent ARPES for clarifying the bulk 3D FS topology of a strongly correlated rare-earth compound, whose 4f electronic states are mutually very different between the bulk and the surface [13].

We have performed the 3D ARPES measurements for CeRu$_2$Ge$_2$ at 20 K which shows a ferromagnetic transition at $T_F \sim 8$ K [16,17]. CeRu$_2$Ge$_2$ crystalsizes into a tetragonal ThCr$_2$Si$_2$-type structure with $a = 4.268$ Å and $c = 10.07$ Å (at 18 K [16]), whose Brillouin zone is shown in Fig. 1. The 4f electrons are thought to be rather localized and have a ferromagnetic alignment because of RKKY interaction under $T_F$ [16,18,19]. On the other hand, isostructural CeRu$_2$Si$_2$ is a typical heavy fermion system which has itinerant 4f electrons [19,20]. The difference between these materials was observed by the bulk-sensitive 3d-4f resonant photoemission [13]. Thermoelectric power

FIG. 1. Brillouin zone of the body-centered tetragonal crystal CeRu$_2$Ge$_2$ with [1−A−Z] = 2\pi/\alpha, in plane [1−Z] = 2\pi/c.
Probing Three-dimensional Electronic Structures and Fermi Surfaces of a Crystalline Solid by High-Energy Angle-Resolved Photoemission

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Introduction

Clarification of electronic structures in crystalline solids is fundamental to understand their physical properties in the field of condensed matter physics and materials science. Especially, the characters and shapes of the Fermi surfaces (momentum distribution of electrons with the highest energy in a metal) are responsible for many intriguing phenomena such as high-temperature superconductivity, metal-insulator transitions, magnetic ordering, and heavy Fermion behavior. Angle-resolved photoemission (ARPES) is known as a powerful tool to simultaneously detect the electronic dispersions (relations between the electron momentum and energy, namely, electronic structures) and the Fermi surfaces [1]. However, conventional low-excitation energy (hv \(< 120\) eV) ARPES mainly reflects the surface electronic states, which are often noticeably deviated from the bulk states for strongly correlated materials. Since the bulk electronic structures are responsible for the physical properties in solids, a technique for probing the bulk electronic structures is very important for studying solids. To overcome the surface sensitivity, high-excitation energy photoemission is promising due to its longer photoelectron escape depth [2] as shown in Fig. 1. Indeed, we have so far measured surface-sensitive low-energy and bulk-sensitive high-energy angle-integrated photoemission spectra reflecting the electronic density of states in the solids for strongly correlated transition metal oxides \(\text{Sr}_2\text{CaVO}_3\) (Fig. 2), demonstrating the qualitative differences in the electronic states between the bulk and surface [3]. Here we show that the power of high-energy ARPES applied for a Ce-based strongly correlated material to clarify the bulk three-dimensional electronic structures and the Fermi surfaces.

Fig. 1. Schematic description for the bulk (surface) sensitivity of high-low-energy photoemission.

Fig. 2. Bulk and surface angle-integrated photoemission spectra of \(\text{Sr}_2\text{CaVO}_3\) obtained by measuring the spectra at \(hv=40.8, 275\) and \(500\) eV [3].

Experimental Details for High-Energy ARPES

In order to achieve a breakthrough for studying the bulk electronic structures in solids, we have constructed a high-energy soft X-ray ARPES system combined with a high-resolution monochromator on a twin-helical undulator beam line BL25SU of SPring-8 [4]. Figure 3 shows an example of the high-energy ARPES data, spectra of \(\text{Sr}_2\text{RuO}_4\) [5], from which the electronic dispersions and the loci on the Fermi surfaces are found along one cut in the momentum space.

Fig. 3. Example of the high-energy ARPES data [5]. About eighty ARPES spectra (Energy distribution curves, EDCs) as well as momentum distribution curves (MDCs) near the Fermi level (binding energy of \(0\) eV) are simultaneously obtained by “one-shot” measurement. The peak positions (momenta) in the MDC at \(E_F\) indicated by blue horizontal arrows represent the loci on the Fermi surfaces.

In the ARPES process, the photoelectron momentum along the perpendicular direction to the sample surface (pz) changes with polar and azimuthal angles. Since the obtained information on the Fermi surfaces is two-dimensional, this technique is useful for layered compounds with “weak” electron correlation and/or ordered surfaces. In the ARPES process, the photoelectron momentum along the perpendicular direction to the sample surface (pz) can be specified as a function of photoelectron kinetic energy (nearly the same as the excitation energy hv in the case of the electron near the Fermi level) by using a nearly free photoelectron model, which is applicable for the high-energy ARPES. In addition, due to its long photoelectron mean free path, the high-energy ARPES has another advantage in well resolving pz by controlling the excitation energy hv.
energy. As schematically shown in Fig. 4, we can detect three-dimensional bulk Fermi surfaces of solids by combination by conventional two-dimensional Fermi surface mapping at fixed hv and another mapping as a function of hv and one polar angle along one direction.

**Bulk Fermi Surfaces of CeRu$_2$Ge$_2$ at a "High" Temperature**

In rare-earth compounds, 4f electrons tend to be localized compared with the other orbital electrons due to strong Coulomb repulsive interaction (electron correlation) although the 4f electron energy is close to the Fermi level. Ce-based compounds with the 4f electron number of ~1 show varieties of the 4f states due to different hybridization between the 4f and valence electrons. For instance, CeRu$_2$Si$_2$ shows the heavy Fermion behavior with enormous effective electron mass enhancement [6]. On the other hand, CeRu$_2$Si$_2$, which is isostuctural to CeRu$_2$Si$_2$, undergoes a ferromagnetic transition at ~8 K for which the localized 4f electronic magnetic moment is responsible [7]. Although the Fermi surfaces of CeRu$_2$Si$_2$ have been revealed at low temperatures in the magnetic phase by a quantum oscillation measurement [8], its electronic structures as well as the Fermi surfaces have not been clear in the paramagnetic phase above ~8 K. In order to reveal them, we have performed the high-energy ARPES for CeRu$_2$Ge$_2$ at a "high" temperature of 20 K.

**Figure 5 shows the high-energy ARPES spectra (EDCs) of CeRu$_2$Ge$_2$ in the paramagnetic phase.** We can recognize that the energy positions of many peaks and shoulders in the spectra are changed depending on momentum. These peak energies as a function of momentum reflect the electronic dispersions. In addition, these structures change three-dimensionally in the momentum space, which is indication of the bulk electronic dispersions since the surface electrons are expected not to disperse along the $p_z$ direction. From the ARPES measurements, we have obtained the cross-sections of the Fermi surfaces for CeRu$_2$Ge$_2$ at the high temperature (20 K) along the [110] and [001] planes including the Z (0,0,0) point in the momentum space as shown in Fig. 6. From these cross-sections, small and large ellipsoidal Fermi surfaces centered at the Z point, and small cylinder-like Fermi surfaces centered at the $X$ (1/2a,1/2a,0) point are expected. Based on the high-energy ARPES results displayed here, we can obtain the qualitative shapes of the three-dimensional Fermi surfaces for CeRu$_2$Ge$_2$ in the paramagnetic phase as shown in Fig. 7. Although the ellipsoidal Fermi surfaces are similar to those in the magnetic phase [8] and in a theoretical result based on a localized 4f model [9], it is found that the cylinder Fermi surfaces are qualitatively different from those in the different phase and in the theoretical result.

**Outlook**

We have developed the new technique for probing three-dimensional electronic dispersions (structures) and Fermi surfaces by using high-energy ARPES, which has been applied for the strongly correlated electron system CeRu$_2$Ge$_2$. To date, such quantum oscillation measurements as observation of de Haas-van Alphen effect are known as powerful tools to investigate the Fermi surfaces of solids although they require low temperatures as several K and almost detect-free high-quality stoichiometric single crystals. The high-energy ARPES can be applicable also for non-stoichiometric doped materials such as high-temperature superconductors even at high temperatures. We are convinced that the high-energy ARPES will become another complementary and powerful technique for probing the bulk Fermi surfaces in solids in near future.

**References**

Data Replication for Improving Data Accessibility in Ad Hoc Networks

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Abstract—in ad hoc networks, due to frequent network partition, data accessibility is lower than that in conventional fixed networks. In this paper, we solve this problem by replicating data items on mobile hosts. First, we propose three replica allocation methods assuming that each data item is not updated. In these three methods, we take into account the access frequency from mobile hosts to each data item and the status of the network connection. Then, we extend the proposed methods by considering aperiodic updates and integrating user profiles consisting of mobile users’ schedules, access behavior, and read/write patterns. We also show the results of simulation experiments regarding the performance evaluation of our proposed methods.

Index Terms—Ad hoc networks, replication scheme, data accessibility, data update.

1 INTRODUCTION

Recently, there has been an increasing interest in ad hoc networks, which are constructed by mobile hosts [21] [5]. In ad hoc networks, every mobile host plays the role of a router, and they communicate with each other. Even if the source and destination mobile hosts are not in the communication range of each other, data packets are forwarded to the destination mobile host by relaying transmission through other mobile hosts which exist between the two mobile hosts. Since no special infrastructure is required, in various fields such as military and rescue affairs, many applications are expected to be developed for ad hoc networks.

In ad hoc networks, since mobile hosts move freely, disconnections occur frequently, and this causes frequent network partition. If a network is partitioned into two networks due to the migrations of mobile hosts, mobile hosts in one of the partitions cannot access data items held by mobile hosts in the other. Thus, data accessibility in ad hoc networks is lower than that in conventional fixed networks. In ad hoc networks, it is very important to prevent the deterioration of data accessibility at the point of network partition. A possible and promising solution is the replication of data items at mobile hosts which are not the owners of the original data.

Since mobile hosts generally have poor resources, it is usually impossible for them to have replicas of all data items in the network. For example, let us suppose a situation where a research project team engaged in excavation work constructs an ad hoc network on a mountain. The results obtained from the investigation may consist of various types of data such as numerical data, photographs, sounds, and videos. In this case, although it is useful to have the data that other members obtained, it seems difficult for a mobile host to have replicas of all the data.

In this paper, we assume an environment where each mobile host has limited memory space for creating replicas and each data item is not updated. First, we propose three replica allocation methods for improving data accessibility. Then, we extend these three methods by considering aperiodic data updates since, in a real environment, updates do occur aperiodically. These extended methods also accommodate user schedule and emergency objects. In this paper, a mesoscale ad hoc network consisting of a few dozen mobile hosts is assumed, and our proposed methods are mainly designed for such a mesoscale network. We verify the effectiveness of our proposed methods by simulation experiments. Note that the mobile hosts that are connected to each other by one-hop or multihop wireless links are simply called connected mobile hosts. That is, connected mobile hosts do not only represent those connected by one-hop links (i.e., neighbors), but also represent those connected by multihop links.

The remainder of this paper is organized as follows: In Section 2, we introduce some related works. In Section 3, we propose replica allocation methods. In Section 4, we extend the methods proposed in Section 3 to adapt to environments with data update. In Section 5, we show the results of simulation experiments. In Section 6, we discuss how we can reduce the number of accesses to old replicas. Finally, in Section 7, we summarize this paper. We note that some of the results of this paper have been reported in [8] and [10].

2 RELATED WORKS

Many routing protocols in ad hoc networks have been proposed in IETF (Internet Engineering Task Force) and other research groups [16], [20], [21], [22]. These protocols improve connectivity among mobile hosts at the network level. Thus, these protocols are useful for applications where users equipped with mobile hosts directly communicate.
Data Replication for Improving Data Accessibility in Ad Hoc Networks

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INTRODUCTION

Recently, there has been an increasing interest in ad hoc networks which are constructed by mobile hosts[1]. In ad hoc networks, every mobile host plays the role of a router, and they communicate with each other in a multi-hop manner by relaying transmission through other mobile hosts which exist between the source and destination. In ad hoc networks, since mobile hosts move freely, disconnections occur frequently, and this causes frequent network partition. If a network is partitioned into two networks, mobile hosts in one of the partitions cannot access data items held by mobile hosts in the other. Since there are many applications where mobile hosts access data items held by other mobile hosts, this problem is very significant in ad hoc networks. Thus, to solve this problem, in this paper, we first propose three replica allocation methods for improving data accessibility assuming that each data item is not updated. Then, we extend these three methods by considering aperiodic data updates. These extended methods also accommodate user schedule.

Replication Schemes

Since mobile hosts generally have poor resources, it is usually impossible for them to have replicas of all data items in the network. Moreover, since mobile hosts move freely and the network topology dynamically changes, the static optimal allocation of replicas does not exist. Thus, efficient and effective replica relocation is crucial for system performance[2],[3].

In this paper, we take the following heuristic approaches: (i) Replicas are relocated in a specific period (relocation period), and (ii) During every relocation period, replica allocation is determined based on the access frequency from each mobile host to each data item and optionally the network topology at the moment. Based on this approach, we proposed three replica allocation methods which differ in emphasis put on access frequency and network topology:

SAF (Static Access Frequency) method: Each mobile host allocates replicas of data items in descending order of its access frequencies to these items within the limit of its own memory space. Fig. 1 shows an example of executing the SAF method.

In the SAF method, mobile hosts do not need to exchange information with each other for replica allocation. Moreover, replica relocation does not occur after each mobile host allocates all necessary replicas. As a result, this method allocates replicas with low overhead and low traffic. On the other hand, since each mobile host allocates replicas based only on its access frequencies to data items, mobile hosts with the same access characteristics allocate the same replicas. Therefore, SAF provides low data accessibility when many mobile hosts have the same or similar access characteristics.

DAFN (Dynamic Access Frequency and Neighborhood) method: To solve the problem with the SAF method, the DAFN method eliminates the replica duplication among neighboring mobile hosts. First, this method preliminarily determines the replica allocation in the same way as the SAF method. Then, if there is replica duplication of a data item between two neighboring mobile hosts, mobile host with the lower access frequency to the data item changes the replica to another replica. Since the neighboring status changes as mobile hosts move, the DAFN method is executed during every relocation period.

DCG (Dynamic Connectivity based Grouping) method: The DCG method shares replicas in larger groups of mobile hosts than DAFN. At every relocation period, each mobile host broadcasts its host identifier. After all mobile hosts complete the broadcasts, every host knows the connected mobile hosts and the network topology from the received host identifiers. In each set of mobile hosts connected to each other, the mobile host with the lowest host identifier executes an algorithm to find bi-connected components with the network topology known by received messages. Even if a mobile host belongs to more than one bi-connected component, it can only belong to one group in which the corresponding bi-connected component was found first. By grouping mobile hosts as bi-connected components, the group is not divided even if one mobile host disappears from the network or one link is disconnected in the groups. Thus, it is assumed that the group has high stability.

Next, replicas of data items are allocated on mobile hosts in each group in descending order of the access frequencies in the group. More specifically, in each group, the mobile host with...
the lowest host identifier becomes the coordinator of the group, who calculates the group’s access frequency to each data item as a summation of access frequencies of mobile hosts in the group to the item. Then the coordinator determines replica allocation in the group according to the calculated access frequencies. Fig. 2 shows an example of executing the DCG method.

Compared with the DAFN method that shares replicas among neighboring hosts, the DCG method shares replicas in larger groups of mobile hosts that have high stability. Thus, data accessibility is expected to be higher. However, both the overhead and the traffic are higher than the other two methods because at each relocation period, mobile hosts exchange information and widely relocate replicas.

Extensions considering Data Update

The methods proposed in the previous section assume that data items are not updated. In a real environment, it is more likely that data items are randomly updated. Therefore, we also propose three extended replica allocation methods, E-SAF+, E-DAFN+, and E-DCG, that are adapted to an environment where data items are randomly updated.

To cope with data update, we use Read/Write Ratio (RWR = R/W) in the algorithms of the SAF, DAFN, and DCG methods instead of access frequencies. Here, Rj denotes the probability that an access request for data item Dj from mobile host Mi is issued at a unit of time (Read operation); Wj denotes the probability that an update for data item Dj from the mobile host who owns the original is issued at a unit of time (Write operation). RWR denotes the ratio of Read probability to Write probability for data item Dj at a unit of time. If the RWR value is higher, more Read and less Write operations have occurred for the data item at a unit time, consequently replicate the data item as the data accessibility is expected to be higher.

We also use profiles[4] where users’ mobility schedules, access behaviors and read/write patterns are recorded, and can actively reconfigure the replicas to adjust to the changes in user locations and system status. Thus, our algorithm can be tailored to satisfy as closely as possible each individual user’s information requirement.

Performance Evaluation

In this section, we show results of simulation experiments regarding performance evaluation of our proposed methods. We assume a mesoscale ad hoc network in which 40 mobile hosts exist and move based on the users’ schedule (and randomly move with a certain probability) in a square area and data items are randomly updated with a certain frequency.

Fig. 3 shows the simulation results when varying the radio communication range of mobile hosts. From the results, we can confirm that our proposed methods can improve the data access ability but there is basically a tradeoff relationship between data accessibility improvement and traffic reduction. Specifically, from Fig. 3(a), as the radio communication range gets larger, the data accessibility also gets higher in every method. In most cases, the E-DCG+ method gives the highest data accessibility.

When the communication range is very small, every method gives almost the same data accessibility. This is because the number of mobile hosts connected to each other is small, and thus replica relocation rarely occurs. When the communication range is very large, every method also gives almost the same data accessibility. This is because most mobile hosts are connected to each other, and thus mobile hosts can access original data items in most cases.

From Fig. 3(b), as the radio communication range gets larger, the traffic produced by each method also gets higher at first, but it gets lower from a certain point. When the radio communication range is very small, every method produces low traffic. This is because the number of mobile hosts connected to each other is small, and thus replica relocation and replica refreshment do not cause high traffic. When the radio communication range is very large, the DCG and E-DCG+ methods give lower traffic than the DAFN and E-DAFN+ methods. This is because in the DCG and E-DCG+ methods, most mobile hosts belong to one big group and thus replica relocation rarely occurs.

Conclusions

In this paper, we proposed replica allocation methods in ad hoc networks to improve data accessibility. The simulation results showed that the proposed methods work well. Since there is a trade-off relationship between the improvement of data accessibility and the reduction of traffic, there cannot be one universal optimal method for replica relocation. Therefore, in a real environment, an appropriate method should be chosen among our proposed methods according to the situation.

The implementation of our scheme calls for careful consideration of several issues such as the maintenance of access histories and user profiles. In this paper, we assume a mesoscale ad hoc network, and thus, we neglect the effect of broadcasting small messages. In order to apply our proposed methods in a large scale ad hoc network, some extensions can be incorporated to prevent broadcast storms, e.g., using gossiping protocols and setting TTL (Time To Live) for broadcasting.

References

Discrete Sandwich Compounds of Monolayer Palladium Sheets

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Despite the abundance of “sandwich” complexes, in which two cyclic aromatic hydrocarbon ligands flank a metal center, this motif has not been extended to sheets of multiple metal atoms. We prepared and isolated two such compounds. In the first, three palladium centers form a planar triangular array, capped by chlorides, between two cyclopentadienyl groups. In the second, a pentapalladium sheet adopts an edge-sharing triangle-trapezoid skeleton between two naphthalene rings. The compounds were characterized by X-ray crystallography and nuclear magnetic resonance spectroscopy. The nature of bonding in the clusters was analyzed by quantum calculations.

The chemistry of metal sandwich complexes has developed extensively since the work of Woodward (C6H5)Fe was elucidated in 1952 (1, 2). The motif now plays an important role in catalysis and materials sciences (3, 4). Most of the discrete sandwich complexes prepared a molecule-metal-metal center between two small aromatic carbocyclic ligands, such as cyclopentadienyl or benzene (Fig. 1A). In contrast, compounds in which the carbon rings flank a molecule of multiple metal atoms have not been isolated as discrete molecules, despite the fascinating implications of such layered sheet structures (Fig. 1B). The potential existence of these compounds was implicated by early observation of facial coordination of cyclopentadienyl or benzene ligands to triangular trilobed cores in a half-sandwich manner (5, 6). More recently, a Ni6(benzene)6 species was detected through mass spectroscopy in a mixture of Ni2(benzene)4 clusters generated in the gas-phase by laser vaporization (7). Stable structures of discrete metal mononuclear sandwich complexes have also been discussed in theoretical studies (8). Moreover, formation of metal monosheets between graphene layers has been observed through transmission electron microscopy (TEM) (9, 10), which further stimulates the search for this carbon sheet–metal sheet–carbon sheet interaction in discrete molecules.

We sought to prepare palladium compounds that adopt this layered motif. Palladium is one of the most versatile transition metal catalysts for transformations of organic and inorganic substrates (11). Although monocyclic 2,9-bis(cyclopentadienyl)- and bisbenzene palladium complexes are unknown, polyatomic palladium frameworks seem likely to exist stably between extended unsaturated hydrocarbon ligands, in view of the isolation of bisbenzene dippalladium complexes (12, 13) as well as the efficient formation of Pd sandwich chain compounds (14, 15). Here, we report the successful isolation and structural characterization of two discrete metal monolayer sandwich compounds: [Pd2(C5H5)2]Cl2[PPPh3] (1) and [Pd2(naphthalene)4]Cl2[Na2][Cu][PPh3] (4-toluenesulfonate), where Na[Cu][Cu][PPh3] (4-toluenesulfonate), where Na[Cu][Cu][PPh3] (4-toluenesulfonate), where Na[Cu][Cu][PPh3] (4-toluenesulfonate), where Na[Cu][Cu][PPh3] (4-toluenesulfonate). The cycloheptatrienyl (Tr) cation [C7H6]+ has been widely studied as a transition metal ligand (16-18) but has rarely been used in palladium chemistry (19, 20). Surprisingly, the reaction of [Pd2(NAH)] (bpa = 1,5-diphenyl-1,4-pentadien-3-one) and [Cu][PPh3] in the presence of [PPPh3]Cl in C6H6 afforded the cycloheptatrienyl trilobed complex [Pd2(Tr)2]Cl2[PPPh3] (1) almost quantitatively after 10 min (Fig. 2A). The product C7 was isolated as wine-red microcrystals in 72% yield after recrystallization from hot acetone. The structure of C7 was determined by a single-crystal X-ray diffraction analysis (Fig. 2B). The triangular trilobed core is sandwiched between two planar cycloheptatrienyl ligands. The Pd–Pd bond (2.74 Å to 2.76 Å) are within the range of normal Pd–Pd bond length (21). The two cycloheptatrienyl rings are slightly deviated from the mutually eclipsed position of the seven carbons in each ring, two pairs C1–C2 and C3–C4 or C10–C11 and C12–C13, are located within the bonding distance (2.15 to 2.28 Å) from Pd1 and Pd2 or Pd2 and Pd3, respectively. The remaining carbon sets, C5, C6, C7 or C8, C9, C14, are bound rather irregularly to Pd3 or Pd1, respectively, with the shorter Pd3–C6 and Pd1–C8 lengths and the longer Pd3–C5, Pd3–C7, Pd1–C9, and Pd1–C14Insights. The C–C bond Insights of

Fig. 1. Illustrated models of (A) metalloccene and (B) hypothetical metal monolayer sandwich compounds.

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Image from Science, 313, Manabashi, T. et al., Discrete Sandwich Compounds of Monolayer Palladium Sheets, 1104-1107, 2006. Reprinted with permission from AAAS.
Discrete Sandwich Compounds of Monolayer Palladium Sheets

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(Graduate School of Engineering)

Introduction

Half a century ago, the sandwich structure of ferrocene Fe(C5H5)2 was elucidated by G. Wilkinson, R. B. Woodward, and E. O. Fischer (Scheme 1). Since then, sandwich complexes have been synthesized for nearly all metallic elements in the periodic table. The structural concept of the molecular sandwich revolutionized the chemistry of metal complexes, and brought us the principle of π coordination of cyclic unsaturated hydrocarbons to a metal center. Typical examples of known sandwich complexes are shown in Scheme 1. Most of the known sandwich complexes are the mononuclear complexes in which cyclic unsaturated hydrocarbon ligands flank a single metal atom. Some dinuclear sandwich complexes are also known. In light of the fact that metal atoms tend to gather by themselves to form a metal cluster; it is of great interest to verify whether three or more metal atoms can be incorporated into the sandwich structure. Thus, we hypothesized the existence of stable multinuclear metal sandwich complexes, and aimed at synthesizing the unprecedented metal sheet sandwich complexes, of which the illustrated images are shown in Fig. 1. In our paper, we described the successful isolation and structural characterization of the first discrete sandwich complexes containing metal sheets.

Scheme 1. Typical examples of known mono- and dinuclear sandwich complexes (From left, Fe(C5H5)2 (ferrocene), Cr(C6H6)2, and Pd2(C6H6)2(AlCl4)2).

SYNTHESIS AND STRUCTURE

The first stable sandwich complexes of triangular trimetal core were synthesized by the reaction of a zerovalent Pd complex Pd2(dba)3 (dba = dibenzylideneacetone) and a tropylum salt [C7H7][BF4], which is the seven-membered aromatic cation, in the presence of PPh4Cl at ambient temperature (Fig. 2A). The molecular structure of the complex 1 was determined by X-ray crystallographic analysis (Fig. 2B). The seven-membered hydrocarbon rings flank the triangular array of three palladium atoms capped by Cl ligands. The Pd–Pd distances (2.7550(5) Å, 2.7446(5) Å, 2.7889(5) Å) are in the range of normal Pd–Pd bond distances (cf. the Pd–Pd length of bulk Pd metal is 2.76 Å). The complex 1 is air- and moisture stable, and no decomposition was observed even after heating in solution at 60 °C, while the mononuclear sandwich complexes of Pd such as Pdη3(allyl)2 are usually unstable in solution. Substitution of Cl ligands with other ligands proceeded without decomposition of the bis-cycloheptatrienyl-Pd3 sandwich framework: [Pd3(μ3-C7H7)2(CH3CN)3][BF4]2 (1-CH3CN) was formed by treatment of 1 with AgBF4 in CH3CN, and [Pd3(μ3-C7H7)2(PPh3)3][BF4]2 (1-PPh3) was formed by addition of excess PPh3 to 1-CH3CN. The trinuclear sandwich structure of 1 was analyzed by theoretical studies. The structural optimization of [Pd3(μ3-C7H7)2Cl]4− using density functional theory (DFT) calculations reproduced well the structure of 1. MO-based fragment overlap population analysis suggested that dπ–dσ antibonding orbitals of [Pd3Cl]4− fragment mainly participate in back-donation to the [C7H7]+ ligands, while dσ–dσ bonding orbitals are also involved in the back-donating interaction. Donating interaction from the π orbitals of the [C7H7]+ ligands to the Pdπ orbitals...
also contribute to the bonding interaction between the C7H7 ligands and the Pd5 center. The Wiberg bond indices (WBIs) indicate the presence of weak Pd–Pd bonding interaction in the triangular tripalladium core.

The palladium sheet sandwich complex was also obtained by using polycyclic aromatic hydrocarbon ligands. The pentapalladium sandwich sheet complex 4-toluene was formed by the reaction of [Pd5(CH2CN)6][BF4]2 (3), naphthalene, and NaBArf (BArf = B{3,5-(CF3)2C6H3}4) in refluxing 1,2-dichloromethane, followed by recrystallization from CH2Cl2/toluene solution. The toluene ligand dissociated during precipitation from CH2Cl2/n-hexane to give a sandwich complex 4 (Fig. 3). The structure of 4-toluene was determined by X-ray crystallographic analysis (Fig. 4). The two naphthalene ligands flank the Pd5 sheet through μ3-η2:η1:Pd3-η2:η1:Pd3-coordination mode. Pd–Pd distances are in the range of normal Pd–Pd bond distances, while Pd4–Pd5 length is somewhat long (Fig. 4C). 13C{1H} NMR chemical shifts indicate that the Pd5(naphthalene)2 sandwich structure in the crystalline state is maintained in solution, although it is not clear whether toluene ligand is bound to the apical Pd in the solution. Theoretical analyses of [Pd5(naphthalene)2]2+ suggested that the donation from naphthalene ligands and back-donation from the [Pd5]2+ moiety contribute to the bonding between [Pd5]2+ fragment and naphthalene ligands. In the Pd5 sheet, there are very weak Pd–Pd interactions.

**CONCLUSION**

This work showed for the first time that metal sheet sandwich complexes exist as the stable, discrete molecules. We propose that sandwich compounds containing different sizes and shapes of metal sheet can be synthesized by employing different extended π-conjugated carbon frameworks, with aid of their template effect. Also, other metal elements may be used in the metal sheet sandwich complexes.
Chemical identification of individual surface atoms by atomic force microscopy

Yoshiaki Sugimoto1, Pablo Pou2, Masayuki Abe1,2, Pavel Jelínek3, Rubén Pérez2, Seizo Morita4 & Óscar Cunstante1

Scanning probe microscopy is a versatile and powerful method that uses sharp tips to image, measure, and manipulate matter at surfaces with atomic resolution1,2. At cryogenic temperatures, scanning probe microscopy can even provide electron tunnelling spectra that serve as fingerprints of the vibrational properties of adsorbed molecules3 and of the electronic properties of magnetic impurity atoms4, thereby allowing chemical identification. But in many instances, and particularly for insulating systems, determining the exact chemical composition of surfaces or nanoscale structures remains a considerable challenge. In principle, dynamic force microscopy should make it possible to overcome this problem: it can image insulators, semiconductor and metal surfaces with true atomic resolution5,6 by detecting and precisely measuring7-10 the short-range forces that arise with the onset of chemical bonding between the tip and surface atoms11-13 and that depend sensitively on the chemical identity of the atoms involved. Here we report precise measurements of such short-range chemical forces, and show that their dependence on the force microscope tip used can be overcome through a normalization procedure. This allows us to use the chemical force measurements as the basis for atomic recognition, even at room temperature. We illustrate the performance of this approach by imaging the surface of a particularly challenging alloy system and successfully identifying the three constituent atomic species silicon, tin, and lead, even though these exhibit very similar chemical properties and identical surface position preferences that render any discrimination attempts based on topographic measurements impossible.

The chemical identification of single atoms and molecules at surfaces has been pursued since the invention of both the scanning tunnelling microscope and the atomic force microscope (AFM). Particularly promising in this quest is dynamic force microscopy, which achieves true atomic imaging resolution11-13 by detecting the short-range forces associated with the onset of the chemical bond between the outermost atom of the tip apex and the surface atoms being imaged14-15 (see Fig. 1 for schematic illustration of the method and imaging examples). Moreover, dynamic force spectroscopy11-13 makes it possible to quantify these forces.

Figure 1a shows five sets of dynamic force spectra measured on a single atomic layer of Sn grown on a Si(111) substrate. Each set of force curves was obtained over an Sn atom and an Si atom having the same local surface configuration as the corresponding atoms highlighted in the topographic image shown in Fig. 1a, always using identical acquisition and analysis protocols (see Methods). However, the sets were collected over multiple measurement sessions, using tips that had different apex terminations. These tips apices presumably differ in both structure and composition (Sn or Si), as sometimes slight tip–surface contacts were intentionally produced before the acquisition of each set of force curves. The force curves show to share only one feature: curves measured over the Si atoms are characterized by a stronger attractive interaction force. Given the high degree of stability, lateral positioning accuracy, and reproducibility provided by our acquisition protocol16-17, we attribute the variability seen in the data in Fig. 2a to a strong tip–dependence of both the registered

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Figure 1: Dynamic force microscopy with atomic resolution. Schematic illustration of AFM operation in dynamic mode (a), and of the onset of the chemical bonding between the outermost tip atom and a surface atom (highlighted by the green stick) that gives rise to the atomic contrast (b). However, the tip experiences not only the short-range force associated with this chemical interaction, but also long-range force contributions that arise from van der Waals and electrostatic interactions between tip and surface (through the offset of the latter is usually minimized through appropriate choice of the experimental set-up). c, d, e. Curves obtained with analytical expressions for the van der Waals and electrostatic interactions between tip and surface (through the offset of the latter is usually minimized through appropriate choice of the experimental set-up). c, d, e. Curves obtained with analytical expressions for the van der Waals and electrostatic interactions between tip and surface (through the offset of the latter is usually minimized through appropriate choice of the experimental set-up). c, d, e. Curves obtained with analytical expressions for the van der Waals and electrostatic interactions between tip and surface (through the offset of the latter is usually minimized through appropriate choice of the experimental set-up).
Chemical Identification of Individual Surface Atoms by Atomic Force Microscopy

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Atomic force microscopy

Atomic force microscopy [1] is one of the most versatile and widely used scanning probe techniques, as it provides access to the characterization of processes taking place at insulator, semiconductor or metal surfaces in environments ranging from vacuum to liquid or air conditions. An atomic force microscope (AFM) basically consists in a sharp tip placed at the end of a flexible, microscopic cantilever that bends under the presence of an interaction force between the AFM tip and the probed sample surface when scanning the cantilever over a surface area. AFM is currently used in material characterization and testing at the micro- and nano-scale by imaging surface structures and studying their mechanical properties such as friction, adhesion or hardness. It has also found important applications in biology, since it enables, for instance, to study the mechanical properties of proteins, or directly image and interact with biologic material in a liquid environment.

In this work, we have operated the AFM in dynamic mode, in a technique known as dynamic force microscopy (DFM) [2]. Under this scheme, the tip at the end of the cantilever is oscillated at a given amplitude and frequency, and these two magnitudes do change under the presence of a tip-surface interaction force [3]. In our case, we keep the oscillation amplitude constant and record the variations on the oscillation frequency with changes in the tip-surface interaction force. In the most refined experimental set ups, this technique allows one to detect and quantify the short-range chemical interaction force between two atoms [4, 5]. If the oscillating tip is driven close enough to the surface, so that the apex of the AFM tip gets closer than 5 angstroms during the turning point of the oscillation, the onset of the chemical bonding between the outermost atom of the AFM tip and the individual atoms of the surface takes place. This is, indeed, the mechanism behind the capability of DFM to truly image the atoms at insulator, semiconductor, and metal surfaces.

Measurement of chemical bonding forces toward chemical identification

The chemical identification of single atoms and molecules at surfaces has been pursued from the invention of both the scanning tunnelling microscope (STM) and the AFM, since it could multiply the already outstanding capabilities of these techniques. The intrinsic detection nature of the STM and the AFM has hindered, until now, most of these efforts, and single atom chemical identification still remains a challenge. On this quest for single-atom chemical identification, DFM may have an advantage since the imaging mechanism is based on detecting the short-range forces associated with the onset of the chemical bonding between the outermost atom of the AFM tip and the atoms at the surface.

Forces associated with the chemical bonding between two atoms are related to the nature of the atomic species involved. Thus, the short-range chemical forces we are measuring over the different surface atoms when exploring a heterogeneous surface with DFM should contain information about these surface atoms' chemical nature. However, to extract this information is not trivial at all since, as we demonstrate in this paper, these short-range chemical forces present a strong variability upon the tip used to probe the surface, that is, for different AFM tip terminations we obtain unlike short-range chemical forces. This variability is illustrated in Fig. 1, where the short-range chemical bonding force between the outermost atom of the AFM tip and two different atomic species at a surface, namely tin (Sn) and silicon (Si), is shown. The typical behaviour of these short-range chemical forces when approaching the tip towards the surface is a curve with an initial reduction of the force values from zero down to a minimum, from which the forces start increasing towards positive values: here, negative forces mean an attractive interaction between the atoms. As it can be seen in Fig. 1, the set of force curves in Fig.1a is completely different from the set shown in Fig. 1b, even when they were very precise measured over a Sn atom and a Si atom with exactly the same acquisition an analysis protocols [6, 7]. The only difference between the two sets of curves depicted in Fig. 1a and 1b, respectively, is that they were measured using two different AFM tip terminations.

Finding the "atoms fingerprint"

We have found a magnitude that remains nearly constant independently of the AFM tip termination we used. This magnitude is the relative interaction ratio of the minimum values of the short-range chemical forces measured over two different atomic species probed with the same tip (relative interaction ratio for short in the following). This can be also seen in Fig.1.
If we take the minimum force value for the curves measured over the Si atoms as 100%, the minimum force value for the curves measured over the Sn atoms will be in both cases close to the 77%. We have corroborated this finding also for other atomic species like lead (Pb) and indium (In). We have measured the short-range chemical forces over Pb and Si (mixing the two atoms over the same surface) using different tips, and then we have quantified the relative interaction ratio for these two species, resulting in a value of 59%. The same procedure, mixing In and Si on a surface, yielded a ratio of 72% for In and Si. In both cases (Pb-Si and In-Si), we found that the values of relative interaction ratio were almost independent of the AFM tip. This property makes it possible to use the relative interaction ratio as a fingerprint for the chemical identification of atoms at surfaces.

Chemical identification of individual surface atoms

The identification method we report in this paper consists in measuring the short-range chemical interaction force over each of the atoms in a surface area using the same AFM tip, and then to compare the ratio of the minimum force values between pairs of atomic species with the previously tabulated relative interaction ratio for the expected atoms to be contained at the surface. To demonstrate this method, we have used a surface alloy mixing Si, Sn, and Pb in equal proportions, and identified each of the atoms in the imaged surface area (Fig. 2). When looking at the topography of this alloy (Fig. 2a) only one of the three species seems to present a different contrast while the other two cannot be differentiated. After systematically measuring the tip-surface, short-range chemical bonding force over each of the atoms, we can see that the minimum force values registered over these atoms can be clearly classified into three groups, as it is shown in the histograms in Fig. 2b. When taking the previously tabulated values of the relative interaction ratio into account (77% for Sn and Si, and 59% for Pb and Si), these groups can be assigned to forces obtained over Sn, Pb, and Si atoms, and therefore each surface atom can be associated with the corresponding chemical element (Fig. 2c).

Outlook

As mentioned above, this capability of identifying atoms at surfaces could multiply the already outstanding possibilities that DFM offers. This method might be of relevance in surface chemistry, material science, nanoscience and nanotechnology, and even in semiconductor technology: in particular, when combining this identification method with the ability of DFM for the manipulation of individual atoms at surfaces [8, 9].

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Although the authorship of this report is limited to two authors, Oscar Custance and Seizo Morita—both of them personnel of Osaka University—should also sign as authors due to their fundamental contributions to both the Nature paper and this report.
Persistent Electron-Transfer State of a π-Complex of Acridinium Ion Inserted between Porphyrin Rings of Cofacial Bisporphyrins

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Abstract: A free-base cofacial bisporphyrin, H2DPO, forms a π-complex with acridinium ion (AcH+) by π-π interaction in benzotrifluoride (b-TFA). Formation of the H2DPO-AcH+ π-complex was probed by UV−vis and NMR spectroscopy. The binding constant between AcH+ and H2DPO is determined as $9.7 \times 10^4$ M$^{-1}$. Photoinitiated electron transfer (ET) from the H2DPO to the AcH+ moiety occurred efficiently in the π-complex to form the ET state (H2DPO$^{−}$−AcH$^+$). The ET state is successfully detected by laser flash photolysis. The lifetime of the ET state is 16 μs in PhCN at 208 K, and the quantum yield of the ET state is 90%. The temperature dependence of the ET state lifetime has been examined in the range from 273 to 353 K. The ET state lifetime exhibited a large temperature dependence, and the linear plot of ln(τ) vs $1/T$ in accordance with the Marcus equation, affords the ET reorganization energy (0.54 eV). As a result, a remarkably long-lived ET state has been attained at low temperature, and virtually no decay of the ET state was observed at 77 K. Such an extremely long-lived ET state is indeed detected by steady-state UV−vis absorption spectroscopy.

Engineering

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Persistent Electron-Transfer State of a π-Complex of Acridinium Ion Inserted between Porphyrin Rings of Cofacial Bisporphyrins

Paper in journals: This is the first page of a paper published in Journal of the American Chemical Society.


Introduction

The natural photosynthetic system consists of light-harvesting antenna units and reaction center units. In the photosynthetic reaction centers of purple bacteria, there are four bacteriochlorophylls, two bacteriopheophytins, two ubiquinones (Q$_{a}$ and Q$_{b}$), and a non-heme iron atom. Light-initiated charge separation occurs between the special pair and the neighboring pigments, finally leading to a special pair excited state and radical-anion Q$_{a}^−$. The electron-transfer process is found to occur very rapidly, with nearly 100% quantum yield. The charge-separated state efficiently converts into chemical energy, because this lifetime is quite long (~1 s). Each component of the photosynthetic reaction center is located at the right position by noncovalent bonding in the protein matrix to optimize the charge-separation efficiency. The use of noncovalent bonding, such as metal−ligand coordination, electrostatic interaction, hydrogen bonds, and rotaxane formation, has recently merited increasing attention as a simpler but more elegant way to construct electron donor−acceptor ensembles mimicking the efficient biological electron-transfer systems.


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American Chemical Society
Persistent Electron Transfer State of a \( \pi \)-Complex between Cofacial Bisporphyrin and Acridinium Ion

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**Introduction**

In the initial process of photosynthesis, electrons are transferred from chlorophyll dimer (special pair) excited by light energy to quinone A and quinone B. The lifetime of charge-separated state is quite long, about 1 second. The energy of long-lived charge-separated state efficiently converts into chemical energy. Extensive efforts have so far been devoted to construct electron donor-acceptor ensembles, which mimic the photosynthetic electron transfer systems by using non-covalent bonding such as metal-ligand coordination, electrostatic interaction and hydrogen bonds and rotaxane formation. In particular, \( \pi \)-\( \pi \) interaction has merited increasing attention as one of the most important types of non-covalent binding because of its important role in biological systems, such as for \( \pi \)-stacking of double-strand DNA. For example, a bisporphyrin has been shown to form a \( \pi \)-complex with fullerene, which is inserted between two porphyrin rings. A DNA intercalator can also be inserted between two porphyrin rings of a water-soluble bisporphyrin. However, there has been no report on the photodynamics of such \( \pi \)-complexes to attain long-lived charge-separated state.

We report herein formation of a \( \pi \)-complex between a free-base cofacial bisporphyrin (H\textsubscript{4}DPOx) and acridinium ion (AcH\textsuperscript{+}), and also the photodynamics in benzonitrile (PhCN) as shown in Scheme 1. H\textsubscript{4}DPOx is regarded as a model compound of a special pair, which has two cofacial porphyrin rings and flexible spacer. The formation of the electron-transfer (ET) state is studied by laser flash photolysis experiments. The decay of the ET state is highly temperature dependent. As a result, a remarkably long-lived ET state has been attained at low temperature and virtually no decay of the ET state was observed at 77 K.

**\( \pi \)-Complex Formation between H\textsubscript{4}DPOx and AcH\textsuperscript{+}**

Formation of the H\textsubscript{4}DPOx-AcH\textsuperscript{+} complex was probed by the UV-vis and NMR spectra. From the spectral titration, it is found that AcH\textsuperscript{+} is inserted between the two porphyrin rings of H\textsubscript{4}DPOx. The formation constant of the 1:1 \( \pi \)-complex is determined as 9.7 \( \times \) 10\textsuperscript{-4} M\textsuperscript{-1} in PhCN. The following is a comment on the published paper shown on the preceding page.

**Figure 1.** (a) Structure of H\textsubscript{4}DPOx AcH\textsuperscript{+} optimized by DFT calculation. (b) HOMO and (c) LUMO orbitals calculated by a DFT method at the B3LYP/6-31G*//B3LYP/3-21G level.

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**Figure 2.** (a) Transient absorption spectrum of H\textsubscript{4}DPOx in the presence of AcH\textsuperscript{+} in deaerated PhCN at 273 K taken at 20 \( \mu \)s after laser excitation at 520 nm. (b) Time profiles of the absorption at 520 nm of H\textsubscript{4}DPOx\textsuperscript{+}+AcH\textsuperscript{+} obtained with different laser power.

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The decay time profiles of the absorption at 520 nm with the different laser intensities are shown in Figure 2b. The first-order plots afford good linear correlations with the same slope irrespective of the different laser intensities. Such a first-order decay with the same slope strongly indicates that the back electron transfer electron transfer (BET) from AcH⁺ to H₄DPOx⁺ occurs in the π-complex and that no bimolecular decay is involved. The lifetime of the ET state is determined as 18 μs at 298 K (k_BET = 5.5 × 10⁴ s⁻¹). On the other hand, the rate constant (k_ET) of ET from the singlet excited state of H₄DPOx to AcH⁺ in the π-complex was determined as 2.9 × 10⁸ s⁻¹ by measuring the time-resolved fluorescence.

The ET state lifetimes in PhCN have also been determined in the range from 273 to 353 K by laser flash photolysis. The lifetime of the ET state exhibits a large temperature dependence. The temperature dependence of the rate constant of back electron transfer (k_BET) is in accordance with the Marcus equation (eq 1), where λ is the reorganization energy, V is the electronic coupling matrix element, h is the Planck constant, k_B is the Boltzmann constant, T is the absolute temperature, and ΔG_ET is the free energy change of back electron transfer to the ground state. The plot of ln(k_BETT¹/²) vs T⁻¹ (eq 1) is linear, as shown in Figure 3, and from the slope and the intercept, the λ and V values are determined as 0.54 eV and 1.6 cm⁻¹, respectively.

The ET state lifetime at 77 K, extrapolated from the linear plot in Figure 3, is obtained as 360 days. Such an extremely long-lived ET state is indeed detected by the steady-state photoradiation of glassy 2-MeTHF containing 10% butyronitrile solution of H₄DPOx AcH⁺ by a 100 W high-pressure mercury lamp at low temperature. The new absorption bands due to H₄DPOx⁺ and AcH⁺ are clearly observed as shown in Figure 4. The observed ET state exhibits no decay for 200 min at 77 K (inset of Figure 4). The color change on going from the ground state of the H₄DPOxAcH⁺ complex to the ET state is also shown in the inset of Figure 4. The color and the absorption spectrum of the ET state go back to the original ones when the temperature is increased to 298 K. The large temperature dependence of the rate constant of BET results from the small λ value in the π-complex, when the BET is deeply in the Marcus inverted region.

**Conclusion**

In conclusion, a 1:1 π-complex is formed between H₄DPOx and AcH⁺ in PhCN with a large binding constant. The surprisingly persistent ET state of the π-complex has been attained upon photoexcitation of the π-complex at low temperature. The small reorganization energy of electron transfer has made it possible to attain fast photoinduced electron transfer but extremely slow back electron transfer in the π-complex.

References

Toll-like receptor–mediated regulation of zinc homeostasis influences dendritic cell function

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Zinc is a trace element that is essential for the function of many enzymes and transcription factors. Zinc deficiency results in defects in innate and acquired immune responses. However, little is known about the mechanism(s) by which zinc affects immune cell function. Here we show that stimulation with the Toll-like receptor 4 agonist lipopolysaccharide (LPS) altered the expression of zinc transporters in dendritic cells and thereby decreased intracellular free zinc. A zinc chelator mimicked the effects of LPS, whereas zinc supplementation or overexpression of the gene encoding Zip6, a zinc transporter whose expression was reduced by LPS, inhibited LPS-induced upregulation of major histocompatibility complex class II and costimulatory molecules. These results establish a link between Toll-like receptor signaling and zinc homeostasis.

Like calcium, zinc is a ‘non-redox’ active ion and is essential for cell growth, development and differentiation. Zinc functions mainly as a cofactor for cellular proteins, nucleic acids, carbohydrates and lipids. More than 500 proteins contain zinc-interacting regions such as zinc-finger motifs, RING fingers or LIM domains that influence cellular responses by coordinating zinc ions.3,4 Zinc homeostasis is maintained mainly by a balance in the expression of two kinds of zinc transporters as well as zinc-binding metallothionein proteins5. Human genome sequencing has shown that the Zip (encoded by the Slc39 genes) family of zinc transporters consists of 14 members, which function as zinc importers, and that the Znt (encoded by the Slc30 genes) family consists of 9 members, which function as zinc exporters.6–10 During gastrulation in zebrafish, expression of Zip5 is regulated by the transcription factor STAT3 in organizer cells, and ZIP6 is essential for the nuclear localization of Snail, a zinc-finger-containing repressor of E-cadherin. Therefore, Zips are essential for cell movement and the epithelial-mesenchymal transition during gastrulation in zebrafish.11,12 Zinc deficiency causes growth retardation13,14 and cognitive impairment.15,16 In addition, zinc deficiency in humans is correlated with increased susceptibility to bacterial and/or viral infections17–19, suggesting that zinc is important in immune responses in vivo. Consistent with this hypothesis, patients with zinc deficiency also show defective cellular immunity, lymphopenia and abnormalities in hematopoietic cells, including T cells,20 natural killer (NK) cells21 and monocytes.22 Despite those findings, the precise mechanisms by which intracellular zinc homeostasis influences immune cell function are not clear.

Here we investigated the relationship between intracellular free zinc and surface expression of MHC class II on dendritic cells (DCs), which are important in presenting antigen to T cells23–25. In the absence of maturation signals, such as Toll-like receptor (TLR) stimulation, few MHC class II molecules are expressed on the surface of DCs. Maturation signals induce intracellular trafficking of MHC class II-containing vesicles and result in increased surface expression of MHC class II molecules.26,27 Endocytosis of MHC class II molecules from the cell surface also influences expression of MHC class II molecules on the plasma membrane of activated DCs.28,29

We found dynamic changes in the expression of zinc transporters (both importers and exporters) during lipopolysaccharide (LPS)–induced DC maturation. LPS stimulation resulted in a decrease in free zinc in DCs. The addition of a zinc chelator increased the surface expression of MHC class II and costimulatory molecules on DCs, just as LPS did, and zinc supplementation or overexpression of the zinc importer Zip6 inhibited the LPS-induced upregulation of MHC class II and costimulatory molecules. These observations demonstrate that zinc homeostasis, which is regulated by zinc transporter expression, is involved in at least some DC maturation events and may affect the magnitude of adaptive immune responses.

RESULTS

Intracellular zinc homeostasis influences DC maturation

To investigate the relationship between LPS-induced surface expression of MHC class II and costimulatory molecules and the abundance of intracellular zinc in DCs, we first measured intracellular free zinc.

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Toll-Like Receptor-Mediated Regulation of Zinc Homeostasis Influences Dendritic Cell Function.

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Zinc is an essential nutrient required for cell growth, development, and survival, and zinc deficiency causes growth retardation, immunodeficiency, and other health problems. Therefore, zinc homeostasis must be tightly controlled. Zinc is known to be important in the immune system, although its precise roles and mechanisms have not been resolved. It has not been known whether zinc itself might act as an intracellular signaling molecule, i.e., a molecule whose intracellular status is altered in response to an extracellular stimulus, and that is capable of transducing the extracellular stimulus into an intracellular signaling event. Here, we showed that TLR-mediated signaling induces a decrease in the intracellular free zinc in dendritic cells, and that this decrease is required for dendritic cell activation followed by CD4+ T-cell activation.

Intracellular zinc homeostasis influences DC activation

We investigated zinc homeostasis after extracellular stimulations in DC. We employed a zinc ion-sensitive fluorescent probe and measured intracellular free zinc level before and after stimulation of DC by LPS. LPS stimulation induced a decrease in the intensity of Newport green DCF staining (Figure 1). These data indicated LPS stimulation decreases intracellular free zinc in DC.

To confirm the LPS-mediated zinc reduction affected DC activation, we analyzed the surface expression of MHC class II and CD86, in DC, both of which are critical for CD4+ T cell activation, treated with chemicals that modulate intracellular zinc homeostasis. We first used the zinc-chelating reagent TPEN (N,N,N',N'-tetrakis (2-pyridylmethyl)ethylenediamine).

Figure 1. LPS signal reduces intracellular free zinc in DC.

Figure 2. Chelating of zinc alone increases surface expression of MHC class II, while zinc supply inhibits LPS-mediated MHC class II expression on DC.

Figure 3. TRIF pathway of LPS is critical for zinc transporter expression (increase of Zip, decrease of Znt family members) followed by intracellular free zinc reduction.
intracellular zinc by modulating the expression of zinc transporters. LPS stimulation suppressed the expression of mRNA transcripts encoding zinc transporters of the Zip family but upregulated the expression of some Znt family members in DC (Figure 3). Because Zip proteins function as zinc importers and Znt proteins act as zinc exporters, these results indicated that LPS-induced alterations in zinc transporter expression are likely to favor a net increase in zinc export. LPS stimulates TLR4, and the resulting signals are transduced by two distinct pathways, each dependent on either MyD88 or TRIF. LPS-mediated alterations in expression of the Zip family and of Znt1 depended mainly on TRIF, and those of Znt4 and Znt6 depended on both TRIF and MyD88 (Figure 3). Consistent with these results, LPS-induced reductions in intracellular zinc did not occur in TRIF-deficient DCs (Figure 4). These results suggested that LPS-mediated regulation of intracellular zinc in DC was dependent on at least TRIF-mediated TLR signaling.

We hypothesize that overexpression of zinc transporters whose expression is downregulated by LPS should inhibit LPS-induced decreases in intracellular zinc and DC activation. To test this, we overexpressed Zip6, a zinc transporter whose expression was downregulated by LPS. Expectedly, Zip6 overexpression significantly inhibited LPS-mediated reductions in intracellular free zinc, as assessed by Newport green DCF in DC (Figure 5). These data suggested that Zip6 is one zinc transporter involved in LPS-mediated decreases in intracellular free zinc. DC infected with Zip6 gene-expressing retrovirus had less LPS-mediated upregulation of MHC class II and CD86 surface expression than did mock-infected DCs (Figure 5), as well as less CD4+ T cell stimulatory activity (Figure 5). These results supported the idea that LPS-induced decreases in intracellular free zinc are a critical step in DC maturation and that these decreases in intracellular free zinc can be induced by altering the expression pattern of zinc transporters.

Zinc influences DC activation in vivo

After LPS or TPEN injection, intracellular free zinc in splenic CD11c+ DC decreased, and surface expression of MHC class II molecules increased (Figure 6). Notably, LPS injection also reduced the expression of mRNA transcripts encoding Zip6 in these same splenic CD11c+ DC (Figure 6). These data demonstrated that LPS stimulation decreases intracellular free zinc and alters zinc transporter expression in splenic CD11c+ DC populations in vivo.

Conclusions

Our data demonstrates that TRIF-dependent TLR signaling alters the expression of zinc transporters and results in decreased intracellular free zinc in DC. Thus, our results establish a link between TLR signaling and intracellular free zinc quantities. They also demonstrate one mechanism by which zinc homeostasis influences the adaptive immune response, activation of CD4+ T cells (Figure 7 left). Moreover, we hypothesize that zinc functions as an intracellular signaling molecule to alter the functions of proteins in the TLR signaling after binding to or depart from them (Figure 7 right).

Figure 4 TRIF pathway is critical for free zinc reduction.

Figure 5 Zip6 overexpression blocks LPS-induced reduction of intracellular zinc level, surface expression of MHC class II followed by inhibition of CD4+ T cell activation.

Figure 6 LPS administration reduces intracellular zinc and Zip6 expression in splenic DC population in vivo.

Figure 7 Summary (left) and our working hypothesis (right).
Crystal structures of a multidrug transporter reveal a functionally rotating mechanism

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AcrB is a principal multidrug efflux transporter in Escherichia coli that cooperates with an outer-membrane channel, TolC, and a membrane-fusion protein, AcrA. Here we describe crystal structures of AcrB with and without substrates. The AcrB-drug complex consists of three protomers, each of which has a different conformation corresponding to one of the three functional states of the transport cycle. Bound substrate was found in the periplasmic domain of one of the three protomers. The voluminous binding pocket is aromatic and allows multi-site binding. The structures indicate that drugs are exported by a three-step functionally rotating mechanism in which substrates undergo ordered binding change.

Multidrug resistance caused by export proteins is a serious problem, not only in the chemotherapy of cancer but also in the antibiotic treatment of numerous different bacterial infections. AcrB is a principal multidrug exporter, expressed almost constitutively and confers intrinsic drug tolerance to E. coli. AcrB belongs to the resistance-nodulation-division (RND) family of transporters, and its homologues occur in many pathogenic species of Gram-negative bacteria. It cooperates with both an outer-membrane channel, TolC, and a membrane-fusion protein, AcrA, and exports a wide variety of toxic compounds— including anionic, cationic, zwitterionic, and neutral compounds— directly out of the cell by bypassing the periplasm.

Our previous crystal structures of AcrB showed that it exists as a trimer, consisting of three layers parallel to the membrane: a transmembrane domain, a porter domain (formerly named the “pore” domain), and a TolC docking domain (Fig. 1a)14. In the porter domain, there is a functionally important helical bundle—the central helices—composed of three α-helices from each protomer (Fig. 1b)15. In the TolC docking domain, AcrB opens like a funnel that fits to the proximal end of TolC, indicating the direct docking of AcrB and TolC (Fig. 2a). TolC is also a trimer, and forms a tube extending across the periplasm and the outer membrane to provide an export pathway to the outside of the cell16. The structures indicate that AcrB takes substrates from the periplasm through the opening at the membrane–periplasm boundary between each protomer, and extrudes them from the top funnel into the TolC channel17. The structures of the membrane-fusion protein AcrA, and its homologue MerA from Pseudomonas aeruginosa, suggest that the AcrB–TolC complex is surrounded by AcrA18,19.

In the AcrA–AcrB–TolC trypartite complex, AcrB determines the substrate specificity and actively exports a wide variety of structurally dissimilar drugs and toxic compounds using the proton motive force. The question that remains is how the multidrug efflux transporter recognizes such a wide variety of toxic compounds, and how it actively exports them out of the cell. In order to address these questions, we needed to determine the structure of the AcrB–substrate complex. Such attempts have been made previously, but there was a possible artefact arising from the crystal symmetry20. We now have AcrB crystallographic data obtained from the C2 space group, which allows each protomer to take a different conformation. These protomers that constitute a trimer are now clearly differentiated in the electron density map at 2.8 Å resolution (Supplementary Fig. S1), and only one of them has a bound substrate in a phenylalanine-rich pocket in the periplasm region21. We propose that the three different conformations of the protomers represent the three states of drug export: access, binding and extrusion. Presumably, the drugs are exported by a functionally rotating mechanism for ordered binding change.

Structure determination

The C2 crystals of substrate-free AcrB and AcrB–substrate complex were grown (see Methods). The structure of the unliganded form was solved at 2.8 Å resolution by the multiple isomorphous replacement method (Supplementary Table S1). Bound substrates were located by difference Fourier calculation, and the bromine atom of the brominated substrate by anomalous difference Fourier map (Fig. 2a).

Overall structure of the asymmetric AcrB–drug complex

Our new crystal structure solved with a C2 crystal is basically consistent with our previous structure of a trigonal R32 crystal, having crystallographic three-fold symmetry; except for some crucial differences in the conformation of each protomer. The root-mean-square deviation value of the superposition of main-chain traces of these two structures is 1.4 Å. The substrate is bound to only one of the three protomers, named the ‘binding’ protomer (Fig. 1, blue). No substrate is found in the central cavity, even at the maximally dissolvable concentration of drugs for co-crystallization.

The other conspicuous difference is observed in the triplet of central helices in the porter domain. One of the three helices (Fig. 1, red) is inclined nearly 15° towards the binding protomer, compared with the other two helices. The potential exit between the distal part...
Crystal Structures of a Multidrug Transporter Reveal a Functionally Rotating Mechanism

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Introduction

The emergence of multidrug resistance is an increasing problem, not only in the chemotherapy of cancer but also in the antibiotic treatment of numerous different bacterial infections. The major cause for the multidrug resistance of bacteria is a multidrug efflux that mediated by membrane transporter proteins, which exports drugs out of the cells. AcrB and its homologues are the major multidrug efflux transporter in Gram-negative bacteria, which confer intrinsic drug tolerance and multidrug resistance when they are overproduced. AcrB exports a wide variety of antibiotics, antiseptics, anti-cancer chemotherapeutics and toxic compounds - including anionic, cationic, zwitterionic, and neutral compounds - directly out of the cells bypassing the periplasm driven by proton motive force. It cooperates with membrane fusion protein AcrA and outer membrane channel TolC (Fig. 1). The X-ray crystal structure of AcrB was first solved by our group in 2002 [1]. It was the first structure of not only a multidrug efflux transporter but also a secondary active transporter, driven by proton motive force [2].

Asymmetric structure of AcrB

Crystal structure of AcrB showed that it exists as a trimer as same as in the membrane. In the previous crystal structure, trimer has three-fold symmetry due to the crystallographic three-fold rotation axes in the unit cell of the crystal. Therefore, we could not discuss about the stoichiometry of substrates transported by the trimer and about the cooperativity within the protomers on the basis of this symmetric structure. In 2006, we solved the crystal structures of AcrB with and without substrates in the new crystal form at 2.8 Ångström resolution (Fig.2) [3]. The crystal used in this study has lower crystallographic symmetry than the previous crystal. And it has no three-fold crystallographic symmetry. The new crystal structure solved with the new crystal form is asymmetric. The AcrB-drug complex consists of asymmetric three protomers, each of which has different conformation corresponding to one of the three functional states of the transport cycle. Substrate binding is also asymmetric and only one of three protomers has a bound substrate in a phenylalanine-rich pocket in the hydrophilic part of the protein. Three protomers have different conformation corresponding to the different transport states. They are, “access” in which substrate is incorporated (colored green in Fig.2), “binding” to which substrate binds (colored blue in Fig.2), and “extrusion” by which substrate is extruded (colored red in Fig.2).

Mechanism for multidrug recognition

We successfully solved the complex structure with antibiot-
ics, minocycline and anticancer agent doxorubicin (Fig. 3). In both cases, bound substrates are observed in a phenylalanine-rich pocket of the protomer named as “binding” protomer. The binding pocket is composed of four subdomains. Only in case of the “binding” state, this binding pocket is expanded by the movement of subdomains. Expanded binding pocket of “binding” protomer has an enough room in which substrates are fitted with aromatic-aromatic interactions. Different substrates interact with different sets of amino acid residues while partially overlapped. In other words, different combination of side chains can be used to bind different substrates in the same binding pocket. This mechanism is known as the “multi-site binding mechanism” which was first found in the multidrug binding transcription factor, QacR [4]. In case of the multidrug efflux transporter, the multi-site binding pocket can control the binding affinity by expanding and shrinking the size of the pocket.

Substrate translocation

The expanded binding pocket in the binding protomer has a gap between subdomains. This gap is a possible exit from the pocket. However, in the binding protomer, the inclined helix from the “extrusion” protomer fills this gap and sterically blocks exit from the binding pocket. (Fig. 2b: dotted circle) [5]. At the same time, this inclination of the helix creates an opened space from the shrink binding pocket to the exit in the “extrusion” protomer. We suggest that this protomer represents the situation just after extrusion of the substrates from the pocket to the exit of the AcrB, which connects to the outer membrane channel, TolC, and named it the “extrusion” protomer [6]. The remaining protomer “access” also has a shrink binding pocket, but opened “vestibule” exposed to the periplasmic space.

Substrates are incorporated from the opened vestibule in the “access” state, and then bind to the respective sites in the voluminous aromatic pocket in the “binding” state. Then, in the “extrusion” state, the vestibule is closed and the exit is opened. At this state, the bound substrate is squeezed out into the TolC channel by shrinking the pocket.

All these structural changes are coupled with proton translocation across the membrane (Fig. 4). The ion pairing of three functionally important charged residues (Asp407, Asp408 and Lys940) is changed by protonation and deprotonation during the transport cycle and the resulted conformational change is transmitted to the substrate binding sites and cause the alternation of the accessibility and the affinity of the substrates.

Three step rotational binding change mechanism

Based on the asymmetric crystal structure, we propose that drugs are exported by a three-step functionally rotating mechanism in which drugs undergo ordered binding change (Fig. 4) [3]. Such an ordered binding change mechanism in a trimer is similar in principal to the mechanism of the trimeric F1ATPase, except that AcrB has no central stalk that undergoes mechanical rotation.

Conclusion

In this study, we reexamined the crystal structure of AcrB. As a result, asymmetric property is very important to understand the molecular mechanism of this transporter protein. In oligomeric proteins, biological (pseudo) symmetry axes could sometimes undesirably correspond to crystallographic rotation axes. Resulting structure also has undesirable symmetry. In many cases, an oligomeric protein complex purposely breaks the symmetry in order to facilitate their function. Allostery and cooperativity are very common examples and smart strategies to control the protein function through the asymmetric property of the homo-oligomeric protein complexes.

References

The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress

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Autophagy, an evolutionarily conserved process for the bulk degradation of cytoplasmic components, serves as a cell survival mechanism in starved cells1,2. Although autophagy has been observed in various heart diseases, including cardiac hypertrophy3,4 and heart failure5-8, it remains unclear whether autophagy plays a beneficial or detrimental role in the heart. Here, we report that the cardiac-specific loss of autophagy causes cardiomyopathy in mice. In adult mice, temporarily controlled cardiac-specific deficiency of Atg5 (autophagy-related 5), a protein required for autophagy, led to cardiac hypertrophy, left ventricular dilatation and contractile dysfunction, accompanied by increased levels of ubiquitination. Furthermore, Atg5-deficient hearts showed disorganized sarcomere structure and mitochondrial misalignment and aggregation. On the other hand, cardiac-specific deficiency of Atg5 early in cardiogenesis showed no such cardiac phenotypes under basal conditions, but developed cardiac dysfunction and left ventricular dilatation one week after treatment with pressure overload. These results indicate that constitutive autophagy in the heart under basal conditions is a nemojistic mechanism for maintaining cardiomyocyte size and global cardiac structure and function, and that upregulation of autophagy in failing hearts is an adaptive response for protecting cells from hemodynamic stress.

The autophagy and the ubiquitin-proteasome pathways are responsible for the degradation of intracellular components. In autophagy, cytoplasmic proteins and dysfunctional organelles are sequestered in an autophagosome, a double-membrane vesicle, delivered to the lysosome by fusion and then degraded12. The principal role of autophagy is to supply nutrients for survival18. In addition, a low level of constitutive autophagy is also important for controlling the quality of proteins and organelles, in order to maintain cell function12. Thus, autophagy functions as a cell-protective mechanism. However, autophagy also has a causative role in cell death11; autophagic structures are present in dying cells in neurodegenerative diseases, myopathies and liver injury14. Autophagic vacuoles are found in cardiomyocytes in ischemic hearts15,16, and in human2 and hamster2 cardiomyopathic failing hearts. Mitochondria, which are the major source of reactive oxygen species, are surrounded by autophagic membranes and lipid rafts18. The role of autophagy in the progression of cardiac hypertrophy14,19. The precise role of autophagy in the heart, however, remains to be elucidated.

To determine first the role of basal constitutive autophagy in adult mouse hearts, we generated temporally controlled cardiac-specific Atg5-deficient mice. We crossed mice bearing an Atg5fl/fl14 with transgenic mice (MerCreMer) which express the Cre recombinase in a tamoxifen-inducible and cardiomyocyte-specific manner15. The resulting Atg5fl/fl;MerCreMer+ mice were indistinguishable in appearance from age-matched control Atg5fl/fl;MerCreMer− littermates. In Atg5fl/fl;MerCreMer− mice that had been treated with tamoxifen for 7 d, we observed an approximately 70% reduction in Atg5 protein levels in whole heart homogenates (Fig. 1a) and an approximately 90% reduction of Atg 5 protein levels in a partially purified adult cardiomyocyte preparation (Fig. 1b). Successful recombination occurred 3 d after tamoxifen injection (Fig. 1c). Suppression of Atg5-dependent conversion of microtubule-associated protein 1 light chain 3 (LC3-I) to LC3-II (a phosphatidylethanolamine conjugate13,15) and accumulation of the p62/sequestosome16 indicated a reduction in autophagy levels in tamoxifen-treated Atg5fl/fl;MerCreMer+ hearts (Fig. 1d). Echocardiographic analysis of tamoxifen-treated Atg5fl/fl;MerCreMer+ demonstrated left ventricular dilatation and severe contractile dysfunction (Fig. 1e,f). The heart-to-body and lung-to-body weight ratios were increased in tamoxifen-treated Atg5fl/fl;MerCreMer− mice (Fig. 1f). Atg5-deficient hearts exhibited no abnormal histological findings; that is, no myocardial disarray, vacuole formation or enhanced intermuscular fibrosis, but did show an increase in the cross-sectional area of cardiomyocytes (Fig. 2a-c). We observed typical changes in the...
The Beneficial Role of Autophagy in Cardiomyocytes in the Basal State and in Response to Hemodynamic Stress

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Introduction
The autophagy and the ubiquitin-proteasome system are responsible for the degradation of intracellular components. In autophagy, cytoplasmic proteins or dysfunctional organelles are sequestered in an autophagosome, a double-membrane vesicle, delivered to the lysosome by fusion and then degraded. The principal role of autophagy is to supply nutrients for survival. In addition, a low level of constitutive autophagy is also important for the quality control of proteins and organelles to maintain cell function. Thus, autophagy functions as a cell protective mechanism. In contrast, the presence of autophagic structures in dying cells in neurodegenerative diseases, myopathies and liver injury led to the hypothesis that autophagy has a causative role in cell death.

Role of autophagy in the heart at the basal state
We, first, generated temporally controlled cardiac-specific Atg5-/- mice to determine the role of basal constitutive autophagy in adult mouse hearts. Mice bearing an Atg5floxed allele, were crossed with transgenic mice (MerCreMer) expressing a Cre recombinase in a tamoxifen-inducible and cardiomyocyte-specific manner. The resulting Atg5floxed; MerCreMer+ mice were indistinguishable in appearance from age-matched control littermates bearing Atg5floxed; MerCreMer-. In Atg5lox/lox; MerCreMer+ mice that had been treated with tamoxifen for 7 d, we observed an approximately 70% reduction in Atg5 protein levels in whole heart homogenates and suppression of the Atg5-dependent conversion of microtubule-associated protein 1 light chain 3 (LC3)-I to LC3-II (phosphatidylethanolamine-conjugate) and the accumulation of p62/sequestosome indicated a reduction in autophagy level in tamoxifen-treated Atg5loxdelox; MerCreMer+ hearts. An echocardiographic analysis of tamoxifen-treated Atg5loxdelox; MerCreMer+ hearts demonstrated left ventricular (LV) dilatation and severe contractile dysfunction (Fig. 1). The hypertrophic response in tamoxifen-treated Atg5loxdelox; MerCreMer+ hearts exhibited no abnormal histological findings, i.e. no myofibrillar disarray, vacuole formation, and enhanced intermuscular cardiomyocytes (Fig. 2a, b).

Polyubiquitinated protein levels and proteasome activity increased in tamoxifen-treated Atg5loxdelox; MerCreMer+ hearts (Fig. 3a, b). Ultrastructural analyses of Atg5-deficient hearts revealed a dysorganized sarcomere structure, dysalignment and aggregation of mitochondria, and the appearance of aberrant concentric membranous structures similar to those observed in Atg7-deficient hearts (Fig. 3c). The observed hypertrophy is not only due to the accumulation of abnormal proteins, but also to activation of molecular maneuvers engaged with cardiac hypertrophy.

The accumulation of ubiquitinated proteins is known to induce endoplasmic reticulum (ER) stress, GRP78 and GRP94 protein levels were significantly increased in Atg5-deficient hearts (Fig. 3d). The protein level of cleaved caspase-12, which mediates ER stress, significantly increased in cardiomyocytes by tamoxifen-positive cells, identified as cardiomyocytes by α-sarcomeric actin staining.
Role of autophagy in the heart in response to pressure overload

We then attempted to confirm these observations using another line of cardiac-specific Atg5-deficient mice. Atg5lox mice were crossed with knock-in mice expressing Cre under the control of myosin light chain 2v (MLC2v) promoter to produce Atg5lox; MLC2v-Cre mice. In these mice, Cre is expressed in cardiomyocytes after embryonic day eight. Atg5floxflox; MLC2v-Cre+ littermates were used as controls. In contrast to tamoxifen-treated Atg5floxflox; MerCreMer+, Atg5floxflox; MLC2v-Cre+ showed no cardiac hypertrophy or dysfunction, suggesting alternate compensatory mechanisms function to cancel the phenotypes. In Atg5floxflox; MLC2v-Cre+, the reduction in autophagy may be too acute for compensatory mechanisms to be effective. This helped clarify the role of autophagy in response to stress such as pressure overload.

Pressure overload by means of a thoracic transverse aortic constriction (TAC) induced cardiac hypertrophy 1 week after the operation and heart failure 4 weeks later in wild-type mice. While autophagy was suppressed in TAC-induced hypertrophied hearts, as detected by decreased LC3-II levels, it was up-regulated in failing hearts, as evidenced by increased LC3-II levels. To elucidate the role of autophagy in cardiac remodeling, we performed TAC operations on Atg5floxflox; MLC2v-Cre+. Autophagy was suppressed in sham- or TAC-operated Atg5floxflox; MLC2v-Cre+ hearts compared to the corresponding controls. The Atg5floxflox; MLC2v-Cre+ showed severe cardiac dysfunction and LV dilatation 1 week after TAC (Fig. 4a) and died of heart failure thereafter. Pressure overload activated the S6 kinase in the heart, but the activation of p70S6 kinase was greater in TAC-operated Atg5floxflox; MLC2v-Cre+ than that in controls. Polyubiquitinated protein and the expression levels of GRP78 and caspase-12 were significantly increased in TAC-operated Atg5floxflox; MLC2v-Cre+ hearts (Fig. 4b).

Proteasome activity in Atg5floxflox; MLC2v-Cre+ was higher than in controls after TAC. These findings suggest that reduced autophagy resulted in the enhancement of both protein synthesis and proteasome-dependent protein degradation in pressure-overloaded hearts. The number of TUNEL-positive cardiomyocytes increased in Atg5floxflox; MLC2v-Cre+ hearts after TAC.

Discussion

In the basal state, autophagy mediates the essential and continuous turnover of intracellular proteins and organelles in the heart. The downregulation of protein turnover could cause abnormal proteins to accumulate, promoting ER stress, leading to apoptosis and cardiac dysfunction. It is also possible that the accumulation of abnormal proteins or organelles may directly cause cardiac dysfunction. Basal autophagy could be a homeostatic mechanism for the maintenance of normal cardiac function and morphology.

As to the role of autophagy in the stress response, our results indicate that autophagy plays a beneficial role in the heart in response to pressure overload. Autophagy is necessary for accelerated protein turnover in remodeling hearts and important for preventing the accumulation of abnormal proteins or damaged organelles, which can disrupt cardiac function. Since autophagy is a mechanism for maintaining energy homeostasis during starvation1, it is also possible that autophagy is necessary to compensate for increased energy demand during remodeling. Finally, autophagy may be an active adaptive intervention for protecting cardiomyocytes under stress by regulating cardiomyocyte death and function.

References
Semaphorin 7A initiates T-cell-mediated inflammatory responses through α1β1 integrin

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Semaphorins are axon guidance factors that assist growing axons in finding appropriate targets and forming synapses. Emerging evidence suggests that semaphorins are involved not only in embryonic development but also in immune responses. Semaphorin 7A (Sem7A; also known as CD108) is a glycosylphosphatidylinositol-anchored semaphorin, promotes axon outgrowth through β1-integrin receptors and contributes to the formation of the lateral olfactory tract. Although Sem7A has been shown to stimulate human monocytes, its function as a negative regulator of T-cell responses has also been reported.

Thus, the precise function of Sem7A in the immune system remains unclear. Here we show that Sem7A, which is expressed on activated T cells, stimulates cytokine production in monocytes and macrophages through α1β1 integrin (also known as very late antigen-1) as a component of the immunological synapse, and is critical for the effector phase of the inflammatory immune response. Sem7A-deficient (Sem7A−/−) mice are defective in cell-mediated immune responses such as contact hypersensitivity and experimental autoimmune encephalomyelitis. Although antigen-specific and cytokine-producing effector T cells can develop and migrate into antigen-challenged sites in Sem7A−/− mice, Sem7A−/− T cells fail to induce contact hypersensitivity even when directly injected into the antigen-challenged sites. Thus, the interaction between Sem7A and α1β1 integrin is crucial at the site of inflammation. These findings not only identify a function of Sem7A as an effector molecule in T-cell-mediated inflammation, but also reveal a mechanism of integrin-mediated immune regulation.

We first examined whether Sem7A could bind and stimulate monocytes and macrophages through integrins. T Eph-1 cells, a human monocyteic cell line, were treated with a panel of function-blocking monoclonal antibodies against Integrins, and subjected to adhesion assays on plates that were coated with Sem7A fused with the Fc portion of human IgG1 (Sem7A-Fc). Pre-treatment of T Eph-1 cells with anti-β1 monoclonal antibody strongly inhibited binding of the cells to Sem7A-Fc (Fig. 1a), which is consistent with a previous observation that Sem7A-induced axon growth is blocked by anti-β1 monoclonal antibody. Notably, treatment with anti-β1 monoclonal antibody also resulted in significant inhibition of binding, and blocking both α1 and β1 integrins reduced the number of bound cells to background levels. In contrast, monoclonal antibodies against α2, α3, α4, α5, α6 and αv subunits—all of which form functional heterodimers with the β1 subunit—and monoclonal antibodies recognizing the αvβ3 and αvβ5 complexes had no inhibitory effects. Although it has been proposed that plexin C1 is a Sem7A receptor, the effect of Sem7A on neuronal cells is independent of plexin C1 (ref. 5). Anti-plexin C1 monoclonal antibody did not affect cell binding to the Sem7A-Fc-coated surface, indicating that plexin C1 is not a binding partner for Sem7A in monocytes. As previously reported, Sem7A-Fc induced the production of pro-inflammatory cytokines in both human peripheral blood monocytes and mouse bone-marrow-derived macrophages (BMDMks) (Supplementary Fig. 1). Sem7A-induced production of interleukin (IL)-6 from monocytes was also significantly inhibited by anti-α1 or anti-β1 monoclonal antibody alone, and the combination of these antibodies resulted in further inhibition (Fig. 1b). These results suggest that Sem7A binds and stimulates monocytes through α1β1 integrin, which is known to be a collagen receptor. The RGD sequence, which is frequently found in proteins recognized by integrins, is necessary for the activity of Sem7A on neuronal cells. Site-directed mutagenesis of the RGD sequence in Sem7A-Fc or treatment of T Eph-1 cells with synthetic RGD peptides blocked cell adhesion (Supplementary Fig. 2), suggesting that α1β1 integrin recognizes the RGD sequence of Sem7A. We next tested the ability of Sem7A to stimulate BMDMks from α1-integrin-deficient (Igα−/−) mice. On Sem7A-Fc-coated surfaces, cytokine production and adhesion were significantly reduced in Igα−/− BMDMs as compared with wild-type cells (Fig. 1c; see also Supplementary Fig. 3). These observations reinforce that α1β1 integrin is the major receptor for Sem7A.

We further examined the interaction between Sem7A and α1β1 integrin using a soluble form of integrin heterodimer. Treatment of the Sem7A-Fc-coated surface with soluble α1β1 integrin, but not soluble α2β1 or α3β1 integrin, significantly inhibited adhesion of T Eph-1 cells (Fig. 1d). We also confirmed binding of Sem7A-Fc to α1β1 integrin by an enzyme-linked immunosorbent assay (ELISA) and co-immunoprecipitation assay (Supplementary Fig. 4a,b). The interaction between these proteins was inhibited with anti-β1 and/or anti-α1 monoclonal antibodies or RGD peptide (Supplementary Fig. 4c). These observations indicate that Sem7A directly interacts with α1β1 integrin.

Once bound to their ligands, integrins form clusters on the cell surface, leading to the recruitment of signalling and cytoskeletal proteins to form multimolecular signalling modules called focal adhesion complexes. When T Eph-1 cells were stimulated with Sem7A-Fc, both α1 and β1 integrins were redistributed to the cell periphery, as was focal adhesion kinase (FAK), which is an essential
Semaphorin 7A Initiates T-cell-mediated Inflammatory Responses through α1β1 Integrin

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Introduction

Semaphorins have been identified as axon guidance factors that assist growing axons in finding appropriate targets and forming synapses (1). Emerging evidence suggests that semaphorins play diverse roles not only in embryonic development but also in immune responses (2). Semaphorin 7A (Sema7A), which is a glycosylphosphatidylinositol (GPI)-anchored semaphorin and also known as CD108 (3, 4), promotes axon outgrowth through β1-integrin receptors and contributes to the formation of the lateral olfactory tract (5). Although Sema7A has been shown to stimulate human monocytes (6), it has remained elusive how Sema7A functions in physiological or pathological immune responses. Here we show that Sema7A, which is expressed on activated T cells, stimulates monocytes and macrophages to produce cytokines through α1β1 integrin, also known as very late antigen-1, as a component of the immunological synapse, and is critical for the effector phase of inflammatory immune responses. These findings not only identify a role of Sema7A as an effector molecule in T cell-mediated inflammation, but also provide a novel mechanism of integrin-mediated immune regulation.

α1β1 integrin is the functional receptor for Sema7A

We first examined whether Sema7A could bind and stimulate monocytes and macrophages through integrins. THP-1 cells, a human monocytic cell line, were treated with a panel of function-blocking monoclonal antibodies (mAbs) against integrins, and subjected to adhesion assays on plates that were coated with Sema7A fused with the Fc portion of human IgG1 (Sema7A-Fc). Pretreatment of THP-1 cells with anti-β1 mAb inhibited binding of the cells to Sema7A-Fc (Fig. 1a), which is consistent with the previous observation that Sema7A-induced axon growth is blocked by anti-β1 mAb (5). Notably, treatment with anti-α1 mAb also resulted in significant inhibition of binding, and blocking both α1 and β1 integrins reduced the number of bound cells to background levels. By contrast, mAbs against several other α subunits that form functional heterodimers with the β1 subunit (7), and mAbs recognizing the complexes of αvβ3 and αvβ5 had no inhibitory effects. Sema7A-induced production of proinflammatory cytokines from monocytes was also blocked with anti-α1 and/or anti-β1 mAbs. In line with these observations, bone marrow-derived macrophages from α1-integrin-deficient (Itgα1−/−) mice showed impaired cytokine production upon stimulation with Sema7A-Fc (Fig. 1b). Direct binding of Sema7A-Fc to soluble α1β1 integrin was also confirmed in an ELISA assay. Therefore, Sema7A binds and stimulates monocytes through α1β1 integrin, which is known to be a collagen receptor (8).

Sema7A stimulates macrophages at the immunological synapse

Its predominant expression on activated T cells and its potent stimulation of macrophages suggest that Sema7A might be involved in macrophage activation by T cells, which is an important step in the inflammatory process. Indeed, Sema7A-deficient (Sema7A−/−) T cells induced considerably lower levels of IL-6 and TNFα from macrophages than wild-type T cells (Fig. 2a), indicating that the importance of Sema7A as an effector molecule on activated T cells. We then analyzed the localization of Sema7A in T-cell-macrophage interactions. Ovalbumin-specific CD4+ T cells, which were stimulated with anti-CD3 and anti-CD28 mAbs to induce Sema7A expression, were conjugated with ovalbumin-peptide-pulsed macrophages. Notably, Sema7A clustered at the contact site between T cells and macrophages along with the lipid raft marker, GM1 glycolipid (39).
cospingolipid (Fig. 2b). Accumulation of α1β1 integrin at the contact site was also observed in macrophages (Fig. 2c). These observations indicate Sema7A and α1β1 integrin are components of the immunological synapse between T cells and macrophages.

Sema7A-/- mice are defective in Tcell-mediated immunity

We investigated the role of Sema7A in pathological immune responses using two different experimental models: contact hypersensitivity (CHS) and experimental autoimmune encephalomyelitis (EAE). Wild-type mice, which had been sensitized with 2,4-dinitrofluorobenzene (DNFB), mounted typical CHS responses characterized by ear swelling and infiltration of mononuclear cells upon rechallenge with DNFB. By contrast, Sema7A-/- mice were defective in eliciting CHS responses to DNFB (Fig. 3a, b). Moreover, Sema7A-/- mice were highly resistant to EAE induction when immunized with myelin oligodendrocyte glycoprotein peptide, and exhibited very few activated cells in the spinal cords (Fig. 3c, d).

Both CHS (9) and EAE (10) are presentations of two consecutive phases of T-cell-mediated immunity. The priming phase corresponds to the processes in which naive T cells are activated with antigen and then differentiated into effector T cells. In the following effector phase, the primed T cells migrate into the antigen-localized sites and induce inflammatory responses. Therefore, the depressed T-cell-mediated responses in Sema7A-/- mice might be attributed either to failure in T-cell priming, or to ineffective trafficking or effector function of primed T cells.

Sema7A promotes regional inflammation

The involvement of Sema7A in each step of the T-cell-mediated immune response was examined utilizing CHS as a model system. To determine the contribution of Sema7A to T-cell priming, T cells were isolated from draining lymph nodes after epicutaneous DNFB sensitization, and stimulated with antigen and then differentiated into effector T cells. Corresponding to the processes in which naive T cells are activated with antigen and then differentiated into effector T cells, the primed T cells migrate into the antigen-localized sites and induce inflammatory responses. Therefore, the depressed T-cell-mediated responses in Sema7A-/- mice might be attributed either to failure in T-cell priming, or to ineffective trafficking or effector function of primed T cells.

We next examined the effects of Sema7A deficiency on the recruitment of effector T cells into inflamed skin. T cells from DNFB-sensitized wild-type or Sema7A-/- mice were fluorescently labeled with 5,6-carboxyfluorescein diacetate, succinimidyl ester (CFSE), and injected intravenously into recipient mice. Both wild-type and Sema7A-/- donor cells were effectively recruited into DNFB-treated ears in wild-type and Sema7A-/- recipients (Fig. 4b). This indicates that Sema7A is not necessary for T-cell trafficking to inflamed cutaneous sites.

The normal recruitment of Sema7A+/+ T cells into inflamed tissues indicates that Sema7A+/+ T cells might be impaired in the capacity to induce inflammation. To test this possibility, T cells from DNFB-sensitized wild-type or Sema7A-/- mice were injected intraocularly into the ear tissue of wild-type recipients, followed by DNFB challenge. Notably, the intensity of the ear-swelling response induced by sensitized Sema7A+/+ T cells was markedly attenuated as compared with that induced by wild-type cells (Fig. 4c). Thus, Sema7A on antigen-primed T cells is required for the optimal effector function of these cells to enhance inflammation at the site of CHS.

Conclusion

Our findings have established that integrin-mediated signaling is a common mechanism by which Sema7A functions in both the nervous and immune systems, and that Sema7A plays an essential function in the effector phase of T-cell-mediated immunity through α1β1 integrin on macrophages. Clinical symptoms of autoimmune and chronic inflammatory diseases are direct manifestations of the effector phases of aberrant inflammatory reactions. Therefore, the interaction between Sema7A and α1β1 integrin is a potential therapeutic target for these immune disorders.

References

Reactivation of Physical Motor Information in the Memory of Action Events

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No. 2 in 100 Papers Selection (p. 59)

When attempting to memorize action sentences (e.g., open an umbrella), performing the action of the sentence (enacted encoding) results in better memory performance than simply memorizing the sentences (verbal encoding). This memory enhancement is called the enactment effect. Magnetoencephalography (MEG) was used to elucidate whether the enactment effect is due to physical motor information or whether movement representation is the critical factor in the enactment effect. Physical motor information, which is implicated in the primary motor cortex, represents the speed, form, and kinesthetic sense of a movement, while movement representation indicates semantic and conceptual information such as movement images, movement ideas, and movement imagery, which are especially associated with the parietal cortex. We measured activities within the motor region and parietal cortex during a recognition test and compared activities during recognition with enacted and verbal encoding condition. The results showed that recognition performance was better for enacted encoding (Table 1, Fig. 1). The MEG data indicated that the left primary motor cortex with enacted encoding condition was activated in all subjects (Fig. 2, A), though with verbal encoding condition, this activation appeared in only one subject. These activities were observed between 150 and 250 ms after recognition stimuli. Moreover, activities in the right parietal cortex following enacted encoding were greater than those following verbal encoding, and the activities appeared 600-700 ms after onset of the recognition stimulus (Fig. 2, B, C). These results suggest that the enactment effect occurs by the reactivation of the physical motor information and that this information facilitates activities related to movement representation.

Table 1 – Mean proportion of false alarms across recognition with enacted and verbal encoding

<table>
<thead>
<tr>
<th>Encoding condition</th>
<th>Pr</th>
<th>Hit</th>
<th>False alarms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enacted encoding</td>
<td>0.59</td>
<td>0.55</td>
<td>0.06</td>
</tr>
<tr>
<td>Verbal encoding</td>
<td>0.34</td>
<td>0.38</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Note: The table above shows significant differences between the 2 conditions. M: mean, SE: standard error.

Long-Term Grooming Partnerships Between Unrelated Adult Females in a Free-Ranging Group of Japanese Monkeys (Macaca fuscata)

NAKAMICHI Masayuki and YAMADA Kazunori
(Graduate School of Human Sciences)
No. 4 in 100 Papers Selection (p. 69)

Fig. 1 Long-term grooming relationships that have been maintained between same-aged unrelated females over a 10-year period. Females were 23 and 22 years old, respectively, in A and B.

Japanese monkeys (Macaca fuscata), females generally remain in their natal group throughout their lives and tend to continuously maintain affiliative relationships with related females such as mothers, sisters, grandmothers, and granddaughters for years. Such affiliations are also found between unrelated females. However, there are few studies on the continuity of long-term affiliative relationships between unrelated females.

Social grooming is a common and frequently observed affiliative behavior in Japanese monkeys, more than half the grooming bouts in groups occur among closely related females. Although the number of available females in a group increases, the number of female grooming partners does not increase. Therefore, not only related female grooming partners but also unrelated female grooming partners that are included among the limited number of grooming partners should be considered to be important.

In order to examine the prevalence of long-term grooming relationships among unrelated females, we recorded grooming interactions of 18 adult females (16 to 32 years) in a free-ranging group of Japanese monkeys at Katsuyama in 2003 and compared them with those recorded 10 years prior, i.e., in 1993. In 2003, on an average, each female had survived the 10 years had grooming interactions with 2 surviving old partners with whom she was recorded to have grooming interactions in 1993, indicating that each of the females had maintained grooming relationships with some surviving unrelated old partners over the 10 years. As the age difference in grooming dyads of such surviving old partners was usually three years or less, affiliative relationships that had developed through social play when females were immature might be maintained through social grooming after animals grew up (Fig. 2). In 2003, moreover, each female had grooming interactions with closely related females of the surviving old grooming partners. In 2003, however, each female had grooming interactions with several unrelated females who were other than the surviving old grooming partners or their related females.

These findings indicate that with regard to grooming relationships, female Japanese monkeys are basically conservative, showing a tendency to concentrate their grooming interactions on closely related females and certain familiar unrelated females such as surviving old partners and some females closely related to these partners. At the same time, however, female Japanese monkeys also showed a progressive trait for grooming since they did form grooming relationships with new partners.
Structure and Function of a Hexameric Copper-containing Nitrite Reductase
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Proceeding of the National Academy of Sciences of the United States of America, 104, 4315-4320 (2007)
No. 11 in 100 Papers Selection (p. 59)

The terrestrial nitrogen cycle sustained by some bacteria plays an important role in all organism kingdoms. Inorganic nitrogen is introduced into the biosphere by the biological fixation of atmospheric nitrogen to produce N\textsubscript{2}, and is finally removed from there again through the process of denitrification. Denitrification is the dissimilatory reduction of NO\textsubscript{3} or NO\textsubscript{2} to produce N\textsubscript{2} via NO and N\textsubscript{2}O. Copper-containing nitrite reductase (NIR) catalyzes one-electron reduction of NO\textsubscript{2} to NO. We have determined the X-ray crystal structure of novel hexameric NIR (HdNIR) from a methylotrophic denitrifying bacterium, *Hyphomicrobium denitrificans*. In Fig. 1, the overall structure of HdNIR reveals a trigonal prism-shaped molecule, in which the monomer consisting of 447 residues and three Cu atoms is organized into a hexamer (a dimer of trimers). Each monomer is made up of three structurally similar Greek-key barrel folding domains (cupredoxin domains I to III); the domains I and II bind one type 1 Cu (domain I, type 1 CuN; domain II, type 1 CuC) and are combined with an unusual long loop comprising 31 amino acid residues (Fig. 1c). As shown in Fig. 2, the type 1 CuN binds five ligands (Glu, Cys, Met, and backbone carbonyl group), but the type 1 CuC binds four ligands (Glu, Cys, and Met). The type 2 Cu having 3His ligands is located at the interface formed by the domain II of one monomer and the domain III of an adjacent monomer. The distance between the type 1 CuC and type 2 Cu connected through the sequence segment (-Cys-His-) is 12.6 Å. The enzyme receives one electron at the type 1 CuN from an electron donor protein (cytochrome c550) and catalyzes one-electron reduction of NO\textsubscript{2} to NO at the type 2 Cu, which intramolecularly accepts an electron from the reduced type 1 CuC. Moreover, the type 1 CuN is essential for dimerization of the trimers. The hexameric structure of HdNIR is also maintained in a solution and the enzyme containing the six catalytic centers in one molecule behaves as a multi-active site enzyme in the periplasm.

Charge Transfer Through DNA Nanoscaled Assembly Programmable with DNA Building Blocks
KAWAI Kiyohiko and MAJIMA Tetsuro
(Institute of Scientific and Industrial Research)
No. 13 in 100 Papers Selection (p. 59)

DNA has been used extensively to form nanoscale structures that may be used as nanotechnology sensors and devices in the future. Despite recent advances in understanding the charge transfer (CT) through DNA, the CT through DNA nanoscaled assembly has not been clarified. A further understanding not only of the CT through DNA nanoscaled assembly, but also the kinetic mechanisms, which provide information about the CT processes, is of fundamental importance in order to create functionalized nanometer-scaled DNA wires and arrays. In this study we have reported photoinduced long-range CT of over 140 Å through a programmable DNA nanoscaled assembly based on guanine-cytosine alternating sequence (GCG) by using time-resolved transient absorption measurements. We revealed that the CT rate through the DNA with the GCG sequence actually is rapide. We also demonstrated that the DNA block system makes it possible to achieve the CT over 140 Å through the DNA nanoscaled assembly based on the GCG CT through the GCG sequences. Moreover, the CT through the nanoscaled DNA assembly sequence is programmable by using DNA blocks.
Change in the Burgers Vector of Perfect Dislocation Loops without Contact with External Dislocations

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\( \text{N} \times 20 \times 130 \) Planview Selectroff (p. 49)

The irreversible plastic deformation of crystalline materials is often governed by the generation and motion of linear defects, termed dislocations. Knowledge of the structure and dynamic processes of dislocations in a crystal is important for understanding the origin of the hardness and toughness of a crystal. These defects connect two parts of a crystal that are sheared on a plane with respect to each other by an atomic translation called the Burgers vector.

The Burgers vector of a dislocation is a major factor controlling the displacement field and the strain energy associated with the dislocation, moving direction, mobility of the dislocation, and so on. Dislocations always obey dislocation laws for the Burgers vector, according to which the total Burgers vector measured in a closed circuit enclosing simple or multiple dislocation lines is always conserved even at their nodes. Furthermore, according to the law, the Burgers vector of a dislocation can change if it joins another dislocation or branches out.

In this paper, we presented a new process for introducing a change in the Burgers vector of nanometer-sized interstitial-type perfect dislocation loops—aggregations of self-interstitial atoms on a habit plane—without contact with external dislocations in bcc Fe, upon high-energy electron irradiation or simple heating, by using in situ transmission electron microscopy (TEM). Two types of loops were formed upon high-energy electron irradiation—those with the Burgers vectors of \( \frac{1}{2}<111> \) and \( <100> \). The Burgers vector of the mobile \( \frac{1}{2}<111> \) loops occasionally changed to that of another \( \frac{1}{2}<111> \) loop without the coalescence of the loop with an external loop. Other types of the changes in the Burgers vector of loops, such as that from \( \frac{1}{2}<111> \) to \( <100> \) and its reverse, occasionally occurred. Figure 1 shows an example of the transformation of a \( \frac{1}{2}<111> \) loop to a \( <100> \) loop.

It should be noted that the \( \frac{1}{2}<111> \) loops without stacking faults transformed although the energy of the system appeared to remain constant or to increase due to this change. The change in the Burgers vector of a prismatic loop without coalescing with external dislocations can be expressed as the nucleation and propagation of a proper shear loop, in which the habit plane is identical to that of the original prismatic loop and only the shear component exists in the Burgers vector inside the prismatic loop.

Mannmade Diamond Structure Opens Photonic Band Gap for Terahertz Waves

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Journal of American Ceramic Society, 90, 92-96 (2007)
\( \text{N} \times 22 \times 130 \) Planview Selectroff (p. 50)

Artificial crystals made of dielectric medium called photonic crystals can inhibit the propagation of electromagnetic waves or light with wavelengths corresponding to the periodic variation of dielectric constant in Bragg scattering and open photonic band gaps. Many photonic crystals were made for infrared and visible optical ranges; however, there has been a little work on 3D photonic crystals with the features of micron scale for terahertz frequencies (100 GHz-10 THz). Development of photonic crystals applications is an emergent issue for bio-imaging and risk management like remote detection of gunpowder, drugs, counterfeit IC cards besides advanced communication, physical and chemical characterization.

Recently, we have developed micro-stereolithography with companies to enable free-forming 3D micro or meso scale structures and its system is sold as the first commercial machine in the world since 2005. Its system can build complex 3D micro and nano structures by layer polymerization of liquid photopolymer resin with precise laser projection of two dimensional figures using DMD (Digital Micro-mirror Device) under ODMAS (Computer Aided Design and Computer Aided Manufacturing) system. Micro ceramic green parts are formed by using resin filled with nanometer sized ceramic particles. Dense micro ceramic components can be obtained by sintering these green parts.

Figure 1(a) is a micro diamond structure made of TiO2 (~170 nm, diameter of 500 nm). The structure is built layer by layer with 5 μm in thickness like a natural crystal growth as seen in Fig. 1(c) and (d). The transmission spectrum along \( <100> \) direction shows a sharp band gap between 280 GHz and 3000 GHz in a THz range as shown in Fig. 2, where the transmission of incident waves goes down below 1%. Such diamond structure has a common band gap opened for all directions. Various micro ceramic devices like photonic crystals having cavities, channels, connectors, and other parts can be freely designed and produced by micro-stereolithography and successive sintering. The digital process which we named “Smart processing” can be linked to internet and applied to future remote and just-in-time manufacturing with saving energy and low environmental impact.

Fig. 1 (a) A micro diamond structure made of 40vol% TiO2 resin with 70μm unit cells. (b) Top view at 1000 plane. (c) Cross linked part of lattice nodal, (d) Side view of stacking layers.

Fig. 2 Transmission spectrum of terahertz waves as a function of frequency.
Compressive Properties of Lotus-Type Porous Stainless Steel

TANE Masakazu and NAKAJIMA Hideo

Lotus-type porous stainless steel (Fig. 1), possessing cylindrical pores aligned in the direction, was fabricated by a continuous zone melting technique in a pressurized mixture gas of hydrogen and helium. Compression tests were carried out on the lotus stainless steel not only in the directions parallel and perpendicular to the elongated-pore direction but also in other directions to reveal its anisotropic compressive behavior. The macroscopic deformation modes depend on the porosity and the angle $\theta$ between the elongated-pore direction and compression direction, which is a unique characteristic resulting from its anisotropic porous structure. The yield stress in the pore direction, $\sigma_{||}$, decreases almost linearly with increasing porosity, while that in the perpendicular direction, $\sigma_{\perp}$, decreases more rapidly (Fig. 2). The yield stress in the direction of $\theta$ from the elongated-pore direction, $\sigma_{\theta}$, decreases monotonically with increase in $\theta$ (Fig. 3). The yield behavior of lotus stainless steel was described using micromechanical mean-field theory based on Ishibashi’s inclusion theory and Mori-Tanaka mean-field theory.

Quantification of Annealed Microstructures in ARB Processed Aluminum

KAMIKAWA Naoya and TSUJI Nobuhiro

When metallic materials are plastically deformed up to ultrahigh strain, ultrafine-grained (UFG) structures with mean grain sizes around 100 nm or nanocrystalline structures with grain sizes of 10 nm are formed. The UFG metals are expected as future structural materials because of their excellent mechanical properties. For example, the UFG metals show strength 2-4 times higher than conventionally coarse-grained ones with the same chemical compositions, so that aluminum can be as strong as steel. Furthermore, the UFG metals sometimes show surprising properties which have not yet been known in conventional metallic materials. One of such peculiar properties the present authors have found is hardening by annealing, and softening by deformation behavior, which is totally opposite to the common sense of previous knowledge in materials science (Science, 312, 249-251 (2006)).

In the present paper, the microstructure and crystallography of the UFG Al fabricated by the ARB (accumulative roll bonding) process, that is a kind of ultrahigh straining process originally developed in Osaka University in 1998, were quantified by advanced electron microscopy techniques (TEM/HAADF imaging and EBSD analyses) in details. The main results are summarized in Fig. 1. Firstly, it was found that the as-ARB processed Al had an unique microstructure: the ultrafine grains certainly have large misorientation to each other and at the same time the structure involves high density of low-angle boundaries as well as dislocations. That is, the UFG structure fabricated by ultrahigh strain has characteristics of "grain" as well as "deformed metals". The ARB Al was annealed at various temperatures for various periods, and then the microstructures were again observed in details. Recovery and grain growth happened during annealing. Microstructure coarsening was quantitatively determined by the present investigations. It was found that the fraction of low-angle boundaries greatly decreased by low temperature annealing. It was suggested that such a structural change is responsible for the occurrence of the unique mechanical property in the UFG metals, hardening by annealing, and softening by deformation.
Periodic Structures Consisting of Germanium Nanoparticles in Buried Channel Waveguides

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(Graduate School of Engineering)
Optics Express, 15, 2047-2054 (2007)

Periodic structures consisting of Ge nanoparticles were formed in buried channel waveguides. Such periodic structures were created in GeO2-B2O3-SiO2 glass films by the combination of exposure to interference patterns of ultraviolet laser light and thermally induced phase changes of the glasses. Figures 1(a) and 1(b) respectively show scanning electron microscope images of the channel structure before and after HF etching. The images of the structures from above and with an enlarged view are shown respectively in Figs. 1(c) and 1(d). Figure 1(a) shows that surfaces of the channel structure were rather smooth in spite of the precipitation of Ge nanoparticles. In particular, the sidewall surface roughness was almost not observed. It is readily apparent from Figs. 1(b) and 1(c) that periodic relief patterns appeared on the channel surfaces after HF etching. The nanoparticles are visible in the convex regions.

Isolated Electrodeless High-Frequency Quartz Crystal Microbalance for Immunosensors

OGI Hirotsugu and MOTOHISA Kazuma
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Analytical Chemistry, 78, 6903-6909 (2006)

Various immunosensors have been studied because of two principal purposes. First, they are used to detect specific protein markers that are excreted by corresponding disorders, such as, glypican-3 protein for hepatocellular carcinoma and amyloid-β peptide for Alzheimer’s disease. Early detection of such protein markers increases the probability of their permanent cure. Second, they are expected to determine the limit of detection related to biochemical reactions, yielding affinity between biomolecules, which significantly contributes to the development of an effective antibody for a specific antigen, that is, in the drug discovery. Among many immunosensors, the quartz-crystal microbalance (QCM) technique has been extensively studied because it allows absolute and quantitative measurement of the affinity. However, it shows lower sensitivity for proteins with smaller molecular masses.

This paper then proposes an ultrahigh-sensitive QCM immunosensor with the wireless-electrodeless technique. The shear bulk wave resonance frequencies of the isolated quartz crystal were measured in a flow cell with the noncontacting manner by the line antenna placed outside the cell (Fig. 1). Exact vibrational analysis predicts higher frequency sensitivity to the adsorbed material at higher modes when the electrode layer is removed. The 13th overtone (72-MHz resonance frequency) was used to detect human immunoglobulin G with concentrations between 0.1 and 20 μg/mL captured by Staphylococcus-aureus protein A immobilized on one side of the crystal (Fig. 2). The real-time monitoring of the binding and dissociating reactions was made via the frequency response (Fig. 3), which yielded the equilibrium constant Kd of 5.21x10⁻⁵ M⁻¹. The much higher sensitivity was confirmed by this novel technique.
Protective Layer Formation during Oxidation of Cu₃Au(100) using Hyperthermal O₂ Molecular Beam

OKADA Michio
(Graduate School of Science)
No. 46 in 100 Papers Selection (p. 60)

Corrosion wastes more than a few percent of the world’s GDP. The initial stage of the corrosion is one of the central topics in material science. The oxidation is a major corrosion process of metals. The growth of a protective thin surface layer, which prevents further oxidation into bulk of a metal, requires the formation of a homogeneous film. One simple way for the protection of underlying metals is surface alloying, combining different substances to form multicomponent surfaces.

We report results of our detailed studies on the initial oxidation process of Cu₃Au(100) with a hyperthermal O₂ molecular beam (HOMB) with varying its incident energy. From the O-uptake curves (middle of Figure), which were determined from a series of O-1s X-ray photo-emission spectroscopy (XPS) measurements in conjunction with synchrotron radiation (SR), it was found that the dissociative sticking probability of O₂ is much lower on Cu₃Au than on Cu. Low-energy electron diffraction (LEED) observations and surface core-level shift (SCLS) measurements suggest that the dissociative adsorption of O₂ occurs accompanied with the Cu segregation on the surface. No obvious growth of Cu₂O was observed even for the prolonged doses of 2.3 eV HOMB on Cu₃Au(100) (Compare the top and bottom of Figure). The combination of the surface Cu-O layer and the second Au-rich layer works as a perfect protective layer even for the energetic O₂.

Single Gadolinium Atoms Observed by Aberration-Free TEM

TAKAI Yoshizo and TSUJI Toshiyuki
(Graduate School of Engineering)
No. 52 in 100 Papers Selection (p. 63)

Recent electron microscopic methods using aberration correction techniques have led to revolutionary improvement in understanding structural and compositional details on a single atomic scale on surfaces, interfaces, and even in bulk crystals. Among them, aberration-free transmission electron microscopy (TEM) based on wave-field restoration techniques has great potential for determining localized atomic structures because complex wave fields on the exit surface of the sample can be restored without image delocalization due to the correction of lens aberrations.

In the present paper, single gadolinium atoms in fullerene encapsulated in a single-wall carbon nano-tube (Gd@C₈₂@SWCNT) are observed by wave field restoration processing based on three-dimensional Fourier filtering method as schematically shown in Fig. 1. In the imaginary part image (phase contrast image) of Fig. 2(a), the single gadolinium atoms encaged in fulleranes F₁ and F₃ can clearly be seen with sharp spot-like contrast, as indicated by the white arrows. Surprisingly, dark spots are also slightly visible in the real part image (amplitude contrast image), which is due to an improved signal-to-noise ratio by Fourier filtering and resolution enhancement by correcting spherical aberration and two-fold astigmatism. These reconstructed images correspond to aberration-free TEM images observed with and without an ideal phase plate, respectively. This result indicates that the present method has high potential to clarify compositional details of the sample on a single atom level by using their image contrasts.
Cu-Catalyzed Cross-Coupling Reaction of Grignard Reagents with primary-Alkyl Chlorides: Remarkable Effect of 1-Phenylpropyne

TERAO Jun and KAMBE Nobuaki
(Graduate School of Engineering)


The copper-catalyzed cross-coupling of alkyl halides or sulfonates with Grignard reagents has become one of the most straightforward methods for constructing methylene chains. A serious drawback of this reaction is its non-applicability toward alkyl chlorides, which are promising alkylating reagents because of their wide availability and low cost relative to their iodo and bromo analogues. This lack of reactivity is probably due to the strong C-Cl bond relative to the C-I and C-Br bonds. We have recently reported that Cu catalyzes the cross-coupling reaction of non-activated alkyl fluorides with Grignard reagents in the presence of 1,3-butadiene additives under mild conditions; however, the corresponding alkyl chlorides gave only poor yields of the cross-coupling products. During the course of this study, we have developed that the Cu-catalyzed alkyl-alkyl cross-coupling reaction between alkyl chlorides and Grignard reagents proceeds efficiently in the presence of 1-phenylpropyne as an additive, and is applicable to alkyl fluorides, mesylates, and tosylates. For example, tert-nonyl chloride reacted with tert-BuMgCl in the presence of catalytic amounts of CuCl2 (2 mol %) and 1-phenylpropyne (10 mol %) in THF under reflux for 6 h to give tetradecane in greater than 98 % yield along with a trace amount of a reduction product, nonane (<1 %). The present Cu-catalyzed cross-coupling reaction proceeds efficiently with sec-butyl, tert-butyl, and phenyl Grignard reagents. It should be noted that alkyl fluorides and mesylates can also undergo the present cross-coupling reaction to give rise to the corresponding products in almost quantitative yields.

Texturization of Multicrystalline Silicon Wafers for Solar Cells by Chemical Treatment Using Metallic Catalyst

MATSUMURA Michio
(Research Center for Solar Energy Chemistry)


In order to increase the efficiency of silicon solar cells, the surface reflectivity must be lowered so that incident photons are absorbed as much as possible. In the case of single crystalline solar cells, the ideally textured surface is produced by utilizing the anisotropic etching in alkaline solution. However, this method cannot be applied to multicrystalline silicon wafers, which are now used most commonly in practical solar cells due to their low production cost. This is because different crystalline faces are exposed to the surface. In order to lower the surface reflectivity of multicrystalline silicon wafers, we have developed a new method for texturizing their surface using a chemical reaction catalyzed by nano-sized metal particles, such as silver particles. The multicrystalline silicon solar cells made of the wafers textured by this method showed lowered reflectivity and increased photodcurrent density. As a result, the efficiency of the cell reached 16.7 %, which was about 1.4 % (absolute) higher than the efficiency of the cell with a surface treated by the conventional method.
Structure of the Light-Harvesting Bacteriochlorophyll c Assembly in Chlorosomes from Chlorobium limicola Determined by Solid-State NMR

FUJWARA Toshimichi and AKUTSU Hideo
(Institute for Protein Research)

We have determined the atomic structure of the bacteriochlorophyll c (BChl c) assembly in a huge light-harvesting antenna, the chlorosome of green photosynthetic bacteria, for the first time by solid-state nuclear magnetic resonance (NMR). Previous electron microscopic and spectroscopic studies indicated that chlorosomes have a cylindrical architecture with a diameter of about 10 nm consisting of layered BChl molecules. Assembly structures in the huge noncrystalline chlorosomes have been proposed mainly from static images of high-resolution electron micrographs and a few distances acquired by solid-state NMR, but these studies did not provide a definite structure. Our approach is based on the 13C dipolar spin-diffusion solid-state NMR of uniformly 13C-labeled chlorosomes under magic-angle spinning (Fig. 1). About 90 intermolecular C-C distances were obtained by the simultaneous assignment of distance correlations and structure optimization preceded by the polarization-transfer matrix analysis. These distances revealed that BChl c molecules form piggyback-dimer based parallel layers in the antenna (Fig. 2). This result rules out the well-known monomer-based structures. We have built a molecular model of the cylinder in the chlorosomes with the parallel layers in reference to the electron micrographs (Fig. 3A and B). This antenna structure provided insights into the mechanisms of efficient light harvesting and excitation transfer to the reaction centers (Fig. 3C). This work constitutes an important advance in the structure determination of huge intact systems that cannot be crystallized.

Genome-wide Identification of Replication Origins in Fission Yeast

HAYASHI Makoto and MASUKATA Hisao
(Graduate School of Science)

DNA replication is a fundamental biological phenomenon, by which genetic information of living organisms is propagated to their descendants. DNA replication of eukaryotic chromosomes initiates at a number of discrete loci, called replication origins. Distribution and regulation of origins are important for complete duplication of the chromosomes. However, precise genome-wide localization of origins has not been determined in most eukaryotes including fission yeast, Schizosaccharomyces pombe.

The process of initiation of replication is composed of two major steps, licensing of replication origins in G1 phase and activation of the origins in the following S phase. The licensing is done by forming pre-RC (pre-Replisome Complex) composed of ORC and MCM on each origin. Subsequently, some of the pre-RC are activated and replication initiation occurs. Thus, we mapped ORC and MCM localization sites in G1 phase as well as nascent replicated DNA in S phase by using high-resolution DNA microarray covering almost the entire genome of fission yeast (Fig. 1). By comparing those genome-wide information, we have successfully identified 460 pre-RC sites, some of which have initiation activity in the early S phase (called early origin) while the others do not (called late origin) (Fig. 2B). Early and late origins tend to distribute separately in large chromosome regions, implying that activation of pre-RCs may be regulated in large chromosome regions.

As replication is likely to be coupled with various chromatin functions such as sister chromatid cohesion, condensation, DNA repair, checkpoint and chromosome structures, the data on replication machinery assembly sites and its activity will help to identify locations and movement of relevant proteins.

Fig. 1. A part of the micro-array results, showing localization of ORC (Orc1) and MCM (Mcm6) in G1 phase as well as newly replicated DNA (HirD) in S phase.

Fig. 2. A genome-wide map of early origins (red diamonds) and late origins (blue diamonds) in fission yeast chromosomes I, II and III.
Amyloid fibrils are highly-ordered filamentous aggregates formed by the self-assembly of peptides or proteins. There are currently approximately 20 known diseases associated with deposition of amyloid fibrils, including Alzheimer’s disease, Parkinson’s disease, and dialysis-related amyloidosis. Additionally, numerous peptides and proteins not directly related to diseases can also form amyloid-like fibrils in vitro, suggesting that amyloid fibril formation is a generic property of the polypeptide chain. To obtain further insight into protein folding and misfolding, it is crucial to clarify the mechanism of fibril formation and the structural stability of amyloid fibrils.

We have combined solid-state nuclear magnetic resonance (NMR), X-ray fiber diffraction, and atomic force microscopy to reveal the 3D structure of amyloid protofilament-like fibrils formed by a 22-residue K3 peptide (Ser27-pro41) of β2-microglobulin, a protein responsible for dialysis-related amyloidosis. Although a uniformly 13C, 15N labeled sample was used for the NMR measurements, we could obtain the 3D structure of the fibrils on the basis of a large number of structural constraints, leading to an atomistic-level understanding of the protofilament structure and the types of interactions required to stabilize this structure. The conformation of K3 fibrils was found to be a β-strand-rich β-sheet, with each K3 molecule stacked in a parallel and staggered manner. The fibrillar conformation was stabilized by intermolecular interactions, rather than intramolecular hydrophobic packing as seen in globular proteins. Together with thermodynamic studies of the full-length protein, formation of the fibrils is likely to require side chains on the intermolecular surface to pack tightly against those of adjacent monomers. By revealing the structure of β2-microglobulin protofilament-like fibrils, this work represents technical progress in analyzing amyloid fibrils in general through solid-state NMR.
A Toxic Monomeric Conformer of the Polyglutamine Protein

Yoshitaka NAGAI and Tatsumi TODA

PGC7/Stella Protects Against DNA Demethylation in Early Embryogenesis

Toshinobu NAKAMURA and Toru NAKANO

DNA methylation is one of the pivotal mechanisms in the epigenetic regulation. However, the molecular mechanisms for the control of DNA methylation remain by and large unclear. In this study, we clearly demonstrated that PGC7/Stella is a modifier of DNA methylation in early embryogenesis. PGC7/Stella is a gene which is expressed in only early embryos, primordial germ cells (very immature germ cell precursors), and oocytes. Previous gene-targeting analysis has revealed that PGC7/Stella-null zygotes derived from control and PGC7/Stella-null eggs was demethylated while that from the control egg was methylated (Fig 1). These results clearly show that PGC7/Stella is required to protect against DNA demethylation of the maternal genome after fertilization, i.e. PGC7/Stella is essential for the establishment of epigenetic asymmetry. Next, we examined the methylation status of two types of genes, retrotransposons and genomic imprinted genes, which are not affected by the global DNA demethylation. Slit2 gene sequencing analysis clearly demonstrated that the DNA methylation of some of these genes was abrogated in the PGC7/Stella-null zygotes. Taken together, PGC7/Stella is considered to be an essential for protecting against DNA demethylation during early embryogenesis (Fig. 2).

PGC7/Stella is a gene which is expressed in only early embryos, primordial germ cells (very immature germ cell precursors), and oocytes. Previous gene-targeting analysis has revealed that PGC7/Stella-null zygotes derived from control and PGC7/Stella-null eggs was demethylated while that from the control egg was methylated (Fig 1). These results clearly show that PGC7/Stella is required to protect against DNA demethylation of the maternal genome after fertilization, i.e. PGC7/Stella is essential for the establishment of epigenetic asymmetry. Next, we examined the methylation status of two types of genes, retrotransposons and genomic imprinted genes, which are not affected by the global DNA demethylation. Slit2 gene sequencing analysis clearly demonstrated that the DNA methylation of some of these genes was abrogated in the PGC7/Stella-null zygotes. Taken together, PGC7/Stella is considered to be an essential for protecting against DNA demethylation during early embryogenesis (Fig. 2).
Crystal Structures of γ-Glutamyltranspeptidase from Escherichia coli, a Key Enzyme in Glutathione Metabolism, and Its Reaction Intermediate

WADA Kei and FUKUYAMA Keiichi
(Graduate School of Science)

Complementation of Placental Defects and Embryonic Lethality by Trophoblast-Specific Lentiviral Gene Transfer

OKADA Yuka and IKAWA Masahito
(Research Institute for Microbial Diseases)

Placental dysfunction underlies many complications during pregnancy, and better understanding of gene function during placentalization could have considerable clinical relevance. However, the lack of a facile method for placenta-specific gene manipulation has hampered investigation of placental organogenesis and the treatment of placental dysfunction. We have previously shown that transduction of fertilized mouse eggs with lentiviral vectors leads to transgene expression in both the fetus and the placenta. Here we report trophoderm- and placenta-specific gene incorporation by lentiviral transduction of mouse blastocysts after removal of the zona pellucida (Fig. 1a). All of the placentas analyzed, but none of the fetuses, were transgenic. Histological analysis revealed uniform and ubiquitous transgene expression in all 3 major layers of the placental labyrinth, spongiotrophoblast, and giant cells (Fig. 1b). Application of this method substantially rescued mice deficient in Ets-2, Mapk14 and Mapk11 from embryonic lethality caused by placental defects. Ectopic expression of Mapk11 also complemented Mapk14 deficiency during placentalization (Fig. 2).
The Mouse Embryo Autonomously Acquires Anterior Posterior Polarity at Implantation.

TAKAOKA Katsuyoshi and HAMADA Hiroshi

(Graduate School of Frontier Biosciences)


No. 92 in 100 Papers Selection (p. 67)

The earliest recognizable sign of patterning of the mouse embryo along the anterior-posterior (A-P) axis is the migration of the distal visceral endoderm (DVE) toward the future anterior side. We report a molecular asymmetry in the mouse embryo that precedes the onset of DVE migration. The gene for Lefty1, a nodal antagonist that influences the direction of DVE migration, was found to be asymmetrically expressed in the primitive endoderm of the implanting blastocyst. Lefty1 is initially randomly localized in the inner cell mass (ICM), but is regionalized to one side of the tilted ICM shortly after implantation. Asymmetric expression of Lefty1 can be established by in vitro culture, indicating that it does not require interaction with the uterus. The asymmetric Lefty1 expression is induced by nodal signaling, although nodal and genes for its effectors are expressed symmetrically. This asymmetry in molecular patterning of the mouse embryo pushes back the origin of the A-P body axis to the peri-implantation stage.

Fig. 1 Asymmetric expression of Lefty1 transgenes in peri-implantation mouse embryos.

Fig. 2 Mouse embryos harboring the Lefty1-9.5 lacZ transgene were recovered at the indicated stages and stained with X-gal.

Fig. 3 Lack of asymmetry in the expression of nodal, foxh1, and cripto in mouse embryos between E3.5 and E4.5.

Reprinted from Developmental Cell, 10, Takaoka, K. et al., The mouse embryo autonomously acquires anterior-posterior polarity at implantation, 451-459, Copyright 2006, with permission from Elsevier.

Triggering Neural Differentiation of ES Cells by Subtype Switching of Importin-α

YASUHARA Noriko and YONEDA Yoshihiro

(Graduate School of Frontier Biosciences)


No. 99 in 100 Papers Selection (p. 67)

Eukaryotic cell nuclei are separated from cytoplasm by lipid bilayer of nuclear envelope. A cell must respond to various cellular events with regulated signal exchanges between cytoplasm and nucleus. Nuclear-cytoplasmic transport of functional proteins is a selective transport mediated by specific transport systems through the nuclear pore. In this paper, we report that a transport receptor family plays a significant role in neural differentiation of ES cells. This is the first study to propose that nuclear transport factors should be considered as major players of cell-fate determination.

Importin-α, a receptor of cargo proteins imported into the nucleus, is classified into three subtypes, importins-α1, α3 and α5, which are differentially expressed in tissues, suggesting a possible role in tissue-specific regulation of cellular processes. Although there was an indication that various types of importins-α contribute to embryonic development and cell differentiation in Drosophila melanogaster and Caenorhabditis elegans, little has been known about how the mammalian importins-α subtypes are involved in the regulation of cell differentiation. This paper shows that the expression of importin-α subtypes is modulated during neural differentiation of mouse ES cells induced by retinoic acid. Whereas undifferentiated ES cells are characterized by high importin-α1 and low importin-α5 expression, the induction of neural differentiation causes an increase of importin-α5 expression with a concomitant decrease of importin-α1 expression. Reproducing this pattern of importin-α expression, by inhibiting importin-α1 expression with siRNAs and exogenously overexpressing importin-α5, induced neuronal differentiation in LIF-containing medium without retinoic acid. This neuronal differentiation triggered by the switch in importin-α subtype was accompanied by modulation in expression of transcription factors such as Oct4/2, Rex1 and Sox2, which play important roles in ES cell-fate determination. In addition, we demonstrated that these transcription factors are differentially imported into the nucleus by importin-α subtypes.

Fig. 1 Downregulation of importin-α1 expression and upregulation of importin-α5 expression induced neuronal differentiation of ES cells cultured in LIF+ medium.

Fig. 2 A model for cell fate determination by the interdependent regulation of nuclear transport machineries and transcription factors.

Fig. 3 Reprinted from Nature Cell Biology, 9, 72-79 (2007). Copyright 2007 Nature Publishing Group.
100 Papers Selection

* Researchers in bold italic letters are faculty members of Osaka University, and their institutions are indicated in parentheses.
* Green shaded papers are the “100 Papers Selection” with short abstracts written by the authors.
* Blue shaded papers are included in the “10 Papers Selection.”
* Red shaded papers are included in the “24 Graphics Selection.”

Humanities & Social Sciences

1. Kasugai, T. (Graduate School of Human Sciences)
   Rethinking of Economic Growth and Life Satisfaction in Post-WWII Japan-A Fresh Approach
   *Social Indicators Research, 81, 79-102 (2007)*
   - GDP has been utilized by academies and policy makers to indicate the economic well-being of the people. However, economic growth measures cannot capture fully the overall well-being of the people. This paper has tested quality of economic growth in Japan after World War II as to whether it has brought about positive outcome in the well-being of its citizens. It has shown that there are clear gaps between objective measures and subjective measures to indicate the overall well-being of the people.

2. Masumoto, K.1; Yamaguchi, M.; Sutani, K.; Tsumoto, S.; Fujita, A.1; Ionoi, M.1
   *Reactivation of Physical Motor Information in the Memory of Action Events*
   *Brain Research, 1101, 102-109 (2006)*

3. Mino, Y.
   (Graduate School of Human Sciences)
   The Political Element in the Works of W. Arthur Lewis: The 1954 Lewis Model and African Development
   *The Developing Economies, XLIV, 328-353 (2006)*
   - The Nobel Prize economist W. Arthur Lewis was actively involved in politics and policymaking. Through a careful rereading of Lewis's original texts including archival records, the article demonstrates that his emphasis on peasant-led agricultural development and advocacy of political pluralism were consistently manifested in his writings on tropical regions, especially on Africa, contrary to the conventional interpretation of his 1954 model of “Economic Development with Unlimited Supplies of Labour,” which has tended to pin much faith on top-down industrialization.

4. Nakamichi, M.; Yamada, K. (Graduate School of Human Sciences)
   Long-Term Gronning Partnerships Between Unrelated Adult Females in a Free-Ranging Group of Japanese Monkeys (Macaca fuscata)
   *American Journal of Primatology, 69, 652-663 (2007)*

   Why Commercial Banks Held Excess Reserves: The Japanese Experience of the Late 1990s
   *Journal of Money, Credit and Banking, 39, 241-257 (2007)*

   Long-run Specialization
   - Using a dynamic, international trade model, we find that even a slight difference in technology causes at least one country to specialize in an industry. Thus, a country's industrial policy that realizes even slight technological superiority in an industry either drives the other country out of this industry or leads the home country to specialize in this industry. Each country's specialization pattern is found to depend on the subjective discount rate, preference parameters, labor endowments and technological conditions.

7. Shigehiro, S. (Institute of Social and Economic Research)
   Pairwise Strategy-Proofness and Self-Enforcing Manipulation
   - In this paper, we advocate "effective pairwise strategy-proofness" for social choice rules. It is the requirement that the social choice rule should be immune to unilateral manipulation and "self-enforcing" pairwise manipulation in the sense that no agent or a pair has the incentive to betray his partner. We apply this axiom to three types of economies: public good economy, pure exchange economy, and allotment economy. Although effective pairwise strategy-proofness is seemingly a much weaker axiom than group strategy-proofness, effective pairwise strategy-proofness characterizes social choice rules that are analyzed by using different axioms in the literature.

8. Takii, K. (Osaka School of International Public Policy)
   The Value of Adaptability-Through the Analysis of a Firm’s Prediction Ability
   *Journal of Economics and Business, 59, 144-162 (2007)*
   - In this paper, we define a firm's adaptability by its ability to correctly predict and, therefore, appropriately adapt to an unexpected change in the environment. Given this definition of adaptability, we develop a model that allows for empirical examination of the impact of a firm's adaptability on its expected profits. The theory shows that a firm's adaptability can be estimated by the squared correlation between an unexpected change and the firm's reaction. The estimates show that adaptability has a positive impact on the average profit rate and the market value of a firm. We also find that an increase in risk is correlated with a rise in adaptability.
Graduate School of Science
Strong Consistency and Asymptotic Efficiency for Adaptive Quantum Estimation Problems
Identifying the state of a quantum system under investigation is one of the most fundamental issues in quantum statistics and quantum information theory. In this paper, it is shown that for an adaptive quantum estimation scheme based on locally unbiased measurements, the sequence of maximum likelihood estimators is strongly consistent and asymptotically efficient. This result settles the long-standing open question as to how one could interpret the quantum Cramer-Rao lower bound from an operational point of view.

(Institute for Protein Research)
Development of a Technique for the Investigation of Folding Dynamics of Single Proteins for Extended Time Periods
A new technique was developed for the detection of fluorescence signals from free single molecules for extended periods. 1-1-cysteine (cyt c) labeled with fluorescent dyes were slowly injected into a capillary. The fluorescence from single molecules of cyt c was imaged as traces, which reflect the time-dependent changes of the sample conformation. Intensity histograms of the traces revealed two distributions, which correspond to the unfolded and intermediate states. The technique was expected to reveal dynamics of proteins along the folding processes without artifacts caused by immobilization.

(Graduate School of Science)
Structure and Function of a Hexamer Copper-containing Nitrite Reductase

12. Ono, Y.; Shikata, T.
(Graduate School of Science)
Hydration and Dynamic Behavior of Poly(N-isopropylacrylamide) in Aqueous Solution: A Sharp Phase Transition at theLower Critical Solution Temperatures
We precisely determined the number of hydrated water molecules, \( n \), per monomer unit of poly(N-isopropylacrylamide), PNIPAm, to be 11 in aqueous solution below the lower critical solution temperature, LCST, of 32 °C using high frequency dielectric relaxation measurements up to 20 GHz. The value of \( n \) decreases dramatically at the LCST and the solution becomes turbid above that. The reason for the sharp phase transition at the LCST is the cooperative dehydration of water molecules hydrated to each PNIPAm unit.

(Institute of Scientific and Industrial Research)
Charge Transfer Through DNA Nanoscale Assembly Programmable with DNA Building Blocks

(Graduate School of Science)
Aromativity of the Pancake-Bonded Dimer of Neutral Phenalenyl Radicals as Studied by MS and NMR Spectroscopies and NICS Analysis
This paper reports on the first discovery of experimental evidences on the structure of neutral radical aggregate in a solution state at low temperature probed by cold-spray ionization mass and solution NMR spectroscopies with help of quantum chemical calculations, demonstrating an unprecedented chemical bonding of 12-centers-2-electron nature. Discussion on a local magnetic environment of the neutral radical aggregate in terms of aromaticity contributes to the breakthrough in material designs and conceptual advances in the field of molecular magnetism.

(Graduate School of Pharmaceutical Sciences)
Chemical Communications, 3606-3608 (2006)
A novel nano-sized molecular cage was developed just neutralizing of the calix[6]arene amino acid derivative by potassium carbonate. The structure of the cage was revealed at an atomic level by X-ray crystallographic analysis using synchrotron radiation. In the structure, the amino acid residues of the calixarene derivatives were intermolecularly aggregated around potassium ions. The cage, which was formed eight calixarene derivatives, possessed large inner space of which the volume was about 1 nm³.

(Graduate School of Engineering Science)
Three-Dimensional Bulk Ferromagnetism of FeRuGe2 in the Paramagnetic Phase by Soft X-Ray In-Dependemnt (700-900 eV) ARPES
pp. 15-17

17. Yokota, K.; Taniguchi, M.; Kawai, T.
(The Institute of Scientific and Industrial Research)
Control of the Electrode-Molecule Interface for Molecular Devices
We systematically investigated the bonding property and the electronic states of the monolayers of benzamidostilbene, benzamidostilbene, and benzamide disulfide. To date, Au-S bonds have often been used in molecular devices as an extension of self-assembled films. However, we found that the Au-Sic bond was more suitable for molecular devices than the other bonds. This result is expected to contribute to improving the electronic conduction property of currently developed molecular devices and to enhancing the device properties.

(Graduate School of Science)
Origin of the Two-color Irresidence in Rock Dove's Feather
The iridescence of the rock dove's neck feather has peculiar optical characteristics; the color change with angle is limited only in two colors, green and purple. We have discovered that this two-color iridescence originates from the surprisingly simple physical mechanisms: thin-layer interference and the color sensitivities of human eye.


  * (Research Center for Solar Energy Chemistry)
  **(Research Center for Ultra-Voige Electron Microscopy)
  *** (Graduate School of Engineering)
Ligand-Free Platinum Nanoparticle Encapsulated in a Hollow Porous Carbon Shell as a Highly Active Heterogeneous Catalyst in Hydrogenation Reactions
A ligand-free platinum (Pt) nanoparticle encapsulated in a hollow porous carbon shell was fabricated. Because the carbon shell not only acts as a barrier to prevent coalescence between Pt nanoparticles but also provides a void space where organic transformation occurs on the naked surface of the Pt nanoparticle, the composite material works as a robust and reusable heterogeneous catalyst for hydrogenation reactions.

31. Isokzaki, K.; Takaya, H.; Naota, T.
(Graduate School of Engineering Science)
Ultrasound-Induced Gelation of Organic Fluids with Metalated Peptides
The remotely switchable gelation of stable organic fluids could be performed by brief ultrasound irradiation of a dilute, homogeneous, and stable solution of newly designed metalated peptides. This is the first case of a reversible, remotely controlled, and rapid sol–gel transition by H-bonding aggregates. The features of the method have been examined to give precise control of the gelation rates and heat-resistant properties of the aggregates by tuning the sonication time.

32. Izumi, T.; Masazawa, T.
(Graduate School of Information Science and Technology)
Condition Adaptation in Synchronous Consensus
The consensus problem is one of the fundamental building blocks for designing highly fault-tolerant distributed systems. In the consensus problem, each process proposes a value and all nonfinal processes have to agree on a common value. This paper presents an input-sensitive solution of the consensus problem. We first introduce a criterion for measuring the difficulty of inputs to consensus algorithms, and show the consensus algorithms whose running time depends on the difficulty of actual inputs. This algorithm achieves the best time complexity of all existing synchronous consensus algorithms.

33. Kamikawa, N.; Tsuji, N.
(Graduate School of Engineering)
Quantification of Anodized Microstructures in ARB Processed Aluminum

(Institute of Scientific and Industrial Research)
Tunnel Magnetoresistance in GeCrN/AlN/GeCrN Ferromagnetic Semiconductor Tunnel Junctions
GeCrN/AlN/GeCrN trilayer structures consisting of room temperature ferromagnetic semiconductor GeCrN and nonmagnetic semiconductor AlN were grown by molecular-beam epitaxy. Well-defined hysteresis loop was observed in the magnetization vs. magnetic field curves even at room temperature. For these trilayer tunnel junction diodes, clear hysteresis characteristic of tunnel magnetoresistance effect was observed in the resistance vs. magnetic field curves at 77 K when the current flow and the applied magnetic field were perpendicular to and parallel to the junction plane, respectively.

35. Kimizuka, H.; Oguta, S.; Shibutani, Y.
* (Graduate School of Engineering Science)
** (Graduate School of Engineering)
Complete Set of Elastic Constants of α-Quartz at High Pressure: A First-Principles Study
Physical Review B, 75, 035109/1–6 (2007)
Using density functional theory (DFT), we calculate all the independent elastic constants of α-quartz under hydrostatic pressure up to 20 GPa, in order to fill a gap that presently exists between experiment and model calculations. The predicted pressure-dependent elastic behavior differs significantly from a recent Brillouin spectroscopy measurement, but is consistent with X-ray data in the literature. Our data analysis provides insight into the elastic behavior of quartz at high pressure.

* (Research Center for Solar Energy Chemistry)
** (Graduate School of Engineering Science)
Temperature-Driven Oxygenation Rate Control by Polymeric Photosensitizer
A polymeric photosensitizer, poly(NIPAM-co-IP), consisting of N-isopropylacrylamide (NIPAM) and benzoporphyrine (BP) units, demonstrates a temperature-controlled oxygenation activity in water. The system promotes a heat-induced oxygenation enhancement at <17 °C and suppression at >22 °C. This unprecedented photocatalytic oxygenation activity is triggered by a heat-induced phase transition of the polymer from coil to micelle, and then to a soluble state, cleverly controlling the stability and diffusion of singlet oxygen and the location of substrate.

(Graduate School of Engineering Science)
Evaluation of Carboxylic Acid-Induced Conformational Transitions of β-Lactoglobulin: Comparison of the Alcohol Effect on β-Lactoglobulin
Biochemical engineering journal, 28, 79–85 (2008)
Conformational transitions of bovine β-lactoglobulin (β-LG) induced by carboxylic acid were systematically studied by steady-state tryptophan (Trp) fluorescence. The behavior of β-LG denaturation depends upon the species and concentration of carboxylic acid, as well as on the pH of solutions. The hydrophobic acyl chain and hydrophilic carboxyl group of carboxylic acid molecule independently contributed to the protein denaturation. The contribution of carboxyl group of carboxylic acids was corresponding to that of hydroxyl group of alcohol. Results in this paper suggest that the conformational transition of protein due to carboxylic acids and alcohols can be explained both by hydrophobicity as well as clustering effects of each carboxylic acid and alcohol molecule.

(Institute of Scientific and Industrial Research)
Formation of Single Quantum Dot in Single-walled Carbon Nanotube Channel Using Focused-Ion-beam Technique
We fabricated a single quantum dot (QD) in a single-walled carbon nanotube (SWNT) channel using focused-ion-beam technique. We formed two tunnel barriers, constituting a single QD, by introducing two damaged regions with a separation of 50 nm into an SWNT channel. Source drain current oscillation was clearly observed at room temperature, resulting from the Coulomb blockade effect. Charging energy of the single QD was estimated to be 255 meV, which is approximately ten times larger than thermal energy at room temperature.
(Graduate School of Engineering)
Synthesis and Characterization of New Promoters Based on
CeO₂-ZrO₂-Bi₂O₃ for Automotive Exhaust Catalysts
▶ New oxidation catalysts based on CeO₂-ZrO₂-Bi₂O₃ solid solutions were synthesized for the effective oxidation of the soot particulates in the automotive emissions. The CeO₂-ZrO₂-Bi₂O₃ solid solution showed oxygen release below 300 °C. The low temperature redox behavior of the CeO₂-ZrO₂-Bi₂O₃ solid solution was promoted by the addition of silver, which is an oxygen permeable component. Furthermore, the reactivity of oxygen in the bulk of the catalyst greatly improves the soot combustion activities at low temperatures.

40. Moeer, A. J.¹,²; Wada, Y.¹; Jiang, K.-J.; Masaki, N.²; Yanoigita, S.²; Morii, S. N.²
¹(Graduate School of Engineering)
²(RIACS, Ritsumeikan University)
Efficient Dye-sensitized Solar Cells Based on a 2-thienyl-2-yl
vinyl conjugated Ruthenium Photocatalyst and a Conjugated
Polymer Hole Conductor
Applied physics letters, 89, 043309/1-3 (2006)
▶ Efficient dye-sensitized TiO₂ solar cells based on a 2-thienyl-2-yl-vinyl-conjugated ruthenium photocatalyst and a conjugated polymer poly(3,4-ethylenedioxythiophene) have been fabricated. A maximum power conversion efficiency of 2.6% is achieved when the mesoporous TiO₂ layer is 5-6 μm. The high fill factor (0.74), the open circuit voltage (0.78 V), and the linear light intensity dependence of the short circuit current density (4.5 mA cm⁻² at 100 mW cm⁻²) make these devices promising for solid state photovoltaic applications.

(Graduate School of Engineering)
Discrete Sandwich Compounds of Monolayer Palladium Sheets
Science, 313, 1104-1107 (2006)
▶ pp. 21-23

42. Nishiyama, H.; Miyamoto, I.; Hirata, Y.; Nishii, J.
(Graduate School of Engineering)
Periodic Structures Consisting of Germanium Nanoparticles in
Buried Channel Waveguides
Optics Express, 15, 2047-2054 (2007)
▶ p. 48

43. Ogi, H.; Motohisa, K.; Matsumoto, T.; Hatanaka, K.; Hiroa, M.
(Graduate School of Engineering Science)
Isolated Electrodeless High-Frequency Quartz Crystal
Microbalance for Immunosensors
Analytical Chemistry, 78, 6903-6909 (2006)
▶ p. 48

44. Ogi, H.; Fujii, M.; Nakamura, N.; Yasui, T.; Hiroa, M.
(Graduate School of Engineering Science)
Stiffened Ultrathin Pt Films Confirmed by Acoustic-Phonon
Resonances
▶ Resonances of coherent acoustic phonons were excited and detected by femtosecond light pulses for determining the normal elastic constant of ultrathin platinum films. The elastic constant increases with the decrease of the film thickness, exceeds the bulk value at the thickness near 5 nm, and significantly increases at low temperatures. It shows a correlation with the normal lattice distance. Thus, this Letter provides evidence of the stiffness enhancement in ultrathin films caused by lattice anharmonicity.

45. Ogoshi, S.; Nagata, M.; Kurowsawa, H.
(Graduate School of Engineering)
Formation of Nickel-Induced Cyclopropyl Ketone by Oxidative Addition
of Cyclopropyl Ketene. Key Intermediate in Nickel-Catalyzed Cyclodehydration
▶ Cyclopropyl phenyl ketone underwent the oxidative addition to Ni(CO)₅ to give a nickelacyclopropane, which is an key intermediate for the nickel-catalyzed homo- or heterocyclodehydration to give cyclopenantane compounds having two carbon substituents at 1,3-positions. In fact, the isolated nickelacyclopropane underwent the insertion of (E)-3-penten-2-one quantitatively to give an [Ni(en)]-catalyzed enolatoalkylnickel complex. The treatment of the [Ni(en)]-enolatoalkylnickel with carbon monoxide led to the formation of cycloaddition products.

(Graduate School of Science)
Protective Layer Formation during Oxidation of Cu₂Au(100) using
Hyperthermal O₂ Molecular Beam
▶ p. 49

41. Ozawa, N.; Roman, T.¹; Nakamichi, H.²; Diño W. A.³, ⁴; Katui, H.⁵, ⁶
¹(Graduate School of Engineering)
²(Graduate School of Science)
³(Center for the Promotion of Research on Nanoscience and Nanotechnology)
⁴(Graduate School of Science)
Quantum States of a Hydrogen Atom Arisen on Cu(100) and (110) Surfaces
▶ In this study, we investigate the behavior of adsorbed hydrogen atoms on Cu(100) and Cu(110) based on wave functions and eigen energies calculated via first principles quantum calculations and discuss the preferred adsorption sites, vibrational states, excitation paths and isotope effects shown by the adsorbed hydrogen atoms. Our calculated eigen energies of hydrogen atom motion on Cu(100) and Cu(110) are fairly in agreement with their corresponding experimental findings.

48. Piao, X.-O.¹; Horikawa, T.²; Hanzawa, H.²; Machida, K.¹⁰
¹(Center for Advance Science and Innovation)
²(Graduate School of Engineering Science)
Characterization and Luminescence Properties of Sr₂Sb₂N₂:Eu²⁺
Phosphor for White Light-emitting-diode Illumination
▶ Good-quality Eu²⁺:Sr₂Sb₂N₂:Eu²⁺ is prepared by the carbothermal reduction and nitridation method. Two main absorption bands are peaking at around 330 and 420 nm, so that the resultant phosphor is effectively excited by InGaN LEDs. The emission peak position of (Sr₂Sb₂N₂:Eu²⁺) varies from 618 to 690 nm with increasing Eu²⁺ ion content. The redshift behavior of emission spectra is due to the covalent Eu-N bond nature and the energy transfer among neighboring Eu²⁺ ions.

49. Sekimoto, T.; Kurowski, K.; Matu, H.; Yamanaka, S.
(Graduate School of Engineering)
LnPdSb (Ln = La, Gd): Promising Intermetallics with Large Carrier
Mobility for High Performance P-type Thermoelectric Materials
▶ We studied the thermoelectric properties of LaPdSb and GdPdSb. The values of the electrical resistivity were on the order of 10⁴ Ωcm, which is unexpectedly low in view of the large values observed for the thermoelectric power (60-100 μV/K). This unusually low electrical resistivity was caused by the large carrier mobility. LaPdSb indicated a large power factor of 20 μW/K²cm at 327 K and a large dimensionless figure of merit of 0.26 at around room temperature.

51. Takai, Y.; Nomaguchi, T.; Matsushita, S.; Kinura, Y. (Graduate School of Engineering) Molecular-Scale Imaging of Unstained Deoxyribonucleic Acid Fibers by Phase Transmission Electron Microscopy Applied Physics Letters, 89, 113902-1-3 (2006) The molecular structure of a deoxyribonucleic acid (DNA) fiber was observed by a phase reconstruction method using a 200 kV transmission electron microscope. The characteristic helical structure and the spacing of adjacent base pairs of DNA were partially resolved due to an improved S/N ratio and resolution enhancement. In the spherical aberration-free phase images, the arrangement of single atom-sized spots forming sinuoidal curves were sometimes observed, which seem to be the contrast originating in the sulfur atoms along the main chains.


53. Takenoto, T.; Takenoto, M. (Institute of Welding Research Institute) Dissolution of Stainless Steels in Molten Lead-free Solders Soldering & Surface Mount Technology, 18, 21 30 (2006) A dissolution test on stainless steels in molten lead-free solders was performed to determine the endurance of stainless steels used for wave soldering container materials in molten lead-free solders. The Sn-Ag lead-free solder showed faster dissolution than did the conventional Sn-Pb eutectic. A severe dissolution rate was also observed for the Sn-Zn system. This paper clarified the effect of basic factors on the dissolution rate of stainless steels in molten solder.

54. Tanaka, M.; Ohkubo, K.; Fukuzumi, S. (Graduate School of Engineering) Reductive DNA Cleavage Induced by UVA Photolirradiation of NADH without Oxygen Journal of the American Chemical Society, 128, 12372-12373 (2006) UVA irradiation of dihydrobiopterin oxidase (NADH), which plays a key role in a number of biological redox processes, results in effective DNA cleavage without oxygen via photooxidation of NADH and the subsequent reaction of hydrated electron with DNA as well as photoinduced electron transfer from NADH to DNA.


56. Taniguchi, M.; Nojima, Y.; Yokota, K.; Terao, J.; Sato, K.; Kambe, N.; Kawai, T. (The Institute of Scientific and Industrial Research) (Graduate School of Engineering) Self-Organized Interconnect Method for Molecular Devices Journal of the American Chemical Society, 128, 13962-13963 (2006) We developed an interconnect method to program three kinds of component molecules with their own functions, and to wire a molecular device between nano-scale electrodes in a self-organized manner. We wired a conductive wire and optical switching device between a 30-nm inter-electrode spacing with this method, and demonstrated their device behaviors. We expect that this interconnect method will be used as an innovative method to control various device characteristics by making various combinations of the three molecular components.


59. Tsuru, T.; Shibutani, Y. (Graduate School of Engineering) Anisotropic effects in elastic and incipient plastic deformation under (001), (110), and (111) nanoindentation of Au and Al Physical Review B, 75, 035415 (2006) Anisotropic effects under nanoindentation are investigated by quasi-static atomic simulation. Two kinds of materials namely single crystalline aluminum and copper, are taken up because of their large different anisotropic properties. As a result, copper, which has much larger anisotropic factor than aluminum, shows the remarkably anisotropic mechanical behaviors observed in the indirect load-depth relation and the indent induced stress distribution. In addition, we found that the critical mean pressure of dislocation nucleation is intrinsically constant regardless of the mechanical boundary conditions.

60. Vi Van, D.; Miyamoto, M.; Nishiyama, N.; Egashira, Y.; Ujyama, K. (Graduate School of Engineering Science) Selective Formation of para-Xylene over H-ZSM-5 Coated with Polycrystalline Silicate Crystals Journal of Catalysis, 243, 369-374 (2006) H-ZSM-5 crystals were coated with polycrystalline silicate-1 layers by a repeated hydrothermal synthesis. Applied to the alkylation of toluene with methanol, the silicate coating enhanced para-selectivity significantly up to 99.9%. The enhanced para-selectivity may originate from diffusion resistance through the inactive silicate layer on the H-ZSM-5, resulting in an increased dimension value. The silicate coating on the H-ZSM-5 catalysts not only improved para-selectivity but also prevented deactivation of the catalysts.


We solved the atomic structure of a virus-like particle isolated from a hyperthermophile, Pyrococcus furiosus, (PV) by X-ray crystallography. The coat protein of PV is encoded in a genome of P. furiosus. The PV particle showed that it retained a 7-fold icosahedral symmetry, as is often the case in spherical viruses. An examination of capsid structures suggested strong evolutionary links among PV and other various virus particles. It provides a previously undescribed example of viral relationships across the three domains of life.


We present the structure of a light-harvesting bacteriochlorophyll a assembly in chlorosomes from Chlorobium limicola determined by solid-state NMR. The structure of the assembly is determined to be a stable, well-ordered unit that provides insights into the function and evolution of light-harvesting systems in photosynthetic bacteria.


A published article on the role of a novel Adenylate Cyclase-activating Polypeptide in Associated with Schizophrenia Molecular Psychiatry, advance online publication, 21 March 2007 (in press)

We provide evidence that genetic variants of putative adenylate cyclase-activating polypeptides (PACAP), a neuropeptide, and its receptor, PAC1, are associated with schizophrenia. The allele of the PACAP gene overexpressed in schizophrenia was associated with reduced hippocampal volume and poorer memory performance, neurobiological traits related to risk for schizophrenia. Abnormal behaviors in PACAP knockout mice, including elevated locomotor activity and deficits in prepulse inhibition, were reversed by an antipsychotic treatment. These data suggest that alterations in PACAP signaling might contribute to the pathogenesis of schizophrenia.


We identified a novel interacting protein of Disrupted-In-Schizophrenia 1 (DISC1), which has relation to major psychiatric disorders, termed DISC1-binding zinc finger protein (DBZ). PACAP caused markedly the dissociation between DISC1 and DBZ in PC12 cells and the inhibition of the DISC1-DH2 dissociation reduced the neurite length in PC12 cells after PACAP stimulation and in primary cultured hippocampal neurons. The present results provide some new molecular insights into the mechanisms of neuronal development and neuropsychiatric disorders.


Here, we investigated the cerebral oscillatory changes during emotional word reading. We found that the emotional connotations increase the activities in the Broca area and the anterior cingulate area. Negative emotional connotations activate the left prefrontal cortex, whereas positive emotional connotations activate the right. Negative and positive emotional words may be processed by different mechanisms. This is the first report documenting, with magnetoencephalography how and where the emotional connotations in words effect on the brain.


Structural Similarity between the Flagellar Type III ATPase Fil and F-ATPase Subunits Proceedings of the National Academy of Sciences of the United States of America, 104, 255-256 (2007)

Construction of the bacterial flagellum in the cell exterior is a highlyordered process. The component proteins are sequentially exported through the central channel of the growing flagellum by the flagellar type III export apparatus in an ATPase-driven manner. We determined the structure of Fil, the ATPase component, at 2.4 Å resolution. The whole structure shows a striking similarity to the subunits of F1-ATPase, implying evolutionary relation between the flagellum and F1-ATPase and a similarity in the mechanism between Fil and F-ATPase.
71. Ito, T., Fujii, Y., Takahashi, K.; Azuma, J. (Graduate School of Pharmaceutical Sciences)
Degradation of NFAT5, a Transcriptional Regulator of Osmostic Stress-Related Genes, is a Critical Event for Dockorulin-Induced Cytotoxicity in Cardiac Myocytes
► NFAT5, a novel member of the NFAT family proteins, functions as a transcriptional factor responsible for the adaptation to hyperosmotic stress. In this paper, we found that anti cancer drug doxorubicin which is also known as a cardiotoxic agent enhances the degradation of NFAT5 through the proteasome activation and downregulates its targeted genes in cultured cardiomyocytes. Inhibition of NFAT5 by using either dominant-negative form of NFAT5 or siRNA decreased myocyte viability. Thus, NFAT5 is a positive regulator of cardiomyocyte survival.

3D Structure of Amyloid Protifilaments of β2-Microglobulin Fragment Probed by Solid-State NMR
Proceeding of the National Academy of Sciences of the United States of America, 103, 18119-18124 (2006)
► p. 52

"(Graduate School of Science)
Structure of the Human GINS Complex and Its Assembly and Functional Interface in Replication Initiation
Nature Structural & Molecular Biology, 14, 398-396 (2007)
► The eukaryotic GINS complex is composed of four subunits, Sld5, Psf1, Psf2 and Psf3, and is essential for the establishment of DNA replication forks and replisome progression. Crystallographic studies showed that they pack together into a two-layered trapezoidal structure, with Sld5 and Psf1 on one level above Psf2 and Psf3 respectively on the other. The data suggest that the core complex enables a stable platform for the C-terminal domain of Pst1 to act as a key interaction interface for other proteins in the replication-initiation process.

Allelic Gene Regulation of Pch3 and μ Clusters Involving Both Monolecular and Biallelic Expression in Single Purkinje Cells
► The Pch3 and μ genes encode multiple membrane-spanning proteins. We investigated total allelic gene regulation in the Pch3-μ and μ clusters in single Purkinje cells. Using split single-cell RT-PCR analysis, almost all of the Purkinje cells biosynthetically expressed all the C-type isomers, whereas the Pch3-μ and μ-β isomers showed both monolecular and biallelic expression. The multiple gene regulation in the Pch3-μ and μ clusters had a potential mechanism for increasing the diversity of individual neurons in the brain.

"(Graduate School of Frontier Biosciences)
Toll-Like Receptor-Mediated Regulation of Zinc Homeostasis Influences Dendritic Cell Function
Nature Immunology, 7, 971-977 (2006)
► pp. 30-32

Aurora Kinase is Required for Chromosome Segregation in Tobacco BY-2 Cells
► p. 52

77. Kurooka, M., Kaneda, Y. (Graduate School of Medicine)
Inactivated Sendai Virus Particles Eradicate Tumors by Inducing Immune Responses through Blocking Regulatory T Cells
► We report that inactivated Sendai virus particle (HVJ-E) alone has vigorous anti-tumor effects. Intra-tumor injections of HVJ-E eradicated tumors in 60-80% of mice. With HVJ-E, dendritic cells (DCs) were matured, immune cells were infiltrated into tumor bed, and tumor-specific cytotoxic T lymphocytes were activated. Moreover, IFN-γ secreted from HVJ-E-matured DCs rescued effector T cell proliferation from regulatory T cell (Treg)-mediated suppression. Thus, by both enhancing anti-tumor immunity and attenuating Treg-mediated suppression, HVJ-E may open the way for effective cancer immunotherapy.

(Research Institute for Microbial Diseases)
Fatty Acid Remodeling of GPI-anchored Proteins Is Required for Their Raft Association
Molecular Biology of the Cell, 18, 1497-1506 (2007)
► The nature that GPI-anchored proteins (GPIs) are enriched into lipid rafts is critical, because rafts modulate various biological functions of GPI-APs. How are GPI-APs accumulated in rafts? Here we report that GPI-APs become competent to be incorporated into lipid rafts by PGAP3 and PGAP2-mediated fatty acid remodeling, replacement of the unsaturated chain with saturated one in FL membrane. The remodeling occurs mostly in the Golgi and requires the preceding PGAP3-mediated desaturation from monounsaturated GPI-APs in the ER.

Synergistic Effects of Recombinant Human Soluble Thrombomodulin and Fluid-Volume Resuscitation in a Rat Lethal Crush Injury Model
Shock, 26, 591-596 (2006)
► Severe crush injury results in a high mortality rate due to acute circulatory failure and hyperkalemia. We evaluated whether administration of prolylactic recombinant human soluble thrombomodulin (rHTM) and/or fluid volume resuscitation prior to reperfusion ameliorates severe crush injury in rats, whose both hindlimbs were compressed. Combined administration of rHTM and volume resuscitation significantly decreased hemocoagulation and hyperkalemia, and also improved the serum interleukin-6 level and mortality. We propose that this prolylactic combined therapy may be effective for severe crush injury patients.

(Graduate School of Frontier Biosciences)
Crystal Structures of a Multidrug Transporter Reveal a Functionally Rotating Mechanism
► pp. 33-35
81. Nagai Y.1; Imai T.; Popiel H.A.2; Fujikake N.3; Hasegawa K.; Urade Y.; Goto K.4; Nakai H.; Tod A.5
1Graduate School of Medicine; 2Institute for Protein Research
A Toxic Monomeric Conformer of the Polyglutamate Protein
> p. 33

82. Nakagawa, T.1; Uozumi, N.2; Nakano, M.3; Mizuno Horibara, Y.4; Okuyama, N.4; Taguchi, T.5; Gu, J.6; Kondo, A.7; Taniguchi, N.8; Miyoshi, E.9
1Graduate School of Medicine
2Research Institute for Microbial Diseases
Fucosylation of N-Glycans Regulates the Secretion of Hepatic Glycoproteins into Bile Ducts
> Fucosylated alpha-fetoprotein (AFP) is a highly specific tumor marker for hepatocellular carcinoma (HCC). However, the molecular mechanism by which prostatic acid phosphatase (PAP) increases in patients with HCC is largely unknown. This paper shows that the fucosylation of glycoproteins including AFP would be a possible signal for secretion into bile ducts in normal liver. Our results suggest that a disruption in this system might involve an increase in fucosylated AFP in the serum of patients with HCC.

Graduate School of Medicine
The Role of Autophagy in Cardiomyocytes in the Basal State and in Response to Hemodynamic Stress
> pp. 36-38

84. Nakamura, S.1; Oda, M.; Kato, S.; Ueda, S.; Uchiyama, S.7; Yoshida, T.8; Kobayashi, Y.9; Okubo, T.10
1Graduate School of Pharmaceutics
2Graduate School of Engineering
Apoptosis- and Hypoxia-Induced Apoptosis in Pseudomonas sp. B-0831: A NUCLEUS TRANSITION INDUCED BY COENZYME BINDING
> We performed an in vitro analysis of the crystal of 3-hexosyltransferase dehydrogenase complexed with NADH. The resulting structure showed that the enzyme exists as a structural homodimer composed of apo- and holo-subunits. A distinct structural difference between them was found in the substrate binding loop region, where the structure in the apo-subunit is disordered while that in the holo-subunit consists of two α helices. This fact proved that the NADH binding allows the helical structures to form the substrate binding pocket even in the absence of the substrate.

85. Nakamura, T.1; Arui, Y.; Umehara, H.2; Masahara, M.; Kimura, T.3; Taniguchi, S.; Sekimoto, T.4; Ikawa, M.5; Yoneda, Y.6; Okabe, M.7; Tanaka, S.; Shiota, K.; Nakano, I.8
1Graduate School of Frontier Biosciences
2Research Institute for Microbial Diseases
4Genome Information Research Center
PGC7/Stella Promotes Apoptotic DNA Damage in Early Embryogenesis
Nature Cell Biology. 9, 64-71 (2007)
> p. 53

Graduate School of Science
Crystal Structures of a Glutamytransferase from Escherichia coli, a Key Enzyme in Glutathione Metabolism, and its Reaction Intermediate
> p. 54

87. Okada, Y.; Ueshin, Y.; Isotani, A.; Saito-Fujita, T.
Research Institute for Microbial Diseases
Complementation of Placental Defects and Embryonic Lethality by Trophoblast-Specific Lentinul Lactim Gene Transfer
> p. 54

88. Oshima, K.; Takezawa, Y.; Sugimoto, Y.; Kobayashi, T.; Thomas C. Irving; Wakahayashi, K.
Graduate School of Engineering Science
Axial Dispositions and Conformations of Myosin Crossbridges Along Thick Filaments in Relaxed and Contracting States of Vertebrate Striated Muscles by X-Ray Fiber Diffraction
> Muscle contraction takes place by the interaction of motor proteins, actin and myosin, coupled by the hydrolysis of ATP. We have investigated structural changes of myosin crossbridges in the transition of relaxed state to isometric contraction by x-ray fiber diffraction. At the relaxed state, head-head interactions of crossbridges occurred, relating to a switching off mechanism for the actomyosin interaction. During contraction, one head of a crossbridge oriented more perpendicularly to the actin filament and the partner head turned axially, leading to the concept that two heads of a crossbridge could play alternate roles for the force generation.

Graduate School of Medicine
Integration of Integrin αβ3 with Nectin: Implication in Cross-Talk Between Cell-matrix and Cell-cell Junctions
> An immunglobulin like cell cell adhesion molecule nectin plays an essential role in the formation of cell-cell junctions. In this study, we have newly demonstrated that nectin interacted with a cell-matrix adhesion molecule integrin αβ3 at the cell-cell adhesion sites. These two cell adhesion molecules cooperatively promote the formation of cell-cell junctions through the activation of a tyrosine kinase Src and small G proteins Rap1, Cdc42, and Rac. These findings provide a novel mechanism insights into the cross-talk between cell-cell and cell-matrix junctions.

Graduate School of Medicine
Lung Tissue Engineering Technique with Adipose Stromal Cells Improves Surgical Outcome for Pulmonary Emphysema
> Hepatocyte growth factor (HGF) is a potent mitotrophic and regenerative factor, and adipose tissue-derived stromal cells (ASCs) produce a large amount of angiogenic factors including HGF. In a rat emphysema model, ASCs cultured on a polyglycolic acid felt (PGA) sheet was implanted onto the remnant lung tissue following lung volume reduction surgery. Both alveolar and vascular regeneration were significantly accelerated as compared with surgery alone. ASCs with PGA felt sheet might be a promising strategy of regenerative medicine of the lung.

91. Suzuki, K.1; Okano, T.2; Yamamoto, M.3; Pasterkamp, R.J.; Takekawa, H.4; Takamatsu, H.5; Kato, I.6; Takagi, J.7; Rennert, P.D.; Koloddin, A.L.; Kumanogoh, A.8; Rihm, H.9
1Graduate School of Medicine
2Institute for Protein research
Semaphorin 7A Initiates T-cell-mediated Inflammatory Responses through α4β1 integrin
> pp. 39-41
92. Takaoka, K.; Yamamoto, M.; Shiratori, H.; Meno, C.; Rossant, J.; Saitoh, Y.; Hamaeda, H.
(Graduate School of Frontier Biosciences)
The Mouse Embryo Autonomously Acquires Anterior-Posterior Polarity at Implantation

**(Graduate School of Medicine)** *(Health Care Center)*
*(Research Institute for Microbial Research)*
Smooth Muscle α-Actin Deficiency In Myofibroblasts Leads to Enhanced Renal Tissue Fibrosis

(Graduate School of Medicine)
Prostaglandin D2 Protects Neonatal Mouse Brain from Hypoxic Ischemic Injury
The Journal of Neuroscience, 27, 4301-4314 (2007)

95. Terao, Y.; Yamaguchi, M.; Hanada, S.; Kowatasu, S.
(Graduate School of Dentistry)
Multifunctional Glyceraldehyde-3-phosphate Dehydrogenase of Streptococcus pyogenes Is Essential for Evasion from Neutrophils

**(Graduate School of Medicine)** *(Research Institute for Microbial Diseases)*
Neprocycin, a Novel HEV Sialomucin, Mediates L-selectin-dependent Lymphocyte Rolling and Promotes Lymphocyte Adhesion under Flow

(Graduate School of Dentistry)
Leukotriene B4 and Lipoxin A4 Are Regulatory Signals for Neural Stem Cell Proliferation and Differentiation

(Graduate School of Medicine)
App-1, a Novel Protein Inducing Cytochrome P-450-dependent but Bax/Bak-related Channel-independent Apoptosis

**(Graduate School of Medicine)** *(Graduate School of Frontier Biosciences)*
Triggering Neural Differentiation of ES Cells by Subtype Switching of Importins
Nature Cell Biology, 9, 72-79 (2007)

**(Graduate School of Medicine)** *(Graduate School of Pharmaceutical Sciences)*
Antisense to Cyclin D1 Inhibits VEGF-Stimulated Growth of Vascular Endothelial Cells: Implication of Tumor Vascularization
Circulation Research, 12, 4720-4729 (2006)

In cultures of human umbilical vein endothelial cells (HUVEC), adenoviral antisense to cyclin D1 (AS CyD1) inhibited growth even in the presence of VEGF and suppressed in vitro tube formation by VEGF-treated HUVEC and in vivo microvessel formation. The xenografts treated with AS CyD1 showed less vessel density and displayed smaller tumor size in eithen cancer cell lines. Our results suggest that AS CyD1 could be potentially useful for targeting both cancer cells and their microenvironment of tumor vessels.